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Biophotonics: Electrochemiluminescence at Microelectrodes During PCR Amplification of DNA

Rosemary L. Smith
Principal Investigator; University of Maine, Orono, rosemary.smith@maine.edu

Scott Collins
Co-Principal Investigator; University of Maine, Orono, scott.collins@maine.edu

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Principal Investigator: Smith, Rosemary L. **Award ID:** 0352401
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Title:
Biophotonics: Electrochemiluminescence at Microelectrodes During PCR Amplification of DNA

Project Participants

Senior Personnel

Name: Smith, Rosemary
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Graduate Student

Name: Tang, Sophia
Worked for more than 160 Hours: Yes
Contribution to Project:
Performed study at Sandia National Laboratory of Carbon film deposition and properties.

Name: Zhong, Guixiong
Worked for more than 160 Hours: Yes
Contribution to Project:
Packaged the microarrays and performed electrochemical characterization.

Name: Ramanajam, Anusha
Worked for more than 160 Hours: Yes
Contribution to Project:
Performed electrochemical characterization of ECL of Rubipy at carbon electrodes.

Name: Baron, Robert
Worked for more than 160 Hours: Yes
Contribution to Project:
Imaged ECL of carbon/platinum microarray, studied effect of surface preparation of carbon on voltammogram.

Name: Steeves, Michael
Worked for more than 160 Hours: Yes
Contribution to Project:
Characterized sputtered carbon films, measuring 4 point probe resistivity, Hall mobility, thickness, and Xray diffraction.

Undergraduate Student

Name: Buchanan, Peter
Worked for more than 160 Hours: Yes
Contribution to Project:
Performed Carbon film sputter depositions.

Technician, Programmer

Name: Wallace, Andrew
Worked for more than 160 Hours: Yes

Contribution to Project:
Fabricated microarrays and performed preliminary bench testing for quality of insulation.

Name: Call, Mike

Worked for more than 160 Hours: Yes

Contribution to Project:
Trained students in thin film deposition and microfabrication, installed and maintained equipment, and performed some film depositions for students during training period.

Other Participant

Name: Collins, Scott

Worked for more than 160 Hours: Yes

Contribution to Project:
Scott Collins was co-PI on this grant. It was transferred from UCDavis to UMaine in 2003, coinciding with our move to UMaine. Prof. Collins was responsible for the training of students in electrochemical analysis and characterization, and for evaluating the resulting data.

Research Experience for Undergraduates

Name: Langeley, Sarah

Worked for more than 160 Hours: Yes

Contribution to Project:
Received training in microscope operation and assisted with microscope imaging.

Years of schooling completed: Sophomore

Home Institution: Other than Research Site

Home Institution if Other: Colby College

Home Institution Highest Degree Granted(in fields supported by NSF): Bachelor's Degree

Fiscal year(s) REU Participant supported: 2005

REU Funding: REU supplement

Organizational Partners

Sandia Laboratories-Livermore

Interest in carbon electrode material deposition was expressed by Douglas Chin at Sandia in 2002, prior to transfer of grant to UMaine (from UCDavis). One graduate research assistant on this NSF project, from UCDavis, was hired during her last semester at Sandia, during which the collaboration began on the Carbon thin film sputter deposition.
Several staff persons at Sandia assisted in the characterization of the carbon films, performing TEM and voltammetry on films deposited there. When the project transferred to UMaine, the data from Sandia was compiled and it was found that film resistivity had no clear dependence on deposition parameters. Hence, depositions commenced at UMaine and those results confirmed that film resistivity was varying with thickness. This concluded the effort at Sandia, but the collaboration continues in the preparation of a journal manuscript.

Other Collaborators or Contacts

Activities and Findings

Research and Education Activities:

This project was entirely experimental, requiring effort in three different research arenas: microfabrication technology, materials science, and electrochemistry. Graduate research assistants had to be trained in one or more of these areas. A short course (3 weeks) on basic

electrocchemistry and electrochemical instrumentation was developed and delivered to two graduate assistants at UMaine by the PI (Smith) and co-PI Collins. The graduate students had BS degrees in Electrical Engineering and Physics, so neither had prior training in electrochemistry. A graduate course on microfabrication and microfluidics was developed by the PI and offered for the first time in 2004-05. Two graduate assistants on this project at the University of Maine, with no prior training in microfabrication, took this course. One was a graduate student in chemistry, the other a graduate student in physics.

Experiments on carbon thin film sputtering were performed at both Sandia National Laboratory in Livermore, CA and at the University of Maine, Laboratory for Surface Science and Technology. At both laboratories, it was observed that the resisitivity of the films varied greatly (orders of magnitude) with deposition parameters, and surprisingly with film thickness. The latter effect resulted in the abandonment of most of our collected data from Sandia and the restart of film deposition characterization at UMaine.

Establishment of an electrode array fabrication process for using thin film carbon in electrochemiluminescence was the subject of a one year effort, which concluded with a working array. However, the direct ECL of Rubipy at carbon has not been successfully reproduced for over a year. A great deal of experimental effort was spent on determining the source of this unexpected problem. A satisfactory explanation was not forthcoming by the end of the project but it is still being investigated.

Findings: (See PDF version submitted by PI at the end of the report)

Training and Development:

A total of six graduate students, one post-doctoral associate, and one undergraduate student were trained in either electrochemical analysis techniques, thin film deposition and characterization, or micro-fabrication during the course of this project. All student participants learned skills in fields outside their formal academic training and background. The project provided a truly interdisciplinary training venue in which researchers from many different fields worked together to achieve a common goal. In weekly group meetings, each student was required to present his/her work to the rest of the project team, followed by questions/answer session. Two students have graduated with MS degrees. Two more will be completing their MS degrees in the coming year.

Outreach Activities:

I have given numerous presentations and tours to visitors and to a group of female high school students, presented at the local IEEE meetings and student conferences and we are in the process of writing two journal articles based on the results of this project. During the course of the project, I sponsored an NSF REU student and a local high school student who worked in my laboratory alongside the graduate research assistants.

Journal Publications

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

Very little is known about how and why glassy carbon thin films are deposited to have high electrical conductivity. This work will contribute to the experimental know how, and may provide insights to understanding the physics behind its unique properties.
Direct ECL of Rubipy in water at Carbon interdigitated microelectrodes was demonstrated by the PI and collaborators in 1997, but there has not been any subsequent reports. The reproduction of direct ECL of Rubipy at carbon electrodes in water has not been achieved, even with trials employing the original, interdigitated electrodes. Clearly, the electrochemical understanding of the processes that take place are incomplete and warrant further investigation. Nevertheless, the ECL of Rubipy labeled DNA at carbon electrodes after hybridization was demonstrated in the presence of TPA, and a new microelectrode array with carbon as one electrode and Pt as the other was successfully fabricated and demonstrated. The advantage of this microelectrode design is that submicron electrode spacing can be achieved without submicron patterning.

Contributions to Other Disciplines:

The unusual result that the carbon film resistivity varies greatly with film thickness has led to a new, collaborative project with Prof. Robert Lad, a materials scientist at UMaine, to explore the underlying mechanism. Also, a new DNA sensor construct is being explored utilizing ECL for signaling hybridization.
Finally, the results from thermal annealing of thin carbon films is being applied to the fabrication of nanoelectrodes.

Contributions to Human Resource Development:

Five graduate students have been trained and educated in multidisciplinary research as an outcome of this project. Two have graduated with MS degrees and are now working in US companies. Two are continuing for PhD degrees at UMaine, and one is pursuing a Phd at UC Berkeley. A high school student who was hired during the summers of 2004 and 2005 has now entered UMaine as an electrical engineering undergraduate, and is continuing to work as a research aid in my laboratory.
In addition to the graduate research assistants, this project had several short term participants, e.g. an NSF REU student from Colby College, a biology professor from Italy, and two undergraduate students in engineering.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Any Journal

Any Book

Any Web/Internet Site

Any Product

Contributions: To Any Resources for Research and Education

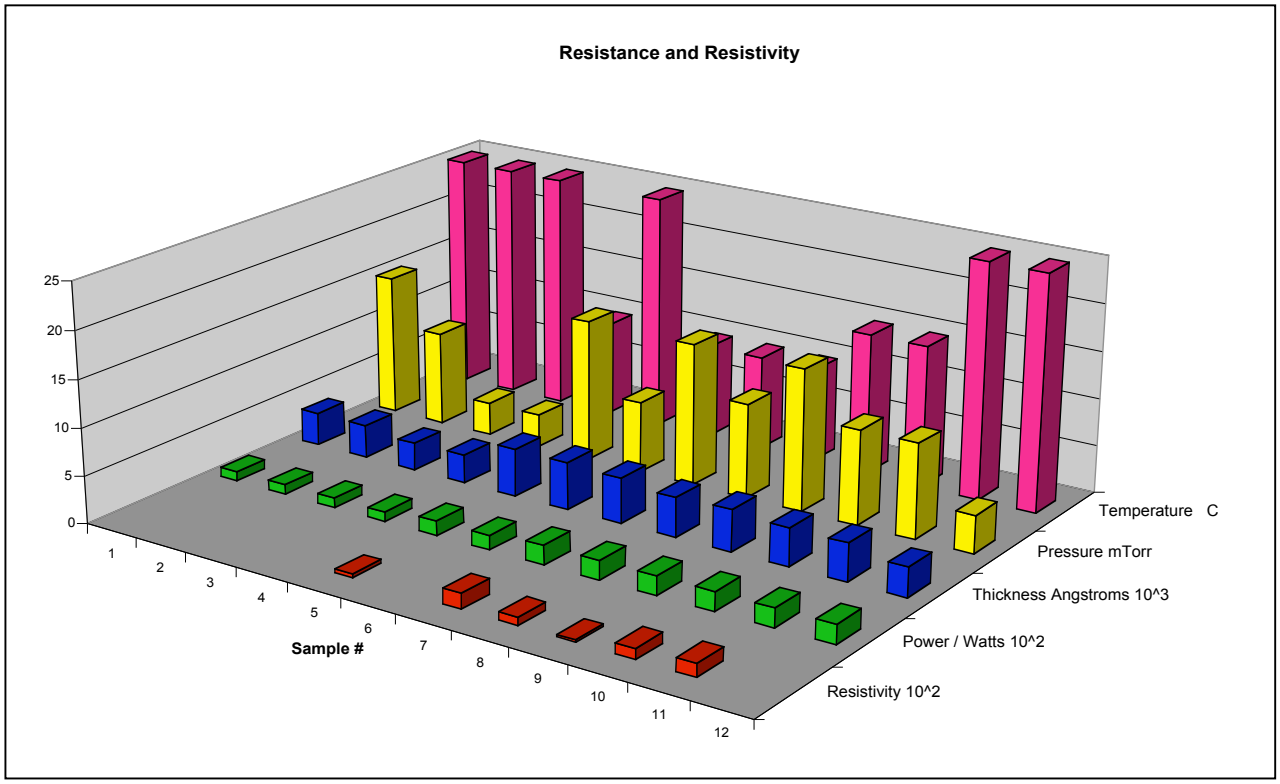
Contributions: To Any Beyond Science and Engineering

The major findings to date are reported in three sections: 1) Carbon thin film deposition/characterization and 2) ECL electrode array fabrication and 3) ECL of Rubipy using carbon electrode.

Carbon thin film deposition/characterization

Carbon thin films were sputter deposited at Sandia National Laboratory in Livermore, CA and at the University of Maine, Laboratory for Surface Science and Technology. Carbon films were obtained by RF sputtering and by Pulse DC sputtering. The project goal was to produce films with resistivity ≤ 50 mΩ cm, so that a 500 nm thick film would have sheet resistance of ≤ 1KΩ per square. This is a maximum resistance that can be tolerated for the carbon electrode, where electrochemiluminescence of Rubipy will be generated.

Substrate temperature, pressure, and power were all found to have effects on the film resistivity and stress. Stress free films could only be obtained by sputtering at very low rates, e.g. < 1 angstrom per second. Some films, although showing no signs of stress immediately after deposition, would crack and/or peel after several days. Variation of resistivity with pressure and RF sputter power were found to vary in an inconsistent and inexplicable manner. Many samples had resistivity that was too high to be measured with the 4 pt probe instrument, i.e. over 1 MΩ-cm. The results obtained by RF sputtering for 12 samples are shown in Figure 1. After compiling the data from over 100 depositions, it was discovered that the film resistivity varied dramatically with the thickness of the deposited film. This was an unexpected result, since resistivity is a property of the material, and is generally considered constant with thickness beyond the first 10-50 nm. In exploring the deposition parameter space of temperature, pressure and power, one parameter was varied while the others were kept constant. Consequently, few samples were generated with the same sputter deposition parameters and with different thicknesses (i.e. different deposition time), obscuring the changing resistivity. Some trends were observed: increased sputter power and decreased pressure tend to decrease resisitivity.



Results obtained by DC pulse sputtering have been more reproducible and have less stress than the RF sputtered samples. The resistivity of three samples, with the same temperature, power and pressure are shown in Figure 2, showing the dramatic change with thickness. A possible explanation for the varying resistivity with film thickness is a change in the molecular structure of the film with thickness. Study of film structure by TEM, AFM and Xray diffraction only indicate that the films are amorphous, with grain size of approximately 50 nm.

Annealing the films in nitrogen, at elevated temperature, reduces the film resistivity and improves adhesion. The effect is shown in Figure 3, for samples annealed for 2 hours in nitrogen at the indicated temperature. The effect is dramatic for T > 1000 K = 730 C , and exhibits an Arhenius behavior, with activation energy of 2.2 eV. This procedure will work well when the carbon film is deposited early in the process, but not for adding carbon electrodes to existing structures or circuits, where low temperature processing is necessary.

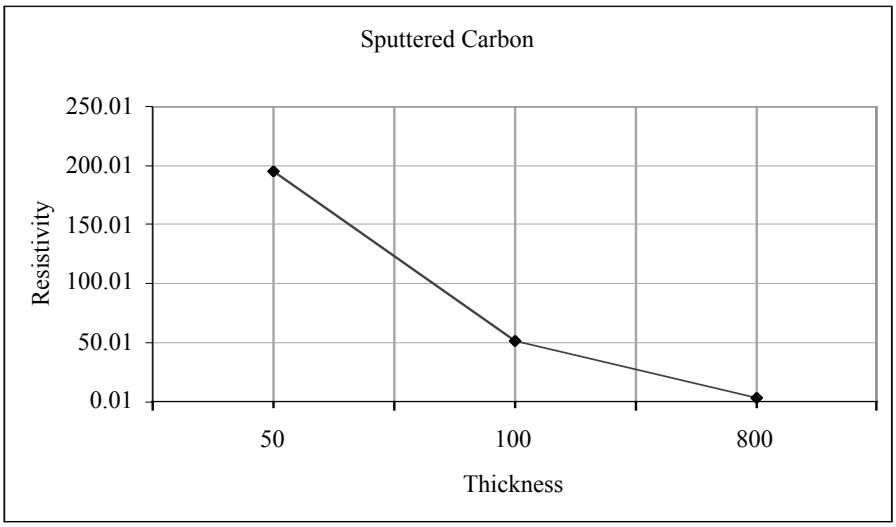


Figure 2: Sputtered film resistivity versus film thickness.
Substrates are Esco 2930 glass microscope slides. The sputter target is graphite, and the Argon flow rate is 4 sccm. Target-to-substrate distance is 19 cm. Pulsed DC power is 250 W.

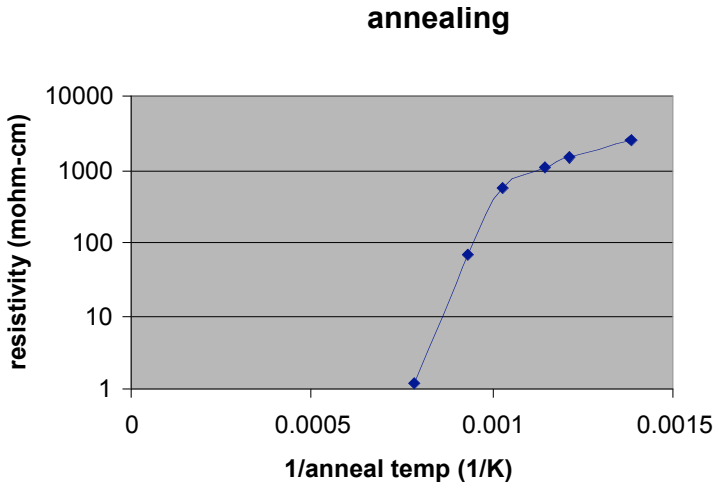


Figure 3: resistivity versus anneal temperature

Microelectrode Array Fabrication

Process Overview

The process depicted in Figure 4 was developed to yield a 3 material stack of Pt/nitride/Carbon. Wafers were purchased with a carbon film sputtered over nitride/oxide insulator on silicon substrates. Another layer of PECVD silicon nitride was then grown on top of the carbon. Photoresist was then spun and patterned, and the platinum electrode was evaporated using a lift-off process. Finally, the PECVD nitride insulator was etched with an RIE plasma using platinum as a mask material.

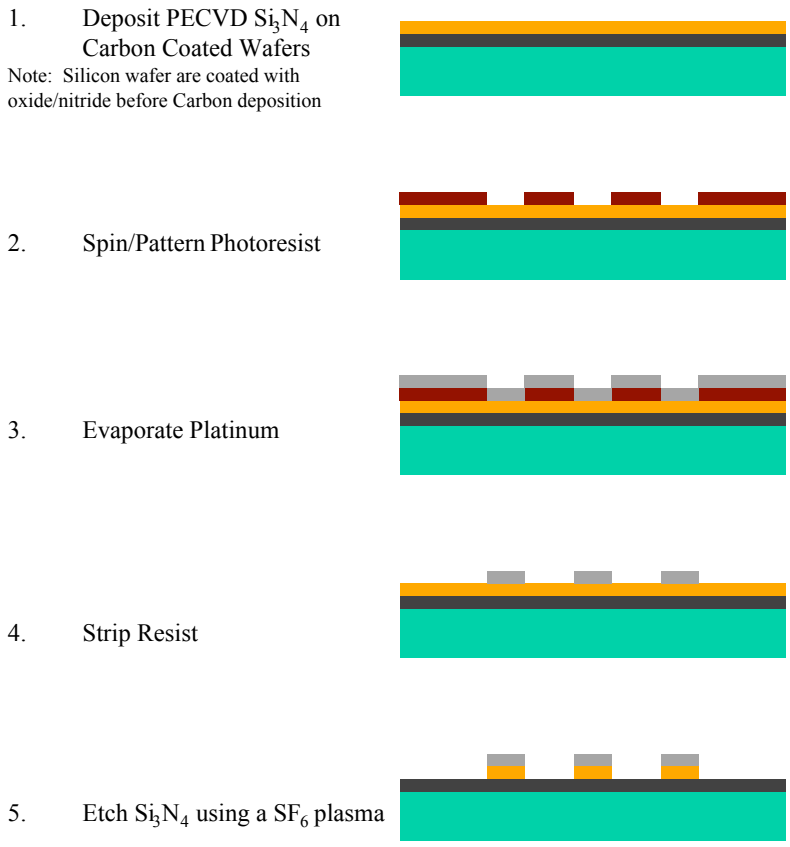


Figure 4: Process for Microelectrode Array

In the last step of the electrode fabrication process, the PECVD silicon nitride layer is patterned by a reactive ion etch. When the nitride film is removed, the carbon layer is exposed to the etching plasma. No O_2 was used in the RIE of the silicon nitride, since an O_2 plasma will etch the Carbon. Instead, only SF_6 is used with the following conditions: 15sccm flow rate, 200W, 150mT, for 16min.

Mask Geometry

To simplify this process for testing purposes, the file dna_grid.tdb was created. An example of one die in this file and as referenced to the entire mask is as shown in Figure 5.

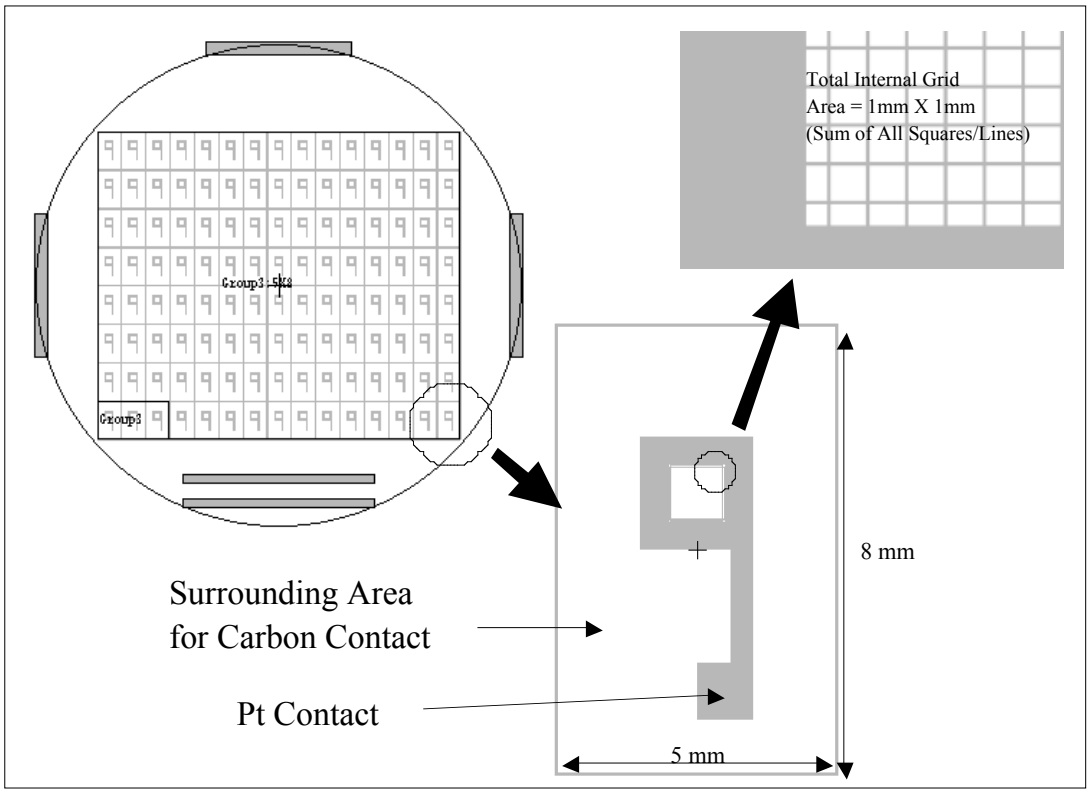


Figure 5: DNA Electrode Grid Mask

Table 1: Diimensions for Electrode Grid Mask

Square Side	Square Area	Line Width	Eff Line Area/Square Area (%)
50	2500	5	10.5%
25	625	5	22.0%
50	2500	10	22.0%

ECL of Rubipy labeled DNA

ECL instensity measurements versus bulk concentration of Rubipy labeled oligos were made at carbon electrode surfaces using Tripropyl Amine (TPA) as the reducing agent. The electrode surface area was 7 mm2. Figure 6 shows the results of two experiments, on different but identical electrodes. First major finding is that the intensity is not proportional to concentration. The second major finding is the very low sensitivity. This is especially noteworthy when compared to the ECL intensity obtained when the Rubipy labeled oligomer is physi-adsorbed (allowed to dry) at the electrode surface. After drying, the electrodes were placed into the buffer solution, and immediately ECL excited by voltage pulse. These results are shown in Figure 7. The ECL intensity (sensitivity) is more than two orders of magnitude greater than for that of oligo that is suspended in solution. Also, the ECL increases nearly linearly with adsorbed oligo. This led us to consider binding the Rubipy labeled DNA to the electrode surface by hybridization. Hybridization at the electrode surface would ensure molecule alignment and spacing from the electrode surface as well as fix the DNA in place at the surface, but could slow down the detection (hybridization could take hours). This method would probably be most

suitable after, not during PCR amplification, and performed in an adjacent chamber, which the amplified sample would enter, similar to the Affymetrix instrument.

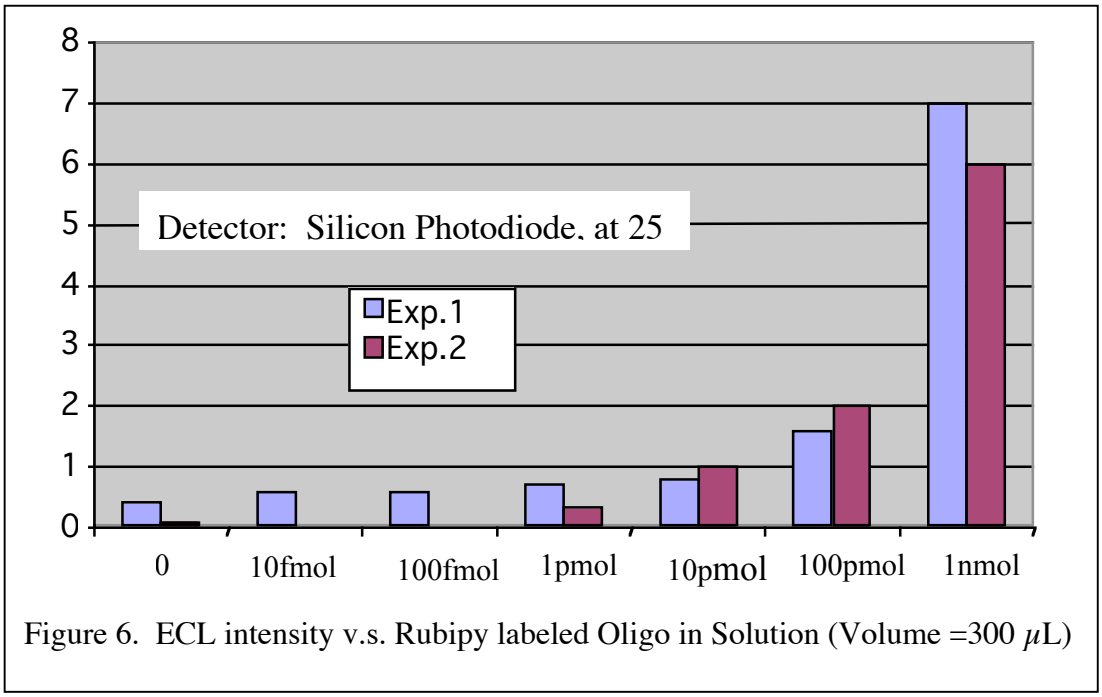


Figure 6. ECL intensity v.s. Rubipy labeled Oligo in Solution (Volume =300 μL)

First, an effective method of attachment of amino-modified DNA to glassy carbon electrode material had to be established. Cyclic voltammetry from 0V to 1.9 V at 2 mV/sec was performed in a Britton-Robinson buffer between the carbon electrode and a pseudo reference platinum electrode in the presence of the oligo 5' amine ACT GGC CGT CGT TTT ACA .

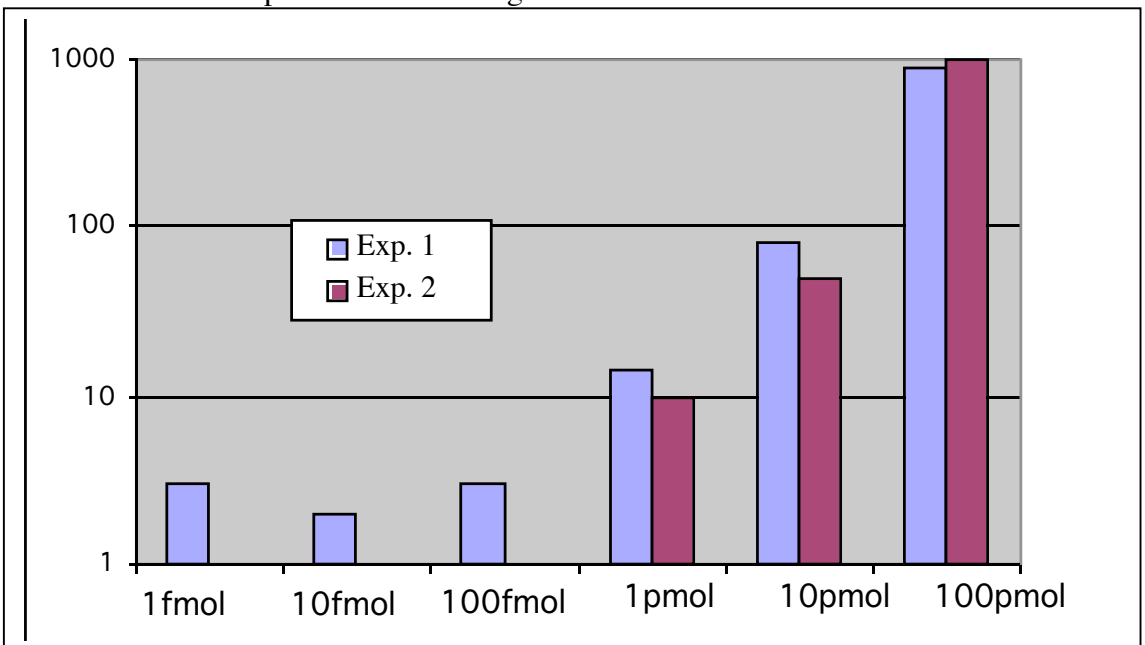


Figure 7. ECL intensity recorded at Carbon electrode v.s. Rubipy labeld oligo concentraton that was physi-adsorbed onto the electrode surface.

TBR labelled oligos , sinthetized by Baron Biotech, were then hybridized to the coated carbon electrode by heating in a 5X SSC for 10 minutes at 80C, followed by slowly cooling to room temperature and incubating at room temperature, with agitation for 2 - 48 hrs. The first tests used DNA oligo 5' -TBR- TGT AAA ACG ACG GCC AGT as the labeled oligo. The electrode was removed from the hybridization solution, and inserted into 0.2 M phosphate buffer with 50 mM NaCl and 4% TPA. ECL was excited by applying a single 10 msec pulse of 2.3 V. Results are plotted in Figure 8. Four major findings were concluded from these preliminary experiments:

1. The ECL intensity is generally higher for Rubipy labeled oligomer that is fixed at the electrode surface than for oligomer which is attracted from solution to the electrode by diffusion or drift (electrophoretic attraction).
2. Electrophoretic attraction from solution resulted in some probe attachment to the electrode, as evidenced by a non-zero ECL after removal of electrode from oligomer solution, and pulsing electrode in buffer solution.
3. The ECL intensity increased linearly with concentration for the physi-adsorbed Rubipy labeled oligomer, but not for the hybridized nor solution suspended probe.
4. Hybridization time had to be extended to 48 hours (long time) before any concentration dependence was observed, and even then, response was not linear with concentration of probe. With long hybridization time, the sensitivity to hybridized probe was about 1 order of magnitude higher than for probe suspended in solution.

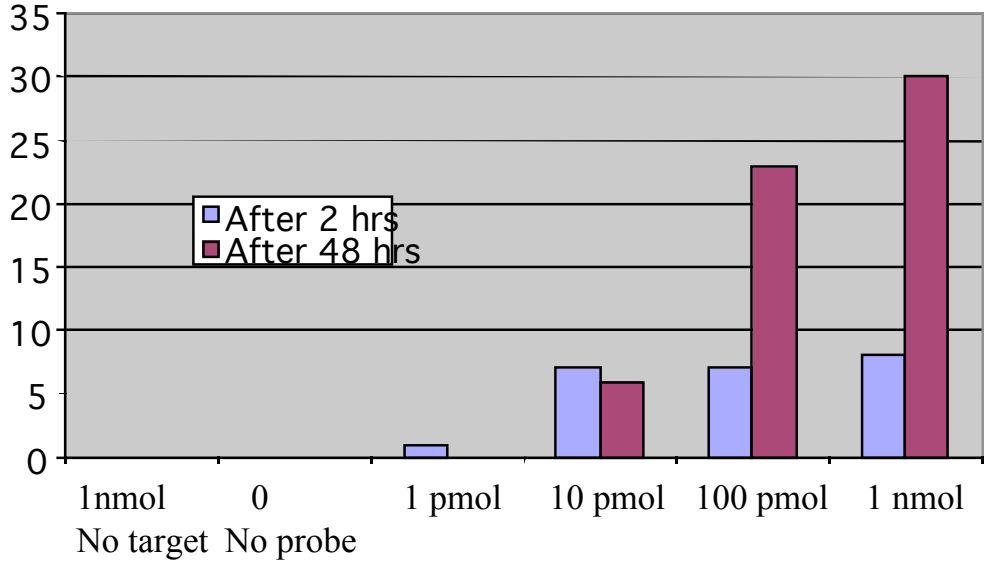


Figure 7. ECL Response of Hybridized Rubipy-Oligo at Carbon Electrode.

Sample volume was found to have a pronounced effect on the ECL intensity, with all other factors kept constant. This means that electrophoretic effects may well be an important factor in establishing sensitivity.

ECL at Carbon /Pt elatectrode array

In the presence of Tripropyl Amine, ECL could be produced with the Carbon/Pt electrode array device. Oxidation was performed at the carbon, and hence light is only emitted there. Figure 8

shows the device under bias, with a square wave potential applied between the carbon and platinum, with magnitude of 2 V.

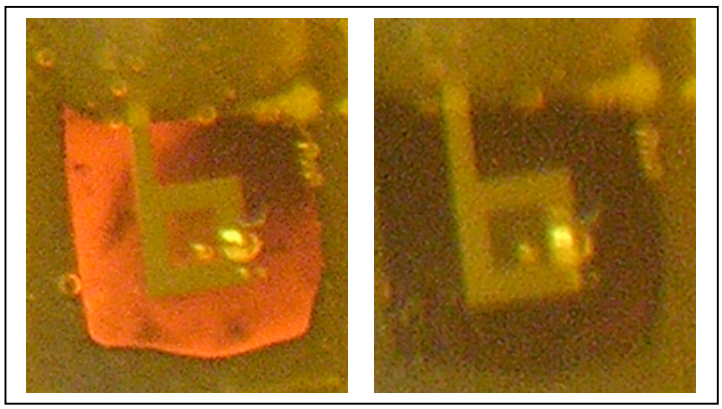


Figure 8. ECL produced at the carbon electrode.

The production of ECL through the generation of $Rubipy^{+1}$ (reduction) at the carbon electrode while generating Rubipy $^{+3}$ (oxidation) at the Pt electrode, so as to produce direct ECL, could not be achieved. Nor could direct ECL be reproduced on all carbon microelectrode arrays. The reason for this has not yet been determined. Voltammograms on the carbon films show the reduction peak at –1.3 Volts versus a Ag/AgCl reference electrode. However, no light emission was detected. Experiments to determine the problem are underway.