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(54) **NONDIFFRACTING BEAM DETECTION DEVICES FOR THREE-DIMENSIONAL IMAGING**

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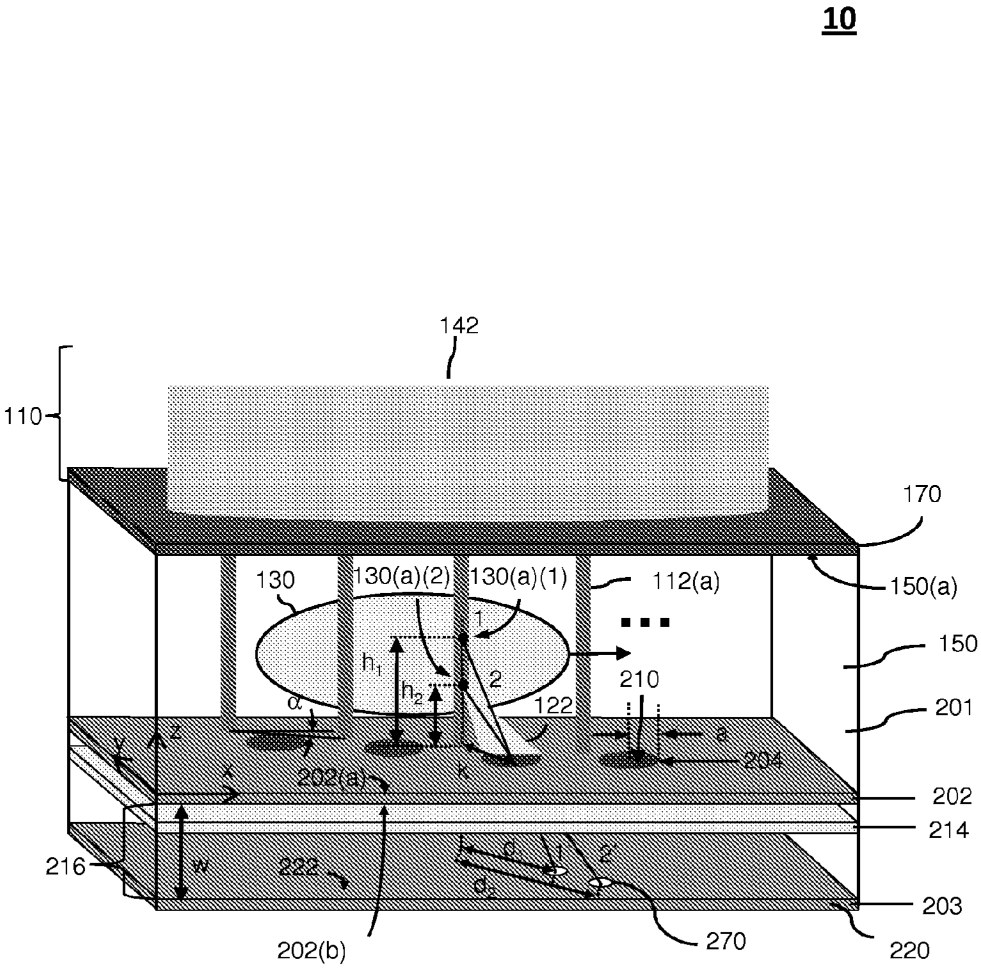
(57) **ABSTRACT**

Embodiments of the present invention relate a nondiffracting beam detection module for generating three-dimensional image data that has a surface layer having a first surface and a light transmissive region, a microaxicon, and a light detector. The microaxicon receives light through the light transmissive region from outside the first surface and generates one or more detection nondiffracting beams based on the received light. The light detector receives the nondiffracting beams and generates three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received. In some cases, the light detector can localize a three-dimensional position on the object associated with each detection nondiffracting beam received. In other cases, the light detector can determine perspective projections based on the detection nondiffracting beams received and generates the three-dimensional image data, using tomography, based on the determined perspective projections.



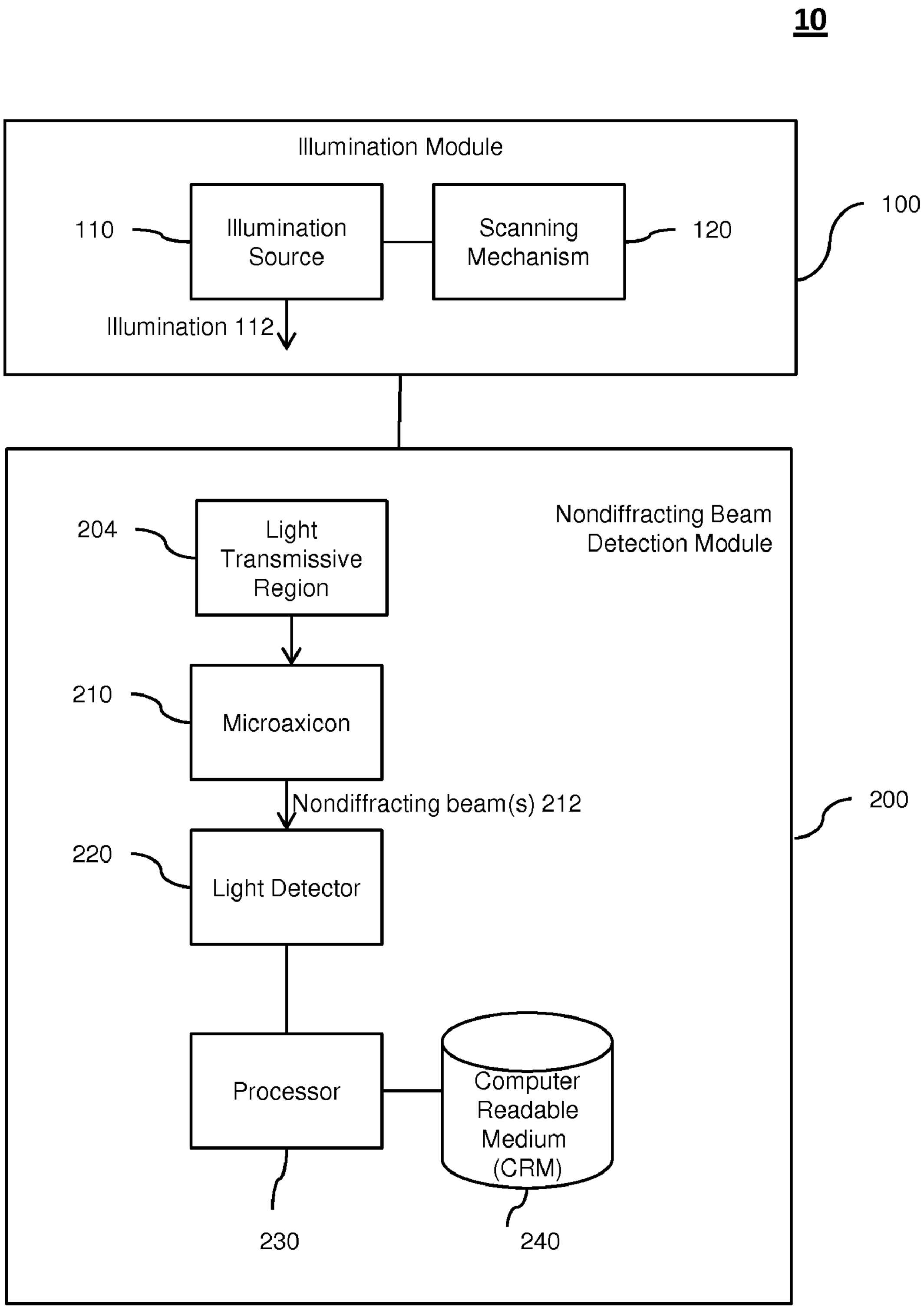

10
Illumination Module
110
Illumination Source
Scanning Mechanism
120
100
Illumination 112
204
Light Transmissive Region
Nondiffracting Beam Detection Module
210
Microaxicon
Nondiffracting beam(s) 212
200
220
Light Detector
Processor
Computer Readable Medium (CRM)
230
240

FIG. 1

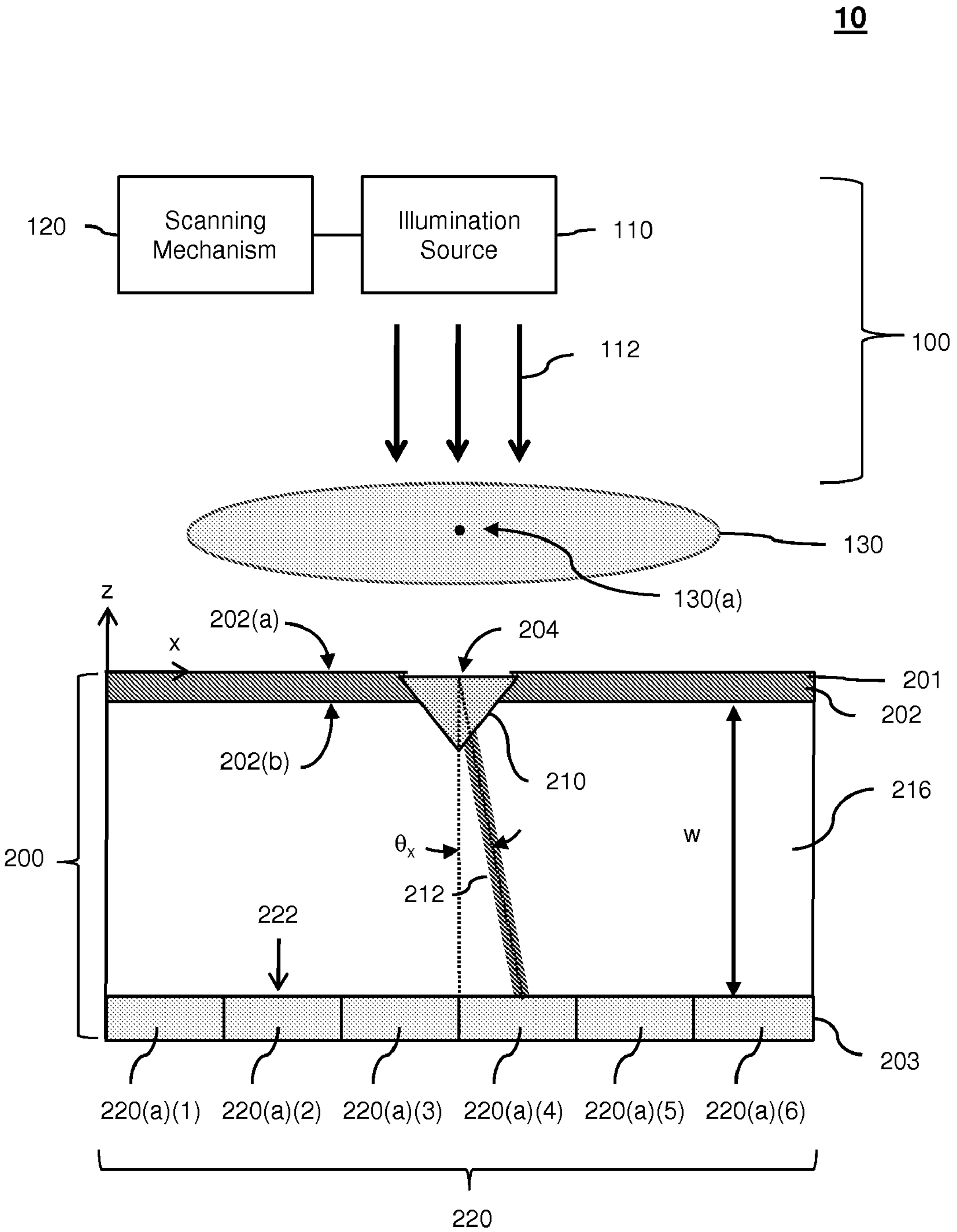
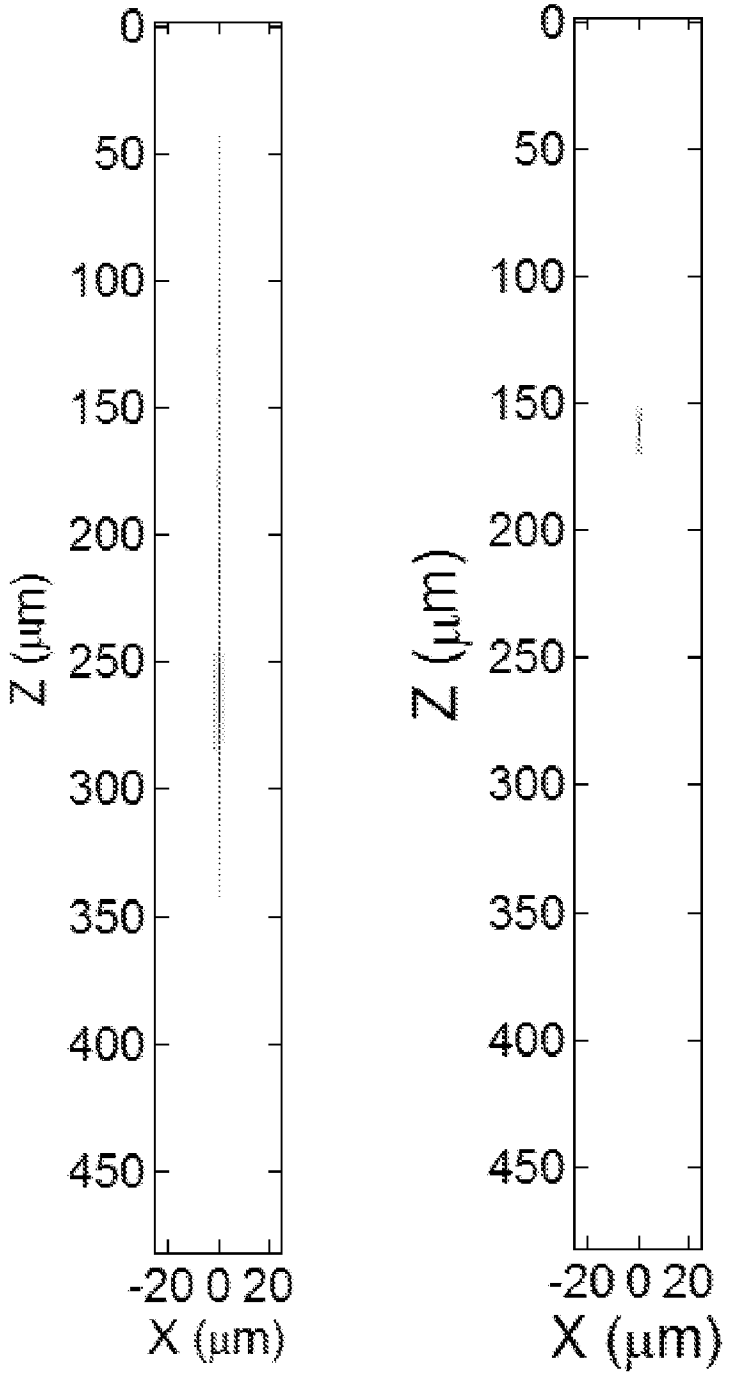

10
Scanning Mechanism
Illumination Source
120
110
112
100
130
130(a)
z
x
202(a)
204
201
202
202(b)
210
216
w
200
θ_x
212
222
203
220(a)(1)
220(a)(2)
220(a)(3)
220(a)(4)
220(a)(5)
220(a)(6)
220

FIG. 2


0
50
100
150
200
250
300
350
400
450
Z (µm)
-20 0 20
X (µm)
0
50
100
150
200
250
300
350
400
450
Z (µm)
-20 0 20
X (µm)

(a) (b)

FIG. 3

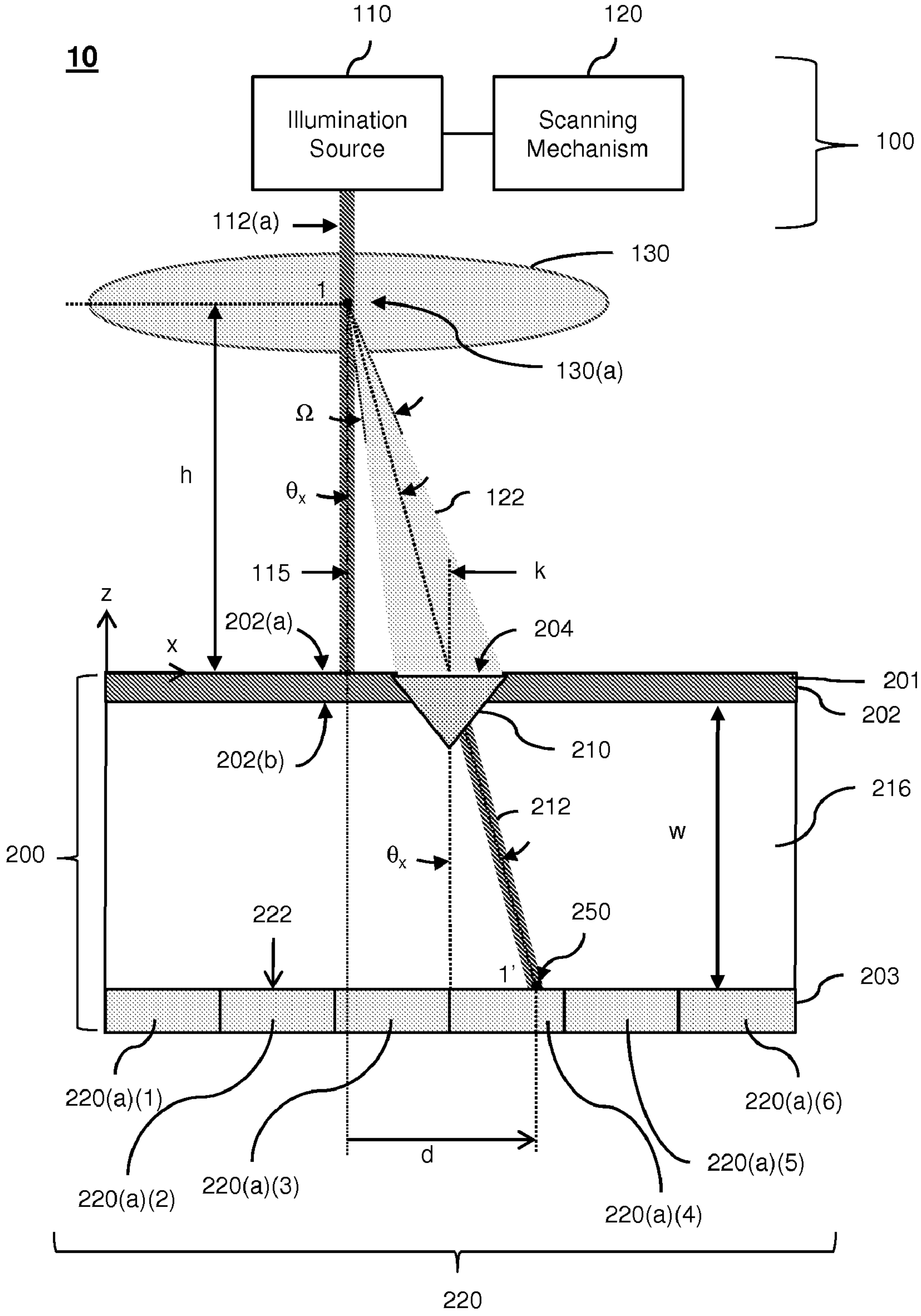

10
110
120
Illumination Source
Scanning Mechanism
100
112(a)
130
1
130(a)
Ω
h
θx
122
115
k
z
x
202(a)
204
201
202
202(b)
210
200
212
216
w
θx
222
250
1'
203
220(a)(1)
220(a)(2)
220(a)(3)
220(a)(4)
220(a)(5)
220(a)(6)
d
220

FIG. 4

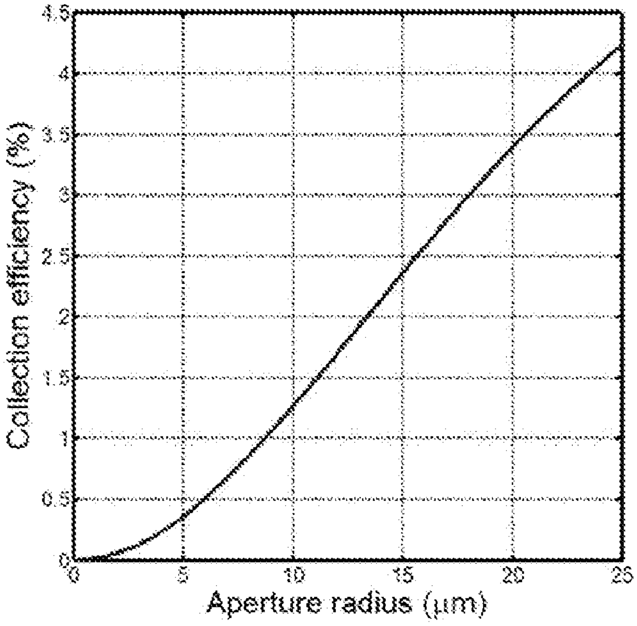

Collection efficiency (%)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Aperture radius (μm)

FIG. 5(a)

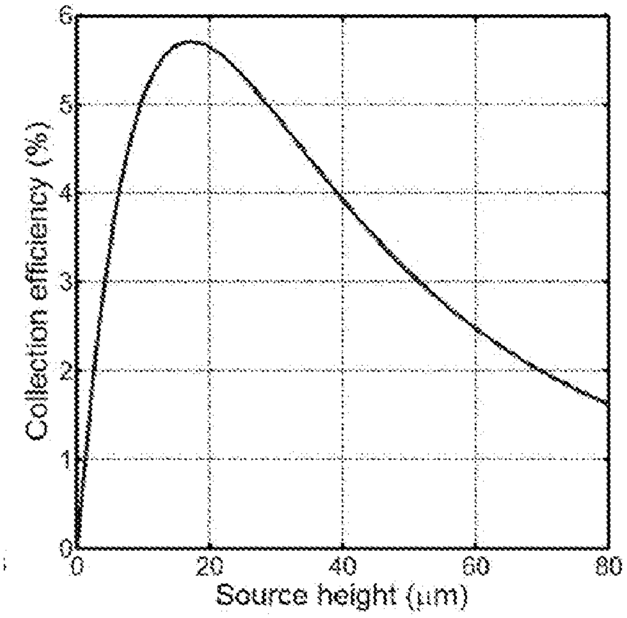

Collection efficiency (%)
6
5
4
3
2
1
0
0
20
40
60
80
Source height (μm)

FIG. 5(b)

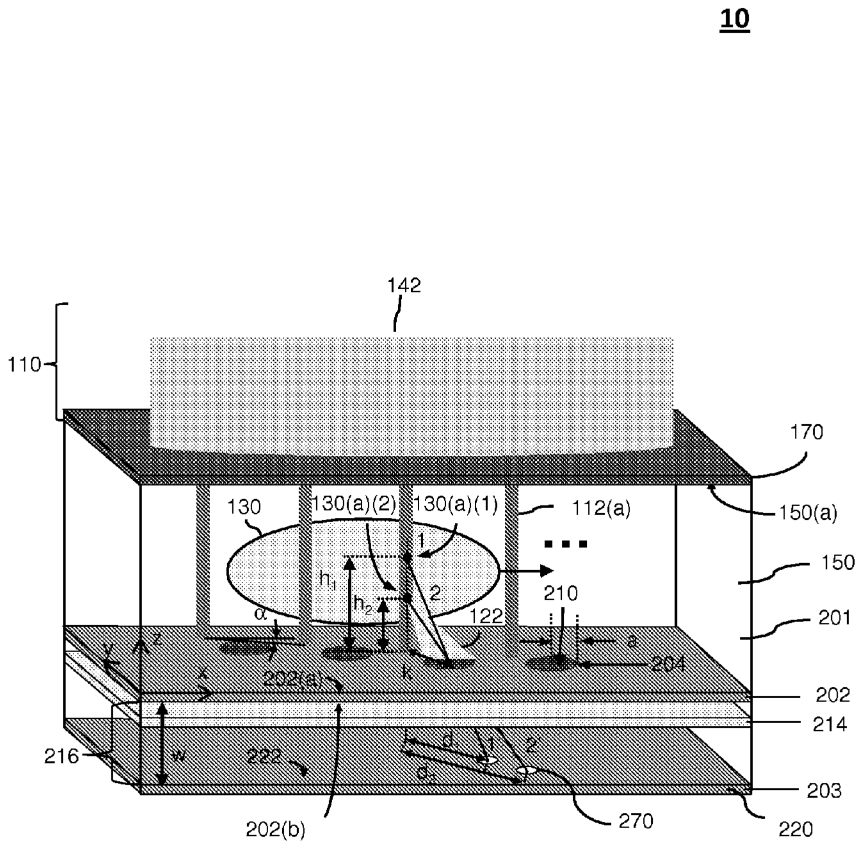

10
142
110
170
150(a)
150
201
130
130(a)(2)
130(a)(1)
112(a)
1
2
h_1
h_2
α
122
210
a
204
k
z
y
x
202(a)
202
214
216
w
222
d_1
d_2
1
2
203
270
220
202(b)

FIG. 6

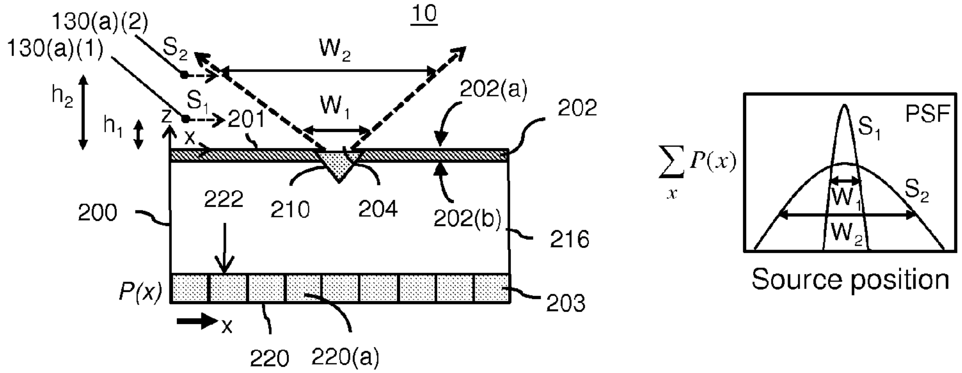


FIG. 7(a)

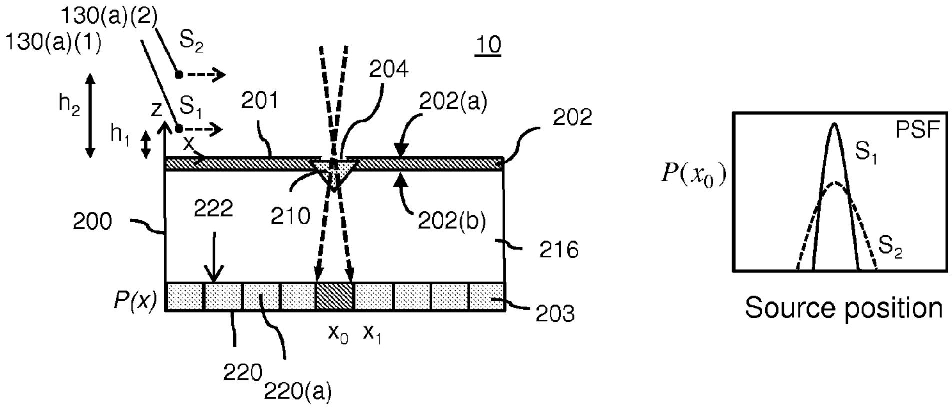


FIG. 7(b)

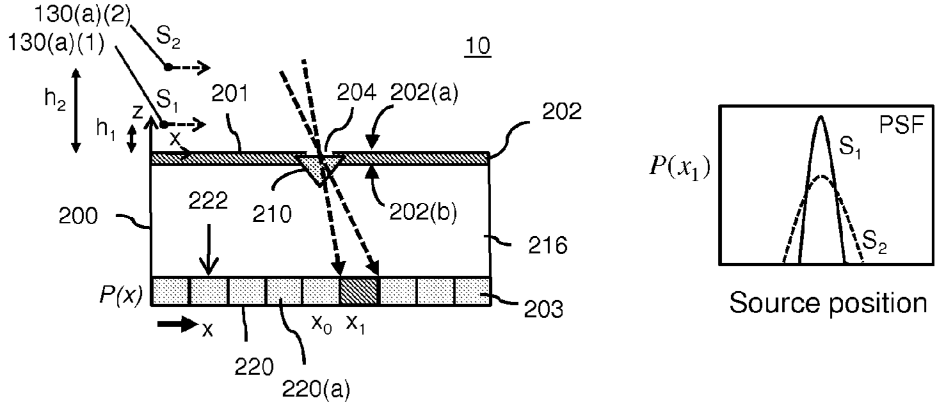


FIG. 7(c)

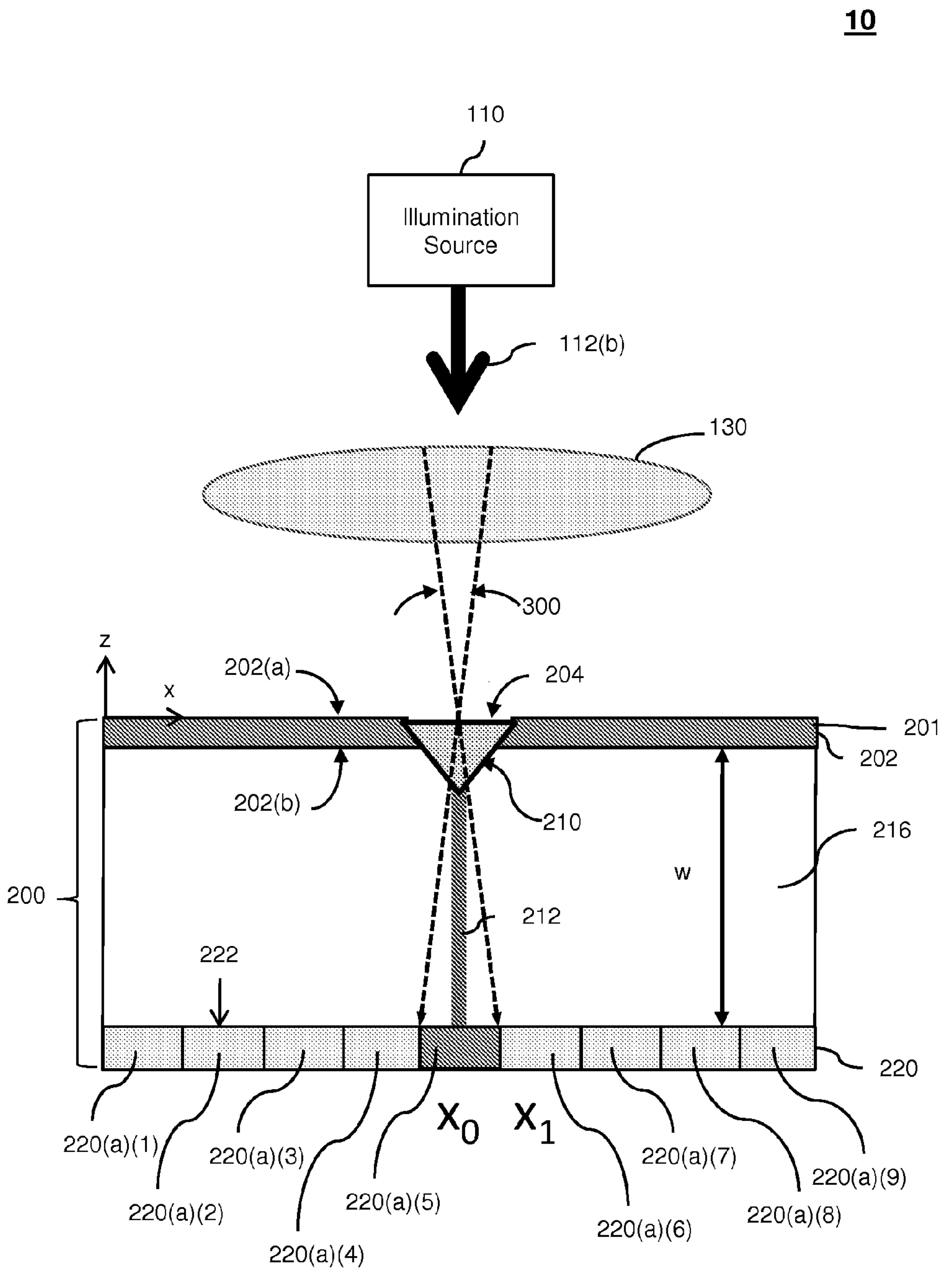

10
110
Illumination Source
112(b)
130
300
z
x
202(a)
204
201
202
202(b)
210
216
w
200
212
222
220
220(a)(1)
220(a)(2)
220(a)(3)
220(a)(4)
220(a)(5)
X_0
X_1
220(a)(6)
220(a)(7)
220(a)(8)
220(a)(9)

FIG. 8

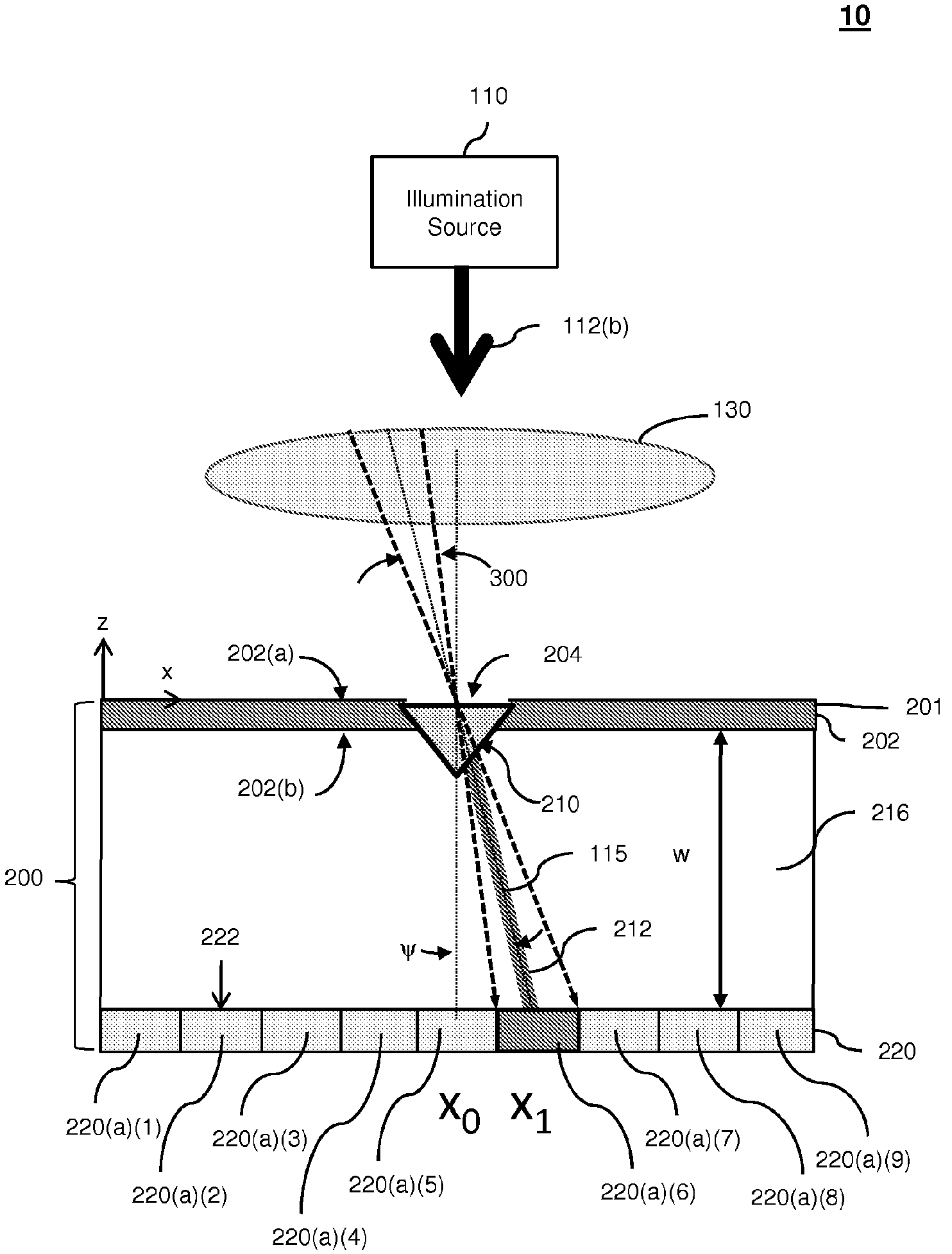

10
110
Illumination Source
112(b)
130
300
z
x
202(a)
204
201
202
202(b)
210
216
200
115
w
212
222
ψ
220
220(a)(1)
220(a)(2)
220(a)(3)
220(a)(4)
220(a)(5)
X_0
X_1
220(a)(6)
220(a)(7)
220(a)(8)
220(a)(9)

FIG. 9

10

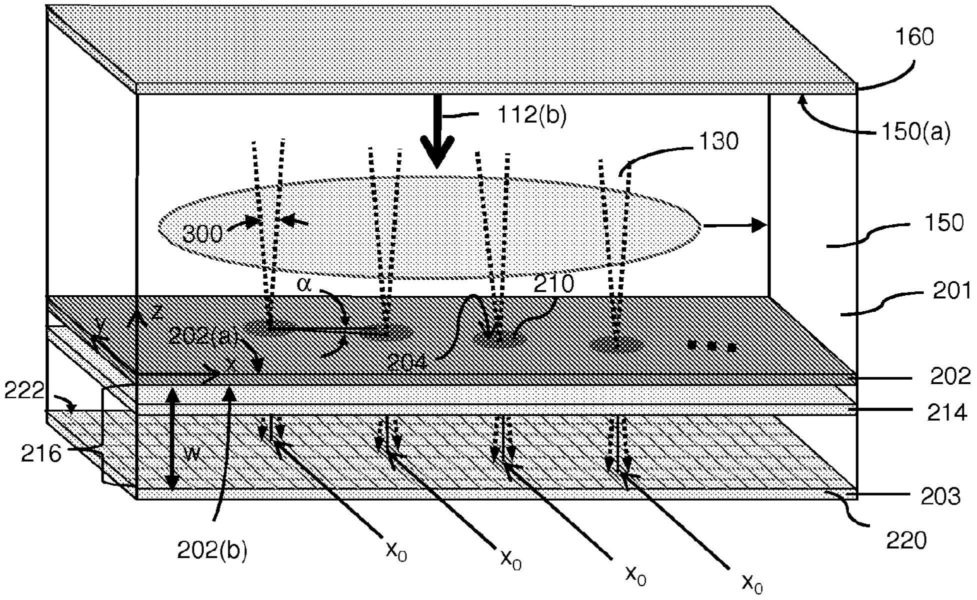

160
112(b)
150(a)
130
300
150
α
210
201
z
y
202(a)
204
x
202
222
214
216
w
203
202(b)
x0
x0
x0
x0
220

FIG. 10

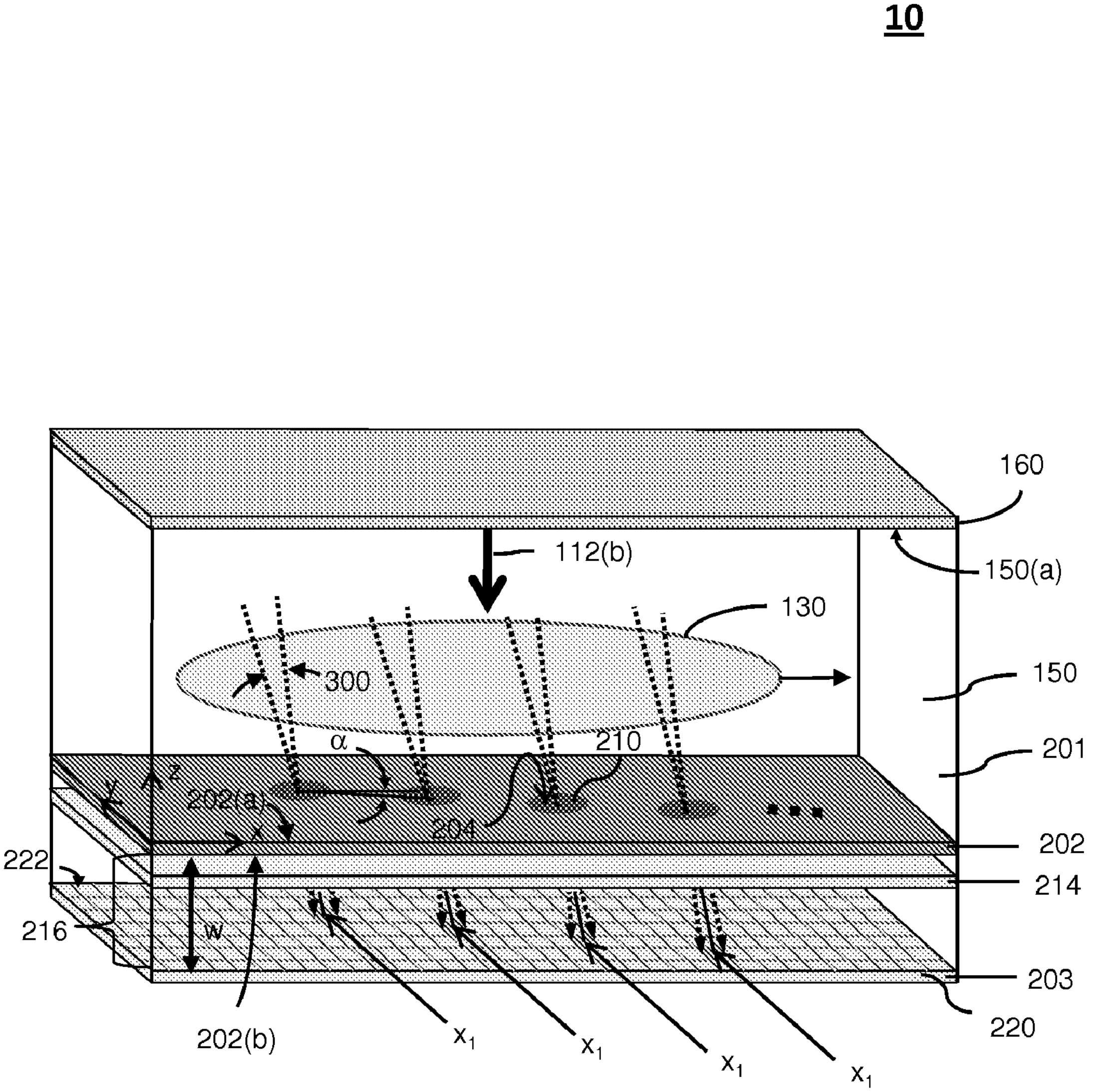

10
160
112(b)
150(a)
130
150
300
201
α
210
z
y
202(a)
x
204
202
222
214
216
w
203
202(b)
x1
x1
x1
x1
220

FIG. 11

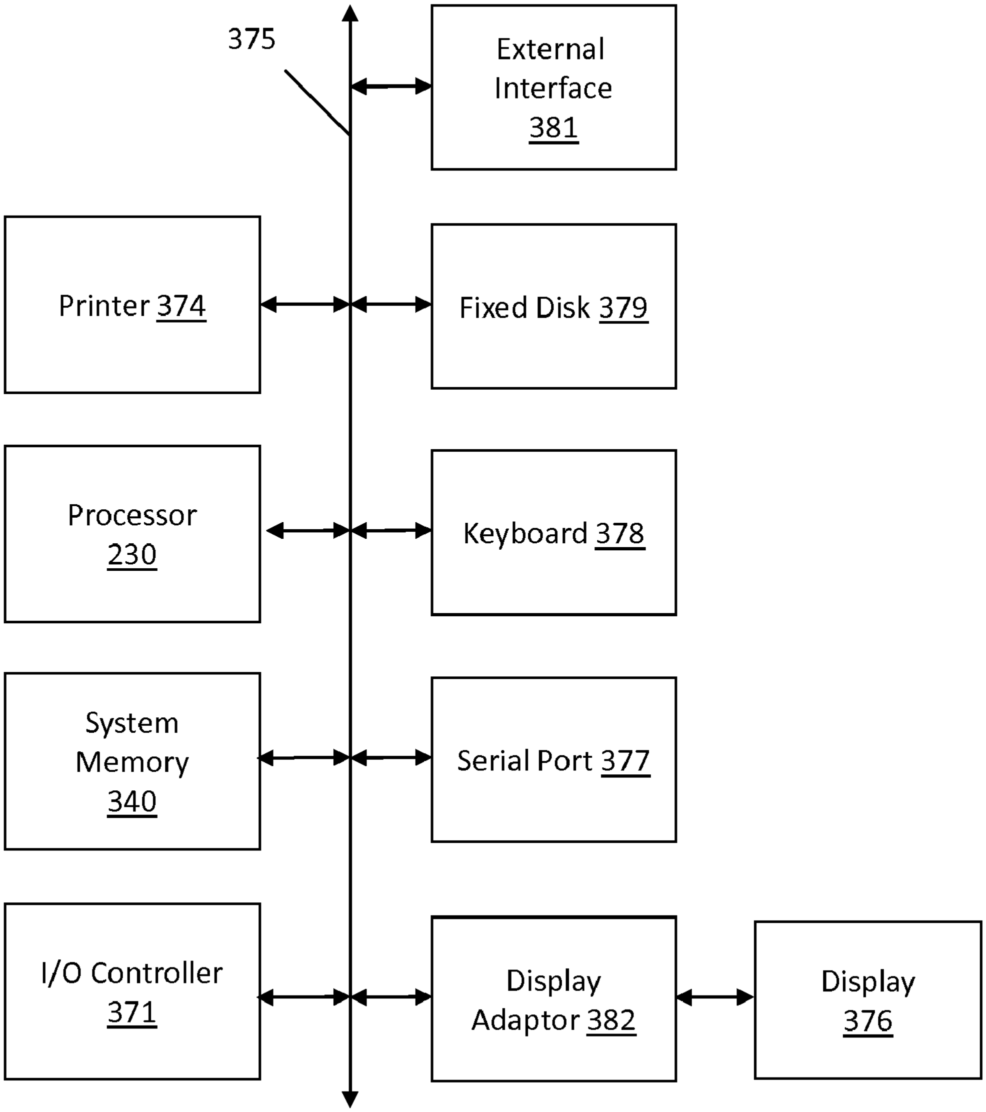

375
External Interface 381
Printer 374
Fixed Disk 379
Processor 230
Keyboard 378
System Memory 340
Serial Port 377
I/O Controller 371
Display Adaptor 382
Display 376

FIG.12

NONDIFFRACTING BEAM DETECTION DEVICES FOR THREE-DIMENSIONAL IMAGING

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This is a non-provisional application of, and claims priority to, U.S. Provisional Patent Application No. 61/307,324 entitled "Three Dimensional Imaging with Optofluidic Microscopes" filed on Feb. 23, 2010 and U.S. Provisional Patent Application No. 61/307,328 entitled "High-Resolution Microscopy with a Wide Field and Extended Focus" filed on Feb. 23, 2010. These provisional applications are hereby incorporated by reference in their entirety for all purposes.

[0002] This non-provisional application is related to the following co-pending and commonly-assigned patent application, which is hereby incorporated by reference in its entirety for all purposes:

[0003] U.S. patent application Ser. No. ______ entitled "High Resolution Imaging Devices with Wide Field and Extended Focus" filed on ______.

BACKGROUND OF THE INVENTION

[0004] Embodiments of the present invention generally relate to imaging devices. More specifically, certain embodiments relate to nondiffracting beam detection devices for generating high resolution three-dimensional bright field and photoluminescence (e.g., fluorescence or phosphorescence) images.

[0005] Optical microscopes have become indispensable tools for modern scientific investigations. They have evolved to appear in different forms and modalities for catering to a wide variety of imaging needs. For example, bright field, dark field, epifluorescence, confocal, two photon, phase contrast, and differential interference contrast (DIC) are now well-known names in biological research labs. In the future, modalities such as stimulated emission and depletion, structured illumination, and photo activated localization are expected to join the list. While these techniques offer improved capabilities, unfortunately, these improvements almost always come with systems that are bulky, complicated, and expensive.

[0006] In addition, conventional microscopes can only focus on a single plane at a setting. Typically, a knob is used to move the optics up and down to focus on the plane of interest.

BRIEF SUMMARY OF THE INVENTION

[0007] Embodiments of the present invention relate to nondiffracting beam detection devices (NBDs) for three-dimensional imaging used in applications such as microscopy or photography.

[0008] One embodiment is directed to an NBD for three-dimensional imaging, comprising a nondiffracting beam detection module (NBDM) and a processor. The NBDM comprises a surface layer having a first surface, a second opposing surface, and a light transmissive region. The NBDM also comprises a microaxicon in the light transmissive region. The microaxicon is configured to receive light through the light transmissive region and generate one or more detection nondiffracting beams based on the received light. The NBDM further comprises a light detector that receives the one or more detection nondiffracting beams and generates three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received. The processor is in communication with the light detector to receive the three-dimensional image data from the light detector. The processor generates a three-dimensional image of a portion of the object based on the three-dimensional image data received. In some cases, the NBD can also include an illumination source for generating an illuminating nondiffracting beam through the object. In these cases, the light detector can localize a three-dimensional position of one or more point sources on the object, associated with the one or more detection nondiffracting beams received. The processor generates the three-dimensional image using the three-dimensional position of the one or more point sources. In other cases, the NBD can also include an illumination source that provides uniform illumination outside the first surface. In these cases, the light detector also determines one or more perspective projections having different viewing angles. The processor estimates, using tomography, the three-dimensional image of the object from the one or more determined perspective projections.

[0009] Another embodiment is directed to an NBDM for generating three-dimensional image data that comprises a surface layer having a first surface and a light transmissive region. The NBDM also comprises a microaxicon that receives light through the light transmissive region from outside the first surface and generate one or more detection nondiffracting beams based on the received light. The NBDM also comprises a light detector that receives the one or more detection nondiffracting beams and generates three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received.

[0010] Another embodiment is directed to an NBD for three-dimensional imaging comprising a body having a surface layer having a first surface, a plurality of NBDMs, and a processor. Each NBDM comprises a light transmissive region in the surface layer, a microaxicon in the light transmissive region, and a light detector. The microaxicon receives light through the light transmissive region and generates one or more detection nondiffracting beams based on the received light. The light detector receives the one or more detection nondiffracting beams and generates three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received. The processor generates a three-dimensional image of the object based on the three-dimensional image data received from the light detectors of the plurality of nondiffracting beam detection modules.

[0011] Another embodiment is directed to an optofluidic NBD for three-dimensional imaging. The optofluidic NBD comprises a body including a fluid channel having a surface layer with a first surface. The optofluidic NBD also comprises an array of light transmissive regions in the surface layer of the body. The array of light transmissive regions extends from a first lateral side to a second lateral side of the fluid channel. The optofluidic NBD also comprises an array of microaxicons in the array of light transmissive regions. Each microaxicon receives light through the associated light transmissive region and generates one or more nondiffracting beams based on the received light. The optofluidic NBD also includes a light detector comprising one or more light detecting elements. The light detector receives the one or more detection nondiffracting beams and generates time varying light data associated with the received one or more detection nondiffracting beams received as an object passes through the fluid channel. The optofluidic NBD also includes a processor configured to generate a three-dimensional image of the object based on the time-varying light data.

[0012] These and other embodiments of the invention are described in further detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. **1** is a block diagram of components of an NBD for three dimensional imaging comprising an illumination module and a nondiffracting beam detection module, according to embodiments of the invention.

[0014] FIG. **2** is a schematic drawing of a cross-sectional view of components of an NBD for three-dimensional imaging, according to embodiments of the invention.

[0015] FIG. **3**(*a*) is an intensity graph of a slice in an XZ plane of a Bessel beam, according to embodiments of the invention.

[0016] FIG. **3**(*b*) is an intensity graph of a slice in an XZ plane of a focusing spherical wave with 0.3 numerical aperture.

[0017] FIG. **4** is a schematic drawing of a cross-sectional view of components of a nondiffracting beam detecting device for three-dimensional imaging that can use the first approach to generate three dimensional image data, according to embodiments of the invention.

[0018] FIG. **5**(*a*) is a plot of the collection efficiency of a nondiffracting beam detecting device for three-dimensional imaging as a function of aperture radius, according to an embodiment of the invention.

[0019] FIG. **5**(*b*) is a plot of the collection efficiency of a nondiffracting beam detecting device for three-dimensional imaging as a function of the height, h, of the object point source, according to an embodiment of the invention.

[0020] FIG. **6** is a schematic drawing of components of a nondiffracting beam detecting device for three-dimensional imaging using optofluidic flow with the three-dimensional position localization approach, according to embodiments of the invention.

[0021] FIG. **7**(*a*) is a schematic drawing of a nondiffracting beam detecting device for three-dimensional imaging which measures light data from a wide viewing angle and an associated plot of the calculated point spread function (PSF) as a function of source position, according to an embodiment of the invention.

[0022] FIGS. **7**(*b*) and **7**(*b*) are schematic drawings of a nondiffracting beam detecting device for three-dimensional imaging, according to embodiments of the invention, which measures perspective projections over narrow viewing angle, and the associated plots of calculated PSF as a function of point source position.

[0023] FIGS. **8** and **9** are schematic drawings of a cross-sectional view of components of a nondiffracting beam detecting device for three-dimensional imaging of an object based on perspective projections, according to embodiments of the invention.

[0024] FIGS. **10** and **11** are schematic drawings of components of an NBD using optofluidic flow and takes the perspective projections approach, according an embodiment of the invention.

[0025] FIG. **12** shows a block diagram of subsystems that may be present in a nondiffracting beam detecting device for three-dimensional imaging, according to embodiments of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Embodiments of the present invention will be described below with reference to the accompanying drawings. Some embodiments include an NBD for three-dimensional imaging of an object. The NBD includes a multi-layer body having a surface layer with a light transmissive region fitted with a microaxicon. The light transmissive region passes light altered by the object. The microaxicon converts light passing through the light transmissive region into nondiffracting beams (e.g., Bessel beams) propagating at different angles. A light detector measures the point of detection and intensity of each nondiffracting beam associated with an extended depth of field through the object. The NBD can generate a high resolution three-dimensional image of the object based on the measured point of detection and intensity associated with an extended depth of field.

[0027] In one approach, the NBD localizes the three-dimensional position and intensity of object point sources (e.g., scattering particles) in an object and uses the information to generate a three-dimensional image of the object. An illumination module generates an illuminating nondiffracting beam providing a narrowly structured illumination path through the object to illuminate object point sources along a beam axis. The microaxicon receives light through the light transmissive region. The microaxicon converts light altered by each object point source into a single detection nondiffracting beam having a single propagation angle. The propagation angle uniquely corresponds to the height of its corresponding object point source. The NBD determines the propagation angle based on the measured point of detection (location of the light detecting element) on the light detector. The NBD can then determine the height of the object point source from the propagation angle determined. The NBD localizes the three-dimensional position of each object point source from the propagation angle and the known location of the illuminating nondiffracting beam. The NBD can then generate three dimensional image data and images based on the three-dimensional position and measured intensity of each object point source.

[0028] In another approach, the NBD measures several two-dimensional perspective projections of the object at various narrow viewing angles and uses tomography to combine the perspective projections to estimate a three-dimensional image. Each perspective projection has an extended depth of field at an associated narrow viewing angle. In this approach, uniform illumination is used. The microaxicon receives light through the light transmission region. The microaxicon converts light from different narrow viewing angles into separate detection nondiffracting beams. Each detection nondiffracting beam propagates at a different projection (propagation) angle so that the nondiffracting beams can be directed to different light detecting elements on the light detector. Each light detecting element receives a detection nondiffracting beam with a projection angle associated with a narrow viewing angle (field of view) of the object. That is, each light detecting element uniquely corresponds to a perspective projection of a narrow viewing angle of the object. The NBD generates several perspective projections over various viewing angles using the light data (e.g., intensity data) measured by the light detecting elements in the light detector. The NBD determines the projection angle of each perspective projection from the point of detection of each nondiffracting beam received. Using tomography algorithms, the NBD combines the perspective projections from different projection angles to estimate a three-dimensional image of the object.

[0029] Embodiments of the invention provide one or more technical advantages. One advantage is that the NBD can perform three-dimensional imaging with few components in a simple multi-layered arrangement (on-chip application). Since the body is a multilayered structure, the device can be fabricated inexpensively using standard semiconductor and micro/nanofabrication procedures. Also, a multilayered structure can be easily miniaturized. Another advantage is that the NBD includes a scanning mechanism such as a raster scanner or optofluidic flow that allows for three-dimensional imaging at high throughput rates. Another advantage is that multiple NBDs (e.g., 100, 200, etc.) can be combined in parallel since they are relatively compact and simple devices. The multiple NBDs can provide high throughput three-dimensional imaging of large sample volumes.

[0030] I. NBD

[0031] FIG. **1** is a block diagram of components of an NBD **10** comprising an illumination module **100** and a nondiffracting beam detection module **200** (NBDM), according to embodiments of the invention. Although the illustrated example shows a single NBDM **200** in communication with the illumination module **100**, any suitable number of NBDM modules **200** can be in communication with to the illumination module **100**.

[0032] The illumination module **100** includes an illumination source **110** for providing illumination **112** (light) in a suitable form (e.g., uniform illumination, nondiffracting beam(s), etc.) and a scanning mechanism **120**. Illumination **112** from the illumination source **110** is altered (blocked, reduced intensity, modified wavelength/phase, polarization) by an object **130** (shown in FIG. **2**), or otherwise remains substantially unaltered with the exception of scattering by particle in the sample.

[0033] The scanning mechanism **120** includes any suitable scanning device that can be associated with the illumination source **110**, an object **130** (shown in FIG. **2**) being imaged, or a specimen stage (e.g., slide) holding an object **130**. In the first case, the scanning device (e.g., raster scanner) can be associated with the illumination source **110** such that it is able move the illumination source **110** relative to the object **130** to provide illumination **112** across the object **130**. In the second case, the scanning mechanism **120** can be associated with the object **130** such that it is able to move the object **130** being imaged relative to the illumination **112**. In this case, the scanning mechanism **120** may be a similar to the scanning mechanism used in optofluidic microscopy, where an image is acquired as an object **130** moves through a fluid channel. An example of a scanning mechanism used in optofluidic microscopy can be found in X. Heng, D. Erickson, L. R. Baugh, Z. Yaqoob, P. W. Sternberg, D. Psaltis, and C. H. Yang, “Optofluidic microscopy—a method for implementing a high resolution optical microscope on a chip,” Lab on a Chip 6, 1274-1276 (2006), which is hereby incorporated by reference in its entirety for all purposes. In the third case, the scanning mechanism **120** can be associated with the specimen stage (e.g., slide) holding the object **130** such that it is able to move the object **130** being imaged relative to the illumination **112**.

[0034] The NBDM **200** includes a light transmissive region **204**, a microaxicon **210**, a light detector **220**, a processor **230** communicatively coupled to the light detector **220**, and computer readable medium (CRM) **240** (e.g., memory) communicatively coupled to the processor **230**. Although the illustrated example shows the NBDM **200** the processor **230** and computer readable medium (CRM) **240**, the processor **230** and CRM **240** may be separate components from the NBDM **200** in other embodiments.

[0035] The NBDM **200** is in communication with the illumination module **100** such that it can receive illumination **112** from the illumination source **110** through the light transmissive region **204**. The microaxicon **210** receives light from the illumination source **110** through the light transmissive region **204** and converts light received into one or more nondiffracting beams **212** (e.g., Bessel beams) propagating at different angles. The light detector **220** receives the one or more nondiffracting beams **212** and generates signals with light data associated with the received beams **212**. The processor **230** is in communication with the light detector **220** such that it can receive signals with light data from the light detector **220**. The processor **230** generates three-dimensional image data of the object **130** based on the light data. The processor **230** can also generate a three-dimensional image of the object **130** based on the three-dimensional image data.

[0036] An illumination source **110** refers to any device(s) or other source of light (e.g. ambient light) capable of providing illumination **112** in a suitable form and with a suitable wavelength, intensity, polarization, and/or phase. The illumination source **110** and/or scanning mechanism **120** may be components of the NBD **10** or may be separate from the NBD **10**. The illumination source **110** may be placed in any suitable location to provide light which can pass through the object **130** and to the microaxicon **210**.

[0037] Some suitable forms of illumination **112** include uniform illumination and one or more nondiffracting beams. In a photoluminescence (e.g., fluorescence or phosphorescence) embodiment, the illumination source **110** provides excitation light of any suitable light property (e.g., a single wavelength, single polarization, etc.) for exciting fluorophores in the object **130**. Some examples of excitation light include fluorescence, two-photon or higher order fluorescence, Raman, second harmonic or higher order, or other emission mechanism that results in emissions at a different wavelength or other light property than the excitation light. The illumination **112** provided by the illumination source **110** may be modulated over time.

[0038] The illumination source **110** may include any suitable device or combination of devices. Some suitable illumination sources **110** are naturally and/or commercially available. In some embodiments, the illumination source **110** includes a combination of devices arranged to generate one or more appropriately separated nondiffracting beams (e.g., Bessel beams). Examples of such devices are described in the Nondiffracting Beam Generator Section IA below. In other embodiments, the illumination source **110** includes a source of uniform illumination.

[0039] The scanning mechanism **120** may be a component of the NBD **10** or may be separate component from the NBD **10**. A scanning mechanism **130** refers to any suitable device (s) or components(s) capable of moving the object **130** or specimen stage (e.g., slide) relative to the illumination **112** or the illumination source **110** relative to the object **130** or specimen stage in order to illuminate at least a portion of the object **130**. The scanning mechanism **120** can be based on any suitable method including, for example, microfluidic flow methods, optical tweezing methods, and scanning methods (raster scanning, linear scanning, etc.). An example of a scanning mechanism **120** employing a microfluidic flow method includes a fluid channel **150** (shown in FIG. **6**) having a fluid flow with the object **130** being imaged. In another example, the scanning mechanism **120** may include a raster scanning device for moving the object **130** or specimen stage (e.g., slide) with the object **130** relative to the illumination **112** or raster scanning the illumination **112** relative to the object **130**. The scanning mechanism **120** can be in any suitable location that does not block the light from the illumination source **110**. In some embodiments, the NBD **10** may omit the scanning mechanism **120**.

[0040] Any suitable object **130** (shown in FIG. **2**) or portion of an object **130** may be imaged by the NBD **10**. Suitable objects **130** can be biological or inorganic entities. Examples of biological entities include whole cells, cell components, microorganisms such as bacteria or viruses, cell components such as proteins, etc. Inorganic entities may also be imaged by embodiments of the invention.

[0041] As used herein, a microaxicon **210** refers to a conical microlens or other suitable lens that can convert light received by the microaxicon **210** into one or more nondiffracting beams. The microaxicon **210** can be of any suitable size and may be in any suitable location in the NBDM **200**. Although many embodiments include a microaxicon **210**, other nondiffracting beam generators (e.g., a holographic element) can be used to convert light into nondiffracting beam(s) in other embodiments. Suitable nondiffracting beam generators are described in Section IA. In many illustrated embodiments, a microaxicon **210** is used to convert light altered by the object **130** into one or more nondiffracting beams **212** with different propagation angles, which are measured by the light detector **220** at different points of detection. In some illustrated embodiments, the illumination source **110** includes a microaxicon **210** to generate an illuminating nondiffracting beam **112**(*a*) (shown in FIG. **4**).

[0042] As used herein, a nondiffracting beam, either the illuminating nondiffracting beam **112**(*a*) (shown in FIG. **4** and FIG. **6**) or the detection nondiffracting beam **212** (shown in FIGS. **2**, **4**, **6**, **8**, **9**, **10**, and **11**), refers to a field of electromagnetic radiation that has little to no diffraction with propagation. For example, as a nondiffracting beam propagates, the beam does not substantially diffract and maintains a tight focus confining photons with a narrow width and sustaining constant width over a relatively long section of the beam's axis. An example of a nondiffracting beam is a Bessel beam.

[0043] Referring to FIGS. **3**(*a*) and **3**(*b*), the intensity graphs illustrate a side by side comparison of a Bessel beam and a focusing spherical wave with 0.3 numerical aperture. FIG. **3**(*a*) is an intensity graph of a slice in an XZ plane of a Bessel beam and FIG. **3**(*b*) is an intensity graph of a slice in an XZ plane of a focusing spherical wave with 0.3 numerical aperture, according to embodiments of the invention. The Bessel beam in FIG. **3**(*a*) has a narrow width for an extended length (z-distance) whereas the focusing spherical wave in FIG. **3**(*b*) has a relatively short beam length in comparison. That is, the Bessel beam or other nondiffracting beam confines photons within a narrow width through an extended depth of field in comparison with the relatively small depth of field of a the beam of the focusing spherical wave. By comparing beam lengths, FIGS. **3**(*a*) and **3**(*b*) illustrate that a Bessel beam or other nondiffracting beam can provide an extended depth of field relative to the beam of the focusing spherical wave. In addition, the Bessel beam or other nondiffracting beam can propagate with a relatively narrow width for an extended length in comparison with the focusing spherical wave.

[0044] Referring back to FIG. **1**, the light detector **220** is in communication with the microaxicon **210** to receive one or more nondiffracting beams **212** generated by the microaxicon **210**. As used herein, a light detector **220** (e.g., a photosensor) refers to any suitable device capable of generating a signal with light data based on light received by the light detector **220**. The signals with light data may be in the form of electrical current from the photoelectric effect. Each light detector **220** includes one or more discrete light detecting elements **220**(*a*) (shown in FIG. **2**). In many embodiments, each light detecting element **220**(*a*) (shown in FIG. **2**) may receive a nondiffracting beam **212** and can generate a signal with the light data associated with the nondiffracting beam **212** received.

[0045] The light detecting elements **220**(*a*) (shown in FIG. **2**) can be arranged in any suitable form such as a single light detecting element, a one-dimensional array of light detecting elements **220**(*a*), a two-dimensional array of light detecting elements **220**(*a*), or a multiplicity of one-dimensional and/or two-dimensional arrays of light detecting elements **220**(*a*). The arrays can be in any suitable orientation or combination of orientations. Some examples of light detectors having a single light detecting element **220**(*a*) include a photo-diode (PD), an avalanche photo-diode (APD) and a photomultiplier tubes (PMT). Some examples of light detectors having one-dimensional or two-dimensional arrays include a charge coupled device (CCD) array, a complementary metal-oxide-semiconductor (CMOS) array, an APD array, a PD array, a PMT array, etc. Other suitable light detectors **220** are commercially available. Each light detecting element **220**(*a*) may be of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square). For example, a complementary metal-oxide-semiconductor (CMOS) or charge coupled device (CCD) light detecting element **220**(*a*) may be 1-10 microns and an APD or PMT light detecting element **220**(*a*) may be as large as 1-4 mm.

[0046] Each light detecting element **220**(*a*) can generate a signal with light data based on light received. As used herein, light data refers to any suitable information related to the light received by the light detecting element **220**(*a*). Light data may include, for example, information about the properties of the light detected such as the intensity of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the light detected by the light detecting elements **220**(*a*) (as shown in FIG. **2**) of the light detector **220**. Light data may also include the location of the light detecting elements **220**(*a*) (shown in FIG. **2**) associated with the light data and the time that the light was detected by the light detecting elements **220**(*a*). Light data may be data based on a single time, based on multiple times, or based on a time varying basis. In some embodiments such as the microfluidic flow embodiments, the light data may be time varying light data.

[0047] Light data from a light detecting element **220**(*a*) may include data associated with a point of detection. The point of detection refers to the location on the light detector plane **222** (shown in FIG. **2**) of the light detector **220** that receives the detection nondiffracting beam **212**. In some cases, the point of detection may be estimated as the center or other specific location of the light detecting element **220**(*a*) receiving detection nondiffracting beam **212**.

[0048] The processor **230** (e.g., microprocessor) receives signals with light data from the light detector **220** associated with the light received by the light detecting elements **220**(*a*). The processor **230** can generate three-dimensional image data based on light data received from the light detector **220**. As used herein, three-dimensional image data refers to any suitable data that can be used to generate a three-dimensional image. Some suitable data includes the three-dimensional position of the object point source **130**(*a*) (shown in FIG. **4**) a propagation angle of the detection nondiffracting beam **212** (shown in FIG. **4**), perspective projections (two-dimensional images), etc.

[0049] The processor **230** executes code stored on the CRM **240** to perform some of the functions of NBD **10** such as interpreting the light data from the light detector **220**, performing analyses of the light data, generating three-dimensional image data from the light data, and/or generating one or more three-dimensional images of the object **130**.

[0050] The CRM (e.g., memory) **240** stores code for performing some functions of the NBD **10**. The code is executable by the processor **230**. In embodiments, the CRM **240** may comprise: a) code for interpreting light data received from the light detector **220**, b) code for generating three-dimensional image data of the object **130** based on light data, c) code for determining the propagation angle of a detection nondiffracting beam **212**, d) code for generating two-dimensional perspective projections of the object **130** based on light data, e) code for determining the three-dimensional position of an object point source **130**(*a*) (shown in FIG. **4**) (e.g., scatterer) based on light data, f) code for generating a three-dimensional image of the object **130** based on the three-dimensional image data, g) code for displaying the three-dimensional images, h) and/or any other suitable code for performing functions of the NBD **10**. The CRM **240** may also include code for performing any of the signal processing or other software-related functions that may be created by those of ordinary skill in the art. The code may be in any suitable programming language including C, C++, Pascal, etc.

[0051] Modifications, additions, or omissions may be made to NBD **10** without departing from the scope of the disclosure. For example, other embodiments of the NBD **10** may include a display **230** communicatively coupled to the processor **230** to receive output data such as a three-dimensional image data and provide output such as a three-dimensional image or three-dimensional image data to a user of the NBD **10**. In addition, the components of NBD **10** may be integrated or separated according to particular needs. For example, the processor **230** or other suitable processor may be integrated into the light detector **220** so that the light detector **220** performs one or more of the functions of the processor **230** in some embodiments. As another example, the processor **230** and CRM **240** may be components of a computer separate from the NBDM **200** and in communication with the NBDM **200**.

[0052] FIG. **2** is a schematic drawing of a cross-sectional view of components of an NBD **10**, according to embodiments of the invention. The NBD **10** comprises an illumination module **100**, an NBDM **200**, and an object **130** being imaged by the NBD **10**. The illumination module **100** generates illumination **112** to illuminate the object **130**. The NBDM **200** generates three-dimensional image data of the object **130** and/or a three-dimensional image of the object **130** based on the illumination **112**. Although a single NBDM module **200** and single NBDM module **200** are shown, any suitable number of modules may be included in the NBD **10** in other embodiments.

[0053] The illumination module **100** includes an illumination source **110** providing illumination **112** in any suitable form (e.g., uniform illumination, nondiffracting beam(s), etc.) and having any suitable wavelength, phase, polarization, and intensity. Some suitable forms include uniform illumination and one or more nondiffracting beams. In a photoluminescence (e.g., fluorescence or phosphorescence) embodiment, the illumination source **110** provides excitation light of any suitable light property (e.g., a single wavelength, single polarization, etc.) for exciting fluorophores in the object **130**. Some examples include a fluorescence, two-photon or higher order fluorescence, Raman, second harmonic or higher order, or other emission mechanism that results in emissions at a different wavelength or other light property than the excitation light. The illumination source **110** may be placed in any suitable location to provide light which can pass through the object **130** and to the microaxicon **210**. The illumination **112** provided by the illumination source **110** may be modulated over time. Some suitable illumination sources **110** are naturally and/or commercially available.

[0054] The illumination source **110** may include any suitable device or combination of devices. In some embodiments, the illumination source **110** includes a combination of devices arranged to generate one or more appropriately separated nondiffracting beams (e.g., Bessel beams). Examples of such devices are described in the Nondiffracting Beam Generator Section IA below. In other embodiments, the illumination source **110** includes a source of uniform illumination.

[0055] The illumination module **100** also includes a scanning mechanism **120** associated with the illumination source **110** such that it is able to move the illumination source **110** relative to the object **130** to provide illumination **112** across the object **130** or a portion of the object **130**. In other embodiments, the scanning mechanism **120** may be associated with the object **130** or specimen stage (e.g., slide) holding the object **130** such that it is able the object **130** relative to the illumination source **110**. The scanning mechanism **120** can be based on any suitable method such as microfluidic flow, optical tweezing, scanning (raster scanning, linear scanning, etc.). An example of a scanning mechanism **120** employing a microfluidic flow method includes a fluid channel **150** (shown in FIG. **6**) having a fluid flow with the object **130** being imaged. In another example, the scanning mechanism **120** may include a raster scanning device for moving the object **130** or specimen stage (e.g., slide) with the object **130** relative to the illumination **112** or scanning the illumination **112** relative to the object **130**. The scanning mechanism **120** can be in any suitable location that does not block the light from the illumination source **110**. In some embodiments, the NBD **10** may omit the scanning mechanism **120**.

[0056] Any suitable object **130** (shown in FIG. **2**) or portion of an object **130** may be imaged by the NBD **10**. Suitable objects **130** can be biological or inorganic entities. Examples of biological entities include whole cells, cell components, microorganisms such as bacteria or viruses, cell components such as proteins, etc. Inorganic entities may also be imaged by embodiments of the invention. Examples of inorganic entities include mineral fibers, and crystals.

[0057] The object **130** includes one or more object point sources **130**(*a*) in the volume of the object **130**. As used herein, an object point source **130**(*a*) refers to a scatterer/fluorophore in the object **130** that can alter (e.g., scatter and/or absorb and re-emit) light of the illuminating nondiffracting beam **112**(*a*). Each object point source **130**(*a*) has a three-dimensional position (X, Y, and Z position).

[0058] The NBD **10** includes a body **201**. In some embodiments, the body **201** may be a single, monolithic structure. In other embodiments, the body **201** may be a multi-layer structure. The layers of a multi-layer body may be of any suitable material or combination of materials having any suitable thickness or thicknesses. The layers of the multi-layer body may also include any suitable devices (e.g., light detector **220**).

[0059] In the embodiment shown in FIG. **2**, the body **201** is a multi-layer structure including a surface layer **202** with a first surface **202**(*a*) and a second surface **202**(*b*), a light detector layer **203** having a light detector **220** with six light detecting elements **220**(*a*)(**1**)-**220**(*a*)(**6**), and a separation layer **216** between the light detector layer **203** and the surface layer **202**. The surface layer **202** may be a thin metallic layer in some cases. The separation layer **216** may be made of a transparent material, or a void. The separation layer **216** has a thickness, w. Any suitable thickness can be used. In some cases, the thickness of the separation layer **216** may be designed to maximize the efficiency of the NBD **10**. The body **201** may optionally include a transparent protective layer (not shown) outside the first surface **202**(*a*) to isolate the surface layer **202**. Although FIG. **2** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. As another example, the multi-layer body **201** may optionally include a filter layer **214** (shown in FIG. **6**).

[0060] The multi-layer body **201** may be fabricated using standard semiconductor and micro/nanofabrication procedures. During an exemplary assembly of the multi-layer body **201**, the separation layer **216** can be placed on top of the light detector **220**. Then, the surface layer **202** with the light transmissive region **204** fitted with the microaxicon **210** can be placed on top of the separation layer **216**.

[0061] In some embodiments, the illumination module **100** and/or NBDM **200** may include one or more layers of the body **201**. As shown in FIG. **2**, for example, the NBDM **200** includes the surface layer **202**, the light detector layer **203**, and the separation layer **216**. As another example, as shown in FIG. **6**, a body **201** includes the holographic element **170** of the illumination module **100**.

[0062] The surface layer **202** also includes a light transmissive region **204**. The light transmissive region **204** may be of any suitable size (e.g., 0.1 μm, 0.5 μm, 1 μm, 5 μm, etc.) and have any suitable cross sectional shape (e.g., circular, rectangular, oval, etc.). In many cases, the light transmissive region **204** is a hole or a slit. In some of these cases, the holes/slits may be at least partially filled with a transparent material.

[0063] The surface layer **202** also includes a microaxicon **210** that is fitted within the light transmissive region **204** to receive light passing through the light transmissive region **204**. Although some illustrated embodiments of the NBD **10** include a microaxicon **210** that is located in the light transmissive region **204**, other embodiments may include a microaxicon **210** that is partially located in the light transmissive region **204** or located outside of the light transmissive region **204** in a location that can receive light passing through the light transmissive region **204**. In FIG. **2**, microaxicon **210** receives light, which may be altered by the object **130**, through the light transmissive region **204** and converts the light received into a detection nondiffracting beam **212** at a propagation angle, e_x. Although a single detection nondiffracting beam **212** is shown, two or more detection nondiffracting beams **212** may be generated by the microaxicon **210** in other embodiments. Although a single light transmissive region **204** with a single microaxicon **210** is shown in the illustrated embodiment, any suitable number of light transmissive regions **204** and microaxicons **210** may be used in other embodiments.

[0064] The NBD **10** also includes an x-axis, a y-axis (not shown), and a z-axis. The x-axis and y-axis lie in the plane of the first surface **202**(*a*) of the surface layer **202**. The z-axis is orthogonal to this plane.

[0065] In FIG. **2**, the light detector **220** includes six light detecting elements **220**(*a*)(**1**), **220**(*a*)(**2**), **220**(*a*)(**3**), **220**(*a*)(**4**), **220**(*a*)(**5**), and **220**(*a*)(**6**) arranged in the X direction. In other embodiments, the light detector **220** may further include light detecting elements **220**(*a*) in the Y direction. In the illustrated example, the fourth light detecting element **220**(*a*)(**4**) receives the detection nondiffracting beam **212** and can generate a signal with light data associated with the nondiffracting beam **212** received. In other embodiments, the microaxicon **210** may generate two or more detection nondiffracting beams **212**, which are received by two or more light detecting elements **220**(*a*).

[0066] Light data may include, for example, information about the properties of the light detected such as the intensity of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the light detected by the light detecting elements **220**(*a*) of the light detector **220**. Light data may also include the location of the light detecting elements **220**(*a*) associated with the light data and the time that the light was detected by the light detecting elements **220**(*a*). Light data may be data based on a single time, based on multiple times, or based on a time varying basis. In some embodiments such as the microfluidic flow embodiments, the light data may be time varying light data.

[0067] Light data from a light detecting element **220**(*a*) may include the three-dimensional position of the point of detection **250** (shown in FIG. **4**). The point of detection refers to the location on the light detector plane **222** of the light detector **220** that receives the detection nondiffracting beam **212**. In some cases, the point of detection may be estimated as the center or other specific location of the light detecting element **220**(*a*) receiving detection nondiffracting beam **212**.

[0068] The NBDM **200** can generate three-dimensional image data based on the light data. Some suitable three-dimensional data includes the three-dimensional position of the object point source **130**(*a*) (shown in FIG. **4**), the propagation angle of the detection nondiffracting beam **212** (shown in FIG. **4**), perspective projections (two-dimensional images), etc.

[0069] Although not shown in FIG. **2**, the NBD **10** also includes a processor **230** (shown in FIG. **1**) communicatively coupled to the light detector **220** and a CRM **240** in communication with the processor **230**. The processor **230** executes code stored on the CRM **240** to perform some of the functions of NBD **10**. For example, the processor **230** may receive a signal with the light data from the light detector **220** and generate three-dimensional image data of the object **130** based on the light data. The processor **230** may also generate one or more three-dimensional images of the object **130** or portions of the object **130** based on the three-dimensional image data.

[0070] In operation, the illumination source **110** and scanning mechanism **120** provide illumination **112** across the object **130**. Light from the illumination source **110** is altered (blocked, reduced intensity, modified wavelength/phase, etc.) by the object **130**, or otherwise remains substantially unaltered with the exception of scattering by other particles. The microaxicon **210** receives light passing through the light transmissive region **204**. The microaxicon **210** generates one or more nondiffracting beams **212**. Each beam propagates at a different propagation angle. The light detector **220** receives the one or more nondiffracting beams **212** and generates a signal with light data associated with the one or more beams **212** received. The processor **230** receives the signal with the light data and generates three-dimensional data of the object **130** based on the light data. The processor **230** also generates a three-dimensional image of the object **130** based on the three-dimensional image data.

[0071] In one embodiment, the NBD **10** can generate three-dimensional photoluminescence (e.g., fluorescence or phosphorescence) images of the object **130**. In this embodiment, the body **201** includes an additional filter layer **214** between the object **130** and the light detector **220**. Typically, the filter layer **214** is sandwiched within the separation layer **216** (as shown in FIG. **6**) at either the second surface **202**(*a*) or the light detector plane **222**. Other locations would be readily apparent to one skilled in the art. The filter layer **214** includes any suitable device(s) (e.g., optical filter) that can reject the excitation light and pass emission light from the fluorophores. In this embodiment, the illumination source **110** (e.g., laser) provides an excitation light having an excitation wavelength that can excite fluorophores in the object **130**. The excited fluorophores can emit an emission light. The light data would be associated with the emission light from the fluorophores in the object **130**. The processor **230** generates three-dimensional image data associated with the emission light data and generates three-dimensional photoluminescence images of the object **130** based on the three-dimensional image data.

[0072] In some embodiments, the NBD **10** may omit the scanning mechanism **120**. In one embodiment, for example, the NBD **10** may include an illumination source **110** that provides uniform illumination across the object **130** without the need for a scanning mechanism **120**. In another embodiment, the NBD **10** may include an illumination source **110** having a nondiffracting beam generator as described in Section 1A that generates a two-dimensional array of illuminating nondiffracting beams **212** (shown in FIG. **2**) of a suitable size to span the object **130** and having a suitable array dimension (e.g., 500×1000, 2000×2000, 10000×10000, etc.). In this embodiment, the NBD **10** can illuminate the object **130** with the array of nondiffracting beams **212** and a scanning mechanism **120** may not be required. The NBD **10** can take a snapshot three-dimensional image of the object **130** using light data measured at a single time.

[0073] Modifications, additions, or omissions may be made to NBD **10** without departing from the scope of the disclosure. For example, other embodiments of the NBD **10** may omit the scanning mechanism **120**. In one such embodiment, the NBD **10** provides uniform illumination across the object **130** without the need for a scanning mechanism **120**. In another such embodiment, the NBD **10** includes an illumination source **110** generates a two-dimensional array of nondiffracting beams **212** of a suitable size to span across the object **130**. The two-dimensional array may have any suitable dimension (e.g., 500×1000, 2000×2000, 10000×10000, etc.). In this embodiment, the NBD **10** may take a snapshot image of the object **130** at a single time. As another example, other embodiments may include a display, a processor **230** and/or a CRM **240**. In addition, the components of NBD **10** may be integrated or separated according to particular needs.

[0074] A. Nondiffracting Beam Generators (e.g., Bessel Beam Generators)

[0075] A nondiffracting beam generator refers to any suitable device or combination of devices that generates one or more nondiffracting beams (e.g., illuminating nondiffracting beams **112**(*a*) in the illumination module **100** or detection nondiffracting beams **212** in the NBDM **200**). Some examples of suitable nondiffracting beam generators include: 1) a computer generated hologram (CGH) or other holographic element **170** coupled to an excitation beam source, 2) a microaxicon, and 3) an optical fiber.

[0076] A nondiffracting beam generator including a CGH or other holographic element **170** (shown in FIG. **6**) coupled to an excitation beam source for providing an excitation beam **142** is the preferred device for generating one or more illuminating nondiffracting beams **112**(*a*) in the illumination module **100** of many embodiments. In some cases, a CGH can be designed so that adjacent illuminating nondiffracting beams **112**(*a*) are sufficiently separated to avoid crosstalk/multiplexing between beams, which can affect the quality of the nondiffracting beam illumination. An example of a nondiffracting beam generator comprising a CGH can be found in "The generation of an array of nondiffracting beams by a single composite computer generated hologram," S H Tao et al 2005 J. Opt. A: Pure Appl. Opt. 7 40, which is hereby incorporated by reference in its entirety for all purposes. In this example, a CGH includes an N×N array of holograms, each hologram generating an individual Bessel beam. The CGH in this example can be used to generate a two-dimensional (N×N) array of Bessel beams. In this example, the holograms are designed to generate Bessel beams with sufficient separation to avoid crosstalk. Another example of a nondiffracting beam generator including a CGH can be found in "Holographic generation of diffraction-free beams, Jari Turunen, Antti Vasara, and Ari T. Friberg, Appl. Opt. 27, 3959-3962 (1988)," S H Tao et al 2005 J. Opt. A: Pure Appl. Opt. 7 40, which is hereby incorporated by reference in its entirety for all purposes.

[0077] In some embodiments, such as the embodiment illustrated in FIG. **6**, the illumination module **100** includes an illumination source **110** including a custom designed holographic element **170** (e.g., custom designed CGH). The illumination source **110** also includes an excitation beam source providing an excitation beam **142** (e.g., plane wave excitation beam). The holographic element **170** produces one or more illuminating nondiffracting beam **112**(*a*) (e.g., Bessel beams), upon being illuminated by the excitation beam **142**. In FIG. **6**, the holographic element **170** produces an array of illuminating nondiffracting beams **112**(*a*) that are separated to reduce or prevent crosstalk. The custom designed holographic element (e.g., custom designed CGH) is custom designed to generate one or more illuminating nondiffracting beams **112**(*a*) that do not diffract or only minimally diffracts within a limited region of space. The holographic element **170** is computationally designed by interfering a conical wave front with a plane wave. The spot size and the focal plane of each illuminating nondiffracting beam **112**(*a*) are controlled by adjusting the width and the peak phase retardation of the conical wave front. Depending on resolution requirements, the custom designed holographic element **170** can either be printed with grayscale graphics printers, with photoplotters as halftone images, or can be fabricated as chrome/iron oxide binary photomasks. Some examples of a suitable custom-designed CGHs can be found in "Holographic generation of diffraction-free beams," Jari Turunen, Antti Vasara, and Ari T. Friberg, Appl. Opt. 27, 3959-3962 (1988) and "The generation of an array of nondiffracting beams by a single composite computer generated hologram," S H Tao et al 2005 J. Opt. A: Pure Appl. Opt. 7 40.

[0078] In FIG. **6**, the holographic element **170** can be made of any suitable materials. Some examples of suitable holographic materials include photographic emulsions, dichromated gelatin, and photoresists. The holographic element **170** can have any suitable dimensions (e.g., 1 mm×1 mm, 2 mm×2 mm, 5 mm×2 mm, 10 mm×10 mm, 10 mm×50 mm, etc.). The holographic element **170** can be made using any holographic recording technique capable of encoding (recording) data about the focal array of light spots. Some examples of suitable holographic recording techniques include in-line (Gabor) and off-axis (Leith-Upatnieks). To play back the recording, the holographic element **170** can be illuminated by the plane wave excitation beam **142** having the same wavelength, same spatial distribution, but not necessarily the same intensity, as the reference beam used to record the holographic element **170**.

[0079] A nondiffracting beam generator including a microaxicon **210** is the preferred device for generating one or more nondiffracting beams **212** in the detection module **200** of many embodiments. An example of a nondiffracting beam generator comprising optical fibers having axicon microlens can be found in "Nearly diffraction-limited focusing of a fiber axicon microlens," Sang-Kee Eah, Wonho Jhe, and Yasuhiko Arakawa, Rev. Sci. Instrum. 74, 4969 (2003), which is hereby incorporated by reference in its entirety for all purposes. In this example, the nondiffracting beam generator includes commercially available single-mode optical fibers. In this example, a microaxicon is fabricated in the end of each optical fiber by selective chemical etching method that allows fine control of the cone angle of the fiber. Light passing through each optical fiber passes through the microaxicon generating a nondiffracting beam and nearly diffraction-limited focused spot.

[0080] A nondiffracting beam generator including a multimode optical fiber is another device that can be used to generate one or more nondiffracting beams. An example of a nondiffracting beam generator comprising a multimode optical fiber device can be found in "Generation of controllable nondiffracting beams using multimode optical fibers," Appl. Phys. Lett. 94, 201102 (2009), which is hereby incorporated by reference in its entirety for all purposes. In this example, the multimode optical fiber device includes a laser coupled to a single mode fiber which is coupled to a multimode fiber generating a nondiffracting beam.

[0081] In addition, embodiments of the NBD **10** include a microaxicon **210** (shown in FIG. **2**) for converting light collected by the microaxicon **210** into one or more nondiffracting beams **212** for measurement by the light detector **220**. An example of a suitable microaxicon **210** that can generate one or more nondiffracting beams **212** can be found in "Nearly diffraction-limited focusing of a fiber axicon microlens," Sang-Kee Eah, Wonho Jhe, and Yasuhiko Arakawa, Rev. Sci. Instrum. 74, 4969 (2003).

[0082] II. Approaches to Generating Three-Dimensional Image Data

[0083] The NBD **10** may use different approaches to generate three-dimensional image data and three-dimensional images. Two different approaches are described below. A first approach uses the NBDM **200** in combination with illuminating nondiffracting beams **112**(*a*) (shown in FIG. **4**) to localize the three dimensional position and brightness of object point sources in the object. The three dimensional position and brightness of object point sources can be used to generate a three-dimensional image of the object. In contrast, a second approach uses the NBDM **200** together with uniform illumination to measure several perspective projections of the object **130** taken at various angles. The perspective projections are combined, using tomography, to form a three-dimensional image data and a three dimensional image of the object **130**.

[0084] A. Three-Dimensional Position Localization Approach

[0085] FIG. **4** is a schematic drawing of a cross-sectional view of components of an NBD **10** that can use the first approach to generate three dimensional image data of an object **130** having an object point source **130**(*a*), according to embodiments of the invention. In the illustrated example, the NBD **10** includes an illumination module **100** for generating an illuminating nondiffracting beam **112**(*a*) and an NBDM **200** for localizing the three-dimensional position of an object point source **130**(*a*) that alters (e.g., scatters) light of the illuminating nondiffracting beam **112**(*a*). The NBD **10** uses the three dimensional position and brightness of the object point source **130**(*a*) to generate three-dimensional image data and a three-dimensional image of the object **130**. The NBD **10** also includes an x-axis, a y-axis (not shown), and a z-axis.

[0086] In this first approach, each illuminating nondiffracting beam **112**(*a*) has an NBDM **200** associated with it. In the illustrated embodiment, a single illumination module **100** providing a single illuminating nondiffracting beam **112**(*a*) is associated with a single NBDM **200**. In other embodiments, the NBD **10** may include a plurality of illumination modules **100** associated with a plurality of NBDMs **200**, each illumination module **100** generating a single illuminating nondiffracting beam **112**(*a*) and associated with a single NBDM **200**.

[0087] In FIG. **4**, the illumination module **100** includes an illumination source **110** and a scanning mechanism **120**. The illumination source **110** generates an illuminating nondiffracting beam **112**(*a*) having a narrowly structured illumination path along a beam axis **115**. The nondiffracting beam **112**(*a*) propagates normal to the XY plane in the illustrated example. In other examples, the nondiffracting beam **112**(*a*) may propagate at an angle, β, from the x-axis and/or at an angle, γ, from the y-axis. The illumination source **110** may include any suitable nondiffracting beam generator to generate the illuminating nondiffracting beam **112**(*a*). Some examples of suitable nondiffracting beam generators (e.g., a custom designed CGH with plane wave excitation beam) are described in Section IA.

[0088] In FIG. **4**, the scanning mechanism **120** is associated with the illumination source **110** such that it is able to move the illumination source **110** relative to the object **130**. In other embodiments, the scanning mechanism **120** may be associated with object **130** or specimen stage (e.g., slide) such that it is able to move the object **130** relative to the illumination source **110**. The scanning mechanism **120** can be based on any suitable method such as microfluidic flow, optical tweezing, scanning (raster scanning, linear scanning, etc.). Although the illumination source **110** and scanning mechanism **120** are shown as components of the NBD **10** in the illustrated example, one or both of these components may be separate from the NBD **10** in other embodiments.

[0089] In FIG. **4**, the object **130** includes an object point source **130**(*a*). The object point source **130**(*a*) alters (e.g., scatters and/or absorbs and re-emits) light from the illuminating nondiffracting beam **112**(*a*). The object point source **130** (*a*) has a three-dimensional position (X, Y, and Z position). The height, h, of the object point source **130**(*a*) is the Z position. Although a single object point source **130**(*a*) may be shown, an object **130** may have two or more object point sources **130**(*a*) in other embodiments.

[0090] The NBD **10** includes a multi-layer body **201** having a surface layer **202** with a first surface **202**(*a*) and a second surface **202**(*b*), a light detector layer **203** having a light detector **220**, and a separation layer **216** between the light detector layer **203** and the surface layer **202**. The layers of a multi-layer body **201** may be of any suitable material or combination of materials having any suitable thickness or thicknesses. The layers of the multi-layer body **201** may also include any suitable device (e.g., light detector **220**). The separation layer **216** may be made of a transparent material or a void. The separation layer **216** may have any suitable thickness, w. In some cases, the thickness of the separation layer **216** may be designed to maximize the efficiency of the NBD **10**.

[0091] Although the body **201** in FIG. **2** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. In a fluorescence embodiment, a body **201** may include an additional filter layer **214** (shown in FIG. **6**) within the separation layer **216**. In another example, a body **201** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**. In another example, the body **201** omits the separation layer **216**.

[0092] In FIG. **4**, the surface layer **202** (e.g., thin metallic layer) includes a first surface **202**(*a*), a second surface **202**(*b*), a light transmissive region **204**, and a microaxicon **210** fitted in the light transmissive region **204**. The light transmissive region **204** may be of any suitable size (e.g., 0.1 μm, 0.5 μm, 1 μm, 5 μm, etc.) and have any suitable cross sectional shape (e.g., circular, rectangular, oval, etc.). In many cases, the light transmissive region **204** is a hole or a slit. In some of these cases, the holes/slits may be at least partially filled with a transparent material.

[0093] The microaxicon **210** is located in the light transmissive region **204** in the surface layer **202** to receive light passing through the light transmissive region **204** from the illumination module **100**. The microaxicon **210** generates a single detection nondiffracting beam **212** for each object point source **130**(*a*) (scatterer/fluorophore) illuminated by its illuminating nondiffracting beam **112**(*a*). In FIG. **4**, the microaxicon **210** receives light altered by the object point source **130**(*a*) in the form of a scattering cone **122** and converts the light received into a single detection nondiffracting beam **212** at a propagation angle, θ_x. Although a single detection nondiffracting beam **212** is shown, two or more detection nondiffracting beams **212** may be generated by the a microaxicon **210** in other embodiments having two or more object point sources **130**(*a*). Although a single light transmissive region **204** with a single microaxicon **210** is shown in the illustrated embodiment, any suitable number of light transmissive regions **204** and microaxicons **210** may be used in other embodiments.

[0094] The multi-layer body **201** may be fabricated using standard semiconductor and micro/nanofabrication procedures. During an exemplary assembly of the multi-layer body **201**, the separation layer **216** can be placed on top of the light detector **220**. Then, the surface layer **202** with the light transmissive region **204** fitted with the microaxicon **210** can be placed on top of the separation layer **216**. In another embodiment, the multi-layer body **201** omits the separation layer **216**. In this embodiment, the surface layer **202** with the light transmissive region **204** fitted with the microaxicon **210** can be placed directly on top of the light detector layer **203** having the light detector **220** (e.g., CMOS sensor).

[0095] The illumination module **100** and/or NBDM **200** may include one or more layers of the body **201**. In the illustrated example, the NBDM **200** includes the surface layer **202**, the light detector layer **203**, and the separation layer **216**.

[0096] The illuminating nondiffracting beam **112**(*a*) is offset from the center of the microaxicon **210**, by a beam offset, k. The beam offset, k, is the Euclidean distance on the XY plane between the center of the beam and the center of the microaxicon **210**.

[0097] The NBD **10** also includes an x-axis, a y-axis (not shown), and a z-axis. The x-axis and y-axis lie in the plane of the first surface **202**(*a*) of the surface layer **202**. The z-axis is orthogonal to this plane.

[0098] In FIG. **4**, the light detector **220** includes six light detecting elements **220**(*a*)(**1**), **220**(*a*)(**2**), **220**(*a*)(**3**), **220**(*a*)(**4**), **220**(*a*)(**5**), and **220**(*a*)(**6**) arranged in the X-direction. Each light detecting element **220**(*a*) may be of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square). Although a one dimensional array of six light detecting elements **220**(*a*) is shown, the light detector **220** may include any suitable number of light detecting elements **220**(*a*) in any suitable form (e.g., two-dimensional array), and in any suitable orientation(s) in other embodiments. Each light detecting element **220**(*a*) can receive one or more detection nondiffracting beams **212** and can generate a signal with the light data associated with the one or more detection nondiffracting beams **212** received.

[0099] The light data may include, for example, information about the properties of the light detected such as the intensity (brightness) of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the one or more nondiffracting beams **212** detected by the light detecting elements **220**(*a*). Light data may also include the X location and Y location of the light detecting elements **220**(*a*) receiving the one or more nondiffracting beams **212** and the time that the light was detected by the light detecting elements **220**(*a*). Light data may be data based on a single time, based on multiple times, or based on a time varying basis.

[0100] Light data from a light detecting element **220**(*a*) may include the three-dimensional position of the point of detection **250** of the nondiffracting beam **212** detected by the light detector **220**. The point of detection **250** refers to the point at the intersection detection nondiffracting beam **212** and the light detector plane **222**. In some cases, the three-dimensional position of the point of detection **250** may be estimated as the center or other specific location of the light detecting element **220**(*a*) receiving detection nondiffracting beam **212** at the light detecting plane **222**.

[0101] The NBDM **200** can measure the Euclidean distance, d on the XY plane between the point of detection **250** and a z-directional axis running through the object point source **130**(*a*). In FIG. **4**, the distance, d, is the Euclidean distance on the XY plane between the point of detection **250** and the nondiffracting beam axis.

[0102] The NBDM **200** can generate three-dimensional image data based on the light data. Some suitable three-dimensional data includes the three-dimensional position of the object point source **130**(*a*), the propagation angle of the detection nondiffracting beam **212**, and the distance d.

[0103] Although not shown, the NBD **10** also includes a processor **230** (shown in FIG. **1**) communicatively coupled to the light detector **220** and a CRM **240** communicatively coupled to the processor **230**. The processor **230** executes code stored on the CRM **240** to perform some of the functions of NBD **10**. For example, the processor **230** can receive a signal with the light data from the light detector **220** and generate three-dimensional image data of the object **130** based on the light data. The processor **230** may also generate one or more three-dimensional images of the object **130** or a portion of the object **130** based on three-dimensional image data.

[0104] In FIG. **4**, the object point source **130**(*a*) alters (e.g., scatters and/or absorbs and re-emits) light from the illuminating nondiffracting beam **112**(*a*) in the form of a cone **122** (e.g., scattering cone or diffraction cone). In many other embodiments, the cone **122** is much wider. The cone **122** includes a solid angle subtended by the light transmissive region **204** angle, ω The cone **122** also includes a cone angle in the X-direction, θ_x, between the central axis of the cone **122** and the x-axis. The cone **122** also includes a cone angle in the Y-direction, θ_y (not shown), formed between the central axis of the cone **122** and the y-axis. The microaxicon **210** receives light altered by the object point source **130**(*a*) through the light transmissive region **204** and converts the altered light from the object point source **130**(*a*) into a single detection nondiffracting beam **212** propagating at an angle, θ_x in the XZ plane. The detection nondiffracting beam **212** may also propagate at a propagation angle in the Y-direction, θ_y (not shown) in the YZ plane.

[0105] The propagation angle (in XZ plane), θ_x, of the detection nondiffracting beam **212** uniquely corresponds to the height, h, (Z position) of its corresponding object point source **130**(*a*). It is this phenomenon that enables the NBD **10** to sense the third dimension (Z position). As illustrated in FIG. **4**, the detection nondiffracting beam **212** associated with the object point source **130**(*a*) at point **1** in the object **130** is sensed at point **1'** on the light detector **220**. In other words, the Z position of the object **130** is encoded in the X position of the light detector plane **222**. By estimating the distance, d, of the detection nondiffracting beam **212** on the light detector plane **222**, the height (h; Z position) of an object point source **130**(*a*) can be directly determined as,

$$h = wk\left(\frac{1}{d}\right) \quad \text{(Eqn. 1)}$$

where w is the width of the separation layer, d is the Euclidean distance on the XY plane between the point of detection and z-directional axis passing through the object point source, and k is the beam offset distance between the center of the microaxicon **210** and z-directional axis passing through the object point source **130**(*a*).

[0106] The X position and Y position of the object point source **130**(*a*) can be determined from the location of the illuminating nondiffracting beam **112**(*a*) at the time of detection, which is based on the known location of the illumination module at the time that the object point source **130**(*a*) is illuminated. In FIG. **4**, the illuminating nondiffracting beam **112**(*a*) propagates in the Z-direction so that the X and Y positions of the object point source **130**(*a*) are the same as the known X and Y position of the beam axis **115** at the time at which the object point source **130**(*a*) is illuminated. In other embodiments, the nondiffracting beam **112**(*a*) may propagate at an angle from the x-axis and/or y-axis. In these embodiments, the X and Y positions of the object point source **130**(*a*) can be determined based propagation angle of the illuminating nondiffracting beam **112**(*a*).

[0107] The brightness (intensity) of the object point source **130**(*a*) is obtained from the total number of photons detected by the light detecting element **220**(*a*)(**4**) corresponding to that object point source **130**(*a*). A three-dimensional image of the object **130** can be obtained by plotting the X, Y, and Z positions of all detected object point sources **130**(*a*) along with their corresponding brightness values.

[0108] Based on Eqn. 1, each light detecting element **220** (*a*) in an NBDM **200** and at a particular distance, d is associated with a specific height (Z position) in the object **130**. Since the position of the illuminating nondiffracting beam **112**(*a*) is known at a the time of detection, each light detecting element **220**(*a*) in an NBDM **200** is also associated with a particular X and Y position of the illuminating nondiffracting beam **112**(*a*) at the time of detection. In this way, each light detecting element **220**(*a*) can be assigned a three position at the time of detection. Each light detecting element **220**(*a*) can then measure the intensity (brightness) associated with a particular three-dimensional position in the object at a given time.

[0109] In photon limited applications such as fluorescence imaging, it is important to have good photon collection efficiencies for achieving three-dimensional imaging with high signal to noise ratios. Collection efficiency is defined as,

$$\text{Collection efficiency} = \frac{\text{Solid Angle subtended by light transmissive region}\,(\Omega)}{4\pi} \times 100 \quad \text{(Eqn. 2)}$$

$$\text{Collection efficiency} = \frac{2a^2 h}{\left[\sqrt{h + (k-a)^2} + \sqrt{h + (k+a)^2}\right]^3} \times 100 \quad \text{(Eqn. 3)}$$

where ‘a’ is the radius of the light transmissive region **204** (e.g., aperture).

[0110] In one embodiment, an NBD **10** can be designed to address specific sampling requirements based on Eqn. 3. For example, if the portion of interest of the object is located at a particular height, the NBD **10** can be designed so that the illuminating nondiffracting beam **112**(*a*) offset, k, has a value such that the collection efficiency, based on Eqn. 3, will be maximum for the particular height.

[0111] FIG. **5**(*a*) is a plot of the collection efficiency of an NBD **10** as a function of aperture radius, according to an embodiment of the invention. FIG. **5**(*b*) is a plot of the collection efficiency of an NBD **10** as a function of the height, h, of the object point source **130**(*a*), according to an embodiment of the invention. The point source height, h, is 40 μm and k is (a+2) μm in FIG. **5**(*a*) and the light transmissive region (e.g., aperture) radius is 23 μm and k is 25 μm in FIG. **5**(*b*). The collection efficiency monotonously increases with increasing aperture size, but exhibits a rather interesting non-monotonous behavior when plotted as a function of height. This is because of the fact that the light transmissive region (e.g., aperture) is slightly displaced (by distance k) from the illuminating nondiffracting beam **112**(*a*). With this understanding, the parameters of our NBD **10** can be designed appropriately for addressing sample specific requirements. Also, from FIG. **5**(*b*), note that the detected brightness of an object point source **130**(*a*) is always influenced by the point's height. This displacement creates a brightness modulation that causes a difference between the actual and measured brightness of the sample by the NBD **10**.

[0112] This brightness modulation by varying collection efficiency can be corrected in the light data before generating the three-dimensional image data and the three-dimensional image, in some embodiments. In one embodiment, the brightness modulation can be corrected by calibrating the NBD **10** to correct for the brightness modulation. For a given design of the NBD **10**, calibrations curves can be experimentally generated to determine the brightness modulation of the system. The calibration curves can then be applied to correct the measured light data for brightness modulation to determine the actual light data from the source.

[0113] The transverse (X,Y) resolution of the NBD **10** is not limited by the size of the light transmissive region **204** (e.g., aperture). Instead, it is only limited by the spot size of the illuminating nondiffracting beam **112**(*a*) generated by a suitable nondiffracting beam generator (e.g., CGH). The axial (Z) resolution of the NBD **10** is limited by the spot size of the detection nondiffracting beam **212** produced by the microaxicon **210**. In addition, the inverse relationship between 'h' and 'd' in Eqn. 1 affects the Z sensitivity of the NBD **10**.

[0114] In operation, the illumination module **100** provides an illuminating nondiffracting beam **112**(*a*) across a portion of the object **130**(*a*). Each object point source **130**(*a*) alters (e.g., scatters and/or absorbs and re-emits) light from the illuminating nondiffracting beam **112**(*a*) in the form of a cone **122** (e.g., scattering cone or diffraction cone). The microaxicon **210** receives light altered by each object point source **130**(*a*) through the light transmissive region **204** and converts the altered light from each object point source **130**(*a*) into a single detection nondiffracting beam **212** propagating at an angle, θ_x in the XZ plane. The detection nondiffracting beam **212** may also propagate at a propagation angle in the Y-direction, θ_y (not shown) in the YZ plane.

[0115] The light detector **220** receives each detection nondiffracting beams **212** associated with each object point source **130**(*a*). In FIG. **4**, the fourth detecting element **220**(*a*) (**4**) receives the nondiffracting beam **212** at the point of detection **250**. Each light detecting element **220**(*a*) generates a signal with light data (e.g., intensity reading) and the location of the fourth detecting element **220**(*a*) receiving the detection nondiffracting beams **212** and/or the three-dimensional position of the point of detection **250**. For each object point source **130**(*a*), the processor **230** determines the Euclidean distance, d, in the XY plane between the point of detection and a z-directional axis passing through the object point source **130**(*a*). For each object point source **130**(*a*), the processor **230** also determines the beam offset distance, k. Using Eqn. 1, the determined k and d, and the known width (w) of the separation layer, the processor **230** determines the three-dimensional position (X, Y, and Z position) of each object point source **130**(*a*) associated with the intensity reading at all the times during the operation. The processor **230** combines the intensity data at the determined X, Y and Z positions at the reading times during the operation to generate three-dimensional image data. The processor **230** then generates one or more three-dimensional images of the object **130** based on the three-dimensional image data.

[0116] 1) Optofluidic Application

[0117] Optofluidic microscopes (OFMs) seek to revisit the fundamentals of microscopy, right from the theory of image formation. With an understanding that lens based imaging is primarily responsible for the bulkiness of traditional microscopes, OFMs eliminate the need for lenses by adopting a direct projection method through submicron scale metal apertures fabricated on a complementary metal oxide semiconductor (CMOS) sensor. A two dimensional (2D) image of the object is obtained by first flowing a specimen with the object over these apertures using a fluid channel, and by then combining the one dimensional signals obtained from the pixels (light detecting elements) under these apertures. With a size not bigger than a dime, and a price not more than a few dollars, OFMs can produce images comparable to the state of the art bulky expensive microscopes. Although originally developed for bright field imaging, OFM imaging modalities have now expanded to achieve DIC, dark field, and color imaging, all in two dimensions.

[0118] Three dimensional imaging in conventional OFMs is hindered by the fact that, unlike traditional microscopes, focus is not an adjustable parameter in the OFM imaging process. The best focus plane in a conventional OFM is typically the plane immediately above the apertures. The transverse planes further above the apertures can suffer from intense blurring, with the amount of blurring increasing as a function of the plane's height from the apertures.

[0119] In some embodiments, an NBD **10** uses optofluidic flow as a scanning mechanism **120** to provide a highly compact, inexpensive, and robust device with highly automatable, high-throughput, three-dimensional imaging with submicron scale resolution.

[0120] FIG. **6** is a schematic drawing of components of an NBD **10** using optofluidic flow as a scanning mechanism and takes the first approach to generating three dimensional image data and three-dimensional images of an object **130**, according to embodiments of the invention. The NBD **10** includes an array of illumination modules **100** and an array of NBDMs **200** located adjacent to their illumination counterparts. The NBD **10** also includes an x-axis, a y-axis, and a z-axis. Each of the modules in the arrays is comprised of an x-directional strip having a strip width in the y-direction and a strip length in the y-direction. In some cases, the strip width may be equal to the distance between the light transmissive regions **204** in the y-direction. The strip length may be equal to the length of the NBD **100** in the x-direction. The strips may overlap in the y-direction in some cases. Any suitable number of illumination modules **100** and corresponding NBDMs **200** may be used.

[0121] In FIG. **6**, the NBD **10** senses the three-dimensional position (X, Y, and Z position) of object point sources **103**(*a*) in the object **130** by first using the array of illumination modules **100** to structure the illumination path to exhibit an array of illuminating nondiffracting beams **112**(*a*), and by then using an array of NBDMs **200** located adjacent to their counterparts. In the NBD **10**, each illumination module **100** provides a single illuminating nondiffracting beam **112**(*a*). Each NBDM **200** senses the three-dimensional position (X, Y, and Z position) of one or more object point sources **103**(*a*) illuminated by the corresponding illuminating nondiffracting beam **112**(*a*) from the adjacent illuminating module **100**. Together, the arrays of illumination modules **100** and NBDMs **200** have the ability to localize the three-dimensional position and brightness of each object point source **130**(*a*) in the object **130** of the specimen within a single pass of the specimen over the array of light transmissive regions **204**. This information can be combined to form the three-dimensional image of the object **130**.

[0122] In FIG. **6**, the object **130** includes a first object point source **130**(*a*)(**1**) at point **1** and a second object point source **130**(*a*)(**2**) at point **2**, being illuminated by an illuminating nondiffracting beam **112**(*a*). In other embodiments, the object **130** may include other object point sources **130**(*a*) at one or more illuminating nondiffracting beams **112**(*a*). Each object point source **130**(*a*) has a three-dimensional position (X, Y, and Z positions). The height, h, of the object point source **130**(*a*) is the Z position. In FIG. **6**, the first object point source **130**(*a*)(**1**) at point **1** has a Z position of h_1, and the second object point source **130**(*a*)(**2**) at point **2** has a Z position of h_2.

[0123] The NBD **10** also includes a multi-layer body **201**, which may be fabricated using standard semiconductor and micro/nanofabrication procedures. The body **201** includes a channel layer **160** having a holographic element **170** (e.g., CGH), a surface layer **202** (e.g., thin metallic layer) having a first surface **202**(*a*) and a second surface **202**(*b*), the light detector layer **203** having a light detector **220**, and a separation layer **216** between the holographic element **170** and the light detector **220**. The layers of a multi-layer body **201** may be of any suitable material or combination of materials having any suitable thickness or thicknesses. The layers of the multi-layer body **201** may also include any suitable device (e.g., light detector **220**). The separation layer **216** may be made of a transparent material. The separation layer **216** may have any suitable thickness, w. In some cases, the thickness of the separation layer **216** may be designed to maximize the collection efficiency of the NBD **10**. In FIG. **6**, the x-axis and y-axis lie in the plane of the first surface **202**(*a*) of the surface layer **202**. The z-axis is orthogonal to this plane.

[0124] The separation layer **216** may optionally include a filter layer **214**, which can be used in fluorescence imaging. The filter layer **214** may include any suitable device(s) (e.g., optical filters) capable of selectively transmitting light having select light properties (e.g., polarization, wavelength, frequency, intensity, phase, spin angular momentum, etc.) while substantially removing light the remaining light by any suitable method such as reflection, absorption or interference. Some examples of suitable devices include filters (e.g., interference filters, absorption filters, etc.). Any type of filter can be used such as dichroic filters, monochromatic filters, etc. In one embodiment, a polarization filter may be used. In the illustrated embodiment, the optional filter layer **214** can be used in fluorescence and phosphorescence applications, to transmit emissions from fluorophores in the object **130** and substantially removes excitation light.

[0125] Although the body **201** in FIG. **6** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. For example, a body **201** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**. In another example, the body **201** omits the separation layer **216**.

[0126] The body **201** also defines or includes a fluid channel **150** which has a channel layer **160** having a first channel surface **150**(*a*) and an opposing second channel surface which coincides with the first surface **202**(*a*) of the surface layer **202**. The fluid channel **150** also has a longitudinal axis, a first lateral side, and a second lateral side. The fluid channel **150** may have any suitable dimensions. For example, the width and/or height of the fluid channel **150** may each be less than about 10 microns, 5 microns, or 1 micron. In some cases, the fluid channel **150** may be sized based on the size of the objects **130** being imaged by the NBD **10**. The fluid channel **150** may also include a fluid flow that can carry the specimen with one or more objects **130** in the general direction of the longitudinal axis. During fabrication, the holographic element **170** may be placed directly on top of the fluid channel **150** in some embodiments.

[0127] The surface layer **202** of the body **201** includes an array of light transmissive regions **204** corresponding and offset from the array of illuminating nondiffracting beams **112**(*a*). Each light transmissive region **204** is fitted with a microaxicon **210**. Each light transmissive region **204** may be of any suitable size and cross-sectional shape. Each light transmissive region **204** may be a hole or a slit. In some cases, the hole/slit may be partially filled with a transparent material.

[0128] In FIG. **6**, the surface layer **202** also includes an array of microaxicons **210** corresponding to the array of light transmissive regions **204**. Each microaxicon **210** is located in the light transmissive region **204** in the surface layer **202** to receive light passing through the light transmissive region **204**. Each microaxicon **210** generates a single detection nondiffracting beam **212** for each object point source **130**(*a*) (scatterer/fluorophore) illuminated by its illuminating nondiffracting beam **112**(*a*). In FIG. **6**, a microaxicon **210** receives light altered by a first object point source **130**(*a*)(**1**) at point **1** and a second object point source **130**(*a*)(**2**) at point **2** and converts the light received into two detection nondiffracting beam **212** at two different propagation angles.

[0129] The arrays of light transmissive regions **204**, the array of microaxicons **210**, and the array of illuminating nondiffracting beams **112**(*a*) are oriented at an array angle, α. Each of the arrays extends across or extends substantially across from the first lateral side to the second lateral side of the fluid channel **150** or alternatively extends across an examining portion of the fluid channel **150** where the object **130** being examined moves through.

[0130] The layers of the body **201** may include any suitable material or combination of materials having any suitable thickness, and may include any suitable devices (e.g., light detector **220**). For example, the surface layer **202** may be made of Polydimethylsiloxane (PDMS). Although FIG. **6** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. For example, the body **210** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**.

[0131] As a group, the array of illumination modules **100** includes an illumination source **110** coupled to a scanning mechanism **120**. The scanning mechanism **120** includes the fluid channel **150** which can have a fluid flow for moving the object **130** through the array of illuminating nondiffracting beams **112**(*a*) and across the array of light transmissive regions **204**. The illumination source **110** includes any suitable nondiffracting beam generator for generating the array of illuminating nondiffracting beams **112**(*a*) (e.g. Bessel beams). In FIG. **6**, the illumination source **110** includes a holographic element **170** (e.g., a custom designed CGH) illuminated by a plane wave excitation beam **142**. An example of a suitable holographic element **170** is described in detail in Section IA. Upon being illuminated by the plane wave from the excitation beam **142**, the holographic element **170** produces the array of well separated illuminating nondiffracting beams **112**(*a*). The distance between the illuminating nondiffracting beams **112**(*a*) is designed to reduce or eliminate crosstalk. In other embodiments, other nondiffracting beam generators may be used.

[0132] Each of the illuminating nondiffracting beams **112** (*a*) in FIG. **6** has a narrowly structured illumination path along a beam axis **115** (shown in FIG. **4**). In FIG. **6**, each illuminating nondiffracting beam **112**(*a*) propagates perpendicular to the XY plane. In other embodiments, the illuminating nondiffracting beam **112**(*a*) may propagate at an angle, β, from the x-axis and/or at an angle, y, from the y-axis.

[0133] In each illumination module and NBDM **200** pair, the illuminating nondiffracting beam **112**(*a*) is offset from the center of the microaxicon **210**, by a beam offset, k. The beam offset, k, is the Euclidean distance on the XY plane between the center of the illuminating nondiffracting beam **112**(*a*) and the center of the microaxicon **210**.

[0134] As a group, the array of NBDMs **200** includes the surface layer **202**, the light detector layer **203** having the light detector **220** with a detecting plane **222**, the separation layer **216** between the light detecting plane **222** and the second surface **202**(*b*) of the surface layer **202**.

[0135] The light detector **220** includes discrete light detecting elements **220**(*a*). The light detecting elements **220**(*a*) may be in any suitable arrangement (e.g., one-dimensional array, two-dimensional array, or a multiplicity of one-dimensional and two-dimensional arrays). The arrays can be in any suitable orientation or combination of orientations. Each light detecting element **220**(*a*) may be of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square).

[0136] In FIG. **6**, each of the detection nondiffracting beams **212** projects a light spot **270** onto the light detector **220** at a particular location. The light spot **270** projected to point **1'** is associated with the object point source **130**(*a*)(**1**) at point **1**. The light spot **270** projected to point **2'** is associated with the object point source **130**(*a*)(**2**) at point **2**.

[0137] The light detecting element(s) **220**(*a*) receiving the detection nondiffracting beams **212** can generate a signal with time varying light data associated with the nondiffracting beam **112** received, as the object **130** moves through the fluid channel **150**. The light detecting element **220**(*a*) at point **1'** will measure time varying light data associated with object point source **130**(*a*)(**1**) at point **1**. The light detecting element **220**(*a*) at point **2'** will measure time varying light data associated with object point source **130**(*a*)(**2**) at point **2**.

[0138] The time varying light data may include, for example, information about the properties of the light detected such as the intensity of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the one or more nondiffracting beams **212** detected by the light detecting elements **220**(*a*), at the time of detection. Time varying light data may also include the location of the light detecting elements **220**(*a*) receiving the one or more nondiffracting beams **212** and the time that the light was detected by the light detecting elements **220**(*a*). For example, the time varying light data from the light detecting element **220**(*a*) at point **1'** may include the location of point **1'** and the intensity of the detection nondiffracting beam **212** measured at point **1'** at different times. As another example, the time varying light data from the light detecting element **220**(*a*) at point **2'** may include the location of point **2'** and the intensity of the detection nondiffracting beam **212** measured at point **2'** at different times.

[0139] Time varying light data from a light detecting element **220**(*a*) may include the three-dimensional position of the point of detection **250** (shown in FIG. **4**) of the nondiffracting beam **212** detected by the light detector **220** at the time of detection. In some cases, the processor **230** may estimate the three-dimensional position of the point of detection **250** as the center or other specific location of the light detecting element **220**(*a*) receiving detection nondiffracting beam **212** at the light detecting plane **222**.

[0140] The NBDM **200** can generate three-dimensional image data based on the time varying light data. Some suitable three-dimensional data includes the distance d, the propagation angle of the detection nondiffracting beam **212**, three-dimensional position of the object point source **130**(*a*), and the intensity or other light property associated with each of the object point sources **130**(*a*).

[0141] Although not shown, the NBD **10** in FIG. **6** also includes a processor **230** communicatively coupled to the light detector **220** and a CRM **240** communicatively coupled to the processor **230**. The processor **230** executes code stored on the CRM **240** to perform some of the functions of NBD **10**. For example, the processor **230** can receive a signal with the time varying light data from the light detector **220** and generate three-dimensional image data of the object **130** based on the time varying light data. The processor **230** may also generate one or more three-dimensional images of the object **130** or a portion of the object **130** based on the three-dimensional image data.

[0142] In the embodiment shown in FIG. **6**, the holographic element **170** on top of the fluid channel **150** can be illuminated with a spatially coherent, incoherent, or partially coherent excitation beam **142** to generate the array of illuminating nondiffracting beams **112**(*a*) that propagate through the height of the fluid channel **150** without diffracting or with highly reduced diffraction. The array of illuminating nondiffracting beams **112**(*a*) is oriented at a small angle to the X axis, so as to sample different points along the Y axis. When an object **130** is present in the fluid channel **150**, each illuminating nondiffracting beam **112**(*a*) illuminates a vertical line (along Z) in the object at a given instant of time. As the sample flows (along X), each illuminating nondiffracting beam **112**

(*a*) consequently illuminates a XZ slice of the object **130**. Because the array of illuminating nondiffracting beams **112**(*a*) are designed to sample different Y locations, each illuminating nondiffracting beam **112**(*a*) illuminates in the array illuminates a subsequent XZ slice of the object. Therefore, within a single pass thorough the array of illuminating nondiffracting beams **112**(*a*), the entire three-dimensional object volume is illuminated.

[0143] The microaxicon **210** of the NBDM **200** generates a detection nondiffracting beam **212** for each object point source (e.g., scaterrer/fluorophore) **130** illuminated by its illuminating nondiffracting beam **112**(*a*). The altered light from the object point source **130**(*a*)(**2**) at point **2** received by the corresponding light transmissive region **204** is illustrated as a cone **122**. The propagation angle (in XZ plane) of the detection nondiffracting beam **212** uniquely corresponds to the height of its corresponding point source **130**(*a*). It is this phenomenon that enables the NBD **10** to sense the third dimension (Z Position). As illustrated, the detection nondiffracting beam **212** created by the object point source **130**(*a*)(**1**) at point **1** in the object **130** is sensed at point **1'** on the light detector **220**, and the detection nondiffracting beam **212** from the object point source **130**(*a*)(**2**) at point **2** is sensed at point **2'** on the light detector **220**. In other words, the Z dimension of the object **130** is encoded in the X dimension of the sensor plane. By estimating the distance (d) of the detection nondiffracting beam **212** on the sensor plane, the height (h; Z position) of an object point source **130**(*a*) can be directly determined as,

$$h = wk\left(\frac{1}{d}\right) \qquad \text{(Eqn. 1)}$$

The X position of the object point source **130**(*a*) is estimated from the time at which the point is imaged as the object flows through the fluid channel of the system. And the Y position of the object point source **130**(*a*) is obtained directly from the Y position of the NBDM **200**. The brightness of the object point source **130**(*a*) is obtained from the total number of photons detected by the light detecting element **220**(*a*) corresponding to that object point source **130**(*a*). The three dimensional image is obtained by plotting the X, Y, and Z positions of all detected object point source **130**(*a*) along with their corresponding brightness values.

[0144] In photon limited applications such as fluorescence imaging, it is important to have good photon collection efficiencies for achieving three-dimensional imaging with high signal to noise ratios. Collection efficiency is defined in Eqns. 2 and 3, where 'a' is the radius of the aperture.

[0145] In one embodiment, an NBD **10** can be designed to address specific sampling requirements based on Eqn. 3. For example, if the portion of interest of a specimen is located at a particular height, the NBD **10** can be designed so that the nondiffracting beam **112**(*a*) offset, k, has a value such that the collection efficiency, based on Eqn. 3, will be maximum for the particular height.

[0146] FIGS. **5**(*a*) and **5**(*b*) are plots of collection efficiencies of the NBD **10** as a function of aperture radius and source height, according to an embodiment of the invention. From FIG. **5**(*b*), the detected brightness of an object point source **130**(*a*) is always influenced by the point's height. This displacement creates a brightness modulation that causes a difference between the actual and measured brightness of the sample by the NBD **10**.

[0147] This brightness modulation by varying collection efficiency can be corrected in the light data before generating the three-dimensional image data and the three-dimensional image, in some embodiments. In one embodiment, the brightness modulation can be corrected by calibrating the NBD **10** to correct for the brightness modulation. For a given design of the NBD **10**, calibrations curves can be experimentally generated to determine the brightness modulation of the system. The calibration curves can then be applied to correct the measured light data for brightness modulation to determine the actual light data from the source.

[0148] The transverse (X,Y) resolution of the NBD **10** is not limited by the size of the light transmissive region (e.g., aperture). Instead, it may only limited by the spot size of the illuminating nondiffracting beam **112**(*a*) generated by a suitable nondiffracting beam generator (e.g., CGH). The axial (Z) resolution of the NBD **10** may be limited by the spot size of the detection nondiffracting beam **212** produced by the microaxicon **210**. In addition, the inverse relationship between 'h' and 'd' in Eqn. 1 affects the Z sensitivity of the NBD **10**.

[0149] B. Perspective Projections Approach

[0150] Using the perspectives projections approach, the NBD **10** measures several two-dimensional images (perspective projections) of the object **130** over different fields of view (viewing angles), and uses tomography algorithms to estimate a three-dimensional image of the object **130** from the two-dimensional projections. In this approach, the NBD **10** includes an NBDM **200** in the presence of uniform illumination **112**(*b*).

[0151] FIGS. **7**(*a*), **7**(*b*), and **7**(*c*) illustrate the fundamentals behind obtaining perspective projections. FIG. **7**(*a*) is a schematic drawing of an NBD **10** which measures light data from a wide viewing angle and an associated plot of the calculated point spread function (PSF) as a function of source position, according to an embodiment of the invention. FIGS. **7**(*b*) and **7**(*b*) are schematic drawings of an NBD **10** which measures perspective projections over narrow viewing angle, and the associated plots of calculated PSF as a function of point source position, according to an embodiment of the invention.

[0152] In FIGS. **7**(*a*), **7**(*b*), and **7**(*c*), each NBD **10** includes an NBDM **200** with a multi-layer body **201** having a surface layer **202**, a light detector layer **203** with a light detector **220** comprised of nine light detecting elements **220**(*a*), and a separation layer **216** between the light detector **202** and the surface layer **202**. The surface layer **202** includes a first surface **202**(*a*) and a second surface **202**(*a*), and a light transmissive region **204** fitted with a microaxicon **210**. Two object point sources **130**(*a*)(**1**) (S_1) and **130**(*a*)(**2**) (S_2) (e.g., scattering particles) are shown moving in the x direction above the surface layer **202**.

[0153] In FIG. **7**(*a*), the NBDM **200** sums light data from all the light detecting elements **220**(*a*) receiving light through a microaxicon **210**, as object point sources **130**(*a*)(**1**) (S_1) and **130**(*a*)(**2**) (S_2) are scanned across the light transmissive region **204**. As shown in FIG. **7**(*a*), when light data from all light detecting elements **220**(*a*) (e.g., pixels) is summed, the NBDM **200** in FIG. **7**(*a*) has a wide viewing angle, with spatial resolutions degrading with increase in the distance between each of the object point sources **130**(*a*)(**1**) and the light transmissive region **204**, providing poor depth of field. This can be seen readily by scanning object point sources **130**(*a*)(**1**) (S_1) and **130**(*a*)(**2**) (S_2) located at different heights, h_1 and h_2, over the NBDM **200**. As shown in the associated plot, the width of the PSF increases rapidly with the height of the point source **130**(*a*). That is, the width, w_1 of the PSF of object point source **130**(*a*)(**1**) (S_1) is much narrower than the width, w_2 of the PSF of object point sources **130**(*a*)(**2**) (S_2). In addition, spatial resolutions degrade with increase in the distance between object point source **130**(*a*) (S_1) and the light transmissive region **204** (e.g., aperture) and thus the NBD **10** exhibits poor depth of field. Also, by summing data over a wide viewing angle, the NBD **10** does not retain the direction (angle) of the photons.

[0154] However, the scenario changes when data from each light detecting element **220**(*a*) (e.g., sensor pixels) is used individually to form image data. In this scenario, the NBD **10** can obtain perspective projections from each of the light detecting elements **220**(*a*), each perspective projection having an extended depth of field. As used herein, a perspective projection refers to a two-dimensional image of the object **130** based on a small viewing angle.

[0155] For example, consider a situation where only the center light detecting element **220**(*a*) (X_0) of the light detector **220** is used, as shown in FIG. **7**(*b*). If the two point sources **130**(*a*)(**1**) (S_1) and **130**(*a*)(**2**) (S_2) at different heights, h_1 and h_2, are scanned again, it can be seen that the PSF width does not increase dramatically with height any longer. In other words, the viewing angle of the NBDM **200** has been reduced. Note that this small viewing angle (narrow field of view) also creates an "extended depth of field" image, where a large Z region of the object **130** is imaged in focus.

[0156] When the light detecting element **220**(*a*) at X_1 (adjacent to X_0) is used for image formation, the NBDM **200** "sees" in a slightly different direction (projection angle) as illustrated in FIG. **7**(*c*). In other words, two-dimensional images formed with light detecting element **220**(*a*) at X_1 and X_0 represent different perspective projections of the object **130**. By forming images from each light detecting element **220**(*a*) of the light detector **220** (e.g., sensor array) within the NBDM **200**, the NBD **10** can obtain different perspective projections of the object **130** at different projection angles. Each projection angle refers to the angle formed by the center axis through each viewing angle.

[0157] The basic idea is to use the light data from different light detecting elements **220**(*a*) (e.g., pixels) of the NBDM **200** to obtain perspective projections (two-dimensional images) of the object **130** at different projection angles. These perspective projections, when fed into a tomography reconstruction algorithm, can then be used to create three-dimensional images of the object **130**.

[0158] In FIGS. **7**(*b*) and **7**(*c*), the microaxicon **210** converts light passing through the light transmissive region **204** into two separate nondiffracting beams **212** propagating at different projection angles directing the detection nondiffracting beams **212** to two light detecting elements **220**(*a*) at X_0 and X_1. The NBDM **200** can generate a perspective projection from the light data measured by each of the light detecting elements **220**(*a*) X_0 and X_1 where the light data from each light detecting elements **220**(*a*) X_0 and X_1 is associated with a different small viewing angle. Each generated perspective projection is associated with a projection angle corresponding to a viewing angle. The projection angle can be determined from the point of detection on the light detecting element **220**(*a*). The NBDM **200** can generate a three-dimensional image of the object **130** using the generated perspective projections and the associated projection angles.

[0159] FIGS. **8** and **9** are schematic drawings of a cross-sectional view of components of an NBD **10** for three-dimensional imaging an object **130** based on perspective projections, according to embodiments of the invention. In FIGS. **8** and **9**, the NBD **10** includes an illumination source **110** providing uniform illumination **112**(*b*) and an NBDM **200**.

[0160] The NBD **10** includes a multi-layer body **201** having a surface layer **202** (e.g., thin metallic layer), a light detector layer **203** having a light detector **220**, and a separation layer **216** (e.g., transparent layer) between the light detector layer **203** and the surface layer **202**. The surface layer **202** includes a first surface **202**(*a*), a second surface **202**(*b*), a light transmissive region **204**, and a microaxicon **210** fitted in the light transmissive region **204**. The separation layer **216** may have any suitable thickness, w. In some cases, the thickness of the separation layer **216** may be designed to maximize the efficiency of the NBD **10**.

[0161] The multi-layer body **201** inexpensively using standard semiconductor and micro/nanofabrication procedures. During an exemplary assembly of the multi-layer body **201**, the separation layer **216** can be placed on top of the light detector **220**. Then, the surface layer **202** with the light transmissive region **204** fitted with the microaxicon **210** can be placed on top of the separation layer **216**.

[0162] Although the body **201** in FIGS. **8** and **9** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. In a fluorescence embodiment, a body **201** may include an additional filter layer **214** (shown in FIG. **6**) within the separation layer **216**. In another example, a body **201** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**. In another example, the body **201** omits the separation layer **216**.

[0163] The light transmissive region **204** may be of any suitable size (e.g., 0.1 μm, 0.5 μm, 1 μm, 5 μm etc.) and have any suitable cross sectional shape (e.g., circular, rectangular, oval, etc.). In many cases, the light transmissive region **204** is a hole or a slit. In some of these cases, the holes/slits may be at least partially filled with a transparent material.

[0164] The microaxicon **210** is located in the light transmissive region **204** in the surface layer **202** to receive light passing through the light transmissive region **204**. The microaxicon **210** can generate one or more detection nondiffracting beams **212** associated with different viewing angles. Each detection nondiffracting beams **212** propagates at a specific projection angle, ψ. Each light detecting element corresponds to a given projection angle, ψ. In FIG. **8**, the microaxicon **210** receives light altered by the object **130** over a narrow viewing angle and converts the light received into a single detection nondiffracting beam **212** at a projection angle, ψ=0. In FIG. **9**, the microaxicon **210** receives light altered by the object **130** over a narrow viewing angle and converts the light received into a single detection nondiffracting beam **212** at a projection angle, ψ.

[0165] The light detector **220** includes nine light detecting elements **220**(*a*)(**1**), **220**(*a*)(**2**), **220**(*a*)(**3**), **220**(*a*)(**4**), **220**(*a*)(**5**), **220**(*a*)(**6**), **220**(*a*)(**7**), **220**(*a*)(**8**), and **220**(*a*)(**9**) arranged in the X-direction. Each light detecting element **220**(*a*) may be of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square). Although a one dimensional array of nine light detecting elements is shown, the light detector **220** may include any suitable number of light detecting elements **220**(*a*) in any suitable form (e.g., two-dimensional array), and in any suitable orientation(s) in other embodiments. Each light detecting element **220**(*a*) can generate a signal with the light data associated with the detection nondiffracting beam **212** received by it.

[0166] Each light detecting element **220**(*a*) in the light detector **220** measures light that can be used to generate a perspective projection at a particular projection angle, ψ. As a group, the light detecting elements **220**(*a*) of each NBDM **200** measure the perspective projections from different projection angles. The projection angle, ψ, refers to the angle formed between the beam axis **115** of the detection nondiffracting beam **212** and a z-directional axis through the center of the light transmissive region **204**. The beam axis **115** can be approximated from the location of the center of the light detecting element **220**(*a*) receiving the detection nondiffracting beam **212** and the center of the light transmissive region **204** at the first surface **202**(*a*) of the surface layer **202**.

[0167] The NBDM **200** comprises the surface layer **202**, the separation layer **216**, and the light detector layer **201** of the body **201**. The NBDM **200** combines, using tomography algorithms, the measured perspective projections at different projection angles to generate three-dimensional image data and three-dimensional images of the object **130**. In FIG. **8**, the light detecting element **220**(*a*) (X_0) measures the perspective projection at a 0 degree projection angle. In FIG. **9**, the light detecting element **220**(*a*) (X_1) measures the perspective projection at projection angle, ψ_z, measured from the z-axis in the XZ plane.

[0168] The light data may include, for example, information about the properties of the light detected such as the intensity (brightness) of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the one or more nondiffracting beams **212** detected by the light detecting elements **220**(*a*). Light data may also include the X location and Y location of the light detecting elements **220**(*a*) receiving the one or more nondiffracting beams **212** and the time that the light was detected by the light detecting elements **220**(*a*). Light data may be data based on a single time, based on multiple times, or based on a time varying basis. Light data from a light detecting element **220**(*a*) may include the three-dimensional position of the point of detection **250** of the detection nondiffracting beam **212** detected by the light detector **220**. In some cases, the three-dimensional position of the point of detection **250** may be estimated as the center or other specific location of the light detecting element **220**(*a*) receiving detection nondiffracting beam **212** at the light detecting plane **222**.

[0169] The NBDM **200** can generate three-dimensional image data based on the light data and can use the three-dimensional image data to generate three-dimensional images. Some suitable three-dimensional data includes the perspective projections and associated projection angles.

[0170] Although not shown, the NBD **10** also includes a processor **230** (shown in FIG. **1**) communicatively coupled to the light detector **220** and a CRM **240** communicatively coupled to the processor **230**. The processor **230** executes code stored on the CRM **240** to perform some of the functions of NBD **10**. For example, the processor **230** can receive a signal with the light data from the light detector **220** and generate three-dimensional image data of the object **130** based on the light data. The processor **230** may also generate one or more three-dimensional images of the object **130** or a portion of the object **130** based on three-dimensional image data.

[0171] The NBD **10** also includes an x-axis, a y-axis (not shown), and a z-axis. The x-axis and y-axis lie in the plane of a first surface **202**(*a*). The z-axis is orthogonal to this plane.

[0172] In operation, the illumination source(s) **110** provides uniform illumination **112**(*b*) across the object **130**. The object **130** alters (e.g., scatters and/or absorbs and re-emits) light from the uniform illumination **112**(*b*). The microaxicon **210** receives light through the light transmissive region **204** from different projection angles. The microaxicon **210** converts the light from the different projection angles into different nondiffracting beams **212** propagating at the projection angles. In some cases, the microaxicon **210** converts the light from predefined projection angles to direct the detection nondiffracting beams **212** at the predefined projection to different light detecting elements **220**(*a*). Each of the light detecting elements **220**(*a*) receives a detection nondiffracting beam **212** and generates a signal with light data (e.g., intensity reading). The processor **230** receives the signal with light data and uses the light data to generate the perspective projections at the different projection angles. The processor uses tomography algorithms to combine the perspective projections at the different projection angles to generate three-dimensional data and/or a three-dimensional image of the object **130**.

[0173] The transverse (X,Y) resolution of the NBD **10** can be limited by the size of the light transmissive region **204** (e.g., aperture) of the NBDM **200** in some cases. The axial resolution (Z) of the NBD **10** can be dictated by the number of perspective projections and range of projection angles used in three-dimensional tomography reconstruction algorithm in some cases.

[0174] Any suitable tomographic reconstruction algorithms can be used by the processor **230** (shown in FIG. **1**) to generate the three-dimensional images from the perspective projections and projection angles. Some examples of tomographic reconstruction algorithms include filtered back-projection and iterative reconstruction.

[0175] 1) Optofluidic Application

[0176] FIGS. **10** and **11** are schematic drawings of components of an NBD **10** using optofluidic flow and takes the perspective projections approach to three-dimensional imaging an object **130**, according to an embodiment of the invention. The NBD **10** includes an array of NBDMs **200** and an array of illumination modules **100** (not shown) providing uniform illumination **112**(*b*). Each of the modules in the arrays is comprised of an x-directional strip having a strip width in the y-direction and a strip length in the y-direction. In some cases, the strip width may be equal to the distance between the light transmissive regions **204** in the y-direction. The strip length may be equal to the length of the NBD **10** in the x-direction. The strips may overlap in the y-direction in some cases. Any suitable number of modules may be used in the array. The NBD **10** also includes an x-axis, a y-axis, and a z-axis.

[0177] Each NBDM **200** measures several perspective projections of the object **130**, as the object **130** moves through the fluid channel **150**. The array of NBDMs **100** has the ability to measure perspective projections from various projection angles and having extended depth of field within a single pass of the object **130** over the array of light transmissive regions **204**. The NBD **10** can use tomography reconstruction algorithms to estimate a three-dimensional image of the object **130** based on the perspective projections measured.

[0178] The NBD **10** includes a multi-layer body **201** having a channel layer **160** having a first channel surface **150**(*a*), surface layer **202** (e.g., thin metallic layer) having a first surface **202**(*a*), a second surface **202**(*b*), an optional filter layer **214**, a light detector layer **203** having a light detector **220**, and a separation layer **216** (e.g., transparent layer) between the light detector layer **203** and the surface layer **202**. The layers of a multi-layer body **201** may be of any suitable material or combination of materials having any suitable thickness or thicknesses. The layers of the multi-layer body **201** may also include any suitable device (e.g., light detector **220**). The separation layer **216** may have any suitable thickness, w. In some cases, the thickness of the separation layer **216** may be designed to maximize the collection efficiency of the NBD **10**. In FIGS. **10** and **11**, the x-axis and y-axis lie in the plane of the first surface **202**(*a*) of the surface layer **202**. The z-axis is orthogonal to this plane.

[0179] The separation layer **216** may optionally include a filter layer **214**, which can be used in fluorescence imaging. The filter layer **214** may include any suitable device(s) (e.g., optical filters) capable of selectively transmitting light having select light properties (e.g., polarization, wavelength, frequency, intensity, phase, spin angular momentum, etc.) while substantially removing light the remaining light by any suitable method such as reflection, absorption or interference. Some examples of suitable devices include filters (e.g., interference filters, absorption filters, etc.). Any type of filter can be used such as dichroic filters, monochromatic filters, etc. In one embodiment, a polarization filter may be used. In the illustrated embodiment, the optional filter layer **214** can be used in fluorescence and phosphorescence applications, to transmit emissions from fluorophores in the object **130** and substantially removes excitation light.

[0180] Although the body **201** in FIGS. **10** and **11** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. For example, a body **201** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**. In another example, the body **201** omits the separation layer **216**.

[0181] The body **201** also defines or includes a fluid channel **150** which has a first channel surface **150**(*a*) and an opposing second channel surface which coincides with the first surface **202**(*a*) of the surface layer **202**. The fluid channel **150** also has a longitudinal axis, a first lateral side, and a second lateral side. The fluid channel **150** may have any suitable dimensions. For example, the width and/or height of the fluid channel **150** may each be less than about 10 microns, 5 microns, or 1 micron. In some cases, the fluid channel **150** may be sized based on the size of the objects **130** being imaged by the NBD **10**. The fluid channel **150** may also include a fluid flow that can carry the specimen with one or more objects **130** in the general direction of the longitudinal axis. During fabrication, the holographic element **170** may be placed directly on top of the fluid channel **150** in some embodiments.

[0182] The surface layer **202** of the body **201** includes an array of light transmissive regions **204**. Each light transmissive region **204** is fitted with a microaxicon **210**. Each light transmissive region **204** may be of any suitable size and cross-sectional shape. Each light transmissive region **204** may be a hole or a slit. In some cases, the hole/slit may be partially filled with a transparent material.

[0183] The surface layer **202** also includes an array of microaxicons **210** corresponding to the array of light transmissive regions **204**. Each microaxicon **210** is located in the light transmissive region **204** in the surface layer **202** to receive light passing through the corresponding light transmissive region **204**. Each microaxicon **210** generates a detection nondiffracting beam **212** for each viewing angle. The microaxicon **210** can generate one or more detection nondiffracting beams **212** associated with different viewing angles **300**. Each detection nondiffracting beams **212** propagates at a specific projection angle, ψ. In FIG. **10**, the microaxicon **210** receives light altered by the object **130** over a narrow viewing angle and converts the light received into a single detection nondiffracting beam **212** at a projection angle, ψ=0. In FIG. **11**, the microaxicon **210** receives light altered by the object **130** over a narrow viewing angle and converts the light received into a single detection nondiffracting beam **212** at a projection angle, ψ.

[0184] The arrays of light transmissive regions **204** and microaxicons **210** are oriented at the array angle, cc. Each array extends across or extends substantially across from the first lateral side to the second lateral side of the fluid channel **150** or alternatively extends across an examining portion of the fluid channel **150** where the object **130** being examined moves through.

[0185] The layers of the body **201** may include any suitable material or combination of materials having any suitable thickness, and may include any suitable devices (e.g., light detector **220**). For example, the surface layer **202** may be made of Polydimethylsiloxane (PDMS). Although FIG. **6** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. For example, the body **210** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**. Although the body **201** in FIGS. **10** and **11** has certain layers, other embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **201**. In another example, a body **201** may include a transparent protective layer outside the first surface **202**(*a*) to isolate the surface layer **202**. In another example, the body **201** omits the separation layer **216**.

[0186] The multi-layer body **201** may be fabricated inexpensively using standard semiconductor and micro/nanofabrication procedures. During an exemplary assembly of the multi-layer body **201**, the separation layer **216** can be placed on top of the light detector **220**. Then, the surface layer **202** with the light transmissive region **204** fitted with the microaxicon **210** can be placed on top of the separation layer **216**.

[0187] As a group, the array of illumination modules **100** includes an illumination source **110** coupled to a scanning mechanism **120**. The scanning mechanism **120** includes the fluid channel **150** which can have a fluid flow for moving the object **130** across the array of light transmissive regions **204**. The illumination source **110** includes any suitable source of uniform illumination **112**(*b*).

[0188] As a group, the array of NBDMs **200** includes the surface layer **202**, the light detector layer **203** having the light detector **220** with a detecting plane **222**, the separation layer **216** between the light detecting plane **222** and the second surface **202**(*b*) of the surface layer **202**.

[0189] The NBDM **200** comprises the surface layer **202**, the separation layer **216**, and the light detector layer **201** of the body **201**. The arrays of NBDMs **200** combine, using tomographic algorithms, the measured perspective projections at different projection angles to generate three-dimensional image data and three-dimensional images of the object **130**. In FIG. **8**, the light detecting element **220**(*a*) (X_0) measures the perspective projection at a 0 degree projection angle. In FIG. **9**, the light detecting element **220**(*a*) (X_1) measures the perspective projection at projection angle, ψ_z, measured from the z-axis in the XZ plane.

[0190] The light detector **220** includes a two-dimensional array of discrete light detecting elements **220**(*a*). Although a two-dimensional array of light detecting elements **220**(*a*) is shown, other suitable arrangements (e.g., one-dimensional array or a multiplicity of one-dimensional and two-dimensional arrays) can be used. The arrays can be in any suitable orientation or combination of orientations. Each light detecting element **220**(*a*) may be of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square).

[0191] Each light detecting element **220**(*a*) in the light detector **220** measures light from a detection nondiffracting beam **212** associated with a narrow viewing angle and a projection angle. The measured light from each light detecting element **220**(*a*) can be used to generate a perspective projection associated with the narrow viewing angle and a projection angle. In FIG. **10**, each light detecting element **220**(*a*) at point X_0 of each NBDM **200** measures a perspective projection at a 0 degree projection angle. In FIG. **11**, the light detecting element **220**(*a*) at point X_1 of each NBDM **200** measures the perspective projection at projection angle, ψ.

[0192] Each light detecting element **220**(*a*) can generate a signal with time varying light data associated with the light received, as the object **130** moves through the fluid channel **150**. The time varying light data can be used to a generate perspective projection at an associated projection angle, ψ. As a group, the light detecting elements **220**(*a*) of each NBDM **200** measure the perspective projections from different projection angles, as the object **130** moves through the fluid channel **150**.

[0193] The time varying light data may include, for example, information about the properties of the light detected such as the intensity of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the one or more nondiffracting beams **212** detected by the light detecting elements **220**(*a*), at the time of detection. Time varying light data may also include the location of the light detecting elements **220**(*a*) receiving the one or more nondiffracting beams **212** and the time that the light was detected by the light detecting elements **220**(*a*). Time varying light from a light detecting element **220**(*a*) may include the three-dimensional position of the point of detection **250** of the detection nondiffracting beam **212** detected by the light detector **220**. In some cases, the three-dimensional position of the point of detection **250** may be estimated as the center or other specific location of the light detecting element **220**(*a*) receiving detection nondiffracting beam **212** at the light detecting plane **222**. Time varying light data may include data in any suitable form such as a line scan.

[0194] In the perspective projection approach, each light detecting element **220**(*a*) is associated with a detection nondiffracting beam **212** that propagates along a projection angle, ψ, which is the center of its associated view cone. The projection angle, ψ, can be approximated as the angle formed between the beam axis **115** of the detection nondiffracting beam **212** and a z-directional axis through the center of the microaxicon **210**. The beam axis **115** can be approximated from the location of the center of the light detecting element **220**(*a*) receiving the detection nondiffracting beam **212** and the center of the light transmissive region **204** at the first surface **202**(*a*) of the surface layer **202**.

[0195] The NBDM **200** can generate three-dimensional image data based on the light data and can use the three-dimensional image data to generate three-dimensional images. Some suitable three-dimensional data includes the perspective projections and associated projection angles.

[0196] Although not shown in FIGS. **10** and **11**, the NBD **10** also includes a processor **230** communicatively coupled to the light detector **220** and a CRM **240** communicatively coupled to the processor **230**. The processor **230** executes code stored on the CRM **240** to perform some of the functions of NBD **10**. For example, the processor **230** can receive a signal with the light data from the light detector **220** and generate three-dimensional image data of the object **130** based on the light data. The processor **230** may also generate one or more three-dimensional images of the object **130** or a portion of the object **130** based on three-dimensional image data.

[0197] In operation, the illumination source **110** provides uniform illumination **112**(*b*). When an object **130** is present in the fluid channel **150**, the object is illuminated. The object **130** alters light. The array of microaxicons **210** receives light through the corresponding array of light transmissive regions **204**, as the object moves through the fluid channel **150**. The microaxicon **210** converts the light received into separate detection nondiffracting beams **212** associated with various viewing angles. The detection nondiffracting beams **212** propagate at different projection angles to different light detecting elements **220**(*a*). The light detecting elements receive the detection nondiffracting beams **212** and generate a signal with time varying light data associated with the received light. The processor **230** uses the time varying light data associated with each light detecting element **220**(*a*) to generate a perspective projection and determine an associated projection angle, at each time of detection. The processor **230** uses a tomography reconstruction algorithm to generate a three-dimensional image of the object **130** using the perspective projections at various projection angles and associated viewing angles.

[0198] Any suitable tomographic reconstruction algorithms can be used by the processor **230** (shown in FIG. **1**) to generate the three-dimensional images from the perspective projections and projection angles. Suitable tomography reconstruction algorithms are commercially available. Some examples of tomographic reconstruction algorithms include filtered back-projection and iterative reconstruction.

[0199] III. Subsystems

[0200] FIG. **12** shows a block diagram of subsystems that may be present in the NBD **10**, according to embodiments of the invention. For example, the NBD **10** includes a processor **230** for processing light data and for generating three-dimensional image data and/or three-dimensional images of the object **130**. The processor **230** may be a component of the light detector **220**, in some cases. In other embodiments, the NBD **10** may be in communication with a computer having one or more of the subsystems in FIG. **12**.

[0201] The various components previously described in the Figures may operate using one or more of the subsystems to facilitate the functions described herein. Any of the components in the Figures may use any suitable number of subsystems to facilitate the functions described herein. Examples of such subsystems and/or components are shown in a FIG. **12**. The subsystems shown in FIG. **12** are interconnected via a system bus **375**. Additional subsystems such as a printer **374**, keyboard **378**, fixed disk **379** (or other memory comprising computer readable media), display **376**, which is coupled to display adapter **382**, and others are shown. Peripherals and input/output (I/O) devices, which couple to I/O controller **371**, can be connected to the computer system by any number of means known in the art, such as serial port **377**. For example, serial port **377** or external interface **381** can be used to connect the computer apparatus to a wide area network such as the Internet, a mouse input device, or a scanner. The interconnection via system bus allows the processor **230** to communicate with each subsystem and to control the execution of instructions from system memory **340** or the fixed disk **379**, as well as the exchange of information between subsystems. The system memory **340** and/or the fixed disk **379** may embody a computer readable medium **240**. Any of these elements may be present in the previously described features. A computer readable medium **240** according to an embodiment of the invention may comprise code for performing any of the functions described above.

[0202] In some embodiments, an output device such as the printer **384** or display **376** of the NBD **10** can output various forms of data. For example, the NBD **10** can output a bright-field image and/or a fluorescence image of an object **130** or other results of analysis.

[0203] It should be understood that the present invention as described above can be implemented in the form of control logic using computer software in a modular or integrated manner. Based on the disclosure and teachings provided herein, a person of ordinary skill in the art will know and appreciate other ways and/or methods to implement the present invention using hardware and a combination of hardware and software.

[0204] Any of the software components or functions described in this application, may be implemented as software code to be executed by a processor using any suitable computer language such as, for example, Java, C++ or Perl using, for example, conventional or object-oriented techniques. The software code may be stored as a series of instructions, or commands on a computer readable medium, such as a random access memory (RAM), a read only memory (ROM), a magnetic medium such as a hard-drive or a floppy disk, or an optical medium such as a CD-ROM. Any such computer readable medium may reside on or within a single computational apparatus, and may be present on or within different computational apparatuses within a system or network.

[0205] A recitation of "a", "an" or "the" is intended to mean "one or more" unless specifically indicated to the contrary.

[0206] The above description is illustrative and is not restrictive. Many variations of the disclosure will become apparent to those skilled in the art upon review of the disclosure. The scope of the disclosure should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the pending claims along with their full scope or equivalents.

[0207] One or more features from any embodiment may be combined with one or more features of any other embodiment without departing from the scope of the disclosure. Further, modifications, additions, or omissions may be made to any embodiment without departing from the scope of the disclosure. The components of any embodiment may be integrated or separated according to particular needs without departing from the scope of the disclosure.

[0208] All patents, patent applications, publications, and descriptions mentioned above are hereby incorporated by reference in their entirety for all purposes. None is admitted to be prior art.

What is claimed is:

1. A nondiffracting beam detection module for generating three-dimensional image data, comprising:

a surface layer having a first surface and a light transmissive region;

a microaxicon configured to receive light through the light transmissive region from outside the first surface and generate one or more detection nondiffracting beams based on the received light; and

a light detector configured to receive the one or more detection nondiffracting beams and generate three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received.

2. The nondiffracting beam detection module for generating three-dimensional image data of claim **1**, further comprising a processor configured to generate a three-dimensional image of a portion of the object based on the three-dimensional image data.

3. The nondiffracting beam detection module for generating three-dimensional image data of claim **1**, wherein the light detector is further configured to localize a three-dimensional position on the object associated with each detection nondiffracting beam received.

4. The nondiffracting beam detection module for generating three-dimensional image data of claim **1**, wherein the light detector includes light detecting elements, each light detecting element associated with a different height on the object.

5. The nondiffracting beam detection module for generating three-dimensional image data of claim **1**, wherein the light detector is further configured to determine perspective projections based on the detection nondiffracting beams received, the light detector further configured to generate the three-dimensional image data, using tomography, based on the determined perspective projections.

6. The non nondiffracting beam detection module for generating three-dimensional image data of claim **1**, further comprising a separation layer between the light detector and the surface layer.

7. The nondiffracting beam detection module for generating three-dimensional image data of claim **1**, further comprising a filter located between the light detector and surface layer, the filter configured to pass emission light.

8. The nondiffracting beam detection module for generating three-dimensional image data of claim **1**, wherein the light transmissive region is an aperture.

9. A nondiffracting beam detection device for three-dimensional imaging, comprising:

a nondiffracting beam detection module comprising:

a surface layer having a first surface, a second opposing surface, and a light transmissive region;

a microaxicon in the light transmissive region, the microaxicon configured to receive light through the light transmissive region and generate one or more detection nondiffracting beams based on the received light; and

a light detector configured to receive the one or more detection nondiffracting beams and generate three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received; and

a processor in communication with the light detector to receive the three-dimensional image data, and configured to generate a three-dimensional image of a portion of the object based on the three-dimensional image data received.

10. The nondiffracting beam detection device of claim **9**, further comprising an illumination source for generating an illuminating nondiffracting beam through the object,

wherein the light detector is further configured to localize a three-dimensional position of one or more point sources on the object, associated with the one or more detection nondiffracting beams received, and

wherein the processor generates the three-dimensional image using the three-dimensional position of the one or more point sources.

11. The nondiffracting beam detection device of claim **9**, further comprising an illumination source for generating an illuminating nondiffracting beam propagating through the object,

wherein the light detector includes one or more light detecting elements, each light detecting element associated with a different height,

wherein the processor generates the three-dimensional image using light data generated by each light detecting element receiving a detection nondiffracting beam and the height associated with the light detection element.

12. The nondiffracting beam detection device of claim **9**, further comprising a scanner coupled to the illumination source, the scanner configured to move the illuminating nondiffracting beam through a volume of the object.

13. The nondiffracting beam detection device of claim **9**, further comprising a scanner coupled to the object, the scanner configured to move the object through the illuminating nondiffracting beam.

14. The nondiffracting beam detection device of claim **9**, further comprising an illumination source providing uniform illumination outside the first surface,

wherein the light detector is further configured to determine one or more perspective projections having different viewing angles, and

wherein the processor is further configured to estimate, using tomography, the three-dimensional image of the object from the one or more determined perspective projections.

15. The nondiffracting beam detection device of claim **9**, further comprising a separation layer between the light detector and the surface layer.

16. The nondiffracting beam detection device of claim **9**, further comprising a filter located between the light detector and surface layer, the filter configured to pass emission light.

17. The nondiffracting beam detection device of claim **9**, wherein the light detector includes the processor.

18. A nondiffracting beam detection device for three-dimensional imaging, comprising:

a body having a surface layer having a first surface;

a plurality of nondiffracting beam detection modules, each nondiffracting beam detection module comprising:

a light transmissive region in the surface layer;

a microaxicon in the light transmissive region, the microaxicon configured to receive light through the light transmissive region and generate one or more detection nondiffracting beams based on the received light; and

a light detector configured to receive the one or more detection nondiffracting beams and generate three-dimensional image data associated with an object located outside the first surface based on the one or more detection nondiffracting beams received; and

a processor configured to generate a three-dimensional image of the object based on the three-dimensional image data received from the light detectors of the plurality of nondiffracting beam detection modules.

19. An optofluidic, nondiffracting beam detection device for three-dimensional imaging, comprising:

a body including a fluid channel having a surface layer with a first surface;

an array of light transmissive regions in the surface layer, the array of light transmissive regions extending from a first lateral side to a second lateral side of the fluid channel;

an array of microaxicons in the array of light transmissive regions, each microaxicon configured to receive light through the associated light transmissive region and generate one or more nondiffracting beams based on the received light;

a light detector comprising one or more light detecting elements, the light detector configured to receive the one or more detection nondiffracting beams and generate time varying light data associated with the one or more detection nondiffracting beams received as an object passes through the fluid channel; and

a processor configured to generate a three-dimensional image of the object based on the time-varying light data.

20. The optofluidic, nondiffracting beam detection device of claim **19**,

further comprising an illumination source for generating an illuminating nondiffracting beam in the fluid channel,

wherein the light detector is further configured to localize a three-dimensional position of one or more point sources on the object, with the one or more detection nondiffracting beams received, and

wherein the processor generates the three-dimensional image using the three-dimensional position of the one or more point sources.

21. The optofluidic, nondiffracting beam detection device of claim **19**,

further comprising an illumination source for generating an illuminating nondiffracting beam in the fluid channel,

wherein each light detecting element is associated with a different height, and

wherein the processor generates the three-dimensional image using light data generated by each light detecting element receiving a detection nondiffracting beam and the height associated with the light detection element.

22. The optofluidic, nondiffracting beam detection device of claim **19**,

further comprising an illumination source providing uniform illumination outside the first surface,

wherein the light detector is further configured to determine one or more perspective projections having different viewing angles, and

wherein the processor is further configured to estimate, using tomography, the three-dimensional image of the object from the one or more determined perspective projections.

23. The optofluidic, nondiffracting beam detection device of claim **19**, further comprising a separation layer between the light detector and the surface layer.

24. The optofluidic, nondiffracting beam detection device of claim **19**, further comprising a filter located between the light detector and surface layer, the filter configured to pass emission light.

25. The optofluidic, nondiffracting beam detection device of claim **19**, wherein the light detector includes the processor.

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