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(54) **DNA SEQUENCING AND AMPLIFICATION SYSTEMS USING NANOSCALE FIELD EFFECT SENSOR ARRAYS**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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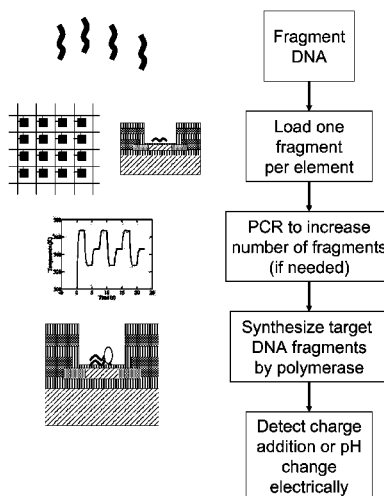
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(57) **ABSTRACT**

In one aspect, described herein are field effect chemical sensor devices useful for chemical and/or biochemical sensing. Also provided herein are methods for single molecule detection. In another aspect, described herein are methods useful for amplification of target molecules by PCR.

80 Claims, 13 Drawing Sheets



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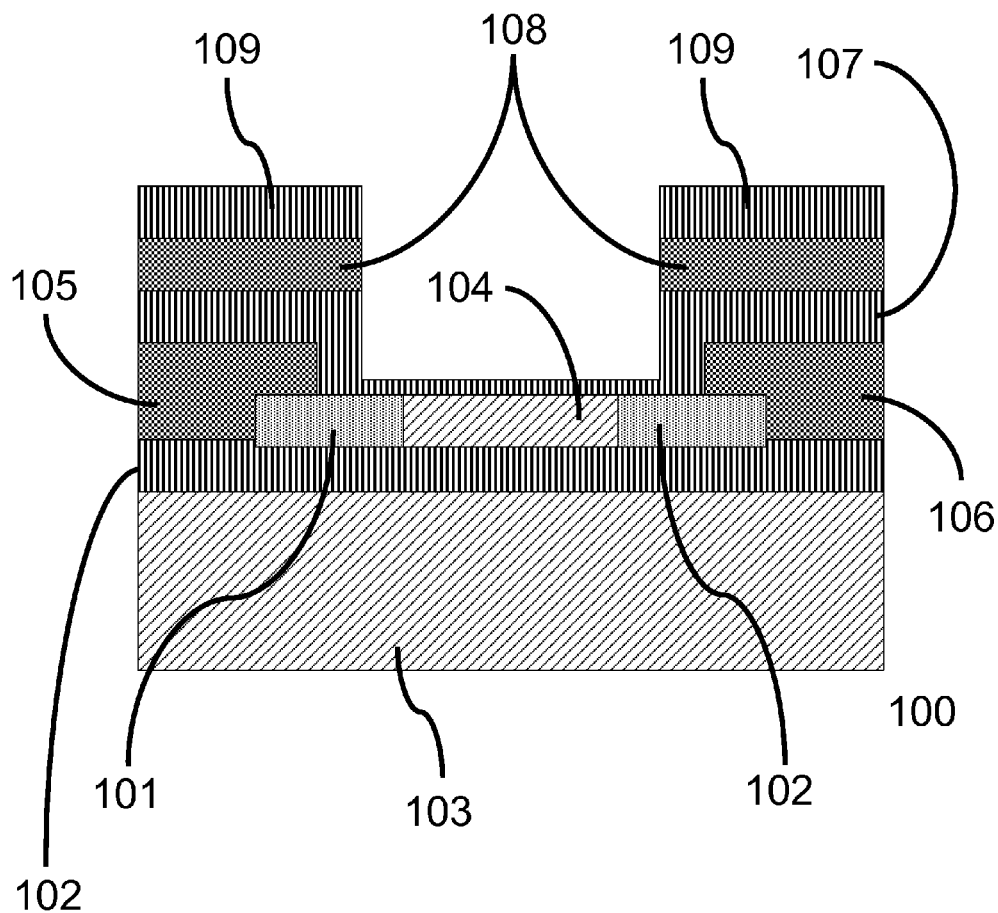


Figure 1

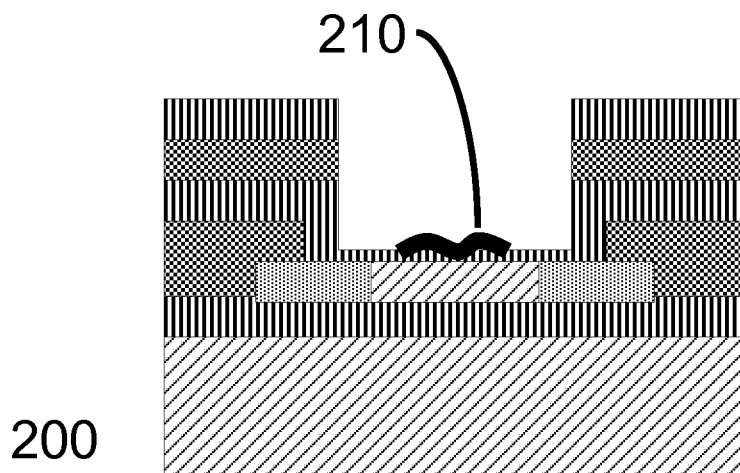


Figure 2A

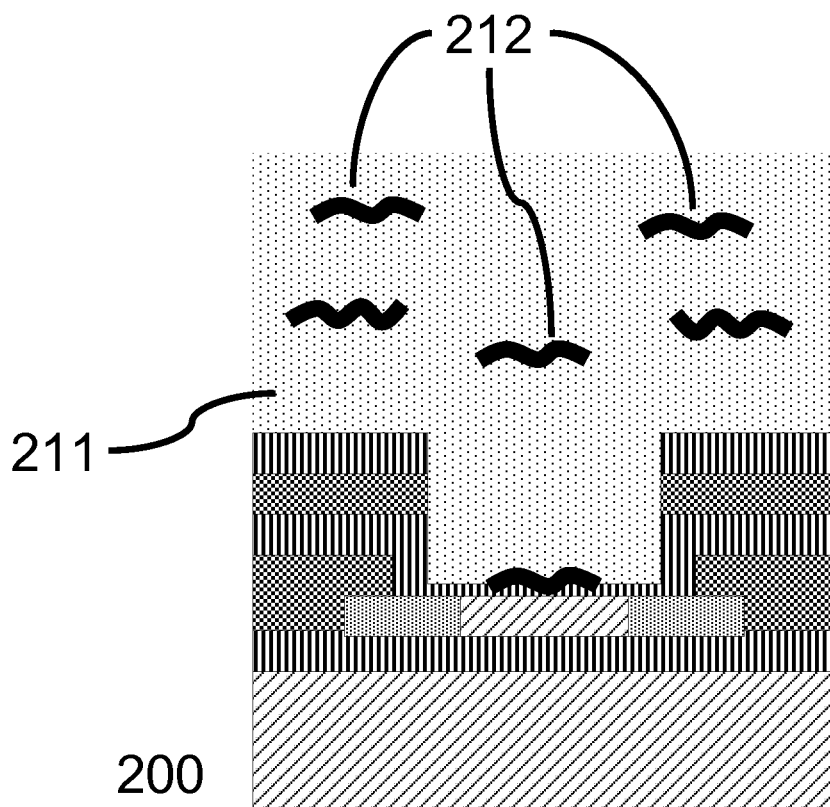


Figure 2B

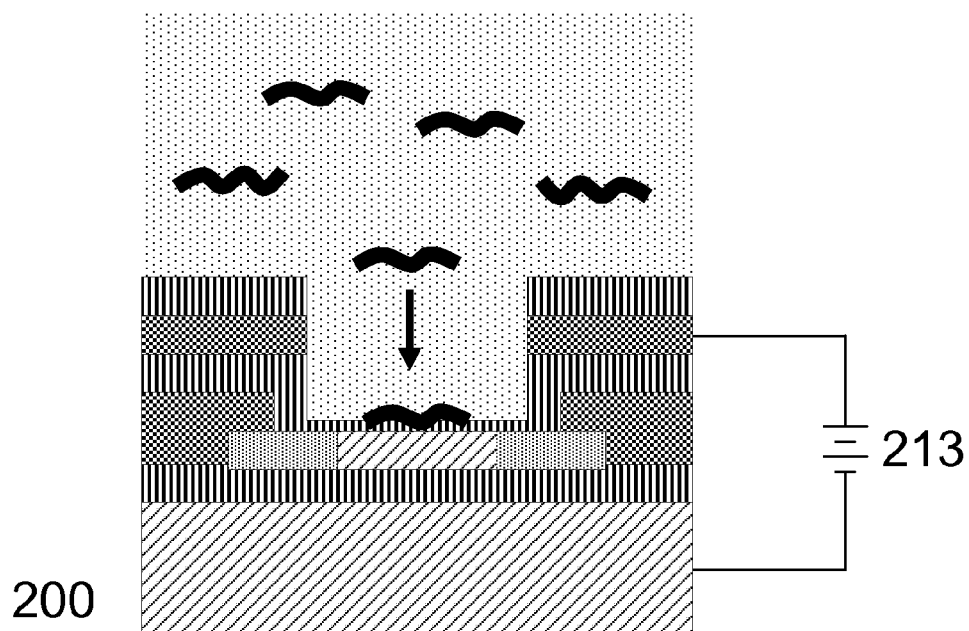


Figure 2C

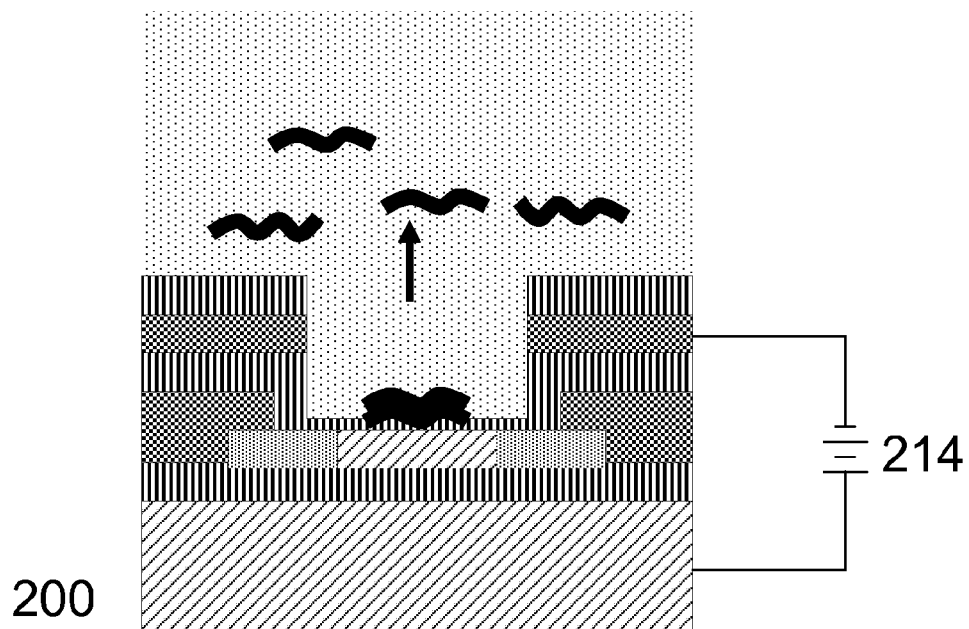


Figure 2D

Figure 3A

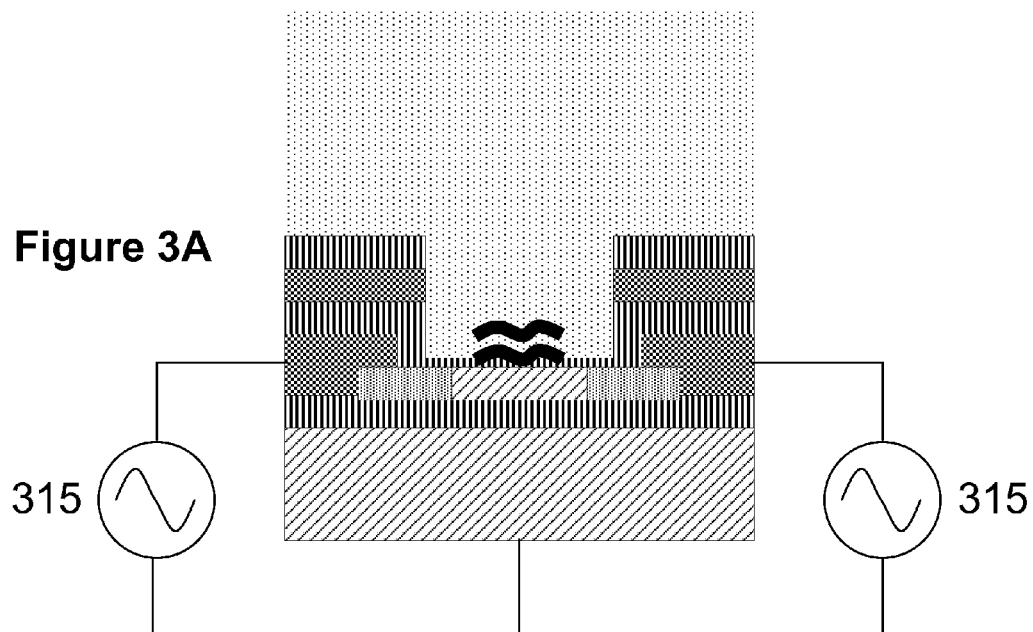


Figure 3B

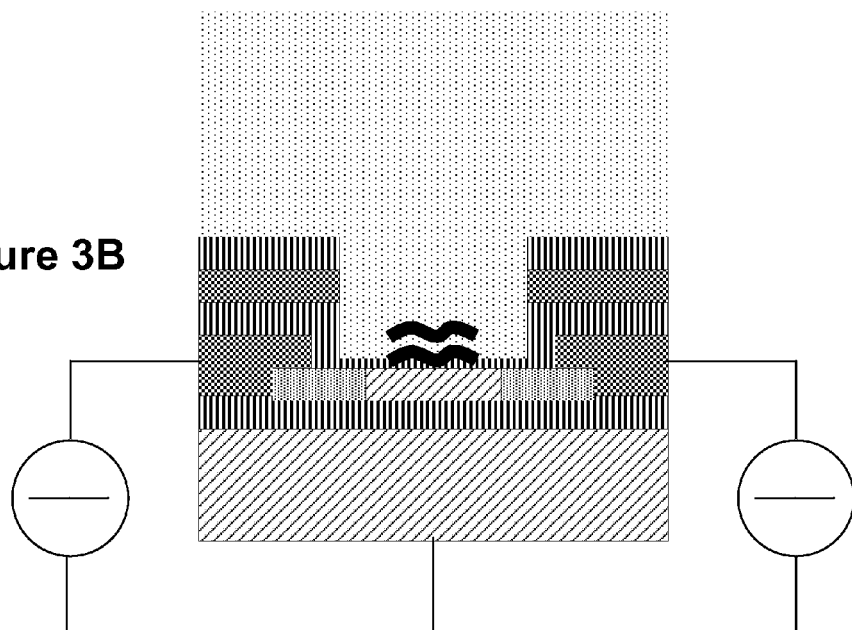


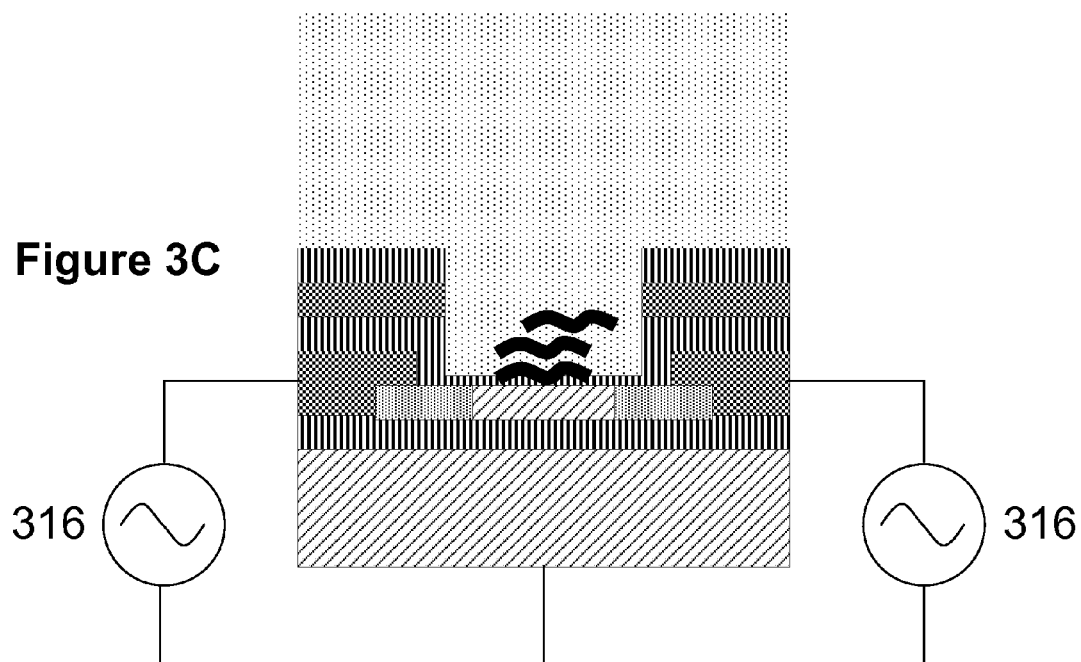
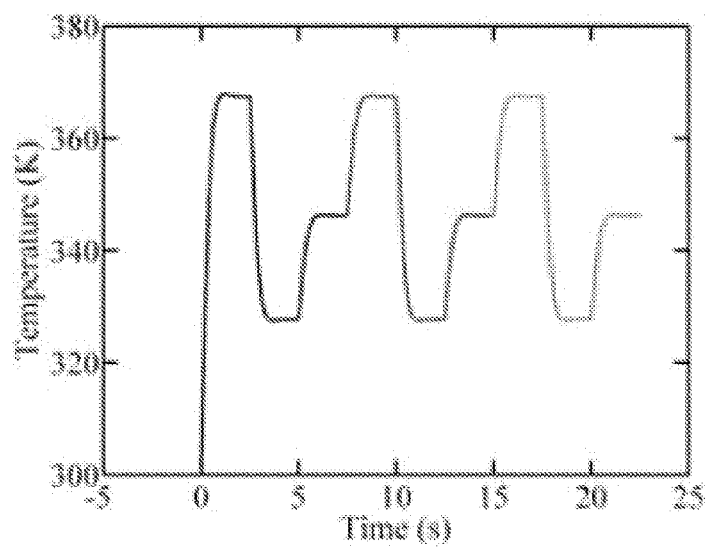
Figure 3C**Figure 3D**

Figure 4A

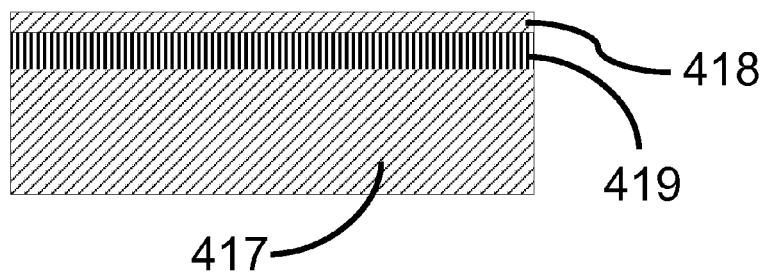


Figure 4B

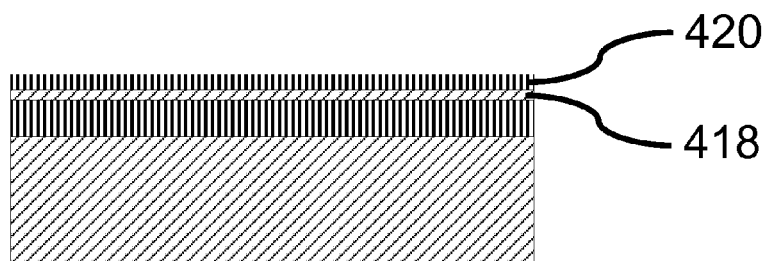


Figure 4C

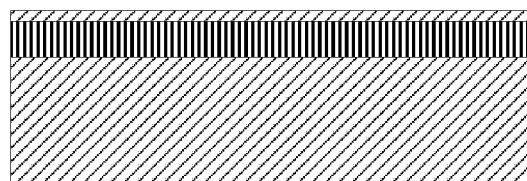


Figure 4D

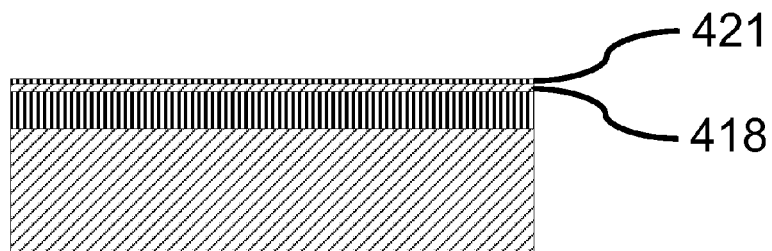


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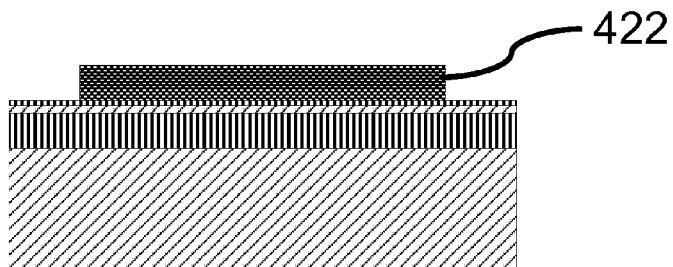


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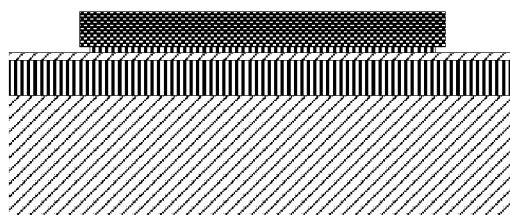


Figure 4G

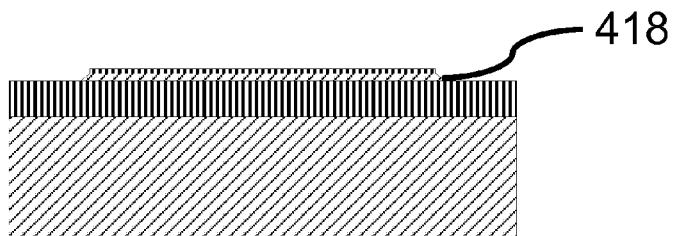


Figure 4H

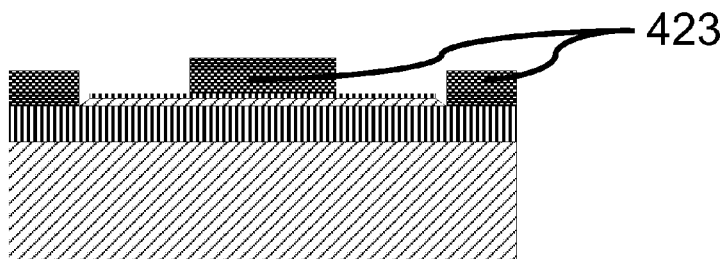


Figure 4I

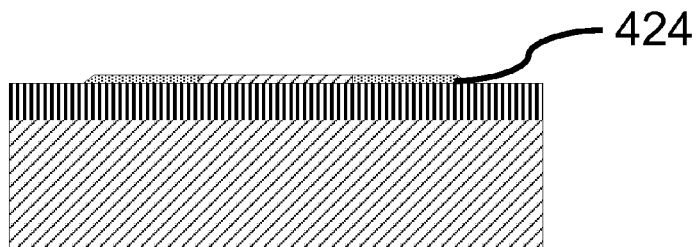


Figure 4J

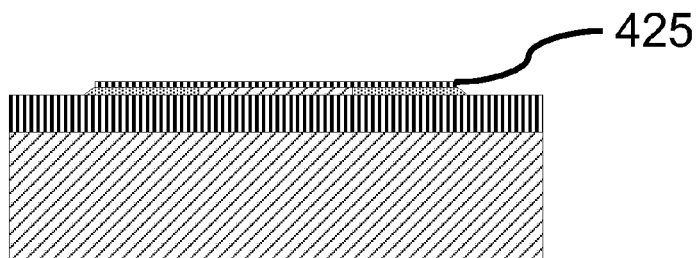


Figure 4K

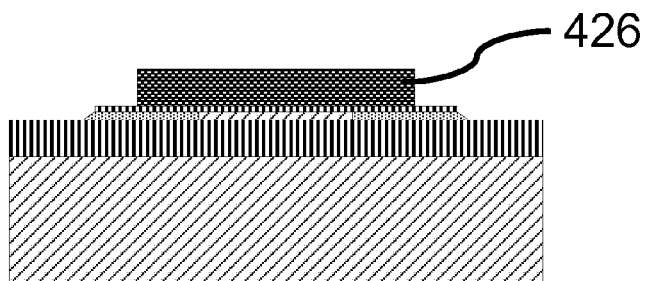


Figure 4L

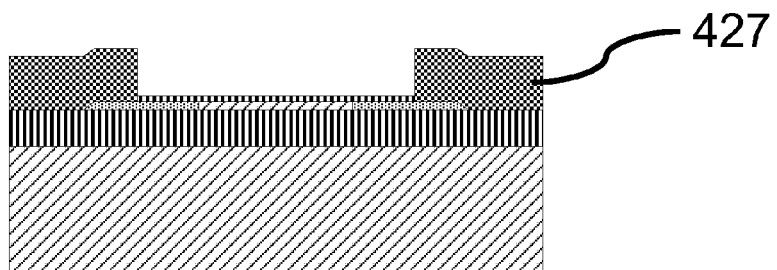


Figure 4M

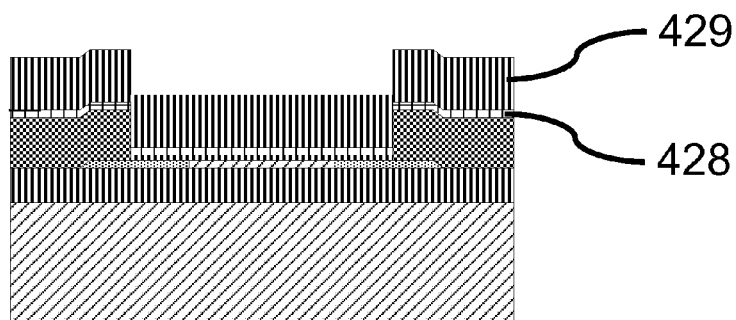


Figure 4N

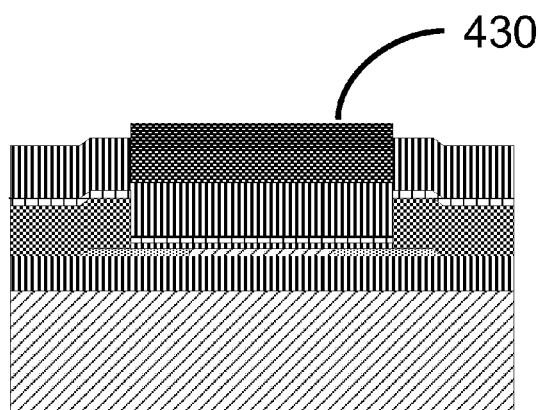


Figure 4O

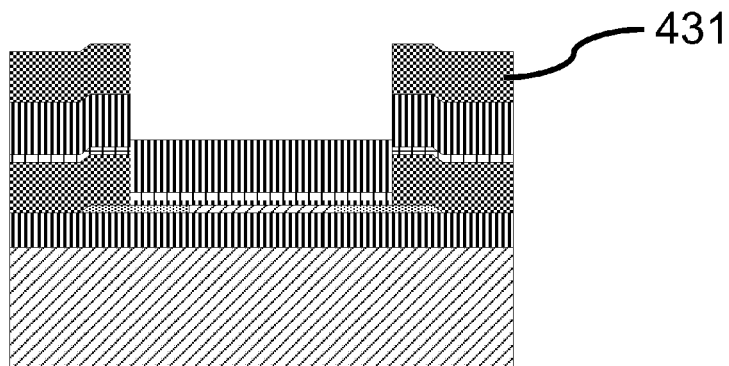


Figure 4P

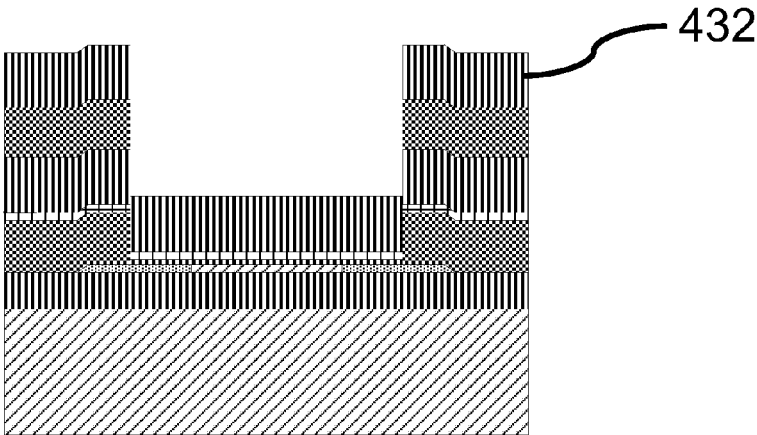


Figure 4Q

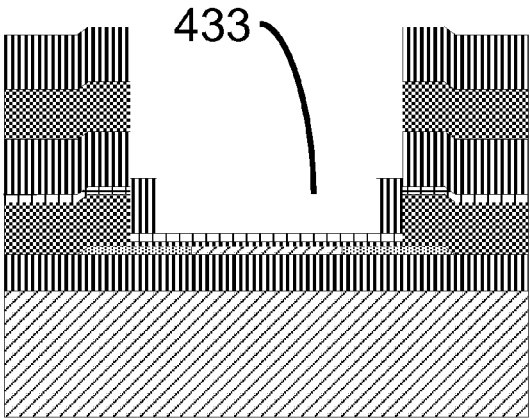


Figure 4R

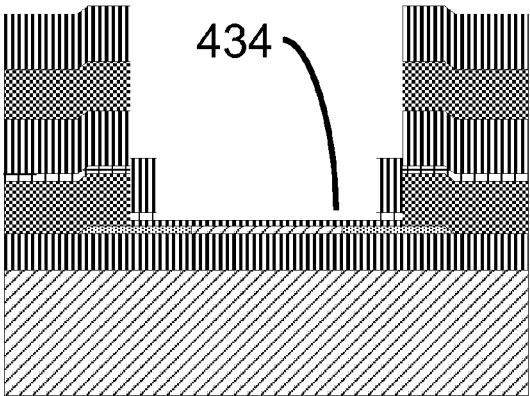


Figure 5A

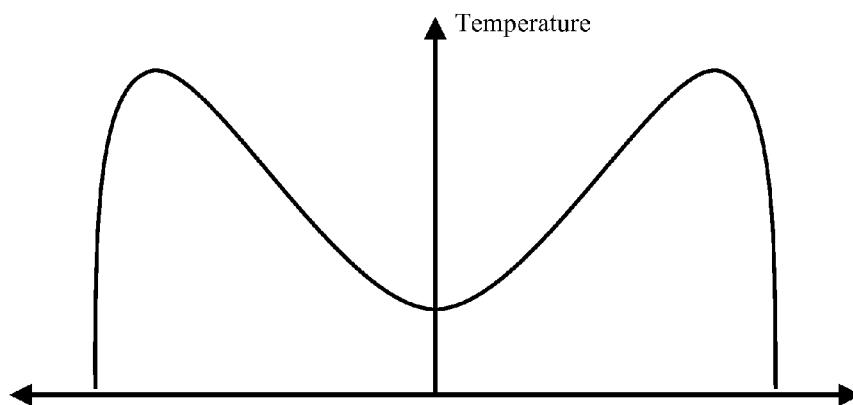
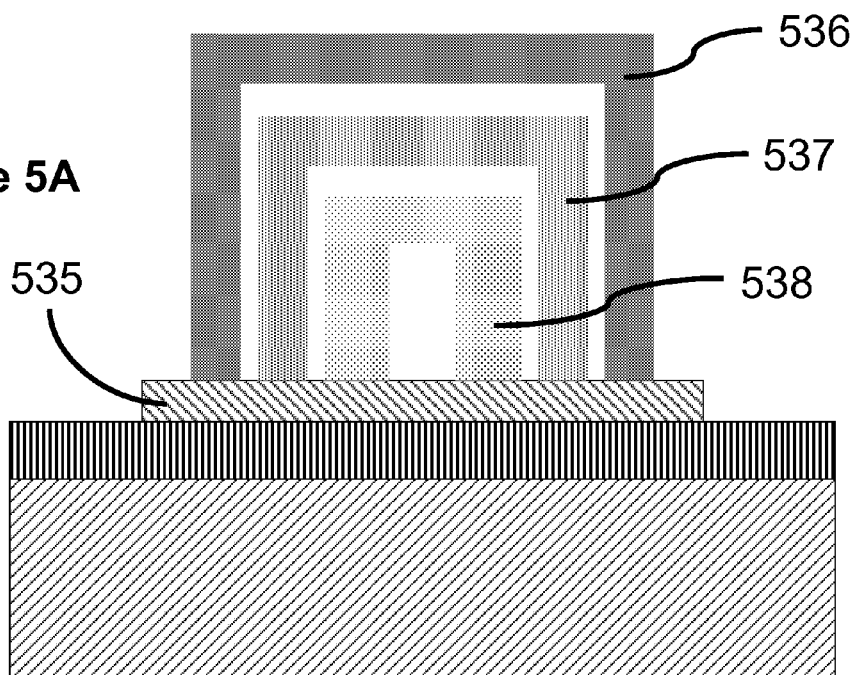


Figure 5B

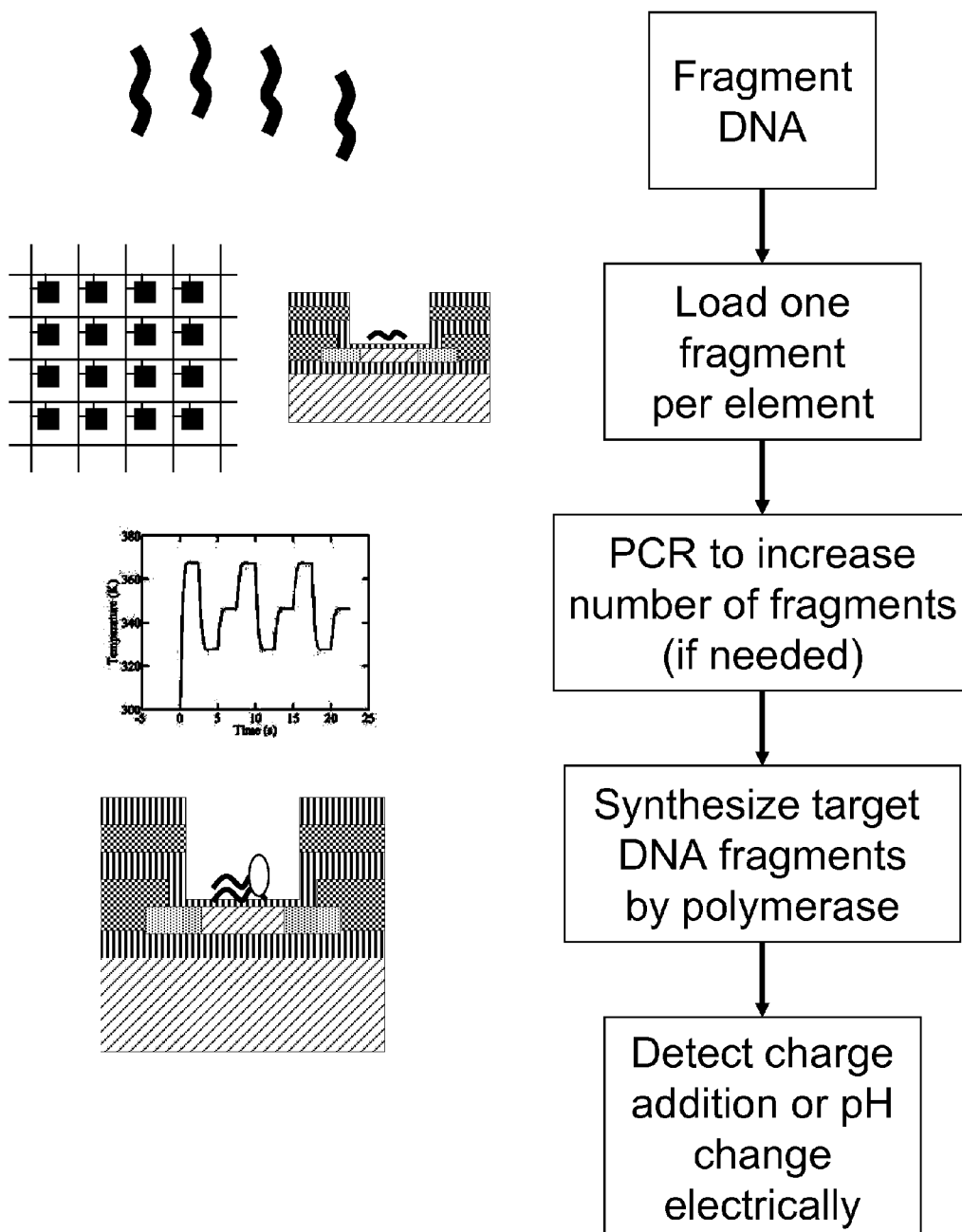


Figure 6

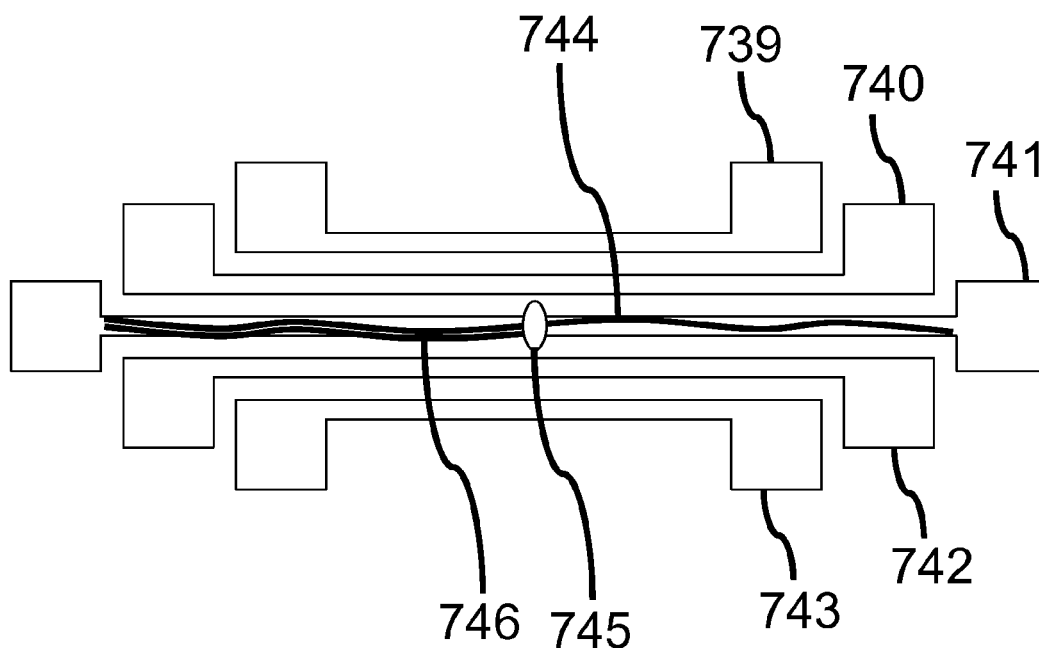


Figure 7

1

DNA SEQUENCING AND AMPLIFICATION SYSTEMS USING NANOSCALE FIELD EFFECT SENSOR ARRAYS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of and priority to PCT International Application PCT/US2009/058739, filed Sep. 29, 2009, and U.S. Provisional Application 61/101,062 filed on Sep. 29, 2008, the disclosures of which are hereby incorporated by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States governmental support under Award No. ECS 0554990 awarded by the National Science Foundation and Award No. 1 R21 EB006308-01 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

BACKGROUND OF THE INVENTION

This invention is in the field of chemical and biological sensing and biotechnology. This invention relates generally devices and methods useful for amplification and/or sensitive detection of molecules, for example nucleic acids.

Recent advances in nanotechnology and integration of top-down and bottom-up fabrication techniques have enabled the realization of integrated devices with nano-scale sensors for the direct, label-free, and electronic detection of biomolecules. These advances are moving towards the vision of personalized and quantitative molecular medicine. Many disease states and disorders in medicine can be attributed to the aberrations and defects in the DNA molecule of individuals. Hence, sequencing the DNA molecule and sequencing of the entire genome in a cost effective, accurate and rapid manner is desirable.

One of the most promising methods being commercialized is the method of 'sequencing by synthesis' where optical detection of each nucleotide added to an existing DNA template molecule that is being polymerized, can be used to obtain the sequence of the starting template.

Recently, the entire genome of a human being, James Watson, was completed within two months by the DNA sequencing company 454 Life Sciences using a novel DNA sequencing approach wherein microfabricated high-density picoliter reactors were used to perform sequencing by synthesis. This method still costs about \$1 million, well above the \$1,000 per genome target of the National Human Genome Research Institute. Various factors determine the current cost structure of the genome sequence technologies.

The current 'sequence by synthesis' approaches consist of the following major components: (i) the original target molecule to be sequenced is digested into smaller fragments, (ii) each smaller fragment is amplified by 30 to 40 cycle PCR to increase their number to have a high signal/noise ratio for detection at a later step, (iii) synthesis of the complementary strands is performed and the addition of each nucleotide is detected using either an optical fluorescence signal generation or conversion of pyrophosphates (PPi) released during DNA polymerization into a chemiluminescence signal (pyrosequencing) with minimum photon count of 10000 from approximately 10 million conjugations, (iv) the information obtained from each original strand (or amplified strands of the

2

same type) is pieced back together (assembly) using sophisticated software and algorithms to obtain the sequence of the original molecule.

Sequencing approaches based on micro to nanoscale technological developments are able to improve sequencing speed and read accuracy as well as reduce costs of sequencing. Wider application of genomics in medicine, from personalized medicine to point-of-care advanced diagnostic and predictive genomic tools, could one day be achieved if such advances reduce the cost of genome sequencing to the \$1000 target of the National Human Genome Research Institute.

A variety of microscale sensor devices have been developed for label-free detection of DNA. For example, U.S. Patent Application Publication No. 2006/0197118 discloses an extended gate field effect transistor sensor device. Probe molecules are immobilized on an exposed metal sensor electrode for detection of target molecules, e.g., DNA, to produce a change in an electrical characteristic of the field effect transistor.

U.S. Patent No. 7,385,267 also discloses a field effect sensor device including a functionalized silicon nanowire serving as a gate electrode for the field effect device. The surfaces of the nanowires are selectively functionalized with reactive binding partners, e.g., for probing DNA molecules.

U.S. Patent Application Publication No. 2007/0292855 discloses a device for electrical detection of molecular binding between a probe molecule and a target molecule. Several independently addressable electrodes positioned above the device serve to attract and trap charged molecules above a channel of the device.

U.S. Patent No. 6,203,683 discloses a device for analysis of polyelectrolytes, such as DNA, which includes electrodes for transporting molecules by dielectrophoresis and a trapping electrode attached to a heating element for heating trapped molecules, for example for thermocycling reactions.

SUMMARY OF THE INVENTION

In one aspect, described herein are field effect chemical sensor devices useful for chemical and/or biochemical sensing. Also provided herein are methods for single molecule detection. In another aspect, described herein are methods useful for amplification of target molecules by PCR.

According to one aspect, a chemical sensor device comprises a semiconductor field effect sensor or transistor having a source, a drain, a gate and a sensing region, wherein the sensing region is positioned between the source and drain and the gate is positioned at least partially below the sensing region, the source, and the drain. For some embodiments, a chemical sensor device is a FIN-FET, a double gate SOI FET, a metal gate FET, an extended gate FET, a strained semiconductor FET or another field effect transistor device.

In some embodiments the semiconductor field effect sensor comprises a semiconductor selected from the group consisting of: Si, Ge, C, any III-V semiconductor, any II-VI semiconductor, and any combination of these materials. In one embodiment, the gate comprises a semiconductor substrate or an electrical contact under a buried oxide. Useful field effect sensors include, but are not limited to: Schottky field effect sensors, field effect sensors fabricated using a top down method and field effect sensors fabricated using a bottom up method, such as nanowires, nanotubes and other nanostructures. The chemical sensor devices may also comprise a gate electrode located below the semiconductor field effect sensor. For some embodiments the source and drain each comprise doped semiconductor, for example, a semiconductor having dopants selected from the group consisting of

phosphorus, nitrogen, boron, aluminum, arsenic, antimony, carbon, oxygen and any combination of these. The sensing region for some embodiments comprises a conductive structure commonly referred to as a nanowire or a nanoplate.

In an embodiment, a chemical sensor device comprises one or more voltage controllers electrically connected to the field effect sensor or other components. In one example, a chemical sensor device comprises an AC voltage controller electrically connected to the gate and the source and drain for providing a preselected AC voltage between the gate and the source and/or drain. In another embodiment, a chemical sensor device comprises a DC voltage controller electrically connected to the electrostatic lens electrode and the gate for providing a preselected DC voltage between the electrostatic lens electrode and the gate.

In a specific embodiment, a chemical sensor device comprises an electrostatic lens electrode positioned over the semiconductor field effect sensor. For some embodiments, a distance between the electrostatic lens electrode and the semiconductor field effect sensor is selected from 0.01 to 5 μm , for example 0.5 μm ; for other embodiments the electrostatic lens electrode is positioned adjacent to the semiconductor field effect sensor. Useful electrostatic lenses include lenses comprising conducting materials, for example gold, platinum, aluminum, doped poly-silicon, silicon, titanium, nickel, chromium or any combination of these or other patternable conducting materials. Useful electrostatic lenses include lenses having a thickness selected from 0.01 to 5 μm .

For specific embodiments, a chemical sensor device comprises a first passivation layer positioned between the electrostatic lens electrode and the source and the drain of the semiconductor field effect sensor for electrically isolating the electrostatic lens electrode from the source and the drain. The first passivation layer may, for example, be at least partially disposed on the sensing region for immobilizing one or more probe molecules above the sensing region. In embodiments, a passivation layer has a thickness selected from 0.01 to 5 μm . The first passivation layer may also have a reduced thickness above the sensing region, for example a thickness selected from 0.001 to 0.1 μm .

For some embodiments, an electrostatic lens electrode includes a first opening above the sensing region for passing the one or more analytes to be sensed to the sensing region. In related embodiments, the first passivation layer optionally includes an opening or a reduced thickness above the sensing region for passing the one or more analytes to be sensed to the sensing region. In a specific embodiment, a chemical sensor device further comprises a second passivation layer located on the electrostatic lens electrode, wherein the second passivation layer includes an opening above the sensing region for passing the one or more analytes to be sensed to the sensing region. The openings above the sensing region may independently have, for example, a cross-sectional dimension selected from 0.1 to 50 μm .

Useful passivation layers include layers comprising insulating materials or other materials. In embodiments, a passivation layer comprises oxygen and/or nitrogen, for example in the form of an oxide or a nitride layer such as SiO_2 or Si_3N_4 . In a specific embodiment, at least a portion of a passivation layer has a composition having a stoichiometry of MO_x , wherein M is a semiconductor and x is selected from the range of 0-2. In a specific embodiment, at least a portion of a passivation layer has a composition having a stoichiometry of MN_y , wherein M is a semiconductor and y is selected from the range of 0-1.33. In embodiments, a passivation layer comprises silicon and/or a silicon rich layer, SiO_2 , Si_3N_4 , SiO_xN_y , Al_2O_3 , HfO_2 , AlN , polyimide, a photoresist, any insulator

that can be deposited or grown on the semiconductor layer and/or any combination of these materials. In another embodiment, a passivation layer comprises a layer having a mixture of aluminum and oxygen. Useful insulating layers also include those comprising sapphire, glasses and/or plastics.

In a specific embodiment, a chemical sensor device comprises one or more probe molecules immobilized on the surface of the sensing region or the surface of the first passivation layer. A variety of probe molecules are useful with the chemical sensor devices described herein for chemical/biochemical sensing. For example, useful probe molecules include molecules selected from the group consisting of DNA, LNA, PNA, RNA, aptamers, other nucleic acid analogs, proteins, amino acids, and any combination of these or other ligands. For a specific embodiment, a chemical sensor device is provided as a pH sensor. For embodiments where the probe molecules are immobilized on the surface of the first passivation layer, the composition of the first passivation layer may be selectively adjusted for immobilization of a particular probe molecule. For example, the first passivation layer may comprise SiO_2 , Si_3N_4 , Si_xN_y , Al_2O_3 , HfO_2 , and/or AlN in varying amounts for selective immobilization of a probe molecule.

The chemical sensor devices provided herein are useful as individual elements in an array of chemical sensors; for example an $N \times N$ array of chemical sensor devices. In a specific embodiment, each chemical sensor device element of the array is independently electrically addressable. For example, the source, drain, gate, and/or electrostatic lens electrode of each chemical sensor device element in an array of chemical sensors may be individually electrically addressable. In another embodiment, the sensing regions of each chemical sensor device element may be independently fluidly addressable; that is, a fluid may be provided to the sensing region of a single chemical sensor device element independently from other chemical sensor device elements.

According to another aspect, a method is provided for single molecule detection for RNA, DNA, LNA, PNA, aptamers, proteins, or other molecules. A method of this aspect comprises the steps of: providing a field effect sensor comprising an electrostatic lens electrode, a gate electrode and a sensing region, wherein the sensing region is functionalized to detect a target analyte molecule; providing a solution to the field effect sensor, wherein the solution comprises a plurality of analyte molecules including at least one target analyte molecule; applying a first potential difference between the electrostatic lens electrode and the gate electrode to attract a target analyte molecule to the sensing region; monitoring an electric parameter of the field effect sensor to determine when a single target analyte molecule is detected by the field effect sensor; and applying a second potential difference between the electrostatic lens electrode and the gate electrode to repel additional analyte molecules from the sensing region. In a specific embodiment, the second potential difference is of the opposite sign of the first potential difference. In a specific embodiment, the electrostatic lens electrode includes an opening above the sensing region for passing the one or more analytes to be sensed to the sensing region.

In another aspect, a method and device are provided for amplifying a single target molecule, for example by PCR. A method of this aspect comprises the steps of: providing a field effect sensor comprising an electrostatic lens electrode, a gate electrode, a source, a drain and a sensing region, and wherein the sensing region is functionalized for detection of a target analyte molecule by the field effect sensor; providing a solu-

tion to the field effect sensor, wherein the solution comprises a plurality of analyte molecules including at least one target analyte molecule; applying a first DC voltage between the electrostatic lens electrode and the gate electrode to attract a target analyte molecule to the sensing region; monitoring an electric parameter of the field effect sensor to determine when a single target analyte molecule is detected by the field effect sensor; applying a second DC voltage between the electrostatic lens electrode and the gate electrode to repel additional analyte molecules from the sensing region, wherein the second DC voltage is of the opposite sign of the first DC voltage; and repeating one or more times the steps of: applying a first AC voltage between the gate electrode and the source and drain to raise the temperature of the target analyte molecule to a first temperature; terminating the application of voltage between the gate electrode and the source and drain and allowing the temperature of the target analyte molecule to relax to a second temperature; and applying a second AC voltage between the gate electrode and the source and drain to raise the temperature of the target analyte molecule to a third temperature. In a specific embodiment, an electric parameter of the field effect sensor is monitored to determine a quantity increase of the target analyte molecule achieved during the repeating steps. In an embodiment, an electric parameter of the field effect sensor is monitored to determine a change in pH achieved during the repeating steps. Certain embodiments further comprise applying a DC and/or AC voltage between the electrostatic lens electrode and the gate electrode, for example to concentrate the molecules and/or keep them at/close to the gate/sensing region during and/or after amplification; for some embodiments, this step is optionally repeated. In a specific embodiment, a negative voltage is applied to the electrostatic lens electrode to keep the amplified molecules at and/or close to the gate electrode and/or sensing region.

For some methods of this aspect, the solution further comprises components and reagents necessary for performing PCR on the single target analyte molecule. Additionally, methods of this aspect may further comprise the step of providing a second solution to the field effect sensor before the repeating steps, wherein the second solution comprises components and reagents necessary for performing PCR on the single target analyte molecule.

Steps of the methods described herein may occur simultaneously or in any order. For example, the monitoring step may occur prior to, during, and after the application of the first potential difference. In an embodiment, the step of applying the second potential difference occurs immediately after the detection of the target analyte during the monitoring step. In a related embodiment, if a target analyte molecule is detected before the application of the first potential difference, the second potential difference is immediately applied.

Useful electric parameters include, but are not limited to, a resistance and/or a conductance across the field effect sensor, a current through the field effect sensor, a potential of the field effect sensor, an AC impedance of the field effect sensor, or any combination of these or other electrical properties. Monitoring of these and other electric parameters is useful, for example, for sensing a change in pH, an increase or decrease in charge at the sensing region and/or the presence or absence of an analyte molecule or a portion of an analyte molecule at the sensing region. A specifically useful electric parameter is the impedance of the solution. The impedance of the solution can be measured, for example, through the electrostatic lens electrodes or the gate electrodes. U.S. Provisional Patent application 61/245,083 filed on Sep. 23, 2009, which is hereby incorporated by reference in its entirety, discloses

methods of detection of amplification of molecules by measuring the impedance of a solution.

In an embodiment, a method of this aspect for amplification of a target molecule by PCR comprises the steps of providing a chemical sensor device, for example as described above; providing a solution containing at least one target molecule to a surface of the chemical sensor device, providing a preselected AC voltage between a gate and a source and a drain of the chemical sensor device to heat the chemical sensor device to a preselected temperature; and transporting the at least one target molecule across the surface of the chemical sensor device.

In a specific embodiment, the transporting step includes moving the at least one target molecule through a spatial temperature gradient. Useful thermal gradients include those which include the temperatures necessary for performing PCR on the at least one target molecule. For example, the spatial temperature gradient can include a denaturation temperature (such as from 90 to 100° C.), an extension temperature (such as from 68 to 75° C.) and an annealing temperature (such as from 45 to 60° C.).

Transport of the target molecules across the spatial temperature gradient provides for sequentially the placing target molecules into the necessary temperatures for amplification. Useful transport processes include active and passive transport, for example transport by diffusion, transport by dielectrophoresis, electro-osmotic flows or any combination of these or other electro-thermal transport processes.

In another aspect, the methods and devices described herein may be utilized in the sequencing of a nucleic acid molecule or group of nucleic acid molecules. A method of this aspect comprises the steps of: providing the nucleic acid to be sequenced; fragmenting the nucleic acid to be sequenced into a plurality of fragments; providing an array of chemical sensors; loading the plurality of fragments into the chemical sensor device elements of the chemical sensor array; and monitoring an electric parameter of each of the chemical sensor device elements to determine the sequence of the plurality of fragments. For example, the monitoring step may include sensing a change in the charge present at the sensing region of each chemical sensor device element as complements to the plurality of fragments are synthesized. A method of this aspect further comprises the step of providing to the chemical sensor device elements the necessary reagents for the synthesis of the complements to the fragments, for example nucleic acids and/or replication enzymes such as a polymerase. In a specific embodiment, an electric parameter of the field effect sensor is monitored to determine a change in pH achieved during synthesis.

For some applications, in the loading step, each sensor device element is loaded with no more than one fragment. In a specific method of this aspect, in the loading step, a first DC voltage is applied between the electrostatic lens electrode and the gate electrode of each chemical sensor device element to attract fragments to the sensing regions of the chemical sensor device elements. This method may further comprise the steps of monitoring an electrical parameter of the chemical sensor device elements to detect when a single fragment is loaded into an individual chemical sensor device element; and applying a second DC voltage between the electrostatic lens electrode and the gate electrode of the individual chemical sensor device element to repel additional fragments, wherein the second DC voltage is of the opposite sign of the first DC voltage.

Depending upon the sensitivity of the chemical sensor device elements, the plurality of fragments loaded into the chemical sensor device elements may be amplified, for

example by PCR. In a specific embodiment of this aspect, an amplification step includes repeating one or more times the steps of: applying a first AC voltage between the gate electrode and the source and drain of the individual chemical sensor device elements to raise the temperature of the fragments to a first temperature; terminating the application of voltage between the gate electrode and the source and drain of the individual chemical sensor device elements and allowing the temperature of the fragments to relax to a second temperature; and applying a second AC voltage between the gate electrode and the source and drain of the individual chemical sensor device elements to raise the temperature of the fragments to a third temperature. When performing PCR on the fragments, in an embodiment, the necessary components and reagents are provided to the chemical sensor device elements. If desired, an electric parameter of the field effect sensors of the individual chemical sensor device elements may be monitored to determine a quantity increase of the fragments achieved during amplification. In an embodiment, an electric parameter of the field effect sensor is monitored to determine a change in pH achieved during amplification.

Some methods of this aspect further comprise the step of heating the chemical sensor device elements of the chemical sensor array to bind the fragments to the sensing regions of the chemical sensor device elements. For example the heating may be achieved by applying a first AC voltage between the gate electrode and the source and drain of individual chemical sensor device elements.

In another aspect, a method is provided for sensing a structure containing nucleic acids, for example a cell (e.g., a plant cell, mammalian cell, or bacterial cell), a spore, and/or a virus. A method of this aspect comprises the steps of: providing a chemical sensor device, for example as described herein; and providing a solution having one or more nucleic acid containing structures to the chemical sensor device. If desired, an electrical parameter of the chemical sensor device is monitored to detect when a nucleic acid containing structure is loaded into the chemical sensor device. In some embodiments one nucleic acid containing structure is loaded into a chemical sensor device; in other embodiments a plurality of nucleic acid containing structures are loaded into a chemical sensor device.

A method of this aspect for trapping a nucleic acid containing structure further comprises the step of providing a first DC voltage between an electrostatic lens electrode and a gate electrode of the chemical sensor device element to attract and/or trap the nucleic acid containing structure. A method of this aspect further comprises the step of providing a second DC voltage between the electrostatic lens electrode and the gate electrode of the chemical sensor device element to repel additional nucleic acid containing structures.

A specific method of this aspect further comprises the step of heating and/or lysing the nucleic acid containing structure to release the nucleic acid, for example by providing an AC voltage between the gate electrode and a source and drain of the chemical sensor device. For example, the nucleic acid containing structure is heated to 95-100° C. for 4-6 minutes to lyse the structure. Once the nucleic acids are released, in an embodiment, they are amplified and/or detected using the methods and/or devices described herein. In a specific embodiment, the nucleic acids are amplified and/or detected using the same chemical sensor device or element used to heat and/or lyse the nucleic acid containing structure.

Without wishing to be bound by any particular theory, there can be discussion herein of beliefs or understandings of underlying principles relating to the invention. It is recognized that regardless of the ultimate correctness of any

mechanistic explanation or hypothesis, an embodiment of the invention can nonetheless be operative and useful.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates an exemplary chemical sensor device.

FIGS. 2A-2D illustrate an embodiment of a method for detection of a single target analyte molecule.

FIGS. 3A-3C illustrate additional steps for amplification of a target analyte molecule by PCR. FIG. 3D shows an example temperature profile achieved by this method.

FIGS. 4A-4R illustrate a cross-sectional process flow of an embodiment of a method for fabrication of a chemical sensor device.

FIG. 5A illustrates three temperature regimes surrounding the active area of a chemical sensor device. FIG. 5B shows a plot of the temperature as a function of the distance from the center of the active area.

FIG. 6 illustrates a method for sequencing DNA, utilizing an NxN array of chemical sensor devices.

FIG. 7 illustrates a top down view of the arrangement of components in a chemical sensor device having multiple sensor portions.

DETAILED DESCRIPTION OF THE INVENTION

In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references and contexts known to those skilled in the art. The following definitions are provided to clarify their specific use in the context of the invention.

“Field effect sensor” refers to a semiconductor device, similar to a field effect transistor, in which the conductivity of a channel in the semiconductor is modified by the presence of analyte molecules near the surface of a sensing region.

“Nanoplate” refers to a sensing region of a field effect sensor having a specific shape, for example a planar or substantially planar and rectangular shape.

“Nanowire” refers to a sensing region of a field effect sensor having a specific shape, for example a cylindrical or substantially cylindrical shape, similar to that of a wire.

“PCR” or “Polymerase chain reaction” refers to the well known technique of enzymatic replication of nucleic acids which uses thermal cycling for example to denature, extend and anneal the nucleic acids.

“Loading” or “loaded” refers to providing a molecule, compound, substance or structure to the sensing region or a well adjacent to or above the sensing region of a chemical sensor device.

The chemical sensor devices disclosed herein are useful, for example, for detection and/or sequencing of nucleic acids. According to one aspect, the devices include an electrostatic lens for attracting and trapping, for example by a DC electric field, a single target nucleic acid chain for selective amplification and detection and/or sequencing. According to another aspect, the devices utilize an AC voltage for dielectric heating of the nucleic acid molecules. The temperature profile above the devices can be utilized in a passive manner, for example, for amplification of nucleic acid molecules by transporting the nucleic acid molecules through the temperatures necessary for amplification by PCR. The temperature above the devices can also, for example, be used in a more active manner by providing a sequence of AC voltages to heat the nucleic acid molecules to the temperatures necessary for amplification by PCR.

The methods and devices described herein are additionally or alternatively useful for nucleic acid amplification in addi-

tion to PCR, for example by using techniques known in the art. For example, Nucleic Acid Sequence Based Amplification (NASBA) and/or Loop mediated isothermal amplification (LAMP) may be used to amplify a target nucleic acid molecule. NASBA is a useful technique, as it is an isothermal amplification technique and does not require the numerous heating and cooling steps that PCR requires. Methods described herein for amplifying a target nucleic acid molecule using a method with one or more heating and/or cooling steps (e.g., a PCR method) can be modified to alternatively use isothermal amplification techniques (e.g., NASBA) by providing the necessary enzymes, synthetic reagents and/or physical conditions (temperature, concentration, pH, etc.).

FIG. 1 shows an embodiment of a chemical sensor device 100. Chemical sensor device 100 includes a field effect sensor having a source region 101, a drain region 102, a gate region 103 and a sensing region 104. Electrodes 105 and 106 respectively allow for electrical connections to be made to source region 101 and drain region 102. A first passivation layer 107 is disposed on the field effect sensor, providing electrical isolation between the field effect sensor and an electrostatic lens electrode 108. Electrostatic lens electrode 108 includes an opening above sensing region 104 to allow target analytes to pass through to sensing region 104. First passivation layer 107 has a reduced thickness portion adjacent to sensing region 104. Such a reduced thickness can, for example, allow target analytes to more strongly interact with and be detected by sensing region 104. A second passivation layer 109 is disposed over electrostatic lens electrode 108.

FIGS. 2A-2D illustrate an embodiment of a method for detection of a single target analyte molecule. FIG. 2A shows a chemical sensor device 200 having the sensing region functionalized with a probe molecule 210 for detection of the target analyte molecule. As shown in FIG. 2B, a solution 211 having analyte molecules is provided to the chemical sensor device. In this example, three target analyte molecules 212 are included in solution 211. In FIG. 2C, a first potential difference 213 is applied between the electrostatic lens electrode and the gate to attract analyte molecules toward the sensing region, as indicated by the arrow. An electric parameter of the field effect sensor is monitored, for example the conductance between the source and the drain, and when it is determined that a single target analyte molecule is detected, a second potential difference 214, having the opposite sign as the first potential difference, is applied between the electrostatic lens electrode and the gate to repel additional molecules from the sensing region, as shown in FIG. 2D.

The method of FIGS. 2A-2D may be further utilized with additional steps for amplification of a target analyte molecule by PCR, as illustrated by FIGS. 3A-3C. Once a single target analyte molecule is detected, as in FIG. 2D, the first subsequent step is application of a first AC voltage 315 between the gate and the source and drain to increase the temperature of the target analyte molecule, as shown in FIG. 3A. After the target analyte molecule reaches a first desired temperature, the voltage is terminated, as shown in FIG. 3B, to allow the temperature of the target analyte molecule to relax. After the target analyte molecule reaches a second desired temperature, a second AC voltage 316 is applied between the gate and the source and drain to increase the temperature of the target analyte molecule, FIG. 3C. After the target analyte molecule reaches a third desired temperature, the steps shown in FIGS. 3A-3C may be optionally repeated to further amplify the target analyte molecule. FIG. 3D shows an example temperature profile which is achieved by this method.

FIGS. 4A-4R illustrate a cross-sectional process flow of an embodiment of a method for fabrication of a chemical sensor

device. Necessary processing materials, techniques and devices will be evident to those having skill in the art.

FIG. 4A: This process begins with a silicon-on-insulator (bonded) wafer with substrate 417 and having a top silicon layer 418 and a buried oxide layer 419. FIG. 4B: A dry oxide 420 is grown on the top silicon layer 418 to thin it down, leaving oxide 420. FIG. 4C: The oxide 420 is etched down to silicon layer 418. FIG. 4D: If desired, dry oxidation of the top silicon layer 418 thins it further, creating oxide 421. FIG. 4E: The active area is defined, for example using e-beam lithography, using a negative resist 422 and oxide 421 as a hard mask. FIG. 4F: The oxide 421 is etched, undercutting the resist slightly. FIG. 4G: The resist is removed and the active area silicon 418 is etched having a slight overcut. FIG. 4H: A photoresist mask 423 is deposited for subsequent implantation of dopants into silicon layer 418. FIG. 4I: Oxide 421 is etched after dopant implantation, exposing the silicon regions 424 having implanted dopants. FIG. 4J: A high quality gate oxide 425 is grown using dry oxidation. FIG. 4K: A mask 426 is placed over the active area. FIG. 4L: Metal contacts 427 are deposited over the implanted regions 424 and the mask 426 is removed. At this point a rapid thermal anneal may be used to anneal the contacts 427. FIG. 4M: A thin layer of silicon nitride 428 and nitride rich silicon oxide 429 are subsequently deposited. FIG. 4N: Another mask 430 is placed over the active area. FIG. 4O: A metal electrostatic lens electrode 431 is deposited over silicon oxide 429 and the mask removed. FIG. 4P: A silicon oxide passivation layer 432 is deposited. FIG. 4Q: The silicon oxide above the active area is removed from region 433, stopping on silicon nitride layer 428. FIG. 4R: The silicon nitride is etched from region 434, stopping on oxide 425.

FIG. 5A illustrates a device useful with embodiments of methods for amplification of a target analyte by PCR. When a preselected AC voltage is applied between the gate and the source and drain of the active area 535 of the device the temperature of the region surrounding the active area 535 is increased. Three general temperature regimes are created: a first temperature region 536, for example having a temperature under which nucleic acids can be denatured; a second temperature region 537, for example having a temperature under which denatured nucleic acids can be extended; and a third temperature region 538, for example having a temperature under which denatured and extended nucleic acids can be annealed. FIG. 5B shows a typical temperature profile of the device of FIG. 5A when a preselected AC voltage is applied between the gate and the source and drain regions.

In an embodiment, a solution containing target analytes to be amplified by PCR and other necessary PCR reagents is provided to the surface of a chemical sensor device, such as depicted in FIG. 5A. When a preselected AC voltage is applied between the gate and the source and drain regions of the chemical sensor device and the target analytes are transported across the chemical sensor device, the target analytes experience the three temperature regimes necessary for amplification by PCR. Transport of the analyte molecules can, for example, be by diffusion and/or dielectrophoresis.

FIG. 6 illustrates the steps of a method for sequencing DNA using an NxN array of chemical sensor devices. Initially, the DNA is fragmented and single fragments are loaded into individual chemical sensor device elements. If needed, the fragments can be amplified using PCR by cycling of the temperature. Next, the complementary target fragments are synthesized using a DNA polymerase. While the target fragments are being synthesized, the charge provided by subsequent bases is monitored and detected, providing the sequence information for the fragments. Finally, the fragment

11

sequence information is reassembled, providing the sequence of the initial DNA (not shown).

In embodiments, multiple field effect sensors are used to isolate, detect, sequence and/or amplify a target molecule, for example a single target molecule. FIG. 7 illustrates a top down view of a specific sensor embodiment showing the arrangement of multiple field effect sensors in a side-by-side fashion, useful for detecting and/or amplifying a single DNA fragment. In this embodiment, five field effect sensors, **739**, **740**, **741**, **742** and **743** are arranged side-by side. A target DNA fragment, **744**, is attracted toward the sensing region where an enzyme **745**, for example DNA polymerase, synthesizes a complementary DNA fragment **746**. For certain embodiments, the complementary DNA fragment **746** is synthesized one base at a time by enzyme **745**. In these and other embodiments, no amplification of the target DNA fragment **744** is necessary. For other embodiments, however, amplification of the target DNA fragment **744** by PCR or other techniques are contemplated.

In an exemplary embodiment, the field effect sensors **739**, **740**, **741**, **742** and **743** are individually biased to attract and stretch DNA fragment **746** along field effect sensor **741**. In one embodiment, field effect sensor **741** is biased at a first potential, V_e , while field effect sensors **739**, **740**, **742** and **743** are biased at a second potential of opposite sign, $-V_e$. In a specific embodiment, field effect sensor **741** is biased with different potentials at opposite sides of the sensor, e.g., $V_e + \Delta V$ at one end and $V_e - \Delta V$ at the opposite end. Without wishing to be bound by any theory, it is believed that when field effect sensors **739**, **740**, **741**, **742** and **743** are biased in this manner, a lensing effect is created, attracting DNA fragment **746** toward and stretched along field effect sensor **741**. In certain embodiments, field effect sensors **739**, **740**, **742** and **743** are biased at different potentials; for example, field effect sensors **740** and **742** can be biased at $-V_e$, while field effect sensors **739** and **743** can be biased at $-2V_e$. In other embodiments, field effect sensors **739**, **740**, **742** and **743** are not field effect sensors but are electrodes and/or electrostatic lenses.

The invention may be further understood by the following non-limiting examples:

EXAMPLE 1

Extremely Localized PCR with Temperature Cycling or PCR without Fluid Movement with Temperature Gradients

Polymerase chain reaction (PCR) is a commonly used technique to amplify the amount of a nucleic acid sample analyzed by taking the sample through 3 temperature steps. These steps are for the annealing of the primer (lowest temperature), extension (the actual amplification, medium temperature) and denaturation of the product, which make up one cycle of the PCR. In each cycle the amount of nucleic acid is amplified twice the value before the cycle. By cycling many times, the nucleic acid at hand can be amplified orders of magnitude.

Dielectric heating capability of the devices described herein can be used for performing PCR, in an extremely localized fashion. A device can simply be cycled through the temperatures needed for the PCR to take place for amplification by changing the magnitude of the applied bias. Extremely localized heating allows for fast cycling due to the extremely low thermal mass associated, enabling nucleic acid amplification of many orders of magnitude in timescales less than a minute.

12

Alternatively PCR on the device can be performed by taking advantage of the thermal gradient that the heating method inherently provides. In this scheme the three temperatures needed for PCR are present on the device at spatially distinct locations, as shown in FIG. 5A. Thus, by passively or actively transporting the molecules to different locations of the device a PCR cycle can be completed. The passive transport of the molecules can be accomplished simply by the diffusion of the molecules. The molecules can be trapped in the device area by the potential barrier already setup due to the alternating electric field caused by dielectrophoresis (DEP). The active transport of molecules can be accomplished by designing the device geometry and in order to balance the thermal and electrical forces.

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STATEMENTS REGARDING INCORPORATION BY REFERENCE AND VARIATIONS

All references throughout this application, for example patent documents including issued or granted patents or equivalents; patent application publications; and non-patent

literature documents or other source material; are hereby incorporated by reference herein in their entireties, as though individually incorporated by reference, to the extent each reference is at least partially not inconsistent with the disclosure in this application (for example, a reference that is partially inconsistent is incorporated by reference except for the partially inconsistent portion of the reference).

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. References cited herein are incorporated by reference herein in their entirety to indicate the state of the art, in some cases as of their filing date, and it is intended that this information can be employed herein, if needed, to exclude (for example, to disclaim) specific embodiments that are in the prior art. For example, when a compound is claimed, it should be understood that compounds known in the prior art, including certain compounds disclosed in the references disclosed herein (particularly in referenced patent documents), are not intended to be included in the claim.

When a group of substituents is disclosed herein, it is understood that all individual members of those groups and all subgroups and classes that can be formed using the substituents are disclosed separately. When a Markush group or other grouping is used herein, all individual members of the group and all combinations and subcombinations possible of the group are intended to be individually included in the disclosure.

Every formulation or combination of components described or exemplified can be used to practice the invention, unless otherwise stated. Specific names of materials are intended to be exemplary, as it is known that one of ordinary skill in the art can name the same material differently. One of ordinary skill in the art will appreciate that methods, device elements, starting materials, and synthetic methods other than those specifically exemplified can be employed in the practice of the invention without resort to undue experimentation. All art-known functional equivalents, of any such methods, device elements, starting materials, and synthetic methods are intended to be included in this invention. Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges and subranges, as well as all individual values included in the ranges given are intended to be included in the disclosure.

As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of elements of a device, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention

15

claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

We claim:

1. A chemical sensor device for sensing one or more analytes, the chemical sensor device comprising:

a semiconductor field effect sensor comprising a source, a drain, a gate and a sensing region, wherein the sensing region comprises a nanowire or a nanoplate positioned between the source and drain and the gate is positioned at least partially below the sensing region, the source, and the drain, wherein the sensing region is a temperature controllable sensing region;

an electrostatic lens electrode positioned over the semiconductor field effect sensor, wherein the electrostatic lens electrode includes a first opening above the sensing region for passing the one or more analytes to be sensed to the sensing region;

a first passivation layer positioned between the electrostatic lens electrode and the source and the drain of the semiconductor field effect sensor for electrically isolating the electrostatic lens electrode from the source and for electrically isolating the electrostatic lens electrode from the drain; and

an AC voltage controller electrically connected to the gate and both the source and the drain for providing a preselected AC voltage between the gate and the source and between the gate and the drain;

wherein application of an AC voltage between the gate and the source and between the gate and the drain causes localized dielectric heating of the sensing region, thereby providing for temperature control of the temperature controllable sensing region.

2. The chemical sensor device of claim 1, further comprising a DC voltage controller electrically connected to the electrostatic lens electrode and the gate for providing a preselected DC voltage between the electrostatic lens electrode and the gate.

3. The chemical sensor device of claim 1, wherein the first opening has a cross-sectional dimension selected from 0.01 to 50 μm .

4. The chemical sensor device of claim 1, wherein a distance between the electrostatic lens electrode and the semiconductor field effect sensor is selected from 0.01 to 5 μm .

5. The chemical sensor device of claim 1, further comprising one or more probe molecules immobilized on the surface of the sensing region.

6. The chemical sensor device of claim 1, wherein the first passivation layer includes a second opening above the sensing region for passing the one or more analytes to be sensed to the sensing region.

7. The chemical sensor device of claim 6, wherein the second opening has a cross-sectional dimension selected from 0.01 to 50 μm .

8. The chemical sensor device of claim 1, wherein the first passivation layer is at least partially disposed on the sensing region for immobilizing one or more probe molecules above the sensing region.

9. The chemical sensor device of claim 1, further comprising a probe molecule immobilized on a surface of the first passivation layer.

10. The chemical sensor device of claim 1, wherein the first passivation layer includes oxygen.

16

11. The chemical sensor device of claim 1, wherein at least a portion of the first passivation layer has a composition having a stoichiometry of MO_x , wherein M is a semiconductor and x is selected from the range of 0-2.

12. The chemical sensor device of claim 1, wherein the first passivation layer comprises nitrogen.

13. The chemical sensor device of claim 1, wherein at least a portion of the first passivation layer has a composition having a stoichiometry of MN_y , wherein M is a semiconductor and y is selected from the range of 0-1.34.

14. The chemical sensor device of claim 1, wherein the first passivation layer comprises silicon.

15. The chemical sensor device of claim 1, wherein the first passivation layer comprises a silicon rich layer, SiO_2 , Si_3N_4 , silicon oxynitride, Al_2O_3 , AlN, HfO_2 , polyimide, photoresist or a depositable or growable insulator.

16. The chemical sensor device of claim 1, wherein the first passivation layer comprises a layer having a mixture of aluminum and oxygen.

17. The chemical sensor device of claim 1, wherein the first passivation layer has a thickness selected from 0.01 to 5 μm .

18. The chemical sensor device of claim 1, further comprising a second passivation layer located on the electrostatic lens electrode, wherein the second passivation layer includes a third opening above the sensing region for passing the one or more analytes to be sensed to the sensing region.

19. The chemical sensor device of claim 18, wherein the third opening has a cross-sectional dimension selected from 0.1 to 50 μm .

20. The chemical sensor device of claim 18, wherein the second passivation layer comprises a material selected from the group consisting of: SiO_2 , Si_3N_4 , silicon oxynitride, Al_2O_3 , AlN, HfO_2 , polyimide and photoresist.

21. The chemical sensor device of claim 18, wherein the second passivation layer has a thickness selected from 0.1 to 5 μm .

22. The chemical sensor device of claim 1, wherein the electrostatic lens electrode comprises a material selected from the group consisting of: gold, platinum, aluminum, doped poly-silicon, silicon, titanium, nickel, and chromium.

23. The chemical sensor device of claim 1, wherein the electrostatic lens electrode has a thickness selected from 0.01 to 5 μm .

24. The chemical sensor device of claim 1, wherein the gate comprises a semiconductor substrate.

25. The chemical sensor device of claim 1, wherein the gate comprises an electrical contact under a buried oxide layer.

26. The chemical sensor device of claim 1, wherein the semiconductor field effect sensor comprises a semiconductor selected from the group consisting of: silicon, germanium, carbon, any III-V semiconductor, and any II-VI semiconductor.

27. The chemical sensor device of claim 1, wherein the source and drain each comprise doped semiconductor.

28. The chemical sensor device of claim 27, wherein dopants in the doped semiconductor are selected from the group consisting of phosphorus, nitrogen, arsenic, antimony, carbon, and oxygen.

29. The chemical sensor device of claim 1, further comprising a gate electrode located below the semiconductor field effect sensor.

30. The chemical sensor device of claim 1, wherein the chemical sensor is a pH sensor.

31. The chemical sensor device of claim 1, wherein the chemical sensor is a FIN-FET, a double gate SOI FET, a metal gate FET, an extended gate FET or a strained semiconductor FET.

17

32. A method for detection of a single target analyte molecule, the method comprising the steps of:

providing the chemical sensor of claim 1, wherein the sensing region is functionalized to detect a target analyte molecule;

providing a solution to the field effect sensor, wherein the solution comprises a plurality of analyte molecules including at least one target analyte molecule;

applying a first potential difference between the electrostatic lens electrode and the gate electrode to attract a target analyte molecule to the sensing region;

monitoring an electric parameter of the field effect sensor to determine when a single target analyte molecule is detected by the field effect sensor; and

applying a second potential difference between the electrostatic lens electrode and the gate electrode to repel additional analyte molecules from the sensing region.

33. The method of claim 32, wherein the electrostatic lens electrode includes an opening above the sensing region for passing the one or more analytes to be sensed to the sensing region.

34. The method of claim 32, wherein the second potential difference is of the opposite sign of the first potential difference.

35. The method of claim 32, wherein the monitoring step occurs prior to the application of the first potential difference.

36. The method of claim 32, wherein the monitoring step occurs prior to, during and after the application of the first potential difference.

37. The method of claim 32, wherein the step of applying the second potential difference occurs immediately after the detection of the target analyte during the monitoring step.

38. The method of claim 32, wherein if a target analyte molecule is detected before the application of the first potential difference, the second potential difference is immediately applied.

39. A method for performing PCR on a single target analyte molecule, the method comprising the steps of:

providing the chemical sensor device of claim 1, wherein the sensing region is functionalized for detection of a target analyte molecule by the field effect sensor;

providing a solution to the field effect sensor, wherein the solution comprises a plurality of analyte molecules including at least one target analyte molecule;

applying a first DC voltage between the electrostatic lens electrode and the gate electrode to attract a target analyte molecule to the sensing region;

monitoring an electric parameter of the field effect sensor to determine when a single target analyte molecule is detected by the field effect sensor;

applying a second DC voltage between the electrostatic lens electrode and the gate electrode to repel additional analyte molecules from the sensing region, wherein the second DC voltage is of the opposite sign of the first DC voltage; and

repeating one or more times the steps of:

applying a first AC voltage between the gate electrode and the source and drain to raise the temperature of the target analyte molecule to a first temperature;

terminating the application of voltage between the gate electrode and the source and drain and allowing the temperature of the target analyte molecule to relax to a second temperature; and

applying a second AC voltage between the gate electrode and the source and drain to raise the temperature of the target analyte molecule to a third temperature.

18

40. The method of claim 39, wherein the solution further comprises components and reagents necessary for performing PCR on the single target analyte molecule.

41. The method of claim 39, further comprising the step of providing a second solution to the field effect sensor before the repeating steps, wherein the second solution comprises components and reagents necessary for performing PCR on the single target analyte molecule.

42. The method of claim 39, further comprising the step of monitoring an electric parameter of the field effect sensor to determine a quantity increase of the target analyte molecule achieved during the repeating steps.

43. The method of claim 39, further comprising the step of monitoring an electric parameter of the field effect sensor to determine a change in pH achieved during the repeating steps.

44. The method of claim 39, further comprising a step of applying a third AC or DC voltage between the electrostatic lens electrode and the gate electrode after raising the temperature of the target analyte molecule to the third temperature.

45. The method of claim 44, wherein the step of applying a third AC or DC voltage is repeated one or more times.

46. The method of claim 39, further comprising a step of measuring the impedance of the solution, thereby detecting amplification of the target molecule.

47. A method for amplification of a target molecule comprising the steps of:

providing a chemical sensor device of claim 1;

providing a solution containing at least one target molecule to a surface of the chemical sensor device;

providing a preselected AC voltage between the gate and the source and drain to heat the chemical sensor device to a preselected temperature; and

transporting the at least one target molecule across the surface of the chemical sensor device.

48. The method of claim 47, wherein the transporting step includes moving the at least one target molecule through a spatial temperature gradient including the temperatures necessary for performing PCR on the at least one target molecule.

49. The method of claim 48, wherein the spatial temperature gradient includes a denaturation temperature, an extension temperature and an annealing temperature.

50. The method of claim 47, wherein the transporting includes passive transport of the at least one target molecule.

51. The method of claim 50, wherein the passive transport is diffusion of the at least one target molecule.

52. The method of claim 47, wherein the transporting includes active transport of the at least one target molecule.

53. The method of claim 52, wherein the active transport is by dielectrophoresis of the at least one target molecule.

54. The method of claim 47, wherein the transporting is by electro-thermal movement of the at least one target molecule.

55. An array of chemical sensors comprising one or more chemical sensor devices of claim 1.

56. The array of chemical sensors of claim 55, wherein each element of the array is independently electrically addressable.

57. A method for sensing a structure containing nucleic acids, the method comprising the steps of:

providing a chemical sensor device of claim 1;

providing a solution having one or more nucleic acid containing structures to the chemical sensor device; and

monitoring an electrical parameter of the chemical sensor device to detect when a nucleic acid containing structure is loaded into the chemical sensor device.

19

58. The method of claim 57, wherein the nucleic acid containing structure is selected from the group consisting of cells, mammalian cells, plant cells, bacterial cells, spores and viruses.

59. The method of claim 57, further comprising the step of providing a first DC voltage between the electrostatic lens electrode and the gate electrode of the chemical sensor device element to attract and/or trap the nucleic acid containing structure.

60. The method of claim 59, further comprising the step of providing a second DC voltage between the electrostatic lens electrode and the gate electrode of the chemical sensor device element to repel additional nucleic acid containing structures.

61. The method of claim 57, further comprising the step of heating the nucleic acid containing structure to lyse the structure and release the nucleic acid into the chemical sensor device.

62. The method of claim 61, wherein the heating step comprises providing an AC voltage between the gate electrode and the source and drain of the chemical sensor device.

63. The method of claim 61, wherein heating comprises heating the nucleic acid containing structure to a temperature selected from about 95 and 100° C. for 5 minutes.

64. The method of claim 61, further comprising the step of amplifying the released nucleic acids.

65. The method of claim 64, wherein amplification of the released nucleic acids is achieved by PCR.

66. The method of claim 64, wherein the amplification step includes repeating one or more times the steps of:

applying a first AC voltage between the gate electrode and the source and drain of the individual chemical sensor device elements to raise the temperature of the fragments to a first temperature;

terminating the application of voltage between the gate electrode and the source and drain of the individual chemical sensor device elements and allowing the temperature of the fragments to relax to a second temperature; and

applying a second AC voltage between the gate electrode and the source and drain of the individual chemical sensor device elements to raise the temperature of the fragments to a third temperature.

67. The method of claim 61, further comprising the step of monitoring an electric parameter of the field effect sensor to determine a quantity increase of the released nucleic acids achieved during the repeating steps.

68. The method of claim 61, further comprising the step of monitoring an electric parameter of the field effect sensor to determine a change in pH achieved during the repeating steps.

69. The method of claim 61, further comprising the step of heating the chemical sensor device to bind the released nucleic acids to the sensing region of the chemical sensor device.

20

70. The method of claim 69, wherein the heating is achieved by applying a first AC voltage between the gate electrode and the source and drain of the chemical sensor device.

71. The method of claim 61, further comprising a step of providing to the chemical sensor device the necessary reagents for the synthesis of the complements to the released nucleic acids.

72. The method of claim 61, further comprising a step of monitoring an electrical parameter to sense a change in the charge present at the sensing region as complements to the released nucleic acids are synthesized.

73. The method of claim 61, further comprising a step of monitoring an electrical parameter to sense a change in the pH as complements to the plurality of fragments are synthesized.

74. The chemical sensor of claim 1, wherein the temperature controllable sensing region includes a nucleic acid denaturation temperature range, a nucleic acid extension temperature range and a denatured and extended nucleic acid annealing temperature range, and wherein:

the nucleic acid denaturation temperature range is from 90° C. to 100° C.;

the nucleic acid extension temperature range is from 68° C. to 75° C.; and

the denatured and extended nucleic acid annealing temperature range is from 68° C. to 75° C.

75. The chemical sensor of claim 1, wherein the temperature controllable sensing region comprises a thermal gradient in the sensing region.

76. The chemical sensor of claim 75, wherein the thermal gradient has a local temperature minimum in a center position of the sensing region.

77. The chemical sensor of claim 1, wherein the temperature controllable sensing region comprises a time varying temperature profile having a nucleic acid denaturation temperature, extension temperature, and an annealing temperature at non-overlapping time points.

78. The chemical sensor of claim 1, wherein the first passivation layer is a continuous layer that separates the semiconductor field effect transistor from the electrostatic lens electrode and that covers the sensing region, wherein the first passivation layer has a reduced thickness over the sensing region that is less than a thickness of the first passivation layer between the field effect transistor and the electrostatic lens electrode.

79. The chemical sensor of claim 78, wherein the reduced thickness of the first passivation layer that covers the sensing region is selected from 0.001 μm and 0.1 μm .

80. The chemical sensor of claim 2, wherein the DC voltage controller has reversible polarity to attract an analyte at a first potential difference and to repel an analyte at a second potential difference having a polarity that is opposite to a polarity of the first potential difference.

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