

# DYNAMICS OF CELL ELECTROPORATION NANOPORES STUDIED BY MEANS OF SECOND HARMONIC GENERATION MICROSCOPY

Dobryna Zalvidea, Enric Claverol-Tinture

Neuroengineering Group, Catalonia Bioengineering Institute (IBEC), c/Baldori i Reixach 15-21, 08028, Barcelona, Spain

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## 1. INTRODUCTION

Electroporation, the electric-field driven introduction of compounds into living cells, is a key technique towards understanding the role of specific genes in disease (electroporation-based gene transfection). Nanopore formation during electroporation is necessary for efficient electric-field driven internalization of compounds, including DNA, and yet limited knowledge is available on the dynamics of nanopores. Living cells can be considered as a conductive body (the cytoplasm) surrounded by a dielectric layer (the membrane). When a transient electric field is applied to a cell population a transient voltage across the membrane ensues. Above a given voltage threshold, the membrane can suffer structural changes and induce pore formation, supporting translocation of ions, drugs, and even DNA fragments into the cell. Nonlinear microscopy has become a standard tool to study living organisms due to its properties: longer penetration depths, out of focus photobleaching and phototoxicity avoidance and intrinsic background reduction [1]. Second Harmonic Generation (SHG) is a second-order nonlinear optical effect that has been successfully used to study action potentials in neurons due to its voltage sensitivity [2]. In this work we propose a method to study the cell membrane subjected to a pulsed external electrical field applied in an imaging microscopy mode.

## 2. METHODS AND RESULTS

The membranes of hippocampal neurons were labeled with the SHG-generating dye Synaptored C1 (Biotium), placed in a custom-made chamber for electroporation and simultaneously bathed in PI (propidium iodide) to confirm electroporation. The experiment was performed in a Leica TCS SP5 MP microscope adapted for collecting SHG. The electroporation electrical signal was generated by the electroporator Genepulser II Capacitance Extender Plus, using two Ag electrodes immersed in the cell medium and separated by a gap of 5 mm.

The SHG signal showed transients correlated with electroporation pulses indicating transient formation of membrane nanopores and in agreement with SHG model. Transient SHG signal was strongly sensitive to membrane location (see Fig. 1). Entry of PI dye in the cell confirms successful electroporation (Fig 2) concomitantly with nanopore formation.

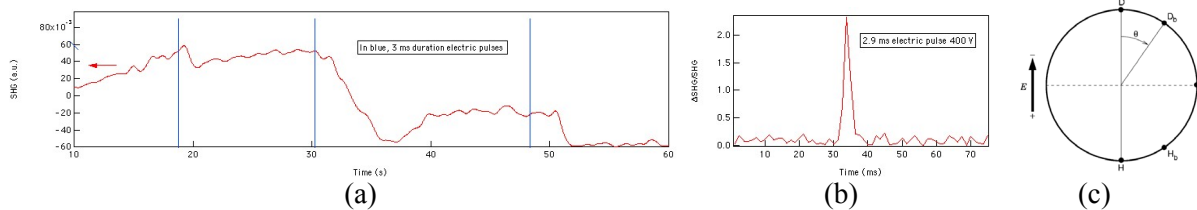


Fig. 1. SHG vs. time at different locations of the cell (a) pole (D in Fig. 1(c)), (b) border location (D<sub>b</sub>). Finally, in (c) the schematic of the cell (poles and equator locations) in the electroporation set-up.

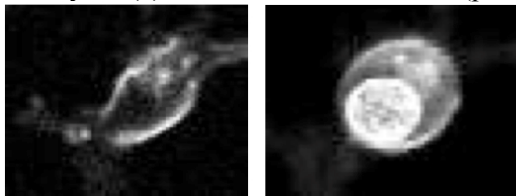


Fig. 2. Two-photon fluorescence from hippocampal neuron, right, before electroporation, and on the left after electroporation.

## 3. CONCLUSIONS

SHG signal has shown to be adequate to study nanopore formation in the cell membrane.

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[1] W. Denk, J. H. Strickler, W. W. Webb, "Two-photon laser scanning fluorescence microscopy", *Science*, **248**, 73 (1990).

[2] M. Nuriya, J. Jiang, B. Nemet, K. B. Eisenthal, R. Yuste, "Imaging membrane potential in dendritic spines", *PNAS*, 103, 786 (2006).