Evanescent light scattering
Optical Tweezers
Random Walk

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Statistical analysis of random walks can be used to measure small forces. This is demonstrated on colloidal particles in an aqueous solution. Optical tweezers were employed to apply controlled forces. Particle trajectories were recorded in all 3 dimensions using transmission light microscopy and total internal reflection microscopy.
Comparison of different analytical methods showed that the measured forces and diffusion coefficients matched expectations, demonstrating the ability to measure forces in the 10^{-15} newton range.

Available at www.github.com/leoole100/fp2.

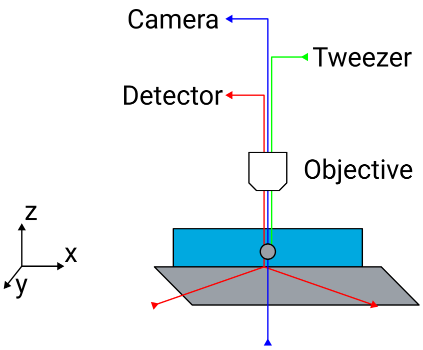

Figure 1 Schematic of the experimental setup. Optical tweezers in green, transmission light microscopy in blue and total internal reflection microscopy in red.

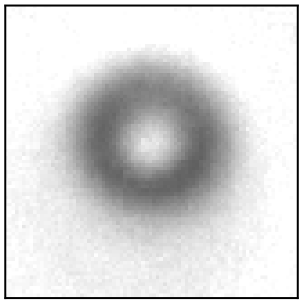

Figure 2 Transmission light microscopy of a observed particle. The radius of the particle is 14(2) px, equivalent to 1.86(27) µm.

1 Introduction

Using the statistics of the random walk small forces can be measured. This is demonstrated here on colloidal particles in an aqueous solution. Forces are applied using optical tweezers and the position is measured with transmission light microscopy and total internal reflection microscopy. Different statistical analysis are explored to determine the forces and diffusion coefficients from the measured trajectories.

2 Methods and Procedures

This experiment operates with light on small particles (in the range of 1 µm [1]) in an aqueous solution. This is shown in Figure 1 as the gray circle in the blue box.

Using a optical tweezers the particle can be trapped. This results in a 3-dimensional harmonic potential, which is proportional to the tweezers laser power. The laser power is continuous (in a used interval) with a unlabeled readout on the laser. This is value is henceforth referred to as *trap strength*.

To directly observe the particle *transmission light microscopy* was used, a blue LED was placed under the sample to illuminate the particle from below. This allows the particle position to measured in x and y direction.

Images with a resolution of 600×800px were recorded with a frequency of 10 Hz for 10 min by a CCD Camera. The magnification of the microscope was assumed to be 0.133 196 72 µm/px [1], this is the main systematic error of this measurement, as the scale changes with focus.

The images were normalized with a black (illumination off) and white (illumination on, particle not in frame) reference image, to remove the influence of dust on the imaging elements.

To determine the trajectory $\vec{r}(t)$ of the particle, an effective center of mass was calculated for each frame:

$$\vec{r}(t) = \iint \vec{r}\ \left(1 - I(\vec{r}, t)\right)^2 d\vec{r} \qquad (1)$$

To measure the position in z direction, *total internal reflection microscopy* was used: A laser beam is totally reflected at the glass-water boundary below the particle. The resulting evanescent field decays exponentially in the z direction with a characteristic length β that is determined by the angle of incidence of the laser beam. The particle scatters the light, depending on the field strength and therefore exponentially on the distance to the wall.

The scattered light, was measured with a sensitive photodetector with a frequency of 1 kHz for 15 min.

3 Results

3.1 Transmission Light Microscopy

In the first section of the experiment, the particles were observed using a transmission light microscope setup. A image of particle the used particle is shown in Figure 2. From this the radius of the particle was estimated to be 1.86(27) µm.

The density of the measured trajectories is shown in Figure 3 for different optical trap stiffnesses. The approximate effective region of the optical trap is drawn as a red circle.

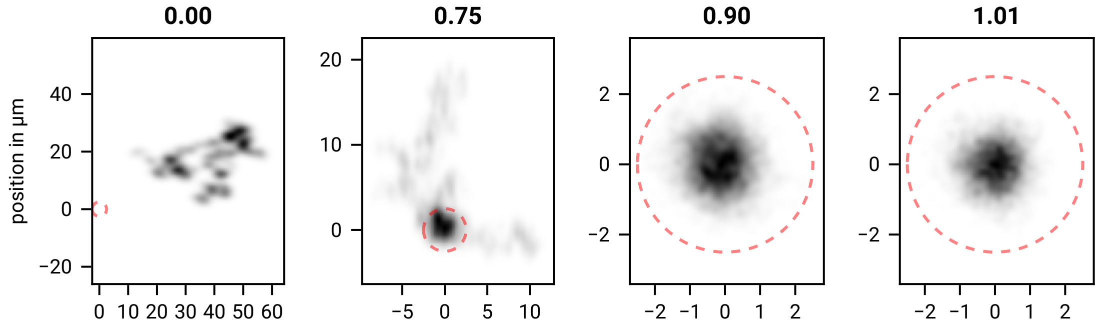

Figure 3 Density of recorded particle positions, grouped by optical trap stiffness. The red circle indicates the approximate radius of the optical trap 2.5 µm.

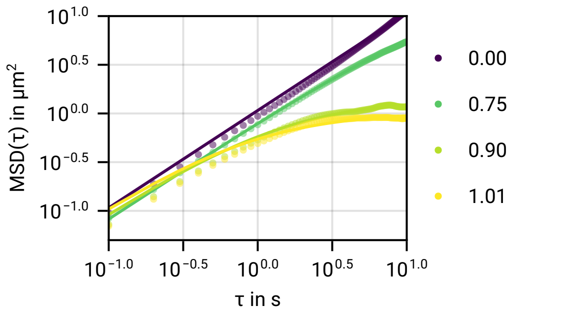

Figure 4 Mean Square Displacement for different optical trap stiffnesses, fit: Equation 3

For the trap stiffness of 0, the particle is free to wander around, for the higher stiffnesses (0.90, 1.01) the particle is mostly confined to the trap.
For a weak trap (0.75) the particle is still mostly confined to the trap, but can escape the linear trap region and randomly wander around, until it is recaptured by the trap. This happened multiple times during the shown measurement.

Mean Square Displacement

A method to describe the trajectory of a random walk is the mean square displacement (MSD) [2]. This is defined as the average of the squared distance of the particle from the starting point [2]:

$$\mathrm{MSD}(t) = \frac{1}{N}\sum_{i}^{N}\left(x_i(t) - x_i(0)\right)^2 \tag{2}$$

Here a different implementation using the autocorrelation of the velocity was used, to achieve a more stable result. This was implemented by [3]. The resulting MSD for different optical trap stiffnesses is shown in Figure 4.

The MSD can be described by the following combination model:

$$\frac{1}{\mathrm{MSD}(\tau)} = \frac{1}{D_0\tau} + \frac{1}{\mathrm{MSD}(\infty)} \tag{3}$$

For a free particle the $\mathrm{MSD}(\infty) = \infty$ and the MSD grows linearly with D_0 over time [1, 2].
The diffusion Coefficient D_0 estimated using the fit is shown in Table 1. With the uncertainty from the fit, the systematic error of the magnification has a square influence and is not included. The measurement is equivalent to the theoretical value of $D_0 = \frac{k_B T}{6\pi\eta r} = 0.122(18)\,\mu\mathrm{m}^2/\mathrm{s}$ [1], with $r = 1.86(27)\,\mu\mathrm{m}$ and $\eta = 0.955\,\mathrm{mPa}\cdot\mathrm{s}$ [4]. The error in the magnification of the microscope does additionally increase the uncertainty in the measurement.

For a confined particle the MSD reaches a plateau at $\mathrm{MSD}(\infty)$ [1]. This happens for the higher trap stiffness (0.90, 1.01). From this the effective spring constant of the optical trap can be estimated $k_{\mathrm{MSD}(\infty)} = \frac{2k_B T}{\mathrm{MSD}(\infty)}$ [1], the values are shown in Table 1 and Figure 6, with uncertainty from the temperature and the statistical error from the fit. The systematic error from the magnification has a order of 2.
For the lower measured spring stiffness (0.75), the MSD does not reach a plateau, as it partially escapes the trap and wanders around (see Figure 3), behaving like a free particle.
Therefore this procedure is not adequate for such low spring stiffnesses.

Potential

A more detailed analysis can be done by looking at the distribution of the particle positions. For this

Stiffness	D_0 in µm^2/s	$k_{\mathrm{MSD}(\infty)}$	k_V
0	0.107 28(93)	NA	0
0.75	0.077 93(3)	NA	1.2743(64)
0.90	0.0678(24)	6.471(62)	6.6718(88)
1.01	0.053 84(8)	8.968(23)	8.514(17)

Table 1 Estimated diffusion constant and spring constant in nN/m.

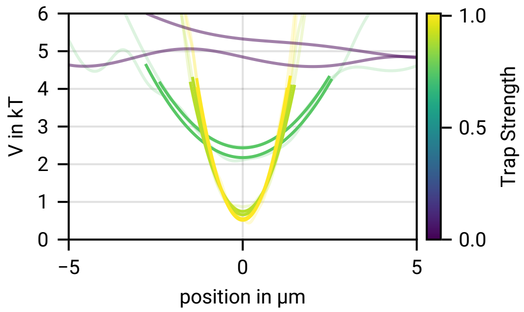

Figure 5 Measured Potential, with quadratic fit.

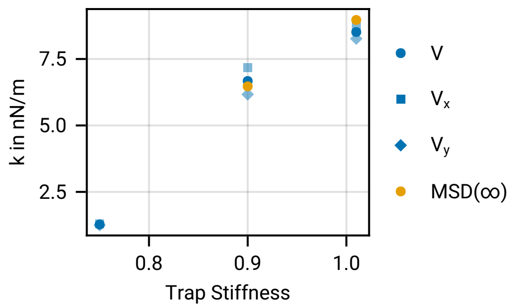

Figure 6 Differently measured spring constants.

the probability density function (PDF) of the particle positions has to be estimated. This is done using kernel density estimation (KDE) with a Gaussian kernel [5]. The resulting 2-dimensional PDF is shown in Figure 3.
As the density is small for large parts of the explored 2d space, the data is reduced by aggregating along the image coordinates x and y. As the Potential should by rotationally symmetric, the data from the x and y axis should not significantly deviate. A factor of $\sqrt{2}$ is to convert x, y to a effective r.

Using the Maxwell Boltzmann relations [1], the potential at a position p can be calculated from the PDF:

$$V(p) = -\frac{\log \mathrm{PDF}(p)}{k_B T} \tag{4}$$

The resulting potential is shown in Figure 5 for

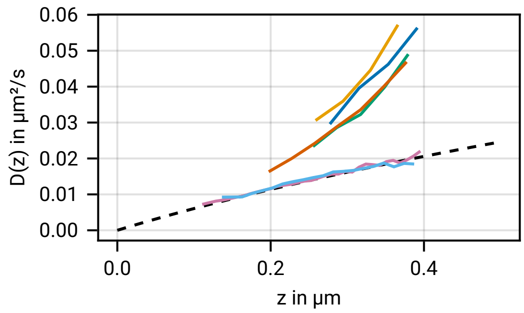

Figure 7 Estimated diffusion coefficient for different measurements. With a theoretical model corresponding to Equation 7.

different trap stiffnesses. The parts of the measurements with a PDF$(x) > 0.05$ are used for a quadratic fit, to estimate the effective spring constant. The results are shown in Figure 6 and Table 1. Here the systematic error from the magnification is not included, it influences the result in order 2, which is the same as in $k_{\mathrm{MSD}(\infty)}$. Assuming that $k_{\mathrm{MSD}(\infty)} = k_V$ a error of 5 % is unaccounted for.

For the lower spring constant of 0.75, the potential begins quadratically, but flattens after approximately 2.5 µm. This is due to the limited size of the trap, but the spring constant can still be estimated.

This method allows for the measurement of a spring constant as low as 0.9011(45) nN/m, which means over the used length scales that forces in the order of 1 fN can be measured.

3.2 Total Internal Reflection Microscopy

Note: Some references in [1] led to nowhere, which caused some confusion.

A promising method to study even small forces is Total Internal Reflection Microscopy, as the controllable exponential decay of the evanescent field allows for precise measurements of the particle position in the z direction.

Position and Diffusion Coefficient

From the measured scattering intensity the z distance between particle and wall can be calculated using the formula [1]

$$z = \beta\left(\log(I_0) - \log(I)\right) \tag{5}$$

where I is the scattered light intensity and I_0 is the maximum possible intensity, i.e. $I(z = 0)$, when the particle touches the glass surface. β is the characteristic length of the evanescent field which can be calculated from the angle of incident of the reflected beam.

The z-dependent diffusion coefficient can be calculated from the position changes Δz in a given z interval. For Brownian motion these should be normally distributed with

$$\sigma^2 = 2 \cdot D \cdot \Delta t \tag{6}$$

where D is the diffusion coefficient [1]. In reality there is an additional component owing to measurement uncertainty which does not depend on Δt. To eliminate it, the derivative with respect to Δt is estimated by calculating Equation 6 for different Δt.

Close to a wall, the diffusion coefficient can be modeled [1] by the equation

$$D(z) = \frac{D_0}{\left(\frac{R}{z}\right) + 0.2 \log\left(\frac{R}{z}\right) + 0.9712} \tag{7}$$

with the radius R of the particle and the free diffusion coefficient D_0 by the previously measured value.

To compute z using Equation 5 the intensity I_0 needs to be estimated. For this, the steps Equation 5 and Equation 6 are calculated iteratively with different I_0 trying to match Equation 7. The distance dependent diffusion coefficient $D(z)$ obtained using this method is shown in Figure 7.

Two measurements yield results matching the theoretical model. For the other measurement series, four of them shown here using $I_0 = 1$, it was not possible to match the theoretical model from Equation 7 adjusting I_0, and even adjusting β did not change that. The provided MatLab script yields the same results.

The success of the I_0 estimation procedure does not depend on the trap stiffness nor the set β value.

To investigate this further, the distributions of the measured variables are shown in figure Figure 8 for a measurement where the procedure was successful and unsuccessful. The measurements were conducted with the same trap stiffness of 1.5 but different β values.

The graphs show the estimated PDF for the measured intensity I, its derivative as well as the PDF of z and its rate of change. As only in one of the two measurements I_0 could be estimated, the other one is drawn using $I_0 = 1$.

stiffness	effective gravity in fN	κ^{-1} in nm
0.70	25.9(26)	260(27)
0.75	13.5(13)	84.2(85)
0.80	29.1(29)	47.3(38)
0.97	46.4(46)	45.6(42)

Table 2 Fit Parameters from the potential.

The derivative of the intensity shows the limited resolution of the sensor. The speed distribution dz/dt is expected to be Gaussian. The peak at 0 and the neighbouring valleys are a result of measurement quantisation: The 0-bin of the histogram collects all values around it and no points fall into the neighbouring bins.

From the distributions no significant differences were found that could explain why the I_0 estimation procedure failed for some measurements.

Potential

From the PDF the potential can be calculated using Equation 4 like in the previous section. The resulting potentials for different optical trap stiffnesses are plotted in Figure 9.

As I_0 could not be estimated the potentials are shifted to center around the the mode of z.

The shape of the potential can be modeled as a combination of a effective gravitational force and a Debye screening:

$$P(z) = A\, e^{-\kappa z} + z\, F_{g,\text{eff}} \tag{8}$$

The characteristic length κ^{-1} of the potential close to the wall is called the *Debye Screening Length*. $F_{g,\text{eff}}$ is the effective force of gravity acting on the particle inside the solution. It is driven by the actual gravitational force but is reduced by buoyancy and increased by the force of the optical trap.

For each measurement, the estimated potential was fitted to Equation 8 to obtain κ and $F_{g,\text{eff}}$. The results are listed in Table 2. The uncertainty is a combination of the statistical uncertainty from the fit and a uncertainty in β of 10 %, corresponding with a 2 mm uncertainty in positioning of the illumination laser. The error in I_0 has no influence, as it is absorbed in the scaling and translation of the model.

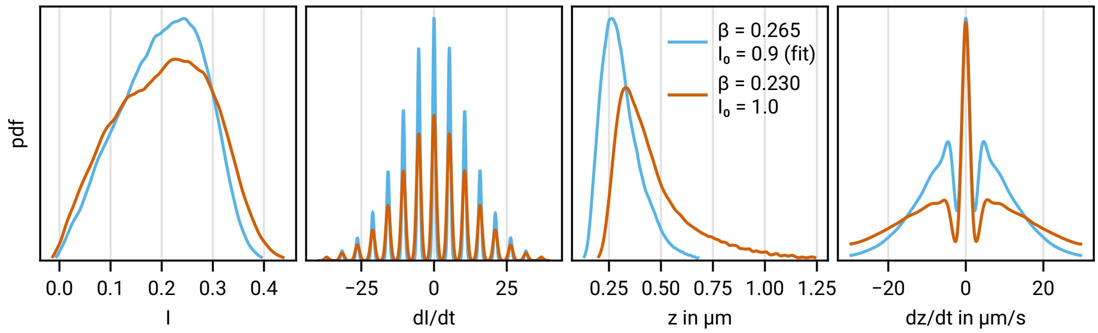

Figure 8 Distribution of values for two measurements for the same trap stiffness 1.5 but with different β. Same colors as Figure 7.

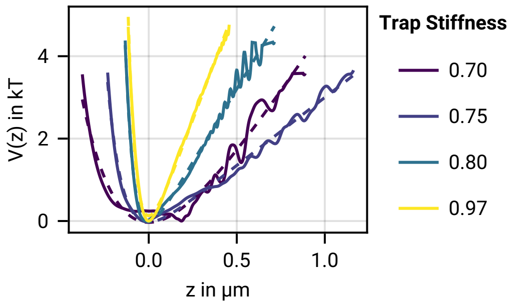

Figure 9 Measured Potential for different optical trap stiffnesses.

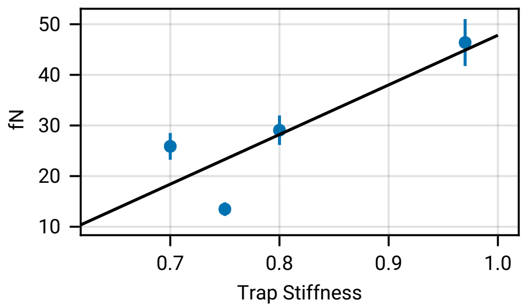

Figure 10 Linear fit of effective gravity for different trap stiffness values.

Effective Gravity The measured effective gravitational force lies in the regime of 10 fN. The linear regression of $F_{g,\mathrm{eff}}$ with respect to trap stiffness shown in Figure 10 can be used to find the mass of the particle.
For this the 0 of the real trap stiffness is needed, which was estimated to be at 0.62(10) units of the non linear trap stiffness. From this the effective weight of the particle can be estimated to be 10(10) fN. The uncertainty is inconclusively high, as the linear regression is not very good.

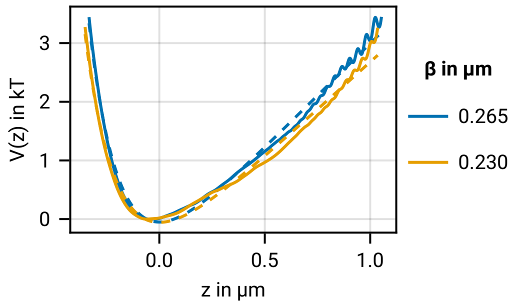

Figure 11 Measured potential at same trap stiffness 1.5, but different illumination angles resulting in different β.

Influence of β The influence of the angle of incidence of the laser beam on the potential was investigated. This should not have a influence on the potential. This is verified by the results shown in Figure 11.

4 Discussion

The measurements based on the transmission light microscopy yielded good results: The diffusion coefficients are in agreement with the theoretical prediction. The effective spring stiffness measured using the MSD and the Maxwell-Boltzmann relation are similar and allow measurements of forces in the 1 fN range.
Limitations of the different analysis techniques were nicely illustrated by observing of a particle that escaped the trap.

The part based on the total internal reflection microscopy were less successful. The measured intensities were small and therefore prone to quantisation errors due to limitations of the measurement equipment. Although the reason for the non convergence of the I_0 estimation procedure could not be found, the results demonstrate that this is a feasible method to measure forces at least in the range of 10 fN.

References

[1] *Evanescent light scattering experiment — Instructions*. Forgeschrittenen Praktikum — University Konstanz. 2023.

[2] Wikipedia. *Mittlere quadratische Verschiebung — Wikipedia*. 2023. URL: https://de.wikipedia.org/w/index.php?title=Mittlere_quadratische_Verschiebung&oldid=234078066.

[3] Riccardo Foffi. *MeanSquaredDisplacement.jl*. 2023. URL: https://github.com/mastrof/MeanSquaredDisplacement.jl.

[4] Anton Paar. IAPWS. 2008. URL: https://wiki.anton-paar.com/en/water/.

[5] JuliaStats. *KernelDensity.jl*. 2024. URL: https://github.com/JuliaStats/KernelDensity.jl.