Supplementary Information for

Single cells and intracellular processes studied by a plasmonic-based electrochemical impedance microscopy

Wei Wang, Kyle Foley, Xiaonan Shan, Shaopeng Wang, Seron Eaton,

Vinay J. Nagaraj, Peter Wiktor, Urmez Patel, and Nongjian Tao*

Center for Bioelectronics and Biosensors, Biodesign Institute,

Department of Electrical Engineering

Arizona State University, Tempe, AZ 85287

*Corresponding author: njtao@asu.edu

Simulation of impedance image of single cells:

We constructed a three-dimensional (3D) model of a cell using COMSOL and modeled the impedance image of the cell attached to a bottom gold electrode using the AC/DC module (Quasi-Statics Electric with COMSOL Multiphysics 3.5a). An electrode was placed on top of the cell. The geometry and dimensions of the cell is shown in Fig. S1a1. The model cell has a membrane, cytoplasm and a nucleus and its envelope. Because the ratio of the cell size to the thickness of the membrane is 4 orders of magnitude, very small grids over a large cellular volume are required, which makes it difficulty for numerical simulations. To overcome this simulation problem, a cell membrane with 50 nm thickness was assumed. Although the membrane thickness in the model was 10 times thicker than that in real situations, the effect was compensated by setting the conductivity of membrane at a value 10 times higher than the actual value. We also included a 50 nm layer of dielectric medium between the bottom of the cell and the top surface of the bottom electrode according to the literatures[1,2]. To simulate the cell apoptosis process, three cell geometries, a flat bottom, and the center portion of the cell bottom detached from the surface over 200 and 400 nm, respectively were used (Figs. S1a1-S1a3).

The electric field in the model is determined by solving the quasi-static form of the Maxwell equations using the finite element method (FEM). The boundary conditions were set to match the real experiment conditions. The bottom electrode was set to ground and top surface was applied -0.1V potential while all the other four surfaces were set to electric insulation. The conductivity and relative permittivity of different layers were list in Table S1[3,4]. Note that the conductivity of cell membrane and nucleus envelope were adjusted due to the adjustment of membrane thickness as described above.

Table S1: The conductivity and relative permittivity of different layers.

Name	Value	Unit	Description
Cs	1.69	S/m	Conductivity of culture media
Cm	1.69e-1	S/m	Conductivity of cell membrane
Cp	0.5	S/m	Conductivity of cytoplasm
Cne	1e-2	S/m	Conductivity of nucleus envelope
Cnp	1.35	S/m	Conductivity of nucleus plasma
Es	79	ε_0	Relative permittivity of culture media
Em	6.2	ε_0	Relative permittivity of cell membrane
Ep	60	ε_0	Relative permittivity of cytoplasm
Ene	28	ε_0	Relative permittivity of nucleus envelope
Enp	52	ε_0	Relative permittivity of nucleus plasma
RIs	1.37		Refractive index of culture

			media
RIm	1.41		Refractive index of cell membrane
RIp	1.38		Refractive index of cytoplasm
RIne	1.41		Refractive index of nucleus envelope
RInp	1.38		Refractive index of nucleus plasma

The electric field ($E_z|_{z=0}$) normal to the bottom surface was calculated by COMSOL Multiphysics, from which the surface charge density was determined using $\rho = \varepsilon E_z|_{z=0}$, a relation based on Gauss' law. The impedance image was obtained by

$$Z^{-1}(x,y,\omega) = j\omega\Delta\rho(x,y,\omega)/\Delta V, \qquad \text{(S1)}$$

where Z^{-1} is the inverse of impedance (admittance), ω is the angular frequency, and ΔV is the voltage perturbation.

The SPR images for the three different cell geometries were also calculated using local index of refraction. SPR response to an object depends sensitively on the distance between the object and the surface, which was simulated by a Matlab program[5]. For example, at 100 nm away from the surface, the SPR sensitivity is 0.45 degree per nm per Refractive Index Unit (RIU), meaning a 1 nm-thick layer at 100 nm away from the surface produces a SPR angle shift of 0.45 degree when the refractive index of the layer is 1 RIU higher than the surrounding media.

The geometries of different cell models were exported to Matlab program, and the SPR sensitivity curve was applied to the geometry of cell model with refractive index values of different layers listed in Table S1. The SPR responses for different cell geometries are shown in Figs. S2(b1-b3).

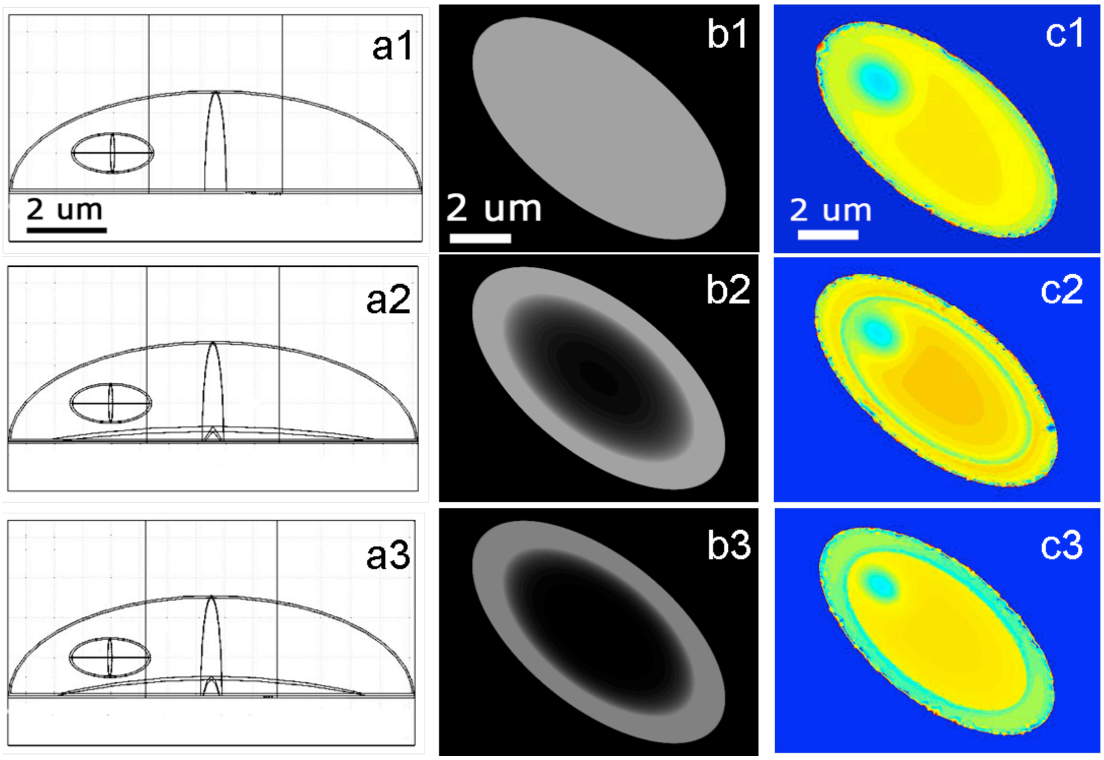

Fig. S1. A cell attached to an electrode surface with its bottom flat on (a1), central portion of the cell bottom detached by 200 nm (a2) and 400 nm (a3) from the electrode, respectively. Corresponding simulated SPR images (b1-b3) and Impedance images (c1-c3).

Electroporation and Recovery:

To minimize permanent damage to the gold surface during electroporation experiments, high frequency AC voltage (e.g., 1 MHz) is often used.[6] This strategy was also used in the present study. As shown in Fig. S2a, a voltage pulse with amplitude 10 V and duration 3 seconds was applied to trigger the electroporation process. We have confirmed that the condition did not result in obvious damage to the electrode surface. The SPR did show a transient response due to polarization of the surface, but the SPR signal recovers after the pulse was over (Fig. S2b). To further prove that the electroporation just wound cells rather than completely killed them, cells were stored in an incubator overnight after the electroporation measurements. The subsequent microscopic observations indicated that most cells were still alive, as shown in Fig. S2(c, d).

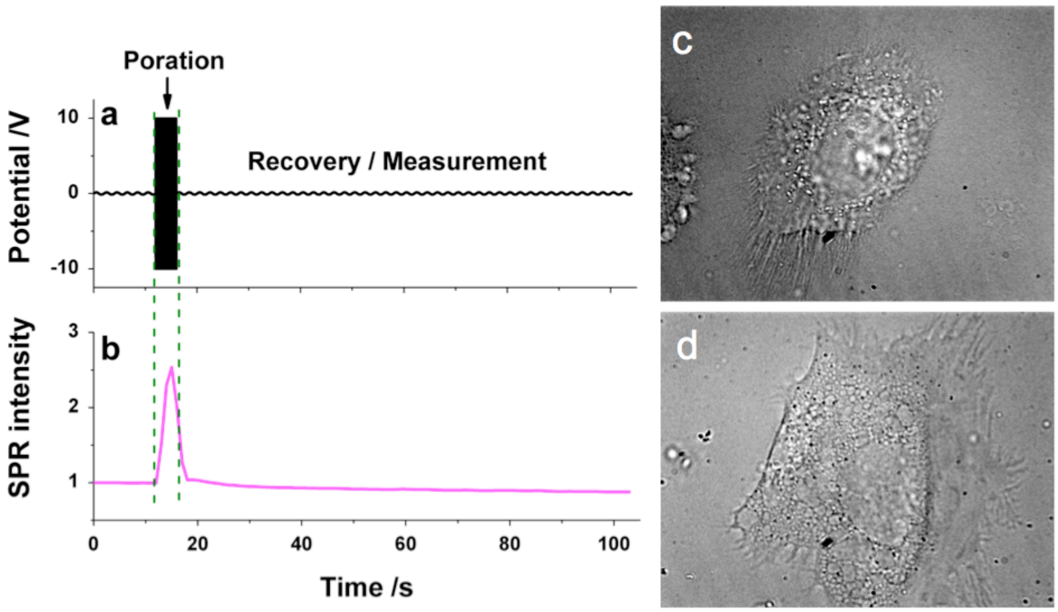

Fig. S2. Potential waveform (a) and SPR response (b) of a bare-gold region in an electroporation measurement. (c) and (d) show the optical images of two cells after electroporation and overnight culture in an incubator.

References:

1 Giebel, K.-F. *et al.* Imaging of cell/substrate contacts of living cells with surface plasmon resonance microscopy. *Biophys. J.* **76**, 509-516 (1999).

2 Lo, C.-M., Keese, C. R. & Giaever, I. Cell–Substrate Contact: Another Factor May Influence Transepithelial Electrical Resistance of Cell Layers Cultured on Permeable Filters. *Exp. Cell Res.* **250**, 576-580 (1999).

3 Griffith, A. W. & Cooper, J. M. Single-Cell Measurements of Human Neutrophil Activation Using Electrorotation. *Anal. Chem.* **70**, 2607-2612 (1998).

4 Pavlin, M. & Miklavcic, D. Effective Conductivity of a Suspension of Permeabilized Cells: A Theoretical Analysis. *Biophys. J.* **85**, 719-729 (2003).

5 Shan, X. *et al.* Measuring Surface Charge Density and Particle Height Using Surface Plasmon Resonance Technique. *Anal. Chem.* **82**, 234-240 (2010).

6 Keese, C. R., Wegener, J., Walker, S. R. & Giaever, I. Eletrical wound-healing assay for cells in vitro. *Proc. Natl. Acad. Sci. USA* **101**, 1554-1559 (2004).

Supporting Online Material Video Clip Files

Movie S1 SPR and EIM videos of a complete apoptosis process (lasted for 8 hours, with a time interval of 15 minutes for each frame). Several snapshots of the videos are shown in Figs. 3a-b of the main text.

Movie S2 SPR and EIM videos of a complete electroporation process (lasted for 103 seconds, with a time interval of 1 second). The electroporation potential was applied at 13th second for an interval of 3 seconds. Several snapshots of the videos are shown in Figs. 4a-b of the main text.