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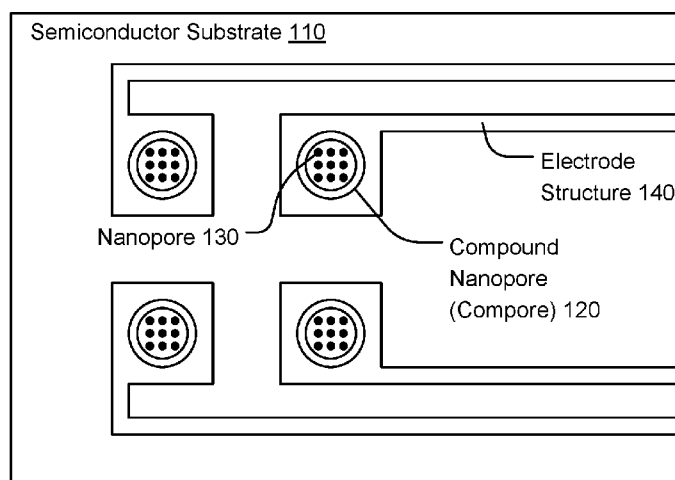
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(54) Title: NANOSENSOR CHIP WITH COMPOUND NANOPORES



Nanosensor
Chip 100

FIG. 1

(57) Abstract: A nanosensor chip for detecting and/or quantifying target molecules in a liquid sample includes a semiconductor or other substrate and one or more electrode structures. The substrate has one or more compound nanopores, referred to as "compores." Each compore is an aperture formed in the substrate and comprises a plurality of nanopores. Each of the nanopores is functionalized with immobilized probe molecules for detecting the target molecules. For each compore, a corresponding electrode structure is laid out on the substrate. The electrode structure has a shape and a position relative to the compore to apply an electric field across all of the nanopores in the compore and to provide a conductive path for an aggregate current through all of the nanopores in the compore. The aggregate current changes in response to target molecules in the liquid sample binding to the probe molecules as a function of the electric field.

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NANOSENSOR CHIP WITH COMPOUND NANOPORES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Patent Application No. 16/383,491, filed April 12, 2019, the entirety of which is incorporated by reference herein.

BACKGROUND

[0001] Nanopipettes have been developed to detect biomolecules in a liquid sample. Current nanopipette sensors have a single funnel-shaped structure with probe molecules on the inner wall of the structure. The nanopipette sensor is immersed in a liquid sample, and an electric field is applied to the sensor. Target molecules bind to the probe molecules when the electric field is applied at a specific voltage. This binding causes a detectable current change across the nanopipette sensor.

[0002] Current nanopipette sensors fashioned from quartz glass capillary tubes suffer from significant shortcomings. They are difficult to fabricate and manufacture at scale. They are also fragile and easily broken. In addition, individual nanopipettes are highly variable and must be individually calibrated for accurate results. Nanopipette tips also show chemical and electrical degradation after repeated use, which seriously compromises performance and limits reuse.

SUMMARY

[0003] A nanosensor chip for detecting and/or quantifying target molecules in a liquid sample is disclosed herein. The nanosensor chip includes a semiconductor substrate with one or more compound nanopores formed in the semiconductor substrate. Each compound nanopore is an aperture that includes multiple nanopores, each of which is functionalized with immobilized probe molecules. The probe molecules are used to detect the target molecules in the liquid sample. A compound nanopore is referred to herein as a “compore.”

[0004] Each compore has a corresponding electrode structure on the semiconductor substrate. The electrode structure has a shape and position relative to the compore that enable the electrode structure to apply an electric field across all of the nanopores in the compore. The electrode structure also provides a conductive path for detecting an aggregate current through all of the nanopores in the compore. The detected aggregate current changes in response to target molecules in the liquid sample binding to the probe molecules, which binding is a function of the applied electric field.

[0005] For example, if the liquid sample includes some of the target molecules (e.g., a particular viral protein in a biofluid sample), when a specific electric field (or voltage) is applied across the compore, the target molecules bind to probe molecules in the nanopores. The probe molecules that functionalize the compore are selected to bind to the particular target molecule when a specific electric field is applied. The binding of the target molecules to the probe molecules changes the electrical characteristics of the nanopore openings, which creates a change in the aggregate current through the compore. A given probe molecule-target molecule pairing binds in the presence of a particular electric field strength or range of electric field strengths. If the liquid sample does not include the target molecule and the electric field is applied, there will be no aggregate current change. In addition, if an electric field different from the particular electric field strength or range is applied, the target molecules do not bind to the probe molecules and there is no aggregate current change.

[0006] The compore structure provides a greater level of reliability than prior nanopipette sensors. For example, if one of the nanopores in the compore is blocked or clogged, an aggregate current change is detected based on the binding of the target molecules to the probe molecules in the other, unblocked nanopores. Furthermore, if the nanopores in a given compore are not uniformly functionalized with the probe molecules, e.g., some nanopores have a higher concentration of probe molecules and other nanopores have a lower concentration of probe molecules, the change in aggregate current across all of the nanopores averages out the variations in concentration when detecting the presence of target molecules in liquid sample. Because of this greater reliability, the compore sensor is more accurate and reliable than prior nanopipette sensors.

[0007] Nanosensor chips may have other advantages over nanopipette technology. In particular, nanosensor chips can be efficiently and inexpensively manufactured at scale. An entire wafer of chips may be functionalized with probe molecules simultaneously, where the prior nanopipettes are individually functionalized. Due to improved consistency, a single chip can be used to calibrate an entire wafer of chips, instead of individually calibrating the nanopipettes.

[0008] Other aspects include components, devices, systems, improvements, methods, processes, applications, and other technologies related to any of the above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure (FIG.) 1 is a top view of a nanosensor chip with multiple compores.

[0010] FIG. 2 is a perspective view of an individual compore.

- [0011] FIG. 3A is a top view of an individual compore.
- [0012] FIG. 3B is a cross section of the compore shown in FIG. 3A.
- [0013] FIG. 4 is a cross-section of a single nanopore within a compore.
- [0014] FIGs. 5A and 5B are cross-sections that show operation of a nanopore.
- [0015] FIGs. 6A and 6B are graphs that illustrate operation of a compore.
- [0016] FIG. 7 is a top view showing a first alternative arrangement of nanopores within a compore.
- [0017] FIG. 8 is a top view showing a second alternative arrangement of nanopores within a compore.
- [0018] FIG. 9 is a cross-section of a compore fabricated using semiconductor technology.
- [0019] FIG. 10 is a block diagram of a detection system that includes a nanosensor chip.

DETAILED DESCRIPTION

[0020] The Figures (FIGs.) and the following description relate to preferred embodiments by way of illustration only. It should be noted that from the following discussion, alternative embodiments of the structures and methods disclosed herein will be readily recognized as viable alternatives that may be employed without departing from the principles of what is claimed.

[0021] Reference will now be made in detail to several embodiments, examples of which are illustrated in the accompanying figures. It is noted that wherever practicable similar or like reference numbers may be used in the figures and may indicate similar or like functionality. The figures depict embodiments of the disclosed nanosensor chip for purposes of illustration only. One skilled in the art will readily recognize from the following description that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles described herein.

[0022] Figure (FIG.) 1 is a top view of a nanosensor chip 100 with multiple conical compound nanopores 120, also referred to as compores 120. The nanosensor chip 100 is formed from a semiconductor substrate 110, e.g., a silicon or gallium arsenide (GaAs) substrate. Alternative substrates can be used instead, such as glass, plastic, film and other materials. One or more compores 120 are formed in the semiconductor substrate 110. A compore 120 is an aperture formed in the semiconductor substrate 110 that includes multiple nanopores 130. The nanopores 130 form holes extending through the semiconductor substrate 110. The nanopores 130 have openings at a top of the compore 120 (shown in FIG. 1) and at a bottom of the compore 120 on the reverse side of the nanosensor chip 100 (not

visible in FIG. 1). The nanopores 130 of the compore 120 are functionalized with immobilized probe molecules and collectively form an aperture through the semiconductor substrate 110. The compore 120 may have a maximum width between 1 micron and 1000 microns. The compore 120 is brought into contact with a liquid sample, for example it may be positioned vertically between sample and buffer reservoirs. It can be used to detect whether or not one or more target molecules are present in the liquid sample and/or to quantify the concentration of target molecules in the liquid sample, based on binding of the target molecules to the probe molecules.

[0023] The compore 120 can be used to assay any liquid sample, e.g., a sample of blood, saliva, spinal liquid, urine, food, beverage, water, etc., in which a target molecule of interest may be present. Different nanosensor chips 100, and different compores 120 within a single nanosensor chip 100, may be configured to assay different types of liquid samples and to detect one or more types of target molecules within the liquid sample. For example, one nanosensor chip 100 may be configured to test for a set of antibodies in blood samples, while another nanosensor chip 100 is configured to test for a set of contaminants in water samples.

[0024] Each of the nanopores 130 in a compore 120 is functionalized with immobilized probe molecules. Each nanopore 130 has a sidewall extending between the two openings, and probe molecules are affixed to the sidewall of the nanopore 130. An example arrangement of probe molecules within a nanopore 130 is shown in FIGs. 5A and 5B. The probe molecules may be selected for detecting a particular type of target molecule or set of target molecules in a liquid sample, e.g., to detect a particular antibody or set of antibodies in a blood sample, or to detect a particular contaminant in water. The probe molecules have a binding affinity to the target molecules, such that in the presence of a specific electric field, target molecules in a sample bind to the probe molecules. The target molecules may reversibly bind to the probe molecules, so that when the electric field is removed, the target molecules release from the probe molecules. Examples of probe molecules include antibodies, antibody analogs, proteins, aptamers, polymers, oligonucleotides, and nanobodies.

[0025] Each compore 120 has a corresponding electrode structure 140 laid out on the semiconductor substrate 110. The electrode structure 140 has a shape and a position relative to the compore 120 suitable to apply an electric field across all of the nanopores 130 in the compore 120. The electrode structure 140 also provides a conductive path for conducting an aggregate current that passes through the nanopores 130 in the compore 120. For example, the electrode structure 140 may include one or more electrodes on the top of the compore 120 (as shown in FIG. 1) and one or more electrodes on the reverse side of the compore 120 (not

visible in FIG. 1). Exemplary electrode structures are shown in FIGs. 3 and 9. The electrode structure 140 may connect to circuitry located on the nanosensor chip 100 for supplying a voltage source for the electric field and/or detecting the aggregate current. Alternatively, the electrode structure 140 may connect to an off-chip source and/or detector.

[0026] When the liquid sample includes the target molecule and the correct electric field is applied across the compore 120, the target molecules bind to the immobilized probe molecules in the nanopores 130. This binding changes an aggregate current that passes through the compore 120. The aggregate current flows through the electrode structure 140 to a current detector on the nanosensor chip 100 or connected to the nanosensor chip 100. A change in the aggregate current through all of the nanopores 130 of a compore 120 indicates the presence of the target molecules in the liquid sample. An amount by which the aggregate current changes may be used to determine a concentration or quantity of the target molecules in the liquid sample. By contrast, if the particular electric field is applied to the compore 120 but no aggregate current change is detected, this indicates that the target molecules are not present in the liquid sample.

[0027] Because each compore 120 includes multiple nanopores 130 that experience the same electric field and the aggregate current through all of the nanopores is detected, the compore 120 has a greater reliability than previous nanosensors. For example, even if one or a few of the nanopores 130 are blocked or clogged, the aggregate current through the set of nanopores 130 in the compore 120 still changes in response to the target molecules in the liquid sample binding to the probe molecules in the presence of the electric field. Similarly, the change in aggregate current through the set of nanopores 130 can still be detected even if the nanopores are not uniformly functionalized, e.g., if some nanopores have more immobilized probe molecules than other nanopores. In addition, using multiple nanopores 130 increases the number of different types of target molecules that a single compore 120 can be used to detect, because the nanopores 130 can be functionalized with multiple different types of probe molecules. Configurations with multiple types of probe molecules are described further in relation to FIGs. 5A and 5B.

[0028] The nanosensor chip 100 depicted in FIG. 1 includes four compores 120. In other arrangements, the nanosensor chip 100 may include any number of compores, e.g., one, four, sixteen, or many hundreds or even thousands of compores. Two compores 120 on the nanosensor chip 100 may test the same liquid sample or different liquid samples. The compores on the nanosensor chip 100 may be identical, or some or all of the compores may be different from each other. For example, two compores 120 on a single nanosensor chip

100 may have different sizes, different shapes, different numbers of nanopores, nanopores with different sizes or shapes, or nanopores with different probe molecules. Including different compores on a single nanosensor chip 100 enables a single nanosensor chip 100 to perform multiple different tests, e.g., to test for multiple different target molecules, to test with different sensitivities, or to include controls to verify the accuracy and to authenticate the nanosensor chip 100. Including multiple identical compores on a single nanosensor chip 100 may be used to improve the accuracy and reliability of a single nanosensor chip 100.

[0029] In FIG. 1, each compore 120 on the nanosensor chip 100 may be individually addressed using its respective electrode structure 140. Because each compore 120 has a separate electrode structure 140, the aggregate current through each compore 120 can be individually measured by a current detector connected to the electrode structure 140. In some embodiments, each electrode structure 140 is also used to individually apply an electric field across each respective compore 120. In other embodiments, the electrode structure for applying the electric field across a compore is distinct from the electrode structure used to measure the aggregate current through a compore. In such embodiments, the electrode structures for applying the electric fields may be connected for two or more compores, so that the same electric field or voltage can be applied to multiple compores simultaneously.

[0030] FIG. 2 is a perspective view of an individual compore 120, but not showing the electrode structure. FIG. 2 shows the structure of the compore 120 in greater detail. The compore 120 has a central region 210 that is thinner than the semiconductor substrate 110. The nanopores 130 are formed within this thinned central region. For example, the central region 210 may be thinned to a thickness of less than 1 micron. The thickness of the central region 210 is also the height of the nanopores 130 formed within the compore 120. In a nanosensor chip with multiple compores, such as the nanosensor chip 100 shown in FIG. 1, the thinned regions of the compores are separated from each other by unthinned regions. If desired, this may be used to maintain separation between the liquid samples of different compores and to maintain isolation between the voltages and currents of different compores.

[0031] Between the top of the central region 210 and the top of the semiconductor substrate 110, the compore 120 has a compore sidewall 220. The compore sidewall 220 is depicted as being sloped, but it may be straight, curved, or have some other arrangement. In some embodiments, the liquid sample may be placed in the depression formed by the central region 210 and the compore sidewall 220, with a buffer solution on the other side of the compore 120. In other embodiments, the nanosensor chip 100 may be positioned vertically

between two reservoirs, one containing buffer solution and the other containing the liquid sample.

[0032] Various other configurations for applying a liquid sample to the compore 120 may be used. For example, the opposite side of the compore 120 may be exposed to the liquid sample instead. In such embodiments, the portion of the compore 120 surrounded by the compore sidewall 220 may receive a liquid buffer.

[0033] FIG. 3A is a top view of an individual compore 120. FIG. 3A shows an exemplary arrangement of nanopores 130 in a compore 120. Adjacent nanopores 130 are separated by some spacing 230. The spacing distance 230 between two adjacent nanopores in a single compore 120 may be, for example, between 1 nanometer and 100 microns. In the example shown in FIG. 3A, the compore 120 has ten nanopores 130. In other embodiments, a compore 120 may have a different number of nanopores 130, e.g., from two to several hundred nanopores. While the nanopores 130 in FIG. 3A shows the nanopores 130 being arranged in three rows, in other embodiments, the nanopores 130 may have a different arrangement.

[0034] FIG. 3B is a cross section of the compore 120 through line A-A' shown in FIG. 3A. The compore cross-section includes three nanopores 130a, 130b, and 130c that pass through the semiconductor substrate 110. In this embodiment, one side of the nanopore structure is characterized by a depression formed by the top of the thinner central region and the compore sidewalls. For convenience, this side will be referred to as the top side, and the reverse side will be referred to as the bottom side. Either the top side or the bottom side may be exposed to the liquid sample, and the other side is typically exposed to a buffer. In FIG. 3B, an upper electrode 330 is formed on the top surface of the compore 120 and a lower electrode 340 is formed on the bottom surface. However, one or both of these electrodes may be mounted externally to the chip, e.g. in the chip receptacle, in alternative embodiments.

[0035] The upper electrode 330 and lower electrode 340 form the electrode structure for applying the electric field to the compore 120 and for conducting the aggregate current through the nanopores 130. Because all of the nanopores 130 in the compore 120 are in close proximity to each other, the electrode structure comprising the upper electrode 330 and lower electrode 340 located to the side of the nanopores 130 may be sufficient to apply an electric field across all of the nanopores 130 and to detect an aggregate current through all of the nanopores 130. In alternative embodiments, the electrode structure may be more complex. For example, the upper electrode 330 may have a portion to the left of the nanopore 130a or encircling all of the nanopores (as shown in FIG. 1). The upper electrode 330 may also

extend into the areas between the nanopores 130a-130c. Similarly, the lower electrode 340 may have an additional portion formed to the left of the nanopore 130a, encircling the nanopores, and/or extending into the areas between the nanopores 130a-130c.

[0036] The upper electrode 330 and lower electrode 340 are connected to a voltage source 350 and a current detector 360. The voltage source 350 supplies a selected voltage to the upper and lower electrodes 340, which creates the electric field across all of the nanopores 130 in the compore 120. The current detector 360 detects the aggregate current flowing through all the nanopores 130 in the compore 120. The voltage source 350 and the current detector 360 may use different electrodes.

[0037] In some embodiments, the voltage source 350 and/or current detector 360 are incorporated into the nanosensor chip 100. In such embodiments, the nanosensor chip 100 may further include a controller for controlling the voltage source 350 and the current detector 360. For example, the controller may control the voltage source 350 to vary the applied voltage, in amplitude or frequency. Different patterns of applied voltages (i.e., electric fields) may be used to probe for different target molecules. From the current detector 360, the controller may determine whether there is a change in the measured aggregate current through the compore 120 as a function of the applied voltage. For example, the controller may instruct the voltage source 350 to apply a series of different voltages across the compore 120 and, for each voltage, detect a level of change in the measured aggregate current. The controller may generate a signal indicating the current detected by the current detector 360 or indicating the determined change in detected current and transmit this signal to an off-chip processor for further processing. The controller may be implemented on the nanosensor chip and/or as part of an external device or component.

[0038] FIG. 4 is a cross-section of a single nanopore 130 of the compore 120. The compore 130 has an upper opening 410 at the top of the nanopore 130 and a lower opening 430 at the bottom of the nanopore 130. The upper opening 410 has an upper diameter 415, and the lower opening 430 has a lower diameter 435. The upper diameter 420 may be in the range of 1 nanometers to 300 nanometers. In this example, the lower diameter 440 is smaller than the upper diameter 420, and may be less than 300 nanometers. However, in other embodiments, the nanopore may be “flipped” so that the upper opening is smaller. The nanopore 130 has a height 450, which is the same as the height of the thinned central region 210 of the nanopore. The height 450 of the nanopore may be 1 micron or less. The nanopore has a sidewall extending between the upper opening 410 and the lower opening 430. The sidewall slope is defined by the angle θ , which may be between 6° and 60° . In other

embodiments, the sidewall is not straight as shown in FIG. 4, but may be curved or have some other shape.

[0039] FIGs. 5A and 5B are cross-sections that show operation of a nanopore. FIG. 5A shows a nanopore 130 having probe molecules 520 affixed to the sidewall. As shown in FIG. 5A, the probe molecules are affixed to the sidewall near the smaller opening, i.e., the lower opening 430 shown in FIG. 4. The probe molecules 520 may extend up the entire sidewall, or may be concentrated in a portion of the sidewall, e.g., along a lower portion of the sidewall near the lower opening 430. The probe molecules 520 may be attached to the sidewall of the nanopore 130 by covalent binding, non-covalent binding, or physisorption.

[0040] In the example shown in FIGs. 5A and 5B, the nanopore 130 is exposed to a liquid sample that includes a target molecule 530. In the example shown in FIG. 5A, the sample with the target molecule is located below the nanopores 130. As described above, in other embodiments, the sample containing the target molecule may be located on the other side of the nanopores 130. FIG. 5A shows the nanopore without the proper electric field applied. In this condition, the target molecules 530 remain separated from the probe molecules 520.

[0041] FIG. 5B shows the same nanopore after a specific electric field has been applied across the compore. In the presence of this electric field, the target molecules 530 are attracted inside the nanopore, and individual target molecules 530 bind to corresponding probe molecules 520 to form probe/target bonds 540. This binding creates an ionic current change in current across the nanopore 130. The other nanopores in the compore are also functionalized with the same probe molecules 520 and exposed to the same sample and electric field, so other target molecules 530 in the sample flow into the other nanopores to form additional probe/target bonds 540 across the compore. The probe/target bonds 540 across the nanopores of the compore create an aggregate ionic current change that is measurable by the current sensor.

[0042] When the electric field is removed or changed, the target molecules 530 release from the probe molecules 520. The target molecules 530 may flow out the nanopore 130, reverting to the arrangement shown in FIG. 5A. The probe/target bond 540 is reversible, so that when the compore is subjected to a varying voltage, the target molecules 530 continually bind and release from the probe molecules 520. The probe molecules 520 remain affixed to the sidewalls of the nanopores after use of the compore 120, e.g., after the target molecules 530 bind and then release, and through resetting the compore 120 with a buffer liquid. Because the probe molecules 520 remain affixed after use, the compore 120 can be reused for multiple samples.

[0043] For some applications, the nanopores of a single compore 120 are functionalized with two or more different probe molecules. The probe molecules may be a same category of molecule (e.g., two antibodies) or different categories of molecules (e.g., one antibody and one protein). This allows a single compore 120 to be used to detect multiple different types of target molecules. In one exemplary application, a first probe molecule pairs with a first target molecule at a first electric field strength (e.g., +0.2 volts), a second probe molecule pairs with a second target molecule at a second electric field strength (e.g., +0.4 volts), and a third probe molecule pairs with a third target molecule at a third electric field strength (e.g., +0.6 volts). A sequence of different electric fields can be applied to the compore 120 to determine if any of the three target molecules are present in the sample. This allows the compore 120 to be used to efficiently perform multiple tests simultaneously on a single sample with a single sensor.

[0044] For other applications, the probe molecules may be selected so that multiple target-probe pairings are able to bind at the same range of voltages. This configuration may be used to detect the presence of any of a set of target molecules, e.g., a set of multiple potential contaminants within a food product, or a set of target antibodies in a blood sample. By using multiple target-probe pairings that bind at the same voltage, the compore 120 can efficiently identify a negative result for a sample. For some applications, if a positive result is obtained, further testing may be performed to determine which target molecule is present after an initial positive result is obtained.

[0045] Including multiple nanopores 130 in a single compore 120 allows the compore 120 to be functionalized with more types of probe molecules than prior sensors. An entire wafer of nanosensor chips may be accurately spotted in parallel with probe molecules using a specialized high-resolution printer. This enables production of multiplex tests and test panels at low cost. Additionally, compores 120 may be functionalized to detect positive and negative controls for validation and calibration, as well as markers to authenticate and verify the integrity of the nanosensor chip and reagents.

[0046] FIGs. 6A and 6B are graphs that illustrate operation of a compore. FIG. 6A is a graph showing the behavior of the compore when there are no target molecules present and FIG. 6B shows the response with target molecules. In this example, the voltage source 350 generates a square-wave current first at a voltage of -400 millivolts (mV), then at -200 mV, at 0 mV, and at +200 mV. Each specific pair of probe and target molecule will have a specific voltage at which they will bind. This changes the electrical characteristics of the nanopore 130 opening, which alters the current, as shown in FIG. 6B where the specific voltage is -200

mV. Compared to FIG. 6A, the strength of the output current changes. This indicates that the target molecules are binding to the probe molecules in the presence of the -200 mV electric field, so the target molecules that bind to probe molecules at -200 mV are present in the sample. The magnitude of the change in current may also indicate the concentration of target molecules in the sample. If a variable voltage is used, the target molecules may bind and release from the probe molecules. Certain target molecules may not bind and release. Instead, these may bind and remain bound.

[0047] While the compore 120 depicted in FIGs. 1-3 has a circular shape and circular nanopores 130, in other embodiments, the compore 120 and/or nanopores 130 may have different shapes and the nanopores may be arranged differently within the compore. FIG. 7 is a top view showing a first alternative arrangement of nanopores within a compore. FIG. 7 depicts a square compore 720 that has square nanopores 730. In other embodiments, the compore 720 may be shaped as an oval, a rectangle, another polygon, or some other shape. Similarly, the nanopores 730 may be oval, rectangular, some other polygon, or have some other shape. The shape of the nanopores 730 may be different from the shape of the compore 720.

[0048] FIG. 8 is a top view showing a second alternative arrangement of nanopores within a compore. While the compores 120 and 720 had nanopores of a consistent size, in other embodiments, the nanopores in a single compore may have different sizes. FIG. 8 depicts a compore 820 that has nanopores 830 of multiple different sizes, including a small nanopore 830A and a large nanopore 830B. The differently-sized nanopores 830 may have sloped sidewalls with the same angle (e.g., each has a sidewall angle of 10°) or different angles. In some embodiments, using multiple different sized nanopores can improve the sensitivity and dynamic range of the nanosensor.

[0049] FIG. 9 is a cross-section of a compore 900 fabricated using semiconductor technology. The structure of the compore 900 is similar to the compore shown in FIG. 3. The compore 900 includes a layer of silicon 910, which is an example of the semiconductor substrate 110. In other embodiments, other semiconductor materials may be used in place of silicon 910. Alternatively, the nanosensor can be implemented on a substrate of glass, plastic, film or other non-conducting or semiconducting material. Various etching processes may be used to thin the substrate to form the compore central regions in the silicon 910, e.g., wet etching or dry etching. A separate process such as ion-beam lithography may be used to form the nanopores. While only one compore 900 is shown in FIG. 9, multiple compores may be formed and an electrode structure laid out, as shown in FIG. 1.

[0050] Two layers of silicon nitride 920 and 930 are deposited on the top and bottom, respectively, of the silicon 910. Various deposition processes for silicon nitride may be used to deposit the two layers of silicon nitride 920 and 930, e.g., chemical vapor deposition or plasma-enhanced chemical vapor deposition. While layers of silicon nitride 920 and 930 for only one compore 900 are shown in FIG. 9, the layers of silicon nitride 920 and 930 may extend across the nanosensor chip for each of the compores included in the nanosensor chip.

[0051] Two layers of electrodes 940 and 950 are deposited on the upper layer of silicon nitride 920 and the lower layer of silicon nitride 930, respectively. The electrode layers 940 and 950 may be formed from any conductive material, e.g., copper, silver or platinum. Various deposition process for depositing the conductive material may be used to deposit the two layers of electrodes 940 and 950, e.g., evaporation or chemical vapor deposition. In this embodiment, the layers of electrodes 940 and 950 are arranged on either side of the compore 900. In other embodiments, the electrodes 940 and 950 may be laid out differently on the compore 900, as described with respect to FIG. 3. For example, in other embodiments, the electrode 940 may also be formed on the silicon sidewalls and/or on the surface of the thinned silicon. Each compore included in the nanosensor chip may have a similar electrode structure. In other embodiments, any of the electrodes may be located off-chip, in a separate component such as the chip receptacle in which the chip is mounted.

[0052] FIG. 10 is a block diagram of a detection system 1000 that includes a nanosensor chip. The detection system 1000 includes a nanosensor chip 1010, a chip receptacle 1020, a voltage source 1030, a current detector 1040, a controller 1050, a user interface 1060, a display 1070, and a communications interface 1080. Other components (not shown) may include processors, memory, digital-to-analog converters, and analog-to-digital converters. In other embodiments, the detection system 1000 has additional, alternative, or fewer components than shown in FIG. 10.

[0053] The nanosensor chip 1010, as described with respect to FIGs. 1-9, has one or more compores. The chip receptacle 1020 is configured to receive and hold the nanosensor chip 1010 and form electrical connections between components of the nanosensor chip 1010 and other components of the detector system 1000. For example, the chip receptacle 1020 may include electrodes configured to connect to the electrode structures 140 shown in FIG. 1. The chip receptacle 1020 may also have one or more fluid connections to the nanosensor chip 1010, e.g., to transfer one or more liquid samples to the compores of the nanosensor chip 1010, or to transfer a buffer liquid to the compores of the nanosensor chip 1010.

[0054] The voltage source 1030 generates the electric field supplied by the electrodes to the compores. The voltage source 1030 may be a variable voltage source that generates a varying current at a range of voltages to one or more compores. In some embodiments, the voltage source 1030 is integrated into the nanosensor chip 1010. The detection system 1000 may have one voltage source 1030 or multiple voltage sources, e.g., one voltage source for each compore included in the nanosensor chip 1010. If the detection system 1000 has fewer voltage sources than compores, the nanosensor chip 1010 may have a switching mechanism to apply the voltage to one compore at a time, or the nanosensor chip 1010 may be configured to apply the same voltage to two or more compores simultaneously.

[0055] The current detector 1040 detects a current through a compore. In some embodiments, the current detector 1040 is integrated into the nanosensor chip 1010. The detection system 1000 may have one current detector 1040, e.g., one current detector for each compore included in the nanosensor chip 1010. Alternatively, it may have multiple current detectors. If the detection system 1000 has fewer current detectors than compores, the nanosensor chip 1010 may include a switching mechanism that allows the current detector to individually address a selected compore.

[0056] The controller 1050 controls the voltage source 1030 and the current detector 1040. The controller 1050 may be similar to the controller described with respect to FIG. 3. The controller 1050 may be integrated into the nanosensor chip 1050, or may be part of a separate component or device. The detection system 1000 may have one controller 1050 for controlling all of the voltage sources, current detectors, and any switching mechanisms included in the detection system 1000. Alternatively, the detection system 1000 may have multiple controllers 1050, e.g., one for each compore. In addition to controlling the voltage source 1030 and current detector 1040, the controller 1050 is also configured to interact with other components of the detection system, e.g., the user interface 1060, the display 1070, and the communications interface 1080.

[0057] The user interface 1060 is configured to receive user input, e.g., a command from a user to start an analysis of a sample, or parameters for analyzing a sample. For example, the user interface 1060 may receive parameters describing one or more voltages to be applied to a compore, or an indication of a testing procedure that is pre-programmed with a set of voltages to be run on the compore. The user interface 1060 passes these commands or parameters to the controller 1050. The user interface 1060 may include buttons, a keyboard, a touch screen, a microphone and voice recognition software, or any other suitable mechanism for receiving input from a user. Alternately, the detection system 1000 may

receive commands and parameters from a mobile phone app, tablet, PC, or web application, or from an automated external control system.

[0058] The display 1070 provides visual output to a user regarding tests run by the detection system 1000. For example, the display 1070 may be used in conjunction with the user interface 1060 and the controller 1050 to display options to a user, which can be selected by the user. The display 1070 may also output test results generated by the controller 1050, e.g., whether a given target molecule is detected in a sample, or a concentration of a target molecule detected in a sample.

[0059] The communications interface 1080 may allow the detection system 1000 to communicate with one or more other devices over a network, e.g., a local network or the Internet, or by means of a serial or parallel, wireless or wired, interface such as Bluetooth, USB or other communication protocols. For example, the communications interface 1080 may upload results of a test performed by the detection system 1000 to another device or component for further processing, or may upload test results to a database.

[0060] Upon reading this disclosure, those of skill in the art will appreciate still additional alternative structural and functional designs for a nanosensor chip and detection system. Thus, while particular embodiments and applications have been illustrated and described, it is to be understood that the described subject matter is not limited to the precise construction and components disclosed herein and that various modifications, changes and variations which will be apparent to those skilled in the art may be made in the arrangement, operation and details of the apparatuses disclosed herein.

[0061] As used herein any reference to “one embodiment” or “an embodiment” means that a particular element, feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. The appearances of the phrase “in one embodiment” in various places in the specification are not necessarily all referring to the same embodiment.

[0062] As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having” or any other variation thereof, are intended to cover a non-exclusive inclusion. For example, a process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. Further, unless expressly stated to the contrary, “or” refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present)

and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0063] In addition, use of the “a” or “an” are employed to describe elements and components of the embodiments herein. This is done merely for convenience and to give a general sense of the disclosure. This description should be read to include one or at least one and the singular also includes the plural unless it is obvious that it is meant otherwise.

What is claimed is:

1. A nanosensor chip for detecting and/or quantifying target molecules in a liquid sample, the nanosensor chip comprising:
 - a substrate with one or more compound nanopores (“compores”), wherein each compore comprises an aperture formed in the substrate with a central region that is thinner than the surrounding substrate and comprises therein a plurality of nanopores, further wherein each of the nanopores has a larger opening and a smaller opening, and each of the nanopores is functionalized with a plurality of immobilized probe molecules affixed to a sidewall of the nanopore; and
 - for each compore, a corresponding electrode structure having a shape that surrounds the plurality of nanopores and is arranged to conduct an aggregate current through the plurality of nanopores, whereby the aggregate current changes in response to binding of target molecules in the liquid sample to the probe molecules.
2. The nanosensor chip of claim 1, wherein the substrate comprises multiple compores.
3. The nanosensor chip of claim 1, wherein the larger openings of the nanopores have a diameter of between 1 nanometer and 300 nanometers.
4. The nanosensor chip of claim 1, wherein the electrode structure for each compore includes a first electrode laid out on one side of the substrate and a second electrode on an opposite side of the substrate, wherein the first electrode surrounds the plurality of nanopores and the first and second electrodes are arranged to create an electric field across the plurality of nanopores and to conduct current through the plurality of nanopores.
5. The nanosensor chip of one of claims 1, 2, 3, or 4, wherein the central region has a thickness that is less than 1 micron.
6. The nanosensor chip of one of claims 1, 2, 3, or 4, wherein at least one compore contains nanopores of different maximum diameters.
7. The nanosensor chip of one of claim 1, 2, 3, or 4, wherein the probe molecules are selected from the group consisting of antibodies, antibody analogs, proteins, aptamers, polymers, oligonucleotides, and nanobodies.

8. The nanosensor chip of claim 7 wherein the substrate comprises multiple compores functionalized with different probe molecules.
9. The nanosensor chip of claim 7 wherein the substrate comprises multiple compores functionalized with the same probe molecules.
10. The nanosensor chip of claim 7, wherein the probe molecules are aptamers.
11. The nanosensor chip of claim 1, wherein the substrate comprises a semiconductor material.
12. The nanosensor chip of claim 1, wherein the probe molecules are attached to the nanopores by covalent binding, non-covalent binding, or physisorption.
13. A detection system for detecting and/or quantifying target molecules in a liquid sample, the detection system comprising:
 - a substrate with one or more compound nanopores (“compores”), wherein each compore comprises an aperture with a central region that is thinner than the surrounding substrate and comprises therein in a plurality of nanopores, further wherein each of the nanopores has a larger opening and a smaller opening, and each of the nanopores is functionalized with a plurality of immobilized probe molecules affixed to a sidewall of the nanopore;
 - for each compore, circuitry comprising a first electrode that surrounds the plurality of nanopores and is arranged to conduct an aggregate current through the plurality of nanopores, wherein the aggregate current changes in response to binding of target molecules in the liquid sample to the probe molecules, the circuitry further comprising a variable voltage source and a current detector in series with the first electrode and a second electrode; and
 - a controller coupled to the variable voltage source and to the current detector, the controller configured to:
 - control the variable voltage source to apply a range of different voltages across the plurality of nanopores in the compore, wherein binding of target molecules to probe molecules is a function of an electric field across the compore; and
 - control the current detector to detect the aggregate current conducted through the plurality of nanopores, wherein binding of target molecules to

probe molecules causes a change in the aggregate current and indicates a presence and/or concentration of the target molecules in the liquid sample.

14. The detection system of claim 13, wherein the substrate comprises multiple compores.
15. The detection system of claim 13, wherein at least one compore contains nanopores of different maximum diameters.
16. The detection system of claim 13 wherein the nanopores have a maximum diameter of between 1 nanometer and 300 nanometers.
17. The detection system according to one of claims 13, 14, 15, and 16, wherein the probe molecules are selected from the group consisting of antibodies, antibody analogs, proteins, aptamers, polymers, oligonucleotides, and nanobodies.
18. The detection system of claim 17, wherein the probe molecules are aptamers.
19. The detection system of claim 17, wherein the substrate comprises a plurality of compores functionalized with the same probe molecule.
20. The detection system of claim 17, wherein the substrate comprises a plurality of compores functionalized with different probe molecules.
21. The detection system of claim 17, wherein the probe molecules are attached to the nanopores by covalent binding, non-covalent binding, or physisorption.
22. A method for detecting and/or quantifying target molecules in a liquid sample, the method comprising:
 - providing a nanosensor chip comprising a substrate with one or more compound nanopores ("compores"), wherein each compore comprises an aperture formed in the substrate with a plurality of nanopores formed therethrough, wherein the nanopores are functionalized with immobilized probe molecules that specifically bind to target molecules, the compore further comprising an electrode structure configured to apply a voltage across all the nanopores in the compore;

allowing target molecules in the liquid sample to bind to the immobilized probe molecules;

using the electrode structure to apply a range of voltages from a voltage source across all of the plurality of nanopores in the compore, such that specific binding of target molecules to probe molecules varies as a function of an electric field applied across the plurality of nanopores; and

detecting an aggregate current flowing through all of the plurality of nanopores in the compore in response to the voltages, using a current sensor connected to the electrode structure, wherein binding of target molecules to probe molecules causes a change in the aggregate current, and thereby indicates a presence and/or concentration of the target molecules in the liquid sample.

23. The method of claim 22 wherein the substrate comprises a semiconductor material.

24. The method of claim 22 wherein the electrode structure comprises a first electrode laid out on one side of the substrate and a second electrode on an opposite side of the substrate, wherein the first electrode surrounds the plurality of nanopores and the first and second electrodes are arranged to apply the voltages across the plurality of nanopores and to conduct the aggregate current through the plurality of nanopores.

25. The method of claim 22 wherein each nanopore has a conical shape with a sidewall slope between 6 and 60 degrees.

26. The method according to one of claims 22, 23, 24, and 25 wherein the nanopores in a compore are in a range of 1nm to 300nm in diameter.

27. The method of claim 22 wherein the substrate comprises a plurality of compores, and nanopores in different compores are functionalized with different probe molecules for detecting different target molecules in the sample, thereby indicating a presence and/or concentration of different target molecules in the sample.

28. The method of claim 27 wherein the step of applying a range of different voltages comprises applying voltages simultaneously to the plurality of compores.

29. The method of claim 22 wherein at least one compore is functionalized with different probe molecules for detecting the same target molecules in the sample.

30. The method of claim 22 wherein the probe molecules are selected from the group consisting of antibodies, proteins, aptamers, polymers, oligonucleotides, and nanobodies.

31. The method of claim 30 wherein the probe molecules are aptamers.

32. The method of claim 22 further comprising the steps of removing any bound target molecules from the nanosensor chip and reusing the nanosensor chip.

33. The method of claim 22 further comprising the step of applying the liquid sample in the form of blood, saliva, spinal liquid, urine, food, beverage, or water.

34. The method of claim 22 further comprising the step of applying an alternating positive and negative voltage across the plurality of nanopores, whereby target molecules dissociate from the probe molecules.

35. The method of claim 22 further comprising the step of measuring change of the aggregate currents at different voltages to determine a concentration of target molecules in the sample.

36. The method of claim 22 further comprising the step of detecting different targets in the sample by using compores containing different probe molecules.

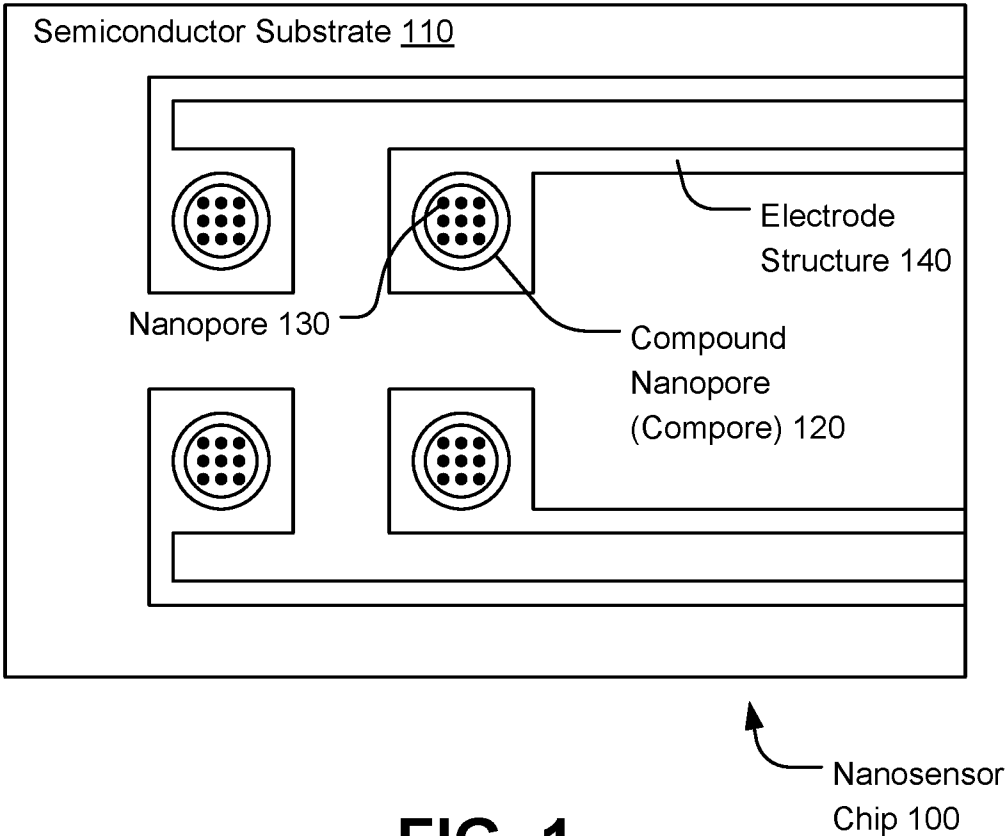


FIG. 1

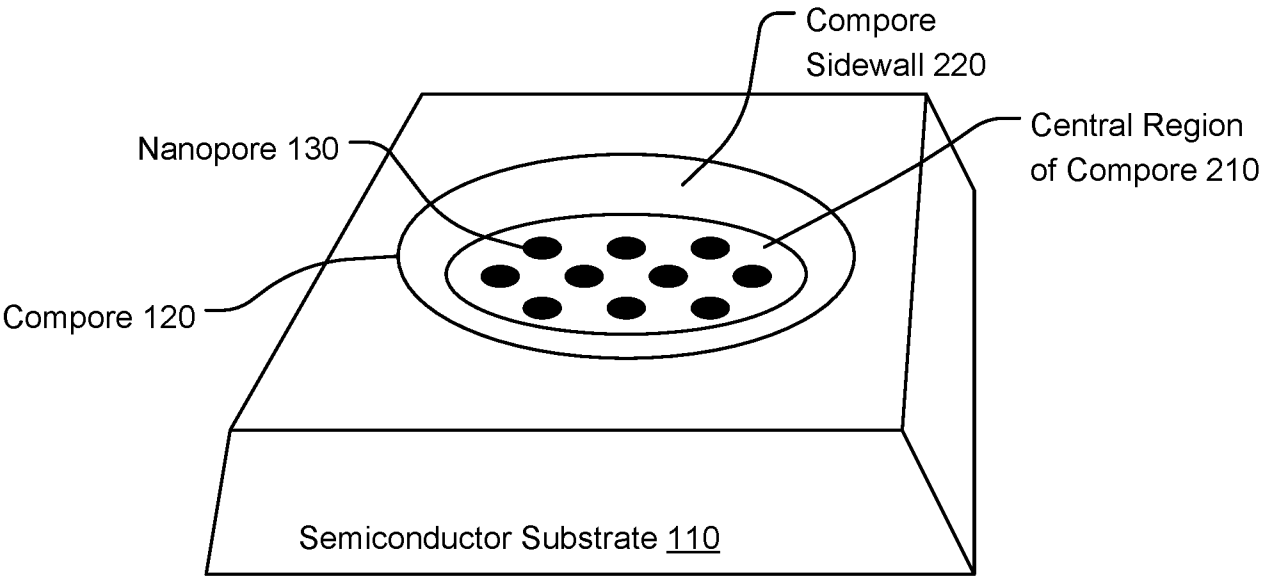


FIG. 2

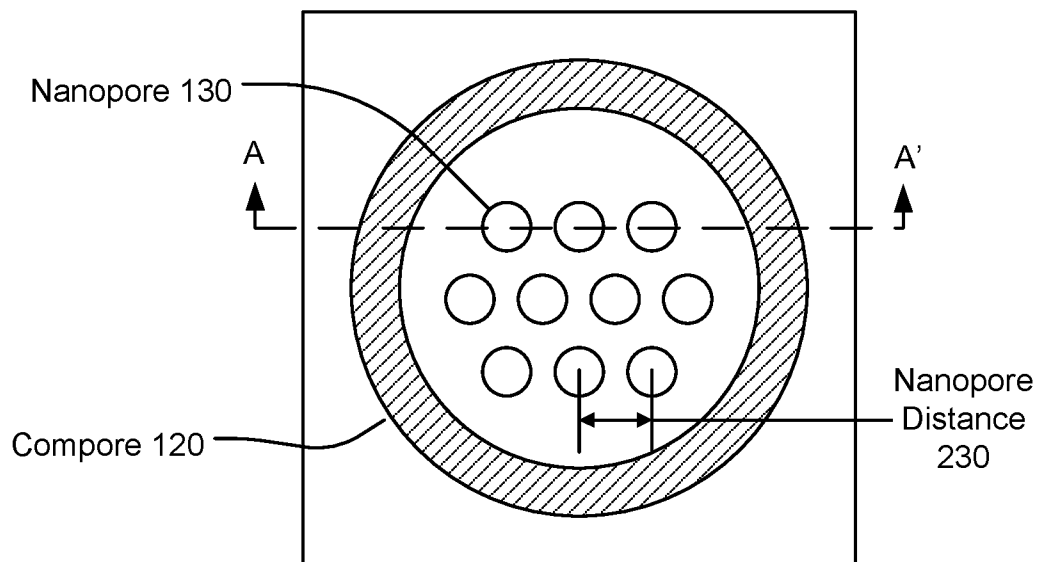


FIG. 3A

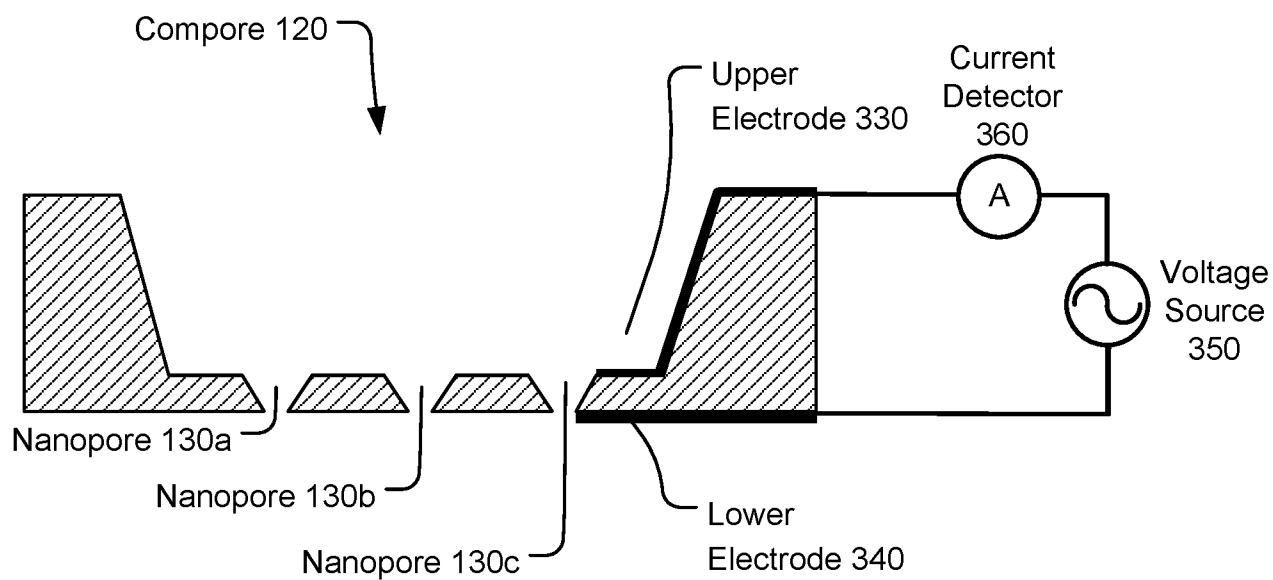
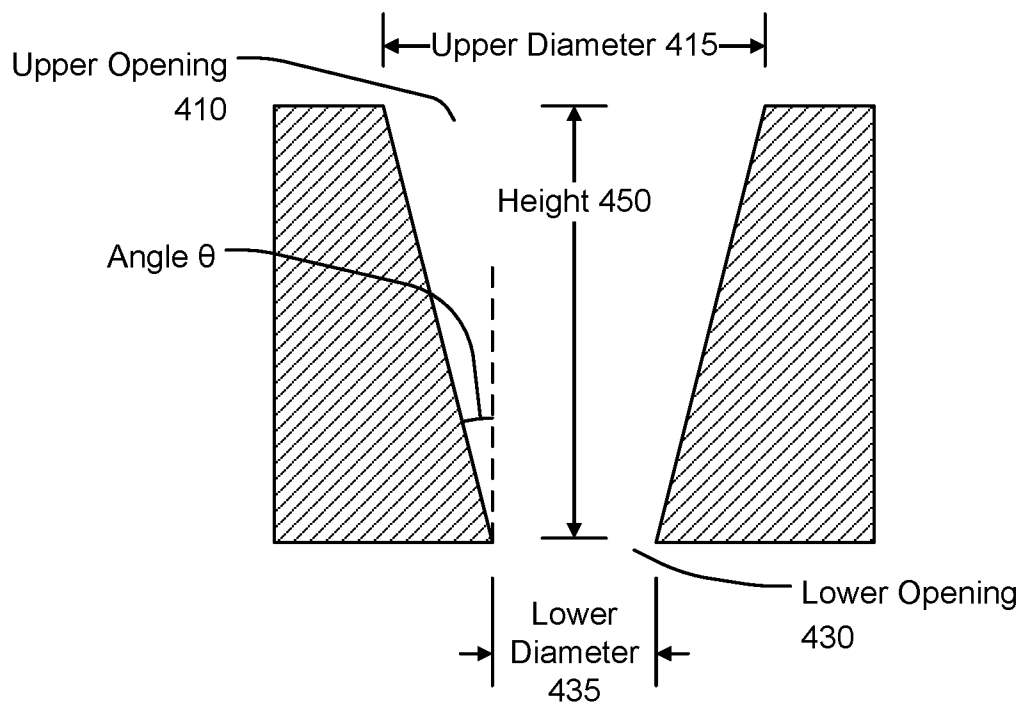
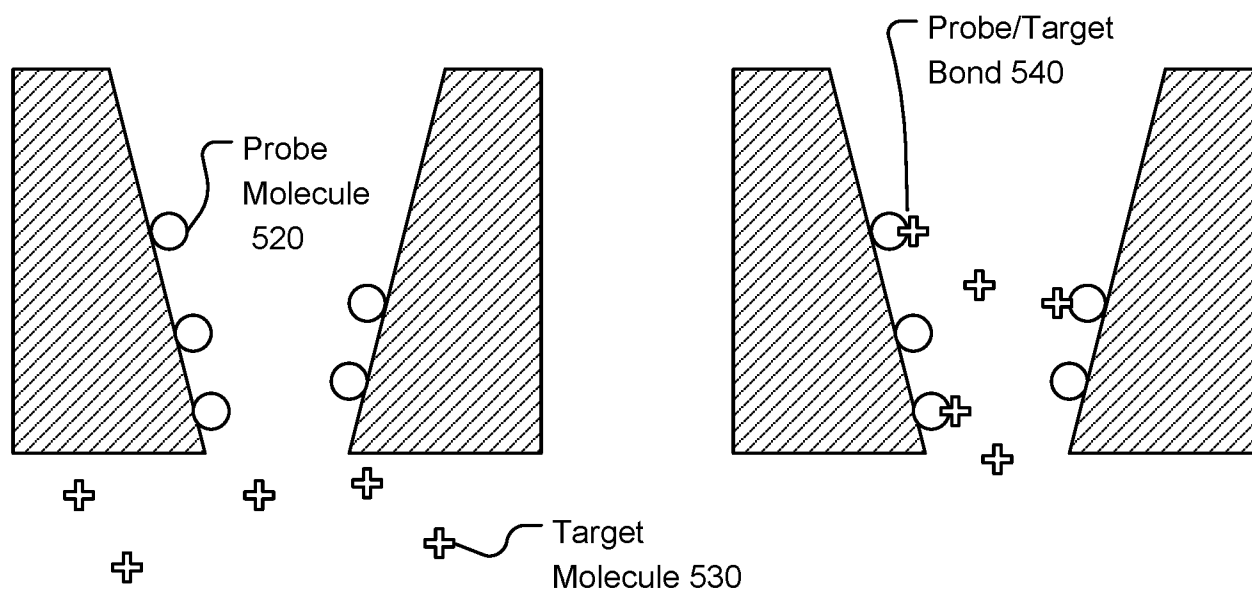


FIG. 3B

**FIG. 4****FIG. 5A****FIG. 5B**

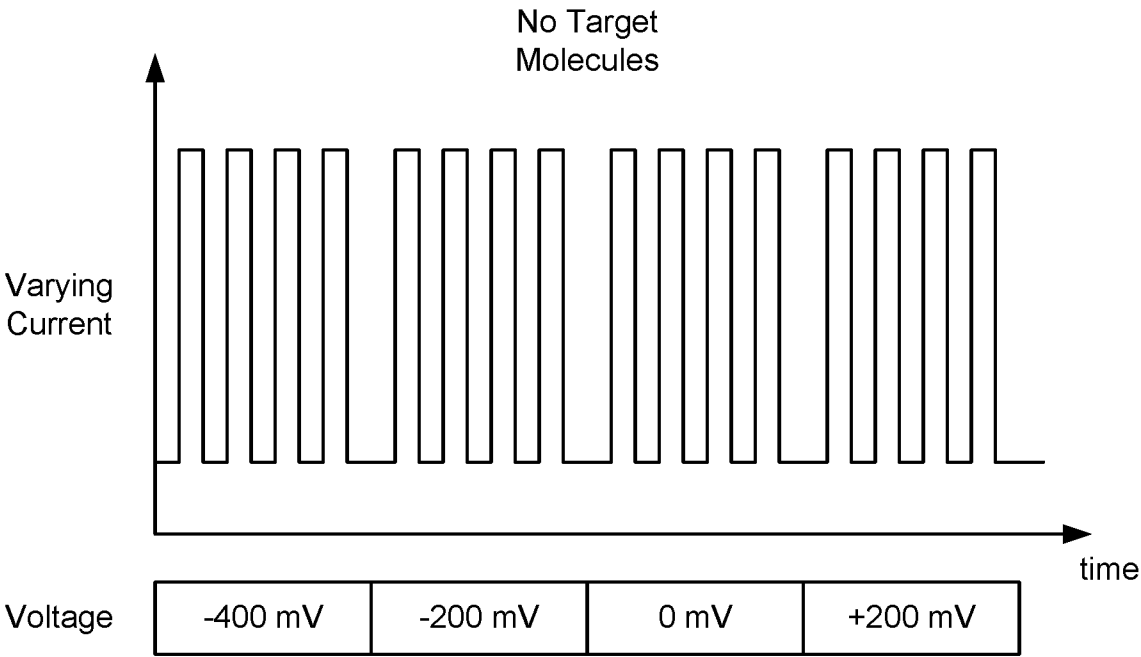


FIG. 6A

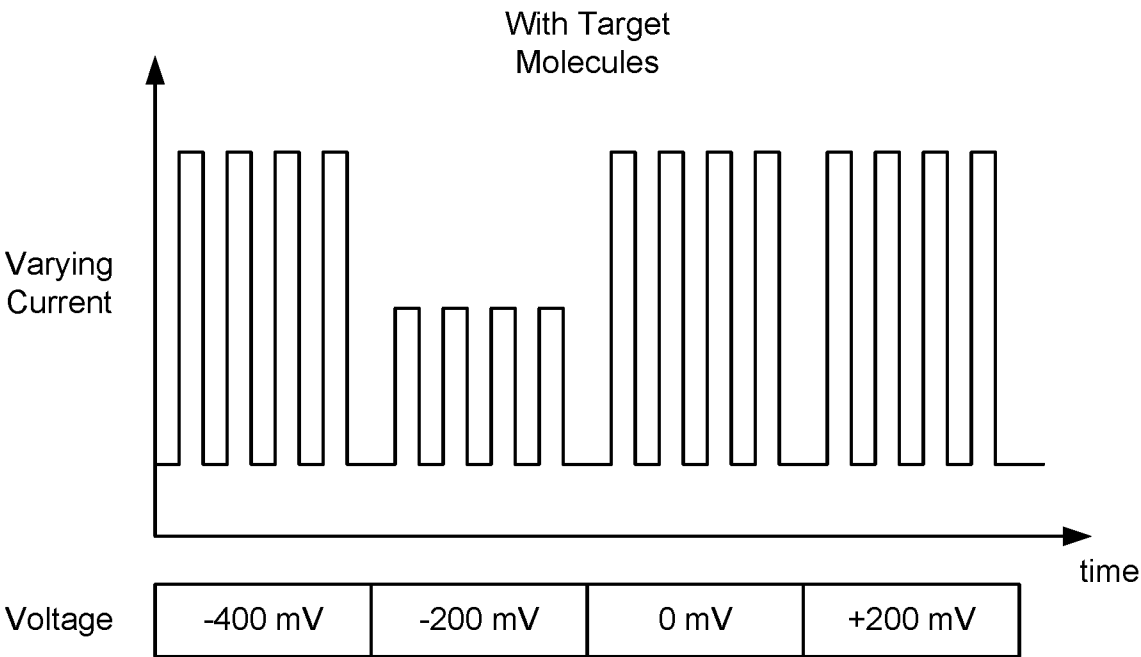


FIG. 6B

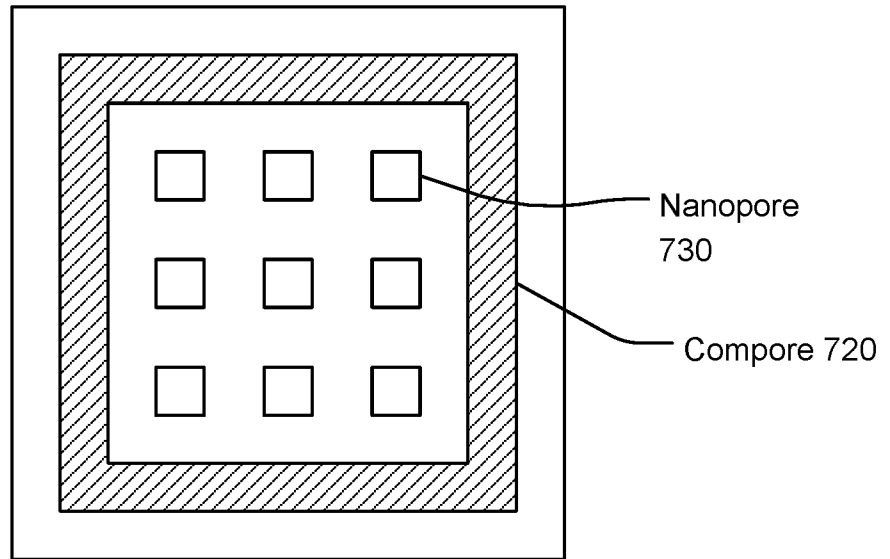


FIG. 7

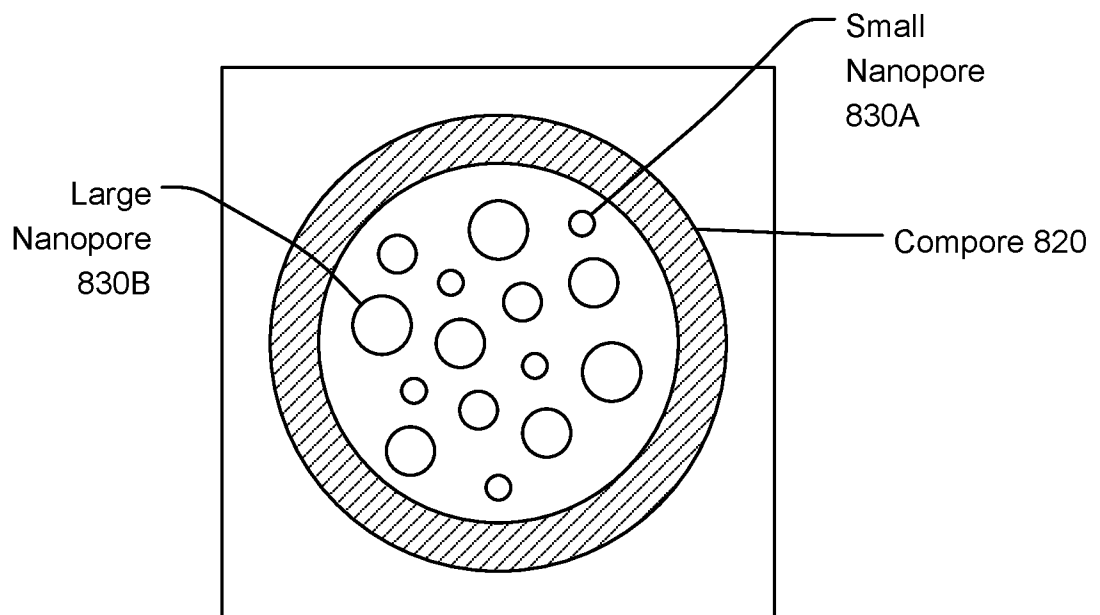
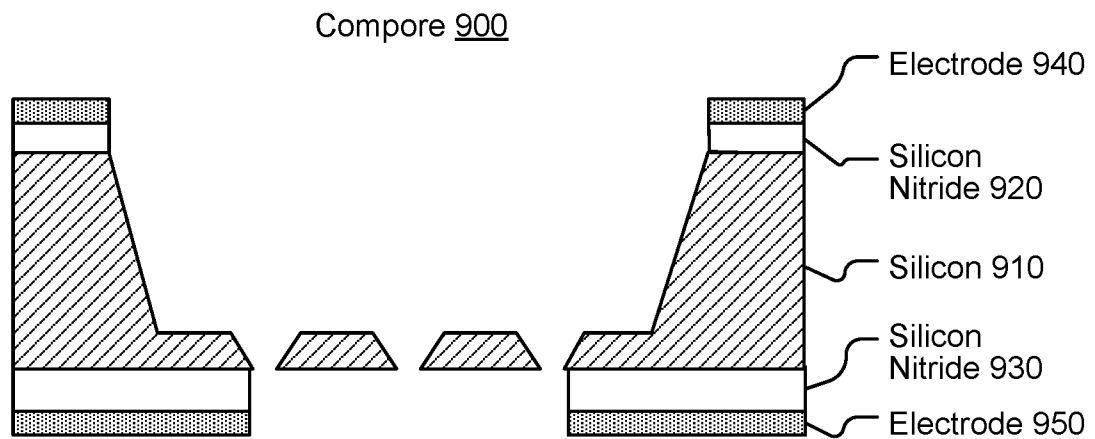
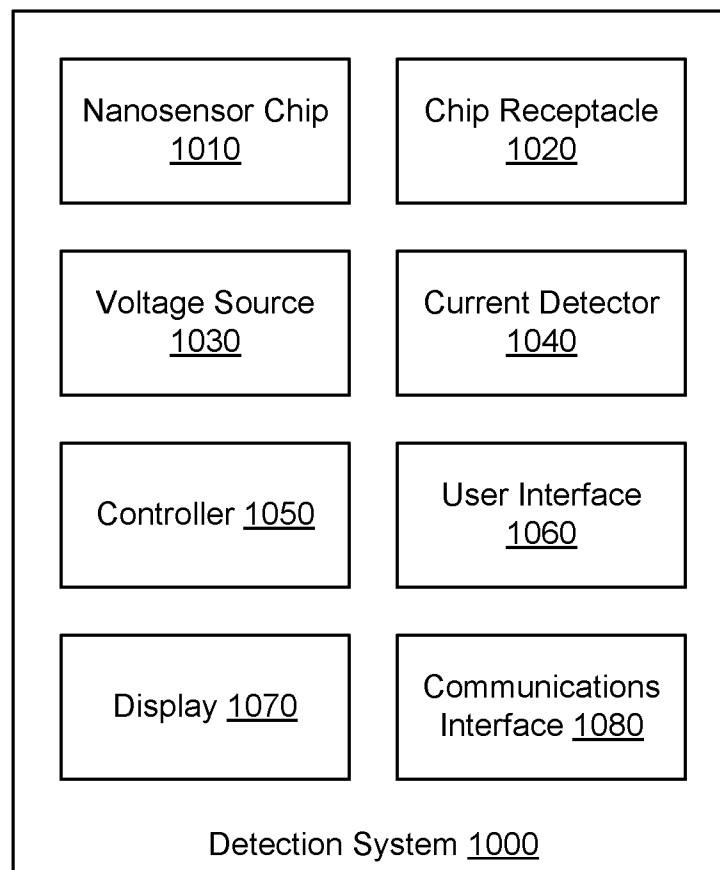


FIG. 8

**FIG. 9****FIG. 10**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/27550

A. CLASSIFICATION OF SUBJECT MATTER

IPC - B01L 3/00; G01N 27/414; G01N 33/53 (2020.01)

CPC - A61B 5/14532; A61B 5/6821; B01L 3/502715; B01L 3/502761

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/0074778 A1 (Letant et al.) 7 April 2005 (07.04.2005) Abstract, para [0006], [0009], [0011]-[0012], [0054]-[0055], Figure 1A; Figure 2; Figure 3	1-36
A	US 2015/0111779 A1 (Genia Technologies, Inc.) 23 April 2015 (23.04.2015) Abstract, para [0052], [0063], [0068], [0071], [0073], [0078], [0085]-[0086], [0088], [0146], Figure 2A; Figure 4.	1-36
A	US 2014/0318968 A1 (International Business Machines Corporation) 30 October 2014 (30.10.2014) Entire Document.	1-36
A	US 2005/0127035 A1 (Ling) 16 June 2005 (16.06.2005) Entire Document.	1-36
A	US 2010/0066348 A1 (Merz et al.) 18 March 2010 (18.03.2010) Entire Document.	1-36

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

21 June 2020

Date of mailing of the international search report

06 JUL 2020

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