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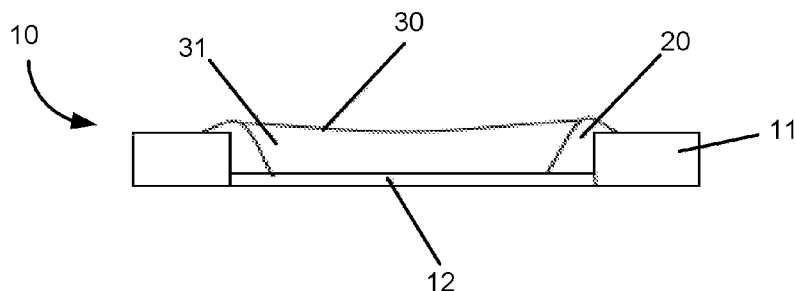
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Fig. 1



(57) Abstract: An apparatus for supporting an array of layers of amphiphilic molecules, the apparatus comprising: a body, formed in a surface of the body, an array of sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells, the sensor wells each containing an electrode for connection to an electrical circuit, and formed in the surface of the body between the sensor wells, flow control wells capable of smoothing the flow of a fluid across the surface.



APPARATUS FOR SUPPORTING AN ARRAY OF LAYERS OF AMPHIPHILIC  
MOLECULES AND METHOD OF FORMING AN ARRAY OF LAYERS OF AMPHIPHILIC  
MOLECULES

5           The present invention relates to an apparatus for supporting an array of amphiphilic molecules and a method of forming such an array. In particular, the invention relates to the efficient formation of arrays of amphiphilic molecules. One area of application is the preparation of lipid bilayers.

10           In one type of known technique, a membrane based layer of amphiphilic molecules may be used as a means of separating two volumes of aqueous solution. The amphiphilic layer resists the flow of current between the volumes when a potential difference is applied between the two volumes. A membrane penetrating protein is inserted into the amphiphilic layer to allow the passage of ions across the layer, which is recorded as an electrical signal detected by electrodes placed in each of the aqueous solutions, such as disclosed in WO2009/077734.

15           In this technique, a target analyte may interact with the membrane penetrating protein to modulate the flow of ions and may be detected by observing the resultant variations in the electrical signal. This technique therefore allows the layer of amphiphilic molecules to be used as a biosensor to detect the analyte.

20           The layer of amphiphilic molecules has a two-fold purpose in this technique. Firstly, the layer provides a platform for the protein that acts as a sensing element. Secondly, the layer isolates the flow of ions between the volumes. The electrical resistance of the layer ensures that the dominant contribution of ionic flow in the system is through the membrane protein of interest, with negligible flow through the layer of amphiphilic molecules, thus allowing detection with single protein channels.

25           A specific application of this technique is in nanopore sensing, where the number of membrane proteins is kept small, typically between 1 and 100, so that the behaviour of a single protein molecule can be monitored electrically. This method gives information on each specific molecular interaction and hence provides richer information than a bulk measurement. However, due to the small currents involved, typically a few pA, this approach relies on the  
30           formation of a very high resistance seal, typically greater than  $1\text{G}\Omega$ , and sufficient electrical sensitivity to measure the current.

          While the requirements for stochastic sensing have been met in the laboratory, conditions and expertise limit its practical application in commercial products. In addition, laboratory methods are laborious and time-consuming and are not scalable easily to the high-density arrays

that are desirable for any commercial biosensor. Furthermore, the fragility of single amphiphilic layer membranes means that they can be difficult to form, so that anti-vibration tables are often employed in the laboratory. Necessitating the use of such anti-vibration tables would not be desirable in a commercial product.

5           There have been great efforts to increase the ease of bilayer formation using micro fabrication. Some techniques have attempted to miniaturise standard systems for folded lipid bilayers or painted lipid bilayers. Other techniques include bilayer formation on solid substrates or directly on electrode surfaces, through either absorption or adsorption. A large proportion of nanopore sensing devices form a bilayer by using a variant of either the folded lipid bilayers  
10           technique, or the painted bilayer technique. To date, most have concentrated either on novel methods of aperture formation, on utilising the emerging technologies in micro fabrication to miniaturise the device, or to create a plurality of addressable sensors such as disclosed in EP2107040 and WO2010/122293.

          There are problems associated with the conventional supported amphiphilic layer  
15           approach that makes the approach unsuitable. The first problem lies with the resistance of the lamellar membrane which typically is about 100M $\Omega$ . While this may be suitable for examining protein behaviour at large protein concentrations, it is not sufficient for a high-fidelity assay based on single molecule sensing. To achieve single-molecule sensing a resistance of at least 1G $\Omega$ , and for some applications one or two orders of magnitude higher, is required. The second  
20           problem relates to the small volume of solution trapped in the small distance between the amphiphilic layer and the solid support, typically of the order of 1nm. This small volume does not contain many ions, and this affects the stability of the potential across the amphiphilic layer and limits the duration for which recording can be performed.

          The techniques used in the silicon chip industry provide an attractive technology for  
25           creating a large number of electrodes that could be used in biosensor applications. This approach is disclosed in the related applications US 7,144,486 and US 7,169,272. US 7,144,486 discloses a method of fabricating a microelectrode device containing microcavities etched into layers of an insulator material. The devices are said to have a wide range of electrochemical applications in which electrodes in the cavities allow measurement of electrical signals.

30           In summary, the known technologies discussed above either present methods of amphiphilic layer formation that cannot reproducibly achieve high resistances; suffer from low ionic reservoirs; are not capable of high duration direct current measurements; and/or require a separate fluidic chamber for each array element. This limits the scale up of the techniques to produce a high-density array device.

WO 2009/077734 describes a simplified apparatus to prepare amphiphilic layers across a recess and to scale the apparatus with multiple recesses forming chambers of a large scale sensor array without any need for a complicated apparatus.

In this method a lipid amphiphilic layer is formed as a layer separating two volumes of aqueous solution, the method comprising: (a) providing an apparatus comprising elements defining a chamber, the elements including a body of non-conductive material having formed therein at least one recess opening into the chamber, the recess containing an electrode; (b) applying a pre-treatment coating of a hydrophobic fluid to the body across the recess; (c) flowing aqueous solution, having amphiphilic molecules added thereto, across the body to cover the recess so that aqueous solution is introduced into the recess from the chamber and so that a layer of the amphiphilic molecules forms across the recess separating a volume of aqueous solution

A key feature of this method is the preparation of high quality amphiphilic layers that are suitable for high sensitivity biosensor applications such as nanopore sensing and single channel recording. The method has been demonstrated to form amphiphilic layers of high resistance, providing highly resistive electrical seals having an electrical resistance of greater than  $1\text{G}\Omega$ , typically  $100\text{G}\Omega$ , which for example, enable high-fidelity recordings from single protein pores.

In this method, formation of a layer of the amphiphilic molecules across a recess simply by flowing the aqueous solution across the body to cover the recess is possible provided that a pre-treatment coating of a hydrophobic fluid is applied to the body across the recess. The pre-treatment coating assists formation of the amphiphilic layer and aids the wetting of the, microcavity forming the sensor well, with aqueous solution.

However, under some circumstances the formation of high quality amphiphilic layers may be compromised. The present invention aims to at least partly address this problem.

According to a first aspect of the invention there is provided an apparatus for supporting an array of layers of amphiphilic molecules, the apparatus comprising: a body, formed in a surface of the body, an array of sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells, the sensor wells each containing an electrode for connection to an electrical circuit, and formed in the surface of the body between the sensor wells, flow control wells capable of smoothing the flow of a fluid across the surface.

This aspect is directed to a body in which inactive flow control wells are provided for increasing uniformity of distribution. That is, the additional wells reduce any stick/slip characteristics, resulting in a more predictably uniform wetted surface. The provision of the additional wells allows the sensor wells to be distributed and function as desired, without needing to account for the wetting characteristics of the system. That is, the desired sensor well

distribution can be selected, and the additional wells can be supplied to account for the system wetting characteristics.

Optionally, the cross-sectional area of a flow control well is less than the area of a sensor well.

5            Optionally, the flow control wells do not contain electrodes. Alternatively, the flow control wells each contain an electrode, the electrodes in the sensor wells being connected to the electrical circuit but the electrodes in the flow control wells not being connected to the electrical circuit. In the embodiments where the flow control wells do not function as active sensor wells, their function is to solely improve the wetting characteristics of the system. As such the  
10            constraint of requiring the flow control wells to be able to function as flow sensor wells is removed and the flow control wells may be provided of dimensions, for example the cross-sectional area of the aperture or shape of the wells, or of a pitch that are/is unsuitable for use as sensor wells.

            Optionally, the apparatus further comprises a cover over the surface of the body defining  
15            a cavity therebetween, and a common electrode arranged in the cavity for connection to the electrical circuit. The cover can have an internal surface facing the surface of the body that is roughened to smooth the flow of fluid thereover.

            Optionally, the array of sensor wells is a regular array, and the flow control wells consist of a regular array of flow control wells. Optionally, the pitch of the array of at least a portion of  
20            the flow control wells is smaller than the pitch of at least a portion of the array of sensor wells. That is, the axial distance between the flow control wells can be smaller than the axial distance between the sensor wells. The flow control wells may be of a different dimension than the flow sensor wells, for example a different size, a different cross-sectional area and/or a different cross-sectional area of the aperture than the sensor wells. Optionally, the sensor wells are  
25            circular, and optionally the flow control wells are square. Optionally, the flow control wells are distributed over a larger area than the sensor wells.

            Optionally, the sensor wells and flow control wells are arranged such that a pre-treatment, being a fluid capable of interacting with the amphiphilic molecules, on the surface would not enter the Cassie-Baxter state. Optionally, the sensor wells and flow control wells are  
30            shaped to provide a surface roughness  $r$ , defined as the total area of the surface and wells divided by the projected area of the surface, and a solid surface area fraction  $f$ , defined as the area of the surface between the wells divided by the projected area of the surface, that meet the requirement in respect of a pre-treatment, that is a fluid capable of interacting with the amphiphilic

molecules, having a contact angle  $\theta$  that  $((\phi - 1)/(r - \phi)) > \cos(\theta)$ . This ensures that the pre-treatment can enter the wells.

Optionally, the wells are formed on the surface with a number density of  $3.2 \times 10^{-5}$  wells/micron<sup>2</sup> or more, optionally  $6.4 \times 10^{-5}$  wells/micron<sup>2</sup> or more, further optionally  $1.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more, and still further optionally  $2.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more.

Optionally, the apparatus further comprises a pre-treatment of a hydrophobic fluid applied to the surface of the body.

According to this aspect, there is also provided a method of preparing an apparatus for forming an array of amphiphilic layers, the method comprising: providing an apparatus as discussed above; delivering across the surface of the body to the wells a pre-treatment coating of a hydrophobic fluid. The pre-treatment coating serves to support the amphiphilic layer such that a highly resistive electrical seal may be formed across the well.

Optionally, the pre-treatment is delivered in a solvent, the method further comprising drying the surface of the body to remove the solvent. Said step of drying the surface of the body to remove the solvent is preferably performed under a pressure below atmospheric pressure.

Optionally, the method is performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values. Further optionally, the method is performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 0.5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values. In this context, the 'average values' refer to the mean values of the rectangularity and the perimeter of the annuli, respectively, as calculated for all the sensor wells.

According to this aspect, there is also provided a method of forming an array of the sensor wells each containing an electrode for connection to an electrical circuit, wherein the wells have a number density of  $3.2 \times 10^{-5}$  wells/micron<sup>2</sup> or more, optionally  $6.4 \times 10^{-5}$  wells/micron<sup>2</sup> or more, further optionally  $1.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more, and still further optionally  $2.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more.

Optionally, all the wells are sensor wells. Alternatively, some of the wells are sensor wells, and the remainder of the wells are flow control wells, formed in the surface of the body between the sensor wells.

Optionally, the area of a flow control well is less than the area of a sensor well.

Optionally, the flow control wells do not contain electrodes. Alternatively, the flow control wells each contain an electrode, the electrodes in the sensor wells being connected to the electrical circuit but the electrodes in the flow control wells not being connected to the electrical circuit.

Optionally, the array of sensor wells is a regular array, and the flow control wells consist of a regular array of flow control wells. Optionally, a pitch of the array of flow control wells is smaller than a pitch of the array of sensor wells. Optionally, the sensor wells are circular, and the flow control wells are square.

Optionally, the flow control wells are distributed over a larger area than the sensor wells.

Optionally, the apparatus further comprises a cover over the surface of the body defining a cavity therebetween, and a common electrode arranged in the cavity for connection to the electrical circuit. Optionally, the cover has an internal surface facing the surface of the body that is roughened to smooth the flow of fluid thereover.

Optionally, the wells have an area density of 0.141 or more.

Optionally, the wells are arranged such that a pre-treatment applied to the surface of the body does not enter the Cassie-Baxter state. Optionally, the sensor wells and flow control wells are shaped to provide a surface roughness  $r$ , defined as the total area of the surface and wells divided by the projected area of the surface, and a solid surface area fraction  $f$ , defined as the area of the surface between the wells divided by the projected area of the surface, that meet the requirement in respect of a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, having a contact angle  $\theta$  that  $((\varphi - 1)/(r - \varphi)) > \cos(\theta)$ .

Optionally, the apparatus further comprises a pre-treatment of a hydrophobic fluid that is applied to the sensor wells.

According to the second aspect, there is also provided a method of preparing an apparatus for forming an array of sensor wells, the method comprising: providing an apparatus of the second aspect, as discussed above; delivering across the surface of the body to the wells a pre-treatment of a hydrophobic fluid.

Optionally, the pre-treatment is delivered in a solvent, the method further comprising drying the surface of the body to remove the solvent. Optionally, the step of drying the surface of the body to remove the solvent is performed under a pressure below atmospheric pressure.

Optionally, the method is performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values. Further optionally, the method is performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 0.5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

According to the second aspect, there is also provided a method of forming an array of layers of amphiphilic molecules, the method comprising: preparing an apparatus by a method of the second aspect, as discussed above; and flowing a fluid containing amphiphilic molecules across the surface of the body to form layers of amphiphilic molecules across at least some of the array of sensor wells.

According to a third aspect, there is provided a method of preparing an apparatus for forming an array of layers of amphiphilic molecules, the method comprising: providing an apparatus comprising a body, and, formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment of a hydrophobic fluid, the sensor wells each containing an electrode for connection to an electrical circuit, and delivering across the surface of the body a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, in a solvent to apply the pre-treatment to the wells; and drying the surface of the body to remove the solvent under a pressure below atmospheric pressure.

According to this aspect, the use of low-pressure drying produces a more uniform dried pre-treatment on the surface of the body.

Optionally, the apparatus is an apparatus according to the first or second aspect, discussed above.

Optionally, the method can be performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values. Further optionally, the method can be performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 0.5%; and the values of



rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

The third aspect of the invention also provides a method of forming an array of layers of amphiphilic molecules, the method comprising: preparing an apparatus by a method according to the method of the third aspect, as discussed above; and flowing a fluid containing amphiphilic molecules across the surface of the body to form layers of amphiphilic molecules across at least some of the array of sensor wells.

According to a fourth aspect of the invention, there is provided a method of preparing an apparatus for forming an array of layers of amphiphilic molecules, the method comprising: providing an apparatus comprising a body, and, formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment of a hydrophobic fluid, the sensor wells each containing an electrode for connection to an electrical circuit, and delivering to the body a pre-treatment of a hydrophobic fluid, the method being performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 5%; and the values of rectangularity and of the perimeter of each of the annuli of pre-treatment around the respective sensor wells falls within 40% of the average values. The visible coverage can be determined with any appropriate light-source. For example, under appropriate lighting conditions, the coverage may be visible in normal light. Alternatively, additives in the pre-treatment may be used to highlight the coverage under particular lighting conditions. For example, in one embodiment of the invention, a green fluorescent dye (a boron-dipyrromethene) is used to highlight the pre-treatment and a red fluorescent dye (sulforhodamine) is used to highlight the buffer under the membrane layer.

Optionally, the method is performed so that each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 0.5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

Optionally, the pre-treatment is applied to the body in a solvent, and the method further comprises drying the surface of the body to remove the solvent, the method being performed so that said conditions are met after said drying.

The fourth aspect further provides a method of forming an array of layers of amphiphilic molecules, the method comprising: preparing an apparatus according to the method of the fourth

aspect, discussed above; and flowing a fluid containing amphiphilic molecules across the surface of the body to form layers of amphiphilic molecules across at least some of the array of sensor wells.

The fourth aspect further provides an apparatus for forming an array of layers of amphiphilic molecules, the apparatus comprising: a body; and formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment of a hydrophobic fluid, the sensor wells each containing an electrode for connection to an electrical circuit, the array of wells being arranged such that after delivery to the body of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values.

Optionally, the array of wells is arranged such that after delivery to the body of a pre-treatment of a hydrophobic fluid, each of the following conditions is met: the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located; the proportion of sensor wells that are filled is less than 0.5%; and the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

Optionally, the apparatus further comprises a pre-treatment of a hydrophobic fluid applied to the sensor wells.

The present invention will be described with reference to exemplary embodiments and the accompanying Figures in which:

Fig. 1 is a diagram of ideal fluid behaviour in a well;

Fig. 2 is a diagram of undesirable fluid behaviour in a well;

Fig. 3 is a diagram showing different wetting behaviours;

Figs. 4a and 4b are of the expected modified contact angles for different 'native' contact angles, for an array of 50 micron wells spaced (a) 63 microns apart and (b) 81 microns apart;

Figs. 5a –c are images that illustrate how changing surface design affects pre-treatment dispersal;

Fig 6 is an image showing pre-treatment dispersal for a first design;

Figs. 7a-d are images showing pre-treatment dispersal for a second design;

Figs. 8a are images showing pre-treatment dispersal for the second design under different conditions;

Figs. 9a-9c are images showing pre-treatment dispersal for a third design;

Fig. 10a is a schematic representation of a fourth design, and Figs. 10b-c are images showing pre-treatment dispersal for the fourth design;

Fig. 11a is a schematic representation of a fifth design, and Figs. 11b-c are images showing pre-treatment dispersal for the fifth design;

Fig. 12a is a schematic representation of a sixth design, and Figs. 12 b and 12c are images showing pre-treatment dispersal for the sixth design; and

Fig. 13a is a schematic representation of a sixth design, and Figs. 13 b and 13c are images showing pre-treatment dispersal for the seventh design;

As mentioned above, the techniques of WO 2009/077734, herein incorporated by reference in its entirety, can result in amphiphilic layers of compromised quality in some circumstances. The present invention has identified that this can be the result of the pre-treatment coating being, in some parts of the array, either greater or less than an optimal level.

Fig. 1 shows a schematic cross section through a microcavity or sensor well 10 of a sensor array. The well 10 is formed in a material 11 such as SU-8 forming a body, and many wells 10 may be formed in close proximity within the material to form an array of sensor wells. Preferably the material in which the wells are formed is itself solid and not porous, so that the wells maintain their integrity and liquid does not leak or leach from the wells. The body may also be made of other materials such as such as a positive or negative photoresists, plastics such as polycarbonate or polyester or solid state inorganic materials such as silicon, glass or silicon nitride. Examples of photoresists that may be used are SU8 2000 or 3000 series materials, poly(methyl methacrylate) (PMMA), poly(methyl glutarimide) (PMGI), phenol formaldehyde resin (DNQ/Novolac), or polyhydroxystyrene-based polymers. At the bottom of the well is an electrode 12 for connection to an electrical circuit, which can be used (in combination with another electrode above the well, not shown in Fig. 1) to monitor the flow of current through the well 10.

In practice, an array of such sensor wells 10 formed in a body will be provided in an apparatus further comprising a cover over the surface of the body, so as to define a cavity between the cover and the body. An electrode is arranged in the cavity for connection to the electrical circuit, and acts a common electrode for the wells in the array.

Fig. 1 shows the ideal configuration, in which a pre-treatment 20 is pinned tightly to the edges of the well 10. This configuration allows for the maximum trapped volume (i.e. volume of amphiphilic layer 30) within the well 10. This configuration results in a biosensor with the longest lifetime.

5 The pre-treatment is a fluid capable of interacting with the amphiphilic molecules. The pre-treatment coating is typically a hydrophobic substance, usually having long chain molecules, in an organic solvent. Suitable organic substances include without limitation: n-decane, hexadecane, isoecoisane, squalene, pristane (2,6,10,14-tetramethylpentadecane), fluorinated oils (suitable for use with fluorinated lipids), alkyl-silane (suitable for use with a glass membrane) and alkyl-thiols (suitable for use with a metallic membrane). Suitable solvents include but are not limited to: pentane, hexane, heptane, octane, decane, and toluene. The material might typically be 0.1µl to 10µl of 0.1% to 50% (v/v) hexadecane in pentane or another solvent, for example 2µl of 1% (v/v) hexadecane in pentane or another solvent, in which case lipid, such as diphantytanoyl-sn-glycero-3-phosphocholine (DPhPC), might be included at a concentration of 15 0.6mg/ml.

Some specific materials for the pre-treatment coating 30 are set out in Table 1 by way of example and without limitation.

Pre-treatment formulation	Volumes applied
0.3% hexadecane in pentane	2x 1µl
1% hexadecane in pentane	2x2x 0.5µl; 2x 0.5µl; 1µl; 2x 1µl; 2x 1µl; 2µl; 2x 2µl; 5µl
3% hexadecane in pentane	2x 1µl; 2µl
10% hexadecane in pentane	2x 1µl; 2µl; 5µl
0.5% hexadecane + 0.6mg/ml DPhPC lipid in pentane	5µl
1.0% hexadecane + 0.6mg/ml DPhPC lipid in pentane	2x 2x 0.5µl
1.5% hexadecane + 0.6mg/ml DPhPC lipid in pentane	2µl; 2x 1µl

Table 1: Examples of pre-treatment materials.

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The amphiphilic layer can be made of any amphiphile that forms a lamellar phase. Amphiphiles include lipids capable of forming lipid bilayers. The amphiphiles are chosen such

that an amphiphilic layer having the required properties, such as surface charge, ability to support membrane proteins, packing density or mechanical properties, is formed. The amphiphiles can comprise one or more different components. For instance, the amphiphiles can contain up to 100 amphiphiles. The amphiphiles may be naturally-occurring or synthetic. The amphiphile may be a block copolymer.

In embodiments where the amphiphile is a lipid, the lipid typically comprises a head group, an interfacial moiety and two hydrophobic tail groups which may be the same or different. Suitable head groups include, but are not limited to, neutral head groups, such as diacylglycerides (DG) and ceramides (CM); zwitterionic head groups, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SM); negatively charged head groups, such as phosphatidylglycerol (PG); phosphatidylserine (PS), phosphatidylinositol (PI), phosphatic acid (PA) and cardiolipin (CA); and positively charged headgroups, such as trimethylammonium-Propane (TAP). Suitable interfacial moieties include, but are not limited to, naturally-occurring interfacial moieties, such as glycerol-based or ceramide-based moieties. Suitable hydrophobic tail groups include, but are not limited to, saturated hydrocarbon chains, such as lauric acid (n-Dodecanolic acid), myristic acid (n-Tetradecanoic acid), palmitic acid (n-Hexadecanoic acid), stearic acid (n-Octadecanoic) and arachidic (n-Eicosanoic); unsaturated hydrocarbon chains, such as oleic acid (cis-9-Octadecanoic); and branched hydrocarbon chains, such as phytanoyl. The length of the chain and the position and number of the double bonds in the unsaturated hydrocarbon chains can vary. The length of the chains and the position and number of the branches, such as methyl groups, in the branched hydrocarbon chains can vary. The hydrophobic tail groups can be linked to the interfacial moiety as an ether or an ester.

The lipid can also be chemically-modified. The head group or the tail group of the lipid may be chemically-modified. Suitable lipids whose head groups have been chemically-modified include, but are not limited to, PEG-modified lipids, such as 1,2-Diacyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000]; functionalised PEG Lipids, such as 1,2-Distearoyl-sn-Glycero-3 Phosphoethanolamine-N-[Biotinyl(Polyethylene Glycol)2000]; and lipids modified for conjugation, such as 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine-N-(succinyl) and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(Biotinyl). Suitable lipids whose tail groups have been chemically-modified include, but are not limited to, polymerisable lipids, such as 1,2-bis(10,12-tricosadiynoyl)-sn-Glycero-3-Phosphocholine; fluorinated lipids, such as 1-Palmitoyl-2-(16-Fluoropalmitoyl)-sn-Glycero-3-

Phosphocholine; deuterated lipids, such as 1,2-Dipalmitoyl-D62-sn-Glycero-3-Phosphocholine; and ether linked lipids, such as 1,2-Di-O-phytanyl-sn-Glycero-3-Phosphocholine.

The lipid may comprise one or more additives that will affect the properties of the lipid bilayer. Suitable additives include, but are not limited to, fatty acids, such as palmitic acid, myristic acid and oleic acid; fatty alcohols, such as palmitic alcohol, myristic alcohol and oleic alcohol; sterols, such as cholesterol, ergosterol, lanosterol, sitosterol and stigmasterol; lysophospholipids, such as 1-Acyl-2-Hydroxy-sn- Glycero-3-Phosphocholine; and ceramides. The lipid preferably comprises cholesterol and/or ergosterol when membrane proteins are to be inserted into the amphiphilic layer.

When pre-treatment oil 20 is not deposited in the optimum configuration of Fig. 1, a smaller trapped volume is the most probable outcome. There is also a higher probability that excess pre-treatment oil will be located on the upper surface of the well 10. This is shown schematically in Fig. 2. In Fig. 2, the pre-treatment 20 is not pinned tightly to the edges of the well 10. As a result, the trapped volume of the amphiphilic molecule 30 is reduced. Further, pre-treatment 20a is also present on the upper surface of the well 10.

In order to form a good contact between pre-treatment 20 and the amphiphilic layer 30, it is preferable to use a hydrophobic material for forming the well 10. This encourages a small contact angle between the pre-treatment 20 and the amphiphilic layer 30. However, this also makes it more likely that pre-treatment oil will form droplets 20a on the surface of the array material unless pinned into the well 10 and collected by Laplace pressures. The appropriate hydrophobic surface properties may be achieved by suitable selection of materials. However, where there are conflicting constraints, for example where the desired surface properties are not available using photoresist material appropriate for fabrication of the required structure, this may not be possible. In this case, commonly, surface treatments are applied to achieve a hydrophilic surface, such as the addition of a chemical coating or plasma modification. These methods are not ideal, typically they are unstable over a long product storage lifetime or may cause interference with the sensor chemical system.

Where there is a desire to form the amphiphilic layers quickly, requiring fast flow rates over the surface or where a very large scale array is used, it has been found that the flow of aqueous solution during the amphiphilic layer formation phase may cause a transfer of pre-treatment 20 to the downstream areas of the array or lead to the creation of an emulsion in the aqueous solution, which is undesirable.. This is more likely in situations where pre-treatment oil 20 is located outside of the well, for example on the SU-8 surface.

In the current invention, the introduction of surface patterning to the bulk surface of the array allows for improved formation of the pre-treatment layer 20 with good uniformity and aids retention of the pre-treatment layer during the subsequent fluid flow associated with amphiphilic layer formation.

5           The uniformity of pre-treatment distribution can be further enhanced by extending the surface patterning beyond the bulk surface of the array to consider the other internal faces of the fluidic flow cell in which the array is contained. In this example, during the pre-treatment application phase, the pre-treatment oil material is also coated onto all other internal surfaces. During the subsequent fluid flow steps this material may also be redistributed, therefore  
10       compromising formation of high quality lipid amphiphilic layers. A surface pattern can be introduced to these other surfaces, and tailored to control the degree of coating with pre-treatment and to enhance retention of the pre-treatment on those surfaces enhancing the overall performance of the apparatus.

          The surface patterning also enables the required surface hydrophobicity, which is  
15       conventionally achieved by surface chemistry modification of the array material, to be achieved through altering the ratio of contribution of surface energies between that of the native material and that of air, or whatever the surrounding bulk medium may be.

          The surface states that may exist for a well-containing surface are defined by the overall thermodynamic position.

20           In the ‘Cassie-Baxter’ state, the hydrophobicity is high enough that the wells are not filled by the wetting fluid, but remain filled with the bulk medium. However, this state is thermodynamically unstable and can, under the correct circumstances, collapse to a lower energy state.

          In the most thermodynamically stable ‘Wenzel’ state, the wells are completely filled by  
25       the wetting fluid. Once achieved it is impossible to revert between the Wenzel and Cassie-Baxter states.

          Fig. 3 illustrates the wetting of (a) a flat surface in comparison to wetting a surface containing microstructures in the (b) Wenzel and (c) Cassie-Baxter states. As can be seen from the Figure, the contact angle  $\theta$  differs in the different states.

30           The modified angles of the Wenzel,  $\theta_w$ , and Cassie-Baxter,  $\theta_{CB}$ , states can be calculated once the contact angle,  $\theta$ , of the native material is known.

$$\cos\theta_{CB} = \phi(\cos\theta + 1) - 1$$

$$\cos\theta_w = r\cos\theta$$

where  $\phi$  is defined as the area of the surface between the wells divided by the projected area of the surface (calculated as: (total area – well area)/(total area)),  $r$  is defined as the ratio of true area of the solid surface to the apparent area.

As such it is possible to calculate the effects for both phenomena over a range of fluid contact angles.

Figs. 4a and 4b show graphs of the expected modified contact angles for different ‘native’ contact angles, for an array of 50 micron wells spaced (a) 63 microns apart and (b) 81 microns apart. These graphs show that there is a significant difference in Cassie-Baxter contact angles between surfaces of 50  $\mu\text{m}$  micro-wells spaced either 63 or 81  $\mu\text{m}$  apart. For example, native SU-8 has a contact angle of around  $76^\circ$ , thus, for 63  $\mu\text{m}$  wells, a Wenzel state would have a modified contact angle of around  $65^\circ$ , whilst the Cassie-Baxter state exhibits modified contact angles in the region of  $115^\circ$ .

As such the surface properties of the array can thus be tailored to specific fluids or to produce a desired surface state, by controlling the surface patterning. In particular, it may be desirable to form the array with additional wells, not intended for sensing, in order to modify the surface properties. Such additional wells may be inactive for sensing, either because they do not contain an electrode or because the electrode is not connected to the sensing circuitry. This approach holds several advantages.

For flow through pre-treatment application on the large-scale, the surface can be controlled to promote pinning of the pre-treatment on the sensor array surface so that pre-treatment does not move during amphiphilic layer formation. Additionally, it is preferable to avoid the Cassie-Baxter state, otherwise the pre-treatment will not fill the wells. That is, it is preferable to design the surface to have a contact angle  $\theta$  for which:  $((\phi - 1)/(r - \phi)) > \cos(\theta)$ .

Using a high density of wells, including inactive wells, over the bulk surface forming the surface pattern also allows maximum flexibility into the design. That is, if it becomes desirable to change the arrangement of the sensing wells, for example to produce a more closely packed electronic array, this can be produced with minimal impact to the overall surface by appropriate ‘balancing’ with inactive wells. That is, the inactive wells can be formed in the surface of the body in which the ‘active’ wells have been formed, adding to the array of active wells to create the desired surface properties. As a result, the surface properties can remain virtually unaltered whilst varying the structure of the active array, and so the optimal fluidic procedure will not need to be changed. The additional ‘flow control’ wells may not contain electrodes, or may contain electrodes that are not attached to the electrical circuit of the sensor wells.



Controlling the hydrophobicity based on the well geometry and placement avoids the need for additional processing steps associated with modifying the surface properties by chemical means. Further, this method of surface control is applicable to all materials, making it unnecessary to tailor a particular chemistry to a particular material.

5 In addition, it has been found that the flow through of pre-treatment is also enhanced by using micro-patterned surfaces. The pre-treatment front can be observed to progress across an array more smoothly in the presence of additional wells, particularly on larger arrays. That is, the additional wells increase the homogeneity of flow across the surface of the body such that the uniformity of wetting is increased. The additional wells are capable of increasing the uniformity  
10 of the distribution of said pre-treatment during deliver across the surface of the body. This smoothing reduces the tendency for the fluid to undergo large scale pinning during flow which results in so-called 'stick/slip' movement of a fluid front. Wetting in this stick/slip fashion is irregular and can result in the fluid being pinned for a period of time before moving to the next pinning position. This can also result in de-wetting of surfaces that have already been wetted as  
15 the shape of the wetting profile changes. To this end, it can also be preferable to roughen the internal surface of the cover, opposite the body, to further smooth the flow of fluid. It can also be preferable to provide the additional wells over a large area than the sensor wells, in order to ensure the edges of the array of sensor wells experience the enhanced flow of pre-treatment.

The pre-treatment distribution is monitored by tagging the pre-treatment oil with a  
20 fluorescent dye. The dye is then imaged using epi-fluorescence microscopy *in situ*.

The images show in Figs. 5a-d show an example of the difference in distributions obtained by introducing additional wells in a surface for otherwise identical fluidic flows of pre-treatment oils dissolved in hexane over an array. Fig. 5a shows an overview of a treated array of active wells, with no additional wells, whilst Fig. 5b shows a close up view of some the wells.  
25 The bright areas indicate the presence of pre-treatment. As is particularly clear in Fig. 5b, many of the wells are completely filled by pre-treatment, and there is much excess pre-treatment on the surface. In contrast, Fig. 5c shows an overview of an array incorporating additional (smaller) inactive wells in addition to the active wells, and Fig. 5d is a close up view of some of the wells. The pre-treatment uniformly forms the 'ideal' ring structure around the wells and no wells are  
30 completely filled. Further, there is much less variation between wells in the quality of pre-treatment (even only considering non-filled wells). It is noted that the brighter areas towards the right hand side of Fig. 5c is excess pre-treatment located on the window of the cell not on the array surface (focal plane).

These images illustrate that the behaviour of fluid flowing over a surface containing wells can be influenced by changing the surface texture in between the wells. The introduced wells may, but need not, also be used as active wells. As such, if it is desired to keep a certain active well spacing, but improve the distribution of the pre-treatment, that is possible by introducing

5 'inactive' wells. These inactive wells help the pre-treatment flow across the surface during the application stage and further aid in the formation of a well distributed pre-treatment during the drying phase.

Exemplary experiments are discussed below.

## 10 Experimental procedures

### Materials Required:

Clean room, Oven, RIE, Hotplate x2, Mask aligner, Resist spinner, Develop dishes x2, Nitrogen supply, Wafer tweezers, Inspection microscope, Silicon Wafers, SU-8 10 photoresist,

15 SU-8 2 photoresist, EC Developer, Photolithography mask 1st layer: 4KCSH51 4201, Photolithography mask 2nd layer: 4KCSH41 4149, Acetone (propan-2-one), IPA (propan-2-ol / 2-propanol).

### Method for preparing wafers with well designs:

To ensure that the surfaces were clean from organic greases and salts from manufacturing

20 and handling, silicon wafers were rinsed with acetone, 2-propanol and deionised water prior to use. The wafers were dried with a gentle supply of nitrogen. Wafers were then placed in a preheated oven for 1 hour at 150°C. The SU-8 solutions (SU-8 2, and SU-8 10) were removed from cold storage and allowed to reach room temperature prior to use. The hotplates were cleaned and allowed to reach stable temperatures of 80°C and 110°C. The spin coater and

25 developer dishes were set-up ready for use. SU-8 2 (9mL) was spun onto oxygen plasma treated (200W, 50mTorr) wafers at 2000 rpm, which was then first placed on a hotplate at 80°C for 1 minute prior to a 2 minute treatment on a hotplate set to 110°C. The soft-baked SU-8 2 layer was then exposed to the electrode-mask for 10 seconds, after suitable alignment to the wafer. A post exposure bake at 80°C for 1 minute and 2 minutes at 110°C for 2 minutes was performed. The

30 wafer was then developed in a two-stage rinsing process, followed by a thorough rinse with 2-propanol. The wafer was dried with nitrogen prior to inspection. The wafer was then re-spun with SU-8 10 (9mL) at 1600 rpm. The wafer was then baked again at 80°C for 1 minute followed by 2 minutes at 110°C. The wafer was then aligned and exposed to UV for 55 seconds under the mask. A further post exposure bake of 3 minute at 80°C followed by a second at

110°C for 7 minutes was performed. The wafer was then developed thoroughly and washed with 2-propanol prior to a de-scumming oxygen plasma process of 1 minute. The wafers were then hard-baked at 150°C for 1 hour. Wafers were then processed for dicing and bonding.

Diced and bonded 128 chips were then examined for surface defects prior to use. A

5 single water wash removed surface dust particles, whilst a single ethanol wash removed surface greases prior to use.

Designs were fabricated on SiO<sub>2</sub>/SU-8 with a well depth of 20 µm.

### Design 1

10 A standard design of ‘active’ wells, Design 1, is a square array of 75 µm wells, pitched at 250 µm along the X and Y axes. Pre-treatment was applied to Design 1 using by dip-coating an SU-8 and silicon piece in a pre-treatment solution of 10% pristane (2,6,10,14-tetramethylpentadecane) in hexane, at a velocity of approximately 1 mm/s.

15 Lipid bilayers were prepared in the following way. The micro-wells were first filled with a solution of lipid vesicles in buffer (3.6 g/L of 1,2-diphytanoyl-sn-glycero-3-phosphocholine in a buffer composed of 400 mM KCl, 25 mM Tris in water). An air-solution interface was then created by slowly retracting the excess lipid solution from the flow cell. The lipid bilayers were then painted by slowly introducing the solution of lipid in the flow cell (the optical dye sulforhodamine 101 (green excitation, red emission) was added to the lipid solution at the  
20 concentration of 0.01g/L). The meniscus of the introduced solution effectively paints lipid bilayers on the micro-wells. The excess lipids were then flushed by a large volume of buffer.

Thereafter, the presence of lipid bilayers was determined by epifluorescence, using the optical dye introduced to the lipid solution, which was trapped in the wells as the bilayer formed. A representative image, giving a general overview of the result (without particular detail of the  
25 wells), is shown in Fig 6, in which brighter areas represent the presence of pre-treatment.

As can be seen, the quality of pre-treatment is variable, with some wells not showing the presence of any pre-treatment at all. Counting a bilayer as present if it covers a micro-well entirely, standard image processing methods of particle counting can be used to analyse the epifluorescence images. An average of 68.5% bilayer formation was found, after 3 tests, with a  
30 standard deviation of 2.7%.

To determine the effect of the design parameters on the quality of bilayer formation, further experiments were conducted.

In the following examples arrays of wells were mounted in flow cell. Pre-treatment (10% pristane in hexane, 100 $\mu$ l) was pushed through the array chip at a flow rate of 100 $\mu$ l/s. The chips were then dried in one of two methods. (1) By removing the connecting pipe-work and placing the array chip in a desiccator for 15 minutes under vacuum, at 200 mBar pressure (i.e. below atmospheric pressure). This allowed the hexane to evaporate leaving behind the pristane in the location it is deposited. (2) By pushing air through the array chip at a constant, but low, flow rate for 15 minutes. This allowed the hexane to evaporate at atmospheric pressure, but the vapour removed which drives the drying process.

## Design 2

The design had 75  $\mu$ m wells, pitched at 250  $\mu$ m along the X and Y axes. These were interleaved with the same design off set 125  $\mu$ m on the X and Y axes, effectively producing a square array of 75  $\mu$ m wells, pitched at 177  $\mu$ m along axes angled at 45° to the X and Y axes. This design, Design 2, doubles the micro-well density on the SU-8 array compared to Design 1.

Representative images of these results are shown in Figs 7a–f, in which Fig. 7a is an overview of a desiccator drying experiment (and does not provide particular detail of the wells), Fig. 7b is close up of a desiccator drying experiment, Fig. 7c is an overview of a pump drying experiment (and does not provide particular detail of the wells), Fig. 7d is a close up of a pump drying experiment.

Using the desiccator drying method, as shown in Figs. 7a and 7b, an acceptable pre-treatment distribution was obtained. The arrays when dried in this way do not show any significant signs of pre-treatment on the surface of the SU-8 (i.e. between the micro-wells). However, it is noted that the arrays did not seem particularly even in intensity, and the overall intensity was low.

Using the pump drying method, as shown in Figs. 7c and 7d, the results were clearly unsatisfactory. Much of the surface was covered in larger pools of pre-treatment and many of the micro-wells were filled with pre-treatment.

Although this may lead to the conclusion that the pre-treatment drying method is the most important factor in obtaining a good pre-treatment distribution, it is not the only consideration. As shown in Figs. 8a and 8b, a sample produced via dip coating the pre-treatment (rather than the painting technique used for Figs. 7a–d), produces a satisfactory but uneven distribution, even though the surface is clear of pre-treatment. Fig. 8a is an overview of the example produced via dip coating (and does not provide particular detail of the wells), and Fig. 8b is a close up of the example produced via dip coating.

### Design 3

A design having: 75  $\mu\text{m}$  wells, squarely pitched at 125 $\mu\text{m}$  on both X and Y axes, was used as Design 3. This effectively represents a grid of 'active' wells as in Design 1, with an additional array of 'inactive' wells also of 75  $\mu\text{m}$  diameter and squarely pitched at (0, 125  $\mu\text{m}$ ), (125  $\mu\text{m}$ , 125  $\mu\text{m}$ ) and (125  $\mu\text{m}$ , 0) on the X and Y axes between the 'active' wells. Design 3 increases the array density by a factor of 4 compared with Design 1.

Representative images of these results are shown in Figs 9a–d, in which Fig. 9a is an overview of a desiccator drying experiment (and does not provide particular detail of the wells), Fig. 9b is close up of a desiccator drying experiment, Fig. 9c is an overview of a pump drying experiment (and does not provide particular detail of the wells), Fig. 9d is a close up of a pump drying experiment. The background brightness running through Figs. 9c and 9d is pre-treatment deposited on the top surface of the viewing cell and not on the chip surface.

As can be seen, desiccator drying resulted in the surface of the chip being completely homogeneous with respect to the pre-treatment. There is little, if any, pre-treatment sat on the SU-8 surface between the micro-wells.

Pump Drying provides an improvement over Design 2 (which has a well density half that of Design 3). However, this design still leads to significant filling of the micro-wells towards the front of the array, less so towards the rear of the chip. This is probably due to flow rate variations over the surface of the chip. Moreover, we can see the pinning effects of the micro-wells; in many cases the pre-treatment is pinned on the top SU-8 surface rather than filling the micro-wells (producing the square looking blobs between wells in Figs. 9c and 9d). This is an unsatisfactory result.

### Design 4

Design 4 utilised wells of different shaped micro-wells, to investigate the effect the well shape has on the quality of the pre-treatment. Changing the well shape changes the aspect ratio of the area covered and also probes if any pinning is due to the shape (and symmetry) of the micro-wells.

Design 4 uses the same pitch as Design 3 (square pitch of 125 $\mu\text{m}$  on both X and Y axes). However as shown in Fig. 10a, instead of an array of only circular wells (as in Design 4), an array based on the repeating pattern of one circular well and three square wells (arranged so that the four wells form a square on the array), was used as Design 4. Each circular well had a diameter of 75  $\mu\text{m}$ , whilst the square wells had a side length of 75  $\mu\text{m}$ .

In this design, the circular wells can be considered as representing ‘active’ wells, whilst the square wells represent ‘inactive’ wells. Therefore, Design 4 corresponds to Design 3, but with the shape of the ‘inactive’ wells changed.

Representative images of these results are shown in Figs 10 b–e, in which Fig. 10b is an overview of a desiccator drying experiment (and does not provide particular detail of the wells), Fig. 10c is close up of a desiccator drying experiment, Fig. 10d is an overview of a pump drying experiment (and does not provide particular detail of the wells), Fig. 10e is a close up of a pump drying experiment. Once again, the bright background in Figs. 10d and 10e is due to pre-treatment deposited on the view cell not on the chip surface.

As can be seen, desiccator drying provided a very similar result to the “all circular” equivalent of Design 3. The shape does not seem to affect the amount of pre-treatment remaining on the surface. That is, the change in shape does not make the quality of pre-treatment worse.

Indeed, the pump drying experiment indicates the change in shape has a positive effect. In the pump dried example (Figs. 10d and 10e), the amount of surface remaining pre-treatment is quite high as in the “all round” counterpart. However, only the square micro-wells have filled. As a result, the quality of pre-treatment is acceptable.

#### Design 5

Design 5 (shown in Fig. 11a) removes a large amount of the SU-8 surface. 75  $\mu\text{m}$  circular wells are arranged on a square pitch of 250  $\mu\text{m}$  on both X and Y axes, as in Design 1. In addition, a ‘background’ pattern of 20  $\mu\text{m}$  squares with a 5  $\mu\text{m}$  border is provided. The use of a square background pattern, closely spaced, provides an efficient pattern for removing as much surface material as possible, whilst providing a texture.

Representative images of these results are shown in Figs 11 b–e, in which Fig. 11b is an overview of a desiccator drying experiment (and does not provide particular detail of the wells), Fig. 11c is close up of a desiccator drying experiment, Fig. 11d is an overview of a pump drying experiment (and does not provide particular detail of the wells), Fig. 11e is a close up of a pump drying experiment. Once again, the bright background in Figs. 11d and 11e is due to pre-treatment deposited on the view cell not on the chip surface.

As expected in view of the results for Designs 3 and 4, desiccator drying of Design 5 provided a surface that is very uniform and free of excess pre-treatment. The small micro-wells make it difficult to see the pre-treatment, but it is very uniform over the whole surface. The variation in background intensity in Fig. 11b is due to pre-treatment on the view cell, not the chip

surface. Further, the bright area at the bottom right was found to be caused by dust on the surface, pinning more pre-treatment, but it is notable no filled wells were produced even in this region.

The pump drying experiment provided an apparently identical result (barring variations due to the presence of pre-treatment on the view-cell) to the desiccator drying experiment.

We look to the designs that we short-listed, namely the 50-81 and 50-63 (which denotes the size of the micro-patterned wells and their pitched spacing - in  $\mu\text{ms}$ ). We know that desiccator drying methods at this scale work well for both designs, since the 125 $\mu\text{m}$  pitched micro-patterned wells performs well under these conditions.

#### Designs 6 and 7

Designs 6 and 7 are also based upon an 'active' array of 75  $\mu\text{m}$  circular wells are arranged on a square pitch of 250  $\mu\text{m}$  on both X and Y axes, as in Design 1. In addition, Design 6 (Fig. 12 a) incorporates a background pattern of 50  $\mu\text{m}$  circular 'inactive' wells between the 'active' wells, on a square pitch of 81  $\mu\text{m}$ , whilst Design 7 (Fig. 13a) incorporates a background pattern of 50  $\mu\text{m}$  circular 'inactive' wells between the 'active' wells, on a square pitch of 63  $\mu\text{m}$ . As such, in these designs the cross-sectional area of the aperture of the additional (or 'inactive' or 'flow control') wells is less than the area of the 'active' or 'sensor' wells.

Only pump drying experiments were performed for these designs, as it can be inferred from the results for Design 3 that desiccator drying will work well.

Representative images of the results for Design 6 are shown in Figs. 12b and 12c, in which Fig. 12b is an overview of a desiccator drying experiment (and does not provide particular detail of the wells), and Fig. 12c is close up of a desiccator drying experiment. Representative images of the results for Design 7 are shown in Figs. 13b and 13c, in which Fig. 13b is an overview of a desiccator drying experiment (and does not provide particular detail of the wells), and Fig. 13c is close up of a desiccator drying experiment.

Satisfactory results were obtained from Design 6. The majority of the surface is uniform, (there is some variation in the pre-treatment over the surface but this may be more related to the surface chemistry of the chip), however there are on average only a few micro-wells that are filled or are non-uniform compared to the majority of the micro-wells for which the pre-treatment forms in a uniform manner..

Good results were obtained from Design 7. Accounting for the obvious view cell variations, there do not appear to be any filled micro-wells, and the distribution appears to be more homogeneous compared to Design 6

The results for the above discussed designs have been quantified, and tabulated in Table 2, by allocating the quality of the pre-treatment distribution a grade. In order to do this, the homogeneity of the pre-treatment distributions were assessed by image analysis, in order to measure the rectangularity and perimeter of the pre-treatment in the wells. The rectangularity is defined as the ratio of the cross-sectional area of pretreatment to the cross-sectional area of a notional inscribed (non-rotational) rectangle within the well (i.e. the rectangle with the largest cross-sectional area which can be inscribed within the pre-treatment cross-sectional area). This ratio is  $\pi/4$  for a perfect circular object and unity for a non-rotated rectangle. To do this, the image being analysed was split into its red, green and blue components. A green fluorescent dye (a boron-dipyrromethene) was used to highlight the pretreatment and a red fluorescent dye (sulforhodamine) was used to highlight the buffer under the membrane layer. The gray-scale image was then threshold filtered just above background level. The duotone image was then subjected to a shape analysis on each object identified. On this basis the following grades were defined:

#### Grade 1:

- the visible pre-treatment coverage of the surface is lower than 5% of the surface of the array in the fluidic cell
- the number of filled wells in the array (both 'active' and 'inactive') is smaller than 0.5%
- the homogeneity of the distribution of the pre-treatment annuli in the wells is high, as quantified by the rectangularity and perimeter being within  $\pm 20\%$  of the average value. For example, if the average value of the perimeter is 140 for a 50  $\mu\text{m}$  well, then all the perimeters measured on the 50  $\mu\text{m}$  wells needs to be in the interval of 112  $\mu\text{m}$  to 168  $\mu\text{m}$ .

#### Grade 2

- $5\% < \text{surface coverage by pre-treatment} < 15\%$
- $0.5\% < \text{number of filled wells} < 5\%$
- $\pm 20\% \text{ of average} < \text{intervals for characteristics of the annuli} < \pm 40\% \text{ of average}$

#### Grade 3

- $\text{surface coverage by pre-treatment} > 15\%$
- $\text{number of filled wells} > 5\%$
- $\text{intervals for characteristics of the annuli} > \pm 40\% \text{ of average}$



	'Additional' Well Diameter ( $\mu\text{m}$ )	X Pitch ( $\mu\text{m}$ )	Y Pitch ( $\mu\text{m}$ )	Drying Method	Grade
Design 2	75	250	250	Vacuum	2
Design 2	75	250	250	Pump	3
Design 3	75	125	125	Vacuum	1
Design 3	75	125	125	Pump	3
Design 4	75 (square)	125	125	Vacuum	1
Design 4	75 (square)	125	125	Pump	2
Design 5	15 (square)	5	5	Vacuum	1
Design 5	15 (square)	5	5	Pump	1
Design 6	50	81	81	Pump	2
Design 7	50	63	63	Pump	1

Table 2: Summary of results for Designs 2-7.

As can be seen from Table 2, and the forgoing discussion, vacuum/desiccator drying provides better quality distributions for similar well geometries than pump/convection drying for less textured (i.e. having larger, more spaced apart additional wells) surfaces. However, for highly textured surfaces the drying method does not affect the grade of pre-treatment obtained (i.e. as shown by Design 5).

It can also be seen that is preferable to have more closely spaced 'additional' wells (e.g. by comparing Designs 2 and 3), to obtain better quality pre-treatment distribution. Preferably the additional wells are spaced at 125  $\mu\text{m}$  apart or less, more preferably 100  $\mu\text{m}$  apart or less, more preferably 81  $\mu\text{m}$  or less, more preferably 63  $\mu\text{m}$  or less. Preferably, the pitch of the additional wells is smaller than the pitch of the array of 'sensor' or 'active' wells.

It is also possible to calculate the number density of wells (wells/ $\mu\text{m}^2$ ), area density of wells (well area/ total area), nearest-neighbour distance between wells for the Designs 1 to 3. These designs represent designs have only one shape of well is present (both in terms of geometry and size). These values are quantified in Table 3.

	Well Number Density (wells/micron <sup>2</sup> )	Well Area Density (-)	Well Nearest Neighbour Distance (microns)
Design 1	$1.6 \times 10^{-5}$	0.071	175
Design 2	$3.2 \times 10^{-5}$	0.141	102
Design 3	$6.4 \times 10^{-5}$	0.283	50

Table 3: Bulk characteristics of Designs 1-3

From the trends in Tables 2 and 3, it is apparent that it is preferable to have a higher density of wells on the surface to provide a better pre-treatment distribution. Preferably, the number distribution of wells (whether active or inactive) is at least  $3.2 \times 10^{-5}$  wells/micron<sup>2</sup>, more preferably  $6.4 \times 10^{-5}$  wells/micron<sup>2</sup>. Preferably, the well area density is 0.141 or more, more preferably 0.283 or more. Preferably the wells are formed so that the distance to the next nearest well is 102 microns away or less, more preferably 50 microns or less.

It is also contemplated that future apparatuses may reduce further in size, in which case the 'additional' wells provided in Designs 6 and 7, may actually be used as a continuous array of active wells. In that case, the Designs would have the characteristics shown in Table 4.

	Well Number Density (wells/micron <sup>2</sup> )	Well Area Density (-)	Well Nearest Neighbour Distance (microns)
Design 6	$1.5 \times 10^{-4}$	0.299	31
Design 7	$2.5 \times 10^{-4}$	0.495	13

Table 4: Bulk characteristics of 'additional' wells of Designs 6 and 7

As such, the number distribution of wells is still more preferably  $1.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more, and still more preferably  $2.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more. Further the well area density is still more preferably 0.299 or more, and still more preferably 0.495 or more. Additionally, the wells are still more preferably formed so that the next nearest well is 31 microns away or less, and more preferably 13 microns away or less.

It is further apparent from Table 1 that is preferable for the wells, whether they are all active or not, to be smaller. Preferably the wells are 75 microns in diameter or smaller, more preferably 50 microns in diameter or smaller.

In practice, the advantage of the present invention may be achieved using arrays constructed either only partially or entirely of active wells. By controlling the surface energy by using the additional wells (whether they are ultimately used for sensing or otherwise) an improved flow of the pre-treatment can be obtained as well as an improvement of the subsequent pre-treatment distribution. Even in the absence of a pre-treatment step, the improved flow control gives more uniform flows that can help bilayer formation.

The present invention has been described above with reference to specific embodiments. It will be understood that the above description does not limit the present invention, which is defined in the appended claims.

## CLAIMS

1. An apparatus for supporting an array of layers of amphiphilic molecules, the apparatus  
5 comprising:  
a body,  
formed in a surface of the body, an array of sensor wells capable of supporting a layer of  
amphiphilic molecules across the sensor wells, the sensor wells each containing an electrode  
for connection to an electrical circuit, and  
10 formed in the surface of the body between the sensor wells, flow control wells capable of  
smoothing the flow of a fluid across the surface.
2. An apparatus according to claim 1, wherein the cross-sectional area of a flow control well is  
less than the area of a sensor well.
- 15 3. An apparatus according to claim 1 or claim 2, wherein the flow control wells do not contain  
electrodes.
4. An apparatus according to claim 1 or claim 2, wherein the flow control wells each contain an  
20 electrode, the electrodes in the sensor wells being connected to the electrical circuit but the  
electrodes in the flow control wells not being connected to the electrical circuit.
5. An apparatus according to any one of claims 1 to 4, further comprising a cover over the  
surface of the body defining a cavity therebetween, and a common electrode arranged in the  
25 cavity for connection to the electrical circuit.
6. An apparatus according to claim 5, wherein the cover has an internal surface facing the  
surface of the body that is roughened to smooth the flow of fluid thereover.
- 30 7. An apparatus according to any one of claims 1 to 6, wherein the array of sensor wells is a  
regular array, and the flow control wells consist of a regular array of flow control wells.

8. An apparatus according to any one of claims 1 to 7, wherein the pitch of at least a portion of the array of flow control wells is smaller than the pitch of at least a portion of the array of sensor wells.
- 5 9. An apparatus according to any one of claims 1 to 8, wherein the sensor wells are circular.  
Claim 9a An apparatus according to any one of claims 1 to 9 wherein the flow control wells are square.
- 10 10. An apparatus according to any one of claims 1 to 9, wherein the flow control wells are distributed over a larger area than the sensor wells.
11. An apparatus according to any one of claims 1 to 10, wherein the sensor wells and flow control wells are arranged such that a pre-treatment of a hydrophobic fluid applied to the surface of the body would not enter the Cassie-Baxter state.
- 15 12. An apparatus according to any one of claims 1 to 11, wherein the sensor wells and flow control wells are shaped to provide a surface roughness  $r$ , defined as the total area of the surface and wells divided by the projected area of the surface, and a solid surface area fraction  $f$ , defined as the area of the surface between the wells divided by the projected area of the surface, that meet the requirement in respect of a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, having a contact angle  $\theta$  that  $((\varphi - 1)/(r - \varphi)) > \cos(\theta)$ .
- 20 13. An apparatus according to any one of claims 1 to 12, wherein the wells are formed on the surface with a number density of  $3.2 \times 10^{-5}$  wells/micron<sup>2</sup> or more, optionally  $6.4 \times 10^{-5}$  wells/micron<sup>2</sup> or more, further optionally  $1.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more, and still further optionally  $2.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more.
- 25 14. An apparatus according to any one of claims 1 to 13, wherein further comprising a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, applied to the sensor wells.
- 30 15. A method of preparing an apparatus for forming an array of layers of amphiphilic molecules, the method comprising:

providing an apparatus according to any one of claims 1 to 13;  
delivering across the surface of the body a pre-treatment of a hydrophobic fluid.

16. A method according to claim 15, wherein the pre-treatment is delivered in a solvent, the  
5 method further comprising drying the surface of the body to remove the solvent.

17. A method according to claim 16, wherein said step of drying the surface of the body to  
remove the solvent is performed under a pressure below atmospheric pressure.

10 18. A method according to any one of claims 15 to 17, wherein the method is performed so that  
each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 15% of the area in  
which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 5%; and

15 the values of rectangularity and the perimeter of all the annuli of pre-treatment around the  
respective sensor wells falls within a 40% of the average values.

19. A method according to any one of claims 15 to 18, wherein the method is performed so that  
each of the following conditions is met:

20 the visible coverage of the surface by the pre-treatment is less than 5% of the area in  
which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 0.5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the  
respective sensor wells falls within a 20% of the average values.

25 20. A method of forming an array of layers of amphiphilic molecules, the method comprising:

preparing an apparatus by a method according to any one of claims 15 to 19; and

flowing a fluid containing amphiphilic molecules across the surface of the body to  
form layers of amphiphilic molecules across at least some of the array of sensor wells.

30 21. A method of forming an array of layers of amphiphilic molecules, the method comprising:

providing an apparatus according to any one of claims 1 to 13; and

flowing a fluid containing amphiphilic molecules across the surface of the body to form  
layers of amphiphilic molecules across at least some of the array of sensor wells.

22. An apparatus for supporting an array of layers of amphiphilic molecules, the apparatus comprising:

a body; and

formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, the sensor wells each containing an electrode for connection to an electrical circuit,

wherein the wells have a number density of  $3.2 \times 10^{-5}$  wells/micron<sup>2</sup> or more, optionally  $6.4 \times 10^{-5}$  wells/micron<sup>2</sup> or more, further optionally  $1.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more, and still further optionally  $2.5 \times 10^{-4}$  wells/micron<sup>2</sup> or more.

23. An apparatus according to claim 22, wherein all the wells are sensor wells.

24. An apparatus according to claim 22, wherein some of the wells are sensor wells, and the remainder of the wells are flow control wells, formed in the surface of the body between the sensor wells.

25. An apparatus according to claim 24, wherein the area of a flow control well is less than the area of a sensor well.

26. An apparatus according to claim 24 or claim 25, wherein the flow control wells do not contain electrodes.

27. An apparatus according to claim 24 or claim 25, wherein the flow control wells each contain an electrode, the electrodes in the sensor wells being connected to the electrical circuit but the electrodes in the flow control wells not being connected to the electrical circuit.

28. An apparatus according to any one of claims 24 to 27, wherein the array of sensor wells is a regular array, and the flow control wells consist of a regular array of flow control wells.

29. An apparatus according to any one of claims 24 to 28, wherein a pitch of the array of flow control wells is smaller than a pitch of the array of sensor wells.

30. An apparatus according to any one of claims 24 to 29, wherein the sensor wells are circular, and the flow control wells are square.
- 5 31. An apparatus according to any one of claims 24 to 30, wherein the flow control wells are distributed over a larger area than the sensor wells.
32. An apparatus according to any one of claims 24 to 31, further comprising a cover over the surface of the body defining a cavity therebetween, and a common electrode arranged in the  
10 cavity for connection to the electrical circuit.
33. An apparatus according to claim 32, wherein the cover has an internal surface facing the surface of the body that is roughened to smooth the flow of fluid thereover.
- 15 34. An apparatus according to any one of claims 22 to 33, wherein the wells have an area density of 0.141 or more.
35. An apparatus according to any one of claims 22 to 34, wherein the wells are arranged such that a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, on  
20 the surface does not enter the Cassie-Baxter state.
36. An apparatus according to any one of claims 22 to 35, wherein the sensor wells and flow control wells are shaped to provide a surface roughness  $r$ , defined as the total area of the surface and wells divided by the projected area of the surface, and a solid surface area  
25 fraction  $f$ , defined as the area of the surface between the wells divided by the projected area of the surface, that meet the requirement in respect of a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, having a contact angle  $\theta$  that  $((\phi - 1)/(r - \phi)) > \cos(\theta)$ .
- 30 37. An apparatus according to any one of claims 22 to 34, wherein further comprising a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, applied to the sensor wells.



38. A method of preparing an apparatus for forming an array of layers of amphiphilic molecules, the method comprising:

providing an apparatus according to any one of claims 22 to 36;

delivering across the surface of the body a pre-treatment that is a fluid capable of

interacting with the amphiphilic molecules to apply the pre-treatment to the wells.

39. A method according to claim 38, wherein the pre-treatment is delivered in a solvent, the method further comprising drying the surface of the body to remove the solvent.

40. A method according to claim 39, wherein said step of drying the surface of the body to remove the solvent is performed under a pressure below atmospheric pressure.

41. A method according to any one of claims 38 to 40, wherein the method is performed so that each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values.

42. A method according to claim 41, wherein the method is performed so that each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 0.5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

43. A method of forming an array of layers of amphiphilic molecules, the method comprising:

preparing an apparatus by a method according to any one of claims 38 to 42; and

flowing a fluid containing amphiphilic molecules across the surface of the body to form layers of amphiphilic molecules across at least some of the array of sensor wells.

44. A method of preparing an apparatus for forming an array of layers of amphiphilic molecules, the method comprising:

providing an apparatus comprising a body, and, formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, the sensor wells each containing an electrode for connection to an electrical circuit, and

delivering across the surface of the body a pre-treatment, that is a fluid capable of interacting with the amphiphilic molecules, in a solvent to apply the pre-treatment to the wells; and

drying the surface of the body to remove the solvent under a pressure below atmospheric pressure.

45. A method according to claim 44, wherein the apparatus is an apparatus according to any one of claims 22 to 36.

46. A method according to claim 44 or claim 45, wherein the method being performed so that each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values.

47. A method according to claim 46, wherein the method is performed so that each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 0.5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

48. A method of forming an array of layers of amphiphilic molecules, the method comprising:

preparing an apparatus by a method according to any one of claims 44 to 46; and

flowing a fluid containing amphiphilic molecules across the surface of the body to form layers of amphiphilic molecules across at least some of the array of sensor wells.

49. A method of preparing an apparatus for forming an array of layers of amphiphilic molecules, the method comprising:

providing an apparatus comprising a body, and, formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, the sensor wells each containing an electrode for connection to an electrical circuit, and

delivering to the body a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules,

the method being performed so that each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values.

50. A method according to claim 49, wherein the method is performed so that each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 0.5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

51. A method according to claim 49 or 50, wherein the pre-treatment is applied to the body in a solvent, and the method further comprises drying the surface of the body to remove the solvent, the method being performed so that said conditions are met after said drying.

52. A method of forming an array of layers of amphiphilic molecules, the method comprising: preparing an apparatus according to any one of claims 49 to 51; and

flowing a fluid containing amphiphilic molecules across the surface of the body to form layers of amphiphilic molecules across at least some of the array of sensor wells.

53. An apparatus for forming an array of layers of amphiphilic molecules, the apparatus

5 comprising:

a body; and

formed in a surface of the body, an array of wells, at least some of which are sensor wells capable of supporting a layer of amphiphilic molecules across the sensor wells after application to the sensor wells of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, the sensor wells each containing an electrode for connection to an electrical circuit, the array of wells being arranged such that after delivery to the body of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, each of the following conditions is met:

15 the visible coverage of the surface by the pre-treatment is less than 15% of the area in which the array of sensor wells is located;

the proportion of sensor wells that are filled is less than 5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 40% of the average values.

20 54. An apparatus according to claim 53, wherein the array of wells is arranged such that after delivery to the body of a pre-treatment that is a fluid capable of interacting with the amphiphilic molecules, each of the following conditions is met:

the visible coverage of the surface by the pre-treatment is less than 5% of the area in which the array of sensor wells is located;

25 the proportion of sensor wells that are filled is less than 0.5%; and

the values of rectangularity and the perimeter of all the annuli of pre-treatment around the respective sensor wells falls within a 20% of the average values.

55. An apparatus according to claim 54, wherein further comprising a pre-treatment, that is a

30 fluid capable of interacting with the amphiphilic molecules, applied to the sensor wells.

Fig. 1

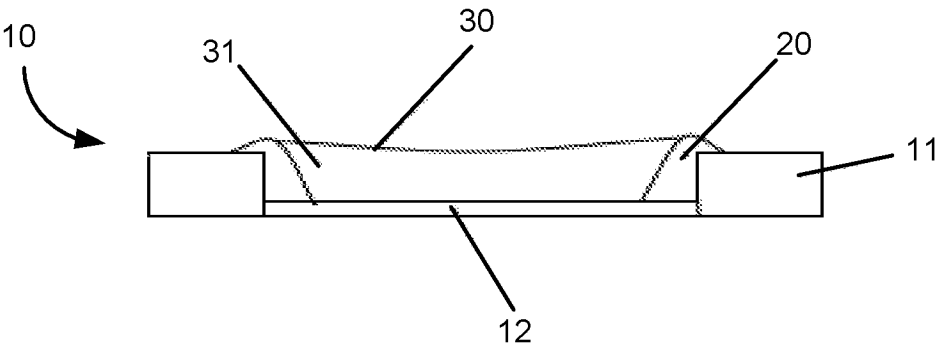


Fig. 2

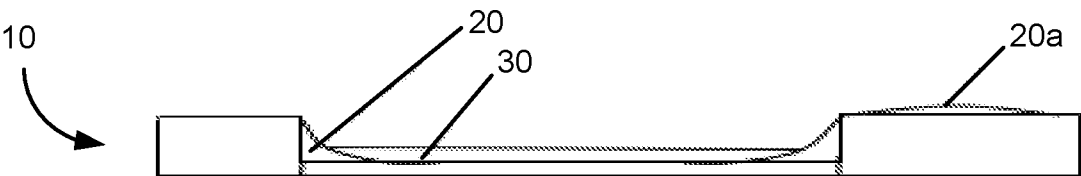


Fig. 3

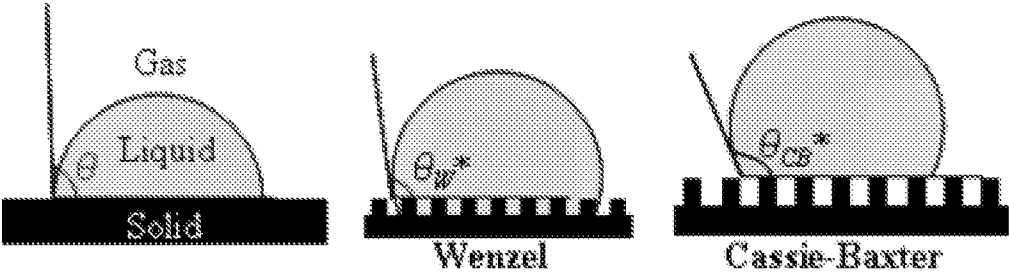


Fig. 4a

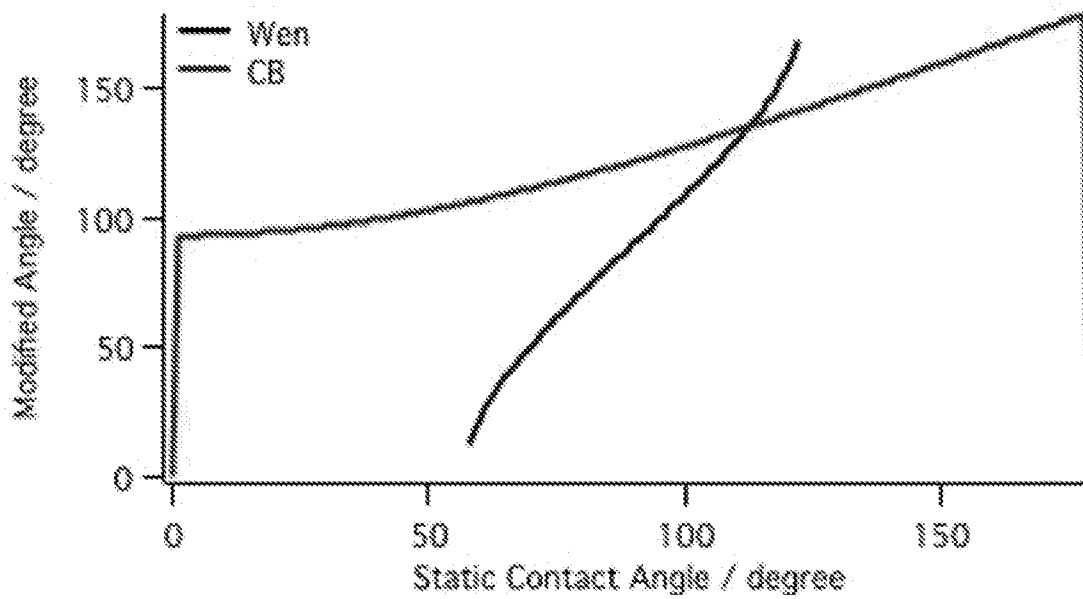


Fig. 4b

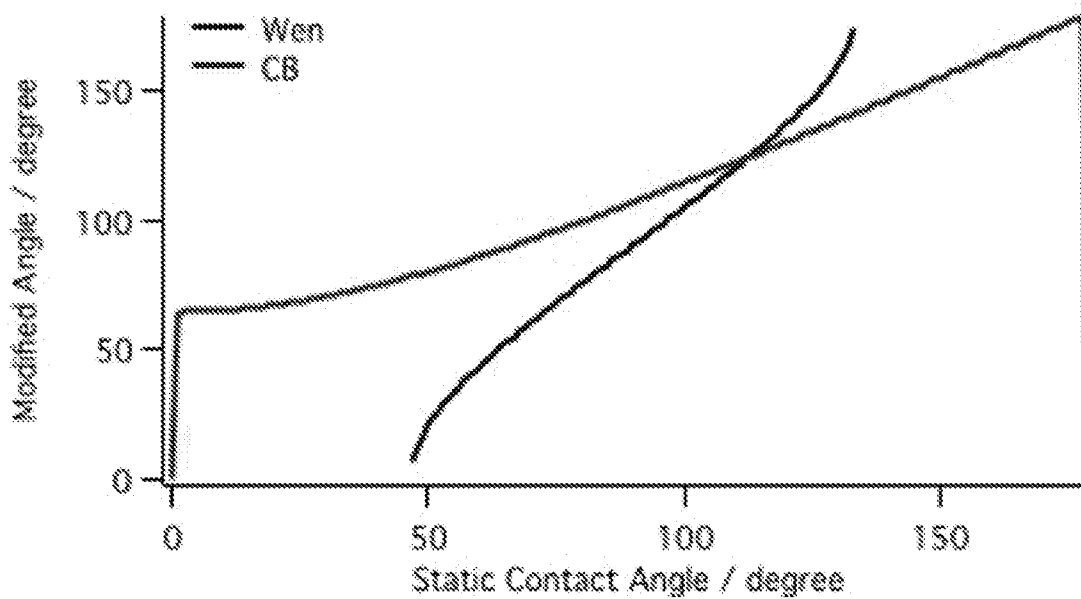


Fig. 5a

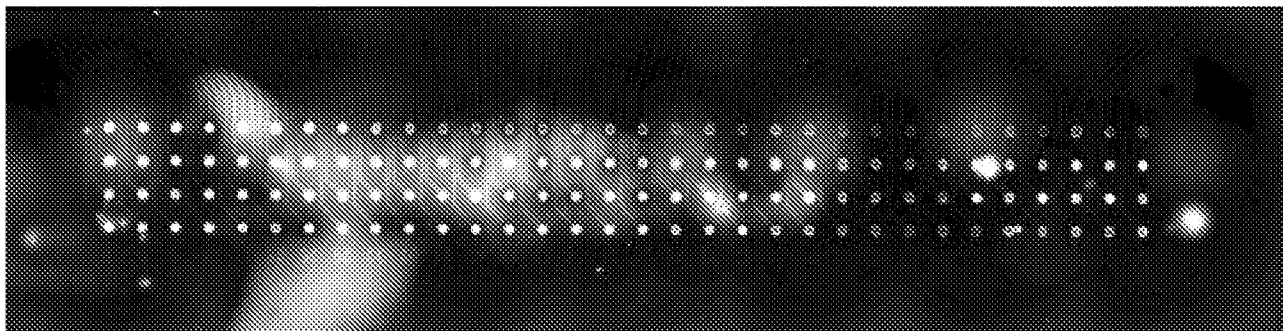


Fig. 5b

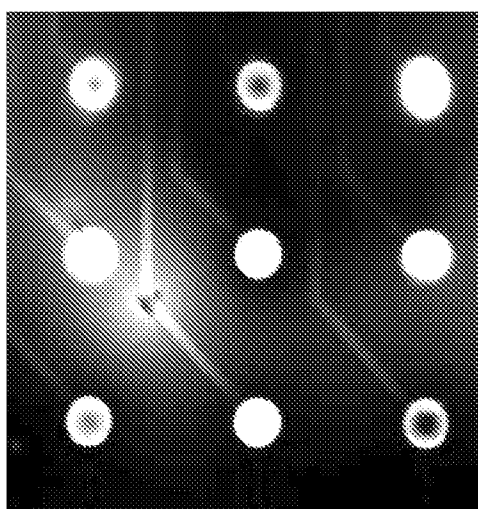


Fig. 5d

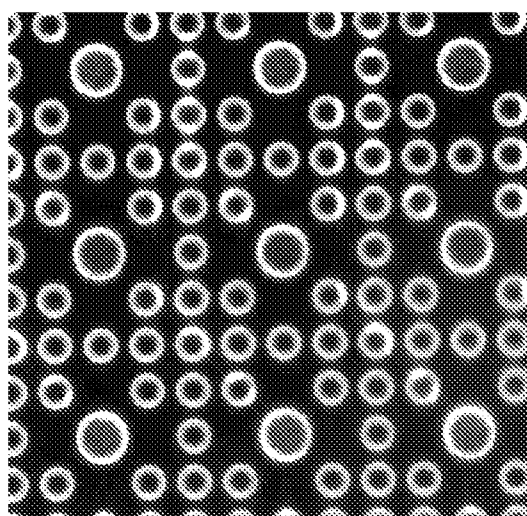
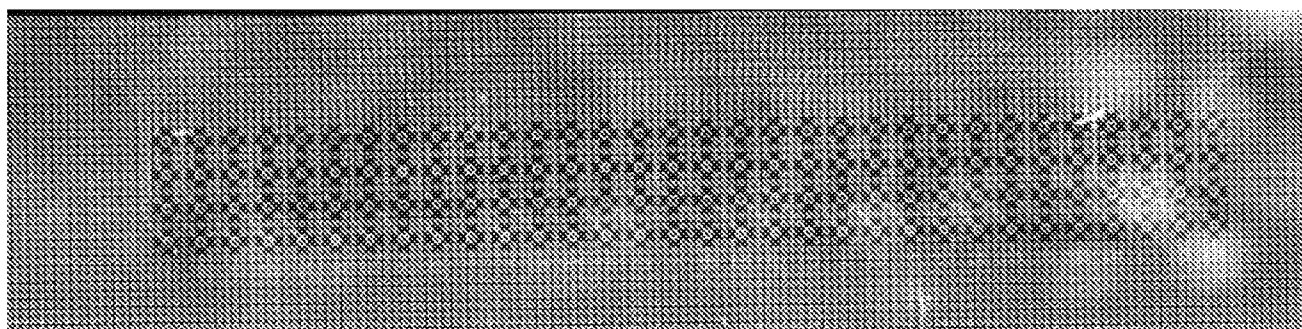


Fig. 5c



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Fig. 6

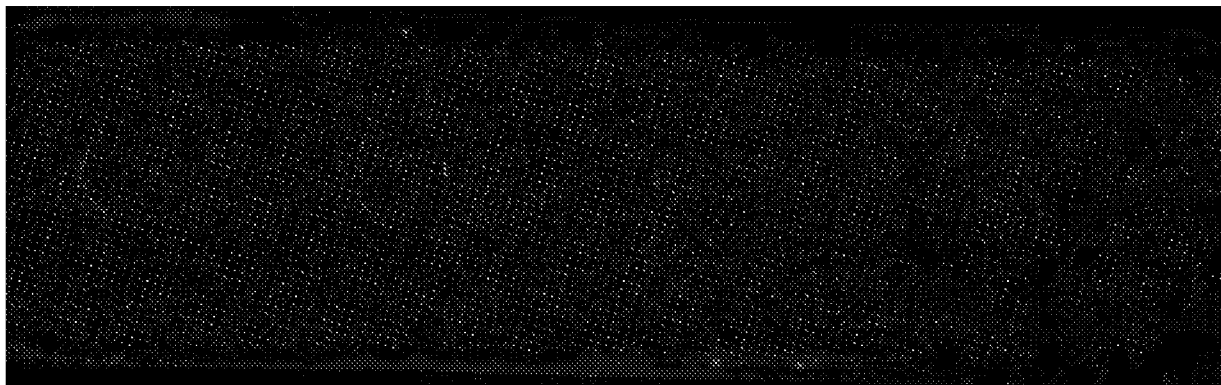


Fig. 7a

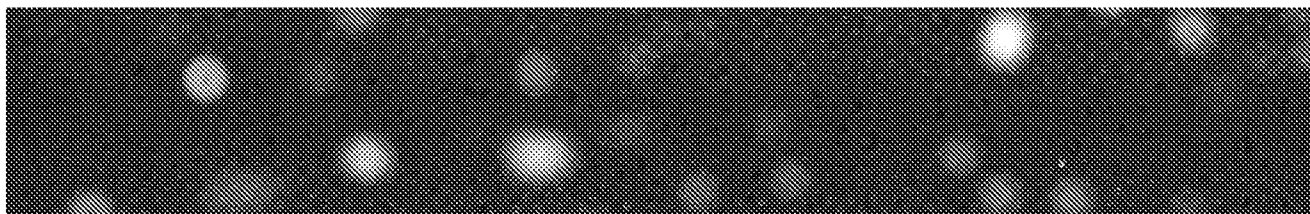


Fig. 7b

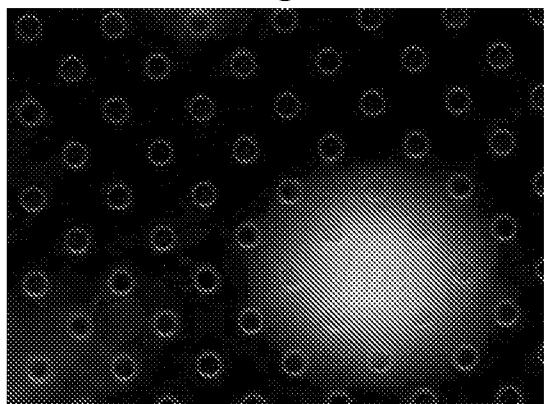


Fig. 7d

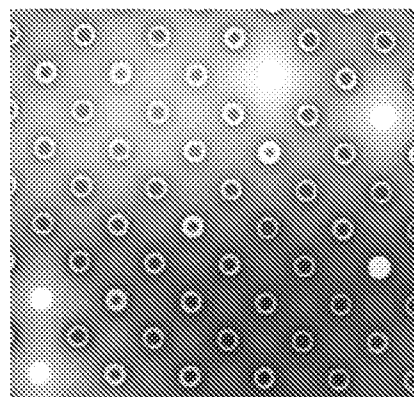
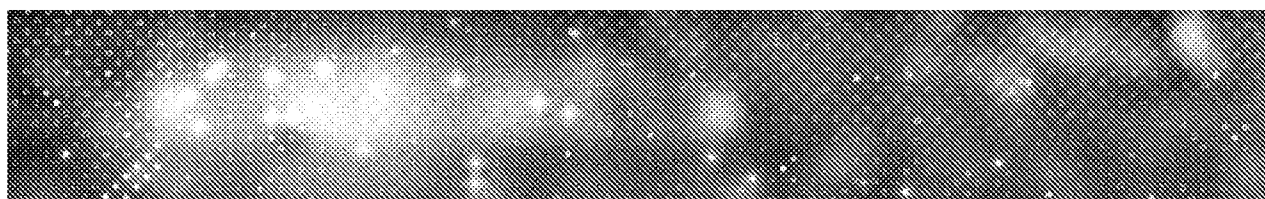


Fig. 7c





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Fig.8a

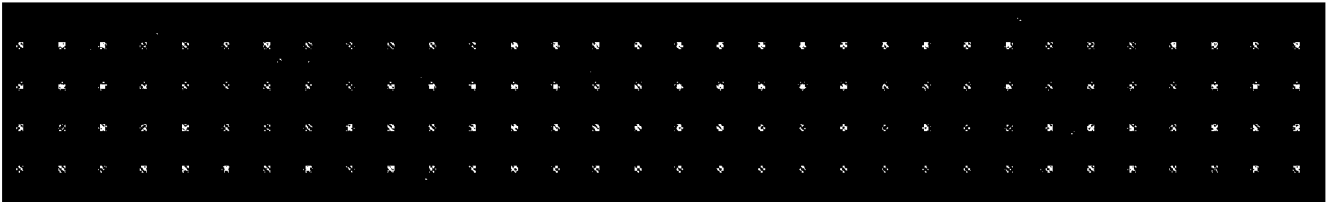


Fig. 8b

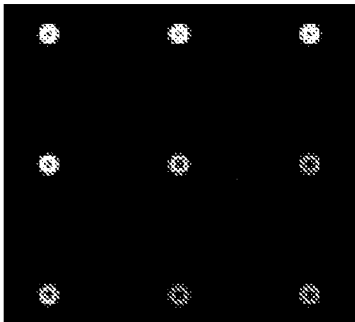


Fig. 9a

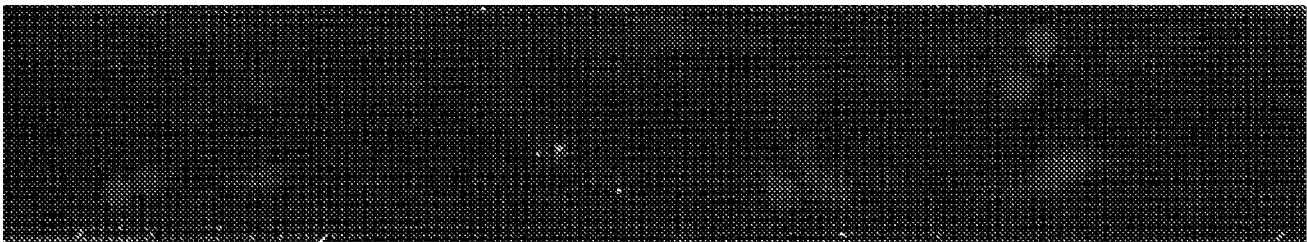


Fig. 9b

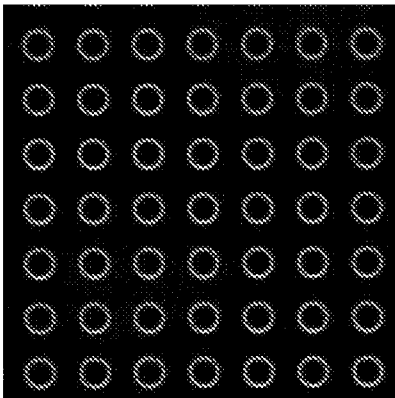


Fig. 9d

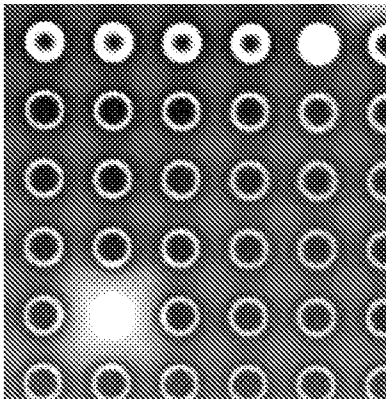


Fig. 9c

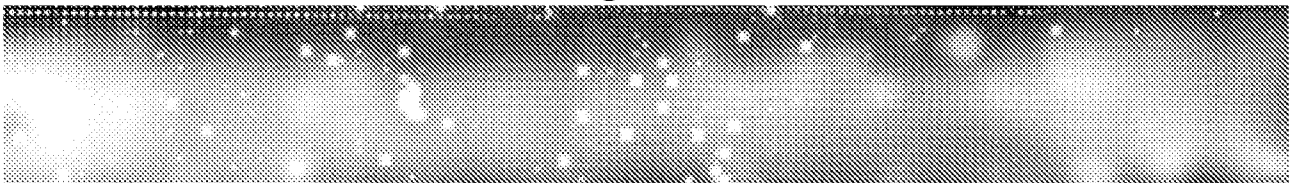


Fig. 10b

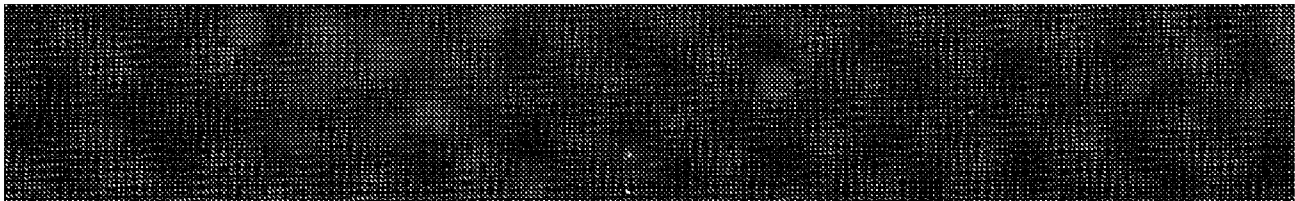


Fig. 10a

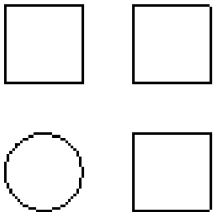


Fig. 10c

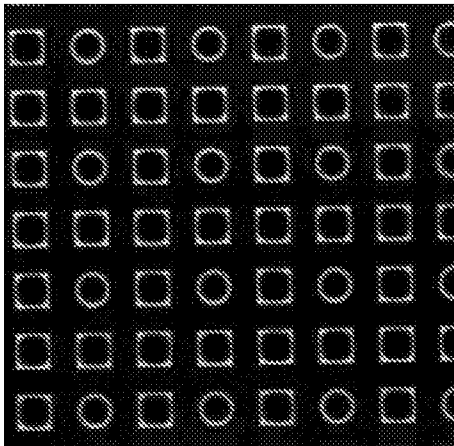


Fig. 10e

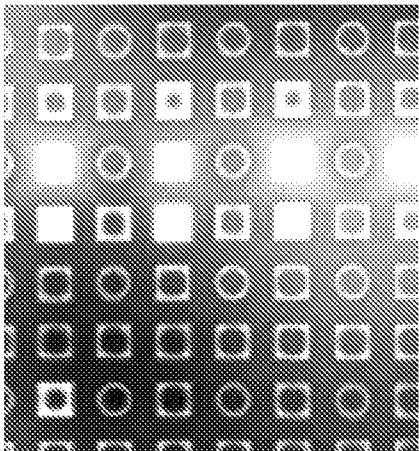


Fig. 10d

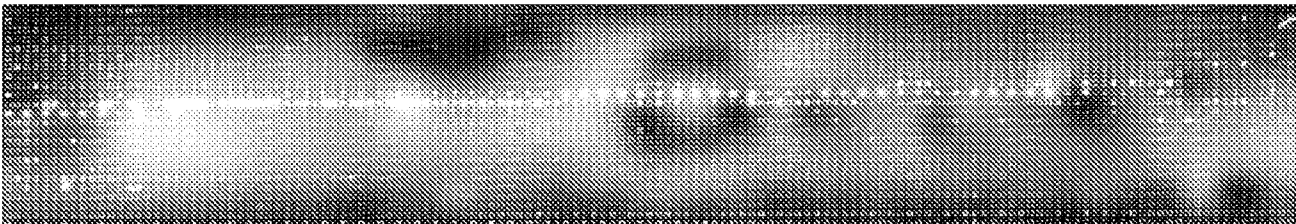


Fig. 11b

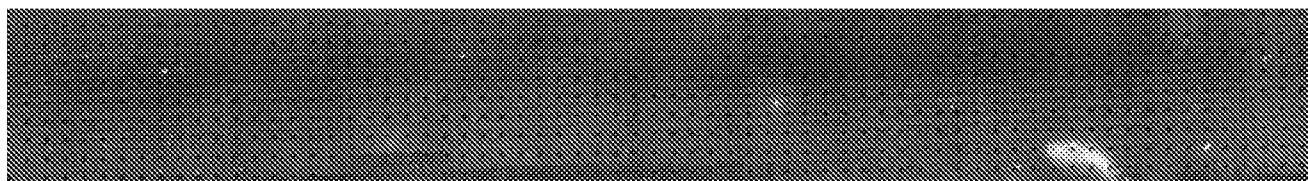


Fig. 11a

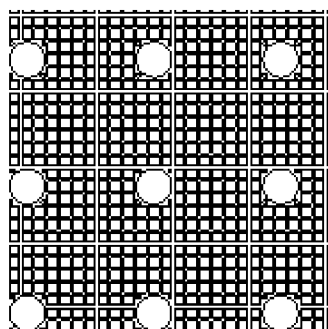


Fig. 11c

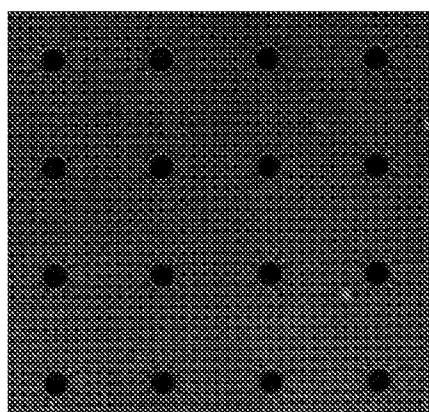


Fig. 11e

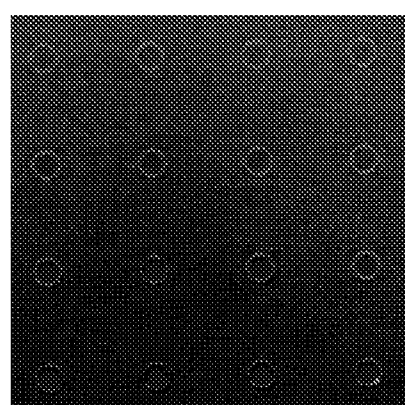


Fig. 11d

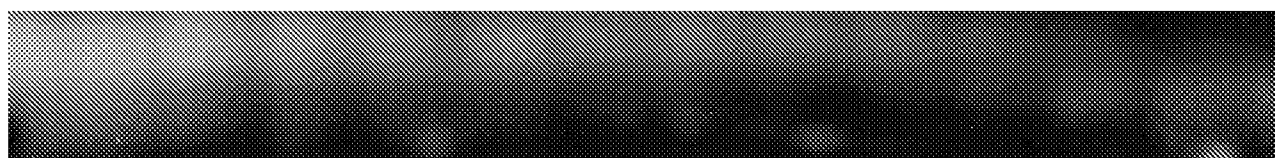


Fig. 12b

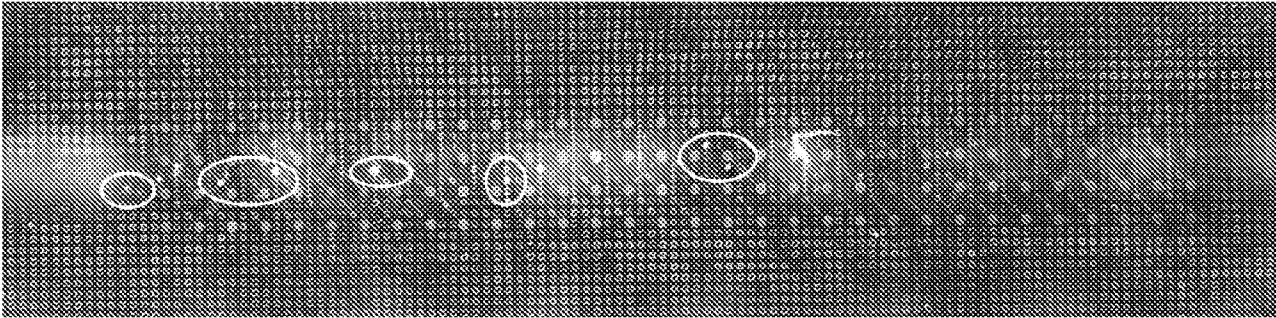


Fig. 12a

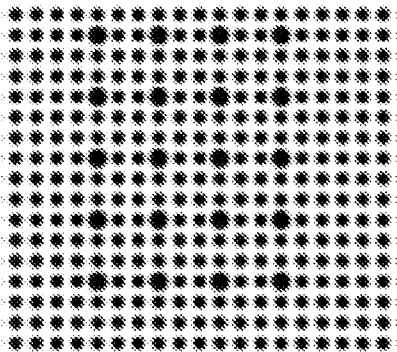


Fig. 12c

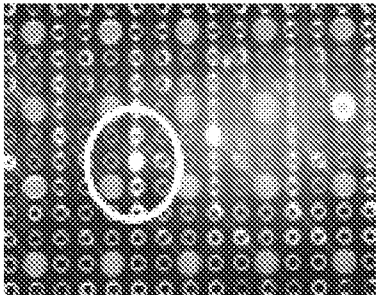


Fig. 13b

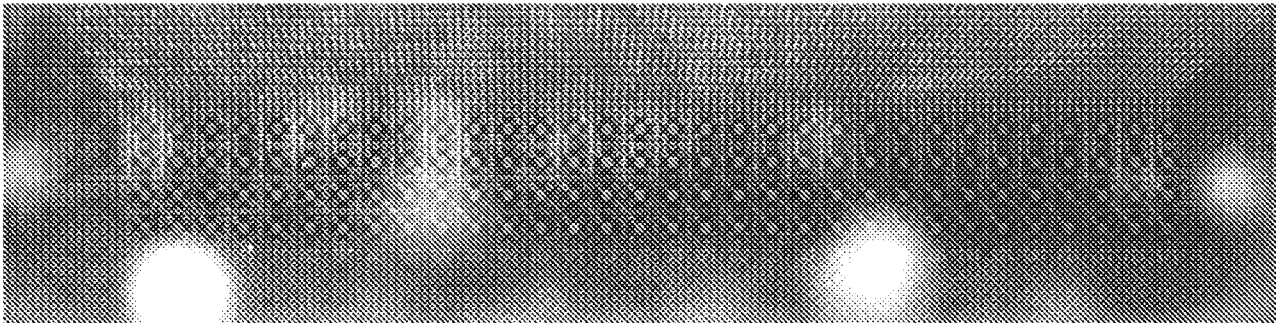


Fig. 13a

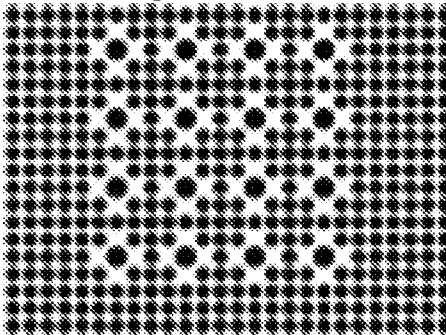


Fig. 13c

