



(86) Date de dépôt PCT/PCT Filing Date: 2002/06/10
(87) Date publication PCT/PCT Publication Date: 2003/05/22
(85) Entrée phase nationale/National Entry: 2003/12/08
(86) N° demande PCT/PCT Application No.: US 2002/018319
(87) N° publication PCT/PCT Publication No.: 2003/042396
(30) Priorités/Priorities: 2001/06/11 (60/297,583) US;
2001/10/02 (09/970,087) US; 2002/05/10 (60/378,938) US

(51) Cl.Int.⁷/Int.Cl.⁷ G01N 27/26, C12Q 1/68, G01N 33/53,
G01N 27/327

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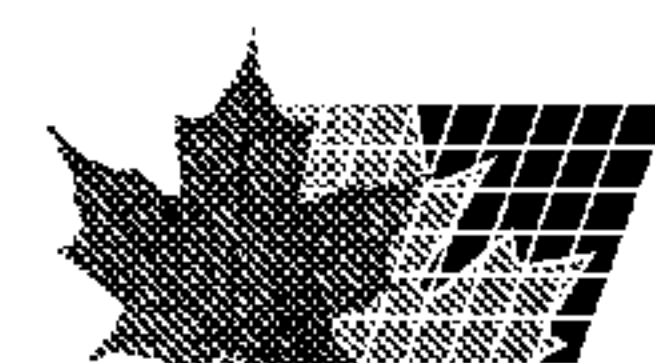
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(54) Titre : DETECTION ELECTRONIQUE DE MOLECULES BIOLOGIQUES AU MOYEN DE FINES COUCHES
(54) Title: ELECTRONIC DETECTION OF BIOLOGICAL MOLECULES USING THIN LAYERS

(57) **Abrégé/Abstract:**

This invention provides novel sensors that facilitate the detection of essentially any analyte. In general, the biosensors of this invention utilize a binding agent (e.g. biomolecule) to specifically bind to one or more target analytes. In preferred embodiments, the biomolecules spans a gap between two electrodes. Binding of the target analyte changes conductivity of the sensor thereby facilitating ready detection of the binding event and thus detection and/or quantitation of the bound analyte.



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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 May 2003 (22.05.2003)

(10) International Publication Number
PCT
WO 03/042396 A2

- (51) International Patent Classification⁷: **C12Q**

(21) International Application Number: PCT/US02/18319

(22) International Filing Date: 10 June 2002 (10.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/297,583 11 June 2001 (11.06.2001) US
09/970,087 2 October 2001 (02.10.2001) US
60/378,938 10 May 2002 (10.05.2002) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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: ELECTRONIC DETECTION OF BIOLOGICAL MOLECULES USING THIN LAYERS

(57) Abstract: This invention provides novel sensors that facilitate the detection of essentially any analyte. In general, the biosensors of this invention utilize a binding agent (*e.g.* biomolecule) to specifically bind to one or more target analytes. In preferred embodiments, the biomolecules spans a gap between two electrodes. Binding of the target analyte changes conductivity of the sensor thereby facilitating ready detection of the binding event and thus detection and/or quantitation of the bound analyte.



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ELECTRONIC DETECTION OF BIOLOGICAL MOLECULES USING THIN LAYERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of USSN 09/970,087, filed on
5 October 2, 2001, which claims priority to and benefit of USSN 60/297,583, filed on June
11, 2001, both of which are incorporated herein by reference in their entirety for all
purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

10 [Not Applicable]

FIELD OF THE INVENTION

[0002] This invention pertains to a biosensor for detecting and/or quantifying
analytes. More particularly, this invention pertains to a biosensor based on a detection
element that is a single macromolecule spanning two electrodes.

BACKGROUND OF THE INVENTION

15 [0003] Biosensors are devices that can detect and/or quantify analytes using known
interactions between a targeted analyte and a binding agent that is typically a biological
macromolecule such as an enzyme, receptor, nucleic acid, protein, lectin, or antibody.
Biosensors have applications in virtually all areas of human endeavor. For example,
20 biosensors have utility in fields as diverse as blood glucose monitoring for diabetics, the
recognition of poisonous gas and/or explosives, the detection of chemicals commonly
associated with spoiled or contaminated food, genetic screening, environmental testing, and
the like..

25 [0004] Biosensors are commonly categorized according to two features, namely, the
type of macromolecule utilized in the device and the means for detecting the contact
between the binding agent and the targeted analyte. Major classes of biosensors include
enzyme (or catalytic) biosensors, immunosensors and DNA biosensors.

[0005] Enzyme (or catalytic) biosensors typically utilize one or more enzymes as the macromolecule and take advantage of the complimentary shape of the selected enzyme and the targeted analyte. Enzymes are proteins that perform most of the catalytic work in biological systems and are known for highly specific catalysis. The shape and reactivity of a given enzyme limits its catalytic activity to a very small number of possible substrates. Enzyme biosensors rely on the specific chemical changes related to the enzyme/analyte interaction as the means for recognizing contact with the targeted analyte. For example, upon interaction with an analyte, an enzyme biosensor may generate electrons, a colored chromophore or a change in pH as the result of the relevant enzymatic reaction.

10 Alternatively, upon interaction with an analyte, an enzyme biosensor may cause a change in a fluorescent or chemiluminescent signal that can be recorded by an appropriate detection system.

[0006] Immunosensors utilize antibodies as binding agents. Antibodies are protein molecules that generally do not perform catalytic reactions, but specifically bind to particular "target" molecules (antigens). Antibodies are quite specific in their interactions and, unlike most enzymes, they are capable of recognizing and selectively binding to very large bodies such as single cells. Thus, in addition to detection of small analytes, antibody-based biosensors allow for the identification of certain pathogens such as dangerous bacterial strains.

20 [0007] DNA biosensors typically utilize the complimentary nature of the DNA or RNA double-strands and are designed for the specific detection of particular nucleic acids. A DNA biosensor sensor generally uses a single-stranded DNA as the binding agent. The nucleic acid material in a given test sample is placed into contact with the binding agent under conditions where the biosensor DNA and the target nucleic acid analyte can form a hybrid duplex. If a nucleic acid in the test sample is complementary to a nucleic acid used in the biosensor, the two interact/bind. The interaction can be monitored by various means such as a change in mass at the sensor surface or the presence of a fluorescent or radioactive signal. In alternative arrangements, the target nucleic acid(s) are bound to the sensor and contacted with labeled probes to allow for identification of the sequence(s) of interest.

30 [0008] While the potential utility for biosensors is great and while hundreds of biosensors have been described in patents and in the literature, actual commercial use of biosensors remains limited. Aspects of biosensors that have limited their commercial

acceptance include a lack the sensitivity and/or speed of detection necessary to accomplish certain tasks, problems with long term stability, difficulty miniaturizing the sensor, and the like. In addition, a number of biosensors must be pre-treated with salts and/or enzyme cofactors, a practice that is inefficient and bothersome.

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SUMMARY OF THE INVENTION

[0009] This invention pertains to the development of a novel molecular sensing apparatus (biosensor) and to methods of use thereof. In preferred embodiments, the particular, the sensing apparatus comprises a first electrode, a second electrode, an insulator between the first and second electrode; and a binding agent (*e.g.* a biological
10 macromolecule) connecting the first electrode to the second electrode. In particularly preferred embodiments, the binding agent is attached to the electrode in a manner that permits charge to flow from the electrode to the binding agent or from the binding agent to the electrode. Preferred binding agents include, but are not limited to biological macromolecules (*e.g.* a nucleic acid, a protein, a polysaccharide, a lectin, a lipid, *etc.*) with a
15 nucleic acid being most preferred. While the nucleic acid can be essentially any length preferred nucleic acids range in length from about 5 to about 5,000 nucleotides, more preferably from about 8 to about 1,000 or 500 nucleotides, still more preferably from about 10 to about 300 nucleotides, and most preferably from about 15, 20, 25, 30, or 50 nucleotides to about 100 or 150 nucleotides in length. Typically the nucleic acid is of
20 sufficient length to specifically hybridize to a target nucleic acid in a complex population of nucleic acids (*e.g.* total genomic DNA) under stringent conditions.

[0010] In preferred embodiments, the biological macromolecule is functionalized with a chemical group thereby facilitating the attachment of the macromolecule to the electrode(s). Preferred chemical groups include, but are not limited to a sulfate, a
25 sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable (labile) group, a group activatable by an electric potential, and the like. In certain embodiments, the biological macromolecule is functionalized with a second biological macromolecule (*e.g.* a receptor, a receptor ligand, an antibody, an epitope, a nucleic acid, a lectin, a sugar, and the
30 like). In preferred embodiments, however, such second biological macromolecules exclude nucleic acids.

[0011] Preferred insulators are insulators having a resistivity greater than about 10^{-3} ohm-meters, more preferably greater than about 10^{-2} ohm-meters, and most preferably greater than about 10^{-1} , 1, or 10 ohm-meters. Suitable insulators include, but are not limited to SiO_2 , TiO_2 , ZrO_2 , quartz, porcelain, ceramic, polystyrene, Teflon (other high-resistivity plastics), an insulating oxide or sulfide of a transition metal in the periodic table of the elements, and the like.

[0012] In certain preferred embodiments, the first electrode and the second electrode are separated by a distance in the range of 1 to 10^9 Angstroms. Typically the first electrode and the second electrode are separated by a distance less than about 300 Angstroms, preferably less than about 150 Angstroms, more preferably less than about 500, preferably less than about 250, more preferably less than about 150, and most preferably less than about 70 Angstroms or less than about 50 angstroms.

[0013] In certain embodiments, the first electrode and/or the second electrode have a resistivity of less than about 10^{-2} ohm-meters, preferably less than about 10^{-3} ohm-meters, more preferably less than about 10^{-4} ohm-meters, and most preferably less than about 10^{-5} , or 10^{-6} ohm-meters. Particularly preferred electrodes comprise a material such as ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon, or a carbon nanotube. In certain preferred embodiments, the first electrode is functionalized to contain a chemical group that can be derivatized or crosslinked (*e.g.*, a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, a group activatable by an electric potential, *etc.*). The first and/or second electrode can bear a self-assembled monolayer (SAM). Particularly preferred SAMs comprise a compound selected from the group consisting of an alkanethiol, a phospholipid, a bola amphiphile, and an oligo(phenylenevinylene).

[0014] In a particularly preferred embodiment, the biological macromolecule is attached to the first and/or to the second electrode directly by a thiol group or through a linker bearing a thiol group. In another particularly preferred embodiment, the biological macromolecule is attached to the first and/or to the second electrode directly by a phosphorothioate and/or a phosphonate, or through a linker bearing a phosphorothioate

and/or a phosphonate. In preferred embodiments, the biological macromolecule is attached to the first and/or to the second electrode by a linker (*e.g.*, DFDNB, DST, ABH, ANB-NOS, EDC, NHS-ASA, SIA, oligo(phenylenevinylene), *etc.*).

[0015] The apparatus can further comprise a substrate (other than the electrode
5 and/or insulator) where the first electrode and the second electrode are integrated with the substrate. In certain embodiments, the first electrode and the second electrode are integrated with the insulator to form a substrate. The electrodes can be formed in essentially any desired shape (*e.g.* convex, concave, textured, corrugated, patterned uniformly, randomly patterned, *etc.*). Certain preferred electrode orientations include
10 annular, planar, and orthogonal. In certain embodiments, the first electrode comprises a first surface and a second electrode comprises a second surface where the first surface and the second surface are not co-planar.

[0016] The apparatus can comprise a plurality of electrode pairs. Thus, in certain
embodiments, the first electrode and the second electrode comprise a first electrode pair,
15 and the molecular sensing apparatus further comprises a second electrode pair comprising a second first electrode and a second second electrode. In certain embodiments, the apparatus comprises at least 3, preferably at least 10 or 20, more preferably at least 50, 100, or 1,000, and most preferably at least 10,000 or at least 1,000,000 electrode pairs. In certain
embodiments, the apparatus can comprise electrode pairs at a density greater than about 10,
20 preferably greater than about 100 or 1000, more preferably greater than about 5,000, 10,000, or 50,000, and most preferably greater than about 100,000 or 1,000,000 electrode pairs per square centimeter.

[0017] In certain embodiments, the apparatus further comprises a measurement
device electrically coupled to the first electrode and to the second electrode of at least one
25 said electrode pair. Preferred measurement devices measure an electromagnetic property selected from the group consisting direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge and
30 magnetic potential. One particularly preferred measurement device is a potentiostat.

[0018] The apparatus can further comprise an electrical circuit electrically coupled to the first electrode and the second electrode. One such circuit comprises an electrical signal gating system (*e.g.* a CMOS gating system), and/or a voltage source, and/or a multiplexor, and/or a computer.

5 [0019] In certain embodiments, the electrodes comprising the first and second electrode pairs have attached the same (species of) biological macromolecule. In certain embodiments, different electrode pairs, have attached different biological molecules.

[0020] In certain embodiments, the first electrode and/or the second electrode comprise a semi-conducting material. Preferred semiconducting materials have a resistivity
10 ranging from about 10^{-6} ohm-meters to about 10^7 ohm-meters. Preferred semiconducting materials include, but are not limited to silicon, dense silicon carbide, boron carbide, Fe_3O_4 , germanium, silicon germanium, silicon carbide, tungsten carbide, titanium carbide, indium phosphide, gallium nitride, gallium phosphide, aluminum phosphide, aluminum arsenide, mercury cadmium telluride, tellurium, selenium, ZnS, ZnO, ZnSe, CdS, ZnTe, GaSe, CdSe,
15 CdTe, GaAs, InP, GaSb, InAs, Te, PbS, InSb, PbTe, PbSe, and tungsten disulfide.

[0021] In one embodiment, the apparatus comprises: a first electrode having a first surface; a second electrode having a second surface coplanar to the first surface; an insulator between said first surface and said second surface; and a nucleic acid joining the first electrode to said second electrode.

20 [0022] This invention also provides a method of making a molecular sensing apparatus. In certain embodiments, the method comprises: providing a first electrode and a second electrode separated by an insulator; contacting the first and the second electrode with a first solution comprising a biological macromolecule (*e.g.*, a nucleic acid); placing a charge on the first electrode to attract the biological macromolecule to the first electrode
25 where the macromolecule attaches to the first electrode to form an attached macromolecule; and placing a charge on the second electrode to attract a portion of the attached macromolecule to the second electrode to attach the macromolecule to the second electrode. Preferred macromolecules, electrodes, electrode configurations, insulators, measurement devices, circuits, and the like, include, but are not limited to those described above. Where
30 the apparatus comprises multiple electrode pairs, the method can further comprise contacting a second electrode pair with a second solution comprising a second biological

macromolecule; placing a charge on the first electrode of the second electrode pair to attract the second biological macromolecule to the first electrode of the second electrode pair whereby the second biological macromolecule attaches to said first electrode to form an attached second macromolecule; and placing a charge on the second electrode of said second electrode pair to attract a portion of said attached second macromolecule to attach said second macromolecule to said second electrode of said second electrode pair. The first and second solution can be the same or different. Similarly, the first biological macromolecule and the second biological macromolecule can be the same or different.

[0023] In still another embodiment, this invention provides a method of detecting an analyte. The method involves i) providing molecular sensing apparatus comprising a first electrode and a second electrode separated by an insulator where said first electrode has a biological macromolecule attached thereto; ii) contacting the attached macromolecule with said analyte whereby said analyte binds to said macromolecule thereby forming a macromolecule/analyte complex; iii) placing a charge on said second electrode attract a portion of said bound analyte to said second electrode where said second analyte is bound to the second electrode such that the macromolecule/analyte complex forms a connection between the first electrode and the second electrode; and iv) detecting the connection between said first and said second electrode. In certain embodiments, the providing comprises: contacting the first electrode with a first solution comprising the biological macromolecule; and placing a charge on the first electrode whereby the charge attracts the biological macromolecule to the electrode and the biological macromolecule attaches to the electrode. Where multiple electrode pairs are present, the method can involve repeating these steps for each electrode pair. The "placing a charge" can, optionally involve placing a charge on the first electrode opposite to the charge on the second electrode. In certain embodiments, the "detecting" comprises detecting an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge, and magnetic potential. Preferred macromolecules, electrodes, electrode configurations, insulators, measurement devices, circuits, and the like, include, but are not limited to those described above.

[0024] In still another embodiment, this invention provides a method of detecting an analyte, where the method involves: i) providing a molecular sensing apparatus comprising a first electrode and a second electrode separated by an insulator where the first electrode has a first biological macromolecule attached thereto and the second electrode has a second biological macromolecule attached thereto; ii) contacting the first attached macromolecule and the second attached macromolecule with the analyte whereby said analyte binds to the first macromolecule and to the second macromolecule thereby forming a macromolecule/analyte complex forming a connection between said first electrode and said second electrode; and iii) detecting the connection between said first and said second electrode. In certain embodiments, the "providing" comprises contacting the first electrode with a first solution comprising the first biological macromolecule; and placing a charge on the first electrode whereby the charge attracts the first biological macromolecule to the electrode and the biological macromolecule attaches to the electrode. Similarly, in certain embodiments, the "providing" comprises contacting the second electrode with a solution comprising the second biological macromolecule; and placing a charge on the second electrode whereby the charge attracts the second biological macromolecule to the second electrode and the second biological macromolecule attaches to the second electrode. In certain embodiments, the "detecting" comprises detecting an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge, and magnetic potential. Preferred macromolecules, electrodes, electrode configurations, insulators, measurement devices, circuits, and the like, include, but are not limited to those described above.

[0025] This invention provides still another method of detecting an analyte. The method involves i) providing a molecular sensing apparatus comprising a first electrode and a second electrode separated by an insulator where a biological macromolecule forms a connection between the first electrode and the second electrode; ii) detecting the connection between said first and the second electrode; iii) contacting macromolecule (binding agent) with the analyte whereby the analyte binds to the macromolecule forming a macromolecule/analyte complex; and iv) detecting a difference in the connection between

the first electrode and said second electrode. In certain embodiments, the "contacting" comprises placing a charge on the first and/or the second electrode whereby the charge attracts the analyte to the biological macromolecule. In certain embodiments, the "providing" comprises contacting the first electrode with a first solution comprising the biological macromolecule; and placing a charge on the first electrode whereby the charge attracts the biological macromolecule to the electrode and the biological macromolecule attaches to the electrode; and placing a charge on the second electrode to attract a portion of the bound macromolecule to the second electrode where the macromolecule is bound to the second electrode such that said macromolecule forms a connection between the first electrode and said second electrode. In certain embodiments, the "placing charge" comprises placing a charge on said first electrode opposite to the charge on said second electrode. The "detecting" can comprise detecting an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge and magnetic potential. In particularly preferred embodiments, the biological macromolecule is attached to said first electrode by an electrically conductive linker. In certain embodiments, the binding agent is a nucleic acid and the analyte is a protein or a protein complex. Preferred macromolecules, electrodes, electrode configurations, insulators, measurement devices, circuits, and the like, include, but are not limited to those described above.

[0026] Any of the methods and devices described herein include embodiments where the binding agents are not joined to the first electrode and/or the second electrodes a second or third nucleic acid. Thus, in such embodiments, where the binding agent is a nucleic acid, a single nucleic acid molecule spans the first and second electrode and linkers or functional groups, if present, are not themselves nucleic acids.

DEFINITIONS

[0027] The term "biosensor" refers to a sensor that uses a biological macromolecule (*e.g.* nucleic acid, carbohydrate, protein, antibody, *etc.*) to specifically recognize/bind to a target analyte. The term "molecular sensing apparatus" is used interchangeably with the term "biosensor".

[0028] The term "biological macromolecule" as used herein refers to a biological molecule such as a nucleic acid, protein, antibody, carbohydrate, polysaccharide, lipid, and the like.

[0029] The term "electrically conductive" wherein used with reference to a linker, molecule or molecular complex refers to the ability of that linker, molecule or molecular complex to pass charge through itself. Preferred electrically conductive molecules have a resistivity lower than about 10^{-3} more preferably lower than about 10^{-4} , and most preferably lower than about 10^{-6} or 10^{-7} ohm-meters.

[0030] The term "electrically coupled" binding agent and an electrode refers to an association between that binding agent and the electrode such that electrons can move from the binding agent to the electrode or from the electrode to the binding agent. Electrical coupling can include direct covalent linkage between the binding agent and the electrode, indirect covalent coupling (*e.g.* via a linker), direct or indirect ionic bonding between the binding agent and the electrode, or other bonding (*e.g.* hydrophobic bonding). In addition, no actual bonding may be required and the binding agent can simply be contacted with the electrode surface.

[0031] The term "sensor element" as used herein refers to a pair of electrodes (*e.g.* first electrode **10** and second electrode **12**) and associated binding agent(s) **14** that, when bound by an analyte form a molecular complex that spans the pair of electrodes.

[0032] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0033] The term "nucleic acid" as used herein refers to a deoxyribonucleotide or ribonucleotide in either single- or double-stranded form. The term encompasses nucleic acids, *i.e.*, oligonucleotides, containing known analogues of natural nucleotides which have similar or improved binding properties, for the purposes desired, as the reference nucleic acid. The term also encompasses nucleic-acid-like structures with synthetic backbones.

DNA backbone analogues provided by the invention include phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl

phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, morpholino carbamate, and peptide nucleic acids (PNAs); see *Oligonucleotides and Analogues, a Practical Approach*, edited by F. Eckstein, IRL Press at Oxford University Press (1991); *Antisense Strategies*, Annals of the New York Academy of Sciences, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Milligan (1993) *J. Med. Chem.* 36:1923-1937; *Antisense Research and Applications* (1993, CRC Press). PNAs contain non-ionic backbones, such as N-(2-aminoethyl) glycine units. Phosphorothioate linkages are described in WO 97/03211; WO 96/39154; Mata (1997) *Toxicol. Appl. Pharmacol.* 144:189-197. Other synthetic backbones encompassed by the term include methylphosphonate linkages or alternating methylphosphonate and phosphodiester linkages (Strauss-Soukup (1997) *Biochemistry* 36: 8692-8698), and benzylphosphonate linkages (Samstag (1996) *Antisense Nucleic Acid Drug Dev* 6: 153-156). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide primer, probe and amplification product.

[0034] The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0035] Antibodies exist *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge

region, thereby converting the F(ab')₂ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, Fundamental Immunology, Third Edition, W.E. Paul, ed., Raven Press, N.Y. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, those synthesized de novo using recombinant DNA methodologies (*e.g.*, single chain Fv), and those found in display libraries (*e.g.* phage display libraries).

[0036] The phrases "hybridizing specifically to" or "specific hybridization" or "selectively hybridize to", refer to the binding, duplexing, or hybridizing of a nucleic acid molecule preferentially to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular) DNA or RNA.

[0037] The term "stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target sequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* part I chapter 2 *Overview of principles of hybridization and the strategy of nucleic acid probe assays*, Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe.

[0038] An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a

0.2x SSC wash at 65°C for 15 minutes (*see*, Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook *et al.*) *supra* for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal.

5 An example of a medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example of a low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids which do
10 not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

[0039] In one particularly preferred embodiment, stringent conditions are
15 characterized by hybridization in 1 M NaCl, 10 mM Tris-HCl, pH 8.0, 0.01% Triton X-100, 0.1 mg/ml fragmented herring sperm DNA with hybridization at 45°C with rotation at 50 RPM followed by washing first in 0.9 M NaCl, 0.06 M NaH₂PO₄, 0.006 M EDTA, 0.01% Tween-20 at 45°C for 1 hr, followed by 0.075 M NaCl, 0.005 M NaH₂PO₄, 0.5 mM EDTA at 45°C for 15 minutes.

20 [0040] A "high resistivity plastic" refers to a plastic with a resistivity greater than about 10⁻³ ohm-meters, more preferably greater than about 10⁻² ohm-meters, and most preferably greater than about 10⁻¹, 1, or 10 ohm-meters.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] Figures 1A through 1D illustrate several variations on a basic biosensor of
25 this invention. The sensor element comprises two electrodes **10** and **12** connected by a binding agent (*e.g.* a biomolecule). Binding of the analyte to the binding agent forms a binding agent/analyte complex spanning the electrodes. The complex is easily detected using, *e.g.* electrical means. The two electrodes can be separated by a spacer **16** and can, optionally be fabricated on a substrate **24** (Figure 1D).

[0042] Figure 2 illustrates the difference between a sandwich configuration and one embodiment of a configuration that is not sandwiched. In the sandwich configuration (device "A") the spacer **16** overlays all or a portion of the first electrode **10**, and the second electrode **12** overlays (or is adjacent) all or a part of the opposite surface of the spacer **16**.

5 In device "B", the two electrodes and spacer do not form a "sandwich configuration". While the spacer **16** overlays all or a portion of the first electrode **10**, the second electrode **12** does not overlay all or a part of the opposite surface of the spacer. In both embodiments, the device is shown on a substrate **24** with a binding agent **14**.

[0043] Figure 3 illustrates two other embodiments where the two electrodes and
10 spacer do not form a sandwich configuration. In device "A" the first electrode **10** and the second electrode **12** are both in contact with the same face of the spacer **16**. In device "B" the first electrode **10** contacts the spacer **16**, while the second electrode **12** does not contact spacer **16** at all.

[0044] Figure 4 illustrates one embodiment where the two electrodes (**10** and **12**)
15 and spacer **16** do not form a sandwich configuration. In the embodiment illustrated, a conductor **10** is disposed on top of spacer **16**, which in turn, is disposed on top of insulator **26** that overlays substrate **24**. The second conductor **12** is disposed adjacent to the conductor **10**/spacer **16** stack and the binding agent **14** connects conductor **10** to conductor **12**. Spacer/insulators **16** and **26** can be the same or different materials or can each be a
20 combination of materials. The device can easily be formed by depositing the various materials (*e.g.* using a photolithographic process) at an angle θ as illustrated.

[0045] Figures 5A and 5B illustrate embodiments where the two electrodes (**10** and **12**) and the spacer **16** do not form a sandwich configuration and the first electrode **10** overhangs the spacer **16**. This is readily accomplished by depositing the electrode material
25 at an angle θ as illustrated (*see, e.g.*, Figure 5A) and/or by etching the spacer **16** out from underneath part of conductor **10** (*see* Figure 5B).

[0046] Figure 6 illustrates a variety of embodiments (devices "A" through "E") that do not have a sandwich configuration. The spacer/insulator layer **26** is optional.

[0047] Figure 7 illustrates a variety of embodiments (devices "F" through "I") that
30 do not have a sandwich configuration. The spacer/insulator layer **26** is optional.

[0048] Figures 8A and 8B illustrate an embodiment of the biosensor comprising two binding agents, **14a** and **14b**, one on each electrode (Figure 8B). The two binding agents are bound by the analyte forming a binding agent/analyte complex spanning the electrodes. The complex is easily detected using, *e.g.* electrical means.

5 [0049] Figures 9A and 9B illustrate an embodiment of the biosensor comprising a binding agent attached to a first electrode **10** of a pair of electrodes (Figure 9A). The analyte binds to the binding agent and to the second electrode **12** analyte forming a binding agent/analyte complex spanning the electrodes (Figure 9B). The complex is easily detected using, *e.g.* electrical means.

10 [0050] Figures 10A and 10B illustrate a simple planar sensor array according to this invention. Figure 10A shows a top view, while Figure 10B illustrates a side view.

[0051] Figure 11 illustrates an aggregation of sensor arrays according to this invention.

[0052] Figure 12A through 12C illustrate various sensor embodiments.

15 [0053] Figure 13 illustrates two sensor elements (*e.g.*, a subset of a sensor array) sharing one common electrode. In the illustrated embodiments, the first conductor **10**, spacer **16**, and second conductor **12** do not form a sandwich configuration. In device B, the spacer **16** has been partially etched out to provide an "air gap" between the electrodes. A sensor array can readily be fabricated containing thousands of such sensor elements.

20 [0054] Figure 14 illustrates regions of a sensor array comprising two sensor elements where each sensor element has an independent first conductor **10** and second conductor **12**. In the illustrated embodiments, the first conductor **10**, spacer **16**, and second conductor **12** do not form a sandwich configuration. In device D, the spacer **16** has been partially etched out to provide an "air gap" between the electrodes.

25 [0055] Figure 15 illustrates a section of a sensor array of this invention where the sensor elements are provided in a stepped configuration.

[0056] Figure 16 illustrates a stepped configuration for a sensor array of this invention. In the illustrated embodiments, each sensor element

[0057] Figure 17 is a schematic a diagram of a support having an array of electrode
30 pairs (sensor elements) controlled by a computer.

[0058] Figure 18 is a schematic diagram of a support having an array of electrode pairs (sensor elements).

[0059] Figure 19 is a schematic diagram of a support having an array of electrode pairs and computer system for controlling the energization of each electrode pair (sensor element).

[0060] Figure 20 is a schematic diagram of a support having an array of electrode pairs and a computer system with a plurality of voltage sources and multiplexers for controlling the energization of each electrode pair (sensor element).

[0061] Figure 21 is a diagram of a support having an array of electrode pairs and a computer system with a plurality of switched voltage sources for controlling the energization of each electrode pair (sensor element).

[0062] Figure 22 illustrates effects of deposition angle on device configuration.

[0063] Figure 23 illustrates device fabrication comprising a deposition step, followed by an etching step, followed by a second deposition.

[0064] Figures 24A, 24B, 24C, and 24D illustrate the deposition of alternating conductor and insulator layers.

[0065] Figure 25 illustrates the use of a biosensor to detect protein/DNA interactions. A biosensor comprising a nucleic acid **14** is hybridized to a second nucleic acid **24** to form a double-stranded nucleic acid spanning two electrodes. Binding of a protein analyte **20** (*e.g.* DNA binding protein) to the nucleic acid changes conductance of the nucleic acid thereby producing a detectable signal.

DETAILED DESCRIPTION

[0066] This invention pertains to a novel sensors (biosensors) that are useful for detecting a wide range of analytes. The sensors utilize a binding agent (*e.g.* a biomolecule) specifically bind to one or more target analytes and thereby confer specificity and selectivity. In preferred embodiments, the binding agent (*e.g.* biomolecule) spans a gap between two electrodes. Binding of the target analyte changes conductivity, or other electrical properties, of the sensor thereby facilitating ready detection of the binding event and thus detection and/or quantitation of the bound analyte. Because the biosensors of this invention provide a change in conductance or charge flow when bound by the target analyte,

they are easily read using electronic/electrochemical means and do not require the use of detectable labels or external electron donors or acceptors.

I. Sensor element configuration.

[0067] One embodiment of a basic biosensor (molecular sensing apparatus) of this invention is schematically illustrated in Figure 1. The sensor comprises a first electrode **10**, a second electrode **12**, and a binding agent (*e.g.* biomolecule) **14** spanning the gap between the two electrodes. The two electrodes can be separated by an air gap, however, in preferred embodiments, the electrodes are separated by a spacer **16** (*e.g.* an insulator, a dielectric, or a semiconductor). The binding agent **14** can be directly bound to the electrodes or it can be coupled to the first electrode **10** and/or the second electrode **12** through one or more linkers or functional groups **18**. The binding agent **14** is attached to the electrodes in a manner that leaves sufficient area of the sensor molecule free to bind with its "cognate" target molecule **20** (the target analyte). When the binding agent **14** binds its cognate target molecule **20** a binding agent/target molecule complex is formed whose conductivity is different than the conductivity of the binding agent **14** alone. This change in conductivity is readily detected indicating the presence and/or concentration of the target molecule **20**.

[0068] It was a surprising discovery of this invention that, in certain instances, where the electrodes are spaced sufficiently close together so that a biological molecule can span the two electrodes (a molecular distance), leakage current through the separating spacer/insulator can interfere with the conductivity measurement of the binding agent. This problem is overcome by the use of configurations in which the first electrode, the spacer (*e.g.*, insulator, dielectric, semiconductor, *etc.*), and the second electrode do not form a sandwich configuration. A sandwich configuration refers to an electrode configuration where the spacer (*e.g.* insulator, dielectric, semiconductor, *etc.*), overlays all or a portion of the first electrode, and the second electrode overlays all or a part of the opposite surface of the spacer (*see, e.g.*, Figure 2). Figure 2 (device "B") and Figure 3 illustrate several electrode/spacer configurations that are not sandwich configurations.

[0069] Figure 4 schematically illustrates one embodiment where the two electrodes (**10** and **12**) and the spacer **16** do not form a sandwich configuration. In the embodiment illustrated, a substrate **24** is overlaid with an insulator **26** (*e.g.* spacer, dielectric, *etc.*).

Insulator **26** is overlayed with spacer **16** which, in turn, is overlayed with conductor **10**. The second conductor **12** is disposed adjacent to the conductor **10**/spacer **16** stack and the binding agent **14** connects conductor **10** to conductor **12**. Spacer/insulators **16** and **26** can be the same or different materials or can each be a combination of materials. The device
5 can easily be formed by depositing the various materials (*e.g.* using a photolithographic process) at an angle θ as illustrated.

[0070] Figures 5A and 5B illustrate embodiments where the two electrodes (**10** and **12**) and the spacer **16** do not form a sandwich configuration and the first electrode **10** overhangs the spacer **16**. This is readily accomplished by depositing the electrode material
10 at an angle θ as illustrated and/or by etching the spacer **16** out from underneath part of conductor **10** (*see* Figure 5B).

[0071] Figures 6 and 7 also illustrate a variety of embodiments (devices "A" through "I") that do not have a sandwich configuration. These embodiments are intended to be illustrative and not limiting. Using the teaching provided herein, numerous other sandwich
15 and non-sandwich configurations can be fabricated by one of skill in the art.

[0072] In one embodiment, the binding agent **14** is a single-stranded nucleic acid. The nucleic acid is derivatized at each terminus with a linker that physically and electrically couples the nucleic acid to the respective electrodes **10** and **12** such that the nucleic acid spans the gap between the electrodes. Single-stranded nucleic acids are essentially non-
20 conductive. However, when the nucleic acid binding agent is contacted with a complementary nucleic acid analyte under conditions that permit nucleic acid hybridization, the analyte nucleic acid binds to the sensor nucleic acid via complementary base pairing to form a double stranded hybrid duplex spanning the electrodes. This double stranded duplex is electrically conductive. The change in conductivity caused by such binding is readily
25 detected using electrical/electrochemical means.

[0073] The binding agent is not limited to a nucleic acid. Any number of other binding agents can also be used in such a biosensor. Generally, binding agents are selected that are capable of specifically binding to a particular target analyte. Such binding agents include, but are not limited to nucleic acids (including, but not limited to single stranded
30 DNA or RNA, double stranded DNA or RNA, peptide nucleic acids, phosphorothioates, and the like), proteins, antibodies, lectins, sugars, lipids, polysaccharides, and the like.

[0074] While, in preferred embodiments, binding agents are utilized that are non-conductive by themselves, but form an electrically conductive complex when bound to the target analyte. The sensors of this invention are not limited to such molecules. In certain
5 conductivity than the binding agent alone.

[0075] Alternatively, where the analyte/binding agent complex shows the same conductivity as the binding agent alone, it is possible to use various chemical agents that intercalate into the analyte/binding agent complex and change the effective conductivity of that complex. There are typically intercalation sites, or fewer sites afforded by the binding
10 agent alone. Thus, the analyte binding complex, by intercalating a greater number of such agents shows a different conductivity.

[0076] Intercalating reagents that change the conductivity of a biomolecule or molecular complex are well known to those of skill in the art. Such intercalators include, but are not limited to redox-active cations (*e.g.* $\text{Ru}(\text{NH}_3)_6^{3+}$ and various transition
15 metal/ligand complexes. Transition metals are those whose atoms have an incomplete shell of electrons. Suitable transition metals for use in the invention include, but are not limited to, cadmium (Cd), magnesium (Mg), copper (Cu), cobalt (Co), palladium (Pd), zinc (Zn), iron (Fe), ruthenium (Ru), rhodium (Rh), osmium (Os), rhenium (Re), platinum (Pt), scandium (Sc), titanium (Ti), Vanadium (V), chromium (Cr), manganese (Mn), nickel (Ni),
20 Molybdenum (Mo), technetium (Tc), tungsten (W), and iridium (Ir). That is, the first series of transition metal, the platinum metals (Ru, Rh, Pd, Os, Ir and Pt), along with Re, W, Mo and Tc, are preferred. Particularly preferred are ruthenium, rhenium, osmium, platinum and iron.

[0077] The transition metals are complexed with a variety of ligands to form
25 suitable transition metal complexes, as is well known in the art. Suitable ligands include, but are not limited to, $-\text{NH}_2$; pyridine; pyrazine; isonicotinamide; imidazole; bipyridine and substituted derivative of bipyridine; phenanthrolines, particularly 1,10-phenanthroline (abbreviated phen) and substituted derivatives of phenanthrolines such as 4,7-
dimethylphenanthroline; dipyrrophenazine; 1,4,5,8,9,12-hexaazatriphenylene (abbreviated
30 hat); 9,10-phenanthrenequinone diimine; 1,4,5,8-tetraazaphenanthrene (abbreviated tap); 1,4,8,11-tetra-azacyclotetradecane; diaminopyridine (abbreviated damp); porphyrins and substituted derivatives of the porphyrin family.

[0078] Such intercalating reagents can also be used to detect mismatches between the binding agent and the target analyte. Thus, for example where the binding agent and the analyte are nucleic acids, intercalating reagents comprising dimeric naphthyridines will specifically intercalate and localize where there is a G-G mismatch between the binding
5 reagent and the target analyte (*see, e.g.,* Nakatani *et al.* (2001) *Nature/Biotechnology*, 19(1): 51-55). Such mismatch specific reagents can be used to detect or screen for single nucleotide polymorphisms (SNPs).

[0079] While Figure 1 illustrates essentially a single sensor element of this invention, various embodiments contemplate the use of a multiplicity of sensor elements.
10 Thus, in various embodiments, there can exist multiple binding agents **14** spanning a single pair of electrodes and/or a multiplicity of electrode pairs **20** each electrode pair being spanned by one or more binding agents **14**. Because of the small size of the sensor element, a large number of sensor elements can be placed in a relatively small area (*e.g.* on a chip) thereby increasing sensitivity and improving signal to noise (S/N) ratio. In addition, assays
15 can be performed using small quantities of sample. A single substrate/chip can incorporate a number of different sensor elements facilitating detection/quantification of a number of different analytes.

[0080] The sensor elements can adopt a wide variety of configurations. Thus, for example, in another embodiment illustrated in Figures 8A and 8B, the electrodes are not
20 spanned by a single binding agent. Rather, a first binding agent **14a** is attached to the first electrode **10** and a second binding agent **14b** is attached to the second electrode **12** (Figure 8A). Binding of the analyte **20** to the two binding agents creates an electrically conductive moiety that spans the gap between the two electrodes allowing current to flow between the electrodes and thereby facilitating detection/quantification of the bound analyte.

25 [0081] Thus, for example, in one embodiment, the first and second binding agents are each nucleic acids complementary to half of the target analyte. When the analyte contacts the binding agents under conditions permitting hybridization, the two binding agents hybridize to the analyte forming a double-stranded nucleic acid spanning the two electrodes (*see, e.g.,* Figure 8B). While this is illustrated with electrodes **10** and **12** and
30 spacer **16** forming a sandwich configuration, a similar approach can be taken in a configuration that is not a sandwich configuration.

[0082] Still another preferred embodiment is illustrated in Figures 9A and 9B. In this embodiment, a binding agent **14** is attached to a first electrode **10** (Figure 9A). The target analyte is tagged with a moiety that causes the analyte to interact with and/or bind to a second electrode. In use, the analyte **20** binds to, *e.g.* the second electrode **12** and is
5 bound by the biological molecule **14**. Together the binding agent **14** and the analyte **20** bridge the gap between the electrodes resulting in a detectable change in conductance. Again, while this is illustrated with electrodes **10** and **12** and spacer **16** forming a sandwich configuration, a similar approach can be taken in a configuration that is not a sandwich configuration.

10 [0083] In certain embodiments, the analyte is allowed to contact the binding agent and form a binding agent/analyte complex. Then application of a charge to the second electrode (and, optionally, an opposite charge to the first electrode) draws the analyte or a portion thereof to the second electrode whereby the analyte, or a linker or functional group of the analyte and/or the electrode causes the analyte to be linked to the second electrode
15 thereby forming the analyte/binding agent complex spanning the two electrodes.

[0084] These configurations are simply illustrative of certain preferred embodiments of this invention. Using the teaching provided herein, other sensor element configurations can be readily developed by one of ordinary skill in the art.

[0085] While each electrode (electrode pair) can bear a single binding agent **14**,
20 typically, each electrode (electrode pair) bears a plurality of binding agents **14**. Thus, in preferred embodiments, each electrode or electrode pair bears at least two, preferably at least 10, more preferably at least 50, still more preferably at least 100, and most preferably at least 1,000, at least 10,000, at least 100,0000, or at least 1,000,000 binding agents (*e.g.*, biomolecules) **14**.

25 [0086] The electrodes comprising an electrode pair (sensor element) can be of any convenient dimension. In preferred embodiments, the electrodes comprising an electrode pair are spaced such that the analyte and/or the analyte/binding agent combination span the gap between the electrodes. In certain embodiments, the electrodes are separated by distance ranging from about of 1 to about 10^{10} Angstroms, preferably from about 10 to
30 about 10^5 Angstroms, more preferably from about 25 to about 10^4 Angstroms, and most preferably from about 40 to about 10^2 angstroms. Preferred interelectrode spacings are less

than about 200 angstroms, preferably less than about 150 angstroms, more preferably less than about 100 angstroms, and most preferably less than about 50, about 40 or about 30 angstroms.

[0087] The gap between the electrodes can be an air gap, filled with oxygen or with an inert gas (*e.g.* argon, *etc.*), a vacuum, or the gap can be filled with an insulator, semiconductor, or a dielectric. In preferred embodiments, the gap between the electrodes is filled with an insulator. Preferred insulators include, elements, compounds or substances that have resistivity greater than about 10^{-3} , preferably greater than about 10^{-2} ohm-meters, more preferably greater than about 10^{-1} ohm meters, and most preferably greater than about 10 ohm meters. Particularly preferred insulators include, but are not limited to SiO_2 , TiO_2 , ZrO_2 , porcelain, ceramic, glass, clay, polystyrene, Teflon, plastics having a resistivity greater than 10^{-3} ohm-meters, and other high resistivity plastics, insulating oxides or sulfides of the transition metals in the periodic table of the elements, and the like.

[0088] The electrodes are conveniently formed from essentially any conductive material. Preferred conductive materials have resistivities of less than about 10^{-3} ohm-meters, preferably less than about 10^{-4} ohm meters, more preferably less than about 10^{-6} ohm meters, and most preferably less than about 10^{-7} ohm meters. In preferred embodiments, the electrodes are formed from materials that include, but are not limited to ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon or carbon nanotubes, and alloys or compounds of these materials.

II. Sensor element arrays.

[0089] Various embodiments of this invention can utilize a single sensor element. However, in preferred embodiments, a plurality of sensor elements are present, optionally forming an array of sensor elements. As used herein, an array of sensor elements refers to a plurality of sensor elements aggregated on a common substrate and/or that share one or more common electrical connections.

[0090] The sensor element arrays can take essentially any conformation that is convenient to the intended application. Thus, in certain embodiments, the sensor element

arrays can comprise planar arrays of sensor elements (*see, e.g.*, Figure 10A and 10B) and/or aggregations of such arrays (*see, e.g.*, Figure 11).

[0091] The sensor element arrays are not limited to planar arrays. Virtually any configuration can be obtained. Thus, for example, sensor elements or arrays thereof can be placed on one or more walls of a capillary, channel, or microchannel, on one or more walls or floor of a sample well (*e.g.* in a multi-well plate such as a microtiter plate), on one or more surfaces of a sensor probe (*e.g.* an insertable or implantable sensor), and the like. In certain embodiments, the sensor arrays can be stacked to provide three-dimensional arrays.

[0092] Certain preferred configurations are illustrated in Figures 10 and 12A through 12C. Thus, for example, Figure 10B illustrates a flush-faced sensor array. The electrodes and insulators are integrated into a multi-layer material presenting a flush surface. Analyte(s) or solutions containing analytes pass across the surface where the analytes are bound by the binding agent(s) **14**. Figure 12A illustrates an embodiment where the electrodes protrude from the intervening insulator and thereby form one or more channels. The channels are useful for guiding reagents/analytes, and the like, *e.g.* in various microfluidics devices. The binding agent(s) attached to the electrodes form convenient "detector domains" in such channels. Such devices are readily fabricated by providing a multi-layer material, *e.g.* as described below, and selectively etching insulator away from the electrodes.

[0093] Still another embodiment is illustrated in Figure 12B. In this embodiment, insulator/support is removed between the electrodes thereby forming channels within the substrate having electrode walls. Optional biasing electrodes **22** are illustrated in these diagrams.

[0094] Figure 12C illustrates a closed channel or well (cross-section) in which sensor element arrays are present in two walls of the channel.

[0095] Figure 13 illustrates two sensor elements sharing one common electrode. In the illustrated embodiments, the first conductor **10**, spacer **16**, and second conductor **12** do not form a sandwich configuration. In device B, the spacer **16** has been partially etched out to provide an "air gap" between the electrodes. A sensor array can readily be fabricated containing thousands of such sensor elements.

[0096] Figures 14 and 15 illustrates a region of a sensor array comprising two sensor elements where each sensor element has an independent first conductor **10** and second conductor **12**. In the illustrated embodiments, the first conductor **10**, spacer **16**, and second conductor **12** do not form a sandwich configuration. In device D, the spacer **16** has
5 been partially etched out to provide an "air gap" between the electrodes. In device F, the second conductor **12** has either been etched away or deposited such that the conductor is not adjacent to the spacer **16**. A sensor array can readily be fabricated containing thousands of such sensor elements by simple photolithographic deposition techniques.

[0097] Figure 16 illustrates a stepped configuration for a sensor array of this
10 invention. In the illustrated embodiments, each sensor element (first conductor **10**, binding agent **14**, and second conductor **12**) comprises an independent set of conductors. The distance between each step of "molecular dimension" (*e.g.* small enough that a molecule can span from one step to the next). The stepped configuration (a configuration that is not a sandwich configuration as illustrated) permits a large number of sensor elements to be
15 distributed in on a relatively small surface. In device "H", the spacer **16** has been etched away to provide an "overhang" between the first and the second conductors.

[0098] These configurations are simply illustrative and not intended to be limiting. Using the teaching provided herein, numerous other configurations will be available to one of ordinary skill in the art.

[0099] Preferred sensor arrays comprise at least two, preferably at least 10, more preferably at least 100, and most preferably at least 1, 000, 10,000 or 1,000,000 sensor elements. The sensor elements can all bear the same biological molecules **14** or various sensor elements can bear different biological molecules and show specificity for different analytes. Thus, in certain embodiments, a single sensor array can detect/quantify two or
20 more, preferably four or more, more preferably 10 or more, still more preferably 100 or more or 1000 or more, and most preferably 10000 more, 100,000 or more, or even 1,000,000 or more different analytes.

[0100] The electrodes comprising the sensor elements of the array(s) can all be separate, or they can be connected in various combinations. Thus, for example the first
30 electrodes **10** of all of the sensor elements or for a subset of sensor elements can be electrically connected to form a common electrode or "switchably connected to form

various electrical connections as desired. Similarly, additional "biasing" electrodes **22** can be connected together or "switchably interconnected."

[0101] Numerous methods may be used for addressing the plurality of sensor elements comprising the sensor element arrays of this invention. Several techniques are schematically illustrated in Figures 17 through 21. Shown in those figures by way of example are four sensor elements **101, 102, 103, 104** and appropriate instrumentation to read them, which typically is a voltammeter incorporating a digital computer.

[0102] In Figure 17, each sensor element (electrode pair) pair **101-104** is individually addressed by a pair of lines connected to the voltammeter **99**. By way of example, lines **105, 106** access electrode/counterelectrode pair **101**. An appropriate voltage may be applied and conductance/resistance measured by the voltammeter at any given time to any one or more of the pairs of lines connected to the various electrode pairs.

[0103] To reduce the number of connections required to address the electrode pairs, alternatives to the direct connection scheme of Figure 17 are provided. For example, a row-and-column accessing scheme is illustrated in Figure 18 for electrically energizing some or all of the electrodes. In this scheme, one of the electrodes **201, 202** in each column of the plurality of electrode pairs is connected to a common electrical conductor **205** on support **200**, and each of the electrodes in each row of the plurality of electrode pairs is connected to conductor **207, 208** on the support **200**. Conductors **205, 206** connect to connections **C1, C2**, respectively, at the edge of support **200** and conductors **207, 208** connect to connections **R1, R2**, respectively. Each of these connections is then connected by a separate line to the voltammeter. As a result, in the configuration of Figure 18, the number of required connections and signal lines from the voltammeter has been reduced from 8 to 4.

[0104] To enable rapid and sequential energizing/reading of each electrode pair, a computer controlled switching device is beneficial. The configuration of Figure 19 shows a plurality of first electrodes connected to a first multiplexer **310**. A plurality of second electrodes are connected to a second multiplexer **320**. The first multiplexer is also connected to a first pole of a voltage source/voltammeter **330** that typically supplies a time varying electrical potential for cyclic voltammetry described herein. The second multiplexer is also connected to a second pole of the voltage source/voltammeter. Using addressing lines **A0-A3** electrically connected to each of the multiplexers and connected to

latch **340**, a computer processor **350** can direct the multiplexers to selectively connect any or all of the first electrodes to the first pole of the voltammeter, and any or all of the second electrodes to the second pole of the voltammeter.

[0105] As shown in Figure 20, a plurality of voltage sources are connected through
5 separate sets of multiplexers to each of the electrodes. If a first electrical potential or range of electrical potentials is required at a particular electrode pair, the multiplexers **410, 420** associated with the voltage source **430** providing that potential are addressed by the computer processor **350**, typically through a latch **340**, thereby connecting that particular voltage source to the electrode pair in question. If a different electrical potential or range of
10 electrical potentials is required for another electrode pair, the multiplexers **440, 450** associated with that different voltage source **460** are addressed by the computer processor, thereby connecting that voltage source through the associated multiplexers **440, 450** to the electrode pair.

[0106] If the electrode array in this embodiment has at least a portion of the
15 electrode pairs independently driveable, as shown in Figure 18 or Figure 19, for example, one electrode pair can be driven by one voltage source/voltammeter while another electrode pair is simultaneously driven with another voltage source/voltammeter. Alternatively, the two voltage sources of Figure 20 can be replaced with a single voltage source/voltammeter connected to both sets of multiplexers in parallel, allowing two electrode pairs to be driven
20 from the same voltage source.

[0107] Instead of a duplicate set of multiplexers for each voltage source as shown in Figure 20, a plurality of voltage sources/voltammeters **520, 530** can be provided as shown in Figure 21. These voltage sources can be connected through a computer controlled electrical switch **510** or switches to a single set of multiplexers **310, 320**. As shown in
25 Figure 21, the computer would direct switch **510** to connect a particular voltage source/voltammeter to the multiplexers, and would also direct the multiplexers (by signaling their address lines **A0-A3**) to connect the selected voltage source to the particular electrode pair desired.

[0108] Alternatively, the electrical potential applied to each of the electrode pairs in
30 any embodiment can be varied. This is of particular benefit when a cassette having a plurality of different sensor elements is used. Such a cassette may require a different range

of applied electrical potential at different sensor elements. Several different embodiments capable of varying the electrical potential applied to each electrode are contemplated.

[0109] Advantageously, a computer controlled voltage source/voltammeter may be used. A computer controlled voltage source/amperometer is one that can be addressed by a computer to select a particular electrical potential/waveform to be supplied. Alternatively it can be programmed to sequentially apply a particular range of electrical potentials over a predetermined time. In such a system, address lines electrically connected to the computer and the voltage source allow the computer to program the voltage source to produce the particular electrical potential to be applied to the electrode pair to be energized.

[0110] Additional methods for addressing the plurality of electrode pairs may also be used. For example, a plurality of reference electrodes may be placed in proximity to each of the plurality of electrode pairs in order to sense the voltage applied thereto. In this way, additional control of the voltage waveform may be maintained.

[0111] While the foregoing discussion was with reference to voltage sources/amperometers, other means of driving/reading the sensor elements can be substituted therefor. Such means include, but are not limited to amperometers, coulometers, and the like.

III. Sensor molecules and target analytes.

A) Preferred sensor molecules and target analytes.

[0112] A wide variety of binding agents (binding reagents) can be used in the devices of this invention and the analytes that can be detected using such binding agents are virtually limitless. The binding agents specifically bind to at least one analyte (ligand) of interest. The binding reagents can be selected from among any molecules known in the art to be capable of, or putatively capable of, specifically binding an analyte of interest.

[0113] Preferred analytes of interest include, but are not limited to a whole cell, a subcellular particle, virus, prion, viroid, nucleic acid, protein, antigen, lipoprotein, lipopolysaccharide, lipid, glycoprotein, carbohydrate moiety, cellulose derivative, antibody or fragment thereof, peptide, hormone, pharmacological agent, cell or cellular components, organic compounds, non-biological polymer, synthetic organic molecule, organo-metallic compounds, or an inorganic molecule present in the sample.

[0114] The sample can be derived from, for example, a solid, emulsion, suspension, liquid or gas. Furthermore, the sample may be derived from, for example, body fluids or tissues, water, food, blood, serum, plasma, urine, feces, tissue, saliva, oils, organic solvents, earth, water, air, or food products. The sample may comprise a reducing agent or an
5 oxidizing agent, solubilizer, diluent, preservative, or other suitable agents.

[0115] Suitable binding agents (biological molecules) 14 include, but are not limited to receptors, ligands for receptors, antibodies or binding portions thereof (*e.g.*, Fab, (Fab)₂), proteins or fragments thereof, nucleic acids, oligonucleotides, glycoproteins, polysaccharides, antigens, epitopes, carbohydrate moieties, enzymes, enzyme substrates,
10 lectins, protein A, protein G, organic compounds, organometallic compounds, lipids, fatty acids, lipopolysaccharides, peptides, cellular metabolites, hormones, pharmacological agents, tranquilizers, barbiturates, alkaloids, steroids, vitamins, amino acids, sugars, nonbiological polymers, biotin, avidin, streptavidin, organic linking compounds such as polymer resins, lipoproteins, cytokines, lymphokines, hormones, synthetic polymers,
15 organic and inorganic molecules, *etc.*

[0116] It will be apparent from the foregoing that the binding agent (*e.g.*, biological molecule) 14 and its target analyte 20 can exist as a pair of "binding partners", *e.g.* a ligand and its cognate receptor, an antibody and its epitope, *etc.* Thus, a biological "binding partner" or a member of a "binding pair" refers to a molecule or composition that
20 specifically binds other molecules to form a binding complex such as antibody-antigen, lectin-carbohydrate, nucleic acid-nucleic acid, biotin-avidin, *etc.*

[0117] The term "specifically binds", as used herein, when referring to a binding agent (*e.g.*, protein, nucleic acid; antibody, *etc.*), refers to a binding reaction that is determinative of the presence binding agent heterogeneous population of proteins and other
25 biologics. Thus, under designated conditions (*e.g.* immunoassay conditions in the case of an antibody, or stringent hybridization conditions in the case of a nucleic acid), the specified ligand or antibody binds to its particular "target" (*e.g.* a protein or nucleic acid) and does not bind in a significant amount to other molecules.

[0118] The binding partner(s) used in this invention are selected based upon the
30 targets that are to be identified/quantified. Thus, for example, where the target is a nucleic acid the binding partner is preferably a nucleic acid or a nucleic acid binding protein or

protein complex (*see, e.g.* Figure 25). Where the target is a protein, the binding partner is preferably a receptor, a ligand, or an antibody that specifically binds that protein. Where the target is a sugar or glycoprotein, the binding partner is preferably a lectin, and so forth.

B) Preparation of binding partners (capture agents).

5 [0119] Methods of synthesizing or isolating suitable binding agents are well known to those of skill in the art as explained below.

1) Nucleic acids

[0120] Nucleic acids for use as binding agents **14** in this invention can be produced or isolated according to any of a number of methods well known to those of skill in the art.

10 In one embodiment, the nucleic acid can be an isolated naturally occurring nucleic acid (*e.g.*, genomic DNA, cDNA, mRNA, *etc.*). Methods of isolating naturally occurring nucleic acids are well known to those of skill in the art (*see, e.g.*, Sambrook *et al.* (1989) *Molecular Cloning -A Laboratory Manual* (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

15 [0121] In a preferred embodiment, the nucleic acid is created *de novo*, *e.g.* through chemical synthesis, *e.g.*, according to the solid phase phosphoramidite triester method described by Beaucage and Caruthers (1981), *Tetrahedron Letts.*, 22(20): 1859-1862, *e.g.*, using an automated synthesizer, as described in Needham-VanDevanter *et al.* (1984) *Nucleic Acids Res.*, 12: 6159-6168. Purification of oligonucleotides, where necessary, is
20 typically performed by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson and Regnier (1983) *J. Chrom.* 255: 137-149. The sequence of the synthetic oligonucleotides can be verified using the chemical degradation method of Maxam and Gilbert (1980) in Grossman and Moldave (eds.) Academic Press, New York, *Meth. Enzymol.* 65: 499-560.

25 **2) Antibodies/antibody fragments.**

[0122] Antibodies or antibody fragments for use in sensor elements of this invention can be produced by a number of methods well known to those of skill in the art (*see, e.g.*, Harlow & Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, and Asai (1993) *Methods in Cell Biology Vol. 37: Antibodies in Cell Biology*, Academic

Press, Inc. N.Y.). In one approach, the antibodies are produced by immunizing an animal (e.g. a rabbit) with an immunogen containing the epitope it is desired to recognize/capture. A number of immunogens may be used to produce specifically reactive antibodies. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein may also be used either in pure or impure form. Synthetic peptides made as well using standard peptide synthesis chemistry (see, e.g., Barany and Merrifield, *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.*, Merrifield *et al.* (1963) *J. Am. Chem. Soc.*, 85: 2149-2156, and Stewart *et al.* (1984) *Solid Phase Peptide Synthesis*, 2nd ed. Pierce Chem. Co., Rockford, Ill.)

[0123] Methods of production of polyclonal antibodies are known to those of skill in the art. In brief, an immunogen is mixed with an adjuvant and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the immunogen. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the immunogen can be done if desired. (See Harlow and Lane, *supra*).

[0124] Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (See, Kohler and Milstein (1976) *Eur. J. Immunol.* 6: 511-519). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse *et al.* (1989) *Science*, 246:1275-1281.

[0125] Antibodies fragments, e.g. single chain antibodies (scFv or others), can also be produced/selected using phage display technology. The ability to express antibody fragments on the surface of viruses that infect bacteria (bacteriophage or phage) makes it

possible to isolate a single binding antibody fragment from a library of greater than 10^{10} nonbinding clones. To express antibody fragments on the surface of phage (phage display), an antibody fragment gene is inserted into the gene encoding a phage surface protein (pIII) and the antibody fragment-pIII fusion protein is displayed on the phage surface (McCafferty
5 *et al.* (1990) *Nature*, 348: 552-554; Hoogenboom *et al.* (1991) *Nucleic Acids Res.* 19: 4133-4137).

[0126] Since the antibody fragments on the surface of the phage are functional, phage bearing antigen binding antibody fragments can be separated from non-binding phage by antigen affinity chromatography (McCafferty *et al.* (1990) *Nature*, 348: 552-554).

10 Depending on the affinity of the antibody fragment, enrichment factors of 20 fold - 1,000,000 fold are obtained for a single round of affinity selection. By infecting bacteria with the eluted phage, however, more phage can be grown and subjected to another round of selection. In this way, an enrichment of 1000 fold in one round can become 1,000,000 fold in two rounds of selection (McCafferty *et al.* (1990) *Nature*, 348: 552-554). Thus even
15 when enrichments are low (Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597), multiple rounds of affinity selection can lead to the isolation of rare phage. Since selection of the phage antibody library on antigen results in enrichment, the majority of clones bind antigen after as few as three to four rounds of selection. Thus only a relatively small number of clones (several hundred) need to be analyzed for binding to antigen.

20 [0127] Human antibodies can be produced without prior immunization by displaying very large and diverse V-gene repertoires on phage (Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597). In one embodiment natural V_H and V_L repertoires present in human peripheral blood lymphocytes are were isolated from unimmunized donors by PCR. The V-gene repertoires were spliced together at random using PCR to create a scFv gene repertoire
25 which is was cloned into a phage vector to create a library of 30 million phage antibodies (*Id.*). From this single "naive" phage antibody library, binding antibody fragments have been isolated against more than 17 different antigens, including haptens, polysaccharides and proteins (Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597; Marks *et al.* (1993). *Bio/Technology*. 10: 779-783; Griffiths *et al.* (1993) *EMBO J.* 12: 725-734; Clackson *et al.*
30 (1991) *Nature*. 352: 624-628). Antibodies have been produced against self proteins, including human thyroglobulin, immunoglobulin, tumor necrosis factor and CEA (Griffiths *et al.* (1993) *EMBO J.* 12: 725-734). It is also possible to isolate antibodies against cell

surface antigens by selecting directly on intact cells. The antibody fragments are highly specific for the antigen used for selection and have affinities in the 1 :M to 100 nM range (Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597; Griffiths *et al.* (1993) *EMBO J.* 12: 725-734). Larger phage antibody libraries result in the isolation of more antibodies of higher binding affinity to a greater proportion of antigens.

3) Binding proteins.

[0128] In one embodiment, the binding partner (capture agent) can be a binding protein. Suitable binding proteins include, but are not limited to receptors (*e.g.* cell surface receptors), receptor ligands, cytokines, transcription factors and other nucleic acid binding proteins, growth factors, *etc.*

[0129] The protein can be isolated from natural sources, mutagenized from isolated proteins or synthesized *de novo*. Means of isolating naturally occurring proteins are well known to those of skill in the art. Such methods include but are not limited to well known protein purification methods including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (*see, generally*, R. Scopes, (1982) *Protein Purification*, Springer-Verlag, N.Y.; Deutscher (1990) *Methods in Enzymology Vol. 182: Guide to Protein Purification*, Academic Press, Inc. N.Y.).

[0130] Where the protein binds a target reversibly, affinity columns bearing the target can be used to affinity purify the protein. Alternatively the protein can be recombinantly expressed with a HIS-Tag and purified using Ni²⁺/NTA chromatography.

[0131] In another embodiment, the protein can be chemically synthesized using standard chemical peptide synthesis techniques. Where the desired subsequences are relatively short the molecule may be synthesized as a single contiguous polypeptide. Where larger molecules are desired, subsequences can be synthesized separately (in one or more units) and then fused by condensation of the amino terminus of one molecule with the carboxyl terminus of the other molecule thereby forming a peptide bond. This is typically accomplished using the same chemistry (*e.g.*, Fmoc, Tbo) used to couple single amino acids in commercial peptide synthesizers.

[0132] Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino

acids in the sequence is the preferred method for the chemical synthesis of the polypeptides of this invention. Techniques for solid phase synthesis are described by Barany and Merrifield (1962) *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.*, Merrifield *et al.* (1963) *J. Am. Chem. Soc.*, 85: 2149-2156, and Stewart *et al.* (1984) *Solid Phase Peptide Synthesis*, 2nd ed. Pierce Chem. Co., Rockford, Ill.

[0133] In a preferred embodiment, the protein can also be synthesized using recombinant DNA methodology. Generally this involves creating a DNA sequence that encodes the binding protein, placing the DNA in an expression cassette under the control of a particular promoter, expressing the protein in a host, isolating the expressed protein and, if required, renaturing the protein.

[0134] DNA encoding binding proteins or subsequences of this invention can be prepared by any suitable method as described above, including, for example, cloning and restriction of appropriate sequences or direct chemical synthesis by methods such as the phosphotriester method of Narang *et al.* (1979) *Meth. Enzymol.* 68: 90-99; the phosphodiester method of Brown *et al.* (1979) *Meth. Enzymol.* 68: 109-151; the diethylphosphoramidite method of Beaucage *et al.* (1981) *Tetra. Lett.*, 22: 1859-1862; and the solid support method of U.S. Patent No. 4,458,066.

[0135] The nucleic acid sequences encoding the desired binding protein(s) may be expressed in a variety of host cells, including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO and HeLa cells lines and myeloma cell lines. The recombinant protein gene will be operably linked to appropriate expression control sequences for each host. For *E. coli* this includes a promoter such as the *T7*, *trp*, or *lambda* promoters, a ribosome binding site and preferably a transcription termination signal. For eukaryotic cells, the control sequences will include a promoter and preferably an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, *etc.*, and a polyadenylation sequence, and may include splice donor and acceptor sequences.

[0136] The plasmids can be transferred into the chosen host cell by well-known methods such as calcium chloride transformation for *E. coli* and calcium phosphate treatment or electroporation for mammalian cells. Cells transformed by the plasmids can be

selected by resistance to antibiotics conferred by genes contained on the plasmids, such as the *amp*, *gpt*, *neo* and *hyg* genes.

[0137] Once expressed, the recombinant binding proteins can be purified according to standard procedures of the art as described above.

5 **4) Sugars and carbohydrates.**

[0138] Other binding agents suitable for sensor elements of this invention include, but are not limited to, sugars and carbohydrates. Sugars and carbohydrates can be isolated from natural sources, enzymatically synthesized or chemically synthesized. A route to production of specific oligosaccharide structures is through the use of the enzymes which
10 make them *in vivo*; the glycosyltransferases. Such enzymes can be used as regio- and stereoselective catalysts for the *in vitro* synthesis of oligosaccharides (Ichikawa *et al.* (1992) *Anal. Biochem.* 202: 215-238). Sialyltransferase can be used in combination with additional glycosyltransferases. For example, one can use a combination of sialyltransferase and galactosyltransferases. A number of methods of using glycosyltransferases to synthesize
15 desired oligosaccharide structures are known. Exemplary methods are described, for instance, WO 96/32491, Ito *et al.* (1993) *Pure Appl. Chem.* 65:753, and U.S. Patents 5,352,670, 5,374,541, and 5,545,553. The enzymes and substrates can be combined in an initial reaction mixture, or alternatively, the enzymes and reagents for a second glycosyltransferase cycle can be added to the reaction medium once the first
20 glycosyltransferase cycle has neared completion. By conducting two glycosyltransferase cycles in sequence in a single vessel, overall yields are improved over procedures in which an intermediate species is isolated.

[0139] Methods of chemical synthesis are described by Zhang *et al.* (1999) *J. Am. Chem. Soc.*, 121(4): 734-753. Briefly, in this approach, a set of sugar-based building blocks
25 is created with each block preloaded with different protecting groups. The building blocks are ranked by reactivity of each protecting group. A computer program then determines exactly which building blocks must be added to the reaction so that the sequences of reactions from fastest to slowest produces the desired compound.

IV. Assembling a sensor.

[0140] The biosensors of this invention can be assembled using methods well known to those of skill in the art. In general two or more electrodes are provided having an inter-electrode spacing sufficiently small that the biomolecule/target analyte complex is capable of carrying charge from one electrode to the other. The electrode(s) are then contacted with the biomolecule(s) **14** in a manner that facilitates the electrical coupling and physical attachment of the biomolecule(s) to one or both electrodes (depending on device configuration). The electrode(s) and/or the biomolecules can be derivatized so that the molecules self assemble/attach to the electrode.

A) Providing two or more electrodes.

[0141] Methods of providing electrodes closely positioned with respect to each other are well known to those of skill in the art. Thus, for example, electrodes can be precisely positioned using micromanipulators, atomic force microscope (AFM) or STM tips, and the like. In preferred embodiments, the plurality of electrodes (optional counter electrodes) and the like are typically placed in registered proximity to one another by mechanical means, *e.g.*, by using guide posts, alignment pins, guide edges, and the like. Other systems using electrical or magnetic registration means are also available.

[0142] In particularly preferred embodiments, the electrodes are fabricated/positioned using micromachining processes (*e.g.* photolithography) well known in the solid state electronics industry. Commonly, microdevices are constructed from semiconductor material substrates such as crystalline silicon, widely available in the form of a semiconductor wafer used to produce integrated circuits, or from glass. Because of the commonality of material(s), fabrication of microdevices from a semiconductor wafer substrate can take advantage of the extensive experience in both surface and bulk etching techniques developed by the semiconductor processing industry for integrated circuit (IC) production.

[0143] Surface etching, used in IC production for defining thin surface patterns in a semiconductor wafer, can be modified to allow for sacrificial undercut etching of thin layers of semiconductor materials to create spaces or gaps. Bulk etching, typically used in IC production when deep trenches are formed in a wafer using anisotropic etch processes, can be used to precisely machine edges or trenches in microdevices. Both surface and bulk

etching of wafers can proceed with "wet processing", using chemicals such as potassium hydroxide in solution to remove non-masked material from a wafer. For microdevice construction, it is even possible to employ anisotropic wet processing techniques that rely on differential crystallographic orientations of materials, or the use of electrochemical etch stops, to define various channel elements.

[0144] Another etch processing technique that allows great microdevice design freedom is commonly known as "dry etch processing". This processing technique is particularly suitable for anisotropic etching of fine structures. Dry etch processing encompasses many gas or plasma phase etching techniques ranging from highly anisotropic sputtering processes that bombard a wafer with high energy atoms or ions to displace wafer atoms into vapor phase (*e.g.* ion beam milling), to somewhat isotropic low energy plasma techniques that direct a plasma stream containing chemically reactive ions against a wafer to induce formation of volatile reaction products.

[0145] Intermediate between high energy sputtering techniques and low energy plasma techniques is a particularly useful dry etch process known as reactive ion etching. Reactive ion etching involves directing an ion containing plasma stream against a semiconductor, or other, wafer for simultaneous sputtering and plasma etching. Reactive ion etching retains some of the advantages of anisotropy associated with sputtering, while still providing reactive plasma ions for formation of vapor phase reaction products in response to contacting the reactive plasma ions with the wafer. In practice, the rate of wafer material removal is greatly enhanced relative to either sputtering techniques or low energy plasma techniques taken alone. Reactive ion etching therefore has the potential to be a superior etching process for construction of microdevices, with relatively high anisotropic etching rates being sustainable. The micromachining techniques described above, as well as many others, are well known to those of skill in the art (*see, e.g.,* Choudhury (1997) *The Handbook of Microlithography, Micromachining, and Microfabrication*, Soc. Photo-Optical Instru. Engineer, Bard & Faulkner (1997) *Fundamentals of Microfabrication*). In addition, examples of the use of micromachining techniques on silicon or borosilicate glass chips can be found in U.S. Patents 5,194,133, 5,132,012, 4,908,112, and 4,891,120.

[0146] In certain embodiments, the electrodes, particularly electrode arrays of this invention are formed as multilayer materials, *e.g.* alternating layers of dielectric and conductor. When etched, cut, or otherwise fractured, the edge of such multilayer materials

affords electrodes separated by dielectric/insulator at extremely high density (close spacing).

[0147] Multilayer materials are widely known in the materials community for scientific study and physics applications and their use has been demonstrated widely (*see*,
5 *e.g.*, U.S. Patents 4,673,623, 4,870,648, 4,915,463 and the like).

[0148] Such electrode arrays are readily fabricated using sputtering techniques (*see*,
e.g. U.S. Patents 5,203,977, 5,486,277, RE37,032, 5,742,471, and the like). Sputtering is a vacuum coating process where an electrically isolated cathode is mounted in a chamber that can be evacuated and partially filled with an inert gas. If the cathode material is an
10 electrical conductor, a direct-current high-voltage power supply is used to apply the high voltage potential. If the cathode is an electrical insulator, the polarity of the electrodes is reversed at very high frequencies to prevent the formation of a positive charge on the cathode that would stop the ion bombardment process. Since the electrode polarity is reversed at a radio frequency, this process is referred to as RF-sputtering.

15 [0149] Magnetron sputtering is a more effective form than diode sputtering that uses a magnetic field to trap electrons in a region near the target surface creating a higher probability of ionizing a gas atom. The high density of ions created near the target surface causes material to be removed many times faster than in diode sputtering. The magnetron effect is created by an array of permanent magnets included within the cathode assembly
20 that produce a magnetic field normal to the electric field. While other sputtering techniques may be used, in particularly preferred embodiments, magnetron sputtering, *e.g.* as described in U.S. Patent 5,486,277, is used to provide the electrode arrays of this invention.

[0150] Figure 22 illustrates how deposition angle can be used to control the location of various conductors (electrodes). With a deposition beam oriented normal to the substrate
25 (conductor set "A"), the insulator and conductors 10 and 12 can be deposited adjacent to each other as illustrated. Use of an appropriate mask during spacer 16 deposition the area covered by the spacer to be precisely delineated. Then deposition of a conductive material produces conductors 10 and 12.

[0151] The deposition angle can be varied to produce alternate patterns. As shown
30 in Figure 22, conductor set "B", deposition of the conductor material at an angle oblique to the substrate can produce a space between conductor 12 and spacer 16.

[0152] As indicated above, the deposition techniques can be combined with etching methods. Conductor set "C" illustrates the result of selectively etching the spacer of conductor set "A". This produces a "cutback" of the spacer with an air gap between conductor 10 and conductor 12.

5 [0153] Figure 23 illustrates another combination of deposition and etching. In this instance, the substrate is uniformly coated first with the spacer material 16 and then with the conductor material 10 (*see*, conductor set "A"). The conductor material 10 and spacer material 16 are then selectively etched in one region to produce a well or channel (*see*, conductor set "B"). A second deposition step with an appropriate mask deposits conductor
10 material 12 in the well or channel (*see*, conductor set "C"). These variations are meant to be illustrative and not limiting. Using the teaching provided herein, one of skill will readily be able to fabricate the devices of this invention.

B) Attachment of biomolecules to electrodes.

[0154] The binding agents (*e.g.* biomolecules) are attached to the electrodes using
15 methods well known to those of skill in the art. Typically the electrode(s) and/or the binding agent(s) are derivatized (functionalized) with reactive moieties (*e.g.* linkers) that facilitate attachment of the electrode to the binding agent. Thus, for example in certain embodiments, the binding agent bears a reactive linker (*e.g.* an aliphatic thiol linker) that reacts with the electrode surface or with a functional group attached thereto, and/or the
20 electrode is derivatized with a linker that binds to the biomolecule.

[0155] The linker can be electrically conductive or it can be short enough that electrons can pass directly or indirectly between the electrode and the biological molecule 14.

[0156] The manner of linking a wide variety of compounds to various surfaces is
25 well known and is amply illustrated in the literature. Means of coupling the biological molecules 14 will be recognized by those of skill in the art. The linkage can be covalent, or by ionic or other non-covalent interactions. The surface and/or the molecule(s) may be specifically derivatized to provide convenient linking groups (*e.g.* sulfur, hydroxyl, amino, *etc.*).

[0157] The linker(s) can be provided as a part of a derivatized binding agent or they can be provided separately. Linkers, when not joined to the molecules to be linked are often either hetero- or homo-bifunctional molecules that contain two or more reactive sites that may each form a covalent bond with the respective binding partner (*i.e.* electrode surface or biological molecule). When provided as a component the biological molecule, or attached to the electrode, the linkers are preferably spacers having one or more reactive sites suitable for bonding to the respective surface or molecule.

[0158] Linkers suitable for joining molecules are well known to those of skill in the art and include, but are not limited to any of a variety of, a straight or branched chain carbon linker, or a heterocyclic carbon linker, amino acid or peptide linkers, and the like. Particularly preferred linkers include, but are not limited to 4,4'-diphenylethyne, 4,4'-diphenylbutadiyne, 4,4'-biphenyl, 1,4-phenylene, 4,4'-stilbene, 1,4-bicyclooctane, 4,4'-azobenzene, 4,4'-benzylideneaniline, and 4,4''-terphenyl, oligophenylene vinylene, and the like (*see, e.g.*, U.S. Patent 6,208,553).

[0159] A wide variety of such linkers comprising surface binding groups are known to those of skill in the art and are often used to produce self-assembling monolayers. Such groups include, but are not limited to thiols (*e.g.* alkanethiols) (which bind gold and other metals), alkyltrichlorosilane (*e.g.*, which bind silicon/silicon dioxide), alkane carboxylic acids (*e.g.*, which bind aluminum oxides), derivatives of ethylene glycol, as well as combinations thereof (*see, e.g.*, Ferguson *et al.* (1993) *Macromolecules* 26(22): 5870-5875; Prime *et al.* (1991) *Science* 252:1164-1167; Bain *et al.* (1989) *Angew. Chem.* 101: 522-528; Kumar *et al.* (1994) *Langmuir* 10: 1498-1511; Laibinis *et al.* (1989) *Science* 245: 845-847; Pale-Grosdemange *et al.* (1991) *J. Am. Chem. Soc.*, 113: 12-20, and the like). In particularly preferred embodiments, the biological molecules 14 are attached to metal electrodes using thiol linkers (*e.g.*, alkanethiol linkers).

[0160] In certain embodiments, the binding agents are functionalized with a chemical group, or a linker bearing a chemical group, that can be activated by the application of an electrical potential. Such groups are well known to those of skill in the art and include, but are not limited to S-benzyloxycarbonyl derivatives, S-benzyl thioethers, S-phenyl thioethers, S-4-picolyl thioethers, S-2,2,2-trichloroethoxycarbonyl derivatives, S-triphenylmethyl thioethers, and the like. In certain embodiments, the binding agents are functionalized with a chemical group, or a linker bearing a chemical group that can be

activated by light of wavelength ranging from 190 nm to 700 nm. Such chemical groups include, but are not limited to an aryl azide, a fluorinated aryl azide, a benzophenone, and (R,S) -1-(3,4- (methylene-dioxy)-6-nitrophenyl) ethyl chloroformate – (MeNPOC), N-((2-pyridyl, ethyl)-4-azido) salicylamide

5 **[0161]** In a particularly preferred embodiment the derivatized biological molecule, in solution, is contacted with the electrode(s). A charge is placed on the first electrode **10** to attract the biological molecule thereto. Upon contact with the electrode, the derivatized biological molecule binds to the electrode. The derivatized biological molecule can bear two linkers, one for attachment to the first electrode and one derivatized for attachment to
10 the second electrode. In such embodiments, the second linker can be blocked to prevent reaction with the first electrode. After the biological molecule has been bound to the first molecule the linker is deprotected permitting binding to the second electrode.

[0162] Thus, for example to span two electrodes with a biological molecule that is a nucleic acid, the nucleic acid is derivatized with two linkers one protected (blocked) thiol
15 and one deprotected (unblocked) thiol. The first electrode **12** is biased positive to attract the nucleic acid thereto whereby the thiol linker binds to the first electrode. The first electrode **10** is then biased negative and the second electrode **12** is biased positive to attract the free end of the nucleic acid to second electrode. The blocked thiol linker is deprotected leaving that linker free to interact with the second. This results in a nucleic acid spanning gap
20 between the first and the second electrode.

[0163] This assembly approach thus uses the device itself, to direct the localization and ultimate attachment of the binding agent. Thus, the devices of this invention are able to electronically self-address each sensor element with a specific binding agent. The device self-assembles itself in the sense that no outside process, mechanism, or equipment is
25 needed to physically direct, position, or place a specific binding agent at a specific location/sensor element/electrode. This self-addressing process is both rapid and specific, and can be carried out in either a serial or parallel manner.

[0164] The device can be serially addressed with specific binding agent by maintaining selected sensor element(s)/electrode(s) in a DC mode and at the opposite
30 charge (potential) to that of a specific binding entity. Other sensor elements/electrodes are maintained at the same charge as the specific binding agent. In cases where the binding

agent is not in excess of the attachment sites on electrode(s), it is necessary to activate only one other micro-electrode to affect the electrophoretic transport to the specific micro-location. The specific binding agent is rapidly transported (in a few seconds, or preferably less than a second) through the solution, and concentrated directly at the specific electrode
 5 where can covalently bonded to the electrode surface.

[0165] The parallel process for addressing sensor elements/electrodes simply involves simultaneously activating a large number (particular group or line) of electrodes so that the same specific binding entity is transported, concentrated, and reacted with more than one specific electrode.

10 [0166] This approach is simply illustrative. Numerous other approaches can be used to attach the biological molecule to the respective electrode(s). Such approaches include, but are not limited to attachment of chemical groups to the surface through the use of photoactivatable chemistries (*see, e.g., Sundberg et al. (1995) J. Am. Chem. Soc.*
 117(49):12050-12057), micro-stamping techniques (*see, e.g., Kumar et al. (1994) Langmuir*
 15 10(5):1498-1511; Kumar et al. (1993) *Appl. Phys. Lett.* 63(14):2002-2004), and the like.

V. Reading the sensor.

[0167] The sensors of this invention are read using standard methods well known to those of skill in the art. In particular, the sensors of this invention provide a signal that is a change in conductivity (resistivity) of the sensor element(s) as target analytes are bound.

20 [0168] In preferred embodiments, the sensors of this invention are read using techniques including, but not limited to amperometry, voltammetry, capacitance, and impedance. Suitable techniques include, but are not limited to, electrogravimetry; coulometry (including controlled potential coulometry and constant current coulometry); voltammetry (cyclic voltammetry, pulse voltammetry (normal pulse voltammetry, square wave
 25 voltammetry, differential pulse voltammetry, Osteryoung square wave voltammetry, and coulostatic pulse techniques); stripping analysis (anodic stripping analysis, cathodic stripping analysis, square wave stripping voltammetry); conductance measurements (electrolytic conductance, direct analysis); time-dependent electrochemical analyses (chronoamperometry, chronopotentiometry, cyclic chronopotentiometry and amperometry,
 30 AC polarography, chronogalvanometry, and chronocoulometry); AC impedance measurement; capacitance measurement; and photoelectrochemistry.

[0169] In a preferred embodiment, monitoring electron transfer through the binding agent/target analyte complex is via amperometric detection. In certain embodiments, a preferred amperometric detector resembles the numerous enzyme-based biosensors currently used to monitor blood glucose, for example. This method of detection involves
5 applying a potential (as compared to a separate reference electrode) between the two electrodes comprising a sensor element of this invention. Electron transfer of differing efficiencies is induced in samples in the presence or absence of target nucleic acid; that is, where the binding agent is a nucleic acid, the single stranded binding agent exhibits a different rate than the probe hybridized to the target sequence. The differing efficiencies of
10 electron transfer result in differing currents being generated in the electrode.

[0170] In preferred embodiments, devices for measuring electron transfer amperometrically involves sensitive (nanoamp to picoamp) current detection and include a means of controlling the voltage potential, usually a potentiostat.

[0171] In other preferred embodiments, alternative electron detection modes are
15 utilized. For example, potentiometric (or voltammetric) measurements involve non-faradaic (no net current flow) processes and are utilized traditionally in pH and other ion detectors. Similar sensors can be used to monitor electron transfer the binding agent/target analyte complex. In addition, other properties of insulators (such as resistance) and of conductors (such as conductivity, impedance and capacitance) can be used to monitor
20 electron transfer through the binding agent/target analyte complex. Finally, any system that generates a current (such as electron transfer) also generates a small magnetic field, which can be monitored in some embodiments.

[0172] In preferred embodiments, the relatively fast rates of electron transfer through the binding agent/target analyte complex can facilitate analysis in the frequency
25 (time) domain and thereby dramatically improve signal to noise (S/N) ratios. Thus, in certain embodiments, electron transfer is initiated and detected using alternating current (AC) methods. In general, the use of AC techniques can result in good signals and low background noise. Without being bound by theory, there are a number of possible contributors to background noise, or "parasitic" signals, *i.e.* detectable signals that are
30 inherent to the system but are not the result of the presence of the target sequence.

[0173] However, all of the contributors to parasitic noise generally give relatively fast signals; that is, the rate of electron transfer through the binding agent/target analyte complex is generally significantly slower than the rate of electron transfer of the parasitic components, such as the contribution of charge carriers in solution, and other "short circuiting" mechanisms. As a result, the parasitic components are generally all phase related; that is, they exhibit a constant phase delay or phase shift that will scale directly with frequency. The binding agent/target analyte complex, in contrast, exhibits a time delay between the input and output signals, which is independent of frequency. Thus, signal produced by analyte binding will remain relatively constant and relatively large as compared to parasitic background. As a consequence, at different frequencies, the phase of the system will change. This is very similar to the time domain detection used in fluorescent systems.

[0174] This difference can be exploited in various methods to decrease the signal to noise ratio. Accordingly, the preferred detection methods comprise applying an AC input signal to a binding agent/target analyte complex. The presence of the binding agent/target analyte complex is detected via an output signal characteristic of electron transfer through the binding agent/target analyte complex; that is, the output signal is characteristic of the binding agent/target analyte complex rather than the parasitic components or unbound binding agent. Thus, for example, the output signal will exhibit a time delay dependent on the rate of electron transfer through the binding agent/target analyte complex.

[0175] In certain preferred embodiments, the input signals are applied at a plurality of frequencies, since this again allows the distinction between true signal and noise. "Plurality" in this context means at least two, and preferably more, frequencies. In general, the AC frequencies will range from about 0.1 Hz to about 10 mHz, with from about 1 Hz to 100 KHz being preferred.

[0176] In certain preferred embodiments, data analysis is preformed in the time domain (frequency domain). Thus, for example, cyclic voltammetry is performed where the signal is analyzed at a harmonic of the fundamental frequency. Such measurements can significantly improve the signal to noise (S/N) ratio.

[0177] In preferred embodiments, a cyclic (*e.g.*, sinusoidal sweeping voltage) is applied to the electrode. The response of the binding agent/target analyte complex to the

sinusoidal voltage is selectively detected at a harmonic of the fundamental frequency of the cyclic voltage rather than at the fundamental frequency. As a result, a complete frequency spectrum can be obtained within one cycle.

[0178] The step of selectively detecting the voltammetric response comprises the
5 step of selectively detecting a current flowing through the binding agent/target analyte complex at a harmonic of the fundamental frequency. Preferably the harmonic comprises at least one harmonic of the current above the fundamental frequency. Typically, the signal is monitored at harmonics at and above the second harmonic of the fundamental frequency. In general, the step of selectively detecting the voltammetric response comprises the step of
10 detecting a plurality of higher harmonics of the fundamental frequency within a frequency spectrum of a current flowing through the analyte, either through the use of multiple lock-in detectors, or via data acquisition in the time domain, followed by, *e.g.*, Fourier transformation and convolution via computer based methods. Methods of cyclic voltammetry are known to those of skill in the art and describe in detail in U.S. Patents
15 6,208,553 and 5,958,215

VI. Analyte detection/quantification.

A) Sample preparation.

[0179] Virtually any sample can be analyzed using the devices and methods of this invention. Such samples include, but are not limited to body fluids or tissues, water, food,
20 blood, serum, plasma, urine, feces, tissue, saliva, oils, organic solvents, earth, water, air, or food products. In a preferred embodiment, the sample is a biological sample. The term "biological sample", as used herein, refers to a sample obtained from an organism or from components (*e.g.*, cells) of an organism. The sample may be of any biological tissue or fluid. Frequently the sample will be a "clinical sample" which is a sample derived from a
25 patient. Such samples include, but are not limited to, sputum, cerebrospinal fluid, blood, blood fractions (*e.g.* serum, plasma), blood cells (*e.g.*, white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

[0180] Biological samples, (*e.g.* serum) may be analyzed directly or they may be subject to some preparation prior to use in the assays of this invention. Such preparation can include, but is not limited to, suspension/dilution of the sample in water or an appropriate buffer or removal of cellular debris, *e.g.* by centrifugation, or selection of particular fractions of the sample before analysis.

B) Sample delivery into system.

[0181] The sample can be introduced into the devices of this invention according to standard methods well known to those of skill in the art. Thus, for example, the sample can be introduced into the channel through an injection port such as those used in high pressure liquid chromatography systems. In another embodiment the sample can be applied to a sample well that communicates to the channel. In still another embodiment the sample can be pumped into the channel. Means of introducing samples into channels are well known and standard in the capillary electrophoresis and chromatography arts.

C) Sample reaction with the binding agent.

[0182] The analyte containing sample is provided to the sensor element in conditions compatible with or that facilitate binding of the analyte to the binding agent comprising the sensor element. Thus, for example, where the sensor element is an antibody or protein, reaction conditions are provided at the sensor element that facilitate antibody binding. Such reaction conditions are well known to those of skill in the art (*see, e.g.,* Techniques for using and manipulating antibodies are found in Coligan (1991) *Current Protocols in Immunology* Wiley/Greene, NY; Harlow and Lane (1989) *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY; Stites *et al.* (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, NY; and Kohler and Milstein (1975) *Nature* 256: 495-497, and the like).

[0183] Similarly, where the binding agent is a nucleic acid the sensor element is maintained under conditions that facilitate binding of the target nucleic acid (analyte) to the binding agent comprising the sensor element(s). Stringency of the reaction can be increased until the sensor shows adequate/desired specificity and selectivity. Conditions suitable for nucleic acid hybridizations are well known to those of skill in the art (*see, e.g.,* Berger and

Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology* 152 Academic Press, Inc., San Diego, CA; Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual (2nd ed.)* Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Ausubel *et al.* (1994) *Current Protocols in Molecular Biology*, , Current Protocols, a joint
 5 venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.; U.S. Patent 5,017,478; European Patent No. 0,246,864, and the like).

[0184] Once the analyte is bound to the binding agent in the sensor element, the sensor is optionally dehydrated and/or stored and/or read.

C) Analyte detection/quantitation.

10 [0185] Once introduced into the sensors of this invention, the sample is detected/quantified using standard methods, *e.g.* as described above, *e.g.* amperometry, voltammetry, coulometry, *etc.* The measurement results can be compared to a standard curve, *i.e.* a series of measurement results plotted as a function of analyte concentration, which permits determination of analyte concentration. The standard curve can be calculated
 15 by/stored in the device performing data acquisition.

V. Cassettes.

[0186] In certain embodiments, this invention provides cassettes comprising one or more sensor elements or sensor element arrays according to this invention. In preferred embodiments, cassettes include one or more biomolecules 14 and/or one or more working
 20 electrodes 10, 12 and/or biasing electrodes 22.

[0187] Thus, for example in certain embodiments, a cassette with comprise a plurality of biomolecules 14, that are each attached to a pair of electrodes. Counter electrodes are optionally provided, *e.g.* integrated in the layer comprising the working electrodes or provided as a component of a second layer comprising the cassette.

25 [0188] In a preferred embodiment, a cassette or apparatus of the invention comprises a sample port and/or reservoir and one or more channels for sample delivery onto the sensor element(s) present in the cassette. The means for sample delivery can be stationary or movable and can be any known in the art, including but not limited to one or more inlets, holes, pores, channels, pipes, microfluidic guides (*e.g.*, capillaries), tubes, and
 30 the like.

[0189] The channel(s) comprising the cassette of this invention can comprise a channel network, *e.g.*, one or more channels, preferably microchannels. Typically included within a given channel network are channels or reservoirs in which the desired analysis is to take place (analysis channels), and thus the sensor elements of this invention are disposed.

5 Also, optionally included are channels for delivering reagents, buffers, diluents, sample material and the like to the analysis channels.

[0190] The cassettes of this invention optionally include separation channels or matrices separating/fractionating materials transported down the length of these channels, for analysis, *i.e.*, size or charged based fractionation of materials, *e.g.*, nucleic acids, proteins etc. Suitable separation matrices include, *e.g.*, GeneScan.TM. polymers (Perkin Elmer-Applied Biosystems Division, Foster City, Calif.). Alternatively, analysis channels are devoid of any separation matrix, and instead, merely provide a channel within which an interaction, reaction *etc.*, takes place. Examples of microfluidic devices incorporating such analysis channels are described in, *e.g.*, PCT Application No. WO 98/00231, and U.S. Pat.
10
15 No. 5,976,336.

[0191] Fluids can be moved through the cassette channel system by a variety of well known methods, for example: pumps, pipettes, syringes, gravity flow, capillary action, wicking, electrophoresis, electroosmosis, pressure, vacuum, *etc.* The means for fluid movement may be located on the cassette or on a separate unit.

20 [0192] The sample can be placed on all of the sensor elements. Alternatively, a sample may be placed on particular sensor elements, *e.g.*, by a capillary fluid transport means. Alternatively, samples may be placed on the sensor element(s) by an automatic pipetter for delivery of fluid samples directly to sensor array, or into a reservoir in a cassette or cassette holder for later delivery directly to the sensor element(s).

25 [0193] The cassettes of this invention can be fabricated from a wide variety of materials including, but not limited to glass, plastic, ceramic, polymeric materials, elastomeric materials, metals, carbon or carbon containing materials, alloys, composite foils, silicon and/or layered materials. Supports may have a wide variety of structural, chemical and/or optical properties. They may be rigid or flexible, flat or deformed,
30 transparent, translucent, partially or fully reflective or opaque and may have composite

properties, regions with different properties, and may be a composite of more than one material.

[0194] Reagents for conducting assays may be stored on the cassette and/or in a separate container. Reagents can be stored in a dry and/or wet state. In one embodiment, dry reagents in the cassette are rehydrated by the addition of a test sample. In a different embodiment, the reagents are stored in solution in "blister packs" which are burst open due to pressure from a movable roller or piston. The cassettes may contain a waste compartment or sponge for the storage of liquid waste after completion of the assay. In one embodiment, the cassette includes a device for preparation of the biological sample to be tested. Thus, for example, a filter may be included for removing cells from blood. In another example, the cassette may include a device such as a precision capillary for the metering of sample.

[0195] A cassette or apparatus of the invention can, optionally, comprise reference electrodes, *e.g.*, Ag/AgCl or a saturated calomel electrode (SCE) and/or various biasing/counter-electrodes.

[0196] The cassette can also comprise more one layer of electrodes. Thus, for example, different electrode sets (*e.g.* arrays of sensor elements) can exist in different lamina of the cassette and thus form a three dimensional array of sensor elements.

VI. Integrated assay device/apparatus.

[0197] State-of-the-art chemical analysis systems for use in chemical production, environmental analysis, medical diagnostics and basic laboratory analysis are preferably capable of complete automation. Such total analysis systems (TAS) (Fillipini *et al.* (1991) *J. Biotechnol.* 18: 153; Garn *et al* (1989) *Biotechnol. Bioeng.* 34: 423; Tshulena (1988) *Phys. Scr.* T23: 293; Edmonds (1985) *Trends Anal. Chem.* 4: 220; Stinshoff *et al.* (1985) *Anal. Chem.* 57:114R; Guibault (1983) *Anal. Chem Symp. Ser.* 17: 637; Widmer (1983) *Trends Anal. Chem.* 2: 8) automatically perform functions ranging from introduction of sample into the system, transport of the sample through the system, sample preparation, separation, purification and detection, including data acquisition and evaluation.

[0198] Recently, sample preparation technologies have been successfully reduced to miniaturized formats. Thus, for example, gas chromatography (Widmer *et al.* (1984) *Int. J.*

Environ. Anal. Chem. 18: 1), high pressure liquid chromatography (Muller *et al.* (1991) *J. High Resolut. Chromatogr.* 14: 174; Manz *et al.* (1990) *Sensors & Actuators B*1:249; Novotny *et al.*, eds. (1985) *Microcolumn Separations: Columns, Instrumentation and Ancillary Techniques J. Chromatogr. Library*, Vol. 30; Kucera, ed. (1984) *Micro-Column High Performance Liquid Chromatography*, Elsevier, Amsterdam; Scott, ed. (1984) *Small Bore Liquid Chromatography Columns: Their Properties and Uses*, Wiley, N.Y.; Jorgenson *et al.* (1983) *J. Chromatogr.* 255: 335; Knox *et al.* (1979) *J. Chromatogr.* 186:405; Tsuda *et al.* (1978) *Anal. Chem.* 50: 632) and capillary electrophoresis (Manz *et al.* (1992) *J. Chromatogr.* 593: 253; Olefirowicz *et al.* (1990) *Anal. Chem.* 62: 1872; *Second Int'l Symp. High-Perf. Capillary Electrophoresis* (1990) *J. Chromatogr.* 516; Ghowsi *et al.* (1990) *Anal. Chem.* 62:2714) have been reduced to miniaturized formats.

[0199] Similarly, in certain embodiments, this invention provides an integrated assay device (*e.g.*, a TAS) for detecting and/or quantifying one or more analytes using the sensor elements, sensor element arrays, or cassettes of this invention.

15 [0200] Thus, in certain embodiments, the cassettes of this invention are designed to be inserted into an apparatus, that contains means for reading one or more sensor elements comprising a cassette of this invention. The apparatus, optionally includes means for applying one or more test samples onto the sensor elements or into a receiving port or reservoir and initiating detecting/quantifying one or more analytes. Such apparatus may be
 20 derived from conventional apparatus suitably modified according to the invention to conduct a plurality of assays based on a support or cassette. Modifications required include the provision for, optional, sample and/or cassette handling, multiple sample delivery, multiple electrode reading by a suitable detector, and signal acquisition and processing means.

25 [0201] Preferred apparatus, in accordance with this invention, thus typically include instrumentation suitable for performing electrochemical measurements and associated data acquisition and subsequent data analysis.

[0202] Preferred apparatus also provide means to hold cassettes, optionally provide reagents and/or buffers and to provide conditions compatible with binding agent/target
 30 analyte binding reactions.

[0203] A preferred apparatus also comprises an electrode contact means able to electrically connect the array of separately addressable electrode connections of the cassette to an electronic-voltage/waveform generator, *e.g.*, potentiostat. The waveform generator means delivers signals sequentially or simultaneously to independently read a plurality of sensor elements in the cassette.

[0204] The apparatus optionally comprises a digital computer or microprocessor to control the functions of the various components of the apparatus.

[0205] The apparatus also comprises signal processing means. In one embodiment, and simply by way of example, the signal processing means comprises a digital computer for transferring, recording, analyzing and/or displaying the results of each assay.

[0206] The sensor element arrays of this invention are particularly well suited for use as detectors in "low sample volume" instrumentation. Such applications include, but are not limited to genomic applications such as monitoring gene expression in plants or animals, parallel gene expression studies, high throughput screening, clinical diagnosis, single nucleotide polymorphism (SNP) screening, genotyping, and the like. Certain particularly preferred embodiments, include miniaturized molecular assay systems, so-called labs-on-a-chip, that are capable of performing thousands of analyses simultaneously

Kits.

[0207] In certain embodiments, this invention provides kits for practice of the methods and/or assembly of the devices described herein. Preferred kits comprise a container containing one or more sensor elements according to the present invention. The sensor elements can be components of a sensor array and/or can comprise a sensor cassette as describe herein. In certain embodiments, the kits, optionally, include one or more reagents and/or buffers for use with the sensors of this invention. The kits can optionally include materials for sample acquisition, processing, and the like.

[0208] The kits can also include instructional materials containing directions (*i.e.*, protocols) for the practice of the assay methods of this invention the use of the cassettes described herein, methods of assembling sensor elements into various instruments, and the like. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and

communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (*e.g.*, magnetic discs, tapes, cartridges, chips), optical media (*e.g.*, CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

5

EXAMPLES

[0209] The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

Sensor element formation.

10 [0210] Alternating layers of insulators and conductors are formed by sputtering or vapor deposition (*e.g.* as described in U.S. Patent 5,414,588.. The layers consist of a substrate (Alkali-free borosilicate glass (Shott AF45)), followed by a first conductor, then an insulator, followed by a second conductor and so forth. The first conductor plus insulator, and the second conductor plus a second insulator comprise one iteration.

15 Iterations are repeated until the desired number of lamina is achieved.

[0211] The position of the conductors and insulators is determined by a mask. Thus, as illustrated in Figure 24A, conductor 1 has a designated mask and/or mask position of the mask determining the location of its deposition. Similarly, the insulator position is determined by the use of a second mask, as illustrated in Figure 24B, and the position of
20 conductor 2 is determined by a third mask as illustrated in Figure 24C. The masks are reused for each subsequent iteration for a total of ten iterations.

[0212] The sputtering process results in a multi-laminar structure of alternating conducting and insulating layers where the first conductor layers are connected to each other and the second layers to be connected to each other, but not to the first conductor
25 layers (*see* Fig. 24D) similar to the capacitor described in U.S. Patent 5,414,588.

[0213] The conductors are fabricated of gold, and the insulator layers are made of glass or polystyrene or teflon.

[0214] The multilayer structure is cut to expose the thin layers of conductors and insulators. The exposed surface is then polished smooth. In selected structures, the insulator layers are etched further to form a channel between the conductive layers.

[0215] The first conductor layers are connected to a first macro-electrode using common semi-conductor etching methods. The second conductor layers are connected to a second macro-electrode also using common semi-conductor etching methods.

[0216] The macro-electrodes are connected to a voltage source and tested for non-conductance using an EG&G High Speed Potentiostat/Galvanostat (PerkinElmer Model 283).

10 **Analyte Detection.**

[0217] The multilayer electrode face is contacted with a capture probe solution comprising 30 mer oligonucleotides. The 5 prime end of the oligonucleotides is derivatized with an electrolabile, an alkyl- or aryl chloroformate, which can be removed at -1.5 volts in the presence of $\text{LiClO}_4/\text{CH}_3\text{OH}$ to reveal a thiol group which can then form a covalent bond with a gold electrode.

[0218] The 3 prime end of the oligonucleotide is derivatized with another electrolabile group such as S-benzyloxycarbonyl moiety which can be removed at -2.6 volts in DMF and tetrabutyl ammonium chloride. Each of the electrolabile groups is cleavable at a unique voltage.

[0219] The first conductor is biased with the activation voltage of the 5 prime electrolabile group on the capture probe thereby exposing the thiol group which then attaches to the first conductor.

[0220] The solution comprising the analyte (a nucleic acid comprising a sequence complementary to the capture probe) is contacted with the capture probe and allowed to hybridize to the capture probe on the electrodes.

[0221] The second conductor is biased with the activation voltage of the 3 prime electrolabile group of the capture probe thereby attaching the probe to connect to the second conductor. The electrodes are then dried under nitrogen or argon.

[0222] The electrodes are connected to a macro electrodes to a voltage source and tested for non-conductance, or a background conductance, is measured using a high-speed potentiostat/galvanostat (*e.g.* Perkin-Elmer, Model 283).

5 [0223] The electrodes are dried again under nitrogen or argon. A voltage (*e.g.*, -6 - +6V,) is applied again to the electrodes and the current is measured. The measured current of the hybridized nucleic acids is significantly greater the current measured for the unhybridized electrodes.

10 [0224] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. A molecular sensing apparatus comprising:
a first electrode;
5 a second electrode;
a spacer between said first and said second electrode; and
a biological macromolecule connecting said first electrode to said
second electrode.
2. The apparatus of claims 1, wherein said first electrode, said spacer,
10 and said second electrode do not form a sandwich configuration.
3. The apparatus of claims 1 or 2, wherein said biological
macromolecule is selected from the group consisting of a nucleic acid, a protein, a
polysaccharide, a lectin and a lipid.
4. The apparatus of claim 3, wherein said biological macromolecule is a
15 nucleic acid.
5. The apparatus of claim 3, wherein said biological macromolecule is
functionalized with a chemical group selected from a sulfate, a sulfhydryl, an amine, an
aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl
group, a bromine, an iodine, a chlorine, a light-activatable group, a group activatable by an
20 electric potential.
6. The apparatus of claims 1 or 2, wherein said spacer comprises an
insulator having a resistivity greater than 10^{-3} ohm-meters.
7. The apparatus of claim 6, wherein spacer comprises an insulator
selected from the group consisting of SiO_2 , TiO_2 , ZrO_2 , quartz, porcelain, ceramic,
25 polystyrene, teflon, and an insulating oxide or sulfide of a transition metal in the periodic
table of the elements.

8. The apparatus of claims 1 or 2, wherein said first electrode and said second electrode are separated by a distance in the range of 1 to 10^9 Angstroms.

9. The apparatus of claims 1 or 2, wherein said first electrode and said second electrode are separated by a distance less than about 500 Angstroms.

5 10. The apparatus of claims 1 or 2, wherein at said first electrode and said second electrode has a resistivity of less than 10^{-2} ohm-meters.

11. The apparatus of claims 1 or 2, wherein at said first electrode and said second electrode has a resistivity of less than 10^{-3} ohm-meters.

10 12. The apparatus of claim 10, wherein the electrodes comprise a material selected from the group consisting of ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon, and a carbon nanotube.

15 13. The apparatus of claims 1 or 2, wherein said first electrode is functionalized to contain a chemical group that can be derivatized or crosslinked.

14. The apparatus of claim 13, wherein the said chemical group is selected from the group consisting of a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a
20 bromine, an iodine, a chlorine, a light-activatable group, and a group activatable by an electric potential.

15. The apparatus of claims 1 or 2, wherein said first electrode bears a self-assembled monolayer (SAM).

25 16. The apparatus of claim 15, wherein said SAM comprises a compound selected from the group consisting of an alkanethiol, a phospholipid, a bola amphiphile, and an oligo(phenylenevinylene).

17. The apparatus of claim 17, wherein the biological macromolecule is attached to the first electrode by a thiol group.

18. The apparatus of claim 17, wherein the biological macromolecule is attached to the first electrode by a phosphorothioate or a phosphonate.

5 19. The apparatus of claims 1 or 2, wherein said biological macromolecule is attached to said first electrode by a linker.

20. The apparatus of claim 19, wherein said linker is selected from the group consisting of DFDNB, DST, ABH, ANB-NOS, EDC, NHS-ASA, and SIA.

10 21. The apparatus of claim 1, further comprising a substrate to support the first electrode and the second electrode, wherein the first electrode and the second electrode are integrated with the substrate.

22. The apparatus of claim 1, further comprising a substrate with the first electrode and the second electrode, wherein the first electrode and the second electrode are integrated with the insulator to form a substrate.

15 23. The apparatus of claim 1, wherein said first electrode comprises a surface with a shape selected from the group consisting of convex, concave, textured, corrugated, patterned uniformly, and randomly patterned.

20 24. The apparatus of claims 1 or 2, wherein said first electrode and said second electrode are oriented in a formation selected from the group consisting of annular, planar, and orthogonal.

25. The apparatus of claims 1 or 2, wherein the first electrode comprises a first surface and a second electrode comprises a second surface wherein the first surface and the second surface are not co-planar.

25 26. The apparatus of claims 1 or 2, wherein the first electrode and the second electrode comprise a first electrode pair, the molecular sensing apparatus further comprising a second electrode pair comprising a second first electrode and a second second electrode.

27. The apparatus of claim 26, wherein said apparatus comprises at least 20 electrode pairs.

28. The apparatus of claim 26, wherein said apparatus comprises at least 100 electrode pairs.

5 29. The apparatus of claim 26, wherein said apparatus comprises about 10^2 to 10^{10} electrode pairs.

30. The apparatus of claim 26, wherein said apparatus comprises at least about 10^2 electrode pairs per square cm.

10 31. The apparatus of claim 26, wherein said apparatus comprises at least about 10^4 electrode pairs per square cm.

32. The apparatus of claim 26, further comprising a measurement device electrically coupled to the first electrode and to the second electrode of at least one electrode pair.

15 33. The apparatus of claim 32, wherein said measurement device measures an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge and magnetic potential.

20 34. The apparatus of claims 1 or 2, further comprising an electrical circuit electrically coupled to the first electrode and the second electrode.

35. The apparatus of claim 34, wherein said electrical circuit comprises an electrical signal gating system.

25 36. The apparatus of claim 35, wherein the said gating system comprises a CMOS gating system.

37. The apparatus of claim 26, wherein the electrodes comprising the first and second electrode pairs have attached the same biological macromolecule.

38. The apparatus of claim 26, wherein the electrodes comprising the electrode pairs have attached different biological molecules.

39. The apparatus of claims 1 or 2, further comprising a computer electrically coupled to the first electrode and the second electrode of at least one electrode pair.

40. The apparatus of claims 1 or 2, wherein at least one of the first electrode and the second electrode comprises a semi-conducting material.

41. The apparatus of claim 40, wherein said semi-conducting material has a resistivity ranging from about $10^{-6} \Omega\text{-m}$ to about $10^7 \Omega\text{-m}$.

42. The apparatus of claim 40, wherein the semi-conducting material is selected from the group consisting of silicon, dense silicon carbide, boron carbide, Fe_3O_4 , germanium, silicon germanium, silicon carbide, tungsten carbide, titanium carbide, indium phosphide, gallium nitride, gallium phosphide, aluminum phosphide, aluminum arsenide, mercury cadmium telluride, tellurium, selenium, ZnS, ZnO, ZnSe, CdS, ZnTe, GaSe, CdSe, CdTe, GaAs, InP, GaSb, InAs, Te, PbS, InSb, PbTe, PbSe, and tungsten disulfide.

43. The apparatus of claim 1, wherein said apparatus comprises:
a first electrode having a first surface;
a second electrode having a second surface coplanar to the first surface;
an insulator between said first surface and said second surface; and
a nucleic acid joining said first electrode to said second electrode.

44. The apparatus of claim 2, wherein said first electrode overlays said insulator and said second electrode is adjacent to said insulator.

45. A method of making a molecular sensing apparatus, said method comprising:
providing a first electrode and a second electrode separated by an insulator;

contacting said first and said second electrode with a first solution comprising a biological macromolecule;

placing a charge on said first electrode to attract said biological macromolecule to said first electrode where said macromolecule attaches to said first
5 electrode to form an attached macromolecule; and

placing a charge on said second electrode to attract a portion of said attached macromolecule to said second electrode where said macromolecule attaches to said second electrode.

46. The method of claim 45, wherein said first electrode, said spacer, and
10 said second electrode do not form a sandwich configuration.

47. The method of claims 45 or 46, wherein said biological macromolecule is selected from the group consisting of a nucleic acid, a proteins, a polysaccharide, a lectin, and a lipid.

48. The method of claims 45 or 46, wherein said biological
15 macromolecule is functionalized with a chemical group selected from the group consisting of a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, a group activatable by an electric potential.

49. The method of claims 45 or 46, wherein said biological
20 macromolecule is selected from the group consisting of a nucleic acid, a protein, a polysaccharide, a lectin and a lipid..

50. The method of claims 45 or 46, wherein said biological macromolecule is a nucleic acid.

51. The method of claims 45 or 46, wherein said spacer is an insulator
25 having a resistivity of greater than about $10^{-3} \Omega\text{-m}$.

52. The method of claims 45 or 46, wherein said spacer comprises an insulator selected from the group containing SiO_2 , TiO_2 , ZrO_2 , porcelain, ceramic, quartz,

high resistivity plastic, and an insulating oxide or sulfide of the transition metals in the periodic table of the elements.

53. The method of claims 45 or 46, wherein said first electrode and said second electrode are separated by a distance range from about 1 to about 10^{10} Angstroms.

5 54. The method of claims 45 or 46, wherein said first electrode and said second electrode are separated by a distance less than about 500 Angstroms.

55. The method of claims 45 or 46, wherein said first electrode and said second electrode have a resistivity of less than about 10^{-3} Ω -m.

10 56. The method of claims 45 or 46, wherein said first electrode and said second electrode comprise a material selected from the group consisting of ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon, and a carbon nanotube.

15 57. The method of claims 45 or 46, wherein said first electrode is functionalized to bear a chemical group capable of being further derivatized or crosslinked.

58. The method of claim 57, wherein the said chemical group is selected from the group consisting of functionalized with a chemical group selected from a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, 20 an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, a group activatable by an electric potential.

59. The method of claims 45 or 46, wherein said biological macromolecule is attached to said first electrode by an electrically conductive linker.

60. The method of claim 59, wherein said linker is selected from the 25 group consisting of DFDNB, DST, ABH, ANB-NOS, EDC, NHS-ASA, and SIA.

61. The method of claim 59, wherein said linker is oligo(phenylenevinylene).

62. The method of claims 45 or 46, further comprising a substrate to support the first electrode and the second electrode, wherein the first electrode and the second electrode are integrated with the substrate.

5 63. The method of claims 45 or 46, further comprising a substrate with the first electrode and the second electrode, wherein the first electrode and the second electrode are integrated with the insulator to form a substrate.

64. The method of claims 45 or 46, wherein the first electrode and the second electrode provide a first electrode pair, the molecular sensing apparatus further comprising a second electrode pair comprising a second first electrode and a second second
10 electrode.

65. The method of claim 64, wherein said apparatus comprises at least 3 electrode pairs.

66. The method of claim 64, wherein said apparatus comprises at least 100 electrode pairs.

15 67. The method of claim 64, wherein said apparatus comprises about 10^2 to about 10^{10} electrode pairs.

68. The method of claim 64, further comprising:
contacting said second electrode pair with a second solution
comprising a second biological macromolecule;
20 placing a charge on a first electrode of said second electrode pair to attract said second biological macromolecule to said first electrode of said second electrode pair whereby said second biological macromolecule attaches to said first electrode to form an attached second macromolecule; and
placing a charge on said second electrode of said second electrode
25 pair to attract a portion of said attached second macromolecule to said second electrode whereby said second macromolecule attaches to said second electrode of said second electrode pair.

69. The method of claim 68, wherein said apparatus comprises a third electrode pair.

70. The method of claim 68, wherein said apparatus comprises greater than 3 electrode pairs.

5 71. The method of claim 68, wherein said first solution and said second solution are the same.

72. The method of claim 68, wherein said first solution and said second solution are different.

10 73. The method of claim 68, wherein said first biological molecule and said second biological molecule are the same.

74. The method of claim 68, wherein said first biological molecule and said second biological molecule are the different.

75. The method of claims 45 or 46, wherein at least one of said first electrode and said second electrode comprise a semi-conducting material.

15 76. The method of claim 75, wherein the semi-conductor material has a resistivity in the range of $10^{-6} \Omega\text{-m}$ to $10^7 \Omega\text{-m}$.

77. The method of claim 75, wherein the semi-conducting material is selected from the group consisting of silicon, dense silicon carbide, boron carbide, Fe_3O_4 , germanium, silicon germanium, silicon carbide, tungsten carbide, titanium carbide, indium phosphide, gallium nitride, gallium phosphide, aluminum phosphide, aluminum arsenide, mercury cadmium telluride, tellurium, selenium, ZnS, ZnO, ZnSe, CdS, ZnTe, GaSe, CdSe, CdTe, GaAs, InP, GaSb, InAs, Te, PbS, InSb, PbTe, PbSe, and tungsten disulfide.

20

78. A method of detecting an analyte, said method comprising:

25 i) providing molecular sensing apparatus comprising a first electrode and a second electrode separated by an insulator where said first electrode has a biological macromolecule attached thereto;

ii) contacting the attached macromolecule with said analyte whereby said analyte binds to said macromolecule thereby forming a macromolecule/analyte complex;

5 iii) placing a charge on said second electrode attract a portion of said bound analyte to said second electrode where said analyte is bound to said second electrode such that said macromolecule/analyte complex forms a connection between said first electrode and said second electrode; and

iv) detecting the connection between said first and said second electrode.

10 79. The method of claim 78, wherein said first electrode, said spacer, and said second electrode do not form a sandwich configuration.

80. The method of claims 78 or 80, wherein said providing comprises: contacting said first electrode with a first solution comprising said biological macromolecule; and

15 placing a charge on said first electrode whereby said charge attracts said biological macromolecule to said electrode and said biological macromolecule attaches to said electrode.

20 81. The method of claims 78 or 80, wherein said placing a charge, further comprising placing a charge on said first electrode opposite to the charge on said second electrode.

25 82. The method of claims 78 or 80, wherein said detecting comprises detecting an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge, and magnetic potential.

83. The method of claims 78 or 80, wherein said biological macromolecule is selected from the group consisting of a nucleic acid, a protein, a polysaccharide, a lectin, and a lipid.

84. The method of claim 83, wherein said biological macromolecule is a nucleic acid.

85. The method of claim 84, wherein said biological macromolecule is functionalized with a chemical group selected from the group consisting of a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, and a group activatable by an electric potential..

86. The method of claims 78 or 80, wherein said spacer comprises an insulator having a resistivity greater than $10^{-3} \Omega\text{-m}$.

87. The method of claims 78 or 80, wherein said spacer comprises an insulator is selected from the group consisting of SiO_2 , TiO_2 , ZrO_2 , porcelain, ceramic, a high resistivity plastic, and an insulating oxide or sulfide of a transition metal in the periodic table of the elements.

88. The method of claims 78 or 80, wherein said first electrode and said second electrode are separated by a distance less than about 500 Angstroms.

89. The method of claims 78 or 80, wherein said first electrode and said second electrode are separated by a distance ranging from about 1 to about 10^{10} Angstroms.

90. The method of claims 78 or 80, wherein said first electrode and said second electrode have a resistivity of less than about $10^{-2} \Omega\text{-m}$.

91. The method of claims 78 or 80, wherein said first electrode comprises a semi-conductor material.

92. The method of claim 94, wherein said semi-conductor material having a resistivity ranging from about $10^{-6} \Omega\text{-m}$ to about $10^7 \Omega\text{-m}$.

93. The method of claim 94, wherein the semi-conducting material is selected from the group consisting of silicon, dense silicon carbide, boron carbide, Fe_3O_4 , germanium, silicon germanium, silicon carbide, tungsten carbide, titanium carbide, indium phosphide, gallium nitride, gallium phosphide, aluminum phosphide, aluminum arsenide,

mercury cadmium telluride, tellurium, selenium, ZnS, ZnO, ZnSe, CdS, ZnTe, GaSe, CdSe, CdTe, GaAs, InP, GaSb, InAs, Te, PbS, InSb, PbTe, PbSe, and tungsten disulfide.

94. The method of claims 78 or 80, wherein said first electrode and said second electrode are formed from a material selected from the group consisting of
5 ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon, and a carbon nanotube.

95. The method of claims 78 or 80, wherein at least said first electrode is
10 functionalized to bear a chemical group capable of being further derivatized or crosslinked.

96. The method of claim 95, wherein the said chemical group is selected from the group consisting of a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, and a group activatable by an electric potential.

97. The method of claim 95, wherein said functionalized biological
15 macromolecule is attached to said first electrode by an electrically conductive linker.

98. The method of claim 97, wherein said linker is selected from the group consisting DFDNB, DST, ABH, ANB-NOS, EDC, NHS-ASA, and SIA.

99. The method of claim 97, wherein said linker is
20 oligo(phenylenevinylene).

100. The method of claims 78 or 80, wherein the first electrode and the second electrode are integrated with a substrate.

101. The method of claims 78 or 80, wherein the first electrode and the second electrode are integrated with the insulator to form a substrate.

102. The method of claims 78 or 80, wherein the first electrode and the
25 second electrode provide a first electrode pair, the molecular sensing apparatus further

comprising a second electrode pair comprising a second first electrode and a second electrode.

103. The method of claim 102, wherein said apparatus comprises at least 3 electrode pairs.

5 104. The method of claim 102, wherein said apparatus comprises at least 100 electrode pairs.

105. The method of claim 102, wherein said apparatus comprises from about 10^2 to about 10^{10} electrode pairs.

10 106. The method of claim 102, further comprising performing steps ii, iii, and iv with said second electrode pair.

107. The method of claim 102, wherein the biological macromolecule on said first electrode pair is different than the biological macromolecule attached to the second electrode pair.

15 108. A method of detecting an analyte, said method comprising:
i) providing a molecular sensing apparatus comprising a first electrode and a second electrode separated by a spacer where said first electrode has a first biological macromolecule attached thereto and said second electrode has a second biological macromolecule attached thereto;
ii) contacting the first attached macromolecule and the second
20 attached macromolecule with said analyte whereby said analyte binds to the first macromolecule and to the second macromolecule thereby forming a macromolecule/analyte complex forming a connection between said first electrode and said second electrode; and
iii) detecting the connection between said first and said second electrode.

25 109. The method of claim 108, wherein, wherein said first electrode, said spacer, and said second electrode do not form a sandwich configuration.

110. The method of claims 108 or 109, wherein said providing comprises:

contacting said first electrode with a first solution comprising said first biological macromolecule; and

placing a charge on said first electrode whereby said charge attracts said first biological macromolecule to said electrode and said biological macromolecule
5 attaches to said electrode.

111. The method of claimss 108 or 109, wherein said detecting comprises detecting an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical
10 impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge and magnetic potential.

112. The method of claims 108 or 109, wherein said biological macromolecule is selected from the group consisting of a nucleic acid, a protein, a polysaccharide, a lectin, and a lipid.

15 113. The method of claim 112, wherein said biological macromolecule is a nucleic acid.

114. The method of claim 113, wherein said biological macromolecule is functionalized with a chemical group selected from the group consisting of a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene,
20 an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, and a group activatable by an electric potential.

115. The method of claims 108 or 109, wherein said spacer comprises an insulator having a resistivity greater than $10^{-3}\Omega\cdot\text{m}$.

116. The method of claims 108 or 109, wherein said spacer comprises an
25 insulator selected from the group consisting of SiO_2 , TiO_2 , ZrO_2 , ceramic, porcelain, a high resistivity plastic, and an insulating oxide or sulfide of a transition metal in the periodic table of the elements.

117. The method of claims 108 or 109, wherein said first electrode and said second electrode are separated by a distance less than about 500 Angstroms.

118. The method of claims 108 or 109, wherein said first electrode and said second electrode are separated by a distance ranging from about 1 to about 10^{10} Angstroms.

119. The method of claims 108 or 109, wherein said first electrode and said second electrode have a resistivity of less than about $10^{-2} \Omega\text{-m}$.

120. The method of claims 108 or 109, wherein said first electrode and said second electrode comprise a material selected from the group consisting of ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon, and a carbon nanotube.

121. The method of claims 108 or 109, wherein at least one of the first electrode and the second electrode comprises a semi-conductor material.

122. The method of claim 121, wherein said semi-conductor material has a resistivity ranging from about $10^{-3} \Omega\text{-cm}$ to about $10^7 \Omega\text{-m}$.

123. The method of claim 121, wherein the semi-conducting material is selected from the group consisting of silicon, dense silicon carbide, boron carbide, Fe_3O_4 , germanium, silicon germanium, silicon carbide, tungsten carbide, titanium carbide, indium phosphide, gallium nitride, gallium phosphide, aluminum phosphide, aluminum arsenide, mercury cadmium telluride, tellurium, selenium, ZnS, ZnO, ZnSe, CdS, ZnTe, GaSe, CdSe, CdTe, GaAs, InP, GaSb, InAs, Te, PbS, InSb, PbTe, PbSe, and tungsten disulfide.

124. The method of claims 108 or 109, wherein said first electrode and said second electrode are formed from a material selected from the group consisting of ruthenium, osmium, cobalt, rhodium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon, and a carbon nanotube.

125. The method of claims 108 or 109, wherein at least one of the said first electrode and second electrode is functionalized to contain a chemical group capable of being further derivatized or crosslinked.

126. The method of claim 125, wherein the said chemical group is selected from the group consisting of an a sulfate, a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, a bromine, an iodine, a chlorine, a light-activatable group, and a group activatable by an electric potential.

127. The method of claim 125, wherein said functionalized biological macromolecule is attached to said first electrode by an electrically conductive linker.

128. The method of claim 127, wherein said linker is selected from the group consisting DFDNB, DST, ABH, ANB-NOS, EDC, NHS-ASA, and SIA.

129. The method of claim 127, wherein said linker is oligo(phenylenevinylene).

130. The method of claims 108 or 109, wherein the first electrode and the second electrode are integrated with a substrate.

131. The method of claims 108 or 109, wherein the first electrode and the second electrode are integrated with the insulator to form a substrate.

132. The method of claims 108 or 109, wherein the first electrode and the second electrode provide a first electrode pair, the molecular sensing apparatus further comprising a second electrode pair comprising a second first electrode and a second electrode.

133. The method of claim 132, wherein said apparatus comprises at least 3 electrode pairs.

134. The method of claim 132, wherein said apparatus comprises at least 100 electrode pairs.

135. The method of claim 132, wherein said apparatus comprises from about 10^2 to about 10^{10} electrode pairs.

136. The method of claim 132, further comprising performing steps ii and iii with said second electrode pair.

137. The method of claim 132, wherein at least one of the biological macromolecule on an electrode of the second pair is different from either of the biological macromolecules on the electrodes of the first electrode pair.

138. The method of claim 132, wherein the biological macromolecules on the electrode of the second electrode pair are different from the biological macromolecules on the electrodes of the first electrode pair.

139. A method of detecting an analyte, said method comprising:

i) providing a molecular sensing apparatus comprising a first electrode and a second electrode separated by a spacer where a biological macromolecule forms a connection between said first electrode and said second electrode;

ii) detecting the connection between said first and said second electrode;

iii) contacting the attached macromolecule with said analyte whereby said analyte binds to said macromolecule forming a macromolecule/analyte complex; and

iv) detecting the difference in the connection between said first electrode and said second electrode.

140. The method of claim 139, wherein said first electrode, said spacer, and said second electrode do not form a sandwich configuration.

141. The method of claims 139 or 140, wherein said contacting comprises placing a charge on said first electrode whereby said charge attracts said analyte to said biological macromolecule.

142. The method of claims 139 or 140, wherein said providing comprises:

contacting said first electrode with a first solution comprising said biological macromolecule; and

placing a charge on said first electrode whereby said charge attracts said biological macromolecule to said electrode and said biological macromolecule attaches to said electrode.

placing a charge on said second electrode to attract a portion of said bound macromolecule to said second electrode where said macromolecule is bound to said second electrode such that said macromolecule forms a connection between said first electrode and said second electrode.

5 143. The method of claims 139 or 140, wherein said placing charge comprises placing a charge on said first electrode opposite to the charge on said second electrode.

10 144. The method of claims 139 or 140, wherein said detecting comprises detecting an electromagnetic property selected from the group consisting of direct electric current, alternating electric current, permittivity, resistivity, electron transfer, electron tunneling, electron hopping, electron transport, electron conductance, voltage, electrical impedance, signal loss, dissipation factor, resistance, capacitance, inductance, magnetic field, electrical potential, charge and magnetic potential.

15 145. The method of claims 139 or 140, wherein said biological macromolecule is selected from the group consisting of a nucleic acid, a protein, a polysaccharide, a lectin or a lipid.

146. The method of claims 139 or 140, wherein said biological macromolecule is a nucleic acid.

20 147. The method of claims 139 or 140, wherein said analyte is a protein or protein complex.

25 148. The method of claims 139 or 140, wherein said biological macromolecule is functionalized with a chemical group consisting of a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, a phosphonate, an alkene, an alkyne, a hydroxyl group, bromine, iodine, chlorine, a chemical group that can be activated by light , and a chemical group that can be activated by the application of an electrical potential.

149. The method of claims 139 or 140, wherein said spacer comprises an insulator selected from the group consisting of elements, compounds or substances that have resistivities greater than $10^{-3}\Omega\cdot\text{m}$.

150. The method of claim 149, wherein said spacer comprises an insulator is selected from the group containing SiO₂, TiO₂, ZrO₂, porcelain, polystyrene, polystyrene, organic compounds produced by polymerization having a resistivity greater than 10⁻³ Ω-m and insulating oxides or sulfides of the transition metals in the periodic table of the elements.

151. The method of claims 139 or 140, wherein said first electrode and said second electrode are separated by a distance less than about 500 Angstroms.

152. The method of claims 139 or 140, wherein said first electrode and said second electrode are separated by a distance in the range of 1 to 10⁹ Angstroms.

153. The method of claims 139 or 140, wherein at least one said first electrode and said second electrode are formed of a material selected from the group consisting of elements, compounds or substances that have resistivities of less than 10⁻² Ω-m.

154. The method of claim 153, wherein the said first electrode and said second electrode are formed from a material selected from the group consisting of, ruthenium, osmium, cobalt, rhodium, rubidium, lithium, sodium, potassium, vanadium, cesium, beryllium, magnesium, calcium, chromium, molybdenum, silicon, germanium, aluminum, iridium, nickel, palladium, platinum, iron, copper, titanium, tungsten, silver, gold, zinc, cadmium, indium tin oxide, carbon or carbon nanotubes or alloys or compounds of these materials.

155. The method of claims 139 or 140, wherein at least one of the first electrode and the second electrode comprises a semiconductor material.

156. The method of claims 139 or 140, wherein said semi-conductor material has a resistivity ranging from about 10⁻² Ω-m to about 10⁹ Ω-m.

157. The method of claim 156, wherein the semi-conducting material is selected from the group consisting of silicon, dense silicon carbide, boron carbide, Fe₃O₄, germanium, silicon germanium, silicon carbide, tungsten carbide, titanium carbide, indium phosphide, gallium nitride, gallium phosphide, aluminum phosphide, aluminum arsenide,

mercury cadmium telluride, tellurium, selenium, tungsten disulfide, ZnS, ZnO, ZnSe, CdS, ZnTe, GaSe, CdSe, CdTe, GaAs, InP, GaSb, InAs, PbS, InSb, PbTe, and PbSe.

158. The method of claims 139 or 140, wherein at least one of the said first electrode and second electrode is functionalized to contain a chemical group capable of being further derivatized or crosslinked.

159. The method of claim 158, wherein the said chemical group is selected from the group consisting of a sulfhydryl, an amine, an aldehyde, a carboxylic acid, a phosphate, an alkene, an alkyne, a hydroxyl group, bromine, iodine, chlorine, a chemical group that can be activated by light of wavelength ranging from 190 nm to 700 nm, such as an aryl azide, a fluorinated aryl azide, a benzophenone, (R,S) -1-(3,4- (methylene-dioxy)-6-nitrophenyl) ethyl chloroformate – (MeNPOC), N-((2-pyridyl, ethyl)-4-azido) salicylamide or a chemical group that can be activated by the application of an electrical potential, such as S-benzyloxycarbonyl derivatives, S-benzyl thioethers, S-phenyl thioethers, S-4-picolyl thioethers, S-2,2,2-trichloroethoxycarbonyl derivatives, S-triphenylmethyl thioethers.

160. The method of claims 139 or 140, wherein said biological macromolecule is attached to said first electrode by an electrically conductive linker.

161. The method of claims 139, or 140 wherein said linker is selected from the group consisting of chemical crosslinkers capable of linking functional groups, such as DFDNB, DST, ABH, ANB-NOS, EDC, NHS, NHS-ASA, SIA.

162. The method of claims 139 or 140, wherein said linker is an oligo(phenylenevinylene).

163. The method of claims 139 or 140, wherein said apparatus further comprises substrate to support the first electrode and the second electrode, wherein the first electrode and the second electrode are integrated with the substrate.

164. The method of claims 139 or 140 wherein said apparatus further comprises a substrate in which the first electrode and the second electrode are integrated with the insulator to form the substrate.

165. The method of claims 139 or 140, wherein the first electrode and the second electrode provide a first electrode pair, the molecular sensing apparatus further comprising a second electrode pair comprising a second first electrode and a second second electrode.

5 166. The method of claim 165, wherein said apparatus comprises at least 3 electrode pairs.

167. The method of claim 165, wherein said apparatus comprises at least 100 electrode pairs.

10 168. The method of claim 165, wherein said apparatus comprises in the range of 10^2 to 10^{10} electrode pairs.

169. The method of claim 165, further comprising performing steps ii, iii and iv with the second electrode pair.

15 170. The method of claim 165, wherein the biological macromolecule attached to said first electrode pair is the same as the biological macromolecule attached to said second electrode pair.

171. The method of claim 165, wherein the biological macromolecule attached to said first electrode pair is different from the biological macromolecule attached to said second electrode pair.

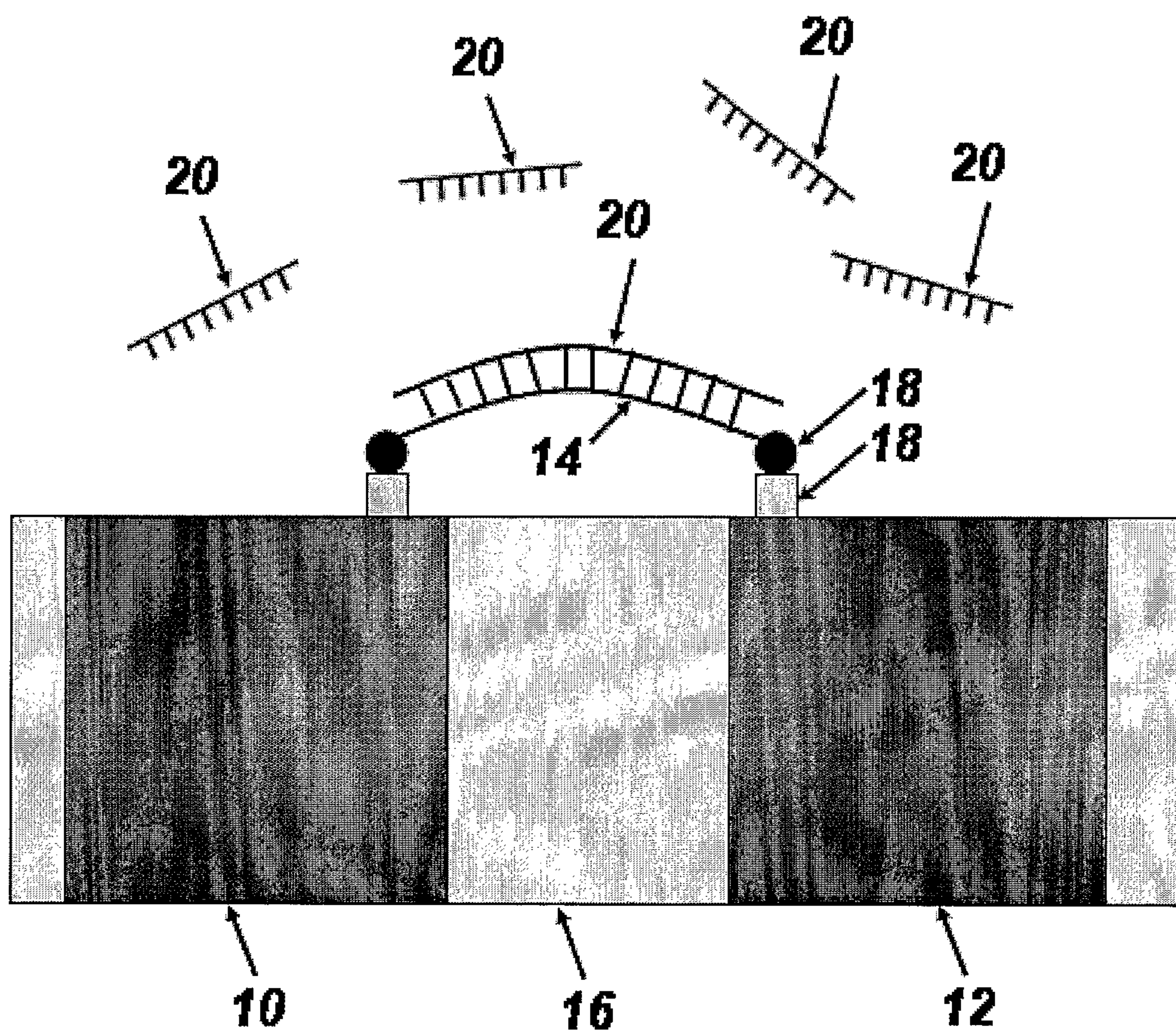
20 172. The method of claim 165, wherein the analyte attached to said first electrode pair is the same as the analyte attached to said second electrode pair.

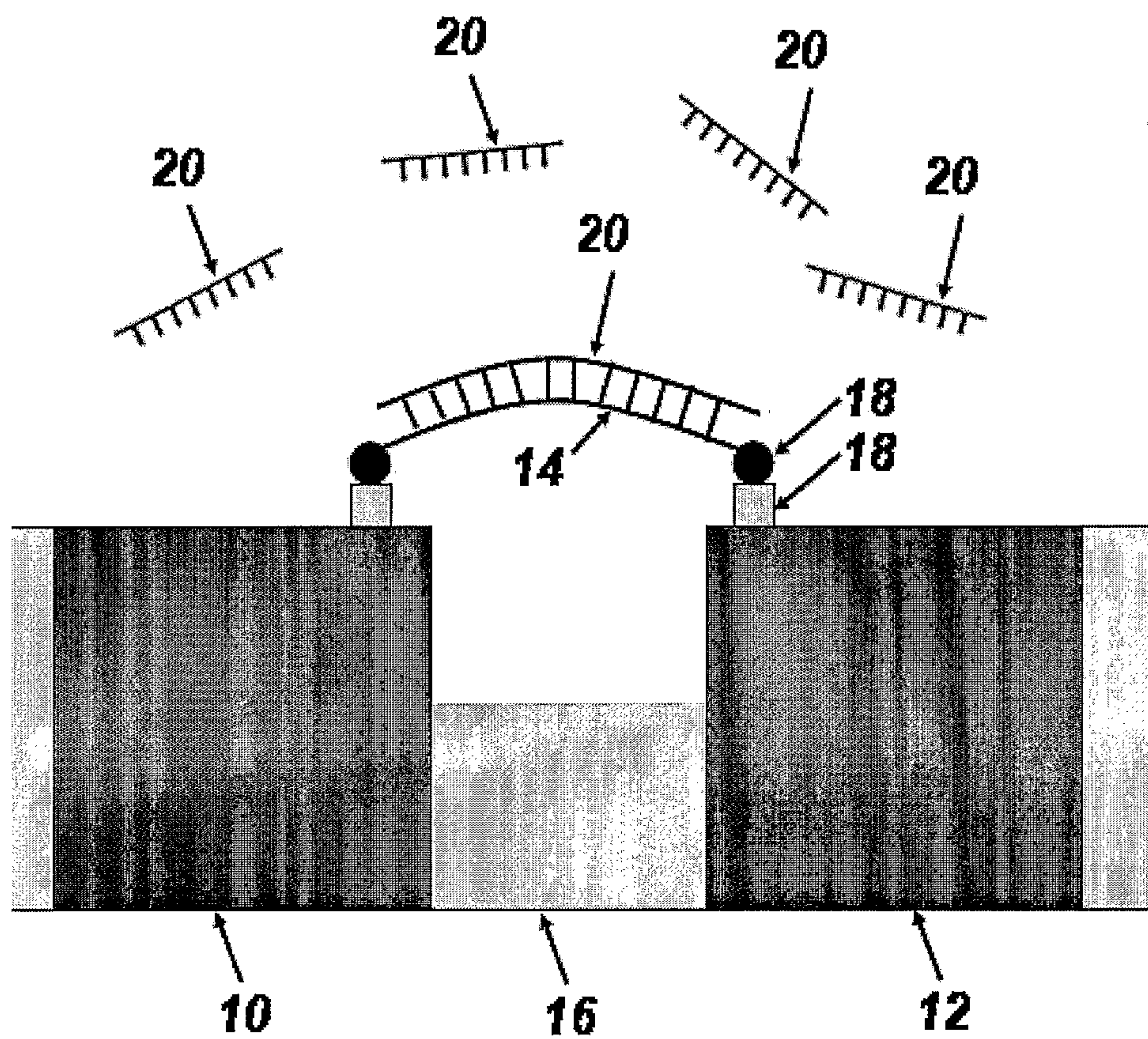
173. The method of claim 165, wherein the analyte attached to said first electrode pair is different from the analyte attached to said second electrode pair.

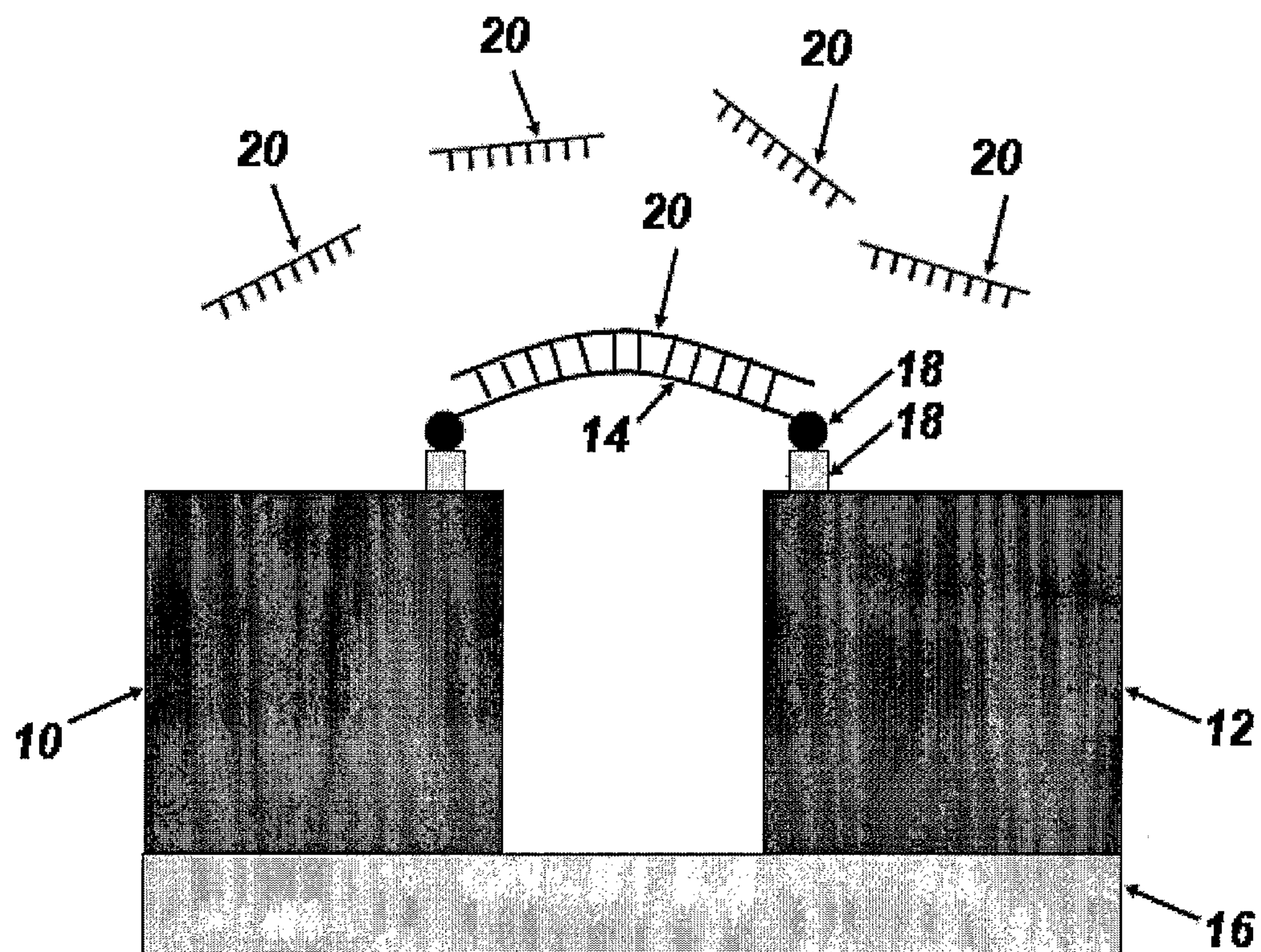
25 174. An array of biological macromolecules, the array comprising:
a solid support comprising a plurality of non-planar surfaces at a density of greater than 500 surfaces/cm²; and
at least one type of biological macromolecule attached to each surface.

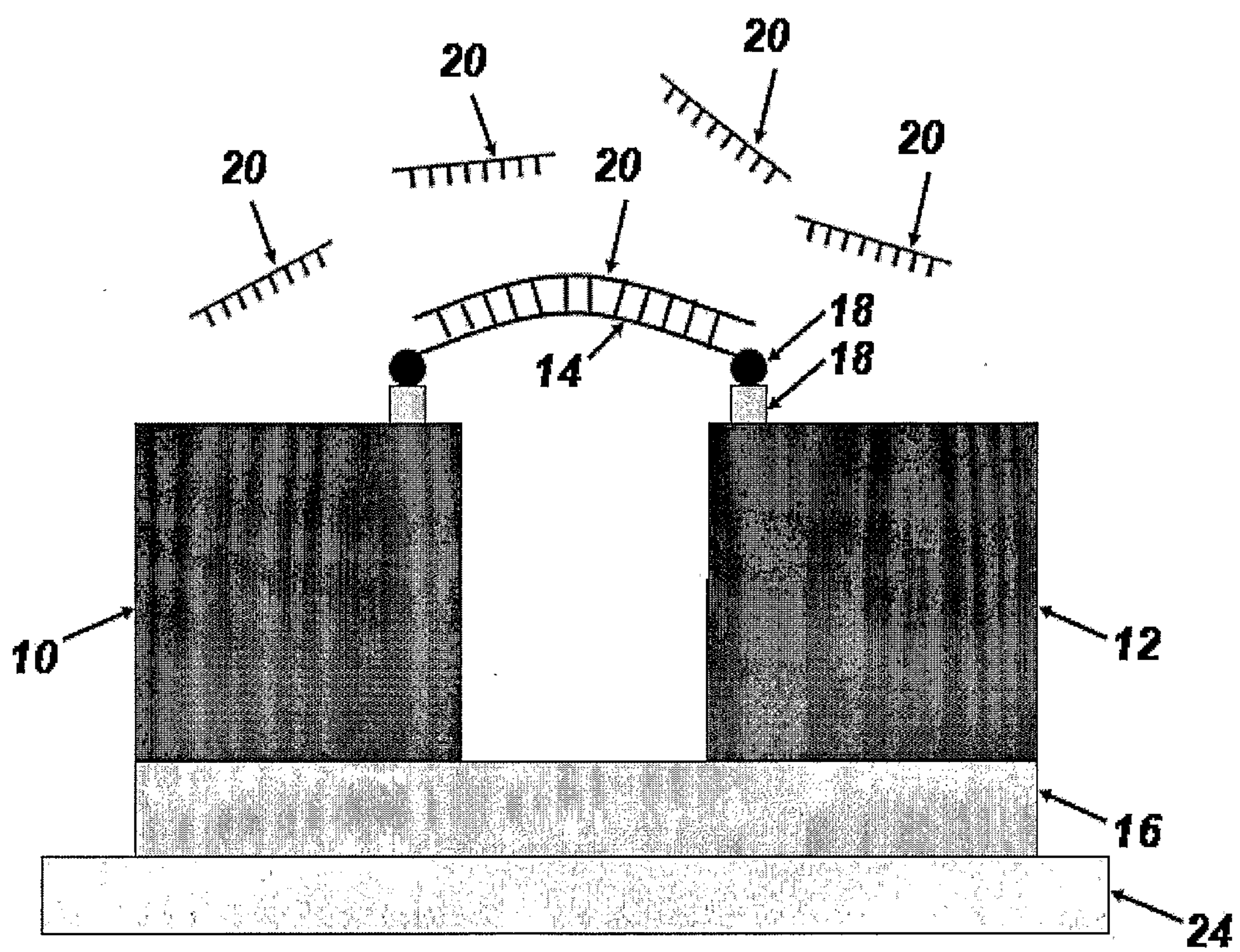
175. The array of claim 174, wherein the biological macromolecule spans two of said non-planar surfaces.

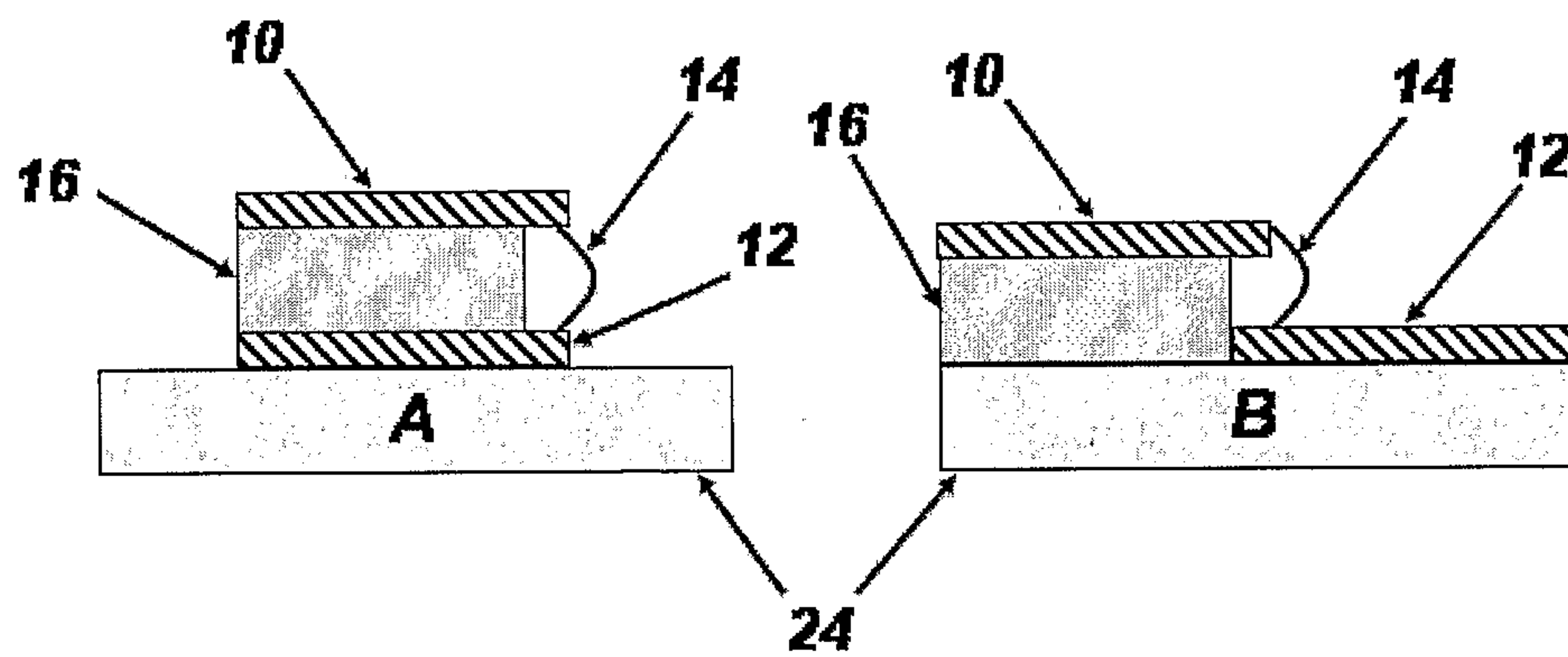
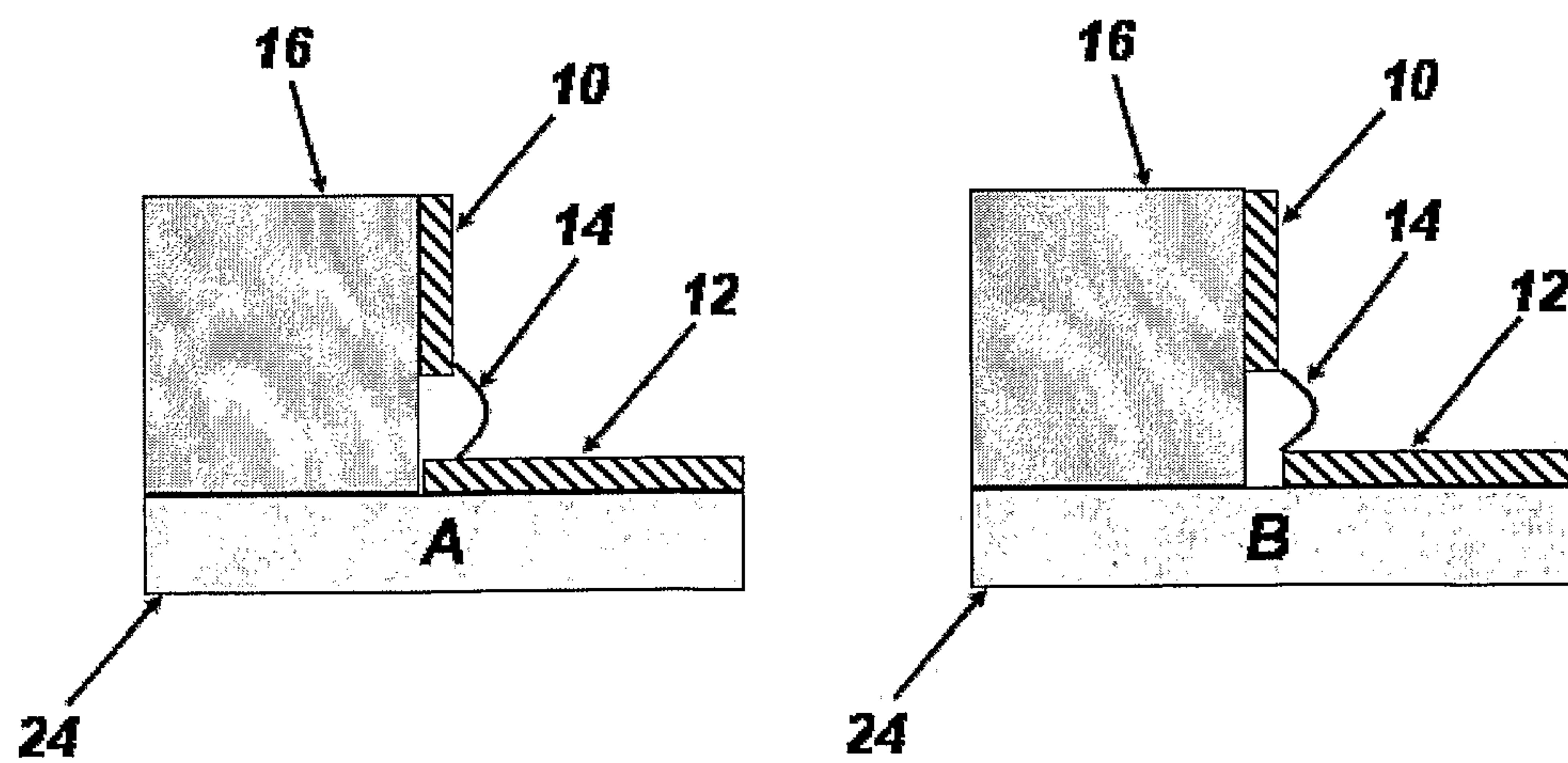
176. The array of claim 174, wherein said non-planar surfaces comprise a stepped configuration.

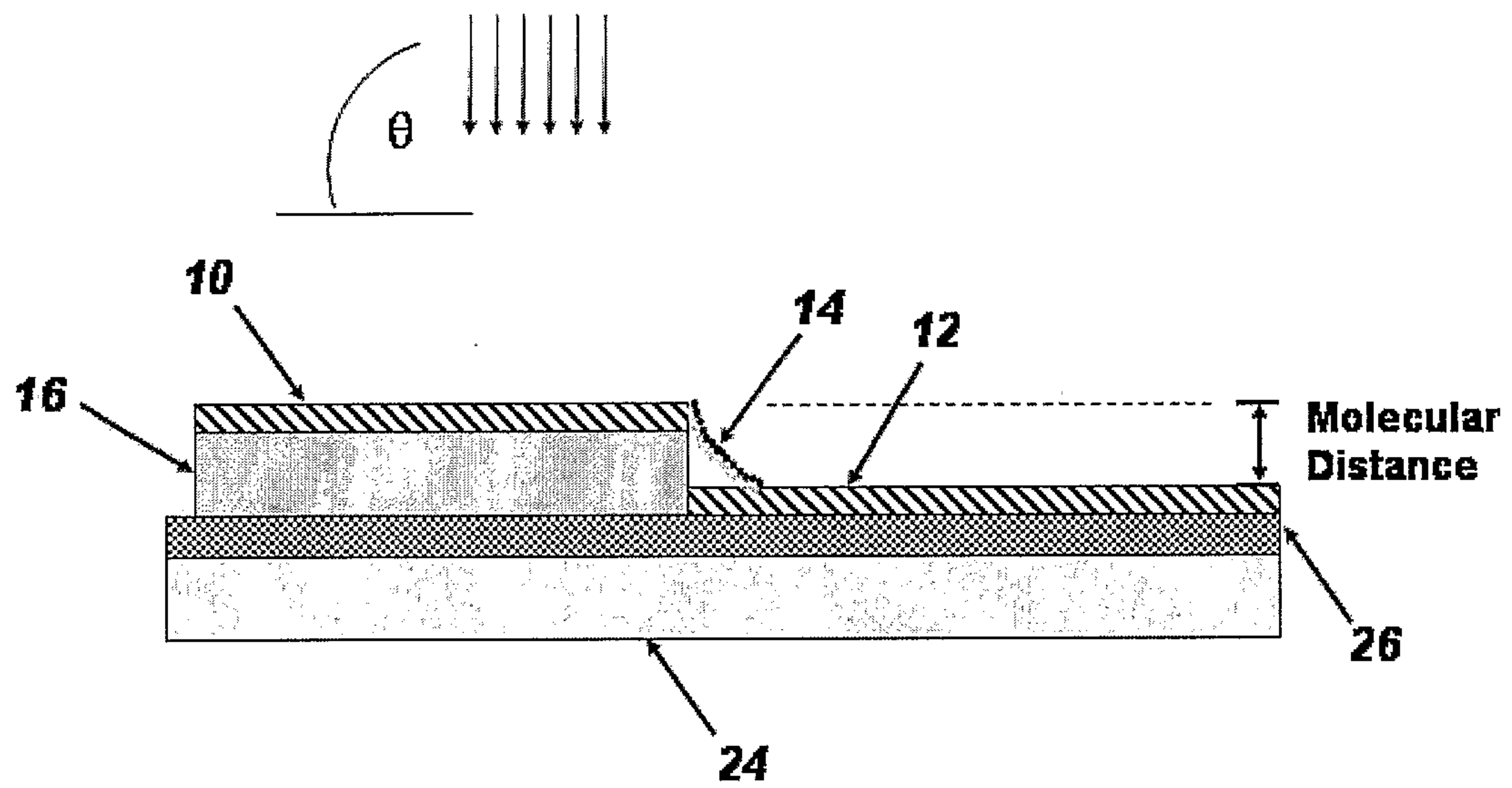
1/30**Fig. 1A**

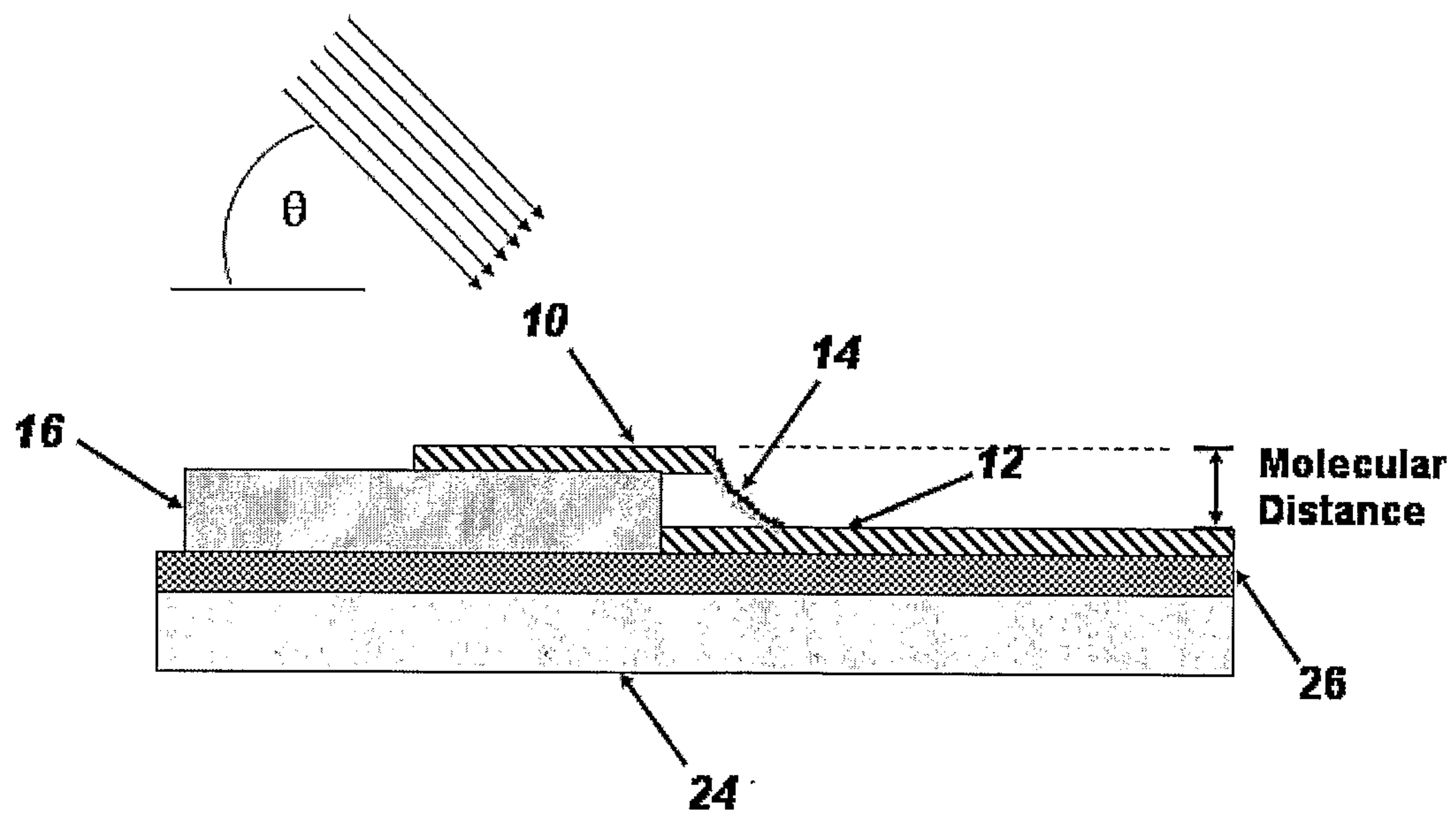
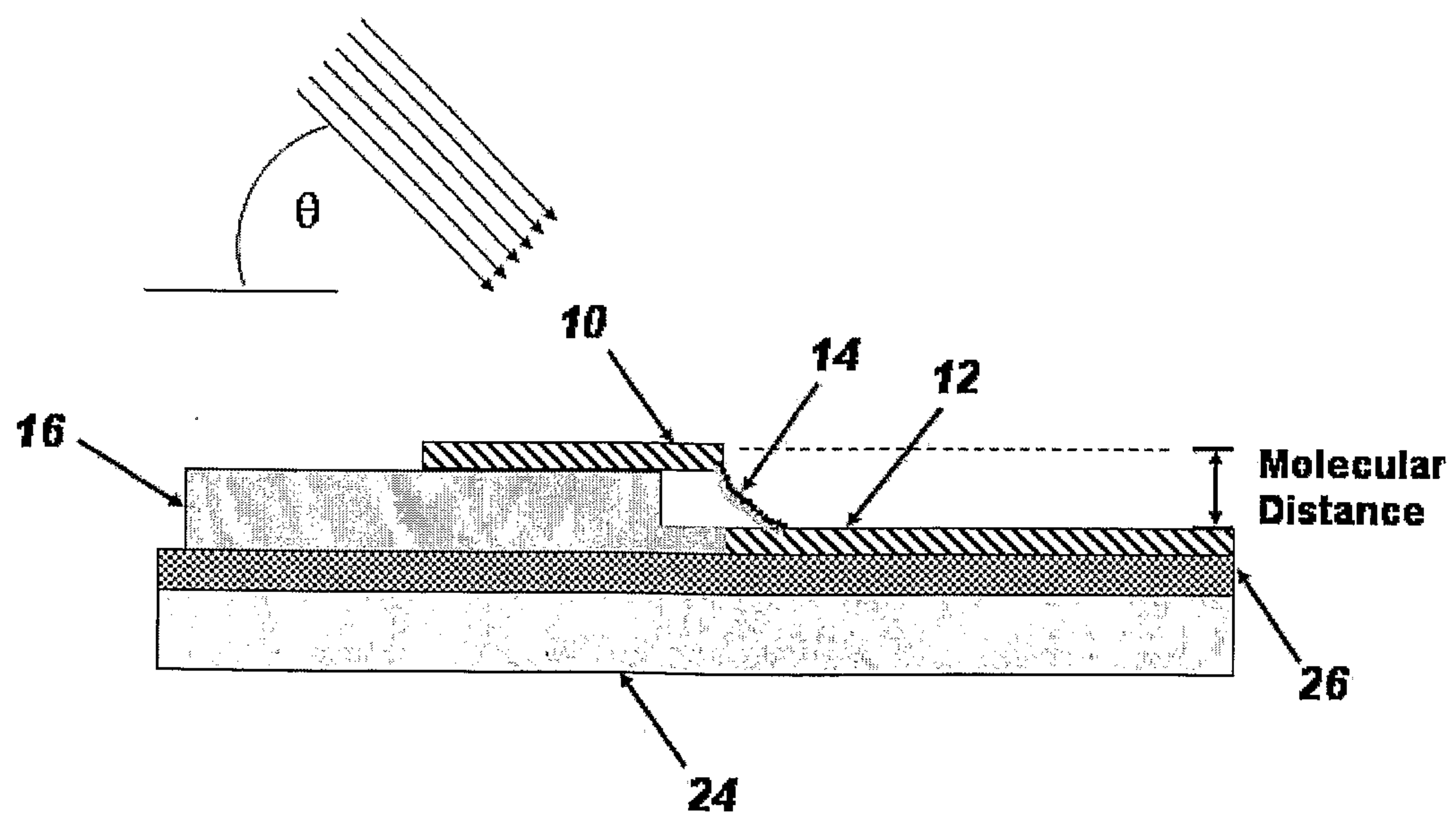
2/30**Fig. 1B**

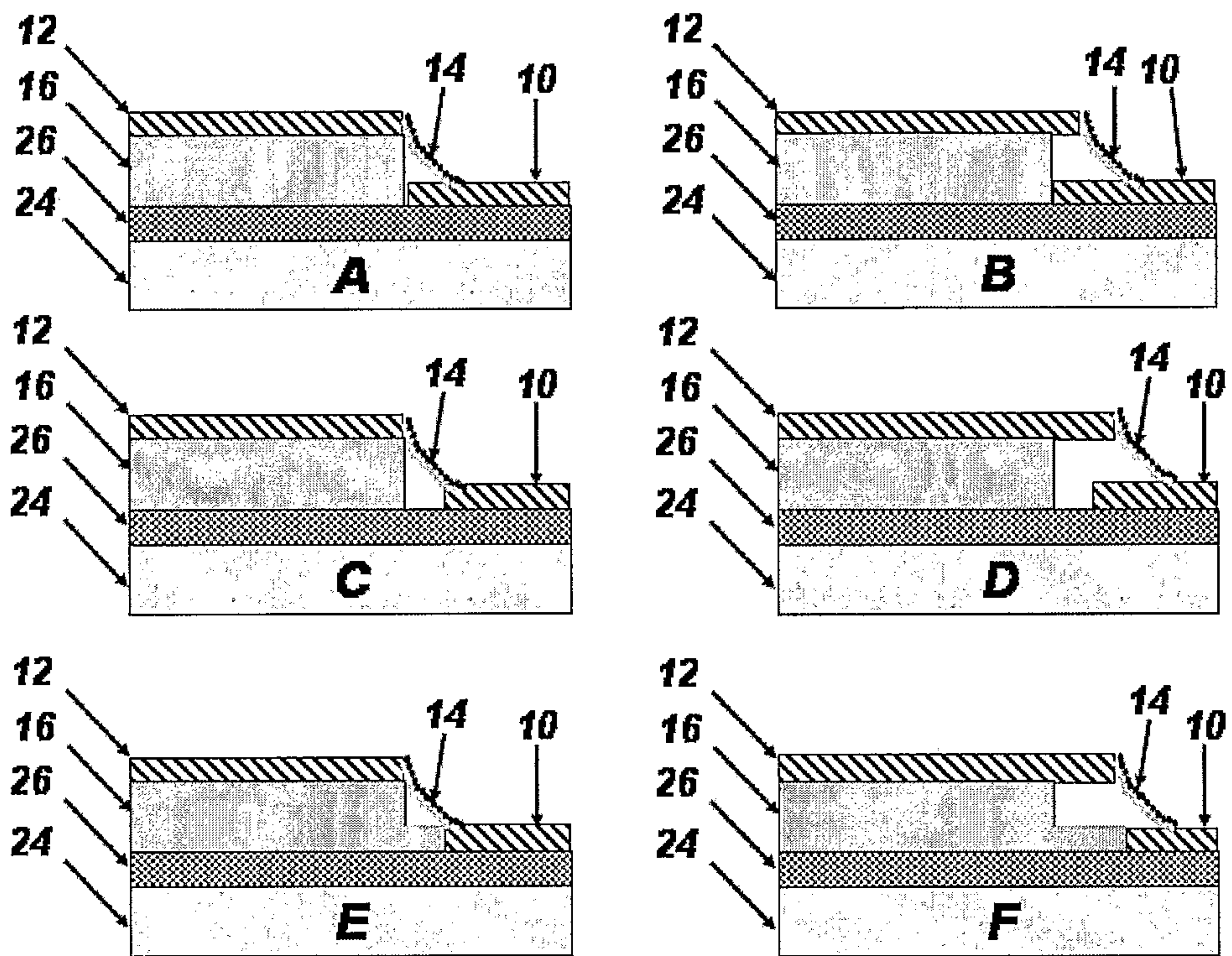
3/30**Fig. 1C**

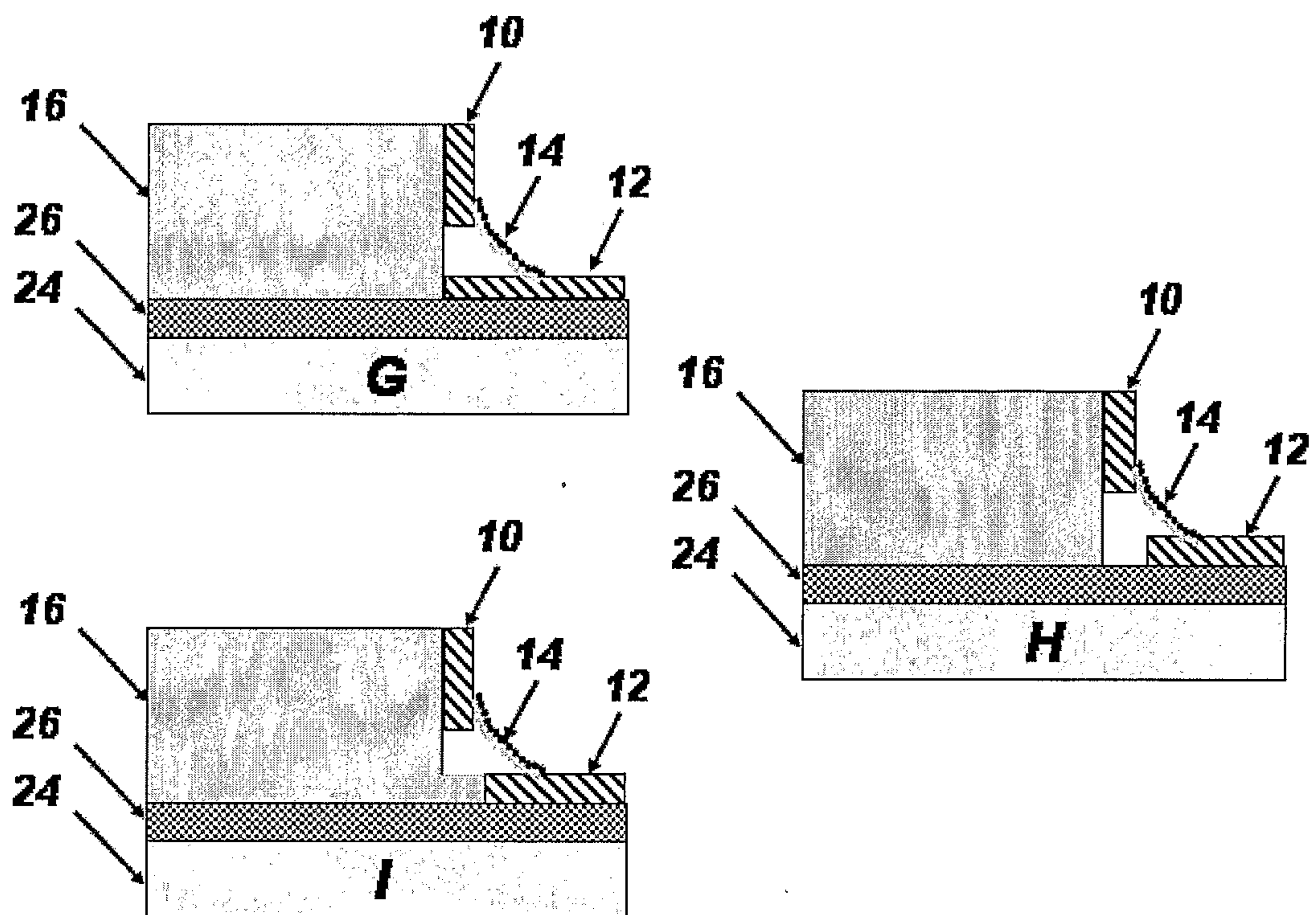
4/30**Fig. 1D**

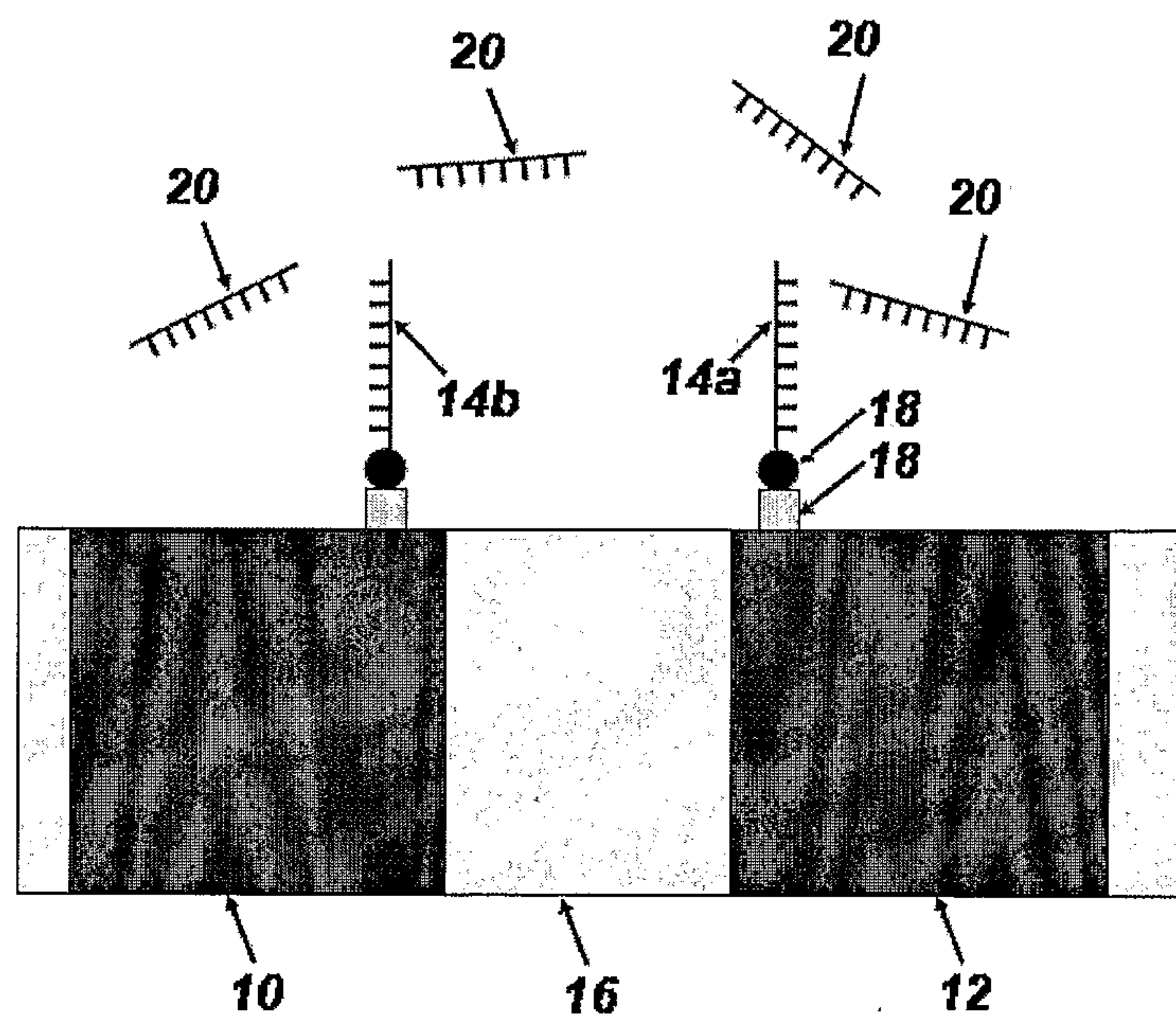
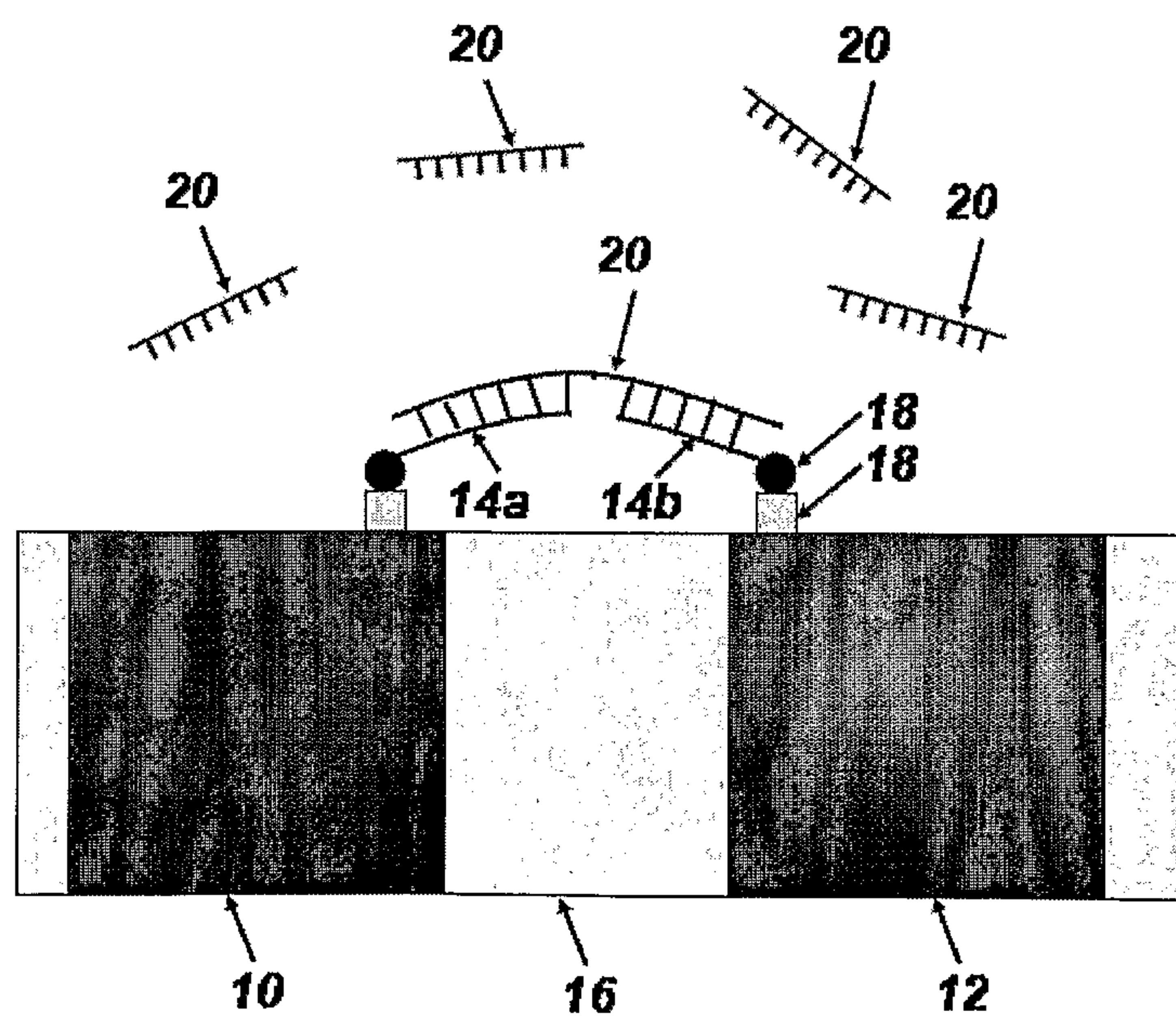
5/30**Fig. 2****Fig. 3**

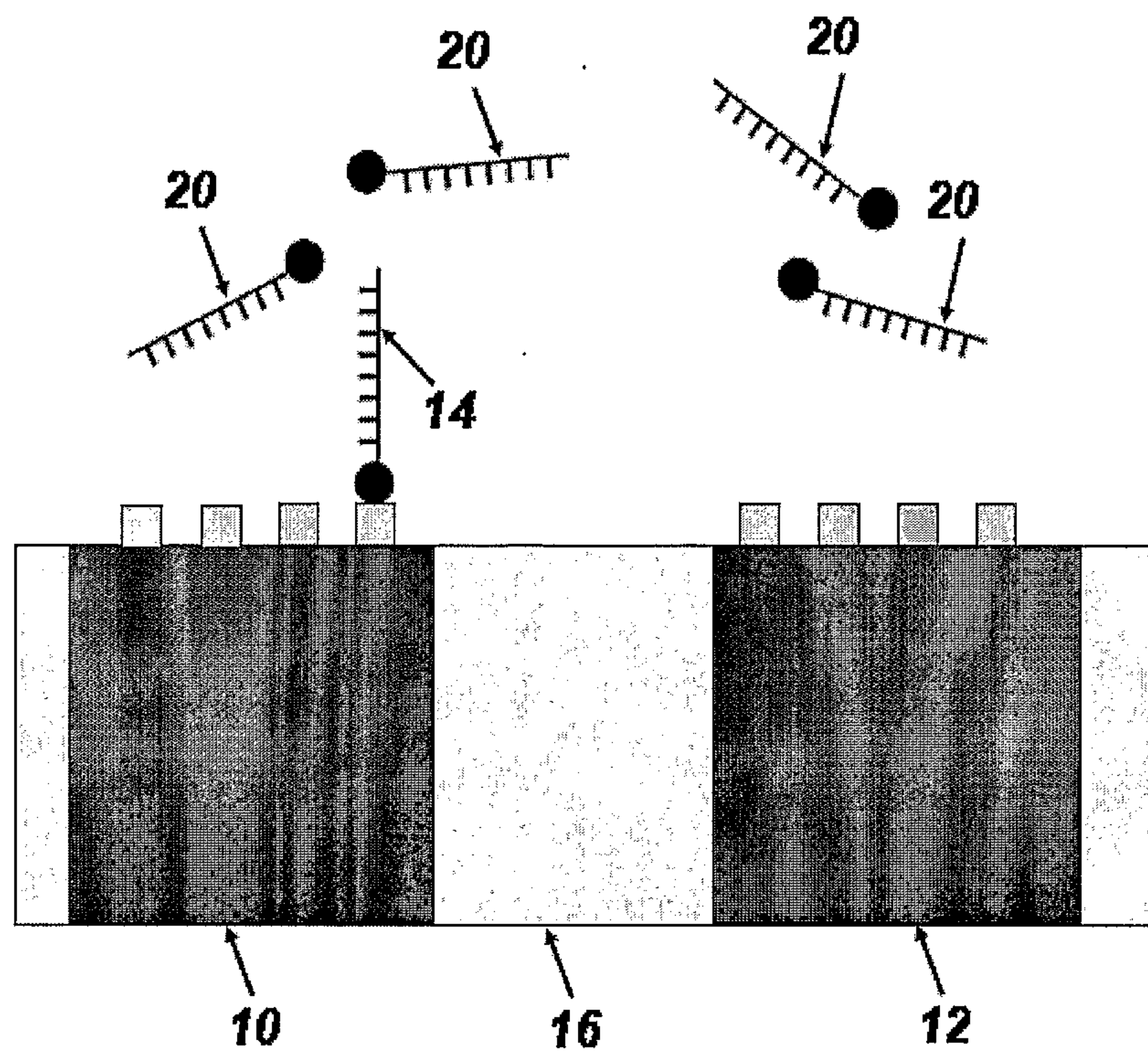
6/30**Fig. 4**

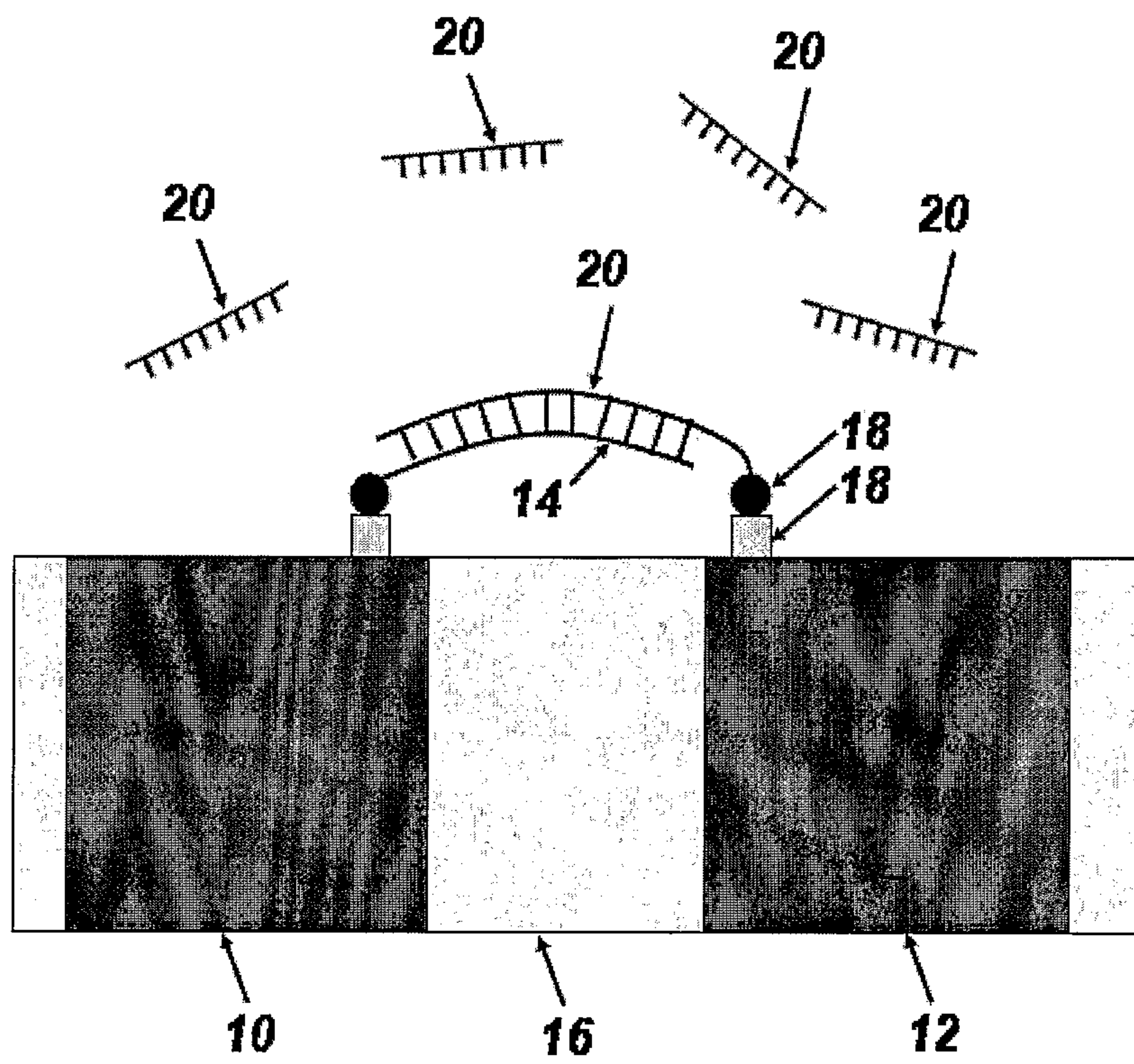
7/30**Fig. 5A****Fig. 5B**

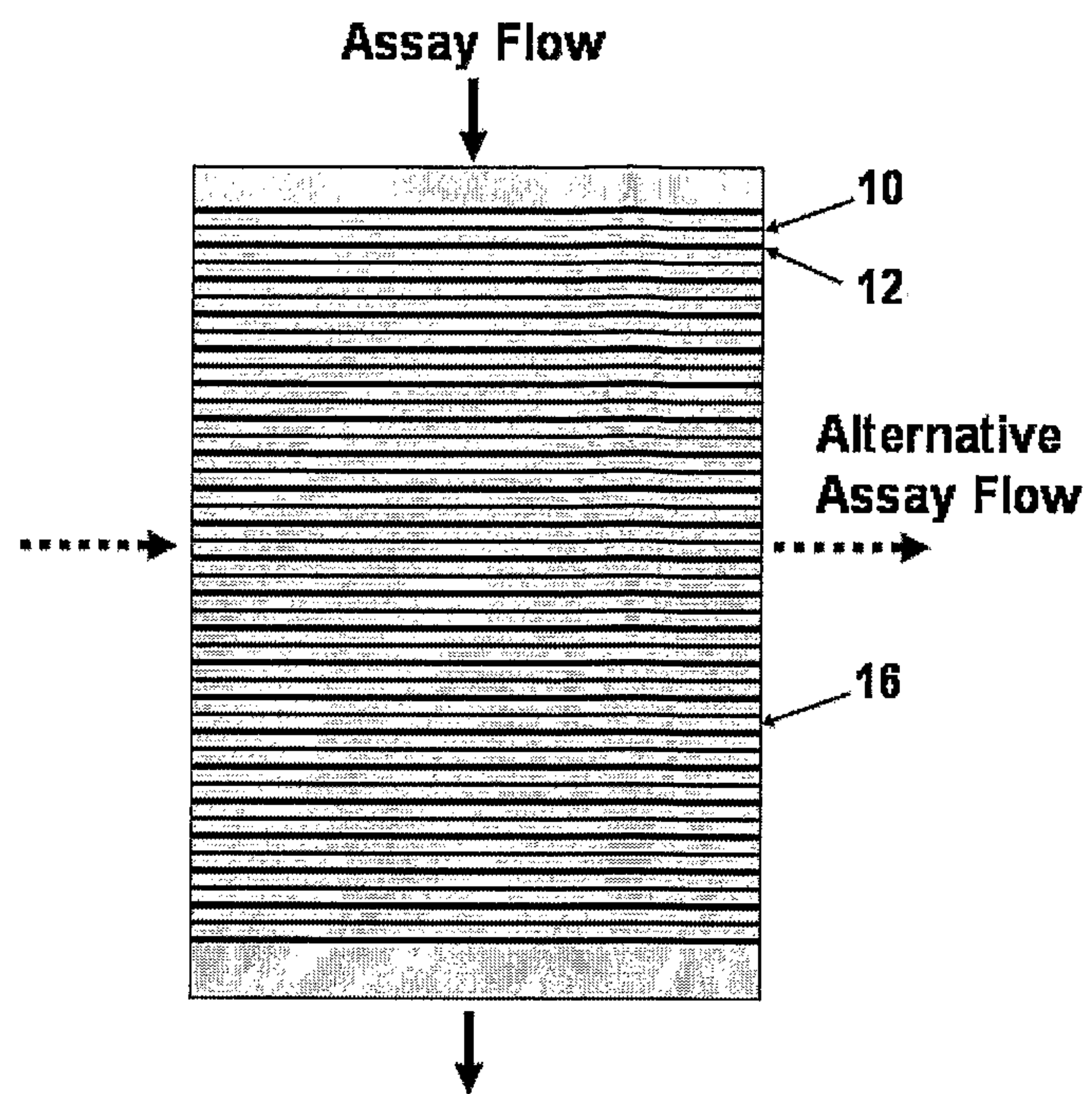
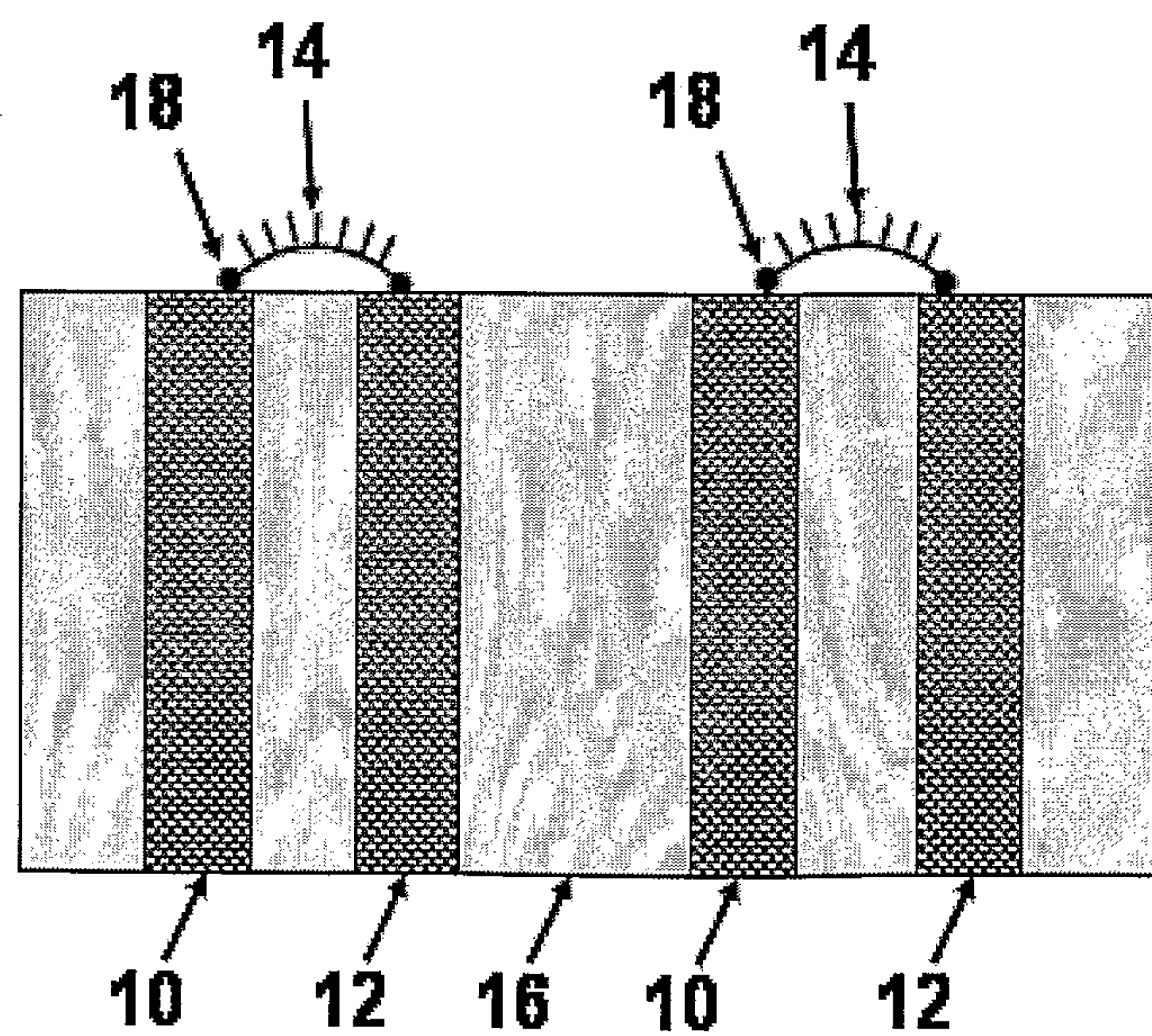
8/30**Fig. 6**

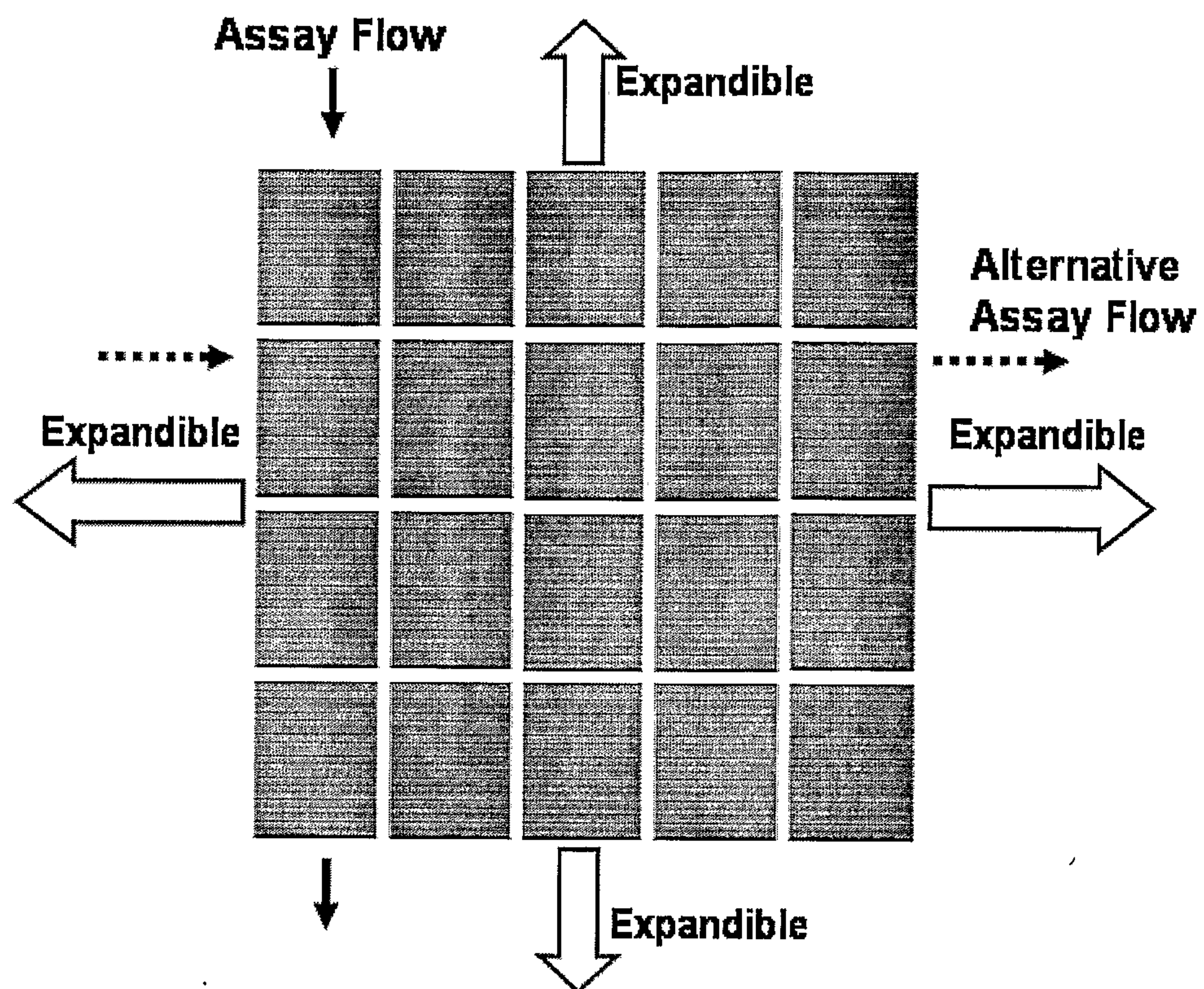
9/30**Fig. 7**

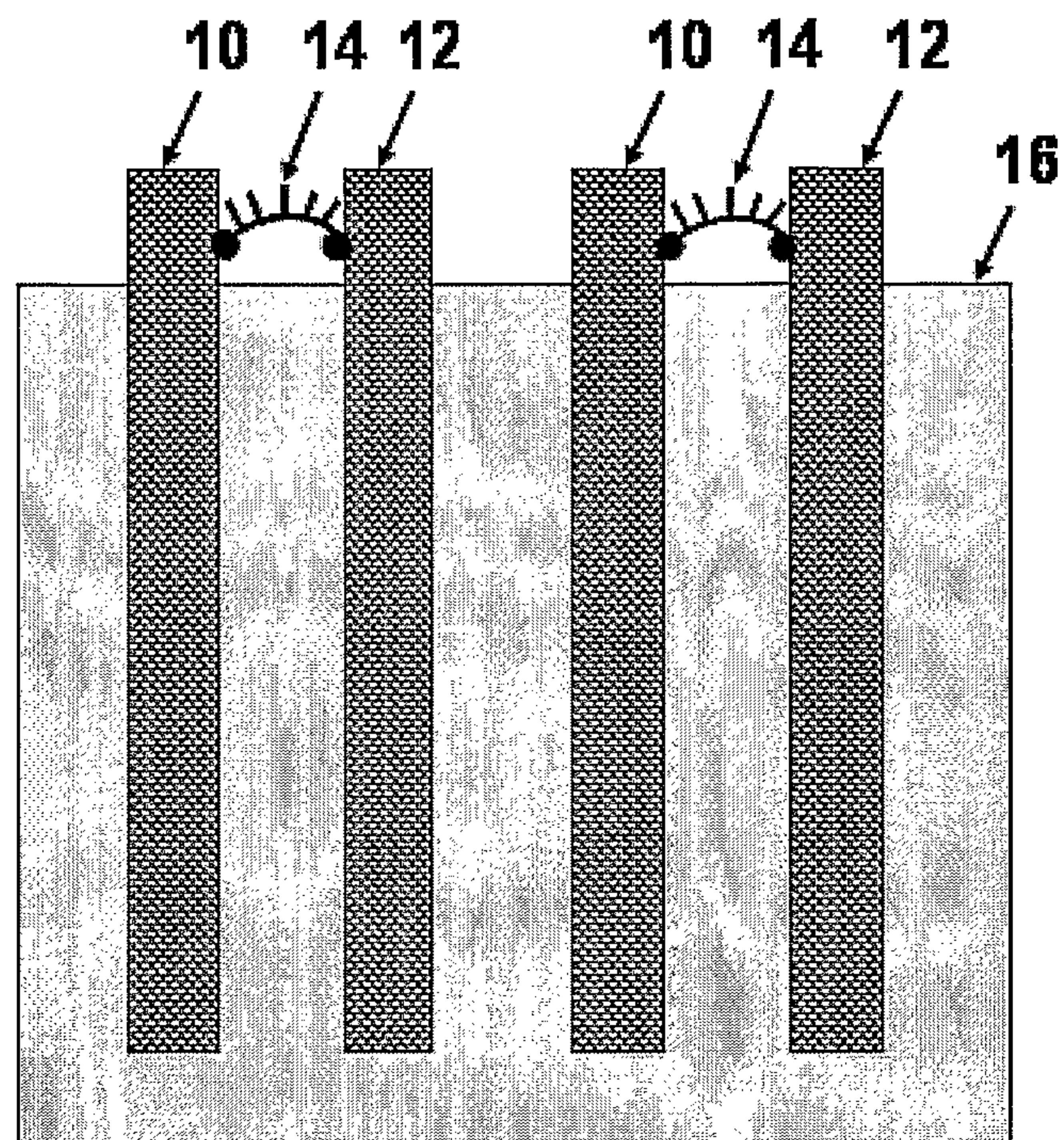
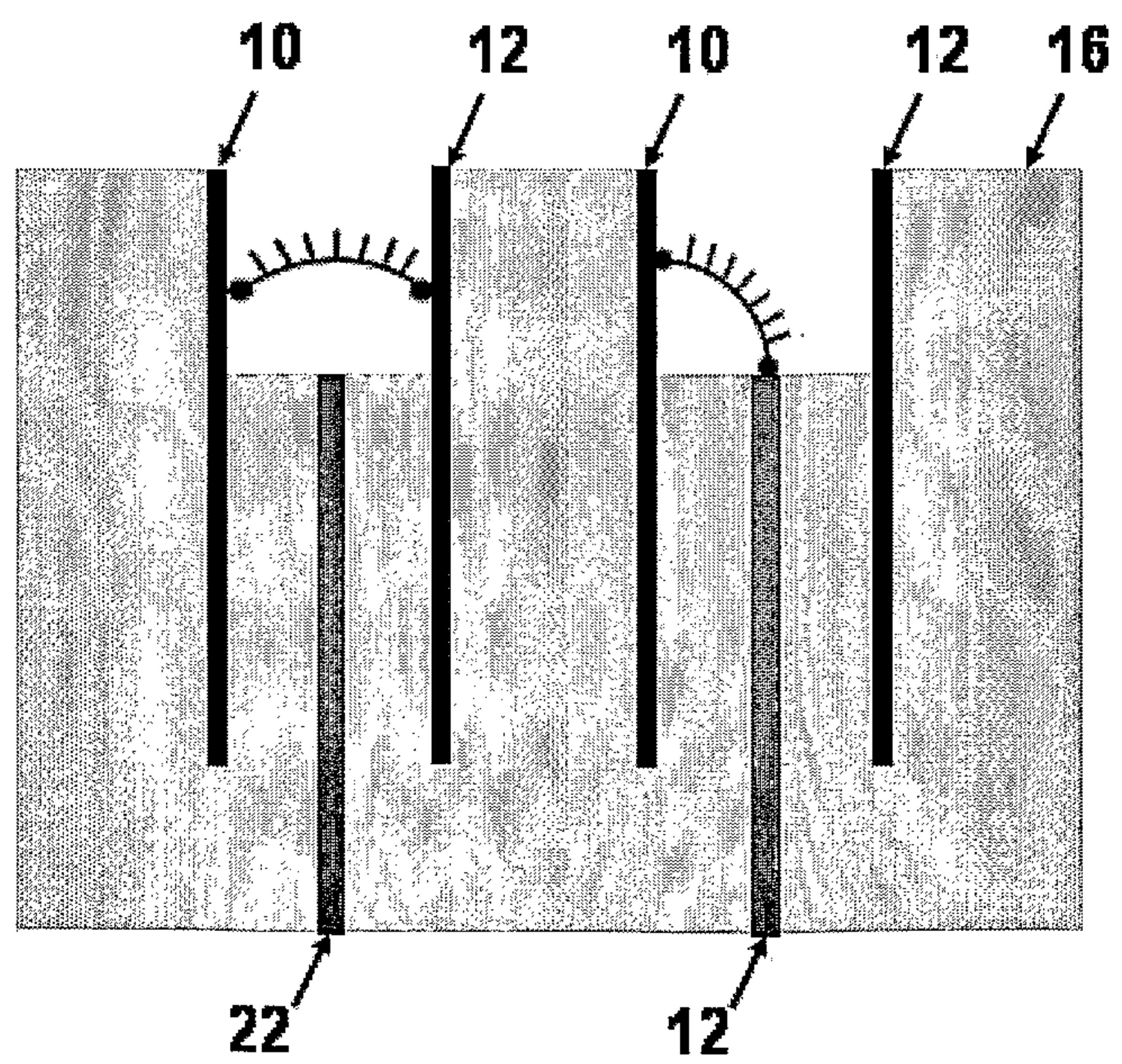
10/30**Fig. 8A****Fig. 8B**

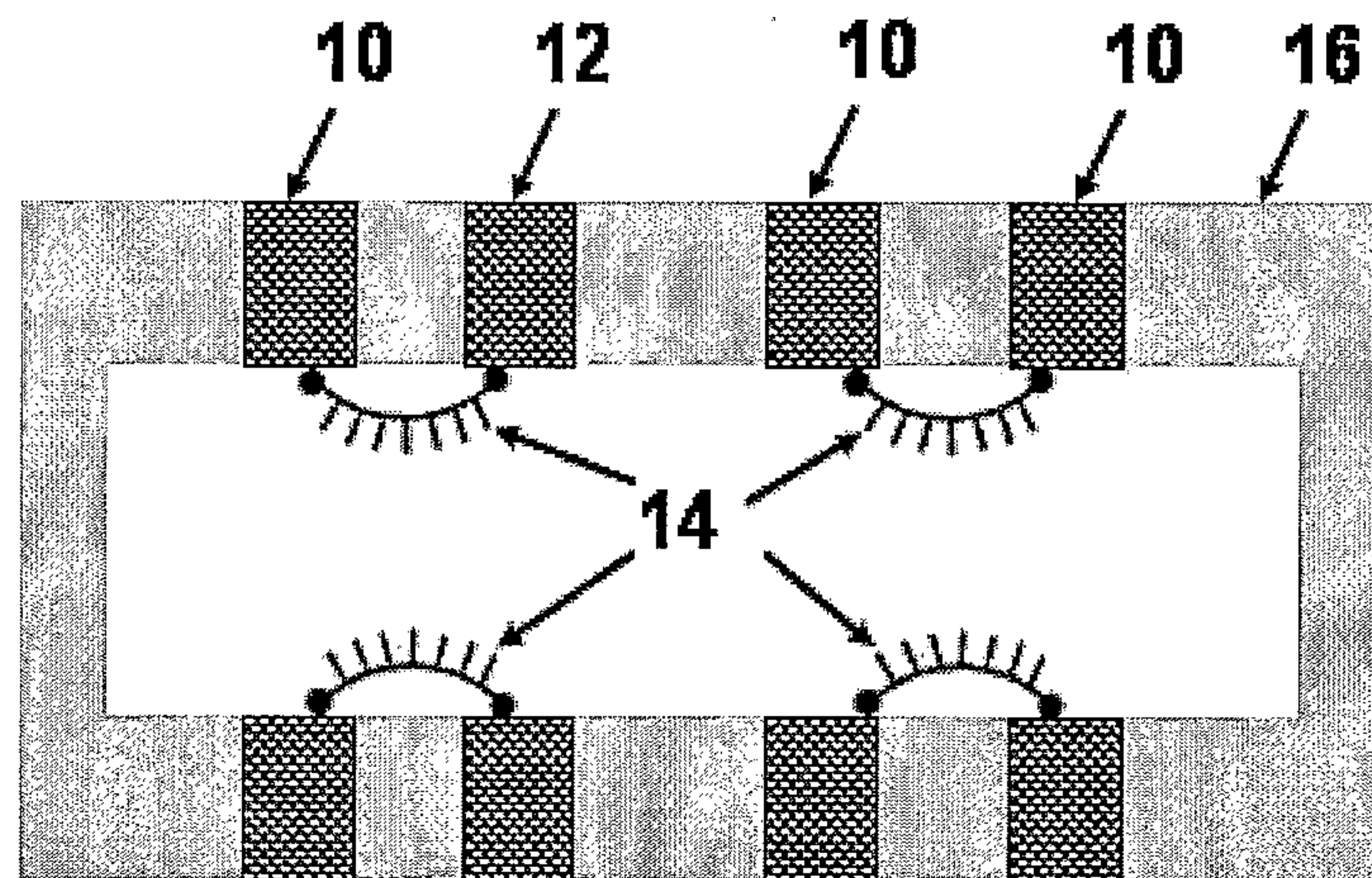
11/30**Fig. 9A**

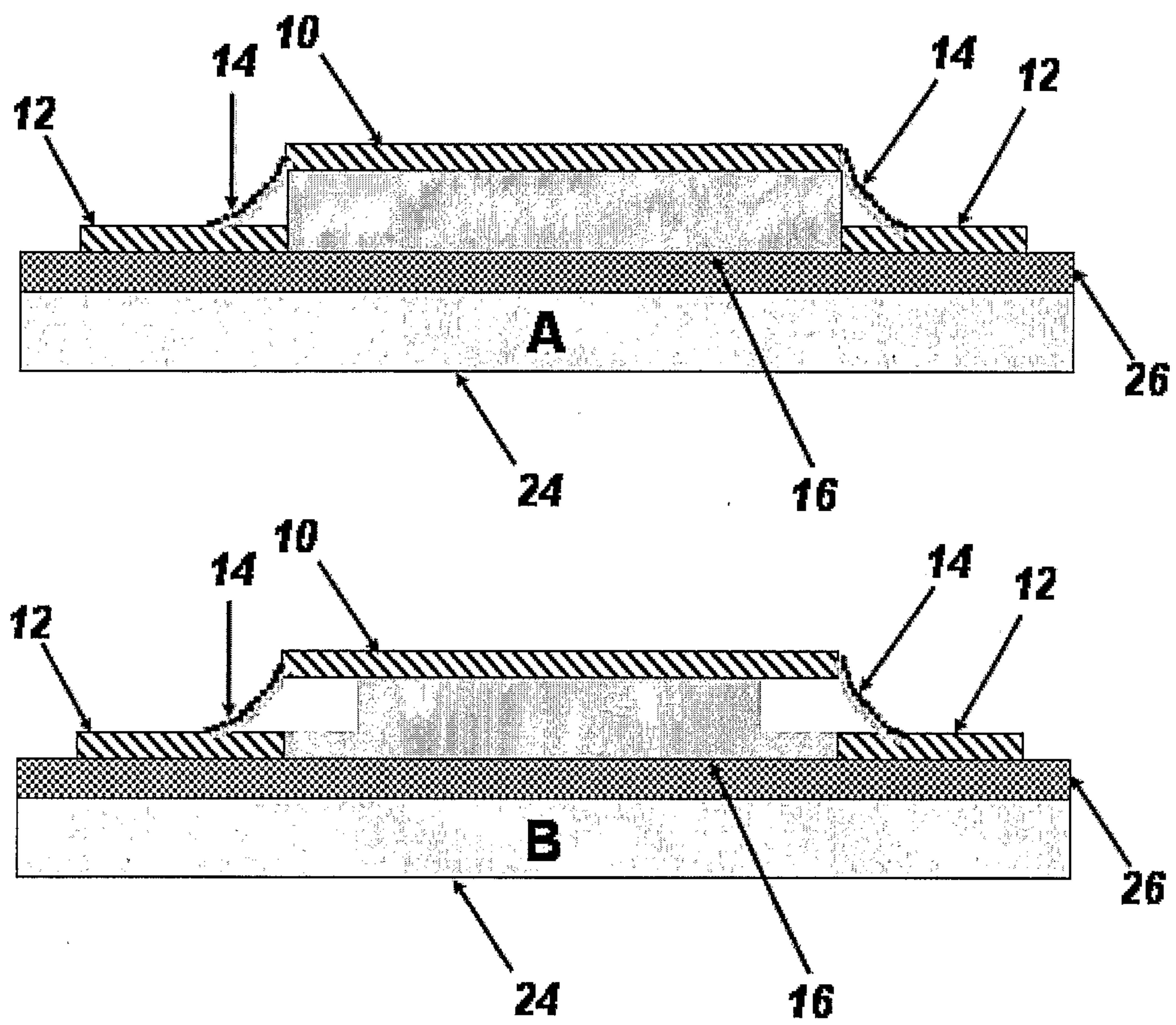
12/30**Fig. 9B**

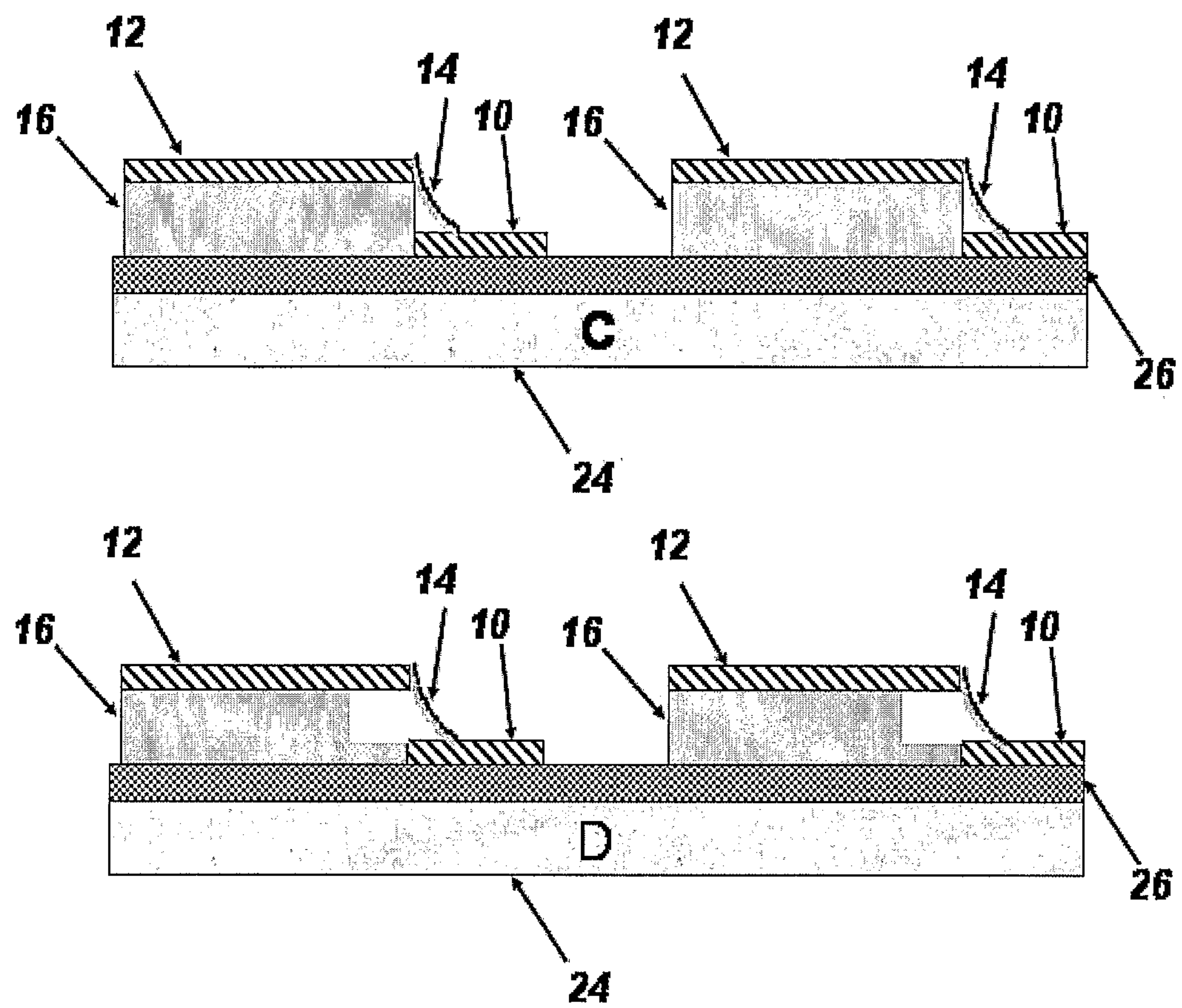
13/30**Fig. 10A****Fig. 10B**

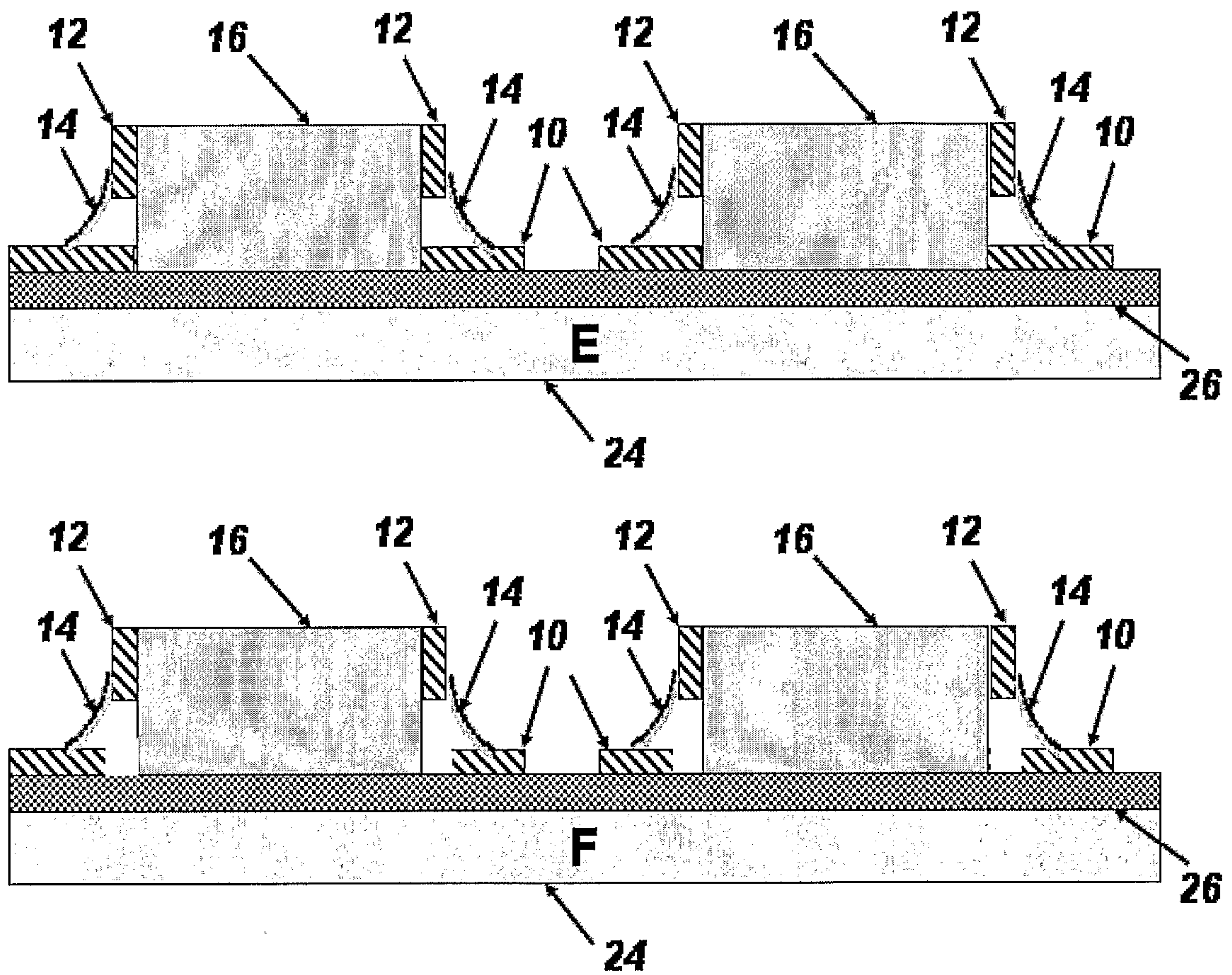
14/30**Fig. 11**

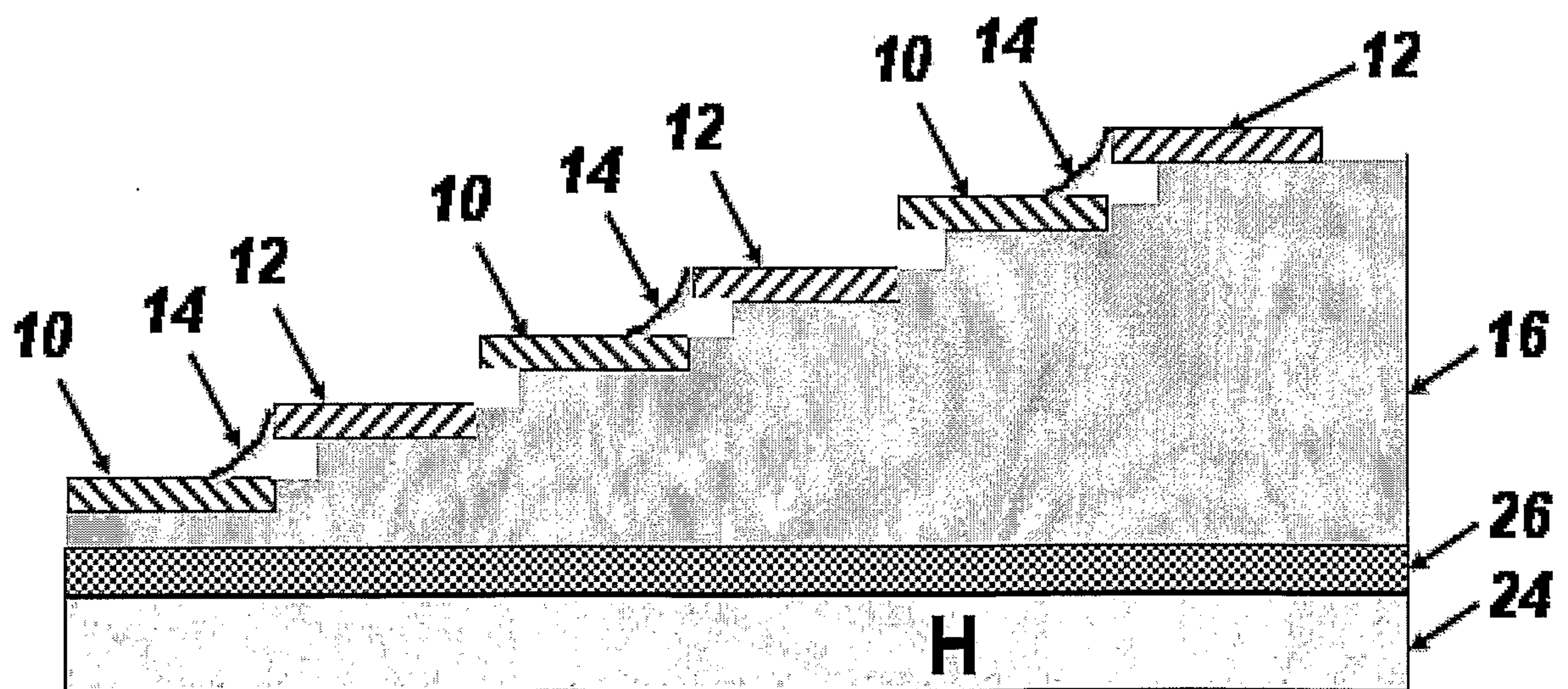
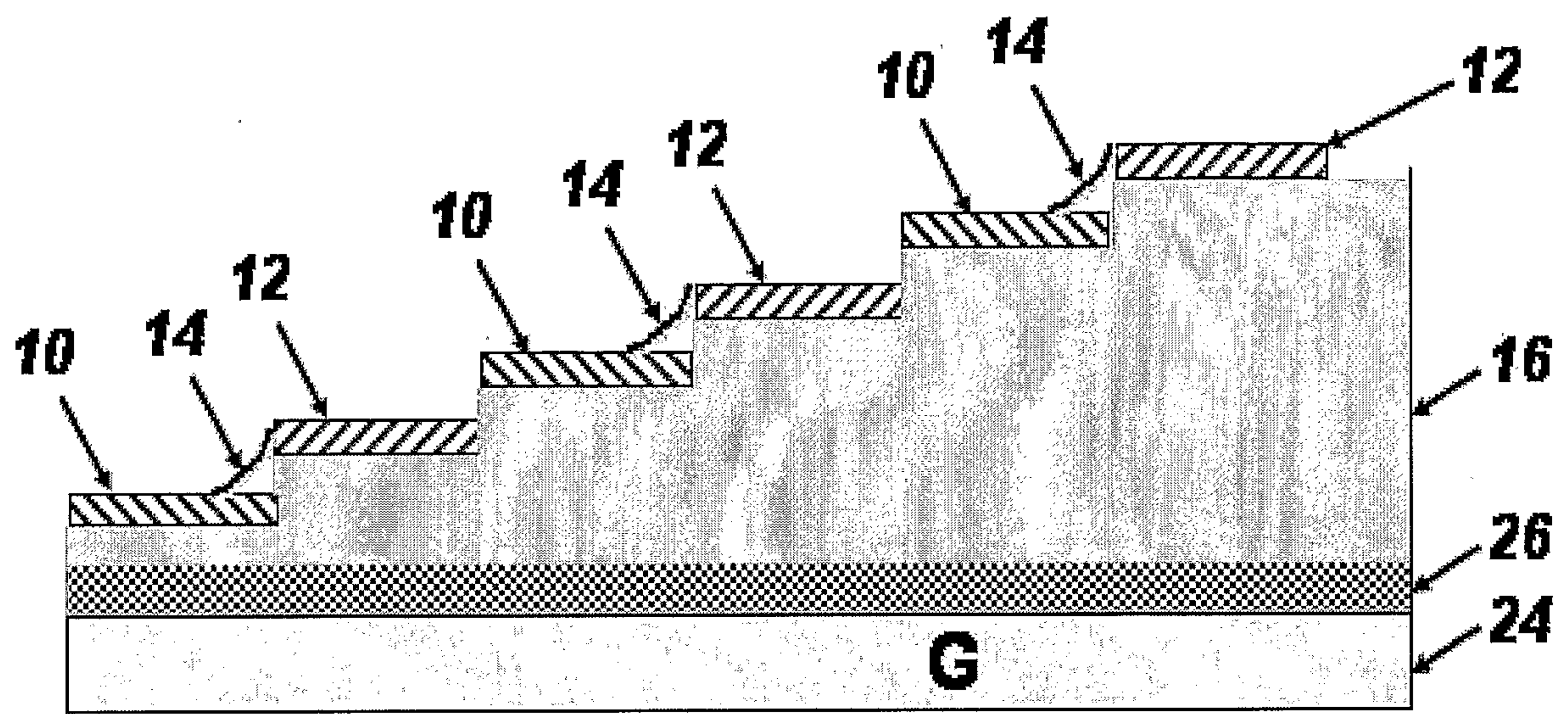
15/30**Fig. 12A****Fig. 12B**

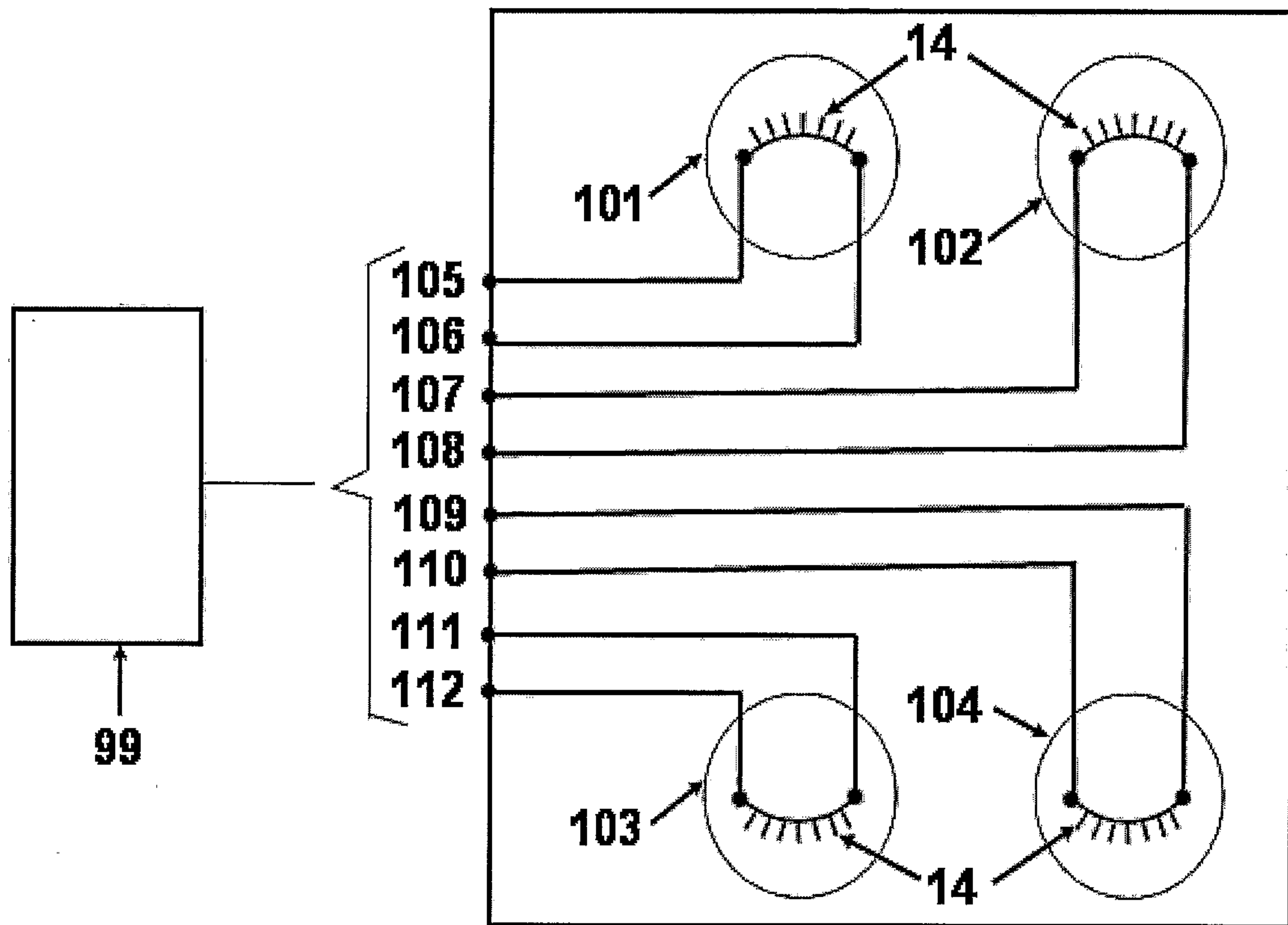
16/30***Fig. 12C***

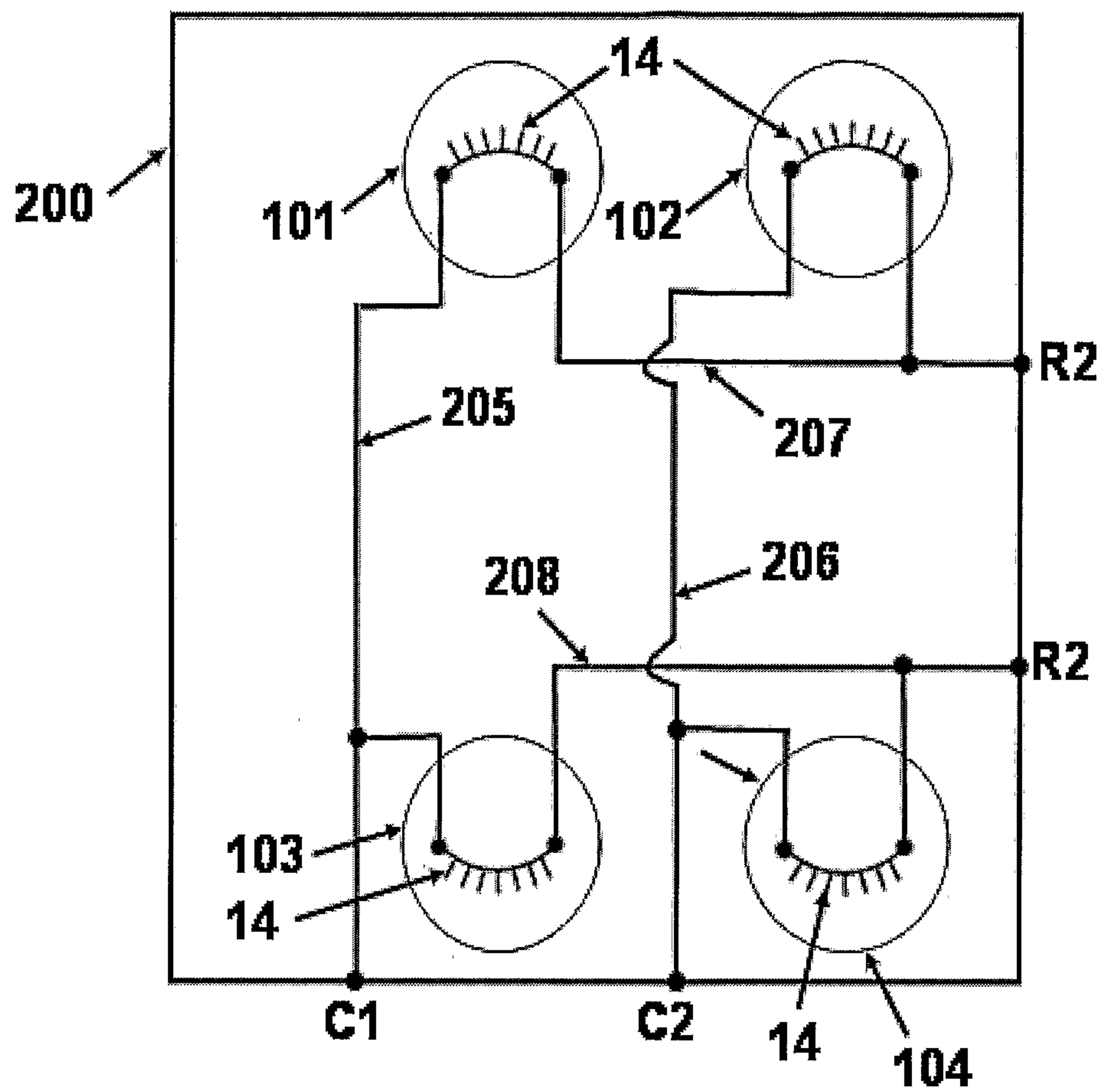
17/30**Fig. 13**

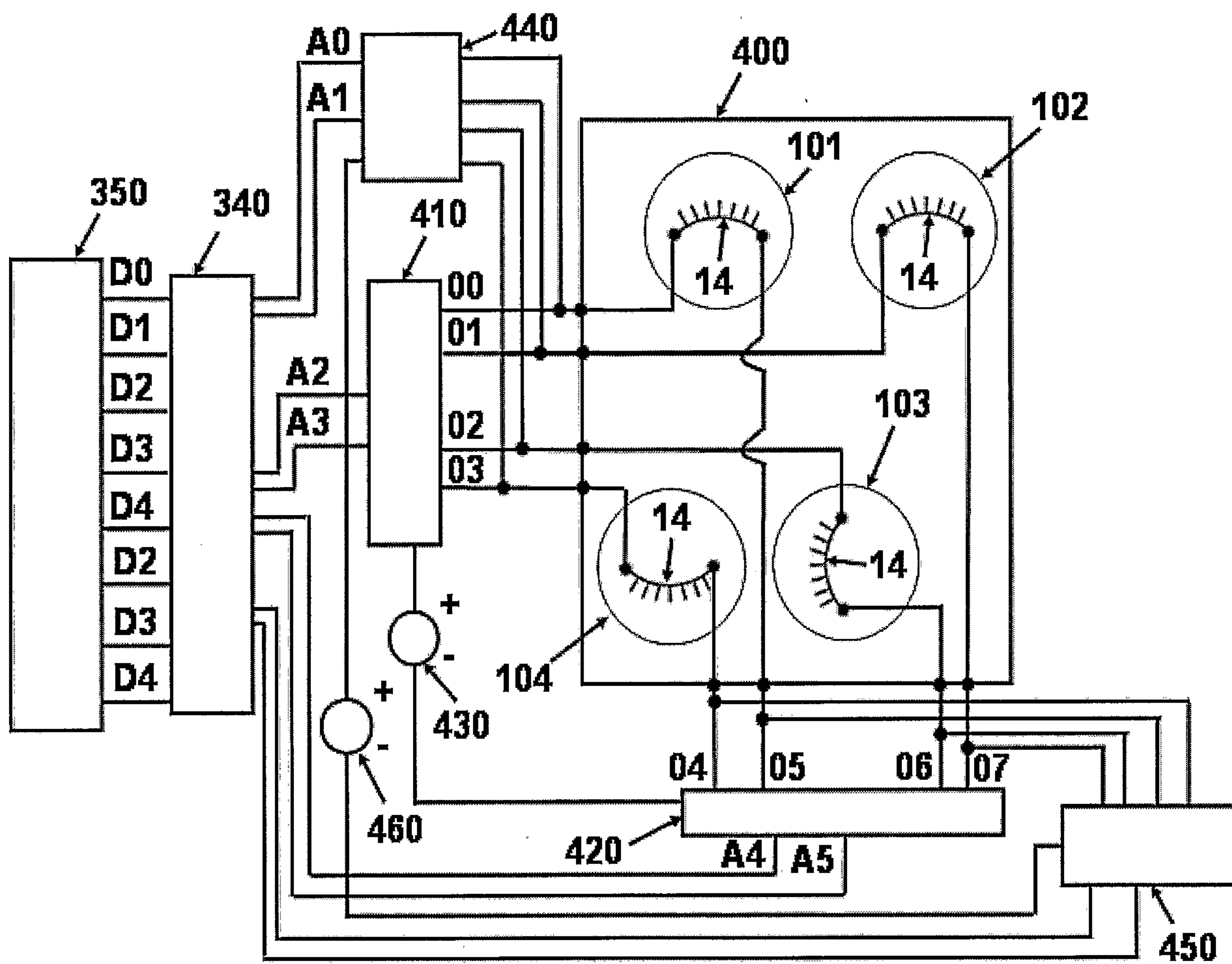
18/30**Fig. 14**

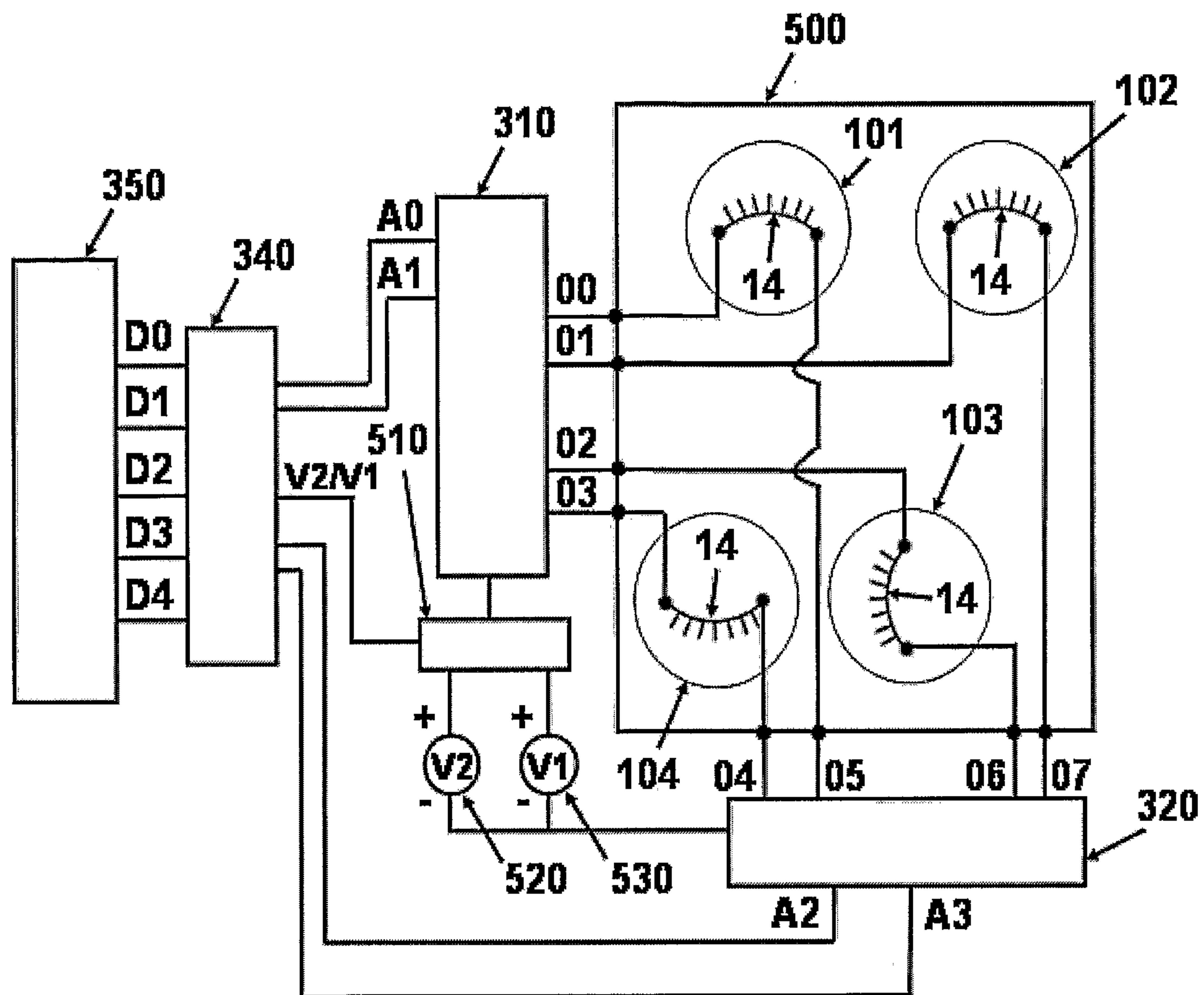
19/30**Fig. 15**

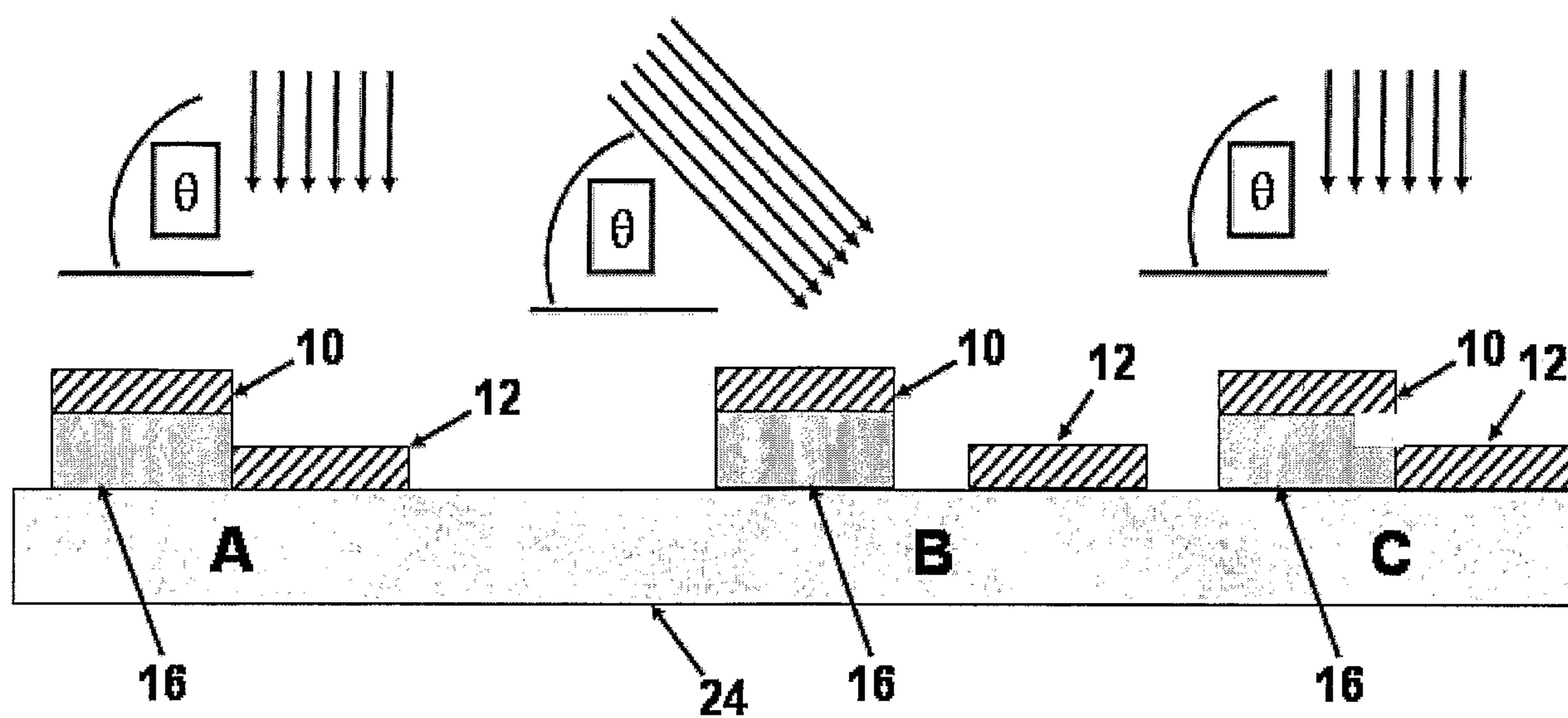
20/30**Fig. 16**

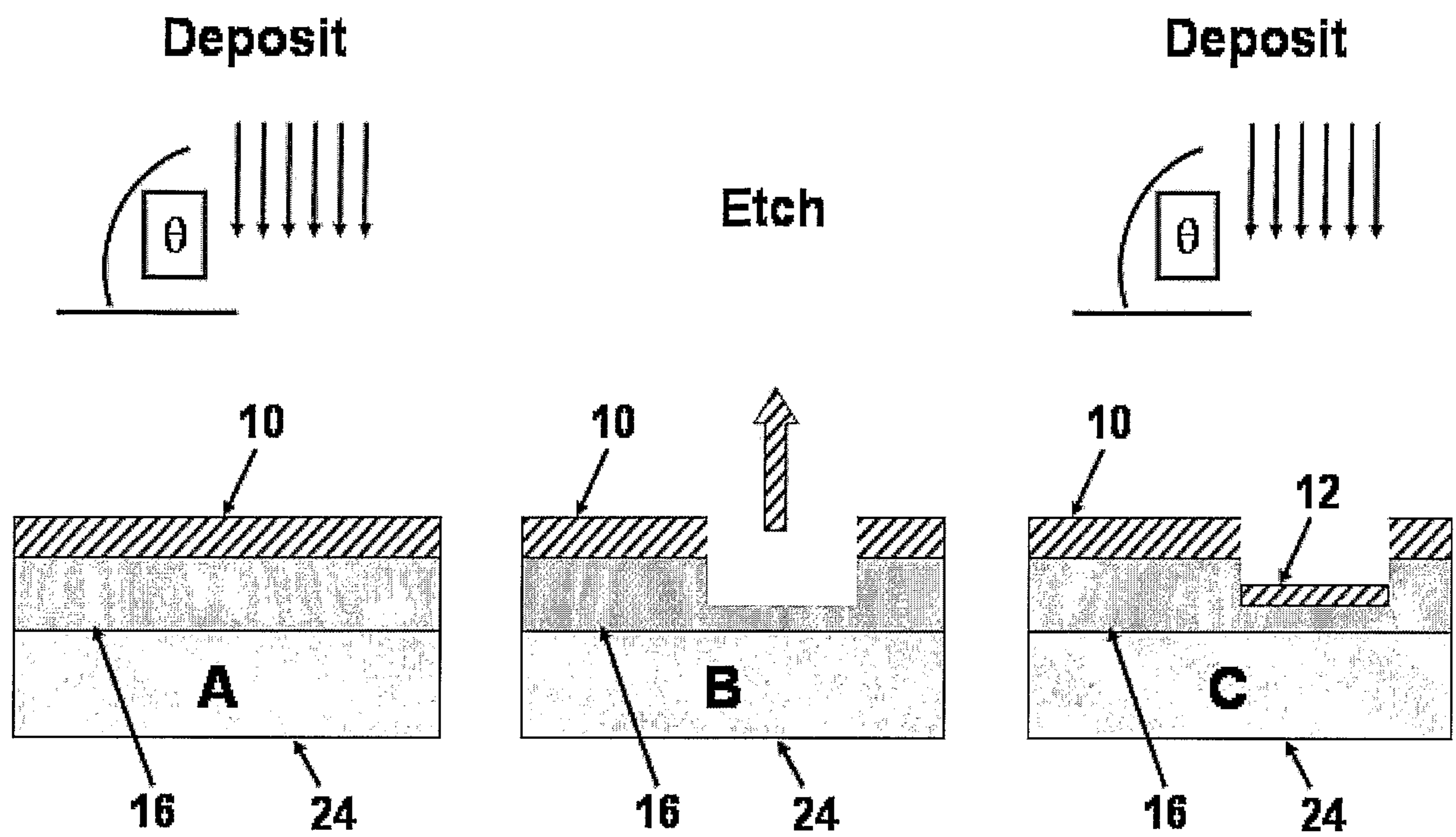
21/30**Fig. 17**

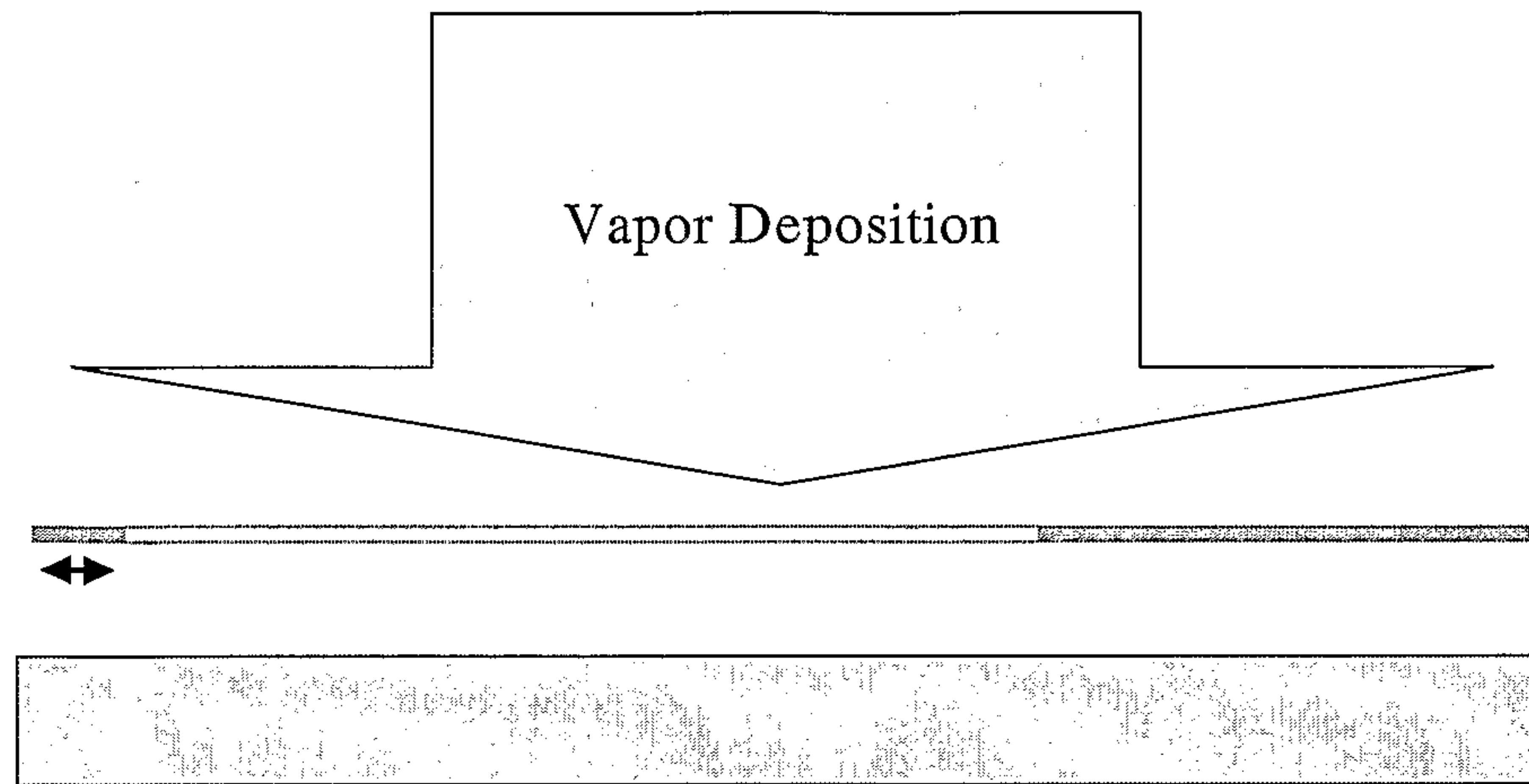
22/30**Fig. 18**

24/30**Fig. 20**

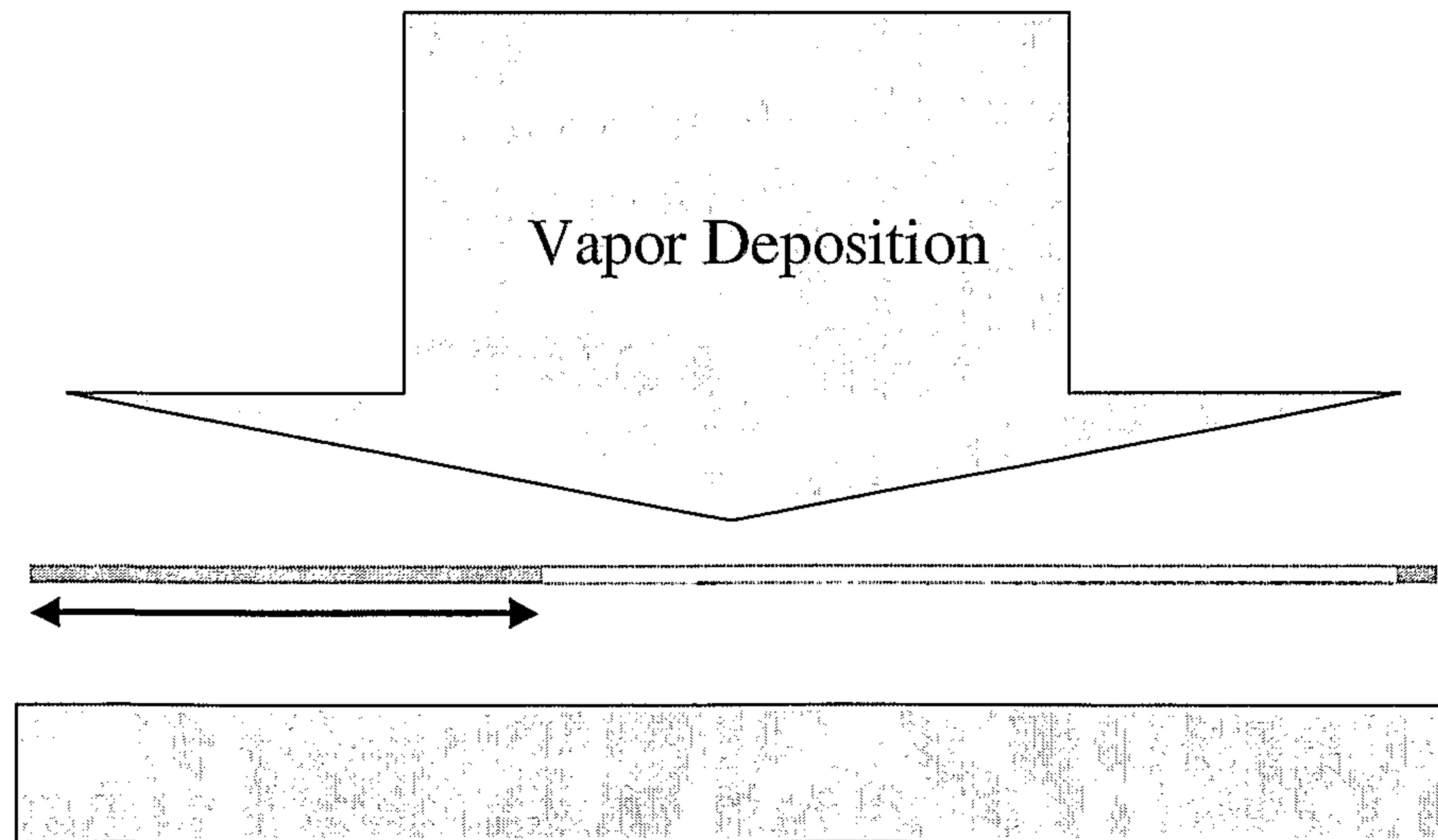
25/30**Fig. 21**

26/30**Fig. 22**

27/30**Fig. 23**

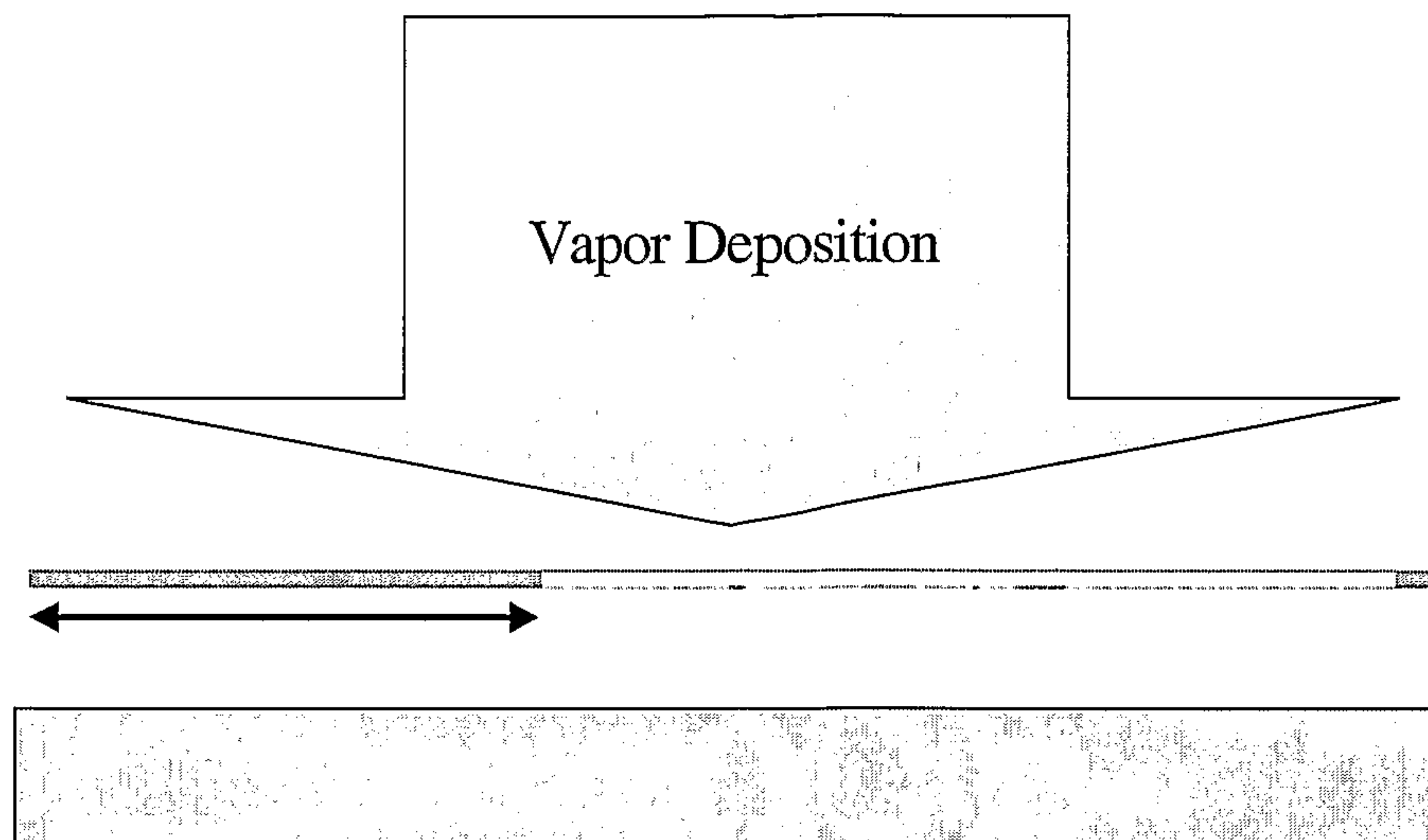
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Conductor 1 position deposition mask

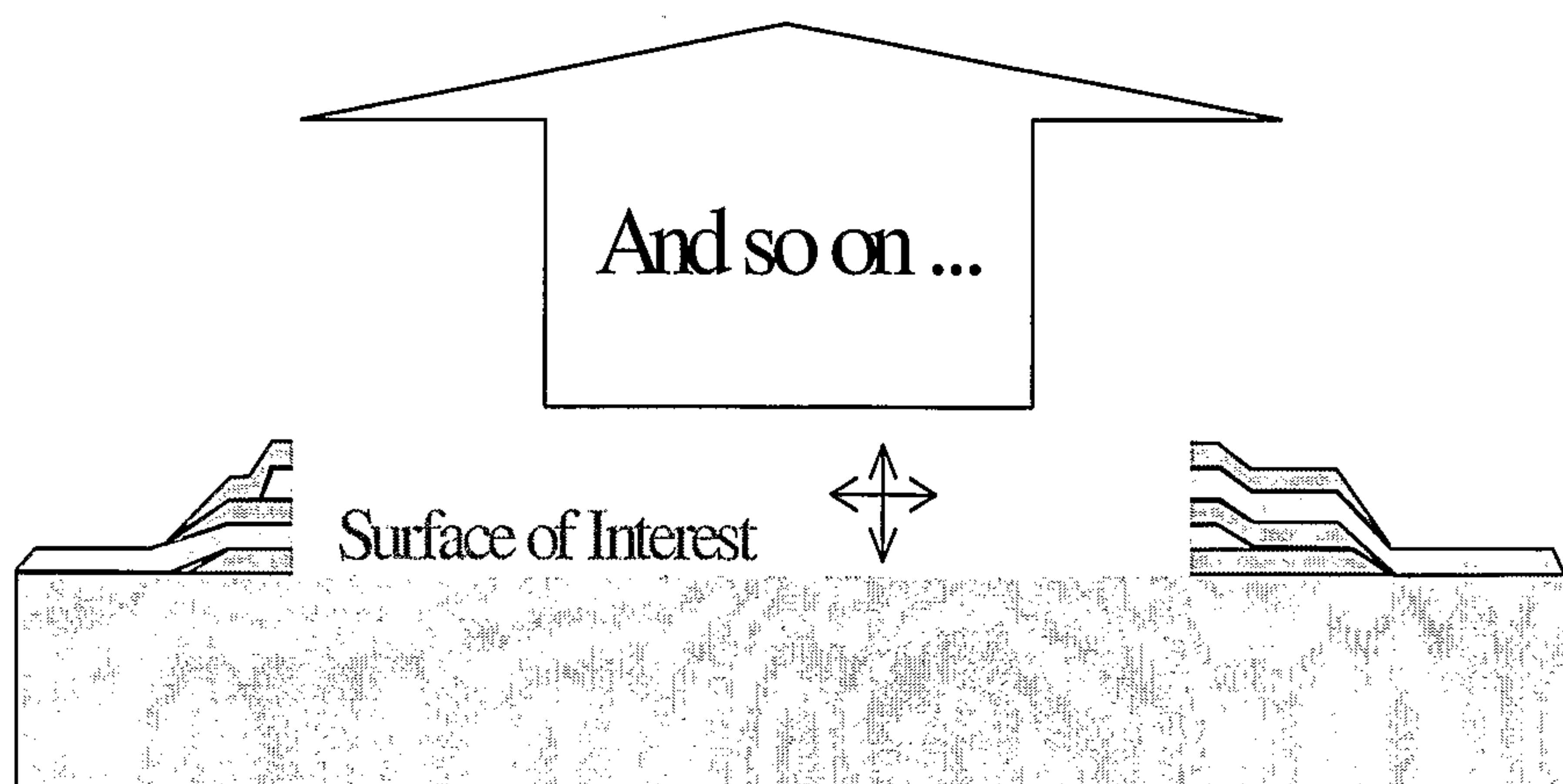
Fig. 24A

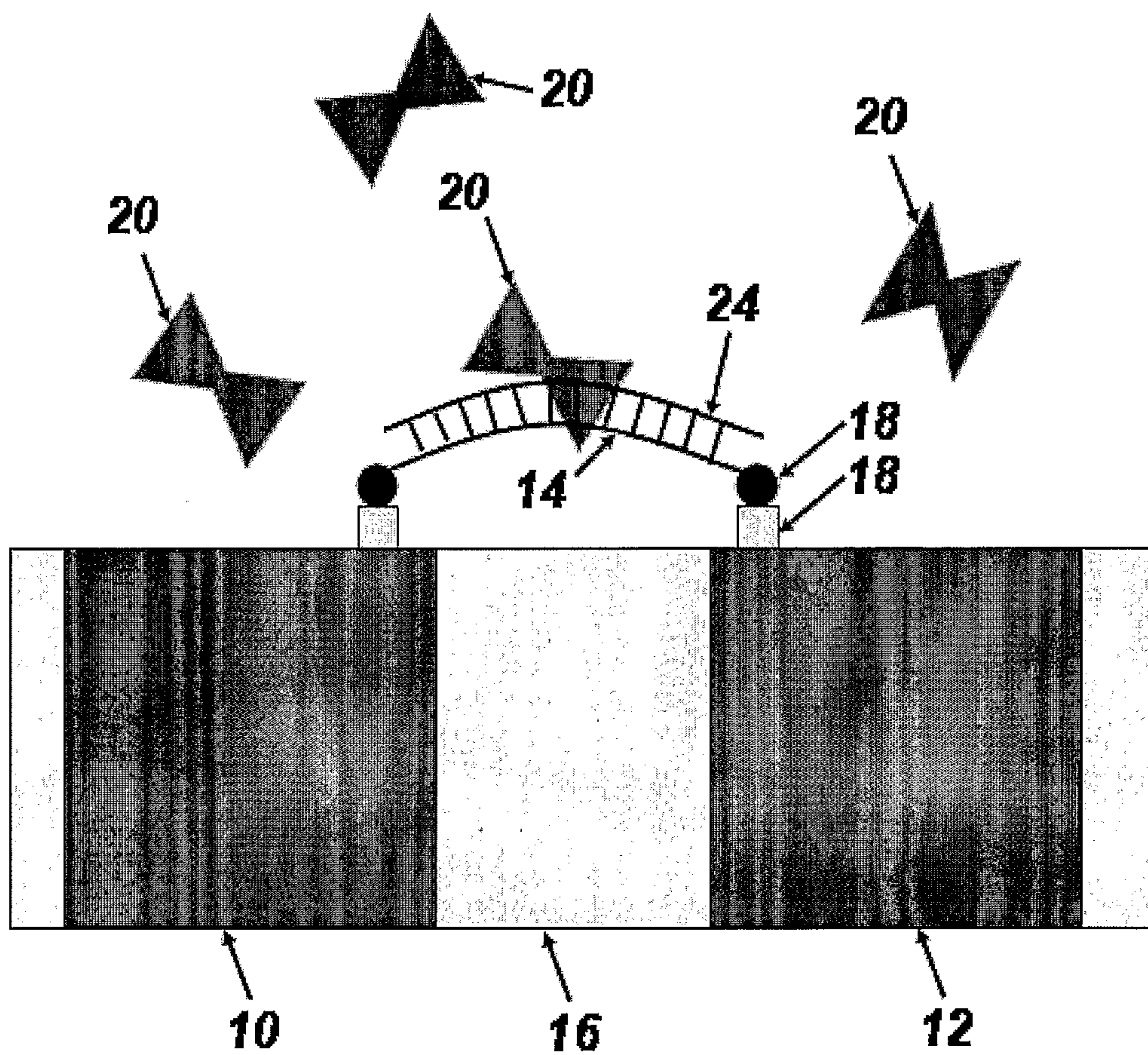
Conductor 2 position deposition mask

Fig. 24B

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Conductor 2 position deposition mask

Fig. 24C**Fig. 24D**

30/30**Fig. 25**