PLAQCHEK

Electrochemiluminescence Immunosensor for Rapid Measurement of Lp-PLA2

Nanotechnology Engineering Fourth Year Design Project Final Report

University of Waterloo: Faculties of Engineering and Science

Departments of Chemical Engineering, Electrical and Computer Engineering, Chemistry

This report is submitted for the FYDP final report requirement for the NE 409 course.

FYDP #12

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Executive Summary

This project aims to address the early detection of the heart disorder, or atherosclerosis, which is the buildup of plaque within one's arteries. The proposed solution utilizes an electrochemiluminescence (ECL) immunosensor device targeted at the Lp-PLA2 enzyme. It will be fitted with integrated signal-processing electronics to deliver a rapid response to the customer. The theory behind this product is based on the enzyme Lp-PLA2 being an indicator of atherosclerosis. Thus, the product's goal is to detect this enzyme concentration through an ECL reaction. The light signal emitted will be processed and interpreted as a concentration of Lp-PLA2. This will provide clinicians with an easier screening method and make it more accessible to the public due to the lack of need for a laboratory. The four primary requirements for this project are: to demonstrate the feasibility of this detection method; to possess a limit of quantitation equal to or less than 2 ng/mL; to possess a detection range between 0 ng/mL to 300 ng/mL; and to produce a result under 30 minutes from sample exposures.

Acknowledgments

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Glossary of Terms and Acronyms

Term	Definition
Lp-PLA2	Lipoprotein-associated phospholipase A2 is a lipoprotein found in blood.
ECL Reaction	An electrochemiluminescent reaction is a reaction that emits light. Species are generated on the surface of an electrode, causing the species to undergo an electron transfer, which emits light.
Biomarker	An indicator of another phenomenon happening in the biosystem.
Immunosensor	A sensor that utilizes a chemical reaction which involves antigens and antibodies.
DLS	Dynamic light scattering is a technique used to determine the size and size distribution of a species.
SEM	Scanning electron microscopy, a technique that focuses a beam of electrons onto a surface of a specimen to produce images of the specimen.
COMSOL	A software application used for simulating the physics of designs.

LTspice	A circuit simulation program which can be used to create circuit designs and analyze their performance.
Photodiode	A semiconductor diode that generates a potential difference once the diode is exposed to light.
SiPM	A silicon photomultiplier, is a solid-state photodetector that produces a current pulse in response to the absorption of a photon.
MRI	Magnetic resonance imaging is a technique that can produce images of the structures inside a body.
ECG	An electrocardiogram is a test where electrodes connected to an ECG machine are attached to the body, which are used to evaluate the electrical activity of the heart.
CoFe PBA	Cobalt Iron Prussian Blue Analogue particles
PEI	Polyethylenimine
APTMS	(3-Aminopropyl) trimethoxysilane
DI Water	Deionized Water

Table of Contents

List of Figures

List of Tables

Introduction

The development of accessible and cost-effective diagnostic tools in the medical industry is crucial for addressing modern-day health challenges. This project aims to create a point-of-care immunosensor device designed to detect Lp-PLA2, an enzyme biomarker that is associated with being an early indicator of coronary artery disease. Through the use of an ElectroChemiLuminescence (ECL) immunosensor, this design will provide consumers with a more affordable and convenient risk assessment method, allowing for rapid detection and early intervention.

Problem Background

Cardiovascular disease remains a significant global health concern and is the leading cause of death worldwide. Among the various forms of cardiovascular disease, coronary artery disease (CAD) is the most common. This disease is caused by the buildup of plaque in the arteries (atherosclerosis), which narrows them and reduces blood flow to the heart. If left undiagnosed and untreated, the blood flow can become completely blocked, resulting in a heart attack.

Unfortunately, symptoms of CAD are commonly unnoticed. Furthermore, current diagnostic methods require expensive and often hospital-based equipment, such as electrocardiography (ECG), echocardiograms, magnetic resonance imaging (MRI), and computed tomography (CT) scans. Therefore, frequent screenings for CAD in the general population are not currently a possible solution for mitigating the risk of CAD in the general population.

High-Level Design Description

This project addresses this issue through a point-of-care device capable of accurately quantifying the enzyme concentration of an atherosclerosis biomarker known as Lp-PLA2 from a small blood sample obtained via a finger-prick device. From this detected enzyme concentration, the concentration of LP-PLA2 will be displayed on the LCD screen of the sensor. Alternatively, if the user chooses to connect the immunosensor to their phone, through an app, the user will be able to track and see what level of risk they are at of suffering from cardiovascular disease.

Design Background

The enzyme lipoprotein-associated phospholipase A2 (Lp-PLA2) has been identified as a highly specific biomarker for atherosclerotic plaque progression, with several studies establishing its significance as an independent risk factor for coronary artery disease (CAD) and ischemic stroke (Cojocaru et al., 2010). It is produced by macrophages during inflammatory reactions associated with the formation of plaque in the walls of the arteries (atherosclerosis), which may cause a stroke (Sertić et al., 2010). However, other studies suggest that the enzyme plays an active role in the plaque buildup cycle. Lp-PLA2 has been shown to trigger the immune system, which causes the plaque buildup cycle.

Although the concentration of the enzyme was not directly correlated to stroke severity, the concentration was shown to be indicative of stroke recurrence (Cojocaru et al., 2010). Furthermore, this correlation was proven to be independent of other known risk factors for CAD, such as elevated cholesterol (Cojocaru et al., 2010). Therefore, the presence of Lp-PLA2 in blood samples is an independent predictor of CAD and ischemic stroke within the general population (Cojocaru et al., 2010).

The methodology of how the immunosensor detects the level of risk of a person suffering from cardiovascular disease also differs from how current methods try to evaluate it. This proposed immunosensor will function by measuring the influence of enzyme Lp-PLA2 on an ECL reaction. Its basic structure consists of a substrate with electrodes, with gold nanoparticles and a chemiluminescent material deposited on the substrate. Lp-PLA2 antibodies are bound to the gold nanoparticles to capture the enzyme. When a pulsed electrolytic potential is applied to the sensor's electrodes, anionic radicals of luminol and reactive oxygen species (ROS) are produced. Once these two products are formed, they react with each other and the chemiluminescent molecules (Luminol) to generate excited anions, which emit photons to release energy. When the target enzyme is captured by the antibody, the steric hindrance of the complex is formed, and the increased mass of the electron transfer channel results in a reduced ECL signal (Al-Sodies et al., 2023). The idea is to measure this reduction in the ECL signal, which will be proportional to the amount of Lp-PLA2 present in the sample. Then, this concentration can be used to determine the level of risk the individual is at for suffering from cardiovascular disease.

This design's main advantage over other current methods used to detect the risk or presence of cardiovascular disease is its significantly lower cost and ease of access for anyone to use. Methods that are currently employed to detect cardiovascular disease include getting an MRI scan, an ECG test, or a CT scan. However, these methods require expensive machinery found in hospitals, and wait times to take these tests can take up to several months. With this design, it is intended to be purchasable for a low price (around $20 - 30 CAD) at pharmacies and be very simple for a person to learn how to detect their risk of suffering from cardiovascular disease. This makes the barrier to being able to test for cardiovascular disease significantly lower and allows for more people to prevent suffering from a stroke or heart attack.

A major challenge in the design is in the fact that the ECL signal is quite weak due to the reaction itself and the fact that blood concentrations of LP-PLA2 are quite low, along with the low sample volume that will be taken to ensure non-intrusiveness of the device. Thus, several techniques have been explored to increase the intensity of the ECL signal. Firstly, some amplification agents may be added in addition to the gold nanoparticles, such as the cobalt iron Prussian blue analogue. This nanoparticle may be coated in gold nanoparticles to form an AuNPs@CoFe PBA nanocomposite as the sensing substrate, which exhibits catalytic activity for luminol, resulting in a 47 times stronger ECL signal compared to bare ITO (Al-Sodies et al., 2023).

Another approach for tackling this challenge is in photodetector selection, which can be optimized. Several high-sensitivity options exist to be explored, including avalanche photodiodes, photomultiplier tubes (PMT), silicon photomultipliers (SiPM), CMOS image sensors, and charge-coupled devices (CCD) (Xu et al., 2023). Luminol has peak emissions at 452 nm and 489 nm (Karabchevsky et al., 2016), thus, the photodetector must have a peak emission around that range.

Customer Requirements

For this project, there are 4 primary and 3 secondary requirements described and defined in Appendix A. For the primary requirements, the thought process began with finding the minimum design requirements to satisfy the customer and address the problem statement. The factors that were chosen to be prioritized are functionality, accuracy, and speed of the immunosensor, which resulted in the 4 primary requirements. To determine the secondary requirements, factors such as ease of use were considered. The secondary requirements aim to make the immunosensor faster, more portable and have a better consumer interface. These requirements are explained and discussed in greater detail in Appendix A.

Requirement	**Importance**	**Functionality**	**Minimum Performance Specification**
The product must demonstrate the ability of Lp-PLA2 detection through the ECL method.	Primary	Non-functional	Through the ECL reaction, the product demonstrates it can detect the difference in the ECL signal before and after the Lp-PLA2 is captured.
The product must have an accurate reading of the Lp-PLA2 concentration level in the given sample.	Primary	Functional	Quantitation reading of less than or equal to ± 2 ng/mL of the Lp-PLA2 concentration.

The product must have a wide range of key Lp-PLA2 concentrations it can detect.	Primary	Functional	The range of detection is at least 0 ng/mL to 300 ng/mL.
The end-to-end response time must be optimized so the user can receive a result in a short amount of time.	Primary	Functional	End-to-end response time of under 30 minutes.
The end-to-end response time could be further optimized so the user can receive a result in a shorter amount of time.	Secondary	Functional	End-to-end response time of under 20 minutes.
The product could have a companion application to make the product even easier to use for the customer.	Secondary	Non-functional	Companion applications have less than 500 ms latency.
The product could be	Secondary	Non-functional	The product could have

portable.			the weight of an average mobile phone and the size of a tissue box.

Table 1: Outlines the Customer Requirements of the design.

Discussion

Design Choices

The general design concept of the immunosensor involves a device that accepts a removable chip sensor that is a part of a three-electrode system, which is optimal for the device's drop delivery setup. There will be a photodiode circuit that will read the light signal emitted from the immunosensor chip. This signal will be processed by the electronics and converted into a prediction of the user's heart disease risk level. All this will be integrated within a portable case featuring an LCD screen that will display the readings to the user. The device will potentially possess wireless communication capabilities and be able to connect to an external device for a more in-depth reading. For a general overview of the entire design, see the figure below.

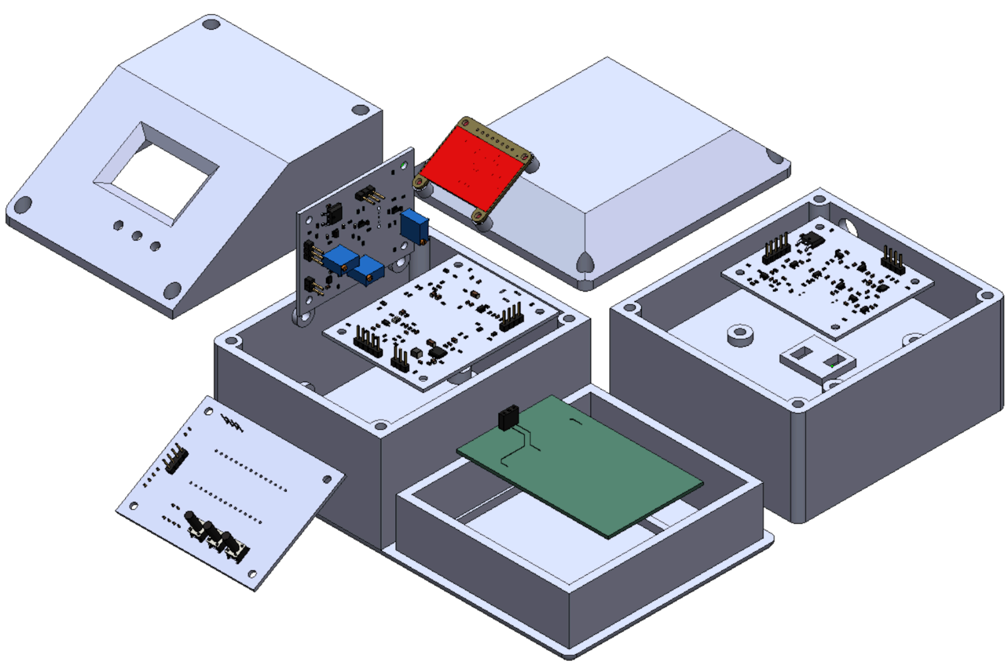


Figure 1: High-Level Exploded-View Diagram

The three-electrode system is integrated onto a PCB that was designed and optimized using COMSOL. The reference and counter electrodes are designed as exposed pads on the PCB, and the immunosensor serves as the working electrode and can be removed from the circuit. The electrode PCB will be printed on FR4 by the manufacturer, and the electrode pads (reference and counter) will be printed with immersion gold (ENIG).

The working electrode is where the ECL reactions occur and is the bulk of our immunosensor. It will be made out of an indium tin oxide (ITO) -coated PEN substrate. The immunosensor will be detachable from the main device body for ease of replacement with a small clip. The PCB electrodes are reusable and stay attached to the device and potentiostat circuit. The placement of the electrodes will be arranged in a fashion shown below.

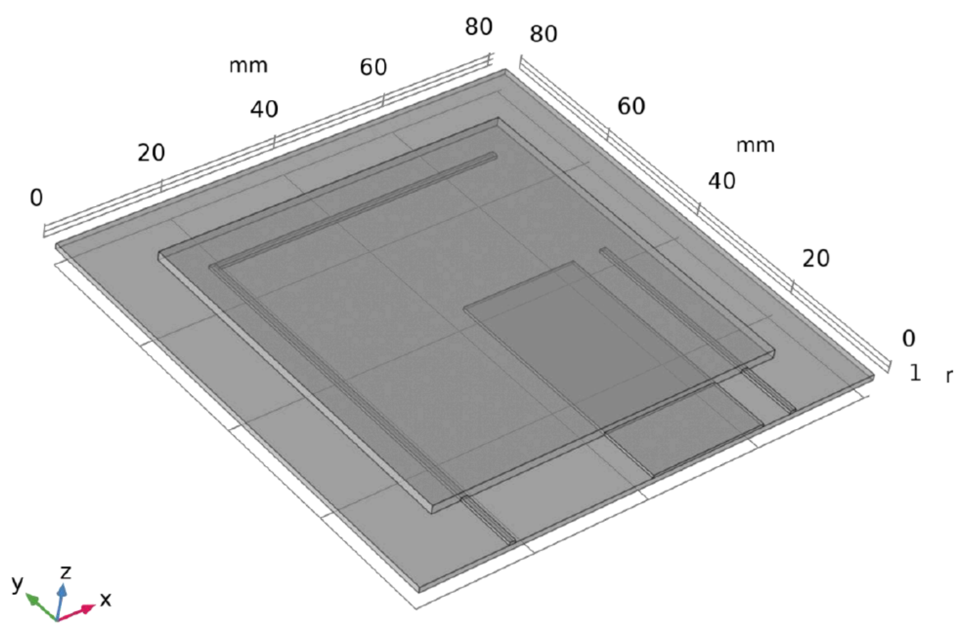


Figure 2: Three-Electrode Design on Chip

As shown in the figure above, the counter electrode will run alongside the working electrode, pass it and loop over it to make an L shape. The reference electrode will be on the other side of the working electrode and will be the same length as the working electrode. The working electrode is in the centre. This placement and electrode geometry were optimized in COMSOL, primarily aiming for a potential distribution where the potential difference between the reference and working electrodes is very small. This is optimal as the reference electrode's purpose is to provide a stable reference potential against which the working electrode is controlled in a feedback circuit.

To align with the customer requirements, the design has been modified to better address these criteria. An ECL reaction was chosen over an ELISA reaction, which is more standard in enzyme detection, because of the decreased cost of antibody procurement. For ELISA, two antibodies are needed to identify one Lp-PLA2 enzyme. In comparison, only one antibody is needed for the same purpose in ECL. This will lower the cost to the consumer. Additionally, the device will be designed to be portable so that it can be more accessible, increasing its reach.

The PEN-coated substrate was chosen over the PET substrate and glass substrate for multiple reasons. A glass substrate was rejected for being too fragile and expensive. Additionally, storing them will take up more space compared to a thinner substrate. As for the PET substrate, it is not chemically resistant enough to survive the fabrication process. Thus, the PEN-coated substrate was chosen for the cartridge design.

Additionally, to support the ease of access for the consumers, the device will have wireless communication capabilities. Ideally, the device will connect to a consumer's mobile device through Bluetooth, and the results from the immunosensor will be displayed on an app. This app will have features to store and track a user's LP-PLA2 levels and display statistics about their risk of heart disease trends. Unfortunately, due to time constraints, this aspect of the project has been assigned as a secondary requirement. As mentioned above, the primary method to view the results of the immunosensor would be through an LCD on the physical device itself.

Custom electronics will be developed for the project to support design requirements. They will be separated into four PCBs, with each PCB serving only one distinct function in the system: a logic board, a power distribution board, a potentiostat board, and a photosensing board. This modular design allowed for easy system bring-up and debugging, as well as allowing for the device to have a small enclosure. Additionally, this separates the switching regulators on the power supply board from the sensitive analog circuitry on the photosensing board, as switching regulators may produce EMI that can be coupled into other circuits.

The first PCB is the main logic board and hosts a microcontroller (Arduino Nano ESP32), buttons for user I/O, an OLED screen, a temperature sensor, and debugging LEDs. It also serves as the master device for receiving ADC data via a serial peripheral interface (SPI) and provides the PWM signal for the potentiostat to modulate the pulse and control the electrode reaction.

For the driving side of the hardware system, a potentiostat circuit was designed to control the potential at the working electrode. Since the optimal electrode potential to get the most luminol reaction out of the sensor cannot be determined until after the sensor has been constructed, this circuit must be designed to support a variable electrode pulse with a varying DC level and amplitude for the electrode potential. To do this, potentiostats are used to tune these variables. As mentioned, the Arduino supplies a PWM signal, which is fed into a circuit which buffers the signal, attenuates or amplifies it, and then adds it to a DC bias via a summing amplifier, which represents the input signal. That signal is then fed to a control amplifier, along with the reference electrode, to set the working electrode potential. The popular TL082 op-amp was chosen for all of the op-amps in the potentiostat.

The bias voltage for the potentiostat is also variable and can either be set to negative or positive via a switch. The bias is held steady and relatively low-noise as it is regulated by TPS7A20, an ultra-low-noise linear voltage regulator. The bias can be set between +5V and 5V, which is plenty of range for ECL reaction.

A near-UV silicon photomultiplier (SiPM) was chosen as the photodiode, as they have high responsivity at luminol's peak wavelength, are very sensitive, cost-effective, and the support circuitry is much simpler than other high-performance photodiodes such as avalanche photodiodes. The SiPM converts the light emitted by the immunosensor into a current, which is then converted into a voltage by the trans-impedance amplifier.

The selection of an op-amp for this circuit must be done carefully to achieve high signal integrity, low noise, and a linear response. It must exhibit very low input current bias, low noise (input-referred noise density and current noise density), high gain-bandwidth product, fast slew rate, low input capacitance, and low offset voltage. Thankfully, there are many op-amps designed for photosensing/photodiode TIA interfaces that fit many of these requirements. Thus, the OPA863A was chosen.

The signal is further amplified by a second-stage amplifier, in case the amplification provided by the transimpedance amplifier is not sufficient. Lastly, the signal is converted to a digital signal by a high-resolution analog-to-digital converter IC. An external ADC will be used as the Arduino’s internal ADC has a resolution of only 10 bits, which may not be sufficient for the characterization of the sensor. Additionally, the ADC must be placed close to the last analog stage to ensure the best signal integrity.

The ADC chosen is the MCP33131, a 16-bit ADC. It requires an external voltage reference, provided by a high-precision voltage reference. Additionally, it was chosen because the front-end circuitry was designed such that the signal spans as much of the ADC range as possible, so that differences in light levels are more noticeable. Therefore, linearity was important. This ADC has a good linearity: a low integral non-linearity (INL) ensuring accurate (linear) conversion at all light levels and low differential non-linearity (DNL) ensuring a smooth response to changes in light intensity.

Lastly, the power supply board consists of four switching power supplies that take the 12V input for the wall adapter plugged into the device and converts it to various potentials needed for the system: 6V and -6V buck regulators for analog supply, 3.3V buck regulator for digital supply, and a 44V boost regulator to supply the SIPM bias.

The SiPM bias supply is a relatively high voltage and must be kept at a very low noise, as any noise in the bias supply will be coupled into the output signal. It is operated at roughly 38V, which is 6V overvoltage. The overvoltage was kept low to reduce the dark count rate (dark current). The power board provides 44V, then an LDO on the photosensing board steps down the voltage to 38V, providing a low-ripple (low noise) supply for the SiPM, which is crucial. The voltage is further filtered with a 2nd-order low-pass filter before reaching the SiPM.

In terms of the other smaller circuit details, buttons and a small (around 2” x 2”) screen will be placed on the device for user input and output. Additionally, the entire device will be powered by a 12V DC wall power adapter with a barrel jack, which is commonly used for many consumer and medical devices. Low-dropout regulators (LDOs) are present on the potentiostat and photosensing boards to reject noise and reduce voltage ripple, ensuring a stable and low-noise supply for the analog circuits. The analog and digital supplies are separated to prevent unwanted coupling.

Trade-offs have also been made for this project. Due to the purpose of our device, its accuracy will be lower compared to conventional methods of heart disease detection. This is because the conventional methods are more sophisticated and more sensitive relative to this device. However, this is acceptable as the project aims to act as an early indicator rather than a full diagnosis. Users will still need to be further tested upon receiving a positive result for heart disease from this device.

As mentioned in Appendix E, the blood acquisition mechanism will be a lancing mechanism, similar to glucose detection devices. This is the result of producing a smaller amount of blood from the user. This may make the detection process harder due to the low quantity of blood. This is an acceptable trade-off for this device, as this will make the test simpler and quicker than using conventional methods of blood drawing, such as a needle.

As shown in Appendix E, the immunosensor will convey its signal as a light emission. As a result, a dark enclosure needs to be made to minimize the background noise from other potential light sources. This involves choosing optimal materials, such as anti-reflective coatings and opaque barriers. The enclosure will also need to be made with tight tolerances to ensure that no light from any other source can be picked up by the photodiode. These requirements will, unfortunately, increase the cost of the device. However, they will be needed to enhance the sensor's detection capabilities.

Design Verification

The goal of the simulation plan was to calculate the optimal placement of the electrodes for the three-electrode system on the sensor chip. Additionally, the dimensions and placement of the electrodes also needed to be optimized for the immunosensor to work optimally. The electronic simulations were done in LTspice, while the dimensional simulations were done in COMSOL. For more information about the exact simulation setup for both, see Appendix C.

The custom electronics in this project require a potentiostat circuit to easily and accurately control the voltage potential at the electrodes. Thus, a simulation was performed in LTspice to aid in designing such a circuit, shown below.

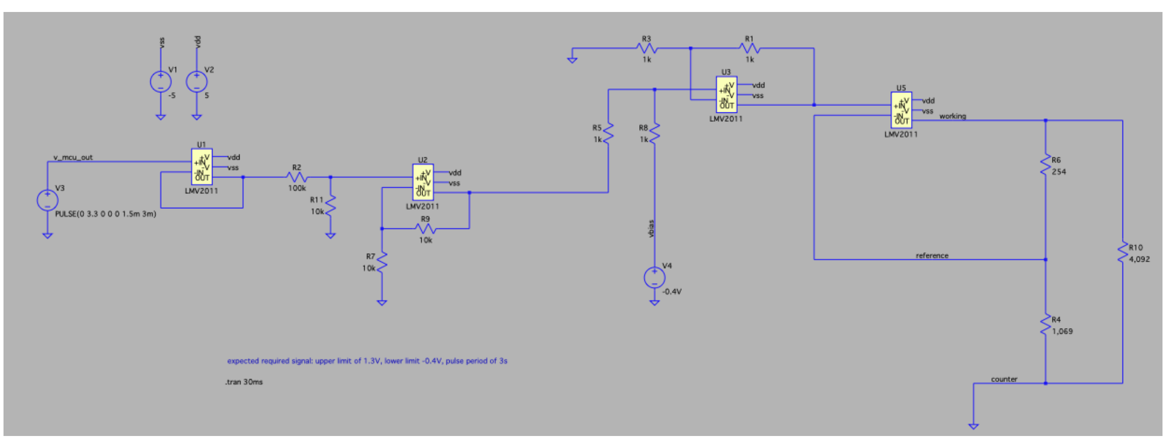


Figure 3: Potentiostat Circuit Design

The input signal is a square wave of 3.3V to 0V, with a pulse period of about 3 μs, which is intended to come from an I/O pin of a microcontroller with a 3.3V logic level. The input is then buffered by U1 to aid in signal integrity, then passes through a voltage divider and an amplifier (U2), allowing the amplitude of the electrode signal to be tuned to both below and above 1.65V (microcontroller's I/O signal amplitude), as needed. A bias voltage is added to the input signal with equal weighting via a summing amplifier U3. The resulting output signal is the signal intended to be pulsed at the working electrode, so it is fed through a control amplifier, provided by U5, which keeps the potential at the reference electrode and the input (working) electrode signal equal. The resulting waveform can be seen in the results of the transient simulation in Figure 1.3.

As seen in the next sections, the reference electrode was designed to be very close to the working electrode so that they see the same potential, as the resistance between them will be minimized. The resistance between the working, counter, and reference electrodes used in the simulation was extracted from COMSOL simulations of the electrode design, described in further detail below. The electrodes will be pulsed at a very low frequency (essentially at DC), so the effect of capacitance was deemed to be negligible, and capacitances between the electrodes were not modelled/represented in the LTspice simulation.

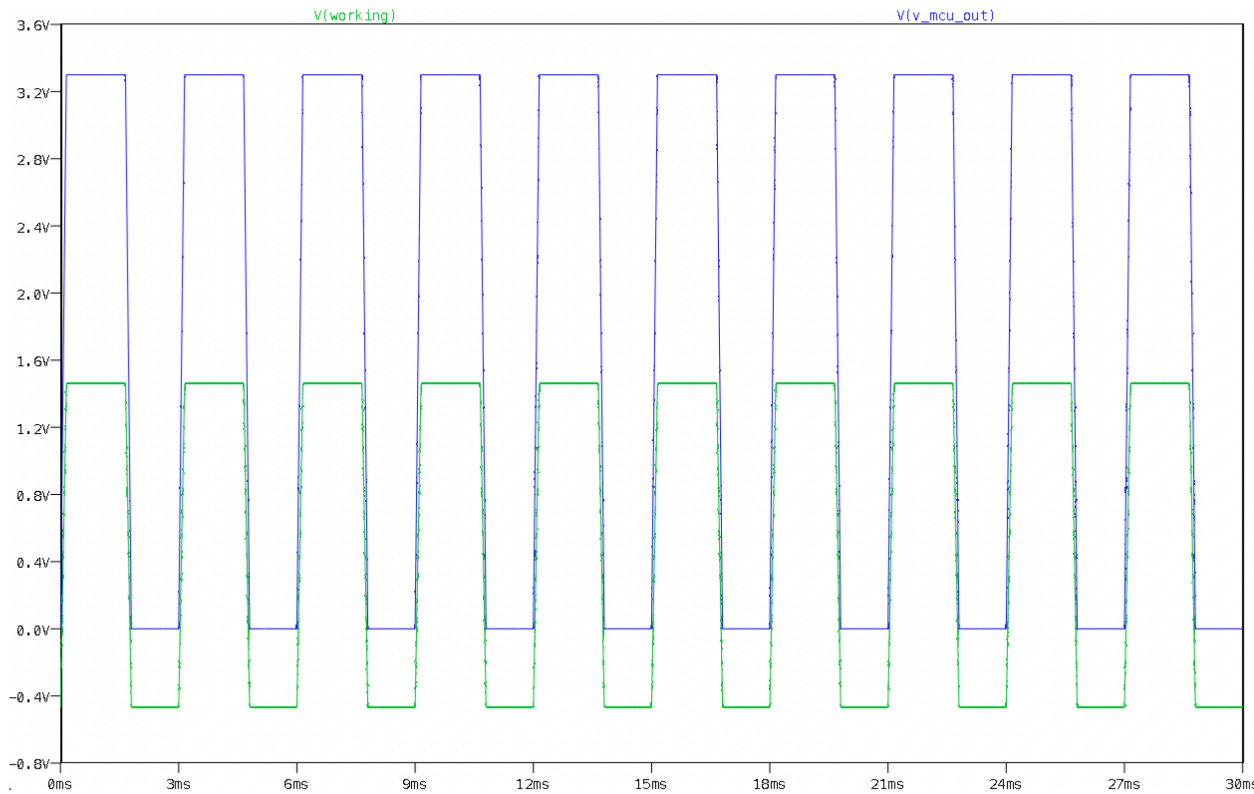


Figure 4: Potentiostat Circuit - Input Pulse From MCU and Output Pulse

The COMSOL simulation recreated the projected sensor with the projected materials. This tool was to aid with the placement of the electrodes on the sensor PCB. For the exact design of the PCB, see Figure 1.1. In addition to the electrodes, the PCB and the electrolyte were added to the geometry. The simulation used the electrostatic and electric current physics interfaces within COMSOL. The centre electrode was labelled as the working electrode and was set as a voltage terminal. The left electrode, or the counter electrode, was set as ground. The reference electrode on the right was not modified.

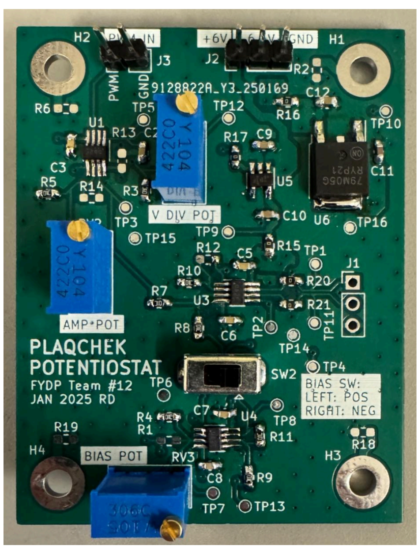
Four stationary studies were conducted on the system. The first study was a general voltage sweep across the working electrode, ranging from 5V to 5V. The other three studies were set as dimensional sweeps. The first of these swept the width of the counter electrode from 0.5 mm to 10 mm in intervals of 0.5 mm. The other two swept the distance between both reference and counter electrodes. They were swept from 1 mm to 5 mm with an interval of 0.5 mm and from 12 mm to 24 mm with an interval of 1 mm, respectively. For the exact results from these studies, see Appendix F.

The resistance of the working electrode to counter electrode, the working electrode to reference electrode, and the reference electrode to counter electrode was simulated and swept with various device dimensions (eg, electrode width, counter electrode distance) to optimize the design and for use in the LTspice simulations described previously. To do this, the voltage potential at each electrode was swept along with the current, and the resistance was determined using this data and Ohm's law.

The dimensions were optimized such that the working electrode and reference electrode saw very similar potentials, as the reference electrode is used to control the working electrode potential. Furthermore, the counter electrode should be placed away from the other electrodes such that the resistance between them is very high, as the counter electrode should not participate in the ECL reaction, and this resistance was found to be the most impactful in the accuracy of the electrode potential control loop.

Prototype Construction

The four custom PCBs were ordered from JLCPCB, and electrical components were purchased from DigiKey and Mouser. The boards were assembled by hand using soldering equipment from the Rapid Prototyping Centre. The boards shown in Figures 5, 6, and 7 were hand-soldered with a soldering iron. There is a mix of surface mount and through-hole components on all the boards, so both soldering techniques had to be used. The power board had some surface mount components with exposed pads underneath them (for heat dissipation), meaning that they could not be soldered by hand with a soldering iron, as those pads are not accessible. Therefore, a reflow oven had to be used instead. To solder the board via reflow oven, solder paste was first applied to the PCB using a stencil (also purchased from JLCPCB), which exposes only the surface mount pads. Next, the components were placed on the PCB one by one. Lastly, a temperature profile was created for the reflow oven, and the PCB was placed inside before running the reflow oven. After it was done and the PCB had cooled down, the through-hole components were soldered on.



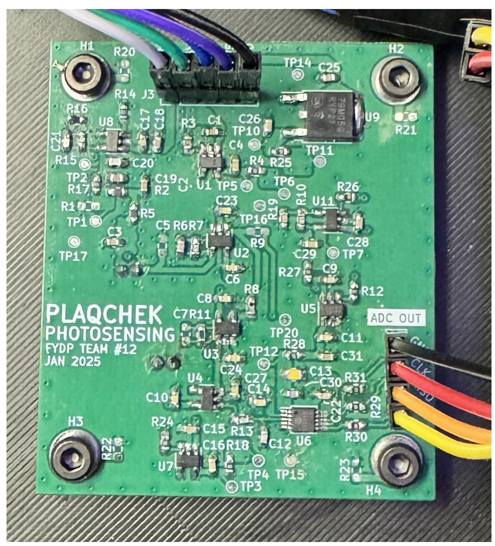
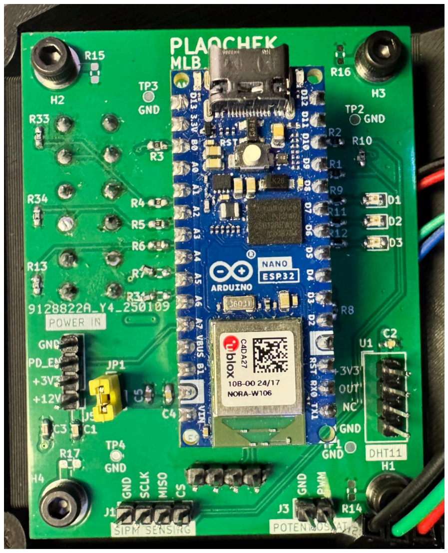


Figure 5. Potentiostat. Figure 6: Optoelectronic sensing board



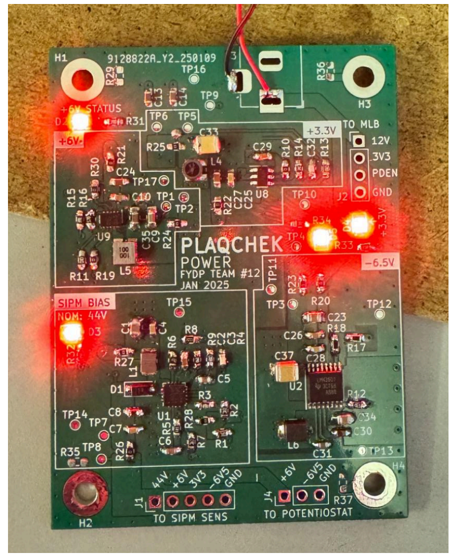


Figure 7: Main logic board. Figure 8: Power distribution board

The device was modelled in SolidWorks, and the 3D CAD model was printed. Within the 3D printed model, all the electronic components of the device would be housed in the device, and where the ITO is placed for blood sample testing would be conducted. On the right-hand side section of the prototype would be the dark box reaction enclosure, where the reading of the light levels would be read when a voltage was applied. On the left-hand side of the prototype, an OLED screen was attached where it would display instructions, progress bar updates, and results for the sample testing process. Buttons were also included to control the starting of tests or the resetting of the device.

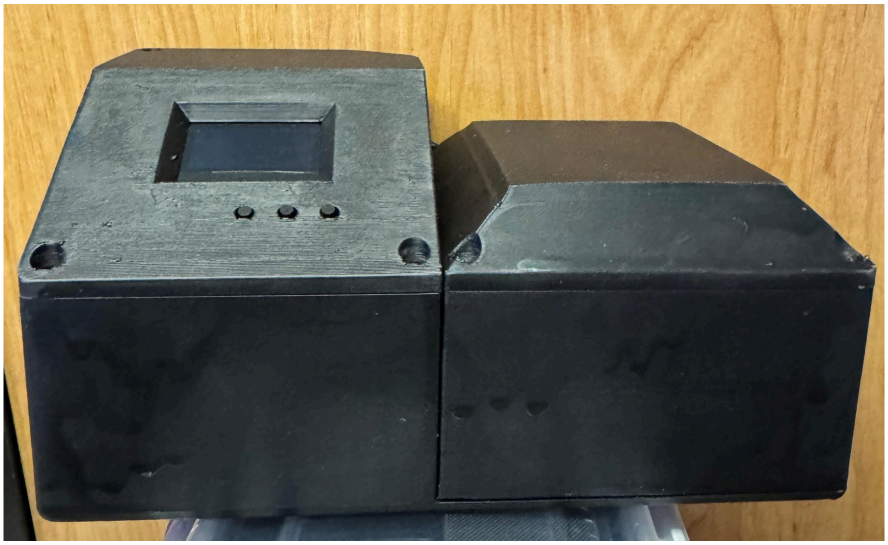


Figure 9: 3D printed CAD model

Multiple ITO-PENs were fabricated to house the reaction with Lp-PLA2. The fabrication process of the immunosensor starts with the fabrication of cobalt-iron particles. This particle will enhance the signal generated by the sensor's electrochemiluminescence (ECL) reaction. This effect is optimized when the cobalt iron is 300 nm in diameter. To fabricate cobalt iron particles, 436.2 mg of cobalt nitrate hexahydrate was mixed with 441.2 mg of trisodium citrate dihydrate in 50 mL of deionized water. This solution is combined with another solution that contains 329.25 mg of potassium ferricyanide in 50 mL of deionized water. The mixture is stirred at 600 rpm for 5 minutes before it is left to age for 24 hours at room temperature. The aged solution is then placed in a centrifuge and spun at 10000 rpm for 5 minutes. This will leave behind the cobalt iron particles. They can be extracted by decanting the green solution and then washing the particles with deionized water and ethanol. The particles are left to dry for 48 hours to evaporate any leftover water and ethanol. The chunks are pulverized and stored in a dry area for safekeeping.

Following this, polyethyleneimine (PEI) must be attached to the particles to act as a medium for the gold nanoparticles. To do this, 10 mg of the cobalt iron particles were dispersed in 10 mL of deionized water alongside 100 μL of PEI. This will be done with a sonicator. Afterward, the mixture is stirred at 800 rpm for 4 hours at room temperature. This should give the PEI enough time to bond with the cobalt iron particles. The solution is spun in a centrifuge at 10000 rpm for 5 minutes to separate the particles. The excess solution is decanted. The particles are washed with deionized water three times to get rid of any unbonded PEI. The particles are resuspended in deionized water so that the concentration is 1 mg/mL. This mixture is stored in a dark fridge at 4°C until the next step is performed.

The gold nanoparticles need to be attached to the PEI-bonded cobalt iron particles. They will act as a medium for the Lp-PLA2 antibodies. The gold nanoparticles must be around 25 nm to maximize the signal strength of the ECL reaction. To synthesize the gold nanoparticles, 200 μL of 1% chloroauric acid was added to 15.3 mL of water. Once added, it was placed on a heating mantle, which was set to 100°C and mixed with a magnetic stirrer at 500 rpm. Once condensation was visually seen in the solution, the reflux continued for 4 minutes. After the 4 minutes were complete, the magnetic stirrer increased to 1400 rpm, and then 300 μL of 1% trisodium citrate dihydrate was added. The solution was to continue boiling for 10 minutes, and the AuNPs were allowed to cool off afterward. DLS was conducted on the synthesized AuNPs, where it was seen to have a diameter of 21.58 nm and a polydispersity of 0.238 nm. To attach the gold nanoparticles, 1 mL of the 1 mg/mL mixture made in the previous step was added to 20 mL of a gold nanoparticle colloid. This solution will be stirred at room temperature for 2 hours. The mixture was later centrifuged at 10000 rpm for 5 minutes to separate the newly created nanocomposites from the solution. The excess liquid will be decanted, and the nanocomposites will be washed with deionized water three times. The nanocomposites will be dispersed in deionized water, such that it has a concentration of 0.2 mg/mL. This solution needs to be stored in a dark fridge at 4°C until the substrate for them is ready.

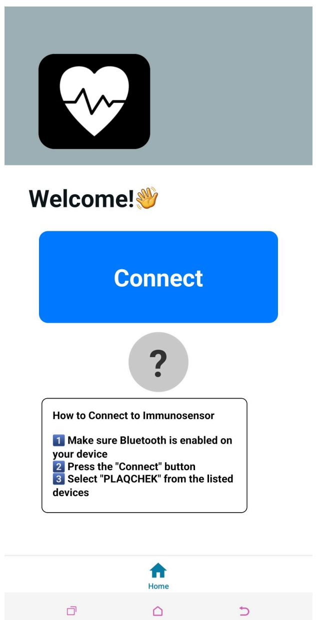
The ITO substrate will be the substrate for the nanocomposites. The substrate will be washed first to remove any impurities that may interfere with the sensor fabrication process. This involves sonicating the substrate with a 1:1 v/v solution of ethanol and 2% sodium hydroxide. This is done for 30 minutes. The substrate also needs to be washed for 30 minutes in acetone and deionized water. Finally, it undergoes sonication with a 30% ammonia solution. The substrate is left to soak in the solution overnight to allow the ITO surface to hydrolyze. It is washed with deionized water and dried with nitrogen gas the following morning.

Next, a thin layer of 3-aminopropyltrimethoxysilane (APTMS) is applied to the ITO surface to act as an insulation layer between the ITO and the gold nanoparticles. To fabricate this, 10 µL of a 0.01% v/v solution containing APTMS in ethanol is dropped onto the ITO surface. The ethanol is allowed to evaporate. The ITO substrate will then be placed in a humid environment at 55°C to allow the APTMS layer to set. The layer-setting process will take approximately four hours. Afterward, the gold nanocomposite solution is dropped onto the ITO surface and allowed to adhere for 5 minutes. The excess will be washed off with deionized water and dried with nitrogen gas. At this point, the base electrode is complete. The ITO needs to be stored in a dark fridge at 4°C until the next step.

The following steps involve bonding the Lp-PLA2 antibodies to the gold nanocomposites. 10 µL of the antibody solution with a concentration of 0.2 ng/mL is dropped onto the finished sensor surface. It is incubated at 30°C for 2 hours. Using 0.01 M of a phosphate-buffered saline (PBS) solution, the remaining unbonded antibodies are washed off. Any remaining unbonded active sites on the gold nanoparticles will be sealed with 10 µL of a 1% v/v bovine saline albumin in deionized water. This is to prevent any unintentional bonding from occurring after the fabrication process. This is incubated as well at 25°C for 1.5 hours. Any excess solution remaining afterwards will be washed off with a 0.01 M PBS solution and dried with nitrogen gas. This completed immunosensor needs to be stored at 4°C until it is ready to be used.

Figure 10: ITO-PEN cartridges with different concentrations of Lp-PLA2 antigen.

A mobile app was created using the open-source platform Expo and written using React Native and TypeScript to interact with the device. Once the app is opened on a mobile device, the app would detect the device’s Bluetooth low energy (BLE) signal and ask the user to connect to it. Once connected to the PLAQCHEK device, the baseline level test and sample test could be started from the app, soft or hard reset the device, or disconnect from the device. After the baseline and sample tests were conducted, the app would display the user’s cardiovascular assessment.



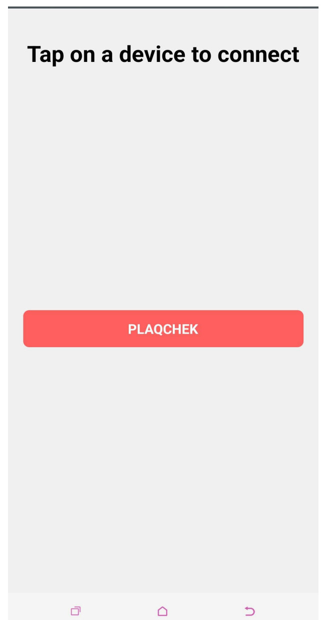
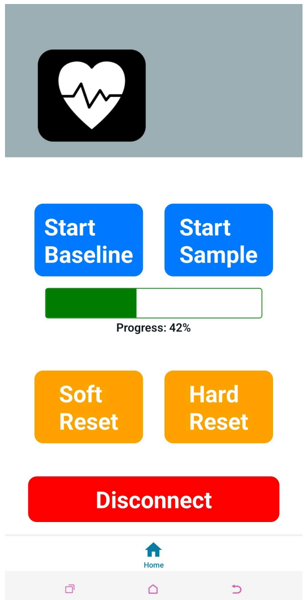


Figure 11: Mobile app home screen. Figure 12: Connecting to the PLAQCHEK device through Bluetooth connection



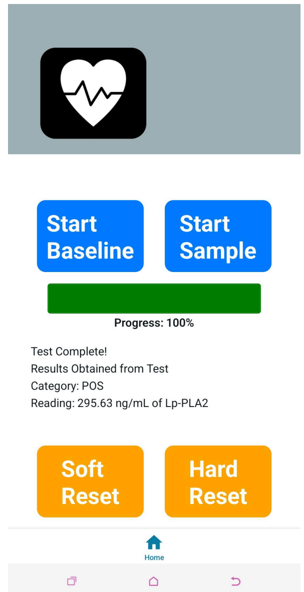


Figure 13: A test in progress. Figure 14: Cardiovascular risk assessment displayed.

Test Results

After the construction of the prototype was completed and all the parts were integrated, the next step was to calibrate the electronics. This step entailed forming a calibration curve to accurately calculate the concentration of Lp-PLA2 from the ADC signals output from the electronics. As mentioned in the previous section, 12 ITO-coated slides were prepped with antigen concentrations between 0 and 300 ng/mL, in intervals of 25 ng/ml. Once the slides were prepared, they were loaded onto the sensor, and through a script, the values from the ADC were recorded. Once all the data was collected, it was then visualized using MATLAB to see any trends.

At first, to confirm if the sensor could differentiate between a blank and Lp-PLA2, a blank ADC value reading was plotted against a 200 ng/ml sample. In Figure 13, a clear shift downwards in ADC values was observed with the antigen-loaded sample when compared to the blank. In Figure 14, 2 slides with different concentrations were tested and plotted together to see if the sensor could differentiate between 2 different concentrations of Lp-PLA2. As seen in the graph, there was a clear shift downwards when comparing the higher concentration curve with the lower concentration curve. To account for any noise, a dark reference result was recorded, and the first 2000 values were averaged. Next, the results from the remaining antigen-loaded samples were measured, and the first 2000 values recorded at each concentration were averaged. The averaged ADC values from the antigen-loaded samples had the average dark reference value subtracted from them and were plotted against their respective concentrations.

From the scatter plot graph, a line of best fit was fitted to the data points, and an equation was found for the calibration curve. This equation was then inverted to make the dependent variable the concentration and was used in the Arduino to calculate the concentration from the ADC values. The final equation used was $y = -0.0538x + 295.63$, where x is the average of the first 2000 ADC values minus the dark reference average, and y is the concentration of Lp-PLA2 found.

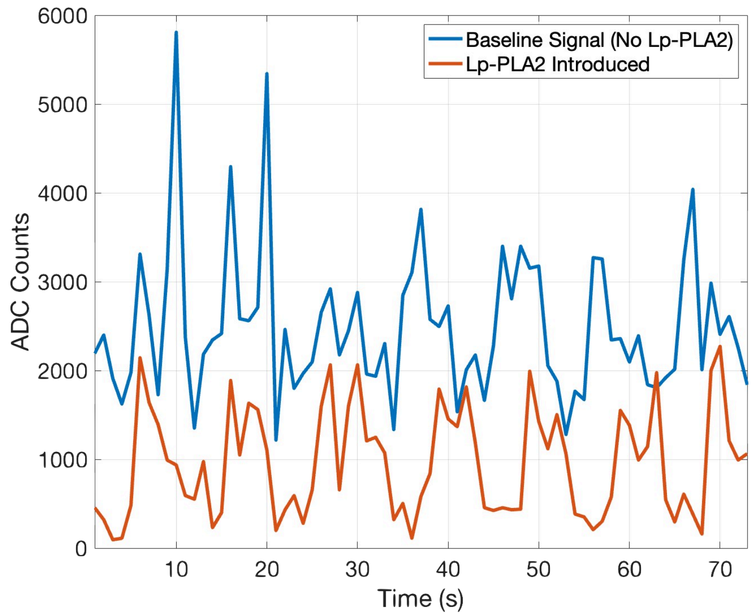


Figure 15: Plotted preliminary data showing ADC value shift in the presence of Lp-PLA2

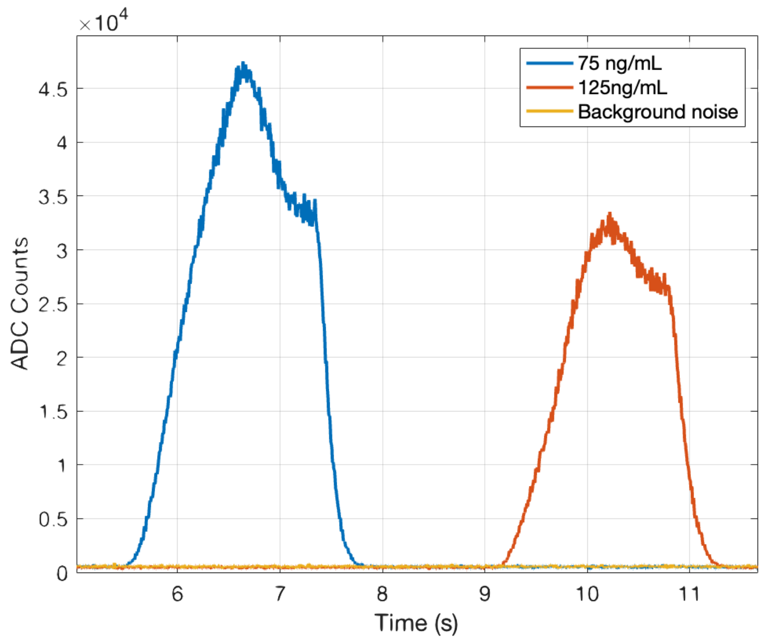


Figure 16: Plot of two different concentrations of Lp-PLA2

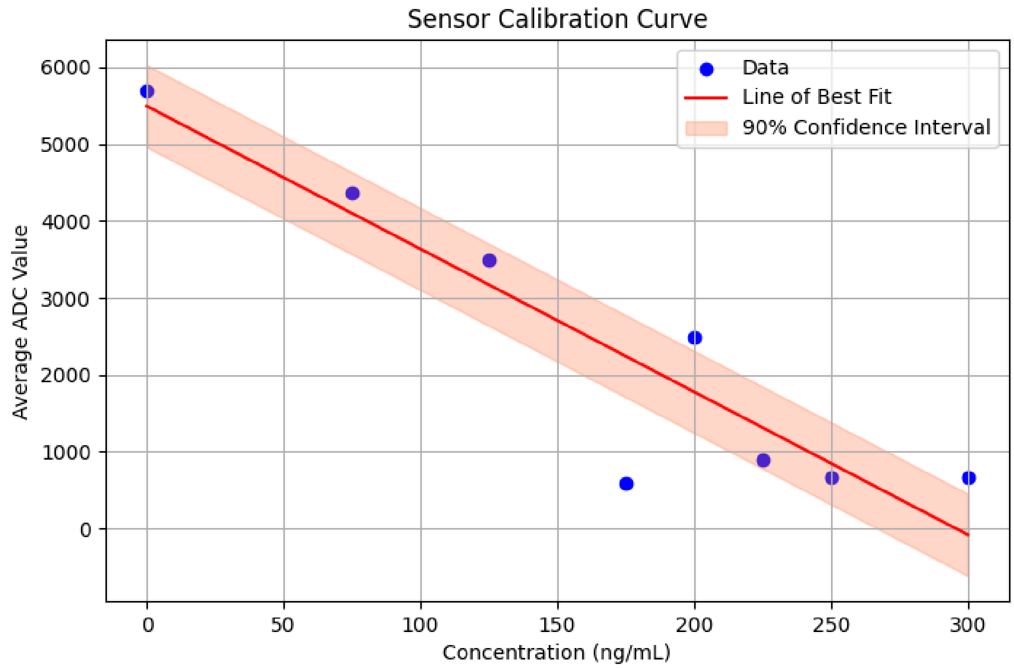


Figure 17: Lp-PLA2 sensor calibration curve

Customer Requirements	Test Results	Level of Success
Detect Lp-PLA2	As seen in Figure 13, the sensor was able to detect Lp-PLA2	This customer requirement was successfully met
Limit of Quantization ≤ 2ng/ml	As seen in Figure 15, the LoQ was 25 ng/mL.	The customer requirement was unfortunately not met, however, a LoQ of 25 ng/ml was achieved.
Detection Range between 0 and 300 ng/ml	From the calibration equation used, the detection range is theoretically from 0 ng/mL to 295.63 ng/mL.	The customer requirement is considered to be achieved as although a maximum of 295.63ng/mL was achieved, the 4.34ng/mL difference is negligible.
Response Time under 30 minutes	After testing, the response time was consistently under 5 minutes.	Since the response time for the device was 5 minutes, it successfully

Response Time under 20 minutes		meets both the primary and secondary requirements of being under 30 and 20 minutes, respectively.
The Mobile App has a latency under 500ms	After multiple tests, the latency was consistently under 450ms.	This customer requirement was successfully met.
The Sensor is Lightweight and Portable	The final sensor had the dimensions of 19cm x 9cm x 10cm and a weight of 275g.	This customer requirement was successfully met as the weight and dimensions of the final product were lightweight and portable.

Table 2: Customer Requirements and their completion

Deviations from Original Design / Shortfalls

Originally, the design was intended to be a small, handheld device that could fit in the palm of a person's hand, so it would be very easy to transport and use. However, after solidifying the design of the product and which specific components would be used, the several electronic components that will be implemented slightly exceed the originally designed size of the product. Thus, the goal of the product has been changed from being a small handheld device to a portable device with the size of a tissue box and with a weight similar to an everyday mobile phone device.

With this design, it is still very easy to transport and properly fit all components of the new design. Furthermore, it more closely aligns with the needs of the primary customers: primary care physicians and health clinics. In these settings, the device only needs to be small enough to fit on a physician's desk (similar to blood pressure machines), and light enough to be portable, but not necessarily 'handheld'.

Originally, an avalanche photodiode was intended to be used for the design, as their internal gain makes them a suitable candidate for this design, where a low amount of light must be detected, as well as their reasonable price ($30-50) and high sensitivity around luminol's peak emission wavelength. However, the required biasing, compensation, and other support circuitry for the avalanche photodiode were deemed to be too risky for the scope of this project. Thus, more research was done, and a similar device was found from a company (Broadcom) that had previously sponsored a design team from one of our members. This device is a silicon photomultiplier and also meets all the design requirements, but it costs slightly more.

The device was originally intended to have a limit of quantitation of less than 2 ng/mL of Lp-PLA2 concentration; however, after testing, the device was determined to have a limit of quantization of 25 ng/mL. The last deviation from the original design of the prototype was excluding the heating element from the sensor. Once a sample is dropped on the slide, it needs to be heated at 30℃ for 1 hour before being inserted into the sensor. The initial design plan had a heating element inside the sensor; however, due to circuit complexities, the heating element module was removed. In the current prototype, the sample is left outside for an hour before being placed inside the sensor to be tested.

Summary

The project of creating an immunosensor for the rapid measurement of concentrations of Lp-PLA2 using ECL reactions has several primary objectives. The product was designed to be able to demonstrate the ability to detect the concentration of Lp-PLA2 through the ECL method from a given solution, have a limit of quantitation of less than or equal to +/- 2 ng/mL of the Lp-PLA2 concentration present, be able to detect a concentration range of at least 0 ng/mL to 300 ng/mL, and have an end-to-end response time under 30 minutes. The design was also designed with the secondary objectives of having the device portable, having interactivity with a mobile app that allows the customer to operate the product through the app with a latency of less than 500 ms, and improving the end-to-end response time to under 20 minutes.

The main design approach taken to achieve the primary and secondary requirements was a research-first approach. Before beginning the design process, numerous hours went into the research behind each component of the sensor and how they could be integrated. Once thorough research was completed, simulations and calculations were done to verify these designs before building the prototype. After the prototype was completed, the results from the design were compared against expected values from several papers. Thus, throughout the entire lifecycle of the project, there was a constant emphasis on making decisions backed by research.

The prototype produced was able to meet the primary customer requirements of being able to detect the presence of Lp-PLA2 in different concentrations of solutions tested, was able to detect the presence and absence of Lp-PLA2 in concentrations ranging from 0 ng/mL, incrementally increasing by 25 ng/mL for each concentration until

300 ng/mL, and had a device response time of approximately 4 minutes. The only customer requirement that the prototype wasn't able to fully meet was having a limit of quantization of +/- 2 ng/mL, but instead achieving a limit of quantization of +/- 25 ng/mL. The prototype was able to meet all of the secondary requirements by having the device meet the size constraints of 19 cm x 9 cm x 10 cm, being lightweight by having a weight of 275 grams, successfully implementing an app that allows the user to interact with the device, and once again having a device response time of 4 minutes.

For a future outlook, several suggestions can be made to improve the prototype if it were to continue development. More concentrations of Lp-PLA2 solutions could be tested to develop a stronger calibration curve for determining the numerical relationship between the level of light detected and the concentration of the protein in the sample. From the limited time in the process of creating the prototype, each concentration of solution could be tested once; thus, being able to test each concentration multiple times for data verification would solidify the calibration curve's validity. Through this, a more precise limit of quantitation and detection range of the prototype could be determined. Beyond that, the prototype could be tested with actual samples of blood and confirmed if the results align with the Lp-PLA2 solutions tested within the lab. In the next iterations, the prototype can implement a heating element housed within the sensor so samples could be heated and then tested within the device rather than having to be heated externally before testing. Currently, the alligator clips are used to connect ITO to the electrodes for testing, but this caused some of the ITOs to burn, and thus, these ITOs couldn't be retested. This could be improved by an alternate method of having several points of contact between the ITO and electrodes.

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Appendices

Appendix A: Customer Requirements

4 Primary Requirements

Detect Lp-PLA2 Concentration in Blood Using ECL

The main goal of the project is to demonstrate that the prediction of the concentration of enzyme Lp-PLA2 through an electrochemiluminescence (ECL) reaction is feasible when put into practice in a sensor on a standalone device and can produce relevant results when real samples are provided. It should be able to predict Lp-PLA2 through an ECL reaction where the Lp-PLA2 enzymes are captured by antibodies bound onto gold nanoparticles on a substrate; when they are captured, the steric hindrance reduces electron transmission, resulting in a reduced ECL signal. This reduced ECL (light) signal can be measured and used to determine the concentration of Lp-PLA2 in the sample.

Limit of Quantitation Less than or Equal to ± 2 ng/mL

The ability to detect a change in Lp-PLA2 concentration in a sample is highly crucial for the product. This feature will allow customers to get an accurate and precise reading of their Lp-PLA2 concentration, thus allowing them to quantify their risk level with atherosclerosis. For the project's goal, we will aim to give the product the ability to sense concentration changes with an uncertainty of less than or equal to ± 2 ng/mL.

Limit (Range) of Detection of 0 to 300 ng/mL

The device must reliably detect Lp-PLA2 concentrations in the range of 0 to 300 ng/mL, with an acceptable signal-to-noise ratio of at least 3:1 (in this range). This detection limit was selected to cover the range of expected Lp-PLA2 concentrations in humans without atherosclerosis or heart disease. One study of 569 subjects found that the concentration of Lp-PLA2 in subjects with coronary artery disease (CAD) was, on average, 136.0 ± 60.5 ng/mL, and the concentration in subjects without CAD was, on average, 113.2 ± 65.6 ng/mL (Ling et al., 2020). Another study measured Lp-PLA2 levels of 256.2 ± 46.8 ng/mL in patients with heart failure (Charniot et al., 2013). Thus, an upper limit of 300 ng/mL was chosen for the specifications. The lower limit of 0 ng/mL was chosen as ideally, the device will be able to detect the lack of Lp-PLA2 or very low concentrations to report failed samples/measurements, collect data to perform device calibrations if needed, and perform dark correction and other baseline corrections to process the ECL signal. This specification (along with the implied SNR specification) must be considered when designing the sensor itself, as well as the optics, electronics, and signal processing (post-processing in software).

Response Time of Under 30 Minutes

The product aims to provide rapid detection of Lp-PLA2 and its concentration in the sample provided. For a similar ECL sensor as the target for this project, the reported response time was approximately 27 minutes for output from their testing (Wang et al., 2023). Thus, to match the findings, while accounting for possible inefficiencies in the product flow and/or design, the product aims to respond to the user within 30 minutes or less to provide results to the user.

3 Secondary Requirements

Response Time of Under 20 Minutes

Ideally, the response time of the product can be decreased to under 20 minutes to decrease the wait time the user has to abide by before seeing the results of the sample. The faster the product works without compromising accuracy, the more convenient the product becomes in the market and for users.

Optimize Device Design to be Portable

The product should have a small enough form factor to be easily carried by the consumer. The primary goals focus on the functionality and accuracy of the sensor, regardless of the structure of the final product, however, a more compact design will encourage consumers to use the sensor more consistently. This will also make the product more discreet and portable, which would add to the convenience of monitoring the consumer’s cardiovascular health.

Mobile App Connection Latency of Less than 500 ms

Although the final device should have a display to report to the user the levels of LP-PLA2 found in the sample, we would like to add a method of tracking and viewing the results through a phone application. There has been a huge increase in personal health monitoring in the last decade with smartwatches. Recently, new glucose sensors have the functionality to connect via Bluetooth to consumer phones, to help them track their health. If possible, the sensor will have the same functionality to provide a better experience to the consumers. We have set a goal of having a response time between the sensor and the phone of less than 500 ms to ensure that there is no delay in conveying the results.

Final Overview

Primary Requirements
The product must demonstrate the ability of Lp-PLA2 detection through the ECL method.
The product must have a limit of quantitation of less than or equal to ± 2 ng/mL.
The project must have a range of detection of at least 0 ng/mL to 300 ng/mL.
The end-to-end response time must be under 30 minutes.
Secondary Requirements
The end-to-end response time could ideally be under 20 minutes.
The product could have a companion application with a latency of less than 500 ms.
The product could be portable.

Table A-1: Customer Requirements Overview

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Appendix B: Verification Plan for Conceptual Design

Verification Plan

For the design verification, the plan is to use COMSOL to simulate the electrode placement at the component level to optimize its layout. LTspice will also be used for component-level verification to simulate the circuit designs used for driving the electrode potential and the circuit for receiving and processing the photodiode's output signal. At the system level, COMSOL and LTspice will be used together. The electrical characteristics of the electrodes will be extracted using COMSOL and then used to model the electrodes' impedance and their impact on the electrode driver circuit using LTspice.

The ECL sensor design requires a 3-electrode system consisting of a working electrode, a reference electrode, and a counter electrode. The working electrode provides the site for carrier exchange and is where the electrochemical reactions take place. The reference electrode serves as a reference to measure and control the potential of the working electrode precisely. This electrode should not interfere with the ECL reaction, and so it should not draw any current. The counter electrode completes the circuit with the working electrode. The placement of these electrodes relative to each other is important. COMSOL can be used to model the effect of the size, placement, and material of the electrodes on the impedance of the electrodes and the performance of the electrochemical system.

The 3-electrode system will be modelled as a 3-D system (the height of the electrodes will be ignored), and the PBS buffer solution can also be accounted for in some of the COMSOL simulations. Firstly, the distribution of the electric field must be uniform for each electrode and will be simulated and verified in COMSOL. Furthermore, the electrodes should demonstrate sufficient stability in their impedance across varying frequencies of interest. COMSOL may also be utilized to simulate the impedance of the electrodes and verify their stability. The frequency response may also be optimized to increase the speed of the electrochemical reaction, which will optimize the response time requirement.

The placement of the electrodes is also important, as they must not interfere with each other once a potential is applied, among other considerations. The shape of the reference electrode and its placement/isolation relative to the working electrode are especially important for the response of the electrodes. Thus, special care will be taken to simulate and optimize the shape and position of the reference electrode using COMSOL.

The circuitry required to interface with the three-electrode system consists of a waveform generator to pulse the working electrode, a control loop to measure the reference electrode potential and adjust the pulse to the working electrode, and, optionally, a current measurement to measure the current through the cell (via the counter electrode). Operational amplifiers, MOSFETs, and discrete components will be used in the design of these circuits, and these analog circuits must be optimized to meet the system requirements. Thus, LTspice will be used to select circuit components and values and gain confidence in the circuit design, meeting system requirements before building and testing it on the prototype.

Since the circuits interfacing with the 3-electrode system are all analog, the electrical characteristics of the electrodes themselves are important to characterize to understand their impact on the circuit's performance and to gain confidence that the circuit design/architecture is suitable to drive and sense the electrodes. Thus, the electrical impedance (resistance, capacitance, inductance) of the final design for the 3-electrode system will be simulated and extracted using COMSOL. Then, the electrodes will be modelled in the LTspice circuit simulations using capacitors, resistors, and inductors that have values extracted from the COMSOL simulations. These simulations together will verify the integration of the hardware and electrodes, allowing the design to meet system requirements.

Empirical Data

sample no	ECL immunosensor (ng mL^{-1})	ELISA (ng mL^{-1})	RD (%)
1	276	266	3.8
2	425	410	3.7
3	401	383	4.7

Table B-1: Empirical data table detected concentration comparison between ECL immunosensor and ELISA techniques (Wang. et al, 2024)

The above table is taken from a research paper that describes the fabrication of the ECL sensor. The table compares the concentration of LP-PLA2 results for randomized samples between the developed ECL sensor and the industry-standard ELISA method. These results indicate that the ECL sensor that was designed was able to detect LP-PLA2 with an accuracy of roughly ± 5% error compared to the industry standard. This supports the claim that the ECL sensor we have developed will be able to produce accurate LP-PLA2 concentration results.

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Appendix C: Test Plan

Customer Requirements

Primary Requirements
The product must demonstrate the ability of Lp-PLA2 detection through the ECL method.
The product must have a limit of quantitation of less than or equal to ± 2 ng/mL.
The project must have a range of detection of at least 0 ng/mL to 300 ng/mL.
The end-to-end response time must be under 30 minutes.
Secondary Requirements
The end-to-end response time could ideally be under 20 minutes.
The product could have a companion application with a latency of less than 500 ms.
The product could be portable.

Table C-1: Customer Requirements Overview

There haven't been any deviations between the current customer requirements stated above and the requirements previously mentioned. The customer requirements are still aligned with the project's primary and secondary goals.

Testing Procedure

After the synthesis of the cobalt iron and gold nanoparticles, the UV-Vis and SEM techniques will be used to verify their diameters. The ideal diameters will be 300 and 25 nm for the cobalt iron and gold nanoparticles, respectively, and once these are verified, the next steps of the procedure may continue.

To test the circuitry of the sensor, an oscilloscope will be used to probe and measure signals to validate their functionality. The electronics consist of analog circuits whose performance must be observed and validated after assembly, for example, the light sensing and processing circuits and the potentiostat drive and control circuitry. Multiple test cases and scenarios will be produced to ensure that the circuit configuration will operate as expected.

Once the Lp-PLA2 reaction is conducted to be captured by the antibody protein and verified if it was successfully captured, the ECL signals will be measured before and after the reaction. If it was successfully captured, the ECL signal should decrease after the Lp-PLA2 was captured, compared to the initial state where it was not captured. Once this behaviour is observed, this will confirm that the reaction was successful.

In the testing phase of the product, a calibration curve will be created based on concentrations of solutions of Lp-PLA2. The concentrations will be increasing in intervals of 25 ng/mL; so, for example, the solutions tested will be 0 ng/mL, 25 ng/mL, and 50 ng/mL, all the way to the designed maximum range of 300 ng/mL of Lp-PLA2. From this, the decrease in ECL signal will be detected for each concentration, and will be plotted out to determine a calibration curve from this data. Then the plot will be analyzed and confirmed if it follows the expected theoretical trend, which would be that as the Lp-PLA2 concentration increases, the drop in ECL signal also increases. This will confirm if the product follows the theory in practice.

Quantitative Value Breakdown

To verify if the customer requirements are met through quantitative values, multiple tests will be used to determine if the designed product has passed the requirements.

Once the calibration curve is completed, more solutions with different varying concentrations of Lp-PLA2 (e.g. 20 ng/mL or 70 ng/mL) will be tested through the product and their decreased ECL signal values. The calibration curve will then be applied to the test data to predict the concentration of Lp-PLA2 in the sample based on the resulting ECL signal change in intensity. This value, derived using the calibration curve, will be compared to the actual concentration of Lp-PLA2 used to generate the test data. The predicted concentration values will be compared to the actual concentration values used in the test solutions to see if they demonstrate a ± 5% accuracy to meet this customer requirement.

Another aspect to be tested will be the response time for the product. The time will be recorded from once the solution is introduced to the product until an output of the measured concentration is displayed from the product. If this time is under 30 minutes, this will verify that the product meets a response time of under 30 minutes. To achieve further efficiency and meet the secondary requirement, the product will be tested to have a response time of under 20 minutes.

When all the components of the product are fabricated, assembled, and enclosed in an outer layer, the device will be safe and easy to use for any customer. The product will then be weighed to see if it weighs less than the average smartphone, which is 250 grams, and smaller than the average smartphone, which is 80 x 165 x 9 millimetres (W x L x H), to meet the secondary requirement of being portable and lightweight.

Another component of the project is the companion application that the user will interface with to view the results of the sensor. Although the device will have an LCD to show the consumer the concentration of LP-PLA2 in the sample, it is beneficial to have a mobile app to display and track the concentrations. Thus, as a secondary requirement, the connection between the sensor and the phone needs to have a latency of less than 500 ms to reduce delay. This will be tested by including a JavaScript ping function within the mobile app to display the connection strength and response time of the sensor. While performing multiple test cases with the sensor, the response times will be recorded with the sensor at varying distances under 2 meters from the phone. These response times should all be under 500 ms.

Appendix D: Final Design Specification

System Level

The project design aims to be a small, portable device that will accept blood samples through a lancing mechanism and introduce them to the ECL immunosensor through an electrolyte. Then, the device will measure the resulting ECL signal using a photodetector and a black box to block out any foreign light source. The resulting data will be displayed on a miniature LCD screen. It may also be sent to an external device (computer or smartphone) that will display the results in greater detail to the user. The system will be operated with three buttons: a dark reference button, a start button, and a reset button. The device contains various parts which must be integrated, such as the photodiode circuit, user interface circuit, heating components, and main sensor chip interface.

Component Level

The ECL sensor is mounted onto a heating plate, which is needed for incubation after coating the Lp-PLA2-containing sample onto the sensor. The first (bottom) layer of the sensor is the working electrode, which is the electrode where the ECL reaction occurs, generating the signal. Mounted close by is the reference electrode, which is used to maintain a known reference potential to compare against the working electrode. On the opposite side of the sensor and further away from the working electrode is the counter electrode, which is biased to balance the current at the working electrode.

The upper layer of the sensor contains the CoFe PBA with gold nanoparticles, bound with the APTMS layer below it. The Lp-PLA2 antibodies bound to the gold nanoparticles. The last important component is the luminol, which will be present within the electrolyte alongside PBS. It will undergo a redox reaction, and the excited state of luminol results in light emission. When the antibodies have detected and bonded with the Lp-PLA2 enzyme, the light emitted will be hindered and lowered. This will be the detection mechanism.

This project contains four custom PCBs. Firstly, it includes the photosensing board, which converts the light emission by the ECL sensor to a current, then converts it to a voltage, filters, amplifies, and then converted to a digital signal by an ADC and processed by a microcontroller unit. The electrode potentials will be controlled by a potentiostat circuit. The circuit will handle the user interface components, such as the buttons, the power switch, and the LCD screen. The PCB will also contain a Bluetooth module connection to allow users of the device to access the results conveniently on external applications. These applications will be able to convert the data measured and processed by the device into an Lp-PLA2 concentration using a calibration curve that will be developed after the device has been fabricated.

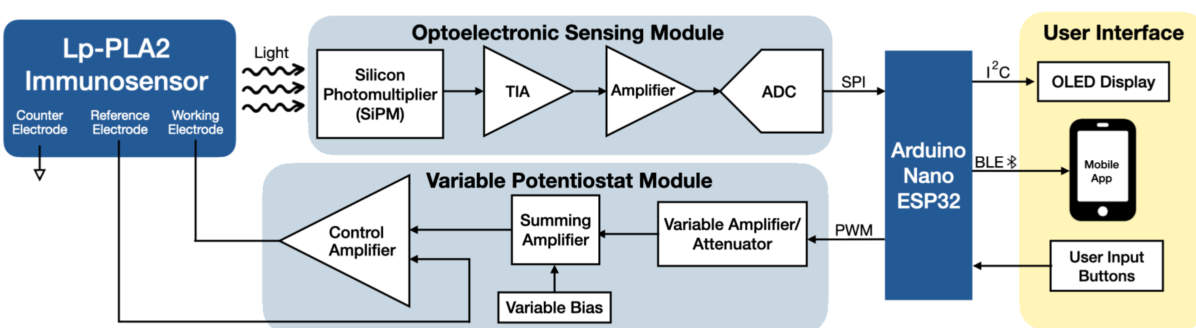

Figure D-1. Lp-PLA2 ECL immunosensor device system block diagram. The components of the ECL sensor are shown in more detail in Figure 2 below.

The schematics for the PCBs can be seen below.

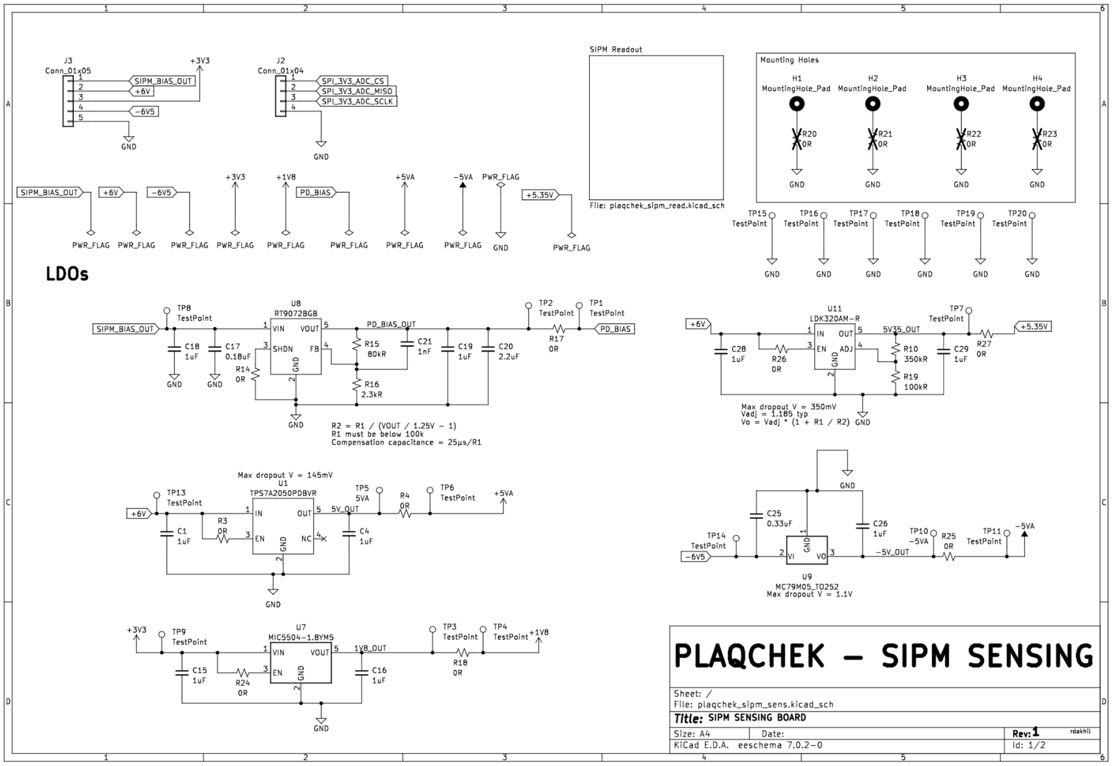

Figure D-2: Optoelectronic Sensing Circuit Schematic Page 1/2

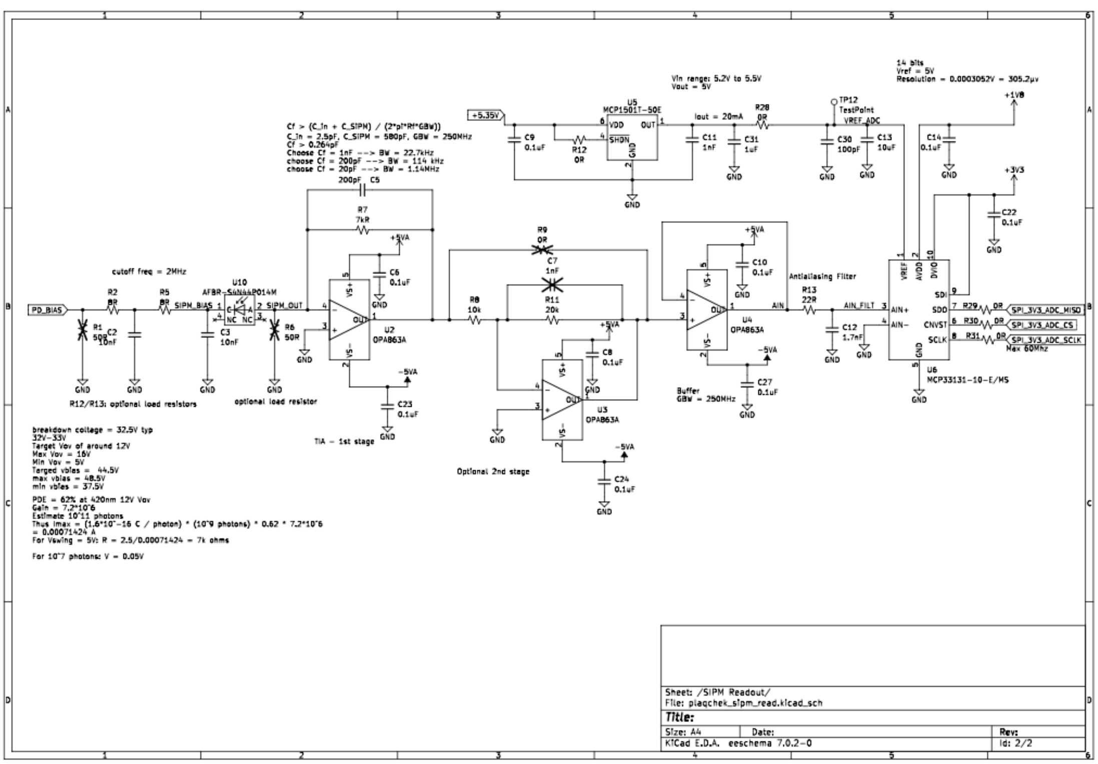

Figure D-3: Optoelectronic Sensing Circuit Schematic Page 2/2

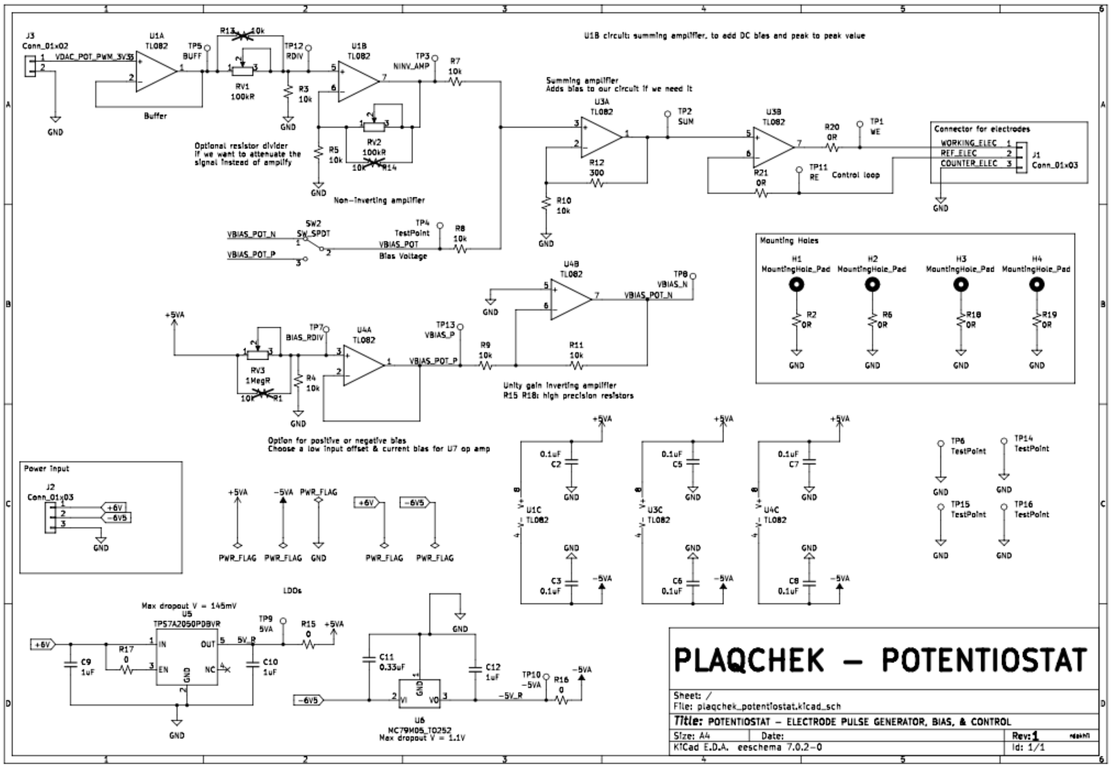

Figure D-4: Potentiostat Circuit Schematic

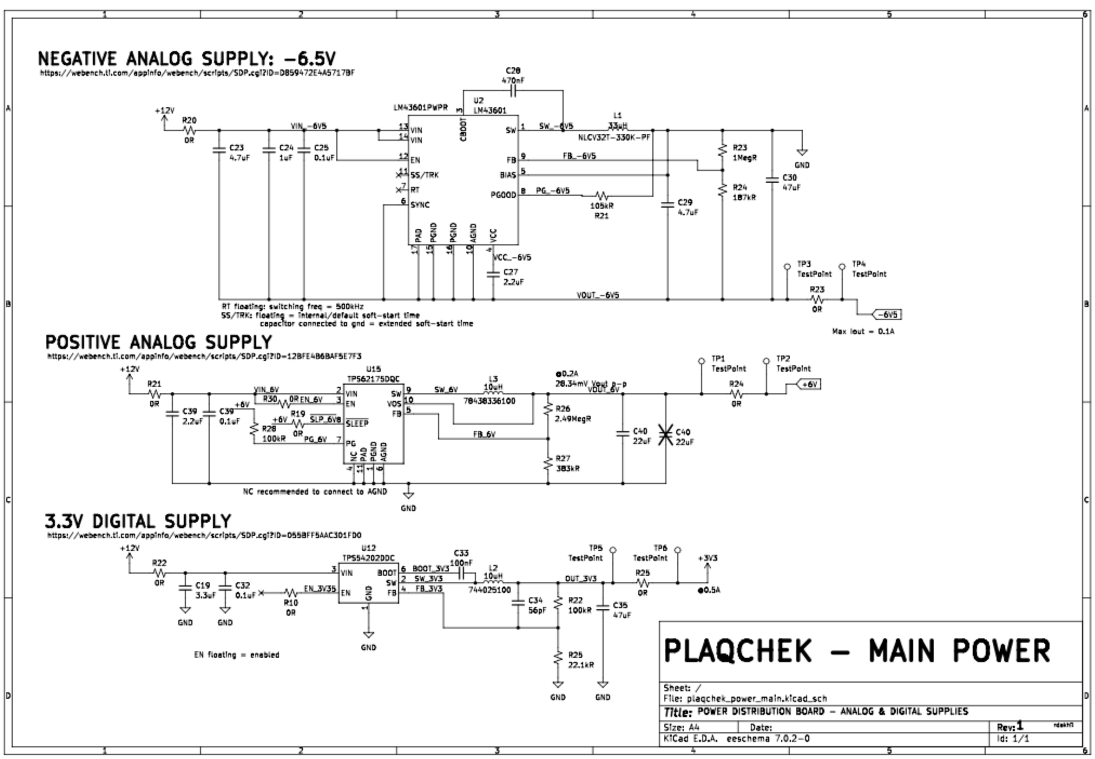

Figure D-5: Power Supply Circuits Schematic Page 1/2

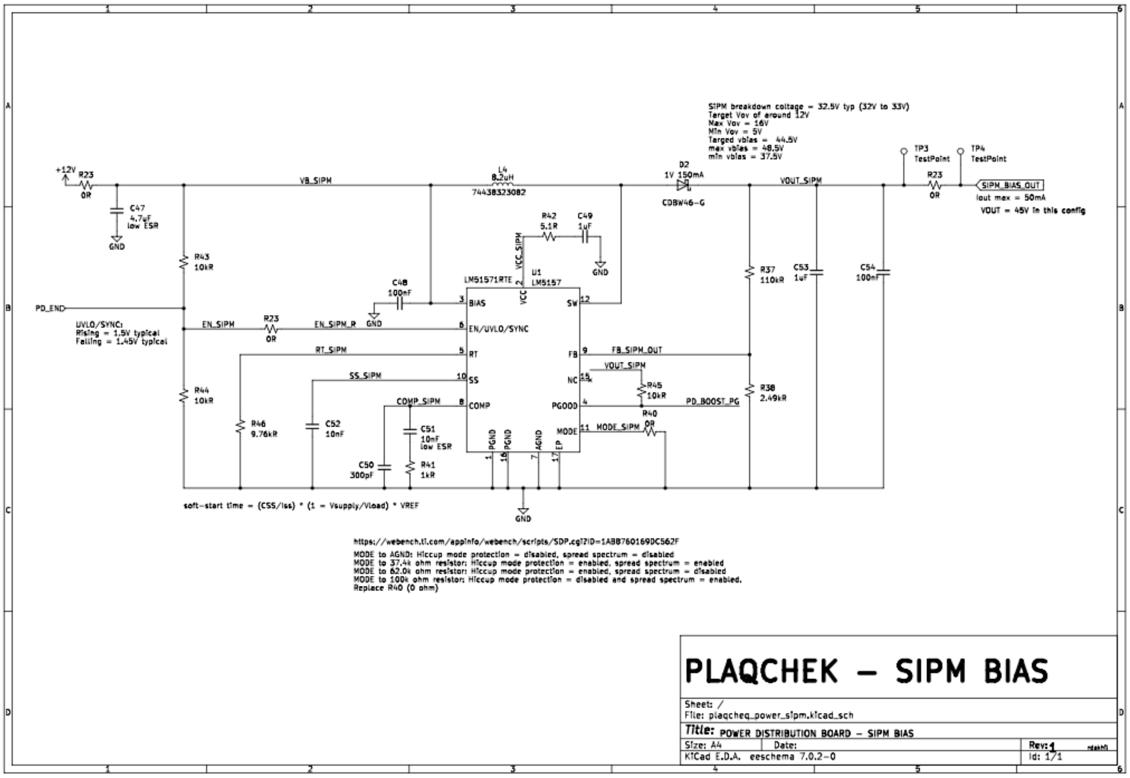

Figure D-6: Power Supply Circuits Schematic Page 2/2

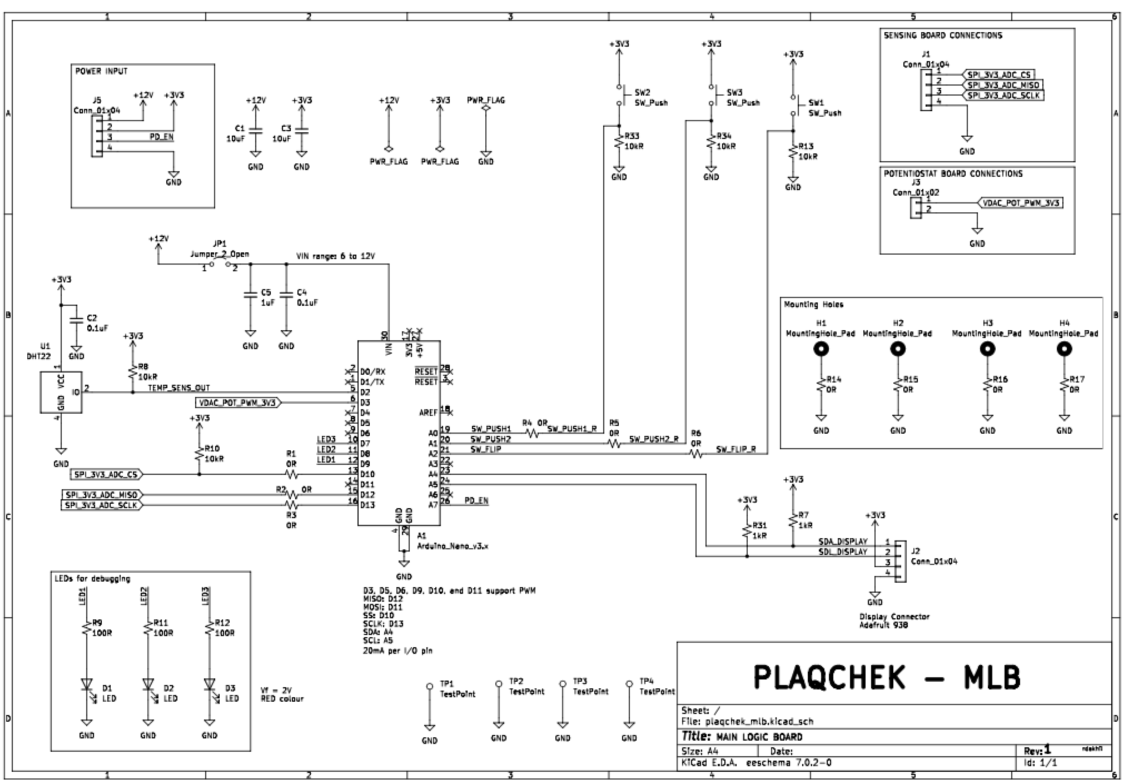

Figure D-7: Main Logic Board Circuit Schematic

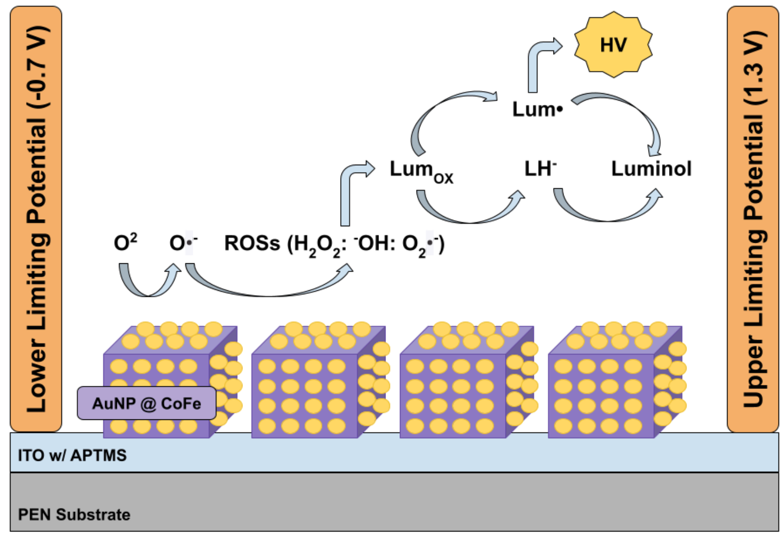

Figure D-8. Cross-sectional diagram of ECL immunosensor for measuring Lp-PLA2 concentrations in samples.

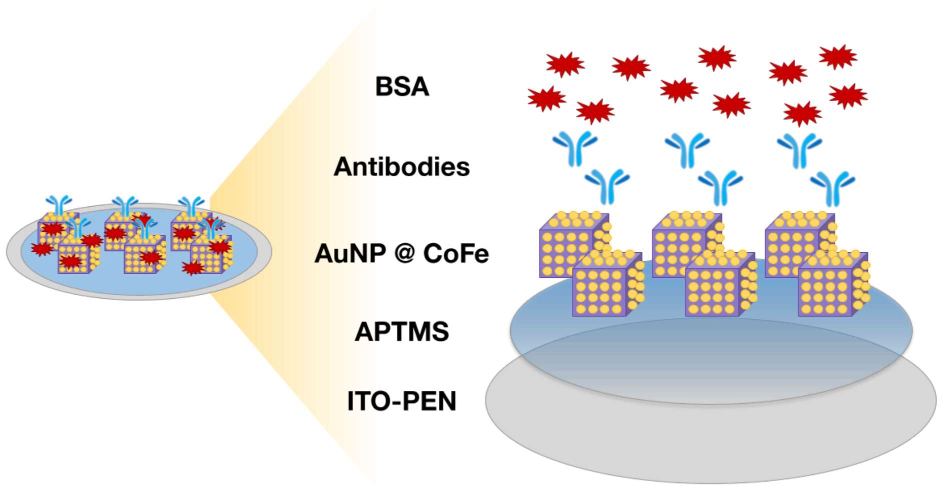

Figure D-9. Breakdown of how the ITOs were fabricated for Lp-PLA2 antigen detection.

Design Choices

The following design choices were made:

- The accuracy and sensitivity range of the sensor.
- Choosing an ECL reaction-based immunosensor
- Designing a 3-electrode system over a 2-electrode system
- ITO-PEN substrate over ITO-PET and ITO-Glass substrate

Electrochemiluminescence was chosen as an approach for this design because its properties will allow us to best meet our customer requirements. Firstly, it has a high sensitivity due to repeated excitation cycles, a wide detection range, and a low detection (quantitation) limit (Mahadevarao and Zubair, 2024), which targets some of our more important specifications, and the concentration of Lp-PLA2 in the blood is low. Secondly, ECL light signals are produced by luminophores, which are highly stable (Mahadevarao and Zubair, 2024), which will allow the device to be used outside a controlled laboratory setting (Mahadevarao and Zubair, 2024).

To enhance the ECL signal, we will also consider the use of catalysts and model the reaction kinetics in MATLAB to optimize reaction speed. An important customer requirement is that the results must be available within 30 minutes, with a more stringent (secondary) requirement of 20 minutes. Thus, the use of catalysts to increase the speed of the ECL reaction will be considered.

For the immunosensor, the PEN plastic substrate with an ITO coating was chosen as it is chemically resistant enough to last the fabrication process. Additionally, it is not as fragile or as expensive as glass.

Lastly, a secondary requirement is for the device to be small and potentially mobile on the scale of any other tabletop medical device. Thus, the sensor and PCB must have smaller footprints, and the device enclosure materials must be chosen to be lightweight and durable.

References

Kerr, E., Hayne, D. J., Soulsby, L. C., Bawden, J. C., Blom, S. J., Doeven, E. H., Henderson, L. C., Hogan, C. F., & Francis, P. S. (2021). A redox-mediator pathway for enhanced multi-colour electrochemiluminescence in aqueous solution. *Chemical science*, *13*(2), 469–477. https://doi.org/10.1039/d1sc05609c

Mahadevarao Premnath, S., & Zubair, M. (2024). Electrochemiluminescence Method. In *StatPearls*. StatPearls Publishing.

Wang, L., Liu, Y., Yan, J., Li, H., & Tu, Y. (2023). Novel Electrochemiluminescent Immunosensor Using Dual Amplified Signals from a CoFe Prussian Blue Analogue and Au Nanoparticle for the Detection of Lp-PLA2. *ACS sensors*, *8*(7), 2859–2868. https://doi.org/10.1021/acssensors.3c00858.

Appendix E: Verification Data

COMSOL Results

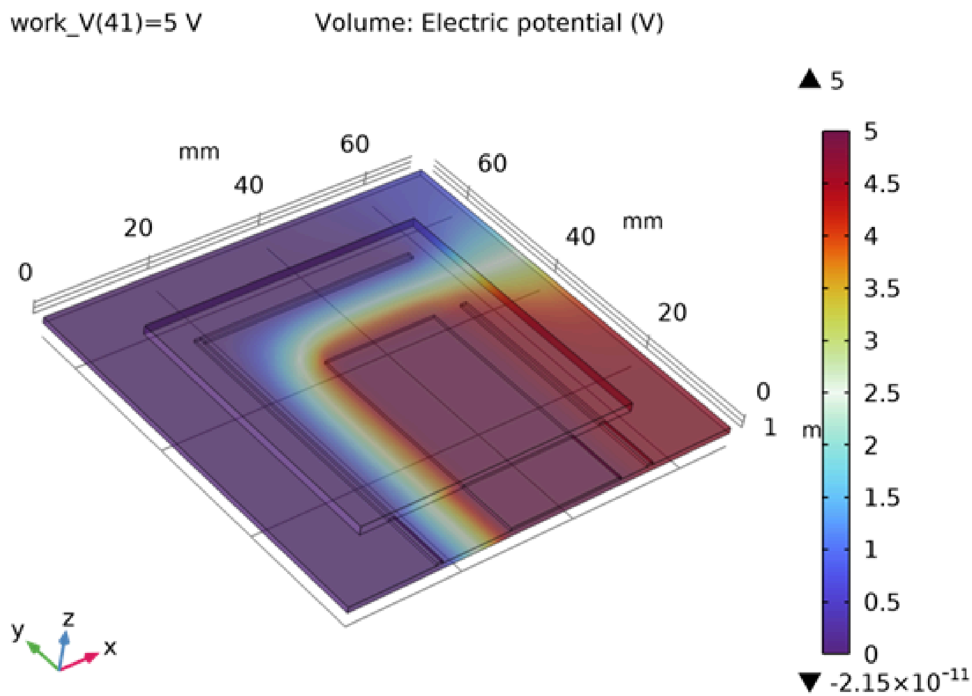

Figure E-1: Electric potential simulation at 5 V

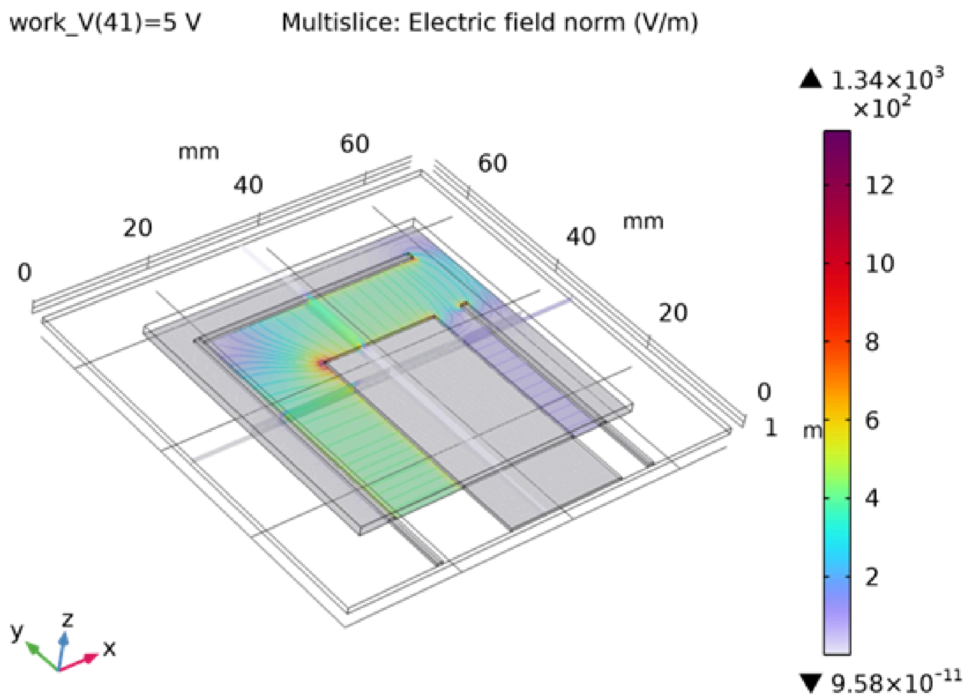

Figure E-2: Electric field simulation at 5 V

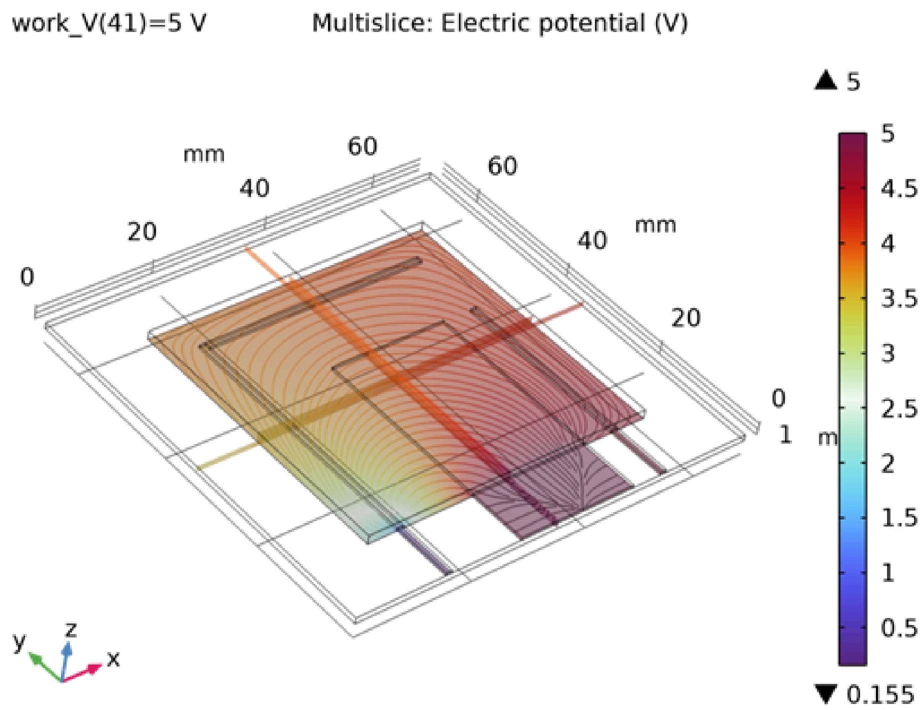

Figure E-3: Electric potential simulation with multislice at 5 V

work_V (V)	Current density, x-component (A)	Current density, x-component (A)	Current density, y-component (A)
-5	0.019738	-0.0046782	-0.001222
-4.75	0.018752	-0.0044443	-0.0011609
-4.5	0.017765	-0.0042104	-0.0010998
-4.25	0.016778	-0.0039765	-0.0010387
-4	0.015791	-0.0037426	-9.7757E-4
-3.75	0.014804	-0.0035087	-9.1647E-4
-3.5	0.013817	-0.0032748	-8.5537E-4
-3.25	0.01283	-0.0030409	-7.9427E-4
-3	0.011843	-0.0028069	-7.3317E-4
-2.75	0.010856	-0.002573	-6.7208E-4
-2.5	0.0098692	-0.0023391	-6.1098E-4
-2.25	0.0088823	-0.0021052	-5.4988E-4
-2	0.0078954	-0.0018713	-4.8878E-4

-1.75	0.0069085	-0.0016374	-4.2769E-4
-1.5	0.0059215	-0.0014035	-3.6659E-4
-1.25	0.0049346	-0.0011696	-3.0549E-4
-1	0.0039477	-9.3565E-4	-2.4439E-4
-0.75	0.0029608	-7.0174E-4	-1.8329E-4
-0.5	0.0019738	-4.6782E-4	-1.222E-4
-0.25	9.8692E-4	-2.3391E-4	-6.1098E-5
0	0	0	0
0.25	-9.8692E-4	2.3391E-4	6.1098E-5
0.5	-0.0019738	4.6782E-4	1.222E-4
0.75	-0.0029608	7.0174E-4	1.8329E-4
1	-0.0039477	9.3565E-4	2.4439E-4
1.25	-0.0049346	0.0011696	3.0549E-4
1.5	-0.0059215	0.0014035	3.6659E-4
1.75	-0.0069085	0.0016374	4.2769E-4
2	-0.0078954	0.0018713	4.8878E-4
2.25	-0.0088823	0.0021052	5.4988E-4
2.5	-0.0098692	0.0023391	6.1098E-4
2.75	-0.010856	0.002573	6.7208E-4
3	-0.011843	0.0028069	7.3317E-4
3.25	-0.01283	0.0030409	7.9427E-4
3.5	-0.013817	0.0032748	8.5537E-4
3.75	-0.014804	0.0035087	9.1647E-4
4	-0.015791	0.0037426	9.7757E-4
4.25	-0.016778	0.0039765	0.0010387
4.5	-0.017765	0.0042104	0.0010998
4.75	-0.018752	0.0044443	0.0011609
5	-0.019738	0.0046782	0.001222

Table E-1: Current Density Simulation with Voltage Sweep

work_V (V)	Electric potential (V)	Electric potential (V)
-5	0	-4.1121
-4.75	0	-3.9065
-4.5	0	-3.7009
-4.25	0	-3.4953
-4	0	-3.2897
-3.75	0	-3.0841
-3.5	0	-2.8785
-3.25	0	-2.6729
-3	0	-2.4673
-2.75	0	-2.2617
-2.5	0	-2.0561
-2.25	0	-1.8505
-2	0	-1.6448
-1.75	0	-1.4392
-1.5	0	-1.2336
-1.25	0	-1.028
-1	0	-0.82242
-0.75	0	-0.61682
-0.5	0	-0.41121
-0.25	0	-0.20561
0	0	0
0.25	0.18902	0.23048
0.5	0.37804	0.46097
0.75	0.56705	0.69145
1	0.75607	0.92194
1.25	0.94509	1.1524
1.5	1.1341	1.3829
1.75	1.3231	1.6134
2	1.5121	1.8439
2.25	1.7012	2.0744
2.5	1.8902	2.3048

2.75	2.0792	2.5353
3	2.2682	2.7658
3.25	2.4572	2.9963
3.5	2.6463	3.2268
3.75	2.8353	3.4573
4	3.0243	3.6878
4.25	3.2133	3.9182
4.5	3.4023	4.1487
4.75	3.5913	4.3792
5	3.7804	4.6097

Table E-2: Electric Potential Simulation with Voltage Sweep

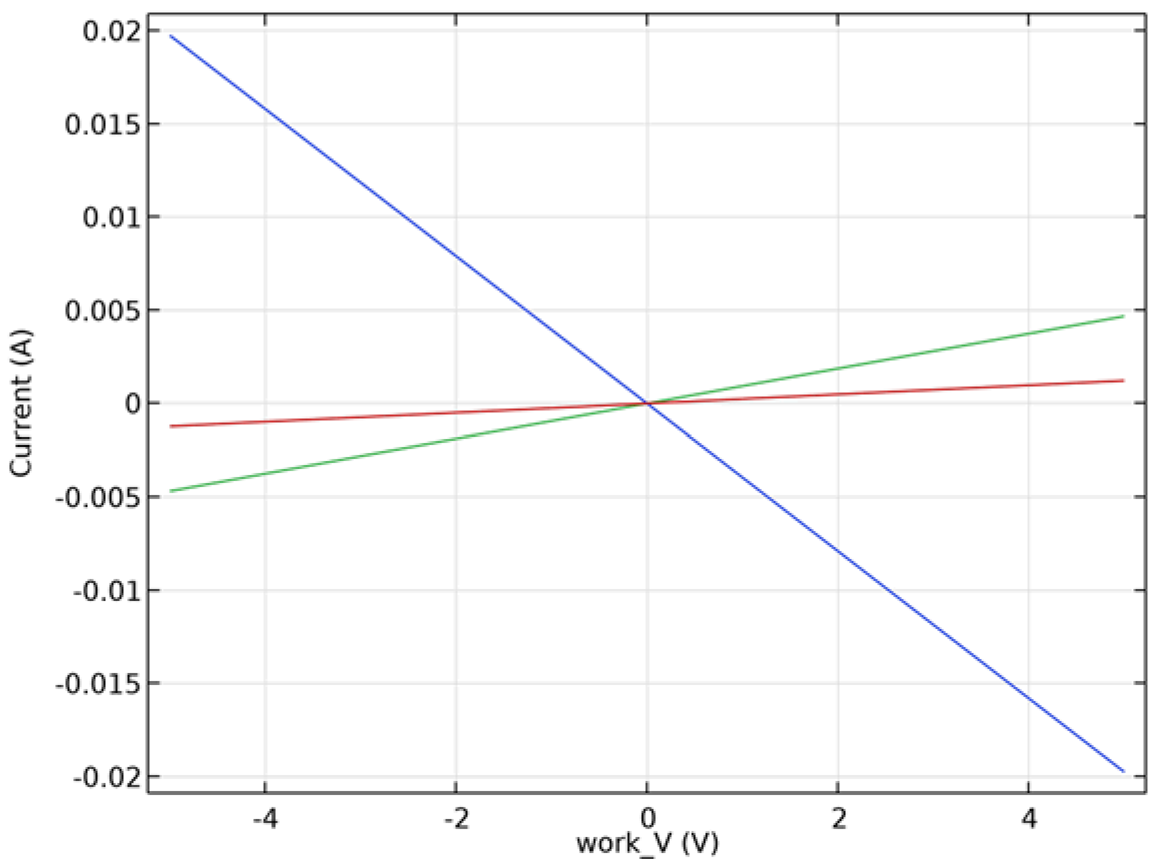

Figure E-4: Current (Voltage Sweep)

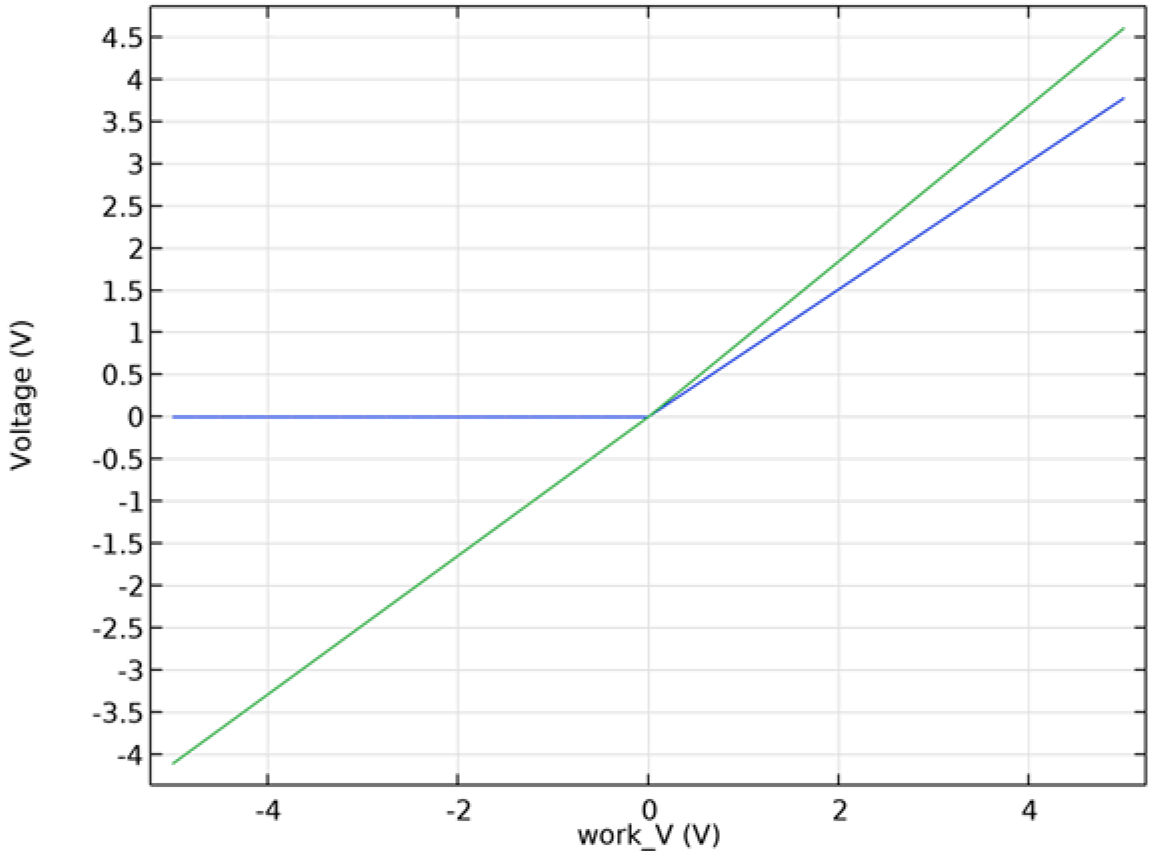

Figure E-5: Voltage at Electrodes (Voltage Sweep)

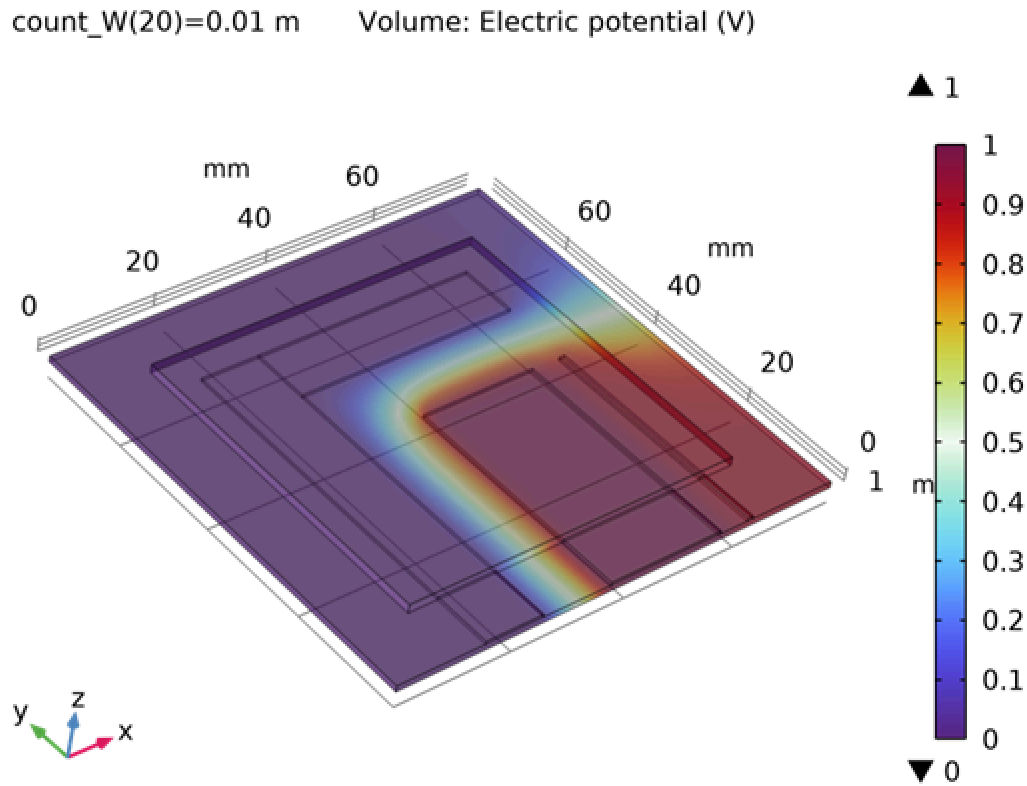

Figure E-6: Voltage potential simulation with counter displacement of 0.01 m

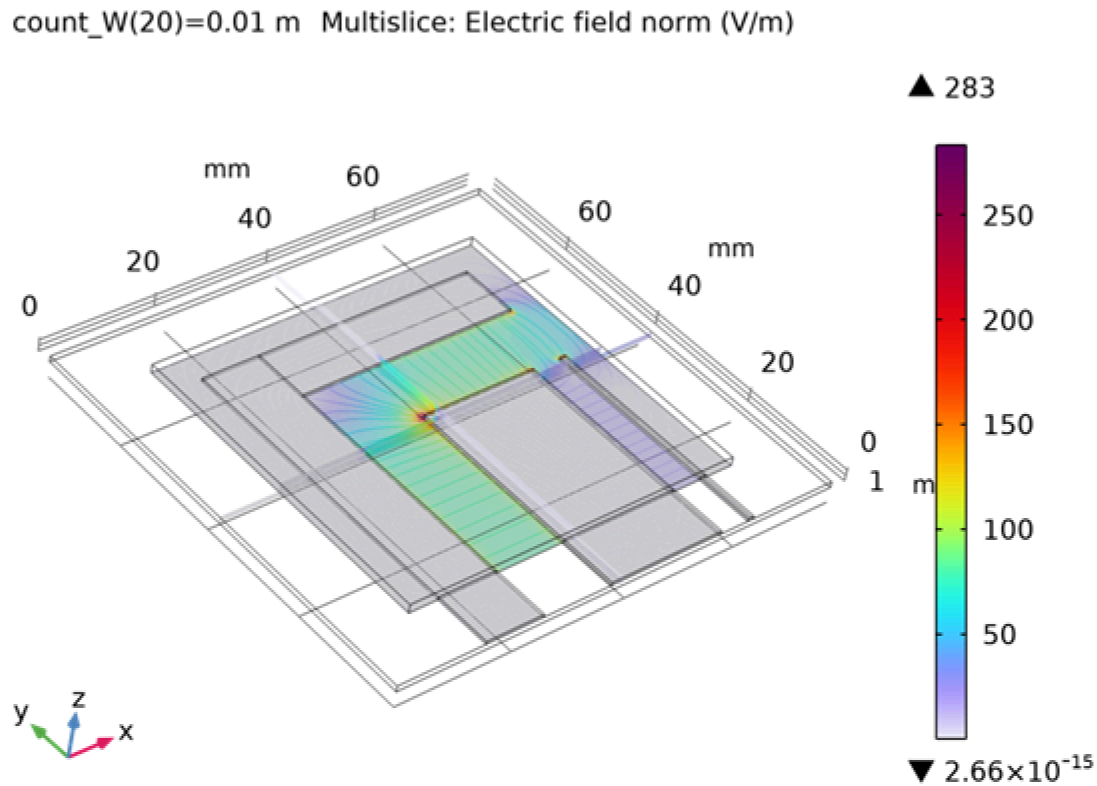

Figure E-7: Electric field simulation with counter electrode at 0.01 m

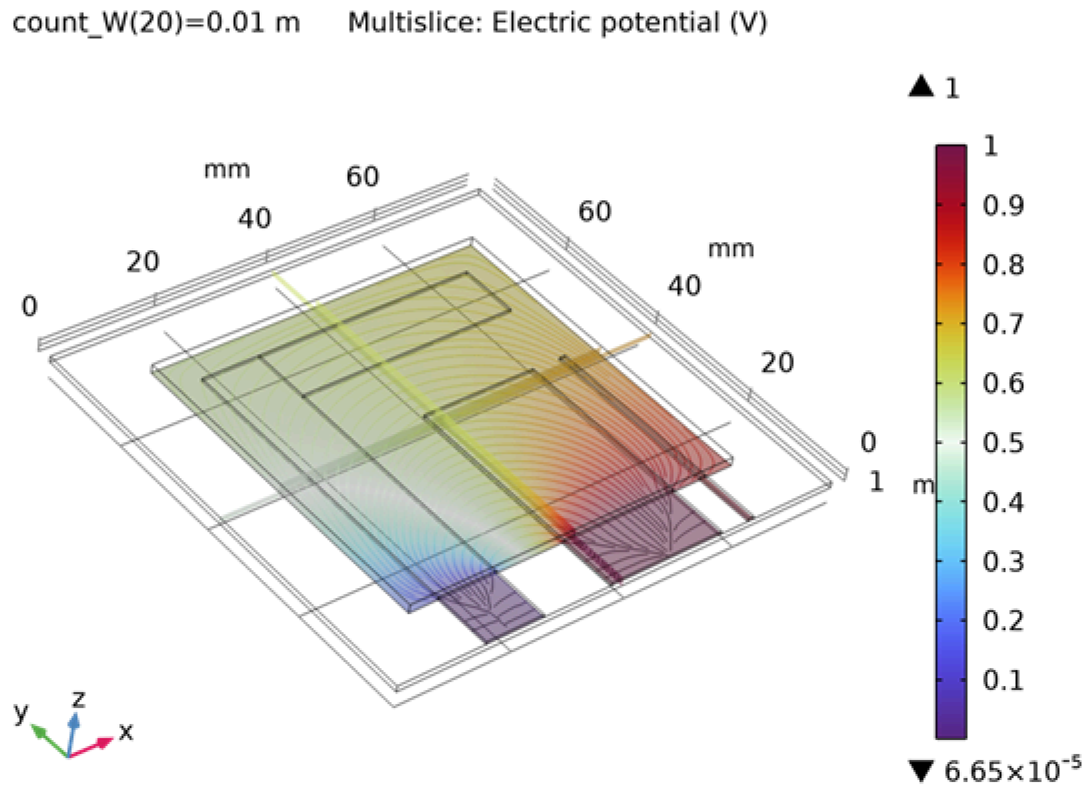

Figure E-8: Electric potential simulation with multislice with counter electrode at 0.01 m

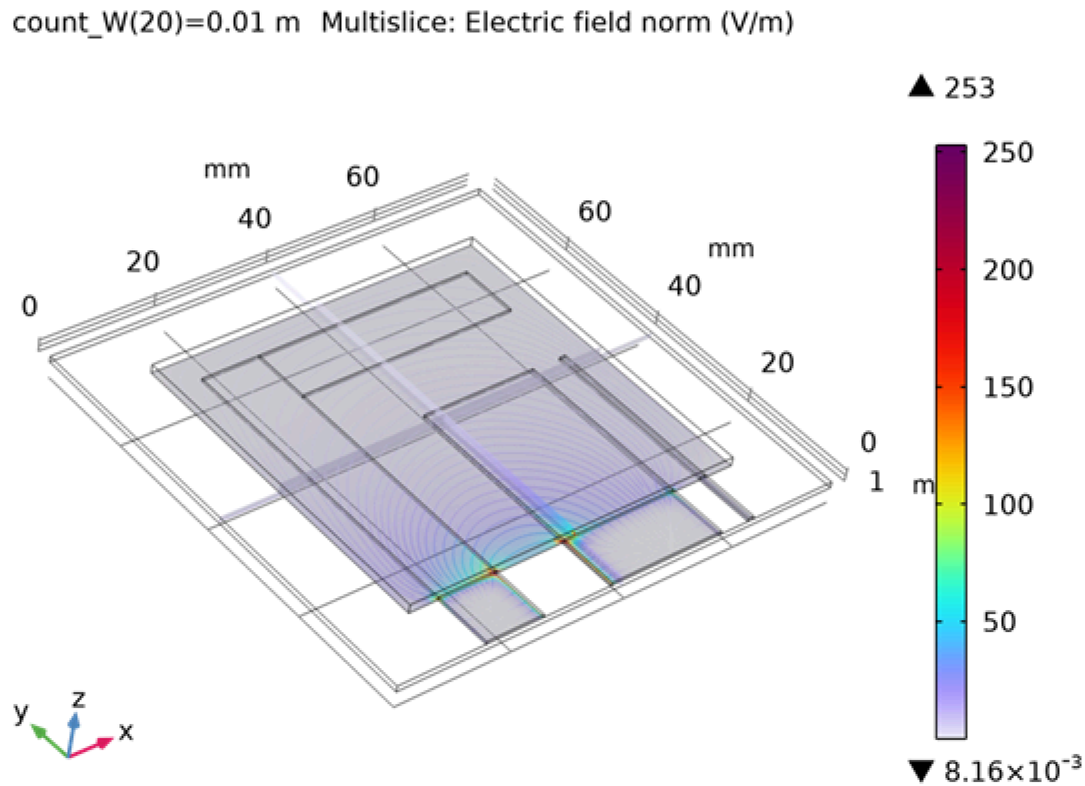

Figure E-9: Electric field simulation with multislice with counter electrode at 0.01 m

count_W (m)	Current density, x-component (A)	Current density, x-component (A)	Current density, y-component (A)
5E-4	-0.0039193	0.0017845	8.6014E-4
0.001	-0.0039291	0.0017097	8.5099E-4
0.0015	-0.0039812	0.0017697	8.4191E-4
0.002	-0.0039746	0.001801	8.9901E-4
0.0025	-0.0039434	0.0018071	9.0357E-4
0.003	-0.0038733	0.0018343	9.1712E-4
0.0035	-0.0039727	0.0017856	9.1631E-4
0.004	-0.0039967	0.0017763	8.6604E-4
0.0045	-0.0040288	0.0017647	9.4409E-4
0.005	-0.0040053	0.0018	9.3262E-4
0.0055	-0.0039828	0.0017825	9.0535E-4
0.006	-0.004003	0.0017855	9.3034E-4

0.0065	-0.0040197	0.0017901	9.3139E-4
0.007	-0.003943	0.0017526	9.0822E-4
0.0075	-0.0039212	0.0017608	9.2172E-4
0.008	-0.003983	0.0017445	8.7674E-4
0.0085	-0.0040188	0.0018222	9.2622E-4
0.009	-0.0040455	0.0018009	9.4851E-4
0.0095	-0.0039665	0.0017629	9.2264E-4
0.01	-0.0040026	0.0017681	9.0291E-4

Table E-3: Current density simulation with counter electrode sweep

count_W (m)	Electric potential (V)	Electric potential (V)
5E-4	0.78772	0.90741
0.001	0.76242	0.89708
0.0015	0.74379	0.88958
0.002	0.73245	0.8847
0.0025	0.71949	0.87981
0.003	0.70428	0.873
0.0035	0.69283	0.8689
0.004	0.68083	0.86411
0.0045	0.67453	0.86136
0.005	0.66694	0.85847
0.0055	0.65829	0.85513
0.006	0.64851	0.85036
0.0065	0.64152	0.84876
0.007	0.63431	0.84606
0.0075	0.62931	0.84461
0.008	0.62233	0.84176
0.0085	0.61555	0.84031
0.009	0.60962	0.83692
0.0095	0.60465	0.83601
0.01	0.59905	0.83289

Table E-4: Electric potential with counter electrode sweep

count_W (m)	Maxwell capacitance (F)
5E-4	3.9438E-13
0.001	4.38E-13
0.0015	4.6538E-13
0.002	4.816E-13
0.0025	5.0095E-13
0.003	5.2167E-13
0.0035	5.3785E-13
0.004	5.548E-13
0.0045	5.6062E-13
0.005	5.6924E-13
0.0055	5.8037E-13
0.006	5.8836E-13
0.0065	5.9744E-13
0.007	6.0535E-13
0.0075	6.0814E-13
0.008	6.1577E-13
0.0085	6.2648E-13
0.009	6.3155E-13
0.0095	6.3552E-13
0.01	6.4112E-13

Table E-5: Maxwell capacitance with counter electrode sweep

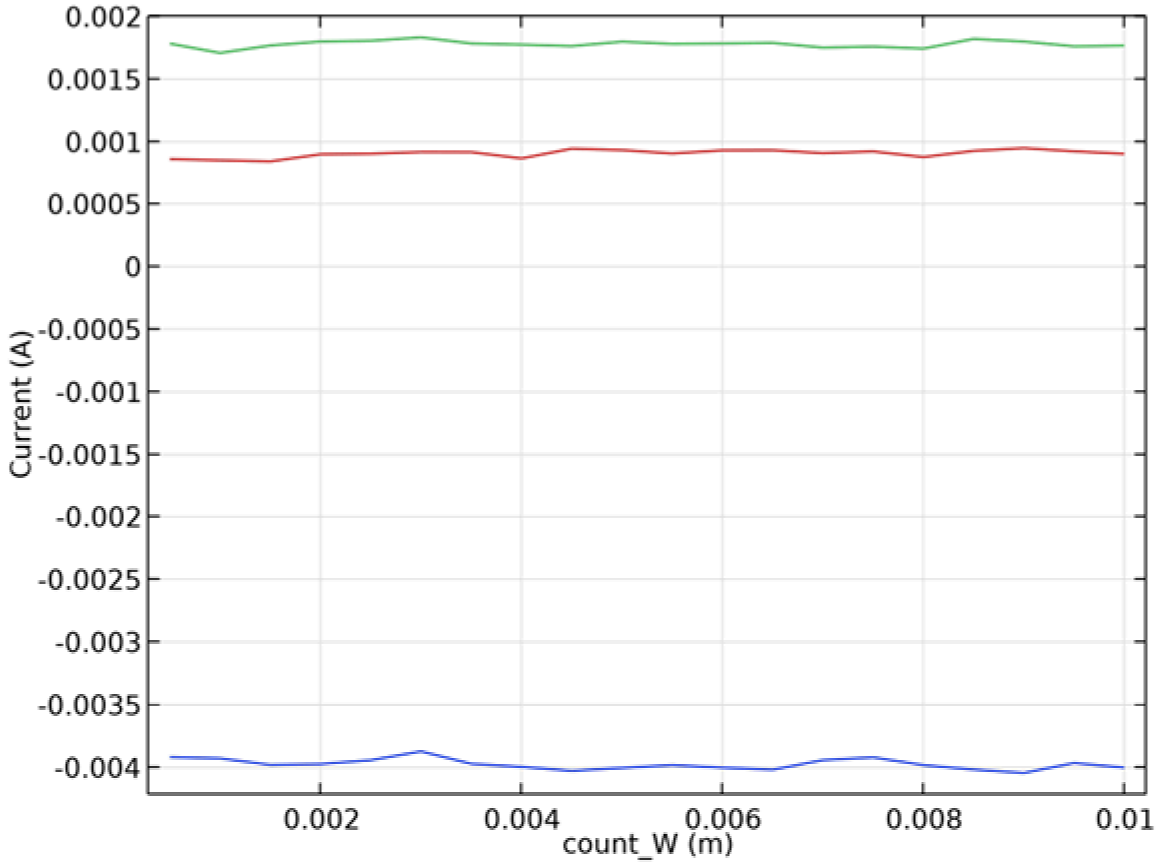

Figure E-10: Current (Dimension Sweep)

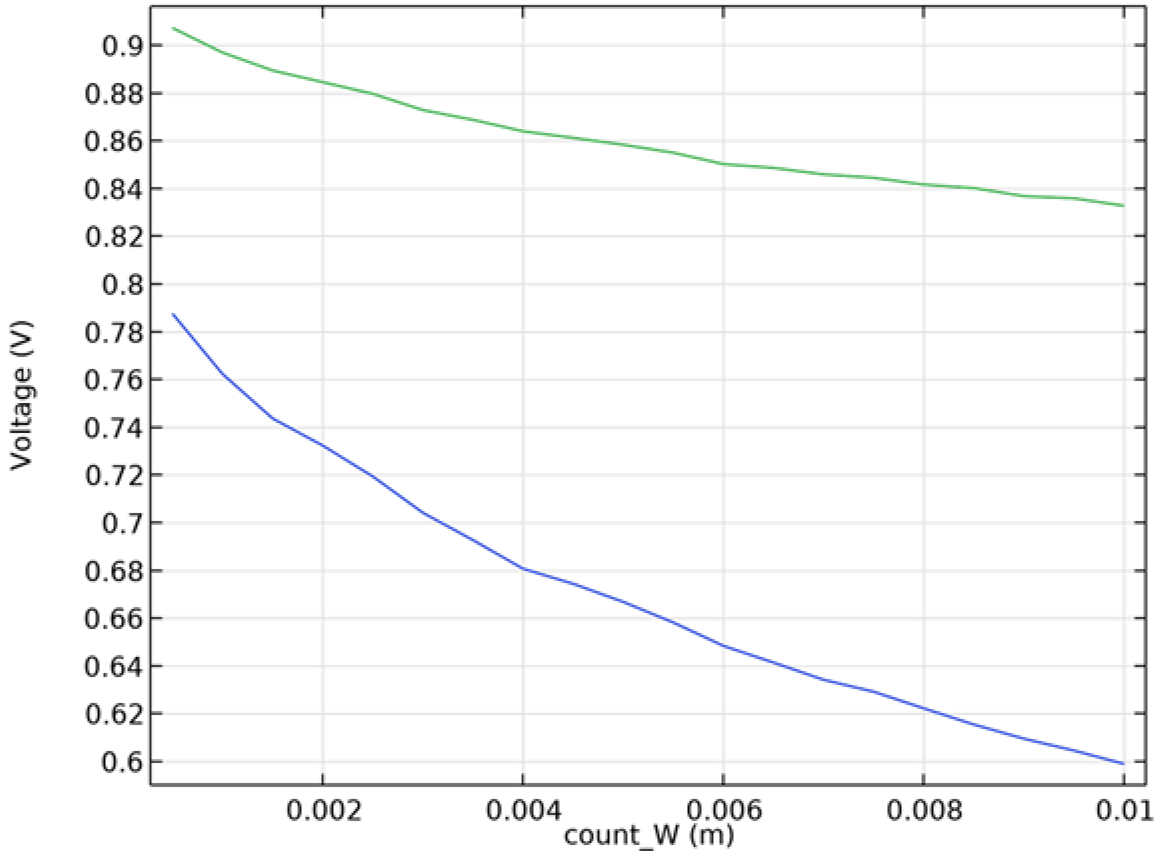

Figure E-11: Voltage at Electrodes (Dimension Sweep)

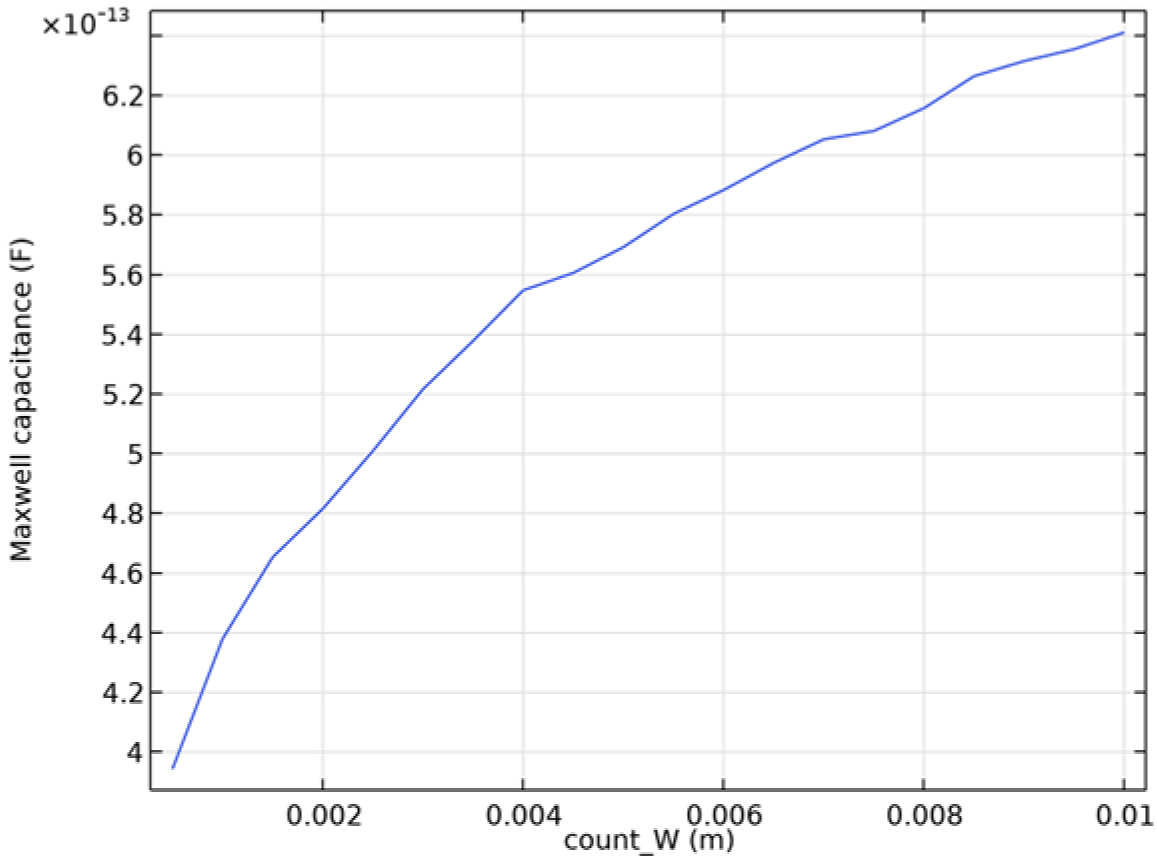

Figure E-12: Capacitance (Dimension Sweep)

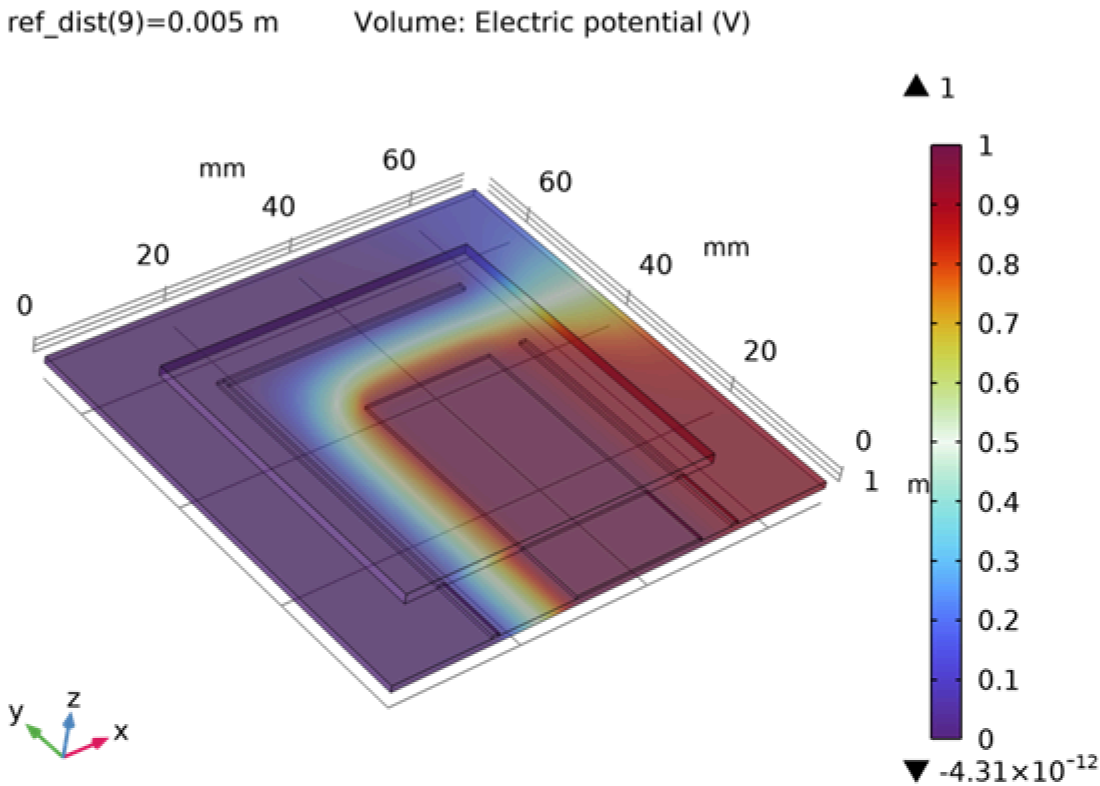

Figure E-13: Electric potential simulation with reference electrode displacement at 0.005 m

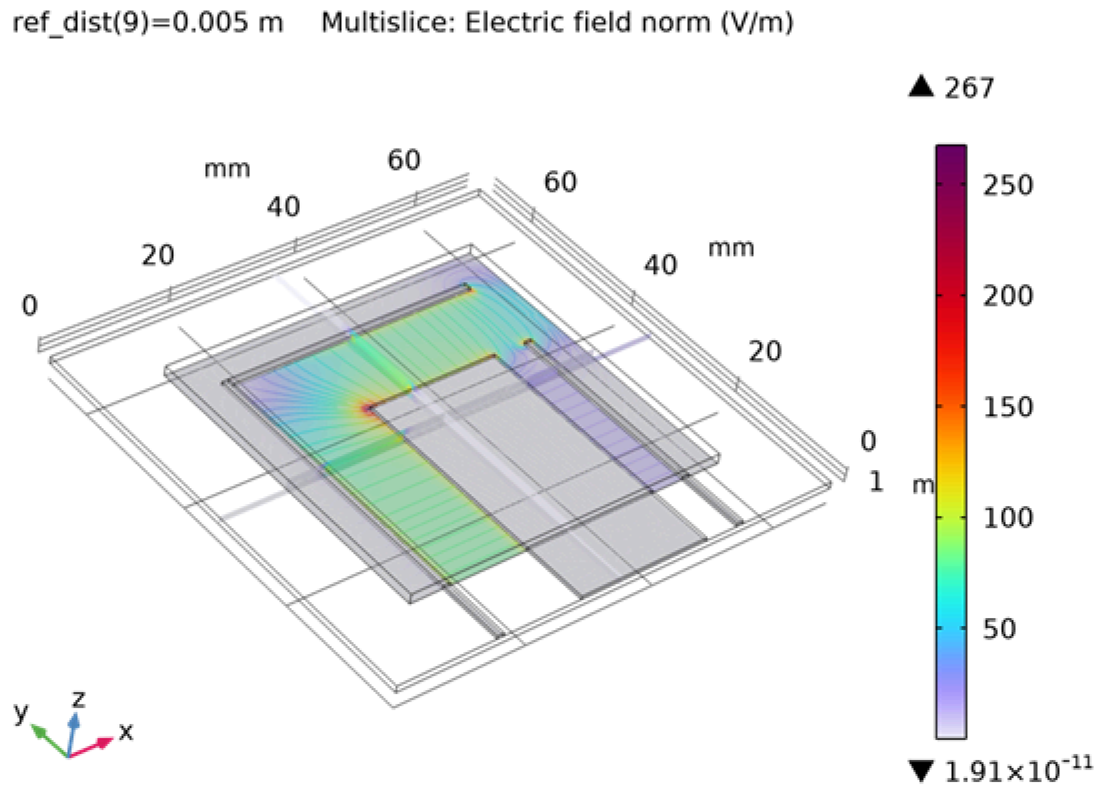

Figure E-14: Electric field simulation with reference electrode displacement at 0.005 m

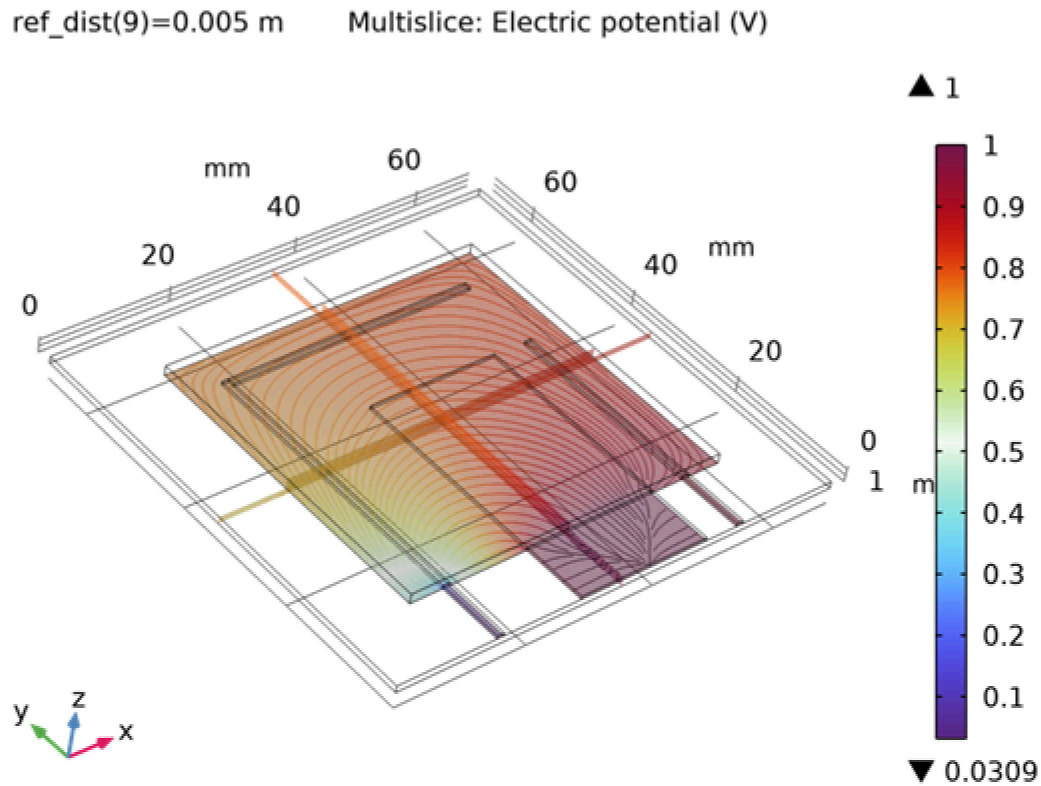

Figure E-15: Electric potential simulation with multislice with reference electrode displacement at 0.005 m

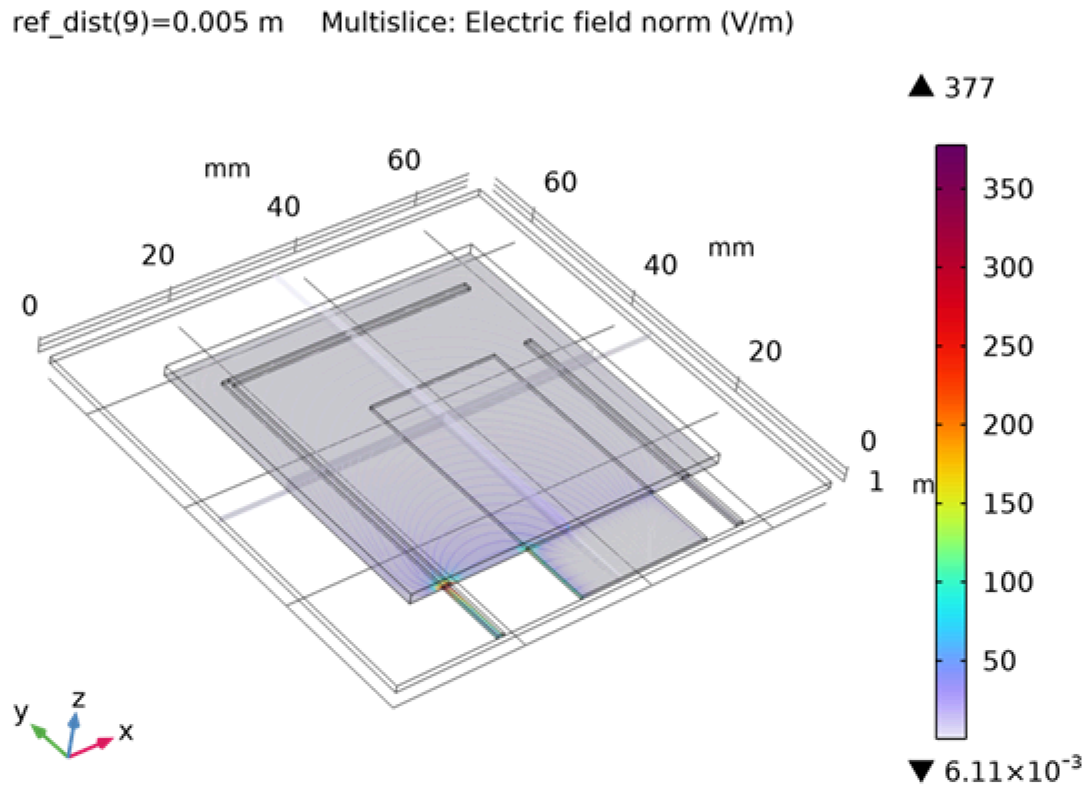

Figure E-16: Electric field simulation with multislice with reference electrode displacement at 0.005 m

ref_dist (m)	Current density, x-component (A)	Current density, x-component (A)	Current density, y-component (A)
0.001	-0.0039571	6.6084E-4	2.5956E-4
0.0015	-0.0039515	6.8153E-4	2.524E-4
0.002	-0.003966	7.2716E-4	2.4576E-4
0.0025	-0.0039655	7.6618E-4	2.4697E-4
0.003	-0.0040095	8.0091E-4	2.5952E-4
0.0035	-0.0039667	8.3796E-4	2.6444E-4
0.004	-0.0039636	8.759E-4	2.7505E-4
0.0045	-0.0038988	8.683E-4	2.5885E-4
0.005	-0.0039477	9.3565E-4	2.4439E-4

Table E-6: Current density simulation with reference electrode displacement sweep

ref_dist (m)	Electric potential (V)	Electric potential (V)
0.001	0.74855	0.9582
0.0015	0.75064	0.95009
0.002	0.7468	0.9438
0.0025	0.7471	0.93923
0.003	0.74997	0.93392
0.0035	0.75403	0.93212
0.004	0.75569	0.92906
0.0045	0.75623	0.92486
0.005	0.75607	0.92194

Table E-7: Electric potential simulation with reference electrode displacement sweep

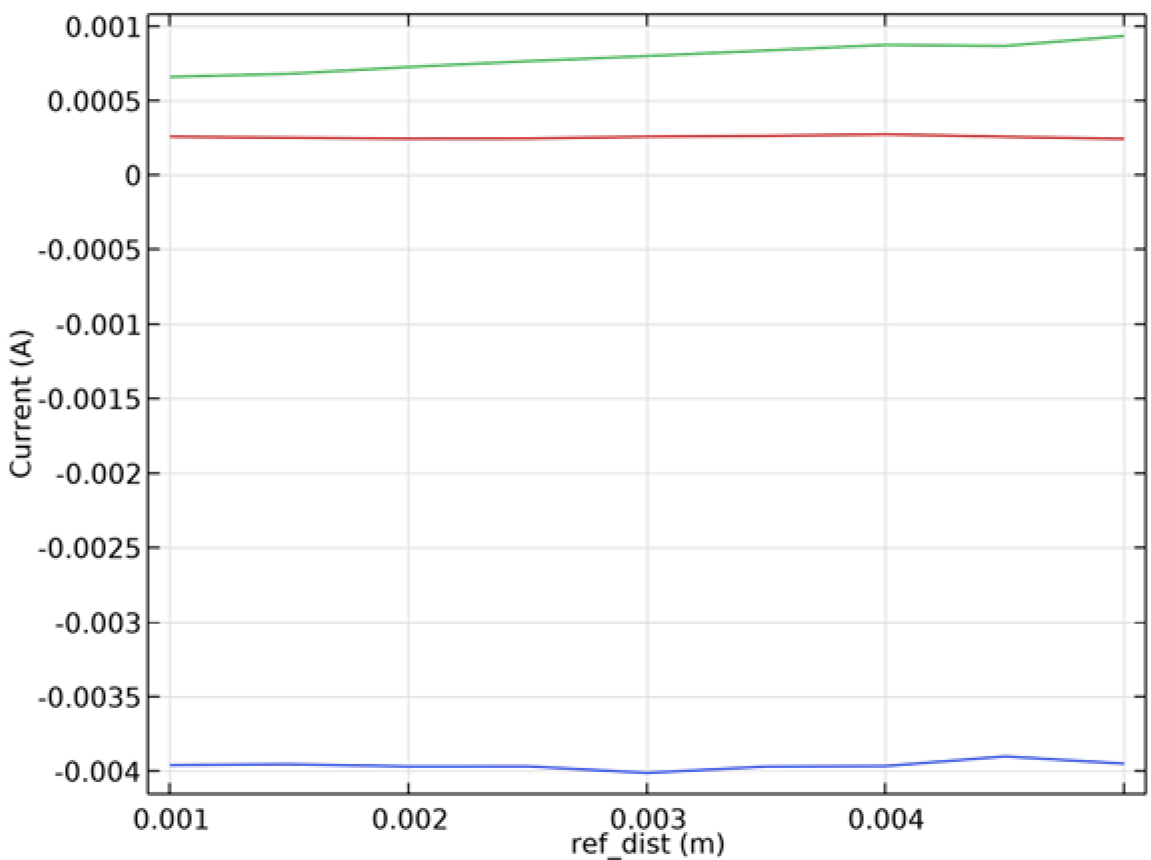

Figure E-17: Current (Ref Displacement Sweep)

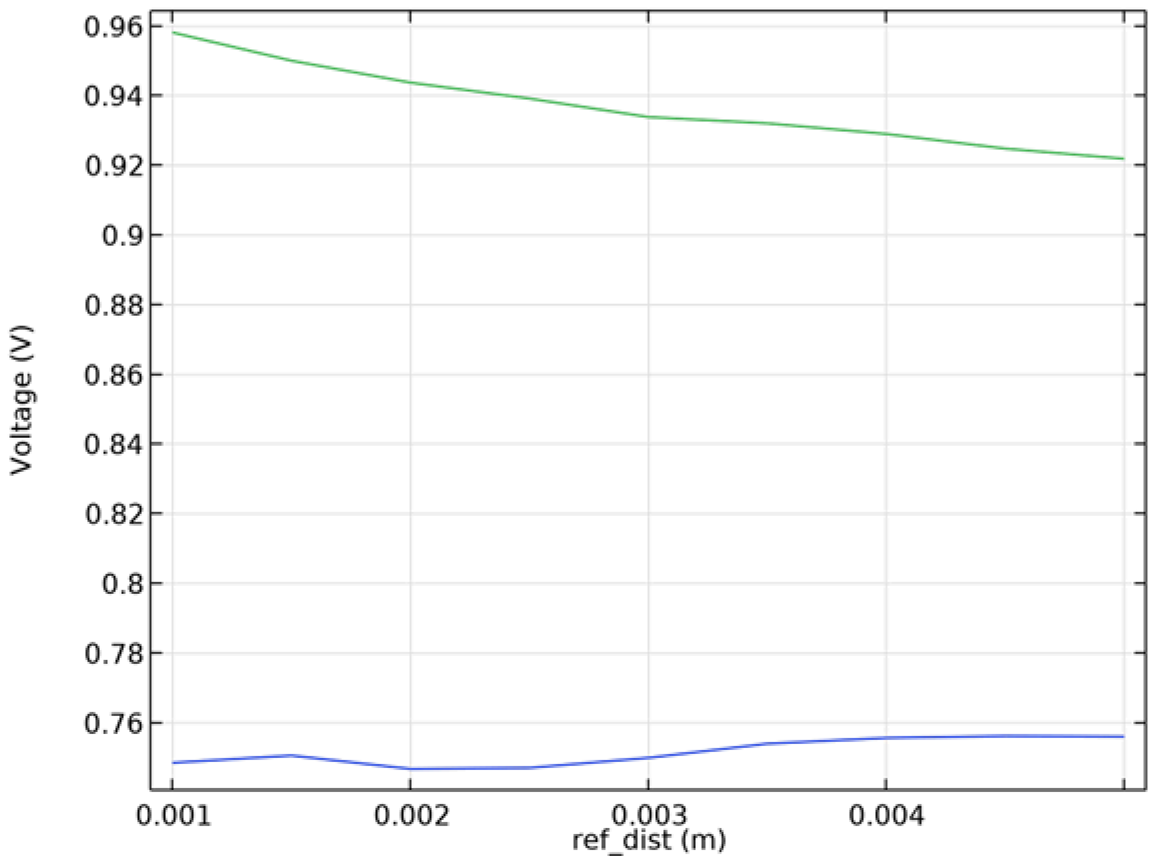

Figure E-18: Voltage at Electrodes (Ref Displacement Sweep)

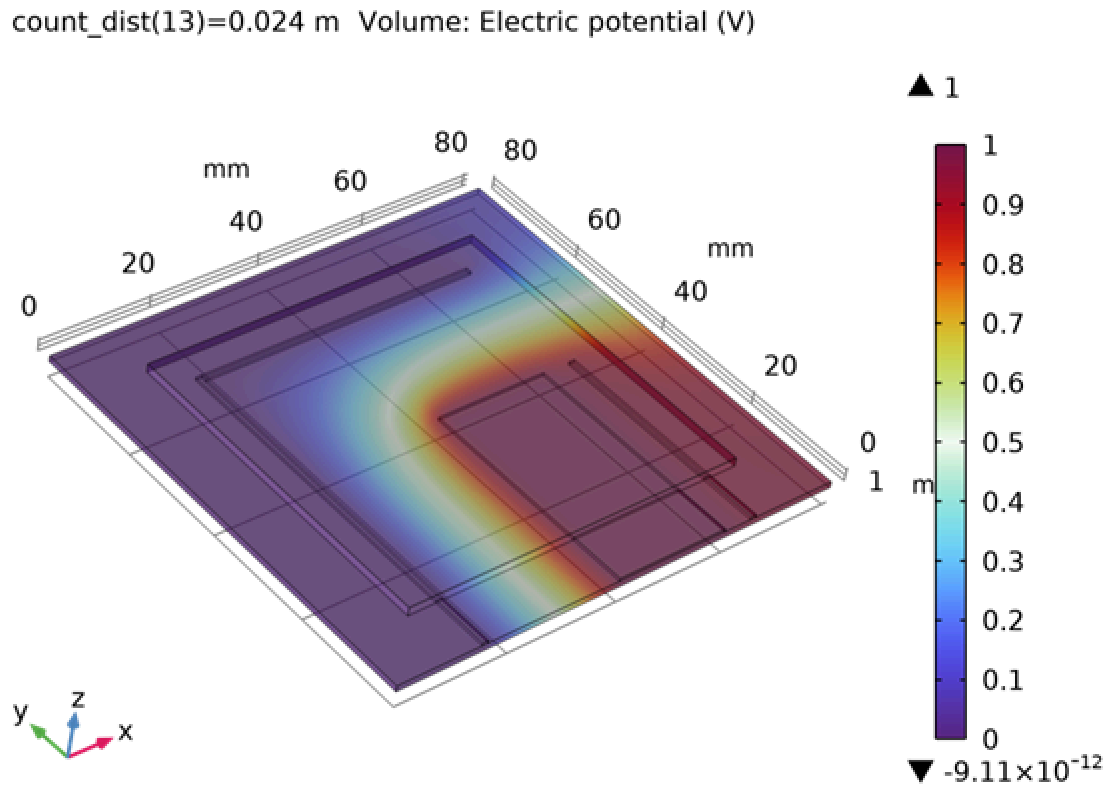

Figure E-19: Electric potential simulation with counter electrode displacement at 0.024 m

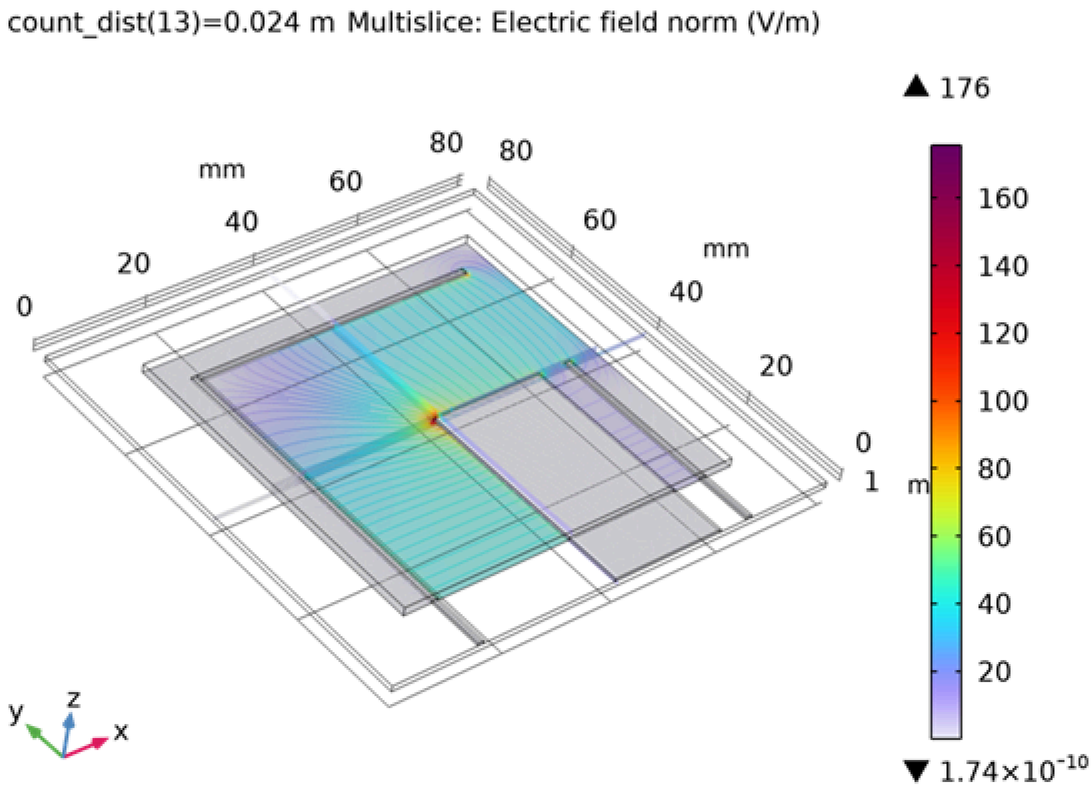

Figure E-20: Electric field simulation with counter electrode displacement at 0.024 m

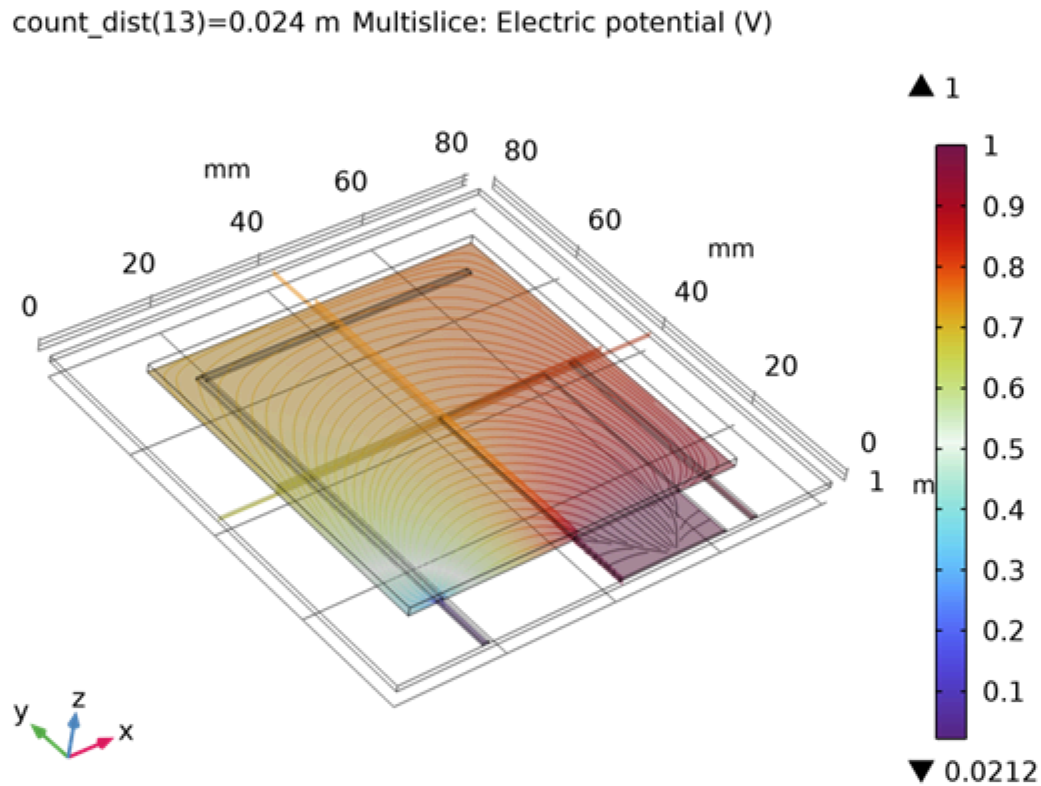

Figure E-21: Electric potential simulation with multislice with counter electrode displacement at 0.024 m

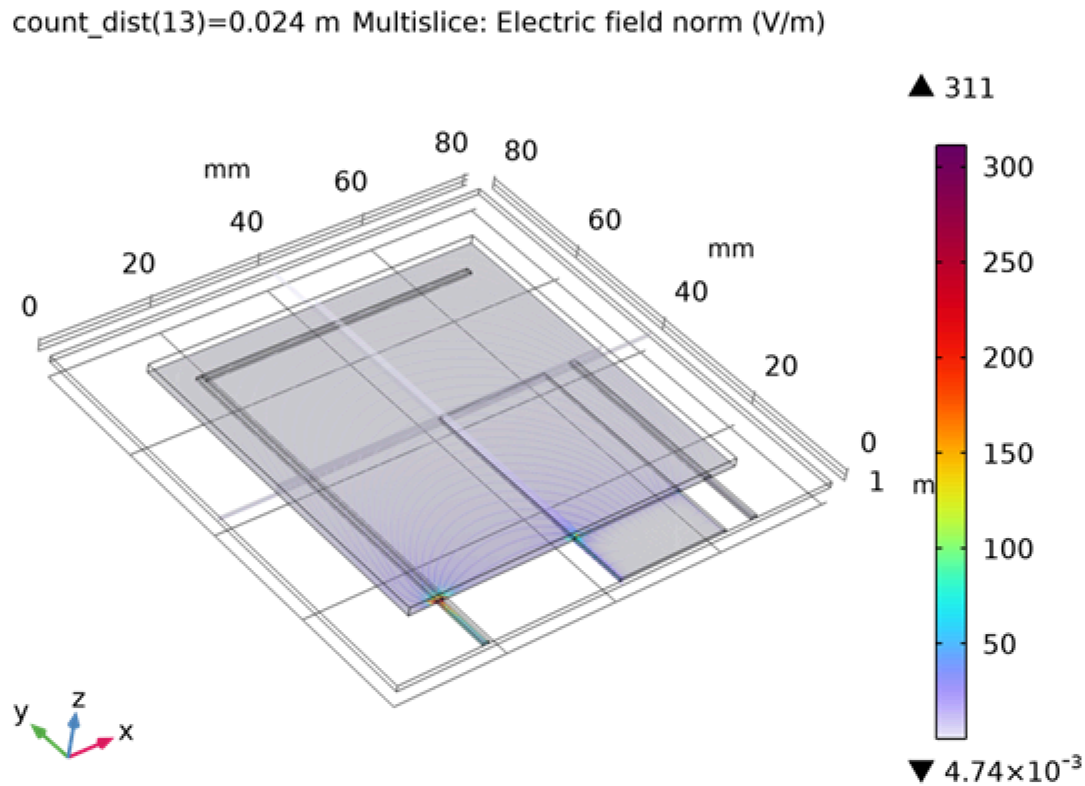

Figure E-22: Electric field simulation with multislice with counter electrode displacement at 0.024 m

count_dist (m)	Current density, x-component (A)	Current density, x-component (A)	Current density, y-component (A)
0.012	-0.0039477	9.3565E-4	2.4439E-4
0.013	-0.0036654	8.7162E-4	2.3992E-4
0.014	-0.0033944	8.1992E-4	2.2022E-4
0.015	-0.003204	7.8058E-4	2.0913E-4
0.016	-0.0029848	7.3463E-4	1.9903E-4
0.017	-0.0029953	7.1081E-4	2.1462E-4
0.018	-0.0027671	6.4838E-4	1.8175E-4
0.019	-0.0026763	6.299E-4	1.7164E-4
0.02	-0.0025883	6.2152E-4	1.4893E-4
0.021	-0.0024795	6.0058E-4	1.7146E-4
0.022	-0.0023784	5.7535E-4	1.5494E-4
0.023	-0.0023254	5.4413E-4	1.5831E-4

0.024	-0.0022231	5.3354E-4	1.1807E-4

Table E-8: Current density simulation with counter electrode displacement sweep

count_dist (m)	Electric potential (V)	Electric potential (V)
0.012	0.75607	0.92194
0.013	0.7535	0.92254
0.014	0.74671	0.92076
0.015	0.74215	0.92064
0.016	0.73526	0.91819
0.017	0.73617	0.91933
0.018	0.7331	0.91921
0.019	0.72971	0.91897
0.02	0.72743	0.91896
0.021	0.72709	0.92025
0.022	0.71855	0.91782
0.023	0.72016	0.91882
0.024	0.71607	0.91921

Table E-9: Electric potential simulation with counter electrode displacement sweep

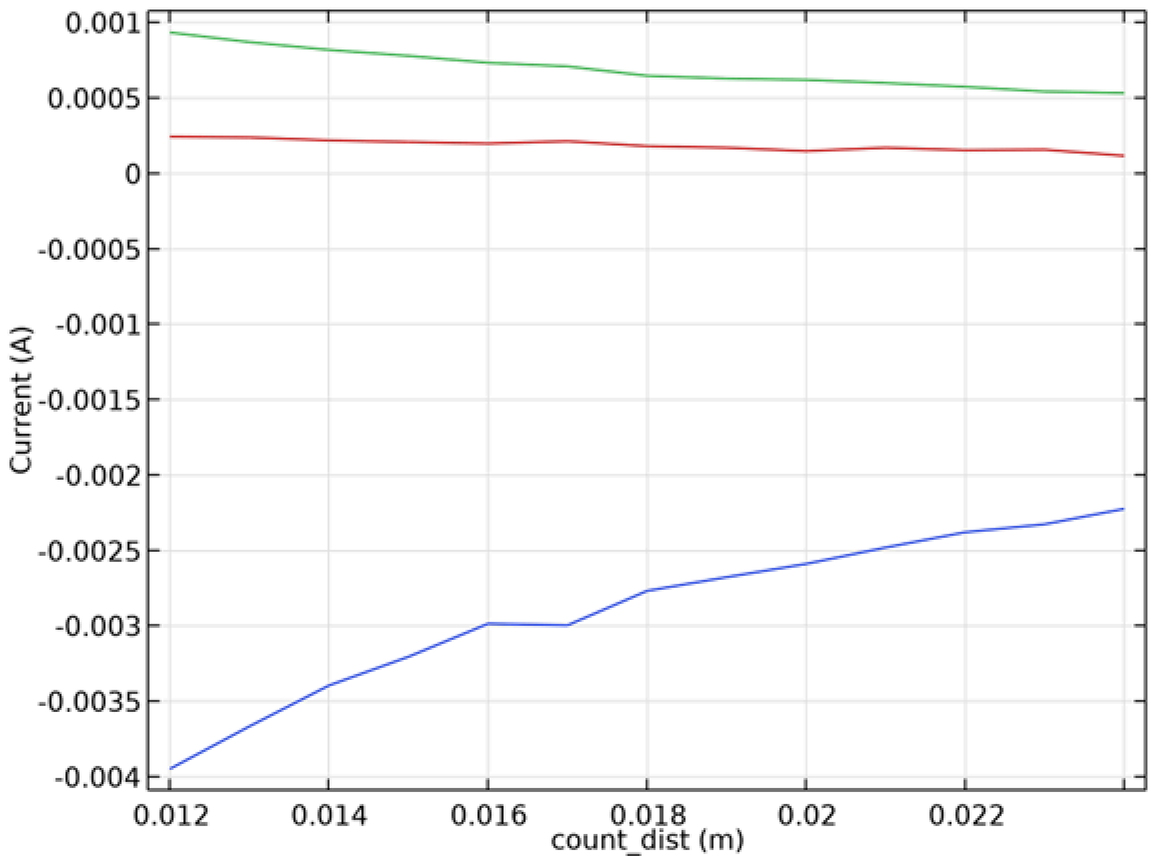

Figure E-23: Current (Counter Displacement Sweep)

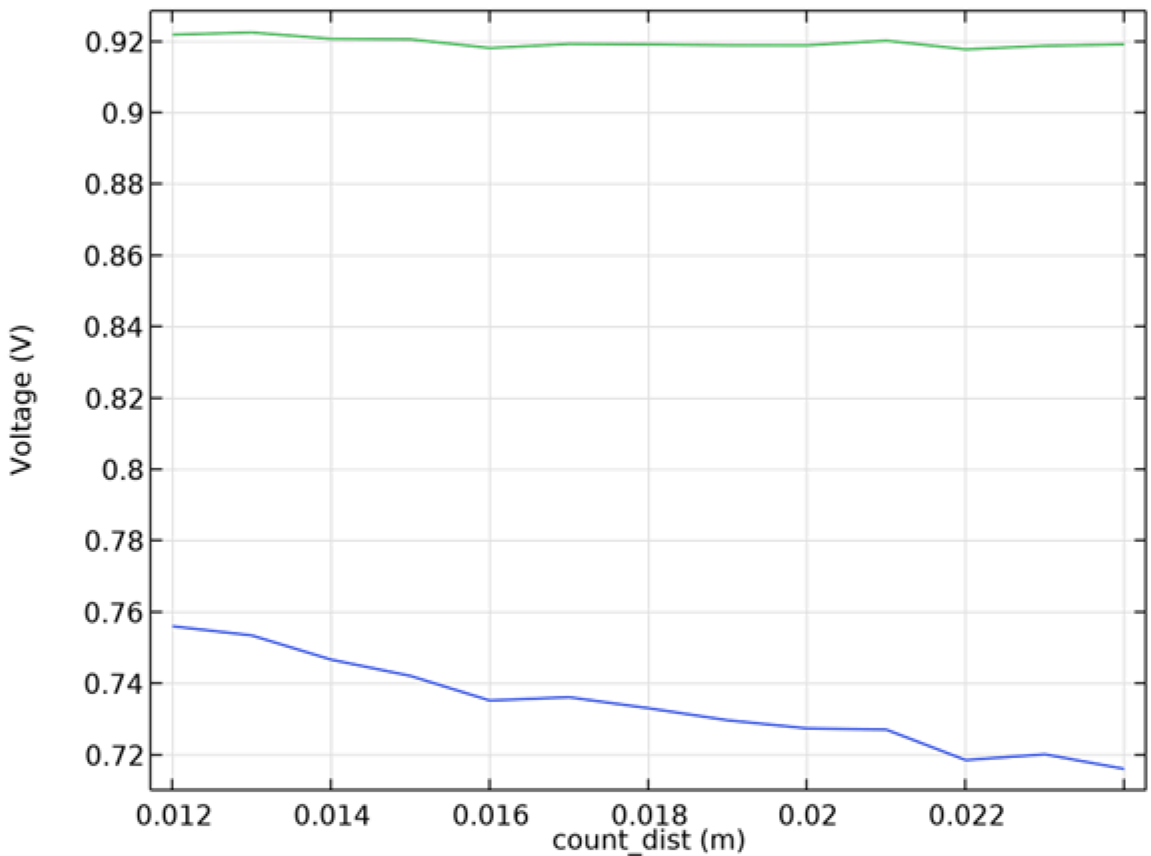

Figure E-24: Voltage at Electrodes (Counter Displacement Sweep)

LTspice Results

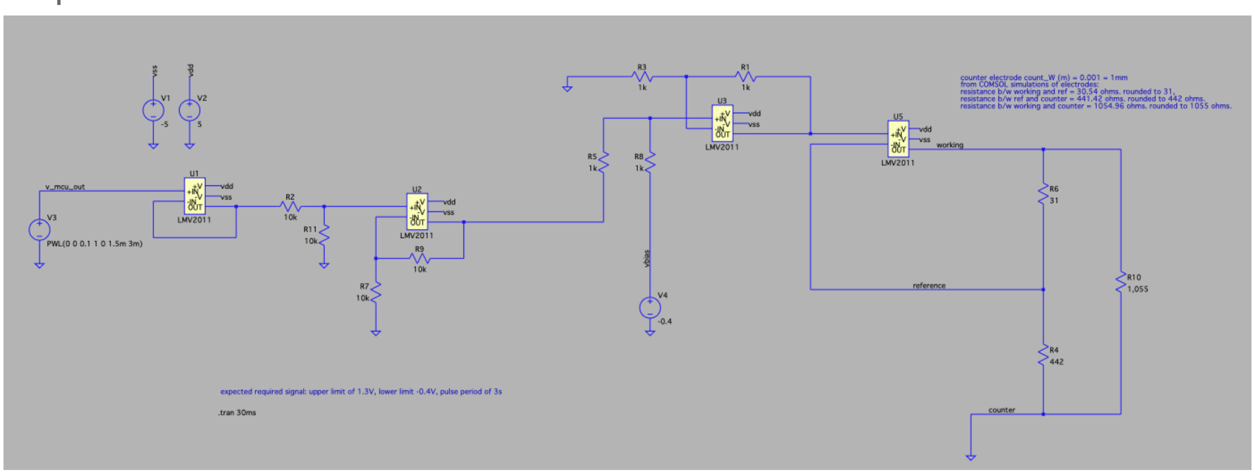

Figure E-25: Potentiostat schematic simulation in LTspice

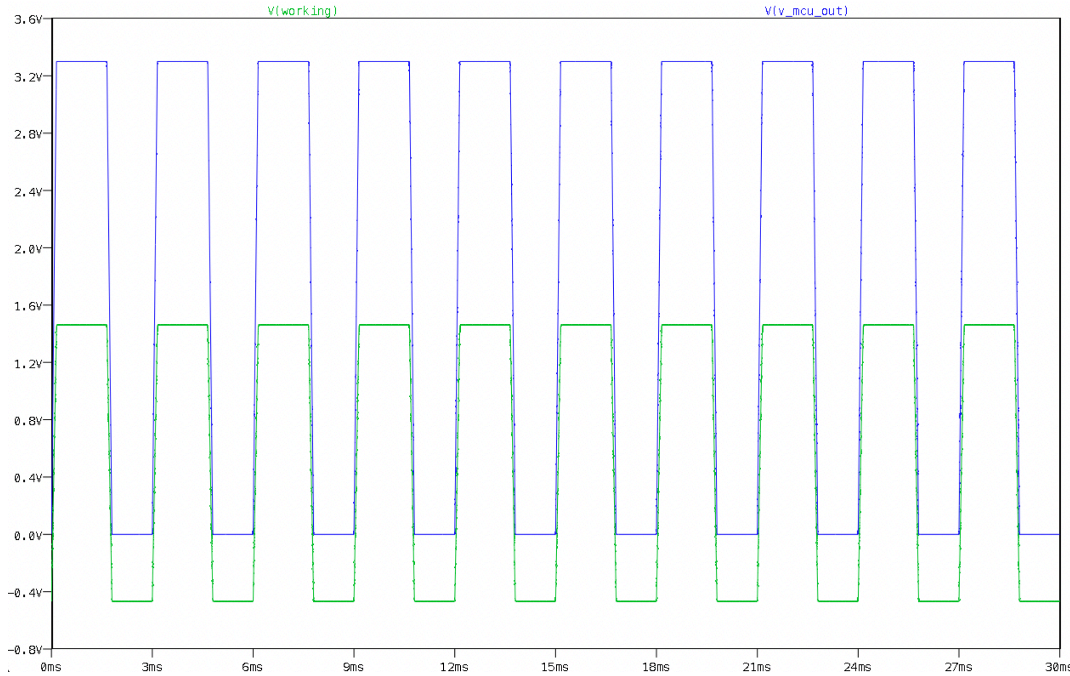

Figure E-26: Input 5V Pulse vs. Output Working Electrode Voltage, Gain & Offset Tuned

Appendix F: Prototype Test / Measurement Data

Raw ADC data values for various concentration points used in the calibration curve are provided below. The data is laid out from left to right, downward from the initial time of 0 seconds to approximately 10 seconds.

First 2000 Raw ADC values for Dark Reference (Noise)									
471	376	176	679	551	336	583	999	591	767
800	311	431	391	719	696	528	575	287	551
327	511	352	671	360	488	495	1264	664	400
512	343	1231	544	567	527	584	447	568	952
432	168	383	431	384	343	616	319	455	440
447	431	1000	296	511	392	728	448	472	528
336	528	472	488	648	912	696	824	455	520
536	512	336	792	367	488	552	519	687	511
472	656	567	392	343	375	343	199	408	575
727	423	215	583	583	247	463	447	751	360
159	415	239	503	536	368	544	567	960	319
568	224	560	344	400	527	231	592	447	247
199	383	624	855	583	496	647	471	503	527
399	968	528	423	575	536	744	423	543	951
503	624	984	1384	784	623	287	655	351	367
760	368	639	383	495	319	655	663	695	720

487	312	272	648	791	583	567	1096	799	967
416	287	528	240	583	391	496	712	368	367
607	464	384	967	872	559	559	368	688	376
511	615	359	312	496	312	400	695	351	232
423	423	272	151	375	655	536	816	551	751
632	495	495	487	576	520	559	304	743	415
256	792	327	711	503	463	672	248	416	583
400	591	248	247	391	640	271	544	375	783
376	472	224	584	567	240	200	320	591	503
503	792	504	472	359	847	327	719	655	255
608	151	208	960	527	783	272	183	367	263
151	255	335	832	503	527	496	759	424	424
600	488	280	383	639	456	432	599	399	423
463	568	272	632	407	279	296	431	927	503
184	136	335	416	480	464	336	504	536	639
512	871	216	528	416	343	423	456	255	368
696	648	600	503	383	608	296	199	455	504
727	831	575	735	615	335	264	263	335	455
407	663	480	704	279	559	487	391	288	416
543	736	632	368	399	360	471	456	296	751
367	455	376	631	856	335	824	543	527	432
359	471	311	504	880	463	223	567	608	400

432	311	616	335	216	807	616	1016	463	767
512	527	583	703	680	504	599	607	440	407
423	192	632	631	623	959	527	720	255	575
567	511	503	327	711	240	911	200	272	416
480	488	399	824	440	655	416	888	376	352
167	312	608	416	648	743	855	432	567	231
1136	360	576	640	456	368	424	335	791	487
352	240	664	415	656	632	727	1128	415	360
776	816	583	495	248	632	504	456	856	824
351	496	527	671	647	367	591	655	504	432
320	592	368	472	567	255	447	375	583	207
287	407	471	535	711	752	431	479	504	576
1008	320	327	568	752	568	584	287	535	359
904	447	904	751	567	239	303	335	832	207
719	527	832	504	448	864	583	415	720	383
688	1024	295	175	319	536	615	823	799	559
336	496	408	328	327	151	416	328	520	520
616	423	295	655	303	824	648	360	296	440
520	216	615	519	344	239	592	336	391	335
431	311	784	408	360	199	832	296	551	743
615	352	912	488	591	984	767	735	400	288
256	592	503	383	712	472	671	615	823	280

1007	527	360	568	423	199	720	648	696	560
855	616	687	320	512	704	407	399	240	504
432	631	456	536	592	592	560	440	567	528
783	320	552	311	375	991	840	824	495	479
192	287	560	447	552	800	111	431	311	768
176	591	335	439	463	631	607	648	856	232
519	272	455	712	311	904	191	392	384	183
256	703	751	703	1023	503	519	264	431	183
464	1184	687	440	600	808	263	703	536	983
384	272	520	599	263	391	295	487	463	464
223	479	1000	664	415	360	384	263	671	727
375	432	264	583	312	464	304	703	360	552
280	472	400	416	664	400	599	576	608	504
600	864	552	512	632	391	455	520	983	728
544	327	631	848	312	528	375	704	479	304
383	728	408	335	528	631	560	623	504	863
535	159	520	487	655	663	511	391	855	687
687	632	224	431	344	904	384	455	280	512
416	800	455	640	472	599	719	599	143	551
431	711	184	999	431	1167	592	463	551	888
255	295	895	575	447	447	527	800	607	599
520	615	807	448	632	392	639	296	624	488

576	639	336	655	559	232	279	599	632	415
504	919	512	328	624	384	495	520	272	279
343	591	327	792	792	175	360	559	575	303
304	928	552	440	328	191	368	239	495	591
1071	303	975	1383	479	495	671	320	456	623
327	568	272	728	632	479	120	608	791	512
616	576	519	631	431	247	632	760	511	528
760	488	632	671	672	496	407	183	464	736
415	368	183	927	224	424	239	744	784	248
495	344	495	319	583	311	632	383	760	648
343	359	351	344	376	464	512	1343	488	831
183	271	536	1055	784	479	223	632	447	503
488	679	488	503	368	216	688	663	487	648
272	279	728	472	735	751	319	503	392	456
271	743	792	328	488	496	456	231	623	519
968	559	423	487	1047	455	536	455	480	327
535	576	383	567	552	552	848	919	560	631
599	328	472	751	352	472	104	375	472	448
463	287	543	615	143	679	216	471	552	616
616	200	648	784	392	368	816	279	687	808
352	567	800	615	584	512	680	695	359	568
719	248	1048	312	191	439	575	679	159	656

719	319	352	935	472	511	416	320	1007	575
768	608	608	1095	448	704	744	608	295	432
959	624	711	575	264	503	335	607	503	367
696	223	432	479	303	303	631	575	239	415
296	599	471	487	560	487	360	728	448	599
319	471	895	343	359	839	399	471	480	423
295	767	567	376	288	807	368	615	872	543
432	455	424	456	280	407	407	248	319	927
544	295	752	496	752	512	416	728	711	799
280	552	376	632	1047	784	535	679	664	704
584	559	215	992	592	408	208	631	775	872
639	423	392	416	1079	456	567	272	335	504
567	632	392	536	512	448	391	303	439	176
584	711	736	967	664	607	335	536	847	664
728	159	343	168	664	544	440	544	567	543
215	296	159	319	480	663	296	368	592	183
424	455	392	471	904	256	480	384	656	639
264	743	416	503	576	359	208	824	312	312
392	544	928	496	448	632	512	639	559	368
495	255	591	631	328	735	719	631	288	591
447	679	287	264	312	591	591	248	304	719
616	320	439	383	336	535	328	463	640	503

511	447	359	607	567	519	496	264	352	775
464	487	855	432	575	312	696	256	535	407
319	671	744	559	568	592	407	584	423	312
528	240	456	415	335	511	400	151	423	568
471	327	936	416	576	223	704	512	504	423
663	272	632	280	656	408	511	975	736	655
480	728	247	600	527	384	432	792	288	495
471	504	576	112	207	712	328	728	472	527
352	344	479	840	255	440	416	760	456	600
431	271	664	695	655	567	368	776	647	448
207	247	215	527	543	384	320	439	344	359
535	607	95	527	184	608	391	519	799	600
360	704	447	351	512	512	624	648	512	528
752	487	504	536	376	239	375	551	472	471
744	415	480	271	600	519	655	344	176	280
976	960	448	480	248	503	311	288	439	616
656	624	479	263	488	399	447	448	472	519
215	23	367	488	319	527	559	255	464	536
303	303	367	664	535	192	575	416	447	440
687	744	600	560	264	264	431	992	415	599
359	776	352	775	583	671	463	575	447	967
351	471	607	344	856	671	536	607	279	608

304	272	551	535	207	407	368	360	543	536
671	375	320	583	792	503	608	408	639	383
279	416	255	367	1016	1111	376	336	1008	456
368	488	607	527	128	520	439	624	399	568
416	400	448	656	736	279	631	399	224	424
528	568	623	319	816	376	383	343	655	615
295	703	424	415	527	839	575	472	600	384
376	784	863	392	639	344	632	624	720	280
295	967	536	552	768	567	680	679	327	464
1016	535	664	295	440	375	599	616	351	407
575	192	208	352	496	447	583	544	624	535
359	440	247	536	320	351	408	575	584	408
920	608	448	392	511	791	384	656	351	399
720	368	543	696	432	336	552	832	848	472
543	287	704	327	320	271	296	303	663	368
1072	576	424	192	384	455	680	392	767	424
295	664	567	279	616	535	975	704	584	519
759	327	504	615	319	383	647	415	479	767
615	599	527	407	392	440	287	719	336	512
544	512	543	704	335	503	751	456	679	232
599	216	639	784	359	712	455	480	496	599
664	504	880	464	536	488	623	416	583	568

303	431	376	687	384	839	551	839	544	303
488	479	496	455	383	751	615	232	663	312
463	520	504	1143	536	536	335	447	184	295
327	535	960	407	248	447	520	431	1031	447
631	463	304	416	423	431	576	583	607	543
432	519	367	319	536	871	416	408	495	423
319	616	335	463	320	343	791	479	495	512
648	752	719	248	280	415	368	255	416	463
391	367	727	936	823	88	399	120	1216	487
679	672	543	695	279	528	327	943	311	448
384	143	328	415	192	367	640	608	376	711
447	344	535	535	472	311	471	448	351	551
343	447	944	416	287	455	440	455	583	672
599	423	519	343	384	152	543	640	143	472
584	615	408	503	287	567	296	1000	623	792
592	263	479	480	303	432	463	191	424	400
520	552	352	823	247	704	608	608	375	359
567	655	432	551	360	647	272	271	239	415
224	431	623	783	143	872	911	648	416	1055
623	256	711	447	815	455	159	199	791	520
295	440	319	320	567	368	519	431	423	439
607	520	312	559	567	255	664	775	679	343

656	536	416	856	527	927	760	264	671	767
544	752	591	456	431	575	520	592	679	519
567	536	215	679	712	752	320	391	943	607
760	488	303	368	744	527	663	784	455	511
591	560	744	479	1024	632	320	352	783	368
639	504	320	183	288	304	376	352	656	768
512	391	463	343	511	240	592	576	440	224
631	216	911	655	551	712	496	616	535	591

Table F-1: First 2000 Raw ADC values for Dark Reference (Noise)

First 2000 Raw ADC values for 75ng/ml Concentration of Lp-PLA2									
360	496	655	384	175	544	344	183	592	807
632	456	775	1040	503	415	200	407	536	479
631	312	831	576	799	576	327	527	376	359
527	143	512	208	272	664	655	712	416	391
599	264	784	295	575	440	448	415	856	544
432	599	671	392	408	735	455	943	335	231
703	392	591	264	647	472	392	567	672	240
1207	599	551	119	671	511	888	303	407	559
247	855	599	567	279	616	439	839	616	720
456	519	567	704	647	968	312	632	440	255
528	399	712	536	592	319	536	503	791	248

288	824	567	703	351	271	775	888	135	624
695	607	639	815	568	327	567	359	256	856
264	592	360	712	575	551	359	448	456	607
464	544	327	903	384	928	599	327	728	360
576	415	359	544	591	256	687	1375	408	392
840	383	487	511	928	303	415	599	472	175
632	152	303	383	424	248	647	464	680	351
271	456	159	423	464	488	919	808	343	280
760	383	536	359	391	599	376	335	672	479
559	567	424	696	240	464	503	399	535	823
344	775	432	687	480	439	383	487	223	504
319	895	615	239	471	911	192	615	727	311
656	383	848	296	439	528	759	608	416	695
776	647	735	959	328	448	1032	336	559	464
567	336	631	407	592	575	1135	872	807	519
408	207	679	479	744	687	487	504	511	175
232	392	680	615	584	496	608	335	1271	415
383	711	487	432	815	247	616	360	384	687
304	208	447	376	400	520	512	511	159	528
544	799	352	439	399	496	399	535	335	575
175	239	279	224	215	216	623	1215	360	544
847	599	247	295	807	272	504	543	135	784

584	455	631	567	464	784	472	272	184	320
375	927	519	672	720	584	608	544	647	176
224	704	448	528	431	728	512	831	543	399
527	672	536	359	423	407	391	464	864	599
839	407	496	1015	463	663	496	743	351	928
416	599	559	479	640	584	464	448	431	536
416	407	303	448	591	431	768	776	576	439
528	599	368	424	303	759	640	319	207	560
336	199	168	576	375	928	671	504	704	424
376	495	568	1263	392	496	344	663	560	584
624	272	536	415	352	760	720	704	567	343
399	719	415	823	271	223	799	824	543	952
567	439	192	167	335	247	535	455	615	399
591	264	623	695	384	767	271	328	583	791
344	560	599	823	296	455	351	704	487	583
479	384	503	520	680	191	903	568	367	312
1064	960	632	527	664	543	536	167	575	463
928	407	304	567	399	511	431	448	503	807
448	560	560	616	583	336	343	903	383	351
784	407	312	712	464	351	527	159	583	592
512	199	263	472	351	216	712	423	399	831
495	656	263	319	471	647	743	288	151	616

471	471	456	567	255	384	599	447	447	200
511	288	431	488	799	368	447	623	503	431
447	903	384	415	816	824	768	296	351	600
488	527	320	672	384	528	471	576	712	944
464	423	367	471	319	232	376	376	312	456
856	239	655	336	544	472	416	472	263	392
511	271	527	864	328	159	600	623	408	391
848	543	312	639	519	576	600	567	416	271
871	455	792	391	584	327	287	679	272	160
192	247	551	776	432	304	640	575	567	696
751	327	807	599	496	199	408	735	311	743
320	592	448	591	231	463	1088	376	319	223
743	351	448	535	255	615	248	319	720	520
640	664	264	416	416	527	344	815	487	432
336	415	791	727	336	463	880	480	464	776
456	648	615	416	408	344	455	584	296	408
208	631	399	616	983	456	552	239	615	816
343	528	576	559	592	472	432	511	544	968
655	311	487	592	703	400	359	568	279	696
543	679	312	352	575	624	559	271	591	479
528	352	840	592	447	375	415	640	519	719
375	439	672	343	543	176	503	335	439	664

360	480	263	384	360	823	392	319	415	680
328	479	272	487	328	232	479	623	623	551
592	464	551	791	528	535	375	871	840	288
591	231	527	495	279	328	352	783	247	599
647	479	415	543	287	375	207	903	456	335
376	703	280	464	1071	471	304	871	624	320
608	504	672	567	488	479	311	751	400	688
648	808	496	496	480	472	663	679	599	383
768	743	463	424	639	911	360	391	624	815
456	447	743	536	704	407	447	696	255	423
567	360	319	488	495	463	304	463	319	895
416	336	376	591	584	551	479	552	591	328
360	336	664	287	911	487	592	295	479	199
640	911	559	568	656	768	231	319	400	591
592	711	368	432	472	375	551	328	599	312
615	167	535	848	215	640	439	319	760	383
840	520	463	648	591	816	520	439	600	280
815	296	544	280	391	504	343	320	424	447
248	423	624	895	943	320	583	399	327	544
360	488	327	207	648	399	592	504	512	264
519	959	904	631	544	392	687	424	776	432
520	631	200	535	535	448	608	503	519	568

176	416	704	360	471	688	312	688	696	503
384	535	423	464	552	247	423	472	512	712
407	431	919	295	367	351	392	440	527	656
263	671	768	255	432	911	848	343	559	407
240	328	447	983	664	448	407	648	1047	744
880	264	520	375	463	615	776	423	695	448
367	519	520	391	591	672	671	583	560	416
584	711	399	544	487	344	231	271	392	672
400	303	624	383	280	424	656	496	464	655
376	279	647	663	312	487	800	431	408	951
600	544	464	144	551	392	407	535	767	295
695	375	223	560	447	319	639	752	503	272
399	1000	567	535	352	576	376	447	367	735
608	712	455	559	448	512	463	640	240	504
480	584	632	479	440	327	608	664	751	399
455	624	319	495	727	448	535	328	559	343
1088	671	448	695	527	407	552	679	232	239
736	943	232	439	504	943	712	719	344	552
784	463	647	335	448	704	575	303	223	423
583	127	535	503	423	608	536	447	519	816
856	287	319	399	376	487	656	776	648	408
519	520	663	240	392	751	504	383	368	727

480	423	520	295	648	456	328	224	840	384
831	311	800	447	359	455	552	479	247	304
656	519	776	295	712	456	415	360	520	840
447	543	1359	255	848	416	456	592	855	471
447	623	407	632	287	744	519	447	295	327
360	375	280	303	623	920	447	383	448	608
247	472	344	391	559	399	328	360	447	392
360	408	359	487	496	303	367	328	423	583
232	1151	488	679	807	231	503	463	432	424
871	448	448	399	591	479	336	496	679	679
768	224	511	408	760	351	640	815	368	223
376	312	543	519	240	567	639	615	279	392
360	464	439	584	616	736	240	184	663	527
528	480	623	496	672	151	399	239	359	648
543	640	335	328	207	416	447	560	455	360
608	456	648	599	463	408	575	791	904	559
480	351	472	503	504	535	463	416	512	416
536	727	864	584	359	616	208	312	472	495
391	784	343	295	376	407	447	455	336	208
304	439	248	527	519	655	591	240	279	615
552	752	264	632	464	488	359	327	432	167
519	296	864	328	311	504	311	391	472	383

728	688	800	439	495	615	896	656	423	312
583	351	544	984	360	736	351	416	320	295
655	456	407	743	744	360	263	479	560	376
495	495	407	511	431	311	415	359	695	591
272	279	607	231	368	672	679	320	216	567
183	447	911	335	623	528	680	440	440	679
464	680	1263	871	727	375	311	376	791	407
231	471	367	408	304	455	639	560	672	399
248	471	255	167	432	208	751	640	1071	423
655	1128	824	727	296	263	800	231	551	639
215	200	616	599	400	288	416	376	616	512
279	535	375	799	488	456	376	263	671	407
151	512	408	719	807	304	295	519	840	408
464	416	216	816	408	271	784	503	656	431
359	599	496	503	519	303	672	720	648	368
335	272	359	368	463	383	743	376	567	448
160	456	495	407	255	855	720	344	383	175
792	608	647	583	535	392	592	568	376	304
295	391	480	1431	463	295	775	608	392	327
279	607	799	383	575	424	616	631	871	424
584	455	464	135	471	320	511	664	327	368
327	872	560	432	543	488	319	223	855	528

719	432	479	271	175	511	512	392	695	432
768	280	424	415	391	815	360	447	351	792
575	271	544	495	335	232	512	400	408	175
984	288	159	543	727	512	447	472	504	471
919	440	88	519	735	232	480	240	472	791
464	656	631	560	519	368	528	528	376	903
368	336	872	687	752	535	295	840	455	600
152	447	527	615	560	224	583	719	368	536
448	415	792	344	295	448	544	527	543	351
751	488	271	496	319	256	607	431	600	376
599	375	559	359	832	528	463	528	767	520
423	496	568	320	368	424	935	584	167	336
520	279	592	463	576	520	736	1215	655	351
399	432	664	407	528	320	464	416	344	599
240	824	360	407	399	376	407	512	360	320
240	424	680	471	407	679	487	608	304	528
799	1095	376	207	279	416	839	423	680	400
592	624	327	560	583	360	679	376	671	471
535	415	336	656	631	1207	400	175	416	464
407	360	727	376	455	831	488	776	496	359
519	335	599	359	824	287	448	391	423	272
591	936	384	504	319	432	480	567	640	751

552	872	336	463	344	672	592	512	151	872
423	816	248	488	287	552	655	383	751	455
560	551	631	287	432	119	768	687	279	448
224	280	847	343	520	727	400	535	567	1000
592	559	263	679	752	672	360	656	383	336
784	407	663	440	247	536	424	431	184	608
455	1016	231	183	440	1039	448	480	296	215
671	632	480	279	560	287	504	464	560	655
784	800	408	432	855	599	880	472	551	623
263	727	528	631	679	503	1208	224	311	232
672	304	248	520	584	639	272	455	599	247
688	536	599	272	295	607	247	399	431	335
303	439	616	360	264	832	223	295	591	656

Table F-2: First 2000 Raw ADC values for 75 ng/ml Concentration of Lp-PLA2

First 2000 Raw ADC values for 125 ng/ml Concentration of Lp-PLA2									
8816	9248	7872	8423	8040	3031	471	455	415	615
6848	8704	9384	8607	7904	3031	479	392	407	360
5640	7775	8976	8127	9743	3143	895	664	583	831
4679	7296	9727	7560	9368	2999	536	447	592	207
5928	6880	8991	7271	8639	2160	823	368	336	272

6192	8263	8984	6896	7288	2559	823	864	423	415
7071	6215	8584	9256	9063	2863	488	415	752	200
6376	7816	9368	9223	8864	1872	488	424	464	328
6631	7871	8055	8424	8039	3559	359	368	1103	576
6328	7488	8960	8448	9448	1967	407	984	871	208
5240	6816	9408	7160	8727	2911	368	367	344	184
6392	7703	7616	8192	7752	2848	512	928	160	799
5952	7568	9223	7359	7272	2567	560	743	711	559
6328	8783	7392	8992	8320	2504	239	440	559	712
6952	7527	8919	8440	8064	2640	896	624	671	200
7304	7615	8343	8407	6471	2512	760	640	256	191
7864	7391	8600	6432	7111	2368	552	720	344	984
6184	7824	9072	8687	8823	2439	623	567	184	400
5991	7567	8575	8255	9416	1984	471	456	440	103
6215	7400	8071	9184	7192	2415	327	503	680	191
6527	7919	8551	9119	9640	2423	592	504	264	576
5839	7255	7479	8207	7039	2599	672	656	263	168
7279	6319	7056	8391	9368	2335	744	176	479	480
6967	8096	9496	7784	7336	1960	431	375	647	416
6231	7703	9343	9527	7464	2128	192	680	392	256
6183	8832	7640	7840	9575	1968	495	223	575	384
6023	7688	9415	9560	7824	1928	472	280	439	255

7320	8287	8584	7471	8439	2551	615	480	408	368
5912	9680	8391	8352	7648	1831	888	184	376	447
6504	8440	8167	8840	8399	2048	383	384	472	823
6304	8288	6983	7144	8104	2263	680	551	479	271
6927	7408	8559	8024	8544	2600	863	144	687	272
6615	8608	10120	8271	7440	1895	392	624	496	543
6023	7471	7735	10087	6951	2456	615	272	448	576
7248	7448	7975	8863	9327	2032	351	256	567	471
5495	7280	9536	9039	6895	1559	919	575	447	360
6736	7480	10072	8367	6399	1752	239	712	328	664
6216	10448	8935	7575	7864	2136	615	464	983	527
6920	8207	9127	8439	6671	1432	159	479	655	583
6944	9376	8056	7824	8176	2239	951	456	623	224
6136	8680	9520	9095	7304	1639	376	423	407	95
6704	8648	8920	8152	7608	1967	688	415	503	288
6480	8543	9128	8472	7951	2064	703	344	479	399
6415	8328	7751	7207	7055	1848	872	320	568	400
6447	7415	8696	6847	7159	1647	423	583	391	472
7447	8024	8983	9447	8096	2264	552	399	503	256
7143	9016	9615	8639	6647	2207	855	631	727	488
6128	8495	8103	10080	6880	1512	296	736	160	800
6880	8632	8495	7824	8687	1983	479	727	568	536

7287	8256	8968	9344	6023	1223	695	184	391	719
7175	8679	7928	7600	8719	1832	352	519	383	576
6072	8391	8536	8311	6168	1704	816	640	383	391
7615	7544	7759	8360	7551	1728	399	535	487	319
6488	8319	9311	7664	7311	1751	368	639	303	231
6239	7216	9679	8376	6271	1424	496	511	471	463
6712	8767	9039	7920	6735	1576	607	224	335	287
6840	7879	8208	7447	6280	1799	1439	624	312	527
7647	8679	10423	9287	7432	1607	440	344	504	680
5736	9655	8783	7176	6751	1600	743	384	664	640
7583	8327	7871	8408	7111	1599	879	447	432	295
7104	8408	11592	8495	6664	1720	207	279	663	559
6583	7527	8536	7863	6783	887	376	415	391	648
7912	9567	7735	8415	7615	1967	519	247	696	448
6640	8376	8551	9911	5304	1391	335	215	543	231
7016	8744	9311	8599	7015	2087	464	528	439	511
7720	8416	8015	6808	6032	1568	760	367	543	599
7744	7608	7719	6887	6351	1559	351	368	176	696
6840	8488	10143	8207	6327	1704	448	488	608	568
6400	7680	7456	8911	6175	1351	919	359	711	711
6703	8279	6551	7847	6711	1527	336	671	200	399
7408	7944	8759	8600	5895	1639	383	688	360	815

5944	7703	7472	7495	5607	1391	383	647	440	319
6720	9383	8559	9120	6663	1128	312	431	487	648
7399	8600	8328	9335	6463	1256	583	392	487	312
6343	10376	9784	8991	5863	1679	440	552	591	271
8151	8767	7919	7991	5639	1600	703	248	535	352
6343	8311	9255	7631	6712	1600	439	799	215	751
6656	7616	8352	7663	6415	928	487	368	351	424
6632	8879	8623	7767	6759	1416	584	176	703	295
7456	8303	8271	8568	5519	943	224	503	247	655
6576	7384	8447	8824	5432	1151	576	615	423	672
7224	9423	7911	8439	6784	911	424	639	447	256
6304	7071	8008	8064	6735	679	392	527	383	544
7760	8743	7247	7311	5775	1399	551	687	552	352
6624	7552	8152	9128	5543	1471	384	480	312	544
6527	9064	9047	7615	6631	1544	607	464	287	271
7383	7335	9311	8792	5447	1096	615	455	448	751
8191	8448	7935	9168	5312	687	472	647	336	399
7519	8688	8944	7783	5256	1175	792	368	455	304
7400	8503	7071	9055	6135	744	304	479	407	511
6904	9616	10359	7703	6503	1359	351	231	623	528
8104	8431	8359	8104	6064	1703	200	303	424	560
6367	9199	8560	7471	7096	1567	600	247	208	432

6071	8063	8976	7895	5791	1064	655	447	656	360
8143	8168	8767	8447	5936	1711	576	440	455	327
6712	8543	9424	7351	4880	1839	448	519	583	327
9736	8783	7352	7624	6295	1112	615	512	559	272
6848	9992	8975	8639	5583	863	759	408	327	496
7408	7007	7960	8631	5152	1072	1151	376	368	792
6647	8263	8104	7831	5896	959	504	480	183	415
8679	8015	9768	8504	5496	1016	632	344	744	711
7792	8592	8248	7639	5192	1511	416	1008	215	815
7167	7416	8904	10144	5159	624	367	504	487	536
6983	8232	9016	8847	6135	975	199	463	184	279
6992	8584	8504	8399	6559	1368	287	512	135	456
8311	8048	7727	8400	6519	1255	199	352	383	560
8264	7536	8264	6656	5248	720	784	535	648	423
6991	8920	7896	8271	4151	1192	687	608	583	576
7823	8703	7768	7831	4615	1400	375	703	183	559
7008	8752	8407	7656	4056	1408	472	248	319	399
8735	9432	8807	7967	4904	1144	408	391	488	535
7207	9231	9640	8695	5608	847	616	552	752	343
8783	8511	8479	9072	5279	1279	319	192	424	463
7384	8359	8071	9008	4671	1304	471	480	479	375
7040	7680	9295	7303	4776	1087	680	431	455	392

7559	9448	8416	8263	5071	1352	559	375	736	592
7664	8216	9431	7752	5151	1168	671	335	343	623
7496	7704	7888	8479	5800	1272	319	727	279	263
7288	8575	8752	9424	5248	1008	487	736	591	631
9687	8496	7704	8207	5024	775	327	375	592	664
6520	8440	9016	8760	5863	1592	319	487	231	920
7455	6967	7328	8560	4263	1079	576	375	207	328
7151	8543	7679	9112	5184	888	704	151	392	472
6991	8127	8320	9007	4912	1215	543	520	863	791
8927	8967	8655	8447	5000	1103	520	383	455	464
7856	8479	8399	8280	6016	1072	479	1063	280	320
10112	8279	7535	8688	3728	1184	400	423	440	520
7623	8528	9607	8320	4071	1176	367	463	400	328
6703	9944	9095	8543	5487	1063	679	440	352	263
7000	8655	7808	8327	3919	984	383	536	303	703
6231	7488	8431	9240	4080	880	296	152	655	311
8151	8039	7880	7144	5407	1368	760	160	312	656
7272	7424	8872	9528	3832	1135	632	536	407	344
7528	7968	7519	7424	3488	1144	759	528	536	472
7263	8936	9135	8400	4656	943	288	600	536	552
8360	8048	8615	10040	4935	896	375	263	208	375
8623	8776	8479	7239	4984	784	543	111	568	431

7055	7591	9912	10096	4752	807	680	191	360	296
8039	9352	7424	7655	4735	600	488	575	624	623
8711	8239	8807	9295	5279	816	320	416	375	176
9423	8208	8647	7455	4623	720	456	231	256	568
6704	10080	7311	8056	3551	456	287	288	279	536
7487	7943	9063	7679	4120	1056	671	399	264	624
6543	9431	9872	7824	4215	1440	560	344	367	208
6183	7431	8679	7568	3671	1111	512	624	423	304
7736	8808	8784	8039	6055	647	456	320	504	519
6599	8767	7672	8455	3976	639	607	255	312	664
7759	9752	7607	8336	4839	784	432	1039	384	248
7448	8615	9104	7903	4127	1015	375	383	407	375
8095	8343	8536	7703	3935	336	375	719	408	416
8047	8831	9735	7992	4183	1119	863	280	680	280
7616	8552	8487	8751	4096	983	199	343	632	384
7976	8167	8496	9039	4175	472	784	135	535	816
7056	7983	9575	10136	3799	679	496	303	584	464
7048	8240	9055	8112	4408	623	784	359	375	376
7487	9104	10759	7735	4031	1192	199	768	351	471
7015	8096	7623	9847	4743	503	456	535	439	368
7967	8552	8071	10120	3088	655	439	407	191	696
8808	10231	8623	8360	4600	807	480	447	439	424

7368	10039	10279	8655	4023	608	576	551	872	456
8840	9016	9216	9488	3896	656	296	504	448	504
8151	8839	8111	6999	3855	823	151	199	360	159
7815	7719	7791	9207	3623	1127	672	1063	463	520
7192	8488	7824	9656	5128	1576	551	695	480	208
6343	8072	9784	8647	3783	495	559	623	304	400
7464	8007	8424	9231	3127	680	104	311	247	607
7952	9567	6759	8199	4400	495	503	863	519	567
7215	8016	10720	9703	3399	840	183	431	391	488
8920	9296	8327	8096	4079	1015	784	568	575	535
8200	7535	7863	7840	3016	407	343	328	320	615
9056	8047	8343	8263	3504	464	487	271	560	360
7943	9079	8608	8424	3784	696	295	719	504	527
9000	8600	8751	8824	3663	655	287	287	111	815
7776	10695	8416	8376	2344	503	487	440	288	240
7768	9303	7760	8640	2696	591	232	336	327	639
9215	8239	7983	7512	3248	423	392	615	280	296
6815	8072	8992	9120	4167	1247	575	224	264	799
7871	8703	8976	7455	4224	463	456	512	592	200
8720	7631	9176	7903	3311	591	608	215	192	311
8583	8296	6552	8216	2871	959	199	271	383	816
9040	8688	7800	8887	2864	672	423	375	647	335

9072	8472	8015	10135	2456	728	528	455	343	255
6696	10000	8263	8471	2647	424	503	327	552	415
7096	7031	9232	8224	3736	360	175	456	704	375
6999	8071	7759	8551	3024	439	224	647	439	303
8280	9368	9175	9015	3103	400	584	472	559	303
9231	7312	8112	8687	3152	607	496	272	751	176
6608	8728	6856	8384	3095	592	415	335	279	399
7952	8344	7496	7704	2328	520	520	679	511	183
7655	10423	7672	8271	3455	240	303	215	400	88
8264	9855	8327	8727	2031	431	384	447	1151	432
9071	8887	7592	6976	3072	928	647	448	576	223
6288	8447	9440	9735	3247	728	280	303	456	415
8623	8999	7688	9304	3240	1192	519	296	591	536
8392	8328	9472	8231	2831	352	480	239	704	184
8199	8223	7624	8975	3343	472	432	544	320	920
6360	7855	8503	8207	2959	575	279	479	239	440
7287	8208	10271	7607	2839	879	272	359	463	711
7919	8160	7695	7552	2864	760	568	279	455	487
8136	8184	8367	9384	3135	375	639	568	575	271

Table F-3: First 2000 Raw ADC values for 125 ng/ml Concentration of Lp-PLA2

First 2000 Raw ADC values for 175 ng/ml Concentration of Lp-PLA2									
663	528	623	680	360	703	272	663	336	528
775	320	543	647	376	479	319	607	448	264
352	519	527	767	511	167	295	416	359	223
479	320	576	335	512	303	264	360	352	599
768	199	856	464	592	432	519	495	216	392
207	952	568	591	544	639	447	367	384	791
847	408	232	239	520	519	439	423	784	271
287	600	280	487	543	376	176	687	391	456
800	487	359	487	424	416	176	104	552	376
848	743	328	855	352	303	247	640	592	416
928	391	127	527	583	216	264	967	295	399
480	375	584	480	472	487	71	727	232	367
440	544	415	504	535	832	807	279	287	160
1024	552	543	280	519	527	384	407	720	384
472	312	504	567	752	375	191	216	480	255
712	463	239	703	615	679	512	464	439	376
711	432	415	295	495	543	256	743	432	376
592	471	695	583	639	528	823	311	456	519
1104	464	791	504	392	503	504	336	400	671
975	647	648	224	720	456	792	367	800	327
768	567	440	688	407	455	504	488	823	296

679	719	408	528	399	464	376	512	168	471
495	560	375	559	303	399	280	552	408	600
872	535	751	704	623	655	439	480	431	231
528	735	567	239	495	567	272	352	975	663
760	503	456	192	783	496	440	208	712	135
520	567	679	503	943	232	359	336	224	423
967	527	504	664	424	600	512	1216	751	304
559	144	568	280	703	888	336	368	399	664
791	296	639	240	488	264	504	743	472	391
519	728	648	279	679	239	568	400	608	615
375	335	679	447	400	336	863	375	535	647
704	615	423	440	560	367	120	432	351	815
1048	248	503	503	640	575	399	280	295	304
536	768	319	271	232	535	264	776	255	320
536	360	688	415	528	360	559	904	327	327
463	336	359	248	463	415	615	536	368	216
583	423	240	191	295	831	567	327	775	647
928	568	679	264	471	488	312	535	384	623
200	552	287	559	439	655	463	776	688	791
775	447	615	872	704	512	656	664	616	720
447	608	384	327	232	312	351	528	599	320
712	567	592	472	399	376	416	711	423	392

512	584	287	607	280	783	487	231	520	327
848	303	600	248	256	335	519	736	287	415
415	488	583	432	768	200	511	231	688	823
776	255	687	455	383	640	520	152	455	559
407	728	352	431	408	632	719	399	839	648
368	216	744	424	456	576	528	775	303	687
599	599	543	367	239	1184	296	535	615	479
511	527	496	399	648	375	416	496	344	536
879	415	487	216	239	247	248	495	640	623
1208	424	231	416	495	455	623	831	247	384
448	527	359	224	432	175	375	624	176	496
608	576	376	503	312	591	312	159	447	175
408	399	192	415	367	344	471	279	672	271
447	208	815	480	519	176	815	359	759	384
567	559	536	631	480	616	407	647	544	352
719	359	527	311	232	375	208	368	296	463
1144	823	104	800	455	647	327	488	303	407
655	416	360	511	424	80	488	423	248	416
599	327	896	384	480	136	600	264	464	271
280	719	383	392	192	368	376	567	279	343
359	263	439	351	296	408	456	392	344	751
415	439	495	383	351	576	495	600	327	367

656	631	975	512	488	663	375	456	432	376
311	704	432	407	504	392	272	360	696	583
535	759	424	472	631	495	376	311	439	431
631	744	423	631	360	423	559	136	319	520
471	520	392	816	311	696	496	736	223	1071
471	567	319	472	584	391	400	407	432	367
719	615	327	848	472	559	568	551	447	407
847	591	456	279	615	232	551	847	248	351
759	471	287	920	344	312	807	335	263	504
304	503	472	879	519	856	351	559	759	256
687	159	320	1055	536	359	600	319	264	607
343	800	575	344	432	559	359	463	471	520
791	536	607	311	200	288	287	319	264	287
519	703	184	503	776	432	375	527	311	136
615	272	247	224	760	343	336	624	560	224
1000	624	223	552	312	264	359	287	472	400
711	439	559	456	232	536	592	536	672	431
384	479	648	359	439	1055	728	215	208	992
591	448	359	896	663	431	399	343	559	552
463	632	471	432	576	1191	575	463	415	303
376	775	823	704	288	183	839	256	543	511
559	487	920	712	559	352	231	519	487	720

632	448	616	687	312	295	584	543	383	655
568	352	472	439	488	736	551	615	463	439
528	368	720	536	607	327	256	672	335	319
600	575	328	559	815	407	375	287	280	376
880	304	823	336	224	512	488	543	352	207
888	408	575	415	727	400	408	448	295	279
720	479	207	448	264	224	335	455	375	320
439	535	359	223	527	239	768	768	607	183
768	800	439	679	591	824	416	384	432	392
808	800	655	527	375	343	480	624	767	848
792	567	224	296	1015	656	455	895	488	615
560	816	695	271	480	672	415	295	472	776
344	616	280	679	352	639	671	448	352	792
703	264	391	328	447	400	359	559	479	632
519	399	376	656	496	344	447	448	496	176
472	632	640	352	351	584	264	184	295	671
344	503	319	695	376	432	735	335	672	247
655	528	359	456	575	807	279	368	543	247
872	375	535	744	536	440	528	263	319	496
336	1319	952	464	640	535	808	543	360	591
263	632	568	344	344	296	599	360	623	247
327	719	200	528	695	384	399	487	816	887

399	639	615	376	496	479	512	240	112	351
784	744	199	567	359	384	351	831	447	639
559	727	399	368	391	344	456	311	520	616
343	527	448	560	143	272	216	167	775	312
855	735	487	416	592	160	455	607	599	752
559	375	248	312	551	431	359	439	472	495
199	800	480	376	343	407	800	736	607	528
527	399	407	607	248	327	488	480	336	279
407	559	687	407	512	544	471	312	431	335
208	527	271	512	543	520	160	391	416	400
375	343	512	343	503	775	512	679	303	583
479	583	607	496	424	487	415	447	592	359
383	304	775	968	503	671	367	399	519	664
752	440	280	175	383	672	360	544	815	416
968	504	679	415	775	544	480	296	464	431
640	479	623	567	471	343	904	656	616	408
367	287	559	679	296	487	952	184	600	504
616	287	319	711	519	376	439	503	623	368
191	352	952	592	520	527	304	311	303	512
1223	880	607	1056	775	480	303	376	192	448
328	168	248	503	463	359	192	471	255	471
479	271	264	600	535	263	1071	584	335	231

543	599	391	304	535	679	200	576	183	344
352	440	1007	215	519	376	591	279	464	351
576	367	152	440	199	464	280	471	479	879
416	407	95	800	447	360	264	455	319	607
311	303	344	479	831	191	480	431	376	751
607	391	519	536	352	391	576	968	656	839
639	552	559	447	415	223	415	712	399	375
344	184	584	400	295	528	328	391	343	527
663	304	423	400	263	383	576	391	623	1111
511	424	471	496	616	280	327	343	384	351
504	303	359	688	552	176	567	359	664	431
320	431	591	303	448	311	440	624	583	799
295	879	320	280	552	751	599	527	423	648
1007	399	936	439	287	352	287	920	351	616
584	1088	416	663	224	568	559	432	224	584
152	767	343	567	407	687	416	391	391	392
624	327	527	903	959	199	519	111	648	559
496	575	423	1000	407	168	1016	456	503	608
807	319	1015	264	199	408	488	616	407	383
719	967	512	527	151	423	752	536	623	447
799	319	416	200	447	560	759	343	159	823
359	335	367	303	272	664	351	648	823	984

720	568	455	416	432	559	288	680	599	271
800	424	640	447	511	304	167	464	816	744
503	775	127	432	647	776	295	463	439	255
1039	736	463	256	151	200	487	448	407	504
215	807	440	264	392	752	479	743	568	296
640	327	288	471	552	552	287	447	480	631
888	535	559	191	223	448	295	600	472	256
328	464	287	800	264	463	447	327	344	391
159	303	400	431	559	448	239	584	447	384
759	416	535	1064	503	335	359	512	480	431
808	568	496	207	207	591	840	352	335	487
560	512	455	880	464	568	616	447	680	591
871	560	272	232	751	559	551	400	295	583
336	320	215	520	440	1392	615	536	663	799
623	247	216	399	575	423	583	311	559	359
864	432	392	416	551	759	576	223	728	775
496	704	615	192	304	336	400	568	311	672
336	792	535	591	391	328	415	495	264	656
704	184	543	239	416	855	935	704	391	519
103	687	136	760	304	624	639	720	320	752
448	544	335	375	343	632	696	528	200	504
520	312	512	551	471	416	736	207	472	352

407	456	647	575	335	360	607	415	455	968
567	1295	471	184	368	751	519	768	1055	360
671	816	448	511	456	407	567	592	431	240
559	463	360	703	495	448	335	295	480	304
368	664	271	400	368	216	703	647	167	336
335	304	591	479	615	879	735	735	640	248
999	663	408	535	176	336	400	792	479	215
568	232	448	951	327	632	599	351	303	568
367	703	608	432	967	623	680	392	87	544
807	447	432	368	544	512	823	535	240	304
863	456	359	551	304	768	640	575	503	559
511	527	760	320	384	576	592	207	415	375
336	399	336	296	823	471	495	752	632	520
407	432	447	1096	280	456	375	615	367	312
383	504	616	336	464	599	591	479	320	408
488	472	367	711	431	615	423	575	439	576
639	496	663	944	632	536	327	168	336	464
1184	415	303	583	303	639	295	192	608	280
511	503	304	544	560	592	664	503	383	512
1032	591	568	368	583	655	832	584	655	135
775	424	104	391	391	352	512	615	311	215
512	528	663	655	400	640	359	215	808	336

575	240	495	592	503	511	216	335	800	448
760	392	479	376	255	375	487	255	256	719
415	264	375	463	640	759	600	376	527	559

Table F-4: First 2000 Raw ADC values for 175 ng/ml Concentration of Lp-PLA2

First 2000 Raw ADC values for 225 ng/ml Concentration of Lp-PLA2									
832	1519	791	1536	1488	664	967	887	624	1087
2255	800	903	1416	735	1344	1471	375	607	1904
1759	928	1256	568	680	1384	768	399	215	1576
975	1207	1063	615	1167	927	1047	399	512	2512
824	1191	1575	1216	1568	1279	1007	287	608	1255
1304	1423	1103	1359	1303	1136	695	599	304	2119
920	1303	1335	1152	840	1424	880	832	472	2512
1271	1343	1431	1080	639	959	2007	335	487	1903
1744	1759	927	1200	1648	1855	1056	247	919	2015
1567	1232	984	936	1495	1080	695	568	672	1120
1064	1264	1087	504	1192	1712	1247	327	735	1744
1216	1111	991	1471	743	712	871	280	480	1824
1240	1288	1871	919	1200	823	1064	415	575	2335
1288	1592	1087	1319	1584	967	1128	512	519	2191
1199	1127	1240	743	1592	792	1248	608	592	1976

871	1488	935	880	1327	1007	967	191	712	1799
1167	815	983	1208	920	536	736	520	543	1440
608	919	1935	1440	1159	576	703	719	416	2551
1544	1296	1095	1039	1344	1055	511	263	648	2063
687	904	1128	1488	1288	1136	632	560	551	1679
1120	992	1024	847	688	1263	840	640	776	2215
1104	1031	1200	695	936	831	1159	96	384	1911
1120	1072	1496	871	863	992	1047	215	304	1848
1296	1128	968	1448	1127	720	1119	456	351	2319
1088	1112	1367	888	968	952	840	591	824	1872
1544	1087	1408	1495	1072	1624	880	407	567	1511
1232	1472	952	1216	1208	1167	679	720	839	2232
791	1223	1111	952	648	1255	776	295	592	1599
672	663	984	791	1495	1016	1176	399	608	2080
728	1391	1023	928	1464	1712	591	255	855	1647
1327	1152	1456	991	783	768	1367	920	400	1759
1071	767	1240	1423	1192	1184	624	440	807	2144
991	1135	1080	1255	951	1120	936	407	815	1983
1231	1215	1272	1592	1559	1191	1159	328	663	2232
952	1160	776	663	840	1351	1408	560	391	2800
664	608	1888	1008	1103	871	775	967	591	2231
1231	1007	504	1072	680	992	1160	711	320	1975

1391	1071	1184	1599	1200	863	1040	376	327	2200
1559	1527	799	1143	912	959	752	696	400	2087
1168	1344	1111	471	1071	968	1559	463	391	2303
1119	1096	1263	1032	1080	544	1207	272	584	1792
1255	1072	1552	1216	1880	1432	976	544	536	2327
1199	1464	1096	1184	1255	856	1143	495	719	2024
1816	1288	615	1312	1415	1480	823	232	703	3088
1192	927	816	1039	1080	968	1023	360	808	2432
1312	1216	1247	1320	1272	615	527	951	407	2056
1432	1128	1343	840	1439	943	1144	679	792	2663
1216	1080	919	864	1024	1360	1311	799	496	2303
1015	1168	1471	1200	927	1039	975	352	743	2927
1175	1311	1391	1463	839	815	1088	616	415	1967
1520	1503	1231	1240	831	1240	1104	760	536	2688
1720	863	952	1623	1336	1320	1320	792	520	2240
1336	1240	1136	1023	1119	1840	903	463	335	2680
1023	1295	1680	831	1055	1288	1455	439	424	1991
975	1128	1127	1520	959	1071	1032	552	640	2935
1448	1519	1311	1183	624	815	1104	168	392	2279
800	1039	1112	1031	999	871	943	271	504	1296
976	1008	1423	1271	895	1167	688	208	431	2135
759	1400	927	1536	983	1168	928	463	423	1415

1031	1136	960	1551	1144	1063	808	655	320	2575
1159	1327	1320	1287	1311	1335	1072	552	368	2240
1008	1456	1856	1464	1056	1327	975	343	488	2135
1360	1295	551	807	975	855	776	184	391	2839
991	599	1368	1687	1295	760	1439	695	743	1887
1136	1239	1399	1232	1087	991	719	456	712	2920
887	1231	768	919	767	1888	904	527	967	2272
1079	847	1056	1263	792	1112	1656	487	567	2383
1127	1375	1424	967	1247	888	560	399	512	2096
1343	1360	1127	999	935	1208	968	496	448	2640
1495	1167	1448	1080	1104	1272	1647	176	351	2831
1175	1175	687	1264	1263	903	1312	288	1024	2424
1311	816	1103	639	663	984	1079	407	799	2768
887	1247	976	1047	1048	743	855	456	440	2527
927	1432	1384	880	1183	655	1296	519	776	3544
1487	1112	1367	847	1176	592	992	432	871	3119
967	1008	1048	1024	1295	1007	888	1040	480	2592
872	1951	1535	1591	1087	1359	1119	279	559	2767
1439	960	1000	696	1023	1167	800	688	967	2496
1495	983	887	831	1032	848	847	448	744	2663
1095	1015	1215	1104	920	807	1559	735	592	2791
935	1191	759	1208	991	1232	1200	463	487	2903

1559	1855	1111	1056	784	759	1040	399	1007	2632
1559	1455	1184	1103	855	960	1303	319	536	2519
1320	912	1119	1143	887	1248	1231	407	1359	2743
1431	927	1335	1039	1159	680	1039	375	656	3184
775	1360	1600	823	1232	648	1216	327	536	2440
1391	1311	1007	855	1263	1095	1167	519	368	2335
1160	1760	1512	1192	807	608	735	344	752	3384
944	1336	1095	983	504	1015	1096	432	695	3135
1640	1192	888	1328	656	711	1208	527	1080	3935
1111	1207	1272	864	743	1079	1088	671	736	2495
1015	1144	1376	879	1208	1319	888	615	1248	2335
1568	1231	1200	1120	703	1119	976	504	487	2208
1447	2624	632	888	983	1295	1248	640	655	2552
1232	952	1072	783	783	1144	1136	399	856	3655
1320	1248	680	1207	928	1384	1344	623	735	2584
872	1272	1376	719	864	1031	1087	431	903	2127
1176	1248	1120	671	735	1376	1231	416	832	3207
1255	904	1199	1247	1080	896	1279	399	496	3168
1656	1504	719	1063	991	1136	799	247	840	2840
1487	1287	1584	1032	1440	1095	1624	568	944	1967
1671	960	591	855	711	1143	935	407	616	2736
2192	1192	984	648	1056	1064	1200	647	487	2663

560	1103	968	711	952	551	623	624	895	2127
928	1536	1320	1024	647	864	784	319	559	3471
832	1256	1247	1127	711	1079	1152	375	895	2432
1095	1296	2183	1159	1136	1600	727	943	1407	3584
1119	895	791	1807	1151	608	1376	608	727	2616
1351	664	1223	855	880	1103	1144	615	863	3016
1671	1360	1112	759	720	1184	1016	664	912	2887
1207	863	911	1464	1399	1640	936	375	952	3336
928	1072	719	1064	1240	1584	1119	440	568	2743
1055	1288	1471	1032	1215	1208	1368	640	760	2824
1047	983	1103	984	1119	1087	1111	264	735	3280
1416	872	1120	799	1079	799	1152	160	1008	2928
952	887	1104	1447	944	1024	584	343	1032	2847
935	1232	1087	727	991	943	935	583	608	3008
927	656	1064	1431	1368	648	1272	368	1960	3871
687	1128	727	872	831	1048	911	327	1064	2823
1879	975	880	1319	1247	920	559	488	959	3528
1200	856	879	1159	1071	1168	1248	376	992	3416
1687	735	1319	1007	1215	1327	640	575	1231	2912
1136	1152	1071	1247	912	1223	623	576	983	2623
624	1047	1079	1943	791	1128	1047	280	639	3287
1199	1479	1407	1055	679	1504	879	352	759	3615

1983	1263	863	1392	1023	831	415	480	1039	2791
1015	1088	1631	592	1032	1096	872	1007	1080	3447
1591	1119	1591	999	1175	960	1311	248	695	3559
952	1112	960	1391	775	616	1056	327	1328	3559
1287	767	1775	2055	903	695	1447	328	1048	3271
944	1064	1240	912	1119	864	1007	544	696	2615
855	895	1256	1095	1263	1143	1296	543	984	2720
984	967	1471	592	1087	1103	1175	432	1223	3304
1176	1008	959	904	1808	784	847	392	999	2648
904	1135	639	544	1360	1024	1167	440	719	3672
1664	1503	1392	1223	1647	1087	896	232	1040	2455
1183	1375	879	799	1216	903	1175	351	1560	3311
968	1031	952	1448	1152	1439	1048	696	1264	2751
1087	1343	1199	1487	1128	1032	1672	623	944	3208
751	1248	1176	623	807	1223	1144	416	615	3496
1623	1167	1463	1127	1215	1472	1264	696	1000	3008
1464	1143	1095	1303	815	1272	712	552	1207	3535
1664	608	1055	928	728	1320	1159	464	1512	3136
847	967	1815	1128	976	928	768	295	1032	3192
687	824	624	1359	1328	832	1352	336	1200	2807
1288	1175	1175	1151	1176	1328	448	535	864	2848
1407	880	664	1287	1192	1312	695	431	1040	3663

1063	823	808	967	1432	1232	560	495	1471	3943
1471	768	1200	1383	1191	919	1231	487	1072	3616
1312	1447	1039	1368	1295	1128	1127	815	960	2808
1640	1095	1016	896	879	535	600	1031	1208	3479
704	1775	759	1063	1143	680	560	591	824	3527
1504	951	824	1160	1183	1047	912	328	1400	3055
824	1495	1263	1544	1152	1096	1464	448	1407	3023
912	1055	903	1103	775	1528	1159	543	824	3656
1231	1368	1223	864	1256	1352	1295	367	1056	4216
951	1215	1264	1087	1032	864	1256	696	1232	3023
1112	1255	1679	928	712	1080	1128	488	1184	3679
1031	928	1392	952	1008	775	911	783	671	3047
1591	1183	664	999	1071	1232	1024	727	1184	3527
960	688	951	712	1320	1519	1064	847	727	2767
1671	927	1263	1088	1567	904	1895	512	1183	4319
1255	1728	951	1712	1176	1528	815	327	1159	4023
911	1352	1352	1279	1528	1447	1384	344	1584	2519
1336	991	1063	1008	1055	1112	984	400	1575	3447
1448	1384	1440	568	983	1263	815	431	1367	2983
1176	1415	1328	1168	1151	1039	784	624	1575	2968
1480	984	1752	1183	1023	1407	823	832	1408	2904
1256	1231	1416	1079	1528	656	1040	344	1208	4528

928	1216	1143	1175	911	871	648	384	1144	3776
1192	999	711	895	1287	1064	1656	528	1688	2920
1183	1184	864	784	792	1168	952	207	1623	3911
1711	1304	1079	1271	1216	1223	1152	807	1120	3320
888	608	1031	1416	1056	984	1295	544	1136	3695
728	800	1295	952	1015	928	864	376	1535	2800
1135	1304	960	1207	591	872	768	600	1464	3223
880	1143	848	1688	1535	743	1135	912	1983	3816
1375	1151	1440	872	936	1287	880	567	1431	2768
1136	999	1007	2215	936	1143	704	712	1184	2552
1384	1103	951	1168	872	1328	447	600	1279	4440
1063	1136	1111	1535	912	968	688	303	1271	3840
984	1568	824	831	591	1199	839	471	1239	3400
1295	1720	1367	928	1407	847	512	608	2000	3712
1368	824	1431	583	1127	1167	888	368	1791	4063
1511	1248	1528	1039	1032	879	728	432	1591	4071
1168	943	1391	1296	904	927	656	319	1384	3239
1095	1640	1376	1104	1183	1264	448	368	1336	3647
871	1472	736	920	1535	904	367	760	975	4496
1024	1344	1527	1103	1095	1447	583	568	1600	3559
1376	1039	631	1751	935	672	559	615	1871	4287
1168	872	808	799	919	896	439	136	1439	3887

1583	1952	1287	1152	1112	487	951	720	2175	3336
639	1527	1184	984	616	975	359	792	1456	3352
1271	1144	1663	1032	1088	983	343	656	1032	3791
1127	1143	735	991	1152	736	487	320	1776	4464
1152	1119	1216	1655	863	1072	879	536	1288	3456
968	1503	735	1071	999	752	712	871	1807	3207
799	703	1040	920	1055	767	696	879	1271	2911
1495	1519	1064	1032	1256	959	455	544	928	3911
1655	1127	1135	1000	919	759	592	536	1976	3951

Table F-5: First 2000 Raw ADC values for 225 ng/ml Concentration of Lp-PLA2

First 2000 Raw ADC values for 250 ng/ml Concentration of Lp-PLA2									
351	776	783	759	688	704	584	320	760	760
687	1120	631	303	760	1040	647	447	527	312
800	768	671	399	311	432	687	520	1167	680
711	863	447	752	695	767	488	463	799	807
648	719	640	600	567	623	839	471	1120	791
695	864	296	551	695	776	783	887	551	536
951	727	463	527	568	904	423	551	943	832
679	503	823	464	391	584	280	1367	751	600
631	424	1008	792	1007	863	879	527	703	463

856	551	312	968	559	887	895	495	920	680
664	559	631	496	903	639	408	511	895	1127
392	631	728	976	688	464	599	519	767	527
760	904	423	216	568	431	559	1080	695	424
639	431	631	432	496	575	375	479	775	728
664	920	687	815	536	992	464	703	400	792
360	592	752	1480	480	679	855	1072	327	728
543	232	664	368	600	712	568	848	527	904
775	1048	711	295	663	487	856	431	616	751
847	703	487	1143	768	816	935	544	631	607
575	768	568	648	1144	623	599	807	687	696
528	664	688	672	520	783	848	896	808	575
904	440	624	768	552	832	568	391	519	559
679	720	632	799	608	991	471	1096	616	535
336	392	392	423	752	704	631	967	968	527
520	711	463	959	480	871	416	456	447	799
367	743	368	1039	471	383	407	359	712	896
792	511	807	704	536	799	703	1047	495	503
327	743	743	416	671	720	543	1032	400	720
879	895	759	455	792	576	471	672	695	512
503	896	768	680	559	703	639	312	767	1296
815	471	736	440	960	1064	496	503	575	967

447	936	759	1376	439	368	808	711	568	776
647	775	368	936	744	791	599	800	823	1023
624	943	568	528	664	504	431	800	648	392
608	735	456	679	1007	423	927	720	407	488
328	903	696	528	743	840	583	543	832	415
871	712	688	703	664	632	816	935	752	1143
711	568	671	840	567	464	671	704	1023	399
551	735	616	1007	584	496	408	760	584	408
664	447	711	792	608	503	824	632	263	584
663	1023	823	600	823	936	463	1104	1000	447
399	743	511	751	647	479	343	760	744	752
336	711	288	607	335	759	327	799	536	984
704	552	632	927	568	607	1128	480	936	384
711	863	1023	656	719	863	511	704	671	1000
624	439	512	1039	1176	535	760	615	423	928
439	503	744	600	623	792	839	832	519	872
647	887	455	568	639	775	808	592	392	544
608	464	815	863	815	776	760	839	783	656
1280	1056	487	439	368	1312	672	816	799	391
607	1023	1175	696	424	960	495	807	559	359
559	816	815	776	1112	495	855	655	624	696
847	535	784	496	504	824	575	567	528	567

543	839	720	392	1135	655	440	519	551	512
599	791	727	464	847	519	695	864	800	575
623	799	400	832	959	736	991	431	272	640
424	1128	760	743	672	775	815	631	448	608
375	1047	880	512	744	895	1000	751	639	799
808	599	751	727	415	696	527	807	695	447
1200	392	759	536	807	672	672	1024	983	567
727	231	608	568	583	768	1143	912	1304	1064
688	927	800	504	776	584	567	344	856	567
192	840	448	735	856	663	647	552	552	888
863	1160	471	480	696	768	687	911	575	767
815	767	327	760	623	648	647	1047	664	712
608	775	855	312	407	903	680	768	624	1104
680	479	695	687	583	880	703	623	687	624
591	679	1015	608	648	471	752	807	1040	760
680	735	727	416	816	895	551	559	456	376
487	839	847	400	328	704	479	375	800	840
488	552	679	688	568	735	552	640	303	832
527	680	495	583	911	496	704	623	504	376
648	728	527	624	455	880	543	887	440	528
1104	751	975	584	880	399	680	319	895	664
519	551	768	815	423	912	456	351	712	647

863	311	823	999	351	528	735	392	463	568
1079	440	791	736	575	448	1032	480	511	632
424	920	624	1191	736	479	311	631	728	535
671	608	392	1120	799	680	743	416	1183	968
536	584	535	775	671	1279	687	655	903	567
464	488	359	632	592	487	432	512	855	296
1039	584	544	704	392	551	864	1023	360	816
567	648	208	616	552	248	775	600	759	448
848	1071	648	568	656	903	775	919	895	368
567	744	496	671	1016	647	871	640	663	407
272	1024	584	335	832	967	624	639	480	767
896	479	576	856	743	639	776	584	800	856
991	856	544	608	599	759	767	655	808	431
495	1055	784	584	831	639	560	959	640	920
591	343	528	784	279	303	424	871	464	632
568	824	448	320	792	1016	479	624	671	767
608	535	504	816	464	503	543	575	719	400
616	839	888	951	727	736	807	560	696	463
1312	519	712	743	959	392	455	680	759	640
1256	615	559	1047	455	1128	551	719	856	624
415	480	751	1047	831	999	519	536	720	639
416	744	536	679	311	1127	856	520	560	695

664	736	951	384	559	791	1160	856	456	623
1128	695	504	367	471	792	431	704	807	968
823	631	568	408	1040	655	656	1064	615	568
1040	831	600	439	464	783	583	680	751	927
1063	1039	376	439	743	703	567	512	479	600
695	952	1103	551	744	855	824	464	423	671
696	360	575	424	832	552	848	455	591	1112
999	519	592	615	1072	808	512	599	424	767
416	440	1416	623	823	360	511	367	712	639
760	647	575	815	559	799	703	911	976	672
736	279	600	727	560	1144	487	391	864	567
920	840	1159	408	895	608	527	344	336	920
520	608	703	616	456	800	824	687	464	503
712	816	727	615	743	207	247	544	495	655
935	696	600	728	543	495	615	1080	671	488
584	664	352	1039	863	743	536	792	727	607
423	655	1352	720	479	816	904	527	432	888
623	703	432	855	575	639	1176	551	559	1264
1032	1039	623	584	712	359	560	840	631	1079
543	848	583	488	1183	511	528	935	720	856
319	672	616	464	1127	1480	599	744	615	719
720	616	560	647	495	712	1287	839	423	711

431	799	279	543	551	751	215	776	688	416
720	928	727	247	967	680	664	488	615	511
720	719	584	664	823	872	952	1248	840	1135
1280	455	607	535	1135	735	712	607	447	432
432	752	983	775	391	1215	655	752	976	560
488	960	736	512	544	895	663	776	567	1007
1208	1232	688	567	407	336	592	575	536	528
440	743	319	527	1000	560	303	639	800	239
808	607	351	576	496	600	543	639	919	775
376	295	423	1080	1176	448	896	256	1271	1032
495	504	1119	823	472	600	696	536	167	359
792	527	351	479	680	551	560	599	536	479
495	264	759	607	704	727	783	416	671	608
760	647	647	631	655	575	480	624	544	440
592	712	424	352	1111	503	535	663	968	424
392	568	608	576	407	503	911	479	775	968
696	839	1000	751	463	784	583	632	751	871
272	839	384	615	592	1056	327	592	527	528
936	984	560	599	464	471	680	367	416	1024
815	471	447	583	504	655	711	464	567	184
487	399	664	607	751	1079	935	560	792	248
575	519	943	679	952	520	1056	751	455	536

767	623	719	663	808	304	704	608	751	167
592	704	792	520	703	575	551	599	375	768
568	448	704	544	463	639	520	520	647	983
303	264	871	400	568	744	471	503	559	872
528	295	359	375	447	664	751	1008	727	568
864	975	583	904	335	655	455	968	783	1328
480	1200	552	1031	496	592	487	1079	887	359
527	671	352	687	568	487	1023	464	839	504
688	743	1064	848	711	432	1112	584	783	624
287	647	359	383	1047	736	743	1087	575	624
680	423	560	712	1439	768	944	368	503	368
680	936	351	671	520	631	479	640	1743	495
448	576	880	480	496	648	808	808	831	600
231	512	624	696	352	767	712	583	551	712
1144	776	384	752	535	1127	575	704	760	928
991	512	951	528	536	616	495	1024	192	768
607	856	519	671	647	792	1400	535	687	696
744	1496	760	487	655	775	671	831	272	799
655	983	575	520	824	903	544	511	504	943
303	799	768	791	1064	456	319	903	800	800
743	192	471	456	535	823	447	1376	680	808
1271	752	848	888	240	480	776	248	599	967

567	655	760	648	616	655	495	904	791	895
560	511	663	664	664	960	560	879	1056	840
791	775	543	888	439	823	711	735	647	608
680	991	272	872	352	383	560	320	631	1671
1031	752	895	535	399	536	944	784	527	824
776	984	495	416	568	1007	1072	776	1103	711
768	776	543	536	480	479	728	608	1376	1072
736	432	264	576	672	928	487	528	759	767
183	287	768	639	895	680	663	1055	952	816
440	327	528	415	263	623	584	751	687	816
1072	504	392	1360	304	624	1008	439	584	736
871	920	968	447	815	864	712	639	832	1087
479	655	544	647	487	487	559	1111	607	423
648	360	488	791	1167	896	927	888	784	599
872	608	456	704	551	911	488	840	592	439
488	632	376	679	520	536	320	1095	799	648
583	560	1015	752	488	439	407	528	447	800
975	767	240	728	576	527	879	536	464	928
743	1064	536	607	584	439	416	575	1120	480
544	743	736	895	552	543	487	679	800	688
391	360	671	624	663	392	383	648	663	824
767	479	424	576	319	599	527	999	551	176

399	895	855	816	464	927	503	895	583	568
543	632	576	583	784	632	679	592	615	1000
600	207	680	896	695	927	792	680	496	615
959	471	720	432	591	344	759	456	1048	487
999	544	511	415	911	303	487	599	303	1055
1127	272	479	992	311	288	543	1063	544	487
616	1151	304	615	728	504	776	400	680	335
960	407	632	855	568	392	1231	727	1008	720
1056	704	360	760	575	1104	488	687	704	423
488	263	648	887	624	912	664	704	608	471
423	680	1023	903	727	520	343	591	775	583
999	512	799	432	408	647	536	376	879	704
783	783	632	272	424	687	471	375	664	263
664	624	575	1055	472	935	600	528	623	784
327	664	479	351	951	951	616	983	287	512

Table F-6: First 2000 Raw ADC values for 250 ng/ml Concentration of Lp-PLA2

First 2000 Raw ADC values for 300 ng/ml Concentration of Lp-PLA2									
551	936	720	631	495	752	680	560	439	455
999	600	552	807	520	519	512	631	408	591
367	463	856	376	903	311	1015	456	391	952

256	848	376	583	943	631	408	688	816	567
703	463	656	871	888	727	343	423	600	264
631	1080	792	608	535	287	576	567	527	552
239	791	752	664	800	672	568	784	759	432
1008	832	384	776	352	727	816	599	487	424
287	512	696	712	543	792	632	792	680	488
679	640	592	688	975	528	840	216	376	656
367	799	639	656	464	495	671	608	904	560
575	504	424	415	864	680	744	599	231	480
887	943	1071	759	591	303	559	696	959	544
448	543	1175	808	952	392	823	503	759	504
512	887	560	535	775	647	423	728	527	448
544	592	656	248	815	880	1016	872	703	415
320	800	663	408	439	535	408	552	719	344
552	712	711	631	791	847	704	320	647	392
415	536	679	864	920	640	503	456	416	1095
703	808	647	879	256	776	543	479	1047	736
1048	415	759	487	407	496	1143	560	903	416
615	432	720	568	535	728	464	855	864	695
528	640	631	767	840	583	463	576	711	575
648	776	432	615	984	607	632	752	528	599
543	880	471	1031	1535	679	335	599	567	615

727	280	639	552	872	567	879	327	736	968
1031	751	911	567	848	559	863	871	1280	712
712	488	440	1064	648	487	696	911	696	568
463	303	496	1120	615	776	568	624	1279	639
808	759	640	567	528	360	368	527	463	480
968	296	455	1023	487	1040	936	672	743	1303
663	607	512	415	656	743	631	664	623	576
552	264	464	392	480	647	535	663	559	1112
568	895	743	352	384	487	519	536	696	543
496	552	871	927	680	383	632	607	1152	575
632	247	815	648	559	712	303	520	727	624
528	439	536	999	536	703	583	719	640	896
687	496	704	672	719	415	871	848	624	1063
495	856	863	759	991	743	823	944	640	816
511	384	640	720	736	591	679	512	928	527
904	959	400	616	623	383	808	416	639	648
528	319	440	704	824	368	376	408	600	632
743	863	615	832	775	575	343	728	648	591
768	728	711	336	647	768	559	648	1408	231
512	1095	511	607	760	584	768	536	607	400
783	440	607	432	631	399	456	888	680	464
752	415	543	703	271	608	392	736	552	352

927	448	447	648	680	599	400	480	776	688
1055	640	735	695	759	568	1047	1248	704	559
1527	1191	496	759	759	1039	599	736	840	767
496	384	551	487	319	776	655	639	591	880
1031	496	479	624	535	424	1015	495	775	1016
535	488	823	527	360	671	399	543	719	463
1095	743	335	496	608	1280	719	624	863	696
959	583	567	584	544	1408	784	592	776	767
760	631	1056	711	928	520	1303	503	1159	903
671	200	800	791	799	879	895	879	672	400
551	880	543	903	1151	415	567	671	831	767
664	679	552	831	775	360	863	783	631	912
832	607	695	696	679	600	752	720	583	775
856	464	359	855	967	831	135	391	280	335
656	456	472	552	448	703	391	727	616	591
760	439	735	1039	503	423	807	575	352	736
1184	503	552	663	560	560	1024	664	727	840
567	624	847	416	704	1184	536	1056	303	800
511	640	831	463	687	664	855	775	855	631
775	527	527	608	328	680	1175	776	512	359
1600	464	495	616	632	1087	552	752	791	367
695	471	711	592	599	671	600	495	351	319

552	608	519	608	743	496	520	360	655	663
536	767	864	920	287	711	368	864	847	576
935	552	863	600	848	704	576	544	488	799
872	479	592	1104	383	759	1488	567	655	600
511	351	543	775	632	991	616	1215	688	808
648	480	407	343	768	1071	528	856	631	671
784	799	919	591	256	455	984	424	439	416
1032	360	632	840	991	520	815	879	664	311
528	664	528	599	1072	463	327	599	607	591
655	896	488	456	568	512	896	856	583	519
327	431	471	672	679	751	1183	304	511	887
312	263	376	919	663	888	599	911	464	512
624	647	1368	423	463	695	208	440	583	831
664	1247	432	408	503	760	911	455	903	615
1192	800	448	503	831	991	384	488	639	744
744	551	327	783	792	1023	424	648	640	391
968	600	775	696	904	656	544	496	720	767
560	120	584	567	568	768	935	327	880	520
496	560	624	671	688	487	687	575	815	639
712	863	879	679	456	743	496	519	872	679
479	544	632	488	847	951	872	447	872	631
623	831	1119	663	887	928	743	1167	863	631

791	863	472	1160	696	399	200	528	503	831
1087	1015	687	743	808	1071	568	591	424	992
552	1280	687	736	608	440	720	303	391	288
664	448	888	656	744	471	839	648	912	304
416	856	335	576	599	783	336	480	535	1015
639	776	584	471	567	751	663	784	904	680
560	896	744	495	520	648	752	567	408	631
760	551	527	407	1184	960	512	576	688	568
376	1040	519	423	767	519	671	991	983	672
591	575	640	1071	623	512	207	271	568	527
752	503	1255	1112	983	255	455	767	743	599
752	815	672	135	407	744	704	959	375	783
679	672	1599	576	528	631	567	1047	855	479
392	487	824	511	695	816	463	1024	656	896
656	744	583	879	903	839	823	591	904	455
1008	656	575	719	480	712	607	719	455	696
600	728	903	872	656	656	440	543	424	1152
791	1024	744	672	1239	727	967	488	455	464
735	607	1079	1103	863	1160	368	768	600	679
688	775	952	1047	839	728	968	559	424	512
656	479	575	768	863	1064	359	799	504	735
447	448	695	424	1016	1271	983	983	855	528

631	615	695	831	1008	447	551	607	639	968
447	840	792	464	1024	744	807	623	760	520
600	608	720	1167	743	696	247	616	631	639
911	895	719	560	552	639	623	559	960	759
487	695	288	367	575	623	752	551	551	607
696	664	1431	448	712	888	319	447	1031	663
680	719	288	696	728	448	863	711	464	607
775	1032	1088	551	567	639	319	592	392	535
656	1111	615	967	1080	935	663	944	592	840
1047	616	431	720	879	279	656	695	823	759
696	680	375	536	760	559	559	488	456	672
800	695	447	488	840	472	447	855	664	759
655	935	1304	944	671	496	319	944	647	719
351	1103	495	1031	832	527	719	656	455	608
863	1040	735	847	1095	799	864	832	984	896
624	455	1104	504	391	472	511	759	752	639
520	784	600	712	703	527	440	640	695	631
703	343	495	792	800	503	519	400	344	471
904	583	487	431	600	287	736	895	776	911
655	1183	663	288	792	279	368	616	816	416
328	624	848	623	711	1063	648	711	464	656
1127	520	528	328	920	903	983	736	632	463

551	720	703	504	736	504	416	743	920	815
695	592	752	936	439	511	599	463	872	712
1200	560	440	767	800	592	984	432	687	967
872	527	800	704	1032	936	559	944	583	751
855	464	431	495	359	783	488	655	479	535
648	664	464	912	688	799	703	712	608	696
624	575	399	1119	783	543	976	488	536	687
407	424	943	455	647	415	655	759	687	640
495	679	504	599	752	687	727	512	576	663
832	1000	824	479	735	632	375	816	487	920
1079	495	240	647	824	511	751	943	528	640
640	815	832	735	519	695	943	272	568	847
823	1135	871	560	535	791	359	935	880	855
679	823	504	559	616	536	431	544	856	392
808	520	768	880	623	856	616	519	864	1183
808	471	543	640	767	368	680	1016	1224	847
975	616	823	1112	1119	416	1095	1112	511	400
671	480	640	560	599	759	544	880	583	496
592	576	488	640	639	823	912	432	808	567
728	847	656	632	680	831	871	679	480	591
576	687	511	1287	712	768	704	551	936	672
1039	503	616	568	495	911	959	519	791	655

736	680	863	455	407	735	935	511	647	535
687	984	264	464	1191	512	639	576	783	239
1183	511	624	447	392	479	703	1008	456	623
664	688	432	631	888	592	695	1023	464	655
392	559	704	623	783	768	1023	839	471	480
528	488	968	391	384	376	527	720	544	799
1095	552	504	919	560	855	647	783	1216	664
464	728	959	711	839	360	615	479	864	480
1023	1232	112	648	351	976	527	544	631	544
800	632	863	520	760	600	575	639	431	759
1119	735	392	728	807	687	871	503	640	536
679	656	512	599	344	832	855	823	624	719
552	352	407	743	671	464	623	591	824	687
959	736	760	639	600	632	791	616	648	824
416	511	863	1023	519	911	759	855	647	935
815	1200	736	855	880	815	599	743	832	519
600	688	431	712	488	599	831	1152	760	368
584	511	744	623	584	560	447	471	608	704
640	799	600	888	727	776	648	1040	783	711
752	680	752	519	759	664	848	871	1136	512
776	888	319	583	584	615	792	527	464	1071
832	687	600	495	424	671	343	552	808	680

607	783	904	232	776	543	320	687	495	328
983	1295	520	1223	1248	816	432	640	704	592
728	295	512	752	431	575	559	568	463	943
799	624	279	831	383	567	591	816	656	624
759	527	855	832	424	535	607	495	640	856
487	983	576	536	671	1104	568	1040	871	263
920	495	576	672	560	527	831	832	543	728
1151	495	856	431	1063	400	375	559	816	527
599	624	792	760	959	519	567	783	608	448
584	608	975	704	720	872	696	576	615	759
880	943	663	1351	792	456	616	855	400	504
680	519	1136	823	384	479	543	768	599	487
671	424	392	1111	735	551	551	783	447	407
927	864	535	504	567	575	695	1119	368	823
896	744	1072	895	495	1359	704	848	768	712
559	408	423	591	752	536	776	735	984	632
1216	800	375	704	672	503	816	871	463	856
640	736	503	791	1063	840	871	1127	839	688
768	839	687	503	928	720	816	1080	647	615
520	575	775	919	919	648	215	664	903	703
727	552	608	703	959	672	455	783	824	768

Table F-7: First 2000 Raw ADC values for 300 ng/ml Concentration of Lp-PLA2

Customer Requirements	Test Results	Level of Success
Detect Lp-PLA2	As seen in Figure 13, the sensor was able to detect Lp-PLA2	This customer requirement was successfully met
Limit of Quantization ≤ 2ng/ml	As seen in Figure 15, the LoQ was 25 ng/ml.	The customer requirement was unfortunately not met, however, a LoQ of 25 ng/ml was achieved.
Detection Range between 0 and 300 ng/ml	From the calibration equation used, the detection range is from 0 to 295.63 ng/ml theoretically.	The customer requirement is considered to be achieved as although a maximum of 295.63ng/ml was achieved, the 4.34ng/ml difference is negligible.
Response Time under 30 minutes	After testing, the response time was consistently under 5 minutes.	Since the response time for the device was 5 minutes, it successfully meets both the primary and secondary requirements of being under 30 and 20 minutes respectively.
Response Time under 20 minutes		
The Mobile App has a latency under 500ms	After multiple tests, the latency was consistently under 450ms.	This customer requirement was successfully met.
The Sensor is Lightweight and Portable	The final sensor had the dimensions of 19cm x 9cm x 10cm and a weight of 275g.	This customer requirement was successfully met as the weight and dimensions of the final product was lightweight and portable.

Table F-8: Customer Requirements and their completion

Appendix G: FYDP Resources

Final Budget Breakdown

Item	Supplier	Cost	Purchaser
PCBs & Stencil	JLCPCB	$175.82	Magilan + Andrew
Final Electronic Components	Digikey	$98.34	Magilan
First Electronic Components	DigiKey	$188.48	Randy
First Electronic Components	Mouser	$36.43	Randy
Lp-PLA2	Bio-Techne	$719.60	NE FYDP Budget (Professor Ghavami)
Misc. (screws etc)	Amazon	$40.59	Andrew
Luminol	Amazon	$39.53	NE FYDP Budget (Professor Ghavami)
APTMS	Sigma-Aldrich	$90.06	NE FYDP Budget (Professor Ghavami)
PET ITO	Adafruit	$52.27	NE FYDP Budget (Professor Ghavami)
3D Printing	Waterloo RPC	$43.90	NE FYDP Budget (Professor Ghavami)
USB 90 Degree Adaptor	Amazon	$9.03	Brandon
Paint Spray	J&J	$41.78	Brandon
	Total Paid:	$1,535.83	
	Budget Left:	$864.17	

Table G-1: Final Budget Breakdown

External Funding

Sponsor	Received
Baylis Medical Technologies	CAD 1000

Table G-2: Sponsorship Records

Equipment Used

Equipment	Location
Hot Plate & Stir Bar	NE UG Labs
Centrifuge	NE UG Labs
Micro Pipettes	NE UG Labs
Glassware	NE UG Labs
DLS	NE UG Labs
Fume Hood	NE UG Labs
Fridge & Freezer	NE UG Labs
SEM	DC Nano Labs
3D Printer	Rapid Prototyping Centre
Soldering Iron	Rapid Prototyping Centre
Reflow Station	Rapid Prototyping Centre

Table G-3: Lab Equipment Used

Contribution Breakdown

Team Member	Activities	Activity Date Range	Hours Spent on Activity	Team Member Signature	Total Hours
A) Andrew Wang	synthesis of AuNPs and DLS, preparation of ITOs, collecting test data, firmware programming, CAD enclosures, 3D printing, prototype assembly, poster creation, slideshow preparation, final report writing, and lab cleaning	Feb. 27 - March 31	synthesis of AuNPs and DLS: 4 hours, preparation of ITOs: 40 hours, collecting test data: 9 hours, firmware programming: 5 hours, CAD enclosure: 5 hours, 3D printing: 5 hours, prototype assembly: 2 hours, poster creation: 7 hours, slideshow preparation: 8 hours, final report writing 5 hours, lab cleaning: 2 hours		~90 hours
B) Magilan Varatharuban	synthesis of AuNPs and DLS, preparation of ITOs with antibodies and known concentrations of antibodies, SEM, collecting test data, mobile app	Feb. 27 - March 31	synthesis of AuNPs and DLS: 4 hours, preparation of ITOs with antibodies: 40 hours, SEM: 2 hours, collecting test data: 20 hours, mobile app development:	Magilan	~90 hours

	development, poster creation, slideshow preparation, and final report writing		4 hours, poster creation: 7 hours, slideshow preparation: 8 hours, final report writing: 5 hours		
C) Randy Dakhil	SiPM validation/testing, PCB integration testing, PCB debugging/resoldering, collecting test data, calibration curve testing, poster creation, symposium prep, final prototype construction, and final report writing	Feb. 27 - March 31	SiPM validation/testing: 8 hours, PCB integration testing: 20 hours, PCB debugging/resoldering: 12 hours, collecting test data: 25 hours, calibration curve testing: 5 hours, poster creation: 7 hours, symposium prep: 6 hours, final prototype assembly: 2 hours, final report writing: 5 hours	Randy Dakhil	~90 hours
D) Brandon Kong	synthesis of AuNPs, preparation of ITOs, calibration curve testing, mobile app development, poster creation, slideshow preparation,	Feb. 27 - March 31	AuNPs synthesis: 3 hours, ITO preparation: 35 hours, calibration curve testing: 5 hours, mobile app development: 20 hours,	Brandon Kong	~90 hours

	and final report writing		poster creation: 9 hours, slideshow preparation: 9 hours, final report: 9 hours,		

Table G-4: Final Contribution Breakdown since last progress update