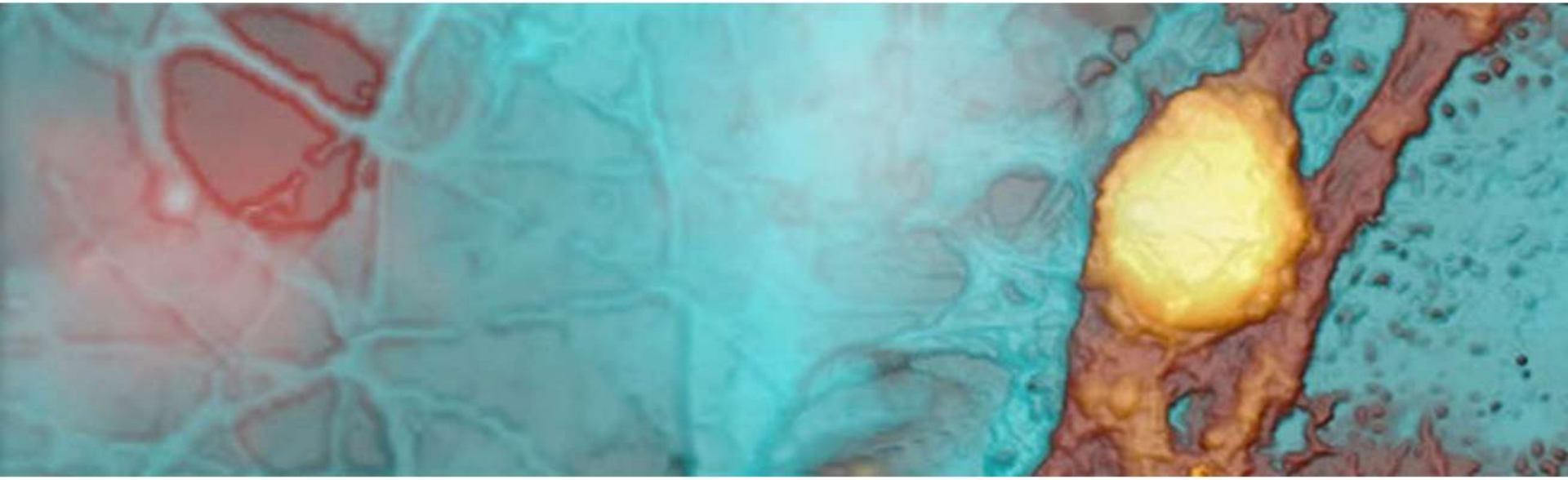
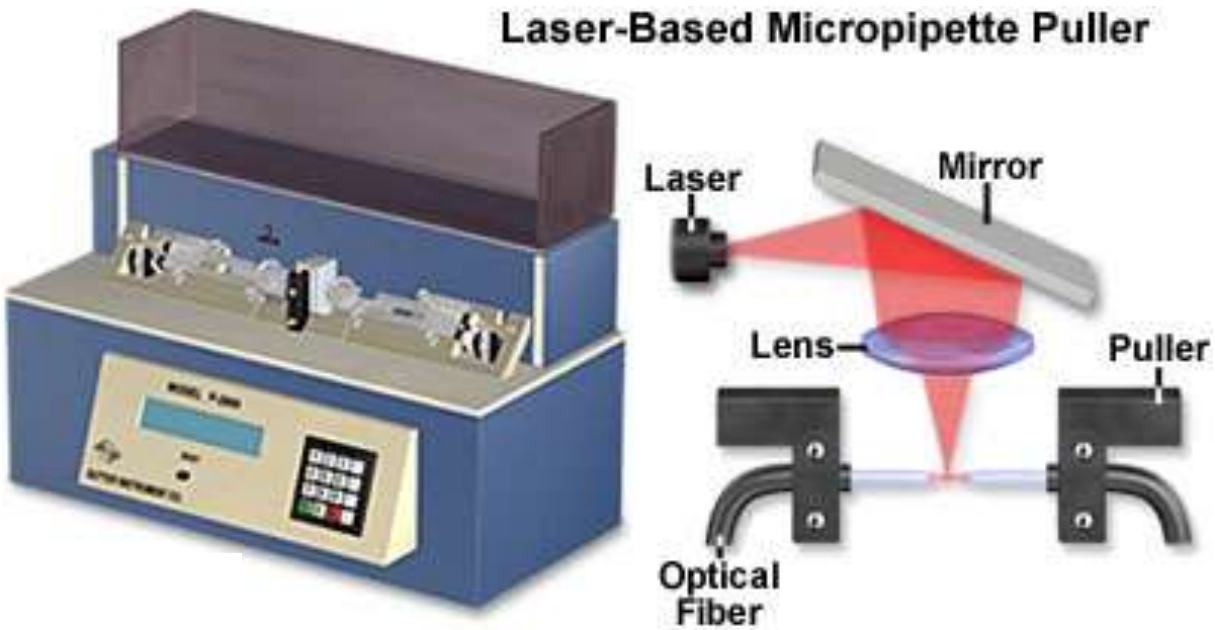
Ион-проводящая микроскопия в высокоскоростном скрининге лекарственных средств in vitro

Ерофеев Александр Сергеевич,
к.ф.-м.н

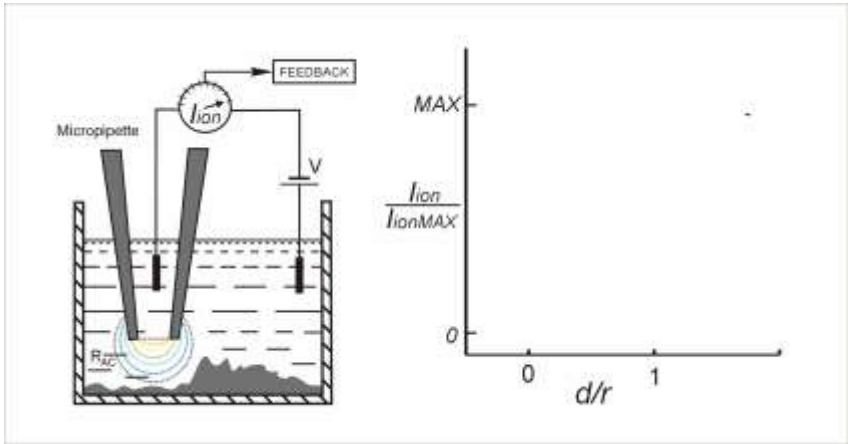


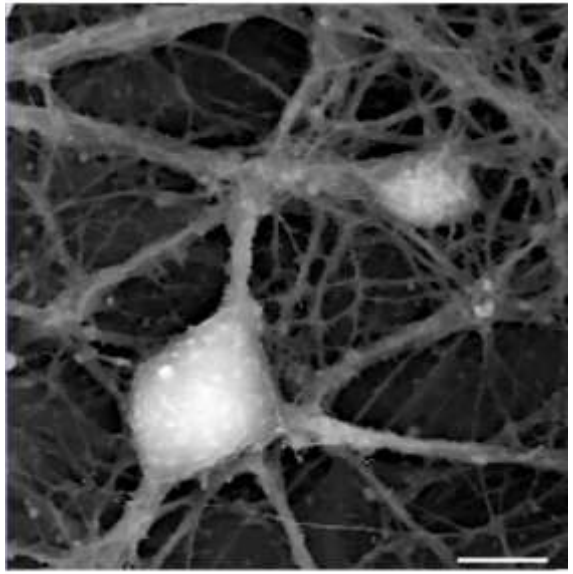
2018

Nanopipette Probe

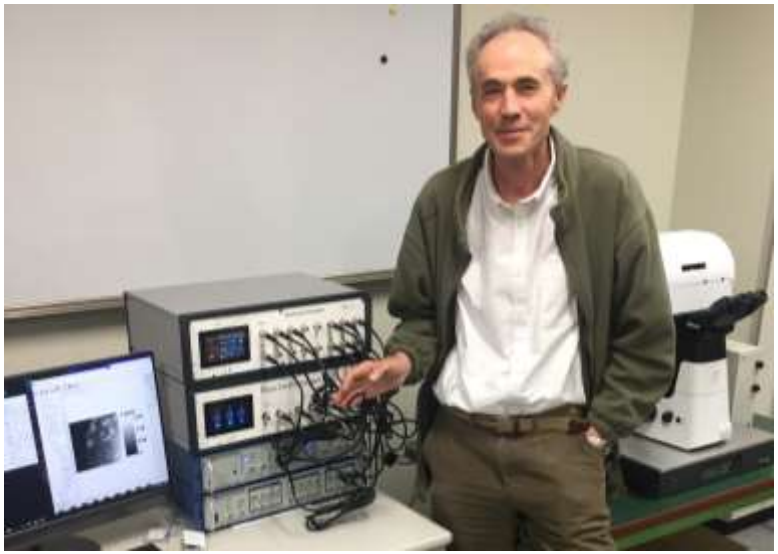


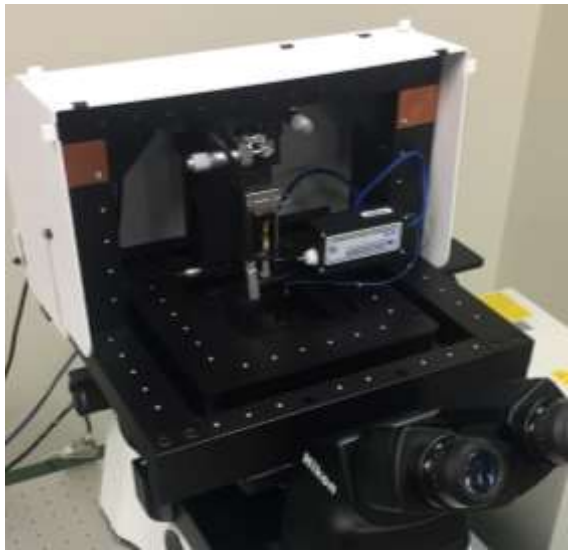
Scanning ion conductance microscopy





Prof. Korchev



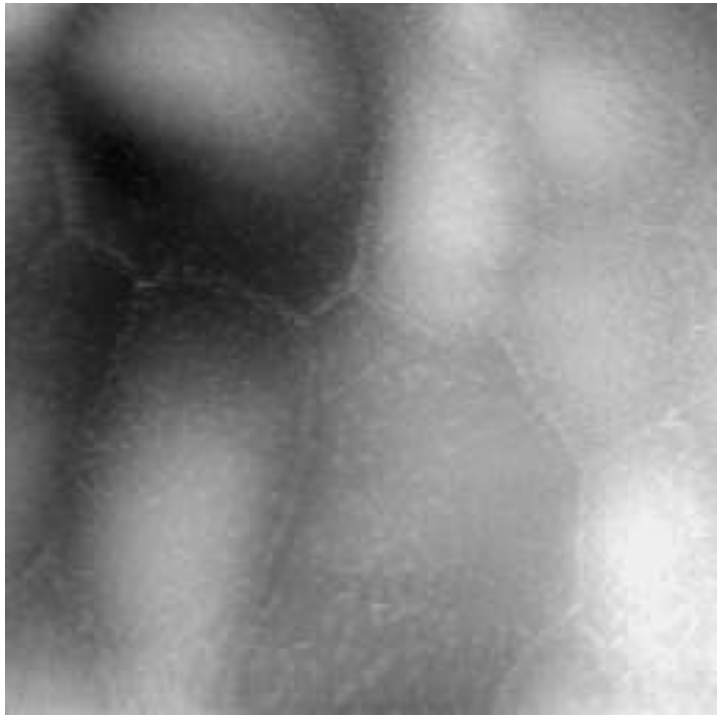


IC APP IC Ion Current Apparatus for Investigation of Cells
www.icappic.com

SICM imaging of live cells

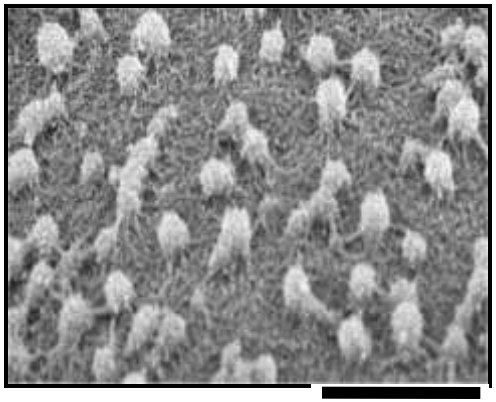
SICM imaging of live cells

SICM

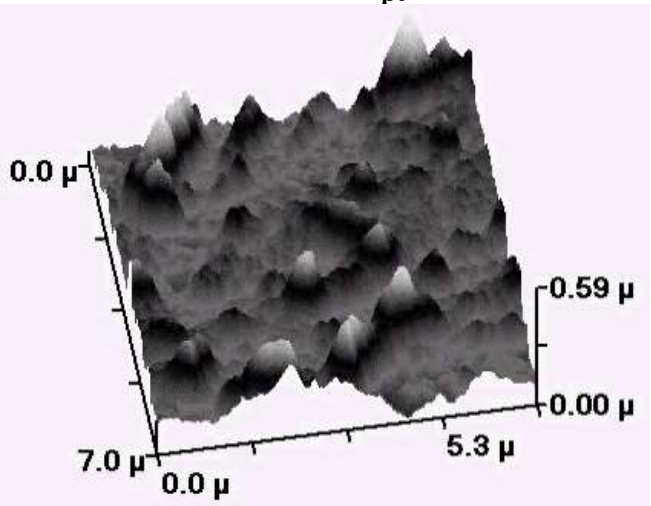


40 x 40 um
Time of measurement 24 h

SEM

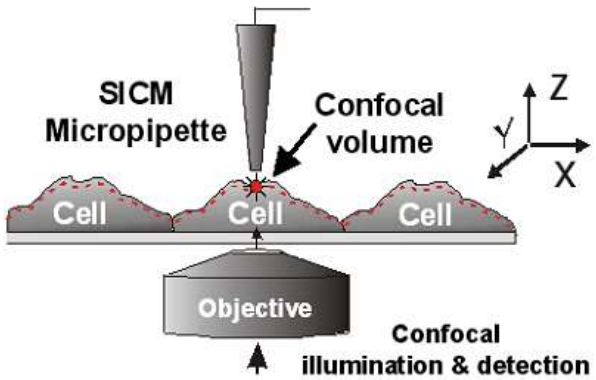


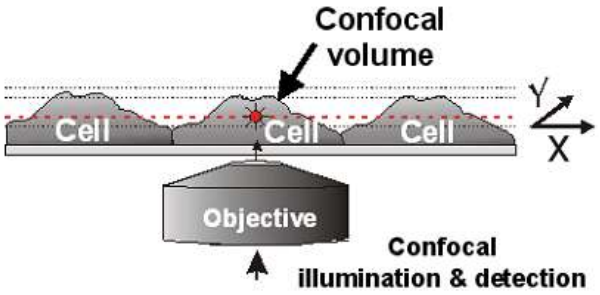
SICM

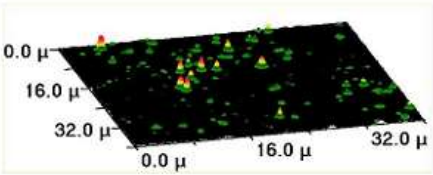


Fast microvillar dynamics
(50 sec/frame)

Scanning Surface Confocal Microscope

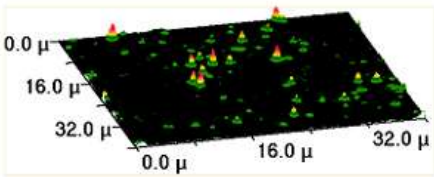




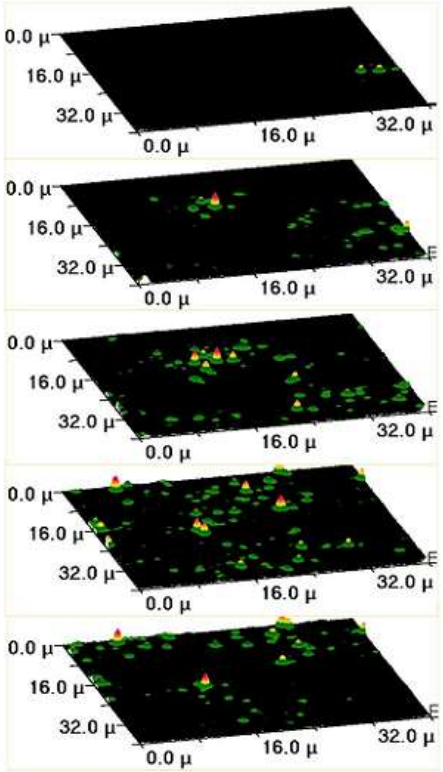


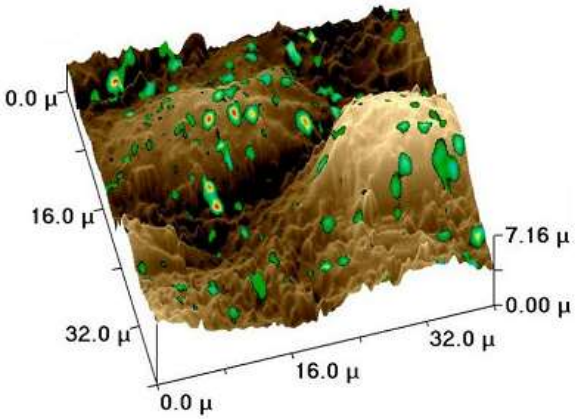
Single scan

=



Sum of scans

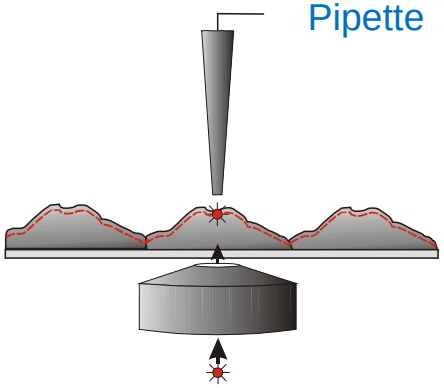




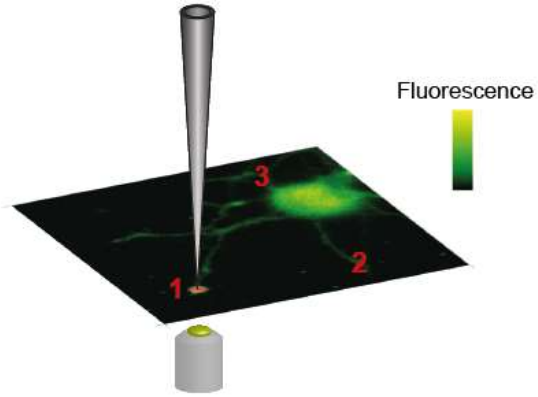
Polyoma virus capsids

Dose response test

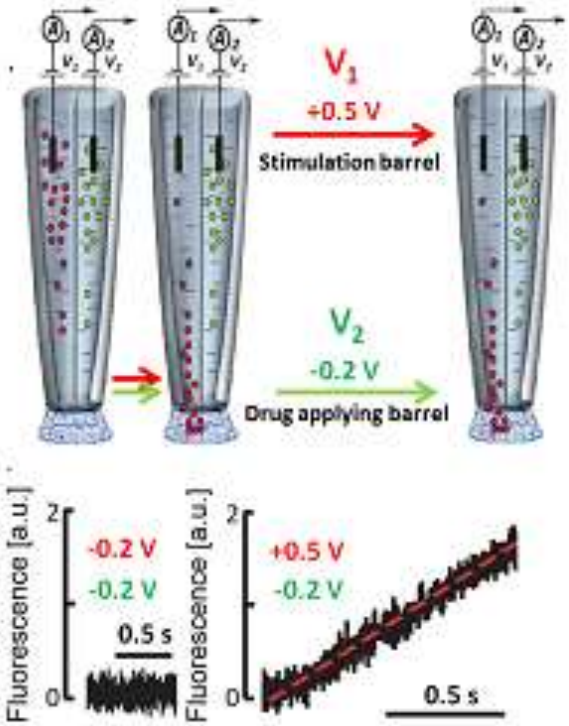
1) Scanning



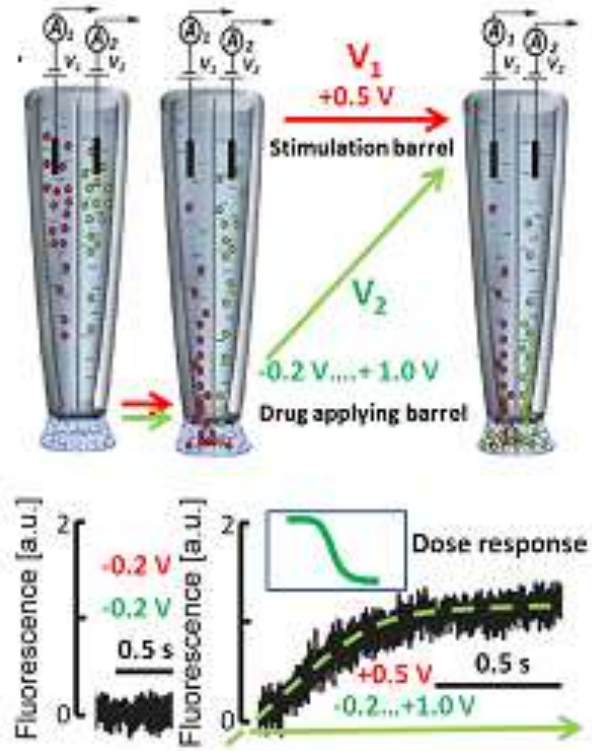
Local fluorescence measurements



2) Stimulation

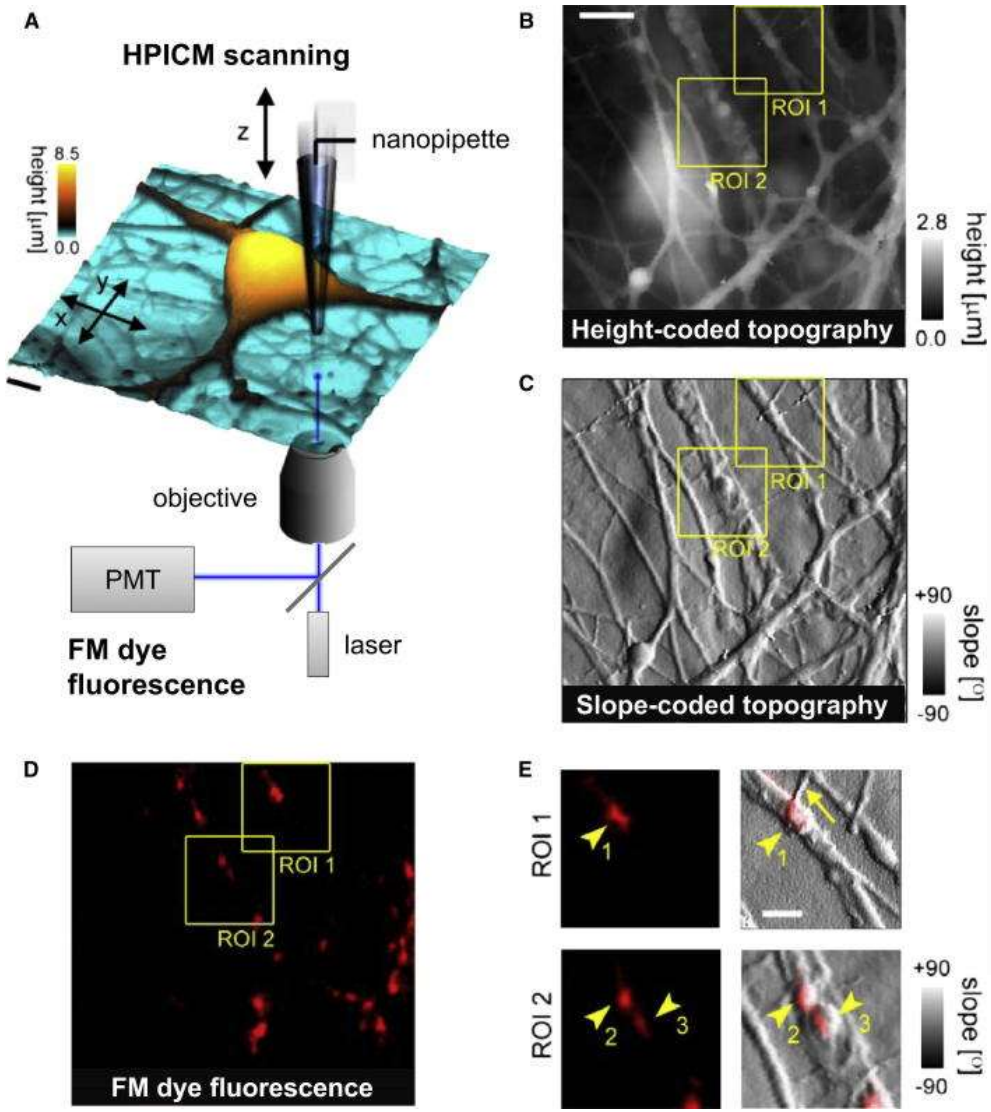


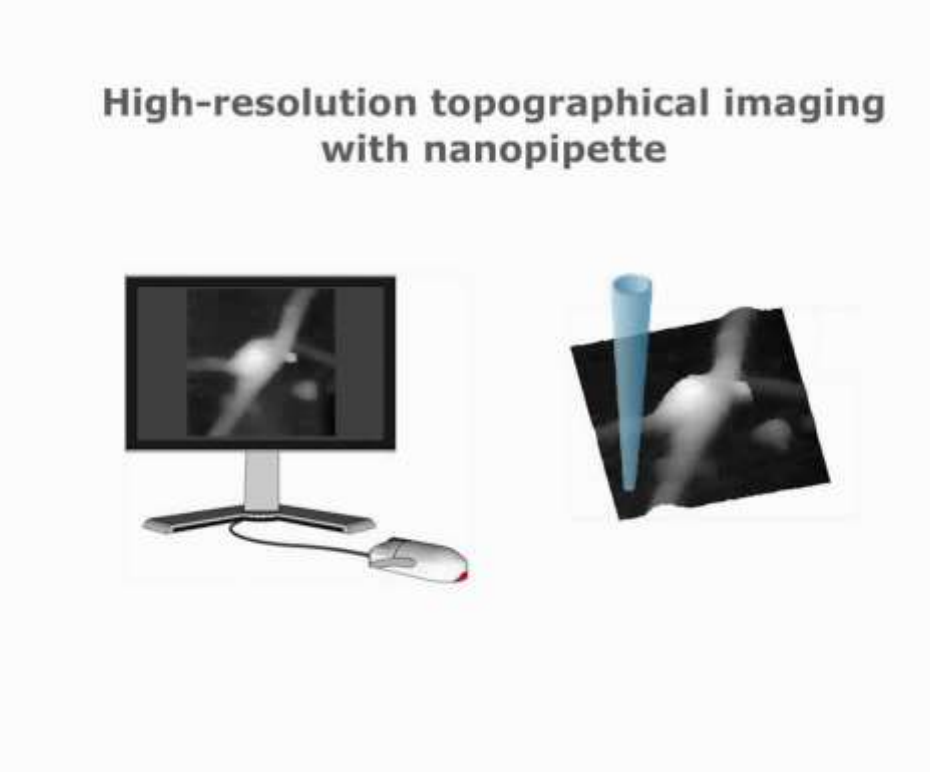
3) Pain-killer efficiency detection



Drug response for 1 second

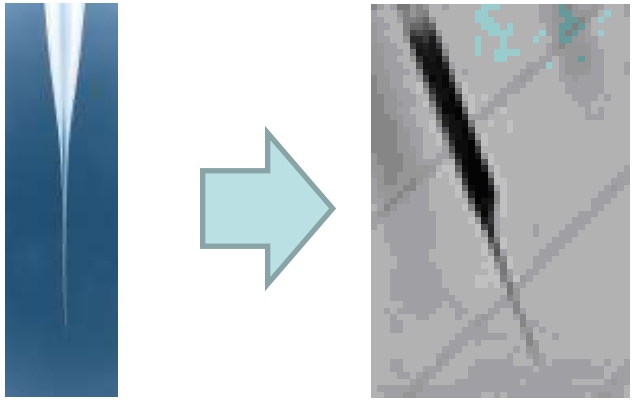
Automated patch clamp





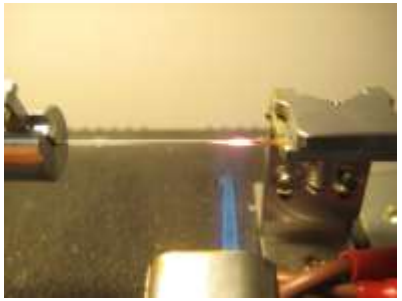
Neuron 79, 1067–1077, September 18, 2013

Nanoelectrode fabrication



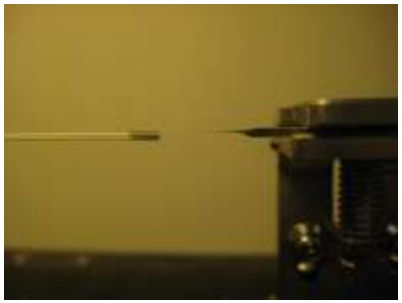
I

II

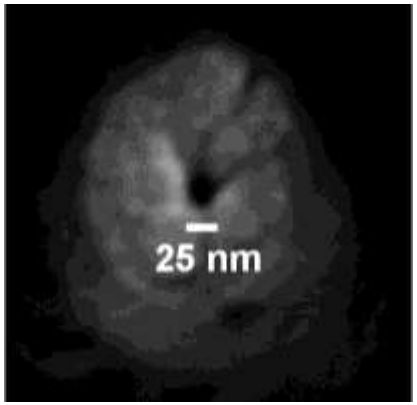


III

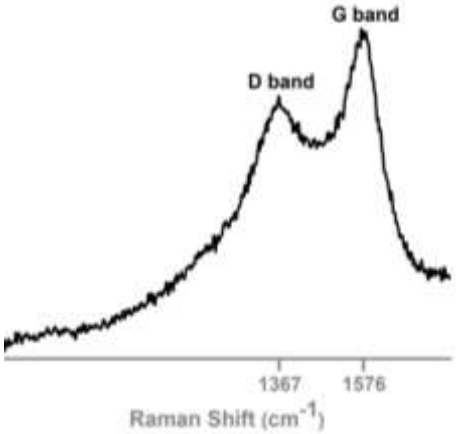
IV



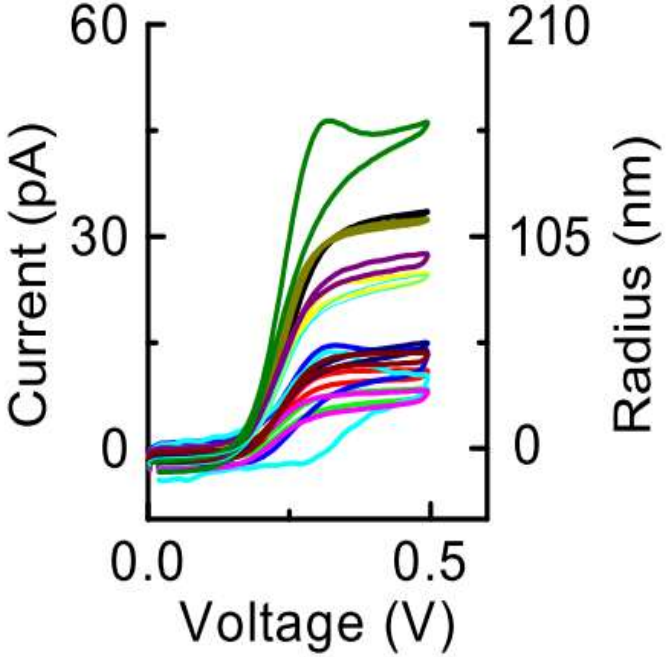
Carbon Nanoelectrode



SEM micrograph of a nanoelectrode tip

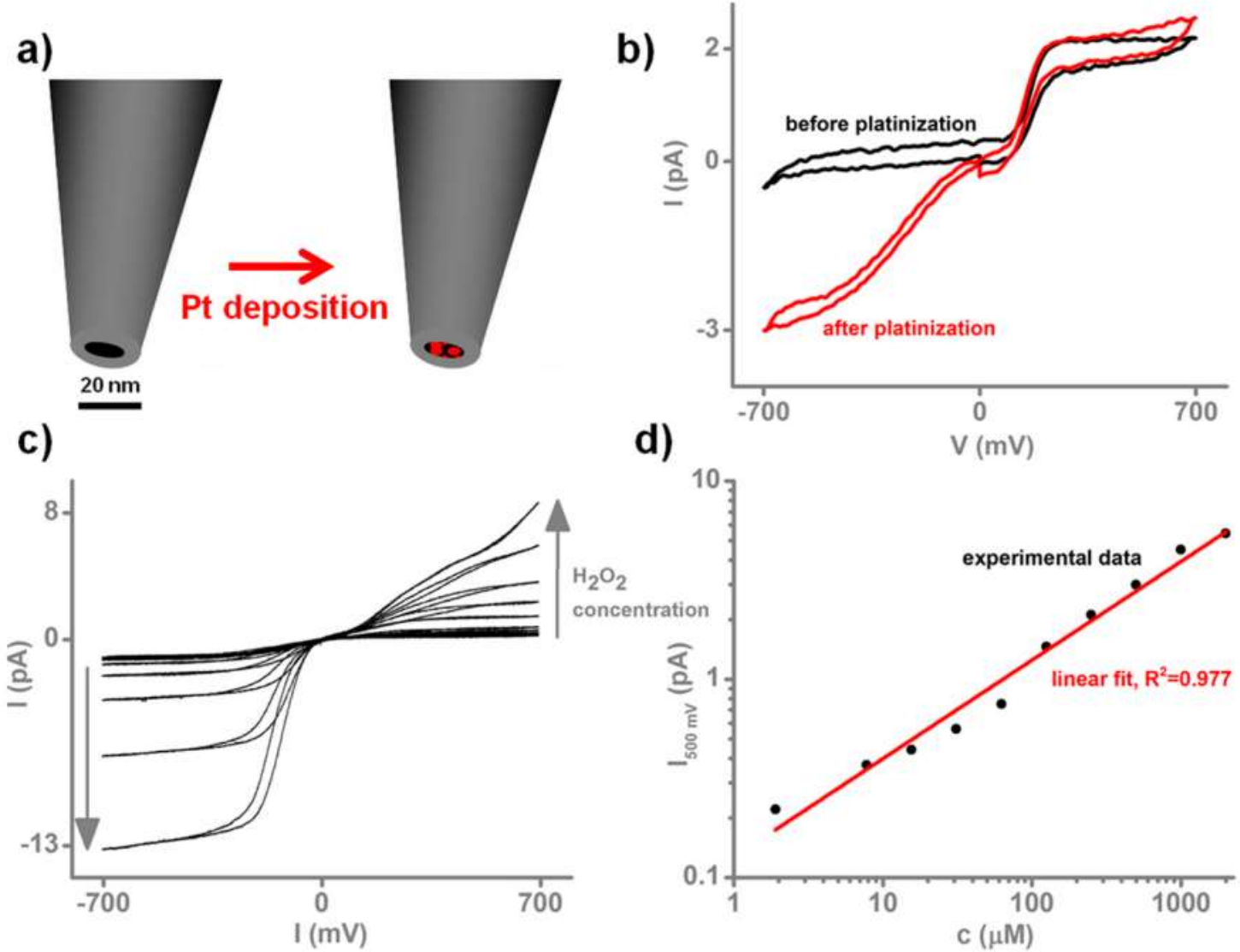


Typical Raman spectrum of the carbon nanoelectrode tip

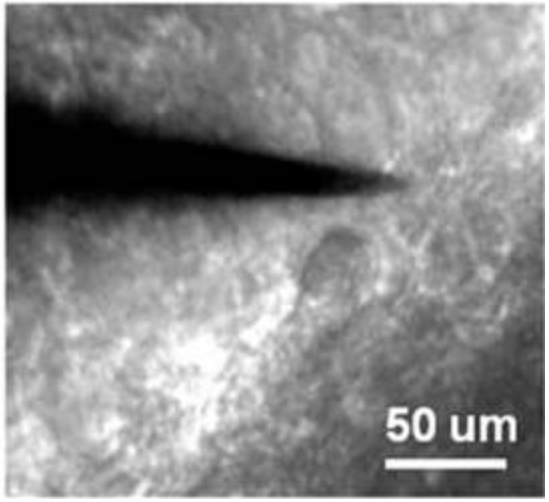


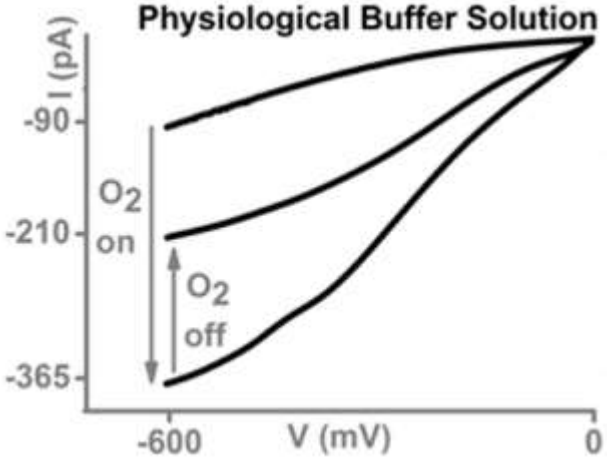
FcMe 1 mM in PBS,
vs Ag/AgCl

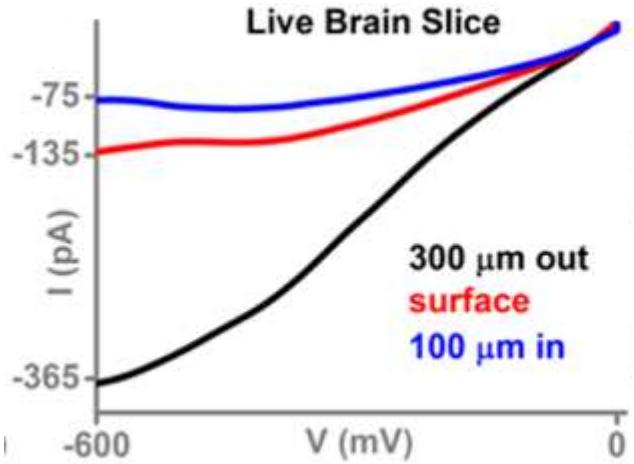
Nanoelectrode Functionalization

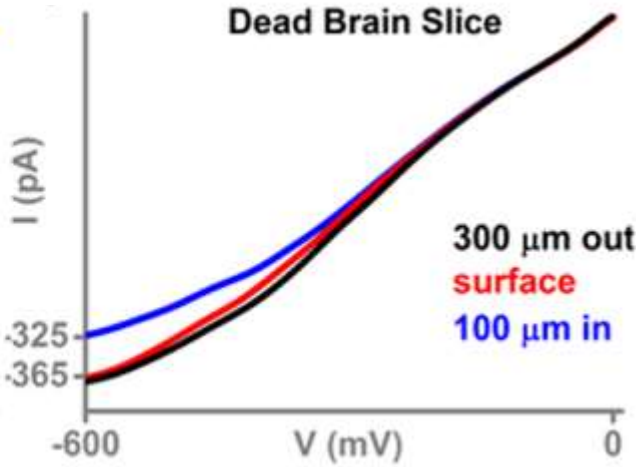


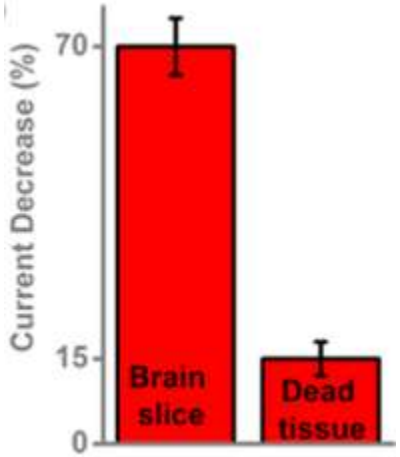
Oxygen Detection in Brain Slices



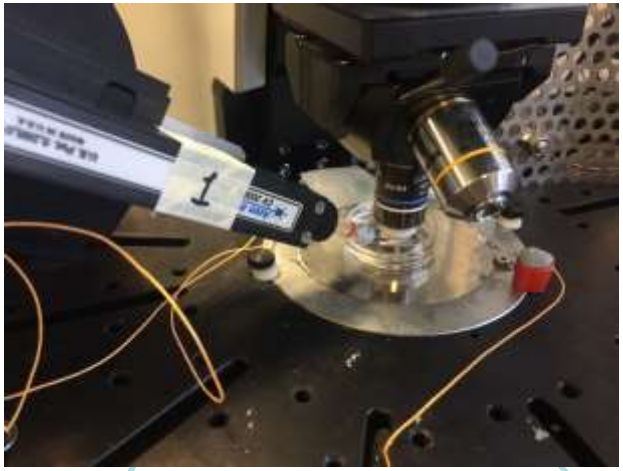


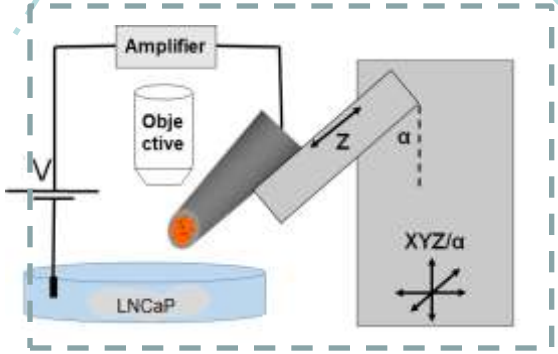


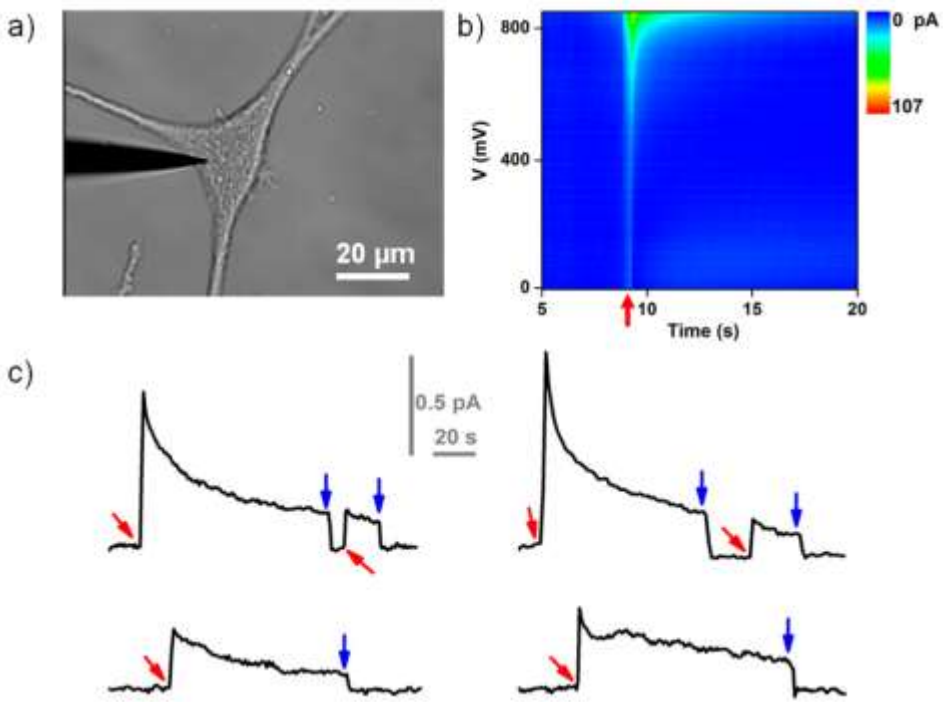




Intracellular Measurements in Melanoma Cells







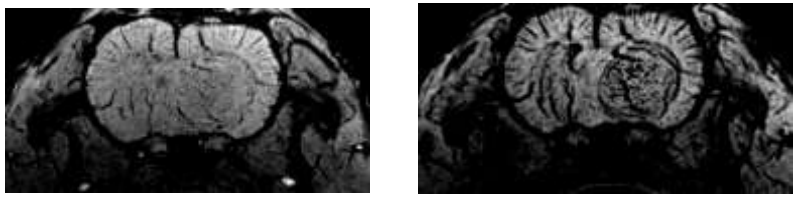
ROS in melanocytes **ROS in melanoma cells**

[Electrochemical Nanoprobes for Single-Cell Analysis **ACS Nano**, Vol 8, №1, P. 875-884]

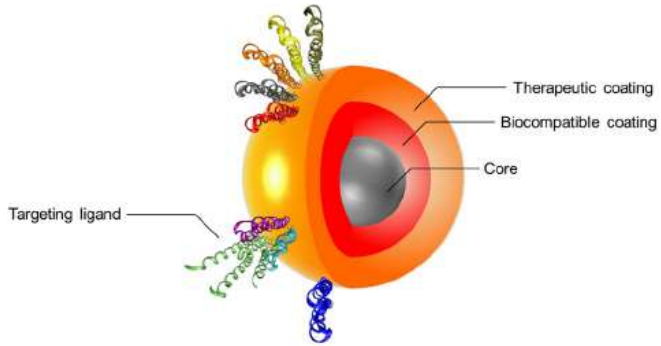
Method for rapid screening of magnetic nanoparticles toxicity

Magnetite nanoparticles in medicine:

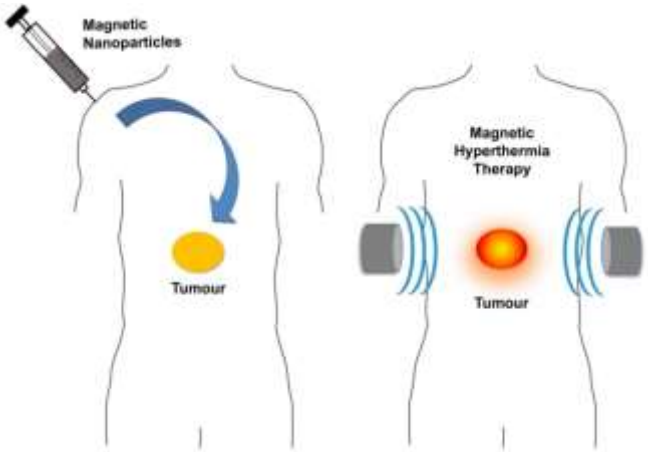
- *Contrast agent for MRI:*



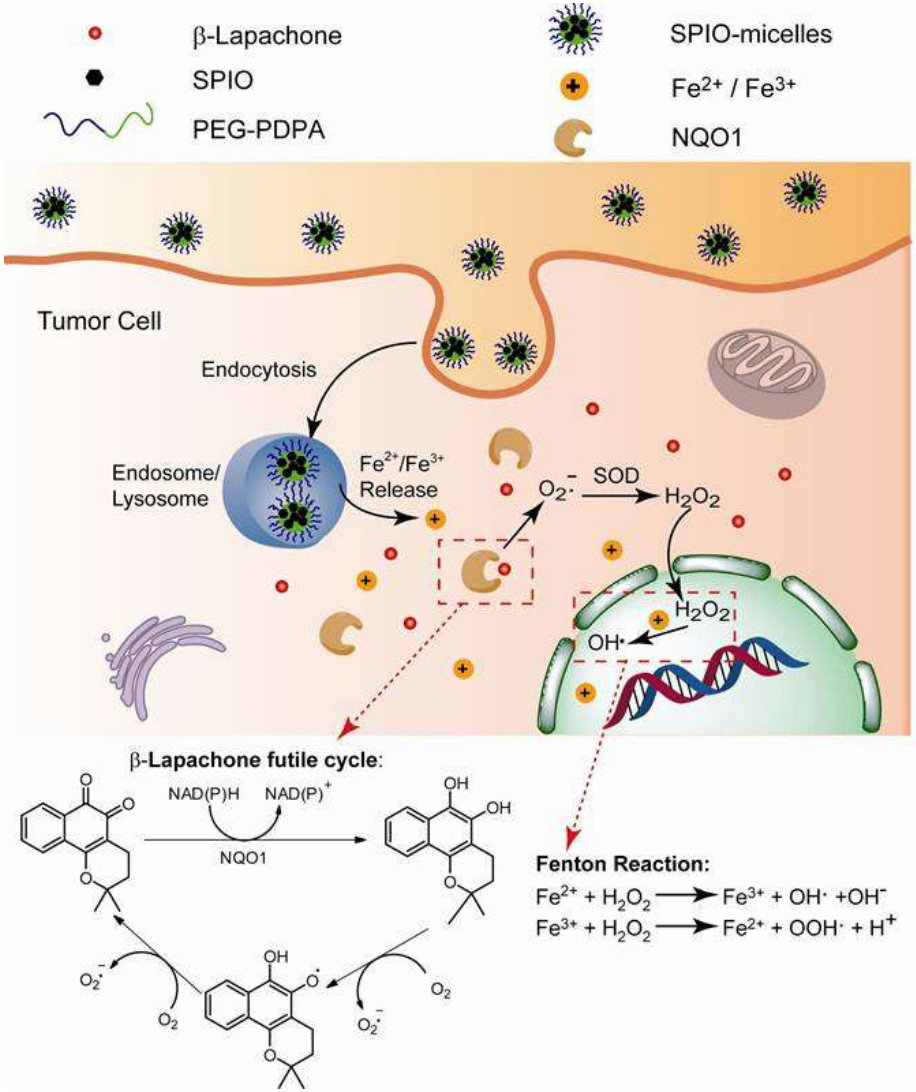
- *Delivery of medicines*



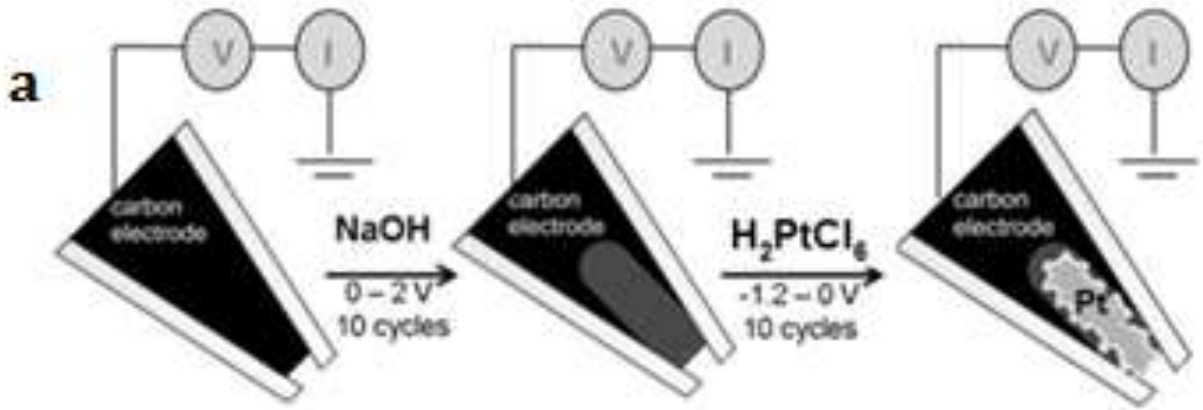
- *Hyperthermia*

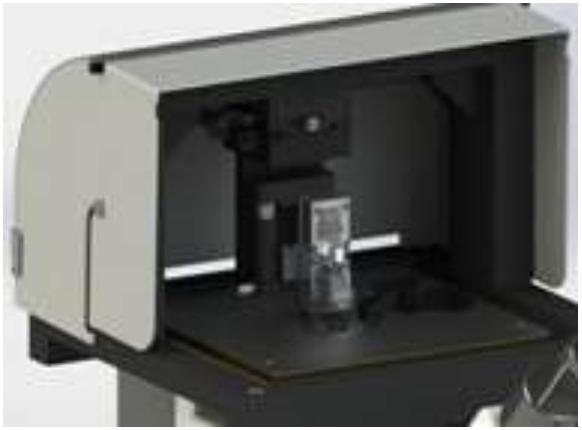


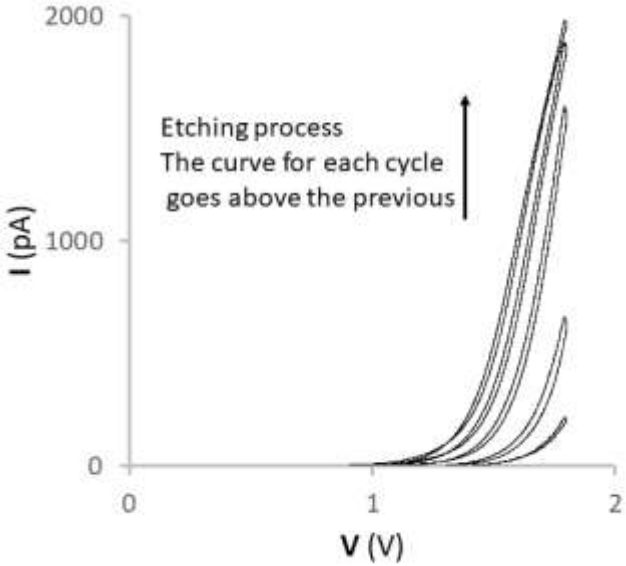
The mechanism of toxicity of magnetite:



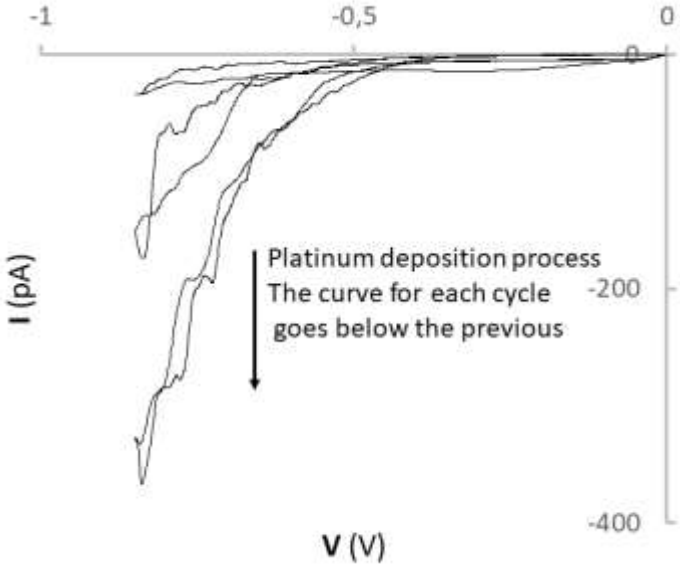
Production of platinized electrodes with increased adhesion of platinum





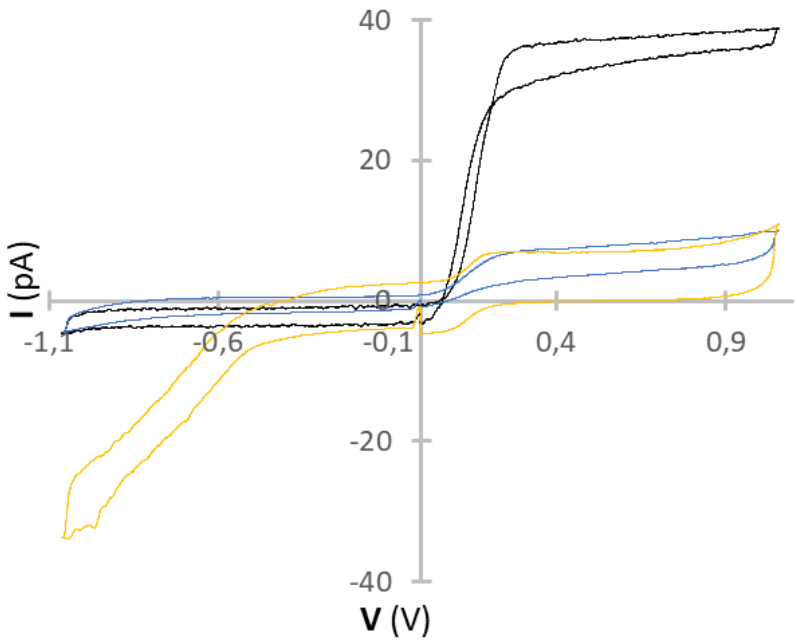


NaOH etching

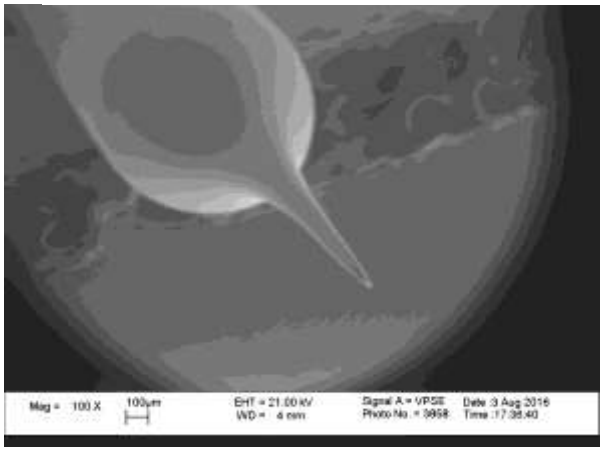


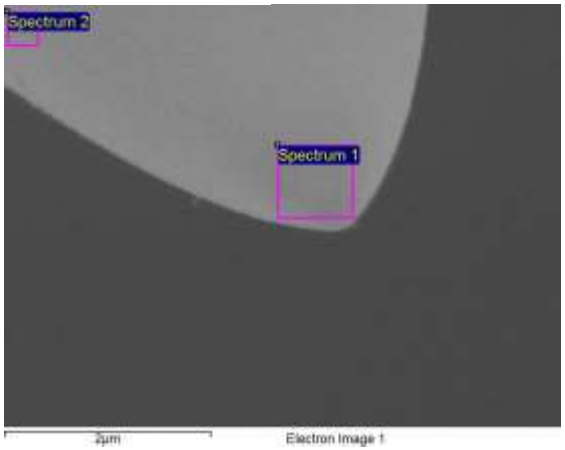
Platinum deposition

Production of platinized electrodes with increased adhesion of platinum



Cyclic voltammograms in 1mM ferrocene methanol in PBS of a nanoelectrodes: black graph – disk shape initial carbon electrode; blue graph – electrode after etching in NaOH solution; yellow graph – platinized electrode.

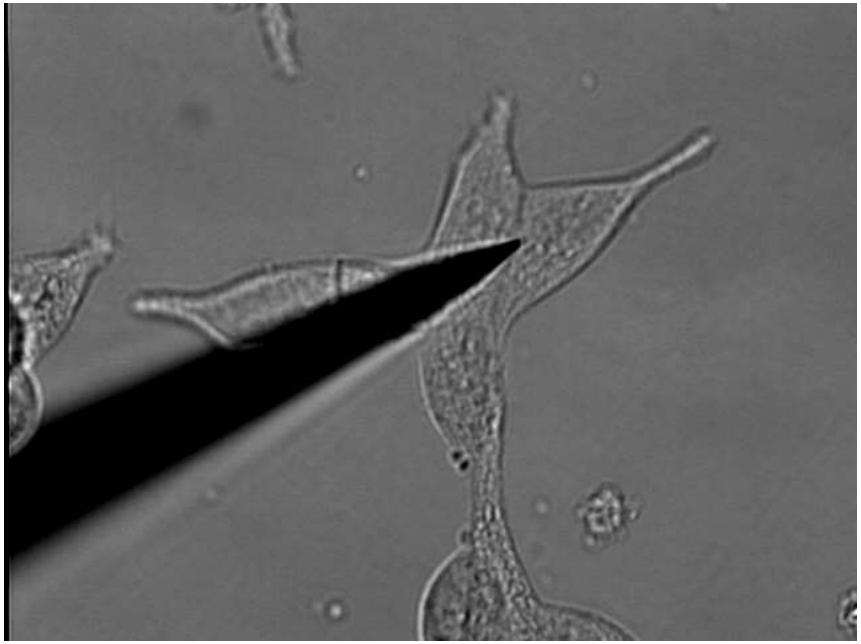




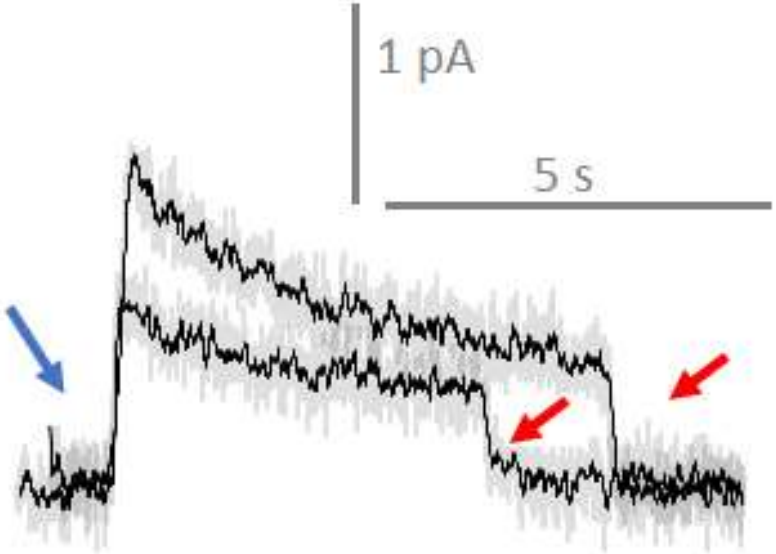
SEM images of platinized electrode is presented (a-100x, b – 50000x). In figure b shown zones of EDX analysis. Platinum was detected only in zone 1 on the tip of electrode.

Intracellular ROS Measurements after incubation with NP

Cells were incubated in HBSS buffer with 8,53 ug/ml Fe3O4 Nanoparticles (10 nm)



Microphotograph of intracellular ROS measurements

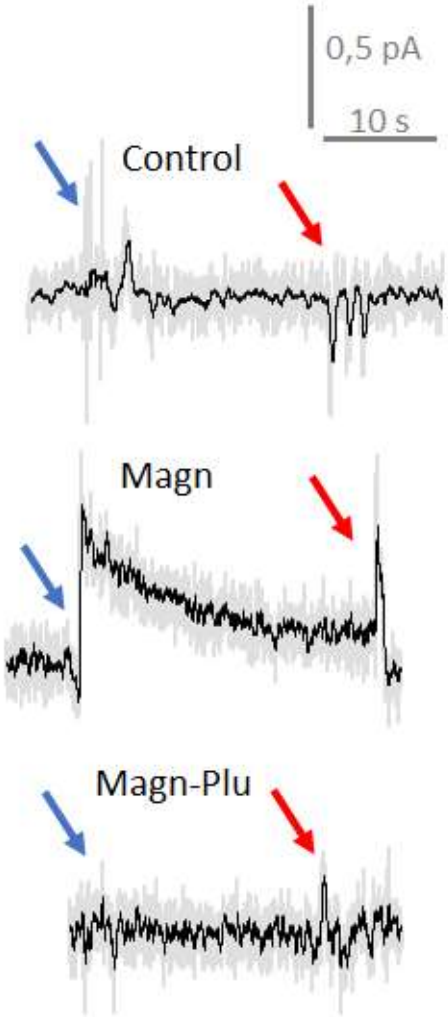


Representative current traces of a nanoelectrode polarized at +800 vs Ag/AgCl inside and outside HEK293 cells. Red and blue arrows indicated, respectively, the moment of penetration and retraction

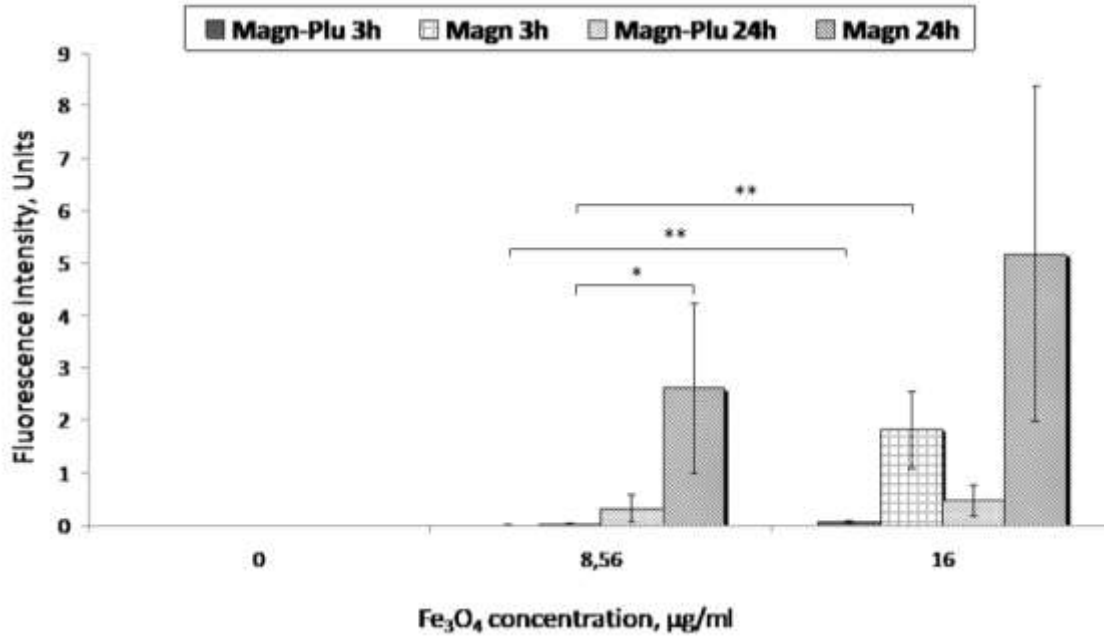
Novel Method of Toxicity Screening of NP

Electrochemical method

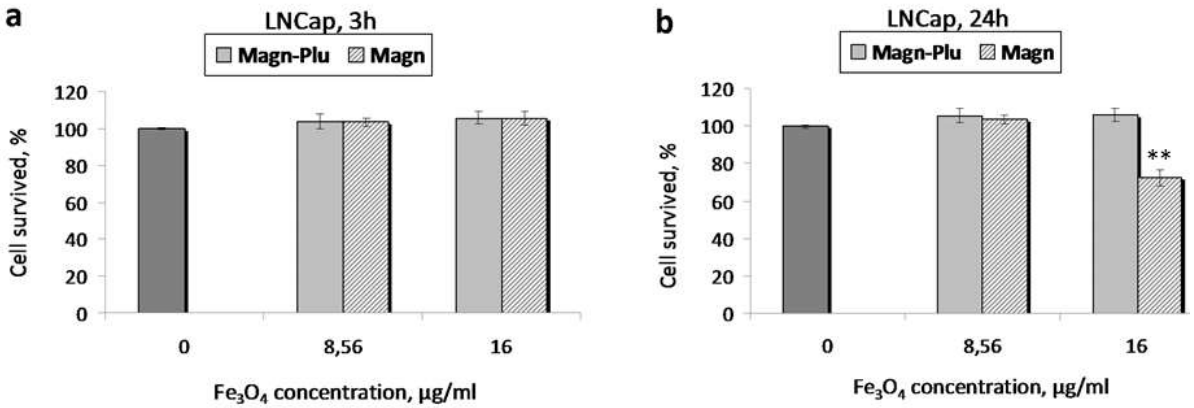
8 ug/ml, 3 h incubation



Fluorescent detection of ROS with H2DCFDA dye

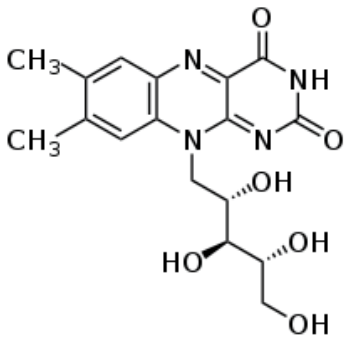


MTS test



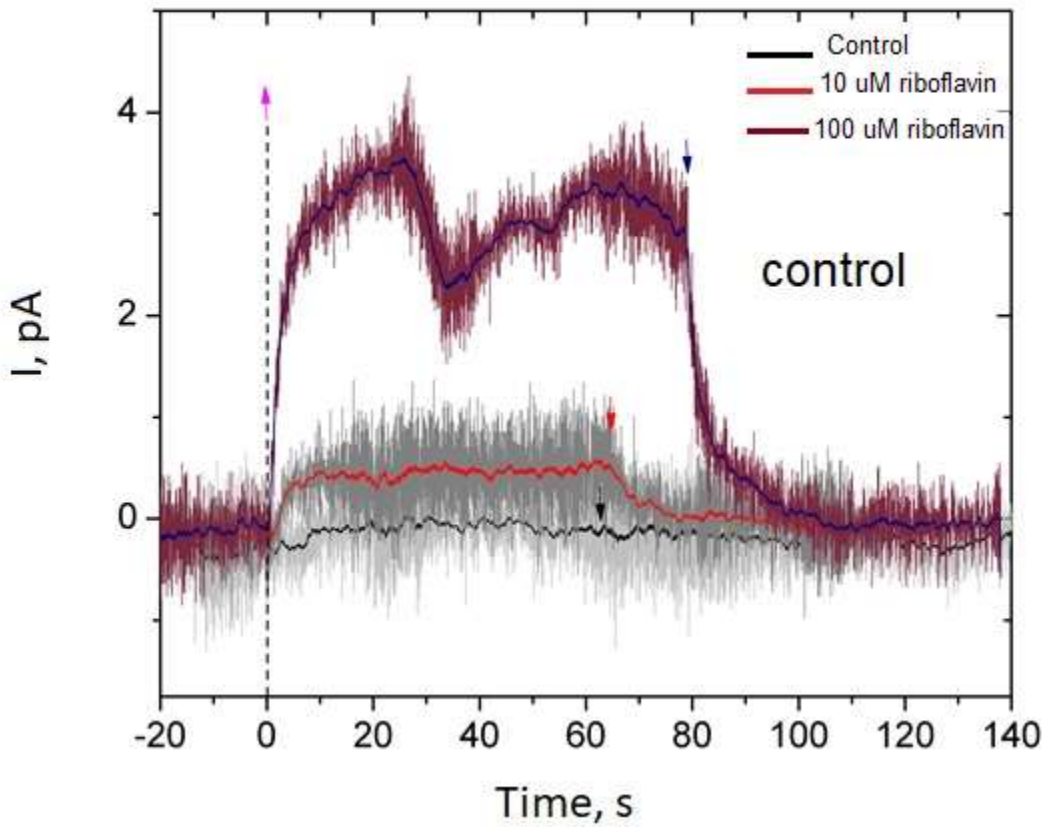
Erofeev et. al. Scientific Reports. 2018

ROS generation by photoactivation of Riboflavin with UV light



UV light

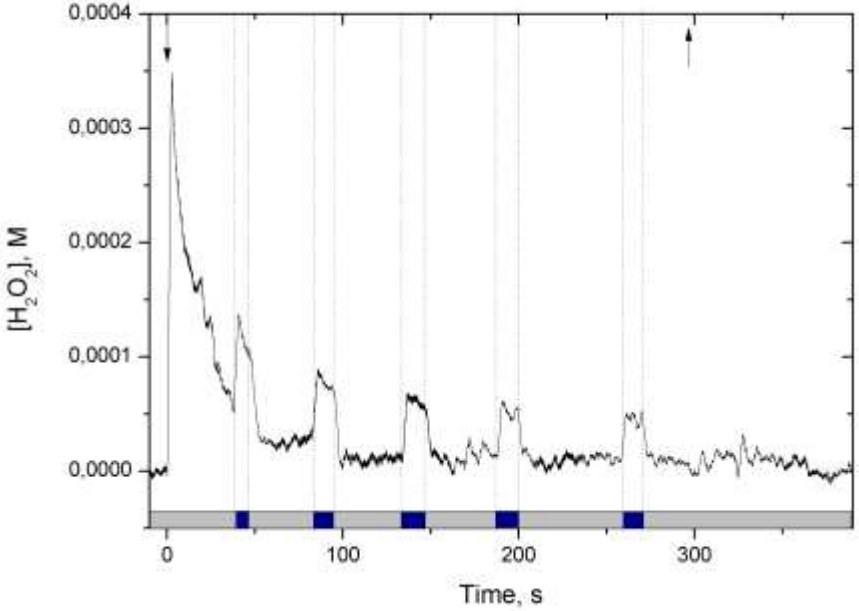
$O_2^{\bullet -}$



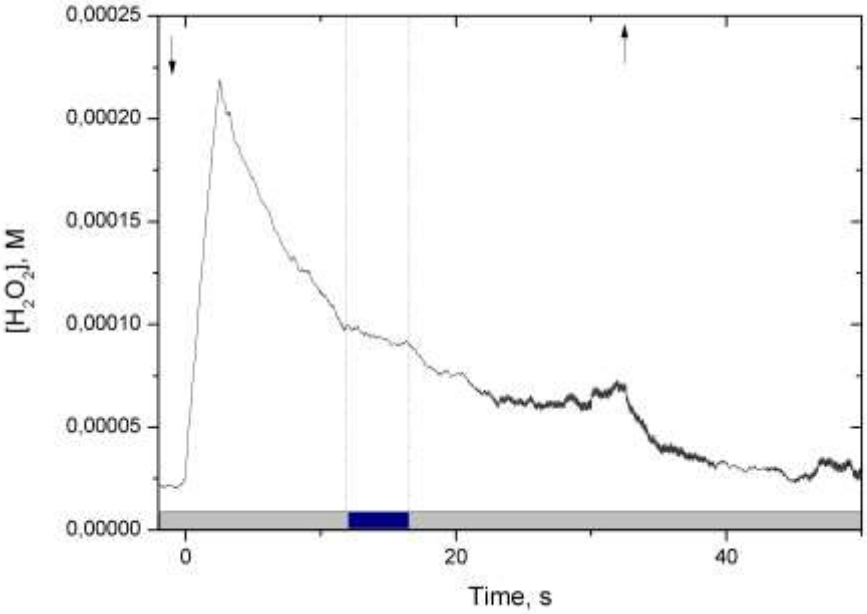
Representative current traces of a nanoelectrode polarized at +800 vs Ag/AgCl

Intracellular Measurements of ROS Production by Photoactivation of Riboflavin

With riboflavin



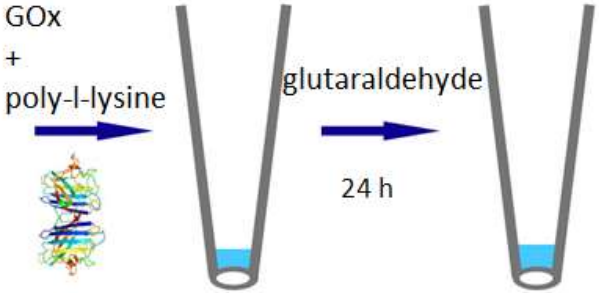
Without riboflavin

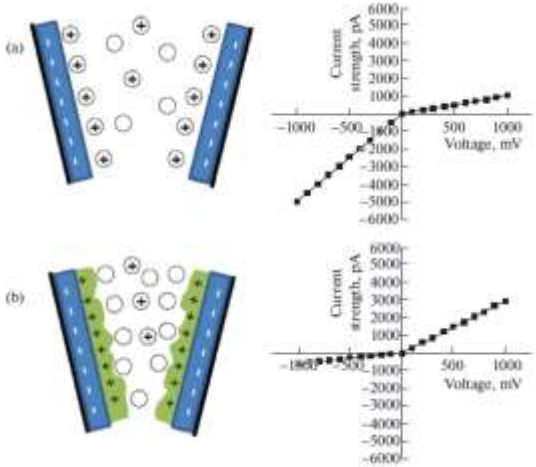


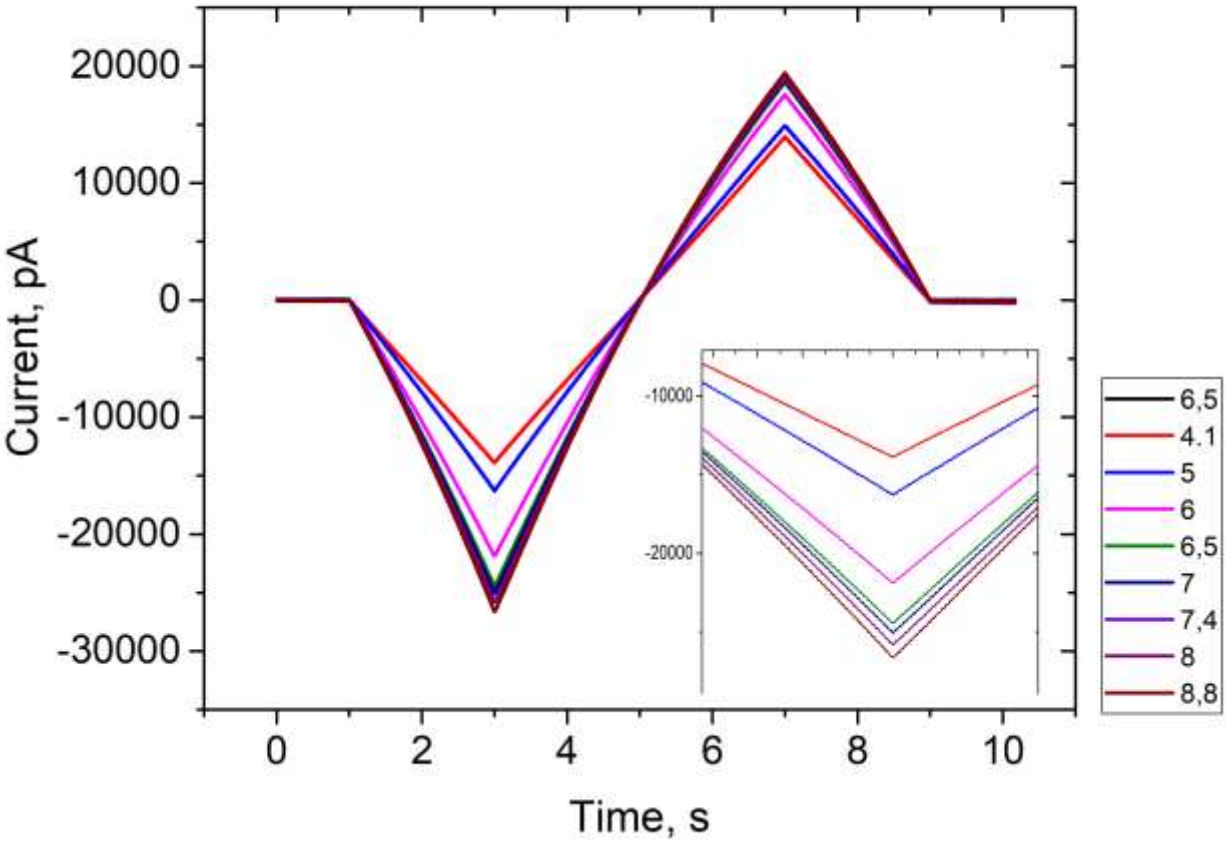
Light 400-500 nm

Melanoma (Mel_IL) cells were incubated in riboflavin solution for 30 min

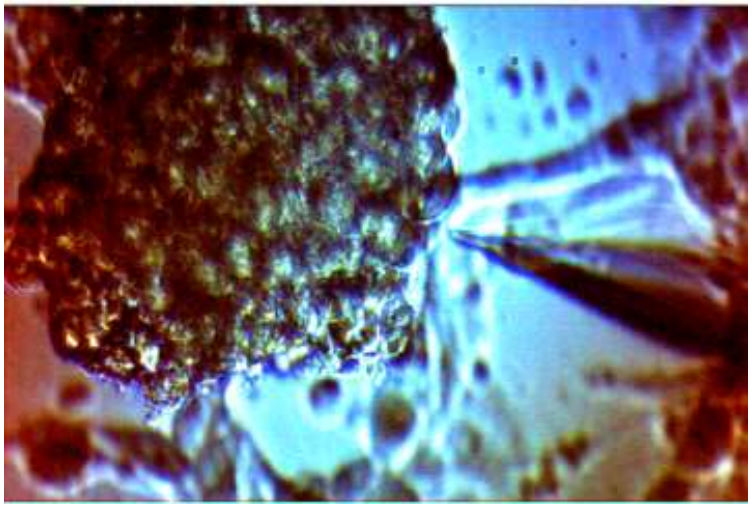
pH Nanopipette Sensor

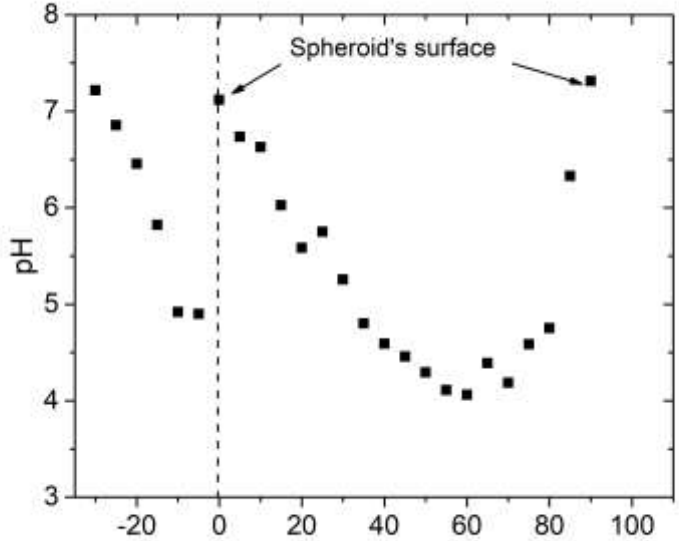


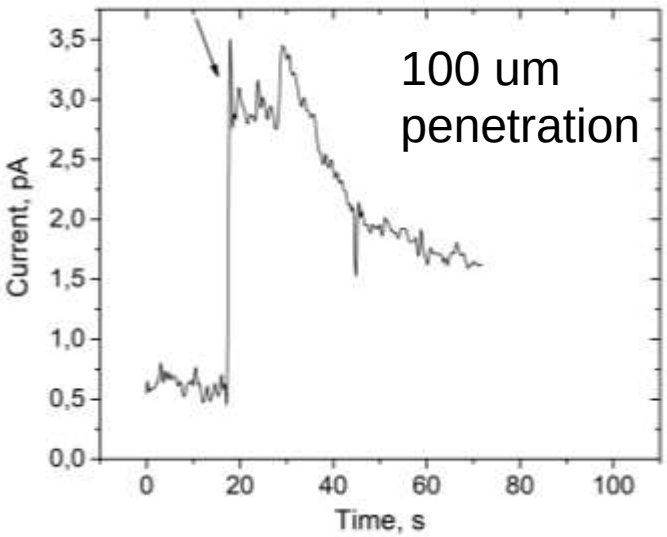




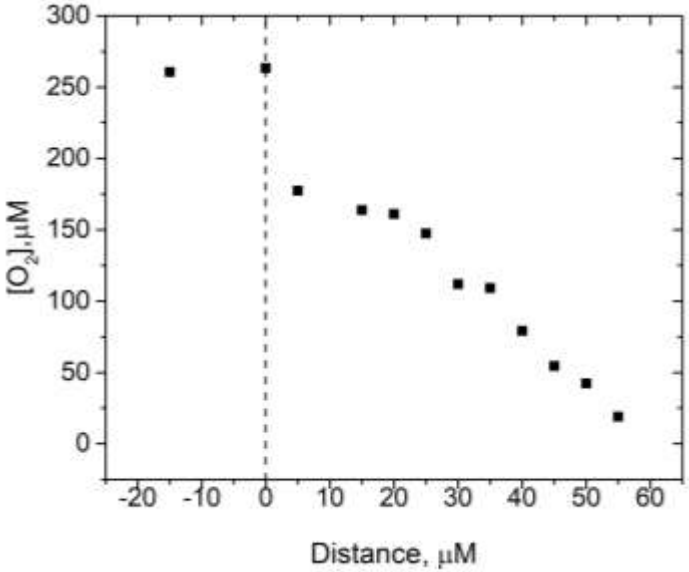
ROS, pH and O2 Measurements in Spheroids (U-87 MG)





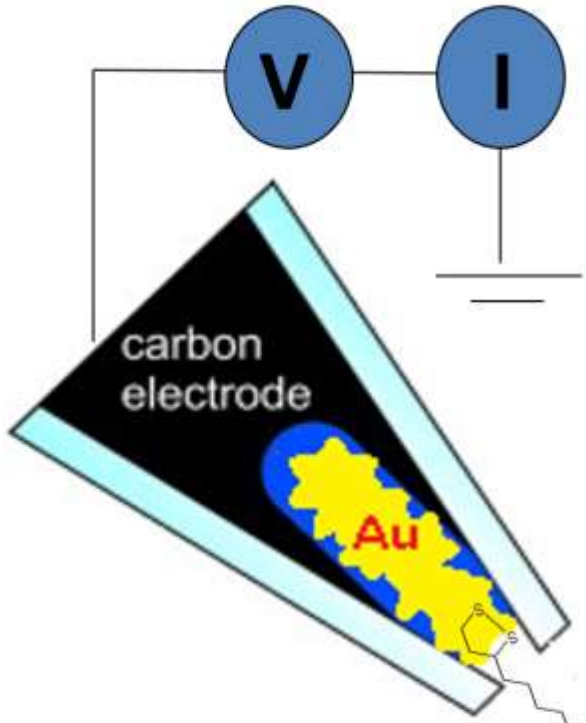


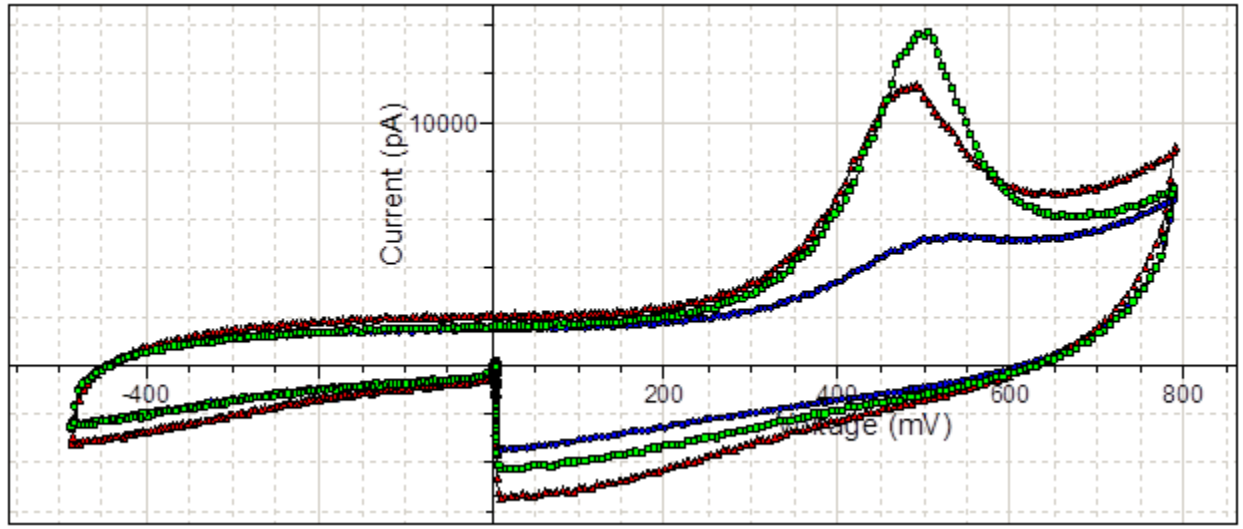
+800 vs Ag/AgCl

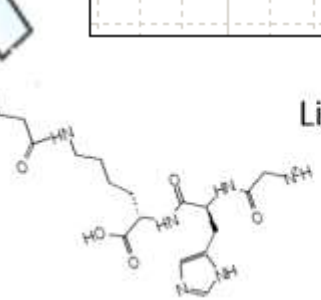


-650 vs Ag/AgCl

Electrochemical copper sensor







Ligand specific to Cu

V =10^3 V/s

Blue- PBS,

Red - $3 \cdot 10^{-7}$ M copper,

Green - 10^{-6} M copper.

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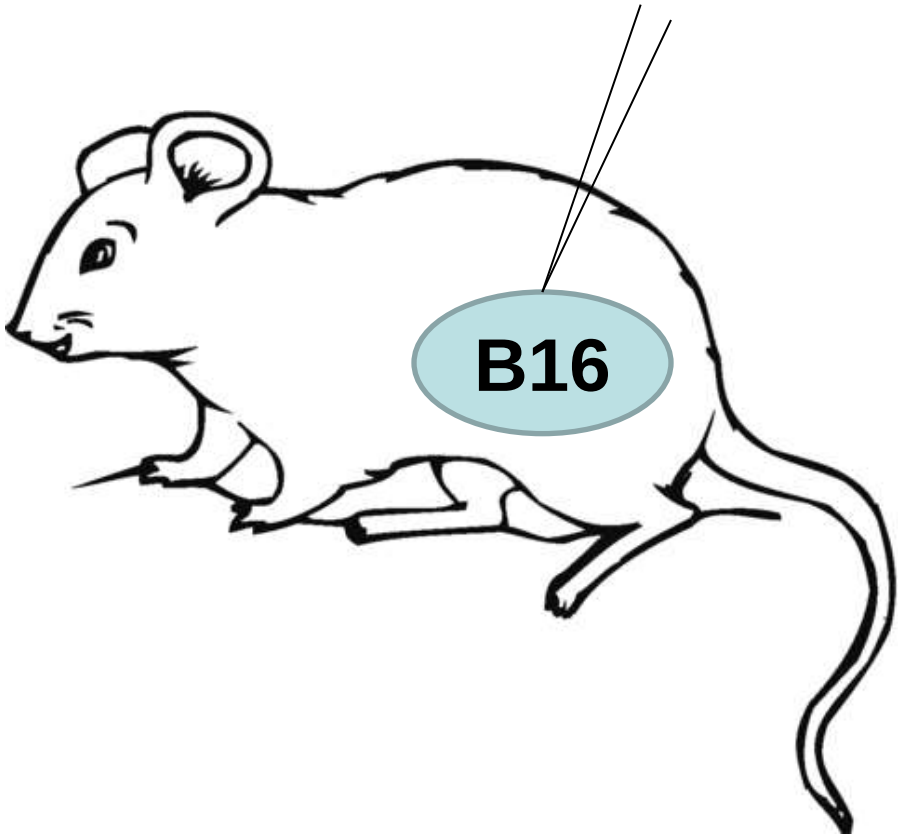
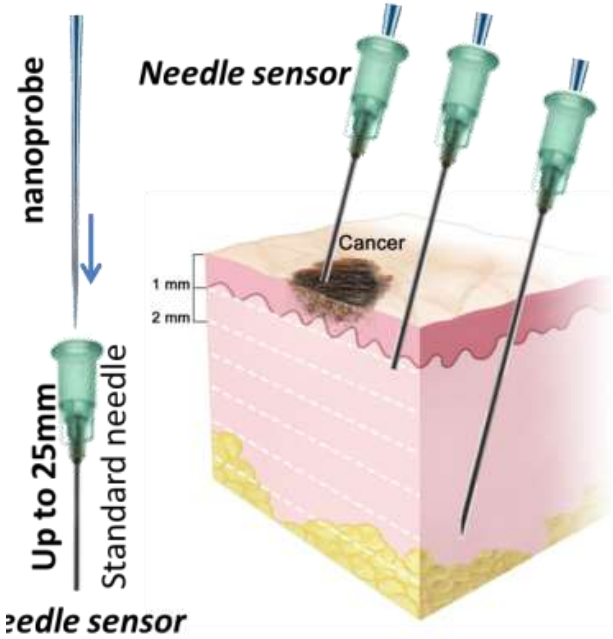
In situ analysis of metabolites





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