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(54) **DEPTH OF FIELD EXTENSION FOR OPTICAL TOMOGRAPHY**

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(75) Inventors: **J. Richard Rahn**, Sammamish, WA (US); **Jon W. Hayenga**, Redmond, WA (US)

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(73) Assignee: **Visiongate, Inc.**, Gig Harbor, WA (US)

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Widjanarko, T., et al., A post-processing technique for extending depth of focus in conventional optical microscopy, *Optics & Laser Technology* 34, pp. 299-305 (2002).

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(Continued)

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G01J 1/00 (2006.01)

Primary Examiner—Gregory J Toatley, Jr.

Assistant Examiner—Tri T Ton

(52) **U.S. Cl.** **356/213**; 356/234

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

(58) **Field of Classification Search** 356/213–236; 250/455.11, 461.2; 382/131–134; 435/447–448, 435/40.5; 600/309–310; 378/6–11, 21–28; 702/19, 21, 28–29; 377/10–12; 73/53.01

(57) **ABSTRACT**

See application file for complete search history.

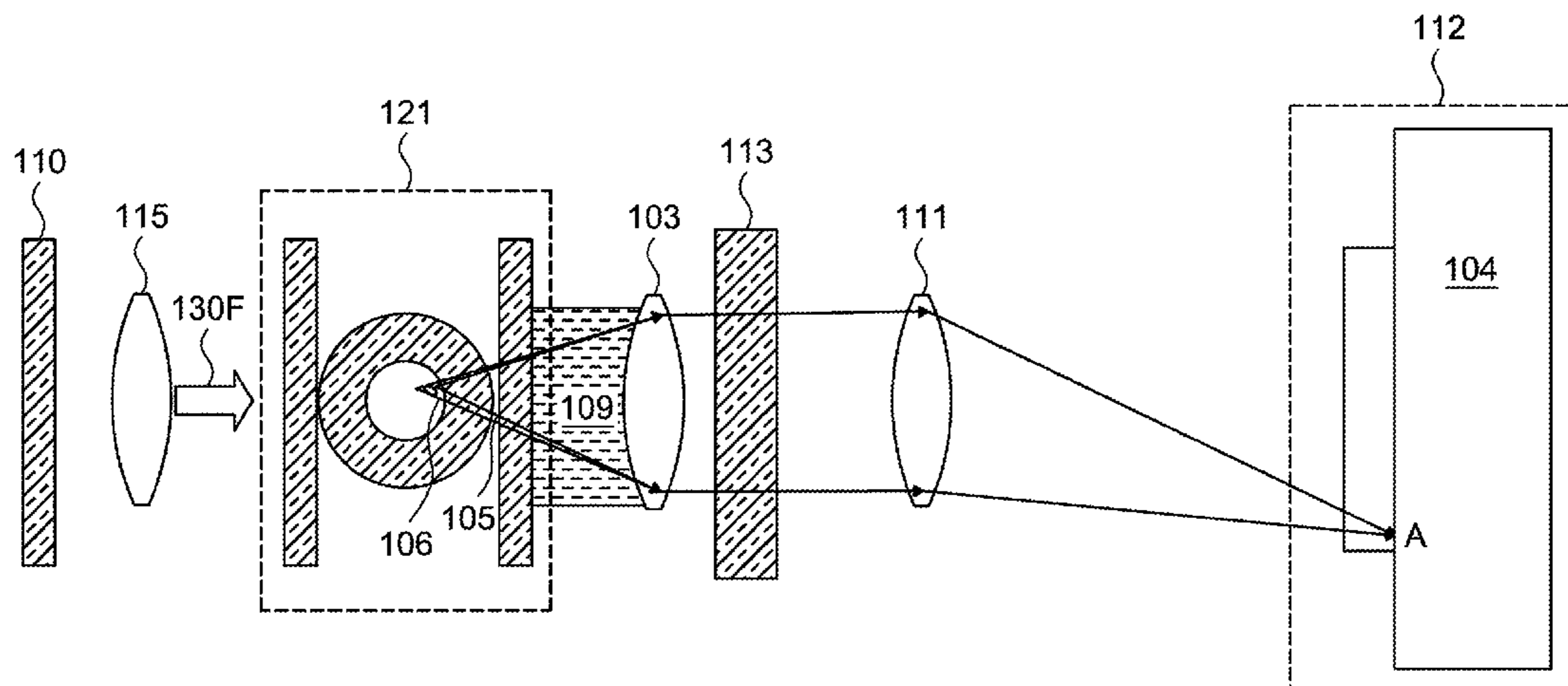
An optical projection tomography system is illuminated with a light source. An object-containing tube, a portion of which is located within the region illuminated by the light source, contains an object of interest that has a feature of interest. A detector is located to receive emerging radiation from the object of interest. A lens, including optical field extension elements, is located in the optical path between the object region and the detector, such that light rays from multiple object planes in the object-containing tube simultaneously focus on the detector. The object-containing tube moves relatively to the detector and the lens operate to provide multiple views of the object region for producing an image of the feature of interest at each view.

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47 Claims, 26 Drawing Sheets



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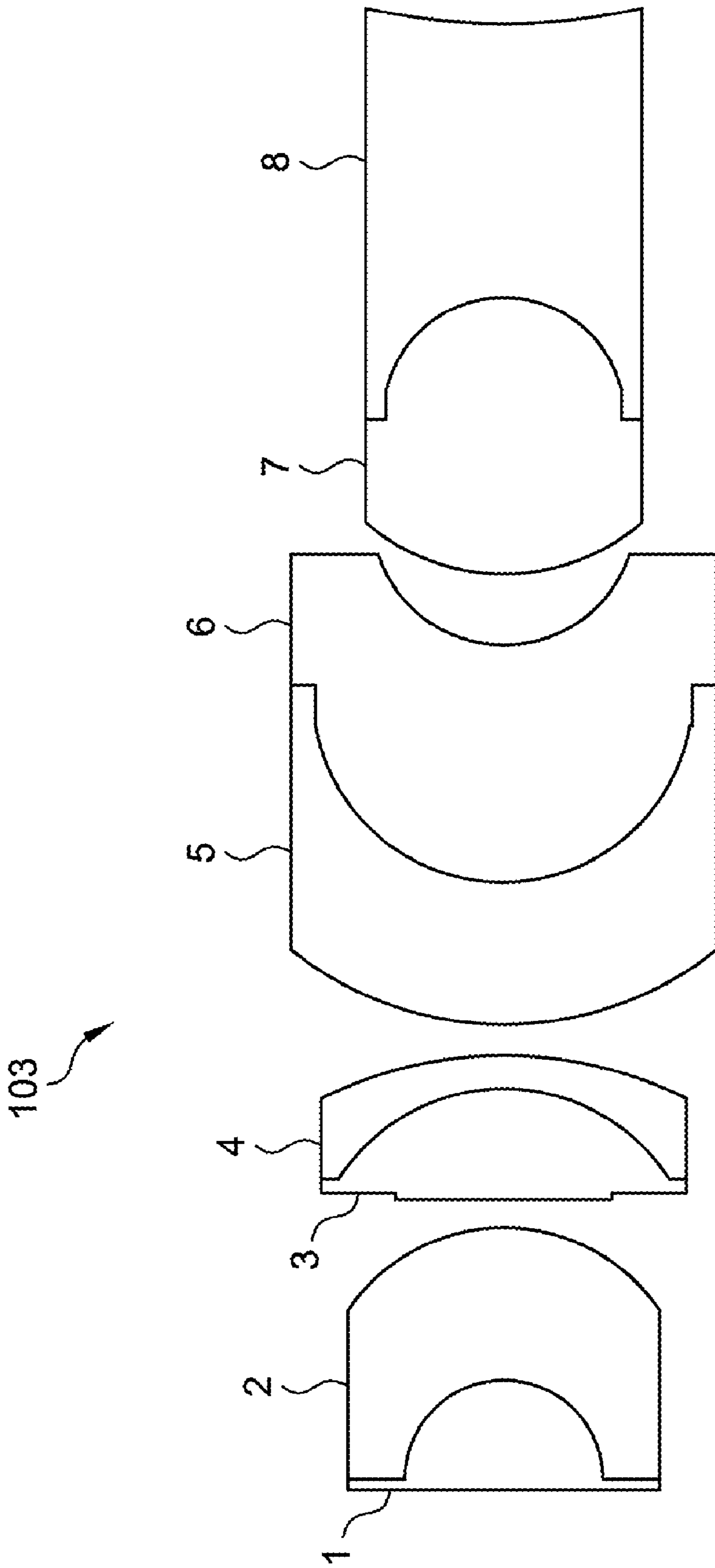


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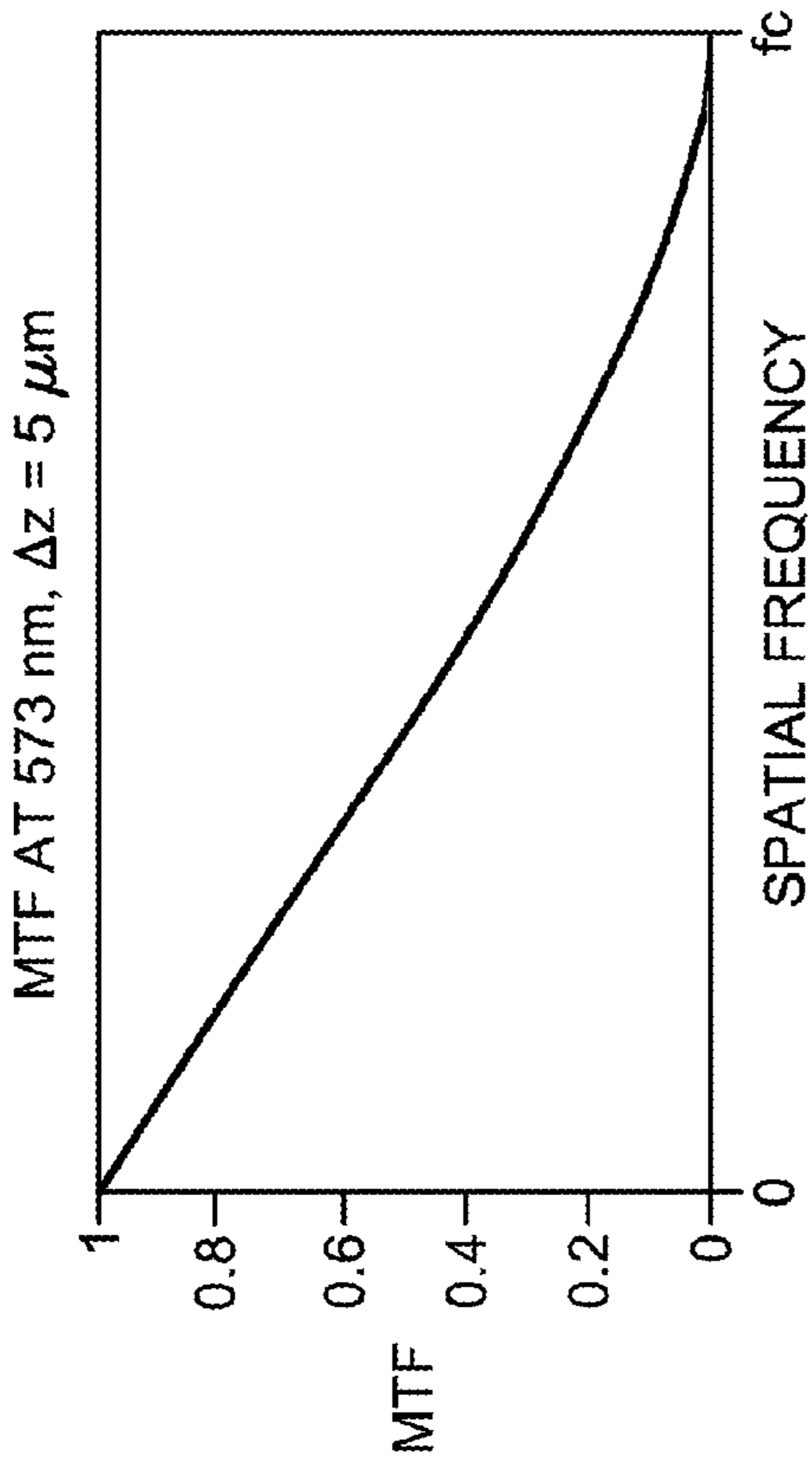


Fig. 2B

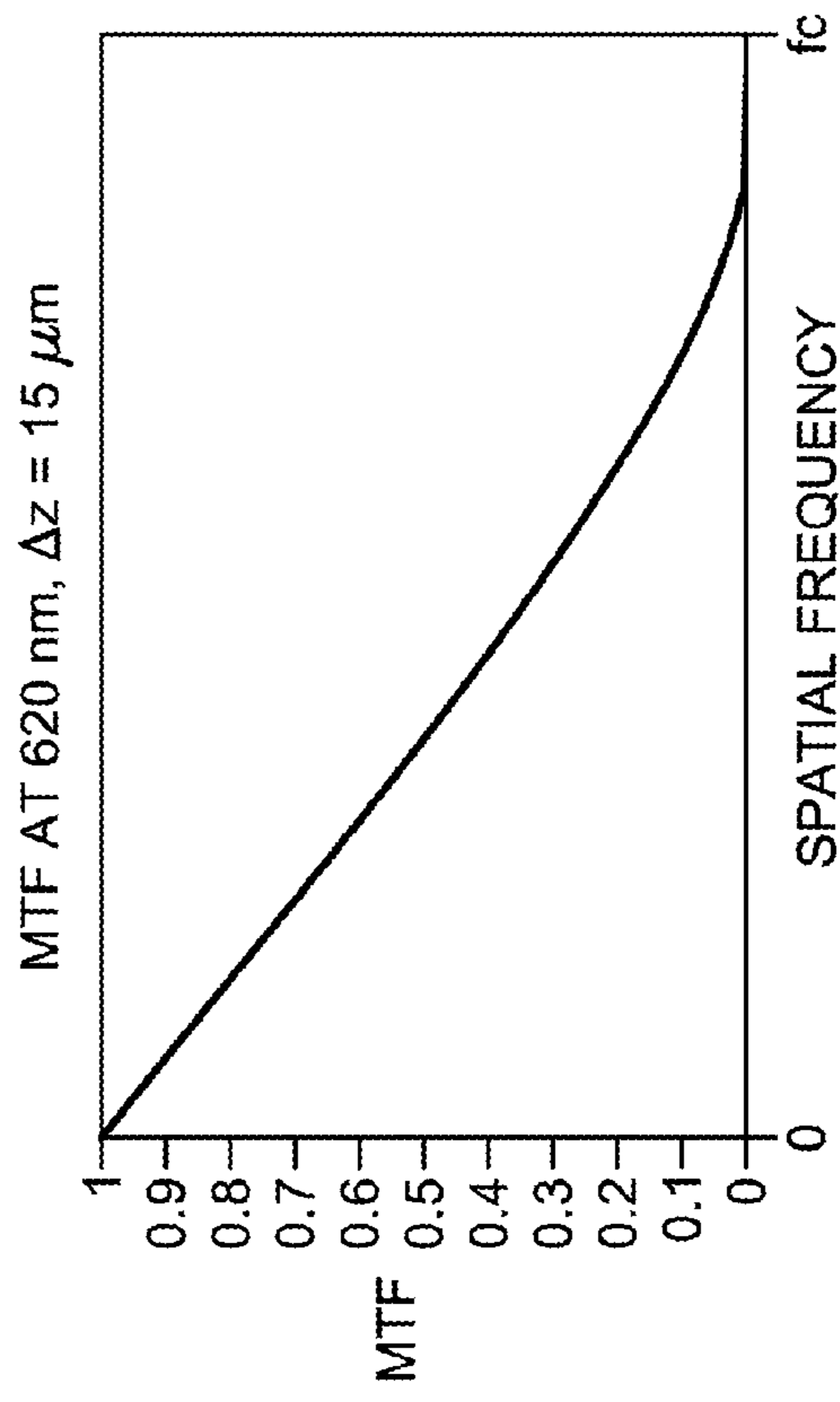


Fig. 2D

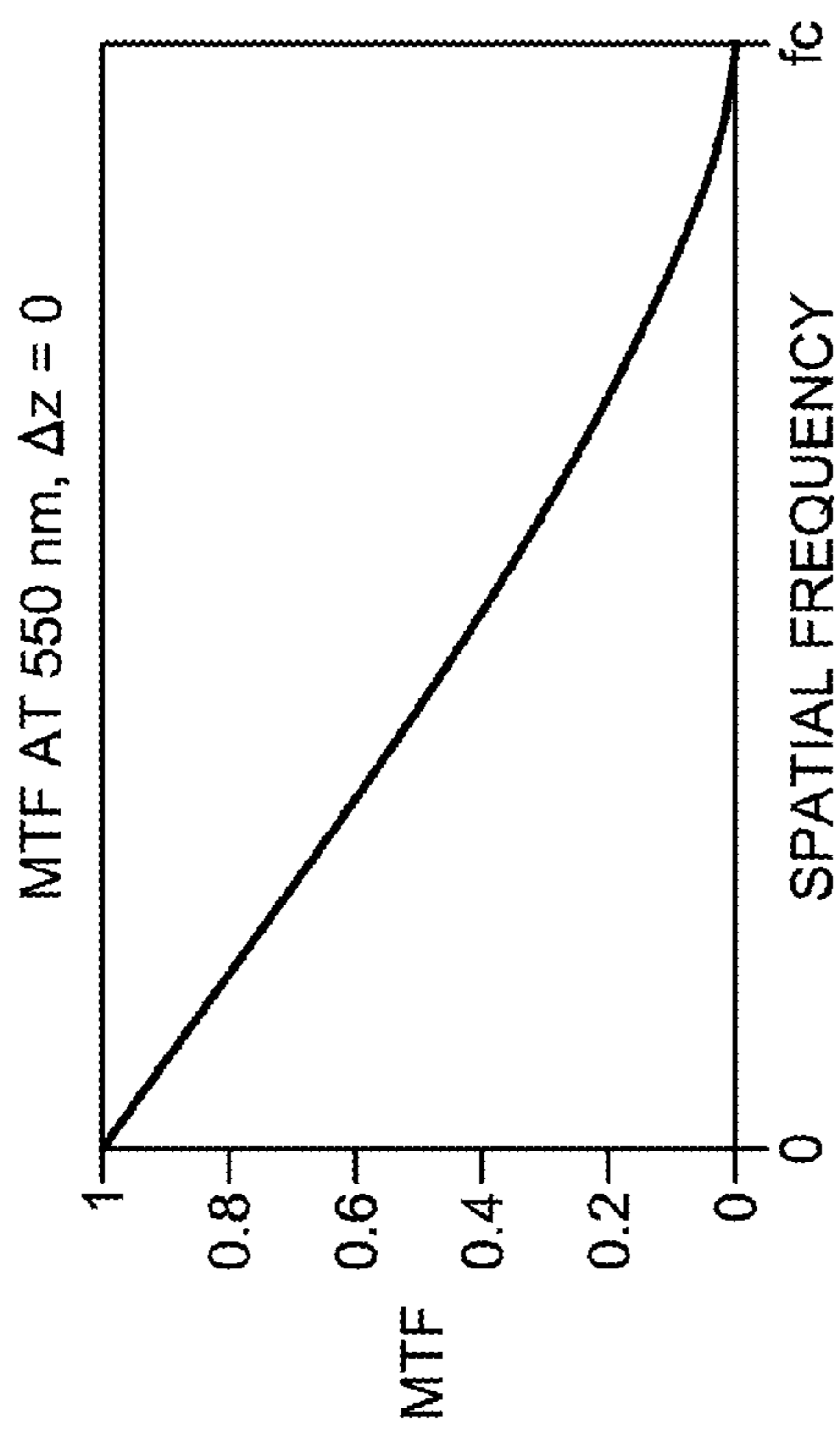


Fig. 2A

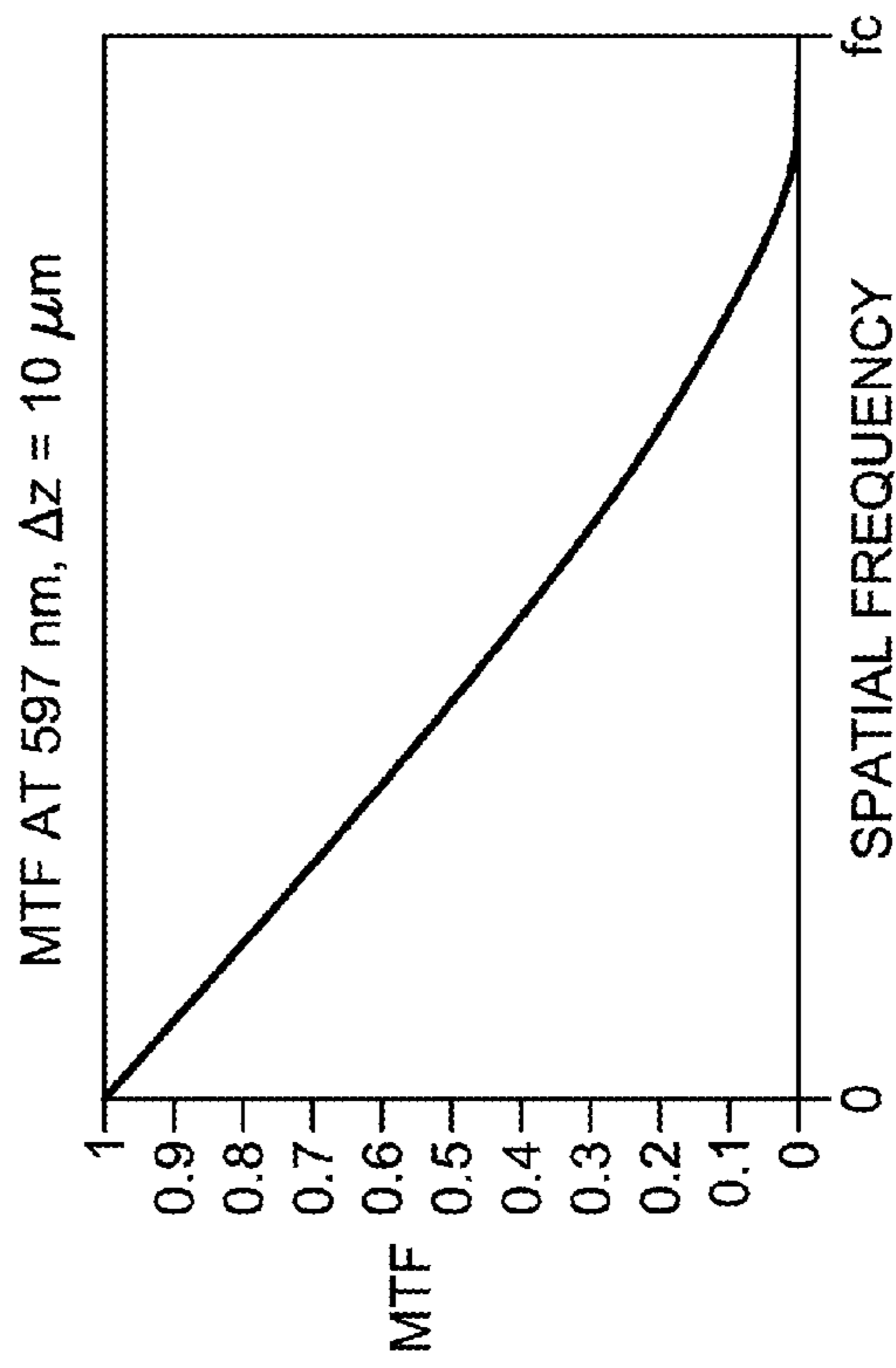


Fig. 2C

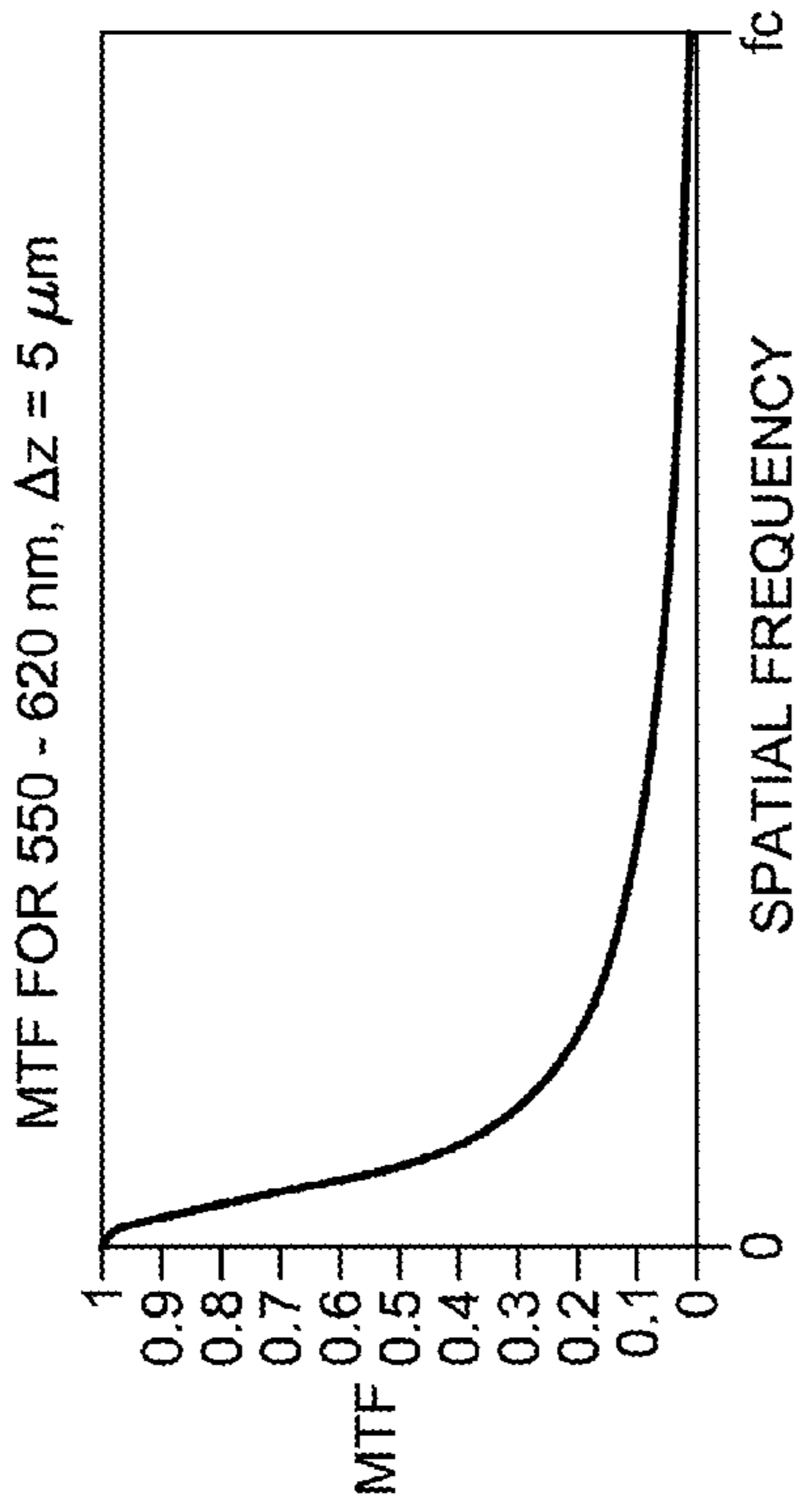


Fig. 3B

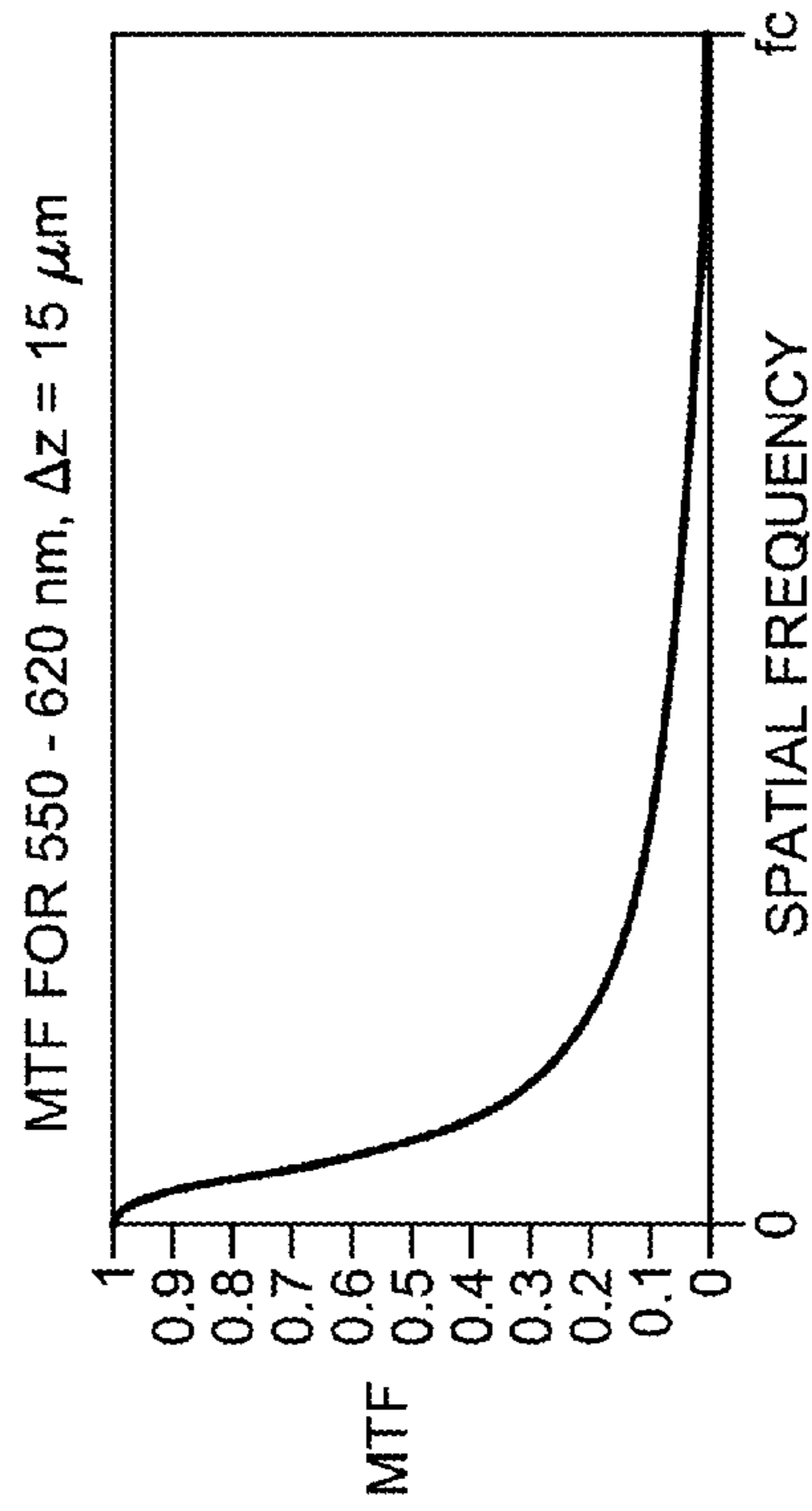


Fig. 3D

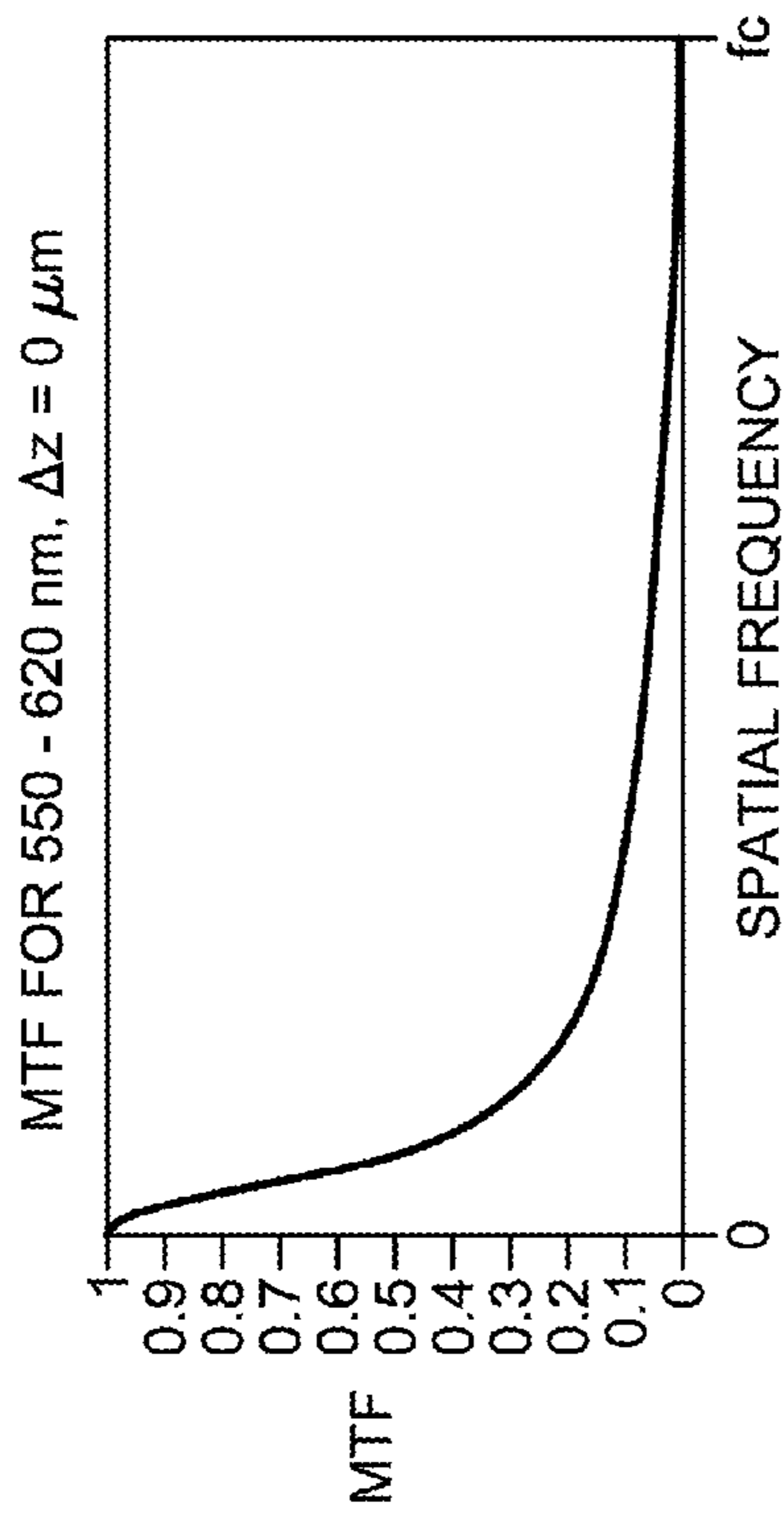


Fig. 3A

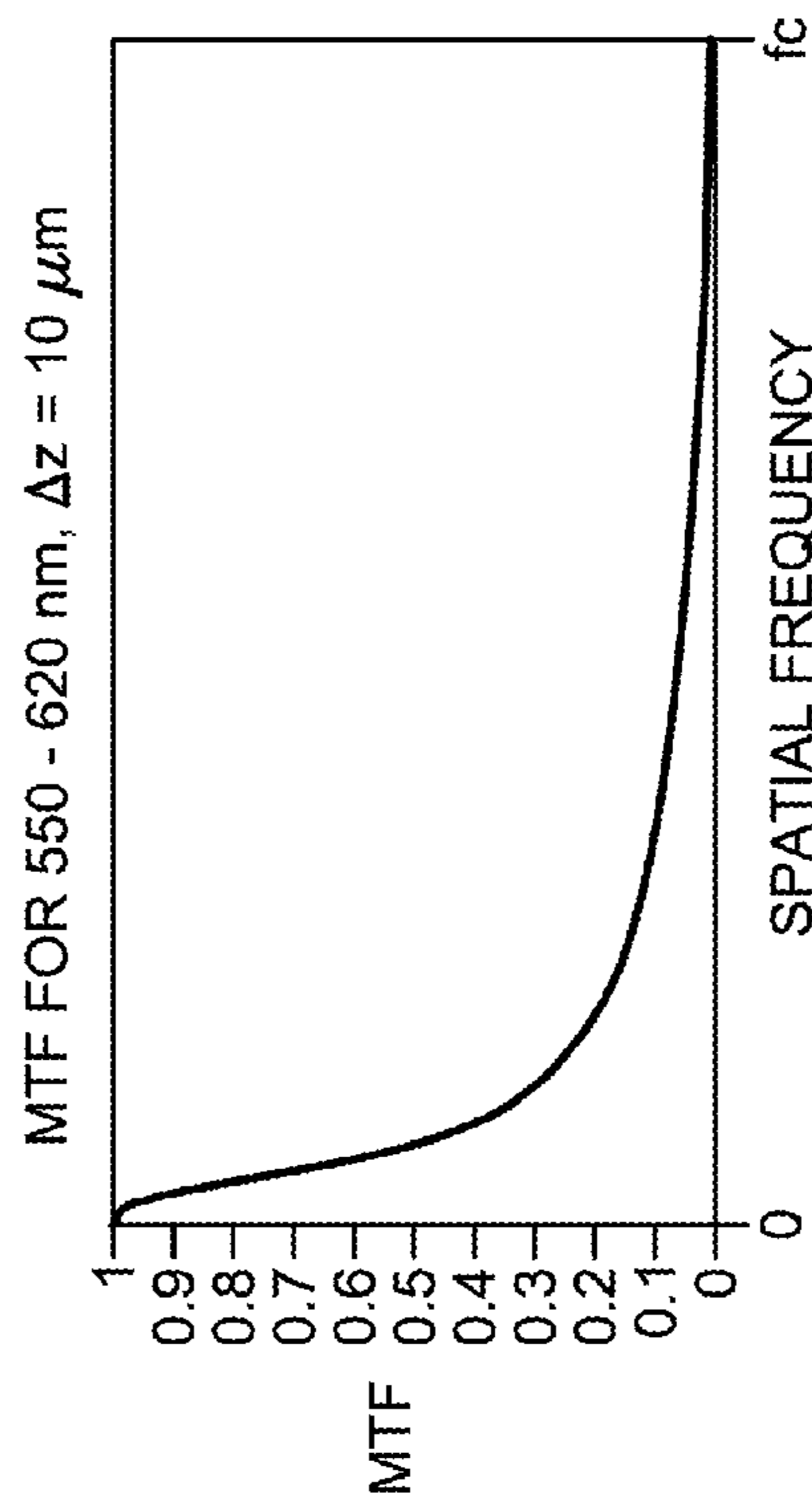


Fig. 3C

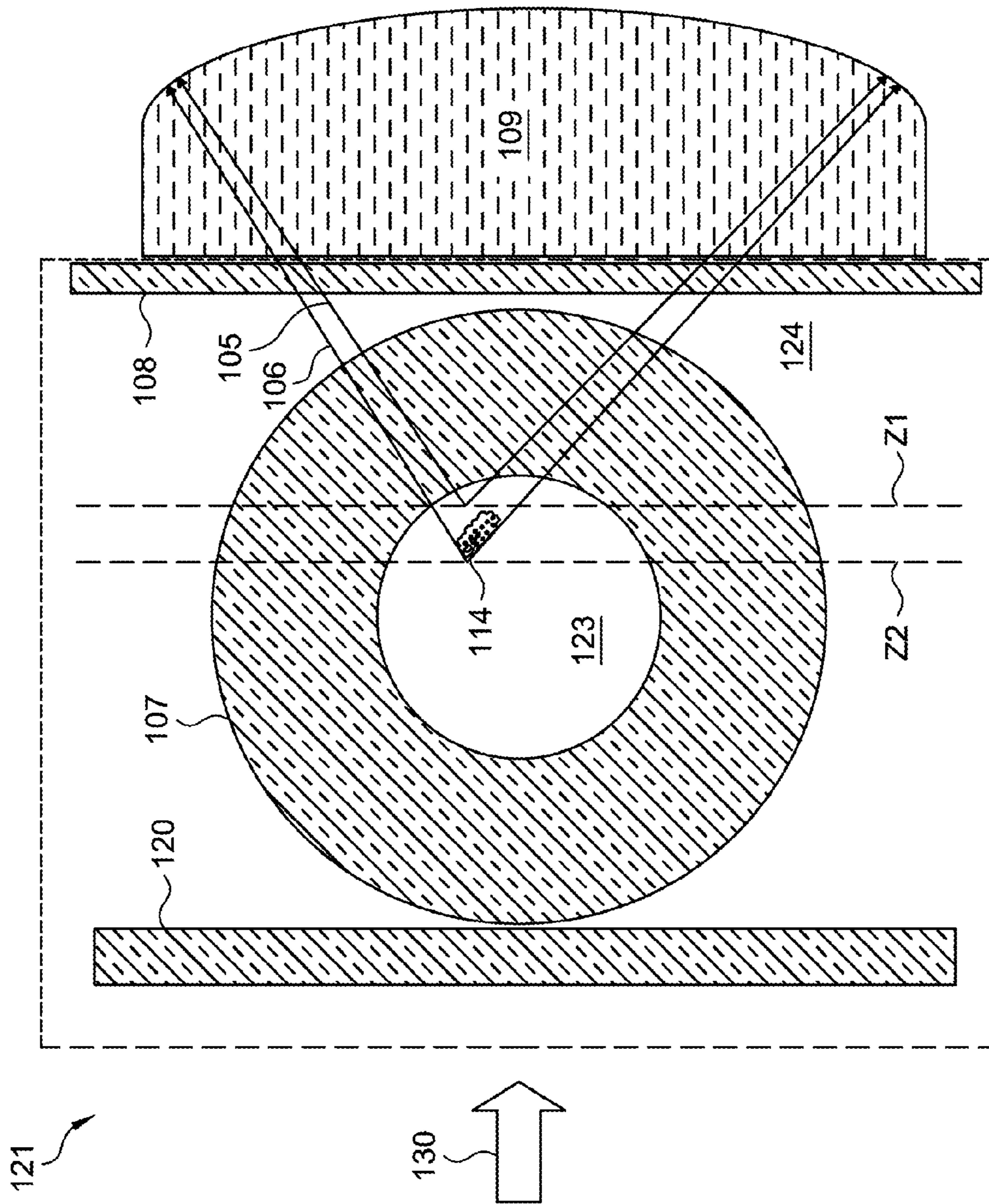


Fig. 4A

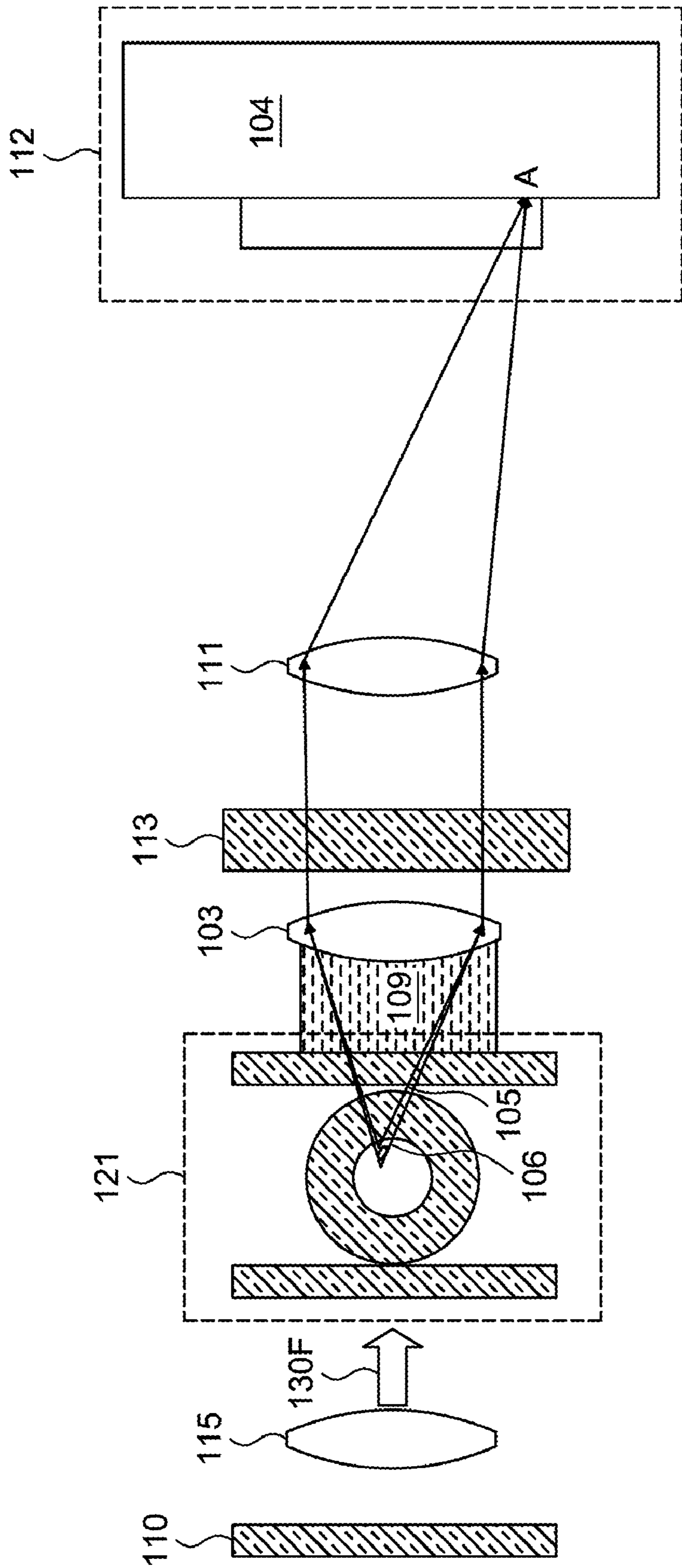


Fig. 4B

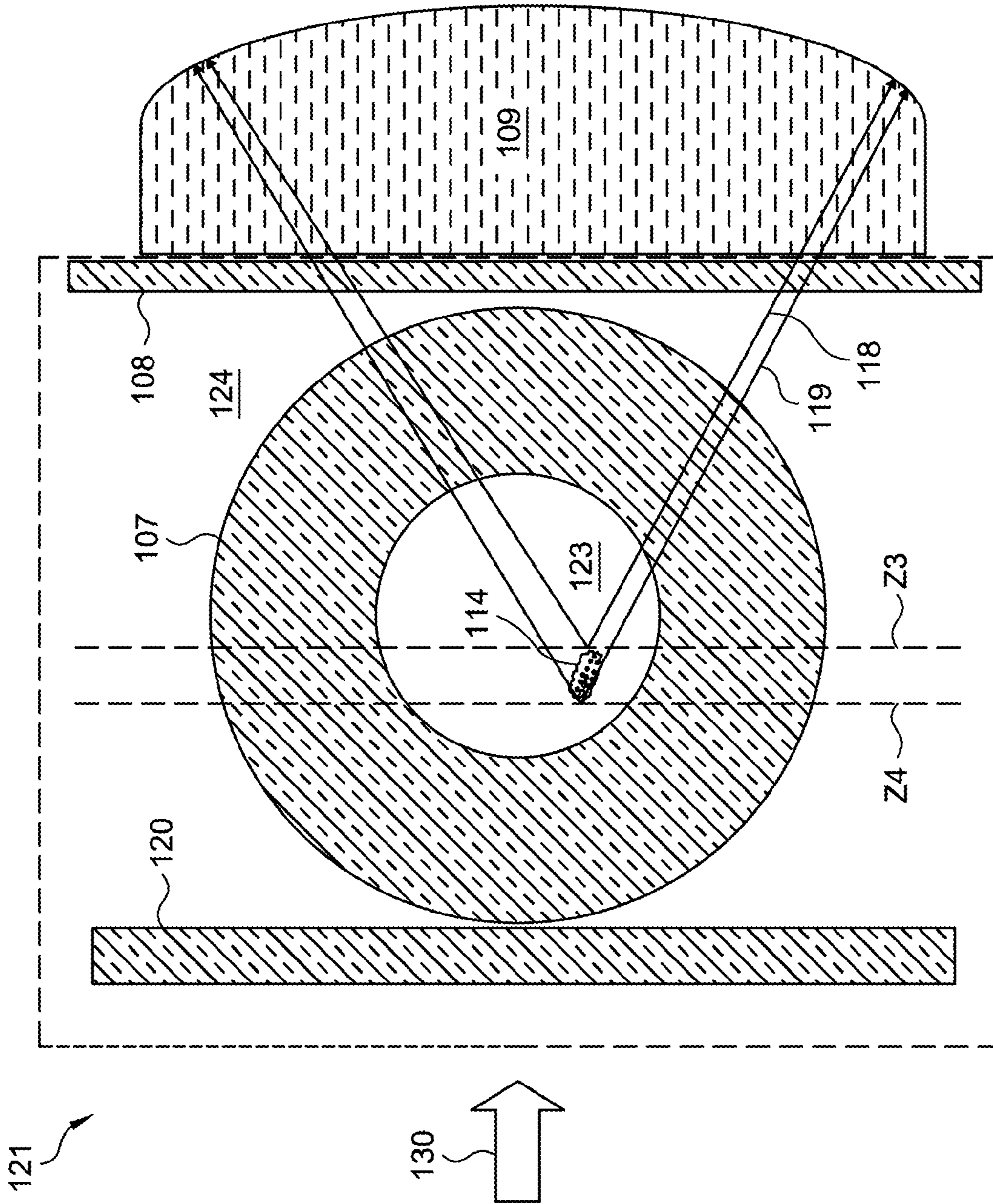


Fig. 4C

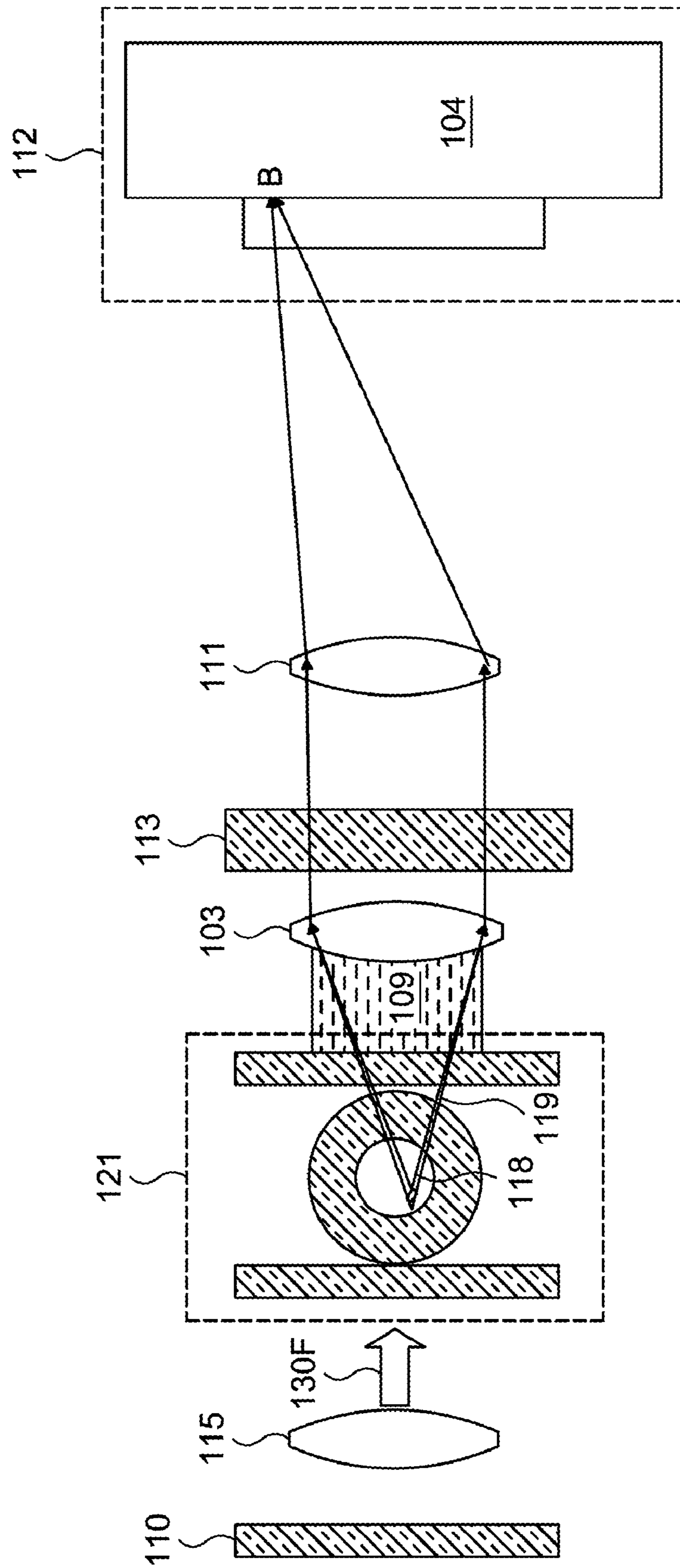


Fig. 4D

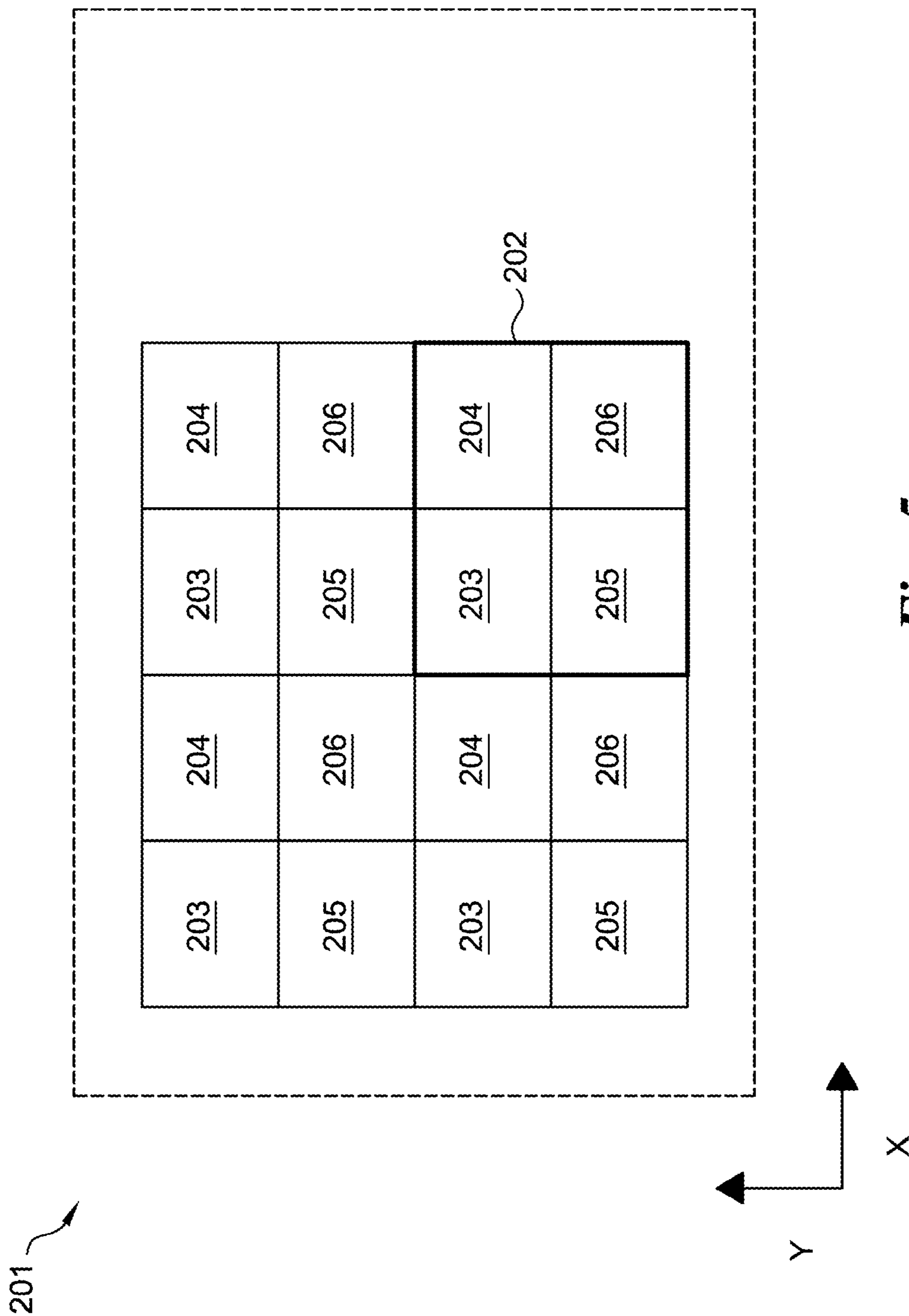


Fig. 5

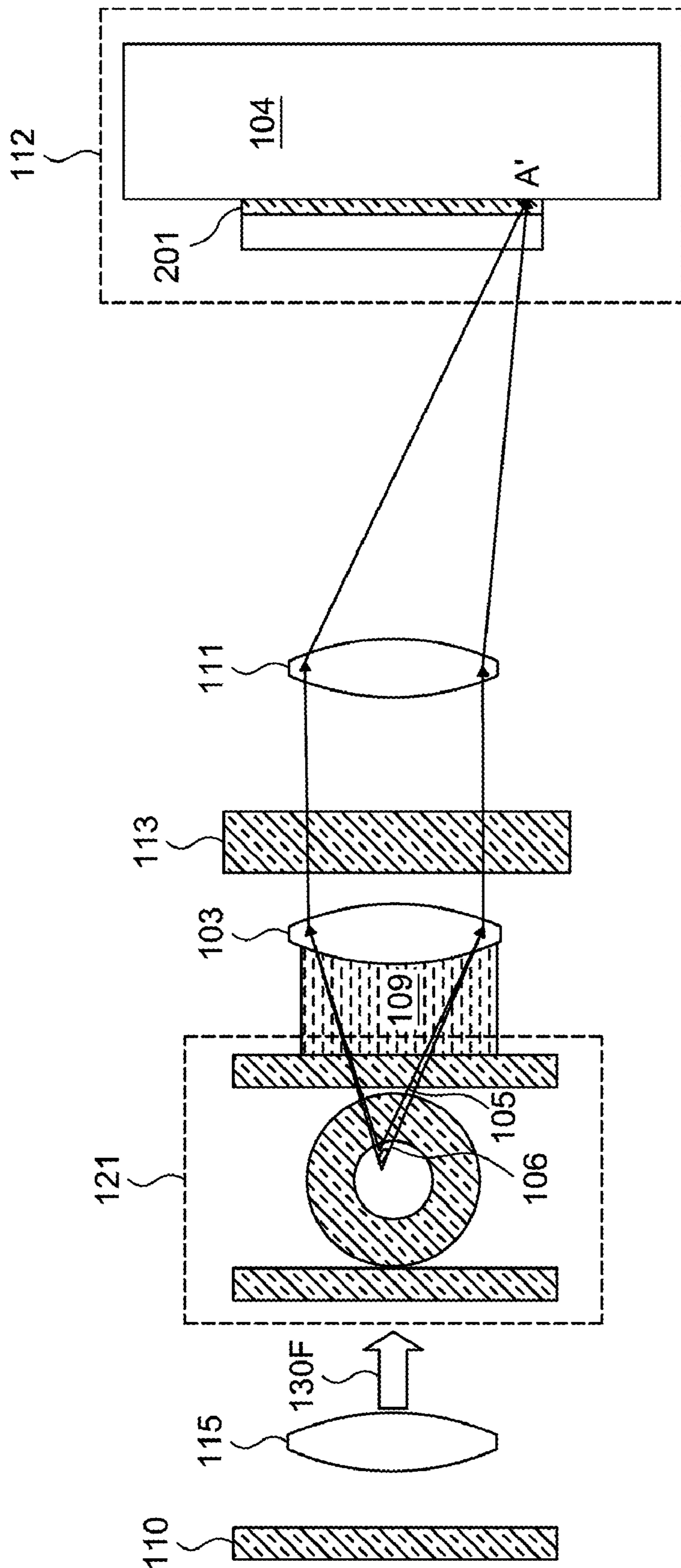


Fig. 6A

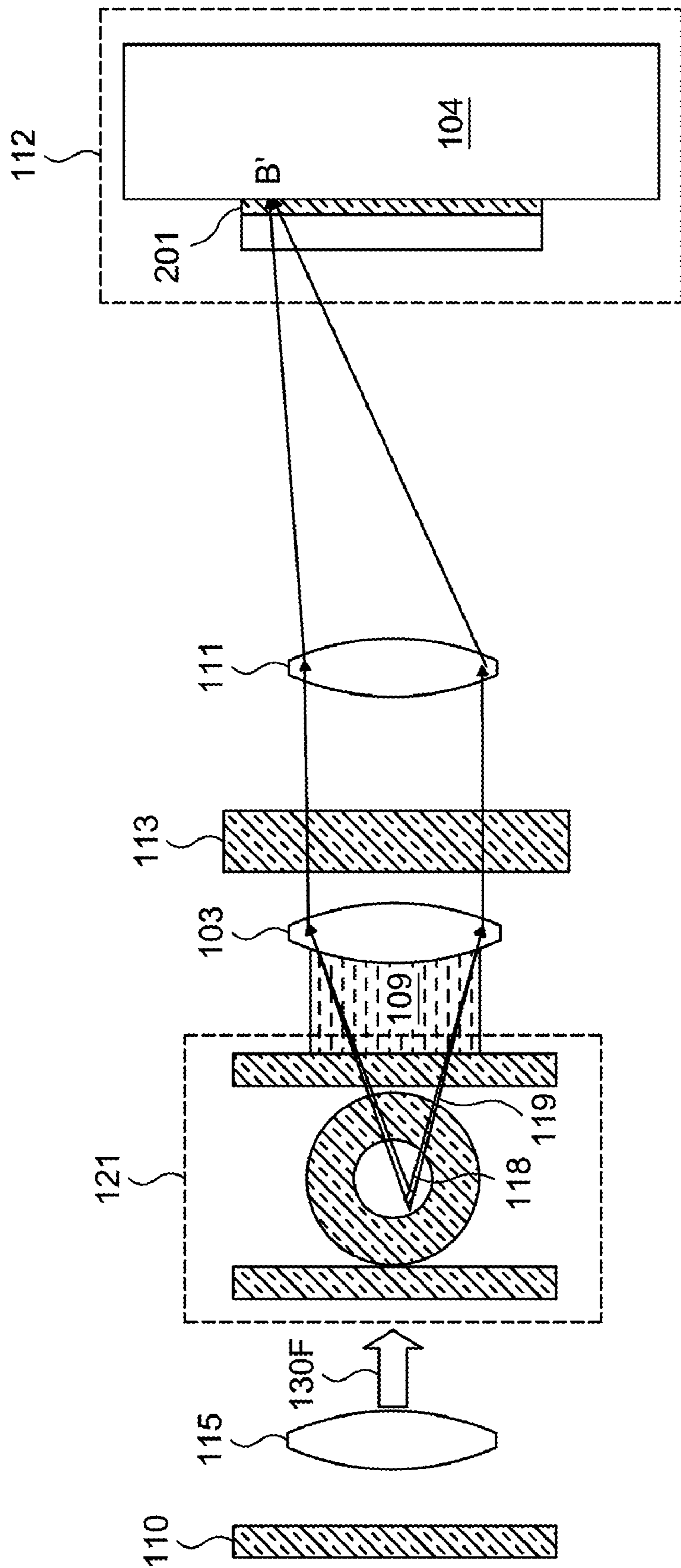
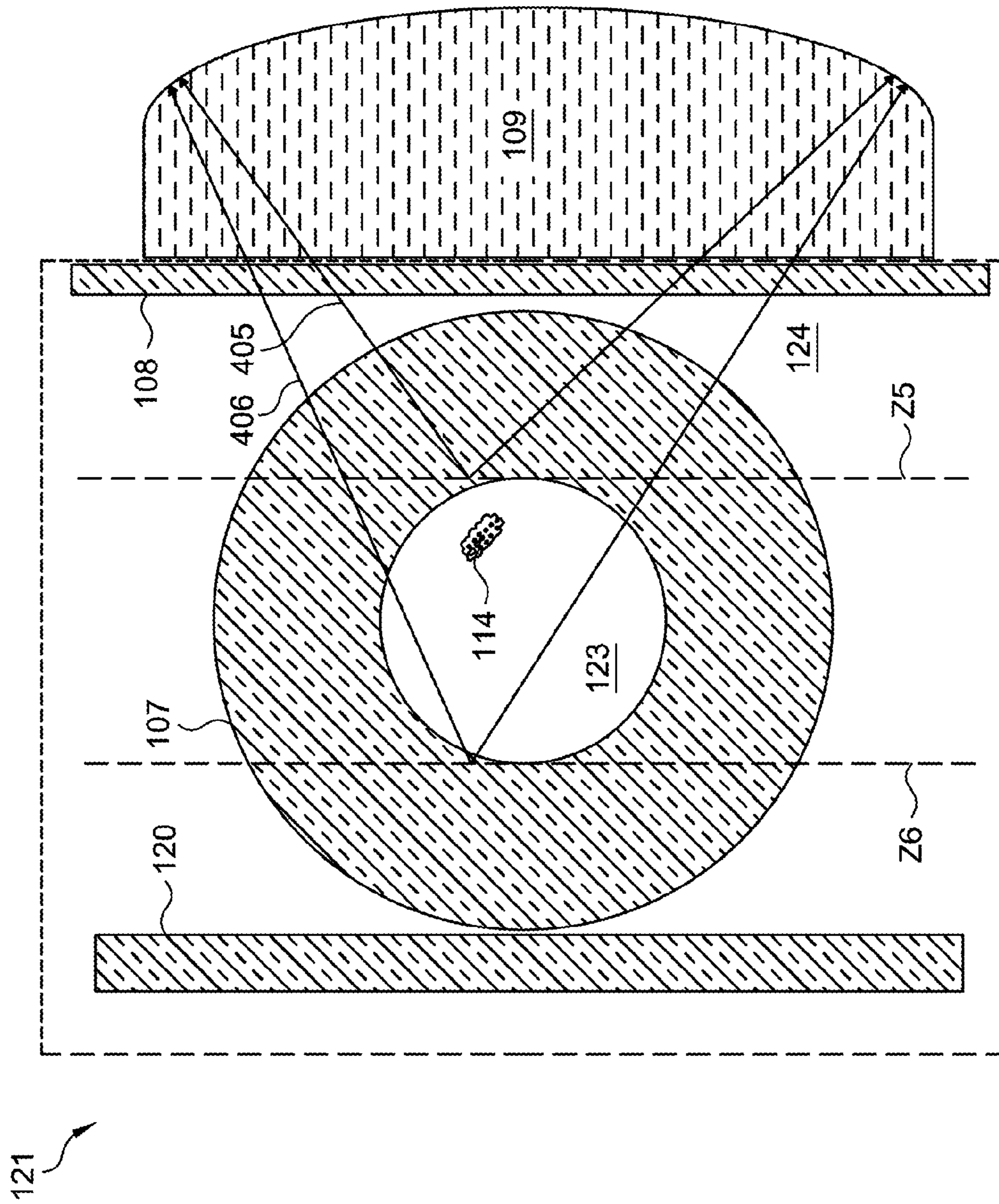


Fig. 6B



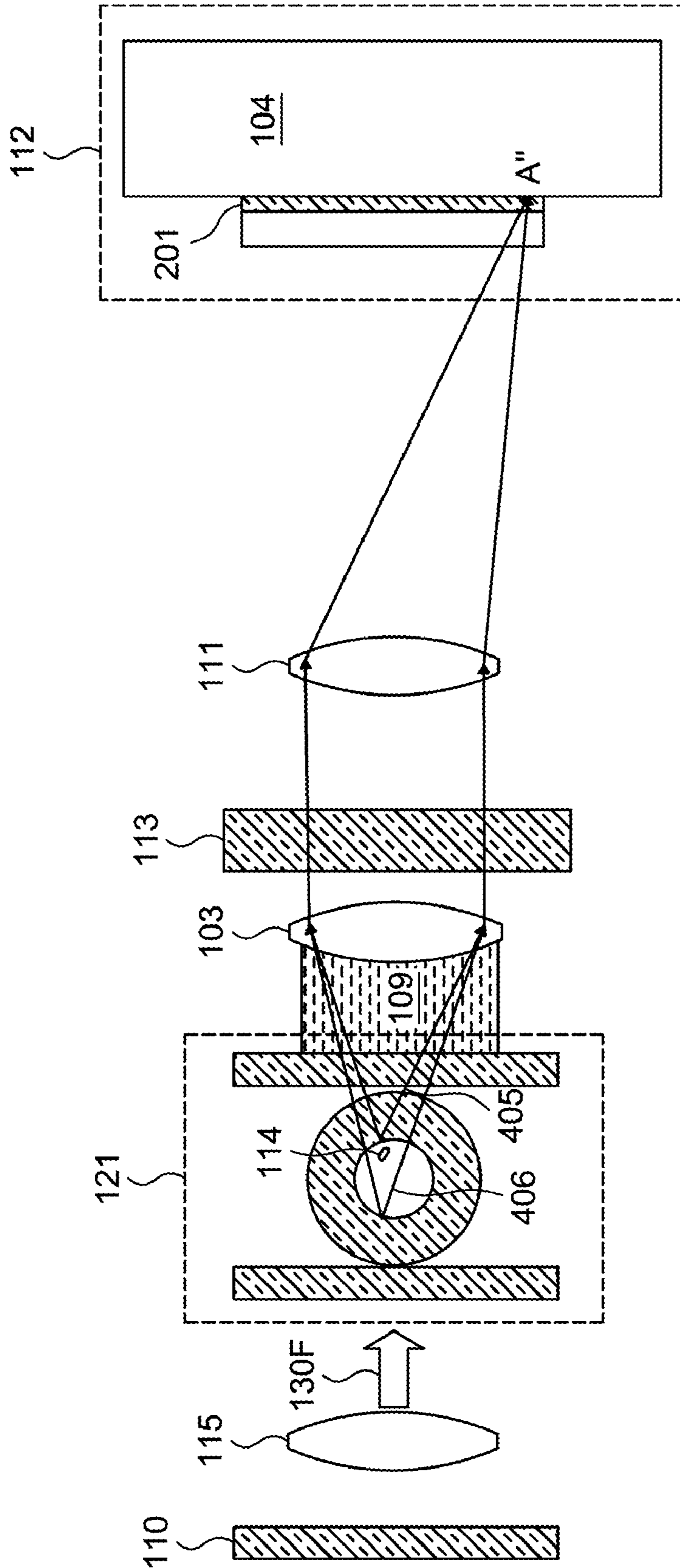


Fig. 7B

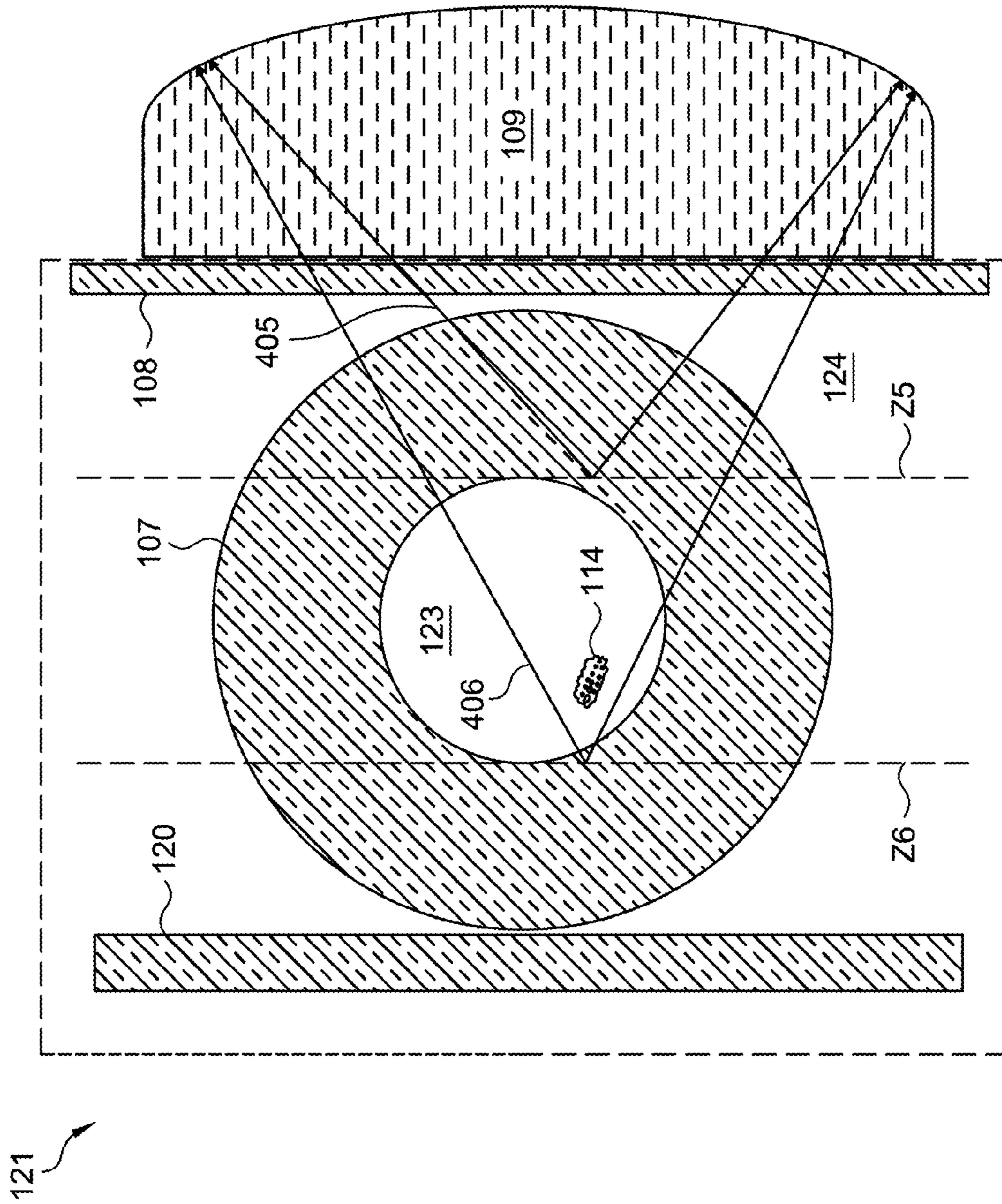


Fig. 7C

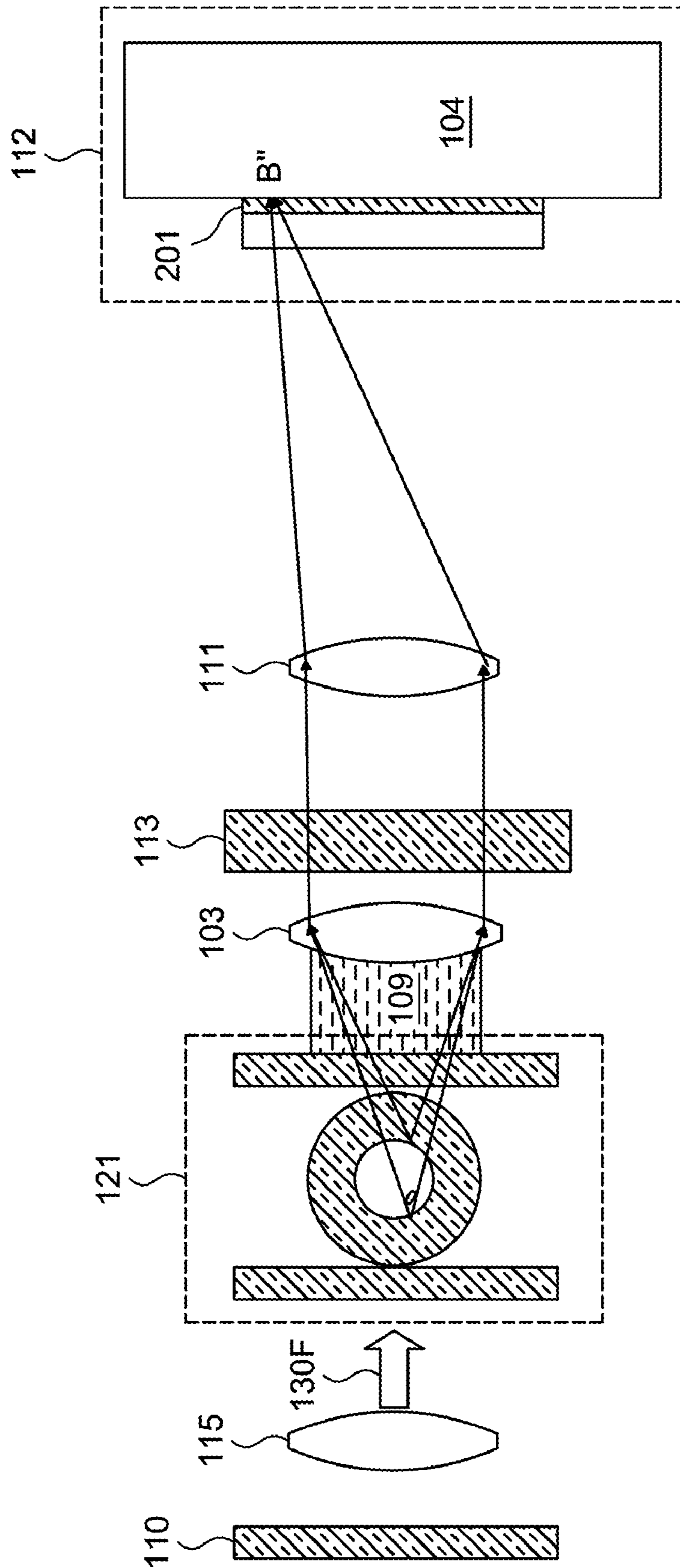


Fig. 7D

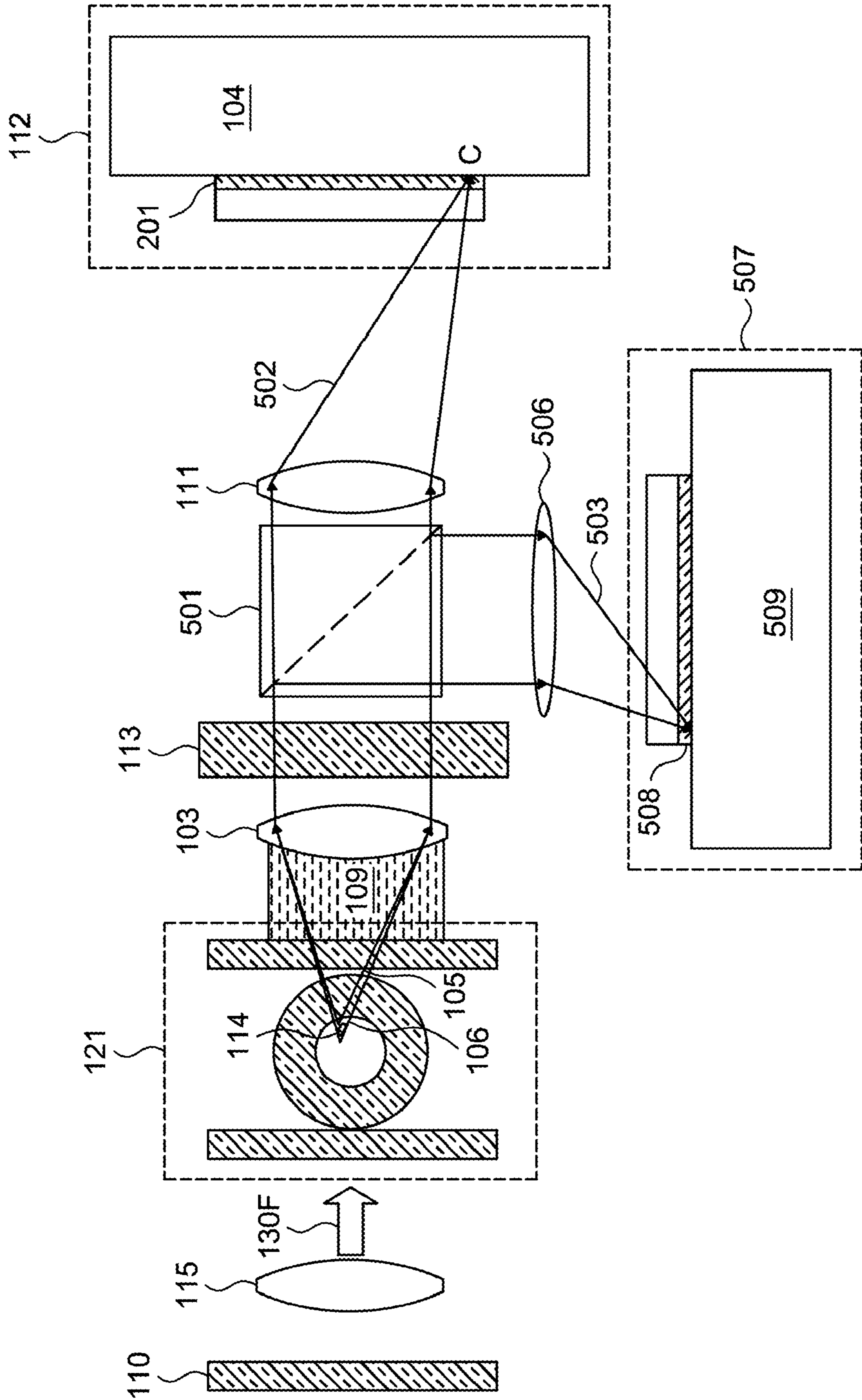


Fig. 8A

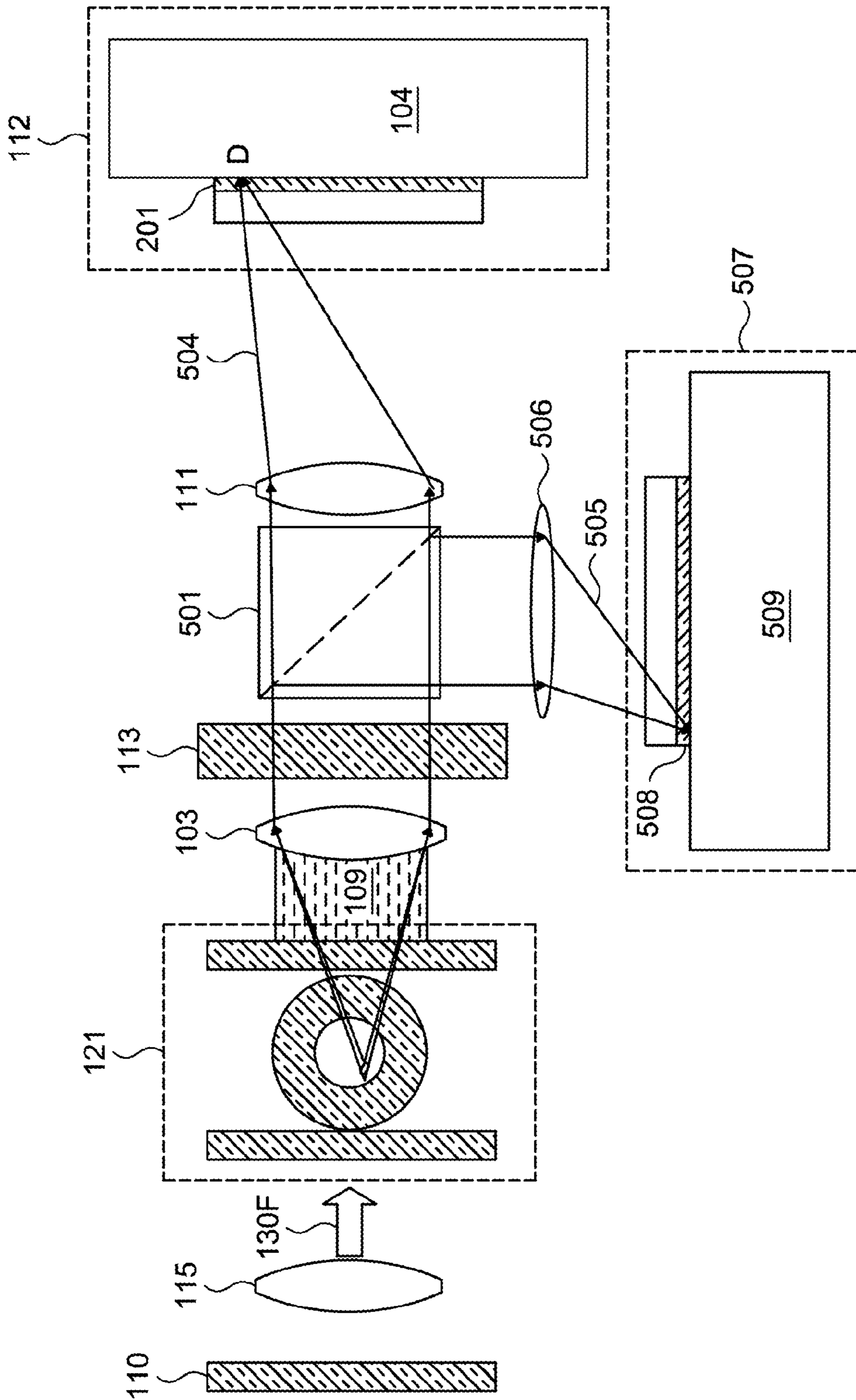


Fig. 8B

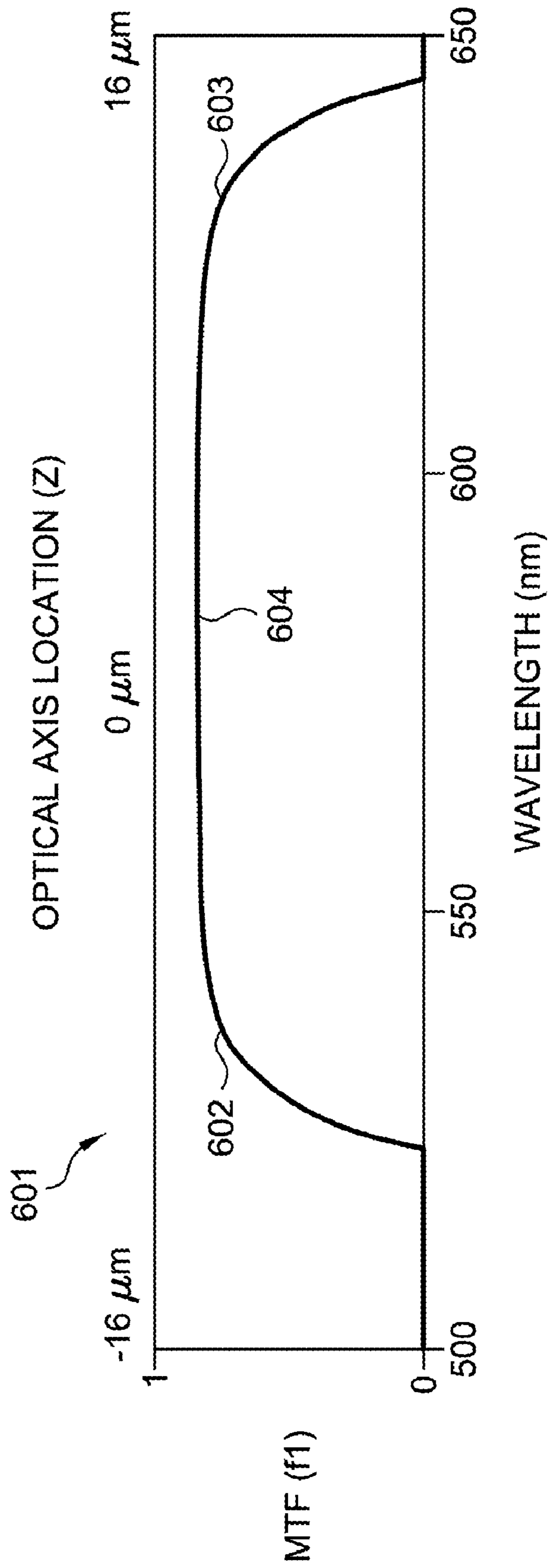


Fig. 9

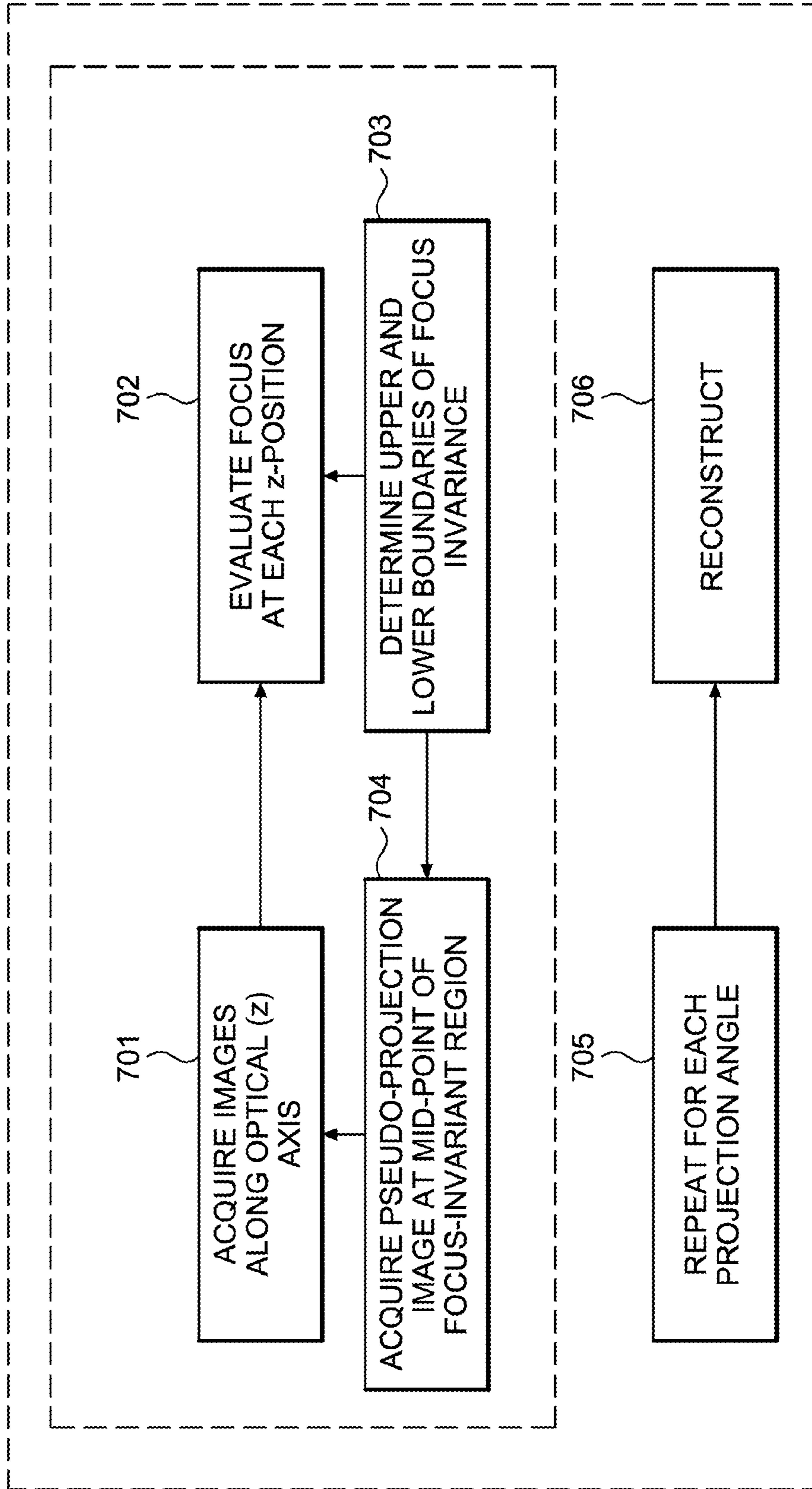


Fig. 10

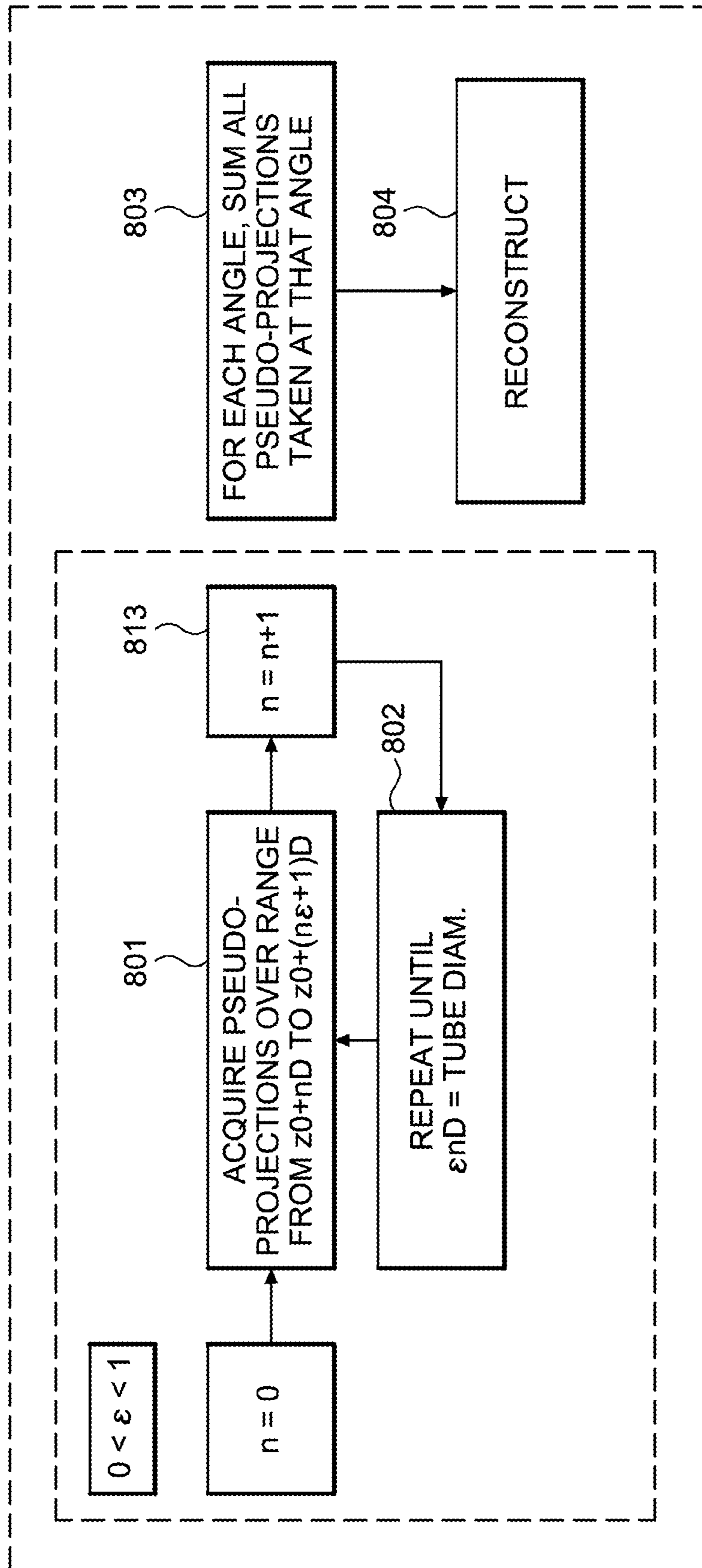


Fig. 11A

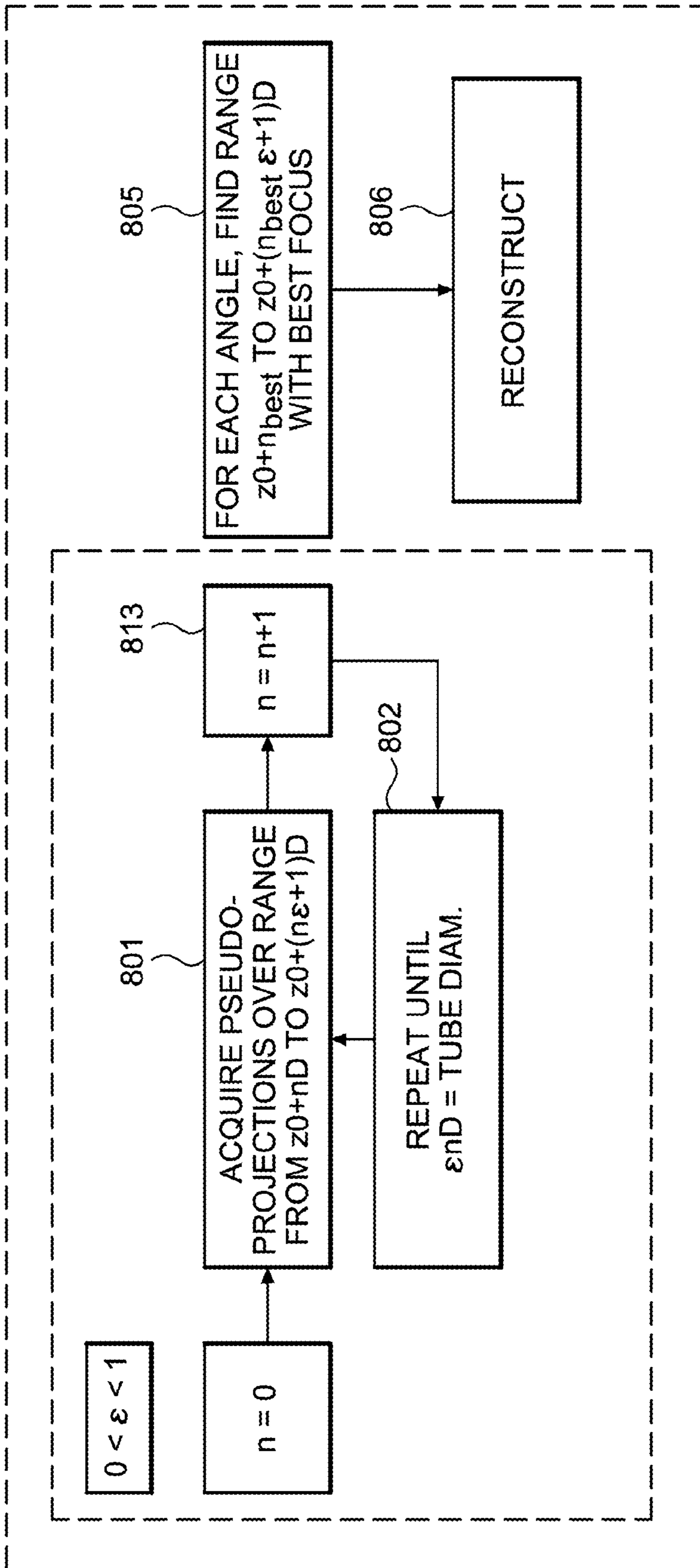


Fig. 11B

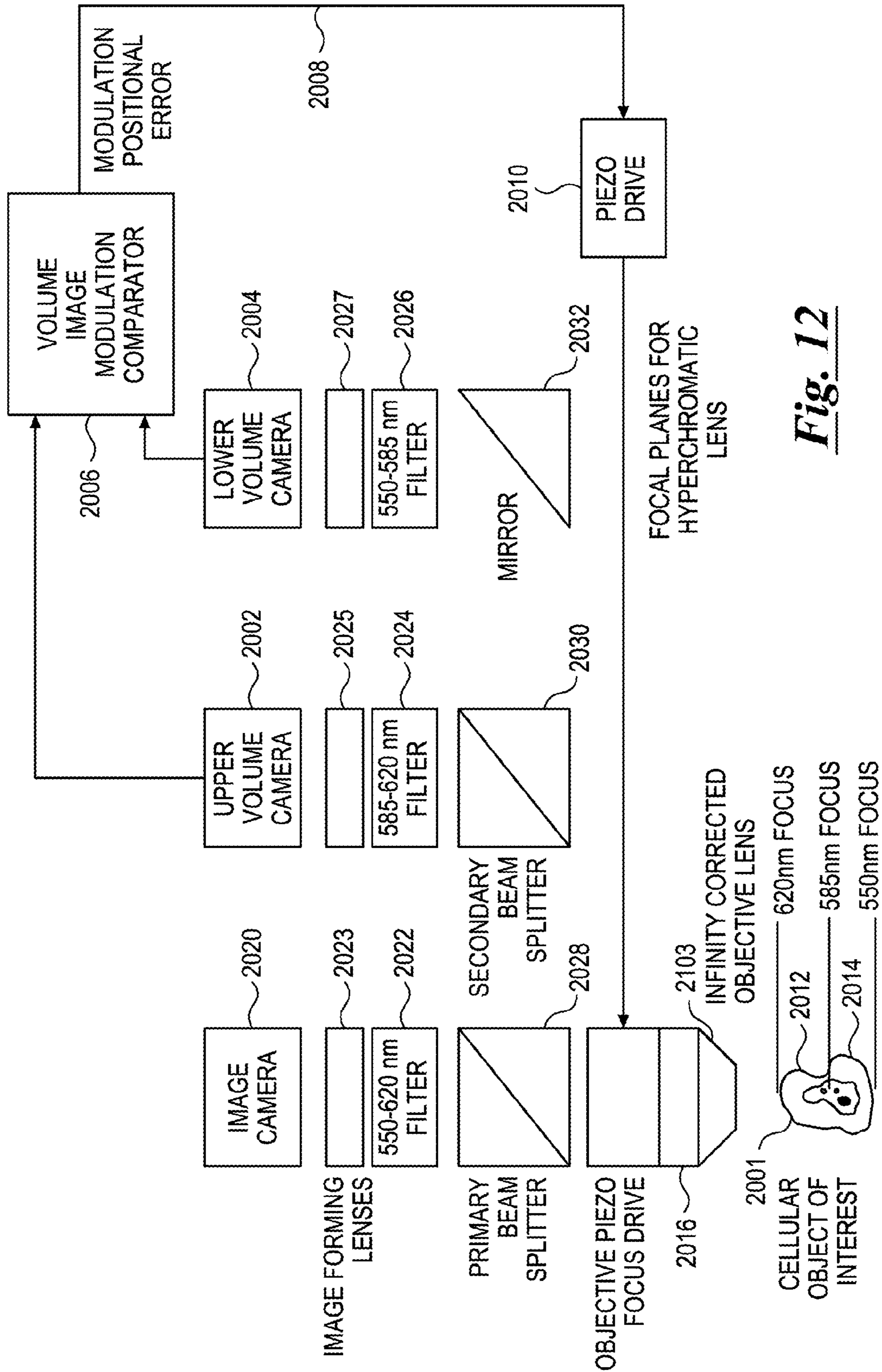


Fig. 12

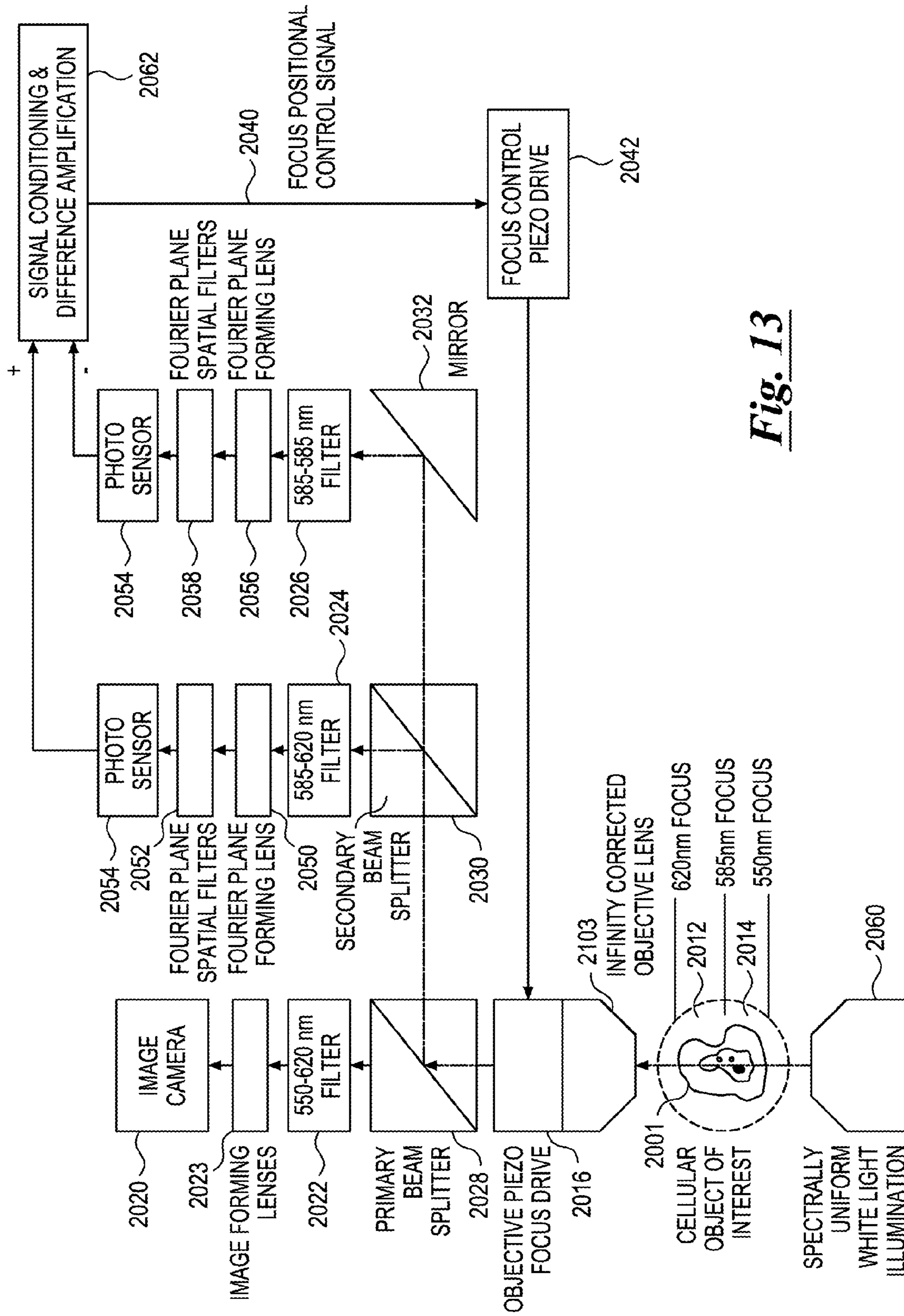


Fig. 13

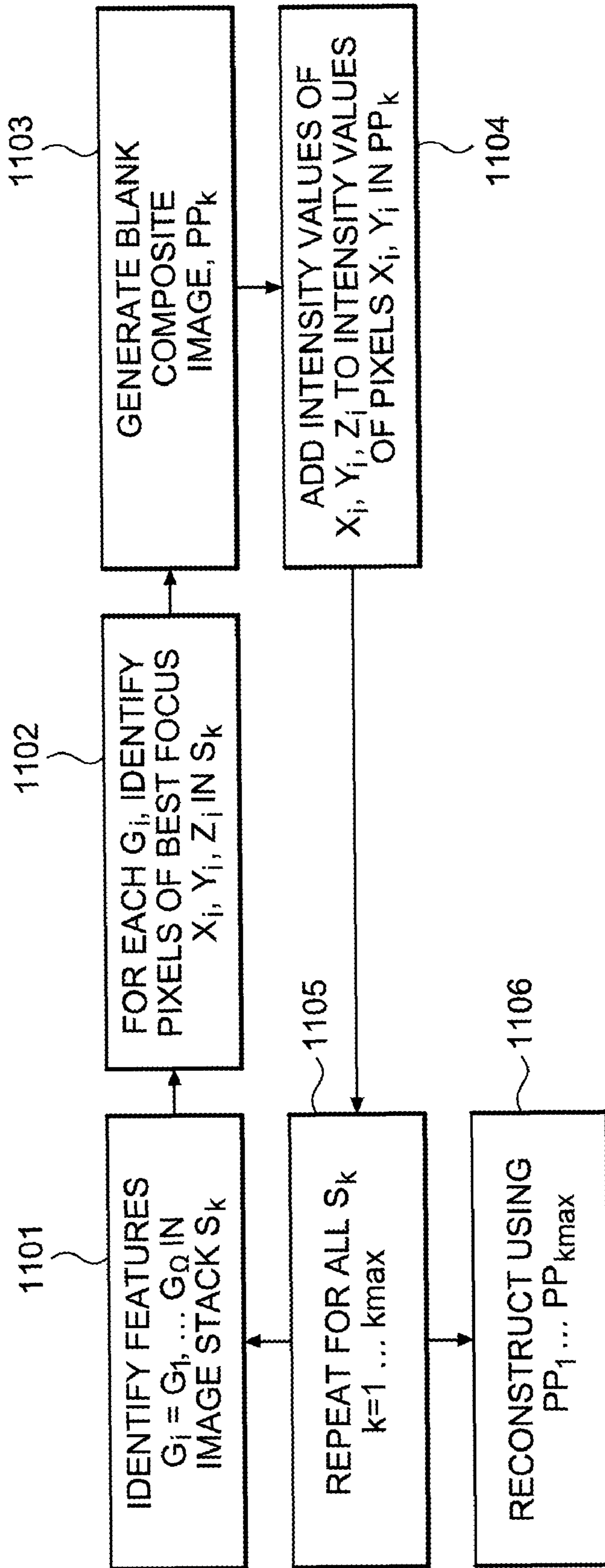


Fig. 14

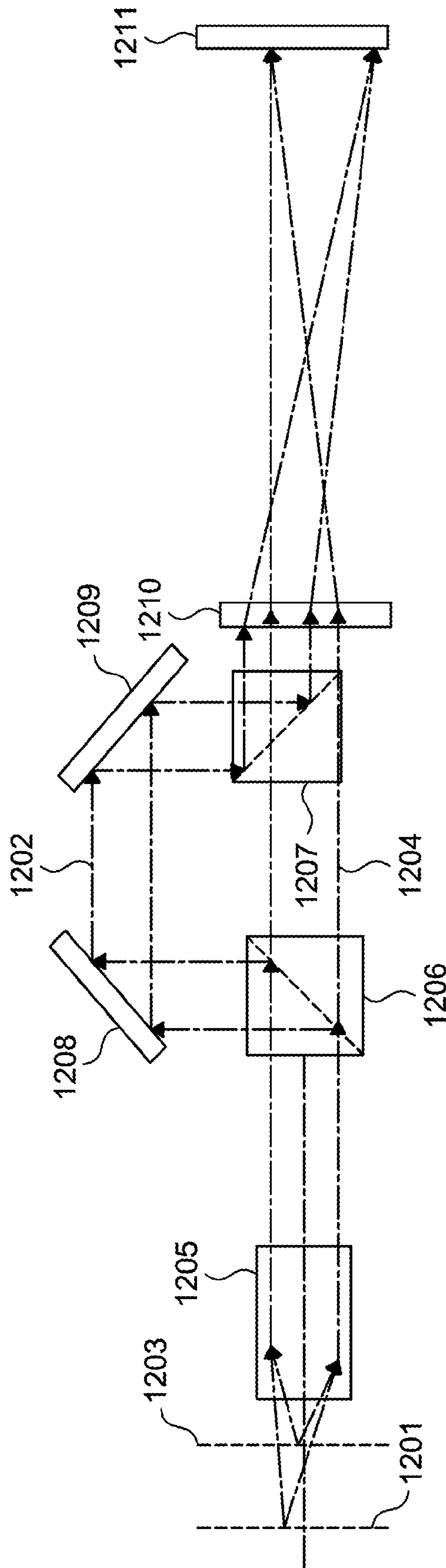


Fig. 15

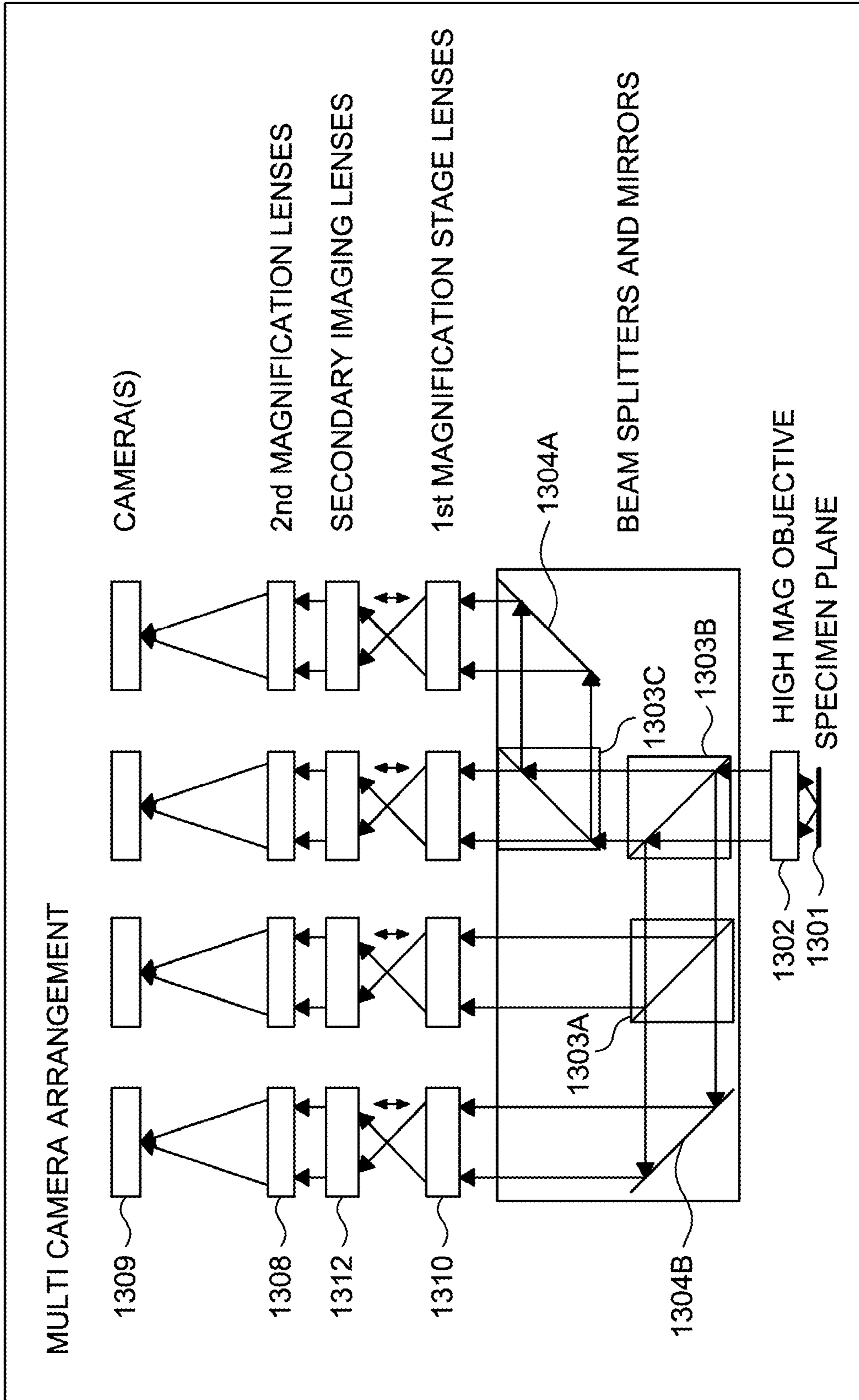


Fig. 16

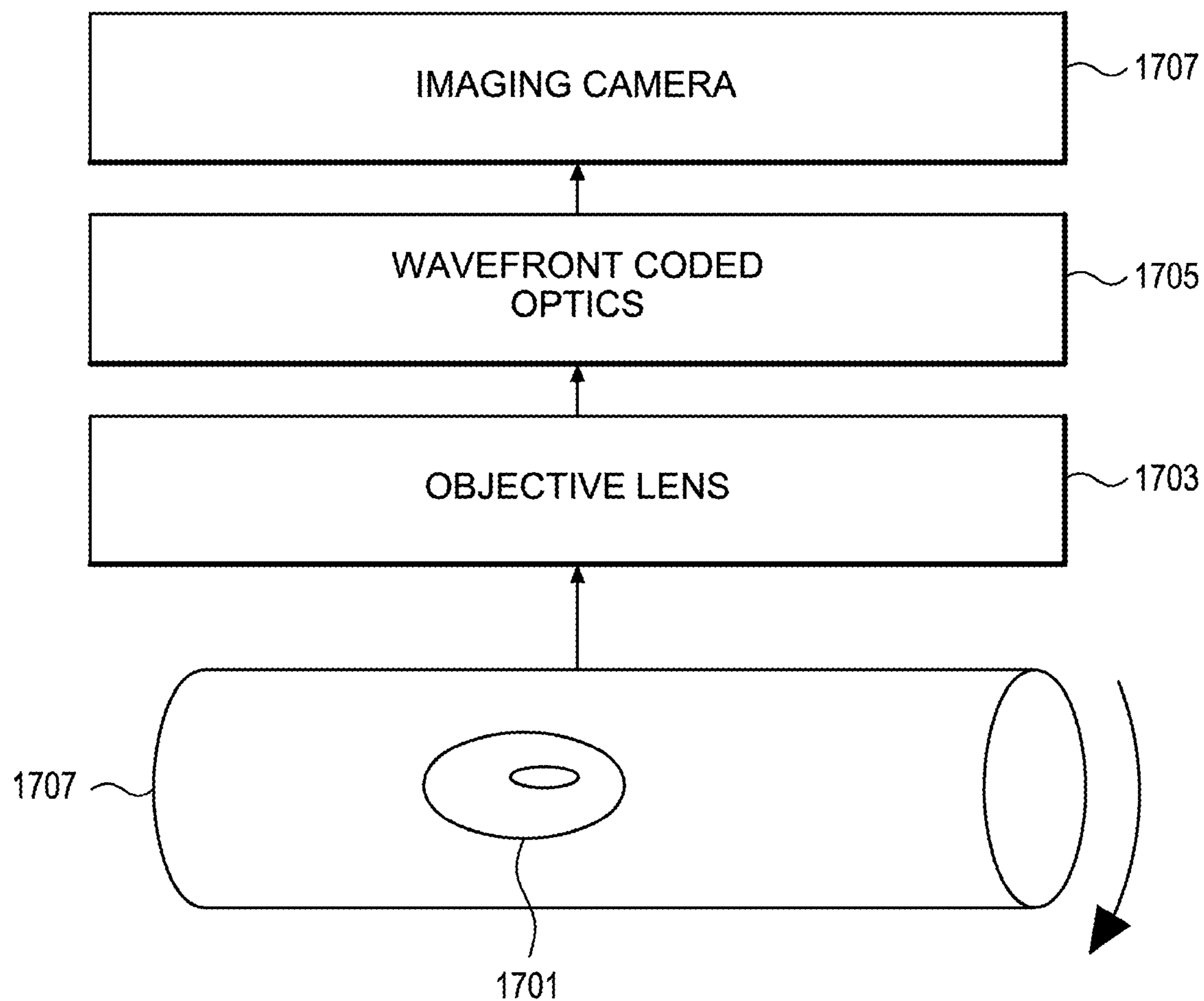


Fig. 17

DEPTH OF FIELD EXTENSION FOR OPTICAL TOMOGRAPHY

TECHNICAL FIELD

The present invention relates to optical tomographic imaging systems in general, and, more particularly, to optical projection tomography, in which a small object, such as a biological cell, is positioned in a capillary tube for imaging by a microscope.

BACKGROUND

Advances in imaging biological cells using optical tomography have been developed by Nelson as disclosed, for example, in U.S. Pat. No. 6,522,775, issued Feb. 18, 2003, and entitled "Apparatus and method for imaging small objects in a flow stream using optical tomography," the full disclosure of which is incorporated by reference. Further developments in the field are taught in Fauver et al., U.S. patent application Ser. No. 10/716,744, filed Nov. 18, 2003 and published as US Publication No. US-2004-0076319-A1 on Apr. 22, 2004, entitled "Method and apparatus of shadowgram formation for optical tomography," (Fauver '744) and Fauver et al., U.S. patent application Ser. No. 11/532,648, filed Sep. 18, 2006, entitled "Focal plane tracking for optical microtomography," (Fauver '648) the full disclosures of which are also incorporated by reference.

Processing in such an optical tomography system begins with specimen preparation. Typically, specimens taken from a patient are received from a hospital or clinic and processed to remove non-diagnostic elements, fixed and then stained. Stained specimens are then mixed with an optical gel, inserted into a micro-capillary tube and images of objects, such as cells, in the specimen are produced using an optical tomography system. The resultant images comprise a set of extended depth of field images from differing perspectives called "pseudo-projection images." The set of pseudo-projection images can be reconstructed using backprojection and filtering techniques to yield a 3D reconstruction of a cell of interest.

The 3D reconstruction then remains available for analysis in order to enable the quantification and the determination of the location of structures, molecules or molecular probes of interest. An object such as a biological cell may be labeled with at least one stain or tagged molecular probe, and the measured amount and location of this probe may yield important information about the disease state of the cell, including, but not limited to, various cancers such as lung, breast, prostate, cervical and ovarian cancers.

In Optical Projection Tomography Microscopy (OPTM) systems as described, for example, in Fauver '648, about 250 sample images taken over a 180-degree rotation are required to adequately sample the volume of a cell nucleus randomly distributed in a flow stream within a 50 micron capillary tube.

The present disclosure provides new and novel techniques for providing higher resolution and improved signal to noise ratio in order to reduce sampling requirements while maintaining acceptable resolution.

In one type of optical tomography system, as described in Fauver '744 and constructed by VisionGate, Inc., the depth of field of the imaging optics is extended by scanning an objective lens transverse to a capillary tube containing a specimen. A piezoelectric transducer (PZT) actuator transversely moves the objective lens sinusoidally several times per second in order to scan a series of focal planes through a specimen. By using a PZT actuator to move the objective lens, a focal plane

moving through the specimen has its speed limited by inertia inherent in moving the objective lens mass rapidly along the optical axis through the specimen. Typically, an upper limit of the scan rate is roughly 60 cycles per second. With well-synchronized rotation and objective scanning, an image can be acquired on the down-stroke as well as the up-stroke of the PZT actuator, allowing up to 120 images per second to be acquired. While this is a useful acquisition rate, it can be significantly improved through the apparatus, systems and methods disclosed herein.

BRIEF SUMMARY OF THE DISCLOSURE

An optical projection tomography system is illuminated with a light source. An object-containing tube, a portion of which is located within the region illuminated by the light source, contains at least one object of interest that has at least one feature of interest. At least one detector is located to receive emerging radiation from the object of interest. A lens, including optical field extension elements, is located in the optical path between the object region and the detector, such that light rays from multiple object planes in the object-containing tube simultaneously focus on the at least one detector. The object-containing tube moves relatively to the at least one detector and the lens operate to provide multiple views of the object region for producing at least one image of the at least one feature of interest at each view.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically shows an example of a design for a hyperchromatic optical lens system.

FIG. 2A-FIG. 2D schematically show qualitative examples of simulated monochromatic modulation transfer functions (MTFs) for each of four wavelengths, as they might be evaluated at four separate image planes.

FIG. 3A-FIG. 3D schematically show qualitative examples of simulated polychromatic modulation transfer functions over a range of wavelengths, as they might be evaluated at four separate image planes.

FIG. 4A schematically shows a detail of the object space at one viewing angle in an optical tomography system incorporating a hyperchromatic optical lens.

FIG. 4B schematically shows the operation of the optical tomography system depicted schematically in FIG. 4A.

FIG. 4C schematically shows a detail of the object space at a second viewing angle in an optical tomography system incorporating a hyperchromatic optical lens.

FIG. 4D schematically shows the operation of the optical tomography system depicted schematically in FIG. 4C.

FIG. 5 schematically shows an example of a design for a chromatic filter array.

FIG. 6A schematically illustrates a first viewing angle for a hyperchromatic optical tomography system incorporating a chromatic filter array.

FIG. 6B schematically illustrates a first viewing angle for a hyperchromatic optical tomography system incorporating a chromatic filter array.

FIG. 7A schematically illustrates object space for a first viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field.

FIG. 7B schematically illustrates a first viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field.

FIG. 7C schematically illustrates the object space for a second viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field.

FIG. 7D schematically illustrates a second viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field.

FIG. 8A schematically illustrates a first viewing angle for a hyperchromatic optical tomography system incorporating multiple detection paths.

FIG. 8B schematically illustrates a second viewing angle for a hyperchromatic optical tomography system incorporating multiple detection paths.

FIG. 9 illustrates an example of focus invariance in an MTF range.

FIG. 10 shows a block diagram of a method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system.

FIG. 11A shows a block diagram of another method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system.

FIG. 11B shows a block diagram of yet another method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system.

FIG. 12 schematically depicts an embodiment of an auto-focusing system using chromatic balance.

FIG. 13 shows another method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system, using two photo-diodes with spatial-frequency filtering.

FIG. 14 schematically shows a block diagram of a method for 2.5-D imaging in a focus-invariant optical tomography system.

FIG. 15 illustrates an example of a folded optical system allowing simultaneous imaging of two focal planes on a single camera.

FIG. 16 schematically illustrates a multiple-camera device for acquiring a range of focal planes in an optical tomography system.

FIG. 17 illustrates a schematic diagram of an OPTM system including wavefront coded optics.

In the drawings, identical reference numbers identify similar elements or components. The sizes and relative positions of elements in the drawings are not necessarily drawn to scale. For example, the shapes of various elements and angles are not drawn to scale, and some of these elements are arbitrarily enlarged and positioned to improve drawing legibility. Further, the particular shapes of the elements as drawn, are not intended to convey any information regarding the actual shape of the particular elements, and have been solely selected for ease of recognition in the drawings.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following disclosure describes several embodiments and systems for imaging an object of interest. Several features of methods and systems in accordance with example embodiments of the invention are set forth and described in the Figures. It will be appreciated that methods and systems in accordance with other example embodiments of the invention can include additional procedures or features different than those shown in Figures. Example embodiments are described herein with respect to biological cells. However, it will be understood that these examples are for the purpose of illustrating the principals of the invention, and that the invention is not so limited.

Additionally, methods and systems in accordance with several example embodiments of the invention may not include

all of the features shown in these Figures. Throughout the Figures, like reference numbers refer to similar or identical components or procedures.

Unless the context requires otherwise, throughout the specification and claims which follow, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense that is as “including, but not limited to.”

Reference throughout this specification to “one example” or “an example embodiment,” “one embodiment,” “an embodiment” or various combinations of these terms means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

Definitions

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

“Capillary tube” has its generally accepted meaning and is intended to include transparent microcapillary tubes and equivalent items with an inside diameter of 100 microns or less.

“Depth of field” is the length along the optical axis within which the focal plane may be shifted before an unacceptable image blur is produced.

“Object” means an individual cell or other entity.

“Pseudo-projection” includes a single image representing a sampled volume of extent larger than the native depth of field of the optics.

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

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

Chromatic Aberration Depth of Field Extension

Most simple lenses will produce wavelength-dependent focal positions known as chromatic focal shift. Chromatic aberrations are typically undesirable in a lens. However, for a sufficiently broad absorption spectrum in a biological sample, the dispersion of chromatic aberration can in effect extend the depth of field image of an absorptive object or feature.

Wavelength-dependent lens material will produce a lens with chromatic aberrations. Nearly all lens materials can have both positive and negative index shifts with wavelength. Lens designers typically choose lens materials to compensate for the chromatic focal plane shifts, resulting in a net chromatic focal shift near zero. For an example of an immersion microscope objective which is corrected for spherical and axial chromatic aberrations see U.S. Pat. No. 5,517,360 issued May 14, 1996 to T Suzuki, entitled “Immersion microscope objective.”

Changing the design parameters to emphasize, rather than minimize, the chromatic focal shift can create large chromatic, or hyperchromatic, aberrations in the optical path. Such hyperchromatic aberrations can simultaneously focus

multiple focal depths on a detector, with each optical wavelength forming an image at the detector of a separate focal plane within the object. This widens the range of focal positions over a limited desired wavelength range. For a specimen with a narrow absorption peak in the stain or contrast agent, a lens can be designed to include optical field extension elements to extend the dispersion over many microns to form an extended depth of field optical system for a narrow range of wavelengths. The optical components and materials are chosen to optimize chromatic dispersion within the stain absorption range.

When employing chromatic aberration, it may be advantageous to alter the relative makeup of the spectral components to compensate for factors that may affect the composition of the image. These may include, but are not limited to, wavelength dependencies of the contrast agent or stain, the camera response, and transmission through the optical materials. The spectral composition may be altered by, for example, incorporating in the illumination, collection, and/or imaging optics a filter that attenuates some wavelengths more than others.

As an example, a limited extension of the depth of field can be achieved for a narrow range of wavelengths accommodating existing stain absorption curves, such as the hematoxylin family of stains. Stains in the hematoxylin family exhibit a peak absorption in the wavelength range from 550 to 620 nanometers.

Example 1

One example of a hyperchromatic objective lens **103**, suitable for use in a hyperchromatic system, is depicted in FIG. 1. This compound lens comprises eight optical elements **1-8** of which optical elements **1** and **2** are cemented together to form a first doublet, and optical elements **3** and **4** are cemented together to form a second doublet, **5** and **6** are cemented together to form a third doublet, and **7** and **8** are cemented together to form a fourth doublet. The first surface of **1** is flat or slightly convex, so as to avoid trapping air in a cavity when this surface comes in contact with an immersion liquid such as oil or water. An example prescription for the objective **103** follows.

Element	Material (Schott designation)	Front Radius of Curvature (mm)	Back Radius of Curvature (mm)	Center Thickness (mm)
1	SF57	200	-3.17	3.54
	LAK14	3.17	-5.92	5.08
	Air	—	—	1
3	KZFSN4	104.5	-6.55	3.64
	Air	—	—	1
4	SF6	6.55	-13.77	1
	Air	—	—	1
5	SF64	10.73	6.27	4.75
	LASF40	-6.27	4.47	7.88
6	Air	—	—	2.24
	SK2	7.23	-3.95	9.05
7	F2	3.95	19.37	8.83

The location of the aperture stop may be chosen to provide telecentricity, and to minimize lateral color (also known as chromatic difference of magnification). Component materials are commercially available from, for example, Schott North America, Inc. Elmsford, N.Y. 10523.

As shown in the examples described hereinbelow with reference to the figures, lens system **103**, when placed in front of a tube lens having a focal length of 180 mm, will provide 60× magnification at numerical aperture (NA) equal to 0.9

over a wavelength range from 550 nm to 620 nm, provided that the space between the front surface of the first element **1** and the top of a cover slip positioned in the field of view of the lens is filled with water. The cover slip is typically about 130 microns thick, while the water-filled space between the cover slip and the lens may be about 200 microns thick. An object is focused on the imaging plane of the camera over a range of 15 microns at separate wavelengths over a 200-micron diameter field of view. In this example embodiment, the portion of the object in a first plane is focused by the 550-nm portion of the incident light a second plane located 5 microns below the first plane is focused by the 573-nm portion of the incident light, a third plane located 10 microns below the first plane is focused by the 597-nm portion of the incident light, and a fourth plane located 15 microns below the first plane is focused by the 620-nm portion of the incident light.

System MTFs

Referring now to FIG. 2A through FIG. 2D qualitative examples of simulated monochromatic modulation transfer functions (MTFs) for each of four wavelengths, as evaluated at four separate image planes. Throughout FIG. 2A-FIG. 3D the vertical axis represents system MTF and the horizontal axis represents frequency ranging from 0 to a cutoff frequency, f_c , where f_c is the highest frequency with a non-zero MTF. An MTF similar to the one shown in FIG. 2A could, in principle, be measured by placing a 2D optical test target in the object space, illuminating it with narrowband light having a wavelength of about 550 nm, and finding the best focus. Changing the wavelength to about 573 nm and moving the focal position by 5 microns may produce the MTF shown in FIG. 2B. Repeating this process for about 597 nm and again for about 620 nm yields the MTFs shown in FIG. 2C and FIG. 2D, respectively. The in-focus information from the entire volume of an object may be acquired simultaneously, for an object thickness up to 15 microns.

Referring now to FIG. 3A through FIG. 3D qualitative examples of simulated polychromatic modulation transfer functions (MTFs) are shown for an optical projection system where the illumination consists of a band of wavelengths between about 550 nm and 620 nm are shown. An MTF similar to the one shown in FIG. 3A could, in principle, be measured by placing a 2D optical test target in the object space, illuminating it with broadband light having a band of wavelengths in the range of about 550-620 nm, and finding the best focus, $\Delta Z=0$. Moving the focal position by 5 microns to focus $\Delta Z=5$ microns qualitatively yields the MTF shown in FIG. 3B. Moving the focal position by 10 microns, $\Delta Z=10$ microns, and by 15 microns, $\Delta Z=15$ microns, qualitatively yields the MTFs shown in FIG. 3C and FIG. 3D respectively. It is to be understood that other prescriptions and optical designs may be employed without departing from the spirit of this embodiment.

The hyperchromatic optical system may advantageously be incorporated into an OPTM system. A translation device, such as, for example, a piezoelectric transducer (PZT) may be used to apply a single, low-speed translation of the objective lens over the course of a 360-degree set of scans. The lens translation keeps the object of interest within a focus interval of about 15 microns, even while the tube rotation causes the object to translate along the optical axis by as much as the internal diameter of the rotating capillary during the 360-degree scan. In contrast to earlier embodiments, a high-speed scan taken at each perspective is no longer required. As a result, image acquisition speed is no longer limited by the speed of the PZT translation device. In addition, synchronization between the tube rotation and translation motion of the

lens no longer needs to be as precise, thereby reducing the complexity of the OPTM instrument control system.

Now referring to FIG. 4A a detail of object space at one viewing angle in an optical tomography system incorporating a hyperchromatic optical lens is schematically shown. A cell **114** lies between a first object plane **Z1** and a second object plane **Z** inside a microcapillary tube **107**. The tube **107** may have, for example, an inner diameter of 50 microns, and the separation between the first object plane and the second object plane may be, for example, 15 microns. The microcapillary tube **107** is preferably filled with an optical matching medium **123** matching the internal index to the tube's index of refraction.

In one example embodiment, an assembly **121** preferably includes the microcapillary tube **107** placed in a viewing area between a first flat optical surface **120**, which may comprise a standard microscope slide, and a second flat optical surface **108**, which may comprise a standard microscope coverslip. The interstices between the tube **107** and the flat surfaces **108**, **120** are filled with optical oil **124**, or an equivalent, having an index of refraction that also substantially matches those of the tube **107**, the flat surfaces **108**, **120**, and the optical gel **123**. The assembly **121** can be mounted on a microscope, and an optical immersion fluid **109**, comprising, for example, oil, water, or air, is placed on the side of the assembly **121** that faces hyperchromatic optics (as shown in FIG. 4B). The outer diameter of the tube **107** may be, for example about 250 microns, the thickness of the coverslip **108** may be about 170 microns, and the thickness of the immersion fluid **109** may be between about 100 and 300 microns.

Broadband light **130** having wavelengths between a first wavelength λ_1 (e.g., λ_1 =about 550 nm) and a second wavelength λ_2 (e.g., λ_2 =about 620 nm) is transmitted into the tube **107** by means of, for example, a condenser lens system. A first set of ray paths **105** of light having wavelength λ_1 travel from the first object plane **Z1** and into the immersion fluid **109**. A second set of ray paths **106** of light having wavelength λ_2 travel from the second object plane **Z2** and into the immersion fluid **109**. Although not depicted in FIG. 4A, it may be understood that light having wavelengths λ_n , where λ_n is a wavelength between λ_1 and λ_2 , travel from intermediate object planes, Z_n , located between the first object plane and the second object plane, along ray paths similar to **105** and **106**.

With reference to FIG. 4B, the operation of this system may be more fully understood. A chromatic filter **110** and a condenser lens **115** provide illumination **130F** having the desired upper and lower wavelength limits (λ_1 , λ_2). The incident light passes through the tube assembly **121**, containing, for example, a biological cell **114**. The ray paths **105** and **106**, having wavelengths λ_1 and λ_2 and beginning near the cell **114** at object planes **Z1** and **Z2**, respectively, pass through the immersion fluid **109** and the hyperchromatic objective lens system **103**, and are substantially collimated when they reach the tube lens **111**. They then pass through the tube lens **111** which may, for example, have a focal length of about 180 mm, and achieve focus **A** on the image plane **104** of a CCD camera **112**. The objective lens **103** is mounted on a PZT **113**, which is capable of moving the objective **103** further from the tube lens **111** and closer to the object planes **Z1** and **Z2**.

Light having a wavelength λ_n , where the λ_n wavelength is a wavelength having a value between λ_1 and λ_2 , will travel from intermediate object planes, Z_n , located between plane **Z1** and plane **Z2**, along ray paths similar to **105** and **106**, and also come to a focus on image plane **104**. The wavelength of λ_n , relative to λ_1 and λ_2 , determines where the intermediate

object plane is located, relative to object planes **Z1** and **Z2**, in order for it to be focused on image plane **104**.

Now referring to FIG. 4C, the system of FIG. 4A is shown after the microcapillary tube **107** has rotated, causing the cell **114** to change its location and orientation as well. To compensate for this motion, the PZT **113** (as shown, for example in FIG. 4B) moves the objective lens **103** by an amount sufficient to cause the focused light on the image plane **104** to originate from the a second set of object planes **Z3** and **Z4**, via ray path **118** from object plane **Z3** for light of wavelength λ_1 , and via ray path **119** from object plane **Z4** for light of wavelength λ_2 . Those skilled in the art and having the benefit of this disclosure will understand that light having varying wavelengths λ_n between λ_1 and λ_2 will travel from intermediate object planes, Z_n , located between object planes **Z3** and **Z4** along ray paths between ray paths **118** and **119** will also come to a focus on image plane **104**. The wavelengths λ_n , relative to λ_1 and λ_2 , determines where object planes Z_n must be located, relative to **Z3** and **Z4**, in order for it to be focused on image plane **104**. FIG. 4D shows the system detailed in FIG. 4C, in particular, illustrating that the PZT **113** has moved the objective **103** so that the focused image is shifted to focus **B** on image plane **104**.

Those skilled in the art and having the benefit of this disclosure will appreciate that the system depicted in FIG. 4A-FIG. 4D allows the camera **112** to acquire an image similar to the defined pseudo-projection produced by scanning a well-corrected objective lens over the entire thickness of the cell **114** and further permits the acquisition of multiple pseudo-projections from multiple angles, without the necessity of moving the objective lens **103** at a high speed via high-frequency drive of the PZT scanning mechanism **113**.

Another embodiment employs the hyperchromatic optical path described previously, having an aberration that produces focus over the thickness of the object (e.g., 15 microns) for wavelengths within the range of constant absorption by the stain. This embodiment further includes a Chromatic Filter Array (CFA) in the optical path, preferably located just before the image plane **104**. The CFA may consist of two or more types of pixels, each pixel having a size corresponding to the pixel size of the camera **112** imaging surface **104**. Each type of pixel passes a separate range of wavelengths. An example of a CFA, having much wider bandpass ranges than the one described as part of this invention, is the Bayer filter, as described in U.S. Pat. No. 4,081,277, "Method for making a solid-state color imaging device having an integral color filter and the device" issued on Mar. 28, 1978 to Brault, et al.

Referring now to FIG. 5, an example of a design for a chromatic filter array is schematically shown. CFA **201** includes a plurality of pixel types in a unit cell **202**. In one example, the plurality of pixel types includes four pixel types **203**, **204**, **205**, **206** included in the unit cell **202**. The four pixel types pass only wavelengths, of 550-567 nm, 567-584 nm, 584-601 nm, and 601-618 nm, respectively. The CFA **201** must comprise a sufficient number of unit cells **202** to cover a substantial portion of the image surface **104**. For example, if the image plane **104** comprises 900x1600 pixels, then the CFA **201** may advantageously comprise 450x800 unit cells **202**, each unit cell **202** in turn comprising a 2x2 matrix of one each of filter pixel types **203**, **204**, **205**, and **206**. This design may limit the spatial resolution by a factor of two, due to the use of only one-fourth of the pixels for each wavelength. However, this is in practice not an issue, as long as the pixel-limited resolvable size (equivalent, with the CFA included, to $4 \times [\text{pixel size}]/\text{magnification}$) is less than the desired optical resolution (in this example, 500 nm). Under typical conditions of camera pixel size=7.4 microns and magnifica-

tion=60, this requirement is fulfilled. The light intensity reaching each CCD pixel in this embodiment is reduced by a factor of four due to the four-wavelength CFA. This reduction is also not a problem in practice, as the source intensity can be increased to provide higher light levels without requiring longer camera exposure times.

With reference jointly to FIG. 6A and FIG. 6B, another example of the operation of a hyperchromatic system incorporating a CFA 201 is illustrated. The system is similar to the one depicted in FIGS. 4A and 4C, with the addition of the CFA 201 on the image surface 104 of the camera 201. Due to translation of the objective 103 along the optical axis of the system, the focus point shifts from focus point A' in FIG. 6A to focus point B' in FIG. 6B.

The inclusion of the CFA 201 makes it possible to separate the signals from two or more (in this example, four) focal ranges, thereby decreasing the amount of defocusing that contaminates the in-focus signal. By saving each focal range separately, they may be combined digitally during post-acquisition processing, permitting an increase in the dynamic range of the combined images, and consequently improving the spatial resolution and contrast of the combined images. Alternatively, the images that result from each wavelength can be processed as two or more separate sets of data, and not combined until after each has been separately tomographically reconstructed, thus providing an improvement in spatial resolution and contrast.

Referring now jointly to FIG. 7A and FIG. 7B, object space for a first viewing angle, Θ_1 , for a hyperchromatic optical tomography system incorporating a long depth of field is schematically illustrated. The extended limits of the focal range, combined with the CFA 201, make a lens transducer, such as PZT 113, unnecessary for the operation of the system. Another pair of object planes Z5 and Z6 corresponds to wavelengths λ_1 and λ_2 , respectively. The object planes are located at the extrema of the inner diameter of the microcapillary tube 107. Because object planes Z5 and Z6 are located at the extrema of the inner diameter of the microcapillary tube 107, the location of object planes Z5 and Z6 remain constant relative to the objective lens even as the tube 107 rotates, causing the cell 114 to change its location relative to the objective lens. For a microcapillary tube 107 having an inner diameter of 50 microns, the separation between object planes Z5 and Z6 should, preferably, be at least 50 microns. The ray paths 405 and 406, comprising light of wavelengths λ_1 and λ_2 , respectively, travel through object planes Z5 and Z6, respectively, and reach the CFA 201, where λ_1 is transmitted only through, for example, CFA pixels of the first type 203, and λ_2 is transmitted only through, for example, CFA pixels of the last type 206.

Owing to the existence of the multiple pixel types in the unit cell of the CFA, each pixel type only collects light from a portion of the interval between the object planes Z5 and Z6. For the four-color CFA 201 shown in FIG. 5 as described above, each interval is, preferably, non-overlapping, and therefore only one-fourth of the total interval is transmitted through any pixel type and collected by the camera 112.

As an example, if the focal plane separation is 50 microns, and the wavelength range is 550 to 618 nm, then camera pixels lying directly behind pixel type 203 will detect only light having wavelengths between 550 and 567 nm, corresponding to object planes between object plane Z5 and Z5+12.5. In a similar manner, camera pixels lying directly behind pixel type 204 will detect only light having wavelengths between 567 and 584 nm, corresponding to focal planes between object planes located between Z5+12.5 microns and Z5+25 microns. Camera pixels lying directly

behind pixel type 205 will detect only light having wavelengths between 584 and 601 nm, corresponding to object planes between Z5+25 microns and Z5+37.5 microns; and camera pixels lying directly behind pixel type 206 will detect only light having wavelengths between 601 and 618 nm, corresponding to object planes between Z5+37.5 microns and Z6 (i.e., Z5+50 microns).

Referring now to FIG. 7B, a first viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field is schematically illustrated. The components comprising the system are similar to those of FIG. 6B, except that a mechanical translator for the objective lens, such as a PZT, is no longer necessary. The ray paths 405 and 406 originate at opposite sides of the tube 205 and follow similar paths to the image sensor 104 at focus point A". A CFA 201 is also shown, although it is optional in this embodiment.

Referring now to FIG. 7C, there schematically illustrated is the object space for a second viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field. Here the cell 114 is rotated to a second viewing angle Θ_2 . Because the cell 114 is always within the boundaries of the extended depth of field (i.e., between planes 15 and Z6), it is not necessary to employ a PZT to move the objective lens 103.

Referring now to FIG. 7D, a second viewing angle for a hyperchromatic optical tomography system incorporating a long depth of field is schematically illustrated showing the system detailed in FIG. 7C. In contrast to FIG. 7B, it should be noted that the focus of objective 103 has shifted to focus point B" on image plane 104. A CFA 201 is also shown although it is optional in this embodiment.

Referring now to FIG. 8A, an example of multiple camera system where a capillary tube holding a specimen is at a first rotation angle is shown. A multiple camera system includes a chromatic filter 110 and a condenser lens 115, and a tube assembly 121, containing, for example, a biological cell 114 substantially as described hereinabove with reference to FIG. 4A and FIG. 4B. Ray paths beginning near the cell 114 at object planes Z1 and Z2, respectively, pass through the immersion fluid 109 and the hyperchromatic objective lens system 103. In a departure from the system described above with reference to FIG. 4A and FIG. 4B, the multiple camera system here incorporates a dichroic beamsplitter cube 501 to split a first plurality of ray paths 502 and 503. The first plurality of ray paths 502 and 503 originate in object planes similar to object planes Z1 and Z2. Each camera may optionally be filtered by a chromatic filter array 201, 508. In an alternate embodiment, a polarization filter array may be substituted for each chromatic filter array. If a wide depth of field is desired, then another embodiment, similar to this one, would employ CFAs 201 and 508 while eliminating the translational mechanism 113 for moving the objective lens similarly to other embodiments described above.

Referring now to FIG. 8B, an example of the multiple camera system of FIG. 8A where the capillary tube holding a specimen is at a second rotation angle is shown. Here the dichroic beamsplitter cube 501 splits a second plurality of ray paths 504 and 505. The second plurality of ray paths 504 and 505 originate in object planes similar to object planes Z3 and Z4.

Referring now jointly to FIG. 8A and FIG. 8B, the rays 502 and 504, being of the same wavelength, travel through the first tube lens 111 to the first camera 112. The rays 503 and 505, being of a wavelength different from rays 502 and 504, travel through the second tube lens 506 to the sensor area 509 of a second camera 507. Additional dichroic beamsplitters and cameras may be readily envisaged.

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Focus Score

One characteristic of an OPTM system incorporating extended depth of field optics is that a fixed-focal plane image can no longer be acquired through the extended depth of field optical path. The focus quality of a flat object is retained over a wide range of focal positions. This property is sometimes referred to as focus invariance.

For an object that is not confined to a single focal plane it is still necessary to find the midpoint of the object of interest so that it may be kept within the focus interval throughout the data acquisition. One method of accomplishing this is to split the optical path prior to introducing the chromatic aberration, so that a separate optical path, incorporating a detector, is available. This separate optical path, being free of chromatic aberration, allows the system to acquire fixed-focal plane images. In a similar method that can be incorporated into a hyperchromatic imaging system, the optical path can be split and one arm chromatically filtered to near-monochromaticity, so that a single focal plane can be imaged by a separate camera, while the other arm provides the pseudo-projection. Another approach includes panning the objective lens over a wide range prior to beginning the scan, acquiring an image at each position, and assigning a focus score to each image. Focus scoring methods may employ autocorrelation, entropy, and/or other equivalent methods.

Referring now to FIG. 9, an example of focus invariance in an MTF range is illustrated. A focus-invariant range for the MTF at one spatial frequency, MTF (f1) 601 is bounded on the optical axis by an upper focal plane 602 and a lower focal plane 603. Within these boundaries the MTF 601 remains at a roughly constant plateau value before dropping down to a much lower level. Using such an approach, as described further below, the two end-points of the plateau 602, 603 in the MTF (f1) 601 can be identified, and the preferred focus chosen by, for example, the mid-point 604 between the end-points 602, 603.

Repetitive focus scoring is not necessary for a system having a depth of field exceeding the inner diameter of a microcapillary tube, provided that the upper and lower boundaries 602, 603 of the focus invariance region do not pass through the interior of the microcapillary tube. This condition can be verified by an initial focus scoring when the instrument is first configured.

Referring now to FIG. 10, a block diagram of an example method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system is schematically shown. According to the example method the midpoint is found and used to compute a 3D reconstruction of an object by:

1. panning through the optical axis and acquiring multiple images of an object in a microcapillary tube while panning (701);
2. evaluating the focus quality at each position along the optical axis (702);
3. determining two break points on the optical axis where the focus quality begins to degrade (703), where the two break points correspond to the upper and lower boundaries 602, 603 of the focus invariance region;
4. acquiring a pseudo-projection image (704), with the center of the pseudo-projection's scanning range centered between the upper and lower boundaries 602, 603 of the focus invariance region;
5. rotating the microcapillary tube to a next projection angle;
6. repeating steps 1-5 until a plurality of pseudo-projections have been acquired at a plurality of projection angles (705);

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7. computing a 3D reconstruction using the acquired pseudo-projections (706).

Referring now to FIG. 11A, a block diagram of another method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system is shown. According to this alternate example method the midpoint is found and used to compute a 3D reconstruction of an object by:

1. for a first viewing angle, acquiring pseudo-projections at a plurality of focal planes 801 by stepping the focus (i.e. by moving the objective lens a short distance represented by $n=n+1$, where n is an incremental step) and acquiring pseudo-projection data at each focal plane 813;
2. moving to a next viewing angle and repeating the acquisition of pseudo-projections 801 until a target volume is covered by stepping the focus as above 802;
3. summing all pseudo-projections for each viewing angle 803 to produce a set of summed pseudo-projections; and
4. computing a 3D reconstruction using the set of summed pseudo-projections 804.

In one example, pseudo-projections are acquired until the following formula is met or exceeded at 802:

$$\epsilon n D = \text{tube diam, where } 0 < \epsilon < 1.$$

Referring now to FIG. 11B shows a block diagram of yet another method for finding the midpoint of the focus-invariant region in a focus-invariant optical tomography system. According to this alternate example method the midpoint is found and used to compute a 3D reconstruction of an object by:

1. for a first viewing angle, acquiring pseudo-projections at a plurality of focal planes 801 by stepping the focus (i.e. by moving the objective lens a short distance represented by $n=n+1$, where n is an incremental step) and acquiring pseudo-projection data at each focal plane 813;
2. moving to a next viewing angle and repeating the acquisition of pseudo-projections 801 until a target volume is covered 802 by stepping the focus as above 802 according to a limiting formula;
4. performing a 2.5-D focus evaluation 805 to determine a best focus pseudo-projection for each viewing angle; and
5. computing a 3D reconstruction using a set of best focus pseudo-projections acquired at the best focus for each angle 806.

The method above is similar to that of FIG. 11A except for the 2.5-D focus evaluation.

Referring now to FIG. 12, an embodiment of an autofocus system using chromatic balance is schematically shown. Here two autofocus cameras 2002, 2004 provide digitized input to an image comparator 2006, which in turn provides a feedback signal 2008 to a transducer drive 2010, such as a PZT controller. As an object of interest 2001 is rotated within a microcapillary tube (as described above), separate images from an upper object volume 2012 and a lower object volume 2014 are captured by the autofocus cameras 2002, 2004. The images are compared and analyzed by the comparator 2006. The feedback signal 2008 from the comparator 2006 drives the transducer drive 2010 which, in turn, controls an objective lens focus drive 2016 so that the focus range of an objective lens 2103 moves closer to the region of poorer focus quality. When the difference in focus quality between the two images becomes sufficiently small, the transducer drive is no longer required for shifting the focus range. This process can

be repeated for each projection angle, which may be necessary as the planes containing the object of interest move up and down the optical axis as the tube rotates. Images are acquired by an image camera **2020**.

In one embodiment, light rays are directed by a primary beam splitter **2028**, a secondary beam splitter **2030** and a mirror **2032**. Light rays directed to the image camera **2020** are filtered by a first filter **2022**, where the first filter passes light having wavelengths between 550 nm and 620 nm through a first image forming lens **2023**. Light rays directed to the first autofocusing camera **2002** are filtered by a second filter **2024**, where the second filter passes light having wavelengths between 585 nm and 620 nm through a second imaging lens **2025**. Light rays impinging mirror **2032** are directed to the second autofocusing camera **2004** after being filtered by a third filter **2026**, where the third filter passes light having wavelengths between 550 nm and 585 nm through a third imaging lens **2027**.

Referring now to FIG. **13**, another embodiment of an autofocusing system using chromatic balance is schematically shown. A source of spectrally uniform light illumination **2060** illuminates an object of interest **2001** which is imaged by image camera **2020**. Similarly to the autofocusing system described above, light rays are directed by a primary beam splitter **2028**, a secondary beam splitter **2030** and a mirror **2032**. Light rays directed to the image camera **2020** are filtered by a first filter **2022**, where the first filter passes light having wavelengths between 550 nm and 620 nm through a first image forming lens **2023**. A portion of light rays passing through the secondary beam splitter **2028** are filtered by a second filter **2024**, where the second filter passes light having wavelengths between 585 nm and 620 nm. Light rays impinging mirror **2032** are filtered by a third filter **2026**, where the third filter passes light having wavelengths between 550 nm and 585 nm. A first Fourier plane forming lens **2050** transmits light from the second filter **2024** through a first Fourier plane spatial filter **2052** to a first photo sensor **2054**. A second Fourier plane forming lens **2056** transmits light from the third filter **2026** through a second Fourier plane spatial filter **2058** to a second photo sensor **2054**.

The Fourier spatial filters **2052**, **2058** operate on two focus paths to provide analog feedback to the focus control controller **2042** via the photo-diodes **2054**. Spatial filtering ensures that the photodiodes only receive the high-spatial frequency components of a focal plane. High spatial frequency content is associated with well-focused objects. The high frequency content of the upper and lower halves of the focal range, **2012**, **2014** respectively, is compared in signal conditioning and difference amplification processor **2062**. The difference amplification processor **2062** provides output **2040** which is used as above to control drive **2042** to cause the transducer **2016** to position the objective lens **2103** until the high-frequency intensities of the two focal regions are sufficiently similar. Under continuous illumination this method has the advantage of tracking motion of an object keeping it in focus balance at all times.

Polarization-Dependent Optics (Birefringent) for 3D Imaging

The location of the focal plane is dependent on the polarization of the light. This system can be implemented using birefringent optics, in which the index of refraction varies according to the electric-field polarization of the optical wavefront. An example of a birefringent optical material is calcite (CaCO_3), for which the index of refraction at 590 nm is either 1.658 or 1.486, depending on the polarization.

Embodiments analogous to those of the hyperchromatic systems described above may be employed. With these techniques, the polarization of the imaged light will depend on the object focal plane from which it originated. For example, the horizontally-polarized (electric-field vector at zero degrees) component of the light may provide the in-focus image for an object plane Z_H , whereas the vertically-polarized (electric-field vector at 90 degrees) component of the light may provide the in-focus image for an object plane Z_V , located, for example, 15 microns closer to the detector than plane Z_H . Light having polarizations between zero and 90 degrees would provide in-focus images for object planes between Z_H and Z_V .

The polarization of the illuminating light can be varied over time by using a spinning polarizing filter, the collected (unpolarized) light passes through a polarizing filter before it reaches the image sensor, or the entire focal range can be collected simultaneously.

In one embodiment, the focal range may be comparable to the thickness of the object, e.g., 15 microns. In this embodiment, a PZT can be incorporated to compensate for rotation-induced translation of the cell, in a system analogous to that depicted in FIG. **6A** and FIG. **6B**.

In another embodiment, analogous to that depicted in FIG. **7A**-FIG. **7D**, the range of the focal planes can be equivalent to the diameter of the microcapillary tube (e.g., 50 microns), and a Polarization Filter Array (PFA; the polarization analog of the chromatic filter array illustrated in FIG. **5**) is incorporated into the system in place of the CFA shown in FIG. **7A**-FIG. **7D**.

In yet another embodiment, the range of the focal planes can be equivalent to the diameter of the microcapillary tube, and the polarization of the light varied over time while a series of synchronized camera exposures acquires the object planes as they come into focus on the detector.

2.5-D Imaging

In any OPTM system incorporating extended depth of field optics, post-acquisition processing may be incorporated to perform pixel-by-pixel analysis to compute a mosaic of in-focus features in the field of view. An example of one type of 2.5-D imaging is found in R J Pieper and A Korpel, "Image processing for extended depth of field," *Applied Optics* 22, 1449 (1983). The 2.5-D imaging approach may be most advantageously employed in those embodiments that make use of a Chromatic or Polarization Filter Array (CFA or PFA) and covering a wide focal range, and in the embodiments that make use of multiple camera exposures. In these systems, the weight assigned to an element type can vary from one pseudo-projection to the next, as the object is rotated through different focal plane regions.

To accomplish this, individual features are identified in the collection of short-focal-plane images that form an image stack. The same feature may appear in several images within the stack, but only a subset of those images will contain a well-focused representation of that feature.

Referring now to FIG. **14**, a block diagram of a 2.5-D focusing method is schematically shown. Features, $G_i = G_1, \dots, G_\Omega$ in an image stack S_k are identified **1101**. For each feature G_i in an image stack S_k , the images for which G_i is in best focus are identified **1102**. A blank composite image PP_k is generated **1103**. The pixels that make up that best-focused feature (X_i, Y_i, Z_i) are added to the composite image PP_k **1104**. This process is repeated for all features ($G_1, G_2 \dots G_\Omega$) until all features have been incorporated into PP_k . Since a single feature may span two or more images in S_k , a single pixel in PP_k may accumulate two or more intensity values,

one for each image in S_k that contains a well-focused representation of G_i . Furthermore, two or more features may share the same X-Y coordinates (but different Z-coordinates), which may result in some pixels in PP_k accumulate intensity values from two or more features. Note that subscripted letters l,k etc. represent index numbers.

The process is repeated for all the image stacks, until all image stacks ($S_1, S_2 \dots S_{kmax}$) have been analyzed and their associated composite images ($PP_1, PP_2 \dots PP_{kmax}$) have been computed **1105**. The tomographic reconstruction can then be computed, using the set of PP_k as the input images **1106**. In one example using this method, each 2x2 block of a 4-color CFA or PFA can be processed by selecting the single pixels containing the best focus, or as a weighted sum of two or more pixels.

Beam Split Multiple Focal Plane

There are several fundamental advantages of shorter integrated pseudo-projections for OPTM performance. First, smaller magnitude pseudo-projections (integrated optical axis scans) reduce the effect of the low frequency information dominating in the spatial spectrum. Second, adding more images that sample the same volume improves the signal to noise proportionally to the square root of the number of images used. Third, multiple images enable the detection and compensation for unusual hot spots in images due to refractive contrast.

The separation of the depth of field into segments allows many other depth of field extenders to work to supply a more limited solution, working better with less complication.

A reduced range of motion or an extended depth is possible with direct objective scan and multiple camera focal planes.

The creation of multiple focal ranges does not necessarily require multiple cameras. With adequate camera sensor area it is possible to merge the images and capture them on a single sensor. This can be done using a fiber optic faceplate splitting the sensor into zones, or a folded optical system merging the multiple images onto a single CCD.

Referring now to FIG. 15, an example of a folded system is depicted. The substantially collimated light exiting the objective **1205** is divided by a first dichroic beam-splitting cube **1206**. One arm **1202** has a wavelength λ_1 and originates in a first focal plane **1201**. It passes through a first tube lens, through a second beam-splitter cube **1207**, and onto the right half of the camera sensor's active area **1211**. The other arm **1204** has a wavelength λ_2 and originates in a second focal plane **1203**. It reflects off two mirrors **1208**, **1209**, passes through the tube lens **1210**, reflects within the second dichroic beam-splitter cube **1207**, and onto the left half of the camera sensor's active area **1211**. The two halves of the camera will acquire focused images having substantially identical magnifications, but originating from different focal planes **1201**, **1203** in object space. The relative lateral shift in the images is achieved by laterally shifting the second dichroic beam-splitter cube **1207**, so that the reflected light of the second arm **1204** is laterally shifted relative to the first arm **1202** and to the tube lens **1210**.

Two-Stage Magnification

Acquiring images separated by 10 microns in object space would require, for a 100x lens, a difference in image-space path length proportional to magnification squared (i.e., 100 mm). If the tube lenses have the same focal lengths, but different back focal planes, then the two halves of the camera will acquire focused images having substantially identical magnifications, but originating from different focal planes in object space. As an illustration, placing the camera 100 mm closer to the second tube lens than to the first tube lens will

result in a difference in focal planes of $100/M^2$ microns, where M is the lateral magnification. If $M=100$, then $100/M^2=10$ microns.

However, a much more modest change in optical axis can be achieved using two 10x magnification stages and changing the focal plane of the secondary objective only slightly. A 10-micron shift in the specimen plane at 10x magnification image is achieved with a one-millimeter shift of the intermediate image plane.

Using a split focal plane approach allows two or more cameras (four are shown in the example of FIG. 16) to each collect a range of the focal depth of the specimen. In the limit the number of cameras is practically limited by the amount of light that can be brought to illuminate the specimen and the cost and complexity of the optical path. A system incorporating more cameras improves signal to noise, assuming that the cameras' well capacities are sufficiently close to full, and reduces the range that each image must deliver in field depth. A shorter field depth aids in producing better representation of high spatial frequencies in a resultant pseudo-projection.

In an example of this embodiment, shown in FIG. 16, an optical system comprises a primary objective **1302**, first and second mirrors **1304A**, **1304B**, three beam-splitters **1303A**-**1303C**, four primary tube lenses **1310**, four secondary objective lenses **1312**, four secondary tube lenses **1308**, and four CCD cameras **1309**. The primary objective **1302** and the primary tube lenses **1310** provide at least 10x magnification, and the secondary objectives and tube lenses provide at least an additional 10x magnification, for a total of at least 100x magnification.

Each ray path passes through two beam-splitter thicknesses, and each ray path undergoes either two or zero reflections, either through the beam-splitters or by the mirrors. The equivalence of the ray path-lengths through the beam-splitters means that the aberrations due to passing through the glass are equivalent. The number of reflections being always even (or always odd) means that all four images retain the same orientation at the image planes of the four cameras **1309**. Space between first tube lens and secondary objective differs for each ray path, so that a different object plane is focused on each camera. A reduced range of motion or an extended depth is possible with focal plane scanning behind the objective and multiple camera focal planes.

Extending the multiple camera optics to greater than 20 focal planes can, in theory, sample a ten-micron depth of field every 500 nm. The arrangement of multiple cameras allows two simultaneous modalities of volumetric sampling that can each be used to contribute their relative strengths to a more accurate volumetric reconstruction. Specifically, the contrast generated by refractive and diffractive effects in the sample media interfaces may be sorted out from the purely absorptive effects and all data captured rapidly and without focal plane motion or rotational blur.

Wavefront Coded Optics

Referring now to FIG. 17, a schematic diagram of an OPTM system including wavefront coded optics is shown. As above, a microcapillary tube **1707** holds an object of interest **1701** and is rotated through various viewing angles as viewed by an objective lens **1703**. Light transmitted through the objective lens **1703** impinges on wavefront coded optics **1705** which are located between the objective lens **1703** and an imaging camera **1707**. The use of wavefront coded optics provides a method of pre-distorting the optical wavefront so that an object of interest is contained within an extended depth of field producing a low but consistent frequency response throughout its volume. Thus all focal planes, within a limited range along the optical axis, are equally defocused.

This constitutes the wavefront coding. Wavefront coding elements are available from CDM Optics, Inc. (Boulder, Colo.), and are described in, for example, E R Dowski, "Wavefront coding optics," U.S. Pat. No. 6,842,297 (2005).

The limit of wavefront coding is about a 12:1 improvement in the depth of field. For an optical tomography application such an improvement will provide about half of the required depth. Thus wavefront coding may advantageously be combined with one of the many other embodiments described herein to deliver a complete solution.

The point of the first contrast-reversal (MTF less than zero) occurs, for matched condenser and objective NA's, at 0.64 waves of defocus, as detailed in V N Mahajan, "Aberration Theory Made Simple" (Bellingham, Wash.: SPIE Press, 1991). This point is readily expressed in terms of the change in the optical depth, Δz , as

$$\Delta z = \pm 1.28 \lambda n / (NA_{obj})^2$$

where λ is the wavelength of the light being collected, n is the refractive index of the region between the objective lens and the object, and NA_{obj} is the numerical aperture of the objective lens. For $\lambda=550$ nm, $n=1$, and $NA_{obj}=0.9$, this distance is $\Delta z = \pm 0.87$ microns. Then for a 12-micron-deep object, we require at least a 5 \times improvement in the depth of field to avoid contrast reversal at 6-micron defocus (roughly 4.4 waves of defocus).

Another embodiment of imaging with wavefront coding incorporates digital enhancement of the image with a complementary transfer function to boost the suppressed high frequency components to recover a sharply focused image while retaining the extended depth.

Another embodiment uses multiple cameras, such as is shown above, that take advantage of the wavefront coded optics approach to extended depth of field by coding each optical path with lens transfer function, thus extending the depth of field from one segment to the next. This mechanism allows for a single brief exposure such as a strobed illuminator to quickly sample a wide depth of field without mechanical motion.

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

What is claimed is:

1. An optical projection tomography system for imaging an object of interest, the optical projection tomography system comprising:

- (a) a light source;
- (b) an object-containing tube, a portion of which is located within the region illuminated by the light source, wherein the object of interest has at least one feature of interest located within the object-containing tube;
- (c) at least one detector, wherein the at least one detector is located to receive emerging radiation from the object of interest; and wherein the object of interest is stained to impart an absorption coefficient of at least on wavelength; and
- (d) at least one lens located in the optical path between the object region and the at least one detector, wherein the at

least one lens includes at least one optical field extension element such that light rays from multiple object planes in the object-containing tube simultaneously focus on the at least one detector.

2. The system of claim 1 wherein the object moves relatively to the at least one detector and the at least one lens operates to provide multiple views of the object region for producing at least one image of the at least one feature of interest at each view.

3. The system of claim 1, wherein the object of interest comprises a biological cell.

4. The system of claim 1, wherein each of the multiple object planes that come to a focus on at least one of the at least one detectors registers light of a different optical wavelength on the at least one detector.

5. The system of claim 1, wherein an interval spanned by the multiple object planes comprises an interval spanning the thickness of the object of interest.

6. The system of claim 1, wherein an interval spanned by the multiple object planes comprises an interval spanning the thickness of the diameter of the object-containing tube.

7. The system of claim 1, wherein the system further comprises a chromatic filter array located between the at least one lens and the at least one detector.

8. The system of claim 1, wherein the system further comprises a piezoelectric transducer coupled to the at least one lens for moving the at least one lens along an optical path between each of the multiple views of the object region, such that the object of interest remains within an interval spanned by the multiple object planes.

9. The system of claim 1, further comprising a wavefront-coded optical element located between the at least one lens and the at least one detector.

10. The system of claim 1, wherein the at least one detector comprises a detector selected from the group consisting of charge coupled devices, complementary metal-oxide-semiconductor devices, solid-state image sensors, and solid-state image sensor detector arrays.

11. The system of claim 1, wherein the object of interest is stained with hematoxylin.

12. The system of claim 1, wherein the light from multiple object planes includes light having a wavelength range of 550 nm to 620 nm spanning a focus interval of up to 50 microns.

13. The system of claim 1, wherein the object-containing tube has a diameter of at least 50 microns.

14. The system of claim 2, wherein the system further comprises a piezoelectric transducer attached to the at least one lens for moving the at least one lens along an optical path between each of the multiple views of the object region, such that the object of interest remains within the focus interval.

15. The system of claim 1, wherein the system further comprises a chromatic filter array located between the at least one lens and the at least one detector.

16. The system of claim 1, wherein an interval spanned by the multiple object planes comprises a focus interval of at least 12 microns.

17. The system of claim 1, wherein the light from multiple object planes includes a wavelength range of 550 nm to 620 nm.

18. The system of claim 1, further comprising a band pass filter for passing light in the range of 550 nm to 620 nm located to band limit light reaching the at least one detector.

19. The system of claim 1, wherein the light source includes a chromatic filter located to filter light reaching the object-containing tube.

20. The system of claim 1, wherein the at least one lens comprises a hyperchromatic lens.

21. The system of claim 1, wherein the at least one optical field extension element comprises a polarizing filter array located between the at least one lens and the at least one detector.

22. The system of claim 1, wherein the at least one optical field extension element comprises a wavefront-coding element located between the hyperchromatic lens and each of the at least two detectors.

23. The system of claim 1, wherein each of the multiple object planes that come to a focus on the at least one detector is formed by light having a different polarization.

24. An optical projection tomography method for imaging an object of interest, the optical projection tomography method comprising:

- (a) staining an object of interest to impart an absorption coefficient of at least one wavelength when illuminated;
- (b) illuminating the object of interest;
- (c) locating at least one lens in the optical path between the object of interest and at least one detector, wherein the at least one lens includes at least one optical field extension element such that light rays from multiple object planes in the object of interest simultaneously focus on the at least one detector.

25. The method of claim 24 further comprising filtering the light rays to pass only light rays having wavelengths within the range of 550 nm to 620 nm.

26. The method of claim 25 wherein the filtering further comprises passing the light through a band pass filter located to limit light reaching at least one detector.

27. The method of claim 24 further comprising delivering the object of interest for imaging from an object containing tube; wherein the object containing tube comprises a tube selected from the group consisting of a capillary tube, a microcapillary tube, a microcapillary tube bundle, and a microcapillary tube cassette.

28. The method of claim 27 wherein the object containing tube has a diameter of at least 50 microns.

29. The method of claim 24, wherein simultaneously focusing comprises moving at least one lens of a hyperchromatic lens system relative to at least one detector.

30. The method of claim 24 further comprising splitting a first plurality of light ray paths via a beamsplitter.

31. The method of claim 24 wherein the simultaneous focusing comprises focusing a hyperchromatic lens.

32. The method of claim 29 wherein the simultaneous focusing further comprises filtering the light by transmitting the light through at least one chromatic filter array.

33. The method of claim 24 wherein the at least one detector comprises a detector selected from the group consisting of

charge coupled devices, complementary metal-oxide-semiconductor devices, solid-state image sensors, and solid-state image sensor detector arrays.

34. The method of claim 32, further comprising locating the at least one chromatic filter array between the hyperchromatic lens and the at least one detector, so that light coming to a focus on the detector is separated into two or more wavelength bands, each wavelength band being transmitted through the chromatic filter array to a separate set of pixels on the at least one detector.

35. The method of claim 32, wherein the at least one chromatic filter array is substituted with at least one polarization filter array.

36. The method of claim 24, wherein simultaneously focusing of the multiple object planes comprises extending focusing through a focus interval spanning the thickness of the object of interest.

37. The method of claim 24, wherein the simultaneously focusing of the multiple object planes comprises extending focusing through a focus interval of at least 12 microns.

38. The method of claim 24, wherein the simultaneously focusing of the multiple object planes comprises extending focusing through a focus interval of up to 50 microns.

39. The method of claim 24 further comprising impinging the light rays onto a wave front-coded optical element.

40. The method of claim 24, wherein the staining of the object of interest is performed with hematoxylin.

41. The method of claim 24, wherein simultaneously focusing of the multiple object planes comprises simultaneously focusing with an autofocusing system.

42. The method of claim 39, wherein the simultaneously focusing with an autofocusing system comprises focusing chromatic balance.

43. The method of claim 24, wherein simultaneously focusing light rays comprises transmitting the light rays through a polarizing filter array.

44. The method of claim 24, wherein simultaneously focusing light rays comprises transmitting the light rays through a wavefront-coding element.

45. The method of claim 24, wherein simultaneously focusing light rays comprises focusing each of the multiple object planes on the at least one detector by light having a different polarization.

46. The method of claim 24, wherein simultaneously focusing light rays comprises performing a 2.5-D focus evaluation to determine a best focus.

47. The method of claim 24, wherein the object of interest comprises a biological cell.

* * * * *