

SECOND HARMONIC MICROSCOPY TO STUDY EFFECTS OF ELECTRICAL PULSES ON CELLS

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1. INTRODUCTION

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 the easiness of background signal filtering [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. This can be done using membrane markers, such as FM4-64, that are excellent SHG chromophores [2].

Living cells can be considered as a conductive body (the cytoplasm) surrounded by a dielectric layer (the surface membrane). When an external electric field is applied to a cell, the resulting current causes accumulation of electrical charges at the cell membrane and, consequently, a voltage across the membrane. Above some voltage threshold, the membrane can suffer structural changes and induce pore formation changing, therefore, the membrane permeability. This can be used to transport particles from outside to inside the cell. In this work we propose a method to study the cell membrane subjected to an pulsed external electrical field applied in an imaging microscopy mode.

2. METHODS AND RESULTS

Pheochromocytoma (PC12) cells were extracellularly marked with a membrane marker FM4-64 and placed in a home-made chamber designed to electrically excite the cells during imaging. The sample illumination was done with 180 fs pulses at 868 nm from a MIRA 900f laser (Coherent) sent through a scanning system (Cambridge Tech.) to a Nikon Eclipse Ti Confocal Microscope. The SHG signal was collected with a 0.5 NA condenser, in the backward direction in a PMT (Hamamatsu H9305-04) after filtering the signal with a 434/17 nm bandwidth filter (Semrock). The chamber comprises a glass-bottom dish (MakTek) with an adapted top cover holding the electrodes and a hole to collect the forward signal. The electrical signal was generated by a pulse generator (TTi-RS Components) and with Ag electrodes immersed in the cell medium with a gap of 5-10 mm.

The measurements were done with a single electrical pulse of 1 s. Without external electrical excitation, the SHG signal from the marked cell membrane is shown in Figure 1a. The SHG was monitored during the process at different times, after 1, and 2 minutes (Fig. 1b, 1c).

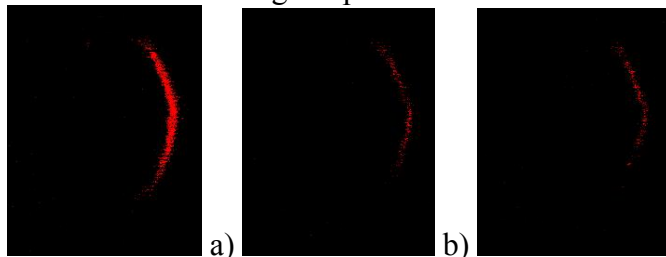


Fig. 1. SHG from PC12 cell membrane.

3. CONCLUSIONS

SHG signal has shown to be adequate to study the effects of external electrical fields over the cell membrane in an imaging mode.

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