Evanescent lightfield

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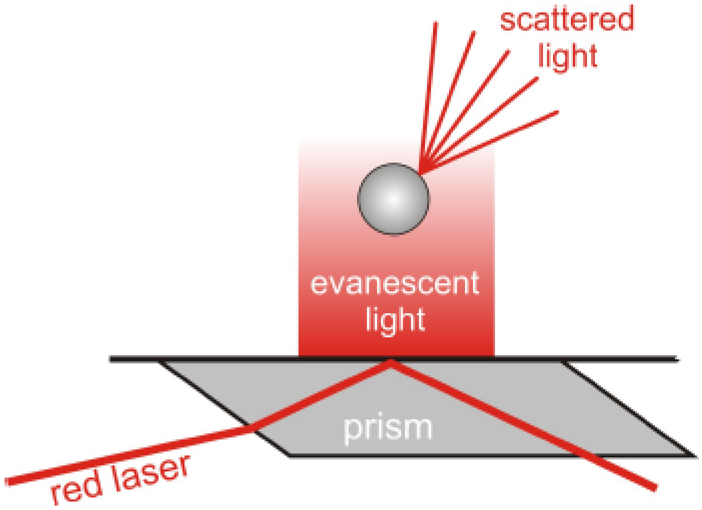


Abstract

In this experiment, a method for the measurement of very small forces between a few micrometers large colloidal particle and a wall is presented. For such 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, or in 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 can still be 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 attached, which then interacts with the surface. The force resolution is limited mainly by the thermal noise of the cantilever used for the measurement.

In order to achieve an even higher order of force resolution, this thermal noise can be analyzed. For individual colloidal particles interacting with a glass wall, the TIRM method (TIRM=Total Internal Reflection Microscopy) offers the possibility to determine the trajectory of the particle with a spatial resolution of a few nanometers, to statistically evaluate it and to determine interaction forces in the range of a few femtonewtons.

The trajectory, ie the temporal course of the distance between particle and wall, is determined by the mean of an evanescent light scattering on a single, spherical micrometer-sized particle in the TIRM method. The particle is suspended in aqueous solution so that its interaction with the wall is altered by changes in the solvent, e.g. by the addition of salts.

Colloidal particles are described by the Brownian motion ie the interaction between the solvent molecules and the particle. This can be described by the Langevin equation, a stochastic differential equation. It should be noted that the particle is located in a potential well 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.

1 Required knowledge

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

- Colloids and interactions of colloidal particles
  - Gravitation force
  - Electostatic double layer force
  - Van der Waals force
  - Light force with an optical tweezer : gradient force and radiation pressure
- Dynamic of a colloidal particle
  - Brownian motion, diffusion coefficient
  - Brownian motion close to a wall, location-dependant diffusion coefficient, correction from Brenner [2]
  - Langevin equation (overdamped, with potential term and location-dependent diffusion)
  - Solution of the Langevin equation, drift term and diffusion term
- TIRM method
  - Total reflexion, evanescent fields, critical angle and penetration depth
  - Light scattering from evanescent waves, intensity-distance behavior
  - Boltzmann distribution
  - Data evaluation for potentials: raw data, distance histogram, potential curve
  - Data evaluation for diffusion coefficients: trajectory, histogram of the position changes, determination of diffusion and drift from the distribution, dependence on the time interval

2 Literature for preparation

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

- An overview article on the TIRM measurement method by D.C. Prieve, the inventor of the method [3]. Here, the measurement method, the standard evaluation and the interactions of a colloidal particle with a wall are discussed.

- The article [4] 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 [5].

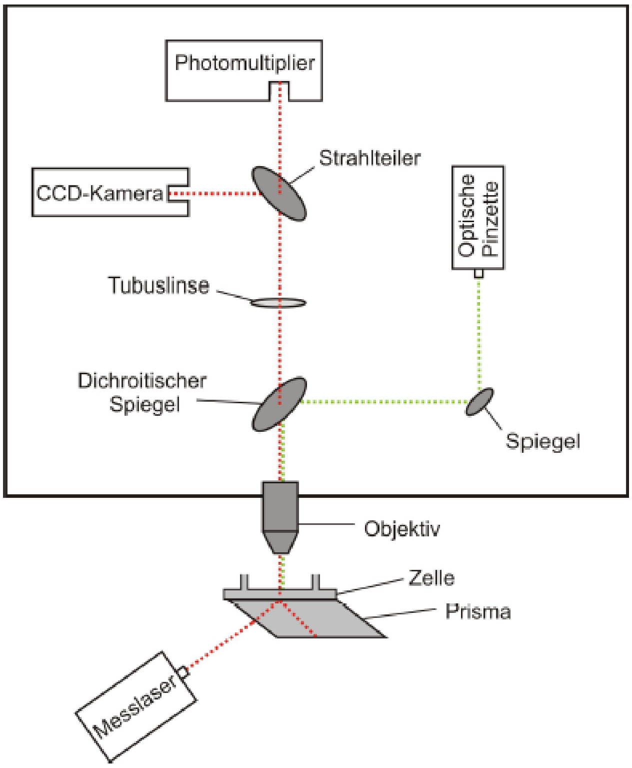
- A general introduction to the physics of the soft condensed matter, to which the colloid physics is added, can be found in [6] or [7]. Brownian motion and interactions of the colloids are described.

- Most of the basic knowledge of this experiment are also dealt with in the dissertation by Laurent Helden [8].

3 Safety instructions

The black box at the experiment, which contains the laser for 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 ≥ 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.

4 Experimental setup



A measuring laser of wavelength $\lambda = 658nm$ (red), with an output power of 25 mW is totally reflected into a prism. This laser is mounted on a swivel arm so that the angle of incidence can be precisely adjusted. The measuring cell, a Helma cell consisting of a glass cuvette with 2 closable nozzles for filling, is located on the prism. The liquid layer within the measuring cell has a thickness of $200\mu m$ and a volume of approximately $150\mu L$. The cuvette is filled with an aqueous, highly dilute dispersion of polystyrene particles with a diameter of $d = 4,28\mu m$. The position fluctuations of one of these particles is examinated. The prism and measuring cell are mounted on a 2D translation platine so that the measuring position within the cell can be selected.
Above the measuring cell, there is an optical microscope in an aluminum housing. The microscope optics consist of a 50x objective with NA = 0.55 and a lens tube. A 10x lens can also be used to locate the particles. If a particle scatters light from the evanescent field of the measuring laser in the microscope, a part of this light is decoupled via a beam splitter and sharply imaged with a CCD camera. This allows to select the position in the measuring cell and the particle. The intensity of the other part of the scattered light is detected by a photomultiplier and recorded in the measuring

computer via a measuring amplifier and an A / D converter card.
For the optical tweezers, the laser's wavelength is $\lambda = 532nm$ (green) with a variable output power of maximum 100mW. It is coupled into the detection beam path by means of a telescope optics (not shown on the figure) and a dichroic mirror. The beam divergence of the laser is adjusted via the telescope in such a way that it is precisely focused by the microscope objective in the image plane of the microscope. This laser focus functions as an optical tweezer.

5 Procedure

- **Adjustment before each measurement**
  Before each measurement, the setup must first be adjusted. At the beginning of the Praktikum, you will receive detailed instructions from the assistant. So here is just a brief list of the tasks to be done :

  1. Adjust the focus:
     With the program 'VirtualDub' the image of the CCD camera is displayed on the measuring computer. A z-platine allows you to change the distance between the objective and the measuring cell. The focus plane is then adjusted so that the particles are focused on the bottom of the measuring cell. When the setting is correct, you can also see the focus of the optical tweezers on the measuring plane. (You can also use it to find the right image plane).

  2. Select the measuring position: Check for particles and make sure there are no other particles or impurities in the environment. The measuring laser has to be positionned in such a way that the evanescent fiels and the frontal focal plane of the objective coincide roughly (when the particle starts to scatter light). This can be done by tuning the to displacement-screws on the measuring laser.

  3. Determination of the critical angle and adjustment of the penetration depth:
     By moving the swivel arm with the micrometric 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 thickness) 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.

4. 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.

- **General information on TIRM measurement**
After adjustment, the actual measurement can be started. To record the data points, the program 'TIRMGetHisto' is used. Under the 'Acquisition Settings' item, the measurement frequency is set to 1 kHz. A measurement duration of 20 minutes corresponds to 1 200 000 data points. This number (at least 20) must be entered at the bottom left of the programm. At the end of the measurement, the data must be stored manually. To do this, the assistent will create a folder with your group number.

6 Evaluation

- **Determination of the potential form**
The scattering intensity $I(z)$ depends, in the following manner, on the distance z between the particle and the wall:

$$I(z) = I_0 \exp(-\beta z) \longleftrightarrow z = -\beta^{-1} \ln\left(\frac{I_z}{I_0}\right) = -\beta^{-1} \ln I(z) + \underbrace{\beta^{-1} \ln I_0}_{z_0} \tag{1}$$

Where β^{-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 arbitraily. The Boltzmann equation combines the distance-dependent probability distribution $p(z)$ with the interaction potential $V(z)$ between particle and wall:

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

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

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

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

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

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 measurement 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 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.

- **Hydrodynamic 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:

$$D_0 = \frac{k_B T}{6\pi\eta R} \tag{5}$$

Where η is the viscosity of the liquid and R corresponds to the particule's radius. In the vicinity of a wall, where the liquid molecules can not 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 $z < R$ to:

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

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 (I0 in (1)). The maximum input voltage of the A/D card of 10 V is for example a realistic starting value for I0.

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 Δz_i is generated within a certain time interval Δt. 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 δt_{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, $D_{(zi)}$ can already be determined from the fit parameter $\sigma_{(z_i,t)}$ for a certain Δt. However, a smaller measurement error is obtained if $\sigma_{(z_i,t)}$ is plotted for different time intervals Δt, $\sigma^2_{(z_i,t)}$ is plotted against Δt, and $D_{(zi)}$ is determined from the slope [4].

3. The unknown parameter $I0$ is now adapted such that the distance-dependent diffusion coefficient D_z coincide with the theoretical predictions from (6) (η 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 $I0$ has to be adapted as a parameter.

7 Tasks

The measurements are made on sillica particles of $4.3\mu m$ diameter, suspended in a $200\mu M$ K Br solution. The measuring frequency is 1 kHz, so that the measured data have a time interval of $\delta t_{mess} = 1ms$.

- **Determination of the potential form**
  The Brownian motion 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.

  1. First, the measured scatter intensity I (t) (after background deduction) is recorded.
  2. A histogram with approx. 100 columns is created from the measured data.
  3. Using the Boltzmann distribution, the potential is calculated and plotted from the probability distribution of the intensities.

- **Distance dependence of the diffusion coefficient and absolute distance**
  For the above measurement, the distance-dependent diffusion coefficient is determined and the absolute particle wall distance is determined by optimization of I_0. Deviations from the theoretical prediction are to be discussed.

- **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, ie 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. A typical display on the trapping laser is 0.50-0.55. At the highest power of the optical tweezers, the particle should only fluctuate about 150 nm in the potential well. How can you recognize this already at the signal on the oscilloscope?

The display of the trapping laser has to be recorded. It can be converted into the output power of the laser with the MatLab function 'Laserpower'. The power within the measuring cell is about half of this output power.

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. ($\rho_{PS} = 1.05gcm^{-3}, \rho_{H_2O} = 1.00gcm^{-3}$)

- **Variation of the screening length**
  By adding ions (here in the form of KBr as a monovalent salt), the Debeye shielding length is changed, ie the length scale of the electrostatic interaction changes. This is illustrated by measurements at different salt concentrations. For each concentration, a potential is measured for two different (but small) powers of the optical tweezers.
  Salt concentration to use :

  1. $50\mu Mol/L$
  2. $200\mu Mol/L$ (Already done in previous measurements)
  3. $600\mu Mol/L$
  4. $1500\mu Mol/L$

  Measure the potentials after deducting the linear contribution (i.e., the effective weight force). The Debey shielding length has to be determined from the potentials. Estimate the error by comparing the measurements with different powers of the optical tweezers. Compare the values with the theoritical Debey's screening length calculated from the salt concentration. Are van der Waal's forces recognizable in the potential?

References

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