

In Vitro Imaging and Molecular Characterization of Ca²⁺ Flux Modulation by Nanosecond Pulsed Electric Fields

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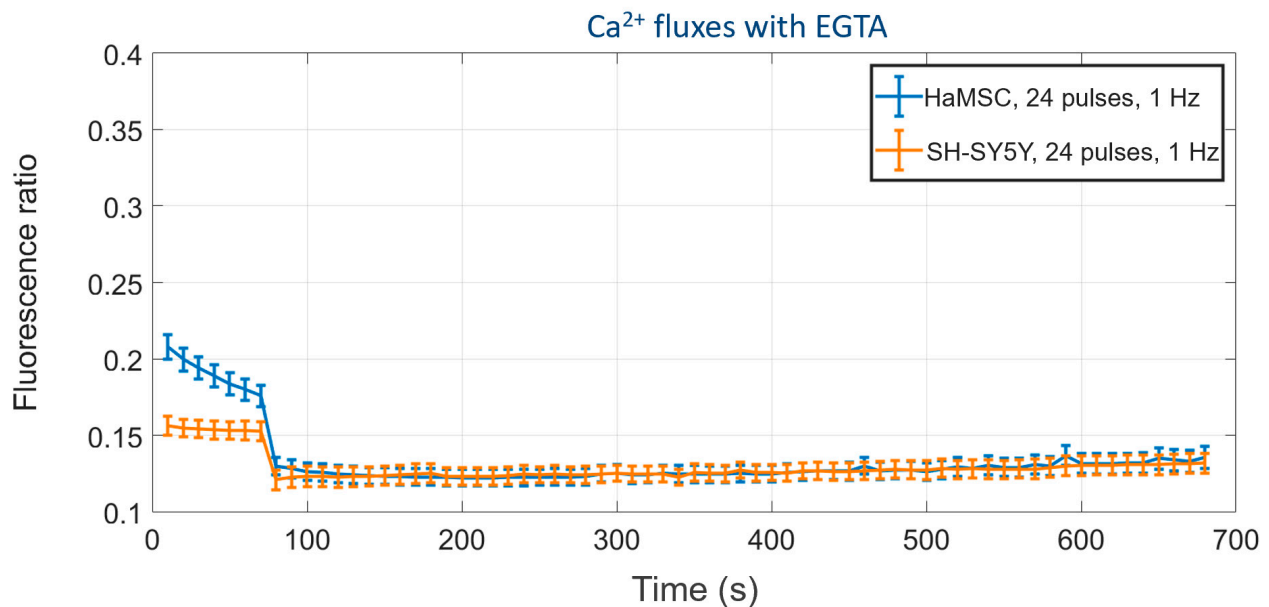


Figure S1. Ca²⁺ fluxes in HaMSC and SH-SY5Y cells in presence of EGTA in the extracellular medium. Fluorescence ratio of FURA-2-AM dye measured by time-lapse before and after pulses delivery using for both cells the same protocol: 24 pulses at 1 Hz delivered consecutively ($p < 0.05$). Acquisition for 11 minutes, 1 image each 10 seconds. The lack of calcium uptake when Ca²⁺ was absent in the extracellular medium indicates that mitochondrial membranes are not affected by the short electric pulses.

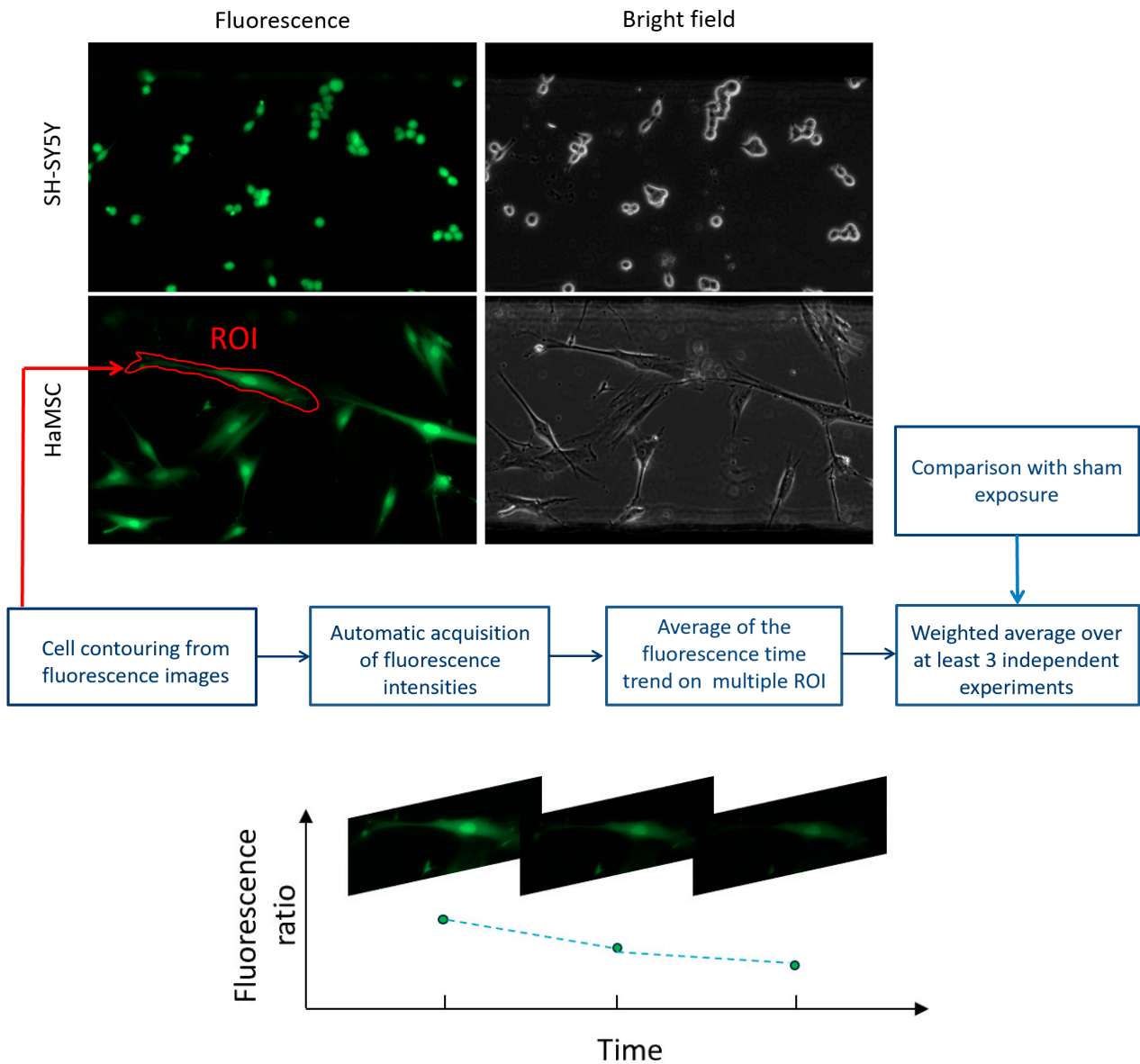


Figure S2. Block diagram of Ca^{2+} fluxes analysis and cell images (fluorescence and bright fields). This is an example of a fluorescence image acquired, in which the green intensity is correlated with the effective calcium concentrations present within the cells. In the images, the following steps were performed: 1) Cells were manually outlined to define a Region Of Interest (ROI); 2) The green intensity in that ROI was automatically acquired and tracked over time; 3) The fluxes obtained from each ROI were averaged, and 4) A further weighted average was calculated over at least 3 independent experiments performed under the same exposure conditions. In the insert on the right, the time trend of the fluorescence ratio for a HaMSC cell is presented; these cells have an average radius dimension of around $36 \mu\text{m}$. In the insert on the left, the time trend of the fluorescence ratio for a SH-SY5Y cell is shown; these cells have an average radius dimension of around $12 \mu\text{m}$.