Total Internal Reflection Microscopy

TIRM

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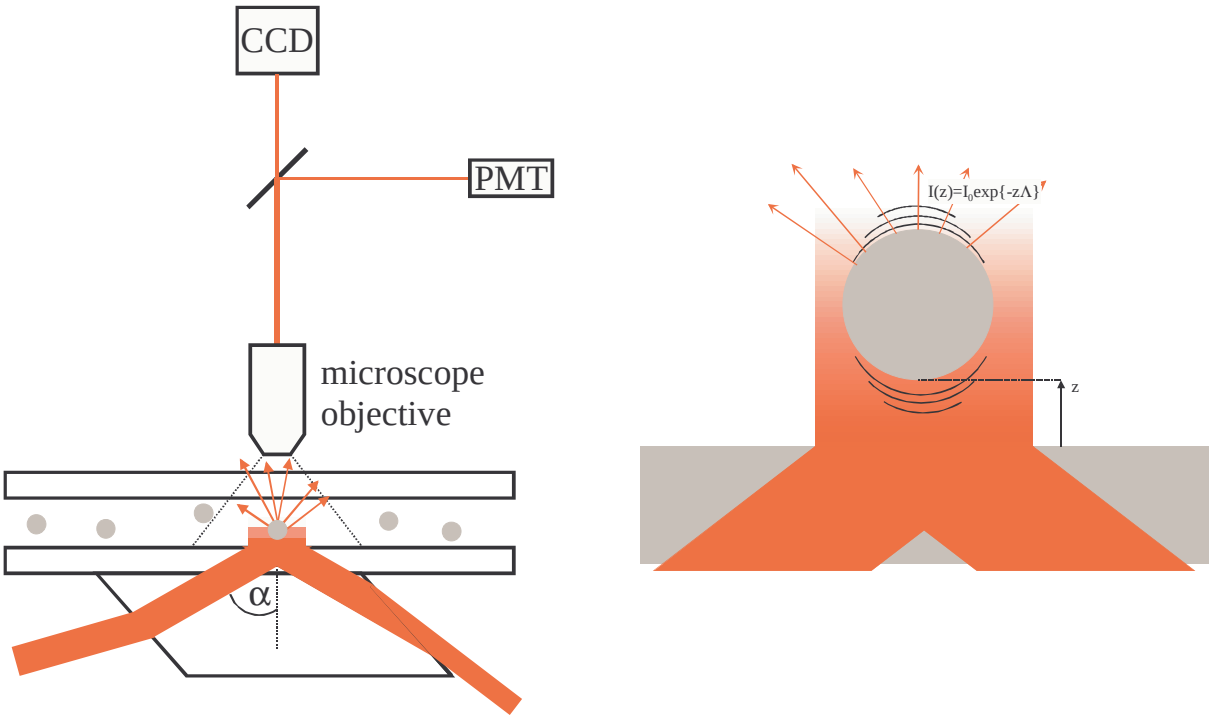

Fig. 1: *Sketch of a TIRM setup. Left: Light scattered from the evanescent wave by a single colloidal sphere is collected with a microscope objective and imaged to a CCD–camera and a photo multiplyer tube via a beam splitter. Right: Dependence of the scattered light on the particle wall separation.*

1 Basic Principles

In a typical TIRM set up a laser beam is directed via a prism to the interface between a glass wall and a highly dilute colloidal suspension at an angle large enough to grant total reflection as sketched in Figure 1. In this case the electric field of the beam leaks into the solution in the form of an evanescent wave, which has an exponentially decaying field strength in the direction normal to the interface. A particle located in this evanescent field will scatter it with an intensity which to a good approximation depends only on the particle separation from the wall

$$I(z) = I_0 \exp\{-\Lambda z\}\,. \tag{1}$$

Here I_0 is the intensity scattered from a particle at zero separation and Λ^{-1} is the $1/e$–penetration depth of the evanescent field intensity

$$\Lambda = \frac{4\pi}{\lambda}\sqrt{(n_1 \sin\alpha)^2 - n_2^2} \tag{2}$$

with λ the vacuum wavelength of the laser, n_1 and n_2 the index of refraction of the glass and the solution, respectively, and α the angle of incidence.
On the other hand, if the particle is subject to a potential profile with a shallow minimum (as shown for example in the far right of Figure 2.) the probability density to find the particle at a certain separation is determined by Boltzmann's law

$$p(z) = p(z_m) \exp\left\{-\frac{\phi(z) - \phi(z_m)}{k_B T}\right\}, \tag{3}$$

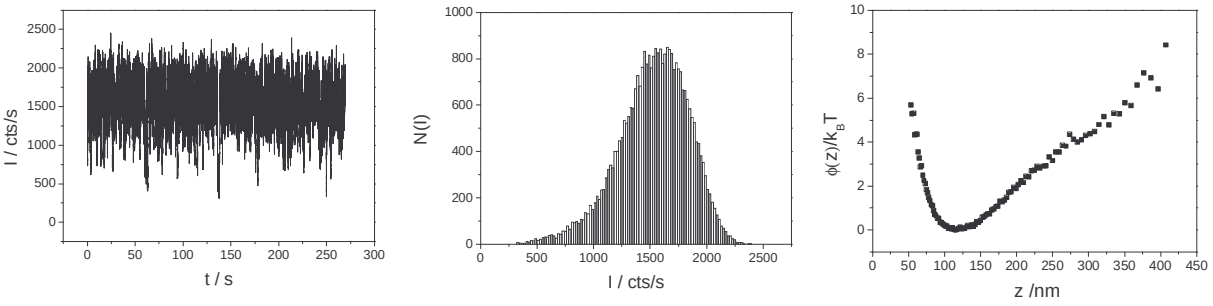

Fig. 2: *From raw data to potential profiles in TIRM: The intensity trace (left) is transformed into a histogram (middle) which can be converted into the potential profile by eq. 7.*

where $p(z_m)$ is the probability density in the potential minimum. The probability density distribution can be measured by recording the scattered intensity from the particle with a time resolution in the millisecond range, and converting the intensity trace into a the histogram $N(I(z))$. For a sufficiently high number of data points the histogram converges to the probability density distribution of scattered intensities $p(I(z))$. Since the probability to observe a certain intensity $I_S(z)$ is equal to the probability to find the particle at the separation z

$$p(I(z))dI(z) = p(z)dz. \tag{4}$$

we can obtain $p(z)$ from experimentally accessible quantities as

$$p(z) = -N(I(z))\frac{1}{\Lambda}I(z) \tag{5}$$

where we used $p(I(z)) \approx N(I(z))$. The route from the original data to a potential profile is sketched in Figure 2. The histogram $N(I(z))$ is constructed from the intensity trace and the potential is calculated using the combination of eqs. 3 and 5

$$N(I(z_m))\frac{1}{\Lambda}I(z_m)\exp\left\{-\frac{\phi(z)-\phi(z_m)}{k_BT}\right\} = N(I(z))\frac{1}{\Lambda}I(z). \tag{6}$$

Here $I(z_m)$ is the scattered intensity from the particle when it is located in the potential minimum, which according to Boltzmann's law is the intensity that hast the highest frequency in the histogram. After a slight rearrangement we get

$$\begin{aligned}\frac{\phi(z-z_m)}{k_BT} &= \ln\frac{N(I(z_m))I(z_m)}{N(I(z))I(z)} \\ &= \ln\frac{N(I(z_m))}{N(I(z))} + \frac{z-z_m}{\Lambda}\end{aligned} \tag{7}$$

where we defined $\phi(z_m) = 0$. To obtain absolute separation distances it is necessary to determine I_0, which in the case of DLVO-potentials can be done by screening the repulsive part of the potential adding high amounts of salt to the solution. Knowing I_0, it is possible to determine the position of the potential minimum through

$$z_m = \Lambda \ln\frac{I_0}{I(z_m)}. \tag{8}$$

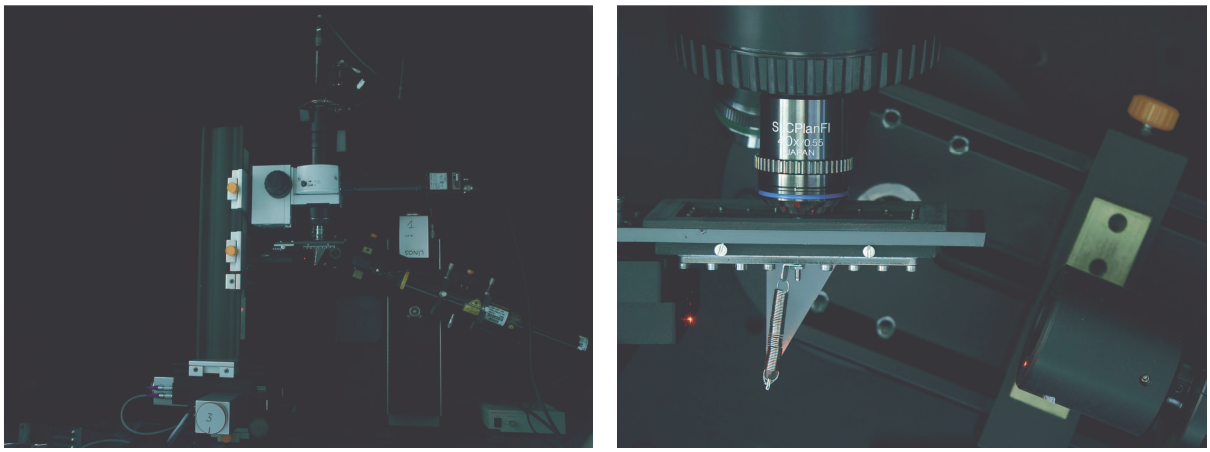

Fig. 3: *Left: Complete view of the TIRM setup. Right: Sample cell of the TIRM*

2 Experimental Setup

Our TIRM setup, which is shown in Figure 3, consists of a dual port unit (Olympus U-DP) with a beam splitter to which a photon counting head (Hamamatsu H7421-40) and a CCD camera (JAI M1) are connected as detectors. The Microscope is equipped with a revolver carrying two long working distance objectives (Olympus SLCPLFL 20× and SLCPLFL 40×). The sample cell was constructed in house and consists of a main body made from carbonized PTFE with an open area $20 \times 60 \times 2$ mm^3 in the center. The clearance is sandwiched between two glass slides and the sample solution is introduced through two filling holes either with a syringe or via tubes by peristaltic pump. To the lower glass slide, a 30/60/90 or a rhombohedral prism is attached to allow for total reflection of the laser beam. We use a 15 mW HeNe Laser as the light source which may be attenuated if necessary. Additionally we use a Verdi V2 Laser (Coherent) as optical tweezers to prevent the particle from leaving the field of view laterally. The angle of incidence can be varied using a motorized linear stage. The entire microscope unit is mounted on a motorized xy-table. This allows to adjust a sphere which is in the field of view of the microscope into the foot print of the laser beam without changing the position of the beam at all. Data acquisition is achieved with a National Instruments Counter and for data analysis we use a Delphi software package which was written in house.