

Contents

1	Motivation	3
2	Theoretical Background	3
2.1	AFM setup	3
2.2	Imaging modes	3
2.3	Problems of the AFM	4
3	Experimental setup day 1	5
4	Experimental setup day 2	5
5	Results Day 1	5
5.1	Blue ray	5
5.2	Elastomer	10
6	Results Day 2	11
6.1	Collagen	11
6.2	DNA	13
7	Discussion Day 1	14
7.1	Blue-ray disk	14
7.2	Elastomer	15
8	Discussion Day 2	15
8.1	Collagen	15
8.2	DNA	15
9	Conclusion	16

1 Motivation

In the past decades, examinations of small surface structures as well as measuring material properties in small scales became more and more important. One method to measure those properties is the atomic force microscope (AFM). It belongs to the scanning tunneling microscopes and was developed in 1986. Nowadays it is used in various scientific areas to image samples and investigate their stiffness [1].

2 Theoretical Background

2.1 AFM setup

The key element of the AFM is the cantilever. The tip of the cantilever interacts with the sample by different forces. Two types of forces play a role in the tip sample interaction. On the one hand there are attractive forces as Van-der-waals and capillary forces. On the other hand there are repulsive forces as coulomb forces because of overlapping electron clouds which deflect the cantilever [2]. The setup of an AFM is similar to other scanning microscopes. The sample is placed on a table which is in most cases controlled by piezo elements while the cantilever is fixed. The position of the cantilever and by that the tip is monitored by a laser, which sends a beam onto the cantilever which is then reflected and detected by a photodiode [3]. Depending on the position of the cantilever, the reflection changes and by that the height position of the cantilever can be detected. When a picture is taken, the cantilever scans the surface of the sample in lines, which then results in the complete image[4].

2.2 Imaging modes

2.2.1 Constant height mode

In constant height mode, the cantilever is lowered in the direction of the sample, until a repulsive force can be measured. After that the height of the cantilever stays fixed for the scan. The sample is then moved line by line and an image is made. The main advantages of this method is the imaging speed and the simplicity of the setup, since only one height is used for the whole scan. Hence, disadvantages are many artifacts because of the vertical scan which are explained later[1][2].

2.2.2 Constant force mode

Constant force mode or contact mode is more complicated than constant height mode. Here, a constant force resulting of the tip-sample interaction is set, which is then controlled by a feedback loop. Here the sample is also scanned line by line. Since the tip is in direct contact with the sample, the wear of the tip is very high. Furthermore damage to the sample can occur[2].

2.2.3 Dynamic modes

Dynamic mode is the most frequent used imaging mode. Here, the cantilever is oscillated with a frequency, which is close to its resonance frequency. When the tip approaches the sample, the frequency changes due to the interaction with the sample which can then be monitored by the detector [5]. It makes sense to use a frequency which is close to the resonance frequency since a small change in the frequency of the cantilever leads to a high change in its amplitude, which then can be measured easily [4]. Because of the gently working mode, the dynamic mode can be used with sensitive samples. A disadvantage is the longer scanning time compare to the other imaging modes. In tapping mode, the cantilever is oscillating and briefly touches the sample, which then results in a change of the frequency. A feedback loop controls this decrease and keeps it constant. By that, a topographic 2D image of the sample can be recorded [6]. In non contact mode, the tip is not contacting the sample surface. A change of the frequency still occurs due to the interaction forces which already exist at a small distance between sample and surface. This mode conserves the sample very well [7]. Despite of only recording topographic images, the AFM has further applications. One is the recording of force curves which is used e.g. in material sciences or biology. By the use of these curves it is possible to find out about material interactions or material properties as the Young's modulus [8].

2.3 Problems of the AFM

Common problems that can occur when using the AFM is the problem of artefacts. Due to the tip geometry, the AFM always has a limited resolution and can not record every part of the surface of the sample. As it can be seen in figure 1, large steps in surfaces often can not be fully recorded by the AFM due to tip geometries. Further problems while using the

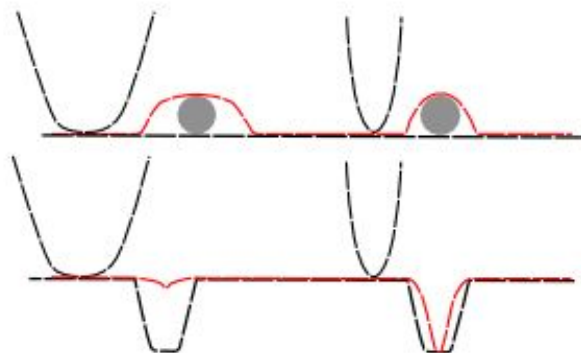


Figure 1: Demonstration of the origin of artefacts in AFM [2]

AFM can be the tip abrasion. Due to tip sample interactions, the tip can be damaged very fast or even be fully destroyed while mounting it [1][2]. Furthermore, the technology is very expensive and needs accurate working, as well as trained people to operate the AFM.

3 Experimental setup day 1

To conduct the intended measurements, first the AFM had to be set up. A fresh cantilever was assembled into the cantilever holder, which was then put into the AFM setup. Next, the laser had to be positioned, so it reflected at the tip of the cantilever. This way, a movement of the cantilever can be detected by the optical system. To finish the calibration, the tilt mirror was adjusted so that the sum signal was around 7V and the photodiode was adjusted so the vertical deflection signal was zero. The sample of the first measurement series was a blue-ray disk. It was then imaged in contact mode with various deflection setpoints and integral gain. After that, force curves were recorded. The second sample was PDMS which is an elastomer. Three different mixing ratios of PDMS base ratio to cross linker (5:1, 10:1, 15:1) were used. Force curves were also recorded for this sample.

4 Experimental setup day 2

The second day of experiments also started with the calibration of the AFM and the assembly of a new cantilever. First, a tune of the cantilever was measured to gain knowledge about the resonance frequency. The first sample, which was observed was a collagen type 1 sample. It was first inserted into a ethanol solution, to improve the sample quality. Then it was transferred into an acetone solution. After that a small amount of the collagen was transferred onto a glass plate. A cross section profile was recorded in tapping mode. The second sample was DNA, which was stored on mica. The sample was previously prepared, so no further preparation was necessary. An overview image of multiple DNA molecules was taken and some molecules were further investigated.

5 Results Day 1

5.1 Blue ray

First, the influence of the deflection setpoint and the integral gain was measured while investigating the blue ray pits. The deflection setpoint is the force which is applied by the tip onto the sample surface in contact mode. In tapping mode it is the amplitude of the cantilever. The integral gain describes the value of the feedback loop and by that how fast it reacts to a change in the sample surface. Figure 2 shows two different deflection setpoints and integral gains. Figure 3 displays the measured trace and retrace image as well as the deflection error for one example.

To estimate the total data capacity of the blue ray disk, the different dimensions of the blue ray pits were measured. The total capacity is then defined as the area of the whole disc (diameter: 12cm and diameter of the hole in the middle: 4cm) divided by the area of one bit, which is defined by the track distance which was calculated to be $0.37\mu\text{m}$ times the width of a pit which was $0.25\mu\text{m}$ as shown in equation 1.

$$C = \frac{\pi \cdot (0.06\text{m})^2 - \pi \cdot (0.02\text{m})^2}{(0.37 \cdot 10^{-6}\text{m}) \cdot (0.25 \cdot 10^{-6}\text{m})} \approx 108.682.124.232\text{bit} \approx 13.5\text{GB} \quad (1)$$

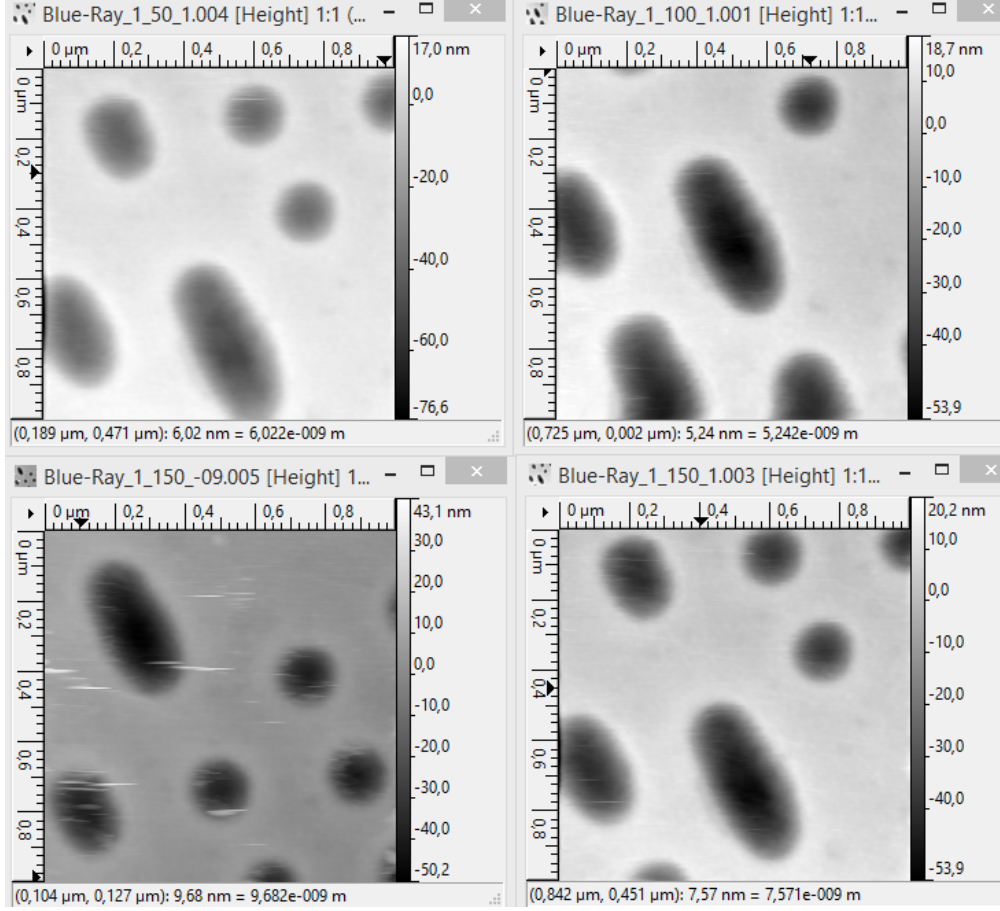


Figure 2: Top left: Integral gain 50, deflection setpoint 1
Top right: Integral gain 100, deflection setpoint 1
Bottom left: Integral gain 150, deflection setpoint -0.9
Bottom right: Integral gain 150, deflection setpoint 1

The optimal pit depth regarding interferometric optical detection is calculated by equation 2 with wavelength $\lambda=405\text{nm}$ and refractive index of the foil $n_{\text{foil}}=1.6$.

$$d = \frac{\lambda/4}{n_{\text{foil}}} = 62.5\text{nm} \quad (2)$$

Figure 4 shows the experimental pit depth measurements which were approximately 55nm.

Next, the resulting Fourier spectra of the disc with different image sizes were calculated which can be seen in figure 5. After that, force curves were recorded to gain knowledge about material properties of the blue-ray disk and calculate the radius of the cantilever tip. Figure 6 shows the force curve with deflection and vertical position. The sensitivity of the LBD system was 100.16nm/V . With the adhesion force of 66nN and the surface tension of water $\gamma= 0.073 \frac{\text{J}}{\text{m}^2}$, the radius of the tip can be calculated with equation 3.

$$R = \frac{F}{4\pi\gamma} = 72\text{nm} \quad (3)$$

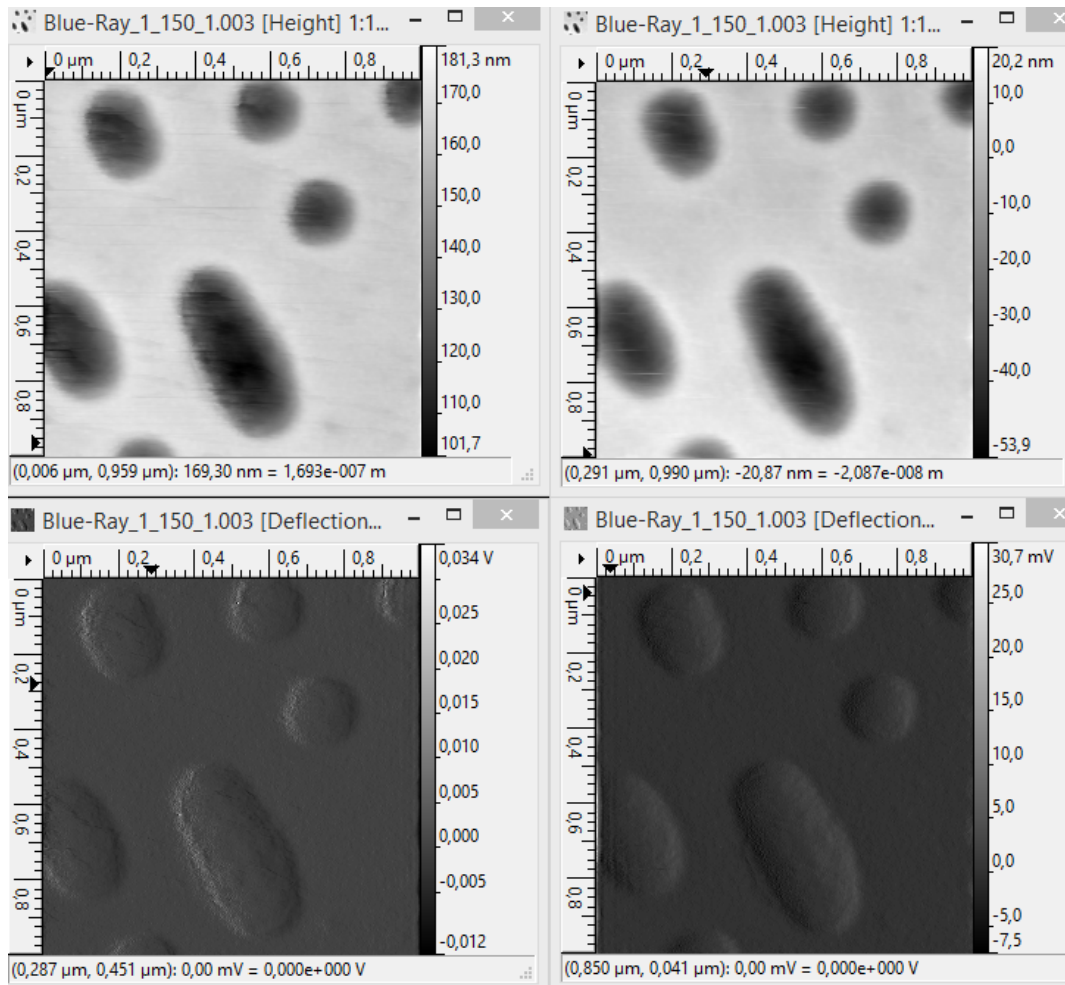


Figure 3: Trace (top left) and retrace as well as the deflection error (bottom left) of the blue ray disk

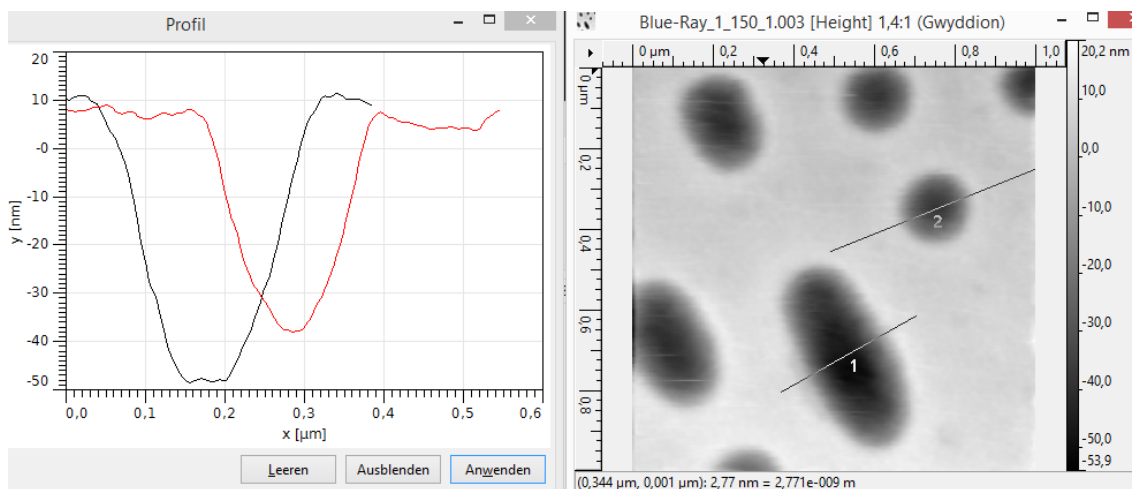


Figure 4: Pit dimensions of the blue ray disk

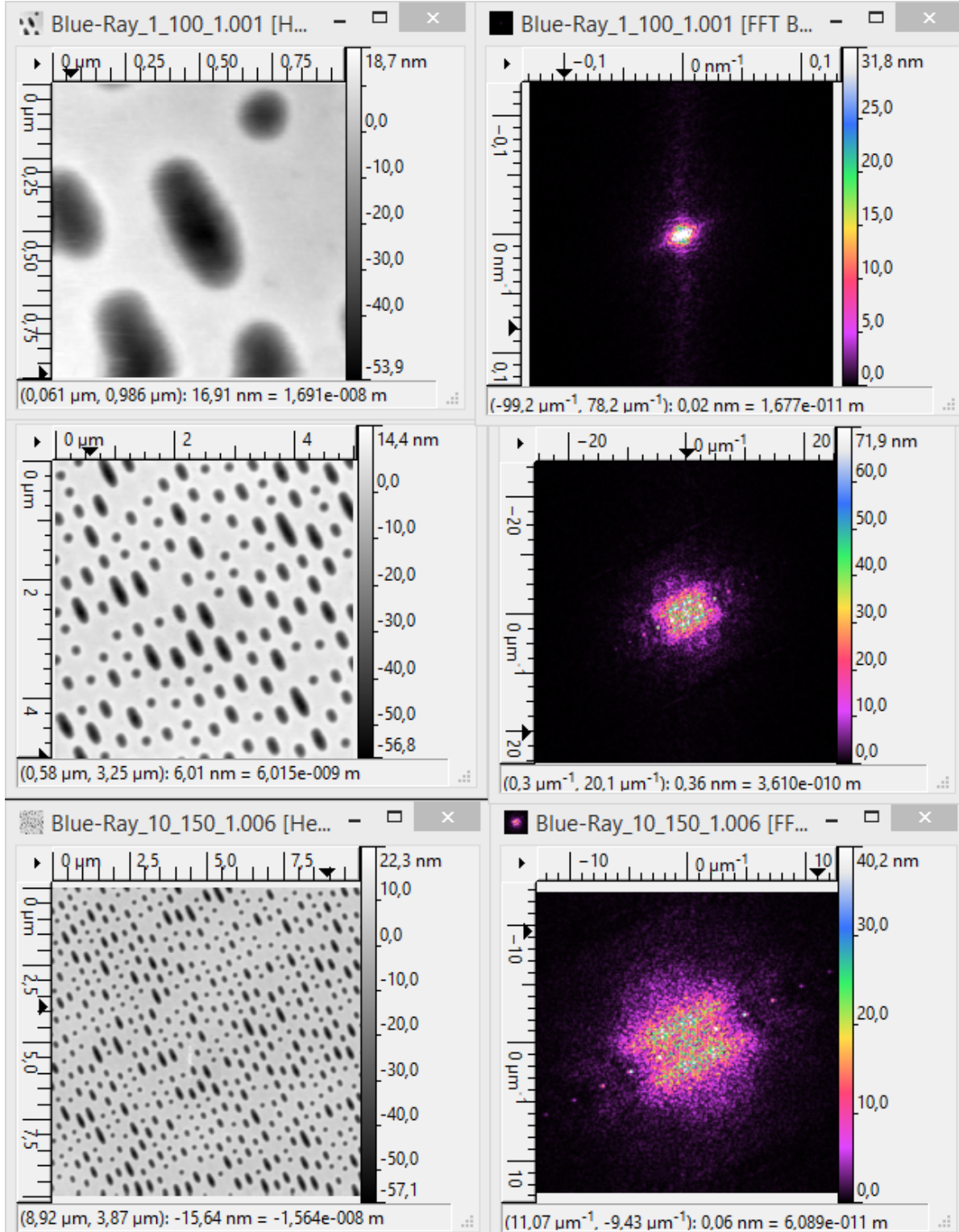


Figure 5: Images of the blue ray (left) and the respective Fourier spectra(right). The different image sizes are 1, 3 and 10 μm .

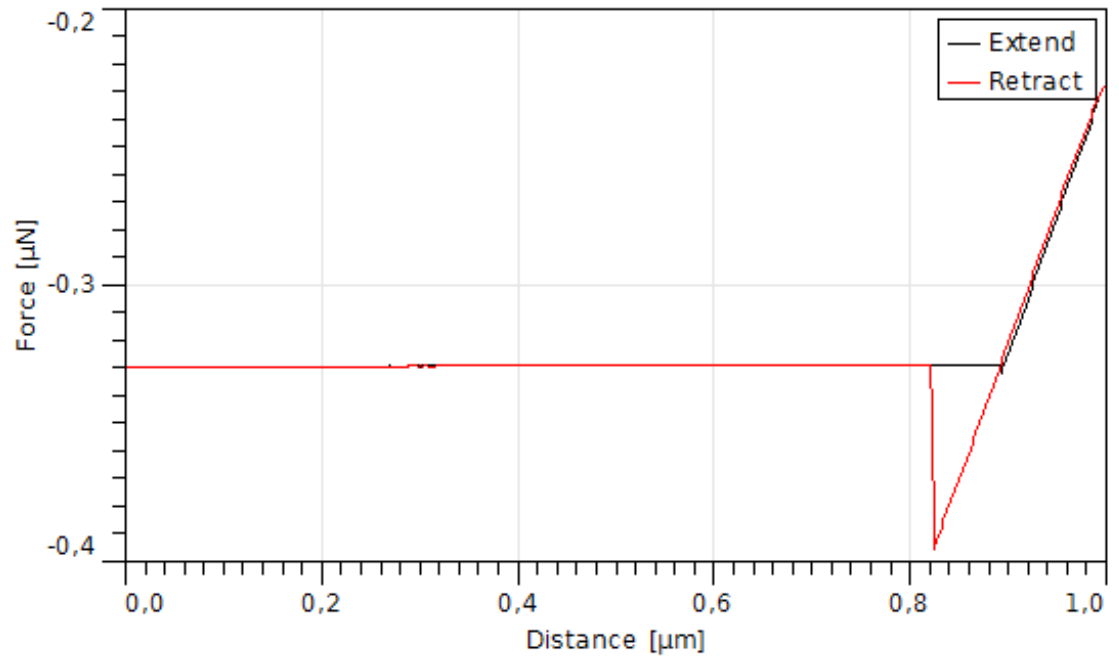


Figure 6: Force curve of the blue ray indentation test with force and vertical position on the axis.

Tip geometries	Values
Length	450 μm
Width	50 μm
Thickness	2 μm
Tip height	17 μm
Young's modulus	180 GPa
Shear modulus	70 GPa

Table 1: Tip geometries of the cantilever.

Out of the geometries of the cantilever, the spring constant was then calculated with equation 4. The tip geometries are displayed in table 1.

$$k = \frac{Et^3w}{4l^3} = 0.198 \frac{N}{m} \quad (4)$$

The spring constant as well as the sensitivity of the LBD system can now be used to calculate the lateral displacement with the assumption that $F_x \approx F_z$. Equation 5 calculates the force.

$$F = k \cdot x = 0.198 \frac{N}{m} \cdot 100nm/V \cdot 1V = 20nN \quad (5)$$

The lateral displacement can now be calculated with equation 6 with $\phi=1.6 \times 10^{-5}m$

$$\Delta x = h \cdot \sin\phi = 4.75pm \quad (6)$$

5.2 Elastomer

In the second part of the practical PDMS as a sample was used. Different force curves for the different mixtures were measured and the Young's moduli of the three samples were calculated by using equation 7 and are displayed in table 2. The Poisson's ratio was assumed to be 0.5. The maximum indentation and the maximum force was read off the force curves. An example for one force curve is shown in figure 7.

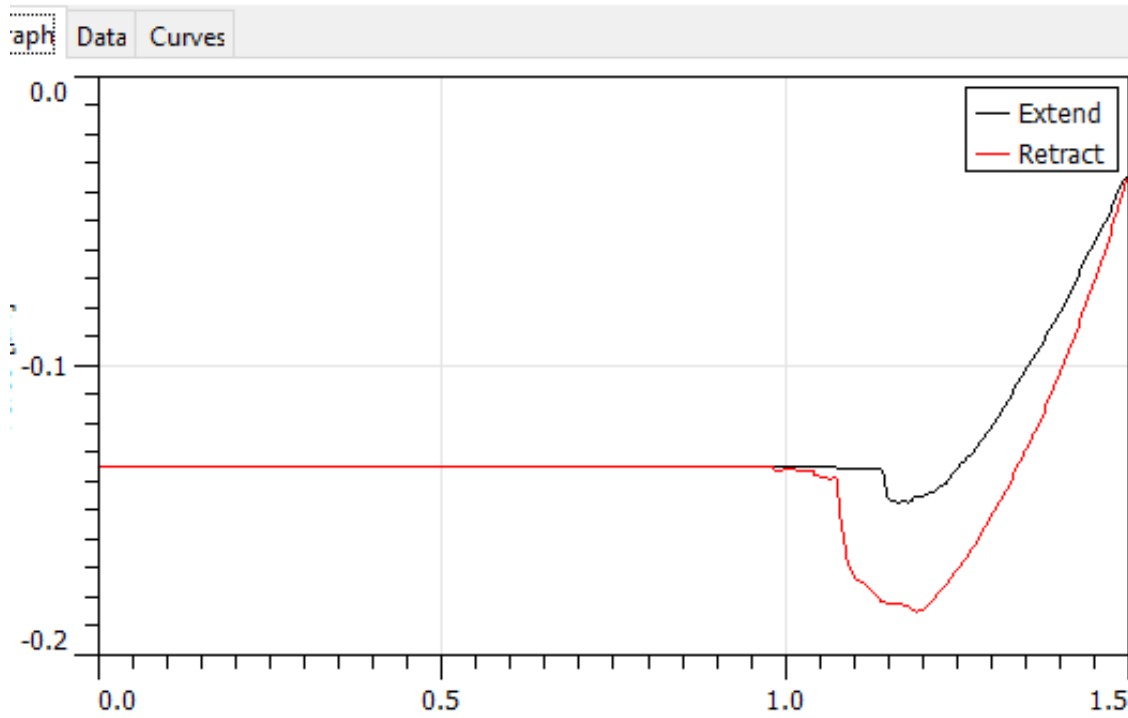


Figure 7: Force curve of the PDMS sample with 1:15 mixture.

$$E = \frac{F(\delta) \cdot \pi(1 - v^2)}{2E \cdot \tan\alpha \cdot \delta^2} \quad (7)$$

PDMS Sample	Young's Modulus [MPa]	Indentation depth in μm
5:1	2.3	0.287
10:1	2	0.355
15:1	1.6	0.435

Table 2: PDMS samples with respective calculated Young's moduli

6 Results Day 2

On day 2, a different, smaller cantilever was used. Here, also the spring constant was calculated according to equation 4. Table 3 shows the dimensions of the cantilever. The spring constant was $k = 22.26 \text{ N/m}$. The resonance frequency was calculated based on equation 8.

$$f_0 = 0.16 \sqrt{\frac{E \cdot t^2}{\rho l^4}} = 204,58 \text{ kHz} \quad (8)$$

Next, a tune of the cantilever from day 2 was performed and the resonance frequency was

Tip geometries	Values
Length	160 μm
Width	40 μm
Thickness	3.7 μm
Young's modulus	180 GPa
Density	2.3 g/cm^3

Table 3: Tip geometries of the cantilever.

read off to be 307kHz. By that, the Q-factor of the cantilever could be calculated according to equation 9 with $\Delta f = 0.5 \text{ kHz}$

$$Q = \frac{f_0}{\Delta f} = 414 \quad (9)$$

6.1 Collagen

Figure 8 shows the cross section profile of the fibril, which was used to calculate the average height (20nm) and full width at half maximum (FWHM)(200nm) of the fibril. To gain knowledge about the amount of triple helices contained in the fibril, an assumption was made, that the diameter of one triple helix is around 1nm [9]. Equation 10 shows the

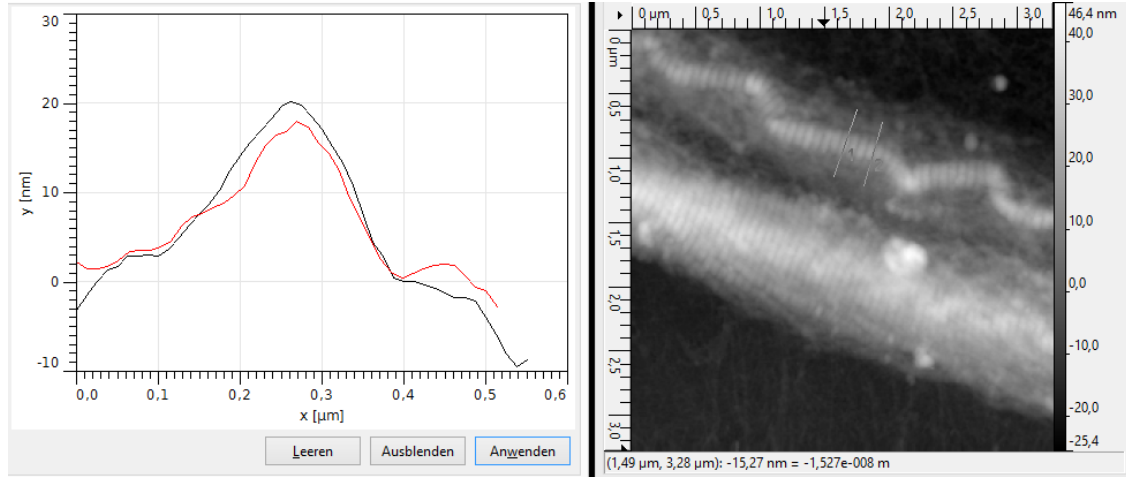


Figure 8: The cross section profile of the collagen fibril of image size $3 \mu\text{m}$ with the height and width measurements on the left.

calculation of the total amount of triple helices. The area of the fibril was treated as an ellipse.

$$N_{\text{th}} = \frac{A_{\text{total}}}{A_{\text{th}}} = 16.000 \quad (10)$$

Furthermore the D-periods of the collagen were measured and compared to the theoretical values of 67nm. Figure 9 shows the collagen fibre and the profile of the measured line. The

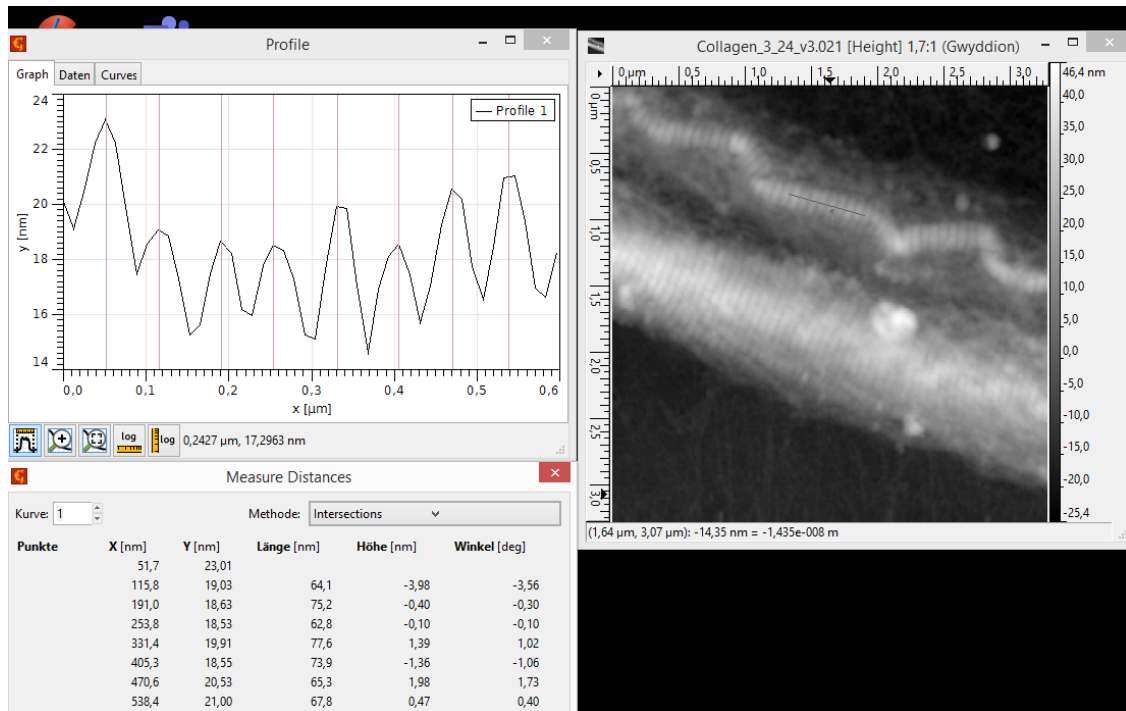


Figure 9: The measurement of the collagen D-period lengths

practical length of the D-period sections ranged from 62.8nm to 77.6nm and had an average value of 69.5nm.

6.2 DNA

The DNA measurements were done with a new cantilever which showed a resonance frequency of 307.8kHz. A high resolution image of the DNA was taken and the height and the diameter of DNA molecules was calculated. It is shown in figure 10. The FWHM averaged 12nm and

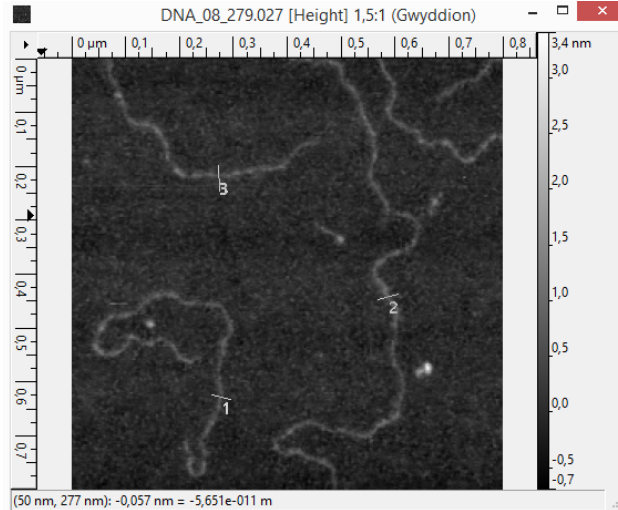


Figure 10: DNA overview picture with the different width measurement markers.

ranged from 10 to 16nm. The average height of the molecules was 0.5nm and ranged from 0.45 to 0.53nm. To measure the contour length and the persistence length of the DNA molecules, ImageJ was used. Figure 11 shows the measured DNA molecules. Also parts of molecules were measured since otherwise only two molecules could be analyzed. Table 4 shows the results of the measured values. The average persistence length was 140.862nm and calculated following equation 10 with R^2 being the squared end-to-end distance and L the contour length of the molecule.

$$P = \frac{R^2}{4L} \quad (11)$$

Figure 11 shows the measured DNA molecules which are analyzed in table 4.

Molecule	contour length [nm]	end-to-end distance [nm]	Persistence length [nm]	Young's modulus [GPa]
1	945.292	524.217	72.677	0.371
2	926.599	155.812	6.550	0.033
3	1030.733	404.658	39.716	0.203
4	3411.493	1879.559	258.885	1.321
5	3305.874	2176.976	358.394	1.828
6	1889.167	1130.473	169.118	0.863
Average	935.946	340.015	150.890	0.77
Standard error			56.084	

Table 4: Calculated persistence lengths and Young's moduli of the DNA molecules

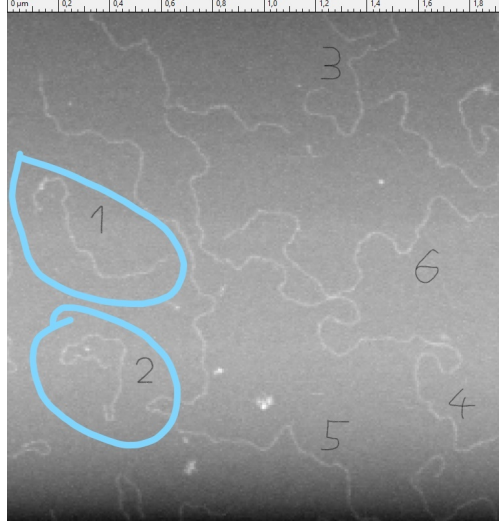


Figure 11: DNA overview picture with the different measured molecules.

7 Discussion Day 1

In general awareness needs to be raised to the fact, that the possibility of statistical and systematic errors can not be neglected totally, because the experiments were only performed once and not multiple times.

7.1 Blue-ray disk

The comparison of variations of the integral gain and deflection setpoint which is shown in figure 2 showed that both have influence on the image quality. The integral gain of 100-150 showed optimal results, while the image with the integral gain of 50 was a bit blurry. The deflection setpoint was set to 1 for most of the images which showed optimal results, while a deflection setpoint of -0.9 showed some lines in the picture and the signal vanished with a deflection setpoint of -1.

The trace and retrace as well as the deflection error images do not show great deviations, which implies a succesful calibration of the AFM as it can be seen in figure 3. The measured pit depth of 55nm compared to the optimal, expected pit depth of 62.5nm showed a small deviation of 7nm. However, these deviations could have occurred due to measurement inaccuracies, since the software did not show clear borders of the pits in the diagrams.

The Fourier spectra which are shown in figure 5, are used to show periodicity. In the 10 μm image one can see bright spots which are diagonal from the bottom left to the top right. These spots mean periodicity in the blue ray disc. It can be seen that the periodicity can be best seen in the largest image size.

The calculated lateral displacement of $\Delta x = 4.75 \text{ pm}$ does not have a large impact on the outcome of the measurements, since the magnitude is at least 10^3 times smaller than the values of interest. Furthermore it can be said, that the AFM was setup manually and most of the results were calculated by graphical software which could have resulted in some deviations.

7.2 Elastomer

The elastomer samples were produced prior to the experiment which is why the mixture and quality of the samples can only be assumed but not assured. The Young's modulus values of around 2 MPa fit to theoretical values, although the impact of the mixture is higher in literature compared to experimental values [10]. According to literature, the Young's modulus for the 5:1 mixture of PDMS has the highest value. Furthermore the contact model assumes a perfect cone and not a rounded tip which could also lead to deviations. With the tip radius being around 72nm and the indentation values, depending on the sample being around 200nm, the tip geometry might have an impact on the results. However, the manufacturer suggests, that the tip radius is smaller than 10nm, which would mean, that the radius has no impact since it is much smaller. However, the two force curves in figure 6 and 7 show very clear the differences between stiff and soft samples when it comes to indentation tests. The blue ray has a linear increase of distance when increasing the force, while the PDMS does not show a linear response.

8 Discussion Day 2

The theoretical values by the manufacturer for the cantilever used on the second day were 200-400kHz for the resonance frequency and the spring constant $k = 8.4-57\text{N/m}$. With the experimental values of 207kHz and 307kHz for the two used cantilevers and a calculated spring constant of $k = 22.26\text{N/m}$, the values are in range. Furthermore awareness needs to be raised to the fact, that the possibility of statistical and systematic errors can not be neglected totally, because the experiments were only performed once and not multiple times.

8.1 Collagen

The collagen measurements showed nice pictures, where the structure of collagen as well as the D-periods could be well investigated. The theoretical value for the D-period of 67nm only deviates by 2nm from the experimental values of 69.5nm which suggests, that a nice collagen fiber was found which was not damaged too much during extraction and preparation.

8.2 DNA

The DNA molecules showed different geometries compared to the theoretical values of $d = 2\text{nm}$. Since the height was much smaller (around 0.5nm) and the FWHM was larger (around 12nm), it can be assumed, that the DNA molecules were flattened or else influenced during preparation. Another reason could be the drying of the molecules which also can change the geometries of the DNA. Furthermore, the persistence length showed large deviations from the theoretical value of 50nm, which also could origin from a damaged sample. Otherwise, the measurement method could also be the reason for the large deviations, because only a few molecules were measured and analyzed. Furthermore, the measurement of the end-to-end distance and the contour length was done manually. This might have lead to the great deviations in persistence lengths and the Young's modulus. Also, the cantilever

geometry leaves out artifacts, which can not be measured, which also can lead to inaccurate results as shown in figure 1.

9 Conclusion

In conclusion, this experiment dealt with the basic principles of AFM and gave a brief introduction to some methods and possibilities. The measurements were conducted on two days while the first day was about the blue-ray disk and the elastomer measurements and the second day was about DNA and collagen measurements. The results mostly accorded to the expected theoretical values, although for some of the measurement, the deviations were very high. It can be expected, that further techniques and applications of AFM will develop in the next years, since it is a very common and highly used method. The goal of this experiment, to understand the basic principles and applications of AFM was achieved.

References

- [1] Franz J. Giessibl. Advances in atomic force microscopy. *Reviews of Modern Physics*, 75(3):949–983, 2003.
- [2] Peter Eaton and Paul West. *Atomic force microscopy*. Oxford university press, 2010.
- [3] E. Meyer. Atomic force microscopy. *Progress in Surface Science*, 41(1):3–49, 1992.
- [4] Daniel Rugar and Paul Hansma. Atomic force microscopy. *Physics today*, 43(10):23–30, 1990.
- [5] R. García. Dynamic atomic force microscopy methods. *Surface Science Reports*, 47(6-8):197–301, 2002.
- [6] Constant AJ Putman, Kees O Van der Werf, Bart G De Grooth, Niek F Van Hulst, and Jan Greve. Tapping mode atomic force microscopy in liquid. *Applied physics letters*, 64(18):2454–2456, 1994.
- [7] I Yu Sokolov, GS Henderson, and FJ Wicks. The contrast mechanism for true atomic resolution by afm in non-contact mode: quasi-non-contact mode? *Surface science*, 381(1):L558–L562, 1997.
- [8] Brunero Cappella and Giovanni Dietler. Force-distance curves by atomic force microscopy. *Surface Science Reports*, 34(1-3):1–104, 1999.
- [9] Matthew D. Shoulders and Ronald T. Raines. Collagen structure and stability. *Annual review of biochemistry*, 78:929–958, 2009.
- [10] Qinwei Xu, Cheng Li, Yuejun Kang, and Yilei Zhang. Long term effects of substrate stiffness on the development of hmsc mechanical properties. *RSC Adv.*, 5, 12 2015.