BE/APh161: Physical Biology of the Cell
Homework 4
Due Date: Wednesday, February 7, 2024

"We must travel in the direction of our fear." - John Berryman

1. Estimating the diffusion constant.

In this problem, we are going to use the observed trajectories of diffusing GFP molecules to estimate the diffusion coefficient. You already did much of this in class.

(a) Conventional microscopy to observe individual fluorescent proteins moving freely in cytoplasm won't work. In this part of the problem, we are going to work out why. During a traditional experiment, the microscope shutter is open during some time interval of order 10s to 100s of milliseconds. By assuming a diffusion constant of 10 μm^2/s, work out how far the fluorescent protein will move during the time that the shutter is open and compare that distance to the size of the cell itself and comment on how this limits our ability to measure the diffusion constant. Perform the estimate a second time, this time using the 0.3 ms exposure time shown in Figure 1(A).

(b) Using the trajectories shown in Figure 2 and our simple rule of thumb that $t_{diffusion} = L^2/D$ to estimate the diffusion constant for GFP. Explain your reasoning carefully.

NOTE: to do this problem, the vignettes "Scaling of Diffusion Time," "Diffusion Time by the Numbers" and "Diffusion: Coin Flips" will be helpful.

2. Fluorescence Recovery After Photobleaching by Pencil and Paper and by Computation.

This part of the course and this homework problem are all part of the general theme that I am really trying to push you hard to think about which is what I have called $stuff(t)$, how quantities of interest to us vary in time. I have argued that the $F = ma$ paradigm of classical mechanics was extremely powerful in a broad array of circumstances. However, problems such as heat flow and Brownian motion both seemed to defy understanding in terms of ideas

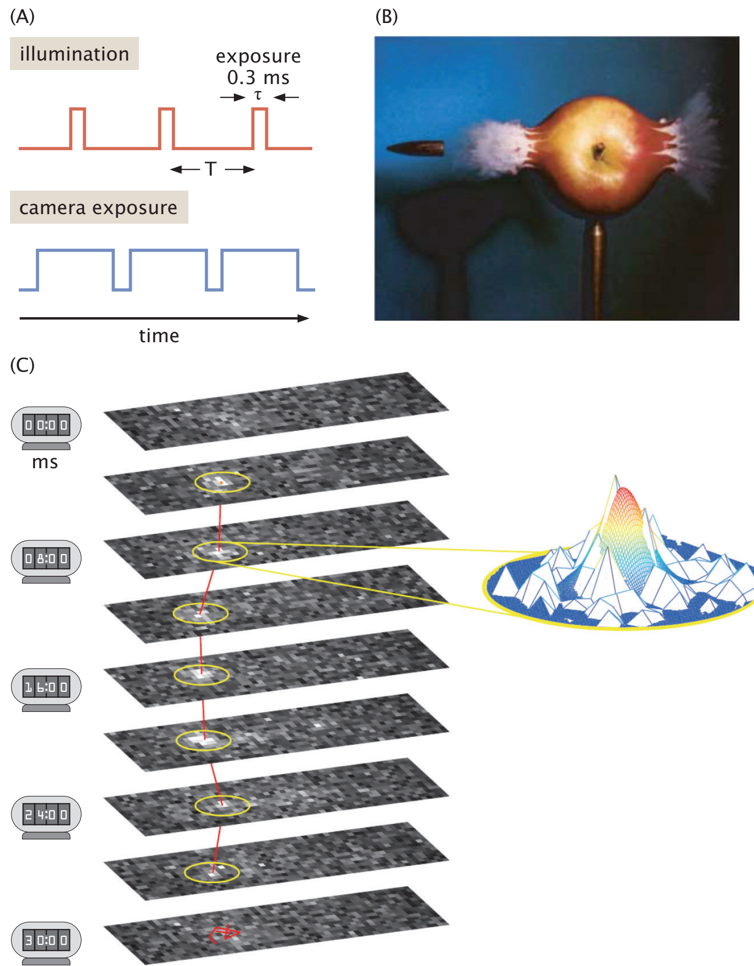

Figure 1: Stroboscopic illumination to capture fast protein dynamics. (A) By only illuminating a sample for a small fraction of the exposure time of a camera, it is possible to capture phenomena that would otherwise be blurred out. (B) A classic photo from MIT legend Harold Edgerton who pioneered stroboscopic photography for science and fun.Capturing the piercing of a bullet through an apple using stroboscopic illumination. (C) Measuring the position of an individual GFP molecule inside *E. coli*. (A, adapted from Harold and Esther Edgerton Foundation, 2006, courtesy of Palm Press, Inc.)

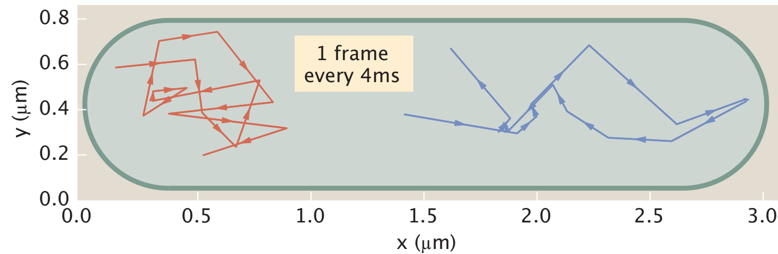

Figure 2: Time series showing positions of diffusing GFP molecules at different times. The red and blue traces correspond to different molecules. The lines are a guide to the eye. Adapted from BP English *et al.* Proc. Nat. Acad. Sci., 108:E365-E373, 2011.

from classical mechanics, resulting in new categories of dynamics such as the diffusion equation and the Langevin equation. In this problem, you will explore the diffusion equation (and in next week's continued homeworks). After we finish this class of approaches to dynamics, we will turn to a uniquely biological example of purposeful dynamics. NOTE: relevant vignettes to watch are those about diffusion.

In this problem, we are going to consider a "one-dimensional" cell. Of course, this sounds contrived, but really we are saying that the fluorescence only depends upon a single coordinate. We will consider the long axis of bacterial cells as the region to be photobleached. So, we will think of a region of length $2L = 4\ \mu m$ that initially has uniform fluorescence. We then photobleach (i.e. destroy the fluorescence) between $-a$ and a, with $a = 0.5\ \mu m$. Consider the concentration in the unbleached region to be $c_0 = 1\ \mu M$, and let the diffusing molecules have a diffusion coefficient of 10 $\mu m^2/s$. For each section below, we will use a different approach to working out the dynamics of the recovery process.

(a) FRAP by coin flips. In this part of the problem, you are going to write a simulation code that takes random walkers that start either in the region $-L$ to $-a$ or a to L and flip coins and let them jiggle around. For each such walker, the only rule you will need is that if on a given flip they try to leave the region from $-L$ to L, you will reflect them off the walls. The goal is to do 100s of such simulations and then plot the concentration as a function

of position for different time points. After one time step, almost all of the walkers will be in the unbleached regions. But over time, more and more molecules will have ventured into the photobleached region. Your goal is to get the full profile of the independently diffusing molecules. Make plots of the concentration as a function of the number of steps. If the lattice parameter you use is $d = 40\ nm$, this will mean that you have 100 such lattice points. You can reconcile your simulation time step, the lattice parameter and the diffusion coefficient through the relation $D = d^2/\tau$, where τ is the time step.

(b) FRAP by math. For this part of the problem, I am going to explicitly walk you through the steps and your job is to really carefully demonstrate that everything works and holds together, showing all of the steps. To compute the recovery curves, we first solve the diffusion equation

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{1}$$

for the concentration of fluorescent molecules $c(x,t)$, with the initial concentration after photobleaching given by

$$c(x,0) = \begin{cases} c_0 & \text{for } -L \text{ to } -a \\ 0 & \text{for } -a \text{ to } a \\ c_0 & \text{for } a \text{ to } L. \end{cases} \tag{2}$$

We also impose the boundary condition $\partial c/\partial x = 0$ for $x = -L$ and $x = L$, which says that the flux of fluorescent molecules vanishes at the boundaries of the one-dimensional cell (no material flows in or out). This mimics the real-life situation with fluorescent proteins confined to the volume of the cell, to the cell membrane, or to some other subcellular structure.

As your first step, use the method of separation of variables where you assume a solution of the form

$$c(x,t) = X(x)T(t) \tag{3}$$

and plug this into the diffusion equation. Show that this leads to two ordinary differential equations, one for $X(x)$ and one for $T(t)$. Show that the solutions to the $X(x)$ equation are of the form

$$X(x) = cos\left(\frac{n\pi x}{L}\right). \tag{4}$$

Similarly, solve the $T(t)$ equation to show that the solutions are of the form

$$T(t) = \mathrm{e}^{-(Dn^2\pi^2/L^2)t}. \tag{5}$$

Now we need to write the full solution by summing over the different solutions labeled by the integer n.

Another way of thinking about the problem is to solve the diffusion equation with the prescribed initial and boundary conditions by expanding the concentration profile $c(x,t)$ in terms of cosine functions using "Fourier series,"

$$c(x,t) = A_0(t) + \sum_{n=1}^{\infty} A_n(t)\cos\left(\frac{x}{L}n\pi\right). \tag{6}$$

This expansion guarantees that the boundary conditions are met, namely each of the functions $A_n(t)\cos(n\pi x/L)$ has vanishing first derivatives with respect to x at $x = \pm L$. Furthermore, since the initial concentration profile takes the same values for positive and negative x, it is readily expanded in cosine functions since the concentration profile is symmetric about the origin. The solution of the diffusion equation now boils down to finding the functions $A_n(t)$ such that both the diffusion equation and the initial condition are satisfied.

To proceed, we substitute the series expansion of $c(x,t)$ into the diffusion equation. This yields

$$\frac{\partial A_0}{\partial t} + \sum_{n=1}^{\infty} \frac{\partial A_n(t)}{\partial t}\cos\left(\frac{x}{L}n\pi\right) = D\sum_{n=1}^{\infty}\left[-A_n(t)\frac{n^2\pi^2}{L^2}\right]\cos\left(\frac{x}{L}n\pi\right), \tag{7}$$

which, due to the orthogonality property of the cosine functions for different n (see Equation 10 below), turns into a set of independent differential equations,

$$\begin{array}{l} \frac{\partial A_0}{\partial t} = 0 \\ \frac{\partial A_n}{\partial t} = -\frac{Dn^2\pi^2}{L^2}A_n(t) \quad (n \geq 1) \end{array} \tag{8}$$

Show that the solution to each one of these (infinite in number) equations is an exponential function

$$A_n(t) = A_n(0)\mathrm{e}^{-(Dn^2\pi^2/L^2)t}, \tag{9}$$

which when substituted into Equation 5 gives

$$c(x,t) = A_0(0) + \sum_{n=1}^{\infty} A_n(0)\mathrm{e}^{-(Dn^2\pi^2/L^2)t}\cos\left(\frac{x}{L}n\pi\right). \tag{10}$$

Make sure you demonstrate this. The final piece of the puzzle is the determination of the constants $A_n(0)$.

To compute the initial amplitudes of the cosine functions, we resort to the orthogonality property of these functions, namely,

$$\int_{-L}^{L} \cos\left(\frac{x}{L} n\pi\right) \cos\left(\frac{x}{L} m\pi\right) \mathrm{d}x = L\delta_{n,m}. \tag{11}$$

In particular, multiply both sides of Equation 9 by $\cos(m\pi x/L)$ for different values of m, and then integrate over x to derive the equations

$$\begin{array}{c} A_0(0) = \frac{1}{2L} \int_{-L}^{L} c(x,0) \mathrm{d}x \\ A_n(0) = \frac{1}{L} \int_{-L}^{L} c(x,0) \cos\left(\frac{x}{L} n\pi\right) \mathrm{d}x \quad (n \geq 1) \end{array} \tag{12}$$

for the initial amplitudes. Substitute the initial concentration profile, $c(x,0)$, into these equations, and perform the integrals, to show that

$$\begin{array}{c} A_0(0) = c_0 \frac{L-a}{L} \\ A_n(0) = -2c_0 \frac{\sin(n\pi a/L)}{n\pi} \quad (n \geq 1) \end{array} \tag{13}$$

Put these results back into the derived formula for $c(x,t)$, Equation 9 and show that the solution for the concentration profile as a function of time is given by

$$c(x,t) = c_0 \left[1 - \frac{a}{L} - \sum_{n=1}^{\infty} \frac{2\sin(n\pi a/L)}{n\pi} \mathrm{e}^{-(Dn^2\pi^2/L^2)t} \cos\left(\frac{x}{L} n\pi\right)\right]. \tag{14}$$

Make a plot of your resulting concentration profile as a function of time for several different times. Also, make sure you illustrate how your result depends upon how many terms you keep in the series. Obviously, you can't do an infinite number of terms. Note that at long times, such that t is much greater than L^2/D, which is the diffusion time for a box of length L, the concentration profile tends to a constant value equal to $c_\infty = c_0(1 - a/L)$. This can be understood in a very simple way. Namely, at long times, we expect diffusion to make the concentration profile uniform over the $2L$ interval. Show that the fact that the number of fluorescent molecules does not change in time leads to the equation

$$c_\infty(2L) = c_0[2(L-a)], \tag{15}$$

which gives the computed value of the concentration at long times.

(c) FRAP by chemical master equation. In class I wrote down the evolution equation

$$p(x,t+\Delta t) = p(x,t)+(k\Delta t)p(x-a,t)+(k\Delta t)p(x+a,t)-(k\Delta t)p(x,t)-(k\Delta t)p(x,t). \tag{16}$$

I argued that the equation as written is the basis of a very nice way to numerically investigate diffusion problems. Here you will consider a 4 μm long cell that is discretized into 100 boxes. As you did in the previous two parts of the problem, you are going to integrate the chemical master equation by starting with the initially bleached profile and then plotting the concentration as a function of time. Make sure you explain all of your steps.