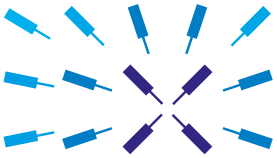
Application Note



Zurich Instruments

Monitoring of Cell Membrane Permeabilization in a Electroporation Device

Instruments: HF2LI, HF2TA

Release date: October 2017

Introduction

Electroporation is a widely used, non-viral, safe approach to deliver foreign vectors into many different cell types. When a cell is exposed to an electric field of the appropriate strength, the membrane undergoes reversible electrical breakdown in which transient pores form, allowing molecular transport into the cell. The controlled intracellular delivery of biomolecules and therapeutics enables the study and engineering of fundamental cellular processes, and has therefore been a major focus in biomedical research and clinical medicine. To this end, a novel microfluidic device had been developed to monitor single cell-level electroporation in a flow environment via impedance monitoring using the Zurich HF2LI Lock-in Amplifier [1].

Successful cell transfection represents the rate-limiting step in numerous biomedical research and bio-production workflows, including cell based therapies, RNA interference screening, and stem cell research. The challenges include variable and poor transformation efficiency, especially with hard-to-transfect cells such as primary cells and stem cells. One of the traditional bottlenecks to electroporation has been obtaining efficient delivery without compromising cell viability. Successful electroporation involves the optimization of a wide range of parameters, such as the buffering solution, molecular payload, and electric field pulse design, balancing the efficiency of transfection (i.e. how much is delivered) with the damage produced (i.e. how many cells are damaged or die). Protocols are often identified through costly trial-and-error, and can vary significantly from cell type-to-cell type, lab-to-lab, and application-to-application [2].

The Zurich Instruments Lock-in Amplifier has been essential in enabling the impedance detection of membrane permeabilization in a continuous flow environment at unprecedented sensitivity, an accomplishment not previously reported in literature. By monitoring changes in the electrical characteristics of individual cells when exposed to short, high-strength electric fields, the ability to identify when a cell became permeable and determine the conditions that lead to molecular deliver while preserving cell viability. This technology will expedite the transfection process by eliminating trial-and-error electroporation protocol development in a safe and effective manner across cell types and applications.

Method

The degree of change in cell impedance as a result of electric field-mediated membrane permeabilization can be assessed numerically using a cell/electrolyte circuit model (Figure 1). The resulting overall impedance equation for describing a membrane permeabilized cell can be found in the supplemental information of Zheng et al. The simulated cell impedance was plotted as a function of frequency for both the unexposed and electroporated conditions. The membrane capacitance Cm was 0.01 F/m^2 and is not affected by the electropolation. Whereas the membrane conductance Gm is affected and is changing

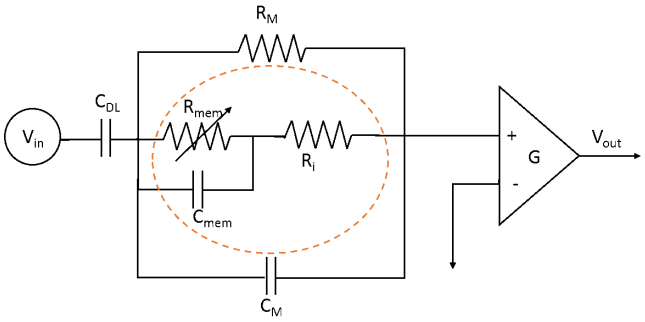


Figure 1. Electrical circuit representation of a suspended cell during detection. V_{in} is the excitation voltage input, C_{DL} is the double layer capacitance, C_{mem} is the capacitance of the cell membrane, and R_i is the cell's internal resistance. R_m and C_m are the resistance and capacitance of the extracellular media, respectively. To reflect the overall impedance change as a result of cell-membrane permeabilization by electroporation, a variable R_{mem} was introduced in parallel to C_{mem}. Lastly, G represents a pre-amplifier that converts current into voltage for digital processing.

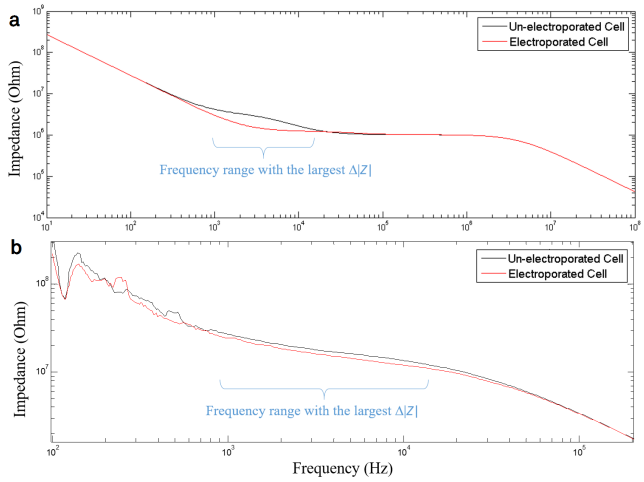


Figure 2. (a) Simulated cell impedance as a function of frequency for an unexposed (black curve) and electroporated (red curve) cell. The region of significant change in $\Delta|Z|$ is shown from 1 to 10 kHz. (b) Frequency sweep from Zurich measuring changes in cell impedance under the same conditions and parameters as that of the simulation.

by several order of magnitude from 1e-4 – 1 S/m^2 to 1e4 – 1e5 S/m^2 after the electropolation. The model was then verified experimentally using the frequency sweep function of the HF2LI (Figure 2).

In Figure 2, the frequency range that results in the largest impedance change when a cell undergoes electroporation is shown, approximately 1kHz to 10kHz. These results guided the experimental conditions for membrane permeabilization detection of single cells sin a continuous flow environment.

Real-time impedance monitoring of cell membrane permeabilization

The micro-device is manufactured using standard microfabrication techniques. Both the microfluidic channel and electrode geometry are defined using photolithography. By means of physical vapor deposition, titanium is first sputtered over the entirety of the glass slides containing the electrode patterns, followed by the platinum over-top. Titanium serves as an adhesion layer as platinum does not adhere to glass. The glass slides are then immersed into an acetone bath for the metal lift-off process, dissolving the photoresist and releasing undesired material, resulting in the metal patterned electrodes. Polydimethylsiloxane (PDMS) is poured over the silicon wafer containing the negative molds for the microfluidic channels. Upon PDMS curing, the devices are cut off the molds resulting in a recess where the channel features are, and inlet and outlet holes are punched through the PDMS using 1 mm biopsy punches. Both these open PDMS channels and glass slides containing electrodes are placed in an oxygen plasma chamber. Following treatment, the electroporation region of the device is aligned with the electrodes using a stereomicroscope and irreversibly bonded together, resulting in a sealed microfluidic channel. Lastly, wire leads are bonded to the electrode bond pads using conductive epoxy to allow for electrical connections.

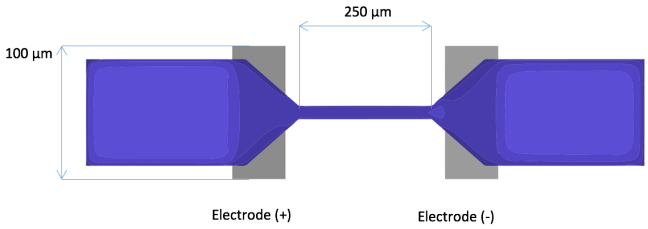


Figure 3. Microfluidic device with channel diameter channel constriction is 250 × 25 × 10 µm^3, electrodes are 100 µm wide separated by 300 µm.

The electroporation microdevice consists of a converging microfluidic constriction measuring 250 × 25 × 10 µm, which is flanked by a single pair of planar electrodes separated by 300 µm as shown in Figure 3. The electrodes are 100 µm in width and are made out of platinum, spanning across the width of the channel, are responsible for both the electrical sensing and electroporation pulse application to the cell in transit. Switching between sensing and pulsing was enabled by a CMOS switch which is controlled by an external function generator and placed in series with the current pre-amplifierinput to prevent measurement artifacts from the electroporation pulse.

The HF2LI Lock-in Amplifier was used to dynamically extract the impedance signal and to apply the electroporation pulse. A custom-built LabVIEW control algorithm was loaded onto the lock-in amplifier's embedded system for real-time processing. A frequency of 1.224 kHz was chosen to provide both optimal cell detection via the derivative-based, peak-detection algorithm and the most sensitive cell-membrane permeabilization detection with the highest SNR of 37dB.

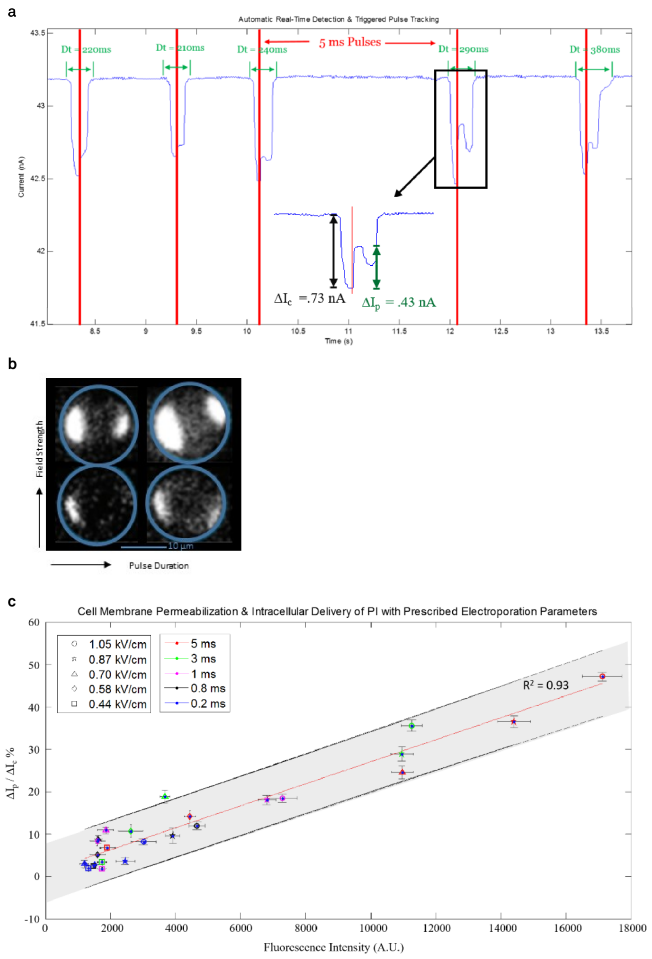


Figure 4. (a) Electrical signals from single cells recorded by the Zurich Instruments HF2LI Lock-in Amplifier. A representative individual cell impedance response is highlighted, which depicts the ΔI_p and ΔI_c values. (b) Representative images of PI fluorescence for increasing pulse strength (y-axis) and pulse duration (x-axis). (c) Electrically-measured cell membrane permeabilization magnitudes ($\Delta I_p/\Delta I_c$) plotted as a function of PI fluorescence intensity in single cells following electroporation treatment with varying pulse strengths and duration. A linear curve fit applied to the ensemble data shows a positive correlation between the electrical and optical characterizations of the cell membrane permeabilization, with the shaded region indicating ± 1σ intervals.

When a cell is detected within the electroporation zone, denoted by a significant drop in current reading (Figure 4a), a digital output from the HF2LI is sent to a function generator to trigger the electroporation pulse. The pulse was programmed in the function generator and fed to a high-voltage amplifier to supply electric field pulses ranging from 0.44 to 1.05 kV/cm with a duration between 0.2 and 5.0 ms. Immediately following the pulse application, the device returns to the sensing state to assess the degree of membrane permeabilization. A schematic of the experimental set-up can be seen in Figure 5.

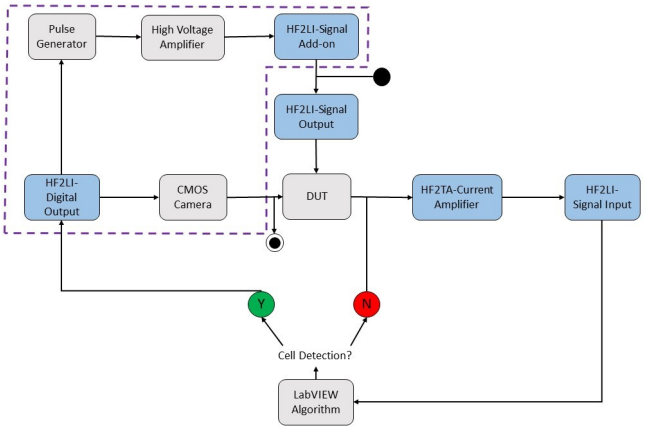


Figure 5. Schematic illustrating the experimental set-up. The lock-in frequency signal is generated by the HF2LI and delivered to the device-under-test (DUT) via the signal output. The voltage in the channel is then measured and sent to the HF2TA current amplifier, and this current reading is then sent into the HF2LI signal input channel. The current is then monitored by a custom-built LabVIEW program. Upon cell detection, a digital output is generated to trigger both the electroporation pulse and image capture.

Experimental example: Analysis and validation of cell membrane permeabilization

By varying the strength and duration of the electroporation pulse, we demonstrate changes in cell impedance that are characteristic of the degree of cell-membrane permeabilization. A full characterization of the cell membrane response as a function of both electric field strength and duration was performed. This response was validated by delivering a fluorescent probe, propidium iodide (PI), through the cell membrane. PI, typically used for live-dead stains, is often used in electroporation validation experiments. PI is impermeable to a fully intact cell membrane. However, when the membrane has been compromised, such as the formation of transient pores via electroporation, PI can enter the intracellular space, where it binds to nucleic acids and emits fluorescence.

Images were taken by synchronizing the electroporation pulse with a microscope-mounted CMOS camera. Image processing was performed to determine the intensity of the fluorescence emission from PI inside the cell. The same electric field pulse strength and conditions were performed with the optical monitoring to correlate the cell impedance characterization to the molecular delivery results. A quadrant of PI images is shown in Figure 4b with increasing pulse duration and pulse strength on the x and y axis, respectively.

The membrane impedance values were represented as a normalized percentage, denoted as $\Delta I_P/\Delta I_C$, where ΔI_P is defined as the permeabilization current reading (i.e. the difference between the bottom-most current reading when the cell passes and the current reading immediately following the pulse application), and ΔI_C

is defined as the cell current reading (i.e. the difference between the current baseline and the bottom-most current reading when the cell passes). This is represented in Figure 4a for better understanding.

The membrane impedance response was normalized to ΔI_C to account for cell-to-cell size variations, as the drop in current from the baseline is proportional to the volume of the passing cell. The degree of membrane permeabilization ($\Delta I_P/\Delta I_C$) has a strong dependence on the strength and duration. Although not shown here, it was reported in Zheng et al. that the transition from 0.8 to 1.0 ms resulted in a significant increase in the membrane permeabilization impedance response for the various electric-fields that were applied.

The optical validation results followed the same dependence on the characteristics of the electric field, showing an increase in the fluorescence intensity of the PI delivered to the cell as field strength and duration were increased. An increase in fluorescence intensity was seen in the transition from 0.8 to 1.0 ms for the larger field strengths (0.87 kV/cm and 1.05 kV/cm). The results of the parametric studies of the membrane impedance and PI-generated fluorescence intensity are strongly correlated, $R^2 = 0.93$ (Figure 4c.).

Key Advantages

Real-time monitoring of the degree of electroporation of individual cells in a continuous flow environment, as reported in this document, has not previously been realized due to the extremely small electrical signals that are generated. However, by utilizing the Zurich Instruments Lock-in amplifier and the HF2TA current preamplifier, these impedance responses can be measured consistently with high sensitivity (37dB). Thus, more reliable and practical approach to single cell-level electroporation can be performed, enabling more controlled biological experiments and aiding in the development of therapeutics.

Conclusion

The ability to monitor the membrane impedance response in a continuous flow environment during the electroporation process has not been previously accomplished. The development of this new technique has the potential to be applied in numerous applications in biomedical and clinical research. Each application will bring tremendous benefits in enabling studies with highly controlled cell electroporation events, ultimately leading to high molecular delivery rates and achieving the apex of cellular viability.

Acknowledgements

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References

[1] Mingde Zheng; Joseph J. Sherba; Jerry W. Shan; Hao Lin; David I Shreiber; Jeffrey D. Zahn. Continuous-flow, electrically-triggered, single cell-level electroporation. Technology, 5:1–11, 2017.

[2] Mohamed M. Sadik; Miao Yu; Mingde Zheng; Jeffrey D. Zahn; Jerry W. Shan; David I. Shreiber; Hao Lin. Scaling relationship and optimization of double-pulse electroporation. Biophysical Journal, 106:801–812, 2014.