Monitoring changes in membrane potential by energy transfer efficiency via fluorescence polarization measurements

In the framework of this research, the transfer of excitation energy was examined between the molecules coumarin (CC2-DMPE) and oxonol (DiSBAC2(3)), that served as donor and acceptor, respectively. The evaluations were carried out in two experimental types; the first type, in a homogeneous solution, and the second type, on the membrane surface of a kidney cell (HEK-293), which I grew. In the experiments that were conducted on the cell types, changes were observed in the "E" values influenced by changes in the membrane potential that were induced by means of potassium ions and hydrogen peroxide. In all the cases, the changes in the "E" values were calculated according to the intensity of the fluorescence polarization.

The first stage of the experiment entailed calibrating the values of the measuring system of the experiment, fluorophors in solutions and the biological model types. At the end, I portrayed the cytoplasmic membrane potential of the kidney cells by means of measuring with the Patch-Clamp system.

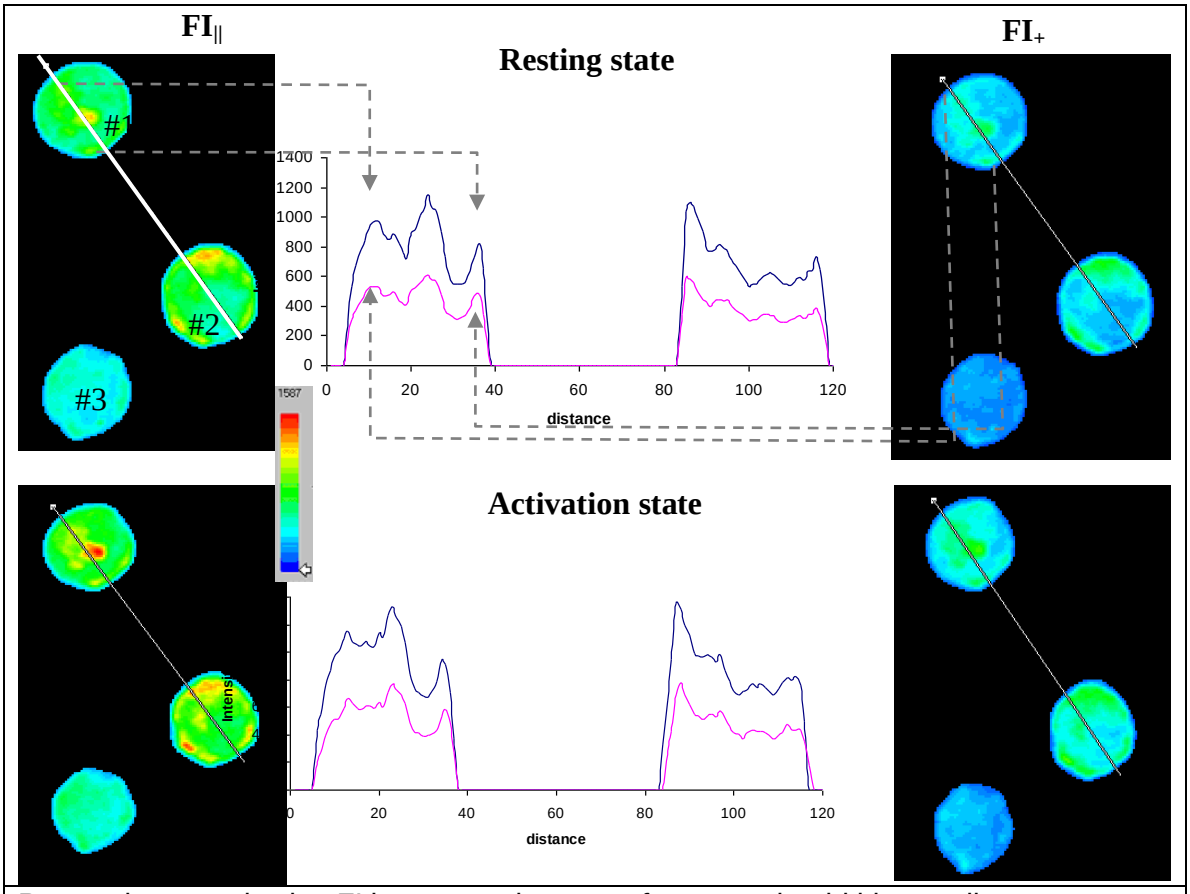
In the kidney cells, energy transfer was measured in two different states: the first state, control – resting potential, meaning the electrically polarized membrane, and the second state, activated – the membrane has undergone electrical depolarization. The analysis was conducted in a sub-cellular resolution through the use of programs for interpreting images, and part of these programs I created myself for the purpose of this research.

Briefly, from the resulting cell images that were obtained as intensity data, maps of intensity and polarization were consolidated for every physiological state. From these maps, I produced a map of energy transfer on the level of a single cell.

The immediate feasible aspect of the success of the evaluation of "E" in solutions and in membranes, between coumarin (CC2-DMPE) and oxonol (DiSBAC2(3)), I examined in an evaluation kit based on a cell model for testing for apoptosis. The cell models were kidney cells HEK-293 in which changes were measured in the cytoplasmic membrane potential in the apoptosis process, using the fluorophor di-BA-C_4-(3); as control, the cells were also monitored through the fluorophors annexin-V and PI.

In my research, I demonstrated that reducing the distance between the donor and the acceptor increased fluorescence polarization of the donor, and that decreasing fluorescence polarization of the donor (courmarin) may be considered as a very early indicator of the beginning of the apoptosis process. In addition, for the first time, changes were measured in "E" in the interior population of the same single

cell, before, during and after the cell's biological activation through fluorescence polarization of the donor in real time.



Pre- and post-activation FI images and curves of same stained-kidney cells.

Up to down: before and after introduction of VSP-2.

Left to right: left panel: $FI_{\parallel}$ images; middle panel: 4 curves of FI (blue: $FI_{\parallel}$, red: FI_{+}) versus pixel number (distance) along profile lines (overlaid on images); right panel: FI_{+} images. Gray dashed arrows correlate between corresponding locations on curves and on images. Color scale indicates levels of intensity. Cell numbers are marked in the left and upper panel.