Real time in-situ monitoring of in-flow electroporation using impedance cytometry

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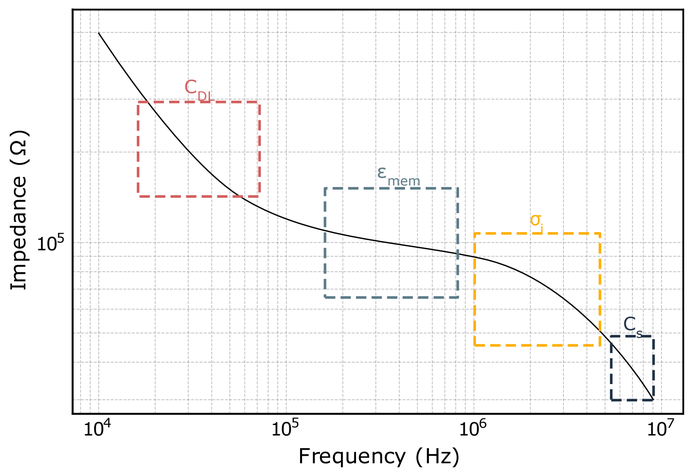
Introduction

Electroporation has become a widely adopted method for the intracellular delivery of exogeneous cargo. Its **integration with microfluidics** and further miniaturization has further consolidated its usefulness, offering more control, efficiency, and viability by performing electroporation on individual cells [1].

This works aims to **monitor the in-flow electroporation process in real time using the impedance cytometry** principle. The combination of electroporation and impedance cytometry has been shown before using macroscopic wire electrodes and a constriction channel geometry for DC excitation signals [2]. Our device further miniaturizes this process by using embedded electrodes inside a microfluidic channel and deconstructs the bipolar square electroporation signal to monitor the impedance across a broad frequency range [3].

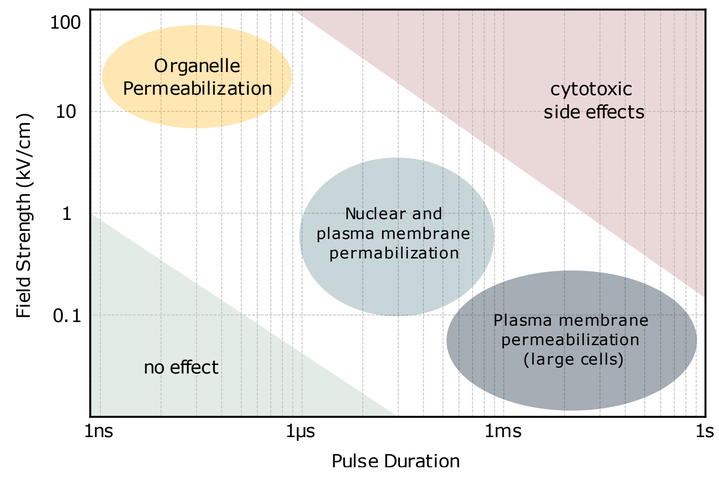
Impedance Cytometry

Impedance cytometry is a label free characterization tool for analyzing the dielectric properties of a cell. Traditionally, it uses a superposition of 3 to 4 sinusoidal excitation signals to probe the cell at different frequencies, and measures the current response using lock-in amplifiers to extract the frequency components. The frequencies are typically chosen in such a way that it probes certain dielectric properties of the cell, like the membrane permittivity and cytosol conductivity. The values of these dielectric properties can be extracted by fitting an equivalent circuit model at each frequency component.



In-Flow Electroporation

In-Flow Electroporation is the formation of nanopores in a cell membrane in the presence of an external field as cells are flowing through a microfluidic channel. Defining and delivering the optimal electric pulse to each cell is of critical importance for efficient transfection while avoiding cytotoxicity[4]. So far, impedance analysis during electroporation has only been limited to DC excitation signals. We propose that by using the underlying harmonics of the bipolar square excitation signal typically used for electroporation, it is possible to monitor the impedance at various frequencies

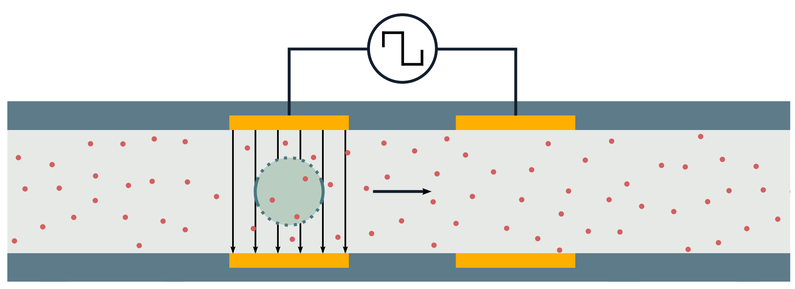


Setup & Methodology

Both impedance cytometry and in-flow electroporation use the same setup: a pair of electrodes at top and bottom of a microfluidic channel. By decreasing the dimensions of the channel and electrodes, it is possible to perform measurements on individual cells.

COMSOL was used to create a digital twin model of the device. Appropriate material properties were chosen for the electrodes, cytosol, and channel medium; while the cell membrane and electrode-medium interface were represented by contact impedance boundary conditions.

A 200 kHz bipolar square excitation wave with a 3V amplitude is applied at the top electrodes while the bottom electrode set is grounded. Using Fast Fourier transform, the frequency spectrum for both the excitation signal and current response are computed. Finally, the impedance is obtained by calculating the voltage-current ratio at the harmonic frequencies.

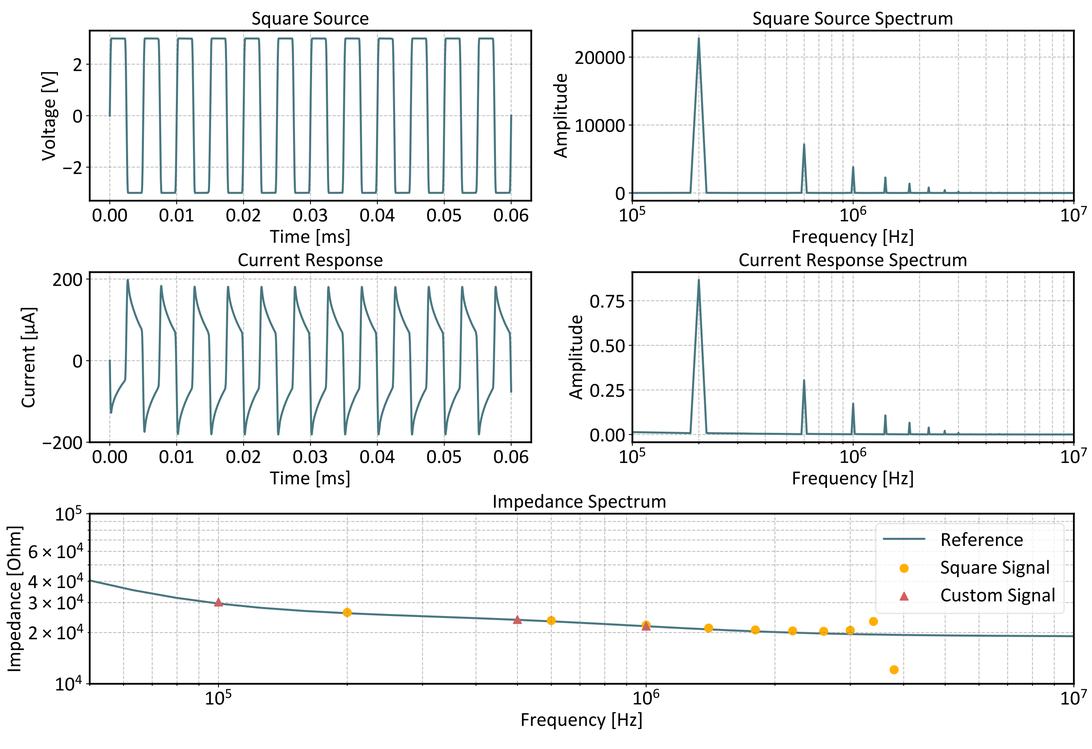


Results & Conclusion

The frequency spectrum of the current response shows the presence of the same underlying harmonics as the excitation signal. It allows us to calculate the impedance at various points across the 100 kHz – 10 MHz frequency range, which is of particular interest for dielectric properties of cells.

For the first 6 harmonics, the obtained impedance values are in close accordance with the reference impedance spectrum of the system. For higher harmonics, the error does increase, most likely due to high frequency noise in the current response signal.

Alternatively, we can construct “custom pulses” with predetermined harmonics, similar to the sinusoidal signals used in standard impedance cytometry (red triangles in the figure below)



References

[1] Duckert, B., Vinkx, S., Braeken, D. & Fauvart, M.. Journal of Controlled Release 330, 963–975 (2021).
[2] 1. Ye, Y. et al. Single-Cell Electroporation with Real-Time Impedance Assessment Using a Constriction Microchannel. Micromachines 11, 856 (2020).
[3] 1. López-Alonso, B., Sarnago, H., Lucía, Ó., Briz, P. & Burdío, J. M. Real-Time Impedance Monitoring During Electroporation Processes in Vegetal Tissue Using a High-Performance Generator. Sensors (Basel) 20, 3158 (2020).
[4] 1. Sung-Eun Choi, Harrison Khoo & Hur, S. C. Recent Advances in Microscale Electroporation. Chemical Reviews (2022) doi:10.1021/acs.chemrev.1c00677.

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