Measuring Transmembrane Potential of Mouse Hypridoma cell culture in Radio Frequency Spectrum

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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grow by rapid production of new parts) potential is correlated with unique ranges of V_{mem} : quiescent cells (cells withdrawn from the cell cycle and do not proliferate) tend to be hyperpolarized (more negative), whereas highly plastic cells such as embryonic cells,adult stem cells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

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

III. Calculating Membrane Potential

We model the impedance of the cell suspension as a resitor $R = d/\sigma A$ 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.

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

$$C = \frac{1}{sZ}, s = j\omega$$
$$\frac{1}{sZ} = \frac{\epsilon A}{d}$$
$$\epsilon = \frac{d}{sAZ}$$

$$\epsilon(\omega) = \frac{d}{\omega AZ} \tag{2}$$

$$\mathcal{Z}^S - Z^P = Z^O \tag{3}$$

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

IV. Distance Between Plates:

With regards to near and far field transmission. The most agreed upon definition of near field transmission is less than one wavelength(λ) away [3]. If we consider a sinusoidal wave traveling at a constant speed, we can calculate wavelength with the following formula ...

$$\lambda = \frac{v}{f} \tag{4}$$

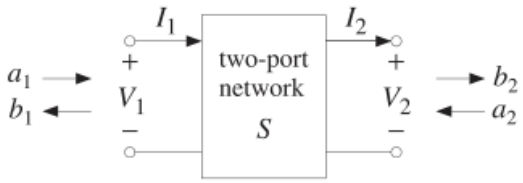
Where v is the magnitude of the phase velocity and f is the frequency of the sinusoid. It is difficult to determine the phase velocity of our electromagnetic wave while propagating through our cell suspension, but if we consider water, we can make a prediction of the wavelength. The velocity of EM waves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

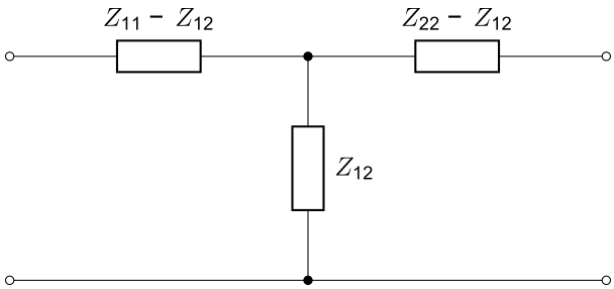
$$\lambda = \frac{343.2 \cdot 4}{10^9} = 1.3728 \times 10^{-6} m \tag{5}$$

Water is a good basis for the phase velocity as the cell suspension is made from distilled H_2O and mouse hybridoma cells. I used $10^9 Hz$ as our frequency, which is in the radio spectrum. Our micrometer is sensitive to $10 \mu m$, thus our 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 just about 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.





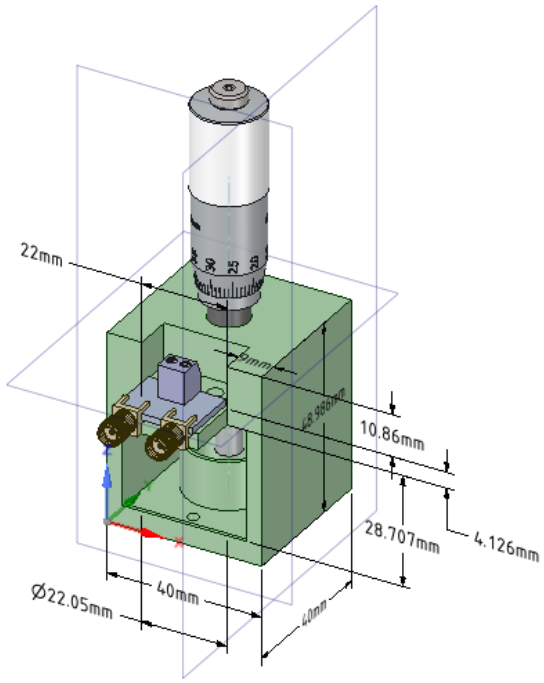
$$Z_{11} = Z_0 \frac{(1+S_{11})(1-S_{22}) + S_{12}S_{21}}{(1-S_{11})(1-S_{22}) - S_{12}S_{21}}$$
$$Z_{12} = Z_0 \frac{2S_{12}}{(1-S_{11})(1-S_{22}) - S_{12}S_{21}}$$
$$Z_{21} = Z_0 \frac{2S_{21}}{(1-S_{11})(1-S_{22}) - S_{12}S_{21}}$$
$$Z_{22} = Z_0 \frac{(1-S_{11})(1+S_{22}) + S_{12}S_{21}}{(1-S_{11})(1-S_{22})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

We can convert the found S-Parameters to Z parameters and get the model of our 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 soidium ions on the outside.The dielectric response of live cells is fundamentally different then 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 you introduce 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 dieletric permittivity of live cell suspensions can be as high as 10^6. This phenomenon is know as the $\alpha-$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

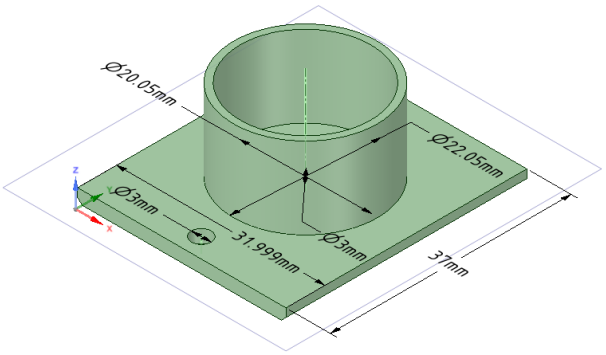
Provide a base for the beaker as well as an accurate and stable micrometer mount.This item will be 3D printed with ABS plastic.

F. Cells

SP 2/0 myelomas hypridoma cell line. These are nonadherent cells, and are essentially 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 peronnel and the environment. Reasearch 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

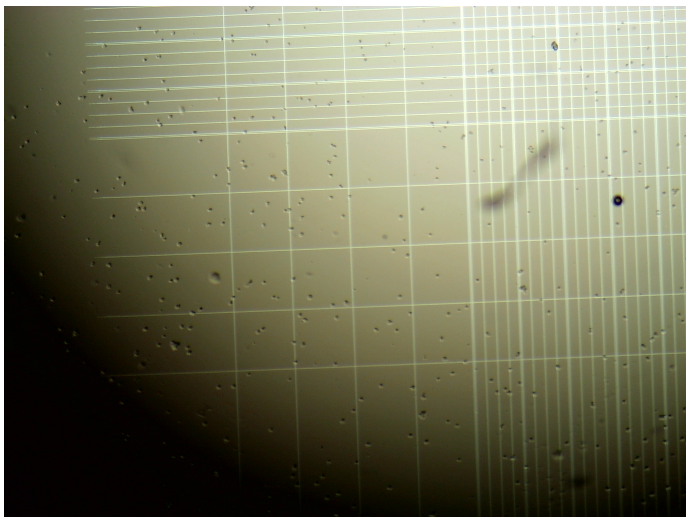
Used with this network analyzer was the Agilent 11857D 7mm test port returns(50Ω). This network analyzer was used to gather our scattering parameters. This version features the 006 option which give it a 6 GHz upper frequency range.
Resolution: $1Hz$
Stability: typically $\pm 7.5ppm$
Accuracy: $\pm 10ppm$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



VIII. Methods

A. Cell Concentration

The cell concentration will be measured using a hemocytometer. An example of the process of counting the cells can be seen in figure



IX. Procedure for splitting cells for regrowth and testing

A. 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

B. Personal Safety Equipment

For bio safety level 2, use of hood, gloves, and lab coat for person protection. With the hood shield down, protective eye ware was not needed. The hood should be turned on, and the shield lowered so only your hands can fit underneath it, before any work with the cells is done. The cells are not to leave the bio safety level 2 lab room.

C. Notes

- The cell suspension will become more acidic over time, and thus, the more yellow or orange than dark pink (the color of the cell food).
- The cells are being kept under conditions of 37 $\circ C$ and 4.9% CO2 in the incubation chamber when not in use.
- The food is being stored in a standard refrigerator in a bio safety lab room.
- The process does not need to leave the room to be completed.

D. Procedure

Centrifuging:

1) Remove the flask from the incubation unit and place it in the hood
2) Using the pipettes, put 1 ml of cell solution from the cell culture flask into each of the centrifuge vials until no more solution in the flask. 10 vials should be full.
3) Seal the vials, and evenly distributed them in a centrifuge which is then run at 1.7krpm for 5 min. Once complete, there will be a pellet of cells in the bottom of those vials. If there is an odd number of vials, do not use the odd one, place it aside on the rack for later disposal.
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 to regrow the cell population.

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. The tip of the pipette will 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 cell culture 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, phosphate saline buffer solution is added to the vials
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 already in the disposable test tube.

Clean up:

If the disposable test tube has not had bleach added to it, several milliliters should be added to it. The liquid should turn light blue to clear, and now can safely be disposed. All surfaces in the hood and materials that are not disposable should be wiped down with 70% ether solution. All equipment used that is disposable should disposed of in bio-safe bins which should be located near the hood.

X. Experiment Procedure

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

Measurements of S-Parameters:

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

Count Cell concentration Using the method as described earlier with the hemocytometer, count the cell concentration.

XI. Conclusion

We hypothesised that using dielectric spectroscopy, on mouse hybridoma cells when exposed to a range of radio frequencies will 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 spectrosopy is not a viable solution to measure the trans-membrane potential at radio frequncies.A reason this may not be a viable solution is the fact the the radio-frequencies are passing through our dielectric as a short. If you look at the impedance for a capacitor $Z = \frac{1}{j2\pi fC}$ you will notice that as the frequency increases the impedance lowers. This means the electromagnetic waves will pass through the capacitor like a short, or in our case will pass through our cells. 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.

XII. Acknowledgement

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