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Microwave Dielectric Spectroscopy of a Single Biological Cell with Improved Sensitivity up to 40 GHz

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Abstract — This paper presents the sensitivity optimization of a microwave biosensor dedicated to the analysis of a single living biological cell from 40 MHz to 40 GHz, directly in its culture medium. To enhance the sensor sensitivity, different capacitive gap located in the center of the biosensor, below the cell position, have been evaluated with different beads sizes. The best capacitive and conductive contrasts have been reached for a gap width of 5 µm with beads exhibiting diameters of 10 and 20 µm, due to electromagnetic field penetration in the beads. Contrasts improvement of 40 and 60 % have been achieved with standard deviations in the order of only 4% and 6% for the capacitive and conductive contrasts respectively. This sensor therefore permits to measure single living biological cells directly in their culture medium with capacitive and conductive contrasts of 0.4 fF at 5 GHz and 85 µS at 40 GHz, and associated standard deviations estimated at 7% and 14% respectively.

Index Terms — Microwave dielectric spectroscopy, biosensor, coplanar waveguide, biology, cell.

I. Introduction

Actual routine instrumentations for cellular analysis are based on optical detection methods. Microscopy and cytometry involves staining or fluorescent techniques, which are indeed very efficient especially in terms of precision and specificity. They however suffer from invasivity with respect to the living analyzed cells, cost and time-efficiency [1].

Among complementary cellular analyzing techniques [2], electrical ones present important advantages. Microwave dielectric spectroscopy is particularly noticeable due to its numerous assets [3]: the technique is naturally non-invasive for the cells, as well as contact free, harmful free and non-ionizing. It is also miniature and therefore compatible with lab-on-chip applications [3]-[5]. Cells may also be directly analyzed in their culture medium during biological processes [6]. Microwave dielectric spectroscopy constitutes therefore a promising candidate for non-invasive cellular analysis in real time.

Both analyses at the population and at the single cell level are of interest for the biologist and medical communities [7]. So far, the microwave sensing technique has been evaluated mainly at the cell population level [3], [8]-[9] and more recently at the single cell level with a microwave interferometric architecture [10], or in broadband with a capacitive topology from 40 MHz up to 40 GHz [11].

This paper therefore deals with a similar capacitive sensor topology for broadband single cell analysis with microwave dielectric spectroscopy and focus on the optimization of the biosensor sensitivity and repeatability of measurements.

II. Biosensor Architecture and Microfabrication

The device developed to perform the microwave dielectric spectroscopy of a single biological cell is presented in Fig. 1. It includes a coplanar waveguide with a capacitive gap in the center. Perpendicularly placed on top is a microfluidic channel, with a mechanical trap in its center. This trap is used to precisely immobilize a biological cell right above the capacitive gap, where the electromagnetic fields are focused.

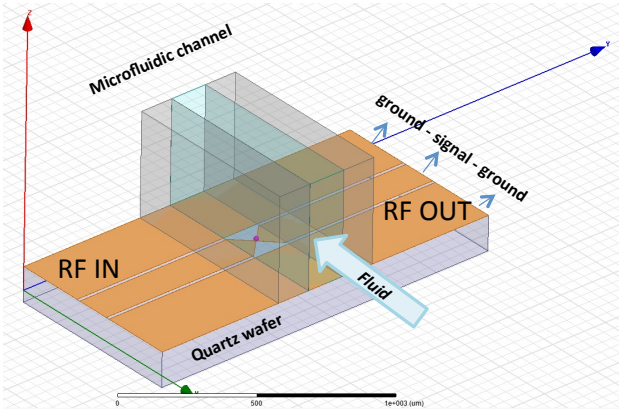

Fig. 1. HFSS view of the biosensor composed of a coplanar waveguide with a microfluidic channel perpendicularly placed on top, which integrates a mechanical trap. The simulated cell is in red.

Fig. 2 presents a zoomed photography of the trap with a polystyrene bead blocked inside. Due to the liquid in the channel, it is not possible to clearly visualize both bead in the blocker and metallization. The waveguide is realized with a 0.3um thick gold layer, whereas trap and microfluidic channel are constituted of SU-8, which has been polymerized after photolithography.

Several biosensors configurations have been fabricated. Both gap and trap sizes have been modified in order to optimize the sensor in terms of sensitivity and trapping efficiency. This paper focuses on the study of sensitivity and repeatability of microwave measurements.

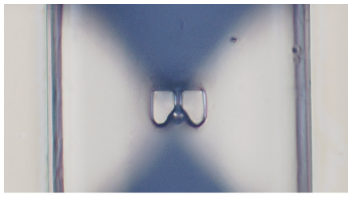

Fig. 2. Photography of a single bead blocked in the trap, located on top of the capacitive gap.

III. Sensitivity Optimization and Repeatability of Measurements with Polystyrene Beads

To assess the sensitivity, biosensors with different size of capacitive gaps have been simulated, fabricated and measured and evaluated with polystyrene beads in de-ionized water. Beads present the interest of being a simple model with constant permittivity and conductivity values compare to biological cells. Gap widths correspond to 5 and 10 µm respectively and have been defined due to the averaged 15 µm diameter of the targeted biological cells. Fig. 3 and 4 present the extracted capacitive and conductive contrasts (similar extraction method as in [11]) for two beads diameters: 10 and 20 µm. Contrasts have been calculated compare to the host liquid medium, DI water. Therefore, the 'zero' line in Fig. 3 and 4 corresponds to the reference DI water. Repeatitivity of the measurements have also been performed in order to extract the standard deviation for all cases.

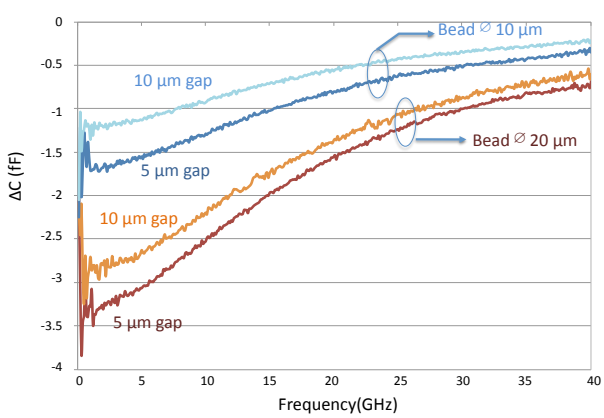

Fig. 3. Capacitive contrast of polystyrene beads of 10 and 20 µm of diameter for the 5 and 10 µm widths of sensor's gap.

The results are summarized in Table 1. As one may expect due to size reduction of the beads, the 10 µm diameter one presents lower capacitive and conductive contrasts compare to the 20 µm diameter bead. Measurements are also very repetitive as the standard deviations are in the order of 4% and 6% for the capacitive and conductive contrasts respectively. Moreover, in all configurations, the 5 µm wide gap presents the largest contrast compare to the 10 µm one. Improvements close to 40% and 50% are reached on the capacitive and conductive contrasts respectively in the case of the 10 µm diameter bead.

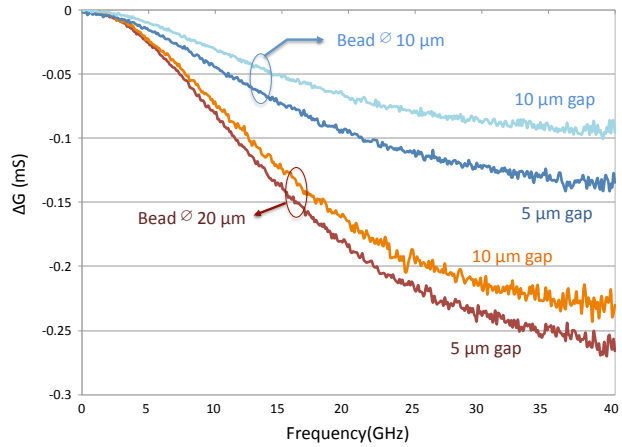

Fig. 4. Conductive contrast of polystyrene beads of 10 and 20 µm of diameter for the 5 and 10 µm widths of sensor's gap.

Table 1. Summary of capacitive and conductive contrasts at 5 and 40 GHz respectively, with respect to gap and bead sizes. Standard deviations in bracket.

	ΔC (fF) @ 5 GHz		ΔG (mS) @ 40 GHz	
	Φ 10 µm	Φ 20 µm	Φ 10 µm	Φ 20 µm
Gap 5 µm	-1.53 (0.06)	-3.05 (0.05)	-0.135 (0.009)	-0.225 (0.009)
Gap 10 µm	-1.12 (0.05)	-2.53 (0.09)	-0.09 (0.009)	-0.26 (0.01)

This result may be explained by the variation of electromagnetic field penetration inside the bead and host medium, as shown in Fig. 5 with simulations performed with the finite element software HFSS© for both 5 and 10 µm wide gaps configurations. Placement of different circles with diameters ranging from 10 to 20 µm is indicated on the figure in order to better estimate the bead location and fields distribution. The strength intensity of the electromagnetic field presents an increase of 63% with the 5 µm gap configuration compare to the 10 µm one, in the 10 µm circle.

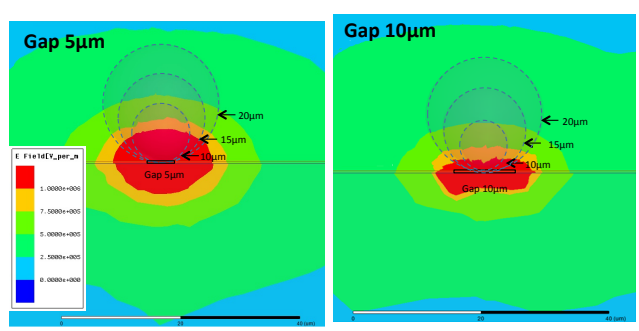

Fig. 5. Electromagnetic field distribution depending of gap width.

Based on this study and to maximize the sensitivity of the biosensor, the capacitive gap size of 5 µm is prefered and employed in the next section, which is dedicated to the RF measurement of single biological cells directly in their culture medium.

IV. Microwave Dielectric Spectroscopy of a Single Bead and Biological Cell in Culture Medium

RF measurements of single biological cells have been performed. Used cells correspond to the THP1, a human monocytic cell line for leukemia investigations. They present an average cell diameter of 15.4 µm. The traditional cell culture medium is the Roswell Park Memorial Institute one (RPMI) with 10% of Fetal Bovine Serum (FBS).

Several individual living cells have been measured and their average contrasts are given in Fig. 6, as well as for 10 µm polystyrene beads in the same host medium as the cells. The reference used in these cases is the culture medium, which in Fig. 6 corresponds to the "zero" line.

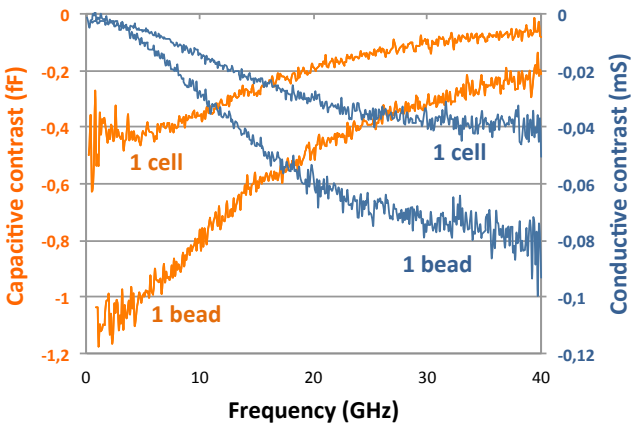

Fig. 6. Capacitive and conductive contrasts of a single polystyrene bead and a single biological cell in cell culture medium (RPMI added with 10% of FBS).

The capacitive contrast of the cell is lower than the bead's one. This is related to their respective relative effective permittivities, which is close to the culture medium one in the case of cell, and thus to water (from 80 to 30 depending on the frequency), whereas polystyrene beads present a permittivity close to 2.4 - 2.7.

Table 2 indicates the summary of measured data for both single beads and cells in culture medium. Standard deviations for the capacitive and conductive contrasts have been estimated at 7% and 14% respectively in the case of the single cells. These values are larger than the ones obtained in the sensitivity optimization section. This may be explained by the heterogeneity of the cells, which exhibit intrinsic variations in terms of diameter and position in the cell cycle. The RF measurement of individual living biological cells is therefore particularly challenging and achieved however with an excellent repeatability.

Table 2. Summary of capacitive and conductive contrasts at 5 and 40 GHz respectively of a single polystyrene bead and a single biological cell. Standard deviations in bracket.

In culture medium	ΔC (fF) @ 5 GHz	ΔG (mS) @ 40 GHz
Single bead	-1 (0.04)	-0.085(0.008)
Single cell	-0.44 (0.03)	-0.044 (0.006)

V. Conclusions

The sensitivity optimization of a microwave biosensor dedicated to the single biological cell analysis directly in its culture medium has been performed and led to an important design rule to further focalize the electromagnetic fields in the cell and reach an improvement of 40% to 60% on capacitive and conductive contrasts respectively. Even if RF measurement of individual living biological cells is particularly challenging due to their ultra-small dielectric contrasts, excellent repeatability of results has been demonstrated with dielectric spectroscopy up to 40 GHz.

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