I. Before you come to lab:

1. Read this handout and the supplemental.

II. Learning 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

III. Materials

* 1 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 ± 0.010) μm
- Viscosity of microsphere solution = (9.5 ± 0.5) x 10^{-4} Pa s

* Biological sample (pond water, basically)

* Onion, flat microscope slides, tweezers, and vacuum grease for BONUS.

IV. Warm up

This lab does not have a warm up.

V. Procedure

Tell us who you are (names, pictures, and emails, please)

A:

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

Prepare a sample as described in the supplemental. Observe the sample under the microscope and collect a video of the Brownian motion of the spheres. (Further details on the setup can be found in the supplemental.)

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.

Once you have a "good" video, use it to mark the position of your microsphere **every 5 frames.** To do this, double-click on the movie. 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.

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?**
A:

Paste a copy of the graph here:
Graph:

In your Video Analysis data set, insert a new calculated column named "Delta x" with a definition function of delta("X"). Follow the same process to create a "Delta Y" column.

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

Obtain the following statistics for "**Delta x**":
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?**
A:

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.

Try fitting a PS2 Gaussian to your histogram. **Obtain the following parameters from the fit**:
Mean =
Standard deviation =

Paste a copy of your histogram with Gaussian fit here:
Histogram:

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?**
A:

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.

Try fitting a PS2 Gaussian to your histogram. **Obtain the following parameters from the fit**:
Mean =
Standard deviation =

Paste a copy of your histogram with Gaussian fit here:
Histogram:

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 $\sigma^2 = 2Dt$. This is an easier and more reliable way of determining *D* than actually calculating the mean squared displacement for each walk.

Using your data for the motion in the *x*-direction, **what is the diffusion constant?** Use the standard deviation obtained from statistics; since you have few data points for the Gaussian fit, using the value from statistics will likely be a better estimate of the real value than the fit. Don't forget uncertainty. *Hint:* You will need the uncertainty of the standard deviation; see supplemental for details.
D (from x) =

Using your data for the motion in the *y*-direction, **what is the diffusion constant?** Don't forget uncertainty.
D (from y) =

Do the two values of D agree? (Hint: How does one evaluate if two values agree or not?)
A:

Calculate the average value for the diffusion constant, using your two values above (one from *x* and one from *y*). Don't forget uncertainty.
Average D =

Use the Einstein-Smoluchowski equation to **find the value of Boltzmann's constant**, k_B. Diameter of the microspheres and viscosity of the microsphere solution are given in the Materials section. Don't forget uncertainty.
k_B =

The accepted value of Boltzmann's constant is 1.38×10^{-23} J/K. (Assume this value has no uncertainty.)
How does it compare with your measured value?
A:

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

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

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?**
A:

Export your "Delta x" and "Delta y" as a csv file and upload it to the folder called "Lab7 Data" on isites. You only have to do this once.

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

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

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?**
A:

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

BONUS: Is it alive?
Make a slide with a very thin slice of onion, as described in the supplemental, and observe it under the microscope. You will see small dots moving around; these are vesicles inside the onion cells. **Are the cells in the onion layer alive? How can you tell?**
A:

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.

VI. Conclusion

What is the most important thing you learned today?
A:

Preparing an onion slice sample
For the BONUS part, you will observe a single slice of onion under the microscope. You will find all you need in the sample preparation station.

- Carefully peel a very thin inside layer of onion using the tweezers. The onion peel will be nearly transparent.
- Place the onion peel on a coverslip.
- Cut the excess onion with a razor blade. This step is not necessary, but it will make handling your sample easier.
- Using an application stick, smear some vacuum grease on the edges of the cover slip. Do not apply too much pressure on the cover slip; it might break.
- Place a clean, flat microscope slide on top of the cover slip with the onion peel and gently press down. The cover slip should now be stuck to the microscope slide.