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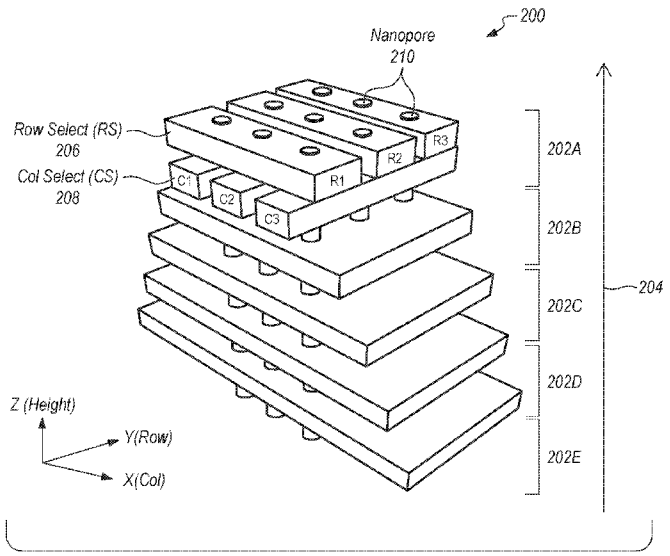

FIG. 2A

(57) Abstract: A 3D nanopore device for characterizing biopolymer molecules includes a first selecting layer having a first axis of selection. The device also includes a second selecting layer disposed adjacent the first selecting layer and having a second axis of selection orthogonal to the first axis of selection. The device further includes an third electrode layer disposed adjacent the second selecting layer, such that the first selecting layer, the second selecting layer, and the third electrode layer form a stack of layers along a Z axis and define a plurality of nanopore pillars.

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NANOPORE DEVICE AND METHOD OF MANUFACTURING SAME

FIELD

[0001] The present disclosure relates generally to systems, devices, and processes for characterizing biopolymer molecules, and methods of manufacturing such systems and devices.

BACKGROUND

[0002] Nucleic acid (e.g., DNA, RNA, etc.) sequencing is one of the most powerful methods to identify genetic variations at the molecular level. Many signatures of genetic diseases can be diagnosed by information collected through genome-wide single nucleotide polymorphisms ("SNPs") analysis, gene fusion, genomic insertion and deletion, etc. These techniques and other molecular biology techniques require nucleic acid sequencing at some point. Current technologies to sequence nucleic acids at the single molecule level include a nanopore sequencing technology that has advantages over previous sequencing techniques because nanopore sequencing technology has the characteristics of a label-free and amplification-free technique that also has improved read lengths, and improved system throughput. Accordingly, nanopore sequencing technology has been incorporated into high-quality gene sequencing applications.

[0003] Early experimental systems for nanopore based DNA sequencing detected electrical behavior of ssDNA passing through an α-hemolysin (αHL) protein nanopore. Since then, nanopore based nucleic acid sequencing technology has been improved. For instance, solid-state nanopore based nucleic acid sequencing replaces biological / protein based nanopores with solid state (e.g., semiconductor, metallic gates) nanopores, as described below.

[0004] A nanopore is a small hole (e.g., with a diameter of about 1 nm to about 1000 nm) that can detect the flow of electrons through the hole by the change in the ionic current and/or tunneling current. Because each nucleotide of a nucleic acid (e.g., adenine, cytosine, guanine, thymine in DNA, uracil in RNA) affects the electric current density across the nanopore in a specific

manner as it physically passes through the nanopore, measuring changes in the current flowing through a nanopore during translocation results in data that can be used to directly sequence a nucleic acid molecule passing through the nanopore. As such, Nanopore technology is based on electrical sensing, which is capable of detecting nucleic acid molecules in concentrations and volumes much smaller than that required for other conventional sequencing methods. Advantages of nanopore based nucleic acid sequencing include long read length, plug and play capability, and scalability. However, current biological nanopore based nucleic acid sequencing techniques can require a fixed nanopore opening (e.g., with a diameter of about 2 nm), have poor sensitivity (i.e., unacceptable amount of false negatives), high cost that renders production worthy manufacturing a challenge, and strong temperature and concentration (e.g., pH) dependency.

[0005] With advancements in semiconductor manufacturing technologies, solid-state nanopores have become an inexpensive and superior alternative to biological nanopores partly due to the superior mechanical, chemical and thermal characteristics, and compatibility with semiconductor technology allowing the integration with other sensing circuitry and nanodevices. However, current nanopore DNA sequencing techniques (e.g., involving biological and/or solid-state nanopores) continue to suffer from various limitations, including low sensitivity and high manufacturing cost. Fig. 1 schematically depicts a state-of-art solid-state based 2-dimensional ("2D") nanopore sequencing device 100. While, the device 100 is referred to as "two dimensional," the device 100 has some thickness along the Z axis.

[0006] Many of the limitations of nanopore DNA sequencing techniques result from the intrinsic nature of nanopore devices and techniques that must overcome the fast translocation speed and small size (e.g., height of about 0.34 nm and diameter of about 1 nm) of a single nucleotide. Conventional electronic instrumentation (e.g., nano-electrodes) cannot resolve such fast moving and small nucleotides using conventional nanopore based DNA sequencing techniques. Also high manufacturing cost prevents wider applications of nanopore based DNA sequencing.

[0007] Many efforts have been made to overcome these drawbacks, including the use of many different types of biological, solid-state and hybrid

(biological and solid-state) nanopores and nanopore sensors. However, none of these efforts has been successful in mass production.

[0008] There is a need for nanopore based sequencing systems and devices that address the shortcomings of currently-available configurations. In particular, there is a need for nanopore based sequencing systems and devices with acceptable sensitivity and manufacturing cost.

SUMMARY

[0009] Embodiments described herein are directed to nanopore based sequencing systems and methods of manufacturing same. In particular, the embodiments are directed to 3D nanopore based sequencing systems and methods of manufacturing same.

[0010] In one embodiment, a 3D nanopore device for characterizing biopolymer molecules includes a first selecting layer having a first axis of selection. The device also includes a second selecting layer disposed adjacent the first selecting layer and having a second axis of selection orthogonal to the first axis of selection. The device further includes an third electrode layer disposed adjacent the second selecting layer, such that the first selecting layer, the second selecting layer, and the third electrode layer form a stack of layers along a Z axis and define a plurality of nanopore pillars.

[0011] In one or more embodiments, the first selecting layer includes a first plurality of inhibitory electrodes. The second selecting layer may include a second plurality of inhibitory electrodes. The first and second pluralities of inhibitory electrodes may form an array partially defining the plurality of nanopore pillars therein. The third electrode layer may include an electrode configured to modulate an electrical bias and to detect a current modulation. The device of may also include one or more electrode layers disposed adjacent the third electrode layer.

[0012] In one or more embodiments, the device also includes a top chamber disposed adjacent the first selecting layer. The device further includes a bottom chamber disposed adjacent a bottom electrode layer, such that the plurality of nanopore pillars fluidly couple the top and bottom chambers and provide a translocation channel when multi-electrodes are

present in the device. The device may also include an electrolyte solution in the top and bottom chambers and surrounding the first selecting layer, the second selecting layer, and the third electrode layer. The electrolyte solution may include KCl or $LiCl_2$.

[0013] In one or more embodiments, the third electrode layer includes a metal rate-control electrode. The third electrode layer may include metals, such as Ta, Al, Cr, Au-Cr, Ti, Graphene, or Al-Cu. The third electrode may include highly doped (either n+ or p+ type) polysilicon or salicided polysilicon. The third electrode layer may have a thickness from 0.2 nm to 1000 nm. The third electrode layer may include a sensing electrode. The sensing electrode may operate by ion blockade, tunneling, capacitive sensing, piezoelectric, or microwave-sensing.

[0014] In one or more embodiments, the device also includes an inner membrane layer configured to modify an inner diameter of the plurality of nanopores. The inner membrane layer may include low stress silicon rich nitrides, such as Si_3N_4 and is coated with dielectrics, such as Al_2O_3, SiO_2, ZnO, or HfO_2. The inner membrane layer may have a thickness of about 10 nm to about 50 nm. Each of the plurality of nanopores may have respective diameters from about 0.2 nm to about 1000 nm. The device may also include a top membrane layer. The top membrane layer may include Si_3N_4, Al_2O_3, SiO_2, 2D dielectrics (e.g., MoS2 or hBN), and polymer membranes (e.g., polyimide and PDMS). The top membrane layer may have a thickness of about 5 nm to about 50 nm.

[0015] In another embodiment, a method of manufacturing a 3D nanopore device includes depositing a first Si_3N_4 layer on the first Si substrate or the first dielectric base layer. The method includes depositing a first dielectric layer on the first Si_3N_4 layer. The method also includes depositing a first metal or polysilicon layer on the first dielectric layer. In one or more embodiments, the method also includes etching and patterning the first metal or polysilicon electrode layer. The method also includes depositing a second dielectric layer on the patterned first metal or polysilicon electrode layer.

[0016] The method also includes depositing a second metal or polysilicon layer on the first metal or polysilicon electrode layer. The method further includes depositing a second Si_3N_4 layer on a second dielectric layer. In one

or more embodiments, the method also includes etching and patterning the second metal or polysilicon electrode layer. The method further includes depositing and patterning the multiple layers of metal or polysilicon electrode layers.

[0017] The method includes etching the first Si or dielectric substrate base layer from the backside to create a channel from the backside.

[0018] The method includes a patterning the nanopore channel from the surface on the multiple stack of Si_3N_4 layers, dielectric layer, and metal or polysilicon layers to form a nanopore therethrough. The method may also include disposing each metal or polysilicon electrodes in every channel and electrically coupling the metal or polysilicon electrode layers.

[0019] In one or more embodiments, the method also includes etching a second channel into the bottom Si_3N_4 layer, where the first and second channels are orthogonal to each other. The method may also include disposing a second inhibitory electrode in the second channel and electrically coupling the second inhibitory electrode to the bottom Si_3N_4 layer. The method may also include depositing a third dielectric base layer on the second metal layer, and etching the third dielectric base layer to form the nanopore therethrough. The method may also include etching the third dielectric layer, and electrically coupling a third electrode to the third dielectric layer. The method may also include etching a substrate, and fluidly coupling a bottom chamber to the plurality of nanopore pillars.

[0020] In one or more embodiments, the method also includes disposing the first dielectric base layer, the first Si_3N_4 layer, and the first metal in a middle chamber between top and bottom chambers, where the top, middle, and bottom chambers contain an electrolyte solution, such that the top and bottom chambers are fluidly coupled by the nanopore. Deposition of the first Si_3N_4 layer, the first metal layer, and the dielectric coating layer may use ALD or CVD. Etching the first dielectric base layer, the first Si_3N_4 layer, and the first metal layer to form the nanopore may use high aspect ratio etching.

[0021] In still another embodiment, a method of detecting a charged particle uses a 3D nanopore device having top, middle and bottom chambers, and a 3D nanopore array disposed in the middle chambers such that the top and bottom chambers are fluidly coupled by a plurality of nanopores in the 3D

nanopore array. The method includes adding electrolyte solution including the charged particle to the top, middle, and bottom chambers. The method also includes placing top and bottom electrodes in the top and bottom chambers respectively. The method further includes applying an electrophoretic bias between the top and bottom electrodes. Moreover, the method includes applying first and second selection biases to first and second selection electrodes in the 3D nanopore device to select one or more nanopores of the plurality of nanopores through which the charged particle will be directed. In addition, the method includes applying a rate control bias to a rate control electrode in the 3D nanopore device to modulate a translocation rate of the charged particle through the one or more nanopores. The method also includes applying a sensing bias to a sensing electrode in the 3D nanopore device. The method further includes detecting a change in a current in the sensing electrode.

[0022] In one or more embodiments, the current is an electrode current or a tunneling current.

[0023] In yet another embodiment, a method of manufacturing a sensor including a 3D nanopore channel pillar array, a plurality of electrodes, a top chamber, and a bottom chamber, includes placing the 3D nanopore channel pillar array in an electrolyte solution including biomolecules and DNA. The method also includes placing an electrode in the electrolyte. The method further includes applying a bias to the electrode in the electrolyte. Moreover, the method includes placing cross patterned column and row inhibitory electrodes surrounding nanopore pillars on top of the 3D nanopore channel pillar array. In addition, the method includes placing metal plane electrodes surrounding the nanopore pillars in the 3D nanopore channel pillar array, the metal plane electrodes including a rate-control electrode and a sensing electrode. The method also includes applying a rate-control bias in the rate-control electrode. The method further includes applying a sensing bias in the sensing electrode. Moreover, the method includes detecting a change in an electrode current in the electrolyte. In addition, the method includes detecting a change in a tunneling current in the electrodes.

[0024] In one or more embodiments, the rate-control electrode has a thickness ranges from about 2 nm to about 1000 nm. The rate-control

electrode may include Ta, Cr, Al, Au-Cr, Graphene, or Al-Cu. It may include heavily doped (n- or p-type) polysilicon or salicided polysilicon . The 3D nanopore channel pillar array may include a biological layer having the rate-control electrode, such that the 3D nanopore channel pillar array is a hybrid. The top and bottom chambers may contain at least some of the electrolyte solution. The electrolyte solution may include KCl and $LiCl_2$. The electrodes for top and bottom chamber may include $Ag/AgCl_2$. The cross patterned column and row inhibitory electrodes may enable array operation by selecting and deselecting the column and row by applying an inhibitory bias to stop ionic current flow vertically. The sensing electrode may utilize ion blockade sensing, tunneling sensing, capacitive sensing, piezoelectric sensing, and/or wave-sensing.

[0025] In one or more embodiments, the 3D nanopore channel pillar array includes a plurality of dielectric-electrodes in a dielectric-electrode stack. The dielectric-electrode stack includes a membrane layer, a dielectric layer to modify a nanopore channel opening width, an array of nanopore channel pillars, a stack of rate-control dielectric-electrode layers, a stack of sensing dielectric-electrode layers, and a source select dielectric-electrode layer. The membrane layer may include a dielectric material and have a thickness from about 10 nm to about 50 nm. The dielectric material may be Si_3N_4, Al_2O_3, or SiO_2. The membrane layer may modify the nanopore channel opening width. The nanopore channel opening width may be from about 2 nm to about 100 nm patterned by standard optical lithography and ion beam (e.g., FIB, TEM) techniques.

[0026] In one or more embodiments, the 3D nanopore channel pillar array is manufactured using ALD and/or CVD deposition of dielectric layers, High aspect ratio Reactive Ion Etch deep trench process (nanopore channel opening etch), ALD and/or CVD deposition of trimming dielectric layers, and/or ALD and/or CVD deposition of membrane dielectric layers.

[0027] In one or more embodiments, the dielectric-electrode stack also includes a bottom dielectric layer. The bottom dielectric layer may have a thickness of about 100 nm to 1000 nm. The bottom dielectric layer may include SiO_2, glass, or SOI to reduce substrate coupled low level noise. The dielectric-electrode stack may also include a top dielectric layer. The top

dielectric layer may have a thickness of about 5 nm to about 50 nm. The top dielectric layer may include SiO_2, Si_3N_4, or Al_2O_3. The top dielectric layer may determine a final nanopore channel opening width.

[0028] In one or more embodiments, the method also includes forming a nanopore channel pillar using high aspect ratio etching to provide a sharp shape to a trench profile of the nanopore channel pillar. The high aspect ratio etching may have an aspect ratio of greater than 5.

[0029] In one or more embodiments, the 3D nanopore channel pillar array facilitates multiplex sequencing applications using high density low cost nanopore channels. A number of electrodes in the 3D nanopore array may be selected depending on the required sequencing applications to provide a Time of Flight ("TOF") technique to control a translocation speed by controlled biasing. Controlling the translocation speed may improve reading of DNA molecules and improves a sensitivity of the sensor.

[0030] In one or more embodiments, the 3D nanopore channel pillar array is integrated in a CMOS flow, thereby facilitating embedded biosensor solutions for CMOS technology. The CMOS flow may include a 2-dim well for electrochemical reactions. The CMOS flow may include an ion-sensitive filed effect transistor technology.

[0031] In one or more embodiments, the 3D nanopore channel pillar array incorporates a hybrid type of nanopore technology including a biological component and a solid-state component in a 3D configuration. The 3D nanopore channel pillar array may facilitate an electro-chemical, thermal, or electro-optical reaction to take place in an enlarged, separated nanopore well with a multi-electrode system to enhance electrochemical and/or sequencing reactions. The 3D nanopore channel pillar array may facilitate multiplex sequencing using a multi-array configuration where individual nanopore channel pillars are addressable. The 3D nanopore channel pillar array may facilitate standard qPCR within a nanopore channel pillar. The 3D nanopore channel pillar array may facilitate probe-mediated targeted sequencing. The 3D nanopore channel pillar array may facilitate tuning of a nanopore channel opening width for different applications.

[0032] In one or more embodiments, the nanopore channel opening width is tunable from about 1 nm to about 100 nm. The nanopore channel opening width may be electronically tunable during manufacturing.

[0033] In one or more embodiments, the method also includes forming a hybrid nanopore channel to enhance stability of the sensor. Forming the hybrid nanopore may include inserting a stable biological component to construct a semi-synthetic membrane porin. The stable biological component may be an αHL molecule. The αHL molecule may be inserted into a SiN based 3D nanopore.

[0034] In one or more embodiments, the method also includes using a top inhibitory electrode to induce a structure in the stable biological component to ensure alignment of the stable biological component and hybrid nanopore.

[0035] The aforementioned and other embodiments of the disclosure are described in the Detailed Description which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The foregoing and other embodiments are described in further detail with reference to the accompanying drawings, in which the same elements in different figures are referred to by common reference numerals, wherein:

[0037] Figure 1 schematically illustrates a prior art solid-state 2D nanopore device;

[0038] Figures 2A-2D schematically illustrate a 3D nanopore device according to one embodiment from perspective, top, front, and right views, respectively.

[0039] Figure 3 schematically illustrates a 3D nanopore device according to one embodiment including some details of its operation.

[0040] Figure 4 is a table summarizing the voltage operation of the nanopore device depicted in Figure 3.

[0041] Figure 5 schematically illustrates a 3D nanopore device according to one embodiment including some of the electrodes therein.

[0042] Figures 6A-6E illustrate a method for manufacturing a 3D nanopore device according to one embodiment.

[0043] Figures 7A-7E illustrate a method for manufacturing a 3D nanopore device according to another embodiment.

[0044] In order to better appreciate how to obtain the above-recited and other advantages and objects of various embodiments, a more detailed description of embodiments is provided with reference to the accompanying drawings. It should be noted that the drawings are not drawn to scale and that elements of similar structures or functions are represented by like reference numerals throughout. It will be understood that these drawings depict only certain illustrated embodiments and are not therefore to be considered limiting of scope of embodiments.

DETAILED DESCRIPTION OF ILLUSTRATED EMBODIMENTS

Exemplary Nanopore Devices

[0045] As described above, current state-of-art nanopore devices are limited at least in terms of sensitivity and manufacturing cost. The nanopore device embodiments described herein address, *inter alia*, these limitations of current nanopore devices.

[0046] Figure 2A-2D schematically depict various views of a nanopore device 200 incorporating solid-state nanopore technology with a three dimensional ("3D") array architecture according to one embodiment. As shown in Figure 2A, the device 200 includes a plurality of 2D arrays or layers 202A-202E stacked along a Z axis 204. While the 2D arrays 202A-202E are referred to as "two dimensional," each of the 2D arrays 202A-202E has some thickness along the Z axis. Figure 2B depicts a top view of the top 2D array 202A depicted in Figure 2A. Figures 2C and 2D schematically depict front and right side views of the nanopore device 200 depicted in Figure 2A.

[0047] The top 2D array 202A includes first and second selecting (inhibitory electrode) layers 206, 208 configured to direct movement of charged particles (e.g., biopolymers) through the nanopores 210 (pillars) formed in the first and second selecting layers 206, 208. The first selecting layer 206 is configured to select from a plurality of rows (R1-R3) in the 2D array 202A. The second selecting layer 208 is configured to select from a plurality of columns (C1-C3) in the 2D array 202A. In one embodiment, the

first and second selecting layers 206, 208 select from the rows and columns, respectively, by modifying a charge adjacent the selected row and column and/or adjacent to the non-selected rows and columns. The other 2D arrays 202B-202E include rate control/current sensing electrodes. Rate control electrodes may be made of highly conductive metals, such as Au-Cr, TiN, TaN, Pt, Cr, Graphene, Al-Cu, etc. The rate control electrodes may have a thickness of about 2 to about 1000 nm. Rate control electrodes may also be made in the biological layer in hybrid nanopores.

[0048] Hybrid nanopores include a stable biological/biochemical component with solid state components to form a semi-synthetic membrane porin to enhance stability of the nanopore. For instance, the biological component may be an αHL molecule. The αHL molecule may be inserted into a SiN based 3D nanopore. The αHL molecule may be induced to take on a structure to ensure alignment of the αHL molecule with the SiN based 3D nanopore by apply a bias to an electrode (e.g., in the top 2D array 202A).

[0049] The nanopore device 200 has a 3D vertical pillar stack array structure that provides a much larger surface area for charge detection than that of a conventional nanopore device having a planar structure. As a charged particle (e.g., biopolymer) passes through each 2D array 202A-202E in the device, its charge can be detected with a detector (e.g., electrode) in some of the 2D arrays 202B-202E. Therefore, the 3D array structure of the device 200 facilitates higher sensitivity, which can compensate for a low signal detector/electrode. Further, the highly integrated small form factor 3D structure provides a high density nanopore array while minimizing manufacturing cost.

[0050] In use, the nanopore device 200 is disposed in a middle chamber separating top and bottom chambers (not shown) such that the top and bottom chambers are fluidly coupled by the nanopore pillars 210. The top, middle, and bottom chambers include an electrolyte solution (e.g., Ag, $AgCl_2$, etc.) containing the charged particles (e.g., DNA) to be detected. Different electrolyte solutions can be used for the detection of different charged particles.

[0051] Electrophoretic charged particle translocation can be driven by applying a bias to electrodes disposed in a top chamber (not shown) adjacent

the top 2D array 202A of the nanopore device 200 and a bottom chamber (not shown) adjacent the bottom 2D array 202E of the nanopore device 200. In some embodiments, the nanopore device 200 is disposed in a middle chamber (not shown) such that the top and bottom chambers are fluidly and electrically coupled by the nanopore pillars 210 in the nanopore device 200. The top, middle, and bottom chambers may contain the electrolyte solution.

[0052] Figure 3 schematically depicts a nanopore device 300 according to another embodiment. Figure 3 depicts the top 2D array 302 in a cross-sectional (x-z plane) view showing the 3D nanopore 310 and nano-electrode schemes. Each nanopore 310 is surrounded by nano-electrodes 312, allowing the nanopore 310 channel to operate under an electric bias field condition generated using the nano-electrodes 312. Cross-patterned nanogap nano-electrodes 312CS-312Cn, 312RS-312Rn are disposed in two layers on top of the nanopore device 300. These nano-electrodes 312CS-312Cn, 312RS-312Rn are column and row inhibitory nano-electrodes 312CS-312Cn, 312RS-312Rn for the nanopore array, respectively. The cross-patterned nano-electrodes 312CS-312Cn, 312RS-312Rn as shown in the top 2D array 302 (x-y plane view) may be formed/patterned at the metal lithography steps. Nano-electrodes 312 in the remaining 2D arrays in the 3D stack may be formed by plane depositing metals. The nanopore 310 hole pillars are surrounded by the metal nano-electrodes 312CS-312Cn, 312RS-312Rn, and thus may operate under the full influence of the electrical bias applied to the multiple stacked nano-electrodes 312.

[0053] By applying an inhibitory electrical bias (0V-VCC) to select nanogap nano-electrodes 312CS-312Cn, 312RS-312Rn in the top 2D array 302, biomolecular translocation (e.g., electrophoretic) through one or more nanopores 302 in the top 2D nanopore array 302 can be inhibited to control nanopore array operation according to one embodiment. The electrical bias applied to the nano-electrodes 312CS-312Cn, 312RS-312Rn can generate an electric field sufficient to suppress ionic translocation of charged particles (e.g., nucleic acids) from a top chamber (not shown) to a bottom chamber (not shown) in a direction orthogonal to the nano-electrodes 312CS-312Cn, 312RS-312Rn. Nano-electrode 312 mediated ionic translocation suppression can be substantially complete or the electrical bias can be modulated to only

reduce the rate of ionic translocation. In one embodiment, after one or more nanopores 310 are selected (e.g., for DNA biomolecules translocation and sequencing), the electrical biases in a stack of 3D nanopore nano-electrodes 312 can be modulated to control the biomolecular translocation speed. In one embodiment, the inhibitory electrical bias reduces/stops ionic current flow in the vertical direction to thereby select and/or deselect various columns and rows defined by the nanogap nano-electrodes 312CS-312Cn, 312RS-312Rn . At the same time, the nano-electrodes 312 can detect current modulations resulting from passage of charged particles (e.g., DNA biomolecules) through the 3D vertical nanopore 310 pillars. In some embodiments, the nano-electrodes 312 can detect current modulations using a variety of principles, including ion blockade, tunneling, capacitive sensing, piezoelectric, and microwave-sensing.

[0054] Figure 4 is a table 400 illustrating the voltage operation of the nanopore device 300 depicted in Figure 3. As shown in Figure 4, the nanopore device 300 can be operated in both translocation and read (sense) mode by modulating the voltage applied to various electrodes 312.

[0055] Figure 5 schematically illustrates a single 3D nanopore sensor 520 in a nanopore device according to one embodiment. The sensor 520 has a column inhibitory electrode layer 522, a row inhibitory electrode layer 524, and a plurality of rate control/sensing electrode layers 526, 528, 530. These layers are stacked on top of each other and separated by an insulator layer 532 (e.g., SiO_2) to define a vertical nanopore 510 hole pillar. Each layer may have a polysilicon or metal (e.g., Ta, Al, Cr, Au-Cr, Ni, Graphene, etc.) top sub-layer and various other sub-layers (e.g., Al_2O_3, Si_3N_4, n^+ or p^+ polysilicon, etc.) The 3D nanopore sensor 520 can operate on a variety of principles, including ion blockade, tunneling, capacitive sensing, piezoelectric, and microwave-sensing. Rate control/sensing electrode layers 526, 528, 530 can be activated by applying a rate control or a sensing bias to the respective electrode layers 526, 528, 530. The sensing electrode layers 526, 528, 530 can detect a change in an electrical characteristic (e.g., an electrode current and/or a tunneling current).

[0056] 3D nanopore devices (e.g., 200, 300) allow either direct or targeted sequencing in an array while minimizing form-factor overhead, because the

2D arrays 202, 302 in the nanopore devices 200, 300 can be stacked vertically instead of positioned horizontally, thereby allowing for high density applications. Further, 3D nanopore devices (e.g., 200, 300) are scalable, with medium to large 3D nanopore devices having more than 1,000 nanopore 210, 310 pillars. Consequently, a larger number of sequencing sensors can be accommodated within the same form-factor. 3D nanopore devices (e.g., 200, 300) can also incorporate biological nanopore or hybrid nanopore technologies to provide more architectural flexibility to accommodate a user's needs.

[0057] In 3D nanopore devices (e.g., 300), each nanopore 310 pillar is composed of a stack of nano-electrodes 312 defining a plurality of nanopores 310. As such, the effective surface area of the sensors in each nanopore 310 column can be orders of magnitude greater than the surface area of a single sensor. In one embodiment, the effective sensor surface area can be 2-3 orders of magnitude greater than the surface area of a single sensor. This increase in effective sensor surface area can significantly improve the sensor signal to noise ratio and sensitivity, while minimizing manufacturing costs.

Exemplary Nanopore Device Manufacturing Methods

[0058] 3D nanopore devices (e.g., 200, 300) can be manufactured utilizing many different methods. In one embodiment, a semiconductor technology (e.g., CMOS process, described below) is used to manufacture 3D nanopore devices 200, 300. The CMOS process also allows nanopore 310 width to be tunable using a large nanopore array. In one embodiment, nanopore 310 width can be controlled during manufacturing using software with a look up table, allowing for mass production manufacturing. Using a CMOS process can embed biosensor solutions in CMOS technology. In various embodiments, the CMOS process includes a 2-dim well for electrochemical reactions and/or an ion-sensitive filed effect transistor technology. Microfluidic channels can be integrated into the 3D nanopore devices 200, 300 (e.g., within a die), therefore reducing the cost of the devices 200, 300.

[0059] Figures 6A-6E illustrate a method 600 of manufacturing a nanopore device according to one embodiment. As shown in Figure 6A, a first dielectric base layer (e.g., SiO_2, Al_2O_3, etc.) 602A, a first base layer of Si_3N_4 604A and a first layer of a metal (e.g., Au-Cr, Al, Graphene, etc.) or polysilicon layer 607A

are deposited on top of each other. Then second and third layers of dielectric base, base, and metal 602B, 604B, 607B, 602C, 604C, 607C are deposited on top of each other and the previous layers. For instance, these deposition steps can be performed using chemical vapor deposition ("CVD") and/or Atomic Layer Deposition ("ALD") of base dielectric layers 602, trimming dielectric layers, and/or membrane dielectric layers (see Figure 6C below). The first dielectric base layer 602A may have a thickness of about 100 nm to 1000 nm to reduce substrate coupled low level noise.

[0060] As shown in Figure 6B, a nanopore 610 is then etched into the deposited layers (e.g., a using high aspect ratio (above 5) nanopore hole trench etching process). The high aspect ratio etching can provide a sharp shape to a trench profile of the nanopore 610 channel pillar. Total depth of the nanopore pillar can be few hundred nanometers to several microns depending on the applications.

[0061] Next, as shown in Figure 6C, thin layers of a dielectric coating 612 (e.g., Si_3N_4, Al_2O_3, SiO_2, etc.) are deposited (e.g., by atomic layer deposition "ALD") on the inner surface of the nanopore 610 to determine the width of the nanopore 610. The dielectric coating 612 may vary in thickness (e.g., from about 10 nm to about 50 nm). By controlling the amount of dielectric coating 612 deposited on the inner surface of the nanopore 610 (e.g., using ALD) target nanopore 610 widths of about 2 nm to about 100 nm can be achieved. Accordingly, the width/diameter of the nanopore/trench 610 can be controlled using ALD of a dielectric coating 612 to suit a variety of applications. A top dielectric coating 612 may have a thickness of about 5 nm to about 20 nm. Depending on the applications and required nanopore 610 opening dimensions, various lithography techniques (e.g., those used in the mass volume production) can be used to etch the nanopore 610 opening. In addition, the depth of the nanopore 610 channel can be selected for the required sensitivity and accuracy with ease using the manufacturing methods described herein.

[0062] As shown in Figure 6D, the vertical nanopore channel and the stacked layers are etched (see "steps" on right side of stacked layers) to form the horizontal (X axis) and vertical (Y axis) nanopore channels to provide access for electrodes 614 (e.g., row and column inhibitory electrodes) on top

and addressing circuits. The base Si_3N_4 604A (or Al_2O_3) layers are selectively wet etched to provide electrical access to all of the horizontal electrodes. Finally, the remaining space is filled with metal.

[0063] Figure 6E depicts the manufactured 3D nanopore device in use for sequencing a biopolymer (e.g., DNA).

[0064] Figures 7A-7E illustrate a method 700 of manufacturing a nanopore device according to another embodiment. The methods 600, 700 depicted in Figures 6A-6E and 7A-7E are similar and share many of the same techniques.

[0065] As shown in Figure 7A, multiple layers of dielectric films (such as SiO_2, Al_2O_3, ZnO, HfO_2, etc.; 10 nm – 100 nm) low stress nitride film (Si_3N_4) and metal (Ta, Al, Cr, Ti, Au-Cr, Graphene, etc.; few nm to 100's of nm) and inter-metal layer (SiO_2) collectively 702 are stack deposited on a Si or Quartz substrate using CVD (Low Pressure/Plasma Enhanced) or Atomic Layer Deposition (ALD). Next, a bottom chamber opening 704 (5x5~100x100 μm^2) is etched by a Deep Reactive Ion Etching (RIE) or KOH wet etching.

[0066] As shown in Figure 7B, a top metal layer 706 is deposited. Next, a top chamber opening 708 is defined using a high aspect ratio nanopore hole deep trench etching process by Reactive Ion Etching. This process can generate nanopore trench etch opening diameters from a few nm to about 100 nm. Lithography techniques using colloidal mask (e.g., Nano-dots, Quantum-dots, or Graphene oxide pores) can also be used instead of conventional tools.

[0067] As shown in Figures 7C and 7D, Thin layers of dielectrics 610 (e.g., films) also can be deposited by ALD to trim the nanopore width to achieve target nanopore widths (using ALD) from about 2 nm to about 1000 nm. Nanopore channel widths can also be controlled to have variable trench width, allowing variable nanopore width for the target diameter.

[0068] Figure 7E depicts the manufactured 3D nanopore device in use for sequencing a biopolymer (e.g., DNA).

[0069] The 3D nanopore device may facilitate multiplex sequencing applications using high density low cost nanopore channels. A number of electrodes in the 3D nanopore device may be selected depending on the required sequencing applications to provide a Time of Flight ("TOF")

technique to control the translocation speed by controlled biasing. Controlling the translocation speed may improve reading of DNA molecules and improves a sensitivity of the sensor. The 3D nanopore device may also facilitate an electro-chemical, thermal, or electro-optical reaction to take place in an enlarged, separated nanopore well with a multi-electrode system to enhance electrochemical and/or sequencing reactions. The 3D nanopore device may further facilitate multiplex sequencing using a multi-array configuration wherein individual nanopore channel pillars are addressable. Moreover, the 3D nanopore device may further facilitate standard qPCR within a nanopore channel pillar and/or probe-mediated targeted sequencing. In addition, the 3D nanopore channel opening width is tunable for different applications. In one embodiment, the nanopore channel opening width is tunable from about 1 nm to about 100 nm. The nanopore channel opening width may be electronically tunable during manufacturing. The 3D nanopore devices described herein can be used in the detection of various charged particles, including but not limited to biomolecules such as nucleotides, nucleic acids, and proteins (direct detection). The 3D nanopore devices can also be used in DNA sequencing and detection of protein-DNA interactions.

[0070] Manufacturing metal or polysilicon plane based nanopore arrays using lithographic processes (e.g., Through-Silicon Via ("TSV") fabrication) minimizes manufacturing cost and line resistance (which significantly reduces IR drop and RC delay limitations to scaling).

[0071] The corresponding structures, materials, acts and equivalents of all means or step plus function elements in the claims below are intended to include any structures, materials, acts and equivalents for performing the function in combination with other claimed elements as specifically claimed. It is to be understood that while the disclosure has been described in conjunction with the above embodiments, the foregoing description and claims are not to limit the scope of the disclosure. Other advantages and modifications within the scope to the disclosure will be apparent to those skilled in the art to which the disclosure pertains.

[0072] Various exemplary embodiments of the disclosure are described herein. Reference is made to these examples in a non-limiting sense. They are provided to illustrate more broadly applicable embodiments of the

disclosure. Various changes may be made to the embodiments described and equivalents may be substituted without departing from the true spirit and scope of the disclosure. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process act(s) or step(s) to the objective(s), spirit or scope of the present disclosure. Further, as will be appreciated by those with skill in the art that each of the individual variations described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosures. All such modifications are intended to be within the scope of claims associated with this disclosure.

[0073] Any of the devices described for carrying out the subject diagnostic or interventional procedures may be provided in packaged combination for use in executing such interventions. These supply "kits" may further include instructions for use and be packaged in sterile trays or containers as commonly employed for such purposes.

[0074] The disclosure includes methods that may be performed using the subject devices. The methods may comprise the act of providing such a suitable device. Such provision may be performed by the end user. In other words, the "providing" act merely requires the end user obtain, access, approach, position, set-up, activate, power-up or otherwise act to provide the requisite device in the subject method. Methods recited herein may be carried out in any order of the recited events which is logically possible, as well as in the recited order of events.

[0075] Exemplary embodiments of the disclosure, together with details regarding material selection and manufacture have been set forth above. Other details of the present disclosure, these may be appreciated in connection with the above-referenced patents and publications as well as generally known or appreciated by those with skill in the art. The same may hold true with respect to method-based embodiments of the disclosure in terms of additional acts as commonly or logically employed.

[0076] In addition, though the disclosure has been described in reference to several examples optionally incorporating various features, the disclosure is not to be limited to that which is described or indicated as contemplated with

respect to each variation of the disclosure. Various changes may be made to the embodiments described and equivalents (whether recited herein or not included for the sake of some brevity) may be substituted without departing from the true spirit and scope of the disclosure. In addition, where a range of values is provided, it is understood that every intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure.

[0077] Also, it is contemplated that any optional feature of the inventive variations described may be set forth and claimed independently, or in combination with any one or more of the features described herein. Reference to a singular item, includes the possibility that there are plural of the same items present. More specifically, as used herein and in claims associated hereto, the singular forms "a," "an," "said," and "the" include plural referents unless the specifically stated otherwise. In other words, use of the articles allow for "at least one" of the subject item in the description above as well as claims associated with this disclosure. It is further noted that such claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0078] Without the use of such exclusive terminology, the term "comprising" in claims associated with this disclosure shall allow for the inclusion of any additional element--irrespective of whether a given number of elements are enumerated in such claims, or the addition of a feature could be regarded as transforming the nature of an element set forth in such claims. Except as specifically defined herein, all technical and scientific terms used herein are to be given as broad a commonly understood meaning as possible while maintaining claim validity.

[0079] The breadth of the present disclosure is not to be limited to the examples provided and/or the subject specification, but rather only by the scope of claim language associated with this disclosure.

What is claimed is:

1. A method of manufacturing a 3D nanopore device, the method comprising:

depositing a first Si_3N_4 layer on a first silicon substrate or dielectric base layer;

depositing a first dielectric layer and a first metal or polysilicon layer on the first Si_3N_4 layer;

etching and patterning the first Si_3N_4 layer, the first dielectric layer, and the first metal or polysilicon layer to form a first gate electrode pattern.

2. The method of claim 1, further comprising etching a first metal or polysilicon electrode channel into the first metal or polysilicon layer.

3. The method of claim 2, further comprising disposing a first metal or polysilicon electrode in the first channel and electrically coupling the first metal or polysilicon electrode to the first gate electrode pattern.

4. The method of claim 3, further comprising:

depositing a second dielectric layer on the first metal or polysilicon layer;

depositing a second Si_3N_4 layer and a second metal layer on the second dielectric layer;

etching and patterning the second dielectric layer, the second Si_3N_4 layer, the second metal or polysilicon layer, and the first Si_3N_4 layer to form a nanopore therethrough.

5. The method of claim 4, further comprising etching a second metal or polysilicon electrode channel into the first Si_3N_4 layer, wherein the first and second metal or polysilicon electrode channels are orthogonal to each other.

6. The method of claim 5, further comprising disposing a second metal or polysilicon electrode in the second channel and electrically coupling the second metal or polysilicon electrode to the first Si_3N_4 layer.

7. The method of claim 5, further comprising etching the first silicon substrate or dielectric base layer from an opposite side relative to the first Si_3N_4 layer and fluidly coupling a bottom chamber to a plurality of nanopore pillars.

8. The method of claim 4, further comprising:

depositing a third dielectric layer on the second metal or polysilicon layer; and etching the third dielectric layer to form the nanopore therethrough.

9. The method of claim 8, further comprising:

etching the third dielectric layer; and

electrically coupling a third electrode to a third gate electrode layer.

10. The method of claim 1, further comprising disposing the first silicon substrate or dielectric base layer, the first Si_3N_4 layer, and the first metal or polysilicon in a middle chamber between top and bottom chambers,

where the top, middle, and bottom chambers contain an electrolyte solution, such that the top and bottom chambers are fluidly coupled by a nanopore.

11. The method of claim 1, further comprising depositing, after nanopore patterning, top and inner surface dielectric coatings to functionalize an inner surface of a nanopore for a biomolecular interaction, and to form a gate electrode dielectric for sensing, and to adjust a width of the nanopore.

12. The method of claim 1, wherein a stack of multiple dielectric layers, Si_3N_4 layers, and metal or polysilicon layers is etched to form a nanopore using high aspect ratio reactive ion etching.

13. A method of detecting a charged particle using a 3D nanopore device having top, middle, and bottom chambers, and a 3D nanopore array disposed in the middle chamber such that the top and bottom chambers are fluidly coupled by a plurality of nanopores in the 3D nanopore array, the method comprising:

adding electrolyte solution including the charged particle to the top, middle, and bottom chambers;

placing top and bottom electrodes in the top and bottom chambers respectively;

applying an electrophoretic bias between the top and bottom electrodes;

applying first and second selection biases to first and second selection electrodes in the 3D nanopore device to select one or more nanopores of the plurality of nanopores through which the charged particle will be directed;

applying a rate control bias to a rate control electrode in the 3D nanopore device to modulate a translocation rate of the charged particle through the one or more nanopores;

applying a sensing bias to a sensing electrode in the 3D nanopore device; and

detecting a change in a current in the sensing electrode.

14. The method of claim 13, wherein the current is an electrode current.

15. The method of claim 13, wherein the current is a tunneling current.

16. A 3D nanopore device for characterizing biopolymer molecules, comprising:

a first selecting layer having a first axis of selection;

a second selecting layer disposed adjacent the first selecting layer and having a second axis of selection orthogonal to the first axis of selection; and

an third electrode layer disposed adjacent the second selecting layer, such that the first selecting layer, the second selecting layer, and the third electrode layer form a stack of layers along a Z axis and define a plurality of nanopore pillars.

17. The device of claim 16, wherein the first selecting layer comprises a first plurality of inhibitory electrodes.

18. The device of claim 17, wherein the second selecting layer comprises a second plurality of inhibitory electrodes.

19. The device of claim 18, wherein the first and second pluralities of inhibitory electrodes form an array partially defining the plurality of nanopore pillars therein.

20. The device of claim 16, wherein the third electrode layer comprises an electrode configured to modulate an electrical bias and to detect a current modulation.

21. The device of claim 16, further comprising one or more electrode layers disposed adjacent the third electrode layer.

22. The device of claim 16, further comprising:

a top chamber disposed adjacent the first selecting layer; and

a bottom chamber disposed adjacent a bottom electrode layer, such that the plurality of nanopore pillars fluidly couple the top and bottom chambers and provide a translocation channel when multi-electrodes are present in the device.

23. The device of claim 22, further comprising

an electrolyte solution in the top and bottom chambers and surrounding the first selecting layer, the second selecting layer, and the third electrode layer; and

top and bottom chamber electrodes,

wherein the top and bottom chamber electrodes each comprise $Ag/AgCl_2$.

24. The device of claim 23, wherein the electrolyte solution comprises KCl or $LiCl_2$.

25. The device of claim 16, wherein the third electrode layer comprises a metal rate-control electrode.

26. The device of claim 25, wherein the third electrode layer comprises metals, such as Ta, Al, Cr, Ti, Au-Cr, and Al-Cu, Graphene, or n^+ or p^+ polysilicon.

27. The device of claim 25, wherein the third electrode layer has a thickness from 2 nm to 1000 nm.

28. The device of claim 16, wherein the third electrode layer comprises a sensing electrode.

29. The device of claim 28, wherein the sensing electrode operates by ion blockade, tunneling, capacitive sensing, piezoelectric, or microwave-sensing.

30. The device of claim 16, further comprising an inner membrane layer configured to modify an inner diameter of the plurality of nanopores.

31. The device of claim 30, wherein the inner membrane layer comprises low stress silicon rich nitrides, such as Si_3N_4 and is coated with dielectrics, such as Al_2O_3, SiO_2, ZnO, or HfO.

32. The device of claim 30, wherein the inner membrane layer has a thickness of about 10 nm to about 50 nm.

33. The device of claim 16, wherein each of the plurality of nanopores has respective diameters from about 2 nm to about 100 nm.

34. The device of claim 16, further comprising a top membrane layer.

35. The device of claim 34, wherein the top membrane layer comprises Si_3N_4, Al_2O_3, or SiO_2.

36. The device of claim 34, wherein the top membrane layer has a thickness of about 5 nm to about 50 nm.

37. A method of manufacturing a sensor comprising a 3D nanopore channel pillar array, a plurality of electrodes, a top chamber, and a bottom chamber, the method comprising:

placing the 3D nanopore channel pillar array in an electrolyte solution comprising biomolecules and DNA;

placing an electrode in the electrolyte;

applying a bias to the electrode in the electrolyte;

placing cross patterned column and row inhibitory electrodes surrounding nanopore pillars on top of the 3D nanopore channel pillar array;

placing metal plane electrodes surrounding the nanopore pillars in the 3D nanopore channel pillar array, the metal plane electrodes comprising a rate-control electrode and a sensing electrode;

applying a rate-control bias in the rate-control electrode;

applying a sensing bias in the sensing electrode;

detecting a change in an electrode current in the electrolyte; and

detecting a change in a tunneling current in the electrodes.

38. The method of claim 37, wherein the rate-control electrode has a thickness ranges from about 2 nm to about 1000 nm.

39. The method of claim 37, wherein the rate-control electrode comprises Ta, Al, Au-Cr, Graphene, n^+ or p^+ polysilicon, or Al-Cu.

40. The method of claim 37, the 3D nanopore channel pillar array comprising a biological layer having the rate-control electrode, such that the 3D nanopore channel pillar array is a hybrid.

41. The method of claim 37, wherein the top and bottom chambers contain at least some of the electrolyte solution.

42. The method of claim 41, wherein the electrolyte solution comprises KCl or $LiCl_2$ and the electrode comprises $Ag/AgCl_2$.

43. The method of claim 37, wherein the cross patterned column and row inhibitory electrodes enabling array operation by selecting and deselecting the column and row by applying an inhibitory bias to stop ionic current flow vertically.

44. The method of claim 37, wherein the sensing electrode utilizes a sensing method selected from the group consisting of ion blockade sensing, tunneling sensing, capacitive sensing, piezoelectric sensing, and wave-sensing.

45. The method of claim 37, wherein the 3D nanopore channel pillar array comprises a plurality of dielectric-electrodes in a dielectric-electrode stack, the dielectric-electrode stack comprising:

a membrane layer;
a dielectric layer to modify a nanopore channel opening width;
an array of nanopore channel pillars;
a stack of rate-control dielectric-electrode layers;
a stack of sensing dielectric-electrode layers; and
a source select dielectric-electrode layer.

46. The method of claim 45, wherein the membrane layer comprises a dielectric material and has a thickness from about 10 nm to about 50 nm.

47. The method of claim 46, wherein the dielectric material is Si_3N_4, Al_2O_3, or SiO_2.

48. The method of claim 45, wherein the membrane layer modifies the nanopore channel opening width.

49. The method of claim 45, wherein the nanopore channel opening width is from about 2 nm to about 1000 nm.

50. The method of claim 45, wherein the 3D nanopore channel pillar array is manufactured using a process selected from the group consisting of:

ALD or CVD deposition of dielectric layers;

High Aspect Ratio Deep Trench (nanopore channel opening etch);

ALD or CVD deposition of trimming dielectric layers; and

ALD or CVD deposition of membrane dielectric layers.

51. The method of claim 45, the dielectric-electrode stack further comprising a bottom dielectric layer.

52. The method of claim 51, wherein the bottom dielectric layer has a thickness of about 100 nm to 1000 nm.

53. The method of claim 51, wherein the bottom dielectric layer comprises SiO_2, glass, Si_3N_4, or SOI to reduce substrate coupled low level noise.

54. The method of claim 45, the dielectric-electrode stack further comprising a top dielectric layer.

55. The method of claim 54, wherein the top dielectric layer has a thickness of about 5 nm to about 50 nm.

56. The method of claim 54, wherein the top dielectric layer comprises SiO_2, Si_3N_4, or Al_2O_3.

57. The method of claim 54, wherein the top dielectric layer determines a final nanopore channel opening width.

58. The method of claim 45, further comprising forming a nanopore channel pillar using high aspect ratio etching to provide a sharp shape to a trench profile of the nanopore channel pillar.

59. The method of claim 58, wherein the high aspect ratio etching has an aspect ratio of greater than 5.

60. The method of claim 37, wherein the 3D nanopore channel pillar array facilitates multiplex sequencing applications using high density low cost nanopore channels.

61. The method of claim 37, wherein a number of electrodes in the 3D nanopore array can be selected depending on the required sequencing applications to provide a Time of Flight ("TOF") technique to control a translocation speed by controlled biasing.

62. The method of claim 61, wherein controlling the translocation speed improves reading of DNA molecules and improves a sensitivity of the sensor.

63. The method of claim 37, wherein the 3D nanopore channel pillar array is integrated in a CMOS flow, thereby facilitating embedded biosensor solutions for CMOS technology.

64. The method of claim 63, wherein the CMOS flow includes a 2-dim well for electrochemical reactions.

65. The method of claim 63, wherein the CMOS flow includes an ion-sensitive filed effect transistor technology.

66. The method of claim 37, wherein the 3D nanopore channel pillar array incorporates a hybrid type of nanopore technology comprising a biological component and a solid-state component in a 3D configuration.

67. The method of claim 37, wherein the 3D nanopore channel pillar array facilitates an electro-chemical, thermal, or electro-optical reaction to take place in an enlarged, separated nanopore well with a multi-electrode system to enhance electrochemical or sequencing reactions.

68. The method of claim 37, wherein the 3D nanopore channel pillar array facilitates multiplex sequencing using a multi-array configuration wherein individual nanopore channel pillars are addressable.

69. The method of claim 37, wherein the 3D nanopore channel pillar array facilitates standard qPCR within a nanopore channel pillar.

70. The method of claim 37, wherein the 3D nanopore channel pillar array facilitates probe-mediated targeted sequencing.

71. The method of claim 37, wherein the 3D nanopore channel pillar array facilitates tuning of a nanopore channel opening width for different applications.

72. The method of claim 71, wherein the nanopore channel opening width is tunable from about 1 nm to about 1000 nm.

73. The method of claim 71, wherein the nanopore channel opening width is electronically tunable during manufacturing.

74. The method of claim 37, further comprising forming a hybrid nanopore channel to enhance stability of the sensor.

75. This method of claim 74, wherein forming the hybrid nanopore comprises inserting a stable biological component to construct a semi-synthetic membrane porin.

76. The method of claim 75, wherein the stable biological component is an αHL molecule.

77. The method of claim 75, wherein the αHL molecule is inserted into a SiN based 3D nanopore.

78. The method of claim 75, further comprising using a top inhibitory electrode to induce a structure in the stable biological component to ensure alignment of the stable biological component and hybrid nanopore.

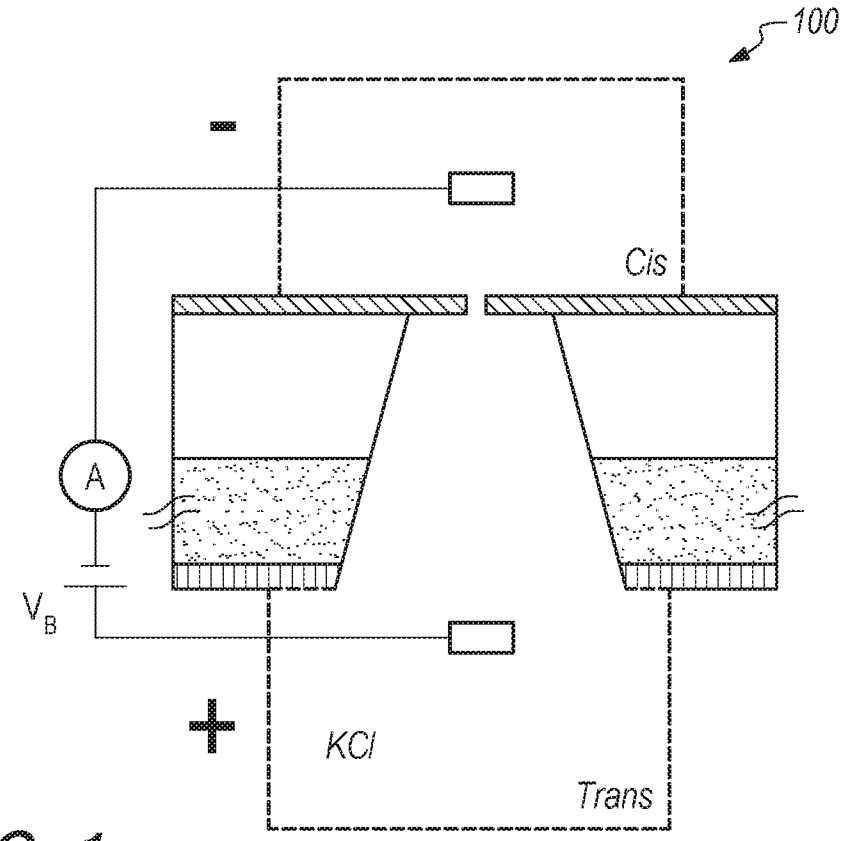


FIG. 1
(PRIOR ART)

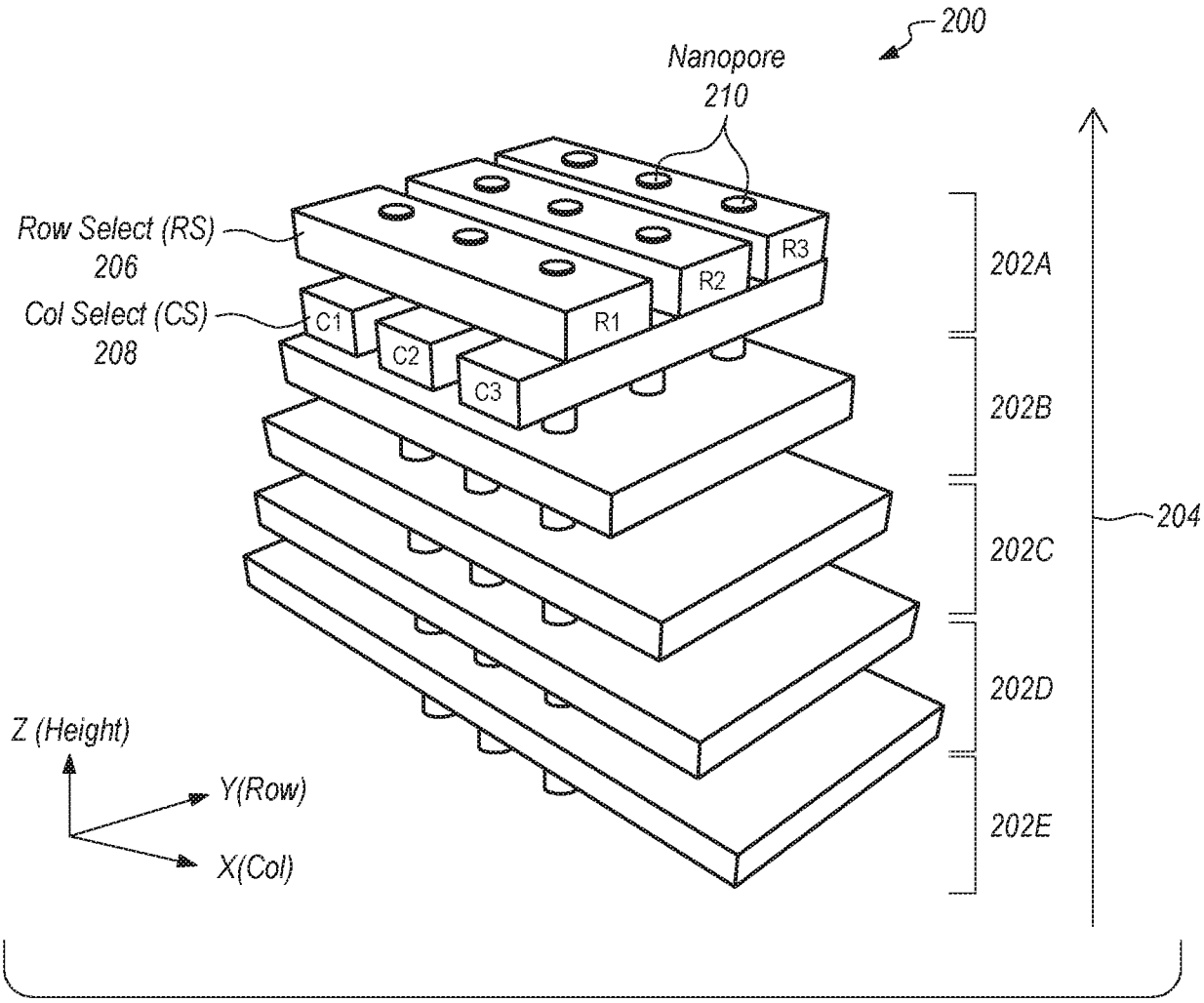

200
Nanopore
210
Row Select (RS)
206
Col Select (CS)
208
R1
R2
R3
C1
C2
C3
202A
202B
202C
202D
202E
204
Z (Height)
Y(Row)
X(Col)

FIG. 2A

FIG. 2B

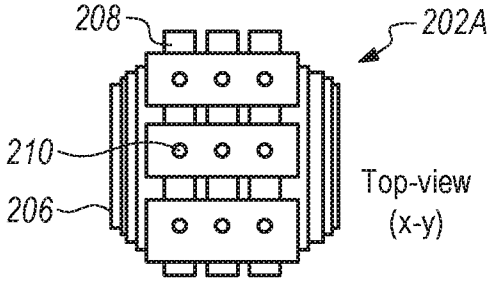


FIG. 2C

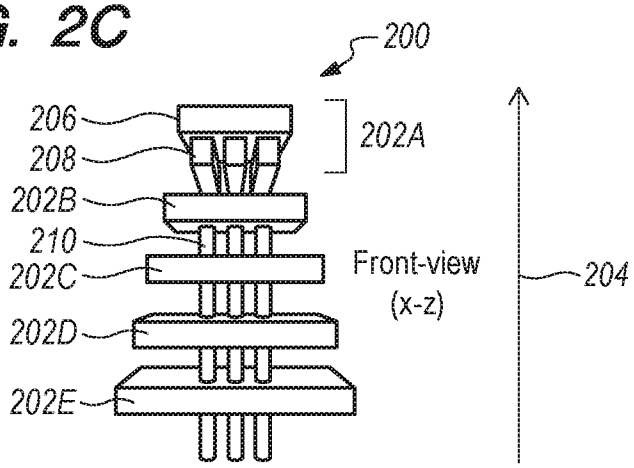


FIG. 2D

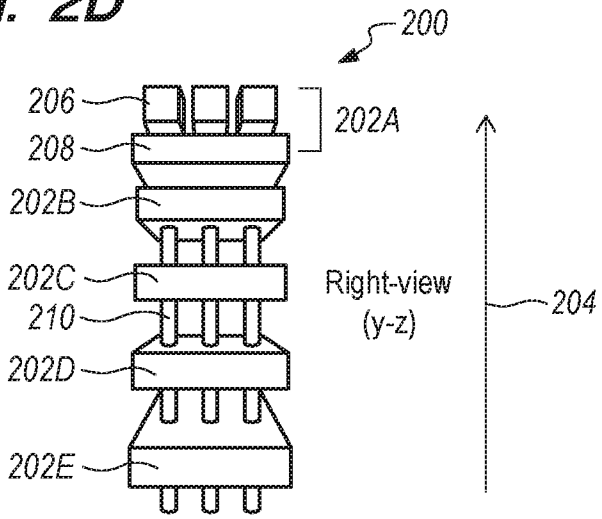
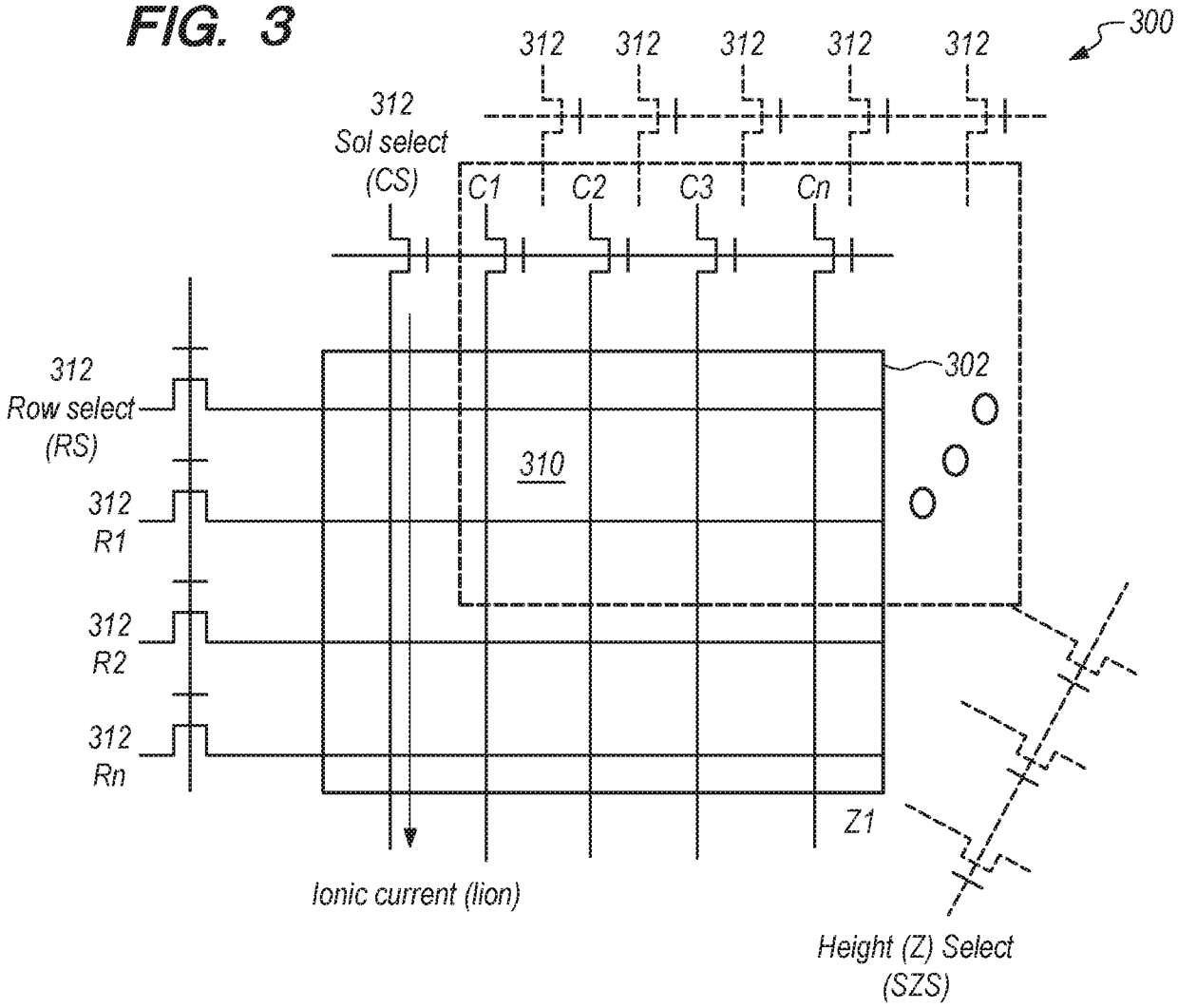


FIG. 3


300
312
312
312
312
312
312
Sol select
(CS)
C1
C2
C3
Cn
312
Row select
(RS)
302
310
312
R1
312
R2
312
Rn
Z1
Ionic current (lion)
Height (Z) Select
(SZS)

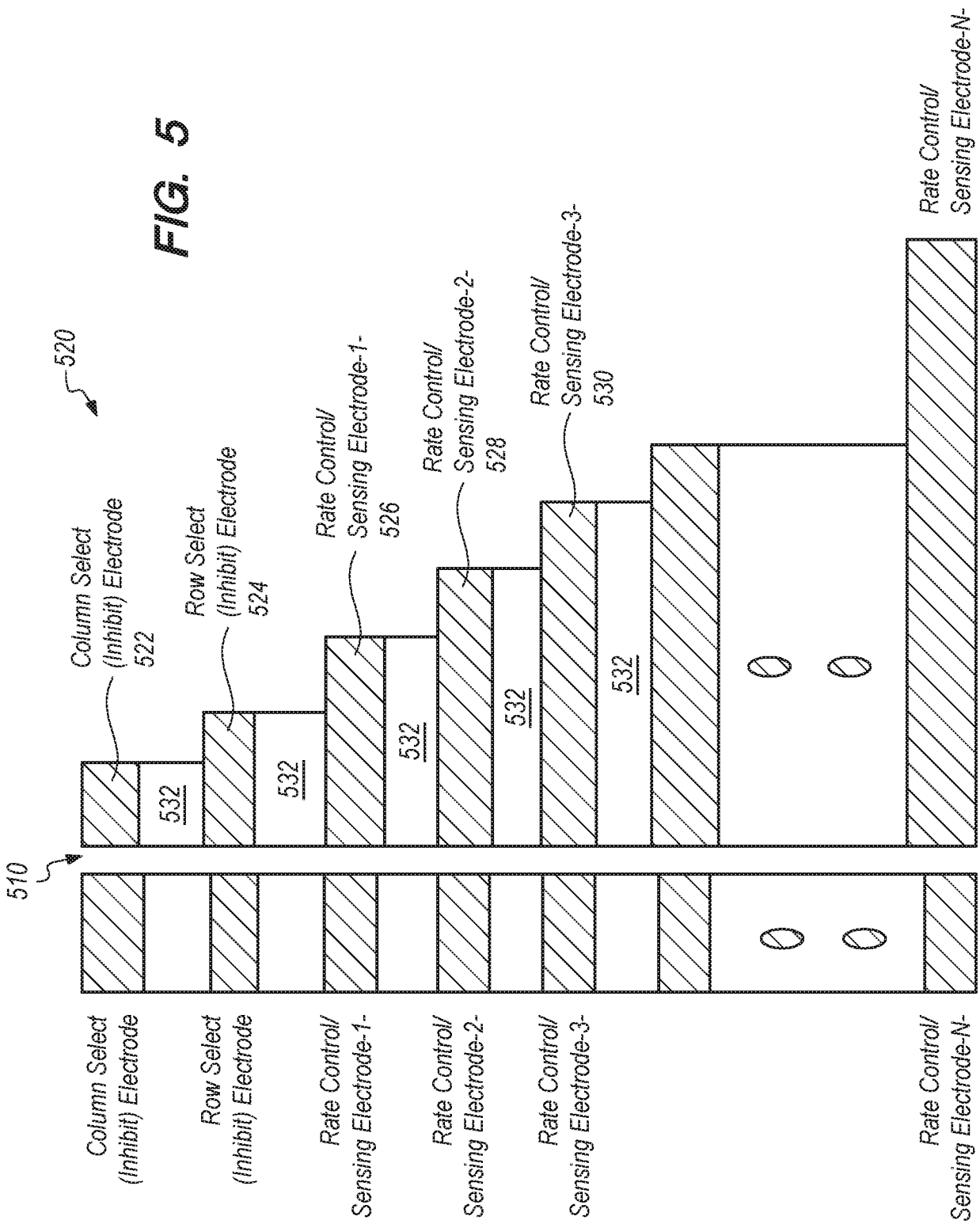
400

VPP=0V-3.3V, VCC=0V-2.8V, VSE=0.1V-1.5V,
other electrodes = GND unless specified otherwise

SZS=VCC (selected plane), OV (unselected plane)			
		VR	VC
INHIBITORY OPERATION	V(SR/SC)	0	0
	V(SR/UC)	0	VPP
	V(UR/SC)	VPP	0
	V(UR/UC)	VPP	VCC
NORMAL OPERATION	V(SR/SC)	VPP	VPP
	V(SR/UC)	VPP	0
	V(UR/SC)	0	VPP
	V(UR/UC)	0	0
SENSING OPERATION	V(SR/SC)	VCC	VSE
	V(SR/UC)	VCC	0
	V(UR/SC)	0	0
	V(UR/UC)	0	0

FIG. 4

FIG. 5


510
520
Column Select (Inhibit) Electrode
522
Row Select (Inhibit) Electrode
524
Rate Control/ Sensing Electrode-1-
526
Rate Control/ Sensing Electrode-2-
528
Rate Control/ Sensing Electrode-3-
530
532
Rate Control/ Sensing Electrode-N-

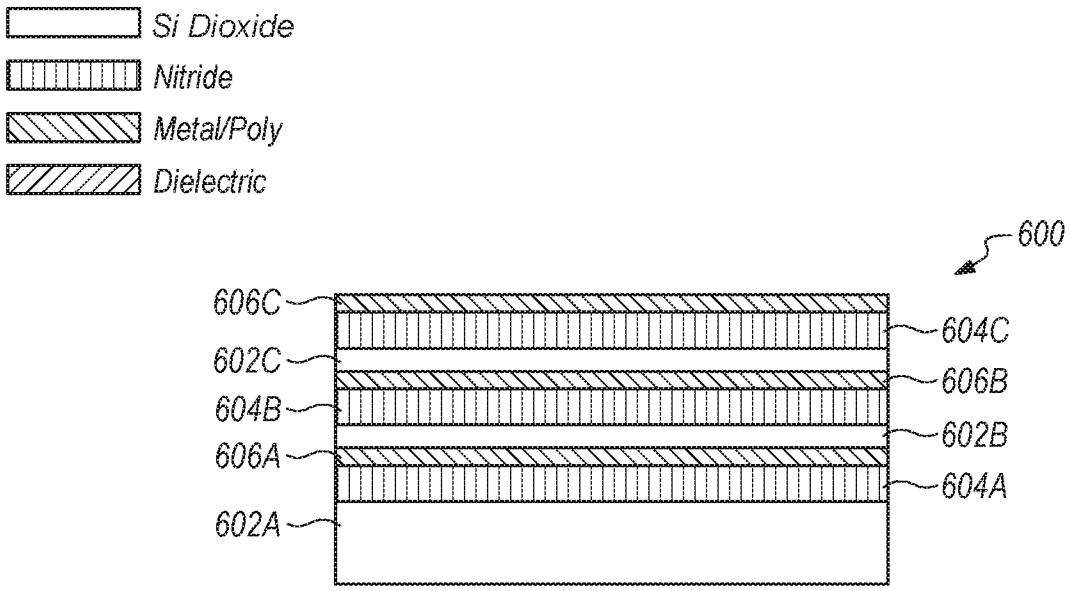


FIG. 6A

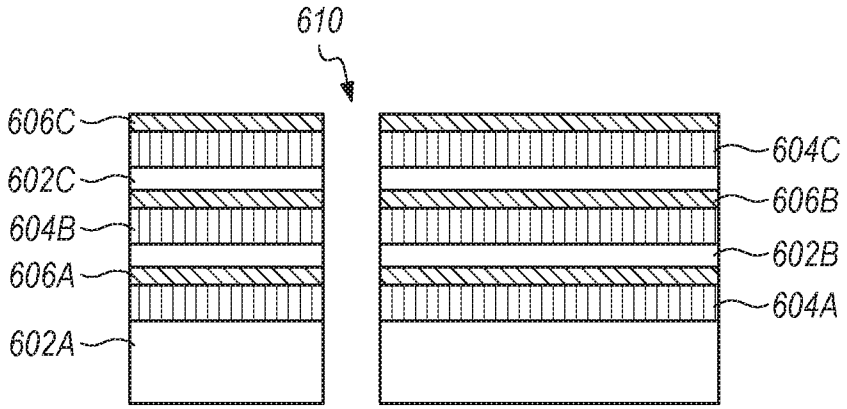


FIG. 6B

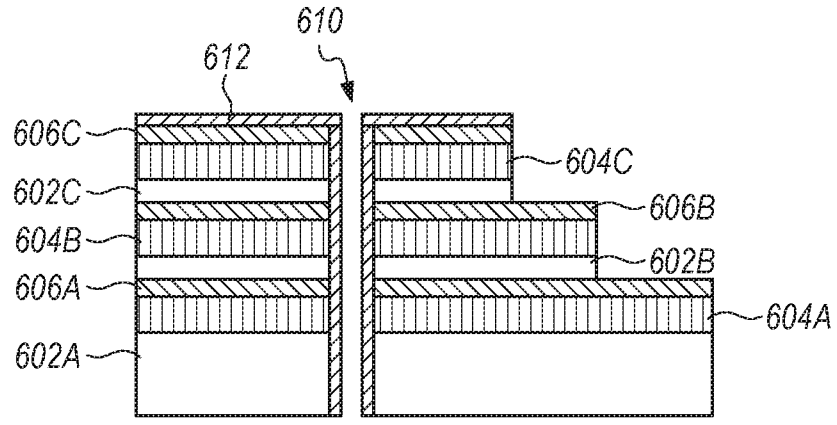


FIG. 6C

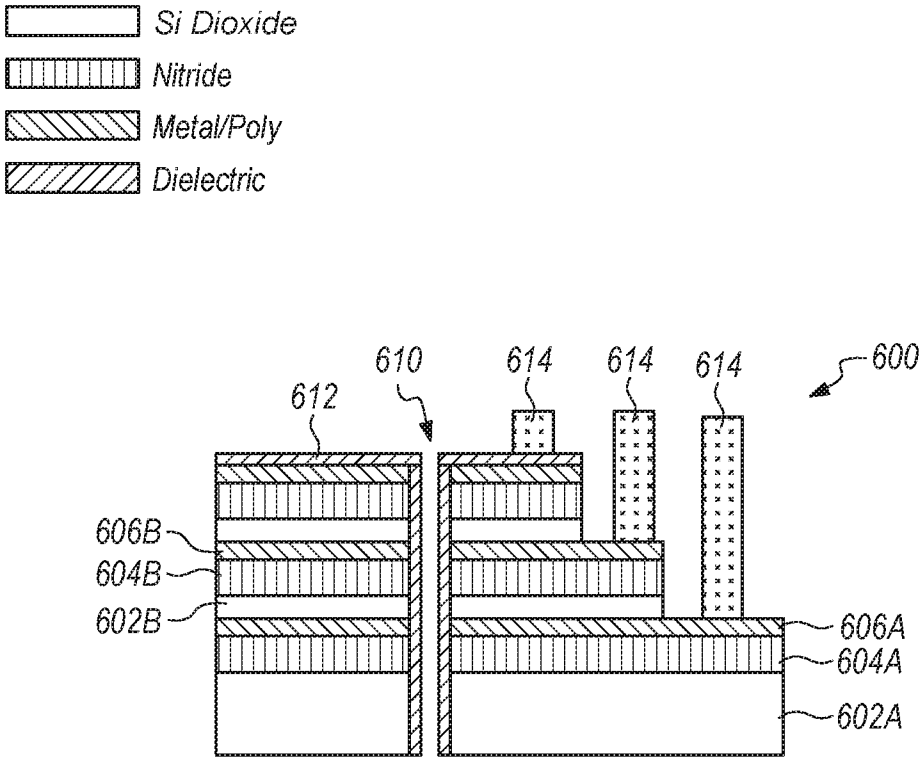

Si Dioxide
Nitride
Metal/Poly
Dielectric
610
614
614
614
600
612
606B
604B
602B
606A
604A
602A

FIG. 6D

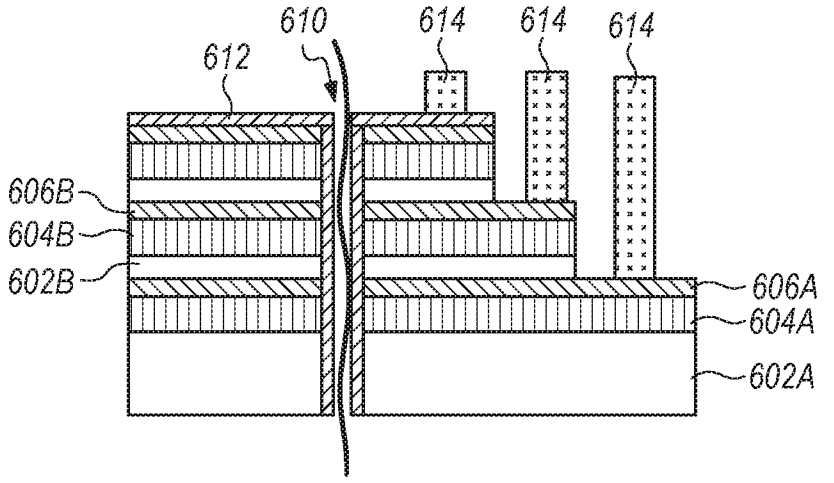

610
614
614
614
612
606B
604B
602B
606A
604A
602A

FIG. 6E

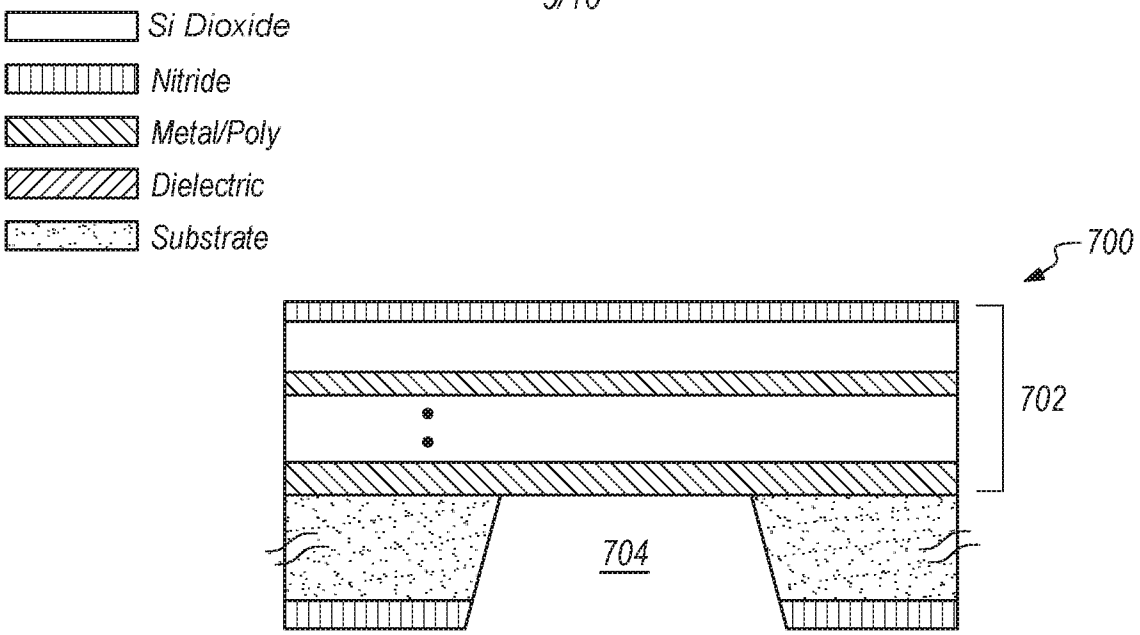


FIG. 7A

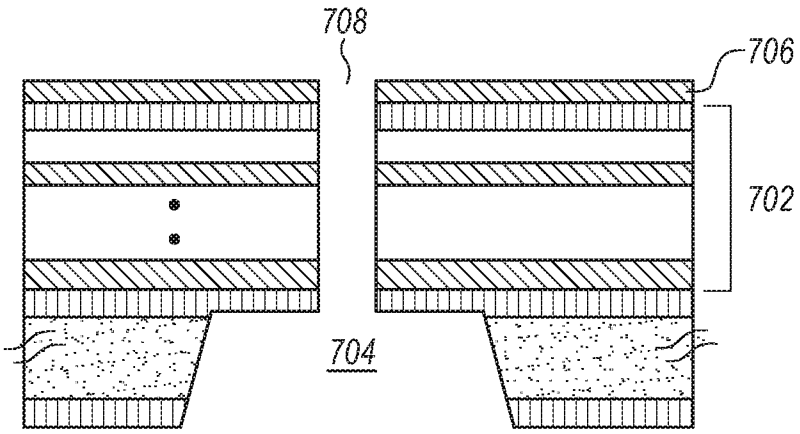


FIG. 7B

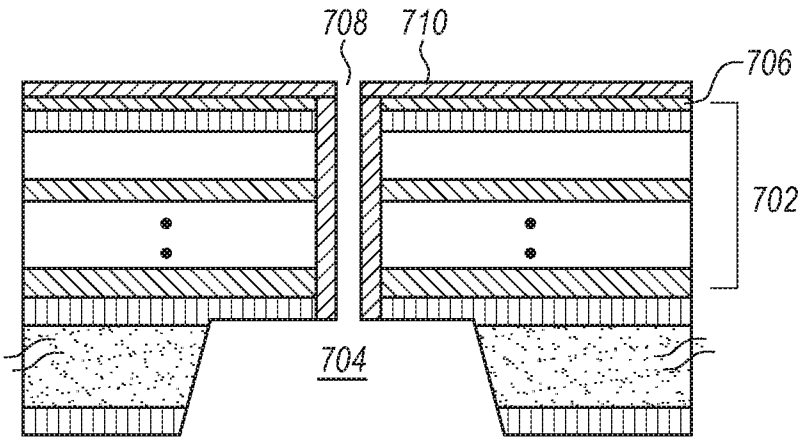


FIG. 7C

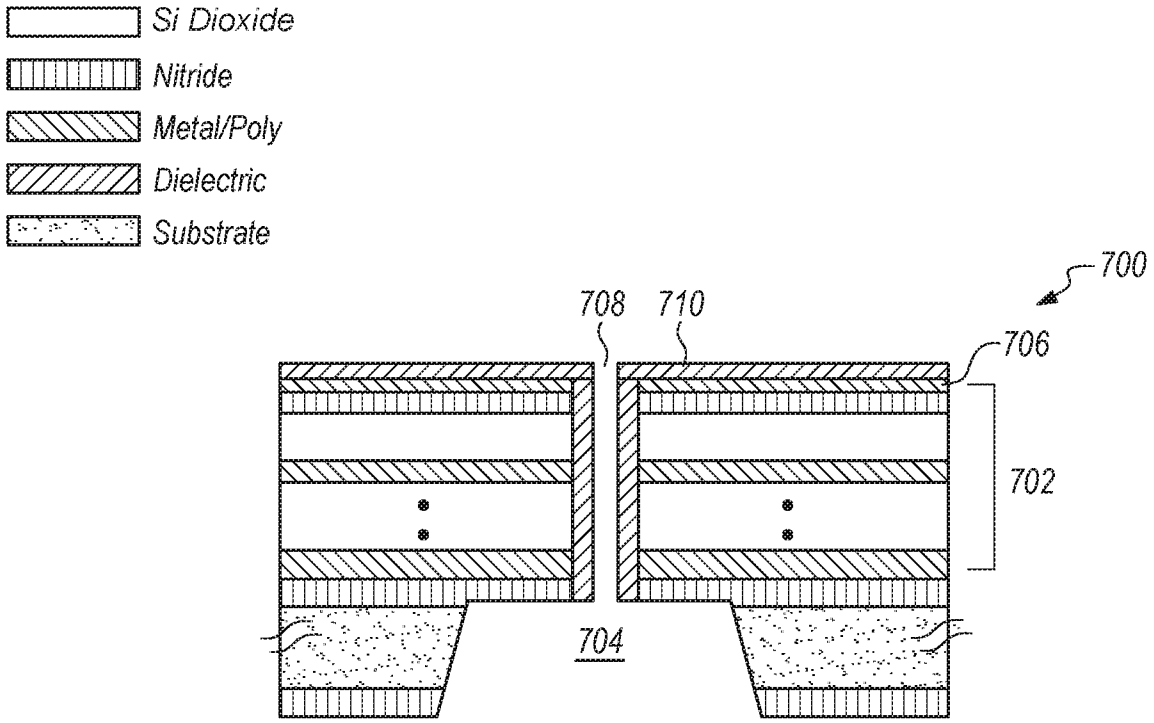

Si Dioxide
Nitride
Metal/Poly
Dielectric
Substrate
700
708
710
706
702
704

FIG. 7D

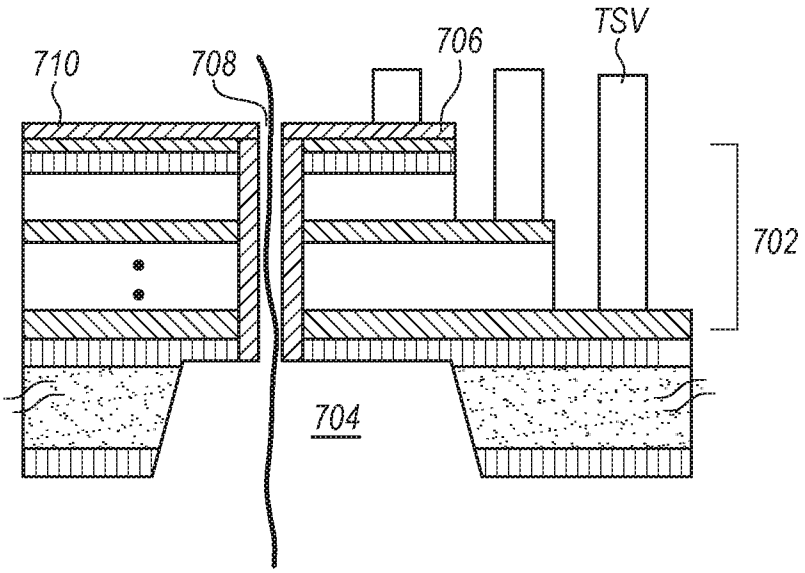

710
708
706
TSV
702
704

FIG. 7E