Study of the Activity of Neurological Cell Solutions Using Complex Permittivity Measurement

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Abstract — **Experimental results of the exposure of neurological cells to radio frequency are presented. The exposure is quantified by the mean of the complex permittivity of cell solutions as a function of frequency. A set-up is used for measurement of complex permittivity of live and dead neurological cell cultures from 20 to 40 GHz. Differences are observed between the two cultures and are compared against the expected measurement error. The statistical significance of these differences is also studied and reported in this paper.**

I. INTRODUCTION

Evaluation of the interaction of electromagnetic energy at microwave and millimeter wave frequencies with biological tissues is of great interest [1]. Majority of works in this area address the potential health hazards due to the advent of wireless communications [2] and therapeutic applications [3]. However, there have been interests in investigating the impact of interaction with biological solutions [4] and cell cultures [5] aiming at observing specific signatures. These signatures can be useful in biomedical research where microwave and millimeter waves can be used to probe a target medium and identify certain biological activities.

From the physical point of view, knowledge of the complex permittivity of the biological target is an appropriate way to quantify the tissue interaction with microwave. An approach was already reported by the authors based on a two-port microstrip test fixture for characterization of biological samples up to millimeter wave frequencies [6]. A procedure for complex permittivity extraction from transmission (S_{21}) measurement for the Tissue Under Test (TUT) has been introduced and applied to extract the complex permittivity of brain tissues up to 50 GHz [7].

This paper presents the results of applying the established technique to extract the complex permittivity of solutions of neurological cells in live and dead states to observe their differences. Cells were cultured from neurological cells taken from mouse brain. The study was performed from 20 to 40 GHz on several identical samples. The results are compared against expected measurement errors and a statistical significance analysis is also performed on the extracted data.

II. EXPERIMENTAL PROCEDURES

The design issues for a test fixture operating up to 50 GHz have been reported previously by the authors [7]. The test fixture is composed of open circuited microstrip transmission lines (realized on fused silica, $\varepsilon_r = 4.1$), which are to couple to the biological sample under test through two small circular apertures. Through-Reflect-Line (TRL) calibration is used to remove the error networks associated with the Automatic Network Analyzer (ANA) internal circuitry, cables, launchers, and the microstrip line length. Then, the reference planes for ports 1 and 2 are set at the middle of the apertures, along their central axes. A finite element modeling of the structure for discrete values of relative permittivity, ε_r, loss tangent $\tan\delta$ (i.e., $\varepsilon''/\varepsilon'$), and operating frequency, f, within the expected range of these quantities for biological materials, is performed. The complex permittivity is extracted from the measured values compared against the simulation results.

Neurological cells have a concentration of 1 million per cubic centimeter and are kept in saline buffer with nutrient. For measurement, the solution is injected in a sample container that involves a rectangular acrylic frame of 0.4" (1.02 mm) thick. The frame has a rectangular opening at the middle and two small holes at the ends (Fig. 1). Two 0.15 mm thick glass cover slips (Corning Zinc Borosilicate glass, $\varepsilon_r = 6.7$) 22×50 mm^2 are glued to the two sides of the frame. The solution is injected inside the container through the holes.

To acquire reliable results, several sample containers were fabricated and 10 containers that had the closest thickness were selected for measurement. They were divided into two groups in random (5 for live and 5 for dead cell cultures). The two groups had thickness of 1.335 ± 0.011 mm and 1.352 ± 0.011 mm, where mean and standard deviation are implied in this representation.

An Anritsu 37397C vector network analyzer was used in the experiment. The major concern in the measurement was

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providing an air gap free contact from the circular apertures to the glass surfaces by gently approaching the two microstrip cavities.

The first set of sample holder was used for the live cell population. Measurement of each container would take about 1-2 minutes including the time for injection of the sample inside. The cells were exposed to −70 °C to create the dead state (second population). Since the solution inside the container would reach the room temperature very quickly, the second population could be evaluated immediately after the first set. Therefore, a measurement session could be finished less than 20 minutes from the beginning to the end. Note that the ambient temperature was monitored 27 °C throughout the experiment and was quite stable.

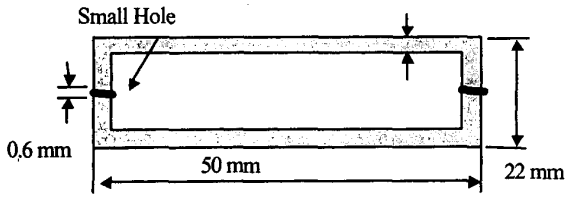

Fig. 1. Mechanical structure of the biological sample holder.

III. RESULTS AND DATA ANALYSIS

Three experiment sessions are performed. First session (session #1) involves two measurements from each sample (a total of 10 measurements for each population), where for the second measurement the sample holder is turned over with respect to the first. This session is conducted immediately after filling the containers with the cells. The second session (session #2) is performed just after the first one involving 1 measurement for each sample container. To provide a clearer validation, the containers are emptied by removing the seals, and refilled with water. Then, the third session is conducted (session #3). In this session, the same procedure as session #1 is repeated for the two groups of containers.

Fig. 2 shows the extracted complex permittivity for session #1. The results are for mean complex permittivity values of the live and dead populations and are compared with the water complex permittivity. A pronounced difference is observed for the mean values of the two populations.

To observe these differences more clearly, the relative differences between the two groups for the real and imaginary parts are shown in Fig. 3, where the results for the three sessions are rendered. The figure also includes the expected measurement error due to the differences in the mean thickness of the sample container for the two groups as well as random errors.

Since the measurements were done quickly (10 – 20 minutes), no significant variation of the room temperature is observed during each session. The equipment drift was also insignificant during the sessions since the network analyzer has been on for a minimum of two hours before the start of the experimental sessions. Note that since the difference measurement results are presented, the systematic sources of error such as those due to the TRL calibration standards' fabrication tolerances, lack of repeatability of the standards' coaxial to microstrip launchers, numerical modeling of the test fixture, complex permittivity extraction procedure, and temperature monitoring device reading have no impact on the result of Fig. 3. Nonetheless, they are either reduced to an insignificant level or can be quantified as well [7].

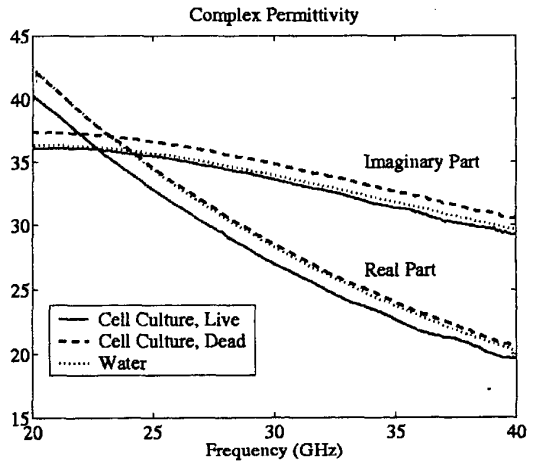

Fig. 2. Complex permittivity of live and dead cell cultures for the first measurement session compared with the water complex permittivity.

Two sources of random error are relevant to the difference measurement. The first is due to the distribution of the container thickness (standard deviation of 11 microns). A more dominant source of the random error originates from the random change in the measured S_{21} due to the random air gap of the contact between the aperture and the sample container's glass surface. This air gap can be a total of 20-50 microns for the two aperture-glass contacts.

To study the effect of thickness variation, two sets of simulations with the containers with slightly different thickness are performed that yield the S_{21} variation with respect to the thickness. The random contact effect was evaluated experimentally by placing a water filled sample

container and measuring it 10 times, and obtaining mean and standard deviation of the real and imaginary parts of S_{21}.

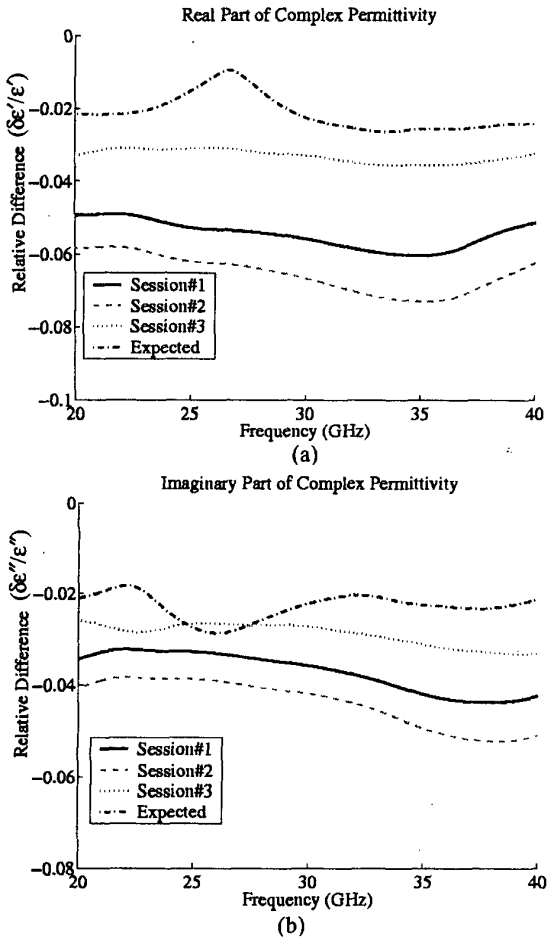

Fig. 3. The relative difference between the two groups and sessions #1, 2, and 3 compared with the expected difference; a) real part and b) imaginary part of the complex permittivity.

Knowing the S_{21} variation of $\delta S_{21,i}$ (i = 1, 2 for the first and second sources of random error), variation in the $\delta\varepsilon_i'$ and $\delta\varepsilon_i''$ are obtained through the following relation

$$\delta\varepsilon_i = \delta\varepsilon_i' - j\delta\varepsilon_i'' = \frac{\delta S_{21,i}}{\left(\frac{\partial S_{21}}{\partial \varepsilon}\right)} \qquad (1)$$

where $\partial S_{21}/\partial\varepsilon$ can be easily obtained since S_{21} is given in a functional form by a procedure that fits the modeling data to a proper function [7]. Because two different groups are being considered for the mean differences, and the sources of random errors are independent the total error can be evaluated as:

$$\delta\varepsilon_{tot}' = \sqrt{2\sum_{i=1,2}(\delta\varepsilon_i')^2}\ ,\ \delta\varepsilon_{tot}'' = \sqrt{2\sum_{i=1,2}(\delta\varepsilon_i'')^2} \qquad (2)$$

The standard deviation of mean of S_{21} is employed for $\delta S_{21,i}$ evaluation, from which the error in the complex permittivity are evaluated through Eq. (1).

To find the difference that can be expected between the two groups given that they are identical, the random error explained above is added to a non-random difference between the mean values of the two groups (i.e., a 17 microns difference that is obtained by the subtraction of the mean thickness for the two container sets). The final results of the error analysis is shown in Fig. 3 as the expected relative difference in that figure.

An alternate approach to the problem is also studied by investigating the statistical significance of the difference in mean values. In this more formal approach, the initial assumption (null hypothesis) is that the two groups represent similar media. The probability of observed result being happened (i.e., the mean values for both real and imaginary parts of the complex permittivity are higher for the second population) under the null hypothesis being true is the so-called statistical significance of the observation. A Student's t-test is appropriate for this evaluation and is performed using Matlab Statistics Toolbox, where the extracted complex permittivity values are assumed to have a normal distribution.

The statistical significance of the difference in the extracted complex permittivity of the two populations for sessions #1 and #3, and for three frequencies of 20, 30, and 40 GHz are listed in Table I. The results given in the table are the probability of the observed differences between the real and imaginary parts of the complex permittivity for the two sessions with the assumption that the two populations are in fact identical. Note that the probability is very small (less than 0.001) for session #1, whereas for session #3 it can be more than 0.01.

TABLE I
THE RESULT OF STATISTICAL ANALYSIS OF COMPLEX PERMITTIVITY

	Session #1		Session #3	
	Real	Imaginary	Real	Imaginary
20 GHz	0.0003	0.0007	0.007	0.002
30 GHz	0.0004	0.0004	0.013	0.003
40 GHz	0.0001	0.0004	0.003	0.003

IV. CONCLUSIONS

The results of the first analysis (cf. Fig. 3.) highlight a difference that exists between the two groups. However, the difference is more pronounced in the case where the two groups represent the two different cell cultures (i.e., live and dead cell cultures) for sessions #1 and #2. Furthermore, for session #3, where the two groups contain the same material (water), the difference is comparable to the expected error in the measurement. The deviation can be justified by noting that after refilling the sample containers with water instead of the cell solution, some left over cells might still be present adhered to the glass.

The statistical results as given in Table I, indicate that the probability of the current observation with the assumption that there is no difference between the two groups is extremely small for session #1 (cell experiment). However, the existence of a difference for session #3 (cells are replaced with water may be rejected at significance level of 1% (confidence level of 99%).

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