MICROELECTRODE ARRAY FOR CAPACITIVE TRANSDUCTION OF RETINAL RESPONSES

Brittany Branch

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Dedication

I dedicate this work to my loving husband.

Without his support this journey would have never been possible.
Achknowledgements

I would like to thank my advisor, Dimiter Petsev, for teaching me the fundamentals in both colloid and material science. I thank you for sharing your knowledge as a mentor and a friend. I would also like to thank my co-advisor, Andrew Dattelbaum for his support during the experimental research conducted in this dissertation. If he did not believe in me from the first day, I would not have succeeded. I would like to thank my committee members, Scott Sibbett and Steven Graves for their time and support over the years. I also would like to thank my colleagues and mentors including John George, Jennifer Schei, Kateryna Artyushkova, Gautam Gupta, Aditya Mohite, Jen Martinez, Aaron Anderson, Manish Dubey, Kevin Baldwin, John Nogan, Edward Gonzales, Don Bethke and Denise Webb for sharing their expertise and support during different stages of this project.

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Neural degenerative diseases and traumatic injuries to the eye affect millions of people worldwide, motivating the development of neural prosthetic interfaces to restore sensory or motor function in affected individuals. Advances in neural sensing and stimulation interface technology will allow a more comprehensive understanding of neural function while leading to the development of hybrid biological-electronic sensor devices for robust, functioning neural prosthetic systems. Current techniques of neural activity sensing employ multi-electrode arrays (MEAs) that typically incorporate metal electrodes
and measure currents via an electrochemical junction, leading to corrosion and charge transfer across the electrode-tissue interface. High-density neural interface technology will require active circuitry within the implant; the device must withstand corrosion and induce minimal damage at the electrode/tissue interface. The work shown here demonstrates a prototype neural interface device based on capacitive coupling through hafnium oxide encapsulation of a novel 3D device architecture, advancing neural sensing technology toward long-term implantable neural interfaces.

The functionalization of biosensors interfaced with neural tissue is important to ensure that the active components of the sensor are fully protected from the surrounding biological environment. Self-assembled monolayers (SAMs) have been extensively studied as coatings for implantable devices due to their ability to tailor surface properties and relative ease of film formation. We report a series of studies aimed at investigating the stability of phosphonate self-assembled monolayers, octdecylphosphonic acid (ODPA) or perfluorophosphonic acid (PFPA) on various oxide surfaces (SiO$_2$, TiO$_2$, Al$_2$O$_3$ and HfO$_2$) to serve as the biotic-abiotic interface of the prototype neural device developed here. The monolayers were deposited by a series of techniques including self-assembly from solution, tethering by aggregation and growth and Langmuir-Blodgett (LB). SAMs prepared by LB were primarily used in our stability investigations because they were found to be the most uniform and reproducible. All films deposited on oxide-coated substrates were characterized by means of water contact angle measurements, spectroscopic ellipsometry, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). XPS data conclusively showed covalent phosphonate formation on
all substrates except SiO$_2$, which had background spectra that interfered with the data analysis. AFM images of SAMs formed on SiO$_2$ and TiO$_2$ showed significant surface reorganization upon exposure to water within 30 minutes. SAMs formed on Al$_2$O$_3$ and HfO$_2$ were much more stable upon exposure to water. PFPA SAMs on HfO$_2$ were found to be the most stable SAMs studied here in either water or phosphate buffer at room temperature. This is the first report of a SAM-oxide system showing stability for an extended period of time, greater than 20 days. These data suggest that phosphonate SAMs should be considered for implantable neural devices that require longer-term stability under aqueous conditions.

To examine the encoding and processing of information by networks of neurons, microelectrode arrays (MEAs) have been developed and applied, but evolving scientific questions and biomedical applications require higher density sampling and wider spatial coverage. The integration of 3D electrodes can provide closer contact with neurons to facilitate detection and resolution of single cell action potentials. The fabrication methods implemented here allows reliable fabrication of a novel MEA consisting of probes with dimensions of a few microns, unlike most other approaches to 3D electrode arrays, which produce structures on the scale of tens of microns or more. The device incorporates over 3,800 micro pillar electrodes, grouped into 60 independent sensors for compatibility with existing electronics, spread over an area of 750 $\mu$m$^2$; each sensor site consists of an 8x8 array of micropillars, interconnected by a lead to an output pad of the device. Individual 3D pillars are 3 $\mu$m in diameter with a height of 8 $\mu$m. Our experience has suggested that such microstructured probes can achieve more intimate contact with
the surface of neural tissue, and enhance the quality of neuronal recordings. Electrochemical impedance spectroscopy (EIS) at 1 kHz measured average magnitude and phase shift of 710 W and 17°, respectively, for a single sensor site. These values confirm the robustness of our fabrication process for developing highly conductive 3D microelectrodes. The results shown here demonstrate high-density, three-dimensional microfabrication technology that was applied to the development of an advanced capacitive sensor array for neural tissue.

Applications in sensing technology now require electro-neural interface devices to withstand corrosion and induce minimal damage at the electrode/tissue interface. We have developed a platform suitable for hermetic sealing and have shown encapsulation through atomic layer deposition of hafnium oxide over the active components of the device to overcome the direct current limitations of existing MEA technology. EIS was used to study the oxide deposition on the 3D micro pillar sensor array to ensure a pinhole-free dielectric coating. The characteristic impedance magnitudes increase up to 3 orders of magnitude upon oxide deposition and the phase indicates fully capacitive sensor sites. The fabrication process and electrochemical impedance study shown here, demonstrates the usefulness of such techniques for building high-density 3D arrays that can be fully encapsulated with a protective dielectric coating. This work advances the technology towards capacitive sensing of retinal neurons with a robust, non-invasive sensing device.
Sensing retinal neurons with the 3D micropillar array developed here was performed for direct current and capacitive configurations of the device. Electroretinograms (ERGs) were recorded and the overall performance of the device was analyzed. The devices showed good consistency across all 60 Pt electrode clusters during characterization and when interfaced with retinal tissue. ERGs were recorded by more than 80% of the direct current electrode sites and the performance was evenly distributed around the mean response. This performance surpasses previous reports of 3D electrode arrays interfaced with retinal tissue, where typically 1-6 electrode signals are recorded successfully. Encapsulation of the device platform was achieved and successful recordings of ERG signals were shown. This work is the first report of sensing the overall electrical behavior of retinal tissue with a coupled capacitive MEA.
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**Table 2.** Contact angles and film thicknesses determined by ellipsometry of ODPA or PFPA monolayers deposited by LB onto different oxide surfaces. The calculated tilt angle of the deposited layer for each film with respect to the surface normal is also reported.
Chapter 1. Introduction

Retinal degenerative disease such as retinitis pigmentosa or age-related macular degeneration affects millions of people worldwide. The leading cause of vision loss in America is age-related macular degeneration, which affects the delicate layer of tissue that lines the back of the eye. This part of the eye known as the retina is essential for vision. The retina converts a light image into a set of electrical signals that travels to the optic nerve ending at the brain, providing us with knowledge of the visual world in real time. The conversion of electrical signals occurs abnormally in people with retinal degenerative diseases and in some cases blindness can occur. In the past several decades, neural activity imaging and sensing techniques have been developed to record individual and networks of neuron activity. The goal is to detect neuron activity at the system level or the collective activity of all neurons in the retina. Advancements in sensing technology will allow a more comprehensive understanding of the retina ultimately allowing the manipulation of the dynamic function of the retinal neurons, leading to the development of hybrid biological-electronic sensor systems or robust, functioning retinal prosthetics.

1.1 Retina Anatomy and Physiology

The most vital part of the eye is the retina, which is essentially brain tissue that receives direct stimulation from the outside environment. The understanding of its structure and function began as early as 1892 with Santiago Ramon y Cajal’s anatomical descriptions of cell types that constitute the retina in vertebrate species. By 1960 visual scientists
developed a basic understanding of how the retina may be organized and functioning\textsuperscript{4-9}. To progress further, the detailed information of neural circuits that underlie the function of the retina needed to be explored. With the developments of electron microscopy, pharmacology, and microelectrode recording techniques, an era of huge advancement in understanding the organization of the vertebrate retina and its functions began and continues today.\textsuperscript{10-14}

The relatively simple organization of the human retina is shown in Figure 1. Light must travel through the thickness (500 microns) of the retina to the photon absorbing visual pigments in the photoreceptors. Light that is not captured by the retina is absorbed in the retinal pigment epithelium layer to prevent reflection back into the eye. Retinal photoreceptors consisting of both rods and cones are responsible for vision during dim illumination and bright illumination, respectively. Light stimulation in the visual pigments in the rods and cones triggers a biochemical response that leads to a change in ionic fluxes across the cell membrane. In the dark, steady-state current (mostly sodium ions Na\textsuperscript{+}) flows into the photoreceptor cells by means of membrane transport proteins. The influx of dark current results in a more positive cell membrane potential that results in depolarization of the photoreceptor membrane potential. Upon photon absorption closure of the cation channels (cGMP-gated) of the photoreceptor membrane occurs stopping the dark current and causing the photoreceptor cell membrane potential to become more negative or hyperpolarized.\textsuperscript{15}
Figure 1. Cellular organization of the human retina, where light (yellow arrows) must pass through the thickness of the retina to the photon absorbing visual pigments in the photoreceptors. Image adapted from reference 15.

The changes in membrane potential of the rods and cones signal neurons in the vertical (photoreceptor to bipolar) and lateral pathways (photoreceptor to horizontal cell to bipolar to amacrine) that combine signals from several photoreceptors into a unique spatial and temporal pattern. The structure of a typical sensory neuron is illustrated in Figure 2. The cell body is the metabolic center of the cell and usually gives rise to two processes: receiving and transmitting of electrical signals. Dendrites are the main structure for receiving signals, while the axon extends away from the cell body and is the main apparatus for carrying signals to subsequent neurons. At rest neurons maintain a difference in electrical potential on either side of the plasma membrane where the net
charge inside is more negative than outside. This difference is established by an unequal
distribution of Na\(^+\) and potassium ions (K\(^+\)) and negatively charged amino acids and
proteins on either side of the cell membrane along with selective permeability of the
membrane to K\(^+\) ions. Once excited the membrane potential reduces making the
membrane more permeable to Na\(^+\) ions. The influx of ions tend to neutralize the negative
charge inside the cell resulting in an even greater reduction in membrane potential, also
known as an action potential.\(^{16}\)
The individual action potentials produced by horizontal, amacrine, and bipolar cells are conducted to the ganglion cells. The axons of ganglion cells are capable of passing the electrical signal to the retinal recipient areas of the brain as trains of action potentials.\textsuperscript{15,16} Physiological investigations have shown that a great deal of insight into the response characteristics of neural networks and individual neurons is encoded in the behavior of action potentials including the frequency of oscillations and the presence of synchronous firing.\textsuperscript{17-21}
As early as 1865 it has been known that light stimuli causes a measurable change in the electrical potential of the eye. Light-induced electrical activity of the entire retina is called the electroretinogram (ERG). Analysis of the time-course of the ERG response began in 1903 when Gotch first reported the ERG consisted of two waves. The experimental setup is shown in Figure 3, where a ‘modified hydraulic motor was used to allow light to fall on the eyeball’ of a frog contained in a ‘special black box’. The eyeball (labeled E in Figure 3) of the frog previously kept in the dark for 24 hours was excised in a dimly lit room and then transferred to the black box (labeled F in Figure 3) and placed between kaolin (mineral that causes blood to clot) pads moistened with 0.6% NaCl. The two pads were associated with non-polarisable electrodes through thread moistened with saline and these with a capillary electrometer (Lippmann Electrometer). His conclusions were first the cornea became negative and then a positive wave of larger amplitude appeared.
Figure 3. Experimental setup for the first account of time relations recorded during an ERG, where a modified hydraulic motor was used to control a series of shutters and mirrors to allow light to fall on a frog eyeball. Image adapted from 22.

Later Einthoven and Jolly (1908) divided the ERG response into three waves; the first wave occurring immediately after light stimulation (arc lamp) was negative on the cornea, followed by a positive wave and a final slower wave that was also positive. The electrical response was measured by a string galvanometer, which utilizes silver-coated quartz filaments as electrodes. Einthoven and Jolly (1908) concluded that light stimulus triggered a chain of reactions within the retina leading to the formation of three products A, B and C, and that each wave of the ERG was a change from one product to the next. 23

This work formed the basis for the analysis of the ERG used today, which consists of
three waves commonly called the a-, b- and c-waves. An additional corneal-positive wave (d-wave) exists at the termination of the light stimulus, but is rarely recorded because the c-wave typically masks the d-wave making it difficult to resolve. Figure 4 shows a standard ERG measurement for frog retina, where the respective waves are illustrated. The amplitude and pattern of the ERG vary between species due to the respective densities of rods and cones. The duration and intensity of light stimulation as well as the recording approach, also affect the waveform.

![Figure 4](image-url)

**Figure 4.** ERG response of isolated frog retina to light stimulation with the a-, b- and c-waves designated by arrows. Note that the retina characteristically responds to both the onset (ON yellow arrow) and offset (OFF black arrow) of light stimulus.

Physiological and pharmacological methods have been used to study the cellular origins of the major ERG waves. The physiological experiments are based on the hypothesis that
the initiators of specific ERG components are located in specific retinal layers, while the pharmacological approaches to understanding the ERG components are based on retinal physiology and biophysics.

The a-wave is the leading element of the ERG and can be divided further into fast and slow components.24,25 The source of these components was obtained from ERG recordings with intra-retinal microelectrodes. Based on these measurements, it was concluded that the a-wave reflects light-induced activity of the photoreceptors and closure of the cGMP-gated cationic channels.26-30 Once the identity of the neurotransmitter of the cGMP-gated cationic channels was determined to be L-glutamate, effective blocking of the synaptic transmission from the photoreceptors was achieved through pharmacological approaches, and the contribution of the photoreceptors to the ERG was eliminated, further validating the origin of the a-wave.31

The b-wave has been studied extensively because it is the major wave of the human ERG and is used in clinical and experimental analysis of retinal function. The b-wave originates in retinal cells that are post-synaptic to the photoreceptors. Initial studies showed that the b-wave results from changes in the membrane potential of the Müller cells due to light-induced changes of extracellular potassium concentration.32,33 This hypothesis has been tested extensively with different species using intracellular recordings from Müller cells, measurements of extracellular concentrations of potassium and recording the ERG at different depths in the retina.34-36 The response of the Müller cells was confirmed in these studies. In addition, an increase in extracellular potassium in
the outer and inner plexiform layers was shown to occur after light stimulation. It was understood that the potassium increase in the outer plexiform layer was a result of depolarization of the bipolar cells. Further, an increase in extracellular potassium in the inner plexiform layer is a result of the light-induced activity of amacrine and ganglion cells. Recent pharmacological studies have further confirmed the contribution of amacrine and ganglion cells to the amplitude and kinetics of the ERG b-wave.

The final, generally studied, component of the ERG is the c-wave, which stems from the pigment epithelium layer of the retina. The first indication of this ERG component was reported in 1954. The concept was further proven in 1970 when intracellular recordings were made from pigment epithelial cells. The potential changes that were recorded in response to light stimuli were identical in shape and temporal pattern to the ERG c-wave.

Although a great deal of research has been done to understand the complexity of retinal function in a variety of species, ERG responses including individual neural responses known as action potentials continue to be investigated. These light-induced responses can be recorded with an active extracellular electrode positioned at different levels in the retina. An ERG of salamander retina with action potentials resulting from low light stimulation is shown in Figure 5.
Figure 5. Responses of isolated salamander retina to light stimulation with action potentials designated by brackets. Image adapted from reference 43.

1.2 Microelectrodes for Investigation of Retina Electrophysiology

Microelectrodes are the basis of the techniques used for measuring the ERG or action potentials described in the previous chapter. A microelectrode, shown in Figure 6, typically consists of a glass micropipette filled with electrolyte solution. Such an electrode was first pioneered by Ling and Gerard in 1949, where cell membrane potentials of muscle fiber of frog were measured with a capillary electrode tip of 1 µm diameter. 44
Although microelectrodes have been the most comprehensive tool for measuring independent electrical responses of the retina, more recent studies suggest that neurons do not act as independent sources of information but instead signal in a concerted fashion.\textsuperscript{46-48} To examine the effects of connectivity on the processing of neural signals, higher densities of electrodes are needed.\textsuperscript{49} The emergence of microelectromechanical systems (MEMS) has enabled the fabrication of several microelectrodes incorporated onto a single chip. This device is known as a microelectrode array (MEA) and allows simultaneous electrical recording of populations of neurons in the retina. The current experimental technique used in most laboratories to measure populations of signals is based on the pioneering work of Meister et al.\textsuperscript{50} Live retinal tissue that is dissected from an animal’s eye is placed in a chamber containing physiological saline solution (Ringers medium) on the top of a MEA. The ERG of the retina and action potentials generated by
the individual neurons in response to light stimulation are recorded by the electrodes.

A typical MEA, which is shown in Figure 7A, consists of a glass slide or ‘‘biochip’’ into which an array of microelectrodes are photoetched, each insulated from each other and coated with conductive materials such as indium-tin oxide (ITO), platinum (Pt) or gold (Au). Each electrode is connected to an external amplifier, which relays the electrical signal to a computer, where it is converted into a digital signal, filtered, detected, stored, and analyzed. The spatial resolution and temporal pattern of neuron signaling are preserved, which gives MEAs a large advantage over other electrophysiological techniques. Figure 7B and 5C illustrates the active electrode array of a MEA biochip. Currently, multiple manufacturers provide hardware and software for commercial MEA systems (e.g., Alpha MED Sciences, Osaka, Japan; Axion Biosystems, Atlanta, GA; Qwane Biosciences, Lausanne, Switzerland; 3-Brain, Landquart, Switzerland; MultiChannel Systems, Reutlingen Germany; Plexon, Inc., Dallas TX; Tucker-Davis Technologies, Alachua, FL).\textsuperscript{51}
Figure 7. Top view of a MEA (A) consisting of a high density of electrode sites (B) with the insulated leads shown in detail (C). Image adopted from reference 49.

To measure the electrical activity of the retina, high spatial resolution microelectrodes are required. The resolution depends on the size, spacing, and number of electrodes in a single biochip. To date, the highest density MEA system reported consists of 4096 planar microelectrodes.52 The platform consists of metallic electrodes integrated with high-speed circuitry and combined with a real-time acquisition system providing the capability to record the entire array and to process the acquired signals. Although this system allows the study of networks of cells through the position-sensitive recording of electrical activity, planar arrays are limited in making good electrical contact with neurons in the anatomical layers of the retina.
With the continuous developments in MEMS technology, 3D MEAs that penetrate the retinal layers have become an important method for simultaneous analysis of neurons beyond the outer retinal layers. Several commercial sources (referenced above) offer 3D MEAs consisting of 60-100 linear probes with multiple electrode contacts and probe shafts on the order of 20-100 µm. Such probes rarely sample neuron signals and increase the likelihood of neuronal damage due to their incompatibility in size. This limitation triggered the development of electrode arrays with probe diameters comparable in size to a single neuron (2-5µm). Several academic sources report fabrication of 3D microelectrode arrays with a variety of geometries and successfully demonstrate the recording of multi-site ERG responses or action potentials of the retinal ganglion neurons.\textsuperscript{53-60}

\textbf{1.3 Limitations of Current Microelectrode Array Technology}

Existing fabrication approaches to MEA technology produce 3D electrodes on the scale of tens of microns or more. Reliable fabrication methods need to be developed for producing probes with dimensions of a few microns to provide closer contact with neurons to facilitate detection and resolution of single cell action potentials. Typically, these MEAs employ electrode arrays that are made of metal and record direct current signals.

Basic science and biomedical applications require electro-neural interface devices to
withstand corrosion, resist innate enzymatic activity, nonspecific surface binding of
cells and macromolecules, and induced immune response, while promoting adhesion and
health of retinal cells.\textsuperscript{61} Although there has been a great deal of research in organic and
biocompatible coatings that address the above requirements,\textsuperscript{62-64} more robust coatings
need to be developed for chronic in-vivo measurements.

Direct current measurements pose an additional problem for chronic in-vivo neuron
recordings. Charge transfer across the electrode-tissue interface can induce neural
damage through irreversible Faradaic reactions. The electrochemically significant
components of extracellular tissue fluid (Ringers medium used in the case of retina
electrophysiology) are water (55 M), sodium chloride (107 mM), sodium bicarbonate (22
mM), carbon dioxide (0.05 atm) and various organic materials such as glucose (10 mM).
Considering these components and the metal electrode, the types of potentially harmful
reactions that can occur are: \textsuperscript{65}

- Electrolysis of water at high anodic and cathodic potentials causes physical
damage to the tissue from the gases produced and from the local pH change near
the electrode.
- Oxidation of saline Cl$^-$ most commonly produces hypochlorite (ClO$^-$), which is
undesirable due to the fact that it is a strong oxidizing agent.
- Oxidation of the metal electrode leads to dissolution products (heavy metal
cations) that have been shown to be toxic to tissue.\textsuperscript{66}
- Oxidation of organic species such as glucose to gluconic acid (or complete
oxidation to CO$_2$) poses special problems if sensitive tissue is attacked or if the oxidation intermediate products should prove toxic.

To overcome the direct current limitations in existing MEA technology, capacitive charge transport between the electrodes and neural tissue should be employed. Encapsulation of MEAs in a protective coating can eliminate corrosion and charge transfer across the electrode-tissue interface, which induce circuitry failure and neural damage through irreversible Faradic reactions. Capacitive coupling has been used for noninvasive measurements in a neuronal culture environment on a semiconductor chip.$^{67}$ Our work aims to develop a novel 3D device architecture and encapsulation coatings that will enable capacitive coupling, advancing the technology towards a robust and noninvasive neural platform.
Chapter 2. Objective of the Research

Advancements in neural sensing technology will allow a more comprehensive understanding of the retina ultimately allowing for the manipulation of the dynamic function of the retinal neurons towards a retinal prosthesis. Basic science and biomedical applications require electro-neural interface devices to withstand corrosion and induce minimal damage at the electrode/tissue interface. The primary objective of this work is to introduce a novel sensing approach and to develop a prototype device, which relies on capacitive coupling between the electrode probes and neural tissue to measure electrical activity in the retina due to light stimulation. Such a sensor will revolutionize the basic studies of neural encoding by eliminating the drawbacks associated with direct current techniques and employing degradation resistant coatings for long-term applications.

2.1 Specific Aims of the Research

To achieve the projects general objective the technical work in this dissertation was organized into the following specific aims:

1. **Design of the Sensor Biotic-Abiotic Interface:** Self-assembly techniques will be employed to deposit organic monolayers as a protective coating on dielectric materials. Such materials may eventually be used to coat the dielectric layer of our proposed 3D capacitive sensing MEA. Silane-based organic films and
organophosphonic acid films will be investigated for monolayer formation using three different deposition techniques: Langmuir-Blodgett, Tethering By Aggregation and Growth method, and Self-Assembly from Solution. Monolayer formation will be confirmed and characterized by contact angle measurements, ellipsometric measurements, atomic force microscopy and x-ray photoelectron spectroscopy. The stability of these monolayers will be investigated on flat surfaces of SiO$_2$, TiO$_2$, Al$_2$O$_3$, and HfO$_2$ in competition with water and PBS at room temperature to determine a suitable dielectric material for further use in the capacitor prototype device and for assessing the utility of organic monolayers deposited on dielectric materials for neural sensing applications. This work is described in detail in Chapter 4.

2. **Microfabrication of the Sensing Device Prototype:** MEMS technology will be implemented to fabricate a novel sensor device for capacitive recording of the electrical activity of the retina. The device design will begin to address the following requirements: higher density sampling, integration of 3D electrodes, and structural compatibility for capacitive coupling through encapsulation. A three-dimensional pillar architecture will be fabricated through lithography techniques to first produce a mask of the desired pillar density. The lithography process will be optimized for different pillar dimensions and spacing to achieve a configuration with maximal capacitance through increased surface area. A unique etching process will be designed using deep reactive-ion etching (DRIE) to construct pillars ~10 µm in height with smooth, straight sidewalls. Insulation of
the 3D platform will be performed through low-pressure chemical vapor
deposition (LPCVD) of SiO₂. Film formation of the active electrode material (Pt)
on the insulated pillar architecture will be investigated using physical vapor
deposition (PVD) and atomic layer deposition (ALD). An array of electrodes will
be realized through precise photolithography masking techniques and wet etching.
Integration of contact pads will be done with electron-beam metal evaporation to
incorporate the device into existing electronics during testing. The fabrication
process will be characterized by scanning electron microscopy (SEM). The
microfabrication of the prototype device is depicted in Chapter 5.

3. **Dielectric Encapsulation of the Sensing Device Prototype:** An insulating
material determined from the Biotic-Abiotic effort will be employed to maintain a
direct charge transfer barrier between the active electrode array realized in the
Microfabrication effort and the excised retinal tissue. The deposition of thin,
conformal layers of dielectric material will be studied through ALD. The ALD
process, including reactor temperature, surface pretreatment, precursor and purge
pulse times and flow rates, will be optimized to produce a uniform pinhole free
dielectric film. The overall capacitance of the insulating layer will be maximized
through the high surface area design of the electrode sensor sights and by
determination of the minimum deposition rate required to coat the working
electrode area. The uniformity of the oxide coating and the electrical properties of
the dielectric material will be characterized by SEM and Electrochemical
Impedance Spectroscopy (EIS). A detailed description of the encapsulation of the
prototype device can be found in Chapter 6.

4. **Evaluation of the Capacitive Sensing Device Interfaced with Retina:** The utility of the microfabrication effort will be assessed through direct current (DC) measurements of retinal electrical responses to light. The performance of the DC device will be quantified through statistical assessment of the overall ERG recording at each sensor site. Durability of the device will be tested to ensure sufficient mechanical strength of the pillar design. With satisfactory performance, incorporation of the insulating material in the dielectric encapsulation effort will lead to capacitive coupling of the device to live animal retina. The microfabricated device will act as a single electrical terminus (electrode). A dielectric film (developed to serve as the biotic-abiotic interface) will encapsulate the active electrode area as an insulating layer. The dissected retina will be placed on the device acting as the opposing (counter) electrical terminus to complete the capacitor device. The assembled sensing device will be evaluated for retinal response to light stimulus, with the physiological studies shown in Chapter 7.
Chapter 3. Experimental Methods

The major experimental techniques employed in this project are discussed in detail below. Although many other important techniques were used during the technical work of this project, the experimental techniques described in the subsequent sections were critical to achieve the overall objective of the project.

3.1 Langmuir-Blodgett

The Langmuir-Blodgett (LB) technique is a standard method used to transfer films of amphiphilic molecules onto a solid substrate. This method enables the precise control of monolayer thickness, allows homogeneous deposition of the monolayer over large areas and gives the potential for multilayer structures with varying layer composition. The LB technique utilizes a LB trough shown in Figure 6 that consists of a dipping device to raise or lower the solid substrate through the monolayer, a movable barrier that adjusts during deposition to maintain the surface pressure of the suspended monolayer, and a pressure sensor that monitors this pressure.
The basis of LB deposition is the self-organization of oil on water as first described by Benjamin Franklin in 1774. The systematic study of monolayers on water by Irwing Langmuir beginning in the late 1910s and his pioneering work with Katherine Blodgett on the transfer of fatty acid molecules onto solid substrates resulted in a deposition technique that has been widely used for many applications over a variety of fields. It is now well known that the phenomenon of self-assembly occurs when there is a difference in the cohesive energies between the monolayer and the dispersed states of a molecule. For the case of LB, a large stable aggregate or Langmuir film (LF) will form on the liquid interface (Figure 7A) due to the chemical potential of the Langmuir film (\(\mu_{LF}\)) being less than the chemical potential of the monomer (\(\mu_m\)) dispersed in a solvent. This relationship is described by the following equation:

\[
\mu_{LF} = \mu_\infty + \frac{ckT}{N^2} \leq \mu_m
\]
where $\mu_\infty$ is the bulk energy of a molecule in an infinite monolayer, $\alpha$ is a constant characteristic of the monomer-monomer, monomer-solvent and monomer-solid interaction, $kT$ is the thermal energy of the system and $N$ is the aggregation number.\textsuperscript{71} It is hypothesized that a combination of convective flow and attractive forces due to surface tension holds these molecules together before transfer of the film onto the solid substrate.\textsuperscript{72}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{A floating monolayer of amphiphilic molecules on water before (A) and after (B) deposition by Langmuir-Blodgett.}
\end{figure}

A continuous film is adsorbed on the solid substrate due to lateral surface forces that act between the molecules during deposition. These are weaker interactions such as van der Waals, hydrophobic, hydrogen-bonding and screened electrostatic interactions. Thus, surface pressure of the LB trough, solvent used for the assembly of the Langmuir monolayer, physical and chemical properties of the amphiphilic molecules can affect the forces within the monolayer itself leading to incomplete coverage or aggregate formation.
The concentration of solution used in self-assembly of the Langmuir monolayer is crucial for the transfer of a LB monolayer. The solution should be below its critical micelle concentration (CMC) to achieve a layer one molecule thick. The forces between the film and the solid surface can be strong covalent or ionic bonds depending on the head group and the solid surface. This interaction can affect the coverage of the surface and also the stability of the monolayer.\textsuperscript{73}

\subsection{3.2 Deep-reactive Ion Etching}

Since the invention of integrated circuits (ICs) in 1958, high aspect ratio (HAR) silicon etching has been an important technology for the semiconductor industry. Silicon etching mainly depended on isotropic wet etching before the invention of Deep-reactive Ion etching (DRIE) in the mid 1990s by Bosch. Today, DRIE based on fluorine plasma chemistry is the method of choice for the development of HAR silicon MEMS.\textsuperscript{74} A standard DRIE system is shown in Figure 10, where the two gases are supplied separately to the reactor through mass flow controllers and a high throughput pumping system maintains low pressure enabling sufficient gas flow in the chamber to replenish depleted reactants near the substrate surface.
The process as shown in Figure 11, alternates between deposition of a fluoropolymer protective film (C4F8) and plasma etching (SF6) with first the removal of polymer at the bottom of the trench and sequential Si-etching. The plasma is generated through supplying RF power to the coil surrounding the chamber that produces a time-varying magnetic field inside the chamber. This field induces an electric field that accelerates electrons to high-energy states while being confined within the plasma to produce higher plasma densities. High plasma densities increase the probability that electrons will undergo ionizing collisions with SF6 before leaving the plasma. By decreasing the chamber pressure, while maintaining the plasma, there is an increase in the mean path of the electrons between collisions with the gas molecules, therefore enabling acceleration of electrons to higher energies increasing the likelihood that a collision will result in breakage of the molecule to form highly reactive radicals (atomic Fluorine). In parallel, the substrate is bombarded with energetic ions by applying an AC voltage between the
RF power at the coil and an additional electrode in contact with the substrate. The alternate attraction of ions and electrons result in less massive electrons having higher mobility towards the substrate resulting in a negative potential with respect to the plasma. This negative bias potential creates a gradient between the plasma and the substrate causing ions to strike the substrate at normal incidence enabling highly anisotropic etching at the Si surface for the realization of HAR features.

Although the basic operation of a DRIE system is relatively straightforward many conditions affect the resulting etch performance. Optimization of source power, bias power, gas flow rate, flow cycle time, substrate temperature, and chamber pressure are important for sufficient etch rates and minimization of scalloping. Scalloping is an inherent result of alternating between passivation and etching steps in the DRIE process. It is the undulation observed along the sidewall of the microstructure as shown in the second etch Figure 11. In some cases the scallop depth can become significant compared to the microstructure diameter of interest and may even result in breakage. Therefore process development is crucial for the desired application.
**Figure 11.** Basic steps in deep-reactive ion etching also known as the Bosch process. Image adapted from reference 77.
3.3 Atomic Layer Deposition

Atomic layer deposition (ALD) was first developed in Finland for the deposition of sulfide thin films. Although this technique was first called atomic layer epitaxy, films grown by this method are usually polycrystalline or in some cases amorphous, thus the name was later replaced with ALD. With the development of thin film high-\( k \) dielectric materials for field effect transistors, Intel introduced ALD for the deposition of thin films in ICs with hafnium oxide used as gate insulating layers. This introduction of new generation ICs led to the vast interest of ALD growth for an array of applications in the electronics industry including microelectronics, transparent electronics, optoelectronics, photovoltaics and spintronics.

The most important feature of ALD is atomic level control of thin film deposition. This control is achieved by sequential self-limiting surface reactions as illustrated in Figure 12. Typically, ALD processes are based on binary reaction cycles where two surface reactions occur independently and deposit a film containing two distinct elements.
Characteristic of ALD are the purge steps, typically N₂, between each surface reaction. This ensures adsorption of a single species as described by the Langmuir adsorption isotherm:

\[ \theta_A = \frac{K_{eq}^A p_A}{1 + K_{eq}^A p_A} \]

where \( \theta_A \) is the fraction of surface sites covered with precursor A, \( K_{eq}^A \) is the equilibrium adsorption constant and \( p_A \) is the partial pressure of precursor A over the surface. The same kinetic derivation can be applied for precursor B. Due to the self-limiting nature of
growth where a finite number of surface sites react to give an equivalent number of surface species, the two reactions may proceed in sequence to deposit a thin film with atomic level control. 80

3.4 Microelectrode Fabrication

The fabrication of the sensing device prototype developed in the scope of this project involved the use of complex microfabrication operations in a clean room environment. Although immense optimization was required during each phase of fabrication, the details of each process will not be described specifically due to the standard operation for each tool utilized. Figure 13 illustrates a process flow diagram for each stage of the microfabrication development including the critical techniques described above.

Figure 13. Process flow diagram of the microfabrication techniques used for the development of the sensing device prototype.
3.5 Electrochemical Impedance Spectroscopy

At an interface physical properties, such as compositional, mechanical and specific electrical properties, change rapidly and charge distributions reduce the overall electrical conductivity of a system. These phenomena are particularly important in solid-state technology, which shifts the emphasis in electrochemistry, from time and concentration dependency to frequency-related dependency through small-amplitude AC studies known as electrochemical impedance spectroscopy (EIS). Impedance spectroscopy is a relatively new and powerful method used to investigate the dynamics of charges in the bulk or interfacial regions of any kind of solid or liquid material. \(^8\) An important concept in impedance spectroscopy is the electrical double layer. The electrical double layer spontaneously occurs at the interface as a phenomenon of compensating the charges through their redistribution and mobility.

In electrochemical systems, a capacitance may arise due to charge distribution at the interface of a flat solid surface in contact with solution. Figure 14 illustrates the charge distribution of ions at a flat interface known as the electrical double layer.
Figure 14. Illustration of electrical double layer capacitance resulting from the local ion distribution at a solid-liquid interface.

The electrostatic potential resulting from this charge distribution is given by the Poisson-Boltzmann equation:

$$\nabla^2 \Psi = \frac{-e}{\varepsilon \varepsilon_o} \sum_i z_i n_i^o \exp \left( -\frac{z_i e \Psi}{kT} \right)$$

where $\Psi$ is the electrostatic potential, $e$ is the elementary charge, $\varepsilon$ and $\varepsilon_o$ are the relative dielectric permittivity and the dielectric constant in vacuum, $z_i$ is the charge number, $n_i^o$ is the number (bulk) concentration of the $i^{th}$ ionic species, and $kT$ is the
thermal energy. The electrostatic potential can be related to charge (i) and the double layer capacitance (ii) can be found by

\[(i) \quad \sigma = \varepsilon \varepsilon_0 \frac{\partial \Psi}{\partial x}\]

\[(ii) \quad C = \frac{\partial \sigma}{\partial \Psi}\]

During an EIS measurement, electrical stimulation is applied to a cell enabling the segregation of the overall electrical response into the fundamental microscopic processes occurring in the cell. These processes include the transport of electrons through the electronic conductors, the transfer of electrons at the electrical double layer, and the flow of charged atoms via defects in the electrolyte. Any intrinsic property that controls the microscopic processes of the electrode system, such as conductivity, dielectric constants, capacitance of the interface region, etc., can be determined by an EIS spectrum.

The structure of the $Z$ (impedance) vs. $\omega$ (frequency) response of the electrode-material system can be determined with an excitation signal given as a function of time and the relationship between radial frequency (radians/second) and frequency (Hertz):

\[E_t = E_0 \sin(\omega t)\]

\[\omega = 2\pi f\]

where $E_t$ is the potential at time $t$, $E_0$ is the amplitude of the signal, and $\omega$ is the radial frequency. The electrical current can be described with a shift in phase and amplitude:

\[I_t = I_0 \sin(\omega t + \phi)\]

Impedance is a measure of the ability of a circuit to resist the flow of electrical current and can be written analogous to Ohm’s Law:
\[ Z = \frac{E_i}{I_i} = \frac{E_o \sin(\omega t)}{I_o \sin(\omega t + \phi)} = Z_o \frac{\sin(\omega t)}{\sin(\omega t + \phi)} \]

The impedance is therefore expressed in terms of a magnitude \( Z_o \) and a phase \( \phi \).\(^{82}\)

Typically the impedance is presented in the form of a Bode plot illustrated in Figure 15.

The impedance is plotted with log frequency on the X-axis and both the absolute values of the impedance and the phase-shift on the Y-axis. This plotting method is useful in deducing information about the physical system such as solution resistance, absolute impedance, capacitance of the double layer, etc.\(^{83}\)
Figure 15. Bode plot presenting absolute impedance vs. log frequency (top) and phase-shift vs. log frequency (bottom). Image adapted from reference 83.

Once impedance behavior is known a direct connection can be made between the behavior of a real system and that of idealized model circuits consisting of discrete electrical elements. Therefore impedance data can be fit or compared to an equivalent
circuit (EC) converting electrochemical processes into physical understanding of the system.

An important EIS building block known as the Debye response describes the electrical double layer mentioned above. The equivalent circuit shown in Figure 16 includes a resistor describing the electrical leads and solution resistance within the cell. An interfacial constant phase element (CPE) in parallel with a resistive element accounts for a solid interface in water where the separation of charge at the solid-liquid interface creates a space-charge capacitance with a delay in polarization at the interface.

![Diagram of equivalent circuit element](image)

**Figure 16.** Equivalent circuit element describing the formation of a electrical double layer at a solid-liquid interface.

Similar electrical analogies can be made for much more complex systems and modern software enables equivalent circuit modeling of such systems with relative ease. In most cases there is not a unique equivalent circuit that describes any given impedance spectrum; therefore, an equivalent circuit that produces a good fit to a given data set must represent an accurate physical model of the cell. Although electrochemical impedance
spectroscopy in combination with EC modeling is an important analytical tool, many limitations are associated with the ambiguity of interpretation of fundamental electrochemical and electronic processes that lead to complications in analysis and inadequate representation of electrical behavior.\textsuperscript{81}
Chapter 4. Design of the Sensor Biotic-Abiotic Interface

4.1 Background

The functionalization of surfaces with thin films that are stable under aqueous conditions is important for a number of applications including microelectronics, lab-on-a-chip devices, micro-Total Analytical Systems and (bio)sensors.\textsuperscript{84-89} For biosensors interfaced with neural tissue it is crucial that the active components of the sensor are fully protected from the surrounding saline environment. Self-assembled monolayers (SAMs) have been extensively studied as coatings in the above mentioned fields due to their ability to tailor surface properties and relative ease of film formation.\textsuperscript{90, 91} Molecules capable of forming monolayer-type thin films usually consist of a head group, which binds to the solid surface, a functional tail group that allows the tailoring of the chemical and physical properties of the interface, and a chain group, usually an alkyl chain, which connects the two. SAMs are ordered assemblies of such molecules that are formed spontaneously by the specific adsorption of the head group onto a solid surface while organizing the molecules into dense layers, which is usually due to weaker interactions (hydrophobic attraction, Van der Waals forces) between adjacent chains.\textsuperscript{92, 93}

Thiols deposited on gold surfaces are a model SAM system extensively used due to their ease of preparation and well-defined packing order dictated by the metal lattice.\textsuperscript{94} They are used for a wide range of applications, but lack long-term stability due to oxidation under ambient conditions or removal from the surface in aqueous environments over
time, and are limited to certain metal surfaces that react with the thiol head group. For some applications, SAM formation on oxide surfaces is preferred. In these cases, alkylsilanes have been extensively studied. Silanes have an advantage over thiol-based chemistries because silane terminated molecules and the thin films obtained from them show higher physical and chemical stability and can be deposited on a wide variety of oxide substrates. However, silane-based monolayer deposition can be more complex depending on parameters such as water content, solvent, deposition time and temperature. Silane molecules are also very reactive, thus more care must be taken during deposition.

In an effort to circumvent the disadvantages of silanes, SAMs of organophosphonic acids have more recently been explored. Phosphonate SAMs are readily prepared and have affinity towards a variety of oxide surfaces, an important feature for the design and implementation in a capacitive system that incorporates oxide thin films for the detection of neuron activity. The formation of an organophosphonate film on an oxide surface is based on a condensation reaction with the phosphonic acid head group reacting with the hydroxyl groups displayed on a hydrated oxide surface that act as reactive sites for covalent attachment after annealing. It is currently believed that organophosphate molecules have up to three sites for attachment and that the films conform to the surface topography rather than forming a two-dimensional network above the surface. This attachment chemistry is most suitable for the capacitive 3D architecture that will be developed for interrogation of the inner anatomical layers of retinal neurons. In recent literature, monolayer formation of phosphonic acids have been reported on several oxide
surfaces of practical importance including Al$_2$O$_3$, TiO$_2$, NiO, SiO$_2$, ITO, HfO$_2$ and ZrO$_2$.\textsuperscript{68, 110, 112-124} Although some work has been done to explore the stability of organophosphonate films on a limited number of oxide coatings,\textsuperscript{125-128} no long-term stability on oxides in aqueous solutions has yet been reported.

In this study we explored three different deposition techniques of two phosphonic acids, octadecylphosphonic acid (ODPA) and perfluorophosphonic acid (PFPA), on various oxide layers. The phosphonic acid SAMs were characterized by water contact angle, ellipsometry, x-ray photoelectron spectroscopy (XPS) and atomic force microscopy. The stability of these monolayers was investigated on a variety of substrates, including SiO$_2$, TiO$_2$, Al$_2$O$_3$, and HfO$_2$, by immersion in deionized water and a phosphate buffer saline (PBS) solution with periodic characterization of the thin films. The stability of these phosphonate films was compared to an octadecyltrichlorosilane film on SiO$_2$/Si substrates. We found that PFPA deposited on HfO$_2$ substrates were the most stable in water and PBS among the alkylsilane and organophosphonate films studied here. These results provide valuable information about phosphonate SAM deposition and stability, and indicate that phosphonate SAMs should be considered for device coatings on the 3D capacitive system developed here.
4.2 Experimental Approach

4.2.1 Oxide Layer Preparation

Oxide coatings were deposited onto (100) silicon wafers (Desert Silicon, Glendale, AZ). The surface of the silicon wafers displayed a native oxide (SiO$_2$) with a thickness of ~1.9 nm as determined by spectroscopic ellipsometry. Aluminum oxide (Al$_2$O$_3$) and hafnium dioxide (HfO$_2$) were deposited onto these wafers by atomic layer deposition (ALD) using a Picosun SUNALE$^\text{TM}$ R-150B ALD system (Detroit, MI). Liquid trimethylaluminum (TMA, SAFC Hitech (USA)) and deionized water (resistivity 18 M$\Omega$/cm), both preheated to 20$^\circ$C, were used as precursors for the deposition of a 25 nm Al$_2$O$_3$ film. TMA was introduced into the reaction chamber at a rate of 150 standard cubic centimeters per minute (sccm) with a pulse time of 0.1 seconds and water followed at 200 sccm for 0.1 seconds with a purge of nitrogen at 150 sccm for 6.0 s between each pulse of precursor. The chamber temperature was maintained at 300$^\circ$C during the completion of 250 cycles, where one cycle consisted of TMA and water pulses with nitrogen purges in between. Tetrakis-dimethylamido hafnium (IV) (TDMAH, SAFC Hitech (USA)) was used as the Hf metal precursor with deionized water as the oxygen source for the 250-cycle ALD deposition of a 25 nm HfO$_2$ film. TDMAH preheated to 75$^\circ$C was delivered to the reaction chamber heated to 200$^\circ$C at 100 sccm with a pulse time of 1.9 seconds. The water precursor and nitrogen purge conditions were the same as those used for the Al$_2$O$_3$ deposition. Titanium dioxide (TiO$_2$) was deposited by electron beam evaporation (Model EV-6, Leybold-Heraeus, Germany) using a TiO$_2$ source at a base pressure of 2x10$^{-7}$ torr.
An internal quartz crystal micro-balance (Model XTC, Inficon, Syracuse, NY) was used to measure the film thickness during deposition of a 30 nm film.

4.2.2 Synthesis of Perfluorophosphonic Acid (PFPA)

Scheme 1: Synthesis of Perfluorophosphonic Acid (PFPA)

Iodofluoroalkane (1.0 g, 2.05 mmol, FluoroFlash) and triisopropylphosphite (0.85 g, 4.1 mmol, Sigma-Aldrich) were combined in a microwave vial with a stir-bar. The vial was capped and sealed, heated to 170°C (30 min) with microwave irradiation (Biotage Initiator, Charlotte, NC) and cooled to room temperature. The crude reaction mixture was filtered through a FluoroFlash column using their published procedure. Briefly, the column was equilibrated with water/methanol (1:4) and loaded with the reaction mixture. The column was eluted with water/methanol to remove non-fluorinated reagents and products, followed by elution with 100% methanol to remove fluorinated products from the column. The methanol wash was concentrated and the residue was reconstituted in chloroform. This solution was dried over solid Na₂SO₄, filtered and concentrated to yield the isopropyl protected intermediate product shown in Scheme 1. ¹H NMR (CDCl₃) d 1.31 (d, 12H), 1.77 (m, 3H), 1.92 (m, 3H), 2.20 (m, 3H), 4.72 (m, 2H. ³¹P NMR(CDCl₃) d 29.1 (s). MALDI-MS: M+1 calc’d: 627.29, found: 627.02. The isopropyl protected phosphonate and trimethylsilyl bromide (TMS-Br, 2.5 g, 16.4 mmol, Sigma-Aldrich) were dissolved in CH₂Cl₂ in a microwave vial. The vial was capped and sealed, heated to
100 °C (10 min) with microwave irradiation (Biotage Initiator) and cooled to room temperature. The reaction mixture was concentrated, evaporated twice from CH₂Cl₂ to remove residual TMS-Br, reconstituted in 1:4 water/methanol and filtered through a FluoroFlash column as described above. The methanol was concentrated to the final PFPA product as a white solid. ¹H NMR (CD₃OD) δ 1.80 (q, 2H), 1.95 (m, 2H), 2.35 (m, 2H). ³¹P NMR (CD₃OD) δ 29.2 (s). MALDI-MS M+1 calc’d: 543.13, found: 542.94. PFPA was used without further purification for the investigation of monolayer formation.

4.2.3 Silane Monolayer Preparation

In all cases substrates were cleaned prior to deposition by sonicating in methanol (ACS, 99.8%) for 10 mins, drying under a stream of air and exposure to deep-UV light for 30 minutes to remove residual organic contamination. A film of n-octadecyltrichlorosilane (OTS, Gelest Inc., Morrisville, PA) was deposited by immersing cleaned SiO₂-coated substrates into vials containing a solution of 1 mM OTS dissolved in n-hexadecane (99%, ACROS Organics). The vials were sealed and allowed to incubate for 24 hours at room temperature. Samples were removed and promptly rinsed first for 10 minutes in chloroform (HPLC, 99.9%), followed by isopropanol (ACS, 99.5%) and deionized water. The samples were then heat treated at 115°C for 10 minutes.

4.2.4 Phosphonate Monolayer Preparation

Three different deposition techniques were used to coat a monolayer of octadecylphosphonic acid (ODPA, Sigma-Aldrich) or perfluorophosphonic acid (PFPA).
Films were deposited onto SiO$_2$, TiO$_2$, Al$_2$O$_3$ and HfO$_2$-coated substrates for further investigation. In all cases substrates were cleaned prior to deposition as described for silane monolayer prepared substrates above. Phosphonate films were deposited using the following techniques:

**Self-Assembly from Solution.** Films of ODPA and PFPA were formed by immersing cleaned substrates into vials containing a 1mM tetrahydrofuran (THF, anhydrous, ACROS Organics) solution of either ODPA or PFPA. The vials were immediately sealed to prevent water absorption and allowed to incubate for 24 hours at room temperature. Samples were promptly removed from solution, heated at 140°C for 24 hours, rinsed and cleaned by sonicating in THF for 2 minutes and methanol for 3 minutes.

**Tethering by Aggregation and Growth (T-BAG).** The evaporation-induced self-assembly of ODPA referred to as the Tethering By Aggregation and Growth (T-BAG) method was reported previously.$^{68}$ This technique was used to deposit ODPA and PFPA films by holding the substrate vertically in a 25 µM solution in THF (total volume of 100 ml). Note that different concentrations of ODPA and PFPA solutions (25 µM - 1 mM) were investigated for the transfer of molecules onto solid substrates and a 25 µM solution gave us the most consistent results. In all cases, the solvent was allowed to evaporate slowly with the meniscus traversing along the surface of the substrate resulting in deposition of a uniform film. Once the meniscus traversed the entire sample and all the solvent evaporated from the substrate, the samples were heated, rinsed and sonicated as described above for films prepared by self-assembly from solution.
**Langmuir-Blodgett Deposition.** THF was used as a solvent for spreading Langmuir-Blodgett (LB) monolayers on a sub-phase of ultrapure water (Millipore Milli-Q UV System, resistivity 18.4 mΩ·cm) using a gas-tight microliter syringe. LB monolayers of both ODPA and PFPA were prepared by consecutive drop-wise addition of solution at the air-water interface in a commercial LB trough (Nima Technology, Linthicum Heights, MD). A total of 100 µL of 1 g/L solution was added for the formation of ODPA films and 350 µL of 2 g/L for the formation of PFPA films. During spreading of the monolayers the surface pressure was monitored to ensure sufficient time between each addition to maintain a starting surface pressure below 1mN/m. After spreading, the monolayers were left to equilibrate for 20 minutes to make sure complete evaporation of the solvent had occurred. LB films were transferred onto freshly cleaned oxide surfaces by the upstroke mode (with a pulling rate of 3 mm/min) of the vertical dipper. A range of surface pressures was explored for the formation of a film on the solid surfaces (10-40 mN/m). AFM analysis of the resulting ODPA and PFPA films revealed that the number of aggregates on the surface grew as the deposition pressure increased for both molecules (data not shown). Based on these tests, a lower surface pressure (10 nM/m) was selected to achieve uniform films on various oxide surfaces studied here. Thus, all films reported here involved compression of the barrier (35 cm²/min) to maintain surface pressures of P=10 mN/m for deposition of both molecules. Once the transfer of monolayers was complete samples were heated and rinsed as described above.

All ODPA and PFPA films were characterized by water contact angle measurements (Tantec, model CAM-MICRO contact angle meter, Schaumburg, IL), spectroscopic
ellipsometry (VASE, J.A. Woollam, Co. Lincoln, NE) and atomic force microscopy (AFM, Veeco EnviroScope now Bruker, Model RTESP, Santa Barbara, CA) equipped with Veeco model RTESP MPP-11100-10, 1-10 Ω·cm Phosphorus (n) doped Si cantilevers (Bruker AFM Probes, Camarillo, CA). XPS data was acquired on a Kratos AXIS Ultra photoelectron spectrometer using an Al Kα source operating at 300W. The base pressure was 2x10⁻¹⁰ torr, and operating pressure was 2x10⁻⁹ torr. Charge compensation was accomplished using low energy electrons. Standard operating conditions for good charge compensation are -3.1 V bias voltage, -1.0 V filament voltage and a filament current of 2.1 A. Reference powders of PFPA and ODPA were analyzed at normal take-off angles (8-10 nm depth). Clean substrates and substrates coated with phosphonate films were analyzed at the same time at 0°, 30° and 75° take-off-angles, which correspond to approximately 8-10, 5-6 and 2-3 nm, respectively, sampling depth. P 2p spectra were acquired for TiO₂ and HfO₂ substrates, while P 2s spectra were acquired only for Al₂O₃ substrates due to overlap between the Al 2p and P 2p spectral lines. A linear background was used for elemental quantification of all spectra. Quantitative analysis was based on sensitivity factors provided by the manufacturer. All spectra were charge referenced to the aliphatic carbon at 284.8 eV. Curve fitting analysis of O 1s spectra was carried out using individual peaks of constrained width, position and 70% Gaussian/30% Lorentzian line shape. Peaks were constrained to a full half width maxima of 1.1 ± 0.2 eV, which was based upon analysis of similar reference materials (e.g., PTFE and SiO₂/Si wafers) using a monochromatic Al source. Curve fitting analysis was first completed on the high-resolution spectra of substrates and reference powders using the above parameters and then similar constraints were applied to curve fitting spectra from
phosphonate film samples. The binding energy of peaks was used to identify the type of chemical bonding between the phosphonate molecules and the various oxide surfaces. The Scienta ESCA300 and NIST Standard Reference Databases were used for identification of possible chemical environments of elements. Two areas per sample were analyzed.

4.3 Results and Discussion

Three different deposition techniques – Langmuir-Blodgett, T-BAG and self-assembly from solution – were initially investigated to coat films of ODPA or PFPA onto SiO$_2$-coated silicon substrates. Static contact angles and ellipsometric measurements were collected for all films (see Table 1). All films on silica were found to be hydrophobic (CA ~90°). For ODPA the calculated molecule length is approximately 2.5 nm (all trans bond configuration) compared to 1.7 nm for the shorter perfluorophosphonic acid.$^{68,133}$ The ellipsometrically measured thicknesses for the ODPA films on SiO$_2$ varied between 0.8 to 2.0 nm for the three deposition techniques. These thicknesses are less than the estimated geometrical thicknesses for a closely packed monolayer with orthogonal (normal) orientation of the aliphatic chains on the surface, which indicates incomplete coverage or that the organic adlayer is tilted with respect to the surface normal. The data found for LB and T-BAG deposition techniques (Table 1) are consistent with a monolayer of ODPA with the organic adlayer arranged at an estimated tilt angle between 37° and 47°. $^{26,47}$
Table 1. Contact angles and film thicknesses determined by ellipsometry for ODPA and PFPA monolayers deposited by Langmuir-Blodgett, T-BAG and self-assembly from solution deposition techniques on SiO$_2$. The calculated tilt angle of the deposited layer for each film with respect to the surface normal is also reported.$^{68, 133}$

<table>
<thead>
<tr>
<th>SiO$_2$ Deposition Technique</th>
<th>ODPA SAMs</th>
<th>PFPA SAMs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact Angle</td>
<td>Film Thickness (nm)</td>
</tr>
<tr>
<td>Langmuir-Blodgett</td>
<td>91°±1</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>T-BAG</td>
<td>89°±3</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>Solution Assembly</td>
<td>90°±2</td>
<td>0.8±0.2</td>
</tr>
</tbody>
</table>

The thicknesses determined for PFPA films deposited by LB and T-BAG ranged between 0.9 nm and 1.0 nm, which corresponds to a monolayer with the perfluorinated adlayer tilted estimated at an angle between 54° and 58°. The film thicknesses and corresponding tilt angles reported here for LB and T-BAG are in agreement with previous reports of monolayer formation of ODPA and a molecule of similar composition to PFPA on SiO$_2$.$^{68, 133}$ However, films deposited by self-assembly from solution appear to have resulted in incomplete coverage of the SiO$_2$ surface as indicated by the extremely thin film thicknesses (and confirmed by AFM as described below).

AFM was used to characterize differences in film topography and surface coverage among the films studies here. AFM images of PFPA films obtained by LB, T-BAG, and self-assembly from solution deposition techniques are shown in Figure 17. Similar results
were observed for the ODPA films (data not shown). As seen in Figure 17A and B, there were few differences in film topography for ODPA films deposited by either the LB or T-BAG method. The resulting films had a uniform surface with no specific features or divergences from the average height observed. A roughness analysis of the two-dimensional images was performed based on a calculation of the standard deviation of all the height values within the given imaged area (root-mean-square \((rms)\) roughness). The \(rms\) roughness value of the control SiO\(_2\) substrate before deposition was found to be 0.14 nm. After film deposition by LB technique or T-BAG method the \(rms\) roughness remained the same. However, for films produced by self-assembly from solution, the \(rms\) roughness increased to 0.43 nm (Figure 17C). These data indicate that a uniform SAM of either ODPA or PFPA was deposited over the entire SiO\(_2\) surface by the LB or T-BAG techniques, while aggregates formed on the surface using the self-assembly from solution technique. Further, while the T-BAG method yielded uniform coverage, we found it difficult to consistently reproduce because it relies heavily on the rinsing procedure rather than the deposition conditions. From these observations we concluded that the LB technique would be used for all subsequent film depositions based on its superior control and reproducibility of the surface modification and coating technique. However, in the absence of a LB trough, T-BAG can be readily used for obtaining good quality films.
The ODPA or PFPA monolayers were also deposited onto TiO$_2$, Al$_2$O$_3$, and HfO$_2$-coated silicon using LB technique. Static contact angle measurements again indicated formation of a hydrophobic film on all oxide surfaces (CA $\sim$ 90-100°). Uniform SAM formation was confirmed by ellipsometric thickness measurements and AFM height images. Before deposition of the oxide and SAMs, nine independent ellipsometric measurements were taken to determine the native silicon dioxide thickness for each wafer used. After deposition of the oxide layer another nine measurements were taken to verify the deposited oxide thickness. After subsequent SAM deposition, three ellipsometric measurements were taken per sample to determine the thickness (height) of the resulting film. The initial contact angles and thicknesses along with the calculated tilt angle with respect to the surface normal of the organic or perfluorinated adlayer are summarized in Table 2. The measured thicknesses for the ODPA films on TiO$_2$, Al$_2$O$_3$, and HfO$_2$, range from 1.8 to 2.2 nm. These data are consistent with a monolayer of ODPA with tilt angles ranging between 44° and 28°, respectively.
Table 2. Contact angles and film thicknesses determined by ellipsometry of ODPA or PFPA monolayers deposited by LB onto different oxide surfaces. The calculated tilt angle of the deposited layer for each film with respect to the surface normal is also reported. 68, 133

<table>
<thead>
<tr>
<th>Oxide Surface</th>
<th>ODPA SAMs</th>
<th>PFPA SAMs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact Angle</td>
<td>Film Thickness (nm)</td>
</tr>
<tr>
<td>Silicon Oxide</td>
<td>91°±1</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>Titanium Oxide</td>
<td>101°±1</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Aluminum Oxide</td>
<td>103°±1</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Hafnium Oxide</td>
<td>101°±2</td>
<td>1.8±0.2</td>
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</table>

The thicknesses determined for PFPA films ranged between 1.0 nm and 1.2 nm, which corresponds to a monolayer with the perfluorinated adlayer tilted between 54° and 45°. The film thicknesses and corresponding tilt angles reported here are in agreement with previous reports of monolayer formation of ODPA and a molecule of similar composition to PFPA on SiO₂, TiO₂ and HfO₂. 68, 122, 128, 133 On HfO₂ coated substrates, the tilt angle for both molecules (ODPA and PFPA) on the surface are in reasonable agreement, so it can be deduced that a complete PFPA monolayer is formed on the hafnium oxide surface. The larger tilt angles for the PFPA film on Al₂O₃ suggest a less compact monolayer or result from a lower coordination bonding mechanism (i.e., mono or bidentate instead of tridentate) for the phosphonate molecules to the surface. To better understand the bonding between the SAMs and the various oxide surfaces studied here, we performed a variety of XPS measurements.
For confirmation of SAM attachment on the substrate, clean substrates and those with SAMs were analyzed by angle-resolved XPS (ARXPS). In ARXPS, the sample is rotated with respect to the analyzer to probe various sampling depths. At larger take-off angles (TOA) more shallow depths are analyzed to allow for the study of concentration gradients, overlayers and attachment mechanisms. Atomic % for shallow probing depths of 2-3 nm at 75° TOA are shown in Table S1 from the Supplemental Material. In the presence of SAMs, an attenuation of signal from the elements present in clean substrates, i.e., Si, Ti, Al and Hf and the appearance of peaks due to P and F (in the case of PFPA) due to SAM formation is expected. None of the clean substrates have a detected P signal, while some F contamination is present in the Al₂O₃ and SiO₂ samples.

Carbon/phosphorus (C/P) ratios for both reference samples of ODPA and PFPA are close to those reported in the literature.¹²⁵ XPS elemental composition provides an average of 2 areas of 300x700 microns in size. Poor molecule packing of ODPA and PFPA SAMs may result in a slightly lower elemental signal obtained from fluorine and phosphorous in the films. For the TiO₂ substrate, increase in C, P and F intensities indicates attachment of SAMs to the oxide surface for both molecules. The C/P ratio as a function of sampling depth indicates attachment of the SAM with the P-O moiety pointing “down” towards the substrate (P-O-X) (data as function of sampling depth are not shown). For Al₂O₃ and SiO₂, similar elemental composition changes to those observed for TiO₂ are detected (no data for ODPA on SiO₂ are shown due to charging during the XPS measurement, which interferes with reliable data analysis.). A decrease in the Al/Si ratio, an increase in C and F and presence of P in the samples confirms the formation of SAMs on both Al₂O₃ and SiO₂, respectively. In the case of HfO₂, surface contamination on the clean substrate was
quite significant, so no expected decrease of Hf signal upon SAM attachment is observed. The presence of F 1s for PFPA and P 2p for both PFPA and ODPA, however, confirm monolayer formation of SAMs on the substrate. Change in the atomic % with TOA as determined by ARXPS confirms monolayer formation for all substrates.

High-resolution XPS spectra provide chemical speciation information for each element that reflects the type of bonding that is present upon SAM formation on the different substrates. Figures 18 and 19 illustrates a subset of the P 2p (P 2s for Al₂O₃) and O 1s XPS high-resolution spectra for both reference PFPA and ODPA samples as well as SAMs supported on TiO₂, Al₂O₃ and HfO₂. The phosphorus (P 2p) peak for both PFPA and ODPA is detected at 135 eV.¹⁰⁹ If there is bonding between P-O- of the SAM and the substrate, the BE of the phosphorous peak will be shifted to a position lower than that for the phosphonic acid. Different bonding mechanisms of phosphonate to the substrate, such as mono-, bi- and tridentate, contribute to the same BE in both the P 2p and P 2s spectral lines, so distinguishing between types of attachment from these signals is not viable. However, we were able to analyze the O1s spectrum to determine the attachment type of ODPA and PFPA to most of the oxides surfaces as illustrated in Figures 18 and 19. Deconvolution of the ODPA reference O 1s spectrum yields four individual peaks that may be assigned to a P-O single bond (532.4 eV), P=O double bond (534.1 eV), P-O⁻ (536 eV), and -OH (537.7 eV).¹⁰⁹ The O speciation for PFPA is slightly different yielding four individual peaks in the O spectra associated with a P-O single bond (532.4 eV), P=O double bond (534.1 eV), P-O⁻ (536.0 eV) and hydrogen bonded O (537.7). For pure ODPA and PFPA, two peaks due to two types of oxygen are expected to be detected, i.e.,
from a P-O bond at 532.4 eV and a P=O species at 534.1 eV. However, the actual references analyzed show the presence of two additional types of oxygen. One peak may be assigned to charged P-O- bonds, while the other may be assigned to either a hydrogen bond between individual ODPA/PFPA molecules or –OH groups present as contamination on the surface. In any event, these groups do not interfere with the peaks used for determining the type of bonding between the phosphonate molecules and the substrate, as discussed further below. If all phosphonate molecules are bound to the oxide surface, the high binding energy peaks (534-536 eV) will not be detected in the O 1s spectra. Formation of a bond between the substrate and P-O--, such as P-O--Ti, P-O--Si, P-O--Al, P-O--Hf, will be confirmed by the presence of a peak at 531.3-531.5 eV, while the presence of free P-OH is confirmed by a peak between 532.4-533 eV. Absence of this peak between 532.4-533 eV in the O 1s spectra, which is due to free P-OH, is a major indication that all three P-O-- groups of phosphonate are bound (tridentate bonding) to the surface. Presence of the free P-OH peak is an indication of some amount of mono- and bidentate binding to the surface. Both bidentate and monodentate contribute to exactly the same BE, so qualitative separation between them is not possible.\textsuperscript{109,125} For 100% of the phosphonate SAM molecules bound through a bidentate linkage, a peak due to free P-OH will be half the intensity of the peak due to P-O-X, where X is Ti, Al, Hf or Si. For 100% of SAM molecules bound through a monodentate linkage, a peak due to free P-OH will be twice as large as the peak due to a P-O-X linkage.

Figure 18 (ODPA) and Figure 19 (PFPA) shows the P 2p spectra for SAMs supported on TiO\textsubscript{2} and HfO\textsubscript{2} as well as the P 2s spectra for SAMs supported on Al\textsubscript{2}O\textsubscript{3}. The shift in
binding energies for both HfO₂ and TiO₂ substrates to about 133.5 eV corresponds to formation of a bond between PFPA/ODPA and the oxide surface. Phosphate-like species are confirmed by binding energies of the P 2s peak for Al₂O₃. For the SiO₂ substrate, the XPS P 2p spectra cannot be reliably used for bonding analysis due to the overlap of the binding energies of the P 2p and P 2s peaks with the corresponding Si peak. The O 1s spectra for SAMs formed on TiO₂ have a low BE peak (530 eV) due to the titania itself. A new peak due to formation of an intermolecular bond between the SAM and substrate P-O--Ti at 531.3 eV was detected. For both SAMs there is small peak due to free P-OH bonds (532.8 eV) indicating that some of the SAMs are bound via a monodentate or bidentate type of bonding. This peak is smaller than what we would expect to observe for 100% mono- or bidentate attachment indicating that some of the phosphonate molecules may be attached via a tridentate linkage as well.

Very similar results are observed for ODPA on the HfO₂ substrates, i.e., there is a small peak at high BE (533.1 eV) due to free P-OH bonds. This peak is smaller than would be expected for 100% mono- or bidentate formation; therefore, a combination of attachment is detected for ODPA on HfO₂. For all other samples, i.e., PFPA on HfO₂ and both SAMs on alumina, the absence of a peak due to free P-OH bonds at high BE (533 eV) confirms 100% tridentate attachment of both ODPA and PFPA to these surfaces.

To summarize the XPS data, elemental XPS analysis and shifts in binding energy of phosphorous spectra confirms SAM formation and attachment to the substrates. Bonding to SiO₂ coated substrates was not determined due to interference from the substrate.
However, deconvolution of the O 1s spectra was assertive in identification of 100% tridentate attachment, in which all three P-O-- bonds are bound to the substrate, of both ODPA and PFPA SAMs on Al₂O₃ and of PFPA on HfO₂. Free P-OH bonds in amounts lower than would be expected for mono- or bidentate attachment were detected for both SAMs on TiO₂ and ODPA on HfO₂ indicating that some intermix among all three binding mechanisms is present.
Figure 18. XPS P 2p and O 1s spectra of the ODPA standard powder and the respective monolayers on TiO$_2$, HfO$_2$ and (P 2s) Al$_2$O$_3$. The deconvoluted peaks of each coordination species are labeled and shown within the spectra.
**Figure 19.** XPS P 2p and O 1s spectra of the PFPA standard powder and the respective monolayers on TiO$_2$, HfO$_2$ and (P 2s) Al$_2$O$_3$. The deconvoluted peaks of each coordination species are labeled and shown within the spectra.
After characterizing the thin-films on various oxide surfaces by XPS to better understand bonding to the surfaces, we investigated the stability of ODPA and PFPA SAMs prepared by LB deposition under aqueous conditions. The initial topography of ODPA and PFPA SAMs on SiO$_2$ substrates before exposure to water is shown in Figure 20A and 20D, respectively. Both films were then immersed in DI water for 30 minutes and dried in a stream of air. Immersion in water for a duration of 30 minutes resulted in reorganization of ODPA and PFPA molecules on the surface as indicated by the formation of islands (~2.2 nm in height) in the AFM images as shown in Figure 20B/E. Interestingly, the PFPA film formed spherical structures 4.4 nm high, which is consistent with a micelle-like structure formation. The size of aggregates on the surface indicates that the PFPA film is desorbing faster than the ODPA film on silica, which may potentially be explained by stronger hydrophobic interactions between neighboring perfluoro groups compared to the interactions between neighboring hydrocarbon chains. We note that similar aggregate behavior was reported for ODPA SAMs on an Al$_2$O$_3$ (0001) single-crystalline surface after exposure to water.$^{126}$

The stability of the ODPA and PFPA films deposited on silica in water was continuously followed for another 10 days. During this time, aggregates on the surface continued to increase in size (Figure 20C/F), which indicates that the phosphonate head group of ODPA and PFPA did not form an immobile chemical bond with the oxidized silicon used in this investigation. More likely a hydrogen bonded phosphonic acid structure was formed on these silica surfaces, which is also consistent with the relatively lower contact angle values observed indicating poor surface ordering. This work is in contrast with
previous reports that have shown strongly bound phosphonate monolayers on SiO$_2$ substrates when the T-BAG deposition method is used.$^{68,128}$ Others have shown that alternative deposition methods result in the headgroup of ODPA only weakly binding to the native oxide layer of silicon likely via hydrogen bonding.$^{111}$ Our results are consistent with the later report where aggregate formation of ODPA and PFPA on SiO$_2$ substrates after immersion in water indicates weak interactions between the silica surface and bound phosphonate molecules. This weaker bonding mechanism may have resulted from the LB deposition technique used in our investigation.

![Figure 20. AFM images of ODPA (A-C) and PFPA (D-F) SAMs on a Si/SiO$_2$ substrate before and after immersion in water for 30 minutes and 10 consecutive days.](image)

We further investigated the stability of ODPA and PFPA films under aqueous conditions on the remaining oxide coated substrates (TiO$_2$, Al$_2$O$_3$, and HfO$_2$). Stability of the ODPA and PFPA films on the various oxide surfaces in water were examined by AFM analysis
after immersion in DI water for 30 minutes. The initial topography of ODPA and PFPA films on TiO$_2$-coated substrates before exposure to water are shown in Figure 21A/D. Uniform coverage was observed with a $rms$ roughness value of $\sim$0.15. The samples were immersed in DI water for 30 minutes, and then dried in a stream of air. As shown in Figure 21B, ODPA films were found to undergo some minor reorganization with the observance of spherical structures on the surface upon 30 minute immersion in water. For PFPA films (Figure 21E), no spherical aggregates formed during the first 30 minutes in water, although the surface did roughen slightly as observed by an increase in the $rms$ to 0.54. The samples were then soaked in water continuously for 10 days and characterized by AFM (Figure 21C/F). Although the XPS data were consistent with a covalent phosphonate linkage to the TiO$_2$ surface, the ODPA SAMs were found to almost completely desorb during this time, while the PFPA significantly reorganized to form large aggregates that remained on the surface after drying. Other reports have shown stability of ODPA on a titanium alloy in water at room temperature for up to 7 days.$^{127}$ However, Mani et al. recently reported that covalently bound phosphonate SAMs of methyl and hydroxyl-terminated phosphonic acids on titania exposed to Tris-buffered saline at $37^\circ$ degraded in $<1$ day.$^{125}$ These results indicate that the stability of ODPA and PFPA in water on titanium is highly dependent upon the nature of the supporting titanium oxide layer and storage conditions.
Figure 21. AFM images of ODPA (A-C) and PFPA (D-F) SAMs on a Si/TiO$_2$ substrate before and after immersion in water for 30 mins and 10 consecutive days.

In contrast to the SiO$_2$ and TiO$_2$ surfaces, we observed significantly greater stability for SAMs deposited on amorphous Al$_2$O$_3$ and HfO$_2$ coatings. AFM images of an ODPA film on Al$_2$O$_3$ and a PFPA film on HfO$_2$ immediately after deposition are shown in Figure 22A and D, respectively. Similar results were observed for PFPA on Al$_2$O$_3$ and ODPA on HfO$_2$ (data not shown). Complete coverage was found for both ODPA and PFPA SAMs on the oxide surfaces. The $\textit{rms}$ roughness value of the control Al$_2$O$_3$ substrate was 0.36 nm; after deposition of ODPA or PFPA films it slightly increased to 0.38 nm. Likewise, the $\textit{rms}$ roughness value of the HfO$_2$ substrate was 0.32 nm but increased to 0.36 nm after deposition of either ODPA or PFPA. The stability of each film on the respective oxide layers was tested by immersing the samples in water for 30 minutes, then completely dried and characterized using AFM. No large changes (i.e., aggregate formation) were observed in the SAM topography or the average height of the monolayer for either the
ODPA or PFPA on Al₂O₃ or HfO₂ (Figure 22B/E). Because both films showed short-term stability in an aqueous environment on Al₂O₃, which agrees with previous reports for ODPA on amorphous and single-crystalline Al₂O₃ surfaces, the samples were continuously immersed for 10 days to test their longer-term stability. AFM height images for ODPA SAMs on Al₂O₃ after this time indicated significant reorganization of the SAMs as shown in Figure 22C. However, this topographic contrast was not present for the phosphonate SAMs on HfO₂ exposed to the same conditions. Although some minor reorganization is observed for PFPA on HfO₂, the majority of the monolayer is intact as seen by comparing the SAM before (Figure 22D) and after (Figure 22F) exposure to water for 10 consecutive days. These data indicate that SAMs deposited by LB on HfO₂ layers are more stable under aqueous conditions than SAMs deposited on other oxide surfaces studied here.
Figure 22. AFM images of (A) an Al₂O₃-coated surface with an as-prepared ODPA SAM and a (D) HfO₂-coated surface with an as-prepared PFPA SAM. AFM images are also shown for (B, C) ODPA on Al₂O₃ and (E, F) PFPA on HfO₂ after immersion in water for 30 minutes and 10 consecutive days.

The studies presented here show a large contrast in stability of phosphonate films on different oxide coatings. As described above, the ODPA and PFPA films on silica are likely weakly bound to silica given their instability in water and past literature results. However, our XPS data show covalently bound phosphonate SAMs on the TiO₂, Al₂O₃, and HfO₂ substrates. Differences between mono or bidentate and tridentate cannot be used to explain the stability differences of, for example, PFPA on Al₂O₃ (tridentate) and HfO₂ (tridentate). We believe that the stability behavior may alternatively be rationalized by the oxide surface reactivity with water. The solubility in aqueous solution of various oxide layers has been reviewed previously. TiO₂, Al₂O₃, and HfO₂ are virtually insoluble in aqueous solution, although it is known that they react with water
resulting in a modification to their surface structure.\textsuperscript{136,137} Our data indicates that the modified TiO\textsubscript{2} surfaces appear to react with water more readily than the Al\textsubscript{2}O\textsubscript{3} or HfO\textsubscript{2} coatings. Thus, the extent of water reactivity appears to determine the overall stability of the phosphonate monolayers.

After examining film stability in water, we immersed the most durable SAMs in PBS (pH=7, 10 mM NaCl) at room temperature. Changes were monitored by measuring the contact angles and collecting AFM images as a function of immersion time in buffer. For comparison, we also studied an OTS film on SiO\textsubscript{2}, which was previously shown to have some stability in water at room temperature.\textsuperscript{102} The OTS SAM was deposited on SiO\textsubscript{2} by a standard solution assembly process.\textsuperscript{130} The OTS film on silica was found to be a monolayer with a thickness of 2.6±0.1 nm determined by ellipsometry that was hydrophobic (contact angle 104°), which are values consistent with previous reports of monolayer formation for OTS on SiO\textsubscript{2}.\textsuperscript{138,139}

Contact angle measurements of ODPA and PFPA SAMs deposited on HfO\textsubscript{2}-coated surfaces and OTS on SiO\textsubscript{2} as a function of immersion time in PBS at room temperature are shown in Figure 23. While all SAMs exhibited an initial drop in contact angle, the phosphonate coatings were found to degrade less quickly over the first 5 days of immersion in PBS. After 10 days, the OTS films had contact angles below 80°, which continued to drop over the next 10 days. ODPA also exhibited a drop in contact angle although less quickly than the OTS sample. However, both ODPA and PFPA SAMs maintained a high level of hydrophobicity through 10 days of immersion in PBS. The
integrity of the PFPA coating was stable until day 20, after which it began to decline as illustrated by the steady decrease in the contact angle measurement (data not shown).

![Graph showing contact angle measurements over immersion days]

**Figure 23.** Static contact angle measurements for ODPA (green squares) and PFPA (red circles) monolayers deposited onto HfO$_2$-coated substrates as well as an OTS (blue diamonds) monolayer deposited onto a SiO$_2$-coated substrate. Substrates were immersed in PBS at room temperature for 20 days. The error bars represent the double standard deviation of the average value from three independent measurements for each data point.

SAMs on HfO$_2$ were also examined by AFM analysis after immersion in PBS for 10 days (Figure 24). The initial topography of the ODPA and PFPA films on HfO$_2$-coated substrates as well as OTS on a SiO$_2$ layer is shown in Figure 24A-C. The samples were then immersed in PBS at room temperature for 10 consecutive days, dried in a stream of air and then characterized by AFM. Significant differences in the film topography were
observed for OTS SAMs after continuous exposure to buffered conditions (Figure 24F), while both ODPA (Figure 24D) and PFPA (Figure 24E) monolayers on HfO$_2$ remained largely intact. We also made stability measurements of PFPA SAMs on HfO$_2$ in PBS at 37°C. However, under such conditions, a rapid (<24 hrs) degradation of all three coatings occurred, which is consistent with similar reports for phosphonate monolayers on TiO$_2$.\textsuperscript{125}

![AFM images](image)

**Figure 24.** AFM images of as-prepared (A) ODPA and (B) PFPA films on HfO$_2$-coated substrates as well as (C) OTS on a SiO$_2$ layer. AFM images of (D) ODPA and (E) PFPA on HfO$_2$, as well as (F) OTS on SiO$_2$ after immersion in PBS for 10 consecutive days.

These AFM images, in combination with the contact angle measurements, lead us to believe that the initial drop in hydrophobicity is due to relatively minor SAM reorganization (perhaps in chain orientation) at the surface for the phosphonate monolayers, whereas OTS aggregates significantly which is consistent with previous reports in PBS.\textsuperscript{140} The enhanced stability of the PFPA SAMs relative to ODPA SAMs
may be due to the movement of water through the films. Water may move slower through the perfluorocarbon film, because it has a more hydrophobic tail than the hydrocarbon tail of ODPA, which slows down the hydrolysis reactions at the surface. These data are consistent with the trend observed for the stability studies presented above in that water reactivity with the oxide substrate may be used to explain the overall SAM stability.

4.4 Conclusions

This report details stability studies of two phosphonate SAMs, ODPA and PFPA, on various oxide surfaces that are important for the design of the abiotic-biotic interface of the capacitive prototype device developed within the scope of this project. We found that films of ODPA and PFPA formed the most uniform coatings when deposited by LB techniques on all substrates, including SiO$_2$, TiO$_2$, Al$_2$O$_3$, and HfO$_2$. The estimated thickness of the SAMs from ellipsometry and angle resolved XPS indicated monolayer formation with covalent attachment to the TiO$_2$, Al$_2$O$_3$, and HfO$_2$ substrates. However, XPS data could not be used to conclusively determine the phosphonic acid bonding to the silica surfaces due to binding energy overlap between film and substrate. Subsequent stability tests indicate that the SAMs on silica prepared here were only weakly bound by hydrogen bonds to the surface. AFM data shows that the phosphonate films deposited on SiO$_2$ and TiO$_2$ formed aggregates upon immersion in water for a duration of 30 minutes. In contrast, SAMs deposited on Al$_2$O$_3$ showed short-term stability (<10 days) in water, while stability of SAMs on HfO$_2$-coated surfaces were monitored up to 10 days under
aqueous conditions with no significant changes to the film integrity. Further, PFPA SAMs deposited on HfO$_2$ were found to be the most stable in aqueous PBS conditions among the phosphonate SAMs studied here. We also show that an OTS SAM on SiO$_2$ is less stable under similar storage conditions. Thus, this work indicates that the choice of substrate to support the phosphonate SAM is critical to determining the overall stability of the deposited film. Based on these findings, hafnium oxide was integrated as the capacitive layer in the prototype device as shown in Chapter 6.
Chapter 5. Microfabrication of the Sensing Device Prototype

5.1 Background

In the past several decades, neural sensing techniques have been developed to record responses of individual neurons or small ensembles of neurons. Recent studies suggest that neurons do not act as independent encoders of information but instead signal in a specific pattern across populations of neurons, making multi-site recordings more crucial. To examine the encoding and processing of information by networks of neurons, microelectrode arrays (MEAs) have been developed and applied, but evolving scientific questions and biomedical applications require higher density sampling and wider spatial coverage. The integration of 3D electrodes, and capacitive coupling to facilitate hermetic sealing of active readout electronics will ultimately enable high density, minimally invasive, chronic recordings of neuronal populations.

Advances in MEMS technology have made it possible to greatly increase the number of electrodes per MEA to thousands of probes, allowing for high spatio-temporal resolution imaging. Such high-density sensor arrays are incompatible with the use of individual passive conductors linking electrodes with external amplifiers and data acquisition electronics; however onboard active readout circuitry has only proven practical for short-term recordings. High-aspect ratio three-dimensional probes can provide closer contact with neurons to facilitate detection and resolution of single cell
action potentials. Our methods allow reliable fabrication of probes with dimensions of a few microns, unlike most other approaches to 3D electrode arrays, which produce structures on the scale of tens of microns or more.

Although MEAs have revolutionized electrophysiological experimentation, most advanced MEAs consist of metal electrodes and utilize direct measurements of neurophysiologic currents. Given inherent limitations of a perforated encapsulating dielectric, metal electrodes in contact with tissue saline lead to inevitable corrosion of embedded active electronics required for very high-density neural interfaces. Direct charge transfer across the electrode-tissue interface also can cause neural damage through irreversible electrochemical/ Faradaic reactions.

Capacitive coupling utilizes a dielectric material to encapsulate all sensors and active circuitry, allowing for corrosion-free integrated electronics and elimination of electrochemical charge transfer at the electrode-tissue interface. Recently there has been a tremendous effort to develop high-k dielectric materials such as hafnium oxide, but little effort has been made to design neural sensors to effectively exploit capacitive coupling. In this study we develop MEA microfabrication techniques that attempt to address the requirements of higher density sampling, integration of 3D sensors, and device designs compatible with capacitive sensing.

The device consists of three-dimensional micro pillar sub-arrays in an 8x8 matrix. Individual micro pillars are comparable in size to a single neuron (3 µm). Although we
demonstrate the fabrication of more than 3,800 pillar electrodes, the pillars are clustered into 60 individual sensing sites to enable active readout with a commercial acquisition system already in place. Ultimately, multi-probe sensor sites may prove useful to compensate for signal loss during capacitive sensing. The cluster configuration of the present device maximizes the surface area of each sensor site, increasing the sensitivity of future capacitive sensors.

The impedance of the device was characterized and shows the fabrication process developed here provides a robust strategy to build high-density microstructures suitable of recording physiological responses when interfaced with live retina. The prototype device fabricated here provides a platform that can be encapsulated in a dielectric coating to eliminate most identified drawbacks of current MEA technology.

5.2 Experimental Approach

5.2.1 Device Fabrication

This study employed a three-mask lithography process using a silicon wafer substrate to fabricate the 3D MEA and integrate signal traces and bond pads on a single device. Figure 25 shows a schematic of the fabrication processes employed to develop the device. Silicon wafers (Silicon Quest, San Jose, CA), n-type with a .005 ohm resistivity and (100) orientation were used as a substrate for the device fabrication. The wafers were first cleaned and a layer of ZEP520A photoresist (Zeon Chemicals, Louisville, KY) was spun at 5000 rpm on the wafer and baked at 170°C for 3 minutes. Figure 25A illustrates the arrays of dots that were exposed in the resist layer using electron beam (e-beam)
lithography (JEOL JBX-6300ES). After e-beam exposure, the pattern was developed in n-amyl acetate (ACROS Organics, 99%) for 60 seconds and thoroughly rinsed in isopropanol (IPA) at room temperature. A metal mask was produced by e-beam evaporation of 100 nm of nickel using the developed pattern as a template. Finally, a lift-off process was performed resulting in arrays of nickel dots 3 µm in diameter with a pitch of 4.5 µm as also shown in Figure 25A.

The nickel dots acted as a mask during a deep reactive-ion etch (DRIE, Plasma-Therm SLR Series ICP Bosch), which alternates between etch and passivation steps resulting in three-dimensional (3D) pillar arrays (Figure 25B). The pulsed DRIE process alternates between two gases, sulfur hexafluoride (SF₆) for the chemical etching of silicon and octafluorocyclobutane (C₄F₈) for depositing the passivation layer. The directionality of the plasma etch removes the passivation layer from the base of the pillar being etched, but not the sidewalls. The first etch step lasted 2 seconds with a gas flow of 10 standard cubic centimeters per minute (sccm). The second etch step lasted 7 seconds with an increased gas flow of 20 sccm. The passivation step lasted 7 seconds with a gas flow of 40 sccm. Both processes were performed at 20 °C, at low pressure (about 23 mTorr) with an inductively coupled plasma (ICP) power ranging from 10-45 W and a reactive ion etching power of 700 W for each step. The etching and passivation steps were repeated continuously for 65 cycles resulting in 8 µm high features. The DRIE process developed here allows the fabrication of features 10’s of microns high (data not shown), by adjusting the number of etching cycles. Figure 25C shows that after the etching was complete the nickel mask was removed with nitric acid (HNO₃) followed by a 30 min dry
oxygen anneal at 1000°C and a hydrofluoric acid (HF) etch to smooth out the pillar sidewalls.

Low-pressure chemical vapor deposition (LPCVD, Trion Technology, Orion III) was used to line the pillar arrays with a 300 nm layer of SiO₂ to electrically insulate the silicon platform as shown in Figure 35D. A conformal layer of platinum (Pt) was deposited onto the insulated 3D platform by physical vapor deposition (PVD) sputtering (Kurt J. Lesker PVD 75) of a 10 nm adhesion layer of titanium (Ti) prior to a 60 nm layer of Pt.

AZ4330 photoresist (AZ Electronic Materials USA Corp.) was used to mask the Pt coated 3D pillar arrays and the electrical leads were integrated by wet etching the conductive layer to insulate each pillar array (Figure 25E). Two photoresist layers were spun at 2000 rpm with soft bakes after each layer of 90 seconds and 5 minutes at 90°C, respectively. Edge bead removal was done and an exposure time of 40 seconds (wavelength of 365nm) was required due to the thickness (~9 um) of the photoresist. The pattern was developed and the masked substrate was hard baked at 110°C for 1 hour 15 minutes before wet etching with aqua regia (3 hydrochloric acid:1 nitric acid: 2 dI water at 85°C for 75 seconds). After etching the mask was removed with acetone and O₂ plasma.

A negative resist (AZ nLOF 2070) was used to mask bond pads terminating each of the Pt leads as shown in Figure 25F. The bond pads were produced by e-beam evaporation of
20 nm titanium and 100 nm gold layer. The Pt traces were insulated with a photoresist (AZ4330) mask, and an opening was made to expose the 3D micro pillar array for further experimentation. Fabrication of the device was characterized by scanning electron microscopy (FEI Nova Nanolab 600, Dual Beam System).

**Figure 25.** Illustrations of the three-mask lithography process using silicon wafer substrate to fabricate the 3D MEA and integrate signal traces and bond pads on a single device.

### 5.2.2 Device Testing

Electrical properties of the device were characterized by electrochemical impedance spectroscopy (EIS) using a CH Instruments (Austin, TX) impedance analyzer. The impedance was recorded along with the phase shift versus frequency sweeping from 10 Hz to 10 kHz at 0.0 V to 5.0 mV, with the frequency of interest being 1 kHz for retinal action potentials. A single pillar array was individually connected as the working
an electrode and tested in amphibian Ringer solution (107mM NaCl, 2.5mM KCl, 1.6mM MgCl₂, 1mM CaCl₂, 22.0mM NaHCO₃, 10mM D-glucose) serving as the electrolyte at room temperature, consistent with the experimental conditions of the electrophysiology measurements (described in Chapter 7). A Ag/AgCl electrode was used as the reference electrode and a gold pad incorporated separately on the device substrate during fabrication was used as the counter electrode. Each individual Pt electrode array of the device was tested this way for quality control and basic electrochemical characterization.

5.3 Results & Discussion

The DRIE process was optimized to fabricate 3D pillar structures with minimum scalloping, which is an inherent effect due to passivation of the sidewalls during the etching process. Figure 26A shows SEM micrographs of a sector of an individual pillar sidewall after etching the silicon substrate. One can see the characteristic inhomogeneities or scalloping on the pillar sidewall that result from an increased gas flow of both passivation (70 sccm, 7 seconds) and etching gases (Step 1: 35 sccm for 2 seconds, Step 2: 70 sccm for 7 seconds) along with higher ICP power (1000 W) of the DRIE process. The average roughness due to scalloping was 50 nm. Minimization of the pillar sidewall scalloping to ~17nm as shown in Figure 26B was accomplished by lowering the gas flows and decreasing the power (see Device Fabrication Section), while maintaining the same step time. Overall the optimized conditions decreased the depth of each etching cycle producing a smoother sidewall, but also decreased the etching rate per cycle from 0.35 µm/cycle to 0.12 µm/cycle.
Figure 26. SEM images of a three-dimensional pillar with (A) significant scalloping of the sidewalls; and (B) after optimizing the Bosch process to minimize scalloping and decrease the roughness of the sidewall.

To further smooth the sidewall of the 3D micro pillar, oxygen annealing combined with an HF etch was performed. Figure 27A and B shows SEM images of the pillar sidewall before and after oxygen annealing, respectively. It can be seen in Figure 27A inset that before annealing the sidewalls are covered with debris from the DRIE process. The observed debris may be due to a combination of the polymer passivation layer and Si debris accumulation on the sidewalls. After oxygen annealing and HF etching the debris is removed and a smooth sidewall results as shown in the inset of Figure 27B. This processing was imperative for subsequent fabrication steps because the roughness became more pronounced after subsequent coating of SiO$_2$ and Pt, which affected the overall uniformity of each layer.
Figure 27. (A) SEM images of a three-dimensional pillar with the nickel mask intact and (B) after removal of the mask and smoothing of the pillar sidewall after oxygen annealing and a HF etch. Insets show magnified sidewall of each pillar, respectively.

Once the device platform was fabricated, a SiO$_2$ layer was grown over the entire surface of the device including the pillar structures, to insulate the Si platform. Figure 28A shows a 3D pillar with 300 nm of SiO$_2$ coating the surface. The intention was to have the Pt layer as the sole electrical component of the device that is actively measured.

Immediately following the oxide deposition, Pt was sputtered onto the device platform, covering the entire surface. Sputtering times were varied to uniformly cover the 3D pillar sidewall. Shorter deposition times resulted in a higher number of defects in the Pt film. Deposition of a 60 nm film provided a consistent Pt layer along the sidewall of the pillars, while still maintaining a thin enough layer to be wet etched in subsequent fabrication steps (Figure 28B).
Figure 28. (A) SEM images of a three-dimensional pillar coated with a 300 nm layer of SiO$_2$ grown by LPCVD that was (B) subsequently coated with a 10 nm adhesion layer of titanium and a 60 nm conformal layer of platinum deposited by PVD sputtering.

To electrically insulate each Pt electrode of the 3D micro pillar array, the device was masked with photoresist and insulating lines were etched with aqua regia. A two-layer photoresist masking process (see Device Fabrication) was developed to withstand the etching conditions, while still protecting the 3D micro pillar array and Pt traces. Figure 29A shows the minimum line width of 3 µm that was achieved with the photoresist masking process. As a result of the masking procedure demonstrated here, all the traces and pillar arrays remained uniformly coated with Pt following the aqua regia etching as shown in Figure 29B. Although etching thin Pt films has been reported previously,$^{175}$ the masking process shown here is unique in that high aspect ratio features are successfully coated with a thick (~9 µm) photoresist protective layer while still resolving 3 µm line widths during a wet etch process. Unlike previous reports$^{176}$ of photoresist
masks degrading during the aqua regia etch, the integrity of the mask remained throughout the etching period of 1 min 15 seconds at 85˚C.

Figure 29. SEM images tilted at 55° of a (A) 3 µm Pt trace leading to the (B) three-dimensional pillar array after etching.

The overall view of the 3D micro pillar array is shown in Figure 30A. Each Pt electrode (60 total) consists of an 8x8 array of three-dimensional pillars 8 µm high with a 3 µm diameter evenly spaced with a 4.5 µm pitch (Figure 30B). Platinum traces patterned from each pillar electrode terminate at Ti/Au bond pads on the outside edge of the device are shown in Figure 30C. The bond pads were configured to connect to a Tucker-Davis Technologies RZ2 multi-channel neurophysiology workstation to further test the device. In addition, a Ti/Au counter electrode was integrated on the device platform (not shown) for electrochemical impedance spectroscopy measurements.
Figure 30. SEM images of the (A) 60-electrode device (B) with three-dimensional pillar electrodes connected by Pt metal traces (C) terminating at bond pads on the outside edge of the device.

EIS was used to study the electrical properties of the as-fabricated high surface area 3D pillar electrodes. Figure 31A shows the Bode plot of an individual Pt electrode sensor site consisting of 64 pillars. To parameterize and quantify the electrochemical behavior obtained by EIS, we used a modified R (CR) circuit. The equivalent circuit used to model the impedance data is shown in the inset of Figure 31A and consists of the resistance of both the solution and the electrical leads ($R_s$) in series with an interfacial constant phase element (CPE) and polarization resistance ($R_p$) circuit in parallel. The CPE was introduced instead of a capacitor to increase the accuracy of fitting the data. CPE elements are generally associated with a non-homogenous surface and variable current density at the electrode. This element accurately describes the electrode surface due to the nature of sputtering onto high aspect ratio features. From the circuit analysis (performed using Z-view software) of the deposited metal film, the values of $R_s$, $R_p$ and CPE that best fit the data were 225.1 $\Omega$, 632.1 $\Omega$, and 5.5685E-7 F, respectively. There is a significant deviation from the pure capacitive behavior of electrode due to the high
surface area morphology (n value 0.8459), which is expected for these electrodes.

Figure 31B shows the phase behavior of a single electrode (~8.7x10^-5 cm^2) and it clearly indicates that the circuit is dominated by resistive element as the phase is predominantly 0 degrees at lower frequencies. The overall impedance is Rs + Rp and is 857.3 Ω indicating that these electrodes are highly conductive as expected for high surface area metal electrodes. Specifically, in the range of frequencies between 10 Hz - 100 kHz with the frequency of interest being 1 kHz for neural action potentials, the behavior of the 3D pillar electrode is characterized with low impedance (710 Ω). Overall, the electrodes were very stable in aqueous conditions and exhibited a uniform behavior across the whole device.
Figure 31. Interface impedance (A) and phase shift (B) of a single Pt electrode array vs. frequency, along with the corresponding equivalent circuit (EC) model as shown in inset.
5.4 Conclusions

A novel three-dimensional micro pillar array has been fabricated and the future application of probing electrical responses of retinal neurons has been confirmed through impedance spectroscopy. We developed a three-mask lithography process using a silicon wafer substrate to fabricate the high aspect ratio 3D micro pillar array with probe dimensions on the order of a few microns, with integrated signal traces and bond pads on a single device. The device consisted of over 3,800 micropillars structured as 60 independent cluster sensors in the present device. The work demonstrated the feasibility of producing high-density arrays. Likewise, the impedance values confirm the robustness of the fabrication process for developing highly conductive 3D microelectrodes. The device showed good consistency of the Pt electrodes during preliminary characterization. The prototype device fabricated here provides a platform that can be extended to capacitive coupling through encapsulation with an insulating material, providing a powerful means to study neural encoding and processing by networks of neurons and may serve as the basis of a functional neural interface for stimulation and recording for neural-electronic prosthetic systems.
Chapter 6. Dielectric Encapsulation of the Sensing Device Prototype

6.1 Background

Neural degenerative diseases and traumatic neurological injuries affect millions of people worldwide, motivating the development of neural prosthetic interfaces to restore sensory or motor function in affected individuals. Most existing systems are based on a relatively small number of relatively simple electrodes, but many applications require higher density arrays able to selectively record from or stimulate individual neurons. Such systems will ultimately require incorporation of active circuitry into packages that can withstand chronic implantation. Advances in neural sensing and stimulation interface technology will lead to the development of hybrid biological-electronic sensor devices for robust, functioning neural prosthetic systems, and also provide a more comprehensive understanding of neural function.

As a specific example, degenerative disease such as retinitis pigmentosa or macular degeneration affects millions of people worldwide. The leading cause of vision loss is age-related macular degeneration, which affects the photoreceptors of the retina, while to some extent sparing the neuronal circuitry, which processes, encodes and transmits visual information to the brain. Recently the FDA has approved a prosthetic retinal implant that can restore limited vision in such patients. However, the underlying technology of present implants will support at most a few hundred electrodes. For high-resolution vision, thousands or even hundreds of thousands of electrodes are probably
desirable. Further, our increasing ability to control the spatio-temporal patterns of activity of extended populations of neurons underscores the limitations of our present knowledge of retinal encoding.

In the past several decades neural activity imaging and sensing techniques have been developed to record the activity of individual neurons and small networks. An emerging goal is to simultaneously measure the activity of all neurons in the retina or a limited region of brain tissue. Advancements in sensing technology will allow a more comprehensive understanding of retinal processing and encoding leading to the development of hybrid biological-electronic sensor systems for robust, functioning retinal prosthetics. We anticipate that such knowledge will also serve as the foundation for more sophisticated electronic systems that will ultimately emulate the perceptual function of biological vision.

Basic science and more importantly biomedical applications require electro-neural interface devices to withstand corrosion and induce minimal damage at the electrode/tissue interface. Current techniques of neural activity sensing employ multielectrode arrays (MEAs) that are typically made of metal electrodes and utilize direct current measurements. Encapsulation of MEAs in a protective coating can eliminate corrosion and minimize electrochemical charge transfer across the electrode-tissue interface, which induce circuitry failure and neural damage through irreversible Faradic reactions.
To overcome the direct current limitations in existing MEA technology, capacitive charge transport between the electrodes and neural tissue can be employed. Capacitive sensing could alleviate most of the drawbacks of the Faradic current stimulation, including corrosion and evolution of chemically reactive species. Recent work has advanced the technology of capacitive stimulation of neurons\textsuperscript{174} and this method will likely prove important for designing future electroneural prosthetic systems. Capacitive coupling has been used for noninvasive measurements in a neuronal culture environment on a semiconductor chip, but the development of this technology has been limited,\textsuperscript{67} and present systems do not support implantation and chronic recording. Advances in the design and fabrication of systems for high-density capacitive coupling to neural tissue will advance prosthetic applications as well as our knowledge of systems neuroscience.

We have developed a three-dimensional micro pillar sensor array that utilizes capacitive coupling in order to sense electrical activity in excised retinal tissue. The device is fully insulated by atomic layer deposition of hafnium oxide. Electrochemical impedance spectroscopy was used to study the oxide deposition on the 3D micro pillar sensor array to ensure a pinhole-free dielectric coating. The characteristic impedance magnitudes increase up to 3 orders of magnitude upon oxide deposition and phase measures indicate fully capacitive sensor sites.

These fabrication processes and electrochemical impedance measurements demonstrate the usefulness of such techniques for building high-density 3D arrays that can be fully encapsulated with a protective dielectric coating. The work shown here advances the
technology towards capacitive sensing of neurons with a robust, non-invasive sensing device, providing a base for studying neural encoding and processing by networks of neurons and for the future development of neural prosthetics.

6.2 Experimental Approach

6.2.1 Hafnium Oxide Deposition

Atomic layer hafnium oxide coatings were deposited onto the fabricated Pt pillar electrodes using a Picosun SUNALE™ R-150B ALD system (Detroit, MI). Pretreatment of the electrode surface with oxygen plasma in the ALD chamber was done prior to HfO₂ deposition. Tetrakis-dimethylamido hafnium (IV) (TDMAH, SAFC Hitech (USA)) was used as the hafnium metal precursor with deionized water as the oxygen source for the ALD deposition of a HfO₂ film. TDMAH preheated to 75°C was delivered to the reaction chamber at 100 standard cubic centimeters per minute (sccm) with a pulse time of 1.9 seconds. The water precursor preheated to 25°C followed at 200 sccm for 0.1 seconds with a purge of nitrogen at 150 sccm for 6.0 s between each pulse of precursor. The chamber temperature was maintained at 200°C during the completion of the ALD cycles, where one cycle consisted of TDMAH and water pulses with nitrogen purges in between. The coverage versus the number of cycles was characterized by X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and impedance spectroscopy.
6.2.2 Impedance Spectroscopy Characterization

The electrical properties of the HfO$_2$ capacitive layer were characterized by electrochemical impedance spectroscopy (EIS) using a CH Instruments (Austin, TX) impedance analyzer. EIS was performed by applying an A.C. potential of 5 mV with a frequency range of 0.1 Hz to 10 kHz. The HfO$_2$ coated electrodes were individually connected as the working electrode and tested in amphibian Ringer solution (107mM NaCl, 2.5mM KCl, 1.6mM MgCl$_2$, 1mM CaCl$_2$, 22.0mM NaHCO$_3$, 10mM D-glucose) serving as the electrolyte at room temperature, consistent with the experimental conditions of the electrophysiology measurements (described below). A Ag/AgCl electrode was used as the reference electrode and a Pt wire as the counter electrode.

6.3 Results & Discussion

Impedance is a method to assess the charge flow between two surfaces as a function of excitation frequency. The characterization of the impedance through equivalent circuit modeling can provide structural information about the material or interface of the system. EIS was used to study the electrical properties of hafnium oxide growth over 3D metal-coated structures. A similar pillar array described in the previous chapter, completely coated in Pt, was used for investigation of atomic layer deposition of hafnium oxide on 3D structures. Figure 32 shows the Bode plot of a 3D Pt electrode before and after HfO$_2$ deposition. A sequential increase in impedance is observed at frequencies lower than 100
Hz, with increasing number of HfO$_2$ cycles (Figure 32A). This impedance behavior is characteristic of ALD growth. The layer-by-layer deposition method exposes the growth surface to two half-reactions keeping the precursors separate during the coating process resulting in atomic scale deposition. Sequential reaction cycles result in larger oxide coverage leading to a more resistive interface. This growth mechanism is further shown by the phase behavior being dominated by a capacitive element with increasing oxide coverage, as indicated by the shift in phase towards 90 degrees for the frequency range between 0.1 and 100 Hz (Figure 32B).
Figure 32. Interface impedance (A) and phase shift (B) vs. frequency of a 3D Pt electrode coated with 100-150 cycles of HfO$_2$. 
To parameterize and quantify the electrochemical behavior obtained by EIS, we used a modified R (CR) circuit. The equivalent circuit (EC) used to model the impedance data for each deposition is shown in Figure 33. The 3D Pt electrode, shown in Figure 33A, consists of the resistance of both the solution and the electrical leads ($R_s$), which remained constant for each measurement, in series with an interfacial constant phase element (CPE) and polarization resistance ($R_{Pt}$) circuit in parallel. This EIS building block is known as the Debye response involving a single time constant accounting for the delay in polarization at the interface. The Debye response accounts for a metal interface in water where the separation of charge at the solid-liquid interface creates a space charge capacitance. The space charge capacitance of non-homogenous surfaces with variable current density at the interface is expressed by the Helmholtz model and can be accounted for by the CPE circuit element. This element accurately describes the electrode surface due to the nature of sputtering Pt onto high aspect ratio features. From the circuit analysis (performed using Z-view software) of the deposited metal film, the values of $R_s$, $R_{Pt}$ and CPE$_{Pt}$ that best fit the data were 281.4 Ω, 9.128x10$^4$ Ω, and 1.349x10$^{-5}$ F, respectively. There is a significant deviation from the pure capacitive behavior of the electrode due to the high surface area morphology ($n$ value 0.8488). The phase behavior of the electrode, depicted by the right axis of Figure 33A, clearly indicates that the circuit is dominated by a resistive element as the phase is predominantly 0 degrees at lower frequencies. The phase behavior along with the overall impedance ($R_s + R_{Pt}$) indicates the 3D metal surface is highly conductive.
With sequential deposition of hafnium oxide we would expect the impedance of the resulting surface to increase. Figure 33B shows that upon deposition of 100 HfO$_2$ cycles the Pt surface is coated by an insulating layer as indicated by the higher impedance. The phase behavior indicates the Debye response remains and an additional capacitive response is present before the phase shift towards 0 degrees as seen previously for a conductive surface. This complex phase behavior is a result of the metal surface coated with an oxide layer containing defects. The EC model accounting for defects in the oxide layer is shown in the inset of Figure 33B. From the circuit analysis of the deposited oxide film, the values of $R_{\text{Oxide}}$, $CPE_{\text{Oxide}}$, $R_{\text{Defect}}$, and $CPE_{\text{Defect}}$ that best fit the data were $5.975 \times 10^4$ $\Omega$, $1.549 \times 10^{-6}$ F, $4.146 \times 10^5$ $\Omega$, and $9.024 \times 10^{-6}$ F, respectively. The true capacitance can be calculated using the relationship:

$$\frac{1}{C} = CPE^a \cdot R^{-n}$$

Therefore the capacitance of the oxide is $4.841 \times 10^{-6}$ F/cm$^2$ compared to $7.581 \times 10^{-5}$ F/cm$^2$ for the electric double layer within the defect. These values reflect what is expected of an oxide film (0.1-20 $\mu$F/cm$^2$) and electric double layer (80-200 $\mu$F/cm$^2$) on a rough surface, validating the EC model proposed here.\textsuperscript{186}
Figure 33. Interface impedance (left axis) and phase shift (right axis) vs. frequency of a 3D Pt electrode (A) coated with ALD of 100 (B) 125 (C) and 150 (D) cycles of HfO$_2$, along with the respective equivalent circuit models.

From the EIS analysis of 100 HfO$_2$ cycles it can be concluded that nucleation and growth of the dielectric layer occurred over the metal microstructures, but increased cycles are required to produce a defect-free film encapsulating the 3D surface. Increasing the deposition to 125 cycles it can be seen in Figure 33C the phase begins to shift (~80°) towards capacitive like behavior, with a single time constant signifying the relaxation time of the dielectric interface. The EIS behavior is evidence for the 3D Pt electrode fully
encapsulated in HfO$_2$. The EC model includes a circuit consisting of an interfacial constant phase element (CPE) and oxide resistance ($R_{\text{oxide}}$) in parallel (Figure 33C inset). From the circuit analysis of the deposited oxide film, the $R_{\text{oxide}}$, $CPE_{\text{oxide}}$ and the n value was used to calculate the capacitance of the oxide film ($3.844 \times 10^{-6}$ F/cm$^2$). Although the film is uniform and capacitive in nature, the ALD deposition was increased to 150 cycles to demonstrate an ideal capacitive layer (90° phase). Figure 33D shows a slight increase in impedance as a function of frequency and a perfect capacitive phase response. The overall capacitance of the oxide layer was found to be $1.265 \times 10^{-6}$ F/cm$^2$, decreasing slightly due to the thicker oxide produced at higher deposition cycles.

To further validate the proposed EC models described above, scanning electron microscopy (SEM) was used to characterize the HfO$_2$ films at varying degrees of deposition time (100-150 cycles). Figure 34 shows SEM micrographs of the bare Pt surface (Figure 34A) and after subsequent deposition of hafnium oxide (Figure 34B-D). After 100 cycles of HfO$_2$ ALD, it can be seen that defects occur on the surface as illustrated in Figure 34B. Increasing the deposition time by 25 or 50 cycles, the defects are eliminated and oxide uniformly covers the surface as seen for both cases Figure 34C and D.
Figure 34. SEM images of the Pt electrode surface (A) coated by 100 cycles of HfO$_2$ (B) resulting in an oxide coating with defects, 125 cycles of HfO$_2$ (B) completely covering the Pt surface and 150 cycles (C) resulting in a thicker layer of oxide.

XPS analysis was performed to confirm hafnium oxide growth and quantify the thickness of the resulting films. XPS spectrum shows an increase in Hf to Pt ratio as the number of cycles increases. For 100 cycles, 0.76 at.% of Pt is detected, while for 125 cycles this amount drops to 0.02 at.% and no Pt is detected for samples with HfO$_2$ coated using 150
cycles. Although it is inherent during these measurements to propagate into the distal Pt layer, the larger detection of Pt after 100 cycles of HfO$_2$ can be attributed to a combination of this propagation and the presence of defects within the layer, this amount drops after 125 cycles of HfO$_2$ due to the lack of defects on the surface and increasing thickness of the oxide film preventing propagation of the beam into the underlying Pt layer. This trend is in good correspondence with SEM data shown previously. Thickness of HfO$_2$ calculated from angle-resolved x-ray photoelectron spectra was between 5.5-5.9 nm for 100 cycles and 10.6-10.9 nm for 125 cycles. These results along with the EIS analysis provide evidence that the growth mechanism for atomic layer deposited HfO$_2$ on Pt microstructures occurs through initial nucleation sites that with subsequent deposition cycles grow together to form a continuous film. As evident from the XPS results, the film thickness rapidly increases upon film formation, but the initial nucleation and spread of the film takes significant time before this occurs. For 150 cycles, the thickness of hafnium oxide becomes larger than sampling depth of underlying Pt; therefore no calculations could be made.

For the application of capacitive sensing, it was imperative that not only the device was uniformly coated with oxide, but also the oxide layer was minimized to increase the sensitivity of the device. After observing the film properties for 100 cycles it was concluded that increasing the number of cycles slightly will allow enough reaction time at the surface to fill the defects and ensure complete coverage, while minimizing the thickness of the film as shown by XPS. Figure 35 shows the Bode plot of a 3D Pt electrode coated with 105 ALD cycles of HfO$_2$. It can be seen that two time constants
exist in the phase behavior, representing two interfaces within the system. The shift in phase towards two capacitive elements indicates the absence of the Debye response and rather two dielectric interfaces on the solid surface. This type of phase behavior was accounted for in the EC model consisting of two circuits in series with a constant phase elements in parallel with a resistor (Figure 35). The model represents a HfO2 film with thicker (8.832x10^{-6} F/cm²) and thinner (9.257x10^{-5} F/cm²) regions resulting in capacitive values calculated from the respective ROxide₁, CPEOxide₁, ROxide₂, CPEOxide₂, and n values as shown previously. The EC model was confirmed with characterization of the oxide film using scanning electron microscopy. Figure 35 inset shows the oxide film with varying thickness as indicated by the arrows.
Figure 35. Bode plot and equivalent circuit model of a oxide film varying in thickness as a result of 105 ALD cycles of HfO$_2$ and the corresponding SEM micrograph (inset) showing the thicker and thinner regions of the film (red arrows).

Having established the upper and lower limits of deposition cycles that yield complete coverage of high aspect ratio Pt pillar microstructures, we deposited HfO$_2$ onto a working device (illustrated in Chapter 5) consisting of 60 individual Pt electrodes with an 8x8 array of three-dimensional pillars 8 μm high with a 3 μm diameter evenly spaced with a 4.5 μm pitch. The device was limited to 60 individual electrodes in order to maintain active readout using a system already in place, but the integration of over 3,800 pillars on a single platform proved the fabrication method developed here would allow building high-density MEAs with thousands of active electrodes. To maximize the capacitance,
each sensor site was designed as a cluster of pillars. Upon oxide deposition, the slightest dielectric film was deposited, while ensuring a defectless film. Figure 36 shows the Bode plot and the respective EC models of a single 3D Pt pillar electrode before and after 140 HfO$_2$ growth cycles.
Figure 36. Interface impedance (A) and phase shift (B) vs. frequency of a 3D Pt pillar electrode measured from the working device before (black diamonds) and after HfO$_2$ deposition (blue circles), along with the respective equivalent circuit models. The error bars represent the double standard deviation of the average value from three independent capacitive electrode measurements for each data point shown.
As seen previously, there was up to three orders of magnitude increase of the impedance upon oxide deposition and the phase indicates a capacitive element. The two time constants remain, with the absence of the Debye response seen previously for an oxide film with defects, thus confirming an oxide layer with thinner and thicker regions, maximizing the capacitance of the device. The EC model again includes two circuits in series consisting of an interfacial constant phase element (CPE) and oxide resistance ($R_{\text{oxide}}$) in parallel (Figure 36). At larger frequencies it can be seen that the experimental data diverges from the model, resulting in higher than expected impedance and phase behavior. This behavior is usually an indication of Warburg impedance and is observed when diffusion limitations occur at the electrode interface. From the circuit analysis of the deposited oxide film the average capacitance of the 3D pillar device was calculated to be 4.369x10^-3 F/cm^2. The EIS data presented thus far establishes the method of fabrication developed here results in a 3D microstructured device that behaves as a capacitor.

6.4 Conclusions

We have developed a novel three-dimensional micro pillar sensor array that utilizes capacitive coupling for the detection of neural activity in the retina. A systematic study of atomic layer deposited HfO$_2$ on 3D Pt microstructures was conducted with EIS, SEM, and XPS. The analysis of this data characterized the capacitive properties and confirmed the uniformity as a function of ALD deposition cycles of the oxide film. Integration of a
highly dense 3D micropillar array that is fully encapsulated with HfO$_2$ is the first step toward hermetically sealed devices that incorporate metal electrodes and active electronics onto a single platform. This illustrates the potential use of this capacitive technology for interrogation of specific cell types or relatively small collections of neurons and improving the selectivity of neural detection, while facilitating mapping of the high spatial detail in visual images.
Chapter 7. Evaluation of the Capacitive Sensing Device Interfaced with Retina

7.1 Background

The electroretinogram (ERG) was first introduced in 1945 as a routine clinical assessment technique in ophthalmology. Since then, many new stimulation and recording techniques have given insight into the sources and components of the electrical signals associated with physiological and pathophysiological events in the retina. Reduction in light-induced electrical activity of the ERG is useful in determining deteriorating retinal function, and these abnormalities are the main criterion for diagnosis of degenerative retinal diseases.

Retinal conditions examined through ERG function include but are not limited to, congenital stationary night blindness, congenital achromatopsia, retinitis pigmentosa (RP), cone–rod dystrophies, cancer-associated retinopathy, melanoma-associated retinopathy, and toxic retinopathies. Most retinal disorders are detected by considerable reduction in the ERG amplitude and time variation in the ERG response. For individuals with RP, amplitudes of the a- and b- waves can be either moderately reduced or almost non-detectable, and time intervals from stimuli are prolonged. ERG amplitudes are accurate for not only diagnosing the disease, but for assessment of severity, to follow the course of disease, to provide a visual prognosis, and for measurement of responses to treatments.
To probe the wealth of information encoded in the ERG, more advanced sensing techniques are required. We have shown the development of a prototype device that addresses the requirements of capacitive coupling and integration of robust 3D micropillar electrodes, while demonstrating a microfabrication process capable of realizing high-density MEAs. The current study aims to first demonstrate the utility of the device through electrophysiological monitoring by direct current means in order to characterize the overall device performance and ensure the current design successfully records retinal response to light stimulation. Finally, capacitive coupling with retina is shown and the characteristic waves of the ERG are resolved. The device performance established here suggests the usefulness of the device for detection of neural activity and can be further extended to detection of single unit action potentials.

7.2 Experimental Approach

7.2.1 Electrophysiology Testing

To evaluate the capability of the direct current and capacitive 3D micro pillar array in sensing the retinal response to light stimulation, electrophysiology measurements were performed as depicted in Figure 37. First, the direct current device was interfaced with retina to fully characterize the measured ERG response. Leopard frogs (*Rana pipiens*) were obtained from Kons Scientific (Germantown, WI) and housed in aquaria under a 12 hour dark/12 hour light cycle. Prior to an electrophysiology experiment, the frogs were dark-adapted for at least 30 minutes. The frogs were rapidly decapitated, double pithed,
and the eyes removed in accordance with standards approved by the Los Alamos National Laboratory Institutional Animal Care and Use Committee. Each eye was hemisected and the retina was carefully removed in a bath of amphibian Ringer under dim red light illumination. The retina was placed ganglion side down on the 3D micro pillar array and a holder of Lucite covered in nylon mesh (Lucite holder) held the retina in contact with the electrodes to ensure sufficient electrical contact with the 3D micro pillar array. The retina was bathed in room temperature Ringer solution contained in a reservoir attached to the MEA using biocompatible epoxy. A 2-second prestimulus recording of baseline neural activity was followed by a 4 second stimulus pulse consisting of a white LED light source shining through an opaque material patterned with 25 randomly placed holes. A rest period of 2-10 minutes took place between recordings and experiments lasted 2-4 hours in duration depending on experimental conditions. All recordings were made inside a Faraday cage at room temperature. Data were amplified (10^5 Ohms input impedance, PZ2, Tucker-Davis Technologies, Alachua, FL), filtered 2-3200 Hz, and digitized at 24.4 kHz using a multi-channel neurophysiology workstation (RZ2, Tucker-Davis Technologies, Alachua, FL).

Similar experiments were conducted to evaluate the performance of the 3D micro pillar array in capacitive sensing of retinal response to light stimulation. Conditions remained constant through both experiments apart from the light stimulus. Initially, an 8 second prestimulus recorded baseline neural activity before a 4 second stimulus pulse consisting of a white LED light source shining through an opaque material with a 50 µm spot size.
7.2.2 Statistical Calculations

The overall device performance was evaluated by the signal-noise-ratio (S/N) over the electrodes that successfully recorded ERGs. The noise was calculated by averaging the first 100 positive and negative points of baseline and taking the absolute difference. The signal was considered the first peak in the ERG response and included the minimum point of the a-wave to the maximum point of the b-wave. The S/N was taken to be the ratio of the two.

Figure 37. Schematic of the 3D micro pillar array interfaced with frog retina during electrophysiology measurements. The 3D micro pillar sensing sites (left insert) are in immediate contact with frog retina where the photoreceptors can be seen from the top (right insert).
7.3 Results & Discussion

7.3.1 Direct Current Device

Successful demonstration of ERG detection with the direct current device, prior to dielectric encapsulation, was imperative for characterization of the device interfaced with the existing multi-channel neurophysiology workstation. We employed the Pt electrode array to measure responses of the frog retina to visible light stimulation. During preliminary experiments it was found that sufficient contact between the retina and the 3D micro pillar array was required to measure a significant potential difference as a result of light stimulus. To propagate the 3D microstructures sufficiently into the retina to maintain enough recording area that results in a signal, a novel Lucite holder (described in Experimental Approach) was employed. Figure 38A and B illustrates the placement of the holder. The nylon mesh allowed adequate Ringer solution around the retina to provide nutrients during testing and maintained enough pressure to ensure contact.

![Figure 38. Schematic of the 3D micro pillar array interfaced with frog retina (A) utilizing a Lucite holder (B) for sufficient contact between the device and tissue.](image)

When measuring ERG responses of the retina, it can be seen that without the Lucite holder a signal much lower in amplitude as shown in Figure 39A is recorded. Upon
application of the hold down device, the amplitude of the recorded ERG signals increased 10 times (Figure 39B), providing evidence that the 3D micropillar array penetrated into the retinal tissue and a larger contact area was made between the retinal tissue and the cluster of micropillars.

Figure 39. Response of isolated frog retina to light stimulation from an individual Pt electrode pad of the 3D micro pillar array with (A) and without (B) the Lucite holder in place.

Ensuring good physical contact, the 3D micro pillar electrode arrays were tested and the simultaneously recorded ERGs from all active electrodes are shown in Figure 40. Most of the Pt electrodes on the fabricated device recorded similar responses with the exception of 9 electrodes, most likely due to insufficient contact at the bond pads. Two electrodes recorded small amplitude signals, but the characteristic ERG waves could be resolved. Robust electroretinogram signals were recorded by 82% of the electrode sites without further signal amplification. After testing 3 separate MEA devices (device resulting in most robust ERG recordings are shown in Figure 40), 80-90% of the electrode sites
recorded ERG signals. For comparison, commercial MEAs were tested under similar conditions and the number of electrode sites that recorded ERG signals varied from 60-98%, so it can be concluded that the number of electrode sites recording is well within the normal range of operation for commercial MEAs. The variability of recording sites depends on many factors including retina preparation, MEA cleaning and handling, and MEA connections to the physiology workstation.

**Figure 40.** Responses of isolated frog retina to light stimulation from all electrode sites within the 3D micro pillar array. Larger insets show scale and stimulation starting at 2 seconds and remaining on for 4 seconds for two of the electrodes. Note that the retina characteristically responds to both the onset and offset of light stimulus.

The performance of the 3D micro pillar array was statistically assessed by the signal-to-noise ratio (S/N) over the electrodes that successfully recorded a signal. This analysis
included 51 of the total 60 electrode sensor sites over the entire device. The threshold of S/N was chosen as the most critical figure of merit. Such threshold for an array of individually addressable electrodes that are aiming to process parallel signal channels can be defined by the “worst case” scenario performance. To determine such a figure of merit we analyzed S/N of each electrode. Figure 41A shows a distribution of the S/N for the 3D micro pillar array. We defined the threshold performance to be the upper limit of the lowest performing 10% of the individually addressable electrodes. The threshold S/N for the 3D micro pillar array as shown in Figure 41B is 11.1. The novel 3D micro pillar array is advantageous because the channels’ performance is evenly distributed amongst the mean response.

![Figure 41](image_url)

**Figure 41.** The (A) signal-to-noise ratio distribution for 51 of the 3D micro pillar electrodes and the (B) threshold S/N electrode response of the upper limit of the lowest performing 10% of the 3D micro pillar array after light stimulation of retina beginning at 2 seconds for a duration of 4 seconds.

The 3D micropillar recorded ON response was compared to a commercial MEA in Figure 42. It can be seen that although the amplitude is a factor of 2 lower, due to impedance...
mismatch of the electronics, the waveform is indistinguishable. This validates the usefulness of the 3D microelectrode array for studying neural encoding.

**Figure 42.** The ON response of isolated frog retina to light stimulation from a commercial electrode and a 3D micropillar electrode.

The durability of the 3D micro pillar array was assessed by placing retina on the 3D microstructures and utilizing a hold down device that was designed to enable penetration of the microstructures into the ganglion cell layer. After placing and removing two separate retinal tissues on the same device, the micro pillars show no sign of mechanical degradation as shown in Figure 43A. During experimentation the performance of the 3D micro pillar remained robust through the lifetime of the retina. After cleaning (Figure
43B) with an acetone air brush (~20 psi) the electrode array was sufficiently cleaned with no sign of mechanical damage and all tissue residue was removed. The results shown here confirm the fabrication of a 3D micro pillar structured array that is robust and reliable for interfacing with retina for multiple experiments.

![SEM images of a (A) 3D micro pillar electrode after experimentation with retina and following (B) cleaning with acetone airbrushing.](image)

**Figure 43.** SEM images of a (A) 3D micro pillar electrode after experimentation with retina and following (B) cleaning with acetone airbrushing.

### 7.3.2 Capacitive Device

Following atomic layer deposition (ALD) of hafnium oxide, the capacitive device was interfaced with retina and Figure 44A shows simultaneously recorded ERGs from 6 of the sensing sites with their respective location within the array. A single ERG measurement is shown in Figure 44B illustrating the characteristic waves of an ERG response.\(^{30}\) No response was recorded in the absence of retina or the absence of light.
Figure 44. Responses (left axis) of isolated frog retina to light stimulation from 6 individual capacitive sensors with their respective location within the array (A) and a single capacitive sensor response (B). Stimulation (right axis) starts at 8 seconds and remains on for 4 seconds. Note that the retina characteristically responds to both the onset and offset of light stimulus.

Fourier analysis of the capacitive response recorded previously, results in the frequency distribution for the ERG response. Analysis places both the onset and offset retinal responses in the frequency range of ~3 Hz. The physiological response frequency is well in the range of the capacitive regime of the sensor developed here. The results shown here of sensing retinal activity through a novel device platform fully encapsulated with HfO$_2$ is the first report integrating 3D electrodes and capacitive coupling.

7.4 Concluding Remarks

We have shown that upon interfacing the 3D micropillar electrode array with retina, ERG responses can be measured and the resulting signals are suitable for in depth physiological analysis, as apparent from the presence of the a-, b-, and c-wave components of the ERG. The integration of 3D micropillars not only improves device-
tissue proximity to target cells within the retina, but increased surface area maximizes the capacitance for a non-invasive sensor. The realization of over 3,800 micropillars on a single platform provides evidence that high-density electrode arrays can be fabricated using the microfabrication techniques presented in this work, which will improve the selectivity of neural detection and facilitate mapping the high spatial detail of visual images. Encapsulation of the 3D micropillar array with HfO$_2$ is the first step towards the future of hermetically sealed devices that incorporates metal electrodes and active electronics onto a single platform. The dielectric coating employed here provides long-term stability of the Pt electrodes while minimizing tissue damage at the interface. Reduced tissue damage increases the excitability of neurons during light stimulation and the characteristic waves of the ERG can readily be detected with the hermetic coating incorporated over the active area of the device. Functionalization of the device interface creates enabling technology for long-term implantation of fully integrated neuro-physiology devices with improved biocompatibility.

The integration of high density, penetrating electrodes that are fully insulated as shown here, supports the effort to understand the transformation of visual scenes into electrical images within the retina. The comprehensive understanding of retinal function requires mapping of numerous sensory neurons in spatially correct order and remains a daunting task. The accurate encoding of neuron function will lead to electrical devices that support or replace the function of defective retinal layer, restoring vision in the blind. With multiplications of the number of sensor arrays within the same technology platform developed here, one can reach neural prosthesis with approximately 10,000 pixels that is
already an image that enables basic environment, orientation, and even facial feature resolution. In simple words, when developed in full, this technology may help blind people see for the first time.
Chapter 8. Future Outlook

Although extensive electrophysiological studies were outside the scope of this project, the device developed here addresses the requirements of higher density sampling, integration of 3D sensors, and device designs compatible with capacitive sensing. Our new MEA sensor allows for the first time, a more comprehensive study of targeted cell populations within the inner anatomical layers of the retina, while providing resistance from corrosive biological fluid and avoiding adverse reactions at the tissue-electrode interface. This level of technology provides a platform that is suitable to elicit action potentials of individual neurons.

The microfabrication techniques developed here can readily be tailored to meet the needs of action potential recording through optimization of the size and spacing of the 3D micro pillar array to provide an adaptable platform for measuring action potentials of specific neuron types within targeted layers of the retina. The realization of high-density electrode arrays can be extended to thousands of sensor sites through multiplexing, providing widespread physiology studies that will enable high acuity image mapping of the retina. Finally, the studies exploring dielectric encapsulation in combination with surface functionalization as a means of hermetically sealing active electrode materials, leads to the development of fully packaged sensors with onsite electronics for implantable visual prosthetics.
The prototype device developed here is a step towards comprehensive sensing tools that enable biomedical and clinical research to establish the system requirements for a retinal prosthesis. With a better understanding, the ability for people to read, recognize faces and navigate may be achieved through electrical impulses generated by subretinal or epiretinal devices. Once such devices are available a great deal of research must be done to advance the utility of the devices through visual psychophysical experiments in order to develop the stimulus algorithms that result in restored site. The level of research and technology development required seems a daunting task, but a retinal prosthesis represents the best hope for curing blindness.
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