Brownian motion

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The purpose of this assignment is to learn some basic statistical physics, microscopy, imaging, and image analysis.

I. BACKGROUND

A. The early days of statistical physics

In the early 1900s, it became increasingly clear to physicists that the "molecular-kinetic theory of heat" (which described a statistical microscopic basis for thermodynamics) had to prevail. One of the final nails in the coffin for those who resisted was one of Einstein's 1905 articles: "Investigations on the theory of the Brownian movement". Einstein had been made aware of microscopic observations by the biologist Brown, where small pollen particles in a liquid moved around in a random manner. Einstein took this as a starting point and created a quantitative model where measurable quantities could be compared. It didn't take many years before experimentalists managed to measure the various quantities independently and thus confirm Einstein's model and, through it, the microscopic theory of heat that we now take for granted.

B. Nobel Prize in 2009

Electronic cameras surround us everywhere and have been used scientifically since they were invented. In 2009, Willard S. Boyle and George E. Smith at Bell Laboratories were awarded the Nobel Prize in Physics "for the invention of an imaging semiconductor circuit - the CCD sensor."

II. LANGEVIN EQUATION: FLUCTUATING FORCES AND RANDOM WALK

In 1908, Langevin published a simpler and more elegant derivation of the model for random walk[1]. His approach has become a recurring theme in statistical physics[2]. Both Einstein's and Langevin's articles are available on Fronter in English, and they are worth a look.

a. Molecular movements and the equipartition principle are fundamental to the microscopic theory of heat: In equilibrium, the kinetic energy of each degree of freedom of movement for the molecules in the liquid and particles floating in the liquid is: $kT/2$ where k is Boltzmann's constant and T is the temperature. In the x-direction, $v_x = dx/dt$:

$$m < v_x^2 >= kt, \tag{1}$$

where $< ... >$ means "ensemble average" or the mean value over many particles.

b. Viscous damping For particles that are much larger than the mean distance between the liquid molecules, the liquid can be treated as a continuum, and the hydrodynamic damping of the particle motion can be considered. The resistance to motion in liquids is covered in another exercise, but for a sphere in a viscous liquid at low Reynolds numbers, Stokes found that the drag force F_d was

$$F_d = 6\pi\mu v_x r, \tag{2}$$

where μ is the shear viscosity of the liquid and r is the radius of the sphere.

c. The Langevin equation The macroscopic particles are subject to a *rapidly varying random force* F_r due to the thermal movements of the liquid molecules and a *slowly varying damping force* due to the viscosity of the liquid. Langevin combined these in an equation of motion for the particles:

$$m\frac{d^2x}{dt^2} = F_r - F_d = F_r - 6\pi\mu r\frac{dx}{dt}. \tag{3}$$

This type of equation of motion for systems that can be divided into a rapidly varying and a slowly varying force is called a Langevin equation. By multiplying both sides by x and using the product rule for derivation, equation (3) can be rewritten as

$$\frac{1}{2}m\left(\frac{d^2x^2}{dt^2} - 2v_x^2\right) = xF_r - 3\pi\mu r\frac{dx^2}{dt}. \tag{4}$$

d. Take the ensemble average and solve the equation If you take the ensemble average of (4), the direct contribution from F_r disappears because $< xF_r >= 0$, meaning the force F_r is random and not correlated with itself in space or time. Equation (4) can now be written as

$$\frac{1}{2}m\frac{d^2 < x^2 >}{dt^2} - kT = -3\pi\mu r\frac{d < x^2 >}{dt}. \tag{5}$$

This equation can be solved:

$$\frac{d < x^2 >}{dt} = \frac{kT}{3\pi\mu r} + Ce^{6\pi\mu rt/m}, \tag{6}$$

where the last term disappears when $t >> m/6\pi\mu r \sim 10^{-8}$ s. This means that if we can track the position of many particles and calculate the mean square displacement per unit time, and we know the viscosity and radius of the particle, we can find Boltzmann's constant, k. Such a determination of k and a comparison with other ways to determine k was the first test of Einstein's model for Brownian motion.

A. Numerical random walk

Random walk and its variants are fundamental and important stochastic models used for many physical phenomena. The Matlab script `virrevandring.m` is a very simple realization of random walk where a number of random walkers are placed randomly in a part of the xy-plane and then they are moved in a random direction with a normally distributed step size (the width of the distribution is 'stepsize') for a given number of time steps. The step size is chosen so that the visualization of the simulation resembles what you will see in the microscope in the laboratory exercise. The mean square displacement shown at the end is calculated based on about 4 times as many particles as what is shown in the box. The positions of the particles shown in the box are also saved. This list of positions at different time steps has the same structure as the list of particle positions you will create from the experiment in the laboratory exercise.

III. OPTICAL MICROSCOPE

We will not say much about the microscope. The microscope is a stable stand that allows for small, controlled movements of what you are looking at relative to the light source and the objective. The illumination is at least as important for what you see with a microscope as the type of objective you use. If the illumination is correct, the resolution of the microscope is determined by the numerical aperture (NA) of the objective. The resolution is given by the radius of the diffraction pattern from luminous points and is at best $r = \lambda/(2NA)$ where λ is the wavelength of the light. We have two objectives with NA=0.4 and NA=0.65, and the resolution of the objectives for green light is then $D_{20X} = 0.7$ μm and $D_{40X} = 0.4$ μm.

IV. IMAGING WITH ELECTRONIC CAMERAS

The objective in the camera sends out an almost parallel beam of rays that must pass through an eyepiece for us to get an image on the retina, or through an imaging lens to form an image on the image plane in a camera. In an electronic camera, electrons are released where light falls on a semiconductor chip. The chip is divided into many pixels, each of which functions as a light sensor. The spatial resolution of a camera is determined by how many pixels the chip is divided into. What is often just as important is the intensity resolution of the camera, i.e., how many intensity levels each individual pixel is divided into. Intensity depth is affected by the size of each pixel (the larger the pixel in μm^2, the more electrons can be generated), the temperature of the sensor (the lower the temperature, the fewer (noise) electrons are generated thermally), and the readout noise (which usually gets larger the faster you read out). Most simple cameras and most image formats operate with $256=2^8$ intensity levels, also called 8 bits. Good SLR cameras have a raw format with 12-bit resolution, i.e., 2^{12}=4096 intensity levels. Good scientific cameras have chips that are cooled down to -80 to -180 C and have expensive readout electronics that provide 2^{16}=65536 intensity levels. Then each individual pixel in the camera is an accurate light meter with high sensitivity. There are a few more factors that affect how we choose cameras for scientific purposes. It is an advantage that the pixels in the camera are square. Then the conversion from the number of pixels to the object size is the same in the horizontal and vertical directions of the image. If you are not going to use the color information provided by a color camera for anything scientific, it is better to choose a grayscale camera. The reason is that you lose spatial resolution and intensity resolution during color filtering. The last consideration is which chip technology to use, CCD or CMOS.

V. IMAGE ANALYSIS

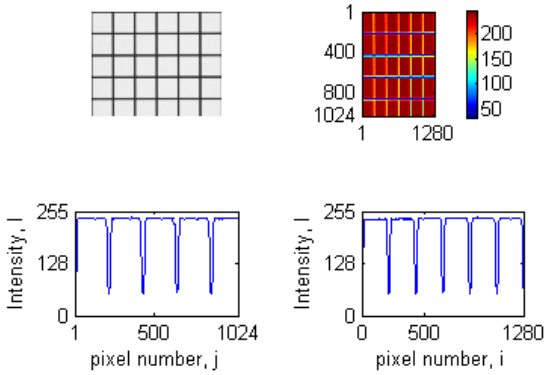


FIG. 1: Analysis of an imaged square grid. **Top left:** Monochrome image of a square grid with 50 μm between the lines and 5 μm wide lines. **Top right:** The same image represented in the matrix I and shown in Matlab with the command 'imagesc(I)'. Note that the image element $I(1,1)$ is in the upper left corner. **Bottom:** Vertical cross-section $I(:,500)$ on the left and horizontal cross-section $I(500,:)$ on the right.

In this assignment, we will only use monochrome cameras, so we will not deal with colors. A monochrome image is a 2-dimensional matrix with elements $\{I_{ij}\}$, $i = 1 \ldots N_x$, $j = 1 \ldots N_y$. For an 8-bit image, I_{ij} has values between 0 and 255 where 0 is completely black and 255 is completely white. The spatial image coordinates i and j (called pixels) correspond to object coordinates $x_i = is_x$ and $y_j = js_y$ where s_x and s_y are the magnifications in the x- and y-directions. One of the first things to do when using a new imaging system is to find s_x and s_y by imaging an object with known dimensions and determining how many pixels it corresponds to. In figure 1, we show a grayscale image that has 1024x1280 pixels and intensity values between 50 and 240. By finding the positions j of the horizontal lines (4 minima in the lower left plot) and the positions i of the vertical lines (5 minima in the lower right plot), we can calculate the magnifications s_x and s_y since we know that the distance between the lines is 50 μm.

VI. PRE-ASSIGNMENT

This pre-assignment is not mandatory but is intended as a help to prepare you for the laboratory assignment.

A. Numerical random walk

Download the Matlab scripts `virrevandring.m`, `virreanalyse.m` and `track.m`. The first script is described above. `virreanalyse.m` reads a position list and step size generated by `virrevandring.m` to analyze it. This position list has the same format as the position lists we will generate from the experiments. The first thing that needs to be done is to track particles from one time point to the next to create continuous particle tracks.

1. Run `virrevandring.m` a few times to see how much the result varies.

2. Try changing 'timesteps', 'numpart', and 'stepsize' to see how it changes the simulation.

3. Read the explanations in `track.m` to find out what to set the parameters to when the routine is called in `virreanalyse.m`.

4. Try out some sensible values for 'maxdisp' in `virreanalyse.m` and run the script. Compare the result with the result of the simulation `virrevandring.m` that generated the position list. (`track.m` does not like the value I have set!)

VII. LABORATORY EXERCISE

A. Imaging quality

Begin by getting help to place the test piece in the microscope, turn on the light, open the camera program, and set the camera to take pictures continuously and show them on the screen. Be careful never to touch the surface of the test piece!

- Use the 20X objective, find a field with a square grid, and focus while looking in the eyepiece of the microscope.

- Open a window that shows the histogram and a window that allows you to determine the shutter speed of the camera. The histogram shows how many pixels register which intensity. You should see two peaks, one corresponds to the white stripes and one to the black stripes.

- Set a light intensity that is comfortable for the eyes and then adjust the focus on the microscope and the shutter speed of the camera to get the largest possible distance between the two peaks in the histogram. You now have maximum contrast.

- Take a still image of the grid, save it as a jpeg, and open it in Matlab with `I=imread('filnavn.jpg');`.

- Proceed as in figure 1 to determine the magnifications s_x and s_y.

- Repeat the procedure for the 40X objective.

- We are interested in knowing whether the resolution of the imaging is limited by diffraction or by the number of pixels in the camera. Use the theoretical (diffraction-limited) resolutions to calculate D_{20X}/s_x and D_{40X}/s_x. What do these ratios say about the imaging system?

- We will now characterize the imaging system better by calculating the "Modulation Transfer Function" (MTF). Begin with the 20X objective, focus on the field with f =60 LP/mm (line pairs per millimeter) and set the intensity, shutter speed, and focus to get the best possible contrast.

- Take one still image of the stripes (60 LP/mm). Open the still images in Matlab and plot an intensity profile normal to the stripes. Note the maximum and minimum values of the intensity, I_{min} and I_{max}.

- Repeat the same with 380 LP/mm, 500 LP/mm, and 600 LP/mm.

- Switch the objective to 40X, set the intensity, focus, and shutter speed, and repeat as for the 20X objective.

- Plot MTF(f) = $(I_{max}(f) - I_{min}(f))/(I_{max}(f = 60) - I_{min}(f = 60))$ for the two objectives.

B. Brownian motion

Get help from a supervisor to prepare a microscope slide with a small volume of liquid with small particles in it. Use the 20X objective and normal illumination to find an area in the preparation with particles. Switch to dark-field illumination if it is difficult to find the particles.

- Take one picture of the particles with normal illumination and one with dark-field illumination, making sure to get as good contrast as possible. Describe what the difference between the two images is and how they are produced.
- Determine the mean particle size in pixels and in micrometers.
- Look at the intensity values in the dark-field image, I, using `imagesc(I), colorbar` and choose a threshold to separate particles from the background. Look at the binary image with some different threshold values: `Ibw=im2bw(I,threshold/255);, imagesc(Ibw)`.
- With the best possible contrast and focus in dark-field, record a 30-second film at 15 frames per second.
- Open the film in ImageJ and run it a few times to find out approximately how far the most mobile particles move during the film.
- Run the script `finn_spor.m` to extract the position of the particles in each frame, save the list of positions, and make a first attempt at calculating the particle tracks.
- Run the script `vis_spor.m` to check if the tracking algorithm managed to create sensible tracks.
- If the tracks were not convincing, look at the last 7 lines in `finn_spor.m` to retrieve the position data (poslist.mat) and do a new tracking with other parameters until the tracks look sensible.
- Create a criterion to remove the tracks of particles that are stuck on the glass surfaces. Insert the criterion into `vis_spor.m` to check that it works and then insert it into `anal_spor.m`.
- With the best possible contrast and focus in dark-field, record a 5-minute film at 15 frames per second.
- Run `finn_spor.m`, `vis_spor.m`, and `anal_spor.m` to calculate the mean square displacement of the Brownian particles.
- How does the result agree with the theory and the value of the other physical quantities in the model for Brownian motion?

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[1] Random walk, also known by many other names.

[2] Einstein's and Langevin's contributions illustrate a scientific-sociological point: if you are the first, it is not so important how tidy or elegant you do it. If you come after, your contribution might become known if it is more elegant, general, and easier to use.