

# Miniature microwave sensor suitable for real-time dielectric analysis of multicellular spheroids

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Microwave dielectric spectroscopy is an analytical technique used to study the dielectric properties of materials at microwave frequencies (300 MHz to 300 GHz). The method involves exposing a sample to a low power microwave field and analyzing the changes in transmitted or reflected waves to measure its complex permittivity. This non-invasive, non-destructive, label-free and cost-effective technique is valuable for characterizing biological samples directly into their culture medium, complementary with the mainly used characterization techniques as optics (1–3) and low frequency electrical methods such as impedancemetry (4). At this frequency range, the electromagnetic waves are able to bypass the plasma membrane, enabling the intracellular content probing (5-6). This technique is able to differentiate cells (cancerous or not, different phenotypes of lymphoma cells for example), as well as cell health (alive, dead, treated, etc), at various biological scales from tissues, cells in suspension down to single cells (1–3). Although, as far as 3D biological models, such as multicellular spheroids, are concerned, microwave dielectric spectroscopy is now addressing this intermediate scale localized between *in vitro* and *in vivo* investigations with a dedicated sensor, as shown in Fig1 (7-8). This bio-model provides a more physiologically relevant environment, exhibiting gradients of nutrients, oxygen, and waste products similar to those of *in vivo* tissues (9–11).

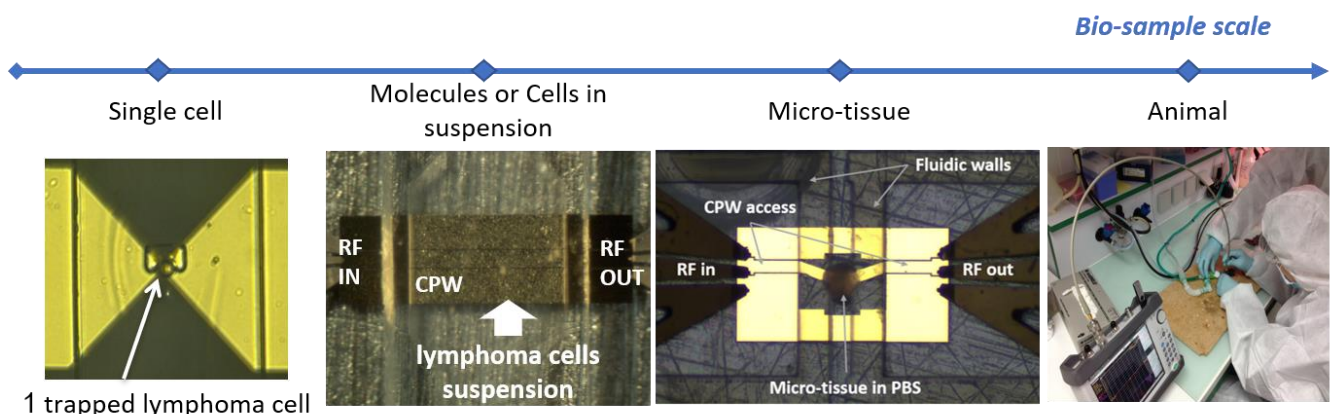
A microfabricated chip, which combines a microwave dielectric spectroscopy sensor and a appropriate microfluidic structure able to maintain a 300 $\mu\text{m}$ -diameter multicellular spheroid in its culture medium has been developed. The microwave sensor includes a coplanar waveguide presenting a 10 $\mu\text{m}$ -width capacitive gap in the center on the structure Fig. 2 a). The top microfluidic structure is based on a regular channel with two inlets. A mechanical trap in a hydrodynamic flow is used to locate the spheroids specifically in the center of the structure over the capacitive sensing area. The device under test is connected to a Vector Network Analyser (VNA) using two coplanar probes and two coaxial cables, which allows to get the S parameters from 500 MHz to 20 GHz. First, a standard short-open-load-through (SOLT) calibration is applied to locate the microwave reference planes at the ends of the RF probes. A second de-embedding method described in (12) is applied to extract from the measured S parameters the dielectric properties through capacitive and conductive contrasts of the multicellular spheroid on the frequency spectrum.

Measurements of several multicellular spheroids from human hepatocarcinoma cell line (HepG2) have been performed during 15min. The mean value and standard deviation of both capacitive and conductive contrasts are presented in Fig. 2 b) and c), as well as the zero level of the culture medium. These results validate the microwave sensor to non-invasively characterize living spheroids. The small standard deviations also demonstrate the stability of the microwave measurements. It makes it suitable for real-time monitoring of 3D biological models while giving access to intracellular modifications enabled by the microwave frequency range, and long-term drug screening.

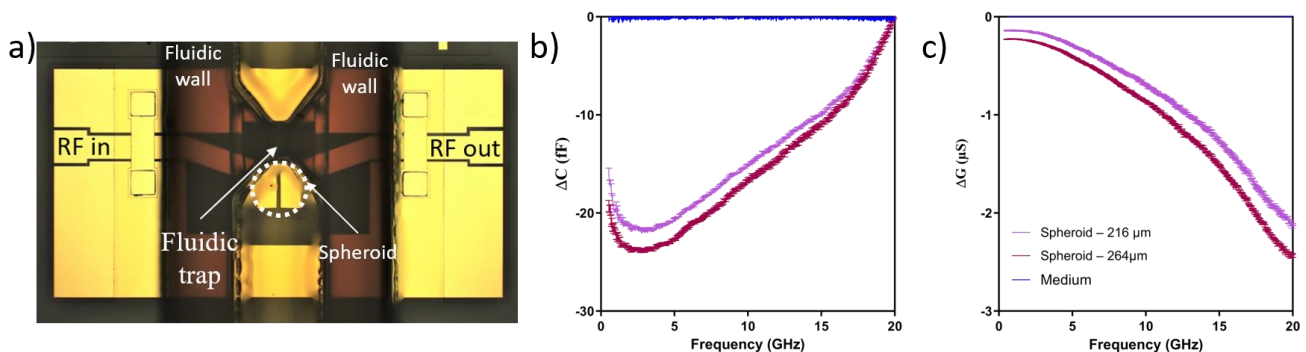
## References

1. Mehrotra P, Chatterjee B, Sen S. EM-Wave Biosensors: A Review of RF, Microwave, mm-Wave and Optical Sensing. *Sensors*. 2019 Feb 27;19(5):1013.
2. Mertens M, Chavoshi M, Peytral-Rieu O, Grenier K, Schreurs D. Dielectric Spectroscopy: Revealing the True Colors of Biological Matter. *IEEE Microw Mag*. 2023 Apr;24(4):49–62.

3. Heileman K, Daoud J, Tabrizian M. Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis. *Biosens Bioelectron.* 2013 Nov;49:348–59.
4. Maino N, Bertsch A, Renaud P. Impedance spectroscopy of the cell/nanovolcano interface enables optimization for electrophysiology. *Microsyst Nanoeng.* 2023 May 16;9(1):62.
5. Artis F, Chen T, Chretiennot T, Fournie JJ, Poupot M, Dubuc D, et al. Microwaving Biological Cells: Intracellular Analysis with Microwave Dielectric Spectroscopy. *IEEE Microw Mag.* 2015 May;16(4):87–96.
6. Schwan HP. Electrical properties of blood and its constituents: Alternating current spectroscopy. *Blut.* 1983 Apr;46(4):185–97.
7. Peytral-Rieu O, Dubuc D, Grenier K. RF sensor dedicated to the dielectric characterization of spheroids between 500MHz and 20GHz. *Int J Microw Wirel Technol.* 2023;
8. Peytral-Rieu O, Dubuc D, Grenier K. Microwave-Based Sensor for the Non-Invasive and Real Time Analysis of 3D Biological Microtissues: Microfluidic Improvement and Sensitivity Study. *IEEE Trans Microw THEORY Tech.* 2023;
9. Costa EC, Moreira AF, de Melo-Diogo D, Gaspar VM, Carvalho MP, Correia IJ. 3D tumor spheroids: an overview on the tools and techniques used for their analysis. *Biotechnol Adv.* 2016 Dec;34(8):1427–41.
10. Nunes AS, Barros AS, Costa EC, Moreira AF, Correia IJ. 3D tumor spheroids as in vitro models to mimic in vivo human solid tumors resistance to therapeutic drugs: NUNES ET AL. *Biotechnol Bioeng.* 2019 Jan;116(1):206–26.
11. Pinto B, Henriques AC, Silva PMA, Bousbaa H. Three-Dimensional Spheroids as In Vitro Preclinical Models for Cancer Research. *Pharmaceutics.* 2020 Dec 6;12(12):1186.
12. Peytral-Rieu O, Grenier K, Dubuc D. Microwave Sensor Dedicated to the Determination of the Dielectric Properties of 3D Biological Models from 500MHz to 20GHz. In: 2021 IEEE MTT-S International Microwave Symposium (IMS). Atlanta, GA: IEEE; 2021. p. 4.



*Fig1: Examples of sensors based on microwave dielectric spectroscopy according to the scale of the bio-sample.*



*Fig2: a) Picture of the microwave biosensor. Mean value and standard deviation of capacitive contrast b) and of conductive contrast c) of two living multicellular spheroids (red and purple) and of culture medium (representing the zero value in blue) during 15min over the frequency range.*