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The Use of Photoresist Derived Carbon as Microelectrodes for Genetic Assays

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The Use of Photoresist Derived Carbon as Microelectrodes for Genetic Assays

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Abstract

Using a photoresist derived carbon and two surface modification techniques, a genetic assay was developed for small sequence DNA hybridization biosensing using an electrochemical method. The use of electrochemical impedance spectroscopy provided a useful and meaningful technique for the measure of surface alteration on an oxygen plasma treated carbon surface. The use of 4-Aminobenzoic acid was explored as an alternative technique to surface modification for single stranded deoxyribose nucleic acid attachment and found to be ineffective.

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1. Introduction

The past decade has brought many advances in the detection and testing for genetic diseases. However, these methods are typically extremely expensive, time consuming, and labor intensive. The purpose of our research was to develop a simpler, routine, and cheap method of analyzing genetic compounds, specifically deoxyribonucleic acid (DNA) and the antibody with its reaction with the protein.

Current electrochemical processes have been developed to both evaluate genetic material through the use of an active microelectronic devise that controls the biological affinity reactions. This current system uses electrode arrays that facilitate the transport of charged molecules to selected locations. The negative aspect to the process relates to the electrode surface needing to be coated in a permeation layer to protect against adverse electrochemical reactions. This permeation layer lengthens the overall process time and makes the experiment very tedious.

The new processes involved the development of a low-cost DNA hybridization or an antibody and protein interaction on a superior electrode. While the process was developed specifically for genetic probe diagnostics, ultimately this experimentation could be developed for all assays in which a reaction is conducted between two molecules.

The nanostructured carbon-based electrodes are a simply produced structure with excellent biocompatibility. The use of electrochemical methods to monitor changes in surface chemistry have been utilized by the bonding of molecules and probe DNA sequences to the functionalized carbon surface as well as the hybridization process. The exploration of different functionalization techniques produced a clearly superior and more stable biosensor. The ultimate result of the entire project is that an oxygen plasma treated surface was able to produce a

biosensor for which a genetic mutation can be detected via the coding DNA or the interaction of the produced protein with the complementary antibody.

2. Background

Nanotechnology has developed many goals, but most recently a great deal of the research has been devoted to the development of biosensors for biomolecular recognition elements (1). Recent developments have proven that carbon nanotubes are the most successful at stabilizing biomolecules for biosensing systems as well as remaining chemically inert. Using carbon surfaces as bases for microelectrochemical bioprocessing, however, is a new field of research. Focusing on creating a simple process for genetic testing requires extensive research into the fundamentals of biochemistry and electrochemistry.

2.1 Fundamentals of Deoxyribonucleic Acid and Genetics

Deoxyribonucleic Acid (DNA) is the core molecule from which the central dogma of biology begins. The central dogma describes the process through which DNA is converted first to ribonucleic acid (RNA) through transcription and from RNA to protein through translation. This process is used to explicate the constant processes any organism routinely performs to maintain life and the control of these processes.

2.1.1 DNA as the Heredity Material

It was not until 1928 that it began to be recognized that a genetic material for organisms existed. However, it was not until the late 1940's and early 1950's that a true acceptance of DNA as the hereditary material began. Prior to the discovery of DNA as the hereditary material it was widely accepted that genes were composed of proteins (2).

In1928, F. Griffith used two forms of *Streptococcus pneumoniae*, smooth with the ability to kill a mouse and rough without the ability. However, when he heat killed the smooth strain (destroying the cells) and injected a mixture of the rough and smooth cells the mouse was killed by transference of the genetic material from one organism to another (3). The technology in

1928 could not determine what biomolecule was the source of genetic material, so it was not until 1944 when O. Avery, C. MacLeod, and M. McCarty undertook the process of killing all of one of the biomolecules (proteins, lipids, polysaccharides, RNA, and DNA) at a time in the smooth form. After each individual biomolecule was destroyed, an injection of the altered smooth and the rough was implanted in the mouse. It was displayed that the mouse only did not die when DNA was the molecule that was destroyed. This proved that genetics and heredity must be controlled by DNA (4).

DNA is a biomolecule that contains all the genetic and heredity information for an organism. DNA is commonly known for the phenotypic effects it displayed universally in human beings such as: eye color, hair color, and height; however, DNA is also the central molecule through which all bodily processes are derived. Modern genetics first began in 1953 with the publication of the known structure of DNA by J. D. Watson and F. H. C. Crick. Their

Figure 1 - Double Helix Structure of DNA

structure described the "double helix" (Figure 1) and determined that the nucleic acids were in the interior of the molecule while the sugar and phosphate linkages formed the exterior backbone of the biomolecule (5).

Genetics is the study of how traits are passed from one generation to the next. Prior to 1953 and the discovery of the structure of DNA, genetics could only described physical

properties of the organism. Gregor Mendel, the Austrian Augustinian priest, first developed the basic principles of genetics in 1865 using the phenotypic properties of pea color and plant size. The principles he developed are still in use today because they are true for both physically visible traits as well as all others.

The discovery of DNA as the genetic information carrier in all species was slow to be determined, but since this determination in the nineteen-fifties, great strides have been made in the structure, mutations, and processes of DNA.

2.1.2 The Structure of DNA

DNA is composed of four different bases connected to a backbone composed of sugars (deoxyribose) and phosphates. These four bases pair with one another with extreme selectivity and precision. The sequence in which these bases fall determines the structure and function of proteins. It is through genetic variations in DNA that proteins differ and phenotypic diversity

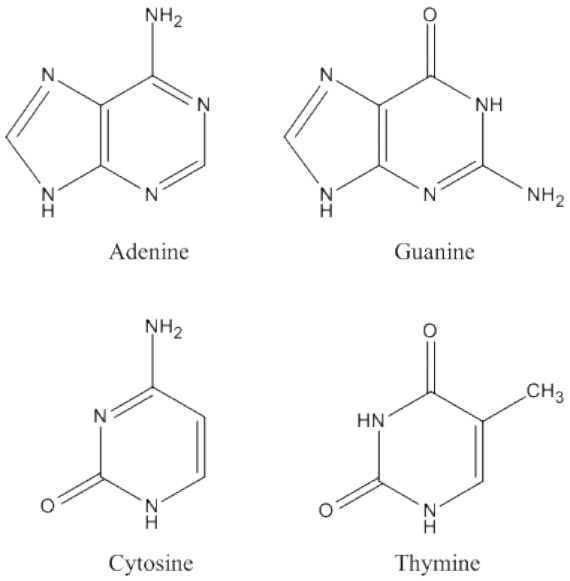

Figure 2 - DNA Components and Their Structures

occur. The bases that compose DNA are adenine, guanine, cytosine, and thymine (Figure 2). These bases are further classified into two categories based on structure: purines which are adenine and guanine and pyrimidines which are cytosine and thymine. Purines only hydrogen

bond with Pyrimidines; however there is greater specificity in that adenine and thymine will only bond with one another and cytosine and guanine will only pair with one another. This binding of adenine with thymine and cytosine with guanine was first discovered in the late 1940's by Edwin Chargaff and is known as Chargaff's Rule (2).

The bases are attached to deoxyribose at the 1' carbon through a nitrogen-carbon bond. The structure is built with the alcohol bound to the 3' carbon reducing to bind to form a phosphate group connected to the 5' carbon of another sugar. This is where the common terminology of five prime and three prime structure of DNA is developed from (Figure 3).

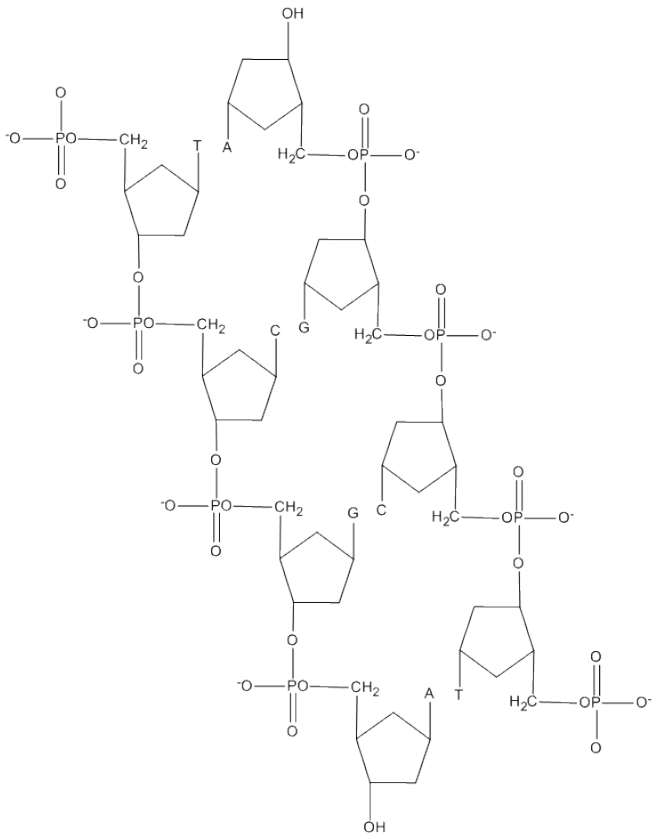

Figure 3 - Sugar and Phosphate Backbone to DNA

The DNA is stabilized by the formation of stacking interactions. As the DNA molecule is built, it naturally curves the structure of the DNA molecule to the point of forming a double helix. This double helix provides a rigid structure for DNA that both protects it and preserves it. The double helix and the DNA itself is protected through even more advanced coiling of the DNA via the use of proteins called Histones. Histones provide the backbone for which DNA is supercoiled onto itself in areas not being transcribed.

2.1.3 DNA-Protein Relationship

Proteins are created through the central dogma of biology. DNA is first transcribed into messenger RNA (mRNA) in the nucleolus. The mRNA is then transferred to endoplasmic reticulum where ribosomes capture the mRNA. The ribosome then uses a set of three nucleic bases, referred to as a codon, to build the protein one amino acid at a time. This is done by using transfer RNA (tRNA), which is a special RNA complex that holds an amino acid at the opposite end to a specific site that recognizes a specific codon. The ribosome takes in the tRNA and transfers the amino acid to the amino acid chain in formation. It is through the sequence of the nucleic acids in DNA that code for the amino acid order in proteins. Each amino acid is coded for by three nucleic bases. There are three codons that are "stop" codons. When the ribosome reaches these codons, the ribosome complex detaches from the mRNA and the protein is released for either further post-translation modification or for use by the cell. For a complete table of the codons that code for the amino acids of proteins see Appendix A.

2.1.4 Genetic Mutations

Genetic mutations in humans are infrequent, only one in 10^7 to 10^{10} mutations occur during transcription. These mutations come in various forms and can have several effects upon an organism. Mutations to the coding sections of DNA for proteins vary in the amount of

nucleic acids affected. Mutations are created through several different processes. Some mutations occur naturally in the process of DNA synthesis or transcription, while others are induced by mutagens that affect the genetic structure of cells. Mutagens include but are not limited to ultraviolet light, ionizing radiation, viruses, and chemical reagents. Mutagens tend to cause extreme damage in the DNA structure and sequence - those in which large sections of the DNA sequences are altered, moved, or destroyed, while mutations that occur in the transcription or translational processes tend to be small mutations - those in which one or two nucleic bases are altered.

Point Mutations

Point mutations change one nucleic base in the sequence, these come in two forms. These can be transition, in which a base on one category is replaced with one of the same category (a purine with a purine or a pyrimadine with a pyrimadine). These mutations can also be transversion. Transversion replaces a purine with a pyrimadine or a pyrimadine with a purine.

While there are two forms of point mutations, there are three results that a point mutation can have on the protein produced. A synonymous mutation changes a nucleic base but the codon still codes for the same amino acid. As a result this mutation does not effect the protein created; the same amino acid is maintained in the protein, which does not alter protein structure of chemical properties.

The second kind of point mutation, a missense mutation, changes one nucleic base to another which changes the codon to one that codes for a different amino acid. Missense mutations can either be conservative or nonconservitive. Conservative mutations change the amino acid to one with similar properties as the original. These changes result in negligible changes to the protein structure or chemical properties. When the structure and chemical

properties of the protein are maintained, the protein is still able to function in similar if not the exact manner it would without the mutation. A nonconservitive mutation, one where the replaced amino acid is drastically different from the original, produce severe changes in the protein structure or properties. As a result of a nonconcervitive mutation the protein is often ineffective or unstable. The protein is rendered completely impaired of its ability to perform its specified functions.

The final type of point mutation changes the codon from one that codes for an amino acid to a termination codon. In this case, the produced protein will be terminated prematurely in protein synthesis. This typically results in a defective, nonfunctional protein.

Frameshift Mutations

In this form of mutation, a nucleic acid is either inserted into the DNA sequence or deleted (Figure 4).

Normal Code									
ATG	TTT	GCG	AGT	CAA	ACC	TGG	AAT	AAA	TGA
Methionine	Phenylalanine	Alanine	Serine	Glutamine	Threonine	Tryptophan	Asparagine	Lysine	STOP
Base Deletion									
ATG	TTT	**GCA**	**GTC**	**AAA**	**CCT**	**GGA**	**ATA**	**AAT**	**GAT**
Methionine	Phenylalanine	Alanine	Valine	Lysine	Proline	Glycine	Isoleucine	Asparagine	Aspartic Acid
Base Insertion									
ATG	TTT	GCG	**ACG**	**TCA**	**AAC**	**CTG**	**GAA**	**TAA**	**ATG**
Methionine	Phenylalanine	Alanine	Threonine	Serine	Asparagine	Leucine	Glutamine Acid	STOP	Methionine

Figure 4 - Examples of Frame Shift Mutations Effect on Sequencing Code

These result in a complete shift of all amino acids after the mutation. This type of mutation results in greatly altered proteins. As displayed in Figure 4, a base deletion or insertion can create a premature stop codon or delete a stop codon, in addition to completely changing the amino acid sequence of a protein. A synonymous mutation is present in the base deletion example where GCG is replaced by GCA. This deletion created a new codon that still codes for alanine; however, this is really irrelevant in the entire scheme of the base deletion. The deletion and insertion methods completely alter every aspect of the protein after the frameshift mutation. These mutations often result in proteins that are ineffective in their ability to perform their necessary functions.

2.1.5 Breast Cancer Genes and Cancer Risk

Women of the United States have a twelve percent chance of developing breast cancer in a ninety-year lifetime. However, patients who exhibit abnormalities in the BRCA1 or BRCA2 genes (the two most common breast cancer genes), have an eighty-five percent chance of developing breast cancer by the time they are seventy. In addition to the increased breast cancer risk, up to fifty-five percent of women with the gene will be diagnosed with ovarian cancer (6).

Table 1 - Common Mutagenic Mutations in the BRCA1 Gene (7), (8)

Codon	Coding Effect	Nucleotide Change	Mutation
64	Missense	TGT → GGT	Cysteine 64 replaced with Glycine
392	Frameshift Deletion	Deletes nt 1294-1333	Deletes nucleotides 1294 through 1333
1250	Nonsense	GAG → TAG	Glutamic Acid 1250 replaced with a STOP
1252	Frameshift Deletion	Deletes nt 3875-3878	Deletes nucleotides 3875 through 3878
1443	Nonsense	CGA → TGA	Arginine 1443 replaced with a STOP
1443	Missense	CGA → GGA	Arginine 1443 replaced with Glycine
1656	Frameshift Deletion	Deletes nt 5085-5103	Deletes nucleotides 5085 throught 5103
1773	Frameshift Insertion	ACC → ACCC	Inserts a C after Threonine 5438

As displayed by Table 1, the BRCA1 gene on chromosome 17 has several different types of mutations that can cause cancer. The majority of these mutations cause cancer to develop in

women well below age fifty, periodically below age forty. The detection of increased risk earlier is valuable because of near non-existent breast cancer screening for women below age forty (9). If simple methods could be used to detect mutations in the BRCA1 gene, a screening for increased risk could take place at any point in the development of a woman; however, an accurate method for determining a point mutation within the DNA sequence is virtually non-existent as a diagnostic tool when compared to the ability to detect frameshift mutations. It is for genes such as the BRCA1 and BRCA2, which have a high number of mutations possible, that a diagnostic method is needed. The ability to determine a person's increased risk for serious disease as well as the presence of a disease will ultimately lead to healthier patients with prolonged life spans.

2.1.6 Current Methods of Genetic Testing

Current methods of genetic testing are laborious and quite complicated. The clinician may choose from one of three options to perform a genetic mutation diagnosis and examination. The first method involves the sequencing of the entire DNA for a patient from a blood or tissue sample. The second is to design a molecular probe (fluorescent, radioactive, etc.) which selectively attaches to the mutation and can be visualized within the laboratory. The final option is for a genetic mutation to be tested via functional or biochemical tests. This is used to determine the presence of an abnormal protein or the absence of a protein.

The first two kinds of genetic tests require that the placement of the gene within the genome is known and that the sequence has been cataloged as well as mutations have been cataloged. For genes, such as the BRCA1 and BRCA2 genes, which possess several variations the first test of sequencing the entire genome is conducted to confirm diagnosis, where as other

genes such as cystic fibrosis which have a relatively few number of mutations only require sequencing of the gene to take place.

The third type of genetic test can be used to diagnose Malignant Hyperthermia Susceptibility (MHS), which is a pharmacogenetic disorder resulting in extreme miss regulation of muscle contraction in relaxants. This is caused be a protein deficient in muscle calcium regulation. To confirm a diagnosis of the disease requires a biopsy a piece of the skeletal muscle, which is then is subjected to a contraction test in the presence of caffeine and anesthetic halothane (10). If the anesthetic responds in a similar manner to the caffeine, then a diagnosis of MHS can be confirmed.

The more complicated genetic testing methods, the first two types, are very cumbersome to complete as well as extremely expensive for a diagnostic test. The price alone is partially why a new biosensor needs to be developed for genetic testing; however, the error associated with genetic tests is at a much higher rate than is acceptable to many clinicians for the generation of accurate diagnosis. Genetic biosensing methods need to be developed for both a low cost alternative and a higher accuracy alternative.

2.2 Biosensors

Biosensors are at the forefront of simple methods for genetic and mutation testing (11), (12), (13). The use of a biosensor has been utilized to detect the progress of a reaction, in determining the presence of a specific protein, and in detection of food-, air-, and blood- borne pathogens. Biosensors are a broad category of methods used to analyze or monitor biological systems. Biosensors are determined to be unique by the base for the sensor, the method for detection, and the system molecules that are being screened for. The use of biosensors have provided the ability for various applications in biomolecular research.

2.2.1 Photolithography

The creation of a thin carbon film uses a pyrolysis process on photoresist layers. A photoresist is a photoreactive polymer that after radiation exposure, in the form of light, changes solubility. The advantages to photoresists include the fact that is material is relatively inexpensive, quite simple to work with, and can be used extensively in microfabrication. The pyrolysis – heating of the material in an inert atmosphere – allows for the conversion of the photoresist to a carbon substrate which behaves similarly to graphite or glassy carbon.

Photolithography is the fabrication of micrometer scale structures using photoresists. Using a silicon wafer as a substrate, the photoresist is applied to the surface of the wafer using a spin coating method. Spin coating involves the placement of the liquid photoresist on the surface of the wafer and applying a circular rotation to spread the material from the center of the wafer to the entire disc.

As mentioned above, photoresist can undergo specific reactions when exposed to radiation, specifically a certain frequency of ultraviolet (UV) light. This changes the solubility of the polymer via either the formation of cross-links between the photoresist molecules or the breaking of cross-links. A positive photoresist will undergo cross-link formation at the aromatic ring present on the material during UV exposure, while a negative photoresist will break the cross-links between the epoxides. One photoresist, SU-8, used in this project was of the negative nature, while the other, S-1813, was of the positive nature. Figure 5 displays an SU-8 monomer.

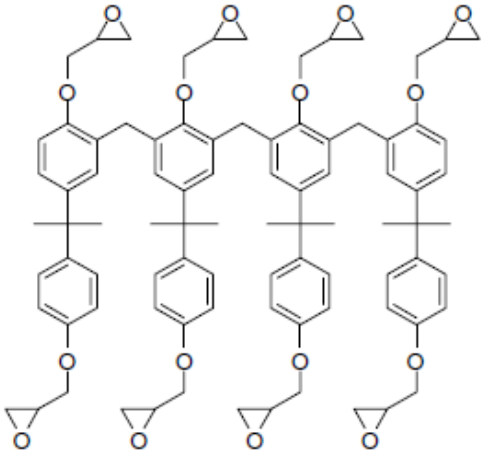

Figure 5 - A SU-8 Monomer (14)

SU-8 is a commonly used photoresist in MEMS applications for its low cost, ability to be easily patterned, and capacity to form high aspect ratio structures. Monomers of SU-8 consist of eight highly reactive epoxide groups that cross link very readily in the presence of an acid. Also in the supplied solution is a photoacid generator which, upon exposure to UV light, creates a strong acid in low concentrations. The acid catalyzes cross-linking of the photoresist, stabilizing regions that are exposed to light. Copolymers of SU-8 and photoacid generating complexes constitute the functional part of the photoresist, while a solvent (gamma-butyrolactone) makes spin coating this polymer possible (14).

The photoresist used for the bulk of this investigation is S-1813. As a positive photoresist, S-1813 has a structure based largely on polymers of aromatic rings. It has a low viscosity and is able to form very thin and very uniform layers when spin cast. It is very soluble in acetone, and stable in deionized water. S-1813 has been observed to adhere poorly to a substrate when subjected to force after soft bake, exposure, and developing. It is capable of being pyrolyzed, and produces a thin carbon film as would be expected (15). In addition to functioning as a carbon preparation for biosensing, the use of photoresist derived carbon has provided capabilities in neurite growth and cell regeneration (16).

2.2.2 Pyrolysis

In order to convert the photoresists discussed above into a carbon surface, a pyrolysis needs to be conducted. Pyrolysis is the chemical decomposition of a substance via a heating process. A. M. Lyons, L. P. Hale, and C. W. Wilkins Jr. at AT&T Laboratories in 1985 conducted one of the first studies in which carbon was created using a photoresist pyrolysis procedure to create microstructures for analysis. Rather than undergo the process of etching a conductive or semi-conductive layer covered by photoresists, the group used a method of modifying the common materials (photoresist) to create the desired conductive properties. The group documented the capability to create insulating, semiconductive, and semimetalic structures while maintaining the quality of the pattern (17).

Pyrolysis has become the process that involves heating a sample in an inert atmosphere. In most cases, the atmosphere used is a vacuum, forming gas, or nitrogen. Experiments by Madou et al. illustrate the affect of atmosphere selection on material loss during the pyrolysis process. Findings indicate that a vacuum is best at preventing film thickness reduction, and that nitrogen is better than forming gas (18). Maintaining a high vacuum demands special equipment considerations for the pyrolysis furnace and forming gas reacts with the carbon film at temperatures in excess of 800°C (17). Nitrogen is thus a reasonable compromise between performance availability and material considerations for this study.

Heating a photoresist to high temperatures forms cross links in polymer chains. X-ray photoelectron spectroscopy (XPS) spectra of pyrolyzed positive photoresist shows a clear shift from C-O bonds to C=O (or C-O-C) bonds with increases in temperature (19). Additional results by Ranganathan et al. demonstrated a lowering of oxygen to carbon ratio with increasing pyrolysis temperature. An increase in O/C ratio was observed after letting samples rest in air for

several days. Forming gas has demonstrated the ability to create the lowest O/C ratios, indicating the purest carbon film because the hydrogen acts as an oxygen scavenger, but at the cost of a great deal more surface loss (18). Experiments have demonstrated that O/C ratios level out to about 0.05 at temperature above 1000C. This tends to indicate that little benefit may be gained by pyrolyzing to temperatures in excess of 1000C.

Analysis has demonstrated that the film left after pyrolysis of photoresist is largely made of carbon. The declining O/C ratio demonstrates an increase in purity of the carbonized residue with an increase in pyrolysis temperature. Electrochemical studies have compared pyrolyzed photoresist to a polished glass carbon standard. Ranganathan et al. demonstrated ΔE_P values that are up to within 17% of those for the glass carbon standard (18). Glassy carbon contains no amorphous carbon and a higher likeness to fullerene-related structures thus suggests lower amorphous carbon content and varying chemical and physical properties (20).

Raman spectroscopy has also demonstrated parallels between pyrolyzed photoresist and a glassy carbon standard. Raman shift observations at 1360 cm^{-1} and 1582 cm^{-1} demonstrates a shift from sp^2 hybridized carbon to sp^3 hybridized carbon with increases in temperature. The structural changes demonstrated by XPS and Raman spectroscopy coordinate very well with electrochemical observations (18). The suface of a spin cast photoresist layer is virtually uniform prior to the pyrolysis process. The pyrolysis process appears to not drastically change the surface topography. One particular procedure produced a surface with a 15 angstrom peak to peak variation and a root mean square roughness of 3.3 angstroms (21).

2.2.3 Transducing Mechanisms

Several methods of detection and monitoring of nanosize interactions are included in the category of nanoparticle biosensors. The use of optical, fiber optic, mechanical, spectroscopy,

and electrochemical methods have all been applied as variations of the biosensor. All use some fundamental property on the nanoscale level to indicate the presence of a biomolecule or a change on the surface of the biosensor.

Optical biosensors utilize refractive index to test for the presence of a molecule. In one special class of optical biosensors, Localized Surface Plasmon Resonance (LSPR), a concentration of a molecule can also be found. The other two classes of optical biosensors are only able to test for the presence of a specific biomolecule, most typically proteins. When a protein is bound to the surface of the biosensor and light is passed over the surface, the speed of light is reduced in the form of wavelength which bends the light. The fact that light is bent determines that the biomolecule is present; however, with LSPR, the extent to which the light is reduced is a direct correlation of the concentration of a biomolecule in a specified volume (22). Optical biosensors have also been used to detect the presence of Methicillin-resistant *Staphylcoccus aureus* (MRSA), concentration of a protein believed to be linked to Alzheimer's disease, and for detection of the avian influenza virus (23), (24), (25).

Mechanical biosensors are another broad category of devices used to manipulate or monitor effects on a surface. The first broad category of mechanical biosensors are cantilever based devices, which are capable of weighing a few Xenon atoms. Analogous to the role cantilevers play, acoustic devices measure changes in resonance frequency of the surface as molecules are absorbed through the use of a piezoelectric crystal (22). A surface acoustic wave is very sensitive to changes in its environment, which when built into a feedback loop can determine changes on the surface of the biosensor. The drawback to this type of sensors is that adjustments in temperature, humidity, and other environmental forces can cause drastic changes in the oscillation frequency of the wave. These changes can cause a false positive situation in

some cases (26). Acoustic devices have been used on a thin gold layer with an immobilized 15-mer oligonucleotide DNA probe for the detection of Hunter syndrome, a genetic disorder that inhibits the males ability to breakdown specific mucopolysaccarides, or specific glycosaminoglycans. Upon binding of the compliment mutated strand, the frequency of the wave changes which a sensor then can detect. This method had a noticeable shift in frequency with a sensitivity level down to 1.55ng/ml/Hz, where sensitivity is defined as the ratio of DNA concentration to frequency shift of the sensor. With an increase frequency of the sensor, this method can be further improved (27). Another disadvantage with these biosensors is that once the probe binds to the DNA, the sensor is unable to be used again.

2.2.4 Electrochemical Methods for Biosensors

Typically, electrodes for electrochemical or electrobiochemical processes have been silicon based. With the expansion of biochemical systems and the necessity of bonding large biochemical polymers it becomes fundamental to use a simpler surface in biosensors. Carbon has become an obvious choice because of abundance of the element and the electrocatalytic properties displayed by carbon surfaces in biosensor systems (28). Studies have proved that pyrolized carbon surfaces have properties appealing for biochemistry and genetic testing (29) (30) (31). Properties such as a low background current, an oxygen to carbon ratio relatively low when compared to glassy carbon (GC), and an extremely flat surface make it ideal for electrochemistry and ultimately biochemistry (18). The ultimate attraction of pyrolized carbon is the ability to create patterns or varying assays on the surface of the biosensors, whose capacity for further advancement are remarkable and infinite.

Cyclic Voltammetry

This electrochemical method is derived from the use of alternating current (AC) and the reaction of the surface of the biosensor to the change in current intensity. A three electrode potentiostat is the standard in which a reference is used as an auxiliary electrode. The other two electrodes are a working electrode which provides the change in current to an electrochemical cell through a copper connection and a counter electrode which measures the current in the solution. Both the counter electrode and the reference electrode are placed within the solution of the electrochemical cell (32).

This is only a valid approach if the reference electrode is physically large enough for a large current to maintain a small current density within the reference electrode. This allows for the potential between the solution and the reference electrode to be nearly constant. The potential difference of a working electrode is described by the equation: $\Phi_W = \Phi - \Phi_{CE} - R_i$, where Φ_W is the potential provided by the working electrode, Φ_{CE} is the potential measured by the counter electrode, R_i is the resistance of the solution, and Φ is the potential of the surface (32). The potential of the working electrode is given because it is induced on the system, the reference electrode measures the resistance of the solution and the counter electrode measures the potential through the solution. The potential of the surface can then be equated, which allows for changes of the surface to be induced, monitored, and detected. By using cyclic voltammetry, where a sinusoidal current is placed on the electrochemical cell, the relative oxidation and reduction peaks for a molecule can be found. When the surface changes, these peaks change to signify this change.

The surface changes that occur during a cyclic voltammetry process are induced by the current and the use of an ion rich solution for conductance. As the voltage is applied in an

increasing and decreasing manner, or a decreasing and then increasing manner, two peaks in the surface potential should be recognized. As the ions in solution become solids on the surface of the electrode a cathode peak will form, and as the ions reform into solution an anode peak will form. These two peaks are unique to the surface material and therefore can be used as a characterization method for most materials. Additionally the association of molecules onto the surface can be conducted by applying a cathode peak and omitting the anode peak.

Electrochemical Impedance Spectroscopy

The second electrochemical method is based on the fact that a simplified relationship between resistance, current, and voltage does not exist for the majority of circuits. The simplified method is based on Ohm's law, where the relationship is simplified to: $E = I \cdot R$ where voltage is current multiplied by resistance. This is based on two premises, those being that resistance is independent of frequency and that the AC current and voltage signals are in phase with one another (33). However, applications in electrochemistry are far more complicated that those to which Ohm's law is capable of describing.

Impedance, another measure of the resistance of a material to current flow is instead used. When a sinusoidal current is applied to an electrochemical cell, the voltage can be described as: $E(t) = E_0 \cos(\omega t)$, where E_0 is the maximum voltage applied (amplitude of the voltage), ω is the radial frequency, and t is the time. Because the voltage and the current are not in phase, and the response signal is reduce compared to the voltage applied, the response signal is defined as: $I(t) = I_0 \cos(\omega t - \phi)$, where I_0 is the new amplitude and ϕ is the phase shift. In an expression similar to Ohm's law, impedance can be described as: $Z = \frac{E(t)}{I(t)} = \frac{E_0 \cos(\omega t)}{I_0 \cos(\omega t - \phi)} = Z_0 \frac{\cos(\omega t)}{\cos(\omega t - \phi)}$. Impedance can then be used to determine changes in the surface of the biosensor.

In Ohm's law, impedance can replace resistance so that $E = I \cdot Z$, and a better description of the system is obtained (34).

A sinusoidal applied voltage has a distinct and universal look. As displayed in Figure 6, the phase shift of the response signal, φ, is displayed by the dash line and the decrease in amplitude is shown by the dotted line. Impedance is ultimately a function of this phase shift and amplitude decrease, while resistance is only a function of amplitude decrease. This allows for a greater understanding of the system and a sophisticated analysis of the biosensor.

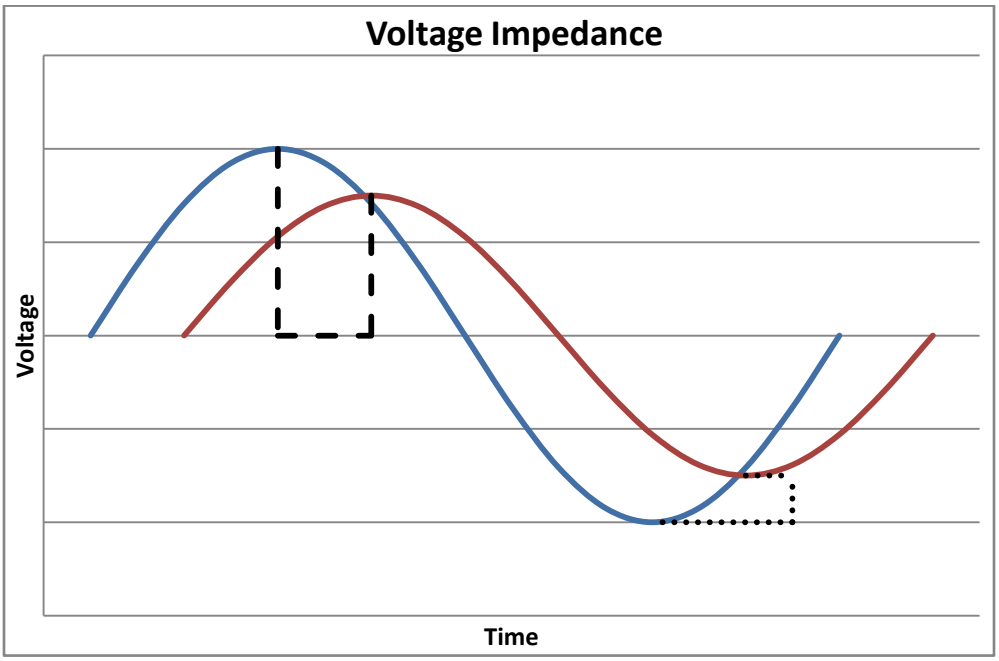

Figure 6 - Display of Sinusoidal Impedance

The principle behind using Electrochemical Impedance Spectroscopy (EIS) in biosensors is to change the surface electrochemistry of the biosensor so that a change in impedance is recognized. Using working electrodes and labeling the materials that attach to the biosensor, changes in surface electrochemistry can be monitored and reported as binding of the material occurs.

The data collected from EIS is present in several types of plots; however, the most useful for understanding the impedance of the situation is the Nyquist plot. The Nyquist plot is derived from the transformation of the applied and responding equations being expressed as complex functions: $E(t) = E_0 exp(i\omega t)$ and $I(t) = I_0 \exp(i\omega t - i\phi)$. The new expression for the induced equation would be: $Z = Z_0 \exp(i\phi)$. With the use of Euler's relationship, $\exp(i\phi) = \cos(\phi) + i \sin(\phi)$, the impedance would be defined as $Z = Z_0[\cos(\phi) + i \sin(\phi)]$. The Nyquist plot is simply the imaginary part of the equation against the real part of the equation. Therefore, each frequency has a unique point. All values on the y-axis or the imaginary axis are negative, while the real axis or the x-axis are positive numbers.

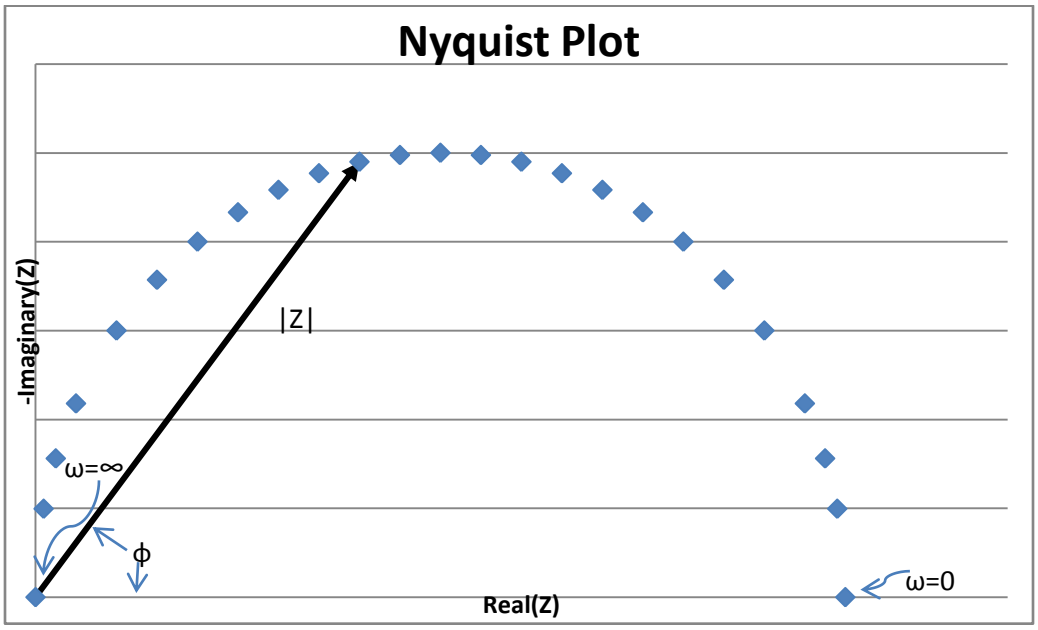

Figure 7 - Example of a Nyquist Plot

Above the example of the Nyquist plot displays how the collected data is used and interpreted in the plot. For any given values of the frequency, ω, the absolute value of Z, can be determined as the vector formed between imaginary and real points. The higher the frequency, the closer the plot approaches zero. Finally the phase shift is the angle that is formed by the Z vector and the x-axis. While the plot does not display the frequency, it does present the fact that

as the shift becomes larger, which is represented by ϕ getting larger, the real part will become smaller and the imaginary will become larger.

Previous Uses of EIS and Cyclic Voltammetry

Typically, electrodes for nucleic acid research have used gold, glassy carbon (GC), or carbon nanotubes. The use of gold have been explored because the DNA is bound to the surface through alkanethiol self-assembly methods (35). Gold has been displayed as a method in which high amounts of hybridization occur and has an easy reproducibility using probe-modified surfaces (35). The thiolated-DNA can and typically is monolayered on the gold surface, which provides both a stabile and a structurally electrochemical interface (36). The thiol-gold bond is also the cause of many issues associated with the use of gold as a biosensor. The thiol bond is only stable in a short range of potentials, and the bond itself is prone to damage due to oxidation and thermal desorption (37). Modifications of the gold surface have shown promise when the gold surface is sputtered on nanoporous niobium oxide. The sensor then has a three times increased sensitivity and 2.4 times greater resolution when compared to standard thick gold electrodes (36). However, the gold surface and metal surfaces in general are weak candidates for electrochemical biosensors.

Glassy carbon and carbon nanotubes have also shown promise as biosensors. Methods have varied, but the process of choice is to use 4-aminobenzoic acid (ABA) to modify the carbon surface. A nitrogen-carbon bond is formed between the surface and the acid, which has a good stability even in electrochemical applications. Further tests have proved that electrochemical impedance and cyclic voltammetry experiments have been successful at measuring changes in surface potential of a biosensor with and ABA/carbon surface (38). While the surface chemistry

does change with the addition of ABA, this molecule provides a self-assembling bond formation between the DNA and the surface of the biosensor.

2.3 Surface Energy Measurements

Surface interaction with a fluid can be completed using the contact angle of a droplet of fluid. The smaller the angle (the flatter the droplet) indicates that surface-liquid interfacial interactions dominate over the gas-liquid interactions. For example, a flat droplet of water on a surface indicates that the surface has the capacity to form hydrogen bonds with the fluid, where a round droplet with a greater contact angle corresponds to a reduced surface affinity for the droplet.

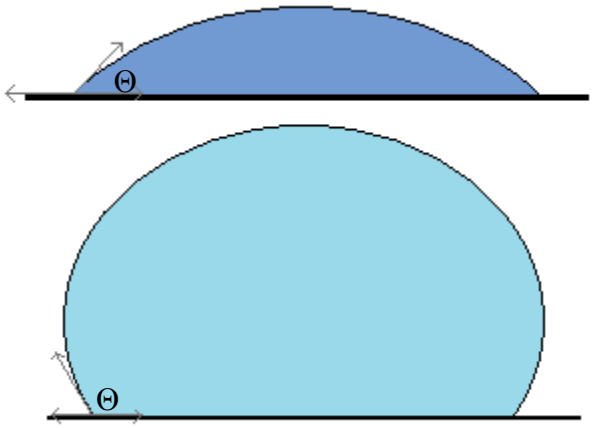

Figure 8 - Depiction of Droplet-Surface Interactions for Different Surface Energies

Droplet contact angle is marked by a "Θ" on the inside of the angle measured. The surface with less energy is displayed at bottom, with a much larger contact angle than the high energy surface at top. The droplet's shape is determined by the ratio between the fluid's surface tension at the fluid-gas interface (the diagonal force vector in grey) and the fluid-surface interaction (solid-liquid surface tension illustrated by the horizontal grey vector pointing inward toward the droplet) and the solid-gas surface tension (the horizontal vector pointing outward away the droplet).

A lower surface energy results in domination of surface tension forces at the fluid-gas interface and a rounder droplet with a higher contact angle formed. The strength of the solid-liquid interfacial stress is directly proportional to the cosine of the contact angle, which is to the horizontal component of the diagonal vector. A higher energy surface has a greater solid-liquid interaction force than liquid-gas interaction (surface tension), the result is a lower contact angle

and greater wettability. By contrast, the low energy surface sees domination of liquid-gas interaction (surface tension) and the droplet is more spherical with a larger contact angle.

2.4 Aspects of the Biochip

Creating a carbon surface for the binding of DNA has proved challenging. The use of a photoresist to create a carbon surface has been analyzed and certain restrictions apply to all photoresists. If the photoresist exceeds temperatures of 1000°C, reactions between hydrogen and carbon occur, with hydrogenation rates becoming substantial over 1000°C. Phenolic polymers have shown several reactions that result in increased density and weight loss of the surface (17). This densification and reduction of the surface leads to epoxy based photoresists being more suitable to DNA biosensor applications.

Study of SU-8 photoresist, has shown that a sufficient carbon surface was formed by spinning 20 mL of photoresist at 3000 rotations per minute for forty seconds. This coverage created about a 3.25 μm photoresist covering on the silicon wafer. The wafers were then baked from 20°C to 900°C in two hours, held at 900°C for one hour and then cooled for 14 hours. This surface provided a suitable carbon environment for the ability of growing cells, which would be sufficient for the binding of ABA to the carbon surface (21). SU-8 has proven to be adherent to silicon surfaces at a rate of 5°C/min, which provides a solid base for modification of the carbon surface (39).

Studies of photoresist derived carbon have shown that within increasing temperature of pyrolysis the oxygen to carbon ratio decreases, eventually reaching a minimum at of 0.05. This determines that the necessary temperature a photoresist must reach to provide a suitable carbon surface must be greater than 700°C but less than 1000°C. Increased temperatures also changes the peak profile from a majority of C-O bonds to C=O bonds (19). The increased strength of the

carbon-oxygen bond makes the surface more suitable for bonding of ABA or other materials to the biosensor.

2.5 Basic Science of Oxygen Plasma

Oxygen plasma treatment is a method for which a surface is decreased to an extremely low pressure and subjected to a relatively high electrical power supply. Plasma is the state of matter for which the gaseous state has been ionized to form very reactive species. Oxygen plasma simply uses oxygen as the gas for the treatment. At the reduced pressure, the gas is more likely to produce an increased amount of reactive oxygen species. Ions produce include: O^+, O^-, O_2^+, and O_3^+ (40). Any material can be treated with oxygen plasma; however, when carbon is treated it has been shown to produce the following species in order of abundance: graphite, hydrocarbons, hydroxide, carbonyl groups, and carboxyl groups (41). Oxygen plasma will only alter the surface, typically covering 10 to 15 percent of the surface with oxygen species and usually decreasing the oxygen content by 25% with each nanometer into the surface (41) (42).

Oxygen plasma is a useful technique to replace the ABA attachment step without the use of the electrochemical method for alteration and to create the oxygen attached directly to the carbon surface. This would replace the presence of the aromatic ring between the oxygen group and the oxygen, allowing for more uniform and reduced surface. Ultimately, the goal of using the oxygen plasma treated surface is to replace a fairly complicated and slow process with an application technique that can be expanded for delivery to several biosensors at once in a quick and relatively simple manner.

2.6 Methods Used to Detect Carcinogenic DNA Mutations

Early detection of a carcinoma is essential for treatment to be effective and for overall patient safety. Current diagnostics for cancers are time consuming, labor intensive, and require

an advanced stage of the carcinoma. Using a glass carbon surface, short sequences of DNA associated with Chronic Myelogenous Leukemia (CML) were attached to the carbon surface in a phosphate buffer solution. Afterwards a 10 μL of complimentary, single mutation, or non-complimentary sequence were allowed to immerse the biosensor surface. After a half hour period, the surface was washed clean and differential pulse voltammograms were completed between 0.1 to -0.6 volts. With the complimentary sequence DNA, the current conducted by the surface reduced by nearly fifty percent; however, with a single base mutation, the current also reduced, but only to seventy-five percent of the original current of the bonded target DNA (43).

3. Materials and Methods

The use of a photoresist derived carbon substrate for genetic testing has two fundamental areas of division. The first division of the research involves the characterization and analysis of the surface to ensure a continuous, level, and stable carbon surface. The second being to characterize how the surface changes as the molecules are attached for proper interpretation of the data presented.

3.1 Chemical Specifications

All chemicals used during the analysis and project were freshly prepared except the 5mM $K_3Fe(II/III)(CN)_6$ in PBS and the 0.1 M KCl and 1 mM ABA in deionized and distilled water solution. Please see Appendixes L through S for the MSDS sheets on all chemicals used.

S1813 was purchased from the Shipley Corporation. Sigma-Aldrich supplied the Potassium hexacyanoferrate(II) trihydrate, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride, and *N*-hydroxysulfosuccinimide. Acros Organics provided the Potassium hexacyanoferrate(III) trihydrate. The Phosphate Buffer Solution (pH 7.20) was purchased from Fisher Scientific. Aldrich also supplied the 4-Aminobenzoic acid (99%). The DNA was ordered from Integrated DNA Technologies (IDT), with the sequence 5'-NH2-C_6-ATC TAC GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC C-3'.

3.2 Preparing the Carbon Surfaces

The carbon surface is created in a two step process. First the spin coating application must be conducted for photoresist presence to then be converted to carbon using the pyrolysis methods. The use of photoresist and the spin coating uses a force to create a fairly level and constant surface that is maintained via equal reduction during pyrolysis to create a level carbon substrate surface.

3.2.1 Covering of the Silicon Discs with Photoresist

The coating process uses cleaned silicon disks and occurs in a clean room. These disks are cleaned in an ultrasound machine using acetone, methanol, and finally DI water for five minutes each. After being washed with the water, the disk is blown dry using nitrogen gas. Finally, the disc is placed in a 110° Celsius oven for a minute to ensure the disk surface is dry and free of all moisture. The disk is then placed in a spin coater, followed by the application of approximately 2 mL drop of S1813 photoresist and the wafer is spun at 3000 rpm for ninety seconds. The disk is immediately heated in the 110° Celsius oven for three minutes to ensure the photoresist material will maintain an unaltered surface composition.

After the initial heating, the wafer is allowed to cool back to room temperature, typically about one minute out of the oven. The disks are then blow with nitrogen gas to ensure no foreign particles are present on the surface of the wafer. After being placed back on the spin coater an additional 2 mL drop of the photoresist is placed in the center of the wafer and the spinning process is repeated. The heating process for three minutes in the 110° Celsius oven is completed immediately after the disk is covered. The process of recovering and reheating takes place two more times, for a total of four coats on the surface.

The wafers are stored in a container that is wrapped in tin foil to ensure that no ultraviolet light damages the photoresist coatings. They are also placed an stored in a light-free environment at all times.

3.2.2 Heating of Photoresist Covered Disks to Create a Carbon Surface

The Carbon surface is first prepared by the heating (baking) of the surface in the Lindberg / Blue M quartz tube furnace HTF55000 Series and the CC58814C control console manufactured by Thermo Electron Corp. The photoresist covered disks are cut into 10 mm by

22 mm rectangles using a diamond-tipped pen. These rectangles are placed on an alumina plate (0.025" × 4.5" × 2.25") sterilized using acetone and deionized water. The tube furnace is prepared for the heating of the carbon covered plate by creating a nitrogen atmosphere in a hood vent. The nitrogen environment is created by a flow of 100 sccm through the quartz tube. Prior to heating, the nitrogen is allowed to flow for a minimum of ten minutes to remove all oxygen from the quartz tube and create a 99.99% Nitrogen atmosphere. Throughout the heating process the nitrogen environment is maintained.

The plate holding the rectangles of photoresist covered silicon is heated to 300 degrees Celsius at a rate of two degrees Celsius per minute followed by a rate of ten degrees Celsius per minute until the furnace reaches one-thousand degrees Celsius. After reaching 1000 degrees Celsius, the furnace is turned off and the plates are allowed to cool to room temperature in the maintained nitrogen environment. After the tube furnace has been at room temperature for one hour, the nitrogen flow is stopped and the alumina plate is removed from the furnace. The rectangles are placed in sterilized Petri dishes wrapped in kim wipes. This ensures that the carbon surface is protected from ultraviolet light and from damage due to interactions with other carbon surfaces.

3.3 Carbon Surface Characterization

Three examinations of the surface were conducted to ensure that a stable and level carbon surface is present. The surface was examined to determine if the substrate produced was carbon, how level the carbon surface was, and the general thickness of the carbon over the wafer.

3.3.1 Substrate Determination

Using the surface of the wafer as the working electrode through a connection with a thin copper strip, the characterization of the surface will be conducted using a 3-electrode potentiostat

with Ag/AgCl as the reference electrode using the PGSTAT12 manufactured by AUTOLAB and utilizing the General Purpose Electrochemical System for Windows version 4.9.004. The surface in the water solution will be treated and monitored using one cycle of cyclic voltammetry scanning between 0.0 and +0.6 V at a rate of 10 mV/s in a 5mM $K_3Fe(II/III)(CN)_6$ in PBS. In addition to analyzing the carbon surface using cyclic voltammetry, the plain silicon wafer surface and a glassy carbon reference were also analyzed using the same technique.

3.3.2 Surface Thickness Analyses

Two analyses were conducted of the surface to determine variations in substrate thickness and percent loss of the mass during the heating procedure. First, analysis of the surface thickness was conducted using a Nikon microscope using a white light source and a 10X Mirou type double beam CF Plan EPI DI objective on a Physik Instrumente E-500.00 piezoelectric controller and measuring device. The wafer is aligned at a slight tilt so that it is not perpendicular to the microscope. The tilt of the sample makes phase contrast lines appear and distances may be measured by observing differences in focal length as reported by the piezoelectric controller while focusing on the top of the film or the bare wafer. This technique was first used to determine if a ramped speed or a constant speed application of the photoresist during the spin coating procedure enabled the creation of a more level and constant thickness surface.

Next the thickness of the photoresist and carbon were obtained prior and after the heating process to determine the variations in surface thickness. The heating process should remove some of the mass of the surface, and this examination was conducted to see how the mass removal effects the surface thickness of the carbon substrate.

Finally, as an additional determination of the mass loss, the mass of the photoresist covered silicon, the mass of the carbon covered silicon, and the mass of the silicon itself were all obtained and a percent mass loss calculation was conducted.

3.3.3 Surface Energy Analysis

Contact angles are estimated by placing a droplet on the surface and taking a photograph. Water, ethanol and toluene are used on bare silicon, silicon coated in S1813, an ordinary thin carbon film and an oxygen plasma treated thin carbon film. For water, a 25 µL droplet is used, 12 µL for ethanol and 8 µL for toluene. The differences in surface tension between the fluids allows for larger droplets to be more stable on the surface, where a larger droplet eases the task of measuring the contact angle. Photographs were taken of the droplets with a Nikon D80 with a Nikon AF Nikkor 50mm 1:1.8D reverse mounted lens. A tangent line is drawn on each end of the droplet using Microsoft Paint and the angle is measured with a protractor.

3.4 Carbon Surface Functionalization with 4-Aminobenzoic acid

Several methods were preformed to alter the carbon surface so that ssDNA is able to attach to the surface and function as a probe for complimentary and non-complimentary ssDNA. By performing the alterations, the carbon surface is first converted to have a free carboxylic acid, and then a free nitrogen group. A schematic of the molecular modifications using the ABA treatment is presented in Appendix B.

3.4.1 Altering the Carbon Surface

Using the pyrolized carbon surface, the rectangular surfaces will first be cleaned by sonication in water, ethanol, and water again for five minutes each using the B25500A-MTH Ultrasonics Cleaner manufactured by VWR North America. The wafers are then allowed to dry in air. Using 3 mM 4-aminobenzoic acid (ABA) in a 1 M KCl deionized and distilled water

solution, the surface will then be treated to create a proper surface for the binding of single stranded DNA (ssDNA). The carbon surface will act as a working electrode using a 3-electrode potentiostat with Ag/AgCl as the reference electrode using the PGSTAT12 manufactured by AUTOLAB and utilizing the General Purpose Electrochemical System for Windows version 4.9.004. The surface in the water solution will be treated and monitored using four cycles of cyclic voltammetry scanning between 0.0 and +1.40 V at a rate of 10 mV/s. For a detailed depiction of the molecular changes occurring during the process, please see Appendix B.

3.4.2 Creating the Carboxcylic Acid for DNA Attachment

After being treated with cyclic voltammetry, the ABA/Carbon electrode will be immerged in 50 mM phosphate buffer solution. The phosphate solution will contain 2mM 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC) and 5mM *N*-hydrosulfosuccinimide (NHS). After two hour of immersion, the electrode will be rinsed with more solution to remove excess NHS or EDC from the surface. The alteration and activation of the free carboxylic acid group allows for easy attachment of the target DNA strands.

3.4.3 Attachment of ssDNA

The prepared carboxylic acid group available for bonding of the single stranded DNA (ssDNA) will then be bind target DNA strands. 50 µl of 50 mM of prepared target ssDNA will then be pipetted onto the electrode and allowed to sit for 24 hours at 20° Celsius (room temperature). After the 24 hour waiting period, the electrode is washed with deionized water to remove oligonucleotides that are not bound to the surface.

3.5 Carbon Surface Functionalization with Oxygen Plasma Treatment

As an alternate method to creating a reactive oxygen group on the surface of the biosensor, an oxygen plasma treatment was conducted. This process used O_2 plasma treatment at

150 mTorr and fifty watts for thirty seconds. This step replaced the ABA functionalization step described above. The oxygen plasma treated surface was further modified in the EDC and NHS method described above. Attachment of ssDNA was also conducted in the method described above.

3.6 EIS Detection

An EIS analysis of the surface was performed using the 3-electrode potentiostat as above, except scanning with 2 volts from 10000 Hz to 1 mHz with 201 frequencies. The surface was analyzed using EIS for: the plain silicon wafer, the carbon surface, the ABA treated surface, the oxygen plasma treated surface, and the EDC/NHS for both the ABA and oxygen plasma treatments. Additionally, the surface was analyzed after the ssDNA had been attached for both methods.

4. Results and Discussion

4.1 Carbon Surface Heating Procedure Determination

The first step of the process was to determine a baking procedure for which the carbon on the silicon disks was stable and maintained stability over time. The use of multiple photoresists required that slight alterations of baking procedure were conducted for each of the differently obtained photoresists. As displayed in Appendix C the SU8 and S1813 required unique heating procedures. The new SU8 obtained from Harvard performed and reacted to the heating process drastically different from the samples of SU8 created within our lab at the initiation of the project. Our conclusion about this is derived from the type of silicon disk used. We believe that the new SU-8 used a fused silica disk, which has a heating coefficient one fourth that of Silicon and Carbon. This would require the heating procedure to be 1°C/minute throughout the entire procedure in order for a stable carbon surface to be created. Instead of the slow procedure using SU8, the non-standard use of multiple layers of S1813 was determined to be most efficient for our process (44; 39). For detailed images of what the various problems were with some of the pyrolysis methods please see Appendix D.

4.1.1 Initial Heating Process

The heating process used by Ranganathan et. al. was the basis for our beginning method of pyrolizing the carbon. This method, however, failed to produce any carbon on the surface. This is due to a final heating temperature that is too low. For the next heating process the final temperature was raised from 600°C to 900°C. The time that the heating process took was also very excessive for this process, therefore the heating rate was also increased from 2°C/minute to 10°C/minute.

4.1.2 Second Heating Process

This heating process created a mostly stable carbon on the silicon wafer. As a basic test of the stability of the carbon surface, the wafer was submerged in tap water. This proved that this carbon had a mostly stable adherence to the wafer. The edges of the rectangles are more susceptible to minor scratching – especially after further cutting for modification and placement in the electrochemical cell. The exact cutting of the wafer is necessary for the wafer to remain completely stable after the pyrolysis process. Several papers suggest that the carbon's stability and the adherence bond's strength increase if the photoresist is allowed to acclimate at a lower temperature. For this purpose, the third heating process introduced a temperature hold at 300°C. To increase the amount of carbon present on the surface, the final pyrolyis temperature was held for 1 hour during the third pyrolysis method.

4.1.3 Third Heating Process

As above this produced a carbon surface that is extremely uniform and stable. The surface is solid carbon. However, this carbon is prone to damage in storage. The simple interaction with other sections of wafer in storage damages the carbon surface via scratches and thinning. The carbon that is produced is preferable for electrochemical methods, therefore this process that is used as a standard for our work.

4.1.4 Fourth Heating Process

Using the Ranganathan et. al. as a source, the carbon they produced is of a thicker nature than the carbon which we were getting. To explore this method further, the initial process was repeated. While there was slight conversion to carbon in a spotty fashion, the carbon was very unstable in water and formed a gel in the water as in the initial process. In an attempt to slightly decrease the time for pyrolysis the fifth heating process was increased to a rate of 3°C/minute

and the temperature was raised to 800°C for a final temperature to create a more uniform carbon conversion.

4.1.5 Fifth Heating Process

While this carbon was stable in water, the physical storage of the carbon in the kim wipe caused significant peeling. This peeling is a known result of not allowing the carbon to acclimate at a lower temperature. For the next heating procedure the photoresist is allowed to heat and maintain temperature at 300°C to prevent this storage damage. To increase the speed of the pyrolysis process without damaging the carbon produced, a split technique of heating rates was used for the sixth process. Using a heat rate of 2°C/minute for the initial heating to 300°C and then a higher rate of 10°C/minute was used for the final heating to 800°C.

4.1.6 Sixth and Seventh Heating Processes

This produced a very stable carbon. There was some minor peeling on the edges, which are most likely a result of the cutting process. This process was repeated for the next method and again there was one small section of peeling but overall the method produces a very stable and uniform carbon surface. To remove the slight peeling, the final temperature was raised to 900°C for the eight heating process.

4.1.7 Eighth, Ninth, and Tenth Heating Processes

This raising of the final temperature produced a perfect film. There was no cracking, no peeling, the carbon was stable for extended lengths of time, and has a good adherent layer. The tenth process changed the photoresist from SU-8 to S-1813. The new photoresist is known to have a lower conversion temperature for pyrolysis; therefore, the eleventh process lowered the final temperature back to 800°C.

4.1.8 Eleventh Heating Process

This produced a consistent, shiny carbon surface. There was minor scratching and spotting which was more pronounced along the edges of the wafers. This processes used one layer of S-1813 spin coated onto the surface. This one layer produced a thin layer of carbon which was too thin for the applications of the electrochemical methods. A multilayered (four photoresist coats of S-1813) wafer was produced. The final heating temperature was raised to 1000°C to allow for the conversion of all coats of the photoresist to carbon during the twelfth heating process.

4.1.9 Twelfth Heating Process

This produced a carbon surface that has no obvious silicon and was very reflective. However, some areas produced were darker than others. This is either due to inconsistent heating or to variations in the thickness which is a direct result of the photoresist spin coating application. If it was a result of the spin coating process, there are no methods available to correct this during the pyrolysis procedure. However, if the inconsistence in color is due to the heating process, it could be an effect of the acclimation step where the lower layers of photoresist are not as acclimated as the upper layers of the photoresist. To correct this acclimation step is removed for the thirteenth pyrolysis procedure.

4.1.10 Thirteenth and Fourteenth Heating Processes

This produced a more consistent carbon surface. There was minor edge effect on two of the wafers; this is most likely the result of the cutting procedure than of the heating process because of the consistencies from the previous four heating methods. The fourteenth method used a wafer which was spin coated for 45 seconds at 600 rpm followed by 45 seconds at 3000 rpm. This was an attempt to increase the thickness of the photoresist on the wafer without

sacrificing the consistency of the surface. This variable spin coating speed resulted in significant cracking and peeling and an overall poor adhesion of the carbon to the wafer.

4.1.11 Fifteenth Heating Process

Due to the success of the heating process on the S-1813, the same process was used on the new SU-8 samples obtained from Harvard University. The only modification was to remove the one hour waiting period at the end of the procedure. This was because the SU-8 was never raised to 1000°C, and it was thought that this higher temperature may remove the need to hold the temperature for an hour at the end of the pyrolysis. This alteration in the SU-8 procedure produced inconsistent surfaces between the wafers. The edge effect was much more pronounced on these samples than any previous samples. The edge effect included disintegration of some of the corners, which was never present before. Other than the corners, the wafers appeared suitable for use. For the sixteenth pyrolysis the one hour hold at the end of the pyrolysis was reintroduced to see if it would eliminate the corner and edge effects by providing an equilibration time before cooling the wafer.

4.1.12 Sixteenth Heating Process

The one hour hold at the end of the pyrolysis procedure produced significant cracking and peeling. The destruction of the surface was so pronounced that the carbon formed bubbles on the surface, where the adherence bonds were completely removed. The adherence bonds were weak throughout the surfaces because immediate disassociation occurred once the wafers were placed in water. The peeling in the fifteenth process was thought to be a result of the cutting process after the results of the sixteenth method. The temperature produced disassociation throughout the wafer, therefore where bonds are already weakened, as with cutting, the disassociation

occurs. A more precise cutting of the materials was conducted and the fifteenth heating procedure was repeated for the seventeenth method.

4.1.13 Seventeenth Heating Process

Even with a precise and less invasive cutting technique, the material was unstable in water and had odd line formations (as in a single crack) on all surfaces. The heating rate is the variable that is most likely to cause cracking, as a result the eighteenth procedure reduced the heating rate throughout the process to 2°C/minute. Cracking can also occur because of an overly accelerated cooling rate, the cooling rate was controlled at 2°C/minute. Because the edge is area which peels first and this is a result of the final temperature, the final temperature for the eighteenth method was also reduced to 800°C.

4.1.14 Eighteenth Heating Process

The edge effect still remains with the decreased heating rate, cooling rate, and final temperature. The wafers disassociate in water; however, less quickly and less pronounced than those of the sixteenth procedure. When the material is exposed to electric current during cyclic voltammetry the carbon readily dissociates. The control of the cooling rate was thought to initiate the dissociative ability of the carbon surface. For the nineteenth heating procedure, the control of the cooling rate was removed.

4.1.15 Nineteenth Heating Process

The results were still inconsistent with the original SU-8 procedure. There was bad cracking, bubbling throughout the surface, and the flow rate of nitrogen over the surface removed carbon on several of the samples. Because of the inconsistencies produced by these SU-8 samples, it is theorized that the silicon wafer is not plain silicon but rather fused silica. Fused silica has a heating coefficient one fourth that of carbon and plain silicon. This variation

in heating coefficient would require that the heating procedure occur at 1°C/minute or less throughout the process. To avoid such a slow and susceptible heating process, the project was continued with the multi-layer S-1813.

4.1.16 Twentieth, Twenty-first, and Twenty-second Heating Processes

For the twentieth and twenty-second heating procedures, the successful heating procedure developed in the thirteenth heating method was repeated. However, in the twenty-first heating procedure the heating rate was raised to 10°C/minute for the entire procedure. This produced significant cracking and peeling. The thirteenth procedure was repeated for the twenty-second process. The results from this process are most consistent and was selected as the standard for the entirety of the project.

4.2 Surface Substrate Electrochemical Analysis

To characterize the surface produced during pyrolysis, cyclic voltammetry (CV) was performed to compare the surface to a glassy carbon reference as well as to the bare silicon surface. All CV's were performed between 0.0 and +0.6 V at a rate of 10 mV/s. Figure 11 displays the cyclic voltogram produced by the carbon substrate. Notice that no characteristics are held in common with the cyclic voltammogram produced by silicon in Figure 9. However, the carbon surface did produce characteristics very similar to that of the glassy carbon cyclic voltogram produced in Figure 10. The anode and cathode peaks appear in similar voltages as well as have similar amplitudes. This would indicate that the carbon substrate is similar in physical and chemical structure to that of glassy carbon. These physical and chemical similarities signify the ability of the photoresist derived carbon to function as an electrochemical biosensor in a similar manner and in similar techniques to the glassy carbon biosensors.

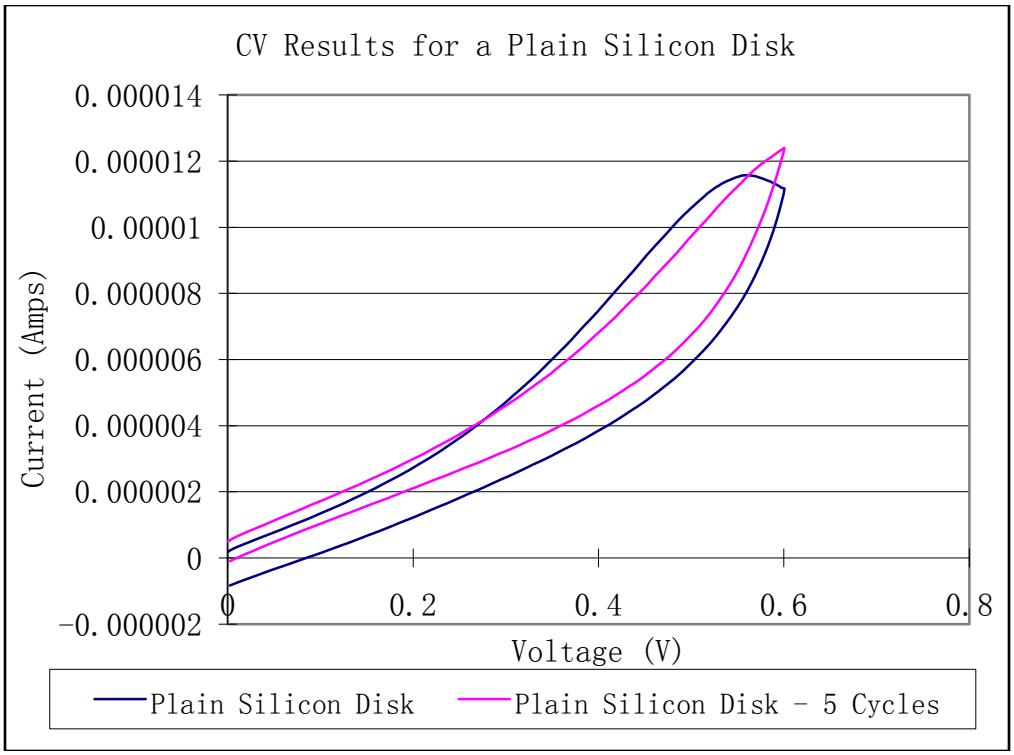

Figure 9 - Silicon Standard Cyclic Voltammogram

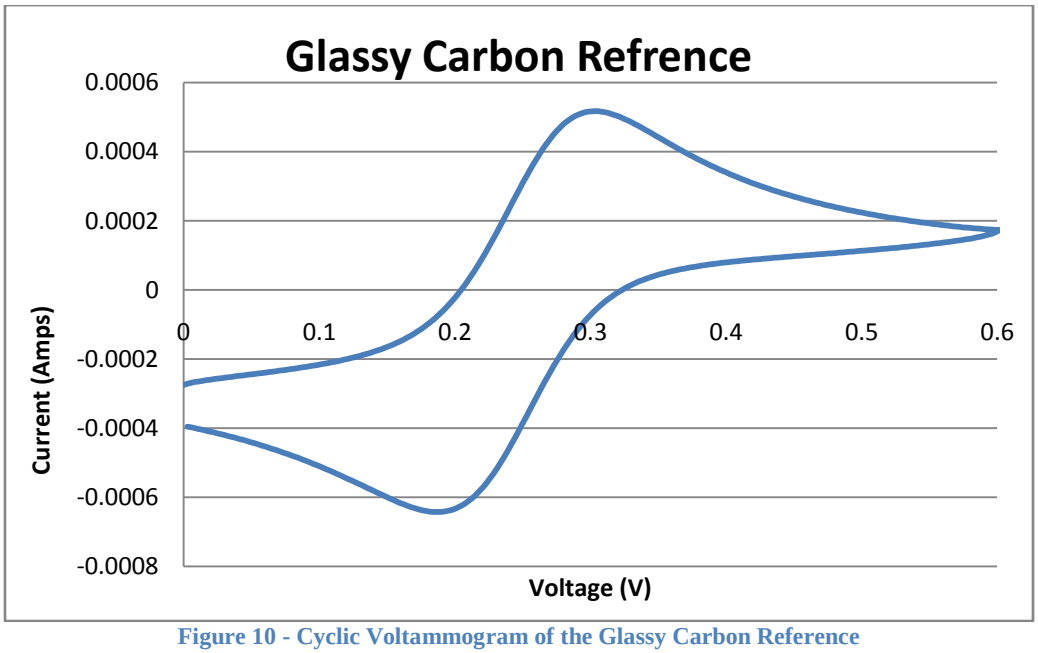

Figure 10 - Cyclic Voltammogram of the Glassy Carbon Reference

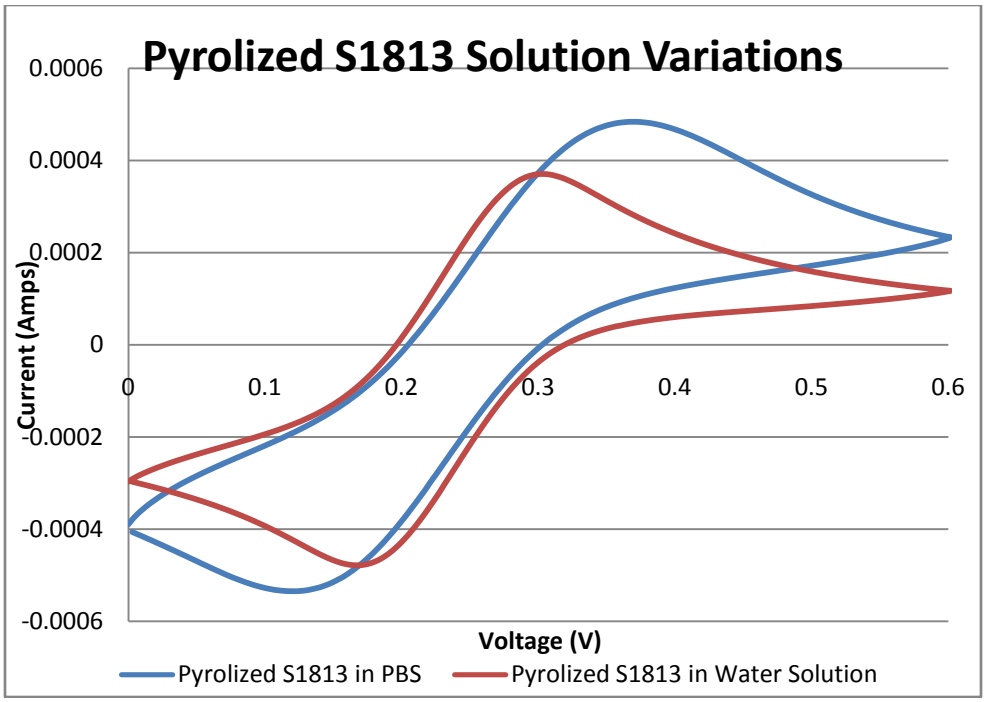

Figure 11 - Cyclic Voltammograms of Carbon Produced by S1813 Photoresist

As Figure 10and Figure 11 display, the derived carbon is akin to the glassy carbon standard. ΔE_p values of 118mV and 137mV for glassy carbon and PPF respectively. The difference of 19mV between ΔE_p values for each material is very similar to findings from procedures using similar temperature ranges (18). Peaks appear at 302mV and 168mV for cathode and anode respectively for the photoresist derived carbon and at 304 mV and 184 mV for the glassy carbon standard. Resulting ΔE_p values are 2mV on the cathode side and 16 mV. This is excellent agreement in peak values, allowing for the conclusion that the photoresist derived carbon contains electrochemical and physical characteristics similar to the glassy carbon standard.

4.3 Surface Thickness and Mass Loss Analysis

To determine the best process for spin coating the S1813 photoresist onto the silicon wafer, a rough analysis of surface thickness was conducted using a Nikon microscope with a white light source. The first test was conducted by spinning the material at 3000 rpm for 90 seconds, while the second test used a ramped speed from 500 rpm to 3000 rpm over a 90 second

period. The constant speed wafer displayed a more constant thickness throughout the wafer than that of the ramped speed (Table 2).

Table 2 - Thickness of Photoresist S1813 on the Silicon Disks

Wafer	Measurement	Center	Half way to Edge	Edge
A (Constant Speed)	Silicon	105.35	118.7	125.6
	Photoresist	112.8	126.3	150
	Thickness (μm)	7.45	7.6	24.4
B (Ramped Speed)	Silicon	149.8	147.7	142.9
	Photoresist	165.3	167.5	167.4
	Thickness (μm)	15.5	19.8	24.5

The ramped speed, while producing a thicker surface throughout the wafer, does not produce a continuous and level surface. The goal of the spin coating process is to create a surface which is as free of inconsistencies as possible. The constant speed wafer had a surface which was within 0.15 μm from the center where minimal force is exerted on the photoresist during the spinning process to the middle. Both wafers produced a thick outer edge. This is assumed to be edge effect caused by the resistance of the photoresist. Because both the constant and ramped speed wafers produce this edge effect, it is assumed to be unavoidable and present after all spin coating procedures. The constant speed is selected for a spin coating application so that during the pyrolysis procedure the inconsistent carbon thickness derived in pyrolysis method twelve are avoided.

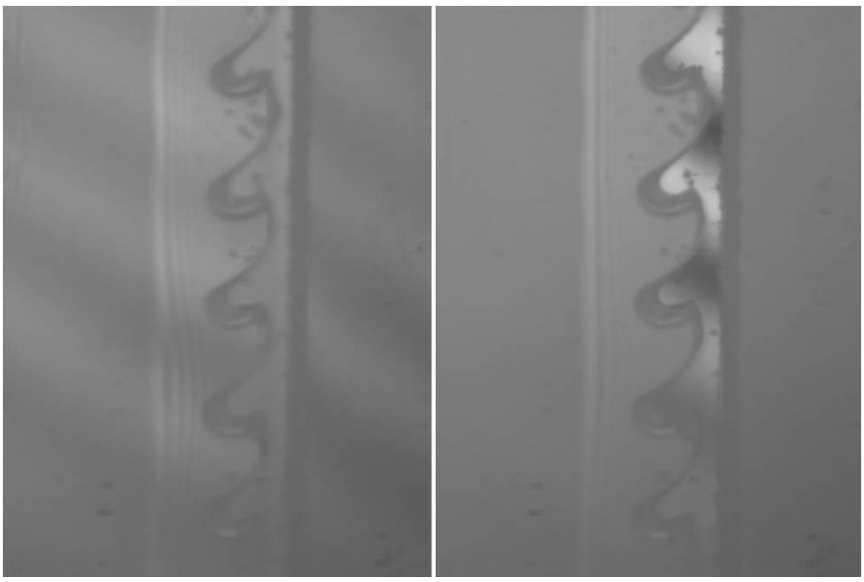

Figure 12 - Example of Microscope Image

Figure 12 is an example of how the piezoelectric controller and measuring device works. Using the light source, the exposed silicon portion of the wafer can be excited. This produces the band formation in the upper portion of the figure. Notice that the photoresist at the bottom of the figure shows no bands and is a continuous color. This machine provides a standard of error of 1 μm.

In addition to measuring the thickness of the photoresist prior to heating, additional measurements of the carbon surface were conducted after heating and plasma treatment to determine overall thickness lost. Table 3 displays the results. This thickness loss amounts to a nearly eighty-two percent loss in thickness as a result of the heating process. This thickness loss is similar to data previously determined (80.51%) (18). The oxygen plasma process removed nearly an additional sixty percent of the surface. The application of four coats of photoresist prevents the carbon surface from being disintegrated and completely removed during the plasma treatment. The loss of a significant thickness is consistent with oxygen plasma treatment (45).

Table 3 - Thickness Loss during Heating Process

	Photoresist	Carbon	Oxygen Plasma
Silicon Surface	136.2	153.6	100.1
Material Surface	125.7	151.8	99.34
Thickness (μm)	10.5	1.8	0.76

While thickness is an acceptable indicator of percent loss, in addition the mass of the wafer was analyzed prior and after the heating process, and once after all carbon had been removed from the wafer. This calculation of mass loss presents data that is of greater understanding to the surface composition. Table 4 displays how the mass of the surface varies throughout the process. Notice that the mass remaining is above thirty-five percent, while the thickness remaining is below twenty-five percent. The surface density of carbon is greater than that of the photoresist. This increased density of the carbon allows for the surface to increase in stability as well as the ability to treat the surface as a carbon substrate. These results signify the fact that the carbon produced is of a thickness with the ability to conduct biosensing via electrochemical methods.

Table 4 - Mass Loss Analysis

Wafer	Pre-Pyrolysis Mass	Post-Pyrolysis Mass	Mass Lost during Pyrolysis	Percent Mass Remaining	Mass of Silicon	Mass of Photoresist	Mass of Carbon	Percent Mass Loss	Percent Mass Remaining
A	0.6934	0.6877	0.0057	99.18	0.6826	0.0108	0.0051	52.8	47.2
B	0.6789	0.6741	0.0048	99.29	0.6712	0.0077	0.0029	62.3	37.7

4.4 Surface Energy Analysis

Contact angles are used to compare surface energy between materials. For this experiment, contact angles are qualitatively estimated as a means to understand adhesive properties of the carbon and oxygen plasma surfaces. Contact angles are estimated by placing a droplet on the surface and captured in a photograph. Water, ethanol, and toluene are used on bare silicon, silicon coated in S-1813, an ordinary thin carbon film and an oxygen plasma treated thin carbon film. For water, a 25 µL droplet is used, 12 µL for ethanol and 8 µL for toluene. The differences in surface tension between the fluids allows for larger droplets to be more stable on the surface, where a larger droplet eases the task of measuring the contact angle.

As may be seen in Figure 13, the contact angle of water is greatest on the plain carbon surface. Contact angles are less on the unmodified S1813 surface, followed by the silicon surface, while the oxygen plasma treated carbon surface has the lowest contact angle. A greater contact angle indicates a more hydrophobic surface, while a lower contact angle correlates to a hydrophilic surface. The bare carbon surface was not expected to be more hydrophobic than the untreated S1813 nor the silicon wafer. It was expected that unmodified S1813 would probably have the greatest contact angle because it is composed of aromatic polymer chains and it is cast in an organic solvent.

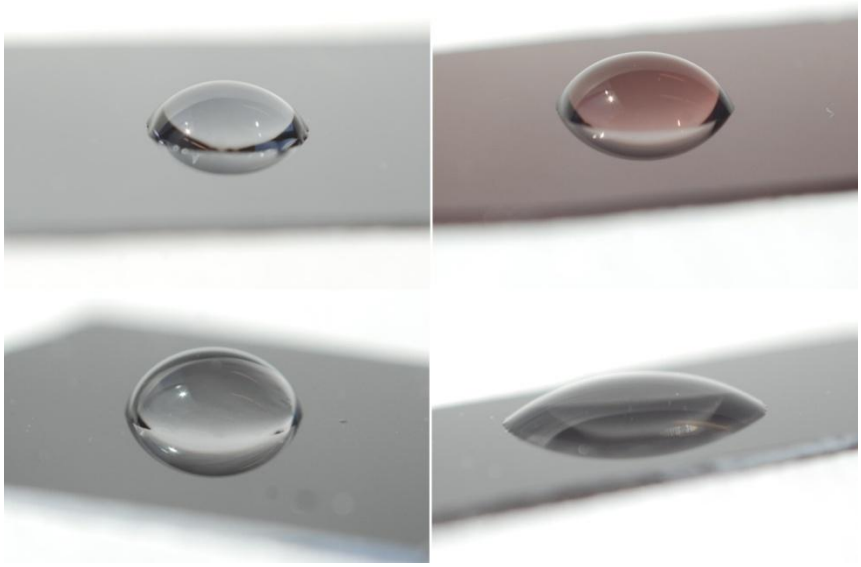

Figure 13 - Image of Droplet Contact with Surface Samples

Image of droplet contact with surface samples. Silicon (top left), untreated S1813 (top right), bare carbon (bottom left) and oxygen plasma treated carbon (bottom right) are pictured. Bare carbon clearly exhibits the largest contact angle, followed by S1813, silicon and oxygen plasma treated carbon in descending order. The larger contact angle indicates greater hydrophobic behavior, while a smaller contact angle indicates more hydrophilic character.

Angles are measured by drawing a tangent line and using a protractor to estimate the contact angle of the droplet. Surface energies are calculated with the use of Young's Equation, where the surface energy S is calculated as $S = \gamma_{LG}(\cos(\theta) + 1)$, where γ_{LG} is the contact energy between the liquid and the gas surroundings (or surface tension) and θ is the contact angle between the solid and fluid. The surface energy, S, is calculated in dynes/cm (or mN/m), forming an analogue to surface tension between the fluid and the solid phases, indicating an attractive force between the two substances. Using measured contact angles and a surface tension of 71.9 dyne/cm (based on a temperature of roughly 25°C in the laboratory), surface energies are measured. These values are used for relative comparisons of surface energy between samples. It is important to note that the method of measuring angles is accurate only to within about ±2 degrees, resulting in an potential error of about 5%.

The calculation of surface energy from measured contact angle is an analysis of the horizontal force between the droplet and the solid surface. Thus, the cosine of the measured

angle will be directly proportional to the horizontal force, and the surface tension at the fluid-vapor interface accounts for the droplet's inherent forces holding the droplet's shape.

Qualitative observations of droplet shape yield insight regarding the interaction between the fluid and the surface, and calculation of relative surface energy values confirms qualitative observations. Within the degree of accuracy of the measuring methods, silicon and unmodified S1813 are effectively equal, while bare carbon has a much higher energy and oxygen plasma etched carbon has a much lower energy.

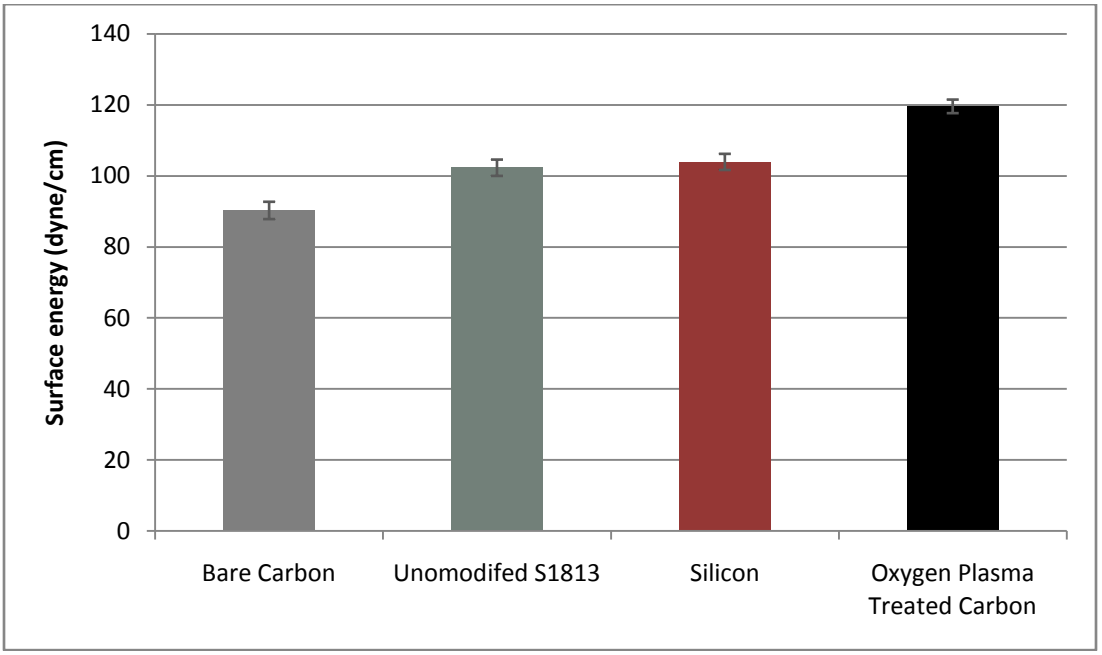

Figure 14 - Energy of interaction between a water droplet and surface samples

A greater surface energy correlates to a greater hydrophilic surface. Within the level of accuracy of measuring angles (±2) there is a 5% margin of error, meaning that there is no significant difference between silicon surfaces and unmodified S1813. Oxygen plasma treated carbon and bare carbon, however exhibit substantially different levels of surface-droplet interaction.

Surface energy has been demonstrated to have a profound effect on cell-surface interactions. A 2006 paper by Kennedy et al. demonstrated a relationship between surface energy and cell migration and proliferation. A surface of graded surface energies was coated in fibronectin and mouse fibroblasts cells were grown on the surface. It was observed that cells on hydrophobic regions had a higher rate of proliferation and a higher density per unit area.

Coating the surface in fibronectin and the choice of fibroblast cells (which adhere via a fibronectin mediated mechanism) prior to seeding cells permits this study to be used draw conclusions only about fibronectin-mediated cell adhesion and proliferation (46).

4.5 Attachment of ABA to the Surface

Prior to the addition of NHS and EDC to functionalize the carbon surface for ssDNA attachment and detection, the carbon surface must first be treated so that a free carboxylic acid is produced and available. This is accomplished through the use of ABA. Using a cyclic voltammetry technique, the electric current attaches the ABA to carbon surface with the carboxylic acid of the molecule oriented away from the surface.

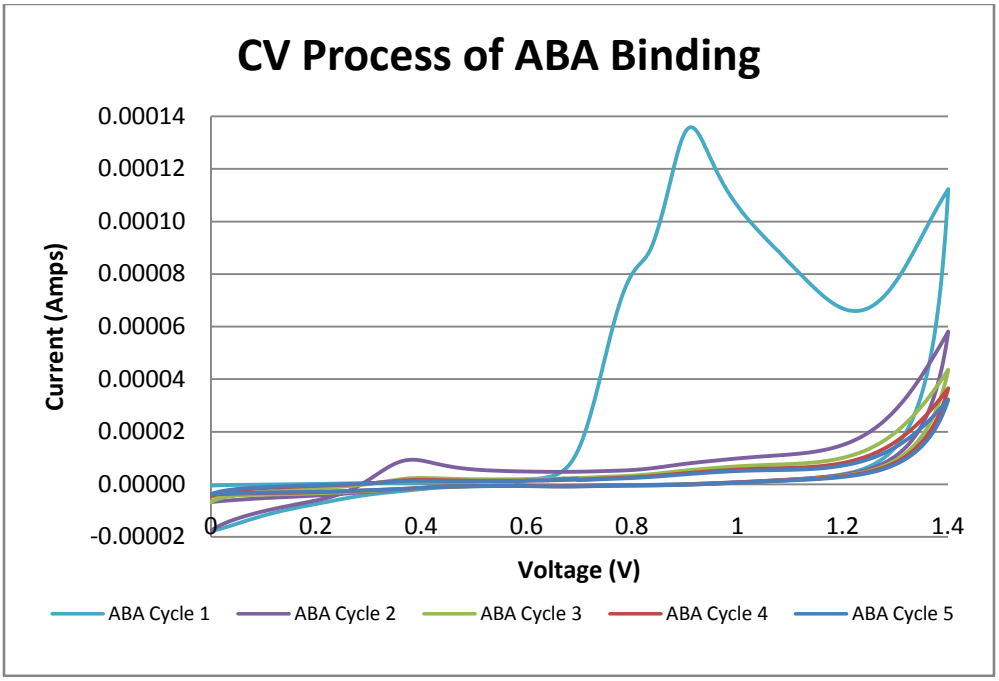

Figure 15 - Cyclic Voltammogram of ABA Treatment

The process of ABA attachment is conducted over four scans, depicted in Figure 15. This figure also shows how minimal the attachment of ABA to the surface changes between a four and five scan technique, as a result the scan number selected was four. Similar graphs of cyclic voltammetry treatment for ABA have been reported (38). This is supported by Figure 16

in which the EIS after every scan within the ABA treatment was conducted and recorded. Scan 4 is not shown in the diagram because it is nearly identical to scan 3. Notice that after 5 scans, the EIS begins to reduce. This plot corresponds to the removal of the ABA solution and the placement of the $K_3Fe(II/III)(CN)_6$ solution for EIS analysis. This would therefore increase the concentration of free ABA with each additional treatment. The increased concentration of ABA will drive the pH of the solution down, ultimately causing the ABA to disassociate. Additionally at a more acidic pH, ABA EIS results have tended to develop smaller radii, as supplemented by current research (38).

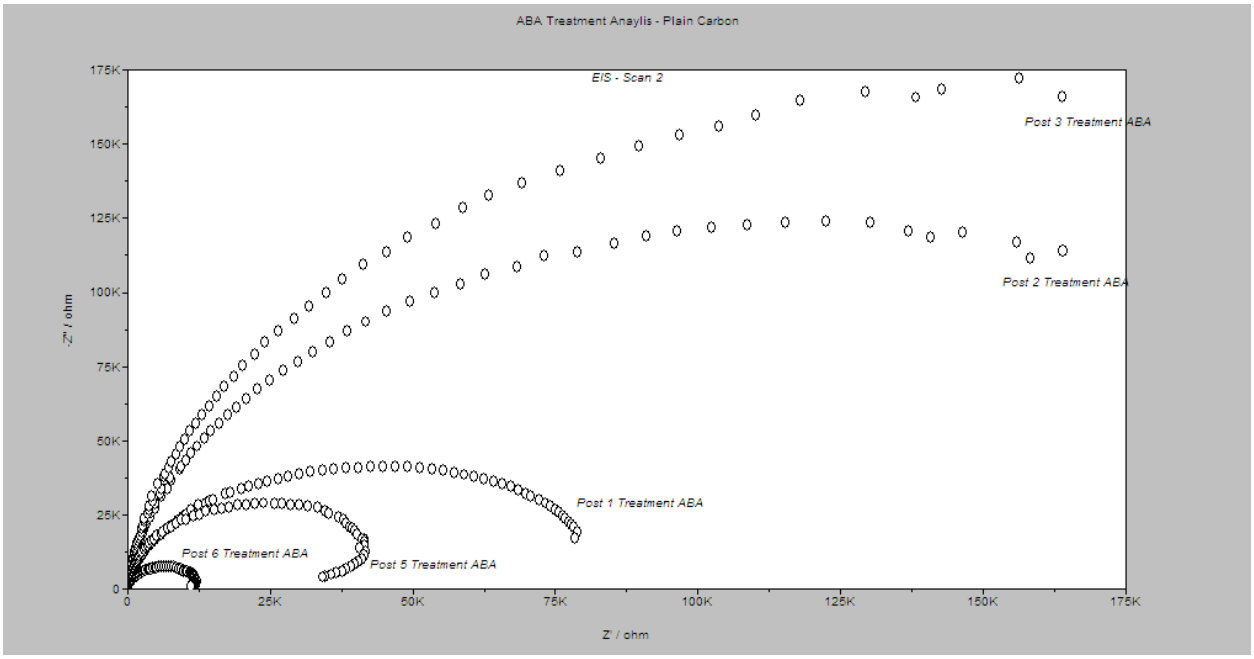

Figure 16 - ABA Treatment Electrochemical Impedance Spectroscopy Analysis

4.6 Alteration of the Carboxylic Acid using EDC and NHS

In order to convert the free carboxylic acid to an amine group for attachment of nitrogen capped ssDNA, the post-ABA treated surface was immersed in a 2mM EDC and 5mM NHS solution of phosphate buffer solution (pH 7.40). The results of alteration with EDC and NHS are shown in Figure 17. As the surface becomes more insulated through treatment with additional

chemicals, the EIS is predicted to increase. The modification with ABA, as shown in Figure 16 and Figure 17, performs as expected; however, the alteration conducted with EDC and NHS reduces the EIS. This is most likely a result of an unstable carbon surface. The EDC and NHS are able to access the carbon surface, and reacts with the carbon surface disassociating the ABA. The removal of material from the surface allows for the EIS to decrease because of a decrease in surface material. This would mean that the carbon produced is not well bound to the surface and that the ABA is ineffective at protecting the carbon surface from reactions. Additionally displayed in Figure 17 is the stability of ABA over time. After two days immersion in water, the ABA EIS does not alter to any great extent. This implies that in the absence of strong chemicals such as EDC and NHS and in a stable pH, such as 7.0 produced by water, the ABA remains bound and protects the surface.

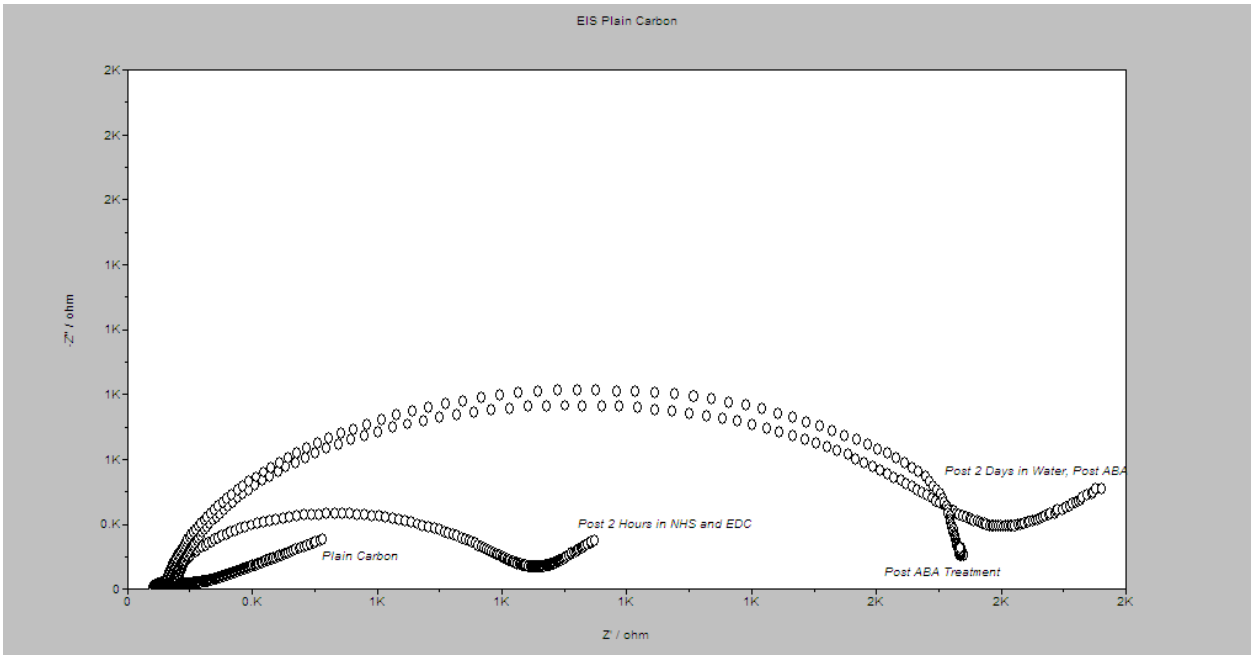

Figure 17 - Electrochemical Impedance Spectroscopes of NHS and EDC Treatment Process

4.7 Attachment of ssDNA Probe to the Carbon Surface

To determine if the NHS and EDC only removed carbon and ABA which was only weakly bound the surface and if what remained was extremely stable, ssDNA was attached to the surface. Figure 18 shows the EIS results. As with the NHS and EDC alterations, the EIS is reduced. The carbon surface produced through pyrolysis and the alteration with ABA ultimately is susceptible to disintegration and disassociation when treated with chemicals and biochemicals.

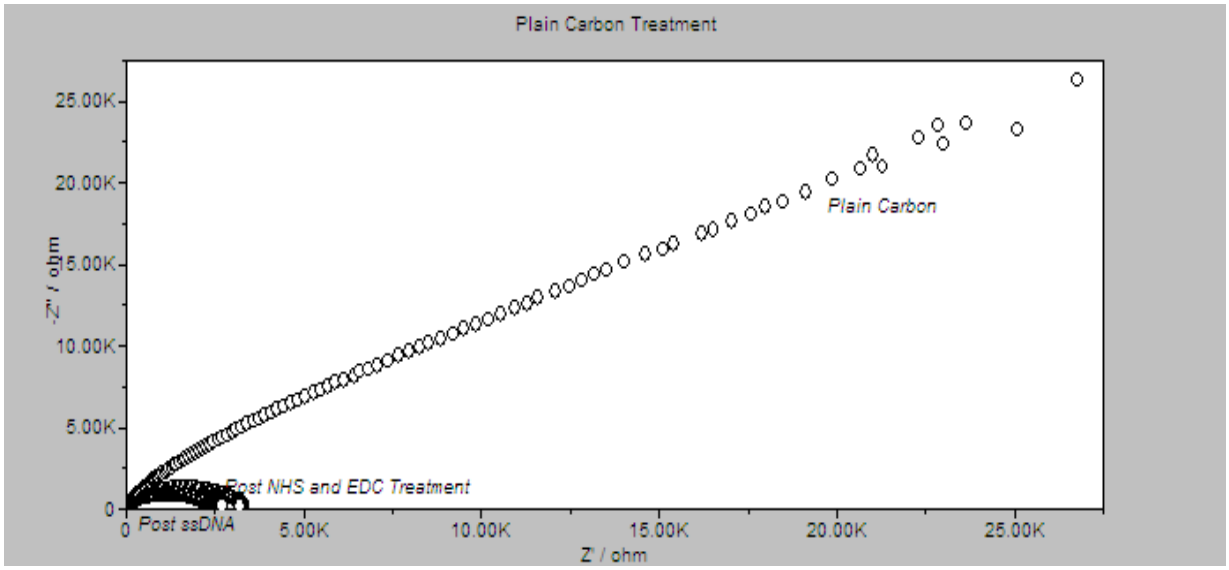

Figure 18 - Electrochemical Impedance Spectroscopy of ssDNA Attachment

4.8 Oxygen Plasma Treated Surface Analysis

In an attempt to quantify if the carbon itself was unstable or if the ABA was the material destroying the ability of the carbon to function as a biosensor, the ABA treatment process was replaced with an oxygen plasma treatment. Figure 19 shows the shift in the CV of the oxygen plasma treated surface and the glassy carbon reference. The oxygen plasma treated surface has an increased ability for electron transfer and exchange, therefore, the increase in amplitude and shift in the cathode and anode peaks are as expected for a surface of increased oxygen content. The increase in shift of the cathode peak is a result of an increased ability to oxidize materials, and the decrease in shift of the anode peak corresponds to a decreased ability to reduce materials.

When compared to the cyclic voltammogram of the photoresist derived carbon in Figure 11, the oxygen plasma produces a similar cyclic voltammogram with slight variations comparable with those of the glassy carbon reference.

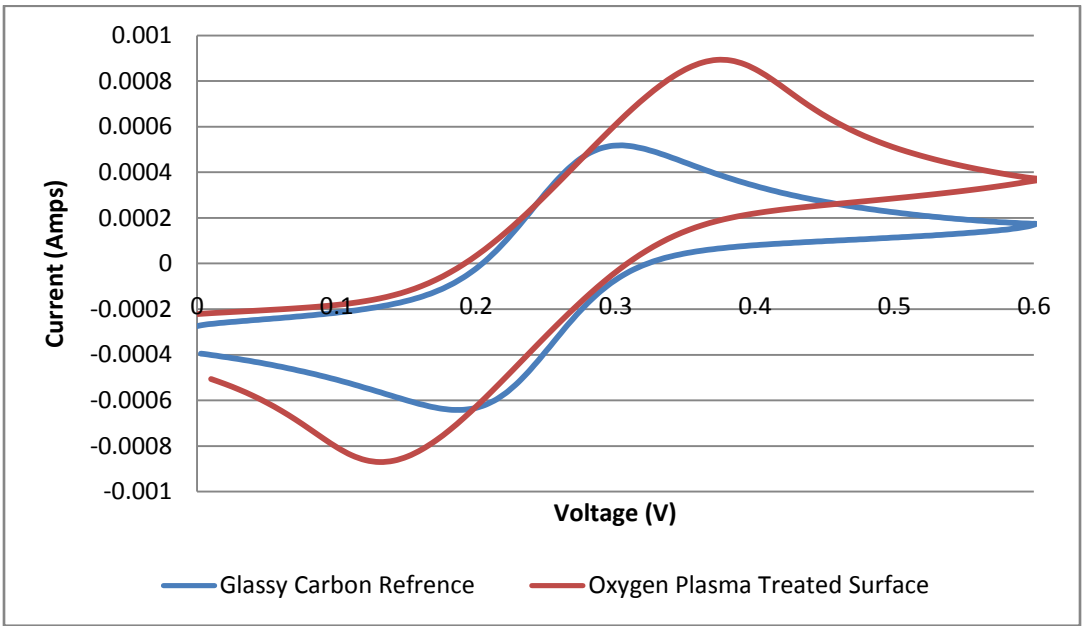

Figure 19 - Cyclic Voltammograms of Oxygen Plasma Treated and Glassy Carbon Surfaces

The ability of the oxygen plasma treatment to protect the carbon surface and produce a functional biosensor required the oxygen plasma treated surface to undergo the same alterations as the ABA treated surface. The electrochemical impedance spectrograms obtained for the plain surface, the surface after EDC and NHS alteration and the ssDNA attachment are shown in Figure 20. Unlike the ABA treated surface, the obtained EIS for each step follows the accepted and expected trend. The ABA treated surface produced a reduced EIS peak with the addition of the EDC / NHS solution and the ssDNA; however, the oxygen plasma treated surface produced increased EIS, and therefore increased impedance, with each addition. The three curves shown, display how the addition and alteration process slightly increases the overall resistance of the system present as a biosensor. The EIS obtained for the ssDNA is slightly increased than the post EDC and NHS EIS, which is itself increased from the plain oxygen plasma treated surface.

Ultimately the oxygen plasma treated electrode protects the photoresist derived carbon from disassociation and reaction with the chemicals added to the system.

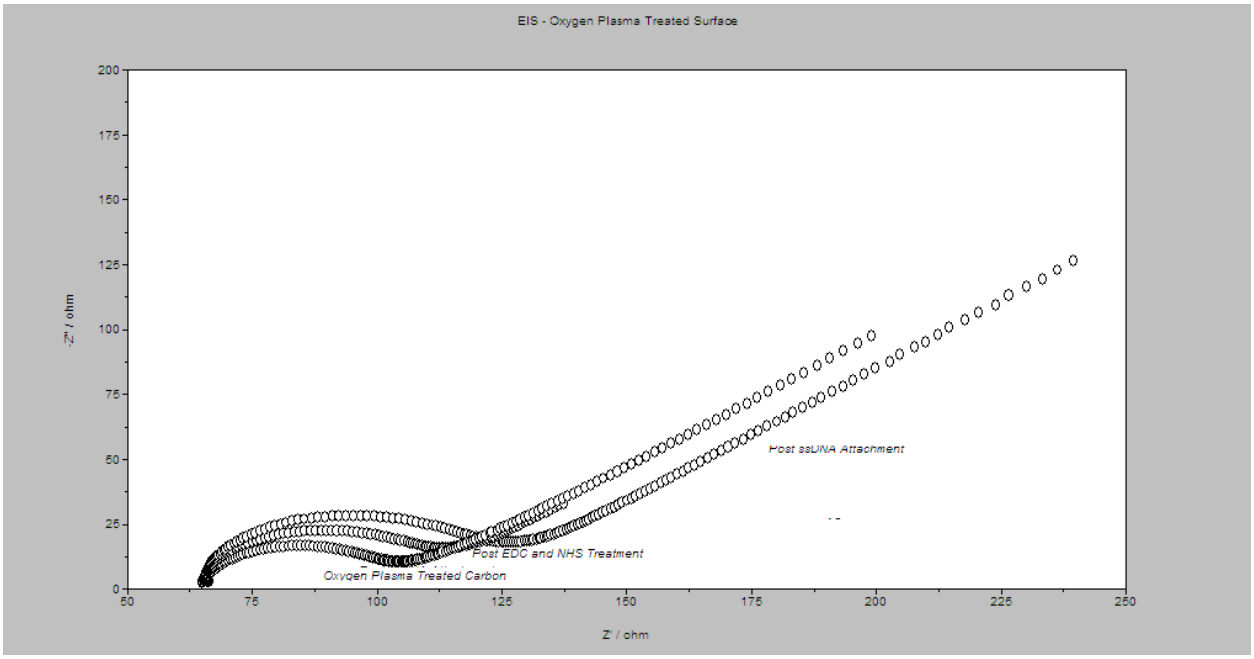

Figure 20 - Electrochemical Impedance Spectrograms for the Oxygen Plasma Treated Surface

5. Conclusion

The use of photoresist derived carbon has been proven to provide the ability for genetic mutation detection. The use of ssDNA as a probe was successful. The use of an oxygen plasma treated surface was required for the signal to be consistent with other sources. The oxygen plasma provided a firmly bound oxygen barrier for modification; the use of 4-Aminobenzoic acid (ABA) destroyed the carbon surface and provided weak bonds for ssDNA attachment. These weak bonds were removed during treatment with 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (NHS). Further treatment with ssDNA on the ABA surface again removed more surface material. The oxygen plasma treated surface provided a standard and acceptable method of detection through consistent and comparable data to those who have done electrochemical impedance spectroscopy with other materials for genetic testing.

In addition to determining the ability for photoresist derived carbon to function as a means for genetic experimentation, the spin coating process provided the ability to examine the multiple-layered ability of photoresist. The constant speed (3000 rpm) application of four layers of S-1813 provided a constant thickness both after spinning and post-pyrolysis. The edge effects from the constant speed application were less pronounced than those of the ramped application.

The surface chemistry and physical characteristics of all of the various types of surface were explored through surface tension methods, cyclic voltammetry, mass loss, and thickness loss. The mass loss was significant, but not as significant as that of the thickness. The carbon produced is of a dense nature. The carbon is confirmed by the use of cyclic voltammetry and comparison to a glassy carbon reference. Surface tension analysis proved the hydrophilic nature

of the oxygen plasma treated surfaces and the hydrophobic nature of the plain carbon, photoresist, and silicon surfaces.

The overall process proved the ability to use a simply derived photoresist carbon possesses the ability to function as a genetic assay through the use of an oxygen plasma treatment. The use of chemicals to alter the carbon surface tends to occur in reduction and removal of the carbon on the surface.

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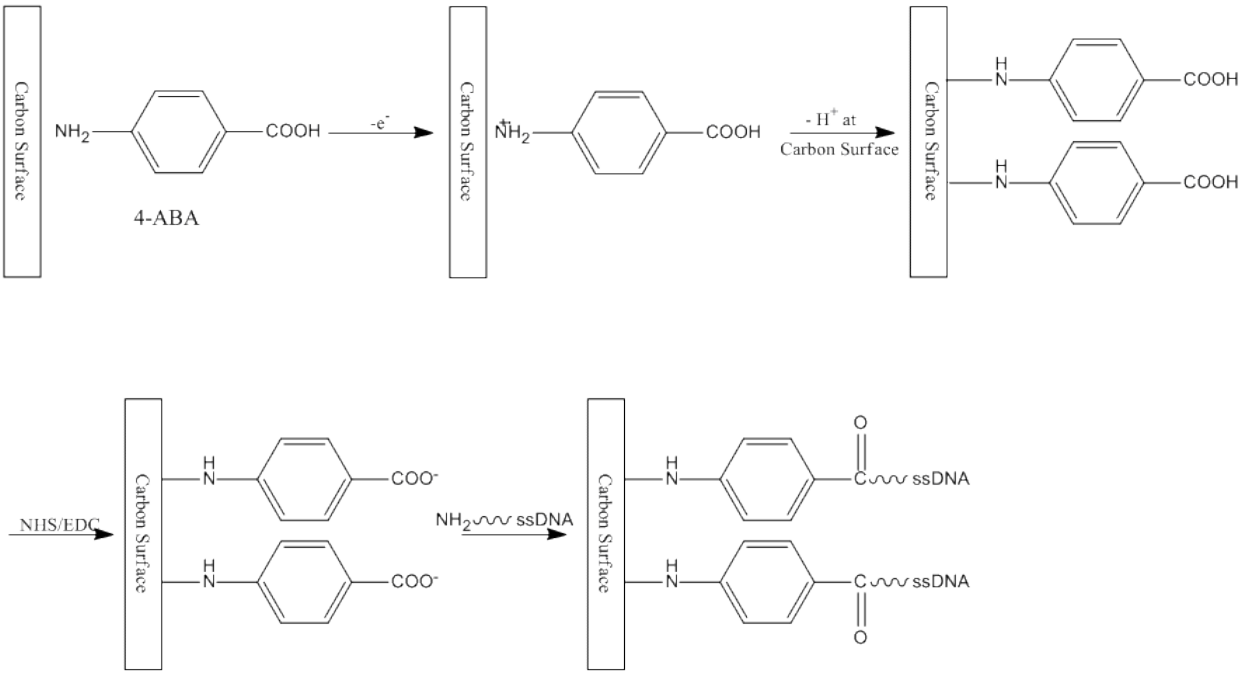
Appendices

Appendix A: Table of Codons and Amino Acids

First Position	Second Position								Third Position
	T		C		A		G		
T	TTT	Phenylalanine	TCT	Serine	TAT	Tyrosine	TGT	Cysteine	T
	TTC		TCC		TAC		TGC		C
	TTA	Leucine	TCA		TAA	STOP	TGA	STOP	A
	TTG		TCG		TAG		TGG	Tryptophan	G
C	CTT	Leucine	CCT	Proline	CAT	Histidine	CGT	Arginine	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	Glutamine	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	Isoleucine	ACT	Threonine	AAT	Asparagine	AGT	Serine	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	Lysine	AGA	Arginine	A
	ATG	Methionine	ACG		AAG		AGG		G
G	GTT	Valine	GCT	Alanine	GAT	Aspartic Acid	GGT	Glycine	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	Glutamic Acid	GGA		A
	GTG		GCG		GAG		GGG		G
Key	Nonpolar		Basic		Acidic		Polar		

Appendix B: 4-ABA Process of Carbon Surface Modification Schematic



Appendix C: Pyrolysis Process Determination

Table 5 - Pyrolysis Results Summary

Procedure Number	Date	Photoresist	Procedure	Results
1	November 29, 2007	SU-8	0 → 600°C @ 2°C/min Hold 1 hr Cool to Room Temperature	● Not pyrolized ● Not stable in water – forms a gel in water
2	December 6, 2007	SU-8	0 → 300°C @ 10°C/min 300 → 900°C @ 10°C/min Cool to Room Temperature	● Little scratching near edges – probably from cutting. ● No obvious splotching or pealing ● Stable in water
3	December 6, 2007	SU-8	0 → 300°C @ 10°C/min Hold 40 min 300 → 900°C @ 10°C/min Hold 1 hr Cool to Room Temperature	● No Visible Flaws ● Some scratching in storage
4	February 20, 2008	SU-8	0 → 600°C @ 2°C/min Hold 1 hr Cool to Room Temperature	● Spotty ● Not stable in water – forms a get in water
5	March 20, 2008	SU-8	0 → 800°C @ 3°C/min Hold 1 hr Cool to Room Temperature	● Peeling when placed in cloth (Damaged in storage)
6	April 8, 2008	SU-8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 800°C @ 10°C/min Hold 1 hr Cool to Room Temperature	● Some peeling on edges, otherwise appears very good
7	April 17, 2008	SU-8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 800°C @ 10°C/min Hold 1 hr Cool to Room Temperature	● 1 small area of peeling – appears cutting
8	June 25, 2008	SU-8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 900°C @ 10°C/min Wait 1 hr Cool to Room Temperature	● Perfect Film ● No visible flaws

9	July 10, 2008	SU-8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 900°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• Stable Carbon • Good adherent layer
10	August 4, 2008	S-1813	0 → 300°C @ 2°C/min Hold 1 hr 300 → 900°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• No problems • Very stable
11	September 4, 2008	S-1813	0 → 300°C @ 2°C/min Hold 1 hr 300 → 800°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• Appears consistent • Some spotting and scratching, more pronounced along an edge • Shiny surface
12	September 11, 2008	S-1813	0 → 300°C @ 2°C/min Hold 1 hr 300 → 1000°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• Very consistent • Reflective • No obvious silicon viewable • Some areas darker than others
13	September 13, 2008	S-1813	0 → 300°C @ 2°C/min 300 → 1000°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• Some edge effects on two of the samples, probably cutting • Consistent • Reflective
14	September 17, 2008	S-1813	0 → 300°C @ 2°C/min 300 → 1000°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• 45/45 Very unstable, significant cracking • Also unstable in water • Film cast – Peeling, very loose structure
15	September 18, 2008	New SU-8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 1000°C @ 10°C/min Cool to Room Temperature	• Inconsistent surface between wafers • Bad edge effects – disintegration along some corners • Looks suitable otherwise
16	November 4, 2008	New S-U8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 1000°C @ 10°C/min Hold 1 hr Cool to Room Temperature	• Significant cracking and peeling • Not stable in water • Bubbling up at points

17	November 6, 2008	New SU-8	0 → 300°C @ 2°C/min Hold 1 hr 300 → 1000°C @ 10°C/min Cool to Room Temperature	• Unstable in water • Some odd line formations on surface
18	November 12, 2008	New SU-8	0 → 800°C @ 2°C/min 800 → 25°C @ 2°C/min	• Some edge from cutting • Very unstable in water and when CV is performed
19	November 13, 2008	New SU-8	0 → 800°C @ 2°C/min Cool to Room Temperature	• Bad cracking • Flow removal • Bubbling up
20	December 11, 2008	S-1813	0 → 300°C @ 2°C/min 300 → 1000°C @ 10°C/min Cool to Room Temperature	• Peeling in water after significant time in water • Striations on surface – appear to be from spinning process • Otherwise very stable carbon • Bubbled areas are easily removed
21	December 14, 2008	New SU-8	0 → 300°C @ 10°C/min 300 → 1000°C @ 10°C/min Cool to Room Temperature	• Significant cracking and peeling
22	December 16, 2008	S-1813	0 → 300°C @ 2°C/min 300 → 1000°C @ 10°C/min Cool to Room Temperature	• Cracking in middle of one wafer; however, rest appear stable • One has small bubbling

Appendix D: Detailed Images of Pyrolysis Results

The pyrolysis methods result in several distinct problems, each unique to a certain variable in the pyrolysis process. The following images are to serve as a guide to the types of problems discussed in pyrolysis results (Section 4.1) and the pyrolysis summary table (Appendix C).

Figure 21 - Wafer Displaying a "Cracking" Result

Figure 22 - Wafer Displaying a "Peeling" Result

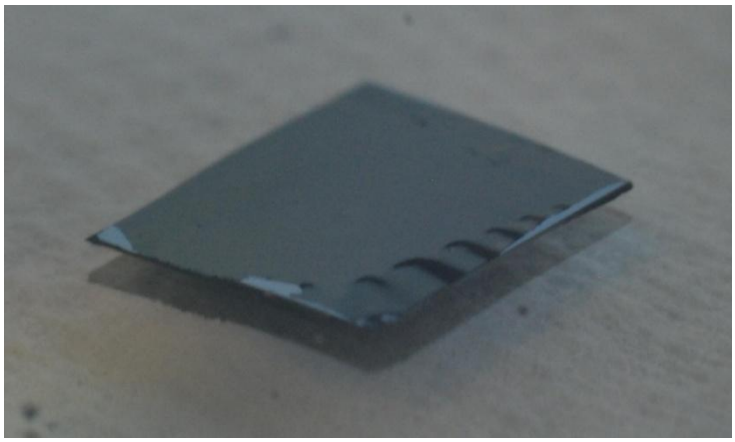


Figure 23- Wafer Displaying "Edge Effect" Result

Figure 24 - An Example of an Obliterated Wafer

Figure 25 - An Example of Electrically Unstable Carbon Wafer

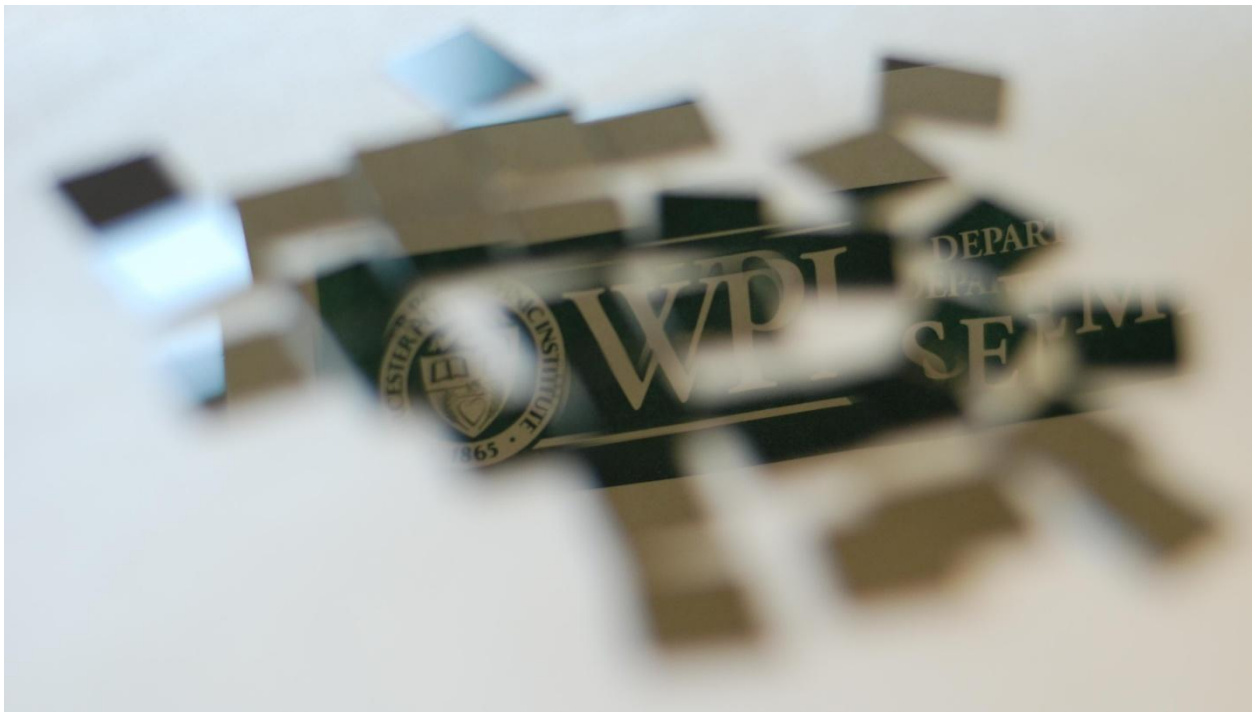


Figure 26 - Image Displaying the Reflective Nature of the Photoresist Derived Stable Carbon

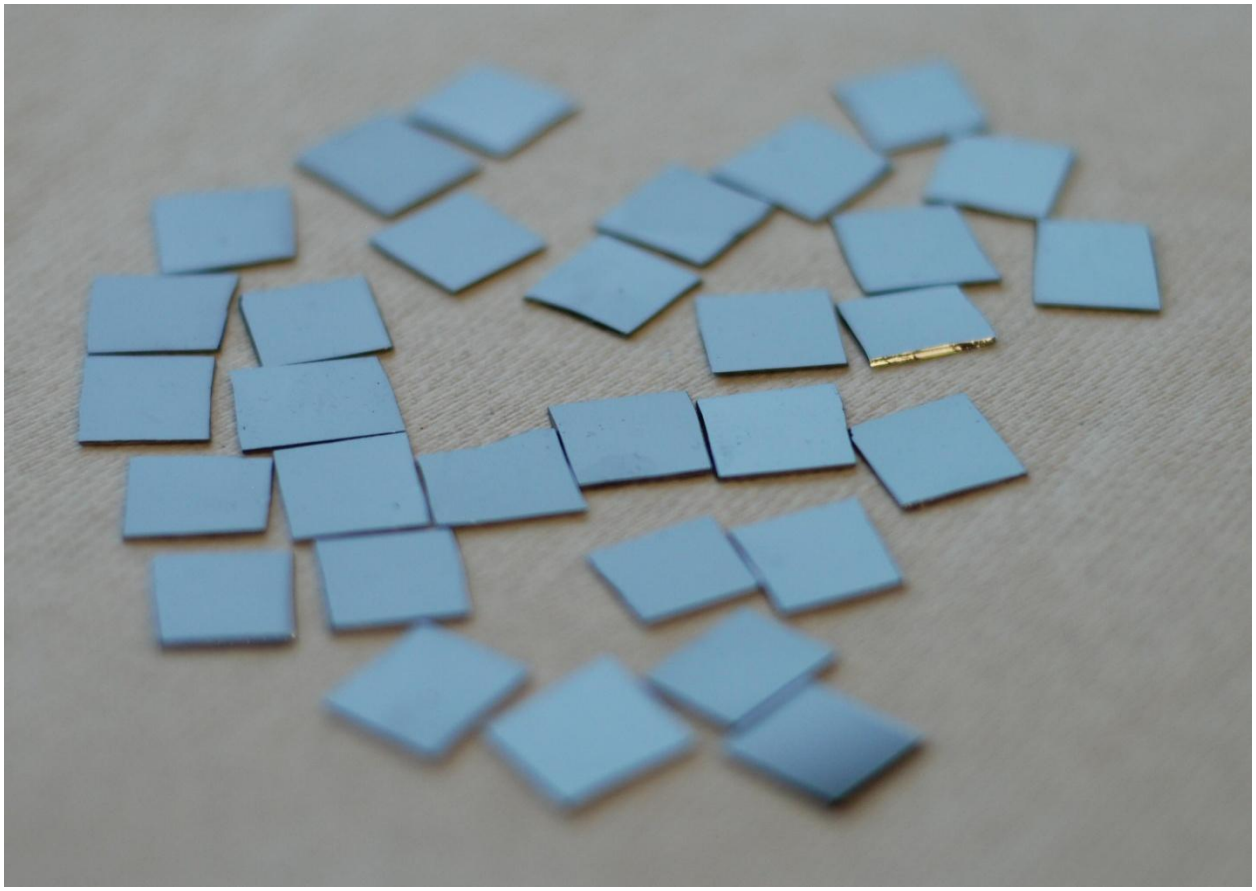


Figure 27 - Example of Stable Carbon Derived via Pyrolysis

Appendix E: Detailed Clean Room Setup

Prior to being able to spin coat within the clean room, some basic procedures need to be observed for functionality. Initially, the nitrogen gas, water, and vacuum supplies must be opened and initiated. On the side of the clean room, a small inlet is present in which the ddH_2O pump, the vacuum, and the nitrogen tanks are kept (Figure 28).

Figure 28 - Clean Room Supply Inlet

First, the water supply should be turned on and opened. The pump's power supply is disconnected after every use to prevent overheating. To begin, plug the pump in (behind the nitrogen tanks), followed by opening the water supply and turning the power switch on the pump to "on." All water pump and water supply areas that need adjusting are displayed in Figure 29.

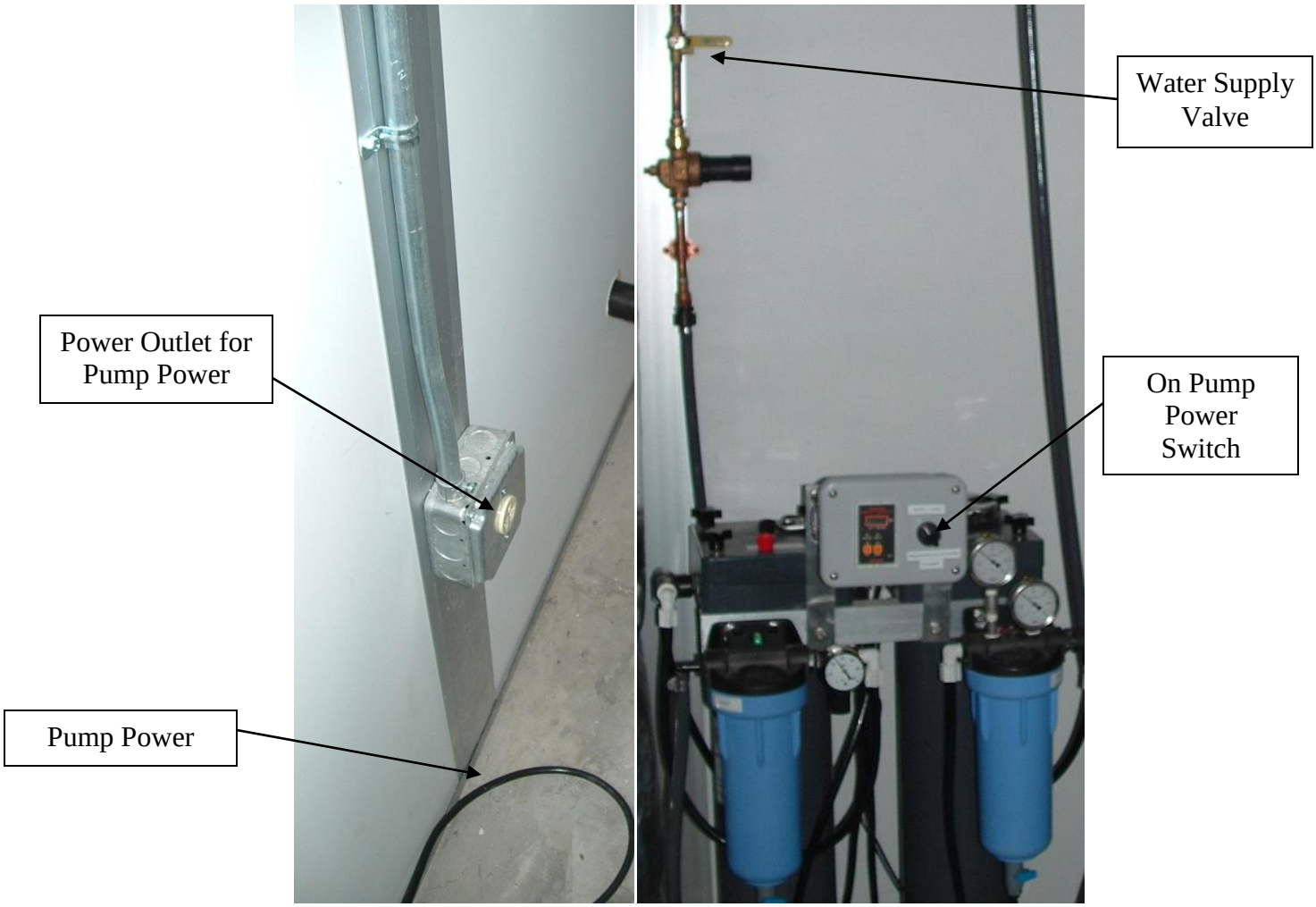


Figure 29 - Water Supply and Pump Points of Interest

The nitrogen gas supply and the vacuum are easily activated. The vacuum uses a simple switch and the nitrogen gas requires two valves to be opened fully. All of these activators are displayed in Figure 30.

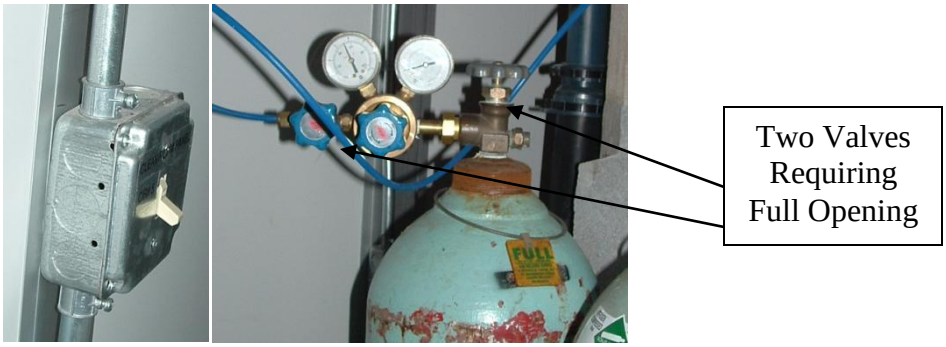


Figure 30 - Vacuum Switch and Nitrogen Gas Valves

After suiting up in the clean suits and entering the clean room, several machines and valves need to be immediately started so that spin coating can run smoothly and cleanly. First the supplies of water, nitrogen, and the vacuum must be opened to the hood and the spin coater. The supplies to the hood are located at the far left behind the hood and the supplies to the spin coater are located directly above the machine. All valves need to be opened for the equipment to function (Figure 31).

Figure 31 - Hood Supply Lines (Left) and Spin Coater Supply Lines (Right)

Next, the water within the hood should be opened and allowed to flow for ten minutes before being used. Additionally the oven should be turned on so that the operating temperature of 110°C can be reached. Figure 32 shows the oven's power switch and control panel.

Figure 32 - Oven Control Panel

At this time the air analyzer within the clean room can be started to determine the quality of air content within the room. To do this, the air collector on the top of the machine must be

opened and the cone must be inserted into the opening. Additionally the green power button followed by the blue arrow must be pressed for the analyzer to begin its process.

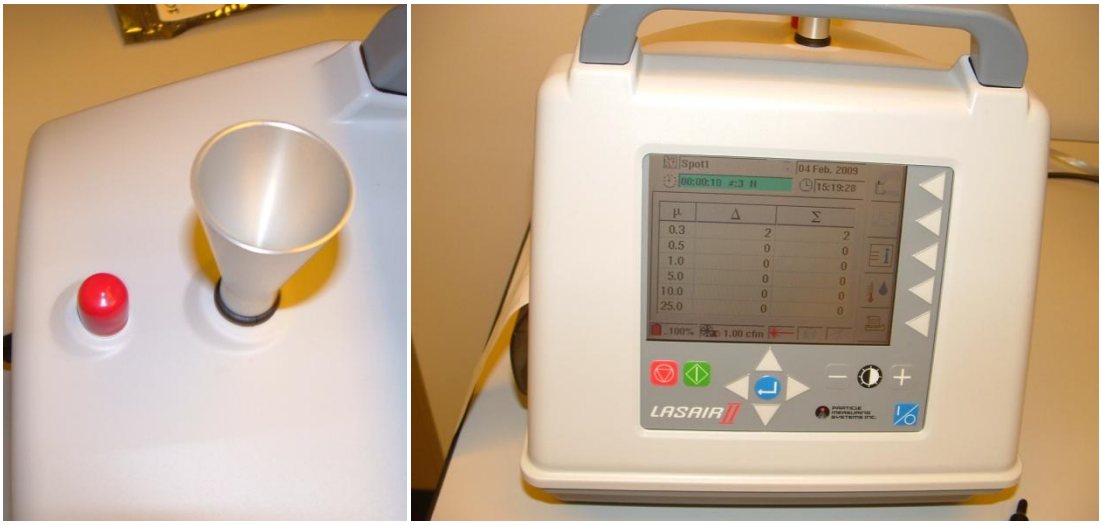


Figure 33 - Clean Room Air Analyzer

Finally the methanol and acetone must be set up for the spin coating process. This requires methanol to be placed in the sonicator within the hood, acetone be placed in a petri dish, and a Petri dish to be placed under the water flow in the hood sink. Figure 34 displays the approximate amount to put in each of the containers.

Figure 34 - Spin Coating Fluid Setup.

The far left is the methanol within the sonicator. The middle picture contains acetone within a large Petri dish. Enough of both materials should be placed into the containers so that a wafer is guaranteed to be covered. Finally, the right picture displays the water flow rate and the placement of the Petri dish under the water.

Appendix F: Counts Obtained for Air Quality within the Clean Room

8/14/2008				
Particle Size	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	1264	1306	3532	459
0.5µ	685	641	1766	141
1.0µ	261	151	459	71
5.0µ	71	50	106	0
10.0µ	49	40	106	0
25.0µ	7	16	35	0

12/10/2008				
Particle Size	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	530	261	742	212
0.5µ	297	170	494	106
1.0µ	141	114	283	35
5.0µ	28	16	35	0
10.0µ	14	19	35	0
25.0µ	0	0	0	0

9/23/2008				
Particle Size	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	261	201	565	35
0.5µ	170	104	318	35
1.0µ	85	59	177	35
5.0µ	35	35	71	0
10.0µ	21	32	71	0
25.0µ	7	16	35	0

1/12/2009				
	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	8434	3064	12291	4732
0.5µ	615	230	954	388
1.0µ	106	79	212	0
5.0µ	21	32	71	0
10.0µ	7	16	35	0
25.0µ	0	0	0	0

9/24/2008				
Particle Size	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	650	1010	2437	0
0.5µ	148	199	494	0
1.0µ	49	54	106	0
5.0µ	7	16	35	0
10.0µ	7	16	35	0
25.0µ	0	0	0	0

1/30/2009				
	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	247	251	600	35
0.5µ	127	159	318	0
1.0µ	71	100	212	0
5.0µ	28	46	106	0
10.0µ	14	32	71	0
25.0µ	0	0	0	0

9/25/2008				
Particle Size	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	388	185	671	212
0.5µ	155	81	247	71
1.0µ	64	30	106	35
5.0µ	7	16	35	0
10.0µ	0	0	0	0
25.0µ	0	0	0	0

2/2/2009				
	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	318	556	1307	0
0.5µ	170	321	742	0
1.0µ	92	205	459	0
5.0µ	14	32	71	0
10.0µ	14	32	71	0
25.0µ	0	0	0	0

12/10/2008				
Particle Size	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	134	58	212	71
0.5µ	92	40	141	35
1.0µ	71	43	141	35
5.0µ	0	0	0	0
10.0µ	0	0	0	0
25.0µ	0	0	0	0

2/4/2009				
	Mean (N/cm)	SD (N/cm)	Max (M/cm)	Min (N/cm)
0.3µ	205	116	388	106
0.5µ	64	72	177	0
1.0µ	49	47	106	0
5.0µ	35	43	106	0
10.0µ	14	19	35	0
25.0µ	0	0	0	0

Appendix G: Detailed Spin Coating Process

The spin coating process begins with removing the silicon wafer from the storage container and rinsing the wafer in an acetone bath for five minutes. After being rinsed in acetone, the wafer is sonicated in methanol for an additional five minutes. These two processes are conducted to ensure the remove of all contaminates on the wafer surface. Following the sonication, the wafer is blown dry with nitrogen gas and placed in a flowing water bath for another five minutes.

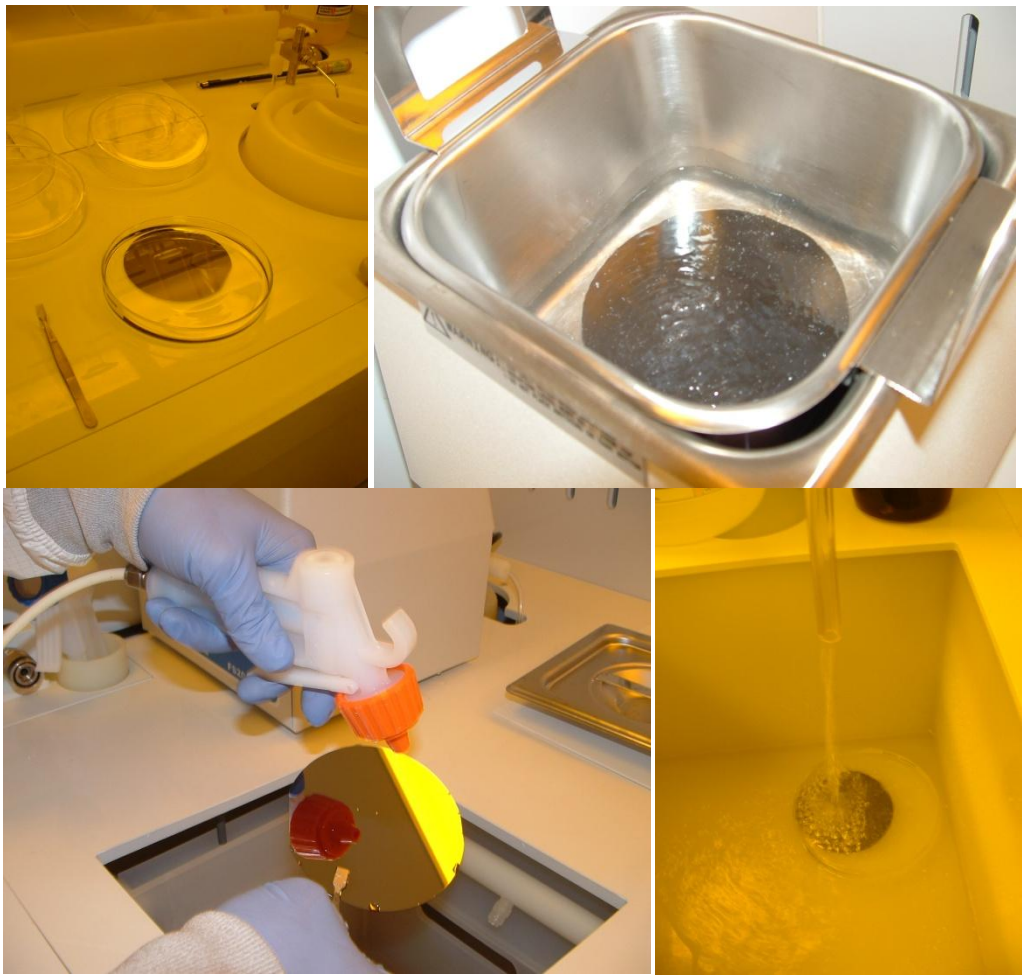


Figure 35 - Spin Coating Liquid Cleaning Treatment

Upper left depicts the acetone bath. Upper right is the sonicator with methanol as the fluid for cleaning the wafer. The bottom left is the method for cleaning all cleaning fluids off of the wafer before being placed in the water bath (bottom right). All liquid treatments are conducted for five minute intervals.

After being cleaned in the water bath, the wafer is first blown dry with the nitrogen gas as after the acetone and methanol treatments. Additionally, the wafer is dried in the 110°C oven for one minute to complete remove all fluids from the wafer surface. The goal of the cleaning process is to ensure a smooth, contaminate-free surface for photoresist attachment. After removing the wafer from the oven, it is placed on a cooling rack for one minute to ensure that the photoresist does not begin to be heated during the spin coating process.

First begin by supplying power to the spin coater via the power switch (Figure 39). Prior to beginning spin coating, the rotations per minute has to be set along with the spinning time. To adjust the RPM, under the "SPIN" heading there is a circular dial, this must be slightly pressed inward and turned in a clockwise direction to increase the RPM. The time for total spincoating is shown below the RPM adjustment knob, and is in seconds.

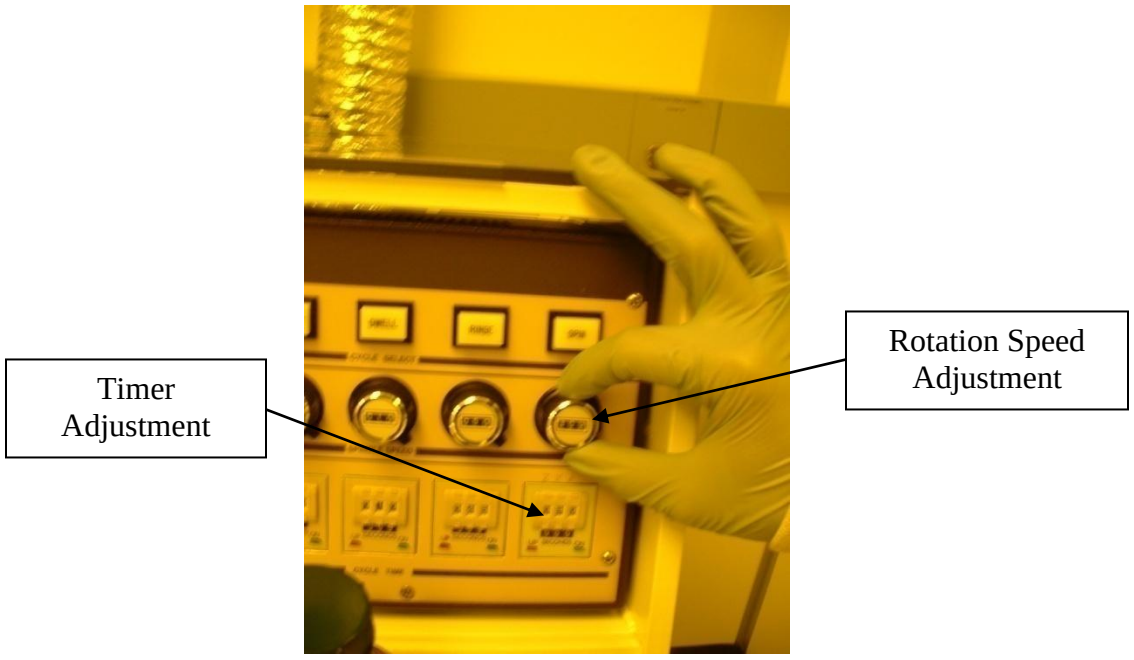


Figure 36 - Spin Coater Rotation Setup Control Panel

Now the actual spin coating process can be completed. First, the vacuum supply to the spin coater must be turned off, otherwise positioning the wafer will be impossible. This is done

by turning the vacuum valve above the spin coater (shown in Figure 31) to off. Next, using the right angled wafer placement bar (Figure 37), the wafer is centered on the spin coater and the vacuum supply is restored. In order to center the wafer you must not use the side which is partially linear, only circular edges should be placed against the right angled placement bar.

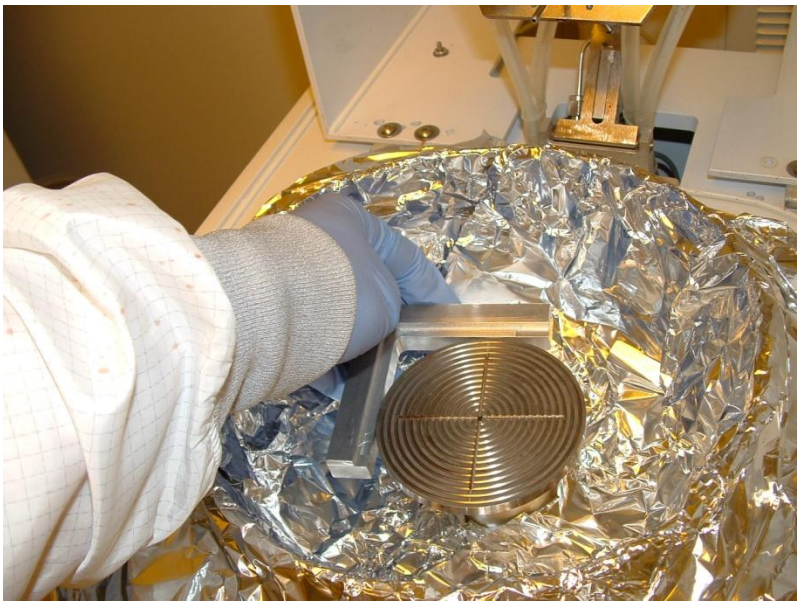


Figure 37 - Placement of the Right Angled Wafer Positioning Bar

After the wafer has been placed on the spin coater, the photoresist can be pipetted onto the surface. This is done using two pipettes almost entirely filled with photoresist (Figure 38). Both are emptied simultaneously in the center of the wafer and the spin coater is activated immediately after the photoresist is completely emptied onto the surface.

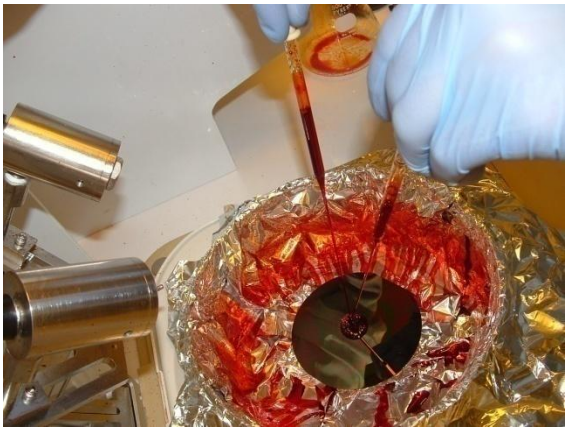


Figure 38 - Spin Coating Photoresist Application Technique

The spin coater is activated by hitting the "START" button on the control consol. The spin coater will follow the predetermined and entered procedure and will automatically stop. After the spin coater has stopped and the applicator head has risen, remove the vacuum supply to the spin coater.

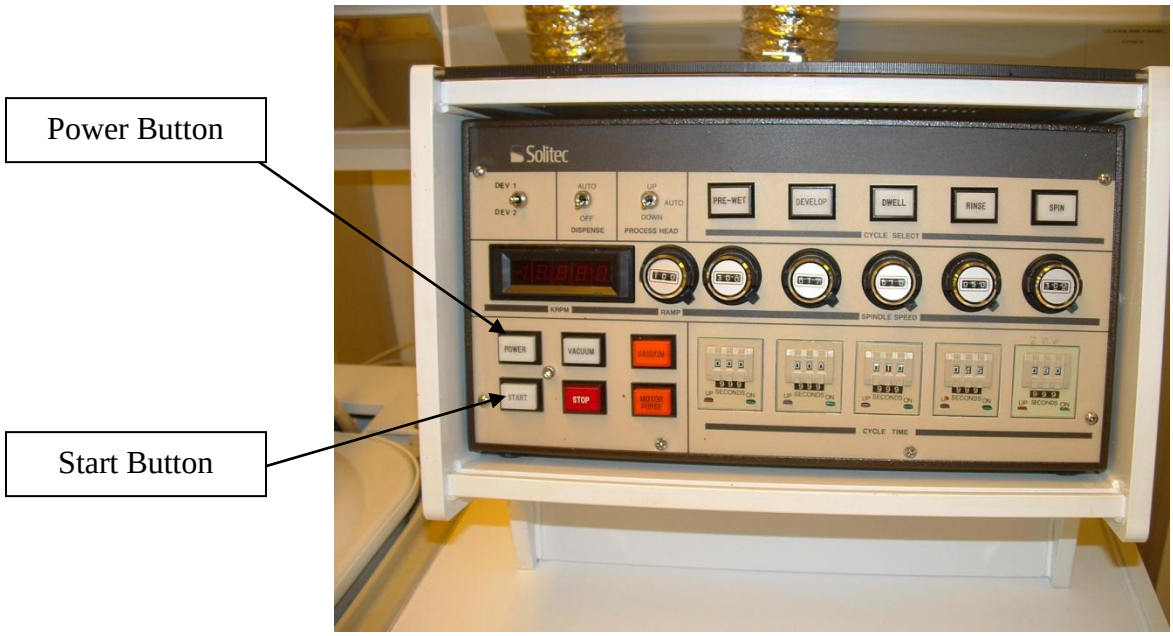


Figure 39 - Spin Coater Consol

Transfer the wafer to the oven for three minutes of heating. Again cool to room temperature, and apply the photoresist as necessary following the same application, heating, and cool procedures described above.

Appendix H: Detailed Pyrolysis Process

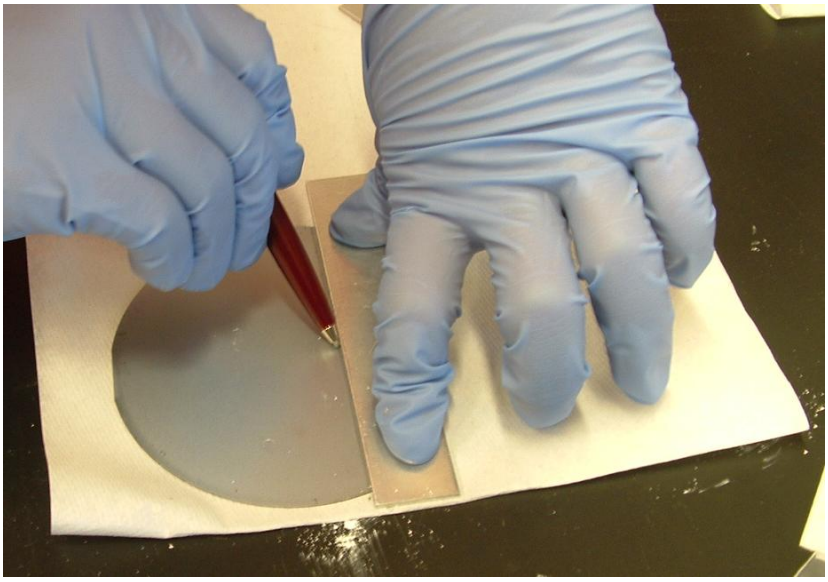


Figure 40 - Wafer Cutting Procedure

First, the wafers are cut to the correct size. For cell growth, the wafer is cut into 22mm by 22mm squares, and into 11mm by 22mm rectangles for electrochemical studies. The wafer is positioned on a paper cloth with the photoresist side facing downward and the wafer is scored with a diamond-tipped pen. An aluminum cutting guide (Figure 40) is aligned with the larger flat cut on the wafer's edge and the wafer is scored from end to end. After scoring, the wafer is broken along the scored line and the process is repeated to yield a number of small chips of the desired size.

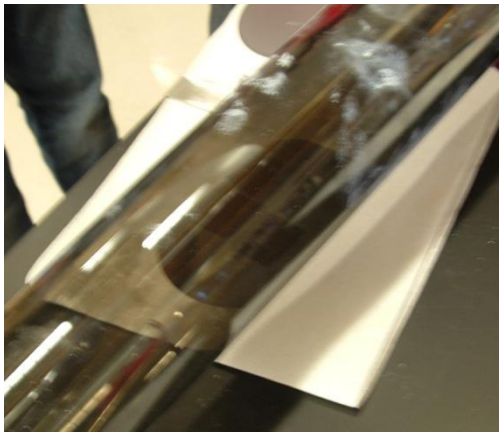


Figure 41 - Wafer Tray Placement

Wafer pieces are placed on a silicon sled and are slid into the open end of the fused quartz furnace tube. The sled is placed slightly past half way in the tube to place it close to center of the heating elements where the most consistent heat is and to place it far from the gas inlet to be sure that the nitrogen reaches the system temperature before being flowed over the wafer pieces (Figure 41).

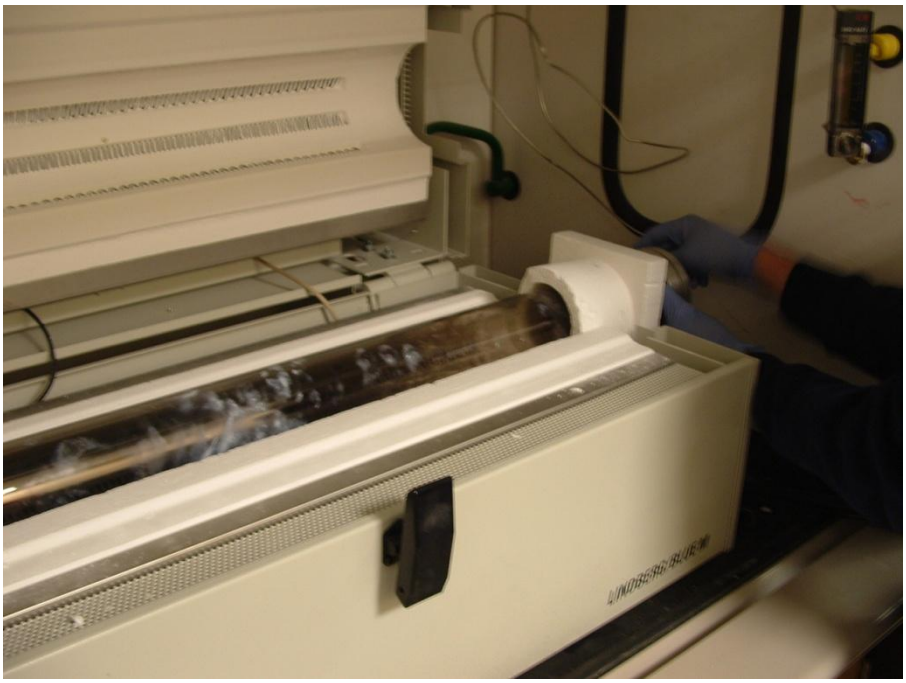


Figure 42 - Tube Placement in the Furnace

The tube is placed in the furnace as shown and the end is capped with the cover attached to the nitrogen gas line (Figure 42). The furnace is closed and the nitrogen is turned on.

Figure 43 - Nitrogen Supply Valves

Nitrogen is then flowed through the furnace tube for 10 minutes to evacuate all oxygen. Valves on the nitrogen tank and regulator are opened (Figure 43 top left) and the valve on the distribution line is opened all the way (Figure 43 top right). The blue valve on the hood (Figure 43 bottom left) is adjusted so that the flow rate indicated by the flow meter (Figure 43 bottom right) reads 100 sccm. After at least 10 minutes of flow, the furnace is turned on and the heat process begins.

Figure 44 - Tube Furnace Control

The furnace controller is turned on with the switch on the left of the machine's front side (Figure 44 left). The target temperature is set by simply pressing the arrow keys on the control unit (Figure 44 right). For this procedure, the maximum temperature is set to 1000°C. For the two step heat procedure, the initial heat rate must be set to 2°C/minute. This is done by holding down the blue button for 5 seconds until the options display appears and then pressing the blue button to cycle through the options until LoC (level of control) is displayed (Figure 45 left). Using the arrow keys, set LoC to -1 (Figure 45 left) and then press the blue button repeatedly to cycle through the options until UPr (up rate) is reached. The arrow keys are used to adjust the heat rate to the desired value. For the initial heat rate, UPr will be set to 2 (Figure 45 center). The blue button is then held down to return to the normal display screen (Figure 44 right). Once the furnace reaches 300°C (displayed in the red letters on the display, shown as 21 in Figure 44 right), UPr is then changed to 10 (Figure 45 right).

Figure 45 - Tube Furnace Adjustments

Once the furnace reaches 1000°C, the controller is turned off by flipping the switch used previously to turn it on and is left to cool for several hours with the gas still flowing. Once the furnace has reached room temperature (which may be confirmed by turning the furnace on for a brief period and observing the temperature reading), the tube may be removed from the furnace and the samples removed.

Appendix I: Electrochemical Cell Assembly and Preparation

To prepare the electrochemical cell for the cyclic voltammetry and the electrochemical impedance spectroscopy, the cell must first be cleaned. The cell, including the connection screws and the copper working electrode supply, are sonicated for ten minute intervals of water, ethanol, and water once more. This is done in a 250 mL beaker. The cell then is allowed to air dry on a paper towel until all moisture is free of the surface.

Figure 46 - All Aspects of the Electrochemical Cell Being Allowed to Dry

The cell is assembled by placing the copper working electrode supply on the upper half of the plate, and placing the 10 mm by 22 mm carbon electrode over this and covering the o-ring in the center of the cell.

Figure 47 - Three Initial Steps to the Electrochemical Cell Assembly

The far left picture displays the placement of the o-ring within the cell, followed by the copper supply in the center photograph. Finally the carbon rectangle is placed on top of both the o-ring and the copper supply. The carbon rectangle must be centered both horizontally and vertically to ensure that the cell is completely sealed when assembled.

The cell is assembled by placing the two halves of the cell together, and tightening each of the four screws and equal distance (this means to rotate through each screw periodically so that all are tightened within one turn of one another).

After assembling the cell and if the cell is going to be store for an extended period of time before use, the interior of the cell should be filled with some pH stable solution (PBS) to maintain the integrity of the cell and the carbon within the cell.

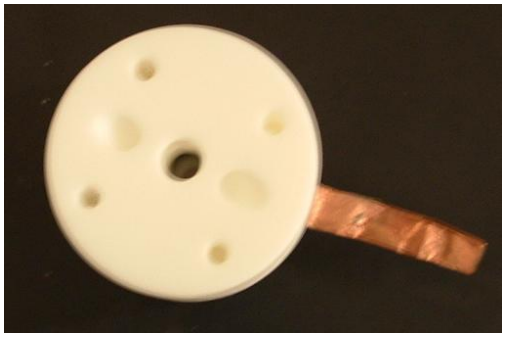


Figure 48 - Assembled Electrochemical Cell

If the cell is going to immediately attached to the potentiostat, the potentiostat needs to be turned on, simply by pressing the "POWER" button. Afterwards, the three electrode system should be assembled as shown in Figure 49. The reference electrode serves to negate the noise presented by the fluid, while the working and counter electrodes function as the current supply and return. This setup is maintained for both cyclic voltammetry and electrochemical impedance spectroscopy.

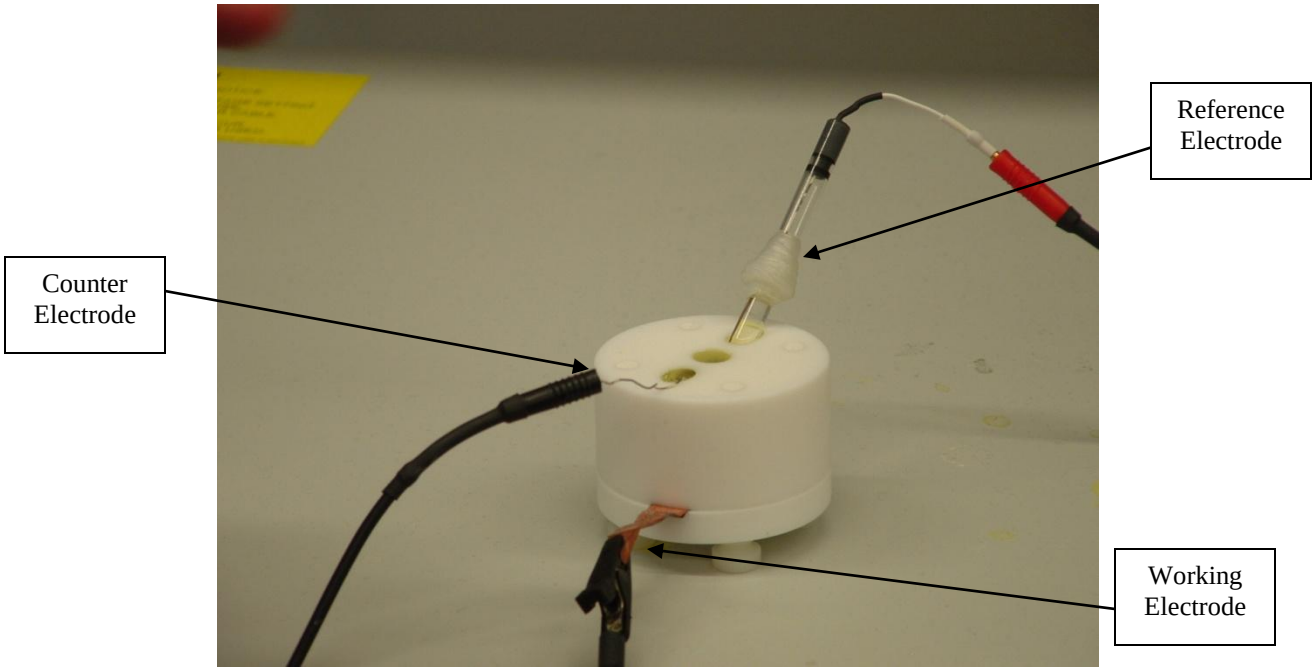


Figure 49 - The Three Electrode Assembly for the Electrochemical Cell

Appendix J: Detailed Cyclic Voltammetry Process

The computer system will not allow the use of the potentiostat software without the credentials of an administrator. In order to use the machine an "ADMIN" must log onto the computer.

To being cyclic voltammetry, open the GPES module within the programs menu (Figure 50).

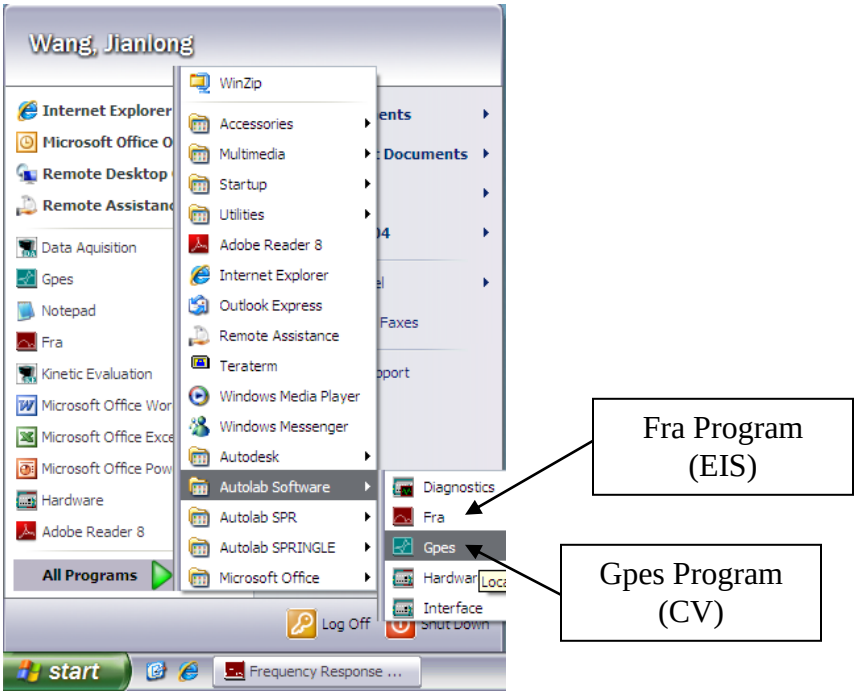


Figure 50 - Programs Menu Depicting CV and EIS Programs

The screen that appears (Figure 51) contains all of the options that are capable for manipulation. The "Edit Procedures" window is the area in which the cyclic voltammogram scan is predefined. "Pretreatment" is created to hold the electrochemical cell at a certain potential before scanning, for our purposes this was not done. An equilibration time of 5 seconds was routinely used. "Measurement" allows for the possibility of running many sequential scans on the same cell, and to adjust the potential that the cell is held at after the scan is complete, for the purposes of these experiments the potential was 0.

"Potentials" is where the user is allowed to truly alter the scan. Under this tab, the scan potentials can be altered as well as the scan rate and the step the machine takes while scanning. The cyclic voltammetry scan encompasses beginning at one potential (Start Potential), scanning at a certain voltage step (Step Potential) to another potential (First Vertex Potential), and finally continuing to a final potential (Second Vertex Potential). Figure 51 displays the values used for the general scanning of the biosensor. For the ABA treatment, the first vortex potential was changed to 1.4 and the scan rate was changed to 0.01.

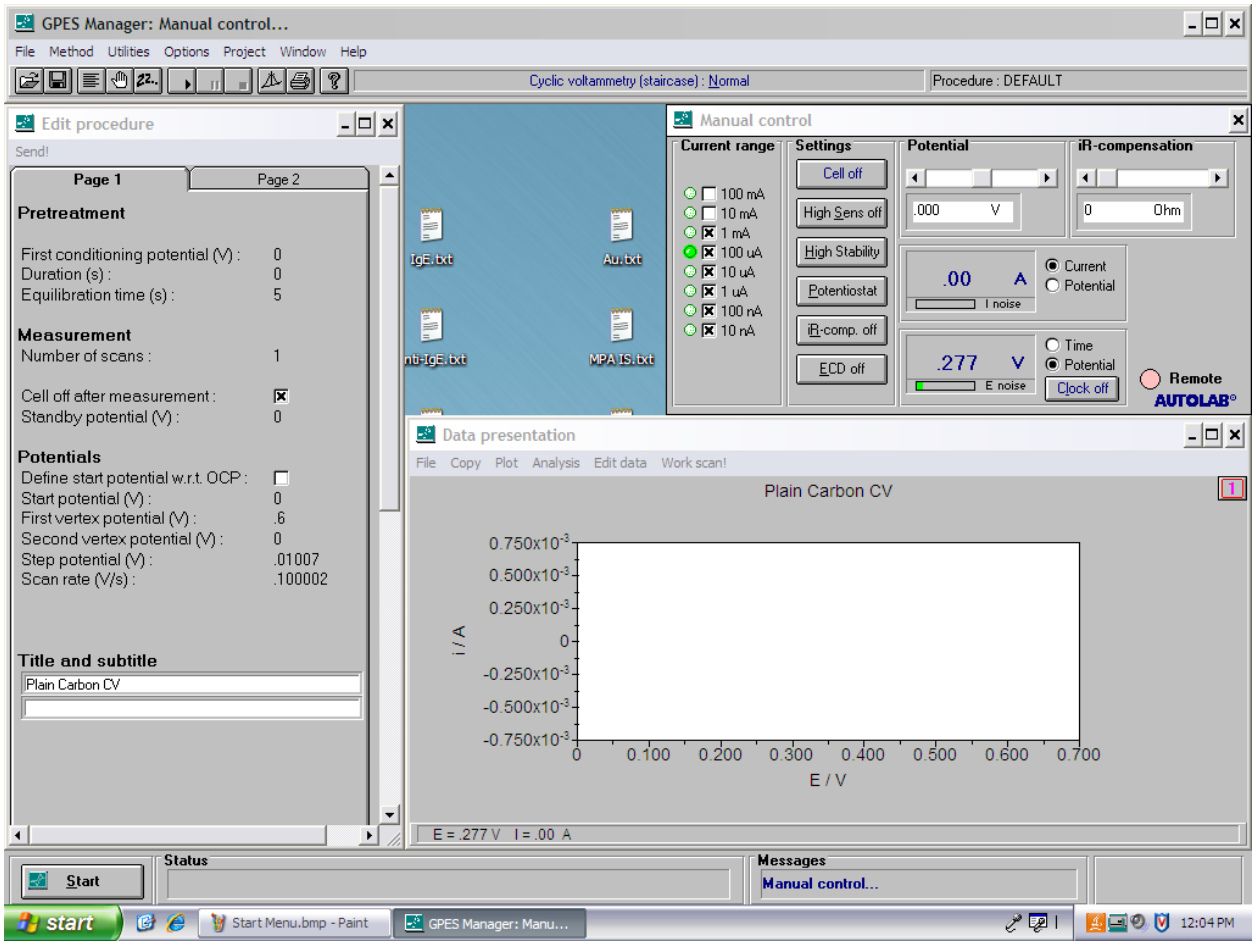


Figure 51 - Gpes (CV) Program Menu and Base Screen

Appendix K: Detailed Electrochemical Impedance Spectroscopy Process

The computer system will not allow the use of the potentiostat software without the credentials of an administrator. In order to use the machine an "ADMIN" must log onto the computer.

To being electrochemical impedance spectroscopy, open the Fra module within the programs menu (Figure 50). Once opened the module has similar adjustments that can be made as in the Gpes module. The "Pretreatment" options remain the same and are described in Appendix I. The "Measurement" menu has changed. The mode for this type of analysis is always single sine. The final adjustment is potential. With EIS, the system is measuring changes due to frequency at a constant voltage, the voltage must be supplied by the user. This can vary for purposes, but as long as one potential is maintained throughout the analysis of sequential comparative experiments, the option is the users.

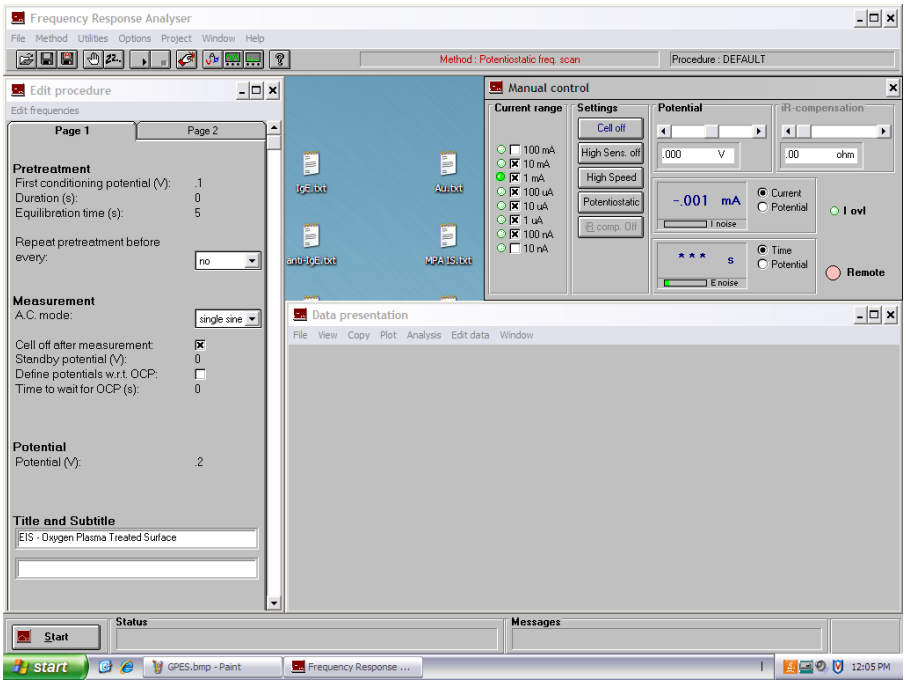


Figure 52 - Fra (EIS) System Module and Base Screen

To alter the frequencies the system performs at, the "Edit Frequencies" Tab in the upper left corner of the "Edit Procedures" menu should be selected. Almost all systems will have their peaks occur between 0.1 and 10000 Hz. However, after sequential scanning it is found that much lower frequencies will suffice, this is the menu where those values can be adjusted. The "Number of Freq." is the number of divisions that the computer will make between the beginning and end frequencies. The larger the number the slower the scan. However, the larger the number the more accurate and precise the data is.

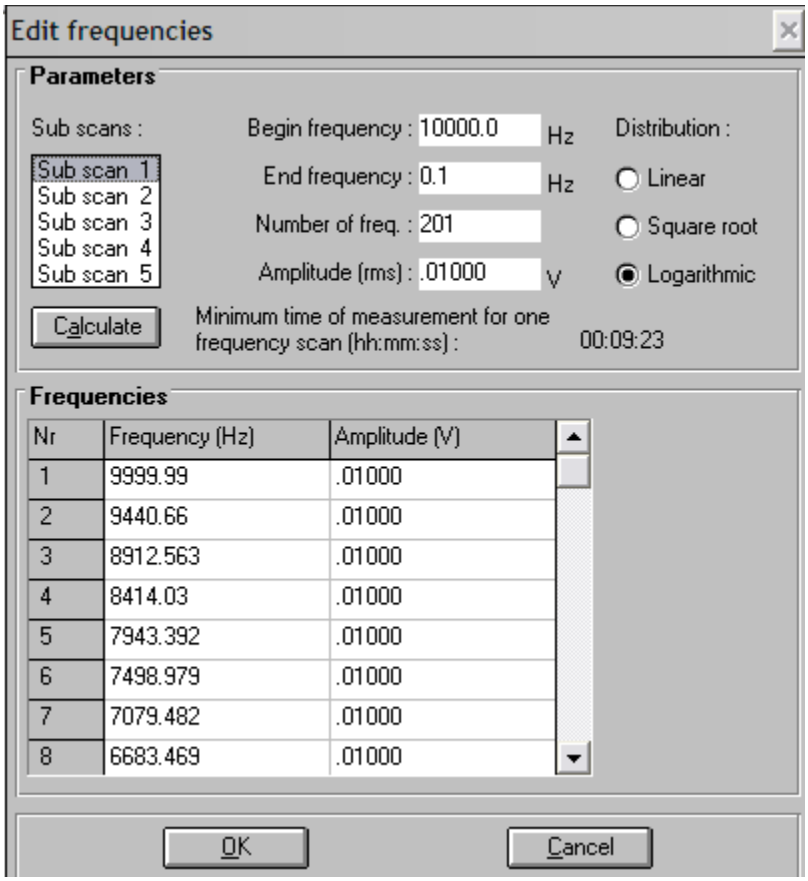


Figure 53 - Edit Frequencies Menu

The final adjustment is the display of a plot for the EIS. The Nyquist plot is called -Z" versus Z' (Figure 54). This is viewed by selecting "View" in the "Data Presentation" window.

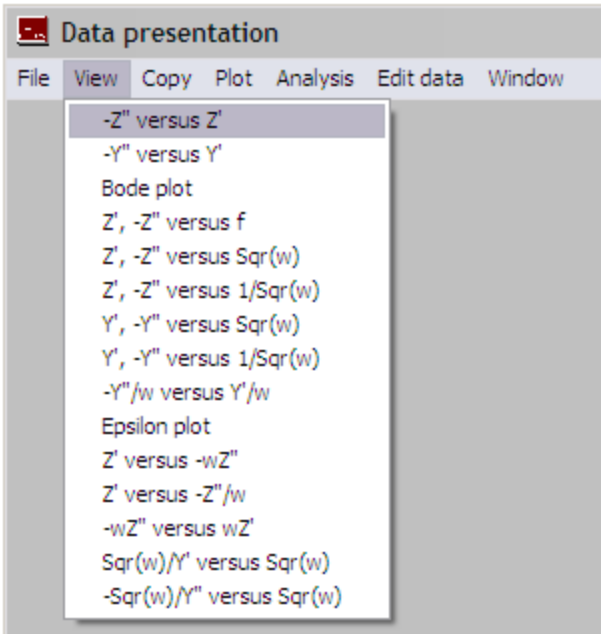


Figure 54 - Nyquist Plot Option

Appendix L: MSDS for 4-Aminobenzoic Acid

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 04/08/2009
Date Updated: 02/06/2009
Version 1.4

Section 1 - Product and Company Information

Product Name	4-AMINOBENZOIC ACID, 99%
Product Number	100536
Brand	ALDRICH
Company	Sigma-Aldrich
Address	3050 Spruce Street SAINT LOUIS MO 63103 US
Technical Phone:	800-325-5832
Fax:	800-325-5052
Emergency Phone:	314-776-6555

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
4-AMINOBENZOIC ACID	150-13-0	No

Formula: C7H7NO2

Synonyms: Acido p-aminobenzoico (Italian) * Amben * Aminobenzoic acid * gamma-Aminobenzoic acid * p-Aminobenzoic acid * 4-Aminobenzoic acid * 1-Amino-4-carboxybenzene * Anticanitic vitamin * Anti-chromotrichia factor * Bacterial vitamin H1 * Benzoic acid, 4-amino- * p-Carboxyaniline * 4-Carboxyaniline * p-Carboxyphenylamine * Chromotrichia factor * Kyselina p-aminobenzoova (Czech) * PAB * PABA * Pabacyd * Pabafilm * Pabanol * Paraminol * Paranate * Romavit * Sunbrella * Trichochromogenic factor * Vitamin BX * Vitamin H'

RTECS Number: DG1400000

Section 3 - Hazards Identification

EMERGENCY OVERVIEW
Harmful.
Harmful if swallowed. Irritating to eyes, respiratory system and skin. May cause sensitization by skin contact.

HMIS RATING
HEALTH: 1
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING
HEALTH: 1
FLAMMABILITY: 0
REACTIVITY: 0

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE
In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL
Evacuate area.

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear respirator, chemical safety goggles, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING
User Exposure: Do not breathe dust. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

STORAGE
Suitable: Keep tightly closed.

SPECIAL REQUIREMENTS
Light sensitive. Air sensitive.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator.
Hand: Compatible chemical-resistant gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES
Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance	Color: Faintly beige Form: Powder	
Property	**Value**	**At Temperature or Pressure**
Molecular Weight	137.14 AMU	
pH	3.5	20 °C Concentration: 5 g/l
BP/BP Range	N/A	
MP/MP Range	186 °C	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	1.374 g/cm3	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	Log Kow: 0.68	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.
Conditions of Instability: May discolor on exposure to light.
Conditions to Avoid: Light. Air.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS
Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Nitrogen oxides.

HAZARDOUS POLYMERIZATION
Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE
Skin Contact: Causes skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: Causes eye irritation.
Inhalation: May be harmful if inhaled. Material is irritating to mucous membranes and upper respiratory tract.
Ingestion: Harmful if swallowed.

SENSITIZATION
Skin: May cause allergic skin reaction.

SIGNS AND SYMPTOMS OF EXPOSURE
The chemical, physical, and toxicological properties of this product have not been thoroughly investigated.

TOXICITY DATA

Oral
Rat
> 6000 mg/kg
LD50

Intraperitoneal
Rat
>3450 MG/KG
LD50

Oral
Mouse
2850 mg/kg
LD50
Remarks: Behavioral:Somnolence (general depressed activity). Behavioral:Muscle weakness.

Oral
Dog
1000 mg/kg
LD50

Oral
Rabbit
1830 mg/kg
LD50

Intravenous
Rabbit
2 GM/KG
LD50

IARC CARCINOGEN LIST

Rating: Group 3

CHRONIC EXPOSURE - MUTAGEN

Species: Mouse
Route: Intraperitoneal
Dose: 1 GM/KG
Mutation test: DNA damage

CHRONIC EXPOSURE - REPRODUCTIVE HAZARD

Species: Rat
Dose: 2500 MG/KG
Route of Application: Oral
Exposure Time: (1-22D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT
Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA
Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: Xn
Indication of Danger: Harmful.
R: 22-36/37/38-43
Risk Statements: Harmful if swallowed. Irritating to eyes, respiratory system and skin. May cause sensitization by skin contact.
S: 26-36
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Harmful.
Risk Statements: Harmful if swallowed. Irritating to eyes,

respiratory system and skin. May cause sensitization by skin contact.
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No
TSCA INVENTORY ITEM: Yes

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: Yes
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Appendix M: MSDS for Potassium Hexacyanoferrate(II) Trihydrate

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 04/08/2009
Date Updated: 02/06/2009
Version 1.10

Section 1 - Product and Company Information

Product Name	POTASSIUM HEXACYANOFERRATE(II) TRIHYDRATE, REAGENTPLUS TM, 99%
Product Number	P9387
Brand	SIAL
Company	Sigma-Aldrich
Address	3050 Spruce Street SAINT LOUIS MO 63103 US
Technical Phone:	800-325-5832
Fax:	800-325-5052
Emergency Phone:	314-776-6555

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
POTASSIUM FERROCYANATE	14459-95-1	No

Formula C6FeK4N6·3H2O

Section 3 - Hazards Identification

EMERGENCY OVERVIEW
Contact with acids liberates very toxic gas.
Caution: Avoid contact and inhalation. Target organ(s): Blood.

HMIS RATING
HEALTH: 1*
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING
HEALTH: 1
FLAMMABILITY: 0
REACTIVITY: 0

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

DERMAL EXPOSURE
In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Noncombustible. Use extinguishing media appropriate to surrounding fire conditions.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Exercise appropriate precautions to minimize direct contact with skin or eyes and prevent inhalation of dust.

METHODS FOR CLEANING UP
Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING
User Exposure: Avoid inhalation. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

STORAGE
Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks.
Hand: Protective gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES

Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance: Physical State: Solid
Color: Light yellow
Form: Fine crystals

Property	Value	At Temperature or Pressure
Molecular Weight	422.41 AMU	
pH	8.0 - 10.0	
BP/BP Range	N/A	
MP/MP Range	70 °C	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	1.85 g/cm3	
Bulk Density	1.2 kg/l	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	Solubility in Water:complete, yellow 0.5 M in H2O, 20°C	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY
Stable: Stable.
Materials to Avoid: Strong acids, Strong oxidizing agents Avoid contact with acid.

HAZARDOUS DECOMPOSITION PRODUCTS
Hazardous Decomposition Products: Nitrogen oxides, Hydrogen cyanide Carbon monoxide, Carbon dioxide.

HAZARDOUS POLYMERIZATION
Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE
Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.

Inhalation: Material may be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled.
Ingestion: May be harmful if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)
Blood.

SIGNS AND SYMPTOMS OF EXPOSURE
May cause cyanosis (blue-gray coloring of skin and lips caused by lack of oxygen).

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT
Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA
Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
R: 32
Risk Statements: Contact with acids liberates very toxic gas.
S: 22-24/25
Safety Statements: Do not breathe dust. Avoid contact with skin and eyes.

US CLASSIFICATION AND LABEL TEXT
Risk Statements: Contact with acids liberates very toxic gas.
US Statements: Caution: Avoid contact and inhalation. Target organ(s): Blood.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER

For R&D use only. Not for drug, household or other uses.

WARRANTY

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Appendix N: MSDS for Potassium Hexacyanoferrate(III) Trihydrate

ACROS ORGANICS

Material Safety Data Sheet

Potassium ferri(III)cyanide, reagent ACS, 99%

MSDS# 95765

Section 1 - Chemical Product and Company Identification

MSDS Name:	Potassium ferri(III)cyanide, reagent ACS, 99%
Catalog Numbers:	AC424120000, AC424120050, AC424125000
Synonyms:	Red prussiate; Red potassium prussiate; Potassium ferricyanide; Potassium hexacyanoferrate(III); Potassium iron(III) cyanide; Iron potassium cyanide; Potassium ferricyanate; Tripotassium iron hexacyanide; Tripotassium hexacyanoferrate.
Company Identification:	Acros Organics BVBA Janssen Pharmaceuticalaan 3a 2440 Geel, Belgium
Company Identification: (USA)	Acros Organics One Reagent Lane Fair Lawn, NJ 07410
For information in the US, call:	800-ACROS-01
For information in Europe, call:	+32 14 57 52 11
Emergency Number, Europe:	+32 14 57 52 99
Emergency Number US:	201-796-7100
CHEMTREC Phone Number, US:	800-424-9300
CHEMTREC Phone Number, Europe:	703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#:	13746-66-2
Chemical Name:	Potassium ferri(III)cyanide
%:	> 99
EINECS#:	237-323-3
Hazard Symbols:	None listed
Risk Phrases:	32

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Caution! Contact with acids liberates hydrogen cyanide, a very toxic, flammable gas or liquid. May cause eye, skin, and respiratory tract irritation. Target Organs: None known.

Potential Health Effects

Eye:	May cause mild eye irritation.
Skin:	May cause skin irritation. May be harmful if absorbed through the skin.
Ingestion:	May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May be harmful if swallowed. May cause effects similar to those of acute inhalation.
Inhalation:	May cause respiratory tract irritation. May cause anoxia, characterized by weakness, headache, dizziness, confusion, cyanosis (bluish discoloration of skin due to deficient oxygenation of the blood), weak and irregular heart beat, collapse, unconsciousness, convulsions, coma and death. May be harmful if inhaled.
Chronic:	Prolonged or repeated skin contact may cause dermatitis.

Section 4 - First Aid Measures

Eyes:	Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.
Skin:	Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
Ingestion:	Get medical aid immediately. Do NOT induce vomiting. If conscious and alert, rinse mouth and drink 2-4 cupfuls of milk or water.
Inhalation:	Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Notes to Physician:	

Section 5 - Fire Fighting Measures

General Information:	As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Dusts at sufficient concentrations can form explosive mixtures with air. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Use water spray to keep fire-exposed containers cool.
Extinguishing Media:	In case of fire, use water, dry chemical, chemical foam, or alcohol-resistant foam. Do NOT use carbon dioxide.
Autoignition Temperature:	Not available.
Flash Point:	Not applicable.
Explosion Limits: Lower:	Not available
Explosion Limits: Upper:	Not available
NFPA Rating:	health: 1; flammability: 1; instability: 1;

Section 6 - Accidental Release Measures

General Information:	Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks:	Vacuum or sweep up material and place into a suitable disposal container. Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Avoid generating dusty conditions. Provide ventilation.

Section 7 - Handling and Storage

Handling:	Minimize dust generation and accumulation. Avoid breathing vapors from heated material. Avoid contact with eyes, skin, and clothing. Use only with adequate ventilation. Avoid breathing dust. Avoid temperatures above 210°C (410°F).
Storage:	Store in a cool, dry, well-ventilated area away from incompatible substances. Store protected from light. Isolate from oxidizing materials and acids.

Section 8 - Exposure Controls, Personal Protection

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Potassium ferri(III)cyanide	1 mg/m3 TWA (as Fe) (listed under Iron salts (soluble)).	1 mg/m3 TWA (as Fe) (listed under Iron salts (soluble)).	5 mg/m3 TWA (listed under Cyanide anion).

OSHA Vacated PELs: Potassium ferri(III)cyanide: 1 mg/m3 TWA (as Fe) (listed under Iron salts (soluble))5 mg/m3 TWA (as CN) (listed under Cyanide anion)

Engineering Controls:

Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.

Exposure Limits

Personal Protective Equipment

Eyes:	Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin:	Wear appropriate protective gloves to prevent skin exposure.
Clothing:	Wear appropriate protective clothing to prevent skin exposure.
Respirators:	Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: powder and chunks
Color: red - orange to red
Odor: odorless
pH: ~6 (5% aq. sol.)
Vapor Pressure: Negligible
Vapor Density: Not available
Evaporation Rate: Not available
Viscosity: Not available
Boiling Point: Not available
Freezing/Melting Point: Decomposes
Decomposition Temperature:
Solubility in water: 464 g/l water (20°C)
Specific Gravity/Density: 1.85 g/cm3
Molecular Formula: C6FeK3N6
Molecular Weight: 329.26

Section 10 - Stability and Reactivity

Chemical Stability:	Decomposes when heated. Light sensitive.
Conditions to Avoid:	High temperatures, incompatible materials, light, dust generation.
Incompatibilities with Other Materials	Oxidizing agents, acids, ammonia, fluorine, sodium nitrate, nitrides (e.g. potassium nitride, sodium nitride), chromium trioxide.
Hazardous Decomposition Products	Nitrogen oxides, carbon monoxide, carbon dioxide, cyanides, oxides of potassium.
Hazardous Polymerization	Has not been reported.

Section 11 - Toxicological Information

RTECS#:	CAS# 13746-66-2: LJ8225000
LD50/LC50:	RTECS: **CAS# 13746-66-2:** Oral, mouse: LD50 = 2970 mg/kg; . Other:
Carcinogenicity:	Potassium ferri(III)cyanide - Not listed as a carcinogen by ACGIH, IARC, NTP, or CA Prop 65.
Epidemiology:	Not available
Teratogenicity:	Not available
Reproductive:	Not available
Neurotoxicity:	Not available
Mutagenicity:	See actual entry in RTECS for complete information.
Other:	The hazards associated with cyanide may be seen in this product.

Section 12 - Ecological Information

Ecotoxicity:	Fish: Fathead Minnow: LC50: 100 mg/l; 96H Fish: Water Flea: LC50: 80 mg/l; 96H
Other:	Do not empty into drains. Acute and long-term toxicity to fish and invertebrates: LC50/96hr for fathead minnow: GT 100mg/L; LC50/96hr for water flea: 80mg/L. Toxicity to aquatic and terrestrial plants: No plan germination adverse effects at 10mg/L for ryegrass, radish and lettuce.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification. RCRA P-Series: None listed. RCRA U-Series: None listed.

Section 14 - Transport Information

US DOT
Shipping Name: Not Regulated.
Hazard Class:
UN Number:
Packing Group:
Canada TDG
Shipping Name: Not available
Hazard Class:
UN Number:
Packing Group:

Section 15 - Regulatory Information

US Federal

TSCA

CAS# 13746-66-2 is listed on the TSCA Inventory.

Health & Safety Reporting List	None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules	None of the chemicals in this product are under a Chemical Test Rule.
Section 12b	None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule	None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs	None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances	None of the chemicals in this product have a TPQ.
Section 313	No chemicals are reportable under Section 313.
Clean Air Act:	This material does not contain any hazardous air pollutants. This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.
Clean Water Act:	None of the chemicals in this product are listed as Hazardous Substances under the CWA. CAS# 13746-66-2 is listed as a Priority Pollutant under the Clean Water Act. CAS# 13746-66-2 is listed as a Toxic Pollutant under the Clean Water Act.
OSHA:	
STATE	Potassium ferri(III)cyanide can be found on the following state right to know lists: California, (listed as Iron salts (soluble)), California, (listed as Cyanides, inorganic salts), New Jersey, (listed as Cyanide anion), New Jersey, (listed as Cyanides, inorganic salts), Pennsylvania, (listed as Iron salts (soluble)), Pennsylvania, (listed as Cyanide anion), Minnesota, (listed as Iron salts (soluble)), Massachusetts, (listed as Cyanide anion).
California Prop 65	
California No Significant Risk Level:	None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:Not available

Risk Phrases:

R 32 Contact with acids liberates very toxic gas.

Safety Phrases:

S 50A Do not mix with acids.

WGK (Water Danger/Protection)

CAS# 13746-66-2: 2

Canada

CAS# 13746-66-2 is listed on Canada's DSL List

Canadian WHMIS Classifications: Not available

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

CAS# 13746-66-2 is not listed on Canada's Ingredient Disclosure List.

Section 16 - Other Information

MSDS Creation Date: 6/27/2000

Revision #5 Date 6/27/2007

Revisions were made in Sections: 3, 4, 7, 9, 10, 14

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantibility or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall the company be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential, or exemplary damages howsoever arising, even if the company has been advised of the possibility of such damages.

Appendix O: MSDS for Potassium Chloride

Fisher Scientific

Material Safety Data Sheet
Potassium chloride

MSDS# 19310

Section 1 - Chemical Product and Company Identification

MSDS Name:	Potassium chloride
Catalog Numbers:	AC193780000, AC193780010, AC193780050, AC196770000, AC196770010, AC424090000 AC424090000, AC424090030, AC424090250, S77375, S77375-1, S77375-2, S79807, 42409-0010, BP366-1, BP366-500, NC9545334, P217-10, P217-250LB, P217-3, P217-500, P217-500LC, P330-250LB, P330-3, P330-500, P333-250LB, P333-3, P333-500, P335-12, P335-212, P335-SAM1, P33512LC
Synonyms:	KCl.
Company Identification:	Fisher Scientific One Reagent Lane Fair Lawn, NJ 07410
For information in the US, call:	201-796-7100
Emergency Number US:	201-796-7100
CHEMTREC Phone Number, US:	800-424-9300

Section 2 - Composition, Information on Ingredients

CAS#:	7447-40-7
Chemical Name:	Potassium chloride
%:	99+
EINECS#:	231-211-8

Hazard Symbols:	None listed
Risk Phrases:	None listed

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Caution! Hygroscopic (absorbs moisture from the air). May cause eye, skin, and respiratory tract irritation. Target Organs: None known.

Potential Health Effects

Eye:	May cause eye irritation.
Skin:	May cause skin irritation. Low hazard for usual industrial handling.
Ingestion:	May cause irritation of the digestive tract. Low hazard for usual industrial handling.
Inhalation:	May cause respiratory tract irritation. Low hazard for usual industrial handling.
Chronic:	No information found.

Section 4 - First Aid Measures

Eyes:	Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
Skin:	Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
Ingestion:	Do not induce vomiting. Get medical aid if irritation or symptoms occur.
Inhalation:	Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
Notes to Physician:	Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information:	As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Substance is noncombustible.
Extinguishing Media:	Substance is noncombustible; use agent most appropriate to extinguish surrounding fire.
Autoignition Temperature:	Not applicable.
Flash Point:	Not applicable.
Explosion Limits: Lower:	Not available
Explosion Limits: Upper:	Not available
NFPA Rating:	health: 1; flammability: 0; instability: 1;

Section 6 - Accidental Release Measures

General Information:	Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks:	Vacuum or sweep up material and place into a suitable disposal container. Avoid generating dusty conditions. Provide ventilation. Do not let this chemical enter the environment.

Section 7 - Handling and Storage

Handling:	Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. Use with adequate ventilation.
Storage:	Store in a cool, dry place. Store in a tightly closed container.

Section 8 - Exposure Controls, Personal Protection

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Potassium chloride	none listed	none listed	none listed

OSHA Vacated PELs: Potassium chloride: None listed

Engineering Controls:

Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Personal Protective Equipment

Eyes:	Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin:	Wear appropriate protective gloves to prevent skin exposure.
Clothing:	Wear appropriate protective clothing to prevent skin exposure.
Respirators:	A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Section 9 - Physical and Chemical Properties

Physical State: Solid
Color: white
Odor: odorless
pH: Not available
Vapor Pressure: Not available
Vapor Density: Not available
Evaporation Rate: Not available
Viscosity: Not available
Boiling Point: 1420 deg C @ 760 mmHg (2,588.00°F)
Freezing/Melting Point: 770 deg C (1,418.00°F)

Decomposition Temperature: Not available
Solubility in water: 340 g/L (20°C)
Specific Gravity/Density: 1.987
Molecular Formula: KCl
Molecular Weight: 74.54

Section 10 - Stability and Reactivity

Chemical Stability:	Hygroscopic: absorbs moisture or water from the air.
Conditions to Avoid:	Incompatible materials, dust generation, excess heat, exposure to moist air or water.
Incompatibilities with Other Materials	Strong oxidizing agents, strong acids, bromine trifluoride, sulfuric acid, potassium permanganate.
Hazardous Decomposition Products	Hydrogen chloride, chlorine, carbon monoxide, carbon dioxide, potassium fume.
Hazardous Polymerization	Will not occur.

Section 11 - Toxicological Information

RTECS#:	CAS# 7447-40-7: TS8050000
LD50/LC50:	RTECS: **CAS# 7447-40-7:** Draize test, rabbit, eye: 500 mg/24H Mild; Oral, mouse: LD50 = 1500 mg/kg; Oral, rat: LD50 = 2600 mg/kg; .
Carcinogenicity:	Potassium chloride - Not listed as a carcinogen by ACGIH, IARC, NTP, or CA Prop 65.
Epidemiology:	Not available
Teratogenicity:	Not available
Reproductive:	Not available
Neurotoxicity:	Not available
Mutagenicity:	Not available
Other:	See actual entry in RTECS for complete information.

Section 12 - Ecological Information

Other: Do not empty into drains.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification. RCRA P-Series: None listed. RCRA U-Series: None listed.

Section 14 - Transport Information

US DOT
Shipping Name: Not regulated.
Hazard Class:
UN Number:
Packing Group:
Canada TDG
Shipping Name: Not regulated as a hazardous material
Hazard Class:
UN Number:
Packing Group:

Section 15 - Regulatory Information

US Federal

TSCA

CAS# 7447-40-7 is listed on the TSCA Inventory.

Health & Safety Reporting List	None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules	None of the chemicals in this product are under a Chemical Test Rule.
Section 12b	None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule	None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs	None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances	None of the chemicals in this product have a TPQ.
SARA Codes	CAS # 7447-40-7: acute.
Section 313	This material contains Potassium chloride (listed as Water Dissociable Nitrate Compounds), 99%, (CAS# 7447-40-7) which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 372.
Clean Air Act:	This material does not contain any hazardous air pollutants. This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.
Clean Water Act:	None of the chemicals in this product are listed as Hazardous Substances under the CWA. None of the chemicals in this product are listed as Priority Pollutants under the CWA. None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:	
STATE	Potassium chloride is not present on state lists from CA, PA, MN, MA, FL, or NJ.
California Prop 65	
California No Significant Risk Level:	None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:Not available

Risk Phrases:

Safety Phrases:

S 24/25 Avoid contact with skin and eyes.

WGK (Water Danger/Protection)

CAS# 7447-40-7: 1

Canada

CAS# 7447-40-7 is listed on Canada's DSL List

Canadian WHMIS Classifications: D2B

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

CAS# 7447-40-7 is not listed on Canada's Ingredient Disclosure List.

Section 16 - Other Information

MSDS Creation Date: 7/15/1999

Revision #8 Date 10/10/2007

Revisions were made in Sections: 2, 3, 4, 5, 6, 7, 9, 10, 11, 1

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantibility or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall the company be liable for any claims, losses, or damages of any third party or for lost profits

or any special, indirect, incidental, consequential, or exemplary damages howsoever arising, even if the company has been advised of the possibility of such damages.

Appendix P: MSDS for Phosphate Buffer Solution

Material Safety Data Sheet
Buffer Solution (Phosphate) pH 7.2

ACC# 41121

Section 1 - Chemical Product and Company Identification

MSDS Name: Buffer Solution (Phosphate) pH 7.2
Catalog Numbers: SP341-1
Synonyms: None
Company Identification:
Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410
For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
7732-18-5	Water	95.07	231-791-2
7758-11-4	Potassium phosphate dibasic	2.2	231-834-5
12125-02-9	Ammonium chloride	2	235-186-4
7558-79-4	Sodium phosphate dibasic anhydrous	1.7	231-448-7
7778-77-0	Potassium phosphate monobasic	0.8	231-913-4

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: not available liquid.
Caution! May cause irritation. This is expected to be a low hazard for usual industrial handling.
Target Organs: None.

Potential Health Effects
Eye: May cause eye irritation.
Skin: Non-irritating to the skin. Low hazard for usual industrial handling.
Ingestion: Not available.
Inhalation: May cause respiratory tract irritation. Low hazard for usual industrial handling.
Chronic: Not available.

Section 4 - First Aid Measures

Eyes: Get medical aid. Gently lift eyelids and flush continuously with wate r.
Skin: Get medical aid if irritation develops or persists. Flush skin with plenty of soap and water.
Ingestion: If victim is conscious and alert, give 2-4 cupfuls of milk or water. Get medical aid.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration.

Notes to Physician: Treat symptomatically and supportively.
Antidote: None reported.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear.
Extinguishing Media: Substance is noncombustible; use agent most appropriate to extinguish surrounding fire.
Flash Point: Not applicable.
Autoignition Temperature: Not applicable.
Explosion Limits, Lower: Not available.
Upper: Not available.
NFPA Rating: (estimated) Health: 1; Flammability: 0; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container.

Section 7 - Handling and Storage

Handling: Use with adequate ventilation. Avoid contact with eyes. Keep container tightly closed. Avoid ingestion and inhalation.
Storage: Store in a cool, dry place.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Good general ventilation should be sufficient to control airborne levels.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Water	none listed	none listed	none listed
Potassium phosphate dibasic	none listed	none listed	none listed
Ammonium chloride	10 mg/m3 TWA (fume); 20 mg/m3 STEL (fume)	10 mg/m3 TWA (fume)	none listed
Sodium phosphate dibasic anhydrous	none listed	none listed	none listed
Potassium phosphate monobasic	none listed	none listed	none listed

OSHA Vacated PELs: Water: No OSHA Vacated PELs are listed for this chemical. Potassium phosphate dibasic: No OSHA Vacated PELs are listed for this chemical. Ammonium chloride: 10 mg/m3 TWA Sodium phosphate dibasic anhydrous: No OSHA Vacated PELs are listed for this chemical. Potassium phosphate monobasic: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to minimize contact with skin.
Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if

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irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: not available
Odor: none reported
pH: 7.2
Vapor Pressure: Not available.
Vapor Density: Not available.
Evaporation Rate: Not available.
Viscosity: Not available.
Boiling Point: 100 deg C
Freezing/Melting Point:0 deg C
Decomposition Temperature: Not available.
Solubility: Soluble in water.
Specific Gravity/Density: Not available.
Molecular Formula: Mixture
Molecular Weight: Not available.

Section 10 - Stability and Reactivity

Chemical Stability: Stable.
Conditions to Avoid: None reported.
Incompatibilities with Other Materials: There is no information for any incompatibilities for this substance.
Hazardous Decomposition Products: None.
Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:
CAS# 7732-18-5: ZC0110000
CAS# 7758-11-4 unlisted.
CAS# 12125-02-9: BP4550000; BP4570000
CAS# 7558-79-4: WC4500000
CAS# 7778-77-0: TC6615500
LD50/LC50:
CAS# 7732-18-5:
Oral, rat: LD50 = >90 mL/kg;
.

CAS# 7758-11-4:
.

CAS# 12125-02-9:
Draize test, rabbit, eye: 500 mg/24H Mild;
Draize test, rabbit, eye: 100 mg Severe;
Oral, mouse: LD50 = 1300 mg/kg;
Oral, rat: LD50 = 1650 mg/kg;
.

CAS# 7558-79-4:
Draize test, rabbit, eye: 500 mg/24H Mild;

Draize test, rabbit, skin: 500 mg/24H Mild;
Oral, rat: LD50 = 17 gm/kg;
.

CAS# 7778-77-0:
Skin, rabbit: LD50 = >4640 mg/kg;
.

Carcinogenicity:
CAS# 7732-18-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7758-11-4: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 12125-02-9: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7558-79-4: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7778-77-0: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No data available.
Teratogenicity: No data available.
Reproductive Effects: No data available.
Mutagenicity: No data available.
Neurotoxicity: No data available.
Other Studies:

Section 12 - Ecological Information

Ecotoxicity: No data available. No information available.
Environmental: No information reported.
Physical: No information available.
Other: None.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	Not Regulated	No information available.
Hazard Class:		
UN Number:		
Packing Group:		

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 7732-18-5 is listed on the TSCA inventory.

CAS# 7758-11-4 is listed on the TSCA inventory.
CAS# 12125-02-9 is listed on the TSCA inventory.
CAS# 7558-79-4 is listed on the TSCA inventory.
CAS# 7778-77-0 is listed on the TSCA inventory.

Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.

Section 12b
None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.

CERCLA Hazardous Substances and corresponding RQs
CAS# 12125-02-9: 5000 lb final RQ; 2270 kg final RQ CAS# 7558-79-4: 5000 lb final RQ; 2270 kg final RQ

SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.

SARA Codes
CAS # 12125-02-9: immediate, delayed.
CAS # 7778-77-0: immediate.

Section 313 No chemicals are reportable under Section 313.

Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.

Clean Water Act:
CAS# 12125-02-9 is listed as a Hazardous Substance under the CWA. CAS# 7558-79-4 is listed as a Hazardous Substance under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.

STATE
CAS# 7732-18-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.
CAS# 7758-11-4 is not present on state lists from CA, PA, MN, MA, FL, or NJ.
CAS# 12125-02-9 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.
CAS# 7558-79-4 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Massachusetts.
CAS# 7778-77-0 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:
Not available.

Risk Phrases:

Safety Phrases:

WGK (Water Danger/Protection)
CAS# 7732-18-5: No information available.
CAS# 7758-11-4: 1
CAS# 12125-02-9: 1
CAS# 7558-79-4: 1
CAS# 7778-77-0: 1

Canada - DSL/NDSL
CAS# 7732-18-5 is listed on Canada's DSL List.
CAS# 7758-11-4 is listed on Canada's DSL List.
CAS# 12125-02-9 is listed on Canada's DSL List.
CAS# 7558-79-4 is listed on Canada's DSL List.
CAS# 7778-77-0 is listed on Canada's DSL List.

Canada - WHMIS
This product has a WHMIS classification of Not controlled..
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List
CAS# 12125-02-9 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 6/08/1998
Revision #4 Date: 11/07/2007

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

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Appendix Q: MSDS for S-1813 Photoresist

SHIPLEY

MATERIAL SAFETY DATA SHEET
MICROPOSIT S1813 PHOTO RESIST
41280 4.00 US US 11.06.1998 MSDS_US

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Code	41280
Trade Name	MICROPOSIT S1813 PHOTO RESIST
Manufacturer/Supplier	Shipley Company
Address	455 Forest St. Marlborough, Massachusetts 01752
Phone Number	(508) 481-7950
Emergency Phone Number	(508) 481-7950
Chemtrec #	(800) 424-9300
MSDS first issued	2 July 1996
MSDS data revised	11 June 1998
Prepared By:	Amy C. Nichols
Local Sales Company	Shipley Company, 455 Forest Street, Marlboro, MA 01752 (508-481-7950)

2. COMPOSITION/INFORMATION ON THE INGREDIENTS

Components in Product

Component Name	CAS# / Codes	Concentration
Electronic grade propylene glycol monomethyl ether acetate	108-65-6	71.00 - 76.00
Mixed cresol novolak resin		10.00 - 20.00
Fluoroaliphatic Polymer Esters		0.01 - 1.00
Diazo Photoactive Compound		1.00 - 10.00
cresol	1319-77-3	0.01 - 0.99

3. HAZARD IDENTIFICATION

Main Hazards	- Irritant - Combustible - Nervous System - Skin - Eye - Kidney - Liver
Routes of Entry	Inhalation, ingestion, eye and skin contact, absorption.
Carcinogenic Status	Not considered carcinogenic by NTP, IARC and OSHA
Target Organs	- Nervous System - Skin - Eye - Liver - Kidney
Health Effects - Eyes	Liquid or vapor may cause pain, transient irritation and superficial corneal effects.
Health Effects - Skin	Material may cause slight irritation on prolonged or repeated contact. Repeated and/or prolonged contact may lead to: -

SHIPLEY

MATERIAL SAFETY DATA SHEET
MICROPOSIT S1813 PHOTO RESIST
41280 4.00 US US 11.06.1998 MSDS_US

3. HAZARD IDENTIFICATION

	drowsiness - liver damage - kidney damage
Health Effects - Ingestion	A large dose may have the following effects: - drowsiness - liver damage - kidney damage
Health Effects - Inhalation	Exposure to vapor at high concentrations may have the following effects: - irritation of nose, throat and respiratory tract - liver damage - kidney damage

4. FIRST AID MEASURES

First Aid - Eyes	Immediately flush the eye with plenty of water for at least 15 minutes, holding the eye open. Obtain medical attention if soreness or redness persists.
First Aid - Skin	Wash skin with water. Obtain medical attention if blistering occurs or redness persists.
First Aid - Ingestion	Wash out mouth with water. Obtain medical attention.
First Aid - Inhalation	Remove from exposure. If there is difficulty in breathing, give oxygen. Seek medical attention if symptoms persist.
Advice to Physicians	Treat symptomatically.

5. FIRE FIGHTING MEASURES

Extinguishing Media	Use water spray, foam, dry chemical or carbon dioxide. Keep containers and surroundings cool with water spray.
Special Fire-Fighting Procedures	This product may give rise to hazardous vapors in a fire. Vapors can travel a considerable distance to a source of ignition and result in flashback.
Unusual Fire & Explosion Hazards	Pressure may build up in closed containers with possible liberation of combustible vapors.
Protective Equipment for Fire-Fighting	Wear full protective clothing and self-contained breathing apparatus.

6. ACCIDENTAL RELEASE MEASURES

Spill Procedures	Contain and absorb using earth, sand or other inert material. Transfer into suitable containers for recovery or disposal. Finally flush area with plenty of water.
Personal Precautions	Wear appropriate protective clothing. Wear respiratory protection. Eliminate all sources of ignition.
Environmental Precautions	Prevent the material from entering drains or water courses.

7. HANDLING AND STORAGE

Handling	Use local exhaust ventilation. Avoid contact with eyes, skin and clothing. Keep container tightly closed when not in use.
Storage	Store in original containers. Store away from sources of heat or ignition. Storage area should be: - cool - dry - well ventilated - out of direct sunlight

Other

Proprietary photoresist film contains approximately 2-4% of 2,3,4-trihydroxybenzophenone(THBP), which may sublime during soft-bake or hard-bake processing. THBP has low acute toxicity (LD50>5g/kg). Contact with eyes, skin or mucous membranes cause irritation.

To prevent accumulation of THBP on equipment surfaces and ventilation ducts, preventative maintenance program including regular cleaning should be implemented. Wipe surfaces using an appropriate cleaning solvent when possible. Provide adequate general or local exhaust ventilation during the cleaning process. In situations where this is not possible or where solvent or dust concentrations become excessive, use an air purifying respirator with an organic vapor/toxic particulate cartridge. When cleaning residual THBP, wear protective gloves and adequate protective clothing to prevent skin contact. Practice good personal hygiene to prevent accidental exposure. Clean all protective clothing and equipment thoroughly after each use.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Occupational Exposure Standards

Electronic grade propylene glycol monomethyl ether acetate	Manufacturer recommends 30ppm 8h TWA and 90ppm 15 min STEL.
cresol	ACGIH: TLV 5ppm (22mg/m3) 8h TWA. OSHA: PEL 5ppm (22mg/m3) 8h TWA. UK EH40: OES 5ppm (22mg/m3) 8h TWA. Can be absorbed through skin.
Engineering Control Measures	Engineering methods to prevent or control exposure are preferred. Methods include process or personnel enclosure, mechanical ventilation (local exhaust), and control of process conditions.

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MATERIAL SAFETY DATA SHEET
MICROPOSIT S1813 PHOTO RESIST
41280 4.00 US US 11.06.1998 MSDS_US

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Respiratory Protection	Respiratory protection if there is a risk of exposure to high vapor concentrations. The specific respirator selected must be based on the airborne concentration found in the workplace and must not exceed the working limits of the respirator.
Hand Protection	Butyl rubber gloves.
Eye Protection	Chemical goggles.
Body Protection	Normal work wear.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Viscous liquid
Color	Red
Odor	Sweet
VOC (g/l)	764.7
Specific Gravity	1.04
pH	Neutral
Boiling Range/Point (°C/F)	145.8/295
Flash Point (PMCC) (°C/F)	40.5-46.1 / 105-115
Explosion Limits (%)	Lower limit 1.5 at 20 °C. Upper limit 7.0 at 20 C..
Solubility in Water	Insoluble.
Vapor Density (Air = 1)	Heavier than air.
Evaporation Rate	Slower than ether
Vapor Pressure	Propylene Glycol Monomethyl Ether Acetate: 3.7 mmHg at 20 °C.

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Conditions to Avoid	- High temperatures - Static discharge
Incompatibilities	- Oxidizing agents
Hazardous Polymerization	Will not occur.
Hazardous Decomposition Products	- oxides of carbon - oxides of nitrogen - acrid smoke and irritating fumes - phenols - carbon monoxide - toxic fluorine compounds

10. STABILITY AND REACTIVITY

11. TOXICOLOGICAL INFORMATION

Acute Data	Propylene Glycol Monomethyl Ether Acetate: Oral LD50 (rat) 8532mg/kg. Dermal LD50 (rabbit) 5000mg/kg.
Chronic/Subchronic Data	No data.
Genotoxicity	It was not mutagenic when tested in bacterial or mammalian systems.
Reproductive/Developmental Toxicity	Developmental effects were seen in laboratory animals only at dose levels that were maternally toxic.
Additional Data	None known.

12. ECOLOGICAL INFORMATION

Mobility	Propylene Glycol Monomethyl Ether Acetate: Koc is 0 - 50.
Persistence/Degradability	The product is partially or slowly biodegradable. BOD20 greater than 40%
Bio-accumulation	No data.
Ecotoxicity	The product is rated as practically non-toxic to aquatic species. Tests on the following species gave a LC50 of 161mg/litre: - fathead minnows Tests on the following species gave a LC50 of 408mg/litre: - daphnia

13. DISPOSAL CONSIDERATIONS

Product Disposal	Incineration is the recommended method of disposal. Dispose of in accordance with all applicable local and national regulations.
Container Disposal	Labels should not be removed from containers until they have been cleaned. Empty containers may contain hazardous residues. Dispose of containers with care.

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MATERIAL SAFETY DATA SHEET
MICROPOSIT S1813 PHOTO RESIST
41280 4.00 US US 11.06.1998 MSDS_US

14. TRANSPORT INFORMATION

DOT Ground:	Not Regulated per 49 CFR 173.150(f)(2)
UN Proper Shipping Name	Flammable liquid, n.o.s.
UN Class	(3) Flammable Liquid
UN Number	UN1993
UN Packaging Group	III
N.O.S. 1:	Propylene Glycol Monomethyl Ether Acetate
N.O.S. 2:	
Subsidiary Risks	None.
ADR/RID Substance Identification Number	CLASS 3 - 31(c)
CERCLA RQ	Cresol (100#)
Marine Pollutant	No.

15. REGULATORY INFORMATION

TSCA Listed	Yes
TSCA Exemptions	
WHMIS Classification	D.2.B B.3
MA Right To Know Law	All components have been checked for inclusion on the Massachusetts Substance List (MSL). Those components present at the de minimus concentration have been identified in the hazardous ingredients section of the MSDS.
California Proposition 65	This product does not contain materials which the State of California has found to cause cancer, birth defects or other reproductive harm.
SARA TITLE III-Section 311/312 Categorization (40 CFR 370)	Immediate, delayed, flammability hazard
SARA TITLE III-Section 313 (40 CFR 372)	This product does not contain a chemical which is listed in Section 313 at or above de minimis concentrations.

16. OTHER INFORMATION

NFPA Rating- FIRE	2
NFPA Rating- HEALTH	2
NFPA Rating- REACTIVITY	0
NFPA Rating- SPECIAL	None.
Revisions Highlighted	Flash Point (PMCC) (°C/F)

16. OTHER INFORMATION

Abbreviations

CAS#: Chemical Abstract Services Number
ACGIH: American Conference of Governmental Industrial Hygienists
OSHA: Occupational Safety and Health Administration
TLV: Threshold Limit Value
PEL: Permissible Exposure Limit
STEL: Short Term Exposure Limit
NTP: National Toxicology Program
IARC: International Agency for Research on Cancer
R: Risk
S: Safety
LD50: Lethal Dose 50%
LC50: Lethal Concentration 50%
BOD: Biological Oxygen Demand
Koc: Soil Organic Carbon Partition Coefficient.
TLm: Median Tolerance Limit

Disclaimer

The data contained herein is based on information that Shipley Company believes to be reliable, but no expressed or implied warranty is made with regard to the accuracy of such data or its suitability for a given situation. Such data relates only to the specific product described and not to such products in combination with any other product and no agent of Shipley Company is authorized to vary any of such data. Shipley Company and its agents disclaim all liability for any action taken or foregone on reliance upon such data.

Appendix R: MSDS for N-hydroxysuccinimide

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.0
Revision Date 06/25/2007
Print Date 04/20/2009

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : **N-Hydroxysuccinimide**

Product Number : 130672
Brand : Aldrich

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052
Emergency Phone # : (314) 776-6555

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : 1-Hydroxy-2,5-pyrrolidinedione
HOSu

Formula : C4H5NO3
Molecular Weight : 115.09 g/mol

CAS-No.	EC-No.	Index-No.	Concentration [%]
N-Hydroxysuccinimide			
6066-82-6	228-001-3	-	-

3. HAZARDS IDENTIFICATION

Emergency Overview
OSHA Hazards
No OSHA Hazards

HMIS Classification
Health Hazard: 0
Flammability: 0
Physical hazards: 0

NFPA Rating
Health Hazard: 0
Fire: 0
Reactivity Hazard: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

4. FIRST AID MEASURES

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIRE-FIGHTING MEASURES

Flammable properties
Flash point no data available

Ignition temperature no data available

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Further information
Prevent fire extinguishing water from contaminating surface water or the ground water system.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid dust formation. Avoid breathing dust.

Environmental precautions
Do not let product enter drains.

Methods for cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal. Pick up when dry.

7. HANDLING AND STORAGE

Handling
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage

Moisture sensitive.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
For prolonged or repeated contact use protective gloves.

Eye protection
Safety glasses

Hygiene measures
General industrial hygiene practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	white

Safety data

pH	no data available
Melting point	95 °C (203 °F)
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Conditions to avoid
Avoid moisture.

Materials to avoid
Strong oxidizing agents, Acid chlorides, Acid anhydrides, Strong bases

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions.
Carbon oxides, nitrogen oxides (NOx)

11. TOXICOLOGICAL INFORMATION

Acute toxicity

no data available

Irritation and corrosion

no data available

Sensitisation

no data available

Chronic exposure

no data available

Signs and Symptoms of Exposure

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

no data available

Ecotoxicity effects

no data available

Further information on ecology

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
No OSHA Hazards

TSCA Status
On TSCA Inventory

DSL Status
All components of this product are on the Canadian DSL list.

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard

Massachusetts Right To Know Components
No Components Listed

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
N-Hydroxysuccinimide	6066-82-6	

New Jersey Right To Know Components

	CAS-No.	Revision Date
N-Hydroxysuccinimide	6066-82-6	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth, or any other reproductive defects.

16. OTHER INFORMATION

Further information
, The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Appendix S: MSDS for 1-Ethyl-3-(3-dimethylaminopropyl)carbodimmide Hydrochloride

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.2
Revision Date 03/09/2009
Print Date 04/20/2009

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride

Product Number : E7750
Brand : Sigma-Aldrich

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA
Telephone : +1 800-325-5832
Fax : +1 800-325-5052
Emergency Phone # : (314) 776-6555

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : EDAC
EDChydrochloride
N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimidehydrochloride
WSChydrochloride

Formula : $C_8H_{17}N_3 \cdot HCl$
Molecular Weight : 191.7 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
1-(3-(Dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride			
25952-53-8	247-361-2	-	-

3. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
Irritant

HMIS Classification
Health Hazard: 2
Flammability: 0
Physical hazards: 1

NFPA Rating
Health Hazard: 2
Fire: 0
Reactivity Hazard: 1

Health Hazard: 2
Fire: 0
Reactivity Hazard: 1

Potential Health Effects

Inhalation May be harmful if inhaled. Causes respiratory tract irritation.
Skin May be harmful if absorbed through skin. Causes skin irritation.
Eyes Causes eye irritation.
Ingestion May be harmful if swallowed.

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Flammable properties
Flash point not applicable
Ignition temperature no data available
Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation.

Environmental precautions
Do not let product enter drains.

Methods for cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: -20 °C

Moisture sensitive. Store under inert gas.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves.

Eye protection
Safety glasses

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Colour	white

Safety data

pH	no data available
Melting point	110 - 115 °C (230 - 239 °F)
Boiling point	no data available
Flash point	not applicable
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	soluble

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Materials to avoid
Strong oxidizing agents, Strong acids

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

11. TOXICOLOGICAL INFORMATION

Acute toxicity
no data available

Irritation and corrosion
no data available

Sensitisation
Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

Chronic exposure

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Potential Health Effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	May be harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.
Ingestion	May be harmful if swallowed.

Additional Information
RTECS: FF2200000

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)
no data available

Ecotoxicity effects
no data available

Further information on ecology
no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
Irritant

DSL Status
All components of this product are on the Canadian DSL list.

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard

Massachusetts Right To Know Components
No Components Listed

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
1-(3-(Dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride	25952-53-8	

New Jersey Right To Know Components

	CAS-No.	Revision Date
1-(3-(Dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride	25952-53-8	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth, or any other reproductive defects.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.