

## Brownian bead test 24.09.2019

Microslides 0.2x4 ID Vitracom

Tried ImagingSource cameras: IC Capture AVI uncompressed Y800 could not be opened by VideoReader in Matlab => Switched to PointGrey camera

From image grid50um\_pointgrey\_40x.tif of 50um grid: number of pixels between 5 grid lines= 1361  
=> magnification  $m=5 \cdot 50/1361=0.1837 \text{ um/px}$

Analysis:

$$k_B = \frac{6\pi\eta r D}{T}, \eta = 10^{-3} \text{ Pa s}$$

Literature value:  $k_B=1.38e-23$

### Software:

Corrected lack of drift correction in analysis software:

```
msdx2=(sumdx2-sumdx.^2/numpart)/numpart;  
(previously msdx2=sumdx2/numpart;)
```

I have not corrected this everywhere in the analyse\_sporny.m

Do not use movieobj.FrameRate because this is only a number set by the software writing the movie to file and you do not know (unless you have carefully controlled it) if movieobj.FrameRate is the real framerate!!!

To be really sure one should calibrate the framerate like in the drag experiment!

To handle different video formats:

```
if movieobj.VideoFormat(1:3)=='RGB'  
    a=double(frames(:,:,2,1));  
else  
    a=double(frames);  
end
```

### p5um\_bead\_pointgrey40x\_2019-09-24-143759-0000.avi

0.51um beads, 40X magnification, I set the framerate to 15fps.

movieobj.FrameRate= 15.0002

```
>> analyse_sporny
```

149 tidssteg, 138 partikler

Midlet total lineær forflytning: (138 partikler)

x-retning: 3.9 +- 2.0 piksler

y-retning: -1.4 +- 2.2 piksler

Midlet total kvadratisk forflytning per tidssteg: (138 partikler)

x-retning: 3.65 +- 0.42 piksler<sup>2</sup>/tidssteg

y-retning: 4.27 +- 0.48 piksler<sup>2</sup>/tidssteg

The errors just overlap. error estimate  $\Delta r/r=0.4/4/\sqrt{2}=7\%$

$4D=15 \cdot 0.1837^2 \cdot (3.65+4.27)/2=2.00 \text{ um}^2/\text{s}$

$D=0.50 \text{ um}^2/\text{s}$

$$k_B = \frac{6\pi \cdot 10^{-3} \cdot 0.5 \cdot 10^{-6} \cdot 0.5 \cdot 10^{-12}}{273} = (1.73 \pm 0.12) \cdot 10^{-23}$$

The deviation from the literature value is 3 times our error estimated from each msd.

```
dx=tr(I,1)-tr(I(1),1);
sumdx=sumdx+dx;
sumdx2=sumdx2+dx.^2;
sumdx4=sumdx4+dx.^4;
msdx2=(sumdx2-sumdx.^2/numpart)/numpart;
msdx4 = sumdx4/numpart;
errdx = sqrt(msdx4-msdx2.^2)/sqrt(numpart);
```

[p5um\\_bead\\_poingrey40x\\_2019-09-24-135353-0000.avi](#)

0.51um beads, 40X magnification, I set the framerate to 15fps.

```
>> analyse_sporny
```

301 tidssteg, 122 partikler

Midlet total lineær forflytning: (122 partikkler)

x-retning: 4.1 +- 2.6 piksler

y-retning: -0.8 +- 2.9 piksler

Midlet total kvadratisk forflytning per tidssteg: (122 partikkler)

x-retning: 2.84 +- 0.39 piksler<sup>2</sup>/tidssteg

y-retning: 3.30 +- 0.44 piksler<sup>2</sup>/tidssteg

But this is without drift correction! With drift correction  $d\langle x^2 \rangle / t = 2.9916$ ,  $d\langle y^2 \rangle / t = 3.3177$

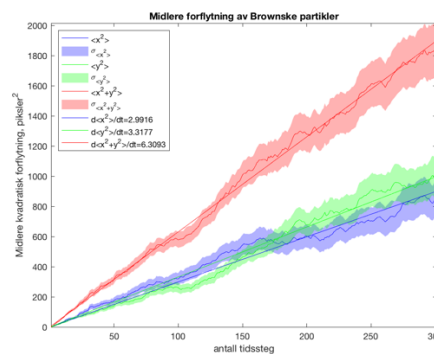
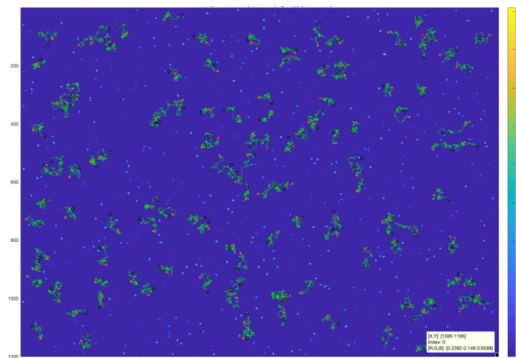
error estimate  $\Delta r / r = 0.4 / 4 / \sqrt{2} = 7\%$

$D = 15 * 0.1837^2 * (2.99 + 3.31) / 2 = 0.3986 \text{ um}^2/\text{s}$

$D = 0.389 \text{ um}^2/\text{s}$

$$k_B = \frac{6\pi \cdot 10^{-3} \cdot 0.3986 \cdot 10^{-6} \cdot 0.5 \cdot 10^{-12}}{273} = (1.38 \pm 0.09) \cdot 10^{-23}$$

Spot on literature value.



[1um\\_bead\\_pointgrey40x\\_2019-09-24-135606-0000.avi](#)

299 tidssteg, 160 partikler

Midlet total lineær forflytning: (160 partikkler)

x-retning: 65.1 +- 1.8 piksler

y-retning: -6.8 +- 1.8 piksler

Large drift in x-direction! Corrected for drift:

$d\langle x^2 \rangle / t = 1.966$ ,

$d\langle y^2 \rangle / t = 1.918$

$D = 15 * 0.1837^2 * (1.966 + 1.918) / 2 / 4 = 0.2458 \text{ um}^2/\text{s}$

$k_B = 6 * \pi * 1e-21 * .2458 * 0.99 / 273 = 1.6802e-23$

deviation from literature: +20%

Once again: deviation is much larger than estimated standard deviation of fit.

### 30s\_40x\_lav\_save\_2019-09-04-101249-0000.avi

This measurement was done on a sample many weeks ago. 0.99 $\mu$ m beads. 40x means magnification is 0.18  $\mu$ m/px

120 tidssteg, 100 partikler

Midlet total lineær forflytning: (100 partikler)

x-retning: -0.8 +- 2.9 piksler

y-retning: -2.9 +- 2.7 piksler

Midlet total kvadratisk forflytning per tidssteg: (100 partikler)

x-retning: 7.17 +- 0.90 piksler<sup>2</sup>/tidssteg

y-retning: 6.00 +- 0.82 piksler<sup>2</sup>/tidssteg

$D=15*0.1837^2*(6.9+6.1)/2/4= 0.8226$

$kB= 6*\pi*1e-21*0.8226*0.99/273= 5.6229e-23$

This is a factor 4 too large!

The image size is 2736 x 2192 which is the maximum frame size of the camera.

At this frame size the camera is not able to capture at 15fps even if it says so in the AVI file. The frame rate control of the pointgrey cameras are quite tricky. There are 3 places where the frame rate can be set, but only one of the three places really controls the frame rate!

## Conclusions

### Fate of this exercise

This exercise is very illustrative of some very basic principles in statistical physics and our understanding of matter. In periods when there have been no technical problems and the results were good the students of FYS2150 have liked it very much. It is also intimately linked to the content of two other courses: FYS2160 Thermal physics and FYS4715 Biological physics. I do not know who will use it in the future, but it will be used!

### Student reports

Because the cameras have several options in terms of frame rate and resolution the experiments are "vulnerable". Neither the camera software nor the microscope write to file what the exact settings are. If the students have not received exact instructions about all settings and made equally stringent note of them it is impossible to know what the cause of deviations from the literature value of  $k_B$  is due to. In general:

- If deviations are less than 30% the settings that were used were probably correct
- Slightly lower  $k_B$  may be due to measurements close to the glass surface where the effective viscosity is higher.
- Larger deviations are probably due to incorrect use of the formulae or mismatch between real frame rate or magnification and what was used in the calculations.

### Error estimates

The error estimates are too low. The deviations between experiments on the same sample are larger than the error estimates of each experiment. I am not sure why this is the case. There is no reason to believe that there are correlations between particles except of short duration between neighbouring particles. Inertia of the particles only last for microseconds, thus each increment of motion recorded

is surely uncorrelated with the previous. This calls for performing a large number of measurements on the same sample to analyse the statistics more carefully.

### **Lab text**

The lab text should be translated into English.

### **Software**

The basic tracking scripts (that perform the band pass filtering, segmenting and identifying the centroids of the particles in each frame and connecting positions into tracks) are old but good. Lots of new software have emerged the later years, many of the ones I have tried are certainly not better.

The scripts that the students use are a packaging to make the tracking scripts adapted to the exact student exercise. They were originally written by me more than 10 years ago and have been corrected and improved by others several times.

The cameras should be run by Micro-Manager (<https://micro-manager.org/>). This software saves information about camera settings and microscope magnifications etc. It runs with the same interface irrespective of which specific camera hardware is present.

I suggest that a student is employed this summer to

- Review available tracking software adapted to this exercise and report this to interested teachers.
- In collaboration with teachers decide which software to use
- Adapt a software "shell" script to adapt the software to the exercise.
- Install micro-manager to run with the cameras used in the lab