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Brownian Motion

Lab Report 3

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Abstract

In this experiment we investigate the Brownian motion of microscopic particles suspended in a fluid by tracking their movement under a microscope. Using Image-J tracking software to measure the position of the particles at regular intervals, we were able to determine the Boltzmann constant and the diffusion constant of the particles by two different approaches - particle ensemble analysis and time ensemble analysis. Additionally, the Boltzmann constant and diffusion constant were calculated from the step length distribution of the particles, assuming their movement follows a two-dimensional random walk model.

Introduction

Brownian motion refers to the random movement of colloidal particles suspended in a fluid, first observed by the botanist Robert Brown in 1827 while studying pollen grains in water. Though it was initially attributed to biological activity, Albert Einstein later provided a theoretical explanation in 1905 that demonstrated that the motion was caused by collisions between the particles and the molecules of the surrounding fluid. This discovery played a significant role in confirming the existence of atoms and molecules, bridging the gap between kinetic theory and thermodynamics. Brownian motion is now widely understood as a fundamental example of stochastic processes and serves as a basis for studying the thermal energy of particles. The aims of this experiment are to:

- Observe and track the Brownian motion of particles under a microscope.
- Determine the Boltzmann constant and diffusion constant through both particle ensemble and time ensemble analysis.
- Calculate the Boltzmann constant and diffusion constant from the step length distribution of the particle (assuming a 2D random walk).

Theoretical Background

Consider a particle relatively large particle ($\approx 1\ \mu$m in diameter) in a fluid with much smaller molecules. Due to the thermal energy of the system, all the particles will have kinetic energy which they will constantly exchange via collisions. Let us assume that there are no interactions besides collisions between the particles.

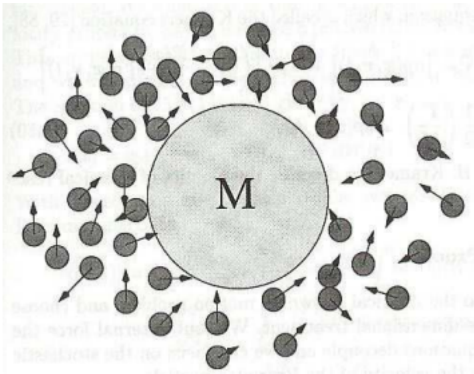

Figure 1: The Brownian particle of mass M will move relatively slower than the fluid molecules around it which will exert a random fluctuating force on it from all directions.
Source: p.76, Non-equilibrium Statistical Mechanics, Zwanzig, R. (2001)

In such a solution, the large particle will experience a drag force as it moves through the fluid with a velocity v. In addition to the drag, let us assume that the molecules exert a random fluctuating force $F(t)$ on the particle from all sides (figure 1). This allows us to write the equations of motion (known as the Langevin equations) for this particle:

$$\frac{dx}{dt} = v \tag{1}$$

$$m\frac{dv}{dt} = -\gamma v + F(t) \tag{2}$$

Where γ is the Stokes law drag coefficient for a spherical body, given by:

$$\gamma = 6\pi\eta R \tag{3}$$

In which η is the dynamic viscosity of the fluid at temperature T and R is the radius of the particle. Since the radius of the particle is much greater than the size of the fluid molecules, we can see that the drag force will dominate for the particle, causing it to move far slower than the molecules. Continuing from equation 2,

$$mx\frac{dv}{dt} = m\left[\frac{d(xv)}{dt} - v^2\right] = -\gamma xv + xF(t)$$

Taking the time average of both sides,

$$m\left[\frac{d\langle xv\rangle}{dt} - \langle v^2\rangle\right] = -\gamma\langle xv\rangle + \langle xF(t)\rangle$$

$$\implies m\frac{d\langle xv\rangle}{dt} = m\langle v^2\rangle - \gamma\langle xv\rangle + \langle xF(t)\rangle \tag{4}$$

Since the force $F(t)$ is independent of the position of the particle x, we can say $\langle xF(t)\rangle = \langle x\rangle\langle F(t)\rangle$. But $\langle x\rangle = 0$ for a particle Maxwellian fluid, so $\langle xF(t)\rangle = 0$.

We also know that for a Maxwellian fluid, the velocity distribution function for 1-dimension is given by:

$$f(v) = \left(\frac{m}{2\pi KT}\right)^{1/2} \exp\left(-\frac{mv^2}{2KT}\right)$$

Where m is the mass of the particle, K is the Boltzmann constant and T is the temperature of the fluid.

Using this, we can determine $\langle v^2 \rangle$:

$$\langle v^2 \rangle = \int_0^\infty v^2 f(v) dv = \frac{KT}{m}$$

$$\implies m\langle v^2 \rangle = KT$$

Hence, equation 4 simplifies to:

$$m\frac{d\langle xv \rangle}{dt} = KT - \gamma\langle xv \rangle$$

$$\implies \frac{m}{2}\frac{d^2\langle x^2 \rangle}{dt^2} + \frac{\gamma}{2}\frac{d\langle x^2 \rangle}{dt} = KT$$

Let $d\langle x^2 \rangle/dt = u$,

$$\frac{du}{dt} + \frac{\gamma}{m}u = \frac{2KT}{m}$$

The solution to this first order ordinary linear differential equation is:

$$u = C\exp\left(-\frac{\gamma t}{m}\right) + \frac{2KT}{\gamma}$$

If we consider the long term behaviour of a Brownian particle, $t \to \infty$ and m is small. So:

$$\frac{d\langle x^2 \rangle}{dt} = \frac{2KT}{\gamma}$$

$$\implies \langle x^2 \rangle = \frac{2KT}{\gamma}t$$

However, for 2-dimensions, the mean square displacement $\langle r^2 \rangle = 2\langle x^2 \rangle$ if we consider an isotropic system, so:

$$\langle r^2 \rangle = \frac{4KT}{\gamma}t$$

Substituting equation 3 into this, we finally get:

$$\langle r^2 \rangle = \frac{2KT}{3\pi\eta R}t \tag{5}$$

Let us define a constant $D = KT/6\pi\eta R$ which is known as the diffusion coefficient of the particle. Hence, we find:

$$\langle r^2 \rangle = 4Dt, \text{ where } D = \frac{KT}{6\pi\eta R} \tag{6}$$

From equation 6 it is clear that for a given particle of radius R in a fluid with dynamic viscosity η at temperature T, the mean square displacement (MSD or $\langle r^2 \rangle$) varies linearly with respect to time t.

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Experimental Setup

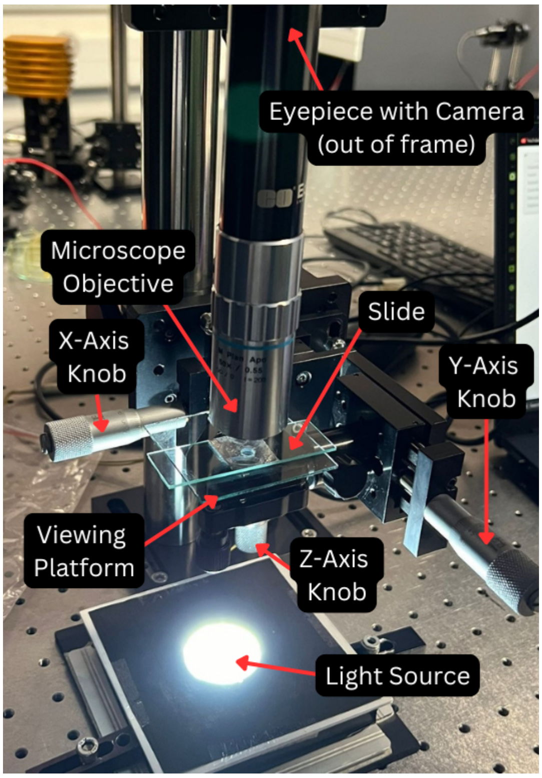

Figure 2: Experimental setup for Brownian motion

Instruments and materials used:

- Microscope
- Vibration isolation table
- Thor Labs CCD USB Camera
- Laptop with Micro-Manager and Image-J software
- Glass slides
- Aluminum tape
- Punch machine
- Cover slips
- Dropper
- Tweezers
- Falcon tubes
- Concentrated solution of 1 μm particles
- Distilled water
- Isopropyl alcohol (IPA)
- Clear nail polish
- Plane spirit level

- 1 mm scale slide with 100 div
- Gloves
- Thermometer

Least count of thermometer = 0.1°C

The setup for this experiment consists of a microscope with a 3-axis adjustable viewing platform and a Thor Labs CCD USB camera attached to the eyepiece (figure 2). The entire setup is placed on a vibration isolation table to dampen external perturbations from the floor. The glass slides are prepared by creating a viewing chamber from aluminum tape in which a small drop of diluted particle solution is put. The chamber is sealed with a cover slip and nail polish before being observed under the microscope. A laptop is connected to the camera via a USB cable and a live feed from the microscope can be viewed and recorded using the Micro-Manager software.

Procedure

Preparing Slides

1. Wear gloves before using tap water and tissue paper to rinse a glass slide. After the initial wash, wet a tissue with isopropyl alcohol and clean the slide. Rinse the alcohol off with distilled water and let the slide dry. Similarly rinse a cover slip.
2. Cut a small square of aluminum tape and use a punch machine to make a small hole in the center. Expose the adhesive side and stick the square in the middle of the slide.
3. Using a dropper, mix 1ml of the concentrated particle solution with 1ml of distilled water in a narrow falcon tube. Close the tube and shake it well until the solution is homogeneous.
4. Using a new dropper (to avoid the mixing of solutions) put a single small drop of the diluted solution in the hole of the tape on the slide.
5. Using tweezers, carefully lower the cover slip over the viewing chamber such that there are no air bubbles or fluid leaks. Apply clear nail polish along the edge of the cover slip to seal it and hold it in place. This prevents evaporation from affecting the motion of the particles in the solution.
6. Allow to slide to dry and the particles to settle into equilibrium before mounting them in the microscope.

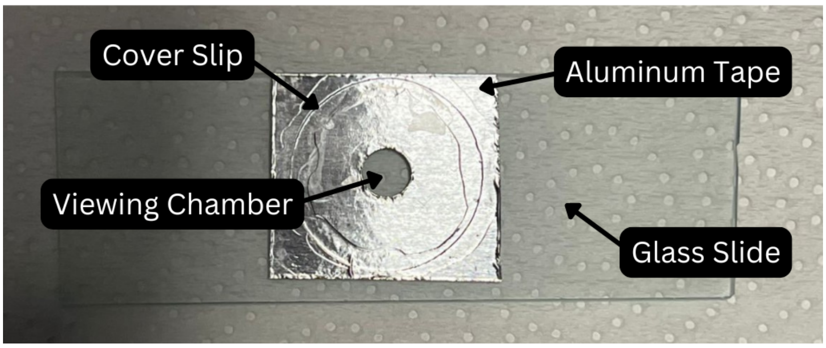

Figure 3: A fully prepared slide with a dilute solution of 1 μm particle in the viewing chamber

Calibration

1. Place the plane spirit level on the viewing platform and adjust the knobs under the platform until the bubble is centered.

2. Connect the USB cable of the camera to the laptop and launch Micro-Manager. Switch on the power supply for the microscope light source and click on 'Live' to get a live view of the microscope (figure 4).

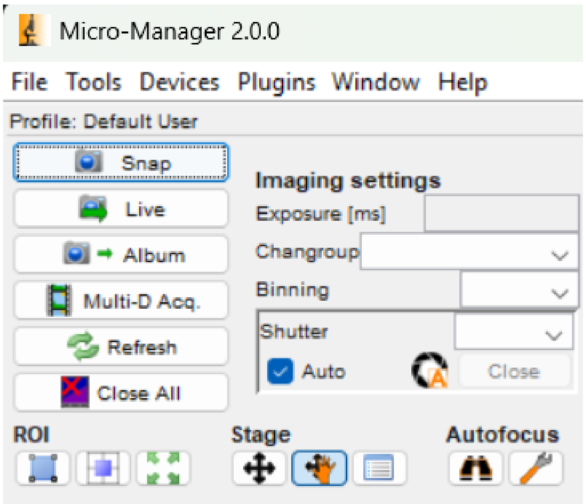

Figure 4: User interface of the Micro-Manager application

3. Place the 1 mm calibration scale slide under the objective of the microscope. Use the x and y axis screw gauge knobs to move the slide until the edge of the circle surrounding the scale comes into view.
4. Slowly adjust the z-axis knob until the edge of the circle comes into focus. Once the slide is in focus, use the x and y knobs to follow the circumference of the circle until you reach an intersecting radial line. Follow the radial line inwards until you locate the divisions of the scale (figure 5).

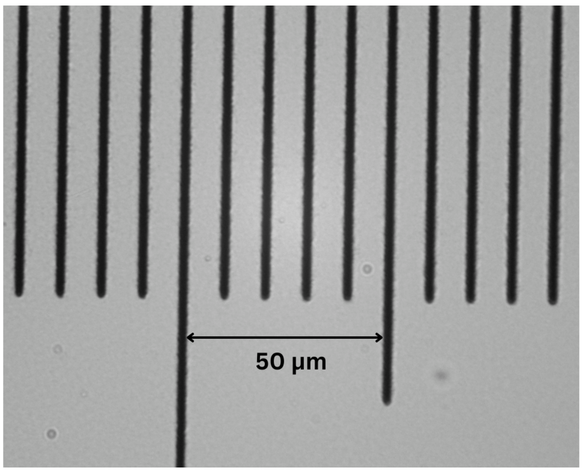

Figure 5: Calibration scale as viewed under the microscope. Each small division is equal to 10 microns.

5. Keeping several divisions of the scale within the frame, capture a photo of the live view with the 'Save' button. Each division of the scale is equal to 10 microns.
6. Using Image-J, find the average pixel to distance scale using the calibration image as a reference. This can be done using the line tool along with the 'Measure' function. Make several measurements on the calibration image and take their average to determine the mean conversion value.

7. Go to Analyse–>Set Scale to calibrate the software. Input the average pixel distance that corresponds to the real distance in microns. Select the global scale checkbox and click 'Ok' to complete calibration.

Observing Brownian Motion

1. Once the microscope has been levelled and calibrated, mount the slide with the micron-sized particle solution. Move the cell in the xy-plane until the edge of the viewing chamber comes into view and use the z-axis knob to sharpen the focus on the particles and slide.

2. Locate a region in which several particles seem to be performing Brownian motion. If the particles appear to drift in a single direction, check the levelling of the viewing platform and allow the slide to settle for some time. If the drift persists, prepare a new slide.

3. Before collecting data, measure the room temperature with the thermometer.

4. Keeping 3-4 particles in the center of the frame (to ensure they remain in frame until the end), click 'Multi-D Acq.' to intiate the data collection process (figure 6). Select the 'Time Points' checkbox and set the count to 600 and interval to 1s before clicking 'Acquire!'. This will capture 600 frames at the rate of 1 fps, meaning that the camera will record for a total of 10 min. If needed, change the exposure for the live feed while recording to improve the contrast between the particles and the background.

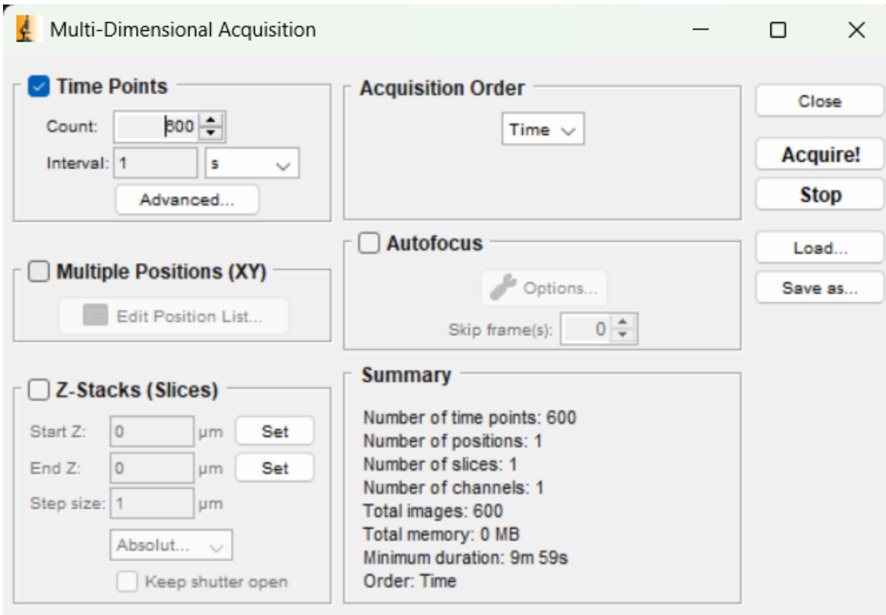

Figure 6: Multi-Dimensional Acquisition interface

5. Save the data as in image stack in a TIF file and repeat the above steps for at least 10 more slides. Every new data collection session requires fresh calibration and levelling prior to data collection.

Tracking Trajectories

1. Import the image stack to Image-J and go to Process–>Binary–>Make Binary–>Ok. This will transform the images into black and white based on contrast, effectively highlighting the particles.

2. Slide through the image stack and pick out the particles that remain in the frame for the entire duration of the video. Ensure that the particle that you choose do not get coupled at any point during the video and remain in focus so that it is easy to determine their position.

3. Go to Plugins–>Tracking–>Manual Tracking. Select 'Add Track' and click on the center of the particle you want to track (figure 7). This will cause the image stack to automatically move to the next frame and a new window recording the x and y position of the particle will open. Continue to click on the center of the particle and record the data for as many frames as possible.

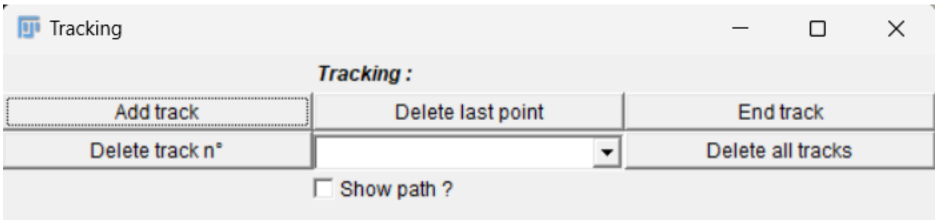

Figure 7: Manual tracking interface

4. Once the particle has been tracked for the maximum number of frames, select the raw data from Image-J and copy it into an CSV file. You can now delete the old track from Image-J and start a new trajectory.

5. In the CSV file, ensure that the values have been converted to microns using the calibration information. Also, set the initial position of every particle to zero by subtracting the initial x and y position from the entire data set. This will make your calculation of the particle's net displacement (r) simpler.

Observations and Analysis

We were able to track a total of 25 particles for roughly 500-600 frames each. Figure 8 shows the 2-dimensional trajectories of these particles as tracked using Image-J. We eliminated any data that displayed major signs of drift which was indicated by a strong bias towards one direction. Nonetheless, the distribution of the trajectories about the origin is slightly asymmetric since we are considering a finite number of particles. If we were to increase our sample size, we should expect the paths to approach a symmetric circular distribution around the origin.

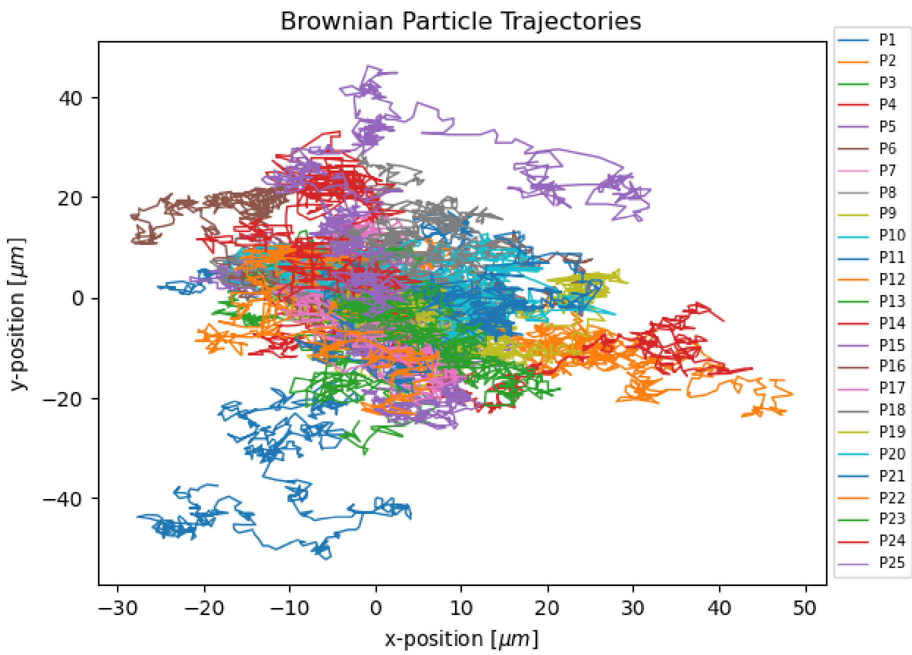

Figure 8: Though most of the trajectories appear to lie close to the origin, certain outliers stray outward as well. Though these outliers may stray far from the origin over long time scales, unlike particles with drift, they display unbiased motion over short time scales.

Particle Ensemble

Since each particle was tracked for a different number of frames depending on how long it was in view and in focus, we first trimmed the data to the length of the shortest data array to create a uniform dataset of both position and time. The net displacement of the particle r from the origin was found from the sum of the squares of the x and y position data (Note that we do not take $(x - x_0)$ and $(y - y_0)$ since we have already subtracted the initial position while creating our data sets):

$$r = \sqrt{x^2 + y^2}$$

For a particle ensemble, we consider the trajectories of several particles and find the mean square displacement (MSD) by taking the square of the displacement of all the particles and finding their mean.

$$\text{Mean Square Displacement} = \langle r^2 \rangle = \frac{\sum_{i=0}^{N} r_i^2}{N}$$

Note that the root mean square (RMS) displacement is the square root of the mean square displacement (i.e. $r_{rms}^2 = \langle r^2 \rangle$). This has been used in the labelling of some of the graphs. The standard deviation of the square displacement was also found in order to determine the error in r_{msd}.

$$\text{Standard Deviation of } r^2 = \sigma_{r^2} = \sqrt{\frac{\sum_{i=0}^{N} (r_i^2 - \langle r^2 \rangle)^2}{N}}$$

An MSD vs time graph was plotted using the data from 25 particles for 250 frames (figure 9).

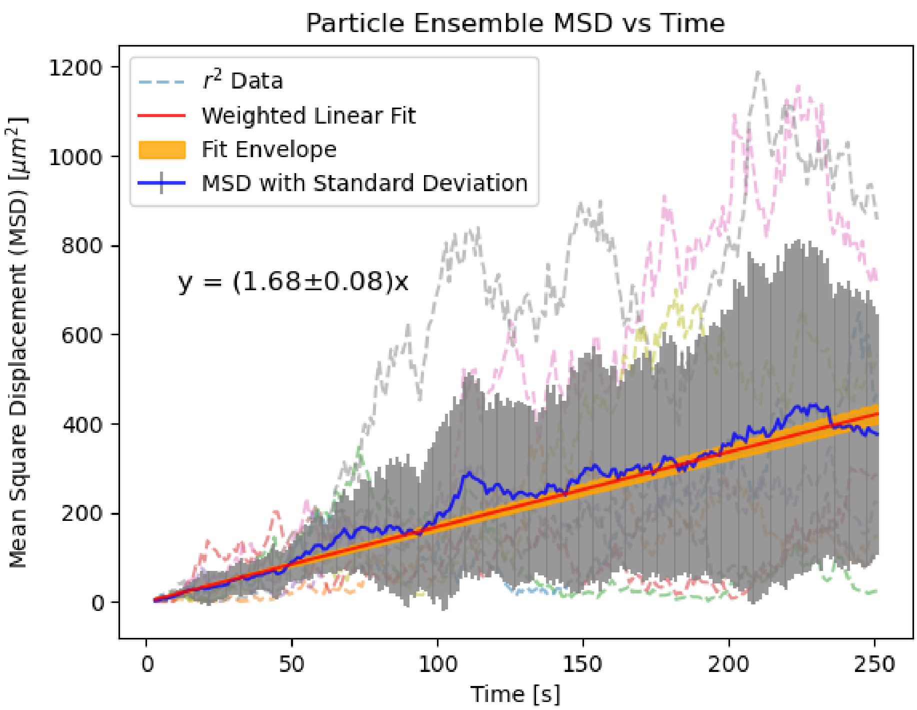

Figure 9: The fluctuations in the MSD have an upwards linear trend with respect to time. Note that the fluctuations and error increase for larger time since as the particle moves further away from the origin, it has a larger number of states that it can attain and therefore its mean can be determined with lower certainty.

We fit the data with the weighted curve-fit function from the `scipy.optimize` package using the single parameter model function $y = ax$ and the standard deviation error in the square displacement as Δy. This gave us a value of $a = 1.68 \pm 0.08$ μm^2/s (where the error in a was determined from the covariance matrix calculated by the curve-fit function). Hence,

$$\frac{\langle r^2 \rangle}{t} = 1.68 \pm 0.08 \ \mu\text{m}^2/\text{s}$$

Let us assume that the particles are performing an unbiased random walk in 2-dimensions. From the diffusion coefficient equation for a 2-dimensional random walk, we can show:

$$D = \frac{\langle r^2 \rangle}{4t} = 4.19 \times 10^{-13} \ \text{m}^2/\text{s}$$

Einstein's relation can be now be used to estimate the value of the Boltzmann constant:

$$K = \frac{6\pi\eta r D}{T} = 1.21 \times 10^{-23} \ \text{J/K}$$

We measured the room temperature to be 23.9°C, so $T = 296.9$ K and the dynamic viscosity of water at 24°C is $\eta = 9.1 \times 10^{-4}$ Pa·s. The radius of the particles was estimated to be $r = 0.5$ μm. This gave us the above value of the Boltzmann constant as found by considering an ensemble of particles performing Brownian motion.

Time Ensemble

Brownian motion is equivalent to an unbiased random walk which implies that the average behaviour of a single particle over a long time period should be equivalent to the behaviour of many particles over a short time period. We can convince ourselves of this by looking at the long term behaviour of a single particle. For an ensemble of particles, we expect the mean displacement to tend towards zero for a large number of particles. Similarly, for a single particle we observe that after a long time, the displacement of the particle reduces and the particle returns close to its origin (figure 10).

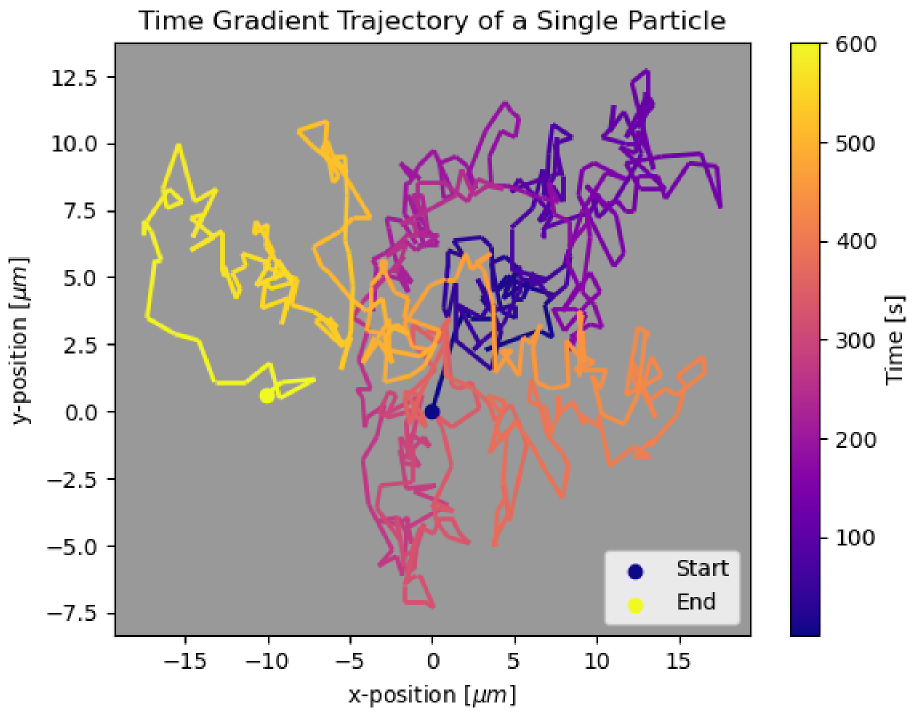

Figure 10: The colour gradient of the trajectory indicates how much time has elapsed since the particle started. We can see that after 600 seconds, the particle ends up quite close to its initial position at (0,0).

In the previous section, we determined the MSD to time ratio by averaging the behaviour of several different particles for a relatively short time period (250 seconds). Instead, we can consider the motion of a single particle over 600 seconds and determine the MSD to time ratio by considering sections of its path as the trajectories of different particles. This provides us with an alternate, less time intensive method of finding the diffusion coefficient and Boltzmann constant.

In order to create such an ensemble of particles in time, we split the 600 frames of a single particle into 60 sets of 10 frames each. Since the particle is performing a random walk throughout, it does not matter where we consider the start of the recording. Hence, we can set the initial value of each dataset to zero such that we have 60 sets of position data for 0-10 seconds. We now perform the identical analysis as the previous section by considering each data set a separate particle and plotting the MSD vs time for 10 seconds (figure 11).

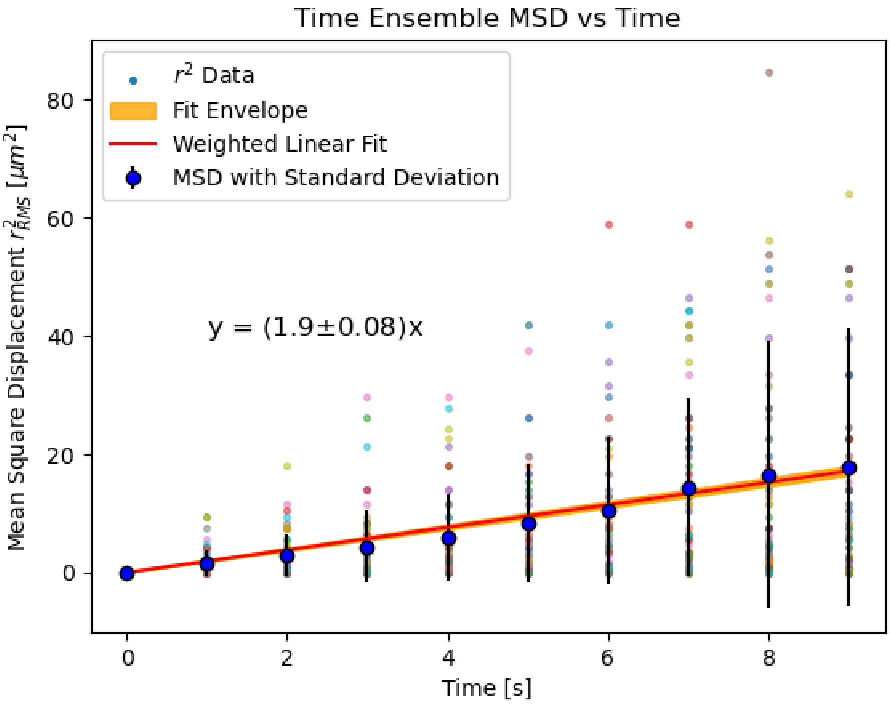

Figure 11: Though the time period over which the MSD varies appears to be lower, we have more 'dummy particles' to consider in the time ensemble compared to the ordinary particle ensemble. This compensates for the length of each data set, giving us a reasonable value for the slope.

A weighted linear fit was used to find the slope of the MSD with respect to time. This gave us:

$$\frac{\langle r^2 \rangle}{t} = 1.90 \pm 0.08 \ \mu\text{m}^2/\text{s}$$

Putting this value into the diffusion coefficient equation we get:

$$D = \frac{\langle r^2 \rangle}{4t} = 4.75 \times 10^{-13} \ \text{m}^2/\text{s}$$

Using the Einstein relation to find the value of the Boltzmann constant:

$$K = \frac{6\pi\eta r D}{T} = 1.37 \times 10^{-23} \ \text{J/K}$$

Where the constants are the same values as used in the previous section.

Probability Distribution

Alternatively, we can use the fact that the probability distribution of the step lengths is given by a Gaussian distribution to determine the diffusion coefficient of the particles:

$$P(\Delta r) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(\frac{-(\Delta r - \mu)^2}{2\sigma^2}\right)$$

Where the standard deviation $\sigma = \sqrt{2D\tau}$. In order to find this distribution, we first determine the step lengths by storing the difference between consecutive steps $\Delta r_i = r_{i+1} - r_i$ for all steps across all particles. We can then plot a histogram of these step lengths and fit a Gaussian function based on the mean and standard deviation of the data (figure 12).

$$\text{Mean Step Length} = \langle \Delta r \rangle = \frac{\sum\limits_{i=0}^{N} \Delta r_i}{N}$$

$$\text{Standard Deviation of Step Length} = \sigma_{\Delta r} = \sqrt{\frac{\sum\limits_{i=0}^{N} (\Delta r_i - \langle \Delta r \rangle)^2}{N}}$$

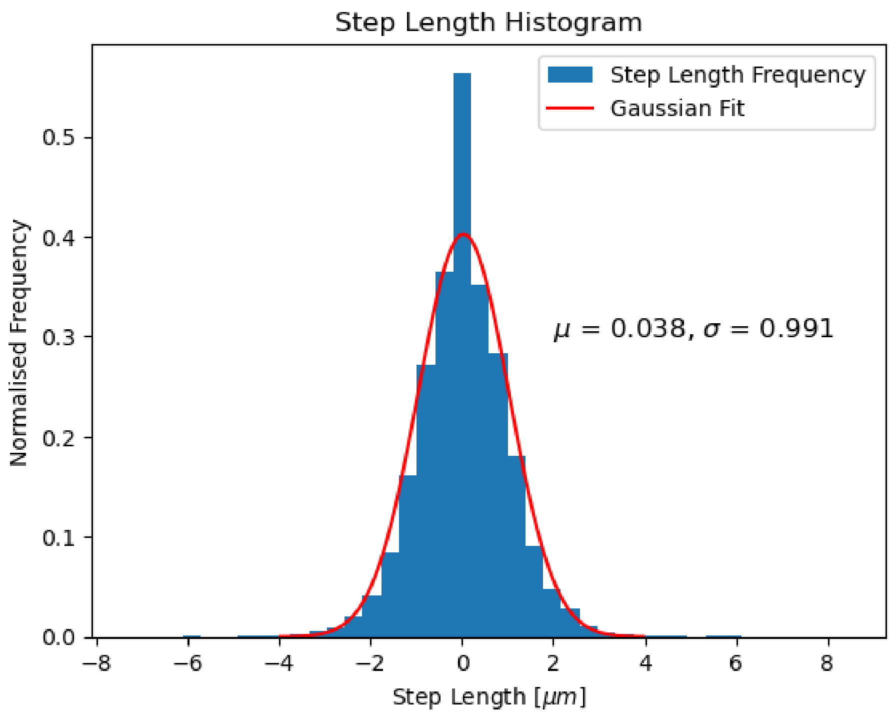

Figure 12: The mean of the distribution is not exactly zero since the data set is finite and hence there might be a slight bias. However, due to the large amount of steps considered (12500 in total), the data clearly has a Gaussian distribution.

We know that $\sigma = \sqrt{2D\tau}$ and for us, $\tau = 1$ s since it refers to the interval between each step. Therefore:

$$D = \frac{\sigma^2}{2\tau} = 4.91 \times 10^{-13} \text{ m}^2/\text{s}$$

And the Boltzmann constant calculated from this value of the diffusion coefficient is:

$$K = \frac{6\pi\eta r D}{T} = 1.42 \times 10^{-23} \text{ J/K}$$

Where the constants are the same values as used in the previous section.

Error Analysis

For both the particle and time ensemble, the error in the diffusion coefficient and Boltzmann constant originate from the error in the measurement of the slope $\langle r^2 \rangle / t$. This error was determined from the covariance matrix of the weighted linear fit using the curve-fit function of the `scipy.optimize` package. From quadrature,

$$\frac{\Delta D}{D} = \frac{\Delta \text{slope}}{\text{slope}} \tag{7}$$

For the particle ensemble we find,

$$\Delta D_1 = 0.2 \times 10^{-13} \text{ m}^2/\text{s}$$

For the time ensemble we find,

$$\Delta D_2 = 0.2 \times 10^{-13} \text{ m}^2/\text{s}$$

The Boltzmann constant K also contains the error in the temperature T and the viscosity η. The error in T is simply the least count error (0.1°C).

$$\frac{\Delta K}{K} = \sqrt{\left(\frac{\Delta D}{D}\right)^2 + \left(\frac{\Delta \eta}{\eta}\right)^2 + \left(\frac{\Delta T}{T}\right)^2}$$

Since $\eta \propto T \implies \frac{\Delta \eta}{\eta} = \frac{\Delta T}{T}$:

$$\frac{\Delta K}{K} = \sqrt{\left(\frac{\Delta D}{D}\right)^2 + 2\left(\frac{\Delta T}{T}\right)^2} \tag{8}$$

For the particle ensemble,

$$\Delta K_1 = 0.07 \times 10^{-23} \text{ J/K}$$

For the time ensemble,

$$\Delta K_2 = 0.07 \times 10^{-23} \text{ J/K}$$

The probability distribution method depends on the step length measurements and any any error in these measurements would propagate into the standard deviation of the Gaussian distribution. By quadrature,

$$\frac{\Delta D}{D} = \sqrt{2\left(\frac{\Delta \sigma}{\sigma}\right)^2}$$

Where we can approximate the error in the standard deviation to be:

$$\Delta \sigma = \frac{\Delta x}{\sqrt{2N}} \tag{9}$$

During calibration, we took several measurements of the scale and found their mean value to determine the calibration constant. The standard deviation of these measurements δx can be assumed to be the error in the measurement of x, so $\Delta x = 2\delta x$. For our calibration, $\delta x = 0.03$ μm, so $\Delta x = 0.06$ μm. The number of measurements for this histogram is $N = 12500$. The error in the diffusion constant is therefore:

$$\frac{\Delta D}{D} = \sqrt{2\left(\frac{\Delta x}{\sigma\sqrt{2N}}\right)^2} \tag{10}$$

For the probability distribution,

$$\Delta D_3 = 0.03 \times 10^{-13} \text{ m}^2/\text{s}$$

and from equation 8,

$$\Delta K_3 = 0.04 \times 10^{-23} \text{ J/K}$$

Results and Discussion

After tracking the 2-dimensional Brownian motion of colloidal particles in a solution under a microscope, we were able to determine the diffusion coefficient and Boltzmann constant by three separate methods - particle ensemble, time ensemble and probability distribution. Each of these methods gave us a value for both constants, tabulated below:

Results	Diffusion Constant (D) [m^2/s]	Boltzmann Constant (K) [J/K]
Particle Ensemble	$(4.2 \pm 0.2) \times 10^{-13}$	$(1.21 \pm 0.07) \times 10^{-23}$
Time Ensemble	$(4.8 \pm 0.2) \times 10^{-13}$	$(1.37 \pm 0.07) \times 10^{-23}$
Probability Distribution	$(4.91 \pm 0.03) \times 10^{-13}$	$(1.42 \pm 0.04) \times 10^{-23}$

Table 1: The probability distribution method appears to give us the highest precision

The percentage error of the Boltzmann constant for the particle ensemble, time ensemble and probability distribution are 12.3%, 0.5% and 2.8%, respectively. However, it should be noted that the absolute error is the least for the probability distribution since it has a larger sample size ($N = 12500$).

Additional sources of error could be:

1. The drift of the particles due to the presence of air bubbles, leaks or evaporation related flows (due to incorrect sealing of the viewing chamber) could potentially shift the mean displacement of the particles, affecting the long term MSD behaviour. However, we removed any data that appeared to display a bias towards one direction in order to remove drift.
2. Since Brownian motion is a stochastic process, a finite sample size will naturally contain random errors. Increasing the sample size would improve the MSD as it would converge onto the linear trend that we theoretically expect. By increasing the number of tracked particles we would be able to increase the sample size for the particle ensemble. The sample size for the time ensemble can be increased by recording a longer video which would give us a larger set of 'dummy particles' to analyse.
3. External perturbations due to vibrations from the table or floor during the recording process may have introduced some external forces to the system, changing the trajectories of the particles. These should be prevented and damped by using a vibration isolated setup and avoiding contact with the setup while recording.

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