Broadband Electrical Impedance Spectroscopy of Single Cells for Viability Assessment

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Principal Investigator(s): Alireza Abbaspourrad
User(s): Amirhossein Favakeh, Amir Mokhtare, Mohammad Javad Asadi, James C. M. Hwang

Affiliation(s): Department of Food Science, Cornell University, Ithaca 14853, New York, USA; School of Electrical and Computer Engineering, Cornell University, Ithaca, New York 14853, USA; School of Electrical and Computer Engineering, Cornell University, Ithaca, New York 14853, USA

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Contact: am2964@cornell.edu, ma2297@cornell.edu, jch263@cornell.edu

Primary CNF Tools Used: Heidelberg DWL2000 Mask Writer, ABM Contact Aligner, SUSS MA6 Contact Aligner, Oxford 82 Etcher, SCVC Even-Hour Evaporator

Abstract:

Single-cell analysis plays an important role in disease diagnosis. However, many characterization methods are labor-intensive, costly, and time-consuming. Electrical impedance spectroscopy (EIS) offers a label-free, non-invasive method for probing the biophysical characteristics of cells and assessing their viability. Here, we have designed and fabricated a coplanar waveguide (CPW) integrated with microfluidics that can precisely capture a single cell between the gaps of the CPW electrodes. By sweeping the frequency from low (30 kHz) to high (6 GHz) through the cell, we successfully extracted the cellular bilayer electrical properties in real-time monitoring and assessed the cell viability through modeling each layer of the cell with a suggested electrical equivalent circuit.

Summary of Research:

To fabricate the electrode (Figure 1a), first, we spin-coated AZ nLOF 2020 photoresist on a 4-inch fused silica wafer with a thickness of 500 µm. We created the photomask using Heidelberg DWL2000 Mask Writer. Then, we patterned the design using the SUSS MA6 Contact Aligner on the photoresist of the fused silica wafer. After developing with AZ 726 MIF, we removed residual resist by descumming the wafer using the Oxford 82 Etcher. Next, metal layers (20 nm titanium and 500 nm gold) were deposited onto the substrate using an electron beam evaporator. This was followed by a lift-off process to remove unwanted metal and remaining photoresist (Figure 1b).

Photolithography was used to fabricate the microfluidic channel. First, we used SU8-2025 negative photoresist to spin-coat a 20-micron-thick layer onto a silicon wafer. After soft baking, exposure was done using the ABM contact aligner, followed by post-baking and developing with SU8 developer. We then hard-baked the pattern. Afterward, the PDMS was poured over the master mold and placed in the oven at 65°C for 2 hours. Next, we peeled the PDMS off and bonded the microfluidic channel with the CPW intersection gap, ensuring a leak-proof seal. Finally, the CPW input and output were terminated with SMA coaxial connectors (Figure 1c).

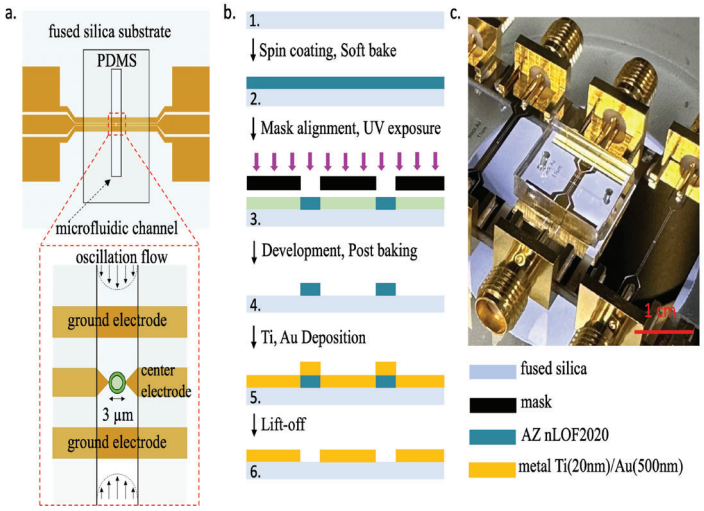

Figure 1: Electrical-impedance microfluidic platform to probe the single-cell biophysical characteristics. (a) Coplanar waveguide (CPW) design. (b) CPW fabrication process. (c) Photograph of the assembled platform.

The platform was connected to the vector network analyzer (VNA), and yeast cells suspended in a low-conductivity 8.5% sucrose solution were injected through the microchannel for final measurements.1 Using dielectrophoresis (DEP), we captured a single cell at 4 MHz and 0 dBm power between

the CPW gap (Figure 2a). With EIS, most of the electric field passes through the cell; 2 therefore, by switching the trapping mode to characterization mode from 30 kHz to 6 GHz and using -18 dBm power, we successfully probed the cell's intracellular properties. Using a two-port measurement, we measured both the membrane and cytoplasm electrical properties, and we assessed cell viability through scattering (S) parameter measurement. The impedance data were then validated by fitting

S-parameters to the proposed equivalent circuit for the cell (Figure 2b) via Advanced Design System (ADS) software.

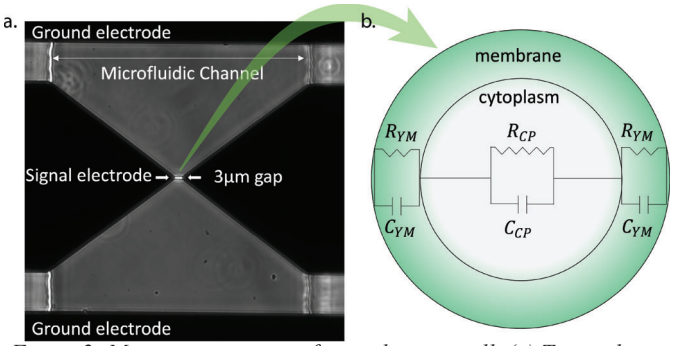

Figure 2: Microwave sensing of a single yeast cell. (a) Trapped single yeast cell between the coplanar waveguide (CPW) electrode gap. (b) Suggested equivalent circuit for each layer of the cell.

Conclusions and Future Steps:

We introduced a high-throughput electrical-impedance microfluidic platform that successfully measures the intracellular electrical properties of single cells. It can distinguish cell viability at high frequencies (3 GHz), where cytoplasm capacitance is dominant. The system enables real-time differentiation between live and dead cells with high accuracy, demonstrating a cytoplasmic capacitance of 3.6 fF for live cells. This platform is fast, accurate, non-invasive, and label-free, enabling real-time monitoring of single cells. It can be used for different electrode configurations and cell types, including mammalian and reproductive cells, for precise single-cell analysis. Future work will focus on EIS measurements of oocytes using this platform to select the best oocyte candidate for assisted reproductive technology (ART) purposes.

References:

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[2] J. C. M. Hwang, IEEE Microwave Mag., 2021, 22, 78–87.