Thermal Motion

1 Introduction and Historical Facts

The most famous description of Brownian Motion was given by the botanist Robert Brown in 1828.His first speciments were of 'live' pollen grains (he believed that pollen grains were alive). To his credit, he did show that inorganic as well as organic objects produced the same movements, and more importantly, demonstrated that the phenomenon was not due to convective currents or other experimental artifacts. Nevertheless, Brown still clung to the notion that the cause of the motion was some unknown active molecules internal to the particles and not in the surrounding fluid [1].

In 1905, Einstein, then an industrious patent clerk, produced a series of work on the elementary theory of Brownian motion (and also found time to hypothesize the photon and invent the theory of special relativity). Remarkably, he completed the first paper without ever having seen Brownian motion himself. The paper begins [2]:

.. "*according to the molecular-kinetic theory of heat, bodies of microscopically visible size suspended in a liquid will perform movements of such magnitude that they can be easily observed in a microscope, on account of the molecular motions of heat. 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.*"

Einstein's intuition was indeed the correct physical picture. The particle is randomly bombarded by the molecules of the surrounding fluid and these random forces impart velocity changes to the particle. The particle's velocity immediately gets damped by viscous forces and so ultimately these impulses generate a change in the position, causing the particle to undergo a random walk. Einstein's theory of Brownian motion requires some knowledge of the diffusion equation, which we have yet to discuss. So we present the relevant results for this lab without further comment, but give alternate derivations of the same results in the next section. Einstein showed theoretically that the mean squared distance of a Brownian particle increases linearly with time, $< x^2 >= 2Dt$, where D is the diffusion coefficient, and that the diffusion coefficient of the particle is equal to the ratio of the thermal energy and Stokes drag, $D = \frac{kT}{\gamma}$, where k is Boltzmann's constant.

Jean Perrin experimentally verified Einstein's results in 1908. Perrin projected magnified images of Brownian particles using a *camera lucida*, and traced their complicated trajectories over time. The figure below shows three such traces of $0.53\mu m$ mastic (tree resin) particles where the position was marked every 30 seconds (1 division = $50\mu m$). By averaging a large number of positions, Perrin showed that the mean squared distance of these particles increased linearly with time [3].
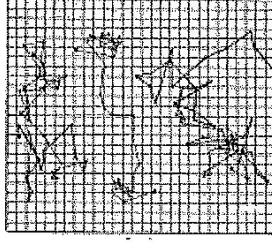
Figure 1: Successive displacements of $0.53\mu m$ mastic particles (Perrin [3])

Another striking verification was obtained by shifting each displacement in space so to give them all the same origin as shown below in Figure 2. This distribution should be Gaussian with a spread that depends on the diffusion coefficient, which Perrin verified quite readily. After much sweat and labour, Perrin came within about 20% of the currently accepted value of Avogadro's number. Can we do better?
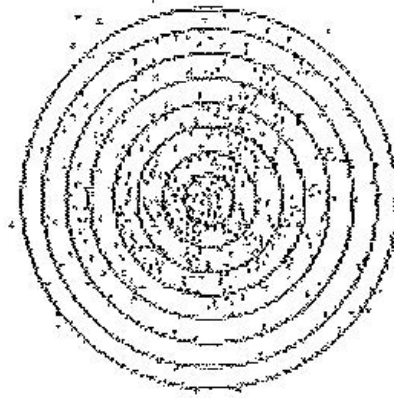

Figure 2: Steps with common origins for $0.37\mu m$ particles, 30 s time interval, $< r^2 > \approx 64\mu m^2$

2 Theory

As you have learned, the motion of an object due to thermal energy is known as Brownian motion. One of the characteristics of this motion is that the mean displacement of the particle is zero $< x >= 0$, while the mean squared displacement increases linearly with time $< x^2 >= 2Dt$, where D is the diffusion coefficient.

1-D random walk

One way to see this relationship is through a simple random walk model. Picture an ensemble of particles stepping randomly left and right in $1-dimension$. Every τ seconds, each particle steps a distance δ in either direction with equal probability. The position of the $i-th$ particle after n steps is:

$$x_i(n) = x_i(n-1) \pm \delta \tag{1}$$

If we assume we have N particles, the average position of the ensemble of particles is:

$$< x(n) >= \frac{1}{N}\sum_{i=1}^{N} x_i(n-1) \pm \delta =< x(n-1) > \tag{2}$$

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Note that the $\pm$ term averages to zero. The mean position from step to step is constant. The average mean squared position is given by:

$$< x^2(n) >= \frac{1}{N} \sum_{i=1}^{N} [x_i^2(n-1) \pm 2\delta x_i^2(n-1) + \delta^2] =< x^2(n-1) > +\delta^2 \tag{3}$$

The $\pm$ term in the brackets averages to zero. Since $< x^2(1) >= \delta^2$, $< x^2(2) >= 2\delta^2$, $< x^2(3) >= 3\delta^2$, etc., we have $< x^2(n) >= n\delta^2$. Since $t = n\tau$, we have:

$$< x^2(t) >= \frac{\delta^2}{\tau} t = 2Dt \tag{4}$$

Newton's equations with external thermal force

Another way to derive this relation is to add an external *thermal* force to the equation of motion:

$$m\frac{d^2x}{dt^2} = -\gamma\frac{dx}{dt} + X \tag{5}$$

In equation (5), X is the *thermal* force, positive or negative. We assume that the viscous drag is Stokes drag $\gamma = 6\pi\eta r$, where η is viscosity of water and r is radius of the sphere. Note that we are looking for the mean square distance the particle travels in some time interval t. Multiplying equation (5) by x, we obtain:

$$mx\frac{d^2x}{dt^2} = -\gamma x\frac{dx}{dt} + xX \tag{6}$$

Using:

$$mx\frac{d^2x}{dt^2} = \frac{m}{2}\frac{d^2x^2}{dt^2} - m\left(\frac{dx}{dt}\right)^2 \tag{7}$$

And noting that: $x\frac{dx}{dt} = \frac{1}{2}\frac{d(x^2)}{dt}$, we obtain:

$$\frac{m}{2}\frac{d^2x^2}{dt^2} - m\left(\frac{dx}{dt}\right)^2 = -\frac{\gamma}{2}\frac{d(x^2)}{dt} + xX \tag{8}$$

Take the time average of both terms. The term $< xX >$ averages to zero.
Setting $z = d(x^2)/dt$ and using equipartition of energy, we obtain:

$$\frac{m}{2}\frac{dz}{dt} + \frac{\gamma}{2}z = kT \tag{9}$$

The solution to equation (9) is:

$$z = \frac{2kT}{\gamma} + Ce^{\frac{-\gamma}{m}t} \tag{10}$$

The time constant of the exponential is small: therefore we ignore this term. We obtain:

$$\frac{d(x)^2}{dt} = \frac{2kT}{\gamma} \tag{11}$$

Equation (11) can be re-written as:

$$< x^2 >= \frac{2kT}{\gamma}t \tag{12}$$

Note that $D = kT/\gamma$ is Einstein's relation, therefore equation (12) becomes $< x^2 >= 2Dt$. The case above is for one dimension, but since the movement along each dimension is independent, the mean squared distance in three dimensions would be simply:

$$< r^2 >=< x^2 > + < y^2 > + < z^2 >= 6Dt \tag{13}$$

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We would be verifying that equation (13) does in fact hold for Brownian motion, by measuring the motion of small polysryrene beads diffusing in water. Plotting the mean squared distance as a function of time should give us a linear relation, with a slope proportional to the diffusion coefficient D.

Having D and assuming that the viscous drag is Stokes drag $\gamma = 6\pi\eta r$, where η is viscosity of water and r is radius of the sphere, we will be able to calculate Boltzmann's constant k using Einstein's relation.

Distribution of steps in a constant time interval

An alternate method to calculate Boltzmann's constant is to measure the distance the bead travels over a specific time interval. The probability distribution for the distance traveled in time is Gaussian:

$$P(x,t) = \frac{1}{(4\pi Dt)^{1/2}} e^{-\frac{x^2}{4Dt}} \tag{14}$$

Where D is the diffusion coefficient. Fitting a histogram of the distances that the beads travel during a given time interval will allow you to calculate D and k. Note that you have in mind the bin width dx when fitting the discrete probablity distribution to the continuous distribution $P(x)$.

Avogadro's number

The connection between the microscopic statistical model of molecular motion and the macroscopic gas laws is done by the Avogardro number N_A.

The gas laws come from empirical results, while the derivation of the gas laws from the kinetic theory of gases is a theoretical result. The ideal gas law can be written as $pV = nRT$ where p is the pressure, V is the volume , n is the number of moles, R is the gas constant and T is the temperature. Alternatively, $pV = kNT$ where N is number of molecules, k is Boltzmann's constant and N_A is Avogadro's number $N_A = R/k$.

3 Experimental Procedure

In this experiment, you will measure the thermal motion of micron-sized spheres in water. The diffusion constant of the beads can be calculated by tracking position over time using video microscopy.

Boltzmann's constant k can be determined from Einstein's relation $D = kT/\gamma$, assuming Stokes drag: $\gamma = 6\pi\eta r$.

You will have two sessions to complete everything below. On the first day, you will track single particles undergoing Brownian motion and start the data analysis. On the second day, you will finish by writing the Python program to help with data analysis.

Exercise 1

Setting up the microscope

1. Switch on the microscope (bottom left switch) and turn up the illumination intensity (botton right dial).

2. Turn the phase ring to (Ph1)

Focus on a piece of printed paper by turning the large focusing knob at the left bottom side until you visualize the shapes of ink sprayed on paper to form letters.

Place an empty slide on the microscope stage and get familiar with moving the slide in 2 dimensions.

CAUTION: excessive pressure from the objective can break the slide and damage the lens. Do not lower the objective too quickly. When approaching the focusing limit, use the inner part of the large knob for a finer adjustment

Preparing the sample

1. On a microscope slide, delimit a long (3-4 cm) rectangular space of about 1.2 cm width using two layers of double sticky tape on either side. Press down the tape to avoid creases and air bubbles, Seal off a space of about 2 cm length with a dot of grease at either end, to prevent the leak of the beads suspension.

2. Pipette out 50 μl of the diluted bead solution and dispense it into the sealed slide space. Gently place a cover glass over the space.

Setting the microscope for fluorescence illumination

1. Switch on the fluorescence illumination (X-Cite box).

2. Rotate the GFP fluorescence cube into place with the dial on the upper right side of the scope. The fluorescence cube should be in Position 2 indicated on the dial (alligned with the mark on top of the filter housing).

3. Make sure that the fluorescence shutter is set to open(O) (front lever just below the eyepieces). You should be able to see the blue excitation light coming from the objective.

4. Carefully place the prepared slide on to the microscope stage so that *grease does not touch the objective*. Leave the sample still for a couple of minutes to ensure equilibrium of particles against flow, evetually created by moving the slide around.

5. Focus the image using phase microscopy (Ph2) with the 40× objective. Use the focusing knob so that you are approximately imaging the center of the preparation depth. You should see bright, star-like points on a darker green background.

6. Note the difference in motion between the large and small particles.

Data acquisition

1. Run the Microscope Camera Controller (LabView application) and switch to camera imaging (lever in/out on the right-top part of the microscope). The acquisition software allows you to acquire a single image, multiple images or AVI movie. Get familar with camera adjustments: Gain Value and Brightness Value. The are found under Acquire/Adjust Image tab. Set up the ajustment values that provide the best contrast between beads and the background.

2. Your goal is to capture images with a few *energized* beads in the field. Too many beads will make the analysis difficult because the paths of the beads will interfere over time. Too few beads will make it difficult to find good candidates for tracking. If necessary, prepare a new slide.

3. Search for beads located in nearly the same focal plane and spaced not too close to each other. If the beads being tracked get dimmer, you can refocus the microscope while the images are being captured. Set up the Microscope Camera Controller on Multiple Image Capture. Select Number of Images: 120 frames and Images per Second: 2 frames. Save the data set as a folder on your memory stick. Each folder will consist of an image sequence. Make a new folder every time you take a new recording.

4. Take at least 10 data sets with different beads. Try to stay on the same focal plane. If the beads seem to be less energetic (motions are not swift anymore) or if it becomes difficult to find any moving bead in the field of view, prepare a fresh slide.

Image analysis

In Image Object Tracker, click on *Click here to select Folder* and open the first image. Click

in the middle of the bead you want to track and the data will be saved as a text file.

Exercise 2

Data analysis with Python: Mean Squared Distance vs. Time

Some constants:

Bead diameter: $1.9 \pm 0.1 \mu m$

Viscosity: 1.00 ± 0.05 centipoise (poise= g/cms) at $20^o C$ but decreases 2% with each degree increase in temperature.

Temperature: $296.5 \pm 0.5K$

Distribution of steps in a constant time interval The probability distribution for the *displacement* of a particle in 2D (or 3D) is also a Gaussian, but now a 2D Gaussian,

$$p(x, y; t) = \frac{1}{4\pi Dt} e^{-\frac{x^2+y^2}{4Dt}} . \tag{15}$$

This differs from Equation (14) in the scaling factor, and that the exponent is now $x^2 + y^2$.

The analysis is complicated by the fact that we now have two independent variables: the distribution is a surface, not a curve. We can convert this distribution into a curve by observing that the *direction* doesn't matter, only the *distance*. Thus, we substitute $r^2 = x^2 + y^2$.

There is one more important step to this variable substitution, because probabilities use integration. For example,

$$P(x < a, y < b; t) = \int_0^b \int_0^a \frac{1}{4\pi Dt} e^{-\frac{x^2+y^2}{4Dt}} dx dy \tag{16}$$

Changing variables of integration requires more care. For polar coordinates, $dxdy \rightarrow rdrd\theta$. Now, we can calculate the probability that a particle has moved up to some distance R,

$$P(r < R; t) = \int_0^{2\pi} \int_0^R \frac{1}{4\pi Dt} r e^{-\frac{r^2}{4Dt}} dr d\theta = \int_0^R \frac{r}{2Dt} e^{-\frac{r^2}{4Dt}} dr d\theta \tag{17}$$

The probability density function for the step size in a 2D diffusion process is therefore,

$$p(r; t) = \frac{r}{2Dt} e^{-\frac{r^2}{4Dt}} \tag{18}$$

This is a Rayleigh distribution. The same derivation in 3 dimensions provides the Maxwell-Boltzmann equation. In other words, if a position (or momentum) follows a Gaussian distribution, its *magnitude* follows the Maxwell-Boltzmann distribution.

Some assumptions of least-squares (`curve_fit()`) are violated when data is put into a histogram. Notably, changing the bin size has a significant effect on the calculation. Instead, when fitting a *pdf*, one should use *the maximum likelihood* to determine parameters. For the Rayleigh distribution, the maximum-likelihood estimate gives the formula,

$$(2Dt)_{\text{est}} = \frac{1}{2N} \sum_{i=1}^{N} r_i^2 . \tag{19}$$

Probability distribution of step lengths

From your bead position data, calculate the distance between each time point, plot the data histogram, using the hist module from SciPy and then fit the probability density function in Equation to the histogram. From the fit, you can determine D.

1. Calculate the distance travelled (the step size) between each time point.

2. Repeat for all of your data sets.

3. Compile all the step size data into a single 1D array.

4. Plot the step size data in a histogram with an appropriate number of bins.

5. Fit Equation (18) to the data using the `curve_fit()` function from SciPy. Refer back to the theoretical discussion; calculate k.

6. Use the maximum likelihood estimate to calculate k.

7. Plot the Rayleigh distribution using each estimate on the same axes as the histogram.

8. Calculate the percent difference from the accepted value of $k = 1.38 \times 10^{-23} J/K$.

The bead tracking program outputs position (x, y) and time in an array format. The distance are in pixel. Convert values to micron, using $0.1155 \mu m/pixel$. The uncertainty in position is $10^{-1} \mu m$. The uncertainty in time is $0.03s$
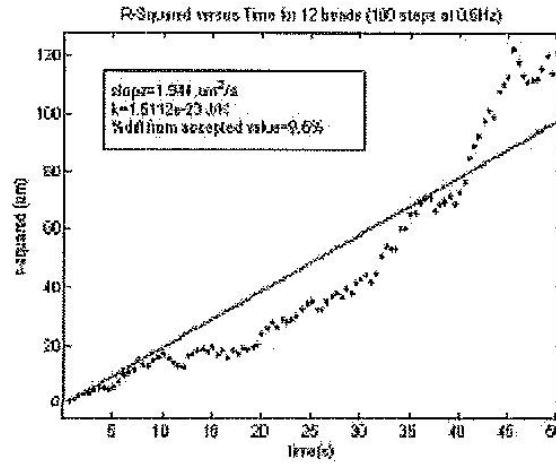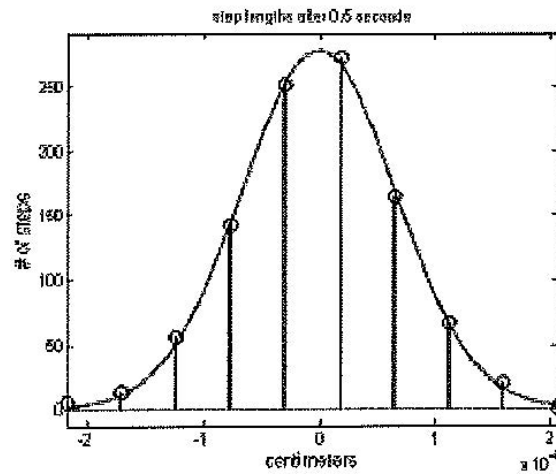

Figure 3: Sample plot: Mean-squared distance vs. time

Figure 4: Sample plot: Probability distribution of step lengths

4 References

[1] E. Nelson *Dynamical Theories of Brownian Motion*, Princeton Univ. Press, Princeton NJ, 1st edition, 1967.

[2] A. Einstein *Investigations on the Theory of the Brownian Movement*, Dover Publ. Inc. New York, 1st edition, 1956.

[3] J. Perrin *Atoms* Ox Bow Press, CT, 1st edition, 1990.

[4] P. Langevin *Sur la théorie du mouvement brownien*, C. R. Acad. Sci, Paris, 146 (530), 1908.

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The LabView Microscope Camera Controller and also the Image Object Tracker were written by Larry Avramidis