Deposition and characterization of hermetic, biocompatible thin film coatings for implantable, electrically active devices

Robyn K. Sweitzer

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Deposition and Characterization of Hermetic, Biocompatible Thin Film Coatings for Implantable, Electrically Active Devices

by

Robyn K. Sweitzer

A DISSERTATION

Submitted in partial fulfillment of the requirements For the degree of Doctor of Philosophy in The Materials Science Program to The School of Graduate Studies of The University of Alabama in Huntsville

HUNTSVILLE, ALABAMA

2016
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Signed

Date

19 Aug 2014
DISSERTATION APPROVAL FORM
Submitted by Robyn K. Sweitzer in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Materials Science and accepted on behalf of the Faculty of the School of Graduate Studies by the dissertation committee.
We, the undersigned members of the Graduate Faculty of the University of Alabama in Huntsville, certify that we have advised and/or supervised the candidate on the work described in this dissertation. We further certify that we have reviewed the dissertation manuscript and approve it in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Material Science Program.

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ABSTRACT
The School of Graduate Studies
The University of Alabama in Huntsville

Degree: Doctor of Philosophy
Name of Candidate: Robyn Kay Sweitzer
Program: Material Science
Title: Deposition and Characterization of Hermetic, Biocompatible Thin Film Coatings for Implantable, Electrically Active Devices

Retinal prostheses may be used to support patients suffering from Age-related macular degeneration or retinitis pigmentosa. A hermetic encapsulation of the poly(imide)-based prosthesis is important in order to prevent the leakage of water and ions into the electric circuitry embedded in the poly(imide) matrix. The deposition of amorphous aluminum oxide (by sputtering) and diamond like carbon (by pulsed laser ablation and vacuum arc vapor deposition) were studied for the application in retinal prostheses. The resulting thin films were characterized for composition, thickness, adhesion and smoothness by scanning electron microscopy-energy dispersive spectroscopy, atomic force microscopy, profilometry and light microscopy. Electrical stability was evaluated and found to be good. The as-deposited films prevented incursion of salinated fluids into the implant over two (2) three month trials soaking in normal saline at body temperature. Biocompatibility was tested in vivo by implanting coated specimen subretinally in the eye of Yucatan pigs. While amorphous aluminum oxide is more readily deposited with sufficient adhesion quality, biocompatibility studies showed a superior behavior of diamond-like carbon. Amorphous aluminum oxide had more adverse effects and caused more severe damage to the retinal tissue.
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I would like to dedicate this work to:

Dana and our kids – for putting up with my late nights and ambition

The Boston Retinal Implant Team –
Dr. Joseph Rizzo III, Dr. Douglas Shire, the late Dr. John Wyatt, Dr. John Lowenstein, Dr. Sandra Montezuma and all others who welcomed and encouraged us in these adventures.

My advisor, Dr. Carmen Scholz, who mentored, encouraged, befriended and worked with me to bring this effort to a successful end.
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INTRODUCTION

In its 2015 Report on World Population Prospects, the United Nations (UN) predicted that the global population aged 60 and up will increase from 901 million to more than 1.4 billion by the year 2030, which represents a population increase of 56%\(^{(1)}\). This growth in the percentage of population over 60 years of age brings with it an increase in medical conditions that are age-related. More than 10 million people in the US are affected by retinal degenerations (RDs)\(^{(2)}\). The World Health Organization states that, globally, cataracts are the leading cause of low vision, glaucoma rates second and AMD, third. In highly developed countries, AMD is the leading cause of blindness, due to increased population longevity\(^{(3)}\). Of these, approximately 90% have Age-Related Macular Degeneration (AMD), and 10% lose vision to other types of RD, including retinitis pigmentosa (RP). These diseases take an enormous toll, both on the affected individual and society, in the form of lost personal independence and increasing dependence on social services. While rapid advances have been made recently in identifying their molecular origins and disease mechanisms, few therapies are available to the RD patient.

Blindness is a grave and mostly irreversible disease that severely curtails the professional and social opportunities of those afflicted. RP and AMD deprive those afflicted of more than their vision. As vision degenerates, patients lose the ability to work, pursue hobbies, read, and drive a car. They become dependent on others to accomplish the most mundane tasks, such as grocery shopping or ordering from a restaurant menu. As independence is lost, so, too is their human spirit. Many blind people tend to limit their social contacts and retreat into an ever-shrinking universe filled with fear of the unseen.
Efforts to create prostheses to enable artificial vision for patients affected by AMD and RP are ongoing across the globe. While it has been determined that visual percepts may be elicited in these patients, the problems of long-term biocompatibility and electrical integrity remain to be solved.

This dissertation project was a collaboration of the Boston Retinal Implant Project (BRIP), consisting of faculty and students of the Harvard Medical School and the Massachusetts Eye and Ear Infirmary (MEEI), the Massachusetts Institute of Technology (MIT), Cornell University and the University of Alabama at Huntsville (UAH), along with nationwide and global partners. The study goal was to produce Amorphous Aluminum Oxide and Diamond-like Carbon films, and demonstrate biocompatibility and hermetic sealing properties for a prototype retinal implant. These features were needed to hide the device from the human immune system, and prevent incursion of salinated body fluids into the implant matrix. Coordinated efforts included prototype production at Cornell University, prototype coating and characterization at UAH, biocompatibility studies at the Mass Eye and Ear Infirmary and electrical integrity studies at MIT.

UAH coatings were shown to be smooth and fault-free, exhibit appropriate compositions and perform well in other studies. Intermediate biocompatibility was demonstrated in Harvard-based studies, and very low leakage currents were observed in salinated soak testing at MIT.
CHAPTER ONE.

The Human Eye and the Effects of AMD and RP

The human eye is an extension of the brain. The gross and some fine anatomy are detailed in Figure 1. The human retina contains an array of photoreceptor cells called rods and cones (Figures 1 and 2). Light impinging on these cells is converted into electrical signals which are transmitted by the optic nerve to the visual cortex in the brain, where they are processed and interpreted. The most dense population of such cells, including those that allow critical color and fine detail vision, are found in a construct at its center, called the macula\(^4\).
Figure 1. Anatomy of the Human Eye

In the image above, the macula, found along the visual axis to the right of the optic nerve, is the portion of the eye responsible for high resolution vision. In the human eye, this structure is approximately 2.5 – 3.0 mm in diameter. The fovea centralis is located at the macula center, and occupies a 0.3 mm diameter.

The macula houses some 150,000-180,000/mm² highly packed cones\(^5\), the photoreceptors that process detailed color vision, and no rods, or photoreceptors that function best in dimly lighted conditions, and therefore do not provide color perception. Cone concentration is highest at the fovea centralis and drops in density as one traces the visual field from the macula to the eye’s periphery. Rods process low-light images, and do not sense color. Rod density is highest just outside the macula at 150,000/mm², thinning toward the retinal perimeter to about 30,000-40,000/mm². Additionally, the
A healthy human eye has a visual field of approximately 200 degrees, but the acuity over that field is not sharp. At the macula, the visual field is limited to about 15 degrees, so the eyes move rapidly to help the macula collect as much information as possible, to help fill in details that are absent in the peripheral vision field.

The eye is supported internally by a viscous fluid called the vitreous, and the retina is located on the inner eye’s posterior (epiretinal) surface, bounding the vitreal space (Figure 2). Just below the retina, retinal ganglion cells are found, followed by bipolar cells, then rods and cones. The ganglion cells transfer the signals to the bipolar cells that interface with amacrine cells (shown in Figure 1) to access and excite both rods and cones to blend the sharp central vision with less sharp visual inputs from the rods, and transmit these signals along the optic nerve to the visual cortex, where they are interpreted to form the image. The subretinal space contains photoreceptors and the retinal pigment epithelium (RPE). RPE functions include phagocytosis of elements shed from the photoreceptors, nutrient transport into the rods and cones, and waste transport/disposal from them. The RPE also functions in pigment transport and pigment regeneration \(^{(6)}\).
There are two manifestations of AMD – Dry and Wet. Some 90% of AMD patients present with the dry form sometimes called Drusenoid AMD (Figure 3). In this case, drusen, consisting of very small protein and fat particles, build up inside a thin layer called the Bruch’s membrane underneath the macula. As this layer thickens, the macula is separated from the capillary bed called the retinal choroid, resulting in photoreceptor cell damage and central vision loss over time.
In some people, the blood vessels feeding the macula and Bruch's membrane harden, making oxygen and waste transport difficult. Old cells from the Retinal Pigment Epithelium (RPE) are less easily disposed of, leading to accumulation of waste material that contributes to drusen.

About 10 percent of Dry AMD patients progress to the point that they develop Wet AMD, a form in which abnormal neovascularization occurs. This new blood vessel growth can penetrate into the macula, where fluid and blood leakage damage the cells within it, as well as in the retina\(^7\). The histology slides captured in Figure 4 depict fan-like features that are choroidal neovascularizations (CNVs). Note that cell death as shown by the arrowheads, precedes the encroaching CNC. In these slides the acronym NH refers to the optic nerve head.
Figure 4. Histology Slides showing Wet AMD.

A submacular fan-shaped Choroidal Neovascularization (CNV) is shown (arrowheads) using direct-illumination to analyze RPE (A) and transillumination (B) to analyze viable blood vessels. Areas of choriocapillaris dropout are located ahead of the CNV (asterisks). Percent RPE and vascular area measurements were made (C) to show that capillary dropout is remarkable (<20% vascular area) immediately in advance of the fan and present beyond the CNV area. (NH = optic nerve head, scale bar = 2 mm)

Credit: NIH NLM\(^7\)

RP is caused by genetic mutations\(^8-9\). Defective genes code photo receptor cells to make the wrong proteins, too much, or too little of proteins needed for proper cell functions. More than 150 proteins have been identified that can become denatured on the retina’s surface resulting in cell damage leading to cell death\(^10\). This process leaves
pigmentation on the damaged/destroyed areas, hence the name Retinitis Pigmentosa. This disease causes initial loss of peripheral vision, that progresses with time until the patient has small islands of vision, and eventually becomes completely blind (Figure 5)\(^{(11)}\).

A process common to all degenerative retinal diseases is the abnormal rate of apoptosis (cell death), which affects the macula in patients with AMD and the rods and cones of the surrounding retina in patients with RP. Patients affected by AMD and RP experience vision loss due to specialized retinal cell death while the underlying neurons, known as retinal ganglia, remain relatively intact. The presence of these ganglia provides an opportunity for artificial stimulation, which may produce an artificial vision\(^{(12,13)}\).
CHAPTER 2

Evolution of Retinal Implants

The use of implants to restore lost biological function is not new to the medical community. Pacemakers were introduced in 1958 to electrically stimulate weak hearts, producing muscle contractions to pump blood at a steady rate, and have saved the lives of millions of people\textsuperscript{(14)}. Modern pacemakers have been refined to the point that they are capable of sensing and averting cardiac arrhythmias.

More recently, a cochlear implant was devised to restore lost hearing to the deaf. When first introduced in the early 1970s, single-channel implants used only a single electrode. A decade later, multi-channel implants were introduced. Current cochlear implants operate with electrode arrays consisting of 12 to 22 electrodes\textsuperscript{(15-17)}. Approximately 120,000 patients who have received cochlear implants have experienced new or restored hearing. The learning curve for cochlear implant recipients can stretch for as long as 10 years, indicating a persisting plasticity of the brain to adapt to the ‘artificial hearing’\textsuperscript{(18)}.

Encouraged by the success of these neural implants and progress in micro- and nano-fabrication technologies for electrical devices, the BRIP set out to develop the most sophisticated neural implant to date – a retinal prosthesis envisioned to restore some useful vision to people blinded by AMD and RP. There are several conceptually different approaches to retinal implants. Three major research groups have been pressing toward the implementation of first and second-
generation devices: Optobionics Corporation, Second Sight Medical Products and the BRIP.

Optobionics Corporation in Chicago, IL was operated by Drs. Alan and Vincent Chow. They developed a passive sensor, consisting of a silicon based microphotodiode array that converts incident light into electrical signals which are then transmitted via the remaining retinal ganglia and bipolar cells to the visual cortex. Unfortunately, due to lack of funding, this effort failed and no longer exists\(^{19, 20}\).

Second Sight Medical Products, Sylmar CA, is another research company working toward ongoing development of a retinal prosthesis. Their first generation device consisted of a small camera and transmitter mounted on a pair of eyeglasses, an implanted receiver and a 5-by-6 mm silicone-platinum 16-electrode array, which is implanted epiretinally, or on the inner surface of the retina, that can be viewed through the lens of the patient’s eye\(^{21}\).

The device was powered by a wireless microprocessor and battery pack which are worn on a belt. Digital images recorded by the camera were converted into electrical signals which were transmitted wirelessly to the microphotodiode array\(^{22, 23}\). Selected electrodes were activated to stimulate underlying retinal ganglia. These electrical impulses were carried via the patient’s optic nerve to the visual cortex. Second Sight recently reported results of chronic (average of 3 years/combined duration of 16 years) implantation of their device. Patients with only bare light perception or worse visual acuity were chosen for the trial. According to the publication, every patient could perceive discrete spots of light, called phosphenes, and all could perform visual spatial tasks. Per Clements and Humayan, patients have been able to find and count objects and recognize differences between objects found in common table settings (plate, cup, fork, knife, etc).
Second Sight Prosthesis System Overview: The Implant

The second generation implant is an epiretinal prosthetic surgically implanted in and on the eye that includes an antenna, an electronics case, and an electrode array (Figure 6). External components are depicted in Figure 7.

![Figure 6. Second Sight Implantation Methodology](Images courtesy of Second Sight Website)

Figure 6. Second Sight Implantation Methodology

![Figure 7. The most recent model, the Argus II Second Sight Product.](Images courtesy of Second Sight Website)

Figure 7. The most recent model, the Argus II Second Sight Product.

The prospects of operational retinal ganglia and brain plasticity helping to assist with visual percept sensitivity\(^{(24-26)}\) motivates the Boston Retinal Implant Project (BRIP) to develop a retinal prosthesis to improve the quality of life for patients affected by varying degrees of blindness. The device envisioned by the BRIP is similar in design to that of
Second Sight Corporation. The first generation prosthesis (Figure 8) consisted of a flexible electrode array embedded in a poly(imide) matrix. The electrode array can be inserted into the subretinal space through a flap in the sclera, the tough white layer surrounding the eye that is visible adjacent to the iris. The second generation design concept is shown in Figure 9. The implant receives power and instruction for electrode stimulation sequencing wirelessly through an inductively coupled coil\(^{(27)}\)

An externally mounted digital camera captures visual information for transfer to the system, and a microchip affixed to the implant close to the retinal interface converts the digital input to electrical stimuli in the locations previously occupied by photoreceptors. This placement enables stimulation of the retina’s inner nuclear layer’s cellular network (bipolar cells, \textit{e.g.}) that mediate vision as well as epiretinal ganglia. There is risk with implant placement because the foreign body is in near contact with the retinal choroid, the eye’s blood supply.

![Diagram](image)

\textbf{Figure 8. First generation retinal implant.}

Suture indicators represent the location of sutures that will secure the inserted implant in the subretinal space.
While others were responsible for implant design, UAH was responsible for the development and characterization of hermetically sealing, biocompatible coatings for the implant to protect the implant from incursion of body fluids which would corrode the delicate electronic circuitry, and to limit unfavorable immune response to the foreign body, which could harm both the device as well as the patient\(^\text{(28)}\).

Immune responses include adhesion of scar tissue, such as glial cells, or biofouling, which is caused by proteins or other cells accumulating on the sensor’s surface. Biofouling is characterized by absorption and adhesion of biological debris on the implant’s outer surface. It can cause device failure due to fibrous encapsulation of an implant, enzymatic decay of the surface, membrane delamination and electrode failure, among other causes\(^\text{(29)}\).

Metal corrosion or bio-corrosion of polymers from the implant could lead to toxic build-up of these materials in biological tissues, and opportunistic microbes could adhere to the surface, inducing infections\(^\text{(30)}\). When biomaterials can be developed that do not cause deleterious effects such as long-term inflammation or any and all of the issues discussed previously, the implant may eventually be implanted into the host for chronic, trouble-free use\(^\text{(31)}\).
CHAPTER 3

The Nature of the Problem and Materials

The human body requires moisture to survive, and the subretinal space contains salinated fluids by necessity. Additionally, the retinal choroid, a high flow capillary bed that supplies nutrients and oxygen to the retina is located in this space. These conditions put the implant at risk of saline incursion, which will corrode the delicate circuitry, destroying electrical function. PI has been reported to absorb up to 5.08 weight per cent of water at diffusion rates of $1.7 \times 10^{-10} \text{ cm}^2/\text{s}$ to $10.5 \times 10^{-10} \text{ cm}^2/\text{s}$\textsuperscript{(32)}.

Proximity to blood flow may put the patient at risk of an immune reaction that could cause infection and possibly loss of the entire eye. Both are serious problems that must be solved.

Biomaterials for use in the human retina have not been well characterized in the literature\textsuperscript{(33)}; however, amorphous aluminum oxide (AAO), and diamond-like carbon (DLC) have been characterized as biomaterials for many implant applications and a wealth of information concerning biocompatibility with respect to other human tissues such as dental applications and bone allografts\textsuperscript{(34, 35)} is available. It is noted that the more hydrophylc a film is, the higher the biocompatibility tends to be, in general, while hydrophobic films tend to be less biocompatible\textsuperscript{(36)}. 
Thin film AAO and DLC are characterized by high hardness and smoothness. They function as good barrier films, tend to be dielectric, and have optical properties that are dependent on deposition method, incident radiation and film thickness\(^{(37-50)}\). Optical properties and desired surface morphology are detailed in Table 1.

<table>
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<th>Material</th>
<th>(n^*)</th>
<th>(k^*)</th>
<th>Surface Morphology</th>
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<tr>
<td>AAO</td>
<td>1.63 - 2.59</td>
<td>0.0029 - 1.67</td>
<td>Smooth, fault free</td>
</tr>
<tr>
<td>DLC</td>
<td>0.01 - 2.1</td>
<td>0.35 - 2.1</td>
<td>Smooth, fault free</td>
</tr>
</tbody>
</table>

In this table, \(n\), the refractive index, and \(k\), the extinction coefficient, are dependent on deposition methodology as well as film thickness\(^{(46-50)}\).

The AAO structure is typically composed of a mix of four- and five-coordinated aluminum atoms and crystalline \(\alpha\)-\(\text{Al}_2\text{O}_3\)\(^{(51)}\) (Figure 10). It is characterized by a density of 3.99 g/cm\(^3\) and electrical resistivity of \(10^{20}\) \(\mu\text{ohm cm}\) making it an excellent insulator. It also possesses chemical stability and thermal stability due to its high melting point of 2043\(^{\circ}\)C.

**Figure 10. Four-and Five-Coordinated AAO Structures**

AAO consists of Four- and Five-coordinated structures interspersed with crystalline \(\text{Al}_2\text{O}_3\) in the Thin Film Product\(^{(52)}\).
Of particular importance to the BRIP is the blood compatibility of AAO. Devices coated with AAO have been tested in high blood-throughput environments, such as stents, heart valves and implanted coronary pumps and sufficiently high blood compatibility was found\(^\text{(53-55)}\). The blood interaction of AAO was studied extensively by Yuhta and it was found that sputter-deposited aluminum oxide produces a durable and blood compatible coating\(^\text{(56)}\). In fact, it was observed that platelet adhesion on AAO was reduced by 50% compared to medical grade poly(urethane). Fewer platelet morphologic changes were observed on the AAO surface than on the comparison polymeric sample\(^\text{(57)}\).

In general, the biocompatibility of DLC coatings is reviewed positively throughout the literature. No toxicity towards living cells, no inflammatory response or loss of cell integrity were reported\(^\text{(58-64)}\). The blood interaction of DLC is described as superior. Yamazaki et al. used DLC for the construction of an implantable blood pump\(^\text{(49, 50)}\). DLC was also tested in coatings for coronary stents\(^\text{(65-66)}\), heart valves\(^\text{(67)}\) and cora rotary pumps\(^\text{(68)}\) and proved to be very beneficial, since the complete, fault-free DLC coating significantly reduces the release of metal ions (nickel and cobalt) from the stent material, as determined by atomic adsorption analysis of the blood plasma. Flow-cytometric analyses also revealed a significantly lower intensity for activation-dependent antigens\(^\text{(69)}\). Based on its durability, inertness, wear- and corrosion resistance, DLC has also been studied in detail for orthopedic implant materials and generally, no adverse effects on a variety of macrophages, fibroblasts and osteoblast-like cells were detected\(^\text{(70-71)}\).

The DLC composition consisting of sp\(^2\) and sp\(^3\) hybridized carbon and hydrogen make it an interesting candidate for this application. The proposed structure is shown
below in Figure 11. DLC, better described as amorphous diamond, is a meta-stable, amorphous phase of carbon, with low coefficients of friction and generally high electrical resistivity\(^{72-74}\).

![Figure 11. Proposed structure for Amorphous DLC](http://www.iws.fraunhofer.de/de/geschaeftsfelder/pvd_nanotechnik/kohlenstoffschichten.html)

The structure is formed by the interspersion of two face centered cubic lattices, displaced by one-fourth of the cube diagonal. Some of the bonding is tetrahedral where \(\text{sp}^3\) hybridization is found. \(\text{sp}^2\) hybridization is noted as graphitic structures within the film\(^{75}\).

The material is smooth, hard and transparent to brownish in color depending on the extent of \(\text{sp}^2\) bonding, and can be deposited by chemical or physical vapor deposition. Chemical vapor deposition processes which typically require high temperatures are not feasible for coating these implants due to the low thermal stability of poly( imide); however, ambient temperature DLC deposition processes such as chemical vapor deposition, using radio-frequency energy to generate plasma from methane and hydrogen gases\(^{76}\) may be used. Additionally, physical vapor deposition by methods such as pulsed laser ablation (PLA) of a graphite target allows for ambient deposition.
temperatures. When using PLA, an ultra-pure graphite target is ablated with a high-powered laser and the substrate (implant) is placed in the plume of reactive carbon species\(^{(77)}\). Deposition processes must be carefully controlled to optimize the percentage of sp\(^3\) and sp\(^2\) species, as these can limit the flexibility of the resulting film if not properly balanced.

In the case of amorphous diamond-like carbon, (a-C:H), film properties are largely determined by the ratio of sp\(^3\) to sp\(^2\) hybridized carbon atoms in the material\(^{(78-79)}\). Diamond-like properties are dominant when the films are mainly sp\(^3\) hybridized (DIAM), and tend to be more polymeric (POLY) when sp\(^2\) hybridization is higher than sp\(^3\)in the composition, or the sp\(^2\) to sp\(^3\) ratio approximates 1:1 (MID). Because of the reported material properties and biocompatibility data, these two materials were selected for this study.
CHAPTER 4

Experimental

4.1 Poly(ime)de film production

Encapsulation experiments were conducted on 2.0 x 0.5 mm x 10 µm PI implant strips that are not electrically functional and intended to be used in subsequent biocompatibility studies, and on 0.7 x 10.0 cm x 10 µm electrically functional implants intended for subsequent testing of electrical stability. PI films were produced at the Cornell University Microfabrication laboratory by Dr. Douglas B. Shire by spin coating PI prepolymer PI-2611 (S-biphenyl dianhydride/p-phenylenediamine, HD Microsystems) onto a silicon disc at 1,200 rpm for 30 seconds on a Brewer Science CEE 6000 spin coater with subsequent soft baking at 140° C on a hotplate, followed by full curing at 350° C for one hour under a 1.0 SCFM/min nitrogen stream in a Y.E.S. Poly(ime)de Bake Oven. Dr. Shire cut the PI specimens into the desired configuration and provided these with instructions to soak these in deionized water to loosen them from the silicon substrate. All PI specimens were cleaned with HPLC grade acetone prior to deposition experiments in order to ensure a clean surface to enhance film adhesion.
4.2 Implant Configurations to be Coated

A variety of electrode array configurations were provided for deposition and characterization. Figure 12 depicts an electrode array used in clinical trials, in which the small end contains a 2X4 electrode array. The small end was surgically placed in a patient’s eye on an island of vision and various combinations of electrodes were activated for a period of four hours. The patient reported and described visual percepts as they occurred.

Figure 12. Experimental Electrode Array used in Clinical Trials

Poly(amide) strips (0.5 mm x 5.0 mm x 10mm) were provided by the BRIP (Figure 13) and were coated for biocompatibility studies to be performed by the BRIP. Second generation implant blanks (Figure 14) were also provided and coated.

Figure 13. 2x10mm PI film Coated and Used in Biocompatibility Studies
4.3 Selected Coating Methods – Pressure Regimes

Thin film coatings have generally been deposited in vacuo \(^{(80, 81)}\), with deposition pressures, temperatures, platen rotational velocities, and distance from the plasma source as well as voltage bias all affecting film properties. Figure 15 demonstrates pressure regimes based on deposition methodology. The processes chosen for this work reside in the microTorr to milliTorr ranges.
4.4 AAO Deposition

Figure 15. Schema for Various Deposition Processes Based on Pressure Regimes
Figure 17 Denton Discovery 18: Used for sputtering thin film coatings.

The cartoon that follows, Figure 17, demonstrates the reactions that occur within the deposition chamber. Here, an Ar-ion beam releases Al$^{+3}$ ions from the target, which react with oxygen to produce AAO cations. These cations bombard the substrate surface, subplanting themselves into the PI.

Figure 18. Schematic of sputtering operation.
Here, an Argon ion beam was trained on a 99.999% Al target in the presence of oxygen at a flow rate of 5 sccm. Al\textsuperscript{3+} cations then reacted with the oxygen, forming AAO\textsuperscript{+}, which then deposited on the cathodic PI films.

4.4.1 AAO Film Thickness Characterization

A Tencor P-10 Profilometer was used to determine film thickness and light-microscopy was used to confirm the minimum film thickness that has no pinholes, cracks or other faults that would expose human tissue to the implant material and fail to protect the embedded electronics from fluid incursion.

4.5 DLC Depositions

Two DLC deposition methods were performed in the investigation of this biomaterial for use in retinal implants: Pulsed laser ablation (PLA), available at the University of Alabama at Birmingham, and Vacuum Arc Vapor Deposition (VAVD), available at UAH. Both are plasma-based depositions and require reduced pressure for operation. Materials properties as a function of deposition type and conditions were investigated.

4.5.1 DLC -Tertiary Amorphous Carbon Deposition

A form of DLC, known as tertiary amorphous carbon (t-aC) was deposited using pulsed laser ablation (PLA). Depositions were performed using a custom-built Neocera instrument (University of Alabama, Birmingham,) Figure 18, at ambient temperatures and pressures between 1.1 and 3.0 \times 10^{-6} \text{Torr}. A KrF excimer laser (\lambda= 248 \text{nm}, V= 14 \text{Kev}) operated for 10,000 pulses at 20 Hz was focused on a pyrolytic graphite target mounted in an inert nitrogen atmosphere. Laser fluence was adjusted to 6.20 \text{J/cm}^2.
Material was then ablated from the surface of the target, generating a plume of activated carbon ions with a diameter of approximately 2 cm. Specimens for biocompatibility studies were placed into this plume on a glass slide, and each side coated in successive order. Optimization of this process included variation of substrate distance from the plume source and variation of applied voltage, and variation of laser fluence from 6.20 J/cm$^2$ to 8.33 J/cm$^2$. A schematic (Figure 19) is a pictorial demonstration of the process.

Figure 19. Custom Built Neocera Instrument for Pulsed Laser Ablation
4.4.3 DLC Film Thickness Characterization

Profilometry was used to determine film thickness and light-microscopy was used to confirm the minimum film thickness that has no pinholes, cracks or other faults that would expose human tissue to the implant material and protect the embedded electronics from fluid incursion.

4.5.2 VAVD Diamond Deposition

Vacuum arc vapor depositions were conducted at the University of Alabama at Huntsville, using a custom build vacuum chamber powered by two 440V 3-phase Square D welders. An ultra-high purity pyrolytic graphite target was targeted with the energy provided by a hollow tungsten anode, producing a carbocation plasma stream in a vacuum chamber that had been evacuated to 1mTorr pressure. The plasma stream was directed to the PI target, and depositions were conducted for 5 minutes and for 10 minutes to determine the best conditions for this application.
4.6 Characterization

Coatings were characterized using the techniques listed below to evaluate their properties and performance. Where it was possible, identical methodologies were used.

- Film thickness as a function of deposition time
- Film adhesion
- Film composition
- Surface morphology by Atomic Force and Scanning Electron Microscopy
- Electrical Stability
- Biocompatibility
- XPS Spectroscopy
4.6.1 Test Methodologies

4.6.1.1 Film Thickness as a Function of Deposition Time

Deposition of all coatings was performed with the PI specimen mounted on a glass slide. Because the polymer is soft and expected to pose profile problems after deposition, it was assumed that the coatings of choice deposited on the glass slide beneath the PI substrate at the same rate, and hence exhibit the same thickness as the film on the substrate. This allowed more accurate measurements of film thickness than if the profilometer probe was used on a less firm surface than the glass. The area on the slide that was occupied by the polymer during deposition was coating-free, and served as the baseline for the measurements. Film thickness was measured for all coatings with a Tencor P-10 profilometer (Figure 21), and five (5) measurements were performed for each specimen. This method employed a nm-dimensioned stylus that was pulled across a surface. When the stylus encountered the ridge formed by the edge of the applied thin film, it was forced to change its position relative to the surface of the glass slide. A plot of the horizontal and vertical distances traveled was generated and the film thickness determined by the vertical distance traveled by the stylus.

Figure 22. Image of Tencor P10 Profilometer
4.6.1.2 Surface Morphology

4.6.1.2.1 Atomic Force Microscopy (AFM)

AFM images were captured using a Dimension 3000 atomic force microscope equipped with a NanoScope IIIa controller (Digital Instruments, Figure 22) and commercially available tapping mode Nanosensors (Digital Instruments). The instrument was operated in tapping mode such that, the cantilever was suspended within a few angstroms of the surface. In tapping mode, the cantilever tip interacts with atomic forces emanating from those surfaces, rather than directly with the film, preventing damage to the film and the soft PI substrate. Tapping mode also enhances the life expectancy of the sensors as the sharp tip could pierce the thin film coating underlying substrate. This occurrence would create tremendous drag on the tip, resulting in inaccurate measurement and/or breakage. These sensors have a nominal spring constant of 45 N/m and resonance frequency of 385 kHz. Coated samples were mounted as coated (without any cleaning procedures) and images recorded at room temperature in air at a scan rate of 0.803 Hz.

Figure 23. Dimension 3000 Atomic Force Microscope (close-up is cantilever tip)
4.6.1.2.2 Scanning Electron Microscopy (SEM)

Specimen surface morphology and chemical composition was determined simultaneously, using a Leo 430i scanning electron microscope equipped with an Oxford Energy Dispersive Spectrometer (SEM-EDS) operated at 10^{-5} Torr. Prior to SEM measurements, samples were sputter-coated with gold to serve as conductive layer if necessary, using an Ernest Fullam instrument for sputtering. A typical SEM is shown in Figure 24.

![Figure 24. Typical SEM](image)

4.6.1.3 Chemical Analysis

4.6.1.3.1 Energy Dispersive X-Ray Analysis

The Leo 430i Scanning electron microscope at the Aerospace Materials Function (AMF), Redstone Arsenal, AL was utilized. The instrument had been upgraded with an Oxford Inca X-Sight 7059 EDS (SEM-EDS). Typical components of the EDS are shown in Figure 25.
The specimen, a poly(imide) implant that has been coated with DLC or AAO thin film was seated firmly on an aluminum platen. A thin layer of gold was sputtered on top of the film to provide a conductive surface and prevent charging effects from obscuring the image during the study. A piece of copper tape was placed on the edge of the sample holder, and was used to calibrate the Energy Dispersive Spectrometer (EDS) prior to collecting an EDS spectrum. Calibration was performed every two hours the instrument was in use. The spectrum was analyzed used to semi-quantitatively determine the film composition.

4.5.1.4 Film Adhesion

The adhesion of AAO and DLC-films were determined with a standard Scotch™ tape test. Adhesion measurements were performed only on large specimens. Tests were performed at different locations along the specimens, with sample sizes of 0.5 cm². Identical spots on the specimen were not be used for two or more successive adhesion tests. The tape was inspected visually for evidence of film delamination, and the results recorded on a pass/fail basis.
4.6.1.5 Electrical Integrity

To ensure that the coatings will enable implant survival in the hostile environment in vivo, electrically functional implants were coated and sent to others responsible for soak testing the implants. The thin film-coated implants provided by the graduate student were soaked in normal saline at body temperature in a Soak Test Station developed at MIT’s Draper Labs, with an electrical bias of 1V for a period of three (3) months, and leakage current was measured. The specimens were allowed to dry for a period of time and the test sequence was repeated at 3 month intervals. To secure the samples while in test, a special jig was designed and produced by one of our collaborators (Figure 26).

Figure 26. Clamping system designed for soak test
Credit: Dr. Ofer Ziv, BRIP

4.6.1.6 Biocompatibility Studies

Biocompatibility studies were performed at the Mass Eye and Ear Infirmary in Cambridge, Massachusetts by Ophthalmologist Dr. Sandra Montezuma, using specimens coated with various materials, to include AAO and PLA DLC. “Biocompatibility” is a
relative term. It is generally evaluated in different environments, based upon implant interaction with the immune system via the degree of exposure to biological specimens, such as tissue, cells, serum, blood etc. Higher exposure offers higher opportunity for interaction that can result in implant encapsulation in fibrous tissue, the formation of potentially lethal blood clots or the onset of infection. Implantation of any device in the subretinal space has the potential to injure the delicate tissue, if for no other reason, denial of nutrients from the retinal choroid through the implant and into the already diseased retina\(^{(82)}\). Such injury is typically accompanied by the release of glial fibrillary acidic protein, GFAP, and Mueller cell glia\(^{(83)}\).

These reactions can result in glial cell scar tissue encapsulating the implant over time, and thickening. In previous experiments, glial cell adhesion proved to be one of the bigger problems associated with the implant presence in the subretinal space. This scar tissue can build to depths of approximately 100 \(\mu m\). It poorly conducts electrical signals, but rather adds to electrical impedance, and hence interferes with the transduction of impulses meant to stimulate artificial vision. As this implant is only 10 \(\mu m\) thick, glial cell adhesion of 100 \(\mu m\) would preclude any signal transmission to the retinal ganglia, rendering the device impotent.

A biocompatibility study protocol was approved by the Animal Care Committee of the Massachusetts Institute of Technology (MIT), and included provisions for control procedures to monitor effects of surgical technique. 5.0 x 0.5 mm x 10.0 \(\mu m\) thick PI specimens were implanted in the subretinal space of Yucatan miniature pigs (20 kg). All animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research. Animals were
anesthetized and a vitrectomy performed to create a retinal detachment in the superior-nasal retina of the right eye by subretinal injection of a balanced saline solution. Surgery was stopped at that point in 2 animals that served as non-implanted control subjects. A second pair of control animals was treated in the same manner, except that a single strip of uncoated PI was placed into the retinotomy for two minutes before removal. Others in the UAH research team had submitted specimens coated with Parylene and with Poly(vinyl) pyrrolidone. In total, PI strips coated with each of 5 materials were tested and one bare PI strip was tested as a control in one animal from each experimental group. A single strip of coated PI was implanted in the subretinal space through the retinotomy of 24 animals (5 animals per coated material type). After 3 months of chronic implantation, the animals were euthanized and histological slides prepared for evaluation of the tissue adjacent to the implants\(^{(84)}\). Biocompatibility was evaluated by analysis of the histology slides for 15 criteria, and paired, two-tailed Student’s t-tests were used for statistics development.

Post-surgically, the animals were treated with antibiotics and pain medications as appropriate. ERGs were conducted at one (1) week, three (3) weeks and three (3) months to monitor retinal electrical activity, and fundus images were collected at one month intervals to monitor the condition of the post-implant fundus.
Chapter 5  RESULTS AND DISCUSSION

5.1  AAO

5.1.1  Film thickness

Data were gathered for two (2) deposition times to determine which deposition time would produce the thinnest suitable film for the application. The first time was 1890s, with a target film thickness goal of 10nm. This film was so poor that additional characterization was not performed. Table 2 presents the data for trials at 1890s and 2835s. It is important to note that deposition rates for both time trials are identical at 0.06 nm/s. This indicates that one could determine a desired film thickness and adjust the time parameter to achieve the target result.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Thickness (nm)</th>
<th>Trial</th>
<th>Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112.30</td>
<td>1</td>
<td>176.40</td>
</tr>
<tr>
<td>2</td>
<td>111.70</td>
<td>2</td>
<td>179.10</td>
</tr>
<tr>
<td>3</td>
<td>112.80</td>
<td>3</td>
<td>173.10</td>
</tr>
<tr>
<td>Mean</td>
<td>112.27</td>
<td>Mean</td>
<td>176.20</td>
</tr>
<tr>
<td>σ</td>
<td>0.55</td>
<td>σ</td>
<td>3.00</td>
</tr>
<tr>
<td>Rate(nm/s)</td>
<td>0.06</td>
<td>Rate(nm/s)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

As-deposited films were examined using light microscopy and film condition characterized, per Table 3.
Table 3. AAO Deposition Results: Film Condition

<table>
<thead>
<tr>
<th>Deposition Times (s)</th>
<th>Target thickness (nm)</th>
<th>Actual Thickness (nm)</th>
<th>Visual Results by Light Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>189</td>
<td>10</td>
<td>Not determined</td>
<td>Cracks and pinholes</td>
</tr>
<tr>
<td>1890</td>
<td>100</td>
<td>112.7</td>
<td>Cracks and pinholes</td>
</tr>
<tr>
<td>2835</td>
<td>150</td>
<td>176.2</td>
<td>Fault-free</td>
</tr>
</tbody>
</table>

AAO film adhesion was evaluated by a simple Scotch® tape test. The tape was applied to the coated, dry surface and flattened to ensure good contact between the tape and the coating. The tape was quickly pulled away and visually inspected for the presence of delaminated film. Twenty-one (21) samples were tested in this manner. The PI implant material for seven (7) specimens tore without evidence of adhesion failure. Only one (1) specimen lost the coating from .04% of its surface area. It can be concluded that this deposition process is capable of producing an efficient and stable coating.
5.1.3 Chemical Composition

The Scanning electron microscope used for this study was equipped with an Energy Dispersive Spectrometer, which was used to analyze the AAO chemical composition. The EDS spectrum (Figure 27) is shown below. A small piece of conductive carbon tape was affixed to the edge of surface and extended to the platen, to prevent charging effects that could obscure the surface. Note there is a carbon peak present in the spectrum, due to the presence of this tape.

The spectrum was integrated by sectioning the peaks for aluminum and oxygen. Each was weighed on an analytical balance (Table 4) and the data used to calculate the empirical formula and the chemical composition (Table 5).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Al (g)</th>
<th>O (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0221</td>
<td>0.0188</td>
</tr>
<tr>
<td>2</td>
<td>0.0221</td>
<td>0.0190</td>
</tr>
<tr>
<td>3</td>
<td>0.0223</td>
<td>0.0191</td>
</tr>
<tr>
<td>4</td>
<td>0.0221</td>
<td>0.0191</td>
</tr>
<tr>
<td>5</td>
<td>0.0222</td>
<td>0.0191</td>
</tr>
<tr>
<td>Mean</td>
<td>0.02216</td>
<td>0.0190</td>
</tr>
<tr>
<td>σ</td>
<td>8.94E-05</td>
<td>1.30E-04</td>
</tr>
</tbody>
</table>
Table 5. Empirical and Chemical Composition

<table>
<thead>
<tr>
<th>Total Mass</th>
<th>Atomic Mass (g/mol)</th>
<th>Empirical Formula</th>
<th>Calculated Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass % Al</td>
<td>53.81</td>
<td>26</td>
<td>1.99</td>
</tr>
<tr>
<td>Mass % O</td>
<td>46.19</td>
<td>16</td>
<td>2.89</td>
</tr>
</tbody>
</table>

For a 100 g sample

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>53.81 g</td>
<td>26 g/mole</td>
<td>2.069</td>
</tr>
<tr>
<td>O</td>
<td>46.19</td>
<td>16 g/mole</td>
<td>2.887</td>
</tr>
</tbody>
</table>
Figure 27. EDS Spectrum for AAO.

Note the presence of a peak denoting the presence of gold in the film. A very thin layer of gold was sputtered on the surface of the specimen to enable performance of semiquantitative analysis.

5.1.4 Surface Morphology

5.1.4.1 AAO AFM

The AFM was operated in tapping mode, as this is the most stable mode of operation in air. In this mode, the oscillating cantilever actually taps the surface during each oscillation. Because of this contact, the oscillation slightly slows and changes phase with reference to the starting phase. This phenomenon produces what is called a phase image. AFM studies were performed on the AAO coated surface and detailed a smooth, pebbled surface morphology, as reported by Henry, et al. (Figure 28). The film was found to be fault free as no pinholes or cracks were observed. The height bar to the right of the image details surface height measurements. Many areas appear to be baseline,
while others appear to be a high as approximately 56.3 nm, compared to the color coded scale. This indicates that the most difference in topological height from the baseline is roughly 56.3 nm.

Figure 28. AFM Generated Surface Morphology

Figure 28 consists of an AFM image of AAO detailing film height based on data collected in tapping mode.

5.1.4.2 AAO SEM

Initial AAO depositions were performed for 1890 seconds with a target film thickness of 10 nm. SEM imaging of the resultant film at 2000X revealed a cracked, partially delaminated surface with pinholes (the small dark spots) as depicted in Figure 29.
Deposition time was increased to 2835 s, resulting in a film thickness of 176.2 nm. The films were shown to be smooth and fault free at 4.45x10^3 magnification as shown in Figure 30. When sputtered for 2835 s, the surface morphology became smooth and fault free, as is typical for films of appropriate thickness\(^{(85)}\). Note that the light objects on the surface are probably dust particles, and are out of focus when the AAO surface has been properly focused.

**5.1.5 Spectroscopic Ellipsometry for AAO**

Spectroscopic ellipsometry was performed at the NASA Materials Laboratory at Marshall Space Flight Center. AAO was characterized for refractive index, \(n\), and
extinction coefficient, $k$. The data were used to generate charts detailing these features over a range of wavelengths, where $300 \text{ nm} \leq \lambda \leq 1400 \text{ nm}$, at three angles of incidence: $75^\circ$, $80^\circ$ and $85^\circ$.

As shown in Figure 30 (below), over the 300-375 nm range, the index of refraction, $n$, for all three angles of incidence is seen to follow nearly identical slopes, representing values of $0.465 - 2.24$. Refractive index is expressed as the group velocity of light in air divided by the group velocity in a different medium, in this case, the thin film under study. Various data are reported in the literature for refractive indices, and these appear to be dependent on deposition methodology as well as ellipsometric angles of incidence. For AAO, index of refraction is reported in values between 1.63 and 2.59. These results are in agreement with the published values$^{(86)}$. As the wavelength approaches 400 nm, the curves seem to diverge, possibly due to angle of incidence, or perhaps due to irregularities or steps in the film surface.

![Aluminum Oxide Ellipsometry](image)

*Figure 31. Ellipsometric Spectrum for AAO*
Figure 31, a plot of extinction coefficient vs wavelength derived by spectroscopic ellipsometry, demonstrates overlapping curves that place the extinction coefficient for AAO in the range of 0.897-0.037\(^{(87)}\). French, Müllejans, and Jones place the extinction coefficients in the range of 0.0029 – 1.67, so that the experimental values appear to be in agreement. In this case, the curves for all three angles of incidence appear to loosely coincide. The extinction coefficient is a measure of how quickly the signal returned to a detector is damped, due to inelastic collisions with subsurface atoms causing scattering.

![Aluminum Oxide Ellipsometry](image)

**Figure 32. Ellipsometric Spectrum: \(k\) vs wavelength**

5.2 DLC Deposited by PLA

5.2.1 Film Thickness

Data were gathered for two (2) deposition times to determine which deposition time would produce the thinnest suitable film for the application. The first deposition time was 5000 pulses at 20 hertz, with a target film thickness goal of 10nm. This film
was so poor that additional characterization was not performed. The film produced on 5,000 pulses exhibited pinholes and some cracking, indicating that film thickness was not adequate to completely encapsulate the surface. In addition, the potential for crack growth to failure in such a film could result in severe damage to the retina and underlying structures. Table 6 presents the data for trials at 5,000 and 10,000 pulses. The film produced at 10,000 pulses was visually smooth and pinhole free, suggesting this film thickness was more suited to the application and was less likely to damage the eye.

Table 6. Film Thickness and Deposition Rates PLA DLC

<table>
<thead>
<tr>
<th>Trial</th>
<th>Thickness (nm)</th>
<th>Deposition Rate (nm/s)</th>
<th>Trial</th>
<th>Thickness (nm)</th>
<th>Deposition Rate (nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>117.67</td>
<td>0.24</td>
<td>1</td>
<td>64.40</td>
<td>0.26</td>
</tr>
<tr>
<td>2</td>
<td>117.63</td>
<td>0.24</td>
<td>2</td>
<td>59.86</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>110.5</td>
<td>0.22</td>
<td>3</td>
<td>64.46</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>118.95</td>
<td>0.24</td>
<td>4</td>
<td>58.93</td>
<td>0.24</td>
</tr>
<tr>
<td>5</td>
<td>111.07</td>
<td>0.22</td>
<td>5</td>
<td>60.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean</td>
<td>115.16</td>
<td>0.23</td>
<td>Mean</td>
<td>61.58</td>
<td>0.25</td>
</tr>
<tr>
<td>σ</td>
<td>4.04</td>
<td>0.09</td>
<td>σ</td>
<td>2.65</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Note that the deposition rates for 5,000 and for 10,000 pulses are practically identical, indicating that the deposition process itself is quite stable.

5.2.2 Film Adhesion

Specimens for biocompatibility testing, 0.5 mm X 5.0 mm X 10 μ were coated using the PLA methodology. These specimens exhibited smooth fault-free films with excellent adhesion. Adhesion was tested in the same manner as the AAO films, and no adhesion failures were noted; however, this excellent adhesion was not exhibited for coatings on larger implant designs. It is theorized that the small size of these specimens precluded full development of the internal stresses known to occur with this deposition method.
The PLA process is known to produce films with high internal residual compressive stresses that result in film delamination \(^{(88-89)}\). Figure 32 below shows dark areas of disturbances due to film separation from the surface \(^{(90)}\). The internal compressive forces are even more evident on the Surgical Experiment Implant type (Figure 32), in that in addition to the film curling and warping, coating delamination occurred. Young Kyu Hong, et. al. report that thermal annealing at temperatures up to 800\(^{\circ}\)C can relieve the residual compressive forces \(^{(91)}\). Unfortunately the underlying PI would undergo thermal degradation at that temperature, and the implant would be destroyed. Additionally, the delamination was observed to occur within moments of removal from the vacuum chamber, and so would not survive for the thermal relaxation process.

It is interesting to note that these thin films on stiff surfaces, such as glass, appeared to adhere tightly to the glass substrate. It could be that the flexibility of the PI films contributes to the delamination process by warping and folding with the stress, rather than resisting deformation.

![Disturbances](image)

*Figure 33. Disturbances on the surface of an implant, possible evidence of film adhesion failure.*
The implant in Figure 34 above had only been coated on one side. It was hypothesized that, if one could rotate the specimen in the plume, and coat both sides during one deposition session, the residual compressive forces would balance one another, and the film integrity might be preserved. An aluminum sample holder (Figure 35) was machined for the second generation implant blanks and additional depositions were attempted.

Note that the specimens were secured to the holder with small screws at the tabs designed for retinal tacks.
In spite of changing the method to coat both sides of each implant in a single session, once the specimens were removed from the sample holder, film delamination occurred rapidly, lending support to the thought that film flexibility may contribute to adhesion failures.

Other methods were employed to attempt problem resolution. The distance from the target to the substrate was reduced in an attempt to have more incoming cations strike the film perpendicular to the surface, embedding more securely into the PI substrate. Next, the flux density was varied from 6.00 J/cm² to 8.33 J/cm², to impart additional energy to the cations to improve cation penetration into the PI. Deposition temperature was raised to 100°C, to partially soften the PI substrate enough to enhance penetration, to no avail. Because adhesion was so problematic, and the only reported, proven methods to relieve this stress involved temperatures detrimental to PI stability, PLA DLC was abandoned as a candidate for this application.

In summary, changing deposition parameters to increase the energies of impinging cations, to limit the distance from target to substrate to control cation flight paths for normal surface impingement, and coating both sides by rotating the specimen in the plume all failed to produce an adherent film on the flexible substrate. Earlier attempts to coat specimens mounted on glass slides produced a film that adhered tightly to the glass. PLA methodology appears to be inappropriate for coating flexible films.

5.2.3 PLA DLC Film Composition

The chemical composition was semi-quantitatively analyzed by Energy Dispersive Spectroscopy (EDS). The EDS spectrum (Figure 36) is shown below.
pronounced carbon peak was noted at approximately 0.25 KeV. A contaminant level oxygen peak was noted in the spectrum, possibly due to oxygen adsorbed on the chamber walls prior to evacuation, and released once the chamber reached equilibrium. Because EDS is insensitive to light elements, the presence of hydrogen in the film, while expected, cannot be demonstrated by this technology.

![Figure 36. EDS Spectrum of PLA DLC](image)

### 5.2.4 PLA Film Morphology

#### 5.2.4.1 PLA DLC AFM

The AFM images shown in Figure 37 appear to be striated, rather than pebbled. Some specimens were suitable for additional studies, but most deposition attempts resulted in poor quality films. PLA-derived DLC is known to develop internal compressive stresses during deposition due to the limited mobility of previously deposited film as new ions bombard the surface, subplanting existing atoms\(^{(92-93)}\).
The image to the left measured the film height, while the image on the right details the phase shift caused by intermittent contact between the cantilever tip and the surface.

5.2.4.2 PLA DLC Scanning Electron Microscopy

SEM images were taken using a LEO 430i scanning electron microscope. Image 38 details a PLA DLC film for deposition time = 1890 seconds. This film exhibits cracks, delamination and pinholes in its surface. Bubbles of various sizes were observed on the surface, possibly representative of micro-scale film delamination. One can also see out of focus particles that are probably dust on the surface. It was expected that the film would be dielectric, and a thin film of gold would have to be sputtered onto the surface to prevent charging effects from obscuring the image; however, this was not the case. One explanation could be that there was a slightly higher graphite content in the film that usually produced by this technology, and the presence of double bonded carbon species allows for some electron mobility. Another explanation could be that the presence of
hydrogen atoms near the surface or at the surface could impart a p-type surface conductivity\textsuperscript{(94)}. The p-type conductivity is attributed to the flow of electrons from near the Fermi level into the conduction band for a given material.

Note the black pinholes and delaminated fragments on the surface in Figure 38.

The SEM image, Figure 39, of a PLA film deposited over 10,000 pulses shows a smooth, pinhole-free surface\textsuperscript{(95)}. One can see several very small areas that appear to be raised. These may be evidence of micro-delamination, although this is not a certainty. The smallest PI samples intended for biocompatibility did not delaminate, possibly because they were too small for these stresses to accumulate to the point that stress risers remained inactive.
Note areas that appear to be raised, possibly representing micro-delamination. Smaller, less focused particles are probably superficial dust.

5.2.5 Ellipsometry Studies

Ellipsometry was not performed because this material was abandoned due to poor film adhesion. Methodology changes were employed to attempt problem resolution, but residual compressive forces within the coated implants resulted in total adhesion failure.

5.3 VAVD DLC

5.3.1 Film Thickness and Deposition Rate

VAVD DLC depositions were performed for five (5) minutes and for ten (10) minutes. The film thickness and deposition rates were determined as in all other material trials. Table 7 details the results of the five minute trials, and Table 8 shows results of the 10 minute trials. It is important to note that the deposition rates for each time period are in agreement when the standard deviation is taken into account.
Table 7. Film Thickness and Deposition Rate Five Minute Depositions

<table>
<thead>
<tr>
<th>Trial</th>
<th>Thickness (nm)</th>
<th>Deposition Rate (nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65.07</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>52.12</td>
<td>0.17</td>
</tr>
<tr>
<td>3</td>
<td>61.52</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>51.69</td>
<td>0.17</td>
</tr>
<tr>
<td>5</td>
<td>53.70</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean</td>
<td>56.82</td>
<td>0.19</td>
</tr>
<tr>
<td>σ</td>
<td>6.09</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 8. Film Thickness and Deposition Rates Ten Minute Depositions

<table>
<thead>
<tr>
<th>Trial</th>
<th>Thickness (nm)</th>
<th>Deposition Rate (nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85.86</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>95.59</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>96.43</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>122.63</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>106.91</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean</td>
<td>101.48</td>
<td>0.17</td>
</tr>
<tr>
<td>σ</td>
<td>13.97</td>
<td>0.02</td>
</tr>
</tbody>
</table>

5.3.2 VAVD Adhesion

VAVD adhesion was ascertained in the same manner as that for AAO and PLA DLC. The results of these trials demonstrated the presence of an adherent film, with two (2) partially delaminated specimens in which the delaminated areas represented 0.05% of the total surface area. The area from which coating was removed was measured and that area was divided by the total surface area tested to derive the percent of the total film area that was lost during this test.

5.3.3 VAVD Chemical Composition

The chemical composition was semi-quantitatively analyzed by EDS. The EDS spectrum (Figure 40) is shown below. The surface was sputtered with a thin layer of gold prior to testing, to prevent charging effects that could obscure the surface. As expected, a
predominant carbon peak was noted at about 0.25 KeV, along with a small oxygen peak was noted in the spectrum, possibly due to oxygen adsorbed on the chamber walls prior to evacuation, and released once the chamber reached equilibrium. A contaminant level gold peak was also observed, caused by the sputtering process. Because EDS is insensitive to light elements, the presence of hydrogen in the film, while expected, cannot be demonstrated by this technology.

Figure 40. EDS Spectrum for VAVD DLC
5.3.4 VAVD DLC Surface Morphology

5.3.4.1 VAVD AFM

AFM studies of the VAVD DLC (Figure 41) proved a smooth, fault free surface with an orange-peel morphology. Note that the surface features appear to be smaller than those shown in the AAO image. This morphology is consistent with images found in the literature (96, 97). The image to the left showed that the height of the cantilever above the film is only 10 nm on a 1.0 µM x 1.0 µM field. The image to the right demonstrated the phase difference over the same field of study.

![AFM Images of VAVD Diamond](image)

Note the height scale on the left image is 10 nm. At lower magnification two steps were observed (Figure 42). The step height analysis (Figure 43) suggests that the film height dropped by approximately 60 nm before the second step was measured at 45 nm. It would appear then, that the second step ended, suggesting that the second step rests at -15 nm from the first step surface. These features may be a result of cations approaching at less than optimal angles of incidence and embedding themselves unevenly.
across the surface\textsuperscript{(98)}, or due to the length of the deposition time being inadequate to fill in the steps, as often happens in ion-bombardment processes. As this method has not been studied for use in the retinal subspace, it is unknown if the multiple steps would present a surface roughness problem that could be damaging to the delicate tissue in contact with it.

The image above represents a 1.0 \( \mu \) X 1.0 \( \mu \) scan of the surface, using a cantilever height of 10 nm.
2 steps: *
Step-height investigation
1. Step: 23 nm
2. Step: 45 nm

The Figure 44 image was taken after sputtering a thin film of gold onto the VAVD DLC surface. The as-deposited surface was non-conductive, and gold application was necessary for SEM imaging. Note the scant coverage, yet it is enough to prevent charging effects from obscuring the underlying coating during SEM analysis. The image is a 1.0 mm X 1.0 mm field of view at a cantilever height of 10 nm.
5.3.4.2 VAVD SEM

As before, the LEO 430i was used to image the VAVD diamond surface. The surface was sputtered with a thin layer of gold using an Ernest Fullam instrument. Per Figure 45, The film was found to be smooth and fault-free. Particles of dust were noted on the surface.

5.3.5 VAVD Ellipsometry Studies

Spectroscopic ellipsometry was performed at the NASA Materials Laboratory at Marshall Space Flight Center. VAVD DLC was characterized for refractive index, \( n \), and
The data were used to generate charts detailing these features over a range of wavelengths, where $300 \text{ nm} \leq \lambda \leq 1400 \text{ nm}$, at three angles of incidence: $75^\circ$, $80^\circ$ and $85^\circ$.

Figure 46 depicts the refractive index dependence on wavelength and ellipsometric angle of incidence. The index of refraction is plotted versus wavelength as shown below, for all three angles of incidence. The data are seen to follow nearly identical curves, representing values of 9.63 to 0.232. Various data are reported in the literature for refractive indices, and these appear to be dependent on deposition materials and methodology as well as ellipsometric angles of incidence. Values reported in the literature vary from 0.01 – 2.1$^{(99-101)}$. The abnormally high refractive index that was calculated could be due to fringe effects, or perhaps to interference due to the step height changes in the film. The refractive index measures the change in the group velocity of light in air to that within the thin film matrix.

Figure 46. Ellipsometric Spectrum: VAVD Diamond
Figure 47 depicts the extinction coefficient’s dependence on wavelength and ellipsometric angle of incidence. The extinction coefficient demonstrates the damping effect of inelastic collisions of the incoming energy with atoms or other scattering centers in the solid film. Calculated values for $k$ vary from 0.078 to 2.076. Table 1 reports the published range(s) for this material between $0.35 - 2.1^{(99-101)}$. It was difficult to find a paper that reported data for this method using these materials, over the range of wavelengths that were used in this study. For that reason, it is possible that the results of this study are in agreement with previously published data.

![Figure 47. Ellipsometric Spectrum: VAVD DLC](image)

**5.3.6 X-ray Photoelectron Spectroscopy (XPS)**

XPS was performed using a KRATOS instrument equipped with a 1253.6 eV Magnesium source (Mg Kα$_{1,2}$) and 1486.6 eV Aluminum source (Al Kα$_{1,2}$). This method typically allows for analysis of sp$^2$/sp$^3$ ratios in DLC films via binding energy analysis. Soft X-rays were generated at the selected source. These X-rays bombarded the film, causing ejection of core electrons, followed by electronic rearrangement to
accommodate the film’s return to the lowest energy state possible. This rearrangement was accompanied by emissions of photons of characteristic energies and Auger electrons. Figure 47 depicts the XPS spectrum recorded for VAVD DLC while Figure 48 shows and enlarged portion of the spectrum with features of interest. There appear to be contaminant levels of sodium and oxygen in this analysis, but the main features of interest are related to the C1s transitions.

![XPS Spectrum](image)

**Figure 48. XPS Spectrum of VAVD DLC**

![XPS Spectrum Enlarged Area of Interest](image)

**Figure 49. XPS Spectrum Enlarged Area of Interest**

Park, *et. al.* report the binding energy of C–C bonds at 284.8–285.1 eV and that of C=Cs at about 284.4 eV\(^{(101)}\). In either case, the C1s electrons will be ejected from the
carbon atoms at approximately 285.0 eV. This spectrum is in agreement with the published values.

5.4 Electrical Stability

A dormant soak test station was refurbished at MIT’s Draper Labs and a highly specialized clamp was designed to secure specimens and ensure proper electrical connections were maintained throughout the test periods. The system was expanded to twenty (20) channels, and one of the 20 was assigned a 10 GOhm +/- 1% resistor to serve as a control. Wiring verification was performed and initial hardware testing was conducted without electrical loading. Leakage current was measured in the $10^{-13}$ to $10^{-14}$ amp range.

Soak testing began July 30, 2006\textsuperscript{(102, 103)}. Specimens that had been coated with VAVD diamond and AAO were soaked in normal saline for a period of three (3) months at 37°C under constant bias. Leakage current was monitored throughout the process. Per a presentation given by Mr. Ken Karcich at the BRIP 2003 meeting, all measurements were found to be in the 0.5 pA range. All specimens were removed from the test station, thoroughly dried and stored for three (3) months. Soak testing under the conditions identical to those described above was repeated, and yielded the same results. Kelly and Shire et al. report that the 256 channel implant currently in development is equipped with a chip that delivers charge in 1 µA increments up to 250 µA. The reported leakage current of 0.5 pA, is 6-7 orders of magnitude below the stimulation current, and should not affect data transmission signals from reaching the retinal ganglia. Specimens were judged to be electrically stable, and therefore, fit to proceed to biocompatibility studies.
5.5 Biocompatibility Studies for AAO and PLA DLC

Biocompatibility studies were performed at the Mass Eye and Ear Infirmary (MEEI) by Dr. Sandra Montezuma. Prior to surgical implantation an electroretinogram (ERG) was performed for each pig to measure and record normal retinal activities and to establish baseline functions for each animal. Noise recordings were performed so that these could be subtracted from the ERG recordings, to produce more accurate records for post-surgical analysis.

ERGs were conducted at one week post-surgery were found to be at or above the baseline developed prior to surgery for the control animals and all but AAO, PEG and Parylene; however, these were statistically insignificant differences that resolved over time. At the 12-week ERG, all materials were judged to have no negative impacts on electro-retinal function. This was confirmed by comparison of both raw and normalized data and performing the paired, 2-tailed Students t-tests ($P = 0.2$ and $0.8$, respectively).

Fundus images collected monthly confirmed that the surgical retinal detachments healed as expected and no retinal detachments developed over the course of the study period. All images showed some cellular disruption, but it is not known whether these results were caused by the surgery, the presence of the implant, or both. Representative images are shown below for PI, AAO and PLA DLC.
Figure 50. Pig Retina with PI Implant 3 months

Figure 51. Pig retina with AAO coated implant at 3 months.
Migration of RPE cells was noted for this coating as well as the AAO coating.

At three (3) months after surgery, the animals were euthanized and the implanted eye enucleated. Histology slides were prepared and evaluated for fifteen (15) criteria to include, “inflammation, hemorrhage; outer nuclear layer or inner nuclear cell loss or disorganization; Müller cell hypertrophy, continuous or discontinuous reactive tissue and disruption, proliferation, metaplasia or migration of the RPE on both sides of the implant. These data were tabulated and analyzed using paired two-tailed Student’s t-tests.

Results for all tested materials were analyzed, and the number of defects on 1100 control animal histology slides were tabulated along with the number of defects on 1500 histology slides for each type of coating. Table 9 presents the summary data.
Table 9. Tabulated Defect Data for Histology Detailing Five (5) Coatings and Controls

<table>
<thead>
<tr>
<th>Controls or Implanted Materials</th>
<th>Number of slides with abnormalities</th>
<th>Statistical Significance: Paired Student t-tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>96/1100</td>
<td></td>
</tr>
<tr>
<td>AAO</td>
<td>697/1500</td>
<td>$P = 0.00005$</td>
</tr>
<tr>
<td>PLA DLC</td>
<td>515/1500</td>
<td>$P = 0.00200$</td>
</tr>
<tr>
<td>PI</td>
<td>588/1500</td>
<td>$P = 0.00004$</td>
</tr>
<tr>
<td>PVP</td>
<td>347/1500</td>
<td>$P = 0.00500$</td>
</tr>
<tr>
<td>Parylene</td>
<td>307/1500</td>
<td>$P = 0.02200$</td>
</tr>
<tr>
<td>PEG</td>
<td>300/1500</td>
<td>$P = 0.01900$</td>
</tr>
</tbody>
</table>

In terms of this study of fifteen histological criteria, the materials producing the fewest anomalies, in order of preference would be PEG > Parylene > PVP > a-C > PI > AAO. Montezuma, et. al. concluded that all of these implant materials caused some impact to the tissue with which they were in contact, but that the study does not determine whether the clinical abnormalities would negatively impact long-term implant performance, or if the coatings would deteriorate in the saline environment in the eye over time. Additional studies need to be conducted to evaluate these potential issues. Histology slides provided by MEEI are shown below (Figures 54-56) to demonstrate some of the criteria that were evaluated for PI, DLC and AAO.
Figure 53. 10X histology slide of post mortem pig retina.

Obvious cellular disruption is shown, although no retinal detachment occurred. Discontinuous RPE cell clumping over the implant was noted along with were INL and ONL disorganization and cell loss. Müller cell hypertrophy, RPE disruption, metaplasia, migration and proliferation were also observed.

Figure 54. 10x Postmortem pig retina implanted with PLA DLC coated PI.
No retinal detachment was observed, but some slides demonstrated discontinuous RPE cells above the implant. INL and ONL disorganization and cell loss were also observed. Müller cell hypertrophy, RPE disruption, metaplasia, migration and proliferation were also noted.

INL and ONL cell disorganization and loss of greater than three (3) layers were found along with RPE disruption, cellular metaplasia, cell migration and proliferation.

5.6 Conclusions and the Path Forward

The use of AAO and DLC are not new to the medical community for use as biocompatible surface modifiers for implant applications; however, there is little information on the deposition and characterization of these films on polymeric substrates for use in the retinal subspace. There is even less to be found where these substrates are
to be used to provide both biocompatibility – protecting the host from immune response to a foreign body - and hermeticity – protecting the embedded electronic circuitry from corrosion that would be caused by the incursion of saline-containing fluids. This project provided an important contribution to the longevity of the implant *in vivo*, allowing for an improved quality of life for patients suffering a heretofore irreversible loss of vision and resulting loss of independence and self-confidence.

One cannot base absolute compliance with research goals on any one study. It would be prudent to repeat the biocompatibility study, using the same test protocol and materials. Should the test data demonstrate reproducibility, it would be wise to down-select to three or four materials for further research.
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Appendix A
Use of the Scanning Electron Microscope and Energy Dispersive Spectrometer
The Basics
Operation of the Scanning Electron Microscope/Energy Dispersive Spectrometer

Two dedicated computers operate each of the instruments that comprise the SEM-EDS. To use the instrument, both computers must be booted. The SEM is activated initially by the following steps:

- Open the LEO UIF program
- When the program has loaded, a toolbar is observed. The toolbar has the following icons
  - A Stop Light to activate the macro that controls the SEM
  - A Light Bulb that activates the tungsten cathode
  - A Lightning Bolt used to control the probe current
  - A Magnifying Glass with a light bulb in it to adjust the image brightness
  - A Magnifying Glass used to control zoom
- A window below the toolbar presents a single message at a time from a drop-down menu. The messages are: “pump,” “vent,” “ready,” “shut down,” and “at air.” At start up, the “ready” message is displayed, as the instrument is kept at vacuum even when not in use to minimize the concentration of adsorbed gases on chamber surfaces that will interfere with its function.
- Progress toward operating pressure can be monitored by selecting the Status option from the “View” menu. It typically takes about 4 minutes to reach operating pressures.
- Unlock the EDS chamber clamp, select “vent” from the menu and wait for the EDS to come to atmospheric pressure. At this time the chamber will open.
• The sample holder is secured to the SEM stage and the chamber is closed and locked.

• Select “pump” and wait for the instrument to develop a vacuum of 9.5 x 10^-6 Torr.

• The Stop Light is clicked using the right mouse button and the macro executes. The macro sets the initial probe current, and positions the specimen at a distance of 16-18 mm from the probe. This distance is usually maintained for the duration of the experiment.

• Click on the Light bulb. The cathode current is displayed in a window to the upper right of the monitor.

• Use the right arrow key to adjust the probe current. It is usually set at 500 milliamps (mA) at start up. Raise the current in increments of 500 mA, and allow the tungsten filament to equilibrate for 15-20 seconds before proceeding to the next increment. Repeat until the filament current is 2 A.

• At this point, continue to slowly increase the filament current until the image brightens, then darkens. Continue until the image brightens a second time, and then reduce the probe current slightly, until no significant change occurs in the image brightness. At this point, the filament is saturated and the instrument is ready for use.

• When new, the filament typically becomes saturated at about 2.7 A. As the filament ages, saturation occurs at lower and lower amperage. Pausing between each incremental increase in current prevents shocking the filament, which will cause it to “blow out” prematurely.
The instrument has a joystick control to allow the user to locate a site of interest by adjusting x, y and z coordinates of the stage. Use the joystick to survey the sample.

Click on the magnifying glass, then hold the left mouse button down and drag the mouse to the right to increase magnification, or to the left to decrease it. Usually, one wants to increase magnification in order to observe very small sections of the specimen at a time.

As magnification is increased, the focus will blur. Click the center mouse button and drag to refocus the image. If the surface is very smooth, it is helpful to find a speck of dust or other debris on the surface on which to focus. Use the joystick to keep the dust speck positioned at the center of the monitor until a good focus is achieved as you continue to magnify the image.

When the desired magnification is achieved, select a site of interest.

Turn your attention to the second computer, and load the INCA software.

Select the option to acquire an image.

Return your attention to the first computer and select “tools.” From the drop-down menu, select “External Controls”. A dialog box appears. Click “On.” This freezes the image on the first computer’s screen. The second computer’s monitor will then acquire the image. At the bottom of the image, a micron marker is displayed along with the magnification at which the image has been acquired.

The INCA software will prompt the user to log a project name, which is used to maintain a file system of images and spectra. The images and spectra are automatically saved in separate sub-files. The user has an option to rename the images and spectra; otherwise the system automatically names images as “Site of
Interest 1”, “Site of Interest 2,” etc. Similarly, the system names the spectra as “Spectrum 1,” “Spectrum 2,” etc.

- After acquiring the image, position and focus the copper strip. From the INCA software, select “Calibrate” and then “Run”. A dialog box will appear.

- This dialog box monitors the detector’s “dead time.” In order to achieve a high resolution spectrum, it is necessary to click the lightning bolt in the Leo software, and adjust the probe intensity until the dead time is set at 30 - 40%. This allows the detector to count the Auger electrons that reach it. If dead time is too high or too low, the detector will not be able to accurately count the Auger electrons or determine their energy properly, and the resulting spectrum will not be reliable.

- After the dead time is properly set, stop acquisition, and then re-start. The system will acquire a spectrum of the copper strip and use the Copper K-α line to calibrate the spectrometer.

- Reposition the sample in the EDS and select a site of interest and appropriate magnification on the first computer. Return to the INCA software and select “Acquire Spectrum.” Check and adjust the dead time if necessary, then stop and re-start acquisition.

- After completing the experiment, return to the Leo software and click the light bulb icon to select the filament current.

- Use the left arrow to reduce filament current to 500 mA.

- The menu window adjacent to the pump control window controls the filament. Select the drop-down menu and turn the filament off.

- Select the pump control window, and select “Vent” from the drop-down menu.
• Unlock the EDS chamber clamp. When the chamber reaches atmospheric pressure, it will open. Remove the sample and holder. Close and lock the chamber.

• Select pump and wait for the chamber to reach operating pressures.

• Click on the “File” menu and select “Shutdown.”

**Imaging System**

The scanning electron microscope contains a complex imaging system which consists of an electron gun and several magnetic lenses. The electron gun produces high energy electrons, which are focused into a collimated beam by the magnetic lenses before illuminating the specimen under study.

The electron gun is generally subdivided into a filament (cathode) which is held at a negative electric potential with respect to ground, a shield with a preset aperture which is held at a slightly positive electric potential, and an anode, held at very high positive potential with respect to the filament. Used in concert, these three components function as an electrostatic lens.

The most commonly used cathode is a tungsten hairpin filament, which is operated at $10^{-4} - 10^{-5}$ Torr and has a typical operating brightness of $\sim 10^5$ A/cm$^2$ steradian and an operating lifetime of 40-60 hours. A steradian is defined as conical in shape, as shown in the illustration. Point $P$ represents the center of the sphere. The solid (conical) angle $\theta$, representing one steradian, is such that the area $A$ of the subtended portion of the sphere is equal to $r^2$, where $r$ is the radius of the sphere (Figure 1).
The tungsten filament is capable of producing a minimum beam diameter of about 50 Å. Other electron sources include lanthanum hexaboride (LaB₆) and Field Emission (FE) guns. These are brighter (~10⁶ A/cm²·steradian and 10⁷-10⁸ A/cm²·steradian, respectively) and longer lived electron sources than the tungsten filament (~3000 hours and Indefinite, respectively), but are far costlier and require ultrahigh vacuum to operate efficiently than does the tungsten filament. The LaB₆ gun is operated in conditions lower than 10⁻⁵ Torr while the FE gun requires 10⁻⁹ Torr or better. The source can produce a beam diameter of about 25 Å, while that of the FE gun can be less than 10 Å.

The Leo 430I SEM is equipped with a tungsten hairpin filament cathode. When in use, electrons are produced by passing a current through the cathode, heating it to a point at which the voltage gradient between the filament and anode produces electrons which
are accelerated by the potential difference between anode and the cathode. A typical schematic of the SEM imaging system is shown in Figure 2.

In this schematic, the shield is labeled as a “Grid cap.” Another synonym for the shield is the Wehnelt cylinder. It is a slightly biased cup containing a round opening that is centered over the filament tip at a distance of approximately 1-2 mm. This opening serves to collimate the beam of electrons from the cathode, and direct them towards the anode. The boas, or slight voltage supplied to the shield serves to allow only electrons emitted from the tip of the filament to reach the anode. For this reason, the separation between the shield and the filament tip is critical. If the shield is positioned too far from
the tip, a wider beam of lower current is allowed to pass, resulting in poor imaging. If
these are too close, the filament will burn out quickly due to overheating.

Upon exiting the aperture in the shield, the electron beam is approximately 25,000-
50,000 Å in diameter. It is accelerated by the large potential difference between the anode
and cathode. A typical SEM can achieve an accelerating voltage between 1 and 30 KeV.
An experienced operator can choose the accelerating voltage based on specimen type and
the sort of data he/she wishes to acquire.

If the specimen to be evaluated is conductive, a higher accelerating voltage is
desired, while a nonconductive specimen must be imaged at much lower accelerating
voltages to prevent charging effects. This phenomenon will be discussed more fully in a
later paragraph.

Because the electron gun behaves like an electrostatic lens and optimal operating
conditions are based on the incandescent heating of the filament, current and filament
temperature are essentially identical. An increase in current requires an increase in the
filament temperature. If the filament is operated at lower than optimum temperatures,
only selective crystalline sections of the filament will produce electrons, effectively
producing multiple beam sources. This condition is referred to as “undersaturation,” and
a filament operated at these temperatures will fail prematurely. As filament temperature
is increased, more and more crystalline areas emit until the tip of the filament
symmetrically emits the electron beam. At this point, the filament is said to be
“saturated.” The operator is trained to observe both undersaturation and saturation by
monitoring the image display. On the Leo 430i, the initial filament current is 0.5A to
prevent electrical shock and premature filament failure. At initial conditions, the image is dark and cannot be observed. As amperage is increased, the image will brighten (undersaturation) and then fade. The current is increased slowly until maximum image brightness is observed. At this point, the operator will reduce the current slightly and the filament is saturated, and its tip will emit the electron beam in a manner that allows for maximum filament life, and optimal image production.

As previously mentioned, the beam diameter, also referred to as the spot size, exiting the gun assembly is 25,000 – 50,000 Å in diameter, nowhere near the beam diameter required to produce an acceptable image. The beam diameter is systematically narrowed by a series of convergent magnetic lenses to approximately 100 Å at the sample level. In terms of current, the beam is reduced from $10^{-4}$ A at the gun to $\sim 10^{-13}$ A at the sample surface. Spot size is critical to image resolution. In general, the smaller the spot size, the higher the resolution. A spot size of 50 Å produces the best resolution one can expect from a tungsten hairpin filament equipped SEM. Larger spot size is selected when one wishes to perform energy dispersive spectroscopy (EDS) with the instrument.

Magnetic lenses are also used to focus and magnify the image. The image is focused by varying the current passing through the final (objective) lens, thereby changing its focal length. Magnification is defined as the ration of the size of the final display to the distance the probe is scanned. One changes the magnification on the SEM by changing the area of the scan. This is accomplished by the use of a deflection coil, which may be part of the objective lens, or located just below it in the imaging system. The deflection coil serves to move the electron beam across the sample surface in a
square or rectangular pattern. The deflection coil is controlled by a scan generator and
deflection yoke. These are synchronized with the image display to produce a 1:1
correspondence between the position of the spot on the surface and the observed image. It
is this correspondence that produced the high depth of field that SEM achieves.
The surface of the specimen is scanned repeatedly during an SEM session. The scan rate
can be varied between 100-100,000 lines per scan. Very high scan rates produce nearly
static images, and are used to survey the specimen surface and select sites of interest, to
focus the image or perform maintenance related tasks such as column alignment.
Extremely slow scan rates (~30-120s/frame) are used to photograph the image because
the slow rate allows the electron beam enough time to interact more fully with the
specimen, which, in turn emits more data signals.

Stigmators are incorporated into the final lens to correct imperfections
(astigmatism) in the image that may be produced by minute flaws or inhomogeneities in
the magnetic lenses. These flaws produce astigmatism, or optical aberrations in the
images. The stigmators are weak lenses that oppose the asymmetric field that would
otherwise produce astigmatism.

An aperture made of platinum or molybdenum is located below the final lens. It
serves to stray electrons that are not part of the imaging beam, and reduces electronic
noise in the final image. The final aperture also precludes the formation of spherical
aberrations in the image, which would arise due to the fact that the fields imposed by
magnetic lenses are stronger at the periphery than at their centers. Unfortunately, the
aperture also reduces the final resolution of the image by limiting the angle of the
“extreme rays” that are allowed to pass. Some SEMs may have several of these apertures
beneath the objective lens. They are usually held in a column liner tube which extends
from the anode to the bottom of the column. All prevent the passage of stray electrons. Resolution, depth of field and image clarity are enhances by the use of small apertures. Larger apertures are useful for performing X-ray analysis, because they allow the use of a more intense probe.

In summary, the imaging system of the SEM is composed of an electron gun, an anode, magnetic lenses, stigmators and apertures. The electron gun produces the electron beam that illuminates the image and probes the surface of the specimen. A large potential difference between anode and cathode accelerate the electrons emerging from the gun, directing the beam toward the sample. The magnetic lenses serve to focus the electron beam into an effective probe. The stigmators are used to correct astigmatism that may be produced by flaws or inhomogeneities in the magnetic fields imposed by the magnetic lenses, and the apertures serve to block stray electrons and prevent them from reaching the specimen surface, minimizing electronic noise in the signal. Stigmators, magnetic lenses and apertures control image clarity. Small apertures enhance image clarity and large apertures allow for X-ray analysis of the specimen of interest. Very fast scan rates are used to select sites of interest and perform maintenance functions such as column alignment while very slow scan rates are used to photograph sites of interest as they allow more interaction between the electron beam and the sample surface, hence the release of more information from the surface.

**Data Signals and Detectors**

The SEM information system consists of the sample, which emits a variety of signals when probed with an electron beam, and a series of detectors. As previously mentioned, most samples to be imaged and analyzed using SEM are mounted on a conductive sample holder, usually an aluminum stub. Highly conductive specimens are
interrogated using high (20-30 KeV) probe currents while nonconductive samples must be sampled at much lower probe currents (~1-2KeV) to minimize overcharging of the substrate and loss of image clarity.

When probed at the proper current, the substrate surface atoms release a variety of signals, dependent on the type on interaction that exists between them and the primary electrons within the electron beam (probe). If collisions between the surface atom’s nucleus and probe electrons are elastic, backscattered electrons (BSE) are emitted. If collisions are inelastic, several phenomena occur simultaneously. The surface atoms absorb some of the energy from the probe, and emit discrete quanta of energy as secondary electrons, X-rays and photons of light (cathodoluminescence). Heat is also generated, in the form of phonons, or strain waves that travel through the substrate’s atomic lattice. Analysis of all of these data signals requires that appropriate detectors be incorporated into the instrument; however, few microscopes exist with enough different detectors to analyze all of the signals. Figure 1 provides a basic schematic a system with a number of potential detectors.

Figure A-3. Schematic of SEM Equipped with a Variety of Detectors
Backscattered electrons are used to generate the topography of the surface under investigation, and to gather some compositional information. In an elastic collision, the primary electron rebounds from the nucleus with a negligible energy loss of about 20%, and is slightly deflected (Rutherford Scattering) from its original path.

The angle of Rutherford Scattering is dependent on atomic weight of the atoms in the substrate. Atoms of low atomic weight (low “Z”) will tend to scatter the incoming electrons at a shallower angle, reducing the probability that these BSE will reach the detector, as depicted in Figure 2. Atoms of low Z are said to release few BSE (Figure 2). An atom of high atomic weight is said to release a greater number of BSE (Figure 32).

![Diagram of Backscattering by Low Atomic Weight (Low Z) Atoms](image)

Figure A-4. Schematic of Backscattering by Low Atomic Weight (Low Z) Atoms

Note that in Figure 1, the Back-scattered Electron (BSE) Detector is mounted just below the final lens of the microscope. The reduced probability of detecting such a scattering event renders SEM a rather insensitive tool for analysis of atoms of low atomic weight.
The probability for elastic scattering increases as the square of the atom’s atomic number, and inversely as the square of the energy. The backscattered coefficient, \( \eta \), describes the fraction of the beam’s electrons that escape from the specimen (Equation 1):

\[
\eta = \frac{(Z-1.5)}{6}
\]  

(1)

The backscattered coefficient increases with atomic mass, \( Z \), and is independent of accelerating voltage, if it is between 10 and 40 KeV. Figure 4 depicts the relationship of \( \eta \) with respect to \( Z \).

Figure A-5. Schematic of Backscattering by High Atomic Weight (High Z) Atoms

Figure A-6. Emission of Back-scattered Electrons as a Function of Atomic Mass, Z
As with any scanning technology, SEM probes the surface of a specimen to a depth that is dependent on the surface composition. This depth coupled with the beam width defines an “excitation volume,” also known as an information depth. The excitation volume defines the volume of the specimen from which data signals arise. Surface composition of high Z atoms prevent deep penetration of the specimen, resulting in a hemispherical excitation volume, while low Z surfaces allow more penetration and can attain an elongated tear drop shape (Figure 4).

![Figure A-7. Relative Excitation Volumes for Low Z and High Z Surface Compositions; the dashed lines indicate the application of low accelerating voltage while the solid lines are indicative of high accelerating voltage.](image)

The depth of penetration $d_p$, is related to $Z$ and the accelerating voltage as defined by equation 2.

$$d_p \propto \frac{W_a V_o}{Z \rho} \quad (2)$$

where $W_a$ is the atomic mass, $V_o$ is accelerating voltage, $Z$ is atomic number and $\rho$ is the density. Obviously, the penetration depth decreases with increasing $Z$.

Electron and other signal emission from a specimen are also influenced by the electrical conductivity of the sample. Metals readily emit electrons, but substrates such as plastics or ceramics generally absorb the primary beam, and accumulate a net negative charge. As this charge increases, the primary beam can be deflected, and the resulting image is poor. One generally sees large bright areas with no resolution of surface details due to these so-called “charging effects.” The operator can compensate for charging effects by applying a thin layer of conductive material such as gold, to provide the charge...
with a path to ground, thereby eliminating the charging effect and improving image resolution, but compromising the ability to determine surface composition. Newer instruments, such as that at AMF can be programmed to ignore signals from added conductive layers.

Primary electrons can also penetrate to atomic nuclei. In this case, core electrons can be ejected from the sample provided the primary beam energy exceeds the electron’s binding energy. Atomic electrons from higher energy levels (think of the Bohr model of the atom) fall into the empty space in a sort of cascade, and photons are emitted from the specimen in the form of X-rays with energies equal to the energy difference between the two shells. X-rays originate from deep within the excitation volume, and because their energies equate to the energy difference between atomic orbitals, they serve as fingerprints, and enable the identification of elements in the surface and calculation of their percent composition. Figure 5 shows a schematic of this process, and illustrates the logic for identifying the shells from which the X-rays originate.

Figure A-8. Bohr Model of the Atom Identifying X-ray Source and Nomenclature

Electrons that normally occupy the K shell are closer to the nucleus and therefore more tightly held than those in the L shell, which are more tightly held than electrons in
Additionally, no two electrons occupying an orbital can have identical energies, according to the Pauli Exclusion Principle. For X-ray analysis purposes, when ejected from the atom core, these electrons may be labeled Kα, Kβ, Lα, Lβ, and so on.

When an electron in the K shell is ejected from the core by the primary beam, an electron from the L orbital may drop into its place and emits a photon of light with a wavelength in the X-ray region of the spectrum. The electronic transition is referred to as a KLL transition. Kα X-rays are the most intense of all emitted X-rays and originate from KLL transitions. And have the highest rate of emission, resulting in more prominent peaks in the X-ray spectrum than other transitions. Kβ X-rays are the most energetic, originating from KMM transitions, but generally appear with lower peak heights than Kα X-rays. Lα X-rays are generated by LMM transitions and Mα from MNN transitions. The Lα and Mα transitions are not usually detected for elements lighter than iron. Figure 7 defines the relationship between atomic number and emission of characteristic X-ray lines.

![Figure A-9. Relationship Between Atomic Number and Characteristic X-Ray Lines](image)
Secondary and back-scattered electrons (BSE) used for common imaging are detected by an Everhart-Thornley (ET) detector (Figure 8), which is mounted at a 90° angle to the optical axis. Secondary electrons are induced to follow a curved trajectory to the detector by a positively charged (40 - 200V) Faraday cage. Inside the Faraday cage is an aluminum scintillator. Incoming electrons are accelerated by a potential difference of 10-12.5 KeV toward the scintillator, which then converts the electron’s energy into a proportional number of photons. These photons follow a light pipe to a photomultiplier tube, which converts them back into an electrical signal for display on the computer monitor, as an image of the surface.

BSE follow a line of sight trajectory, and so are only detected if their path coincides with the detector’s position. BSE may be collected by the ET detector and secondary electrons rejected simply be reducing the potential of the Faraday cage to approximately 50V.

Because BSE follow a line of sight trajectory, detectors designed specifically to detect them are generally mounted beneath the final lens and surround the optical axis. Two common BSE detectors are the solid state BES detectors and the Robinson detector.

Solid state detectors (Figure 9) are composed of doped silicon wafers (semiconductors) equipped with thin film electrodes. Collision of BSE with these materials creates electron-hole pairs which are separated by an applied electrical bias.
This creates an electrical current which is directly proportional to the energy of the incident BSE. This current is used in turn to create the SEM image. BSE retain approximately 80% of the primary beam energy, while secondary electrons are generally of lower energy. Because of this, solid state detectors are not useful for detection of secondary electrons.

![Figure A-11. Schematic of a Solid State Detector](image)

The Robinson detector (Figure 10) was created to image wet biological samples and is generally moved toward or away from the specimen, and cannot be used in conjunction with other detectors. It is equipped with a plastic scintillator that is connected to a photomultiplier tube.

![Figure A-12. Schematic of a Robinson Detector](image)
In order to obtain an image using a solid state or Robinson detector, the sample must be mounted perpendicular to the beam and parallel to the detector, and ideally, must be close to the detector. Because of these limitations, successful imaging may require that the sample be tilted, especially if the surface is rough. In general, an angle between $15^\circ$ and $45^\circ$ is used to study rough surface topography. Peaks on a rough surface generally appear brighter than valleys in the final image. Crevices or pits may be hard to image, as secondary electrons produced in these areas tend to be absorbed by the sample.

The combination of signals from BSE and secondary electrons provide SEM images with extraordinary depth of field, allowing for three-dimensional imaging of the specimen surface. X-rays emitted as a result of core electron ejection and resulting KLL, LMM, MNN transitions are detected to provide a microanalysis of the chemical composition of the SEM specimen.

Many SEMs are equipped with Energy Dispersive Spectrometers (EDS) that are used to analyze the X-rays. These detectors are composed of a semiconductor, or solid state detector, a field-effect transistor (FET) and a cold finger, kept at liquid nitrogen temperatures. Figure 11 depicts a schematic of the EDS detector.

![Figure A-13 Schematic of a Typical EDS X-ray Detector](image-url)
The semiconductor is typically a lithium drifted silicon diode (SiLi, pronounced “silly”). The diode is doped with boron, and the “drifted zone” contains equal numbers of Li\(^+\) donors and B\(^-\) acceptors. A thin layer of gold covers the front of the diode. During operation, the gold layer is reverse biased to 1000V, eliminating the electron-hole pairs that are usually present in semiconductors, thereby establishing a depletion zone. Incoming X-rays generate electron-hole pairs that are separated by the bias, and reappear as a pulse of electronic charge on the opposite side of the diode. The FET simultaneously integrates the total charge of the pulse and converts it to a proportional voltage signal. It essentially serves as a preamplifier for the signal. Both the SiLi and the FET are kept at liquid nitrogen temperature to prevent free migration of Li\(^+\) ions and to reduce electronic noise in the signal. A thin beryllium window protects the SiLi crystal from mechanical damage and ensures vacuum integrity. Beryllium was chosen as the window material because it is of low enough atomic weight to permit the transmission of some of the weaker X-rays.

After leaving the detector, the signals are further amplified and electronically modulated to improve the signal to noise ratio. Signal amplification takes time, and if pulses to the amplifier are too frequent, they may pile up, and produce a distortion of peaks in the X-ray spectrum. To prevent pulse pile up and produce a cleaner spectrum, the operator of the Leo 430i usually monitors and adjusts detector “dead time” to approximately 30%. This action allows the detector and amplifier to perform their jobs in the most efficient manner and minimizes distortion of peaks in the X-ray spectra produced. Finally, the shaped and amplified signal is converted into a digital signal by an analog-to-digital converter, then sorted, stored and displayed on a multichannel analyzer.
Appendix B
Use of the Atomic Force Spectrometer
The Basics
AFM: The Basics

The Atomic Force Microscope (AFM) was developed in response to the inability of the Scanning Tunneling Microscope to image non-conductive surfaces. The instrument uses a very sensitive cantilever that raster-scans the surface of interest. The AFM utilized a cantilever with a very sensitive integrated tip. Typical probe radii vary from 5 to 20 nm, but can be larger depending on application. As the specimen is scanned, the tip senses the atomic forces on the sample’s surface and can be deflected by repulsive forces, or settle lower in the absence of repulsion. A laser trained on the cantilever measures vertical bending and deflection and send the information to a photodetector. The various deflections are mapped, resulting in a topographical map of sorts, showing the surface fine details in three dimensions.

One benefit of AFM as compared to Tunneling Electron Microscopy or Scanning Electron Microscopy is the simplicity of operation in many environments including vacuum, air, water, gases and other aqueous media. While resolution in the X-Y plan is approximately 1nm, resolution in the z-direction can be on the order of Angstroms.

There are several scanning modes: contact, non-contact and tapping mode. When operated in contact mode, the tip and the surface being probed are positioned angstroms apart. At this distance, the overlap of electronic orbitals in the surface produces a repulsive force, which, when dominant, define contact mode. In contact mode, the deflection of the cantilever is kept constant throughout the scan. As distance increases between the tip and the surface, attractive Van Der Waals forces begin to dominate. Contact mode sampling can be influenced by frictional and
adhesion forces, causing damage to specimens, and image distortion. Non-contact mode is performed by oscillation of the tip at the resonance frequency, while keeping amplitude constant. As the distance from the surface is increased, the quality of the resulting image is typically poor and may be affected further by the presence of humidity contamination between the tip and the specimen. Tapping mode is defined to be “between contact and non-contact” modes. As a “blended” mode, the method takes advantage of the benefits of contact and non-contact modes while eliminating the pitfalls associated with each. As the oscillating cantilever periodically allows the tip to touch the surface, the AFM can image steps that may be present in thin film coatings and measure the depth of each step. It can also detect sample surface structure and elasticity properties.

Imaging mode, oscillation frequencies and size of the cantilever tip all affect the quality of the final image. For nm sized features, a nm size tip must be used that is as close to the size of the feature as is practicable.

**AFM: Operation**

**Turn on DI Dimension 3000 AFM**

1. Turn on the Nanoscope controller.
2. Turn on the lamp for the optics.
3. Click the "Nanoscope" icon.
4. The following windows are opened on the left and right PC monitors.
5. Click the following icon in the menu.
6. The measurement windows appear.
1.1 Load AFM Tip onto Holder

1. Load AFM tip (cantilever) onto cantilever holder
2 Mount Holder to AFM Head.

*Note: Make sure that the sample chuck is located on the right bottom side for the safety of the AFM head.

1. Rotate-in the lock knob to unlock the AFM head.
2. Remove the AFM head protector.
3. Take the AFM head out of the tool.

**WARNING: NEVER drop the AFM head off!!** The accident often happens at this stage.

4. Mount the tip holder to the head.
5. Put the AFM head back to the tool.

**WARNING: NEVER drop the AFM head off!!** The accident often happens at this stage.

6. Rotate-out the lock knob to lock the AFM head.

**Align Laser Position with AFM Tip**

1. You can see the He-Ne laser spot on the stage.
2. Put a piece of paper on the stage to clearly see the spot.
3. Rotate the knobs on the AFM head to adjust the laser position, in order for the laser to cross the AFM tip.
4. When the laser crosses the AFM tip, the laser spot becomes dim.
5. Make sure that the light intensity reflected on the AFM tip is maximum on the Sum indicator on the left bottom side of the left PC screen.
6. Rotate the knobs on the left side of the AFM head to move the red spot to ~ the center of the window on the AFM head, and move the red spot to the center of the Detector box on the left PC screen.

2.0. Load Sample

1. Place the sample on the sample chuck.
2. Turn on sample vacuum to secure the specimen to the platen.
3. Using the XY-stage knobs, move the sample beneath the AFM tip.

3.0 Locate Tip

1. Choose "Locate Tip" in the drop-down menu on the "Stage" in the menu of the right PC screen.
2. Click the "Zoom In" button in the "Locate Tip" dialog box, if necessary.
3. Use the trackball to focus the tip.
4. Align the tip position with the crosshair in the "Vision System" box, using the knobs at the left of the optics.
5. Click the "OK" button in the "Locate Tip" dialog box.

4.0 Focus Surface

1. Choose "Focus Surface" in the drop-down menu on the "Stage" in the menu of the right PC screen.
2. The "Focus Surface" dialog box appears in the right screen, so that the vertical motion of the AFM Head is activated for focusing and zooming.
3. Click the "Zoom In" button in the "Focus Surface" box, if necessary.
4. Move down the AFM head and focus on the sample surface in "Vision System" box in the left screen, using the trackball.

**WARNING: Do not crash tip into the sample (the travel distance for fine engage is ~200um)!!**

5. Move the sample location, using the XY-stage knobs for the sample chuck, if necessary.

### 5.0 Tune Resonance Frequency

1. Click the capture icon in the menu.

2. Click the "Auto Tune" button in the Auto Tune Controls" box to automatically obtain the drive frequency of the maximum peak of amplitude.

**Note:** You must tune the frequency of the maximum peak of amplitude. On the other hand, the frequency obtained by auto-tuning is not necessary to be the same as that of the actual maximum peak of amplitude. You want to confirm it by manual tuning.

- Target amplitude in the "Auto Tune Controls" box: 1~2V

4. Click the following icon on the right top side of the right PC screen to go back to the measurement windows.
6.0 Adjust Scan Parameters.

1. Adjust scan rate, scan size, XY offset, and so on in the "Scan Controls" box of the right screen.
   - Default scan rate: 1.0 Hz for 1 μm scan size
   - The tip location can be moved using XY offset (< 20 μm).

7.0 Start Scanning

1. Click the following icon in the menu of the right screen.

2. Start scanning on Height, Amplitude, and Phase.

3. If the Z center position is retracted too much, click the Scope Trace icon to switch the screen to Scope Trace.

4. The scope trace screen appears.

5. Adjust the integral and proportional gain, and the Amplitude setpoint, if necessary.
   - Amplitude setpoint: If retracted too much, reduce the Amplitude setpoint, and vice versa.
   - Integral and Proportional gain: Reduce the noise as much as possible.

6. Adjust the scales of Height, Amplitude, and Phase in the right screen, if necessary.

7. If it is OK, then click the following icon to go back to the scanning screen.

8. Make sure that the image is in focus.
   - Leveling the background or removing the shadow can be done using graphic software.
8.0. Capture the image

1. Click the camera icon to capture the image.

2. When the capturing starts, the capturing status is shown on the bottom of the right screen.

3. Click the following icon to check the saved data.

9.0 Stop Scanning

1. Click the tip up icon to stop scanning.

10.0 Analyze Data

1. Click the following icon on the desktop menu of the left PC screen.

2. Open the data.

3. Choose the Height data, if necessary.

4. Choose Flatten to level the background or to remove the shadow.

5. Mark the structures, and execute.

6. Save the data.
11.0 Unload Sample and AFM tip

1. Choose "Focus Surface" in the drop-down menu on the "Stage" in the menu of the right PC screen.

2. The "Focus Surface" dialog box appears in the right screen, so that the vertical motion of the AFM Head is activated for focusing and zooming.

3. Move up the AFM head at least 1 cm above the chuck, using the trackball.

4. Click the "OK" button, if necessary.

5. Move the sample chuck to the right bottom side for the safety of the AFM head.

6. Remove the sample.

7. Remove the AFM tip holder from the AFM head.

8. Put the AFM head back to the tool, and the AFM head should be covered with the head protector.

WARNING: NEVER drop the AFM head. The accident often happens at this stage.

9. Remove the AFM tip from the holder.

10. Put the tip back in the tip box.

12.0 Stand-by

1. Turn off the Nanoscope controller.

2. Turn off the lamp for the optics.
Appendix C
Ellipsometry
The Basics
Ellipsometry: The Basics

Ellipsometry is a non-destructive optical technique used to characterize thin films by probing the dielectric properties of a specimen. A HeNe (λ = 632.8 nm) laser is set to emit a beam of linearly polarized light toward the substrate under study. Upon striking the substrate, the reflected light undergoes a phase shift and change in amplitude caused by differences in the refractive index between the intermediate medium (usually air) and the substrate. Reflected light is then elliptically polarized. Analysis of the state of polarization of light reflected from the sample allows the determination of substrate thickness, even if the layer is thinner than the wavelength of the light, for single layer or multilayer stacks ranging from a few angstroms to several microns.

In order to understand ellipsometry, one must first understand the properties of electromagnetic (EM) radiation. The equation of motion for an EM wave in one dimension is:

\[ E(z, t) = E_0 \sin \left( -\frac{2\pi}{\lambda} (z - vt) + \xi \right) \]  

where \( E \) is the electric field strength, \( E_0 \) is the maximum field strength (amplitude) of the wave, \( z \) is the distance along the direction of travel, \( \lambda \) is the wavelength and \( \xi \) is an arbitrary phase angle. The last parameter allows the operator to offset one wave from another when combining them. Figure C-1 depicts a one-dimensional wave with its electric field oscillating with the wave.
If two light beams with the same frequency and amplitude that are in phase and traveling the same path are combined, linearly polarized light is produced (Figure 2). The bottom image in Figure 3 is a representation of the vector sum of the components of the two waves. Here, the resultant wave is linearly polarized at an angle 45° to the vertical.

**Figure C-1** The electric field is shown schematically as a function of position, at a fixed time.
The combination of two waves of the same frequency and amplitude that are traveling in the same direction, but are 90° out of phase produces circularly polarized light (Figure C-3).
The resultant wave is depicted by the bold-faced arrows, the tips of which, if viewed end-on, would appear to trace out a circle in space.

The combination of two waves that are of the same frequency and amplitude, traveling in the same direction, that are out of phase by anything other than 90° would produce resultant vectors whose tips would appear to trace out an ellipse in space. This is called elliptically polarized light.

When ordinary white light strikes a surface, some of the light may be transmitted into the substrate, and some of the light will be reflected from the surface. The angle of reflection is identical to the angle of incidence, designated as $\phi_1$ in Figure C-4.
This light is characterized by a complex index of refraction according to equation 2:

\[ \tilde{N} = n - jk \]  

(2)

Where \( n \) is called the index of refraction, \( k \) is the extinction coefficient – a measure of how quickly the intensity of light decreases as it passes through a material, \( j \) is the imaginary number and the remaining variable is the complex index of refraction. The variable \( n \) is an inverse measure of the phase velocity of light in free space (Equation 3).

\[ n = \frac{c}{v} \]  

(3)

Where \( c \) is the speed of light and \( v \) is the phase velocity. For most materials, the law of refraction, Snell’s law in its general form is shown in Equation 4.

\[ \tilde{N}_1 \sin \phi_1 = \tilde{N}_2 \sin \phi_2 \]  

(4)

For a dielectric material, \( k \) (the extinction coefficient) = 0, and Snell’s law simplifies to Equation 5:
\[ n_1 \sin \phi_1 = n_2 \sin \phi_2 \] (5)

As stated previously, an ellipsometer functions by reflecting a beam of linearly polarized light off of a sample, and measuring the polarization change upon reflection. The exact nature of the polarization change is determined by the sample's properties.

Ellipsometry is a specular optical technique (the angle of incidence equals the angle of reflection). In Figure C-5, the angle of incidence is the angle between the light beam and the normal to the surface. \( E_s \) is the amplitude of the associated electric wave in the plane of incidence and \( E_p \) is the amplitude of the associated electric wave perpendicular to the plane of incidence.

\[ E_s \] is defined as an “s-wave” and of \( E_p \) as a “p-wave.” When the beam of light strikes the surface of interest, each type of wave may be affected differently, producing changes in the polarization of light as it makes a reflection from the surface. To effectively analyze the data from the elliptically polarized wave, two parameters that can be represented graphically must be defined: \( \Delta \) and \( \Psi \).
The difference in phase shift between the p-wave and the s-wave prior to reflection is defined as $\delta_1$ and the phase shift difference between them after reflection as $\delta_2$. Then,

$$\delta_2 - \delta_1 = \Delta$$  \hspace{1cm} (6)

In order to define $\Psi$, a quantity known as reflectance, $\mathcal{R}$ must be defined, which is related to the ratio of the amplitude of the outgoing light compared to that of the incident light. This ratio of light intensity is defined by the Fresnel reflection coefficients, given in their simplest form for a single interface in Equation 7. It should be noted that one must consider that the light must travel through a medium, usually air, prior to striking the surface, and that there are changes in both the p-waves and s-waves as the light traverses each medium.

$$r_{12}^p = \frac{\hat{N}_2 \cos \phi_1 - \hat{N}_1 \cos \phi_2}{\hat{N}_2 \cos \phi_1 + \hat{N}_1 \cos \phi_2} \hspace{1cm} r_{12}^s = \frac{\hat{N}_1 \cos \phi_1 - \hat{N}_2 \cos \phi_2}{\hat{N}_1 \cos \phi_1 + \hat{N}_2 \cos \phi_2}$$  \hspace{1cm} (7)

Here, $r_{12}^p$ represents the reflection coefficient for the p-wave between medium 1 (air) and medium 2 (the surface), and $r_{12}^s$ is that of the s-wave. The reflectance is equal to the square of the reflection coefficient (Equation 8).

$$\mathcal{R}_s = (r_{12}^s)^2 \hspace{1cm} \mathcal{R}_p = (r_{12}^p)^2$$  \hspace{1cm} (8)

At normal incidence, the cosine function = 1 and $\mathcal{R}_s = \mathcal{R}_p$.

This research will investigate what happens when a substrate has been coated with a thin film. In this case, the light must pass through two media (air and the thin film) and at least strike a third (the polyimide substrate. In this case, the Fresnel equation becomes more complicated, as follows:
$R^p = \frac{r^p_{12} + r^p_{23} \exp(-j2\beta)}{1 + r^p_{12} r^p_{23} \exp(-j2\beta)}$ \hspace{1cm} $R^s = \frac{r^s_{12} + r^s_{23} \exp(-j2\beta)}{1 + r^s_{12} r^s_{23} \exp(-j2\beta)}$

where

$$\beta = 2\pi \left( \frac{d}{\lambda} \right) \tilde{N}_2 \cos \phi_2$$

(9)

$R^p$ are the total reflection coefficients for the p-waves and the s-waves, and represent reductions in amplitude of these waves. It is important to note that the amplitude reduction for each type of wave may not be the same. These total reflectance coefficients define the ratio of the amplitude of the incoming wave to that of the reflected wave. When $k \neq 0$, $\cos\phi$ is a complex number, as is $\beta$. To make the final leap from the total reflection coefficients to reflectance, which is defined as the ratio of the intensity of the incoming wave to that of the reflected wave, the magnitude of the reflection coefficients must be squared (Equation 10).

$$\mathcal{R}_s = |R^p|^2 \quad \text{and} \quad \mathcal{R}_p = |R^s|^2$$

(10)

$\Psi$ is defined as the angle whose tangent is the ratio of the magnitude of the total reflection coefficients. Note that $\tan\Psi$ is a real number and can have values from $0^\circ$ to $90^\circ$.

$$\tan\Psi = \left| \frac{R^p}{R^s} \right|$$

(11)

While the value defined by Equation 11 is a real number, complex solutions often arise, and so a variable must be defined to allow calculation of those solutions:

$$\rho = \frac{R^p}{R^s}$$

(12)

Then the Fundamental Equation of Ellipsometry is written as follows:

$$\rho = \tan\Psi e^{i\Delta} \quad \text{or} \quad \tan\Psi e^{i\Delta} = \frac{R^p}{R^s}$$

(13)
The magnitude of \( \rho \) is contained in the \( \tan \Psi \) part of the equation while phase shift information is contained in the exponential.

It is important to note that ellipsometers measure \( \Psi \) and \( \Delta \). Assuming the instrument is operating correctly, these quantities are always correct. Whether the calculated quantities such as film thickness, \( n \) and \( k \) are correct depends on whether the correct model is chosen for the calculations.
Appendix D

X-ray Photoelectron Spectroscopy (XPS)

Also known as Electron Spectroscopy for Chemical Analysis (ESCA): The Basics\(^{(1)}\)

Figure E 1. Typical Instrument
X-ray Photoelectron Spectroscopy: The Basics

XPS, also known as ESCA is used to perform surface analysis on specimens of interest. The instrument consists of an external chamber equipped with a vacuum pump, an ultra-high vacuum test chamber, a hemispherical analyzer, an 1253.6 eV Magnesium source (Mg K\(\alpha_{1,2}\)) or 1486.6 eV Aluminum source (Al K\(\alpha_{1,2}\)) and various detectors all connected to an on-board computer used for data analysis and spectrum generation.

The concept begins with Einstein’s 1905 proof of the photovoltaic effect, \(E = h\nu\), where \(h\) is Planck’s constant and \(\nu\) is the frequency of incident radiation. If high energy light is shone on a surface, photoionization occurs and characteristic photoelectrons are emitted along with Auger electrons. The kinetic energies of emitted electrons can be determined by use of an electron energy analyzer.

Figure 1 presents the Bohr Model of the atom. This is a thermodynamic model, where the letter “n” designates the energy level of orbitals, starting at \(n = 1\) for the “K” shell, \(n = 2\) for the “L” shell, and so on.
Figure E 2. Bohr Model of an Atom. The letter “n” is used to designate quantum energy levels. When n=1, the lowest energy level, the orbital is referred to as the “K” shell. When n=2, the orbital is named the “L” shell, and When n=3, the “M” shell.

XPS sources typically emit X-rays in the range of 5-20 keV. These are considered “soft” X-rays. They penetrate to the core electrons at the “K” shell, imparting sufficient kinetic energy to eject them from their atoms (Figure E-3).
Figure E.3. Schematic depicting the ejection of a “K” electron after exposure to soft X-rays. The remaining atom components are in a higher energy state than thermodynamics allows. The atom attempts to stabilize by transitioning an “L” shell electron to the K level. This first transition to lower energy transfers excess energy to the outgoing electron (Figure 3). These transitions from higher energy levels to lower ones results in emission of Auger electrons.
Figure E 4. Schematic depicting an “L” electron dropping into the “K” shell. This action requires that the demoted electron transfer its excess energy to a neighboring electron, which is then emitted as an Auger electron.

The ejected electrons travel to a hemispherical analyzer, where they are essentially sorted by their energies and counted/analyzed by the electron detector at the other end of the analyzer. Figure 4, below, shows the basic features of the instrument, to include the hemispherical analyzer.
When the X-ray source is Aluminum Kα, additional X-rays may be released (Kα₃ and/or Kα₄). These additional lines can produce a continuous spectrum called Bremsstrahlung, instead of the desired discrete spectrum. A quartz crystal is imposed in the electron path to filter unwanted radiation and increase spectral resolution.

XPS data can be used to calculate the binding energies of the photoelectrons and the kinetic energy of the Auger electrons, according to Equation 1.

1. \( KE = h\nu - BE - \phi_{\text{spec}} \)

Where:
- \( BE \) = Electron Binding Energy
- \( KE \) = Electron Kinetic Energy
- \( \phi_{\text{spec}} \) = Spectrometer Work Function
Note that photoelectron line energies are dependent on incident photon energy, while Auger electrons are not. Rearrangement of Equation 1 to yield Equation 2 allows the calculation of the Binding Energy:

2. \( \text{BE} = h\nu - KE - \phi_{\text{spec}} \)

where definitions for each term are identical to those in Equation 1. In this case, note that photoelectron energy is not dependent on the incident photon energy, but is dependent on the energy state from which it was ejected; however, Auger electron energy is dependent on the incident energy. Calculation of binding energies enables one to determine and compare chemical states of the ejected core electrons. The carbon 1s electron (K-shell electron) has a binding energy of 285.0 eV. This attraction between the electron and its nucleus allows the identification of the element. As the distance from the nucleus to outer electron shells increases, the outer shells are held less tightly, and knowing the binding energy of each also allows identification of the parent atom.