(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 June 2006 (01.06.2006)

PCT

(10) International Publication Number
WO 2006/058246 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2005/042800

(22) International Filing Date:
28 November 2005 (28.11.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/631,526	29 November 2004 (29.11.2004)	US
60/672,366	18 April 2005 (18.04.2005)	US
60/679,263	9 May 2005 (09.05.2005)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

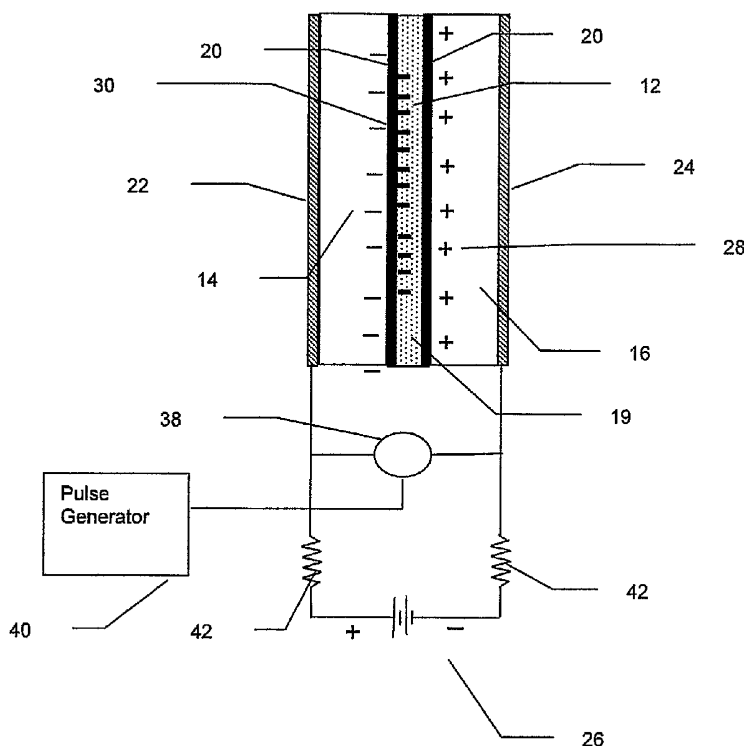
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SYSTEM AND METHOD FOR REPLICATING A BIO-MOLECULAR MICRO-ARRAY



(57) Abstract: A method for producing a complementary, replica array from a master array of bio-molecules. The replica substrate is separated from the master array by a layer of liquid. The master array has master bio-molecules covalently attached to its confronting surface, and complementary bio-molecules bound to those master bio-molecules. Opposing electrical charges applied to electrodes located close to the non-confronting surfaces of the master and replica substrates, result equalizing electric charges accumulating on the confronting surfaces. The complementary bio-molecules are un-bound from the master bio-molecules by heating, and the electrodes shorted out so that the opposing electrical charges cancel. The equalizing electric charges, however, remain for a while on the inner, confronting surfaces and now provide an electric field in the separating, liquid layer. The freed, complementary bio-molecules have a natural charge and are, therefore, moved toward the replica substrate by this electric field.

WO 2006/058246 A2

TITLE: System and Method for Replicating a Bio-Molecular Micro-Array.

INVENTORS: Roy Rosser

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CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Patent application no. 60/631,526 filed on 29 November 2004 by Roy J. Rosser entitled "System and Method for Producing a Nucleic Acid Micro-array", U.S. Provisional Patent application no. 60/672,366 filed on 18 April 2005 by Roy J. Rosser and Edward Cox entitled "System and Method for Replicating and Reading our a Nucleic Acid Micro Array", and U.S. Provisional Patent application no. 60/679,263 filed on May 9, 2005 by Roy J. Rosser entitled "System and Method for Pulsed Electro-transfer of Charged Molecules across a Film of Weakly Conducting Polar Liquid" the contents of all which are hereby incorporated by reference.

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FIELD OF THE INVENTION

[0002] The present invention relates to the replication of bio-molecular micro-arrays and particularly to methods of producing and using pulsed electric fields for replicating nucleic acid micro-arrays.

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BACKGROUND OF THE INVENTION

[0003] A bio-molecular micro-array is a collection of microscopic bio-molecules attached to a solid surface, such as glass, plastic or silicon chip, forming an array. The bio-molecules, which may be, but are not limited to, strands of Deoxyribonucleic acid (DNA), proteins, tissue or other bio-molecular material, are typically used to detect the presence or absence of other bio-molecules having complementary binding characteristics.

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[0004] A well-known bio-molecular micro-array is the DNA micro-array, also known as a DNA chip, in which different nucleic acid sequences are immobilized at precise locations on the surface via in situ solid phase synthesis or covalent immobilization of nucleic acids to the surface. The nucleic acids serve as probes for detecting complementary nucleic acid sequences. The array can have from hundreds to thousands of immobilized nucleic acids. A dense array may have more than 1000 nucleic acid sequences per square cm.

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[0005] To use a DNA micro-array, fluorescently labeled DNA or RNA sequences (either synthetic or obtained from a cell of interest) are contacted with the array. The hybridization pattern of the fluorescently labeled fragments can provide a wealth of information.

5 [0006] For instance, DNA micro-arrays have the unique ability to track the expression of many of a cell's genes at once, allowing researchers to view the behavior of thousands of genes in concert. Thus, bio-molecular micro-arrays are useful for diagnostics. Detection of unique gene expression patterns may, may for instance, assist a physician in pinpointing the onset of diseases such as cancer, Alzheimer's, osteoporosis 10 and heart disease. Bio-molecular micro-arrays are also useful for understanding which genes are active in a particular disease. Bio-molecular micro-arrays are also useful for pathogen identification and forensic applications.

[0007] DNA micro-arrays can be manufactured using a variety of techniques. For example, the various oligonucleotides can be manufactured by solid phase synthesis on 15 the array surface using photo-lithographic techniques.

[0008] Alternately, DNA micro-arrays can be manufactured using robotic or ink-jet printer technology to deposit pre-existing nucleic acids onto the array surface, and then immobilizing the nucleic acid. For example, arrays may be manufactured by applying polylysine to glass slides, and then printing pre-prepared nucleic acid sequences (also 20 known as oligomers) onto the coated slides. The printed slides are then exposed to UV light to crosslink the nucleic acid with the polylysine, thereby immobilizing the oligomers to the array.

[0009] These methods of manufacturing require many precise steps and tend to be time consuming. Proposals have been made to replicate bio-molecular micro-arrays from 25 a master array. Some of these proposals include transferring charged bio-molecules across a film of electrolyte by an electric field, as outlined in, for instance, PCT patent application PCT/US2003/031490 entitled "Manufacturing Method and Readout System for Bipolymer Arrays" by McGrew et al. and published as WO 2004/031366, the contents of which are hereby incorporated by reference. The methods proposed by McGraw, 30 however, requires an additional electrode to be placed on both the master and the replica micro-array substrates as shown in figure 1. Not only is this an additional step, but it complicates the surface chemistry of both the master and the replica array and may compromise the use of them both.

[0010] What is needed is a system that allows the replication of nucleic acid micro-arrays from a master array that does not require additional electrodes to be part of either the master or copied micro array.

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SUMMARY OF THE INVENTION

[0011] The present invention provides a method and apparatus for producing a complementary, replica array of bio-molecules from a master array of bio-molecules.

[0012] In a preferred embodiment of the invention, the replica substrate is
10 positioned proximate to the master array such that the confronting surfaces of the master array and the replica substrate are separated by a layer of liquid. The master array has one or more master bio-molecules attached to its confronting surface, and there are one or more complementary bio-molecules bound to the master bio-molecules.

[0013] Electrodes are located proximate to the non-confronting surfaces of the
15 master and replica substrates, and opposing electric charges are applied to the electrodes. As a result of the opposing charges on the electrodes, equalizing electric charges accumulate on the confronting surfaces of the master and replica substrates. These charges are the result of ions and electrons being conducted across the layer of liquid separating the confronting surfaces of the master array and the replica substrate.

20 [0014] The complementary bio-molecules are then separated, or unbound, from the master bio-molecules, typically by heating or suitable change of ph value.

[0015] The two electrodes are then conductively connected to each other, so that the opposing electric charges on them cancel each other out. This leaves the equalizing electric charges that have built up on the inner, confronting surfaces of the master array
25 and the replica substrates un-equalized, and so provides an electric field in the separating, liquid layer. The original charges to the electrodes are selected so that this electric field moves the now free, complementary bio-molecules toward the replica substrate, using the natural charge of the bio-molecule.

[0016] In a preferred embodiment, the liquid layer separating the master array and
30 the replica surface is slightly conducting, and the equalizing electric charges dissipate with time. The electrodes are, therefore, isolatingly disconnected, and the steps of relatively slowly applying opposing charges, and then rapidly shorting out the electrodes is repeated to provide a series of pulses of correctly oriented electric field in the liquid layer to move the charged bio-molecules all the way to the replica surface.

[0017] In a further preferred embodiment of the invention, the master bio-molecules are strands of nucleic acid co-valently bonded to the confronting surface of the master array. The complementary bio-molecules bound are complementary strands of nucleic acid hybridized to the master strands of nucleic acid. At the appropriate time in
5 the procedure, the hybridized complementary and master strands of DNA are separated by heating them to a temperature sufficient to denature them.

[0018] These and other features of the invention will be more fully understood by references to the following drawings.

10 BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1 is a schematic cross sectional drawing showing the application of an electric field capable of transferring charged bio-molecules across a film of electrolyte using electrodes on the confronting sides of the proximate substrates.

[0020] FIG. 2 is a is a schematic cross sectional drawing showing the application
15 of an electric field not capable of transferring charged bio-molecules across a film of electrolyte or a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0021] FIG. 2A is a graph showing the electric field corresponding to the schematic cross sectional drawing of FIG. 2 showing the application of an electric field
20 not capable of transferring charged bio-molecules across a film of electrolyte or a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0022] FIG. 3 is a is a schematic cross sectional drawing showing the charging stage of an apparatus capable of supplying an electric field capable of transferring
25 charged bio-molecules part way across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0023] FIG. 3A is a graph showing the electric field corresponding to FIG. 3 and the charging stage of an apparatus capable of supplying an electric field capable of transferring charged bio-molecules part way across a film of a weakly conducting polar
30 liquid using electrodes on the non-confronting sides of the proximate substrates.

[0024] FIG. 4 is a is a schematic cross sectional drawing showing the discharging stage of an apparatus capable of supplying an electric field capable of transferring charged bio-molecules part way across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0025] FIG. 4A is a graph showing the electric field corresponding to FIG. 4 and the discharging stage of an apparatus capable of supplying an electric field capable of transferring charged bio-molecules part way across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

5 [0026] FIG. 5 is a is a schematic cross sectional drawing showing a pulsed charging apparatus capable of supplying a series of electric field pulses capable of transferring charged bio-molecules across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0027] FIG. 5A is a graph showing the electric field pulses corresponding to FIG. 10 5 and a pulsed charging apparatus capable of supplying a series of electric field pulses capable of transferring charged bio-molecules across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0028] FIG. 6A is a circuit diagram showing an equivalent circuit corresponding to the charging stage a pulsed charging apparatus capable of supplying a series of electric 15 field pulses capable of transferring charged bio-molecules across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0029] FIG. 6B is a circuit diagram showing an equivalent circuit corresponding to the discharging stage of a pulsed charging apparatus capable of supplying a series of 20 electric field pulses capable of transferring charged bio-molecules across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0030] FIG. 7 is a schematic cross section showing a further embodiment of a pulsed charging apparatus capable of supplying a series of electric field pulses capable of 25 transferring charged bio-molecules across a film of electrolyte using electrodes on the non-confronting sides of the proximate substrates.

[0031] FIGS. 8a-d are a schematic representation of an exemplary method of forming and replicating a master array suitable for replication, having master bio-molecules attached to the master array surface and complementary bio-molecules 30 attached to the master bio-molecules.

[0032] FIG. 9 is a schematic view of a master bio-molecule attached to a master array surface preparation.

DETAILED DESCRIPTION

[0033] The present invention relates to methods and apparatus for replicating bio-molecular micro-arrays by providing means for transferring charged bio-molecules across a film of a weakly conducting polar liquid, such as, but not limited to, distilled water.

5 [0034] Previous methods aimed at replicating bio-molecular arrays from master arrays have typically tried to create direct contact between the master array and the replica substrate. Because the microscopic irregularities of macroscopically flat surfaces such as, but not limited to, optically polished glass slides, are large compared to the bio-molecules being replicated, these have either required constructions such as the use of 10 sprung beads on pins as described in, for instance, US Patent 5,795,714 issued to Cantor et al. on August 18, 1998, entitled "Method for replicating an array of nucleic acid probes", the contents of which are hereby incorporated by reference, or have required that either the master or the replica has a deformable surface, as described, for instance, in US Patent 6,432,360 issued to Church on August 13, 2002, entitled "Replica 15 amplification of nucleic acid arrays", the contents of which are hereby incorporated by reference. A further method is to use some medium such as glass beads, to effect the transfer, as described, for instance, in US Patent 6,514,768 issued to Guire et al. on February 4, 2003 entitled "Replicable probe array", the contents of which are hereby incorporated by reference.

20 [0035] Proposals have been made to facilitate replication from a macroscopically flat solid master array to a macroscopically flat solid replica substrate using electric fields to transport the bio-molecules across any microscopic gaps as detailed, for instance, in PCT patent application PCT/US2003/031490 entitled "Manufacturing Method and Readout System for Bipolymer Arrays" by McGrew et al. and published as WO 25 2004/031366, the contents of which are hereby incorporated by reference. These methods, however, require additional electrodes on or close to the surfaces to which the master array bio-molecules are attached, complicating the surface chemistry.

[0036] In order to replicate bio-molecular micro-arrays, it is highly desirable to be able to transfer charged bio-molecules across a film of a weakly conducting polar liquid 30 using electrodes placed on the non-confronting sides of the proximate substrates. This allows conventional micro-arrays to be produced without the need for additional electrodes that are an integral part of either the master or copied array.

[0037] A preferred embodiment of the present invention will now be described in more detail by reference to the accompanying drawings in which, as far as possible, like numbers describe like elements.

[0038] Figure 1 is a schematic cross section of showing the application of an electric field capable of transferring charged bio-molecules across a film of electrolyte using electrodes on the confronting sides of the proximate substrates. In order to transfer a pattern of replicated nucleic acid 12 from a master array substrate 14 to a replica array 16 across a gap comprised of a film of electrolyte 18, electrodes 22 and 24 are placed on the confronting sides of the proximate substrates. This allows an electric circuit to be set up using electric power supply 26, which may be a battery of appropriate voltage and polarity. Because the thin coating 20, necessary to covalently bond the nucleic acid, has relatively high conductance, a current flows through the circuit allowing the electric field to be maintained indefinitely and so allows the migration of the charged bio-molecules 12. The thin coating 20 may be one of many preparations that allow unmodified or amino-modified oligonucleotides to be covalently attached to a substrate including but, not limited to, the 3-gylcidoxypropyltrimethoxysilane epoxide coatings applied to glass substrates, supplied commercially as Corning® Epoxide coated slides by Corning Incorporated Life Sciences of Acton, MA or a gamma amino propyl silane coating, supplied commercially as GAPS™ or UltraGAPS™ coated slides by Corning Incorporated Life Sciences.

[0039] A drawback of the circuit of figure 1 is that the electrodes are on the confronting surfaces. This means that an optically transparent conducting film has to be added to the glass slide. Although such films are well known in the art, they are an additional step, add cost and any variability in their thickness or conductivity may introduce further complications not only in the transfer of the charged bio-molecules across the electrolyte gap between the covalent bonding surfaces, i.e., the thin epoxide or lysine coating 20. A preferable solution is to effect the migration of the charged bio-molecules using electrodes that are on or adjacent to the non-confronting sides of the proximate substrates. This allows a standard master micro-array to be copied to a conventional micro-array copy with no additional conduction films as part of either the master or copy. There are, however, practical problems with using electrodes on the non-confronting sides that are described in detail below.

[0040] Figure 2 is a is a schematic cross sectional drawing showing the application of an electric field not capable of transferring charged bio-molecules across a

film of electrolyte or a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates. The problem with the circuit of figure 2, is that the micro-array substrates 14 and 16 are non-conducting dielectrics typically made of glass, or non-conducting plastics, that may be 1 to 3 mm thick. When the power supply
5 26 applies a voltage to the electrodes 22 and 24 on the non-confronting sides of the proximate substrates 14 and 16, a current flows through the circuit until capacitive charges 30 and 28 build up on both the confronting and no-confronting surfaces of the substrate. The charges 28 and 30 that build up on the confronting surfaces are the result of current flowing through the electrolyte film 18. The charges 28 and 30 that build up on
10 the confronting surfaces also mean that there is no significant net electric field within the electrolyte film 18, and so no force to move the charged bio-molecule across the gap. As detailed below, even for the least conducting polar solvent, distilled water, the time for this charge to build up is of the order of micro-seconds. Even if kilovolt plus voltages are applied to the external electrodes, the electric field across the gap is too short lived to
15 effect the transfer of a charged molecule such as a 30 mer strand of nucleic acid across a reasonable sized gap of even 0.5 micron to 10 microns.

[0041] Figure 2A is a graph showing the electric field corresponding to Figure 1 and the application of an electric field not capable of transferring charged bio-molecules across a film of electrolyte or a weakly conducting polar liquid using electrodes on the
20 non-confronting sides of the proximate substrates. As discussed above, the result of capacitive charges 30 and 28 building up on both the confronting and no-confronting surfaces of the substrate is that there is an electric field 32 present across the glass, plastic or other dielectric portion of the substrates 14 and 16, and a net electric field 34 that is substantially equal to zero across electrolyte film 18.

25 [0042] Figure 3 is a is a schematic cross sectional drawing showing the charging stage of an apparatus capable of supplying an electric field capable of transferring charged bio-molecules part way across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0043] With switch 36 in position 1, a voltage is supplied to the electrode 22 and
30 24. A current initially flows through the circuit resulting, in a very short time, in the build up of capacitance charges 24 and 28 and the electric field of figure 3A. As can be seen from figure 3A, the electric field 38 across the dielectric substrate are of the opposite sense of the fields in figure 2A. There is, however, still no net electric field 34 across the film of a weakly conducting polar liquid 19,

[0044] Figure 4 is a is a schematic cross sectional drawing showing the discharging stage of an apparatus capable of supplying an electric field capable of transferring charged bio-molecules part way across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

5 [0045] Switch 36 is now in position 2, which disconnects the electrodes 22 and 24 from the supply 26, and short circuits or discharges them. There is now substantially no electric charge on the electrodes 22 and 24. There are, however, still capacitive charges 28 and 30 on the confronting surfaces of the substrates 14 and 16 and there is now, as shown in figure 4A a net electric field 34 across the weakly conducting polar liquid 19

10 that is of the correct orientation to transfer charged bio-molecules part way across the gap of film 19. The field 34 is, however, short lived in a practical set up. The least conduction polar solvent is distilled water, and even with pure distilled water forming the liquid film gap 19, the charges forming appropriately oriented field 34 dissipate in micro-seconds, when they need to be applied for about 300 milliseconds to effect a transfer

15 across a 10 micron gap, if the voltage of power supply 26 is about 60 volts. For the transfer to work the voltage would need to be increased about 5 orders of magnitude, i.e. to about 6 MV. While technically possible, this is a very high voltage and the result would not be the most practical system.

[0046] Figure 5 is a is a schematic cross sectional drawing showing a pulsed

20 charging apparatus capable of supplying a series of electric field pulses capable of transferring charged bio-molecules across a film of a weakly conducting polar liquid using electrodes on the non-confronting sides of the proximate substrates.

[0047] Charging of the electrode 22 and 24 is done by supply 26 through resistor 42. Resistor 42 is chosen to be high compare to the resistance across weakly conducting

25 polar liquid 19. A 10 micron film of distilled water of area 1 cm by 1 cm has a resistance of about 1 K ohm, so resistors 42 should be about 10 to 100 K ohm or higher.

[0048] Pulse generator 42 supplies pulses to switch fast switch 38 at an appropriate frequency. Fast switch 38 may for instance be, but is not limited to, a high-voltage power MOSFET switching transistor such as those supplied by Infineon which

30 switch in nanoseconds and have an on-state resistance of 100 mille-ohms or less.

[0049] Figure 5A shows the electric field across the weakly conducting polar liquid 19 circuit of figure 5. The electric field has two components. The first, shown by the dotted lines 44 and 50 is the electric field due to the opposite capacitive charges on the outer electrodes. The second is shown by solid lines 46 and 48 and is due to the

opposite capacitive charges on the proximate surfaces of the substrate. Because charging occurs through resistors 42, they limit the rate of charging and so both electric fields 44 and 46 build up at a substantially equal rate with little net electric field. Because discharging of the outer electrodes occurs through essentially a short circuit, the discharge 50 is much more rapid than the discharge of the capacitive charges on the proximate surfaces 48, which occurs through weakly conducting polar liquid 19. The result is a net electric field of the correct orientation to cause charged bio-molecule to migrate part way across the film 19. By repeating the charging and discharging process, the bio-molecule can be made to migrate the complete way across the gap of film 19. A 30 mer single strand of nucleic acid may, for instance, migrate across a gap of about 10 microns in about 1 second using an external voltage of about 60 volts switched at a frequency of about 200 KHz, at a temperature of around 95 degrees C.

[0050] In a preferred embodiment, the charging time constants RC of all the capacitances through the resistors 42 is set approximately equal to the discharging time constant of the film 19. For 1 mm glass slides having a dielectric constant of 8 and a distilled water gap of 10 microns, this means that resistor 42 should have a resistance value about 2000 times higher than the resistance across the film 19. If the film 19 is distilled water, the resistance is about 1K ohm per cm square, so the resistors 42 would need to be about 2 M ohm resistors. For a glass slide have an area of about 17 cm squared, the total resistance would only need to be about 100 K ohms.

[0051] Figure 6a is an equivalent circuit for the charging stage of the method. In a preferred embodiment, the two capacitors C_1 and C_2 formed by the glass slides with the distilled water acting as a resistor R_2 between them. This simplification is possible because R1 is significantly higher than R_2. In a preferred embodiment,

[0052] $R_1 C_1/2 = R_2 C_3$

[0053] Figure 6b is an equivalent circuit for the discharge stage of the method. In a preferred embodiment, a capacitor formed by the surfaces of the glass slide filled by distilled water is being discharged through the distilled water. For a capacitor having surface area of 1 cm by 1 cm, and a thickness of 10 microns, the following apply. Extremely pure, de-ionized and degassed distilled water may have a conductance as low as 10 micro siemens per cm at 95 degree centigrade. (i.e. the resistance of a cube of water 1 cm on all sides is as high as 1 M ohm). So a slab of water 1 cm square and 10 mico-meters thick may have a resistance of as much as 1 K ohm. The capacitance of this 10 micron slab of water, with a relative permittivity of 80 is given by:

[0054] $C = \varepsilon_0 \varepsilon_r A / d$

With $\varepsilon_0 = 8.85 \times 10^{-12}$ F/m, $\varepsilon_r = 80$ for distilled water, $A = 1 \times 10^{-2}$ m. 1×10^{-2} m. and $d = 10 \times 10^{-6}$ m

$C = 8.85 \times 10^{-12} \times 80\ 1 \times 10^{-4} / 10 \times 10^{-6}$ m

5 [0055] $= 7 \times 10^{-9}$ Farads

[0056] Or 7 pico Farads

[0057] With 1 K ohm resistance, the time constant RC is 7×10^{-6}seconds, i.e. 7 micro-seconds. For a 15 v/cm field, nucleic acid drift speed is estimated to be about 35 microns/ second. So in 7 micro-seconds, nucleic acid would only be moved about 0.2 10 nanometers. Instead a pulsed system of this invention having a 200 KHz switching time needs to be used for about 0.6 seconds.

[0058] Figure 7 is a schematic cross section showing a further embodiment of a pulsed charging apparatus capable of supplying a series of electric field pulses capable of transferring charged bio-molecules across a film of electrolyte using electrodes on the 15 non-confronting sides of the proximate substrates.

[0059] External electrodes 22 and 24 are encapsulated in suitable dielectric material 54 and 56 which may for instance be, but is not limited to, a suitable polymer. Container 52 may contain a suitable polar liquid solvent 50, which may be distilled water.

[0060] One of ordinary skill in the art will readily appreciate that the master array 20 may be constructed using any of the standard methods of micro-array production including, but not limited to, robotic spotting of nucleotides, ink-jet printing of nucleotides, photo-lithographic, solid phase synthysis on the array surface or by ink-jet printing, insitu synthysis some of which are described in, for instance, U.S. Patent 6,600,031 issued to Fodor et al. on July 29, 2003 entitled "Methods of making nucleic 25 acid or oligonucleotide arrays", the contents of which are hereby incorporated by reference.

[0061] Similarly, one of ordinary skill in the art will appreciate that the initial copying of the master bio-molecules of micro-array, prior to separation and transport of the replica bio-molecules to the replica substrate, may be performed in a number of ways. 30 For instance, when the master bio-molecules are stands of DNA, the initial copying may be done by first annealing a universal primer to part of all the DNA strands, then extending the primer by submersing the micro-array in a suitable buffer solution containing a suitable mixture of nucleotides (also known as dNTPS) and polymerase. Unlike the high temperature polymerase required in a conventional DNA amplifying

Polymerase Chain Reaction (PCR), the polymerase used in extending the primer need not be stable at high temperatures as only one round of extension is required.

[0062] Similarly, one of ordinary skill in the art will appreciate that a master strand of DNA may have three sequence portions. A part of the master strand may be complementary to the primer strand and common to all the stands of DNA on an array. A second part of the master DNA strand may be unique and correspond to a complementary sequence required on the replica array. Finally, there may be a third part of the sequence which is a spacer to allow enough physical room between the substrate and a portion desired to be copied for the polymerase molecule to fit. The spacer may be the portion of the sequence that is complementary to the primer. The spacer may also be a non-DNA structure.

[0063] Figures 8a-b are a schematic representation of an exemplary method of forming a master array suitable for replication, having master bio-molecules attached to the master array surface and complementary bio-molecules attached to the master bio-molecules.

[0064] Figure 8a shows a master array to which master bio-molecules have been covalently attached, comprising a substantially flat, solid substrate 14, lengths of single stranded nucleic acid 66 that are complementary to the oligomers required on the replicated array, optional spacer strands of nucleic acid 64, and short strands of common nucleic acid 62, that are common to the ends of all the nucleic acid on the master array.

[0065] Figure 8b shows the master array further having complementary bio-molecules bound to the master bio-molecules. The complementary bio-molecules may be produced by what are essentially the annealing and extension stages of a PCR reaction. The buffer used for the PCR reaction includes, in addition to the required polymerase enzyme, nucleotides 70 and primer strands 68, the primer strands having a complementary sequence to the common ends 62 on the nucleic acid on the master array. Strands of nucleic acid 74 having the required sequences for the replica array are formed in conjunction with optional spacer strands 64 and the primer 68. Because only one cycle of copying is required, the polymerase enzyme used in creating the master array with bound replica bio-molecules need not be resistant to high temperatures.

[0066] Figure 8C shows the replica bio-molecules 84 being separated, or unbound, from the master bio-molecules 82. This separation may be done by heating to a suitable temperature as in, for instance, the denaturing step of a PCR reaction, or it may be effected by a suitable change in ph value of the solution containing the bio-molecules.

The negatively charged, replica bio-molecules 84 are in the process of migrating to the replica substrate 16 under the influence of the positive charge 80 on the proximate surface of the replica substrate 16.

[0067] Figure 8D shows the replica bio-molecules 84 having reached the surface of the replica substrate where they may be attached using, for instance, the same or similar surface chemistry used in constructing the master micro-array such as, but not limited to, covalent attachment to a 3-gylcidoxypropyltrimethoxysilane epoxide coating or a gamma amino propyl silane coating.

[0068] Figure 9 is a schematic view of an exemplary master bio-molecule attached to a master array surface preparation. The master bio-molecule comprise an optional spacer 76, a length of single stranded nucleic acid 66 that are complementary to the oligomers required on the replicated array and short strands of common nucleic acid 62, that are common to all the master bio-molecules on the master bio-molecule array.

[0069] Industrial applications of replicating bio-molecular micro-arrays include making micro-arrays cheaper and, therefore, of use in clinical as well as research environments. Low cost, high fidelity replication may also have considerable use in the sequencing of genetic material. For instance, a genome, or long strand of DNA material, may be broken down into thousands of strands each a few hundred base pairs long. The material may be used to form a master array by for instance, attaching a common space/primer complementary sequence suitable for covalent bonding to a suitably prepared glass surface. A few hundred replicas of the master array may be made using the methods of this invention. Each of the replicas will contain a complementary copy of the same master strand in the same place. Each array may then be subjected to repeated cycles of polymerase extension, with a single nucleotide extension for each cycle. However, each array will receive a set of tagged nucleotides after a different number of cycles. For instance, a first array may receive a set of nucleotides in which each of the bases is fluorescently tagged with a different wavelength dye on the first cycle of extension after annealing the primer. By examining the first array under suitable lighting conditions, the first nucleotide in each of the tethered DNA stands will be identified. The second array may have untagged nucleotides on the first cycle of extension and tagged nucleotides on the second cycle. As the arrays are all copies of the same master, combining the results of the first and second arrays will give the first two nucleotides of all the tethered DNA stands. By repeating these steps, eventually all the hundred or so arrays will, in combination, give the full sequence of all the stands of tethered DNA. If

there are sufficient overlaps between the stands, the complete sequence of the long strand of DNA may then be reconstructed by computer.

[0070] Although the invention has been described in language specific to structural features and/or methodological acts, it is to be understood that the invention defined in the appended claims is not necessarily limited to the specific features or acts described. Rather, the specific features and acts are disclosed as exemplary forms of implementing the claimed invention.

What is claimed:

1. A method for replicating an array of bio-molecules, said method comprising the steps of:

5 positioning a replica substrate proximate to a master array such that the confronting surface of said master array and the confronting surface of said replica substrate are separated by a layer of liquid, and wherein said master array comprises one or more master bio-molecules attached to said confronting surface of said master array and one or more complementary bio-molecules bound to said master bio-molecules;

10 locating a first electrode proximate to the non-confronting surface of said master substrate;

locating a second electrode proximate to the non-confronting surface of said replica substrate;

applying opposing electric charges to said first and second electrodes such that 15 equalizing electric charges accumulate on said confronting surface of said master substrate and said confronting surface of said replica substrate;

separating said one or more complementary bio-molecules from said master bio-molecules; and

conductively connecting said first electrode to said second electrode such that said 20 opposing electric charges on said first and second electrodes cancel each other, said equalizing electric charges on said confronting surfaces provide an electric field in said liquid layer and said electric field moves said one or more complementary bio-molecules move toward said confronting surface of said replica substrate.

25 2. The method of claim 1 further comprising the steps of isolatingly disconnecting said first and second electrode; and then repeating said step of applying opposing charges, and then repeating said step of conductively connecting said first and second electrodes.

3. The method of claim 1 wherein said one or more master bio-molecules attached to said 30 confronting surface of said master array comprise master strands of nucleic acid co-valently bonded to said confronting surface of said master array.

4. The method of claim 3 wherein said one or more complementary bio-molecules bound to said master bio-molecules comprise one or more complementary strands of nucleic 35 acid hybridized to said master strands of nucleic acid.

5. The method of claim 4 wherein separating comprises heating to a temperature sufficient to denature said hybridized complementary and master strands of nucleic acid.

6. The method of claim 1 wherein said replica substrate comprises a sheet of dielectric material having a substantially flat confronting surface.

7. The method of claim 6 wherein the thickness of said replica substrate is greater than
5 0.5 mm.

8. The method of claim 7 wherein said dielectric material is glass.

9. The method of claim 8 wherein said steps of isolating and connecting comprise
10 switching a transistor using a pulse generator.

10. The method of claim 9 wherein said switching occurs at a rate greater than 20 KHz.

11. The method of claim 9 wherein said switching occurs at a rate greater than 200 KHz.
15

11. The method of claim 1 wherein said layer of liquid comprises distilled water having a thickness less than 1 micron.

12. The method of claim 1 wherein said layer of liquid comprises distilled water having a
20 thickness between 1 and 10 microns.

13. The method of claim 1 wherein said layer of liquid comprises distilled water having a thickness greater than 10 microns.

25 14. An apparatus for replicating an array of bio-molecules, comprising:

a replica substrate located proximate to a master array such that the confronting surface of said master array and the confronting surface of said replica substrate are separated by a layer of distilled water, and wherein said master array comprises one or more master strands of nucleic acid co-valenty bonded to said confronting surface of said
30 master array and one or more complementary strands of nucleic acid hybridized to said master strands of nucleic acid;

a first electrode located proximate to the non-confronting surface of said master substrate;

a second electrode located proximate to the non-confronting surface of said
35 replica substrate;

a charging unit connected to said first and second electrodes such that opposing electrical charges are applied to said electrodes and equalizing electric charges accumulate on said confronting surfaces of said substrates; and

a switch that, when closed, conductively connects said first electrode to said second electrode such that said opposing electrical charges cancel and said equalizing electric charges provide an electric field in said layer of distilled water that move said one or more complementary strands of nucleic acid toward said confronting surface of said replica substrate when said complementary strands of nucleic acid are separated from said master stands of nucleic acid by denaturing.

15. The apparatus of claim 14 wherein said switch comprises a transistor, and further comprising a pulse generator capable of switching said transistor from an open state in which said first and second electrodes are isolated and connected to said charging unit to said closed state.

16. The apparatus of claim 15 wherein said pulse generator switches said transistor at a rate greater than 20 KHz.

17. The apparatus of claim 14 wherein said replica array comprises a sheet of dielectric material having a substantially flat confronting surface and wherein the thickness of said sheet is greater than 0.5mm.

18. The apparatus of claim 14 wherein said layer said layer of distilled water has a thickness less than 1 micron.

19. The apparatus of claim 14 wherein said layer said layer of distilled water has a thickness greater than 1 micron.

20. A device for replicating an array of bio-molecules, comprising:

means for positioning a replica substrate proximate to a master array such that the confronting surface of said master array and the confronting surface of said replica substrate are separated by a layer of distilled water, and wherein said master array comprises one or more master bio-molecules attached to said confronting surface of said master array and one or more complementary bio-molecules bound to said master bio-molecules;

means for locating a first electrode proximate to the non-confronting surface of said master substrate;

means for locating a second electrode proximate to the non-confronting surface of said replica substrate;

means for applying opposing electric charges to said first and second electrodes such that equalizing electric charges accumulate on said confronting surface of said master substrate and said confronting surface of said replica substrate;

means for separating said one or more complementary bio-molecules from said master bio-molecules; and

means for conductively connecting said first electrode to said second electrode such that said opposing electric charges on said first and second electrodes cancel each other and said equalizing electric charges on said confronting surfaces provide an electric field in said layer of distilled water, said electric field providing means for moving said one or more complementary bio-molecules toward said confronting surface of said replica substrate.

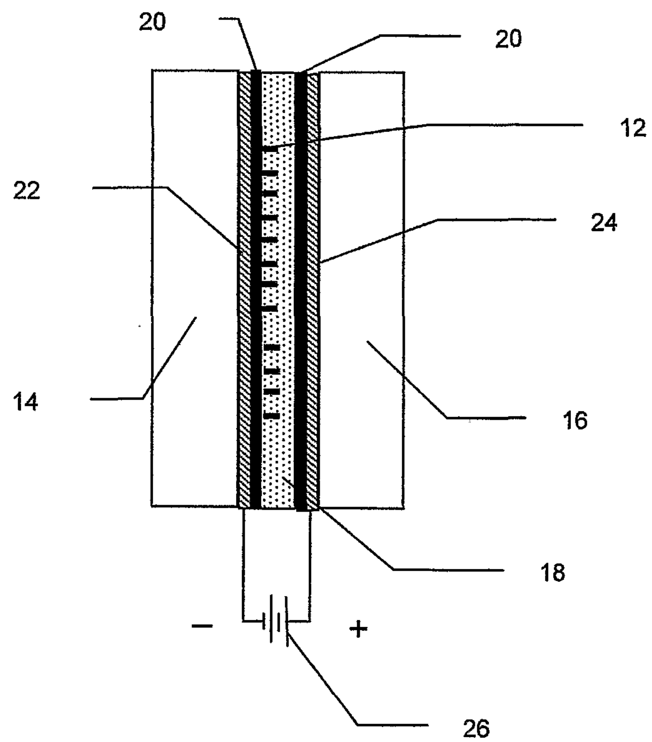


FIG. 1

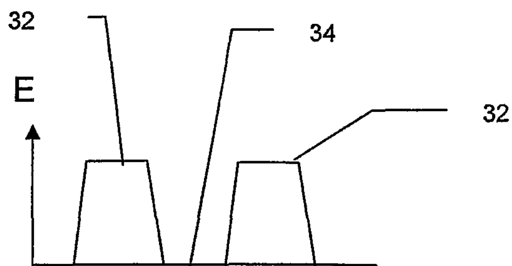


FIG 2A

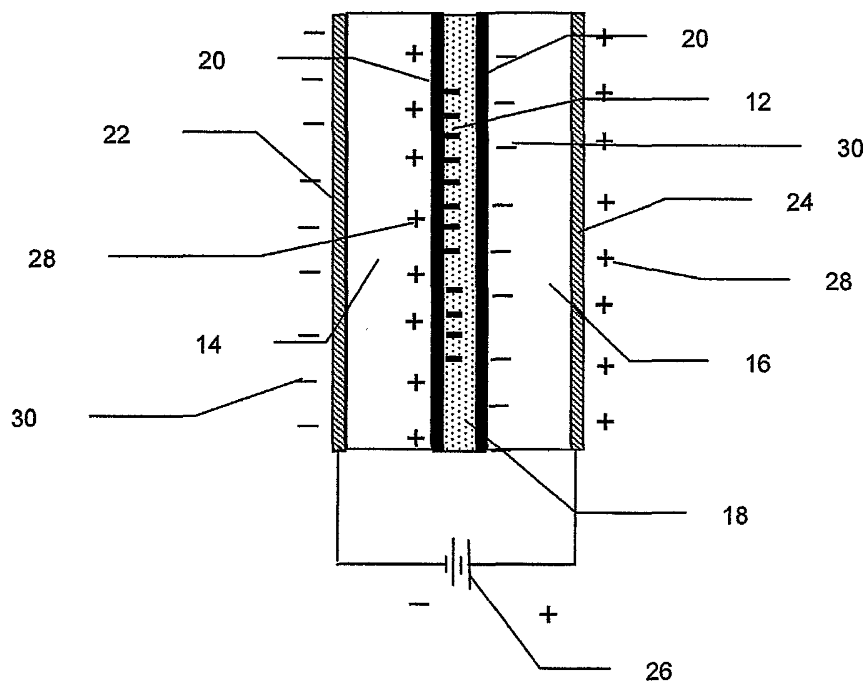


FIG. 2

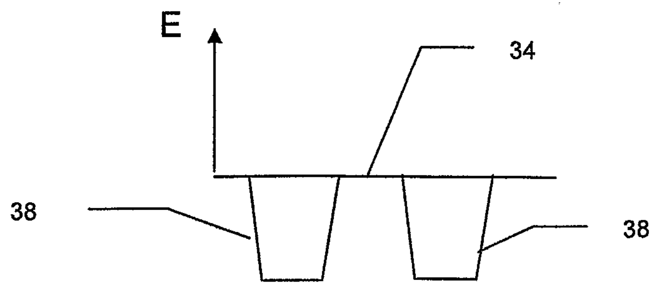


FIG 3A

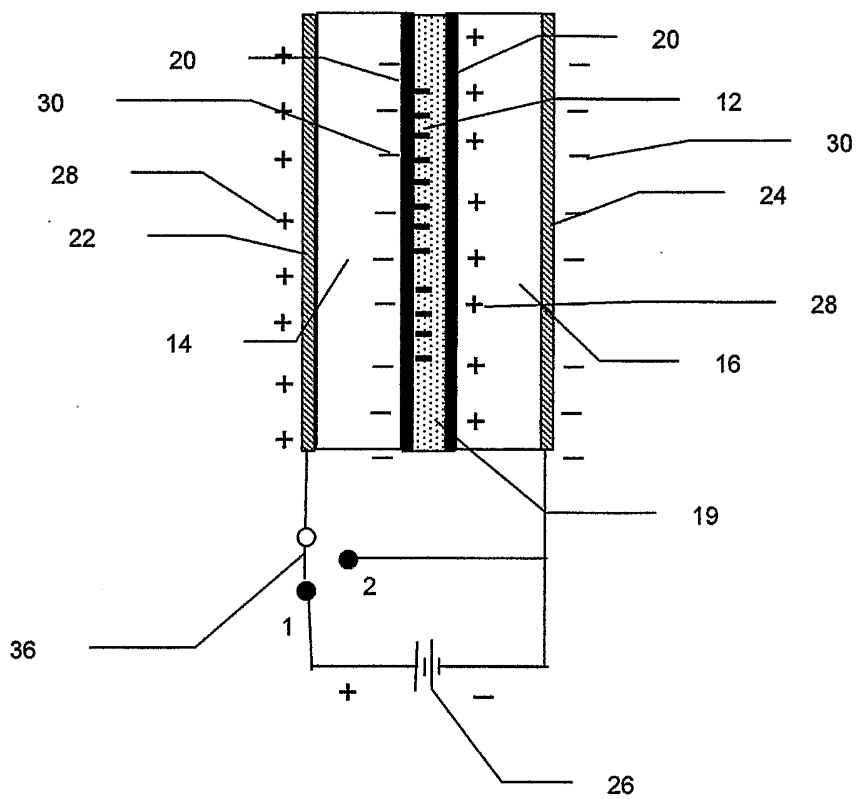


FIG. 3

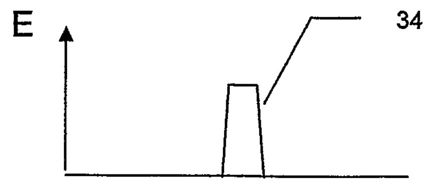


FIG 4A

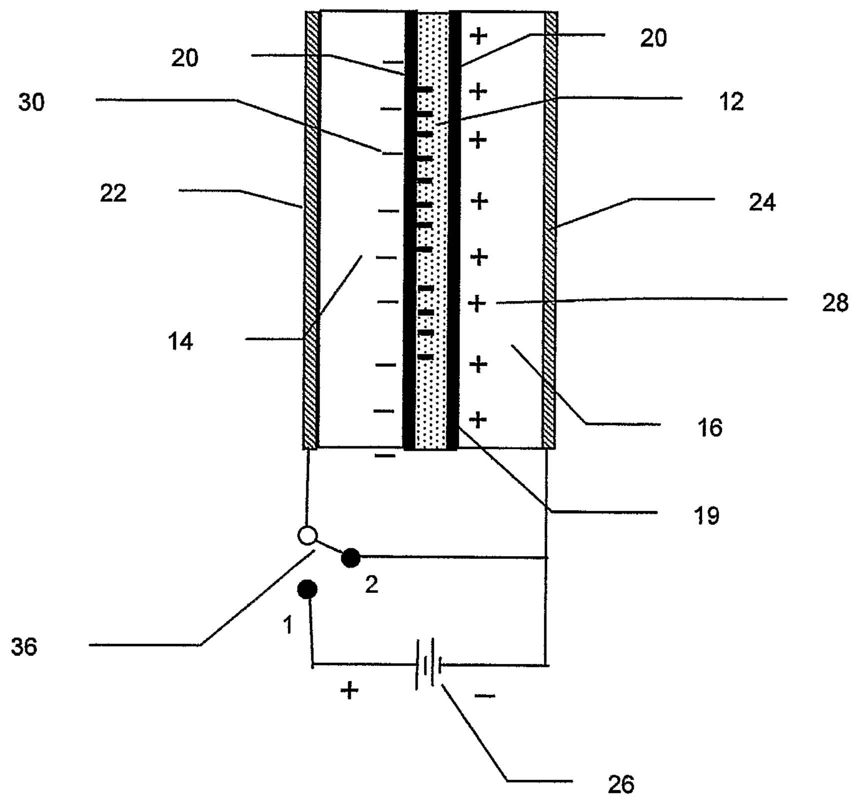


FIG. 4

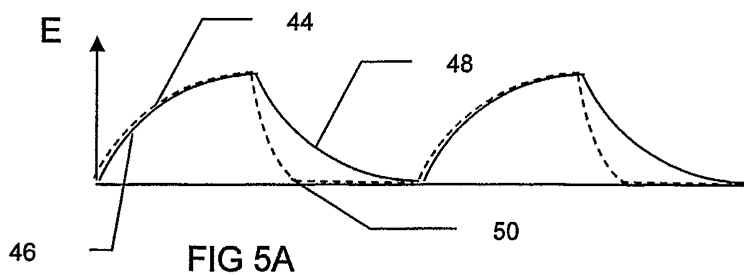


FIG 5A

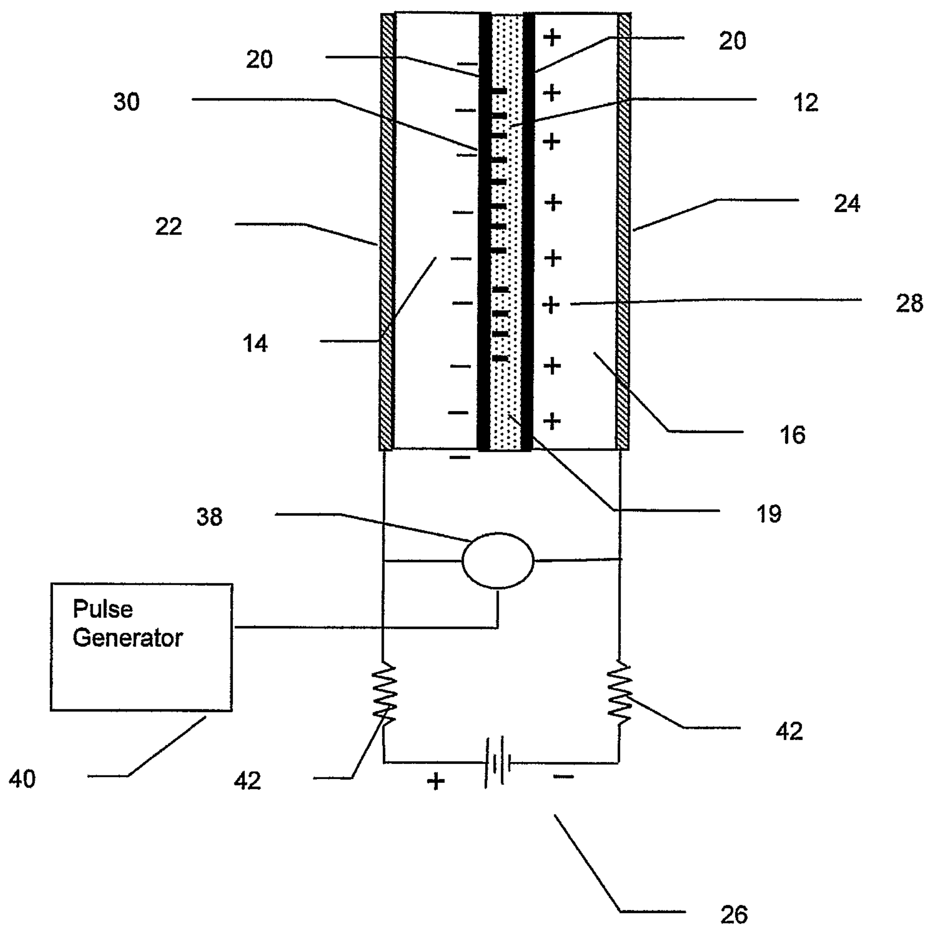


FIG. 5

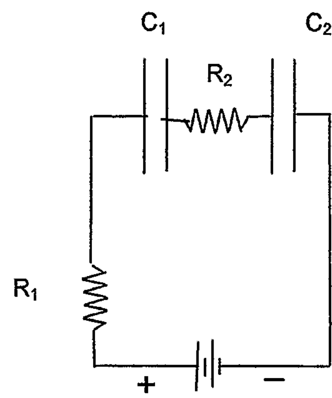


FIG. 6A

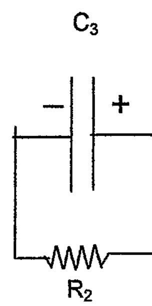


FIG. 6B

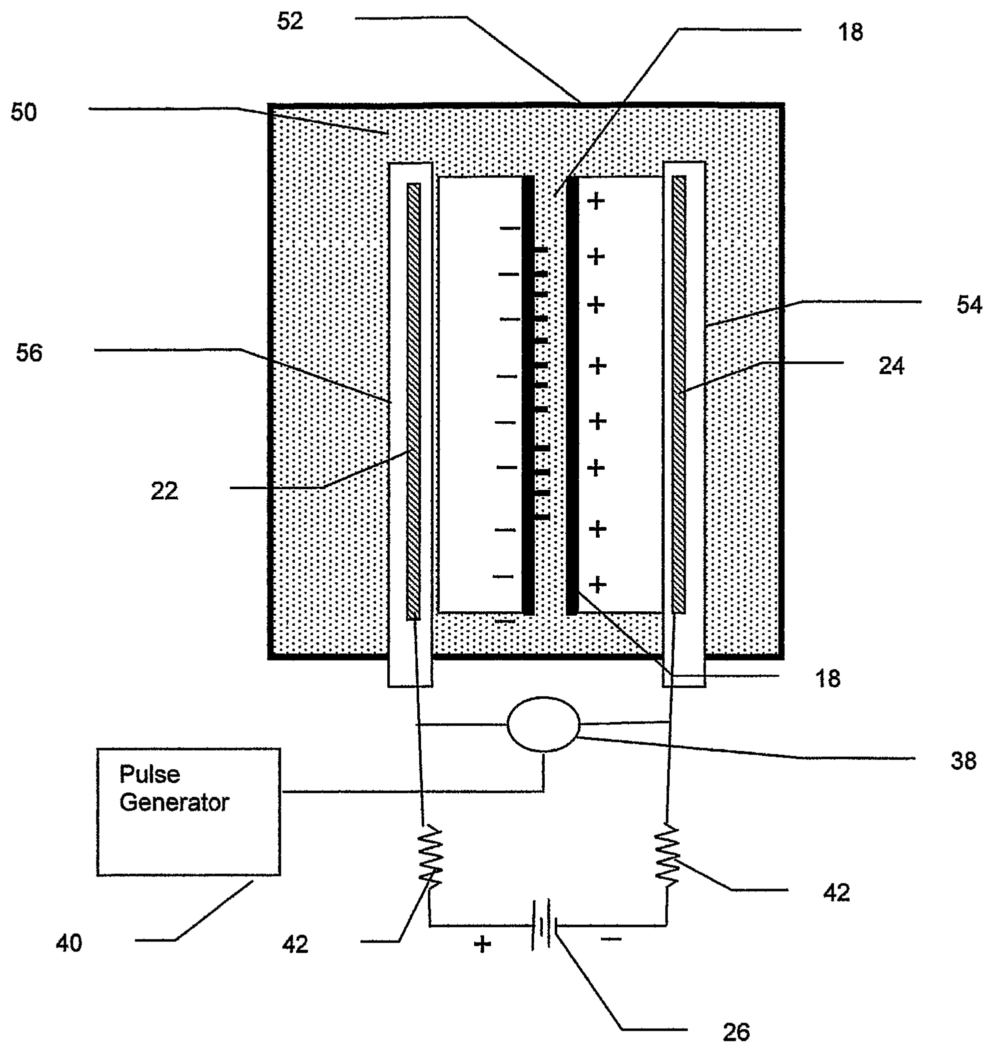


FIG. 7

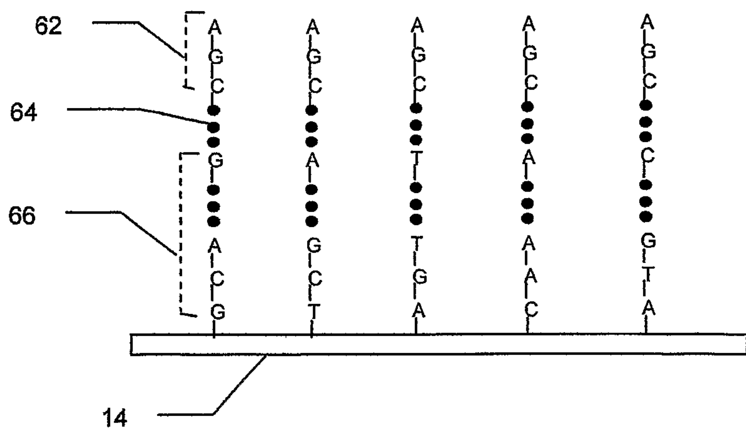


FIG. 8A

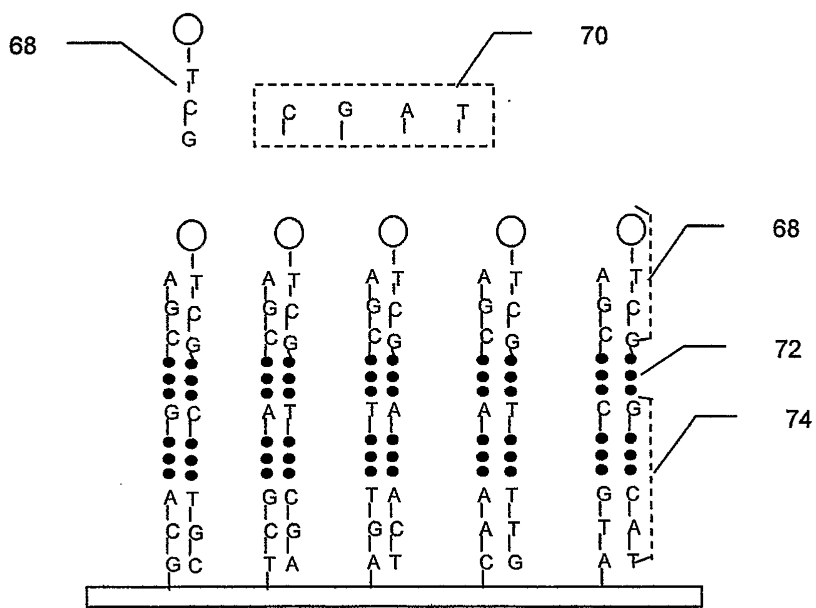
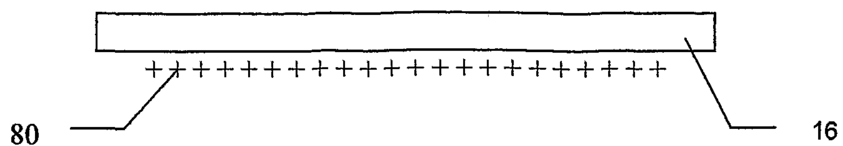


FIG. 8B



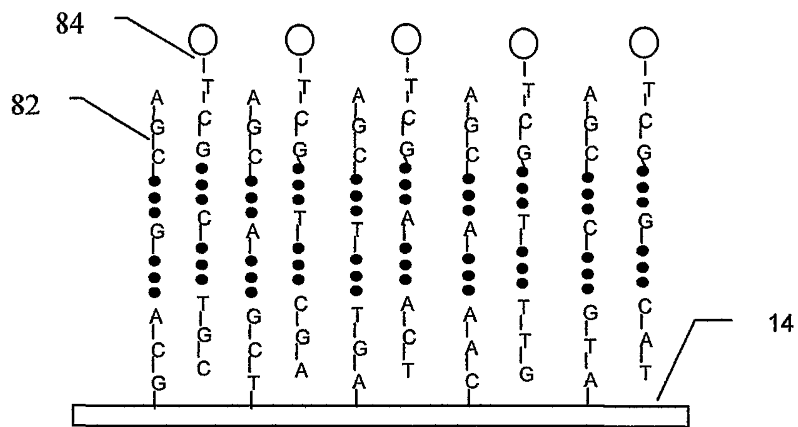


FIG. 8C

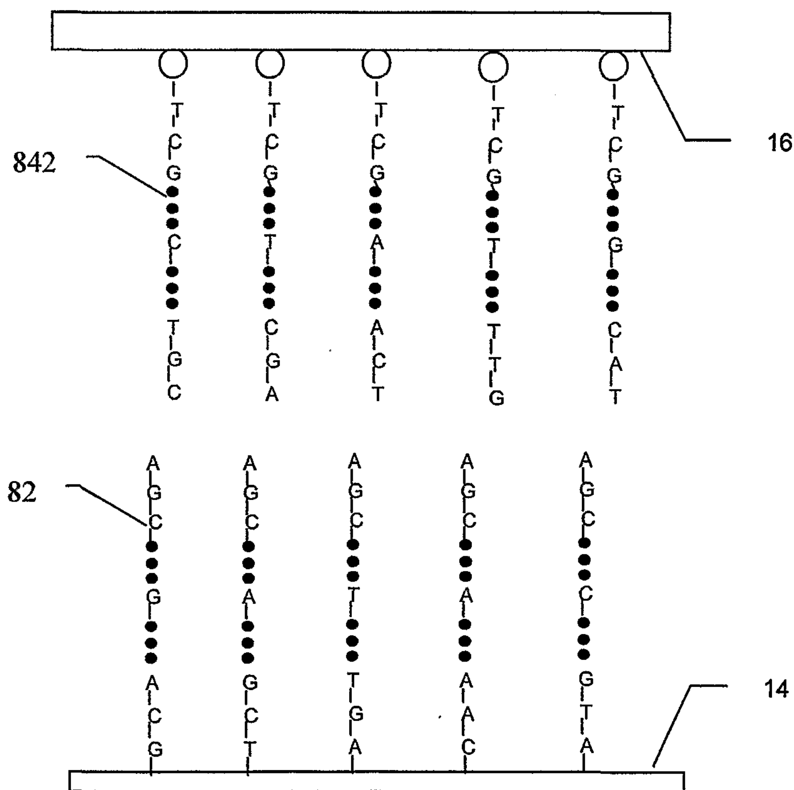


FIG. 8D

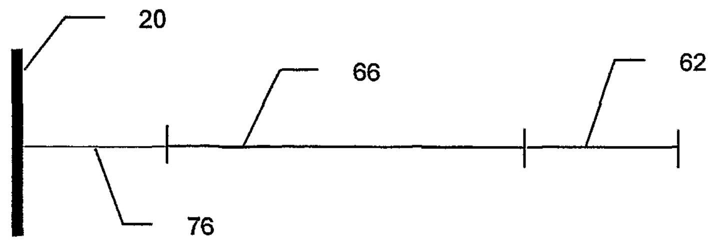


FIG. 9