Single Cell Impedance Measurements Using Microfabricated Electrodes and
Labview Graphical Programming

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Master of Science in Engineering, Biomedical Concentration

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ABSTRACT

Single Cell Impedance Measurements using Microfabricated Electrodes and Labview Graphical Programming Stephanie Sophia Hernandez

This Master's Thesis project consists of the research, design, and fabrication of a system that could perform broadband impedance measurements (1kHz-20Mhz) of single cells using National Instruments Labview data acquisition and programming in coordination with a single cell capture device. Presented first is the background information on cells and their electrical properties, along with background in micro-total-analysis systems as well as impedance spectroscopy. Experimental Methods are then discussed for the electrode design, cellular modeling in COMSOL, fabrication methods, and Labview 8.0 Set-up and programming. Measurements were performed using the single-cell capture device on saline, yeast cells, and a polystyrene bead. Analysis of the impedance data showed a clear visual and statistically significant difference between live yeast, the bead, and saline. A comparison of live yeast cells to nutrient-starved yeast cells was also performed and a distinct difference in spectra was observed.

Keywords: Single-cell analysis, Impedance Spectroscopy, μ -Tas, Lab-on-a-chip

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1- Introduction

1.1 - Purpose

The overall goal of this project was to provide a proof of concept for a device that would capture a single cell from solution and take an impedance measurement of that single cell. This type of device is desired due to its potential use in assessing fluids/air cellular response to the presence of toxins or disease. It could also easily be made portable, which could be particularly important for use for soldiers or civilians at sea, or for use when in other countries and advanced labs and technologies are unavailable. Owing to the simplicity of the design, its applications could be modified to spread as far as detection of virus or cancer in cells.

1.2 - Objective

This project was divided into two projects due to the complexity of the device. The first half (cell capture) of the project was done by Josh Fadriquela at Cal Poly San Luis Obispo, in conjunction with design work for the impedance measurement. In order to complete the impedance spectra project, a project management session using the Stanford IPSolutions Project Management was completed and six deliverables were established. They were to create a model-assisted design; fabricate an impedance chamber with electrodes; set-up, program, and test drive a Labview interface to drive the Impedance sensor; set-up an Impedance test station including a final impedance program; using the established cell protocol, characterize yeast viability; and finally fulfill all MS requirements.

II. Background

2.1- Cell properties

2.1.1 - Basic Cell Structures

The cell is commonly known as the basic unit of life. It is usually made up of an outer protective surface known as a membrane, and inside carries genetic material and all the necessary mechanisms to sustain an organism. The cytoplasm of a cell refers to all the cellular fluids and materials in between the nucleus and the cell membrane. It is made up of organelles and the cytosol, which is a fluid containing salts, proteins, and nucleic acids that suspends the other constituents of the cytoplasm. The compartmentalized cytoplasmic organelles consist of the endoplasmic reticulum, mitochondria, the Golgi apparatus, lysosomes, peroxisomes, and the cytoskeleton, all of which work together to maintain homeostasis within the cell. Shown below in Figure 1 is a detailed representation of a generalized Eukaryotic cell.

Figure 1 – Cell Structure
(http://www.biomedicalphysics.org/PhysCircCourse/chapter_1.htm)

Many of these organelles are highly dependent on conditions such as pH (lysosomes) and ion concentrations (cytoskeleton & smooth endoplasmic reticulum), and disruption inside the cytoplasm can lead to serious problems including cell death. For example, introduction of viruses can cause the formation of viral capsids in the cytoplasm which can detrimentally affect cell function [1]. Changing the cytosol pH would halt lysosome hydrogen ion pumps, and could lead to a buildup of cell debris and bacteria.

2.1.2 – The Cell Membrane

The cell membrane is an important structure that serves a number of important function including mediating cell/extracellular interactions, cell/cell interactions, cell signaling, and ion channel conductance. In Eukaryotic cells, this cell membrane (also called the plasma membrane) is made up of a structure called a lipid bilayer that contains proteins that move within the bilayer which is described most accurately by the fluid mosaic model [2]. This lipid bilayer is made up of mostly phospholipids that contain a round-shaped polar hydrophilic head, and two fatty acid chain hydrophobic tails. Because the heads are hydrophilic and attract to water, they naturally are attracted to both extracellular and intracellular fluid which are largely water in composition. The end result is a formation of a sort of selectively permeable sandwich around the cell, with the hydrophilic tails neatly fitting together in rows in the center, with the polar heads facing the towards the outside and inside of the cell. Within this structure are a number of membrane proteins (Figure 2) that facilitate most of the functions mentioned earlier including transport and signaling. The functions most relative to this study is the plasma

Figure 2 – The Cell Membrane & its Structures

membrane's role in transport. Both active and passive transport take place across the plasma membrane. Diffusion and osmosis occur passively while primary and secondary active transport carry materials against their gradients. Active transport is particularly important, and involves a sodium (Na^+) and potassium (K^+) pump that works to drive Na^+ against its concentration gradient and pumps K^+ into the cell. What this does is create a resting membrane potential (a voltage) across the plasma membrane. Since there are more charged ions (Na^+) on the outside, the inside of the cell is considered negative relative to the outside, so the resting membrane potential will range from -5 to -100 millivolts [2]. Maintaining these delicate ion concentrations is very important for normal cells so that they can maintain their fluid volume, and it's important to excitable tissues such as muscles and nerves so they can function.

There are a large number of ways to perturb this balance the cell membrane maintains and kill the cell, such as toxins, osmotic shear, temperature, etc. Most commonly studied is programmed cell death; apoptosis or necrosis. A variety of factors can cause a cell to undergo apoptosis. External information controlling apoptosis includes the presence or absence of interactions with other cells as well as the presence or absence of soluble signaling molecules, for example, calcium ions can act as

extracellular inducers of apoptosis [3]. Other inducers of apoptosis include UV irradiation, cytotoxic drugs, virus, thermal radiation (which induces necrosis), and cell “arrest” inducing chemicals. These have all been studied in depth using a number of different cell viability tests.

2.2 - Cell viability

The definition of cell death differs in different applications, but usually refers to mechanisms of programmed cell death such as necrosis or apoptosis. Viability is another term used to assess a cell, and is commonly used in microbiology to make a distinction on whether a cell is alive or dead [1]. For the purpose of this paper, non-viable is used to describe a cell whose membrane is no longer intact due to apoptosis or necrosis.

Optical measurements have been a predominant method for evaluating cell viability; this is because the morphological changes a cell undergoes can be monitored, and will give information on whether apoptosis or necrosis is occurring. A common method for studying this is electron microscopy. Electron microscopy uses highly focused beams of electrons to investigate the nucleus, specifically changes in the size of chromatin (which form chromosomes), cytoplasm morphological changes, and the presence of apoptotic bodies. It also visualizes necrosis by identifying indicators such as surface blebbing, mitochondrial swelling, chromatin crowding in the nucleus, and absence of ribosomes [3]. In standard light microscopy, it's more difficult to detect apoptosis since well-preserved apoptotic bodies must be present for detection, and with less magnification it is difficult, but possible. Necrosis is visible again by the presence of condensed chromatin and nuclei (in late stage degeneration) are only visible as faint outlines. These methods, and others such as exclusion dyes

and fluorescent labeling all share a common drawback which is expensive machinery and time consuming preparation of cells [4].

2.3 – Fluid Mechanics Factors

In microfluidics, you're dealing with scaled down systems and in order to design accordingly, you have to understand the related flow concepts including the viscous forces, surface-to-volume ratios, and Brownian motion. The Reynolds number (a scaling factor) is important since viscous forces factor into the Navier-Stokes momentum equation for Newtonian Fluids (Where ρ = Density, u = Velocity vector, η = Fluid viscosity, and b = Body force unit). It's also an important factor for designing

Reynolds equation: $Re = \rho v l / \eta$

v = Inlet Fluid

l = Characteristic dimension size of device

Navier-Stokes equation: $\rho \left[\frac{\delta u}{\delta t} + (u \cdot \nabla)u \right] = -\nabla P + \eta \nabla^2 u + b$

(Inertial Forces) = (Applied Air, Viscous, & Body Forces)

microfluidic sub-systems, such as a value can tell you whether the fluid motion is experiencing turbulent or laminar flow. This is relevant information since turbulent flow is usually experienced only with the macroscopic length scale, while at the microscale level flow is usually laminar [5].

Another factor that can affect design is surface to volume ratios. In some conditions this measure channel size and radius can affect what species and conditions can be put through the system, particularly when dealing with protein adsorption [6]. An additional important fluid factor is diffusion of the sample into the surrounding media, which can be either a useful or harmful tool depending on what

the application is. It's dependant on flow rate, diffusion coefficients, and sizes of particles of different species.

2.4 - Electrical Background

2.4.1 Electric Circuit Fundamentals

In order to design a system capable of performing impedance measurements, a basic knowledge of electricity and circuit theory is required. In order to electrically excite a particle, an electrical current can be run through it and the loss of signal measured. Current flow can be described by the relationship between an applied voltage and opposition to current flow, called resistance. This relationship was formulated by George S. Ohm and says that current can be measured in a unit called an ampere (A) which is a movement faster than one coulomb per second (a coulomb being a measure of charge). Ohm said that one ampere was equal to the applied voltage divided by the measure of opposition to flow, resistance (measured in a unit called an Ohm Ω) [7]. Other important electrical factors include conductance,

$$I = V/R$$

$$\text{Amperes} = \text{volts/ohms}$$

capacitance, reactance, and impedance. Conductance is simply measured to be the reciprocal of resistance, while capacitance and reactance are more complex.

Capacitance is a store of charge that is dependent on a material's dielectric constant, ϵ_r , also called relative permittivity ($\epsilon_r = \epsilon/\epsilon_0$) with ϵ_0 = permittivity of a vacuum. The equation for capacitance is

$$C = \frac{\epsilon A}{d} * \frac{1}{4\pi k}$$

where d is the thickness of the dielectric, A is the area of the conductor, and k is a constant = 9×10^{11} cm/F. Conductance (G) is also a measure of resistance, and is simply the inverse of the capacitance with units of inverse Ohms.

$$G = 1/R$$

Capacitance is also directly related to reactance (X_c), and is dependent on frequency of an Alternating Current (AC) signal.

$$X_c = \frac{1}{2\pi fC}$$

Another important characteristic of AC signals is the resistance, which for AC analysis is called Impedance (Z). In AC analysis, the excitation signal changes from a steady state to an alternating state, usually a sinusoidal state. Ohm's equation becomes

$$i(t) = v_s(t)/R$$

where the current and voltage are complex numbers with magnitude and a phase [8].

The resistance is also complex, and since $i(t)$ and $v_s(t)$ are dependent on frequency when converted to phasor notation, the impedance Z is as well. Using Ohm's law again to solve for Z

$$V = I * Z \quad \text{so} \quad Z = I/V$$

Impedance contains both a phase and a magnitude, and these can be described in terms of the relationship between voltage and current in a graphical form. This is

called the parallelogram method, which graphs 1/Reactance (capacitance) against 1/Resistance (conductance) with a slope of 1/Z. This gives the equation

$$Z = \sqrt{X_c^2 + R^2}$$

2.4.2 – Electrical Properties of Cells

Cell electrical properties can be directly linked to the status of the cell internal and external environment. On the surface of cells, there is an attraction of positive ions due to the negative charge of surface structures. This attraction of charged particles creates a phenomenon known as an electric double layer. The thickness of this layer can range from 10-1000 nm [9], and can have small to large effect on the fluid motion, as well as electric signaling depending on the field that is applied. The double layer has been studied using a number of different methods including Mean-field theory (which was been shown to have limitations unless verified experimentally [10]), Poisson-Boltzmann, and matched asymptotic expansion. These studies generally conclude that the double layer does create a resistance that is not insignificant, particularly in the case of gram-negative and gram-positive bacteria [11], but can be tedious to solve unless the parameters at the double layer are all lumped into surface effects [9]. The next layer down into a cell is the cell wall (for plant cells and special cells such as yeast), whose properties will vary depending on external changes as it is responsible for exchanging ions with the solution it's suspended in. The membranes of cells are slightly more complicated as they have a number of different electrical properties, specifically the transmembrane conductance g_m , membrane conductance C_m , and permittivity. The thickness, which is typically 4-10nm [12], effective area which can vary considerably, dielectric constant, and conductivity all have an effect on permittivity which typically for a membrane ranges between 2-10

[M]. Under the membrane is the cytoplasm, which as discussed previously is assumed to be a highly conductive salt solution. With all organelles and debris included, the value of permittivity of the cytoplasm tends to be between 50-200.

These electrical properties of cells change as they undergo various forms of stress and cell death, and have been studied in depth. For example, it has been shown that often initial exposure to toxins that in general cell membrane capacitance increases. Depending on cell type, however, cell membrane capacitance decay back to fatal levels at different rates [1]. In addition to toxic stress, it has been shown that temperature stresses on cell electrical properties occur, e.g. conductivity seems to be affected when temperature is varied, both of the membrane and the internal systems. Finally, viruses can also affect electrical properties as they attack cells, causing an increase in ion channels, resulting in changes in membrane capacitance and cytosol conductance.

2.5 - Lab-on-a-chip Design

2.5.1 - μ -TAS Systems

In order to get more direct measurements, there have been a number of advances made in microsystems in the form of μ -TAS (Micro Total Analysis Systems) and devices known as “Labs-on-chip” devices. These specialized systems aim to scale down measurement chambers to the cellular level and combine both microfluidic designs with modern cytometry to get detailed information about cells and their environment. Designing these total analysis systems requires a number of factors to be taken into account. One of the most important considerations is the sensing and

detection of cells/liquids/other signals. Some standard sensing methods include direct measurement using electrochemical transducers, electrochemical sensing of cellular events, and optical detection methods [5]. Another important aspect of design for microsystems is the controls for the flow, including valves and pumps, as well as packaging/integration materials.

2.5.2 Flow Cytometry

Until recently, one of the only methods of sorting, counting, and examining microscopic particles has been traditional flow cytometry. Flow cytometry systems typically can measure multiple parameters including physical and chemical characteristics for example, pH and Ca^{2+} measurements of cells in a flow through environment. The drawback to this method is that it's limited to electronic detection or investigation by optical methods such as emission ratio dyes [3,7].

Figure 3- Flow Cytometry System [13]

The figure above shows an example of a flow cytometry system, highlighting the electrodes and the electric field lines as they send and receive signal across the sample.

2.5.3 – Electrorotation

Another form of analysis and separation of particles is electrorotation (ROT). This type of characterization of dielectric properties (conductivity, capacitance, etc) does so without measuring impedance. Instead, a rotating electric field creates a torque that directly affects the particle. As the frequency of the electric field is increased, the rate of rotation of the particle is measured. Since the rate of rotation is a function of the Clausius-Mossotti factor (discussed in the next section), dielectric properties can be established. This technique can give precise information, for example in depth studies have been done on liposomes to compare experimental measurements with actual cell structure and found this method could demonstrate this well [14]. The biggest drawback to this method is the amount of time necessary to take the measurements.

2.6 - Dielectrophoresis

Dielectrophoresis and electrophoresis are widely used in μ -TAS systems since they have the ability to manipulate particles in the range of 10nm-100 μ m and thus can be used to study cells, proteins, viruses, and DNA.

Electrophoresis has mostly been used for particle detection and separation, specifically for proteins and DNA. It does so based on the native charges of particles and differences in size to separate particles from each other. The drawback to this method is the lack of specificity, which prevents particles from separating from the solution [15]. More specifically, most biological matter of interest, e.g, proteins,

nucleic acids, is negatively charged, and in many cases they have very similar charges.

Dielectrophoresis is also used in cell/particle separations, but uses electric fields, specifically non-uniform electric fields, to move particles. This is accomplished as the non-uniform electric field will elicit a polarization response that is dependent on particle and it's suspending medias physical properties. Peter Debye and Herbert A. Pohl [16] performed the early work on Dielectrophoresis (DEP) and showed that using this DEP Force, particles could be moved in specific directions, towards areas of higher or lower field strength, and called these forces positive DEP and negative DEP (respectively). Other factors that contribute to the DEP field strength are electrodes that either drive or sense the electric field $\vec{E}$, as well the electrolyte or fluid suspension that helps prevent cells/particles from sticking to the surfaces of the system. Pohl also concluded from his early studies on DEP forces that the permittivity, ϵ , had a great effect on the DEP force. Permittivity is a measure of how polarizable a particle is, and is generally expressed in terms of the permittivity of a vacuum ϵ_0 (8.854×10^{-12} F/m).

$$\epsilon_r = \epsilon / \epsilon_0$$

He found that particles with permittivity greater than the permittivity of the suspension media are attracted to regions with stronger fields, and are alternately repelled from regions of stronger field strength if the permittivity was less than the permittivity of the media [17].

The simplest mathematical model for DEP force on a particle is a lossless spherical particle. The DEP force can be written as

$$F_{DEP} = 2\pi\epsilon_1 R^3 K \nabla E_0^2$$

with ε_1 representing the permittivity of the particle, R is the radius of the particle, K is the Clausius-Mossotti Factor, and E_0 is the applied electric field. The most studied part of this equation is the Clausius-Mossotti Factor and is dependent on both the permittivity of the particle, ε_1 and of the medium, ε_2 .

$$K = (\varepsilon_1^* - \varepsilon_2^*)/(\varepsilon_1^* + 2\varepsilon_2)$$

$$\varepsilon^* = \varepsilon - j\sigma/\omega$$

Using knowledge of dielectrophoretic forces, a number of different systems have been created to sort, trap, transport, and detect particles at the single and multiple cell level. Standard particle separation takes advantage of the differences in cell frequency-dependent properties to direct certain cells in predetermined ways. It is often done by applying positive/negative dielectrophoresis at a certain frequency to a flow of particles, which separates the desired particles, though to remove them, they must be flushed. Improved methods of separation include traveling-wave DEP and DEP field flow fractionation (DEP-FFF). In traveling wave DEP, the real part of the Clausius-Mossotti factor is used to levitate particles for transport [18]. With Field Flow Fractionation, DEP is combined with fluid factors such as density and the velocity gradients to separate particles. Particle trapping has also been investigated using DEP techniques. This can be done by physically trapping a particle in a chamber to take measurements, or suspending a particle based on its dielectrophoretic properties. Negative DEP can be used to suspend particles in a chamber [18] or in a spiral electrode array [11] for further analysis. A more advanced model for trapping was designed by Voldman [19] who designed and fabricated a device with quadrupole electrodes that had been extruded much higher than typical planar electrodes. A similar model was created using a trap with three micropillars on glass in order to obtain impedance measurements from a cell [20] (Figure 4).

Figure 4- Cell Trapping using Micropillars

This device shows the three pillar design for capturing the cell as well as the gold planar electrodes that perform the DEP measurements.

2.7- Impedance Spectroscopy

Impedance spectroscopy can be divided into two categories; electrical impedance spectroscopy (EIS) and other impedance spectroscopy (IS). EIS usually refers to measurement and study of materials with ionic conduction (such as electrolytes) whereas IS is performed on dielectric materials (non-conductors). This technique is typically used to characterize systems with interfaces between these materials, as the properties at interfaces can change fairly drastically [21]. As in dielectrophoretic studies, two electrodes are typically used to create a signal and measure the response. In Impedance Spectroscopy, there are three types of electrical signaling used; transient step voltage, $v(t)$ composed of white noise, and third is to apply a single frequency voltage to measure impedance properties which was used in these experiments.

In general there are two ways of modeling cellular impedance; a classical Coulter apparatus or Maxwell's Mixture Theory. The biggest difference between the two methods is the type of impedance measured. The Coulter counter is a device that has long been in use to study volumes of particles [22]. What these devices study is the direct current (DC) resistance due to a steady applied current between two electrodes. To solve for the resistance change using Coulter counters, Laplace equations are often used and produce the equations

$$\Delta R_{\left(\frac{D_m}{L}\right) < 1} = \left(\frac{4\rho d^3}{\pi D_m^4}\right) F\left(\frac{d^3}{D_m^4}\right)$$

$$\text{with } F\left(\frac{d^3}{D_m^4}\right) \cong 1 + 1.268 \frac{d^3}{D_m^3} + 1.17 \frac{d^6}{D_m^6}$$

which then simplifies to $\Delta R = 4\rho d^3 / \pi D_m^4$ where D and L are the diameter and length of a cylinder containing the cell, d is the particle diameter, and p is the liquid resistivity [13]. Downsides to these systems include a lower signal to noise ratio, and also the equations don't account for polarization activities. They are mostly used to give you scaling information. The second method of modeling is a more complex method known as Maxwell's Mixture Theory (MMT). Maxwell's equation for a spherical cell solved for the complex permittivity or a mixture where f_{CM}

$$\varepsilon_{mix} = \varepsilon_m * (1 + 2\varphi f_{CM}) / (1 - \varphi f_{CM})$$

represents the Clausius-Mossotti factor, φ is a volume fraction, and ε_{mix} , ε_m are the complex permittivities of the mixture and medium. Substituting in the Clausius-Mossotti factor, the cell permittivity (ε_p) can be solved for [23]. ($\gamma = R/(R - d)$)

$$\varepsilon_p = \varepsilon_{mem} * ((\gamma^3 + 2 * (\varepsilon_i - \varepsilon_{mem}) / (\varepsilon_i + 2\varepsilon_{mem})) / (\gamma^3 - (\varepsilon_i - \varepsilon_{mem}) / (\varepsilon_i + 2\varepsilon_{mem})))$$

The advantage to using this mathematical model is that frequency dependent characteristics can be measured including the β -relaxation. This equation does however have a limitation in that the particle must remain still to ensure a homogenous field is applied.

Another important aspect of impedance spectroscopy is the use of equivalent circuits to model materials and their surroundings. Typical factors in an equivalent circuit are capacitance and resistance, which combined with voltage/current sources, amplifiers, filters, or transformers can give you the necessary information to solve for impedance. Some common circuits include simple RC circuits and bridges, which are more complex and combine potentiostats and oscillators to detect signal. Some limitations

Common graphical methods for impedance include the Cole-Cole plot which graphs what's called the Debye response [24, 25] which is governed by permittivity and a time constant (shown below in Figure 5). Other methods include graphing impedance by splitting it into the real (Re) and imaginary (Im) parts of the response

Figure 5 - Cole-Cole Plot of Impedance

and plotting them on x- and y- axis (respectively), usage of oscilloscopes, and the automated, more common method is to plot the impedance using a Frequency response analyzers to plot Bode diagrams of frequency vs the impedance response.

Common materials that are studied using impedance spectroscopy include colloids [25], cancerous tissue [20], bovine chromaffin cells [26], yeast [27], red blood cells [28], and many other cell types. Even dielectric properties of single stranded DNA and double stranded DNA can be studied by suspending the strands and measuring impedance [29]. When combined with other types of analysis such as confocal microscopes, dielectrophoresis, or electrophoresis, even more information can be provided. For example, a study combining impedance spectroscopy with cell culturing can give information about the growth process of skin cells [30]. The advantage to these systems is that they can easily be automated, can be modified to measure single cells, and give data that can be correlated to other physical properties of materials [21].

2.8 – Microfabrication

Photolithography is a well-studied method of patterning something basic like a simple low resolution pattern to something complex like an intricately small pattern of metal onto substrates such as silicon, as used in the semiconductor industry, or glass in microfluidic devices. The main components involved are masks, photoresists, exposure to light, development techniques, and stripping. Masks are used to generate the pattern wanted and come in two polarities, dark and light field (also known as positive and negative masks). How masks project their image for patterning differs as well. They either come in hard (physical) contact with the substrate, or soft contact, where they hover slightly above the substrate and project an image [31] Since one of the goals of this project was to maintain a low budget, a photomask was used to

create the pattern. Photomasks utilize laser plotters to print a pattern onto a transparency, which is then transmitted onto the substrate during exposure. Photoresists are extremely important in lithography, since they allow for the pattern of the mask to be created on the substrate. They are viscous liquids made up of an organic polymer, a sensitizer, and a casting solvent, and are sensitive to light like film in cameras. They come in two forms, positive tone (positive resist) or negative tone (negative resist). Positive resists are made soluble to developers by a process called chain scission in which the bonds break when they are exposed to DUV light (~220-240nm wavelength). Negative resists work in the opposite manner in that after exposure to light, they are rendered insoluble due to cross linkage of polymers, making it more stable. Typically the resist layers are deposited in microns, and must be free of defects, so that when deposition and patterning occur, defects aren't manufactured onto the substrate. The initial step is to distribute the thin layer evenly onto the cleaned substrate by using what is called a photoresist spinner. Spinners can be programmed to very accurately spin thin layers of resist. The thickness of the layer (T) of photoresist is governed by the equation

$$T = \frac{KC^{\beta}\eta^{\gamma}}{\omega^{\alpha}}$$

Where T = Thickness of the layer

K = Calibration constant

C = Polymer concentration in g/100mL solution (measure of molecular weight)

η = intrinsic viscosity

ω = rotations per minute (rpm)

Following the spinning step, the resist that has been distributed still contains some amount of solvent, as well as internal stresses from the motion. In order to eliminate

leftover solvent and internal stresses, medium heat is required (~75-100°C) and the resist-coated wafer must be baked at this temperature to ensure the pattern will adhere well and be free of defects [31].

Like photography film, the resist must next be exposed to a light source. Light sources in photolithography are in the UV ranges (10-500 nm wavelength) and different types are used based on the sensitivity of the resist, that is, how easily the resist will undergo a photochemical reaction. In order to expose the pattern, the mask that has been created is put into a machine called a contact aligner. Aligners can put the mask into direct, soft, or vacuum contact with the resist-covered substrate, depending on what amount of space is desired between the wafer and the mask. The wafer is then exposed to the UV light, either strengthening or weakening the photoresist depending on its tone. The wavelength of the light is important in fabrication in that it can dictate the size of the smallest feature. The wavelength it is exposed to is usually the limit of the smallest feature size (Ex: 150 nm wavelength will only properly create features over 150nm in size). Following exposure, it's necessary to develop the resist in a bath of developer. This will ensure only the pattern is left so that during metal deposition no excess material will be deposited.

Metal deposition can be done a number of ways, but is most commonly done by sputtering or electron beam deposition. Both methods involve a metal target (Titanium, Chrome, Gold, etc) that is used to essentially eject metal ions onto a collecting surface (where the wafer would be placed). In sputtering, the wafer is placed on an anode, while the cathode that holds the target is bombarded with positive argon ions. Most materials can be used with a sputter system, whereas electron beam deposition uses electrons to knock loose metal ions in very precise layers. Another

advantage to these systems is they often can deposit metal on a number of wafers at once.

Following metal deposition, the final step in photolithography is to strip away the resist layers in order to remove the unwanted metal to create the desired pattern. This can be done with oxygen plasma (dry stripping) or with liquid strippers.

III. Experimental Methods

3.1 –Electrode Design

Electrodes for the single cell impedance measurements were first designed in SolidWorks following PDMS chamber design (Figure 6). As the LabSmith Video Microscope that was to be used required a clear substrate, glass was chosen as the substrate that the electrodes were to be fabricated on. The PDMS fluidic chamber design included two flush channels, the inlet for samples, and a suction chamber. Electrodes were to cross the cell chamber in order to take readings.

Figure 6- The PDMS chamber design

Fabrication itself was done using the Nanotech Facility at University of California, Santa Barbara with the final work being done in the Microfabrication Lab at Cal Poly San Luis Obispo. Initial tests done at Cal Poly for proof of concept involved aluminum, but further research showed that gold is the standard for these types of

impedance measurements as it is highly conductive and biocompatible [19-20, 30]. Gold however, will not adhere well to glass and thus requires a second metal layer for adhesion, such as chrome or titanium, which was chosen in this study. After further research and consulting with faculty at UCSB, electron beam deposition in conjunction with photolithography was chosen as the fabrication methods.

3.1.1- Initial tests

Prior to Fabrication, a photomask was made by CadArt Services using SolidWorks and AutoCad models that had been drawn. Initial designs for a multi-chamber device were used to test multiple electrode layouts as well as test resolution of the CadArt photomasks. These early designs experimented with electrodes following fluid paths and varied in size and height in order to find an optimal size for features. It was found that features $10\mu\text{m}$ and smaller did not print accurately and were often missing entirely from the mask. Thinner features with sharp angles or small gaps (less than $10\mu\text{m}$) suffered from a bleeding effect. Two final concepts for electrode configuration were chosen (Figure 7). The parallel electrodes

Figure 7- Parallel and Planar Electrode Configurations

were chosen to allow for some play in alignment and also maximize signal through the solution. Distance was chosen by modeling the electrodes in COMSOL.

3.1.2 - COMSOL Modeling

COMSOL Multiphysics is a useful tool for modeling complex systems that involve electricity, fluid flow, thermodynamics, and includes other factors such as material composition. The purpose of the models was to study electric field lines at different differences and arrangements to maximize the signal across the cell, and also to provide a comparison measurement of the impedance across the channel. In order to create the model, the Electric Currents Mode of the AC/DC Module was used. This mode allows you to measure the electric field strength based on permittivity and conductivity, which as discussed earlier, can give you dielectrophoretic information about the cell and the suspending medium. It also neglects the effects of coupling between electric and magnetic fields which mathematically means that you can solve specifically for the electric field $\mathbf{E}$ [32]. The equation for $\mathbf{E}$ in terms of the scalar potential (V) is $\mathbf{E} = -\nabla V$. To solve the continuity for electric fields, the equation is

$$\nabla \cdot \mathbf{J} = -\delta\rho/\delta t$$

With $\mathbf{J}$ = Current density, and ρ = charge density. Since we are examining the transient case, the equation for continuity is:

$$-\nabla \cdot \left(\frac{\delta}{\delta t} \right) (\epsilon_0 \nabla V + \mathbf{P}) - \nabla \cdot (\sigma \nabla V - \mathbf{J}^e) = 0$$

Where σ = conductivity, ε_0 = permittivity of a vacuum, and $\mathbf{P}$ = the Electric Polarization vector.

For this model, the cell capture chamber was modeled as a cube with a double-layered sphere inside representing a yeast cell. As discussed previously, the simplest model to find the dielectrophoretic force is a single layer, spherical, lossless sphere. However, as a yeast cell is a more complex structure with a cell wall and thin cell membrane, a more complex model is required. Multi-shell, lossy models have been derived to describe a walled cell such as yeast [17]. The purpose of using these shell models is to as closely model particle structure to understand specific inner workings of the cell. Specifically, individual shells have different DEP forces depending on the size of the cell [11]. One method of creating a shell model involves combining shell permittivity values into a homogeneous permittivity ε'_2 in place of ε_2 in the equation for the Clausius-Mossotti factor by taking into account surface capacitance, however the simpler model is to give the shells their own properties and thicknesses.

Figure 8 – Multi-shell Model

Both Boundary and Subdomain settings needed to be defined in order to solve the equation. Boundary conditions were defined to be the PDMS, the two electrodes, the layers of the cell, and the faces that were open to fluid flow. The Boundary

condition for PDMS was treated as electric insulation, the driving electrode as a Port, the sensing electrode as a ground, the cell membrane layers as electric shielding, and the open walls as continuity. Each boundary also had to be defined in terms of conductivity, permittivity, and thickness. From [16,33] the yeast cell was modeled with a cytoplasm with conductivity $\sigma = .25$ S/m, permittivity $\epsilon_{rel} = 50$, a 200nm thick cell wall with conductivity $\sigma = 10$ mS/m, permittivity $\epsilon_{rel} = 60$, an 8nm thick membrane with conductivity $\sigma = .1$ mS/m, permittivity $\epsilon_{rel} = 8$, and saline solution was modeled with conductivity $\sigma = 1.4$ S/m, permittivity $\epsilon_{rel} = 78$. The two electrode distances were tested by running the Linear Solver to solve the continuity equation in COMSOL, followed by Post-processing to display the field lines. Due to lack of memory on the computer hard drive, both models were cut in half to be able to solve the complex equations.

Figure 9 – Isometric View and Side view of large gap, Electric Field Lines

Figure 9 shows the Field lines of the electrodes with a large gap, ($10\text{ }\mu\text{m}$) which show some distortion in the field lines, particularly near the top of the cells. This distortion is mostly likely due to the distance between electrodes. The model with a small gap

between electrodes (5 μm apart) displayed much smoother field lines across the fluid and the cell membrane (Figure 10) so the final designs included a much smaller gap between electrodes.

Figure 10 – Small Gap between electrodes, Electric Field Lines

3.1.3 -Final Design

The finalized single-chamber device drawing was modified from beginning designs in order to create larger electrode pads in order to make better connections with wires and resistor as well as incorporate the design from COMSOL. The outer design was based on a 4" wafer that could fit into typical aligners to project the image.

Figure 11 – AutoCAD Model of Electrodes

Cross and L-shaped alignment marks were included to match the PDMS design done by Josh Fadriqueles in order to ensure the layer of PDMS would align to the glass. Electrode and ground pad design were drawn to fit a 2"x3" typical glass slide which served as the 'wafer'. Dimensions for the electrode arms that cross into the cell chamber are shown below. The arms were initially designed to be 10 μm thick and perfectly match the length of the chamber; however the final design (shown below) shows the electrode arms to be 11.5 μm thick, with an extra 3 μm past the chamber. This was decided from the results of the preliminary multi-chamber designs which showed that features 10 μm and under were extremely difficult for the laser-plotter to recreate, and other initial tests of the resist showed that some over-exposure was

likely to occur and cause a small amount of shrinkage of the design to prevent bleeding of features.

Figure 12 – Chamber and Alignment markings

Figure 13 – Zoom view of electrodes over chamber

3.2 – Fabrication

3.2.1 - Photolithography

Fabrication at UCSB included deposition of electrodes using electron-beam deposition of titanium and gold, followed by testing of plasma treating of the PDMS and glass in order to get a strong bond. For e-beam deposition, a dual-layer lift off process was used using an Image Reversal resist, an underlayer and a CHA Industries Multi-Wafer Evaporator. Dual layer lift-off began with thorough cleaning of the glass slides in Piranha solution (3:1 OH to H₂SO₄). Next, the underlayer and negative photoresist were deposited. To spin the layers on, a Pure Aire Corporation Spin Wet Bench was used. Heights are programmed into spinners at UCSB based on the spin speed curves of resists.

Figure 14 – Key Steps for AZ5214-E IR development

Step 1) In order to get the desired height and shape for the chamber of approximately 10µm, a lift-off layer called Shipley LOL2000 was pipette onto the

cleaned wafers and it was recommended to be spun at 2000 rpm for 30 seconds. Following the underlayer spin step, the wafer was hard baked at 200°C for 3 minutes to remove the residual resist and internal stresses. Step 2) Following deposition of the lift-off layer, the AZ-5214-EIR resist was pipette on top and suggested to be spun at 4000 rpm for 30 seconds. After spinning, a soft bake was done at 95°C for 1 minute.

Step 3) The next step was to project the image from the mask onto the photoresist. A Karl-Suss Aligner MJB-3 aligner was used to achieve the contact alignment of the photoresist mask. The resist was then exposed to UV light for 6 seconds total. This time was chosen after an initial test to ensure features did not bleach or shrink.

A unique property of AZ-5214-E IR resist is that it can be used as either a negative or positive tone resist. Following projection of the mask, exposed areas can be cross linked (made insoluble to developers) by performing a post bake. Step 4) The glass wafer was then baked at 110°C for 90 seconds which cross linked exposed areas. Step 5) To ensure the non-exposed areas would become soluble, the wafer was put into the Karl-Suss aligner without a mask present, and exposed to flood light for 60 seconds. Step 6) The next step was to develop the resist, which was done by placing the wafer in AZ 300 MIF Developer for 60 seconds, followed by a rinse step in deionized water.

Next, the metal layers were to be deposited using the electron beam evaporator using the attached protocol (Appendix A). After literature review, a height of the electrodes was chosen. Step 7) To balance between a smaller desirable height for PDMS bonding purposes and the need for sufficient electrical signaling, a 15 nm layer of Titanium with a 150nm top layer of Gold was chosen, bringing total height of

electrodes to 200 nm. Step 8) was to strip the remaining resist structures by placing the glass wafer into a solution of Shipley 1165 stripper into a 75-80°C ultrasonic bath for 5-10 minutes. Once dried, the glass wafer was left with only a thin film of electrodes in the shape of the photomask pattern.

3.2.2 - Bonding

A Poly-di-methyl-siloxane (PDMS) chip was created using photolithography by J. Fadriquela with a cell solution introduction port, flush chamber, and suction port (Figure 6). This final design included a single chamber to capture a cell so that measurements could be taken. To bond the glass wafer with electrodes to the PDMS channel, a Reactive Ion Etcher with Oxygen plasma was used at Cal Poly San Luis Obispo with the help of Materials Engineering Graduate student Ryan Rivers. Treating a surface with plasma prior to bonding greatly increases the bonding strength by cross linking the surface, which is particularly important for PDMS. PDMS will adhere much stronger when the polymers on its surface are cross linked. A notebook study was performed to test optimal settings for bonding PDMS to glass, based off J. Fadriquela's protocol [34]. The settings tested were gas pressure in the chamber, power, and exposure time. Gas pressure in the chamber of a reactive ion etcher is important, because it determines the number of ions present. This can affect the amount of power required to ignite the plasma. The amount of time the plasma is treating the wafer can affect bond strength as well. Slight changes from the protocol were found to be required due to the necessary alignment step which would be performed under a microscope after treatment. The optimal settings found for the RIE were at 50mmTorr, with 20 Watts of power and anywhere between 1 to 3 seconds of Oxygen plasma treatment. Also important was the amount of time between plasma

treatment and physical bonding time. Parts were tested at optimal machine settings and then bonded after five and ten minutes. Parts bonded within five minutes of treatment had a very even bond compared to ten minutes, so five was chosen to be the maximum time allowed between plasma treatment and bonding. To further promote correct alignment after plasma treatment, a thin layer of methanol was deposited on the surface of the two surfaces that were to be bonded, to allow some movement of the surfaces before bonding. Once the PDMS channels and electrode features were aligned under microscope, the parts were immediately put in a 60°C oven for an hour. Alignment of electrodes over the chamber shown below (Figure 15).

Figure 15 – Aligned electrodes over capture chamber

3.3 – Labview Programming

In order to source the signal, perform measurements, and output a data file and a waveform, Labview 8.0 was chosen for its ease of use. The style of programming is known as graphical programming, since it utilizes different types of icons instead of blocks of text code. There are two panels (windows) in a given

project, the Block Diagram and the Front Panel. Both panels contain a Tools Palette, which allow you to alternate from wiring to selecting to probing, and a Functions Palette which allows you to place the icons that create the code or create the buttons that allow you to control inputs. The Block Diagram (an example is shown below) is where the code itself is written, and the Front Panel (Figure 16 shown below with both palettes open) is where user inputs/outputs are displayed. For example, to create a graph with a sinusoidal wave may show a button in the Front Panel to control amplitude and a graph, while in the Block diagram you would physically wire the button to control a sinusoidal wave. Labview maintains the same types of programming functions as other common languages such as C++ and Java such as “If” Statements, arrays, and “while loops” but also has some built in advanced signal processing tools. Since the goal of this project was to create a voltage source, measure a voltage drop and in turn find an impedance value for a fluid and a cell, a Labview Program titled Function Generator was created. Interacting with the Labview Program was two data acquisition cards (DAQ cards); a National Instruments Scope (NI5124) and a National Instruments Function Generator (NI-5421). These two DAQ cards come with software that has built in functions for data analysis including waveform initialization, waveform configuration, and tone measurements for frequency, phase, and amplitude. An external factor in this set-up was the use of a $1\text{k}\Omega$ resistor connecting the sensing electrode to the ground pad. The purpose of this resistor was to create a current phasor; a current is necessary to solve for impedance in the circuit, and $1\text{k}\Omega$ was chosen over lower resistances to ensure the current was a small enough value to prevent electrically damaging the cells.

The Impedance Generator code is in some ways similar to the National Instruments Bode Analyzer as far as requiring initialization and iteration of a while

loop, however the rest of the code was written specifically for this application. The code in the Block Diagram consists of initialization of both the Scope and Function Generator, including creation of a waveform. Following initialization, the waveform is configured, and the Scope is configured to read the specific waveform, in this case a sinusoidal waveform. The amplitude of the wave was given a button on the Front Panel to allow the user to change the voltage if signal was too low or high. The next body of code that executes is a loop that samples (takes a reading of) the waveform at specific intervals and frequencies. Since double layer effects were not taken into account in this study and are mostly predominant in the frequencies below 1kHz[35], impedance measurements were taken starting at 1kHz. At each frequency, the data is collected into an array and stored for further analysis. The output of a data set was a graph on the Front Panel of magnitude as well as phase with fitted lines. Additionally, three excel files with magnitude of the signal, phase, and the frequency were output. Note: for further information on the programming including step-by-step information on individual functions, please see Appendix 2.

Figure 16- The Front Panel of the Impedance Generator (Palettes shown)

Figure 17- Impedance Generator Block Diagram

3.4 – Electrical Connections

Several options were considered for connecting electrode pads to Labview Data Acquisition Cards. To minimize costs, a simple set up with standard BNC cabling along with solder was chose. BNC cables were connected to the National Instruments Function Generator and Scope. A splitter was run from the output of the Function Generator in order to measure output signal and also provide signal to the signal driving electrode. The BNC cable attached to the Scope was to be attached to the sensing electrode. BNC female jacks with solder ground pads were connected to the cables that would drive & sense the electrodes. Initial tests were performed with spare wires, connectors, and glass wafers with electrodes to verify if the wires could be soldered directly to the electrodes. The wires did successfully solder to the BNC jacks and ground tabs, however because of the extreme heat of the solder gun and the thinness of the electrodes, the solder melted through the metal layers and could not connect successfully. A polymeric electrically conducting tape was chosen due to its flexibility, conductivity, and tackiness to attach the wires to the electrode pads, as well as attach the external resistor. For sections that had trouble adhering to the metal, copper conducting tape was used to secure the wires down (Figure 18).

Figure 18 – Chip Set up and Microfluidic Test Station

The pictures above show the final set up of the test station. On the left, the picture shows the fluid connections (the flush/suction ports) along with the connections to the electrodes including wires soldered from the BNC jack to the electrode pads. Also shown is the external 1k Ω resistor.

3.5 – Interfacing with Microfluidic Station

In order to successfully introduce cells and fluids and determine when a cell was captured, the Microfluidic Test Station developed by J. Fadriqueela was used. This system incorporated a LabSmith Video Microfluidics Component Starter Kit, three syringe pumps, and Small Parts Tygon tubing. Figure 18 above shows the two computer systems connected to the device (illuminated in blue light). Using this configuration, the cell protocol and Labview programming, we were able to coordinate cell introduction with electrical activities and successfully demonstrated a capture of a bead, followed by a grouping of yeast cells.

IV. Results

4.1 - Fabrication Results

After fabrication, two devices were chosen to perform experiments with based on their alignment over the cell capture chamber. One observation of the devices was that there appeared to be micron sized gold particles spread on the glass slides. If glass is not properly cleaned in Piranha solution prior to lithography, portions of the photoresist may cling to the glass, and following metal deposition, may leave a thin layer.

Figure 19- Aligned electrodes

Figure 20- Gold particle observation

4.2 - COMSOL Modeling Results

The COMSOL model of the cell in a chamber was run from 1kHz to 100MHz to capture the full impedance curve. In this module, there are three scalar variables that can be changed; the permittivity of a vacuum, permeability of a vacuum, and the frequency. In order to get a full range of impedance values, the scalar frequency value must be manually changed each time the simulation is run. An example of an Isosurface plot output by the model is shown below (Figure 21). The

red signifies a strong electric field, while the blue and darker purple color represent areas with little electric field present. In order to get an impedance value/graph in COMSOL, a post-processing step must be taken. Since the desired measurement is the impedance of the ground electrode (the signal sensing electrode), it was necessary to plot the entire electrode domain, in this case, the face of the electrode itself. This produced the plot on the right below (Figure 22).

Figure 21- Isosurface Voltage Plot

Figure 22- Impedance Plot

For each frequency, a data point was recorded and plotted (Figure 23 below). The data forms an exponential line ranging from .15M Ω at 1kHz to approximately .13M Ω at 100MHz, with a drop off occurring around 2 MHz.

Figure 23 – COMSOL Impedance Graph

4.3 - Experimental Results

After the adjustments to the Labview program to ensure sufficient data points were taken at the lower frequencies, a full range of impedance was returned up to 40MhZ for the empty chamber, a saline filled chamber, a chamber with live yeast cells, and a chamber with a polysterene bead, and a chamber filled with dead yeast cells. For each situation, three measurements were taken and recorded in a spreadsheet for impedance, and for reference, input voltage, output voltage, and phase. The averages were plotted over time, and a fitted line was applied to the results.

4.3.1 - Initial Results

The first experiment was to run the device electrically connected to the chip with no fluid or cells in the chamber. Next, saline was injected into the chamber and measurements were taken. Both devices were tested to create a comparison, however after taking saline measurements in both, one device showed a small leak between the PDMS and glass, so final measurements were taken with one device

only. The results for saline were very similar for both devices, which was promising to show repeatability of the experiment. (Figure 24). During this initial testing, it was

Figure 24- Baseline Saline Measurement of Device 1&2

observed that the input voltage which was set to .2V, was reading at less than .1V. A noise filter was being used in Labview, however after testing signal with the filter removed, there appeared to be no change to the impedance curve. Since there seemed to be no benefit to the output and the input signal seemed to be attenuated, the filter was removed.

4.3.2- Final Test Results

The first measurement was taken of the chamber with no fluid present to confirm connections and plotted. The impedance ranged from approximately 3.2 M Ω

at 1 kHz to 2.3 k Ω at 30 MHz, and followed an exponential fitted line plot. After 30MHz, the response had considerable noise, which is possibly due to reaching the maximum range for the data acquisition card. Saline solution was then tested across the same frequency range, and impedance values ranged from 6.2M Ω at 1kHz to 7.25k Ω at 30MHz. Polysterene beads (10 micron diameter) were then introduced into the chamber until a single bead was captured (Figure 20). The impedance values for the polysterene bead ranged from 15 M Ω at 1kHz to 1.39k Ω at 30MHz. After readings were taken, the suction port was reversed to flush the chamber, and the other two flush chambers were also turned on. Once all beads had been removed, the suction port was used to remove saline left in the chamber.

To test yeast cells, several types of yeast were examined to find a species with sufficient size (10micron). The closest particles in size were Bakers Yeast (*Saccharomyces cerevisiae*) at around 6 microns in diameter. The yeast was then cultured and put into solution. Due to the size of the yeast cells, a slight deviation was taken from the cell protocol. A whirlpool effect in the inlet port was seen, so the syringe port was turned off once cells were seen in the base of the inlet port to allow flow into the device. After approximately a minute, a collection of cells had gathered in the cell capture chamber (Figure 25).

Figure 25 – Collection of Yeast Cells in Capture Chamber

Impedance values for the collection of yeast cells were higher than the previous two; they ranged from $19.7\text{ M}\Omega$ at 1 kHz , to $1.8\text{ k}\Omega$ at 30 MHz . The data also fit an exponential curve. After the yeast was measured, the same procedure was done to clean the device, however this time the initial flush of the chamber was not performed. The side flush chambers cleared the yeast cells from the inlet to the device, followed by a removal of all fluids at a very low speed in order to keep the yeast cells in the chamber. The device ports were covered so no foreign material could enter, and was let sit at room temperature for two weeks. The idea was to essentially starve the yeast cells and test if a change in viability (i.e, a change in impedance) could be measured. After two weeks, the device was set up and again tested without any fluid to ensure connections. Once verified, saline was slowly pumped into the chamber and measurements were taken of the starved yeast cells. The impedance ranged from $4.7\text{ M}\Omega$ at 1 kHz to $.42\text{ k}\Omega$ at 30 MHz , which was closer to the saline values. Data for all five sets was compiled and is shown in the table below. Of note is the amount of data points taken at the lower frequency ranges compared to the upper. This was due to the fact that there was a balance in the minimum record length, sampling rate, and the

rate of iteration of the while loop. In order to prevent timeout of the program but also get sufficient data points at the lower frequency to capture a full waveform, there had to be a high sampling rate and also a long minimum record length. To prevent timeout, the iteration of the while loop was chosen to be a smaller value ($e^{[frequency=(i+1)/7]}$), with a minimum record length of 10000 points per channel.

Table 1 – Impedance testing Results

Frequency (Hz)	Air Impedance (Ohms)	Dead Yeast Impedance (Ohms)	Saline Impedance (Ohms)	Polysterene Impedance (Ohms)	Yeast Impedance (Ohms)
1.10E+03	3.20E+06	4.72E+06	6.26E+06	1.57E+07	1.97E+07
1.27E+03	3.03E+06	4.53E+06	5.90E+06	1.40E+07	2.37E+07
1.46E+03	3.31E+06	4.81E+06	6.58E+06	1.50E+07	2.16E+07
1.68E+03	3.05E+06	4.59E+06	6.44E+06	1.46E+07	2.47E+07
1.94E+03	3.15E+06	4.68E+06	6.21E+06	1.42E+07	2.36E+07
2.24E+03	3.30E+06	4.69E+06	6.23E+06	1.46E+07	2.14E+07
2.58E+03	3.05E+06	4.78E+06	6.13E+06	1.56E+07	2.63E+07
2.98E+03	3.11E+06	4.67E+06	5.63E+06	1.64E+07	2.90E+07
3.44E+03	3.13E+06	4.83E+06	6.11E+06	1.55E+07	2.64E+07
3.97E+03	3.23E+06	4.72E+06	6.44E+06	1.51E+07	2.35E+07
4.58E+03	3.04E+06	4.85E+06	6.17E+06	1.53E+07	2.14E+07
5.28E+03	3.10E+06	4.77E+06	6.28E+06	1.57E+07	2.03E+07
6.09E+03	3.23E+06	5.08E+06	6.04E+06	1.47E+07	2.31E+07
7.02E+03	3.07E+06	4.98E+06	5.52E+06	1.53E+07	2.52E+07
8.10E+03	3.12E+06	4.63E+06	6.54E+06	1.37E+07	1.96E+07
9.35E+03	3.31E+06	4.53E+06	6.41E+06	1.51E+07	1.96E+07
1.08E+04	3.16E+06	4.35E+06	6.36E+06	1.66E+07	2.54E+07

1.24E+04	3.13E+06	4.37E+06	5.96E+06	1.44E+07	2.25E+07
1.43E+04	3.28E+06	4.66E+06	6.59E+06	1.45E+07	2.10E+07
1.66E+04	3.23E+06	4.48E+06	5.64E+06	1.48E+07	2.50E+07
1.91E+04	3.02E+06	4.64E+06	6.00E+06	1.50E+07	2.13E+07
2.20E+04	2.91E+06	4.50E+06	5.94E+06	1.53E+07	2.35E+07
2.54E+04	3.29E+06	4.71E+06	6.40E+06	1.54E+07	1.83E+07
2.93E+04	3.31E+06	4.52E+06	6.65E+06	1.57E+07	2.96E+07
3.38E+04	3.17E+06	4.60E+06	6.43E+06	1.48E+07	2.73E+07
3.90E+04	2.94E+06	4.54E+06	6.56E+06	1.47E+07	2.64E+07
4.50E+04	3.05E+06	4.69E+06	6.26E+06	1.62E+07	2.44E+07
5.19E+04	2.93E+06	4.53E+06	6.57E+06	1.52E+07	2.33E+07
5.99E+04	3.21E+06	4.40E+06	6.03E+06	1.60E+07	2.41E+07
6.91E+04	2.87E+06	4.49E+06	6.12E+06	1.59E+07	2.40E+07
7.97E+04	3.10E+06	4.53E+06	6.21E+06	1.56E+07	2.15E+07
9.19E+04	3.07E+06	4.98E+06	6.27E+06	1.46E+07	2.20E+07
1.06E+05	3.01E+06	4.30E+06	5.64E+06	1.44E+07	2.08E+07
1.22E+05	2.93E+06	4.63E+06	6.03E+06	1.54E+07	1.89E+07
1.41E+05	3.01E+06	4.70E+06	6.29E+06	1.49E+07	1.56E+07
1.63E+05	2.87E+06	4.54E+06	6.63E+06	1.53E+07	1.48E+07
1.88E+05	3.04E+06	4.84E+06	5.19E+06	1.30E+07	1.72E+07
2.17E+05	3.11E+06	4.73E+06	6.07E+06	1.34E+07	1.57E+07
2.50E+05	2.99E+06	4.96E+06	5.71E+06	1.42E+07	1.40E+07
2.88E+05	3.14E+06	4.84E+06	5.38E+06	1.17E+07	1.13E+07
3.32E+05	2.98E+06	4.70E+06	5.86E+06	1.26E+07	1.02E+07
3.84E+05	2.90E+06	4.55E+06	5.85E+06	1.16E+07	1.16E+07
4.42E+05	2.99E+06	4.21E+06	5.26E+06	1.63E+07	9.75E+06

5.10E+05	2.66E+06	4.41E+06	4.03E+06	1.23E+07	9.94E+06
5.89E+05	3.39E+06	3.77E+06	4.83E+06	9.62E+06	8.90E+06
6.79E+05	2.87E+06	4.18E+06	4.72E+06	1.28E+07	7.78E+06
7.83E+05	2.93E+06	4.71E+06	3.86E+06	1.14E+07	7.87E+06
9.04E+05	2.98E+06	4.27E+06	3.85E+06	1.25E+07	6.68E+06
1.04E+06	2.71E+06	3.84E+06	3.95E+06	1.29E+07	6.02E+06
1.20E+06	3.02E+06	3.67E+06	3.73E+06	1.08E+07	4.81E+06
1.39E+06	2.34E+06	3.64E+06	3.47E+06	1.11E+07	4.10E+06
1.60E+06	2.69E+06	3.47E+06	3.38E+06	1.01E+07	3.61E+06
1.85E+06	2.23E+06	3.30E+06	3.10E+06	9.85E+06	3.14E+06
2.13E+06	2.41E+06	3.18E+06	3.09E+06	9.24E+06	2.12E+06
2.46E+06	2.35E+06	2.94E+06	2.85E+06	1.05E+07	1.46E+06
2.83E+06	2.26E+06	2.73E+06	2.77E+06	8.96E+06	6.76E+05
3.27E+06	2.16E+06	2.77E+06	2.14E+06	7.85E+06	1.59E+05
3.77E+06	1.94E+06	2.33E+06	2.44E+06	8.03E+06	1.70E+05
4.35E+06	1.86E+06	2.64E+06	2.31E+06	6.25E+06	1.68E+05
5.02E+06	1.65E+06	1.62E+06	2.11E+06	6.31E+06	6.22E+04
5.79E+06	1.42E+06	2.38E+06	2.18E+06	6.85E+06	2.92E+04
6.68E+06	1.40E+06	1.81E+06	1.61E+06	4.28E+06	1.22E+04
7.70E+06	1.26E+06	1.77E+06	2.21E+06	5.76E+06	1.22E+05
8.89E+06	1.21E+06	1.85E+06	1.73E+06	5.19E+06	1.49E+05
1.03E+07	1.06E+06	1.40E+06	1.72E+06	3.19E+06	7.63E+04
1.18E+07	7.13E+05	1.40E+06	1.71E+06	3.30E+06	1.25E+05
1.36E+07	5.57E+05	1.06E+06	1.32E+06	2.72E+06	1.32E+05
1.57E+07	3.82E+05	5.89E+05	1.34E+06	1.51E+06	1.47E+05
1.82E+07	2.16E+05	3.58E+05	1.46E+06	8.99E+05	8.25E+04

2.09E+07	1.09E+05	1.38E+05	8.00E+05	3.30E+05	8.93E+04
2.42E+07	2.98E+04	1.81E+04	4.32E+05	4.57E+04	2.86E+04
2.79E+07	2.35E+04	4.29E+03	7.25E+04	1.39E+04	1.84E+04

Figure 26 – Impedance Curves

The above tables show the magnitude of the impedance values of all five tests. Figure 26 shows a graphical representation of the data, with an exponential curve fit applied to each testing case.

Another way of visually identifying the data to show a difference would be using a boxplot, with whiskers describing the standard deviation of the populations. This graph shows a large difference between the different data sets. For air, starved yeast, and polystyrene beads, there were several outliers at the lower end of the

frequency range. These could be due to noise in the signal due the length of samples required to obtain a full cycle

Figure 27- Boxplot of Impedance

4.4 - Statistical Analysis

In order to try to show statistical significance between saline, live yeast, and starved yeast, a t-test would normally be used to test the statistical difference between the means of the three populations. However, there are some issues with this for something like a frequency response of materials. As seen in the impedance curves, there are areas of the curves (the higher frequency ranges) where we would expect values to be much closer together, if not converge completely. Further, the data as in Table 1 is not randomized, but instead taken at the same frequencies for all three populations, and because of the trend in the data, difficult to fit to a normal curve. In order to deal with these problems, usage of nonparametric functions was necessary.

Nonparametric functions in MiniTab are used for data that fits this description; data you would not expect to fall into a normal distribution but you need to compare. In order to make further comparison, the values were cut off at approximately 0.8MHz due to the inflection of the data past this frequency. The prerequisite that then needed to be filled was randomization. In order to do this, the random data generator function in Minitab was used to randomize the frequencies in one column and place them into another column. The next step was to ensure that the magnitude values matched the correct frequencies, so a vlookup function was used in excel and applied to all columns. (Table 2)

Table 2- Randomized Impedance Data

Frequency	Air Impedance	Starved Yeast Impedance	Saline Impedance	Polysterene Impedance	Yeast Impedance
3.84E+05	2.900E+06	4.551E+06	5.845E+06	1.157E+07	1.162E+07
9.35E+03	3.307E+06	4.528E+06	6.410E+06	1.509E+07	1.965E+07
6.79E+05	2.868E+06	4.176E+06	4.721E+06	1.283E+07	7.776E+06
1.08E+04	3.160E+06	4.351E+06	6.361E+06	1.657E+07	2.538E+07
6.91E+04	2.866E+06	4.487E+06	6.125E+06	1.589E+07	2.398E+07
8.10E+03	3.117E+06	4.633E+06	6.544E+06	1.367E+07	1.963E+07
1.10E+03	3.203E+06	4.725E+06	6.259E+06	1.575E+07	1.973E+07
1.24E+04	3.133E+06	4.375E+06	5.959E+06	1.442E+07	2.249E+07
6.09E+03	3.228E+06	5.076E+06	6.037E+06	1.474E+07	2.310E+07
2.58E+03	3.053E+06	4.782E+06	6.126E+06	1.561E+07	2.630E+07
1.22E+05	2.930E+06	4.630E+06	6.033E+06	1.537E+07	1.894E+07
5.10E+05	2.663E+06	4.411E+06	4.035E+06	1.235E+07	9.938E+06
5.19E+04	2.931E+06	4.530E+06	6.575E+06	1.520E+07	2.328E+07

2.24E+03	3.303E+06	4.693E+06	6.227E+06	1.456E+07	2.143E+07
7.83E+05	2.933E+06	4.707E+06	3.863E+06	1.143E+07	7.871E+06
3.97E+03	3.234E+06	4.724E+06	6.436E+06	1.506E+07	2.353E+07
1.41E+05	3.009E+06	4.695E+06	6.288E+06	1.493E+07	1.564E+07
1.46E+03	3.305E+06	4.809E+06	6.579E+06	1.501E+07	2.163E+07
2.88E+05	3.135E+06	4.844E+06	5.377E+06	1.170E+07	1.126E+07
5.99E+04	3.213E+06	4.395E+06	6.031E+06	1.605E+07	2.407E+07
4.58E+03	3.041E+06	4.851E+06	6.168E+06	1.529E+07	2.144E+07
3.32E+05	2.981E+06	4.700E+06	5.864E+06	1.261E+07	1.024E+07
1.88E+05	3.043E+06	4.845E+06	5.188E+06	1.295E+07	1.721E+07
1.63E+05	2.868E+06	4.539E+06	6.635E+06	1.526E+07	1.483E+07
1.06E+05	3.007E+06	4.304E+06	5.636E+06	1.442E+07	2.085E+07
7.97E+04	3.103E+06	4.533E+06	6.205E+06	1.557E+07	2.146E+07
2.54E+04	3.286E+06	4.714E+06	6.402E+06	1.540E+07	1.834E+07
2.98E+03	3.110E+06	4.675E+06	5.628E+06	1.639E+07	2.895E+07
3.90E+04	2.940E+06	4.537E+06	6.557E+06	1.466E+07	2.637E+07
1.94E+03	3.151E+06	4.682E+06	6.207E+06	1.418E+07	2.364E+07
9.19E+04	3.066E+06	4.983E+06	6.269E+06	1.464E+07	2.196E+07
3.38E+04	3.166E+06	4.605E+06	6.430E+06	1.484E+07	2.726E+07
1.66E+04	3.235E+06	4.476E+06	5.643E+06	1.480E+07	2.501E+07
4.42E+05	2.989E+06	4.214E+06	5.259E+06	1.627E+07	9.745E+06
1.68E+03	3.052E+06	4.595E+06	6.438E+06	1.455E+07	2.472E+07
4.50E+04	3.049E+06	4.689E+06	6.256E+06	1.623E+07	2.440E+07
2.93E+04	3.307E+06	4.521E+06	6.649E+06	1.566E+07	2.962E+07
2.17E+05	3.112E+06	4.735E+06	6.073E+06	1.341E+07	1.575E+07

7.02E+03	3.070E+06	4.980E+06	5.521E+06	1.530E+07	2.522E+07
1.43E+04	3.281E+06	4.657E+06	6.585E+06	1.447E+07	2.095E+07
1.91E+04	3.022E+06	4.637E+06	5.998E+06	1.499E+07	2.129E+07
3.44E+03	3.128E+06	4.831E+06	6.113E+06	1.546E+07	2.642E+07
1.27E+03	3.028E+06	4.527E+06	5.897E+06	1.404E+07	2.367E+07
2.20E+04	2.908E+06	4.497E+06	5.937E+06	1.527E+07	2.352E+07
5.28E+03	3.097E+06	4.774E+06	6.282E+06	1.570E+07	2.030E+07
5.89E+05	3.391E+06	3.773E+06	4.827E+06	9.620E+06	8.902E+06
2.50E+05	2.992E+06	4.957E+06	5.713E+06	1.424E+07	1.405E+07

The next step was to decide what values were to be compared. Of most importance was the difference between yeast to saline, yeast to starved yeast, and starved yeast to saline. These columns were subtracted from another to create three new columns with the differences displayed in Table 3.

Table 3- Difference Columns

Yeast-Saline	Yeast-Starved Yeast	Saline – Starved
5.77E+06	7.06E+06	1.29E+06
1.32E+07	1.51E+07	1.88E+06
3.05E+06	3.60E+06	5.45E+05
1.90E+07	2.10E+07	2.01E+06
1.79E+07	1.95E+07	1.64E+06
1.31E+07	1.50E+07	1.91E+06
1.35E+07	1.50E+07	1.53E+06
1.65E+07	1.81E+07	1.58E+06
1.71E+07	1.80E+07	9.60E+05

2.02E+07	2.15E+07	1.34E+06
1.29E+07	1.43E+07	1.40E+06
5.90E+06	5.53E+06	-3.77E+05
1.67E+07	1.87E+07	2.04E+06
1.52E+07	1.67E+07	1.53E+06
4.01E+06	3.16E+06	-8.44E+05
1.71E+07	1.88E+07	1.71E+06
9.35E+06	1.09E+07	1.59E+06
1.51E+07	1.68E+07	1.77E+06
5.89E+06	6.42E+06	5.33E+05
1.80E+07	1.97E+07	1.64E+06
1.53E+07	1.66E+07	1.32E+06
4.38E+06	5.55E+06	1.16E+06
1.20E+07	1.24E+07	3.43E+05
8.19E+06	1.03E+07	2.10E+06
1.52E+07	1.65E+07	1.33E+06
1.53E+07	1.69E+07	1.67E+06
1.19E+07	1.36E+07	1.69E+06
2.33E+07	2.43E+07	9.53E+05
1.98E+07	2.18E+07	2.02E+06
1.74E+07	1.90E+07	1.52E+06
1.57E+07	1.70E+07	1.29E+06
2.08E+07	2.27E+07	1.83E+06
1.94E+07	2.05E+07	1.17E+06
4.49E+06	5.53E+06	1.04E+06
1.83E+07	2.01E+07	1.84E+06

1.81E+07	1.97E+07	1.57E+06
2.30E+07	2.51E+07	2.13E+06
9.68E+06	1.10E+07	1.34E+06
1.97E+07	2.02E+07	5.41E+05
1.44E+07	1.63E+07	1.93E+06
1.53E+07	1.67E+07	1.36E+06
2.03E+07	2.16E+07	1.28E+06
1.78E+07	1.91E+07	1.37E+06
1.76E+07	1.90E+07	1.44E+06
1.40E+07	1.55E+07	1.51E+06
4.08E+06	5.13E+06	1.05E+06
8.34E+06	9.09E+06	7.56E+05

For each of these columns, Run's Test for nonparametric data was performed in Minitab, which verifies if data was randomized (results shown below). As in all hypothesis testing, there must be a null hypothesis (H_0) and an alternative (H_A). In this test, the null hypothesis is that the data is randomized. In order to reject this hypothesis, a p-value must be calculated by Minitab to be less than .05, meaning that there is 95% ($1-.05$) confidence that the data is randomized.

Runs Test: Yeast-Saline

Runs test for Yeast-Saline

Runs above and below K = 14109123

The observed number of runs = 21

The expected number of runs = 23.6383

28 observations above K, 19 below

P-value = 0.419

Runs Test: Yeast-Starved Yeast

Runs test for Yeast-Starved Yeast

Runs above and below K = 15455006

The observed number of runs = 21

The expected number of runs = 23.2128

29 observations above K, 18 below

P-value = 0.489

Runs Test: Saline - Starved

Runs test for Saline - Starved

Runs above and below K = 1345883

The observed number of runs = 27

The expected number of runs = 23.9787

27 observations above K, 20 below

P-value = 0.362

For the first test (Yeast-Saline), the p-value was .419. For the second test (Yeast – Starved Yeast) the p-value was .489, and for Saline – Starved, the p-value was .362. So for all three tests, we failed to reject the null hypothesis (that the data is randomized) for all tests. Once the data was proven to be randomized, a test needed to be run to show that the difference between the three populations was not equal to zero. To investigate if there was a statistical difference, a nonparametric function called a One-Sample Sign test was used to test this hypothesis by comparing the medians of the populations. In other types of data that fit distributions, typically the

means would be compared, however the median is more resistant to distribution and is used in non-parametric analysis.

Sign Test for Median: Yeast-Saline

Sign test of median = 0.00000 versus not = 0.00000

	N	Below	Equal	Above	P	Median
Yeast-Saline	47	0	0	47	0.0000	15255165

Sign Test for Median: Yeast-Starved Yeast

Sign test of median = 0.00000 versus not = 0.00000

	N	Below	Equal	Above	P	Median
Yeast-Starved Yeast	47	0	0	47	0.0000	16733394

Sign Test for Median: Saline - Starved

Sign test of median = 0.00000 versus not = 0.00000

	N	Below	Equal	Above	P	Median
Saline - Starved	47	2	0	45	0.0000	1440030

For all three tests, the p-value was very close to zero meaning the null hypothesis (that the difference between the populations was zero) must be rejected.

V. Discussion

5.1 - COMSOL Model

The graph for the COMSOL model shows an exponential decay in the impedance as the frequency increases. The high value at lower frequencies is expected, since at low frequencies the cell membrane shields the cell [28], preventing it from conducting a signal. Information about the size of the cell at these lower frequencies can be achieved due to the fact that the cell fills the channel, and because of its high membrane impedance the signal will drop occurs across the membrane instead of the fluid [33], so the response is based on the cell. Also important at lower frequencies is the presence of the double layer which causes the impedance of the cell to act like a capacitor [36] and causes a nonlinear relationship between the cell volume and the signal [13]. The flat line in the graph between 1kHz and approximately 2MHz (the intermediate frequencies) can also partially be explained by the behavior of the double layer, which allows more current flow as the frequency increases until the resistance matches the resistance of the solution. At these mid-range frequencies, the depolarization of the membrane begins to decrease, and the charge in the cell membrane begins to build, which is known as dielectric relaxation period or β -relaxtion [23]. Cell membrane capacitance however, will increase and lead to plasma membrane permittivity contributing most. At upper frequencies, past the dielectric relaxation frequency, the membrane no longer provides an impedance barrier. Thus, the signal at these high frequencies gives you information about the inside of the cell, including cytoplasm and organelles.

One of the biggest disadvantages to using this mode has been discovered by others [13] and here; it's difficult to account for the electrode interface impedance. This model is also limited slightly due to the fact that the driving electrode is controlled by a

fixed current density. In COMSOL, this means that the value of the current is either set to 0 or 1, which would prevent the magnitude from matching the live cells. The other factor not included in the model was the external resistor used in the experiments.

Despite some limitations, the COMSOL cell chamber model did give useful information. From the initial models, a distance between electrodes was optimized to create even electric field lines. The results of the model also matched the shape of the curve of the experimental results fairly well. Also, the rate of decay of the impedance past the β -relaxation was similar to the experimental results.

5.2 - Impedance Measurements

The impedance graph of the polystyrene bead, saline, empty chamber, yeast and starved yeast cells shows the highest impedance magnitude was yeast, followed by the polystyrene bead, saline, starved yeast, and the empty chamber. This follows other studies which show that impedance magnitude increases when particles are in the chamber [36, 37]. Saline impedance values were higher than air impedance, The high magnitude of the yeast impedance could be due to the fact that there was a fairly large collection of cells in the chamber as opposed to a single cell. The point of dielectric dispersion for the yeast appears to be approximately $.1\text{M}\Omega$, then begins to decrease rapidly. This is due to the fact that the membrane stops offering resistance at the higher frequencies and lead to high cytoplasmic conductivity. This rapid drop is not present to such a high degree on any of the other graphs.

Further analysis of the graph show that the starved yeast cells and saline are very similar in magnitude throughout all frequencies. This could be due to the fact that as cells are starved from nutrients and undergo cell death, the membranes can

rupture, and the electric field path would essentially see the same impedance as the saline solution.

The statistical analysis examined whether or not there was a statistical significant difference between yeast, saline, and starved yeast. After randomizing, the column differences were taken, and the hypothesis test asked whether or not the differences were equal to zero. For all three tests the hypothesis that the differences were zero was rejected, so all tests had a significant difference. Yeast magnitude would be expected to be higher than saline since the layers of the cell would serve to protect it, and produce a higher resistance to electrical signal. It is also expected that there would be a difference between the two yeast samples since yeast cell viability would be expected to degrade over time without nutrients or solution to suspend it. As the cells degrade over time, it was hypothesized that the values would approach those of saline. While the saline to starved yeast comparison showed a statistical difference, the average median difference was a full order of magnitude smaller than the differences between yeast-saline and yeast-starved yeast.

VI. Major Conclusions

6.1 – Experiment Conclusions

The goal of this project was to capture a single cell from solution and take an impedance measurement of that single cell in order to obtain information about cell properties. To achieve this goal, the project was divided into six objectives; create a model-assisted design, fabricate an impedance chamber with electrodes, create the Labview interface to drive the Impedance sensor, set-up an Impedance test station, differentiate yeast viability, and fulfill all MS requirements.

The first objective was met in several ways. Several generations of SolidWorks model were created and used to optimize the electrical signal across the chamber. This was coupled with 3-D COMSOL designs to actually visualize the field lines and predict a response.

This method of fabrication was also successfully established. By creating a CAD model of the electrodes to characterize any scaling the photomask would require, a successful mask was created. Titanium and gold electrodes were successfully deposited with correct dimensions to match the capture chamber, after characterizing the electron beam deposition and photolithography steps. In particular, exposure time and post baking time investigation helped in creating the correct dimensions. Experiments to find optimal settings to bond to the PDMS device were performed to optimize the process, and several devices were able to be fabricated, demonstrating that this method was repeatable.

Electrical signaling and chip set-up were effectively demonstrated by writing a Labview program and interfacing National Instrument Data Acquisition cards directly to

the chip. This program allowed the user to input voltage and sampling size, and output the impedance measurements graphically and also into a spreadsheet for analysis.

Yeast impedance measurements were also established from both a live healthy population, and a population that had been deprived of nutrients and suspending medium. The impedance data was compared for the two and found to be statistically different. The yeast cells were however in a cluster, as the size of Baker's yeast averages around 6 μm in diameter and the chamber was designed to capture a 10 μm particle. To prove that single cell measurements were plausible, a polystyrene bead (10 μm in diameter) was captured and impedance measurements successfully taken.

The final objective of the project was to fulfill the Masters Degree requirements, and completion and defense of this work will achieve this.

6.2 -Future Research

For this specific design, the next step would be to design the system so it can truly capture a single live particle and measure impedance. Another improvement would be to find an easier, more reliable method of bonding the PDMS chamber to the glass wafer so that the electrodes are aligned to the chamber without having to manually align them before the plasma treatment expires. Further work could be done to better measure the phase values (which for this study were ignored due to large noise that could not be filtered out) to even further characterize cell responses.

Applications for research using this type of device are numerous and exciting. As these micro analysis systems have gained popularity, many are aiming to replace expensive testing procedures with simple devices like the single cell impedance sensor created. For instance, testing water for specific types of bacteria could be

automated by an impedance sensor that can differentiate via electrical signal if a “bad bacteria” is present, as a study [38] has shown to be possible. Other studies in the last few years have started to experiment with tissues and cell types that could help provide quantitative data for conditions the previously were not well-defined. For example, a recent study done on cardiac myocytes with an impedance sensing system can help to prove information on cardiac hypertrophy to help understand sudden cardiac death. This would otherwise involve invasive and expensive procedures to study. Other studies have shown that cancerous cells can be examined using cell sorting [28], trapping [39, 20], and impedance measurements, which could potentially be used to better define stages of cancer with quantitative data. Other possible venues for this research include stem cell differentiation monitoring and viral induced cell changes. By combining impedance measurements with other dielectrophoretic methods, the potential for these devices is immense.

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Appendix

A. E-Beam #4 Operating Procedure

Standby Conditions:

Vacuum Gauge Controller Panel (Granville Phillips 307)

1. Verify status of vacuum system.
 - a. IG1: Cryo pump indicating high vacuum ($\times 10^{-7}$)
 - b. IG2: Chamber vacuum ($\times 10^{-7}$)
 - c. A: Roughing Line ($\times 10^{-3}$).
2. Verify Mechanical Pump Gas Bleed Valve switch is in 'AUTO'.
3. Check Cryo Temperature (10-14K)

Fixture Control Panel

1. Auto/Off/Manual Rocker Switch in 'OFF'.
2. Auto/Closed/Open Shutter Switch in 'AUTO' position.
3. Slow/Fast Potentiometer Knob set for normal application speed.
4. Direction Toggle Switch set for normal application direction.

Deposition Controller (Inficon IC/5)

1. If not in 'READY' press 'STOP' then 'RESET'.
2. Check for correct process selected and correct programmed thickness. (Eight most commonly used processes may be selected using Process Select Control Knob on E-Beam Gun Rotation Control Panel-other processes select using Inficon IC/5).

E-Beam Gun Rotation Controller

1. Auto/Off/Manual Rocker Switch in 'AUTO'.
2. Process Selector Switch set to desired Process Number (check that the IC/5 displays the same process number).
3. Source Select Switch will not necessarily indicate the programmed process material until HIVAC is open and IG2 is ON.

Auto Tech II Panel

1. Rotary Mode Select Knob set for 'AUTO'.
2. Rocker Switch should be set to 'START'.

Process Run Preparation

Vent Chamber

1. Press IG1 on the vacuum gauge controller.
2. Switch Auto Tech IIC rocker to 'VENT' causing chamber to vent with nitrogen.
3. The Granville Phillips should read as follows:
 - a. IG1 (System Ion Gauge): $<2 \times 10^{-7}$
 - b. Convectron Gauge A (Mechanical Pump foreline): $<1 \times 10^{-2}$
 - c. Convectron Gauge B (Chamber): 760 Torr
4. Bell Jar opens automatically.

Inspect and Load

1. Open Shutter
2. Inspect/Clean chamber as required with vacuum.
3. Inspect individual EB Gun pockets for condition of melt and add material as necessary. (To turn turret, place E-Beam Gun Rotation Control Source Select Auto/Off/Manual rocker switch to 'MANUAL'—place back in 'AUTO' when inspection completed.)
4. Close Shutter by pressing Shutter Auto/Closed/Open rocker switch to 'AUTO'.
5. Remove lift off dome to load samples or load samples with dome installed.
6. Place ROTATION Auto/Off/Manual rocker switch to 'AUTO'.
7. When lift off dome reinstalled, switch Auto Tech IIC rocker to 'LOWER/START'. **When bell jar is seated, switch Auto Tech II rocker to 'STANDBY' (within 20 sec.) and verify crystal sensor condition and replace if ACT(activity)<125.**

Deposition Cycle

Automatic Deposition

1. Push the rocker switch on the Auto Tech II from 'STANDBY' to 'START'. After a short Delay (20 sec.), the roughing valve opens. The ROUGHING light will illuminate, the STANDBY light will go off and the AUTOMATIC light will stay lit.
2. When Vacuum in the Chamber (Convectron Gauge 'B') has reached crossover setpoint of **1.2x10⁻¹ Torr**, the Auto Tech II will automatically switch to High Vacuum. The ROUGHING light will go off and the HIVAC and FORELINE lights will illuminate. The sample holder tooling will now begin to rotate and the green ROTATION indicator light will illuminate. (Note: if crossover setpoint is not reached within 6minutes, the 'R' light on Auto Tech II illuminates and the Auto Tech II rocker switch automatically goes to 'STANDBY'. The adjacent 'H' light illuminates if ever the HIVAC valve shuts automatically due to an overpressure condition.
3. Press IG2 on the Granville Philips ion gauge controller.
4. When IG2 indicates **3x10⁻⁶ Torr**, close SR10 mainframe breaker. The INTERLOCK light string on the CHA SR10 Controller front panel will illuminate to allow automatic deposition. A power level will be indicated on the Gun Control Module for the SR10 (LO, MID or HI).
5. Turn KEY to ON, switch H.V. to ON, switch E-Beam Gun to ON and Press Start on the IC/5 Deposition Controller. High Voltage comes on and Deposition current starts to rise according to IC/5 sequence: Ramp 1, Soak 1, Ramp 2, Soak 2, Deposit, Idle Ramp, Stop. While in Ramp 1 through Soak 2 steps, manipulate latitude and longitude stepper knobs to melt any new pellets and adjust position of beam. After Shutter opens for Deposition, watch for constant power levels and deposition rates and position of beam.
6. When Process is completed and IC/5 is indicating IDLE, wait five minutes or more until source material has cooled (not glowing) before venting or starting a new process.
7. Switch E.B. Gun to OFF, switch H.V. to OFF, turn key to OFF, Open SR10 Mainframe breaker, switch to IG1.
8. Push the Auto Tech II rocker switch to 'VENT'. Vent light will illuminate. Bell jar will automatically open.
9. Remove sample.

Restore to Standby Conditions

1. Set the Rotation Rocker Switch on the motor control panel to the 'OFF' position.
2. Push the Auto Tech II rocker switch to 'LOWER/START'.
3. The system will go into the ROUGH PUMPING mode. The ROUGHING light illuminates.
4. Allow the system to pump down to crossover setpoint and then turn on IG2 ion gauge.

5. IC/5 controller should display READY. If it does not, push the 'STOP' and then 'RESET' on the deposition controller.
6. Log Usage.

B. Labview Graphical Program Documentation

Programming Notes:

- All boxes/wires in orange represent numbers that are treated as "double"
- All boxes/wires in blue pass a number as a "single"
- All pink wires are connected error in or error out and pass error messages from one function to the next
- Purple wires ensure that the correct DAQ card and name from that session are passed from one function to the next
- Green text boxes indicate functions that can be controlled or are displayed on the Front Panel

1. Labview Block Diagram Initialization functions and wiring inputs for both Scope and Function Generator.

NI-FGEN Initialization

Creating this pull-down Menu Button on the Front Panel to allow the user to select the appropriate data acquisition card (DAQ card).

Initializes the DAQ card selected.

Configure Output Mode function is necessary to tell the card whether to create an arbitrary waveform or a standard waveform. In this case we want a standard waveform. 0 is wired into the output mode to signify standard.

The next step is to configure the standard waveform. Here the type of waveform can be set (sine, cos, etc) along with the properties of the waveform such as waveform, amplitude, DC offset, frequency, and start phase. Here, a sine wave was used and a Front Panel button was wired to the Amplitude input to allow user control. This same amplitude is relevant to initialization of the NI-SCOPE. The initial frequency was set to e^7 since impedance readings tend to not change until around a kHz and based on the sampling size, taking readings starting at 1Hz could time out the scope.

To commit all the above data to memory, the Commit function must be used.

Finally, the waveform with all the saved properties that are now committed to memory, can be generated.

SCOPE Initialization

This function allows to choose the appropriate DAQ card for the SCOPE. This is created by opening the Front Panel and choosing IVI Logical Name from the Input/Output section. IVIs (Interchangeable Virtual Instruments) include oscilloscopes, function generators, power meters, spectrum analyzers, etc so this was used for both niFGEN and niSCOPE.

Initializes the scope.

After initialization, the scope must be configured. To configure the vertical range (in this case, the amplitude) the range is the user specified amplitude multiplied by 1.25 to ensure the full waveform is captured. The channels (0,1) are also configured at this step.

The next step is to configure the characteristics of the channels that were just chosen. The input impedance of the card must be selected (for this card it is 50 ohms) and the maximum input frequency must be input based on the sampling rate chosen.

Configure Horizontal Timing (chose a minimum record length of 10000 to get that many points per channel to ensure that at lower frequencies getting enough data points). Sampling rate is chosen based on Nyquist Theorem which says the sampling rate should be twice as high as the bandwidth or upper frequency to avoid signal aliasing. The maximum frequency of the function generator is ~40MHz, however the maximum bandwidth of the scope is 60MHz, so the final frequency reading used was lower, around 27 MHz with a sampling rate of 50MHz.

Configure Trigger (chose edge trigger. 0 = when to trigger)

Property node (returns actual value of sample rate) and Commit.

2. Labview Block Diagram While Loop Code.

While Loop

While loop is designed to perform measurements during every iteration, i , until it reaches a set condition, in this case an error. The loop starts at the starting frequency input at NI-FGEN Configure Waveform (e^7) and is iterated by $e^{[frequency=(i+1)/7]}$. This value of 7 was chosen by trial and error to find the optimal amount of data points to get an accurate waveform. The iteration reads the frequency and displays the current value on the front panel.

After initialization, the errors from the Scope and Function Generator are merged, and a property node box was used to set the frequency value.

Following the frequency property node, the error wire was connected to the NI-SCOPE abort function. This function will abort the loop if an error occurs and will output an error message.

Since the frequency value has now been set, data acquisition can begin. This function starts data acquisition.

This block of code designates the number of samples to be taken at this frequency. Number samples here = sample rate * frequency/100. This scope function reads in data from the specified channels (0,1) and returns the data as sets of 1-Dimensional Arrays.

Extraction of waveform information

This block uses the Index Waveform Array (Under Programming -> Waveform) to retrieve data from indices 0 and 1, which have information from channels 0 and 1 at that frequency. Then the Extract Single Tone Function was connected to record the amplitude with the highest value as well as the phase.

After the Extract Single Tone Function outputs amplitude and phase data for each channel, the impedance value could be calculated. From Ohm's Law, voltage (V) is a function of current (I) measured in Amperes and resistance (R) measured in Ohms. For the following equivalent circuit, the Impedance of the cell can be calculated.

Since two voltages are measured (V_{OUT} directly after the cell and input V_S), Kirchhoff's Current law can be applied. At node 1, the current in must equal the current out. Since the current out only experiences a voltage drop across the external resistor R_X , the equation on the right hand side is $I = V_{OUT}/R_X$. To calculate the current in, a resistance

drop is experienced across the cell. Therefore the current here $I = (V_{OUT} - V_S)/Z_C$.

Which gives:

$$V_{OUT}/R_X = (V_S - V_{OUT})/Z_C \quad \text{solving for } Z_C \text{ gives:}$$

$$Z_C = (V_S - V_{OUT})/(V_{OUT}/R_X)$$

Phase data was directly extracted from both channels and the difference subtracted.

3. Labview Program Post-Processing Steps

This group of code takes the calculated magnitude and phase data, groups it along with the frequency to create two dimensional arrays of numbers. Each number is recorded during the loop iteration and stored into this array, and after the loop has completed is output into a spreadsheet.

This block of code following the loop feeds any final errors into the Close functions of both the scope and the function generator. The DAQ cards turn off, and if any errors are found, it outputs the messages to the Front Panel.

This last block of code serves to show the graphs seen in the Front Panel. The magnitude vs. frequency is connected to an array, as well as the phase vs. frequency. Before the array however, an Exponential Curve Fit line function was used so that the final graph wouldn't display all the individual data points but instead the trend.