Advanced Physical Laboratory
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Faculty 18 - Natural Science
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Experiment F09

Imaging of biological samples by means of Scanning Probe Microscopy

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Protocol filing:_____
(Date and signature of the supervisor)

Attestation:_____
(Date and signature of the supervisor)

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1 Fundamentals

1.1 Principles of Scanning Probe Microscopy (SPM)

Scanning Probe Microscopy is a general expression which describes a technique for examining the surface of samples. Although there are a number of alternatives in this technique, they all have in common that a probe with a sharp tip scans an area on a sample. Dependant on the layout of the scanning device, the probe receives some kind of feedback from the surface which is then used to generate a map that displays the desired properties of the surface. These properties may be for example the topography, the electric structure or the magnetic structure. The feedback in these cases is either a force (static or dynamic, contact or non-contact) or an electric current that can be measured. The manner of using the interaction with the surface is called a mode.
The main advantage of SPM is, that the resolution is not limited by diffraction like in optical microscopes. However, the imaging process is comparatively slow due to the line-by-line scanning.

In this experiment, Scanning Tunneling and Scanning Force Microscopy will be presented while only the Scanning Force Microscopy will be used to examine DNA molecules on a surface.

1.2 Scanning Tunneling Microscopy (STM)

Scanning Tunneling Microscopy uses the tunneling effect, that causes electrons to leap from the sample to the scanning tip or vice versa.

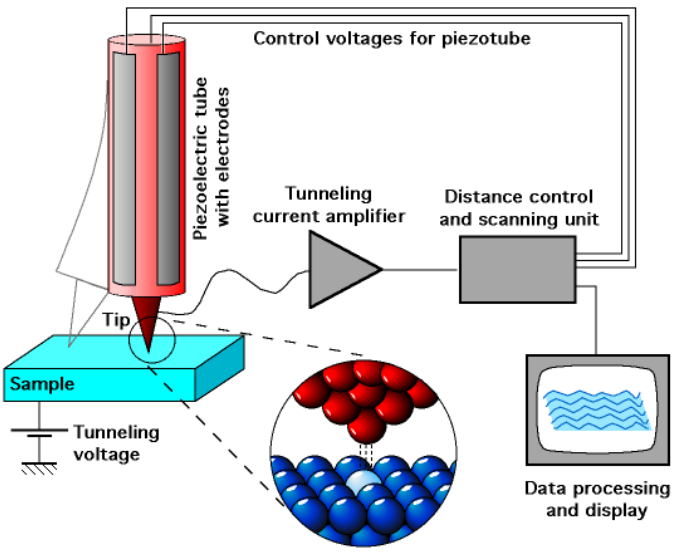


Illustration 1.1: *Schematic view of an STM Microscope. The tunneling current is indicated by the dashed lines between tip and sample in the amplification. (Image taken from en.wikipedia.org)*

For this method, a small voltage is impressed between the sample and the probe. The voltage is far too small to cause a dielectric breakdown, so the current between tip and

sample is only possible when the electrons tunnel through the potential barrier and therefore, the current depends on the tunneling probability. This is why the current-distance dependency is exponenential:

$$I(x) \propto e^{-2x\kappa}$$

with κ as a constant that depends on the voltage, the material etc. The Movement of the probe is controlled by piezoelectric elements in all three dimensions, so it is possible to scan a sample very precisely, but only in a small area. The distance between tip and sample is in the scale of 0,5nm to make the tunneling process possible. A schematic view of the scanning device is seen in ill. 1.1. STM can be performed in different modes:

Constant height mode: The probe is moved while the height of the tip is not changed in relation to the scanning device, so the distance between tip and sample changes according to the topography. In this mode, the variations of the current are used to calculate the topography of the sample.
This mode has the advantage of being relatively fast, but it can only be used on rather flat samples. Otherwise, there is a chance that the probe hits a high elevation and breaks, or that the current depletes when the probe encounters a deep dent.

Constant current mode: A feedback loop adjusts the height of the tip during the scan so that the current stays constant. Thus the probe follows the topography of the surface.
This mode is much slower than the constant height mode, but here, also samples with a more coarse surface can be examined without risking a crash of the tip.

The probe usually consists of noble metals or tungsten. The sample has to be conductive in order to make an electric current possible.
STM has a sufficient resolution to show the atomic structure of a surface. However, it interacts with the electronic states and therefore it has to be thought on what it actually measures. In illustration 1.2 for example, the image shows the electronic density in the quantum corral while there is no actual “surface”.

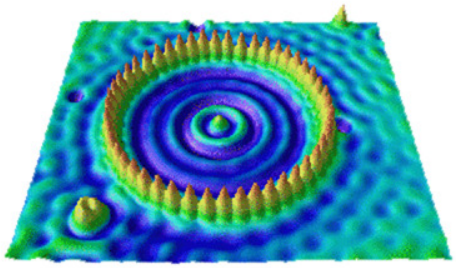


Illustration 1.2: *STM image of a so-called “Quantum Corral” where a Ring of Atoms on a flat substrate force surface Electrons into a ring-shaped quantum state.*

1.3 Scanning Force Microscopy (SFM)

Different from the STM, the Scanning Force Microscopy uses inter-atomic forces to receive a signal from the sample. The occurring forces are the *van der Waals-Force* which result from attracting dipole-dipole interactions and a repulsive force caused by the *Pauli exclusion principle*. The resulting potential is known as a Lennard-Jones-Potential and has the form

$$V = \frac{A}{r^{12}} - \frac{B}{r^6}$$

This Potential determines the force that the probe experiences.
To read out the measured forces, The probe is mounted on the top of a cantilever, as seen in ill. 1.3. The movement of the cantilever changes the angle in which the laser is reflected. The position of the laser is detected by two photodiodes. The signals of these photodiodes are compared and the displacement of the cantilever is calculated depending on which diode is illuminated more.

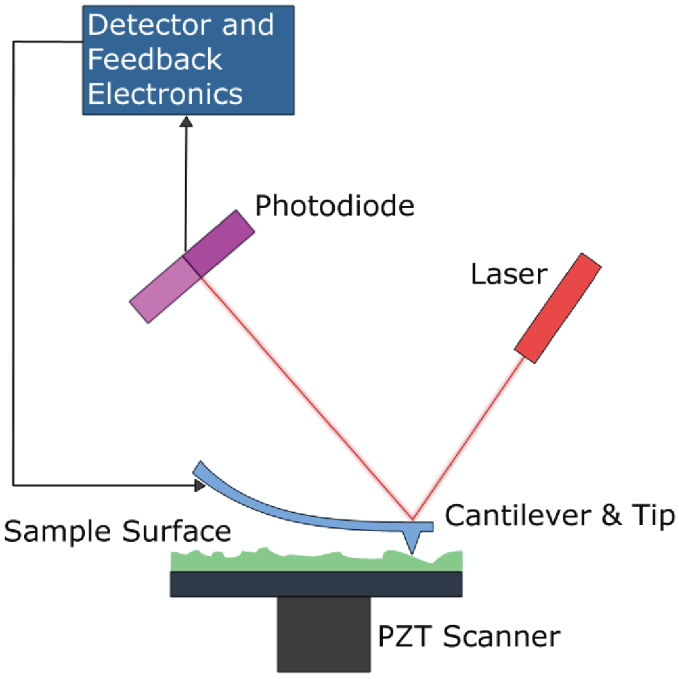


Illustration 1.3: *Operation principle of an AFM microscope. The two different colors of the photodiode signify that there are actually two photodiodes whose signals are compared in order to get a better resolution of the cantilever's position. (Image taken from en.wikipedia.org)*

The scanning modes of an AFM divide into a contact mode where the tip is pressed onto the surface and a non-contact mode where the tip is influenced by the occurring Lennard-Jones-Potantial near the surface:

Contact Mode: Because of the Pauli-exclusion-principle, the probe experiences a strong repulsion from the surface. Like in the STM, the sample is scanned line by line and also in contact mode, there is a constant-height- and a constant force mode which work similar to the STM modes, but of course by measuring

the bending of the cantilever instead of an electric current.
The surface itself may be scratched or damaged by the probe, therefore the possible application of this mode is limited. The force on the surface is smaller in the constant force mode but still existent, the scanning speed is higher in constant height mode because there is no tracking that has to be done.

Non-Contact Mode: This mode is different from the other modes mentioned, because it does not use a static signal. The cantilever is excited to oscillate on its resonance frequency. Any force from the surface that influences the probe changes the oscillation frequency and is a measure for the interaction. This information is used to calculate the structure of the surface. If applied in vacuum or UHV, it is possible to detect single Atoms or molecules on a surface that is does not have to be conductive, unlike an STM sample.

In this experiment, the DNA samples are examined via the AFM non-contact mode.

1.4 DNA

Desoxyribo**n**ucleic-**a**cid is a long polymer that carries the genetic information of all living organisms. The DNA is divided into segments that have different purposes: the genes carry the information to construct parts of cells, for example proteins, other segments regulate the use of these information.

1.4.1 Structure

A DNA molecule is a polymer that forms a double-helix structure with base-pairs in between two backbone chains, see ill. 1.4. The chains consist of two alternating units: phosphoric acid and desoxyribose, a sugar. The two strands are not exactly opposite to each other, so the projection of a DNA molecule shows a major and minor groove. Furthermore, they are arranged antiparallel, so an end or breakage of a DNA-strand has a 5'-end at the phosphate and a 3'-end at the sugar-ended side. As seen in illustration 1.4, the molecule has a diameter of 2.2 to 2.4nm. The Base-pairs have a distance of 0.34nm and an angle of 36° to each other, that means a full turn of the helix is 3.4nm long and contains 10 base-pairs.

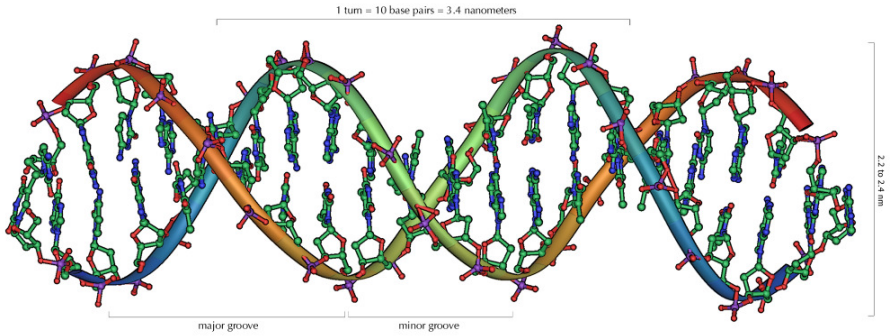


Illustration 1.4: *The structure of a DNA double helix, B-Form. The 5'-ends are colored red, the 3' ends blue.*

Attached to the sugar there is one of the four bases Adenine, Cytosine, Guanine or Thymine. They form groups of either Adenine and Thymine or Guanine and Cytosine at the same elevation between the spiral strands and are connected via Hydrogen bonds. The sequence of the base-pairs is the information that the DNA contains.
In natural biological systems, three different conformations of the DNA have been observed: A-DNA, B-DNA and Z-DNA. The B-form of DNA is the most common conformation and has therefore been chosen for a more detailed description, the other conformations differ in structure and size:

A-DNA: With a diameter of 2.6nm slightly wider than the other conformations, the A-DNA has an average of 11.6 base-pairs in each helical turn. The angle between the pairs is 31° and the distance is 0,29nm. The form major groove is narrower and deeper while the minor groove is shallower and wider compared to the B-DNA. The A-DNA is produced in cells as a hybrid pairing of DNA and RNA strands.

Z-DNA: This form shows a left-handed twist and a much larger distance between the base-pairs: 0.74nm. On the other hand, it is a bit thinner with a diameter of 1.8nm. This unusual form of DNA has been found in connection with B-DNA in 2005 and gave first hints of the biological activity of Z-DNA.

1.4.2 Elements

The helical structure of DNA consists, as already mentioned, of phosphoric acid and a sugar, 2-desoxyribose. These form a supporting structure that contains the base-pairs Adenine-Thymine and Cytosine-Guanine as it is shown in ill. 1.5. The AT-pair is connected by 2 hydrogen bonds, the CG-pair with 3 bonds.

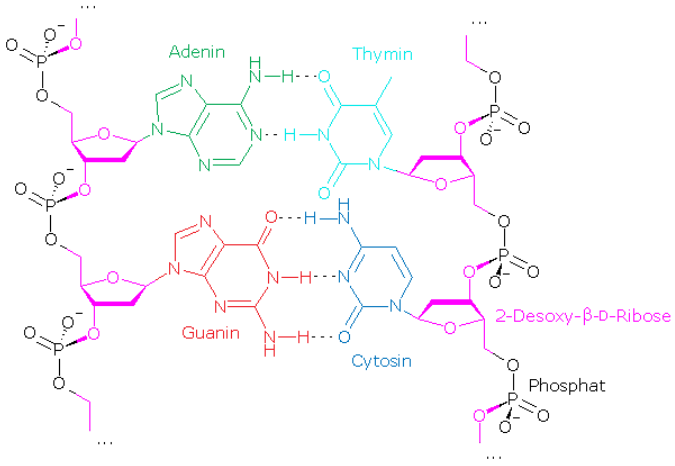


Illustration 1.5: *Chemical structure of the components that form a DNA polymer. Every molecule is marked in a different color, the dashed lines indicate hydrogen bonds.*

Regarding the similar structures of Adenine and Guanine, these molecules belong to the group of Purines while Cytosine and Thymine are Pyrimidines. The phosphate is hydrophil because of its negative charge. It is also responsible for the chemical properties of an acid of the whole structure.

2 Performance

2.1 Experimental Setup

The experiment is set up under normal conditions, that means not in a vacuum and without cooling to reduce the particle movement. The AFM is placed on an active damping table which stands as well as on an optic table. The sample insertion and calibration of the cantilever is done manual, the control of the measurement settings and data readout is done by a measurement software.

2.2 Performance

For sample preparation, $3\mu l$ DNA solution ($0.5\frac{ng}{\mu l}$ DNA in solution of $10mmol$ HEPES buffer, $10mmol\ MgCl_2$ and $400\mu l$) is superimposed on a mica substrate, which was first cleaned by adhesive tape. After deposition the DNA solution the sample is scavenged by double-distilled water and dried with waterless nitrogen.
Ensuing the prepared sample is transferred in the AFM and an autoapproach is performed. By the completion of the approach the sample can be imaged and the results saved on hard disk.

2.3 Measurement Data

The acquired data is saved on the PC connected to the AFM and copied to an USB stick for the evaluation at home.

3 Evaluation and error analysis

In this evaluation the length, height, width and conformation of the used DNA molecules are been analyse by the image-analysis-programs, ProScan 1.7 and ImageJ 1.36b. We select 40 DNA out of eight measured AFM-pictures to make significant statistic. This choosen and numbered DNA are illustrated in the appendix (6.1), page -13-.

3.1 DNA length

The DNA length is determinated by the program ImageJ 1.36b and abstracted in the following table, illustration (3.1).

Image 1

DNA	length[nm]	twists	typ
1	1570	0	open
2	1852	2	closed
3	1818	2	closed
4	1836	1	closed
5	1726	0	closed
6	1824	0	closed
7	1785	2	closed

Image 2

DNA	length[nm]	twists	typ
1	1852	0	closed
2	1802	0	closed
3	1898	1	closed
4	1891	1	closed
5	463	0	open
6	1942	0	closed

Image 3

DNA	length[nm]	twists	typ
1	1499	2	open
2	1294	0	open
3	1608	0	closed
4	748	0	open
5	385	1	open
6	1481	0	closed
7	1572	0	closed

Image 4

DNA	length[nm]	twists	typ
1	3437	4	closed
2	1544	0	closed
3	1863	0	closed
4	1504	1	closed
5	1583	1	closed

Image 5

DNA	length[nm]	twists	typ
1	1590	0	open

Image 6

DNA	length[nm]	twists	typ
1	1955	0	closed
2	1852	0	closed

Image 7

DNA	length[nm]	twists	typ
1	2069	3	closed
2	2044	1	closed
3	1893	0	closed
4	1801	0	closed
5	1903	0	closed
6	1800	0	closed
7	1829	0	closed

Image 8

DNA	length[nm]	twists	typ
1	1791	1	open
2	1905	1	closed
3	1785	0	closed
4	1748	0	closed
5	1859	1	closed

Illustration 3.1: *length and conformation of the selected DNA from appendix (6.1)*

3.1.1 Distribution of the DNA length

If we classify the DNA in length-intervals of 100 *nm*, it is possible to get an statistic distribution of the DNA length on the prepared substrate.
This statistic distribution is shown in the following illustration (3.2) on page -10-.
In the used solution, on average the most frequently DNA length is approximately 1800 *nm* and could directly read off the illustration (3.2).

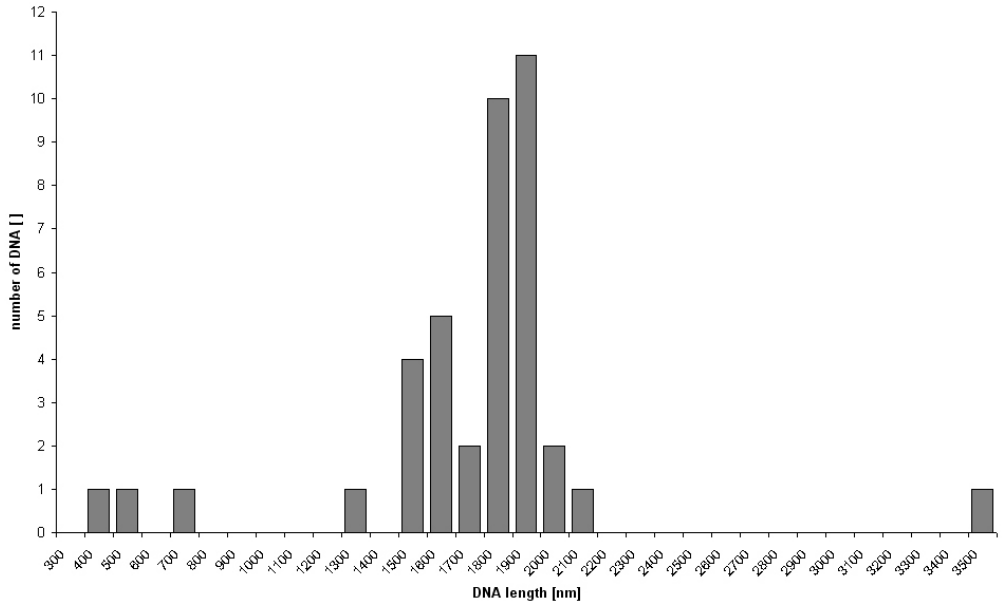
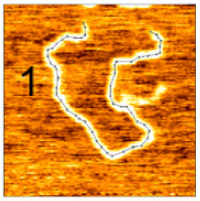
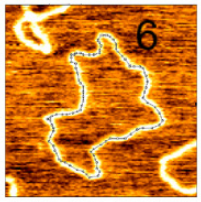


Illustration 3.2: *statistic distribution of DNA length*

3.1.2 Error of the DNA length determination

The error of the DNA length determination is calculated by standard deviation. We measure ten times the length of two typs of DNA, an open and a closed one, to get sensible values for standard deviation. This approach is displaied in illustration (3.3) below.





open DNA	Image 1 DNA 1
measurement	length [nm]
1	1555,4
2	1570,8
3	1568,3
4	1561,6
5	1565,2
6	1570,9
7	1564,4
8	1557,7
9	1607,2
10	1579,4
average	1570,1
deviation	14,8
rel. error	0,009

closed DNA	Image 1 DNA 6
measurement	length [nm]
1	1836,3
2	1798,2
3	1821,2
4	1841,3
5	1807,6
6	1802,1
7	1816,4
8	1858,4
9	1824,9
10	1828,6
average	1823,5
deviation	18,7
rel. error	0,010

Illustration 3.3: *Determination of the DNA length-error*

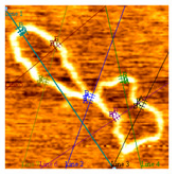
From the average of both standard deviations we get a relative error for the DNA lenght of $\pm 1\%$.

3.2 DNA width and height

The DNA width and height are determinated by the program ProScan 1.7. Three typs of DNA, an open, a closed and a crossed one is analysed representativly for each group of conformation. The results are shown in the illustration (3.4) below.

Image 1 - DNA 4

line	height [nm]	width [nm]
1	0,281	25,36
2	0,515	24,13
3	0,294	24,20
4	0,255	21,93
5	0,258	23,29
6	0,260	22,71
7	0,205	18,29



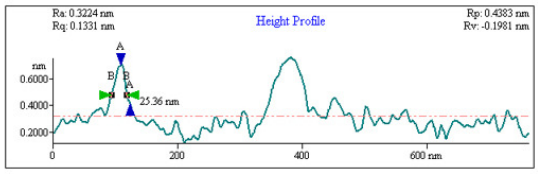
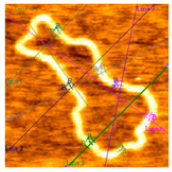


Image 6 - DNA 2

line	height [nm]	width [nm]
1	0,307	33,40
2	0,290	36,38
3	0,310	36,70
4	0,289	29,65
5	0,324	35,94
6	0,336	34,84
7	0,292	31,39



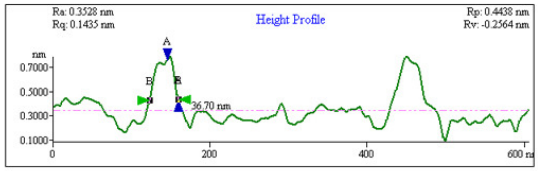
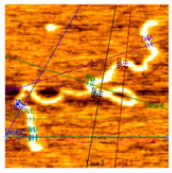


Image 8 - DNA 1

line	height [nm]	width [nm]
1	0,289	24,90
2	0,293	21,78
3	0,236	20,84
4	0,285	23,17
5	0,261	22,09
6	0,204	26,26
7	0,274	29,30



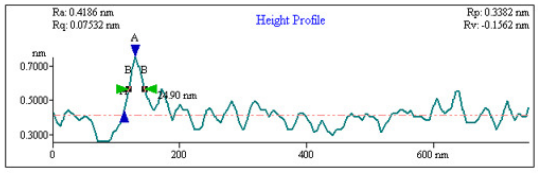


Illustration 3.4: *width and height for three typs of DNA: open, closed and crossed*

It is easily to see that the width and height for all typs of DNA is almost equal. So the average of the height is

$$0.29nm \pm 0.06nm \;=\; 0.29nm \cdot (1 \pm 0.214)$$

and the average of the width is

$$27.0nm \pm 5.7nm \;=\; 27.0nm \cdot (1 \pm 0.213)$$

. From both averages of the standard deviation we get altogether a relative error for the DNA width and height of $\pm 21.4\%$.

3.3 Conformation of the DNA molecules

There exists two types of conformation of the DNA on the substrate surface. One refers to the number of twists and the other on the open or closed ringstructure of the DNA. At first it is statistically possible to say that approximately 80% of the DNA in solution keep hold of their closed ringstructure by preparating the sample and 20% creat a stringstructure.
For the twistnumbers it make sense to make an statistical analyse. The distribution of the number of DNA-twists on the sample is shown in illustration (3.5) below.
According to this twistless DNA's averages at least 60%.

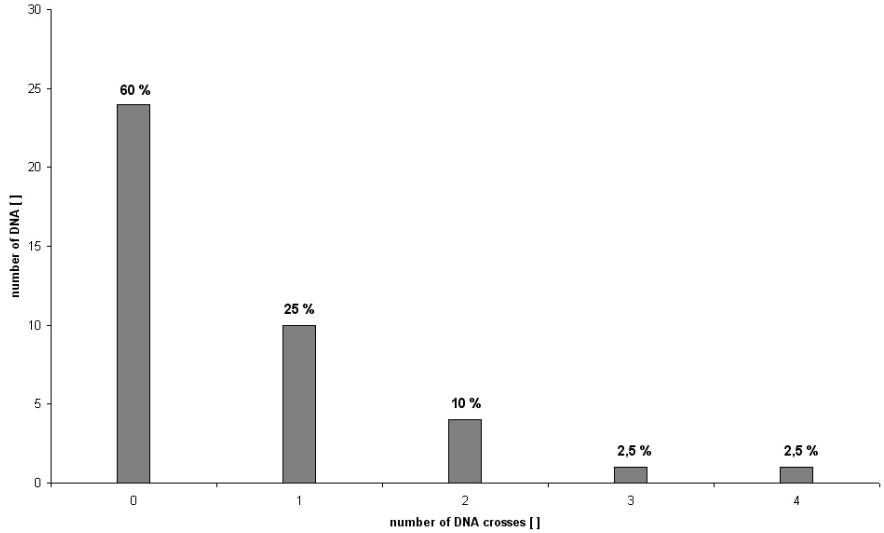


Illustration 3.5: *statistic distribution of the number of DNA-twists*

4 Error discussion

At first we could say that the preparation and AFM-analyse of the DNA-solution on the sample was succesful. The recorded AFM-images could be interpret well and it existed enough different DNA types to make satisfying statistic calculations.
The relative error of only 1% for measuring the DNA-length is pleasant small.
In comparison to the norminal value of $2.37nm$ for DNA diameter the width and height differ strongly. It exists different reasons for this circumstances. Foremost the circumference of the AFM tip is around $10nm$ and therefore five times larger than the DNA diameter, which would be measured. It would never be possible to reach DNA-diameter-resolution with the used tip-types and measuring in no-contact-mode.
An other reason for the hundred times smaller height then width of the outcomes could be put down to the ionic interaction between the negative charged DNA and the positive charged subtrate using $Mg^{2+} + Cl^{-}$ buffer.
Finally the performing and evaluation of the experiment could be judged as good.

5 Outcomes

In the described experiment - Imaging of biological samples by means of Scanning Probe Microscopy - the statistical distribution of length, width, height and conformation of $0.5\frac{ng}{\mu l}$ DNA in solution of $10mmol$ HEPES buffer, $10mmol$ $MgCl_2$ and $400\mu l$ double-distilled water were analysed by atomic force microscopy.
The performing and evaluation of the experimnt are resulted in the outcomes below.

relative error of lenghtmeasurement: $\pm 1\%$
middle height of used DNA: $0.29nm \cdot (1 \pm 0.214)$
middle width of used DNA: $27.0nm \cdot (1 \pm 0.213)$
approx. 60% of the DNA on the subtrat offer no twists
approx. 80% of the DNA on the substrat offer a ringstructure

6 Appendix

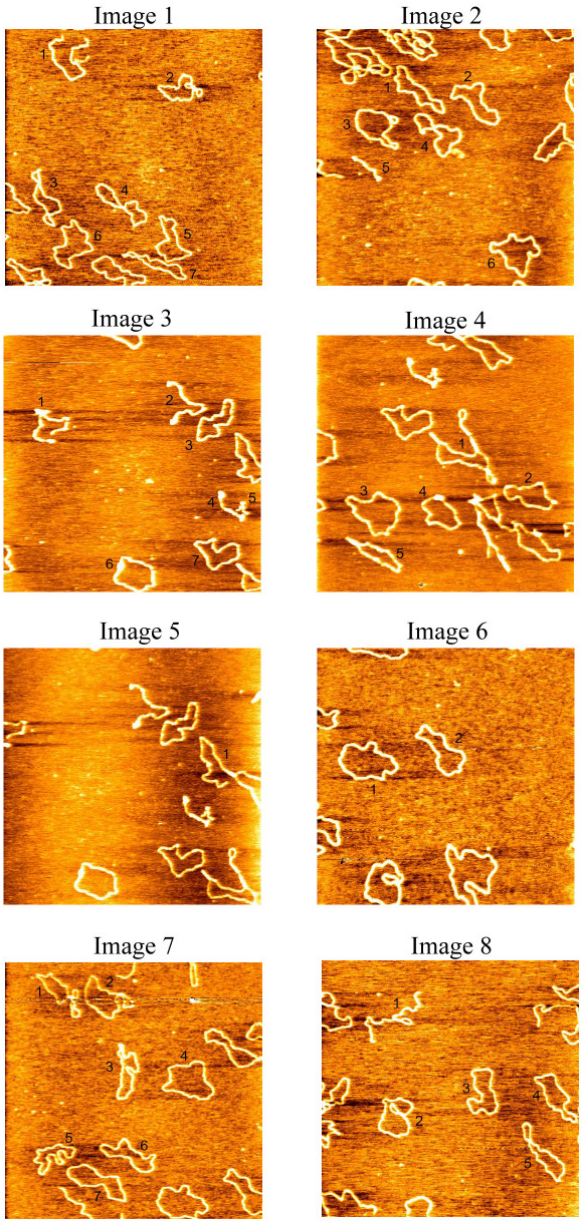


Illustration 6.1: *the 40 selected and numbered DNA*

Literatur

[1] INSTITUT FÜR PHYSIK, ARBEITSGRUPPE TRÄGER, FB 18, UNIVERSITÄT KASSEL:
Versuchsskript: DNA imaging by means of Scanning Probe Microscopy, 2006.

Abbildungsverzeichnis