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(54) **METHOD OF FORMING PLANAR LIPID DOUBLE MEMBRANE FOR MEMBRANE PROTEIN ANALYSIS AND APPARATUS THEREFOR**

VERFAHREN ZUR BILDUNG EINER FLACHEN LIPIDDOPPELMEMBRAN ZUR MEMBRANPROTEINANALYSE SOWIE VORRICHTUNG DAFÜR

METHODE PERMETTANT DE FORMER UNE MEMBRANE LIPIDIQUE DOUBLE PLANE A DES FINS D'ANALYSE PROTEIQUE MEMBRANAIRE

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- **PAUL S CREMER & TINGLU YANG: "Creating Spatially Addressed Arrays of Planar Supported Fluid Phospholipid Membranes" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC.; US, US, vol. 121, 19 August 1999 (1999-08-19), pages 8130-8132, XP002160898 ISSN: 0002-7863**
- **FERTIG N ET AL: "Microstructured glass chip for ion-channel electrophysiology" PHYSICAL REVIEW E. STATISTICAL PHYSICS, PLASMAS, FLUIDS, ANDRELATED INTERDISCIPLINARY TOPICS, AMERICAN INSTITUTE OF PHYSICS, NEW YORK, NY, US, vol. 64, no. 4 I, 1 October 2001 (2001-10-01), pages 409011-409014, XP002381286 ISSN: 1063-651X**
- **SUZUKI ET AL: 'Micro Ryuro o Mochiita Shishitsu Heimen no Saikosei' KAGAKU TO MICRO NANO SYSTEM KENKYUKAI KOEN YOSHISHU vol. 8, 2003, page 61, XP002992134**
- **SUZUKI ET AL: 'PLANAR LIPID MEMBRANE ARRAY FOR MEMBRANE PROTEIN CHIP' IEEE INTERNATIONAL CONFERENCE ON MICRO ELECTRO MECHANICAL SYSTEMS 25 January 2004 - 29 January 2004, pages 272 - 275, XP002992135**



EP 1 712 909 B1

## Description

Technical Field

**[0001]** The present invention relates to methods of forming planar lipid-bilayer membranes for membrane protein analysis and devices therefor. The planar lipid-bilayer membranes are used in fields such as biotechnology, biochips, membrane protein analysis, drug discovery screening, and biosensors.

Background Art

**[0002]** As typical methods for producing planar lipid-bilayer membranes used in analysis of membrane proteins such as ion channels, painting method and Langmuir-Blodgett method (LB method) are conventionally known. Both methods are of forming a planar lipid-bilayer membrane in an aperture opened in a Teflon (registered trademark) sheet in a chamber filled with a buffer solution. The aperture has a size of several-hundred microns. In the painting method, a lipid solution is applied to the aperture with a brush. The LB method utilizes the fact that a monomolecular layer of a lipid molecule is formed on the surface of a solution. In this method, a planar lipid-bilayer membrane is formed by gradually raising the solution surface level at both sides of a Teflon (registered trademark) sheet in a chamber.

**[0003]** FIG. 1 is a schematic diagram showing a method of forming a planar lipid-bilayer membrane by the LB method.

**[0004]** In the drawing, a reference numeral 1 denotes a Teflon (registered trademark) sheet, a reference numeral 2 denotes an aperture formed in the Teflon (registered trademark) sheet 1, a reference numeral 3 denotes a solution on the surface of which a monomolecular layer 4 of lipid is formed, and a reference numeral 5 denotes a buffer solution. A planar lipid-bilayer membrane 6 is formed by gradually raising the surface level of the solution 3 at both sides of the Teflon (registered trademark) sheet 1 in a chamber.

Patent Document 1: Japanese Unexamined Patent Application Publication No. 02-35941
Patent Document 2: Japanese Unexamined Patent Application Publication No. 05-253467
Patent Document 3: Japanese Unexamined Patent Application Publication No. 07-241512
Patent Document 4: Japanese Unexamined Patent Application Publication No. 2002-505007
Patent Document 5: Japanese Unexamined Patent Application Publication No. 2003-511679
Patent Document 6: Japanese Patent Application No. 2003-329667

**[0005]** WO 00/25121A discloses a device for generating an oscillating electrical current, the device incorporating an ion channel. The device has nanoscale dimensions and thus can transform biological processes into an electrical output.

**[0006]** WO 94/25862 discloses a biosensor substrate for mounting a bilayer lipid membrane containing a receptor.

**[0007]** PAUL S CREMER & TINGLU YANG: "Creating Spatially Addressed Arrays of Planar Supported Fluid Phospholipid Membranes" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC.; US, US, vol. 121, 19 August 1999 (1999-08-19), pages 8130-8132, XP002160898 ISSN: 0002-7863 discloses a methodology for the formation of spatially addressed phospholipid bilayers containing multifarious lipids, peptides, receptors and membrane-associated proteins.

**[0008]** WO 02/29402 discloses a system for determining and/or monitoring electrophysiological properties of ion channels or ion channel-containing membranes, typically lipid membrane-containing structures such as cells.

**[0009]** SUZUKI ET AL: 'Micro Ryuro o Mochiita Shishitsu Heimen no Saikosei' KAGAKU TO MICRO NANO SYSTEM KENKYUKAI KOEN YOSHISHU vol. 8, 2003, page 61, discloses a planar lipid membrane chip with a flow process in both the upper and lower flow channels.

Non-Patent Document 1: H. Zhu, et al., "Global Analysis of Protein Activities Using Proteome Chips", Science, Vol. 293, pp. 2101-2105, 2001.

Non-Patent Document 2: B. Alberts, et al., "Molecular Biology of the Cell; 4th Ed.", Garland Science, 2002.

Non-Patent Document 3: C. Miller, ed., "Ion Channel Reconstitution", Plenum Press, 1986.

Non-Patent Document 4: T. Ide and T. Yanagida, "An Artificial Lipid Bilayer Formed on an Agarose-Coated Glass for Simultaneous Electrical and Optical Measurement of Single Ion Channels," Biochem. Biophys. Res. Comm., 265, pp. 595-599, 1999.

Non-Patent Document 5: T. Ide, Y. Takeuchi, and T. Yanagida, "Development of an Experimental Apparatus for Simultaneous Observation of Optical and Electrical Signals from Single Ion Channels," Single Molecules, 3(1), pp. 33-42, 2002.

Non-Patent Document 6: J. T. Groves, N. Ulman, and S. G. Boxer, "Micropatterning Fluid Lipid Bilayers on Solid Supports," Science, Vol. 275, pp. 651-653.

Non-Patent Document 7: M. Mayer, et al., "Microfabricated Teflon Membranes for Low-Noise Recording of Ion Channels in Planar Lipid Bilayers," Biophys. J., Vol. 85, pp. 2684-2695, 2003.

Non-Patent Document 8: Fertig et al., "Microstructured Glass Chip for Ion-Channel Electrophysiology," Phys. Rev. E., Vol. 64, 040901(R), 2001.

Non-Patent Document 9: H. Suzuki, H. Noji, S. Takeuchi, SEIBUTSU BUTSURI (Biophysics), Vol. 43, SUPPLEMENT 1, p. S118, B374, August 2003

Disclosure of Invention

**[0010]** Both methods mentioned above require large chambers of about several centimeters in size. Therefore, the dead volumes are large and microscopic observation cannot be performed. Additionally, in these methods, when a plurality of planar lipid-bilayer membranes are simultaneously formed in a channel by providing a plurality of apertures, adjacent apertures (planar lipid-bilayer membranes) are electrically connected to each other through a buffer solution in the channel and the electrophysiological measurement of each membrane is difficult.

**[0011]** Furthermore, basically, only one planar lipid-bilayer membrane is formed at a time. Therefore, multichannel analysis is impossible. In addition, these methods require an experienced skill and their repeatability is low.

**[0012]** The present inventors have already proposed a method of forming an artificial lipid membrane and a device therefor (the Patent Document 6), in which a first and second microchannels are formed and the flow of a lipid solution in the second microchannel is controlled so that a planar lipid-bilayer membrane is formed.

**[0013]** In the method, firstly, the first microchannel is filled with a buffer solution (aqueous solution), and then the second microchannel having an aperture is filled with a lipid solution. Then, the lipid solution is discharged by infusing air to the second microchannel. A part of the lipid solution remains at the interface of the buffer solution in the aperture at this step. Then, the buffer solution is introduced into the second microchannel to discharge the air. The air is replaced with the buffer solution and a planar lipid-bilayer membrane is thereby formed in the aperture.

**[0014]** However, in this method, the number of the steps for the formation of a planar lipid-bilayer membrane is large and the process is complicated. In addition, it is difficult to control the thickness of the planar lipid-bilayer membrane.

**[0015]** Recently, it has been required to apply different kinds of reagents or different kinds of proteins to a multichamber device and to measure their reaction/binding. However, no existing technologies meet such requirement.

**[0016]** Under such circumstances, it is an object of the present invention to provide a method of forming a planar lipid-bilayer membrane array for membrane protein analysis, which is capable of downsizing, simplifying, and multichanneling of a device therefor.

**[0017]** The present invention provides a method and a device for forming a planar lipid-bilayer as claimed.

Brief Description of the Drawings

**[0018]**

FIG. 1 is a schematic diagram showing a method of forming a planar lipid-bilayer membrane by the LB method.

FIG. 2 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a first embodiment of the present invention.

FIG. 3 is a schematic diagram of a lipid solution according to the present invention.

FIG. 4 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a second embodiment of the present invention.

FIG. 5 is a diagram showing the incorporation of a membrane protein into a planar lipid-bilayer membrane by using a liposome according to the present invention.

FIG. 6 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a third embodiment of the present invention.

FIG. 7 is schematic diagrams of a device for forming a planar lipid-bilayer membrane according to a fourth embodiment of the present invention.

FIG. 8 is cross-sectional views of a well array chip of the devices for forming planar lipid-bilayer membranes in a fabrication process thereof according to the fourth embodiment of the present invention.

FIG. 9 is enlarged plan views of a part of an array of the devices for forming planar lipid-bilayer membranes according to the fourth embodiment of the present invention.

FIG. 10 is a perspective view of a microinjection device of an array of the devices for forming planar lipid-bilayer membranes according to a fifth embodiment of the present invention.

Best Mode for Carrying Out the Invention

**[0019]** According to the present invention, a planar lipid-bilayer membrane is formed: a microchannel is filled with a buffer solution, the microchannel being disposed under a horizontal partition wall having an aperture; a chamber being formed at a position corresponding to the aperture and provided with a liquid trap on the partition wall inside the chamber; a small amount of a lipid solution is applied as a droplet to the aperture filled with the buffer solution to form a thin layer of the lipid solution in a channel,; and a buffer solution is applied as a droplet to the chamber from the upper side of the chamber to thereby form the planar lipid-bilayer membrane. Consequently, the amount of the lipid solution to be introduced to the chamber can be precisely controlled, and the planar lipid-bilayer membrane can be readily formed (reconstituted)

with a high repeatability.

**[0020]** The present invention will now be described in detail with reference to the embodiments.

First Embodiment

**[0021]** FIG. 2 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a first embodiment of the present invention. FIG. 3 is a schematic diagram showing a lipid solution.

**[0022]** In FIG. 2, a reference numeral 11 denotes a glass substrate, a reference numeral 12 denotes a microchannel, a reference numeral 13 denotes a partition wall, a reference numeral 14 denotes an aperture (opening) provided to the partition wall 13, a reference numeral 15 denotes a liquid trap provided on the partition wall 13, a reference numeral 17 denotes a chamber defined by a well 16, a reference numeral 18 denotes a buffer solution which fills the microchannel 12 and the aperture (opening) 14, a reference numeral 19 denotes a microinjection device (microinjector), a reference numeral 20 denotes a lipid solution applied as a droplet from the microinjection device 19, a reference numeral 21 denotes a layer of the lipid solution, a reference numeral 22 denotes a microinjection device (microinjector or pipette) for applying a buffer solution as a droplet, a reference numeral 23 denotes a buffer solution applied as a droplet from the microinjection device 22, and a reference numeral 24 denotes a planar lipid-bilayer membrane.

**[0023]** In the device for forming (reconstituting) a planar lipid-bilayer membrane, as described above, the microchannel 12 and the chamber 17 are separated from each other by the partition wall 13 having the aperture (opening) 14.

**[0024]** Firstly, as shown in FIG. 2(a), the microchannel 12 and the aperture 14 are filled with the buffer solution 18 (KCl or aqueous solution). At this stage, the interface of the buffer solution 18 stops at the aperture (opening) 14 due to the surface tension. Here, the aperture (opening) 14 is provided with a taper 13A so that the diameter of the aperture 14 narrows from the lower side toward the upper side. Thus, the interface of the buffer solution 18 readily stops at the aperture (opening) 14.

**[0025]** Then, as shown in FIG. 2(b), the lipid solution 20 is applied as a droplet to the aperture (opening) 14 by using the microinjection device 19. At this stage, the excess of the lipid solution 20 flows into the liquid trap 15 provided at the periphery of the aperture (opening) 14. Accordingly, the layer (lipid solution layer 21) of the remaining lipid solution 20 at the interface of the buffer solution 18 can be sufficiently thinned (submicrometer order).

**[0026]** Lastly, as shown in FIG. 2(c), the buffer solution 23 is applied as a droplet to the chamber 17 by using the microinjection device 22, and thereby a planar lipid-bilayer membrane (thickness: about 10 nm) 24 is spontaneously formed.

**[0027]** As described above, (1) the microchannel 12 and the aperture 14 are filled with the buffer solution 18, (2) a small amount of the lipid solution 20 is applied as a droplet, and (3) the buffer solution 23 is applied to the chamber 17 as a droplet. As a result, a layer (lipid solution layer 21) of the lipid solution 20 is spontaneously assembled to a planar lipid-bilayer membrane 24.

**[0028]** Here, as shown in FIG. 3(a), the lipid solution 20 includes a component (phospholipids) having a hydrophilic group 20A and a hydrophobic group 20B. By thinning the lipid solution layer, as shown in FIG. 3(b), the hydrophobic group 20B is arranged to face inside, and engaged and bound to each other to form the planar lipid-bilayer membrane 24.

**[0029]** For forming the bilayer, the layer of the lipid solution must be thinned as much as possible (nm order). Then, a means for controlling the thickness of the layer by communication with the lipid trap 15 may be provided, as described below.

Second Embodiment

**[0030]** FIG. 4 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a second embodiment of the present invention.

**[0031]** In a second embodiment, in addition to the components in the first embodiment, a first thin-film electrode 25 is provided on the glass substrate 11 of the microchannel 12 and a second thin-film electrode 26 is provided on the partition wall 13 within the chamber 17 defined by the well 16. Since the thin-film electrodes 25 and 26 are independently provided in the chamber 17 defined by the well 16, the membrane potential and current can be measured.

**[0032]** In order to incorporate a membrane protein to be analyzed into the planar lipid-bilayer membrane 24 formed at the interface of the buffer solution 18, a spherical vesicle (liposome) of the same lipid bilayer is used.

**[0033]** As shown in FIG. 5, a liposome 31 containing Alamethicin 32 which is a channel protein is prepared. Droplets of the liposome is mixed in the buffer solution 23, and introduced to the planar lipid-bilayer membrane 24 as a droplet. The liposome 31 and the planar lipid-bilayer membrane 24 are spontaneously fused to each other by the contact of the liposome 31 with the planar lipid-bilayer membrane 24, and Alamethicin 32 is incorporated into the planar lipid-bilayer membrane 24. The present inventors have succeeded, as a test case, to insert Alamethicin into a planar lipid-bilayer membrane by fusing a liposome containing Alamethicin to the planar lipid-bilayer membrane formed by a known planar lipid-bilayer method. Alamethicin is a peptide that stochastically forms ion channels by oligomerization, changing transiently between its open and close states. The membrane current was measured with the addition of the buffer solution containing Alamethicin to confirm the fusion of the membrane protein (peptide) to the bilayer.

Third Embodiment

**[0034]** FIG. 6 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a third embodiment of the present invention.

**[0035]** In this embodiment, the device is provided with a channel 12A connecting to the liquid trap 15. Therefore, it is possible to control the thickness of the layer of the lipid solution remaining on the interface of the buffer solution 18. In other words, when a thick lipid solution layer 21 is formed by the lipid solution 20 remaining on the interface of the buffer solution 18, the thickness of the lipid solution layer 21 can be decreased by sucking the excess of the lipid solution 20 through the channel 12A connecting to the liquid trap 15. Reversely, when a thin lipid solution layer 21 is formed by the lipid solution 20 remaining on the interface of the buffer solution 18, the thickness of the lipid solution layer 21 can be increased by pushing the lipid solution 20 back through the channel 12A connecting to the liquid trap 15.

Fourth Embodiment

**[0036]** FIG. 7 is schematic diagrams of a device for forming a planar lipid-bilayer membrane according to a fourth embodiment of the present invention. FIG. 7(a) is a perspective view of the top face of chambers in an array. FIG. 7(b) is a cross-sectional view of a well array chip.

**[0037]** In the drawings, a reference numeral 41 denotes a glass substrate, a reference numeral 42 denotes a microchannel, a reference numeral 43 denotes a partition wall formed of silicon, a reference numeral 44 denotes an aperture formed by etching the partition wall 43, a reference numeral 45 denotes a liquid trap formed at the periphery of the aperture 44, a reference numeral 47 denotes a chamber defined by a well 46, a reference numeral 48 denotes a buffer solution which fills the microchamber 42 and the aperture 44, a reference numeral 49 denotes a planar lipid-bilayer membrane, a reference numeral 50 denotes a buffer solution applied as a droplet on the planar lipid-bilayer membrane 49, a reference numeral 51 denotes a first thin-film electrode disposed on the glass substrate 41 at the position under the aperture 44, a reference numeral 52 denotes a second thin-film electrode disposed at the periphery of the liquid trap 45, and a reference numeral 53 denotes a power source with ammeter disposed between the first thin-film electrode 51 and the second thin-film electrode 52. The partition wall 43 may be formed of an acrylic plastic instead of silicon. The acrylic plastic can be mechanically processed.

**[0038]** As described above, in this embodiment, the chambers 47 are each defined by a well 46 and disposed in an array.

**[0039]** Therefore, a plurality of kinds of membrane proteins can be simultaneously measured by applying liposomes each containing a different membrane protein to the respective chambers 47. The different membrane proteins are each incorporated into the respective planar lipid-bilayer membranes formed in an array according to this embodiment with a microinjection device for a reagent. Then, the membrane proteins are simultaneously measured with a multichannel system. For example, membrane proteins A and B are separately incorporated into different planar lipid-bilayer membranes. When a reagent which suppresses or activates either of these membrane proteins is applied to chambers through the channel, electric signals of the membrane proteins A and B are different from that of each other. In addition, another signal can be obtained by applying a reagent having another effect. Thus, a plurality of measurements can be simultaneously performed with a high sensitivity to analyze how the membrane proteins react to which reagents.

**[0040]** A measuring system (not shown) according to the present invention includes a planar lipid-membrane chip, an injection device for injecting a membrane protein (liposome) a syringe pump for injecting a reagent, an amplifier (patch amplifier) for amplifying small membrane current/voltage, and a computer for result analysis. Firstly, planar lipid-bilayer membranes are formed in an array according to the present invention. Liposomes each containing a different objective membrane protein are applied to the planar lipid-bilayer membranes with the microinjection device. The membrane currents/voltages when various reagents are applied to each membrane protein through the microchannel are measured by using the thin-film electrodes, and signals amplified by the amplifier are incorporated into the computer. The computer analyzes the output signals. Thus, identification and functional analysis of each membrane protein can be performed.

**[0041]** In addition, the temperature of each chamber in an array may be independently controlled. Liposomes each containing a different membrane protein are applied to the planar lipid-bilayer membranes. Thus, the proteins different in temperature may be simultaneously measured. In such a case, a heating device (not shown) is provided to each chamber.

**[0042]** FIG. 8 is cross-sectional views of a well array chip of the devices for forming planar lipid-bilayer membranes in a fabrication process thereof according to the fourth embodiment of the present invention.

(1) Firstly, as shown in FIG. 8(a), oxide films 62 are formed on the top and bottom faces of a silicon substrate 61.

(2) Then, as shown in FIG. 8(b), one of the oxide films 62 is patterned and tiny holes (50 to 100 μm in width and 200 μm in depth) 63 are formed by reactive ion etching.

(3) Then, as shown in FIG. 8(c), a microchannel 64 and an aperture 65 are etched using tetramethylammonium hydroxide (TMAH).

(4) Then, as shown in FIG. 8(d), a liquid trap 66 is formed at the periphery of the aperture 65 by etching

the oxide film 62 and the silicon substrate 61.

(5) Then, as shown in FIG. 8(e), the entire chip is coated with Parylene C 67 for the electrical insulation.

(6) Finally, as shown in FIG. 8(f), a lower electrode 68 and a glass substrate 69 are bonded to the bottom side.

On the top side, an upper electrode (Au) 70 is patterned and a well 71 is formed of a resist (SU8: product name) of 40 μm in thickness.

**[0043]** FIG. 9 is an enlarged plan view of a part of an array of the devices for forming planar lipid-bilayer membranes according to the fourth embodiment of the present invention. FIG. 9(a) shows an array chip, and FIG. 9(b) shows an enlarged view of the chip.

**[0044]** In these drawings, apertures 65 are each surrounded by a liquid trap 66 formed in a shape of a square trench. At the periphery of each liquid trap 66 formed in a shape of a square trench, an upper electrode 70 is disposed. SU8 wells 71 are formed so as to define each chamber.

**[0045]** Here, in the drawings, the size of the aperture 65 formed at the center is 200 μm, the size of the well 71 is 900 μm. The size and depth of the liquid trap 66 are 500 μm and 40 μm, respectively. The capacity of the liquid trap 66 is 8 nL (8 nanoliters). The upper electrodes 79 are electrically separated for each chamber and the lower electrode 68 is common to all chambers.

Fifth Embodiment

**[0046]** FIG. 10 is a perspective view of a microinjection device of an array of the devices for forming planar lipid-bilayer membranes according to a fifth embodiment of the present invention.

**[0047]** In this drawing, reference numerals 81 to 89 denote nozzles of the microinjection device. Each of the nozzles corresponds to the respective chambers of a well array chip 92. A reference numeral 90 denotes a cover integrated with the nozzles. The cover is provided for positioning the nozzles 81 to 89 of the microinjection device relative to each chamber of the well array chip 92. A reference numeral 91 denotes an engaging member for engaging the cover with the well array chip 92 for the positioning.

**[0048]** The drying of the planar lipid-bilayer membrane disadvantageously affects the measurement. By immediately installing the cover 90 to the well array chip 92 after the production of the planar lipid-bilayer membranes containing membrane proteins, the drying of the planar lipid-bilayer membranes in an array can be reduced.

**[0049]** During the measurement, the drying of buffer solutions in the chambers can be avoided by optionally applying a buffer solution as a droplet to each chamber through the respective nozzles 81 to 89 of the microinjection device.

**[0050]** The present invention is not limited to the above-mentioned embodiments. Various modifications based on the concept of the present invention are possible and are within the scope of the present invention.

**[0051]** According to the present invention, the following advantageous effects are achieved:

(1) The device for forming a planar lipid-bilayer membrane for membrane protein analysis includes a substrate, a partition wall disposed over the substrate so as to be parallel to the substrate, a microchannel defined by the substrate and the partition wall, and a chamber having an aperture provided to the partition wall and a liquid trap formed at the periphery of the aperture. The amount of a lipid solution can be precisely controlled and injected to the chamber from the upper side of the chamber with a microinjection device (microinjector). Planar lipid-bilayer membranes with high repeatability can be readily formed (reconstituted).

(2) Since the apertures and chambers disposed in an array are independent to each other as a measurement system, many kinds of measurements can be simultaneously conducted. Therefore, fast membrane protein analysis can be achieved.

(3) Since the measurement system and the channel for injecting a reagent are fabricated in a microscale (less than 1 mm), the dead volume is considerably reduced to significantly decrease the amounts of the reagent and the sample of necessary.

(4) Since the size of the measurement system is very small, the measurement is not easily affected by external electric noise. Thus, the electrical measurement can be further precisely performed.

Industrial Applicability

**[0052]** The present invention is suitable for biotechnology, biochips, membrane protein analysis, drug discovery screening, and biosensors, and can be applied to an ultrasensitive membrane-protein analysis device, an ultrasensitive multichannel drug discovery screening device, and an ultrasensitive ion sensor.

**Claims**

1. A method of forming a planar lipid-bilayer membrane (24, 49) for membrane protein analysis, the method using a horizontal partition wall (13, 43) disposed over a substrate so as to be parallel to the substrate, said partition wall having an aperture (14, 44) with a liquid trap (15, 45) formed at the periphery of the aperture (14, 44) on the partition wall (13, 43) inside a chamber (17, 47) defined by a well (16, 46), the chamber (17, 47) being formed at a position corresponding to the aperture (14, 44), the method comprising the steps of:

(a) filling a microchannel with a buffer solution (18, 48), the microchannel (12, 42) being disposed under the partition wall (13, 43);
(b) filling the aperture (14, 44) with the buffer solution (18, 48);
(c) applying a small amount of a lipid solution (20) as a droplet to the aperture (14, 44) to form a thin layer of the lipid solution (21) in the chamber (17, 47); and
(d) applying a buffer solution (23, 50) as a droplet to the chamber (17, 47) from the upper side thereof.

**2.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 1, wherein the thickness of the thin layer of the lipid solution (21) is controlled by a channel connected to the liquid trap.

**3.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 1 or 2, wherein the buffer solution (18, 48) contains a liposome incorporated with an objective membrane protein, and the liposome is fused with the planar lipid-bilayer membrane (24, 49) to incorporate the membrane protein into the planar lipid-bilayer membrane (24, 49).

**4.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 1, wherein a plurality of the chambers (17, 47) are integrally formed.

**5.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 4, wherein the plurality of the chambers (17, 47) are formed in an array.

**6.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 4 or 5, wherein liposomes each containing a different protein are each applied to a different chamber (17, 47), and different kinds of proteins are simultaneously measured.

**7.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 4 or 5, wherein the reaction/binding of different kinds of reagents or different kinds of proteins in each of the chambers (17, 47) are simultaneously measured.

**8.** The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 4 or 5, wherein the temperature of each chamber is independently controlled, liposomes each containing a different protein are each applied to a different chamber (17, 47), and the proteins different in temperature are simultaneously measured.

**9.** A device for forming a planar lipid-bilayer membrane for membrane protein analysis, the device comprising:

(a) a substrate (11, 41);
(b) a horizontal partition wall (13, 43) having an aperture (14, 44) disposed over the substrate (11, 41) so as to be parallel to the substrate (11, 41);
(c) a microchannel (12, 42) defined by the substrate (11, 41) and the partition wall (13, 43);
(d) a chamber (17, 47) defined by a well (16, 46) formed in the partition wall (13, 43) and a liquid trap (15, 45) formed at the periphery of the aperture (14, 44) on the partition wall (13, 43) inside the chamber (17, 47), the chamber (17, 47) being formed at a position corresponding to the aperture (14, 44);
(e) a microinjection device (19) for applying droplets of a lipid solution (20) to the aperture (14, 44) and the liquid trap (15, 45) from the upper side of the chamber (17, 47) to form a thin layer of the lipid solution (21); and
(f) a microinjection device (22) for applying a buffer solution (23, 50) to form a planar lipid-bilayer (24, 49).

**10.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 9, the device further comprising a first thin-film electrode disposed on the substrate (11, 41) at the position corresponding to the chamber (17, 47) and a second thin-film electrode (26, 52) disposed near the liquid trap.

**11.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 9 or 10, wherein the partition wall (13, 43) has a channel (12A) connected to the liquid trap (15, 45) for controlling the thickness of the layer of the lipid solution (21).

**12.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 9 or 10, wherein a plurality of the chambers (17, 47) are integrally formed.

**13.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 12, wherein the plurality of the chambers (17, 47) are formed in an array.

**14.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 12 or 13, wherein the microinjection device (22) further includes a cover (90) for positioning the mi-

croinjection device relative to each chamber (17, 47).

**15.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 12 or 13, the device further comprising a means for applying liposomes each containing a different protein to the respective chambers (17, 47) and simultaneously measuring the different kinds of proteins.

**16.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 12 or 13, the device further comprising a means for independently controlling the temperature of each chamber in an array, applying liposomes each containing a different protein to the respective chamber (17, 47), and simultaneously measuring the proteins different in temperature.

**17.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 9, wherein the aperture is provided with a taper so that the diameter of the aperture (14, 44) narrows from the lower side toward the upper side.

**18.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 9, wherein the partition wall (13, 43) is formed of a silicon substrate and the aperture (14, 44) is formed by etching the silicon substrate.

**19.** The device for forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 10, the device further comprising a means for measuring a property of the membrane protein by applying a voltage between the first thin-film electrode (25, 51) and the second thin-film electrode (26, 52).

**Patentansprüche**

**1.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran (24, 49) zur Membranproteinanalyse, wobei das Verfahren eine horizontale Trennwand (13, 43) verwendet, die über einem Substrat angeordnet ist, so dass sie parallel zum Substrat ist, wobei die Trennwand eine Öffnung (14, 44) mit einer Flüssigkeitsfalle (15, 45) aufweist, die am Umfang der Öffnung (14, 44) an der Trennwand (13, 43) innerhalb einer Kammer (17, 47) ausgebildet ist, die von einer Senke (16, 46) begrenzt ist, wobei die Kammer (17, 47) an einer Position ausgebildet ist, die der Öffnung (14, 44) entspricht und das Verfahren folgende Schritte umfasst:

(a) Füllen eines Mikrokanals mit einer Pufferlösung (18, 48), wobei der Mikrokanal (12, 42) unter der Trennwand (13, 43) angeordnet ist;
(b) Füllen der Öffnung (14, 44) mit der Pufferlösung (18, 48);
(c) Auftragen einer kleinen Menge einer Lipidlösung (20) als Tröpfchen auf die Öffnung (14, 44) zur Bildung einer dünnen Schicht der Lipidlösung (21) in der Kammer (17, 47); und
(d) Auftragen einer Pufferlösung (23, 50) als Tröpfchen auf die Kammer (17, 47) von deren Oberseite.

**2.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 1, wobei die Dicke der dünnen Schicht der Lipidlösung (21) durch einen mit der Flüssigkeitsfalle verbundenen Kanal gesteuert wird.

**3.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 1 oder 2, wobei die Pufferlösung (18, 48) ein Liposom enthält, in das ein Zielmembranprotein aufgenommen ist, und das Liposom mit der flachen Lipiddoppelschichtmembran (24, 49) verschmolzen wird, um das Membranprotein in die flache Lipiddoppelschichtmembran (24, 49) aufzunehmen.

**4.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 1, wobei mehrere der Kammern (17, 47) einstückig ausgebildet sind.

**5.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 4, wobei die mehreren Kammern (17, 47) in einer Anordnung ausgebildet sind.

**6.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 4 oder 5, wobei Liposome, die jeweils ein anderes Protein enthalten, jeweils in einer anderen Kammer (17, 47) aufgetragen und verschiedene Proteinarten gleichzeitig gemessen werden.

**7.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 4 oder 5, wobei die Umsetzung/Bindung unterschiedlicher Reagenzienarten oder unterschiedlicher Proteinarten in jeder der Kammern (17, 47) gleichzeitig gemessen werden.

**8.** Verfahren zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 4 oder 5, wobei die Temperatur jeder Kammer unabhängig gesteuert wird, Liposome, die jeweils ein anderes Protein enthalten, jeweils in einer anderen Kammer (17, 47) aufgetragen werden und die Proteine von unterschiedlicher Temperatur gleichzeitig gemessen werden.

**9.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse, wobei die Vorrichtung umfasst:

(a) ein Substrat (11, 41);
(b) eine horizontale Trennwand (13, 43) mit einer Öffnung (14, 44), die über dem Substrat (11, 41) angeordnet ist, so dass sie parallel zum Substrat (11, 41) ist;
(c) einen Mikrokanal (12, 42), der durch das Substrat (11,41) und die Trennwand (13, 43) begrenzt ist;
(d) eine Kammer (17, 47), die durch eine Senke (16, 46), die in der Trennwand (13, 43) ausgebildet ist, und eine Flüssigkeitsfalle (15, 45), die am Umfang der Öffnung (14, 44) an der Trennwand (13, 43) innerhalb der Kammer (17, 47) ausgebildet ist, begrenzt ist, wobei die Kammer (17, 47) an einer Position ausgebildet ist, die der Öffnung (14, 44) entspricht;
(e) eine Mikroinjektionsvorrichtung (19) zum Auftragen von Tröpfchen einer Lipidlösung (20) auf die Öffnung (14, 44) und die Flüssigkeitsfalle (15, 45) von der Oberseite der Kammer (17, 47) zur Ausbildung einer dünnen Schicht der Lipidlösung (21); und
(f) eine Mikroinjektionsvorrichtung (22) zum Auftragen einer Pufferlösung (23, 50) zur Ausbildung einer flachen Lipiddoppelschicht (24, 49).

**10.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 9, wobei die Vorrichtung weiter eine erste Dünnschichtelektrode umfasst, die auf dem Substrat (11, 41) an der Position angeordnet ist, die der Kammer (17, 47) entspricht, und eine zweite Dünnschichtelektrode (26, 52) umfasst, die nahe der Flüssigkeitsfalle angeordnet ist.

**11.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 9 oder 10, wobei die Trennwand (13, 43) einen Kanal aufweist (12A), der mit der Flüssigkeitsfalle (15, 45) verbunden ist, um die Dicke der Schicht der Lipidlösung (21) zu steuern.

**12.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 9 oder 10, wobei mehrere der Kammern (17, 47) einstückig ausgebildet sind.

**13.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 12, wobei die mehreren Kammern (17, 47) in einer Anordnung ausgebildet sind.

**14.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 12 oder 13, wobei die Mikroinjektionsvorrichtung (22) weiterhin eine Abdeckung (90) zum Positionieren der Mikroinjektionsvorrichtung relativ zu jeder Kammer (17, 47) einschließt.

**15.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 12 oder 13, wobei die Vorrichtung weiter ein Mittel zum Auftragen von Liposomen, die jeweils ein anderes Protein enthalten, in den jeweiligen Kammern (17, 47) und zum gleichzeitigen Messen der verschiedenen Proteinarten umfasst.

**16.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 12 oder 13, wobei die Vorrichtung weiter ein Mittel zum unabhängigen Steuern der Temperatur jeder Kammer in einer Anordnung, Auftragen von Liposomen, die jeweils ein anderes Protein enthalten, in der jeweiligen Kammer (17, 47) und gleichzeitiges Messen der Proteine von unterschiedlicher Temperatur umfasst.

**17.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 9, wobei die Öffnung mit einer Abschrägung versehen ist, so dass sich der Durchmesser der Öffnung (14, 44) von der Unterseite zur Oberseite verjüngt.

**18.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 9, wobei die Trennwand (13, 43) aus einem Siliciumsubstrat ausgebildet ist und die Öffnung (14, 44) durch Ätzen des Siliciumsubstrats ausgebildet ist.

**19.** Vorrichtung zur Bildung einer flachen Lipiddoppelschichtmembran zur Membranproteinanalyse nach Anspruch 10, wobei die Vorrichtung weiter ein Mittel zum Messen einer Eigenschaft des Membranproteins durch Anlegen einer Spannung zwischen der ersten Dünnschichtelektrode (25, 51) und der zweiten Dünnschichtelektrode (26, 52) umfasst.

**Revendications**

**1.** Un procédé de formation d'une membrane lipidique plane bicouche (24, 49) à des fins d'analyse protéique membranaire, le procédé utilisant une paroi de séparation horizontale (13, 43) placée au-dessus d'un substrat de façon à être parallèle au substrat, la paroi de séparation ayant une ouverture (14, 44) avec un réservoir de liquide (15, 45) à la périphérie de l'ouverture (14, 44) sur la paroi de séparation (13, 43) à l'intérieur d'une chambre (17, 47) définie par un puits (16, 46), la chambre (17, 47) se trouvant à

un endroit correspondant à l'ouverture (14, 44), le procédé se composant des opérations suivantes :

(a) remplir un canal de micro-écoulement d'une solution tampon (18, 48), le canal de micro-écoulement (12, 42) se trouvant sous la paroi de séparation (13, 43) ;
(b) remplir l'ouverture (14, 44) de solution tampon (18, 48) ;
(c) appliquer une petite quantité de solution lipidique (20) en tant que gouttelette dans l'ouverture (14, 44) afin de former une fine couche de solution lipidique (21) dans la chambre (17, 47) ; et
(d) appliquer une solution tampon (23, 50) en tant que gouttelette dans la chambre (17, 47) à partir de son côté supérieur.

**2.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit à la revendication 1, dans lequel l'épaisseur de la fine couche de solution lipidique (21) est contrôlée par le canal connecté au réservoir de liquide.

**3.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit aux revendications 1 ou 2, dans lequel la solution tampon (18, 48) contient un liposome incorporé avec une protéine membranaire objective et le liposome est fusionné avec la membrane lipidique plane bicouche (24, 49) pour incorporer la protéine membranaire dans la membrane lipidique plane bicouche (24, 49).

**4.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit à la revendication 1, dans lequel plusieurs des chambres (17, 47) sont intégralement formées.

**5.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit à la revendication 4, dans lequel les différentes chambres (17, 47) sont formées en réseau.

**6.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit aux revendications 4 ou 5, dans lequel des liposomes contenant chacun une protéine différente sont chacun appliqués dans une chambre différente (17, 47) et différents types de protéines sont mesurés simultanément.

**7.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit aux revendications 4 ou 5, dans lequel la réaction/liaison de différents types de réactifs ou de différents types de protéines dans chacune des chambres (17, 47) est mesurée simultanément.

**8.** Le procédé de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire décrit aux revendications 4 ou 5, dans lequel la température de chaque chambre est indépendamment régulée, des liposomes contenant chacun une protéine différente sont chacun appliqués dans une chambre différente (17, 47) et les protéines de température différente sont mesurées simultanément.

**9.** Un dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire, le dispositif se composant de ce qui suit :

(a) un substrat (11, 41) ;
(b) une paroi de séparation horizontale (13, 43) ayant une ouverture (14, 44) disposée au-dessus du substrat (11, 41) de façon à être parallèle au substrat (11, 41);
(c) un canal de micro-écoulement (12, 42) défini par le substrat (11, 41) et la paroi de séparation (13, 43) ;
(d) une chambre (17, 47) définie par un puits (16, 46) formée dans la paroi de séparation (13, 43) et un réservoir de liquide (15, 45) formé à la périphérie de l'ouverture (14, 44) sur la paroi de séparation (13, 43) à l'intérieur de la chambre (17, 47), la chambre (17, 47) étant formée à un endroit correspondant à l'ouverture (14, 44) ;
(e) un dispositif de micro-injection (19) pour l'application de gouttelettes d'une solution lipidique (20) dans l'ouverture (14, 44) et le réservoir de liquide (15, 45) à partir du côté supérieur de la chambre (17, 47) afin de former une couche mince de solution lipidique (21) ; et
(f) un dispositif de micro-injection (22) pour l'application d'une solution tampon (23, 50) en vue de former une membrane lipidique plane bicouche (24, 49).

**10.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon la revendication 9, le dispositif comprenant également une première électrode à film mince sur le substrat (11, 41), à l'endroit correspondant à la chambre (17, 47), et une deuxième électrode à film mince (26, 52) disposée près du réservoir de liquide.

**11.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon les revendications 9 ou 10, dans lequel la paroi de séparation (13, 43) possède un canal (12A) connecté au réservoir de liquide (15, 45) pour

contrôler l'épaisseur de la couche de solution lipidique (21).

**12.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon les revendications 9 ou 10, dans lequel plusieurs des chambres (17, 47) sont intégralement formées.

**13.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon la revendication 12, dans lequel les chambres (17, 47) sont formées en réseau.

**14.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon les revendications 12 ou 13, dans lequel le dispositif de micro-injection (22) comporte également un couvercle (90) afin de positionner le dispositif de micro-injection par rapport à chaque chambre (17, 47).

**15.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon les revendications 12 ou 13, le dispositif comprenant également un dispositif pour appliquer des liposomes contenant chacun une protéine différente dans les chambres respectives (17, 47) et mesurer simultanément les différents types de protéines.

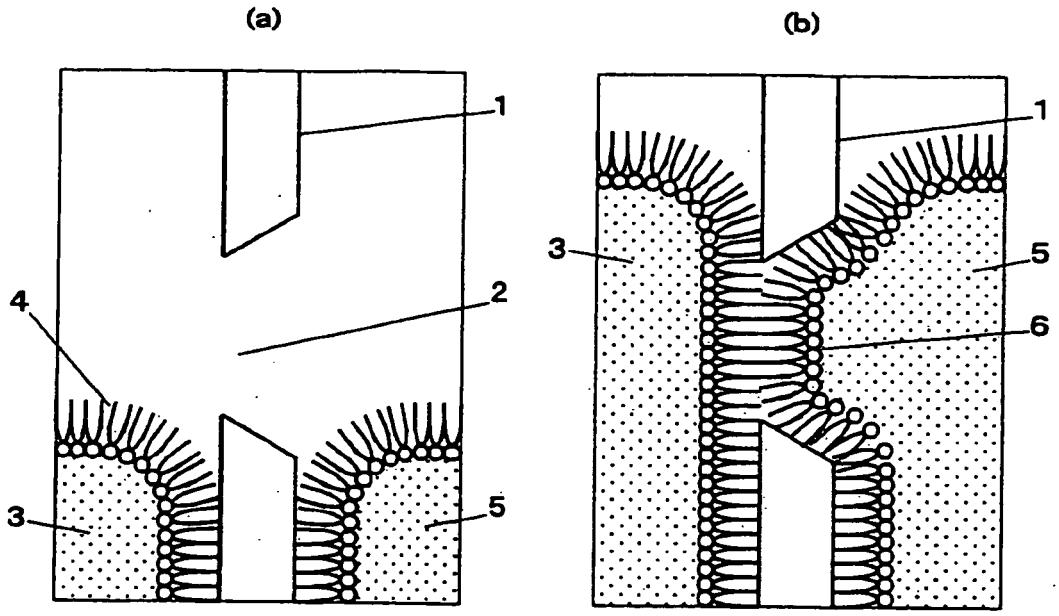
**16.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon les revendications 12 ou 13, le dispositif comprenant également un moyen de réguler indépendamment la température de chaque chambre d'un réseau, en appliquant des liposomes contenant chacun une protéine différente dans la chambre respective (17, 47), et de mesurer simultanément les protéines de température différente.

**17.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon la revendication 9, dans lequel l'ouverture est conique, de telle sorte que le diamètre de l'ouverture (14, 44) se rétrécit du bas vers le haut.

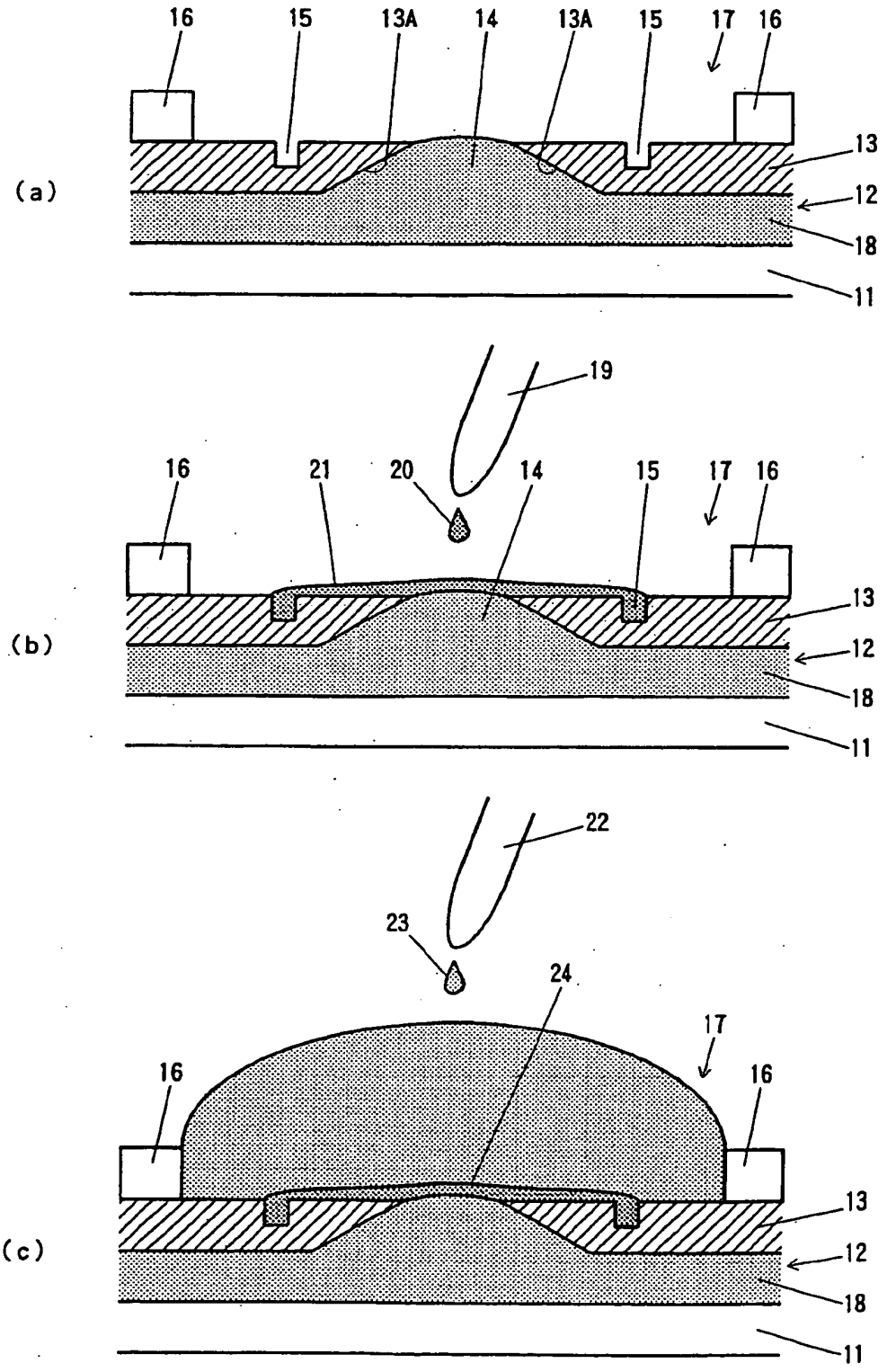
**18.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon la revendication 9, dans lequel la paroi de séparation (13, 43) est formée d'un substrat en silicone et l'ouverture (14, 44) est formée par gravure du substrat en silicone.

**19.** Le dispositif de formation d'une membrane lipidique plane bicouche en vue de l'analyse protéique membranaire selon la revendication 10, le dispositif comprenant également un moyen de mesurer une propriété de la protéine membranaire en appliquant une tension entre la première électrode à film mince (25, 51) et la deuxième électrode à film mince (26, 52).

FIG. 1



# FIG. 2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16
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13A
14
13A
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17
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(b)
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FIG. 3

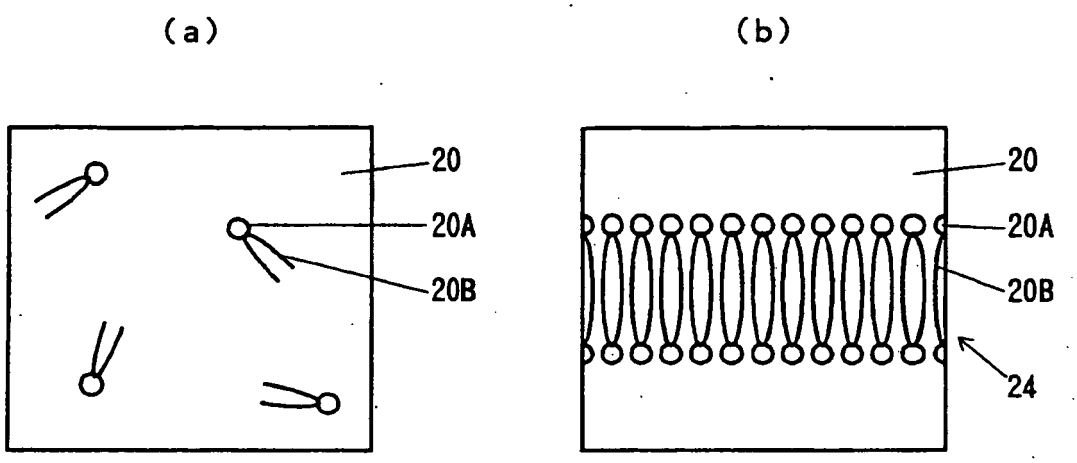
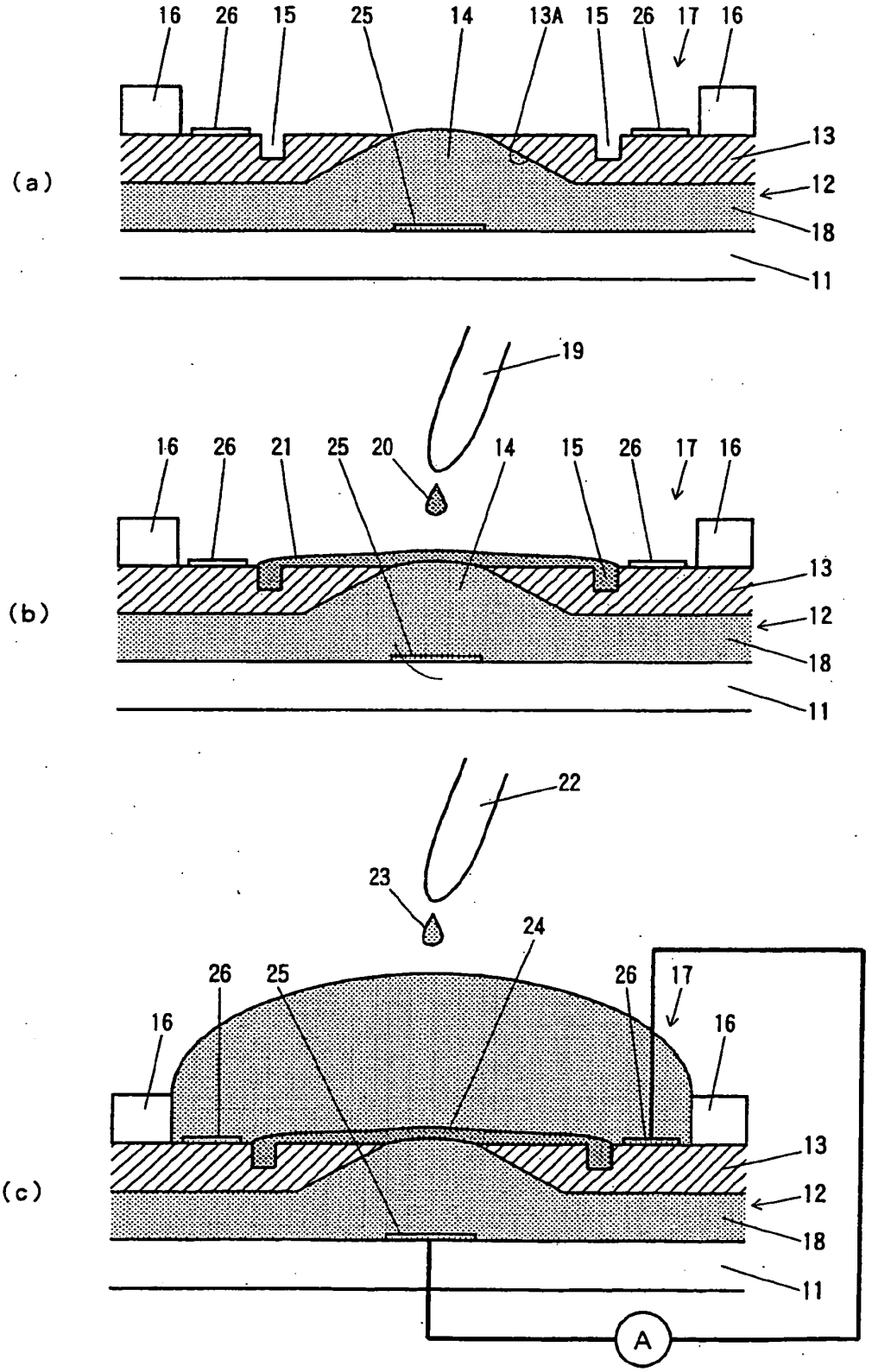


FIG. 4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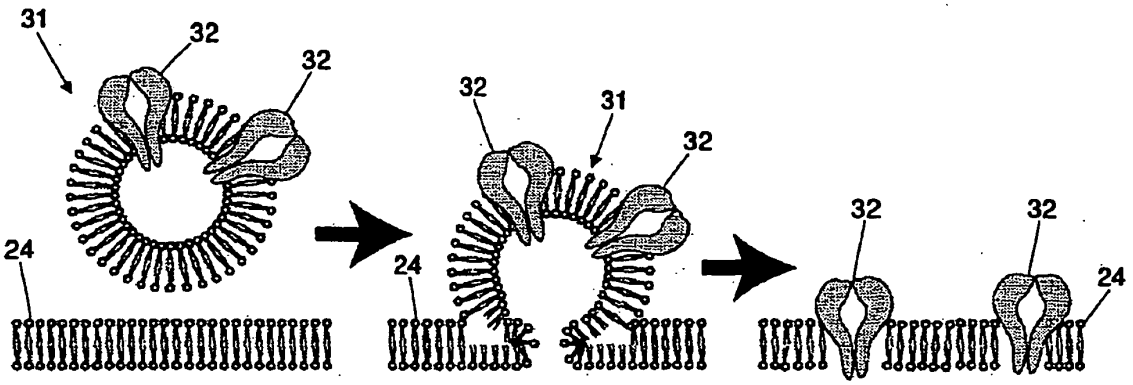
A

FIG. 5




FUSION

FIG. 6

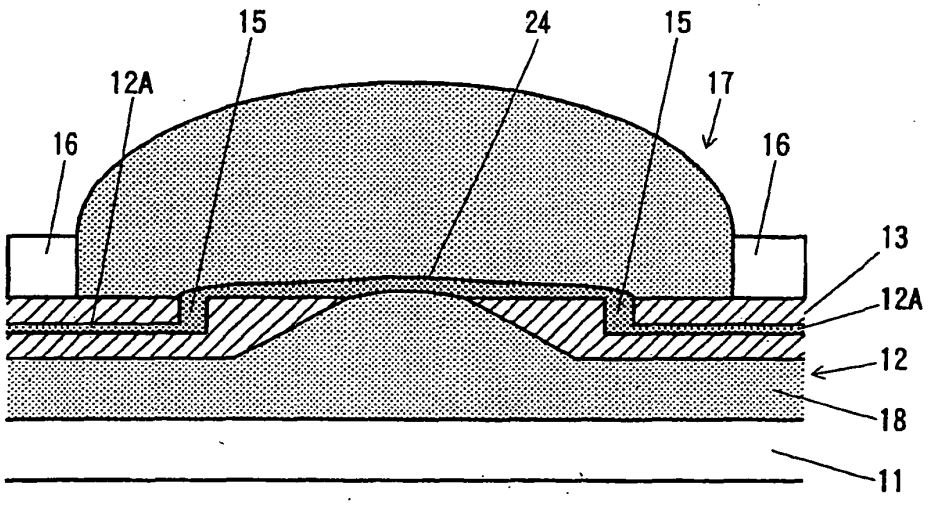


FIG. 7

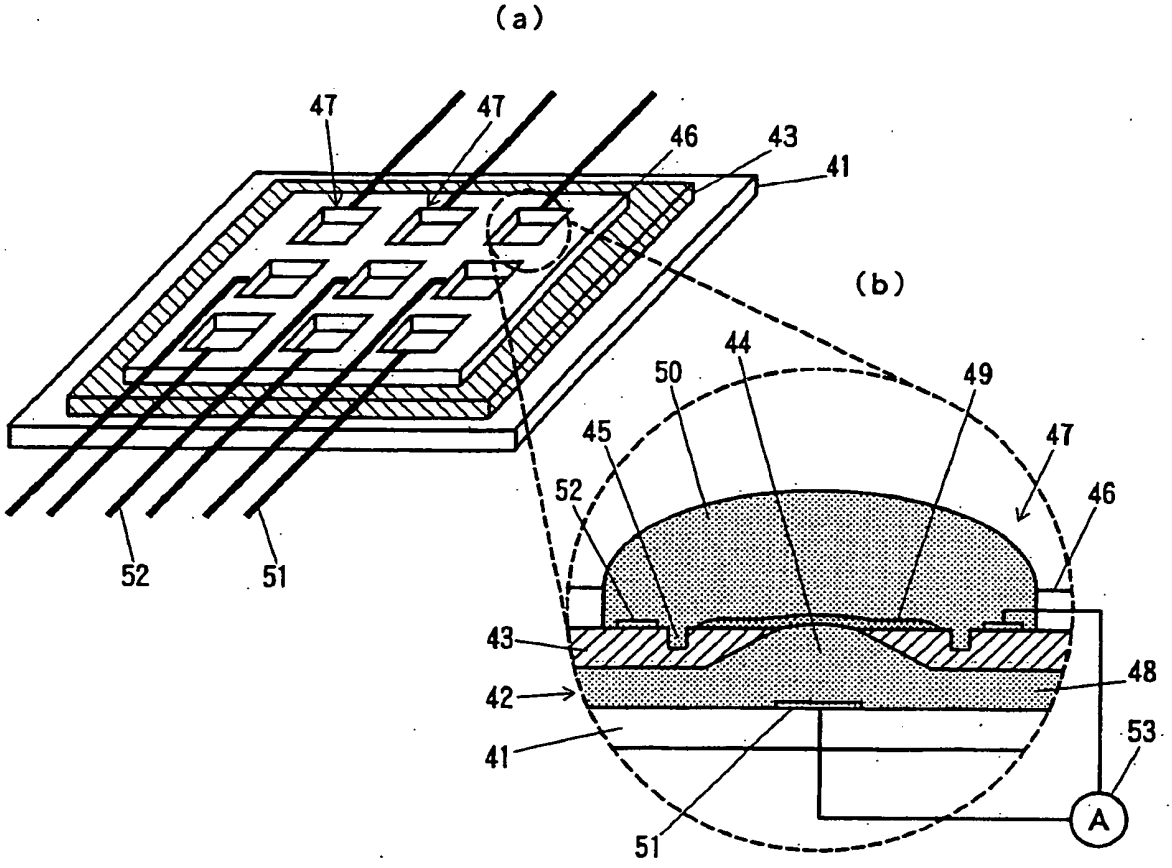


FIG. 8

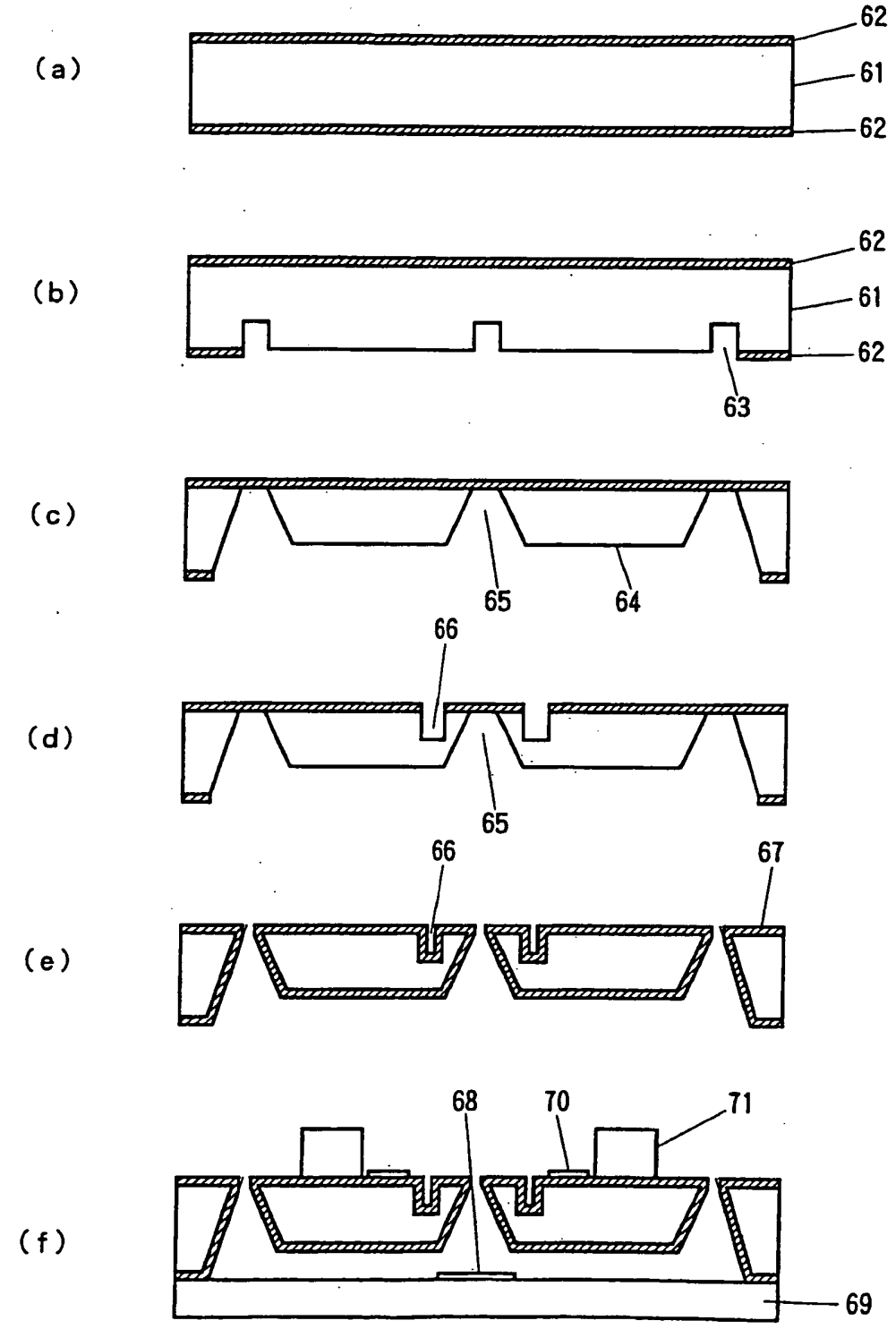


FIG. 9

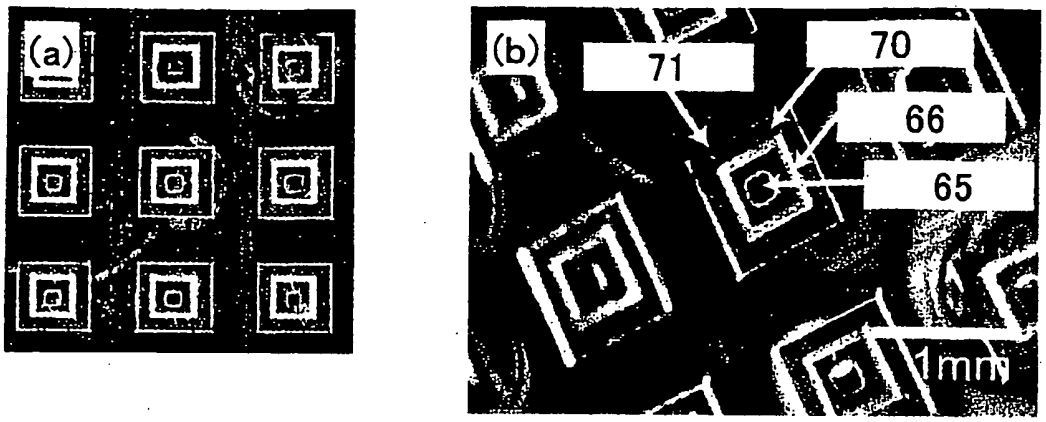
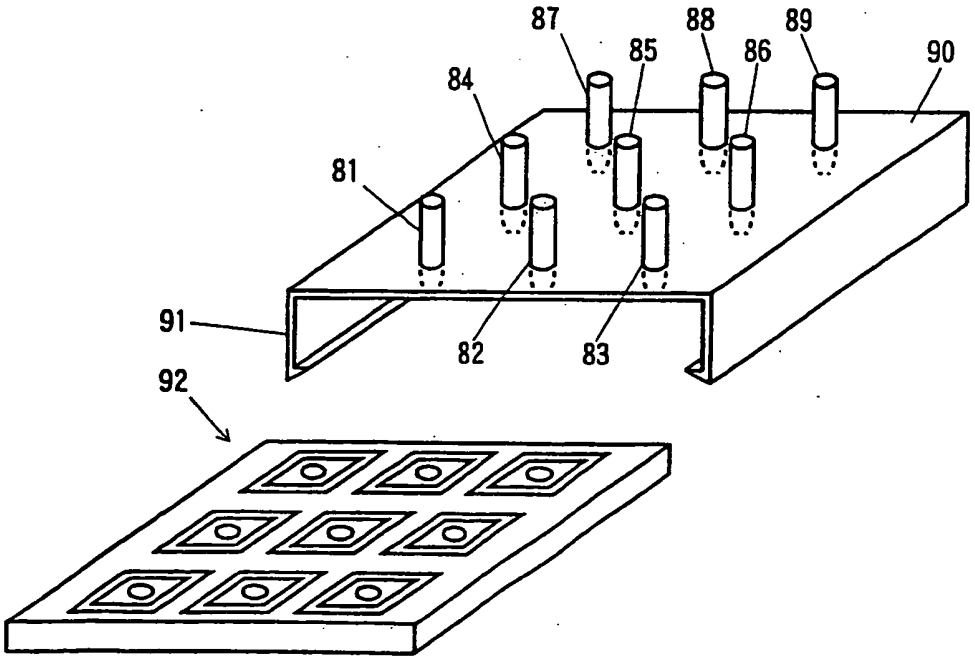



FIG. 10



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