

I realize that some of you have little or no experience with partial differential equations (PDEs). However we think it is important to understand the nature of the diffusion process, especially as it relates to biology, to this end I would like to go through the theory behind the experiment you are about to do. This reading is certainly of the crash-course variety, so feel free to ask Rob, Hernan, or me any questions.

Solution of the 2D Diffusion Equation:

The 2D diffusion equation allows us to talk about the statistical movements of randomly moving particles in two dimensions. By random, we mean that we cannot correlate the movement at one moment to movement at the next, or in other words there is no deterministic/predictive power over the exact motion of the particle. The movement of each individual particle moving in a Brownian (diffuse) way does *not* follow the diffusion equation. However, many identical particles each obeying the same boundary and initial conditions share some statistical properties dealing with their spatial and temporal evolution. It is those statistical properties that the diffusion equation captures. The function  $P(x,y,t)$  gives the probability of finding a perfectly average particle in the small vicinity of the point  $(x,y)$  at time  $t$  (we call this a *probability distribution*). Such properties are for instance the average position of a statistical particle as a function of time. The evolution of some systems does follow the diffusion equation outright: when you put a drop of dye in a beaker, there are millions of dye molecules each exhibiting Brownian motion, but as a group they exhibit the smooth, well-behaved statistical features of the diffusion equation.

Consider the two-dimensional diffusion equation in Cartesian coordinates:

$$\nabla^2 P - \frac{1}{D} \frac{\partial P}{\partial t} = 0 \rightarrow \frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial y^2} - \frac{1}{D} \frac{\partial P}{\partial t} = 0$$

The diffusion equation can be derived from the probabilistic nature of Brownian motion described as random walks (speak with me if you really want to see the derivation). The constant  $D$  is the diffusion coefficient whose nature we will explore in a moment, but for now we are solving a math problem. Notice we have no powers or complicated functions of any derivative of the function  $P$ . Since the derivatives of  $P$  only appear to first power, we call this a *linear PDE*. Also notice that the equation does not mix  $x$ 's,  $y$ 's, or time within a single term, that is there are no multiplicative, divisional, or functional terms in the equation between the independent variables, we call this feature *separability* of the PDE. These two properties, linearity and separability, make the problem vastly more tractable than many other PDE's encountered in physics. I am going to attempt to walk you through a challenging but very powerful method for solving PDE's using the Fourier Transform (FT). Many of you may know that the FT is used in signal analysis and manipulation, but it was first used by Fourier to solve *this* problem. Consider the following integral relations that define the 2D FT in Cartesian coordinates. We will call the function  $\hat{P}$  the FT of our original function  $P$ :

$$\hat{P}(k_x, k_y, t) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} P(x, y, t) dx dy$$

$$P(x, y, t) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{i2\pi(k_x \cdot x + k_y \cdot y)} \hat{P}(k_x, k_y, t) dk_x dk_y$$

Notice the symmetry in going forward and backward in the transform. This is because switching between the normal form of the problem and what we call *Fourier Space*, where the problem exists after the FT, are physically identical. We will require one more fact familiar from calculus, namely integration by parts:

$$\int_a^b u dv = uv \Big|_a^b - \int_a^b v du$$

Using these facts, let's examine the spatial derivatives of the diffusion equation, where we consider the second derivative to be the function of interest. We can integrate these second derivatives by parts, identifying  $u$  and  $v$  as:

$$u = e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \quad \text{and} \quad v = \frac{\partial P}{\partial x}$$

$$du = \frac{\partial}{\partial x} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} dx \quad \text{and} \quad dv = \frac{\partial^2 P}{\partial x^2} dx$$

then integration by parts tells us:

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \frac{\partial^2 P}{\partial x^2} dx dy = e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \frac{\partial P}{\partial x} \Big|_{-\infty(x,y)}^{\infty} - \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{\partial}{\partial x} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \cdot \frac{\partial P}{\partial x} dx dy$$

$$= e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \frac{\partial P}{\partial x} \Big|_{-\infty(x,y)}^{\infty} + 2\pi i k_x \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \cdot \frac{\partial P}{\partial x} dx dy$$

Notice in the first line we effectively 'transferred' one derivative off  $P$  and put it on the exponential of the FT, but since the exponential is an explicit function we can just perform the derivative, giving us the constant on the right most integral of the second line. It should be physically intuitive that at an infinite distance from where we originally introduced our statistically perfect particle there should be zero chance for all time of it appearing. This leads us to the *boundary condition* that the function  $P$  and all of its derivatives are zero at  $|x| = |y| = \infty$ , eliminating the left term of the second line, and allowing us to write:

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \frac{\partial^2 P}{\partial x^2} dx dy = 2\pi i k_x \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \cdot \frac{\partial P}{\partial x} dx dy$$

Looking at the right hand side, it looks like we are taking the FT of a first derivative of  $P$ . We pursue this integral in the same manner:

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \cdot \frac{\partial P}{\partial x} dx dy = P \cdot e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \Big|_{-\infty(x,y)}^{\infty} + 2\pi i k_x \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P \cdot e^{-i2\pi(k_x \cdot x + k_y \cdot y)} dx dy$$

Recall, according to our boundary condition,  $P = 0$  at  $|x| = |y| = \infty$ , and we have:

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \cdot \frac{\partial P}{\partial x} dx dy = 2\pi i k_x \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P \cdot e^{-i2\pi(k_x \cdot x + k_y \cdot y)} dx dy$$

Finally we can say that:

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \frac{\partial^2 P}{\partial x^2} dx dy = (2\pi i k_x)^2 \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P \cdot e^{-i2\pi(k_x \cdot x + k_y \cdot y)} dx dy$$

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-i2\pi(k_x \cdot x + k_y \cdot y)} \frac{\partial^2 P}{\partial x^2} dx dy = (2\pi i k_x)^2 \hat{P}$$

In general, if you continued the above procedure, you would find that:

$$\frac{\partial^n}{\partial x^n} P = (2\pi i k_x)^n \hat{P}$$

Now keep in mind, we took the spatial FT (i.e. dealing with  $x$  and  $y$ ), so the derivative in time does not change under a FT. This means we can write the 2D diffusion equation after our FT as:

$$(2\pi)^2 \hat{P} \cdot (k_x^2 + k_y^2) + \frac{1}{D} \frac{\partial \hat{P}}{\partial t} = 0 \rightarrow \frac{\partial \hat{P}}{\partial t} + D(2\pi)^2 (k_x^2 + k_y^2) \cdot \hat{P} = 0$$

Amazing! We've completely eliminated our spatial dependence; this remaining equation is a simple first order ODE in time, with the solution by separation of variables:

$$\frac{\partial \hat{P}}{\partial t} + D(2\pi)^2 (k_x^2 + k_y^2) \cdot \hat{P} = 0 \rightarrow \hat{P} = A e^{-D(2\pi)^2 (k_x^2 + k_y^2) t}$$

The constant  $A$  is called the normalization; in a moment we will use it to make a statement about conservation. Okay, so we have this weird  $\hat{P}$  thing, what does that tell us? Recall we also have an inverse FT, it was the second integral equation under the definition of the FT. Then our original function,  $P$ , is given by:

$$P = A \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{-D(2\pi)^2 (k_x^2 + k_y^2) t} \cdot e^{i2\pi(k_x \cdot x + k_y \cdot y)} dk_x dk_y$$

One of the features of the original diffusion equation comes into play here; the entire integral is *separable* by spatial variable:

$$P = A \left( \int_{-\infty}^{\infty} e^{i2\pi k_x \cdot x - D(2\pi k_x)^2 t} dk_x \right) \cdot \left( \int_{-\infty}^{\infty} e^{i2\pi k_y \cdot y - D(2\pi k_y)^2 t} dk_y \right)$$

In general, the number of these separable integrals is directly related to the dimensionality of the diffusion equation; in three dimensions there would be three such integrals. This integral is not trivial...it requires completing the square of the exponent, rescaling the integration variable, and changing to polar coordinates. I will not cover it here, but it is just an integral, so Maple<sup>®</sup> it! Using Maple<sup>®</sup> v8.0 I got:

$$P(x, y, t) = A \frac{e^{\left( \frac{-(x^2 + y^2)}{4D \cdot t} \right)}}{4\pi D \cdot t}$$

We're almost there! What about this normalization constant  $A$ ? If we are calling this function  $P$  a probability distribution, then it makes sense that the particle must exist somewhere in the  $x$ - $y$  plane from  $x$  and  $y$  to plus and

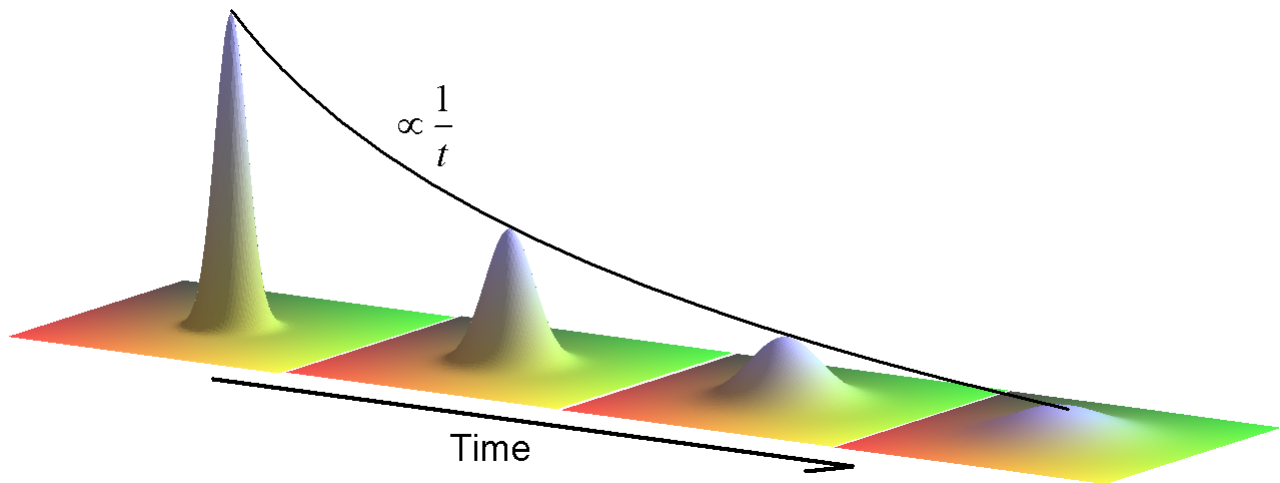
minus infinity. So the constant  $A$  essentially tells us how many non-interacting particles we have in the system (we are considering just one representative particle). We impose this condition by making the total probability sum to one:

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P(x, y, t) dx dy = 1$$

In this case, solving the above equation for  $A$ , tells us that  $A=1$ . Finally notice that  $x^2+y^2$  in the exponent is exactly  $r^2$  in polar coordinates, which tells us this diffusion process is isotropic (independent of direction) on the x-y plane (i.e. only the radial distance from the origin matters). Finally we have a solution to the 2D isotropic diffusion equation:

$$P(r, t) = \frac{e^{\left(\frac{-r^2}{4D \cdot t}\right)}}{4\pi D \cdot t}$$

This is called a normalized Gaussian function. The first plot below shows diffusion at early times; notice how high the peak is, meaning the particles are localized around the area where they were first introduced. The remaining plots demonstrate how the particles spread out from their original entry point with time:



Now that we have some understanding of the diffusion equation and its mathematical results, we will focus our attention on the meaning of the diffusion coefficient and how it is connected to other features of the system. From unit analysis of the diffusion equation, you can deduce that  $D$  has units of length squared over time, and it is indeed related to rate of increase of the mean squared distance of the particles with time, or in other words, it is related to how fast the function spreads out.

In your experiment, you will track the movements of fluorescent beads of different sizes, and by measuring how they exhibit Brownian motion, you will calculate the diffusion coefficient and viscosity of the medium. Let's see if we can physically reason out the form of the diffusion coefficient. It makes sense that if the particles are moving faster in their random motion, they diffuse quicker. Of course temperature is a measure of the average kinetic energy, thus the diffusion coefficient should increase with temperature. If the medium is very viscous, we would expect it to be difficult for the particles to move, and hence the diffusion coefficient would decrease. Finally, it is intuitively obvious that smaller particles should diffuse faster than bigger ones. For a thermal unit of energy ( $k_B T$ ), the corresponding velocity is lower for a larger particle (as confirmed by solving  $2k_B T = mv^2$  for  $v$ ). So to within a constant, we have determined the diffusion coefficient is:

$$D \propto \frac{k_B T}{\eta \cdot R}$$

where  $k_B$  is Boltzmann's constant,  $T$  is the temperature (these two together are *the* thermal energy unit),  $\eta$  is the viscosity, and  $R$  is the size of the diffusing particle. It turns out that the real diffusion coefficient for spherical objects in viscous media is:

$$D = \frac{k_B T}{6\pi\eta \cdot R}$$

where the denominator is called the Stokes drag for a spherical particle and is the viscous drag of a spherical particle in any viscous media. There is one piece of the puzzle that remains. We have not connected our result from the diffusion equation,  $P(r,t)$ , with a real measurable quantity. Recall that  $P(r,t)$  is a probability distribution able to tell us about the statistical features of diffusing particles. In probability theory, a distribution holds its statistical information in the *moments of the distribution*. We already calculated the *zeroth moment* when we demanded the total probability be equal to one. The *first moment* is what we commonly call the mean. Spatially this entails calculating the mean  $x$  and mean  $y$  position of the particle. To calculate such a moment we simply need to integrate our probability distribution over our space of interest:

$$\begin{aligned} \langle x \rangle &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} x \cdot P(x, y, t) dx dy = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{x}{4\pi Dt} e^{\left(\frac{-(x^2+y^2)}{4Dt}\right)} dx dy = 0 \\ \langle y \rangle &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} y \cdot P(x, y, t) dx dy = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{y}{4\pi Dt} e^{\left(\frac{-(x^2+y^2)}{4Dt}\right)} dx dy = 0 \end{aligned}$$

The first moment tells what we already knew, the particle's average position is where we originally introduced the particle ( $x = y = 0$ ). The *second moment* draws a useful relationship about diffusion and what we can observe (we'll take advantage of the radial symmetry and use polar coordinates):

$$\begin{aligned} \langle r^2 \rangle &= \int_0^{2\pi} \int_0^{\infty} r^2 P(r, t) r dr d\theta = \int_0^{2\pi} \int_0^{\infty} \frac{r^3}{4\pi Dt} e^{\left(\frac{-r^2}{4Dt}\right)} dr d\theta \\ \langle r^2 \rangle &= 4Dt \end{aligned}$$

In general for  $n$  dimensional isotropic diffusion the mean squared distance is:

$$\langle r^2 \rangle = 2nDt$$

In the real world experiment you will face a few problems naively applying this formula. The first and most irritating problem in the actual experiment will be the presence of an overall velocity field. Due to natural gradients in temperature, chemical potential, and concentration at the onset, you will most probably notice that the magnitude of the Brownian motion is comparable to some overall directed movement. Often these gradients will naturally disappear in a few minutes. The other big problem is something I already mentioned: each individual particle does not follow the diffusion equation, hence you will see stochastic variations that will add significant noise to your trace of one particle. The last problem will be picking a time step (this just requires some calibration). If you wait too long between images you will not be able to say with any certainty which particle in the first image corresponds to which particle in the second image. It is a competition: on longer time scales the diffusion effect is bigger, but you lose certainty about which particle is which, on shorter time scales the diffusion effect is small, but you will almost certainly be able to correlate particles between images. There is hope! Instead of looking at the movement one particle, we will take an image of many (~200) particles at once, wait an appropriate time step and take a second picture. We'll repeat this a few times. Then we'll measure the mean squared displacement of all the particles from one image to the next. We'll see a distribution of distances, however, that distribution will have a peak somewhere, corresponding to the correct mean-squared displacement for our time step. The rest is algebra.

Then knowing the diffusion coefficient we can deduce the viscosity of the medium. One last fact we need to know is that the glass slide interferes with normal viscous drag, and changes the diffusion coefficient slightly<sup>2</sup>:

$$D = D_o \left( 1 - \frac{9 R}{16 h} \right)$$

where  $D_o$  is our original diffusion coefficient,  $R$  is the particle radius, and  $h$  is the distance from the particle's center to the glass surface. We should note that the units of viscosity are  $Ns/m^2$  (pressure x time), and some typical values are listed in the table below:

Water @ 20C	1 $mNs/m^2$
Water @ 25C	0.898 $mNs/m^2$
Corn Oil @ 20C	71 $mNs/m^2$
Castor Oil @ 20C	102 $mNs/m^2$

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<sup>1</sup> Prepared and written by: Tristan Ursell, 1/19/05

<sup>2</sup> See: <http://www.physics.nyu.edu/grierlab/methods/node11.html>