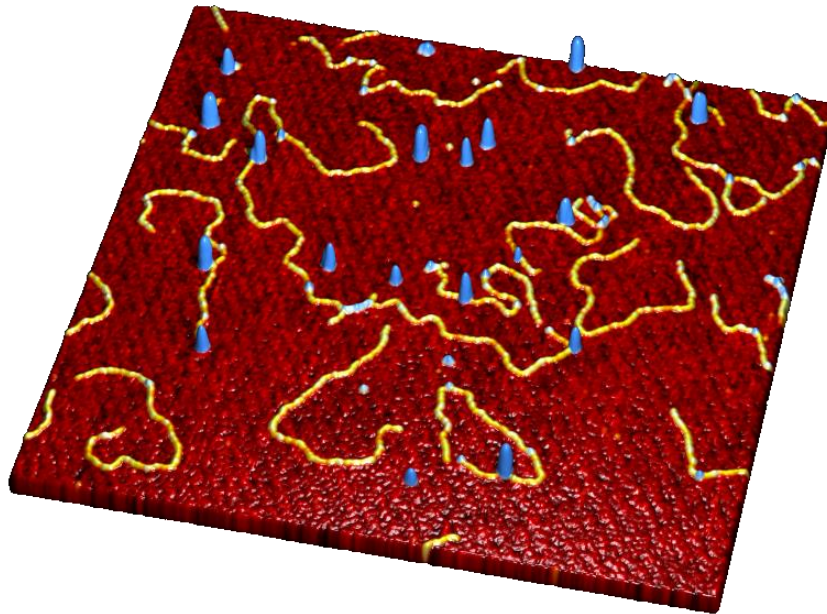




Atomic Force Microscopy (AFM)



Instruction Manual

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I. Preparation (at home)

In order to conduct this experiment, knowledge of the following topics should be acquired by reading this instruction manual:

- General principle and setup of the AFM
- Principle of piezo actuators
- Theory of the optical deflection detection method
- Imaging in contact mode
- Tip-sample interactions
- Interpretation of force curves
- Theory of bending and vibration modes of cantilevers

This knowledge can be acquired using this instruction manual and the following literature, which can be borrowed from the instructor:

- Einführung in die Raster-Kraftmikroskopie. Uni Tübingen, 2013.
- A Practical Guide to Scanning Probe Microscopy. Bruker Corporation, 2005.
- Mironov, V. L., Fundamentals of Scanning Probe Microscopy. The Russian Academy of Science: Nizhniy Novgorod, 2004.
- www.bioforce.uni-tuebingen.de → Lehre/Teaching → Kraftkurven-Simulationen/Force Curve Simulations.
- <http://www.ntmdt.com/spm-principles> (has Flash animations).
- Bhushan, B., Springer Handbook of Nanotechnology. Springer: Berlin, 2004.

You should be able to answer the following questions before the experiment:

- How do an AFM and its components (piezo actuators, optical detection) work?
- Which are the most important imaging modes? What are their advantages / disadvantages?
- How does the tip-sample feedback work during AFM imaging in the contact mode?
- How does the typical shape of a force curve come about, and when do instabilities ("jump-in", "jump-out") occur?
- How does a force curve look on a stiff sample and on a soft sample?

1. Introduction

The era of scanning probe microscopy was launched in 1981 with the construction of the first scanning tunneling microscope (STM) by Binnig, Rohrer, and coworkers.¹ This microscope is based on the measurement of tiny tunneling currents between a sharp metal probe and a flat metal sample. For the first time, individual atoms could be laterally resolved on conducting surfaces. But more than that: the STM has triggered the development of a

large family of new microscopes. These so-called scanning probe microscopes (SPM) are all based on a small, locally confined probe that is sensitive to various kinds of physical quantities. In 1984, Pohl *et al.* invented the near-field scanning probe microscope (SNOM) that enables optical imaging of surfaces with sub-wavelength resolution.² In 1986, the same year when the Nobel Prize in Physics was awarded to Binnig and Rohrer for the invention of the STM, Binnig *et al.* invented the atomic force microscope (AFM), sometimes also denoted as scanning force microscope (SFM).³ This microscope is based on the measurement of minute forces with the help of a flexible cantilever. One of its main applications is the creation of high-resolution topographic images of surfaces, where it has the advantage over STM of not requiring electrically conducting probes and samples. Several related scanning probe microscopes have been developed afterwards, including the magnetic force microscope (MFM),^{4,5} the electric force microscope (EFM),⁶ the scanning electrochemical microscope (SECM),⁷ and the scanning ion conductance microscope (SICM).⁸

Soon after its invention, the AFM was used to image samples in a liquid environment.⁹ Since then it has evolved as a powerful tool not only in physics, but also in chemistry and biology.¹⁰ The AFM has the capability to acquire high-resolution images on length scales from single molecules up to living cells. The great advantage of the AFM over many other high-resolution imaging techniques is its flexibility with respect to the imaging conditions. It is capable of imaging biological objects in their aqueous environment, and it can resolve structural details at sub-nanometer resolution.^{11,12} By repeatedly scanning the same sample area, dynamic processes such as the enzymatic degradation of single molecules,¹³ the activation of human platelets,¹⁴ or polymerase activity¹⁵ have been resolved. But not only the imaging, but also the force sensing capabilities of the AFM have been employed.^{16,17} Force spectroscopy measurements are used, for example, for the characterization of sample stiffness,¹⁸ surface charges,¹⁹ and solvation forces.²⁰ The field of single-molecule force spectroscopy²¹ evolved after it became apparent that the AFM can measure the interaction forces between individual ligand-receptor pairs²²⁻²⁴ and that it can mechanically unfold macromolecules.²⁵⁻²⁷

Methodological improvements have been the key to many of these advances. While the detection of cantilever deflections in the original AFM was achieved with an STM tip placed directly above the cantilever,³ optical interferometry was soon later used to allow for remote deflection sensing.^{28,29} Eventually, the optical beam deflection method emerged,^{30,31} surpassing other methods in simplicity. The vast majority of all commercially available AFMs today are based on the optical beam deflection method (Figure 1). The development of the tapping mode³²⁻³⁴ has enabled the imaging of weakly immobilized and soft samples that could not be imaged before.^{13,35} Single-wall carbon nanotube AFM tips promise to boost the lateral resolution to below 0.5 nm.^{36,37} But while substantial progress in AFM technology and biological applications has been made, there are many intriguing challenges that remain.

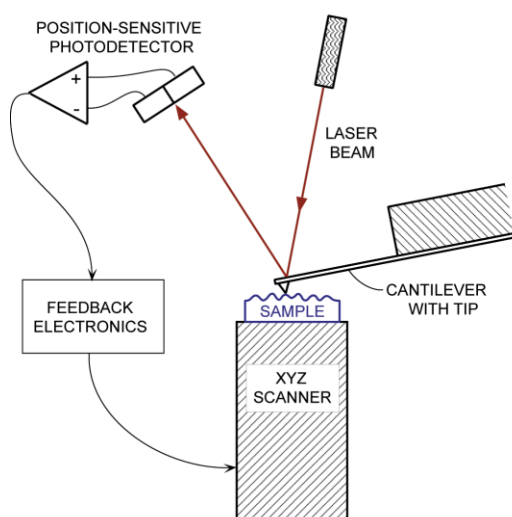


Figure 1. Schematic of an atomic force microscope (AFM). A sharp tip is attached onto the free end of a flexible cantilever. This tip is brought into contact with a sample surface and is scanned across it. Interaction forces between the tip and the sample cause the tip and thus the cantilever to deflect. This deflection is sensed by an optical laser beam, which is reflected by the cantilever and projected onto a position-sensitive photodetector. This detection method allows for the remote and non-invasive detection of tip deflections with sub-nanometer resolution.

A first challenge is to increase the lateral (1), the vertical (2), and the temporal (3) resolution of the AFM. (1) The lateral resolution on biomolecules is currently on the order of 0.5–1 nm.³⁸ It does not only depend on the size of the tip apex that contacts the sample, but also on the properties of the medium between sample and tip¹² and on the sample support.³⁹ Sample preparation is therefore still one of the limiting steps for obtaining high resolution images. (2) Vertical resolution of the tip position is one of the great strengths of the AFM. Values of 0.1 nm are currently obtained on biomolecules.³⁸ Closely related to the vertical resolution is the force resolution, which is relevant for single-molecule force spectroscopy measurements. It is currently limited to 5–10 pN, enough to resolve inter- and intra-molecular unfolding events, but not enough to resolve refolding events that occur at even smaller forces (<2 pN).²¹ The vertical resolution is fundamentally limited by the thermo-mechanical noise of the cantilever⁴⁰ and by the sensitivity of the deflection detector.^{41,42} Low-noise cantilevers and detectors are therefore needed. (3) The temporal resolution of the AFM is usually on the order of minutes, the time required to acquire one image. This is quite slow for the investigation of bio-molecular interaction processes.^{15,43} Fast AFM imaging and the recording of dynamic image sequences is therefore of particular relevance.

2. Fundamentals of the AFM

2.1. Principle

The principle of the AFM is based on the interactions between a force sensor and a surface. The force sensor is usually a micro-fabricated cantilever⁴⁴ brought close to the probe's surface using a micro-positioning device (see Figure 2). The scan distance between sensor and surface depends on the operating mode (see chapter 2.3) and the kind of interacting forces that cause a bending of the cantilever.

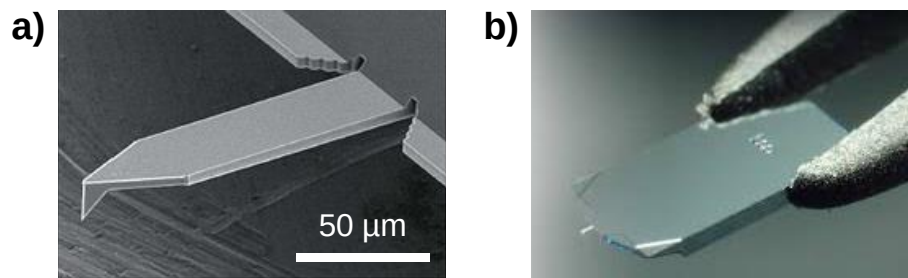


Figure 2. (a) Scanning electron microscopy images of rectangular AFM cantilever (Olympus OMCL-AC160TS) and (b) photograph of tweezers holding a cantilever chip (upside down) (source: www.olympus-global.com).

The cantilever deflection can be determined with Hook's law (see section 2.5.2):

$$F = k \cdot x . \quad (1)$$

Since the forces' magnitude is of the order of $10^{-9} \text{ N} = 1 \text{ nN}$, a deflection of about 1 nm can be expected for a spring constant of 1 N/m. By using different kinds of deflection measurements (e.g. tunnel-current detection,⁴⁵ capacity measurement, deflection of a laser beam,³⁰ and interferometry⁴⁶) the bending of the force sensor can be measured at high sensitivity and bandwidth. If the surface is moved in a raster-pattern in respect to the force sensor and the deflection is kept constant using a control loop, the control loop's output signal mainly represents the surface's topography. Material-dependent contrast and other effects should be ignored at first. The AFM is thereby able to visualize macroscopic structures at a range of 100 μm ⁴⁷ as well as atomic structures.⁴⁵

For the high-resolution measurement of microscopic structures it is necessary to minimize the interaction area between cantilever and surface. Therefore, a micro-tip is attached to the end of the cantilever, to achieve a higher lateral resolution of the AFM.

By choosing a specific type of material for probe and sensor or the scan distance several kinds of forces can be detected (e.g. van der Waals forces,⁴⁶ repulsive forces, or magnetic forces⁴⁶). Even different individual forces can be distinguished, if they occur at the same time, because they often have different distance dependence.

2.2. Types of tip-sample interactions

Many different forces act between tip and sample in AFM measurements. Examples are:

- Long-range electrostatic interactions between tip and sample, which act as a capacitor.
- Van der Waals interactions between the atoms or molecules of tip and sample, which can be further separated into:
 - forces between permanent dipoles: dipole interaction between constantly polarized molecules ("Keesom forces")
 - force between permanent dipoles and induced dipoles: induced forces

- starting at one neutral molecule which gets polarized by the permanent dipole of another molecule (“Debye forces”)
- forces between induced dipoles: statistic changing forces between atoms/molecules which are neutral in the mean over time. But in shorter time frames they show a dipole momentum. This short dipole moment can polarize another neutral atom/molecule (“London forces”)
- Repulsive (Pauli) interactions for tip-sample distances below 0.2 nm, when the electron’s orbitals would overlap, which would violate the Pauli principle.

2.2.1. Adhesion and meniscus force

When performing AFM measurements in air under ambient conditions, the tip-sample interaction is dominated by the water meniscus forming at the contact point (see Figure 3). The adhesion force between a sphere of radius R and a plane is given by⁴⁸

$$F = 2\pi R\gamma(\cos \vartheta_1 + \cos \vartheta_2) \quad (2)$$

with $\gamma = 0.073 \text{ J/m}^2$ being the surface tension of the water at room temperature and ϑ_1 and ϑ_2 are the contact angles of the liquid with the two solids. For hydrophilic surfaces ($\vartheta_1, \vartheta_2 \approx 0$) this simplifies to⁴⁹

$$F = 4\pi R\gamma \quad (3)$$

Some conclusions can be taken out of this:

- (1) The attractive capillary force is another static component of the total force and, therefore, is an additional load for the probe. Since the geometric setup at the tip is not known, the total load of the sample is vague.
- (2) The image formation can be changed on hydrophobic regions of the sample (i.e. areas where water hardly condenses) during scanning.

Thus, the capillary force is the minimal imaging force for AFM in air. This imaging force might be too high for sensitive biological objects, so that they can only be imaged in fluid.

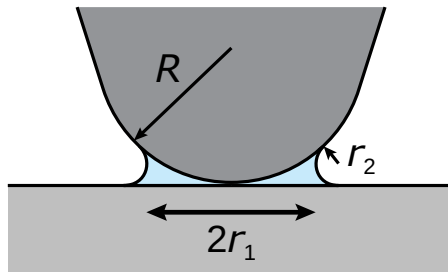


Figure 3. Schematic illustration of the meniscus shape. For small contact angles, the meniscus curvature is given by $r_2 \cong r_1^2/4R$. Using the Young-Laplace equation with $r_2 \ll r_1$, the interface pressure $\Delta p \cong \gamma/r_2$ can be then used to estimate the adhesion force $F \cong \pi r_1^2 \Delta p \cong 4\pi R\gamma$, giving Equation (3).

2.2.2. Force-distance curves

The tip-sample interactions can be studied with the AFM using a so-called force-distance curve (or short “force curve”). During a force curve the tip is approached to the sample until a pre-defined maximum force (“trigger threshold”) is reached and then retraced again, while the deflection of the cantilever is recorded as a function of the vertical position of the sample with respect to the cantilever base (or the cantilever with respect to the sample). The deflection d can then be transformed into the force acting on the cantilever, $F = k \cdot d$,

using the spring constant k . In an AFM force curve on a sample with adhesion, four different regimes can be identified (Figure 4):

- (1) At the beginning, the tip is above surface out of contact and the cantilever deflection is constant (zero).
- (2) After the tip contacts the sample the cantilever deflection increases linearly. Moreover, the deflection increases just as the vertical position (i.e. force curve has a slope of 1). This fact is also commonly used to calibrate the sensitivity of the optical detector (to get a relation between the detector output in Volts and the actual cantilever deflection in nanometers). Sometimes, a small minimum can be observed, when the tip jumps into contact with the surface (“snap in”).
- (3) During retracting the cantilever, the tip sticks to the sample due to the adhesion force and is released from the sample, when the force pulling the cantilever up exceeds the adhesion force (“snap out”).
- (4) Finally, the tip is out of contact and the cantilever deflection is constant (zero) again.

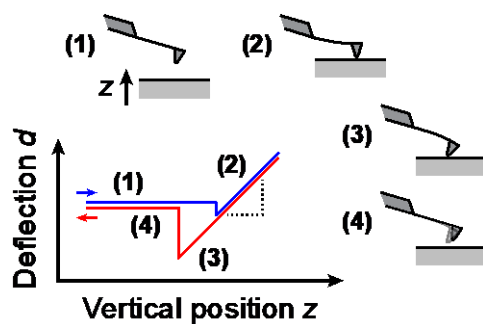


Figure 4. Schematic AFM force curves on a stiff sample with adhesion with approach part in blue and retract part in red (slightly offset for visibility reasons).

2.2.3. The Cone-Plane Contact Model and Force-Indentation Curves

The AFM can also be utilized to measure the mechanical properties of samples⁵⁰ on the nanometer scale using contact models⁵¹ to describe the tip-sample interaction. The contact model for a cone-plane contact (Figure 5) relates the loading force F and the indentation depth δ by

$$F(\delta) = \frac{2}{\pi} \frac{E}{1 - \nu^2} \tan \alpha \cdot \delta^2, \quad (4)$$

where α is the half cone angle of the indenter and E is the Young's modulus (or elastic modulus) and ν the Poisson's ratio of the sample. For the elastomer used here, a Poisson's ratio of 0.5 can be assumed.⁵²

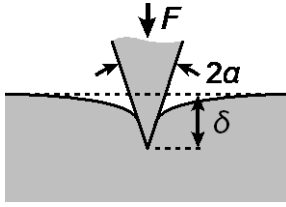


Figure 5. Schematic of the contact model for a cone-plane contact. A rigid cone-shaped indenter with half cone angle α is penetrating an elastic body by a indentation depth δ due to a loading force F .

In AFM, however, the force and the indentation are not accessible directly, but the cantilever deflection and the vertical position of the sample with respect to the cantilever base (or the cantilever with respect to the sample) is measured during a force curve. Figure 6 shows schematic force curves on a stiff (a) and on a soft sample (b). The deflection is constant when the tip is out of contact (left side of the force curve). After the contact, on a stiff sample the deflection increases with a slope of 1 (Figure 6a). On a soft sample, however, the deflection increases more slowly (Figure 6b). To obtain the same cantilever deflection (and therefore the same force) as on a stiff sample, the sample has to be moved by an additional distance δ , which corresponds to the indentation depth of the tip into the sample surface. Therefore, the indentation δ can be calculated (including a constant offset) by

$$\delta = z - d. \quad (5)$$

The force can be easily calculated using Hooke's law from the deflection

$$F = k \cdot d, \quad (6)$$

using the cantilever's spring constant k .

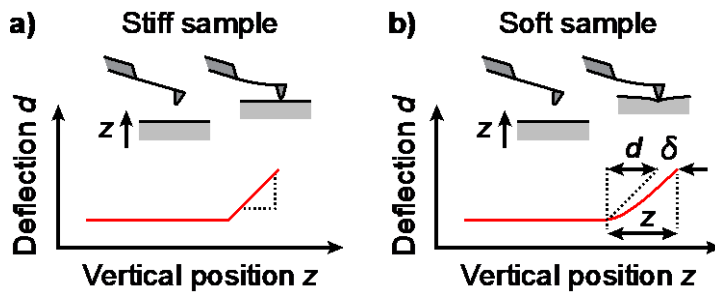


Figure 6. Schematic AFM force curves on (a) a stiff and (b) on a soft sample (for simplicity here shown without adhesion).

2.2.4. Friction Forces

Besides vertical forces, lateral friction forces frequently occur in static AFM in contact mode. They can be categorised by their orientation parallel or perpendicular with respect to the cantilever axis.

Parallel acting friction forces cause an additional vertical bending of the cantilever, depending on the tilt and on the roughness of the sample. The size of the so-called parasitic shift depends on the cantilever stiffness and is in the range of some Å's to some nm's. When

the scan direction is parallel to the cantilever axis (usually defined as 0°), this effect can cause errors in the topography image, especially at topography steps (Figure 7). These errors can be avoided choosing the scan direction as 90° with respect to the cantilever axis.

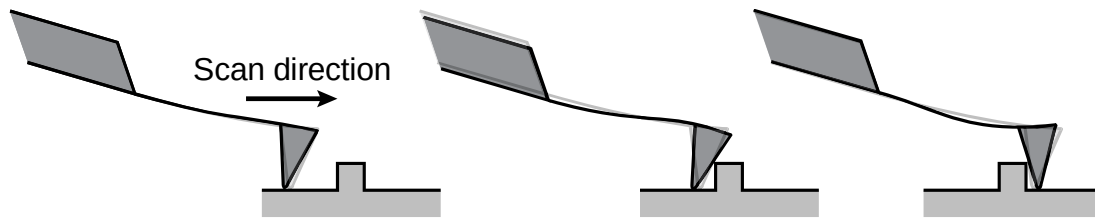


Figure 7. Schematic illustration of a parasitic shift

Lateral friction forces acting perpendicular to the cantilever axis result in a torsion of the cantilever (Figure 8, see section 2.5.2 for details). Since vertical and lateral forces are then decoupled from each other, there is no parasitic shift affecting the imaging. Furthermore, measuring the torsion of the cantilever allows for estimating the friction force during imaging, which is frequently used in AFM and denoted as lateral force microscopy (LFM).

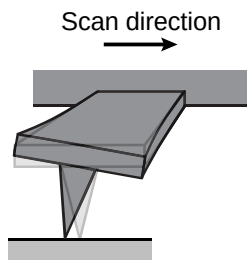


Figure 8. Schematic illustration of the AFM cantilever being twisted by lateral forces.

2.3. Static AFM Imaging Modes

In the static AFM modes cantilever is hold fixed during scanning. The most relevant modes are:

1. Constant height mode
2. Constant force mode

2.3.1. Constant Height Mode

In the “constant height mode”, the cantilever is brought into the repulsive regime of the tip-sample interaction and hold on a constant height while being scanned over the sample. The tip directly follows changes in the sample topography, which is thereby directly represented by the deflection of the cantilever. The advantages of the imaging mode are simple technical implementation and high imaging speed. Some attempts in high-speed AFM imaging are hence performed in the constant height mode, but beyond that it is mainly of historic interest.

The disadvantages of this mode are however:

- The changing imaging force leads to varying elastic deformations of the sample when

imaging soft materials, especially biological materials, which results in errors in the topography image. Thus a quantitative interpretation can be complicated.

- Artifacts can occur due to discontinuities in the tip-sample interaction, which significantly impair the interpretation of the results.
- The resulting imaging force can vary within 2-3 orders of magnitude for samples with large height changes. This can result in a considerably fast tip wear and sample damage.

2.3.2. Constant Force Mode

The next more complicated AFM imaging is the “constant force mode”, also usually simply denoted as “contact mode”, since the the tip is permanently in direct contact with the sample. During scanning, a feedback loop is controlling the vertical cantilever position with respect to the sample to maintain the deflection at a given “setpoint”, which thereby keeps the tip-sample force constant (typically on the order of nN’s). The cantilever is then scanned in a line-by-line, raster-shape pattern across the sample. During that, the vertical cantilever position represents the topography of the sample (there usually denoted as the “height” signal). Advantages are the approximately constant imaging forces, limiting the tip wear and reducing errors due to elastic deformations of the sample. Disadvantages are, however, the limited bandwidth of the feedback system, which is usually dominated by the response of the piezo scanner’s vertical movement, and lateral friction forces acting on the sample.

2.3.3. Dynamic AFM Imaging Modes

In dynamical AFM imaging modes the cantilever is not at permanent contact with the sample, but oscillated at a frequency close to it’s resonance frequency, which is typically in the range of 10’s to 100’s of kHz, and the oscillation amplitude or frequency is used as input for the feedback loop. Compared to the static imaging modes, the vertical and especially lateral imaging forces are reduced, making dynamical imaging modes suitable for delicate samples. Due to experimental challenges it is used in the block course on the second day (for interest see section II.1).

2.4. Imaging Artifacts

Due to the finite size of the cantilever tip, the topography of the sample surface is not imaged without artefacts. Depending on the tip shape, either the sample surface or rather the tip itself gets imaged. These artifacts get the stronger when the sample has steep slopes or when the size of the sample structures are comparable to the tip radius (Figure 9). Since the resulting image is not a mathematical convolution between the sample surface and the tip shape, it is not possible to mathematically completely reconstruct the actual surface topography even when knowing the tipshape.

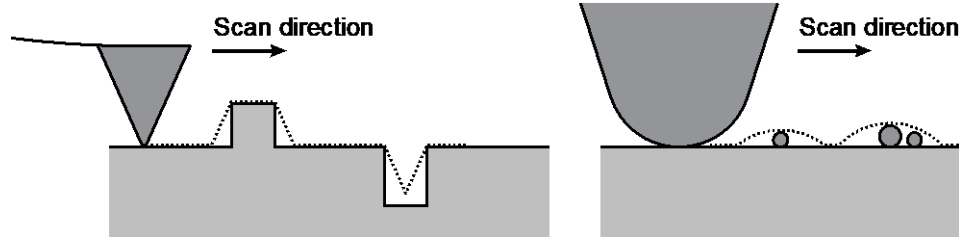


Figure 9. Illustration of imaging artifacts caused by the geometry of the AFM tip shape.

2.5. Experimental Setup

2.5.1. Cantilever

The cantilever is the most important part of the AFM setup and has the following requirements:

- Robust against external mechanical influences
- High lateral stiffness to avoid imaging artifacts
- High sensitivity to vertical forces (i.e. small spring constant)
- Sharp tip and small tip radius for high lateral resolution
- Large optical amplification (i.e. short cantilever) and high reflectivity when using optical detection, usually accomplished with a thin (for low added mass) reflection coating
- Reliable fabrication to allow for reproducible properties
- Availability of different materials and geometries of cantilever and tip for detecting different kinds of interaction forces
- Conducting (or at least not insulating) cantilever material to avoid charging effects

Usually it is not possible to fulfill all requirements with one cantilever. Thus its construction is oriented on the needs of a specific AFM setup or application and therefore also the sample.

2.5.2. Bending Theory of AFM Cantilevers

Rectangular and triangular force sensor types are the most common types for AFM cantilevers nowadays (Figure 10). Triangular force sensors are laterally more stable at small spring constants and, therefore, typically used for routine measurements of soft samples. Rectangular cantilevers are more affected by lateral forces, which on the other hand allows to detect laterally acting forces as friction forces simultaneously to the normal forces. Cantilevers are characterized by their:

- Bending line
- Spring constant (typical 0.01 - 10 N/m)
- Resonant frequency (typical 10 - 1000 kHz)

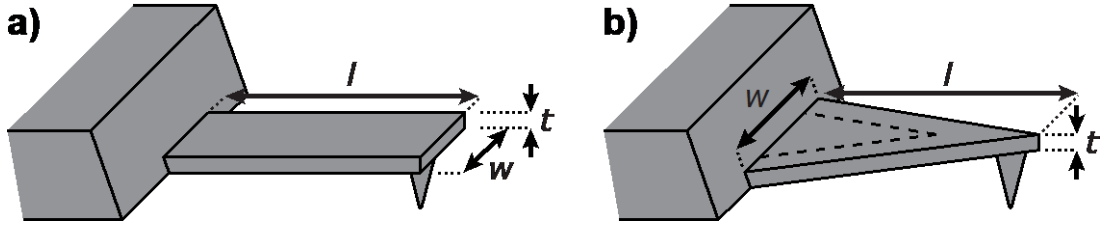


Figure 10. Schematic of (a) rectangular and (b) triangular AFM cantilevers with length l , (base) width w , and thickness t . The inner part of the triangular cantilever is assumed to be present for the analysis here.

Bending line

According to Euler-Bernoulli beam theory the bending line $y(x)$ is the deflection of the cantilever (see Figure 11) and results by integrating the differential equation⁵³

$$y''(x) = \frac{M(x)}{E I(x)} = \frac{F_z \cdot (l - x)}{E I(x)}, \quad (7)$$

where M is the bending moment by a vertical force F_z at the end of the cantilever and E is the Young's modulus of the cantilever material. $I(x)$ is the second moment of area, which can be specified for the cantilevers by:

$$\text{rectangular: } I(x) = \frac{1}{12} t^3 w \quad ; \quad \text{triangular: } I(x) = \frac{1}{12} t^3 w \frac{l - x}{l} \quad (8)$$

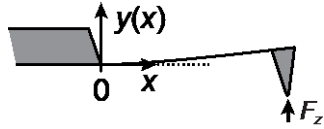


Figure 11. Bending line $y(x)$ of the cantilever with $x = 0$ and $x = l$ correspond to cantilever base and end, respectively, and a vertical force F_z acting on the cantilever end.

Integration of Equation (7) with the boundary conditions $y(0) = y'(0) = 0$ gives the bending lines:

$$\begin{aligned} \text{rectangular: } y(x) &= \frac{F_z}{E t^3 w} (6lx^2 - 2x^3) \\ \text{triangular: } y(x) &= 6 \frac{F_z l}{E t^3 w} x^2 \end{aligned} \quad (9)$$

Spring constant

The spring constant k can be calculated for both cases with help of Hooke's law as the ratio of applied force F_z over deflection of the cantilever end $y(l)$:

$$\text{rectangular: } k = \frac{F_z}{y(l)} = \frac{Et^3 w}{4l^3} \quad ; \quad \text{triangular: } k = \frac{Et^3 w}{6l^3}. \quad (10)$$

Torsional stiffness

A lateral friction force F_x acting perpendicular to the cantilever axis result in a torsion of the cantilever, i.e. an end of the cantilever gets rotated with respect to the other end along the longitudinal axis (Figure 12). The torsion angle φ (in rad) can be calculated⁵³ for a bar-shaped cantilever with

$$\varphi = \frac{M_T l}{G I_T} , \quad (11)$$

where I_T (sometimes also denoted as J) is the torsional (or torsion) constant, which is given⁵⁴ by $I_T \cong t^3 w/3$ for a rectangular cantilever (for $w \gg t$). $M_T = F_x \cdot h$ is the torsion moment of the force F_x acting laterally on the tip of height h and G the material's shear modulus. The lateral displacement is then $\Delta x = h \cdot \sin \varphi \cong h \cdot \varphi$ for small torsion angles.

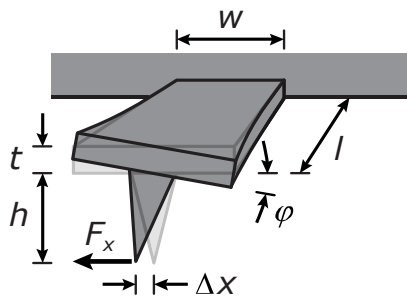


Figure 12. Schematic of rectangular cantilever with torsion by lateral force F_x acting on the cantilevers tip of length h .

2.5.3. Detector Types

The cantilever deflection can be detected by different methods. The oldest detector type is the **tunneling current detection**,³ where a metal tip is arranged at tunneling distance at the back side of the cantilever. A bending of the cantilever changes the tunnel current substantially due to the exponential distance dependence.

In other detector systems the cantilever is part of a capacitive system, whichs **capacity** is changed by the deflection of the cantilever.

The **laser beam deflection (LBD)** is a very reliable detection method,³⁰ which is also used in the AFM setup here. A light beam gets reflected at the mirrored backside of the cantilever and its position change in the observation plane can be registered by a position sensitive detector (PSD). Using a laser diode as light source makes compact and robust AFM setups possible. A small drawback of the LBD method is that the sensitivity has to be calibrated separately for every cantilever used.

Further very sensitive deflection detectors can be realised by **light interferometric systems**. Many different kinds of interferometers can be integrated, such as heterodyne interferometer⁴⁶ or Michelson interferometer. However, the very high detection sensitivity brings along a high experimental effort, compared to LBD.

New developments are pushed towards an indirect integration of the detector system in the cantilever. The so-called “piezolevers” are currently developed, which consist of piezo-resistive material that changes its resistance when it is under mechanical strain (i.e. deflection). The deflection can thereby be measured using the cantilever’s resistance, which is a very elegant way but so far these cantilevers are very expensive and the method is not as precise as the optical methods due to the relatively high resistance noise.

The selection of the best-fitting detector type depends on the AFMs field of application. Decision criterias are:

- Ultrahigh vacuum capability
- Required lateral and vertical resolution
- Maximum deflection range
- Type of force interaction to be measured

2.5.4. Scanners

In scanning probe microscopes usually the sample is moved in all three spatial directions with respect to the tip,⁵⁵ since all kinds of detectors have to be connected to the tip (e.g. in AFM laser and PSD have to be stationary with respect to the cantilever). Translators made of piezo-electric ceramics are used as mechanic adjustment device in the sub-nanometer region (“scanner”). The piezo scanner should meet the following requirements:

- High resonance frequency and Q factor for high raster and feedback speed
- Small hysteresis
- Small piezo creep
- Independent movements in x, y, z direction (no coupling)

All requirements cannot be fulfilled at the same time so always compromises have to be made. The following types of scanners are most commonly used depending on the required maximum scan range and speed.

Tripod

A tripod piezo scanner can be built by combining three piezos together according to the three directions of a Cartesian coordinate system. The piezo ceramics have to be longitudinally polarised. The scanner’s maximum scan range depends on the used piezo ceramics and the maximum operation voltage. The piezo constant is typically in a range from 2 Å/V to 5 Å/V. A maximal operating voltage of about 1000 V is then needed for these piezo ceramics to receive an sufficient scan range.

Tube scanner

The tube scanner is a radially polarised piezo tube with typically one inside electrode and four uniform segmented outside electrodes (Figure 13). An reverse voltage at opposing

segments cause a contraction of one and a extension of the other segment and hence a bending of the tube (x or y direction). A equal voltage on all segments leads to a contraction or extension of the tube (z direction). Thus, movements in all three dimensions can be examined by the tube scanner. The scanner constant SK in the three spatial dimensions x, y, and z is approximatly

$$SK_{xy} = \frac{\sqrt{2}}{\pi} d_{31} \frac{l_t^2}{r_t t_t} \quad \text{and} \quad SK_z = d_{31} \frac{l_t}{t_t}, \quad (12)$$

with tube length l_t , inner tube radius r_t , tube's wall thickness t_t and voltage conversion factor d_{31} . The directions of polarisation orientation are numbered consecutively with 1, 2, and 3 as label of the, and the direction of polarisation is arbirtary set as 3. Therefore, the factor d_{31} labels the length change of a piezo ceramic perpendicular to the polarisation orientation.

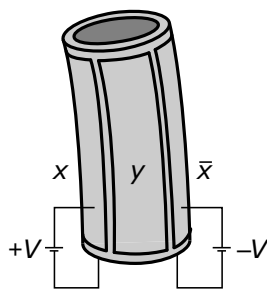


Figure 13. Schematic design of an AFM tube scanner. An reverse voltage at the x and $\bar{x}$ electrodes cause a microscopic bending of the tube.

The mass of the tube scanner is reduced to obtain a high mechanical resonance frequency and hence a high scan speed. The scanning constant varies from 16 Å/V to 400 Å/V, so small operation voltages up to 150 V are sufficient. It is not possible to completely separate the x, y, and z rastering direction because of inhomogenities in the piezo material. However, it is possible to eliminate the errors with using a clever mechanical desing, an electronic compensation circuit, or an offline-correction after the measurement.

Piezo stages

More advanced scanners are piezo stages, where piezo actuators are combined with parallel kinematics and often also translation sensors, to allow for a accurate, reproducible and uncoupled movement of the sample in all three directions. Piezo stages have, however, typically comparably low resonance frequencies and are relatively expensive.

Discussion

Due to its high resonance frequency, decent linearity and hysteresis, and low cost, tube scanner are often used. The small sizes make a very compact setup possible, so that there is often no need for a complicated vibration isolation for some applications.

It might be necessary to measure the disadvantageous properties of piezo translators (like non-linearity, hysteresis or creep) or compensate them directly during imaging using translation sensors. In the setup used in this practical, a correction routine is implemented in the software, which automatically corrects for piezo artifacts.

3. Laboratory

The AFM used for the experiment is an expensive research microscope. The experimental work may therefore be started only after briefing by the instructor. In the experiment, the following issues should be investigated:

1. Set up and adjust the AFM.
2. **Blu-ray disk:**
 - a. Prepare a Blu-ray disk sample and image it in the contact mode in air.
 - b. Discuss the obtained “height” and “deflection error” images. Compare the “trace” with the “retrace” images.
 - c. Measure the lengths, widths, and depths of the pits and the track distance. Estimate the data storage capacity of the Blu-ray disk.
 - d. What is the expected optimum pit depth (concerning the interferometric optical detection)? How do your experimental results compare to this prediction?
 - e. Calculate the 2D Fourier spectra of images with different scan sizes (e.g. 1 μm , 3 μm , and 10 μm). How can the Fourier spectra be interpreted?
 - f. Record some force curves (deflection vs. vertical position) and discuss their shape. How large is the typical tip-sample adhesion force? Estimate the tip radius from the adhesion force using Equation (3).
 - g. Calculate the spring constant for the cantilever used (type ContAI-G, rectangular, length $l = 450 \mu\text{m}$, width $w = 50 \mu\text{m}$, thickness $t = 2 \mu\text{m}$, tip height $h = 17 \mu\text{m}$, made from silicon with $E = 180 \text{ GPa}$ and $G = 70 \text{ GPa}$) using Equation (10).
 - h. Estimate the typical imaging force F_z applied during contact mode imaging. For that you need the selected imaging set point (in Volts) and the sensitivity of the LBD system (e.g. in nm/V), don't forget to note them during the experiment.
 - i. Estimate the lateral displacement of the tip due to friction forces during scanning assuming that $F_x \approx F_z$.
3. **Elastomer:**
 - a. Prepare three samples from elastomers (PDMS Sylgard 184) of three different mixing ratios 10:1, 15:1, and 20:1 (ratio base to cross-linker).
 - b. Record force curves on the elastomer samples and discuss their shape.

- c. Compare the estimated tip radius with the indentation depth. Is it the use of conical contact model (assuming a perfectly sharp cone, see Figure 5) justified?
- d. Determine the Young's modulus of the elastomer samples. For that you have two possibilities:
 - i. From the force curves read off the maximum indentation and the maximum force and calculate the Young's modulus by rearranging Equation (4).
 - ii. Convert the force curves into force-indentation curves using Equations (5) and (6). Then determine the Young's modulus by fitting the contact model $F(\delta)$ from Equation (4) to the experimental data. Note that deflection d and position z are usually offset in the recorded force curves.

4. Protocol

The protocol should briefly discuss the topics listed under "Preparation (at home)". The major part of the protocol should be the presentation of the results obtained during the experiment and their detailed analysis, discussion and interpretation. The protocol must contain a signed declaration that the elaboration of the report was performed by you and your group (see below).

Name:	
Matriculation number:	
I hereby declare that my elaboration (or that of my group) was made independently and is not a copy (even partially) of an existing protocol or of the instruction manual. All external sources (also the instruction manual) have to be cited. I am aware that a violation of this rules is plagiarism and leads to the experiment being graded as failed (grade 5) and can result in an exclusion from the labwork.	
..... Date	 Signature

Do not forget to export your data from the proprietary AFM software for your protocol. Alternatively, the AFM data can also be opened in other freeware software such as Gwyddion (<http://gwyddion.net/>).

II. Further preparation (for the block course only)

Please note: The following topics need only be prepared for the block course in the semester break!

In order to conduct the experiment on the second day, knowledge of the following topics is required:

- Behavior of a (damped) harmonic oscillator
- Basics of the dynamic imaging modes
- Theory of thermal bending of polymers
- Basics of the worm-like chain model

You should be able to answer the following questions before the experiment:

- How does the “resonance curve” of a (damped) harmonic oscillator look like and how can it be interpreted?
- How does the resonance curve of a harmonic oscillator change under the influence of a constant force or a force gradient?
- Which regions of the tip-sample interaction do the different AFM imaging modes work in?
- What are the normal vibration modes of a cantilever?
- How does tapping mode work?
- What is collagen? What is the ultrastructure of collagen fibrils and the “D-period”?
- What is DNA? What is the persistence length of a flexible polymer chain?

1. Dynamic AFM Imaging Modes

In dynamical AFM modes the cantilever is not at permanent contact with the sample, but oscillated at a frequency close to it's resonance frequency, which is typically in the range of 10's to 100's of kHz.

Resonant frequency

The resonance frequency f_0 of a cantilever is given given by

$$f_0 = \frac{\omega_0}{2\pi} = \frac{1}{2\pi} \sqrt{\frac{k}{m_{\text{eff}}}} \quad (13)$$

where k is the spring constant after Equation (10) and m_{eff} is the effective mass of the cantilever. For a rectangular cantilever $m_{\text{eff}} = 0.24 m$, with $m = \rho \cdot twl$ being the full mass of the cantilever from material with density ρ . The factor $m_{\text{eff}}/m = 0.24$ accounts for the

fact that only a part of the cantilever actually oscillates. For a rectangular cantilever Equation (13) therefore simplifies to

$$f_0 \cong 0.16 \sqrt{\frac{E t^2}{\rho l^4}} . \quad (14)$$

If an additional force interaction $F(z)$ is acting on the cantilever, the effective spring constant changes to $k + dF/dz$, resulting in an increase or decrease of the cantilevers resonance frequency

$$\omega_0 \cong \sqrt{\frac{k + dF/dz}{m_{\text{eff}}}} . \quad (15)$$

All dynamic AFM modes have in common that the change in resonance frequency is directly or indirectly employed to control the average tip-sample distance.

Non-contact mode

For example, when the cantilever is excited at it's free resonance frequency, an attractive force causes a decrease of the resonance frequency and thereby a smaller oscillation amplitude at fixed excitation frequency (since the excitation frequency is now further away from the new resonance frequency). Again, a feedback loop can be used to maintain a constant oscillation amplitude and thereby keep the average tip-sample distance constant ("amplitude modulation"). Since it is possible to just use the attractive part of the tip-sample interaction and avoid direct mechanical contact between tip and sample, the mode is often referred as "non-contact mode". It is even more efficient (but also technically more challenging) to detect the change in resonance frequency itself and use it as an input for the feedback loop ("frequency modulation").

Tapping mode

Non-contact mode is only possible, if the tip-sample interaction is very stable and does not vary a lot over the sample, which is practically only with very clear samples and/or in vacuum. In liquid or even air the repulsive regime of the tip-sample interaction is therefore used for imaging. Since the tip just occasionally contacts the sample, the mode is termed "intermittent contact mode" or descriptively "tapping mode". In tapping mode, the cantilever is usually excited at a frequency below its free resonance frequency and amplitude on the order of 100 nm. When the tip approaches the sample, due to the repulsive tip-sample interaction the resonance frequency increases and the oscillation amplitude decreases. During scanning, this amplitude decrease serves as the input for the feedback loop, controlling the vertical cantilever position. Advantages of the tapping mode compared to the contact mode are mainly the reduced lateral forces, which allow imaging of more fragile samples.

Q factor

The damping influences of the surrounding air or water changes the resonance peak of the cantilever. The Q factor is defined by the ratio of the resonance frequency to the width of the resonance peak. The Q factor of force sensors can be bigger than 10000 in vacuum; in air it drops down to around 100. It is an important factor for the force resolution of dynamic AFM.⁴⁶ It has a subsidiary relevance for the static AFM because of its high resonance frequencies above 10 kHz.

2. Thermal Bending of Linear Polymers

For the theoretical background of persistence length, we follow Rheinlaender *et al.*⁵⁶ The conformation of a semi-flexible polymer chain (here: DNA) in thermodynamic equilibrium can be described by the worm-like chain model (WLC).⁵⁷ In the WLC model, the polymer is described as a homogenous elastic rod with a circular cross section. The energy U to bend a polymer segment of length ΔL by an angle ϑ is given by

$$U(\vartheta) = \frac{EI}{2\Delta L} \vartheta^2, \quad (16)$$

where E is the stiffness (Young's modulus) and $I = \pi r^4/4$ is the polymer's cross sectional area moment of inertia, with r being the radius of the cross section.⁵⁸ In thermodynamic equilibrium, the probability p that the segment is bent by the angle ϑ is given by Boltzmann's law:

$$p(\vartheta) \propto e^{-\frac{U(\vartheta)}{k_B T}} = e^{-\frac{P}{2\Delta L} \vartheta^2}. \quad (17)$$

The persistence length of the polymer,

$$P = \frac{EI}{k_B T}, \quad (18)$$

can be interpreted as the distance along the polymer at which its angular orientation becomes uncorrelated. For a long polymer of contour length L that is confined within a plane, the WLC model predicts the mean squared end-to-end distance as⁵⁹

$$\langle R^2 \rangle_{2D} = 4PL \left[1 - \frac{2P}{L} \left(1 - e^{-\frac{L}{2P}} \right) \right]. \quad (19)$$

For $L \gg P$, Equation (19) can be approximated by $\langle R^2 \rangle_{2D} \cong 4PL$.

3. Laboratory

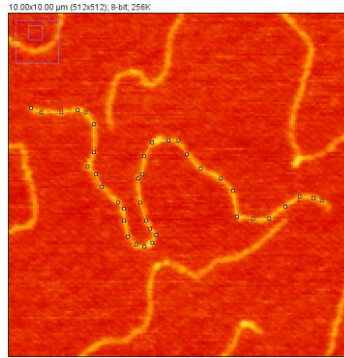
1. Set up and adjust the AFM.

2. Calculate the nominal spring constant k and the resonance frequency f_0 for the cantilever used (type Olympus OMCL-AC160TS, see also Figure 2, length $l = 160\text{ }\mu\text{m}$, width $w = 40\text{ }\mu\text{m}$, thickness $t = 3.7\text{ }\mu\text{m}$, made from silicon with $E = 180\text{ GPa}$ and $\rho = 2.3\text{ g/cm}^3$).
3. Record a tune of the cantilever. What can you read off this measurement? Record the thermal noise spectrum of the cantilever. How can this spectrum be interpreted? What is the approximate resonance frequency and Q factor of the cantilever?
4. **Collagen:**
 - a. Prepare a sample of collagen type I and image it in the tapping mode in air.
 - b. Measure the heights and widths of the collagen fibrils. Of how many triple-helices do the fibrils approximately consist?
 - c. Record a high-resolution image of a fiber and measure the “D-period” banding.
5. **DNA:**
 - a. Prepare a DNA sample and image it in the tapping mode in air.
 - b. Measure the heights, widths, contour lengths, and end-to-end distances of the DNA fragments.
 - c. Record multiple, high-resolution images of single DNA molecules and measure their contour lengths and end-to-end distances using the ImageJ software (s. below). Determine the persistence length of DNA from these measurements by plotting the squared end-to-end distance of each DNA fragment $\langle R^2 \rangle$ vs. its contour length L . Equation (19) (or the approximation) can then be fit to this data to obtain the persistence length of DNA. Estimate the effective Young’s modulus of the DNA molecule (using either your result or a literature value for the radius r).

4. Measurement of Contour Length with ImageJ

ImageJ is a Java-based free software package for image analysis and can be downloaded from <http://imagej.nih.gov/ij>. To measure the contour length / end-to-end distance of DNA fragments follow these steps:

- Open the image in ImageJ.
- Use the Analyze/Set Scale dialog to define the spatial scale: enter the image width in the “Distance in Pixels” field, enter the field width in the “Known Distance” field, enter “nm” as the “Unit of Length”, click “OK”.
- Use the magnifying glass tool to zoom in on the DNA contour to be measured.
- Use the segmented line tool to create a line selection that outlines the DNA contour. The line selection can be adjusted by clicking and dragging the tiny black and white “handles” along the outline.



- Use the Analyze/Measure command (Ctrl+M) to measure the length of the DNA contour.
- Also measure the corresponding end-to-end distance for this DNA contour using the straight line tool.
- Measurements can be transferred to a spreadsheet (right-click in the “Results” window, select “Copy”).

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