Modeling Diffusion in the Lens Capsule

My PhD research centers on the study of the lens capsule, a contiguous basement membrane surrounding the lens of the eye. This membrane is composed of extracellular molecules that self assemble into a heteroporous matrix selectively allowing molecules to diffuse through based on size, shape and charge. Part of my research involves characterizing the mechanisms responsible for this selectivity.

Using a confocal microscope and a technique called <u>F</u>luorescence <u>R</u>ecovery <u>A</u>fter <u>P</u>hotobleaching (FRAP) we are able to quantifiably measure the X-Y dimensional diffusion caused by Brownian movement of fluorescently labeled tracer molecules through a matrix. In brief, an intact lens is placed in a chamber containing tissue media and the fluorescent tracer molecule, which equilibrates into the lens capsule matrix. The confocal microscope is focused into the matrix at a depth of approximately 5μm and a primary and secondary region of interest (ROI), both with 5μm radius, are defined within the field-of-view. A laser scans the sample at a rate of 20 frames per second exciting the tracer molecules which emits a signal in the form of photons that are focused to a detector and subsequently converted into a fluorescence intensity value. After a baseline intensity is measured for several frames in both ROIs the laser is briefly focused at a higher power on the primary ROI for 200ms, thus irreversibly bleaching the tracer molecules located in this region (Figure 1). The secondary ROI is not bleached and is used to normalize the collected data for fluctuations in laser power and any incidental bleaching caused by scanning during image collection. Recovery of fluorescent intensity in the primary ROI represents the passive exchange of bleached and unbleached tracer molecules.

An example of the raw intensity data is plotted (Figure 2) using an analysis tool provided by the manufacturer of the confocal microscope, Carl Zeiss, Göttingen, Germany. The tool can also normalize the data and fit a curve based either on a single (Figure 3) or double (Figure 4) exponential equation. The equations are used to determine the half recovery time of the fluorescence in the bleached ROI, which is used to calculate a diffusion coefficient describing the mobile fraction of the tracer molecule in the matrix. The diffusion coefficient is calculated with the equation $D=\omega^2/(4*\tau_{1/2})*\gamma$ (Axelrod, 1976), where ω is the radius of the ROI, $\tau_{1/2}$ is the half recovery time, and γ is the correction factor, 0.88, for diffusion independent of the amount of bleaching. In our case however, neither the resulting single nor double curves fit the resulting data accurately.

To more accurately quantify our results we are have the following needs:

1. A tool (possibly written with Matlab) capable of using several equations attempting to reliably fit a curve to our raw data.
2. A method to determine which equation best fits the curve to the raw data.
3. Chi-square value as output to determine goodness of fit.
4. Possible assistance with empirical and theoretical modeling of diffusion within the lens capsule.

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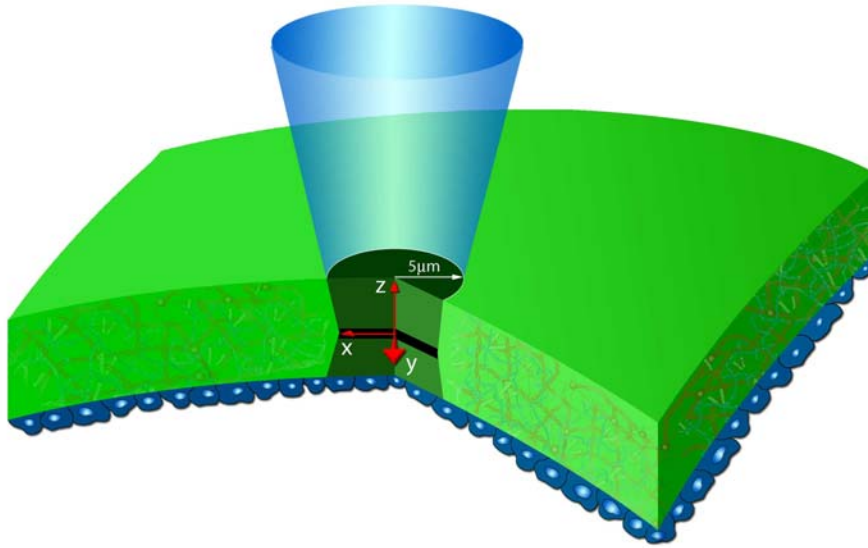# Modeling Diffusion in the Lens Capsule

Figure 1: FRAP performed in the anterior lens capsule on a ROI at a depth of 5μm.

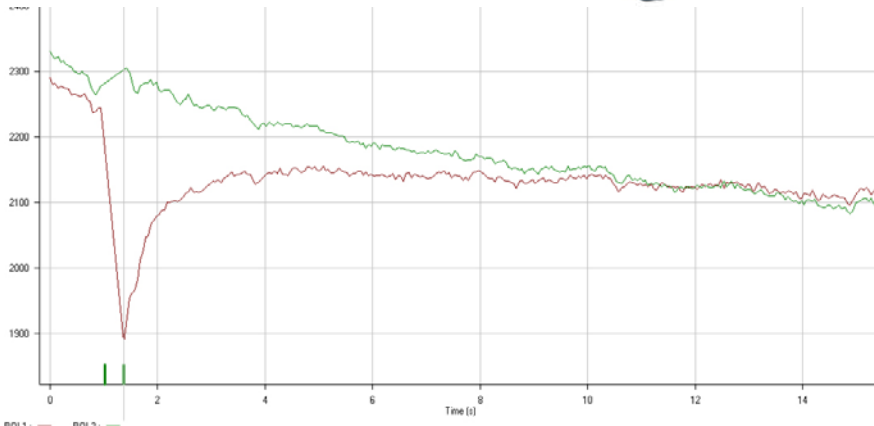Figure 2: Raw intensity data for primary and secondary ROIs plotted over time
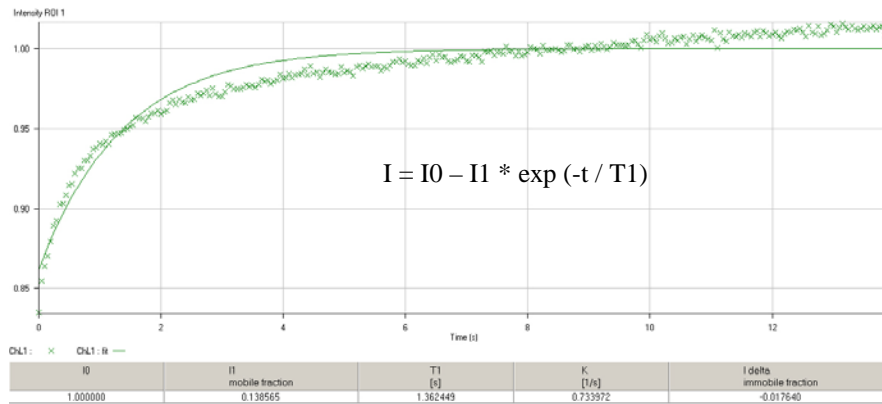

$$I = I0 - I1 * \exp(-t / T1)$$

Figure 3: Intensity data normalized and curve fitted using the double exponential equation

I0	I1 mobile fraction	T1 [s]	K [1/s]	I delta immobile fraction
1.000000	0.138565	1.362449	0.733972	-0.017640

$$I = I0 - I1 * \exp(-t / T1) - I2 * \exp(-t / T2)$$
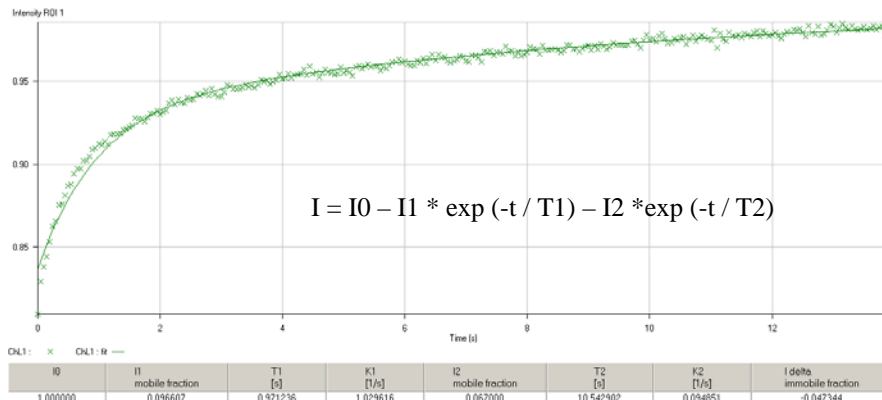
Figure 4: Intensity data normalized and curve fitted using the double exponential equation

I0	I1 mobile fraction	T1 [s]	K1 [1/s]	I2 mobile fraction	T2 [s]	K2 [1/s]	I delta immobile fraction
1.000000	0.096607	0.971236	1.029616	0.067000	10.542902	0.094851	-0.047344

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Additional Information:

The European Advanced Light Microscopy Network (EAMNET) is an EU funded network of researchers assisting scientists in microscopy methods. Their website, (http://www.embl.de/eamnet/html/teaching_modules.html), provides information for fitting a simple exponential equation to the data.

$$I(t) = A \cdot (1 - e^{\tau \cdot t})$$

After determining τ by fitting the above equation to the recovery curve the corresponding halftime of the recovery can be calculated with the following formula:

$$t_{half} = \frac{\ln 0.5}{-\tau}$$
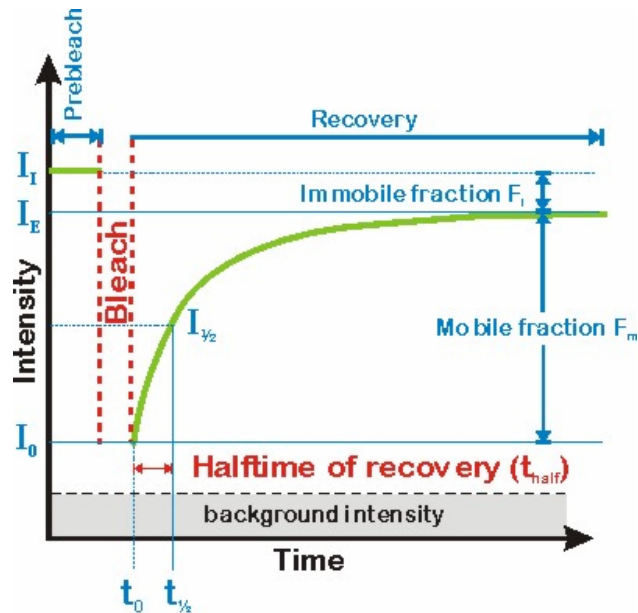
I_I: initial intensity
I_0: intensity at timepoint t_0 (first postbleach intensity)
$I_{1/2}$: half recovered intensity ($I_{1/2} = (I_E - I_0) / 2$)
I_E: endvalue of the recovered intensity
t_{half}: Halftime of recovery corresponding to $I_{1/2}$ ($t_{1/2} - t_0$)
Mobile fraction $F_m = (I_E - I_0) / (I_I - I_0)$
Immobile fraction $F_i = 1 - F_m$