Microwave Interferometry Measurements of Yeast Cell Suspension and Sediment Process

M. Zhang*, T. Markovic** and B. Nauwelaers*
* Department of Electrical Engineering, University of Leuven, Leuven, Belgium
** imec, Heverlee, Belgium
meng.zhang@esat.kuleuven.be

***Abstract* – A microwave interferometry based coplanar waveguide sensor is proposed for cell dynamic process monitoring applications. Combining the ease of microfluidics integration and impedance modeling from coplanar waveguide with high sensitivity and wide frequency range from interferometer structure, the designed setup has proved its potential for biological applications. Based on characteristic of interferometer and sensor modeling, the algorithm for extraction of the material under test complex permittivity from the S-parameters changes has been developed. Measurement and calculation of 2 mg/ml yeast cell suspension have been carried out to validate the setup, which agreed well with reference data in literature. Furthermore, monitoring of yeast cell sediment process was performed to lay the groundwork for future cell growth monitoring.**

Keywords - dielectric spectroscopy, interferometry sensor, millimeter wave, microfluidics, biogenic liquid

I. INTRODUCTION

Cell suspension measurement and cell growth monitoring are of great importance for both academic and industries in biology, biochemistry, and pharmaceutical fields [1], [2]. Among the fast developing and widely used bio-sensing techniques, such as optical, mechanical and chemical methods, electrical detection and property extraction of cell cultures have raised significant interests for label-free, noninvasive and high-sensitivity biosensor designs [3], [4]. One major method is to characterize the material-under-test (MUT) with its frequency dependent permittivity, a physical representation of (di)electric polarization [5]. Biogenic liquid's permittivity contains valuable biological information, especially for dielectric dispersions at microwave and millimeter wave frequencies. However, most of the work studying cell dynamic properties so far mainly focus on kHz frequency range [6], [7] and are often limited on sensitivities.

Many efforts, for example, resonator structures, have been explored to improve electrical biosensor's sensitivity [8], but its frequency range is confined to the resonance point. In order to measure broad frequency range complex permittivity of MUT with high sensitivity, interferometric dielectric spectroscopy has proved its potential for bio-material characterization in recent years [9]. Due to its flexibility regarding sensing structures, many microwave topologies, e.g. coplanar waveguide (CPW), with easy integration of miniaturized microfluidics have led to broad frequency range and high sensitivity measurements of liquid mixtures [10-12], cell cultures [13-14], single cells [15] and even microwave heating at the micro-meter scale [11], [16-17]. And yet, few researches have been carried out on the cell culture monitor, especially, the sediment process.

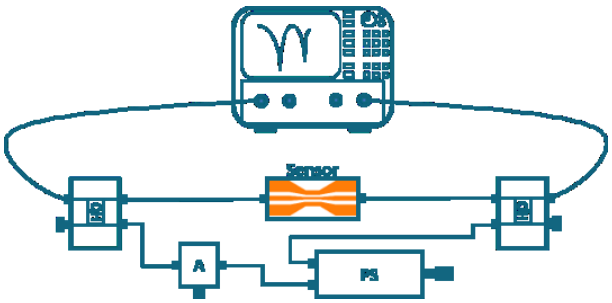

Figure 1. Schematic of measurement setup.

As a capability demonstration of microwave interferometry techniques in measuring and monitoring cell behavior in suspensions, this work illustrates the application of a CPW-based biosensor to extract the dielectric dispersion of yeast cell suspensions and their sediment process at 12-18 GHz. Section II describes the measurement setup, the sensing principle with CPW sensor design, and the permittivity extraction method. In section III, the measurements are conducted with yeast cell to water concentration of 1 g / 50 ml and 1 g / 500 ml. Finally, the paper is concluded in the last section.

II. MEASUREMENT SETUP AND SENSING PRINCIPLE

A. Measurement Setup

Schematic of the system is shown in Fig. 1. Two 90-degree couplers (Quadrature Hybrid (QH), SigaTek, SQ16506) split and combine the sensing signal (e.g. 15 GHz) from Vector Network Analyzer (VNA) into two branches: the sensor channel and the reference/tuning channel with 180-degree phase difference, aiming to form destructive interference. The reference channel is connected to mechanically controlled Attenuator (Narda 4799) to compensate the attenuation and Phase Shifter (PS, ARRA 9428A) to finely tune the phase difference between these two branches. The sensor channel consists of a tapered CPW sensing structure to adapt to miniaturized microfluidics (Fig. 2). Interference null is achieved as S_{21}

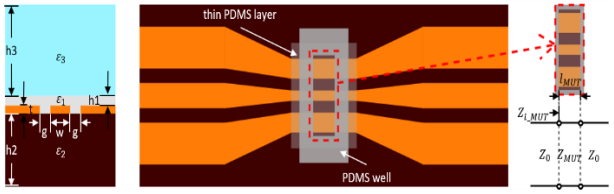

Figure 2. The cross-section (left), top views (middle) and impedance model (right) of the CPW sensing structure, where w = 0.6 mm, g = 0.4 mm, t = 18 µm (copper), h_1 = 20 µm (thin PDMS layer), h_2 = 1.6 mm, h_3 = 5 mm (MUT), ε_1 = 2.69 (PDMS), ε_2 = 2.2 (Duroid 5880), ε_3 = permittivity of MUT.

on VNA, when these two channels are turned to similar attenuation but around 180-degree phase difference.

B. *Sensing Principle and Sensor Design*

Fig. 2 illustrates the cross-section (left), top views (middle) and impedance model (right) of the CPW sensing structure for the measurements. The CPW is made of Duroid 5880 with copper thickness of 18 µm. And to make it biocompatible, a 20 µm polydimethylsiloxane (PDMS) layer is applied in the sensing area, which is indicated with red dashed rectangular. At last, the PDMS microfluidic well used to hold MUT is attached on top. CPW-based sensor was chosen because of its ease of fabrication and connection to the measurement system and high sensitivity to changes of MUT above. The depth of microfluidic well on top of the CPW sensing area are designed relatively deep (h_1 = 5 mm) compared to the gap of CPW (g = 4 mm) to guarantee sufficient height of MUT, so that the detected signal change is caused mainly by MUT, and also reduce errors introduced by manual filling and evaporation. The width of well, l_{MUT}, is 1 mm, resulting an effective volume of 7 µl.

The attenuator and PS are tuned to obtain small S_{21}, inducing better destructive interference, at a certain frequency f_0 = 15 GHz. The smaller S_{21} was tuned, the higher Q-factor the system will achieve, thus, larger sensitivity. Fig. 3 displays the system's S-parameters in frequency domain after tuning S_{21} to below -70 dB and its Q-factor has reached 10^4.

Similar to the permittivity extraction described in [18], with corresponding S_{11} and f_0, the complex permittivity of MUT,

$$\varepsilon = \varepsilon' - j\varepsilon'' \tag{1}$$

can be calculated from the signal propagation constant:

$$\gamma_{MUT} = (\alpha_c + \alpha_c) + j\beta \tag{2}$$

of the CPW sensing area filled with MUT. Specifically, here we apply:

$$\frac{S_{11(MUT)}}{S_{11(CAL)}} = \frac{Z_{l\_MUT} - Z_0}{Z_{l\_MUT} + Z_0} \times \frac{Z_{l\_CAL} + Z_0}{Z_{l\_CAL} - Z_0} \tag{3}$$

$$\frac{S_{21(MUT)}}{S_{21(CAL)}} = \frac{\exp(-\gamma_{MUT} \times l_{MUT})}{\exp(-\gamma_{CAL} \times l_{CAL})} \tag{4}$$

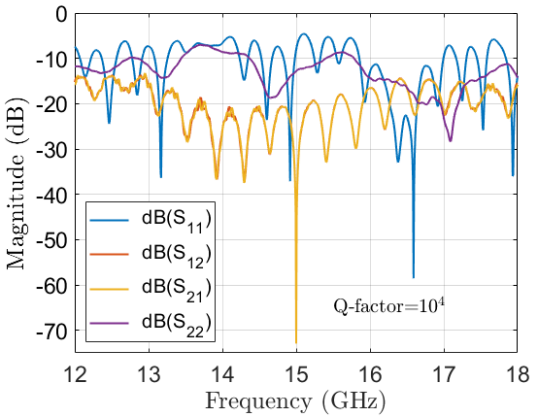

Figure 3. S-parameters of the system with S_{21} (15 GHz) tuned below -70 dB, Q-factor = 10^4.

among which the subscripts MUT and CAL, refer to "material under test" and "calibration liquid" respectively; And $l_{MUT/CAL}$ is the physical length of the sensing area sections; Z_{l_MUT} and Z_0 are impedances marked in Fig.2. Deionized water is used as calibration liquid to help eliminate the signal transfer functions of all other sensor and system components except for the sensing area section.

With the obtained signal propagation constant of the sensing area filled with MUT (γ_{MUT}), described in [19], $Z_{l\_MUT/CAL}$ is determined by the term "tanh(-γ_{MUT} × l_{MUT})". Thus, the real and imaginary part of MUT permittivity can be acquired. Then, repeating the same calculations for each frequency point.

III. Measurements and Discussions

A. *Yeast Cell Suspension Measurement*

To validate the design, yeast cell suspension with concentration of 2 mg/ml is tested first. The measurement was conducted under room temperature of 16.7°C with setup schematic illustrated in Fig. 1 and guided by the following steps:

- Firstly, after connecting all the components and sensor, deionized water was injected into the PDMS well with a pipette.
- Secondly, tuning attenuator and PS alternately at 15 GHz in frequency domain sweep mode until S_{21} reaches below -70 dB for higher sensitivity and recording the S-parameters.
- Thirdly, mixing the Bruggeman dry bakery yeast with deionized water and rotating the container for 5 mins to fully suspend the yeast cells.
- Finally, carefully and completely removing deionized water inside the well and refilling it with 2 mg/ml yeast cell suspension and recording the S-parameters again.

Fig. 4 shows the extracted real and imaginary permittivity of 2 mg/ml yeast cell suspension with error bars. The extracted permittivity correlates well with reference data in [20], validating the sensor's capability of sensitive and accurate permittivity measurements.

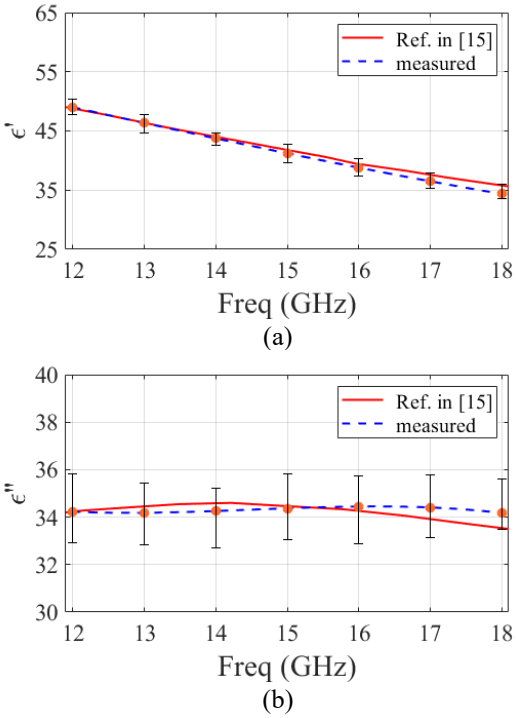

Figure 4. Extracted permittivity of 2 mg/ml yeast cell suspension: (a) real part, (b) imaginary part.

B. *Yeast Cell Sediment Monitoring*

Since the density of yeast cell is slightly larger than deionized water, the sediment process will happen right after filling the PDMS well with yeast cell suspension. Accumulating at the bottom of CPW sensor, the extracted permittivity will decrease for both real and imaginary parts (Fig. 5). From the sediment monitoring measurement, we can see that for 2 mg/ml yeast cell suspension, the cells will settle down in about 23 minutes with a volume of 7 µl.

C. *Discussions*

The error of extracted permittivity is considered relatively large especially for imaginary part. A few methods can be applied to improve its accuracy; firstly, making a better fixation of the system to eliminate the environmental noises, since the system is very sensitive to mechanical movements; secondly, providing temperature control of the MUT to reduce the error caused by temperature difference, noted that the measurement temperature in [18] is at 18.5°C while this work is carried out at 16.7°C; and finally, coming up with more accurate permittivity extraction algorithm, taking the reflection of signal between different components into consideration. Our next step also includes cell growth monitoring on thin layer of cell culture mediums, such as luria agar, lysogeny broth and collagen gel, broadening the horizon of microwave sensors on biological applications.

IV. CONLUSION

This paper presents a broadband, high sensitivity microwave measurement setup for monitoring cell dynamic properties. The system is based on microwave interferometry structure and combined with CPW sensor to enable adaptation to microfluidics and extraction of permittivity. The setup was validated by 2 mg/ml yeast cell suspension measurement with good agreement compared to reference data in [20]. Then, it was applied to sediment process monitoring, providing valuable information about cell settlement for future cell growth monitoring experiments.

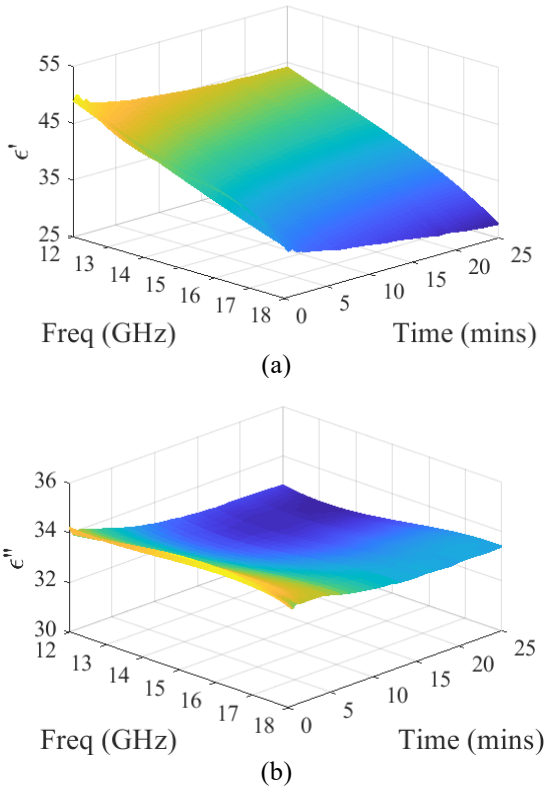

Figure 5. Sediment monitoring of 2 mg/ml yeast cell suspension for 25 minutes: (a) real part, (b) imaginary part.

ACKNOWLEDGMENT

This work was supported in part by the KU Leuven under project C24/15/015 Microwave Microbiology (µ²BIO), and in part by Chinese Scholarship Council for Meng Zhang.

REFERENCES

[1] A. K. Laird, "Dynamics of tumour growth: comparison of growth rates and extrapolation of growth curve to one cell," Br. J. Cancer, vol. 19, no. 2, pp. 278-291, 1965.

[2] M. Mir et al., "Optical measurement of cycle-dependent cell growth," Proc. Natl. Acad. Sci. U.S.A., vol. 108, no. 32, pp. 13124-13129, 2011.

[3] T. Sun and H. Morgan, "Single-cell microfluidic impedance cytometry: a review," Microfluid. Nanofluid., vol. 8, pp. 423-443, 2010.

[4] Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, and P. Wang, "Cell-based biosensors and their application in biomedicine," Chem. Rev., vol. 114, no. 12, pp. 6423-6461, 2014.

[5] F. S. Barnes and B. Greenebaum, Biological and medical aspects of electromagnetic fields, third edition, CRC press, Taylor & Francis Group, 2007.

[6] I. Giaever and C. R. Keese, "Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture," IEEE Trans. Biomed. Eng., vol. BME-33, no. 2, pp. 242-247, 1986.

[7] I. O. K'Owino and O. A. Sadik, "Impedance spectroscopy: a powerful tool for rapid biomolecular screening and cell culture

monitoring," Electroanalysis, vol. 17, no. 23, pp. 2101–2113, October 2005.

[8] D. Wood, S.-H. Oh, S.-H. Lee, H. Soh, and A. Cleland, "High-bandwidth radio frequency Coulter counter," Appl. Phys. Lett., vol. 87, pp. 184106, 2005.

[9] Y. Yang et al., "Distinguishing the viability of a single yeast cell with an ultra-sensitive radio frequency sensor," Lab Chip, vol. 10, pp. 553-555, 2010.

[10] S. Liu et al., "Broadband dielectric spectroscopy calibration using calibration liquids with unknown permittivity," 84th ARFTG Microwave Measurement Conference, Boulder, CO, 2014, pp. 1-5.

[11] T. Markovic et al., "An interdigital capacitor for microwave heating at 25 GHz and wideband dielectric sensing of nL volumes in continuous microfluidics," Sensors, vol. 19, no. 3, pp. 715, 2019.

[12] J. Bao et al., "A 20-GHz microwave miniaturized ring resonator for nL microfluidic sensing applications," IEEE Sensors Letters, vol. 3, no. 6, pp. 1-4, 2019.

[13] S. Liu, I. Ocket, M. Cauwe, D. Schreurs and B. Nauwelaers, "Sensitivity analysis of broadband on-wafer dielectric spectroscopy of yeast cell suspensions up to 110 GHz," IEEE Microw. Wireless Compon. Lett., vol. 25, no. 3, pp. 199–201, 2015.

[14] T. Chen, D. Dubuc, M. Poupot, J-J. Fourni, and K. Grenier, "Accurate nanoliter liquid characterization up to 40 GHz for biomedical applications: toward noninvasive living cells monitoring," IEEE Trans. Microw. Theory Techn., vol. 60, no. 12, pp. 4171–4177, 2012.

[15] H. Li, X. Ma, Y. Ning, X. Cheng, and J. C. M. Hwang, "Correlation between morphology change and microwave property during single-cell electroporation," 2017 IEEE MTT-S Int. Microw. Sym. (IMS) Dig., Honololu, HI, 2017, pp. 869–871.

[16] T. Markovic, I. Ocket, B. Jones and B. Nauwelaers, "Characterization of a novel microwave heater for continuous flow microfluidics fabricated on high-resistivity silicon," 2016 IEEE MTT-S Int. Microw. Symp. (IMS), San Francisco, CA, 2016, pp. 1-4.

[17] T. Markovic, S. Liu, I. Ocket and B. Nauwelaers, "A 20 GHz microwave heater for digital microfluidic", Int. J. Microw. Wirel. T., vol. 9, no. 08, pp. 1591-1596, 2017.

[18] M. Zhang et al., "Broadband interferometric dielectric spectroscopy for aqueous solutions," 2017 IEEE MTT-S International Microwave Workshop Series on Advanced Materials and Processes for RF and THz Applications (IMWS-AMP), Pavia, 2017, pp. 1-3.

[19] Y. Cui, J. Sun, Y. He, Z. Wang, and P. Wang, "A simple, tunable, and highly sensitive radio-frequency sensor," Appl. Phys. Lett., vol. 103, no. 6, pp. 062906, 2013.

[20] S. Liu, I. Ocket, P. Barmuta, A. Lewandowski, D. Schreurs and B. Nauwelaers, "Broadband dielectric spectroscopy calibration for microliter samples of biogenic liquid," 2014 44th European Microwave Conference, Rome, 2014, pp. 279-282.