Microwave biosensor dedicated to the dielectric spectroscopy of a single alive biological cell in its culture medium

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Abstract — This paper presents a biosensor dedicated to the dielectric spectroscopy of a single and living biological cell in its liquid culture medium in the micro and millimeter wave ranges. This detector works in the near field and involves a capacitive gap to perform the electromagnetic sensing, while a microfluidic system has been developed and adapted to the RF circuit to precisely localize the single biological cell under study. Both capacitive and conductive contrasts of a living biological cell measured in its culture medium are accessible. A living B lymphoma cell has then been measured from 40 MHz up to 40 GHz, with a measured capacitive contrast of the order of several hundreds of attofarads.

Index Terms — Microwave, biosensor, biological cell, single cell, dielectric spectroscopy.

I. Introduction

Having access to the features of biological cells, and notably at the single cell level [1] is of prime importance and has led to the development of impressive cellular and molecular analyzing tools (eg. flow cytometers for instance). These are nevertheless invasive and necessitate for a large majority labeling methods with fluorochromes, which may induce cells modifications and thus erroneous or unwanted modifications observations. The development of new non-invasive cellular and molecular analyzing instruments presents consequently a big and attractive challenge for researchers to be applied in biochemistry, biology and biomedical sciences.

Among the possible miniaturized bio-sensing methods (optical, mechanical, chemical), the electrical one, and especially the microwave dielectric spectroscopy performed in liquid constitutes a very attractive and promising candidate, as it does require neither bio-functionalization nor cellular / molecular labeling protocols, while maintaining cell viability and important levels of sensitivity [2-4] and specificity [5-7] of detection. Microwave dielectric spectroscopy in liquid medium has so far been demonstrated with cells suspensions with strong concentrations of millions/ml [3] and down to an amount of 20 cells in suspension [4]. Up to now, microwave measurement of single cells in a liquid environment has been achieved with resonant based RF circuits' configuration [7-8]. The dielectric spectroscopy requires the development of a dedicated RF biosensor compatible with the scale of a single alive cell and with a broadband measurement technique. This constitutes the topic of this paper: a microwave-based biosensor dedicated to the broadband dielectric spectroscopy of single cells in their liquid environment.

First, the microwave biosensor dedicated to the broadband microwave measurement of a single biological cell in its culture medium is described. The capacitive sensor is then evaluated with first polystyrene microspheres, which exhibits a similar size as the investigated cells and constitutes a simplified model to validate the sensor. Finally, the dielectric spectroscopy of a single and living B lymphoma cell in its culture medium is performed and commented.

II. Description of the Microwave Biosensor

The 3D schematic of the microwave biosensor dedicated to single cell analysis is given in Fig. 1. It corresponds to a coplanar line with a capacitive gap located at its center. Two tapers transition are used to locally narrow the central conductor to a dimension compatible with the size of the cell, ie. 10 µm in diameter in the case of this paper.

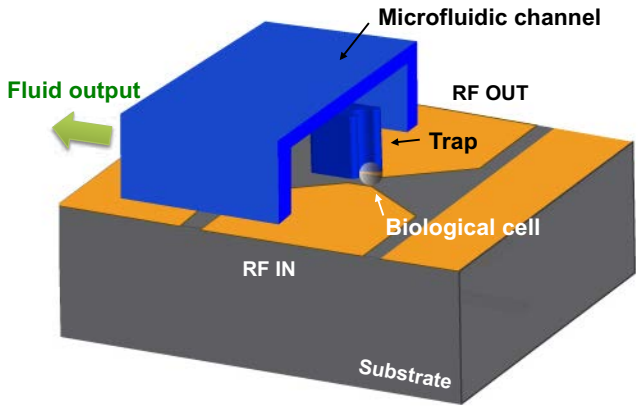

Fig. 1. Schematic of the microwave-based biosensor for living and single cell analysis.

Perpendicularly to the capacitor is placed a microfluidic channel (drawn in blue in Fig. 1), which includes a trap used to precisely localize the cell on the capacitive gap. The fluidic

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channel is crossing the entire microwave structure. However, only half of it is drawn in Fig. 1 in order to allow the visualization of the trap. Such a sensor enables the measurement of the cell in its living pathological state in its traditional culture medium.

Technologically, the coplanar structure is realized with a 300 nm thick gold layer on a quartz substrate. The microfluidic channel and trap are realized in Polydimethylsiloxane (PDMS), a traditional elastomer used in microfluidics, which is biocompatible and transparent for cell observation in the trap.

III. Characterization of the Microwave Biosensor

The characterization is performed from 40 MHz up to 40 GHz. From the measured S parameters may be extracted the complex admittance elements of the capacitor. A first de-embedding step is performed to eliminate the two access transmission lines and the PDMS walls. The admittance corresponds then to a capacitor in parallel with a resistor, which are both modified with the presence or not of a bead or cell due to its relative complex permittivity (ε'_r and ε''). The sensor has then been evaluated with polystyrene microspheres, which constitutes a simple model to calibrate the electrical parameter extraction.

A. Evaluation of the sensor with a single bead

Microbeads with a 10 µm diameter in aqueous solution have been employed. To block a single bead into the sensor, the following protocol has been established. First the beads solution is incorporated in the microfluidic channel. As soon as a bead is trapped, the channel is rinsed with pure DI water in order to remove the other remaining beads in the channel, and especially on the device. The HF measurement is then performed. Fig. 2 presents a photography of a single bead of 10 µm of diameter trapped on the capacitive device.

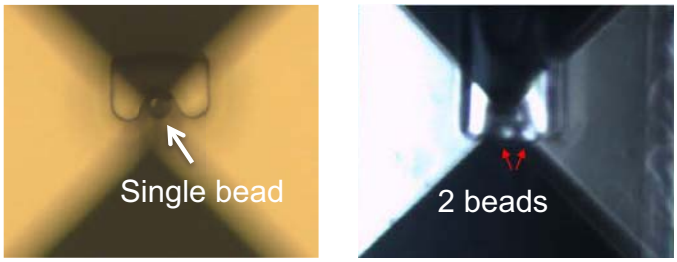

Fig. 2. Photographs of a single bead and two beads trapped on the RF sensor.

The device is also previously measured with DI water, the host medium of the beads. The capacitance is then extracted from the measured S parameters in both cases (bead and host medium), and referred as C_{bead} and $C_{DIwater}$ respectively.

To well distinguish the beads' contribution in the liquid environment, the capacitive contrast of the bead compared to the host medium has been calculated with the equation 1 and is plotted in Fig. 3.

$$\Delta C = C_{bead} - C_{DIwater} \qquad (1)$$

This capacitive contrast has been defined for both one single bead and two trapped beads configurations. The contrast of the

To validate these measurements, simulations with the 3D electromagnetic software HFSS have been performed. The Debye model of DI water has then been implemented as well as a constant relative permittivity of bead (ε_r of 2.6). Fig. 3 shows an excellent agreement between the simulated contrasts (represented in grey) and the measured ones (in black) for both beads' quantities.

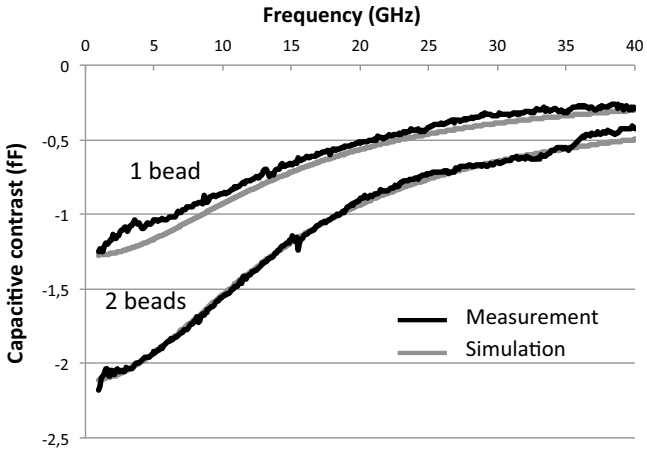

Fig. 3. Capacitive contrast spectra of one and two beads trapped in the biosensor.

The measured and simulated capacitive contrasts of a single bead correspond to 1.2 fF and 1.4 fF at 5 GHz respectively. This discrepancy is related to the position of the bead on the sensor.

In the case of 2 beads, both measured and simulated ΔC are equal to 2.1 fF at 5 GHz. To obtain such an agreement between the two curves, the exact localization of the two beads on the sensor has been taken into account in the simulations after optical observation of the beads (cf. Fig. 2). Moreover, one could expect a doubling of the capacitive contrast due to the two beads. However, one of the beads is well placed at the center of the sensor, whereas the second one is slightly shifted of few micrometers (due to space obstruction by the first bead in the trap). Its dielectric impact is consequently lowered, which leads to a decreased ΔC value of 2.1 fF instead of the 2.4 fF expected one.

Based on this first evaluation with beads, the RF biosensor has been investigated with single biological cells.

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B. Evaluation of the sensor with a single biological cell

Finally, the sensor was evaluated with single and living cells. We have analyzed single B lymphoma cells of RL type, which is a traditional biological model for blood cancer investigations. The cells are prepared and taken out from the incubator just before the RF measurement sequence in order to keep them alive and very healthy. Cells are in suspension in their culture medium, which is composed of Roswell Park Memorial Institute medium (RPMI) with 10 % of fetal calf serum. This medium provides all nutrients and salt required for the viability of cells.

Fig. 3 presents the photography of such a cell blocked in the center of the RF sensor.

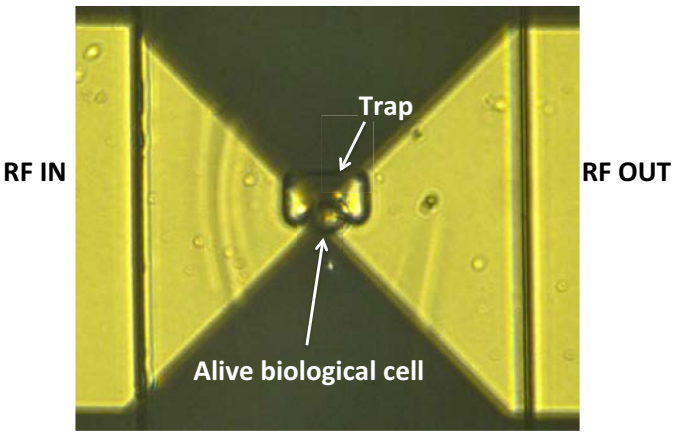

Fig. 4. Photography of a single and live lymphoma cell, which is trapped in the center of the RF sensing area.

Similarly to the beads investigation, the capacitances with the cell in its RPMI liquid environment and without the cell have been extracted from the measurements. The corresponding capacitive contrast has been calculated using the equation 2.

$$\Delta C = C_{cell} - C_{medium} \qquad (2)$$

It has led to the capacitive contrast spectrum up to 40 GHz of Fig. 5, for two single B lymphoma cells, which have been measured one by one.

Two single B lymphoma cells spectra are presented in Fig. 5 to demonstrate the repeatability of the spectrum. Some dispersion is visible between the two spectra, which may be attributed to the heterogeneity into a cell suspension (intrinsic cells differences and cell cycle...).

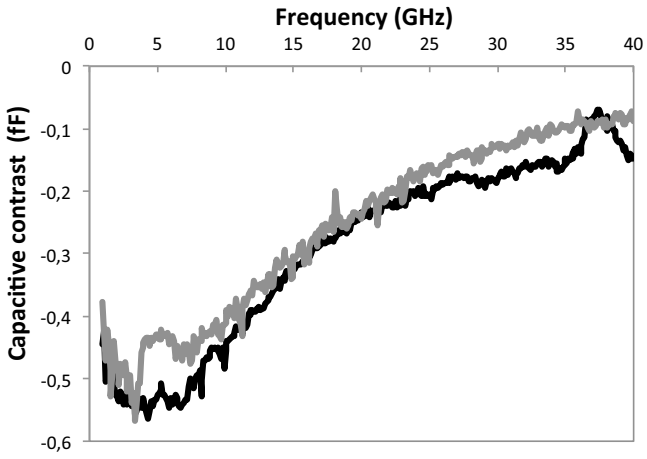

Fig. 5. Capacitive contrast of living single B lymphoma cells trapped in the biosensor.

The maximum contrast is obtained around 5 GHz with a value close to 0.53 fF, which is much larger than the estimated measurement resolution of 0.01 fF. Compared to the beads, the cell contrast spectrum is lower. The cells and the culture medium are indeed not very different, as they are both mainly constituted of water, molecules, ions and other biomaterials in the contrary to beads with a small permittivity value of 2.6.

IV. Conclusion

To the best of our knowledge, this paper presents for the first time the dielectric spectrum of a single and healthy biological cell in the micro and millimeter wave ranges. To achieve such a spectroscopy, a RF biosensor has been specifically defined, realized and successfully evaluated. It includes a capacitive sensing zone associated to a microfluidic channel with a cell's trap. The measured capacitive contrast of a B lymphoma cell is in the order of hundreds of attofarads, far from the measurement resolution. It allows envisaging further non-invasive biological processes studies at the single celling level and getting access to new understanding of biological reactions.

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