Lab 7: Brownian Motion

I. Introduction

A. Objectives for this lab:

1. Learn about statistical physics in a system, specifically the two-dimensional random walk

2. Understand how the motion of self-propelled organisms differs from Brownian motion

B. 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.

II. Background

Brownian motion isn't covered in Bauer & Westfall, so please read the additional handout on viscosity & Brownian motion from the course website for more detailed background information.

A. A little bit more on statistics

1. Standard deviation

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

a. 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 this squared difference over all the data points, and finally take the square root.

b. In equation form, if μ is the mean of the distribution of x_i's, then

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

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

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

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

2. Uncertainty of the standard deviation

a. You might recall that for a data set with N measurements, we can use the mean of the data set as a *best estimate* of the "true" mean of the underlying distribution. The uncertainty of this estimate is given by something called the *standard error of the mean*, which is σ/√N.

b. In the same way, the standard deviation of the set of N measurements is our best estimate of the "true" standard deviation of the underlying distribution. As with estimating the mean this estimate has an uncertainty, which is called the *standard error of the standard deviation*.

(1) Ordinarily, we don't really care that much how accurate our estimate of σ is because we are only using σ to characterize the uncertainty of something else (the mean), and the uncertainty of an uncertainty is not usually of much interest.

(2) However, there are instances in which σ itself is the measured value of some quantity, in which case we would very much like to know the uncertainty in our estimate of σ. This is where the standard error of σ comes in:

$$\mathrm{SE}(\sigma) = \frac{\sigma}{\sqrt{2N-2}}$$

B. Random walks: the quick & dirty summary

Recall the basic premise for a random walk: every τ seconds, you flip a coin. If it's heads, you move to the right by a distance δ; if it's tails, you move to the left by δ. The random walk has no "memory"; each step is independent of everything that has gone before. Here are the results we derived in class:

1. In one dimension

a. Any single random walk is fundamentally not predictable, and also not very informative. It's only when you **average over many random walks** that meaningful quantitative trends start to emerge.

b.

$$\langle x \rangle = 0$$

That is, **on average, you don't go anywhere** (either to the left or to the right), no matter how many steps you take.

c. Now we can directly apply all of the results from our random walk model, using a step size of δ and a time between steps of τ. For one-dimensional Brownian motion, the result 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

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

The factor of 2 in the denominator is part of the definition for convenience.

2. In more dimensions

a. x, y, and z each independently obey 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.

b. 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 $\langle r^2 \rangle = 6Dt$ in 3 dimensions, or $\langle r^2 \rangle = 4Dt$ in 2 dimensions.

C. Brownian motion

1. 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.

2. The bottom line: we can still use the important quantitative result that $\langle x^2 \rangle$ increases as 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.

3. 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, $r^2 = x^2 + y^2$, is equal to 4Dt.

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

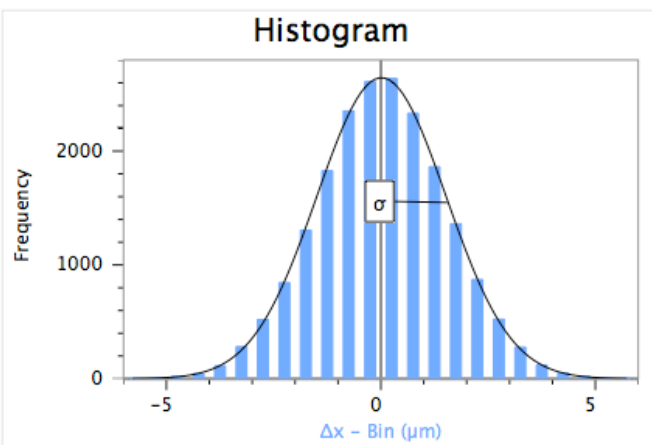
$$Df = k_B T$$

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. $F_{drag} = fv$. Recall that for a sphere of radius r, the Stokes formula gives $f = 6\pi\eta r$.

5. 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.

6. 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:

- **a.** 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 produces a Gaussian 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).

- **b.** 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 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. This method relies on the fact that a random walk has no "memory," so that each displacement is an independent random walk.

III. Materials

A. 1 microscope

1. There are two types of microscopes: the grey Bausch & Lomb microscopes, and the black Spencer microscopes. They are largely equivalent.

Bausch & Lomb

Spencer

2. The microscopes have been fitted with an LED light source and a modified iSight 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 from below the microscope stage by the LED. From this setup, Logger Pro can capture time-lapse video of Brownian motion of particles suspended in solution on the microscope slide.

3. Each microscope has an objective for 10X magnification, and an objective for approximately 40X magnification. (Some of the microscopes may have 43X instead of 40X.)

4. Each microscope also has two focus knobs. The larger/upper knob is for coarse focus adjustments, and the lower one is for fine adjustments.

5. The on/off switch for the LED is located just below the 9-volt battery.

B. Microscope slides

1. 

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

C. Cover slips

D. Microsphere solution

1. 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.
2. Diameter of microsphere = (1.025 ± 0.010) µm
3. Viscosity of microsphere solution = $(9.5 \pm 0.5) \times 10^{-4}$ Pa s

IV. Procedure

A. Take a photo of yourselves...

1. This time, because the external iSight cameras are being used for microscopy, you will need to convince Photo Booth to use the built-in camera above the monitor. To do this:

- **a.** Open the file Lab7.cmbl in Logger Pro.
- **b.** From the Insert menu, select Video Capture...
- **c.** When it prompts you for which camera source to use, choose IIDC FireWire (which is the external iSight camera).
- **d.** If it asks you for a resolution, select 800x600. If it asks you for the audio source, pick any of the options.
- **e.** Now open Photo Booth. Because Logger Pro is using the external camera, Photo Booth will automatically default to using the built-in camera. **Take a picture of your lab group and drag it into the space below:**

2. Tell us your names (from left to right in the above photo):

B. Measure the Brownian motion of a 1-micron polystyrene sphere in water.

1. Prepare a sample.

- **a.** A sample preparation area is next to the sink. There are several bottles of 1-micron spheres (as well as various biological samples).
- **b.** Using the pipette, place a drop or two of solution into one of the well slides and cover it with a cover slip. Make sure not to trap air bubbles under the cover slip.
- **c.** Invert the slide onto a Kimwipe and tap it gently to remove excess solution.
- **d.** After a few minutes the edges will dry, and the cover slip will become lightly stuck to the well slide. This should provide a sample with minimal evaporation. Evaporation would cause an overall drift of particles in the direction of the fastest evaporation, which is something we don't want.

2. Observe the sample under the microscope.

- **a.** Focus an image onto the camera. Use the 10X objective first before switching to the 40X objective.
- **b.** You will collect data using the 40X objective. Be careful! On the 40X objective, it is very possible to break your slide and/or cover slip by trying to move the objective too close to the sample using the coarse focus knob.
- **c.** Before taking a video, observe the microspheres for long enough to convince yourself that the particles are diffusing with minimal overall drift. This step is **crucial** because you will need to retake your five-minute video if there is significant drift.

3. Collect a video capture of the Brownian motion of the spheres as follows:

- **a.** In the Video Capture window, click on the Options button. Set the following options:
  - **(1)** Video Capture Only
  - **(2)** Capture Duration: 300 seconds
  - **(3)** Check **Time-Lapse Capture**
  - **(4)** Capture Interval: 5 seconds
  - **(5)** Capture File Name Starts With: Brownian
  - **(6)** Click on OK.

b. Click on Start Time Lapse. (Time Lapse differs from a regular video capture in that instead of recording video at 30 frames per second, it records only one frame every 5 seconds.)

c. Save your Logger Pro file.

4. Set the scale for your captured video.

a. From the Logger Pro Insert menu, select Picture → Picture Only.

b. In the Lab 7 folder on your computer's desktop is a photo of a scale with 10 micron divisions, taken at 40X on your microscope. Use the photo to set the length scale of your video.

5. Quickly scroll through the entire movie (by dragging the circle through the scroll bar) to see if there is a microsphere that appears to stay in the picture for the duration of the capture. This is preferable for the video analysis; however, if there is no such sphere, that's okay too. 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 just make a new sample and retake your data.

a. Make sure to choose a sphere that appears to be moving freely, rather than one that is stuck to the glass (or is perhaps not a microsphere at all, but just a smudge on the microscope or camera lens!).

b. If no single microsphere is visible for the entire video, just switch to a new one part way through. Since only the *differences* in position between adjacent points are used in the analysis, you will only lose one data point; however, be sure to remember that you did this so you can strike out the phony data point later when you do your analysis. (It will end up looking as if the microsphere jumped all the way across the screen in a single 5-second interval.)

6. Mark the position of your microsphere in every frame.

7. Analyze the data.

a. Make a graph of Y position vs X position.

(1) Double-click on the graph and check the box for Connect Points.

(2) What does this plot represent?

Paste a copy here:

b. Move to page 2 in the Logger Pro file. In your Video Analysis data set, redefine the "X Velocity" column as a calculated column named "Delta x" with a definition equation of delta("X"). Follow the same process to create a "Delta Y" column.

(1) If you had to switch spheres during your video, eliminate the spurious data point by striking through it in the "Delta x" and "Delta y" columns (*not* in the original X or Y column) using Edit > Strike Through Data Cells.

c. Make histograms of the "Delta x" and "Delta y" columns:

(1) Statistics for delta x:

(a) mean =

(b) standard deviation =

(c) standard error of the mean=

(d) 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 SE away from zero, you may have had excessive drift and the rest of the analysis will not work very well. Talk to a TF.

(e) Try fitting a PS2 Gaussian to your histogram.

I. mean =

II. std dev =

(f) Paste a copy of your histogram with Gaussian fit here:

(2) Statistics for delta y:

(a) mean =

(b) standard deviation =

(c) standard error of the mean =

(d) Approximately how many standard errors away from zero?

(e) Try fitting a PS2 Gaussian to your histogram.

I. mean =

II. std dev =

(f) Paste a copy of your histogram with Gaussian fit here:

d. Now, we are going to treat our five-minute random walk as 60 random walks, each lasting five seconds. 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 **σ^2= 2Dt**. This is an easier and more reliable way of determining D than actually calculating the mean squared displacement for each walk.

(1) Calculate D (from x) =

(2) Calculate D (from y) =

(3) Now we'll try to determine the uncertainty in our calculated values of D. Recall that the uncertainty in the standard deviation of a set of N values is: $\delta\sigma = \sigma/\sqrt{(2N-2)}$. Therefore,

$$\frac{\delta D}{D} = \frac{\delta(\sigma^2)}{\sigma^2} = \frac{2\delta\sigma}{\sigma} = \frac{2}{\sqrt{2N-2}}.$$

(a) Uncertainty in D (from x) =

(b) Uncertainty in D (from y) =

(c) Do the two values of D agree with each other? (Hint: error propagation)

If the values don't agree, talk with a TF before continuing.

(d) Average D =

(e) Uncertainty in the average D =

e. Deduce a value for Boltzmann's constant k_B from the Einstein-Smoluchowski equation:

$$Df = k_B T$$

, where $f = 6\pi\eta r$.

(1) Remember the propagation of error equation you will need in this case is:

$$\frac{\delta k_B}{k_B} = \sqrt{\left(\frac{\delta D}{D}\right)^2 + \left(\frac{\delta \eta}{\eta}\right)^2 + \left(\frac{\delta r}{r}\right)^2 + \left(\frac{\delta T}{T}\right)^2}$$

(2) k_B (including correct units and uncertainty) =

(3) The accepted value of Boltzmann's constant is 1.38×10^{-23} J/K. **How does your measured value compare with this?**

f. Finally, using R = 8.31 J/(K*mol), calculate Avogadro's number, $N_A = R/k_B$. (Assume the value of R has no uncertainty.)

(1) N_A with uncertainty =

(2) The accepted value for Avogadro's number is 6.02×10^{23}. **How does your measured value compare with this?**

8. 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? (Hint: Consider the Einstein-Smoluchowski equation.)

C. Observe the non-Brownian motion of a biological sample.

1. At the sample preparation station, there are also solutions containing biological samples. Make up a microscope slide containing one of these solutions.

2. Perform a short video analysis (~30 seconds) on one of the creatures you find. Be sure to uncheck "Time Lapse".

3. What is the creature's typical speed as it moves around?

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

5. You can model a bacterium as a sphere roughly 1 micron in diameter. **If it were not self-propelled, about how far would it diffuse in 1 second (RMS distance)?** (Treat the problem in two dimensions.) In order for self-propulsion to be useful to the bacterium, it must be able to move itself *faster* than diffusion!

6. Approximately how much larger is the creature's "self-propelled" average speed relative to its "diffusion" average speed (per second)?

V. Conclusion

A. Clean-up: place all used slides in the jar at the sample preparation station. All broken glassware, used pipettes, and used cover slips, get deposited in the glass disposal box. **Do not discard the well slides**; instead, clean them off and leave them to dry.

B. Submit your work as usual to sapling. Remember to save your file as a pdf first!