Optical Tweezer Lab Manual – for Physics 2020 Online

1 Introduction

Optical tweezers were first developed in the early 1980s by Art Ashkin when he was working at Bell Labs. He used them to trap polystyrene spheres. The main application is to attach biological molecules to the spheres and to measure their mechanical properties on a single-molecule level. Applications include measuring the polymer physics of individual molecules such as DNA and RNA, looking at DNA-protein and RNA-protein interaction, picking up cells, and many, many others.

The goals of the optical tweezer practicum is to calibrate an optical tweezer setup, by determining the dependency of the trap stiffness on the laser power.

Normally you would come to the lab to (build part of the setup,) trap a bead, do calibration measurements, and analyse your data. With all on-campus education suspended, you will receive instructional videos explaining the setup, and showing the struggles of trapping a bead. You will also receive data, which you have to analyse.

2. Setup overview

A polystyrene bead (radius r=2 µm) is trapped at the location of highest intensity: the focus of the laser beam.

A schematic representation of the sample, containing the polystyrene beads, is given in figure 1a. The focus of the laser beam is inside the bead suspension, trapping the bead.

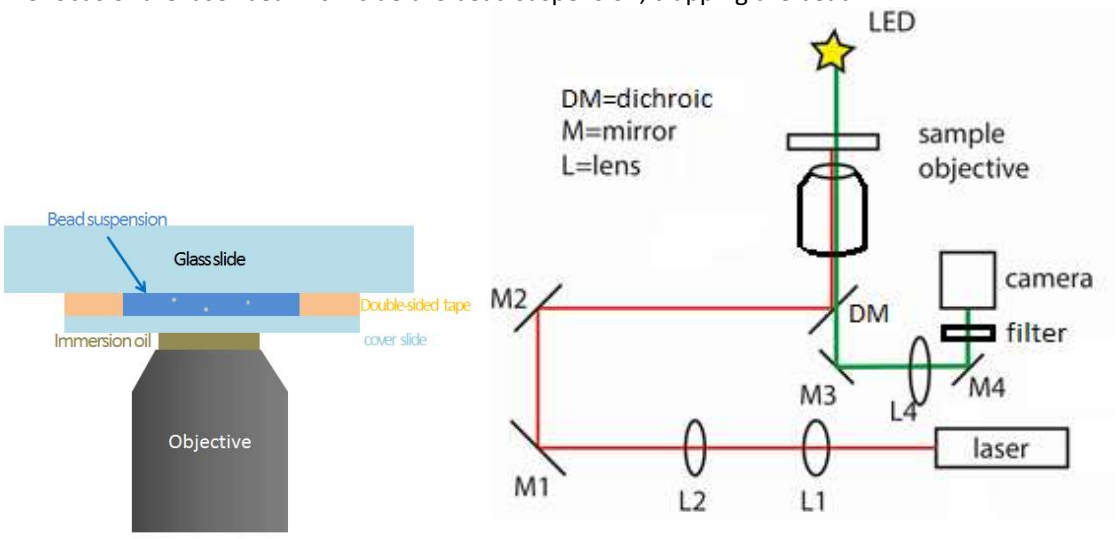

Figure 1. a. schematic representation of the placement of the sample on an inverted microscope, b. optical tweezer schematic. The red line is the trapping laser, the green line represents the light emitting diode (LED) line used for recording the bead image on a camera.

In figure 1b the optical tweezer schematics is shown. The red light from the laser, with wavelength λ=658 nm, and output power P=40 mW, passes through a beam expander (with focal length of the first lens f1=50 mm, and f2=350mm), in order to completely fill the back (back focal plane) of the objective. The mirrors M1 and M2 are used for compacting the beam path, and aligning the beam to the optical axis of the objective. Whenever the laser power is above we have to use laser safety glasses to protect our eyes.

This objective (lens) is meant to be used in an infinity corrected microscope. In such a microscope the objective is placed at focal distance of the objective lens, which results in a parallel collimated beam after the objective. A second lens (figure 1b, L4) focuses the light back to an image at (different) focal distance. The advantage of infinity corrected microscopes is that you can place all kind of filters in the infinite beam path, without affecting the beam path.
In our setup the (front) focal distance of the objective lens is 1.8 mm, and if the second lens has a focal length of 180 mm, the magnification would be 100x. However, we use a 200 mm focal length instead. Given that the pixel size of the camera is 5.2 µm, one can derive the nm/pixel conversion required to calculate distances in the recorded image.

3 Software and calibration: camera and extract position

In order to be able to use the tweezer setup for single-molecule biophysics, the setup needs to be calibrated such that the applied forces are quantitatively known. We use the fluctuation method in real time.

Let's consider the motion of a dielectric bead in the optical tweezer. This bead will fluctuate because it constantly undergoes random collisions from the surrounding water molecules, resulting in Brownian motion with an energy $\frac{1}{2}k_BT$ per translational degree of freedom as stipulated by the equipartition theorem. That implies that the bead excursions in the optical trap along the x-direction, δx, are limited to

$$\delta x^2 = \frac{k_BT}{k_{trap}}$$

where k_{trap} is the spring constant of the optical trap. In order to determine this trap stiffness, all you have to do is monitor the position of the bead long enough.

A typical optical trap stiffness is 10^{-6}-0.1 pN/nm.

3a Matlab

Run GUI6_MDTIF.m in Matlab. You will see the screen (figure 2). Enter your data location of the recorded tif file (optionally Browse). Press START to start the analysis. After the analysis is done, the plots are filled with data. To visually inspect the analysis, hit PLAY and run the recorded bead movie with overlay of a white x, denoting the found xy position.

Figure 2. Matlab GUI.

The found trap stiffness in x and y direction is given at the top right. Run the analysis for each recorded video, and determine the dependency of the trap constant on the laser power.

4 Report

Besides the regular report content, your report should include the following:

1. Theory and description of the setup
2. outcomes of your analysis, one plot per dataset (2 datasets given):
    a. on x-axis the power and on y-axis the resulting trap stiffness (kx & ky individually and combined).
    b. Please remember to label all axis with units, provide captions, legend.
    c. A fit on the above plots. Think about the correct dependency
    d. Error analysis (https://en.wikipedia.org/wiki/Propagation_of_uncertainty): derivation and error bars in plot
3. Discussion on difference kx,ky, on the fit, and on the difference between datasets.
4. Implementation of Matlab code into Python, at the start of your RP you get a certain part of the code appointed.