

Experiment 6: Brownian Motion

- **Learning Goals**

After you finish this lab, you will be able to:

1. Describe (quantitatively and qualitatively) the motion of a particle undergoing a 2-dimensional “random walk”
2. Record and analyze the motion of small microspheres in water using a microscope.

Introduction: Please read all of this BEFORE you come to lab.

Background and introduction

- In this lab, you’ll explore Brownian motion. You’ll observe a micron-sized sphere under a microscope and watch as it undergoes a random walk in two dimensions. You’ll then quantitatively analyze its motion and measure its diffusion constant. Using known properties of the sphere, you can then experimentally determine Boltzmann’s constant and Avogadro’s number, just as Einstein and Perrin did in the early 20th century (which led to Perrin’s 1926 Nobel Prize in Physics). Finally, you’ll get a chance to observe motion that is *not* Brownian, but rather due to self-propelled micro-organisms.

More on Statistics

Standard deviation

- Earlier in the semester, we told you that standard deviation σ is a number that characterizes the “spread” in a distribution or data set. Let’s take a closer look at how the standard deviation is calculated.

The standard deviation is defined as the **RMS deviation from the mean**. RMS stands for “root mean square.” What this definition means is that in order to calculate σ , you first look at every data point in the distribution and figure out how far it is from the mean. Then you square that distance, average over all the data points, and finally take the square root.

In equation form, if there are N samples, and each sample is labeled x_i (for i ranging from 1 to N), and μ is the mean of all of the samples, then

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \mu)^2}$$

Alternatively, using the angle bracket notation for mean or average,

$$\sigma = \sqrt{\langle (x - \langle x \rangle)^2 \rangle}$$

This definition will be useful to us when we consider the distribution of steps in a random walk (Brownian motion).

Random walks: the quick & dirty summary

- Recall the basic premise for a (one-dimensional) random walk:
 - You start at the origin $x = 0$.
 - At regular intervals (every τ seconds) you flip a coin. (τ is the **step time**)
 - If you get heads, you move to the *right* a fixed distance δ (the **step size**).
 - If you get tails, you move to the *left* a fixed distance δ .

As we did (or will) show in class, although each random walk is unpredictable, there are some predictable results that you can derive if you consider the *average* behavior over many random walks. Here are the results we derived in class:

In one dimension

- Since you are equally likely to take a step to the right, as you are to take a step to the left, the average position after a time t is zero:

$$\langle x \rangle = 0$$

That is, **on average, you don't go anywhere** (either to the left or to the right), no matter how many steps you take. If you think of each step as moving in either the $+x$ or $-x$ direction, you can probably imagine that since positive and negative steps are equally likely, *on average* you just end up at the origin.

- But if you *square* the final displacement, and then take the average, the positive and negative directions won't cancel—after all, squaring will always give you a positive number. In class we derive the result that, for one-dimensional Brownian motion, the mean square displacement is:

$$\langle x^2 \rangle = 2Dt$$

That is to say, **you do go somewhere** (away from where you started), but **the average squared distance from the starting point only increases linearly as the number of steps**. It doesn't take long to get a few steps away, but if you want to go twice as far you have to wait four times as long. The *diffusion constant* D is defined by

$$D = \frac{\delta^2}{2\tau}$$

Here, δ is the size of a single step, and τ is the time between consecutive steps. The factor of 2 in the denominator is there for convenience.

In more dimensions

- Each dimension (x , y , and z) independently obeys both of the equations for random walks in one dimension. Therefore, it remains true that no matter how many steps you take, on average you will move neither left nor right, neither forward nor backward, and neither up nor down.

However, r^2 , the distance from the origin, is expected to increase with the number of steps. Since $r^2 = x^2 + y^2 + z^2$, we can write $\langle r^2 \rangle = 4Dt$ in 2 dimensions, or $\langle r^2 \rangle = 6Dt$ in 3 dimensions.

Brownian motion

- Surprisingly, the simple random walk is a very good model for Brownian motion: a particle in a fluid is frequently being "bumped" by nearby molecules, and the result is that every τ seconds, it gets jostled in one direction or another by a distance δ . You could make the model more sophisticated, but this very simple model has all of the important (and correct) features that a more complete analysis would provide.

The bottom line is that we can still use the important quantitative result that $\langle x^2 \rangle = 2Dt$ (in one dimension). This means that if we actually perform a Brownian motion experiment and measure the average squared displacement in a certain time interval, we can determine the diffusion constant D .

For 2- and 3-dimensional Brownian motion, the same equation holds for each of x , y , and z independently. For example, in two dimensions, the mean squared displacement from the origin is equal to $4Dt$.

- In general, D depends on the size and shape of the diffusing particle, as well as on the temperature. In 1905, by thinking about viscous drag and thermal energy, Einstein derived the important relationship:

$$Df = k_B T$$

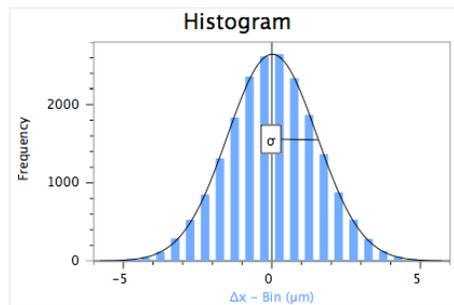
This is known as the **Einstein-Smoluchowski** equation. The constant f on the left-hand side is called the **drag coefficient**; it is the proportionality constant between the viscous drag force on the particle and its speed, i.e. $F_{\text{drag}} = fv$. Recall that for a sphere of radius R ,

the Stokes formula gives $f = 6\pi\eta R$, or in other words, $F_{\text{drag}} = 6\pi\eta Rv$. T is measured in Kelvin.

Because D , f , and T are easily measurable experimentally, the Einstein-Smoluchowski equation gave the first way of making a direct measurement of Boltzmann's constant k_B . Since Boltzmann's constant is just the ideal gas constant (which had been known for over a century) divided by Avogadro's number, this was one of the first measurements of Avogadro's number and a convincing proof of the theory that matter is made of discrete molecules.

Remember that in order to measure the **mean** squared displacement, you need to perform **many random walks**, or equivalently, many Brownian motion experiments. A single random walk won't necessarily resemble the average at all. There are two basic ways of "repeating" a Brownian motion experiment:

Method 1: If you have a large number of particles all at the same starting point, and take a snapshot of where they all end up at a later time, then each particle has undergone an independent random walk. Averaging the values of x^2 for each particle would enable you to determine D . Even better, if you plotted a histogram of the x values (not squared) for each particle, you'd actually see ... our old friend, the Gaussian distribution:



(We'll learn why this is the case when we study the diffusion equation.) The mean of this Gaussian is the average displacement, which is zero. **The standard deviation σ is just the RMS displacement, so $\sigma^2 = 2Dt$ (in one dimension).**

Method 2: If you take a single particle in Brownian motion and measure its position many times at regular intervals, you are effectively performing many short random walks in succession. The key, however, is that each time you measure its position, what you are really interested in is *how far it has moved since the last time you measured it*, not on how far it has moved since its initial position. That way each random walk will have the same (short) time t , so you can figure out the average x^2 during that time t and thus measure D .

Experiment 6: Lab Activity

- Follow along in this lab activity. **Wherever you see a question highlighted in red**, be sure to answer that question, or paste in some data, or a graph, or whatever is being asked. Your lab report will be incomplete if any of these questions remains unanswered.

Who are you? Take a picture of your lab group with Photo Both and paste it below along with your names.

- Materials:**

- Microscope

These custom-built microscopes have been fitted with an LED light source and a camera. The microscope objective will cast an image of the sample on the slide directly onto the CCD array of the camera; illumination is provided by the LED. From this setup, Logger Pro can capture video of Brownian motion of particles suspended in solution on the microscope slide.

Each microscope has an objective for 40X magnification. Each microscope also has one focus knob. The sample holder and the LED are mounted on a movable stage; the knob at the top moves the stage closer to the objective (down) or farther away from the objective (up), allowing the user to adjust the focus.

- Microscope slides

The slides are “well slides,” which means they have a slight depression in the center to hold the sample.

- Cover slips

- Microsphere solution

This is a solution of micron-sized polystyrene spheres in water. You’ll place a drop of this solution onto the well slides and then observe it under a microscope.

Diameter of microsphere = $(1.025 \pm 0.010) \mu\text{m}$

Viscosity of microsphere solution $\eta = (9.5 \pm 0.5) \times 10^{-4} \text{ Pa}\cdot\text{s}$

- Biological sample (pond water, basically)



- **Procedure**

Part 1: Brownian motion of a 1-micron polystyrene sphere in water

Preparing a microsphere sample

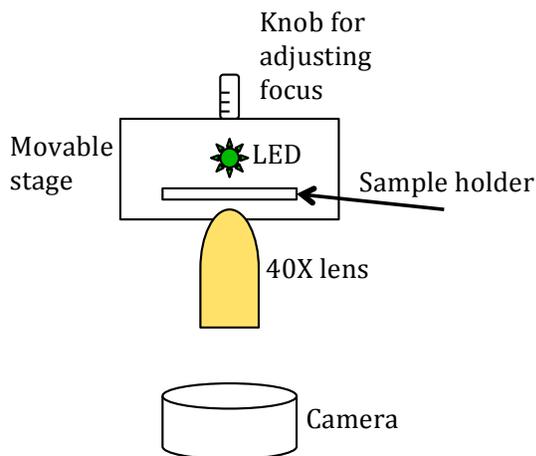
The sample preparation station is at the front of the room. When preparing a slide, first make sure the slide is clean. To clean the slide, rinse with warm water, and then dry. Make sure it is dry before using it.

- Place the well microscope slide on a piece of tissue paper, with the well side up.
- Use the dropper to add two drops into the well, almost overflowing it. Put the dropper back in microsphere solution container.
- Carefully place a single cover slip on top of the sample, making sure there are no air bubbles inside; the sample should overflow.
- Gently blot the excess sample with a tissue paper by pressing it against the edge of the cover slip.

This sample preparation technique is usually enough to seal the cover slip in place and avoid currents inside your sample. However, you still have to be very careful when handling the slide. Keep it horizontal with the cover slip on top, until you are ready to place it in the microscope. Avoid shaking it.

If you get bubbles inside your sample, it is easiest to simply prepare a new one, instead of trying to fix the one you already have.

Using the microscope



These custom-built microscopes shown in the sketch to the left (not to scale) have the basic optical components to allow imaging of small objects. The sample holder and LED for illumination are mounted on a movable stage that allows for focus adjustment. In the middle you will find a 40X objective lens and at the bottom is a Pro Scope CCD camera.

The distance between the lens and the camera is fixed.

Do not move the camera or the lens!

Before placing your sample on the microscope, make sure to move the sample holder away from the objective.

Open up the Logger Pro file on the computer. The first page should be blank. Use “Insert” -> “video capture” to start a video stream to help you focus on your sample, since these microscopes do not have an eyepiece you can look through.

To place the sample on the microscope, turn the slide with **cover slip down** and slip the microscope slide inside the clip holders. You can gently hold the clip holders open while you do this.

Make sure the well in the microscope slide is aligned with the center of the lens.

Slowly move the sample down towards the objective lens, until you can see your sample in the screen.

Sometimes dust can fall on the CCD cover and appear in your image. Ask a TF for help removing this dust if it is getting in the way of your experiments.

Recording a movie of Brownian Motion

- On the movie capture window, click on “Options...”
- On the window that opens, set to record for **20 seconds continuously** (do not select “Time Lapse”); this should give you about 300 frames.
- Click “OK” to close the “Options” window.
- When you are ready to record, click “Start Recording”. Logger Pro may warn you that the video file will be very large; don’t worry about that.

When you are done recording, close the video capture window, and play your movie.

Make sure you have about 300 frames. You can see the frame number on the top right corner. Make sure you have enough frames, and that the microspheres are not all drifting in one direction.

Quickly scroll through the entire movie to **see if there is a microsphere that appears to stay in the picture for most of the duration of the capture.** The other, and more important, thing to **check** for is **that there is minimal overall drift** of the entire sample in the same direction. If there is significant drift, the easiest thing to do is to prepare a new sample and retake your data.

Setting the scale on your movie

In Logger Pro menu bar, select “Insert” and scroll down to “Picture” then “Picture With Photo Analysis”.

Select the file “PhysicsLab##_Scale” file on the Desktop. Click OK.

You will see a window open that shows a series of vertical lines. To correctly set up the scale, you need to measure the entire width of the picture, and determine what that distance is in microns.

Using the “Photo Distance” tool, measure from one line to another; this corresponds to 10 microns.

Measure from the left edge of one line to the left edge of the next line. The distance is in pixels. Call this distance x_1 .

Measure from right edge of same line to the right edge of the same next line. The distance is in pixels. Call this distance x_2 .

Take the average of x_1 and x_2 . This distance is the pixel distance that corresponds to 10 microns (10^{-5} meters).

Using the “Photo Distance” tool, measure the entire width of the picture, in pixels. Using the pixel to micron conversion found in step 3, make a note of what this distance is in microns.

Go to your movie. Click the “Set Scale” button, and trace a line that covers the entire width of the image. Enter the correct distance in meters or microns, as found in step 4 above. This approach works because the width of the image (the “field of view”) of the microscope image is the same whether you are taking a still photo or analyzing a movie.

Analyzing your movie

Once you have a “good” video, use video analysis to mark the position of your microsphere **every 5 frames**. To do this, right-click on the movie and choose “Movie Options”. In the new window that opens, enter 5 next to “Advance the movie” under “Video Analysis”, then click OK. Now you should see the frame number increase by 5 every time you add a point. **You should have about 60 points for one microsphere**. Proceed to analyze the data, which should appear on Page 2 of your Logger Pro file.

Make a graph of Y position vs. X position. Double-click on the graph and check the box for Connect Points.

What does this plot represent?

Paste a copy of the graph here:

Go to Page 2 of the logger pro file. In your Video Analysis data set, insert a new calculated column named "Delta x" with a definition function of ΔX . To do this, go to "Data" -> "New Calculated Column" and fill in the name, short name, and unit fields. Then in the large textbox enter the function: ΔX . Follow the same process to create a "Delta Y" column.

Make histograms of the "Delta x" and "Delta y" columns by going to "Insert"-> "Addition Graphs" -> "Histogram". Double click on the new histogram to choose the bin size and proper column to plot. Obtain the following statistics for "**Delta x**" by clicking the stats icon:

Mean =

Standard deviation =

Standard error of the mean =

If there is no overall drift, we expect the mean to be zero.

Approximately how many standard errors away from zero is it?

If the mean is more than two standard errors away from zero, you may have had excessive drift and the rest of the analysis will not work very well. You may have to take new data. Talk to a TF.

Paste a copy of your histogram for "Delta x" here:

Obtain the following statistics for "**Delta y**":

Mean =

Standard deviation =

Standard error of the mean =

If there is no overall drift, we expect the mean to be zero.

Approximately how many standard errors away from zero is it?

If the mean is more than two standard errors away from zero, you may have had excessive drift and the rest of the analysis will not work very well. You may have to take new data. Talk to a TF.

Paste a copy of your histogram for “Delta y” here:

Now, we are going to treat our 20-second random walk as 60-ish independent random walks, each lasting 1/3 second. With this new interpretation, each value of “Delta x” is really just a final position of a short walk. We are interested in finding the *diffusion constant* D , but we know that this is related to the **standard deviation** of the final position by $\sigma^2 = 2Dt$.

So, you can take the standard deviation you obtained above for motion in the x -direction, use the time $t = 1/3$ of a second, and solve for D in the equation above. For the motion in the x -direction, **what is the diffusion constant?**

D (from x) =

Using your data for the motion in the y -direction, **what is the diffusion constant?**

D (from y) =

Calculate the average value for the diffusion constant, using your two values above (one from x and one from y).

Average D =

Use the Einstein-Smoluchowski equation (see Introduction section on Brownian motion) to **find the value of Boltzmann’s constant**, k_B . (The diameter of the microspheres and viscosity η of the microsphere solution are given in the Materials section.) Note that the temperature T should be measured in Kelvin.

k_B =

The accepted value of Boltzmann’s constant is 1.38×10^{-23} J/K.

How does it compare with your measured value?

Finally, using $R = 8.31 \text{ J/(K}\cdot\text{mol)}$, **calculate Avogadro's number**, $N_A = R/k_B$.

$N_A =$

The accepted value for Avogadro's number is 6.02×10^{23} .

How does your measured value compare with this?

Suppose that instead of 1-micron spheres, you had observed 2-micron spheres.

What diffusion constant would you have measured? Why does this make sense physically?

Part 2: Non-Brownian motion of a biological sample

At the sample preparation station, there are also solutions containing biological samples.

Make up a microscope slide containing one of these solutions.

Perform a short video analysis (~30 seconds) on one of the creatures you find just as we did for the microspheres. We want to choose a creature that moves rapidly across almost the entire field of view, but is easily visible. The video analysis data will appear on Page 2 again with x and y velocities automatically calculated. Plot these velocities and have logger pro compute the mean x and y velocities. To calculate the average speed, add the x and y velocities in quadrature (avg speed = $\text{Sqrt}(V_x^2 + V_y^2)$)

What is the creature's typical (average) speed?

What do you notice qualitatively about the motion of these biological samples? How does it differ from the Brownian motion of the microspheres?

You can model a bacterium as a sphere roughly 1 micron in diameter.

If it were not self-propelled, how far (on average) would it diffuse in 1 second? Assume the viscosity of the solution has not changed drastically.

Approximately how much larger is the creature's "self-propelled" average speed relative to its "diffusion" average speed?

Cleaning up

- Cover slips (small, thin glass squares): drop inside beaker with water
- Well slides (1in x 3in thick glass with depression in center): rinse with water, wipe with isopropyl alcohol (using alcohol wipe or using alcohol and tissue paper), let air dry, store in orange box.
- Regular slides (1in x 3in thick glass): rinse with water, wipe with isopropyl alcohol (using alcohol wipe or using alcohol and tissue paper), let air dry, store in cardboard box.
- Tissue paper: drop in cardboard box labeled trash.

Conclusion

What is the most important thing you learned today?

What was the most confusing aspect of lab today?