Cooking, Looking and Tweezing

December 11, 2024

1 Course Details

This course is divided into 5 parts (see Experiments) over 5 days. The final 2 days of this course are used to catch up or write the report. You will perform the experiments and write the report.

1.1 General Information

This course is open to Science, Chemistry and Physics students. The study load for this practical course is 3 EC (84 hours). This is built up as follows:

- 60 hours laboratory work
- 24 hours individual study (reading and preparation)

A lab-day will start at 08:30 and end at 17:00. Coffee breaks are from 10:10 to 10:30 and 15:10 to 15:30, lunch break is from 12:45 to 13:30. In case you cannot attend as a result of illness you should report yourself as sick by sending an email to ruth.crothers@ru.nl before the start of the practical.

Each pair of students are expected to hand in one report on all experimental parts, including your Matlab code. Reports can be written in your preferred application, however we expect you to adhere to the report template provided via Brightspace. Your final grade will be calculated based on your answers in this handbook (25%), the final report (50%) and your in lab work (25%). In order to pass this course all of the above parts should be graded *pass* ≥ 5.5.

1.2 Supervision and Organisation

This practical is organised and delivered by the Physics and Chemistry of Soft Matter Department.

- Roel Dullens
- Ruth Crothers
- Marieke Reijneveld
- Habib Moradi
- Arran Curran
- Jan van Leeuwen

2 Introduction

The existence of atoms was first experimentally confirmed through the study of colloidal particles. By analysing the Brownian motion of these particles, scientists were able to determine Boltzmann's constant and subsequently Avogadro's number, thereby validating Einstein's theory on Brownian motion.

In this experiment, we will follow similar steps taken by Jean Perrin in the early part of the 20th century, to determine Avogadro's number. We will first synthesise colloidal particles, then use a brightfield microscope to image them. We use particle tracking routines in Matlab to determine the mean square displacement.

2.1 Outline

1. Colloidal Synthesis
2. Building a Brightfield Microscope
3. Imaging Colloids
4. Tracking Colloids using Matlab
5. Determination of Avogadro's number
6. Optical Trapping and Determination of Avogadro's number

3 Prep Work

Define the following terms, you can refer to *Brownian Motion* by *Albert P. Philipse*.

- A Colloid
- Mean Square Displacement (in terms of radial distance)
- Ensemble averaged
- Brownian motion (and explain why a colloidal particle undergoes this motion)
- Translational Diffusion Constant (equation with all terms defined)

Using the same resource, write a paragraph summarising both methods used by Perin to find Avagadro's number. It is best to work on these tasks in tandem with the synthesis.

4 Synthesising Colloids

In this section you will synthesis colloids from the organosilica compound 3-(trimethoxysilyl)propyl methacrylate (TPM). You are expected to complete the preparation work for the rest of the course in between synthesis steps.

4.1 Learning Outcomes

- Understand the mechanism of synthesis of TPM colloidal particles

4.2 Mateirals

1. 10mM hydrochloric acid (HCl)
2. Azobisisobutyronitrile (AIBN)
3. 0.028 % ammonium hydroxide (NH_4OH) (1×base)
4. Ultrapure water (MilliQ)

4.3 Procedure

1. Add 4.75 ml MilliQ + 0.25 ml 10 mM HCl + 0.5 ml TPM to a glass vial.
2. Stir vigorously for 1 hour. The resulting solution is referred to as hTPM.
3. In separate eppendorfs, set up the following;

	1	2	3	4	5	6	7	8	9	10	11
1×base/μl	1000	900	800	700	600	500	400	300	200	100	50
hTPM/μl	500	500	500	500	500	500	500	500	500	500	500

   Note: Add hTPM to the eppendorf first then add the base quickly as one addition.
4. Leave to stand 1 hour
5. Add a spatula full of AIBN and place in oven for 3 hours, tumble every 30 minutes.
6. Leave to cool 30 minutes.
7. Wash and re-suspend with fresh MilliQ by centrifuging at TODO: rpm for TODO: minutes.

4.4 Discussion

1. Write the structure of TPM, identify hydrophobic and hydrophilic groups.

2. Write down the mechanism for steps 1) and 3) in the procedure.

3. What is the mechansim for droplet formation?

4. What is the main difference in the products from 1 to 11? How could you justify this difference?

5. What is the role of AIBN?

5 Building a Brightfield Microscope

In this section you will build a brightfield microscope to capture high magnification images of your colloids.

5.1 Learning Outcomes

- Understand the basic principles of ray tracing.
- Learn how to measure the focal length of a lens.
- Understand image formation in simple optical systems.

5.2 Equipment

- Camera
- Tube lens
- Microscope objective
- A Ruler
- Alignment grid
- Various optomechanical components
- Illumination module

5.3 Basic Principles of a Simple Lens

Most optical systems can be reasonably described with the concept of refraction, and standardised definitions.

- Light rays entering a lens experience a transition from a low refractive index to a high refractive index (lens material). According to Snell's Law, the ray will bend towards the normal to the lens surface at the point of incidence.
- An optical axis is an imaginary line that passes through the centre of the lens and is perpendicular to the lens surfaces.
- All incident rays that are parallel to the optical axis are refracted by the lens to a converging point on the optical axis which is called the focal point.
- The distance from the lens to this focal point is called the focal length, and a plane normal to the optical axis at the focal point is called the focal plane.
- Optical diagrams are drawn from the left to the right. The object is traditionally placed on the left side of the optical system and the image is on the right side.

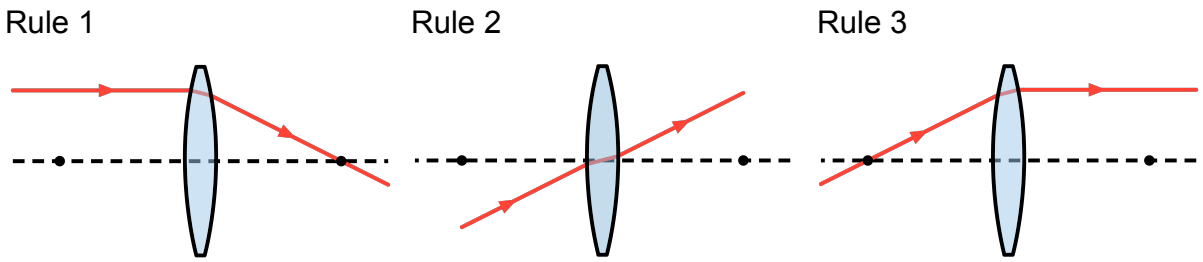

Figure 1: The basic rules of ray tracing for a simple lens.

5.3.1 Ray Tracing

Understanding how light propagates through optical elements can be well approximated by simplifying the light into lines called rays. This method is called ray tracing and is a fundamental concept in optics, where light-matter interactions are described by Snell's Law and rays are drawn along the optical system to give a good approximation of the optical behavior of a given system. The three rules of ray tracing for a simple lens are shown in Figure 1 and described below;

1. Rays parallel to the optical axis (the central line perpendicular to the lens surfaces) will focus at the focal point on the right side of the lens. Rule 1 in Figure 1.
2. Rays that pass through the center of the lens will continue in a straight line without deviation. Rule 2 in Figure 1.
3. Rays that pass through the focal point on the left side of the lens will emerge parallel to the optical axis. Rule 3 in Figure 1.

5.3.2 Thin Lens Equation

The thin lens equation is a key principle in geometrical optics that describes the relationship between the focal length of a lens, the distance from the object to the lens, and the distance from the resulting image to the lens. It is used to predict how a lens will focus light from an object to form an image. You will use this to measure the focal length of your tube lens.

$\frac{1}{f} = \frac{1}{d_o} + \frac{1}{d_i}$.

Where f is the focal length of the lens, d_o is the object distance and d_i is the image distance.

This equation assumes the lens is thin, meaning its thickness is negligible compared to the object and image distances.

5.4 Finding the focal length of a lens

Apply the ray tracing rules to the tip of the a-symmetric arrow in Figure 2. Complete the figure by drawing the image of the object. By measuring the height of the formed imaged, the object distance, d_o and the image distance, d_i calculate the magnification of

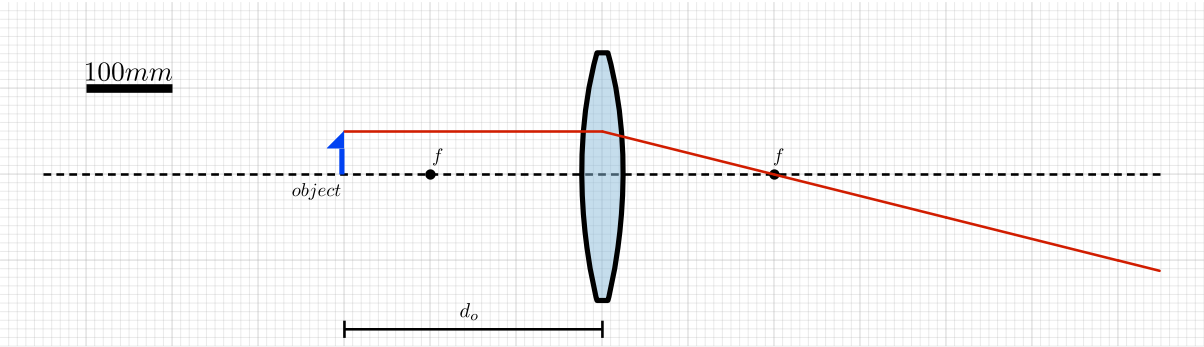

Figure 2: Forming an image with a single lens.

the image using two different methods. Using the thin lens equation, calculate the focal length of the lens in millimetres.

Focal Length =.

5.5 Find the focal length of your tube lens

First we will find a rough approximation of the focal length of the tube lens. This is done by forming an image of an approximated infinite light source (a ceiling light for example) on the surface of the floor. Measure the distance between the lens and the image to find the approximated focal length, $f_{\approx}$.

$f_{\approx} =$

Why is this method an approximate measure of the focal length? Is the measured focal length longer or shorter than the true focal length?

5.5.1 Image Formation with a Single Lens

Install the alignment target onto the end of four of the rods and install your tube lens at a distance of $2f_{\approx}$ from the target. With the naked eye, find the position where the image of the grid is in focus. What do you notice about the image magnification?

Move your tube lens 25% closer to the alignment target (ie a distance of $0.75 \times 2f_{\approx}$). Observe the image again with the naked eye. What do you notice about the image quality?

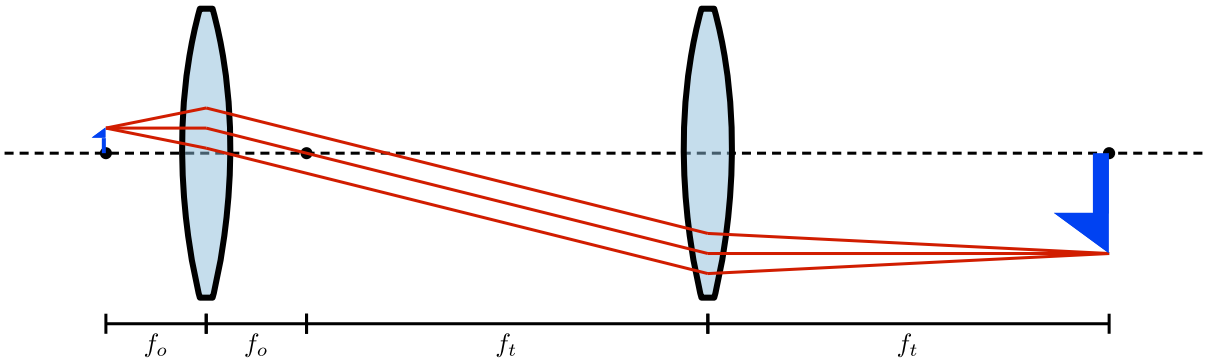

Figure 3: Image formation with an objective and tube lens.

5.6 Image Formation with Two Lens

To form an aberration free, magnified image we need to place the object in the focal plane of the lens, however this would result in an image being formed at infinity from the lens (see lens maker equation). So we use two lenses. The first lens, the objective, collects light from the object and the tube lens forms an image at the focal plane of itself. To do this we need a more accurate measurement of the focal length of the tube lens.

5.6.1 Form same image on camera

Place alignment grid, tube lens and camera on the alignment rail. Using your approximated focal length of the tube lens, place the lens a distance of $2f_{\approx}$ from the camera sensor. Now place the target grid on the opposite side of the lens and adjust its position until you see a clear image on the camera. Measure the distance between the lens and the target to find the true focal length of the tube lens, f_t. Position the tube lens exactly f_t from the camera sensor.

$f_t =$

5.7 Complete scope build

The set up you are building contains an objective and a tube lens which have different focal lengths. Consider the path of light through both lenses in the diagram below what is the significance of using two lens of different focal length?

Place objective roughly a focal length in front of tube lens. Place the adaptor over the objective and switch on the illumination. Adjust the position of objective until an image of the alignment grid is seen on the camera. TODO: This needs revising to account for the fact that the objective can be placed almost anywhere and still produce an image. Once we have the objectives in the lab we can measure their focal length and instruct students to position the objective at a distance of 2f from the tube lens, using a ruler.

5.7.1 Calibrate the camera

Capture an image of the alignment target and measure the size in pixels (px) of the $10\mu m$ grid. This gives you the pixels per micrometre calibration so you can convert your images

to real world units.

Pixel Size =

5.7.2 Measure your microscopes magnification

Knowing the calibration above and the physical size of each camera pixel (2 μm), calculate the magnification of your microscope.

Magnification =

Why is your magnification not that stated on your objective?

What is the focal length of your objective?

6 Imaging Colloids

6.1 Understanding How a Colloid Image is Formed

Depending on the height the colloids relative to the focal plane of the objective, the image formed will be different. For computational tracking of your colloids the position of the sample is crucial. For optimal tracking we want the image of a colloid to have a bright centre with a dark ring around it. Explain why the dark ring is darker than the background level of your image. Draw a diagram of a colloid with collimated incident rays and show how the rays interact with the curvature of the colloid. Indicate on the drawing where the focal plane of the objective should be for optimum imaging.

6.2 Sizing Colloids

- Add 2 μl of each colloidal suspension (1-11) to a glass slide and leave to dry for 30 minutes.
- Using your microscope, find a concentrated section of colloids where a crystal lattice can be identified.
- Draw a line through 3 or more particles to determine the mean diameter in μm.

	1	2	3	4	5	6	7	8	9	10	11
$radius/\mu m$											

6.3 Imaging Parameters

A key in many-particle tracking is linking up particles positions in a time series of images to form a trajectory for each particle. In order to keep track of each particle, the displacement from image to image needs to be significantly smaller than the mean particle diameter. So we have to capture images at a frame rate that meets this requirement.

To estimate our frame rate we need to know the translational diffusion constant of our particles. This can be calculated from the Stokes-Einstein equation,

$$D = \frac{k_B T}{6\pi\eta r}, \tag{1}$$

where D is the translational diffusion constant, k_B is the Boltzmann constant, T is the temperature, η is the viscosity of the solvent and r is the radius of the particle.

Given that $\langle x^2 \rangle = 2D\tau$ calculate the time, τ it takes for a particle to move a distance equal to its diameter, d.

	1	2	3	4	5	6	7	8	9	10	11
τ/s											

What is the lowest frame rate you can use such that the colloid will be trackable from frame to frame?

Select one batch to continue with. What was the reasoning behind this choice?

Create a sample using the instructions in the sample preparation area. Ensure your colloids are diluted such that you end up with a monolayer of colloids when imaging them. Start with a dilution of 1 mL of water to 1 μL of colloid solution. Let your sample sediment for $\approx$ 5 minutes on the microscope then check to see if you have a monolayer.

Once you have a monolayer, capture a series of images at the frame rate you estimated earlier.

TODO: How many images?

7 Tracking and Analysis

In this section you will use Matlab to analyse your microscopy images.

The code can be found at: github.com/Dullens-Lab/Matlab-Particle-Tracking

Particle tracking can be divided into 3 main sections;

- Image Processing
- Particle Location
- Particle Tracking

In words first the raw images are cleaned up, then the particle coordinates in each frame are found and finally these coordinates are linked up to form trajectories connected to an individual particle.

First you will work through a tutorial on image processing and particle location and then you will build these routines into a loop to find particles your own data. Finally the particle coordinates will be tracked.

- In Matlab open the file `Tutorial.m`.
- Use run section to load the example image into the workspace.
- Use the command `imagesc()` or `imshow()` to display the image.

7.1 Image Processing

Particle location relies on having good images where the only features are the colloids themselves. The image filtering step filters high and low frequency noise and applies a fixed background subtraction such that the background equals a pixel value of 0. Open the function file `bpass.m` and read through the code.

- Define the arguments to the function.

- Run the function with one of your images `bpass(image, true, true, 1, true)`.
- Change the value for the background until all the background is removed.
- Change the input for `hpass` and `lpass` to integers and explore the parameter space.

7.2 Particle Location

Once the image is processed the particle locations can be found. This is a two step process, where the brightest pixels are found with `pkfnd.m` then these pixel coordinates are used by `cntrd.m` to calculate the subpixel coordinates of the particles.

- Open the function `pkfnd.m`.

- Define the inputs.

- Define the outputs.

- On what basis is the particle centre found?

- Run this section with the following inputs: `pkfnd(filtered, 10, 3)`

- Find the peaks in the raw and the filtered image, how would tracking unprocessed images affect results?

Once the peak pixels are found, the positions are refined by `cntrd.m`.

- Open the function `cntrd.m`.
- Describe in words how the function calculates the sub-pixel coordinate.

- Define the output.

- Run the function with the following arguments, `cntrd(filtered, est_pks, 5, true, 1)`

Create a figure either in Matlab or PowerPoint of the raw image, filtered image, filtered image with `est_pks` and filtered image with `cntrds`.

7.3 Create Tracking Loop

A convenient way to store information in Matlab is to use structures, where each field within a structure contains an array e.g. if `COORD` is a structure with one field, `cntrds`, the positions for frame t can be stored in `COORD(t).cntrds`.

Rewrite the tracking procedure as a loop over all frames where the final coordinates are stored in a structure called `COORD`.

Note before the coordinates are stored add a fourth column containing the timestamp. Ensure you suppress any image or plot displays during the loop iterations.

7.4 Particle Tracking

Once we have the particle locations in each frame we must link them up to form trajectories.

- Open the tracking function file, `trck.m`.
- Define the inputs.
- Define the outputs.
- Track the particles using the diameter as the maximum displacement.

7.5 Analyse Your Own Data

Run you tracking loop over your own data. (Note you will have to optimise the parameters for each step, you can use the tutorial script for this).

7.6 Mean Square Displacement

What shape do you expect the MSD to be? What function should fit the line?

7.6.1 Calculate the MSD

- Import the MSD function into the correct workspace
- Run this function `input = (x y z t ID)`
- Output of this function is the ensemble averaged MSD, describe in a sentence how this averaging is being carried out.
- Plot the MSD versus time (in seconds)

7.6.2 Fitting the MSD

- Using the Matlab function `fit`, fit the MSD versus $t(s)$ with the correct function.
- From this value determine D_T. What are the units?
- What is the value of D_T after fitting over 10 points vs 1000 points? Which is likely to be more accurate

7.6.3 Calculate Avagadro's Number

Start with Stokes-Einstein equation and using the Ideal Gas Laws, write down the relationship between Avagadro's number, N_A and D_T. From your measured D_T calculate Avagadro's Number.

8 Optical Trapping

In this section you will utilise the technique of optical trapping to again determine Avagadro's number. Optical trapping is a technique used across many fields of science that uses a laser light source to 'trap' a probe which allows direct manipulation and measurements of forces within a sample.

Optical trapping relies on a refractive index mismatch between particle and solvent.

What type of light source is a laser?

Consider the Fig. 4 which shows a colloidal particle trapped within a laser beam and a particle displaced from the centre of the beam. Consider the first scenario:

- What two things happens to the incident ray when it hits the particle?

- Label the two resultant forces on the particle from both effects?

- Therefore explain why a refractive index mismatch between particle and solvent is important.

Consider the second scenario:

- What two things happens to the incident ray when it hits the particle?

- Label the two resultant forces on the particle from both effects?

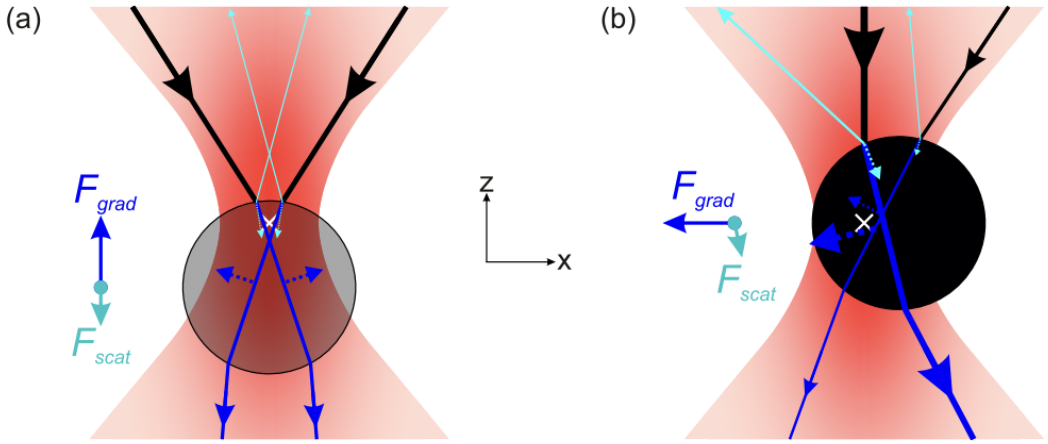

Figure 4: Forces in Optical Trapping.

- For the particle to be restored to the trap which force must be greater?

Sample should be prepped in an identical way to the previous microscopy experiments.

8.1 Safety

In this section you will carry out this experiment in the laser lab of PCSM. This set up includes a Class IV laser, this is the most dangerous type of laser which can cause serious eye damage. You must adhere to the following safety procedures:

- Laser Safety glasses must be worn at all times in the laser lab
- Failure to listen to instructions from supervisors will result in a penalty to your grade.

8.2 Analysis

- Using the particle location and tracking loop you developed previously calculate the MSD for the trapped particle.
- How is is different to the 'free' particle?

A particle on in a trap can be compared to a particle on a spring. We can determine the stiffness of the trap using Hooke's Law.

- What is Hooke's law for the potential energy of a spring?

$U(r)$ can be approximated as the $-ln(P(r))$, where r is the displacement of the particle.

- Copy the code for MSD into a new file and adapt it to plot $-ln(P(r)$.
- Fit this with the relation given by Hooke's Law to determine the trap stiffness.

The trap stiffness can be related to Boltzmann constant by the equation;

$\langle x^2 \rangle = k_b \frac{T}{\alpha}$

where α is the trap stiffness.

- Use this relation to determine Boltzmann constant and in turn Avagadro's number.

- Which method was more accurate and why?

9 Report

You may use either Word or LateX to complete your report. It should follow the following layout:

9.1 Introduction

Describe the work of Perin (Prep work) and state the aim of this Practical.

9.2 Experimental Details and Theory

9.2.1 Colloidal Model System

- Define a Colloid.
- Define Brownian Motion, MSD and how to calculate D_T.
- Describe why colloids can be used as 'big atoms'.

9.2.2 Synthesis

- Explain mechanism of synthesis of colloidal particles from TPM.
- Outline Ppocedure followed.
- Include images and sizes for all batches.
- Include what batch was chosen for further experiments and why.

9.2.3 Micrscopy

- Include the diagram provided and draw the path of light through the set up.
- Define ach component.
- Define the focal length and give the determined value.
- Give the determined pixel size of the camera and magnification of the microscope.
- Give the final image parameters and explain the values.

9.2.4 Tracking

- Include the raw tutorial image as well as the same image after each step.
- Describe the process of image processing, particle location and tracking.

9.2.5 Optical Trapping

- Include the diagram provided with the completed ray diagram.
- Describe the forces acting on the particle.
- Describe how the magnitude of the forces dictate if the particle remains in the trap.

9.3 Results

9.3.1 Determination of Avagadro's Number from MSD

- Show graph of MSD against time.
- Give value of Dt and NA.
- Explain any sources of discrepancy in your answer.
- How is this method an improvement on Perrin's work.

9.3.2 Determination of Avagadro's Number from k

- Show graph of MSD against time.
- Show graph of U vs r.
- Give value of k and NA.
- Explain any sources of discrepancy between your two values.