Supplementary Materials
for
"Optoporation and genetic manipulation of cells using femtosecond laser pulses"
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Model of dye efflux from an optoporated cell

To model the efflux of cytoplasmic dye from a cell after optoporation, we must consider both the contributions from dye diffusion and the net fluid flow due to osmotic gradients. However, since our choice of extracellular media is isotonic with the cellular cytosol, we anticipate *a priori* that osmotic gradients are negligible and are subsequently ignored. This assumption is in good agreement with our findings that cell volume was observed to change only at the site of membrane irradiation, with no bulk swelling or shrinking present. We are therefore justified in considering only diffusive terms in our model.

We used Fick's first law of diffusion, where the diffusion flux, **J**, is given by:

$$\mathbf{J} = -D\nabla\phi \qquad \text{(A1)}$$

where D is the diffusion constant and $\nabla\phi$ is the dye concentration gradient. We approximated the gradient as being one-dimensional across the cell membrane, and we took the concentration of dye, ϕ, inside the cell to be uniform at all times and positions within the cell. We further approximated the derivative as a finite difference over the membrane thickness, e:

$$\frac{d\phi}{dx} \approx \frac{\Delta\phi}{\Delta x} = \frac{\phi}{e} \qquad \text{(A2)}$$

The change in the number of dye molecules, N, inside the cell was equal to the flux through a pore of radius, r:

$$\frac{dN}{dt} = \mathbf{J}\pi r^2 \qquad \text{(A3)}$$

Dividing by the cell volume, V_{cell}, and using Equation A1 and A2, we have:

$$\frac{d\phi}{dt} = -\frac{D\pi r^2}{eV_{cell}}\phi \qquad \text{(A4)}$$

Assuming a constant radius for the pore, this equation is integrated to give:

$$\phi(t) = \phi_0 e^{(-t/\tau)} \quad \text{(A5)}$$

where ϕ_0 is the initial concentration of dye within the cell and τ is the diffusion time constant for the pore and is given by, $\tau = eV_{cell}/D\pi r^2$. From the Stokes-Einstein relation,

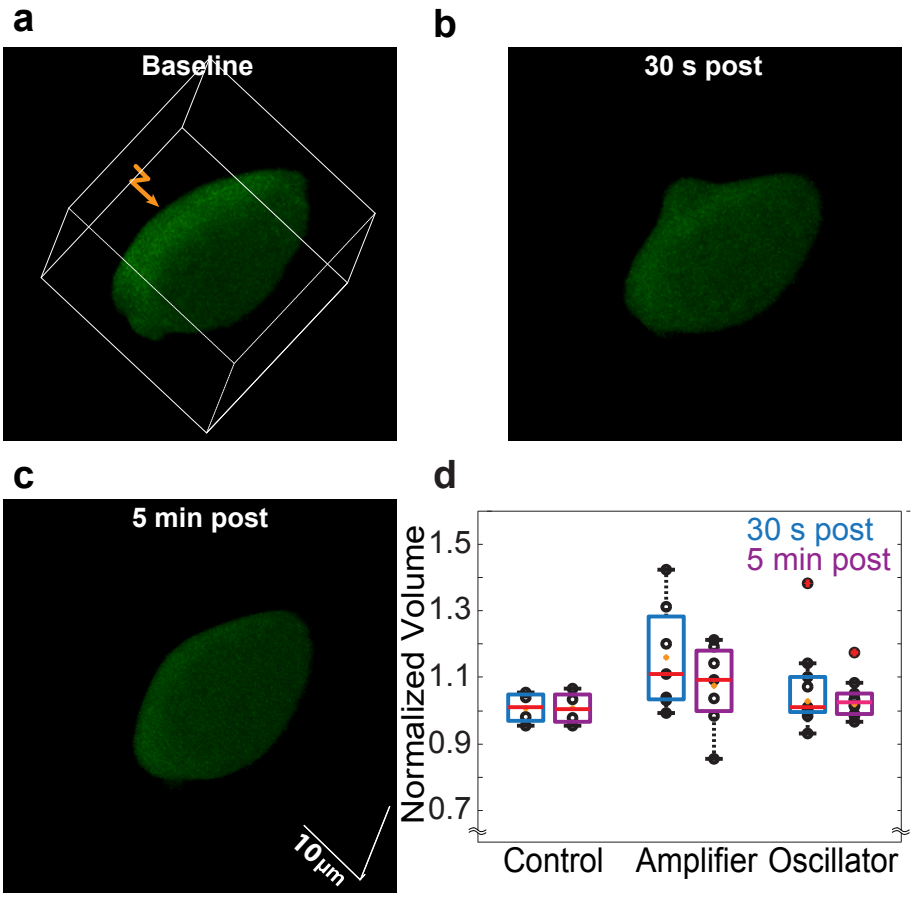
$$D = \frac{k_B T}{6\pi\eta_0 R_g} \quad \text{(A6)}$$

where η_0, is viscosity, R_g is the radius of gyration of the molecular, T is temperature, and k_B is the Boltzmann constant, we arrive at,

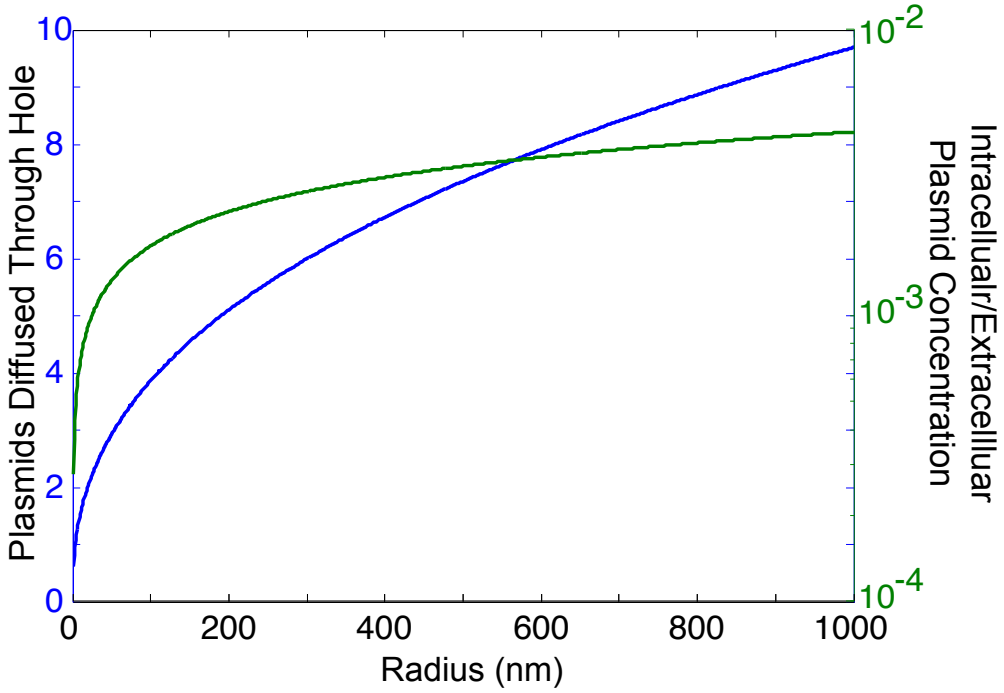
$$\tau = \frac{6\eta_0 R_g e V_{cell}}{k_B T r^2} \quad \text{(A7)}$$

We fit the initial decrease in cellular fluorescence (which is proportional to the concentration) to Equation A5. Using R_g = 0.6 nm for calcein dye, η_0 = 1 cP (nominal value for water), e = 10 nm (nominal membrane thickness), $k_B = 1.38 \times 10^{23}$ J·K^{-1}, T = 298 K, and $V_{cell} = 1.2 \times 10^3$ μm^3 (based on volume measurements from image stacks), we used Equation A7 to determine the initial hole radius, r.

We note that the choice of a meaningful viscosity is complicated by the fact that the viscosity prior to pore formation is a Heaviside step function, resulting in a discontinuity between the intracellular and extracellular media. However, we note that the viscosity of the extracellular media differs only negligibly from that of water, making water an appropriate choice for this side of the membrane. We also note that since we anticipate the intracellular environment to be more viscous than the extracellular environment, any significant mismatch between these viscosities would result in the formation of an intracellular dye gradient, since dye would be able to diffuse much faster out of the cell than within the cell. Since this intracellular gradient was not observed, the two viscosities are likely to be similar, and we are justified in using the viscosity of water as a reasonable parameter in our model. As a final argument in favor of this approach, we note that calcein is a small molecule, and therefore not likely to be encumbered by the intracellular environment in the same way that we would anticipate for large macromolecules.



Supplementary Figure 1: Cell shape and volume changes after optoporation. Cells expressing GFP were irradiated using optimal energy ranges and three-dimensional 2PEF stacks were taken at baseline (a), 30 s (b), and 5 min (c) after irradiation to determine changes in cell volume that resulted from optoporation. Cells exhibited a protrusion (b) at the target location (a, *arrow*) on the membrane for both the amplifier and oscillator laser pulses shortly after membrane irradiation, which then retracted back over time (c). On average, the protrusion produced by the amplifier was larger than that produced by the oscillator, resulting in a larger volume increase after irradiation (d).



Supplementary Figure 2: Calculated diffusive entry of DNA plasmids through laser-created pores. From our measurements, we have excellent estimates of the radius of the pore, r, diffusion time, τ, and the duration the pore is typically open, t_{hole}. We used these data and Equations 1 and 4 to calculate the intracellular concentration of 4.7 kb-DNA plasmid in terms of the fraction of extracellular plasmid concentration (*green*; right-hand axis) assuming only diffusive entry. The blue curve depicts the number of plasmids that enter the cell (left-hand axis), for the extracellular plasmid concentration used in this work (10 μg/ml). Pore radii associated with optimal transfection efficiency were around 100-200 nm, predicting the entry of only 2-3 plasmid copies by diffusion. Because of intracellular barriers that degrade or block cytoplasmic DNA from reaching the nucleus and expressing, it is unclear that just a few plasmids entering the cell via diffusion is sufficient to drive transgene expression.