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Experimental Procedure for Determination of the Dielectric Properties of Biological Samples in the 2-50 GHz Range

ELIAS ODELSTAD[1], SUJITH RAMAN[2], ANDERS RYDBERG[2], (Member, IEEE), AND ROBIN AUGUSTINE[2]

[1]Division of Astronomy and Space Physics, Department of Physics and Astronomy, Uppsala University, Uppsala 75121, Sweden
[2]Angstrom Laboratory, Microwave Group, Solid State Electronics, Department of Engineering Sciences, Uppsala University, Uppsala 75121, Sweden

CORRESPONDING AUTHOR: R. AUGUSTINE (robin.augustine@angstrom.uu.se)

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ABSTRACT The objective of this paper was to test and evaluate an experimental procedure for providing data on the complex permittivity of different cell lines in the 2–50-GHz range at room temperature, for the purpose of future dosimetric studies. The complex permittivity measurements were performed on cells suspended in culture medium using an open-ended coaxial probe. Maxwell's mixture equation then allows the calculation of the permittivity profiles of the cells from the difference in permittivity between the cell suspensions and pure culture medium. The open-ended coaxial probe turned out to be very sensitive to disturbances affecting the measurements, resulting in poor precision. Permittivity differences were not large in relation to the spread of the measurements and repeated measurements were performed to improve statistics. The 95% confidence intervals were computed for the arithmetic means of the measured permittivity differences in order to test the statistical significance. The results showed that for bone cells at the lowest tested concentration (33 500/ml), there were significance in the real part of the permittivity at frequencies above 30 GHz, and no significance in the imaginary part. For the second lowest concentration (67 000/ml) there was no significance at all. For a medium concentration of bone cells (135 000/ml) there was no significance in the real part, but there was significance in the imaginary part at frequencies below about 25 GHz. The cell suspension with a concentration of 1 350 000/ml had significance in the real part for both high (above 30 GHz) and low (below 15 GHz) frequencies. The imaginary part showed significance for frequencies below 25 GHz. In the case of an osteosarcoma cell line with a concentration of 2 700 000/ml, only the imaginary part showed significance, and only for frequencies below 15 GHz. For muscle cells at a concentration of 743 450/ml, there was only significance in the imaginary part for frequencies below 5 GHz. The experimental data indicated that the complex permittivity of the culture medium may be used for modeling of cell suspensions.

INDEX TERMS Biological solutions, osteosarcoma, myoblast, dielectric characterization, open ended probe.

I. INTRODUCTION

The power dissipated in a dielectric medium in a (time-varying) electromagnetic field is dependent on the dielectric permittivity of the medium. Therefore, when examining the potential effects of microwave radiation on cells, it is beneficial to know the permittivity of the cells. There are several applications for millimeter waves in the 30–50 GHz range. Due to low interference with existing systems and high data rates, these frequencies are increasingly gaining importance [1]. Need for miniaturizing antennas and larger communication bandwidth has resulted in strong research interests aimed at developing future wearable networks. Characterization of on-body propagation for developing millimeter wave wearable antennas has been a hot research topic among many research groups [2]. Increasing usage of on-body communication increases the concern of effects of

these signals on human cells. For more than 40 years the biocompatibility issues of this radiation have been investigated. In several of these publications, it has been reported that this radiation can influence biological cells [3]–[6]. Due to current exposure regulations (10 W/m^2 limit for far-field), there will be less direct effects. But millimeter wave radiation could contribute to localized power absorption in a small volume of tissue, thus medium to high power exposures can create thermal effects that can initiate biological responses. The studies being conducted mainly deal with the possibility of synergistic or combined effects, resolution of power levels for any effects to be seen, and detecting triggering mechanisms on overexposure. Most of the established data base for the permittivity and conductivity of biological tissues are provided for frequencies below 20 GHz. Thus, lack of valid data above 20 GHz is one of the major hurdles of millimeter-wave dosimetry. A precise knowledge of these dielectric properties is crucial and directly impacts the accuracy of any numerical dosimetric study [7]. In some of the works [8]–[10], data for millimeter waves are experimentally determined for skin tissue. But still very little data is available on biological solutions and cells [11], [12]. There are some theoretical models for biological samples presented in the literature but they are not experimentally validated, especially for frequencies above 20 GHz. The main purpose of this study was to test and evaluate an experimental procedure for providing data on the complex permittivity and frequency dispersive behavior of cancerous and non-cancerous bone cells and other cell lines, for frequencies up to 50 GHz at room temperature (22 °C). By studying cancerous and non-cancerous cell lines it will be possible to identify cancer cells in a suspension medium which could be used as a viable technique to detect cell type of the tissue. Once diagnosed during dialysis the affected blood can be treated using microwave/millimeter wave hyperthermia and the blood could be reused in the patients.

II. THEORY

A. LOSSES IN DIELECTRIC MEDIA

The total time-average power dissipated in a volume V is given by Joule's law [13]

$$P_t = \frac{\sigma}{2}\int_V |E|^2\, dv + \frac{\omega}{2}\int_V \varepsilon'' |E|^2 + \mu'' |H|^2) dv \qquad (1)$$

where, σ, ε'' and μ'' are the conductivity and imaginary parts of the dielectric permittivity and magnetic permeability, respectively. E is the complex electric field and H is the complex magnetic-field intensity. For non-magnetic materials and equation (1) can be written as,

$$P_t = \frac{1}{2}\int_V (\sigma + \omega\varepsilon'')\, |E|^2\, dv \qquad (2)$$

At high frequencies the dielectric term dominates over the conductive term in equation (2), giving,

$$P_t = \frac{\omega}{2}\int_V \varepsilon'' |E|^2\, dv \qquad (3)$$

Thus, the power dissipated at any point in a dielectric medium is proportional to the imaginary part of permittivity at that point.

B. MAXWELL'S MIXTURE EQUATION

The dielectric permittivity of a suspension of small spherical particles in a homogeneous background medium is given by Maxwell's mixture equation [14]:

$$\frac{\varepsilon_1 - \varepsilon_s}{2\varepsilon_1 + \varepsilon_s} = p\frac{\varepsilon_1 - \varepsilon_2}{2\varepsilon_1 + \varepsilon_2} \qquad (4)$$

where ε_s, ε_1 and ε_2 are the permittivities of the suspension, the background medium and the particles, respectively. p is the volumetric fraction of particles in the suspension. Solving for ε_2 yields

$$\varepsilon_2 = (1 + \frac{\varepsilon_1 - \varepsilon_s}{(\varepsilon_1 - \varepsilon_s) - p(2\varepsilon_1 + \varepsilon_s)}). \qquad (5)$$

If the cells are modeled as small spheres and the culture medium is homogeneous, the dielectric permittivity of the cells can be calculated from measurements of pure culture medium and cell suspension permittivities using equation (5).

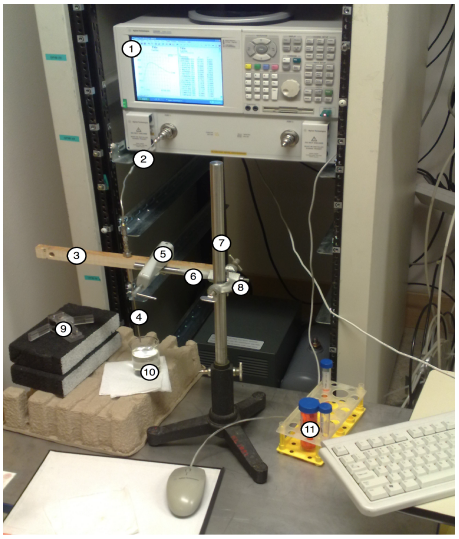

FIGURE 1. Experimental setup for permittivity measurements. (1) Network analyzer, (2) coaxial cable, (3) wooden block, (4) coaxial probe, (5) adjustable clamp, (6) metal rod, (7) stand, (8) dual adjustable clamp, (9) shorting block, (10) water beaker, (11) cell suspension.

III. MATERIALS AND METHOD

A. EXPERIMENTAL SETUP

The permittivity measurements were performed using a 200 mm coaxial probe (Agilent 85070E Dielectric Probe Kit) connected to a vector network analyzer (Agilent E8364B PNA series) via a semi-rigid coaxial cable (Wiltron V120-12). The experimental setup is shown in figure 1. To mount the probe (4), a 10 mm diameter hole was drilled in a rectangular wooden block (3). The probe was then inserted through this hole, thereby fixing it to the block. Using an adjustable clamp (5), the block was attached to a metal rod (6), which in turn was mounted on a stand (7) using a dual

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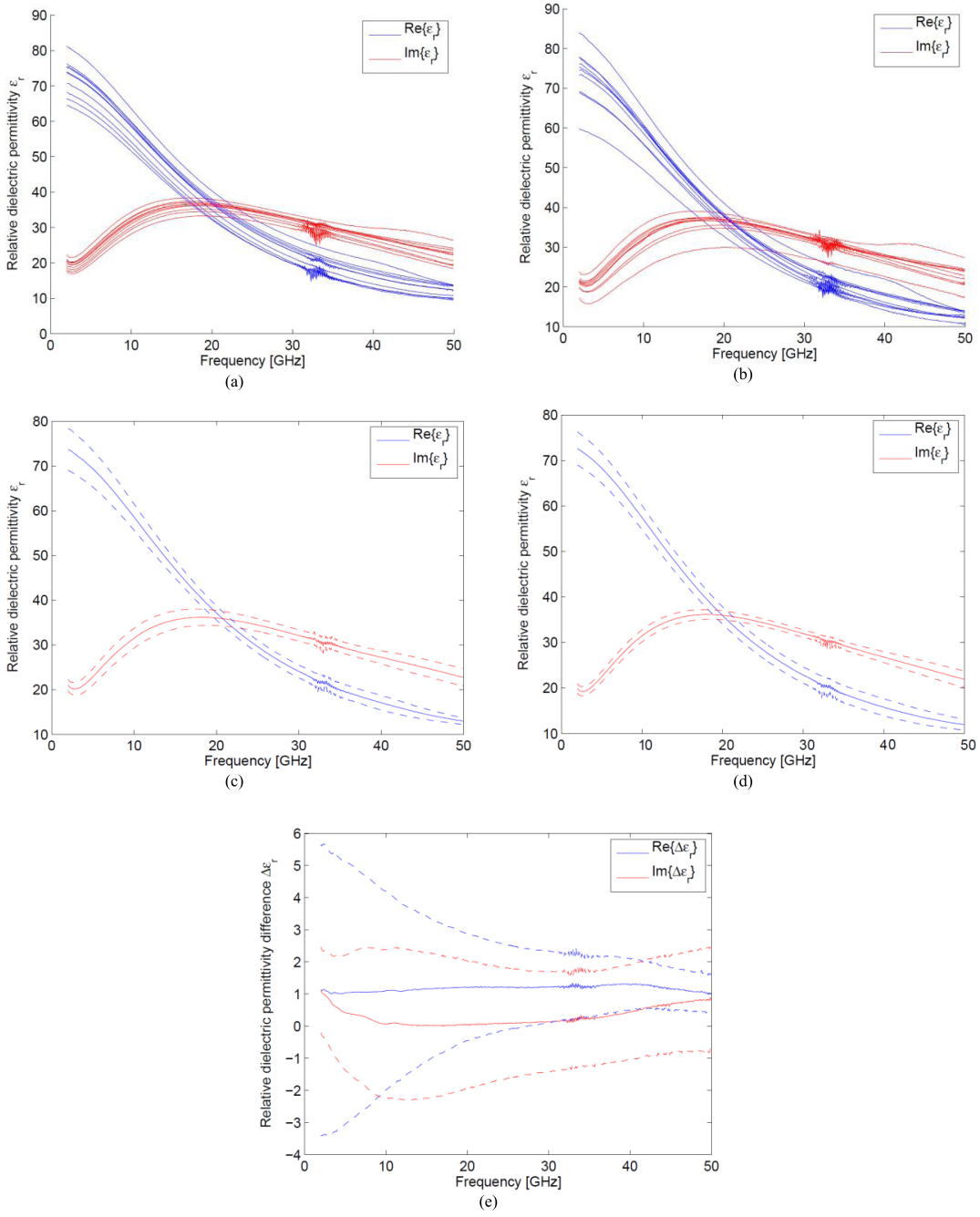

FIGURE 2. **(a) Culture medium: All measurements. (b) hFOB 1.19 - 33500cells/ml suspension: All measurements. (c) Culture medium: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (d) hFOB 1.19 - 33500cells/ml suspension: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (e) hFOB 1.19 - 33500cells/ml suspension: Permittivity differences: Arithmetic means (solid curves) and 95% confidence interval (dashed curves).**

adjustable clamp (8). Measurements could now be performed by simply holding the material under test (MUT) up to the probe so that the tip was immersed (at least 5 mm) into the suspension. The coaxial probe can only be used for liquid samples or soft semi-solids, since the tip has to be inserted into the sample. Therefore, measurements were performed on suspensions of cells in culture medium. The permittivity of the cells is calculated from the difference in permittivity between culture medium and cell suspension using Maxwell's mixture equation (equation (4)).

B. MEASUREMENT PROCEDURE

The coaxial probe measures the reflection coefficient of guided EM waves at its tip and the associated software then calculates the dielectric permittivity of the medium. To get good statistics, ten measurement sets were performed for each

TABLE 1. Different cell lines and their concentrations.

Cell type	Description	Concentration cells/ml
Bone cells	Human osteoblast cell line hFOB 1.19	33500
‘’	‘’	67000
‘’	‘’	135000
‘’	‘’	1350000
Cancerous bone cells	Human osteosarcoma cell line SaOS-2	2700000
Muscle cells	Mouse myoblast cell line C2C12	743450

MUT. Each measurement set started with calibration using the standards air (open circuit), shorting block and water. The air and water standards were then remeasured to verify the calibration. In case of excessive deviation from expected values, the calibration procedure was repeated until acceptable values were obtained. Following verification of the calibration, measurements were performed on culture medium and cell suspension, respectively. The idea was to measure the difference in permittivity between these two MUTs and then compute the permittivity of the cell suspension through addition.

C. MATERIALS UNDER TEST

The cell suspensions tested are presented in table 1. The experiments were carried out at room temperature, which was approximately 22 °C. It was initially intended that many different concentrations of cell suspensions be measured for each cell type, but a preliminary visual inspection of the measurements on the first few samples suggested little or no significant difference in permittivity between pure culture medium and cell suspension, so it was decided that only maximal available concentrations be used.

IV. RESULTS AND DISCUSSION

A. PERMITTIVITY RESULTS

The results of the measurements performed are shown in figures 2 - 7. Subfigures of figure 2(a) and (b) show all measurements plotted in the same diagram for culture medium and cell suspension, respectively. Subfigures (c) and (d) show the arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). Subfigures (e) show the arithmetic mean of the differences in measured permittivity between cell suspension and culture medium, and the associated 95% confidence intervals.

B. STATISTICAL ANALYSIS

As can be seen in figures 2-7, there was a large spread in the measurements, resulting in confidence intervals that were fairly large compared to the measured differences in

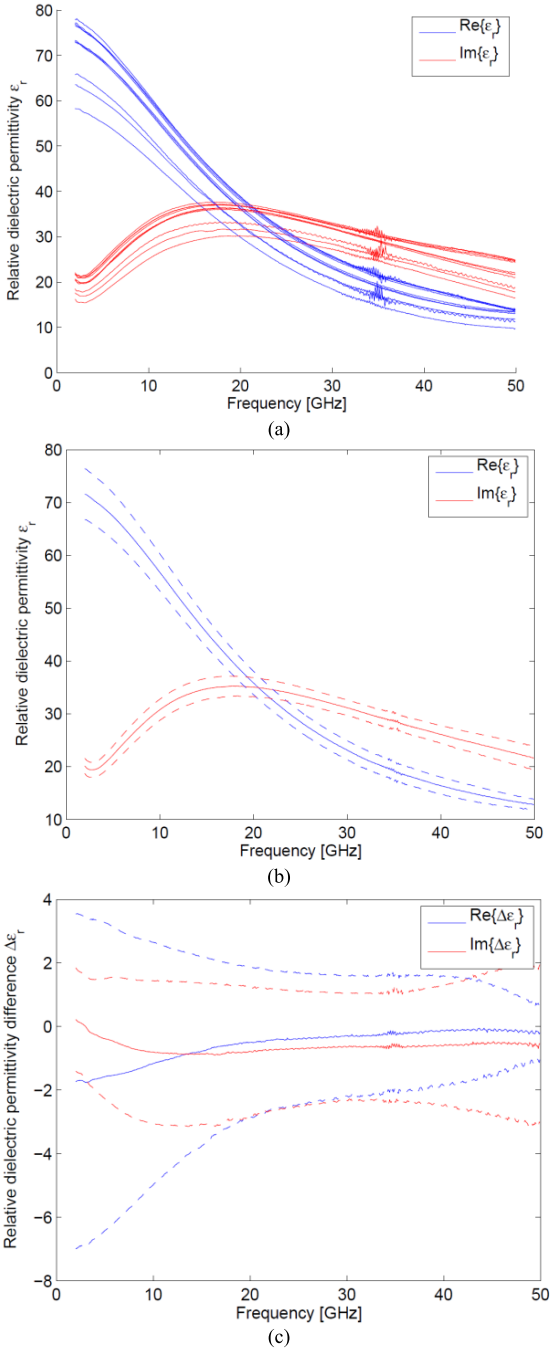

FIGURE 3. (a) hFOB 1.19 - 67000cells/ml: All measurements. (b) hFOB 1.19 - 67000cells/ml suspension: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (c) hFOB 1.19 - 67000cells/ml suspension: Permittivity difference: Arithmetic means (solid curves) and 95% confidence interval (dashed curves).

permittivity between culture medium and cell suspensions. Whether these differences are statistically significant can be checked in figures 2 - 7 (c): If the value 0 is located outside the confidence interval of the permittivity difference, the difference is significant (at a significance level of 95%). For frequency samples where the value 0 is located inside

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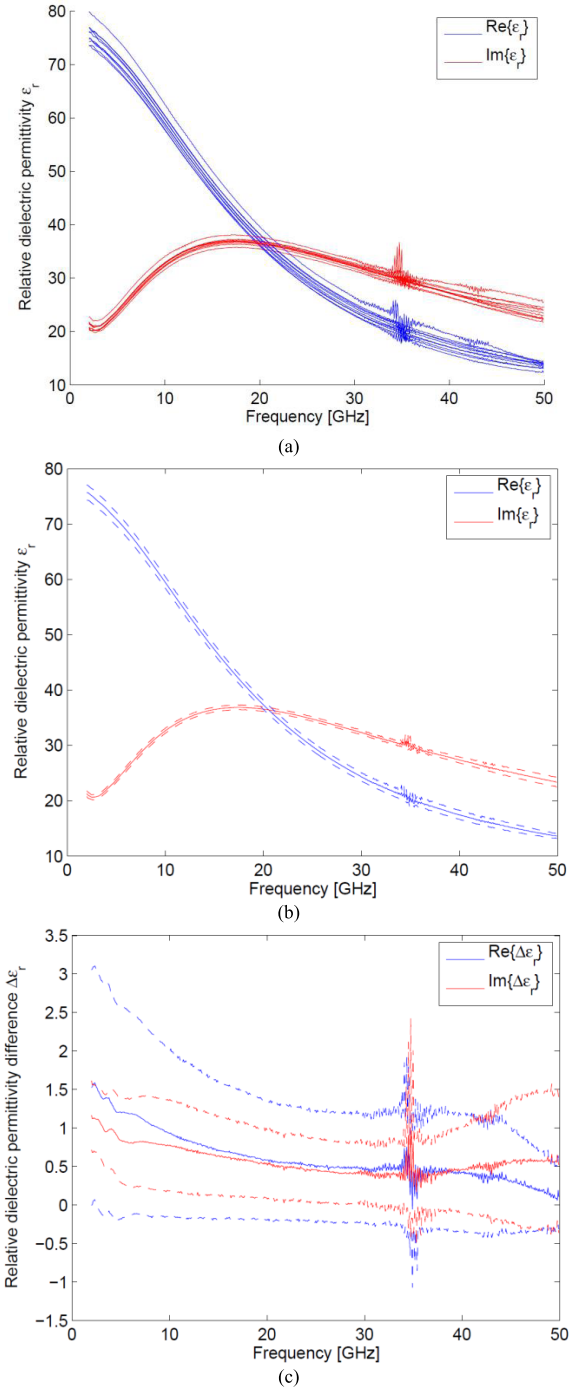

FIGURE 4. (a) hFOB 1.19 - 135000cells/ml: All measurements. (b) hFOB 1.19 - 135000cells/ml suspension: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (c) hFOB 1.19 - 135000cells/ml suspension: Permittivity difference: Arithmetic means (solid curves) and 95% confidence interval (dashed curves).

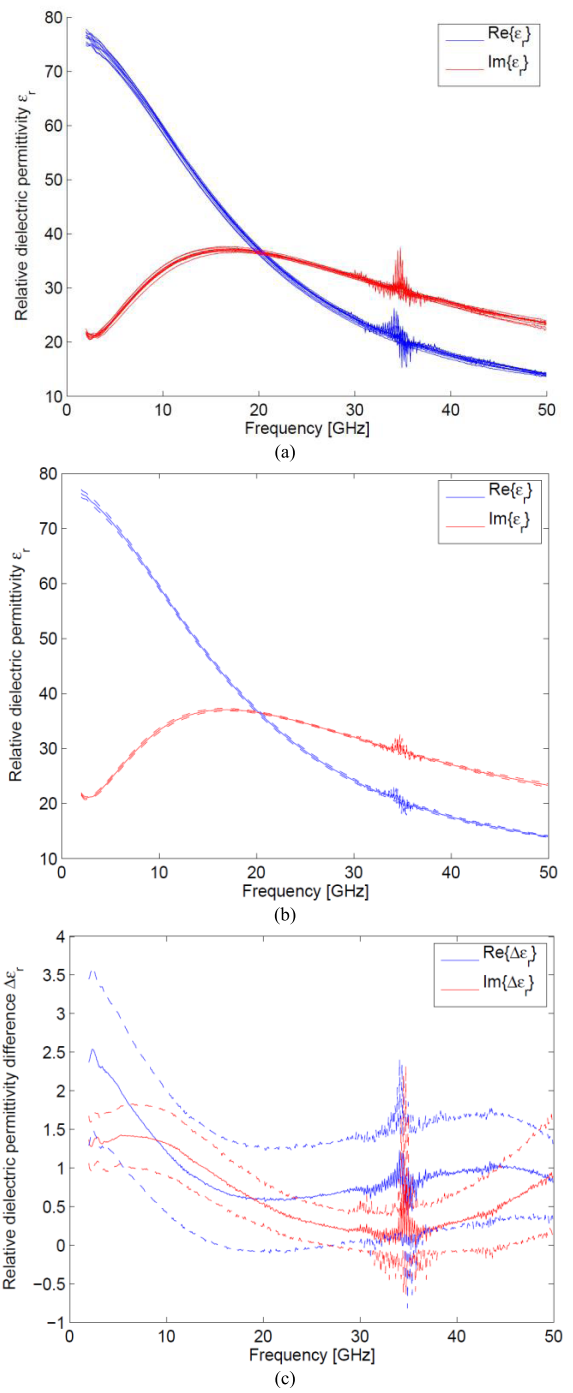

FIGURE 5. (a) hFOB 1.19 - 1350000cells/ml: All measurements. (b) hFOB 1.19 – 1350000 cells/ml suspension: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (c) hFOB 1.19 – 1350000 cells/ml suspension: Permittivity difference: Arithmetic means (solid curves) and 95% confidence interval (dashed curves).

the confidence interval, there is no statistically significant difference. 1) For bone cells at the lowest concentration (33500/ml), the above procedure suggested significance for the real part of the permittivity at frequencies above 30 GHz, and no significance for the imaginary part. 2) For the slightly higher concentration (67000/ml) there was no significance at all. 3) For 135000/ml there was no significance for the real part, but there was significance in the imaginary part at frequencies below about 25 GHz. 4)For the super-high concentration of 1350000/ml, there was significance in the real part for both high (above 30GHz) and low (below 15GHz) frequencies. The imaginary part showed significance

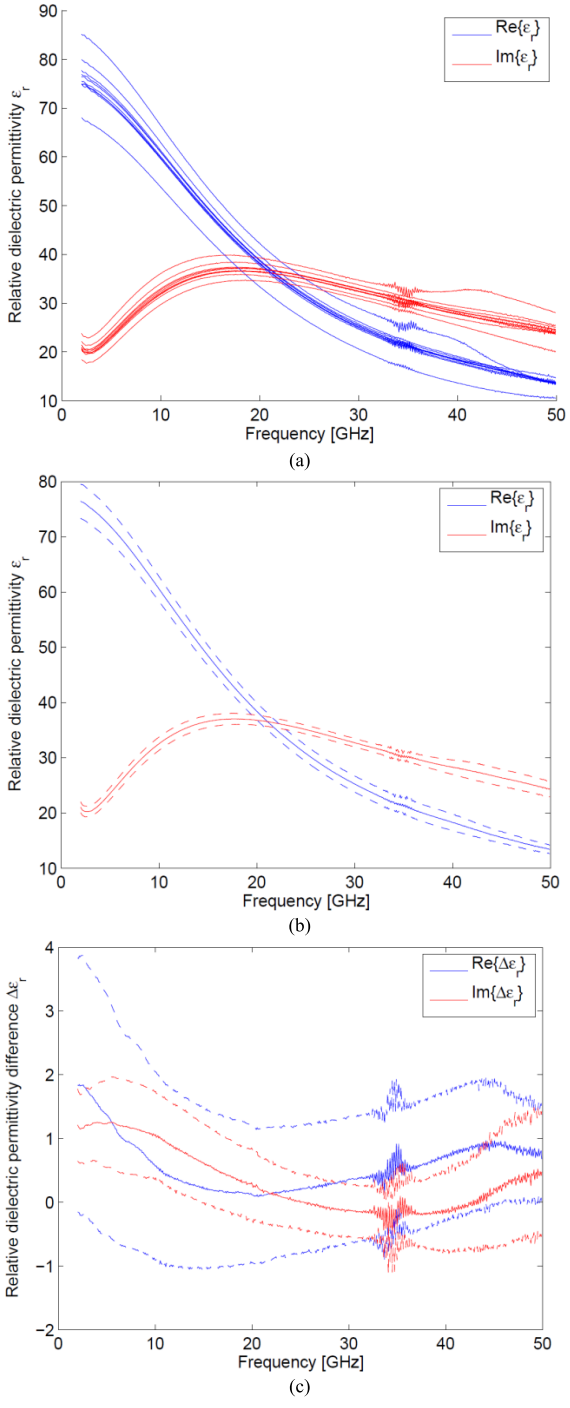

FIGURE 6. **(a) SaOS-2 - 2700000cells/ml: All measurements. (b) SaOS-2 - 2700000cells /ml suspension: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (c) SaOS-2 - 2700000cells /ml suspension: Permittivity difference: Arithmetic means (solid curves) and 95% confidence interval (dashed curves).**

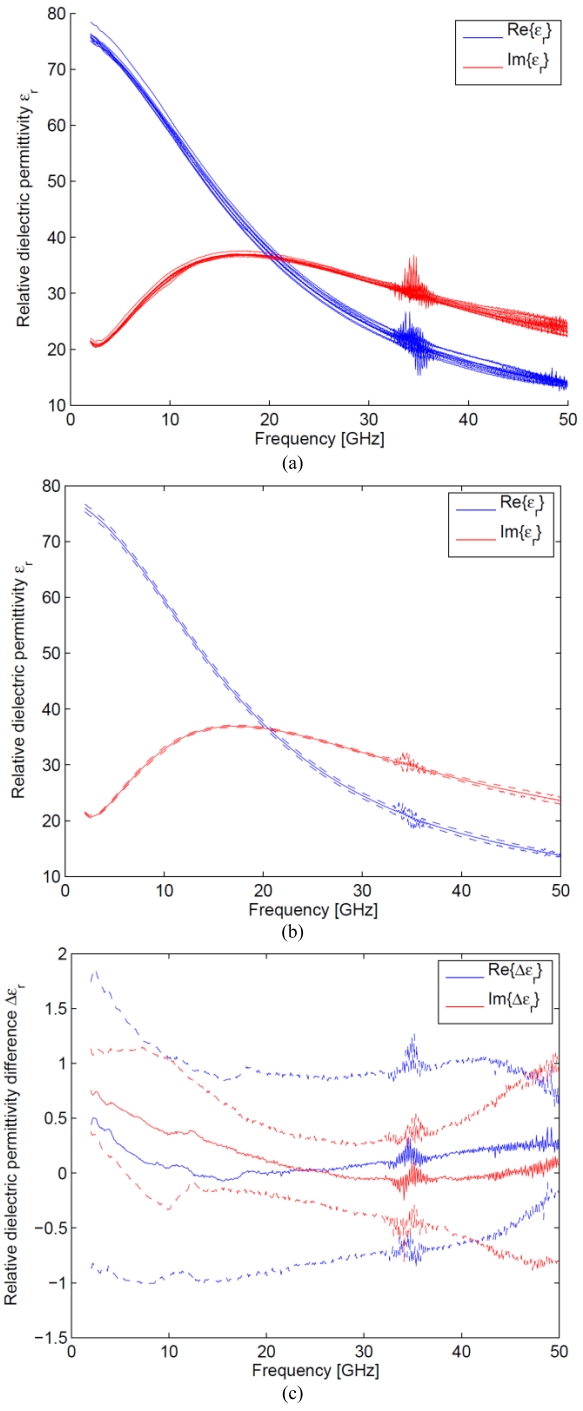

FIGURE 7. **(a) C2C12 – 743450 cells/ml: All measurements. (b) C2C12 – 743450 cells/ml suspension: Arithmetic means of the measurements (solid curves) and 95% confidence interval (dashed curves). (c) C2C12 – 743450 cells/ml suspension: Permittivity difference: Arithmetic means (solid curves) and 95% confidence interval (dashed curves).**

for frequencies below 25 GHz. 5) For cancerous bone cells with a concentration of 2700000/ml, only the imaginary part showed significance, and only for frequencies below 15 GHz. 6) For muscle cells at a concentration of 743450/ml, there was only significance in the imaginary part for frequencies below 5 GHz. There was no significance in the real part.

C. CLINICAL USE

The results demonstrate the method to be a viable technique for distinguishing between different cancerous and non-cancerous cell types. Blood samples containing cancer cells can be analyzed for identifying the phenotype.

IEEE Journal of Translational Engineering in Health and Medicine

The dielectric profile studies presented here are useful in finding appropriate frequencies for hyperthermia applications. For example at 15 GHz where the loss factor is at its maximum hence the conductivity of the cells is also large ($\sigma = \omega\varepsilon_0\varepsilon''$, where ε_0 is permittivity of free space). This implies that at 15 GHz more power will be dissipated in the cells causing rise in temperature. Thus this technique can be used clinically for optimizing exposure frequency for hyperthermia treatment.

D. ERROR SOURCES

1) CALIBRATION ISSUES

During the experiment, validating the calibrations proved to be a difficult and time-consuming task. Control measurements of the calibration standards frequently yielded results that deviated so much from what was expected that the calibration had to be redone. The 'expected' values were $\varepsilon' = 1$ and $\varepsilon'' = 0$ for air. No strict quantitative criteria were set, but in general the control measurements of air were not allowed to deviate more than about 0.01 from these values over most of the spectrum, although at times this requirement had to be relaxed a bit due to time constraints (see below). For water, it was generally required that the curve for start somewhere between 70 and 85 and that both curves be relatively smooth over most of the spectrum. The smoothness criteria proved to be the most difficult to fulfill since the water measurements showed strong oscillatory tendencies around about 30-35 GHz and occasionally such calibrations had to be accepted due to the time constraints.

2) BUBBLES AND PERTURBATION

The reason for the high measurement uncertainty was thought to be either bubbles of air that tended to form at the tip of the probe, a phenomenon which was visually observed on at least one occasion, or perturbations of the experimental setup. The bubbles were as likely to form during calibration measurements, resulting in corrupt calibrations, as during calibration validation or actual measurements (just as for the calibration standards, the MUT curves were expected to be smooth; considerable oscillations, or unprecedented deviations from previous results, were taken as indications of corrupt measurements). In the latter cases, wiping of the probe tip with a paper towel and redoing the measurements would often be sufficient to eliminate the problem without redoing the calibration. This was usually tried a couple of times before the calibration was scrapped, since re-calibrating was much more time consuming. Perturbations of the experimental setup were most likely to occur during calibration, when attaching or removing the shorting block. For this kind of errors, the only solution was to redo the calibration. Of course, for calibration validation it was not possible to distinguish between perturbation errors and deviations due to bubbles, but if the calibration was validated successfully it was usually assumed that any artifacts in subsequent measurements were caused by bubbles, since corrupt calibrations should be visible already in the validation measurements.

3) TIME CONSTRAINTS

Aside from concern of finishing the project in the time available, the risk that the cells would start to aggregate when kept in the suspension for too long added an extra time constraint to the measurements. The cell suspension was carefully turned upside down in its container a couple of times just before each measurement to mitigate this problem, but the large number of measurements that had to be performed took several hours to complete so aggregation is still a likely source of error.

V. CONCLUSIONS

Various cell suspensions were prepared and measured for the complex permittivity using Agilent 85070E open ended coaxial probe. All the results show an increase in the arithmetic mean of the permittivity difference at low frequencies, even though the large measurement uncertainty means that this is not statistically significant for some of the MUTs. As a result, it could perhaps be inferred that any difference in permittivity between cells and culture medium should primarily be sought at the low end of the spectrum. This indicates that the complex permittivity of the culture medium may be used for modeling of cell suspensions.

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REFERENCES

[1] C. M. Alabaster, "Permittivity of human skin in millimeter wave band," *Electron. Lett.*, vol. 39, no. 21, pp. 1521–1522, Oct. 2003.

[2] B. Alberts, P. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*. New York, NY, USA: Garland, 2002.

[3] S. I. Alekseev and M. C. Ziskin, "Human skin permittivity determined by millimeter wave reflection measurements," *Bioelectromagnetics*, vol. 28, no. 5, pp. 331–339, Jul. 2007.

[4] S. I. Alekseev, O. V. Gordiienko, and M. C. Ziskin "Reflection and penetration depth of millimeter waves in murine skin," *Bioelectromagnetics*, vol. 29, no. 5, pp. 340–344, Jul. 2008.

[5] S. I. Alekseev, O. V. Gordiienko, A. A. Radzievsky, and M. C. Ziskin, "Millimeter-wave effects on electrical responses of the sural nerve *in vivo*," *Bioelectromagnetics*, vol. 31, no. 3, pp. 180–190, Apr. 2010.

[6] J. M. Alison and R. J. Sheppard, "Dielectric properties of humanblood at microwave frequencies," *Phys. Med. Biol.*, vol. 38, no. 7, pp. 971–978, 1993.

[7] M. Arai, J. G. P. Binner, and T. E. Cross, "Correction of errors owing to thermal elongation of high temperature coaxial probe for microwave permittivity measurement," *Electron. Lett.*, vol. 31, no. 14, pp. 1138–1139, Jul. 1995.

[8] D. V. Blackham and R. D. Pollard, "An improved technique for permittivity measurements using a coaxial probe," *IEEE Trans Instrum. Meas.*, vol. 46, no. 5, pp. 1093–1099, Oct. 1997.

[9] N. Chahat, M. Zhadobov, R. Augustine, and R. Sauleau "Human skin permittivity models for millimetre-wave range," *Electron. Lett.*, vol. 47, no. 7, pp. 427–428, Mar. 2011.

[10] L.-F. Chen, C. K. Ong, C. P. Neo, and V. K. Varadan, *Microwaveelectronics: Measurement and Materials Characterisation*. New York, NY, USA: Wiley, 2004.

[11] F. A. Duck, *Physical Properties of Tissue*. San Diego, CA, USA: Academic, 1990.

[12] S. Gabriel, R. W. Lau, and C. Gabriel, "The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues," *Phys. Med. Biol.*, vol. 41, no. 11, pp. 2271–2293, 1996.

[13] C. A. Balanis, *Antenna Theory: Analysis and Design*, 3rd ed. New York, NY, USA: Wiley, 2005.

[14] M. Zhadobov *et al.*, "Complex permittivity of representative biological solutions in the 2–67 GHz range," *Bioelectromagnetics*, vol. 33, no. 4, pp. 346–355, May 2012.

ELIAS ODELSTAD received the M.Sc. degree in engineering physics with Uppsala University, Uppsala, Sweden. He is currently pursuing the Ph.D. degree in space and plasma physics with Uppsala University and the Swedish Institute of Space Physics, Kiruna, Sweden.

His current research interests include the evolution of the plasma environment around comets and spacecraft plasma interactions.

SUJITH RAMAN was born in India. He received the B.Sc. degree in physics from Government Victoria College, University of Calicut, Malappuram, India, in 2003, the B.Ed. degree from the University Teacher Education Centre, University of Calicut, in 2005, and the M.Sc. degree in electronic science and the Ph.D. degree in microwave electronics from the Cochin University of Science and Technology, Kochi, India, in 2007 and 2012, respectively.

He was with the Wireless Sensor Applied Research Laboratory, Letterkenny Institute of Technology, Donegal, Ireland, as a Post-Doctoral Researcher. He is currently a Post-Doctoral Researcher in Solid-State Electronics with the Angstrom Laboratory, Uppsala University, Uppsala, Sweden. His current research interests include designing of planar antennas, planar microwave devices, bioelectromagnetics, and microwave material characterization.

Dr. Raman was a recipient of the URSI Young Scientist Award at the 2011 URSI General Assembly and Scientific Symposium, Istanbul, Turkey.

ANDERS RYDBERG (M'89) received the M.Sc. degree from the Lund University of Technology, Lund, Sweden, in 1976, and the Ph.D. degree from the University of Technology, Göteborg, Sweden, in 1988.

He was with the National Defense Research Establishment, Stockholm, Sweden, ELLEMTEL Development Company, Ltd., Stockholm, and the Onsala Space Observatory, Onsala, Sweden, from 1977 to 1983. In 1991, he was appointed as a Docent (Associated Professor) with the Chalmers University of Technology. From 1990 to 1991, he was a Senior Research Engineer with Farran Technology Ltd., Cork, Ireland. He was with Uppsala University, Uppsala, Sweden, as a Senior Lecturer and Professor in Applied Microwave and Millimeter-Wave Technology in 1992 and 2001, respectively. He is heading the Microwave Group with the Department of Engineering Science, Uppsala University. He has been the joint-owner of Integrated Antennas AB and WISENET Holding AB, Uppsala, since 2007. He has authored and co-authored more than 220 publications in the area of micro and millimeter-wave antennas, sensors, solid-state components and circuits, and holds three patents in the areas. He has supervised seven Ph.D. students.

Prof. Rydberg is an Editorial Board Member of the IEEE Microwave Theory and Techniques (MTT), the Chairman of Section D and an Adjunct Member of Section B of the Swedish Member Committee of URSI (SNRV), and the Chairman of the Swedish IEEE MTT/AP Chapter.

ROBIN AUGUSTINE was born in India in 1982. He received the degree in electronics science from Mahatma Gandhi University, Kottayam, India, in 2003, the master's degree in electronics from the Cochin University of Science and Technology, Kochi, India, in 2005, and the Ph.D. degree in electronics and optic systems from Univerisité Paris Est Marne La Vallée, Paris, France, in 2009.

He held post-doctoral positions with Paris University, Paris, and the University of Rennes 1, Rennes, France, from 2009 to 2011. Since 2011, he has been a Researcher with Uppsala University, Uppsala, Sweden. He has authored and co-authored more than 35 publications, including journals and conferences. His current research interests include designing of wearable antennas, BMD sensors, dielectric characterization, bioelectromagnetism, noninvasive diagnostics, and biological healing effects of RF in *in vitro* and *in vivo* systems.

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