ELECTRICAL COMMUNICATION BETWEEN SOLID STATE ELECTRONICS AND BIOCHEMICAL SYSTEMS USING A NANOMATERIAL AND NANOELECTROCHEMICAL STRATEGY

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
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This thesis is dedicated to my mother, Mercia L. Richardson.

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Major Department: Chemistry Department

We investigated a nanomaterial and nanoelectrochemical approach for establishing electrical communication between biochemical processes and solid state electronics. The biochemical processes are in electrical contact with four contact pads on a circuit board. An interface film, with millions of parallel nanowires running throughout its thickness, allows z axis electrical connections between the biochemical processes and the pads of the underlying circuit board. The interface film was prepared by the template method (whereby the 30 nm diameter pores in a nanoporous polycarbonate membrane were filled with gold wires using an electroless Au-plating method). The Au surface layers of the membrane were removed leaving a film with ~10^9 parallel nanowires spanning the thickness of the film. The film was heated to make it impervious to solutions. This important step made the polycarbonate membrane a good interface film, thus separating the wet biochemistry above from the underlying solid-state electronics below (yet

providing 10^9 nanoelectrode per cm^2 film interfaces for communication between these two phases).

Silver epoxy was used to stick the interface film to the mini-circuit board device and to make Ohmic contact between the wires in the film and the contact pads on the circuit board. Au contact pads were sputtered through a mask onto the rough surface of the film. These pads were the same size and orientation as the contact pads on a mini-circuit board. This procedure was necessary to protect the film from solvents in the silver epoxy that would cause the film to crack. The gold pads also protected the film from silver particles in the silver epoxy (that would otherwise puncture the film). This Au sputtering procedure is necessary to make good Ohmic contact between the nanowires in the film and the silver epoxy (which was used to stick the film to the contact pads on the circuit board).

Cyclic voltammetry showed oxidation and reduction peaks that are typical for electroactive species [(trimethylamino)methyl]ferrocene ($TMAFc^+$) perchlorate, similar to results that were obtained previously using nanoelectrodes ensembles (NEE) based on the electroless Au wires. Each electrode area of NEE in our device can be addressed individually, growing electrochemically conductive polymers giving their electroactivities in specific electrolytes with specific shapes of voltammograms (and thus unique signatures).

CHAPTER 1
INTRODUCTION

Recently numerous research efforts aimed to establish electrical communication between biochemical processes and integrated circuits (in areas such as engineering, molecular electronics, biosensors, bioartificial organs, and neural networks) were reported (1-11). The simplest and most relevant example of the need for biochemical/solid-state electronic communication is in the area of biosensors. In the field of biochemical analysis, the need for multiparametric assays has prompted the development of a series of biochemical processes linked to the supports in precise locations (addresses). To reach a high information density, miniaturization of this kind of support has been carried out on microelectrode arrays (1-4). The dual demands for increased range of analytes and decreased size are driving biosensors toward micro arrays. Because an integrated chip can have many circuits, many biochemical processes or biosensors can (in principle) be linked separately to each circuitry.

To open lines of communication between biochemical systems and solid-state electronics some key scientific and technical challenges must be addressed.

- Because biochemical processes usually occur in aqueous solution (which is incompatible with solid-state electronic devices), the first challenge is to establish an interface between these wet and dry phases.
- One of the key advantages of IC technology is the ability to pack many circuit elements onto a single chip. It would be highly desirable if a correspondingly large number of biomedical processes could be packed onto (and communicating with) a single chip. The second challenge then concerns precisely isolating and confining of the biomedical processes near the chip.
- The third challenge concerns signal transduction; the biochemical events must be transduced to electrical currents that can be accepted by the chip.

- The forth challenge concerns control and signal processing.The underlying IC chip must have on board the necessary electronics to control the desired biochemical reactions to process the electronic signals obtained from these reactions and to interface to a simple digital microcontroller.

One of the challenges faced when placing biochemicals directly on a chip is the problem of shorting out the electronics onboard the chip. A recent report claims to have solved the problem of analyte solution seeping around the electrode down to the circuitry onboard an array-based amperometric sensor chip (12, 13). First, the complete analog and digital circuitries were produced. Then two passivation layers (one of silicon dioxide and one of silicon nitride) were deposited. The electrodes were structured into these passivation layers. It is not clear how the passivation layers around the electrode prevented the seepage of solution down to the chip and there is no report of how long such a setup can remain functional before shorting the circuit below.

The objectives of this study were:

- To show proof of concept for the first three challenges outlined above by using a novel nanomaterial/nanoelectrochemical strategy.
- To develop prototype devices that allow for parallel electronic communication between numerous different biochemical processes (confined at the micron scale) and an underlying circuit chip.

To have transduction directly on the chip, our device must have parallel electronic communication between multiple biochemical processes (biosensors), confined at the micron scale, and an underlying integrated circuit chip. We used of an advanced material fabricated and pioneered in the Martin lab (14-16) to interface between the wet biochemical processes and underlying chip. We used the template method to prepare this interface film (17, 18). This method entails using the pores of a microporous membrane (Fig. 1-1) as templates to prepare Au nanowires in the pores. Because the membranes used contain cylindrical pores with monodisperse pores, corresponding cylindrical (and

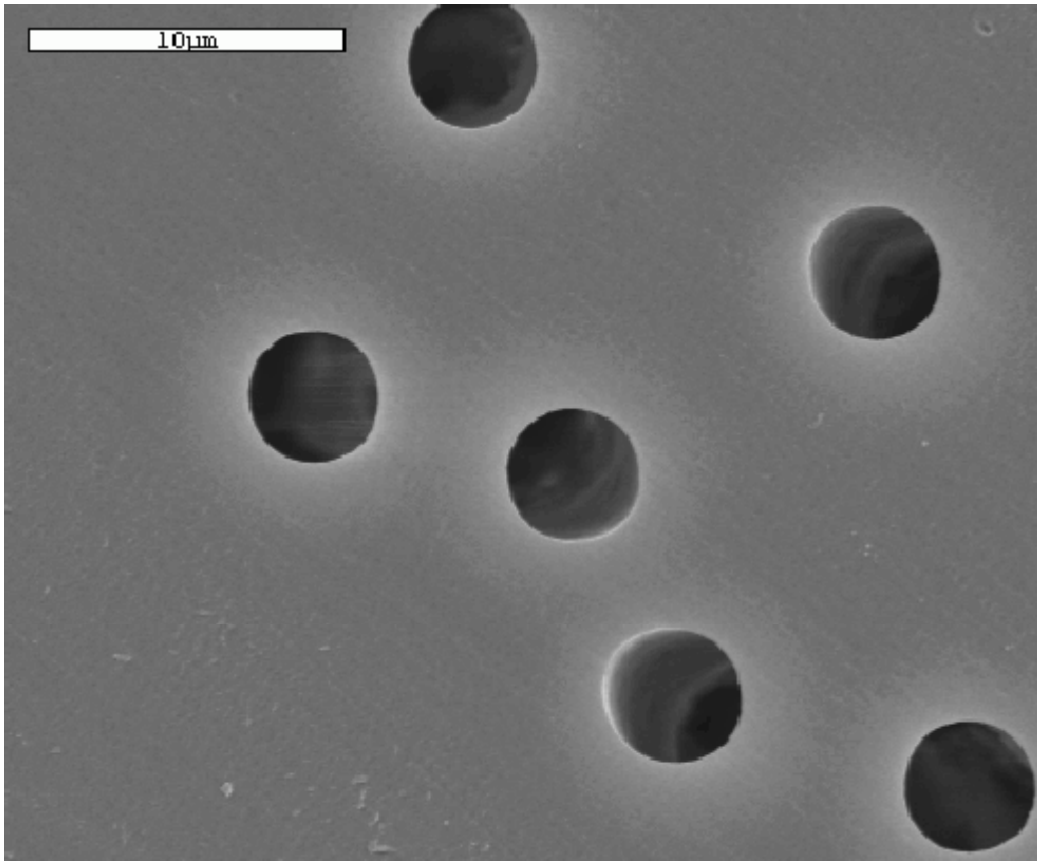

Figure 1-1. Scanning electron micrograph of track-etched polycarbonate membrane with 5 µm diameter pore

monodispersed) nanowires were obtained. We used polymeric film containing an ensemble of monodisperse, cylindrical gold nanowires (diameter = 30 nm) that span the complete thickness (6 µm) of the film. This interface film (Fig. 1-2) contains ~109 nanowires per cm2 of film area. The nanowires in the film transmit electrical signals (generated by the biochemical processes above the film) to the contact pads on the underlying chip. This nanowire-containing film is impervious to solution (15) and thus protects the underlying chip from the wet biochemistry above. In addition, the ends of the nanowires at the upper surface of the interface film constitute an ensemble of nanoscopic disk-shape electrodes (14-16) that are used for control and signal transduction.

As pointed out earlier, the simplest and most relevant need for

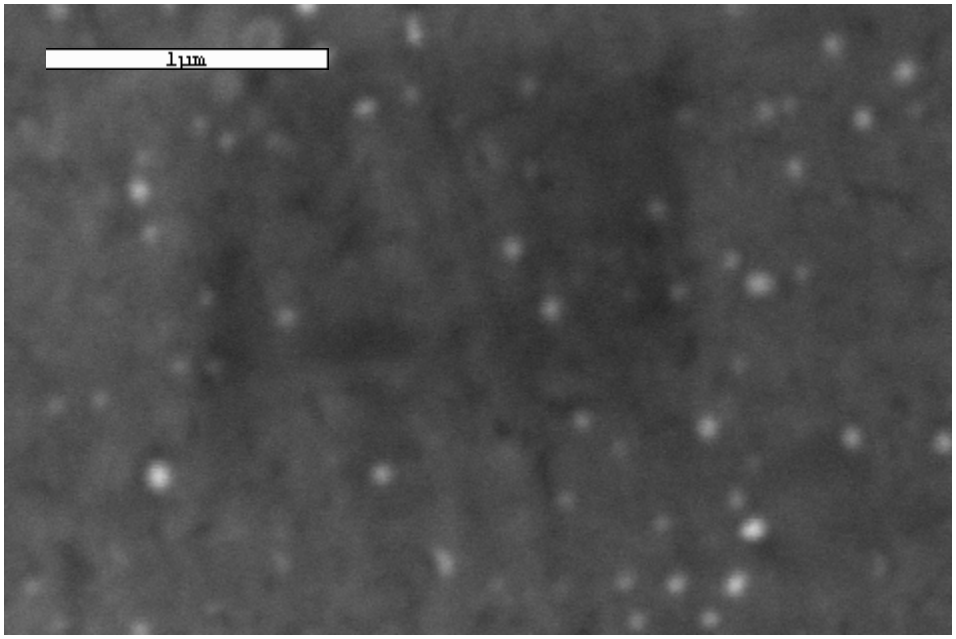

Figure 1-2. Scanning electron micrograph of the surface of the interface film. The circular ends of the nanowires appear as bright spots

biochemical/solid-state electronic communication is in the area of biosensors.

The aim of a biosensor is to produce an electronic signal that is proportional to the concentration of a specific chemical or set of chemicals. Ideally, biosensors are specific, rapid and simple to operate and to fabricate (with minimal sample pretreatment). Biosensor devices combine the specificity and sensitivity of biological systems with signal processing. Biosensors typically consist of a selective interface in close proximity to (or integrated with) a transducer which relays an interaction between the surface and the analyte (either directly or through a mediator) (6) (figure 1-3). The analyte selective interface is often a bioactive substance (e.g. enzyme, antibody or micro-organism) (6). These are capable of recognizing their specific analytes and also regulate the specificity and sensitivity of the device. The transducer converts the biochemical recognition event into a signal that can be measured by a suitable detection system (figure1-3). The transducer forms the link between the biochemical and solid-state world (6).

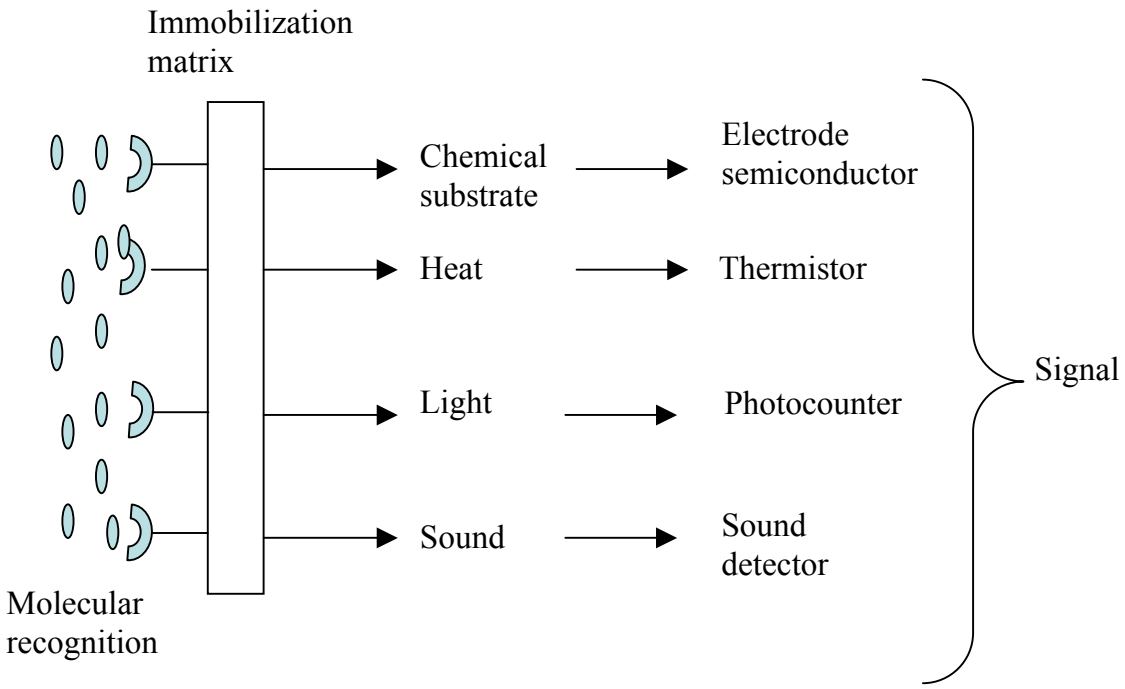

Figure 1-3. Schematic of a biosensor

The biochemical signal can be transduced in a number of ways. These include transduction based on measurement of an electrical potential or current; photon emission or absorption; and measurement of heat associated with biochemical processes. It must be pointed out that in general, the transduction event is removed from the electronic circuitry by a wire (or fiber optic etc). The device we envision will have transduction done directly on board.

This thesis describes proof of concept using a novel nanomaterial (and also a nanoelectrochemical approach) for establishing electronic communication between wet biochemical processes (confined on the micron scale) and underlying solid-state electronics. The device we ultimately propose to make is shown schematically in figure 1-4. We used a mini-circuit board as the model for the integrated circuit chip. In the preliminary studies described here, conducting polymers are used as a surrogate for biochemical processes since conducting polymer can function as immobilization supports

for biosensors, and also can be used to demonstrate that individual electrodes can be modified to function as specific sensing elements.

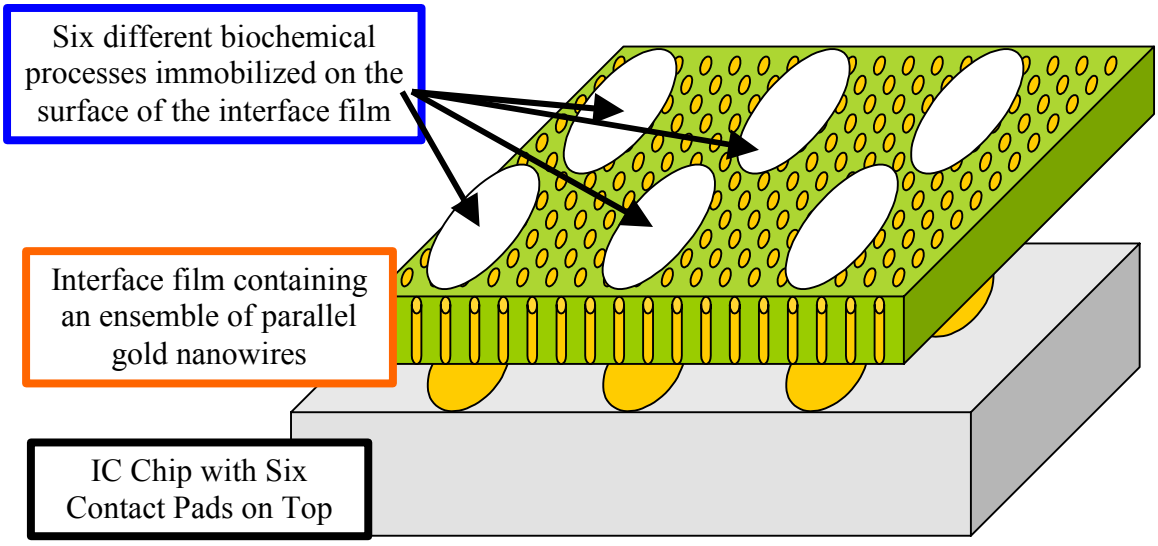

Figure 1-4. Schematic of nanomaterial/nanoelectrochemical approach for establishing electronic communication between confined biochemical processes and an underlying IC chip

Electrically conducting polymers are organic compounds that conduct electricity. They have extended π-conjugated backbones of alternating single and double bonds along the polymer chain. Many contain a ring structure which may include nitrogen or sulfer in the ring. One such example is polypyrrole (7) (Fig 1-5). The general details of electrochemical synthesis of polypyrrole are similar to other polymers such as polythiophene and polyaniline (7). The polymers can be electrochemically synthesized by the oxidation of their monomers at an electrode surface. A film of the polymer adheres to the electrode surface.

This research effort will lead to a new approach based on nanoscale science and technology to interface biochemical processes to integrated circuit chips. While the device that we will use to demonstrate proof of concept will be biosensors, the basic approach is general and applicable to other areas where an interface between biosystems

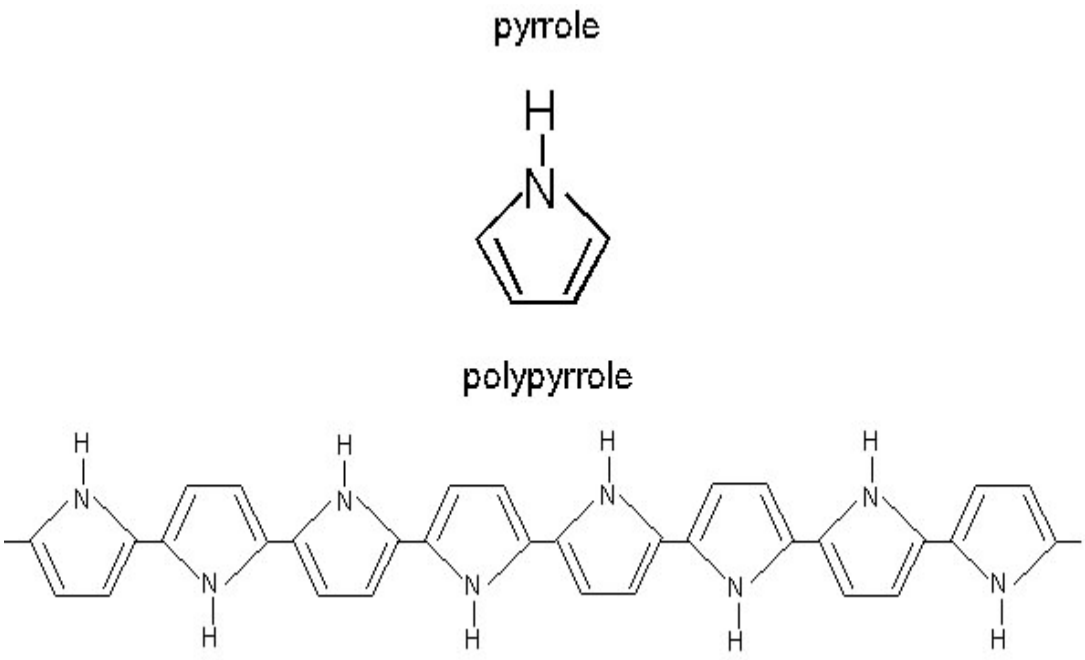

Figure 1-5. Structure of pyrrole and polypyrrole

and IC technology is needed. In addition, this research will make use of a variety of state-of-the-art nanomaterials fabrication, processing and manipulation methods including template synthesis, self-assembly, and bioencapsulation in nanoparticles. Electrochemical synthesis of polypyrrole are similar to other polymers such as polythiophene and polyaniline (7). The polymers can be electrochemically synthesized by the oxidation of their monomers at an electrode surface. A film of the polymer adheres to the electrode surface.

CHAPTER 2
REVIEW OF LITERATURE

Conducting Polymers as Support for Biosensors on Electrodes

Electrodes can be made more selective by modifying them with polymers of various sorts (19). In recent years, extensive research has been done on the modification of electrode surfaces by coating them with different types of polymers (7 - 22). These have been mainly of three types, i.e. conductive polymers, ion-exchange polymers and redox polymers. Often, chemical groups are attached to these polymer coatings (or incorporated into their structures) in order to introduce particular electrochemical effects. Recently, conducting polymers have attracted much interest in the development of biosensors on electrode surfaces (7). The most studied conducting polymers include polypyrrole, polyaniline and polythiophene. Conducting polymers are conjugated polymers, namely organic compound that have an extended π-orbital system, through which electrons can move from one end of the polymer to the other. These can be easily prepared by electrochemically oxidizing the monomer on the electrode surface (19). The solvent used, and more particularly the counter anion in solution, have a major effect on the properties of the polymer, in particular on its selectivity characteristics for use in sensors (19).

Conducting polymers have been used as a suitable matrix for the entrapment of enzymes (23, 24). Unwin and Bard (25) showed that incorporating of enzymes into conducting polymeric films permit the localization of biologically active molecules on

electrodes of any size or geometry which is useful for the fabrication of multi-analyte micro-amperometric biosensors.

Work by Gambhir et al. (26) demonstrated that electrochemical synthesis of conducting polymers allow the direct deposition of the polymers on the electrode surface, while simultaneously trapping protein molecules. They showed that it is possible to control the spatial distribution of the immobilized enzymes, the film thickness and the state of the polymer.

Conducting polymers have the ability to efficiently transfer electronic charge produced by the biochemical reaction to electronic circuit and can be deposited to defined areas of electrodes (27).

Dupont-Fillard et al. (28) reported a versatile and reversible DNA sensor. In this system, biotin grating-units (covalently linked to a polypyrrole matrix) are able to anchor large biomolecules due to biotin/avidin affinity. According to the authors, these grafting units can be regenerated after the "denaturation" of the biotin/avidin link, allowing the reuse of the matrix for the immobilization of another assembly and then the possibility to generate a new sensor.

In a paper by Livache et al. (9), pyrrole monomers were copolymerized with oligodeoxynucleotides, and these were further grafted to polypyrrole chains that had been electropolymerized on multi-microelectrode arrays. These syntheses were carried out on 50 μm electrodes on passive chips or on active (multiplexed) chips bearing 48 or 128 gold microelectrodes respectively. Here, electroconducting polymers were used for the construction of DNA or peptide arrays on microelectrodes on a silicon chip.

N. Lassalle et al. (29) reported the immobilization of oligodeoxynucleotides on polypyrrole through covalent grafting for electrode modification. These biofilms were used for the direct and real-time detection of DNA hybridization by using photocurrent spectroscopy and quartz crystal microbalance (QCM).

Interfacing Biochemical Processes with Solid State Electronics

The introduction of silicon as a substrate with on board field effect transistor connected to remote microelectrodes, specifically meant for electrophysiological measurements (action potentials), started in the late sixties at the Stanford University by Wise et al (10). It was later realized that it was a better idea that the transistor chip itself should form the electrode tip. Because of this device, ion concentration could be measured beside electric potentials. This device was called ISFET, for ion selective field effect transistor in 1970s (10).

Later work on interfacing sensor on a chip is the well known chemically selective field-effect transistor (Chem-FET) (20-22). A FET is a solid state device in which current is controlled between source and drain terminals by voltage applied to a gate terminal, which is insulated from the semiconductor substrate. This device consists of a field effect transistor in which the gate has been coated with a chemically selective film. Interaction of this film with the analyte molecule produces a change in the gate voltage, which in turn, changes the measured source-drain current. In this case the problem of separating the wet biochemical and the dry solid-state worlds is made easy because the gate is an inert dielectric material and only this comes into contact with the solution phase. Chem-FETs where the chemically-selective layer incorporates biomolecular-recognition agents including antibodies, enzymes, and ionophores have been described (21-22). Arrays of chemFETs on a single chip are also well known (20). In one example, a measurement

system with pH-sensitive ISFET for the measurement of extracellular pH-related signals on cells on tissues is presented. Small distances between sensor and cells and the use of ISFET sensors with fast response times allow high signal resolution in space and time. For insulation of the gate on the ISFET SiO_2 or SiO_2/ SiN_3 was used.

Another resent approach entails the use of a phototransistor IC chip to monitor DNA hybridization using fluorescently-labeled single-stranded DNA (30). This device is of interest because these authors had to solve the same problems of isolating the solid-state device from the analyte solution and confining the biochemical processes in proximity to the chip. Since photons were detected, the interface was simply a nitrocellulose membrane that is impervious to liquids that was placed above the photodetectors on the underlying IC chip. To confine the biomedical processes, a microdispensor was used to deposit drops of solution containing the desired single stranded DNA onto the surface of the nitrocellulose film. The DNA was subsequently cross-linked to the film. The size of each spot was on the order of 0.5 to 0.8 mm. This system was used to detect specific sequences of the human immunodeficiency virus (30). IC-based thermal sensors have also been described (31, 32). These sensors detect the heat released when an enzyme turns over its substrate molecule.

There has been a report of an array-based amperometric sensor on a chip (33, 34). This device was fabricated in a modified CMOS process. First, the complete analog and digital circuitry was produced. Then two passivation layers, one of silicon dioxide and one of silicon nitride, were deposited. The electrodes were structured into these passivation layers. The authors state that the passivation layers are “necessary to protect the electronic circuits against humidity while working in liquids”. No evidence for

sealing the circuitry on the chip from solution at any extended period of time has been presented.

Another method using silicon nitride to protect the underlying circuitry on a chip from solution above is reported. Here, a multi-analyte biosensor that uses magnetic microbeads as labels to detect DNA hybridization on a micro-fabricated chip was described (11). The beads are detected by giant magnetoresistance magnetoelectronic sensors embedded in the chip. The prototype device is a tabletop unit containing electronics, a chip carrier with a microfluidic flow cell, and a compact electromagnet and is capable of simultaneous detection of eight different analytes. To facilitate connection with the electronics, the chip is wirebonded to a chip carrier board. The chips are 5 mm × 5 mm and were designed to allow integration with fluidics. The surface of the chip is sputter-coated with a 1μm-thick silicon nitride layer to passivate the surface against the electrolytic solution that is present during an assay.

Interface Film

A general method called the template method pioneered by C. R. Martin to prepare nanomaterials in polycarbonate and other porous membranes has been reported (17, 18). This method entails the use of the pores in a micro or nanoporous membrane as a template to prepare nanoscopic particles of the desired material. Because the membranes used contain cylindrical pores with monodispersed pore diameters, corresponding cylindrical (and monodisperse) nanostructures are obtained. These may be either solid nanowires or hollow nanotubes. In particular, gold has been prepared in the pores of polycarbonate template membrane by electroless Au deposition (15). The membrane is 6 μm thick and has monodispersed 30 nm diameter pores, with pore density of 6×10^8 pores per cm^2 of membrane surface area (35-39). At first, the polycarbonate membrane is

plated with Au for 24 h, filling all the pores with gold nanowires and coating the surface of the membrane with thin Au films. The thin layers of Au on the faces are then removed by applying and removing a piece of Scotch tape (15). This leaves a polycarbonate membrane with Au filled pores. Studies have been done where these Au nanowire-containing membrane were used as ensemble of nanoscopic electrodes for electroanalytical chemistry (14-16).

The results that may suggest that these Au filled membrane may be suitable for an interface between biochemicals processes and solid state electronics are as follows: The ends of the nanowires act as electrochemically well-defined disk-shaped nanoelectrodes when immersed into an electrolyte solution containing a redox-active molecule. The potential of these disk-shaped nanoelectrodes can be controlled by applying a potential at the other end of the nanowire, and the resulting electrochemical current can be measured using conventional potentiostatic circuitry. The seal between the naowires and the surrounding polycarbonate membrane can be made completely watertight such that the solution in contact with the nanoelectrode disk at one face of the membrane does not penetrate through to the other face of the membrane. Electroanalytical detection limits at these nanoelectrode ensembles can be orders of magnitude lower than at electrodes of conventional dimensions. Electron-tranfer mediators that are used to shuttle electrons to redox proteins are electroactive at these nanoelectrodes at orders of magnitude more sensitive than at conventional electrodes. These electrodes are flexible and mechanically strong.

A recent report (40) of another type of advantage for nanoelectrodes as compared to conventional macroelectrodes was demonstrated by using Azure dyes which are

biosensor mediators. Here, it was reported that due to the poorer detection limit of macroelectrodes, the concentration of the dye must be very high and this causes adsorption of the reduced form at the electrode surface to occur. The lower detection limits at the NEE allow for lower concentrations of the Azure dyes to be used, thus preventing adsorption of the dyes to the electrode surface.

Making Ohmic Contact using Electrically Conductive Adhesive

Conductive adhesives are composite materials prepared by incorporation of metal powders, flakes or fibers, or other fillers, such as metal oxides and ceramic powders, into an organic matrix. Electrically conductive adhesives are mainly used as die attach materials in the semiconductor industry. Two other emerging applications are the bonding of electronic drivers to the liquid crystal display devices and the surface-mount assembly of integrated circuits and discrete devices to printed wiring boards. The primary function of these materials is to provide a structural bond between two high energy surfaces and assuring an electrical interconnection or heat transfer, or both, between the adherends (41).

In a review by Rabilloud, electrically conductive adhesives were shown to be available in two forms: pastes and films or tape adhesives. Paste adhesives are purchased either as two-part materials that have to be mixed just before use, or as premixed, degassed, and frozen in small tubes or syringes. Frozen adhesives have a fairly long shelf life when stored at -40ºC, and they are convenient to use since only the number of syringes that have to be used need to be allowed to come to ambient temperature. In contrast, although the two containers of the two part epoxies are degassed by the adhesive manufacturers, air is introduced when the components are mixed by the end-users and, if

not adequately degassed, air bubbles are entrapped in the viscous paste thus creating small voids in the bond line (41).

Silver is the metal filler of choice for the manufacture of conductive adhesives. It is seldom feasible to add more than 50% by volume of most metal and ceramic fillers because of the increase in viscosity far above the level acceptable in the production equipment. A technique to increase particle loading while maintaining low viscosity is to incorporate a solvent that evaporates during cure, but this method has the main disadvantage of causing the formation of voids (41).

According to Rabilloud (41), conductive epoxies typically contain several chemical components, one of the most important being the epoxy resin constituting the base of the adhesive formulation. To the epoxy resins are added a variety of materials including catalysts, co-reactants, reactive diluents, solvents, flexibilising or toughening agents and fillers. All these additives have a considerable effect on the properties of the adhesive composition before, during and after the curing process. Some commonly accepted terms such as single-component or two-component adhesives are misleading because they do not refer to one or two chemical ingredients but to one or two containers. Single or one-part systems contain a base epoxy resin, a hardener and, eventually, an accelerator combined with an adhesion promoter, various additives, and, an electrically conductive filler. Frozen one-part adhesives include highly reactive catalysts and thus have to be preserved at low temperature (ca. -40ºC) before use. In two-part systems, the epoxy resin loaded with conductive filler is delivered in one container and the hardener mixed with a solvent or reactive diluent combined with the same or a different conductive filler from the second component.

The one-part and two-part commercially available conductive adhesive are often claimed as solventless formulations. In fact, many of them contain from 5 to 10% by weight of organic solvents mainly used to lower the viscosity of the mixture.

In a report by Mitchell and Berg (42), it was shown that, except for one epoxy, the volume resistivity of commercial conductive adhesives is in the range of 2.27×10^{-4} Ω cm to a 2.74×10^{-5} Ω cm for two part material.

Silver-filled adhesives are prepared by dispersing silver particles in an insulated polymer matrix to form a metallic network within the organic binder. Electrons can move between the two surface boundaries only across the points of contact between adjacent particles, and the current flow through the conductive adhesive layer is generally described by the percolation theory, introduced by Broadbent and Hammersley (43). As regards to electrical conduction, the percolation model predicts a critical volume fraction Φc of the conducting material randomly distributed within the insulating medium, below which the overall conductivity is zero. In other words, for volume loadings Φf smaller than the percolation threshold (i.e., for $\Phi f > \Phi c$), the probability is zero that a conductive path will cross the thickness of the sample, because the establishment of such conductive channels requires uninterrupted contacts between the particles of the metallic filler.

Klosterman et al. reported (44) on the resistance measured across the interfaces formed within an adhesive joint. It was first establish that, in general, silver filled adhesives have filler volume fractions that exceed the percolation threshold, and therefore, any change in electrical resistance requires an understanding of the conduction characteristics at the interfaces. At the contact points the current flow is constricted because of the small contact area and the bending of the current flow path requires an

added voltage called the constriction voltage. Another additional voltage is necessary if an insulating film, oxide or polymer, separates the conducting particle or their junction with the substrates.

CHAPTER 3
MATERIALS AND METHODS

Materials and Reagents

Anhydrous $SnCl_2$ (Aldrich), $AgNO_3$ (Spectrum), trifluoroacetic acid, Na_2SO_3, $NaNO_3$, NH_4OH, formaldehyde, methanol and concentrated HNO_3 (Mallinckrodt) were used as received. Commercial gold electroless plating solution (Oromerse Part B) was obtained from Technic Inc. Milli Q 18-MΩ water was used for rinsing and preparation for all solutions. [(Trimethylamino)methyl]ferrocene ($TMAFc^+$) perchlorate was prepared by metathesis from the iodide salt and recrystallized (45). The iodide salt was prepared from [(dimethylamino)methyl]ferrocene (Aldrich) according to the established procedure (46). Solutions of Pyrrole, aniline and 3, 4-dioxyethylenethiophene (Aldrich) were purged with nitrogen prior to use. A Two part electrically conductive epoxy (part # 19-2092) was purchased from GE electronics and was prepared and used as directed by the manufacturer.

Membrane

Porous polycarbonate membranes were made by nuclear track-etched technology (47, 48). Heavy particles (such as uranium fission fragments) create single-event "damage tracks" in these polymeric insulators (47-49). These tracks can be chemically etched at a greatly enhanced rate (compared to the bulk material). Irradiation of a membrane with beam of heavy particles followed by chemical etching results in a membrane with randomly distributed pores. These track-etched polycarbonate membrane filters were obtained from Osmonics. Membranes 6 μm thick with pore size of 30 nm in

diameter were used to make the nanoelectrode ensembles (NNEs). The pore density of the membranes is approximately 6×10^8 pores / cm^2. These membranes have a rough and smooth face (50). The rough face has a dull (nonshiny) appearance and the smooth face has a shiny appearance (50). Electron microscopy shows that the pores on the smooth face are more regular in shape and size than those on the rough face. Figure 3-1 is an electron micrograph of a 30 nm pore diameter polycarbonate membrane as received from Osmonics before experimental treatment.

Electroless Gold Deposition

Au nanowires were deposited within the pores of the polycarbonate membrane using an electroless deposition method (Fig.3-2) developed by the Martin group (51). The polycarbonate membrane was immersed in methanol for five minutes and then immersed in a solution of 0.025 M $SnCl_2$ and 0.07 M trifluoroacitic acid causing it to be Sn-sensitized (51). The membrane was then immersed in methanol for 5 minutes twice. Following this, the membrane was immersed into an ammoniacal solution of 0.029 M $AgNO_3$ for five minutes then in methanol for five minutes. Finally, the membrane was placed in a gold-plating bath, consisting of 0.5 mL of the commercial gold plating solution, 0.127 M in Na_2SO_3, 0.625 M formaldehyde, and 0.025 M in $NaHCO_3$. Before placing the plating solution into the bath, the pH of the solution was adjusted from 12 to 10 using 0.5 M H_2SO_4 dropwise while being stirred. The temperature of the bath was maintained at 4°C. The polycarbonate membrane was left in the gold-plating bath for 24 hours. This ensures that the pores were completely filled to form solid gold cylinder or wires (Fig. 3-3). After 24 hours the membrane was removed from the bath and immersed in water for 6 hours. The membrane was then immersed in 25% HNO_3 for 12 hours then

rinsed with water for 2 more hours. After removal from water the membrane was left to dry in air.

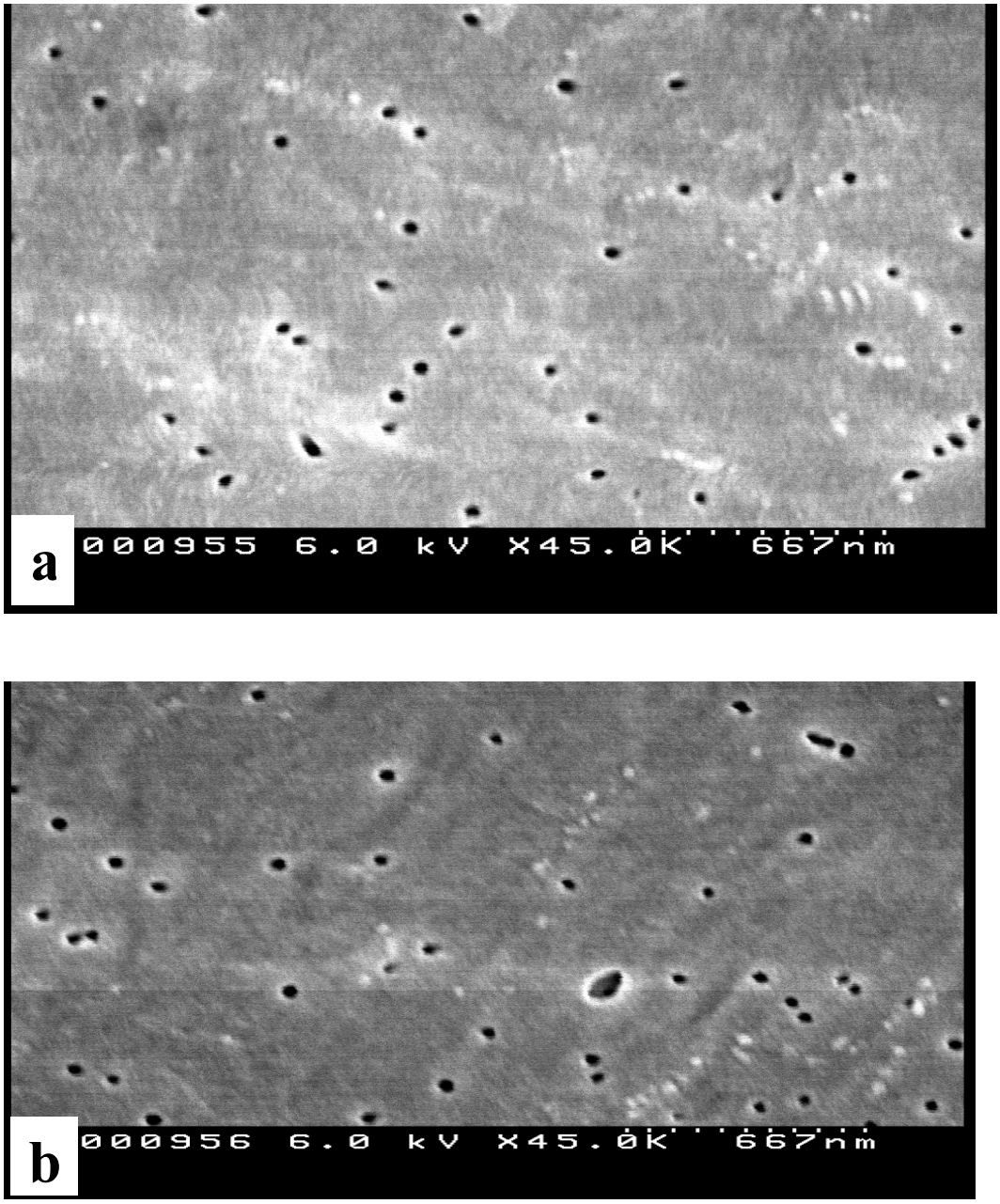

Figure 3-1. Electron micrographs of 30 nm diameter pore polycarbonate membranes as received from Osmonics before experimental treatment showing (a) smooth face and (b) rough face

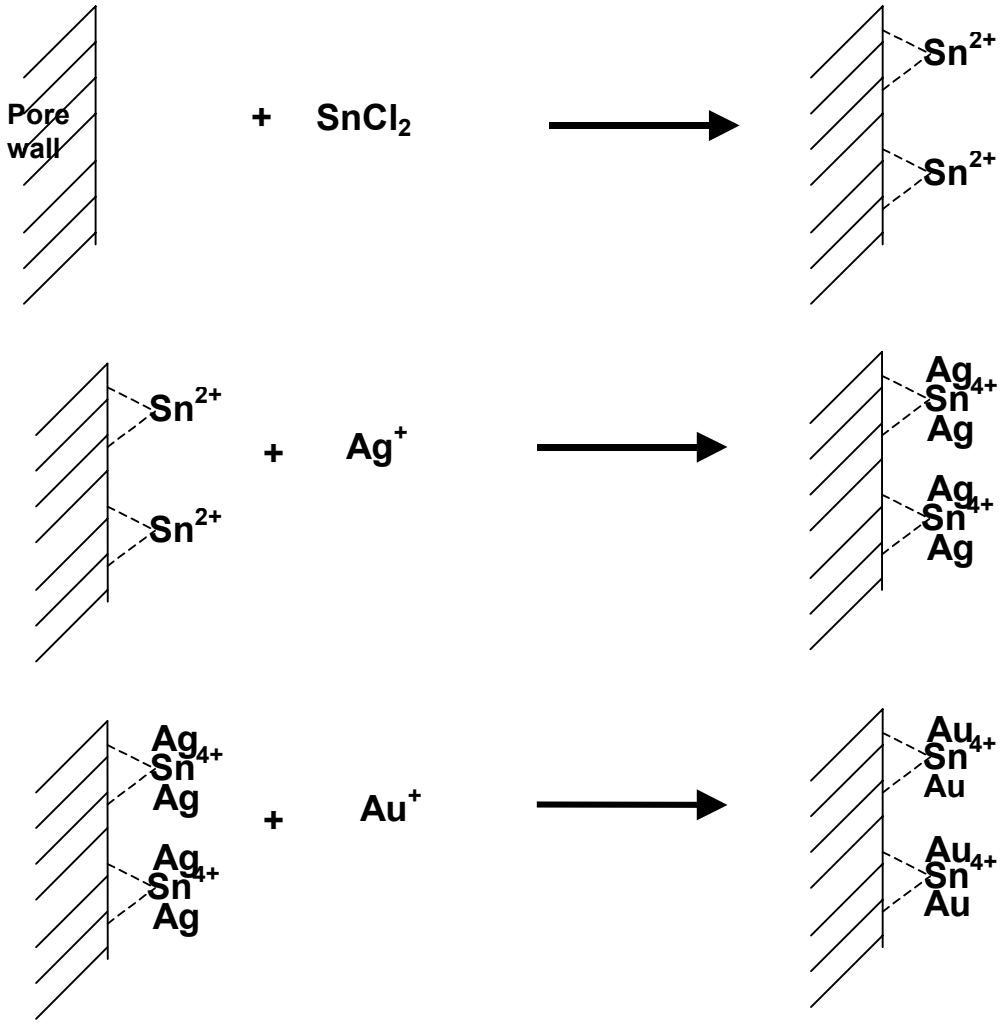

Figure 3-2. Electroless procedure used to deposit gold in the pores of the polycarbonate template membrane

Preparation of the Interface Film

The interface film is the nanowire-containing polycarbonate with both gold surface layers removed. The gold surface layers of the membrane are removed by applying transparent mailing tape (Scotch^{TM}) to both surfaces of the film and then removing them. This leaves a polycarbonate film with monodispersed nanowires running throughout the thickness of membrane. Figure 3-4 shows the schematic for the removal of the gold surface layers.

At this point a heat-treament process is applied to the membrane (Fig 3-5). This procedure produces a water-tight seal between the Au nanowires and the pore walls (51). The membrane is sandwiched between two glass slides, and placed in a glass dish. It is heated (above the glass transition temperature of 150°C) in an oven at 170°C for 15 min. This sandwiching keeps the membrane flat and prevents wrinkling. Without being sandwiched between glass slides, the membrane becomes nonconductive after heat treatment. The membrane is taken out from the oven and left to cool to room temperature.

This next stage of the interface film preparation involves the protection of the membrane from solvent and small silver particles in the electrical conductive epoxy while enhancing electrical conductivity in the areas where it will be in contact with the contact pads of the mini-circuit board. Four 1.5 mm holes are punched in double layers of Scotch tape (Fig. 3-6). The holes are arranged in a square with a gap of 2 mm between them. This arrangement of the holes is identical to that of the contact pads on the mini-circuit board. The heat-treated membrane is placed on nonstick paper with the rough surface facing up. The tape mask is then placed on the membrane. This tape-membrane composite is placed in a sputtering system (Hummer 6.2, Anatech LTD) with the holes facing up. Gold is sputtered for 45 minutes under 60 Pa pressure using 15 Amps. After sputtering, the sputtered membrane is taken out from the sputterer and the upper tape is removed to expose four Au pads. Using a small precision scissors, the membrane is then cut to the appropriate size to be placed on the mini-circuit board.

Mini-Circuit Board

The mini-circuit board (Fig. 3-7) is made from standard circuit board material (laminate of epoxy and glass fibers). It is 2.54 cm^2 and has 4 contact pads (made of steel)

that run perpendicularly throughout its thickness. At the top of the board the pads lie evenly to the surface and are arranged in a square at the center of the board with 2 mm gap between them. The diameter of each pad is 1.5 mm. At the bottom of the board, the pads protrude about 1.5 cm. The board was designed and made by Professor John Harris's group in the electrical and computer engineering department at University of Florida.

Interface Film Mini-Circuit Board Assembly

Conductive epoxy is spread evenly on the contact pads of the mini-circuit board. The membrane is assembled to the circuit board with its Au pads aligned to the contact pad on the board (Fig. 3-8). A glass slide is placed on top of the membrane providing a small pressure to ensure good contact. This assembly is left to cure for 24 hours. After curing, a tape with a punch hole wide enough to surround the electrode area, is placed on the membrane to seal the edges of the interface film. The resistance between the exposed smooth surface of the membrane and the contact pad is measured using a Keithley 2000 multimeter. The resistance averages 3 Ω. The resistance across the contact pad and the epoxy averages 0.7 Ω.

Electrochemical Measurements and SEM Observations

Cyclic voltametry was carried out on 5mM $TMAFc^{+}$ perchlorate in 1mM $NaNO_3$. A 3mL plastic pipette was used to drop the solution onto the electrode area of the interface film circuit board assembly. The contact pads of the assembly were connected to a PAR model 273 potentiostat and corrware software was used to generate the voltamograms. The electrodes were further modified with conductive polymers and cyclic voltamograms for their growth and electroactivity are shown. The electrode arrangement and setup is shown in figure 3-9.

Electropolymerization of pyrrole was carried out on the nanoelectrodes using 20 mM pyrrole in 100 mM $LiClO_4$. The polypyrrole films were synthesized on the nanoelectrodes by potential sweeping between -0.35 and + 0.85 V vs Ag/AgCl at a scan rate of 100 mV/s for 36 cycles. Similar runs were done using only nanoelectrodes as working, reference and counter electrodes. Electroactivity of the polypyrrole electrodes was measured in 0.5 M KCl, with potential sweep from 0.0 to + 0.5 V at a scan rate of 20 mV/s.

Electropolymerization of aniline was carried out on the nanoelectrodes using 0.1 M aniline in 0.1 M H_2SO_4. The polyaniline films were synthesized on the nanoelectrodes by potential sweeping between -0.4 and + 1.2 V vs Ag/AgCl at a scan rate of 20 mV/s for 36 cycles. Similar runs were done using only nanoelectrodes as working, reference and counter electrodes. Electroactivity of the polyaniline was measured in 0.5 M H_2SO_4, with potential sweep from -0.1 to + 0.7 V at a scan rate of 50 mV/s.

Electropolymerization of 3, 4-ethylenedioxythiophene (EDOT) from 3, 4- was carried out on the nanoelectrodes using 0.5 EDOT in 0.07 M SDS + 0.1 M $LiClO_4$. The PEDOT films were synthesized on the nanoelectrodes by potential sweeping between -0.5 and + 1.0 V vs Ag/AgCl at a scan rate of 100 mV/s for 36 cycles. Similar runs were done using only nanoelectrodes as working, reference and counter electrodes. Electroactivity of the PEDOT electrodes was measured in 0.2 M H_2SO_4, with potential sweep from -0.1 to + 0.7 V at a scan rate of10 mV/s.

Scanning electron micrographs of polycarbonate and conductive polymer growth were obtained with a Jeol 6400 microscope.

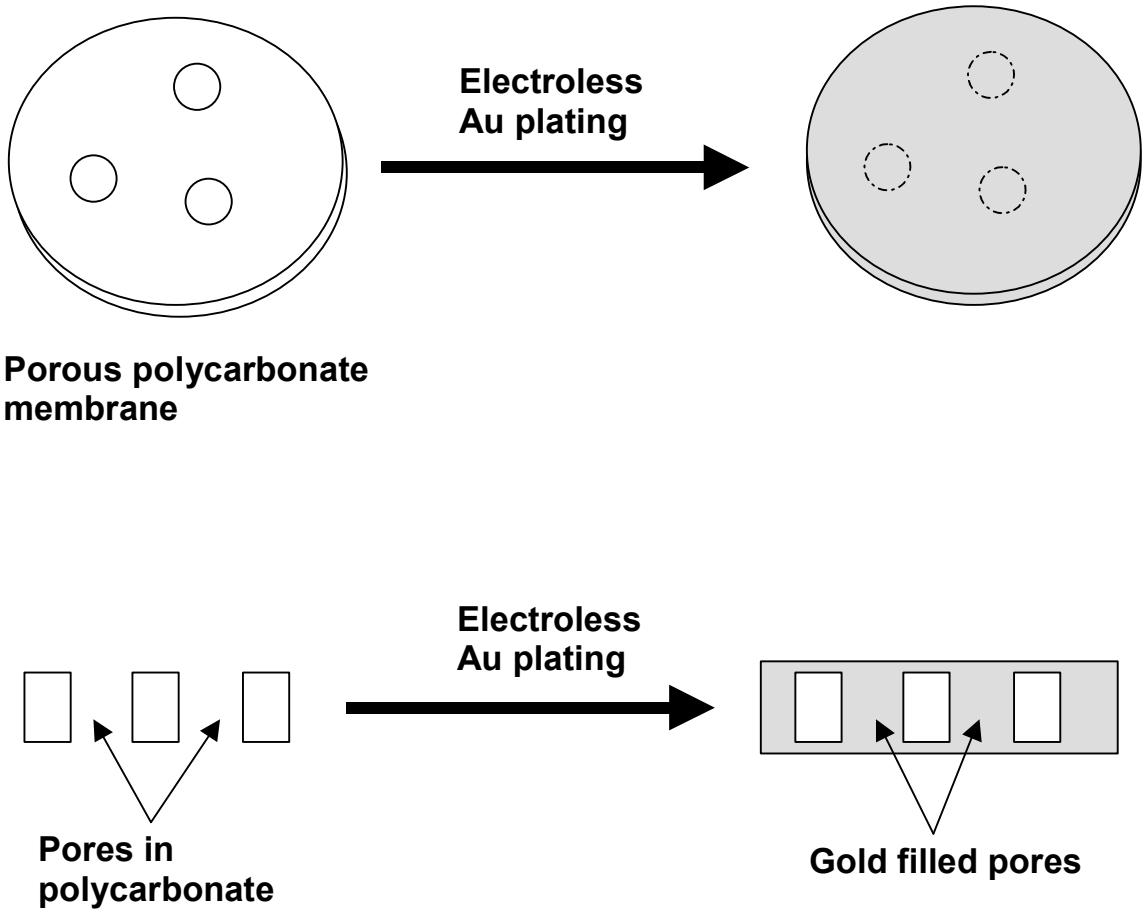

Figure 3-3. Electroless plating of Au in porous polycarbonate membrane

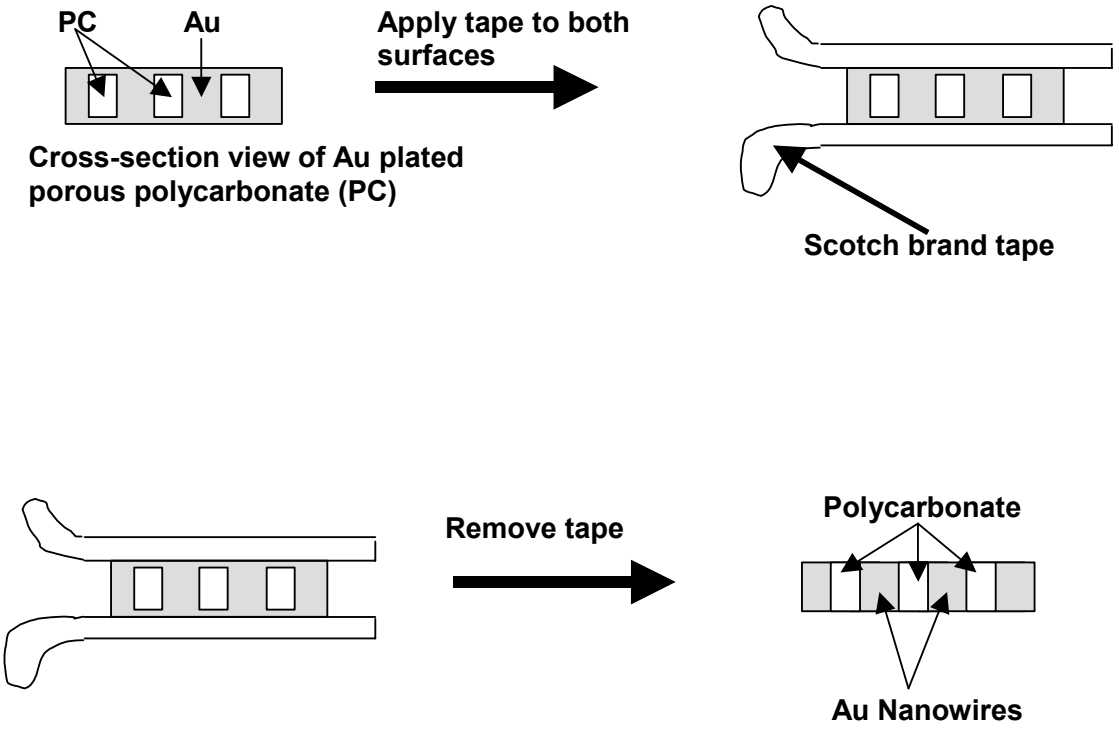

Figure 3-4. Fabrication of nanowire-containing polycarbonate from Au plated polycarbonate.

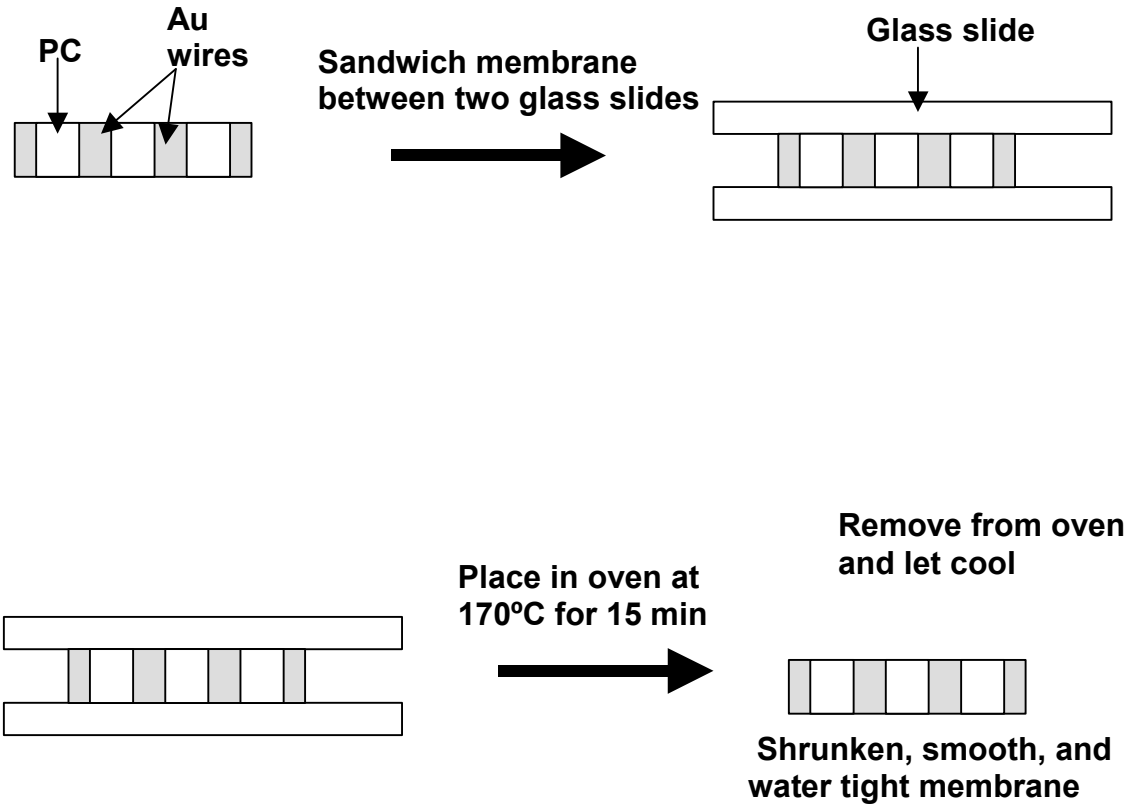

Figure 3-5. Heat treatment of nanowire-containing polycarbonate membrane

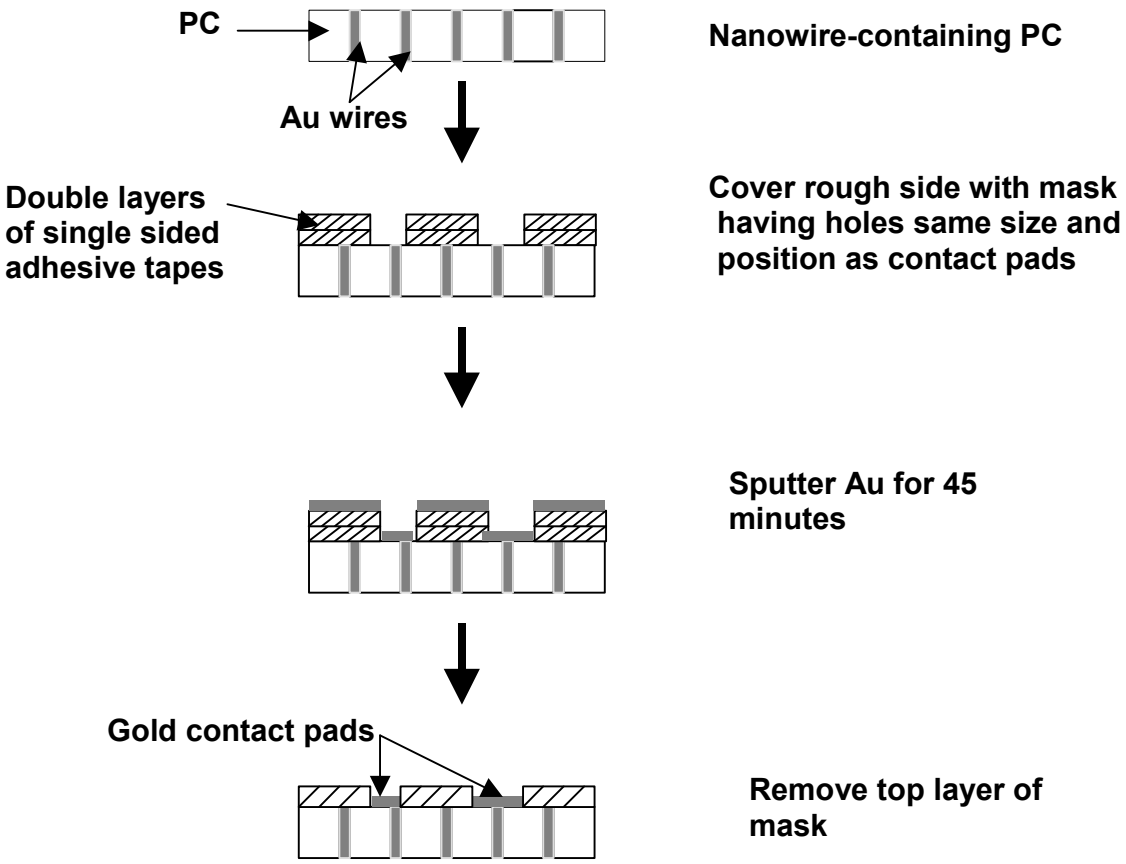

Figure 3-6. Procedure for making Au contact pads on interface film

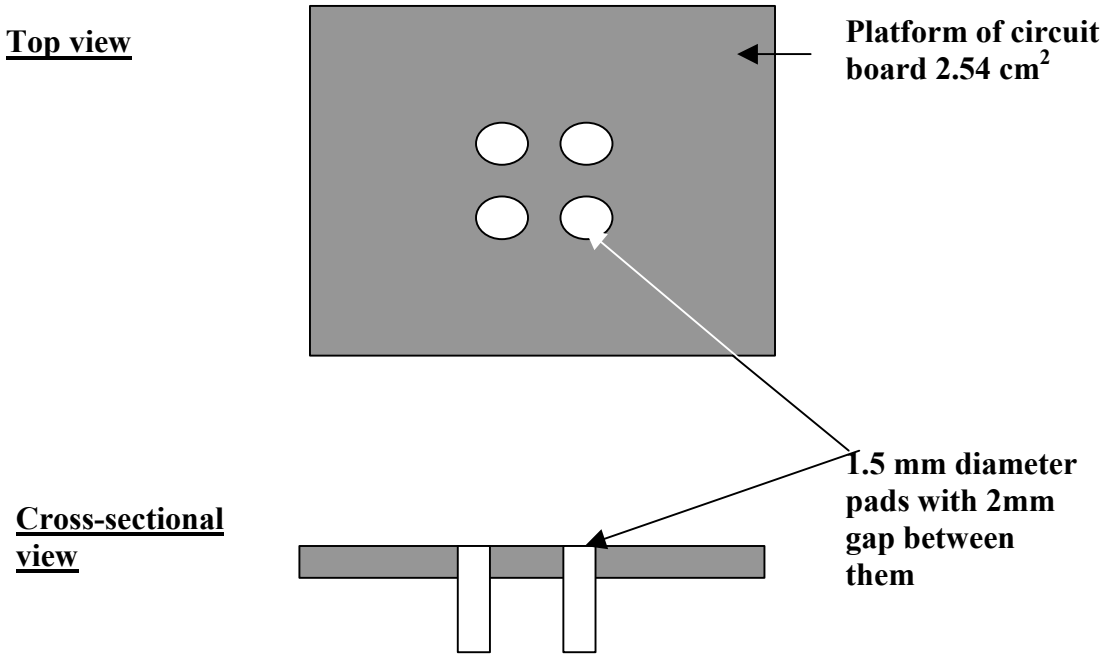

Figure 3-7. Diagram of mini-circuit board

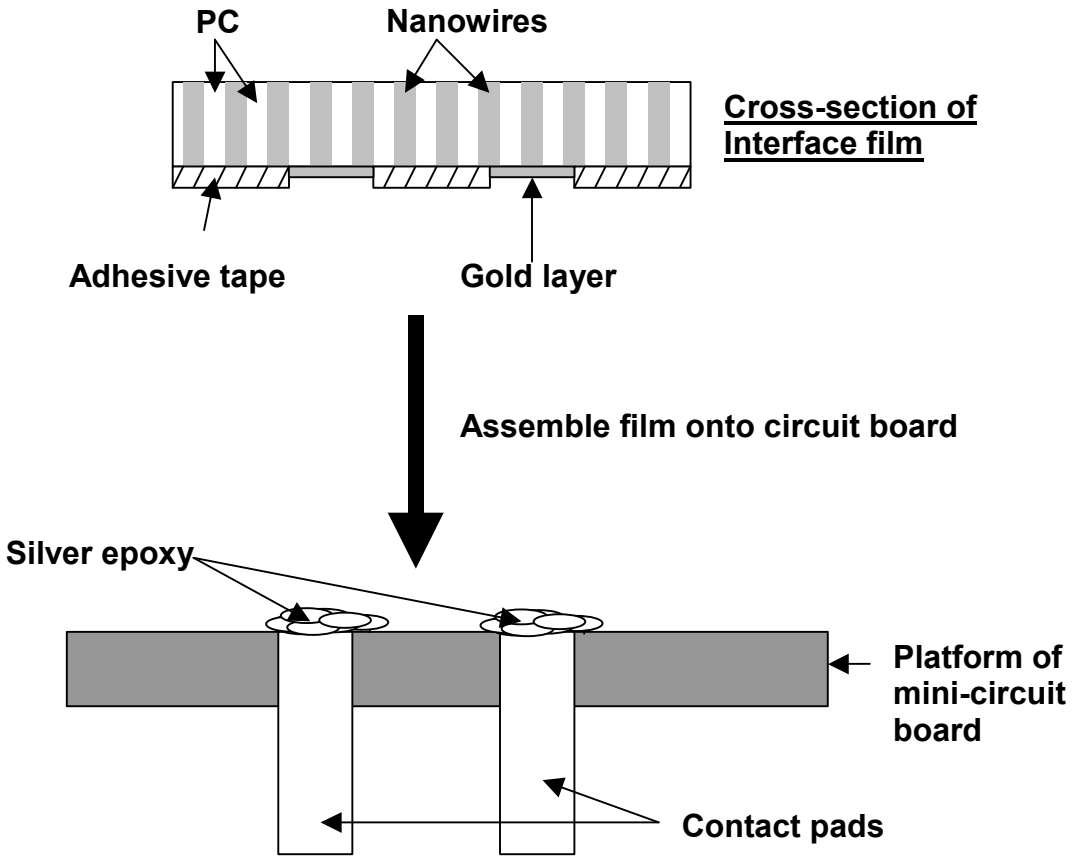

Figure 3-8. Assembly of interface film onto mini-circuit board

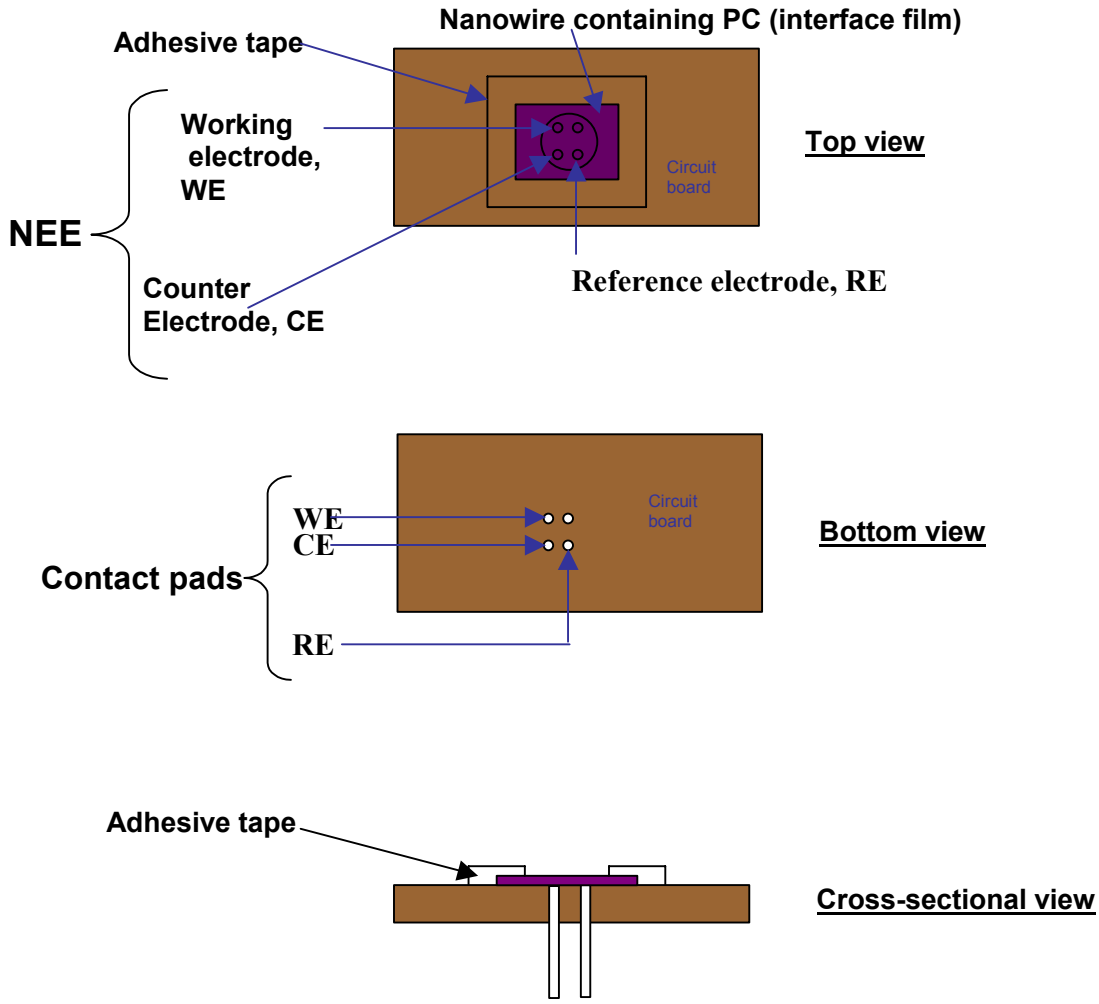

Figure 3-9. Electrode arrangement and setup for electrochemical measurements

CHAPTER 4
RESULTS AND DISCUSSION

Interface Film

Figure 4-1 shows a scanning electron micrograph of a 30 nm diameter pore polycarbonate membrane that has been Au plated for 24 hr and had the Au surface layers removed. As can be seen, numerous (~10^9) nearly circular Au disk (white spots) with diameters of ~30 nm are randomly distributed across the surface with average center to center distance of 200 nm. These disks are the ends of the Au nanowires that run through the pores in the membrane. One of the criteria for the membrane to be useful as an interface film is that, it conducts electricity efficiently across its thickness. This criterion

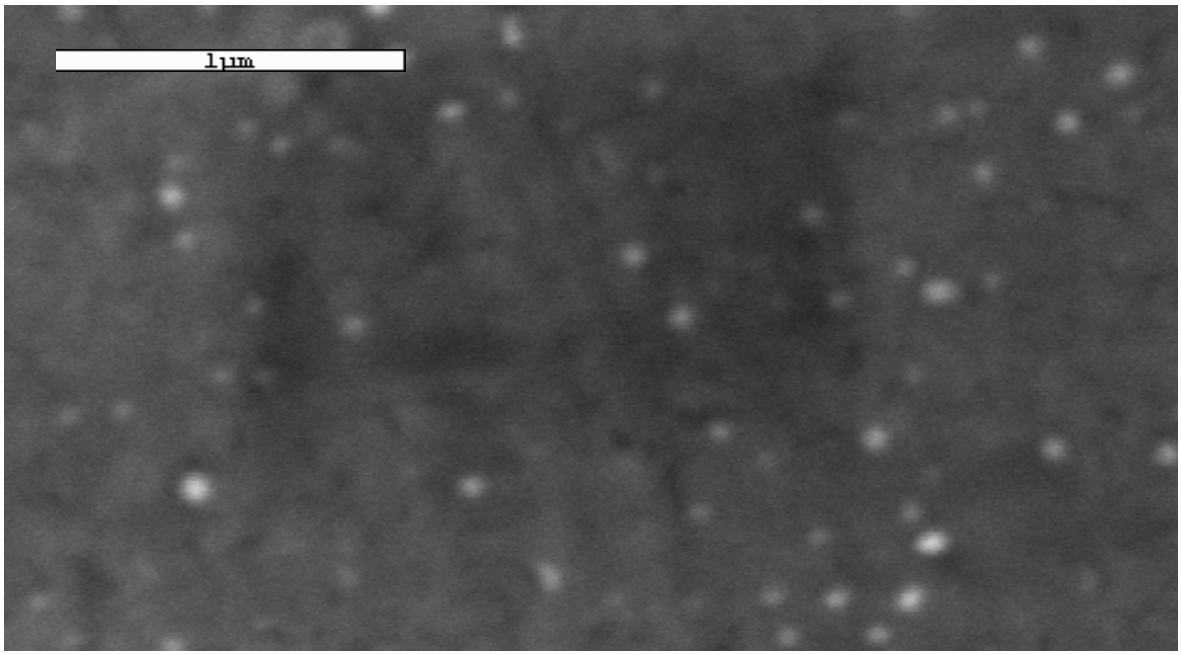

Figure 4-1. Scanning electron micrograph of the surface a 30 nm diameter pore polycarbonate membrane with Au nanowires

of conductivity was met when the resistance was measured across the film thickness giving 50–100 Ohm or less.

Another criterion for the polycarbonate film to be effective as an interface film is that, it must be impervious to solutions and protect the underlying circuitry from wet biochemistry above. As described previously (51), heating the polycarbonate membrane above the glass transition temperature (T_g ~150 ºC) causes it to shrink and seals the junction between the Au nanowires and the polymer membrane. Literature has been cited (12, 13) where, authors claim to have solved the seepage of solution between the electrode and substrate down onto a biochip circuitry using passivation layers of silicon dioxide and silicon nitride. It is difficult to imagine solution applied to the surface of the chip not seeping through the microscopic pores in these passivation layers. No demonstration of long term function was reported, however, our interface film has performed reproducibly without leakage for many months.

Electrical Communication

The nanoelectrode ensemble within the interface film makes electrical communication between the wet biochemical processes and the underlying circuit board. Figure 4-2 shows cyclic voltammograms of the background currents of a typical nanoelectrodes ensemble (NEE) fabricated in the Martin lab, and that of the nanoeletrode ensemble/circuit board assembly (NEE/CBA) fabricated in this work. As can be seen, the background currents, about 10 n Amps, for 1 mM $NaNO_3$ are about the same for both structures. This suggests that detection limits of analytes for our NEE/CBA should be comparable to that of the NEE as reported (19).

Figure 4-3 shows cyclic voltammogram for 5μM [(trimethylamino) methyl] ferrocene ($TMAFc^+$) perchlorate at a NEE/CBA. These voltammograms are complete

analogs to those obtained in fundamental investigations of electrochemistry of these NEE. (19). Here, the peak current is about 50 nAmps and the conventional peak-shaped voltammogram is seen as reported in these previous studies (51). As pointed out by

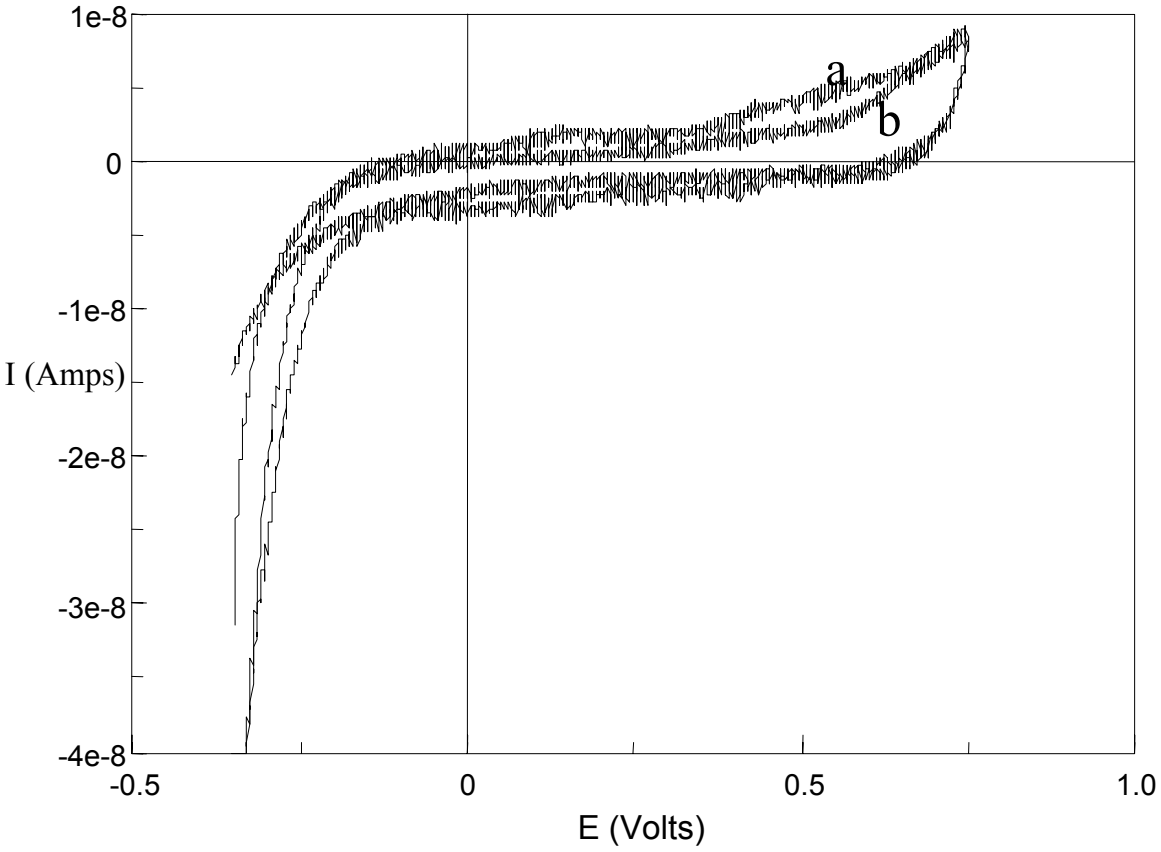

Figure 4-2. Cyclic voltammograms of the background currents of (a) (NEE) and (b) (NEE/CBA) in5 mM $NaNO_3$ at 50 mV/s

Menon and Martin (51), detection limits of nanoelectrode ensembles operating in this so called total overlap mode, can be as much as three orders of magnitude lower than at convention electrodes of the same geometric area. In the total overlap mode, the current signal generated at the NNE is equivalent to that obtained at the conventional macroelectrode of equivalent geometric area. However, the background double-layer charging current is significantly less because these currents are proportional only to the

active Au area. The detection limit depends on the ratio of the background to faradic current.

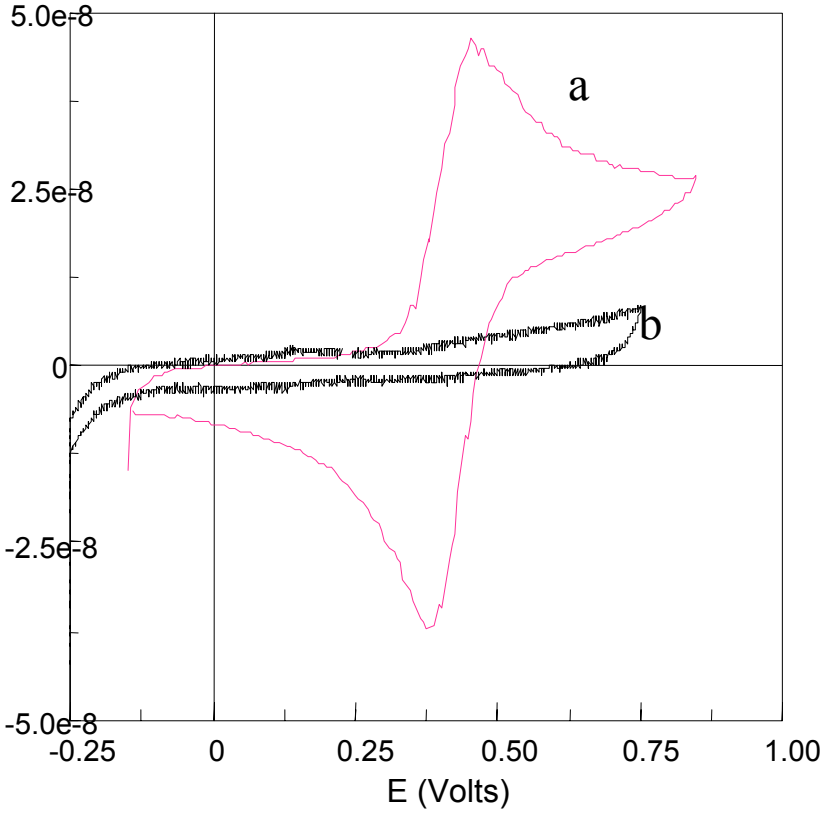

Figure 4-3. Cyclic voltammogram at NEE/CBA for (a), 5µM [(trimethylamino) methyl] ferrocene (TMAFc+) perchlorate in 5mM $NaNO_3$ and (b), 5mM $NaNO_3$

In these studies the reference and counter electrode were immersed into the solution with the NEE/CBA. It is also possible to have collections of the NEE elements function as the counter electrode and as a quasi-reference electrode. The advantage is that this provides a totally self-contained package, and no external electrodes are required. Figure 4-4 shows cyclic voltamogramms for $TMAFc^+$ at NNE/CBA where all three electrode areas are ensemble of nanoelectrodes. As can be seen, the oxidation and reduction peaks are well defined, showing clear reversibility as is well know for this compound. This shows that excellent electrochemical performance is obtained using this self-contained

electrode approach. All future work was carried out using only gold nanoelectrode ensembles as working, counter and reference electrodes.

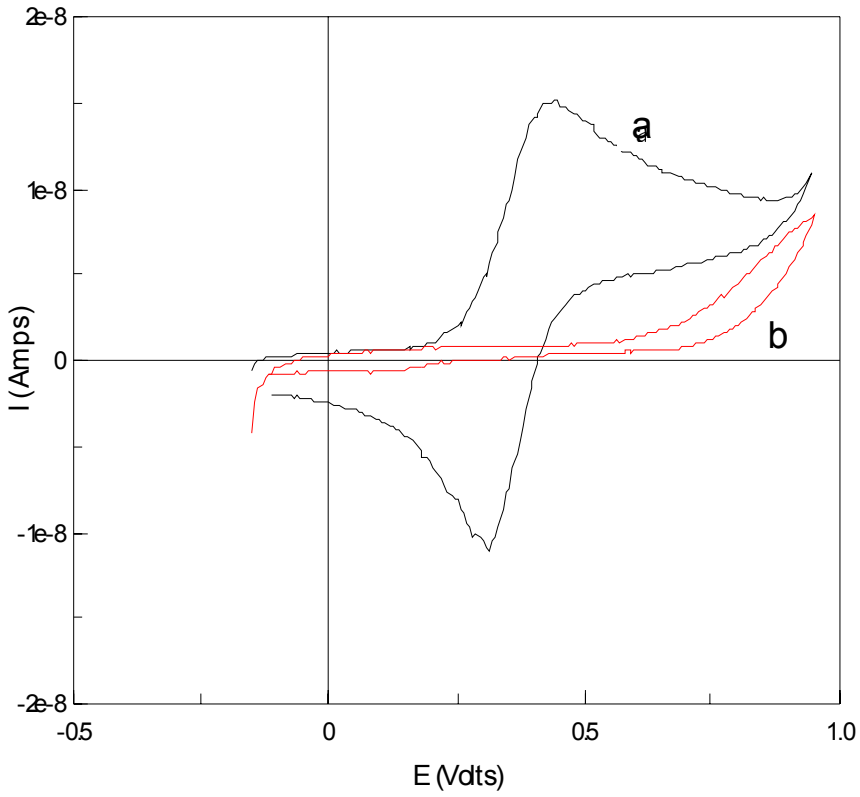

Figure 4-4. Cyclic voltamogramms at NEE/CBA for (a), 5 µM TMAFc+ in 5 mM $NaNO_3$ and (b), 5 mM $NaNO_3$, where all three electrode areas are ensemble of nanoelectrodes

One of the objectives was to establish electrical communication between solid state electronics and biochemical processes (biosensors). A solution of monomers (pyrrole, 3-4-ethylenedioxythiophene and aniline), were individually placed on the surface of the NEE/CB. Each solution was in contact with the Au electrodes of the NEE/CBA. Selecting one set of NEE on the device as working electrode, and sweeping the potential as described in the experimental, electrochemically polymerized the monomers on that particular electrode surface. Addressing of different monomers was achieved by

successive polymerizations on the selected set of NEE on the NEE/CBA. Figure 4-5 shows a photograph of NEE/CBA surface with the film of polypyrrole and polyanilene. It can be seen that polymerization is exactly limited to the selected working electrode surface.

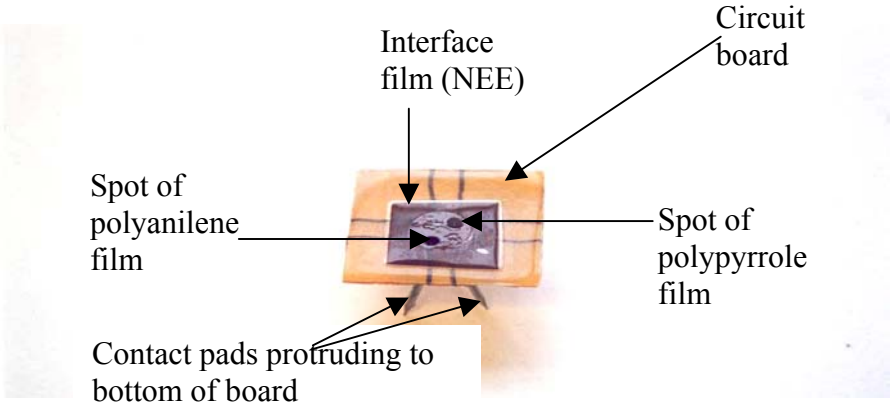

Figure 4-5. Photograph of NEE/CBA with spots of polypyrrole and polyanilene on surface of interface film.

Figures 4-6 shows low magnification SEM images of polypyrrole, polyaniline, and poly(3,4-ethylenedioxythiophene) (PEDOT) that were electrochemically polymerized on the working electrodes of the NEE/CBA. These figures indicate that each set of NEEs in contact with a contact contact pad selected as the working electrode, can

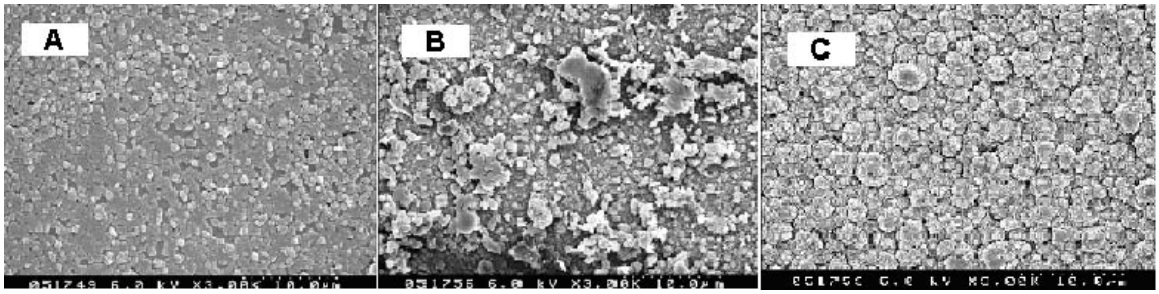

Figure 4-6. Scanning electron micrographs of (a) polypyrrole (b) polyaniline and (c) PEDOT, that were electrochemically polymerized across a portion of the NEE/CBA surface

be addressed individually (a necessary feature for any multielectrode array with multifuntional assay capability). This feature allows for modification of electrodes with different biosensor materials.

Figure 4-7 through 4-8, show cyclic voltammograms for the growth of polypyrrole, polyaniline, and PEDOT, from electrochemical polymerization of their monomers on the surface of the working electrode of the NEE/CBA. Figure 25 through 27, show cylic voltammograms for the electroactivities of these polymers. These cyclic voltammograms are similar to the ones presented in the literature (52-54), and the experimental conditions are similar. There are four contact pads on the NEE/CBA, each making electrical contact with a set of nanowires in the interface film that make up one NEE. When one of these contact pads is selected by the potentiostat as the working electrode connector, nanowires directly above it is in turn selected as the working NEE. Three of these four NEEs have been modified successively by three different electrically conducting polymers when a specific potential sweep had been applied to each NEE. It was shown that each of the four NEEs can be independently selected at will as the working electrode of the NEE/CBA, and that electroactive chemicals (biochemical processes) at the electrode surface are in electrical communication with the device. These modified electrodes have a specific shaped voltammogram for a specific electrolyte. In other word, each recognition element or polymer has a unique signature for a specific analyte. Here, we have demonstrated that our device can reproduce well known electroactivities of these electrically conductive polymers. These results suggest the possibility of immobilizing a series of different biological sensing elements in a precise location (address) on our device for analysis of different analytes.

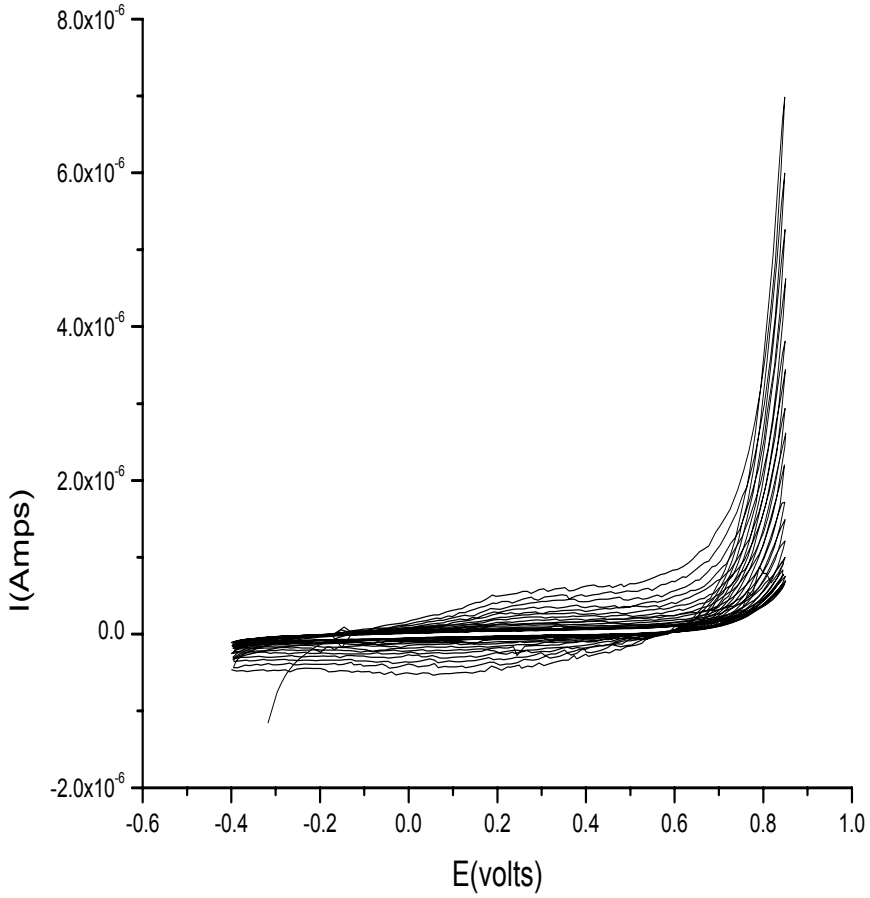

Figure 4-7. Polypyrrole voltammetric waves during electrochemical polymerization of the monomer on the surface of the working electrode of the NEE/CBA

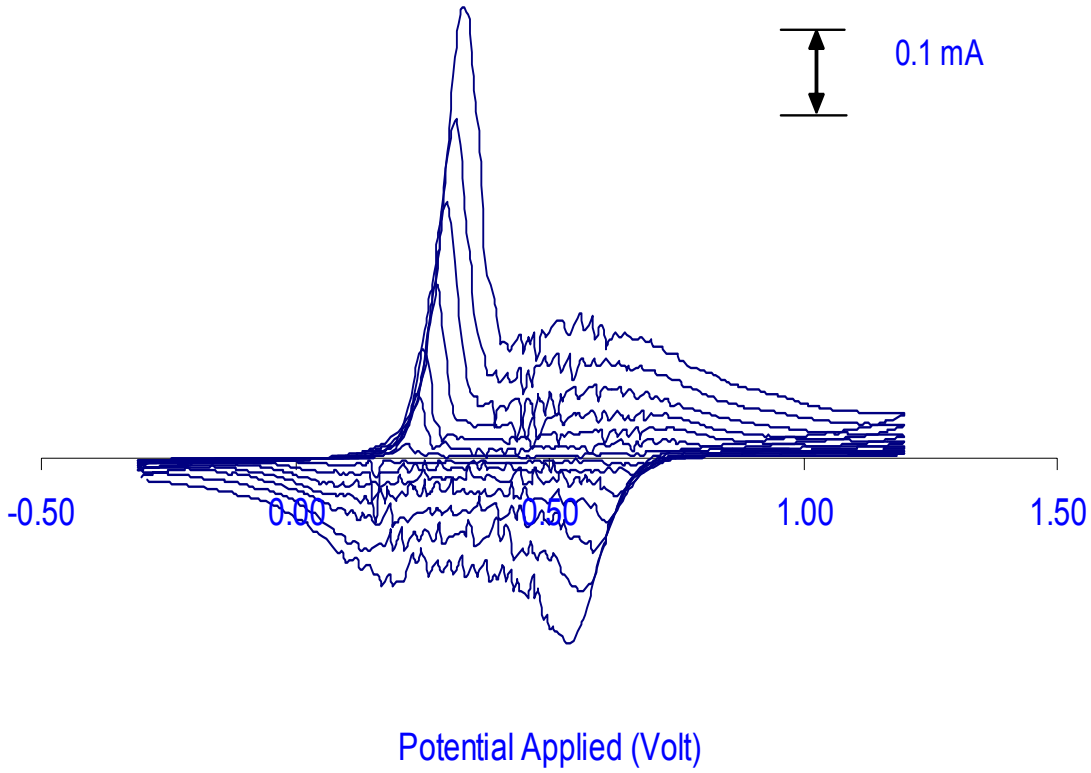

Figure 4-7. Polyaniline voltammetric waves during electrochemical polymerization of its monomer on the surface of the working electrode of the NEE/CBA

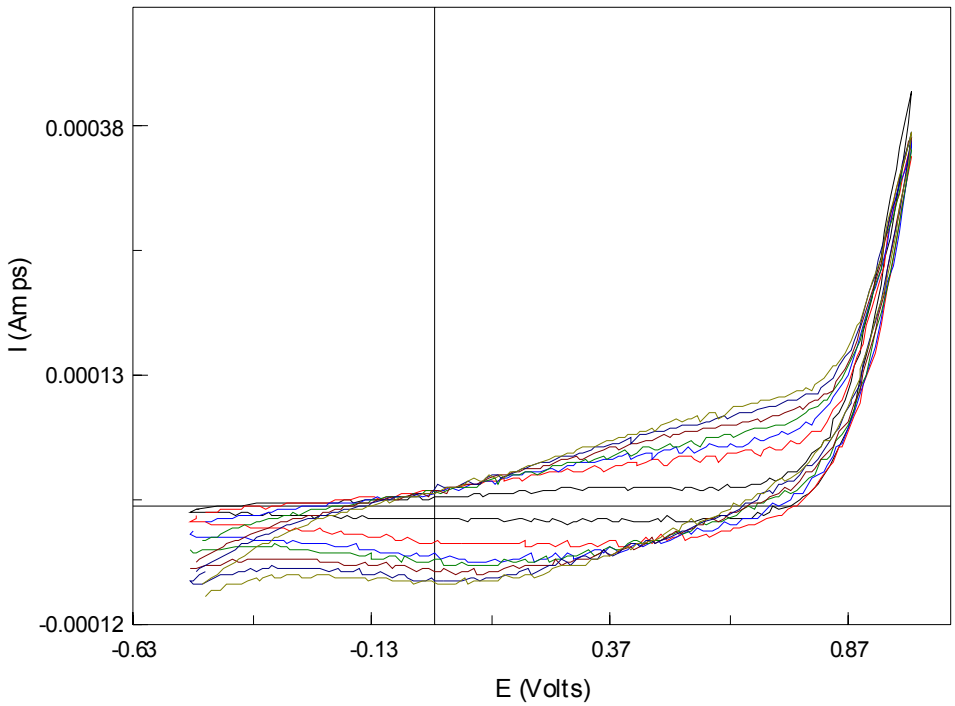

Figure 4-8. Voltammetric waves of PEDOT during electrochemical polymerization of its monomer on the surface of the working electrode of the NEE/CBA

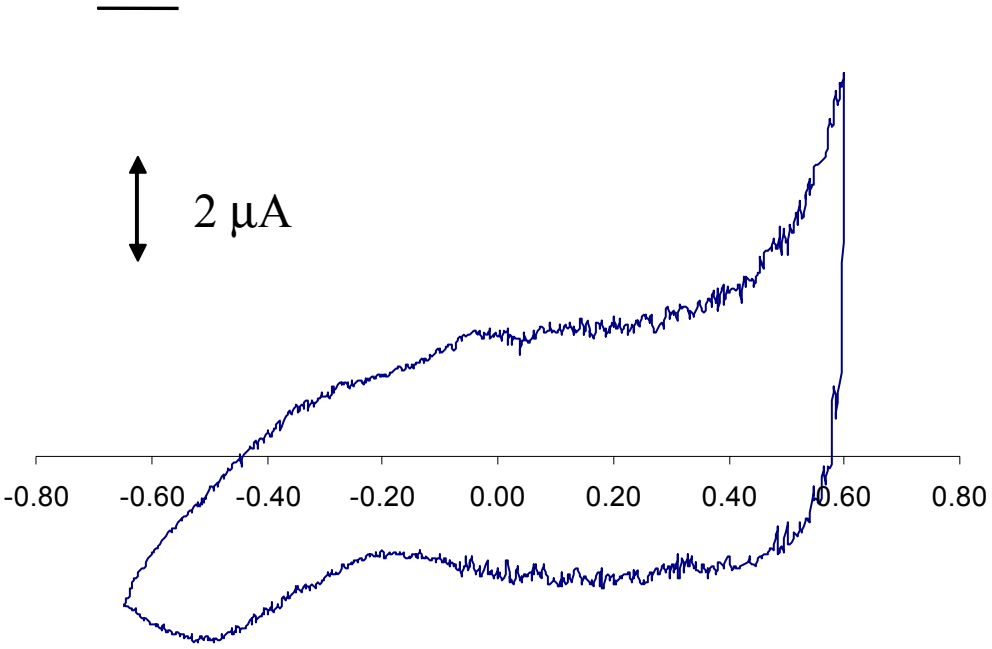

Figure 4-9. Cylicvoltammogram for the electroactivity of polypyrrole film in 0.5 M KCl at 20mV/s

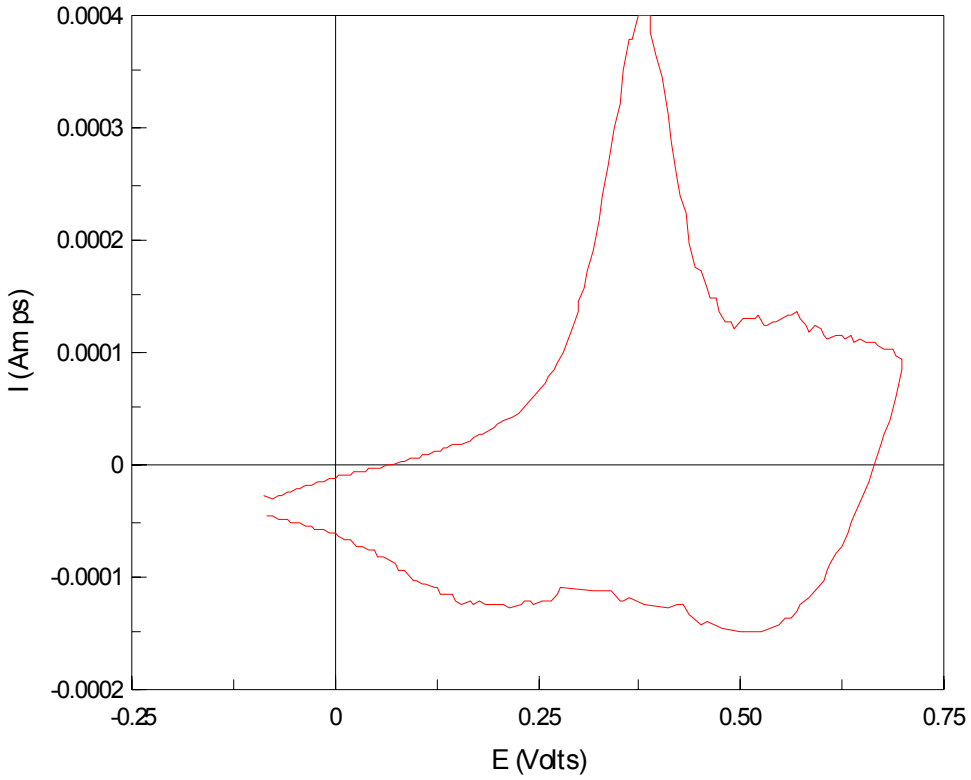

Figure 4-10. Cyclic voltammograms for the electroactivity of PANI film in 2M H_2SO_4 at scan rate of 50 mV/s

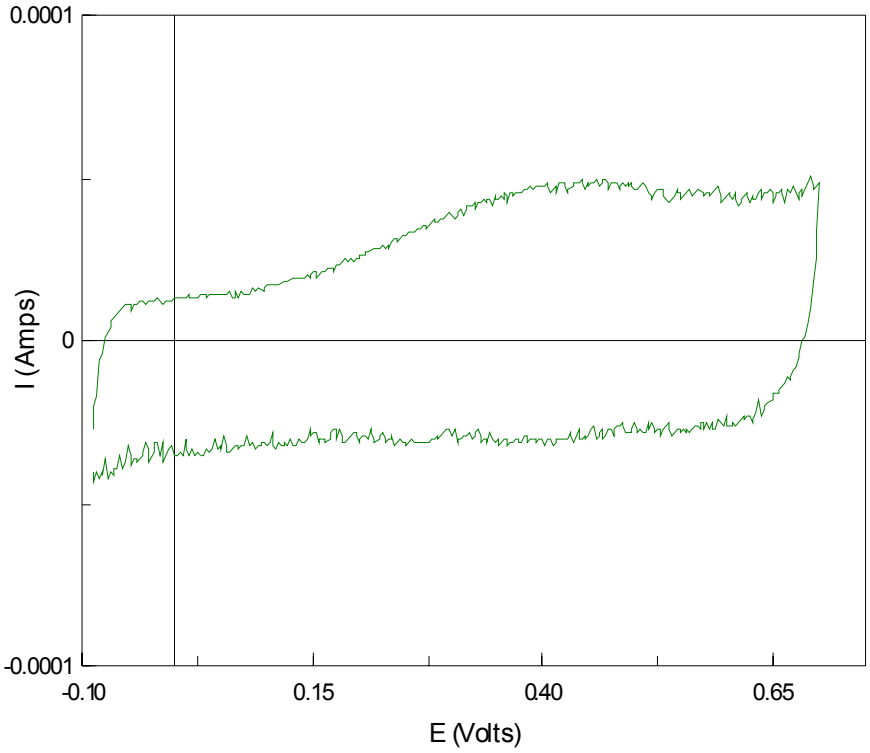

Figure 4-11. Cyclic voltammograms for the electroactivity of the PEDOT film in 0.2 M H_2SO_4 at scan rate of 20 mV/s.

Making Ohmic Contact

One obvious way to ensure proper ohmic contact between the nanowires in the interface film and the contact pad on the mini-circuit board would be to somehow expose the ends of the nanowires on the lower surface of the interface film. The nanowires were in fact exposed by etching the surface of the film with oxygen plasma.

One problem encountered when using the silver epoxy, was that the silver particles punctured the polycarbonate membrane. There was evidence of leakage on the contact pads when the film was removed. Also, the currents were very high for such small electrode area, and the voltammogram was not the typical shape for the oxidation of $TMAFc^{+}$. This suggests that the analyte was in contact with the silver particles. In addition to this problem, the solvents in the silver epoxy created cracks in the polycarbonate film. When a solventless silver epoxy is used, and the film is very gently placed on the contact pads of the circuit board, no cracking or puncturing of the membrane occurred, but the resistance is still very high unless a gold base is sputtered on the film.

Exposing the nanowires by etching did not improve the electrical conductivity between the pads and the film when using silver epoxy as the electrically conductive adhesive. One argument that could be made is that most of the nanowires are buried in epoxy resin. Silver epoxy is usually 30-40% volume fraction of silver particles after cure (41). So, ~70% by volume of the epoxy is resin as represented in figure 4-12. This means that most of the nanowires are surrounded by the insulated resin since the wires are too small to span from one silver particle to the next in areas where the particles are not touching (Figure 4-13).

In addition, it is suspected that the resin in the silver epoxy formed a film around these nanowires, making a high resistive layer. Klosterman et al. (44) pointed out that

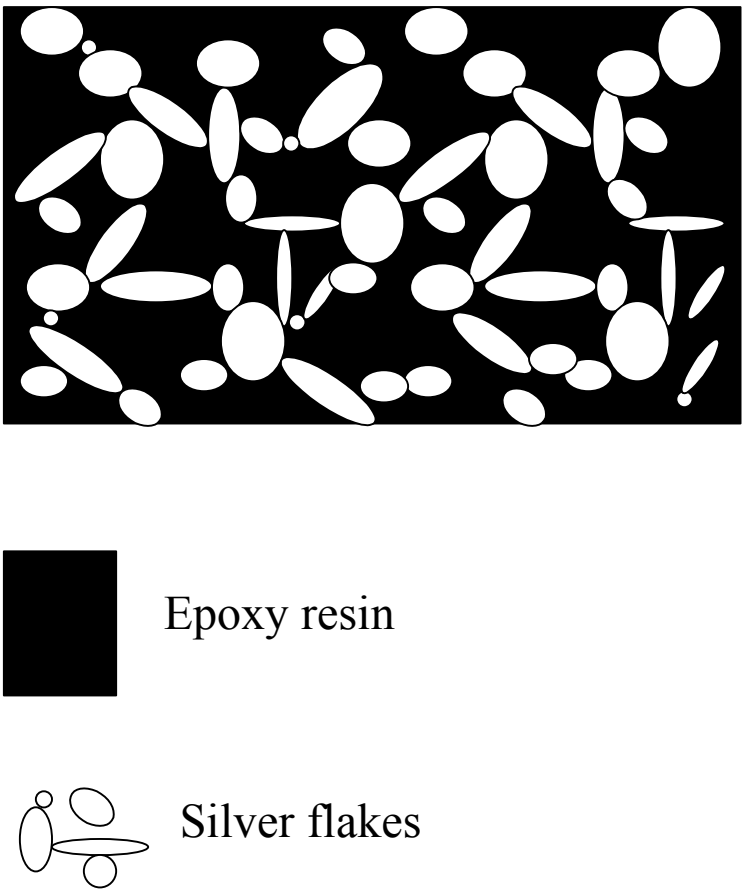

Figure 4-12. Arrangements and volume faction of silver particles (flakes) in silver epoxy

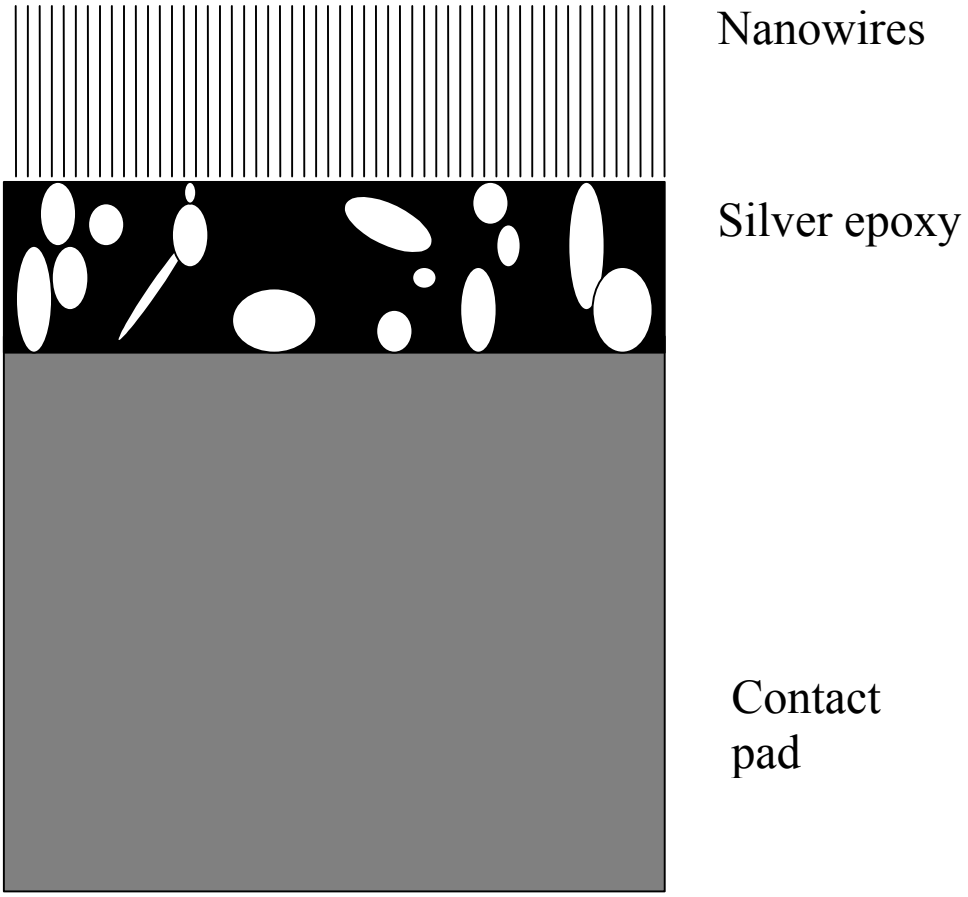

Figure 4-13. Cross- sectional view showing contact between nanowires and silver flakes in silver epoxy

many of the silver particles in silver epoxy that come in contact with a conductive material may be coated with epoxy resin, and this forms an insulating layer thick enough such that electrons cannot hop across the interface. This implies then, that of the nanowires that are in contact with silver particles, only some are actually making Ohmic contact with the contact pads on the circuit board. These drawbacks were reflected in the high resistance (in the KΩ range) across the film when resistance was measured across one face of the film and the other face that was coated with silver epoxy. On the other hand, if the wires have a large gold base (diameter > space between silver particles), then it only needs one silver particle to make electrical contact with thousands of nanowires

(Figure 4-14). This is certainly the case when the contact area is sputtered with gold as described in the experimental.

These problems were overcome by sputtering gold pads on the film through a mask, making a good electrical contact area and protection layer as described in the experimental section. The preparation of the interface film (as describe in the scheme outlined in the experimental section) produced reproducible results, giving cyclic voltammograms from comparable experiments (51). It is interesting to note that the nanoelectrodes ensemble fabricated by Martin and Menon (51), had a complete gold layer on the rough side giving a good electrical contact area. However, when the author removed this gold layer and used the polycarbonate film for the fabrication of nanoelectrodes ensemble, the resistance was too high and no cyclic voltammograms were obtained even after the film was etched, exposing the nanowires.

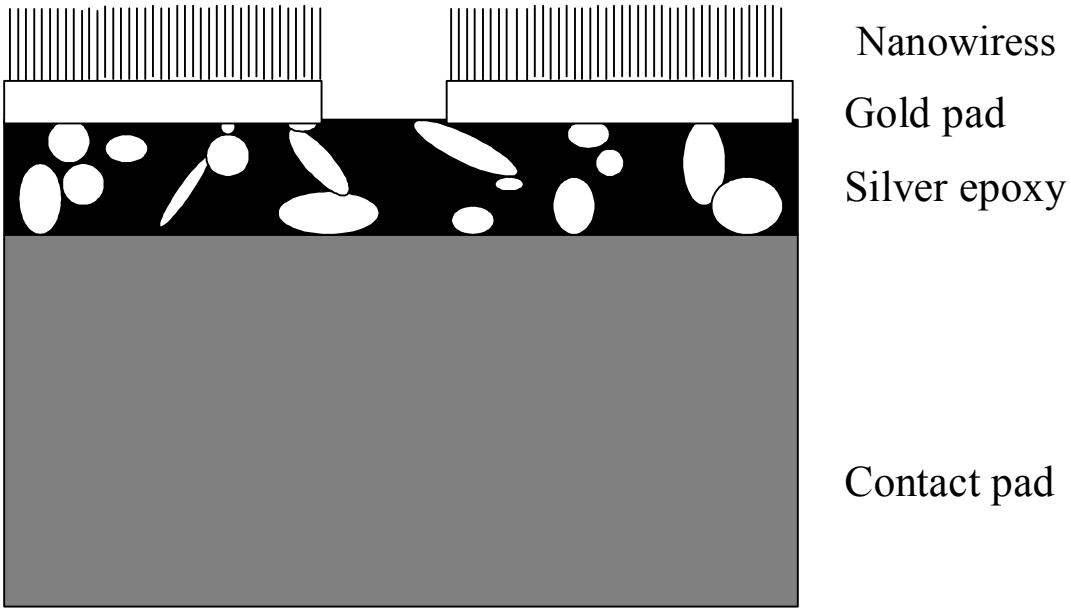

Figure 4-14. Cross-sectional view showing contact between large gold based nanowires and silver flakes in silver epoxy.

CHAPTER 5
SUMMARY AND CONCLUSIONS

The electrochemical communication between biochemical processes and solid state electronics can be established directly on circuit board devices by interfacing the biochemical processes and the electronics, with a template synthesized nanomaterial that is only a few microns thick.

The film is impervious to solutions, so there is no problem of the electronics on board the device shorting out from wet biochemistry above.

This interface film has functioned previously as a nanoelectrode ensemble containing material.It has a low detection limit (three times lower than the limit obtained at conventional macroelectrodes) for the electroactive species $TMAFc^{+}$ and has performed similarly on our device.

It has been demonstrated that our device has individually addressable electrodes that can be modified with conducting polymers (and hence biosensor) to selectively respond to, and give a signal signature to specific analytes, thus function as a multi-analyte sensor.

Our device has potential for parallel electronic communication between different biomedical processes confined at the micron scale, and underlying solid-state electronics. It is expected that this technology will be transferred to an IC chip with many contact pads that will be in parallel communication with many biochemical processes. Work is already underway towards this goal. We are currently exploring other technologies to attach the interface film to the circuit board or chip. One such approach involves the

fabrication of an anisotropic electrically conductive adhesive specifically designed for our task, but should be useful to other applications as well. Present anisotropic electrically conductive adhesives are not suitable for our purpose.

The next stage of this project would be to use blank silicon chips (no circuitry on board) that have contact pads with the same size and gap as those of the mini circuit board. This will be followed by using chips with smaller contact pads from 400μm down to 50μm diameter, but maintaining the same gap between the contact pads. Following this stage, attempts will be made to use chips with pad getting progressively closer down to 300μm apart. At this stage where the pads are so close together, our newly fabricated anisotropic electrically conductive adhesive tape should prove very handy.

LIST OF REFERENCES

1. Yershov, G.; Barsky, V.; Belgovskiy, A.; Kirllov, E.; Kreindlin, E.; Ivanov, I.; Guschin, D.; Drobishev, A.; Dubiley, S.; Mirzabekov, A. **1996**. DNA analysis and diagnostics on oligonucleotide microchips. *Proc. Natl. Acad. Sci*. USA 93, 4913-4918.

2. Blanchard, A. P.; Kaiser, R. J.; Hood, L. E. *Biosens. Bioelectron*. **1996**, 11, 687-690.

3. Pease, A.C.; Solas, D.; Sullivan, E.J.; Cronin, M.T.; Holmes, C.P.; Fodor, S.P., **1994**. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc. Natl. Acad. Sci*. 91, 5022-5026.

4. Livache, T.; Roget, A.; Dejean, E.; Barthet, C.; Bidan, G.; Teoule, R. *Nucleic Acids Res*. **1994**, 22, 2915-2921.

5. Chaubey, A.; Malhotra, B.D. *Biosens. Bioelectron*. **2002**, 17, 441-456.

6. Hall, E. A. H., **1992**. Overview of Biosensors. In: P.G. Edelman, J. Wang (Eds.), Biosensors and Chemical Sensors, Optimizing Performance through Polymeric Materials, ACS Symposium series, 487, pp. 1-5.

7. Gerard, M.; Chaubey, A.; Malhotra, B. D. *Biosens. Bioelectron*. **2002**, 17(5), 345-359.

8. Eggins, B. R. *Chemical Sensors and Biosensors*, John Wiley & Sons, UK, **2002**, pp. 74.

9. Livache, T.; Bazin, H.; Caillat, P.; Roget, A. *Biosens. Bioelectron*. **1998**, 13, 629-634.

10. Bergveld, P. *Sensors and Actuators*, B, **2003**, 88, 1-20.

11. Edelstein, R. L.; Tamanaha, C. R.; Sheehan, P. E.; Miller, M.M.; Baselt, D.R.; Whitman, L. J.; Colton, R. J. *Biosens. Bioelectron*. **2000**, 14, 805-813.

12. Meyer, H.; Drewer, H; Grunding, B.; Cammann, K.; Kakerow, R.; Yiannos, M.; Mokwa, W.; Rospert, M. *Anal. Chem*. **1995**, 67, 1164-1170.

13. Meyer, H.; Drewer, H; Krause, J; Cammann, K.; Kakerow, R.; Manoli, Y.; Mokwa, W.; Rospert, M. *Sens. Act*. B, **1994**, 18-19, 229-234.

14. Ugo, P.; Moretto, L.M.; Bellomi, S.; Menon, V.P.; Martin, C.R. *Anal. Chem*. **1996**, 68, 4160-4165.

15. Menon, V. P.; Martin, C. R. *Anal. Chem*. **1995**, 67, 1920-1928.

16. Hulteen, J. C.; Menon, V. P.; Martin, C. R. *J. Chem. Soc*., Faraday Trans. 1, **1996**, 92(20), 4029-4032.

17. Martin, C. R. *Science*. **1994**, 266, 1961-1966.

18. Hulteen, J. C.; Martin, C. R. *J. Mater. Chem*. **1997**, 7(7), 1075-1087.

19. Martin, C. R.; Foss, C. A. Jr., in *Laboratory Techniques in Electroanalytical Chemistry* (Kissinger, P. T.; Heineman, W. R., eds.), Marcel Dekker, New York, **1996**, pp. 403-468.

20. Baumann, W.H.; Lehmann, M.; Schwinde, A.; Ehret, R.; Brischwein, M.; Wolf, B. *Sen. Act*., B, **1999**, 55, 77-89.

21. Barbaro, A.; Colapicchioni, C.; Davani, E.; Mazzamurro, G.; Piotto, A.P.; Porcelli, F. *Adv. Mater*. **1992**, 4, 402-408.

22. Starodub, N. F.; Torbicz, W.; Pijanowska, D.; Starodub, V.M.; Kanjuk, M.I.; Dawgul, M. *Sen. Act*. **1999**, B, 58, 420-426.

23. Adeloju, S. B.; Wallace, G. G. *Analyst*, **1996**, 121, 699-703.

24. Sung, W. J.; Bae, Y. H. *Anal. Chem*., **200**, 72, 2177-2181.

25. Unwin, P. R.; Bard, A. J. *Anal. Chem*., **1992**, 64, 113-119.

26. Gambhir, A.; Gerard, M.; Jain, S. K.; Malhotra, B. D. Appl. Biochem. Biotech. (in press).

27. De Taxis du Poet, P.; Miyamoto, S.; Murakami, T.; Kimura, J.; Karube, I. *Anal. Chem. Acta*, **1990**, 235, 255-264.

28. Dupont-Filliard, A.; Roget, A.; Livache, T.; Billon, M. *Anal. Chem. Acta*, **2001**, 449, 45-50.

29. Lassalle, N.; Mailley, P.; Vieil, E.; Livache, T.; Roget, A.; Correia, J.P.; Abrantes, L. M. *J. Electroanalytical Chem*. **2001**, 509, 48-57.

30. Vo-Dinh, T.; Alarie, J. P.; Isoloa, N.; Landis, D.; Winterberg, A.L.; Ericson, M. N. *Anal. Chem*. **1999**, 71, 358-363.

31. Xie, B.; Danielsson, B. *Anal Lett*. **1996**, 29, 1921-1923.

32. Muramatsu, H.; Dicks, J. M.; Karube, I. *Anal. Chim. Acta*. **1987**, 197, 347-352.

33. Meyer, H.; Drewer, H.; Grundig, B.; Cammann, K.; Kakerow, R.; Yiannos, M.; Mokwa, W.; Rospert, M. *Anal. Chem*. **1995**, 67, 1164-1170.

34. Meyer, H.; Drewer, H.; Krause, J.; Cammann, K.; Kakerow, R.; Manoli, Y.; Mokwa, W.; Rospert, M. *Sens. Act*. B, **1994**, 18-19, 229-234.

35. Kobayashi, Y.; Martin, C. R. *Electroanal. Chem*. **1997**, 431, 29-33.

36. Jirage, K. B.; Hulteen, J. C.; Martin, C. R. *Science*, **1997**, 278, 655-658.

37. Hulteen, J. C.; Jirage, K. B.; Martin, C. R. *J. Amer. Chem. Soc*. **1998**, 120, 6603-6604.

38. Kobayashi, Y.; Martin, C. R. *Anal. Chem*. **1999**, 71, 3665-3672.

39. Jirage, K. B.; Hulteen, J. C.; Martin, C. R. *Anal. Chem*. **1999**, 71, 4913-4918.

40. Brunetti, B.; Ugo, P.; Moretto, L. M.; Martin, C. R. J. Electroanal. Chem. **2000**, 491, 166-174.

41. Rabilloud, G. *High-Performance Polymers*. Editions Technip, Paris, **1997**.

42. Mitchell, C.; Bergh, H. *Proc. Int. Microelectron. Symp*. **1976**, 52-58.

43. Broadbent, S. R.; Hammersley, J. M. *Proc. Cambr. Phil. Soc*. **1957**, 53, 629-641.

44. Klosterman, D.; Rak, S.; Wille, S.; Dubinski, D.; Desai, P. *Int. Conf. Adhes. Joining Technol. Electron. Manuf*. VDI/VDEIT, **1994**, 1.

45. Szentirmay, M. N.; Martin, C. R. *Anal. Chem*. **1984**, 56, 1898-1902.

46. Lindsay, J. K.; Hauser, C. R. *J. Org. Chem*. **1957**, 22, 355-358.

47. Fischer, B. E.; Spohr, R. *Rev. Mod. Phy*. **1983**, 55(4), 907-948.

48. Fleisher, R. L.; Price, P. B.; Walker, R. M. *Nuclear Tracks in Solids: Principles and Applications* (University of California Press, Berkeley, CA, **1975**).

49. Durrani, S. A.; Bull, R. K. *Solid State Nuclear Track Detection: Principles, Methods and Applications*, 1st ed. (Pergamon Press, Elmsford, NY, **1987**), Vol. 111: International Series in Natural Philosophy.

50. Guillot, G.; Rondelez, F. *J. Appl. Phys*. **1981**, 52, 7155-7164.

51. Menon, V. P.; Martin, C. R. *Anal. Chem*. **1995**, 67, 1920-1928.

52. Yuan, Y. J.; Adeloju, S. B.; Wallace. European Polymer J. **1999**, 35, 1761-1772.

53. Camalet, J.-L.; Lacroix, J.-C.; Nguyen, T. D.; Aeiyach, S.; Pham, M. C.; Petitjean, J.; Lacaze, P.-C. J. Electroanal. Chem. **2000**, 485, 13-20.

54. Bazzaoui, E. A.; Aeiyach, S.; Lacaze, P. C. J. Electroanal. Chem. **1994**, 364, 63-69.

BIOGRAPHICAL SKETCH

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