

# Brownian Motion in Cells

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## Abstract

The diffusion of a particle in a solvent can be described by Einstein's theory of Brownian motion. In this theory, the displacements of particles are Gaussian distributed and the diffusion coefficient of the system can be computed from the mean-square of the displacements of particles in the system. The theory of Brownian motion can be applied to the motion of vesicles in cells. However, diffusion is insufficient to explain the transport of vesicles in cells and active transport methods are required to explain the transport.

## 1 Introduction

In 1827, the when the botanist Robert Brown was studying pollen grains suspended in water, he noticed that small particles were being expelled from the grains [1]. These small particles, suspended in the water, had a random motion, and Brown was unable to determine the cause of their motion. That random and jittery motion and diffusion of the particles later became known as Brownian motion and its cause was explained in great detail by Albert Einstein [2]. In 1908, the experimentalist Jean Perrin confirmed that Einstein's theory of Brownian motion was correct, earning him a Nobel Prize in physics in 1926.

In his 1905 paper, Einstein showed that the displacements of particles from their origins follows the diffusion equation

$$\frac{\partial f}{\partial t} = D \frac{\partial^2 f}{\partial x^2} \quad (1)$$

where  $D$  is the diffusion coefficient [2]. When using the conditions that lead to Brownian motion, (1) has the solution

$$f(x, t) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \quad (2)$$

This distribution is Gaussian, so it has a mean of 0 and variance of  $2Dt$ . Since the mean of the Gaussian in (2) is zero,  $\sigma^2 = \langle x^2 \rangle = 2Dt$  in this case. These mathematical facts about the behavior of Brownian motion will come in handy when constructing simulations and when investigating diffusion.

Brownian motion is useful in explaining certain cellular behavior, and Brownian motion is something that can be measured when observing cells. Two examples of cellular objects that undergo Brownian motion are vesicles and mitochondria. The diffusion through Brownian motion in cells is a passive method of transporting material from one location to another. However, many cellular transport processes are more directed and deterministic, and Brownian motion simply is not enough to explain

cellular transport. In addition to the passive transport method of diffusion, cells also use active transport methods to move material, and these active transport methods can be measured using the same experimental techniques used to measure Brownian motion.

## 2 Simulating Brownian Motion

Brownian motion experiments can be simulated on a computer with Monte Carlo simulations. This is useful because the simulations demonstrate what to expect when taking measurements on a system with certain conditions. In Brownian motion, the total displacement of a particle is the sum of tiny displacements of the particle from its origin. The distribution of these tiny displacements is given by (2), and this makes creating Monte Carlo simulations Brownian motion very straightforward to implement.

### 2.1 The Diffusion Coefficient

The goal of the Monte Carlo is to generate random particle trajectories and use them to compute the diffusion coefficient. The mean-square displacements can be related to the diffusion coefficient since [3]

$$\langle |\vec{r}(t + \Delta t) - \vec{r}(t)|^2 \rangle = 2dD\Delta t \quad (3)$$

where  $d$  is the number of dimensions in the simulation. Each component of displacement in the particle's trajectory is taken by sampling from a normal distribution where  $\sigma^2 = 2D\Delta t$ . This variance comes from the Gaussian described in (2), and this determines the length scale of the simulation. The theoretical value of diffusion is [4]

$$D = \frac{k_B T}{3\pi\eta r} \quad (4)$$

where  $k_B$  is Boltzmann's constant,  $T$  is the temperature of the solution,  $\eta$  is the viscosity of the solution, and  $r$  is the radius of the particles in the solution. This value of the diffusion coefficient is that can be used to sample the displacements of the particles.

At a first glance, the description of the simulation might seem kind of circular. In order to measure the diffusion coefficient from a random sampling, one needs to know what the diffusion coefficient is. The point of the Monte Carlo simulation is to get measurements of the diffusion coefficient from particle tracks and to determine how long a particle track needs to be in order to get a certain precision. A quick example of a Monte Carlo simulation for a one-particle system with particles of radius of 1  $\mu m$  in solution with viscosity of 1 cP can be seen in Figure 1. Another simulation that can be done is a system with many particles (see Figure 2). This simulation is useful because it matches what will be measured in an actual experiment much more closely than the one-particle simulation. The reasons for this is that in an actual experiment, multiple particles will be tracked. Additionally, the particles in an experiment will only be tracked for about 10 to 50 displacements. In a one-particle system, that would not give a very precise value of diffusion. However, adding more particles to the system makes the measured value of diffusion much more precise.

It is important to check how many steps in a particle track is needed to get a certain level of precision. Since the diffusion coefficient is obtained from  $\langle \Delta r^2 \rangle$ , the variance of  $\Delta x^2$  can be computed as

$$\sigma_{\Delta x^2} = \sqrt{\langle \Delta x^4 \rangle - \langle \Delta x^2 \rangle^2} = 2\sqrt{2}D\Delta t \quad (5)$$

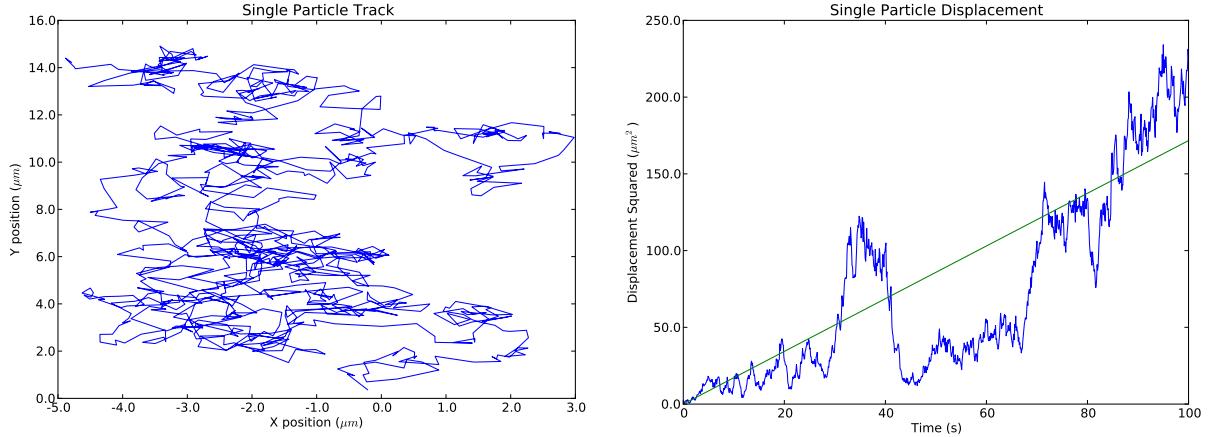


Figure 1: Monte Carlo simulation for a system with a diffusion coefficient of  $0.429 \mu\text{m}^2/\text{s}$ . The measured diffusion coefficient for this simulation was  $0.427 \pm 1.6 \times 10^{-3} \mu\text{m}^2/\text{s}$ . The particle for this simulation had a radius of  $1 \mu\text{m}$  and the viscosity of the simulation was 1 cP. The temperature of the solution was  $20^\circ\text{C}$  and displacement measurements were taken every 0.1 seconds.

It can be seen from the fact that since  $D = \frac{\langle \Delta x^2 \rangle}{2\Delta t}$ , the error for computing  $D$  by averaging  $\Delta r^2$  should be

$$\sigma_D = D \sqrt{\frac{2}{dnN}} \quad (6)$$

where  $d$  is the number of dimensions,  $n$  is the number of tracks, and  $N$  is the number of particles in the system. This theoretical value of the uncertainty can be used to show that if one wants a precision of 1% on the value of the diffusion coefficient for a 2-dimensional system, then one would need to track one particle for 10,000 steps. Alternatively, the same precision could be achieved by tracking 100 particles for 100 steps each or any other combination of  $n$  and  $N$  where  $nN = 10,000$ .

## 2.2 Bulk flow

So far, it has been assumed that diffusion has been the only mechanism to transport particles through a medium. This is not true in general. One example of a transport mechanism for particles in a fluid is a constant bulk flow. Adding bulk flow to the simulations is pretty straightforward. Consider a displacement  $\Delta \vec{r}$ . This displacement can be written as a sum of two mechanisms as

$$\Delta \vec{r} = \Delta \vec{r}_b + \vec{v} \Delta t \quad (7)$$

where  $\Delta \vec{r}_b$  is the displacement due to Brownian motion and  $\vec{v}$  is a constant flow of the solution containing the particles. When taking the average of (7), the term for  $\vec{r}_b$  vanishes, since it is Gaussian distributed and the average displacement then becomes

$$\langle \Delta \vec{r} \rangle = \vec{v} \Delta t \quad (8)$$

This provides a simple mechanism for determining from data whether particles are being transported by a bulk flow by computing the vector components of the flow.

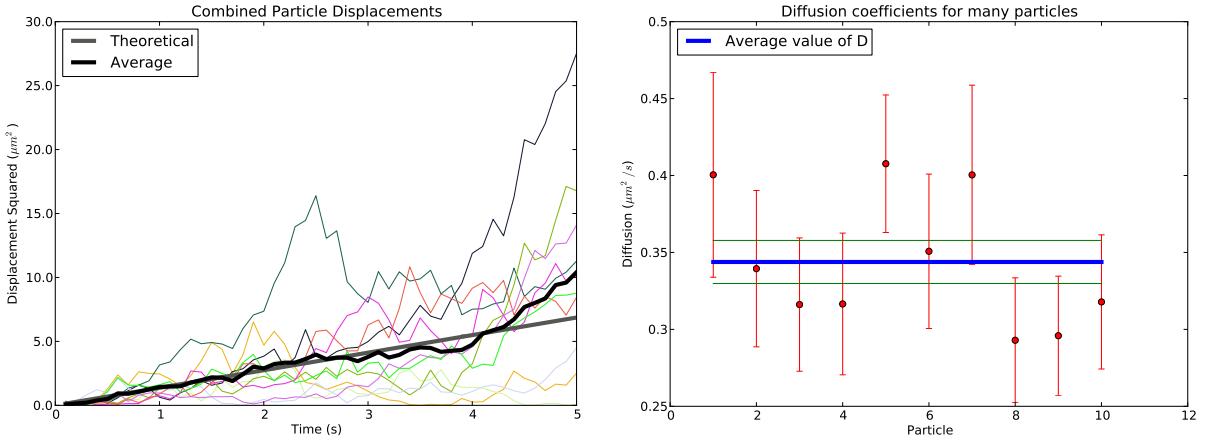


Figure 2: Monte Carlo simulation for a many particle system. The radius of the particles were half a micron and the viscosity of the solution for this simulation was 2.50 cP. This simulation matches the experimental conditions used to measure Brownian motion. The blue line on the right plot is the average of the diffusion coefficients and the green is the uncertainty on that average.

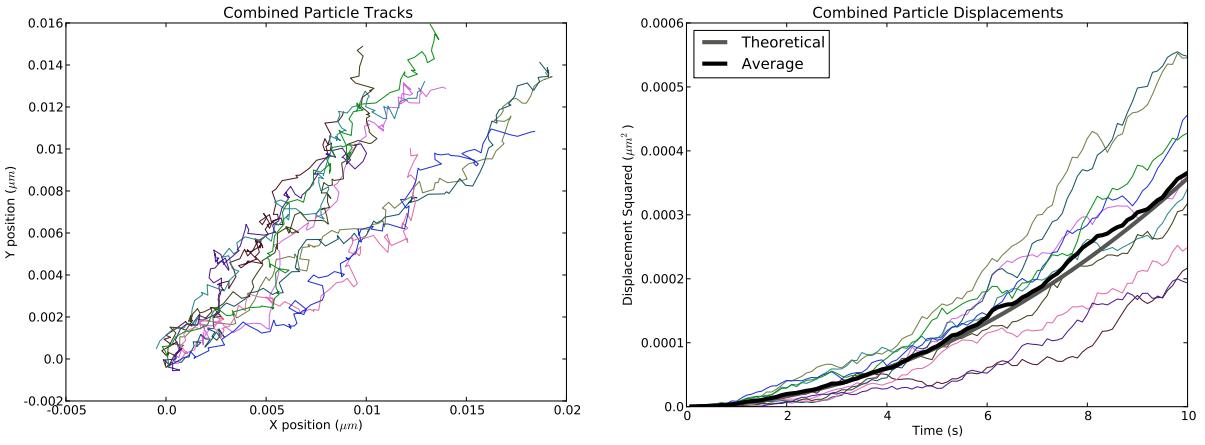


Figure 3: Monte Carlo simulation for a many particle system undergoing a bulk flow. The system is the same as the system in Figure 2, except that an extra flow with velocity  $\vec{v} = \sqrt{\frac{D}{2\Delta t}} (\hat{x} + \hat{y})$  was added to the system.

Bulk flow can easily be added to the Monte Carlo simulations for Brownian Motion. The variance of (2) defines the length scale of the simulation to be  $\sqrt{2D\Delta t}$ . The bulk flow can be added to the simulation as a scaling factor times the length scale, giving displacements as

$$\Delta \vec{r} = \Delta \vec{r}_b + \vec{\epsilon} \sqrt{2D\Delta t} \quad (9)$$

where  $\vec{\epsilon}$  is the scaling parameter that determines the strength of the bulk flow in each direction. Clearly, it can be seen that the theoretical velocity of the flow that drives the Monte Carlo is

$$\vec{v} = \vec{\epsilon} \sqrt{\frac{2D}{\Delta t}} \quad (10)$$

and the simulations can be used to see if the flow velocity can be extracted from data. Another prediction that can be added to the Monte Carlo model is how the total displacement squared will behave. Squaring (7) and taking the average gives

$$\langle \Delta r^2 \rangle = \langle \Delta r_b^2 \rangle + v^2 \tau^2 = 2dD\tau + v^2 \tau^2 \quad (11)$$

which has an extra quadratic term that vanishes in the absence of bulk flow. This prediction can be seen in Figure 3. It should be noted that Figures 2 and 3 outline another method of determining the diffusion coefficient and the bulk flow of a system. This can be done by computing the average displacement squared at each point in time and fitting to either a linear or quadratic fit. When applying the fitting to find the bulk flow, only the magnitude of the flow can be found. The direction of the flow has to be found by averaging the individual displacements.

### 3 Experimental setup

Both of the experiments performed essentially use the same method of tracking particles in a sample. How it works is that a microscope is plugged into a computer, and software is used to track the particles and save data. Once the data is saved, it is then analyzed to find certain quantities of interest and their uncertainties.

#### 3.1 The Microscope

The microscope used for tracking the particles was the Zeiss Axiovert 200. This microscope was used for both bright-field and dark-field microscopy. Bright-field microscopy is used to observe light that is reflected off of objects, whereas dark-field microscopy is done by scattering light off of particles. Bright-field microscopy was not very useful in the context of the experiments performed in this lab since the size of the particles tracked with the microscope were about the size of wavelengths of visible light. Fortunately, dark-field microscopy makes it possible to observe tiny objects in the microscope samples. An illustration of the basic principle behind dark-field illumination can be seen in Figure 4 [5]. This technique makes it possible to observe tiny particles that are not visible by reflecting light.

Before setting up dark-field illumination, Köhler illumination must be first achieved. This also must be done when changing the objective on the microscope. The reason for this is that setting up Köhler illumination creates an illumination on a sample that is very uniform [6]. Setting up Köhler illumination is fairly straightforward. This is done by closing the iris a small amount and adjusting the position of the iris until the center of the iris can be viewed from the eyepiece. This step is

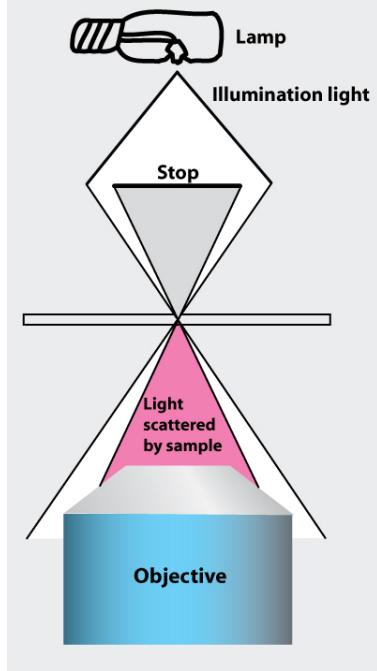


Figure 4: Schematic diagram of dark-field illumination [5]. The stop creates an optical system where light is sent to the side of the sample and gets scattered off of it. This makes it possible for objects smaller than a wavelength of light that cannot be observed using bright-field illumination to be observable.

repeated until the iris is almost closed entirely and the entire open region of the iris is centered with respect to the view of the eyepiece. Once the iris is centered, the iris is then opened so that the edge of the iris is outside of the field of view. After this is done, the microscope can then be set to use dark-field illumination. An example of a sample being viewed with dark-field illumination can be seen in Figure 5.

### 3.2 Particle Tracking

In order to measure the diffusion using (3), the particles in the field of view of the microscope must be tracked. Particle tracking has two main components, blob finding and tracking. Once the trajectories of the particles have been recorded, then statistics can be run on them to compute the diffusion coefficient and its uncertainty [7]. The particles were tracked using software that was unfortunately written in the C# language.

Blob finding is the method of locating particles. This is done by computing the background level of an image by taking the mean brightness and flagging all spots in the image that exceed a certain brightness. The brightness level that determines whether or not a spot is a particle is called the Z-score threshold, and that sets the number of standard deviations above the background a spot must be in order for it to be considered a particle [7]. Of course, in order for a set of pixels to be considered a particle, they must fall within a limit set by the user of the tracking software that dictates the pixel range that a particle can be.

The method described so far has one giant deficiency. Suppose there are static structures, cell walls for example, in the microscope camera's field of view that are much brighter than the objects that are moving. If that happens, it is likely that the moving objects will not be distinguishable from background noise. Additionally, the large static structures would probably be tracked, and that would ruin a set of data being used to compute the diffusion of a system or detect active transport methods occurring in a cell.

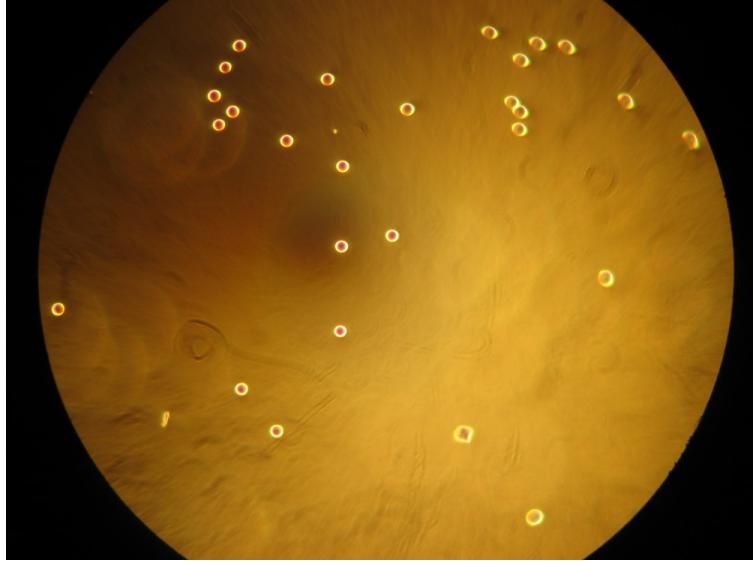


Figure 5: Polystyrene spheres under the microscope viewed using dark-field illumination.

Before computing the mean of a frame to find the background, the average image must be subtracted from the frame to remove noise and static structures like cell walls. The easiest way to do this would be to collect a movie of data frames, take the average of all frames, subtract that average from each frame, and then run the particle tracking algorithm. Unfortunately, this method is undesirable because it cannot run in real time. Fortunately, there is a way to get a real-time average, and that is done with exponential smoothing. Exponential smoothing is defined as such [8]

$$\begin{aligned} s_0 &= x_0 \\ s_j &= \alpha x_j + (1 - \alpha) s_{j-1}, \end{aligned} \tag{12}$$

where  $s_j$  is the average images up to iteration  $j$ ,  $x_j$  is the camera image recorded at iteration  $j$ , and  $\alpha$  is the smoothing parameter, where  $0 < \alpha < 1$ . By default, exponential smoothing was not implemented in the source code for the tracking software, but adding it was easy to do in only 2 lines of code. The smoothing factor that was used was chosen to be 0.05.

In addition to exponential smoothing, another feature that needed to be added to the particle tracking software was an algorithm to find the center of the blobs. Originally, the tracking software used the brightest pixel in a blob as the center. However, for oddly shaped blobs, that will be inaccurate. This can be fixed by using a method analogous to finding the center of mass of a massive object. The center of brightness for a 2-D object is defined as

$$\vec{R}_{CB} = \frac{\int \vec{r} \rho(x, y) dA}{\int \rho(x, y) dA} \tag{13}$$

where  $\rho(x, y)$  is the density of brightness of each point in an image. Of course, when dealing with images from a camera, the integrals turn into sums, since the camera images are discrete.

Blob finding is not sufficient to track particles because it does not address whether or not a blob in one frame and a blob in the next frame are the same blob. The simplest way to track particles is to

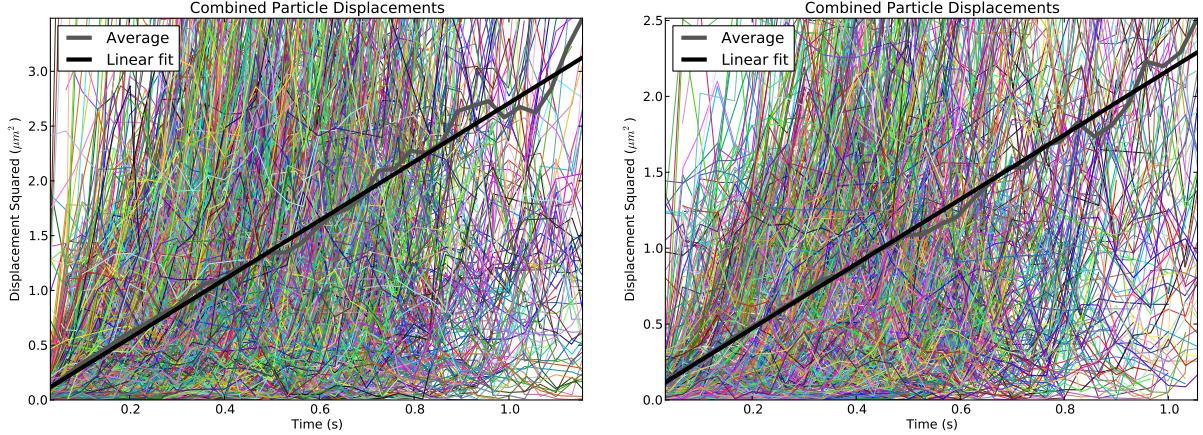


Figure 6: (left) Polystyrene spheres in glycerol solvent. (right) Polystyrene spheres in PVP solvent. Both plots are for spheres with diameters of  $0.47 \mu\text{m}$  and the viscosity of both solvents used is 2.50 cP.

use the nearest neighbor method, which matches particles in one frame to the closest particles in the next frame. While this method is very simple, it only works when particles are loosely packed together and are not moving very fast. Another method to do is create groups of particles and connect particle tracks based on that. All of the particle tracking functionality in the tracking software was already implemented and didn't need to be changed in any way.

Finally, the software must be calibrated so that it can properly convert distances in pixels to distances in meters. This is done by viewing particles of known size in the microscope and measuring how many pixels wide they are for each magnification setting in the tracking software. The conversion factors for each magnification can be seen in the following table.

10x	20x	40x
$0.666 \mu\text{m}/\text{px}$	$0.4 \mu\text{m}/\text{px}$	$0.222 \mu\text{m}/\text{px}$

## 4 Brownian Motion of Micro-Spheres

Einstein's theory of Brownian motion can be tested by tracking spherical particles in a solvent. If Einstein's theory is true, then the data should support the model in (3) where the diffusion coefficient is defined as (4). In order to properly test Einstein's theory, particles of a variety of sizes must be placed in solvents of varying viscosity. This experiment tests Einstein's theory with polystyrene spheres of diameters  $0.47 \mu\text{m}$  and  $1.0 \mu\text{m}$  in solvents of viscosities 1.66 cP, 2.50 cP, and 4.65 cP. The solvents used are glycerol and PVP. The following table shows the values of the diffusion coefficient predicted by (4) at  $20^\circ\text{C}$ .

Diameter	1.66 cP	2.50 cP	4.65 cP
$0.47 \mu\text{m}$	$1.00 \times 10^{-12} \text{ m}^2/\text{s}$	$7.31 \times 10^{-12} \text{ m}^2/\text{s}$	$3.93 \times 10^{-12} \text{ m}^2/\text{s}$
$1.0 \mu\text{m}$	$5.17 \times 10^{-13} \text{ m}^2/\text{s}$	$3.43 \times 10^{-13} \text{ m}^2/\text{s}$	$1.84 \times 10^{-13} \text{ m}^2/\text{s}$

The diffusion coefficient can be computed two ways. The first is to directly use Einstein's relation for 2 dimensions

$$\langle \Delta r^2 \rangle = 4D\tau \quad (14)$$

to compute a value of the diffusion coefficient for each track and then take the weighted mean over all tracks of these values of  $D$  and get the diffusion coefficient that way. Another way to calculate  $D$  is to use (14) and fit the average net displacement at each point in time and compute  $D$  from the slope of the linear fit. In the averaging method, the error on the diffusion coefficient computed for each track is the standard deviation of the displacements divided by the square root of the number of steps recorded for that track. Due to the limitations of the tracking software, the number of recorded steps is not the same for each particle track. The weighted mean diffusion, and its uncertainty are therefore

$$\begin{aligned} \langle D \rangle &= \sigma_{\langle D \rangle}^2 \sum_{i=1}^N \frac{\langle D_i \rangle}{\sigma_{\langle D_i \rangle}^2} \\ \frac{1}{\sigma_{\langle D \rangle}^2} &= \sum_{i=1}^N \frac{1}{\sigma_{\langle D_i \rangle}^2} \end{aligned} \quad (15)$$

where  $\sigma_{\langle D \rangle} = \sigma_{D_i} / \sqrt{n_i}$ . When finding the diffusion coefficient using a linear fit, the uncertainty on the diffusion can be obtained from the covariance matrix of the fit. Each system measured had at least two data recordings, and the results of each method of getting the diffusion coefficient can be combined in a weighted average for each method separately. The following tables show the values of the diffusion coefficients and their uncertainties, calculated from averaging displacements and creating a linear fit. Unless otherwise noted, the solvent is glycerol. It should be noted that the curve fitting method produces results must closer to the theoretical value calculated with (4).

Diameter:  $0.47 \mu m$

Averaging method	1.66 cP	2.50 cP	4.65 cP	2.50 cP (PVP)
Diffusion coefficient ( $\mu m^2/s$ )	0.613	0.424	0.275	0.378
Uncertainty	$3 \times 10^{-3}$	$2 \times 10^{-3}$	$1 \times 10^{-3}$	$2 \times 10^{-3}$

Curve fitting method	1.66 cP	2.50 cP	4.65 cP	2.50 cP (PVP)
Diffusion coefficient ( $\mu m^2/s$ )	1.014	0.645	0.434	0.526
Uncertainty	$8 \times 10^{-3}$	$5 \times 10^{-3}$	$4 \times 10^{-3}$	$4 \times 10^{-3}$

Diameter:  $1.0 \mu m$

Averaging method	1.66 cP	2.50 cP	4.65 cP	2.50 cP (PVP)
Diffusion coefficient ( $\mu m^2/s$ )	0.395	0.386	0.252	0.482
Uncertainty	$2 \times 10^{-3}$	$2 \times 10^{-3}$	$1 \times 10^{-3}$	$3 \times 10^{-3}$

Curve fitting method	1.66 cP	2.50 cP	4.65 cP	2.50 cP (PVP)
Diffusion coefficient ( $\mu m^2/s$ )	0.566	0.592	0.342	0.659
Uncertainty	$3 \times 10^{-3}$	$4 \times 10^{-3}$	$5 \times 10^{-3}$	$7 \times 10^{-3}$

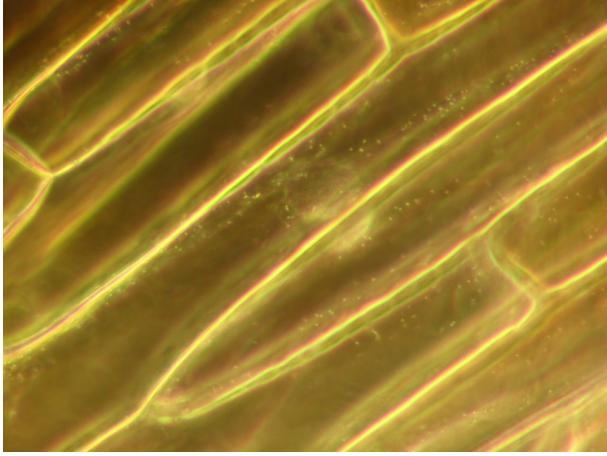


Figure 7: Vesicles moving along transport paths in red onion cells.

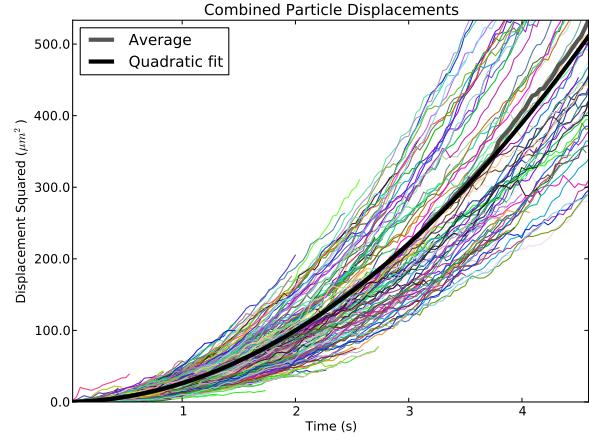


Figure 8: Vesicles in the onion cell undergoing bulk flow. The flow in this sample was very large, and it could be the result of saline flowing to the edge of the slide.

## 5 Intracellular Movement in Onion Cells

The organelle used by cells to transport material is called a vesicle. In the low Reynold's number limit, the amount of work that it takes to transport a vesicle from one part of a cell to another can be computed from Stokes' Law [9]

$$F_d = 6\pi\eta rv \quad (16)$$

where  $F_d$  is the drag force on the particle and  $v$  is the velocity of the particle. Using the definition of the diffusion coefficient (4), it can be shown that the work to transport a particle a small distance is

$$dW = 2v \frac{k_B T}{D} dr \quad (17)$$

This can be computed for each step in a particle track and then summed to get the total work. Since this work is the amount of work needed to resist the drag of the intracellular fluids, this work can come from diffusion or active transport. It should be noted that diffusion is insufficient as a transport mechanism. One reason for this is because diffusion produces random walks for vesicle trajectories, whereas vesicles are often transported along non-random trajectories (see Figure 7).

The techniques used to calculate the diffusion coefficients of the systems with the polystyrene spheres can be used to calculate the diffusion coefficient of the cells, and therefore calculate the viscosity of the cytosol. The diameter of the particles detected in the tracking software was about 4 pixels, which corresponds to 900 nm. By tracking the vesicles in regions where the only motion is Brownian motion, it was found that the diffusion coefficient for the cells was  $0.700(5)\mu\text{m}^2/\text{s}$ , which implies that the viscosity of the cytosol is about 1.38 cP.

The combined particle tracks for vesicles in a red onion can be seen in Figure 9. It is clear from this that there are pathways of cellular transport. This can both be seen from the left figure of the particle tracks, which shows the trajectories, as well as the right figure, which shows a tracks having quadratic displacement, as predicted by (11). Another verification of (11) can be seen in Figure 8.

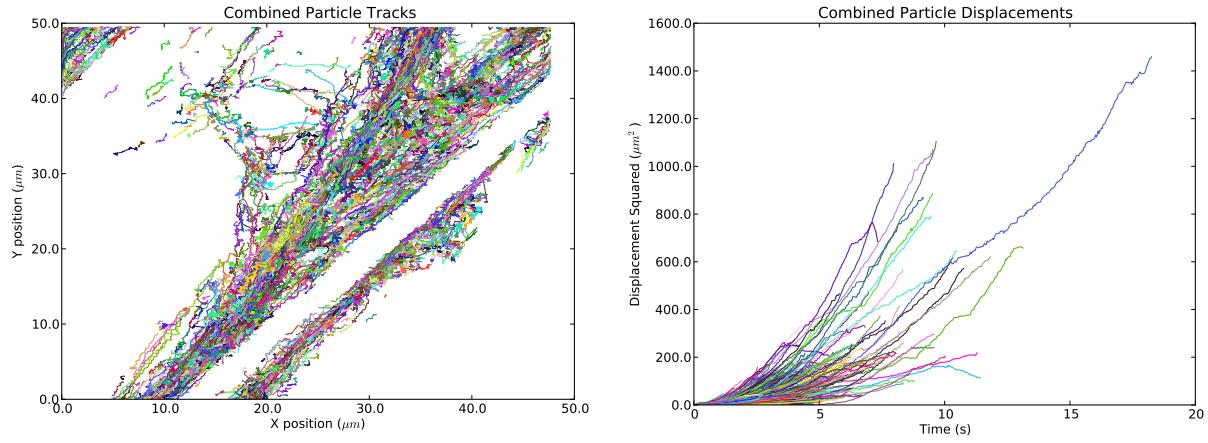


Figure 9: Transport paths and displacements of vesicles in a red onion cell.

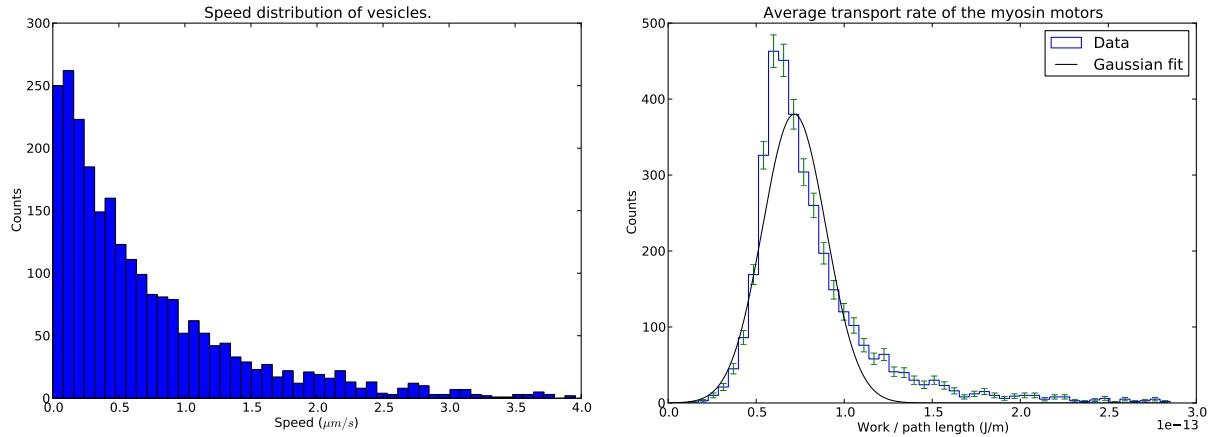


Figure 10: The speed distribution of particles has an exponential drop off rate.

Figure 11: The work required to transport each vesicle is something that is fairly straightforward to calculate. However, since each particle traverses a different trajectory, a more interesting quantity is the work that it takes to transport a vesicle normalized to the line integral of the vesicle's trajectory. This produces a somewhat sharply peaked distribution that behaves like a Gaussian.

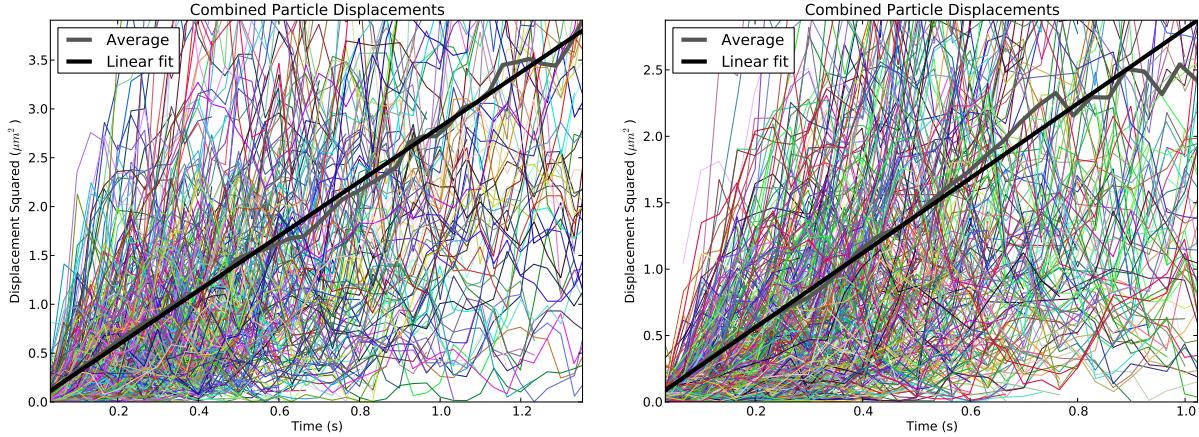


Figure 12: Brownian motion results from onion cells. The diffusion coefficient computed from these two plots implies that the viscosity of the cytosol is about 1.38 cP.

In that figure, there was a strong flow of solution moving the vesicles. One possible cause of that system was saline flowing to the edge of the slide.

In addition to the particle tracks and overall displacements, the speed distribution of the vesicles undergoing active transport is something that should be looked at (see Figure 10). Most vesicles are not moving very quickly, as the drop off in the speed distribution is exponential. In all of the regions measured that contained active transport, the RMS speed was fairly consistent, and the combined average of the RMS speed was  $1.13(2) \mu\text{m}/\text{s}$ .

The measurements of active transport can be used to quantify the amount of molecular motors it takes for certain cellular processes to occur. Stokes' Law can be used to calculate the amount of work that it takes to transport particles over a distance. Since each vesicle travels a different length, the average amount of energy per unit length traveled to transport a vesicle can be found by normalizing the work for transporting each vesicle with the line integral of each vesicle's trajectory. The distribution of the total work per total path length can be seen in Figure 11. The mean of the Gaussian fit of that distribution was fairly consistent over all transport regions, and the combined average of it was  $7.64(5) \times 10^{-14} \text{ J/m}$ . Additionally, the combined average amount of work done on a vesicle was found to be  $3.55(2) \times 10^{-19} \text{ J}$ .

The theory behind cellular transport is that myosin motors are a driver of active transport [10]. The process of a myosin motor cycle converts one molecule from ATP to ADP. This, along with the energy release from converting ATP to ADP and the efficiency of the myosin motors can be used to find the approximate number of motors involved in intracellular transport. The energy of an ATP to ADP reaction is 20.5 kJ/mol [11], and the energy for a single reaction can be found by dividing that number by Avogadro's constant. The peak efficiency of the myosin motors is about 0.4 [12]. This information, plus the average amount of work done on a vesicle can be used to conclude that cellular transport takes about 26,000 myosin cycles.

The source code written for the simulations and analysis has been uploaded to Github and can be found at [13]  
<https://bitbucket.org/domagalski/physics111-advanced-lab/>.

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