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(71) Applicant: **UNIVERSITY HEALTH NETWORK** [CA/CA]; 190 Elizabeth Street, R. Fraser Elliot Building, Room 1S-417, Toronto, Ontario M5G 2C4 (CA).

(72) Inventors: **DACOSTA, Ralph S.**; 37 Anglesey Blvd., Etobicoke, Ontario M9A 3B7 (CA). **GIBSON, Christopher**; 3617-70 Temperance St., Toronto, Ontario M5H 0B1 (CA). **PHOON, Arcturus Cheuk Yiu**; 45 Bruce Farm Dr., Toronto, Ontario M2H 1G4 (CA).

(74) Agent: **NORTON ROSE FULBRIGHT CANADA LLP**; 1 Place Ville Marie, Suite 2500, Montreal, Québec H3B 1R1 (CA).

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(54) **Title:** TISSUE PHANTOMS

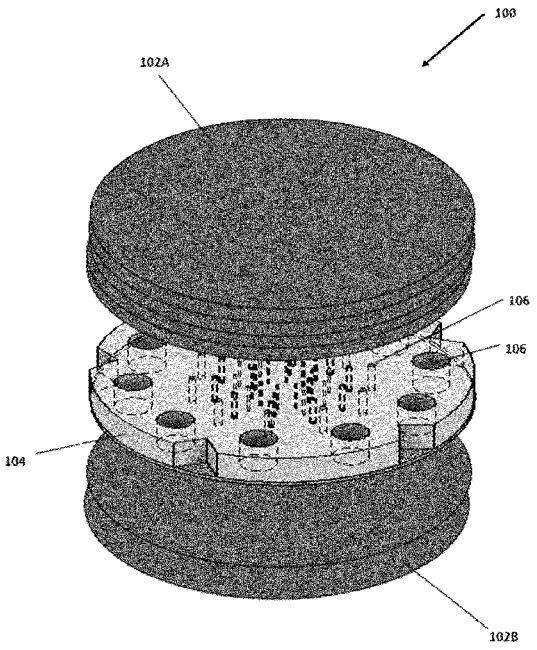

FIG. 1

(57) **Abstract:** A tissue phantom system and methods of making and using the tissue phantom system are provided. The tissue phantom system includes a base, a fluorophore support portion, and a plurality of tissue phantom films. The base portion of the tissue phantom system is configured to simulate fluorescence and optical properties of healthy tissue. The fluorophore support portion includes a fluorophore material exhibiting optical properties of diseased tissue. The plurality of tissue phantom films is configured to form a top portion of the tissue phantom system, the top portion having an adjustable thickness.

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TISSUE PHANTOMS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Application No. 63/453,364, filed on March 20, 2023, the entire contents of which is incorporated by reference herein.

TECHNICAL FIELD

[002] The present disclosure relates to tissue phantoms including, for example, a breast tissue phantom. The phantom may be used to calibrate an imaging device that is used to identify abnormal cells such as one or more of residual cancer cells, precancerous cells, and satellite lesions in a surgical site such as a surgical cavity or in a tissue specimen removed from a surgical cavity, such as breast tissue removed during Breast Conserving Surgery ("BCS").

BACKGROUND

[003] Surgery is one of the oldest types of cancer therapy and is an effective treatment for many types of cancer. Oncology surgery may take different forms, dependent upon the goals of the surgery. For example, oncology surgery may include biopsies to diagnose or determine a type or stage of cancer, tumor removal to remove some or all of a tumor or cancerous tissue, exploratory surgery to locate or identify a tumor or cancerous tissue, debulking surgery to reduce the size of or remove as much of a tumor as possible without adversely affecting other body structures, and palliative surgery to address conditions caused by a tumor such as pain or pressure on body organs.

[004] In surgeries in which the goal is to remove the tumor(s) or cancerous tissue, surgeons often face uncertainty in determining if all cancer has been removed.

The surgical bed, or tissue bed, from which a tumor is removed, may contain residual cancer cells, i.e., cancer cells that remain in the surgical margin of the area from which the tumor is removed. If these residual cancer cells remain in the body, the likelihood of recurrence and metastasis increases. Often, the suspected presence of the residual cancer cells, based on examination of surgical margins of the excised tissue during pathological analysis of the tumor, leads to a secondary surgery to remove additional tissue from the surgical margin.

[005] For example, breast cancer, the most prevalent cancer in women, is commonly treated by breast conservation surgery (BCS), e.g., a lumpectomy, which removes the tumor while leaving as much healthy breast tissue as possible. Treatment efficacy of BCS depends on the complete removal of malignant tissue while leaving enough healthy breast tissue to ensure adequate breast reconstruction, which may be poor if too much breast tissue is removed. Traditionally, tumor margins are visualized under standard white light (WL) in an operating room in order to determine the effectiveness of the BCS procedure.

[006] Imaging devices may also be used to evaluate specimen tissue for the presence of cancer cells. For example, imaging devices may be used to determine the amount of cancer cells, if any, remaining after a BCS procedure, thus determining the efficacy of the procedure. Imaging devices may also be used to provide guidance during the BCS procedure. Such imaging devices may perform white light (WL) or fluorescent (FL) imaging and/or may rely on use of imaging contrast to make such cells visible under the imaging device light source(s). Calibration of the imaging devices, as well as

training and/or education of the persons using the imaging devices to identify residual cancer cells will contribute to the efficacy of removing residual cancer cells during BCS.

SUMMARY

[007] The present disclosure may demonstrate one or more of the above-mentioned desirable features. Other features and/or advantages may become apparent from the description that follows.

[008] In accordance with one aspect of the present disclosure, a tissue phantom system is provided. The tissue phantom system includes a base, a fluorophore support portion, and a plurality of tissue phantom films.

[009] In accordance with one or more example embodiments, the fluorophore support portion may include a plurality of wells.

[0010] In accordance with one or more example embodiments, the plurality of wells may include at least two wells having a first size and at least two wells having a second size different from the first size.

[0011] In accordance with one or more example embodiments, the base may be configured to simulate fluorescence and optical properties of healthy tissue.

[0012] In accordance with one or more example embodiments, the plurality of tissue phantom films may be configured to form a top portion of the tissue phantom system.

[0013] In accordance with one or more example embodiments, the top portion of the tissue phantom system may have an adjustable thickness.

[0014] In accordance with one or more example embodiments, the thickness of the top portion may be adjustable by adding or removing tissue phantom films to the tissue phantom system when assembled.

[0015] In accordance with one or more example embodiments, when the tissue phantom system is an assembled state, the fluorophore support portion may include a first material exhibiting optical properties of diseased tissue and comprising a fluorophore material having a first concentration.

[0016] In accordance with one or more example embodiments, when the tissue phantom system is in an assembled state, the fluorophore support portion may include a second material exhibiting optical properties of diseased tissue and comprising a fluorophore material having a second concentration, different from the first concentration.

[0017] In accordance with one or more example embodiments, the fluorophore support portion may be positioned on the base and one or more of the plurality of tissue phantom films may be positioned on or over the fluorophore support portion when the tissue phantom system is in an assembled state.

[0018] In accordance with one or more example embodiments, at least one of the plurality of tissue phantom films may include a material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue.

[0019] In accordance with one or more example embodiments, at least one of the plurality of tissue phantom films may include a material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

[0020] In accordance with one or more example embodiments, the first material and the second material may include a composition exhibiting fluorescence and optical characteristics of diseased human or animal tissue.

[0021] In accordance with one or more example embodiments, the first material and the second material may include at least one of agar, phosphate buffered saline, water, agarose, dimethyl sulfoxide, or blood tissue or cells.

[0022] In accordance with one or more example embodiments, the fluorophore material may include a protoporphyrin IX (PpIX), indocyanine green (ICG), quantum dots, or other fluorescent dye.

[0023] In accordance with one or more example embodiments, the plurality of wells may be arranged in a concentric pattern in or on the fluorophore support portion.

[0024] In accordance with another aspect of the present disclosure, a customizable tissue phantom system is provided. The customizable tissue phantom system includes a base portion configured to simulate optical properties of healthy tissue, a fluorophore support portion configured to contain fluorophores of varying quantities representative of diseased tissue, and a cover portion configured to be positioned on or over the fluorophore support portion. An uppermost surface of the cover portion may be configured to represent a skin layer of the tissue phantom system and a distance between the uppermost surface of the cover portion and the fluorophore support portion may represents a depth from the skin layer to the diseased tissue. The depth may be adjustable by adjusting a thickness of the cover portion.

[0025] In accordance with one or more example embodiments, the fluorophore support portion may include a tray having a plurality of wells, wherein a quantity of

fluorophores contained in each well is determined by the size of the well and the concentration of the fluorophore.

[0026] In accordance with one or more example embodiments, the cover portion may include one or more tissue phantom films.

[0027] In accordance with one or more example embodiments, each of the tissue phantom films may include a tissue phantom material configured to simulate the fluorescence and optical properties of healthy tissue.

[0028] In accordance with one or more example embodiments, at least one of the tissue phantom films may include a material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue.

[0029] In accordance with one or more example embodiments, at least one of the tissue phantom films may include a material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

[0030] In accordance with one or more example embodiments, at least one of the tissue phantom films may include portions of a first material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue and portions of a second material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

[0031] n accordance with one or more example embodiments, each of the plurality of tissue phantom films may have a thickness between about 100 μm and

about 1 mm.

[0032] In accordance with one or more example embodiments, a thickness of the cover portion may vary between about 100 μm and about 2000 μm.

[0033] In accordance with one or more example embodiments, the fluorophore support portion may include an aperture containing a tubular structure, wherein an interior volume of the tubular structure defines a well within the fluorophore support portion.

[0034] In accordance with one or more example embodiments, the tissue phantom system may also include a fluorophore material in the well defined by the tubular structure, and one or more tissue phantom films positioned over a surface of the fluorophore support portion and over the well to form the cover portion of the phantom.

[0035] In accordance with one or more example embodiments, the fluorophore support portion has a thickness, and the tubular structure may include a length equal to the thickness of the fluorophore support portion.

[0036] In accordance with one or more example embodiments, the aperture may be one of a plurality of apertures in the fluorophore support portion; and the tubular structure may be one of a plurality of tubular structures, each of the plurality of tubular support structure being associated with a respective aperture of the plurality of apertures.

[0037] In accordance with one or more example embodiments, a first tubular structure of the plurality of tubular structures may have an interior volume defined by a

first diameter, and a second tubular structure of the plurality of tubular structures may have an interior volume defined by a second diameter greater than the first diameter.

[0038] In accordance with a further aspect of the present disclosure a method of making a tissue phantom system is provided. The method includes forming a fluorophore support portion having an aperture, moving a tubular structure within the aperture to a partially inserted condition, drawing a fluorophore-containing material into the tubular structure via capillary action, and fully inserting the tubular structure into the fluorophore support portion such that the tubular structure is flush with a top surface and a bottom surface of the fluorophore support portion.

[0039] In accordance with one or more example embodiments, the method may include sealing one or both of the top surface and the bottom surface of the fluorophore support portion with a fused silica wafer.

[0040] In accordance with one or more example embodiments, the method may include positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

[0041] In accordance with one or more example embodiments, positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion may include positioning at least one tissue phantom film comprising a material configured to exhibit fluorescence and optical properties of connective tissue over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

[0042] In accordance with one or more example embodiments, positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the

bottom surface of the fluorophore support portion may include positioning at least one tissue phantom film comprising a material configured to exhibit fluorescence and optical properties of adipose tissue over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

[0043] In accordance with one or more example embodiments, the aperture may be a first aperture and the method may further include forming a second aperture in the fluorophore support portion, coating an interior surface of the second aperture with an opaque material, and introducing a fluorophore-containing material into the second aperture.

[0044] In accordance with one or more example embodiments, drawing a fluorophore-containing material into the tubular structure may include positioning a portion of the tubular structure protruding from the fluorophore portion in the partially inserted condition of the tubular structure into a reservoir containing the fluorophore material.

[0045] In accordance with one or more example embodiments, the method may further include creating a water-tight seal between the fluorophore support portion and the fused silica wafer with a silicone grease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] The present disclosure can be understood from the following detailed description either alone or together with the accompanying drawings. The drawings are included to provide a further understanding of the disclosed teachings and are incorporated in and constitute a part of this specification. The drawings illustrate one or

more example embodiments of the present disclosure and together with the description serve to explain various principles and operations. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color photographs and color drawings will be provided by the Office upon request and payment of the necessary fee.

[0047] FIG. 1 is an expanded perspective view of a connective tissue phantom in accordance with an example embodiment of the present disclosure, showing a plurality of thin connective tissue films positioned above a fluorophore support structure (fluorophore chip) which is in turn positioned above a base including a portion of connective tissue phantom material.

[0048] FIG. 2A is a white light (WL) image of a white connective tissue phantom material.

[0049] FIG. 2B is a fluorescent (FL) image of the connective tissue phantom material of FIG. 2A taken using 405 nm illumination with the connective tissue phantom material fluorescing a green color.

[0050] FIG. 2C is a white light (WL) image of a pink adipose tissue phantom material.

[0051] FIG. 2D is a fluorescent (FL) image of the adipose tissue phantom material of FIG. 2C taken using 405 nm illumination with the adipose tissue phantom material fluorescing an orange/orange-brown color.

[0052] FIG. 3A is a white light (WL) image of a white connective tissue thin film mounted on a fused silica wafer.

[0053] FIG. 3B is a side view of the connective tissue thin film mounted on a fused silica wafer show in FIG. 2A, with the connective tissue thin film having a thickness of 0.9 mm and the wafer having a thickness of 0.7 mm.

[0054] FIG. 3C is a white light (WL) image of a thin section of pink adipose tissue phantom material.

[0055] FIG. 4A is a white light (WL) image of a composite tissue phantom material containing adipose tissue phantom material (pink) and connective tissue phantom material (white).

[0056] FIG. 4B is a fluorescent (FL) image of the composite tissue phantom material of FIG. 4A taken using 405 nm illumination in which the fluorescing adipose tissue phantom material appears brownish and the fluorescing connective tissue phantom material appears green.

[0057] FIGS. 5A and 5B are isometric views of an example fluorophore support structurer (fluorophore chip) including wells configured to contain a fluorophore material.

[0058] FIG. 5C is a plan view of the fluorophore support structure of FIGS. 5A and 5B.

[0059] FIG. 5D is a FL image of the fluorophore support structure of FIGS. 5A-5C in which the wells are filled with 630 nm quantum dots in various concentrations, with all wells of a single radial column of the support structure having the same concentration of quantum dots and each radial column of wells having a concentration of quantum dots different than other radial columns of the support structure.

[0060] FIG. 6A is a side view of the component of FIGS. 5A-5C with capillary tubes partially inserted into the component.

[0061] FIG. 6B is a plan view of a fluorophore reservoir used in a process of creating a tissue phantom in accordance with an example embodiment of the present disclosure.

[0062] FIG. 6C is a side view of the component of FIGS. 5A-5C and the fluorophore reservoir of FIG. 6B.

[0063] FIG. 6D is a side view similar to FIG. 6C showing the capillary tubes introduced into the fluorophore reservoir and the passive uptake of the fluorophore from the reservoir to the fluorophore chip via the capillary tubes.

[0064] FIG. 7 is side view of the component of the tissue phantom of FIG. 6A after rotating 180 degrees and mounted on a fused silica wafer.

[0065] FIG. 8 is a plan view of a tissue phantom according to the present disclosure showing fluorophore wells of the fluorophore support structure being filled according to an example embodiment.

[0066] FIG. 9 is a schematic representation of fluorophore-containing wells of a fluorophore support structure of a tissue phantom, the wells having varying sizes and containing varying fluorophore concentrations according to the present disclosure.

[0067] FIG. 10 is a schematic side view of a fixture for preparing a phantom material portion.

[0068] FIG. 11 is a white light (WL) image of a composition tissue phantom material including portions representing connective tissue (white) and portions representing adipose tissue (pink).

[0069] FIG. 12 shows a workflow for assembling a tissue phantom system according to an example embodiment of the present disclosure.

[0070] FIGS. 13A and 13B are fluorescent (FL) images showing fluorescent response of an example adipose tissue phantom system, the example adipose tissue phantom system of FIG. 13A showing fluorescent response for an adipose tissue thin film depth of 300 μm with the chip loaded with 630 nm quantum dots in which the adipose tissue is fluorescing a dark orangish-brown color and the quantum dots are fluorescing a red color and the example adipose tissue phantom system of FIG. 13B showing fluorescent response for an adipose tissue thin film depth of 1645 μm with the chip loaded with 630 nm quantum dots in which the adipose tissue is fluorescing a dark orangish-brown color and the quantum dots are fluorescing red and are only slightly visible through the depth of the adipose tissue film, according to example embodiments of the present disclosure.

[0071] FIGS. 143A and 14B are fluorescent (FL) images showing fluorescent response of an example connective tissue phantom system, the example connective tissue phantom system of FIG. 14A showing fluorescent response for a connective tissue thin film depth of 900 μm with the connective tissue film fluorescing a green color and the quantum dots are fluorescing a red color and the example connective tissue phantom system of FIG. 14B showing fluorescent response for a connective tissue thin film depth of 409 μm with the connective tissue film fluorescing a green color and the quantum dots are fluorescing a red color according to example embodiments of the present disclosure.

DESCRIPTION OF VARIOUS EXAMPLE EMBODIMENTS

[0072] Tissue phantoms, as discussed herein, may be used to calibrate an imaging device and/or to provide practice for an operating surgeon. For example, the tissue phantoms may represent and mimic the optical properties of “normal” or “healthy” tissue. Additionally, the tissue phantoms may include one or more portions that represent and mimic the optical properties of diseased or abnormal tissue such as “cancerous” tissue.

[0073] In alternative embodiments, the tissue phantom may be configured to include more than one component of a tissue or a tissue structure and can be used to calibrate an imaging device or other device configured to distinguish one tissue component from another or locate one tissue component relative to another in a tissue structure. In addition to calibration, the phantom can also be used for training purposes. For example, the phantom may be configured to represent human breast tissue and may contain different components/tissue types such as adipose tissue, connective tissue, and vasculature and the phantom can be used to train surgeons to locate the blood or vasculature relative to the adipose or connective tissue within a breast with an imaging device.

[0074] In another example, the tissue phantom may be used to train new users of a fluorescence imaging device to correctly identify tissues based on their fluorescence (e.g., identifying red tumor against green/pink connective/adipose tissue background). Or to show how certain tissues would fluoresce when imaged with such a device (blood shows up dark red/black for example, which may not be intuitive to new users).

[0075] As noted above, in accordance with one aspect of the present disclosure, the tissue phantom is configured to include a “normal” or “healthy” tissue portion and one or more “diseased” or “abnormal” tissue portions. The healthy tissue of the tissue phantom can be any type of tissue and the diseased tissue or “target” tissue of the tissue phantom can be chosen to mimic any disease found in the particular type of healthy tissue modeled by the tissue phantom. The examples provided herein discuss a breast tissue phantom having one or more areas of diseased tissue, i.e., cancerous tissue or tumors. It should be understood that these examples are non-limiting examples only and that the concept of a tissue phantom comprising healthy and diseased tissue is applicable to many other types of human and animal tissues and their diseases. Although discussed herein with regard to breast tissue, it is possible to use the present disclosure as a guide to create a tissue phantom representative of any tissue having a disease based on the optical properties for normal tissue and diseased tissue for the particular tissue and disease of interest. For example, knowing the absorption coefficient and reduced scattering coefficient of the chosen tissue type (for both normal and diseased tissues) at the wavelength that is being used for excitation would permit the creation of a phantom for a particular tissue having a particular disease as described herein. To create such a custom tissue phantom, information regarding how the tissue appears when imaged using a particular excitation light source and optical filter combination would be relied upon. For example, a fluorescence emission spectrum and/or fluorescence images of the tissues would provide the information needed. Examples of diseased tissue that have optical properties that may

differ from the optical properties of healthy tissue include inflamed tissue (e.g., rheumatoid arthritis), fibrotic tissue, and ischemic tissue.

[0076] For example, in accordance with the present disclosure, tissue phantoms representative of healthy or diseased tissues that may be created in as disclosed herein may include: spinal cord, brain, skin, limbs (sarcoma), oral cavity, prostate, cervix, colon, thyroid, ovaries, lymph nodes, lungs, pancreas, esophagus, muscle, bone, cartilage, uterus, or vagina. This list is intended to provide examples only and is not intended to limit the range of possible phantoms created in accordance with the present disclosure. In example embodiments of a phantom configured to represent healthy tissue and abnormal or diseased tissue such as “cancerous tissue,” the “cancerous tissue” of the phantom contains a material that will cause the “cancerous tissue” of the phantom, when illuminated with excitation light having a known wavelength, to fluoresce or emit light having a wavelength which will allow detection/visualization of the “cancerous tissue” relative to the healthy tissue. For example, in some embodiments, a tissue phantom in accordance with the present disclosure may comprise “healthy tissue” configured to fluoresce green when illuminated with excitation light having a wavelength of between about 400 nm and about 450 nm. In addition, the tissue phantom may comprise “cancerous tissue” configured to fluoresce red when illuminated with the same excitation light having a wavelength of between about 400 nm and about 450 nm. An example of the material included in the “cancerous tissue” of the phantom that fluoresces a red color when illuminated with excitation light having a wavelength between about 400 nm and about 450 nm is the porphyrin PpIX. Alternatively, other fluorophores can be used to represent tissues that are different from healthy tissue, also

referred to herein as "target tissue.'" For example, indocyanine green (ICG), a green dye such as Pacific Green (https://www.thermofisher.com/ca/en/home/life-science/cell-analysis/fluorophores/pacific-green-dye.html), IRDye 800CW, or other fluorophores of interest may be used. In an example embodiment where blood or vasculature is the "target tissue," ICG may be used in the portion of the phantom that represents the blood or vasculature.

[0077] In the example breast tissue phantoms disclosed herein, PpIX has been selected as the fluorophore of interest. PpIX is a fluorescent molecule that, when excited by the appropriate excitation light, emits a red fluorescence. The PpIX molecule is naturally broken down by healthy tissue (non-cancerous tissue) in a patient to Heme. Thus, healthy tissue does not contain PpIX and therefore does not emit the red fluorescence. However, cancerous tissue is not able to process PpIX and, thus, the PpIX collects in the cancerous tissue. The PpIX collected in cancer cells, when excited by light emitted by an imaging device and having a wavelength of between about 400 nm and about 450 nm, fluoresces red, making the cancerous tissue appear red to the imaging device. This allows a user of the imaging device to determine the presence or absence of cancerous cells based upon the corresponding presence or absence of red fluorescence emitted by the PpIX molecules.

[0078] As disclosed herein, a tissue phantom may be used with an imaging device in order to determine the presence, location and/or amount of the "cancerous" tissue with respect to the "normal" tissue within the tissue phantom. Such results may then allow a user to calibrate the imaging device, if the concentration of PpIX within the tissue phantom is known by the user.

[0079] Example devices, systems, and methods for detecting cancer cells containing PpIX or other induced porphyrins during surgical intervention are disclosed in U.S. Provisional Patent Application No. 62/625,983, filed February 3, 2018 and entitled "Devices, Systems, and Methods for Tumor Visualization and Removal," and in PCT/CA2019/000015, filed February 1, 2019, entitled "Devices, Systems, and Methods for Tumor Visualization and Removal" and published as WO2019/148,268 on August 8, 2019, the entire content of each of which is incorporated herein by reference.

[0080] During use of the tissue phantom for calibration of an imaging device, the imaging device may be inserted at least partially within a tissue phantom, such as a breast tissue phantom in accordance with the present disclosure, or positioned adjacent a tissue phantom, and emit a desired wavelength of light to illuminate the tissue phantom. Illumination with the excitation light causes the "cancerous tissue" within the tissue phantom to fluoresce, as described above, thus making the cancerous tissue of the tissue phantom visible to the imaging device and to those observing the output of the imaging device. As discussed above, the "cancerous" tissue within the tissue phantom may fluoresce due to the presence of PpIX (or another fluorescent dye) contained within portions of the phantom.

[0081] The "normal" or "healthy" tissue of the tissue phantom does not include PpIX (or another red-fluorescing fluorophore) and, therefore, does not fluoresce in the same manner, i.e., does not emit/reflect light at the same wavelength as the "cancerous tissue" when illuminated by the excitation light of the imaging device.

[0082] It should be noted that the healthy tissue phantom material may contain fluorophores endogenous to the reagents used to fabricate the material but none are

similar to or fluoresce in the same manner as PpIX. The “normal” tissue of the tissue phantom is created to mimic normal healthy tissue, which autofluoresces when illuminated with the excitation light. Different healthy tissues emit different wavelengths of light in response to illumination by excitation light. Thus, when illuminating a tissue phantom with excitation light as disclosed herein, the different components of the phantom (healthy tissue, cancerous tissue) will emit different wavelengths of light in response. This allows the light emitted from the cancerous tissue to be distinguished from the light emitted by the healthy tissue of the tissue phantom and, thus, permits the surgeon to identify the presence of cancerous tissue and its location.

[0083] For example, for calibration of an imaging device configured to emit excitation light of between about 400 nm – 450 nm, the tissue phantom has optical properties that allow the phantom to mimic the emission response of tissue illuminated with excitation light of between about 400 nm – 450 nm. The optical properties of the tissue phantom can be narrowly tailored to mimic tissue response (of both healthy tissue and diseased tissue) to excitation by any range of excitation light. For example, the phantom can be formed to have optical properties that allow it to mimic tissue response to illumination by excitation light in the ultraviolet/blue range, near infrared range, and infrared range. For example, the present disclosure contemplates a tissue phantom having optical properties that mimic tissue response to illumination by excitation light in the following example ranges: about 350 nm – about 400 nm, about 400 nm – about 450 nm, about 450 nm – about 500 nm, about 500 nm – about 550 nm, about 550 nm – about 600 nm, about 600 nm – about 650 nm, about 650 nm – about 700 nm, about 700 nm – about 750 nm, about 750 nm – about 800 nm, about 800 nm –

about 850 nm, about 850 nm – about 900 nm, about 900 nm – about 950 nm, about 950 nm – about 1000 nm, and/or various combinations therefor. In certain non-limiting, example embodiments disclosed herein, the tissue phantom is configured to respond to illumination with excitation light in the blue/violet range, for example 405 nm, in a manner the same or substantially the same as human or animal tissue.

[0084] The tissue phantoms disclosed herein also can be used to help identify an optimum amount of PpIX to be collected in cancerous cells in order for the fluorescence of the cancer cells to be detected by the imaging device and/or the surgeon. Using this information, it is possible to then determine the appropriate amount or dose of porphyrin-inducing composition that should be administered to the patient, for example prior to BCS, as well as the timing of the dosage. For example, as disclosed in U.S. Provisional Patent Application No. 62/625,967, filed February 2, 2018 and entitled "Devices, Systems, and Methods for Tumor Visualization and Removal," and in U.S. Provisional Patent Application No. 62/625,983, filed February 3, 2018 and entitled "Devices, Systems, and Methods for Tumor Visualization and Removal," and in PCT/CA2019/000015, filed February 1, 2019, entitled "Devices, Systems, and Methods for Tumor Visualization and Removal" and published as WO2019/148,268 on August 8, 2019, the entire content of each of which is incorporated herein by reference, the surgical subject or patient may be given a diagnostic dose (i.e., not a therapeutic dose) of a compound (imaging/contrast agent) such as the pro-drug aminolevulinic acid (ALA). As understood by those of ordinary skill in the art, dosages of ALA less than 60 mg/kg are generally considered diagnostic while dosages greater than 60 mg/kg are generally considered therapeutic. As disclosed herein, the diagnostic dosage of ALA may be

greater than 0 mg/kg and less than 60 kg/mg, between about 10 mg/kg and about 50 mg/kg, between about 20 mg/kg and 40 mg/kg, and may be administered to the subject in a dosage of 5 mg/kg, 10 mg/kg, 15 kg/mg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, or 55 mg/kg. The ALA may be administered orally, intravenously, via aerosol, via immersion, via lavage, and/or topically. Although a diagnostic dosage is contemplated for visualization of the residual cancer cells, precancer cells, and satellite lesions, it is within the scope of the present disclosure to use the disclosed devices, systems, and methods to provide guidance during treatment and/or removal of these cells and/or lesions.

[0085] The ALA given to the patient induces porphyrin formation (protoporphyrin IX (PpIX)) in tumor/cancer cells present in the patient and, when the cells containing PpIX are illuminated by the appropriate excitation light, an emission having a wavelength that appears as red fluorescence from cells containing the PpIX is captured by the imaging device. These cells are then visible against the green fluorescence emitted by the healthy tissues (which have broken down the PpIX into Heme and, thus, do not fluoresce a red color), which enhances the red-to-green fluorescence contrast between the tumor/cancer tissue cells and normal tissue cells (e.g., collagen) imaged with the device. ALA is non-fluorescent by itself, but PpIX emissions, when excited by excitation light having a wavelength of between 400 nm and about 450 nm, have wavelengths of about 630 nm, about 680 nm, and about 710 nm, with the 630 nm emission being the strongest.

[0086] Tissue phantoms, as discussed herein, may be used to calibrate an imaging device and/or to provide practice for an operating surgeon. For example, the

tissue phantoms may represent and mimic the optical properties of “normal” or “healthy” tissue. Additionally, the tissue phantoms may include one or more portions that represent and mimic the optical properties of diseased or abnormal tissue such as, for example, “cancerous” tissue. In alternative embodiments, the tissue phantom may be configured to include more than one type of tissue and can be used to calibrate an imaging device or other device configured to distinguish one tissue type from another or locate one tissue type relative to another. In addition to calibration, the phantom can also be used for training purposes. For example, the phantom may contain a first tissue type such as adipose tissue or connective tissue, both of which are components of breast tissue, and a second type of tissue such as blood or vasculature (also a component of breast tissue), and the phantom can be used to train surgeons to locate the blood or vasculature relative to the adipose or connective tissue with an imaging device. Similarly, phantoms can be used to train surgeons and clinicians to distinguish between healthy tissue and abnormal or diseased tissue. As the type of tissue (e.g., vascular, adipose, or connective) may impact the appears of the diseased/abnormal tissue relative to the healthy tissue, training with phantoms may promote better surgical outcomes. Tissue phantoms, as discussed herein, may also be used to train users of a fluorescence imaging device to correctly identify tissues based on their fluorescence, such as identifying a red tumor against a green/pink connective/adipose tissue background. Additionally, tissue phantoms, as discussed herein, may be used to show how certain tissues fluoresce when imaged with such a fluorescence imaging device, e.g., to demonstrate the color(s) a particular tissue fluoresces to a user. For example, FIGS. 2A and 2B show images of how a connective tissue phantom material will appear

(white in color) under white light (WL) imaging (FIG. 2A) and how the same connective tissue phantom material will appear (bright green in color) under fluorescent (FL) imaging using 405 nm wavelength illumination. Similarly, FIGS. 2C and 2D show images of how an adipose tissue phantom material will appear (pink in color) under white light (WL) imaging (FIG. 2C) and how the same adipose tissue phantom material will appear (brownish-pink tor brownish-orange color) under fluorescent (FL) imaging using 405 nm wavelength illumination.

[0087] In accordance with one aspect of the present disclosure, the tissue phantom is configured to include a “normal” or “healthy” tissue portion and one or more “diseased” or “abnormal” tissue portions. The healthy tissue of the tissue phantom can be any type of tissue and the diseased tissue or “target” tissue of the tissue phantom can be chosen to mimic any disease found in the particular type of healthy tissue modeled by the tissue phantom. The examples provided herein discuss a breast tissue phantom having one or more areas of diseased tissue, i.e., cancerous tissue or tumors. It should be understood that these examples are non-limiting examples only and that the concept of a tissue phantom comprising healthy and diseased tissue is applicable to many other types of human and animal tissues and their diseases. Although discussed herein with regard to breast tissue, it is possible to use the present disclosure as a guide to create a tissue phantom representative of any type of tissue having a disease, based on the optical properties for normal tissue and diseased tissue for the particular tissue of interest. Examples of other “diseased tissue” for which optical properties may differ from healthy tissue include, but are not limited to, inflamed tissue (e.g., affected by rheumatoid arthritis), fibrotic tissue, and ischemic tissue. For the “diseased tissue” of

interest, the optical absorption coefficient and reduced scattering coefficient of the tissue type, for both normal and diseased tissues, at the wavelength of light that is being used for excitation of the tissue are determined.

[0088] As disclosed herein, a tissue phantom may be used with an imaging device in order to determine the presence, location and/or amount of the “cancerous” tissue with respect to the “normal” tissue within the tissue phantom. In some example embodiments, “cancerous” tissue may comprise a tissue representation including a material such as a fluorophore configured to become visible and differentiated from surrounding tissues under illumination with the excitation light source of the imaging device. For example, a “cancerous” tissue representation may include a fluorophore configured to fluoresce red when illuminated with an excitation light having a wavelength of between about 400 nm and 450 nm. An example of a fluorophore included in the “cancerous tissue” of the phantom that fluoresces a red color when illuminated with excitation light having a wavelength between about 400 nm and about 450 nm is the porphyrin PpIX. Alternatively, other fluorophores such as quantum dots, ICG, IRDye800/IRDye680, and dyes (including blue dyes such as methylene blue, patent blue V, etc.) can be used to represent tissues that are different from healthy tissue, also referred to herein as “target tissue.”

[0089] Such results may allow a user to calibrate the imaging device, if the concentration of PpIX (or other fluorophore) within the tissue phantom is known by the user. Example devices, systems, and methods for detecting cancer cells containing PpIX or other induced porphyrins during surgical intervention are disclosed in U.S. Provisional Patent Application No. 62/625,983, filed February 3, 2018 and entitled

"Devices, Systems, and Methods for Tumor Visualization and Removal," and in PCT/CA2019/000015, filed February 1, 2019, entitled "Devices, Systems, and Methods for Tumor Visualization and Removal" and published as WO2019/148,268 on August 8, 2019, the entire content of each of which is incorporated herein by reference.

[0090] Tissue phantoms as disclosed herein can include the various features, characteristics, materials, and uses as disclosed in detail in Int'l Patent Application No. PCT/IB2020/050379, filed on January 17, 2020, titled "TISSUE PHANTOM," the entire contents of which are incorporated by reference herein. In addition to the features and configurations disclosed in the above-referenced application, tissue phantoms of the present disclosure can include additional features that can provide additional accuracy, precision, and/or resolution as compared to prior designs. Additionally, various features and configurations disclosed herein can facilitate manufacturing tissue phantoms having the desired characteristics noted above

[0091] In some example embodiments, a tissue phantom is a modular system and can include a chip portion having a plurality of wells configured to contain a fluorophore composition. In some examples, the wells can have varying sizes and may contain fluorophore compositions having varying concentrations. The chip portion can be positioned within one or more layers of tissue phantom material configured to approximate the characteristics of biological tissue, such as adipose tissue, connective tissue, or other tissue characteristics. In some example embodiments, the chip portion is refillable and provides a real-time adjustable depth capability using thin film phantoms.

[0092] In some examples, a tissue phantom can be used to test whether an imaging device's overlay performs on different tissue backgrounds and to measure the minimum fluorophore quantities (target size, concentration) that can be successfully detected and overlaid. A tissue phantom may be used device's ambient light imaging capability can be evaluated by imaging the phantom in ambient and dark room conditions and comparing the sensitivity in the two scenarios. In other examples, the tissue phantom can be used to quantify the minimum detectable concentration and target size that an imaging device can detect at multiple depths below the surface.

[0093] In some examples, an imaging system's quantification abilities may be verified by comparing the known vs. measured concentrations, including below the surface and for different well sizes using a tissue phantom. In other examples, fluorophores in the tissue phantom base and the fluorophore within the chip can be different. The tissue phantom material can be molded into the desired anatomy (e.g. breast cavity) to evaluate ergonomics while imaging.

[0094] For example, referring now to FIG. 1, a tissue phantom system or assembly 100 according to an example embodiment of the present disclosure is shown. The tissue phantom system 100 is shown in exploded view to facilitate illustration of the various portions thereof. For example, tissue phantom systems according to the present disclosure can be customized to vary the depth within the tissue phantom system that the “diseased tissue” or “cancerous tissue” is located from a surface of the phantom. In accordance with the present disclosure, the tissue phantom assembly can include one or more tissue phantom portions 102A, 102B, which may comprise materials configured to approximate the characteristics of adipose tissue, connective tissue, or combinations

thereof. For example, each of the tissue phantom portions 102A, 102B may each comprise any material that is sufficient to mimic optical properties of “normal” or “healthy” tissue, i.e., tissue that is not cancerous or otherwise diseased, to provide the tissue phantom 100 with optical properties that approximate the characteristics of normal tissue. In some embodiments, each of the tissue phantom portions 102 may be formed of a tris buffer and a gelatin mixture. The gelatin mixture can include additional materials to approximate the characteristics of healthy tissue. In one example, the gelatin mixture includes hemoglobin and Intralipid to impart the desired optical properties. In one example embodiment, the material(s) of the tissue phantom portions 102A, 102B may be homogenous throughout such that the healthy tissue portion of the tissue phantom 100 comprises no more than a 2% variation in a full spectrum reflectance measurement throughout the tissue phantom system 100.

[0095] The material used for the tissue phantom portions 102A, 102B can include one or more colorants such as dyes to approximate the color of biologic tissue such that the tissue phantom portion mimics the appearance of the type of tissue it represents (e.g., adipose or connective tissue). This allows the appearance of the tissue phantom under fluorescent imaging to closely match the appearance of the corresponding biological tissue, which the tissue phantom represents, when under the same fluorescent imaging conditions. When comparing the color of the imaged tissue phantom to the color of the corresponding biological tissue, comparisons are made using the same imaging device from the same distance, with the same optical power, and with the imaging target (phantom or biologic tissue) placed in the center of the imaging device’s field of view, with a goal of replicating the tumor-to-normal contrast

that would be encountered in the clinic. For example, connective tissues appear bright green under fluorescence imaging, PpIX, a fluorophore used in the tumor material and also found in biologic tumors of patients that have been administered ALA, appears bright red under fluorescence imaging, and adipose tissue appears an orange-brown color under fluorescence imaging. In one example, the tissue phantom portions configured to approximate adipose tissue may include Direct Red 81, yellow food coloring, or other dyes and/or colorants. In one example embodiment, the connective tissue phantom material contains 8% gelatin, 0.135 g/L hemoglobin, and 3.30% intralipid. In another example, the adipose tissue phantom material includes 8% gelatin, 0.041 g/L hemoglobin, 0.030% yellow food coloring, 50 μM DR81, and 2.1% intraplid.

[0096] As shown in FIGS. 3A and 3B, the tissue phantom portions 102A may be formed as thin films of the phantom tissue material. For example, FIGS. 3A and 3B shown an example tissue phantom material thin film 102A which is mounted on a fused silica wafer for ease of handling. A customizable tissue phantom system 100 in accordance with the present disclosure may include a plurality of these tissue phantom material thin films 102A to allow a depth of the healthy tissue phantom material (shown in green in FIG. 1) positioned above the fluorophore support structure (fluorophore chip, discussed below) to be adjusted as desired, by adding or removing tissue phantom material thin films 102A. In the example embodiment shown in FIGS. 3A and 3B, the tissue phantom material thin film has a thickness of 0.9 mm and is mounted on a fused silica wafer having a thickness of 0.7 mm. In some example embodiments, the thickness of the phantom material thin films 102A can range from about 100 μm to about 1000 μm (0.1 mm to 1 mm) as desired. In another example embodiment, the

phantom material thin films may be thicker, for example, having a thickness between about 1 mm to about 1 cm or more.

[0097] The tissue phantom system 100 also includes a tissue phantom portion 102B in the form of a base of the tissue phantom system 100. The tissue phantom base portion 102B is intended to support a portion of the tissue phantom system configured to approximate the optical characteristics of diseased (e.g., cancerous) tissue. The tissue phantom base portion 102B is used to facilitate replication of the fluorescence (FL) of a chosen healthy tissue type in order to reproduce the tumour-to-normal contrast expected when imaging human tissues. Use of the base portion 102B also permits replication of the optical properties of the healthy tissue type so that the light interacting with the base is absorbed/scattered in a similar fashion to human tissues. Thus, in the tissue phantom system 100, the tissue phantom base portion 102B represents the healthy tissue positioned below or underneath the diseased or cancerous tissue found *in situ* in breast cancer patients. In example embodiments, the tissue phantom base portion 102b is at least as large as the chip/fluorophore support portion in diameter and is of sufficient depth to minimize light transmission through the material (since human tissues being imaged are so thick that light will not transmit through the tissue). In the example embodiments discussed herein, the base portion 102B has a diameter of about 10 centimeters and has a depth of about 8mm to about 1cm.

[0098] The tissue phantom system 100 also includes a portion configured to approximate the optical characteristics of diseased (e.g., cancerous) tissue. For example, in the embodiment of FIG. 1, this portion can be a fluorophore chip, fluorophore support, or other supportive structure configured to contain fluorophores. In

the example embodiment of FIG. 1, this portion is as a tray 104 comprising a plurality of wells 106 configured to hold one or more materials different from the material of the tray and different from the tissue phantom materials discussed above. For example, each well 106 may contain one material that approximates the optical properties of diseased tissue. In addition, a second material configured to cause the diseased tissue to fluoresce in response to illumination with excitation light is included. These materials may be in solid or liquid form, or a combination thereof. In accordance with one example embodiment, the first composition may include any material that capable of mimicking the optical properties of “cancerous” tissue to provide one or more portions of the phantom with optical characteristics substantially the same as the optical characteristics of cancerous tissue. The first composition may include, for example, agar, phosphate buffered saline (PBS), water, agarose, dimethyl sulfoxide (DMSO) and/or blood tissue. These components may be used to hold the second material in suspension in the solution.

[0099] The second material may include a fluorophore such as PpIX, indocyanine green (ICG), and/or another fluorescent dye, quantum dots, such as 630 nm quantum dot, or other fluorescent materials such as quantum dots, ICG, IRDye800/IRDye680, and dyes (including blue dyes such as methylene blue, patent blue V, etc.). In one example embodiment, the quantity of fluorophore present in each well is based on the size of the well and the concentration of the fluorophore used, resulting in a plurality of wells of various sizes with varying concentrations of fluorophore (the concentration of fluorophore may vary between wells of the same size). The well sizes may range in representation, for example, from the size of a few cancer cells to the size of a palpable

tumor. Each well 106 may include a solution with a different concentration of PpIX and/or other fluorescent dye, such that the concentration of PpIX or fluorophore in each well 106 can differ from that of an adjacent well 106. In addition, the plurality of wells 106 can include wells of varying sizes (e.g., diameters and/or depths). In this way, the tray 104 includes a variety of sizes and concentrations of materials approximating diseased tissue. Such variations in known size and concentration of materials approximating diseased tissue can be used to calibrate imaging devices by an end user. For example, the tissue phantom 100 can be used to confirm correct calibration of an imaging device by ensuring the fluorescence emissions from the fluorophore in each well 106 matches a standard, expected emission profile determined under controlled conditions.

[00100] Referring now to FIGS. 5A-5C, an example embodiment a fluorophore support or tray 104, which is shown in plan view (FIG. 5C). The tray 104 comprises a plurality of wells 106A-E arranged in descending size from a radially outward positioned well to a radially inward positioned well relative to a center 208 of the tray 104. While the tray 104 in the embodiment of FIG. 5C includes the plurality of wells 106A-E arranged generally in a circular array, other configurations are within the scope of the disclosure, such as rectangular arrays, irregular arrays, or other configurations. Further, in the embodiment of FIGS. 5A-5C, each well of the plurality of wells 106A-E exhibits a circular cross-sectional shape. The cross-sectional shapes disclosed herein are non-limiting, and other cross-sectional shapes are considered within the scope of the disclosure based on various considerations. For example, circular cross-sectional shapes may facilitate manufacturing using commonly available equipment for

subtractive manufacturing, while other manufacturing methods, such as additive manufacturing, may be conducive to other shapes and configurations of the plurality of wells 106A-E.

[00101] The plurality of wells 106 can vary from a larger size (e.g., wells 106A) to a smaller size (e.g., wells 106E). For example, in the embodiment of FIGS. 5A-5C, the size can range from a diameter of about 100 micrometers (μm) to about 5000 μm, with the wells decreasing in size as the distance toward the center 208 of the tray 104 decrease. That is, the wells 106A around a perimeter of the tray 104 can be larger in diameter (such as, for example, 5000 μm) while the wells 106E positioned most radially inward on the tray 104 can be smaller in diameter (such as, for example, 100 μm) while the wells 106B-D in between can exhibit various sizes in between, e.g., generally in proportion to their radial location on the tray 104. In the example of FIGS. 5A-5C, the wells 106A-E form five concentric rings, each ring having ten equal size wells, although more or fewer rings of different sizes are within the scope of the disclosure, such as three concentric rings, four concentric rings, or more than five concentric rings. In one example embodiment, the tray 104 includes five (5) radial arrays (rings) of 10 wells each, the diameters of the wells in each radial array decreasing in size moving from an outer perimeter of the tray to a center of the tray. For example, the outermost ring of wells may have a diameter of 5 mm and the innermost ring of wells may have a diameter of 100 micrometers. In this example embodiment, the well diameters are, from smallest (innermost ring) to largest (outermost ring) 100 μm, 250 μm, 500 μm, 1000 μm (1 mm), and 5000 μm (5 mm).

[00102] The tray 104 can be made from a material that is not autofluorescent and has suitable mechanical characteristics for production and sufficient strength for handling. Various polymer materials can be used to form the tray 104. One example of a suitable polymer material is polydimethylsiloxane (PDMS). The tray 104 can be formed via additive manufacturing methods, subtractive manufacturing methods, or other approaches. In some example embodiments, the tray 104 can be formed of PDMS molded using a mold or may be created through additive manufacturing, such as 3d printing, for example, or through other known techniques. In some embodiments, the tray 104 can have a thickness in the range of millimeters or tens of millimeters. In the embodiment of FIGS. 5A-5C, the tray 104 can have a thickness chosen from a range of from about 1 mm and about 5 mm. The thickness of the tray and the resultant depth of the wells 106 may be selected to correlate with the type of diseased or cancerous tissue the tray represents. For example, a relatively thick tray with relatively deep wells may represent a solid tumor while a relatively thin tray with relatively shallow wells my represent a small area of residual carcinoma. FIG. 5D shows the tray of FIGS. 5A-5C filled with 630 nm quantum dots in various concentrations. The concentrations are the same in each radial column of the chip. The tray 104 may also include alignment features 105, as discussed further below.

[00103] Some of the wells 106 may be sized such that they cannot be filled with the materials approximating the diseased tissue using conventional methods, such as filling via pipette or other device suitable for filling the larger wells. Accordingly, in some embodiments, smaller-sized wells 106 (e.g., wells 106C-E) may be filled using alternative methods. In some example embodiments, wells having a size too small to fill

using a pipette or other device can be filled using capillary action. In some embodiments, such an approach may involve forming wells 106 of the plurality of wells 106 from tubing, such as hypotubing. The hypotubes may be made of steel or other materials, such as for example, transparent fused silica capillary tubes. In such examples, hypotubes having an internal diameter corresponding to the desired diameter of the well can be positioned within apertures in the tray 104 to form the wells 106. The apertures in the tray 104 can have an internal diameter corresponding to an external diameter of the hypotube to be inserted. In some example embodiments, hypotubes can be used to form some of the smaller well sizes, while the larger wells are formed in the PDMS material of the tray 104 itself. For example, in the embodiment of FIGS. 5A-5C, the smallest three concentric rings (i.e., wells 106C-E) can be formed using hypotubes. The smallest (i.e., wells 106E closest to the center 208 of the tray 104) can be formed of tubes having an internal diameter of 100 μm, the second smallest (i.e., wells 106D immediately adjacent and radially outward from the smallest wells) can be formed of tubes having an internal diameter of 250 μm, and the third smallest wells 106C can be formed of tubes having an internal diameter of 500 μm. These configurations and sizes are example in nature only, and a person of ordinary skill in the art would understand that variations to the number, size, type, and arrangement of tubing are within the scope of the disclosure.

[00104] To facilitate filling the wells formed using hypotubes, the hypotubes may be only partially inserted into the tray 104 and then introduced into a reservoir containing fluorescent material, which is then drawn into the hypotubes by capillary action. The tubes can then be fully inserted into the tray 104 such that the tubes are

flush with the tray 104 on both sides of the tray 104, thereby creating filled wells 106 with the desired size.

[00105] For example, with reference now to FIG. 6A, a side view of the tray 104 of FIG. 5C is shown. A plurality of tubes 310, which may be hypotubes having a length equal to a thickness t of the tray 104, partially protrude from the tray 104. In the example embodiment of FIG. 6A, the tubes 310 may comprise fused silica or other materials suitable for hypotubes. Further, each of the tubes 310 can have an inside diameter corresponding to the desired diameter of the specific well 106 (FIGS. 1 and 5C) with which they are associated, as discussed above.

[00106] To fill the tubes 310, the tubes 310 may be introduced into a reservoir containing a fluorophore, such as the materials discussed above to approximate the optical characteristics of diseased tissue. For example, referring now to FIG. 6B, a fluorophore reservoir 412 is shown. The fluorophore reservoir 412 contains a plurality of channels 414 which can be filled with the materials to be drawn into the tubes 310. The channels 414 can be aligned with the wells 106 of the tray 104 to reduce the total volume of fluorophore needed to fill the reservoir 412. For example, the reservoir 412 can include alignment features 415 corresponding to alignment features 105 of the tray 104 (FIG. 2). Alternatively, the channels 414 can be formed as concentric rings corresponding to the rings of the wells 106, and the channels can be in fluid communication with one another or fluidically isolated from one another, as shown in FIG. 6B. In the embodiment of FIG. 6B, each of the channels 414 can be filled with a fluorophore having a different concentration, so that wells 106 of the tray 104 have

varying concentrations in a circumferential direction, as further discussed below in connection with FIG. 9.

[00107] Referring now to FIGS. 6C and 6D, a side view of the fluorophore reservoir 412 and tray 104 are shown. In FIG. 6C, the tray 104 is shown positioned above the reservoir 412. In FIG. 6D, the hypotubes 310 of the tray 104 are introduced into the channels 414 (FIG. 6B), and the hypotubes 310 are filled via capillary action drawing the fluorophore material into the hypotubes 310. Once the hypotubes 310 are filled via capillary action, a fused silica wafer 616 is applied to the PDMS tray 104. In an example embodiment, the silica wafer 616 may be adhered to the tray 104 via frictional force inherent to the PDMS material of the tray 104. In some embodiments, a silicone grease may be used between the silica wafer 616 and the tray 104 to seal the wells and hypotubes 310. In other embodiments, the silica wafer 616 may be bonded to the tray 104 via an adhesive or other method of bonding.

[00108] Referring now to FIG. 7, the tray 104 is inverted from the position shown in FIG. 6D, and the hypotubes 310 are pressed fully into the tray 104 such that the hypotubes 310, which are filled with fluorophore material by the capillary action approach discussed above, are flush with the tray 104. The hypotubes 310 were gently pressed in using forceps, taking care to only contact the side of the capillary tube and not the center which could result in accidental removal of some fluorophore and risk contamination. While the tubes were inserted one by one, it is within the scope of the present disclosure to create a simple device to push in all the tubes at once.

[00109] With the fused silica wafer 616 in place, the larger wells 106A and 106B can be filled via pipette 818, as shown in FIG. 8. In some embodiments, the material of

the tray 104 is translucent to such a degree that fluorescence from material in the larger wells 106 may interfere with other wells. To mitigate any such interference, the inside surfaces of the larger wells 106A and/or 106B may be coated with an opaque material, such as paint or other coating, prior to filling the wells 106A and/or 106B. When using transparent fused silica capillary tubes, it may also be desirable in some cases to coat the exterior surfaces of the tubes in the opaque material.

[00110] As noted above, different wells 106 may be provided with material having different concentrations of fluorophores to facilitate precision and accuracy of imaging device calibration. Referring now to FIG. 9, a schematic diagram showing the varying sizes and concentrations of fluorophore material are shown. Each of the wells 906 positioned along a given radially extending line varies in size, with the sizes increasing in the radially outward direction. The concentration decreases from a highest concentration at the 12 o'clock position of FIG. 9, and gradually decreases in the clockwise direction. It is contemplated that the concentration may decrease to 0 (i.e., there may include a "control" concentration provided in one of the wells).

[00111] The different sizes of fluorophore wells 906, and the gradual decrease in concentration, provides varying levels of fluorescence response to enable calibration and testing of a fluorescence imaging device.

[00112] The tray having the plurality of wells filled as described above forms part of a tissue phantom system. Once the tray is complete and the wells are filled as discussed above, thin tissue phantom films can be positioned, one at a time (or more than one at a time if desired) to increase the fluorophore depth below the imaging surface. Each thin tissue phantom film may represent a particular type of tissue, such as

connective tissue and/or adipose tissue, or a combination of tissue types, such as a combination of connective tissue and adipose tissue. In practice, thin tissue phantom films are placed over the tray (e.g., tissue phantom portions 102 shown in FIG. 1) individually, and on top of any previously placed thin tissue phantom films (to form layers of thin tissue phantom films), and after each film is placed, the surface of the tissue phantom is imaged with the imaging device to provide information regarding the appearance, intensity, and color of the fluorescence of the “cancerous” tissue through the healthy tissue formed by the thin phantom tissue films when illuminated by excitation light of the imaging device.

[00113] Each of the thin tissue phantom films has been created to mimic the optic properties of the tissue the film represents. Thus, for example, the material that forms the thin tissue phantom films mimic the absorption and reduced scattering coefficients at the wavelength of the excitation light (e.g., 400 nm – 450 nm or 405 nm) of the tissue type represented by the phantom. As noted above, the thin tissue phantom films may comprise a mixture of tris buffer and gelatin to provide optical properties that approximate that of healthy adipose tissue or connective tissue. To form the tissue phantom films, tissue phantom material can be heated to a liquid or semi-liquid state and pressed between flat platens, such as metal or other materials, separated by spacers having the desired thickness of the tissue phantom films. The material is then cooled to congeal to a generally stable form and shape. For example, referring now to FIG. 10, the tissue phantom material 1002 is pressed between solid platens 1020 separated by spacers 1022 having the desired thickness of the completed phantom material films. In some example embodiments, the thickness of the phantom material

thin films can range from about 100 μm to about 1000 μm (0.1 mm to 1 mm) as desired. In another example embodiment, the phantom material thin films may be thicker, for example, having a thickness between about 1 mm to about 1 cm or more and a customizable tissue phantom system may include sufficient tissue phantom material thin films of varying thicknesses to allow a depth of "healthy tissue" positioned above the fluorophore support portion to range between 2.5 mm and 10 cm. This may be useful in an application where the imaging illumination is going to be in the infrared range. The ranges of thickness provided herein are examples only and the thickness of phantom material thin films may vary dependent upon the type of illumination and imaging the tissue phantom system will be subjected to. The purpose of the different thicknesses is to measure the maximum depth that each well, containing a unique fluorophore quantity, can be imaged. In one example experiment, a tissue phantom system was imaged with films from 200μm up to 2400 μm before the fluorescence from the largest well with the highest concentration was no longer visible. Wells with lower fluorophore quantities were only visible at lesser depths. For IR applications, the maximum imaging depth may be centimeters deep within tissue, so films can be fabricated at appropriate thicknesses to measure the maximum depth in these cases

[00114] During preparation of the tissue phantom films, the phantom material can be separated from the surface of the platens by a film separator, such as PARAFILM®, available from Bemis Company, Inc. (Neenah, WI, USA). The film separator may facilitate handling of the finished phantom film and prevent the phantom material from sticking to the platens. In some embodiments, the film separator can be removed from the phantom material, and the phantom material can be trimmed to cover a side of the

tray 104. In some embodiments, the phantom material may be placed on a fused silica wafer to provide structure to the phantom material, and the silica wafer and phantom material may be placed on the tray 104. One or more layers of phantom material may be placed on each side of the tray 104 to provide the desired thickness of tissue phantom material over the wells 106 of the tray 104. While fused silica wafers are used in example embodiments of the phantom material thin films to enhance handling of the thin films, it is not necessary to use the wafers. The impact of the fused silica on imaging and light transmission is minimal, with an expected loss of about 2%-10% of light transmitted through the fused silica (in each direction, i.e., the intensity of light transmitted from the imaging device to the fluorophore is decreased, and the light intensity emitted from the fluorophore to the camera is decreased), depending on the thickness of the wafer. When using .7 mm thick wafers as disclosed herein, the expected loss of light transmission is about 2%.

[00115] In some embodiments, one or more layers of tissue phantom material can include both phantom material configured to approximate the optical characteristics of connective tissue and phantom material configured to approximate the characteristics of adipose tissue. For example, the different materials may be slightly “marbled” together to produce a tissue phantom with portions representing adipose tissue and portions representing connective tissue. For example, referring now to FIG. 11, a tissue phantom base portion 1102 including portions representing adipose tissue 1103 (which appear pink in FIG. 11) and portions representing connective tissue 1105 (which appear white in FIG. 11) is shown. The different portions can be created by creating a tissue phantom as discussed above in connection with FIGS. 3A, 3B, and 10 by combining, but not

completely mixing, some material configured to approximate connective tissue and some material configured to approximate adipose tissue, thereby creating a phantom with portions 1103 representing adipose tissue and portions representing connective tissue 1105. In one embodiment, the connective tissue material and adipose tissue material can be combined at a temperature at which the materials are sufficiently viscous to be non-confluent thereby enabling the phantoms to be arranged in patterns without mixing of the different materials. This composite tissue phantom material may take the form of a tissue phantom base portion and/or a tissue phantom material thin film. As certain embodiments of the tissue phantom system use very thin films, the composite thin films can be fabricated in accordance with the method discussed with relation to FIG. 10. For thick base portions of the tissue phantom system, such as the base portion shown in FIG. 11, it is possible to fabricate the composite base portion by pouring the two types of phantom material into a mold such as a petri dish.

[00116] Referring now to FIG. 12, a workflow 1200 for producing a tissue phantom is shown. At 1202, capillary tubes are inserted into apertures in a tray such that the capillary tubes protrude at least partially from the tray. At 1204, the capillary tubes protruding from the tray are introduced into a reservoir containing a fluorophore material. At 1206, the fluorophore material is drawn into the capillary tubes via capillary action. At 1208, the capillary tubes are pressed fully into the tray. Optionally, one or more fused silica wafers are adhered (e.g., via friction or a bonding agent) to the tray. At 1210, one or more tissue phantom portions are placed over one or both of the top and bottom side of the tray to provide a desired thickness of the tissue phantoms. Such

tissue phantom portions can include fused silica wafers as backing material to provide structure and support to the tissue phantom material.

[00117] Referring now to FIG. 13A, an example tissue phantom 1300A using adipose tissue phantom material is shown in use. Fluorescence material in wells (for example, wells 106 as discussed in connection with FIGS. 1 and 5A-5D) fluoresces through the tissue phantom material thin films (for example, thin films 102A discussed in connection with FIGS. 1, 3A, and 3B) upon exposure to excitation light, for example, from a fluorescence imaging device as discussed above. The known, differing well sizes and concentrations of fluorophore material as well as the know differences in the depth (total thickness) of the tissue phantom thin films positioned above the fluorescence material in the wells, as discussed above, provide various levels of fluorescence response, thereby enabling calibration of the imaging device as discussed above, e.g., by comparing fluorescence emitted by the tissue phantom to a known, standardized emission profile expected from the adipose tissue phantom 1300A. In the example shown in the fluorescent image of FIG. 13A, the adipose tissue phantom system 1300A comprises tissue phantom thin films providing a depth of adipose tissue of 300 µm.above the fluorescent material in the wells. In the example shown in the fluorescent image of FIG. 13B, the adipose tissue phantom system 1300B comprises tissue phantom thin films providing a depth of adipose tissue of 1645 µm above the fluorescent material in the wells. In each system the adipose tissue phantom thin film fluoresces a dark orangish-brown color. The difference between the tissue phantom thin film depths in tissue phantom systems 1300A versus 1300B is indicated by the bright red fluorescence visible through the tissue phantom thin films as shown in FIG. 13A versus

the much more subdued red fluorescence visible through the tissue phantom thin films as shown in FIG. 13B.

[00118] FIGS. 14A and 14B show images similar to FIGS. 13A and 13B, except the tissue phantom 1400A in FIG. 14A uses connective tissue phantom material to facilitate calibration of an imaging device. Similar to the approach discussed in connection with FIGS. 13A and 13B, the tissue phantom systems 1400A and 1400 B provide various levels of fluorescence response in situations where connective tissue is present to be compared to a known, standardized emission profile expected from the connective tissue phantom systems 1400A and 1400B. In the example shown in the fluorescent image of FIG. 14A, the connective tissue phantom system 1400A comprises tissue phantom thin films providing a connective tissue depth of 900 μm.above the fluorescent material in the wells. In the example shown in the fluorescent image of FIG. 14B, the connective tissue phantom system 1400B comprises tissue phantom thin films providing a depth of connective tissue of 409 μm above the fluorescent material in the wells. In each system the connective tissue phantom thin film fluoresces a green color. The difference between the tissue phantom thin film depths in tissue phantom systems 1400A versus 1400B is indicated by the subdued red fluorescence visible through the tissue phantom thin films as shown in FIG. 14A versus the much brighter red fluorescence visible through the tissue phantom thin films as shown in FIG. 14B.

[00119] The disclosure of the provisional application to which this application claims the benefit of priority also relies on and incorporates by reference the Chapters of the inventor's thesis, including Chapter 5 "*Optical Tissue Phantom Platform for Standardization of Fluorescence Imaging Devices*" and Chapter 6 "*Characterization of*

Imaging Sensitivity Using Phantoms." This portion of the inventor's thesis is presented in the Appendix of the provisional patent application. The Appendix further includes a draft article that will be submitted for publication after filing of the provisional application, the article is entitled "Customizable Optical Tissue Phantom Platform for Characterization of Fluorescence Imaging Device Sensitivity" which is incorporated by reference in its entirety herein. The Appendix also includes additional photographs and slides which also form part of the disclosure of the provisional patent application and the Appendix is hereby incorporated by reference in its entirety. The chapters of the thesis, draft article, photographs, and slides contained in the Appendix are provided in color to preserve the detail and clarity of the disclosure for features and elements which cannot be accurately represented in grayscale or black and white line drawings.

[00120] Illustrative examples of systems and methods described herein are provided below. An embodiment of a system and/or method described herein may include any one or more, and any combination of, the aspects/embodiments/features described below in any order and/or in any combination:

[00121] 1. A tissue phantom system, comprising: a base; a fluorophore support portion; and a plurality of tissue phantom films.

[00122] 2. The system of any preceding or following embodiment/feature/aspect, wherein the fluorophore support portion comprises a plurality of wells.

[00123] 3. The system of any preceding or following embodiment/feature/aspect, wherein the plurality of wells comprises at least two wells having a first size and at least two wells having a second size different from the first size.

[00124] 4. The system of any preceding or following embodiment/feature/aspect, wherein the base is configured to simulate fluorescence and optical properties of healthy tissue.

[00125] 5. The system of any preceding or following embodiment/feature/aspect, wherein the plurality of tissue phantom films is configured to form a top portion of the tissue phantom system.

[00126] 6. The system of any preceding or following embodiment/feature/aspect, wherein the top portion of the tissue phantom system has an adjustable thickness.

[00127] 7. The system of any preceding or following embodiment/feature/aspect, wherein the thickness of the top portion is adjustable by adding or removing tissue phantom films to the tissue phantom system when assembled.

[00128] 8. The system of any preceding or following embodiment/feature/aspect, wherein, when the tissue phantom system is an assembled state, the fluorophore support portion includes a first material exhibiting optical properties of diseased tissue and comprising a fluorophore material having a first concentration.

[00129] 9. The system of any preceding or following embodiment/feature/aspect, wherein, when the tissue phantom system is in an assembled state, the fluorophore support portion includes a second material exhibiting optical properties of diseased tissue and comprising a fluorophore material having a second concentration, different from the first concentration.

[00130] 10. The system of any preceding or following embodiment/feature/aspect, wherein, the fluorophore support portion is positioned on the base and one or more of

the plurality of tissue phantom films is positioned on or over the fluorophore support portion when the tissue phantom system is in an assembled state.

[00131] 11. The system of any preceding or following embodiment/feature/aspect, wherein at least one of the plurality of tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue.

[00132] 12. The system of any preceding or following embodiment/feature/aspect, wherein at least one of the plurality of tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

[00133] 13. The system of any preceding or following embodiment/feature/aspect, wherein the first material and the second material comprise a composition exhibiting fluorescence and optical characteristics of diseased human or animal tissue.

[00134] 14. The system of any preceding or following embodiment/feature/aspect, wherein the first material and the second material comprise at least one of agar, phosphate buffered saline, water, agarose, dimethyl sulfoxide, or blood tissue or cells.

[00135] 15. The system of any preceding or following embodiment/feature/aspect, wherein the fluorophore material comprises a protoporphyrin IX (PpIX), indocyanine green (ICG), quantum dots, or other fluorescent dye.

[00136] 16. The system of any preceding or following embodiment/feature/aspect, wherein the plurality of wells is arranged in a concentric pattern in or on the fluorophore support portion.

[00137] 17. A customizable tissue phantom system, comprising: a base portion configured to simulate optical properties of healthy tissue; a fluorophore support portion configured to contain fluorophores of varying quantities representative of diseased tissue; and a cover portion configured to be positioned on or over the fluorophore support portion; wherein an uppermost surface of the cover portion is configured to represent a skin layer of the tissue phantom system, wherein a distance between the uppermost surface of the cover portion and the fluorophore support portion represents a depth from the skin layer to the diseased tissue, and wherein the depth is adjustable by adjusting a thickness of the cover portion.

[00138] 18. The system of any preceding or following embodiment/feature/aspect, wherein the fluorophore support portion comprises a tray having a plurality of wells, wherein a quantity of fluorophores contained in each well is determined by the size of the well and the concentration of the fluorophore.

[00139] 19. The system of any preceding or following embodiment/feature/aspect, wherein the cover portion comprises one or more tissue phantom films.

[00140] 20. The system of any preceding or following embodiment/feature/aspect, wherein each of the tissue phantom films comprises a tissue phantom material configured to simulate the fluorescence and optical properties of healthy tissue.

[00141] 21. The system of any preceding or following embodiment/feature/aspect, wherein at least one of the tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue.

[00142] 22. The system of any preceding or following embodiment/feature/aspect, wherein at least one of the tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

[00143] 23. The system of any preceding or following embodiment/feature/aspect, wherein at least one of the tissue phantom films comprises portions of a first material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue and portions of a second material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

[00144] 24. The system of any preceding or following embodiment/feature/aspect, wherein each of the plurality of tissue phantom films has a thickness between about 100 μm and about 1 mm.

[00145] 25. The system of any preceding or following embodiment/feature/aspect, wherein a thickness of the cover portion may vary between about 100 μm and about 2000 μm.

[00146] 26. The system of any preceding or following embodiment/feature/aspect, wherein the fluorophore support portion comprises an aperture containing a tubular structure, wherein an interior volume of the tubular structure defines a well within the fluorophore support portion.

[00147] 27. The system of any preceding or following embodiment/feature/aspect, further comprising a fluorophore material in the well defined by the tubular structure, and one or more tissue phantom films positioned over a surface of the fluorophore support portion and over the well to form the cover portion of the phantom.

[00148] 28. The system of any preceding or following embodiment/feature/aspect, wherein the fluorophore support portion comprises a thickness, and the tubular structure comprises a length equal to the thickness of the fluorophore support portion.

[00149] 29. The system of any one of any preceding or following embodiment/feature/aspect, wherein: the aperture is one of a plurality of apertures in the fluorophore support portion; and the tubular structure is one of a plurality of tubular structures, each of the plurality of tubular support structure being associated with a respective aperture of the plurality of apertures.

[00150] 30. The system of any preceding or following embodiment/feature/aspect, wherein a first tubular structure of the plurality of tubular structures comprises an interior volume defined by a first diameter, and a second tubular structure of the plurality of tubular structures comprises an interior volume defined by a second diameter greater than the first diameter.

[00151] The system of any preceding or following embodiment/feature/aspect, wherein the tissue phantom system is made, formed, customized, or used by the method of any preceding or following embodiment/feature/aspect.

[00152] 31. A method of making a tissue phantom system, the method comprising: forming a fluorophore support portion having an aperture; moving a tubular structure within the aperture to a partially inserted condition; drawing a fluorophore-containing material into the tubular structure via capillary action; and fully inserting the tubular structure into the fluorophore support portion such that the tubular structure is flush with a top surface and a bottom surface of the fluorophore support portion.

[00153] 32. The method of any preceding or following embodiment/feature/aspect, further comprising sealing one or both of the top surface and the bottom surface of the fluorophore support portion with a fused silica wafer.

[00154] 33. The method of any preceding or following embodiment/feature/aspect, further comprising positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

[00155] 34. The method of any preceding or following embodiment/feature/aspect, wherein positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion comprises positioning at least one tissue phantom film comprising a material configured to exhibit fluorescence and optical properties of connective tissue over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

[00156] 35. The method of any preceding or following embodiment/feature/aspect, wherein positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion comprises positioning at least one tissue phantom film comprising a material configured to exhibit fluorescence and optical properties of adipose tissue over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

[00157] 36. The method of any one of any preceding or following embodiment/feature/aspect, wherein the aperture is a first aperture and the method further comprises: forming a second aperture in the fluorophore support portion; coating

an interior surface of the second aperture with an opaque material; and introducing a fluorophore-containing material into the second aperture.

[00158] 37. The method of any preceding or following embodiment/feature/aspect, wherein drawing a fluorophore-containing material into the tubular structure comprises positioning a portion of the tubular structure protruding from the fluorophore portion in the partially inserted condition of the tubular structure into a reservoir containing the fluorophore material.

[00159] 38. The method of any preceding or following embodiment/feature/aspect, further comprising creating a water-tight seal between the fluorophore support portion and the fused silica wafer with a silicone grease.

[00160] It will be appreciated by those ordinarily skilled in the art having the benefit of this disclosure that the present disclosure provides various example devices, systems, and methods related to fluorescent imaging. Further modifications and alternative embodiments of various aspects of the present disclosure will be apparent to those skilled in the art in view of this description.

[00161] Furthermore, the devices and methods may include additional components or steps that were omitted from the drawings for clarity of illustration and/or operation. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the general manner of carrying out the present disclosure. It is to be understood that the various embodiments shown and described herein are to be taken as example. Elements and materials, and arrangements of those elements and materials, may be substituted for those illustrated and described herein, parts and processes may be reversed, and certain features of the

present disclosure may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of the description herein. Changes may be made in the elements described herein without departing from the spirit and scope of the present disclosure and following claims, including their equivalents.

[00162] It is to be understood that the particular examples and embodiments set forth herein are non-limiting, and modifications to structure, dimensions, materials, and methodologies may be made without departing from the scope of the present disclosure.

[00163] Furthermore, this description's terminology is not intended to limit the present disclosure. For example, spatially relative terms—such as "beneath," "below," "lower," "above," "upper," "bottom," "right," "left," "proximal," "distal," "front," and the like—may be used to describe one element's or feature's relationship to another element or feature as illustrated in the figures. These spatially relative terms are intended to encompass different positions (i.e., locations) and orientations (i.e., rotational placements) of a device in use or operation in addition to the position and orientation shown in the drawings.

[00164] For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about" if they are not already. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least,

and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00165] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the present disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass any and all sub-ranges subsumed therein.

[00166] It is noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the," and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

[00167] It should be understood that while the present disclosure has been described in detail with respect to various example embodiments thereof, it should not be considered limited to such, as numerous modifications are possible without departing from the broad scope of the appended claims, including the equivalents they encompass.

What is claimed is:

1. A tissue phantom system, comprising:
a base;
a fluorophore support portion; and
a plurality of tissue phantom films.

2. The system of claim 1, wherein the fluorophore support portion comprises a plurality of wells.

3. The system of claim 2, wherein the plurality of wells comprises at least two wells having a first size and at least two wells having a second size different from the first size.

4. The system of any one of claims 1-3, wherein the base is configured to simulate fluorescence and optical properties of healthy tissue.

5. The system of any one of claims 1-4, wherein the plurality of tissue phantom films is configured to form a top portion of the tissue phantom system.

6. The system of claim 5, wherein the top portion of the tissue phantom system has an adjustable thickness.

7. The system of claim 6, wherein the thickness of the top portion is adjustable by adding or removing tissue phantom films to the tissue phantom system when assembled.

8. The system of any one of claims 1-7, wherein, when the tissue phantom system is an assembled state, the fluorophore support portion includes a first material exhibiting optical properties of diseased tissue and comprising a fluorophore material having a first concentration.

9. The system of claim 8, wherein, when the tissue phantom system is in an assembled state, the fluorophore support portion includes a second material exhibiting optical properties of diseased tissue and comprising a fluorophore material having a second concentration, different from the first concentration.

10. The system of any one of claims 1-9, wherein, the fluorophore support portion is positioned on the base and one or more of the plurality of tissue phantom films is positioned on or over the fluorophore support portion when the tissue phantom system is in an assembled state.

11. The system of any one of claims 1-10, wherein at least one of the plurality of tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue.

12. The system of any one of claims 1-11, wherein at least one of the plurality of tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

13. The system of any one of claims 8-12, wherein the first material and the second material comprise a composition exhibiting fluorescence and optical characteristics of diseased human or animal tissue.

14. The system of claim 13, wherein the first material and the second material comprise at least one of agar, phosphate buffered saline, water, agarose, dimethyl sulfoxide, or blood tissue or cells.

15. The system of any one of claims 8-15, wherein the fluorophore material comprises a protoporphyrin IX (PpIX), indocyanine green (ICG), quantum dots, or other fluorescent dye.

16. The system of any one of claims 2-15, wherein the plurality of wells is arranged in a concentric pattern in or on the fluorophore support portion.

17. A customizable tissue phantom system, comprising:

a base portion configured to simulate optical properties of healthy tissue;

a fluorophore support portion configured to contain fluorophores of varying quantities representative of diseased tissue; and

a cover portion configured to be positioned on or over the fluorophore support portion;

wherein an uppermost surface of the cover portion is configured to represent a skin layer of the tissue phantom system,

wherein a distance between the uppermost surface of the cover portion and the fluorophore support portion represents a depth from the skin layer to the diseased tissue, and

wherein the depth is adjustable by adjusting a thickness of the cover portion.

18. The system of claim 17, wherein the fluorophore support portion comprises a tray having a plurality of wells, wherein a quantity of fluorophores contained in each well is determined by the size of the well and the concentration of the fluorophore.

19. The system of claim 17 or claim 18, wherein the cover portion comprises one or more tissue phantom films.

20. The system of claim 19, wherein each of the tissue phantom films comprises a tissue phantom material configured to simulate the fluorescence and optical properties of healthy tissue.

21. The system of claim 19 or claim 20, wherein at least one of the tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue.

22. The system of any one of claims 19-21, wherein at least one of the tissue phantom films comprises a material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

23. The system of any one of claims 19-22, wherein at least one of the tissue phantom films comprises portions of a first material configured to exhibit fluorescence and optical characteristics of human or animal adipose tissue and portions of a second material configured to exhibit fluorescence and optical characteristics of human or animal connective tissue.

24. The system of any one of claims 19-23, wherein each of the plurality of tissue phantom films has a thickness between about 100 μm and about 1 mm.

25. The system of any one of claims 17-24, wherein a thickness of the cover portion varies between about 100 μm and about 2000 μm.

26. The system of claim 17, wherein the fluorophore support portion comprises an aperture containing a tubular structure, wherein an interior volume of the tubular structure defines a well within the fluorophore support portion.

27. The system of claim 26, further comprising a fluorophore material in the well defined by the tubular structure, and one or more tissue phantom films positioned over a

surface of the fluorophore support portion and over the well to form the cover portion of the phantom.

28. The system of claim 26 or claim 27, wherein the fluorophore support portion comprises a thickness, and the tubular structure comprises a length equal to the thickness of the fluorophore support portion.

29. The system of any one of claims 26-28, wherein:
the aperture is one of a plurality of apertures in the fluorophore support portion; and the tubular structure is one of a plurality of tubular structures, each of the plurality of tubular support structure being associated with a respective aperture of the plurality of apertures.

30. The system of claim 29, wherein a first tubular structure of the plurality of tubular structures comprises an interior volume defined by a first diameter, and a second tubular structure of the plurality of tubular structures comprises an interior volume defined by a second diameter greater than the first diameter.

31. A method of making a tissue phantom system, the method comprising:

forming a fluorophore support portion having an aperture;

moving a tubular structure within the aperture to a partially inserted condition;

drawing a fluorophore-containing material into the tubular structure via capillary action; and

fully inserting the tubular structure into the fluorophore support portion such that the tubular structure is flush with a top surface and a bottom surface of the fluorophore support portion.

32. The method of claim 31, further comprising sealing one or both of the top surface and the bottom surface of the fluorophore support portion with a fused silica wafer.

33. The method of claim 31 or claim 32, further comprising positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

34. The method of claim 33, wherein positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion comprises positioning at least one tissue phantom film comprising a material configured to exhibit fluorescence and optical properties of connective tissue over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

35. The method of claim 33 or claim 34, wherein positioning one or more tissue phantom films over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion comprises positioning at least one tissue phantom film comprising a material configured to exhibit fluorescence and optical

properties of adipose tissue over one of the top surface of the fluorophore portion and the bottom surface of the fluorophore support portion.

36. The method of any one of claims 31-35, wherein the aperture is a first aperture and the method further comprises:

forming a second aperture in the fluorophore support portion;

coating an interior surface of the second aperture with an opaque material; and

introducing a fluorophore-containing material into the second aperture.

37. The method of any one of claims 31-36, wherein drawing a fluorophore-containing material into the tubular structure comprises positioning a portion of the tubular structure protruding from the fluorophore portion in the partially inserted condition of the tubular structure into a reservoir containing the fluorophore material.

38. The method of any one of claims 32-37, further comprising creating a water-tight seal between the fluorophore support portion and the fused silica wafer with a silicone grease.

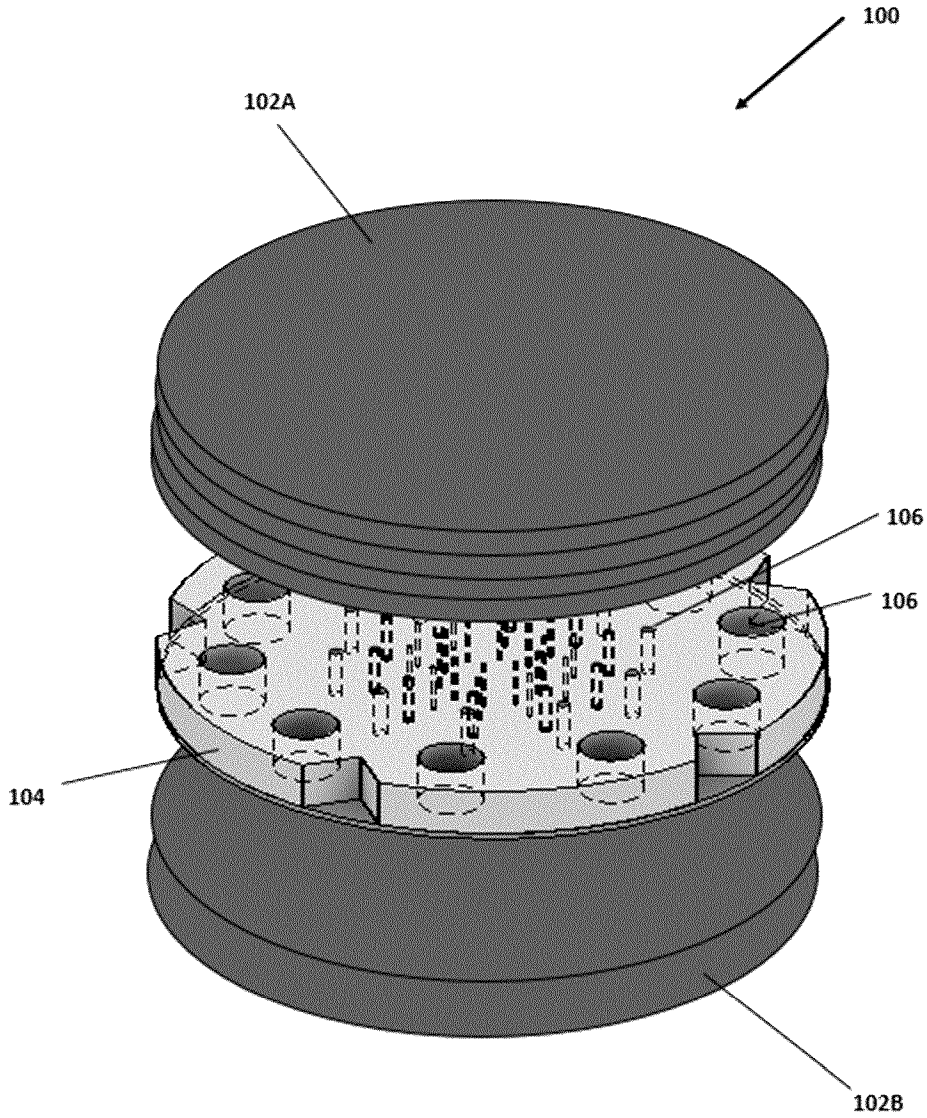


FIG. 1

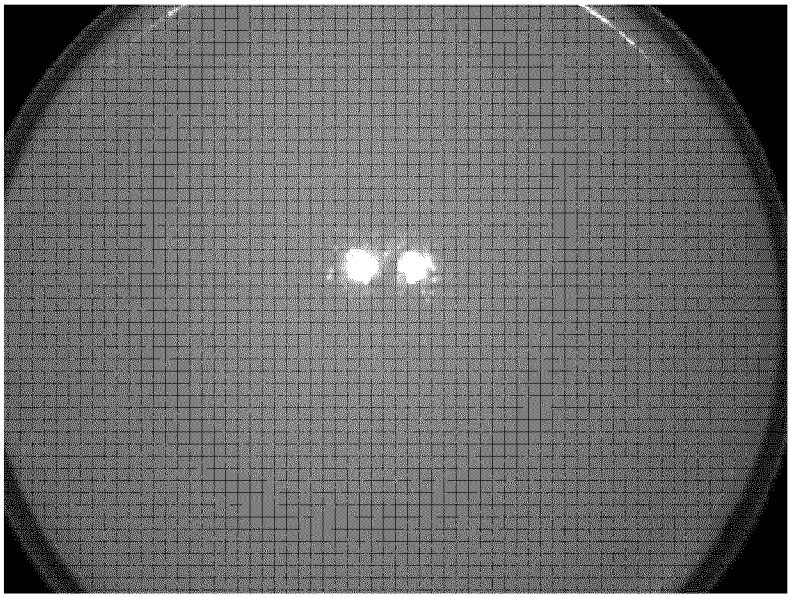


FIG. 2A

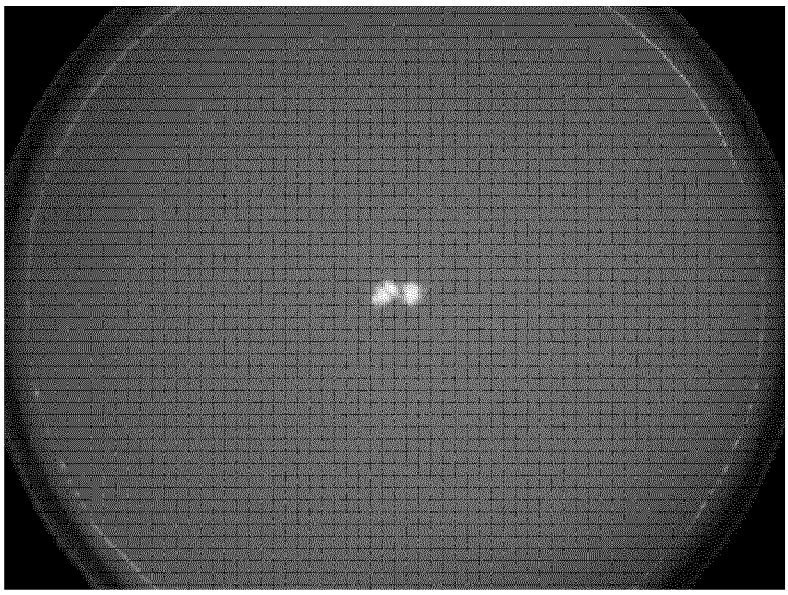


FIG. 2B

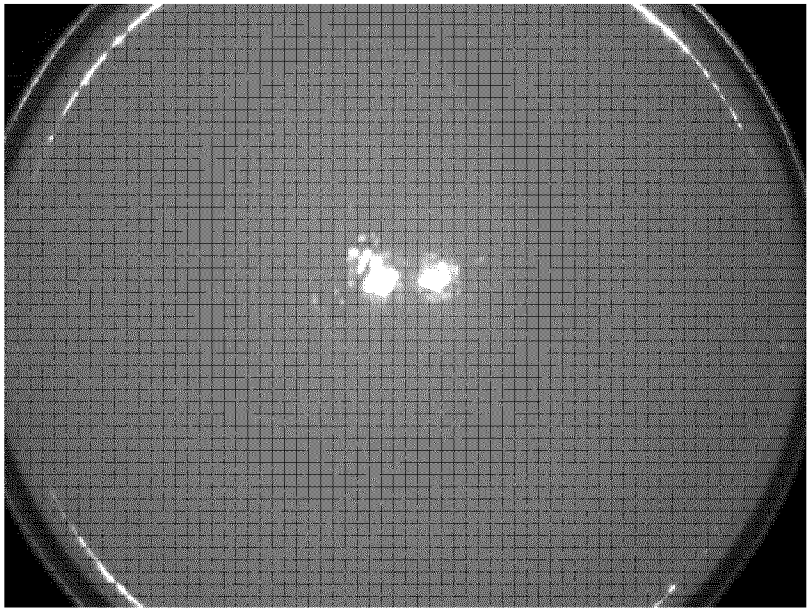


FIG. 2C

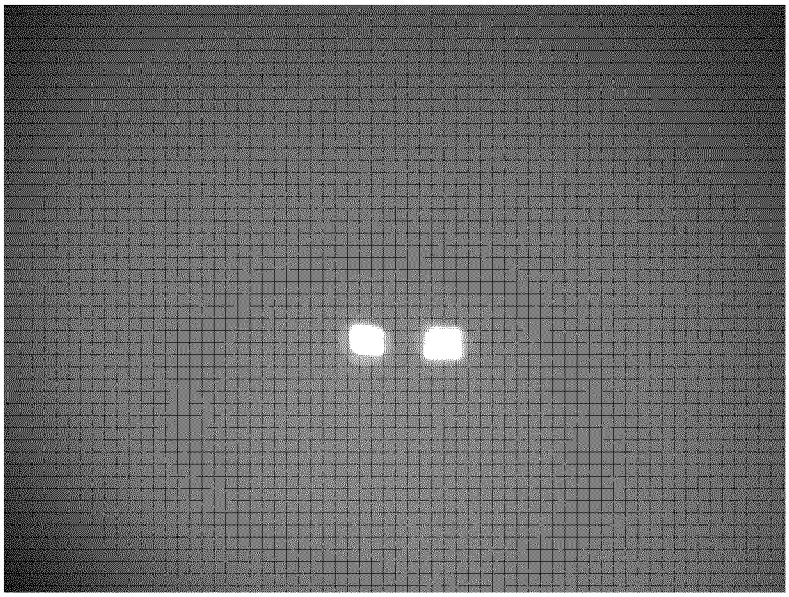


FIG. 2D

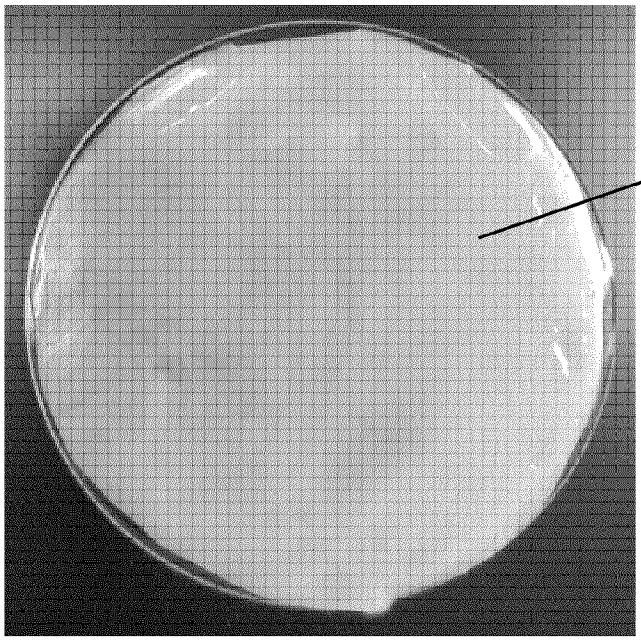

102A

FIG. 3A

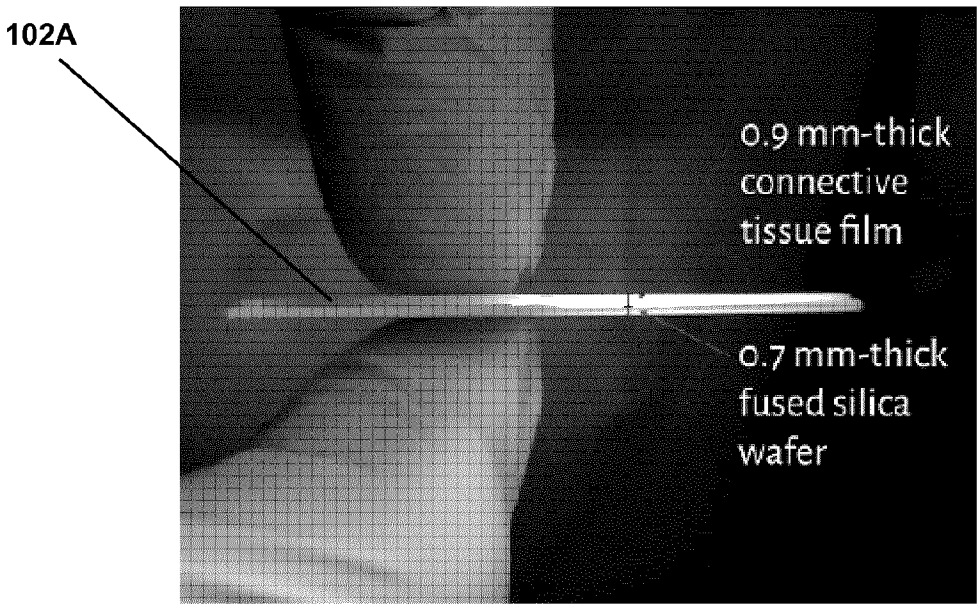

102A
0.9 mm-thick
connective
tissue film
0.7 mm-thick
fused silica
wafer

FIG. 3B

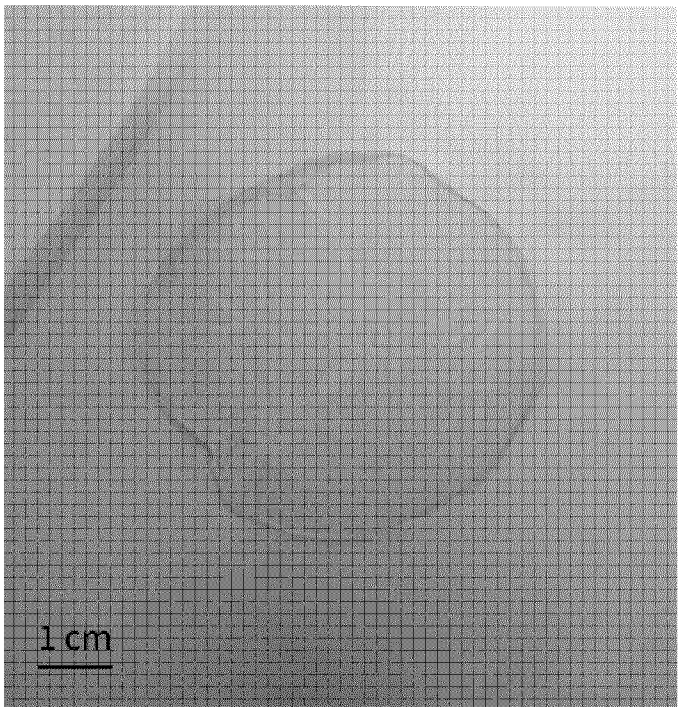


FIG. 3C

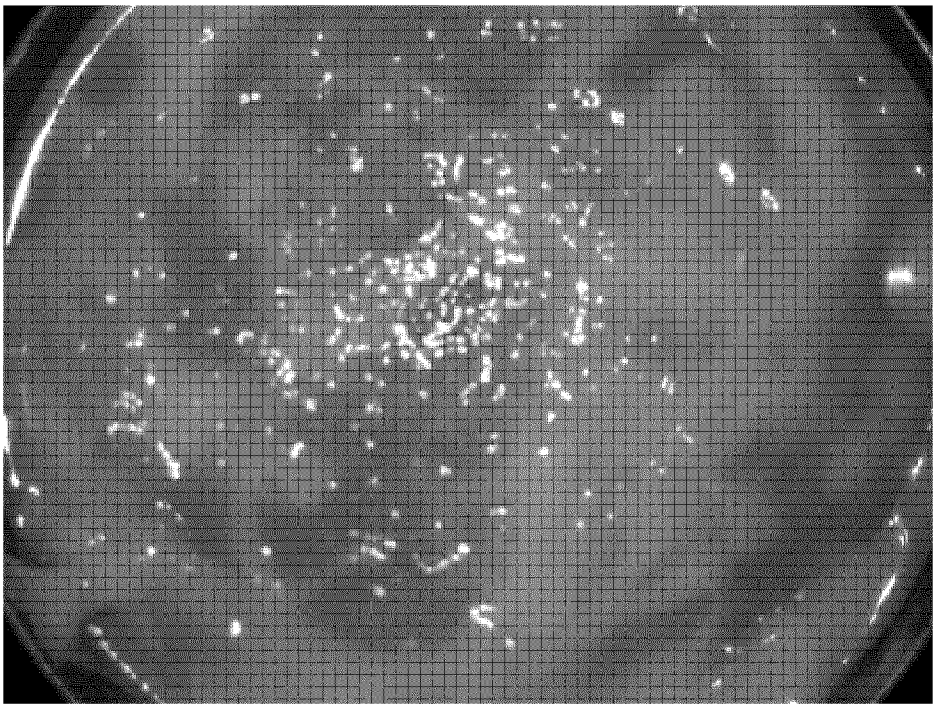


FIG. 4A

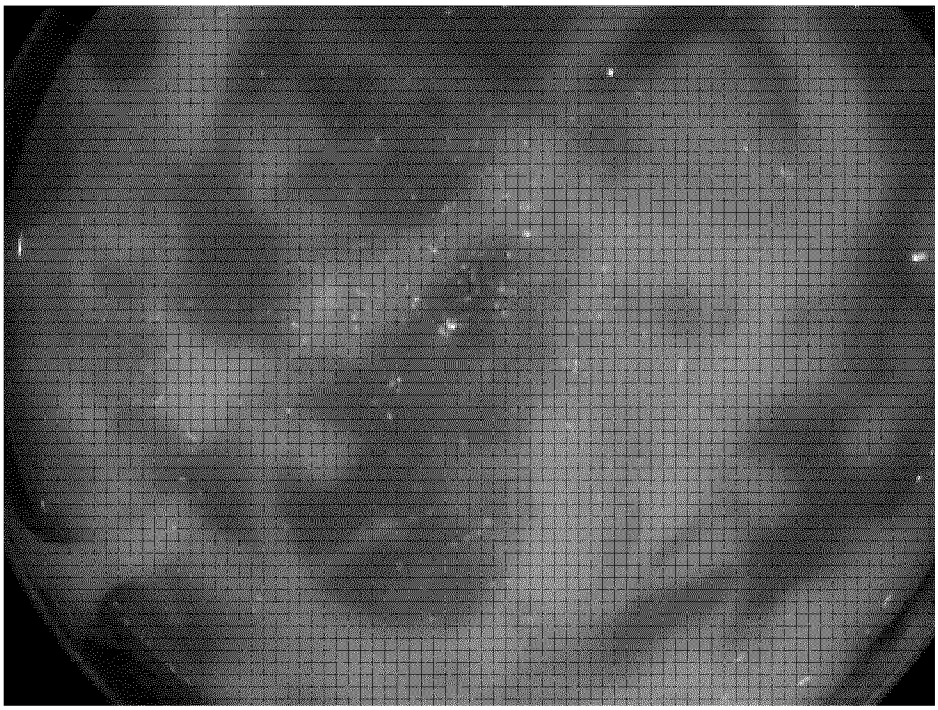


FIG. 4B

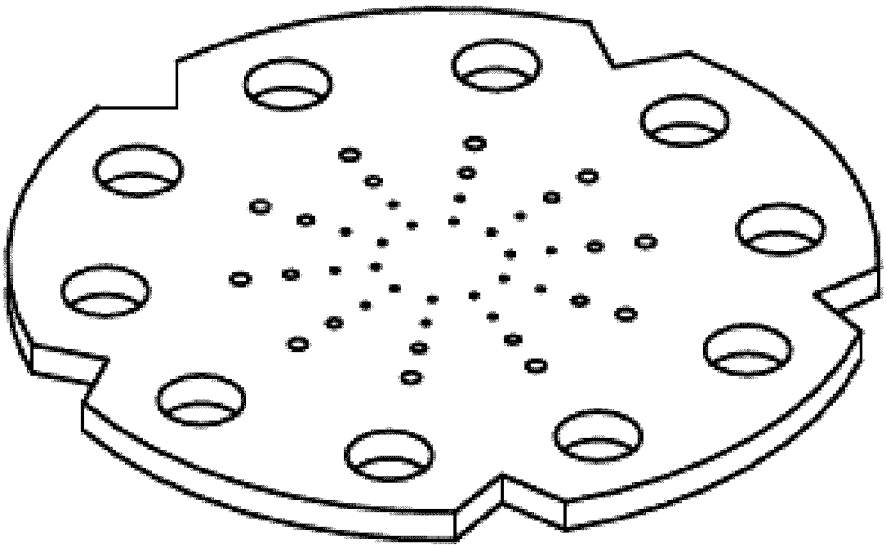


FIG. 5A

Isometric views

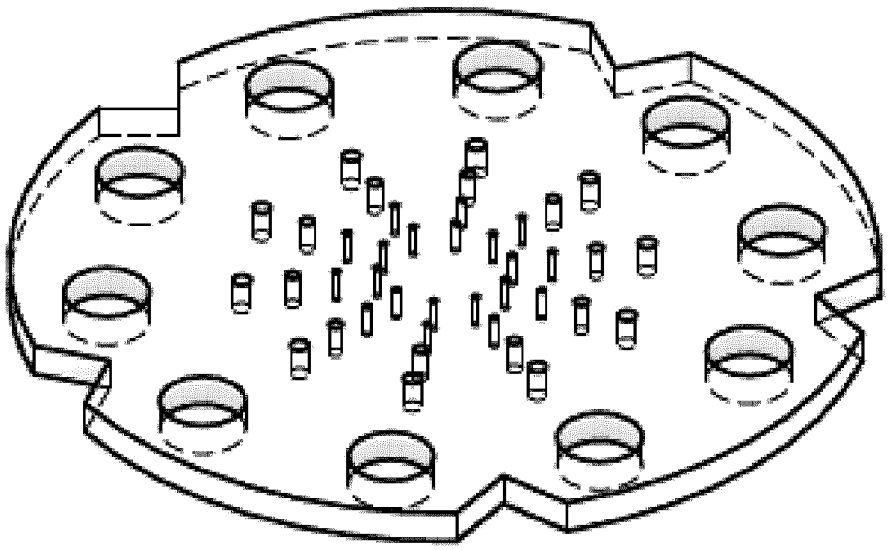


FIG. 5B

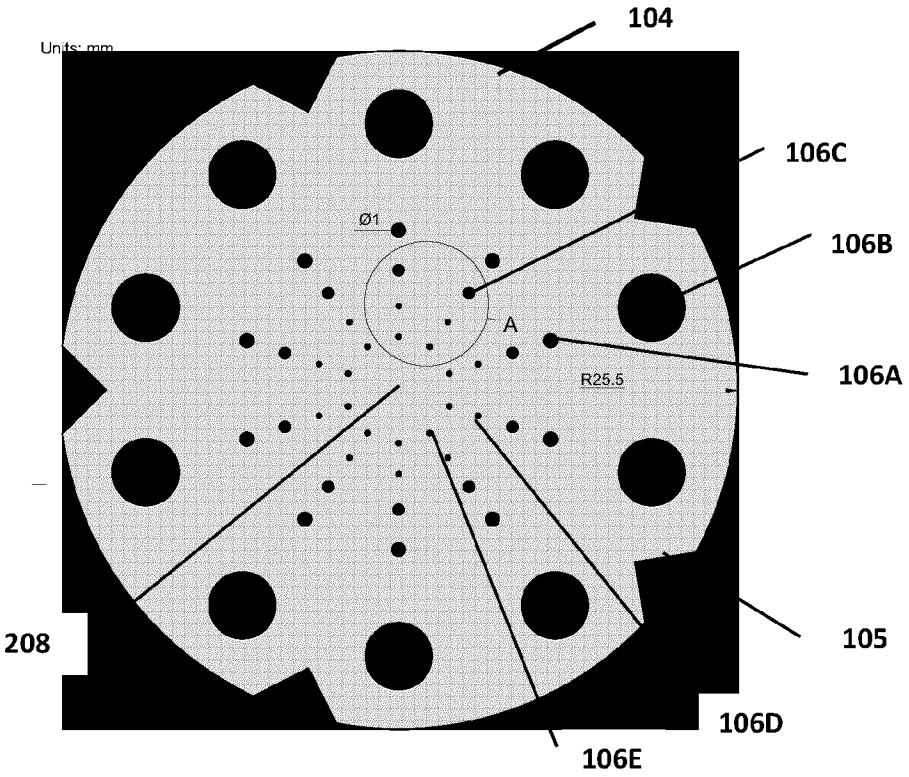


FIG. 5C

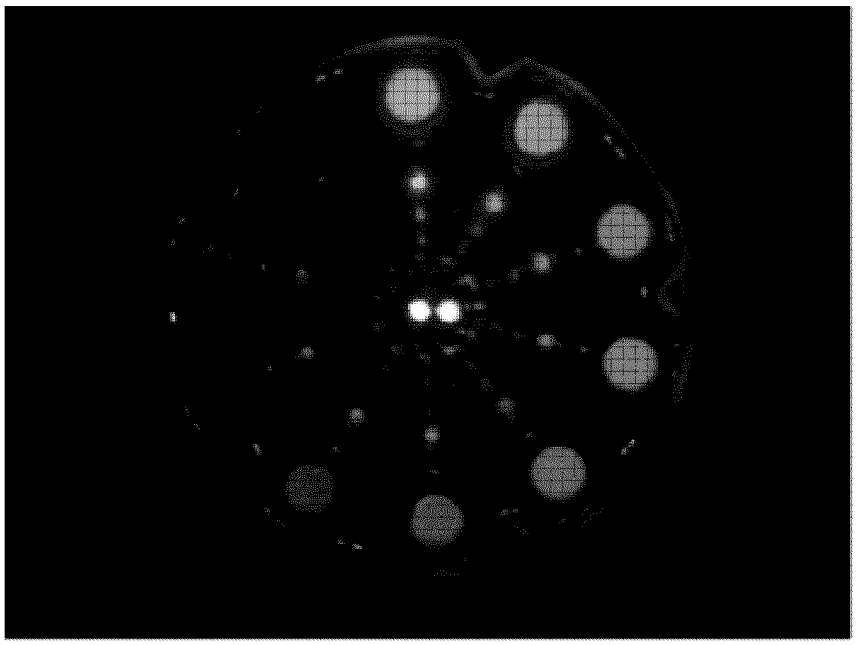


FIG. 5D

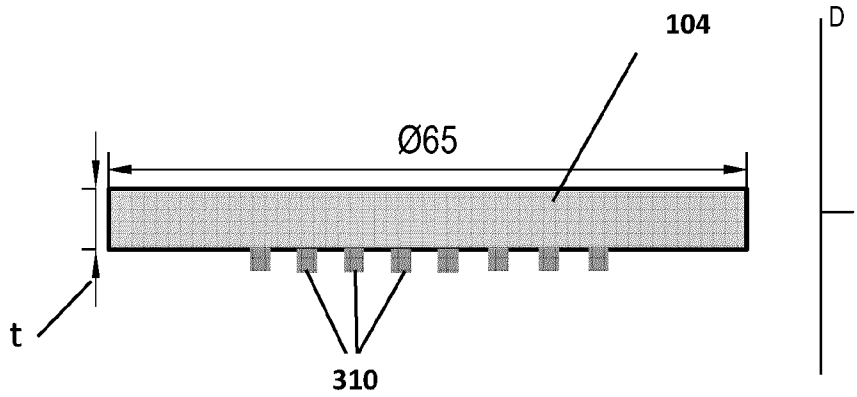


FIG. 6A

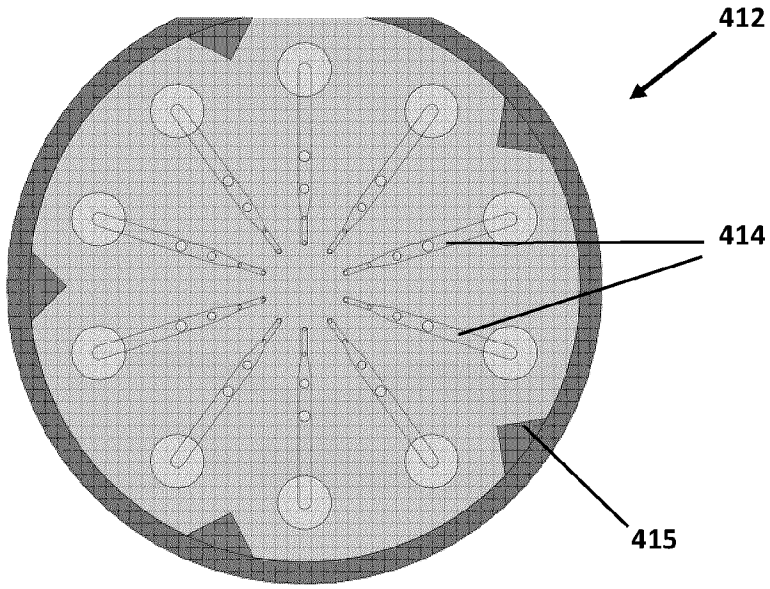


FIG. 6B

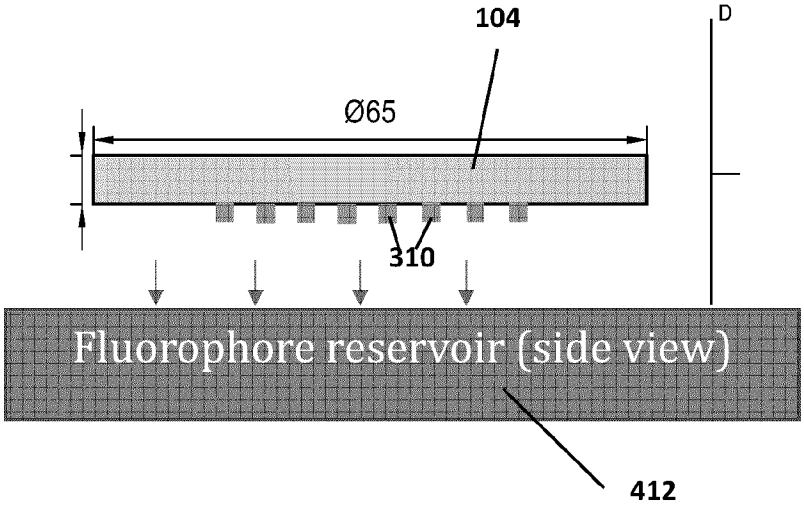

104
D
Ø65
310
Fluorophore reservoir (side view)
412

FIG. 6C

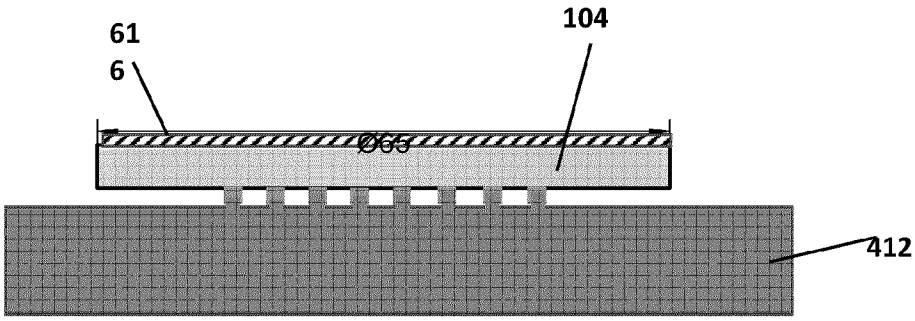

61
6
104
Ø65
412

FIG. 6D

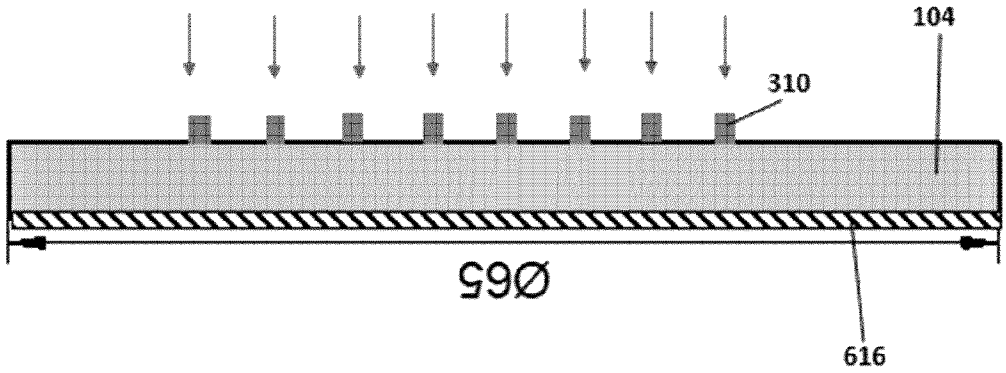


FIG. 7

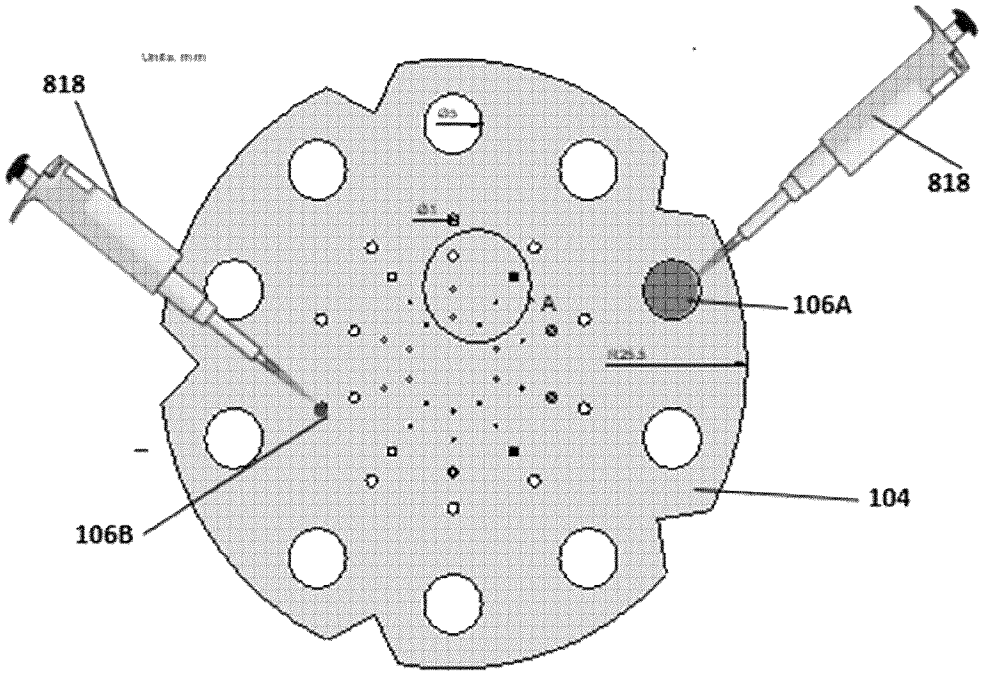


FIG. 8

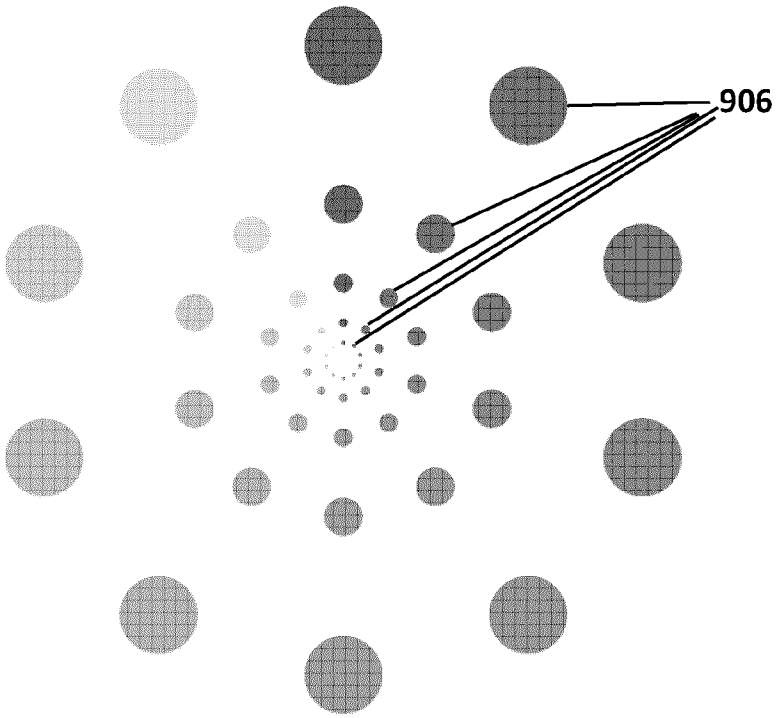


FIG. 9

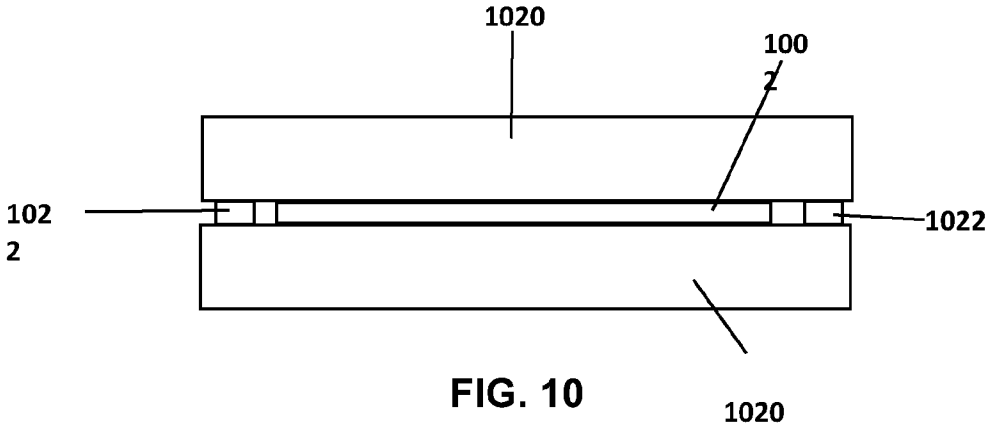


FIG. 10

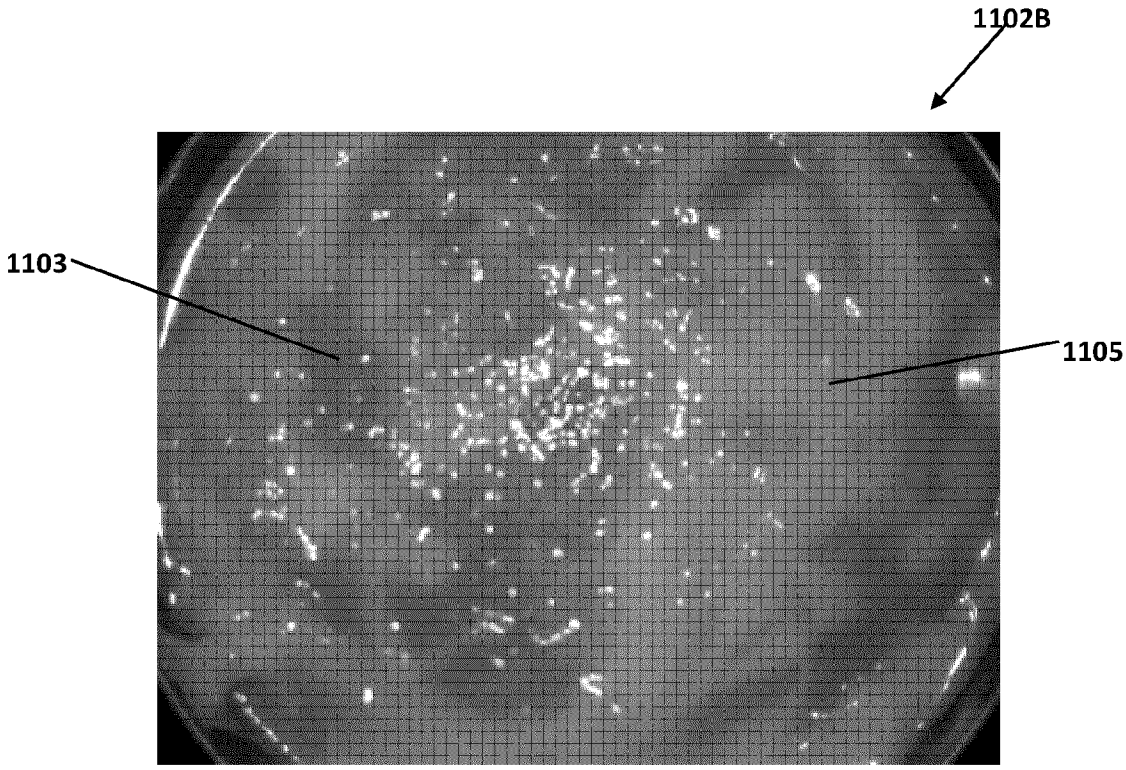


FIG. 11

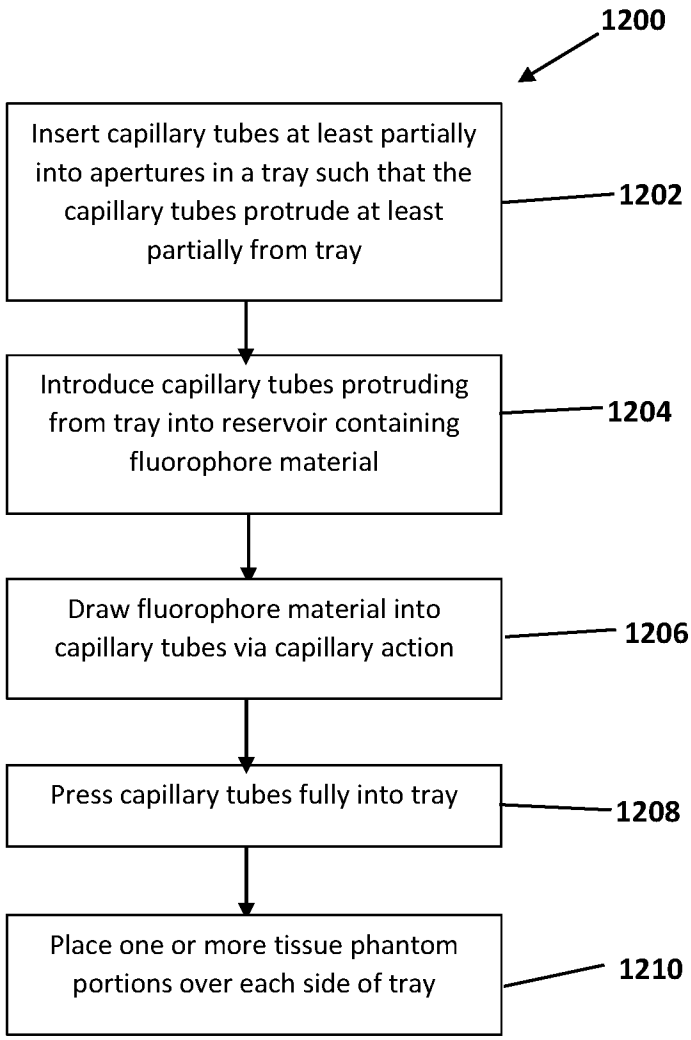
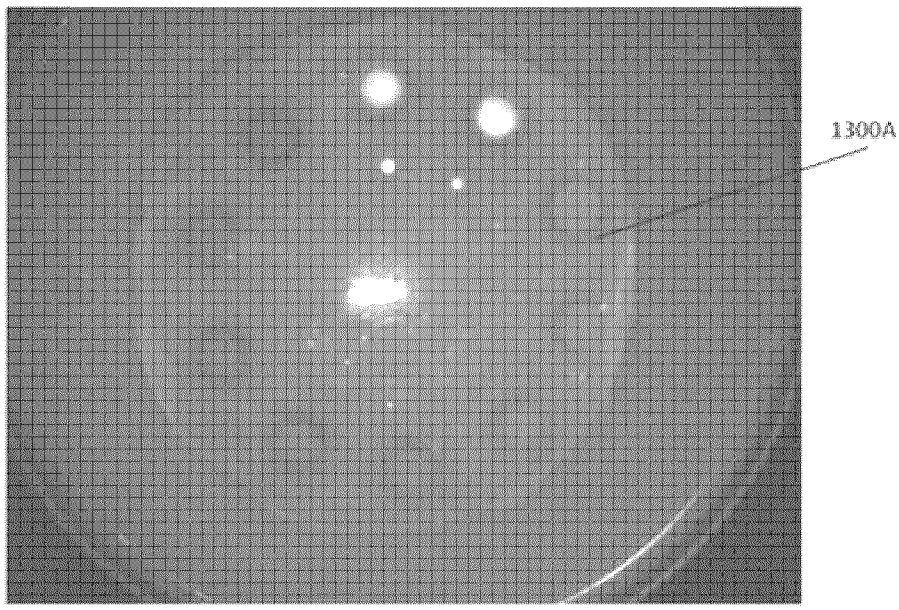

1200
Insert capillary tubes at least partially into apertures in a tray such that the capillary tubes protrude at least partially from tray
1202
Introduce capillary tubes protruding from tray into reservoir containing fluorophore material
1204
Draw fluorophore material into capillary tubes via capillary action
1206
Press capillary tubes fully into tray
1208
Place one or more tissue phantom portions over each side of tray
1210

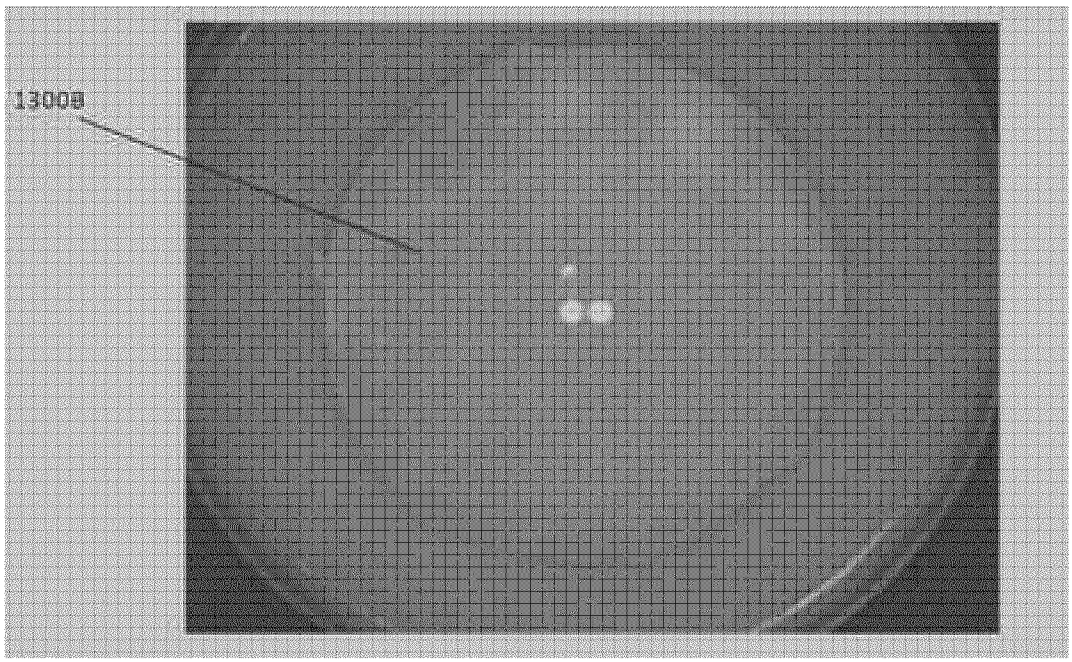
FIG. 12



Adipose tissue phantom FIG. 13A

Imaging distance: 9 cm

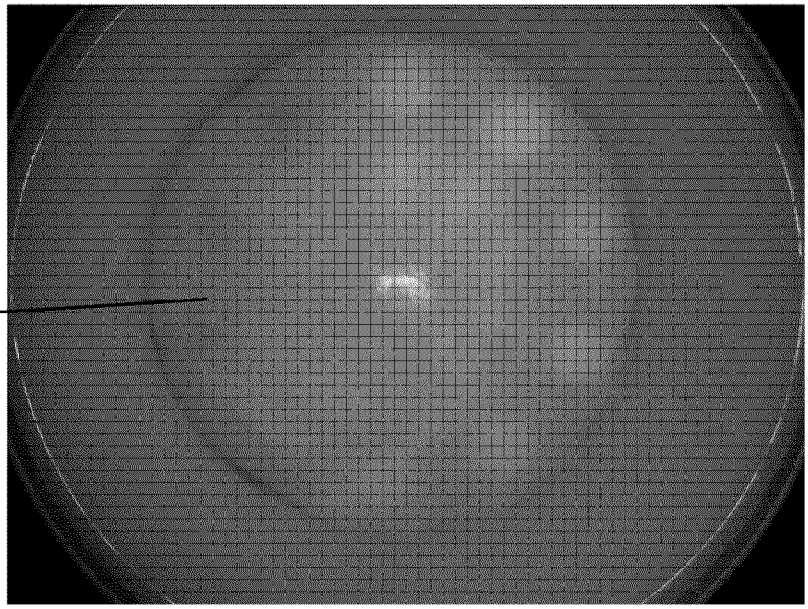
Thin film depth: 300 µm



Adipose tissue phantom FIG. 13B

Imaging distance: 9 cm

Thin film depth: 1645 µm

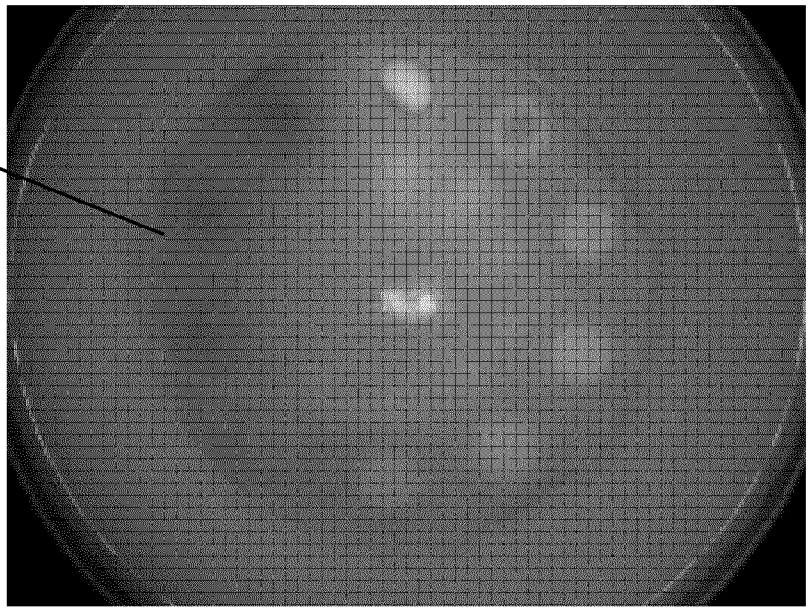


Connective tissue phantom

FIG. 14A

Imaging distance: 9 cm

Thin film depth: 900 µm



Connective tissue phantom

FIG. 14B

Imaging distance: 9 cm

Thin film depth: 409 µm

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2024/050328

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61B 5/00*** (2006.01) , ***A61K 49/00*** (2006.01)

CPC: , A61B 5/0059 (2020.01) , A61B 5/0071 (2020.01) , A61B 5/0091 (2020.01) , A61K 49/0017 (2020.01)

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)
IPC: A61B 5/00 (2006.01), A61K 49/00 (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: Questel Orbit, Google Patents, Google, IEEE
Keywords: tissue phantom, system, tissue, phantom, fluorophore, film, diseased tissue, healthy tissue, skin layer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/148720 A1 (Dacosta et al) 23 July 2020 (23-07-2020) * figures 2A-D, 3A-C; paragraphs [0035], [0055]-[0073], [0081]-[0082]	1-38
A	CA 3 173 719 A1 (Wood et al.) 9 March 2023 (09-03-2023) * entire document	1-38
A	US 2017/0249872 A1 (Piron et al.) 31 August 2017 (31-08-2017) * entire document	1-38

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"D"	document cited by the applicant in the international application	"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
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search	Date of mailing of the international search report 28 May 2024 (28-05-2024)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Sabina Khan (289) 356-4317

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2024/050328

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		CA3190062A1	23 July 2020 (23-07-2020)
		EP3911941A1	24 November 2021 (24-11-2021)
		EP3911941A4	05 October 2022 (05-10-2022)
		US2022082500A1	17 March 2022 (17-03-2022)
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		GB2612886A	17 May 2023 (17-05-2023)
		US2023071061A1	09 March 2023 (09-03-2023)
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		CA2963571C	03 January 2023 (03-01-2023)
		GB201708535D0	12 July 2017 (12-07-2017)
		GB2547594A	23 August 2017 (23-08-2017)
		GB2547594B	12 June 2019 (12-06-2019)
		WO2016074061A1	19 May 2016 (19-05-2016)