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Single cell electric impedance topography: Mapping membrane capacitance

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Abstract

Single-cell electric impedance topography (sceTopo), a technique introduced here, maps the spatial distribution of capacitance (*i.e.* displacement current) associated with the membranes of isolated, living cells. Cells were positioned in the center of a circular recording chamber surrounded by eight electrodes. Electrodes were evenly distributed on the periphery of the recording chamber. Electric impedance measured between adjacent electrode pairs (10 kHz–5 MHz) was used to construct topographical maps of the spatial distribution of membrane capacitance. *Xenopus* Oocytes were used as a model cell to develop sceTopo because these cells consist of two visually distinguishable hemispheres, each with distinct membrane composition and structure. Results showed significant differences in the imaginary component of the impedance between the two oocyte hemispheres. In addition, the same circumferential array was used to map the size of the extracellular electrical shunt path around the cell, providing a means to estimate the location and shape of the cell in the recording chamber.

Introduction

Measurement of the electrical displacement current or capacitance of cell membranes provides a powerful means to examine key biological events such as synaptic transmission,¹ ion-channel gating,^{2,3} cell division and growth,^{4–6} protein-based electromotility,⁷ and membrane flexoelectricity.⁸ Capacitance provides information about cell size and structure resulting from lipid bilayer surface area, ultrastructure and molecular composition.^{9–13} Techniques to measure membrane capacitance, therefore, have significant applications in basic science and can also be used to screen candidate pharmaceutical compounds¹⁴ and genetic manipulations for therapeutic efficacy.

Here we introduce a new technique, single-cell electric impedance topography (sceTopo), that measures the spatial distribution of membrane[†] capacitance around isolated living cells using radio frequency interrogation. The recording system resembles that used in electric impedance tomography (EIT), where current/voltage measurements made by an array of electrodes around the human body are used to generate two- or three- dimensional (2D or 3D) electrical images of the bodies' interior.^{15–17} In the present study, electrode arrays were miniaturized to record electrical impedance values around an isolated, single cell. The method differs from EIT, however, in that it is used to probe the electrical properties of a

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[†]The term “membrane” used herein refers to the multiple layers of lipids, proteins, and sugars on the periphery of the cell.

material near the electrode's surface (in this case, the cell membrane and the extracellular solution) and is not well suited to image the interior of cells. Hence, the present method is referred to as single cell electric impedance topography (rather than tomography).

In sceTopo, isolated cells were positioned within a recording chamber that was surrounded by an array of conducting electrodes. The conducting tips of the electrodes were equally spaced around the periphery of a circular recording chamber with the insulated shanks of the electrodes directed radially. Local electrical properties of the membrane were examined by recording the impedance between adjacent pairs of extracellular electrodes at radiofrequencies, a method based in electric impedance spectroscopy.^{9,18-26} The recording chamber was sized to minimize the distance between the electrodes and the plasma membrane in order to maximize interaction between the electric field and the plasma membrane. Data were used to construct topographical maps showing the spatial distribution of membrane electrical properties. The system was designed/fabricated for use on a microscope stage and facilitated application of chemical solutions, microinjections, or electrophysiological recordings during sceTopo.

Xenopus Oocytes (frog eggs) were used as the model cell to develop sceTopo because of their large size (~1 mm diameter), ease of manipulation, availability, and polarized structure. These cells are a common model for electrophysiological studies because their size facilitates two-electrode voltage clamp and because they can express exogenous proteins (including ion channels) in their membrane.²⁷⁻³⁰ Oocytes have two distinct hemispheres or poles, the animal pole characterized by its dark brown color and the vegetal pole characterized by its yellow color. The types of ion channels, organelles, melanin concentration and microvilli vary as a function of oocyte hemisphere. This endogenous polarization makes the oocyte a natural choice to test the ability of sceTopo to resolve spatial inhomogeneity of membrane properties.

Results shown here specifically demonstrate the ability of sceTopo to resolve spatial differences in electrical impedance around native *Xenopus* Oocyte membranes. Results also show, using phantoms (*i.e.* objects with known electrical properties), that the shape and location of the cell within the chamber can be estimated.

System design and fabrication

Electrode array fabrication

Electrode arrays were constructed using a combination of thick-film, microfabrication, and xurographic techniques.³¹ A knife-plotter (Graphtec 7000, Graphtec America, Santa Ana, CA) was used to pattern a circularly distributed set of 8 electrodes into the top layer of the double-layered Rubylith polymer (Rubylith RU3, Ulano Corp. City). The top layer of the patterned Rubylith served as a mask during metal deposition. A 1.1–1.2 mm diameter hole was also cut in the center of the electrodes through both layers of the Rubylith polymer and acted as a recording chamber. Platinum and titanium (seed layer) were sputtered on the patterned Rubylith (TMV SS-40CIV, T-M Vacuum Products, Cinnaminson, NJ) at the University of Utah's Microfabrication Laboratory. The mask layer of the Rubylith was lifted off to reveal the electrode array. Electrodes were plated using a current density of 10 mA/cm² in a platinum black plating solution (3.3% (by weight) chloroplatinic acid and 0.03% (by weight) lead acetate). Platinization increased the electrode surface area, thereby decreasing the electrode double-layer impedance. The electrodes were individually isolated using a Kapton polyimide adhesive (5314 Kapton, 3M, St. Paul, MN) (Fig. 1B). The volume above the triangular portion of each electrode was filled with either physiological saline (phantom experiments) or Super Barth's Oocyte media (88 mM NaCl, 1 mM KCl, 0.41 mM CaCl₂, 0.33 mM Ca(NO₃)₂, 1 mM MgSO₄, 2.4 mM NaHCO₃, 10 mM HEPES, 1 mM

pyruvate, and 50 $\mu\text{g/ml}$ gentamicin, titrated using NaOH to a pH of 7.4). This geometry increased the electrode surface area contacting the media and further lowered the double-layer impedance of the Pt electrodes. Electrical bond pads, located near the periphery of each electrode array, connected the electrode array to a printed circuit board (PCB) (Fig. 1C). The PCB was interfaced with the electronic instrumentation described below.

The platform

The electrode array was clamped in a polycarbonate interface that allowed for cell loading and positioning (*via* a vacuum port) into the recording chamber (Fig. 1C). The bond pads on the electrode array contacted the PCB through a series of springloaded gold pins (B1363-D4 Interface Contacts, Rika Denshi, Attleboro, MA) (Fig. 1C). The electrical source consisted of either the Tektronix AFG320 or AWG430 (Tektronix, Beaverton, OR). Each source was individually calibrated using a Thévenin equivalent model to compensate for load-dependent loss at frequencies greater than 100 kHz. A voltage-dividing on-board reference impedance was used to calculate the recording chamber impedance.^{18,21,32} High-impedance voltage-follower operational amplifiers (OPA356, Texas Instruments, Dallas, TX) located on the headstage PCB were used to sample the voltage drop across the reference impedance to determine the current. All signals were recorded in quadrature by lock-in amplification (Stanford Research, SR830 SR844, Sunnyvale, CA). Electrodes on the array were individually addressable, and the interrogating signal was directed to the electrode of interest using digital switches (Max4521, Maxim Integrated Products, Sunnyvale, CA) located on the custom headstage PCB (Fig. 1D). Source output, signal recording, and electrode pair selection were automatically controlled *via* a custom computer interface (IGOR Pro, WaveMetrics, Portland, OR). The program remotely controlled the waveform generator and lock-in amplifier recording (GPIB, National Instruments IEEE 488). Analog voltage sampling and digital switching were controlled *via* 16-bit analog–digital converters and digital outputs (ITC 1600, HEKA Inst., Bellmore, NY).

Theory

Simulations

A qualitative 2D finite-element model of the oocyte loaded micro-chamber was developed to gain insight into the nature of electric fringe fields and to guide interpretation of results. We modeled the cell membrane as a highly resistive linear dielectric ($\sigma = 3.14 \times 10^{-9} \text{ S/m}$, $\epsilon = 90 \text{ F/m}$, thickness = 20 nm), the cytoplasm as a simple ionic conductor ($\sigma = 1.4 \text{ S/m}$), and the extracellular space as a conductor ($\sigma = 1.4 \text{ S/m}$, thickness = 2 μm).³³ The 2D Maxwell equations were solved in the frequency domain (Comsol Multiphysics, Los Angeles, CA). Sinusoidal voltage stimuli were applied in the model across adjacent pairs of electrodes to simulate electric fringe-field interaction with the cell membrane in the recording chamber. The electrode–electrolyte interface impedance was not included in the model and finite element simulations pertain only to the impedance associated with the cell and extracellular shunt pathway. A lumped parameter model (Fig. 2) — including the electrode–electrolyte double layer, the conductive shunt extracellular pathway, the plasma membrane and the intracellular impedance — was also used to facilitate interpretation of results.

Experimental

Measurement from passive cells and phantoms

Xenopus Oocytes were chosen as the model cell to develop and test the sceTopo approach. Oocytes are visibly polarized into brown (animal) and white (vegetal) hemispheres, are large in size (1.2 mm in diameter), and have inhomogeneous membranebound protein/channel expression between the animal vs. vegetal hemispheres.^{27,29,34–36} Differences between the

animal and vegetal hemispheres, thought to help in guiding oocyte development, also provide a polarized, easily visualizable, cellular structure ideal for testing the sceTopo platform.^{27,29,34-36} It was hypothesized that the imaginary component of the impedance associated with a resting oocyte would differ between the two hemispheres and would be detectable using sceTopo. Oocytes (defolliculated) and phantoms were individually placed in the recording chamber using transfer pipettes, and impedance measurements between adjacent pairs of electrodes were made around the cell. Data were collected in quadrature with a lock-in filter of 18 dB/octave and a lock-in integration time of 3 ms (SR844, Stanford Research, CA). Results were corrected using a Thévenin source calibration and combined with an on-board reference impedance to obtain complex-valued impedance measurements between adjacent pairs of electrodes. Lock-in amplifier analog outputs were sampled in quadrature at 1 kHz (ITC 1600, InstruTECH, Bellmore, NY). To estimate the contribution of the electrode–electrolyte double-layer impedance, data were also collected under the same stimulus conditions when the chamber was only filled with extracellular media (media-only condition) during each experiment. Since the impedance in the media-only condition was low relative to the tight-fitting cell-loaded condition, subtracting the media-only data from the cell-loaded data removed major contributions of the double-layer impedance from the cell/phantom measurements.¹⁸ One caveat of this approach is that it also subtracts the electrical resistance of the media itself, and therefore the real component of the impedance obtained is shifted by a real-valued constant.

After subtracting the double-layer, the impedance measured between adjacent electrode pairs was divided in half and assigned to chamber positions that corresponded to the center point directly in front of each interrogating electrode. This was done for every electrode-pair measurement, and the values associated with each electrode position were averaged together. The procedure resulted in impedance data for 8 locations around the circumference of the cell, corresponding to the 8 electrode positions in the circular array. Impedance data were interpolated between electrodes and smoothed using a five-term Fourier series in the

form
$$Z(\vartheta) = \sum_{n=0}^5 (A_n \cos(\vartheta - \vartheta_0) + B_n \sin(\vartheta - \vartheta_0))$$
. A_n and B_n , calculated from the data using a least squares regression, are complex-valued Fourier coefficients. Z is the interpolated impedance as a function of polar angle ϑ .

Polar impedance plots were constructed for both toroidal glass washers (phantoms, 1 ± 0.1 mm diameter about its radially symmetric axis, 0.61 ± 0.06 mm thick, 0.44 ± 0.06 mm dia. hole; purchased as seed beads from the Garden of Beaden, Upland, CA) and for single cells (oocytes). The second moment of area and the centroid of $Z(\vartheta)$ were also computed as metrics of the shape and orientation of the phantom. Impedance plots for each phantom were first constructed using $\vartheta_0 = 0$, and subsequently rotated by angle ϑ_0 to align the phantom major axis with the horizontal. The major axis was identified using a photomicrograph of the phantom in the chamber. These rotated results were then averaged across phantoms. Means and standard errors were plotted as a function of angle ϑ . Unpaired, Student's t-tests were used to compare the major and minor axis lengths of the toroidal glass phantoms. Unpaired, Student's t-tests were also used to compare difference in angular position (the angle between the major axes of the phantom to the 0° – 180° axes) of the phantoms in their photomicrographs to the impedance-based phantom representations.

At high frequencies, the capacitive shunt impedance of the electronic head-stage board and cabling was significant. At the frequencies used (<5 MHz), the glass phantoms can be considered nearly perfect insulators. This allowed us to find a correction factor to account for the additional capacitive shunt loss at 1 MHz by comparison to results obtained at 10

kHz. The correction factor was applied to subsequent oocyte data collected using the same array, since the capacitive shunt would be nearly the same in the two cases.

The procedure to measure $Z(\theta)$ described above for phantoms was also used for cells. Cells were positioned in the channel using very small amounts of negative pressure (applied through a vacuum port – see Fig. 1D), so as to minimize any membrane distortion during cell loading. Pressure release and application resulted in coarse manipulation of cell position in the recording chamber, and a constant negative pressure was used to hold the oocytes in place after the meridian of each oocyte was centrally aligned in the electrode array. For each oocyte, a photomicrograph taken during sceTopo interrogation was used to rotate the data by an angle θ_0 (see Fourier series above) so that the meridian separating the animal and vegetal hemispheres for a particular cell was aligned with the horizontal. This allowed for alignment of the gross morphology of different oocytes and comparisons across cells. Real and imaginary components of Z were plotted in polar form to map the impedance as a function of position around the circular electrode array. Means and standard errors were determined across multiple cells, thus providing confidence intervals as functions of angular position around the cell. Unpaired Student's *t*-tests were used to compare spatial differences between the two hemispheres of the oocyte.

Results and discussion

Simulations

Fig. 3 illustrates the results of a 2D finite element model for pairwise fringe-field electrical interrogation at three frequencies: A) 50 kHz, B) 500 kHz, and C) 5 MHz. Voltage magnitudes are shown as shades of gray ranging from maximum (white) to minimum (black). At low frequencies (A) where conduction current dominates, virtually all of the current is shunted in the extracellular space between the two interrogating electrodes. This occurs because the resistance of the plasma membrane is much greater than the surrounding media. While low-frequency fringe-field data is not useful in elucidating information about the membrane, intracellular organelles, or proteins, it does provide information about extracellular shunt path size and therefore can be used to estimate the shape of a cell in the sceTopo recording chamber (see Phantom Data results). As the interrogation frequency increases, the current is divided between displacement current in the membrane and conduction current in the extracellular shunt path (B). The amount of current displaced across the membrane in this mid-frequency range can be used to estimate the effective capacitance of the membrane. At high frequencies (C), displacement current across the membrane dominates; the membrane effectively becomes transparent to the applied radio-frequency signals and more current is transmitted to the intracellular space. These simulations are not intended to be quantitatively accurate since the electrode–electrolyte doublelayer impedance was not included, thereby shifting the magnitude and phase of the electrode voltage relative to that shown. Furthermore, the simulations are two dimensional and cannot accurately capture out-of-plane current paths. Nevertheless, the simulations do illustrate the concept of measuring electric impedance between adjacent electrodes to 1) estimate the shape of the cell, 2) measure the effective dielectric properties of the membrane, and 3) possibly detect or measure effects of nearby intracellular organelle(s). As with the lumped parameter model illustrated in Fig. 2, finite-element simulations show that the size of the extracellular shunt path is the primary experimental parameter that must be minimized to enable measurement of displacement currents in the membrane at the low-to-mid radio frequency range of interrogation. If the extracellular shunt path is too large, the corner frequency can quickly exceed 5 MHz, thus limiting the practical utility of the approach for membrane interrogation. It is therefore advantageous to size the system so cells fit snugly within the chamber and are in close vicinity to the interrogating electrodes.

Phantom data — mapping cell shape via the extracellular conductance

Due to their low conductivity and permittivity, glass phantoms acted as nearly perfect insulators relative to the extracellular media. Therefore, virtually all of the current was conducted in the space around the phantom. This made the phantoms useful to demonstrate the utility of sceTopo in cell/object shape estimation.

Photomicrographs of the phantoms in the array are shown in the left column of Fig. 4 and impedance maps are shown for the same phantoms in the right column. The electrodes are outlined in solid white (numbered 1–8), and the recording chamber is outlined in a dotted white line. The impedance magnitude at each electrode position (after subtraction of the double layer) is plotted in polar form to generate the maps in the right column (see Experimental). Notice the correspondence between the physical shape of the phantom (left) and the electrical map of the shunt conductance (right). The centroid of the impedance data (right, square symbol at intersection of dotted lines) provides the electrical center of the phantom, and the principle directions of the second moment of area tensor provide the orientations of the major and minor axes of the phantom (dotted lines). The electrical center and orientation (right) showed good qualitative comparison to the photomicrographs of the phantoms (left), and no significant difference was measured when comparing the angular position (the angle between the major axes of the phantom to the 0°–180° axes) of the phantoms in the photomicrographs to the impedance-based phantom representations ($p = 0.95$, $n = 4$). Data was not used in the statistical comparison if the inner annulus of the phantom was visible in the photomicrograph. Since the phantoms were highly resistive relative to saline and fit loosely within the chamber, the interrogating currents were shunted in the space around the phantoms and the electrical impedance data reflected the magnitude of this shunt. Phantoms positioned more closely to the electrodes resulted in qualitatively more accurate representations of phantom shape.

Data from individual phantoms were then compared to examine repeatability of impedance maps ($n = 9$). To avoid any systematic errors associated with the array, phantoms were placed with random angular orientation in the recording chamber and the Fourier series representations produced by the data were subsequently rotated to the configuration depicted in Fig. 5A (see Experimental). Since the phantoms fit loosely in the chamber, impedance data were dominated by conduction current in the saline. The corner frequency where displacement currents in the phantom became significant was well beyond the 5 MHz limit of present experiments. Results mapping the magnitude of the impedance (normalized) as a function of angular position around the array are provided in Fig. 5B for data collected at 1 MHz (blue, long dashes, short error bar caps) and 10 kHz (red, short dashes, long error bar caps). Error bars and shaded bands show one standard error of the mean and are plotted around the averaged 10 kHz (longer error bar ends, pink color band) and 1 MHz (shorter error bar ends, blue color band) data. Differences in extracellular shunt path length along the major vs. minor axes were statistically significant ($p = 0.00014$ for the 10 kHz data set). The major conclusion from data shown in Fig. 4 and Fig. 5 is that sceTopo can map the gross shape of an insulating object by measuring spatial variations in the electrical shunt resistance around the outside of the object.

Mapping membrane charge displacement

Xenopus Oocytes were randomly placed in the recording chamber. Cells fit snugly in the array with the electrodes separated from the plasma membrane by only a thin layer of oocyte media (Super Barth's). This tight fit reduced the extracellular shunt pathway and placed the electrical corner frequency of fringe-field interrogation near 100 kHz. Above this corner frequency, displacement currents in the membrane became clearly observable in the data. Below this corner frequency, the impedance was dominated by the extracellular shunt. Thus,

comparison of measured impedance differences between high and low frequencies of interrogation allows for a description of cell electrical properties that accounts for cell location/morphology in the recording chamber. After interrogation, impedance maps ($n = 8$) were rotated using photomicrographs of each cell, such that oocyte meridians were aligned with the horizontal axis (see Fig. 6A, horizontal axis stretched from 0° – 180°) to compare data across cells (see Experimental). Impedance results at 10 kHz (pink, small dashes, long error bar caps) and 1 MHz (blue, long dashes, short error bar caps) are shown in the form of real (Fig. 6B: $\text{Re}(Z)$) and imaginary (Fig. 6C: $\text{Im}(Z)$) components after double-layer subtraction and unit circle normalization. The real-valued impedance map (Fig. 6B) showed no significant difference between the 10 kHz and 1 MHz interrogation as a function of oocyte hemisphere ($p = 0.33$). This was expected, since the real component of the impedance is dominated by the extracellular shunt conductance at these interrogation frequencies and is not very sensitive to the properties of the oocyte membrane. In contrast, the imaginary component of the impedance showed a significant ($p = 0.0058$) difference between the 10 kHz and 1 MHz data when comparing the vegetal (0° – 180°) and animal (180° – 360°) hemispheres of an oocyte (the oocyte meridian is horizontal, the white vegetal pole is at 90° , and the brown animal pole is at 270°). There was very little difference in the imaginary component between the two frequencies on the animal pole of the cell (270°), but a much larger difference on the vegetal pole of the cell (90°).

The imaginary component is dominated by the displacement current in the membrane and includes capacitance contributions from the passive permittivity of the lipid membrane, mobile charges associated with membrane-bound proteins, and excitable contributions associated with voltage-gated ion channel kinetics. Known compositional differences between the animal and vegetal hemispheres of an oocyte include, but are not limited to, concentrations of calcium-activated chloride channels, microvilli ultrastructure, yolk platelets (cholesterol granules), and melanin granules.^{27,29,34,35} The effective dielectric properties associated with these differences presumably underlie the asymmetry in the imaginary-valued impedance observed between the two hemispheres reported here (Fig. 6C). We did not explore which of these potential factors was responsible for the difference, but it is hypothesized that differences in membrane(s) ultrastructure contributed significantly to differences in capacitance measured between the two hemispheres. Future studies could use pharmacological agents to disrupt microvilli ultrastructure or bind ion channels to explore hypothetical contributions of these components to the effective dielectric properties of an oocyte membrane.

Conclusion

Results show that sceTopo can effectively be applied to map 1) cell shape and position in the recording chamber and 2) the endogenous spatial distribution of membrane capacitance around a single cell. The two-dimensional maps reported here provide images around the meridian of a *Xenopus* Oocyte. Spatial resolution is directly dependent on the number of electrodes around the cell in the recording chamber (currently, $\sim 1/8$ cellular circumference). This could be increased, however, by incorporating additional electrodes in the array. Incorporating a pressure control system to precisely position cells and a perfusion system to facilitate exchange of solutions and pharmacological agents would also improve the system.

Furthermore, it is feasible that the sceTopo approach could extend to three dimensions (3D) and that the array could be downsized for smaller cells. Both of these aims are within the scope of current technology. Known thick film laminate technologies, based on heat and pressure sensitive films allow for the simple stacking of individual electrode array layers and should result in a straightforward 3D array structure.³⁷⁻⁴⁰ The large size of *Xenopus* Oocytes allowed the use of simple electrode fabrication techniques, but technology is already

available to fabricate micron-sized metal electrodes that surround 10 μm diameter cells.^{18,26,32} Furthermore, electron-beam and ion-beam lithography have been used to construct nanometer-sized electrodes that can analyze DNA sequences and detect glucose containing analytes.^{41,42} Similar fabrication techniques could potentially be applied to the sceTopo platform to allow for micron-sized cell interrogation with nanometer scale resolution.

Lastly, it is also possible that this approach could offer an alternative means to study the fast kinetics of charged, excitable, membrane-bound proteins in oocytes themselves. Oocytes can express high concentrations of exogenous proteins in their membranes and are commonly used to study the kinetics associated with single proteins. As such, the sceTopo platform can be used to interrogate electrically excited oocytes expressing exogenous, voltage-sensitive, membrane-bound proteins. The high interrogation frequencies of the sceTopo system should timesolve changes in charged protein conformation during cellular excitation, and these differences should manifest as measurable differences in effective capacitance. If true, sceTopo might offer a new window to observe excitable integral membrane protein dynamics.

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Fig. 1. Experimental Setup

A) Platinum was patterned onto a polyester substrate, and the electrodes near the recording chamber were plated with platinum black. B) Insulated, electrode array. C) A polycarbonate interface (C-i) allowed for positioning of the electrode array (C-ii) such that spring-loaded gold pins (C-iii) connected the bond pads on the electrode array to bond pads (C-iv) on a printed circuit board (PCB, C-v). Inset shows the multiple layers of the electrode array (a polyester base (ii₁), recessed platinum-black coated electrodes (ii₂), and insulating Kapton tape (ii₃)). D) PCB and fluidic interface positioned on the stage of an upright microscope showing the headstage amplifiers (D-i), computer controlled digital switches (D-ii) and vacuum-port (D-iii).

Fig. 2. Circuit Model

An electrical lumped parameter model of a cell in the recording chamber. Impedances shown are associated with the electrode–electrolyte interface (Z_{d1} , Z_{d2}), the shunt path around the cell (R_{med}), and the impedance of the cell including the membrane complex and intracellular space (Z_m , Z_{cyt}).

Fig. 3. Quasi-Electrostatic 2D Model

A 2D frequency-domain (50 kHz–5 MHz) finite element simulation of a cell in the recording chamber. Contours show lines of constant voltage. A) 50 kHz: Due to high resistance of the membrane, current flow is restricted to the extracellular space. B) 500 kHz: Current is divided between the cell membrane (displacement current) and the extracellular space (conductive current). C) 5 MHz: Almost all of the applied current is displaced across the highly capacitive cell membrane.

Fig. 4. Glass Toroid Impedance Magnitude Image

Polar plots of impedance magnitude (right column) around glass toroidal phantoms reflect the orientation and shape of the phantom (left column: microphotographs of phantoms) in the recording chamber. Due to the highly resistive properties of glass, the applied current (1 kHz–1 MHz) shunted around the phantom and was not displaced across the phantom. Dotted lines in the polar plots (right column) show the second moments of area indicating maximal and minimal axes for each phantom. Solid lines show the second-order least-squared Fourier series representation of the phantom impedance data.

Fig. 5. Phantom Summary

Data for each phantom ($n = 9$) was rotated based on photomicrographs to have the orientation shown in A (topdown view). Data were then averaged across phantoms, normalized, and plotted in polar form (B: impedance magnitude). Error bars denote 1 standard error of the mean. Data at 10 kHz and 1 MHz are nearly identical, as would be expected since the applied current at these frequencies should shunt around the highly resistive phantom.

Fig. 6. Cell Summary

Data for *Xenopus* Oocytes ($n = 8$) were rotated based on photomicrographs of each cell to have the orientation shown (A). B) Real component and C) imaginary component of the impedance. Results are shown at 10 kHz (pink, longer error bar ends) and at 1 MHz (blue, shorter error bar ends) with error bars denoting one standard error of the mean. Error bars denote 1 standard error of the mean. The difference between the high and low frequency measurement are significantly different when comparing the vegetal hemisphere of the oocyte to the animal hemisphere.