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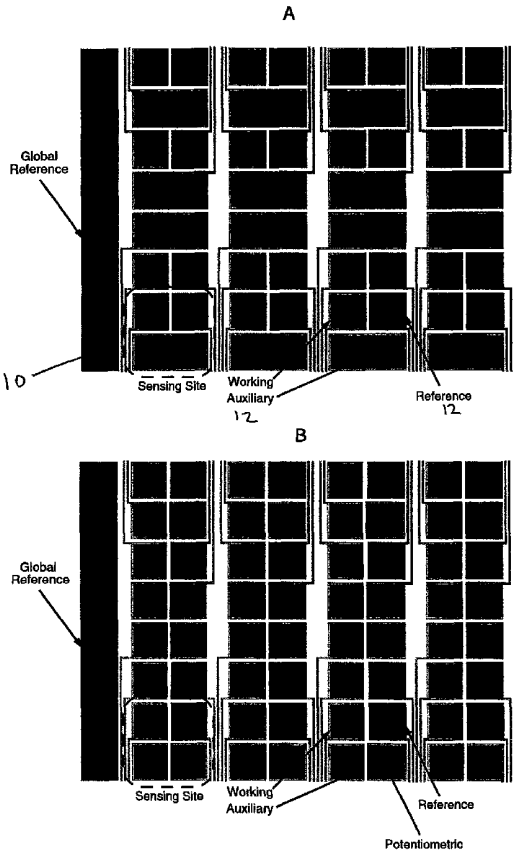

(57) Abstract: There is provided a recording device having at least one potentiometric electrode, at least one amperometric electrode, wherein both electrodes are located on the same recording device. Also provided is a single locus recording device having at least one potentiometric electrode, at least one amperometric electrode, wherein both electrodes are located on the same recording device.

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MICROSCOPIC MULTI-SITE SENSOR ARRAY WITH INTEGRATED CONTROL AND ANALYSIS CIRCUITRY

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention relates to a sensor array. More specifically, the present invention relates to a microscopic multi-site sensor array.

2. DESCRIPTION OF RELATED ART

As sensor array systems grow relatively large, the efficient operation of the systems becomes more of a consideration. Efficient interfacing of sensor array systems with electrical connections off-chip raise pin or contact limitation issues. Further, constraints regarding effective chip or array system size present issues regarding the selection of components, and the size of them, for inclusion on the chip or substrate. Often times, various selections must be made to provide an effective optimization of advantages in the overall design.

One proposed solution for the control of an array of electrodes utilizing less than one individual dedicated connection per electrode or test site is provided in Kovacs U.S. patent application Ser. No. 08/677,305, entitled "Multiplexed Active Biological Array", filed Jul. 9, 1996, incorporated herein as if fully set forth herein. The array is formed of a plurality of electrode sites, a typical electrode site including an electrode, a driving element coupled to the electrode for applying an electrical stimulus to the electrode and a local memory coupled to the driving element for receiving and storing a signal indicative of a magnitude of the electrical stimulus to be applied to the electrode. Multiple embodiments are disclosed for selectively coupling a value signal through coaction of a row line and a column line for storage in the local memory. In this way, the values at the various electrodes in the array can differ from one another.

In Fiaccabrino, G. C., et al., (1994), an array of n^2 electrodes are connected to 2n pins, plus 2 additional pins for signal output and bulk bias. The row and column signals drive series connected transistors to provide a single value to a working electrode. This system does not enable the switching of two or more electrodes simultaneously at different potentials.

In Kakerow, R. et al., (1994), a monolithic single chip sensor array for measuring chemical and biochemical parameters is described. A 20×20 array of individually addessable sensor cells is provided. The sensor cells are serially addressed by the sensor control unit. One horizontal and one vertical shift register control selection of the sensor cells. Only one sensor cell is selected at a time. As a result, multiple sites can not be activated simultaneously.

Yet another concern is the ability to test an electronic device prior to application of a conductive solution on the device. As devices or chips become more complicated, the possibility of a manufacturing or process defects generally increases. While visual inspection of the device can be performed, further electrical and chemical testing can ensure an operational device is provided to the end user.

As is apparent from the preceding discussion, numerous attempts have been made to provide effective techniques to monitor multi-step, multiple molecular, analytical, and biological reactions. However, for the reasons stated above, these techniques are "piece-meal", limited, and have not effectively optimized solutions. In many situations, it is necessary to monitor both potentiometrically and amperometrically over an area to monitor electrochemical signals and concentration gradients. These various approaches are not easily combined to form a system which can carry out a complete cellular metabolic analysis and/or DNA diagnostic assay. Despite the long-recognized need for such a system, no satisfactory solution has been proposed previously.

An understanding of the communication between neurons is key to the advancements in neuroscience. It is important for not only purely scientific reasons, but can provide assistance in studying various neurological injuries and disorders, possibly leading to treatments, and development of neural prosthetics.

Miniature silicon neurosensors have been available to neuroscientists for many years (Wise, K.D., et al., 1970; Kuperstien, M., et al., 1981; Blum, N.A., et al., 1991; Connolly, P. et al., 1990; Valderrama, E., et al., 1995; BeMent, S.L., et al., 1986; Kim, C., et al., 1996). These devices have enhanced the study of neural signaling significantly. Arrays of sites can be easily produced on such sensors, allowing the study of multiple groups of neurons concurrently. In addition, the silicon substrate enables the inclusion of active electronics, providing low noise, on-chip amplification and multiplexing to reduce lead count. Silicon micromachining techniques allow the final shape of the devices to be tailored to the application, from fine needle-like probes to three-dimensional arrays. To date, these devices have provided only neuroelectrical signals but no chemical data.

Carbon fibers affixed in pulled-glass micropipettes are among the most common devices for neurochemical sensing. The pipettes are drawn under heat and broken, exposing the carbon fibers at the tip, which form the electrode (Krüger, J., 1991; Stamford, J.A., 1992). Electronics can be used to allow the detection of both electrical and chemical signals. Unfortunately, their manufacture makes it difficult to insure consistent performance. It is also difficult to create an array, since the sensors are manufactured individually. Further, it is difficult, if not impossible, to place there discrete carbon electrodes stereotactically in close proximity to each other. Amplification must be performed using discrete components, requiring long interconnects that are subject to noise.

SUMMARY OF THE INVENTION

There is provided a recording device having at least one potentiometric electrode, at least one amperometric set of electrodes, wherein all electrodes are located on the same recording device. Also provided is a single locus recording device having at least one potentiometric electrode, at least one amperometric set of electrodes, wherein all electrodes are located on the same recording device. Also provided are on-chip integrated electronics to control and monitor the sensors within the array.

DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention can be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is the schematic representation of a sensor array with rectangular electrode geometry, A) is a 3 electrode conformation where the counter electrode serves the dual function as a potentiometric electrode and B) is a 4 electrode conformation;

Figure 2 is a block diagram for the present invention;

Figure 3 is a schematic of the analog multiplexer used for the amperometric and potentiometric sensors;

Figure 4 A-C are schematics of the ultra-low noise potentiometric electrode instrumental amplifier block, A) Simple Instrumental Amplifier, B) Instrumental Amplifier with high input impedance and variable gain where gain does not affect Common Mode Rejection Ratio (CMRR), C) Buffered differential-input Instrumentation Amplifier;

Figure 5 is a schematic of the ultra-low noise amperometric current-to-voltage conversion block with on-chip control of gain and filter;

Figure 6 is a diagram of the analog-to-digital conversion and digital to analog conversion block with optional sample and hold circuitry;

Figure 7 is a schematic of the amperometric sensor function generator block with on-chip control of amperometric signal parameters;

Figure 8 is a schematic of the amperometer controller function block;

Figure 9 is a diagram of a thin film heater;

Figure 10 is a cross-sectional view of the nitride shelf used to prevent solution from forming a dual contact or short circuit to the electrode;

Figure 11 is a photograph of a packaged device wire-bonded and epoxied into a pin grid array carrier;

Figure 12 is a graph showing the combined chemical and electrical signal from a sensor site demonstrating stimulator and inhibitory potentiometric (action potential) influences on neurotransmitter;

Figure 13 is a graph showing the combined chemical and electrical signal from a sensor site, the amperometric signal reveals the release of neurotransmitter in response to the action potentials shown in potentiometric recordings, demonstrating the phenomenon of neural potentiation;

Figure 14 is a diagram of the active sensor array used as an analyte electrophoretic separation and analysis system;

Figure 15 is a photo micrograph of a packaged device wire-bonded into a pin grid array carrier before sealing with epoxy without circuitry;

Figure 16 is a photograph of a cell culture chamber siliconed to the pin grid array carrier which houses the sensor array;

Figure 17 is a graph of cyclic voltammogram of Dopamine in cell culture medium;

Figure 18 is a graph of a Dopamine does response curve, the neighboring 650mV peak is unaffected by Dopamine concentration and represents a different electroactive compound in the culture media;

Figure 19 is a graph showing the combined chemical and electrical signal from a single sensor neuron, the potentiometric signal provides "action potential" data, the amperometric signal represents the resultant release of neurotransmitter;

Figure 20 is a photograph of dozens of mammalian cell culture chambers with incorporated interdigitated amperometric and potentiometric sensor arrays;

Figure 21 is a is a photograph of the carrier printed circuit board and prototype sensor controlling/monitoring circuitry;

Figure 22 is a photo micrograph of 2 mm x 2 mm "windows" surrounding the sensor array, constructed of inert silicone, intended to constrain the cells over the sensor arrays;

Figure 23 is an image of the computer screen during an experiment employing two sensors; cyclic voltommograms for each sensor are displayed in the windows labeled "Sensor A" and "Sensor B"; the line graphs below the

voltommograms represent current (proportional to concentration) at various voltages (350, 550, and 725 mV, indicated by vertical markers over the cyclic voltammograms, and by color or patter the line graph) versus time;

Figure 24 is a graph of superimposed traces of oxidatively derived current v. time, transduced by all 16 amperometric sensors in the array simultaneously;

Figure 25 is a photograph of a 6 foot Baker EdgeGARD horizontal laminar flow hood encased with stainless steel screen and plastic sheeting to provide a Faraday cage free of electronic noise with constant temperature environment;

Figure 26 is a photo micrograph of nearly confluent hNT neuronal cells cultured on a 4μm electrode size sensor array;

Figure 27 is a photo micrograph of light field and dark field illumination of hNT cells on day 4 of incubation;

Figure 28 contains time-series graphic representations of waves of potentiometric activity (action potentials) recorded in a neural network of hNT cells demonstrating points of "pulse generation";

Figure 29 is a simplified schematic of the circuitry which provides the functionality to utilized the amperometric counter electrode as a potentiometric electrode allowing single-locus cellular recordings;

Figure 30 is a diagram of a two-stage push-pull CMOS operational amplifier; and

Figure 31 is a diagram of a generalized folded cascode CMOS operational amplifier.

DETAILED DESCRIPTION

Generally, the present invention provides a recording device having at least one potentiometric electrode, at least one amperometric set of electrodes, wherein all electrodes are located on the same recording device. More specifically, the present invention provides a device having the amperometric electrodes and potentiometric electrode in close proximity to one another on the same device, and/or a configuration where the amperometric counter electrode serves the dual purpose as potentiometric electrode providing single locus determinations.

By “support substrate”, it is meant a substrate on which a sensor array is placed or integrated. This can be made of, but is not limited to, ceramic, glass, and silicon.

"Coulometry" is the determination of charge passed or projected to pass during complete or nearly complete electrolysis of an analyte, either directly on the electrode or through one or more electron transfer agents. The current, and therefore analyte concentration, is determined by measurement of charge passed during partial or nearly complete electrolysis of the analyte or, more often, by multiple measurements during the electrolysis of a decaying current and elapsed time. Once the hydration shell has been established around the electrodes, the decaying current results from the decline in the local concentration of the electrolyzed species caused by the electrolysis.

A "counter electrode" is an electrode at which analyte is electrooxidized or electroreduced with or without the agency of a redox mediator.

It refers to an electrode paired with the working electrode, through which passes an electrochemical current equal in magnitude and opposite in sign to the current passed through the working electrode. In the context of the invention, the term "counter electrode" is meant to include counter electrodes which can also have the dual function as a potentiometric electrode (i.e. a counter/potentiometric electrode).

An "amperometric electrochemical sensor" is a device configured to detect the presence and/or measure the concentration of an analyte via electrochemical oxidation and reduction reactions on the sensor. These reactions are transduced to an electrical signal that can be correlated to an amount or concentration of analyte.

"Electrolysis" is the electrooxidation or electroreduction of a compound either directly at an electrode or via one or more electron transfer agents. An example of this includes, but is not limited to, using Glucose Oxidase to catalyze Glucose oxidation creating oxidized Glucose and Hydrogen Peroxide, where the Hydrogen Peroxide is being measured.

The term "facing electrodes" refers to a configuration of the working and counter electrodes in which the working surface of the working electrode is disposed in approximate apposition to a surface of the counter electrode.

A compound is "immobilized" on a surface when it is physically entrapped on or chemically bound to the surface.

The "measurement zone" is defined herein as a region of the sample chamber sized to contain only that portion of the sample that is to be interrogated during the analyte assay.

A "non-leachable" or "non-releasable" compound is a compound which does not substantially diffuse away from the working surface of the working and/or counter electrodes for the duration of the analyte assay.

A "redox mediator" is an electron transfer agent for carrying electrons between the analyte and the working electrode, either directly, or via a second electron transfer agent.

A "reference electrode" is an electrode used to monitor and account for voltage and drop due to medium distance in amperometric sensors, and supplies a reference potential for comparison in potentiometric electrodes.

A "second electron transfer agent" is a molecule which carries electrons between the redox mediator and the analyte (See example above).

"Sorbent material" is material which wicks, retains, or is wetted by a fluid sample in its void volume and which does not substantially prevent diffusion of the analyte to the electrode.

The "working electrode" supplies the potential source to affect oxidation/reduction.

A "working surface" is that portion of the working electrode which is coated with redox mediator and configured for exposure to sample.

By way of background, the vast majority of neural monitoring devices designed for use within neural prostheses monitor only the electrical membrane potential of multiple neurons (multi-unit recording), providing data confirming only that neurons have fired action potentials (Stamford, J.A., 1992; Kruger, J., 1991). Many neurons within the central nervous system (CNS) and the peripheral nervous system (PNS) are capable of graded secretion of neurotransmitter. Additionally, the central nervous system is composed of an heterogeneous

population of nerve cell bodies and terminals that are closely apposed and intermingled, each secreting its own class of neurotransmitter.

Recently, a few investigators have employed amperometric electrochemical analysis of neurotransmitter secretion, however, they either did not employ potentiometric monitoring of neuronal action potentials or they did so using separate electrode systems implanted adjacent to each other (Suaud-Chagny, M.F., et al., 1992). It is difficult, if not impossible, to implant an array of more than a few sets of electrodes stereotaxically within sufficiently close proximity of each other that the electrophysiological and electrochemical events can be considered to be directly related (Stamford, J.A., 1990).

The present microscopic multi-site sensor array is capable of monitoring the activity of an individual neuron both potentiometrically, and, most importantly, amperometrically. The potentiometric recordings provide information regarding the dynamics of firing rate and desensitization, while the amperometric sensors monitor the dynamics of neurotransmitter release, degradation, and re-uptake. The multi-site sensor array is constructed in microscopic dimensions on silicon wafers using complimentary metal oxide semiconductor (CMOS) technology, the same technology used to create integrated circuit chips. CMOS manufacture provides economical mass production with great precision and fidelity between batches.

The present invention teaches the incorporation of amperometer and potentiostat circuitry within the sensor's silicon substrate, located within hundreds of microns of the electrodes. The present invention also teaches the incorporation of multiplexers, sample and hold, A/D, serial drivers, etc. As opposed to employing external circuitry located remotely, this can confer to the sensors the greatest sensitivity and limit of detection possible, while minimizing noise due to transmitting high impedance signals long distances.

Further, the present invention includes the incorporation of actuator controllers directly on the sensor substrate to provide closed-loop control of devices (or muscles) based upon voluntary and involuntary neural input. Also provided by the present invention is a miniature chip neural prosthetic device that can provide autonomic and voluntary functions in persons with brain injuries/defects, spinal cord injuries, and sensory organ deficits (e.g. sound, vision, and touch).

Circuitry responsible for controlling and amplifying both amperometric and potentiometric sensors were optimized for the microscopic sensor array. A single, integrated, neurological sensing device with closed-loop control capabilities has been created. The sensor array was tested by culturing a neuronal cell line, hNT (Stratagene, La Jolla, CA, USA), directly on the silicon sensor surface. hNT cells resemble primary neuronal cultures morphologically and in density of process outgrowth and, like primary neurons, provide elaborate processes that differentiate into axons and dendrites (Lee, V., et al., 1992). These cells produce spontaneous action potentials (Personal communication with Dr. Marcus Zeller, Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, FL 33101, USA) and secrete acetylcholine and dopamine. This is an ideal model for testing the ability to monitor and correlate the dynamics between electrical action potential and neurotransmitter release since cell culture work is significantly more economical, repeatable, and controllable than *in vivo* experimentation.

The microscopic multi-site sensor array is capable of single-unit and multi-unit neural potentiometric recording with concomitant rapid neurotransmitter analysis via amperometric sensor technology. The multi-site sensor array enables investigators to address a number of scientifically and clinically important questions, and ultimately, provides the basis for functional neural prosthetic devices. The working hypothesis is that amperometric sensor techniques are capable of monitoring the dynamics of fast neurotransmitter secretory events on-line and in real-time. Currently, scientists employ potentiometric studies to monitor

gross neural activity, and/or utilize amperometric measurements from groups of neurons using relatively large electrodes.

Potentiometric studies are extremely useful for mapping the nervous system using evoked response potentials. These studies generally involve the placement of large potential recording electrodes within a sensory area of the brain, stimulating the sensory organ, and correlating the sensory fields that evoked neural electrical firing.

Electrochemical detection, specifically amperometry, has been used in the past in relatively unsophisticated applications, for example detecting and quantifying eluted molecules at the end of chromatographic columns (Kissinger, P.T. et al., 1984). The main limitations of amperometry are its low specificity and sensitivity. The present invention takes advantage of this technique's speed and overcomes its limited specificity and sensitivity: First, to enable the amperometric sensors to detect multiple neurotransmitters independently, the proposed system employs two particular forms of amperometry; cyclic and constant voltage voltammetry. Second, utilizing a micro-screen printing device, such as a New Long LS-15TV, several different selectivity membranes can be applied over the individual sensors to eliminate background measurement of unwanted compounds (such as ascorbic acid) and impart specificity onto the microscopic electrodes comprising the sensor (Goldberg, H.D., et al, 1994). Finally, by encapsulating the multi-site sensor array leads with silicon nitride, a substrate upon which neurons can be made to readily attach, the sensor array is in very close apposition to the secreting cells, such as neurons, allowing measurement of the relatively high neurotransmitter or other metabolite concentrations in the immediate vicinity of the axon, prior to degradation, dilution, dispersion, and re-uptake.

An amperometric process, cyclic voltammetry, is a technique whereby a cyclically repeated triangular waveform of potential is applied between the working and counter electrodes. Individual analytes, such as neurotransmitters, have characteristic oxidation and reduction potentials based on

their chemical moieties (Adams, R.N., 1969; Dryhurst et al., 1982). When the voltage between the electrodes reaches the oxidation potential of a particular neurotransmitter, that molecule oxidizes. Oxidation is a process whereby an electron is stripped from the molecule. The counter electrode, absorbs the oxidatively produced electrons, effectively transducing chemistry into electricity. The flow of electrons per unit of time is current, which is proportional to the number of molecules being oxidized. The voltage at which this oxidatively produced current is obtained provides information useful for identifying the analyte, such as neurotransmitter, hormone, or cellular metabolite being measured (Dryhurst, G., et al., 1982; Baizer, M.M., 1973).

At solid stationary microelectrodes operating under conditions of cyclic voltammetry the peak current in microamperes, ip, is given for a reversible electrode reaction by the Randles-Sevcik equation (Randles, 1948):

$$i_p=2.687x10^5n^{3/2}AD^{1/2}Cv^{1/2}$$

where n = the number of electrons transferred

A = the electrode area in cm^2

D = the diffusion coefficient of the electroactive species in cm^2 per second

C = the bulk concentration of the electroactive species in millimoles per liter

v = the scan rate of the applied cyclic voltage sweep in volts per second.

Cyclic voltammetry has several advantages over other amperometric techniques such as constant voltage voltammetry. During each cycle, the potential on the working electrode reverses and electrically cleans the electrode of molecules adsorbed during the previous cycle. The technique is quantitative for both oxidation and reduction (i.e. the measurement of biogenic amines or oxygen respectively) (Bard et al., 1980). Cyclic voltammetry is capable of providing further

confirmation of the identity of an analyte by measuring its reduction potential as well as its oxidation potential (Oldham, K., et al., 1989; Heineman, W.R., et al., 1989). As the electrode potential is scanned toward a negative potential, a cathodic peak is obtained due to reduction of the analyte, Ox, to form a reduced metabolite, Red, according to the following equation:

$$Ox + ne \Leftrightarrow Red$$

where one is the number of electrons transferred in the reaction (Hush et al., 1971). The voltage sweep then reverses direction and scans towards a positive potential. If the scan rate is sufficiently rapid, some of the Red produced by the cathodic sweep can still be in the vicinity of the electrodes and are reoxidized to Ox, producing the anodic peak (Adams, 1969). For completely reversible reactions, the anodic and cathodic peak potentials are separated by the potential increment:

$$E_{anodic} - E_{cathodic} = 0.059/ne \text{ Volts}$$

where one is the number of electrons involved in the oxidation and reduction (Oldman et al., 1989). If the electrode reaction is not completely reversible, i.e. stable intermediate reaction products are produced, then the peak potentials are separated by a characteristic, but more than expected value (Oldman et al., 1989; Hush et al., 1971). This is the case for ascorbate: the oxidation of ascorbate produces dehydroascorbate, a fairly stable product (Adams, 1969; Oldman et al., 1989; Rose, R.C., 1989). For totally irreversible reactions, one of the peaks disappears completely. Independent of the extent of reversibility, the anodic/cathodic peak voltage difference is constant for any particular voltage scan rate, and in addition to oxidation voltage, it can be used to determine analyte identity (Bard et al., 1980; Oldman et al., 1989).

Amperometric techniques are very sensitive to the voltage scan rate. When slow scan rates (< 200 mV/sec) are used, all of the analyte in the immediate

vicinity of the electrodes is oxidized. Sensor measurements are then limited by the diffusion of unoxidized analyte to the electrode surface. This leads to diffusion limiting current, and results in defined oxidation peaks centered around the oxidation potential of the analyte. High scan rates (> 500 mV/sec) do not permit enough time for diffusion to occur. When medium to high scan rates are used, the oxidized molecule remains in the vicinity of the electrodes and can be reduced back to its native form. This aids in identification of the analyte as discussed previously.

Cyclic voltammetry provides the ability to measure the concentrations of several molecules sequentially in a single scan, as long as their oxidation potentials differ. For example, the concentrations of dopamine, norepinephrine, serotonin, and ascorbate can all be monitored sequentially from a mixture of these compounds; the value of oxidatively-derived current flow is captured at the characteristic potentials for each analyte. Once the identity of the analyte is confirmed using cyclic voltammetry, high speed measurements (> 20 kHz) can be achieved by utilizing constant voltage voltammetry.

Constant voltage voltammetry, as the name implies, employs a single operating potential to effect oxidation. This technique is used most commonly by investigators since it is the simplest to implement, both in terms of the controlling circuitry, and especially in the data acquisition phase. One major advantage to constant voltage voltammetry is that the sensor can be sampled at a very high rate allowing elucidation of the dynamics of neurotransmitter secretion, degradation, and re-uptake. Unfortunately, the drawback to this technique is that it lacks specificity since all molecules within the vicinity of the electrodes whose oxidation potential is less than that applied to the electrodes oxidize and contributes to the value of the measurement.

To overcome this limitation, selective molecular access to the electrodes can be provided by depositing membranes on them. Several different classes of membranes are available for use. Several ion exchange materials,

such as nafion, poly(vinylpyridine), and poly(ester sulfonic acid) act as ion exclusion membranes (Brazell, M.P. et al., 1987; Baizer et al., 1973; Randles et al., 1998), allowing only uncharged molecules to gain access to the electrodes. Nafion is commonly used to greatly reduce the background signal generated by ascorbate ion, which is ubiquitously present in neural tissue. Additionally, various mixtures of cellulose acetate were prepared which act as size exclusion membranes, allowing only specific molecular weight species to gain access to the electrodes. This is critical when monitoring dopaminergic neurons. Dopamine is quickly degraded to dopac, homovanillic acid, and other break down products (Cahill, P.S., et al., 1996). While only dopamine is active as a neurotransmitter, all of the break down products oxidize at potentials very close to the parent molecule. Using a variety of decreasing size exclusion membranes on the sensors in the array, one can determine the concentration of the parent molecule (i.e. dopamine), as well as each of its break down products, uniquely.

While many electroactive molecules are found in biological systems, very few oxidize at low potentials, i.e. less than 900 mV vs. an Ag/AgCl reference electrode (Dryhurst et al., 1982). Fortunately, most neuronal and endocrine products are among the very few low-voltage oxidizing molecules and can therefore be measured without interference using this technique (Adams, R.N., et al., 1982; Dryhurst, G., et al., 1982; Dryhurst, G., et al., 1982). Neurotransmitters produced by certain neurons are not electroactive; however, they are stored in vesicles and packaged with granins and chromogranins, protective anti-oxidant molecules that sacrificially prevent neurotransmitter degradation (Winkler, H., et al., 1992; Bassetti, M., et al., 1990; Huttner, W.B., et al., 1991; Konecki, D.S., et al., 1987). If the neuronal product is not oxidizable (such as magnocellular neurons in the hypothalamus) the co-secreted molecules can be measured. Fortunately, these protective agents are secreted with the neurotransmitters concomitantly and stoichiometrically (Scammell, J.G., et al., 1993; Hinkle, P.M., et al., 1992).

The incorporation of the amperometer and potentiostat circuitry directly within the same silicon substrate upon which the sensors are created is the primary focus of the present invention. This minimizes the distance that the high impedance signals must travel to submillimeter dimensions and substantially decreases the effect from radio frequency (rf) interference. This also provides the added benefit of making the multi-site sensor array an inexpensive, disposable, stand alone analysis laboratory with direct analog and/or digital output.

The array of the present invention designed, constructed, and verified the functionality of an *in vivo* sensor device which can also be used *in vitro* or *ex vivo*, with an array of 49 potentiometric and amperometric electrodes, that selectively detects neuronal action potentials at potentiometric sites and identifies and quantifies neurotransmitter secretion at amperometric sites (Figure 1). This included the construction and testing of the amperometric/potentiometric multi-site sensor array and demonstration of its ability to uniquely monitor multiple neurotransmitter classes. Performance of the sensor array was evaluated by growing a neuronal cell line, hNT (Stratagene, La Jolla, CA), on the silicon sensor surface. hNT cells resemble primary neuronal cultures morphologically and in density of process outgrowth and, like primary neurons, go on to elaborate processes that differentiate into axons and dendrites (Lee et al., 1992). These cells produce spontaneous action potentials and secrete acetylcholine and dopamine. hNT cells are ideal to test the ability to monitor and correlate the dynamics between electrical action potential and neurotransmitter release. This cell line was selected because it is known to be spontaneously active, and requires no external stimulation. The ability to record data without stimulating neuron activity is important because it allows the study of undisturbed neuron behavior without external influence.

In summary, the prototype multi-site sensor array system demonstrated the capability of monitoring the activity of cultured neurons potentiometrically and, most importantly, amperometrically. The potentiometric recordings provide information regarding the dynamics of firing rate and

desensitization, while the amperometric sensors monitor the dynamics of neurotransmitter release, degradation, and re-uptake.

More specifically, amperometric and potentiometric sensor arrays consist of interdigitated amperometric and potentiometric sensor pads, constructed of metallic materials, such as platinum, iridium, gold, silver, silver/silver chloride, etc.

Representative example of a 4 x 4 interdigitated amperometric and potentiometric sensor arrays (Figure 1). The potentiometric sensor is located between 2 μm and 20 μm away from the amperometric sensor depending upon the size of the array. In another embodiment, the Auxiliary (Counter) electrodes can be utilized to transduce action potentials via the integrated circuitry. In this manner, both amperometric (chemical) and potentiometric (action potential) data can be transduced from a single neuronal locus.

Towards the goal of electrophysiological and electrochemical analysis of neuronal activity at a single locus, on-chip circuitry has been designed which allows the utility of the counter electrode within the amperometric sensor to also serve as a potentiometric electrode (Figure 29). Solid state switches are used to connect the counter electrode to circuitry appropriate for each type of analysis, amperometry and potentiometry, and ground the working electrode to effectively switch the amperometric circuitry off.

On-chip circuitry includes differentiators and integrators, connected to both the amperometric and potentiometric sensing systems. In this manner, digital switches can be controlled based upon several criteria. For example, when monitoring action potentials via the potentiometric sensors, firing rate (indicated by the differentiators) and firing during of episodic firing (indicated by the integrators) can be used to control external devices, such as muscle stimulators (for voluntary and involuntary actions), wheel chair controls, computer mouse movement, etc. Similar controls are constructed for the amperometric sensors, in which case the

integrators detect total amount of neurotransmitters (or hormone) secreted during a predefined epoch of time, with superthreshold values used to control external devices as described above.

The on-chip digital to analog converter circuitry allows an external computer to set the parameters the differentiators and integrators, i.e., levels of threshold, integrator bleed times, and integrator and differentiator sampling time windows.

The potentiometric sensor analog multiplexer block has circuitry which contains megahertz sample and hold circuitry capable of acquiring data from each potentiometric sensor within the array and presenting the data to the Ultra-low Noise Potentiometric Electrode Instrumental Amplifier Block (Figures 2, 3, and 4).

The digital "selects" to the analog multiplexer are utilized to independently select one of hundreds of analog channels to be fed to the output.

The inputs to the multiplexer come from each of the potentiometric sensors within the array, either directly or via signal buffers. To reduce the size, and therefore cost of the circuitry, it can be advantageous to buffer the signal after the multiplexer. However, because of the high impedance characteristics of the sensors, it is often necessary to buffer the sensor signals prior to the multiplexer. The signals can be buffered with single CMOS transistors or operation amplifier (OP AMP) or (OA) buffers (Figures 30 and 31). The impedance of the multiplexer is dependent on the input signal. Generally a multiplexer with very low impedance in the on state is required for the potentiometric signals. When the signals are buffered before the multiplexer, the multiplexer impedance requirements are not as stringent. The use of low impedance multiplexers requires certain circuitry performance trade-offs. As the impedance goes down, the leakage current increases. Due to the nature of these sensors, it is necessary to keep the leakage current low.

An ultra-low noise potentiometric electrode instrumental amplifier block, Figure 4, consists of low noise instrumental amplifiers that amplify potentiometric signals acquired from the potentiometric sensors, comparing them to a reference electrode, for example a silver/silver chloride electrode: A. Simplest Instrumental Amplifier; B. Instrumental Amplifier with High Input Impedance and Variable Gain. Gain does not affect Common Mode Rejection Ratio (CMRR); C. Buffered Differential-Input Instrumentation Amplifier.

The amperiometric sensor analog multiplexer block, Figure 3, has circuitry which contains megahertz sample and hold circuitry capable of acquiring data from each amperometric sensor within the array and presenting the data to the Ultra-low noise amperometric current-to-voltage conversion block.

The inputs to the multiplexer come from each of the amperometric sensors within the array, either directly or via the current voltage converters or signal buffers. To reduce the size and therefore cost of the circuitry, it can be advantageous to buffer the signal after the multiplexer. However, because of the high impedance characteristics of the sensors, and the very low currents being transduced, it is often necessary to buffer and/or amplify the sensor signals prior to the multiplexer. The signals can be buffered with single transistors (bipolar transistors), op amp buffers, or current-to-voltage converters. The impedance of the multiplexer is dependent on the input signal. Generally, a multiplexer with very low impedance in the on state is required for the amperometric signals. When the signals are buffered before the multiplexer, the multiplexer impedance requirements are not as stringent. The use of low impedance multiplexers requires certain circuitry performance trade-offs. As the impedance goes down, the leakage current increases. Due to the nature of these sensors, it is necessary to keep the leakage current low.

An ultra-low noise amperometric current-to-voltage conversion block, Figure 5, consists of low noise instrumental amplifiers that convert the oxidatively

derived currents transduced from the amperometric sensors into low impedance voltage, which can then be amplified.

Also provided is a representative amperometric current-to-voltage converter with voltage amplifier (Figure 5). The CF coverts the oxidatively derived current into low impedance voltage. R1 is used to set the first stage gain, and the optional C1 is used to provide low-pass electronic filtering. The OA then amplifies the low impedance voltage. R3 and R4 determine second stage amplification. The optional C2 is used to provide active low-pass electronic filtering. Valves for R1, C1, R3, R4, and C2 are all digitally selectable using the on-chip circuitry. The output of the OA can be fed to an analog-to-digital converter for digital output, or can be fed directly to a lead for low impedance analog output.

An analog-to-digital conversion unit, Figure 6, converts the analog signals acquired from the potentiometric and amperometric amplifiers and provides a digital, serial stream of data compatible with RS-232 data standards for computer interface. Alternatively, the on-chip circuitry can output low-impedance analog signals that are then fed into a commercial a/d converter, such as one from Texas Instruments, via a MCM, flip-chip configuration, or, since the analog signals have low impedance, a remotely located integrated chip.

Initially, signals from the analog multiplexer are input to the Buffers/Current-to-Voltage converters. These acquire the analog data from the multiplexer, and provide the signals to the sample and hold circuit or the Analog-to-Digital (A/D) converter. Each of these have a low impedance. The sample and hold circuitry can improve the signal in two ways. First, it can eliminate the switching noise caused by the multiplexer as it changes from one channel to another. Second, many channels can be sampled at the same time and later the A/D converter can convert the analog signals to a digital format usable by a computer. High-speed A/D converters can be used if the sampling rate must be very fast, though the number of bits is often reduced (i.e. 12-bits). If extremely high speeds are not required, more accuracy can be obtained (i.e. 16-bits).

The digital-to-analog conversion unit acquires RS-232 standard data from a computer to control:

i) gain, offset, sampling frequency, sampling order, etc. from the potentiometric electrodes, and

ii) voltammetry control signal type (i.e. ramp, cyclic, chrono, pulse voltammetry, etc.) for the amperometric sensor function generator block, as well as controlling gain, offset, sampling frequency, sampling order, etc. from the amperometric electrodes.

An amperometric sensor function generator block, figure 7, acquires data from the Digital-to-Analog converter unit and provides the specified voltammetric stimulation signal to the amperometric controller functional block (Amperometer).

A selected representative amperometric sensor function generator circuit is also provided by the present invention (Figure 7). The circuitry presented is capable of providing triangle waves for cyclic voltammetry (via the Schmidt Trigger) with variable amplitudes (symmetric or non-symmetric) at selected frequencies. The Potential Source can be used to provide an offset voltage in combination with any of the other techniques (such as cyclic voltammetry) as well as being able to provide the signal for chrono- and pulse-voltammetry. The Ramp Integrator can provide ramp voltages to the working electrode with a variety of voltages and frequencies, i.e. sawtooth signals. All parameters are adjustable through the on-chip digital interface to the controlling computer. In addition to the analog circuitry presented, the Amperometric Sensor Function Generation can occur off chip with either standard analog circuitry, or through digital-to-analog conversion generated by a computer.

The amperometric controller functional block (Figure 8) provides the voltage controlling source, with feedback control to adjust for varying osmolarity of

the solution, to provide precise control of the voltage applied to the amperometric sensors within the amperometric sensor array.

In the representative amperometric controller circuitry (Figure 8), F1 serves as an impedance matching follower for the amperometric controlling signal profile (e.g. cyclic voltammetry, chrono potentiometry, pulse voltammetry, etc.). The control amplifier (CA) sends the stimulating voltage to the working electrode. F2 serves as a follower, receiving the potential from the reference electrode, and provides feedback through R1 to the CA to compensate for voltage drops due to alterations in the osmolarity of the solution between the electrodes. S1 is the sampling point to determine the actual voltage applied to the working electrode. This signal can be fed to an analog-to-digital converter for digital output, or can be fed directly to a lead for analog output.

. A thin film heater (figure 9), is used to provide incubation temperatures (37C) to maintain mammalian cells, or higher temperatures to increase reaction rates in non-cellular studies. A capacitor is used to provide power to the thin film during transport from incubator to experimental station when the chip is disconnected from a power source.

A thermal sensor is located directly under the sensor pads (and the cells) to provide closed loop feedback control of the thin film heater. The thermal sensor can be a thermocouple, thermistor, or simple temperature dependant transistor.

Utilizing CAD software that interfaced directly with the CMOS manufacturing hardware, multiple designs were prepared for the potentiometric and amperometric sensors, including rectangular, circular, and concentric circle shapes. The designs were computationally simulated using the CAD packages providing anticipated resistances and capacitances of individual electrode structures and leads. Each electrode conformation was analyzed and re-designed to optimize the electrode structure and interconnect prior to manufacture.

As described in the previous section, prototype chips incorporating rectangular, circular, and concentric circle shaped electrodes were produced (Figures 4 and 5).

Five conformations of sensor arrays, each incorporating 49 electrodes, were constructed using electrode sizes of 2, 4, 8, 32, and 100μm. Three electrodes compose each amperometric sensor (reference, working, and counter). Diagrams of two embodiments of the sensor array are presented in Figure 1.

The resulting sixteen sensor sites are arranged into a 4 x 4 array. The surface of the counter and working electrodes are platinum to provide a polarizable contact to solution, but the surface can also be made of other metals such as iridium, gold, etc. The reference electrode included in each sensor site and the global reference electrode were coated with silver (Ag) and electrolytically chloridized to provide reversible Ag/AgCl electrodes. The electrodes are connected to the active circuitry using Al or Cu interconnects.

The inventors of the present invention developed and implemented an alternate fabrication process to chloridize Ag reference electrodes in a batch-wise manner. The fabrication process entails the use of a reactive ion etch (RIE) plasma as a chloride source. This technique allows wafer level chloridation of all reference electrodes within each sensor array at once, prior to separating the silicon wafer into individual chips. This methodology eliminates the necessity to provide electrolysis current during chloridation and improves the accuracy and precision of the silver chloride fabrication process.

Conventional techniques used to fabricate Ag/AgCl electrodes, described in the literature, generally employ passing a small electrolyzing current through chlorine salt or dilute hydrochloric acid. The current is applied to an

electrode coated with elemental Ag. AgCl is created in a layer on the electrode surface via a redox reaction with the solution containing Cl^- ions.

The electrolytic technique suffers from several limitations. It is difficult to perform in a controlled manner on more than one electrode at a time. This can be quite time consuming in situations where arrays of Ag/AgCl electrodes are to be created. More importantly, it is extremely difficult to produce consistent results from electrode to electrode, requiring that each sensing site be calibrated independently to provide an accurate reference potential during use. The inconsistencies occur because the current flow, and therefore chloridization rate, is dependant on resistance of the electrical conducting line, surface area, and potential. All of these parameters are slightly different for every electrode, especially if several sites are chloridized at the same time. In addition, for the devices that use on-chip readout electronics attached to the Ag/AgCl electrode, these electronics must take into account the electrolytic procedure to prevent circuit damage, or must be designed to participate in the procedure itself, requiring special connections that must later be severed.

An additional technique for the chloridization of the electrodes is to use chemical chloridization. Chloridization can be accomplished in a $FeCl_3$ solution. While this technique can be used to chloridize many sites at the same time, it is not selective, it produces an inferior Ag/AgCl layer, and in has a very fast chloridization rate that is difficult to control with the extremely thin Ag layer associated with planar electrodes. Additionally, small amounts of iron can be left as a contaminant on the sensor, affecting sensor transduction, as well as acting as a cellular toxin.

The schematic diagram in Figure 1 illustrates that electrode orientation was altered from site to site. This permitted combination of electrodes from adjacent sites to act as a single larger electrode providing more flexibility to detect, verify, and analyze the effect of electrode size on the electrochemical response curves.

Subsequent to CMOS fabrication the sensor arrays were fabricated in a three-mask process with two metal layers selected from the following metals: Ag, Au, Ir, Pt, etc. Since these metals are difficult to etch using wet chemistry, a resist lift-off process was used to pattern them. This provided an additional advantage in allowing the use of layered materials in a metal structure to modify electrode properties and still allowed for patterning to occur in one step (see cross section presented in Figure 10).

Previous experimentation has revealed a tendency for the silver to delaminate from underlying metal layers. To prevent this, a thin layer of titanium (Ti) was deposited in order to enhance adhesion. There was some concern that the use of a highly reactive metal that is exposed to solution could effect lifetime, zero offset, and corrosion resistance in the final devices, however, the prototype sensors developed did not significantly suffer from these limitations since the silicon nitride encapsulation prevented exposure of the Ti to solution. The last two sensor masks are reversed, hence sealing the Ti edge and preventing the possibility of these effects.

The metal lift-off technology used was improved with a short undercut etch to promote easier release of excess material. After depositing the lift-off photoresist and patterning it, a short etch was performed on the underlying dielectric. This etch was a wet HF acid etch of the silicon dioxide, in the case of the platinum metallization, and a reactive ion etch (RIE) of the silicon nitride for the Ag sites. This etch performs two functions: First, since the resist tends to be undercut in a wet etch, a small gap is formed under the resist edge. After metal deposition the gap forms a natural breakline during lift-off that prevents metal, coating the sidewalls of the resist, from remaining behind on the device forming spikes or walls. These spikes can be very difficult to cover completely in later dielectric depositions and could form short circuits to solution. Second, if the etch is timed properly it planarizes the sensor topography by submerging the metal layer into the dielectric (Einspruch, N.G., 1987).

The Ag layer is on top of a PECVD silicon nitride encapsulating layer. Since wet etches for silicon nitride are difficult to control and react with metalization used in circuit processes, an RIE machine was used. Due to the vertical directional nature of an RIE, it does not provide the undercut and gap described above in a wet etch. However, the steep sidewalls created in the dielectric enhanced the lift-off effect and the addition of the recessed metal layer results in a device that is more planer than if no etch was performed.

The sensor chips were packaged in ceramic 100-pin, pin grid array (PGA) packages (Spectrum Semiconductor Materials, Inc., San Jose, CA) with gold plated pins and interconnects (Figure 11). The ceramic package body offers several advantages when used in tissue culture. First, ceramic material is heat resistant allowing autoclave temperatures required for sterilization. Second, the thermal properties of ceramic material provide temperature stability when used for *in vitro* studies. During the short period when moving the chips from the incubator to the horizontal laminar flow hood (experimental station) the ceramic material acted as a thermal buffer minimizing temperature fluctuations at the cells.

The completed wire-bonds are quite fragile and can form an electrical connection to the growth media if they were exposed. Therefore, an epoxy material, Epoxy Patch 1C (Dexter Corporation), was used to seal the bonds. The bonded ceramic packages were heated to approximately 130C. Epoxy Patch was carefully applied to the hot packages with the heat causing the material to liquefy and flow easily around the bonds to seal them. After curing at 130C for 30 minutes the result was a hard, durable coating that provided excellent electrical isolation and completely resisted moisture exposure.

Many physiologically important molecules, such as hormones and neurotransmitters, including dopamine and serotonin, are electroactive. The application of a potential causes electrons to be removed from these species as they undergo electrochemical oxidation. The observed current is directly related

to the concentration of the species. The number of species that can be detected is somewhat limited however. For example non-electroactive species, such as acetylcholine, are not detectable using voltammetry; however electroactive metabolites of such species sometimes can be detected. In order to be detected by amperometry, the species need to have oxidation voltages in a narrow range. For platinum electrodes with a silver/silver chloride reference, this range is approximately -0.8 V on the low end, where dissolved oxygen in the solution begins to reduce, and about 1.2 V at the high end, where water begins to oxidize. These signals overwhelm any other response that might otherwise be observed.

Cyclic voltammetry, a form of amperometry, is a technique where a ramped potential is applied to the test solution while recording the induced oxidation current. The potential is applied to the solution using a system of three-electrodes: working, counter, and reference. Using a potentiostat, the potential between the working and counter electrodes is precisely controlled by applying voltage to the working electrode. The circuitry is designed to draw very little current through the reference electrode. By placing the reference electrode in close proximity to the working electrode, the potential drop across the solution can be accounted for. This removes artifacts due to conductivity or osmolarity changes in the test solution, hence increasing accuracy (Bard, A.J., et al., 1980).

As the potential is increased, the current drawn through the counter electrode is recorded. When the oxidation potential of each electroactive species present in the solution is reached, a corresponding current increase, caused by oxidation of the species, is observed. The oxidation potential at which this oxidation current is observed is measured between the reference and the working electrodes. When sites are to be stimulated in unison, they can share a common reference electrode and counter electrode.

Cyclic voltammetry extends the voltage sweep by reversing the potential, causing the newly oxidized species to undergo reduction. The reduction cycle provides additional information for the identification of species and also

mediates some of the toxicity of the chemicals produced during the oxidation scan (Stamford, J.A., 1992; Stamford, J.A., 1990).

Constant voltage voltammetry, as the name implies, employs a single operating potential to effect oxidation. This technique is used most commonly by investigators since it is the simplest to implement, both in terms of the controlling circuitry, and especially in the data acquisition phase. One major advantage to constant voltage voltammetry is that the sensor can be sampled at a very high rate allowing elucidation of the dynamics of neurotransmitter secretion, degradation, and re-uptake. Unfortunately, the drawback to this technique is that it lacks specificity since all molecules within the vicinity of the electrodes whose oxidation potential is less than that applied to the electrodes oxidizes and contributes to the measurement.

Fast sweep rates can provide near real-time analysis. A baseline curve must be obtained before testing begins to record the charging currents of the electrodes, allowing them to be removed from the final results. A separate nearby electrode can be used for recording electrical signaling, or a grounded counter system can provide the capability of recording both signals from the same electrode (Stamford, J.A., 1991; Stamford, J.A., et al 1993).

Data from a single potentiometric sensor, presented in Figure 12, provide evidence of inhibitory as well as stimulatory effects of action potentials on neurotransmitter secretion. The trailing edge of the secretory event, at approximately 0.715 seconds, is much steeper than those observed in other experiments (Figure 13). Potentiometric activity immediately prior to and during the trailing edge of neurotransmitter secretion appear to cause an inhibitory effect, possibly due to axon-axonal input or some other such mechanism. The fact that the potentiometric activity during the trailing edge of secretion is approximately half the magnitude of those that stimulate neurotransmitter secretion indicates that, most likely, at least two different neurons are being monitored. The lower magnitude potentiometric measurements can also indicate that the neuron (or

population of neurons) responsible for inhibition can be further away from the recording electrode than the neuron (or population of neurons) responsible for the stimulatory effect. If this phenomenon was monitored only potentiometrically, the action potentials which caused inhibition of neurotransmitter secretion can have been incorrectly interpreted as stimulatory. These data can be interpreted correctly through correlation of potentiometric with amperometric data, as demonstrated by the sensor array.

The above discussion provides a factual basis for the use of the sensor array. The methods used with and the utility of the present invention can be shown by the following non-limiting examples and accompanying figures.

EXAMPLES

General Methods:

Generally, a substrate 10 supports a matrix or array of electronically addressable microelectrodes (Figure 14). A permeation layer can be disposed above the individual electrodes 12. The permeation layer permits transport of relatively small charged entities through it, but reduces or limits the mobility of large charged entities, such as cellular contaminants or DNA, to preferably keep the large charged entities from easily contacting the electrodes 12 directly during the duration of the test. The permeation layer 14 reduces the electrochemical degradation which can occur due to proteins and/or DNA by direct contact with the electrodes 12, possibly due, in part, to extreme pH resulting from the electrolytic reaction. It further serves to minimize the strong, non-specific adsorption of proteins and DNA to electrodes. Attachment regions 16 are disposed upon the permeation layer 14 and provide for specific binding sites for target materials. The attachment regions 16 can be effectively incorporated into or integrated with the permeation layers, such as by including attachment material directly within the permeation material.

In operation, reservoir 18 comprises that space above the attachment regions 16 that contains the desired, as well as undesired, materials for detection, analysis or use. Charged entities 20, such as homogenized cells and charged DNA are located within the reservoir 18. In one aspect of this invention, the active, programmable, matrix system comprises a method for transporting the charged material 20 to any of the specific microlocations 12. When activated, a microlocation 12 generates the field electrophoretic transport of any charged functionalized specific binding entity 20 towards the electrode 12. For example, if the electrode 12 were made positive and electrode 12 negative, electrophoretic lines of force can run between the electrodes. The lines of electrophoretic force cause transport of charged entities 20 that have a net negative charge toward the positive electrode 12. Charged materials 20 having a net positive charge move under the electrophoretic force toward the negatively charged electrode 12. When the net negatively charged binding entity 20 that has been functionalized contacts the attachment layer 16, as a result of its movement under the electrophoretic force, the functionalized specific binding entity 20 can become covalently attached to the attachment layer 16. Optionally, electrodes can be disposed outside of the array. The electrodes can optionally serve as return electrodes, counterelectrodes, disposal (dump) electrodes or otherwise. Optionally, a flowcell can be provided adjacent the device for fluidic containment.

One embodiment of the invention utilizes focusing electrodes, and optionally, transport electrodes. The device 20 includes a substrate 10, which can be of any sufficiently rigid, substantially nonconductive material to support the components formed thereon. The substrate 10 can be a flex circuit (e.g., a polyimide such as DuPont Kapton, polyester, ABS or other such materials), a printed circuit board or a semiconductive material, preferably with an insulative overcoating. Connectors couple to traces, which in turn, couple to other electrical components of the system. These components can be any form of conductor, such as copper, or gold, or any other conductor known to those skilled in the art. Various connectors are shown unconnected to traces or other electrical components. It is appreciated by those skilled in the art that not every connector

34, such as in a system adapted to mate with an edge connector system is utilized. Additionally, traces can be of differing widths depending upon the demands, especially the current demands, to be made on that trace. Thus, some traces can be wider, such as those being coupled to the focusing electrodes in comparison to those traces coupled to the microelectrodes within the array.

A first collection electrode and counter electrodes are disposed on the substrate. These components generally fit within the footprint of the flow cell, and comprise a relatively large percentage thereof, preferably at least substantially 40%, and more preferably substantially 50%, and most preferably substantially 60%. The counter electrodes (sometimes functioning as return electrodes) and collection electrode are preferably disposed at or near the periphery of the flow cell footprint, and can substantially circumscribe, e.g., to 80%, the footprint perimeter.

Typically, the collection electrode and counter electrodes are disposed on the substrate 10 so that the electrophoretic lines of force are significant over substantially all, e.g., 80% or more, of the flow cell volume. By way of example, the concentration and counterelectrodes can be disposed near the periphery of the footprint of the flow cell. In yet another embodiment, they can be disposed at substantially opposite ends of the flowcell footprint (See, e.g., FIG. 14). In yet another embodiment, the counterelectrode substantially circumscribes the footprint of the flow, with a centrally disposed collection electrode. The relatively large percent of coverage of the flow cell footprint and its position aids in effective electrophoretic interrogation of the flow cell contents.

Returning to FIG. 14, focusing electrodes are disposed on the substrate to aid in focusing materials collected on the collection electrode to the array. The focusing electrodes are preferably disposed in a mirror-image, "Y" or "V" shaped pattern, the open end encompassing, at least in part, the collection electrode. As shown, there are two symmetric focusing electrodes. One focusing electrode can be utilized, or more than two focusing electrodes can be utilized. As

shown, the focusing electrodes include substantially parallel portions (adjacent the array) and angled portions (adjacent the transport electrodes and optionally, the collection electrode) extending in a symmetrical manner enveloping the transport electrodes. Stated otherwise, there are first and second electrodes being disposed at least in part adjacent the array of microlocations, the distance between the first and second electrodes adjacent the array being smaller than the distance between the first and second electrodes in yet another region disposed away from the array. The focusing electrodes can optionally include portions which are disposed on the opposite side of the array from the collection electrode. The focusing electrodes are preferably coupled to leads which are relatively larger than the leads coupled to the array, so as to permit the carrying of effective currents and potentials.

Transport electrodes are optionally included. Electrodes of monotonically decreasing size as they approach the array can be used. A first transport electrode is relatively smaller than the collection electrode, the second transport electrode is relatively smaller than the first transport electrode, and the third transport electrode is yet smaller still. The differential sizing serves to reduce current density mismatches between locations, and aids in reducing or eliminating burn-out which can result if too great a current density mismatch exists. Transport efficiency is maximized. The ratio of sizes of larger to smaller is preferably substantially 2 to 1, more preferably 3 to 1, and can be even greater, such as 4 to 1 or higher.

The seven steps of the field shaping protocol serve to effectively interrogate the sample volume and to collect materials onto the array for analysis. In the first step, interrogation of the sample volume is effected through negative bias of the counterelectrodes and positive bias of the first collection electrode 40. The placement of the counterelectrodes and collection electrode generally near the periphery of the footprint of the flow cell permit the rapid, effective interrogation of that sample volume. Secondly, with the collected material adjacent the collection electrode, that electrode is made negative (repulsive) to materials of

interest, while the first transport electrode is made positive (attractive). The repulsion and attraction effects transport of materials from the collection electrode to the first transport electrode. Additionally, the focusing electrodes are biased negative. Such a negative (repulsive) bias serves to provide a force which can be lateral to the direction of transport, thereby more centrally concentrating material in the solution. Thirdly, with material collected at the first transport electrode, that electrode can be biased negative (repulsive), while the second transport electrode is biased positive (attractive). The focusing electrodes can be biased negatively, which serves to provide a repulsive force on the charged materials, thereby providing a transverse component to their direction of motion and collecting the material within a smaller physical region or volume. Fourth, the second transport electrode can be biased negative, as well as optionally biasing of the first transport electrode, to effect transport away from those electrodes and to the now positively biased third transport electrode. Again, the focusing electrodes can retain their negative bias. The next three steps are optionally separated, as described, to transport materials to various rows or regions of the array.

The field shaping protocol includes currents and biased times. In this embodiment, there is an inversely proportional relationship between the size of the electrode and the amount of current supplied to it. Further, for the collection electrode and transport electrodes, there is an inversely proportional relationship between the electrode size and the bias time, that is, the smaller the electrode, the larger the bias time. Through this protocol, the current density at various devices is kept relatively more uniform, or substantially similar to each other. Further, as the current from a given electrode decreases (relative to a larger electrode) a relatively longer bias time can be required in order to provide transport of effective amounts of charged material between the various electrodes. Stated otherwise, for a given amount of charged material, a relatively longer bias time can be required to effect transport of a given amount of material at a lower current.

The traces can be of the same width or of varying width, such as where a relatively wider trace can be utilized for larger current carrying capacity (e.g., traces to first collection electrode and second collection electrode).

In another embodiment, the first collection electrode is a trapezoid, which has a long base adjacent to and parallel to one side of the array and top, which is preferably shorter than the base, with sloping sides, tapering wider (away from each other) toward the base. The second collection electrode is disposed on the other side of the array, and is similarly (though not necessarily identically) shaped and sized. Top is preferably shorter than base, and accordingly, the sides are non-parallel and slope away from each other, moving towards the array. Optionally, the electrodes can be of different sizes, such as where the area of the first collection electrode is approximately 10% smaller (optionally approximately 20% smaller) than the second collection electrode. Input port electrode and port electrode are optionally included on the substrate, within the footprint of the flow cell. The input port electrodes and port electrode are either of the same size or of different size.

In operation, the flow cell contents are interrogated by placing or biasing one of the first and second collection electrodes attractive (typically positive) to the materials to be collected. Once collected, the materials can be transported away from the first collection electrode towards the array. The materials can be effectively held in place over the array, such as by application of AC fields such as at a frequency in the range from 0.01 to 10^6 Hz, most preferably between 0.1 to 10^3 Hz between the electrodes. Then materials can be transported to the other electrode or can be repeatedly reacted by moving materials from the array to the electrodes.

Optionally, the microelectrodes of the array can be electrically active or passive. The device, substrate, connectors, traces and array are as previously described, with the exception that the array can be arranged concentrically. A concentric return electrode and central concentration electrode, preferably round,

coact to concentrate material at electrode, and then to move it over or position it above the array.

In the above embodiments, capture sequences or probes can be disposed on the devices. Preferably these are at least on the collection or concentration electrodes. Optionally, different sequences are disposed on different devices such as the transport electrode. For example, each sequence as an approach is made to the array can be more specific.

A device includes a support substrate having a first surface (optionally called the top surface) and a second surface (optionally called the bottom surface), which can be of materials suitable for the function of support and conduction, such as flex circuitry, printed circuit board, semiconductive material or like material. Contacts lead to traces, which lead to the second substrate. This second substrate can also be referred to as the flipped chip. This second substrate can optionally be a chip, system or support on which assays or other diagnostic materials are provided. Contacts, such as bump contacts, e.g., solder bumps, indium solder bumps, conductive polymers, or silver filled epoxy, provide electrical contact between traces and the chip or substrate. A sealant is disposed between the second (bottom) surface of the support substrate and the first (top) surface of the second substrate. Generally, the opposing faces of the support substrate and second substrate are those which are placed in fluid-blocking contact via the inclusion of a sealant. An inlet port can be in conductive relation to a sample chamber, which yet further leads to the assay chamber, and on to the outlet port. The lateral width of the via is less than the lateral width of the second substrate.

In the preferred embodiment, the device is formed of a minimum number of components to reduce cost, improve manufacturing simplicity and reliability or the like. One embodiment is achieved in substantially five components. While the device can be fabricated with five components, the addition of components which do not detract from or vary the inventive concept

can be utilized. These components are as follows. First a support substrate having a first surface and second surface, and a via between the first surface and second surface to permit fluid flow through the substrate, the second surface supporting electrical traces. Second, a second substrate including at least a first surface, the first surface being adapted to be disposed in facing arrangement with the second surface of the first substrate, the second substrate including electrically conductive traces connecting to an array of microlocations, the array being adapted to receive said fluid through the via. Third, electrically conductive bumps interconnecting the electrical traces on the second surface of the support substrate and the electrical traces on the first surface of the second substrate. Fourth, a sealant disposed between the second face of the support substrate and the first face of the second substrate, said sealant providing a fluidic seal by and between the first substrate and the second substrate. Fifth, a flowcell is optionally disposed on the first surface of the first substrate. While the number of elements can vary, advantages can be obtained from selection of these five elements.

In operation, a sample is provided to the inlet port and passed to the sample chamber. The sample chamber can serve to house various sample processing functions, including but not limited to cell separation, cell lysing, cell component separation, complexity reduction, amplification (e.g., PCR, LCR, enzymatic techniques, and/or denaturation). Thereafter, the sample flows to the assay chamber. Solution containing sample flows down through a via. A space is formed comprising the via, bounded on the bottom by the second substrate, with sealant or adhesive forming a barrier between the interface of the second surface of the support substrate and the first surface of second substrate.

In the preferred method of manufacture, a light curable sealant is wicked or otherwise provided to the interface between the second surface of the support substrate and the first surface of the second substrate. Light is provided through the via. A dam is formed, stopping the advance of the sealant, thereby maintaining the array substantially free from sealant or adhesive. By the appropriate sizing of the lateral width of the via, the via serves essentially as a

shadow mask for the incident light, which serves to cure the sealant. Alternatively, the sealant can be supplied to the interface between the second surface of the support substrate and the first surface of the second substrate in an amount and with a viscosity such that it does not flow onto the array. Further or final curing of the sealant can be performed as required, such as by heating.

The electrical regions can be contained wholly or partially within the chip or substrate, such as through the provision of semiconductive regions. These semiconductive regions can be controlled in an active manner so as to provide selective connections within the chip or substrate. Typically, the first surface is that surface on which the active biological interactions takes place. Optionally, an edge illumination member can be disposed adjacent to and substantially coplanar with the first surface of the chip or substrate. The illumination sheet preferably includes holes, vias or pathways to permit electrical interconnections to pass therethrough. The illumination sheet can be disposed directly over conductive traces or can be directly affixed to the adjacent supporting sheet. Electrical traces can be included on the first surface of the substrate or chip. An electrically conductive element, such as a solder connection, indium bump, conductive polymer or the like couples the conductive pathway on the substrate to the conductive portion of the contact trace. The contact trace preferably is then contacted by a conductive member, such as a wire, whisker wire, or other electrical contact, for connection to the remainder of the circuit. Sealant is preferably disposed between the substrate or chip and the next layer, such as the flex support layer.

Other conductive members, not disposed in the plane of the cut, are included and provide further mechanical support between the substrate and the trace support layer. Further, the edge illumination layer includes a terminal edge, which is disposed toward the upper surface of the substrate or chip. The edge illumination layer can terminate outside of or inside of the sealant. An adhesive layer is disposed adjacent the trace support layer, and provides adhesive contact to an upper layer. The upper layer can optionally include pathways, indentations, or other cutouts, such as shown for an inlet and an outlet. As shown, the adhesive

layer can optionally be a die-cutable adhesive material, such as one which includes release paper on both the top surface and the bottom surface prior to assembly. Suppliers of such materials include 3M or Dupont. The die-cutable adhesive material can be cut so as to form all or a part of the wall of a chamber. The geometry can be made in any desired shape or flow cell configuration.

Preferably, a top member is provided. The top member can extend substantially over the remainder of the device. Optionally, the top member can form a window or other containment surface at the top of the flow cell chamber. Preferably, the top material is formed from polycarbonate or polystyrene. It is advantageous that the array of test sites are accessed optically through the top member, and accordingly, it is desirable to form the top member from materials which are substantially transparent to both the excitation and emission radiation.

The flow cell chamber region is characterized by substantially parallel sidewalls (Figure 14). Preferably, a first decreasing width region is provided between the flow cell region and the output. Most preferably, the decreasing region begins with a width D', most preferably where D'=D, and decreases to a width d', preferably where d'=d. The height of the inlet chamber decreases from the inlet at a height H to a lesser height h at the inlet to the flow cell chamber. Preferably, the decrease is monotonic, and most preferably, linear.

In the preferred embodiment, the height h of the inlet chamber and the width w are chosen such that a substantially constant flow area is provided, that is, the product of the height h and the width w ($h \times w$) is substantially constant. Thus, at the portion of the inlet chamber adjacent the inlet, while the height h is relative large, the width w is relatively small. Correspondingly, when proceeding through the inlet chamber towards the flow cell chamber, as the width w increases, the height h decreases. Preferably, the outlet chamber includes substantially the same geometry, and preferably the same flow cell area constant.

A support substrate is generally planar, and includes a first face and a second face. A via permits fluid or solution flow from above the support substrate to the second substrate, particularly to the first surface of the second substrate. Sealant is provided between the second face of the support substrate and the second substrate. The sealant provides a preferably fluid tight-seal, so as to permit fluid flow to the array on the second substrate. A source of illumination, such as a laser beam, illuminate the array on the second substrate. Preferably, the system includes a waveguide with an input adapted to receive illumination from the source, and to provide illumination via output. The waveguide is preferably co-planar with the support substrate, and can be secured to it, such as by being adhered to the second surface of the support substrate. Electronics can be included to control the system. Optionally, surface mounted electronic components can be included on the substrates. Fluidics can be provided in combination with the system to aid in provision of the sample to the second substrate.

Typically, the array is formed of rows and columns, more typically an equal number of rows and columns, yet most typically in an orthogonal arrangement for rows and columns. For example, an array of 10×10, 20×20 or more can be formed with these techniques. The individual unit cell of the array of unit cells is selected by action of selectors such as a row selector and a column selector. The selectors can be a memory, such as a shift register memory, or a decoder, or a combination of both. An input for address information receives addresses, typically from off-chip, though on chip address generators can be utilized. In the preferred embodiment, the row selectors comprise shift registers, either in a by one configuration (x1), or in a wider configuration, such as a by four configuration (x4).

In operation, the selection registers are sequentially loaded with values indicating selection, or not, of a unit cell, and optionally, the value of output for that cell. Optionally, memory can be provided to retain those values so as to continue the output from the unit cell.

An array includes a plurality of unit cells. In the preferred embodiment, the unit cells are arranged in rows and columns. The designation row or column can also refer to a group or subset of unit cells, such as a portion of a row or column, or a group or set of unit cells which are not linearly contiguous. In general, there are m rows and n columns of unit cells, typically where m=n, and m=2, 3, 4 . . . . By way of example, a 5×5 matrix of unit cells, a 10×10 matrix of unit cells and a 20×20 matrix of unit cells provides for a total number of unit cells of 25, 100 and 400, respectively.

The unit cells are addressed by action of at least one row selector and at least one column selector. This detailed description begins with the case of a single row selector and column selector, and later describes the use of additional selectors. Row selector receives input information and outputs a row selection signal on one or more row lines. The selection signal on the row line is supplied to the unit cell, and interacts therewith such as through a row contact.

In typical implementation, the row line is electrically continuous, though can be made of any combination of materials. For example, the row line can be one continuous conductive line, such as formed of conductive polysilicon, or can be a combination structure such as where conductive segments are electrically connected via a higher conductivity material, such as metal, i.e. aluminum or copper.

The column selector receives an input for determining the selection of a column, or in the preferred embodiment, the value (or correlated value) of the output at the unit cell. The column selector is coupled to the column lines which serves to provide a column select signal to the unit cells. In the preferred embodiment, the column selector selects more than two states (e.g., four states), preferably voltage states, which are supplied via the column line to the unit cell. The column line is coupled to the unit cell, such as through a column contact. In

the preferred embodiment, the column contact can be a control gate for a transistor, such as a field effect transistor.

If required for activation of the unit cell, a second row selector, input lines, second row lines and column contacts can be included. Likewise, a second column selector can be added, having an input, and being coupled to secondary or supplemental column lines which in turn are coupled to secondary column contacts.

The row selectors and column selectors, optionally include an enable input or chip select. One of the functions of these signals is to permit entry of input information without the activation of a row line, or column line. Further, some or all of the row selectors and column selectors can include an output which can be used for output of information. In one application, the output value can be a signal or bit, such as the most significant bit of a series, indicating that the input data has been successfully loaded into the row selector, or column selector. Optionally, this output information can be utilized to then trigger the enable or chip select signals.

The row selectors and column selectors function to receive row and column input information and to use that to select one or more unit cells, and optionally, to provide signal values indicative of the level of current (potential) to be provided from the unit cell. The selectors can be in the form of memory, such as in the form of a shift register memory, or can be in the form of a decoder circuit, such as where the desired addresses are provided as input information and the output is then in a decoded relationship thereto. Numerous circuits are known to those skilled in the art to effect this functionality.

A current source, such as a current mirror, optionally receives a source of current and a control signal (VCASP). Connections couple the current from the source to column selector, and if present, second column selector. Further, one or more sources of current can be supplied. The value of the current can be static, or can vary over time (such as in the application of a pulsed

waveform, sinusoidal waveform, square wave, sawtooth, etc.). Generally, any desired varying waveform can be utilized.

Each of the unit cells can be activated at a given time. Alternatively, certain unit cells can be activated and yet other unit cells remain inactive. By way of example, if a given column has been selected at a first value, each of the unit cells within that column which are associated with one or more selected rows selected by the row selector can be activated at the value corresponding to that level of the voltage on the column. Yet other unit cells within that same column can be placed at the same or a different level by coupling to the second column selector, where the one or more row lines associated with those unit cells are driven by the second row selector. Thus, within one column of unit cells, each unit cell can be either driven at a value corresponding to the signal on the column associated with the column selector, or with the value on the column associated with the second column selector, or be in an undriven, unconnected, floating or high impedance state. In a like manner, other columns can be set to desired levels of output. In this way, the entire array of unit cells can be placed in the desired state or set of states.

The use of the terms `levels of output` and `desired state` include signals which vary as a function of time. Additionally, more values within a given column can be added, such as through the addition of further column selectors and column lines which are coupled to selected unit cells.

A variable current control element includes an input, an output and a control element. The control element is coupled to a line, such as the column line, which is in turn coupled to the column select. A selector switch includes an input, an output and a control element. The control element is coupled to a control line, such as a row line. The output of the variable current control element is coupled to the input of the select switch. The output of the select switch couples to a node which provides the output current, I_{out}. A first potential, e.g., Vcc, is provided to input of the variable current control element.

In operation, application of a signal on row line to the input of the select switch provides a conductive path between node and output of the variable current control element. The signal value applied to the column line, which is coupled to the input of the variable current control element, serves to provide a variable amount of current flowing through the series connected variable current control element and select switch between the first potential node and the output node. A return electrode serves to complete the circuit, though it can be appreciated that the return electrode can be yet another unit cell.

In the preferred embodiment, the variable current control element is a transistor, such as a field effect transistor, and most particularly a MOSFET. The select switch is preferably a transistor, more preferably a field effect transistor, and most particularly a MOSFET. Various types of particular implementation can be utilized, whether P-MOS, N-MOS, CMOS, bipolar, gallium arsenide, or otherwise, so long as consistent with the functional requirements of the system. Further, in the preferred embodiment, a matching arrangement of a second variable current control element and second select switch couples between a second potential and the output node. Optionally, channel lengths of the various devices can be arranged such that a symmetric arrangement is implemented. For example, in a CMOS implementation, the p-channel select device can have a shorter channel length than the n-channel device, to compensate for the differing electron/hole mobility. (e.g., 80 μm v. 126 μm channel length).

The discussion regarding the circuit, above, applies to the circuitry including the second variable current control element and second select switch.

EXAMPLE 1:

The neural arrays consist of sixteen sites of three-electrodes. Each incorporates platinum working and counter electrodes, and a chloridized silver reference electrode. Two schematics of sensor arrays are shown in Figure 1. Six

different versions of the sensor array were fabricated, with minimum electrode dimensions ranging from 2 to 100 μm, to facilitate the study of electrode size on performance. Individual site orientation is alternated. This allows neighboring sites to be used in unison as a single larger electrode, providing additional information about the effect of electrode surface area on performance.

Fabrication begins on standard 4" <100> silicon wafers. Standard CMOS circuit processes are utilized to fabricate the circuitry. A modified lift-off procedure is used for patterning the Pt electrode sites. A timed etch in hydrofluoric acid submerges the area where the platinum is applied. This results in a nearly planar surface, improving the coverage of the top isolation layer. A 20 nm layer of titanium is used to improve adhesion of the 100 nm platinum layer.

A top isolation layer of 800 nm of PECVD silicon nitride is deposited over the platinum, and a reactive ion etch opens the contacts. The resist for the silver lift-off step is then applied. However, before depositing the silver, a second reactive ion etch is performed to etch 700 nm of the nitride layer. This leaves a shelf of nitride around the bottom of the silver sites as shown in Figure 10. As discovered in previous developmental work using lift-off patterned silver at these dimensions, projections of silver coating the sidewalls of the lift-off resist caused difficulties. These silver projections could break off as the sensors were handled, leaving small pieces of silver behind, which could cause contamination of the platinum electrode surfaces. The nitride etch forms a break line at the resist edge, preventing the formation of the silver projections. This etch is halted prior to reaching the platinum layer to prevent the formation of a seam between the silver sidewalls and the surrounding silicon nitride. This seam could provide a route of encroachment for the test solution, resulting in a dual contact.

Next, a 650 nm thick layer of silver is applied with a 20 nm layer of titanium as an adhesion promoter. The final lift-off is performed in acetone. Early work using Microposit 1112A photoresist remover (Shipley, Marlboro, MA, U.S.A.) caused discoloration of the silver reference electrodes. Acetone is more inert to

the silver, however, it lacks surfactants to prevent re-deposition. Some care is required to insure that all of the waste metallization is completely removed.

Finished chips are wire-bonded into ceramic 100-pin, pin grid array (PGA) packages (Figure 11) (spectrum semiconductor materials, Inc., San Jose, CA, USA). The ceramic package provides both the high I/O count for contact to the array and a large thermal mass. The cultured neurons are very sensitive to thermal disturbances. The ceramic package forms an adequate thermal buffer to allow the neuron cultures to be moved from an incubator into a test apparatus without temperature variation.

Epoxy is used to seal the wire bonds, connecting the sensor array clip to the PGA, from the solution. Figure 15 shows a bonded device before sealing. Culture cylinders are then attached to the surface to provide containment of the culture medium and cells. Finally, a small plastic petri dish with a hole in the bottom, is affixed over the culture cylinder (Figure 16). This outer petri dish provides an isolated sterile environment for the culture media. The resulting package system can be autoclaved for easy sterilization before use.

The culture cylinder was infused with growth medium for initial testing. After initial scans to establish the charging currents for the electrode array, calibrated amounts of neurotransmitter were added to the culture cylinder, and cyclic voltammograms were taken. The temperature was carefully maintained by incubation to match actual neural culture conditions as closely as possible.

Three distinct peaks appeared in the dopamine calibration voltammograms (Figure 17). The peak at 500 mV is related to dopamine concentration. The peak with a potential greater than 1 V could have been caused by the oxidation of the phenol red in the culture medium. The chemical species causing the peak at 650 mV is unknown at this time. As the dopamine concentration was increased, the 500 mV peak responded, while the other two peaks remained unchanged, demonstrating that the devices could distinguish

dopamine from other electrochemically active species present in the culture media (Figure 18). Excellent linearity for the 500 mV peak is observed over the tested range, while the 650 mV peak remains unchanged.

Live cell studies were performed with human neuron cultures (hNT cell line from Stratagene, La Jolla, CA, U.S.A.). This cell line was selected because it is known to be spontaneously active, and requires no external stimulation. The ability to record data without stimulating neuron activity is important because it allows the study of undisturbed neuron behavior without external influence. These hNT neurons are also known to release dopamine, providing chemical signals detectable by amperometry.

Twenty-eight packaged neural arrays were sterilized and prepared with cultures. After six days of incubation, 24 of the cultures were electrically active. The cultures were monitored for 75 days, at which point the experiment was terminated. For these preliminary tests, the internal counter electrode was replaced with a single external electrode. This simplified instrumentation by allowing the internal counter electrodes to be used to detect neuroelectrical signals, but required all the sites to be swept in unison while performing cyclic voltammograms. In another embodiment of the present invention, the electrode configuration is in either a three electrode or four electrode array (Figure 1). This embodiment enables the amperometric and potentiometric electrodes to work either respectively, sequentially or in parallel. Cyclic voltammograms were used to identify species and oxidation voltages, while constant voltage voltammetry was used for concentration measurement over time. The use of constant voltage voltammetry simplifies analysis and provides real-time monitoring capabilities.

The results demonstrate the ability to detect dopamine concentrations and to differentiate between dopamine and other electroactive species. Detection of serotonin and other electroactive neurotransmitters is possible since several are in the oxidation range of the device. The ability to concurrently record both neurochemical and neuroelectrical activity has been

demonstrated (see Figure 13). The figure shows two groups of spike activity representing neuronal action potentials at about 9.21 and 9.25 seconds, each followed by an increase in oxidation current, signifying an apparent neurotransmitter release.

The data show an example of neuron behavior known as potentiation. Potentiation is a behavior in which a neuron fires initially to prime or activate a particular neural system, with subsequent activation causing a heightened response as a result of the initial electrical firing. The capture of such an event demonstrates the capability of the device to study cause/effect relationship between neuronal electrical and chemical communication.

The devices have sufficient resolution to capture the cause-effect interaction of a single action potential and the resulting neurotransmitter release (see Figure 19). Correlations between action potentials across the array were observed as well, showing propagation of signals through the neural network.

The present invention provides an *in vivo, in vitro,* and *ex vivo* sensor device with an array of microscopic potentiometric and amperometric electrodes capable of selectively detecting neuronal action potentials at the potentiometric sites and permits identification and quantification of neurotransmitter secretion at the amperometric sites (Figure 1).

Several hundred sensor arrays were prepared as noted above: 28 were packaged and tested, eight each with 2, 4, and 8μm electrode structures and four with 32μm electrode structures (Figure 20).

Two printed circuit boards (PCB) were designed and constructed to provide a simple and easy to use interface between the sensor bearing ceramic carrier and the potentiometric and amperometric circuitry (Figure 21). The PCBs contain a zero insertion force (ZIF) socket that allows rapid swapping of the ceramic carrier, and a series of connector rails that provide access to the circuitry

within the sensor array (Figure 21). The PCB is a three-layer board with the inner layer constituting a ground plane to help reduce environmental noise.

In an effort to increase the amount of useful data that could be acquired from the sensors, it was decided that a single, platinum electrode can replace the working electrode present in each amperometric sensor. In a likewise manner, the single reference strip was utilized. The only limitation this produced was that each amperometric sensor would have to cycle in concert for the cyclic voltammograms. This was not an issue since concerted cycling was intended. The main advantage provided by this alteration was the availability of each of the former working electrodes to act as potentiometric electrodes. In this manner, it was possible to monitor 16 amperometric sensors (both with constant voltage and cyclic voltammetry) and 16 potentiometric sensors simultaneously. Further, the physical distance between the amperometric and potentiometric electrodes was very small (4 to 20μm depending upon the electrode size) providing the ability to monitor cellular action potentials and neurotransmitter release in extremely close proximity to each other. Alternatively, the amperometric sensors can be cycled independently, in which case the proximity of the electrodes must be carefully monitored to prevent interference between the electrodes.

Initially, the amperometric and potentiometric circuitry was constructed on breadboards allowing manipulation of the components responsible for gain, filtering, etc. (Figure 21). Using this technique, a variety of circuitry parameters were easily altered and optimized to provide relatively noise free data from the individual electrodes within each of the sensor arrays. The optimized circuit layout is constructed on the sensor arrays' silicon substrate, just hundreds of microns away from the electrodes. This significantly reduces the amount of interconnect wires (from meters to microns) and significantly increases the signal to noise ratio. Further, the circuitry responsible for monitoring from 16 potentiometric and 16 amperometric electrodes decreased in area by a factor of approximately 100.

The 16 channels of output from the potentiometric sensor circuitry were connected to 16 channels of input on a high speed, 16-bit, National Instruments a/d board (PCI-MIO16XH) housed in a PowerPC Macintosh computer. Similarly, the 16 channels of output from the amperometric sensor circuitry were connected to 16 channels of input on another high speed, 16- bit, National Instruments a/d board (NB-MIO16XH) housed in a separate Macintosh computer. A NB-MIO16XH board, housed in a second computer, was utilized to achieve the goal of monitoring from 32 total sensors, 16 amperometric and 16 potentiometric, simultaneously. Data synchronization was assured by sharing a single clocking signal between both National Instruments boards. A digital trigger was used to initiate acquisition in the two a/d converters within 100 nanoseconds of each other.

The maximum sampling rate of the NB was less than that of the PCI board. Therefore, the PCI board was used to monitor the potentiometric sensors that transduced the neural action potentials (the highest speed component) while the NB board was used to monitor the amperometric sensors that transduced the secreted neurotransmitters (the slower speed component). The sample rates were maintained at the maximum that each board allows while maintaining whole number multiples of each other, simplifying direct comparison of data from each computer system. When monitoring each of the 32 sensors, a/d sampling rates were 6kHz for the potentiometric sensors and 2kHz for the amperometric sensors. When cellular activity was detected on a potentiometric/amperometric set of sensors, that subset of sensors was monitored at higher sampling rates. For example, 16 sensor (8 potentiometric and 8 amperometric) were monitored at 12kHz for the potentiometric and 4kHz for the amperometric. When a single potentiometric and amperometric pair were monitored, the potentiometric rate was 60kHz and the amperometric rate was 20kHz. A second PCI-MIO16XH board can be placed in a single computer to provide a 1:1 ratio of sampling rate between the potentiometric and amperometric sensors. Alternatively, A/D converters are constructed on-chip for direct R5232 Digital Communication to the PC.

The high speed, 16-bit, d/a converter in the PCI-MIO16XH board was utilized as the voltage source for the amperometric controller circuitry. Software was written to provide a variety of user-selectable schemes for cyclic voltammetry (selectable parameters included control of voltage range and cycle frequency) and for constant voltage voltammetry (selectable parameters included control of initial and final voltage composing the voltage step function as well as delay before onset and duration of the step function). Alternatively, the on-chip circuitry can be utilized to control the amperometric circuitry.

Once the sensor was secured within the ceramic carrier, a layer of inert Dow Corning Silicone RTV Sealant 732 (World Precision Instruments, Inc., Sarasota, FL) or other such biocompatible inert sealant, was applied over the sensor array leaving a 2mm x 2mm window over the actual sensor array (Figure 22). A small, sterile cloning cylinder (Fisher Scientific, Chicago, IL) was placed over this window and attached with silicone. Finally, a 35mm petri dish (Fisher Scientific, Chicago, IL), with a hole drilled in the bottom to accommodate the cloning cylinder, was attached (Figure 16). This arrangement provides several features: 1) the petri is clear allowing observation of the culture medium, sensors, and cells; 2) the ability to transport the cells (cultured within the carrier chip) from the incubator to the horizontal laminar flow hood where the experimentation was conducted without compromising sterility; and 3) provides free transport of metabolic gasses (CO_2, O_2) and, at the same time, minimizes evaporation of the culture medium while housed in the incubator.

The biological testing of the sensors was accomplished using hNT neuronal cells. hNT neuronal cells require a basement membrane matrix for cell attachment to the sensor surface. Poly-D-lysine and Matrigel matrix (Becton Dickinson Labware, Bedford, MA) were used to provide a basement membrane for the cells.

The sensor array was first coated with poly-D-lysine to promote bonding of the Matrigel matrix. Sterile poly-D-lysine, at a concentration of 10µg/ml

in distilled water, was applied to each of the 2mm x 2mm exposed sensor arrays and allowed to incubate at room temperature for 2 hours. The poly-D-lysine solution was then aspirated with a sterile pipette. The sensor arrays were placed at an incline with lids off in a sterile laminar flow hood and allowed to dry for 1.5 hours.

Matrigel matrix was thawed overnight in a refrigerator, and then diluted to 1:40 in cold DMEM/ F12 (Life Technologies, Rockville, MD). 20µl of the Matrigel matrix was applied to each sensor array and spread evenly using a Pasture pipette. The solution was allowed to completely dry at room temperature in a sterile laminar flow hood. The Matrigel application was then repeated.

The sensor arrays could be stored for at least 2 months with one coat of poly-D-lysine and one coat of Matrigel matrix. The final coat of Matrigel matrix must be applied on the day of use. Under the microscope, a dry coated sensor appeared to have a fine, frost-like mesh. Care was taken to avoid opaque clots indicating the Matrigel matrix concentration was too high.

The sensor arrays were tested for their ability to monitor multiple neurotransmitters simultaneously and independently. These measurements were conducted using hNT conditioned DMEM/F12 culture medium (Stratagene, La Jolla, CA), the same medium used throughout the biological experimentation. In all cases, the medium was equilibrated for temperature and CO_2 in a mammalian cell incubator for 30 minutes prior to testing.

Dose response curves for dopamine (Sigma-Aldrich Co., St. Louis, MO) were generated by performing cyclic voltammetry utilizing the microscopic sensor arrays. Oxidatively derived currents generated at the unique oxidation potential for dopamine, approximately 500mV versus silver/silver chloride reference, were stored and plotted as a dose response curve in Figure 18. Since acetylcholine is not oxidized at lower voltages, the dose response curves for dopamine were unaffected by the presence of even super-physiological

concentrations of acetylcholine. Independent of the concentration of acetylcholine, the value of the oxidatively derived current at 500mV varied in a linear manner with dopamine concentration.

Figure 23 is an image of the computer screen showing a single cyclic voltammogram (y-axis oxidatively derived current versus x-axis excitation voltage) in the main window. The peaks in oxidatively derived current can be visually discerned. The peak at 500mV corresponds to the oxidation of dopamine. The other two peaks at 650mV and 1080mV remained essentially constant in amplitude regardless of neurotransmitter concentrations and are most likely attributable to components within the DMEM/F12 culture medium. When phenol red was not present in the culture medium, the peak at 1080mV disappeared. The molecular source of the peak at 650mV is currently under investigation. Again, the value of the oxidatively derived current at these voltages did not significantly vary in any of the dose response, control, or cellular experiments (Figure 17).

During the generation of dose response curves, transduction properties of individual sensors within the sensor array were compared to one another to insure uniformity. Since the electrode structures are created using electron beam lithography, physical parameters of each electrode, such as size and surface area, are nearly identical. The uniform transduction properties afforded by the uniform physical geometries are demonstrated by nearly identical cyclic voltammograms seen at each of the 16 amperometric sensors (Figure 24).

In another embodiment of the present invention, a six-foot EdgeGARD horizontal laminar flow hood (The Baker Company, Sanford, Maine) was modified for use in data acquisition. The hood was lined with stainless steel small mesh screen. The screening, when grounded, provided a nearly electronic noise-free environment within the hood, essentially mimicking an expensive Faraday cage. The front opening of the laminar flow hood was also covered with the screen material. An access door in the form of a retractable flap was constructed for easy access to the experimental setup (Figure 25). This is not

required for the sensor array to function properly, but can be used in association with the sensor of the present invention. This is not required because the internal circuitry of the sensor array of the present invention reduces noise and provides incubation for heat and temperature control.

The laminar flow hood was also modified to provide a heated, constant temperature environment (37C) to maintain normal physiological activity within the cultured cells. Towards this goal, the hood was lined with an additional layer of plastic sheeting to make the inner chamber nearly air tight. A standard hair dryer, controlled by an inexpensive digital temperature regulator (Fisher Scientific, Chicago, IL), was used as the heat source. The hair dryer was located outside of the Faraday cage to minimize electronic noise at the sensor arrays. The heated air was transported to the interior through a 4-inch diameter metal dryer duct. The thermocouple probe for the temperature controller was placed in close proximity to the cells. This inexpensive setup provided a constant temperature environment with approximately 0.5C temperature fluctuations.

Most important, the experimental setup allowed rapid transfer and electrical connection of the culture chambers with integrated sensor arrays to the electronic circuitry. Using this setup, one could easily monitor dozens of culture chambers with integrated sensor arrays daily.

hNT cells were procured from Stratagene (Catalog #204104, Lot #0980822) in a highly purified, frozen state. The hNT cells were thawed and plated, according to Stratagene recommended procedures, on the sensor arrays at a concentration of approximately 8×10^5 cells/cm^2. Several cultures were prepared as previously reported with the exception of plating hNT cells. These cultures served as controls.

Electrochemical monitoring of the cultured hNT cells began the day following plating. At this point, day 1, the cells had spread out into a monolayer with approximately 70-80% confluence (Figure 26). Note that the cells spread

almost exclusively upon the sensor array coated with Matrigel matrix as opposed to the silicone. No measurable activity was observed until day 4.

By day 4, the hNT cells resembled primary neuronal cultures morphologically and in density of process outgrowth and, like primary neurons, exhibited elaborate processes that differentiated into axons and dendrites (Figure 27). On day 4, one culture was observed to spontaneously exhibit action potentials and neurotransmitter release. By day 5, one third of the cultures were observed to spontaneously exhibit action potentials and neurotransmitter release. Finally, by day 6, twenty-four of the twenty-eight cultures were observed to spontaneously exhibit action potentials and neurotransmitter release. Experimentation was continued for a total of 75 days. Approximately every 5 days, one culture was taken apart and observed microscopically. Of the cultures remaining, all those that were previously active remained active for the entire 75 days. Even though the cultures were still active, experimentation had to be terminated at the end of the 75th day due to lack of available funds.

Due to the multitude of electrical connections in the passive devices (wire-bonding of the sensor chip to the ceramic carrier, electrical connections at the zero insertion force socket, cabling to the potentiometric and amperometric circuitry, breadboard connections of the circuitry, and finally cabling to the analog-to-digital converter) there was a component of external electronic interference present in data transduced by the sensors. Through the use of analog bandpass filters and oversampling techniques the noise was significantly reduced. Additionally, the signals were digitally filtered during data analysis. The electronic noise is resolved by incorporating potentiometric and amperometric circuitry directly onto the silicon sensor array substrate utilizing CMOS processing thereby drastically reducing the multitude of required electrical connections previously identified. Alternatively, the analog signals can be converted to digital either using circuitry on chip, or via external A/D converteres located in MCM or flip chip, configurations or remotely located. An additional benefit is reduction in the length of cabling from meters to microns. Upon integration of electronics within the

revised sensor array, the signals emanating from the sensor chip are composed of low impedance amplified voltages. These signals can be transmitted by wire several meters without signal degradation.

The inventors of the present invention created the initial design of a wireless telemetry system for the sensor arrays. The digital, wireless, telemetry system provides enormous benefit when these sensor arrays are utilized *in vivo*. The telemetry system provides a greater degree of patient acceptance, especially when the sensor arrays are used to monitor neurons to provide closed loop control of remote electronics and/or muscles.

Data from a single potentiometric sensor, presented in Figure 12, provide evidence of inhibitory as well as stimulatory effects of action potentials on neurotransmitter secretion. The trailing edge of the secretory event, at approximately 0.715 seconds, is much steeper than those observed in other experiments figure 13. Potentiometric activity immediately prior to and during the trailing edge of neurotransmitter secretion appear to cause an inhibitory effect, possibly due to axon-axonal input or some other such mechanism. The fact that the potentiometric activity during the trailing edge of secretion is approximately half the magnitude of those that stimulate neurotransmitter secretion indicates that, most likely, at least two different neurons are being monitored. The lower magnitude potentiometric measurement can also indicate that the neuron (or population of neurons) responsible for inhibition can be further away from the recording electrode than the neuron (or population of neurons) responsible for the stimulatory effect. If this phenomenon was monitored only potentiometrically, the action potentials which caused inhibition of neurotransmitter secretion can have been incorrectly interpreted as stimulatory. These data can be interpreted correctly through correlation of potentiometric with amperometric data, as demonstrated by the sensor array.

Thousands of episodes were recorded in which neurotransmitter secretion could be observed in response to a single action potential (Figure 19).

In the experiment which generated the data presented in Figure 19, the sensors responsible for the potentiometric and amperometric signals were merely 6μm apart. The potentiometric data were acquired at 12kHz and the amperometric data were acquired at 4kHz. There is less than 1msec delay between the action potential and the resultant secretion of neurotransmitter.

In most of the cultures, intracellular communication was observed between the neurons. Waves of communication were monitored in which a single neuron (or group of neurons) acted as a pulse generator with subsequent cells responding in-kind. These waves of neuronal activity were monitored across the entire span of the sensor array (Figure 28). Figure 28 was generated by dividing the potentiometric signals into 150msec periods and integrating the data within each period. Higher integration values indicate greater action potential activity. The value of each integral was then quantized into six equally weighted categories and assigned a gray scale value: black indicating greatest activity and white indicating no activity. These waves were modeled and simulated in the form of impressive QuickTime™ videos.

The neural array described herein combines the advantages of both the silicon neurosensors and the carbon fiber electrodes, while eliminating most of the complications. The devices are fabricated using standard silicon-processing technology. This provides high yield and consistent performance due to the tight dimensional control of photolithography. High density sensor arrays facilitate the study of small neural systems in addition to individual neurons. Using these devices, real-time neurochemical *and* neuroelectrical activity of the neurons can be recorded. This provides the important capability of studying the correlation of electrical and chemical events in neuronal communication.

Summary

In summary, the microscopic sensor array is able to monitor neuronal activity by capturing action potentials and neurotransmitter secretion simultaneously from

single neurons. There has been identified four potential commercial products that resulted directly from this Phase I effort: 1) the amperometric/potentiometric microscopic sensor array; 2) optimized amperometric circuitry system capable of controlling and monitoring microscopic amperometric sensors; 3) optimized potentiometric circuitry system capable of monitoring microscopic potentiometric sensors; and 4) the software system that provides online and real-time storage, analysis, and display of sensor data.

EXAMPLE 2:

As shown in Figures 2 and 3, the digital "selects" to the analog multiplexer are utilized to independently select one of hundreds of analog channels to be fed to the output.

The inputs to the multiplexer (Figure 3) come from each of the potentiometric sensors within the array, either directly or via signal buffers. To reduce the size, and therefore cost of the circuitry, it can be advantageous to buffer the signal after the multiplexer. However, because of the high impedance characteristics of the sensors, it is often necessary to buffer the sensor signals prior to the multiplexer. The signals can be buffered with single CMOS transistors or OP AMP buffers. The impedance of the multiplexer is dependent on the input signal. Generally a multiplexer with very low impedance in the on state is required for the potentiometric signals. When the signals are buffered before the multiplexer, the multiplexer impedance requirements are not as stringent. The use of low impedance multiplexers requires certain circuitry performance trade-offs. As the impedance goes down, the leakage current increases. Due to the nature of these sensors, it is necessary to keep the leakage current low.

The digital "selects" to the analog multiplexer are utilized to independently select one of hundreds of analog channels to be fed to the output (Figure 3).

The inputs to the multiplexer come from each of the amperometric sensors within the array, either directly or via the current to voltage converters or signal buffers (Figure 3). To reduce the size and therefore cost of the circuitry, it can be advantageous to buffer the signal after the multiplexer. However, because of the high impedance characteristics of the sensors, it is often necessary to buffer the sensor signals prior to the multiplexer. The signals can be buffered with single transistors (bipolar transistors), op amp buffers, or current-to-voltage converters. The impedance of the multiplexer is dependent on the input signal. Generally a multiplexer with very low impedance in the on state is required for the amperometric signals. When the signals are buffered before the multiplexer, the multiplexer impedance requirements are not as stringent. The use of low impedance multiplexers requires certain circuitry performance trade-offs. As the impedance goes down, the leakage current increases. Due to the nature of these sensors, it is necessary to keep the leakage current low.

Figure 5 depicts representative amperometric Current-to-Voltage converter with voltage amplifier. The CF converts the oxidatively derived current into low impedance voltage. R1 is used to set the first stage gain, and the optional C1 is used to provide low-pass electronic filtering. The OA then amplifies the low impedance voltage. R3 and R4 determine second stage amperometric. The optional C2 is used to provide active low-pass electronic filtering. Values for R1, C1, R3, R4, and C2 are all digitally selectable using the on-chip circuitry. The output of the OA can be fed to an analog-to-digital output, or can be fed directly to a lead for analog output.

Initially, signals from the analog multiplexer are input to the Buffers/Current to Voltage converters that take the analog data from the multiplexer and allow the signals to be used in the sample and hold circuit, or the Analog-to-Digital (A/D) converter, which have a lower impedance. The sample and hold circuitry can improve the signal in two ways. For example, it can eliminate the switching noise caused by the multiplexer as it changes from one channel to another. Many channels can be sampled and stored at the same time,

and later the A/D converter can convert the analog signals to a digital format usable by a computer. High-speed A/D converters can be used if the sampling rate must be very fast, though the number of bits is often reduced (i.e. 12-bits). If extremely high speeds are not required, more accuracy can be obtained (i.e. 16-bits). An analog-to-digital conversion unit converts the analog signals acquired from the potentiometric and amperometric amplifiers and provides a digital, serial stream of data compatible with RS-232 data standards for computer interface (Figure 6). Alternatively, the on-chip circuitry can output low-impedance analog signals that are then fed into a commercial A/D converter, such as one from Texas Instruments, via a MCM, flip-chip configuration, or, since the analog signals have low imedpance, a remotely located integrated chip.

Figure 7 depicts a selected representative Amperometric Sensor Function Generator circuit. The circuitry presented is capable of providing triangle waves for cyclic voltammetry (via the Schmidt Trigger) with variable amplitudes (symetric or non-symetric) and frequency. The Potential Source can be used to provide an offset voltage in combination with any of the other techniques (such as cyclic voltammetry) as well as being able to provide the signal for chrono- and pulse-voltammetry. The Ramp Integrator can provide ramp voltages to the working electrode with a variety of voltages and frequencies. All parameters are adjustable through the on-chip digital interface to the controlling computer.

In addition to the analog circuitry presented, the Amperometric Sensor Function Generation can occur off chip with either standard analog circuitry, or through digital-to-analog conversion generated by a computer.

Figure 8 depicts representative Amperometric Controller circuitry. F1 serves as an impedance matching follower for the amperometric controlling signal profile (e.g. cyclic voltammetry, chrono potentiometry, pulse voltammetry, etc.). The CA sends the stimulating voltage to the working electrode. F2 serves as a follower, receiving the potential from the reference electrode, and provides feedback through R1 to the CA to compensate for voltage drops due to alterations

in the osmolarity of the solution between the electrodes. S1 is the sampling point to determine the actual voltage applied to the working electrode. This signal can be fed to an analog-to-digital converter for digital output, or can be fed directly to a lead for analog output.

A thin film heater is used to provide incubation temperatures (37C) to maintain mammalian cells, or higher temperatures to increase reaction rates in non-cellular studies (Figure 9). A capacitor is used to provide power to the thin film heater during transport from incubator to experimental station when the chip is disconnected from a power source.

A thermal sensor is located directly under the sensor pads (and the cells) to provide closed loop feedback control of the thin film heater. The thermal sensor can be a thermocouple, thermistor, or simple temperature dependant transistor.

EXAMPLE 3

Towards the goal of electrophysiological and electrochemical analysis of neuronal activity at a single locus, on-chip circuitry has been designed which allows the utility of the counter electrode within the amperometric sensor to also serve as a potentiometric electrode (Figure 29). Solid state switches are used to connect the counter electrode to circuitry appropriate for each type of analysis, amperometry and potentiometry, and ground the working electrode to effectively switch the amperometric circuitry off.

To provide physiological relevance, the solid-state switches must be able to switch between amperometric and potentiometric mode of operation in timeframes less than 10μsec. Solid state transistors are capable of switching states in times on the order of 350 psec. Further, during an electrochemical scan, the software must automatically diable the potentiometric amplifiers and short the output to ground for the duration of the electrochemical scan (between 2 and 20

msec depending upon the cyclic voltammetry scan rate, or between 0.01 and 4 msec when utilizing constant voltage voltammetry)

When the potentiometric electrode system is activated, immediately after the electrochemical scan, a switching transient is generated by the working electrode as it regenerates the dipole hydration shell. This transient is minimized by the fact that the electrode, and hence the hydration shell, is extremely small. Filtering circuitry has been developed to minimize the effect of the DC transient. Further, software algorithms filter the transient such that valid potentiometric data can be obtained during the transition.

Another addition to the on-chip controlling system includes the development of a triggering system that initiates fast scan cyclic voltammetry (and/or constant voltage voltammetry) when potentiometric signals indicate the presence of neuronal action potentials. This creates a free running system, requiring no user input, which monitors an individual neuron for neurotransmitter secretion in response to action potential activity.

Solid-state, high-speed switches are controlled by either direct digital input to the chip or via the on-chip analog-to-digital converter. A schematic of a representative simplified first state potentiometric amplifier circuit, with incorporated solid state switch and bandpass filter, is presented in Figure 29.

Active high-pass and low-pass filters have been designed and constructed to filter the DC potential switching transient that are generated by the working electrode as it regenerates the dipole hydration shell. Since the signals of interest are composed of AC components, the DC potential offset is removed. This allows reliable measurement from the sensors while the hydration shell re-establishes around the working electrode.

One method of operation employs the use of the time-sharing scheme between the potentiometric and electrochemical analysis of neural

activity. The duration of sampling period is controllable for each analysis technique and adjusted through computer input to the chip.

Thresholds for duration of action potential trains are established as the criterion for switching to electrochemical analysis. An automated triggering system has been developed to initiate fast scan cyclic voltammetry (and/or constant voltage voltammetry) when potentiometric signals indicate the presence of neuronal action potentials.

On-Chip Closed Loop Control Circuitry:

On-chip circuitry includes differentiators and integrators, connected to both the amperometric and potentiometric sensing systems. In this manner, digital switches can be controlled based upon several criteria. For example, when monitoring action potentials via the potentiometric sensors, firing rate (indicated by the differentiators) and firing during of episodic firing (indicated by the integrators) can be used to control external devices, such as muscle stimulators (for voluntary and involuntary actions), wheel chair controls, computer mouse movement, etc. Similar controls are constructed for the amperometric sensors, in which case the integrators detect total amount of neurotransmitters (or hormone) secreted during a predefined epoch of time, with superthreshold values used to control external devices as described above.

The on-chip digital to analog converter circuitry allows an external computer to set the parameters the differentiators and integrators, i.e., levels of threshold, integrator bleed times, and integrator and differentiator sampling time windows.

Calibration Techniques

Using custom developed test circuitry, the instrumentation amplifiers and buffers are performance tested prior to use. During this testing, gain and

offset are specified and adjusted if necessary. The buffer and gain circuitry utilized by the sensors require accurate and precise resistor settings to normalize the gain of each sensor in the sensor array. Normally this is done with analog circuits by laser trimming the resistors. This can also be accomplished using an electronically-programmable read only memory (E-PROM) on-chip or electronically erasable PROM and digitally compensate the resistors with a CMOS transistor switched resistor tree, used to digitally set the value of resistors.

Sensor Section for Recording

Due to the microscopic size of the sensors, i.e., sensors that are approximately 1/20 the size of cells, several of the sensor sites can either provide redundant data (data from a single cell) or no data at all (as in the case when the sensor does not have an apposed cell). As the number of sensors within the sensor array increases (up to thousands), the bandwidth requirements of the transferring data from each sensor to the recording computer can become greater than what is possible using current state-of-the-art technologies. Therefore, data from each sensor can be examined to determine whether it is receiving redundant cellular information, or no cellular information at all. Since each sensor within the array is independently addressable, those sensors receiving either redundant data, or no data, can be ignored by the circuitry, hence alleviating bandwidth limitations. In this manner, extremely densely packed arrays of interdigitated amperometric and potentiometric sensors can be utilized to ensure that every cell within the tissue being examined is exposed to a unique sensor within the array, and still be sampled at sufficiently high rates (required to satisfy the Nyquist criterion) without the burden of having to continuously monitor every single sensor. Using this methodology, the sensors can even be "over-sampled" to aid with digital signal processing algorithms capable of detecting patterns within the cellular generated data and/or filtering of the data.

Data Output From The Sensor Arrays:

Data output from the CMOS sensor array chips can be digital and/or analog. Incorporating a digital to analog converter on chip improves the signal-to-noise ratio, decreases the power requirements of the device, and reduces the overall size of the system. Sensor array chips with digital output provided through low cost parallel or serial interface to the computer or readout device are used. Another alternative employed involves the use of multi-chip modules (MCMs), where analog output from the sensor array chip is fed directly to either AST's custom analog-to-digital (A/D) converter chip or to commercially available A/D chips.

When using MCMs, the sensor array chip decreases in size and increases in yield, thus decreasing manufacturing cost. In this scenario, buffered, multiplexed, analog outputs from the sensor array chip are interfaced with low-cost, high-speed a/d converters located in either a flip-chip or side-by-side configuration, i.e., MCMs.

Once the analog signals are converted to a low impedance voltage and buffered, the signals are nearly un-susceptible to noise. Because of this, commercially available or custom analog-to-digital converters can be placed external to the sensing device. This significantly reduces the cost associated with the sensor arrays with integrated electronic controlling circuitry.

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention can be practiced otherwise than as specifically described.

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CLAIMS

What is claimed is:

1. A recording device comprising:

at least one potentiometric electrode;

at least one amperometric electrode on the device with said potentiometric electrode, wherein said electrodes are located on the same device.

2. The device according to claim 1, wherein said amperometric sensor includes at least one reference electrode, at least one working electrode, and at least one counter electrode.

3. The device according to claim 2, wherein said amperometric sensors cycle in concert with a cyclic voltammogram or independently.

4. The device according to claim 2, wherein said amperometric sensor utilizes constant voltage voltammetry.

5. The device according to claim 1, wherein said potentiometric sensor includes at least two reference electrodes.

6. The device according to claim 1, wherein said amperometric sensor and said potentiometric sensor are in close proximity.

7. The device according to claim 1, wherein said device is made of material selected from the group consisting of platinum, gold, irridium, and silver.

8. The device according to claim 1, wherein said device is made using CMOS technology.

9. The device according to claim 1, wherein said device is etched using a lift-off process.

10. The device according to claim 9, wherein said lift-off process is made by a reactive ion etch.

11. A device for measuring neurochemical and neuroelectrical activity in tissue culture, comprising:

at least one potentiometric electrode;

at least one amperometric electrode interconnected to said potentiometric electrode, wherein said electrodes are located on the same device.

12. The device according to claim 11, wherein said amperometric sensor includes at least one reference electrode, at least one working electrode, and at least one counter electrode.

13. The device according to claim 12, wherein said amperometric sensor cycles in concert with a cyclic voltammogram or independently.

14. The device according to claim 11, wherein said potentiometric sensor includes at least two reference electrodes.

15. The device according to claim 11, wherein said amperometric sensor and said potentiometric sensor are in close proximity.

16. The device according to claim 11, wherein said device is made of material selected from the group consisting of platinum, gold, irridium and silver.

17. The device according to claim 11, wherein said device is made using CMOS technology.

18. The device according to claim 11, wherein said device is etched using a lift-off process.

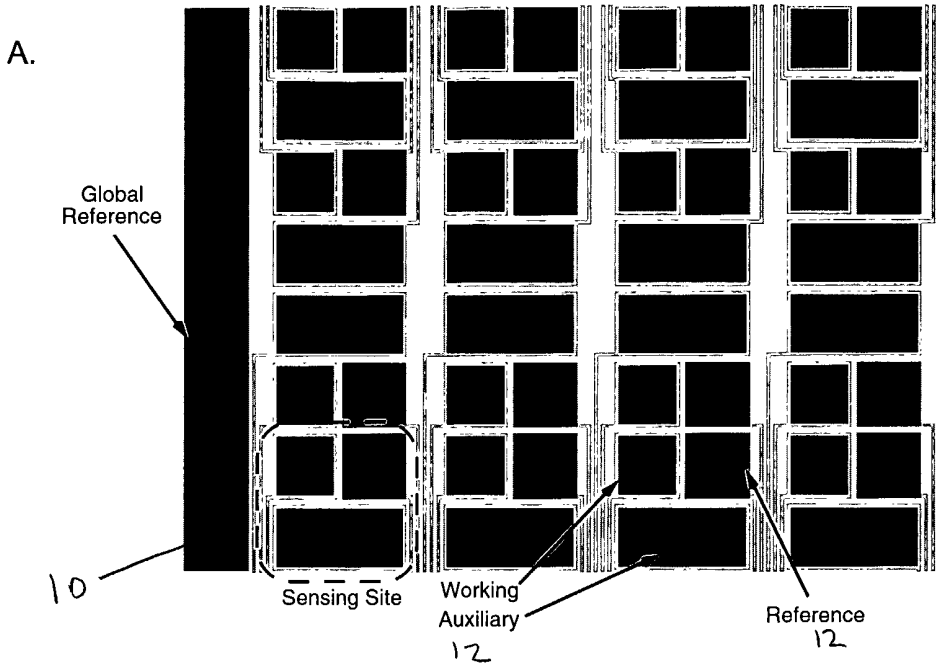
19. The device according to claim 18, wherein said lift-off process is made by a reactive ion etch.

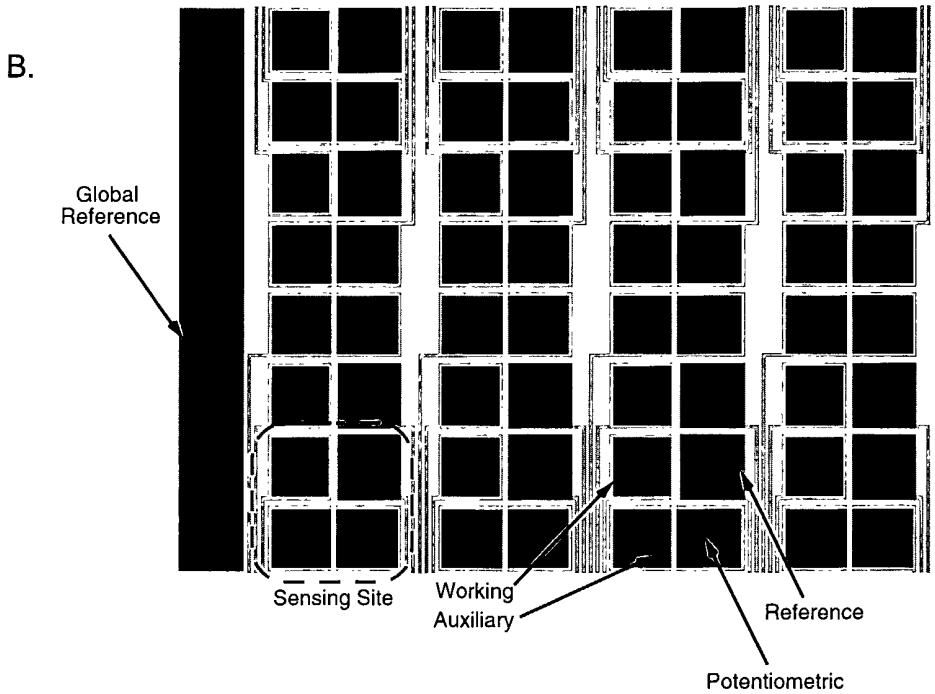
20. A single locus recording device comprising:

at least one potentiometric electrode;

at least one amperometric electrode interconnected to said potentiometric electrode, wherein said electrodes are located on the same device.

21. On-chip closed loop control circuitry comprising an amperometric sensing systems, a potentiometric sensing system at least one differentiator connected to said amperometric and potentiometric sensing systems, and at least one integrator connected to said amperometric and potentiometric sensing systems.


A.
Global Reference
10
Sensing Site
Working
Auxiliary
12
Reference
12


B.
Global Reference
Sensing Site
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Auxiliary
Reference
Potentiometric

Figure 1.

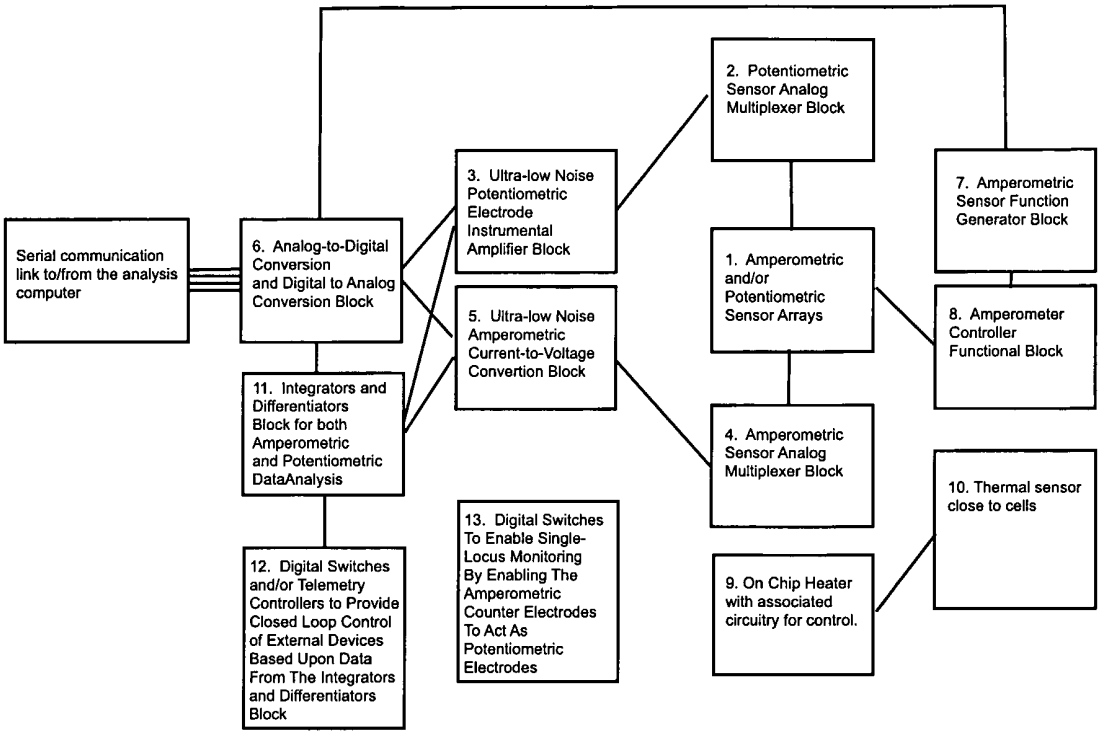

Serial communication link to/from the analysis computer
6. Analog-to-Digital Conversion and Digital to Analog Conversion Block
3. Ultra-low Noise Potentiometric Electrode Instrumental Amplifier Block
5. Ultra-low Noise Amperometric Current-to-Voltage Convertion Block
11. Integrators and Differentiators Block for both Amperometric and Potentiometric DataAnalysis
12. Digital Switches and/or Telemetry Controllers to Provide Closed Loop Control of External Devices Based Upon Data From The Integrators and Differentiators Block
13. Digital Switches To Enable Single-Locus Monitoring By Enabling The Amperometric Counter Electrodes To Act As Potentiometric Electrodes
2. Potentiometric Sensor Analog Multiplexer Block
1. Amperometric and/or Potentiometric Sensor Arrays
4. Amperometric Sensor Analog Multiplexer Block
9. On Chip Heater with associated circuitry for control.
7. Amperometric Sensor Function Generator Block
8. Amperometer Controller Functional Block
10. Thermal sensor close to cells

Figure 2.

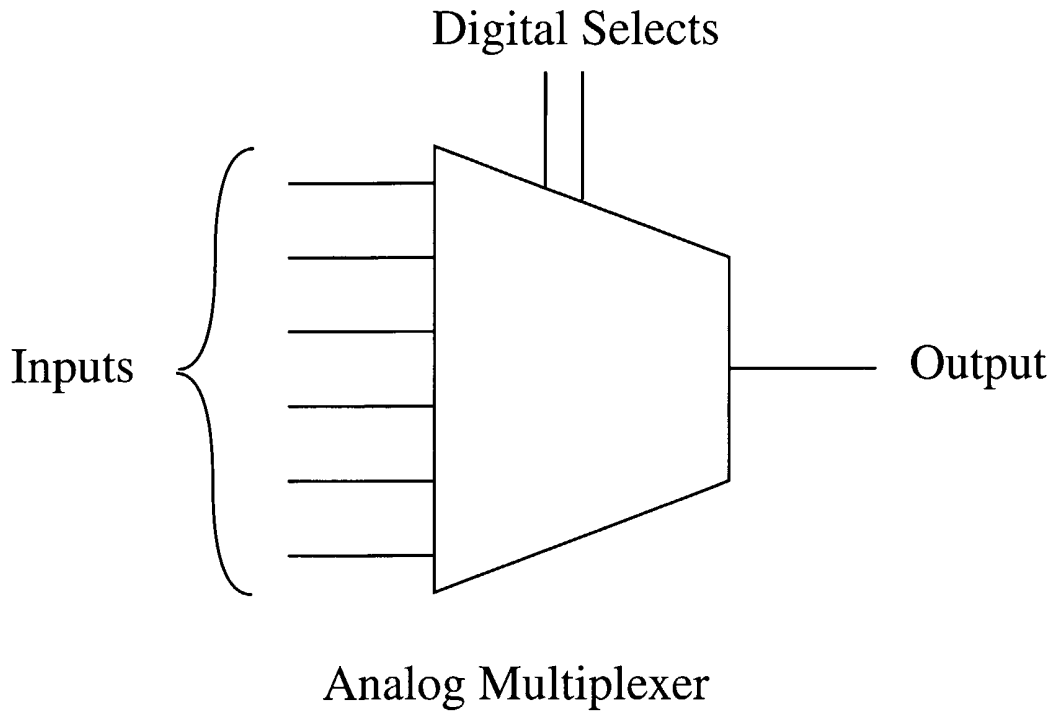
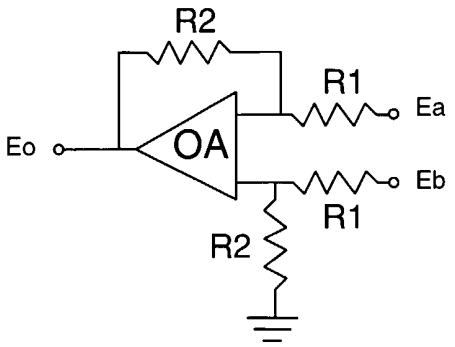


Figure 3.

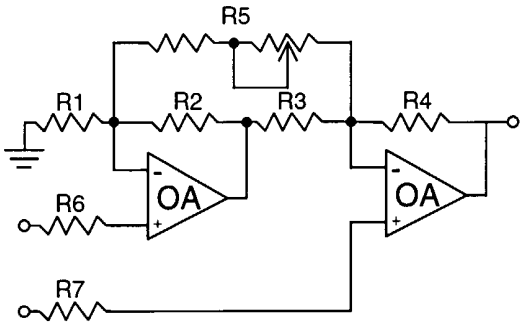
A.



Eo = (Ea - Eb)(R2/R1)

Av = R3/R1

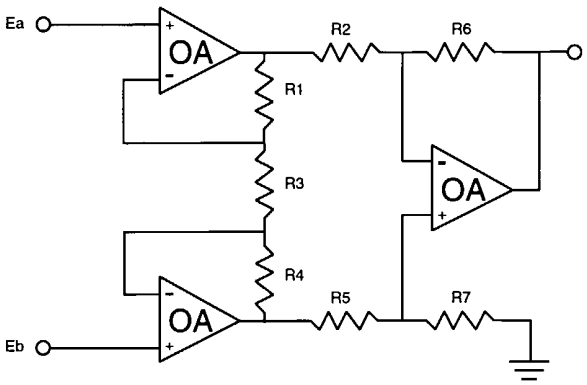
B.



Eo = (Ea - Eb)(1 + R4/R3 + 2R4/R5)

Av = 1 + (R4/R3) + 2(R4/R5)

C.



$E0 = (Ea - Eb)\frac{(R3 + 2R1)(R6/R2)}{R3}$

R1 = R4
R2 = R5
R6 = R7

$Av = \frac{(R3 + 2R1)(R6/R2)}{R3}$

Figure 4.

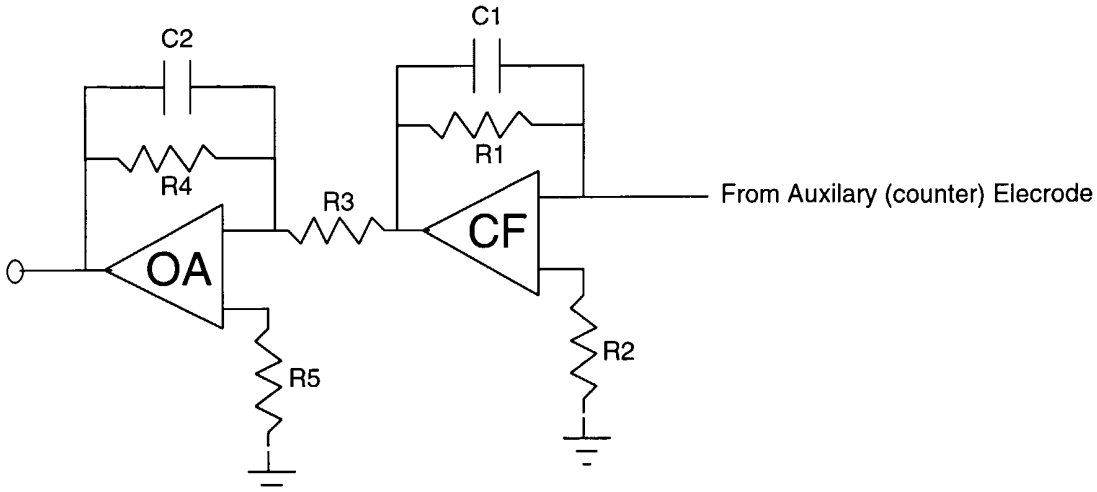


Figure 5.

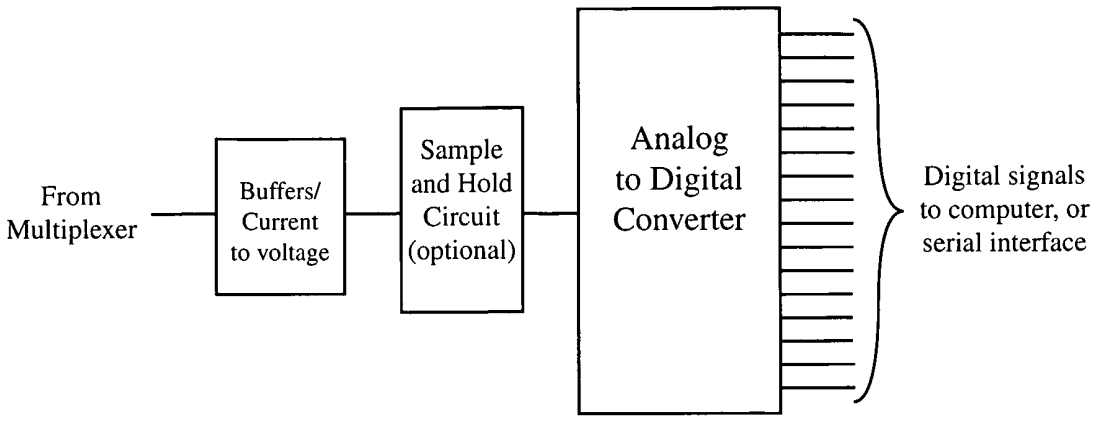

From
Multiplexer
Buffers/
Current
to voltage
Sample
and Hold
Circuit
(optional)
Analog
to Digital
Converter
Digital signals
to computer, or
serial interface

Figure 6.

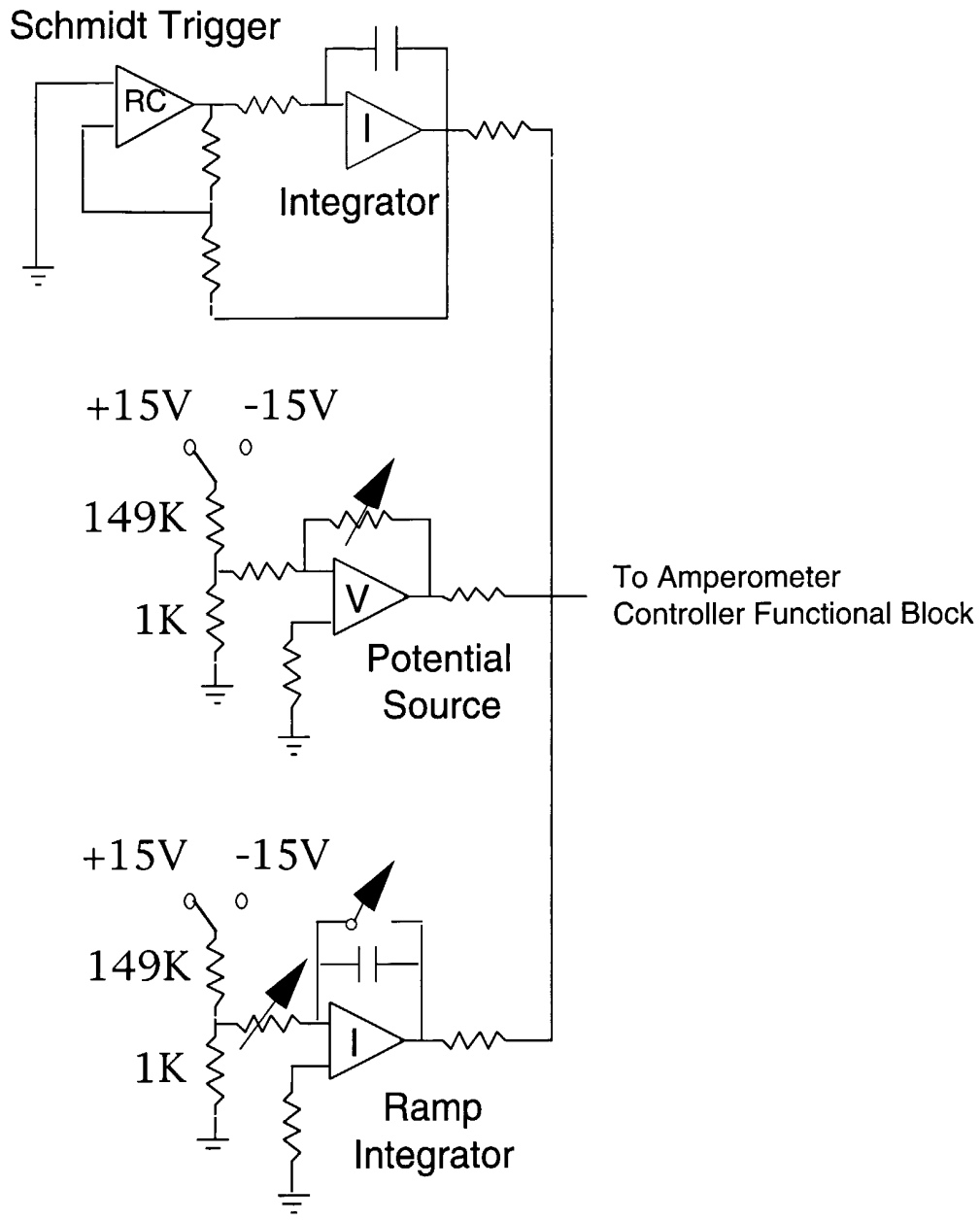

Schmidt Trigger
RC
I
Integrator
+15V
-15V
149K
1K
V
Potential
Source
To Amperometer
Controller Functional Block
+15V
-15V
149K
1K
I
Ramp
Integrator

Figure 7.

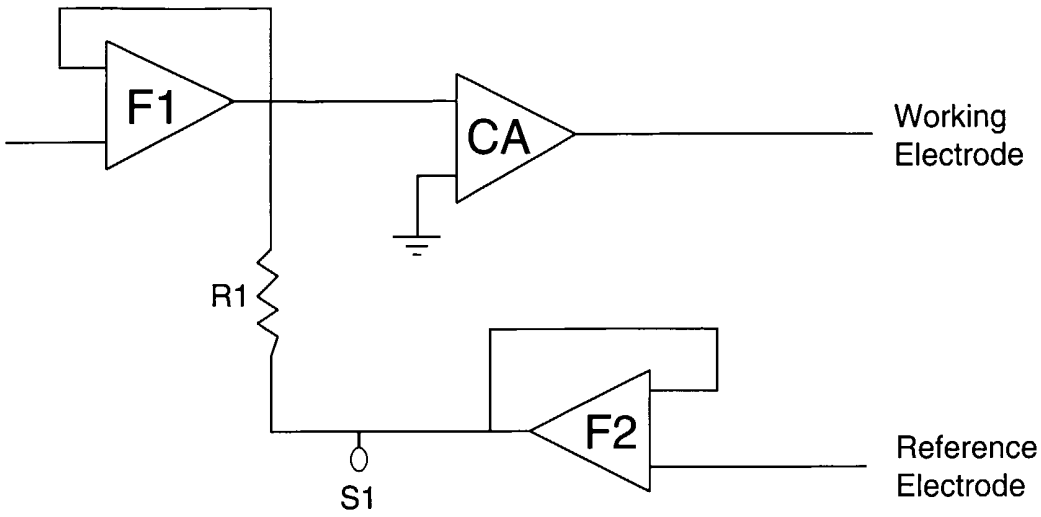


Figure 8.

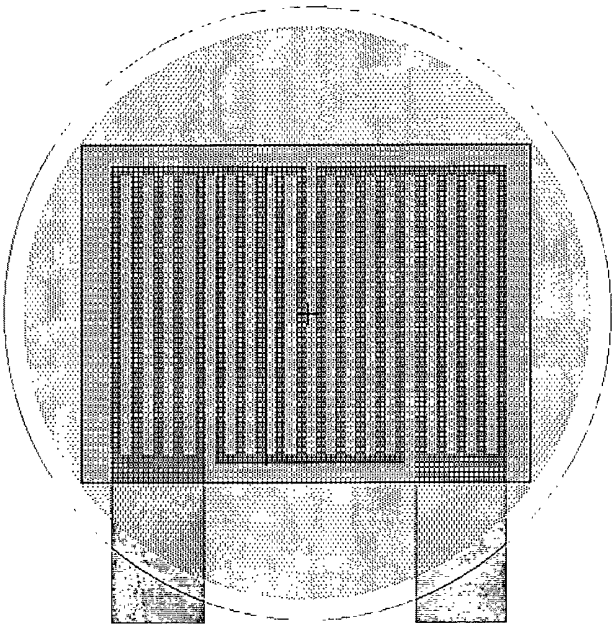


Figure 9.

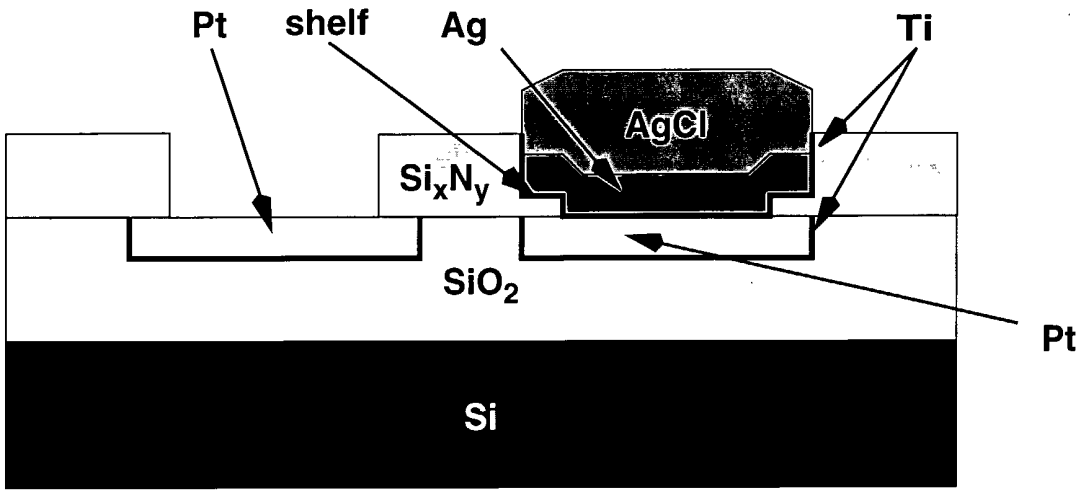


Figure 10.

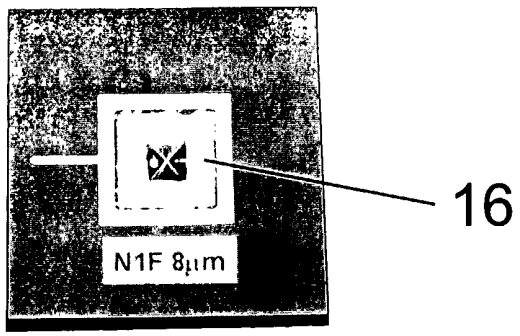


Figure 11.

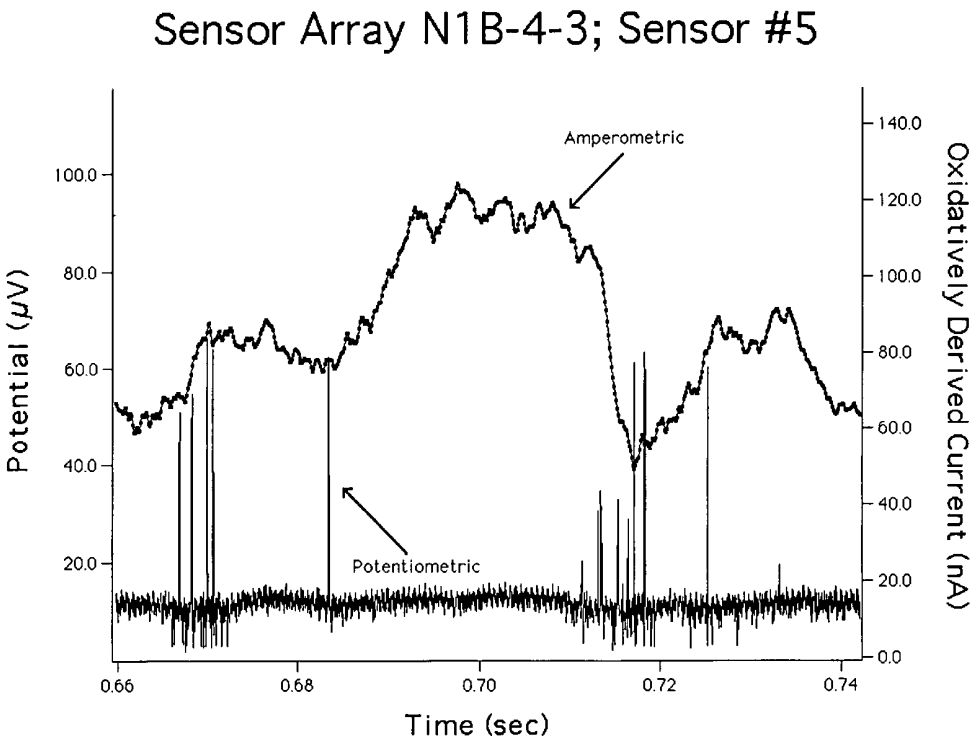

Sensor Array N1B-4-3; Sensor #5
Amperometric
Potentiometric
Potential (μV)
Oxidatively Derived Current (nA)
Time (sec)
100.0
80.0
60.0
40.0
20.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.66
0.68
0.70
0.72
0.74

Figure 12.

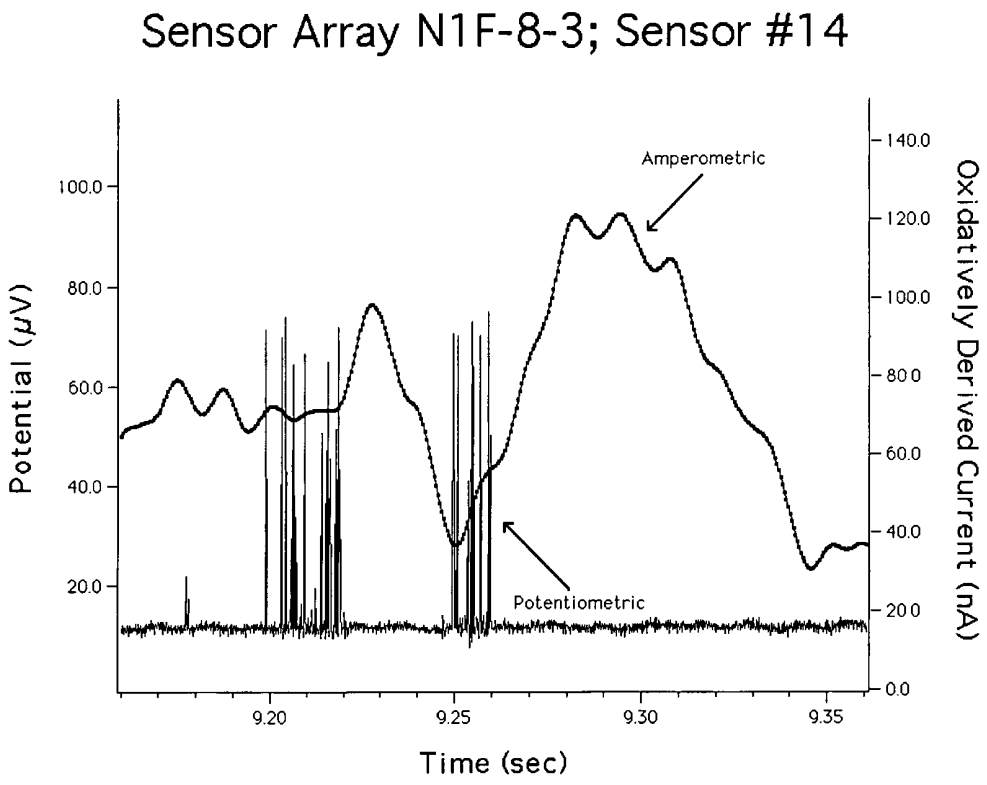

Sensor Array N1F-8-3; Sensor #14
Amperometric
Potentiometric
Potential (μV)
Oxidatively Derived Current (nA)
Time (sec)
100.0
80.0
60.0
40.0
20.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
9.20
9.25
9.30
9.35

Figure 13.

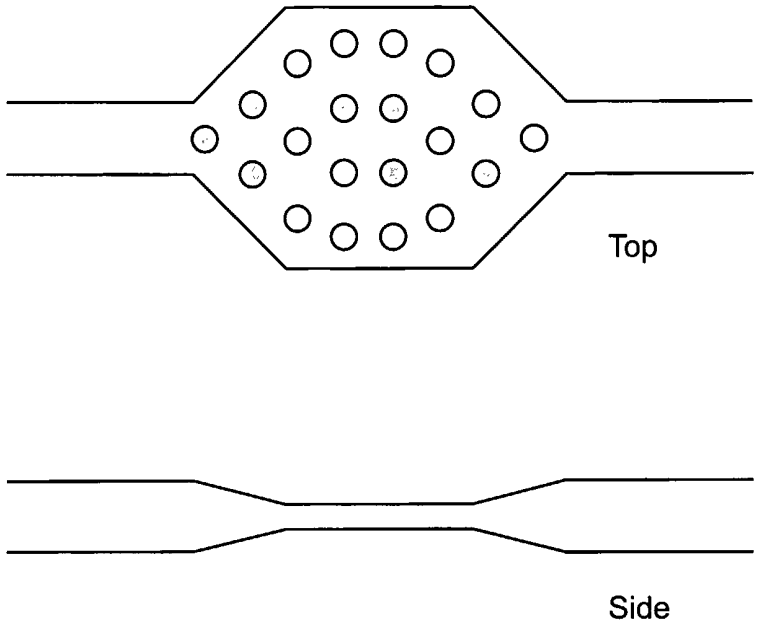


Figure 14.

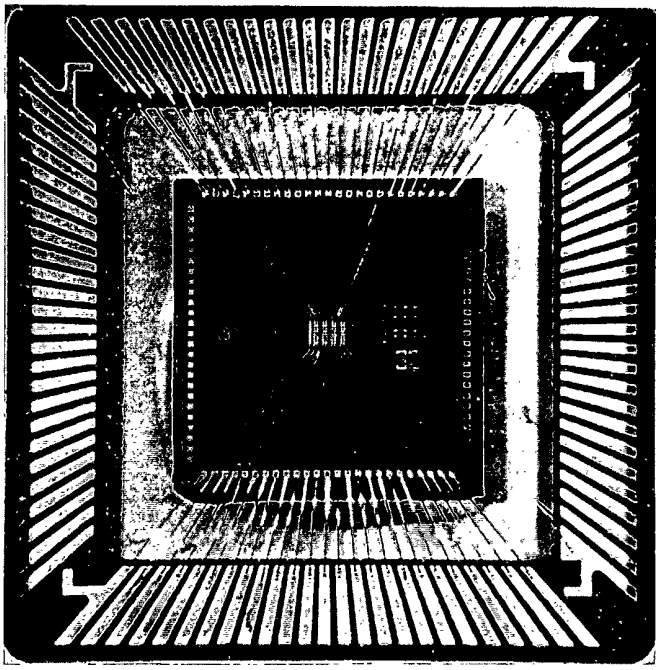


Figure 15.

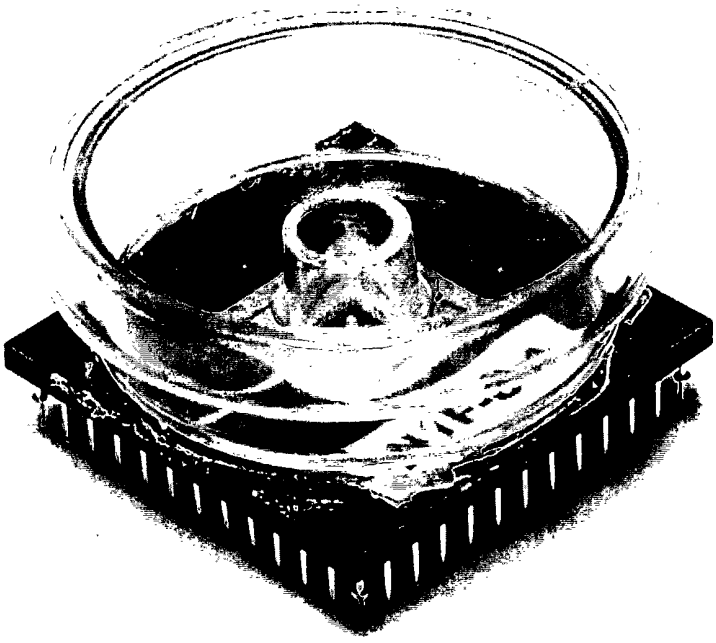


Figure 16.

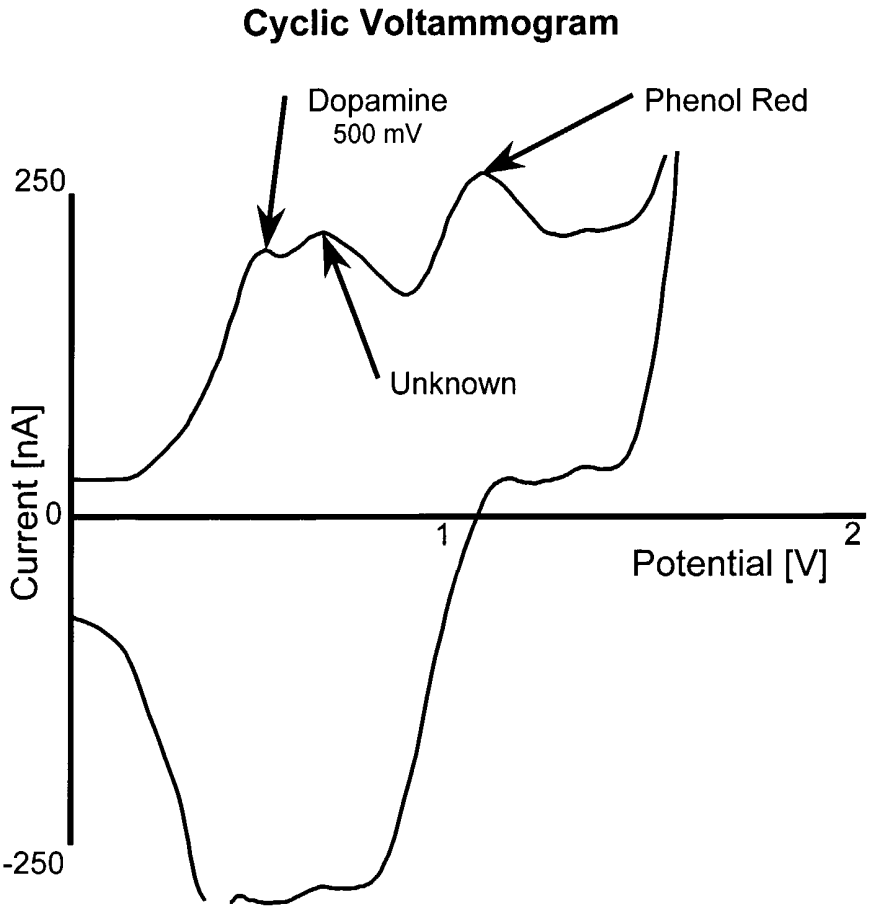

Cyclic Voltammogram
Dopamine
500 mV
Phenol Red
Unknown
250
0
-250
Current [nA]
1
2
Potential [V]

Figure 17.

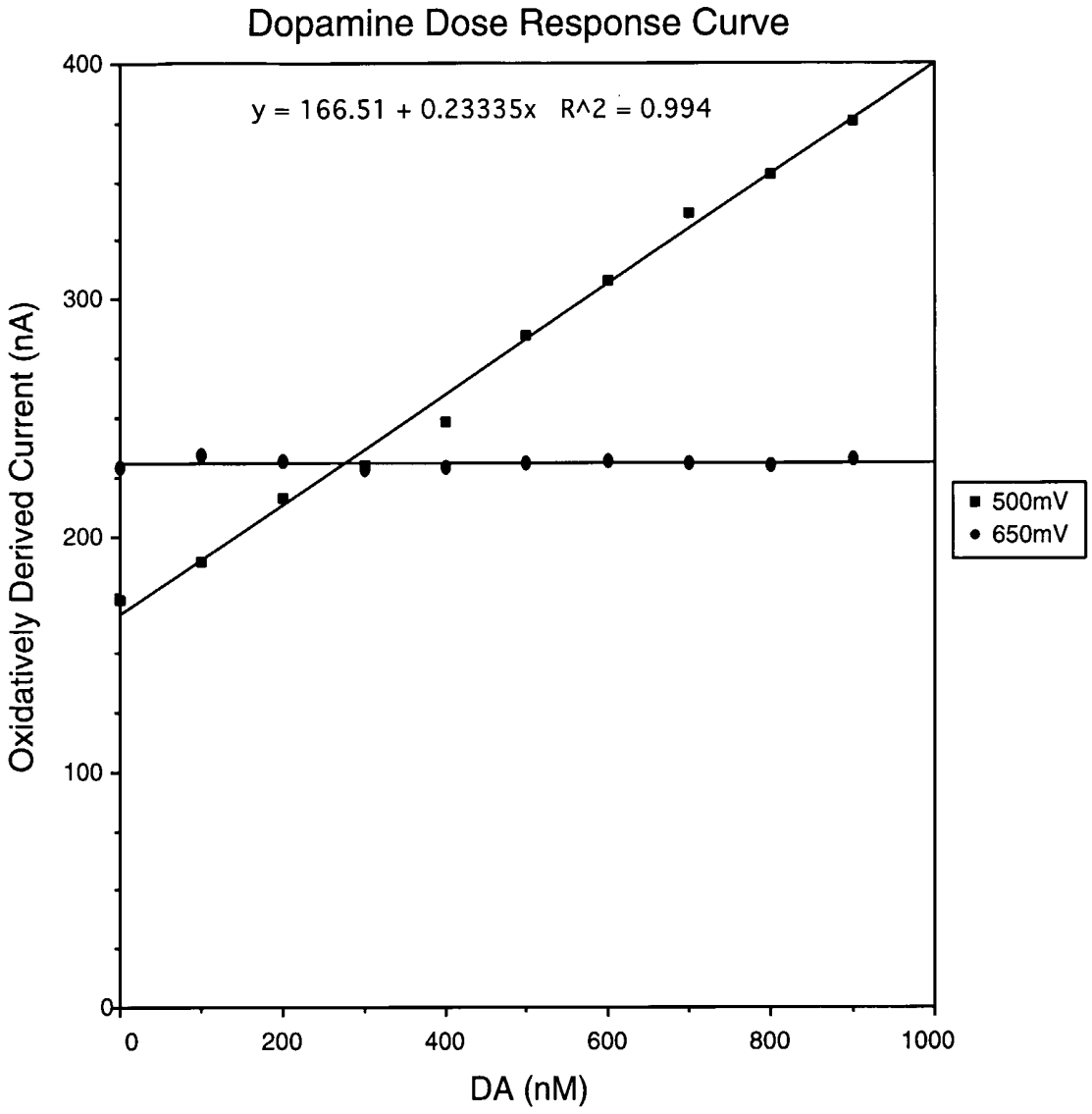

Dopamine Dose Response Curve
y = 166.51 + 0.23335x R^2 = 0.994
Oxidatively Derived Current (nA)
DA (nM)
500mV
650mV
0
100
200
300
400
0
200
400
600
800
1000

Figure 18.

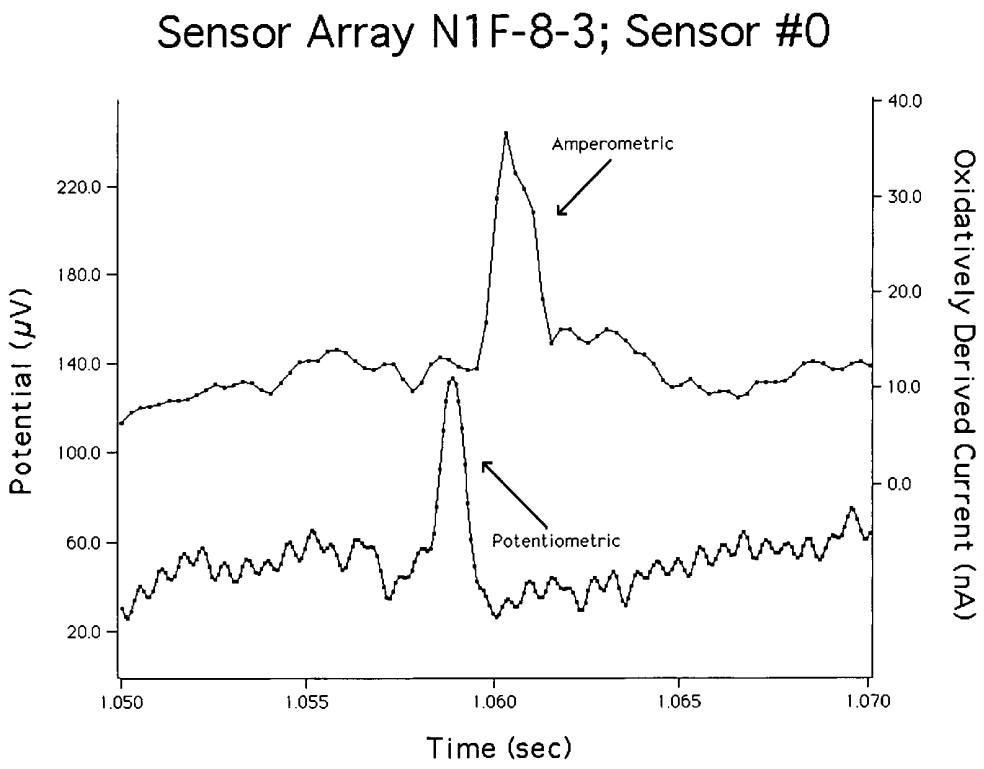

Sensor Array N1F-8-3; Sensor #0
Amperometric
Potentiometric
Potential (μV)
Oxidatively Derived Current (nA)
Time (sec)
220.0
180.0
140.0
100.0
60.0
20.0
40.0
30.0
20.0
10.0
0.0
1.050
1.055
1.060
1.065
1.070

Figure 19.

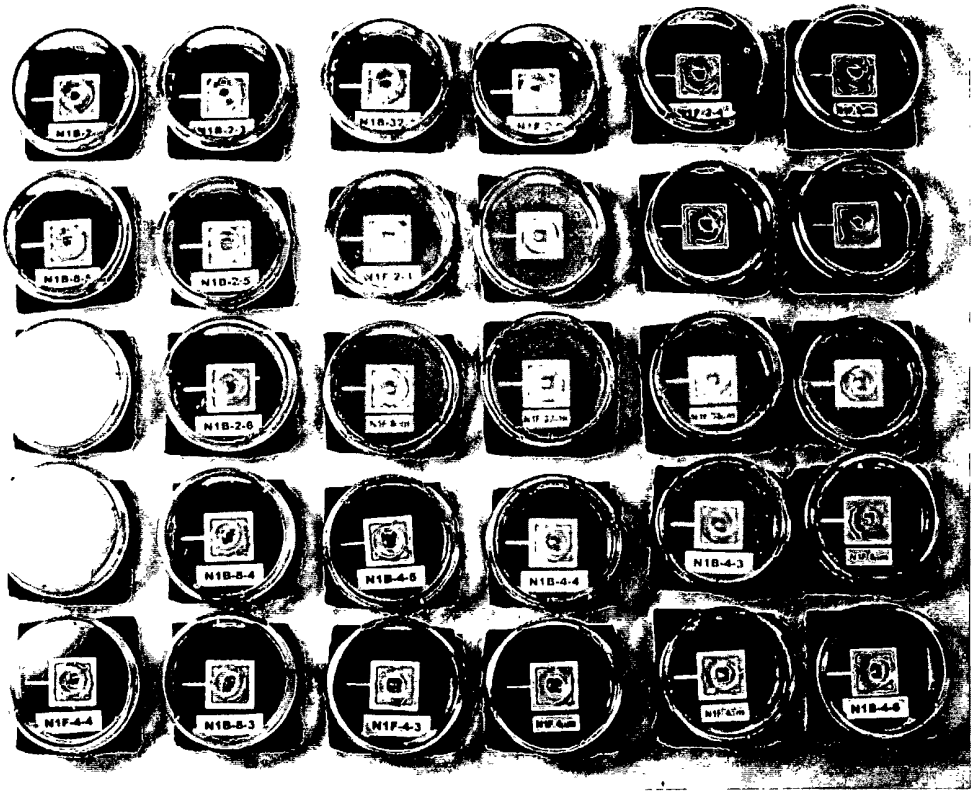


Figure 20.

Figure 20.

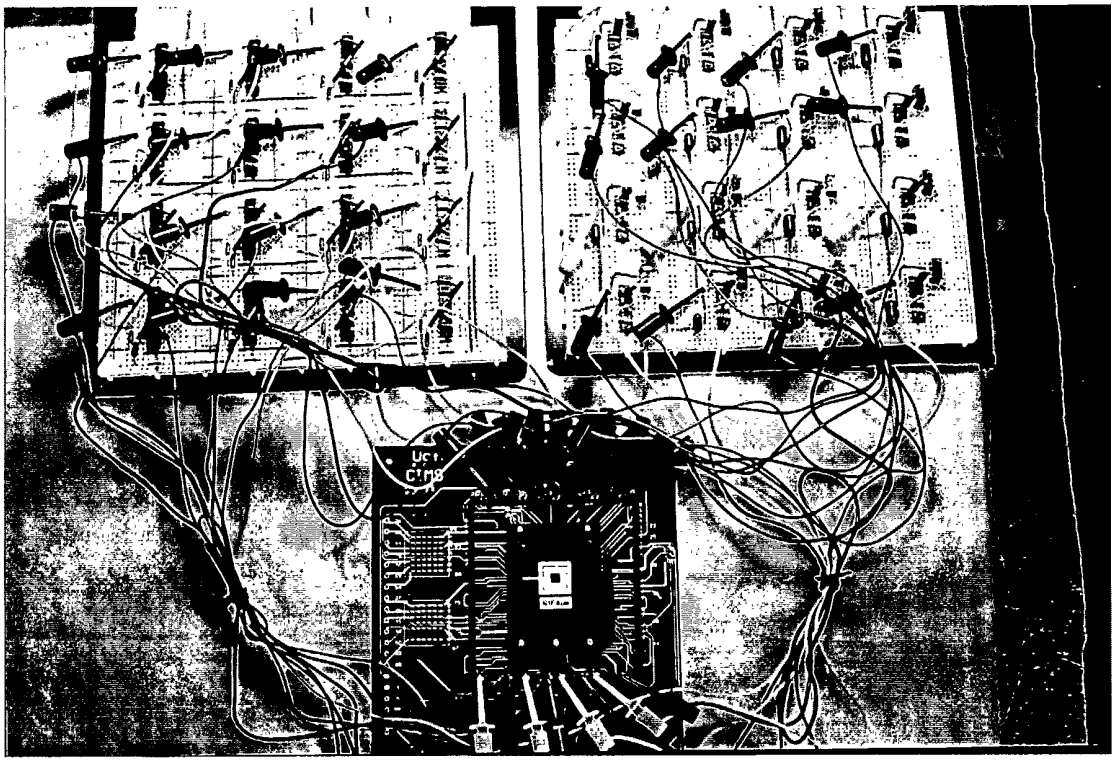
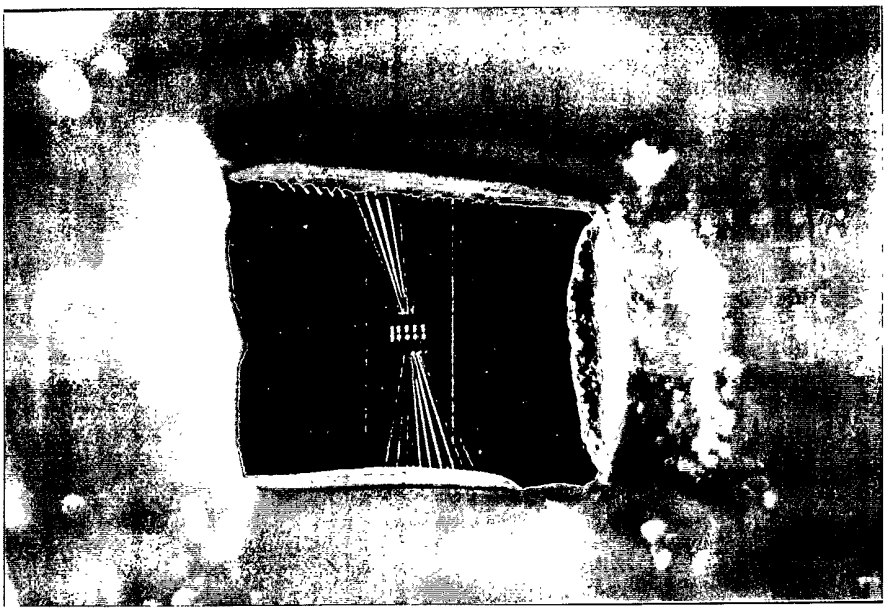


Figure 21.



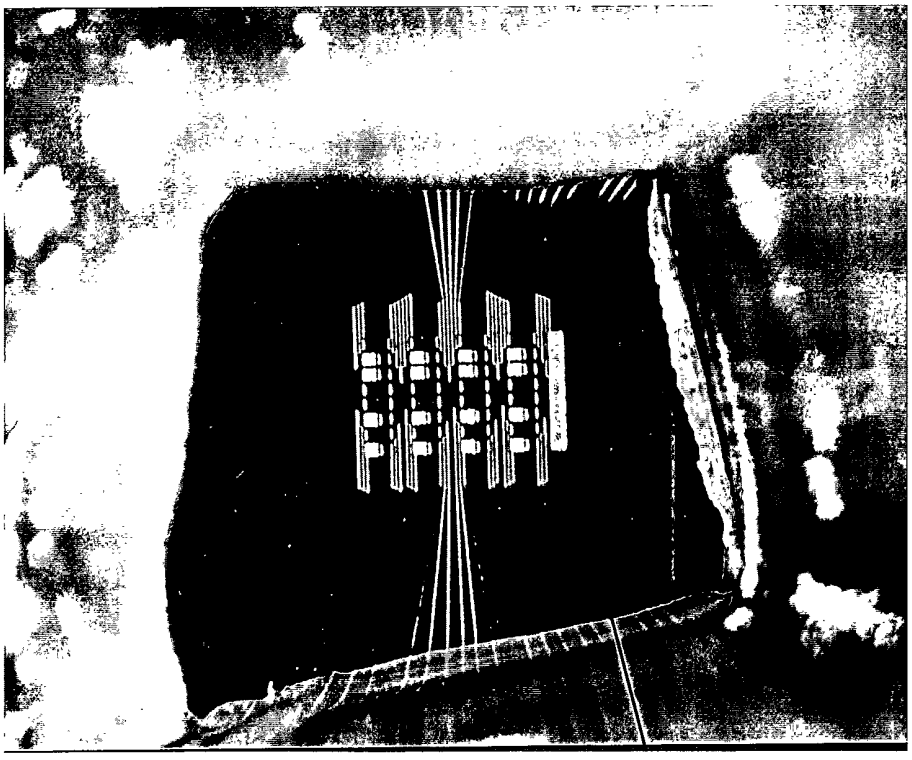


Figure 22.

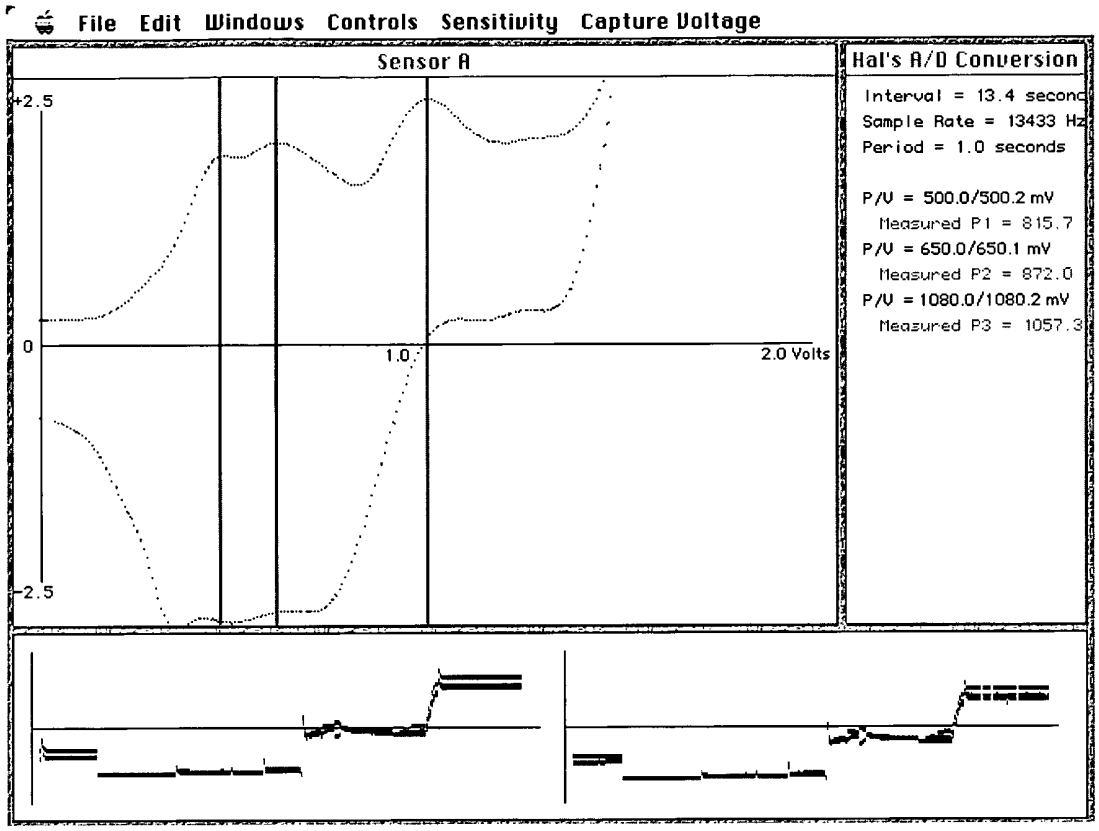

File Edit Windows Controls Sensitivity Capture Voltage
Sensor A
+2.5
0
-2.5
1.0
2.0 Volts
Hal's A/D Conversion
Interval = 13.4 second
Sample Rate = 13433 Hz
Period = 1.0 seconds
P/V = 500.0/500.2 mV
Measured P1 = 815.7
P/V = 650.0/650.1 mV
Measured P2 = 872.0
P/V = 1080.0/1080.2 mV
Measured P3 = 1057.3

Figure 23.

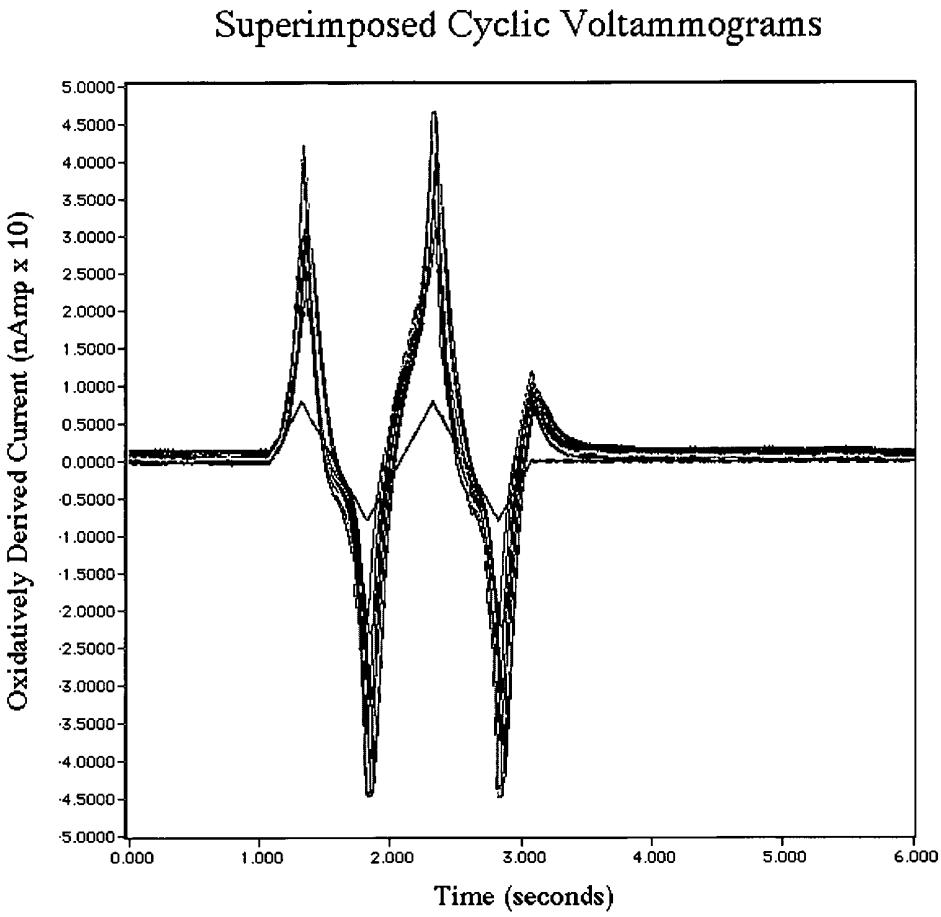

Superimposed Cyclic Voltammograms
Oxidatively Derived Current (nAmp x 10)
5.0000
4.5000
4.0000
3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
-0.5000
-1.0000
-1.5000
-2.0000
-2.5000
-3.0000
-3.5000
-4.0000
-4.5000
-5.0000
0.000
1.000
2.000
3.000
4.000
5.000
6.000
Time (seconds)

Figure 24.

Figure 25.

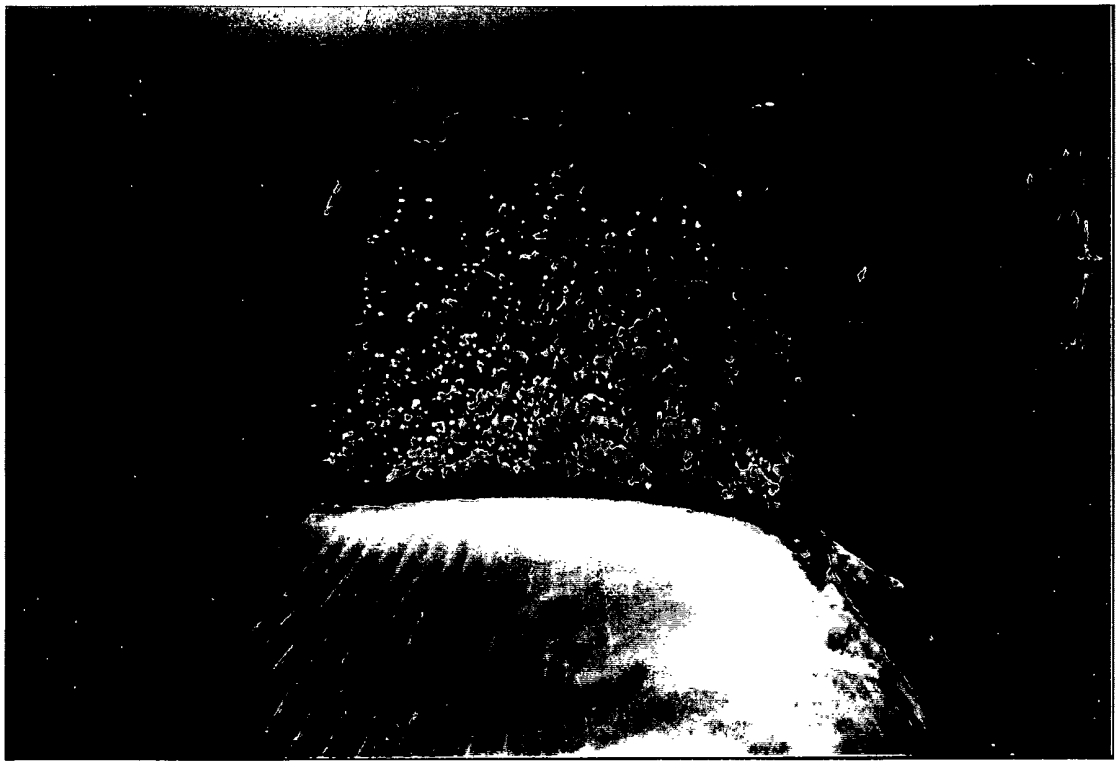
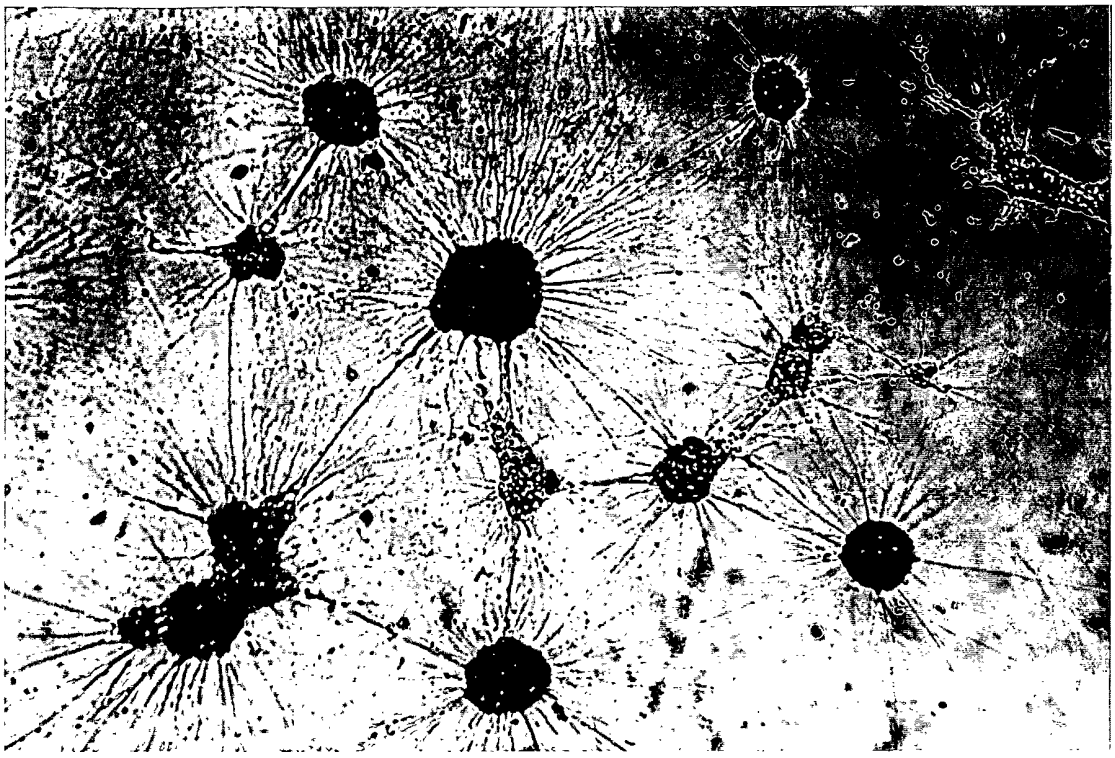


Figure 26.



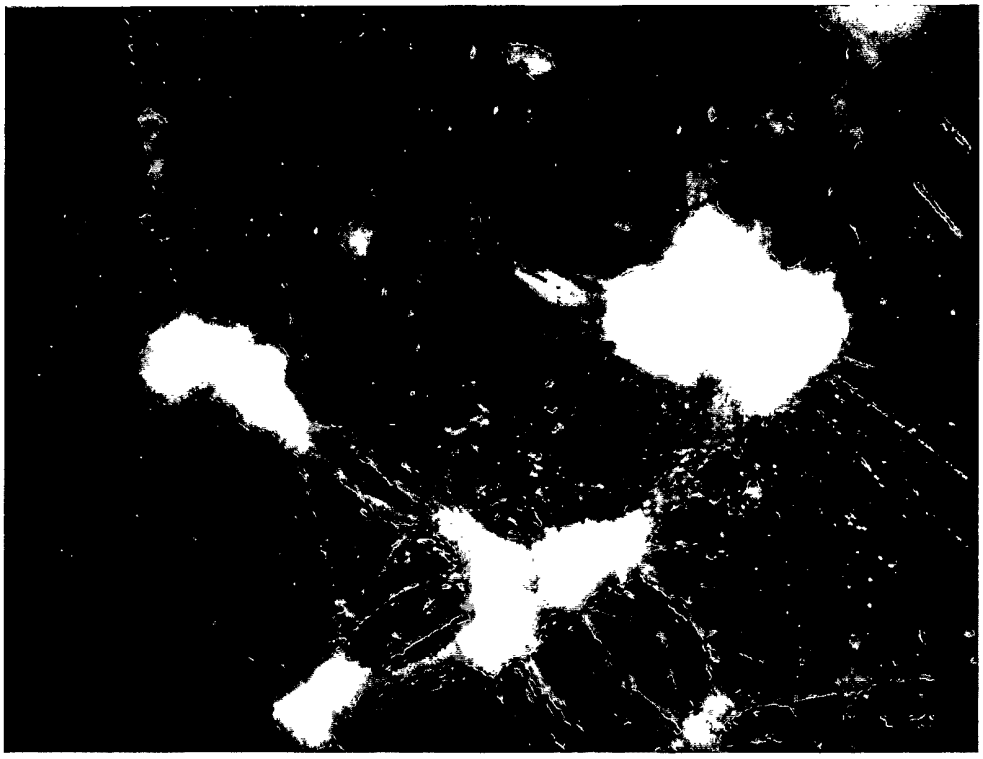


Figure 27.

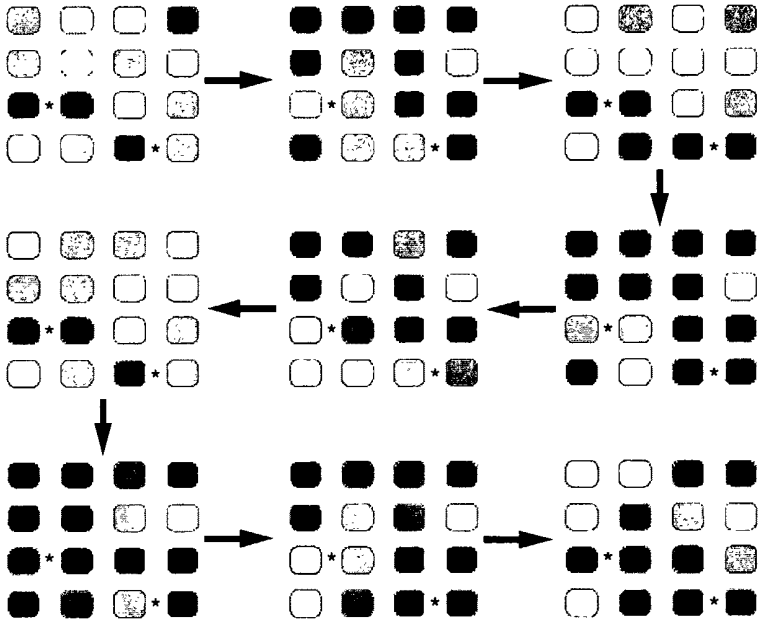


Figure 28.

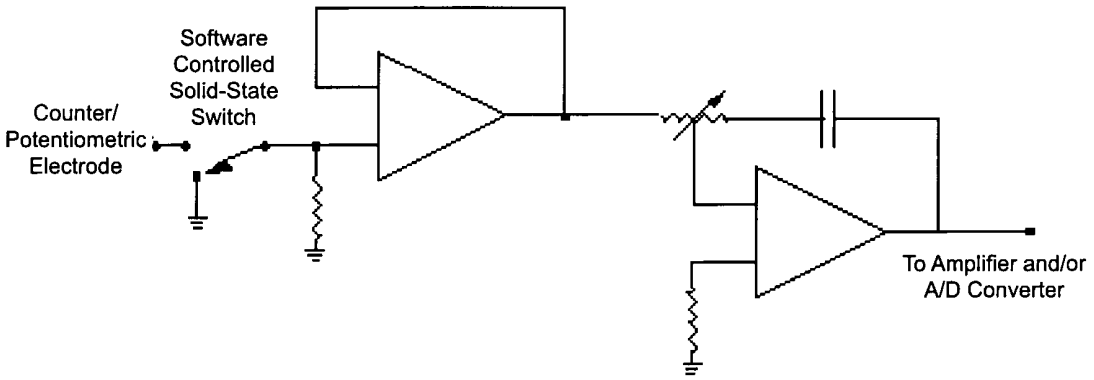

Software
Controlled
Solid-State
Switch
Counter/
Potentiometric
Electrode
To Amplifier and/or
A/D Converter

Figure 29.

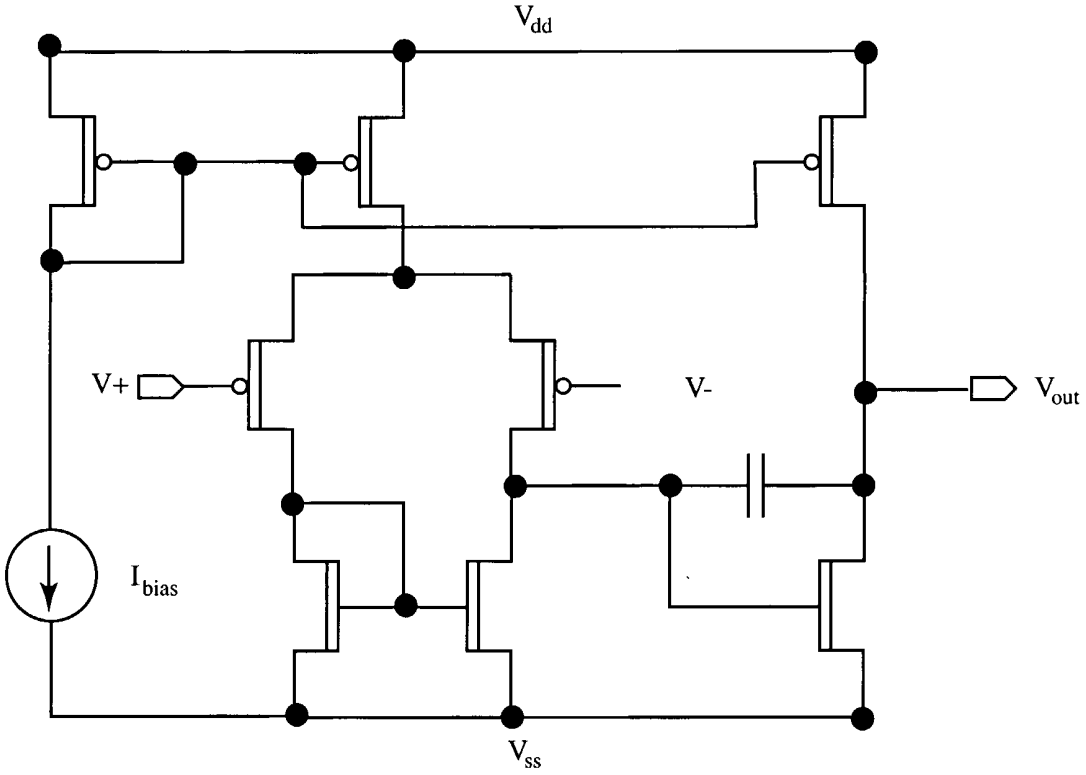


Figure 30.

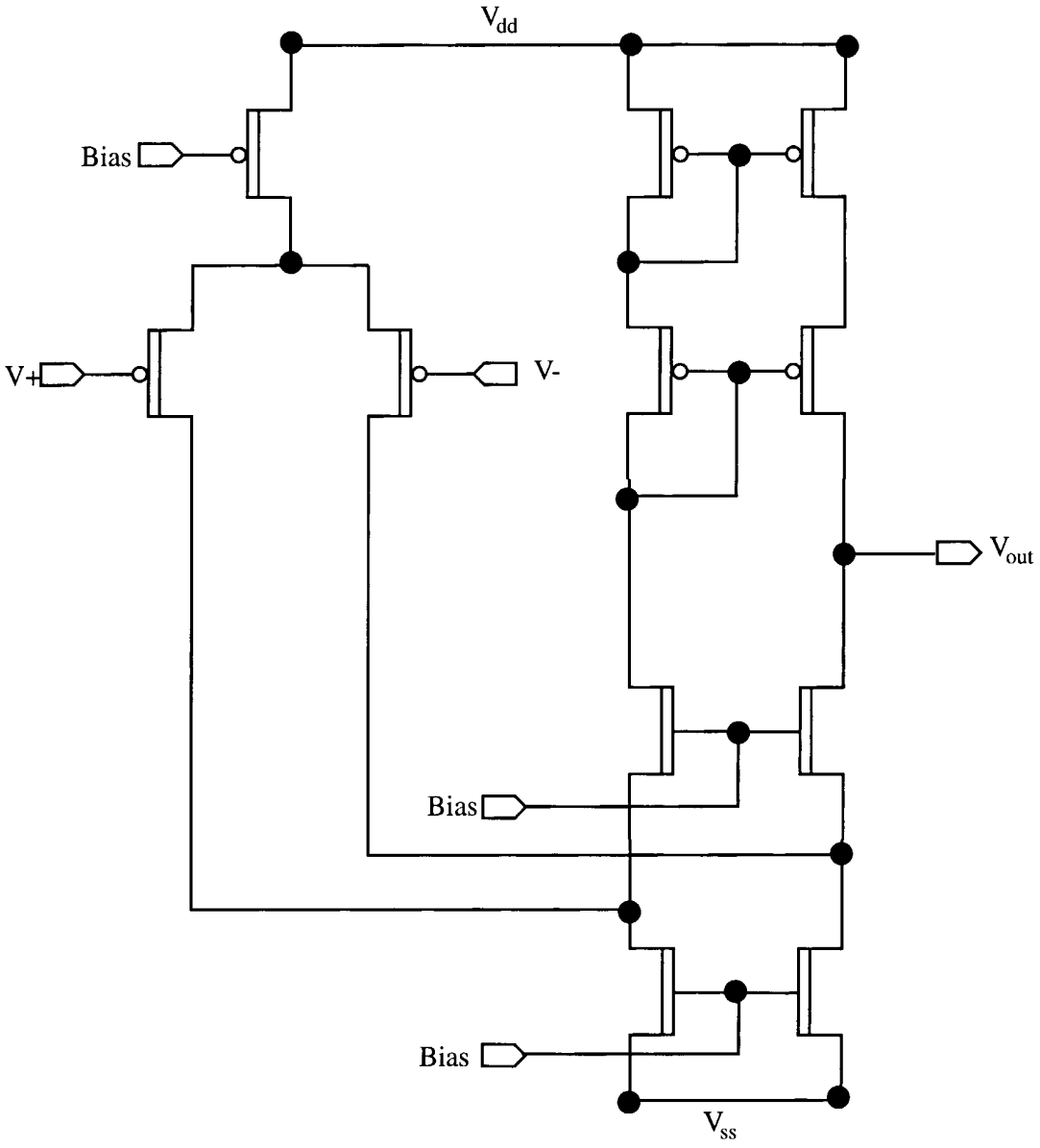


Figure 31.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30484

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 27/26
US CL : 204/ 412
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/ 412, 403, 406

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, EAST(EPO,JPO)
search terms: amperomet? and (potentiomet? or voltamet?)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,683,562 A (SCHAFFAR et al.) 04 November 1997, the abstract; Figure 7a ;and column 5, lines 7-14; and col. 6, ll. 32-36.	1, 2, 6, 7, 20 --- 3-5 and 11-16
X, E --- Y, E	US 6,146,510 A (LEADER et al.) 14 November 2000, the abstract; col. 6, ll. 1-9; col. 7, ll. 13-33; col. 8, ll. 22-35; col. 6, ll. 59-64; col. 12, ll. 47-50; col. 16, ll. 62-65.	1, 2, 6, 7 --- 3, 4, 5, 11-16, 20
X --- Y	US 5,718,816 A (SAVAGE et al.) 17 February 1998, the abstract; col. 10, ll. 14-22; col. 12, ll. 35-48; col. 11, ll. 5-11; col. 11, ll. 25-42; and col. 14, ll. 32-63.	1, 2, 6, 7 --- 3-5, 11-16, 20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 JANUARY 2001	Date of mailing of the international search report 12 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ALEX NOGUEROLA Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30484

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, E	US 6,159,353 A (WEST et al.) 12 December 2000, the abstract; Figure 1A; col. 5, ll. 27-36; col. 6, ll. 13-27; and col. 9, ll. 22-25.	1-7, 11-16, and 20