Computational Modeling and Measurement of Electro-osmosis

NSF Functional Nanostructures Grant 9871994
PIs: **William Brownell**, **John Halter**, **Peter Saggau**
Baylor College of Medicine

Overview - Membranes in the outer hair cell lateral wall form a naturally occurring nanostructure which acts as a microelectromechanical (MEM) system[1]. Computational and optical techniques are being developed to predict, verify and refine the description of nanoscale behavior in the outer hair cell (OHC) lateral wall. The biological, engineering and physics expertise of an interdisciplinary three-member team investigate electro-osmotic fluid movement in the cell's 30 nm extracisternal space. Nature may have co-opted electro-osmosis as the most efficient means of achieving bulk flow in nanoscale structures. The technology developed in describing this phenomenon in the outer hair cell can be applied to man-made nanoscale devices.

Structure-function - The mammalian outer hair cell is found in the inner ear. It is a biological sensor and effector that carries out bidirectional transduction as part of its normal function. All hair cells are specialized mechanosensors and therefore perform mechanoelectrical transduction. The outer hair cell displays a unique electromotility. This electro-mechanical transduction enhances the sensitivity and frequency selectivity of mammalian hearing by generating mechanical energy at acoustic frequencies. OHC electromotility is voltage- (not current-) dependent, occurs in less than .03 ms and does not utilize cellular stores of energy. The motor is known to reside in the outer hair cell's lateral wall, which is a 100 nm thick, three-layer structure composed of two membranous structures with a cytoskeletal network sandwiched between them. The outer and inner structures are the plasma membrane and subsurface cisterna respectively. The 30 nm space between them is called the extracisternal space (ECiS). Coordinated computational and experimental approaches identify how the nanoscale organization of the lateral wall influences electro-osmosis in the ECiS.

*Nanoscale fluid movemen*t - Electro-osmosis is the movement of fluid by an electric field. It is an electrokinetic phenomenon associated with the electrical double layer at the interface between an ionic solution and a charged surface (in this case a membrane). The charged membrane attracts a cloud of counterions which move freely in response to an electrical field applied parallel to the surface of the membrane. Fluid is moved as the hydrated counterions respond to the applied electric field. Electro-osmosis is the only way to achieve fluid movement in biological nanostructures because the pressures required to match the movement hydrodynamically would rupture membranes. Electro-osmosis occurs in the ECiS because the membranes which border it are charged and electric gradients have been demonstrated in the ECiS.

Computational development - The computational model is an adaptation to a previously developed distributed-parameter model of the outer hair cell that allows for a detailed representation of the anatomy and utilizes the Nernst-Planck electrodiffusion relation to compute

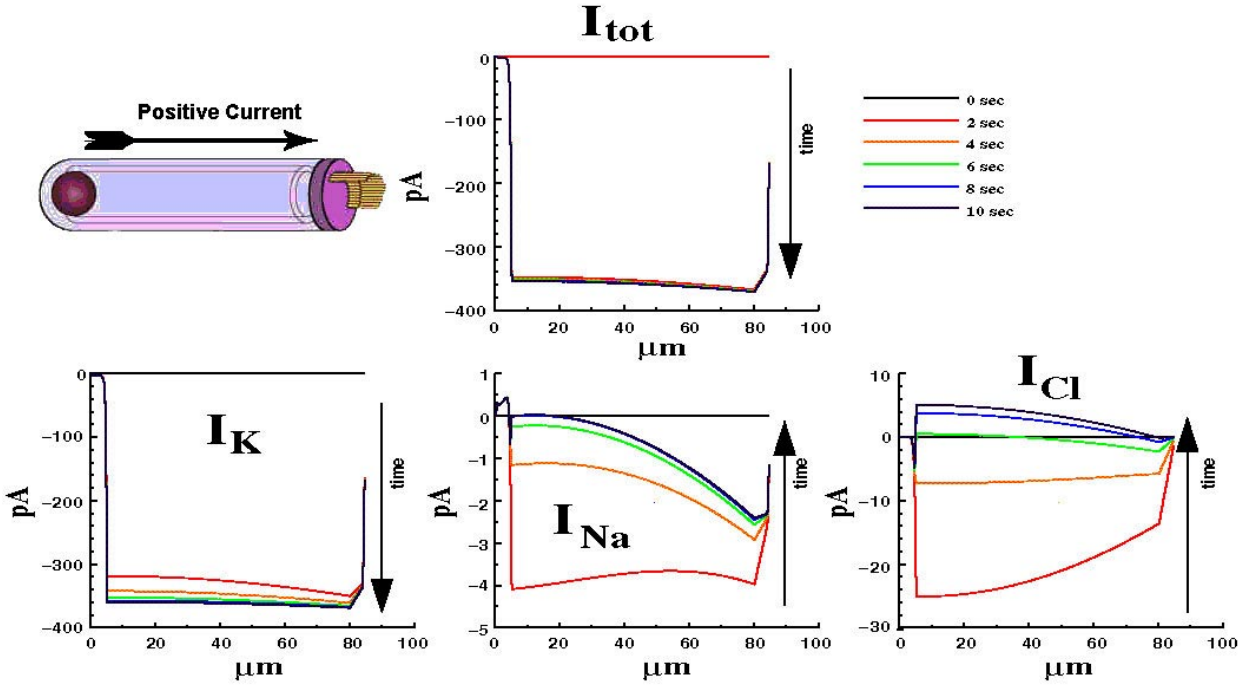
[1] For details see: http://www.bcm.tmc.edu/oto/research/cochlea/index.html
http://hurkle.deas.harvard.edu/nsf/brownell_files/v3_document.htm

the ion concentration dynamics. It takes the form of a coupled system of non-linear partial differential equations (Eq. 1) for which a numerical solution is determined using a finite-difference approximation (please see reference 3 for details).

$$(2r_-C_- + 2r_+C_+)\frac{f\Phi}{ft} = \sum_s z_s F(r_+^2 - r_-^2)\frac{f[s]}{ft} - 2r_+J_{o,r|_{r=r_+}} + 2r_-J_{o,r|_{r=r_-}} +$$

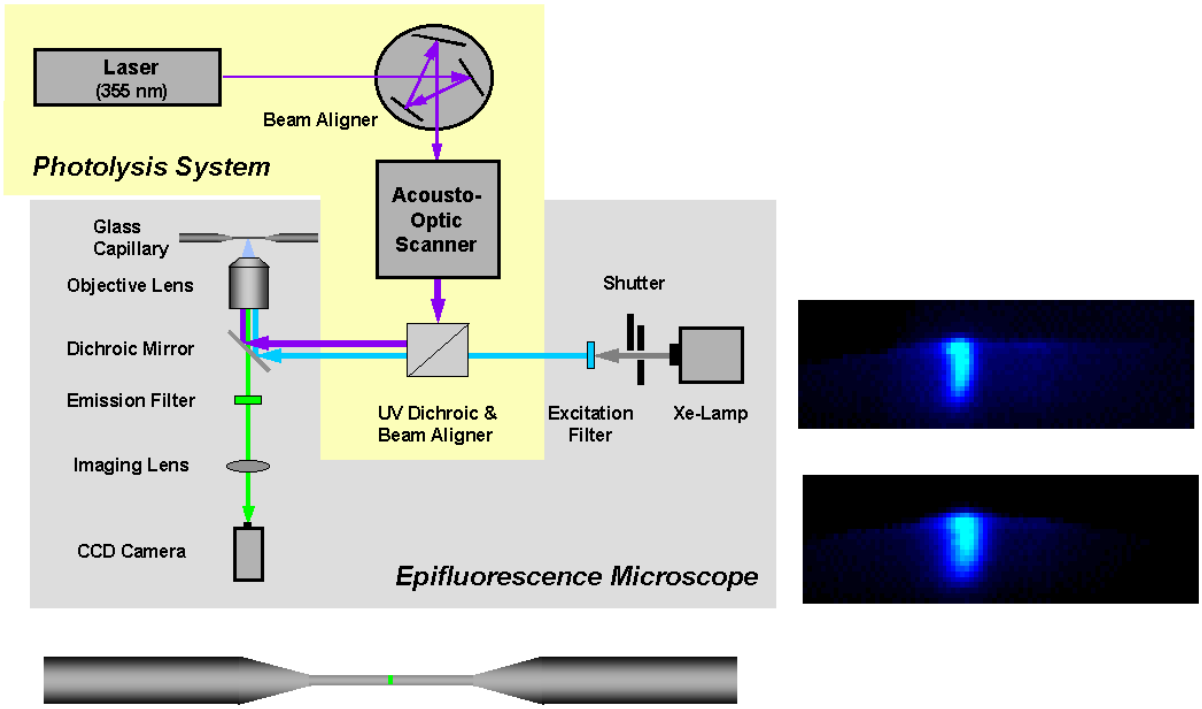
$$+ (r_+^2 - r_-^2)\frac{f}{fx}\left[\sigma_o \frac{f\Phi}{fx}\right] + 2r_-C_-\frac{f\Phi^-}{ft} + 2r_+C_+\frac{f\Phi^+}{ft}. \qquad \text{(Eq. 1)}$$

The model has already predicted potential gradients in the ECiS that have been optically verified using voltage sensitive dyes. The electro-osmotic velocity within the cytoplasm is computed using the linear relation to the potential gradient and compared to the experimental data. An example of the model prediction of ionic 'standing currents' that could be an electro-osmotic driving force for the turgor pressure in the outer hair cell are presented below.



Enhancing optical technology - Molecular approaches in combination with optical techniques have become powerful investigative tools in many biological systems where size is the limiting factor. Although the thickness of the ECiS of 30 nm is too small to be resolved with light microscopy, displacement of a bolus of fluorescent molecules within the ECiS along the axis of the OHC can be optically detected. For this purpose, the ECiS is loaded with a caged compound

that is initially non-fluorescent but becomes instantaneously fluorescent when exposed to UV light. The bolus of fluorescent molecules is induced by flashing with a focused laser beam on the OHC. Then, normal imaging techniques are applied to monitor the fate of the bolus. The results of these measurements are used to refine the computational model.



Combined photolysis and imaging

The scheme on the left illustrates the optical methods currently employed. The photolysis system, including a pulsed UV laser and acousto-optic beam deflection is used to generate a scanning UV pattern, i.e. a line, in the object plane of the epifluorescence microscope. A glass capillary filled with caged fluorescein (non-fluorescent) is located in the object plane. The kinetics of uncaged fluoresein (fluorescent) is imaged with an air-cooled CCD camera (Photometrics). The top image on the right was acquired just after the uncaging pulse train (t=40 msec) and the bottom image 50 msec later. Passive diffusion of the uncaged 100 uM DMNB-caged fluoresecein is observed. Fluoresecein is 10000 MW dextran conjugate in 10 mM phosphate buffer pH~8, Ex: 495+/-10 nm, Em: 530+/-10 nm, uncaged at 355 nm

Relevant publications:

1. Brownell, W.E., Spector, A.A., Raphael, R.M., Popel, A.S., Micro- and nanomechanics of the cochlear outer hair cell. Ann. Rev. of Biomedical Engineering **3** (2001) in press.
2. Halter JA, Kruger RP, Yium MJ, Brownell WE. The influence of the subsurface cisterna on the electrical properties of the outer hair cell. Neuroreport **8** (1997) 2517-2521.
3. Nygren A, Halter JA. A general approach to modeling conduction and concentration dynamics in excitable cells of concentric cylindrical geometry. J Theor Biol **199** (1999) 329-358

4. Oghalai, J.S., Zhao, H-B, Kutz, J.W., Brownell, W.E. Voltage and tension-dependent lipid mobility in the outer hair cell plasma membrane. Science **287** (2000) 658-661.
5. Qian J, Saggau P. Activity-dependent modulation of K+ currents at presynaptic terminals of mammalian central synapses. J Physiol **519** (1999) 427-437
6. Raphael, R.M., Popel, A.S., Brownell, W.E. A membrane bending model of outer hair cell electromotility. Biophysical Journal **78** (2000) 2844-2862.