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(54) **NANOPORE ARRAYS**

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G11C 7/00	(2006.01)
G11C 16/34	(2006.01)

(52) **U.S. Cl.**
CPC ***G01N 33/48721*** (2013.01); *G11C 7/00* (2013.01); *G11C 16/34* (2013.01)

(58) **Field of Classification Search**
CPC G01N 33/48721; G11C 7/00; G11C 16/34
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2011/0193570 A1*	8/2011	Chen	.....	G01N 33/48721 324/654
2015/0111779 A1*	4/2015	Davis	.....	G01N 33/48721 506/9

* cited by examiner

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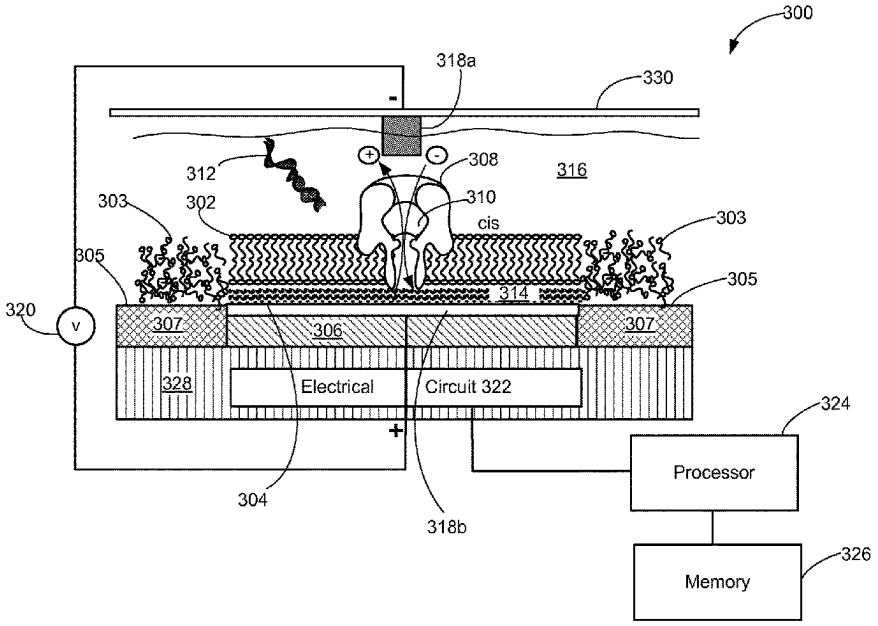
(57) **ABSTRACT**

A method of analyzing molecules using a nanopore array including a plurality of cells included on a chip is disclosed. Nanopores are caused to be formed in at least a portion of the plurality of the cells. A first physical measurement of the nanopores is evaluated. It is determined whether to cause the molecules to interact with the nanopores. At least a portion of the nanopores is caused to interact with the molecules. A second physical measurement of the nanopores that indicates a property of the molecules is evaluated. It is determined whether to cause the nanopores to be reformed so that the cells may be reused to interact with additional molecules.

10 Claims, 7 Drawing Sheets



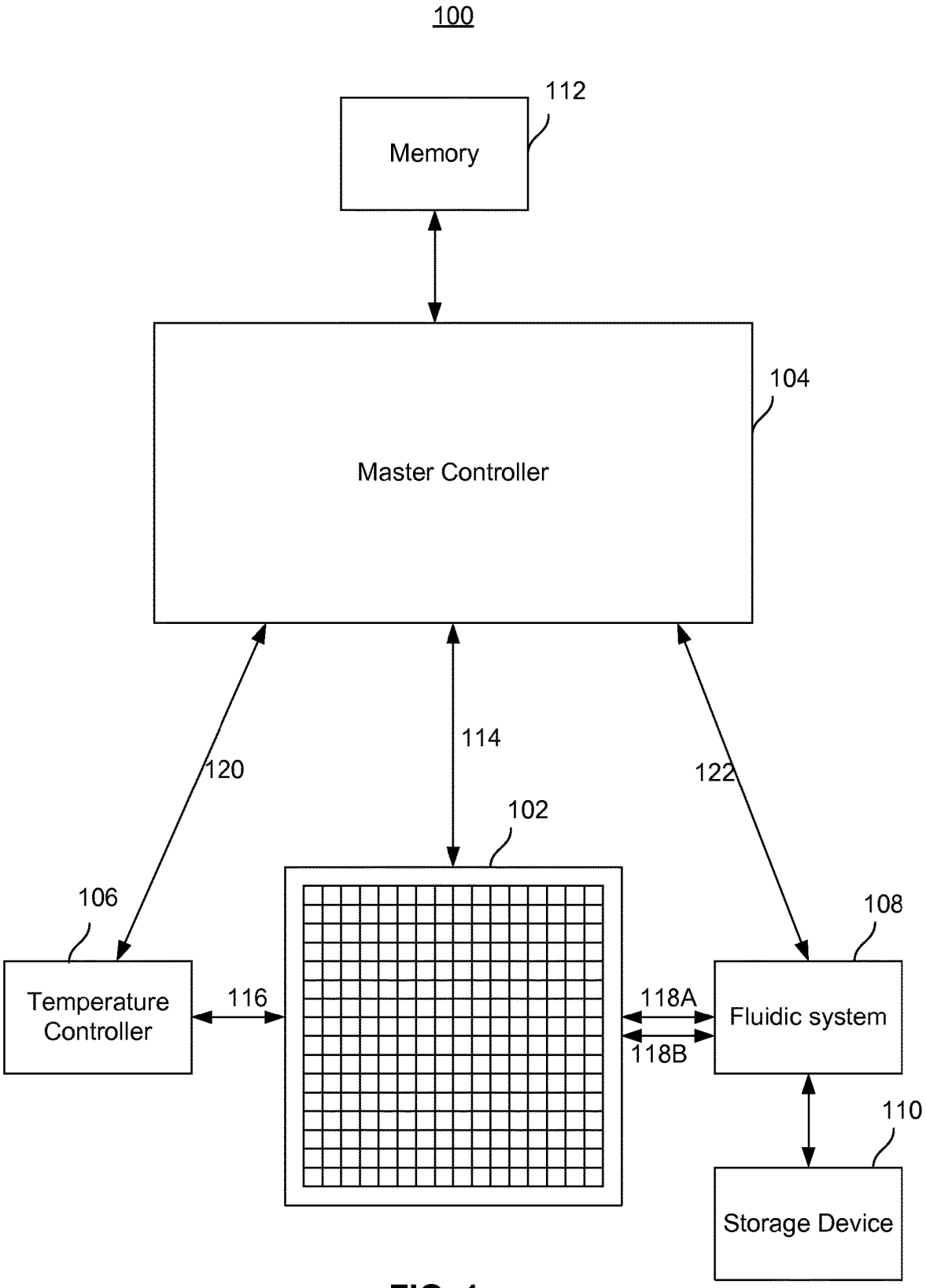

100
112
Memory
104
Master Controller
120
114
122
102
106
108
Temperature Controller
116
118A
118B
Fluidic system
110
Storage Device

FIG. 1

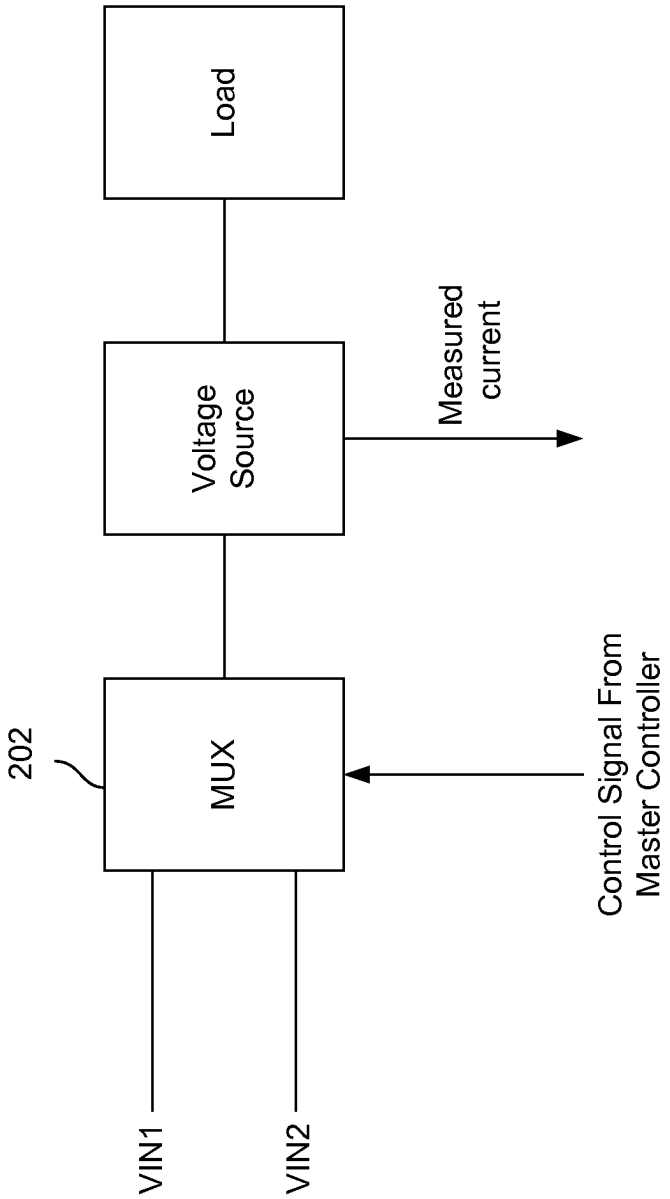

Load
Voltage Source
Measured current
202
MUX
Control Signal From Master Controller
VIN1
VIN2

FIG. 2

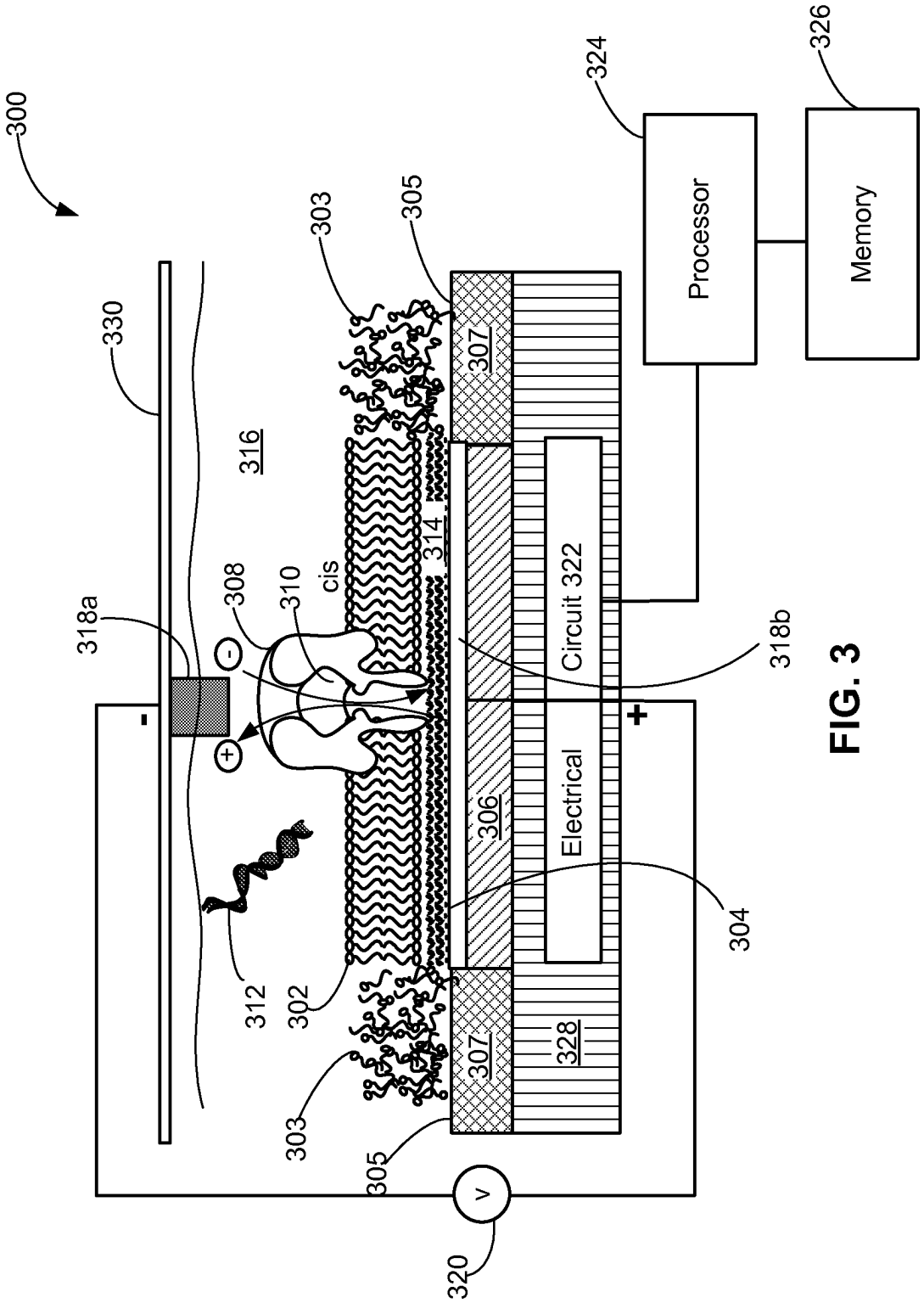

300
324
326
Processor
Memory
303
305
330
307
316
314
cis
308
310
318a
318b
Circuit 322
Electrical
306
304
312
302
328
V
320

FIG. 3

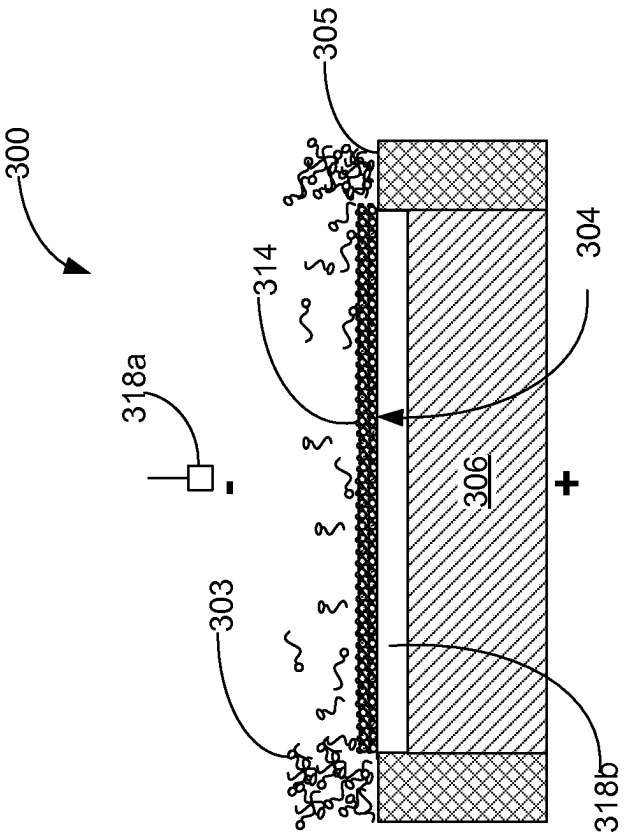


FIG. 4A

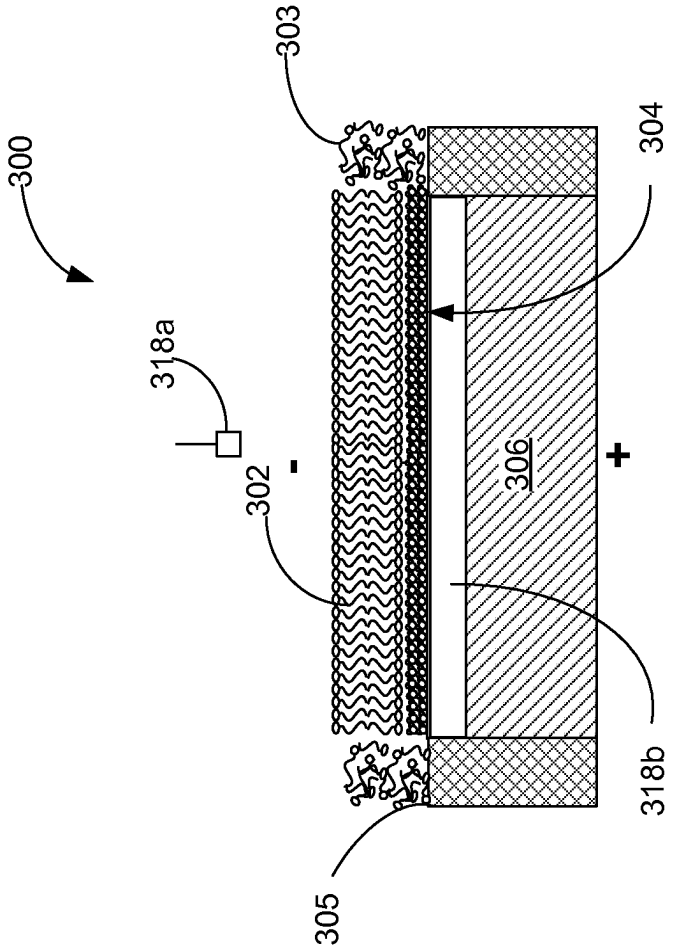


FIG. 4B

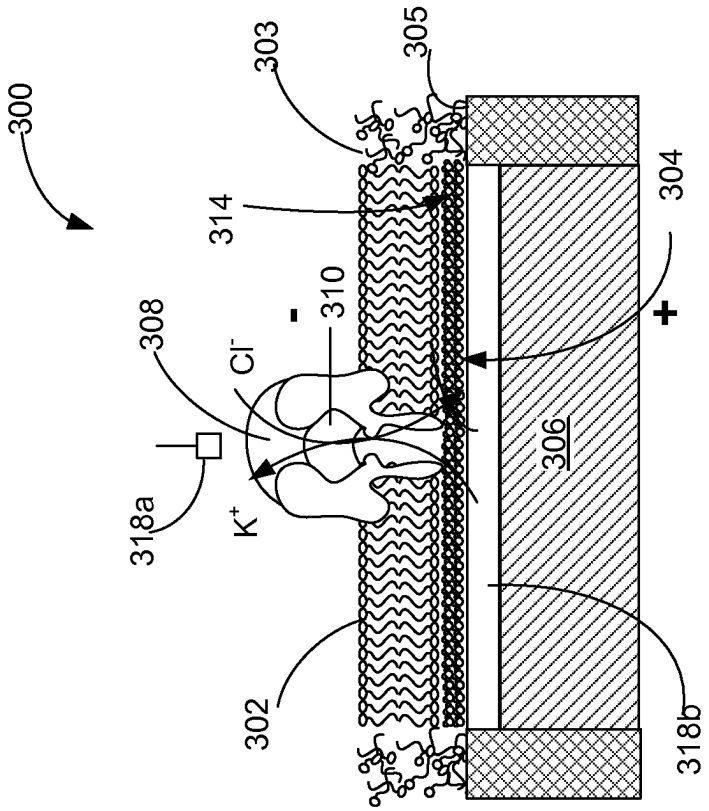


FIG. 4C

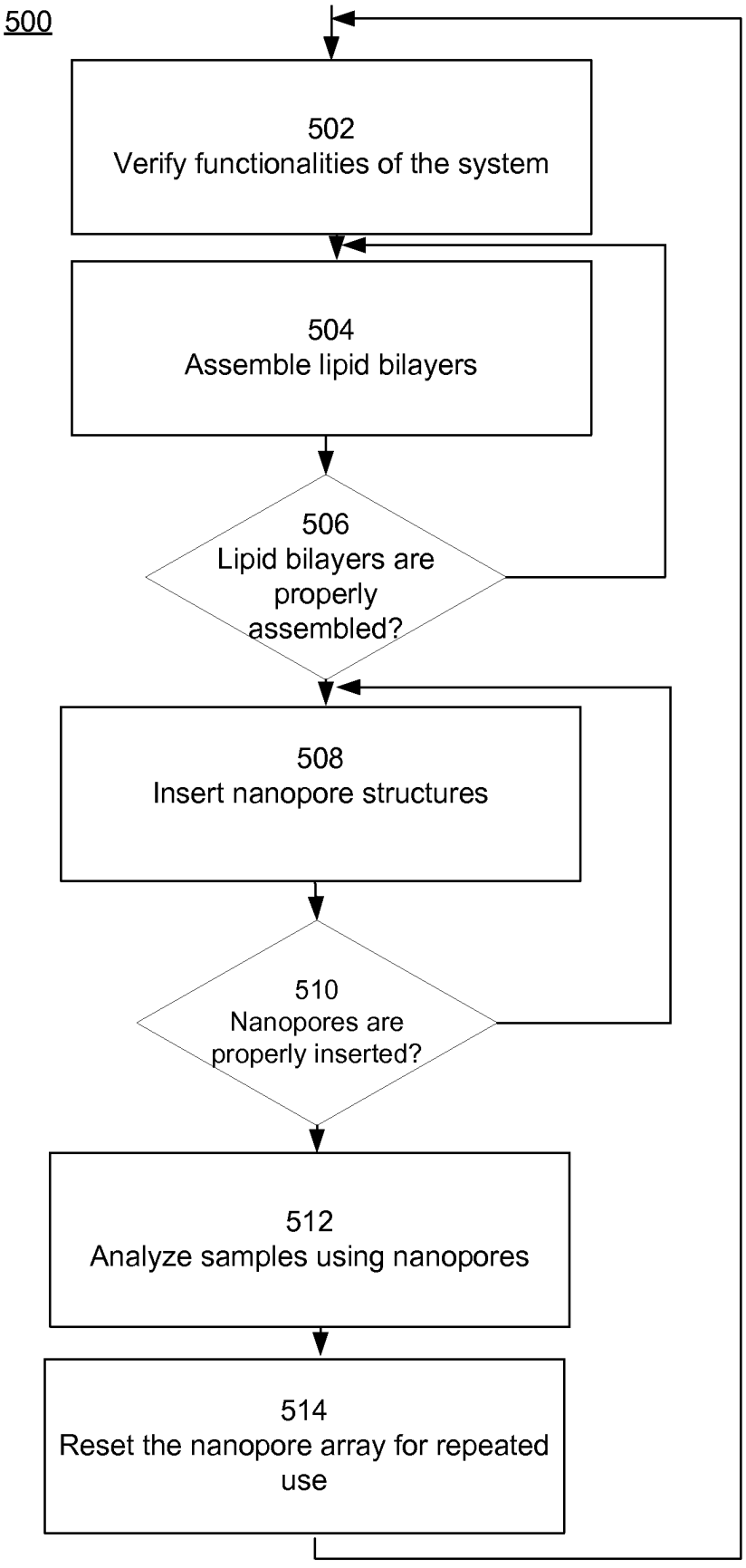

500
502
Verify functionalities of the system
504
Assemble lipid bilayers
506
Lipid bilayers are properly assembled?
508
Insert nanopore structures
510
Nanopores are properly inserted?
512
Analyze samples using nanopores
514
Reset the nanopore array for repeated use

FIG. 5

NANOPORE ARRAYS

CROSS REFERENCE TO OTHER APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 16/948,372, filed Sep. 15, 2020, titled "NANOPORE ARRAYS," which is a continuation of U.S. patent application Ser. No. 15/983,426, filed May 18, 2018, titled "NANOPORE ARRAYS", now U.S. Pat. No. 10,809,244, which is a continuation of U.S. patent application Ser. No. 15/462,483, entitled NANOPORE ARRAYS, filed Mar. 17, 2017, now U.S. Pat. No. 10,012,637, which is a continuation of U.S. patent application Ser. No. 13/759,701, now U.S. Pat. No. 9,759,711, entitled NANOPORE ARRAYS, filed Feb. 5, 2013, each of which are incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

Advances in micro-miniaturization within the semiconductor industry in recent years have enabled biotechnologists to begin packing their traditionally bulky sensing tools into smaller and smaller form factors, onto so-called biochips. It would be desirable to develop techniques for biochips that make them more robust, efficient, and cost-effective.

BRIEF DESCRIPTION OF THE DRA WINGS

Various embodiments of the invention are disclosed in the following detailed description and the accompanying drawings.

FIG. **1** is a block diagram illustrating an embodiment of a system **100** for analyzing molecules using nanopore devices.

FIG. **2** is a block diagram illustrating an embodiment for applying a voltage stimulus to a cell in nanopore array.

FIG. **3** is a diagram illustrating an embodiment of a nanopore device **300** within a cell of nanopore array.

FIG. **4**A is a diagram illustrating that nanopore device **300** is in a state in which a lipid bilayer has not yet been formed.

FIG. **4**B is a diagram illustrating that nanopore device **300** is in a state in which a lipid bilayer **302** has been formed.

FIG. **4**C is a diagram illustrating that nanopore device **300** is in a state in which a nanopore structure **308** with a nanopore **310** has been inserted into lipid bilayer **302**.

FIG. **5** is a flow diagram illustrating an embodiment of a process **500** for analyzing molecules using nanopore devices.

DETAILED DESCRIPTION

The invention can be implemented in numerous ways, including as a process; an apparatus; a system; a composition of matter; a computer program product embodied on a computer readable storage medium; and/or a processor, such as a processor configured to execute instructions stored on and/or provided by a memory coupled to the processor. In this specification, these implementations, or any other form that the invention may take, may be referred to as techniques. In general, the order of the steps of disclosed processes may be altered within the scope of the invention. Unless stated otherwise, a component such as a processor or a memory described as being configured to perform a task may be implemented as a general component that is temporarily configured to perform the task at a given time or a specific component that is manufactured to perform the task. As used herein, the term 'processor' refers to one or more devices, circuits, and/or processing cores configured to process data, such as computer program instructions.

In various embodiments, the techniques described herein are implemented in a variety of systems or forms. In some embodiments, the techniques are implemented in hardware as an application-specific integrated circuit (ASIC) or a field-programmable gate array (FPGA). In some embodiments, a processor (e.g., an embedded one such as an ARM core) is used where the processor is provided or loaded with instructions to perform the techniques described herein. In some embodiments, the technique is implemented as a computer program product which is embodied in a computer readable storage medium and comprises computer instructions.

A detailed description of one or more embodiments of the invention is provided below along with accompanying figures that illustrate the principles of the invention. The invention is described in connection with such embodiments, but the invention is not limited to any embodiment. The scope of the invention is limited only by the claims and the invention encompasses numerous alternatives, modifications and equivalents. Numerous specific details are set forth in the following description in order to provide a thorough understanding of the invention. These details are provided for the purpose of example and the invention may be practiced according to the claims without some or all of these specific details. For the purpose of clarity, technical material that is known in the technical fields related to the invention has not been described in detail so that the invention is not unnecessarily obscured.

Advances in micro-miniaturization within the semiconductor industry in recent years have enabled biotechnologists to begin packing their traditionally bulky sensing tools into smaller and smaller form factors, onto so-called biochips. These chips are essentially miniaturized laboratories that can perform hundreds or thousands of simultaneous biochemical reactions. Biochips enable researchers to quickly screen large numbers of biological analytes for a variety of purposes, from disease diagnosis to detection of bioterrorism agents.

Typically, a biochip includes a large array of cells. For example, a biochip for nucleotide sequencing may contain thousands or millions of single cells in an array. Each cell includes a molecular complex composed of monomers that make up an oligomeric nanopore. Each cell may further include a single strand of DNA, and anything bound to that single strand of DNA. The nanopore is a small hole in an electrically insulating membrane that can be used as a single-molecule detector. A nanopore may be formed using a biological material, such as α-hemolysin or MspA. A nanopore may be formed using a solid-state material, such as a semiconductor material. When a small voltage is applied across a molecular complex containing a nanopore, an ionic current through the molecular complex can be measured to provide information about the structure of a molecule transiting the molecular complex. In a single cell of the array, an electrical circuit may be used for controlling the electrical stimulus applied across a lipid bilayer which contains a nanopore, and for detecting and analyzing the electrical patterns, or signatures, of a molecule passing through the nanopore.

FIG. **1** is a block diagram illustrating an embodiment of a system **100** for analyzing molecules using nanopore devices. System **100** includes a nanopore array **102**, a master controller **104**, a temperature controller **106**, a fluidic system

108, a storage device 110 for storing extracted results, and a memory 112. In some embodiments, some of the modules may be combined together as a single module, and some of the modules may be optional. In some embodiments, the cells of nanopore array 102 and the nanopore devices within the cells are individually controllable and individually addressable by other modules of system 100, including by master controller 104, temperature controller 106, and fluidic system 108. In some embodiments, performance data or other data corresponding to each of the cells may be sent from nanopore array 102 to other modules in system 100. Control, address, performance, or other data signals may be communicated between nanopore array 102 and other modules in system 100 via signal lines 114, 116, and 118A, respectively.

In some embodiments, the cells of nanopore array 102 and the nanopore devices within the cells are individually controllable and individually addressable by master controller 104. This allows master controller 104 to control each of the cells or each group of cells in nanopore array 102 such that the particular cell or particular group of cells performs different functions or transits through different states independently, without affecting the functioning or progress of other cells or other groups of cells in nanopore array 102. In one example, a mal-functioning cell in nanopore array 102 may be put in a state (e.g., disabled state) by master controller 104 such that the mal-functioning cell does not affect the functioning of other cells in nanopore array 102. For example, if a lipid bilayer fails to form in a particular cell, the cell may be disabled such that no electrical stimulus is applied to the cell; otherwise, the cell may draw a large current, which may affect the performance of other cells in nanopore array 102.

In another example, master controller 104 may send control signals to nanopore array 102 such that different stimuli are applied to different cells or groups of cells. For example, a first stimulus (e.g., a voltage) is applied to a first group of cells and a second stimulus is applied to a second group of cells at time t_1. The first stimulus may be a stimulus corresponding to a particular state of a cell, and the second stimulus may be a stimulus corresponding to a different state of a cell. The stimulus that is applied to the first group of cells may vary over time, as the first group of cells transits from one state to another. FIG. 2 is a block diagram illustrating an embodiment for applying a voltage stimulus to a cell in nanopore array 102. As shown in FIG. 2, control signals from master controller 104 may be used as input to a multiplexer 202 to select one of two voltages that can be applied to a cell in nanopore array 102.

In some embodiments, performance or other data corresponding to each of the cells may be received by master controller 104. By monitoring the performance or other data of the cells, master controller 104 may determine any state transitions of the cells. The state information of the cells may be stored in memory 112 by master controller 104. In addition, if the overall performance of nanopore array 102 falls below a certain threshold, master controller 104 may reset and re-initialize nanopore array 102 such that any processes running on nanopore array 102 may be terminated or restarted again. In some embodiments, nanopore array 102 may also be reused multiple times. For example, nanopore array 102 may be used for analyzing different types of samples during different runs. In another example, nanopore array 102 may be reused for analyzing a single type of samples over multiple runs. In some embodiments, nanopore array 102 may be reused after the contents in nanopore array 102 have been flushed out or rinsed out by master controller 104 and fluidic system 108.

In some embodiments, the cells of nanopore array 102 are individually controllable and individually addressable by temperature controller 106 via signal line 116. Temperature or other data corresponding to a cell may be received by temperature controller 106 via signal line 116. Depending on the state or condition of a particular cell or a group of cells, different temperature stimuli may be applied to the cell or group of cells by temperature controller 106. In some embodiments, temperature controller 106 receives state information of the cells via signal line 120 and applies the appropriate temperature stimuli to the cells in nanopore array 102 at least in part based on the state information. In some embodiments, temperature controller 106 receives control signal via signal line 120 from master controller 104, and then temperature controller 106 applies the appropriate temperature stimuli to the cells in nanopore array 102 based on the received control signal.

In some embodiments, the cells of nanopore array 102 are individually controllable and individually addressable by fluidic system 108. The control and address information is communicated between nanopore array 102 and fluidic system 108 via signal lines 118A. Different contents may be delivered in and out of the individual cells of nanopore array 102 via channels 118B. The contents may be any fluids or reagents that are used for the operations within the cells of nanopore array 102, including saline solution for rinsing, samples to be analyzed by nanopore array 102, lipid bilayer forming reagent, nanopore forming reagent, gas catalyst, and the like. The contents delivered out of nanopore array 102 may be any molecules that are extracted from the samples that have been analyzed by nanopore array 102, and the extracted molecules may be further delivered to a storage device 110 by fluidic system 108. The contents may be in any form, including liquid or gas. Depending on the state or condition of a particular cell or a group of cells, different fluids may be delivered to or from the cell or group of cells by fluidic system 108. In some embodiments, fluidic system 108 receives state information of the cells via signal line 122 and delivers the appropriate fluid to or from the cells in nanopore array 102 at least in part based on the state information. In some embodiments, fluidic system 108 receives control signal via signal line 122 from master controller 104, and then fluidic system 108 delivers the appropriate fluid to or from the cells in nanopore array 102 based on the received control signal. In some embodiments, nanopore array 102 may be reused after the contents in nanopore array 102 have been flushed out or rinsed out by master controller 104 and fluidic system 108.

Nanopore array 102 includes a large array of cells. Each cell includes a nanopore device for analyzing and characterizing molecules. Within a nanopore device, a lipid bilayer is formed, and a nanopore structure is then formed on the lipid bilayer. The nanopore structure has a nanopore that is large enough for enclosing at least a portion of a molecule that is being analyzed or passing at least a portion of the molecule between the two sides of the lipid bilayer. The nanopore device also includes a sample chamber for holding a solution of the analyzed molecules. The solution may be provided over the lipid bilayer for introducing the analyzed molecules for characterization. The nanopore device further includes means for providing electrical stimulus, sensing electrical characteristics, detecting and processing signal of the nanopore device.

FIG. **3** is a diagram illustrating an embodiment of a nanopore device **300** within a cell of nanopore array **102**. Nanopore device **300** includes a lipid bilayer **302** formed on a lipid bilayer compatible surface **304** of a conductive solid substrate **306**. Lipid bilayer compatible surface **304** may be isolated by lipid bilayer incompatible surfaces **305**, and conductive solid substrate **306** may be electrically isolated by insulating materials **307**. Lipid bilayer **302** may be surrounded by an amorphous lipid **303** formed on lipid bilayer incompatible surfaces **305**.

In some embodiments, lipid bilayer **302** is embedded with a single nanopore structure **308** having a nanopore **310** large enough for passing at least a portion of a molecule **312** being characterized and/or small ions (e.g., Na^+, K^+, Ca^{2+}, Cl^-) between the two sides of lipid bilayer **302**. A layer of water molecules **314** (also referred to as an aqueous film **314**) may be adsorbed on lipid bilayer compatible surface **304** and sandwiched between lipid bilayer **302** and lipid bilayer compatible surface **304**. Aqueous film **314** adsorbed on the hydrophilic lipid bilayer compatible surface **304** may promote the ordering of lipid molecules and facilitate the formation of lipid bilayer **302** on lipid bilayer compatible surface **304**.

A sample chamber **316** may be provided over lipid bilayer **302** for introducing a sample for characterization. The sample may be a solution of molecule **312** that is being characterized. The solution may be an aqueous solution containing electrolytes and buffered to an optimum ion concentration and maintained at an optimum pH to keep nanopore **310** open. In some embodiments, sample chamber **316** receives the sample from fluidic system **108**. The sample may also be flushed out of nanopore device **300** by fluidic system **108** after the characterization of the sample has been performed. Sample chamber **316** may also be rinsed with saline solution by fluidic system **108** such that nanopore device **300** may be reused again.

Nanopore device **300** includes a pair of electrodes **318** (including a negative node **318***a* and a positive node **318***b*) coupled to a variable voltage source **320** for providing electrical stimulus (e.g., voltage bias) across the lipid bilayer **302** and for sensing the electrical characteristics of the lipid bilayer **302** (e.g., resistance, capacitance, and ionic current flow). The surface of the negative positive electrode **318***b* is or forms a part of the lipid bilayer compatible surface **304**. The conductive solid substrate **306** may be coupled to or forms a part of one of the electrodes **318**. Nanopore device **300** may also include an electrical circuit **322** for controlling electrical stimulation and for processing the signal detected. In some embodiments, the variable voltage source **320** is included as a part of the electrical circuit **322**. The electrical circuitry **322** may include amplifiers, integrators, noise filters, feedback control logic, and/or various other components. In some embodiments, the electrical circuitry **322** may be an integrated electrical circuitry integrated within a silicon substrate **328** and may be further coupled to a computer processor **324** coupled to a memory **326**. For example, computer processor **324** may be a portion of master controller **104**, and memory **326** may be memory **112** that is coupled to master controller **104**. Master controller **104** may control the various components of nanopore device **300** via electrical circuit **322**. Master controller **104** may also receive data collected by nanopore device **300** via electrical circuit **322**.

The lipid bilayer compatible surface **304** can be formed from various materials that are suitable for ion transduction and gas formation to facilitate lipid bilayer formation. In some embodiments, conductive or semi-conductive hydrophilic materials as opposed to insulating hydrophilic materials are preferred because they may allow better detection of a change in the lipid bilayer electrical characteristics. Example materials include Ag—AgCl, Ag—Au alloy, Ag—Pt alloy, or doped silicon or other semiconductor materials.

The lipid bilayer incompatible surface **305** can be formed from various materials that are not suitable for lipid bilayer formation and they are typically hydrophobic. In some embodiments, a non-conductive hydrophobic material is preferred, since it electrically insulates the lipid bilayer regions in addition to separating the lipid bilayer regions from each other. Example lipid bilayer incompatible materials include silicon nitride (e.g., Si_3N_4) and Teflon.

In one particular example, nanopore device **300** of FIG. **3** is a alpha hemolysin (αHL) nanopore device having a single αHL protein embedded in a diphytanoylphosphatidylcholine (DPhPC) lipid bilayer **302** formed over a lipid bilayer compatible silver-gold alloy surface **304** coated on a copper material **306**. The lipid bilayer compatible silver-gold alloy surface **304** is isolated by lipid bilayer incompatible silicon nitride surfaces **305**, and the copper material **306** is electrically insulated by silicon nitride materials **307**. The copper **306** is coupled to electrical circuitry **322** that is integrated in a silicon substrate **328**. A silver-silver chloride electrode placed on-chip or extending down from a cover plate contacts an aqueous solution containing dsDNA molecules.

The αHL nanopore is an assembly of seven individual peptides. The entrance or vestible of the αHL nanopore is approximately 26 Å in diameter, which is wide enough to accommodate a portion of a dsDNA molecule. From the vestible, the αHL nanopore first widens and then narrows to a barrel having a diameter of approximately 15 Å, which is wide enough to allow a single ssDNA molecule to pass through but not wide enough to allow a dsDNA molecule to pass through. At a given time, approximately 1-20 DNA bases can occupy the barrel of the αHL nanopore.

In addition to DPhPC, the lipid bilayer of the nanopore device can be assembled from various other suitable amphiphilic materials, selected based on various considerations, such as the type of nanopore used, the type of molecule being characterized, and various physical, chemical and/or electrical characteristics of the lipid bilayer formed, such as stability and permeability, resistance, and capacitance of the lipid bilayer formed. Example amphiphilic materials include various phospholipids such as palmitoyl-oleoyl-phosphatidyl-choline (POPC) and dioleoyl-phosphatidyl-methylester (DOPME), diphytanoylphosphatidylcholine (DPhPC) dipalmitoylphosphatidylcholine (DPPC), phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylglycerol, and sphingomyelin.

In addition to the αHL nanopore shown above, the nanopore may be one of various other types of nanopores; examples include γ-hemolysin, leukocidin, melittin, and various other naturally occurring, modified natural, and synthetic nanopores. A suitable nanopore may be selected based on various characteristics of the analyte molecule, such as the size of the analyte molecule in relation to the pore size of the nanopore. For example, the αHL nanopore is a nanopore that has a restrictive pore size of approximately 15 Å. It is suitable for analyzing DNA molecules since it allows a single strand DNA (ssDNA) to pass through while restricting a double strand DNA (dsDNA).

FIGS. **4**A-**4**C illustrate three different states of nanopore device **300**. FIG. **4**A is a diagram illustrating that nanopore device **300** is in a state in which a lipid bilayer has not yet been formed. FIG. **4**B is a diagram illustrating that nanopore

device **300** is in a state in which a lipid bilayer **302** has been formed. FIG. **4**C is a diagram illustrating that nanopore device **300** is in a state in which a nanopore structure **308** with a nanopore **310** has been inserted into lipid bilayer **302**.

FIG. **5** is a flow diagram illustrating an embodiment of a process **500** for analyzing molecules using nanopore devices. In some embodiments, process **500** is a process that is performed by system **100** of FIG. **1**.

At **502**, various functionalities of system **100** are verified. In some embodiments, master controller **104** may send test signals to the modules of system **100**, including nanopore array **102**, temperature controller **106**, and fluidic system **108**. In response, each module may perform verification steps at the module. For example, nanopore array **102** may measure the current flowing in a particular nanopore device. After the verification steps are performed at the modules, each of the modules may send a response back to master controller **104** for verification purposes. Depending on the responses received from the various modules, master controller **104** may determine whether further verifications are needed. In some embodiments, the verification results may be stored in a log file. In some embodiments, if master controller **104** has detected any errors, then an alarm may be triggered or process **500** may be terminated.

In some embodiments, verification of the different modules may be performed at different levels, and the levels may be configurable. For example, master controller **104** may verify the functionalities of nanopore array **102** at the printed circuit board level or at the semiconductor chip level. In some embodiments, master controller **104** may verify the functionalities of a group of cells. If the number of cells within the group that are functioning properly falls below a certain threshold, then master controller **104** may determine that the group of cells is mal-functioning and that the group of cells should be disabled.

At **504**, lipid bilayers are assembled. In some embodiments, master controller **104** may cause fluidic system **108** to deliver a lipid forming reagent to the cells of nanopore array **102**. The lipid forming reagent is then deposited on lipid bilayer compatible surface **304** within a cell. As discussed above, the lipid bilayer may be formed using different materials, including different amphiphilic materials. Depending on the type of lipid bilayers to be formed, master controller **104** may cause different stimuli (e.g., electrical, temperature, chemical, or gas) to be applied to the cells to facilitate the assembling of the lipid bilayers.

At **506**, it is determined whether the lipid bilayers are properly formed. Depending on the type of lipid bilayers to be formed, different physical or electrical property measurements (e.g., resistance, current, or capacitance measurements) may be made at the cells and then sent to master controller **104** via signal lines **114** for determining whether lipid bilayers are properly assembled. In some embodiments, steps **504** and **506** are repeated until master controller **104** has determined that lipid bilayers have been properly assembled in a minimum number of cells in nanopore array **102**. In some embodiments, if the number of cells with lipid bilayers properly assembled falls below a certain threshold after a fixed period of time, master controller **104** may terminate process **500**. In addition, an alarm may be triggered or an error message may be written to the log file. In some embodiments, if the number of cells with lipid bilayers properly assembled is above a certain threshold, master controller **104** may cause system **100** to proceed to step **508**.

At **508**, nanopore structures with nanopores are inserted. In some embodiments, master controller **104** may cause fluidic system **108** to deliver a nanopore forming reagent (e.g., a solution containing α-hemolysin) to the cells of nanopore array **102**. Master controller **104** may cause different stimuli (e.g., electrical, temperature, chemical, or gas) to be applied to the cells to facilitate the insertion of the nanopore structures into the lipid bilayers.

At **510**, it is determined whether the nanopore structures are properly formed. Depending on the type of nanopores to be formed, different measurements (e.g., resistance, current, or capacitance measurements) may be made at the cells and then sent to master controller **104** via signal lines **114** for determining whether nanopores are properly inserted. In some embodiments, steps **508** and **510** are repeated until master controller **104** has determined that nanopores have been properly inserted in a minimum number of cells in nanopore array **102**. In some embodiments, if the number of cells with nanopores properly inserted falls below a certain threshold after a fixed period of time, master controller **104** may terminate process **500**. In addition, an alarm may be triggered or an error message may be written to the log file. In some embodiments, if the number of cells with nanopores properly inserted is above a certain threshold, master controller **104** may cause system **100** to proceed to step **512**.

At **512**, samples are analyzed using the nanopores in nanopore array **102**. In some embodiments, master controller **104** may cause fluidic system **108** to deliver samples to the sample chambers **316** in nanopore array **102**. Depending on different factors, including the type of samples that are being analyzed and the type of nanopores formed, master controller **104** may cause different stimuli (e.g., electrical, temperature, chemical, or gas) to be applied to the cells to facilitate the manipulating, detecting, correlating, characterizing, analyzing and/or sequencing of molecules in the nanopores. Different measurements (e.g., resistance, current, or capacitance measurements) may be made at the cells and then sent to master controller **104** via signal lines **114**. Master controller **104** may use the received measurements to detect, correlate, determine, characterize, sequence and/or discriminate various structural and chemical features of a molecule as the molecule stays inside the nanopore, traverses through the nanopore, or interacts with the nanopore.

At **514**, nanopore array is reset and re-initialized for repeated uses. In some embodiments, nanopore array **102** may be reused multiple times. For example, nanopore array **102** may be used for analyzing different types of samples during different runs. In another example, nanopore array **102** may be reused for analyzing a single type of samples over multiple runs. New nanopores may be reformed in nanopore array **102** such that nanopore array **102** may be reused. New nanopores may be reformed in nanopore array **102** after the contents (e.g., lipid bilayers with nanopores inserted, lipid bilayers without nanopores inserted, and samples) in nanopore array **102** have been flushed out or rinsed out (e.g., using saline solution) by master controller **104** and fluidic system **108**.

In some embodiments, master controller **104** may detect and determine whether there are any molecules or other contents of interest remaining in the cells of nanopore array **102**. Master controller **104** and fluidic system **108** may selectively rinse out the contents (e.g., lipid bilayers) within cells in which no molecules or other contents of interest are found. The molecules or other contents of interest in the remaining cells may be retrieved. In one example, the molecules may be retrieved manually. In another example, master controller **104** and fluidic system **108** may deliver the molecules or other contents of interest to storage device **110** before the remaining contents are rinsed out. After **514**, nanopore array **102** is ready for repeated uses again, and

process **500** may be restarted at **502**. In some embodiments, step **514** is performed before a nanopore array **102** is used for the first time. For example, nanopore array **102** is rinsed with saline solution before the functionalities of system **100** is checked at **502**.

Although the foregoing embodiments have been described in some detail for purposes of clarity of understanding, the invention is not limited to the details provided. There are many alternative ways of implementing the invention. The disclosed embodiments are illustrative and not restrictive.

What is claimed is:

1. A method for analyzing molecules using a nanopore array including a plurality of cells included on a chip, the method comprising:

assembling a first set of membranes in the plurality of cells;

inserting a first set of nanopores in the first set of membranes;

analyzing a first sample using the first set of nanopores;

after analyzing the first sample, rinsing out the plurality of cells to remove the first sample, the first set of membranes, and the first set of nanopores from within the plurality of cells;

assembling a second set of membranes in the plurality of cells;

inserting a second set of nanopores in the second set of membranes; and

analyzing a second sample using the second set of nanopores.

2. The method of claim **1**, wherein the first set of membranes and the second set of membranes are lipid bilayers.

3. The method of claim **1**, wherein the step of inserting a first set of nanopores comprises applying an electrical stimulus across the first set of membranes.

4. The method of claim **1**, wherein the step of analyzing the first sample comprises:

applying a voltage across the first set of nanopores; and

measuring an electrical signal in response to the applied voltage across the first set of nanopores.

5. The method of claim **1**, wherein the step of inserting a first step of nanopores comprises flowing a solution of nanopores over the first set of membranes and applying an electrical stimulus to the cells.

6. A system for analyzing a plurality of molecules, the system comprising:

a nanopore array including a plurality of cells included on a chip;

a fluidic system configured to send one or more reagents to the nanopore array;

a data interface configured to receive measurements from the plurality of cells; and

a master controller, the master controller programmed to:

assemble a first set of membranes in the plurality of cells;

insert a first set of nanopores in the first set of membranes;

analyze a first sample using the first set of nanopores;

after analyzing the first sample, rinse out the plurality of cells to remove the first sample, the first set of membranes, and the first set of nanopores from within the plurality of cells;

assemble a second set of membranes in the plurality of cells;

insert a second set of nanopores in the second set of membranes; and

analyze a second sample using the second set of nanopores.

7. The system of claim **6**, wherein the first set of membranes and the second set of membranes are lipid bilayers.

8. The system of claim **6**, wherein the master controller is programmed to apply an electrical stimulus across the first set of membranes during the step of inserting the first set of nanopores.

9. The system of claim **6**, wherein, during the step of analyzing the first sample, the master controller is programmed to:

apply a voltage across the first set of nanopores; and

measure an electrical signal in response to the applied voltage across the first set of nanopores.

10. The system of claim **6**, wherein, during the step of inserting a first step of nanopores, the master controller is programmed to flow a solution of nanopores over the first set of membranes and apply an electrical stimulus to the cells.

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