PERR
Particle density in a gravitational field

The number density of small particles suspended in a fluid depends exponentially on height, just as the density of air molecules and consequently pressure in Earth's atmosphere. The exponential dependence is caused by the gravitational potential and the fact that at larger heights less particles have sufficient thermal (kinetic) energy to overcome this potential. The sedimentation equilibrium is governed by principles of statistical mechanics.
In this experiment, you will investigate the sedimentation behavior of colloidal particles under the influence of gravity. Initially, the colloidal particles are homogeneously distributed throughout the sample. Over time, gravity causes these particles to settle, leading to a higher concentration of particles in the lower regions of the sample compared to the upper regions. You can compare the particle concentration $n(z)$ as a function of height z in a sample under the microscope to an exponential distribution and determine parameters of the problem.

1 Objective and deliverables

Objective Determine characteristic parameters of the height dependence of spherical particles in suspension by studying them in a sedimentation equilibrium, and compare to expected values.

Deliverables

- A lab journal with a compact but complete account of your lab activities.
- One or more (Python) scripts containing the data processing, if necessary accompanied by separate data files.
- A max. 6 page article on your research.

Requirements are described in the rubric for the experimental project. We **strongly** recommend to read the rubric so that you know what is expected from you.

2 Orientation

2.1 Historical Background

At the turn of the 20^{th} century, the scientific community was deeply engaged in a debate about the nature of matter and the existence of atoms. Many prominent scientists, particularly those following the traditions of classical mechanics and thermodynamics, believed in the continuity of matter. They argued that matter could be divided infinitely without ever encountering discrete particles. However, among others, Ludwig Boltzmann and Albert Einstein advocated the (now accepted) hypothesis that matter is composed of indivisible units called atoms.
In 1905, Einstein published a paper on Brownian motion, providing a mathematical explanation that directly supported this hypothesis [1]. His work demonstrated that the random motion of particles suspended in a fluid could be explained by collisions with invisible atoms or molecules. Jean Perrin conducted experiments on Brownian motion and the sedimentation equilibrium of colloidal particles suspended in a fluid. He received the Nobel prize in 1926 'for his work on the discontinuous structure of matter, and especially for his discovery of sedimentation equilibrium'. The full Nobel lecture can be

found online [2]. A short account of Perrin's experiment with its challenges, and a semi-modern adaptation of the experiment can be found in Ref. [3]. In this experiment, you will perform a more advanced version of Perrin's experiments.

2.2 Statistical equilibrium description

Blundell and Blundell Ch. 4 introduces the canonical distribution [4]. In a system with fixed temperature T, the probability of of a state of energy E to be occupied is $\propto \mathrm{e}^{-\frac{E}{k_B T}}$. Here, k_B is the Boltzmann constant. Study Example 4.4 (Section 4.7) about the isothermal atmosphere. This is comparable to the situation of particles suspended in a liquid, with one major difference. Because the particles in suspension experience an upward force due to Archimedes' principle[1], the potential energy of a particle with mass m corresponding at height z (with respect to some arbitrary $z = 0$) is $(m - m_0)gz$, where m_0 is the mass of the displaced liquid and g is the gravitational acceleration. The particle number density (i.e. number of particles per unit volume) at height z can then be written as

$$\begin{aligned} n(z) &= n_0 \mathrm{e}^{-\frac{E}{k_B T}} \\ &= n_0 \mathrm{e}^{-\frac{(m-m_0)gz}{k_B T}}, \end{aligned} \tag{1}$$

where n_0 is the particle number density at $z = 0$.

Exploratory questions

- Introduce the particle diameter d, and rewrite Eq. (1) in terms of d.
- Give an expression for z_0 such that the exponential in your equation becomes e^{-z/z_0}. The parameter z_0 is the distance at which the particle concentration n decreases with a factor $\frac{1}{e}$. It is important in experiments; its value should lie within a range that is accessible experimentally (see also Section 2.4).

2.3 Dynamical description

In your experiment, the suspension of colloidal particles initially is evenly distributed throughout the sample. The (vertical) particle flux is mediated by both gravity and general diffusion. Over time, the equilibrium situation described in Section 2.2 is reached. To get a grip on the dynamics of this process, we will now derive the one-dimensional differential equation describing the evolution of the particle density.

Diffusion is described by Fick's law (Ref. [4], Sec. 9.3 - this is not exam material for the Theory component of the course), which states that the flux due to diffusion[2] $\vec{\Phi}_{\text{diff}}$ across a unit area is proportional to the concentration gradient ∇n:

$$\vec{\Phi}_{\text{diff}} = D \nabla n. \tag{2}$$

Here, D is the diffusion coefficient, measured in $\mathrm{m}^2\mathrm{s}^{-1}$. Φ is the number of particles passing per unit of area and unit of time, measured in $\mathrm{m}^{-2}\mathrm{s}^{-1}$.

[1] https://en.wikipedia.org/wiki/Archimedes'_principle

[2] We follow the notation in Blundell. For the flux, several symbols are in use. In the course Electromagnetism current density or carrier flux was denoted by $\vec{J}$ (and measured in A $\mathrm{m}^{-2}\mathrm{s}^{-1}$). Wikipedia also uses $\vec{J}$, see e.g. https://en.wikipedia.org/wiki/Fick's_laws_of_diffusion

Exploratory questions

- What is the one-dimensional (z dependent) expression of Fick's law? In which cases can one simplify Fick's equation to one dimension?

The particle flux is also affected by gravity. If a particle is travelling at a velocity v in a medium with a certain viscosity η, then the net force on a particle is

$$F = (m - m_0)g - \alpha v, \tag{3}$$

with α being the viscous drag of the particle. For a spherical particle of diameter d, $\alpha = 3\pi\eta d$ ([4], Sec. 33.1).

Exploratory questions

- Given Eq. (3), what is the terminal velocity v_t of a particle in a viscous medium (Hint: assume net force zero so that there is no acceleration)? Why is this a useful quantity? Realise at this point that gravity causes a constant vertical flow of particles.
- If $n(z)$ is the particle density at a certain depth, argue that the total flux can be written as $\Phi_{\text{total}} = -D\frac{\partial n}{\partial z} - nv_t$.
- Explain the physical meaning of the formula $\frac{\partial}{\partial t}\int n dV = -\int \vec{\Phi}\cdot d\vec{S}$ (divergence theorem). Which quantity is conserved?
- Using the divergence theorem, show that the above two formulas lead to the differential equation

$$\frac{\partial n(z)}{\partial t} = D\frac{\partial^2 n(z)}{\partial z^2} + v_t\frac{\partial n(z)}{\partial z} \tag{4}$$

- Derive a result for the steady state solution $n(z)$.
- Compare your result to the result in Section 2.2, and give an expression for D.

We can turn Eq. (4) into dimensionless form ($\tilde{n} = n/n_0$, $\tilde{z} = z/z_0$, $\tilde{t} = t/t_0$)

$$\frac{\partial \tilde{n}}{\partial t} = \frac{\partial^2 \tilde{n}}{\partial \tilde{z}^2} + \frac{\partial \tilde{n}}{\partial \tilde{z}}, \tag{5}$$

where n_0 and z_0 are as defined before and $t_0 = D/v_t^2$ = a characteristic time for reaching equilibrium.
Eq. (5) can be solved numerically. A Python script that does the job for a constant initial density with some small fluctuation[3] can be found on Blackboard.
Figure 1 displays results for $\tilde{n}$ als a function of $\tilde{z}$ for several $\tilde{t}$, assuming polystyrene particles (densities). From Figure 1, you can conclude that it takes at least $\sim 10\,t_0$ for the equilibrium to be approached.

2.4 Experimental setup

Operation via laptop Data collection and image processing can be performed via your laptop. To this end, you need to install some applications (References and Blackboard give download links)[4]:

- The DinoEye CCD camera is used for image (and video) acquisition. You need to install the driver. [5]
- DinoCapture is used to operate the DinoEye and record photos and videos. You need to install the program. [6]

How to use these programs is explained in the following sections.

[3] The fluctuation is required to ensure that $\frac{\partial \tilde{n}}{\partial \tilde{z}}$ and $\frac{\partial^2 \tilde{n}}{\partial \tilde{z}^2}$ are not 0; otherwise $\frac{\partial \tilde{n}}{\partial \tilde{t}} = 0$ and nothing would change. Observe that the dynamics is fluctuation-driven.

[4] We assume you have a version of Python installed on your laptop. If not, check the instructions in the Simulation part of this course.

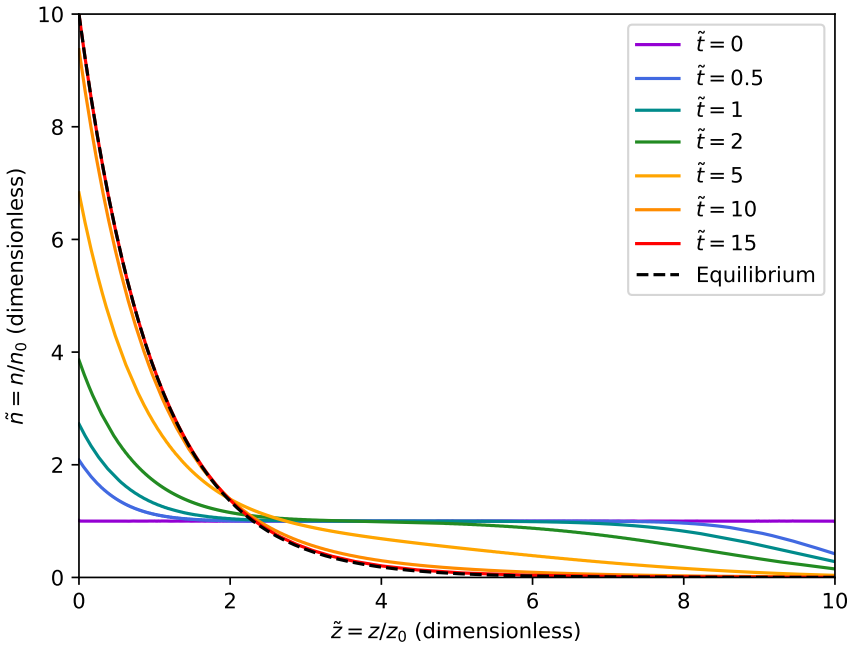


Figure 1: Normalized number density as a function of normalized height for a range of times.

Microscope and camera Your sample is placed under an ordinary optical microscope. A short instruction on the use of the Bleeker microscope (including hints for proper illumination) is added as an appendix to this instruction.

The magnification of a microscope is the multiplication of the magnifications of the objective and the eyepiece. On most microscopes, objective magnifications of 10×, 40× and 90× are available. Eyepieces are available to observe the sample by eye. The DinoEye CCD camera used in measurements has its own imaging lens and magnification (around 70× at 640 × 480 image format). **Note**: Please do not remove the coated lens in the DinoEye CCD or try to clean the lens or CCD yourself! Ask for a technician if you think there is a problem here.

As you want to explore the concentration of particles per depth, you will need to make pictures or videos at multiple depths of your sample. Images (pictures) and videos can be recorded using the program DinoCapture. Video settings and particle counting (i.e. retrieving the particle locations in a series of images) are explained in Section 2.7.

A LED light source is used for illumination. If desired, a lens and diffuser can be used to change the illumination of the sample.

The microscope creates a magnified image of the object plane in the image plane (CCD or retina). The image plane can be displaced vertically in a coarse way by translating the microscope tube with the large black knobs. This is suitable for alignment purposes. Turning away from you brings the tube *down*. Fine adjustment (for the experiment) can be done by translating the object table with the ruled knobs on the table. Each full turn corresponds to 50 lines on the scale. Turning away from you brings the object table *up*. The fine translation requires manual calibration.

Because you record images as a function of height in this experiment, we discuss two optical properties of the microscope in the (vertical) direction perpendicular to the object plane: the working distance and the field of depth.

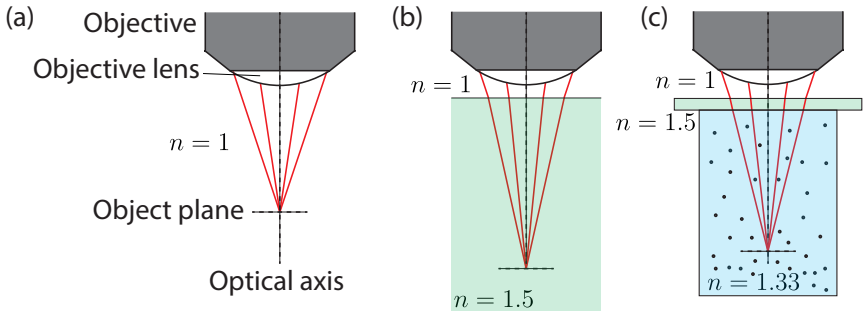


Figure 2: Illustration of the effect of the refractive index on the location of the object plane (a) Rays in case there is only air ($n \sim 1$) between the objective and the object plane. (b) Rays in case the light travels through a layer of thickness d of a second material present with $n > 1$. Refraction at the surface causes the object plane to shift downwards by $(n-1)d$. (c) Realistic situation in the actual experiment: light first travels through air, then through a glass cover slip, and then through water.

Working distance and vertical scanning range The working distance (usually termed W or WD) of the objective lens is the maximal distance between the focal plane and the front of the lens. The 40× objective, which we recommend for this experiment, has a working distance in air of around 700 μm. The addition 'in air' is relevant, because the vertical translation of the object plane depends on both the translation of the object table *and* the refractive index of materials under the objective.
The situation is depicted in Fig. 2. Due to refraction at the interface between air and some other material (water - as in your experiment - or glass - as in your calibration) the object plane is displaced downward. One can show that, under the assumption of small angles with respect to the (veritcal) optical axis, if the refractive index of the material is n and the refractive index of the air above the sample ~ 1, the vertical translation of the focal plane is n times the vertical translation of the sample table. The working distance and the thicknesses of the various materials between the objective and the object plane dictate the maximal scan range in your experiment.

Depth of field Fig. 2 suggests that only a single plane is imaged. In reality, the object (focal) plane is the only plane that is imaged *sharply*. Objects away from the object plane are also imaged, the images becoming more blurry and weaker as you move further away from the object plane. The effective 'thickness' of the object plane (the vertical distance that can be imaged sharply) is termed the *depth of field* (usually termed DOF). The minimum single-sided value for DOF[5] is $\frac{\lambda\sqrt{n^2-\mathrm{NA}^2}}{2\mathrm{NA}^2}$, with λ the wavelength of light, n the refractive index of the material at the object plane and NA the numerical aperture of the objective lens (indicated on the objective, 0.65 for 40×).
In reality, the uncertainty in the vertical direction is larger than the DOF since particles that are not imaged sharply can still be detected by the tracking algorithm (see Section 2.7). Furthermore, the minimal value of the DOF is valid only for optimal setting of the condenser lens (see the Appendix on how to use the Bleeker microscope).

Exploratory questions

- Practice with the microscope using prototype microscope slides with diatoms, first with the eyepiece and then with the DinoEye CCD camera. Practice correct focus and illumination.
  The wrench pictogram in DinoCapture allows you to improve brightness etc. digitally. We rec-

[5] https://dovermotion.com/applications/automated-imaging/microscope-calculations/

ommend to optimize physical illumination (microscope settings) first, and then change digital settings.

- You can make images using the Snapshot button.
- You will need to perform a calibration to determine the 'height' at which pictures are recorded. For this, make and/or use fatty smudges on both the back side and the front side of a microscope slide or cover slip. The thickness of an ordinary microscope slide is ≈ 1 mm, of a cover slip around 150 μm (you can use the HBM micrometer to measure this yourself: ask the TA if this device is not at your setup).

  Get the smudge on the back side in focus with the coarse translator. With the fine translator, determine how many turns / lines it takes to get the front side in focus. Use this information, combined with the thickness of the slide, to determine a calibration factor. Mind the refractive index of glass (a refractometer is available for measurement - ask the TA). Also estimate an uncertainty in your calibration factor.
- Assuming the refractive index of your suspension is $n_{\text{water}} = 1.33$, if you displace the object table by δz, how much will the focal plane displace?

2.5 Sample preparation

Preparing a sample The glass workshop at Instrumentation[6] has developed glass microscope slides with a 6 mm diameter, ~ 600 μm deep wells.
Standard cover slips are available to cover the wells. We do not want the sample to dry out before the sedimentation equilibrium is reached, which can take several days. Therefore, it is necessary to seal the edges of the cover slip, so the sample is sealed watertight. Nail polish was found to be a good sealant.
To practice making a good sample, we suggest that you do a first trial with demineralized water to avoid spilling the nanoparticles. Drops of water (and later your dispersion) can be added by pipettes.
We found the following procedure to work quite well (but since this experiment is in the pilot phase, we welcome improvements):

1. One drop should be sufficient to fill the well. Make sure there is a small top above the well; otherwise the capillary forces will drain your well in the next step. Air bubbles under the cover slit are usually a problem, since they influence diffusion and sometimes cause convection currents.
2. Put a cover slip next to the well and slide it over the well.
3. Wait some time for the slide to dry up around the cover slip (very important, since the nail polish will not attach if the slide is still wet). If the first dry spots appear under the cover slip, proceed to the next step. If necessary, use a tissue to remove remaining wet spots.
4. Close the edges of the cover slip. Take a bottle of nail polish and use the brush to apply the nail polish alongside all of the edges. Apply sufficient nail polish so that the raised edge of the cover slip is completely filled; a stripe of a few mm thick should suffice for this. If the nail polish does not attach, the glass was still wet and you'll have to wait.
5. Wait until the nail polish dries before you test your sample under the microscope, to prevent pollution of the objective lens.
6. If the sample remains properly wet after a longer period of time, you can assume it was prepared properly.

[6] https://www.uu.nl/en/research/scientific-instrumentation

Table 1: Available particle diameters, diameter standard deviations and undiluted mass concentration w/v: the amount of particle mass per unit volume relative to the total mass. This number allows you to calculate the number of particles per unit volume.

Diameter d (μm)	Standard deviation σ_d (μm)	Concentration w/v (%)
0.51	0.01	2.6
0.746	0.022	2.6
0.99	0.03	2.6
1.93	0.054	2.6

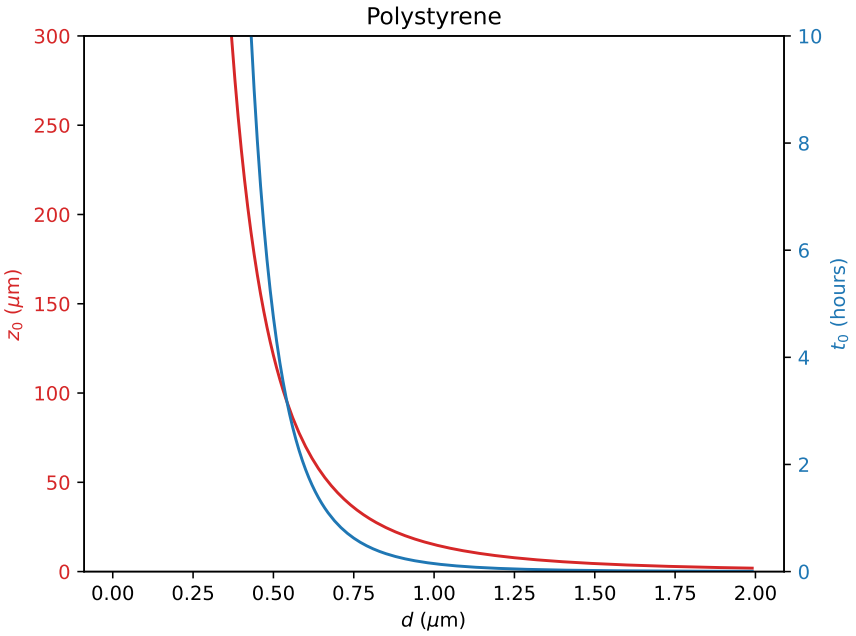


Figure 3: Sedimentation length and time for polystyrene colloids with different diameters.

Microscope slides and cover slips are fragile! Handle with care!

After your tests, you can start working with actual particles.

Selecting particle size and concentration You will use a colloidal suspension (or so-called *dispersion*) of polystyrene particles in water. Particles (at least the ones with the diameters we use) in the object plane appear as dark spots because they scatter incoming light - and this light will not reach the image plane (see also the appendix on use of the microscope).
Table 1 gives an overview of the particle diameters d that are available, including the diameter standard deviation[7]. The concentration of the solutions we acquired from the manufacturer is 2.6% w/v for each particle size. Dilutions of factors of 10 of the standard concentration (1%, 0.5%, 0.1%, 0.05%, 0.01%) are available for the experiment. Note that the bottles give the particle *diameters*!
Both the sedimentation time t_0 and sedimentation length z_0 are dependent on the particle diameter, and the diameter used should therefore be chosen sensibly. Figure 3 displays these parameters for

[7] Data sheet is available on Blackboard. Note: small particles scatter less light (scattering is proportional to d^6)

polystyrene particles in water. Note from Fig. 1 that it typically takes $\sim 10\,t_0$ for the initial distribution to approach the final exponential density distribution.
For the exponential function to be observed within the limits of your experiment, the sedimentation length z_0 of the colloids (Sec. 2.2) must not be larger than the microscope slide well depth. On the other hand, z_0 should not be smaller than the DOF or the uncertainty in the vertical direction, since no meaningful data can be collected in that case.
To prevent pollution of the dilutions we supply, use clean pipettes and never pour particles back. In any case, dispose of your samples and glass work in the appropriate container (ask your TA!); these particles should not leak to the environment.

Exploratory questions

- Choose an appropriate particle diameter and determine an optimal dilution. A good dilution ensures that particles are neither too sparse at the top of your sample nor overly concentrated at the bottom.
- Prepare a sample, using the procedure in the next section (or your own improved version).
- Without waiting for the sample to settle into sedimentation equilibrium, place your sample under a well-prepared microscope. Check whether the microscope settings are satisfactory and whether the sample quality meets your requirements.
- Estimate the uncertainty in the vertical direction σ_z (in which range is a particle observable / sharp?).
- Wait until sedimentation equilibrium has (roughly) been reached, and make some images at the bottom of your sample, and at the top of your sample.

At this point, you might want to ponder about how many pictures you want to take, at which heights, horizontal positions, over which vertical range, with what step size, constant or depending on where you are in the sample, ... Delay your decisions until you explored and tested data processing.

2.6 Safety

What are potential safety risks involved in this experiment? Include precaution measures in your plan of work if required.

2.7 Data processing

DinoEye With DinoEye, you can make snapshots of your sample.

Python: Trackpy and other packages Trackpy is a Python package especially written for particle tracking. It is however also useful for particle counting. For information on how to use Trackpy, we highly recommend to take a look at the tracking tutorial on
http://soft-matter.github.io/trackpy/dev/tutorial/walkthrough.html[8]. In order for this walkthrough to work, Trackpy, and a few other packages need to be installed. This can easiest be done in Anaconda prompt. Within Anaconda prompt, it might be useful to update Anaconda first by typing `conda update conda`. Then, install the packages with the command
`conda install -c conda-forge yourpackagetobeinstalled`. Do this for the packages `trackpy`, `pims` and `moviepy`. Trackpy should automatically install the package `pandas` as a dependency, but if

[8] Information on all commands in the Trackpy package can be found on
https://soft-matter.github.io/trackpy/dev/api.html

that fails, this package can be downloaded with the same steps. Don't be afraid to ask your TA for guidance if you get stuck on this step![9]:

Using Trackpy For using trackpy, the tracking tutorial on http://soft-matter.github.io/trackpy/dev/tutorial/walkthrough.html[10] can be followed. A detailed discussion of the tracking algorithm can be found in Ref. [8].
As the walkthrough is primarily focused on tracking particles, you don't have to follow the walkthrough all the way. The only important parts of the walkthrough are the finding and the filtering of the particles. You therefore only have to look at step 1 and 2 of the walkthrough, and the filtering steps of part 3 that do not involve trajectories.

- If you choose to read the images as a batch (you can read an entire folder of photos in at the same time), be careful with the command `tp.batch()`. `tp.batch` performs object identification on a range of frames.
  Note: from version 0.5.0 on by default processing is done on multiple cpu cores. Unfortunately, simply running the script on Windows systems leads to problems. There are two fixes:

  1. You can prevent errors by the setting `processes=1` in `tp.batch` so that processing is only done on one core.
  2. The second fix is to put *all* your code in an if-loop as `if __name__ == '__main__'`. This way processing is done on multiple cores without error messages.

  Running the script on multiple cores does not reduce the processing time significantly, so we recommend to go for option 1.
- You can also locate particles in separate images using `tp.locate()` and store the intermediate results (use for example package `os` and `os.listdir()` to generate a list of images to be analysed).

Some general suggestions:

- The package was designed to detect and track particles. Therefore, you will sometimes get error messages when no particles are found, and when the concentration is too high, not all particles might be found. This puts limits on the concentrations or vertical scan range.

[9] On Mac systems, installations appear more involved due to clashing dependencies of Python packages. This can be resolved by defining a separate environment in Anaconda. The following procedure usually works; if not consult the instructor.

- Open the terminal via Applications | Utilities | Terminal
- Follow these steps (typewriter font should be entered in the terminal):

  1. `conda update conda` (updates to the newest version of the conda package management system)
  2. `conda create -n trackpy` (creates the new empty environment called trackpy)
  3. `conda activate trackpy` (from now on all your commands act on environment trackpy)
  4. `conda install -c anaconda spyder` (installs spyder editor)
  5. `conda install -c conda-forge trackpy` (installs trackpy but also other packages like matplotlib and scipy)
  6. `conda install -c conda-forge moviepy`
  7. `conda install -c conda-forge pims` (installs pims, for image processing)
  8. `conda install scikit-image` (also for image operations)
- You can now close the terminal (type `exit` and close window)
- Open Anaconda and switch 'Applications on' to trackpy. Then, launch Spyder (in the new environment) and everything should work.

If required, additional packages can be installed in the same way.

[10] Information on all commands in the Trackpy package can be found on https://soft-matter.github.io/trackpy/dev/api.html

- When using incorrect settings, some particles will be detected multiple times. Especially `diameter` and `minmass` can prevent this. Be sure to investigate the influence of trackpy settings on your final results.
- Think about false positives (e.g. background spots that are detected as particles) and negatives (e.g. particles that are not detected). Are they a form of systematic error? Are there ways to improve or prevent or account for this when analysing the data?
- Be mindful that trackpy settings should be used to filter out spurious particles, and not to get the results you want to see (cherrypicking). It is good practice to be consistent in your settings. For example: use the same `minmass` for every snapshot.
- Since you count particles, based on the statistics you can assign an uncertainty to the particle number (right?).

Processing The results for particles, locations etc. are collected in a (pandas) DataFrame. This object can be turned into a `numpy` array by using `np.array()`, but you can also choose to proceed to work with DataFrames. How to process the data into a form that can answer your research question is up to you. Some *Python* commands that might be useful are `np.nanmean` and `np.nanstd` (calculate mean and standard deviation while neglecting `NaN`'s in an array). Think about how you want to fit your results. Do you want to work with a linear scale or a logarithmic scale?

2.8 Test measurements

Test measurements address at least the following issues:

- Uniformity and quality of the illumination.
- Sample preparation / requirements and choice for objective lens.
- Measurements at different heights

Due to refraction effects (see Section 2.4), the measured depth differs from the 'real' depth by a factor $n_{water} = 1.33$. Adjust your height scale accordingly.

3 Plan of work

The plan of work is developed during the preparation phase. The purpose is to fix your decisions and ensure an efficient and faultless execution. Use your test measurements as support for your decisions. The plan of work should contain the following elements:

- Research goal
- Model (relations between the relevant quantities, hypothesis or expected outcome)
- Setup (components, accuracies, operation)
- Method (protocol, time schedule, possible safety measures, checks and recommendations)
- References

More information can be found in the General Instruction on Blackboard. The plan of work does not have to be a separate document, as long as you can find the relevant information in your lab journal. The plan of work is discussed orally with the TA and graded (together with the achievements in the preparation phase).

4 Experiment

Execute the plan of work after discussing it with the TA and making possible revisions. Analyse the data and draw conclusions based on the results. Evaluate your approach and results critically (e.g. which critical factors limit the accuracy?). The results are discussed with the TA before you transfer them to the article.
Evaluate results critically (Do you find an exponential relation, does the value of your measured constant found correspond with the expected value, are results reproducible?) and discuss them with the lab assistant.

References

[1] A. Einstein, *Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen*, Ann. Phys. 17 549 (1905). An English translation can be found at
http://users.physik.fu-berlin.de/~kleinert/files/eins_brownian.pdf

[2] The Nobel Prize in Physics 1926, https://www.nobelprize.org/prizes/physics/1926/perrin/facts/

[3] Michael Horne, Peter Farago and John Oliver, *An Experiment to Measure Boltzmann's Constant*, Am. J. Phys. 41, 344–348 (1973)
https://doi.org/10.1119/1.1987221 (accessible from within soliscom domain)

[4] S.J. Blundell and K.M. Blundell, *Concepts in Thermal Physics*, Oxford University Press, Oxford, 2006.

[5] Download location for DinoEye driver: http://nspracticum.science.uu.nl/STF2024/Practicum/BRWN/Background/dnldrv4.exe

[6] DinoCapture download site: https://www.dino-lite.eu/en/support-download/software Manual: https://www.dino-lite.eu/en/support-download/manuals/dino-lite-manual-2023/download

[7] Trackpy user documentation: http://soft-matter.github.io/trackpy

[8] John C. Crocker and David G. Grier, *Methods of Digital Video Microscopy for Colloidal Studies*, Journal of Colloid and Interface Science, Volume 179, Issue 1, 15 April 1996, pages 298, Access from within soliscom domain: http://www.sciencedirect.com/science/article/pii/S0021979796902179

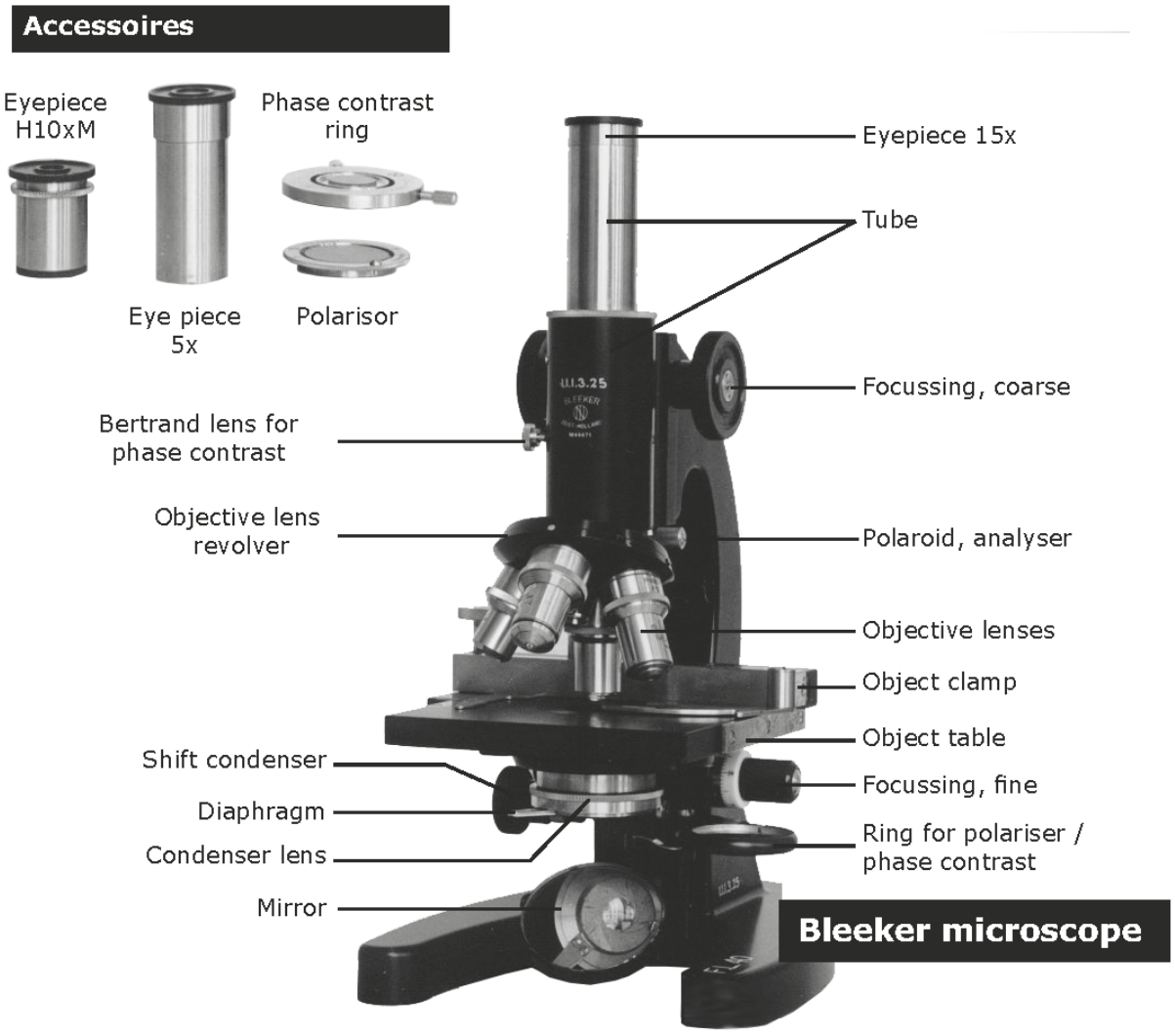
[9] Eric R. Dufresne, David Altman, and David G. Grier, *Brownian Dynamics of a Sphere Between Parallel Walls*, Europhys. Lett. 53, 264 (2001) Available through Arxiv.org: https://arxiv.org/pdf/cond-mat/0008316.pdf

Using the Bleeker microscope

Introduction

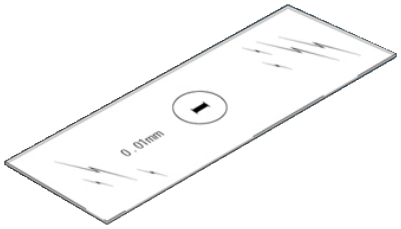
The Bleeker microscope (named after its developer, dr. Caroline Bleeker) is a basic microscope with some advanced options, like polarization-dependent measurements and (in some models) phase contrast (PC) microscopy. The latter techniques are not used in the Statistical Physics practicum. This instruction discusses the most important components.

Microscope



Dino-eye camera USB 1.3M

Micrometer slide



Components

Eyepiece

The eyepieces shown are standard components for each microscope; some have an additional 7x magnification. The eyepiece can be replaced by the DinoEye camera (see below). Make sure that either an eyepiece or a camera is placed in the microscope tube, so that the internal parts of the microscope remain free of pollution.
Hint: Focusing and finding correct illumination is often easier by eye than by the camera.

Objective lens

Most microscopes have four objective lenses in the rotatable revolver. All are equipped with an A2 / A3 wide range objective lens, a 10x and a 40x objective lens. The fourth objective lens is either a 40x phase contrast lens or a 90x (oil immersion) objective. The 90x lens gives a larger magnification, but when used without immersion also gives blurred images due to strong refraction.

Usage

Illumination

In general, more light that enters the eyepiece is better (attenuation can always me done if necessary, and not all samples are equally transparent). The Dino-Eye camera will automatically correct for low light intensity (i.e. a sample that looks well-illuminated may not automatically be so).

Light from the external (LED) light source reaches the microscope via the mirror at the bottom. The light can be aimed on the relevant part of the sample by properly positioning both the light source and the mirror. Place the light source at the desired distance from the mirror, and if necessary place a diffusing glass between the source and the mirror (the intensities of the light that hits the mirror sometimes become more equal, which improves illumination).

Next, direct the mirror:

1. Set the objective to a low magnification (e.g. 10x)
2. Remove the eyepiece or microscope camera from the tube
3. Look directly down into the tube and, by directing the mirror, ensure that the light spot is in the center of the tube (in that case you will see weak scattered light from all sides of the tube).

Note: if the mirror or light source (or microscope) gets shifted, the illumination will no longer be correct.

Condenser

A condenser is then used to achieve good illumination of the sample. It consists of four parts:

- the condenser lens
- a diaphragm
- a polarisation / phase contrast ring (not used)
- and a control knob.

The condenser lens focuses the incoming light on the sample. The diaphragm determines the width of the beam of light falling onto the condenser lens.

Both shifting the condenser lens up and down and opening or closing the diaphragm influence the smoothness of illumination of the sample, the field of depth (which is the part of the sample in the vertical direction that will be imaged – the 'thickness of the object plane' to put it otherwise) and the image contrast.

Hint: Slides containing diatoms (single-celled algae) are available for positioning the condenser and for focusing. These slides contain some paper with a description. Move the paper to the illuminated spot and then ensure that the illuminated spot is as small as possible by moving the condenser. The diaphragm opening is optimal if you maximally attenuate the light around the illuminated spot without weakening the illuminated spot itself.

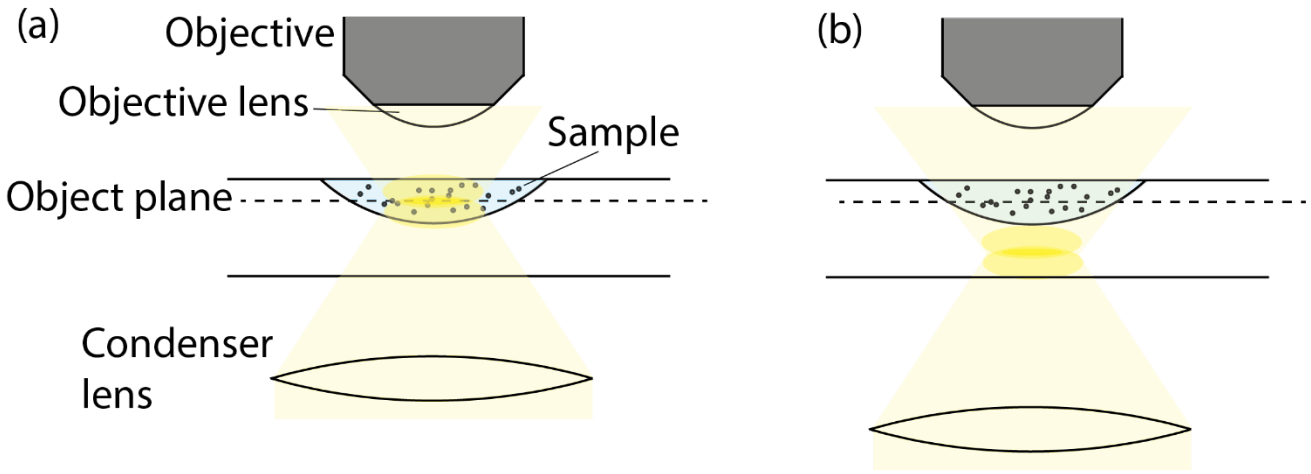


Figure 1 Optimal positioning of the condenser. First, the particles in the object plane (which is sharply imaged) have the best visibility, because light intensity is greatest there. Second, the light hits the particles in that object plane almost vertically, causing scattering to block light very effectively. (b) Poor positioning of the condenser. Now all particles are visible, even those that are not sharply imaged. Moreover, they all become fainter because at each point in the observation plane the scattered light constitutes a smaller part of the total amount of light.

Figure 1 illustrates the cases of optimal and poor positioning of the condenser. Figure 2 shows some images for optimal and suboptimal settings for condenser lens and diaphragm for the same 750-nm particles (but on different positions due to Brownian motion). You see that there is a lot to gain especially when optimizing the condenser lens position. Since the size of the part of the sample that is imaged on the CCD or retina depends on the choice for objective lens, the optimal settings for condenser lens and diaphragm will differ depending on the choice for objective lens.

Note: if the condenser is moved downward too far, it might turn the mirror and ruin correct illumination.

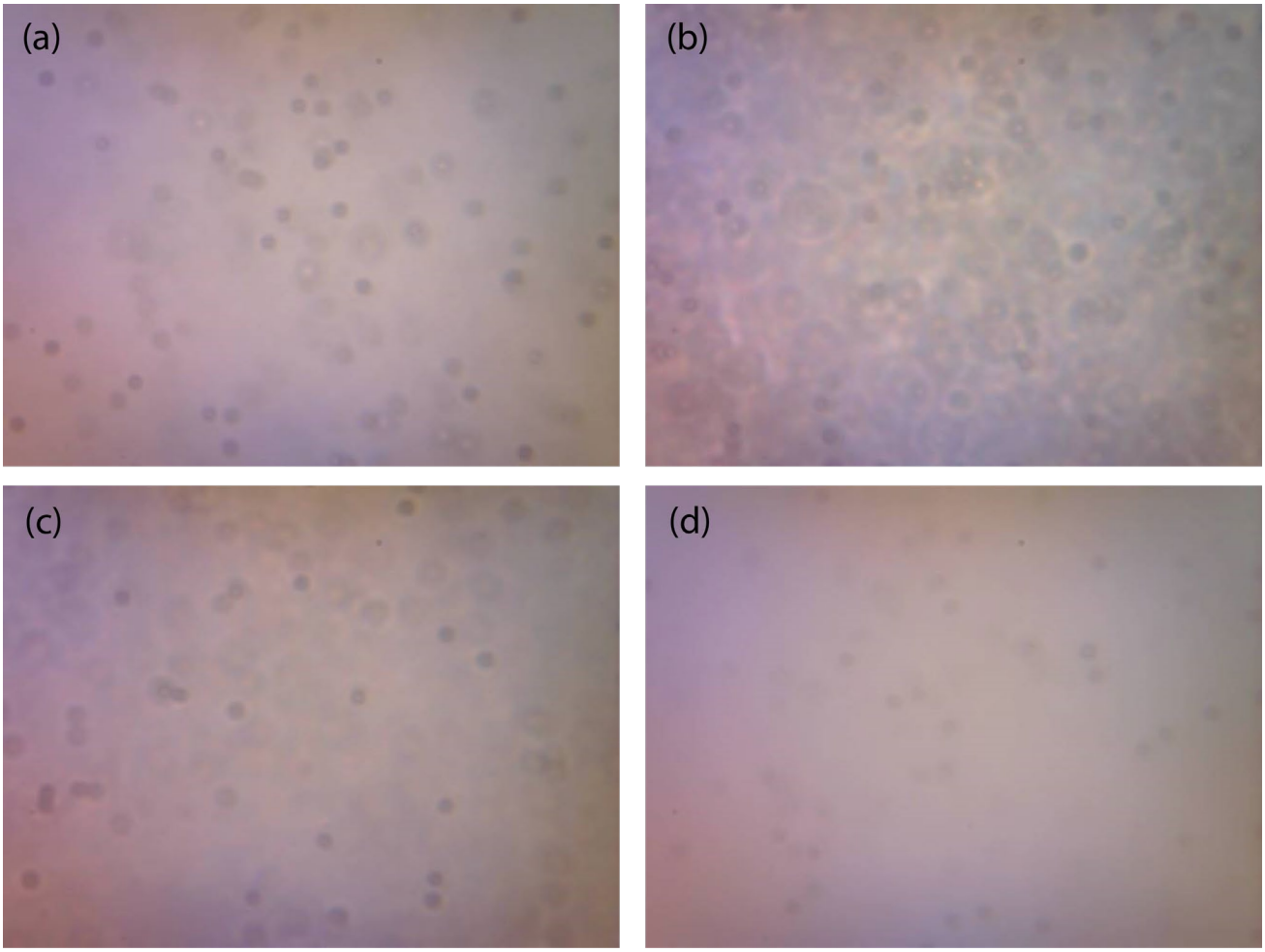


Figure 2 Set of images of the same sample, but for different settings for condenser lens and diaphraghm.
(a): 'ideal': particles are imaged as sharp black dots, and we don't see many particles in the background (which means that the field of depth is small). This is important when trying to track particles.
(b): condenser lens too low (large field of depth). You see a lot of blurry particles; the sharp particles of (a) have faded and others at different depths in the sample have appeared.
(c) condenser lens at the correct position, but diaphragm is almost closed. Diffraction rings have formed around the sharp features of (a) – this is hard to see on this image but very clear when you work with the microscope.
(d) As for (c), but now with diaphragm too far open. Here, the contrast is greatly decreased (overillumination).

Hint: Practice and fix illumination using a sample with sharp features, like the micrometer slide (see below) or the diatom samples (or printed texts on them). Once you have a sharp and properly illuminated sample, keep the settings fixed throughout the experiment.

Focusing

Focusing is done in two steps:

1. Course, by translating. the tube, and
2. Fine, by translating the object table

Hint: The safest way to achieve a focus (i.e. without damaging your sample) for the 40x / 90x objective lenses:

1. Use the 10x objective lens to get a focus using the coarse and fine translations
2. Switch to the 40x objective lens

3. Only use object table translation in the direction upward (away from the object table) to refocus
4. (optional) Repeat 2. and 3. for the 90x objective lens.

Hint: It works best to practice focusing on the paper with letters on the slides with diatoms. On your own slide, a good place to focus is the edge of a cover slip (but you can also mark a line on the slide).

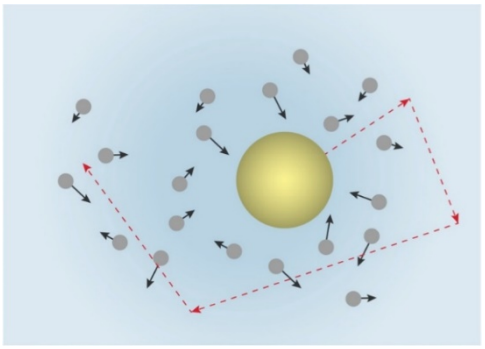
Hint: If you are not able to get a clear picture but you have no idea what is wrong, probably the Bertrand lens is in the tube. Remove it by rotating the small knob on the right of the tube.

Accessories

- *Dino-Eye camera USB 1.3M:* This camera is operated via DinoCapture software on the desktop / laptop. Put the dust cap on the camera if it is not mounted in the microscope.
  https://www.dino-lite.eu/en/software/general-software/dinoxcope-mac (software, Mac)
  https://www.dino-lite.eu/en/software/general-software/dinocapture-windows (software, Windows)
- *Micrometer slide*: This slide (that must be shared amongst groups) can be used to calibrate the scaling of the recorded image. It is a glass slide with a graduation of total range 1 mm, subdivided by 100 µm coarse (long) and 10 µm fine (short) scale marks. Use the circle around the graduation to find it. The micrometer slide is expensive, make sure you don't break it when focusing!

Advanced options

- The microscope has a sheet of polarizing material (the *analyser*) in the tube, that can be placed in the optical path via a knob on the tube. When another sheet of polarizing material (the *polarizer*) is located before the sample, the optical activity of the samples can be investigated.
- Phase contrast (PC) microscopes are indicated by a red line on the box and red rings on some objective lenses. The PC microscope has a so-called Bertrand lens in the tube, that can be placed in the optical path via another knob. Phase contrast microscopy uses optical interference to enhance the visibility of transparent objects.
  For more information, see
  https://en.wikipedia.org/wiki/Phase-contrast_microscopy



Practicum
Brownian motion

Suggestions

- Fill only the narrow part of the pipette; just one or two drops are sufficient for filling the well slide.
- Dispose of pipettes in the glass container
- Rubber squeezers can be reused
- Microscope slides are reused: wipe using paper towels and dispose of the towels in the waste bin*. Optionally rinse your slides with purified water and dry them.
- Dispose of cover slits in the glass container
- You can use markers to write on your slides (ink is not water-resistant).
  Leave the markers at the preparation table.
- Return the diatom specimen after use (it is expensive and vulnerable)
- Alert the TA or technician if the bottles with diluted particle samples are almost empty
- Leave the preparation table clean and organized
- Thanks for your cooperation!

More information on non-diluted Polybead® microbead solutions:

www.polysciences.com

2 µm	cat# 19814
1 µm	cat# 07310
0.75 µm	cat# 07309
0.5 µm	cat# 07307

* Polystyrene microparticles cannot be disposed of via waste water in the Netherlands, via ordinary waste is allowed.