Optics lab 2

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Abstract

In the second optics lab we use light microscopy and computer tracking software to estimate the Boltzman konstant (k_B) by studying the brownian motion of 1μm particles dispersed in water. The experiment showed that when adjusting the equipment correctly relatively accurate values of k_B were estimated , but only specific configurations of the microscope and tracking software could be used to obtain reliable data.

1 Introduction

When small particles are dispersed in a liquid they are subject to brownian motion (background information in section 2.2). The distribution of step sizes in the brownian motion is related to the thermal energy of the system, which again is given by the temperature and the Bolzman constant. Thus by observing and recording the random motion of the particles, tracking the particle walks, and employing statistical models on the data we can estimate the value of the Bolzman constant.

2 Theory

2.1 Optical microscopy

Optical lab 1 was an introduction to optical microscopy and the techniques described there are applicable for this lab. For the general theory and additional details behind optical microscopy see Optics lab 1 by Kai Beckwith, 20.02.08 ([1]).

2.2 Brownian motion

Brownian motion was first discovered and described by Robert Brown in 1827 [2]. He concluded that the movement of the particles later named of him was not caused by external forces such as movement in the liquid, but only the particles themselves. Despite being partly wrong, he did discover this very important effect. Albert Einstein was the first to mathematically characterize the brownian motion, and also give a physical interpretation of the phenomenon [3]. Small particles, such as watermolecules in the Brown's case, are constantly crashing into a large particle, such as the pollen grain, at a very high pace. But each collision causes only a very small displacement of the pollengrain, at the same time as so many collisions happen each second that it would be impossible to observe the motion caused by this. But Einstein solved this problem by combining the two effects with statistical theory: Once in a while there will be a discrepancy in the amount of bombardments from one side compared to the other, and the particle will "lurch" a relatively large step in the opposite direction, which is what we then see as brownian motion. Using thermodynamics, diffusion theory and statistics, he obtained several useful formulas which will be discussed now.

The diffusion constant for spherical particles is given by

$$D = \frac{RT}{N6\pi\eta P}, \tag{1}$$

where R is the universal gas constant (8.315 J/K mol), related to the Bolzman constant by $R = k_B N$. T is the temperature in Kelvin, N is Avogadro's number $(6,022 * 10^{23})$, η is the viscosity of the liquid and P is the radius of the sphere.

The probability of a certain displacement along a given axis can be calculated for a certain time interval τ by assuming the step sizes follow a Gaussian distribution (normal distribution). Relating the step time to the diffusion constant Einstein obtained:

$$\lambda_x = \sqrt{2D\tau}, \tag{2}$$

where λ_x is the expected (mean) dislplacement in the time interval τ. Thus by measuring the mean displacement during a given time interval, we can estimate D and thus find the value of k_B, assuming the other variables are known.

2.3 Measuring k_B

As mentioned in section 2.2 we can find D and thus k_B by measuring the mean distance a particle moves during a given time. If we use several particles and set up a distribution of step sizes, we can get relatively accurate values of k_B. This method is described in a 2002 paper by Paul Nakroshis et. al. [4].

The tracking is done by first taking a small video using the imaging software ImageJ, then using a tracking plugin to obtain particle trajectories. For the tracking plugin to work, the video has to be converted to 8-bit greyscale colouring, inverted, and the contrast and brightness needs to be adjusted until the particles are all that are visible. The trajectories are then output as a text file which gets imported into matlab. In matlab this data is used to compile a histogram of the distribution of step sizes. The histogram is normalized and fit with a gaussian distribution, and from the gaussian distribution we can establish the diffusion constant, and k_B.

The error in the results is estimated using standard statistics approach to measuring the sample variance. The sample deviation is given by

$$S = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(X_i - \bar{X})^2} \tag{3}$$

where N is the amount of samples, X_i is the value of the given sample and $\bar{X} = \frac{1}{N}\sum_{i=1}^{N} X_i$ is the mean of the samples. S is the sample variance, and it's computed value is denoted s.

3 Experiment

3.1 Sample

The sample used was spherical polystyrene particles with a diameter of 1μm. These particles where dispered in clean water at room temperature, 19 degrees C. The sample was put onto a dimpled microscope slide, covered with a cover slide, and put into the microscope for study.

3.2 Adjustment

The microscope was of the model Leica DMLS, a standard optical microscope with magnifications ranging from 10X to 100X. The 100X objective is an oil objective.
The microscope was first adjusted at 20X magnification using Köler's principle[1] to obtain maximum contrast. The condenser aperture[5] was opened to decrease the depth of field (DOF), because in this experiment it was important to view only a limited amount of particles which where in focus. Bright field[1] was chosen as method, due to the type of sample used. The sample was not transparent enough for phase contrast to give a significant advantage, nor was did it scatter enough for dark field to be advantageous. Figure 1 shows the end result after the microscope was adjusted to optimal settings, but before any digital enhancements were done to the image.

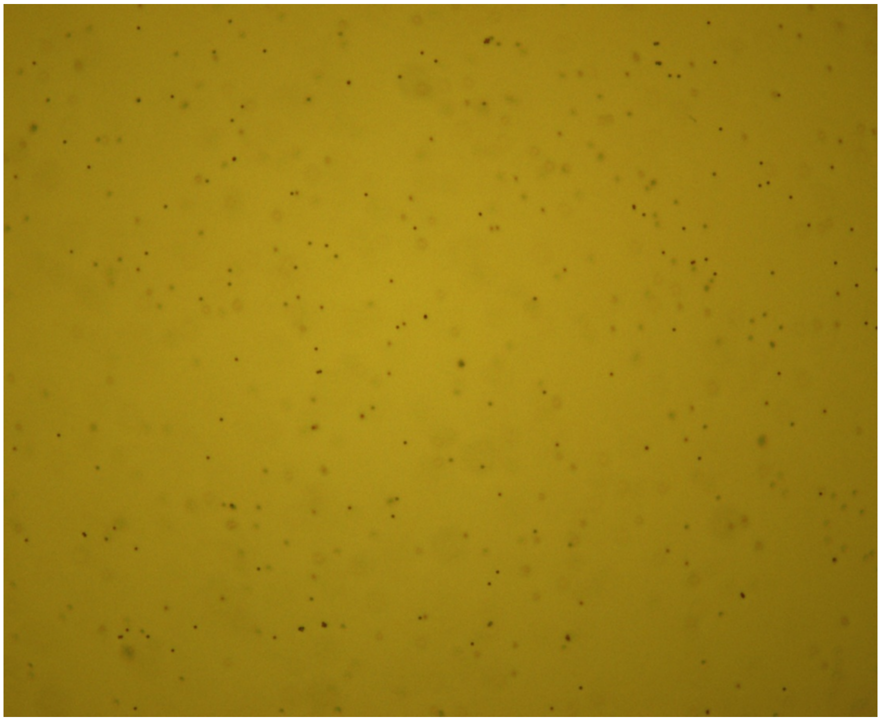

Figure 1: *Bright field image of the 1μm particles at 20X magnification (not to scale, only for comparison purposes). The depth of field was tweaked to obtain maximum detail on particles in a single plane.*

4 Results

4.1 Measurements with 20X magnification lens

The first measurements were done by two videos at 20X magnification, 100 frames at 7,5fps. Figure 2 shows the image after editing to make the tracking software as accurate as possible.

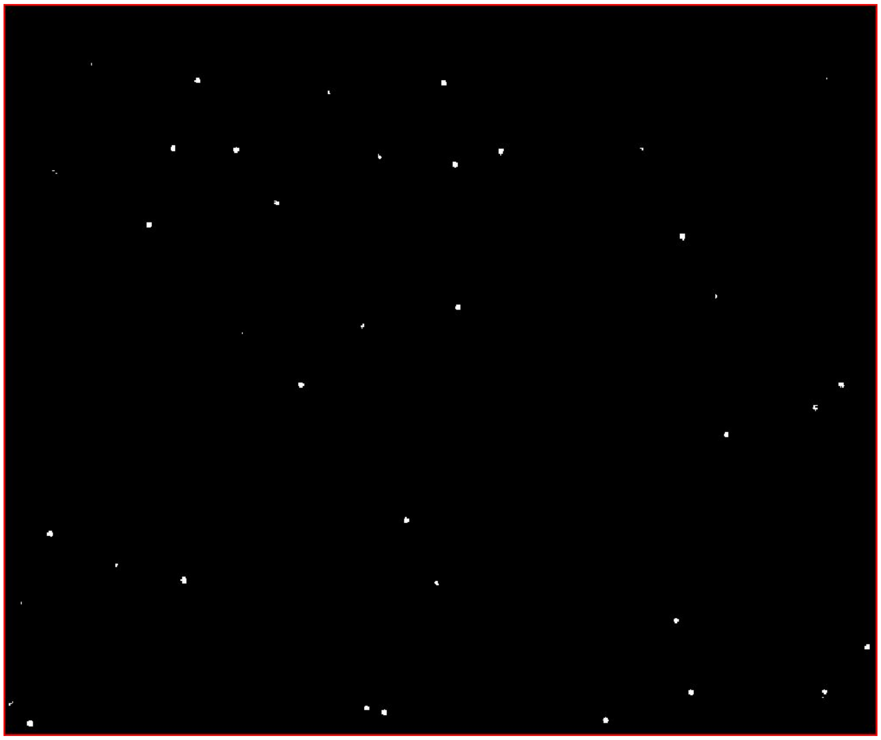

Figure 2: *Image taken under similar conditions as figure 1, but after digital manipulation for maximum contrast.*

Using the methods described in section 2.2 and 2.3 we obtain results for k_B based on steps in x-direction and y-direction. The results were:

$$\begin{aligned} k_B^{x_1} &= 5,919 \cdot 10^{-21} \\ k_B^{y_1} &= 5,129 \cdot 10^{-21} \\ k_B^{x_2} &= 3,064 \cdot 10^{-21} \\ k_B^{y_2} &= 4,002 \cdot 10^{-21} \\ \bar{k_B} &= 4,529 \cdot 10^{-21} \\ k_B(292K) &= 4,06 \cdot 10^{-21}, \end{aligned}$$

where the first four are the actual results, $\bar{k_B}$ is the avarage result, and $k_B(292K)$ is the real value of k_B at 19 degrees C. The error of k_B is calculated using the method in the end of section 2.3, which gives an error (sample deviation) of $\pm 1,61 \cdot 10^{-21}$ for the above data.

4.2 Other measurements

Magnifications of 40X and 10X where tried, using exactly the same technique as above. The results at 40X where very widely spread, giving no conclusive results. In the tracking software the number of trajectories measured varied between 500-1000, despite there only being between 10-20 particles in the video at a given time. The same happened for 10X magnification. See section 5 for discussion as to why this could be.

5 Discussion

For 20X magnification we obtained results that under more controlled experiment conditions and with more repeats could yield accurate results for the Boltzman constant. The totally inconclusive and widely spread results for both 10X and 40X magnification indicates that the tracking program has trouble tracking the particles accuratly. For 40X the reason is probably due to the particles movement along the z-axis. Although the program is supposed to correct for this, it appears not to do so adequately. When the particles move along the z-axis and appear and disappear from the focal plane, they get registered as different particles. This is also indicated by the high trajectory count despite a limited amount of particles.

For 10X magnification a similar, but different problem occurs. Due to lower magnification, the DOF increases, causing more particles to appear and disappear into the focal plane. This was impossible to regulate, despite extensive time spent adjusting the microscope and capturing software. Therefore a huge amount of trajectories was tracked, and we get similarily diverging results.

Other sources of error that can be considered are agglomeration of the polymer particles, especially in regions close to air/water bounderies where the particles have been known to self-assemble. To counteract this areas where no such agglomeration was present was chosen for the samples. Particles near the glass slide might also not move in a completely random brownian motion, du to electrostatic or other influences from the glass. But due to using samples from different depths and diverging results regardless, this problem is likely dwarfed by the tracking problems described above.

6 Conclusion

Our results show that 20X magnification seems to be the optimal setting for this experiment, as 10X proved too low and 40X proved too high. With the correct settings this method gives a way to estimate the Bolzmann constant relatively accurately, and it also proves again Einstein's postulates about random motion and diffusion. Obtaining good results proved to be hard unless optimal settings were used, showing that correct adjustment and use of the equipment is as important for the experiment as a good sample. Knowing the limits of the equipment and possible sources of error can also make the error seeking process easier, and save a lot of time during the experiment.

References

[1] Lab report: Optics lab 1 by Kai Beckwith, 20.02.08. Contact beckwith@stud.ntnu.no for details and access.

[2] Brown, Robert, ”A brief account of microscopical observations made in the months of June, July and August, 1827, on the particles contained in the pollen of plants; and on the general existence of active molecules in organic and inorganic bodies.” Phil. Mag. 4, 161-173, 1828.

[3] Einstein, A. (1905), ”Über die von der molekularkinetischen Theorie der Wrme geforderte Bewegung von in ruhenden Flssigkeiten suspendierten Teilchen.”, Annalen der Physik 17: 549-560

[4] Paul Nakroshis, Matthew Amoroso, Jason Legere, and Christian Smith, Measuring Boltzmanns constant using video microscopy of Brownian motion, Am. J. Phys. 71 (6), June 2003

[5] Pawel Sikorski, Section 2.1, Laboratory in TFY4330: Light Microscopy Exercises 1 and 2, February 1, 2008.