

# Evanescent light scattering experiment

May 2023

## 1 Introduction

In this experiment, a method for the measurement of very small forces between a micrometer-sized colloidal particle and a wall is studied. For macroscopic objects, there are many different methods to measure forces. Perhaps the simplest example is the spring balance, the elongation of which can be traced back to the weight of an object hanging on it. With the increasing miniaturization process of mechanical components for the investigation of microscopic objects such as colloids, forces in the nano, pico, and femtonewton domains are relevant. Even in the measurement of such very small forces, the principle of the spring balance is still used, but it is necessary to build more sensitive systems. Such sensitive device for force measurements is for example the Atomic Force Microscope (AFM), which has a resolution of a few piconewtons [1]. In the AFM, the spring balance is realized by the mean of a cantilever, to which a tip or a colloidal particle is which interacts with a surface that is attached. The force resolution is limited mainly by the thermal noise of the cantilever used for the measurement.

In order to achieve an even higher force resolution, the thermal noise of particles can be analyzed. For individual colloidal particles interacting with a glass wall, the TIRM method (Total Internal Reflection Microscopy) offers the possibility to determine the trajectory of the particle with a spatial resolution of the order of nanometers, and to statistically evaluate it and to determine interaction forces on the order of femtonewtons. The trajectory, i.e; the temporal course of the distance between the particle and the wall is determined by means of the evanescent light scattering method. The particle is suspended in an aqueous solution so that its interaction with the wall is altered by changes in the solvent, e.g. by the addition of salts.

A characteristic of colloidal particles is their Brownian motion due to random disturbances with the solvent molecules. This can be described by the Langevin equation, a stochastic differential equation. It should be noted that the particle is located in a potential pot due to its interaction with the wall and with external fields (gravitation, light forces of an optical tweezer), and that the diffusion coefficient is location-dependent due to the hydrodynamic interaction with the wall.

## 2 Prerequisites

This experiment requires an understanding of the investigated interactions, the measuring principle and the data evaluation. Important aspects are in particular:

1. Colloidal interactions

- (a) Gravitational force
  - (b) Electrostatic double-layer force
  - (c) Van der Waal's force
  - (d) Light force from optical tweezer
2. Dynamics of colloidal particles
- (a) Brownian motion and diffusion coefficient
  - (b) Langevin equation
3. TIRM method
- (a) Total internal reflection, evanescent field, penetration depth
  - (b) Light scattering from evanescent field
  - (c) Boltzmann distribution
  - (d) Evaluation of data for MSD
  - (e) Evaluation of data for potential

### 3 Literature review

For preparation, you can help yourself with the following publications:

- An overview article on the TIRM measurement method by D.C. Prieve [6], the inventor of the method. Here, the measurement method, the standard evaluation, and the interactions of a colloidal particle with a wall are discussed.
- The article [5] deals with the hydrodynamic evaluation for the distance-dependent diffusion coefficient. Here the Langevin equation is at least discussed at a phenomenological level. An alternative detailed discussion can be found in [7].
- A general introduction to the physics of the soft condensed matter, to which the colloid physics is added. Brownian motion and interactions of the colloids are described [4] and [3].
- Most of the basic knowledge of this experiment is also dealt with in the dissertation by Laurent Helden [2].

### 4 Safety instructions

The black box at the experiment, which contains the laser of the optical tweezers and the detection optics has to be kept closed for protection against laser radiation. One must never look directly into a beam. When changing the measuring cell, the TIRM detection laser (red) must be switched off, so that no reflections are produced when the measuring cell holder is lowered. The laser for the optical tweezers (green) must be switched off before switching the cell (current for pump diodes = 0). During the experiments, laser protection goggles or adjustment goggles must be used. The adjustment glasses in the test room weaken the laser radiation by a factor  $\leq 100$  so that they provide the same protection as laser protection goggles for the lasers used ( $< 100$  mW output power). However, you can still see the beam weakly.

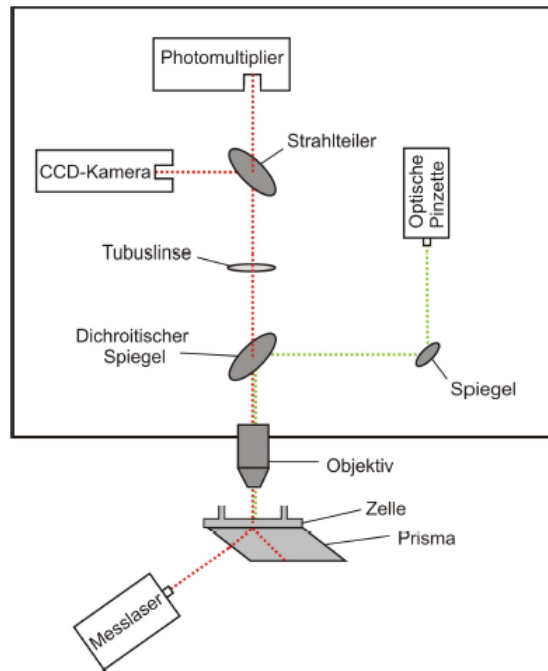


Figure 1: Schematic diagram of the setup

## 5 Procedure

Before each measurement, the setup must first be adjusted. At the beginning of the experiment, you will receive detailed instructions from the assistant. So here is just a brief list of the tasks to be done in which order.

1. Adjust the focus: With the uEye camera software, adjust the position of the objective such that the particles at the bottom of the sample cell is in the focal plane.
2. Select the correct region of interest: Find an isolated particle with no other particles in the field of view and bring it close to the trap position and adjust the region of interest. This is required to prevent additional procedures to filter out unwanted particles while tracking. The sample can be moved in the X-Y plane by using the screws at the right of the sample.
3. Once you have a particle in focus, start recording the motion of that particle with the green laser off. Repeat this again with trap on for different trap stiffness (laser power). These videos are tracked to obtain the particle position at each time frame and MSD is calculated from these trajectories.
4. Determination of the critical angle and adjustment of the penetration depth: By moving the swivel arm with the micrometer screw, it is possible to search the angle at which the transmitted beam just disappears. A defined deviation from the critical angle is achieved by clamping a spacer (aluminum blocks of different thicknesses) between the micrometer screw and the swivel arm. The penetration depth of the evanescent field is given in a table as a function of the thickness of the spacer.

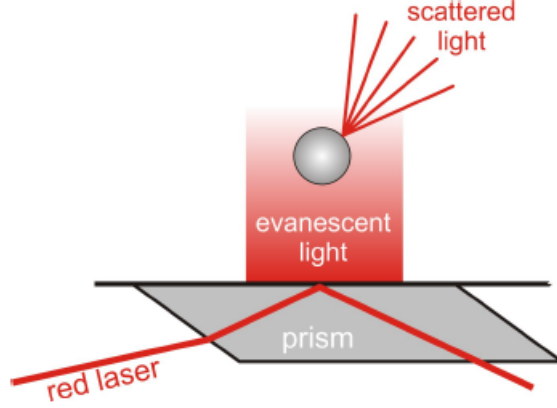


Figure 2: Illustration of evanescent light scattered by a colloidal particle.

5. Fine adjustment of the measuring laser: In order to optimize the scattered intensity, the measuring laser has to be positioned in such a way that the particle is located laterally in the center of the laser beam. Since the particle fluctuates, the best is to adjust when the particle is pressed against the surface by strong light forces.
6. After adjustment, the actual measurement can be started. To record the data points, the output from the photomultiplier is connected to an NI card. The Matlab code-named 'record-tweezer.m' can be used to record the data. A measurement duration of 20 minutes corresponds to 1 200 000 data points. This number must be entered at the bottom left of the program.

## 6 Analysis

### • Determination of potential form:

The scattering intensity  $I(z)$  depends, in the following manner, on the distance  $z$  between the particle and the wall:

$$I(z) = I_0 \exp(-\beta z) \rightarrow z = -\beta^{-1} \ln\left(\frac{I_z}{I_0}\right) = -\beta^{-1} \ln I_z + \beta^{-1} \ln I_0 \quad (1)$$

Where  $\beta^{-1}$  is the penetration depth of the evanescent field and  $I_0$  is the scatter intensity that results when particles and wall are in direct contact. Without knowing this constant, only relative distances can be determined, since  $z_0$  can not be computed. The absolute distance is determined hydrodynamically in the second section of the evaluation; here  $I_0 = 10$  is assumed arbitrarily. The Boltzmann equation combines the distance-dependent probability distribution  $p(z)$  with the interaction potential  $V(z)$  between particle and wall:

$$p(z) = p_0 \exp\left(-\frac{V(z)}{k_B T}\right) \rightarrow \frac{V(z)}{k_B T} = -\ln\left(\frac{p(z)}{p_0}\right) \quad (2)$$

The distance-dependant probability distribution is calculated using (1), and can then be converted into an intensity probability distribution  $N(I)$ :

$$p(z) = N(I) \frac{dI}{dz} = -\beta N(I) I(z) \quad (3)$$

$N(I)$  is obtained directly from the measured data as a histogram. The resulting potential is then:

$$\frac{V(z)}{k_B T} = -\ln \left( \frac{-\beta N(I) I(z)}{p_0} \right) = -\ln(N(I) I(z)) + \ln \left( \frac{-p_0}{\beta} \right) \quad (4)$$

Before creating the histogram, the background must first be subtracted from the measured data. This corresponds to the scatter signal in the absence of the particle. For this purpose, the particle can be pulled out of the field of view with the optical tweezers and the background at the measuring position can be determined directly (approximately 10 to 30s measurements averaged). The number of bins in the histogram determines the number of potential values that are calculated. The error on the calculated potential value decreases with the increasing number of counts per bin. Approximately 100 bins represent a good compromise between a small error of the individual potential values on the one hand and good local resolution on the other. Eq. (4) potential already has the correct shape, but not yet the correct absolute distance from the surface.

- **Hydrodynamics evaluation:** For the determination of the potential shape, only the probability distribution has so far been used. However, much more information is available in the measurement data. An analysis of the dynamics of the measured data provides information on the distance dependence of the diffusion coefficient and also allows the determination of the absolute distance. The 3D diffusion coefficient (far from the surface),  $D_0$  is described by the Stokes-Einstein equation:

$$D_0 = \frac{k_B T}{6\pi\eta R} \quad (5)$$

Where  $\eta$  is the viscosity of the liquid and  $R$  corresponds to the particle's radius. In the vicinity of a wall, where the liquid molecules cannot move (stick boundary conditions), the diffusion coefficient becomes distance-dependent and anisotropic. The distance-dependent diffusion coefficient for diffusion perpendicular to the wall  $D_\perp$  was calculated analytically by Brenner as an infinite series [2]. This series can be approximated very well for small distances  $z \ll R$  to:

$$D_\perp = \frac{D_0}{\left(\frac{R}{z}\right) + 0.2 \ln \left(\frac{R}{z}\right) + 0.9712} \quad (6)$$

Considering the solution of the Langevin equation for a spherical particle near a wall, the distance-dependent diffusion coefficient can also be determined from the measured trajectory of the particle [4]. To do this, proceed as follows:

1. The measured intensity data are converted into distances, whereby an arbitrary value is initially assumed for the scatter intensity at the contact of particle and wall ( $I_0$  in (3)). The maximum input voltage of the A/D card of 10 V is for example a realistic starting value for  $I_0$ .
2. For the analysis of the dynamics, the measured trajectory is divided into about 20 intervals  $a_j$ . For each  $a_j$ , a histogram of the distance changes  $\Delta z_i$  is generated within a certain time interval  $t$ . That under the condition that the  $i$ -th distance value  $z_i$  measured in the trajectory is in the interval  $a_j$ ,  $z_i = z_i + k$   $z_i$  is calculated and entered into the histogram. The time

interval  $t = k\delta t_{mess}$  is necessarily an integer multiple of the measurement interval  $\delta t_{mess}$  and  $k = 1, 2$ , in order not to average over too long time intervals.

Neglecting the effects of the interaction potential (curvature), a Gaussian distribution of the distance changes is to be expected, whose width is  $\sigma_{z_i, \Delta t} = \sqrt{2D_{z_i}\Delta t}$ . In principle,  $D_{(z_i)}$  can already be determined from the fit parameter  $\sigma_{(z_i, t)}$  for a certain  $\Delta t$ . However, a smaller measurement error is obtained if  $\sigma_{(z_i, t)}$  is plotted for different time intervals  $t$ ,  $\sigma_{(z_i, t)}$  is plotted against  $\Delta t$ , and  $D_{(z_i)}$  is determined from the slope [4].

3. The unknown parameter  $I_0$  is now adapted such that the distance dependent diffusion coefficient  $D_z$  coincide with the theoretical predictions from (8) ( $\eta$  and  $a$  are known). From this follows the correct absolute particle wall distance for the potential.

In the practice of the experiment, the described evaluation is implemented by a predetermined MATLAB routine, in which only  $I_0$  has to be adapted as a parameter.

## 7 Tasks

The measurements are made on polystyrene particles of  $4.3\mu\text{m}$  diameter, suspended in a water solution. The measuring frequency is 1 kHz, so that the measured data have a time interval of  $\delta t_{mess} = 1\text{ms}$ .

- **Determination of the diffusion coefficient**

The 2D Brownian motion of a particle is video-recorded during 15-20 min. Several information can be extracted from the analysis of the motion :

1. First, use the given tracking (track-circle.m) program to obtain the trajectory of the particle.
2. Calculate the mean square displacement (MSD-calculation.m). Discuss any deviation from theory.
3. Calculate the experimental diffusion coefficient, slope of the MSD vs time curve, and compare it to the theoretical one.

- **Determination of the influence of the optical tweezers**

You will now study how an optical tweezers created by laser light is confining the 2D motion of the particle.

1. Do 3 measurements with the following laser voltage, for example: 0.55; 0.70; 0.85
2. Compare the different trajectories. Do you see a difference between them?
3. Plot the different MSDs. From this, extract the stiffness of the optical trap.

- **Distance dependence of the diffusion coefficient and absolute distance**

The Brownian motion in the  $z$  direction of a colloid is recorded for 15- 20 minutes at a penetration depth of about 200nm. In this case, the strength of the optical tweezers has to be chosen in such a way that the colloid can not diffuse laterally from the trap while it moves in the vertical direction over the widest possible distance range.

For the above measurement, the distance-dependent diffusion coefficient is determined and the absolute particle wall distance is determined by optimization of  $I_0$  by comparing the theoretical and experimental diffusion coefficients. MATLAB script FP-TIRM-z0.m is used to determine the  $I_0$  value. Deviations from the theoretical prediction are to be discussed.

- **Determination of the potential form**

1. First, the measured scatter intensity  $I(t)$  is converted to  $z$  distances using the  $I_0$  value obtained from the previous script.
2. histogram with approx. 100 columns is created from the measured data for both intensity and  $z$  positions.
3. Using the Boltzmann distribution, the potential is calculated and plotted from the probability distribution of the  $z$  positions (FP-TIRM.m).

- **Measure of the light force**

With the optical tweezers, very precise forces can be exerted on the probe particles. The light intensity along the beam direction, i.e. perpendicular to the surface, depends linearly on the power of the laser used for the optical tweezers. By changing the laser power, the forces can be varied within the range of less than 10 femtonewtons.

1. The interaction potentials are measured with at least 5 different powers of the optical tweezers. The lowest power is to be selected in such a way that the lateral gradient forces of the optical tweezers keep the particle just in the optical trap. At the highest power of the optical tweezers, the particle should only fluctuate about 150 nm in the potential well.
2. Then, the free particle's motion (i.e., when the optical tweezers are turned off) is measured. In this case, the particle diffuses laterally within the measuring cell. In this case, the inner third of the screen should not be left, since otherwise the detection efficiency decreases and the measurement data falsifies. If the particle diffuses too far, it can be moved back into the center by carefully moving the measuring cell during the measurement.
3. The measured potentials are fitted with consideration of double layer forces and a linear contribution of light and gravitational force. The slope of the linear component is plotted as a function of the laser power. Results of the measurements with optical tweezers are extrapolated to for  $P=0$  and compared with both the measurement without optical tweezers and the weight force calculated from density difference and particle volume. ( $\rho_{Si} = 2gcm^{-3}$ ,  $\rho_{water} = 1.0gcm^{-3}$ )

## References

- [1] William A Ducker, Tim J Senden, and Richard M Pashley. "Direct measurement of colloidal forces using an atomic force microscope". In: *Nature* 353.6341 (1991), pp. 239–241.
- [2] Laurent Helden. "Untersuchung von Partikel-Wand-Wechselwirkungen mit evaneszenter Lichtstreuung". PhD thesis. 2002.
- [3] Jacob N Israelachvili. *Intermolecular and surface forces*. Academic press, 2011.
- [4] Richard AL Jones. *Soft condensed matter*. Vol. 6. Oxford University Press, 2002.

- [5] Ratna J Oetama and John Y Walz. “A new approach for analyzing particle motion near an interface using total internal reflection microscopy”. In: *Journal of colloid and interface science* 284.1 (2005), pp. 323–331.
- [6] Dennis C Prieve. “Measurement of colloidal forces with TIRM”. In: *Advances in Colloid and Interface Science* 82.1-3 (1999), pp. 93–125.
- [7] Hannes Risken. *The Fokker-Planck equation, volume 18 of Springer Series in Synergetics*. 1989.