

Brownian Motion in Cells

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Abstract

This lab concerns observation and analysis of the motion in a few different types of particles. Specifically, the first portion of this lab deals with Brownian motion in synthetic microbeads of various sizes suspended in solutions of varying viscosity. We track particles using a microscope and motion tracking software to record their sizes, displacements, velocities, and diffusion coefficients. The second part of this lab consists of similar measurements of particles in onion cells, as well as additional measurements of active transport in and around onion cells along actin filaments. For both parts, we compare measurements to theoretically obtained or known values for diffusion coefficients in Brownian motion and active transport speeds. The diffusion coefficient for granules undergoing Brownian motion in onion cells was found to be $(2.171 \pm 0.038) \times 10^{13} m^2/s$ and the average velocity for granules undergoing active transport in onion cells was determined to be about $9500 \pm 580 \text{ nm/s}$. The work required to transport granules along the length of one cell was further calculated to be about $0.0132 \pm 0.0009 \text{ fJ}$.

1 Introduction

Brownian motion is the term used to refer to the microscopic random motion of free particles in a fluid. This was first theorized by Albert Einstein while describing the kinetic theory of fluids and was later experimentally confirmed by Jean Perrin using microscopic latex spheres suspended in viscous solutions [Lab].

The experimental observation of the Brownian motion of synthetic particles in this lab is conducted on tiny polystyrene spheres, similar to Perrin's original work. Viewing the spheres through a microscope and tracking them with sensitive motion tracking software, the diffusion coefficient for spheres of various sizes in viscous solutions of varying viscosity is calculated. The variations in solution viscosity and bead size serve to build a more complete picture of the variables that the diffusion constant depends on and helps verify the *Stokes-Einstein equation* for Brownian motion, which is the theoretical method used to calculate the diffusion coefficient.

Further, this experiment studies the motion of particles in living cells, identifying Brownian motion in otherwise "still" particles and active transport in particles responsible for inter- and intra-cellular transportation. The observation of active transport along actin fibers particularly is evidence for a more complex model of cell transport than pure diffusion. Specifically, this experiment is conducted on onions, whose extremely thin and fragile epidermal layers provide the advantage of already being a single cell in thickness. These layers, when carefully peeled off and put under a microscope, allow unobstructed observation of the motion of particles within and surrounding the cells [Lab]. This report discusses the qualitative observations of Brownian motion and active transport in the epidermal layers of onions. Additionally, data collected to quantify this motion yields information about the speed of active transport in these particles and the work done by tiny protein "motors" to move them.

2 Theory

2.1 Brownian Motion

Brownian motion was first derived theoretically by Einstein by combining the equation for osmotic pressure with equations describing motion in viscous fluids, concluding that particles colliding frequently results in this effect [Ein05]. Brownian motion refers to the small, random motion of particles

when suspended in a fluid. The fluid can be thought of as a huge collection of particles continuously colliding with the particles suspended in the fluid, causing movements in all directions. The movements of a free particle in a Newtonian fluid, as Einstein derived using Stoke's law, can be represented using the Stokes-Einstein equation:

$$D = \frac{k_B T}{3\pi\eta d} \text{[Ein05]} \quad (1)$$

Here, D is the diffusion coefficient, T is the temperature of the suspension, η is the viscosity of the fluid, and d is the diameter of the (approximately spherical) particle suspended in the fluid. This formula makes intuitive sense and clearly holds when thought of through the lens of the kinetic theory of molecules: at higher temperatures, less viscous fluids, or smaller particle sizes, particles move more freely and vigorously. This leads to increased numbers of collisions, ultimately increasing the amount of diffusion that takes place.

Another way to find the diffusion coefficient is in terms of time t and the root-mean-square displacement λ_x :

$$\lambda_x = \sqrt{2Dt} \text{[Ein05]} \quad (2)$$

This can further be combined with Equation 1 to yield:

$$\lambda_x = \sqrt{\frac{tk_B T}{3\pi\eta d}} \quad (3)$$

A full derivation can be found in [Ein05]. These equations are helpful for calculating the diffusion coefficient experimentally since displacement and time are both observable.

Brownian motion can readily be observed in suspensions of silicon beads in polyvinylpyrrolidone (PVP) solutions created during this experiment and in nature when observing onion cells. Accurately identifying and tracking the motion of particles undergoing Brownian motion should yield fairly small, random-looking tracks centered approximately at the center of the particle.

2.2 Active Transport

Observing onion cells clearly shows many particles drifting from one end of the microscope view to the other, with movement far too deliberate and large-scale to be attributed to Brownian motion. This motion is often characterized as active transport, a form of movement within living cells that transports granules with contents such as food or waste around the cell [Lab].

Active transport is known to concentrate around actin filaments, which are fibers that comprise the more rigid components of onion cell structures. Figure 1 shows an example of this clustering behavior. This process is carried out by proteins known as myosin motors, which carry granules along actin filament highways between and within cells [SK].

With the data collected in this experiment, we hope to calculate the work done by myosin motors while transporting granules along the length of an onion cell. Theoretically, we can calculate an expected value by considering the change in energy due to hydrolysis, the process of converting ATP (chemical energy from soil or photosynthesis) into mechanical work (moving granules a certain distance) [SK]. This change in Gibbs free energy ΔG is not readily known for onions specifically, but is approximately $-30 \frac{\text{kJ}}{\text{mol}}$ on average for plant and animal cells [Wac98].

When considering onions specifically, we must keep in mind that this average is skewed to be of greater magnitude because of animal cells, which can have approximately $-60 \frac{\text{kJ}}{\text{mol}}$ of energy change on average. Thus, an estimate for the change in energy caused by hydrolysis in onion cells can be found by finding an average of the more precise values listed in [Wac98], eventually arriving at approximately $-47 \frac{\text{kJ}}{\text{mol}}$ with a standard error of $4.94 \frac{\text{kJ}}{\text{mol}}$.

The drag force experienced by myosin motors, which can then be used to calculate the work done by them, can be found using Stokes' equation 4:

$$F = 6\pi r\eta v \text{[Ein05]} \quad (4)$$

where r is the radius of the particle, v is its velocity, and η is the viscosity of the fluid it's in.

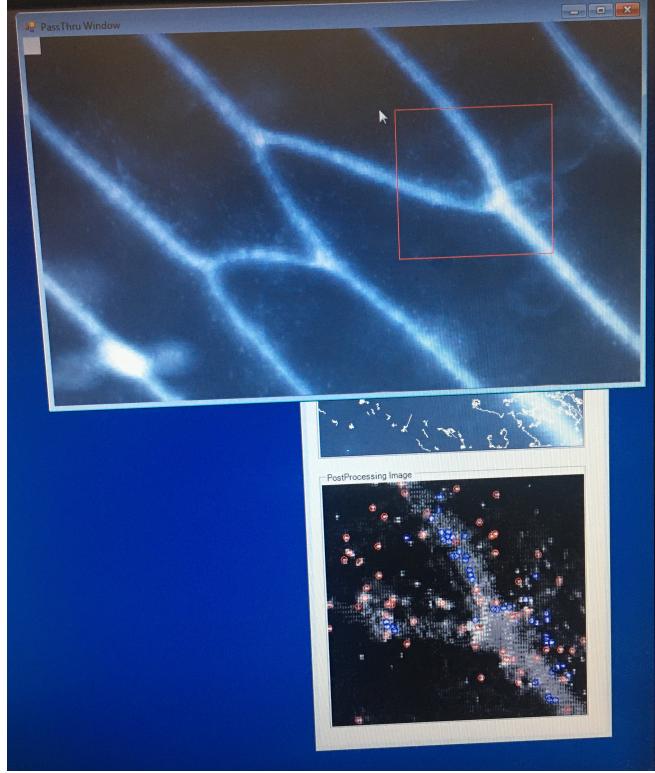


Figure 1: An example of granules clustering around rigid components of onion cells. The long lines in the upper image are cell walls and the colored circles around particles label identified granules. Note how empty the spaces near the center of cells are compared to the amounts of activity at cell walls! The zoomed in picture of an intersection of two walls offers a particularly granule-dense area.

3 Apparatus and Procedure

3.1 Setup

A block diagram detailing the setup for this experiment can be found in Figure 2. We use an Axiovert 200 Microscope for this experiment, with a CCD camera attached to its left sideport. This microscope has a revolving objective nosepiece that offers 5x, 10x, 20x, and 40x objective lenses. The light that illuminates samples on the stage originates at a halogen filament, the brightness of which can be varied [Lab]. The light is then focused through a condenser and can then be observed through the objective lenses.

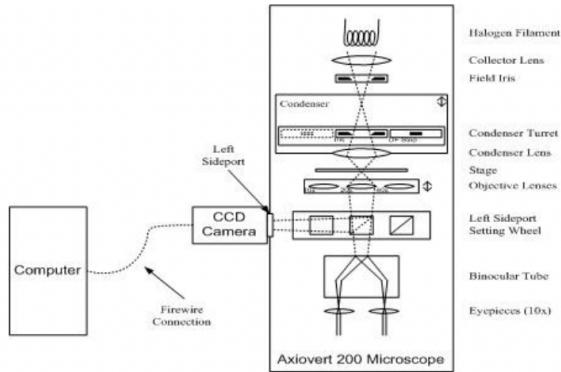


Figure 2: Block diagram for the experimental setup [Lab].

Köhler illumination is achieved to ensure even illumination of the suspension and a clear image of the microbeads, excluding any stray beams imaging the light bulb [Lab]. An example of Köhler illumination in the bright field is shown in Figure 3.

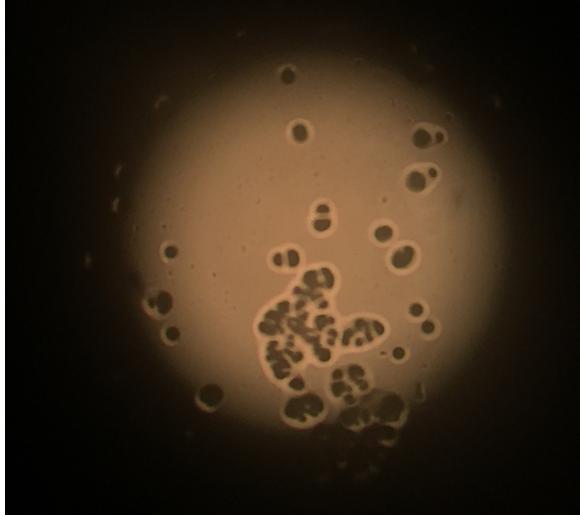


Figure 3: 10 micrometer beads in bright field illumination as seen during the experiment.

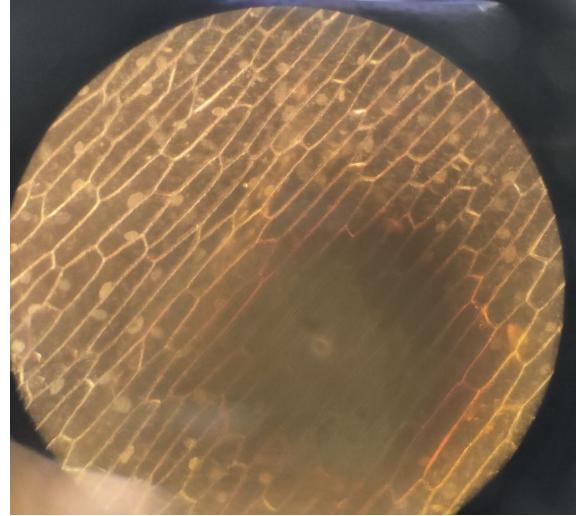


Figure 4: Onion cells in dark field illumination as seen during the experiment.

Note that the objects observed appear to be dark against a bright background in bright field illumination and bright against a dark background in dark field illumination.

Additionally, dark field illumination is used in this experiment. This is a technique that focuses scattered light of the sample and blocks transmitted light in a way that illuminates the sample almost as an opposition to bright field imaging - the particles appear light against a dark background [Bri22].

This is done as follows:

1. First, the light from the light source hits the dark field patch stop, which blocks light from entering the condenser and creates a ring of illumination (easily visible through microscope).
2. Next, light passes through the condenser lens, which focuses the light from the ring of illumination on the sample.
3. Lastly, after the light hits the sample, it scatters from or transmits through the particle. If it is scattered, it enters the objective lens and can be seen by the viewer; if it is transmitted, it is not collected by the objective lens [Bri22].

Thus, opaque objects such as actin filaments scatter light and appear white (or otherwise light in color) when viewed in dark field illumination, while transparent substances such as saline solution or (mostly transparent) viscous fluid transmit light and appear darker in color. Dark field illumination allows for high contrast, making it much easier to see details and small particles moving around in view.

The CCD camera is additionally connected to a computer in lab that runs an application to track particles. This software interprets the imaging captured by the camera in order to identify and track particles. By using particle size, spread, and brightness estimates from user input, the software is able to accurately distinguish bright particles from the dark background and follow their motion.

3.2 Procedure

Before beginning any measurements, it is important to understand the setup and software well and measure the pixel-to-meter conversion rate between the images shown on the computer screen and the actual particles being observed. This can be calculated by measuring the number of pixels taken up by microbeads of known sizes at various magnifications and are found to be $0.72 \mu\text{m}/\text{px}$ for 10x magnification, $0.33 \mu\text{m}/\text{px}$ for 20x magnification, and $0.22 \mu\text{m}/\text{px}$ for 40x magnification.

3.2.1 Synthetic Microbeads

The first part of this experiment involves observing Brownian motion of synthetic microbeads of different sizes suspended in solutions of varying viscosity. These suspensions are prepared by combining Polystyrene microbeads with a viscous polyvinylpyrrolidone (PVP) solution. The PVP solution is a combination of PVP and deionized water with varying amounts of each changing the viscosity of the solution. The combinations of sizes and viscosity used for this experiment are listed in the following table:

Microbead Diameter	Solvents	Viscosity
2.04 μm	PVP and water	2.50 cP
2.04 μm	PVP and water	4.65 cP
2.04 μm	PVP and water	13.2 cP
6.15 μm	PVP and water	4.65 cP
2.04 μm	Glycerol and water	4.65 cP

Table 1: Microbead diameter, solvents, and viscosity for each suspension used in this part of the experiment [Lab].

After creating the solvents, microbeads are added and slides with drops of the suspension are prepared. Microbeads with larger diameters are far, far easier to find with the microscope than smaller ones, but larger microbeads exhibit much less significant Brownian motion. Once the beads are located using the microscope, motion tracking data can be taken through the program. For the smaller beads (which are the ones with significant Brownian motion), upon increasing magnification to be able to observe them in higher resolution, there were issues with observing large enough numbers of beads in one frame once. This unfortunately rendered much of the data collected for these beads too noisy to be used for meaningful data analysis, with the software only being able to pick up a very small handful of particles at a time. In order to carry out more meaningful data analysis, we have instead simulated data with the MATLAB code provided on the Physics 111B experimentation laboratory course website [Lab]. Furthermore, the majority of the experimental conclusions discussed in this report consequently rely on the data collected from onion cells.

3.2.2 Onion Epidermal Layers

The epidermal layers of onions allow a straightforward way to observe microscopic motion in nature. These delicate layers, which are a single cell in thickness, can be found at the inner section of an onion bulb. After carefully peeling off an epidermal layer, a slide can be prepared with a few drops of saline solution.

Onion cells are found to be rich in motion, with some particles exhibiting Brownian motion and others more deliberate movement. Qualitatively, it is quite obvious which particles are experiencing which type of motion – particles exhibiting Brownian motion tend to stay approximately in the same place, with their tracks looking random overall (as seen in Figure 5); granules undergoing active transport move decisively along cell walls and actin filaments in groups, forming “highways” as seen in Figure 6. Data regarding their speed and distances covered is recorded by focusing on areas with cells that predominantly exhibit one of the types of motion, then calculating the diffusion coefficient (for Brownian motion) or measuring the time taken to traverse the length of one cell.

Fortunately, the tracking of granules in the epidermal layers of onions was far more successful than for the microbeads and yielded data that is analyzed in the following sections.

4 Results and Analysis

4.1 Brownian Motion of Synthetic Microbeads

Using Equation 1 and assuming that $T \approx 295\text{K}$ (room temperature), theoretical values of the diffusion coefficient for each set of microbeads can be found. Errors in these theoretical values are found through error propagation of manufacturer-given errors in microbead sizing and the estimated error in measuring pipette volume while making the viscous solution.

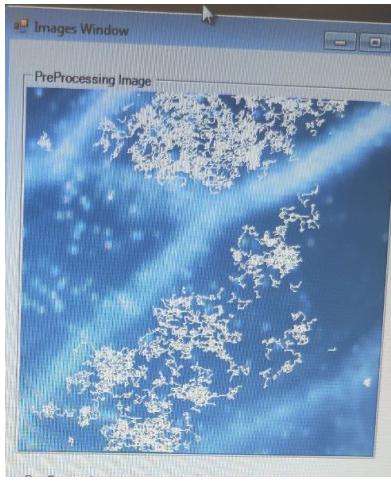


Figure 5: Brownian motion tracks in onion cells.

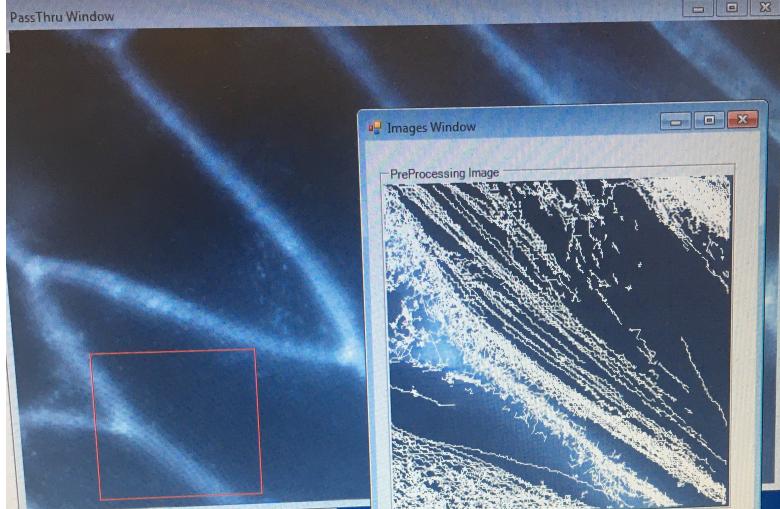


Figure 6: Active transport tracks in and around onion cells.

Brownian motion tracks are easily spotted as small squiggles, with particles moving randomly but overall staying in the same general area the entire time. Active transport tracks are long lines, with each particle moving along a wall or fiber in a consistent direction.

Microbead Diameter	Viscosity	Theoretical D	Simulated D
2.04 μm	2.50 cP	$(8.41 \pm 0.9) \times 10^{-14}$	$(8.18 \pm 0.249) \times 10^{-14} \frac{\text{m}^2}{\text{s}}$
2.04 μm	4.65 cP	$(4.52 \pm 0.5) \times 10^{-14}$	$(4.24 \pm 0.00455) \times 10^{-14} \frac{\text{m}^2}{\text{s}}$
2.04 μm	13.2 cP	$(1.59 \pm 0.18) \times 10^{-14}$	$(1.63 \pm 0.00129) \times 10^{-14} \frac{\text{m}^2}{\text{s}}$
6.15 μm	4.65 cP	$(1.50 \pm 0.23) \times 10^{-14}$	$(1.46 \pm 0.00414) \times 10^{-14} \frac{\text{m}^2}{\text{s}}$

Table 2: Theoretically calculated and simulated values of the diffusion constant for various suspensions. Note that these are all PVP and water solutions, since viscosity is the same regardless of solvent for the simulation.

The theoretical and simulated values of the diffusion coefficient are listed in Table 2.

Though there is no difference between PVP and Glycerol solutions in the simulation, we did tentatively find during the experiment that the measured values of the diffusion coefficient were significantly higher for glycerol (around $1.5 \times 10^{-13} \frac{\text{m}^2}{\text{s}}$) compared to PVP (around $7.2 \times 10^{-14} \frac{\text{m}^2}{\text{s}}$) at the same microbead diameter and viscosity ($2.04 \mu\text{m}$ and 4.83 cP).

Figure 7 plots the theoretical and actual simulated data of time vs the displacement of particles squared at the microbead diameters and viscosities listed in table 2.

The simulated values generally follow the theoretical values fairly well and lend themselves to the following conclusions:

- As viscosity increases, displacement decreases (as seen by the steadily decreasing slopes for higher viscosity solutions in 7).
- At the lowest viscosity (red on the graph), the simulated data diverges from the theoretical data slightly more than for other sets. This may be because beads in a lower viscosity fluid are more susceptible to noise that causes other kinds of motion in addition to Brownian motion.
- Increasing particle size (within the same order of magnitude) does not significantly impact the displacement, though there are not enough attempts with various diameters to conclusively say this for much larger particles.

Using this simulated data for displacement and equation 2, diffusion coefficients can be calculated for each of the cases. These are listed in table 2, with the errors equal to the standard error of the data points. The values for D from the simulation are quite close to the theoretical values, but technically do not agree due to the very, very small error bars of the simulated data. The simulated data has

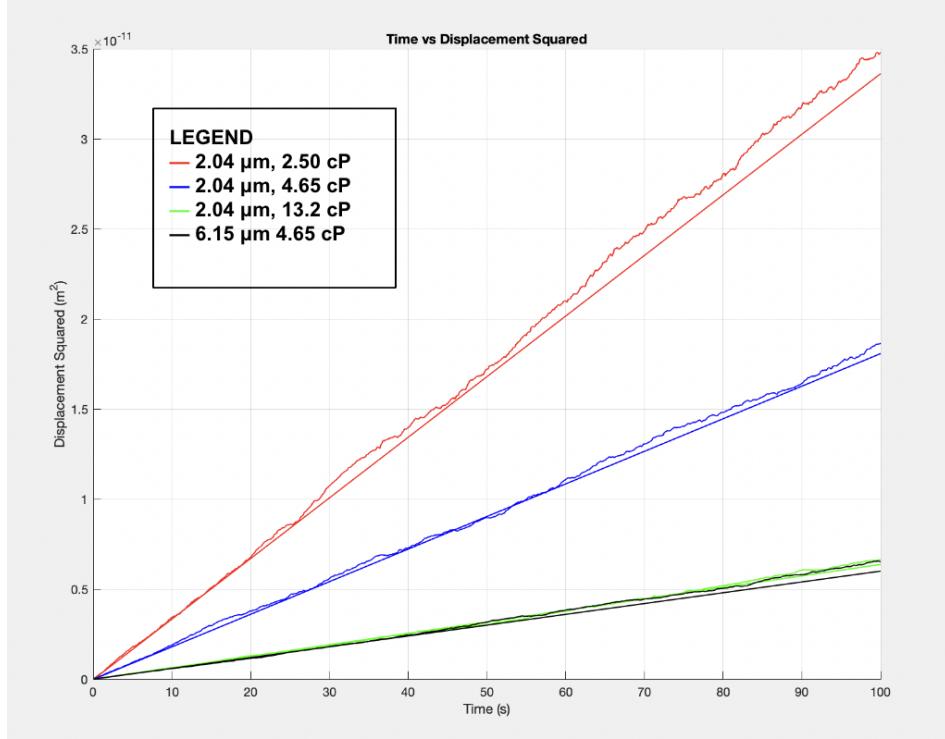


Figure 7: Time vs. displacement squared (simulated and theoretical). Note that the error bars are too small to be visible on this graph.

smaller error bars than otherwise expected with real data because it lacks the sources of error that arise in real experimental conditions. The patterns listed above still hold, though, and these calculated values of D further confirm the Stokes-Einstein equation 1.

Figure 8 provides a closer look at the average error bars (green) on the average calculated diffusion constants (blue) after 100 simulations each. Once again, these correspond to the values listed in table 2. This graph provides a visualization of how rapidly the diffusion coefficient changes with viscosity, with the change between the top three lines being very substantial with each new viscosity. Similarly, this graph highlights the minimal difference between the bottom two lines (where the error bars overlap!), indicating the very modest difference caused by increasing the microbead diameter over threefold.

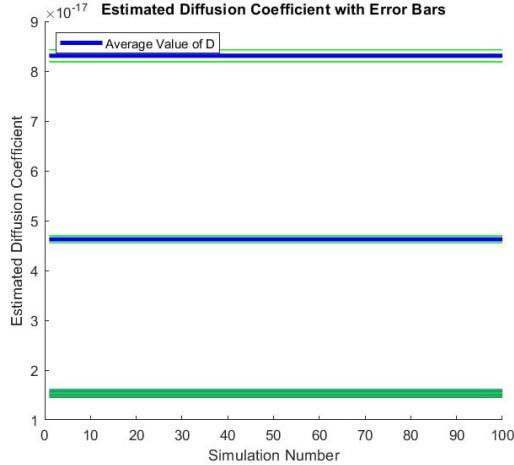


Figure 8: Diffusion constants for each (bead diameter, viscosity) pair plotted with error bars.

4.2 Brownian Motion in Onion Cells

Brownian motion in onion cells was fairly easy to identify, with the majority of it taking place near the center of onion cells and away from the active transport regions by the cell walls. The process of zooming in on an area with particles exhibiting Brownian motion is straightforward and pictured in Figure 9. Additionally, the software is configured to automatically measure diffusion coefficients in given areas. After taking 10 measurements in various areas and of various quantities of particles per area, we find the average diffusion coefficient for particles undergoing Brownian motion inside onion cells to be approximately $(2.171 \pm 0.038) \times 10^{-13} \frac{m^2}{s}$. This provides a first estimate of the viscosity of the insides of an onion cell: it is less than 2.50 cP.

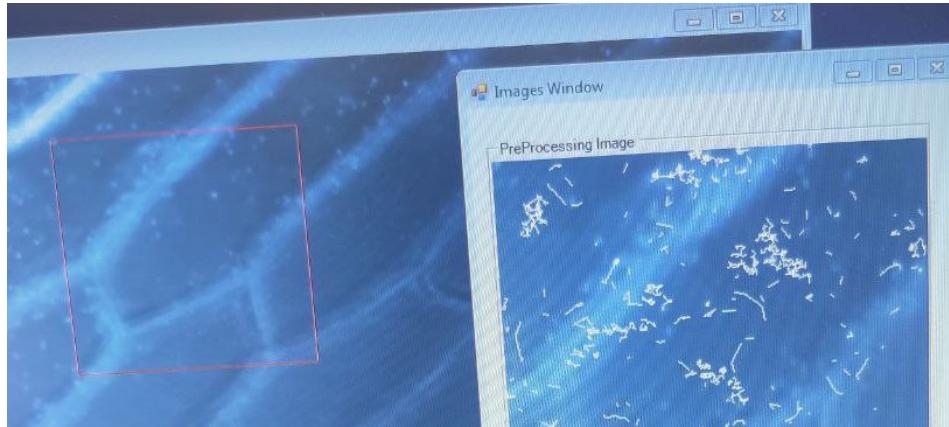


Figure 9: An example of Brownian motion in onion cells. The particles in the center of the pictured cells are great candidates for data-taking, though the area of focus would need to be made smaller to exclude cell walls and actin fibers.

4.3 Active Transport in and around Onion Cells

Particles moving in large groups along a rigid structure in or around an onion cell are evidence of active transport, which helps cells move granules of food or waste from one part of the organism to another.

To specifically measure the velocity of active transport in and around onion cells, we find areas of active transport - these are generally actin fibers or cell walls, as pictured in Figure 10.

With the help of the motion tracking software, which is able to determine average velocities in x and y directions, the average magnitude of the velocity vectors of particles can be determined. In this experiment, it was found to be about $9500 \pm 580 \text{ nm/s}$. This value is on a similar scale to values found in other papers for the speed of myosin motors, $814 \pm 16 \text{ nm/s}$. Our measured value does not completely agree with this known value. This can be due to several reasons, including the software picking up dust or variations in speed in different onion specimen.

To determine the drag force acting on the particles as they move through the cells, equation 4 can be used. First, by using the previously determined pixel-to-meter conversion for the 20x objective lens, the diameter of an average granule is calculated to be $1.82 \pm 0.0245 \mu\text{m}$. The tracking software can further be used to find the diffusion coefficient $(3.171 \pm 0.578) \times 10^{-13}$ and consequently a more accurate estimate of the viscosity of the cytoplasm: $(1.65 \pm 0.031) \times 10^{-3} \text{ Pa*s}$. The viscosity of water is $1 \times 10^{-3} \text{ Pa*s}$ and cytoplasm is mostly water, so the calculated viscosity being close to the viscosity of water makes sense. Finally, with all the required components, the drag force is calculated to be $0.532 \pm 0.033 \text{ pN}$. The minute magnitude of this force is fitting for the observed microscopic and low velocity particles. In literature, comparable calculations have found values of the force exerted by a single kinesin against a viscous load to be about $(4.2 \pm 0.5) \text{ pN}$ [kin]. The value calculated in this experiment is noticeably smaller than this value, potentially due to a slightly dried out onion (at the time that these measurements were made, the slide of the epidermal layer had been exposed to the air for about 25 minutes already). As the slide dries, the motion in the cells slows to a stop [Lab].

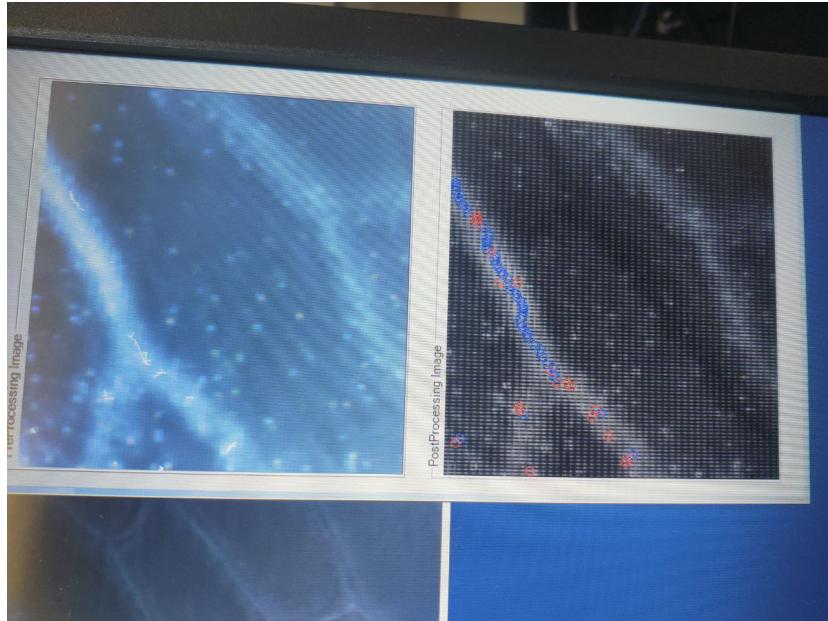


Figure 10: Granules clustered at a cell wall, a great candidate for active transport measurements.

After calculating this average drag force and estimating the length of an edge of the cell wall ($249 \pm 7.31\mu\text{m}$), the work done by myosin motors in onion cells can be estimated. The work done by myosin motors is determined using $W = F \times s$, where s is the distance travelled and F is the force to be overcome. The work required to move a single particle is thus determined to be $0.0132 \pm 0.0009 \text{ fJ}$. The average energy released by hydrolysis is known to be roughly $47.4 \pm 4.94 \text{ kJ/mol}$, which is approximately the amount of energy required for one “step” of a myosin motor [Wac98]. Myosin motors are known to have an efficiency of about 50%, so assuming that the actual energy used by the motors is $23.7 \pm 2.47 \text{ kJ/mol}$ per step and that an even amount of force is required per step in the cell, we find that it takes about 340 ± 42 times the energy of one “step” to move the granule along one long edge of the cell wall [kin].

5 Conclusion

This experiment explored simulated Brownian motion in microbeads suspended in viscous fluids, Brownian motion in onion cells, and active transport in onion cells. The diffusion coefficients determined in this work largely support Einstein’s theoretical work and Perrin’s experimental work on Brownian motion. The diffusion coefficient for granules undergoing Brownian motion in onion cells was found to be $(2.171 \pm 0.038) \times 10^{13} \text{ m}^2/\text{s}$ and the average velocity for granules undergoing active transport in onion cells was determined to be about $9500 \pm 580 \text{ nm/s}$. The work required to transport granules along the length of one cell was calculated to be about $0.0132 \pm 0.0009 \text{ fJ}$, which is about 340 times the energy released by hydrolyzing one molecule of ATP.

References

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