ADDRESSABLE CHEM/BIO CHIP ARRAY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefits from U.S. Provisional Patent Application No. **60/558,115** filed April 1, 2004, the contents of which are hereby incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the electronic or electrochemical detection of bio- and chemical molecules, and more particularly to arrays used in such detection.

BACKGROUND OF THE INVENTION

[0003] Methods for electrical or electrochemical detection of molecular interactions between biomolecules have provided an attractive alternative to detection techniques relying on radioactive or fluorescent labels. Electrical and electrochemical detection techniques are based on the detection of alterations in the electrical properties of an electrode arising from interactions between molecules attached to the surface of the electrode (often referred to as "probe" molecules) and molecules present in a reaction mixture (often referred to as "target" molecules) in contact with the electrode. Examples of methods and devices related to electrical or electrochemical detection of biomolecules are disclosed in U.S. Pat. Nos. 4,072,576, 4,098,645, 4,414,323, 4,840,893, 5,164,319, 5,187,096, and 5,891,630.

[0004] Electrical or electrochemical detection eliminates many of the disadvantages inherent in the use of radioactive or fluorescent labels to detect interactions between probe and target molecules. Electrical or electrochemical

detection is safe, inexpensive, sensitive, and is not burdened with complex and onerous regulatory requirements. The development of microfabricated arrays (microarrays) of bio- and chemical molecules has led to further improvements of traditional analytical techniques. Microarrays of bio- and molecules (e.g. oligonucleotides, nucleic acids, proteins, peptides, or antibodies) have utility in a wide variety of applications in which molecular interactions between target molecules in a reaction mixture and large numbers of distinct probe molecules bound to defined regions of a substrate can be simultaneously assayed using electrical, optical, or radioactive detection strategies. Microarrays address the demands for inexpensive, high-throughput detection of biomolecular interactions. Obviously, microarrays can also provide a low cost and high-throughput platform for detection of chemical species. There are, however, problems associated with electrical or electronic systems as such devices cannot detect the same way as optical scanners do and require multiplexing to scan different detection spots. Electronic detection systems also require an electrolyte (either solid or liquid), making ionic isolation of different detection spots critical.

[0005] Although biochip arrays for the electrochemical detection of molecular interactions between bio- and/or chemical molecules have been proposed, these devices have significant disadvantages. For example, the device disclosed by Egger et al. in U.S. Pat. Nos. 5,670,322 and 5,532,128 cannot be made column-and-row (or "x-y") addressable, thus limiting the density of the test sites in the array and the usefulness of the apparatus. In U.S. Pat. No. 5,653,939, Hollis et al. disclosed an x-y addressable array wherein a solid supporting substrate comprises a plurality of test sites in electrochemical contact with a set of orthogonally oriented electrodes. However, Hollis et al. does not provide an apparatus for eliminating the ionic shortage when an electrolyte is applied in microarray chips, in which case, the array electrodes cannot be addressed.

[0006] US Patent Application No. 20020090649A1 discloses a high density addressable array for electronic detection biochips. The array, however, does not provide for true four-terminal electronic detection for high S/N ratio and

high sensitivity, and does not lend itself for use in high density array chips without isolation channels.

[0007] Impedance measurements could be used to directly detect the impedance changes after biomolecular interaction. Most protein and DNA sensors require labelling (such as florescent labels) to report biomolecular interactions. Impedance can enable labelless detection. However, the sensitivity needs to be further improved.

[0008] Electronic or electrochemical sensors require two electrodes (working and counter/reference electrodes) or three electrodes (working, counter and reference electrodes) for detection measurements. Existing electronic array sensors are designed to have either two or three electrodes at every array detection site, or are designed to have common counter or reference electrodes. Two or three electrodes per test site typically results in many electrodes and high manufacturing cost. Common counter or reference electrodes cause different solution resistance resulting in additional noise and difficult data processing.

[0009] There thus remains a need for an improved column-and-row addressable chem/bio chip array for the electrical or electrochemical detection of molecular interactions that are effective while reducing the cost of performing various analyses, and that can also be easily and cost effectively fabricated. Such devices, and methods for their use, may have wide application in the fields of medicine and genetics and molecular biology research.

SUMMARY OF THE INVENTION

[0010] In accordance with the present invention, addressable bio/chem chips include a plurality of isolated test cells or regions, each addressable by at least two electrodes for measuring electrical characteristics of probe/test molecule interactions. In one embodiment, electrodes are located within a channel to allow for four terminal measurement. In another embodiment,

electrodes are arranged in rows and columns and interconnected with electrolyte pads used as test sites. In yet another embodiment, electrodes are arranged in cells, with each cell including a counter electrode surrounded encircled by working electrodes.

[0011] In accordance with an aspect of the present invention, there is provided a column-and-row addressable chip including a substrate, a plurality of channels formed in the substrate, a plurality of column electrodes, each one of the plurality extending along a length of one of the channels, a plurality of spaced row electrodes, within each of the channels and electrically isolated from the column electrode of the channel, and at least one pair of electrodes for interconnection with a voltage source, electrically isolated from the row and column electrodes, and positioned to generate a widthwise potential across at least a portion of one of the channels.

[0012] In accordance with an aspect of the present invention, there is provided an addressable chip including a substrate having a planar surface, a plurality of row electrodes formed atop the planar surface, a plurality of column electrodes, each formed atop the planar surface proximate a row electrode and electrically isolated from the row electrodes; electrolyte pads covering each of the column electrodes, proximate a row electrode; pairs of the row and column electrodes for interconnection with a voltage source, to generate a potential for detection of chem/biological probe and target molecule interaction proximate the electrolyte pads. Portions of the planar surface not covered by the pads are hydrophobic.

[0013] In accordance with an aspect of the present invention, there is provided an addressable chip including: a substrate; a plurality of counter electrodes formed on the substrate; a plurality of working electrodes, groups of the plurality of working electrodes encircling each of the plurality of counter electrodes and electrically isolated from the counter electrodes; pairs of the counter/working electrodes for interconnection with a voltage source, to generate a potential for detection of chem/biological probe and target molecule interaction proximate a pair of counter/working electrodes.

[0014] In accordance with another aspect of the invention, there is provided a test apparatus including a substrate, a plurality of pads formed of insoluble material for binding to samples of interest, arranged on the substrate; a plurality of working electrodes, each of the plurality of working electrodes electrically interconnected to first groups of the plurality of pads; a plurality of counter electrodes, each of the plurality of counter electrodes electrically interconnected to second groups of the plurality of pads; wherein each of the pads is interconnected to one of the plurality of working electrodes and one of the plurality of counter electrodes; a measuring device for measuring electrical characteristics at a selected pair of the counter and working electrodes, to analyse an analyte proximate the one of the plurality of pads interconnected with the selected pair of the counter and working electrodes.

[0015] Other aspects and features of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] In the figures which illustrate by way of example only, embodiments of the present invention,

[0017] **FIG. 1** is a perspective view of a bio/chem chip array, exemplary of an embodiment of the present invention;

[0018] **FIG. 2** is a top schematic view of a portion of bio/chem chip array of **FIG. 1**;

[0019] **FIG. 3A** is a partial cross-sectional view of bio/chem chip array of **FIG. 1**;

[0020] **FIG. 3B** is a partial cross-sectional view of bio/chem chip exemplary of another embodiment of the present invention;

[0021] **FIGS. 4A** and **4B** are schematic diagrams of a bio/chem chip array, exemplary of another embodiment of the present invention;

[0022] **FIGS. 5A** is a schematic diagram of a bio/chem chip array, exemplary of another embodiment of the present invention;

[0023] **FIGS. 5B** is an enlarged view of the counter-working electrode addressable chip of **FIG. 5A**;

[0024] **FIGS. 6A** and **6B** are schematic diagrams of test cells of the bio/chem chip array of **FIGS. 4A** and **4B**; and

[0025] **FIG. 7** is a graph illustrating the sensitivity of test cells of the bio/chem. chip arrays of **FIGS. 4A** and **4B**.

DETAILED DESCRIPTION

[0026] **FIG. 1** schematically illustrates a four-terminal addressable bio/chem chip array **10**, exemplary of an embodiment of the present invention.

[0027] As illustrated, array **10** includes a substrate **12**, and plurality of width wise extending microchannels **14a** and **14b** (individually and collectively microchannels **14**). Substrate **12** is formed of an insulating material such as plastic, ceramic, glass, rubber, fabric, printed circuit board, silicon or combinations thereof. Microchannels **14** are formed in the top surface of substrate **12**. Example microchannels **14** may be formed using conventional etching or embossing techniques, and may have widths on the order of 0.1 μm to 2000 μm and depths of 0.1 to 2000 μm. For clarity, depicted array **10** only includes two microchannels **14a** and **14b**. However, a suitably sized substrate **12** may include any number of similarly formed microchannels. As well, in the depicted embodiment, microchannels **14** extend from edge to edge of substrate **12**. However, microchannels **14** may only extend partially between edges.

[0028] Exemplary array **10** further includes x-y addressable row and

column electrodes **16, 18** in different microchannels **14**, as further illustrated in **FIG. 2A**. As illustrated, addressable column electrodes **18a, 18b** (individually and collectively column electrodes **18**) are designed as conductive strips, each one running through the length of microchannels **14**. Column electrodes **18** thus provide a relatively large surface area, which can serve as reference/counter electrodes without significant polarization. Addressable row (x) electrodes **16**, on the other hand, are formed within each channel and isolated from column electrodes **18**. Row and column electrodes **16, 18** are electrically isolated from each other. In the depicted embodiment of **FIG. 2A** row electrodes **16** are formed as finite sized generally round conductive pads within microchannels **14**. Example alternate shapes for row electrodes **16** will be readily apparent to a person of ordinary skill. For example, electrodes **16'** and **16"** formed as generally rectangular and square conductive pads are depicted in **FIGS. 2B** and **2C**, respectively. Again, for clarity, depicted array **10** only includes four row electrodes **16**. A suitably sized substrate **12** may include any number of similarly formed row electrodes **16**.

[0029] Row electrodes **16** may be provided at spacings on the order of 0.1 μm to 500 μm. Exemplary row/counter electrodes **16, 18** have sizes on the order of 0.001 μm to 500 μm, which can be fabricated by conventional lithography or nanolithography technologies. Examples of the row/column and voltage electrode materials include solid or porous gold, silver, platinum, copper, titanium, chromium, aluminum, metal oxide, metal carbide, carbon, graphite, fullerene, conductive plastic, conductive polymer, metal impregnated polymers, or combinations thereof.

[0030] Additionally, pairs of electrodes **20, 22** for imposing an applied voltage (referred to as "voltage electrodes") are located in microchannels **14** outside of the addressable row/column (x/y) electrodes **16, 18**. In the depicted embodiment, one of the pair of voltage electrodes **20, 22** is formed on either side of column electrode **18** and on either side of each row electrode **16**. All positive voltage electrodes **20** and negative voltage electrodes **22** of array **10** in microchannels **14** may be electronically interconnected together at

two lines.

[0031] A section view of the row/column electrodes **16, 18** and voltage electrodes **20, 22** which are isolated from the current electrodes for true four-terminal measurements is illustrated in **FIG. 3A**. As will become apparent, each pair of voltage electrodes **20, 22** provides an electrical field for four-terminal impedance measurements, and are isolated from the x-y addressable electrodes **16, 18**.

[0032] Since the function of voltage electrodes **20, 22** is to establish an electric field in channel **14**, they can be isolated from the channel **14** and testing fluid samples. Thus, alternatively, voltage electrodes **20, 22** can be embedded into substrate **12** as illustrated in **FIG. 3B**. This design allows conducting four-terminal electrical and addressable detection with high signal to noise (S/N) ratio and simple multiplexing detection for significantly reducing I/O lines.

[0033] In the arrangements of both **FIGS. 3A** and **3B**, column electrodes **16** are separated by microchannels **14**. As such, as column electrodes are isolated, a pair of arrayed row/column electrodes **16, 18** can be addressed independently, without being influenced or influencing measurements at other pairs of row/column electrodes **16/18**.

[0034] In operation, different probe molecules or sensing layers such as DNA, proteins, peptides, etc may be immobilized at or proximate row electrodes **16** in microchannels **14** by linkers or other modification methods such as adsorption, covalent binding, chemical affinity, surface monolayer self-assembly, etc.

[0035] Samples containing analyte that may contain target molecules is flown into or through channels **14**. Optionally, thereafter, the channels may be washed to remove any excess, non-bound analyte.

[0036] A voltage is applied across electrodes **20/22**, during or after flow of the analyte. This voltage, in turn generates a lateral electric field within each channel between electrodes **20** and **22**, and between electrodes **16** and **18**.

In the presence of immobilized probe molecules bound to target molecules, proximate for example, the i^{th} and j^{th} row and column electrodes **16**, **18**, current may flow between these i^{th} and j^{th} row and column electrodes **16** and **18**. Conventional measuring equipment may detect this current to verify the presence of the immobilized probe molecules and target molecule. Alternatively, impedance changes at the i^{th} and j^{th} row and column electrodes may be detected in other conventional manners. Absent immobilized probe molecule/target molecule combinations, proximate row/column electrode pair **16**, **18** no significant current will flow between proximate row and column electrodes **16**, **18**.

[0037] That is, molecular interactions between the immobilized probe molecules and target molecules that bind specifically to the probe molecules may be detected as a difference in the electrical signal detected at the corresponding row and column electrode **16**, **18**. If a gel or solid electrolyte is applied instead of the liquid electrolyte at these microchannels **14** an addressable array can be fabricated to detect gas compounds.

[0038] As will be appreciated, the number of samples that may be independently tested using array **10** equals the number of row electrodes since the column channels are isolated from each other for detection of different samples.

[0039] Advantageously exemplary array **10** enables true four-terminal impedance measurements with a high S/N ratio for highly sensitive electronic and electrochemical array sensors. The applied electrical field between pairs of voltage electrodes **20/22** may also enhance the diffusion rates of target biomolecules in analytes if the target molecules are charged. As DNA and many other biomolecules such as proteins are charged molecules, array **10** is particularly useful for biomolecule detection. Conveniently the number of I/O lines for multiplexing is reduced and can conduct different electronic detections. Hydrophobic coating or additional isolation parts are not required. The design may thus result in higher automation performance, shorter assay times and lower manufacturing costs.

[0040] **FIG. 4A** schematically illustrates an alternate addressable chem/bio chip array **100** for use as an electronic chem/biochip in test apparatus **99**. Array **100** includes a substrate **110** having a generally planar surface and an array of row and column electrodes **116, 118** formed thereon. Substrate **110** may be hydrophobic, or coated with a hydrophobic coating. An insoluble layer, for example, in the form of solid, semi-solid or polymer electrolyte pads **102** is coated on top of each electrode **116** for formation of a test cell. In this embodiment, each electrolyte pad **102** further covers a proximate column electrode **118**.

[0041] Pads **102** may be used to attach probe molecules. Pads **102** may, for example, be formed of cross-linked hydrogel, and can be formed by printing or dispensing. Alternatively pads **102** may be formed of polyacrylamide on pairs of row/column electrodes followed by the deposition of a mixture solution of monomer acrylamide and streptavidin only onto the arrayed row electrode. After UV-curing, the streptavidin molecules are entrapped in the polymerized polymer matrix and can be used to attach any biotinylated probe. A monomer with functional groups such amine or hydroxyl groups for protein probe attachment can also be used. Alternatively, the electrolyte pad **102** may be formed after probe attachment on top of pairs of row/column electrode.

[0042] Pads **102** can be used in both gas or liquid sample detection. After incubation of samples on pads **102**, excess liquid will flow out. Different arrayed cells are isolated by hydrophobic plastic substrates.

[0043] Conveniently, array **100** may be easily and inexpensively manufactured and reduced numbers of I/O lines are required due to the addressable array. Conveniently, insoluble pads **102** when formed of a polymer electrolyte provide a bioconjugation platform for the attachment of different biomolecular probes for the detection of target molecules, such as proteins, DNA and peptides. Different or like probe molecules may be attached at each pad **102**, as desired.

[0044] As will be appreciated, arrangement of row and column electrodes

116, **118** and pads **102** need not be strictly Cartesian. Instead, rows and columns may arranged in numerous ways, with row electrodes and column electrodes interconnected to groups of pads **102**, with each pad **102** interconnected with a unique electrode pair.

[0045] In an alternate embodiment depicted in **FIGS. 6A** and **6B**, an addressabe bio/chem chip **200** is arranged as a plurality of working/counter electrode complexes **202**. Each working/counter electrode complex **202** includes a single counter electrode **218** surrounded by a group of working electrodes **216**, as best illustrated in **FIG. 5B**. Each group includes a plurlality of working electrodes **216a**, **216b** ... (individually and collectively working electrodes **216**). Optional reference electrode **220** may further surround each counter electrode **218**. In the depicted embodiment, the working electrodes **216** form a partial circle about each counter electrode. Each counter/working electrode pair **218/216** can be uniquely addressed by a single counter electrode **218** C_i and a single working electrode **216** W_n. For example, C_1W_5 addresses a detection at working electrode 5 of counter electrode 1. Conveniently, like positioned working electrodes of all working/counter electrode complexes **202** may be interconnected, as best illustrated in **FIG. 5A**.

[0046] In order to allow independent assays at the various working/electrode complexes **202**, complexes **202** may be physically isolated by one or more upstanding walls or trenches separating each complex **202** from each other complex **202**. Channels (not shown) may also be formed in a substrate **210** to guide liquid analyte to each complex **202**. Alternatively, a solid or semi-solid electrolyte pad (not shown) can be formed on various working/electrode complexes **202**, respectively, and complexes **202** may be isolated from each other by hydrophobic surface between two neighboured complexes.

[0047] Again, any portions of the top surface of the surface of chip **200** not covered by complexes **202** may be covered, or formed of a hydrophobic substance or layer.

[0048] Probe molecules may be immobilized proximate the tip of each working electrode

[0049] Chip **200**, like addressable chip **100**, can significantly reduce the number of I/O lines required for simpler multiplex measurements and thus result in low manufacturing costs. Conveniently, due to the symmetric arrangement of working electrodes **216** about counter electrodes **218**, the electrical background contributions at each working/counter electrode pair **216/218**, such as electrolyte resistance, distribution electronic components, etc. from different detection sites proximate a single counter electrode **218** are identical to each other. This can significantly improve the S/N ratio of measured signals. In addition, every group of working electrodes **216** about a single counter electrode **218** can be used to detect different samples and each working electrode **216** may detect a different target biomolecules for more flexibility. Each working electrode **216**, in turn, can be immobilized with a unique probe molecule.

[0050] Addressable chip **200** may be fabricated using conventional printed circuit board technology on a plastic substrate. In the depicted embodiment, generally round working and counter electrodes **216**, **218** and interconnects are etched in conductive material atop a substrate **210**. Substrate **210** may be of the same material as substrate **104**. Numerous other configurations and fabrication techniques will be understood by persons for ordinary skill. For example, electrodes **216**, **218** may be generally round, square, rectangular or the like. A suitable biochip **200** may be formed using lithography and ceramic, and the substrate materials can be plastic, ceramic, silicon and the like. Working and counter electrodes may be formed of at least one of platinum, gold, metal, carbon, carbides, nitrides, conductive polymers.

[0051] In operation, array **100** (or array **200**) is first incubated with test samples and then washed. Remaining water is removed from the hydrophobic surface, not covered by insoluble pads **102**. Since this surface is hydrophobic, water can easily be removed. Thus, array **100** (or array **200**) can be treated as a free-standing component for electronic detection without the problem of cross assay interference, since every detection site forms a

miniaturized isolated test location, addressable by two electrodes.

[0052] Once array has been incubated in the test samples and washed, array **100** (or **200**) may be used together with impedance spectroscopy to detect bio-molecule binding, such as DNA matching and protein bindings, proximate each addressed row and column electrode pair **116, 118**, or counter/working electrode pair **216, 218**. In such applications, the binding behavior of target molecules causes changes of the solution-electrode interface impedance. By characterizing the impedance spectrum, binding can be detected.

[0053] That is, each addressed row/column electrode pair **116, 118** of array **100 (FIG. 4A)** may be modelled as a two-electrode test cell as shown in **FIG. 6A** and **6B**. Counter/working electrode pair **216, 218** of array **200 (FIG. 5A)** may be similarly modelled. The interface between the bound test sample and the row electrode **116** may be modelled as a double-layer structure. Now, the impedance between the most proximate row and column electrodes **116, 118**, as a function of frequency (ω) of any applied voltage, can be expressed as

$$Z = R - j\frac{1}{\omega C} \qquad (1)$$

where R is resistance, and C is the capacitance between the row/column electrode **116, 118**. As will be appreciated, there is no material inductance term in the impedance expression for the double-layer structure, and the imaginary part is always available.

[0054] When this test cell is used to detect molecule binding, the relative sensitivity is usually characterized by the dependence of the relative change of the resistance, $(R - R_0)/R_0$, on the target molecule concentration, where R_0 is the electrode-solution interface resistance when no binding takes place, and R is the electrode-solution interface resistance in the presence of binding. Bio/chemical molecule interaction on an electrode surface also results in change of the double-layer capacitance. The relative change of double layer capacitance, $(C - C_0)/C_0$ electrode can be used for target bio/chemical

molecule detection.

[0055] However, sensitivity may be improved if an inductor is put in series with the row or column electrode **116**, **118**, as depicted in **FIG. 4B** and **6B**, or counter/working electrode **216**, **218**. The sensitivity of this test cell may be calculated as $[d(|Z - Z_0|)/d\log(f)] / [d|Z_0|/d\log(f)]$. Here, Z_0 is the impedance of the electrode-solution interface and the inductor when no binding takes place, Z is the impedance of the electrode-solution interface and the inductor when binding takes place, and f is frequency.

[0056] **FIG. 7** illustrates the relative sensitivity of a test cell of array **100** in the presence of inductor **120** (**FIG. 6B**), and in the absence of inductor **120** (**FIG. 6A**), using the same analyte (the binding effect of chicken antigen to antibody), for various concentrations of the analyte. It is evident that the relative impedance amplitude change of the row/column electrodes in the presence of an inductor is much higher than the relative resistance change of the system without inductor, and both the results are correlated with each other.

[0057] After addition of an inductor, L, the impedance between row and column electrodes **116**, **118** will be

$$Z = R - j\frac{1}{\omega C} + j\omega L = R + j(\omega L - \frac{1}{\omega C}) \qquad (2)$$

[0058] Thus, there is a specific resonant frequency, f_0, at which Z approaches the minimum value of R, and $[d(|Z - Z_0|)/d\log(f)] / [d|Z_0|/d\log(f)]$ at f_0 will approach to ∞. A measurement frequency, f can be selected to very close to f_0 and to have very small $\Delta f = f - f_0$. Since $[d(|Z - Z_0|)/d\log(f)] / [d|Z_0|/d\log(f)]$ value can be extremely large when f approaches to f_0, the capacitance component change in the impedance (inductance is fixed) from target and probe molecule bindings or interactions is significantly amplified at a measurement frequency close to f_0. Thus, this method can be significantly increased the measurement sensitivity.

[0059] As such, as illustrated in **FIG. 4B**, an addressable array **100** forms part of a test apparatus **99**. A single inductor **120** interconnects a measuring device **122** to selected pairs of row/column electrodes **116, 118** proximate pads **102**. Example measuring device **122** may, for example, take the form of a conventional impedance frequency analyser, and may include a variable frequency voltage source and an ammeter (not shown). Row/column selectors **124** and **126** may control which row/column electrode pair **116, 118** is intereconnected with measuring device **122** at any instance in time.

[0060] Row/column selectors **124** and **126** may then be operated to interconnect measuring device **122** to each row/column pair **116, 118**. For each row/column pair **116, 118** measuring device **122** may be interconnected to that row/column pair **116, 118** and cycled through a range of frequencies to determine the impedance of the row/column pair **116, 118** as a function of frequency. The resulting impedance of each row/column electrode pair **116, 118** may be measured using measuring device **122**, to determine the frequency response of the row/column electrode in the presence of a test solution. Alternatively, changes in impedance at or near f_0 may be measured for each row/column electrode pair **116, 118**. Operation of row/column selectors **124** and **126** and measuring device **122** may be controlled by a general purpose computing device under software control, in manners understood by those of ordinary skill. Results may be tabulated, displayed and/or further processed.

[0061] As well, use of exemplary arrays **10, 100, 200** is not limited to detection of biomolecule interactions; it can, for example, also be also used for detection of chemical analytes. The exemplary arrays **10, 100, 200** may be suitable for a variety of applications including for example diagnostics, drug discovery, pathogen detection, food sensing and environmental protection. The arrays **10, 100, 200** can be used to detect molecular interactions such as nucleic acid hybridization, protein binding and other chemical/electrochemical reactions.

Example 1:

[0062] Electrode preparation using array **100** (**FIG. 4B**): A solution containing 0.1 M pyrrole, purchased from Aldrich), + PBS buffer was prepared as solution A. A solution for probe synthesis was prepared by adding 1 µl of anti-AZT ,281 D, thymidine analog used in treatment of AIDS, at 100 µg/ml, purchased from Sigma, into 99 µl of solution A. A glassy carbon (GC) electrode with 3 mm was used to immobilize polypyrrole/anti-AZT probes by an electrochemical deposition with the precusor solution containing pyrrole and AZT. During the electrochemical deposition, a platinum foil with much larger surface area than that of the GC, and a Ag/AgCl electrode were used as row and column electrodes, respectively. CH 660 potentiostat was used to conduct cyclic voltammetry (CV) over –0.1 to 1.0 vs. Ag/AgCl for four cycles for electrochemical synthesis. The anti-AZT was entrapped in the polypyrrole film as the probe molecule to detect AZT, a low molecular weight protein molecule in solutions. After the polypyrrole/protein probe film deposited on the microelectrode surface and was rinsed with PBS solution, a Solartron Impedance Frequency Analyzer 1260 with Electrochemical Interface 1287 was used to measure the impedance, Z_0 of the GC electrode in PBS buffer. Solutions with concentrations of AZT from 10 pg/ml to 1 µg/ml in PBS were prepared by series dilution method. Polypyrrole/anti-AZT electrodes were submerged in 200 µl of solutions with different concentrations of AZT, respectively, for 1.0 hours. Then the electrodes were washed by PBS to remove unbound AZT and placed in PBS for impedance measurements.

[0063] The impedance change of $(R - R_0)/R_0$ vs. concentrations is shown in **FIG. 7** as denoted. $[d(|Z - Z_0|)/d\log(f)] / [d|Z_0|/d\log(f)]$ values were calculated with step of 0.001 and shown in **FIG. 7** as denoted. As illustrated, $[d(|Z - Z_0|)/d\log(f)] / [d|Z_0|/d\log(f)]$ is more sensitive than $(R - R_0)/R_0$ by almost 1 order of magnitude.

[0064] Alternatively, the sensitivity enhancement by adding an inductor onto the detection circuit can be conducted by introducing a virtual inductor to process the results obtained by conventional ac impedance measurements. For example, in most electrochemical sensors, the components in an equivalent circuit are resistance, the real part, and capacitance, the imaginary

part. An additional imaginary part, the virtual inductor could introduced during processing and calculating the experimental data with the S/N amplification method described above.

[0065] Of course, the above described embodiments are intended to be illustrative only and in no way limiting. The described embodiments of carrying out the invention are susceptible to many modifications of form, arrangement of parts, details and order of operation. The invention, rather, is intended to encompass all such modification within its scope, as defined by the claims.

WHAT IS CLAIMED IS:

1. An addressable chip comprising:

a substrate;

a plurality of counter electrodes formed on said substrate;

a plurality of working electrodes, groups of said plurality of working electrodes encircling each of said plurality of counter electrodes and electrically isolated from said counter electrodes; wherein the working electrodes is at an equal distance from the counter electrode;

pairs of said counter/working electrodes for interconnection with a voltage source, to generate a potential for detection of chem/biological probe and target molecule interaction proximate a pair of said counter/working electrodes.

2. The chip of claim 1, wherein said working and counter electrodes are formed of at least one of platinum, gold, metal, carbon, carbides, nitrides, and conductive polymers.

3. The chip of any of claims 1 to 2, further comprising at least one wall isolating fluids proximate each of said counter electrodes from other ones of said counter electrodes.

4. The chip of any of claims 1 to 2, further comprising at least one trench isolating fluids proximate each of said counter electrodes from other ones of said counter electrodes.

5. The chip of any of claims 1 to 4, wherein said substrate is formed of at least one of plastic, ceramic, glass, rubber, fabric, printed circuit board, and silicon.

6. The chip of any of claims 1 to 5, further comprising a plurality of interconnects allowing independent measurement of current flow between each one of said counter electrodes and one of said working electrodes about said each one of said counter electrodes.

7. The chip of any of claims 1 to 6, wherein one working electrode about each of said counter electrodes is interconnected with one working electrode of each of said groups of working electrodes.

8. The chip of any of claims 1 to 7, wherein each of said counter electrodes is generally rectangular, square or circular in shape.

9. The chip of any of claims 1 to 8, further comprising a plurality of electrolyte pads, each covering one of said counter electrodes and its surrounding group of working electrodes.