

Using Dielectric Spectroscopy in Radio Frequencies to Measure Membrane Potential of Mouse Hypridoma cells

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Abstract—This article explores using dielectric spectroscopy in an attempt to measure the trans-membrane potential of mouse hypridoma cells in the radio frequency spectrum. The purpose of this test is to "mimic" a capacitor with the cell suspension acting as a dielectric. This is a non-invasive way to measure the average membrane potential across your cell suspension and has been proven in [1] for frequencies up to $10^5 Hz$.

I. IMPLICATIONS OF MEMBRANE POTENTIAL

Although there may be many implications of transmembrane potential at radio frequencies, the focus of this example is in disease models. In the late 1930's it was suggested that a relationship lie between cancer and the bioelectric properties of host tissue. Interestingly, membrane voltage (V_{mem}) analysis in many different mammalian cell types reveals that proliferative (to grow by rapid production of new parts) potential is correlated with unique ranges of V_{mem} : quiescent cells (cells withdrawn from the cell cycle and do not proliferate) tend to be hyperpolarized (more negative), whereas highly plastic cells such as embryonic cells, adult stem cells and tumors cells are depolarized (more positive). [4]. During the early stages of tumor formation, V_{mem} is a key regulator of the cell cycle and determines the proliferative state of many different kinds of cells. These statements excite the possibility of non-invasive techniques being used to track bioelectric cell states and detection of tumors, or even mitigation of tumor formation by canonical oncogene (gene that has potential to cause cancer).

II. HYPOTHESIS

Mouse hybridoma cells when exposed to a range of radio frequencies will have a constant relative dielectric permittivity, when using dielectric spectroscopy.

III. CALCULATING MEMBRANE POTENTIAL

Modeling the impedance of the cell suspension as a resistor $R = d/\sigma A$ in parallel with a capacitor $C = \epsilon A/d$, where σ and ϵ are the conductivity and dielectric permittivity of the cell suspension, and A and d are the surface area of the two disk electrodes and the distance between them.

$$\sigma(\omega) = \frac{d}{AZ} \quad (1)$$

$$\begin{aligned} C &= \frac{1}{sZ}, s = j\omega \\ \frac{1}{sZ} &= \frac{\epsilon_r \epsilon_0 A}{d} \\ \epsilon_r &= \frac{d}{s\epsilon_0 AZ} \end{aligned}$$

$$\epsilon_r(\omega) = Im \frac{d}{\epsilon_0 \omega AZ} \quad (2)$$

$$Z^S - Z^P = Z^O \quad (3)$$

Z^S is the sample impedance, Z^P is the unknown device impedance, and Z^O is the measured impedance.

IV. DISTANCE BETWEEN PLATES:

The near and far field transmission will depend on plate distance. The most agreed upon definition of near field transmission is less than one wavelength(λ) away [3]. Considering a sinusoidal wave traveling at a constant speed, the wavelength can be calculated with the following formula ...

$$\lambda = \frac{v}{f} \quad (4)$$

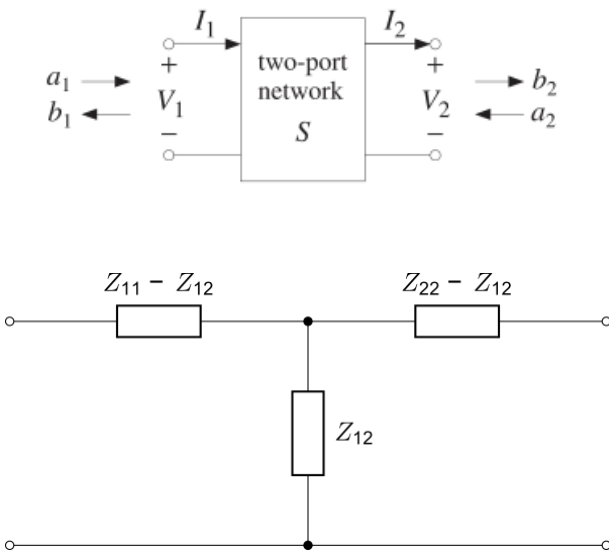
v is the magnitude of the phase velocity and f is the frequency of the sinusoid. It is difficult to determine the phase velocity of our electromagnetic wave while propagating through the cell suspension, but considering water, a prediction of the wavelength can be made. The velocity of EM waves is more than 4 orders faster than acoustic waves according to [2]. Knowing that the speed of sound is $343.2m/s$ we can say ...

$$\lambda = \frac{343.2 \cdot 4}{10^9} = 1.3728 \times 10^{-6}m \quad (5)$$

Water is a good basis for the phase velocity as the cell suspension is made from distilled H_2O and mouse hybridoma cells. $10^9 Hz$ will be used as the minimum radio frequency. The micrometer is sensitive to $10\mu m$, thus the test fixture will be capable of measuring only waves 10 times greater than the fundamental wavelength; meaning far field transmissions will be measured. This is a satisfactory result, as the far field is the "real" radio waves that propagate through space at relatively the speed of light [3].

V. S-PARAMETERS

At low frequencies it is common to use the transfer and impedance matrices, since this experiment uses high frequencies, scattering parameters are more preferable. A linear two-port network is used to characterize the equivalent circuit parameters for our experiment. More specifically, scattering parameters are used to relate the outgoing waves b_1, b_2 to the incoming waves a_1, a_2 . The parameters S_{11}, S_{22} are the reflection coefficients, or the ratio of the amplitude of the reflected wave to the incident wave. The parameters S_{21}, S_{12} are the transmission coefficients, or the amplitude, intensity or total power of a transmitted wave relative to a incident wave (power of propagation through a medium). As a reminder, the incident wave is the wave traveling from source to load, as the reflected wave is the traveling from load to source.



$$Z_{11} = Z_0 \frac{(1 + S_{11})(1 - S_{22}) + S_{12}S_{21}}{(1 - S_{11})(1 - S_{22}) - S_{12}S_{21}}$$

$$Z_{12} = Z_0 \frac{2S_{12}}{(1 - S_{11})(1 - S_{22}) - S_{12}S_{21}}$$

$$Z_{21} = Z_0 \frac{2S_{21}}{(1 - S_{11})(1 - S_{22}) - S_{12}S_{21}}$$

$$Z_{22} = Z_0 \frac{(1 - S_{11})(1 + S_{22}) + S_{12}S_{21}}{(1 - S_{11})(1 - S_{22}) - S_{12}S_{21}}$$

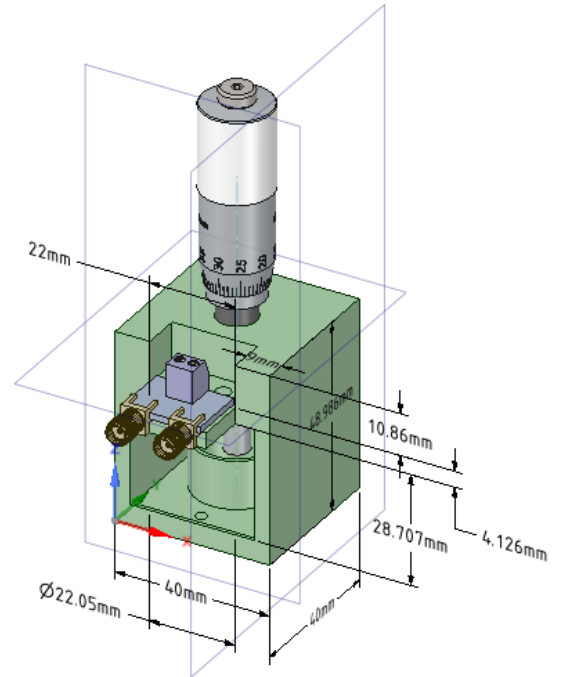
The found S-Parameters can be converted to Z-parameters to get the model of the Device Under Test (DUT) as shown in the resistor network above.

VI. TRANSMEMBRANE POTENTIAL

It is generally accepted that the cell membrane can be represented by a dielectric shell (ϵ_1, σ_1). The core can be described by (ϵ_2, σ_2). These live cells contain negative charges inside and attract the positive charges, mainly potassium and sodium ions on the outside. The dielectric response of live cells is fundamentally different than dead cells. The main difference between the two is the membrane potential in live

cells. The effect of the membrane potential is an accumulation of mobile electric charge carriers at the membrane surfaces. When introducing a time-oscillating electric field, these charges move on the surface of the membrane. Since the mobility of these surface charges is relatively small, this effect appears only at low frequencies, typically $< 10kHz$. In this range the relative dielectric permittivity of live cell suspensions can be as high as 10^6 . This phenomenon is known as the α -relaxation effect[8]. There is a sharp frequency above which the ions can no longer follow the electric field. Above this frequency, the polarizability of the cells drastically decreases and the α -effect disappears.

VII. MATERIALS



A. Copper Plates

Two copper plates $3/4$ inch in diameter will be used. One plate will be fixed to the bottom of the beaker and the other is attached to a micrometer. To create the copper plates a hole puncher will be used.

B. Copper Wire Leads

Two wires will be attached to the copper plates. The wire attached to the fixed bottom plate will be attached to the center, and run through the bottom of the test fixture to later be attached to a network analyzer. The second wire will be attached to top plate on one of the edges and run up the side of the beaker to be attached to a network analyzer. These two wires will provide the radio frequency signal and the probes to be attached to a network analyzer.

C. 3-D printed Beaker

A 3-D printed beaker will be made to perfectly fit the test fixture. The beaker will be made of ABS plastic and is used to hold the cell suspension and two copper plates. It will be placed in the center of the test fixture stand.

D. Micrometer

A micrometer will be used to adjust the height of one of the copper plates. The micrometer will be attached to the test fixture stand, and the drive of the micrometer will be attached to one of the copper plates. A micrometer cap will be 3-D printed in ABS plastic to perfectly fit the micrometer and later be adhered to the copper plate.

E. Test Fixture Stand

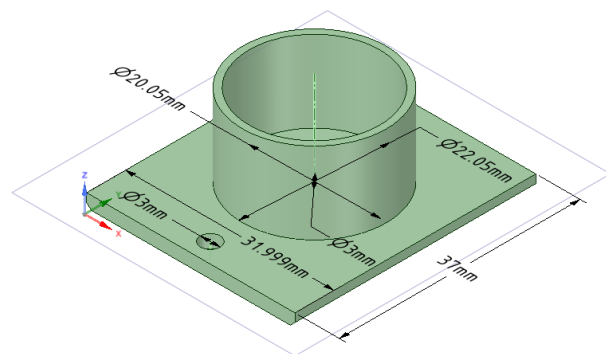
The test fixture stand will provide a base for the beaker as well as an accurate and stable micrometer mount. This item will be 3D printed with ABS plastic.

F. Cells

Cells will consist of a line of SP 2/0 myelomas hybridoma. These are nonadherent cells, and are cancer cells from mice.[6] They are rated at biosafety level 1. This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. Research with these agents may be performed on standard open laboratory benches without the use of special containment equipment, and it is not necessary for Biosafety level 1 labs to be isolated from the general building.[5]

G. Hewlett-Packard 8735E Network Analyzer 30kHz - 6GHz

Used with this network analyzer was the Agilent 11857D 7mm test port returns(50 Ω). This network analyzer will be used to gather our scattering parameters. This version features the 006 option which give it a 6 GHz upper frequency range.
Resolution: 1Hz
Stability: typically $\pm 7.5\text{ppm}$
Accuracy: $\pm 10\text{ppm}$
Resolution: 0.05dB



VIII. METHODS

A. Procedure for splitting cells for regrowth and testing

B. Tools and Equipment

- New cell culture flask
- Centrifuge
- 1ml centrifuge vials
- Pipette with 10ml graduated tips
- Bleach
- Phosphate buffer solution (PBS)
- Cell food (10% FBS PFS solution)
- Vial rack for the centrifuge vials
- 50ml sealable test tube

C. Personal Safety Equipment

For bio safety level 1, the use of gloves and a lab coat is required for personal protection. The cells are not to leave the bio safety level 1 lab room. This is a requirement set forth by those in charge of the biology lab.

D. Notes

- The cell suspension will become more acidic over time, yellow indicates optimal cell growth and orange indicates high acidity. Dark pink is the original color of PBS solution.
- The cells are being kept under conditions of 37 $^{\circ}\text{C}$ and 4.9% CO_2 in the incubation chamber when not in use.

E. Procedure

Centrifuging:

- 1) Remove the flask from the incubator.
- 2) Using the pipettes, put 1 ml of cell solution from the cell culture flask into each of the centrifuge vials.
- 3) Seal the vials and evenly distribute them in a centrifuge. Dispose of any vials that would cause an odd number. Run the centrifuge at 1.7krpm for 5 min. Once complete, there will be a pellet of cells in the bottom of the vials.
- 4) Split the vials into two equal groups, one group will be used to create the solution for testing and the other will be used for regrowth.

For cell regrowth:

- 1) Siphon off the solution in the vials and put it in the test tube.
- 2) Using a new pipette, add 1ml of food to the vials. Note, the tip of the pipette should not touch the cells.
- 3) Shake the vials to dissolve the pellet.
- 4) Using a pipette, add the solution of cells and cell food to a new flask. The flask should be labeled with the type of cells or strain, and what path number they are on.
- 5) The flask is then sealed, and placed horizontally back in the incubation chamber until this process needs to be repeated.

Preparing Cells for testing:

- 1) Repeat step 1 from the Cell regrowth section.
- 2) Using the pipette, add 1 ml of a buffer solution.
- 3) Repeat step 3 of the Cell Regrowth section.

- 4) Using the pipette, the solution can be added as needed to the test fixture. It can also be stored temporarily in test tube or a cell culture flask.

For unused centrifuge vials:

- 1) Repeat step 1 from the Cell regrowth section.
- 2) Using the pipette, add 1ml of bleach to the vials.
- 3) Shake the vials to dissolve.
- 4) Using a pipette, the bleach and cell solution is added to the mixture of old cells in the disposable test tube.

Clean up:

Add several milliliters of bleach to the disposable test tube. The liquid should turn color from light blue to clear and can now be safely disposed. All surfaces and materials that are not disposable should be wiped down with 70% ether solution. All disposable equipment can be discarded in the bio-safe bins located near the hood.

IX. EXPERIMENT PROCEDURE (NETWORK ANALYZER)

Initial Setup

- Set a 10 mm spacing between the copper plates. The first plate is located where the micrometer reads 1 mm.
- Connect the Agilent 11857D 7mm test port returns(50Ω) between the network analyzer and the SMA connectors located on the test fixture. Both wires from the test fixture must be screwed into the lead terminals.
- Before moving the top plate to the desired position, insert 3.15ml of the fluid under test.
- Record all measurements to the floppy disc to be further processed.

Measurements of S-Parameters:

- 1) Measure the S-Parameters of our device with air as a dielectric.
- 2) Measure the S-Parameters with PBS (Phosphate Buffered Saline).
- 3) Measure the S-Parameters with PBS Mouse Hybridoma solution.

X. EXPERIMENTAL PROCEDURE (IMPEDANCE ANALYZER)

Initial Setup

- Set the test beaker to 3 mm spacing between the copper plates. The first plate is located where the micrometer reads 1 mm. (a total of 2mm distance between plates)
- Connect the impedance analyzer leads to wires of test fixture.
- Before moving the top plate to the desired position, insert 0.57ml of the fluid under test.
- Record all measurements on the computer.

Measurements of Impedance:

- 1) Measure the impedance of our device with air as a dielectric.
- 2) Measure the impedance with PBS (Phosphate Buffered Saline).
- 3) Measure the impedance with PBS Mouse Hybridoma solution.

Count Cell concentration

The cell concentration will be measured using a hemocytometer. The equation for finding cell concentration can be found below. The tables below show how many cells were counted in each box.

$$\text{Cell Concentration} = \frac{\text{Number of Cells}}{\text{Volume of Cells Counted}} \quad (6)$$

Experiment Procedure (Network Analyzer)

TABLE I
CELL CONCENTRATION

10	9	11	11
6	11	6	18
11	10	12	8
4	8	7	6

$$\text{Cell Concentration} = 1.48 \text{ cells/nL}$$

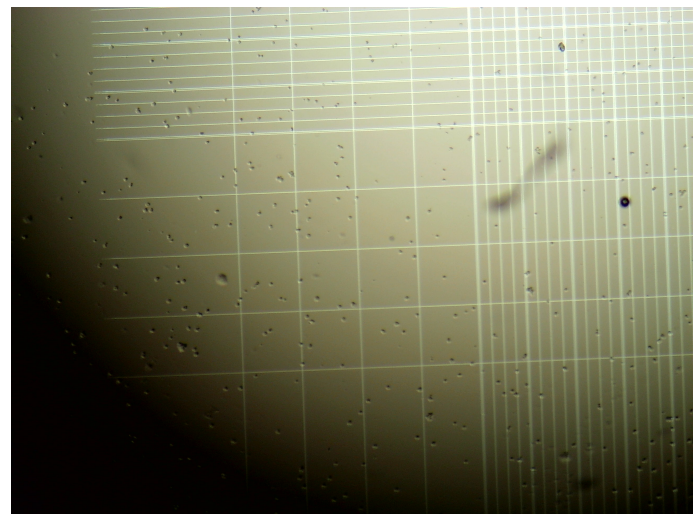
Experiment Procedure (Impedance Analyzer)

TABLE II
CELL CONCENTRATION

18	13	15	19
12	11	9	16
11	12	12	17
9	12	13	6

$$\text{Cell Concentration} = 2.10 \text{ cells/nL}$$

In the picture below an example of a hemocytometer can be observed. We used the largest sized divisions with an area of 0.0625mm^2 , or 6.25nL and a total area of 1mm^2 or 100nL



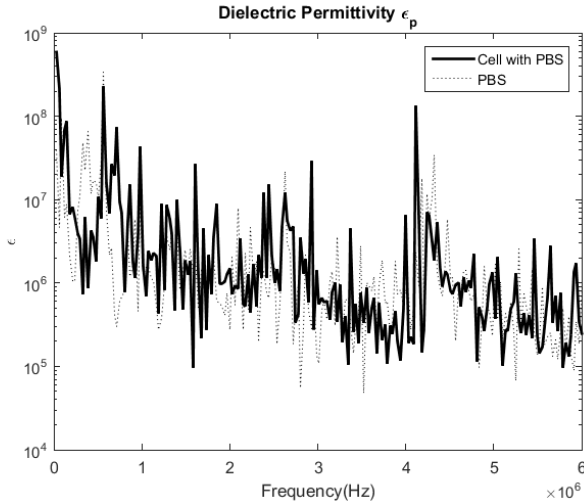
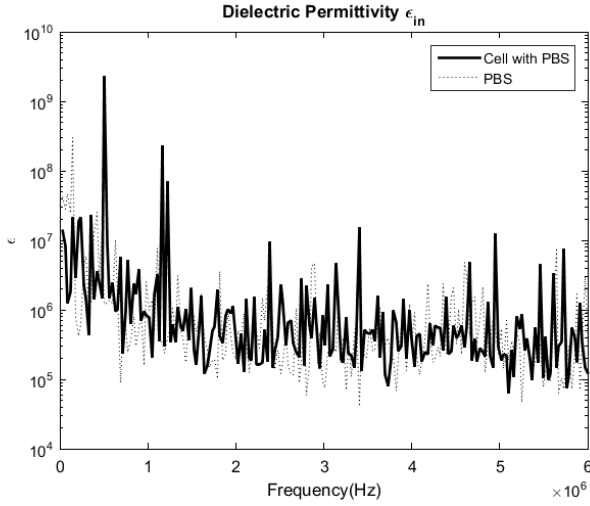
XI. DATA AND ANALYSIS

The first three plots show the relative dielectric permittivity of the device under test. The device has been modeled by a resistor network and each of the permittivities have been calculated in the following figures using equation 2.

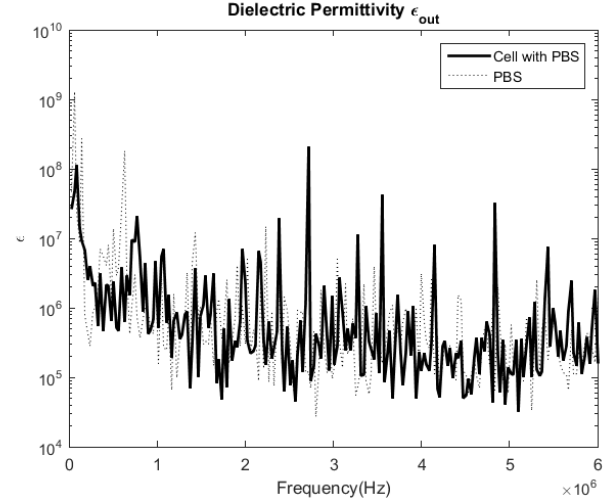
$$Z_{in} = Z_{11} - Z_{12}$$

$$Z_p = Z_{12}$$

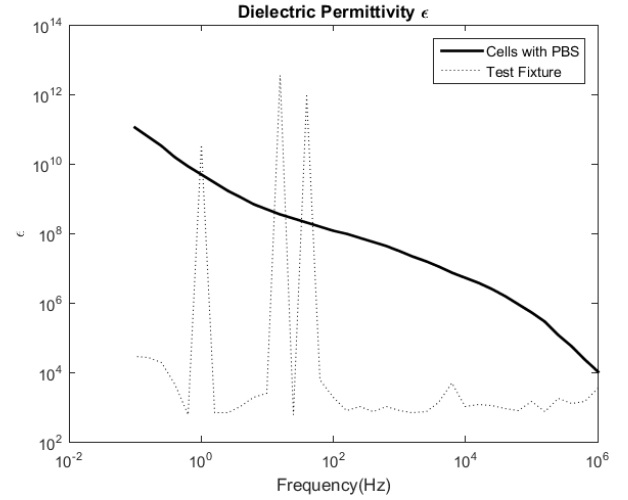
$$Z_{out} = Z_{22} - Z_{12}$$



As illustrated in the graphs, the relative permittivity of the cells in all cases is similar to the relative permittivity of the PBS solution. This implies that only the permittivity of the PBS solution and test fixture is measured. The relative permittivity is noticeably high, where the mean of the three relative permittivities is 7.5538×10^6 . To put this in perspective, air has a relative permittivity of 1.0006. When looking at the results from experiment two at low frequencies, we see how the cells suspended in the PBS solution have a high permittivity and drops down with frequency. This is a trend that has been seen



in research before such as in reference [1]. This area is known as the α -relaxation region. At high frequencies, noise is also noticeable.



XII. CONCLUSION

The hypothesis stated: when using dielectric spectroscopy, mouse hybridoma cells exposed to a range of radio frequencies would cause a constant relative dielectric permittivity. Having this constant relative permittivity would lead to the conclusion that no potential exists at radio frequencies, or that dielectric spectroscopy is not a viable solution to measure the trans-membrane potential at radio frequencies. A reason this may not be a viable solution is the radio-frequencies are passing through our dielectric as a short. When observing the impedance for a capacitor $Z = \frac{1}{j2\pi fC}$ notice that as the frequency increases the impedance lowers. This lower impedance is problematic when trying to use dielectric spectroscopy for measuring trans-membrane potential. It is also known from prior research that an α -relaxation effect occurs at frequencies lower than 10 kHz. Above this frequency the ions can no longer follow the electric field, and the ions in the cell no

longer have time to react to the electric field. Regarding the noise, it can be suppressed in a faraday cage.

XIII. ALTERNATIVE METHODS

A report in 2001 was written on developing coplanar waveguide devices which can perform dielectric spectroscopy on biological samples within a microfluidic channel. These devices yield permittivity spectra across an exceptionally broad range of frequencies from 40 Hz to 26.5 GHz. At low frequencies, the polarization is able to closely follow the applied electric field ($\epsilon = \epsilon_{LF}$), while at high frequencies applied excitations oscillate too fast for the charges to respond ($\epsilon = \epsilon_{HF}$), where generally $\epsilon_{LF} \gg \epsilon_{HF}$. By combining transmission line design with robust thin-film insulation, sensitivity to sample properties can be achieved in both low and high frequency regimes within a single device.

XIV. ACKNOWLEDGEMENT

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REFERENCES

- [1] C.Prodan *et al.*, "Low-frequency, low-field dielectric spectroscopy of living cell suspensions," *Journal of Applied Physics*, 2004.
- [2] Shan Jiang and Stavros Georgakopoulos, "Electromagnetic Wave Propagation into Fresh Water," *Journal of Electromagnetic Analysis and Applications*, 2011, pp. 261-266.
- [3] Lou Frenzel, What is the Difference Between EM Near Field and Far Field *Electronic Design*, 2012.
- [4] Brook T.Chernet and Michael Levin, Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a *Xenopus* model, *Disease Models & Mechanisms* 2013, pp. 595-607.
- [5] Center for Disease Control and Prevention *Recognizing the Biosafety Levels*. www.cdc.gov/training/QuickLearns/biosafety/
- [6] ATCC *Mouse Hybridoma (Sp 2/0)*, IMM010.51.2 (PTA-2360). American Type Culture Collection
- [7] Sophocles J. Orfanidis, *Electromagnetic Waves and Antennas Rutgers University*, 1999
- [8] Emil Prodan *et al.*, "The Dielectric Response of Spherical Live Cells in Suspension: An Analytic Solution," *Biophysical Journal*, 2008
- [9] G.R. Facer *et al.*, "Dielectric spectroscopy for bioanalysis: From 40 Hz to 26.5 GHz in a microfabricated wave guide," *Applied Physics Letters*, 2001