

# BIO435- Lab02: Perrins Experiment to Measure Avogadro's Number

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## Locations:

Day 1: Biology Research Labs, 2nd floor, Main Building

Day 2: LHC303 computer labs

## 1 What you will do as part of the lab

1. Preparation for the DAY1 LAB and DAY2 computational exercise
2. Experimental observations: image analysis, XY particle coordinates
3. Fitting the change in x- and y to a distribution using Jupyter to estimate  $\mathbf{D}$  and  $\mathbf{N}_A$ , Avogadro's number.
4. Saving the Jupyter file and data (XY Trajectories) for submission as a report

## 2 Preparation

### Reading

Refer to previous lectures on **viscosity** and **brownian motion**. Also refer to a Random Walk applet using the open physics engine to see what theoretical expectations might predict [4].

### Pre Lab Reading

(for DAY2) What distribution fits the frequency distribution of the distance covered ( $r$ ) from point of release of particles in the OPE 2D random walk applet? BEFORE you reach lab, prepare a single page document with your name, roll number and the plot of the histogram that you will have run before coming to lab. Include a few lines describing the distribution- hint look up Rayleigh Distribution.

# Background

## Some statistics

We have discussed in the lecture that the standard deviation, i.e. the root mean square deviation from the mean, was a quantity that turned up in diffusion. Thus the standard deviation ( $\sigma$ ) of a distribution  $x_i$  from the mean ( $\mu$ ) can be written as:

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \mu)^2} \quad (1)$$

The alternative angle notation gives us:

$$\sigma = \sqrt{\langle x - \bar{x} \rangle} \quad (2)$$

This definition will be needed to describe the distribution of steps in brownian motion.

The standard error (SE) is the deviation in the standard deviation! It indicates the accuracy of our estimate. This is only useful when  $\sigma$  is being used in itself as a measure of something. Hence:

$$SE(\sigma) = \frac{\sigma}{\sqrt{2N - 2}} \quad (3)$$

## Random walk in brief

The random walk assumes that every  $\tau$  seconds a random choice is made to move either left or right by a distance  $\delta$ . On an average a random walk does not allow the walker to go anywhere, i.e.

$$\langle x \rangle = 0 \quad (4)$$

For a 1D random walk

$$\langle x^2 \rangle = 2Dt \quad (5)$$

and the diffusion coefficient is

$$D = \frac{\delta^2}{2\tau} \quad (6)$$

In 2D and 3D the equation of mean square displacement becomes  $\langle r^2 \rangle = 4Dt$  and  $\langle r^2 \rangle = 6Dt$  respectively, where  $r$  is the distance from where the particles originate.

The relationship between the molecular aspects of molecules and thermal conditions was put forth by Einstein in 1905 and referred to as the Einstein-Smoluchowski relation [2]. It is written as

$$D = \frac{k_B \cdot T}{f} \quad (7)$$

where  $k_B$  is the Boltzmann constant,  $T$  the absolute temperature and  $f$  the frictional drag coefficient. Stokes expression for a spherical object gives the frictional drag coefficient to be:

$$f = 6 \cdot \pi \cdot \eta \cdot r \quad (8)$$

In 1926 Jean-Baptiste Perrin was awarded the Nobel prize for physics for reproducing all of Einstein's predictions. To do so he measured the  $r_{rms}$  of mono disperse resin particles that he himself developed a method to produce [3].

Since  $D$ ,  $T$  and  $f$  (the drag force is related with velocity ( $v$ ) by  $f$  as  $F_{drag} = f \cdot v$ ) are all measurable and  $k_B = \frac{R}{N_A}$ , where  $R$  is the universal gas constant, this method also allows a direct estimation of the Avogadro's number ( $N_A$ ). Historically this estimation helped put the molecular theory of matter on a firmer footing.

## Aim

To measure the diffusion of mono-disperse particles. This will repeat the historic experiment of Perrin. As a second step, you will also measure the movement of non-passive objects, namely bacteria. The first bit will allow you to make an estimate of  $D$  and  $N_A$ . The second will allow a comparison.

## Materials

1. Microscope to view the micron-sized particles (Zeiss Apotome or Nikon TiE with a 40x air lens)
2. Micron sized beads (note the manufacturer's name, specifications about size of beads and the error in the size.)
3. Microscope slides (note the manufacturer, thickness and grade of glass)
4. Microscope coverslip
5. Double-backed tape to form a miniature chamber in which to observe the movement
6. 1 ml Distilled water ( $H_2O$ ) to dilute the bead suspension
7. Pipet and tip to dispense the solution of beads

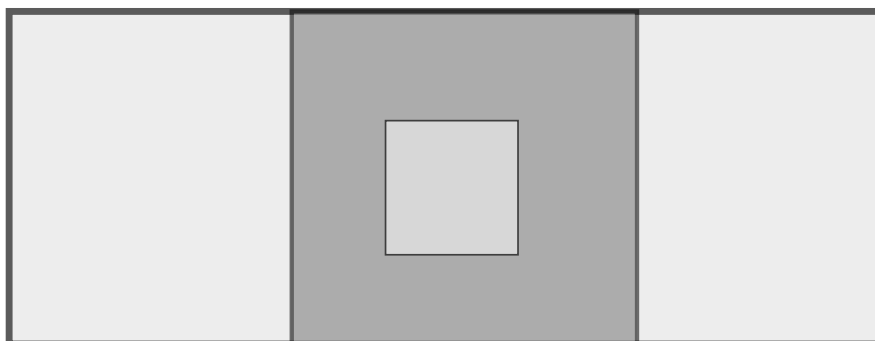


Figure 1: Chamber slide for observing the movement of micron-sized particle in a top view. Light grey is the glass slide, dark-grey is the tape.

## Methods

### Experiment

#### DAY 1

1. Take a picture of your group. Note the names, indicating the picture (left to right) and put it into an electronic document.
2. Create a chamber slide by sticking a double-backed tape on the slide and cutting out a small (0.5x0.5 cm) square in the middle.
3. Expose the second sticky side of the tape by removing the backing.
4. Take a drop of the polystyrene bead solution (or bacteria) prepared for you and put it in the “well”.
5. Seal this chamber by placing the coverslip on it. Ensure no air-bubble is trapped.
6. Place the sealed chamber slide (coverslip first) on a tissue paper to dry it from the outside and clean any dust. Be careful not to put your fingers on the coverslip or area directly under the sample-well.
7. Such a sealed chamber slide will minimize evaporation and prevent evaporation-dependent flows in any one direction from causing movement.
8. To further seal the chamber slide, use some colorless nail-paint provided on the edges of the coverslip sealing the system.
9. Observe the sample under the microscope in the 40x lens. You will be given a brief orientation of the parts of the microscope. This does not

however constitute training on the instrument, for which a more elaborate procedure is in place. Hence you will be allowed to focus and acquire data, but not play around with it much.

10. Using the coarse- and fine-focus knobs, get the beads into focus,
11. The solution should be dense enough so you see  $\geq 5$  particles in the field of view- if not move the slide around using the XY translation knobs for the microscope stage.
12. Using the time-lapse function (with the help of the instructor) acquire a time-lapse dataset with the following parameters:
  - **Total time: 300 s**
  - **Interval between frames: 5 s**
  - **Mode of imaging- Differential interference contrast (DIC)**
  - **Filename to save as ‘brownian\_gXX’ where XX is the group number. Save the files in uncompressed TIF format. A Zeiss .zvi format will also work.**
13. Save the image-data file on the computer. At the very end of the exercise all the data will be put on a DVD and passed around.
14. If no single sphere is visible throughout the whole time series, switch to following another particle.
15. Acquire fresh data if the spheres in your field of view appear to be stuck to the surface.
16. Find the scaling factor (pixels to microns conversion) and make a note of it.
17. Run through the time-lapse captured and make sure that a few particles remain in the field of view in the entire recording
18. There should be minimal (not at all) drift in the sample- if that is observed prepare your chamber slide once again and make another attempt. Else check if there are some fluid leaks or air-bubbles in your preparation and get rid of them.
19. Note the temperature at which you measured the whole experiment (there is a temperature humidity sensor in the lab)

## Video Analysis

### DAYS 1 & 2

1. Download and use the biological image analysis program **ImageJ**<sup>1</sup>. This program has an extensive online help. You will use the program for its file opener and basic analysis routines. It has a much wider functionality and a HUGE global community of users and developers, that you are encouraged to explore.
2. From the ImageJ menu-bar, navigate to “Analyze → Set Measurements” to determine what data to collect- centroid, centre of mass and frame number should be checked. Use the “Circular” tool to outline a particle (Figure 2(b)). Press “Ctrl + M” to record the data (it appears in a little independent window (Figure 2(c))). As the particle moves, move the ROI (yellow outline) with the cursor to fit the particle under observation. This gives you a data-window with x- and y-coordinates and a frame number.
3. As an alternative for improved particle tracking in a semi-automatic manner, download and follow the instructions for using the “**MOSAIC Plugin**”<sup>2</sup>. This allows the detection and tracking of particles with little manual intervention. It is however not optimized for the images we will acquire, so its possible manual tracking is better. Discuss this in your final submission.
4. Save the data-window as a .txt file (ascii delimited text file)

## Observations

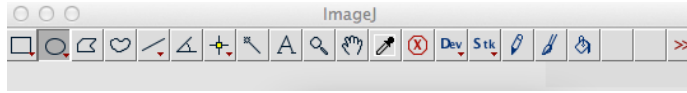
### DAY 2: Computational analysis exercise using Jupyter

- Using **Python (JUPYTER)**, write a script to read in the .txt delimited xy-coordinates data and time data and plot them in an X- and Y- plot with  $\mu m$  scales as the units of the axes.
- In the scripting language of your choice, calculate the  $\Delta x$  and  $\Delta y$  values for each trajectory.
- Plot frequency histograms of the  $\Delta x$  and  $\Delta y$  values.
- Calculate the statistical mean ( $\mu$ ), standard deviation ( $\sigma$ ) and standard error (SE) of the datasets of  $\Delta x$  and  $\Delta y$ .
- If there is no drift,  $\mu = 0$ . If the mean is 2xSE away from zero, excess drift is probable. Discuss this in your result writeup. This might be one **source of error** in your further estimates.
- Does the histogram resemble a Gaussian distribution? If it appears to do so, fit a gaussian using Jupyter (numpy and scipy) fitting routines involving standard normal distribution function. Note the  $\mu$  and  $\sigma$  values.

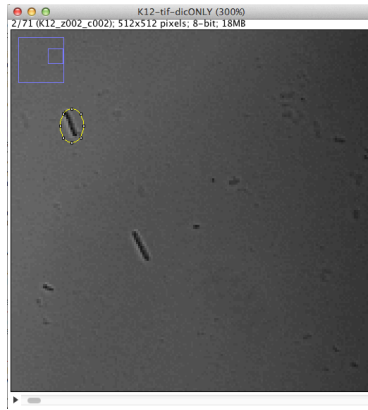
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<sup>1</sup><http://rsbweb.nih.gov/ij/>

<sup>2</sup><http://www.mosaic.ethz.ch/Downloads/ParticleTracker>



(a)



(b)

Results										
	Area	Mean	Min	Max	X	Y	XM	YM	Slice	
1	112	92.723	0	143	363	176	362.800	176.139	1	
2	112	92.884	18	132	363	176	362.860	176.088	2	

(c)

Figure 2: (b) ROI drawn around a cell of interest. (c) The function “Analyze → Measure” measures a set of preset values from the ROI.

- How do the fitted mean and standard deviations compare to the previously calculated values?
- If we treat the 5 minute (300s) random walk as 60 independent RWs,  $\Delta x$  is just the final position of such a walk. To calculate the diffusion coefficient  $D$ , we use the expression of the standard  $\sigma^2 = 2Dt$ .
- Calculate  $D$  from the  $\Delta x$  and  $\Delta y$  values and include units ( $\mu m^2/s$ )
- Calculate the uncertainty in  $D_x$  and  $D_y$  values using the standard error expression in Equation 3.
- Use the mean of the  $D_x$  and  $D_y$  estimates as your “final” value of  $D$  with the uncertainty of  $D$  from the standard error.
- Using the known value of  $T$  and your estimate of  $D$ , estimate  $k_B$  and  $N_A$  using the value of  $R = 8.31 J/molK$ .

## Non-Brownian motion

If we have time, we might use the living bacterial samples to make the same kind of recordings as with the beads and perform the same analysis up to the point of calculating  $\Delta x$  and  $\Delta y$ . If we do plot a histogram of displacement from the start position distributions, what do you expect will be the difference to that obtained from brownian motion? Using a spherical approximation for the bacterium, what should its net displacement have been if it was diffusing?

## Conclusions

1. Clean up- glasses in the glass disposal, biologicals in the bio-disposal and the rest in the regular garbage bins.
2. Save your work from the microscopy and ImageJ. Upload the x,y trajectories of each particle from your group onto the google-drive spreadsheet that will be shared with you (link on the course website).
3. Submit your full report, combining text, code, data and figures in ONE Python notebook file. Name the file as **yourname.ipynb**. Upload it in the GoogleClassroom.

## Acknowledgement

This lab procedure has modified and in places directly taken from the Brownian Motion Lab, Harvard University, Physics [1]. It has been used under the fair use dictum and is for academic purposes only.

## References

- [1] PS2 (Physical Sciences 2), Fall 2007, Harvard University, Boston, USA.
- [2] M.A. Islam (2004) EinsteinSmoluchowski Diffusion Equation: A Discussion. Phys. Scr. 70 120 doi:10.1088/0031-8949/70/2-3/008.
- [3] Jörg Kärger, Farida Grinberg, Paul Heitjans (2005) Diffusion Fundamentals. Leipziger Universittsverlag (Leipzig University Press), Leipzig, Germany.
- [4] 2D random walk applet [Open Physics Engine \(OPE\)](#)