Observing Brownian Motion in 0.7 μm Polystyrene Particles

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Abstract

We investigated Brownian motion, the random thermal movement of particles, in 0.7 μm fluorescent polystyrene particles diluted in a fluid using video microscopy. By tracking particle trajectories across multiple trials, we measured mean squared displacement (MSD) over time and verified the expected linear relationship predicted by diffusion theory. From our data, we found an experimental diffusion constant of $D = (1.68 \pm 0.04) \times 10^{-12} \frac{m^2}{s}$, significantly higher than the theoretical value of $(6.23 \pm 0.47) \times 10^{-13} \frac{m^2}{s}$.

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I. INTRODUCTION

Robert Brown first observed Brownian motion in pollen particles in the 1820's. Einstein later quantified Brownian motion in 1905 [1]. He judged the random thermal movement of fluid molecules responsible, asserting that they collide randomly into the larger pollen particle—causing sporadic motion. Einstein argued that this motion was evidence of the atomic nature of matter. Later, the 1926 Nobel Prize in physics was given to Perrin for precisely measuring Brownian motion [1]. Brownian motion is used today as a probe of a particle's environment, where researchers work backwards from a larger particle's motion to deduce what is happening around it. It has also been used to examine motion in living cells [1].

II. THEORY

A. Deriving the diffusion constant

In our experiment, we used a microscope and a CCD camera to observe the movement of 0.7μm polystyrene particles in a fluid sample. In the following theory, we show how we derive the diffusion constant of the liquid from the mean squared displacement of the polystyrene particles. We first use a simple 1D model from statistical mechanics to explore how particle diffusion changes with its environment's properties. Our particles are subject to random impulses that we shall call $F(t)$ and a dissipative drag force from the fluid. Thus, in one dimension,

$$m\frac{d^2x}{dt^2} = -6\pi a\eta\frac{dx}{dt} + F(t) \tag{1}$$

Here, the drag force is modelled by Stoke's law where a is the particle radius and η is the fluid viscosity. Our goal is to manipulate this equation to find an equation for the mean squared displacement $< x^2 >$ as a function of time. To do this, our first technique is to multiply the equation by x to find:

$$mx\frac{d^2x}{dt^2} = -6\pi a\eta x\frac{dx}{dt} + xF(t) \tag{2}$$

We can rearrange the second derivative to get:

$$mx\frac{d^2x}{dt^2} = m\frac{d}{dt}(x\frac{dx}{dt}) - mv^2 = -6\pi a\eta x\frac{dx}{dt} + xF(t) \tag{3}$$

We can use the equipartition theorem, $\frac{1}{2}mv^2 = \frac{1}{2}kT$ where k is Boltzmann's constant to remove our previous equation's dependence on v^2 which is much harder to measure than temperature T. We can also take the average of our equation over time and since $< xF(t) >= 0$ because $F(t)$ is a random force, we obtain:

$$\frac{m}{2} < \frac{d}{dt}(x\frac{dx}{dt}) > -kT = -3\pi a\eta < \frac{dx^2}{dt} > \tag{4}$$

Rearranging terms, we find:

$$\frac{m}{2} < \frac{d}{dt}(x\frac{dx}{dt}) > +3\pi a\eta < \frac{dx^2}{dt} >= kT \tag{5}$$

Using a substitution for $< \frac{dx^2}{dt^2} >$ and integrating, we obtain our final desired result:

$$< x^2 >= \frac{2kTt}{6\pi a\eta} = 2Dt \tag{6}$$

$D = \frac{kT}{6\pi a\eta}$ is our desired diffusion constant for the 1D model. In order to derive the mean squared displacement of our particles, we approximate them as confined to 2 dimensions. This approximation will be more justifiable to the reader after reading our methods. To extend equation 6 to two dimensions, we can add a factor of 2 because the motion in each direction is not correlated, and thus $< r^2 >=< x^2 + y^2 >=< x^2 > + < y^2 >= 4Dt$ for two dimensions. An important quality of this equation is that the MSD plot of the Brownian motion is linear in time t.

B. Calculating our diffusion constant

For our experiment, we can now estimate a value for D using the result of Equation 6 that $D = \frac{kT}{6\pi a\eta}$. Using a room temperature of $293 \pm 2\,K$, estimating the fluid viscosity to be water's fluid viscosity of $(1.0 \pm 0.05) \times 10^{-3}\ \frac{kg}{ms}$, and taking a, the radius of the polystyrene particles, to be $(0.35 \pm 0.02) \times 10^{-6}\,m$, gives us a diffusion constant of $(6.23 \pm 0.47) \times 10^{-13}\ \frac{m^2}{s}$.

III. EXPERIMENTAL METHODS

A. Sample

We first took prepared sample vials that held a solution with diluted $0.7\,\mu$m polystyrene particles and extracted a small amount of liquid with a Gilson W61244L micropipette. The

sample was diluted with water 100X compared to the original solution. This was done so the polystyrene particles would interact less, and the camera could track individual particles more easily. We then placed one drop on a microscope slide in between two roughly 1 in squares of Scotch tape roughly $\frac{3}{4}$ in apart that act as spacers. We then placed the cover slip flat and centered over the drop so that it was held by both pieces of tape. We used a small drop that did not spread to the edges of the slide, reducing external forces. We then placed the prepared slide under the microscope.

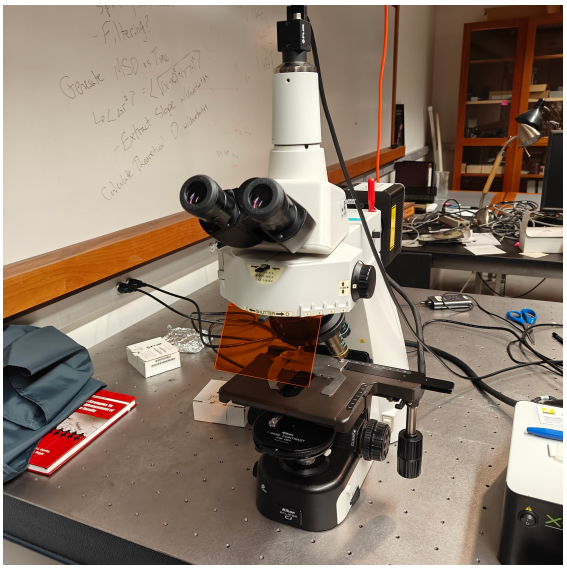

FIG. 1. Nikon Eclipse Ci optical microscope with 10X eyepiece connected to CCD camera visible at the top of the figure, and the objective visible in the middle of the figure.

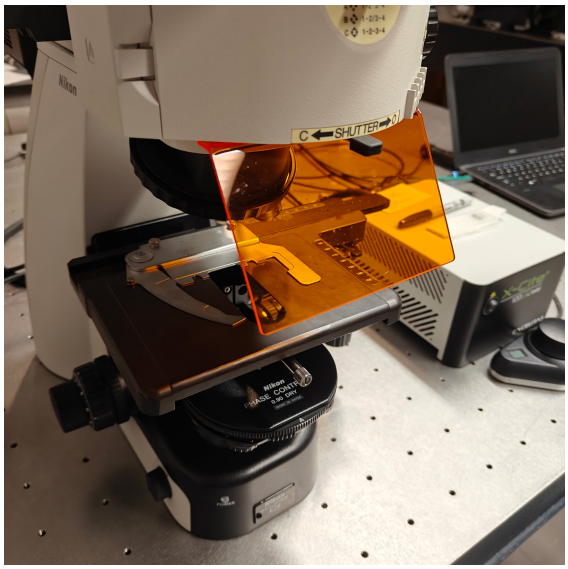

FIG. 2. Close up of Nikon Eclipse Ci optical microscope sample stage.

B. Data Acquisition

We used a Nikon Eclipse Ci optical microscope (shown in Figure 1) to observe our prepared sample. We used a UV light to make the particles easier to see (since they are florescent and shine green under UV light) and viewed the sample through the 10X objective. We adjusted the stage in x and y translation and focus as well as possible. Then, we switched to the 40X objective and used the fine focus to find a field of view of many jiggling bright particles without clumps of unmoving particles.

Once a good visual sample of moving particles was in focus under the microscope, we switched the UV light to a bright white backlight and set the microscope to send the image through a 10X eyepiece coupled to a CCD camera to the computer. We then used Matlab with the runflir.m file to record videos of the particles moving, in which 1 μm corresponded to 7.5 $\pm$ 0.5 pixels in our digital images as determined using a stage micrometer. We set the parameters for acquisition time, frames per second, and the file name to be 10 seconds, 20 fps, and trialx (where x is the number of trial) respectively. We then ran the program 3 times each for 2 different samples. In between each trial, we adjusted the microscope to a different part of the drop.

C. Image Processing

Once we collected the 200 frames per trial, we had scripts to detect particle position by their pixel and use our pixel to μm conversion ratio mentioned before to find the real x, y location of each particle after improving image contrast. Particles were detected if they met a certain brightness and size threshold, and all non-filtered identified particles of a sample frame can be seen as a pink dot in Figure 3.

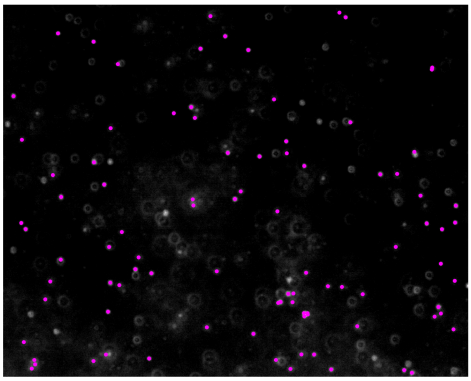

FIG. 3. A single frame of a sample where pink dots represent detected particles before filtering out clusters.

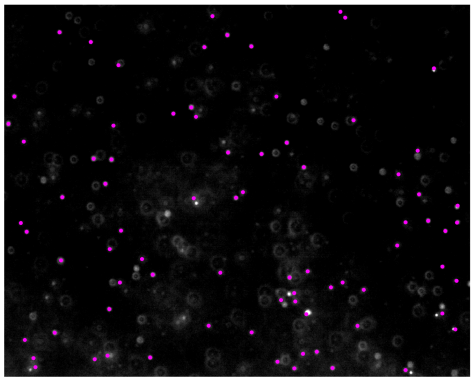

FIG. 4. A single frame of a sample where pink dots represent detected particles after filtering out clusters.

Particles were then tracked across the 200 frames by identifying particles that were in similar locations in neighboring frames. Certain particles were filtered out if they were in clusters as it would be nontrivial to determine their locations in future frames because any

one particle nearby in a future frame could reasonably be any of those in the cluster in the current frame. The remaining non-filtered particles can be seen in Figure 4. Note that clusters are now identified by a single pink dot.

Almost all of the particles drifted in and out of focus throughout our 200 frames. Only particles that were tracked for a minimum number of 150 frames were used in the final data analysis, and it can be seen in Figure 5 that the number of tracked particles rapidly drops off with how long they stayed in focus. Particles that were not moving were also excluded from data analysis. These particles were likely adhered to the slide or the tape, so they did not exhibit Brownian motion like the other particles.

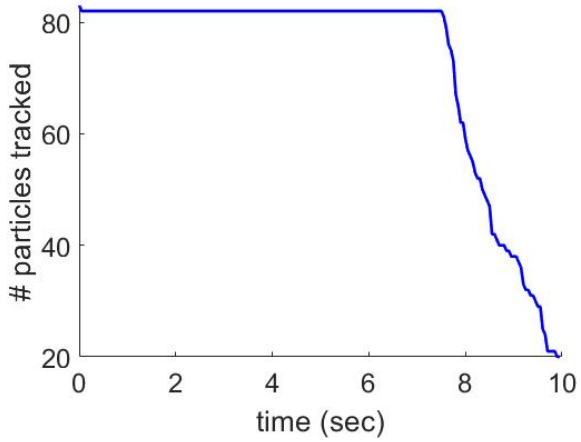

FIG. 5. Number of particle trajectories versus zeroed time. This figure was created after filtering for only particles that were in focus at least 7.5 seconds.

IV. RESULTS AND ANALYSIS

The trajectories of a single particle and all non-filtered particles in a sample trial can be seen in Figures 6 and 7 respectively. It can be seen that each particular particle path appears to be effectively random with any pattern being hard to identify. In Figure 7, some curves are on top of each other because particles could occupy the same space at different times.

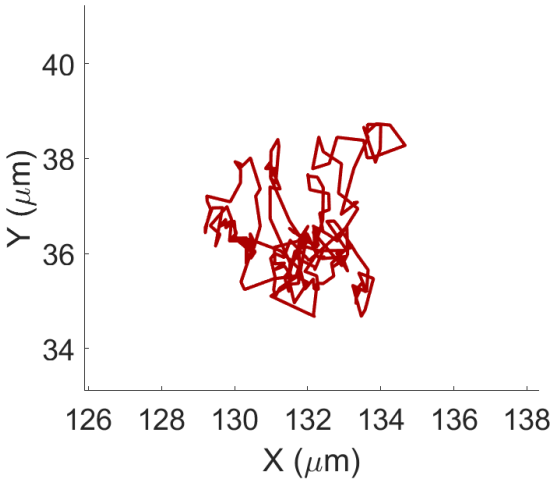

FIG. 6. The trajectory of a single sample particle.

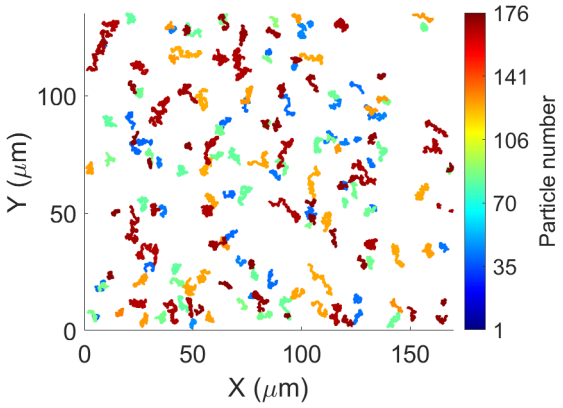

FIG. 7. The trajectories of all non-filtered particles in a trial. Particle number is an arbitrary tracking number used to identify different particles in our software.

Similarly, plotting the mean squared displacement of a single particle over time is illuminating and appears very sporadic. This is shown in Figure 8. This particle stayed in focus for almost all of the 200 frames, and its mean squared displacement is simply its displacement from its location in the initial frame squared.

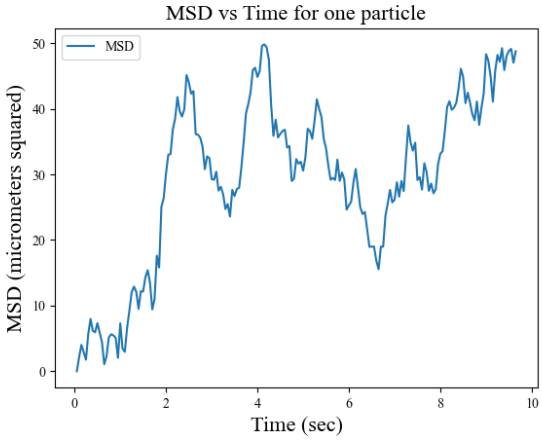

FIG. 8. The mean squared displacement plotted against time for a single sample particle in a trial.

However, when we find the mean squared displacement of all particles across all 12 of our trials over time, the relationship shows a very linear pattern for the first 7.5 seconds as shown in Figure 9. After this point, the data is unclear, this could be due to a range

of factors, perhaps including the rapidly decreasing amount of data at this point or the possibility that our two dimensional assumption begins to break down.

This plot however, allows us to find a linear relationship between MSD and time, and thus, from the theory, we should then be able to find our diffusion constant by simply dividing the slope by four. We found the slope by finding a best fit line for the first 100 data. A slope of $6.73 \pm 0.15 \frac{\mu m^2}{s}$ was measured from the first 100 frames. So, we find that our experimental diffusion constant, $D = (1.68 \pm 0.04) \times 10^{-12} \frac{m^2}{s}$.

This is very different than our theoretically found diffusion constant of $(6.23 \pm 0.47) \times 10^{-13} \frac{m^2}{s}$.

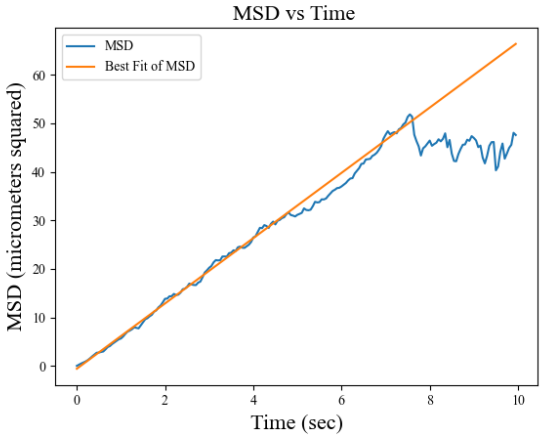

FIG. 9. The mean squared displacement of particles across all trials that were in focus for more than 7.5 seconds plotted against time. A best fit line is fitted to the first 100 data points and is shown. It has a slope of $6.73 \pm 0.15 \frac{\mu m^2}{s}$

V. CONCLUSIONS

The relationship between mean squared displacement and time of particles due to Brownian motion has historically been shown to be linear. We used microscope and CCD camera to investigate the movement of polystyrene particles in a fluid sample and found an experimental relationship between MSD and time. Our results reinforced that the relationship is linear, but our calculated diffusion constant varied significantly from our theoretical value. This error could come from a variety of sources including water currents in the sample or errors in image processing. This experiment could be repeated with other fluid samples with

different viscosities to explore if the linear relationship is maintained in different fluids. The data could also be reprocessed or acquired with alternative methods to determine if there is an error in our data acquisition or image processing. Tracking the particles in 3 dimensions would be a further improvement to ensure that our theory extends to the 3 dimensional reality in which we live, though the setup would likely be significantly more complex.

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[1] *Tracking Brownian motion through video microscopy–Advanced Laboratory*, Physics Department, Swarthmore College, unpublished (2024).