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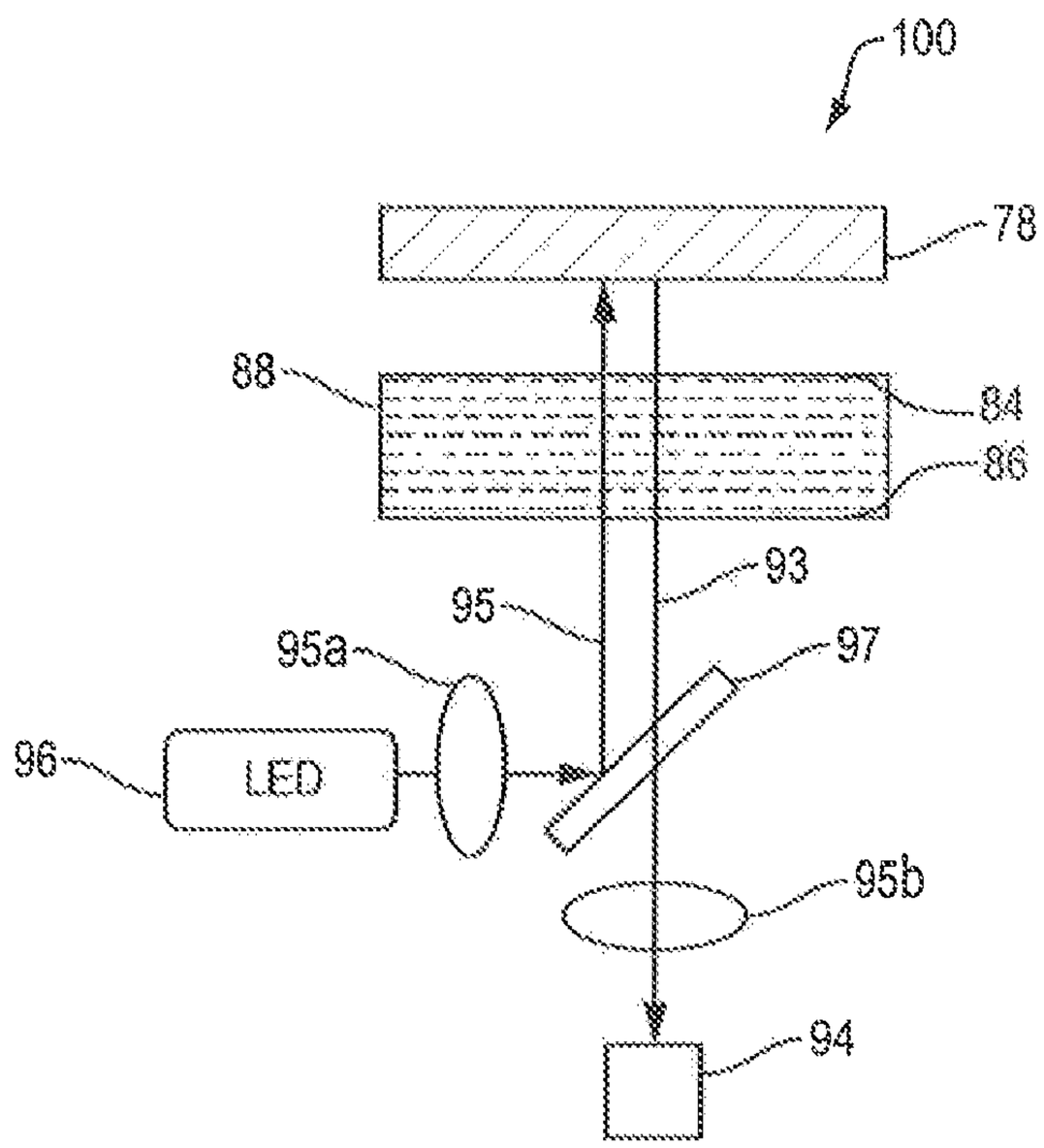


FIG. 11A

(57) Abrégé/Abstract:
This invention relates to an optical system and method for performing turbidity assay, e.g. coagulation of blood or plasma, comprising a standard optical reference (78), a sample handling structure (88), a light source (96) and an optical detection unit

(21) 2 946 877

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(57) Abrégé(suite)/Abstract(continued):
(94). The standard optical reference (78), such as a fluorophore-doped glass, provides constant optical signal under controlled optical conditions. The sample handling structure (88), such as a microfluidic system with reaction chamber, can be placed beneath or above the standard optical reference (78). During operation, the coagulating plasma/blood changes its optical absorbance and reflection properties, which results in changes in optical signal that reaches the optical reading unit (94). The variation of the optical signal, such as fluorescence signal indicates the kinetics of the turbidity varying process, such as plasma/blood coagulation process. This invention is used for performing turbidity assay with optical system, including photometry system, fluorescence system, Raman Spectroscopy system and so on.

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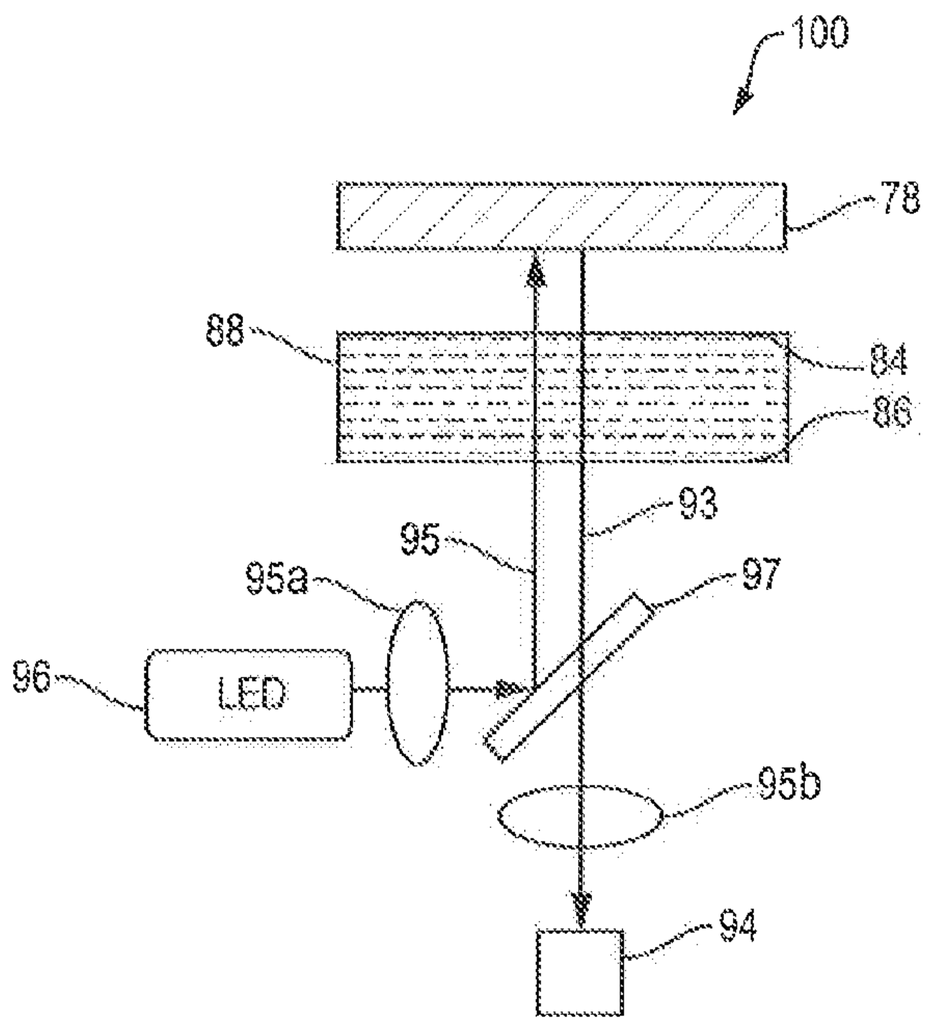


FIG. 11A

(57) Abstract: This invention relates to an optical system and method for performing turbidity assay, e.g. coagulation of blood or plasma, comprising a standard optical reference (78), a sample handling structure (88), a light source (96) and an optical detection unit (94). The standard optical reference (78), such as a fluorophore-doped glass, provides constant optical signal under controlled optical conditions. The sample handling structure (88), such as a microfluidic system with reaction chamber, can be placed beneath or above the standard optical reference (78). During operation, the coagulating plasma/blood changes its optical absorbance and reflection properties, which results in changes in optical signal that reaches the optical reading unit (94). The variation of the optical signal, such as fluorescence signal indicates the kinetics of the turbidity varying process, such as plasma/blood coagulation process. This invention is used for performing turbidity assay with optical system, including photometry system, fluorescence system, Raman Spectroscopy system and so on.

WO 2015/167983 A1

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METHODS AND SYSTEMS FOR POINT-OF-CARE COAGULATION ASSAYS BY OPTICAL DETECTION

FIELD OF THE INVENTION

This invention relates to an optical system and method for detecting coagulation of plasma or blood comprising a standard optical reference, a sample handling structure, a light source and an optical detection unit.

BACKGROUND

Coagulation assays are important tools to monitor a patient's risk of bleeding or thrombosis, both of which could lead to fatal consequences if intervention does not occur promptly and appropriately. This is especially critical in emergency and operation rooms, as a patient's hemostasis health status needs to be understood before proper hemotherapy is administered. Among all the coagulation assays, prothrombin time (PT) and activated partial thromboplastin time (APTT) assays are currently the most popular coagulation tests used in clinics and hospitals.

Instruments performing PT and APTT assays usually contain blood sample preparation mechanisms such as coagulation reagents and optical spectroscopy measurement units. Despite the advantages such as high throughput and good accuracy, these assays have some disadvantages that prevent its application for point-of-care tests. First, (1) due to the complex sample preparation and measurement process, the sampling-to-result time ranges from days to weeks. Such a slow turnaround time cannot meet the near real-time requirement in emergency rooms or other near-patient use. Secondly, (2) a large volume of blood, i.e., more than a milliliter of blood is required with these instruments for proper sample handling and accurate measurement.

Fluorescence-based technologies with state-of-the-art microfluidic sample preparation, such as lab-on-a-chip immunoassay, were developed to solve the above shortfalls. A popular method in the recognized art is to use thrombin or plasmin (both factors are generated during coagulation reaction pathways) specific substrates containing immunoreactive fragments. Upon exposure to thrombin or plasmin, the substrates are cleaved and the immunoreactive fragments are released from the substrate, which generates a fluorescence signal as an

indicator of the kinetics of coagulation process. These technologies suffer from poor reliability due to the low efficiency of the chemical reaction and the stability of the immunoreactive fragments. Additionally, the requirement by the industry for quality control in chemical production, instrument manufacturing, and final usage increases the cost of these prior art coagulation assays.

The invention disclosed herein was developed to successfully solve the problems identified in prior art coagulation assays of slow turn-around-time, large sample size requirement, excessive production costs, lack of reagent stability, and inability of prior art coagulation assays to meet the near real-time requirement in emergency rooms or other near-patient use for immediate coagulation assay results.

SUMMARY OF THE INVENTION

The fluorescence based and other coagulation assays according to the invention described below can be used widely in various clinical situations. Centralized large instruments or point-of-care instruments can be developed around these methods to achieve high throughput coagulation assays. Various assays specific to certain factors involved in the coagulation cascade, for example, can be realized with this technology.

More importantly, compact point-of-care devices according to the invention described herein can be developed for emergency room, surgical suites, intensive care units or a physician's office. The rapid response and small sample size requirement of the disclosed invention allow the technology to be used for continuous monitoring of coagulation kinetics, e.g., when hemotherapy is required. In the meantime, the invention can be used with existing immunoassay systems and/or microfluidic systems that are currently used for the diagnosis of heart diseases and cancers of patients, without the need for extensive new instrument development.

In one aspect, the invention is directed to an assay system comprising a reaction chamber for holding a sample, an excitation light source, an optical reference for providing an optical signal, and an optical receiver. The optical reference is positioned to absorb the excitation light and generates the optical signal to the optical receiver.

The reaction chamber according to the invention is positioned to inhibit or enhance the signal generated from the optical reference and detected by the optical receiver. In one embodiment, the reaction chamber holds a sample in the absence of a colorimetric reagent.

The excitation light source provides a specific wavelength ranging, for example but not limited to, from 20 nm to 5000 nm, 50 nm to 2000 nm, or 100 nm to 1000 nm.

The optical reference according to the assay system is selected from the group consisting of, for example, fluorescence doped glass, stained glass, dyed glass, and materials showing Raman effect. The reaction chamber comprises a lumen, a planar first wall, and a planar second wall. In one embodiment of the reaction chamber, the planar second wall is opposite and parallel to the planar first wall.

In one embodiment of the invention, the planar first wall and the planar second wall are each optically transparent to light in the wavelength range of, for example, about 20 nm to about 5000 nm, or alternatively, in the wavelength range of about 20 nm to about 2000 nm.

In various embodiments of the invention, the reaction chamber is positioned between the optical reference and the optical receiver and excitation light source, or the optical reference is positioned between the reaction chamber and the optical receiver and excitation light source, alternatively the optical reference is positioned between the excitation light source and the reaction chamber, and the reaction chamber is positioned between the optical reference and the optical receiver.

The assay system further comprises an optical receiver that includes a light detector for detecting emission light emitted from the light source or the optical reference, or for detecting reflected or secondary light. In one embodiment, the optical receiver module and light source module are integrated.

In one embodiment, each of the first and second planar walls of the reaction chamber comprise a luminal surface and the first planar luminal surface is coated with one or more reactants. The reaction chamber may further comprise a sample inlet port and a reaction fluid outlet port. The first inlet port may feature a v-shape.

In another aspect, the invention is directed to a method for detecting coagulation. In one embodiment of this aspect of the invention, the method requires:

(i) providing a system comprising an optical reference consisting of a device for generating a calibrated optical signal;

(ii) providing a reaction chamber comprising a chamber for holding a fluid, the chamber comprising a planar first wall and a planar second wall that is opposite and parallel to the planar first wall, and a lumen for holding a fluid, the first and second planar walls of the reaction chamber comprising a luminal surface and the first planar luminal surface is coated with one or more reactants, and an inlet, for example, a V-shaped inlet for introducing a body fluid sample into the reaction chamber;

(iii) transmitting excitation light from a light source through the fluid in the reaction chamber to an optical reference;

(iv) measuring emission light from the optical reference transmitted through the fluid in the reaction chamber to an optical detector;

(v) comparing the measured emission light to a pre-determined standard for determining coagulation time in the system.

In another embodiment, the method requires

(i) providing a system comprising an optical reference consisting of a device for generating a calibrated optical signal;

(ii) providing a reaction chamber comprising a chamber for holding a fluid, the chamber comprising a planar first wall and a planar second wall that is opposite and parallel to the planar first wall, and a lumen for holding a fluid, the first and second planar walls of the reaction chamber comprising a luminal surface and the first planar luminal surface is coated with one or more reactants, and an inlet, for example, a V-shaped inlet for introducing a body fluid sample into the reaction chamber;

(iii) transmitting excitation light from a light source through the optical reference to the fluid in the reaction chamber;

(iv) measuring reflected emission light from the optical reference transmitted to an optical detector; and

(v) comparing the measured emission light to a pre-determined standard for determining coagulation time in the system.

In yet another embodiment, the method requires

(i) providing a system comprising an optical reference consisting of a device for generating a calibrated optical signal;

(ii) providing a reaction chamber comprising a chamber for holding a fluid, the chamber comprising a planar first wall and a planar second wall that is opposite and parallel to the planar first wall, and a lumen for holding a fluid, the first and second planar walls of the reaction chamber comprising a luminal surface and the first planar luminal surface is coated with one or more reactants, and an inlet, for example, a V-shaped inlet introducing a body fluid sample into the reaction chamber;

(iii) transmitting excitation light from a light source through the optical reference;

(iv) the optical reference generates a secondary light which passes through the reaction chamber;

(v) measuring the secondary light by the optical detector; and

(vi) comparing the measured secondary light to a predetermined standard for determining coagulation time in the system.

The foregoing and other objects, features, and advantages of the invention will become apparent from the following, more particular description of the preferred embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is described with particularity in the appended claims. The further advantages of the invention described herein may be better understood by referring to the following description taken in conjunction with the accompanying drawings.

Figure 1 illustrates a double absorbance optical configuration for the coagulation system to absorb both excitation light from a light source and returning light from optical reference during coagulation of a plasma or blood sample according to one embodiment of the invention.

Figure 2 illustrates a reflection optical configuration for the coagulation system to entrap excitation light at the interface between sample and optical reference to enhance optical signal generated in the optical reference during coagulation of a plasma or blood sample according to another embodiment of the invention.

Figure 3 illustrates a transmission configuration for the coagulation system to absorb the light emitted from the optical reference excited by the light source during coagulation of a plasma or blood sample according to another embodiment of the invention.

Figure 4 illustrates that the distance between optical reference and sample fluid (d) can vary from 0 to a large value, typically 0 to 200 mm with all the configurations described with respect to Figure 1, 2 and 3.

Figures 5A-E illustrate a method to make the optical reference with various compositions, including (A) dope optical agents, such as but not limited to fluorescence molecules, particle, dyes, inside a substrate material such as, but not limited to plastics, glass, and silicon; (B) chemically assemble a layer of optical agents on the first surface of the substrate; (C) chemically assemble a layer of optical agents on the opposite surface of the substrate; (D) coating a layer of optical agents on the first surface of a substrate by either physical or chemical method; (E) coating a layer of optical agents on the opposite surface of the substrate by either physical or chemical method.

Figures 6A-G illustrate exemplary configurations for integrating the optical reference with the reaction chamber of the sample handling device: (A) embedding the optical reference in the first wall of the integral reaction chamber; (B) bonding a flat optical reference to form the first wall of the reaction chamber with the cavity of the reaction chamber in the bottom part; (C) bonding the optical reference to the remaining portions of the reaction chamber illustrated in 6B except the bottom portion; (D) placing the optical reference outside the enclosed reaction chamber as a separate part; (E) embedding the optical reference within the wall opposite to the first wall of the integral reaction chamber; (F) bonding the flat optical reference to form the opposite wall of the reaction chamber with the cavity of the reaction chamber in the remaining portion; (G) bonding the optical reference to the remaining portions of the reaction chamber illustrated in 6F except the first wall.

Figure 7A is a view of the bottom of a microfluidic plate with a reaction chamber illustrating one exemplary configuration of the reaction chamber having a fluidic inlet and outlet for the sample, and dry reagent pre-stored in the chamber, and Figure 7B illustrates a side view of Figure 7A;

Figures 8A-D illustrate a liquid handling device including an exemplary configuration of the reaction chamber with two fluidic inlets; one for sample and one for reagent separately, and a fluid outlet. (A) is a perspective view of the reaction chamber; (B) is a top view of the reaction chamber; (C) is a cross-section of Figure 8B; (D) is another cross-section of Figure 8B. Figures 8E-8H illustrate sequential filling of the reaction chamber with reagent from time

= 0 to time = 3, and Figures 8I-8L illustrate the sequential filling of the reaction chamber with sample fluid from time = 4 to time = 7.

Figure 9 illustrates a cross-section of the reaction chamber filled with reagent and sample fluid.

Figure 10A and 10B are top and bottom views, respectively, of an exemplary microfluidic device including a plurality of reaction chambers in accordance with the invention.

Figure 11 shows an embodiment of the invention based on the optical configuration shown in Figure 1 and exemplary assay result. (A) in this specific embodiment, an LED is used as light source, a fluorescence doped glass is used as optical reference, and a quantitative fluorescence detector is used as an optical detection unit. (B) illustrates fluorescence signal from an assay group having abnormal plasma (b) and control group having normal plasma (a) according to one embodiment of the double absorbance configuration of the coagulation system according to the invention shown in (A). The abnormal assay group results show delayed signal change compared to that from the normal control assay group.

Figure 12 shows an embodiment of the invention based on Figure 2 optical configuration and exemplary assay result. (A) in this specific embodiment, an LED is used as light source, a fluorescence doped glass is used as optical reference, and a quantitative fluorescence detector is used as an optical detection unit; (B) illustrates fluorescence signal from an assay group having coagulated plasma (a) and control group having uncoagulated plasma (b) according to one embodiment of the double absorbance configuration of the coagulation system according to the invention shown in A. The assay results shows enlarged signal change from the coagulated plasma (a) compared to that from plasma without coagulation (b);

Figure 13A-D shows an exemplary mathematical method to process the optical data to obtain quantitative coagulation time.

DESCRIPTION

In one aspect, the invention relates to a system for detecting coagulation of a patient plasma or blood sample in a reaction chamber, for example, a chamber of a microfluidic device. The system includes an optical reference part, such as but not limited to a standard

fluorescence element, such as but not limited to a fluorophore-doped glass, a polymer film or sheet containing intrinsic fluorescence that is used to generate a fluorescence reference signal. The positioning of the fluorescence element and the coagulating blood/plasma sample is configured to vary the light energy that reaches and/or leaves the optical reference. With such configuration, the system according to the invention de-couples the fluorescence signal from chemical reactions. The variation of the fluorescence signal indicates the kinetics of the plasma/blood sample coagulation process.

The coagulation detection system according to the invention is used for performing coagulation assays, for example, with fluorescence detection. As a point-of-care (POC) coagulation immunoassay system, the sample preparation can be implemented in a microfluidic cartridge, allowing small sample volume, i.e., less than a milliliter, preferably less than 100 microliters, and low manufacturing cost. The invention can be used for types of wet chemical assays where a change in adsorption, turbidity during the assay is used for detection and quantification of an analyte in a sample. Typical wet chemical assays are immunochemical, enzymatic, clotting assays, affinity based, and nucleic acid based assays. Different optical detection methods may be used in various embodiments such as but not limited to turbidity, absorption, reflectance, fluorescence intensity, time resolved fluorescence, NIR and others. Compared to traditional coagulation assay tools such as optical spectroscopy or lab-on-a-chip assay systems, the coagulation system according to the invention has at a minimum the following advantages:

(1) the enhanced portability of the system and fast turnaround time allowing point-of-care applications;

(2) the system's handling of a sample requires only a small amount, i.e. less than a milliliter of patient blood or plasma, preferably below 100 microliters;

(3) no indicators like those typically required in state-of-the-art fluorescence assays such as fluorophore reagents or colorimetric reagents need to be added into the assay . This simplifies the assay protocol by reducing the assay handling steps which would otherwise require immunoreactive reagents, intra-assay chemicals, and chemical reactions. The fluorescence signal generated according to the invention is only a function of the coagulation reaction and requires no fluorophores added into the sample, resulting in lower cost and lower background interference;

(4) the decoupling of fluorescence signal and chemical reaction, together with using a standard fluorescence element, allows easy and reliable quality control;

(5) the system according to the invention described herein can be realized in any fluorescence system, various liquid handling systems including microfluidics, robotic, and manual liquid transportation systems allowing rapid and cost-effective adoption and integration with other biomarker detection systems, such as, but not limited to solid phase immunoassays for the quantification of other analytes in blood such as cardiac markers like troponin I or markers providing additional information to clotting parameters such as D-Dimer. D-dimer tests are ordered, along with other laboratory tests and imaging scans, to help rule out the presence of a thrombus;

(6) the cost of the cartridge which includes the various embodiments of the optical system according to the invention is sufficiently low to be disposable which reduces the risk of cross-contamination. The cartridge can be manufactured preferably in polymers such as polystyrene or cycloolefines, by manufacturing methods, preferably injection molding or hot embossing;

(7) different wavelengths may be used for the light source and signal detection thereby reducing background interference. The light source may be selected from but is not limited to the group consisting of a laser, a mercury arc lamp, and an LED. Wavelengths range from, for example, about 20nM to about 5000nM, about 50nM to about 2000nM, about 100nm to about 1000nM.

Optical Configuration

Various optical configurations, with different arrangements of optical reference and sample reaction chamber, are disclosed for various turbidity assays, e.g. blood coagulation assays. The schematics of each configuration according to embodiments of the invention are illustrated in Figures 1, 2 and 3, and the operation principles are described below.

Double Absorbance Optical Configuration

As shown in Figure 1, according to one embodiment of the invention, a double absorbance optical configuration system 100 has a fluorescence module 98, a reaction chamber 88, and a fluorescence reference 78. In one embodiment, a fluorescence module 98 integrates both light source 96 and fluorescence detection unit 94, for example, but not limited to a detection system to measure time resolved fluorescence (TRF) using an LED (360nm),

for example, for excitation and a photodetector, such as a photo diode or a multi pixel photon counter (MPPC, to quantify the fluorescence emission.

With continued reference to Figure 1, according to one embodiment of the invention, the double absorbance optical configuration system 100 has a light source 96, an optical detection unit 94, an optical reference 78 and a reaction chamber 88. During operation, the light 95 from the light source 96 and the returning light 93 from the optical reference 78 both transmit through the sample in the reaction chamber 88 and is absorbed due to turbidity change of the sample, plasma or blood, for example. The source or excitation light 95 and returning or emission light 93 can have same or different wavelengths. The optical reference 78 can be realized with various optical technologies, such as but not limited to generic photometry, fluorescence, Raman spectroscopy time-resolved fluorescence, and surface enhanced Raman spectroscopy. In one embodiment, a fluorescence-based method is used for blood coagulation time measurement with light source being LED, optical reference being a fluorescence glass, returning light being emission from the fluorescence element, the sample being plasma, and the optical detection unit being a fluorescence detector. In this embodiment, when the plasma coagulates in the reaction chamber, the fluorescence signal read at the optical detection unit is reduced due to increased optical absorbance by the coagulated plasma.

With continued reference to Figure 1, the reaction chamber 88 encloses a plasma or blood sample and reagent(s) for a particular target coagulation assay. The reaction chamber 88 comprises a first wall 86 and a second wall 84 that is opposite to the first wall and is positioned between the optical reference 78 and the light source 96 and detector 94. The first wall 86 is optically transparent to a light of specified wavelength and is closer to the fluorescence module 98 than the second wall 84. The second wall 84 is optically transparent to the light with specified wavelengths and is positioned opposite and parallel to the first wall 86 and closer to the fluorescence reference 78 than the first wall 86. A reagent added to the plasma or blood sample in the reaction chamber 88 enables the coagulation reaction in the reaction chamber 88.

The optical reference 78 is, for example, but not limited to, a fluorescence-doped glass, or fluorophores immobilized on the surface of the second, opposite wall 84 of the reaction chamber 88. In the double absorbance optical configuration embodiment, the

fluorescence reference 78 is positioned on the side of the reaction chamber 88 that is opposite to the fluorescence module 98 as illustrated in Figure 1. The purpose of the optical reference 78 is to provide a calibrated optical signal at a specific wavelength.

During operation, once the plasma or blood sample coagulation process starts, more and more fibrin is formed thereby increase the turbidity of the plasma or blood sample in the reaction chamber 88. As a result, the transmission of the excitation light 95 through the sample is reduced and the excitation of fluorescence molecules on the optical reference 78 is inhibited. In addition, the reduced emission light 93 from the optical reference 78 is absorbed further when it passes through the sample in the reaction chamber 88 to the fluorescence module 98 where it is detected and measured by the fluorescence detector 94. The combined effect of the two absorbance processes, i.e., the first absorbance as the excitation light 95 passes through the reaction chamber 88 to the fluorescence reference 78, and the second absorbance as the emission light 93 passes from the optical reference 78 through the reaction chamber 88, is expected to produce a signal change detected by the optical detector 94. The signal change indicates the coagulation process of the sample in the reaction chamber 88. As a result, a decrease of the fluorescence signal detected by the optical detector 94 in this double absorbance optical configuration indicates that the coagulation process has begun. The relative change of the signal with time gives information about the coagulation process (kinetics, slope). For the proper calculation of the different coagulation parameters such as PT, APTT, the maximum and minimum signal is determined.

Reflection Optical Signal

Figure 2 illustrates a reflection optical configuration of the turbidity system 100' according to another embodiment of the invention in which the fluorescence reference 78 is positioned between the reaction chamber 88 and the fluorescence module 98.

With continued reference to Figure 2, the reflection optical configuration system 100', like the double absorbance optical configuration system 100' described above, comprises a fluorescence module 98, a reaction chamber 88, and a fluorescence reference 78. In one embodiment of the reflection optical configuration, the fluorescence module 98 integrates both light source 96 and fluorescence detection unit 94, for example, but not limited to a fluorescence reader from Horiba Instruments Inc. (Kyoto, Japan) that has an LED (360nm) light source and a MPPC (a multi pixel photon counter) detector.

The reaction chamber 88 encloses a plasma or blood sample, and reagent(s) for a specified target coagulation assay and typically has a plurality of planar walls, at least two of which are parallel and opposite. The optical reference 78 is positioned between the reaction chamber 88 and the excitation light source 96, and optical receiver 94. For example, the reaction chamber 88 comprises a first wall 86 and a second wall 84 opposite to the first wall 86. In a preferred embodiment the first wall 86 and the second wall 84 are parallel to one another. Alternatively the first wall and the second wall may be placed at an angle to one another, for example, at a 45° angle. In the reflection optical configuration, the first wall 86 is optically transparent to a light of specified wavelength and is positioned closer to the fluorescence reference 78 than the second wall 84. The second wall 84 is positioned opposite and parallel to the first wall 86 and further away from the fluorescence reference 78 than the first wall 86. The second wall 84 may or may not be optically transparent. A reagent added to the plasma or blood sample in the reaction chamber 88 enables the coagulation reaction in the reaction chamber 88.

The optical reference 78 is, for example, but not limited to, a fluorescence-doped glass, or fluorophores immobilized on the surface of the first wall 86 of the reaction chamber 88. In this embodiment, the fluorescence reference 78 is positioned between the reaction chamber 88 and the fluorescence module 98 as illustrated in Figure 2. The purpose of the fluorescence reference 78 is to provide a calibrated fluorescence signal.

During operation, once the plasma or blood sample coagulation process starts, more and more fibrin is formed thereby increase the turbidity of the plasma or blood sample in the reaction chamber 88.

As illustrated in Figure 2, in the reflection optical configuration, the excitation light 95 first reaches the optical reference 78 and then transmits through the sample in the reaction chamber 88. In other words, one portion of the excitation light 95 excites fluorescence of the optical reference 78 before transmission through the reaction chamber 88, while the remaining portion of the excitation light 95 transmits through the sample in the reaction chamber 88. As coagulation of the plasma or blood sample in the reaction chamber 88 initiates and propagates, and the quantity of fibrin increases in the sample, the energy distribution of the two light portions, i.e., transmitted and reflected light, is varied due to the change of the sample's transmission property. Namely, the transmission of the excitation light 95 is

inhibited, and more light is trapped at the interface of the fluorescence reference 78 and the first wall 86 of the reaction chamber 88 to excite more fluorophores. As a result, an increase of the fluorescence signal detected by the optical detector 94 in this configuration indicates that the coagulation process has begun. The clotting time can be determined by, for example, the slope of the clotting curve which is calculated by the first derivative of the clotting curve (maximum value of the first derivative is giving the start time for coagulation) as shown in Figure 12. Maximum (start of the reaction, time point zero) and minimal signal (coagulation completed) are needed to determine the clotting time.

Transmission Optical Configuration

Figure 3 illustrates yet another optical configuration of the system 100". The optical reference 78 is arranged between the light source 96 and sample reaction chamber 88, and the reaction chamber 88 is placed between optical reference 78 and optical detection unit 94. During operation, the optical reference 78 is excited by the source light 96 and emits a secondary light 93, such as fluorescence signal. The secondary light 93 passes through the reaction chamber 88 and is absorbed due to turbidity change of the sample. The optical detector 94 reads the signal of the secondary light 93 from the optical reference 78. The quantitative value of the signal represents the kinetics of the coagulation reaction.

Figure 4 illustrates that the distance (d) between the optical reference 78 and the sample in the lumen 83 of the reaction chamber 88 can vary from about 0 to about 200 mm with each configuration described above with respect to Figure 1, 2 and 3.

Figure 5 illustrates exemplary configurations of the optical reference 78. Using optical reference with fluorescence properties as a non-limiting example, the optical agents 61 can be made by embedding fluorescence molecules, particles or other carriers into a plastic, glass or silicon material substrate 78 (Figure 5A). Alternatively, the optical fluorescence agents can be chemically or physically coated on the surface of the substrate, either on the top 60 or bottom 62 surface, i.e., first surface 60 or second surface 62 opposite the first surface, or both. For example, as illustrated in Figure 5(B) a layer of optical agents 61 may be chemically assembled on the first surface 60 of the substrate by physical means or chemical means; in Figure 5(C) a layer of optical agents 61 may be chemically assembled on the second surface 62 of the substrate by physical or chemical means; in Figure 5(D), a layer of optical agents 61 may be coated on the first surface 60 of the substrate by chemical or physical means; or, in

Figure 5(e), a layer of optical agents 61 may be coated on the second surface 62 of the substrate by chemical or physical means.

Figure 6 illustrates various exemplary arrangements of the reaction chamber 88 and the optical reference 78. The optical reference 78 can be an integral part of the reaction chamber 88, for example, by being embedded in the top or bottom part of the enclosed wall 64 of the reaction chamber 88, or, alternatively, the optical reference can be a separate part placed outside above or outside below the reaction chamber 88 to form suitable optical configurations according to the invention. Preferably the long axis of the optical reference 78 is perpendicular to the excitation light. Alternatively the excitation light may be at an angle to the long axis of the optical reference 78.

Figure 6A illustrates an exemplary planar optical reference 78 embedded in the first wall 65 of the enclosing wall 64 of the reaction chamber 88, according to one embodiment. Alternatively, Figure 6B illustrates a planar optical reference 78 bonded to and forming the first wall 65 of the reaction chamber 88 with the lumen 83 of the reaction chamber 88 on the inside of the first wall 65 of the reaction chamber 88. In a preferred embodiment the long axis of the optical reference 78 is perpendicular to the light source or alternatively at an angle up to about 45°.

In another embodiment, illustrated in Figure 6C, the optical reference 78 forms three walls, 65, 65'', 65''' of the reaction chamber 88 while only the second wall 65', opposite wall 65, is not a portion of the optical reference 78.

In still another embodiment, illustrated in Figure 6D, optical reference 78 is positioned as an element separate from any wall of the reaction chamber 88 and with the long axis of the optical reference 78 parallel to at least one wall of the reaction chamber 88; illustrated in Figure 6E, the optical reference 78 is embedded in the second wall 65' of the reaction chamber 88; illustrated in Figure 6F, the optical reference 78 is planar and bonded to the second wall 65' of the reaction chamber 88; illustrated in Figure 6G, the optical reference 78, forms three walls, 65', 65'', 65''', with only the first wall 65 opposite to wall 65' not a portion of the optical reference 78.

Sample preparation cartridge

According to the embodiments of the coagulation systems 100, 100' and 100" illustrated in Figures 1, 2, and 3, sample preparation in this invention can be realized in

various ways, from manual pippeting to an automatic fluidic control system. Non-limiting examples of microfluidic devices and methods applicable to the coagulation assay systems described above are given below. These devices and methods are not limited to assays for coagulation and can be used for a variety of wet chemical assays where metering, reagent addition, mixing, incubation and quantification of the assay reaction product is needed. Typical assays are using enzymatic reaction to measure metabolites such as lactate or creatinine or turbidimetric assays. Examples of such turbidimetric assays are agglutination assays such as latex agglutination where mono-disperse immune particles are complexing in the presence of an analyte which can be monitored by a change in turbidity.

Flow chamber with dry reagent

Referring now to Figures 7A and 7B, in one embodiment, a reaction chamber 88 of a liquid handling device 120 with a defined volume is formed by a microchannel plate 90 covered with a lid 91. The reaction chamber is used to meter the sample volume, one fluid inlet 68 is used to introduce the sample, e.g., plasma, from the bottom 60b of the reaction chamber 88, and one fluid outlet 66 at the bottom 60b of the reaction chamber 88 is used to discharge the excessive liquid from the lumen 83 of the reaction chamber 88. Dry reagent, such as lyophilized PT/APTT reagent, biotin and etc., is pre-stored in the reaction chamber 88, uniformly coated on the luminal surface of the first wall 86, for example. When the plasma fills the reaction chamber 88, the dry reagent starts to dissolve and then diffuses into the sample along the vertical direction, i.e., from the bottom 60b of the chamber 88 toward the top 60a of the chamber. The dry reagent has a relatively large contact area with the liquid sample and the diffusion distance along the vertical direction is relatively short. This configuration provides a homogeneous coagulation process across the lateral plane of the reaction chamber 88. During operation, once the chamber 88 is filled with sample, the assay process starts and the fluorescence signal acquisition begins to follow the reaction kinetics.

Flow chamber with liquid reagent

Figures 8A-8D illustrate one embodiment of the invention illustrating a liquid handling device 120 for investigation of a sample fluid. The liquid handling device 120 comprises a reaction chamber 88, two inlet ports and channels 66 and 68 to deliver sample fluid and reagent fluid into the lumen 83 of the reaction chamber, respectively, and an outlet channel 64 for venting of the reaction chamber 88 during filling. The device 120 may contain

one or more fluidic structures 68a and 64a, for example, to provide a controlled and bubble free filling of the reaction chamber lumen 83.

According to one embodiment of the liquid handling device 120 illustrated in Figures 8A-8D, the reaction chamber 88 of the device 120 is firstly filled with a metered amount of a liquid reagent via a first inlet 66. A bubble-free liquid filling can be achieved by a capillary stop feature 64a next to the outlet 64. In Figure 8A, for example, a cylindrical groove is acting as a capillary stop 64A. A capillary stop is defined either by a sudden channel opening and by the curvature of the feature 64a or by making the outlet area 64 hydrophobic. Figures 8E to 8H illustrate the sequential filling of reagent into the reaction chamber 88 at different points of time from time=0, to time=3. After a metered amount of reagent has filled into the lumen 83 of the reaction chamber 88, a metered amount of sample fluid (such as plasma and whole blood) is filled into the chamber lumen 83 via the second inlet 68 as illustrated in Figures 8I to 8L.

Additional features of the embodiment shown on Figures 8A-8D follows. The liquid handling device 120 is oriented in the horizontal direction, i.e. the top view of the liquid handling device 120 is as shown on Figure 8B. The v-Shape 68a at the inlet channel 68 has, for example, an opening angle of 30°. This v-Shape 68a could have an angle ranging from 0° to 180°, typically 15° to 120°. It is also noted that, according to this embodiment of the liquid handling device 120, the second inlet 68 and the outlet 64 is positioned on the top side 60a of the liquid handling device 120, while the first inlet 66 is positioned on the bottom side 60b of the liquid handling device 120. Other arrangements of the inlets and outlets on the top and bottom sides of the liquid handling structure are also possible and are not limited by the illustrated embodiment. The flow rate for sample and reagent may range from about 0.5µl/s to 200µl/s, typically 2µl/s to 100µl/s.

Figure 9 illustratively exemplifies the reaction chamber 88 after filling of reagent and sample have been completed. Two layers are shown: a layer of reagent and a layer of sample fluid. The sample layer is spread above the reagent layer across the whole surface of the fluid in the lumen 83 of reaction chamber 88. Therefore, it generates a large contact area between the two liquids, namely reagent and sample. With this large contact area, the mixing and thereby the reaction of the reagent and sample liquids is highly efficient.

In the illustrated embodiments in Figure 8, a v-shaped geometry of the inlet structure 68 is used to support an even distribution of the sample fluid into the reaction chamber. As illustrated in Figures 8c and 8d, the sample inlet 68 is connected to the top 60a of the reaction chamber 88 whereas the reagent inlet 66 is positioned in opposite to the bottom 60b of the reaction chamber 88.

Referring to Figure 10A, a top view of an embodiment of a microfluidic device 50 having four reaction chambers 88 a-d is illustrated. In the illustrated embodiment, the reaction chambers 88 a-d are positioned toward one side of the microfluidic device 50 but could be positioned in the microfluidic card at other positions.

Figure 10B illustrates a bottom view of the microfluidic card 50 including a plurality of channels 67 that are in fluid communication with the reaction chambers 88.

Exemplification/Proof of Principle

The embodiments of the coagulation systems 100, 100', 100" discussed above and their associated assay methods for detecting coagulation of a blood or plasma sample were evaluated with controlled plasma samples and reagents for PT and APTT assays. In the example of the double absorbance configuration described above with respect to Figure 1 and illustrated in Figure 11A, the fluorescence module applied in the method was a PMT-based Time Resolved Fluorescence (TRF) unit, fluorescence reference 78 was a Europium-doped glass, which contained precisely-controlled amount of europium and did not have photo bleach during excitation, and an LED 96 was used as a light source. A filter 95A was placed between the LED 96 and a dichroic mirror 97. A second filter 95B was placed between the detector 94 and the dichroic mirror 97. The plasma samples included normal control plasma (a) and high abnormal control plasma (b) from Instrumentation Laboratory Company (Orangeburg, NY). Coagulation was initiated by introduction of a coagulation initiater.

Referring to Figure 11B, the intensity of fluorescence signal emanating from the fluorescence reference and transmitted to the fluorescence detector in the double absorbance coagulation system described above with respect to Figures 1 and 11A, is represented by curve (a) for normal control and a curve (b) for abnormal control plasma. In both normal and abnormal plasma samples, the fluorescence signal decreased as the coagulation initiated,

propagated, and reached a stable value when coagulation was completed. The abnormal plasma takes a longer time to start and finish the coagulation process than the normal plasma.

Referring to Figure 12A, an embodiment of the invention using reflection configuration described above with respect to Figure 2 is realized. As illustrated in Figure 12A, an LED 96 was used as the light source, a fluorescence-doped glass was used as the optical reference 78, and a quantitative fluorescence detector was used as the optical detector unit 94. A filter 95A was placed between LED 96 and a dichroic mirror 97. A second filter 95B was placed between the detector 94 and the dichroic mirror 97. The plasma samples included a normal plasma sample (a) to which a coagulation reagent was introduced and a control normal sample (b) to which water (no coagulation reagent) was introduced. The optical signal obtained from plasma with coagulation (a) and plasma without coagulation (b) is illustrated in figure 12B. The fluorescence signal increased on sample (a) and reached a stable value as coagulation initiated and propagated and reached a stable value when coagulation was completed. The control (b) used the same plasma sample but with the addition of deionized water (no coagulation happened).

Figure 13 shows an exemplary method to process the optical data to obtain the quantitative coagulation time. In the four steps, the original data is first normalized (Fig. 13A) and filtered (Fig. 13B) to eliminate redundant data and noise. A first order derivative (Fig. 13C) of the original data is implemented to identify the time spot when the quickest change of optical signal locates. The peak position of the first-order derivative (Fig. 13D) is used as the coagulation start time. Other methods can be used to quantitatively study the coagulation process as well.

Various modifications and other implementations of what is described and illustrated herein will occur to those of ordinary skill in the art without departing from the scope and spirit of the invention. The invention is not to be defined only by the preceding illustrative descriptions or drawings.

AMENDED CLAIMS
received by the International Bureau on 18 September 2015 (18.09.2015).

1. An assay system, comprising:

a reaction chamber for holding a sample;

an excitation light source for directing an excitation light through the reaction chamber;

an optical reference for providing an optical signal which absorbs the excitation light having passed through the reaction chamber and emits an emission light responsive to the absorption of the excitation light, said emission light directed towards and passing through the reaction chamber; and,

an optical detector for detecting a signal from said optical reference, said signal conveyed via said emission light or the secondary light passing through said reaction chamber.

2. The assay system of claim 1 wherein the reaction chamber is positioned to inhibit or enhance the signal generated from the optical reference and detected by the optical detector.

3. The assay system of claim 1 wherein the reaction chamber holds the sample in absence of a colorimetric reagent.

4. The assay system of claim 1 wherein the excitation light source provides a specific wavelength ranging from 20 nm to 5000 nm.

5. The assay system of claim 1 wherein the optical reference comprises an optical agent embedded into a plastic, a glass or a silicon material substrate.

6. The assay system of claim 1 wherein said reaction chamber comprises a lumen, a planar first wall, and a planar second wall that is opposite and parallel to said planar first wall.

7. The assay system of claim 6 wherein said planar first wall and said planar second wall are each optically transparent to the excitation light in a wavelength range of about 20 nm to about 5000 nm.

8. The assay system of claim 7 wherein said excitation light is in a wavelength range of about 20 nm to about 2000 nm.

9. The assay system of claim 1 wherein said reaction chamber is positioned between said optical reference and said optical receiver and excitation light source.

10. (Canceled)

11. (Canceled)

12. The assay system of claim 1 wherein said optical receiver and said excitation light source are integrated.

13. The assay system of claim 1 wherein said optical receiver includes a light detector for detecting emission light emitted from said optical reference.

14. The assay system of claim 6 wherein each of said first and second planar walls of said reaction chamber comprise a luminal surface and said first planar luminal surface is coated with one or more reactants.

15. The assay system of claim 1 wherein said reaction chamber further comprises a sample inlet port and a reaction fluid outlet port.

16. The assay chamber of claim 15 wherein said sample inlet port is v-shaped.

17. A method for detecting coagulation, comprising:

(i) providing an optical configuration system comprising an optical reference for generating an optical signal;

(ii) providing a reaction chamber for holding a fluid;

(iii) transmitting excitation light from a light source through said fluid in said reaction chamber to the optical reference which absorbs the excitation light and transmits an emission light;

(iv) transmitting the emission light in (iii) through said fluid in said reaction chamber;

(v) providing an optical detector for detecting a signal from said optical reference, said signal conveyed via said emission light passing through said fluid in said reaction chamber; and,

(vi) comparing said measured emission light to a pre-determined standard for determining coagulation time in said system.

18. The assay system of claim 1 wherein said optical receiver includes a light detector for detecting the emission light from the optical reference or the secondary light which passed through the reaction chamber.

19. The assay system of claim 1 wherein said sample is selected from the group consisting of plasma and blood.

20. The assay system of claim 1 wherein the optical reference can be realized with an optical technology selected from the group consisting of photometry, fluorescence, Raman spectroscopy time-resolved fluorescence, and surface enhanced Raman spectroscopy.

21. The assay system of claim 5 wherein the optical agent is embedded in one or more walls of the optical reference.

22. The assay system of claim 5 wherein the optical agent is chemically or physically coated on a surface of a substrate.

23. The assay system of claim 1 wherein the optical reference is selected from the group consisting of fluorescence doped glass, fluorescence stained glass, fluorescence dyed glass, and fluorescence materials showing Raman effect.

24. An assay system, comprising:

a reaction chamber for holding a sample;

an excitation light source for directing an excitation light towards an optical reference, wherein the excitation light excites an optical agent in the optical reference before a portion of said emission light is transmitted through the reaction chamber, while another portion of the emission light is reflected at said reaction chamber; and

an optical detector for detecting said reflected emission light, wherein an increase in said reflected emission light is indicative of coagulation.

25. The assay system of claim 24 wherein said optical reference is positioned between said reaction chamber and said optical receiver and excitation light source.

26. An assay system, comprising:

a reaction chamber for holding a sample;

an excitation light source for directing an excitation light;

an optical reference for absorbing said excitation light having passed through said optical reference, and emits a secondary light directed towards and passing through the reaction chamber; and,

an optical detector for detecting said secondary light from said optical reference, said signal conveyed via said secondary light passing through said reaction chamber.

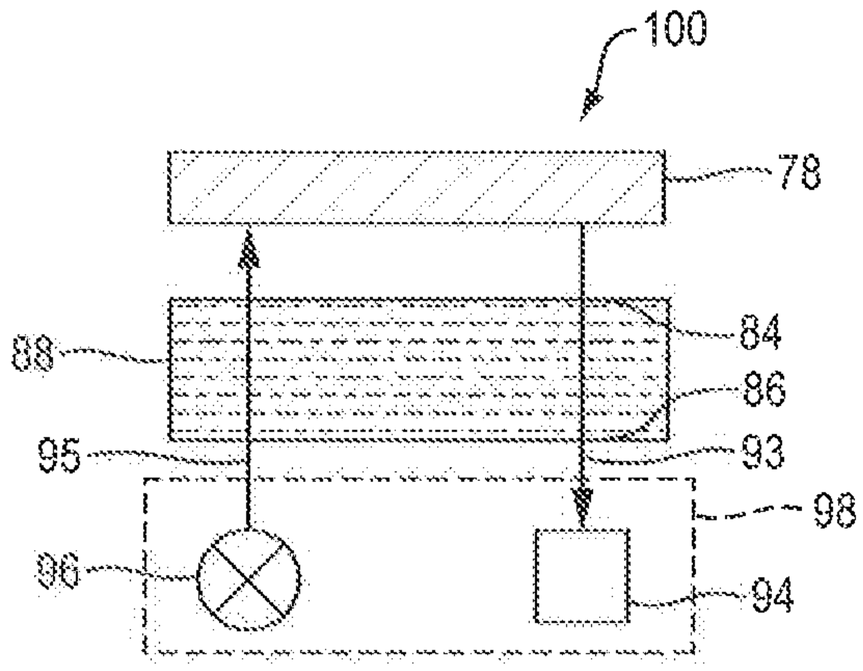


FIG. 1

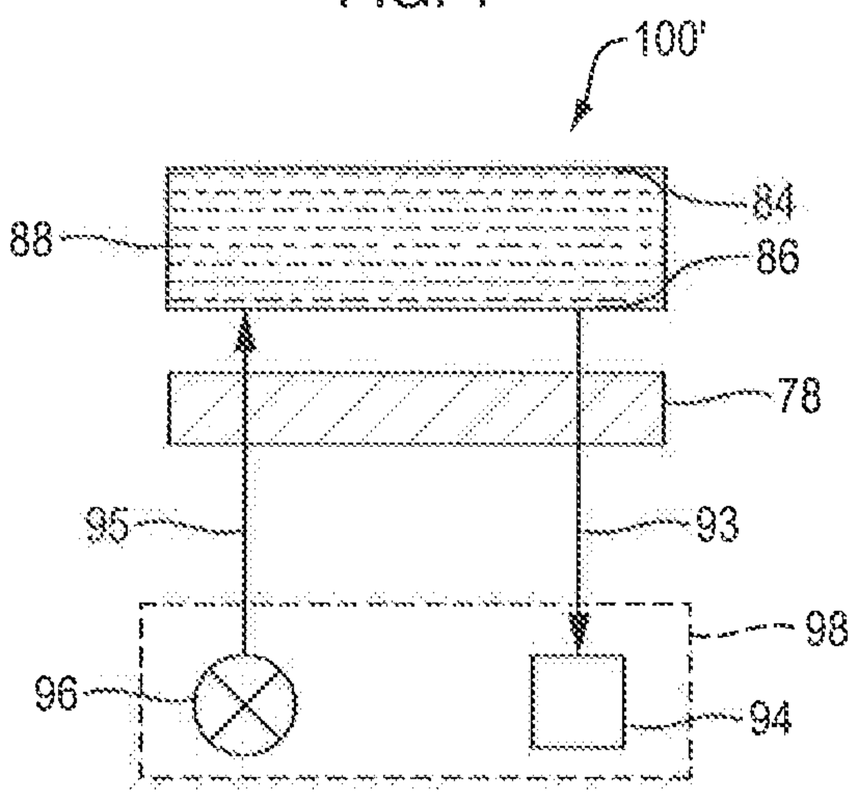


FIG. 2

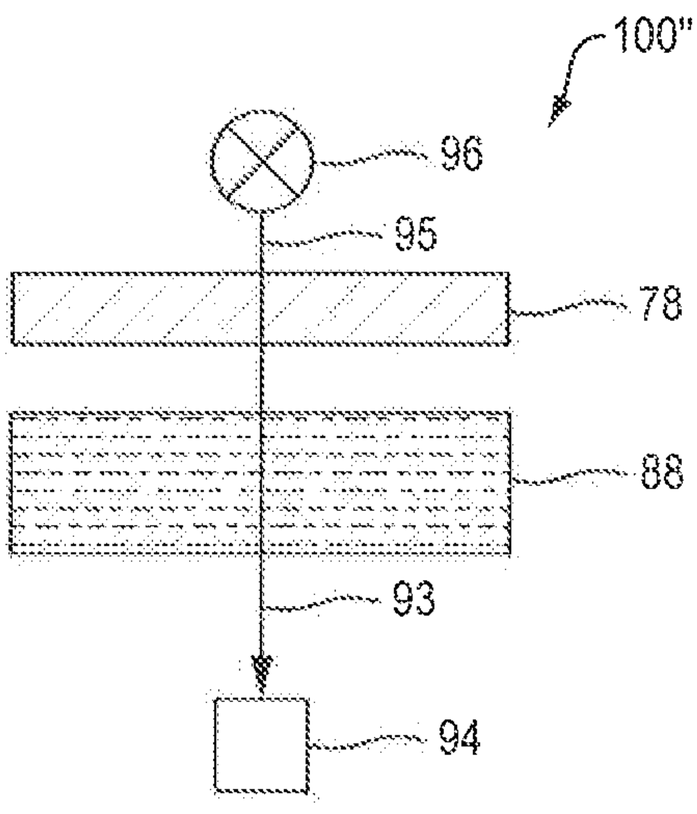


FIG. 3

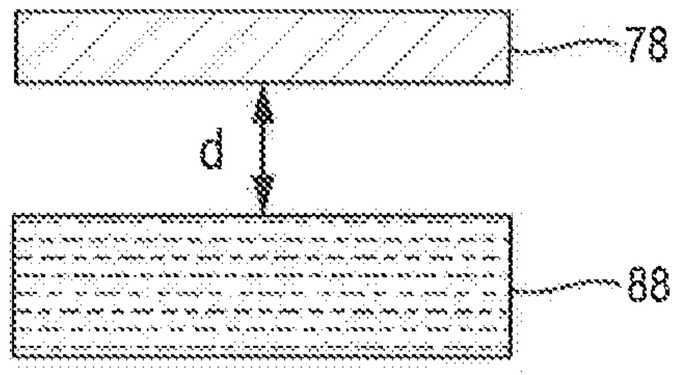


FIG. 4

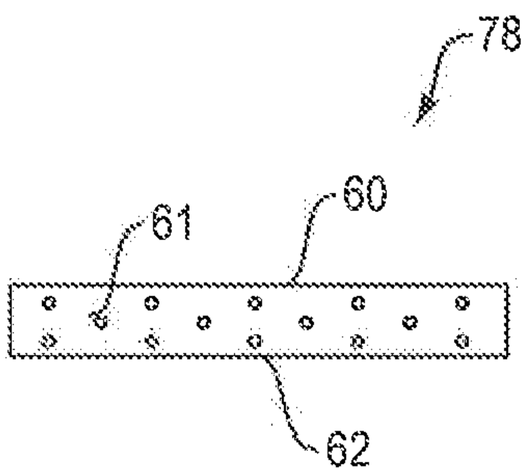


FIG. 5A

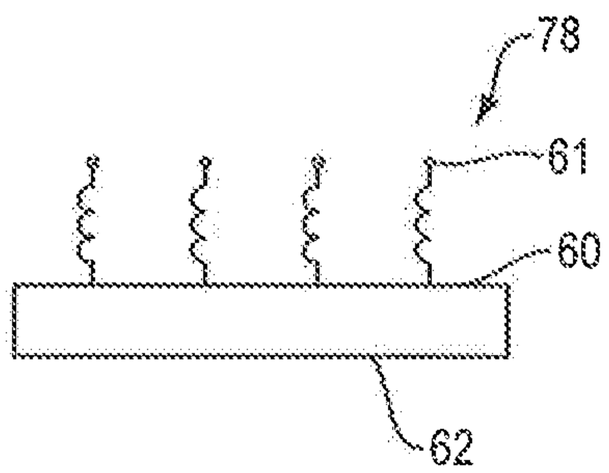


FIG. 5B

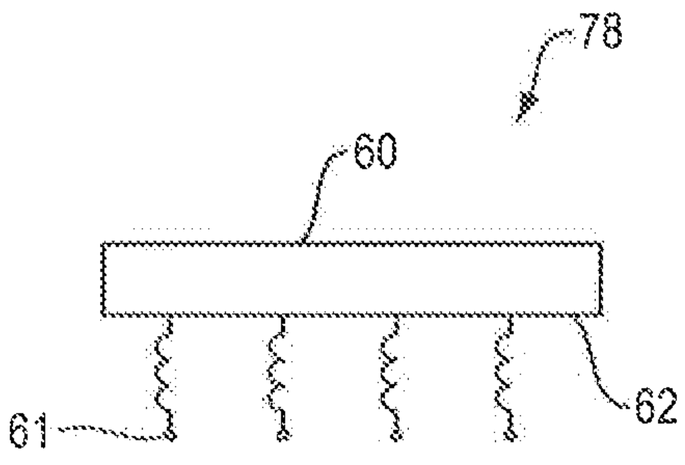


FIG. 5C

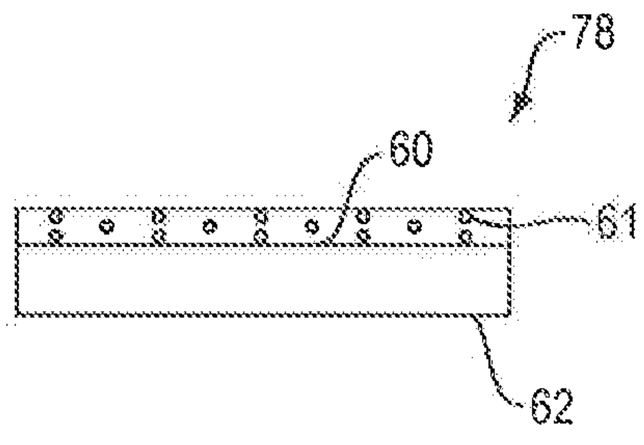


FIG. 5D

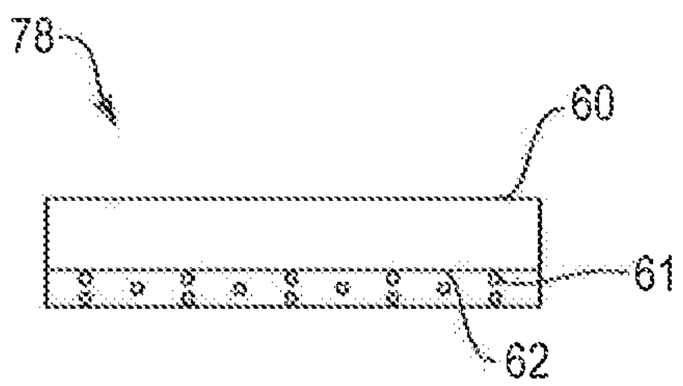


FIG. 5E

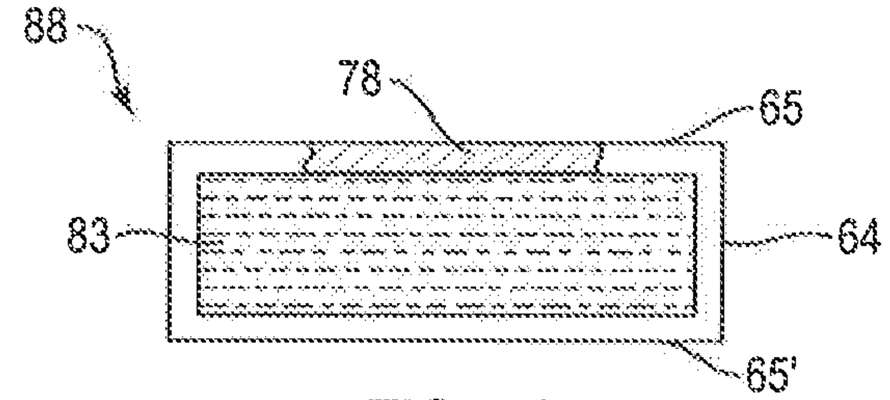


FIG. 6A

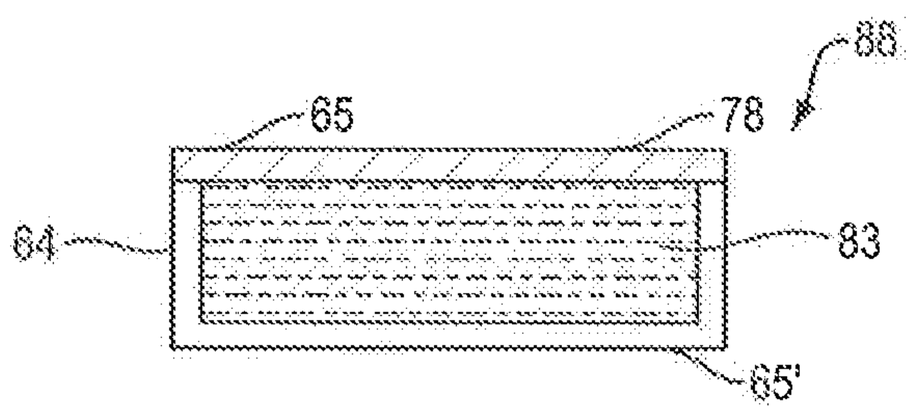


FIG. 6B

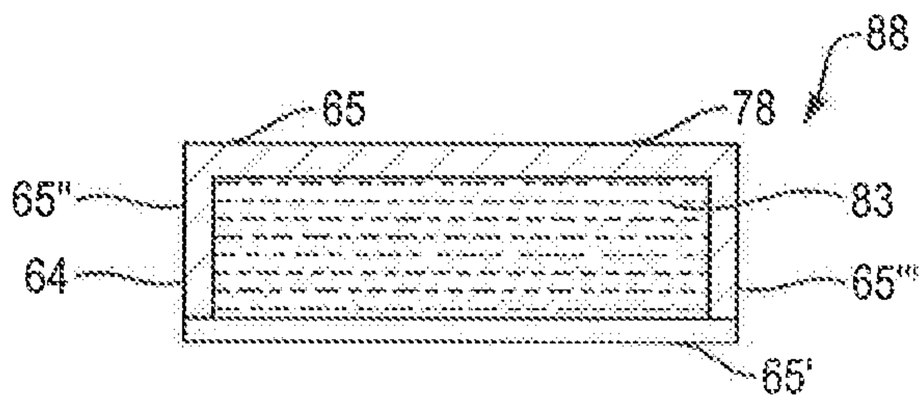


FIG. 6C

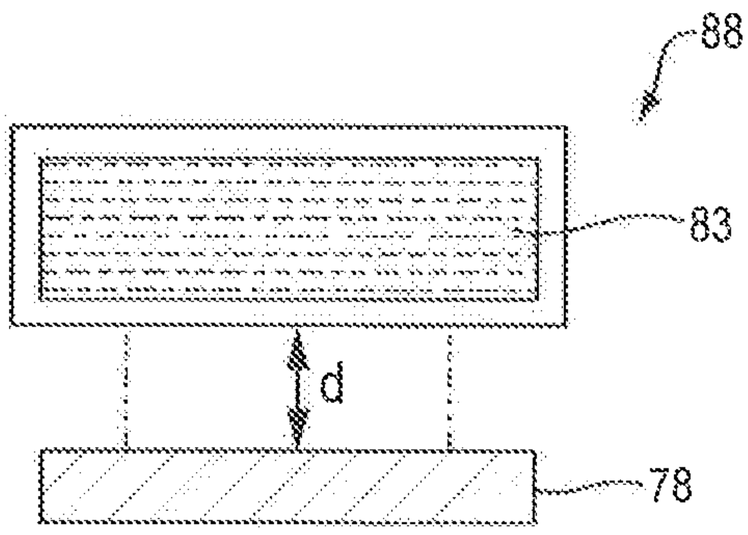


FIG. 6D

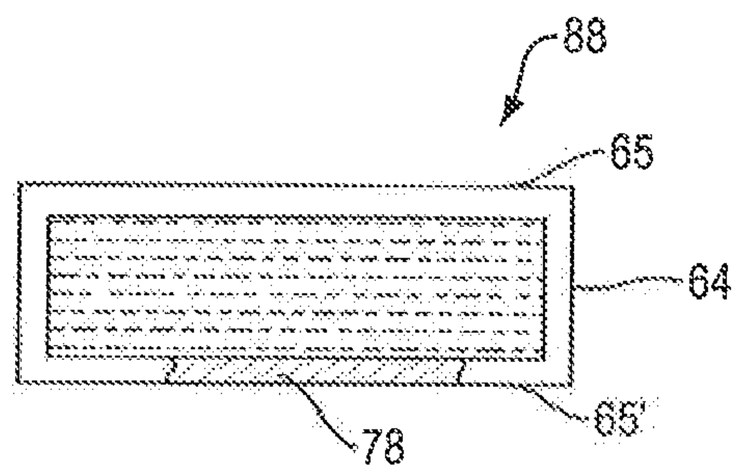


FIG. 6E

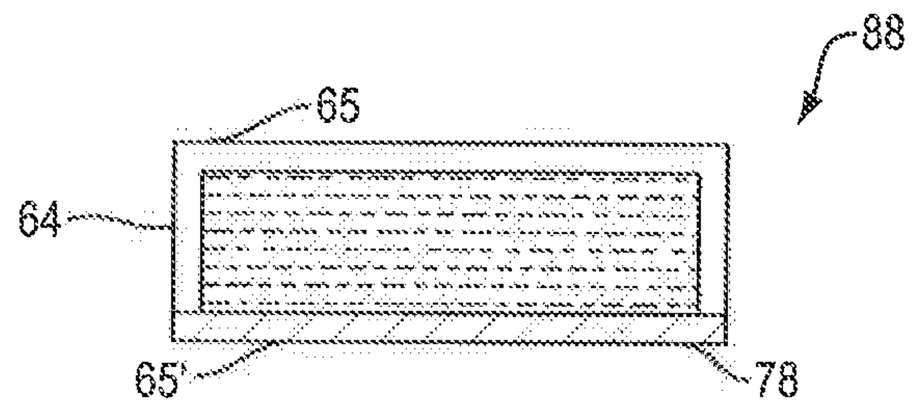


FIG. 6F

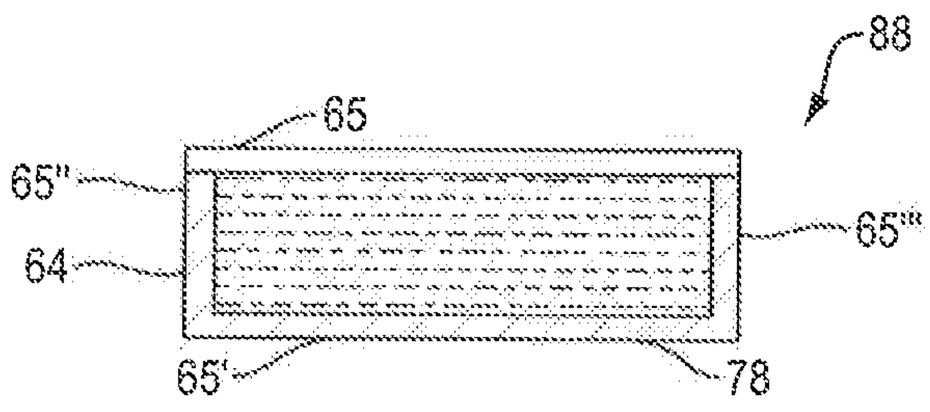


FIG. 6G

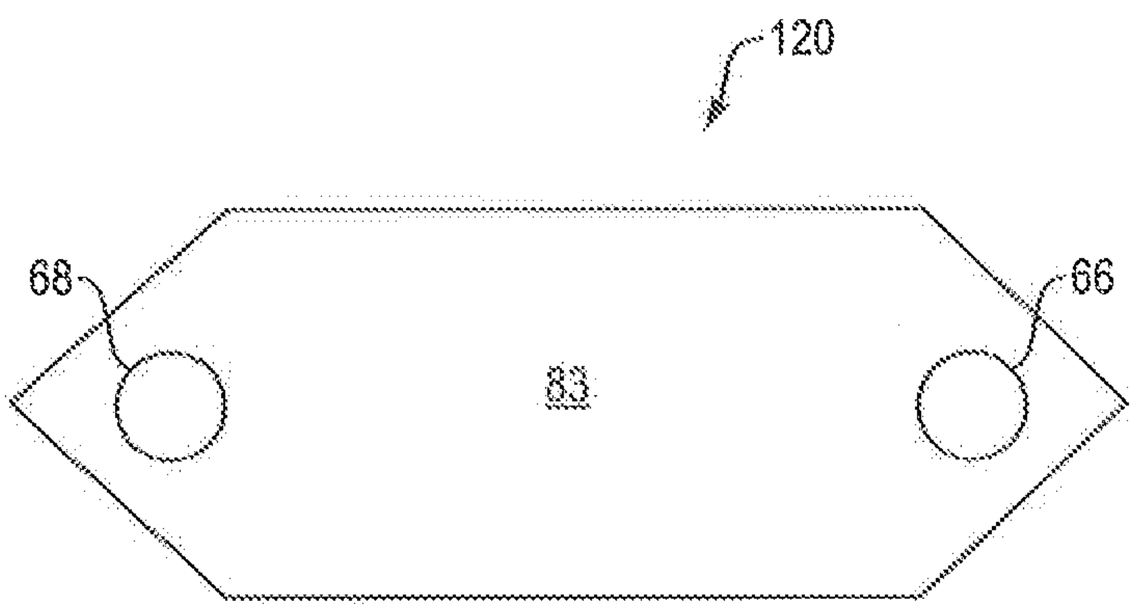


FIG. 7A

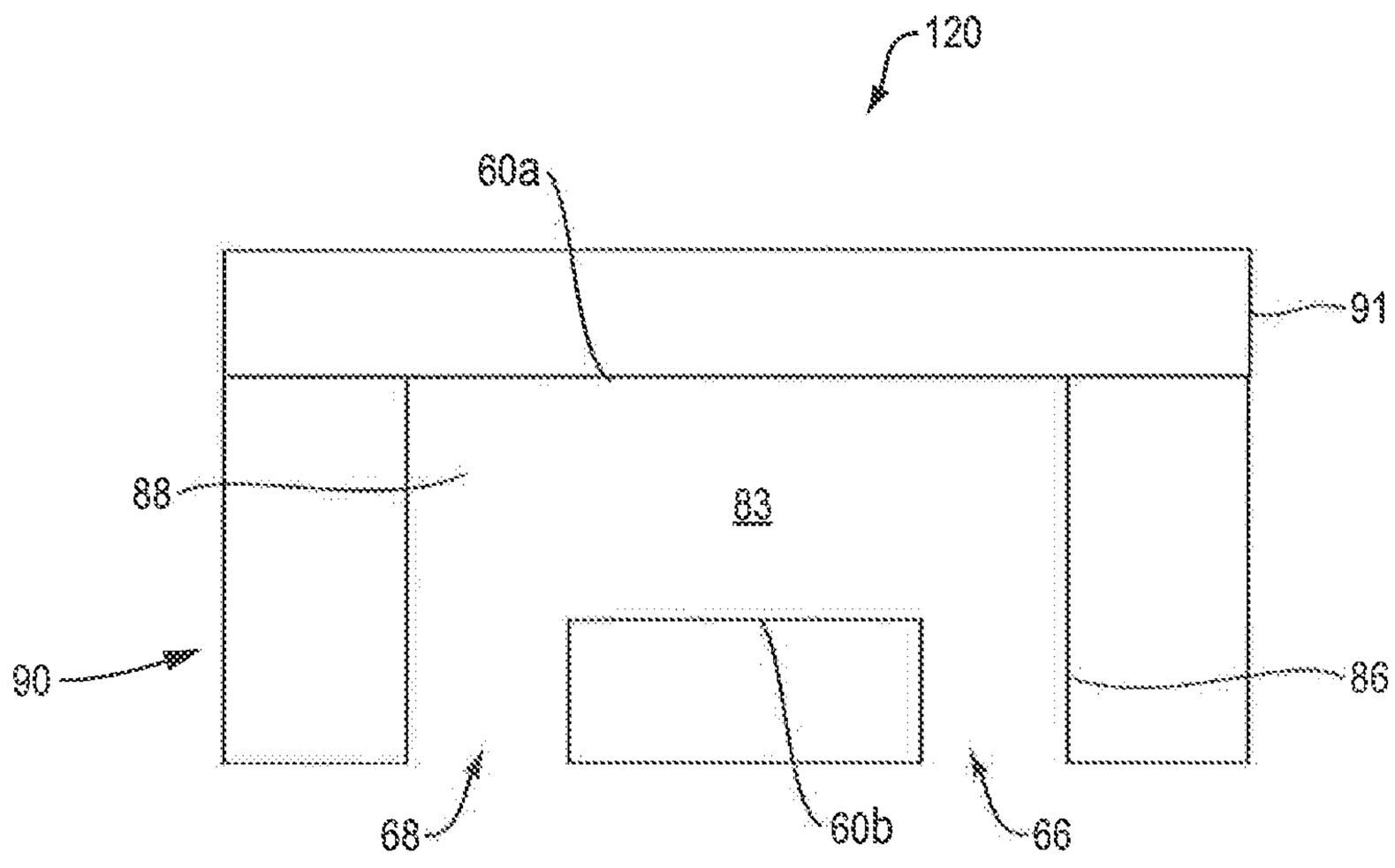


FIG. 7B

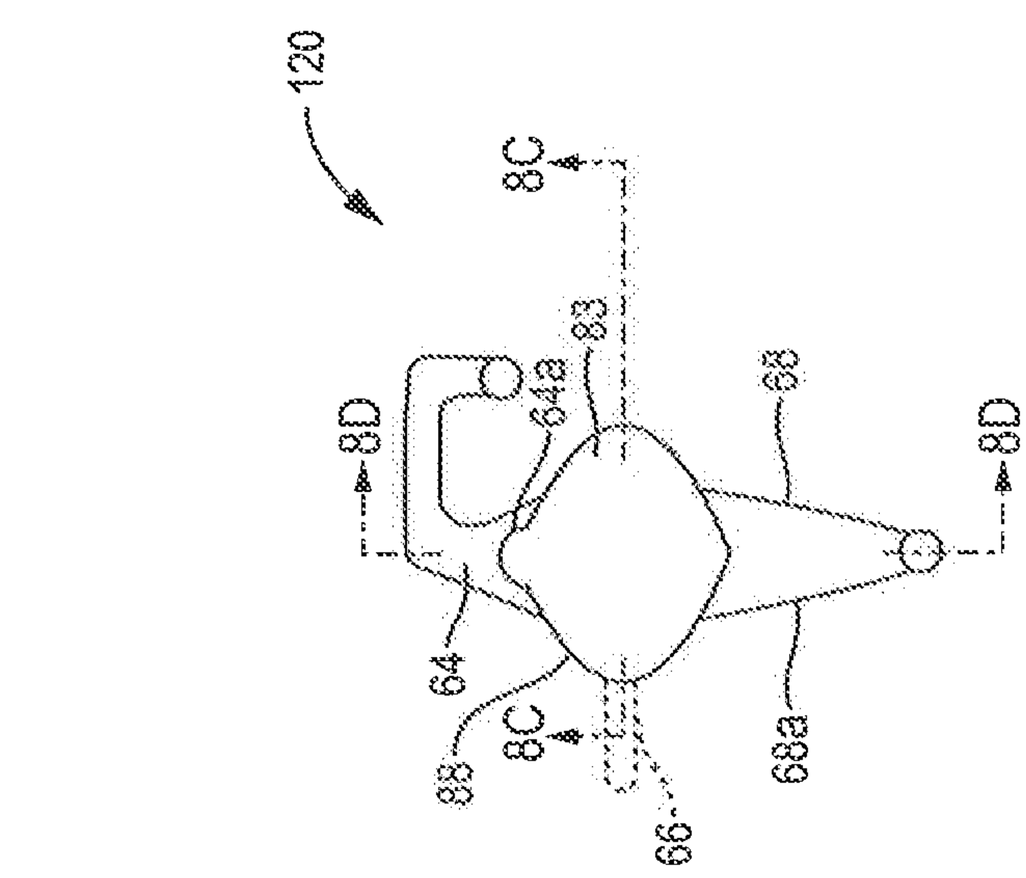


FIG. 8B

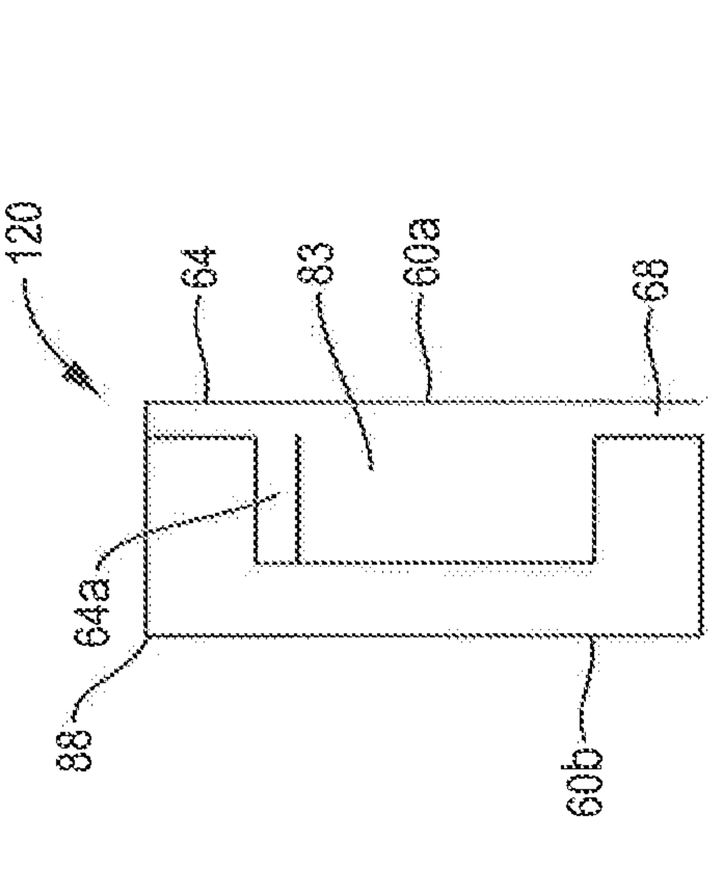


FIG. 8D

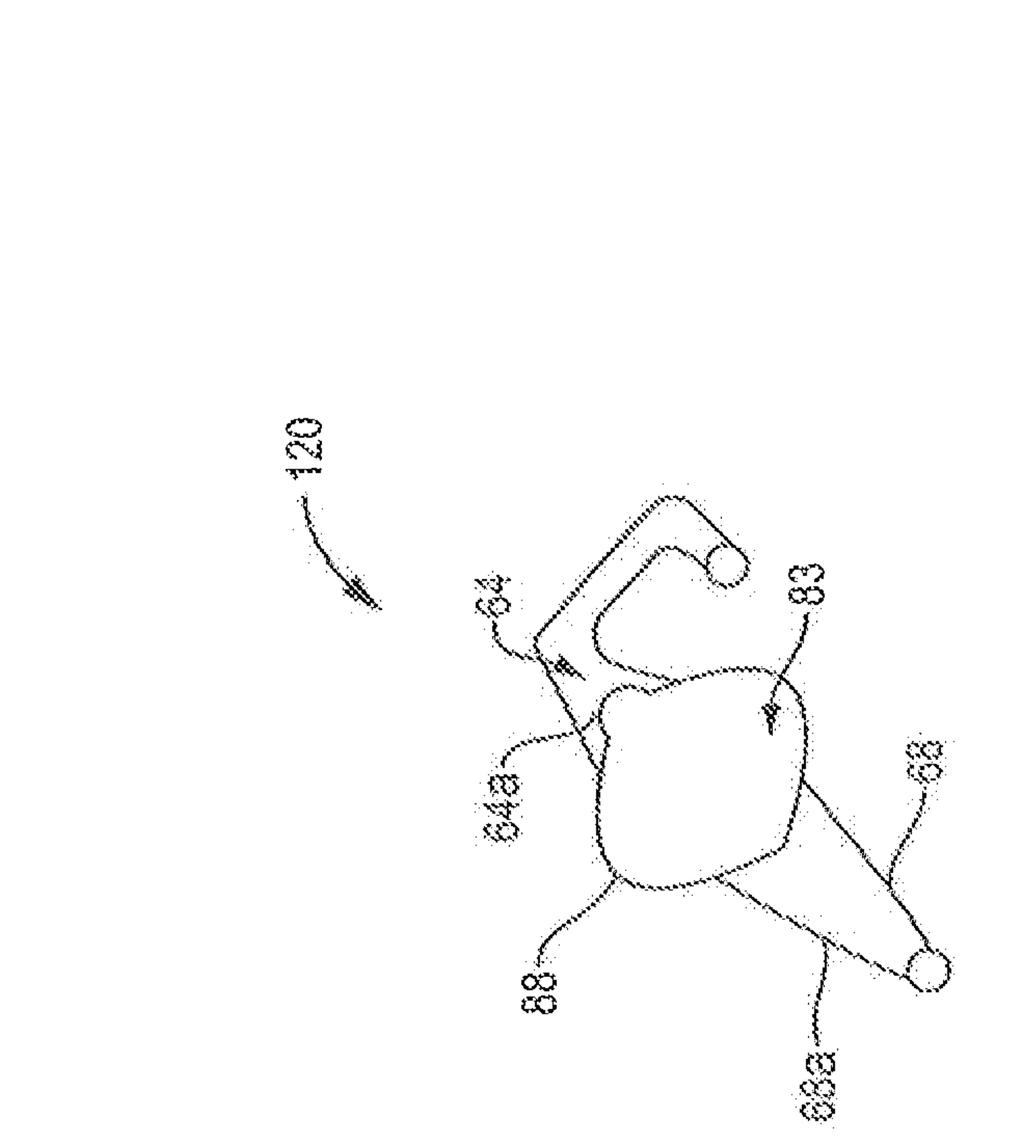


FIG. 8A

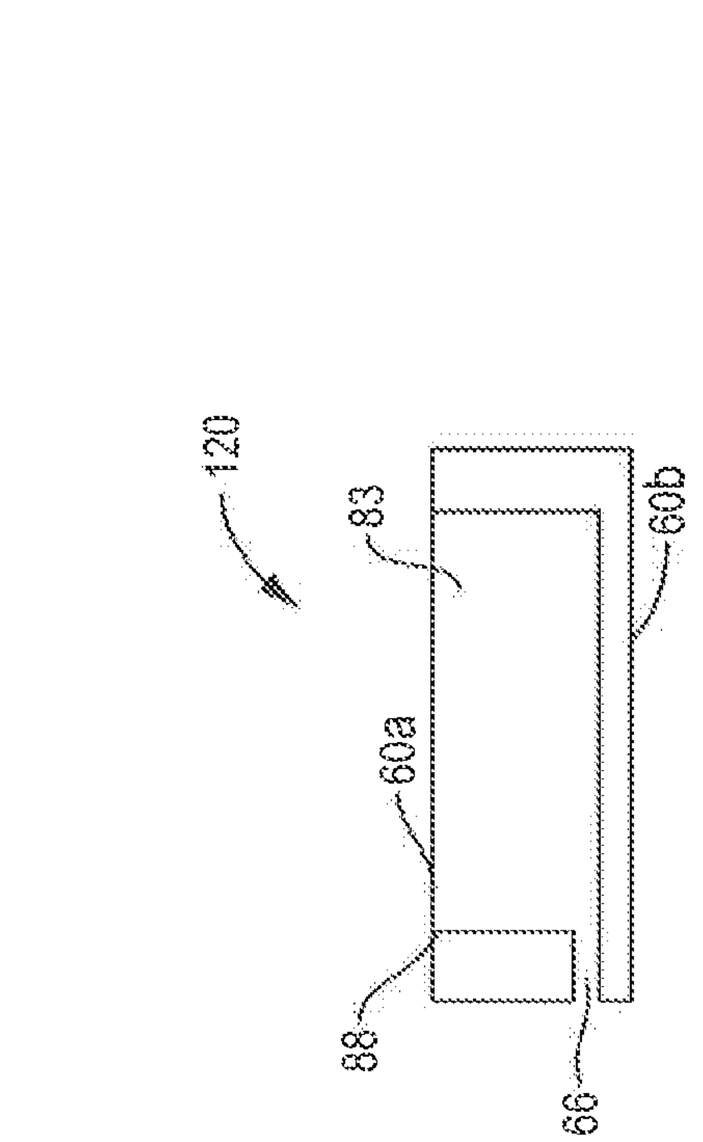


FIG. 8C

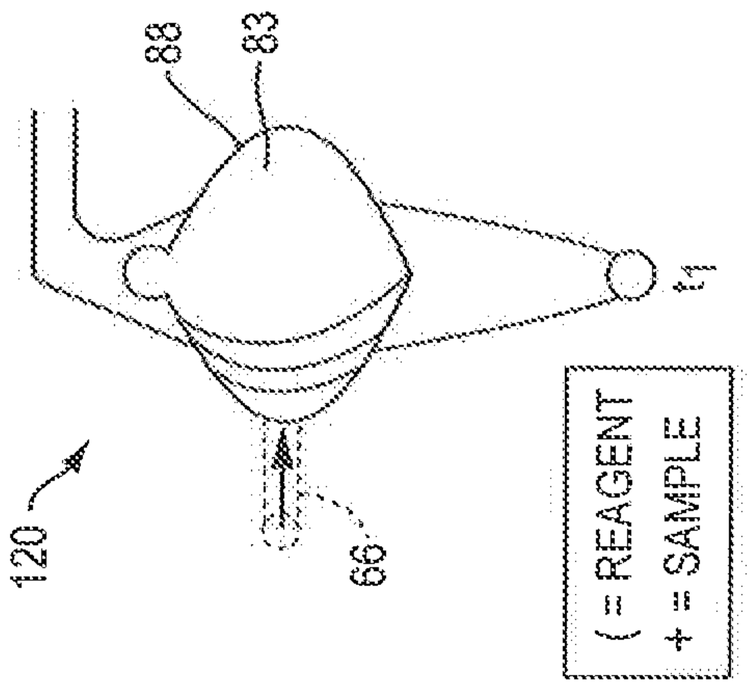


FIG. 8F

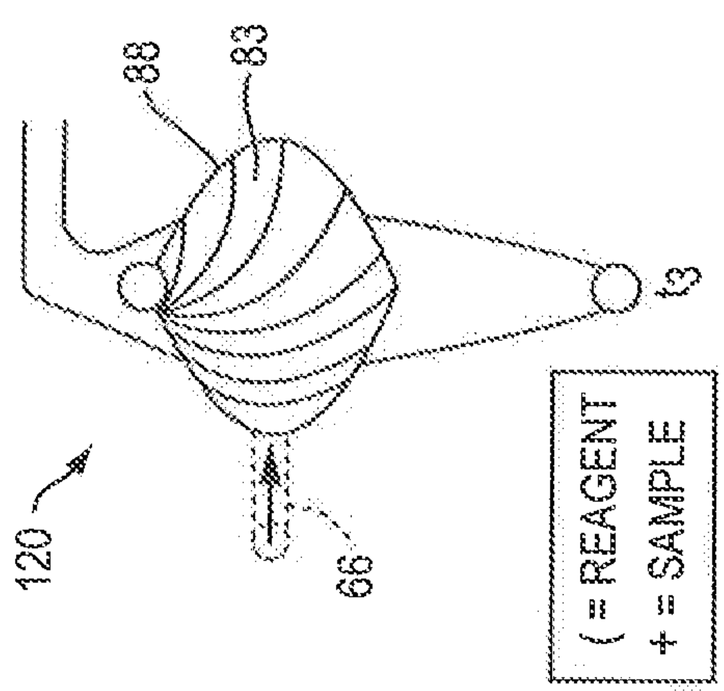


FIG. 8H

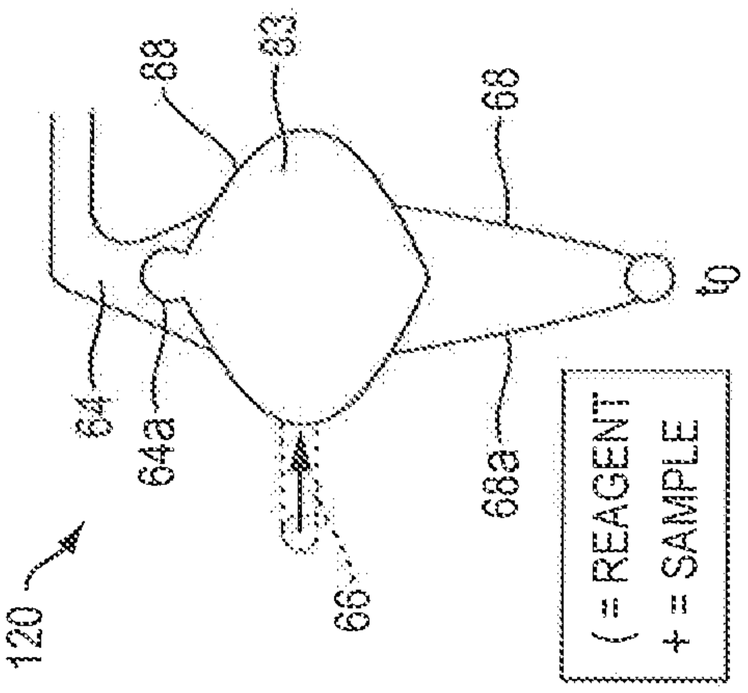


FIG. 8E

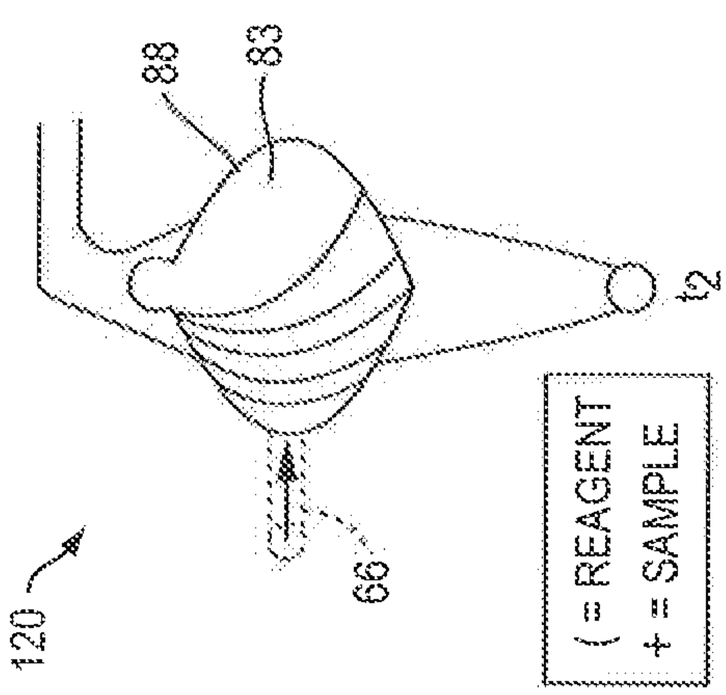


FIG. 8G

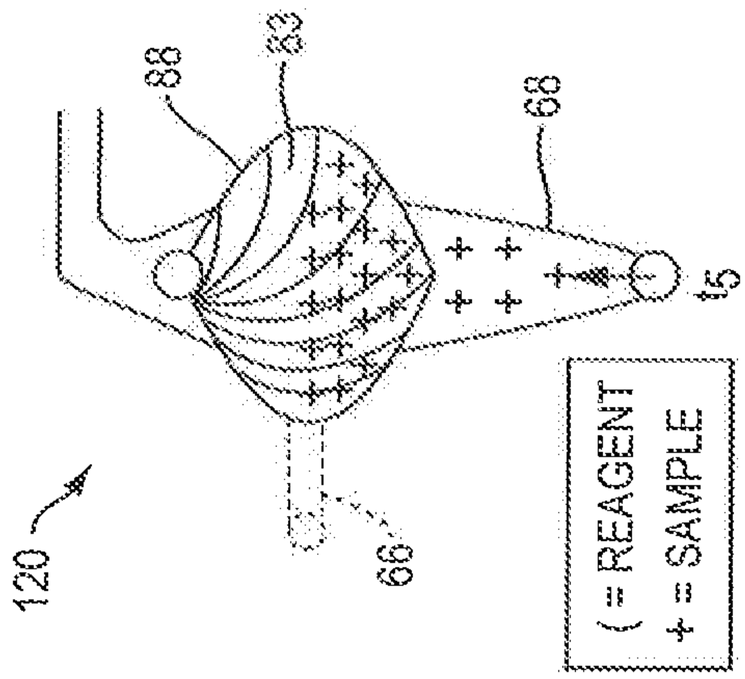


FIG. 8J

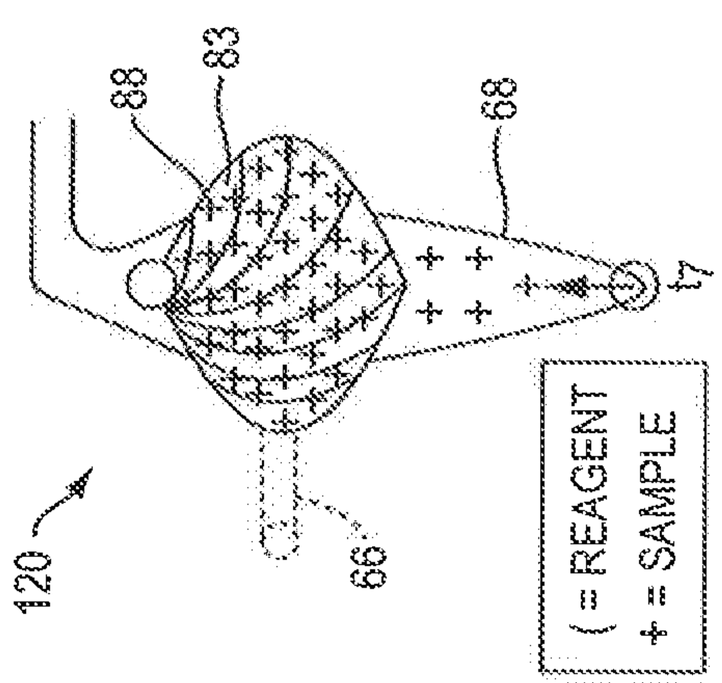


FIG. 8L

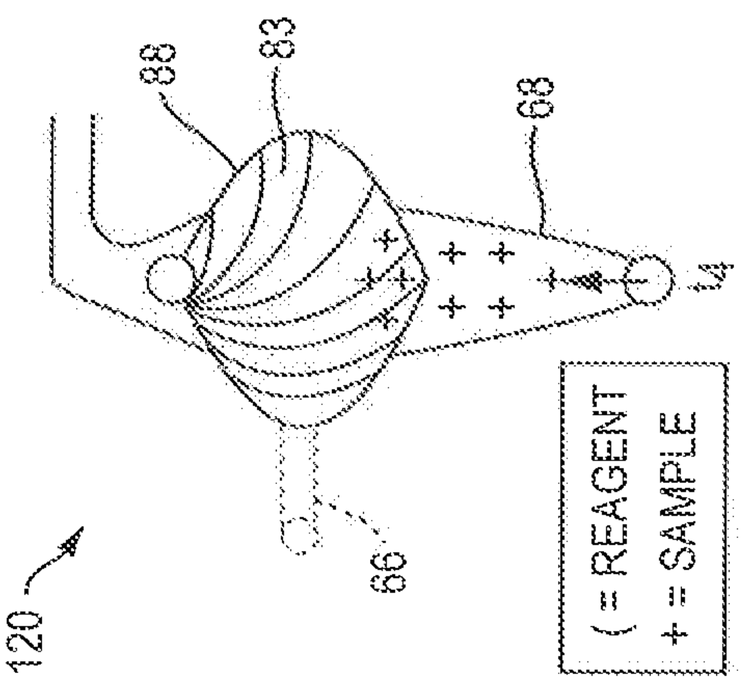


FIG. 8I

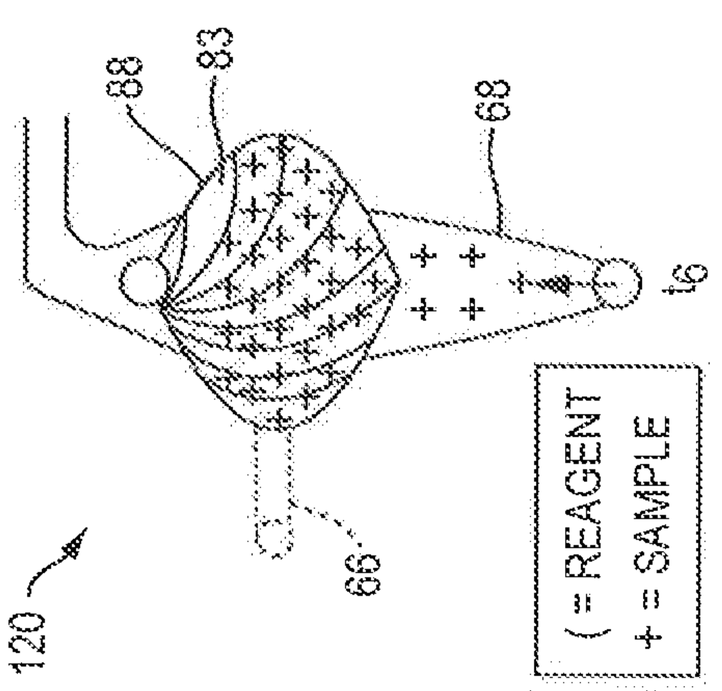


FIG. 8K

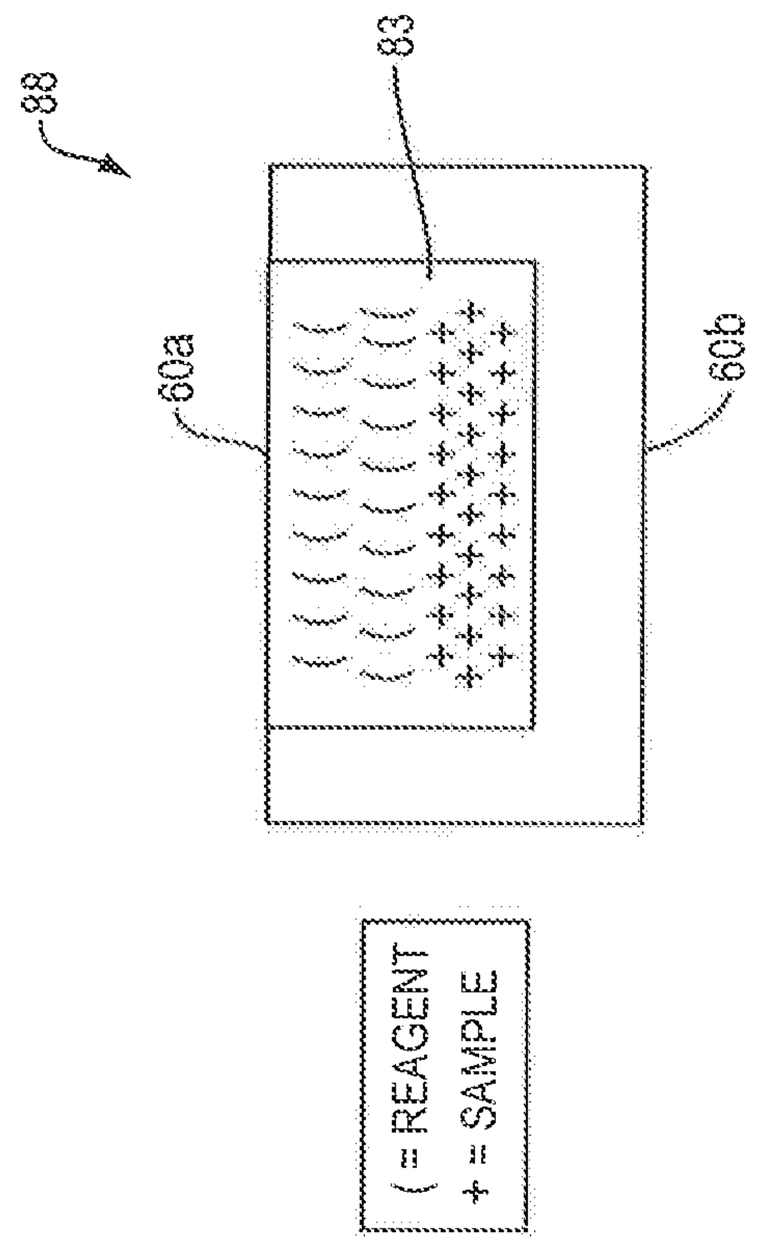


FIG. 9

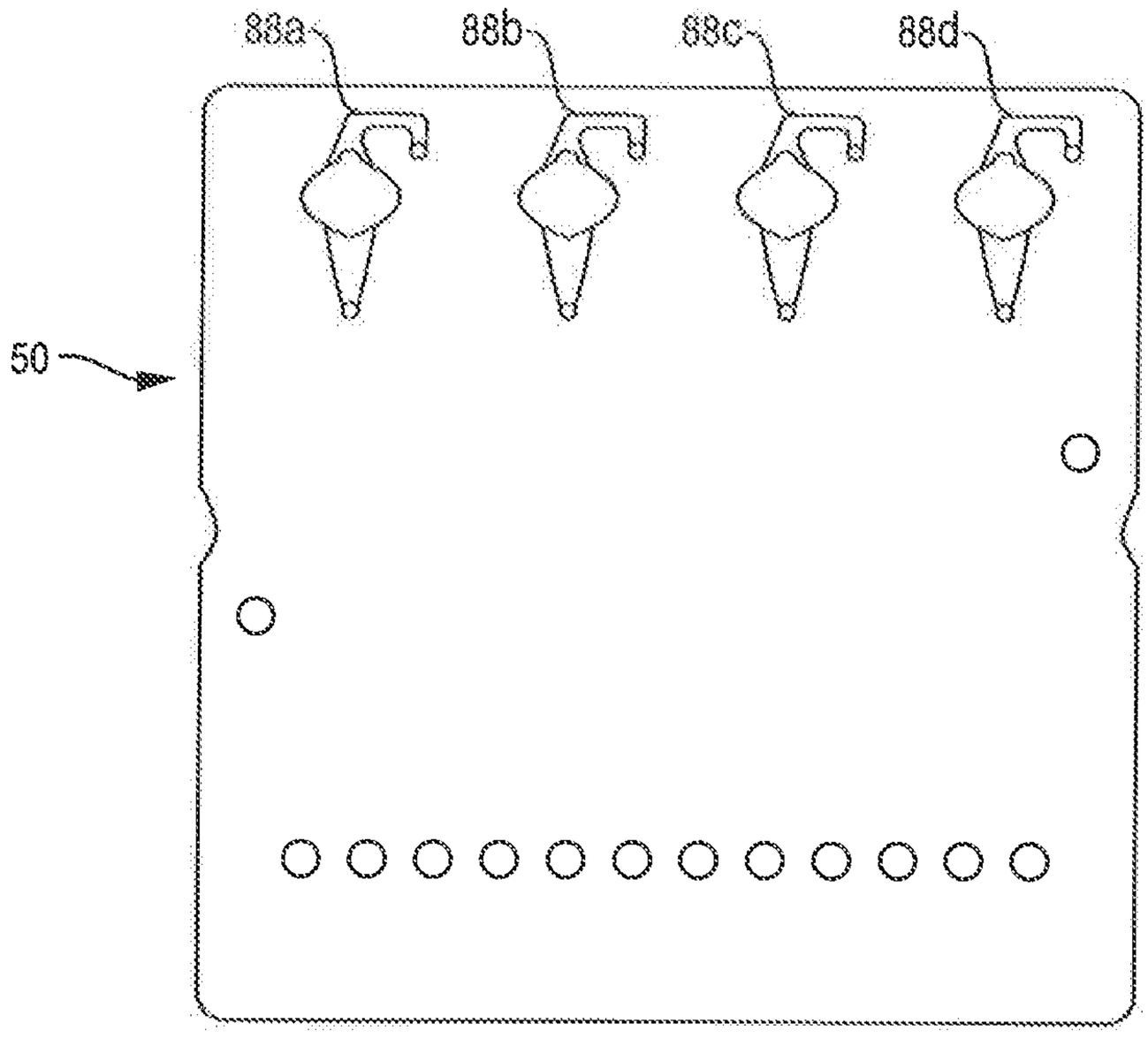


FIG. 10A

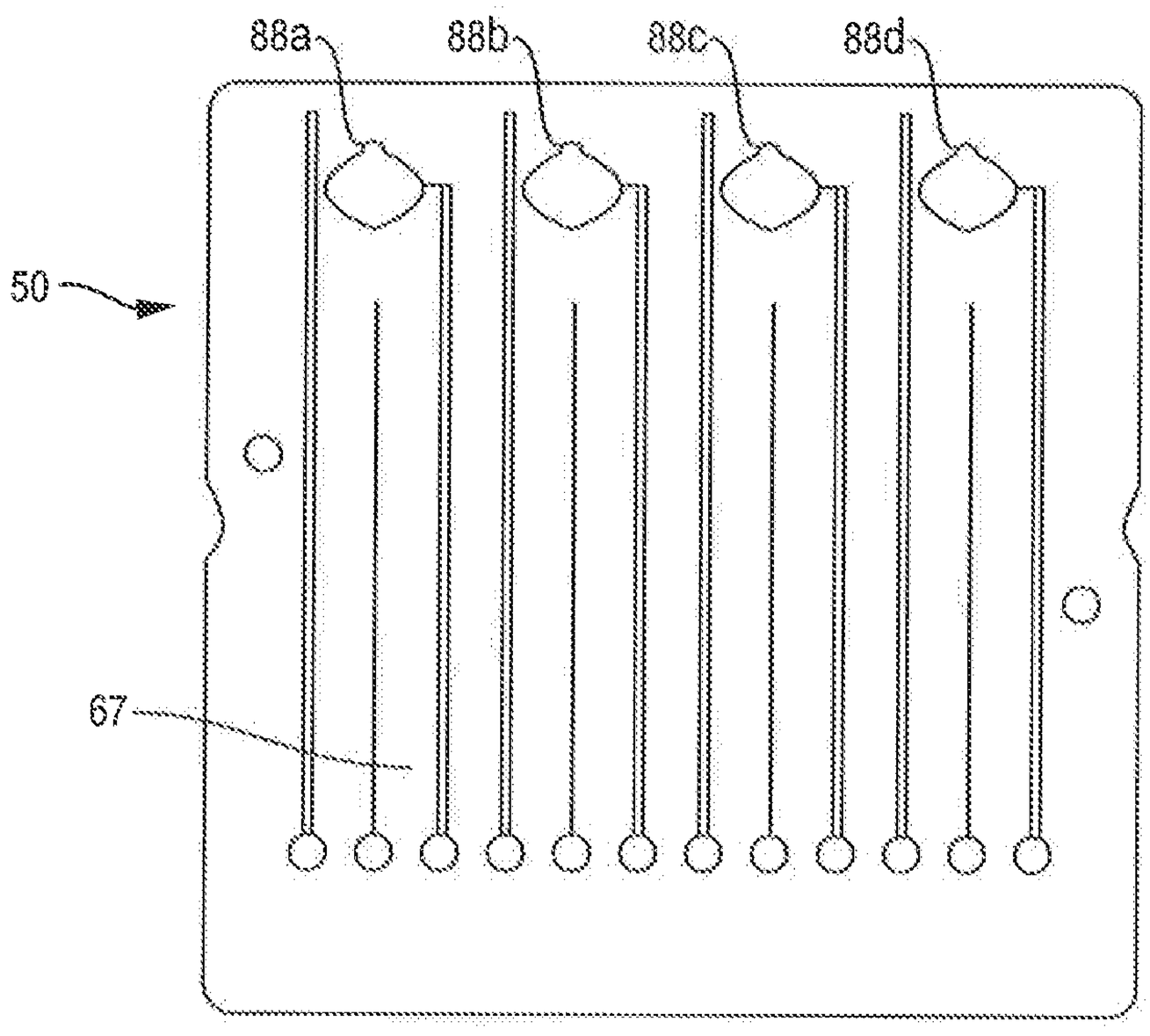


FIG. 10B

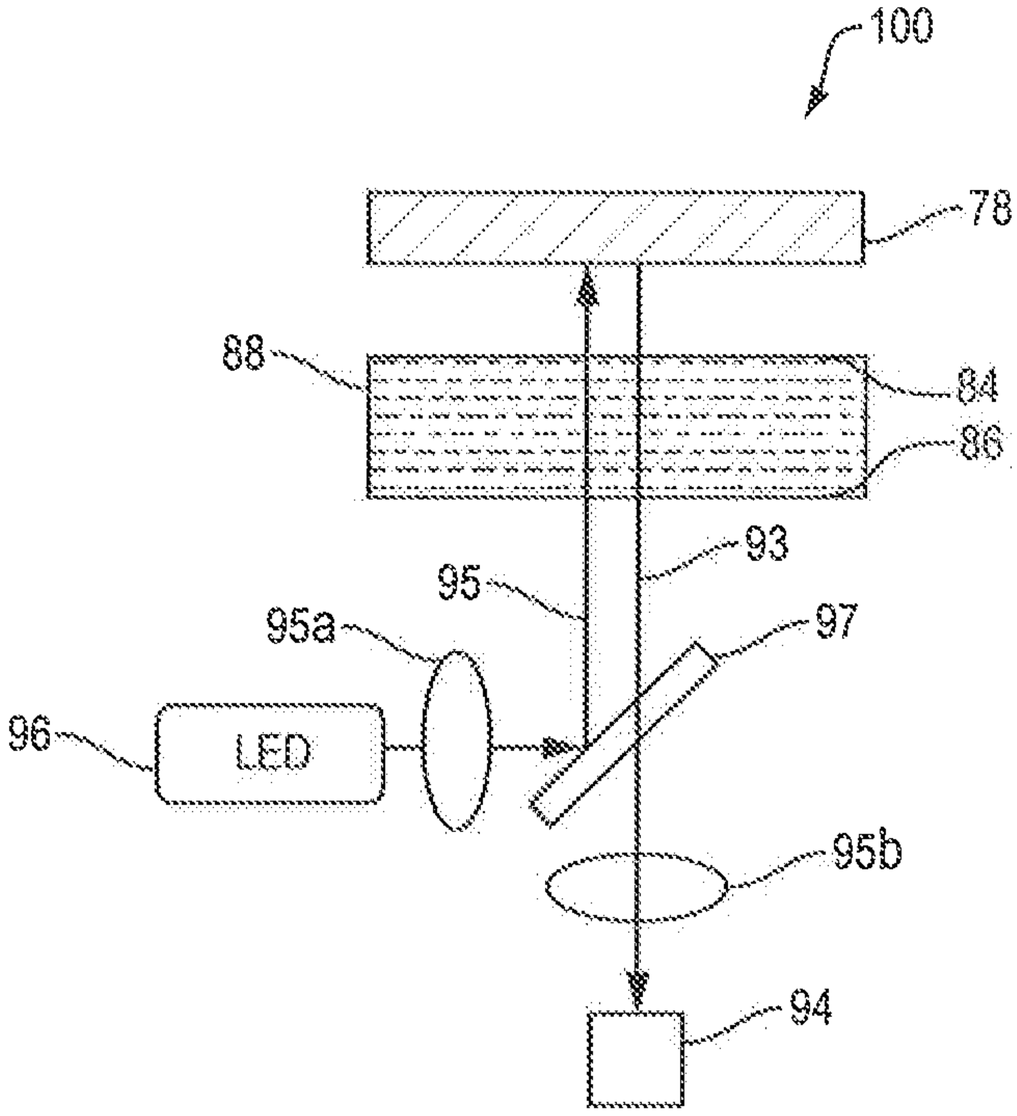


FIG. 11A

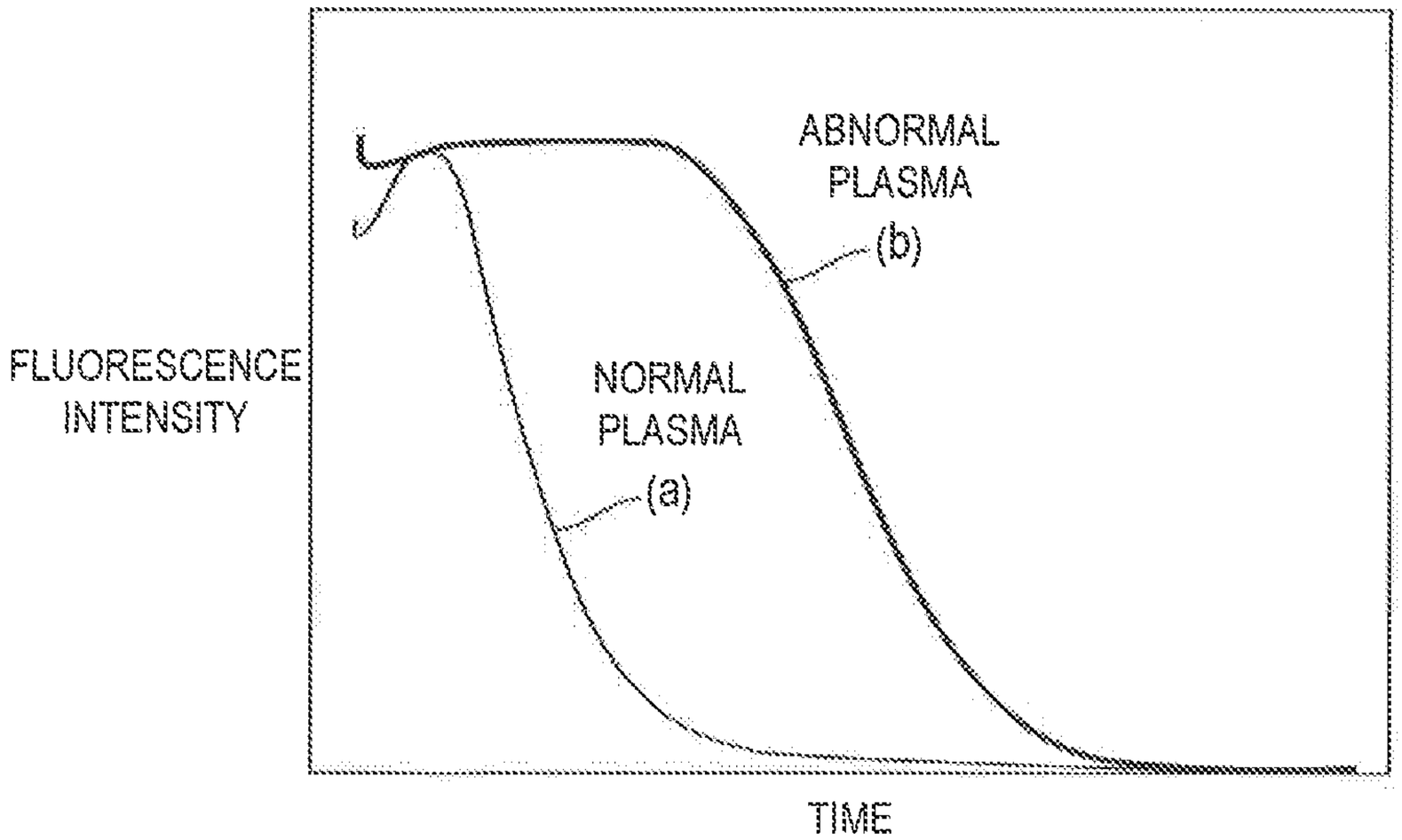


FIG. 11B

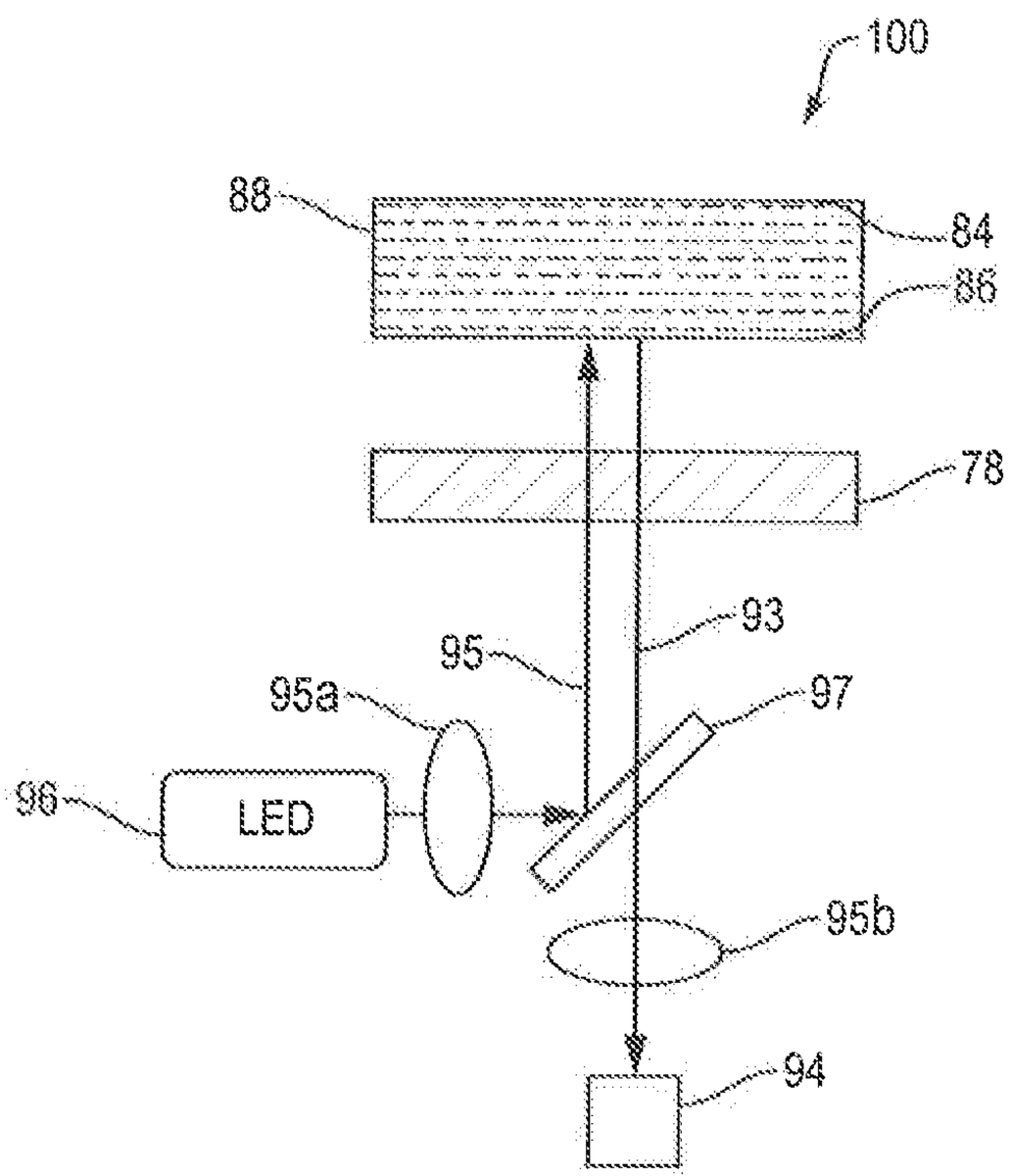


FIG. 12A

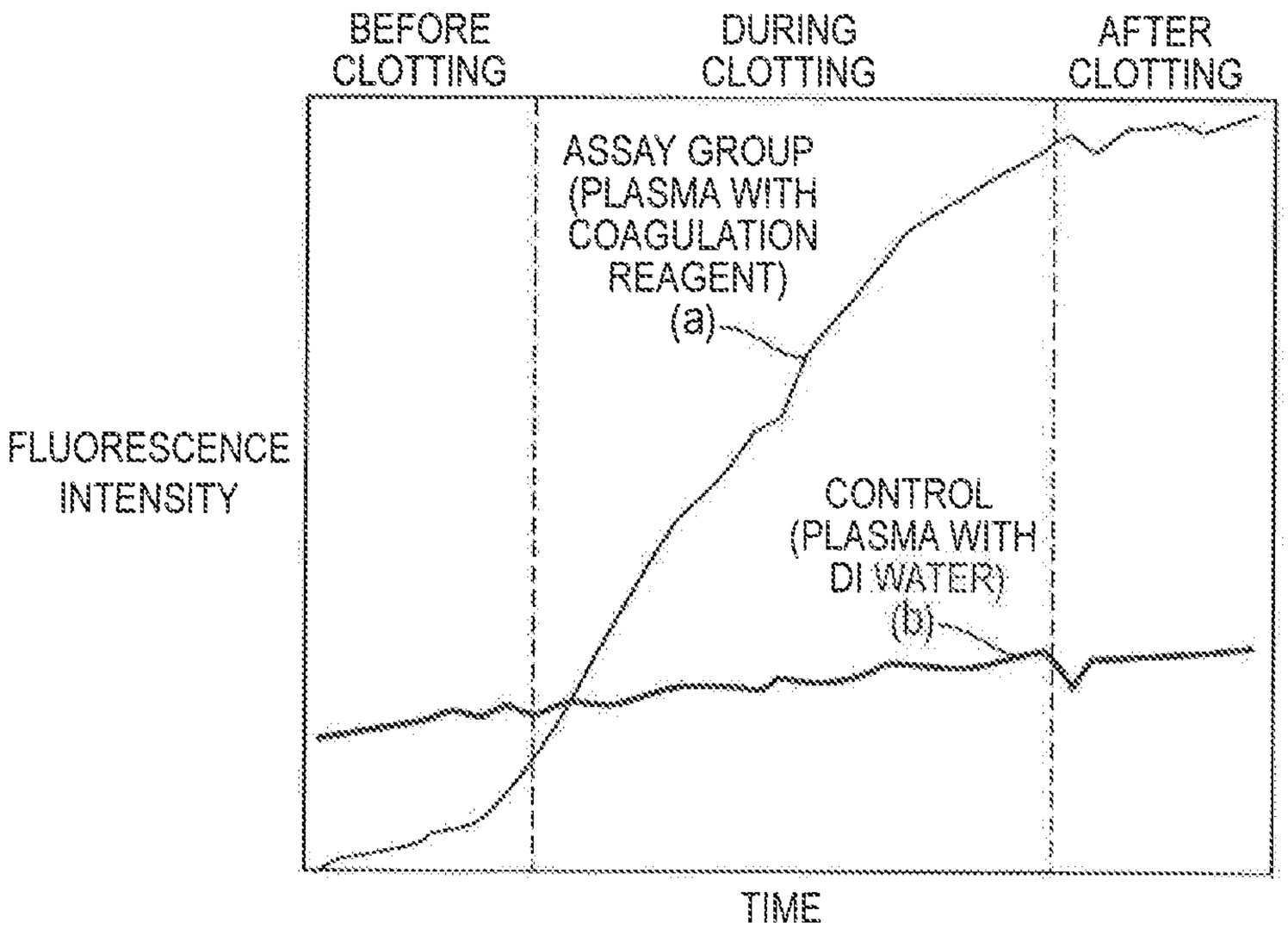


FIG. 12B



FIG. 13B

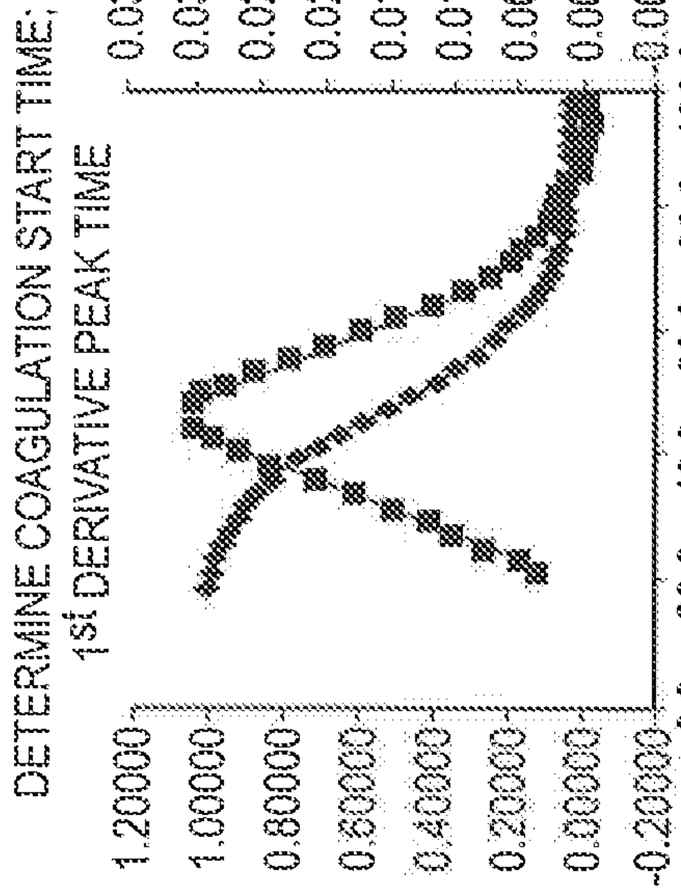


FIG. 13D

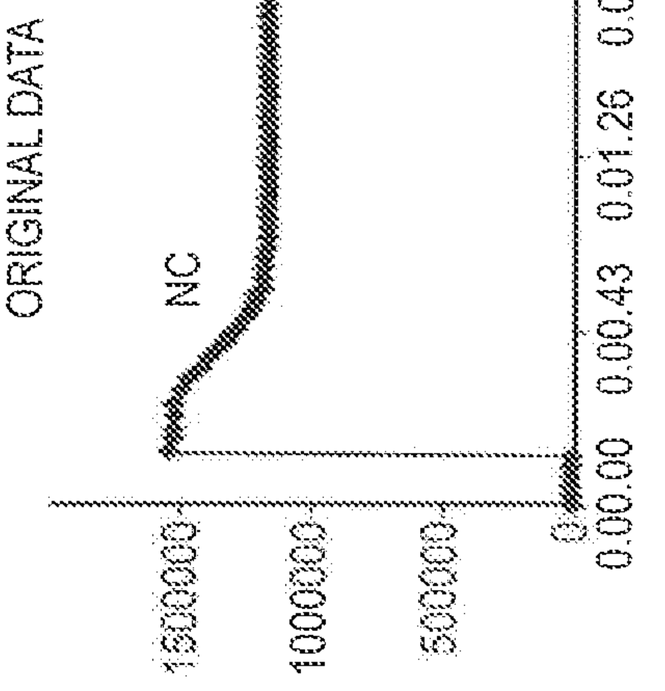


FIG. 13A

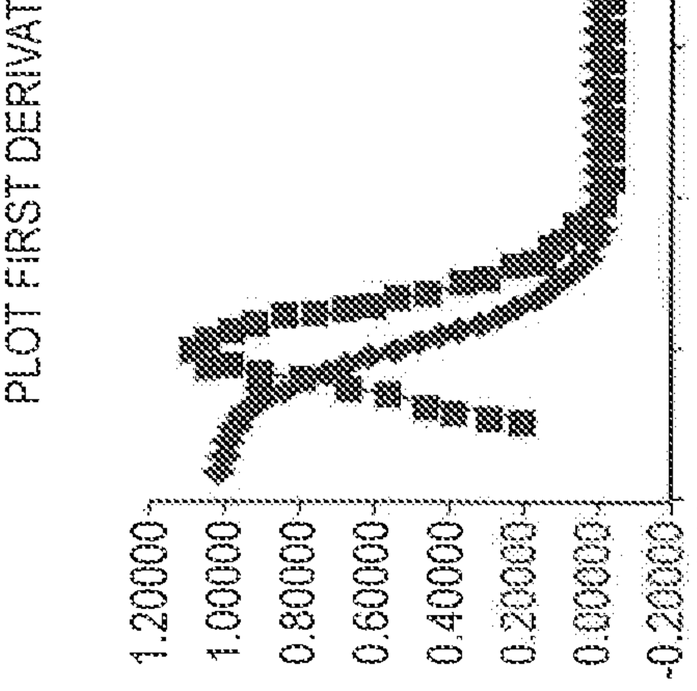


FIG. 13C

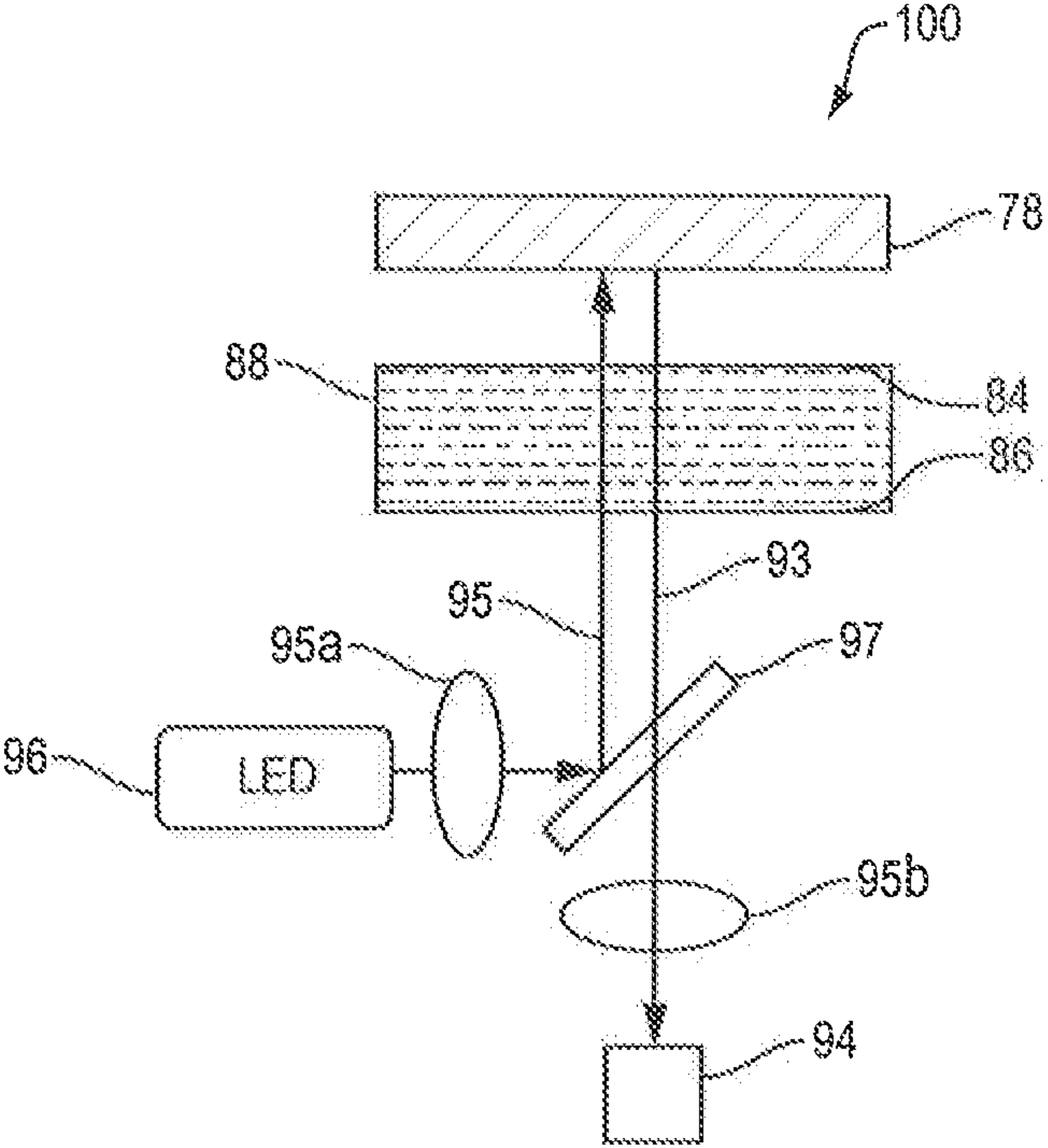


FIG. 11A