Magnetic Tweezers Experiments to Probe the Mechanics of Nucleic Acids

Praktikum: g5b
July 18, 2018

Ludwig-Maximilians-University Munich
Department of Physics

Franziska Kriegel, Philipp Walker and Prof. Jan Lipfert

Contents

Chapter 1

Introduction

The mechanics of single molecules in biological systems play a crucial role in gene regulation. The binding of proteins to DNA molecules often provokes a conformational change. These structural changes decide either the protein is active or not. As all biological processes contain binding and unbinding of molecules, the study of mechanical properties is of huge interest. There are several single-molecule techniques such as the atomic-force microscope (AFM), optical tweezers (OT) or magnetic tweezers (MT) to measure mechanical parameters of molecules such as DNA or proteins.

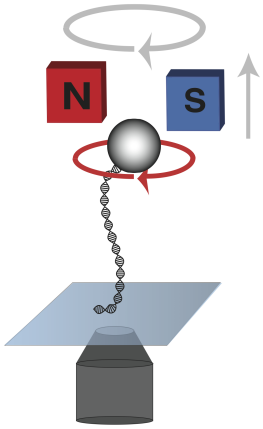


Figure 1.0.0: Principle of a magnetic tweezers setup: A magnetic bead is attached to the DNA molecule using specific binding, while the other end is attached to the bottom of the flow cell trough antibody binding. Permanent magnets are placed just above the flow cell and exert a magnetic force on the bead. The stretching force and the rotation of the molecule can be controlled by controlling the magnets position in translation and rotation. 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 center.

In MT 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 just above the flow cell exert a magnetic field that induces a magnetic force on the magnetic bead and thus the DNA molecule, see Figure 1.0.0. The gradient of the magnetic force is nearly constant over typical molecule sizes, this implies that the magnetic force is constant over the field of view (force-clamp). In contrast to an AFM there is no feed back loop needed to exert constant forces also over a longer period of time.

In MT a CMOS camera is used to track the magnetic bead's movement out of focus. Using its diffraction pattern, one can derive the bead's and such the molecules x,y and z position, from which the molecule's extension as well as the stretching force acting on the DNA molecule is calibrated.

DNA is highly packed in the cell nucleus. Due to its helical structure DNA undergoes conformational transitions upon the addition of stretching forces and torques. In MT molecules can be twisted by rotating the magnets. Freely orbiting magnetic tweezers (FOMT) and magnetic torque tweezers (MTT) additionally allow to measure angular changes within the molecule.

This experiment is about studying mechanical properties of DNA using magnetic tweezers. Here you should learn the principles of a conventional magnetic tweezers setup and perform a standard force-extension measurement of double-stranded DNA and a rotational measurement to observe DNA supercoiling.

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

Chapter 2

Theory

In this laboratory course you are asked to perform force-extension measurements on double-stranded DNA, which means to apply various stretching forces and observe the DNA's tether extension. In a second experiment you are asked to apply turns to the DNA molecule and study its behavior upon analyzing changes in the DNA's length. This Chapter covers briefly the theoretical background that is needed to do this laboratory course.

2.1 Force-Extension Behavior of Molecules

In order to describe the response of constant forces acting on biological macromolecules, we would like to introduce two typical 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, consisting of rigid segments, while the other model (WLC) considers the molecule as an isotropic rod that is continuously flexible.

2.1.1 Freely-Jointed Chain Model (FJC)

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

$$L = N \cdot l$$

$\vec{R}$ is the end- to- end vector of the molecule, describing 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 2.1.1. 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:

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

Where θ is the angle between one monomer and F the force acting in z-direction.

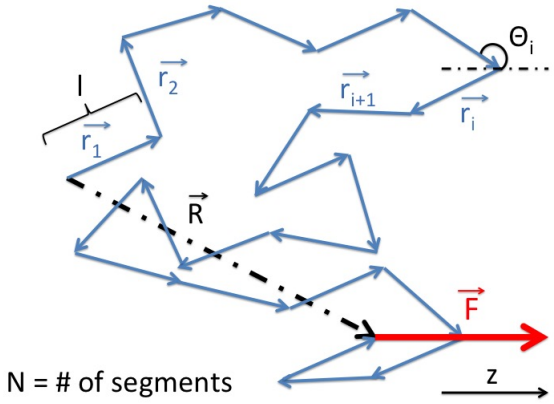


Figure 2.1.1: In the FJC model a polymer is considered to consist of N rigid segments with length l. Also shown in this figure is the definition of the end- to-end vector $\vec{R}$, which which is spanned from the first segment to the last segment of the polymer.

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 and inserting equation (2.1.1) leads to the following result:

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

Where k 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:

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

Inserting this to the definition of the *Gibbs Free Energy (G)*: *-kT · ln(Z)* the extension z of the molecule due to the force $\vec{F}$ acting on it can be calculated:

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

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 at equation (2.1.4) step by step.

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 2.1.4.

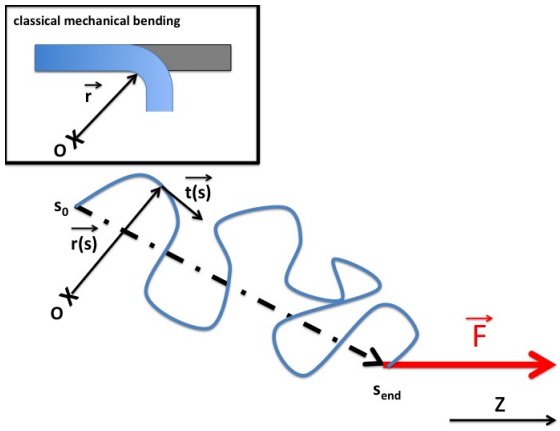


Figure 2.1.4: The worm-like chain is comparable to the classical mechanical bending of a rod, see black box on the top left. The molecule of length L_c is parametrized by points s on the molecule. Vector $\vec{r}$(s) points to the chain whereas $\vec{t}$(s) is the tangential vector to point s.

The chain consists of segments s with length ds, with s element $[0, L_c]$, 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. Therefor the formula of the energy E^{WLC} ends up in an integral, integrating over the whole length of the molecule:

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

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 second, one calculates the partition sum. 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. Here, we sum the mathematics to a minimum. Consider that the polymer behaves like a bar that is bend by a force $\vec{F}$ (classical mechanics), see Figure 2.1.4, 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 A = kT $\cdot$ L_P. L_P is called 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 percent agreement to the exact solution:

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

Where f is the force acting on the molecule, L_P is the persistence length, z is the extension and L_C is the total 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: How many fitting parameters does Equation 2.1.8 have? Is this formula an exact solution or is it an approximated solution?

Question 6: What is the persistence length? Is the persistence length temperature dependent (think of spaghetti: is the persistence length the same before and after cooking it)? What do you think is the persistence length of ds DNA?

2.2 Double-stranded DNA under Force and Torque

In this Chapter we explain how the stretching force on the bead is calibrated in a magnetic tweezers setup and how DNA reacts to induced torques.

2.2.1 Force Calibration in a Magnetic Tweezers Setup

In a magnetic tweezers setup, magnets exert a magnetic field and thus a magnetic force on a magnetic bead. This bead is attached to one end of the DNA molecule (via multiple biotin-streptavidin linkages), while the other end is attached to the bottom of the flow cell via antibody binding, see Figure 2.2.0. The bead transfers the force to the molecule: it can be stretched and twisted simply by controlling the magnets position in translation and rotation.

The stretching force is calibrated using the fluctuations of the magnetic bead in solution, which are caused by Brownian motion. The setup can be seen as an inverted pendulum, see Figure 2.2.0.

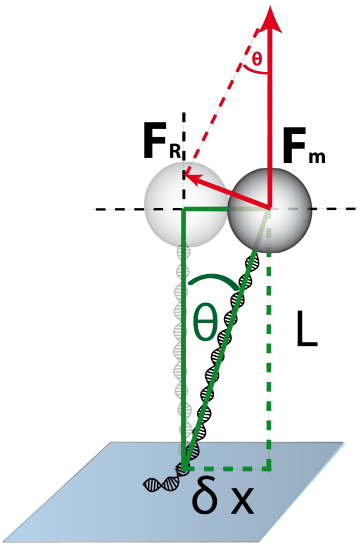


Figure 2.2.0: The magnets exert a force $\vec{F}_m$ on the magnetic bead. The force can be calibrated using the deviation δx of the bead from its original position, which is due to Brownian motion. The length of the molecule is labeled with L, the angular deviation is called Θ and $\vec{F}_R$ is the 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 the force is defined.

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

We find that the bead is in a harmonic potential with spring constant $\kappa = \frac{F_m}{l}$. The system has only one degree of freedom and therefore we derive at:

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

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

$$F_m = \frac{l \cdot k_B T}{(\delta x)^2} \tag{2.2.3}$$

In order to calibrate the force, one has to measure the xy-movement of the bead (several times of its characteristic time).

Question 6: What is Brownian motion?

Question 7: What is the characteristic time of a system? In this case: does the characteristic time of the system depend on the stretching forces?

Question 8: How could one increase the forces in an MT setup?

2.2.2 Inducing Twists on DNA Molecules

MT are able to induce twists on DNA molecules. DNA is a right handed molecule and behaves (for forces larger 1 pN) different upon the direction of applied number of turns n. Positive turns twist DNA with its helical nature, while negative turns twist the molecule against its helical nature. The response of DNA to applied turns depends on the stretching force. For forces smaller 1 pN, DNA behaves symmetric according to the applied number of turns, see Figure 2.2.3A (left column). For a small number of turns the molecule's extension stays constant, while the molecular torque increases linearly. Beyond the buckling transition (indicated with blue squares for 0.9 and 1.1 pN) the molecule's extension decreases linearly, while the molecular torque stays constant. At the buckling transition it is energetically more favorable to form DNA plectonemes as indicated by coarse-grained simulations in 2.2.3B in contrast to staying overwound. In the post-buckling regime every turn of the molecule leads to the formation of a DNA supercoil. For higher stretching forces ($>$ 1pN) the respond of DNA upon the direction of applied turns is asymmetric. While in general the buckling point shifts to a larger number of applied turns with higher stretching forces, there is no buckling point in negative direction, see Figure 2.2.3A (right column). Instead torque-induced melting occurs at -10 pN·nm. Torsional stress is hereby released by local basepair breaks.

At the buckling point (n_b) the DNA's extension is reduced due to the formation of supercoils. The change in the end-to end distance corresponds to 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.

At the buckling point (n_b) the twist energy $E_{torsion}$ of the molecule corresponds to the energy that is needed to form such 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 can be seen as a circle with radius r. The work, needed to reduce the length of the DNA molecule against the force F is

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

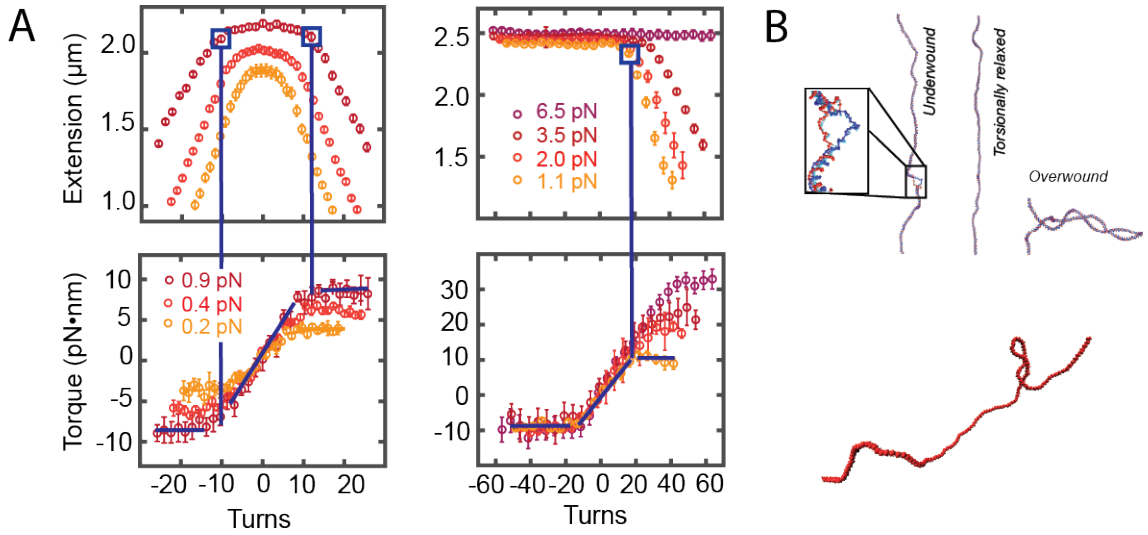


Figure 2.2.3: (A) Extension (top) and torque vs. applied number of turns (bottom) for different stretching forces. Buckling points (n_b) are indicated for 0.9 pN and 1.1 pN in the extension vs. turns plot with a blue square. (B) Coarse-grained DNA simulations show torsionally relaxed DNA, underwound DNA (upon torque-induced melting at -10 pN·nm) and overwound DNA with the formation of DNA plectonemes. References: Kriegel et al. Nucleic Acids Res. 2017 "Probing the salt dependence of the torsinal stiffness of DNA by multiplexed magnetic torque tweezers" and Kriegel et al. Nucleic Acids Res. 2018 "The temperature dependence of the helical twist of DNA".

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.

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

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

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 ds DNA behave (referring to the buckling point)?

Question 11: Where is/where are the buckling point(s) in Figure 2.2.3?

Chapter 3

Setup

This Chapter describes the experimental setup, the software used to control the instrument, and the fabrication of the flow cells used in this experiment.

3.1 Experimental Setup

In MT the molecule of interest is attached to the surface of a flow cell and on the other side to a superparamagnetic bead (Figure 3.1.0). A pair of permanent magnets placed above the flow cell exert magnetic fields, which in turn induce magnetic forces on the superparamagnetic bead. The stretching force can be controlled by changing the permanent magnets position in translation. The molecules rotation can be controlled by rotating the magnets. The flow cell is mounted on a flow cell holder, such that the flow cell is placed on top of the oil objective. The objective itself can be moved up and down very precisely using a piezo, which is in particular needed to perform a "look up table" for z-tracking. To control liquid exchange a pump is connected to the flow cell. A LED monochromatically illuminates the flow cell through the gap (1 mm) of the magnet assembly. Pictures of the illuminated area are taken by a CMOS camera and transferred to a computer where image analysis is performed. The tether itself cannot be seen in MT, that is why the beads are tracked and used for data analysis.

Measurements of the bead are performed out-of focus. The diffraction pattern of the bead is used in order to track the tether extension along z (Figure 3.1.0). A calibration of the bead's diffraction pattern is performed prior to the measurement. Therefore the magnets are brought close to the flow cell to exert large stretching forces which cause small fluctuations of the bead itself. The position of the objective can be controlled by a piezo. In oder to create a "look-up table (LUT)", the objective's position is moved in nanometer steps (e.g. 100 nm) and the bead's diffraction pattern is captured. To get precise information in vertical direction the software interpolates between each picture and creates

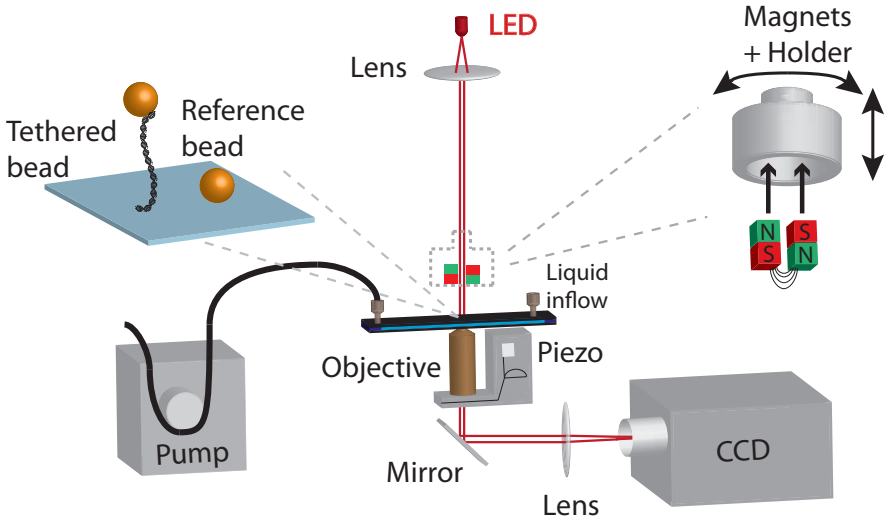


Figure 3.1.0: Overview of a Magnet Tweezers Setup. A pair of permanent magnets is placed on top of the flow cell. The light of the LED illuminates the sample from above. The light is directed to a camera via a mirror and a lense.

the LUT. During the measurement the piezo is kept at a fixed position and the current diffraction pattern of the beads are compared to the LUT, which returns the extension of the molecule. For the lateral position of the bead, the center of the diffraction rings are calculated via cross-correlation.

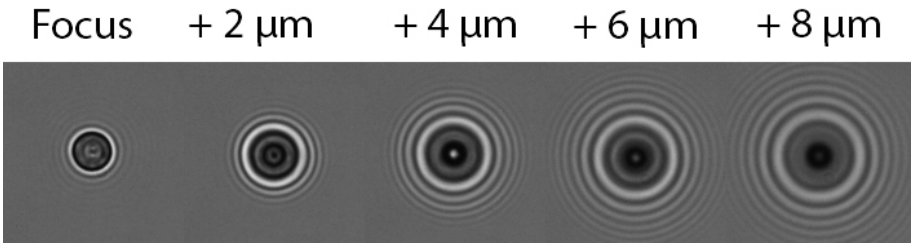


Figure 3.1.0: Tracked pictures of magnetic particles. The pattern of the diffraction rings changes with distance to the focus. This feature is used to determine the molecule's extension.

To reduce effects of mechanical drift, which can disturb the measurements in lateral as well as in vertical direction, a reference bead is tracked. Reference beads are beads that are stuck to the surface of the flow cell. To correct for drift, the recorded trace of the reference bead is subtracted from bead's that are attached to DNA molecules. Note: saved values in this course are without subtraction of the reference bead.

You are asked to perform a force calibration of the MT setup as well as force-extension measurements for DNA molecules. Figure 3.1.0 illustrates the forces for each height of the magnets used in this laboratory course and can be used to write measurement protocols.

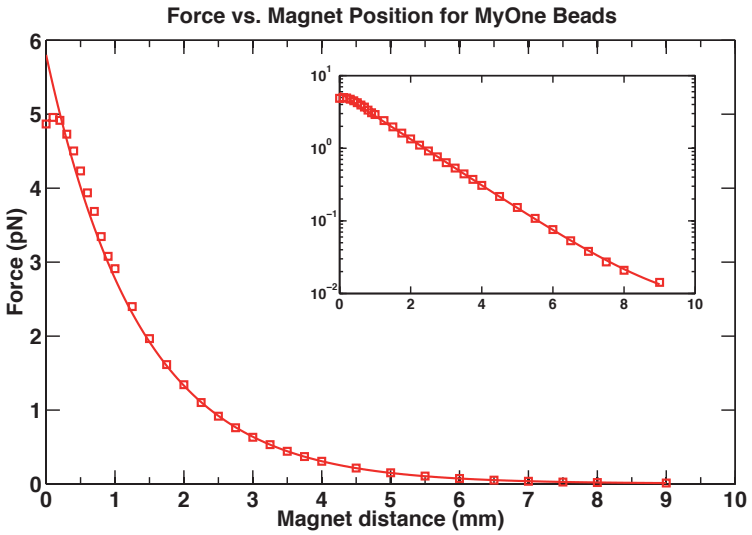


Figure 3.1.0: Force vs. magnet position calibrated for the setup used in this laboratory course and for MyOne beads. The solid line is a double-exponential fit to the measured values.

3.2 Software

The software to control the instrument is written in LabVIEW. The piezo stage and both motors to control the magnets (translation and rotation) can be controlled using the software. The pump to exchange fluids has to be used manually.

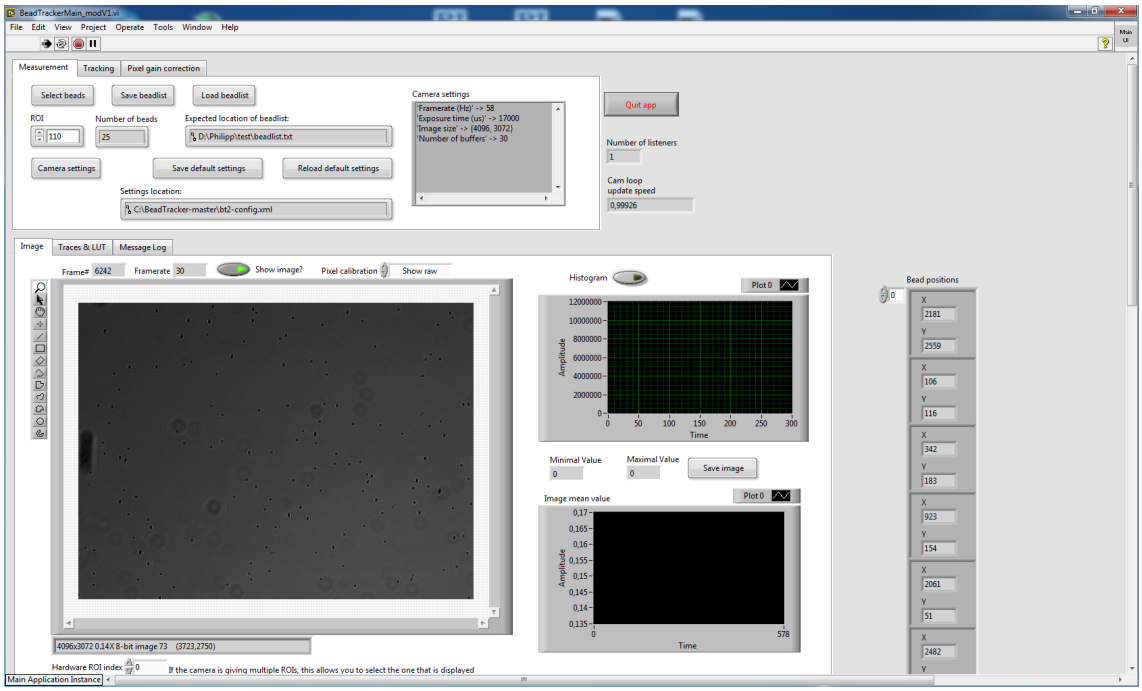
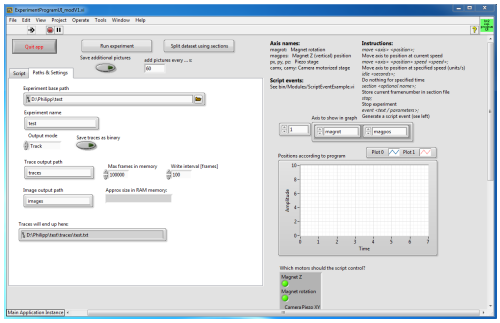


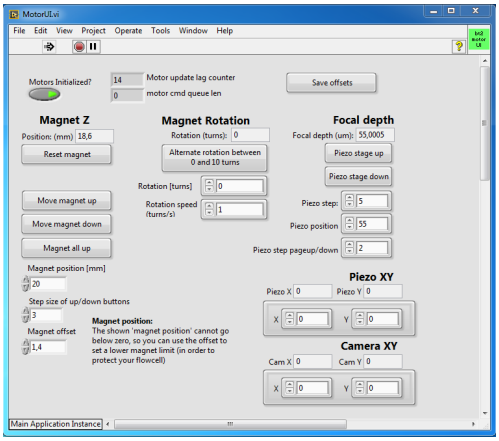
Figure 3.2.0: "BeadTrackerMain.vi" is the main window of the software. It contains a real time image, as well as the option to select for beads and perform a LUT.

The software consists of three separated windows (see Figure 3.2.0 and 3.2.0). A live picture of the field of view (FOV) of the flow cell is shown in "BeadTrackerMain.vi" window. Basic setup configurations, selection of beads and the LUT are made in this window. Motors and the piezo stage are controlled in "MotorUI.vi". "ExperimentProgramUI.vi" contains all options to start and control measurements.

The bead positions are tracked in real time and saved in text files including the motor positions. Another text file contains the "sections" frame number written in a protocol (see Figure 4.2.2).



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

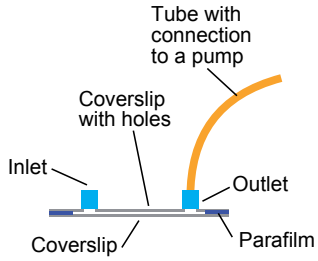


(b) With "MotorUI.vi" the piezo-stage as well as the two motors for the magnets can be controlled manually.

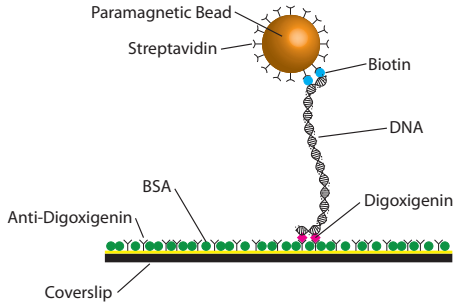
Figure 3.2.0: "ExperimentProgramUI.vi" and "MotorUI.vi" to start measurements and to control motor positions, respectively.

3.3 Flow cell and Attachment Chemistry

The flow cell (Figure 3.3.0 a)) provides the measurement chamber for Magnetic Tweezers. Two glass cover slips separated by a soft melted parafilm spacer provide a measurement chamber. The upper cover slip has two holes for fluid handling. The DNA has two handles serving as attachment fragments. Each handle is approximately 600 bps long and contains several nucleotides with biotin (biotin handle) or digoxigenin labels (dogoxigenin handle). The biotin handle binds to a streptavidin coated superparamagnetic bead and the digoxigenin labeled end of the DNA binds to the anti-digoxigenin coated surface of the flow cell (Figure 3.3.0 b)). To enable for rotation DNA molecules, multiple biotin and streptavidin moieties are used. To avoid unspecific binding of the bead to the surface, the cover slip is passivated with BA blocking solution (or BSA).



(a) A schematic illustration of a flow cell. Two coverslips and a parafilm layer form a measurement chamber. Flow cells are placed in a floe cell holder that is connected to a pump for fluig exchange. reservoir.



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

Figure 3.3.0: Flow cell construction and attachment chemistry for (typical) MT measurements.

Chapter 4

Measurements

This Chapter guides you through the experiments today. Read the text carefully, tray to follow the manuscript and ask the tutor if you have questions.

4.1 Preparation of the Flow cell

The flow cell was already coated with anti-digoxigenin and stored over night with a BA-blocking solution. Before you can start the experiments you have to prepare the flow cell by yourself. You have to prepare a DNA-Bead solution, give this to the flow cell, flush with buffer such that you remove unbound beads from the cell and insert magnets. A step-by step protocol follows.

Make sure a tutor is present and get familiar with the instrument. Before you start with the following protocol, remove and remount the magnet holder. Furthermore, try to move the z-motor and rotation motor. 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 180 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

Get a tutor (if necessary) for the following steps!

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

Within the next step you have to select for beads that are bound to a single, coilable DNA molecule.

- Move the piezo-stage (window "MotorUI.vi") to get the beads in focus (ideally you find two foci one for beads attached to DNA and one for beads unspecifically attached to the surface of the flow cell)
- Choose the beads that have DNA attached
- Move the piezo-stage 8 μm upwards
- Search in the field-of-view in window "BeadTrackerMain.vi" for two beads which are not moving, i.e. unspecifically attached to the surface, and whose interference rings are not overlapping. Remember their positions. These beads 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. Make sure that the squares that the program puts around the beads is fully visible in the field of view. Do not use beads that are too close to the edge. LabVIEW will crash!
- Press "close" to close the window

For z-tracking a LUT has to be calibrated for every single bead:

- If necessary: get a tutor
- 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
- In case you are asked "QTrk DLL path...", press two times "ok" in the windows that popped up

The setup is now ready to track beads. To ensure that tracked beads are attached to a single and coilable DNA tether, we perform three tests prior to the actual measurements:

- 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
- Wait until the camera reacts (frame number in this window) before you click on any other button. LabVIEW is "slow".
- Move the motor to 12 mm distance, wait 10 seconds and drive back to 0.3 mm
- Stop measurement ("abort experiment")
- 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
- What do you expect according to Figure 2.2.3? Check the traces for beads who did not behave as expected and delete them with "Discard bead"
- Change the measurement name for the third test
- Rotate -20 turns at a distance of 4 mm (0.5 pN) and back to 0
- What do you expect according to Figure 2.2.3? Check the traces and discard beads.
- Never discard the last bead. This can crash LabVIEW. If the last bead is not usable in your case, make a note in you lab book and ignore the data in the analysis at home

If you have less than two good beads left, contact your tutor.
Do not discard beads anymore!

4.2 Experiment 1: Force-Extension Measurement

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. Do not discard beads anymore.

In a second file, determine the force for varying magnet heights for all DNA molecules, similar to Figure 3.1.0. 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 $\epsilon = 10\%$.
An example how to write a protocol is given in Figure 4.2.2. Start with the highest force in your protocol. Why is this clever? Before you can run your script, you have to press "Simulate script" in the "ExperimentProgramUI.vi".

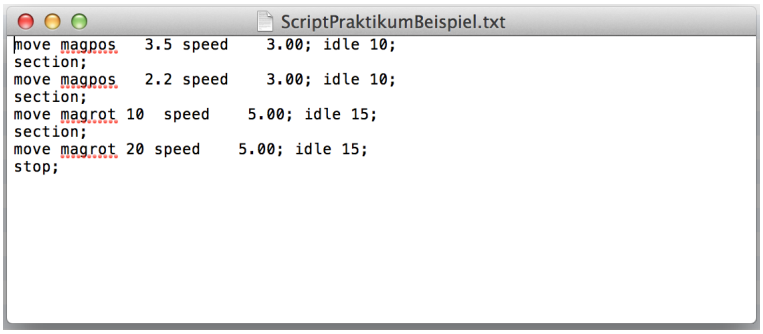


Figure 4.2.2: Example of a script for a measurement protocol. The first two lines move the z-motor of the magnets, the values "magpos" are the absolute distance to the flow cell. The number behind "idle" defines the time to record frames in seconds. The third and fourth line rotate the motors. "magrot" 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 Measurement

Here, you should test DNA tether extension when turning the magnets. 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 0.4pN. Start with the highest force. Why is this clever? Use Figure 3.1.0 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 Measurement

- Create a plot as shown in Figure 3.1.0. 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 basepairs is the DNA molecule supposed to be stiff? Compare your values to literature. Which model describes better the behavior of DNA?

5.2 Analysis Experiment 2: Rotation-Extension Measurement

- 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 DNA