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(54) Title: METHOD AND SYSTEM FOR TREATING BIOLOGICAL SAMPLES

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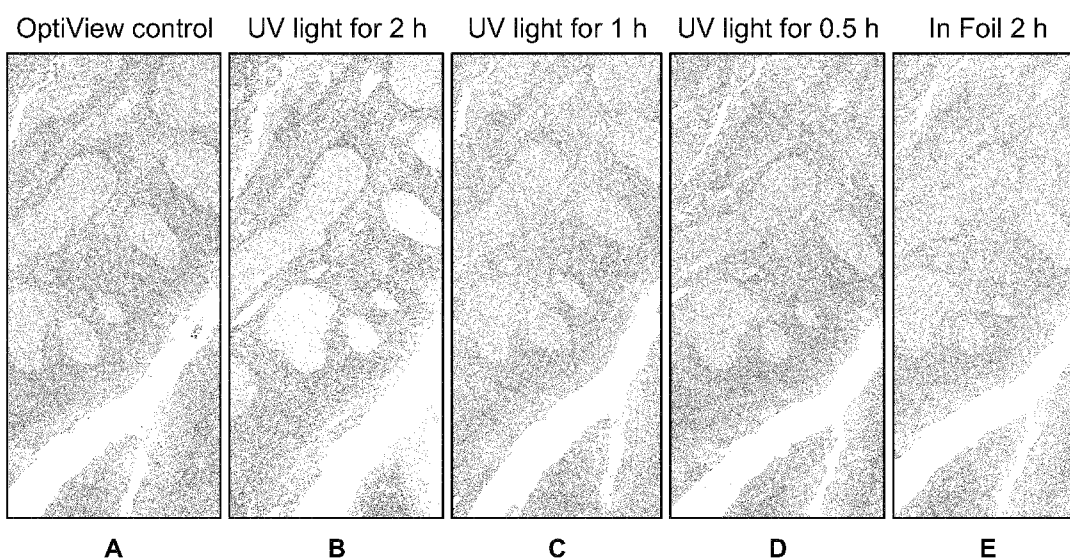


FIG. 2

(57) Abstract: Disclosed are methods and systems for unmasking protein antigens and nucleic acid targets in fixed cell samples using UV light. In some embodiments, UV light exposure is combined with other unmasking methods and agents to help ensure difficult to unmask molecular entities are reliably rendered detectable.

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
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METHOD AND SYSTEM FOR TREATING BIOLOGICAL SAMPLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to and the benefit of the filing date of U.S. Provisional Patent Application No. 62/428,977, which was filed on December 1, 2016, the disclosure of which is hereby incorporated herein by reference in its entirety.

FIELD

[0002] The present disclosure relates to a method and system for unmasking protein antigens and nucleic acid targets from fixed biological samples, and more particularly, to a method and system for unmasking antigens and targets in fixed biological samples using ultraviolet light.

BACKGROUND

[0003] Fixation of tissue and cell samples is used to help ensure that the morphology of the sample and the spatial distribution of biomolecules is preserved and thus used for the basis of a diagnosis by a pathologist. As an adjunct to morphological indicators of disease, the presence of particular proteins and/or nucleic acid sequences can be used to further characterize a disease state and, in some instances, is used by an oncologist to direct therapy of the disease. However, fixation can also interfere with the detection of proteins and nucleic acids in a sample. As such, for immunohistochemical (IHC) procedures and *in-situ* hybridization (ISH) procedures, an unmasking step (also known as "antigen retrieval" or "target retrieval" for IHC and ISH, respectively) is often used make protein antigens or nucleic acid targets accessible to detection reagents such as antibodies or probes.

[0004] Fixation of samples is routinely accomplished using neutral-buffered formalin (NBF). It is believed that the formaldehyde in NBF preserves tissue and cell morphology by forming cross-links between reactive groups on proteins and nucleic acids in the sample, and these cross-links can lead to certain portions of the molecules being rendered undetectable. A variety of methods are used to reverse the effects of formalin fixation and provide access to antigens and

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targets in fixed biological samples. None of these methods, however, is completely successful in all instances.

SUMMARY

5 **[0005]** The present disclosure provides methods and systems that facilitate the detection of protein and/or nucleic acid targets in a sample.

10 **[0006]** In one aspect of the present disclosure is a method for preparing a fixed cell sample for analysis, the method including contacting the sample with a retrieval agent and exposing the fixed cell sample to electromagnetic radiation, the electromagnetic radiation having a wavelength of between about 10 nm and about 400 nm (UV radiation). In some embodiments, the electromagnetic radiation has a wavelength of between about 200 nm and about 400 nm. In some embodiments, the electromagnetic radiation has a wavelength of between about 250 nm and about 400nm. In some embodiments, the electromagnetic radiation includes one or more of UVA radiation (having a wavelength ranging from about 315nm to about 400 nm), UVB radiation (having a wavelength ranging from about 280nm to about 315 nm), and UVC radiation (having a wavelength ranging from about 100nm to about 280 nm).

20 **[0007]** In some embodiments, the retrieval agent is an antigen retrieval agent which includes one or more of water, a buffer, an enzyme, a chaotropic reagent, a chelating agent, a nucleophile, an oxidizing agent, an organic acid/base pair, an electron-deficient compound such as a Lewis acid, and a surfactant. In some embodiments, the antigen retrieval agent includes at least two of water, a buffer, an enzyme, a chaotropic reagent, a chelating agent, a nucleophile, an oxidizing agent, an organic acid/base pair, an electron-deficient compound such as a Lewis acid, and a surfactant. In some embodiments, the antigen retrieval agent includes at least three of water, a buffer, an enzyme, a chaotropic reagent, a chelating agent, a nucleophile, an oxidizing agent, an organic acid/base pair, an electron-deficient compound such as a Lewis acid, and a surfactant. In some

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embodiments, the antigen retrieval agent includes a buffer (e.g. TRIS) and has a pH ranging from about 7 to about 9.

5 [0008] In some embodiments, the method includes exposing the sample to one or more of heat, microwaves and ultrasound. In some embodiments, the sample is heated to between about 60°C and about 150°C. In some embodiments, when the sample is heating, it can be held under pressure to minimize evaporation of the antigen retrieval agent or mitigate or prevent the boiling of the antigen retrieval agent.

10 [0009] In another aspect of the present disclosure is an automated system for treating a fixed cell sample mounted on a substrate, the system including a substrate holder configured to hold the substrate, and a reagent dispenser configured to dispense a retrieval agent, e.g. an antigen retrieval agent, onto the fixed cell sample. In some embodiments, the system includes an electromagnetic radiation source configured to direct UV electromagnetic radiation toward the fixed cell sample. In some embodiments, UV electromagnetic radiation directed toward 15 the fixed cell sample has wavelength of between about 200 nm and about 400 nm. In some embodiments, the system also includes a controller configured to control the dispenser and the electromagnetic radiation source to deliver the retrieval agent and direct the electromagnetic radiation (at a suitable wavelength or range of 20 wavelengths) to the fixed cell sample mounted on the substrate. In some embodiments, the controller directs the operation of the dispenser and/or radiation source to operate according to a predetermined sequence and/or for a predetermined amount of time. In some embodiments, the controller optionally enables the direction of electromagnetic radiation toward the sample at a 25 predetermined intensity or at a predetermined radiation flux. In some embodiments, the substrate holder includes a heating element. In some embodiments, the substrate holder is enclosed in a pressurizable chamber. In some embodiments, the system includes a source of microwave radiation that can be used to heat the sample and/or the retrieval agent. In some embodiments, the microwave 30 radiation employed has a wavelength ranging from between about 7.5 cm to about

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15 cm. In some embodiments, the substrate is glass. In some embodiments, the substrate is a microscope slide.

[0010] In another aspect of the present disclosure is an apparatus for the treatment of a fixed cell sample, the apparatus including a pressurizable chamber, a removable rack for mounting one or more substrates holding one or more fixed cell samples, and a UV light source configured to operate within the pressurizable chamber and direct UV light onto the one or more fixed cell samples. In a particular embodiment, a central UV light source is surrounded by a sample rack that fits around the periphery of the pressurizable chamber.

10 BRIEF DESCRIPTION OF THE FIGURES

[0011] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings(s) will be provided by the Office upon request and payment of the necessary fee.

15 [0012] Further features and advantages of disclosed system and method will become apparent in view of the Detailed Description that follows, and through reference to the accompanying figures in which:

[0013] FIG. 1 is a set of images showing the UV reactor apparatus used to expose the tissue samples shown in FIGS. 1 and 2 to UV light, where panel A is the outside of the UV reactor apparatus, panel B is a view from the top of the reactor showing the UV light sources arranged around the periphery, and panel C shows the tray in which slides were held at the bottom of the reactor apparatus.

20 [0014] FIG. 2 is set of micrographs illustrating the effect of ultraviolet treatment according to a disclosed method on FFPET tonsil tissue samples stained for the protein BCL-2, where panel A shows a positive control sample obtained by conventional heat-induced antigen retrieval using a citrate buffer solution; panel B shows the results for 2 hours of UV light exposure combined with contacting the sample with citrate buffer; panel C shows the results for 1 hour of UV light

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exposure; panel D shows the results for 30 minutes of UV light exposure; and panel E shows a control sample that was covered with foil and placed alongside the UV light exposed slides for the maximum exposure of 2 hours.

5 [0015] FIG. 3 is a set of micrographs at a higher magnification that correspond to the micrographs of FIG. 2, where panel A shows a positive control sample obtained by conventional heat-induced antigen retrieval using a citrate buffer solution; panel B shows the results for 2 hours of UV light exposure combined with contacting the sample with citrate buffer; panel C shows the results for 1 hour of UV light exposure; panel D shows the results for 30 minutes of UV
10 light exposure; and panel E shows a control sample that was covered with foil and placed alongside the UV light exposed slides for the maximum exposure of 2 hours.

[0016] FIG. 4 is a schematic of an embodiment of the disclosed automated system for treating biological samples on microscope slides.

15 [0017] FIG. 5 is a top-view schematic showing an embodiment of the disclosed apparatus for treatment of a fixed cell sample according to a disclosed method.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

20 [0018] As used herein, the singular terms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise.

[0019] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list
25 of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list

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of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0020] The terms "comprising," "including," "having," and the like are used interchangeably and have the same meaning. Similarly, "comprises," "includes," "has," and the like are used interchangeably and have the same meaning. Specifically, each of the terms is defined consistent with the common United States patent law definition of "comprising" and is therefore interpreted to be an open term meaning "at least the following," and is also interpreted not to exclude additional features, limitations, aspects, etc. Thus, for example, "a device having components a, b, and c" means that the device includes at least components a, b and c. Similarly, the phrase: "a method involving steps a, b, and c" means that the method includes at least steps a, b, and c. Moreover, while the steps and processes may be outlined herein in a particular order, the skilled artisan will recognize that the ordering steps and processes may vary unless a particular order is clearly indicated by the context.

[0021] As used herein, the term "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values (e.g., $\pm 5\text{-}10\%$ of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

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[0022] As used herein, the term "substantially" means the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. In some embodiments, "substantially" means within about 20%. In some embodiments, "substantially" means within about 15%. In some
5 embodiments, "substantially" means within about 10%. In some embodiments, "substantially" means within about 5%.

[0023] As used herein, the term "antigen" refers to a compound, composition, or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell
10 receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (e.g., oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (e.g., polysaccharides), phospholipids, nucleic acids and proteins.

[0024] As used herein the term "biological sample" refers to any sample
15 including a biomolecule (such as a protein, a peptide, a nucleic acid, a lipid, a carbohydrate, or a combination thereof) that is obtained from any organism including viruses. Other examples of organisms include mammals (such as humans; veterinary animals like cats, dogs, horses, cattle, and swine; and laboratory animals like mice, rats and primates), insects, annelids, arachnids,
20 marsupials, reptiles, amphibians, bacteria, and fungi. Biological samples include tissue samples (such as tissue sections and needle biopsies of tissue), cell samples (such as cytological smears such as Pap smears or blood smears or samples of cells obtained by microdissection), or cell fractions, fragments or organelles (such as obtained by lysing cells and separating their components by centrifugation or
25 otherwise). Other examples of biological samples include blood, serum, urine, semen, fecal matter, cerebrospinal fluid, interstitial fluid, mucous, tears, sweat, pus, biopsied tissue (for example, obtained by a surgical biopsy or a needle biopsy), nipple aspirates, cerumen, milk, vaginal fluid, saliva, swabs (such as buccal swabs), or any material containing biomolecules that is derived from a first biological
30 sample. In certain embodiments, the term "biological sample" as used herein refers

to a sample (such as a homogenized or liquefied sample) prepared from a tumor or a portion thereof obtained from a subject.

[0025] As used herein, the term "cell sample" refers to a biological sample comprising cells or a morphological portion thereof, which cells can be individual cells or can be arranged together in a tissue structure. In one embodiment, the cell sample is a collection of cells (such as blood or cervical cells) placed onto a microscope slide. In another embodiment, the cell sample is a frozen tissue section placed on a microscope slide. In yet another embodiment, the cell sample is a formalin-fixed paraffin-embedded tissue sample (FFPET sample), prepared by first fixing a collection of cells or a piece of tissue using formalin, which is then processed using known techniques to dehydrate the sample, clear the sample and impregnate the sample in a block of paraffin (or other materials) such that it can be sliced into thin sections that are placed onto a microscope slide for treatment and analysis.

[0026] As used herein the term "fixed cell sample" refers to a biological sample that has been treated to help preserve the morphological and/or biochemical features of the cells as they existed before the biological sample was obtained from the organism or alternatively before the sample was obtained from a culture of material originally obtained from a biological sample (e.g. a culture of bacteria present in mucus or pus). A fixed cell sample can, for example, be prepared by contacting the sample with an aldehyde fixative such as formaldehyde or glutaraldehyde. A fixed cell sample can be prepared by contacting the sample with an alcohol-based fixative such as one including methanol, ethanol or acetic acid. Alternatively, a fixed cell sample can be prepared by contacting the sample with an oxidizing agent such as osmium tetroxide or potassium permanganate, or a fixed cell sample can be prepared using a metallic based fixative such as mercuric chloride or picric acid. In a particular embodiment, a fixed cell sample is prepared by contacting a biological sample comprising cells with a neutral buffered formalin (NBF) solution, which is typically used as a solution that is about 3.7% formalin (10% formaldehyde and 1% methanol).

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[0027] As used herein, the term "slide" refers to any substrate (e.g., substrates made, in whole or in part, glass, quartz, plastic, silicon, etc.) of any suitable dimensions on which a biological specimen is placed for analysis, and more particularly to a "microscope slide" such as a standard 3 inch (7.62 cm) by 1
5 inch (2.54 cm) microscope slide or a standard 75 mm by 25 mm microscope slide. Examples of biological specimens that can be placed on a slide include, without limitation, a cytological smear, a thin tissue section (such as from a biopsy), and an array of biological specimens, for example a tissue array, a cellular array, a DNA array, an RNA array, a protein array, or any combination thereof.

10 [0028] As used herein, the term "target" refers to any molecule for which the presence, location and/or concentration is or can be determined. Examples of target molecules include proteins, epitopes, nucleic acid sequences, and haptens, such as haptens covalently bonded to proteins. Target molecules are typically detected using one or more conjugates of a specific binding molecule and a
15 detectable label.

[0029] As used herein, the terms "unmask" or "unmasking" refer to retrieving antigens and/or improving the detection of amino acids, peptides, and proteins in a fixed tissue.

[0030] In one embodiment, is a method of preparing a fixed cell sample for
20 analysis. In some embodiments, the method includes contacting the sample with a retrieval agent and exposing the sample to UV radiation. In some embodiments, the UV radiation is electromagnetic radiation having a wavelength of between about 10 nm and about 400 nm. In other embodiments, the UV radiation is electromagnetic radiation having a wavelength of between about 200 nm and about
25 400 nm. In other embodiments, the UV radiation is electromagnetic radiation having a wavelength of between about 250 nm and about 400 nm. In some embodiments, the UV radiation is applied to the fixed cell sample for a predetermined amount of time. In some embodiments, the predetermined amount of time is a time period that is sufficient to unmask at least one antigen that was
30 masked during the fixation process. In some embodiments, the predetermined time

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can be established empirically by comparing a staining pattern for the at least one antigen (or target) before and after the exposure to the UV radiation. The staining pattern may be determined using a computer system, such as one coupled to an imaging device, the computer system comprising one or more processors and a memory, such as a non-transitory memory.

[0031] In some embodiments, UV radiation is introduced for a time period of about 2 hours or less. In some embodiments, UV radiation is introduced for a time period ranging from between about 1 minute to about 1 hour. In some embodiments, UV radiation is introduced for a time period ranging from between about 1 minute to about 45 minutes. In other embodiments, UV radiation is introduced for a time period ranging from between about 1 minute to about 30 minutes. In other embodiments, UV radiation is introduced for a time period ranging from between about 2 minutes to about 30 minutes. In other embodiments, UV radiation is introduced for a time period ranging from between about 3 minutes to about 30 minutes. In other embodiments, UV radiation is introduced for a time period ranging from between about 2 minutes to about 15 minutes. In other embodiments, UV radiation is introduced for a time period ranging from between about 5 minutes to about 20 minutes. In other embodiments, UV radiation is introduced for a time period ranging from between about 5 minutes to about 15 minutes. In some embodiments, the time for UV irradiation depends on the frequency of the UV radiation, the angle of incidence from the UV radiation source to the sample, and/or the proximity of the UV radiation source to the sample.

[0032] In some embodiments, the UV radiation is introduced in pulses, i.e. where the UV radiation source is "on" for a period of time, followed by a period of time where the UV radiation source is "off." For example, the UV radiation source may be turned "on" for a period of time ranging from between about 0.5 seconds and about 60 seconds, followed by a period of time in which the UV radiation source is turned "off" for a period of time ranging from between about 0.5 seconds to about 60 seconds.

[0033] In some embodiments, the retrieval agent comprises water and/or an aqueous buffer solution. In some embodiments, the sample is also heated, such as to a temperature ranging from between about 30°C to about 150°C. In some embodiments, the sample is heated for a predetermined amount of time (e.g. 10
5 seconds, 30 seconds, 60 seconds, etc.). In some embodiments, the predetermined time of heating can coincide with a predetermined time of exposure to UV radiation, or could be shorter or longer time period, and can itself be established empirically by comparing a staining pattern for at least one antigen (or target) before and after the combination of UV treatment and heat exposure of the sample.
10 Procedures for heating, such as by microwave heating, are described by Shan-Rong Shi, Antigen Retrieval Immunohistochemistry: Review and Future Prospects in Research and Diagnosis over Two Decades, Journal of Histochemistry & Cytochemistry 59(1) 13–32, the disclosure of which is hereby incorporated by reference herein in its entirety. Another procedure for heat-induced antigen
15 retrieval is described in US Patent No. 9,506,928, the disclosure of which is hereby incorporated by reference herein in its entirety. For example, the '928 Patent describes heating from about 70°C to about 95°C for about 30 minutes to facilitate the unmasking or retrieval of chemical epitopes.

[0034] Another procedure for heat-induced antigen retrieval procedure was
20 disclosed by Ding et al., "Water Bath and Pressure Cooker Antigen Retrieval in Immunohistochemistry: A Comparative Study," Appl Immunohistochem Mol Morphol, Volume 22, Number 3, March 2014. There, the authors disclosed placing dewaxed and rehydrated slides into heat-resistant plastic slide racks and where the racks were immersed in a glass or stainless-steel breaker filled with 300mL of an
25 antigen retrieval solution (e.g. buffer (0.01M citrate buffer, pH 6.0)). The breaker was then placed in a pressure cooker filled with 1500mL of distilled water or antigen retrieval solution. The cooker was finally placed on a conventional electric stove (400 or 1200 W) or an induction cooker (rated at 1200 W), covered with an unlocked lid, and heated to a boil. The slides were immersed in the boiling solution
30 and reheated to a boil.

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[0035] In other embodiments, the fixed cell sample can be exposed to UV radiation and also be exposed to microwave radiation. In some embodiments, the microwave radiation includes electromagnetic radiation having a wavelength ranging from between about 0.1 cm to about 100 cm. In some embodiments, the microwave radiation includes electromagnetic radiation having a wavelength of between about 0.75 cm and about 100 cm. In some embodiments, the microwave radiation includes electromagnetic radiation having a wavelength of between about 7.5 cm and about 15 cm. In some embodiments, the microwave radiation is applied to the sample for a predetermined amount of time. In some embodiments, the exposure time for the microwave radiation can be sufficient to raise the temperature of the sample and/or the retrieval agent in contact with sample, or it could be applied in bursts so as to not significantly raise the temperature of the sample (e.g. pulses of microwave radiation of a predetermined number of seconds followed by pauses between pulses of a predetermined number of seconds). The predetermined time and pattern of exposure to the microwave radiation can be empirically established by comparing a staining pattern for at least one antigen (or target) before and after the particular combination of UV and microwave radiation exposure. In still other embodiments, a combination of UV exposure, microwave exposure (continuous or in bursts), and heating of the fixed cell sample can be applied to sufficiently unmask a particular antigen so that it can be detected in the fixed cell sample.

[0036] In still other embodiments, a fixed cell sample can be exposed to ultrasound energy in addition to the UV radiation and/or microwave radiation for a predetermined amount of time. The time, length and intensity of any or all of the different electromagnetic energies or radiation can be established empirically by comparing a pattern of staining for a particular antigen (or target), as noted herein.

[0037] In particular embodiments, the retrieval agent can be a buffer solution having a pH ranging from between about 5 and about 10. In particular embodiments, the retrieval agent can be a buffer solution having a pH ranging from between about 7 and about 9. In other particular embodiments, the retrieval agent

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can comprise a buffer solution having a pH ranging from between about 7.5 and about 11. Examples of buffers include citric acid, potassium dihydrogen phosphate, boric acid, diethyl barbituric acid, piperazine-N,N'-bis(2-ethanesulfonic acid), dimethylarsinic acid, 2-(N-morpholino)ethanesulfonic acid, tris(hydroxymethyl)methylamine (TRIS), 2-(N-morpholino)ethanesulfonic acid (TAPS), N,N-bis(2-hydroxyethyl)glycine(Bicine), N-tris(hydroxymethyl)methylglycine (Tricine), 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid (TES), and combinations thereof.

[0038] In some embodiments, the retrieval agent includes a tris based buffer with a slightly basic pH. In other embodiments, the retrieval agent includes about 100 mM Tris, the retrieval agent having a pH of between about 8 and about 10. In other embodiments, the retrieval agent includes about 0.05% citraconic anhydride. In other embodiments, the retrieval agent includes about 10 mM citrate with about 2 mM EDTA and with about 0.05% Tween 20, the retrieval agent having a pH of about 6.2. In other embodiments, the retrieval agent includes about 0.01M citrate buffer, the retrieval agent having a pH of about 6.0.

[0039] In some embodiments, the retrieval agent can include a reagent that reacts with any liberated fixative so as to prevent it from reacting again with the sample. Examples a reagent that reacts with any liberated fixative include purpald or dimedone that are used to determine formaldehyde concentrations because they form very stable products. Alternatively, or in addition, the retrieval agent can include a reagent that reacts in a reversible manner with free amino-groups of proteins and thus protects them from reaction with any available formaldehyde. An example of a reagent that reacts in such a reversible manner is citraconic anhydride (CCA).

[0040] In other particular embodiments, the retrieval agent can include a chelator such as ethylenediaminetetraacetic acid (EDTA). Additional examples of chelators include ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic

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acid (EGTA), EGTA/AM (EGTA, Tetra(acetoxymethyl Ester)), (1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid) (BAPTA), BAPTA/AM (EGTA Tetra(acetoxymethyl Ester)), 5,5'-Dimethyl-BAPTA-AM; 1,2-Bis(2-amino-5-methylphenoxy) ethane (MAPTAM), N,N,N',N'-tetrakis(2-pyridylmethyl)ethane-1,2-diamine (TPEN), citrate, or ionophores such as ionomycin or calcimycin, or any combination or mixtures thereof.

[0041] In further embodiments, the retrieval agent can include an enzyme. Examples of an enzyme that can be included in the retrieval agent are a proteinase (such as trypsin, chymotrypsin, pepsin, or proteinase K), a nuclease, a glycanase, and a hyaluronidase.

[0042] In still further embodiments, the retrieval agent can include a chaotropic agent. Examples of chaotropic agents include butanol, ethanol, a guanidinium salt (for example, guanidinium hydrochloride or guanidinium thiocyanate), lithium perchlorate, lithium acetate, magnesium chloride, phenol, propanol, thiourea and urea.

[0043] In even further embodiments, the retrieval agent can include a nucleophile, i.e. a chemical species that donates an electron pair to an electrophile. Examples of nucleophiles include ammonia, primary amines, secondary amines and tertiary amines. Other examples of nucleophiles include hydrazines, alcohols and halogenides.

[0044] In other embodiments, the retrieval agent can include a Lewis acid. Examples of Lewis acids include metal ions such as iron (III) ions, aluminium ions, magnesium ions or other electron-deficient compounds such as toluenesulfonic acid, boric acid, boron trifluoride, and tartaric acid.

[0045] In other embodiments, the retrieval agent can include a surfactant. As used herein, "surfactants" are classified as anionic, cationic, or nonionic, depending on their mode of chemical action. In general, surfactants reduce interfacial tension between two liquids. An example of a surfactant is sodium

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dodecyl sulfate. Examples of suitable nonionic surfactants include polyethylene glycol monohexadecyl ether, Cetostearyl alcohol, Cetyl alcohol, cocamide diethanolamine, cocamide monoethanolamine, Decyl glucoside, Octylphenoxypolyethoxyethanol, Polyethylene glycol monoisohexadecyl ether, 5 Lauryl glucoside, Nonyl phenoxypolyethoxylethanol, 4-Nonylphenyl-polyethylene glycol, 1-(4-Nonylphenyl)-1,4,7,10,13,16,19,22,25-nonaohaheptacosan-27-ol, nonoxynols, Monolaurin, Octaethylene glycol monododecyl ether, Oleyl alcohol, Polyethylene-polypropylene glycol, Polyglycerol polyricinoleate, Polysorbates, Sorbitan monostearate. Sorbitan tristearate; Stearyl alcohol; polyethylene glycol p- 10 (1,1,3,3-tetramethylbutyl)-phenyl ether; Polyoxyethylene (20) sorbitan monooleate. octyl-, decyl, dodecyl-glucopyranoside, -maltoside or deoxycholic acid. Exemplary surfactants are sold under the names: Brij® 35, TWEEN®, Tergitol™, Triton™, Ecosurf™, Dowfax™, polysorbate 80™, BigCHAP, Deoxy BigCHAP, IGEPAL®, Saponin, Thesit®, Nonidet®, Pluronic F-68, digitonin, deoxycholate, 15 and the like. Particular disclosed working embodiments concern using surfactants selected from Brij® 35, TWEEN®, Tergitol™, Triton™.

[0046] In some embodiments, the retrieval agent comprises deionized water, TRIS, and a chelator. In some embodiments, the retrieval agent comprises deionized water, TRIS, and a chelator and has a pH ranging from between about 7 20 to about 9.5. In some embodiments, the retrieval agent comprises deionized water, TRIS, a chelator, and a preservative. In some embodiments, the retrieval agent is CC1, available from Ventana Medical Systems, Inc., Tucson, AZ, USA.

[0047] It is to be understood that in various embodiments the retrieval agent can include one, some or all of the different types of compounds listed above, and 25 that the retrieval agent can be used in combination with UV radiation and one or more of microwave radiation, heat and ultrasound energy.

[0048] Also disclosed is an automated system for the treatment of a fixed cell sample mounted on a substrate. In some embodiments, the automated system includes a substrate holder configured to hold the substrate. A reagent dispenser is 30 configured to dispense a retrieval agent onto the fixed cell sample, and a UV

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radiation source is configured to direct electromagnetic radiation toward the fixed cell sample. In some embodiments, the automated system includes a controller that is configured to control the reagent dispenser to dispense the retrieval agent and to direct UV radiation toward the fixed cell sample in a predetermined sequence and for a predetermined amount of time. The source of UV radiation can be any known or future developed source of UV radiation, including, without limitation, an LED emitting a particular range of UV wavelengths or an array of LED lamps emitting a narrow spectrum of UV light or a broad spectrum of UV light, a mercury vapor lamp, a UV LED laser, or a xenon lamp, or any combination thereof. In particular embodiments, the source of UV radiation can be a source of one or more of UVA, UVB and UVC radiation. In other particular embodiments, the source of UV radiation can include wavelengths of between about 200 nm and about 400 nm.

[0049] In some embodiments, the automated system includes an enclosure surrounding one or more substrate holders, which enclosure can be pressurizable. In even more particular embodiments, the substrate holder can include a heating and/or cooling element (e.g. a conductive heating element or a Peltier heating and cooling element). In other embodiments, the substrate holder can be automatically moveable under the direction of the controller into and out of the enclosure, such as through a door that can be sealed once the substrate holder is moved into the enclosure. Any of the slide holders disclosed in U.S. Patent Nos. 8,663,991; 8,048,373; and 7,468,161; and U.S. Patent Publication No. 2016/0282239 (the disclosures of each are hereby incorporated by reference herein in their entireties) may be adapted for use in the present disclosure. Alternatively, a substrate mover can be used to automatically place the substrate holding the fixed cell sample onto a stationary substrate holder kept inside the enclosure, such as through a sealable door mechanism.

[0050] In other particular embodiments, the automated system can include a source of microwave radiation and/or a source of ultrasound energy, such that microwaves or ultrasound are applied to the sample, such as at predetermined wavelengths or frequencies. In some embodiments, the microwave radiation and or

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the ultrasound energy is applied for a predetermined period of time. In some embodiments, microwave radiation or ultrasound energy is introduced for a time period ranging from between about 1 minute to about 60 minutes. In some embodiments, microwave radiation or ultrasound energy is introduced for a time period ranging from between about 2 minutes to about 30 minutes. In some embodiments, microwave radiation or ultrasound energy is introduced for a time period ranging from between about 3 minutes to about 15 minutes. In some embodiments, microwave radiation or ultrasound energy is introduced for a time period ranging from between about 5 minutes to about 15 minutes.

[0051] It is to be understood that the disclosed apparatus for the treatment of a fixed cell sample could be one module in a much larger system for treating biological samples held on substrates. A larger system, such as a specimen processing apparatus, might include other modules for performing other procedures on the substrate mounted biological samples, such as deparaffinization procedures, the application of antibodies and probes to the sample, drying, coverslipping, incubating and the like. For example, a specimen processing apparatus may be utilized to apply a wide range of substances to the specimen. The substances include, without limitation, stains, probes, reagents, rinses, and/or conditioners (hereinafter collectively referred to as "stains"). The substances can be fluids (e.g., gases, liquids, or gas/liquid mixtures), or the like. The fluids can be solvents (e.g., polar solvents, non-polar solvents, etc.), solutions (e.g., aqueous solutions or other types of solutions), or the like. Reagents can include, without limitation, stains, wetting agents, antibodies (e.g., monoclonal antibodies, polyclonal antibodies, etc.), antigen recovering fluids (e.g., aqueous- or non-aqueous-based antigen retrieval solutions, antigen recovering buffers, etc.), or the like. Probes can be an isolated nucleic acid or an isolated synthetic oligonucleotide, attached to a detectable label or reporter molecule. Labels can include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes.

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[0052] The specimen processing apparatus can be an automated apparatus, such as the BENCHMARK XT instrument and SYMPHONY instrument sold by Ventana Medical Systems, Inc. Ventana Medical Systems, Inc. is the assignee of a number of United States patents disclosing systems and methods for performing automated analyses, including U.S. Pat. Nos. 5,650,327, 5,654,200, 6,296,809, 6,352,861, 6,827,901 and 6,943,029, and U.S. Published Patent Application Nos. 20030211630 and 20040052685, each of which is incorporated herein by reference in its entirety. Examples of commercially available H&E stainers include the VENTANA SYMPHONY (individual slide stainer) and VENTANA HE 600 (individual slide stainer) series H&E stainers from Roche; the Dako CoverStainer (batch stainer) from Agilent Technologies; the Leica ST4020 Small Linear Stainer (batch stainer), Leica ST5020 Multistainer (batch stainer), and the Leica ST5010 Autostainer XL series (batch stainer) H&E stainers from Leica Biosystems Nussloch GmbH. The contactless mixer describes herein may be added to any of the aforementioned specimen processing systems.

[0053] Also disclosed is an apparatus for the treatment of a fixed sample that includes a chamber, which in some embodiments is pressurizable. In some embodiments, the apparatus includes a removable rack configured to fit inside the chamber and to hold one or more substrates holding one or more fixed cells. Examples of suitable apparatuses include those described in United States Patent Nos. 6,534,008, 8,361,388, 8,377,377 and related family members, the disclosures of which are hereby incorporated by reference herein in their entireties.

[0054] Also included in the apparatus is a UV light source (which can be any known or future developed source of UV light, such as noted herein) that is configured to operate within the chamber and direct UV light onto the one or more fixed cell samples.

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[0055] Referring now to FIG. 1, studies were performed to determine the effectiveness of UV radiation in unmasking protein antigens using a Rayonet RPR-200 Photochemical Reactor that is shown in panel A of FIG. 1. As shown in panel B of FIG. 1, the interior of the UV reactor includes a number of UV lamps arranged around the periphery of the chamber. Microscope slides bearing tissue samples were placed into the container shown in panel C of FIG. 1 and covered with a thin layer of cell conditioning solution 1 (CC-1, Ventana Medical Systems, Inc., Tucson, AZ, USA), then exposed to UV light for a predetermined amount of time. Formalin-fixed paraffin-embedded tissue (FFPET) sections of tonsil tissue were first deparaffinized using a standard method performed on a Sakura Tissue-Tek Prisma A1D Automated Slide Stainer, removed and placed into the tray of panel C of FIG. 1 for UV treatment.

[0056] Following UV treatment for a predetermined amount of time (2 hours, 1 hour or 30 minutes), the slides were removed from the UV reactor and were subjected to a procedure for detection of the protein BCL-2 according to a standard protocol on a BenchMark Ultra system (Ventana Medical Systems, Inc., Tucson, AZ, USA). This procedure included application of a peroxidase inhibitor, followed by application of a primary antibody specific for the BCL-2 protein (SP66, Ventana Medical Systems, Inc., Tucson, AZ, USA) and allowed to incubate under a coverslip for 16 minutes. DAB detection (OptiView DAB, Ventana Medical Systems, Inc., Tucson, AZ, USA) of the bound BCL-2 antibodies was then performed. Following the antibody treatment and detection, a counter stain was applied. First one drop of hematoxylin was applied to the sample and allowed to incubate for 4 minutes under a coverslip. Next, a drop of bluing reagent was applied under a coverslip and allowed to incubate for an additional 4 min, and then coverslipped.

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5 [0057] As a pair of controls, an additional sample of tonsil tissue was subjected to a standardized, automated heat-induced antigen retrieval procedure on the followed by BCL-2 detection (BenchMark Ultra system, Ventana Medical Systems, Inc., Tucson, AZ, USA) and another sample was placed into the UV reactor for 2 hours, but covered with aluminum foil to prevent UV light from reaching the sample. All samples where then examined microscopically for staining of the BCL-2 protein, which is a known tonsil protein that requires antigen retrieval to be made visible in tonsil tissue. The results of these studies are shown in FIGS. 2 and 3.

10 [0058] In panel A of FIG 2, a positive control slide (labeled OptiView Control) is shown which is stained according to a standardized heat-induced antigen retrieval procedure (i.e. heating of the sample or antigen retrieval agent), and where no UV light was applied to the sample. In this panel, the brown staining of the BCL-2 protein is clearly evident.

15 [0059] In panel E of FIG. 2 is shown a micrograph of the control slide which received neither heat-induced antigen retrieval treatment according to the standardized procedures, nor any exposure to UV light. Little brown BCL-2 staining is evident in this control slide.

20 [0060] In contrast, panels B, C and D of FIG. 2 are micrographs of tonsil samples exposed to UV light for 2 hours, 1 hour and 30 minutes, respectively. Since the negative control slide was also held in the reactor for the maximum time of 2 hours, it is clear that any antigen retrieval as evidence by BCL-2 staining was due to exposure to UV light and not any heating effect due to the lamps in the UV reactor. A comparison of panels B, C and D clearly demonstrates that the increase
25 in the amount of BCL-2 staining correlates with the amount of time the samples were subjected to UV radiation. FIG. 3 shows micrographs corresponding to those of FIG 2, only at higher magnifications.

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[0061] FIG. 4 shows a schematic diagram of an embodiment of an automated system **100** according to the disclosure that may be used to automatically treat fixed cell samples with UV light according to the disclosed methods. The apparatus of FIG. 4 includes, in this embodiment, enclosure **102** (which may or may not be pressurizable) and UV source **104** that is positioned above substrate holder **108** on which microscope slide (substrate) **110** is placed and on top of which microscope slide **110** is fixed cell sample **112**. Also shown is reagent dispenser **106** which can be used to dispense a retrieval agent onto the fixed cell sample **112**, and thereafter UV source **104** can direct UV radiation onto the fixed cell sample **112** for a predetermined amount of time. An example of a reagent dispenser is described in US Patent No. 8,790,596, the disclosure of which is hereby incorporated by reference herein in its entirety. A controller (not shown) can be used to turn the UV light source on and off, and to actuate a pump, syringe, pipette (or the like) to dispense a retrieval agent onto the fixed cell sample **112**.

[0062] In some embodiments, substrate holder **108** can include a heating or cooling mechanism (such as a conductive heater or a Peltier device). An example of a slide heating device is described in US Patent Nos. 7,425,306 and 6,582,962, the disclosures of which are hereby incorporated by reference herein in their entireties. Alternatively, hot air or steam could be introduced into enclosure **102** to raise the temperature of the fixed cell sample **112**. Where enclosure **102** is able to be pressurized, it is possible to raise the temperature within the enclosure without inducing boiling of the retrieval agent in contact with fixed cell sample **112**.

[0063] FIG. 5 shows a top view of a different embodiment of an apparatus **200** for carrying out the disclosed methods. In this embodiment, apparatus **200** includes housing **202**, which can include a lid (not shown) and can be pressurizable. Removable rack **204** holds substrates **206a**, **206b**, **206c**, **206d**, **206e**, **206f**, **206g**, and **206h**, which although not shown explicitly, could hold fixed cell samples on either the surface facing the UV light source **208** or on the opposite surface. In various embodiments, the apparatus of FIG. 5 can be used for manual treatment of fixed cell samples according to the disclosed methods, or for semi-automated

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5 methods (such as where the device includes a timer for turning UV light source **208** on an off after a pre-determined amount of time. In practice, UV light source **208** would advantageously be enclosed in a liquid-proof housing such that the interior space of the housing **202** could be filled with a retrieval agent solution. As discussed with respect to the system of FIG. 4, the housing **202** could be pressurized to prevent boiling of the retrieval agent during the treatment process, particularly if the housing **202** also includes a heating element for raising the internal temperature of the apparatus. Thus, the advantages of both heat-induced and UV-induced antigen retrieval/target retrieval can be realized.

10 **[0064]** Although described with respect to particular embodiments, it should be clear that the disclosed methods and systems can be modified without departing from the scope as defined by the claims that follow.

PATENT CLAIMS

1. A method for preparing a fixed cell sample for analysis, comprising:
 - a) contacting the sample with a retrieval agent, and;
 - b) exposing the fixed cell sample to electromagnetic radiation, the
- 5 electromagnetic radiation having a wavelength ranging from between about
 10 nm and about 400 nm.
2. The method of claim 1, wherein the retrieval agent comprises water.
3. The method of any of the preceding claims, further comprising exposing the
 fixed cell sample to a temperature of between about 30°C and about 150° C
- 10 4. The method of any of the preceding claims, further comprising exposing the
 fixed cell sample to additional electromagnetic radiation, the additional
 electromagnetic radiation having a wavelength between about 0.1 cm and about
 100 cm.
5. The method of claim 4, wherein the additional electromagnetic radiation has a
- 15 wavelength of between about 0.75 cm and about 100 cm.
6. The method of claim 5, wherein the additional electromagnetic radiation has a
 wavelength between about 7.5 cm and about 15 cm.
7. The method of any of the preceding claims, wherein the electromagnetic
 radiation has a wavelength ranging from between about 200 nm and about 400
- 20 nm.
8. The method of claim 7, wherein the electromagnetic radiation has a wavelength
 ranging from between about 250 nm and about 400 nm.

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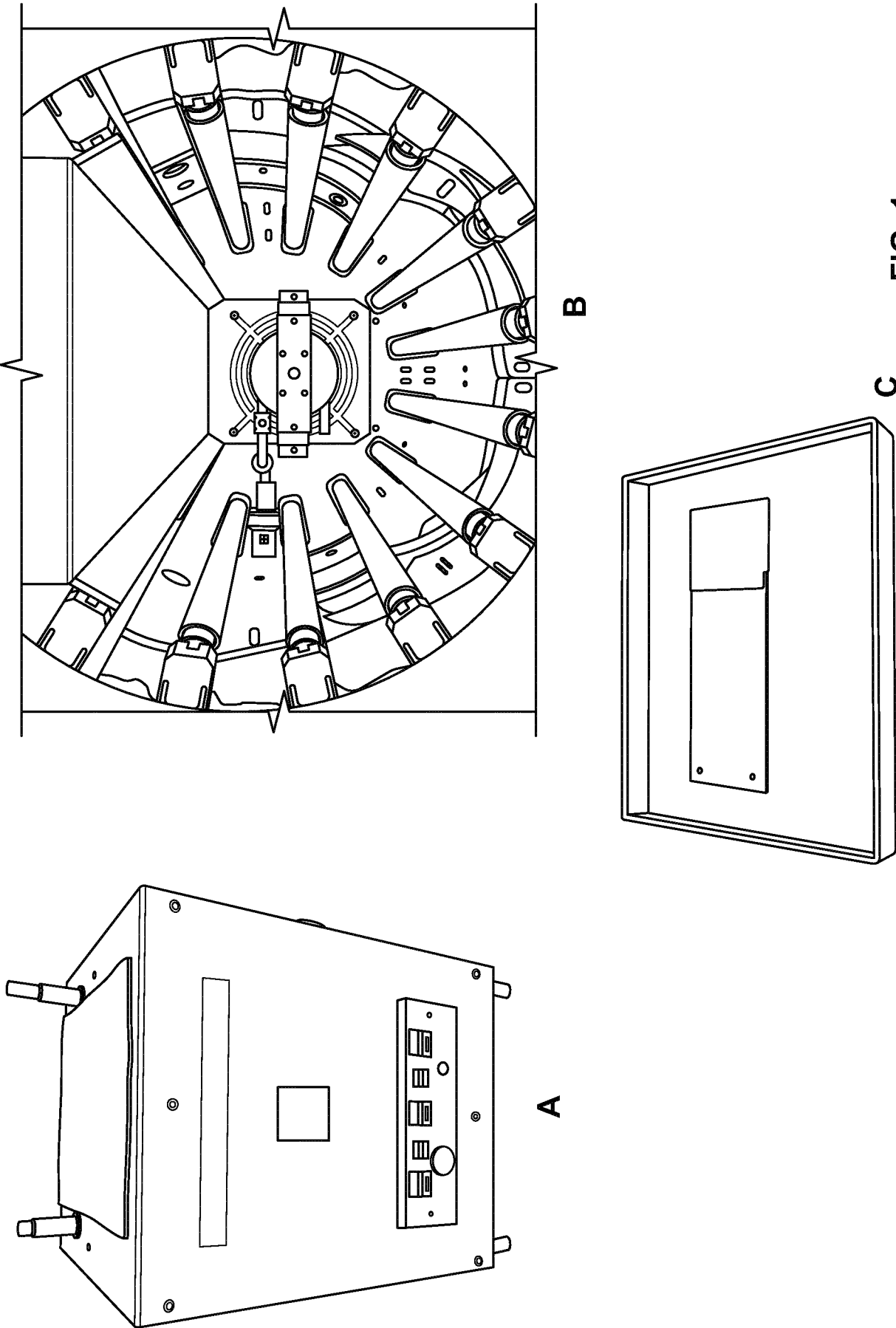
9. The method of any of the preceding claims, wherein the retrieval agent comprises tris(hydroxymethyl)aminomethane and has a pH ranging from between about 6 and about 8.
10. The method of any of the preceding claims, wherein the retrieval agent
5 comprises an enzyme.
11. The method of any of the preceding claims, wherein the retrieval agent comprises a chaotropic agent.
12. The method of any of the preceding claims, wherein the retrieval agent comprises a nucleophilic agent.
- 10 13. The method of any of the preceding claims, wherein the fixed cell sample is a formalin-fixed cell sample, wherein there the method further comprises passing ultrasound through the sample.
14. The method of any of the preceding claims, wherein the fixed cell sample comprises an aldehyde-fixed cell sample.
- 15 15. The method of claim 14, wherein the aldehyde-fixed cell sample comprises a formalin-fixed cell sample.
16. The method of any of the preceding claims, wherein the retrieval agent comprises at least two of a buffer, an enzyme, a chaotropic reagent, a chelating agent, a nucleophile, an oxidizing agent, an organic acid/base pair, an electron-
20 deficient compound, and a surfactant.
17. The method of any of the preceding claims, wherein the retrieval agent consists essentially of a buffer.
18. The method of any of any of the preceding claims, wherein the retrieval agent consists essentially of water and a salt.

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19. An automated system for treatment of a fixed cell sample mounted on a substrate, comprising:
- a) a substrate holder configured to hold the substrate;
 - b) a reagent dispenser configured to dispense a retrieval agent onto the fixed
5 cell sample;
 - c) an electromagnetic radiation source configured to direct electromagnetic radiation toward the fixed cell sample, the electromagnetic radiation having a wavelength of between about 200 nm and about 400 nm; and
 - d) a controller configured to control the dispenser and the electromagnetic
10 radiation source to deliver the retrieval agent and impinge the electromagnetic radiation onto the fixed cell sample mounted on the substrate in a predetermined sequence and for a predetermined amount of time.
20. The automated system of claim 19, wherein the substrate holder further
15 comprises a heating element.
21. The automated system of claim 19, further comprising an additional source of electromagnetic radiation configured to direct additional electromagnetic radiation toward the fixed cell sample, wherein the additional electromagnetic radiation has a wavelength of between about 7.5 cm and about 15 cm.
22. The automated system of claim 19, wherein the substrate holder is enclosed in a
20 pressurizable chamber.
23. An apparatus for treatment of a fixed cell sample, comprising:
- a) a pressurizable chamber;

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- b) a removable rack for mounting one or more substrates holding one or more fixed cell samples; and
- c) a UV light source configured to operate within the pressurizable chamber and direct UV light onto the one or more fixed cell samples.



BCL2 on Tonsil

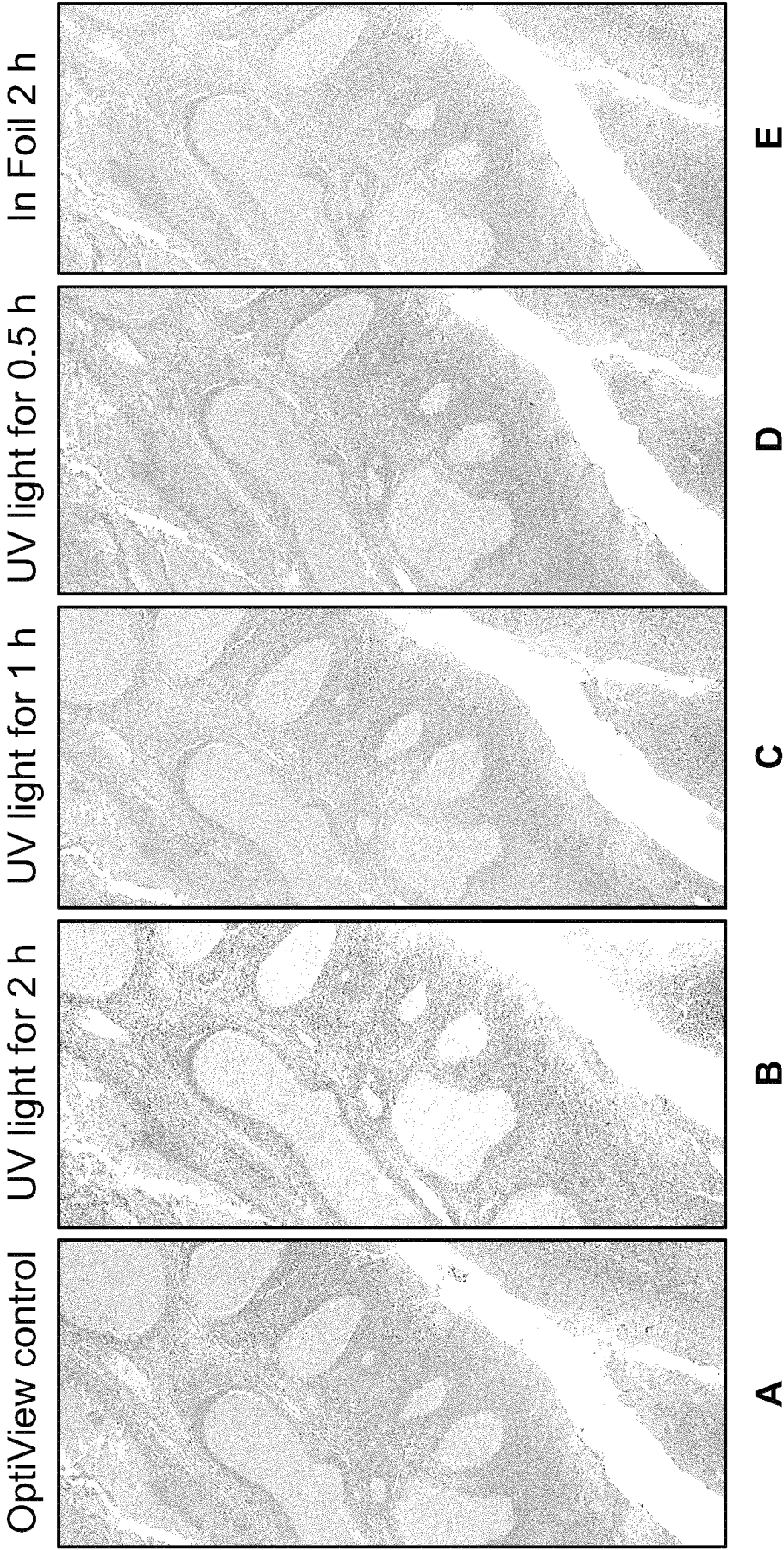


FIG. 2

BCL2 on Tonsil

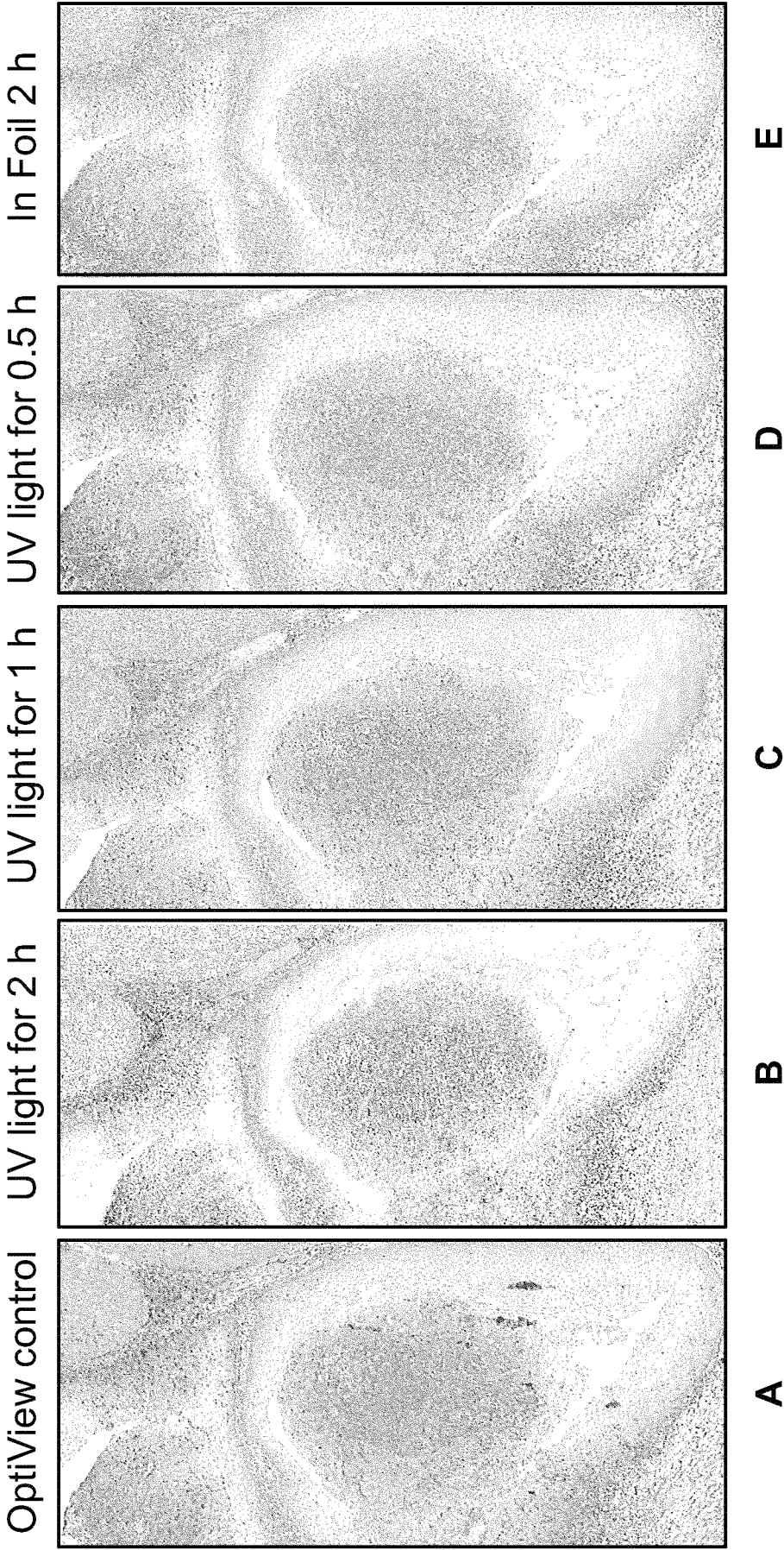


FIG. 3

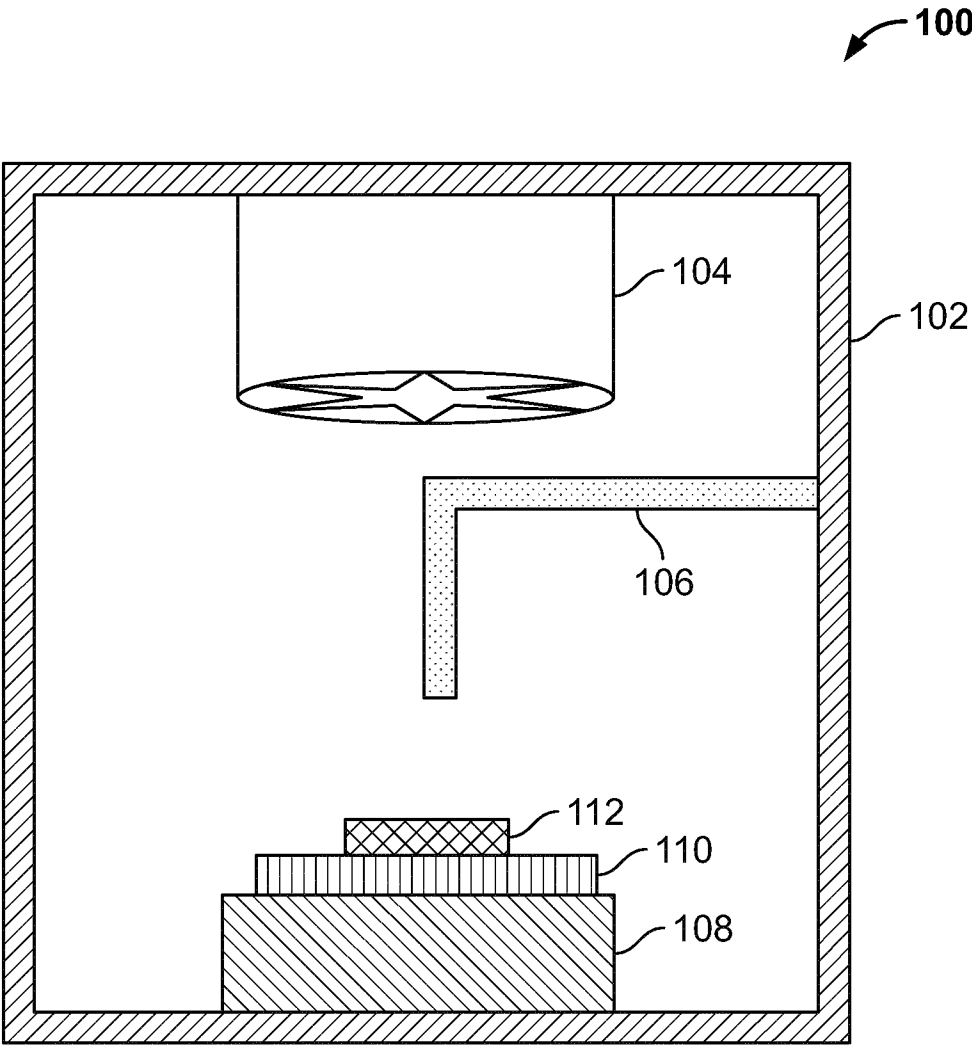


FIG. 4

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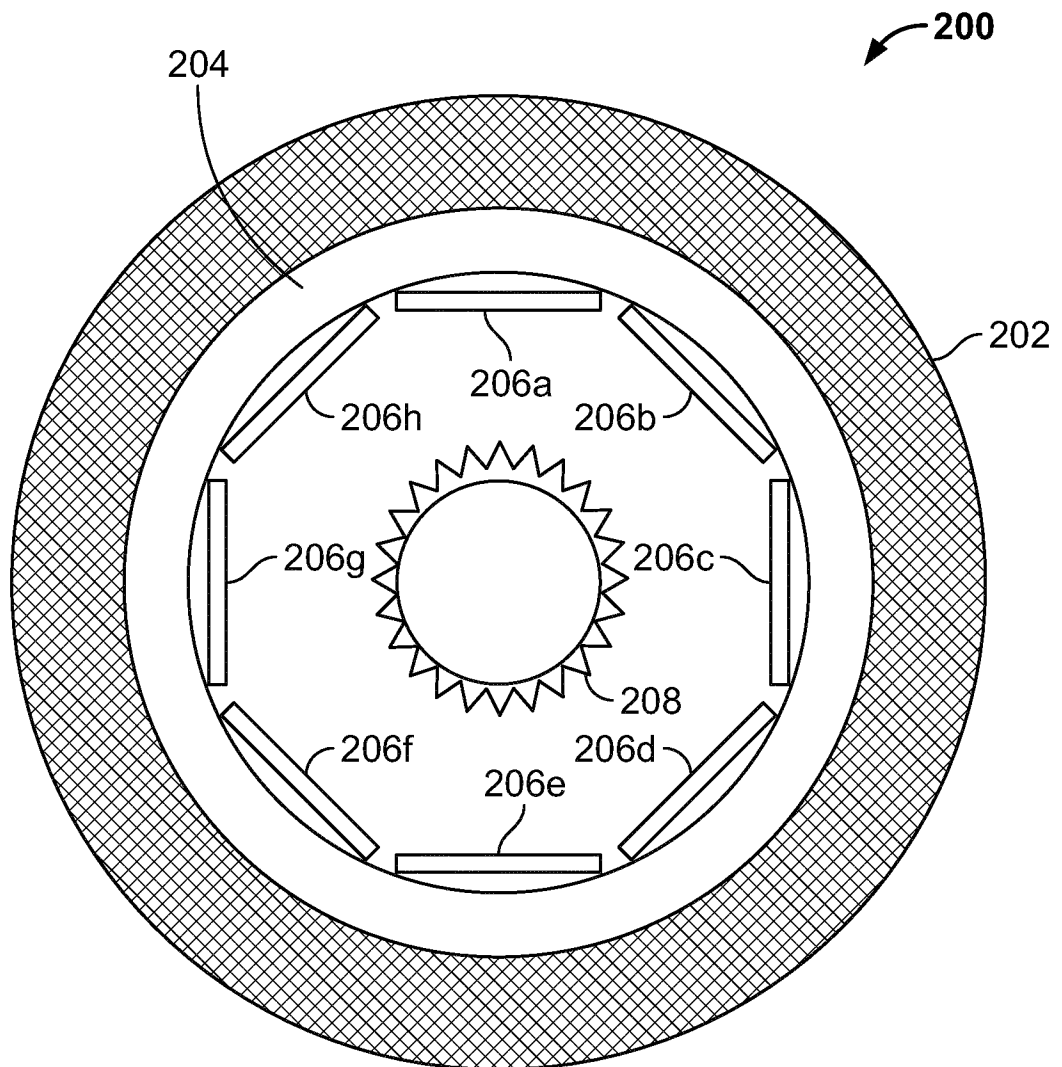


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/080894

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N1/44 G01N1/30 C12Q1/6806
ADD. G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N C12Q C12N C12M A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/148243 A1 (LEE HUN-JOO [KR] ET AL) 28 May 2015 (2015-05-28)	1,7,8
Y	Abstract; paragraphs [0009], [0012], [0060] - [0062]	2-6,9-23
Y	----- US 2010/028978 A1 (ANGROS LEE H [US]) 4 February 2010 (2010-02-04) paragraphs [0005] - [0007], [0067], [0077], [0102], [0106], [0107], [0111], [0112], [0119]; figures 1, 2, 6	2-6,9-23
Y	----- WO 2007/031874 A2 (DAKO DENMARK AS [DK]; WINTHER LARS [DK]) 22 March 2007 (2007-03-22) paragraphs [0078], [0109], [0111] -----	10,13-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 February 2018

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/080894

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2015148243 A1	28-05-2015	KR 20150059449 A US 2015148243 A1	01-06-2015 28-05-2015
US 2010028978 A1	04-02-2010	AU 2009285551 A1 AU 2016200563 A1 AU 2018200116 A1 CA 2772493 A1 GB 2477648 A HK 1160929 A1 US 2010028978 A1 US 2010068757 A1 US 2013302818 A1 US 2016169777 A1 US 2018003601 A1 WO 2010025425 A1	04-03-2010 18-02-2016 25-01-2018 04-03-2010 10-08-2011 18-10-2013 04-02-2010 18-03-2010 14-11-2013 16-06-2016 04-01-2018 04-03-2010
WO 2007031874 A2	22-03-2007	EP 1920073 A2 US 2010105145 A1 WO 2007031874 A2	14-05-2008 29-04-2010 22-03-2007