BROWNIAN MOTION

Introduction

The curious motion of microscopic particles in solution, noted after the development of high-magnification (200x) microscopes by van Leeuwenhoek in the 1600's, was long a puzzle. Initially supposed to be caused by some kind of life force or "vitality," this supposition was convincingly disproved by Robert Brown in 1828 who show that even pieces of ancient granite from the Sphinx displayed such motion when suspended in solution. Explanations for the motion remained unresolved until Einstein in his Ph.D. thesis and a subsequent 1905 paper proposed a theory for the statistical behavior of microscopic particles, and suggested that "it is possible that the movements to be discussed here are identical with the so-called 'Brownian molecular motion'; however the information available to me regarding the latter is so lacking in precision, that I can form no judgment in the matter."
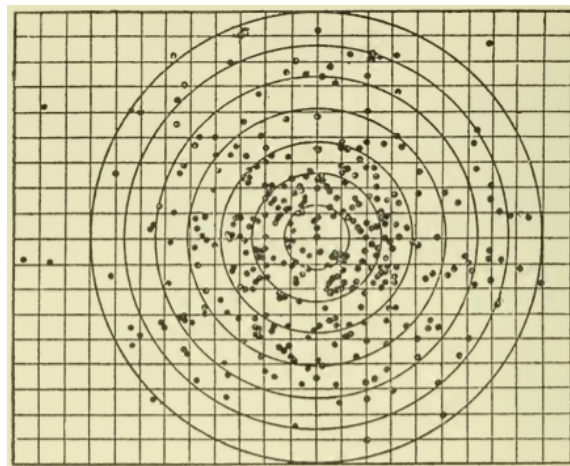

Figure 1 Distribution of 365 particle displacements normalized to a common origin. (Figure 7 in J. Perrin, "Brownian Movement and Molecular reality," Translated by F. Soddy. The original paper appeared in Ann. Chimi Phys. 18 (8me Serie), 5-114 (1909).

Einstein argued that the motion is due to the random collisions of unseen water molecules with the particle and related the motion to the macroscopic concepts of diffusion and viscosity. The derivation led to an expression for the mean square displacement of the particle, σ_{dx}^2, as a function of the particle radius r, the viscosity η of the medium, the time step t, the temperature T, and Boltzmann's constant k_B, as

$$\sigma_{dx}^2 = \frac{k_B T}{3\pi r \eta} t \tag{1}$$

In 1908, Jean Perrin provided the requisite experiments to determine a value for Boltzmann's constant. The experiment was of great importance because it provided direct evidence for the reality of atoms and molecules. Perrin received the 1926 Nobel prize for this work. Einstein's original work, the first reference in Appendix 1, is readable today. The second and third references in that Appendix provide historical background and alternative arguments leading to Equation (1).

In this lab experiment, you will be able to carry out measurements similar to Perrin's using more modern instruments and specimens. You simply have to make repeated measurements of a particle's position at equal time intervals.

Experiment

You have at your disposal the following tools:
- Latex microspheres, 1 μm in diameter, in solution.
- Directions for preparing a microscope slide with a solution of latex microspheres.
- Dimpled microscope slides and cover slips.

- Diffraction gratings and a He-Ne laser for calibrating your measurements.
- A microscope, with a dedicated microscope camera and associated software.
- A computer (but feel free to use your own)
- Fiji, a software package built around ImageJ, widely used for image processing.
- Thermometers (hanging on the wall).

The microscope is fitted with a camera that can take photographs at 1600x1200 resolution with a period that you specify. Fiji/ImageJ can then run through these images, from which you can extract the coordinate (in pixels) of these particles. Using the diffraction grating as a calibration slide, you can calibrate the pixels measurements into lab coordinates (microns, for example). In this manner you can procure a table of particle coordinates as a function of time, which you can further analyze using statistical and uncertainty analyzes.

You might consider carrying out the experiment in two stages. In the first stage, taking just a couple of hours, you figure out how to use the equipment and make an initial set of measurements, from which you can do a preliminary analysis of the data. You can then return for a second stage measurement, where, now that you are familiar with the tools, you can plan for a more careful and systematic measurement and analysis with precise uncertainties.

Your lab guru will quickly run through the steps for making a sample, for using the microscope, the microscope camera and software, and the image processing software. The appendices 2-5 provide specific details on some of the capacities of these tools. They are geared at getting you up and running with a "stage 1" type experiment in case you miss the lab introduction.

Helpful Hints (to avoid impending disaster):

Be sure to periodically save your data! Unload your raw images and processed data to the cloud or to a USB drive so that you have this data secure.

Keep a notebook and record your procedures, thoughts, and calculations along the way. Use your phone and computer screen-capture functions to record important details. Note the serial numbers of the microscopes and diffraction gratings that you use.

Stop and think: keep your procedure logical and systematic. Think about the uncertainties in your measurements at every step, and how you might test for their size.

Don't forget to measure the temperature before leaving the lab.

Take a picture of a diffraction grating under exactly the same conditions as for the images of your specimen when you make your measurements.

Appendix 1: References

Einstein, A. "Investigations on the Theory of the Brownian Movement," Annalen der Physik 17, 1905, p. 549. Translated by A.D. Cowper and edited with Notes by R. Feurth, Dover, 1956. Find it here: http://www.damtp.cam.ac.uk/user/gold/pdfs/teaching/einstein_brownian05.pdf

Einstein, Perrin, and the reality of atoms: 1905 revisited, by Newburgh, Peidle, and Rueckner, (AJP **74**[6], pp 478-481, June 2006).
http://scitation.aip.org/content/aapt/journal/ajp/74/6/10.1119/1.2188962

Measuring Boltzmann's constant using video microscopy of Brownian motion, by Nakroshis, Amoroso, Legere, and Smith (AJP **71**[6], pp 568-573, June 2003)
http://scitation.aip.org/content/aapt/journal/ajp/71/6/10.1119/1.1542619

J. Perrin, "Brownian Movement and Molecular reality," Translated by F. Soddy. The original paper appeared in Ann. Chimi Phys. **18** (8eme Serie), 5-114 (1909).
(http://web.mit.edu/swangroup/footer/perrin_bm.pdf)

Thermometers: Two are hanging in the rear of 478, near the ends of the half-walls.

Instruction Manual for the Nikon E200 Microscope:
http://lecdemo.johnshopkins.edu/E200_IM.pdf

Youtube Nikon E200 Instructional video: https://www.youtube.com/watch?v=-lo-KICXa84

Viscosity Data: http://webbook.nist.gov/chemistry/fluid/

Nelson, E. 1967. *Dynamical Theories of Brownian Motion*, Princeton, N.J.: Princeton University Press (https://web.math.princeton.edu/~nelson/books/bmotion.pdf). Nice historical review as well as a viewpoint from different statistical approaches.

ImageJ user guide: https://imagej.nih.gov/ij/docs/guide/146.html. Many other tutorials and plug-ins are available through the world wide web.

Particle Tracking Scripts: http://tacaswell.github.io/tracking/html/. This is a list of and repository for various particle tracking routines. If you go this route, you may want to review the third reference above (Nakroshis et. al.) for some ideas on how to pre-process the image files.

Appendix 2: Preparation of Brownian Motion Experiment Samples

1. Make up a secondary latex sphere solution in a 100 ml beaker if one is not available (see below.

2. Lay a dimpled microscope slide flat on the table on top of a Kimwipe tissue.

3. Use the Disposable Pipets to remove a portion of the water-diluted Latex sphere secondary solution from the 100 ml beaker, and over-fill the dimple in the slide with the solution.

4. Lay a cover slide gently on top of the dimple, with one edge down on the microscope slide, and gently push the cover slide in place, squeezing out the excess latex sphere solution.

5. Use another Kimwipe to blot up the excess solution. Examine—there should be few or no bubbles in the solution. If there, wash off the dimpled slide and try again.

IF A PREPARED SECONDARY LATEX SPHERE SOLUTION IS NOT AVAILABLE, you will need to make your own secondary solution.

1. Find a clean 100 ml beaker and fill it up (80 to 90 ml) with deionized water.

2. Locate the primary latex sphere solution, in the small bottle from Thermo Scientific and labeled "Latex Microsphere Suspensions."

3. Located the Eppendorf 20-200μl pipet, and put a brand new yellow pipet tip on it.

4. Using the Eppendorf pipet, remove a tiny amount of latex solution from the primary bottle, and drop just one drop into the deionized water—being careful not to touch the water with the pipet. Any remaining latex sphere solution in the pipet can be returned to the primary bottle. Cap the primary bottle, discard the yellow pipet tip off of the Eppendorf pipet, and return the pipet, yellow pipet tips, and primary solution to their box.

5. Using a disposable pipet, stir the secondary solution, and then remove what you need for your experiment.

The secondary solution will last for up to a day. After that, the spheres tend to clump together, and the solution should be discarded in the sink and remade.

After you are done with your Brownian motion experiment, dispose of the slides in the beaker marked "USED SLIDES IN WATER."

Appendix 3: Expert's Guide to Setting up the Microscope

1. Remove the dust cover
2. Turn on the microscope lamp and open the lamp and condenser irises so that the microscope stage is illuminated.
3. Fasten a microscope slide onto the stage.
4. Make sure that the Aperture Selector is set to "A" (for brightfield microscopy)
5. Rotate the revolving nosepiece until the lowest power objective is in place.
6. Push in the Bino/Photo lever, so that the image forms inside the binocular tube.
7. Looking through the eyepiece, rotate the Coarse and Fine focusing knobs until the image is in focus. Rotate one of the two eyepieces until the image is in focus in both eyes.
8. Move the microscope slide around until the sample is in the center of the field of view.
9. Close down the Lamp Iris, and adjust the Condenser Focus Knob until the Lamp Iris is in focus. At this setting, the microscope should have the most even and clearest illumination on the stage.
10. Center the lamp iris, if not already, by means of the lamp Iris Centering Screws and then widen the lamp iris to max.
11. Successively switch objectives to higher powers, adjusting the focus, lamp intensity, and condenser iris as necessary, until the desired magnification is reached.
12. Adjust the condenser iris and lamp brightness until the desired contrast in the image is achieved.
13. Make observations.
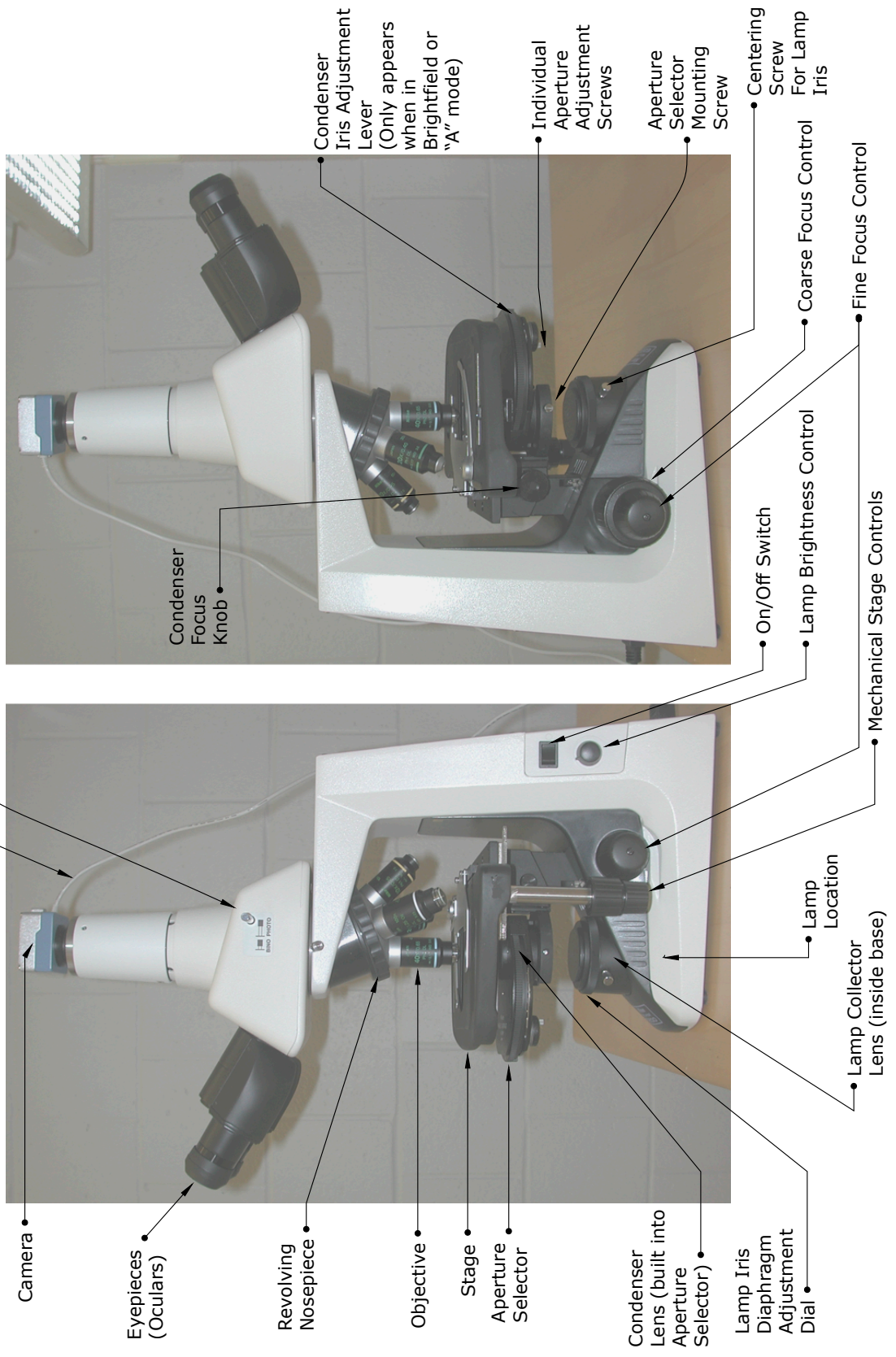14. Clean up, turn off the microscope lamp, and replace the dust cover.

Troubleshooting: common problems

- The microscope is not fully plugged in, lamp is not on or is turned down too low.
- The Bino/Photo lever is not fully pushed in or out.
- The nosepiece containing the objectives is not snapped into place.
- The aperture selector beneath the stage is not snapped into place.
- The aperture selector is not at the correct aperture ("A" for brightfield)
- The condenser iris is closed or open too far.

Notes:

- ❖ Use a coverslip over the sample to project the objective lens.
- ❖ Do not touch the glass part of any lens with your fingers. Use only special lens paper to clean the lenses.
- ❖ Always keep the microscope covered when not in use.

Parts of the Microscope

Camera

Eyepieces (Oculars)

Revolving Nosepiece

Objective

Stage

Aperture Selector

Condenser Lens (built into Aperture Selector)

Lamp Iris Diaphragm Adjustment Dial

Lamp Collector Lens (inside base)

Lamp Location

USB Cable to computer

Bino/Photo Lever (switches between oculars and camera)

Condenser Iris Adjustment Lever (Only appears when in Brightfield or "A" mode)

Individual Aperture Adjustment Screws

Aperture Selector Mounting Screw

Centering Screw For Lamp Iris

Coarse Focus Control

Fine Focus Control

Condenser Focus Knob

On/Off Switch

Lamp Brightness Control

Mechanical Stage Controls

Appendix 4: The camera

Camera: Motic Moticam 2MP.
Sensor: 1/3" size, 1600 x 1200 pixels
Designed to work with "Motic Images Plus 2.0 for PC and Mac" software
Comes with 4-dot calibration slide

Software: Motic Images Plus 2.0 ML.\
Note: this software bundles another piece of software, called "Motic Live Imaging Module" which is the part that interacts with the camera.

Notes on using the Software:
(1) The settings for taking photographs are inside the Motic Images Plus software, under the **Settings** tab. Here you set how frequently and how many photos are taken. We recommend that you initially start with 2 seconds between photos, and that you save the file with the .tiff extension and use the current date and time as the file name.
(2) The Motic camera should be connected to a computer via a USB cable before launching the live imaging module.
(3) On the microscope, the bino/photo lever should be pulled out in order for the image to be projected onto the camera sensor.

In the Live Imagine Module (launched via the **Capture** tab in Motic Images Plus)
(4) In the first tab, **video device** should show "Motic 2.0 MP" if camera is connected.
(5) **Fit to Window**, at the bottom of the first tab, if NOT checked, displays the camera image pixel-to-pixel. That is, one pixel on the camera sensor corresponds to one pixel on the computer. This gives you maximum precision and resolution for examining the image. When checked, the entire camera sensor's image is presented within the confines of the computer's window.
(6) The **exposure** setting, which is related to the time required to take a photograph, may set a lower limit on how fast you can take a sequence of photographs. If you see horizontal bands scrolling down the image, then the exposure setting is too low.
(7) The sequence of photos specified under **Settings** is launched by pressing the "AutoCapture" button in the **Capture** tab of the Live Imaging Module.
(8) The photographs in our computers are stored in C:\Motic\Motic Images Plus 2.0\Capture Folder.

# Appendix 5: The Image Processing Software, Fiji / ImageJ

Goal: to extract the coordinates of a particle as it undergoes Brownian motion from a sequence of images, fairly quickly, to use for preliminary data analysis.

Solution: Many different software packages (including Python) are able to analyze image data. We offer for your use a popular, powerful, free, and open-source package called Fiji, which bundles the core program ImageJ with many interesting add-ons and updating tools. This program will allow you to quickly obtain the data you need.

An easy procedure for using this software:
1. Launch Fiji. No need to run updates if asked.
2. File → Open. Navigate to the folder where your images are stored and select the first image.
3. Image → Properties. Insure that the unit of length is pixel; set the pixel width, pixel height, and voxel depth each to 1. Check the "global" box in the dialog (see box at right).
4. Choose one particle that you will follow through all of your images. Select the "Point" Tool (✛) from the ImageJ menu and place the curser squarely on the center of the particle. You may want to zoom in so to better place your cursor, and use the arrow keys to move the cursor.
5. Analyze → Measure will launch a table that includes the coordinates of the particle you have chosen.
6. File → Open Next will open the next image. *Note: If a dialog box pops up about image calibration, uncheck the box "Disable Global Calibration" and check the box "Disable these Messages."* Use the arrow keys to move the cursor to the new particle location, and then Analyze -- > Measure (again) to add the coordinates to the Results table.
7. Repeat process (using keyboard shortcuts) until the particle is fully tracked.
8. Save your measurement results (as a csv file by default).
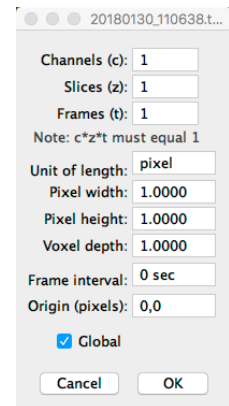
Generally speaking, it is a good idea to separate your calibrations from your measurement data, and to apply the calibration separately. There are uncertainties in both the measurement data (i.e. in your determination of the pixel coordinates) and in the calibration measurements. Both uncertainties should be accounted for in the final set of reported data.

A procedure for making a single calibration determination:
9. Open an image containing your diffraction grating. Use the "Straight Line" tool (⟋) to place a line segment on this slide. The zoom tool is your friend to help you place the endpoints with precision.
10. Analyze→Set Scale. From this you can read off the distance in pixels.
11. Use the Ne-Ne laser and rulers in the adjacent room to determine the spacing of your grating. Because the wavelength of the HeNe laser is so well known (632.8 nm) and so easily produced, it provides a solid number against which to calibrate the gratings.

Appendix 6: Objectives and Goals of this Experiment

To become acquainted with the properties of Brownian motion: to understand what it is, what causes it, and the approximate order of magnitude at which it exists.

To learn how to handle the statistics needed to describe the data and its uncertainties

To learn how to use a microscope camera and associated software to take pictures

To learn how to calibrate the dimensions of a microscope slide, with uncertainties.

To be introduced to image processing software and some of its capabilities

To learn how to figure out how to make measurements: what to measure? how many measurements? At what scale?