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(54) Titre : IMAGERIE EN 3D DE CELLULES VIVANTES PAR UN RAYONNEMENT ULTRAVIOLET
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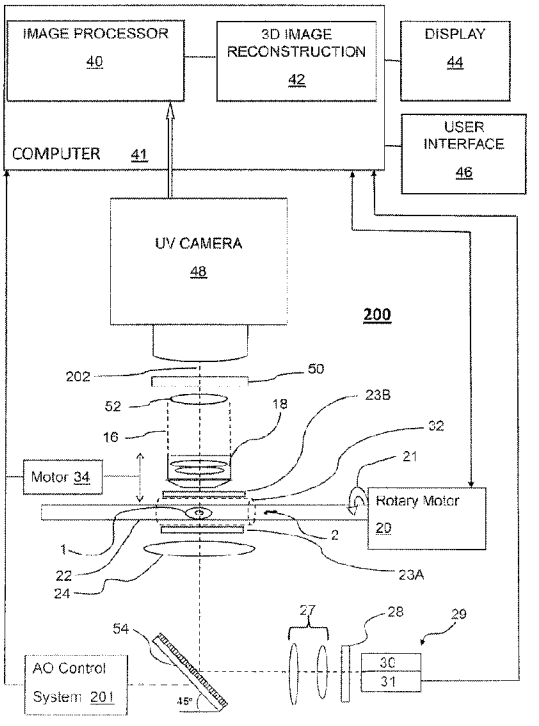

(57) Abrégé/Abstract:
A method for 3D imaging of cells in an optical tomography system (11) includes moving a biological object (1) relatively to a microscope objective (18) to present varying angles of view. The biological object (1) is illuminated with radiation having a spectral bandwidth limited to wavelengths between 150 nm and 390 nm. Radiation transmitted through the biological object (1) and the microscope objective (18) is sensed with a camera (48) from a plurality of differing view angles. A plurality of pseudoprojections of the biological object (1) from the sensed radiation is formed and the plurality of pseudoprojections is reconstructed to form a 3D image of the cell.

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Abstract

A method for 3D imaging of cells in an optical tomography system (11) includes moving a biological object (1) relatively to a microscope objective (18) to present varying angles of view. The biological object (1) is illuminated with radiation having a spectral bandwidth limited to wavelengths between 150 nm and 390 nm. Radiation transmitted through the biological object (1) and the microscope objective (18) is sensed with a camera (48) from a plurality of differing view angles. A plurality of pseudoprojections of the biological object (1) from the sensed radiation is formed and the plurality of pseudoprojections is reconstructed to form a 3D image of the cell.

77501-47D1

3D IMAGING OF LIVE CELLS WITH ULTRAVIOLET RADIATION

This is a divisional of Canadian Patent Application No. 2,715,623 filed on February 3, 2009.

Technical Field

The present invention relates to optical tomographic imaging systems in general, and, more particularly, to optical projection tomography for 3D microscopy, in which a small object, such as a biological cell, is illuminated with ultraviolet radiation for pseudoprojection imaging and reconstruction into a 3D image.

Background

Advances in imaging biological cells using optical tomography have been developed by Nelson as disclosed, for example, in US Patent No. 6,522,775, issued 2/18/2003, and entitled "Apparatus and method for imaging small objects in a flow stream using optical tomography."

Further developments in the field are taught in Fauver et al., US Patent application number 10/716,744, filed 11/18/2003 and published as US Publication No. US-2004-0076319-A1 on 4/22/2004, entitled "Method and apparatus of shadowgram formation for optical tomography," (Fauver '744) and Fauver et al., US Patent application number 11/532,648, filed 9/18/2006, entitled "Focal plane tracking for optical microtomography," (Fauver '648).

Processing in 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 microcapillary 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 "pseudoprojection images." The set of pseudoprojection images can be reconstructed using backprojection and filtering techniques to yield a 3D reconstruction of a cell of interest. The ability to have isometric or roughly equal resolution in all three dimensions is an advantage in 3D tomographic cell imaging, especially for quantitative image analysis.

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 biomarker may yield important information about the disease state of the cell, including, but not limited to, various cancers such as lung, breast, prostate, cervical, stomach and pancreatic cancers.

The present disclosure allows an extension of optical projection tomography to live cell imaging and is expected to advance cell analysis, drug development, personalized therapy, and related fields. Until now, live cell microscopy has traditionally been done by non-labeling 2D imaging techniques such as phase contrast, DIC, and polarization contrast microscopy.

Native absorbance and fluorescence imaging using deep ultraviolet (DUV) at 250 nm to 290 nm wavelengths has been technically challenging and causes phototoxicity in irradiated cells. More recently, vital stains have been used that typically emit fluorescence signals for 3D live cell imaging, because commercial microscopes (of confocal, deconvolution, and multiphoton excitation varieties) rely on fluorescence for building up multiple planar slices for generating 3D images. However, in these cases, the 3D image resulting from a stack of 2D images has about four times less axial resolution as the lateral resolution within each slice, thereby making quantitative analysis imprecise. The ability to have isometric or roughly equal resolution in all three dimensions is a significant advantage in 3D tomographic cell imaging, especially for quantitative image analysis.

One advantage of using DUV illumination for live cells is that native DNA and protein absorb the light at 260nm and 280nm, respectively, without the use of any photochemical label that must permeate the cell membrane and sometimes the nuclear membrane of the cell, which is in a non-normal state. Furthermore, the label or stain is only an intermediary step toward the measurement of target protein or nucleotide (DNA) which adds a large degree of variability in this measurement. Elimination of such exogenous species would potentially improve the accuracy of a quantitative measure of protein or nucleotide (DNA), as well as reduce time, effort and complexity by eliminating steps in the sample preparation. Unfortunately, the use of DUV illumination has demonstrated phototoxicity in the past, due to the high dose of radiation required to stimulate a strong signal.

Recently, however, DUV imaging of live cultured human and mouse cells was demonstrated at 260 nm and 280 nm using DUV light-emitting diodes (LEDs) (See,

for example, Zeskind, BJ, et al., "P. Nucleic acid and protein mass mapping by live cell deep ultraviolet microscopy," Nature Methods 4(7):567-569 (2007)).

The present disclosure describes a new, novel and surprisingly effective 3D imaging system that provides solutions to long felt needs in the field of DUV 3D imaging of cells, and more particularly, live cells.

Brief Summary of the Disclosure

A method for 3D imaging of cells in an optical tomography system is provided including moving a biological object relatively to a microscope objective to present varying angles of view. The biological object is illuminated with optical radiation having a spectral bandwidth limited to wavelengths between 150 nm and 390 nm. Radiation transmitted through, scattered by, or secondarily emitted by the biological object and captured by the microscope objective is sensed with a camera to record images from a plurality of differing view angles. A plurality of pseudoprojection imag es of the biological object from the sensed radiation is formed and the plurality of pseudoprojections is reconstructed to form a 3D image of the cell.

According to one aspect, there is provided an optical tomography system for acquiring 3D images comprising: a tube for containing a biological object; a rotary motor for rotating a biological object relative to a microscope objective; a single optical illumination source for illuminating the entire biological object; a beamsplitter positioned to split radiation transmitted through the biological object into at least two selected wavelengths; a microscope objective lens having a depth of field, the objective lens being located to receive light emanating through the object; an axial translation mechanism coupled to translate the microscope objective lens for axially scanning the entire biological object with the microscope objective lens through the thickness of the biological object; a first ultraviolet camera for sensing a first radiation bandwidth transmitted through the entire biological object and the microscope objective lens during axially scanning, the first ultraviolet camera positioned to separately receive one of the at least two selected wavelengths from

the beamsplitter, wherein the first radiation bandwidth has a first spectral range between 150 nm and 390 nm to produce a first plurality of pseudo-projection images of the biological object onto the first ultraviolet camera, and a second ultraviolet camera positioned to separately receive a different one of the at least two selected wavelengths from the beamsplitter, for sensing a second radiation bandwidth within a second spectral range which is also transmitted through the entire biological object and the microscope objective to the second ultraviolet camera during axially scanning, wherein the second spectral range is also between 150 nm and 390 nm, but different than the first spectral range, to produce a second plurality of pseudo-projection images of the biological object onto the second ultraviolet camera; an image processor coupled to receive data from the first and second ultraviolet cameras; and a reconstruction module coupled to the image processor for reconstructing the plurality of pseudoprojection images to form a reconstructed 3D image.

Brief Description of the Drawings

FIG. 1 schematically shows an example of a system for 3D imaging of cells in an optical tomography system employing ultraviolet radiation.

FIG. 2 schematically shows an alternate example of a system for 3D imaging of cells in an optical tomography system with ultraviolet radiation using a UV camera and optional adaptive optics.

FIG. 3 schematically shows an embodiment of a temperature-controlled housing for use in an optical tomography system.

FIG. 4 schematically shows a side view of an example of a microfluidics cartridge as used in a raceway configuration for imaging cells.

FIG. 5 schematically shows a top view of an example of a microfluidics cartridge as used in a racetrack configuration for imaging cells.

FIG. 6 schematically shows an optical tomography process including separate imaging stages along the same pathway.

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 disclosure. 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 generally of 500 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 for a specified feature is produced.

"Object" means an individual cell, item, thing or other entity.

"Pseudoprojection" includes a single image representing a sampled volume of extent larger than the native depth of field of the optics. The concept of a pseudoprojection is taught in Fauver '744.

"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.

With respect to imaging of live cells, several assumptions are made in this disclosure: (1) submicron isometric resolution is required of the chromatin structure in the nucleus which limits the wavelength of optical radiation to frequencies higher than infrared (less than or equal to near infrared wavelengths, < 1000 nm), (2) individual cells are being imaged or possibly analyzed which may allow for diffraction measurement at multiple perspectives, and (3) harvesting of the cell after imaging is desired with minimal cell damage.

Referring now to **FIG. 1** a system for 3D imaging of cells in an optical tomography system **11** employing ultraviolet radiation is schematically shown. A tube **22**, such as a capillary tube, microcapillary tube or equivalent, is positioned to be viewed by a microscope **16** including a microscope objective **18** and a tube lens element **52**. A rotation mechanism, for example, a rotary motor **20** is attached to the tube **22**. An axial translation mechanism, for example motor **34**, is coupled to the microscope objective. A radiation source **29** is positioned to illuminate a part of the

tube **22** including a biological object **1** held therein. The radiation source **29** generates radiation having a spectral bandwidth limited to wavelengths between 150 nm and 390 nm. In one useful example, the radiation source **29** comprises multiple sources **30**, **31** transmitting at least two selected wavelengths that are detected concurrently by a first light detector **10** and a second light detector **14**. Optional filters **12A, 12B** are selected to block fluorescence having a wavelength longer than the UV limited spectral bandwidth, such as native tryptophan fluorescence, and/or increase separation of differing ultraviolet radiation signals. The radiation source may advantageously be incorporated into a computer-controlled light source and condenser lens assembly **56**. The computer-controlled light source and condenser lens assembly **56** may further include condenser lens optics **24, 26** a light diffuser **28** and the radiation source **29**.

In one example embodiment, the tube **22** is placed in a viewing area between two optically flat surfaces such as a standard microscope slide **23A** and a standard microscope coverslip **23B**. The interstices between the tube **22** and the microscope slide **23A** and coverslip **23B** are filled with optical gel **32** or an equivalent material such as inorganic and organic oils, having an index of refraction that also substantially matches those of the tube **22**, and the microscope slide and coverslip. The tube **22** itself may advantageously be coated with an oil of similar optical properties. The outer diameter of the tube **22** may be, for example about 250 microns. Although not always shown in order to simplify the drawings for clarity, it will be understood that refractive index matching materials are used to match optics in the various embodiments described herein. A typical refraction index, n, matched to capillary tubing used in an optical tomography system is about 1.48, for example, at 590 nm, but the dispersion curve moves sharply upward in the UV. Estimated refractive index of fused silica capillary tube is 1.51 at 250nm, and transmittance of DUV by UV grade fused silica is about 90%.

The biological object **1** may advantageously be selected from the group consisting of a cell, a live cell, a fixed cell, an unfixed cell, a frozen cell, a thawed cell, a desiccated cell, a cloned cell, a mobile cell, an immobilized cell, an encapsulated cell, a cell nucleus, cell parts, an organelle, a sub-cellular component, chromosomes, and equivalent materials. The optical tomographic imaging system **11** may advantageously employ illumination radiation having a frequency that stimulates native fluorescence from the biological object, where the light detectors and image

processor further include modules for measuring the stimulated fluorescence. The biological object is contained in aqueous environment **2**. The aqueous environment **2** comprises physiological buffered saline or other solutions as described below.

A beamsplitter **15** is positioned to split radiation transmitted through the biological object into at least two selected wavelengths. The beamsplitter may advantageously be selected from the group consisting of a polarizing beam splitter, a Wollaston prism, a birefringent element, a half-silvered mirror, a 50/50 intensity beamsplitter, a dielectric optically coated mirror, a pellicle film, a dichroic beamsplitter, mirror, prism, diffractive optical element, grating, and equivalents. The first light detector **10** is positioned to sense radiation transmitted through the biological object **1**, the microscope objective **18**, the beamsplitter **15** and a first set of the optional filters **12A**. Similarly, the second light detector **14** is positioned to sense radiation transmitted through the biological object **1**, the microscope objective **18**, the beamsplitter **15** and a second set of the optional filters **12B**. In one example, the first and second light detectors **10**, **14** may each particularly include a pixel array detector sensitive to ultraviolet light, where each pixel array detector is selected to detect a different one of the two selected wavelengths.

A computer **41** includes an image processor **40** coupled to receive data from the first and second light detectors **10**, **14**. A reconstruction module **42** is coupled to the image processor **40**, where the reconstruction module processes the data to form a 3D image of the cell using reconstruction algorithm techniques such as taught in Fauver '744 for example. The image processor **40** transmits processed image data to the 3D image reconstruction module **42** which may advantageously be coupled to an optical display **44** for operator viewing. User interface **46** can be provided for operator control and information purposes. The user interface **46** may be a GUI interface or the like coupled to the computer **41**.

In one example, the axial translation mechanism **34** comprises a piezoelectric transducer or equivalent device. A controller **35** linked to control the piezoelectric transducer may advantageously be a computer, computer module or the like, where the piezoelectric transducer is controlled to axially move the objective lens **18**.

In one example system, the optical tomographic imaging system **11** is configured through use of filters and radiation sources to image cells using wavelengths limited to between 240 nm and 300 nm. Radiation detected by the first detector **10** may have wavelengths primarily in a first range between 260 nm and

265 nm. Radiation detected by the second detector **14** may have wavelengths primarily in a second range between 280 nm and 285 nm. The first range operates to enhance natural radiation absorbance by DNA and RNA. The second range operates to enhance natural radiation absorbance by protein. The first and second wavelength ranges may be provided using a pair of radiation sources, each source transmitting one of the two selected of wavelength ranges. One of the detectors may be tuned to detect absorbance around 270 nm near hydrophilic surfaces such as DNA and protein.

In one embodiment the radiation may be measured in time series using time to separate signals. The radiation source may be pulsed in a time series to cause pulsed excitation of the cells in order to increase signal to noise, separating signals. For example, a radiation source at 260 nm may be pulsed at a time, T_0, followed by a 280 nm pulse at T_1, followed in turn by one or more laser pulses at n subsequent time increments, T_n, where n is any number denoting a subsequent point in time.

Alternatively, the native tryptophan fluorescence can be measured to obtain a secondary measure of protein and its confirmation and constituents, such as amino acids. A third beam splitter would be required unless time series illumination is used. In this alternative design, beamsplitter **15** would split all DUV light (240-300 nm) to the DUV light detector **14** while the lower frequency fluorescence signal would be detected by a fluorescence light detector **10** (>300 nm). Operation of DUV light sources **30**, **31** can be in time-series so radiation absorbance primarily by nucleotides (260-265 nm) can be captured at time T_0 while radiation absorbance primarily by amino acids (280-285 nm) can be captured at time T_1 using the same detector **14**. Discussion of filters **12A, 12B** is warranted in this example as the set before the fluorescence detector will be the standard long-pass fluorescence emission filters while the set before the DUV detector will be DUV band pass filters or short-pass fluorescence blocking filters.

In yet another example, laser light is incident at an oblique angle relative to the objective lens optical axis, blocking the unscattered light and allowing dark-field measurement of the scattering profile at higher scattering angles. One example of the use of laser scattering at visible wavelengths may be found in U.S. Patent No. 6,741,730, issued 5/25/2004 to Rahn, entitled "Method and Apparatus for Three-Dimensional Imaging in the Fourier Domain."

In still another example, laser illumination parallel to the optical axis is used. A disk of absorbing material is located in the back focal plane of the objective. The diameter of the absorber is only large enough to block unscattered and very low-angle scattered light. The resulting annular aperture permits a dark-field measurement of the scattering profile at higher scattering angles.

In still another example, live stain, either absorbance or fluorescence, in standard bright-field transmission mode (removing diffraction analysis) or antibody/probe and nanoparticle is used in dark-field illumination mode for molecular specific labeling of proteins and/or DNA in the living cell.

In operation the image reconstruction module **42** determines a size of a voxel in the reconstructed 3D image. The reconstruction module **42** may further include a module constructed in accordance with known software engineering techniques for measuring a concentration of molecules absorbing the radiation by measuring the absorbance per voxel.

In one useful embodiment, the optical tomographic imaging system **11** lends itself nicely to DUV absorbance imaging. Using LEDs at 260 nm and 280 nm with bandwidths of 20 nm allows for simple and robust instrumentation without need for excitation filters. The condenser optics **56** may include, for example, a DUV condenser lens (for example, model UV-Kond, from Zeiss, Germany) and objective lens **18** may comprise a lens such as available from Zeiss, 100x, 1.25 NA, Ultrafluar, or a custom 265 nm objective lens, as available from Optics Technologies, Inc., Rochester, NY. To block the ambient and fluorescent light, filters **12A, 12B** may include a band pass filter with a bandpass from 250 nm to 290 nm as available from Chroma Technology Corp. or Omega Optical, both of Brattleboro, VT, before light reaches the UV sensitive CCD camera. Useful CCD cameras include CCD cameras from Sony Corporation of Japan, the PhotonMax model camera from Princeton Instruments, Trenton, NJ, or devices from Sarnoff Imaging, Princeton, NJ.

Live cell imaging often requires the specimen stage and glycerol, oil, or water-immersion objective lens to be temperature controlled. To convert from 2D DUV imaging to 3D Cell-CT DUV imaging, the materials must be UV transparent for the short transmission distances (path lengths) required for imaging isolated cells in a microcapillary tube of 50 microns in diameter. For example, the cell medium should be a physiological buffer solution that may have higher refractive index to help match to the cell plasma membrane. Additives to the aqueous solution may include, but are

not limited to, polyethylene glycol (PEG), glycerol, modified or derivative PEGs, and agarose gel. When the cell medium cannot be well matched to the glass used for the microcapillary tube, then increasing the inner diameter may help reduce the degree of refraction at the inner tube wall. The refractive index should be able to be matched well with the outer tube wall since no biocompatibility needs to be addressed. However, materials that do not fluoresce within the wavelength range of signal 250 nm - 290 nm should be considered when the rotational joint is being selected.

Referring now to **FIG. 2** an alternate example of system for 3D imaging of cells in an optical tomography system with ultraviolet radiation using a UV camera and optional adaptive optics is schematically shown. The requirement for live cell imaging imposes a restriction on the types of aqueous and physiological buffer solutions and thus on the range of refractive index that can be used around the cell. This embedding medium surrounding the cell and within the tube is expected to have sufficient refractive index mismatch with standard dry or oil immersion microscope objectives to cause aberrations in the resulting images. Compensation for this index mismatch can be designed for a specified imaging depth or distance from objective lens to cell that contains physiological buffer. However, even low-order spherical aberration varies with the variation in axial depth, so dynamic compensation of optical wavefront distortion is advantageous for microscopic imaging across axial depths. This technique of dynamic distortion control or compensation is referred to as adaptive optics. The optical component used for such dynamic aberration compensation is often a spatial light modulator or a deformable membrane mirror. An adaptive reflection mirror is the preferred component in a DUV microscope due to the non-optimal transmission properties of DUV light through sophisticated optical components.

A system for 3D imaging of cells **200** includes several components that are the same as or similar to those described above with respect to **FIG. 1**. As described above, a tube **22** is positioned relative to a microscope objective **18** for viewing an object of interest **1**. As described above, a microscope **16** includes an objective lens **18** and a tube lens element **52**. The microscope objective **18** is aligned along an optical axis **202**. In contrast to the system of **FIG. 1**, only a single ultraviolet (UV) camera **48** is used for acquiring images of the object of interest. The UV camera **48** is also aligned along the optical axis **202**. Interposed between the UV camera **48** and the tube lens element **52** is a fluorescence-blocking filter **50**. As above, the

fluorescence blocking filter **50** is selected to block longer wavelength fluorescence and/or increase separation of differing ultraviolet radiation signals.

The aqueous environment **2** and object of interest **1** may cause a sufficiently large refractive index mismatch between microscope objective **18** and tube **22** and optical gel **32** or equivalent to necessitate the use of an adaptive mirror **54** with associated adaptive optics (AO) controller **201** to reduce depth-dependent image aberrations. This adaptive optics component can be an optional element located between the radiation source **29,** optical elements **27** and condenser lens **24.** Whether unpowered or energized at a constant wavefront compensation (2D) profile, the adaptive mirror **54** becomes a static 90-degree turn in the optical system that may compensate for a single depth level.

As described above, images from the UV camera **48** are transmitted to the image processor **40.** The image processor transmits processed image data to the 3D image reconstruction module **42** which may advantageously be coupled to the optical display **44** for operator viewing if desired. User interface **46** is provided for operator control and information purposes. The user interface **46** may be a GUI interface or the like.

Referring now to **FIG. 3**, an embodiment of a temperature-controlled housing for use in an optical tomography system is schematically shown. A temperature-controlled housing **300** contains an object of interest, such as a biological cell **1**, or other biological material, is contained in a tube, capillary tube, or microcapillary tube **22**, that is positioned relative to a microscope objective **18**. The microcapillary tube **22** is rotatable by a rotary motor **20** to allow controlled rotational motion **21** of the cells **1** within the microcapillary tube **22**. The cell **1** and gel **32** can be advanced within the capillary tube **22** along the horizontal axis by positive pressure applied, for example, by a syringe **80**. Another motor **34** controls vertical axial movement of the microscope objective **18**, and tube lens **52**. The microcapillary tube **22** is encased within optical gel or refractive index matching medium **32** and is part of and atop of the sample-condenser light assembly **56**.

A power amplifier **60** provides energy for the temperature controller **64** that responds to at least one sensor **74** and that may be further regulated with computer and electronic input **78** to maintain the desired temperature within specified ranges, such as 5 to 39 degrees C. However, to maintain functions approaching physiological levels, a warm-blooded animal cell such as a human requires tight

temperature control, i.e. 36 degrees C with range of +/- 0.5 degrees C. Regulation of temperature as well as microfluidic conditions facilitates keeping cells alive (i.e. especially labile normal or abnormal cells, pre-cancerous, cancerous, viral infected; or other pathogenic cell populations). In one example, three sensors **74** are positioned near the microscope head **16** and above and below the microcapillary tube **22**. An optional internal fan **68** for air circulation is present in some embodiments to aid in temperature control. Peltier thermoelectric heaters/coolers **70** may be positioned in throughout the system and may be positioned both above and below the microcapillary tube **22** provide thermal energy for fine temperature control. Additional locations for Peltier heaters/coolers **70** may be advantageous in specific embodiments. Alternatives to thermoelectric heater/coolers and fans are the options of temperature controlled water circulator or equivalents around a chamber that encloses the microscope. In some embodiments temperatures of about 35 degrees C to about 36 degrees C are used, in others higher or lower temperatures may facilitate study of specific biological processes or for use of specific reagents in living cells.

Having described the optical tomography system in detail above, a description of the operation of the system will now be presented in order to aid understanding of the disclosure. Biological objects **1**, such as living cells, are injected into the microcapillary tube **22** via the syringe device **80** where pressurized capillary flow **84** moves the biological objects **1** to a viewing window beneath the objective lens **18** of the microscope **16**. At least one radiation source **29** (e.g. DUV and visible light) is positioned to illuminate a part of the microcapillary tube **22** that includes the biological objects **1**. In some embodiments the radiation wavelengths of about 260 nm to about 280 nm are used. The radiation passes through the light diffuser **28** and condenser lens assembly **24**, **26**, as part of the sample-condenser light assembly **56**. The integrated sensors **74**, temperature controller **64** and fan **68** maintain the temperature to maintain and increase cell viability. The system allows numerous variations to study living cells under defined and controlled conditions.

The optical tomography system described above and elsewhere uses temperature control and microfluidics to maintain suitable conditions such that any living biological material may be examined including, but not limited to, cells from humans, as well as cells from any other species. The cells, or other biological material, flow through one or more tubes (e.g. microcapillary tubes) to facilitate

imaging. In some embodiments the microcapillary tube **22** comprises a straight tube of more than one channel. It is recognized that the optical tomography system may be used to harvest cells or sub-cellular material in certain embodiments.

In some embodiments the system senses radiation including imaging signals emanating from macromolecular complexes, nucleoprotein, DNA, RNA, or protein, comprised in living cells, or in some cases non-living cells, or fragments thereof. Cells comprising component DNA, RNA, and protein complexes may be treated with chemicals, biological agents, including, but not limited to biologically active molecules, nanoparticles, modified nanoparticles, microspheres, protein protocells, antibodies, biomarkers, cytokines, other nucleotides, other proteins, or alternately mechanically manipulated by micromanipulation or other treatments (e.g. transfection reagents, viruses, liposomes, and like agents) to alter or facilitate molecular uptake or affect other cellular processes during the imaging process. Biological or chemical agents may be labeled or modified with chromophores and fluorophores. Embodiments also use nanoparticles that are modified by labeling with gold, colloidal gold, iron, and iron oxide, and like molecules that have absorption, fluorescence, and scattering properties acting as optical contrast mechanisms in the 3D image or diffraction pattern. Use of nanoparticles and microspheres in addition to chromophores and fluorophores allows enhanced 3D contrast. For example, cells could be treated with agents that affect the cell cycle, cellular differentiation, infectivity, reduce or increase pathogenicity, or the cells can be further manipulated to alter sub-cellular compartmentalization. The expression and display of cell surface biomarkers or chromatin or other cellular nucleoprotein or macromolecular complexes could be examined during all or some of these treatments.

In certain embodiments the living cells or other biological material are illuminated with multiple wavelengths of radiation. In such cases, a plurality of pseudoprojection images of the cell, or other biological material that are formed from the computer processing of input images may be processed using ratio imaging techniques. In some embodiments the ratio imaging includes images formed from radiation wavelengths of about 260 nm to about 280 nm.

Alternately, in some cases, live cell staining techniques including, but not limited to fluorescence and laser diffraction may be used to advantage for obtaining images.

Referring now to **FIG. 4**, a side view of an example of a microfluidics cartridge **400** is schematically shown. A rotary motor **20** includes a shaft **121** coupled to turn a belt **188**, where a second end of the belt **188** is coupled to rotate a microcapillary tube **22**. The microfluidics cartridge **400** operates with positive pressure and negative pressure **120** to move the cells in a raceway with a secondary channel **504** to supply nutrients and oxygen, remove metabolic waste, and allow drugs to interact with cells in physiological buffer (as best shown in **FIG. 5**). A bearing or friction fit **92** allows the microcapillary tube **22** to rotate while an object, such as a cell, passes through the tube. A microscope **16** including condenser illumination assembly **56** is positioned proximate the cartridge to view the object along the optical axis of the microscope **16**.

Referring now to **FIG. 5**, a top view of an example of a microfluidics cartridge as used in a racetrack configuration for imaging cells is schematically shown. The microfluidics cartridge **400** is coupled in a fluidic racetrack configuration **500**. The racetrack configuration includes an imaging area **116** along the optical axis of the objective lens including an optical window. Also included is an entrance valve **96**, an exit valve **124** and a first channel **502**. The first channel **502** is in fluid communication with a secondary channel **504**. The channels may be joined, for example, with a semi-permeable membrane **104**. The entire racetrack is maintained in a temperature controlled environment such described herein with respect to **FIG. 3** using Peltier heater/cooler elements or equivalents. In some embodiments the racetrack and channels comprise conduit.

Fresh nutrients, oxygen, buffer (pH, osmolarity, etc), optional drugs and the like as needed to maintain cell viability may be introduced through the secondary channel **504** as indicated by flow arrows **108**. However, if microfluidic conditions are right, then the cells won't move laterally, only axially through the first channel **502** while diffusion allows fresh nutrients such as O_2, buffer materials and metabolic waste to move and thus mix along concentration gradients. In one example, the semi-permeable membrane **104** may be replaced by a joined channel with non-turbulent parallel flows allowing diffusion of small molecules and solutions while maintaining cells within their original streamlines of microfluidic flow. Shear stress within physiological range is possible with slow flow rates while channel geometry, fluid viscosity, temperature, and cell type also play a role.

In operation cells are injected through entrance valve **96** into the microfluidics cartridge **400**. A trough **100** serves as a housing for the rotation motor and belt used to rotate the microcapillary tube **22** while cells travel through the tube. Positive and negative pressure **120** is applied to control pressurized flow **84** throughout the racetrack. After imaging, a an exit valve **124** can be used to direct selected cell **1** by flowing fluid into a discard channel or for harvesting the live cell.

The specimen being examined may be a biopsy from a fine needle aspirate (FNA). The resulting sample of live cells may be split into several different racetracks with separate entrance valves (not shown). Each sub-sample being examined may be exposed to different drugs (such as drug A, drug B, drug combination A+B, and control – no drug), and the response may be monitored as real-time feedback for the purpose of personalized drug response for the patient.

In one example, the racetrack configuration is useful as a research/drug discovery instrument. In operation, live cells may be circulated in the racetrack while imaging in 3D. Each live cell in the sample may be exposed to a chemical and environmental protocol and small changes in cellular response may be indicative of a desired cell type. Variations is apoptosis, mitosis, necrosis, secretion, and other programmed cell responses to stimuli can be measured at high sensitivity in real-time. When the live cells exhibit desired characteristics, the cells may be harvested. One such harvesting method is disclosed in co-pending US patent application to Hayenga, entitled, "Cantilevered coaxial flow injector apparatus and method for sorting particles," and published on 9/20/2007 under publication number US 2007-0215528 A1.

In some alternative embodiments, labeled nanoparticles like antibody/DNA labeling of gold or nanospheres can be used with live cells to label specific proteins, chromatin, and DNA. For example, gold nanoparticles or colloidal gold have both absorption and scattering contrast and are biocompatible with living cells. Fluorescently-labeled nanospheres and microspheres can have absorption, fluorescence, and scattering as optical contrast mechanisms in the 3D image or diffraction pattern. Using nanoparticles in addition to chromophores and fluorophores will allow a third contrast enhancement, which is scattering. A means for imaging the scatter signal as high contrast on a "black" background or field is to illuminate with light that is incident at an angle of incidence beyond that of the imaging objective lens, so only the signal scatter is collected. The image is analogous to that of

fluorescence imaging where the illumination photons are rejected from the final image. Live cell imaging in 2D using dark-field microscopy is being conducted at Duke University, see, for example, Curry, A., Hwang, W.L., and Wax, A. (2006), "Epi-illumination through the microscope objective applied to dark-field imaging and microspectroscopy of nanoparticle interaction with cells in culture," Optics Express 14(14): 6535-6542.

Diffraction pattern measurement is a non-imaging technique that is complementary to the above imaging techniques which measure the spatial pattern in 3D of DNA, chromatin, proteins, and their specific labeling enhancements. Disease specific signatures of diffraction may be found at specific spatial frequencies, which are measured at specific scattering angles from the cell. Since the zero order light from the laser beam is orders of magnitude greater than the weakly scattered light from live cells, the technique of oblique illumination of the cell is proposed to greatly reduce this zero order light from reaching the optical detector or camera. This technique is similar to dark-field microscopy using nanoparticles as discussed above.

Examples of each of the techniques above may also be implemented as combinations using some general concepts described below. However, laboratory implementation will most likely be done as examples of the individual techniques for simplicity and lack of confounding variables during the development stage of live cell 3D imaging. Some examples of combining multiple imaging and measurement techniques are presented below.

Referring now to **FIG. 6**, an optical tomography process including separate imaging stages along the same pathway is shown. Separate imaging stages may be processed along the same pathway, such as a single microcapillary tube. For example, visible light diffraction analysis and cell counting **602** may be done at a first stage **611**, followed by visible light imaging **604** at a second stage **612.** In the case of imaging using live stains, 280 nm absorption imaging **606** may be conducted at a third stage **613**, followed by 260 nm absorption imaging **608** at a fourth stage **614**. For this example embodiment the cell should be aligned within the limited field of view at each stage as the cell continuously moves down a single rotating capillary tube. The 280 nm absorption imaging includes illuminating the object **1** with DUV light at a first wavelength in the range of about 275 nm to 285 nm. The 260 nm

absorption imaging includes illuminating the object **1** DUV light at a second wavelength in the range of about 255 nm to 265 nm.

In another example, a single imaging stage that combines one or more image contrast mechanisms, such as absorption at wavelengths of 260 nm and 280 nm, measuring DUV absorption and native fluorescence, or measuring absorption at more than two visible wavelengths for one or more live stains. The components for combining optical imaging techniques can use multiple optical components for beam splitting and combining (dichroic or polarization beamsplitters) and possibly multiple cameras. Alternatively, a single camera and detection pathway can be used if the multiple excitation light sources are pulsed in time series or filter wheels or actual sources are physically moved or shuttered in time series. The single stage for imaging and measurement allow for stopped flow axial transport of the cells for precise alignment with the field of view.

In yet another example, dark-field imaging of live-cell stain with nanoparticle scatterers may advantageously be combined with oblique illumination of the cell with a laser for diffraction pattern analysis. This technique may be run at higher speeds and may be an initial stage before the slower and subsequent 3D imaging stage if initial results warrant a detailed 3D image of a particular cell.

In operation, the system provides an optical tomography process including separate imaging stages along the same pathway. A plurality of biological objects is transported along a pathway **25** to the first stage **611**. At least one object of the plurality of objects is illuminated with visible light at the first stage to produce a diffraction pattern and the diffraction pattern is sensed by a light sensor. Using a computer program or equivalent, the diffraction pattern is analyzed to produce a diffraction analysis. At the second stage **612** the at least one object **1** is illuminated with visible light and the visible light emanating from the at least one object is sensed to produce a first plurality of pseudoprojection images. At the third stage **613** the at least one object **1** is illuminated with DUV light at a first wavelength and the DUV light at a first wavelength emanating from the at least one object is sensed to produce a second plurality of pseudoprojection images. At the fourth stage the at least one object is illuminated with DUV light at a second wavelength that is sensed to produce a third plurality of pseudoprojection images. Based on features derived from the first, second and third pluralities of pseudoprojection images and the diffraction analysis a plurality of objects may be sorted or otherwise classified using a

sorter **610**. The sorter **610** may be any of many types of conventional classifiers, usually embodied in software residing in a computer such as a statistical sorter, adaptive classifier, neural network or equivalents.

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 scope of the present invention.

CLAIMS:

1. An optical tomography system for acquiring 3D images comprising:

a tube for containing a biological object;

a rotary motor for rotating a biological object relative to a microscope objective;

a single optical illumination source for illuminating the entire biological object;

a beamsplitter positioned to split radiation transmitted through the biological object into at least two selected wavelengths;

a microscope objective lens having a depth of field, the objective lens being located to receive light emanating through the object;

an axial translation mechanism coupled to translate the microscope objective lens for axially scanning the entire biological object with the microscope objective lens through the thickness of the biological object;

a first ultraviolet camera for sensing a first radiation bandwidth transmitted through the entire biological object and the microscope objective lens during axially scanning, the first ultraviolet camera positioned to separately receive one of the at least two selected wavelengths from the beamsplitter, wherein the first radiation bandwidth has a first spectral range between 150 nm and 390 nm to produce a first plurality of pseudo-projection images of the biological object onto the first ultraviolet camera, and a second ultraviolet camera positioned to separately receive a different one of the at least two selected wavelengths from the beamsplitter, for sensing a second radiation bandwidth within a second spectral range which is also transmitted through the entire biological object and the microscope objective to the second ultraviolet camera during axially scanning, wherein the second spectral

range is also between 150 nm and 390 nm, but different than the first spectral range, to produce a second plurality of pseudo-projection images of the biological object onto the second ultraviolet camera;

an image processor coupled to receive data from the first and second ultraviolet cameras; and

a reconstruction module coupled to the image processor for reconstructing the plurality of pseudoprojection images to form a reconstructed 3D image.

2. The system of claim 1 wherein the axial translation mechanism comprises a piezoelectric transducer.

3. The system of claim 2 wherein a computer is linked to control the piezoelectric transducer, where the piezoelectric transducer axially moves the microscope objective lens so as to extend the depth of field of the microscope objective lens.

4. The system of claim 1 wherein the single optical illumination source comprises a computer-controlled light source and condenser lens assembly.

5. The system of claim 1 wherein the tube comprises a microcapillary tube.

6. The system of claim 1 wherein the first and second spectral ranges have wavelengths further limited to between 240 nm and 300 nm.

7. The system of claim 1 wherein the first and second spectral ranges have wavelengths further limited to between 260 nm and 265 nm.

8. The system of claim 1 wherein the first and second spectral ranges have wavelengths further limited to between 280 nm and 285 nm.

9. The system of claim 1 wherein the biological object is selected from the group consisting of a cell, cell parts, chromosomes, a live cell, a fixed cell, an unfixed cell, a frozen cell, a thawed cell, a desiccated cell, a cloned cell, a cell nucleus, an organelle, a mobile cell, an immobilized cell, DNA, and protein.

10. The system of claim 1, wherein illumination from the single optical illumination source stimulates native fluorescence from the biological object.

11. The system of claim 1, wherein a size of a voxel in the reconstructed 3D image is known, further comprising means for measuring a concentration of molecules absorbing the radiation by measuring the absorbance per voxel.

12. The system of claim 1 wherein the biological object comprises a live cell.

13. The system of claim 1 wherein the first and second ultraviolet cameras sense radiation including imaging signals emanating from DNA.

14. The system of claim 1 wherein the first and second ultraviolet cameras sense radiation including imaging signals emanating from protein.

15. The system of claim 1 wherein the first and second ultraviolet cameras sense radiation including imaging signals emanating from hydrophilic surfaces.

16. The system of claim 1 wherein the single optical illumination source generates light having multiple wavelengths.

17. The system of claim 1 wherein the reconstruction module includes a ratio imaging process.

18. The system of claim 17 wherein the ratio imaging process includes images formed from wavelengths ranging from 260 nm to 280 nm.

19. The system of claim 1 wherein at least one of the first and second ultraviolet cameras comprises an ultraviolet pixel array detector.

20. The system of claim 1 wherein the first and second spectral ranges are limited to wavelengths selected to enhance natural radiation absorbance by protein.

21. The system of claim 1 wherein the first and second spectral ranges are selected to enhance natural radiation absorbance by DNA.

22. The system of claim 1 wherein the first ultraviolet camera is sensitive to radiation having a wavelength matching the natural absorbance of human DNA.

23. The system of claim 1 wherein the second ultraviolet camera is sensitive to radiation having a wavelength matching the natural absorbance of protein.

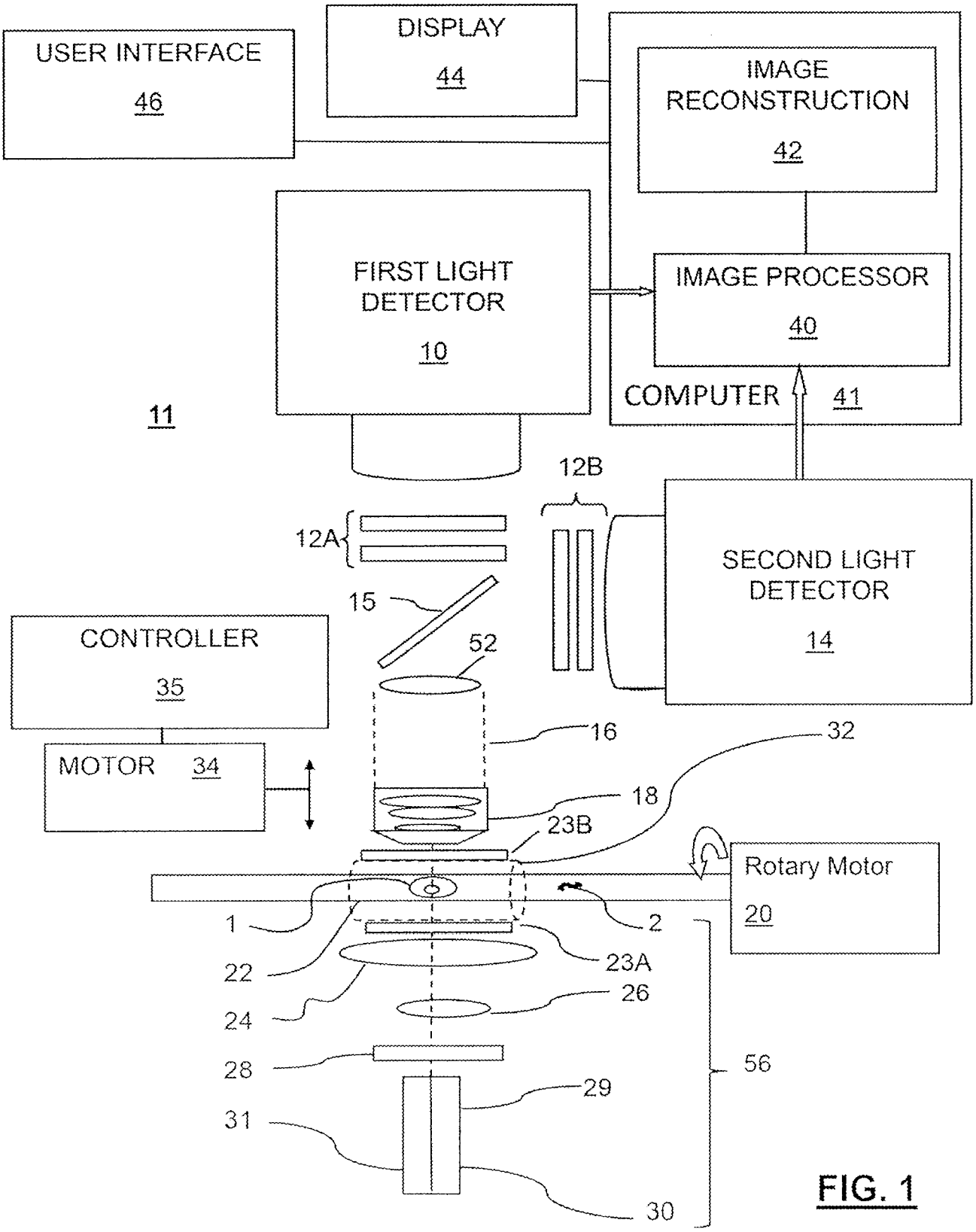
24. The system of claim 1 wherein the first ultraviolet camera is sensitive to radiation having a wavelength that includes imaging signals emanating from hydrophilic surfaces.

25. The system of claim 1 wherein the beamsplitter is selected from the group consisting of a polarizing beam splitter, a Wollaston prism, a birefringent element, a half-silvered mirror, a 50/50 intensity beamsplitter, a dielectric optically coated mirror, a pellicle film and a dichroic mirrored prism.

26. The system of claim 1 further comprising an adaptive mirror positioned to direct the radiation to the microscope objective.

27. The system of claim 26 further comprising coupling the adaptive mirror to an adaptive optics controller.

28. The system of claim 26 wherein the adaptive mirror is selected from the group consisting of an unpowered adaptive mirror and an adaptive mirror energized at a constant wavefront compensation profile.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FIRST LIGHT DETECTOR
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COMPUTER
41
11
12B
12A
SECOND LIGHT DETECTOR
14
15
CONTROLLER
35
52
MOTOR
34
16
32
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23B
Rotary Motor
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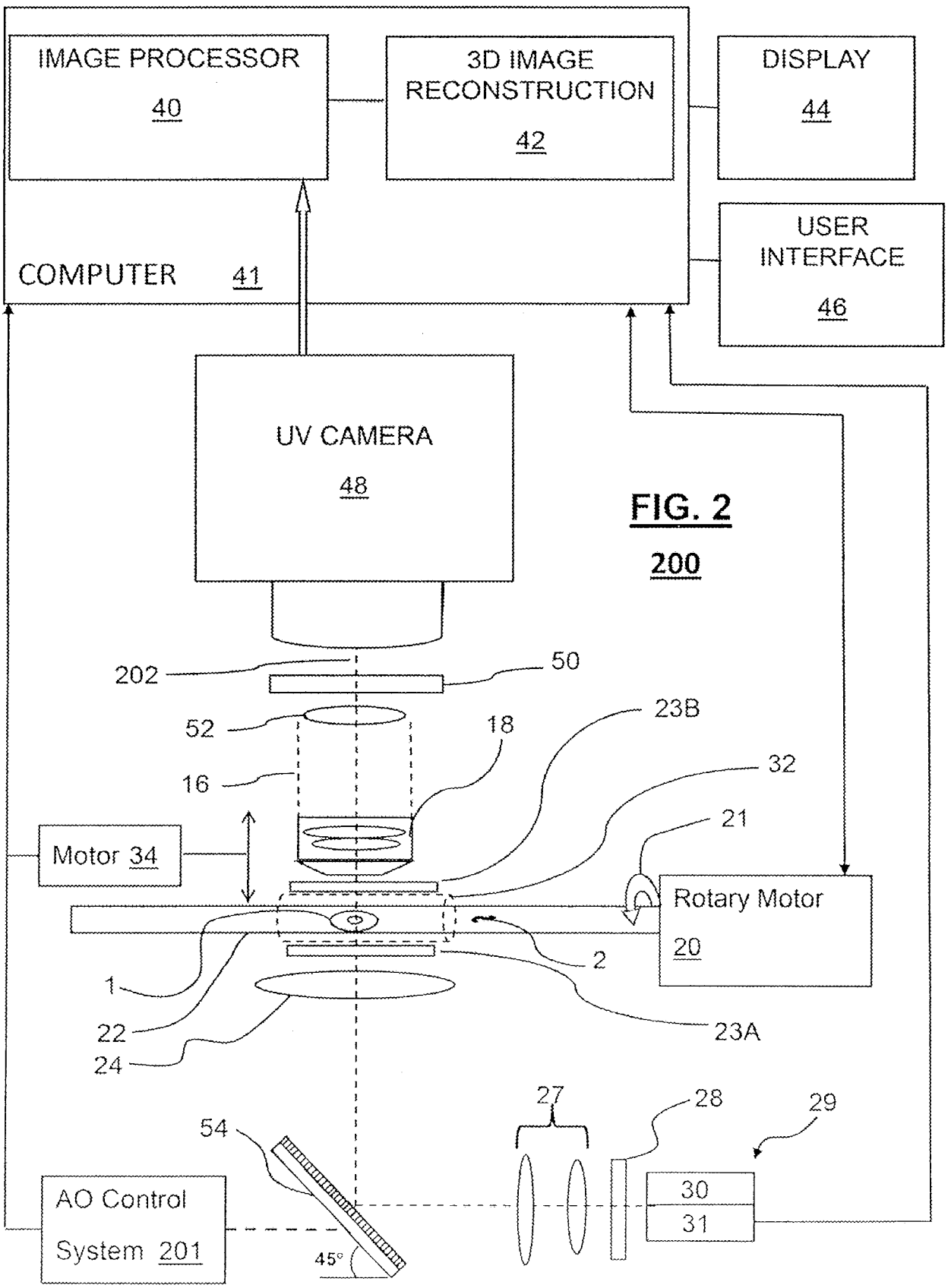
2
22
23A
24
26
28
56
29
31
30

FIG. 1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52
18
23B
16
32
21
Motor 34
Rotary Motor
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22
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23A
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28
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54
AO Control System 201
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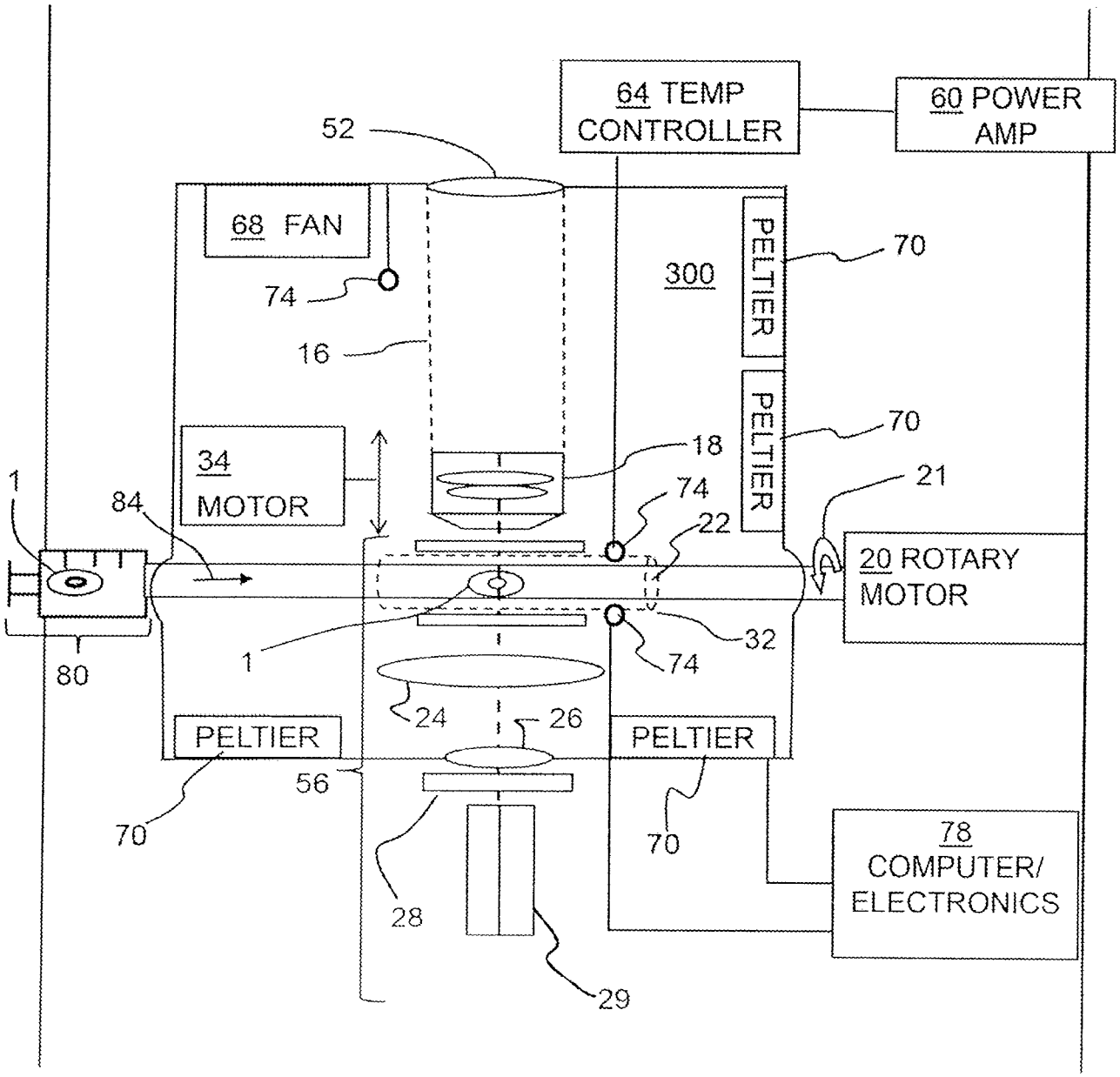
31
45°

FIG. 2

200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21
22
1
84
20 ROTARY MOTOR
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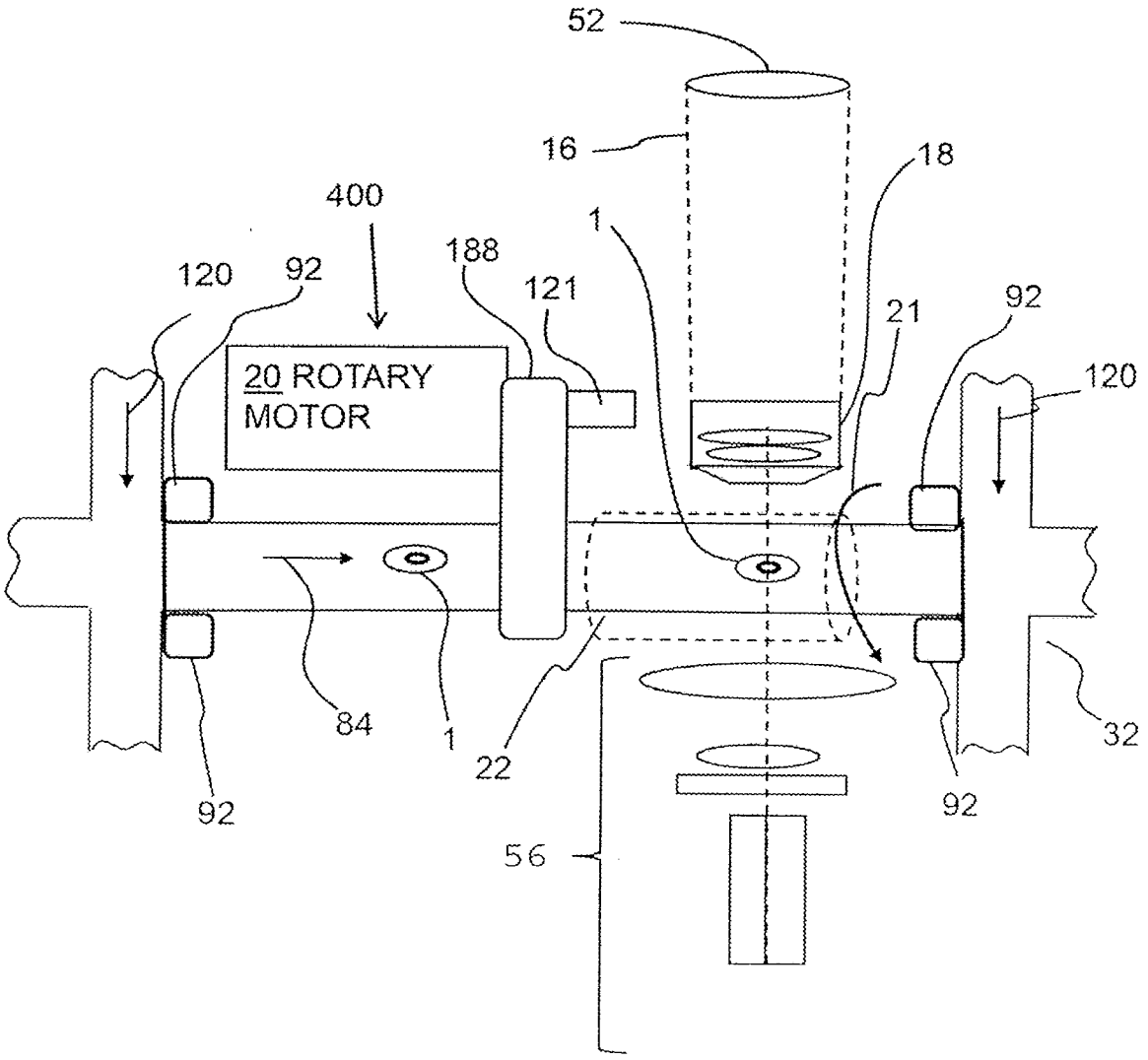
28
29

FIG. 3


52
16
18
400
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121
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20 ROTARY MOTOR
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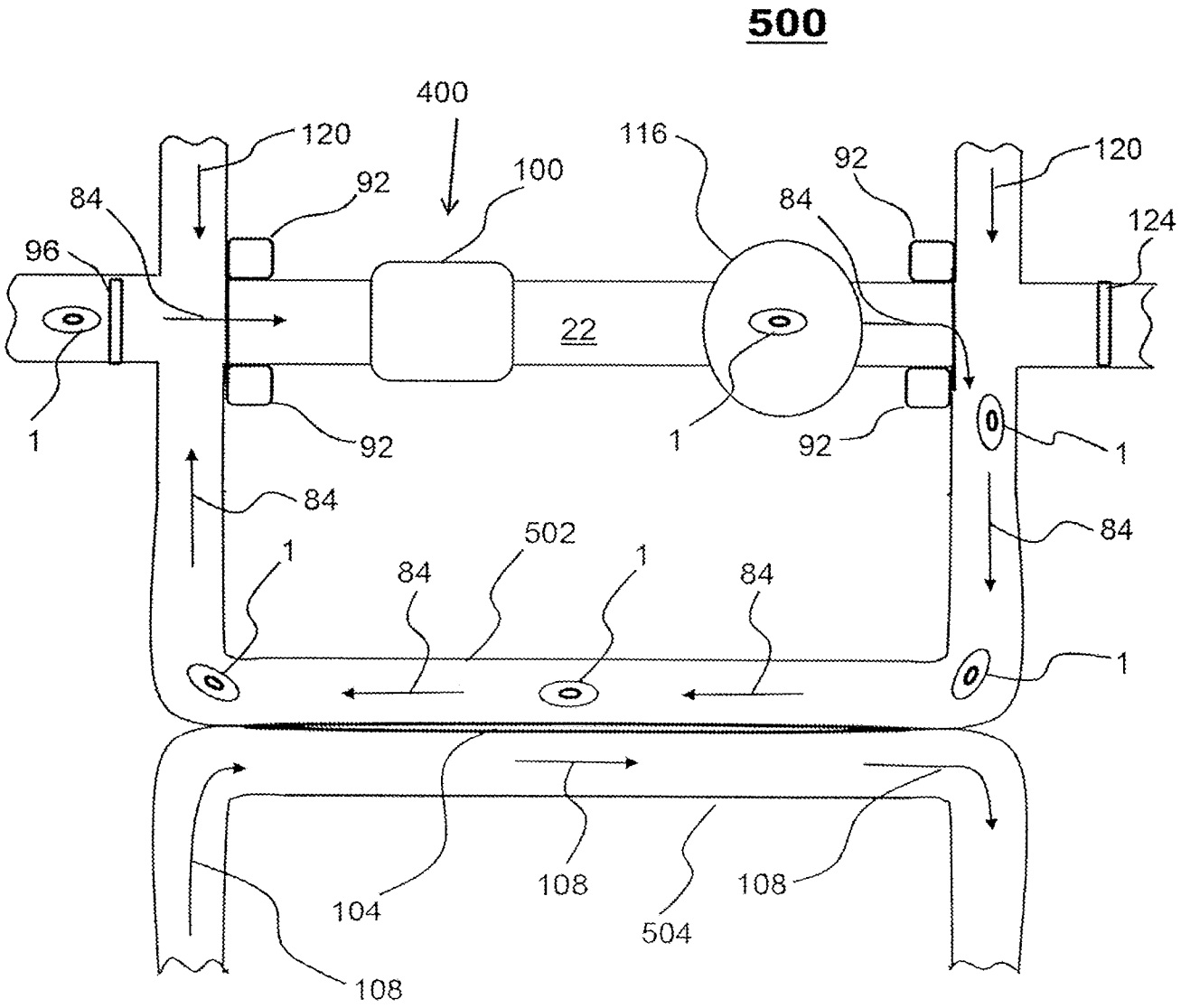
92
32
56

FIG. 4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400
120
92
100
116
92
120
84
84
124
96
22
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92
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84
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1
84
1
104
108
504
108
108

FIG. 5

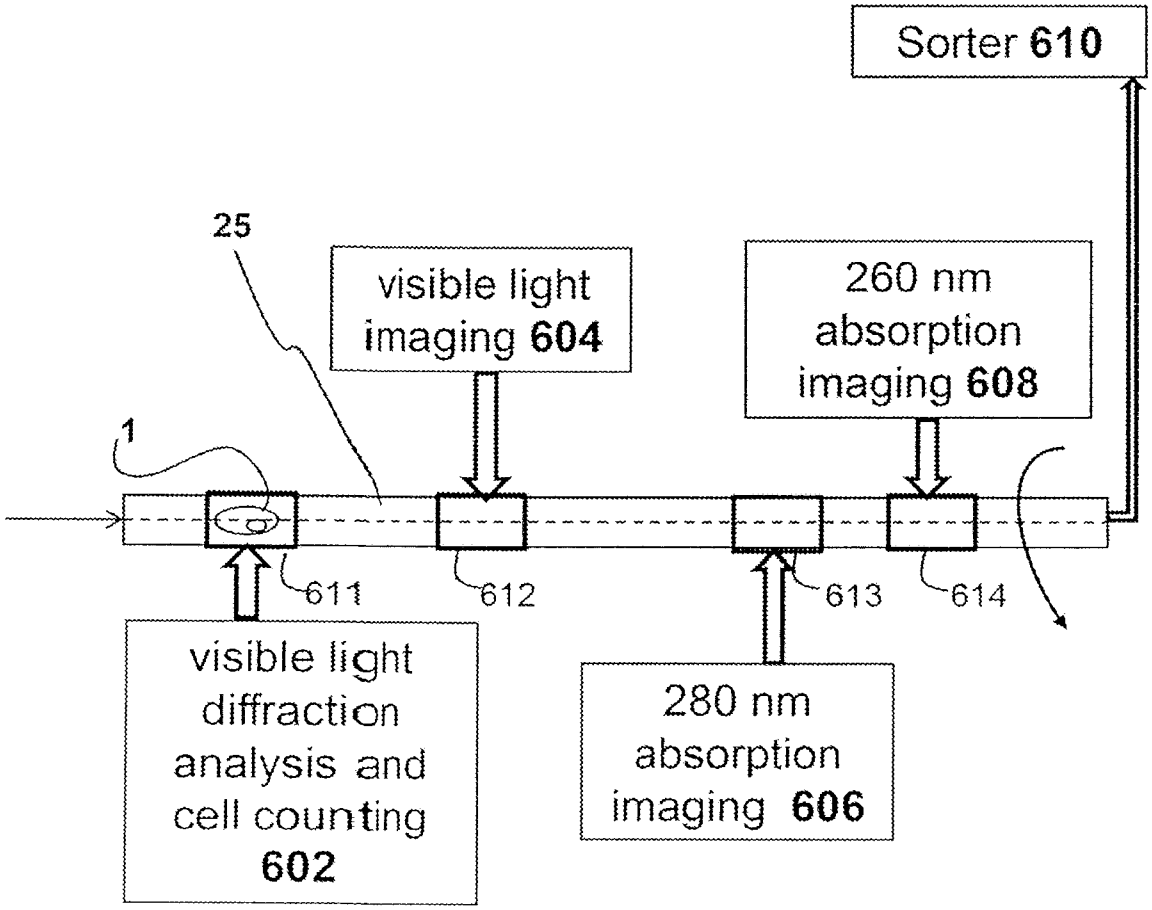
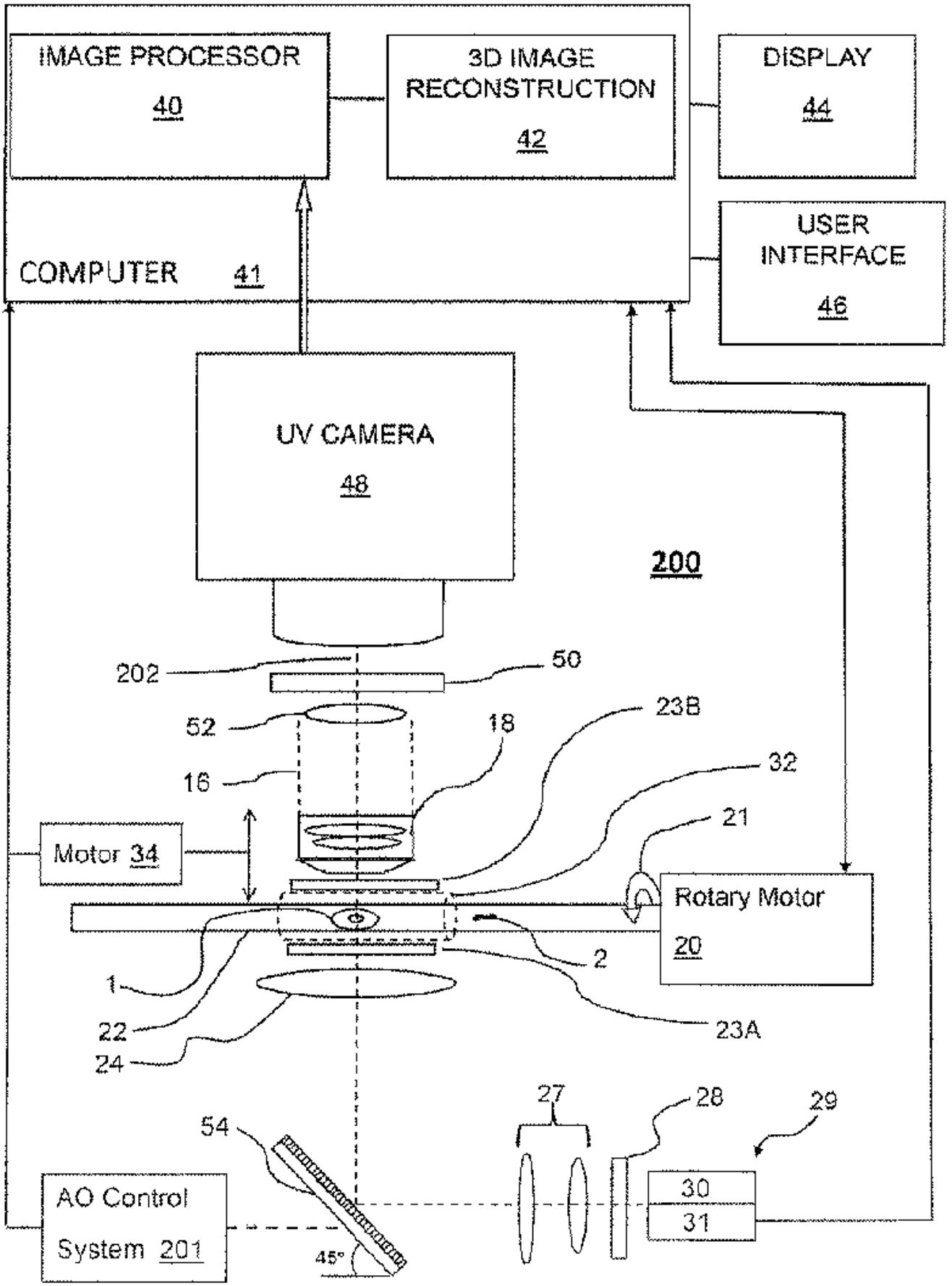

Sorter 610
25
visible light imaging 604
260 nm absorption imaging 608
1
611
612
613
614
visible light diffraction analysis and cell counting 602
280 nm absorption imaging 606

FIG. 6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DISPLAY
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USER INTERFACE
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UV CAMERA
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18
23B
16
32
21
Motor 34
Rotary Motor
20
2
1
23A
22
24
27
28
29
54
30
31
AO Control System 201
45°