Probing the Mechanics of double-stranded DNA using Magnetic Tweezers

Praktikum: g5b
July 30, 2015

Ludwig-Maximilians-University Munich
Department of Physics

Supervisors:
Franziska Kriegel, Philipp Walker, and Prof. Jan Lipfert

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Chapter 1

Introduction

The mechanics of single molecules in biological systems play a crucial role. For example, in human cells 2 m of DNA need to be placed into a $\sim$ 10 μm sized nucleus: at the same DNA is processed, copied, and re-arranged by a number of protein machines. There are several single- molecule techniques, like atomic-force microscopy (AFM), optical tweezers (OT) or magnetic tweezers (MT) to probe the mechanical properties of macromolecules such as DNA or proteins. Fundamentally, we want to characterize the mechanical properties of e.g. bare DNA. Secondly, these properties are altered through interactions with proteins, which often provoke conformational changes.

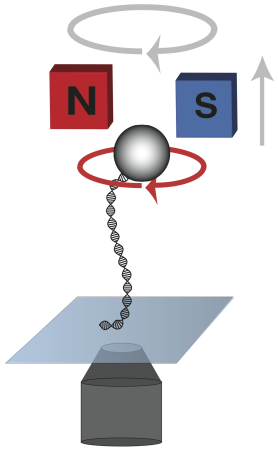


Figure 1.1: Principle of a MT setup: A magnetic bead is attached to the DNA molecule using specific binding, while the other end is attached to the bottom of the flow cell trough antibody binding. Permanet magnets are placed just above and exert a magnetic force on the bead. The diffraction pattern of the bead is observed using an inverted microscope and gives rise of the x, y and z position of the beads centre.

In this experiment you will study the mechanical properties of DNA using magnetic tweezers. You will learn the principles of a conventional magnetic tweezers setup and perform a standard force-extension measurement of double-stranded (ds) DNA and a rotational measurement to observe DNA supercoiling.

In a MT setup, a superparamagnetic bead is attached to the molecule of interest (here DNA), while the other end is attached to the bottom of the flow cell. Permanent magnets, placed above the flow cell exert a magnetic field on the magnetic bead and thus the DNA molecule, see Figure 1.1. The gradient of the magnetic force is nearly constant over typical length scales of molecules and even over the scale of the field of view, which implies that the magnetic force is constant over the field of view (force- clamp). In contrast to an AFM there is no feedback loop needed to exert constant forces also over a longer period of time.

From the diffraction pattern of the bead, observed with a CCD camera, one can track the bead's position in x, y and z over time. From that one can compute the extension z and standard deviation in x and y, which are required for the analysis. In addition to the AFM or the OT, when rotating the magnets in a MT setup one can rotate the bead and this in turn twists the molecule. Due to the helical structure of dsDNA this is an interesting fact when studying DNA or further more the interaction of DNA with other molecules.

Question 1: What is a *force-clamp*?

Chapter 2

Theory

This chapter covers briefly the theoretical background in polymer physics needed to understand and interpret the measurements in the magnetic tweezers lab.

2.1 Force- Extension Behavior of Molecules

In order to describe the response to constant forces acting on biological macromolecules , we introduce two theoretical models, the freely- jointed chain model (FJC) and the worm-like chain model (WLC). The first model (FJC) considers the molecule as a chain of rigid segments, while the second model (WLC) considers the molecule as an isotropic rod that is continuously flexible.

2.1.1 Freely- Jointed Chain Model (FJC)

This model considers a polymer of total length L that consists of N segments each with the same length l (Kuhn-length):

$$L = N \cdot l$$

$\vec{R}$ is the end- to end vector of the molecule, which is a good measure of the actual size of the molecule in its environment. All segments are described by a vector $l \cdot \vec{r}_i$. There are no interactions between the monomers and the bond between two vectors $\vec{r}_i$ and $\vec{r}_{i+1}$ is flexible (random walk), see Figure 2.1.

All conformations of the polymer are equally likely to occur in solution and follow the Maxwell- Boltzmann distribution. If an external force $\vec{F} = f \cdot \vec{e}_z$ acts on the molecule, the potential energy E^{α}_{pot} of the molecule in a particular angular confirmation α is:

$$E^{\alpha}_{pot} = -\sum_{i=1}^{N} \vec{F} \cdot l \cdot \vec{r}_i = -l \cdot f \sum_{i=1}^{N} \cos\theta_i \tag{2.1.1}$$

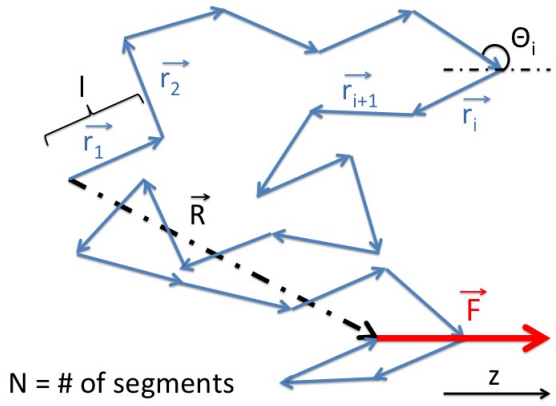


Figure 2.1: In the FJC model a polymer is considered to consist of N rigid segments with length l. Also shown in this figure is the definition of the end- to end vector $\vec{R}$, which is spanned from the first segment to the last segment of the polymer.

Where θ is the angle between a segment and the z- axis and F the force acting in z- direction.

The response (extension) of a molecule to a force acting on it can be derived through statistical mechanics. A canonical ensemble is a statistical ensemble to represent all possible states of a mechanical system that is in thermal equilibrium. In a canonical ensemble the volume V, the number of particles N and the temperature T are considered constant. The canonical partition sum Z can be defined using the following formula. Inserting equation (2.1.1) leads to the following result:

$$Z = \sum_{\alpha} e^{\frac{-E^{\alpha}_{pot}}{k_B T}} = \sum_{\alpha} \prod_{i=1}^{N} e^{\frac{fl\cos\theta_i}{k_B T}} = (\sum_{\alpha} e^{\frac{fl\cos\theta}{k_B T}})^N \tag{2.1.2}$$

Where k_B is the Boltzmann constant and T is the temperature.

Due to the fact that the angle is continuous, the sum can be replaced by an integral, integrating over the whole space:

$$Z = (\int d\Omega \cdot e^{\frac{fl\cos\theta}{k_B T}})^N = (\frac{2\pi k_B T}{fl}\sinh(\frac{fl}{k_B T}))^N \tag{2.1.3}$$

When inserting this to the definition of the *Gibbs Free Energy (G)*:
G= $-k_B T \cdot ln(Z)$ the extension z of the molecule due to the force $\vec{F}$ acting on it can be calculated:

$$z = -\frac{\delta G}{\delta f} = L \cdot (\coth(\frac{fl}{k_B T}) - \frac{k_B T}{fl}) \tag{2.1.4}$$

Question 2: What is a *Kuhn- length* and what is a *random walk*? Where do you find the random walk in the freely- jointed- chain model?

Question 3: What is a *Maxwell- Boltzmann distribution*? What is a *canonical ensemble*?

Question 4: It is always good to do this.....Try to derive from (2.1.3) at equation (2.1.4) step by step.

See also the lecture *Biophysics of Macromolecules* (LMU).

2.1.2 Worm- Like Chain Model (WLC)

In comparison to the FJC model, the WLC model considers the molecule as a continuously flexible chain, see Figure 2.2.

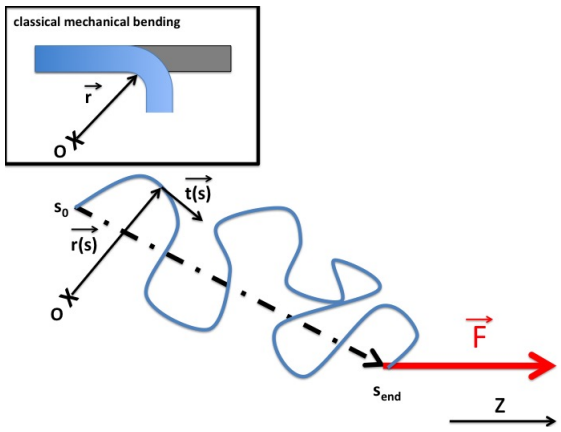


Figure 2.2: The worm- like chain is comparable to the classical mechanical bending of a rod, see black box on the top left. The molecule of length L_c is parametrized by points s along the contour of the molecule. Vector $\vec{r}$(s) points to the chain whereas $\vec{t}$(s) is the tangential vector of this point.

The chain is splitted in small segments s with length ds, s $\in$ [0, L_c], where L_c is the contour length of the molecule. $\vec{t}(s)$ are the tangent vectors to each point s. If a force $\vec{F}$ is acting on the molecule, it acts on each tangential vector. Therefore the formula of the energy E is given by an integral, integrating over the contour of the molecule. For example, the energy due to the external force is given by:

$$E = \int_0^{L_c} ds \cdot \vec{t}(s) \cdot \vec{F} = f \cdot \int_0^{L_c} ds \cdot \vec{t}(s) \cdot \vec{e}_z \tag{2.1.5}$$

Generally the worm- like chain is solved in analogy to the freely jointed chain: first one defines the energy for one conformation (E_α^{WLC}) of the polymer and secondly one calculates the partition sum. From that point all necessary quantities can be defined. In order to integrate over all conformations, one has to perform a path integral $D(\vec{t}(s))$, integrating over all tangent vectors in point s. This becomes very difficult and there exists no analytical solution of the worm-like chain but numerical, which is in good approximation.

Here, we sum the mathematics to a minimum, considering that a polymer that behaves as a worm- like chain is comparable to a bar that is bend by a force $\vec{F}$ (classical mechanics), see Figure 2.2, black box. The energy to deform a bar is proportional to $\frac{1}{R^2}$, where R is the radius of the bending circle. Taking into account that $(\frac{\delta\vec{t}(s)}{\delta s})^2 = \frac{1}{R^2}$ we get:

$$E_\alpha^{WLC} = \int_0^{L_c} ds \cdot (\frac{A}{2} \cdot (\frac{\delta\vec{t}(s)}{\delta s})^2) \tag{2.1.6}$$

Where $\mathrm{A} = k_B\mathrm{T} \cdot L_P$. L_P is called the bending persistence length and defines the rigidity of the chain. Segments smaller than the persistence length behave like a rigid rod. Segments larger than the persistence length are flexible and can be bend. An exact solution of the WLC model is hard to derive, as one has to perform the path integral:

$$\int_{\vec{t}(0)}^{\vec{t}(L)} D(\vec{t}(s)) \cdot exp(-\frac{L_P}{2}) \cdot \int_0^L ds(\frac{\delta\vec{t}(s)}{\delta s})^2 \tag{2.1.7}$$

Doing an interpolation facilitates the solution of the worm-like chain. The relation of force-extension for a worm-like chain behaving molecule is in 90 % agreement to the exact solution:

$$\frac{fL_P}{k_BT} = \frac{z}{L_c} + \frac{1}{4 \cdot (1 - \frac{z}{L_c})^2} - \frac{1}{4} \tag{2.1.8}$$

Where f is the force acting on the molecule, L_P is the persistence length, z is the extension and L_C is the contour length of the molecule.

Question 4: What is the difference between the *end-to end distance* and the *contour length* of a molecule?

Question 5: What is the *persistence length*? Is the persistence length temperature dependent? What do you think is the persistence length of dsDNA?

2.2 Double- stranded DNA under Force and Torque

In this laboratory we will be working with dsDNA. In this chapter we will discuss how the force on the bead is calibrated in a magnetic tweezers setup and how DNA reacts to torques.

2.2.1 Force Calibration in a Magnetic Tweezers Setup

In a magnetic tweezers setup, magnets exert a magnetic field and thus a force on the magnetic beads. One end of the DNA molecule is attached to the magnetic bead, while the other end is attached to the bottom of the flow cell, see 3.6. The magnetic fields exert a force on the bead and thus the DNA molecule. The DNA molecule can be stretched and twisted simply by translating and rotating the magnets.

The force is calibrated using the fluctuations of the magnetic bead in solution, due to Brownian motion. The setup can be seen as an inverted pendulum, see Figure 2.3.

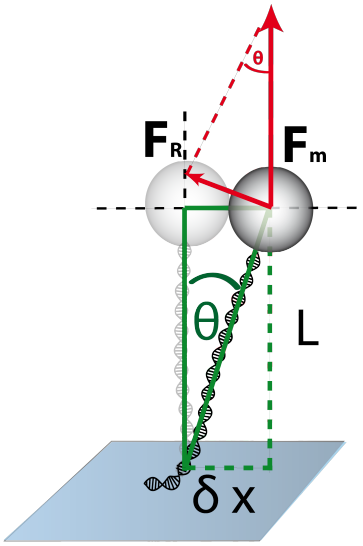


Figure 2.3: The magnets exert a force $\vec{F}_m$ on the magnetic bead. The force can be calibrated using the deviations δx of the bead from its equilibrium position, due to Brownian motion. The extension of the molecule is labeled with L, the angular deviation is called Θ and $\vec{F}_R$ is the restoring force acting on the bead.

The force ($\vec{F}_m$) on the bead is pointing in z- direction. Due to Brownian motion the bead fluctuates around its origin. A restoring force ($\vec{F}_R$) forces the bead always back to its initial position. Using the angular deviation Θ and δx one can define the force.

$$|\vec{F}_R| = \sin\Theta \cdot |\vec{F}_m| = \frac{\delta x}{l} \cdot |\vec{F}_m| \tag{2.2.1}$$

The bead is in an harmonic potential with spring constant $\kappa = \frac{F_m}{l}$. The system, as described above, has only one degree of freedom and therefore

$$\frac{1}{2} \cdot \kappa \cdot < \delta x >^2 = \frac{1}{2} k_B T \tag{2.2.2}$$

Inserting the spring constant and solving the equation for F_m, one can calculate the force acting on the bead:

$$\vec{F}_m = \frac{l \cdot k_B T}{< \delta x >^2} \tag{2.2.3}$$

In order to calibrate the force, one has to measure the xy- movement of the bead, longer than its characteristic time. There can be a variation from bead to bead due to inhomogeneity in the bead size or the magnetization.

(For a better and more exact derivation, see Vilfan et al. 2009.)

Question 6: What is *Brownian motion*?

Question 7: How could one increase the forces in a MT setup?

Question 8: What is the *characteristic time* of a system? Is the characteristic time of this system larger for larger forces?

2.2.2 Inducing Twists on DNA Molecules

In a MT setup one is able to induce twists on a DNA molecule. Due to the helical structure of DNA (right handed DNA) one differentiates between positive and negative turns. Positive turns go with the helical structure, while negative turns go against it.

The response of dsDNA to twist depends on the force, see Figure 2.4. When rotating a torsionally relaxed DNA, the DNA wrings about itself without any decrease in its length. In this case the torque of ds DNA goes linearly with the number of turns n. When crossing a certain number of turns, the so called buckling point (n_b), it is energetically more favorable for the molecule to form supercoils. Thereby the DNA length is reduced. The change in the end-to end distance is reduced by the part of DNA that forms DNA supercoils.

$$E_{torsion} = 2\pi n \cdot \Gamma \tag{2.2.4}$$

$$\Gamma = \frac{C}{L} \cdot 2\pi n \tag{2.2.5}$$

Where Γ is the Torque and C is the torsional stiffness.

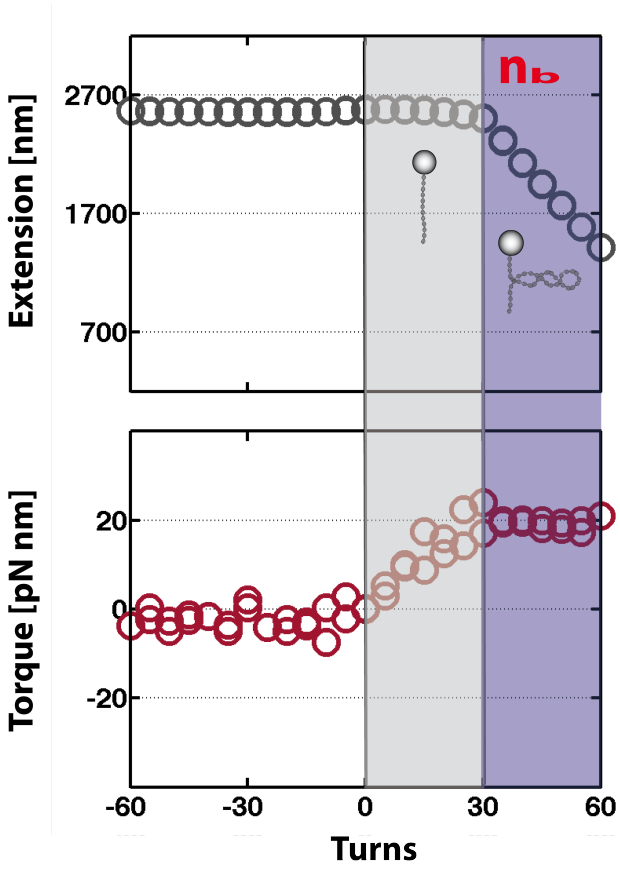


Figure 2.4: The top figure shows a rotation curve of dsDNA at high force. The lower curve shows the corresponding torque. At the buckling point n_b it is energetically more favorable to form DNA supercoils than remaining stretched, in that case the torque remains constant (purple). Whereas the torque increases linearly in the regime where no supercoils are formed (grey).

At the buckling point (n_b) the energy $E_{torsion}$ corresponds to the energy that is needed to form a loop (E_{loop}). E_{loop} has two contributions: the work W that is needed to shorten the DNA and E_{bend} to bend the DNA strand.

$$E_{torsion}(n_b) = E_{loop} = W + E_{bend} \tag{2.2.6}$$

One supercoil is roughly approximated as a circle with radius r. The work needed to reduce the length of the DNA molecule against the force F is

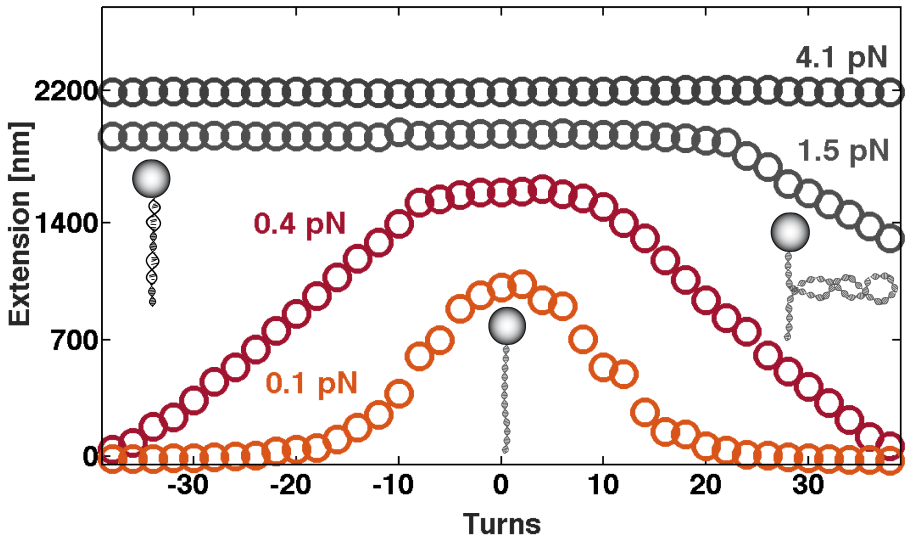


Figure 2.5: Rotation curves of dsDNA for four different forces: 4.1 pN, 1.5 pN, 0.4 pN and 0.1 pN. For low forces (0.1 pN and 0.4 pN) the curves are symmetric for positive and negative turns. For 1.5 pN the curve is asymmetric, no supercoils are formed for negative coils. At 4.1 pN the extension of the molecule stays constant in both directions, no supercoiling occurs.

$$W = -2\pi r F \tag{2.2.7}$$

while the energy for the bending to perform one loop is (see WLC)

$$E_{bend} = \int_0^{2\pi r} ds \frac{A}{2}\frac{1}{r^2} = \pi A \frac{1}{r} \tag{2.2.8}$$

Minimizing the looping energy one can derive the radius r of the loop

$$r = \sqrt{\frac{A}{2F}} \tag{2.2.9}$$

Having the radius, one can calculate the buckling torque Γ_b and the number of turns n_b where the buckling takes place.

$$\Gamma_b = \sqrt{2AF} \tag{2.2.10}$$

$$n_b = \frac{L}{2\pi C} \cdot \sqrt{2AF} \tag{2.2.11}$$

Figure 2.5 shows four rotation curves for dsDNA. For low forces (0.1 pN and 0.4 pN) the curve is symmetric for positive and negative turns. After a certain

number of turns (n_b) supercoils are formed, this leads to a decrease in the end-to end distance of the molecule. With each further turn, one supercoil is formed. For higher forces the curve becomes asymmetric (1.5 pN). No supercoils are formed in negative direction. Instead, torque induced melting occurs.

Question 9: What is the *buckling point*? What happens if the number of turns n is exactly n_b?

Question 10: How does the torque of dsDNA behave (referring to the buckling point)?

Question 11: Where is/where are the buckling point(s) in Figure 2.5?

Chapter 3

Setup

This chapter describes the experimental setup, the software, and the fabrication of the flow cell.

3.1 Experimental Setup

Magnetic Tweezers make use of the fact that paramagnetic beads experience a force in a magnetic field gradient. A molecule of interest is attached to the surface of a flow cell and on the oter side to a superparamagnetic bead (Figure 3.1). Force can be exerted on the bead, stretching or rotating the molecule. The flow cell is mounted on a flow cell holder, so that the flow cell is placed on top of an oil objective. The objective can be moved up and down very precisely with a piezo-stage. A pump is connected to the flow cell, so that the fluid in the flow cell can be exchanged. The magnetic field gradient is produced by a pair of permanent magnets with a small gap between the magnets. Light produced by a LED travels through the gap and illuminates the flow cell with parallel light. Pictures of the illuminated area are taken by a CCD camera and transferred to a computer where image analysis is performed. The tether itself cannot be seen in Magnetic Tweezers, but the beads are tracked.

The magnet holder is attached to a linear and to a rotation motor. The magnetic force acting on the bead is controlled by moving the magnets up or down (Figure 3.2), resulting in a tether stretched at high forces or a more freely moving bead due to Brownian motion at low forces. Rotating the magnets causes a direct rotation of the bead as the torque of the molecule is negligible compared to the torque applied by the magnets. As described above, the change in force and effect of rotation resulting in supercoils can be seen in Figure 2.5.

To get information about the bead in the lateral directions as well as the height of the bead, the bead is out of focus of the objective. Diffraction rings of

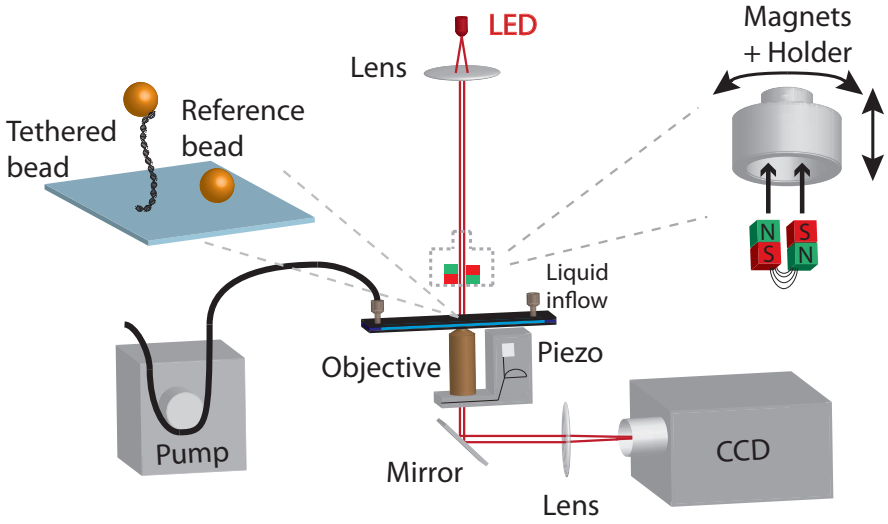


Figure 3.1: Overview of a Magnet Tweezers Setup. A pair of magnets produce a magnetic field gradient, so that paramagnetic beads "feel" a magnetic force, resulting in an pulling force to the DNA molecule. The light of a LED travels through a gap between two magnets and illuminates the sample. On a CCD camera, the interference rings of the beads can be detected.

the bead can be detected with the CCD camera. As can be seen in Figure 3.3 the diffraction rings change at different distances of the focus.
To get precise information in the vertical direction, a look-up-table (LUT) is created for every bead before the actual measurement starts. To obtain the LUT, the objective is moved step wise and pictures are taken at every position (e.g. every 100 nm). During the measurements the piezo is kept at a fixed position and the current diffraction pattern are compared to the LUT. Thus, the height of the bead can be calculated. By interpolation the LUT images, a better resolution than the step size of the LUT can be achieved.
For the lateral position of the bead, the centre of the diffraction rings are calculated via cross-correlation.

To reduce effects of mechanical drift, which can disturb the measurements in the lateral as well as in vertical direction, at least one moving bead with a tether and one reference bead stuck to the ground are tracked. The positions of the stuck reference bead are subtracted from the ones of the bead with a tether, removing the drift of the flow cell. Note: the saved values in this course are without subtraction of the reference bead!

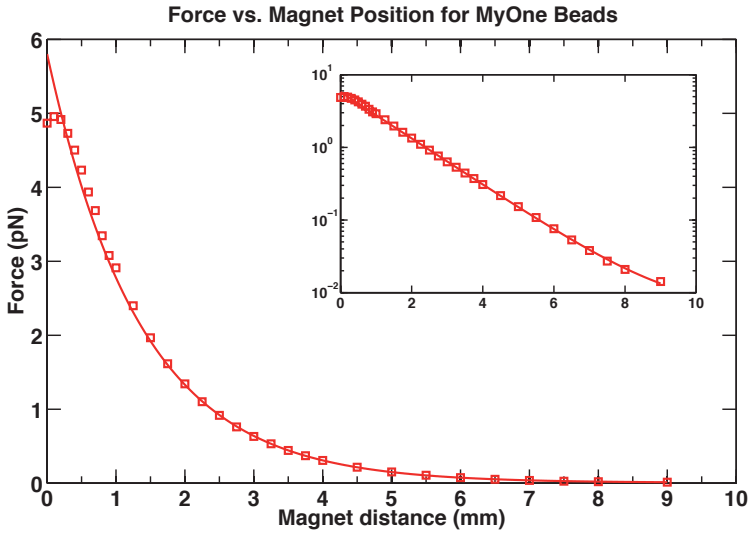


Figure 3.2: Force vs. magnet position for MyOne beads. The solid line is an exponential fit to the measured values.

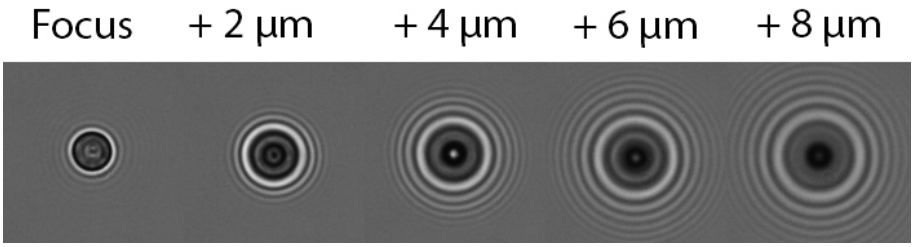


Figure 3.3: Change of diffraction pattern according to the "out of focus" distance.

3.2 Software

The software to perform the measurements is written in LabView. The piezo-stage and both motors to control the magnets (height and rotation) can be controlled by the software. The pump to exchange the fluids has to be used manually, also the position of the flow cell has to be set manually by micrometer screws.
The software consists of three separated windows (see Figure 3.4 and 3.5).
A live picture of the field of view of the flow cell is shown in the "BeadTracker-Main.vi" window. Furthermore, basic setup configurations can be set, the beads to measure can be selected, and a LUT can be performed. With the "MotorUI.vi" window, the motors can be controlled as well as the piezo-stage. The third window "ExperimentProgramUI.vi" contains all options to start and control the measurements.
The bead positions are tracked in real time and saved in text files with the motor positions. Furthermore, another text file is produced which contains the

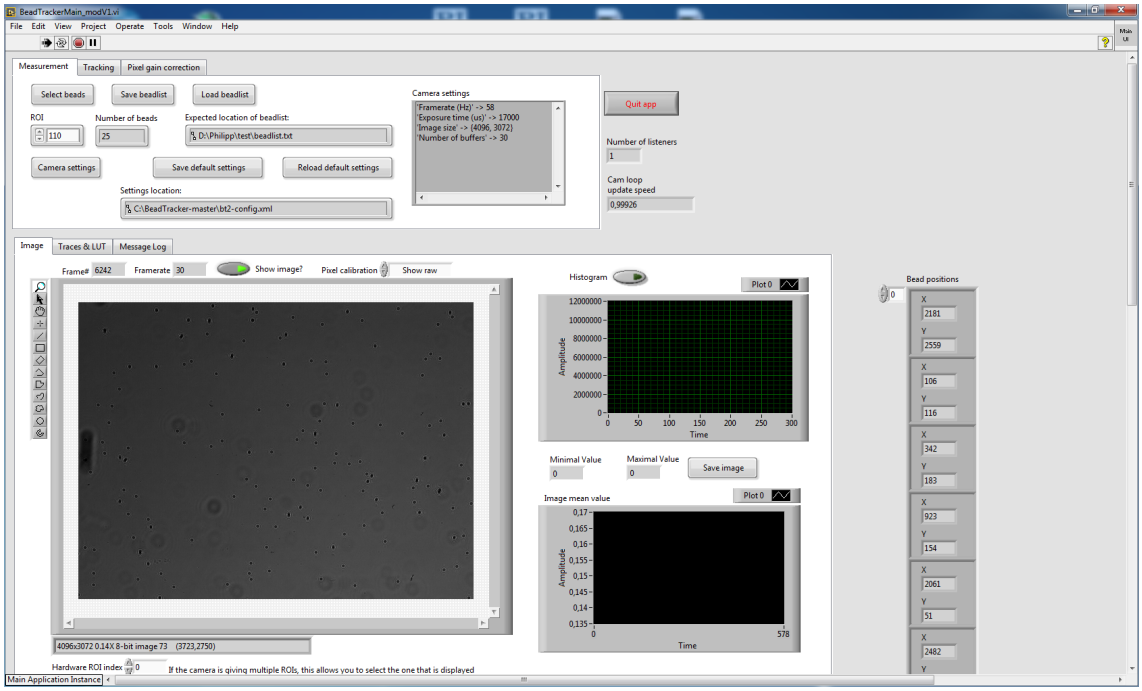
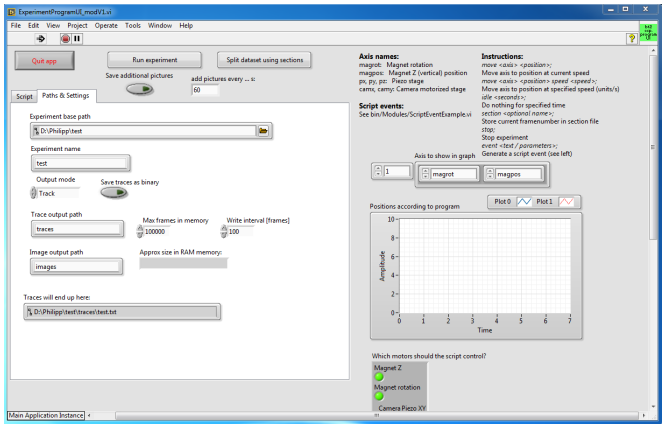


Figure 3.4: "BeadTrackerMain.vi" is the main window of the software. It contains a real time image, as well as the option to select beads and perform a LUT.

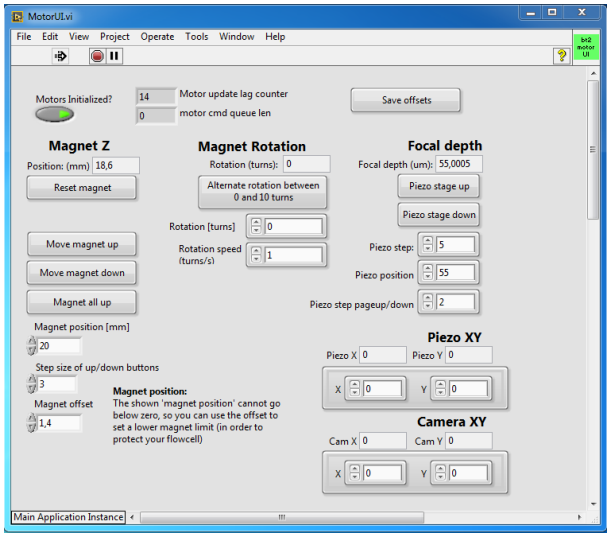
"sections"-frame number written in a protocol (see Figure 4.1).

3.3 Flow cell and Attachment Chemistry

The flow cell (Figure 3.6 a)) contains the biological part of Magnetic Tweezers. Two coverslips are stuck together by a soft melted parafilm as a spacer. Parafilm is cut so as to provide a rectangular chamber between the top and bottom coverslip. The upper coverslip has two holes (inlet and outlet) which makes it possible to change the fluid inside the flow cell. For practical reasons a tube and a pump are used as an outlet, whereas a small reservoir is fixed to the inlet to store fluid. The DNA has two handles serving as attachment fragments. Each handle is approximately 600 bps long and contains several nucleotides with a biotin (biotin handle) or a digoxigenin label (dogoxigenin handle). The biotin handle binds to a streptavidin coated superparamagnetic bead and the digoxigenin labeled end of the DNA binds to an anti-digoxigenin coated coverslip. To prevent the DNA from freely rotating at the biotin or digoxigenin label, there are several biotin and digoxigenin labels at each handle which have the opportunity to bind (Figure 3.6 b)). To avoid unspecific binding of the bead to the surface, the coverslip is passivated with BSA-blocking solution.

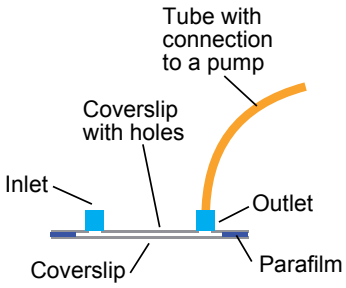


(a) In the window "ExperimentProgramUI.vi" the folder path can be defined, protocols can be written and measurements can be started.

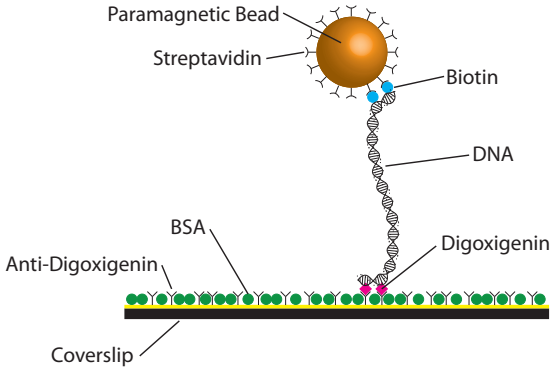


(b) With "MotorUI.vi" the piezo-stage as well as the two motors for the magnets can be controlled.

Figure 3.5: "ExperimentProgramUI.vi" and "MotorUI.vi"



(a) A schematic illustration of a flow cell. Two coverslips, the upper one with two holes as inlet and outlet are bound together by a parafilm. The parafilm forms the wall for the remaining flow cell reservoir.



(b) Schematic illustration of a bead-tether-surface construct. Anti-digoxigenin is fixed to the bottom coverslip by epoxysilan. The bead has a streptavidin coated surface. A DNA containing digoxigenin labels on one site and biotin on the other site binds to the bead and surface. For passivation the coverslip is coated with BSA.

Figure 3.6: Flow cell and attachment Chemistry

Chapter 4

Measurements

This chapter describes the measurements which will be performed in the lab course.

4.1 Preparation of the Flow Cell

The flow cell is already coated with anti-digoxigenin and stored with a BSA-blocking solution. Before the actual measurements are performed, the DNA molecules have to be attached to the bead, incubated in the flow cell and unbound beads have to be flushed out. Before you start, remove and remount the magnet holder to get familiar with the system. Furthermore, try to move the z-motor and rotation motor (make sure a tutor is present). In the end, the magnet holder has to be removed and the motor has to be set to "all up".

- Cleaning the beads: add 2 μl of well mixed MyOne-bead solution to 10 μl 1x PBS and mix
- Use a magnetic holder to keep beads to one side and remove 10 μl
- Redo washing step
- Add 10 μl 1x PBS
- Add 1.0 μl DNA and wait for 15 min. Mix solution by tapping the reservoir every 3 minutes, avoid mixing with a pipette (results in DNA nicks)
- Fill up with 150 μl 1x PBS
- Remove magnets if not done so far
- Flush flow cell with 600 μl 1x PBS (v = 170 $\frac{\mu l}{min}$)

- Fill the chamber with ca. 60 μl DNA-beads and wait for 10 min
- Flush with 800 μl 1xPBS (v = 400$\frac{\mu l}{min}$)
- Mount magnets and drive them down to about 2 mm distance to the flow cell

Note: From this point, the magnets should never have a larger distance as 8 mm from the flow cell for more than 20 s to avoid sticking of the beads.

Within the next steps, the beads to measure will be selected.

- Move the piezo-stage (window "MotorUI.vi") to get the beads in focus
- Move the piezo-stage 8 μm upwards
- Search in the field-of-view which you can see in window "BeadTracker-Main.vi" for two beads which are not moving and their interference rings are not overlapping with other beads. Remember their positions. These beads are unspecifically attached to the surface and can be used as reference beads.
- Press "select beads" and click "remove all". Press "add" and click on the two beads chosen before as reference beads. Now, click on every other bead you can see whose interference rings are not overlapping.
- Press "ok" to close the window

Now all beads are selected and a LUT has to be performed.

- Make sure a tutor is present
- Move magnets to a distance of 0.3 mm to apply a high force, which is necessary to avoid large fluctuations of the beads during the LUT-measurement.
- Click on "Tracking" in the "BeadTrackerMain.vi" window and type the actual piezo position minus 5 μm into the field "ZLUT Start piezo Z". This sets the lowest piezo position for the LUT. The LUT settings are by default 100 steps à 100 nm in the upward direction, so that the actual piezo position is in the middle of the LUT.
- Press "Build ZLUT" to start the LUT measurement and wait until the software has finished

The setup is now ready to track the beads. To avoid a large amount of data of beads which are stuck to the surface or have multiple tethers, a length measurement and a rotation test should be performed.

- Choose a folder to save the measurements and a name for your measurement in window "ExperimentProgramUI.vi"
- Check that the motor position is still at 0.3 mm
- Start the measurement with the button "Run experiment" in the window "ExperimentProgramUI.vi"
- A new window will pop up
- Move the motor to 12 mm distance, wait 10 seconds and drive back to 0.3 mm
- Stop measurement ("abort measurement")
- Activate "Trace & LUT" in window "BeadTrackerMain.vi", click through the traces and delete every bead which has a smaller change in distance as 1.5 μm (not the two reference beads zero and one!). Note: You can change the number of frames which are shown in the trace and you can subtract a reference bead from the traces to see the z- extension more clearly. Ask your tutor if you have problems.
- Change the measurement name for the second measurement
- Check that the motors are at a distance of 0.3 mm ($\approx$ 5 pN)
- Start the measurement
- Rotate the motors to - 30 turns (at 1 turn/s)
- Rotate back to 0 turns
- Stop the measurement
- Check again the traces for beads who did not behave as expected and delete them
- Do the same with a distance of the magnets to the flow cell of 2.5 mm (1 pN) and rotate to + 30 turns and turn back to 0

If you have less than two good beads left, contact your tutor.

4.2 Experiment 1: Force-Extension Curves

Magnetic Tweezers measure only relative length changes, but not the current total length of a tether. Therefore, start with a measurement to get the zero position of the tether, i.e. when the bead touches the surface. To get this value, measure again from 0.3 mm to 12 mm (15 s) to 0.3 mm, magnet height.
In a second file, measure a force-extension curve for this DNA molecule like in Figure 3.2. Therefore write a measurement protocol and consider that the characteristic time of the system can be calculated by

$$\tau_c = \frac{12 \cdot \pi^2 \cdot \eta \cdot R}{F} \cdot l \tag{4.2.1}$$

with η the viscosity ($1.1 \cdot 10^{-3}$ $\frac{kg}{ms}$), l the extension of the tether, R the radius of the bead (0.5 μm) and F the force. The precision of the measurement ϵ depends on how often the characteristic time will be measured. It is defined by

$$\epsilon = \sqrt{\frac{\tau_c}{t}} \tag{4.2.2}$$

with t the measured time of the experiment. You should use an ϵ of 10%.
An example how to write a protocol is given in Figure 4.1. Before you can start your script, you have to press "Simulate script" in the "ExperimentProgramUI.vi".

ScriptPraktikumBeispiel.txt

```
move magpos   3.5 speed    3.00; idle 10;
section;
move magpos   2.2 speed    3.00; idle 10;
section;
move magrot 10  speed    5.00; idle 15;
section;
move magrot 20 speed    5.00; idle 15;
stop;
```

Figure 4.1: Example of a script for a measurement protocol. The first two lines move the z-motor of the magnets, the values "magpos" are the absolute distance to the flow cell. The number behind "idle" defines the time to record frames in seconds. The third and fourth line rotate the motors. "magrot" defines the absolute values of the rotation motor. The speed values should be always used as given in the example (z-motor = 3.0; rotation motor = 5.0). Every "section" will save the frame numbers in a text file, which makes it easy to find motor movements for analysis.

4.3 Experiment 2: Rotation-Extension Curves

The aim of this measurements is to get the data for a determination of the modulus of torsion. Therefore, measure rotation curves from - 40 to + 40 turns in steps of 2 turns at forces of 5.0 pN, 2.0 pN, 1.5 pN, 1.0 pN, 0.7 pN, 0.5 pN and 0.4 pN. Use Figure 3.2 to estimate the z position of the motors. It is recommended to start a new measurement for every force for easier analysis. A measurement time of 10 s for each turn is sufficient.

Chapter 5

Analysis

5.1 Analysis Experiment 1: Force-Extension Curves

- Create a plot as shown in Figure 3.2. Fit an exponential function to your data.
- Plot the force- extension of the DNA molecule and fit the FJC as well as the WLC. Determine the Kuhn length (FJC) as well as the persistence length (WLC). For how many base pairs is the DNA molecule supposed to be stiff? Compare your values to literature. Which model describes better the behaviour of the DNA molecule?

5.2 Analysis Experiment 2: Rotation-Extension Curves

- Plot the extension of the DNA vs. the number of turns for every force
- Explain the difference of the plots for all forces
- Determine the size of one supercoil
- Determine the buckling point n_b and the buckling torque Γ_b for all forces and determine the torsional stiffness of the DNA