Diffusion

1. Find the probability distribution of step lengths of diffusing polystyrene spheres in a time interval of (1/2 sec) and see if it can be fitted to a Gaussian distribution. Determine the width (σ) of the distribution and the diffusion constant of the spheres.
2. Derive from Your measurements the Boltzmanns constant by making use of the known relation between the diffusion constant and the friction coefficient of spheres.
3. Use both EXCEL AND Matlab for the analysis of the distributions.
4. *(optional)* Determine the diffusion constant from the time evolution of the average square of the particle position.

Equipment: Leica microskop; model DMLS
Video camera: Scion Corporation; Model CFW-1312C
Matlab

Theory

When a particle is doing a random walk and is starting from origo (x = 0) at time $t = 0$, the probability of finding the particle at position $\boldsymbol{x}$ at time $\boldsymbol{t}$ is:

$$P(x,t) = \frac{\delta}{\sqrt{\pi D \cdot t}} \exp(-\frac{x^2}{4Dt}) \equiv \frac{1}{\sigma\sqrt{2\pi}} \exp(-\frac{x^2}{2\sigma^2})$$

The left-hand side of this expression follows from a model of diffusion in which the probability for making a right or a left step is equally large and that the step lengths δ are equal (see lecture notes). It is a Gaussian distribution, and the expression to the right is the standard way of writing the Gauss function. $\boldsymbol{\sigma}$ is the standard deviation or the width of the distribution. When You do a fitting of Your measurements to a Gaussian distribution the parameter that is returned from the fitting procedure is $\boldsymbol{\sigma}$, the standard deviation, and accordingly, $4Dt = 2\sigma^2$, or; $D = \frac{\sigma^2}{2t}$.

A video will be used to find the positions of the diffusing particles and the time interval between two successive video pictures is usually set to: $t = \Delta = \frac{1}{2}\sec$. During that time a particle has made a large number of steps and by analyzing two successive video pictures, one can see the distances that the different particles have moved in that time interval. Many particles will be photographed, and for two successive video pictures (frames), the distribution of diffusion lengths of the various particles can be found. About 50 frames are recorded in the video, and accordingly, several distributions can be collected into one distribution, by sampling all frames. This is done by a *Matlab* program called "*particle tracker*".

We can then fit this distribution to a Gaussian function. This is done by comparing our actual distribution with the Gaussian function and change σ until the two graphs are matching. Then we get σ as a result, and we can see if the fitting is satisfactory or not. This is done in Matlab. Then we can find the diffusion constant as;

$$D = \frac{\sigma^2}{2\Delta} \qquad \text{(compare the two functions above)}$$

Furthermore, the diffusion constant and the friction coefficient (***f***) of the diffusion particles are related quantities (see lecture notes);

$D = \frac{kT}{f}$, For a sphere with radius ***a*** moving at constant speed in a liquid with kinematic viscosity $\boldsymbol{\eta}$ the friction coefficient is;

$f = 6\pi \cdot a \cdot \eta$, (see lecture notes; digression on friction)

According to this, we have in this case the following expression for the product of the Boltzmann constant and the absolute temperature;

$$kT = D \cdot f = D \cdot 6\pi\eta a$$

In the analysis to be done, the diffusion constant is found from the distribution of step lengths, the known viscosity of water and the particle size.

Experimental procedure

Video recording and analysis of Brownian motion

Making the Video; Use

Micro-ma

Prepare Video analysis

Use the image processing toolbox in Matlab called Image J1.38

ImageJ1.38 - File open - select a region of interest (ROI)

Image – Crop - Edit - Invert
Image - Adjust brightness/contrast (*apply*)

Plugins - particles detector and tracker -OK

Visualize all trajectories - Save full report (on C/Data/Matlab)

Analysis - using MATLAB

Open MATLAB - change : tracks.in - Traj_ MovieT2.txt (save)

\>> Tracks =loadTracks('tracks.in','',6,5000);

(>> is the command line in Matlab)

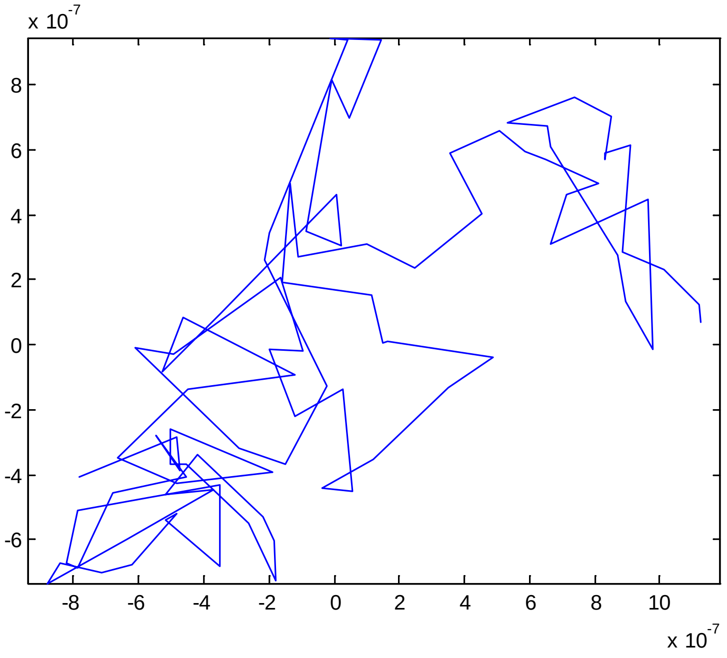
The subroutine Tracks will find the pixel values of all particles in a frame and identify a particle as the nearest neighbour in the next frame.

\>> plot TracksPS(Tracks,1,20,2)

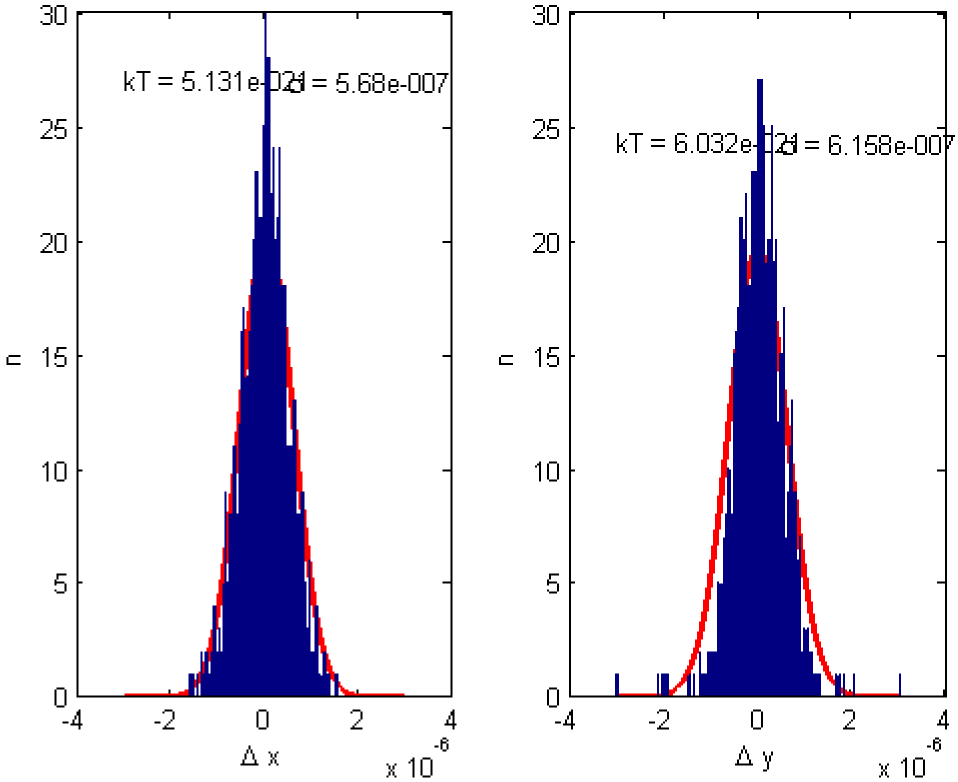
This subroutine will plot the distribution of step lengths and fit the measured distribution to a Gaussion distribution. The subroutine will also return the $\boldsymbol{\sigma}$ and the derived $\boldsymbol{kT}$ of the distribution.
20 is the magnification of the microscope and 2 is the frequency of the video pictures.

An example of particle tracking is shown below:

In the tracking procedure, the coordinates (pixel values) of individual particles are found for successive pictures in the video.



Video tracking of a diffusing particle



Probability distributions of velocities of two dimensional diffusion polystyrene spheres.
Red curve: Matlab fitting of measured distribution, returning the average kinetic energy: $kT = 5.13 \cdot 10^{-21}$ and $\sigma = 5.68 \cdot 10^{-7}$.

USING EXCEL

The camera chip is composed of 1024 x 1024 sensitive elements called pixels. A sensitive element is labelled with an (x, y) value, where x and y is any number between 1 and 1024 in the x and y direction called the pixel elements. The brightness and colour of each pixel element is stored in the camera memory. Whenever a picture is taken, the pixel elements correspond to a certain point in the object itself. In a microscope distances are being magnified and the magnification is the ratio between a pixel distance and a real distance in the object. In order to find the magnification one has to find the relation between pixel distances in the image and real distances at the objective of the microscope. For this purpose, take a picture of a ruler with calibrated distances.

In the ***Save full report*** from the Matlab program the pixel values for the particle positions in each frame are listed. The particles does not move far, and in the ***particle tracker*** program in Matlab the nearest pixel positions in two successive frames are referred to the same particle. At the end of the report is the ***x***- and ***y*** pixels of a particular particle listed. Import the x- and y pixels for a particle that the Tracker was able to follow for a long time.

In the EXCEL sheet, the following calculations can be done:

Find a particle in the Full report that the tracking program has been able to follow for a long time and import the x-and y pixel values into an Excel sheet. In the neighbouring columns You can create the step lengths as differences between two successive x or y pixel values. The actual step lengths have to found by multiplying by the step lengths in pixels by the magnification factor. Use the histogram function to find the distribution of step lengths and compare it to a Gaussian distribution (Use Solver, if available). When σ is found, the diffusion constant *D* is found and finally kT.

$\eta(H_2O)$ = 1.00 E-3 Ns/m^2 (viscosity of water)