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PHYSIC 607: Experiments in Squishy Physics Fall 2025

Lab 1: Brownian Motion

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Abstract

Brownian motion was experimentally demonstrated using a 1:100 solution of 1.0 μm polystyrene microspheres and water. The solution was recorded via a Zeiss AxioObserver Z1 inverted fluorescence microscope and the particles were tracked and processed via 1 particle microrheology software. An experimental diffusion coefficient of $0.5507\ \frac{\mu m^2}{s}$ was calculated against a theoretical value of $0.4916\ \frac{\mu m^2}{s}$.

1 Background

Brownian motion describes the random motion of particles that are suspended in a medium, such as water or helium. It was first observed by Robert Brown (a Scottish Physicist) in 1827 via pollen in water; and was later used by Albert Einstein in 1905 where he modeled Brownian Motion. To describe this kind of motion mathematically, the Stokes-Einstein formula was discovered and is

$$D = \left(\frac{K_B T}{6\eta\pi R}\right)$$

where D is the diffusion coefficient, K_B is the Boltzmann constant, η is viscosity of the medium, R is the radius of the microspheres, and T is the absolute temperature of the room the experiment is performed in.

A theoretical value of the diffusion coefficient was calculated using the parameters from the experiment. Noting that the temperature of the laboratory was

$$T_{room} = 297.5\,K$$

and the viscosity of the solution the polystyrene microspheres were suspended in was

$$\eta_{H_2 0} = 0.893 \times 10^{-3}\,Pa \cdot s$$

at the room's temperature. Using polystyrene microspheres with a radius of

$$R_{spheres} = 0.5\mu m$$

a theoretical diffusion coefficient was calculated to be:

$$D_{theory} = 0.4916\,\frac{\mu m^2}{s}$$

2 Materials and Methods

A suspension of Invitrogen 1.0 µm polystyrene microspheres, fluorescently labeled (2% solids in distilled water), were prepared for imaging. The polystyrene microspheres were dyed to a "yellow-green" color that is able to appear under a microscope while using the green fluorescent protein (GFP) settings. The GFP setting on the microscope applies a filter so only light around the 510 nm range is visible. To create an imaging-compatible solution, 10 µL of the stock suspension was diluted with 990 µL of deinonized water. After, 25 µL of the diluted sample was pipetted using an Accumax pipette onto a 75 x 25 mm glass slide from Fisher Scientific. The sample was then sealed using vacuum grease and covered with an 18 × 18 mm Zeiss glass coverslip. It is important to note that if the grease is applied incorrectly, it can cause an incorrect pressure in the sample, causing systematic drift.

Two videos of Brownian motion were recorded using a Zeiss AxioObserver Z1 inverted fluorescence microscope: one at 14 fps for 320 frames, and another at 28 fps for 500 frames. It was observed that the concentration of microspheres in the sample was higher than in comparable samples prepared by other groups. Image sequences were processed in MATLAB via standard particle tracking routines.

Processing consisted of locating particle centers in each frame, tracking the particle movement across each frame, and using the position of each microsphere as a function of time to calculate the mean squared displacements and diffusion constant.

The data from the Zeiss AxioObserver Z1 microscope was processed and analyzed using a software suite created by Vincent Pelletier and Maria Kilfoil. The software suite was written in 2007 and utilizes MATLAB as its base. Minor adjustments were made to the software to replace legacy code with currently supported MATLAB functions. The main processing element of the code was not changed. The changed elements included updating analysis deliverables to output to .xlsx files to support larger data sets than what was supported when the program was written, as well as updating .tiff file name indexing and calling schemes.

The program was initially run with suggested parameters from the user manual, but the parameters were refined to improve processing power and memory allocation. The first frame was used to find particles that fit the appropriate parameters to track throughout the program. A feature size of 0.5 μm was used with a minimum integrated intensity of 100 and a maximum gyration (squared) of 7 was used. Maximum eccentricity of 0.1 and a minimum ratio of integrated intensity to radius of gyration squared of 1 was used to filter out particles. Further, no minimum intensity of local maxima was considered and a threshold of 30 was used to cut out integrated intensity.

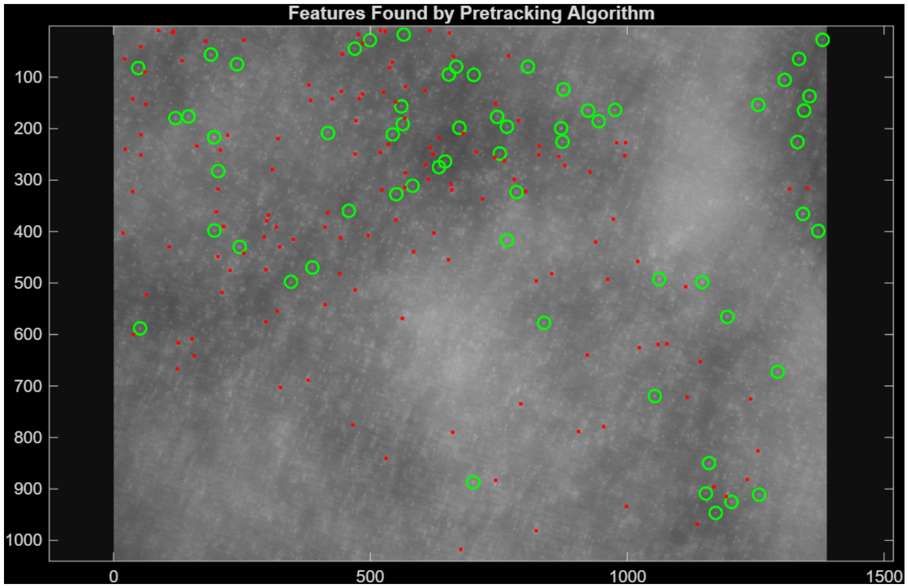

Figure 1: A plot of accepted and rejected beads against the solution of water and 1.0 μm polystyrene microspheres

203 features were found and pretracked using these parameters. Throughout pretracking, 63 features were kept with a minimum intensity of 502.9779. Maximum Rg was 6.9748 and maximum eccentricity was 0.098212, all of which were calculated by the program.

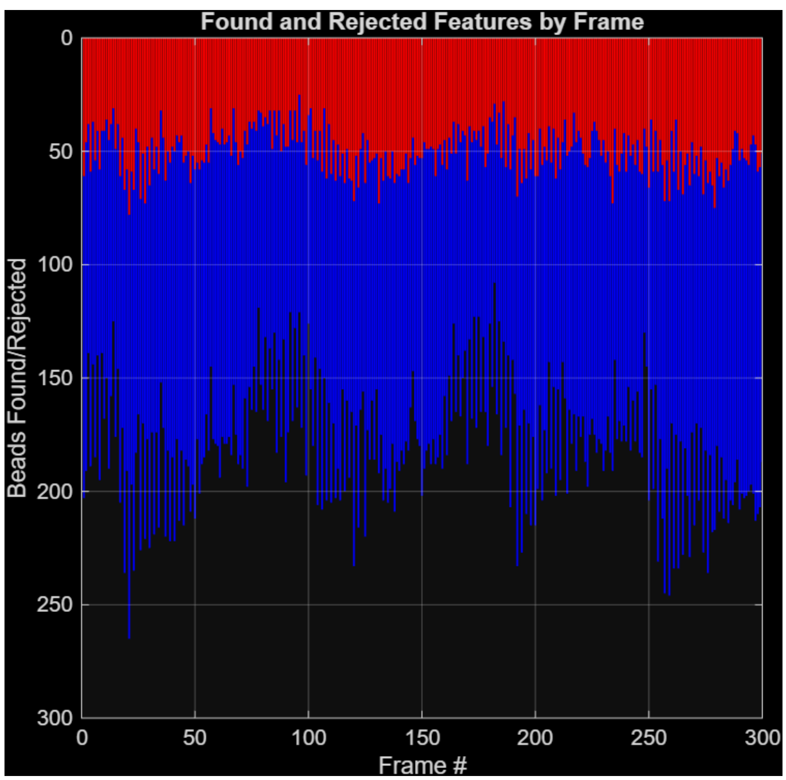

Figure 2: Found and rejected bead counts for each frame. Each bead in each frame was compared against the acceptable parameters to only include beads with a specific intensity, radius, gyration and eccentricity.

The features were then tracked frame by frame and their trajectories were calculated by minimizing the sum of the distances between features for two consecutive frames. For features not found in consecutive frames, their distances were assigned to the maximum allowable displacement parameter when running the code which was set to 10 pixels allowing a possible displacement of one radius of the bead. Additionally, features were only kept if they were found within 3 to 7 consecutive frames. Since the diameter of the bead was 1 μm or 7 pixels, these parameters were used to allow tracking between lost frames within one bead length of distance.

The beads were then dedrifted using one particle microrheology methods to remove non-Brownian motion from their trajectories. Among the 300 frames that were analyzed, a total of 1351 individual beads were found. The mean squared displacement (MSD) and lag time (τ) was then calculated for each bead between the 300 frames.

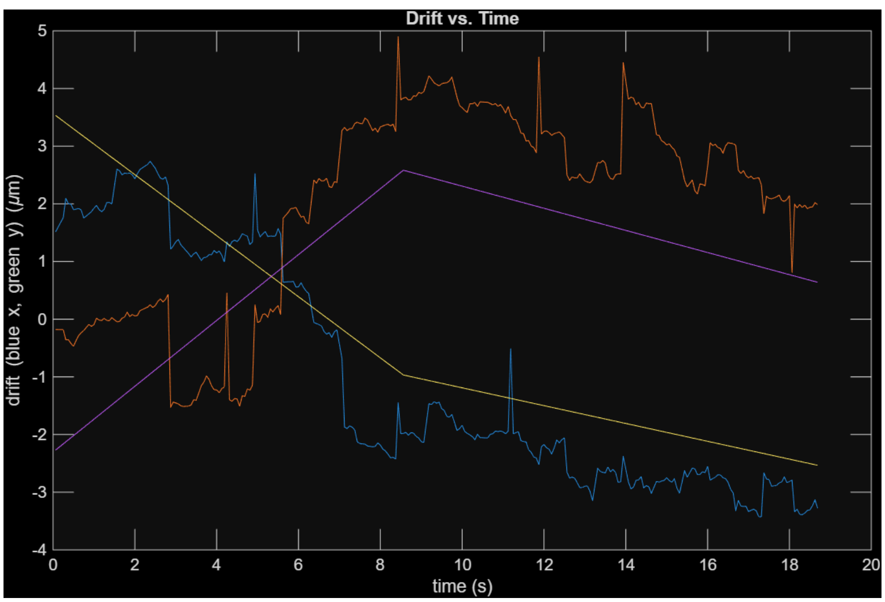

Figure 3: Average drift against lag time for the beads of polystyrene microspheres suspended in distilled water.

3 Experimental Results and Analysis

Individual squared displacements (MSD) were plotted against lag time for the 14 fps movie with an additional means squared displacement against lag time. A linear regression model was fitted over the first 2.42857 seconds of lag time due to the linearity of the MSD vs τ function.

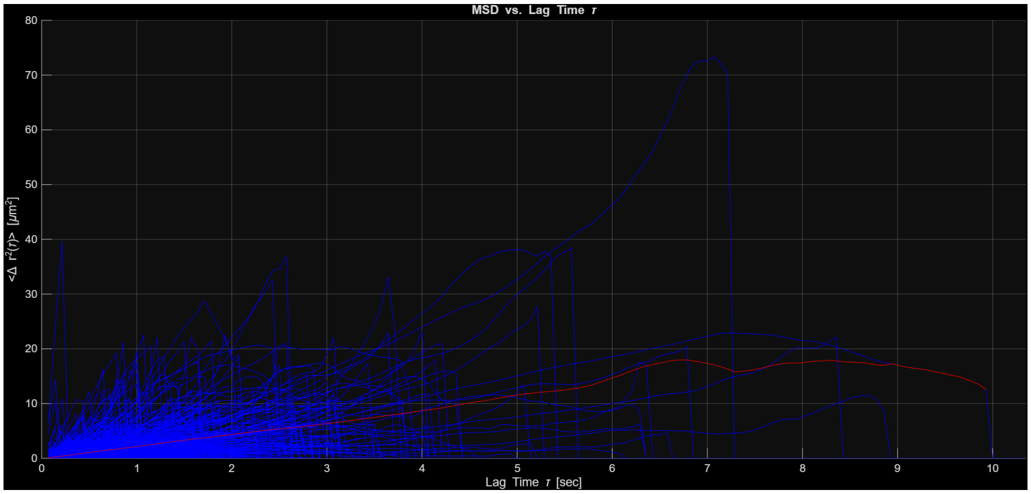

Figure 4: MSD against lag time (blue) for each individual particle along with average MSD versus lag time (red). Only the first 2.42857 seconds of the average MSD vs lag time was used for linear regression.

It was observed that after the 2.42857 second lag time, not enough statistical data was present to continue with in depth analysis due to the low likelihood of higher displacement counts per lag time. The linear regression model calculated a slope of

$$slope\ =\ 2.2029\ \frac{\mu m^2}{s}$$

and an estimated y-intercept of

$$y_{int}\ =\ -\ 0.037856\ \frac{\mu m^2}{s}$$

Noting that two-dimensional particle tracking was conducted, a diffusion coefficient was calculated to be

$$D_{exp}\ =\ 0.5507\ \frac{\mu m^2}{s}$$

with a percent difference of

$$\%_{diff}\ =\ 12.02\ \%$$

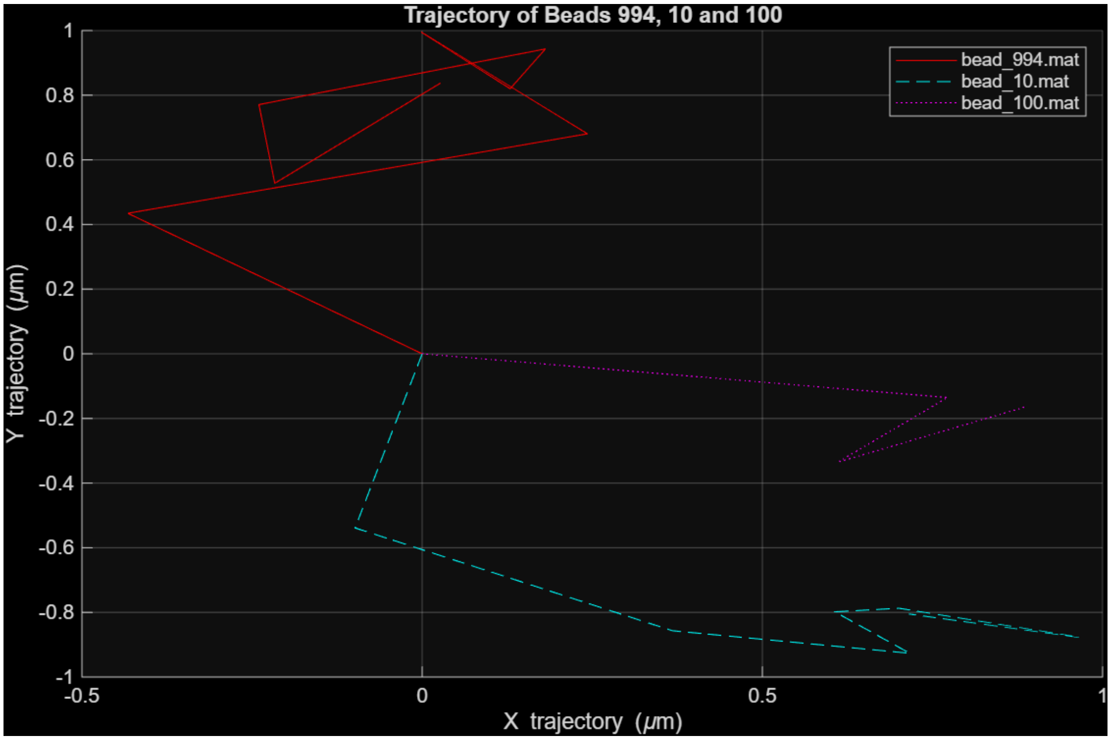

Figure 5: Representative 2D trajectory of microsphere. Plot begins at origin. The random, non-directional nature of the motion is characteristic of Brownian motion in colloidal systems.

In addition to analyzing the MSD, we quantified the step statistics between successive frames for all tracked particle motion. The average step size was found to be

$$\overline{x} = 1.127\ \mu m$$

with an average squared step size of

$$\overline{x^2} = 1.270\ \mu m^2$$

The average velocity was calculated by dividing each step size by the time interval between frames:

$$\overline{v} = -1.495 \times 10^3 \frac{\mu m}{s}$$

and the average squared velocity was

$$\overline{v^2} = 5.36 \times 10^7 \left(\frac{\mu m}{s}\right)^2$$

These statistics provide a direct quantitative measure of the random motion exhibited by the colloidal particles and are in line with the expected nature of Brownian motion.

4 Discussion

This experiment was aimed to observe Brownian motion of polystyrene microspheres in water and calculate the diffusion coefficient for comparison with theoretical prediction. The diffusion coefficient was determined to be $0.5507\ \frac{\mu m^2}{s}$ with a percent difference of 12.02% from the theoretical value of $0.4916\ \frac{\mu m^2}{s}$, which was calculated using the Stoke Einstein equation. The error in the results can be explained by multiple factors including sample preparation concentration, temperature, slide contamination and limitation in data collection.

The solution was observed to have a relatively high concentration of particles compared to other groups. This can lead to particle to particle interactions which could have increased the observed mean squared displacement (MSD) and subsequently the diffusion coefficient. To prevent this in the future, stiring the solution may provide a better sample with less clustering. Environmental conditions also played a role in influencing the results. The room temperature was recorded after the experiment and assumed to remain constant from the time the experiment was conducted to the time the temperature was measured. A small change in temperature would affect the kinetic energy of the microsperes and viscosity of the water which will directly influence the theoretical and experimental value for the diffusion coefficient.

Impurities on the microscope slides also contributed to the slight deviation in the experimental result. The slides used in this experiment were contaminated, then decontaminated, with dust. There is a small possibility that the slides were not fully decontaminated and some beads that were tracked were dust particles instead of polystyrene microspheres which would have introduced additional noise into the data set and potentially altered the diffusion coefficient calculation. Mean Square Displacement (MSD) values showed a clear linear relationship with lag time only within the first 2.4 seconds of measurement. Beyond this point, the reliability of the data decreased due to fewer observed displacements per lag time, limiting the precision of the diffusion coefficient determination. Collecting longer-duration videos or increasing the frame rate of data collection would provide more robust datasets and extend the range over which linear regression could be applied.

The calculated average step size $1.127\ \mu m$ and squared step size $1.270\ \mu m^2$ reflect the expected behavior for micron-scale Brownian particles observed at our experimental frame rate. The negative value of the average velocity $-\ 1.495 \times 10^3 \frac{\mu m}{s}$ suggests the presence of some residual systematic drift or an artifact of the dedrifting procedure since ideal Brownian motion should have a mean velocity close to zero. The high value of the average squared velocity $5.36 \times 10^7 (\frac{\mu m}{s})^2$ is characteristic of the broad distribution of instantaneous velocities seen in Brownian motion, further supporting the nature of the observed trajectories.

The step size and velocity statistics reinforce our observation of random, diffusive behavior and align with the linear growth of mean squared displacement with lag time. Any small deviations from ideal expectations may be attributed to residual drift, sample overcrowding, or uncertainties in the drift removal and particle linking processes. Such factors are inherent in video-based particle tracking experiments and were considered in our analysis. Overall, these results confirm the presence of Brownian motion and the reliability of our experimental and analytical approach.

The experiment successfully demonstrated Brownian motion. Though some errors were observed, the experimental and theoretical diffusion coefficients confirm the nature of Brownian motion and the effectiveness of particle tracking as a method for studying microscopic transport phenomena.

5 Author Contributions

All authors contributed equally to this experiment and the write-up. All authors analyzed the data separately to verify proper analysis techniques within the program. The final analysis deliverables were provided by N. Girard. N. Girard, M. Dunn and R. Tetteh conducted sample preparation. M. Dunn conducted the microscopy and logging materials used. R. Lecuyer was unavailable for data generation due to scheduling conflicts. N. Girard wrote the abstract. R. Lecuyer contributed to the background, materials and methods and the discussion. M. Dunn contributed to materials and methods and experimental results and analysis. R. Tetteh contributed to the abstract and the discussion. N. Girard contributed to the background, materials and methods, experimental results and analysis, and the discussion.