

Brownian Motion Lab

Purpose:

In this experiment you will explore the Brownian motion of microspheres in water. As the water molecules agitate due to the thermal vibrations of the molecules, they correspondingly bump into the microspheres, causing them to move within the liquid. This is Brownian motion. The motion of the microspheres is random, and the probability that a sphere travels a given displacement in any given time

interval obeys a normal (Gaussian) distribution, $P(\Delta x) = A e^{-\frac{(\Delta x)^2}{2(2D\Delta t)}}$, where D is called the diffusion coefficient, $D = kT/6\pi\eta r$, which depends on the temperature, T , the viscosity of the fluid (look up in C.R.C), η , and the radius of the microsphere, r . By solving for the Boltzmann constant, k , you can experimentally measure Avogadro's number N_A , since $k = R/N_A$, and the value of the ideal gas constant is known (8.31 J/mol K).

Resources:

J. Peidle et al. "Inexpensive microscopy for introductory laboratory courses", Am. J. Phys. **77** 931, 2009.

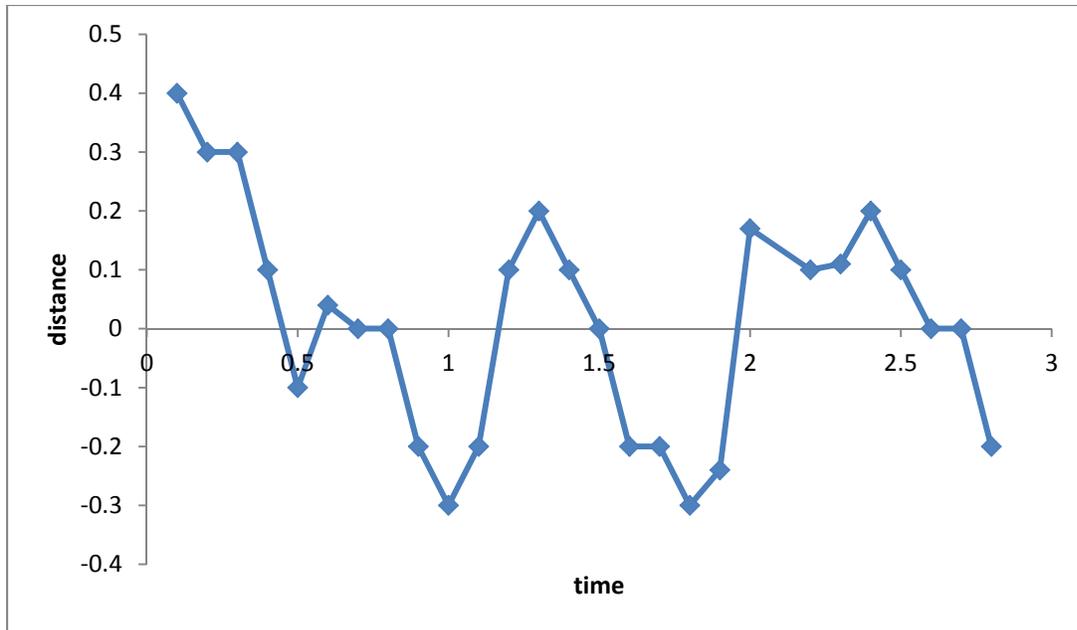
Paul Nakroshis et al. "Measuring Boltzmann's constant using video microscopy of Brownian motion" Am. J. Phys. **71** 568-573, 2003

R. Chabay, and B. Sherwood, "Matter and Interactions", John Wiley and Sons (1st, 2nd or 3rd edition), Chapter 12.

Background:

The goal of this experiment is to use measurements of the motion of microscopic particles to measure a fundamental constant, Avogadro's number.

If we look at a very small particle in a fluid (lets start with a one dimensional fluid), it experiences numerous microscopic collisions with the molecules of the fluid. In between the collisions, the particle will travel with a constant velocity. However, because the particle is being bombarded on all sides by the fluid the *average velocity* of the particle is zero. If the particle is bumped every Δt seconds (at which point the velocity will change), the trajectory of the particle will look something like this:



Notice that the average position of the particle is zero, but that over time the particle wanders around. This *Brownian motion* due to the bombardment of the particle by the fluid molecules allows the particle to move via a *random walk*, which we can describe mathematically.

Because the motion is random, the average position will be zero, $\langle x \rangle = 0$. This is not very instructive because it leads you to believe that the particle is not moving. Rather, we can look at the standard deviation of the motion, or, specifically the *mean squared displacement* $\langle \Delta x^2 \rangle$. In the mean squared displacement, we are actually looking at the average magnitude of the displacement (squared) between each position, so it is a measure of how far the particle moves in time Δt , between collisions. The *Diffusion coefficient* is in fact a measure of exactly this: the mean squared displacement over time Δt :

$$D = \frac{\langle \Delta x^2 \rangle}{2\Delta t}$$

The 2 is added for convenience (see below for explanation). Thus the mean squared displacement of the particle then depends on D and the time interval collisions (Δt). Although we derived this expression by considering the microscopic motion of a particle, the diffusion coefficient is in fact defined and measured by *macroscopic* measurements.

In 1905, Einstein published a groundbreaking paper (no, not the one on special relativity or the one on the photoelectric effect, but another one!) in which he used the above arguments to provide support for the hypothesis that matter is made up of discrete atoms and molecules. It was based on our understanding of the motion of molecules due to thermal energy. The thermal energy of a molecule at temperature T can be related to its average kinetic energy

$$\frac{1}{2} m \langle v^2 \rangle = \frac{1}{2} kT$$

Thermal energy of an atom is related to its kinetic energy by $\frac{1}{2} kT$ of energy for each degree of freedom (for each dimension in a 3 dimensional world), where k is the Boltzmann constant $1.38 \times 10^{-23} \text{ kg m}^2/\text{K/s}^2$. Einstein's paper showed that this same equation works for large particles in addition to atoms. If we do some rearranging, we can find that

$$\langle v^2 \rangle = \frac{kT}{m}$$

and if

$$\langle v^2 \rangle = \frac{(\Delta x)^2}{(\Delta t)^2}$$

Then we have an equation for $\langle \Delta x^2 \rangle$, which you will experimentally measure from which you can calculate D , the *diffusion coefficient*, a microscopic quantity:

$$\langle \Delta x^2 \rangle = \frac{kT}{m} (\Delta t)^2 = 2D(\Delta t),$$

where Δt is the average time between collisions (a calculated quantity).

But how do we measure $\langle \Delta x^2 \rangle$? $(\Delta x)^2$ of many particles in the same fluid can be measured (with a ruler if necessary), but the Δt that we can measure with a camera is much larger than the time between collisions of a particle with a molecule (much much larger). It turns out that for a given experimental Δt_{exp} , the distribution of Δx 's (how far the particle travels in Δt_{exp} is not necessarily the same every time) is Gaussian, as are all random distributions. From statistics we know that a Gaussian function has the form

$$P(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{x^2}{2\sigma^2}}$$

where $\langle x^2 \rangle = \sigma^2$, is the definition of standard deviation. By analogy, we can relate this to the Gaussian distribution of Δx 's measured, where $\sigma^2 = 2D\Delta t_{\text{expt}}$.

Going back to the theoretical background, we note that Δt , the time between actual collisions, is a material-dependent value, and will depend on the drag force of the liquid (which sets the terminal velocity of the particles), as explained below. When a particle is travelling through a viscous liquid it feels a drag force $F_{\text{drag}} = fv$, in the presence of an external force (think gravity for example) $F_{\text{ext}} = F_{\text{drag}} = fv$ at terminal velocity. The displacement of the particle can be described by kinematics

$$\Delta x = x_0 + v_0 \Delta t + \frac{1}{2} (F_{\text{ext}}/m) \Delta t^2$$

and

$$v = \Delta x / \Delta t$$

If we look just at the average velocity and combine the two equations (remember, the *average* of v_0 and x_0 are zero, because they are being bounced both up and down)

$$\langle v \rangle = \frac{1}{2} (F_{\text{ext}}/m) \Delta t = \frac{1}{2} (f \langle v \rangle / m) \Delta t$$

The $\langle v \rangle$'s cancel and we have a relationship between the drag coefficient f and the time between collisions:

$$\Delta t = 2m/f$$

For a spherical particle $f=6\pi\eta r$ (*Stokes' law*), where η , is the viscosity of the fluid, a *macroscopic* quantity, and m is the mass of the particle and r is the radius of the particle.

Once Δt is known, you can use your measurement of D to find the Boltzmann constant k , as

$$k = \frac{2m}{T\Delta t} D$$

$$(\Delta t) = 2Dm / kT = 2m / (6\pi\eta r)$$

$$k = 6\pi\eta r D / T$$

and from there find N_A , since $k = R/N_A$, and the value of the ideal gas constant is known (8.31 J/mol K).

Brownian Motion Lab Experiment

Equipment:

1 mm scale slide, Microscope slide, Hole reinforcement, Coverslip
Microscope
Celestron Webcam with lens removed
Computer with image capture software, ImageJ analysis software, and SigmaPlot

1.03 μm (diameter) polystyrene microspheres (diluted $\sim 100\text{x}$ from manufacturers concentration)
($\rho_{\text{polystyrene}}=1.055\text{g/cm}^3$)
Isopropyl alcohol
Distilled water
Dial caliper (measured in 0.001 inches)
C-clamp
Thermometer
Kimberly Clark precision wipes

Procedure:

1. Turn on the microscope light, and note down the temperature by sticking the bulb of the thermometer where the slide should be. Allow two minutes for this measurement.

Using the microscope and the imaging software and setting the scale

2. Using the micrometer slide, capture images of the scale using the 4x and 40x lens. Through the 4x lens, you should be able to see a circle with a small scale within.
 - a. The camera should be attached to the microscope, and therefore you should not have to remove it.
 - b. To capture still images, go to Start/All Programs/Celestron AMCap/AMCAP.exe . This opens a “live” view of the webcam. (The manual is also in this folder)
 - c. Using both the coarse (big knob) and fine (smaller knob) adjustment screws focus the microscope on the scale.
 - d. In the “capture” menu, go to “still/folder” to set the folder to save your images. Use “Ctrl-S” to “snap” a picture. Make sure you either re-name your image file or write down what each image file corresponds to. These images will be used while measuring distances on the microsphere sample.

Measuring motion due to diffusion

3. To prepare your microsphere sample, clean the slide and the coverslip first with isopropyl alcohol and then with distilled water. Rub clean to remove moisture and all traces of fingerprints with precision wipes. Remove the tube of spheres from the refrigerator *do not use the bottle

from the manufacturer, it is too concentrated* and shake vigorously to re-suspend spheres. Make a slide of spheres by putting one hole reinforcement in the center of a microscope slide. Add one small drop to the center of the reinforcement and put a coverslip on top of the reinforcement. Do this slowly to assure that there are no air bubbles under the coverslip. Also make sure not to touch the middle of the slide/coverslip so that your fingerprints do not cloud the image.

4. Place your sample slide under the microscope, and focus on the inner edge of the hole reinforcement using the 4x lens. You will need to turn the fine/coarse focus knob very gently. If you see bright black edged spheres, those are air bubbles. Try to minimize the number of air bubbles as they may cause drift in the sample (drift will give you a non-zero center to your random motion making the calculation more complex). There may also be stationary black/brown spots which are dirt on the lens. You will not see microspheres using the 4x lens. Move the slide so that you are then looking in the center of the sample and shift to the 40x lens. In the eyepiece you should see the tiny **vibrating** microspheres. In the camera you should see the microspheres as well.
5. Record a movie of the microspheres moving, 5 seconds should be sufficient (movie files are huge!) make sure the file is saved as an .avi.
 - a. Under Options/Video Capture Pin, make sure the streaming frame rate is 15frames/s, Color Space/ Compression is YUY2 and the "output size" is 640/480.
 - b. Go to "file/set capture file" to create a blank file to save your data in.
 - c. Go to "capture/set frame rate" and set this to something <14 frames/s, click "use frame rate". Go to "capture/set time limit", click "use time limit" box and set 7s. (The software captures 2 seconds less than the time limit.) Go to capture/ start capture and allocate 25 Mb.
6. To analyze the motion of the spheres you will need to open the file in ImageJ software.
 - a. Before looking at your movie, open your 40x scale image. Using the "line" button, draw a line of a known length. In the Analyze menu select "set scale" enter your scale bar conversion, and click "global".
7. Open your .avi file and click "convert to greyscale" button.
8. Go to Image/adjust/threshold. In the drop down menu to "black and white"
9. Use sliders to adjust contrast (make sure you ensure the moving spheres are clearly visible).
10. Apply. Check box "black background".
11. Save the movie as a new black and white .avi as a different file.
12. Close original file, and open the new file.
13. Open the Particle tracker program in "plugins/particle detector and tracker/ particle tracker" in ImageJ.
14. Edit the radius, cutoff, and percentile variables and click the "preview detected" button until the particle tracker correctly circles the real microspheres (Start with cutoff=0. This requires a lot of adjusting. You might want to try the tutorial found here <https://weeman.inf.ethz.ch/ParticleTracker/>) This manual is in the public documents directory on the computer. When you are satisfied, run the particle tracking, and save the results. Pay

attention when you run the particle tracking to the resulting traces of the particle motion. If you see a lot of traces that are not for “real microspheres” you will want to adjust the variables until you find that the majority of the traces reported are for “real microspheres”

15. Open the results in Excel using “import text”. The first 3 columns are “frame number, y, x” and are all you need. The frame corresponds to the number of the image in the movie.
 - a. Delete all the traces of motion which are for fake particles (not moving), and ones which are shorter than $\sim 10 \Delta t$.
 - b. In the end you should have ~ 10 -20 traces of particles or at least 100-200 measurements of Δx which cover one Δt . Determine the displacement of each particle at each time point from the previous. You will calculate both the x displacement, and the y displacement (positive or negative values). Delete all displacements that you calculate that skip frames (i.e. In the trace if the particle goes through frames 1,2,3,4,7,8,10, do not calculate the displacement from frame 4 to frame 7 and from frame 8 to frame 10). You will use the x- and y- displacements as independent data sets of particle motion when you recreate your Gaussian distribution. (Remember to use your scaling factor to have your results in say, microns).
16. Using the “frequency” function in excel you can bin the displacements (Δx and Δy combined) into small intervals. You may decide the bin size by finding the difference between the max and min displacements and dividing by the number of bins. You will want somewhere between 20 and 50 bins. For help using the “frequency” function, refer to:
<http://support.microsoft.com/kb/100122>.
Create a histogram of 1-D displacement (not the net displacement) vs. “number of times a sphere moves that distance” (frequency). You may use the x- and y- displacements as independent data and use both in the histogram. This graph is essentially a graph of the probability that a sphere moved a certain 1-D displacement in the time between frames, Δt . (Remember what a Boltzmann distribution should look like and compare with what you obtain!)
 - a. Using SigmaPlot, or some other software determine your experimental value of the Boltzmann constant from the 1-D diffusion. In sigmaplot you will fit a 3-parameter Gaussian function to your data.

Results:

1. You should know the answers to these questions:
 - a. What is a Boltzmann distribution?
 - b. What is diffusion, and why does it occur? How is it related to Brownian Motion?
 - c. What is “mean squared displacement”?
 - d. What is your experimentally measured value for the Boltzmann constant, and what is the uncertainty on this value? Note that this uncertainty is NOT the same as how well your experimental number agrees with the accepted value, though you should also discuss this in your report.

Tips and Tricks:

2. Particle Tracking setting:
 - a. Kernel Radius: a radius of 10 seems to give good results
 - b. Percentile: setting this between 0.15 and 0.4 results in the Particle Tracker finding between 100 to 500 trajectories, through which you will need to sort.
3. Analysis Tips:
 - a. We define a trajectory step (TStep) as: For a given trajectory, a step is from one trajectory measurement in a particular frame, to the next trajectory measurement in a particular frame.
 - b. A good TStep has the following properties:
 - i. The difference between frame numbers bracketing the TStep is one.
 - ii. The total 2-D displacement of the particle during the TStep is greater than 0.2 microns.
 - c. The way to weed out false trajectories is to keep only those trajectories which have more than ± 10 good TSteps.
 - d. Only use the 1-D displacements (both x and y) arising from good TSteps in good trajectories for the frequency analysis.