

### 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  $\sigma$  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  $\sigma$ , 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  $\mu$  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,

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

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

#### Uncertainty of the standard deviation

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  $\sigma / \sqrt{N}$ .

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**.

Ordinarily, we don't really care that much how accurate our estimate of  $\sigma$  is because we are only using  $\sigma$  to characterize the uncertainty of something else (the mean), and the uncertainty of an uncertainty is not usually of much interest. However, there are instances in which  $\sigma$  itself is the measured value of some quantity, in which case we would very much like to know the uncertainty in our estimate of  $\sigma$ . This is where the standard error of  $\sigma$  comes in:

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

### Random walks: the quick & dirty summary

Recall the basic premise for a random walk: every  $\tau$  seconds, you flip a coin. If it's heads, you move to the right by a distance  $\delta$ ; if it's tails, you move to the left by  $\delta$ . Here are the results we derived in class:

#### In one dimension

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.

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.

Now we can directly apply all of the results from our random walk model, using a step size of  $\delta$  and a time between steps of  $\tau$ . 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

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

Here,  $\delta$  is the size of a single step, and  $\tau$  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  $\langle r^2 \rangle = 6Dt$  in 3 dimensions, or  $\langle r^2 \rangle = 4Dt$  in 2 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  $\tau$  seconds, it gets jostled in one direction or another by a distance  $\delta$ . 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—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  $D$ .

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 Einstein, by thinking about viscous drag and thermal energy, derived the important relationship:

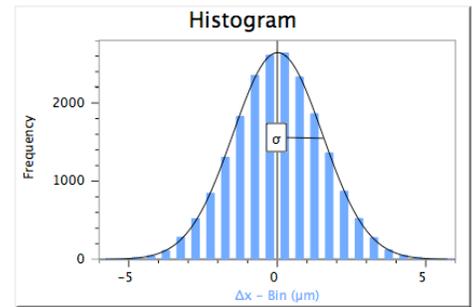
$$Df = k_B T$$

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

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  $\sigma$  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  $D$ .

### Preparing a microsphere sample

The sample preparation station is at the back of the room, next to the sink. When preparing a slide, first make sure the slide is clean. To clean the slide, rinse with warm water, and then clean it with an alcohol pad. Let it dry before using it.

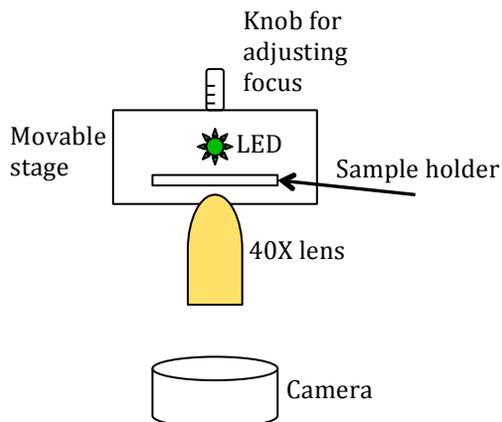
- Place the well microscope slide on a piece of tissue paper, with the well side up.
- Use the glass dropper to add a few drops into the well, overflowing it. Put the dropper back in microsphere solution container.
- Carefully place a single cover slip on top of the sample, making sure there are no air 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.

Use the same technique for the liquid biological sample.

### Using the microscope



These custom-build 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 lens.

Also, make sure you insert a movie capture on Logger Pro, 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 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 for 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”.

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 there is no drift.

#### Setting the scale on your movie

The scale picture does not have the same pixel resolution as the video. The software/hardware automatically adjusts resolution to best fit the selected resolution, but it seems to do it in a different way when taking pictures than when taking video. What remains constant, though, is the actual size (in meters) of the field of view.

To correctly set up the scale, you need to measure the entire width of the picture, and determine what that distance is in microns. To do this:

1. In Logger Pro menu bar, select “Insert” and scroll down to “Picture” then “Picture With Photo Analysis”.
2. Select the file “PhysicsLab##\_Scale” file on the Desktop. Click OK.
3. Using “Photo Distance” tool, measure from one line to another; this corresponds to 10 microns.
  - a. Measure from the left edge of one line to the left edge of the next line. The distance is in pixels. Call this distance  $x_1$ .
  - b. Measure from right edge of same line to the right edge of the same next line. The distance is in pixels. Call this distance  $x_2$ .
  - c. Take the average of  $x_1$  and  $x_2$ . This distance is the pixel distance that corresponds to 10 microns.
4. 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.
5. 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.

#### Using the PS2 Gaussian

In the Logger Pro curve fit window, there should be a custom function called “PS2 Gaussian”. If you do not see it, you can define it yourself. The function has the following form:

$$Ae^{-(x-B)^2/2C^2}$$

This form directly gives you the mean (B) and the standard deviation (C) of a Gaussian distribution.