BROWNIAN MOTION

Goals:

- To become acquainted with the appearance of Brownian motion via direct observation and measurement of the positions of micron-sized spherical particles in water.
- To become acquainted with the statistical distribution of particle displacements.
- To calculate k_B by measurements of a particle's mean squared displacement.

Overview:

The experiment has three parts. In part I, you photograph, at regular intervals, a single particle, using a microscope with a digital camera driven by computer software. In part II, you use a second software package, Image J, to record the position of the particle in each photograph. In part III, you analyze the position data, with the help of Logger Pro software for manipulating and graphing the data.

Read *Einstein, Perrin, and the reality of atoms: 1905 revisited*, by Newburgh, Peidle, and Rueckner, (AJP **74**[6], pp 478-481, June 2006), and *Measuring Boltzmann's constant using video microscopy of Brownian motion*, by Nakroshis, Amoroso, Legere, and Smith (AJP **71**[6], pp 568-573, June 2003) for the theory and useful background information.

Part I: Data collection

Sample Preparation: Turn on the ultrasonic cleaner (under the stairs) and hold the latex sphere solution in it so that the solution level in the bottle is about equal to the water level in the ultrasonic cleaner. Hold for 10 seconds or so, so as to mix up the solution. Place a sheet of lens paper on the table, and place a dimpled microscope slide, dimple side up, on top. Using a small syringe, transfer a drop or so of the latex sphere solution to the center of the dimple. The drop's diameter should be no more than ½ centimeter. Cover the sample with a cover slide, so that the drop is squashed between the dimple and the cover slide, without touching the flat area of the microscope slide (if it does, start over!). Surface tension should hold the cover glass to the slide. Record the particle size and the ambient temperature.

Camera Preparation: Turn the microscope on, and select the 10X objective. Make sure the condenser mask ring is set to "A" (i.e. brightfield microscopy). Place the Motic calibration slide on the microscope stage, and looking through the eyepiece, focus the microscope on the cross-shaped scale at the center of the calibration slide. Adjust the illumination. Slide the microscope's eyepiece/camera switch to the camera position. Unplug, and then replug, the USB connection from the camera into the computer. Start up the Motic Images Plus 2.0 software. Go to File → Setting and set the following parameters:

File Name = `Capture` + Serial Number + `.tiff`
Every `2 Seconds` capture one image
Maximum Capture images: `120`
Image Size: `Auto`
Checkbox `checked`: Using current date and time as file names
Capture Source Select: `MC1001/MC2002`

Click OK. This sets the camera to take 120 photos, 2 seconds apart, when you use Auto Capture. Go to **File → Capture Window**. The right window displays a real-time image of your sample. If it appears all white or all black, click the Auto Exposure button on the settings side of the

capture window. You should see a slightly-out of focus cross. Adjust the microscope focus until the image is sharp. Set

Preview to 800 x 600
Capture Size to 1600 x 1200.

Calibration preparation: Our measuring program will give the location and displacements of the sphere images in units of pixels. The idea here is to take a picture of an object of known length--the separation of the bars on the calibration slide--to convert your pixel lengths into real world units. Now that you've verified that the camera is working, switch back to eyepiece view, and refocus on the crossed scale with the 40X objective. Without changing the aperture, increase the light bulb's intensity to maximum. Switch back to the camera, press Auto Exposure again, and then get the scale back in focus (at 40X). Try to position the scale in such a way that it fills the full width of the screen. Click once on the Capture icon. Note that it blinks and takes about 1 second to take the photo. Verify that that the photo was taken by minimizing the Capture Window. You should see the photo in the upper right corner of the Motic Images Plus 2.0 ML Software. Maximize the Capture window.

Capture Images: Switch back to eyepiece view, remove the calibration slide and place your sample in its place. You may be able to see the latex spheres in Brownian motion at 40X without having to change focus too much. (If not, switch to the 10X objective, and successively move to the 40X objective after getting the image in focus). Note that the spheres move in 3D, and so will appear to move in and out of focus. Move the focus up and down to find top and bottom of sample cell. Return your focus to a plane somewhere in the middle.

Switch over to the camera. You should be able to see the Brownian motion clearly. If there's a bright spot in the middle of your image, try closing the field diaphragm to reduce it.

Move the stage and adjust the focus so that you can clearly see a sphere near the middle plane, not too near other spheres, and near the center of your image.

Click the AutoCap icon to start the photography. It will take 240 seconds to capture 120 images. It is important not to disturb the table holding the microscope while photography is underway. Keep your eyes on your sphere—if it moves out of the plane, you may nonetheless need to move the microscope stage up or down to keep it in focus. Do this carefully, with bumping the table or translating the stage--this will mess up your calibration.

Now you've acquired your raw data. If you close the Capture window, you should see the photographs at right in the Motic Images Plus 2.0 ML software. Close the Motic Images Plus 2.0 ML software. Remove your sample, turn off the microscope, and cover it with its cover. Hold the microscope slide cover slide over the Sharps Container (under the stairs), and slide the cover slide (but not the microscope slide!) into it. Rinse the microscope slide at the sink and set it aside to dry.

The images are stored in
C:\Documents and Settings\Wireless\Application Data\Motic\Motic Images Plus 2.0\Capture Folder. I recommend that you make a new folder, on your desktop, and move the images into this folder for convenience. This is your raw data.

Part II: Preliminary Analysis with ImageJ:

ImageJ is public domain software for analyzing images. Click on the ImageJ icon to start the program. Click on **File → Import → Image Sequence**. In the Open Image Sequence pop-up box, navigate to the folder containing your images, click on the first image of the your sequence (this will be the calibration image), and click Open. In the Sequence Opt… popup box, verify that the correct number of images is selected, and check the "Convert to 8-bit Grayscale" box to minimize memory use.

Your first image should now appear, with a scroll bar at the bottom that allows you to scroll through your images. Scroll through them—does all appear okay? Click on the Magnifying Glass icon  in the toolbar, move to your image, and experiment with right- and left-clicking on the image. For ease in the next steps, try to magnify your image as much as possible, while keeping your object in the field of view in all of the frames.

Calibration:

Click on the line selection tool  from the ImageJ toolbar, click-hold on the center of one of the scale ticks, drag to the center of another scale tick on the other side of your photo, and let go. Select **Analyze → Set Scale** from the menu, and enter the Known Distance. (Recall each scale tick is separated by 10 microns). Enter "microns" as the unit of length, and check the "Global" checkbox so that this scale applies to subsequent images. Record the Distance in Pixels, your Known Distance, and the Scale in your lab write-up. [For example, 6.024 pixels/micron, 1445.749 pixels, 240 microns.]

Particle Tracking:

Move to the next image, and zoom in on your sphere. Click on the point selection tool  , and move to the image; your cursor is now a cross-hair. Click on the center of your sphere.

Go to **Analyze → Measure**, and a table will pop up displaying the coordinates, in microns, of your sphere. Move to the next photo in your sequence by clicking on the right arrow at the right of your scroll bar.

Move the cursor, either with the mouse or using the arrow keys after first clicking on the image, to the center of your particle, and go to **Analyze → Measure** again (you can also click CTRL+M to measure). This will enter the second set of coordinates into your table. Repeat this process for all of your images. It will go pretty fast.

When you've reached the last image, you may want to take a few measurements of the pixels adjacent to each other, to get some idea of the size of these pixels. Save your results to your desktop. The default format, .XLS, is fine. Quit ImageJ, but don't save any changes.

Save your Data. At this point, it is an excellent idea to copy your images and results to a writeable CD for safekeeping and for home analysis if desired.

Part III: Data Analysis:

Two programs on these laptops are available: Excel and Logger Pro. It's probably easier to manipulate your data in Excel, and to plot the results in Logger Pro. Of course, you are welcome to use Super Mongo, Igor Pro, or any other suitable program for your analysis.

1. First, plot a map of the sphere's random walk. Open up your data in Excel. You'll see a data point number in the left-hand column, followed by a column of X-positions and another of Y-positions. Delete any remaining columns to the right. Open up Logger Pro, and copy your X-values and Y-value columns into it. You can change most items by double clicking on them, so as to produce a nice graph. Print it.

2. Recall that for a particle executing a random walk, its probability of moving in the positive x-axis direction in a given step is equal to that for moving in the negative x-direction, and the step lengths are distributed in a Gaussian form. The same is true for the y-direction.

Delete the data in Logger pro. Use Excel to calculate the size Δx for each step, and cut/paste these values into Logger Pro. Select **Insert → Additional Graphs → Histogram**. Double click on the Histogram to bring up the Histogram options, and select appropriate values to produce a histogram.

You can fit a Gaussian (or any other function) to this data by selecting **Analyze → Curve Fi**t. You may need to play around with the histogram bin size and/or define your own function to get a good fit. What does it mean if your histogram is not centered on zero, to within experimental error? Print your histogram, displaying the fit with the fit results, and repeat for the Y-axis data.

According to our analysis of diffusion and the random walk, the distribution of step lengths Δx in the x direction follows a normalized probability distribution of the form

$$P(\Delta x) = \sqrt{\frac{1}{4\pi Dt}} \exp\{\frac{-(\Delta x)^2}{4Dt}\}$$

where $D=k_BT/(6\pi\eta a)$ is the self-diffusion coefficient, k_B is Boltzmann's constant, η is the viscosity of the solution, a is the particle radius, and t is the time between photographs. Look up the viscosity of water η at the room's temperature (this can be found in the CRC Handbook, or from the NIST Chemistry Webbook's Thermophysical Properties of Fluid Systems, at http://webbook.nist.gov/chemistry/fluid/). There are thermometers in the lab that will give you the temperature T. Use these to estimate a value for the spread in the distribution of step sizes in x. Compare to your results.

3. According to the analysis of Brownian motion, the mean squared displacement of the particle's position for diffusion in 2 Dimensions,

$$\langle \Delta r^2 \rangle = \langle \Delta x^2 + \Delta y^2 \rangle = 4Dt,$$

can be combined with the Einstein relation, $\zeta D = k_BT$, and the viscous drag coefficient for a sphere $\zeta = 6\pi\eta a$, to enable a measurement of Boltzmann's constant k_B. Use the mean squared displacement $\langle \Delta r^2 \rangle$ that you calculated for your particle by averaging over all of its displacements to calculate k_B. Compare to the known value. Don't forget to include the uncertainties in η, a, T, and $\langle r \rangle^2$ in your comparison.

Finally--time permitting--find a way to automate the tracking of the spheres in your photographs so that you can quickly generate more data. This will enable you to produce results with much more precision.