Basic methods in imaging of micro and nano structures with atomic force microscopy (AFM)

(Item No.: P2538000)

Curricular Relevance

Area of Expertise: Physics
Education Level: University
Topic: Modern Physics
Subtopic: Nano Physics
Experiment: Basic methods in imaging of micro and nano structures with atomic force microscopy (AFM)

Difficulty	Preparation Time	Execution Time	Recommended Group Size
Very difficult	10 Minutes	2 Hours	2 Students

Additional Requirements:

- PC (Windows)

Experiment Variations:

Keywords:

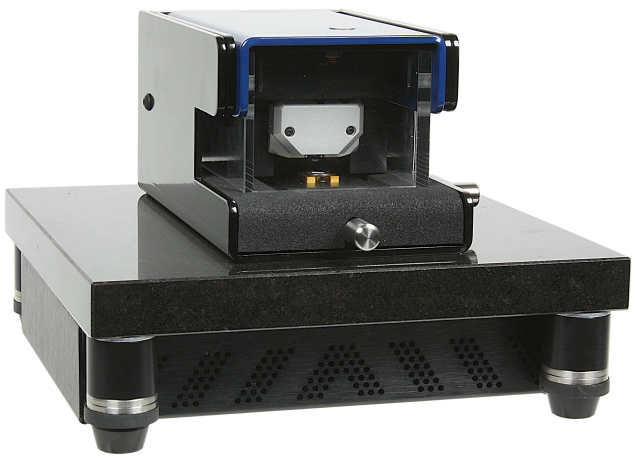
Atomic Force Microscopy (AFM), Lennard-Jones potential, Imaging of nano structures, Static force mode, Dynamic force mode, Feedback loop, Force, Vibrational amplitude

Overview

Short description

Principle

Approaching a sharp silicon tip mounted on a cantilever to a sample surface leads to an atomic scale interaction. The results is a bend of the cantilever which is detected by a Laser. In static mode the resulting deflection is used to investigate the topography of the sample surface line-by-line using a feedback loop. In dynamic mode the cantilever is oscillated at fixed frequency resulting in a damped amplitude near the surface. The measurement parameters (setpoint, feedback gain,...) play a crucial role for image quality. The dependence on the imaging quality is investigated for different nano structured samples.



PHYWE Atomic Force Microscope

Equipment

Position No.	Material	Order No.	Quantity
1	Compact AFM, Atomic Force Microscope	09700-99	1
Additional material			
	PC, Windows® XP or higher		

Tasks

Setup and procedure

Setup

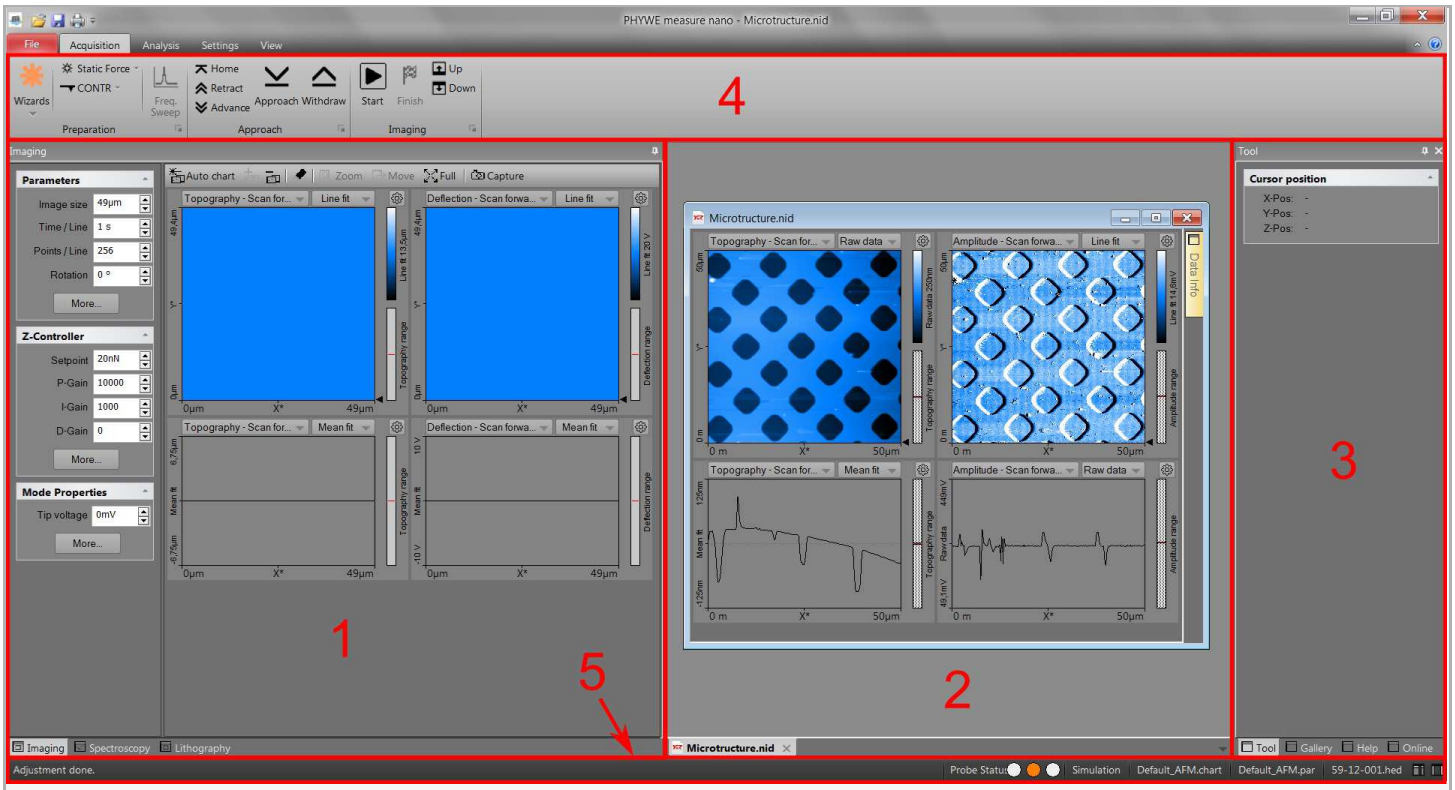
Procedure (task 1)

The software interface

Start the PHYWE *Measure Nano*software and make sure the correct calibration files are loaded:

1. Open the menu item “File” >> “Parameters” >> “Load...”, and load the file “Default_AFM.par” from the directory that holds the default Measure Nano configurations. Usually this is “C:\Program Files (x86)\PHYWE\measure nano\Config”.
2. Open the menu item “File” >> “Chart Arrangement” >> “Load...”, and load the file “Default_AFM.chart” from the directory that holds the default Measure Nano configurations.

Software interface



The Software Interface in “Normal” workspace mode

The software provides all functions to operate the microscope during imaging of surfaces and more advanced operating modes. It also provides data analysis functions for post-processing of measurement data.

The main STM Control Software window (also referred to as workspace) consists of five major areas (Figure 3):
(1) The Measurement pane on the left. This area contains the so-called *Operating windows*, which are used to acquire and display ongoing measurement data
(2) The *Document space*in the middle. This area is used for displaying and analyzing previously stored measurement documents.
(3) The Info pane on the right. This area contains several stacked *Panels*and is used to group a diverse array of functionality and information.
(4) The *Ribbon*at the top. This area is used to access all action functions.
(5) The *Status bar*at the bottom. This area is used to display additional information.

Changing parameters in any panel

- Activate the parameter by clicking it with the mouse pointer, or by selecting it with the “Tab” key.

In case of a drop-down menu selection, change the selection with the mouse, or the “Up” and “Down” arrows on the keyboard. In case of a numerical value, use one of the following methods:

- Use the “Up” and “Down” arrow keys of your keyboard to increase or decrease the value of a parameter. The new value is automatically used after one second.
- Click the arrow buttons next to the parameter’s value with the mouse pointer. The new value is automatically used after one second.

Enter the new value using the keyboard. The entered value is applied by pressing the “Enter”/“Return” key, or by activating another input.
The entered value is discarded by pressing the “Esc” key. The unit prefix can be changed by typing one of the following keyboard keys:

shortcut	prefix
f	femto
p	pico
n	nano
u	micro
m	milli
k	kilo
M	mega
G	giga
T	tera
space-bar	none

Example: If the basic unit is volts, type “m” to change to millivolts, or type “u” for microvolts. Sometimes the program changes an entered parameter value to a slightly different value. This happens when the desired value is outside the digitization range of the Measure Nano Controller, for example due to resolution or timing limits. In such cases, the desired value is automatically changed to the nearest possible value.

Mounting the cantilever (incl. tip)

Sample preparation

Approaching the tip towards the sample

To start measuring, the cantilever tip must come within a fraction of a nanometer of the sample without touching it with too much force. To achieve this, a very careful and sensitive approach of the cantilever is required. This delicate operation is carried out in two steps: *Manual coarse approach*and the *Automatic final approach*. The color of the Status light (at the bottom of the software interface) shows the current status of the approach:
– Orange/yellow
Normal state during approach: the Z-scanner is fully extended toward the sample.
– Red
The approach has gone too far: the tip was driven into the sample, and the Z-scanner is fully retracted from the sample. In this case, the tip is probably damaged and you will have to install a new cantilever again.
– Green
The approach has finished successfully: the Z-scanner is within the measuring range. To prepare for the approach process:

- Select the Acquisition tab

The controls for positioning the cantilever with respect to the sample are located in the Approach group.
During the approach steps described in the following sections, use the side view of the cantilever, accessible from the lens on top of the device, to judge the distance between tip and sample surface:

Manual coarse approach

In this step, the tip is brought as close to the sample surface as possible, without touching it. The closer the two are together, the less time the automatic final approach takes.

1. Observe the distance between tip and sample in the side view of the integrated optics.
2. While observing the tip–sample distance, click and hold the “Advance” button in the Approach group of the Acquisition tab until the tip is close enough to the sample: The tip should not come closer to the sample than a few times the cantilever width (*Figure 10*). Now that the sample is in focus, the top view image from the integrated can be used to find a suitable location to measure on.

To use the top view:

1. Select *video*in the *info pane*of the Sofware interface.
2. If necessary, move the Sample Holder to find a suitable location that is free of dust particles.

Figure 10: Side view of sample and cantilever after manual approach.

Automatic final approach

In this last step, the tip automatically approaches the sample until a given Setpoint is reached. Before starting the automatic approach, select the desired operating mode and cantilever type. To do this:

- In the Preparation group of the Acquisition tab, select an operating mode and cantilever type that match the cantilever installed.

In Dynamic Force mode, the instrument will automatically determine the vibration frequency to be used during imaging. To determine the optimal frequency, the controller performs a coarse and a fine frequency sweep in which the cantilever vibration amplitude are recorded as a function of excitation frequency. It is instructive to see both frequency sweep measurements in all detail at least once. To do this, it is possible to manually perform the frequency sweeps:

1. In the Preparation group of the Acquisition tab, click the “Freq. Sweep” button: The Vibration Frequency Search dialog now opens
2. Click the “Auto frequency set” button. The SPM Control Software now sets appropriate values for the coarse and fine sweeps and performs these sweeps. The fine sweep will overwrite the data of the coarse sweep in the charts displayed in the “Vibration frequency search” dialog. To see the results of the individual sweeps:

- Press the “Coarse sweep” and “Fine sweep” buttons sequentially.

Frequency sweeps can be a used to check if a cantilever is undamaged and was mounted correctly in dynamic mode (It is not necessary to close the AFM to do so). If so you will receive a well define resonance curve and a high vibrational Amplitude (depending on the excitation amplitude).

Before final approach of the sample, it is necessary to set the scanning and feedback parameters of the control software to suitable initial values. The easiest way to do this is to use the “Auto Set” wizard:

1. In the Preparation group of the Acquisition tab, click the “Auto Set” button: A dialog will pop up, which will ask you some basic questions about your sample and your measurement needs.
2. Answer the questions of the wizard to the best of your knowledge.

Now that the initial software settings have been given suitable values, you need to name the measurement series. Each completed measurement (scan/image) will be temporarily saved (automatically) in the History folder under this name, with index numbers (or, optionally, date and time attributes) added to identify the individual measurements. It is best to enter the measurement series’ name now, since the control software will (by default) start measuring as soon as the final approach is done. It is also strongly recommended to move all relevant measurements to a new folder when you are finished, since the files in the History folder will be overwritten over time.

To set the measurement series name:

1. Activate the Gallery panel in the Info pane.
2. Click the History tab at the top of the Gallery panel with the mouse.
3. In the entry box at the top of the panel, enter a name by hand or use the Mask Editor dialog to create the name mask. If no [INDEX] attribute is explicitly added to the name mask, it will be implicitly applied to the end of the file name so that individual measurements can be stored and distinguished.

The automated final approach can now be started. To do this:

Robert-Bosch-Breite 10
D - 37079 Göttingen
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107
info@phywe.de
www.phywe.com

1. In the Approach group of the Acquisition tab, click the “Approach” button:

The cantilever is moved towards the sample via the approach stage, with the ZController turned on. This movement continues until the Z-Controller error becomes zero. From this point onward, the distance between sample and tip is maintained automatically by the electronics. The probe status light changes to a constant green, and a message “Approach done” appears: Click the “OK” button.

Starting a measurement

Now that the tip–sample interaction defined by Setpoint is established between tip and sample, measurements can start. By default, the instrument is set to automatically start measuring after the automatic approach. If this is not the case:

- Start measurements manually by clicking the “Start” button in the Imaging group of the Acquisition tab:

Two representations of the ongoing measurement are drawn in the Imaging panel. One representation is a color coded height image (Topography) called a Color map. The other is a plot of height as a function of X* position called a Line graph. With the current settings, the software automatically adjusts the contrast of the Color map, and height range of the Line graph to the data that have been measured.
To judge the imaging quality, watch the displays until at least one fourth of the measurement has been completed. When a measurement contains large disturbances, or no two scan lines are similar, stop
measuring and try to reduce or eliminate the disturbances or try retracting the tip and re-approaching a different sample position.

Selecting a measurement area

If you were able to prepare your measurement so that the scan line in the Line graph reproduces stably, the color map graph should look similar to the one shown below after the measurement has finished. To zoom in to an interesting part of the measurement:

1. Activate the color map graph by clicking on it.
2. Click the “Zoom” button in the Chart bar: The mouse pointer becomes pen-shaped when moving over the color map.
3. Click on one corner of the region to be selected using the left mouse button, and keep the button pressed.
4. Drag the mouse to the other corner of the region. The size and the position of the square are shown in the Tool results panel of the Info pane.
5. Release the mouse button when the size of the square covers approximately oneperiod of the grid.
6. Confirm the selection by double clicking the color map graph using the left mousebutton. Now the selection is enlarged to the whole display size. You can abort the zoom function by clicking the “Zoom” button again.

Depending on measurement parameters you will come across bad scans as shown in *Figure 11*. In this case the scanning parameters need to be adjusted as shown in this manual.

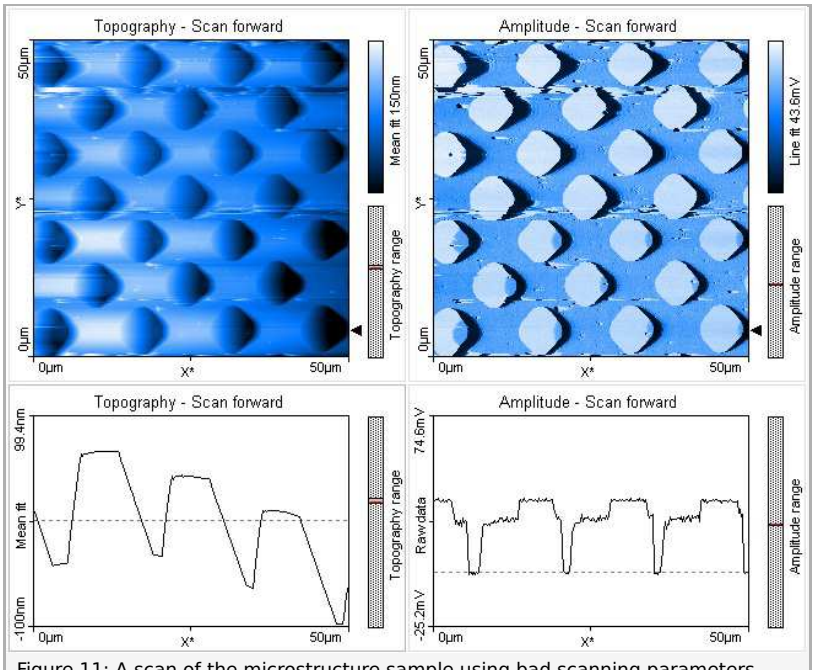


Figure 11: A scan of the microstructure sample using bad scanning parameters

Theory

AFM

PID feedback system

Before starting any AFM measurement it is necessary to understand how the feedback regulation system works. This regulation enables the acquisition of an AFM image. As described previously, the cantilever deflection is detected by a sensor. This position is then compared to a set-point, i.e. a constant value of cantilever deflection chosen by the user. As the deflection of the cantilever is directly related to the tip–sample interaction force, the set point is usually given in Newton (N). Typical forces are in the nN range. The difference between the actual interaction force and the desired force is called the error signal . This error signal is then used to move the tip or sample to a distance where the cantilever has the desired deflection. This movement is then plotted in function of the lateral position of the tip and is the so-called topography. The goal of the feedback system is to minimize the error in a very fast manner so that the measured topography corresponds to the real topography of the sample. Therefore the error signal must be amplified by a PID controller (Proportional Integral Differential). A schematic representation of the feed-back system is shown in *Figure 15: PID controller.*

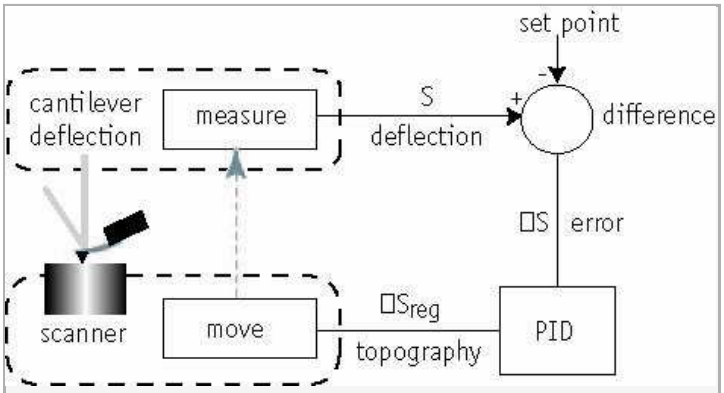


Figure 15: PID controller

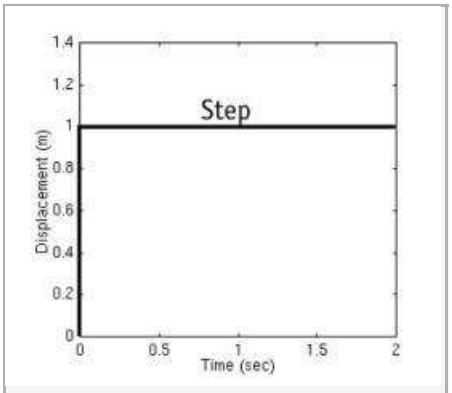


Figure 16: Step

These three gains can be set individually and define how fast and in which manner the error is minimized and the therefore how good the topography of the sample is reproduced in the measurement. Thus it is important to understand its characteristics. To illustrate the effect of the PID gains consider the following experiment. A step signal from 0 to 1 will be measured (see *Figure 16: Step*).

The goal is to reproduce the rectangular step as precisely as possible. Hence the PID gains must be adjusted. Figure 17: P-Gain shows the result when only the proportional gain (P) is turned up. The topography shows a long rise time (slope), an overshoot (peak) and a settling time (wobbles). As next the differential gain (D) will be turned up in addition to P. It can be seen in Figure 18: PD-Gain that the derivative gain reduces both the overshoot and the settling time, and had little effect on the rise time. In order to see the influence of the Integral gain (I) the D gain is turned down and the I gain up. As can be observed in Figure 19: PI-Gain the I controller further reduced the overshoot and decreased the settling time. The response is much smoother now, albeit with an increased rising time. When the P, I and D gains are combined in an appropriate way it is possible to obtain the response shown in Figure 20: PID-Gain with no overshoot, short rise time, and short settling time. The correct PID settings are sample dependent and have to be determined for each measurement.

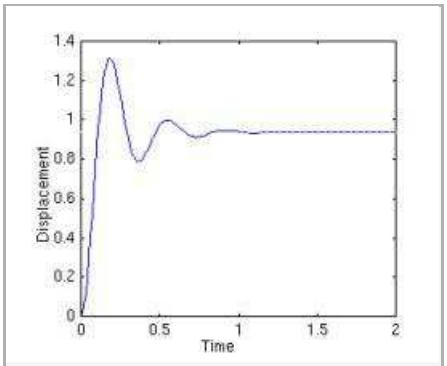


Figure 17: P-Gain

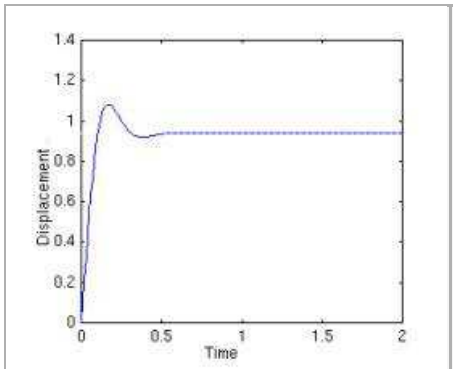


Figure 18: PD-Gain

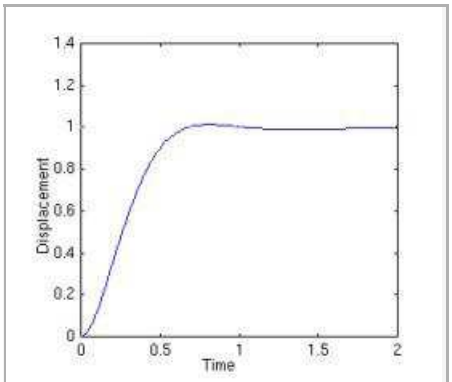


Figure 19: PI-Gain

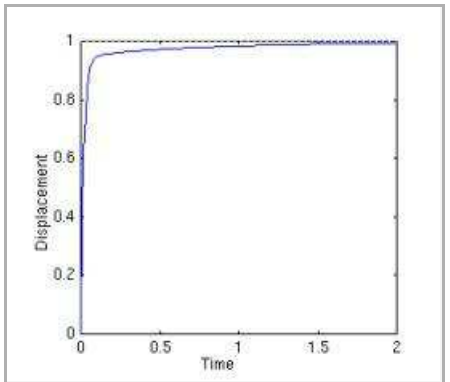


Figure 20: PID-Gain

Operating modes

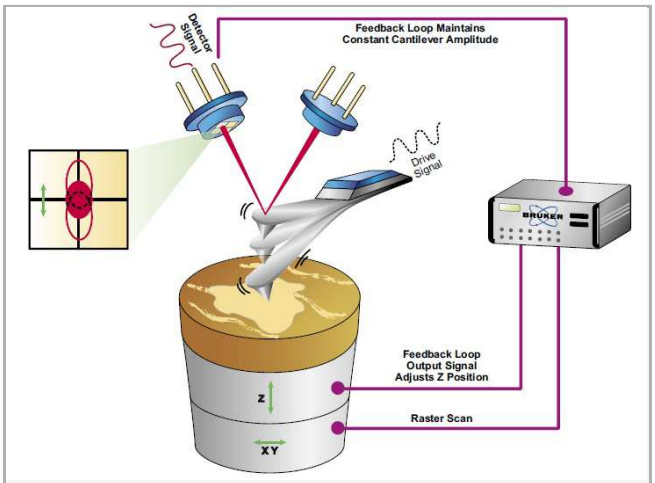


Figure 22: AFM setup in dynamic mode.

Task 2

Task

Influence of PID-gain

Since the chip structures are so well defined, this sample is conducive to testing the effects of your instrument's gain settings. The gain settings play an important role regarding image quality for all measurement modes of the AFM.

Image acquisition

1. Set a large scan range, somewhere between 10 and 80 µm. The chip structure can be clearly seen at this size.
2. Approach the reflective part at the center of the sample. This is the section that contains the most interesting structures of the chip. Note the well-ordered, repeating pattern. The height of the structures (or rather: the depth of the trench) is approximately 1.6 µm.

Caution:

Excessively high or low gains can result in damaged to the tip. Monitor your system carefully when adjusting the gains.

Optimize your gain settings

- If you have not already done so, make sure your gains are set to levels that produce reasonable images. The line trace in *Figure 24: Optimized Gain*represents well optimized gain settings; the tip is accurately tracking the topography of the SCA sample.

Lowering the gain

- Lower the integral gain well below the optimal setting. As you lower the gain, the feedback loop will not work quickly enough to provide high resolution. Note the poorly defined edges in *Figure 25: Low Gain*. At a lowered gain, the feedback loop is not responding quickly enough to respond to changes in height.

Raising the gain

- Gradually raise the gain to well above the optimal settings. At some point, the Z-controller will start to overcompensate for feedback errors when the tip encounters steps in the sample. This overcompensation is also called overshoot.

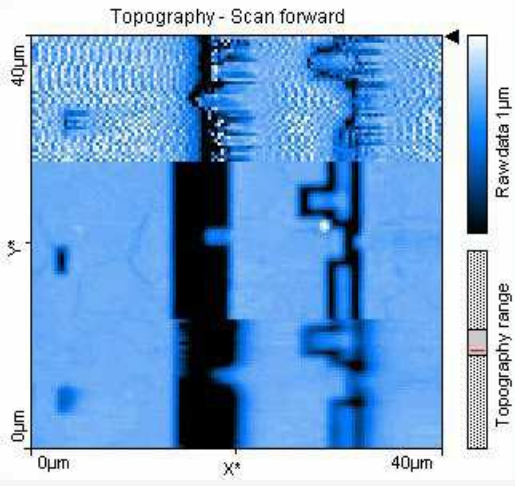


Figure 23: SCA chip structure imaged at different integral gain settings. In the lowest region of the image, the gain is too low; at the center, it is optimized; at the top, it is too high.

Overshoot and Undershoot

When the gain settings are increased further, the controller will react to this overshoot by undershooting; the undershoot will be less than the overshoot. These overreactions initiate an oscillation that eventually subsides. The frequency of this oscillation is either the mechanical resonance frequency of the
scanner or the resonance frequency of the cantilever itself.

At even higher gains, the oscillation will no longer subside. Instead, it will steadily increase, most likely resulting in damage to

the cantilever tip. The oscillation should be visible in both the topography and the error signal (deflection or amplitude, depending on the measurement mode) images. Be sure to monitor your system for indications that the controller is becoming unstable. First it will overshoot, and then it will “ring”, which is represented by a vibration with decreasing amplitude at the step edges. Additionally, the error signal (in this case the cantilever deflection) will start to increase again.

The following scans are performed in dynamic mode using a corresponding cantilever. Remember selecting the right cantilever in the *Acquisition*tab, too. The obervations are basically the same when measuring in contact mode. However, in contact mode the tip or sample might get damaged easier if the gain values are not set correctly.

As seen in the scans too low gain settings result in blurry images of the measured structures and too high gain will result in oscillations. Either extreme might damage the tip. Therefore, it is recommended to use the *Auto Set*function which will provide reasonable gain settings to start with. However, in general further optimization of the gain is necessary to get the best possible results.

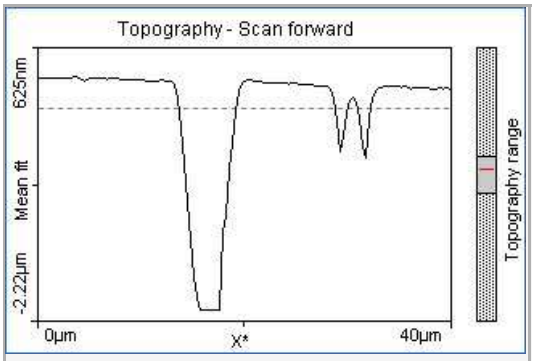


Figure 24: Optimized Gain. Optimized gain setting for SCA chip structure

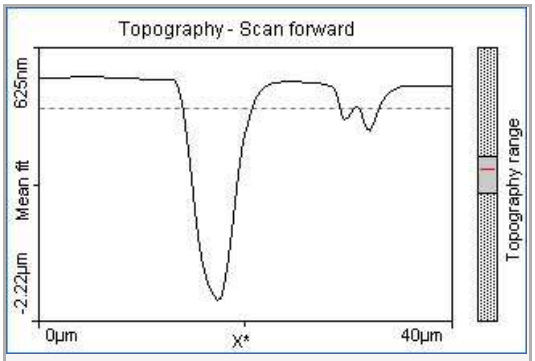


Figure 25: Low Gain. The feedback loop is not responding quickly enough.

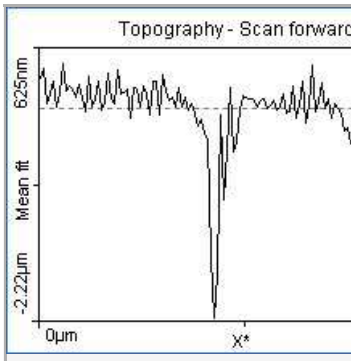


Figure 26: High Gain. Oscillating sig set too high.

Influence of setpoint

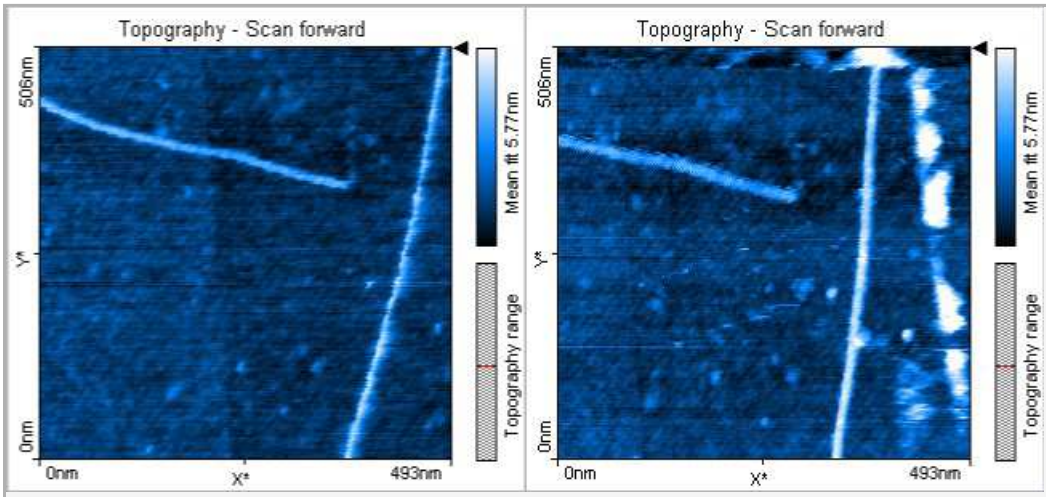


Figure 26: Static mode (Left) Force set point (10 nN) is too high. (Right) Lower force set point (2 nN). Note the dirt moved around by the previous measurement with excessive force set point.

The investigation of the influence of the setpoint can be observed best when measureing the carbon nanotube sample in static mode using a suitable cantilever. This sample represents the group of materials with macromolecules that are used for molecular nanotechnology. Other well-known examples are self-assembled particles, DNA, and nanotubes made of other materials.

AFMs can be used to characterize and manipulate such molecules. The well defined structure of nanotubes makes them ideal for demonstrating the influence the structure of the end of the AFM tip has on the measured image.

The carbon nanotube sample consists of a piece of silicon wafer on which carbon nanotubes are deposited. Nanotubes are less than 10 nm in diameter and can reach lengths of several 100 micrometers.

With this sample, the tip is likely to be damaged, if the scan parameters are not well optimized. Therefore, if you start with a relatively large range (~ 15 µm) and successively zoom in on an area of interest, it may not be possible to measure the nanotubes at high resolution because the tip already has been damaged by the high scan speed in the large scan.

Image acquisition

1. Set a small scan range (2 µm or less).
2. Take a scan.
3. Optimize scanning parameters
4. Zoom out by taking a scan at a relatively large scan range (~15 µm).
5. Identify an area of interest.
6. Zoom back in.

*Figure 26*illustrates an optimization sequence. At first, the set point is too high (10nN), so the nanotube gets pushed around. This makes it appear streaky and not as wide as it should be. With the Set Point lowered, the nanotube is imaged more stably. Note that the dirt that was pushed to the side in the first scan is visible on the side of the second scan. This is and effect typical for contact mode and is the result of scratching the tip across the surface always in the same direction as the scanning takes place.

As seen in the scans any structures that are not tightly aligned on the sample surface will be manipulated by the tip scratching over them in static mode. For hard samples like single crystals usually this is not an issue, however the tip might get damaged on such samples. On the other hand, static mode measurements on soft (e.b. biological) samples damage the samples easily. These effects can be controlled to a certain extend by adjusting the setpoint, but in general for any sample the wear of tip and sample is higher in static mode compared to dynamic mode.

Influence of vibration amplitude in dynamic mode

In dynamic mode, using a suitable cantliver, setting the vibration amplitude is crucial for achieving the best possible resolution. In static mode, the main parameter to regulate the image quality are the PID feedback settings and the Set point. In dynamic mode, the setting of the vibration amplitude additionally plays an important role. In general, the vibration amplitude must correspond to the size of the sample features:

- Low structures require a small amplitude.
- High structures requite a big amplitude.
- Small structures on top of big structures require a small amplitude and a slow scan speed.

Image acquisition

1. Find a clean spot on the sample
2. Approach the sample
3. Start the measurement
4. Adjust PID gains
5. Find the optimum vibration amplitude

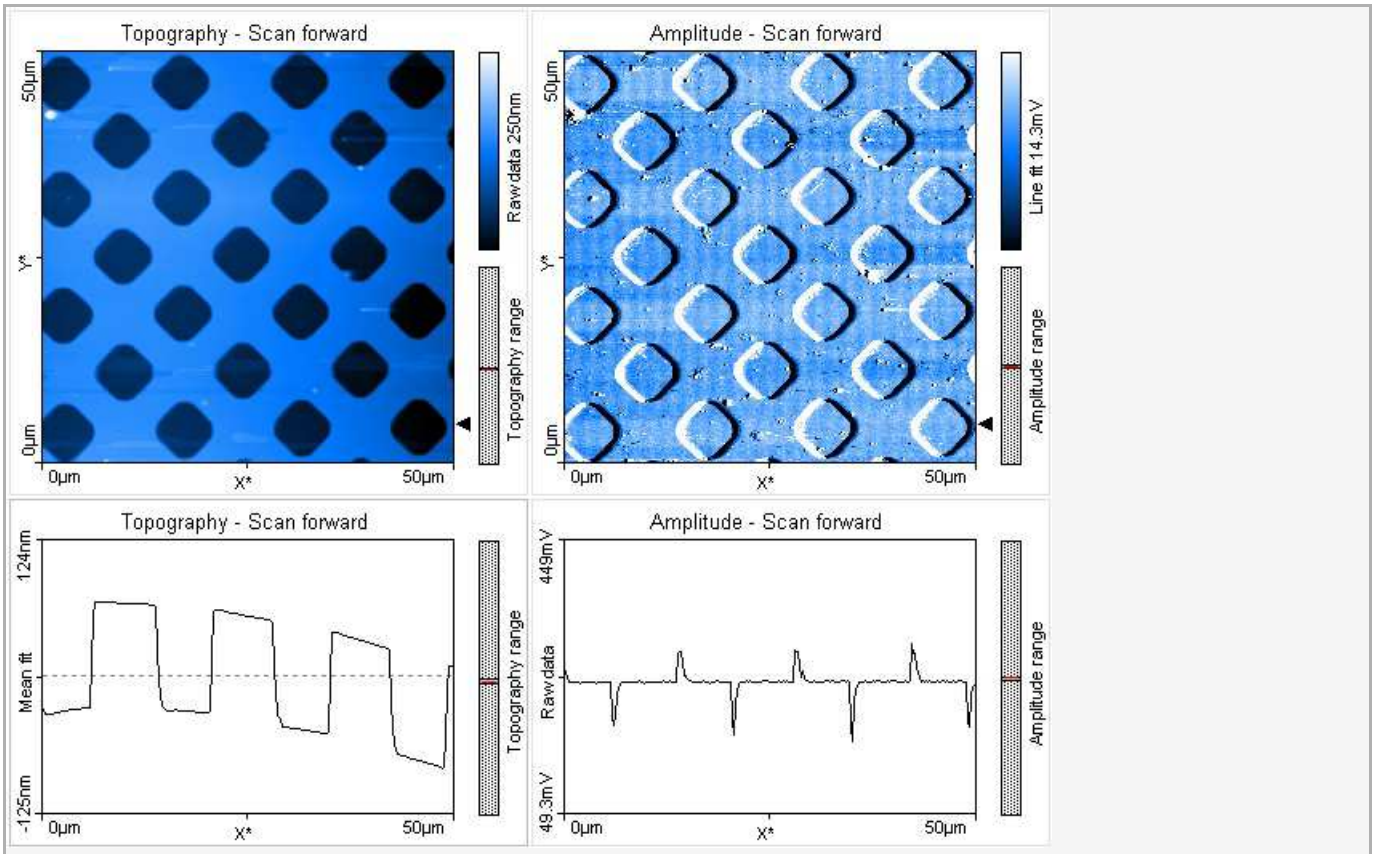


Figure 27: Large Amplitude. Topography and amplitude image of the microstructure sample. The line graphs show a cross section of the images above at the position indicated by the arrow. The vibration amplitude was set to 400 mV.

*Figure 27: Large Amplitude*shows the topography and amplitude image of the microstructure sample. The line graphs show a cross section of the images above at the position indicated by the arrow at the right side of the sacn. It is clearly visible that the in the topography the slopes are steep. After each perturbation the amplitude signal is also corrected to the Set point value very quickly.

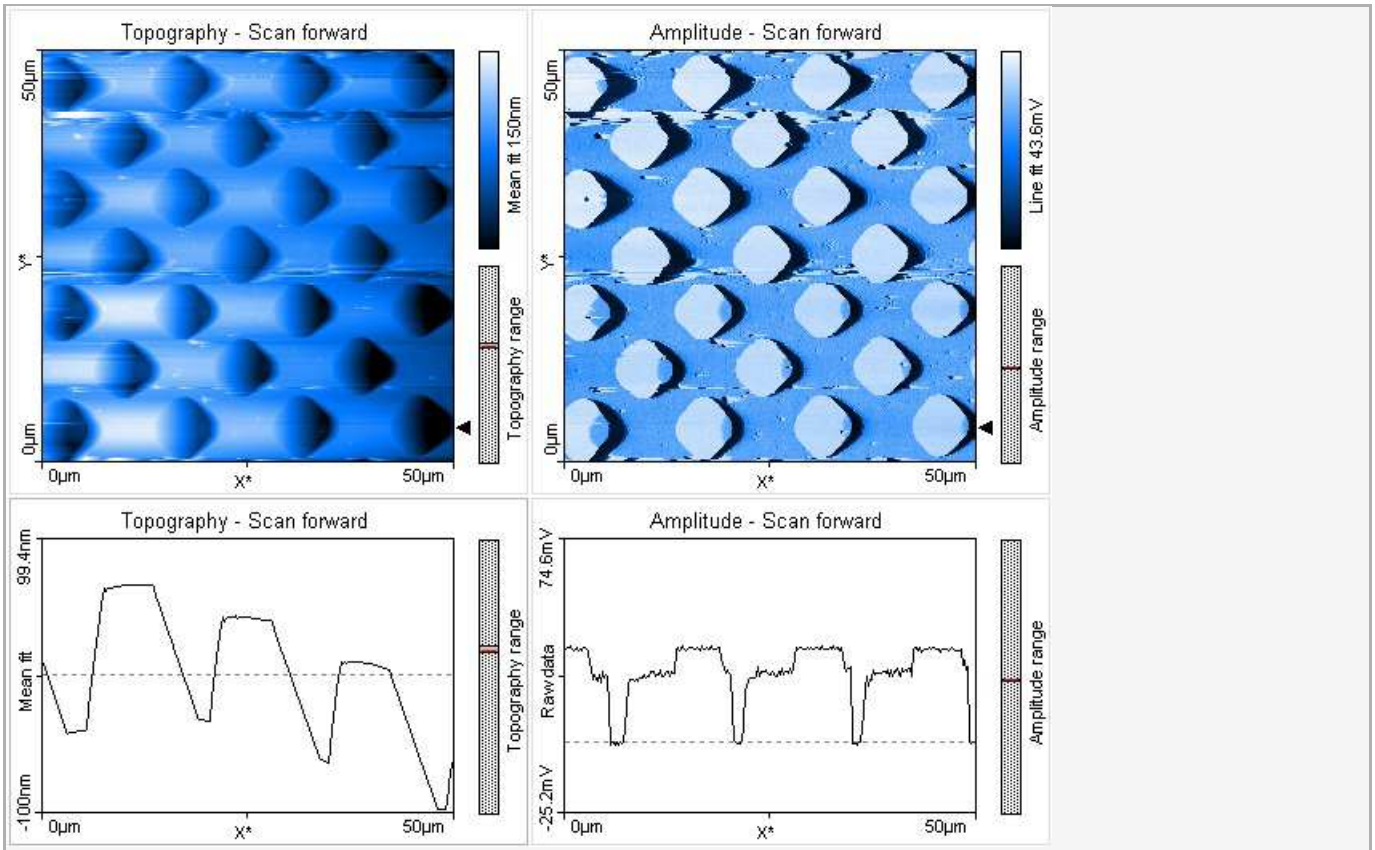


Figure 28: Small Amplitude. Topography and amplitude image of the microstructure sample. The line graphs show a cross section of the images above at the position indicated by the arrow. The vibration amplitude was set to 40 mV.

*Figure 28: Small Amplitude*shows the topography and amplitude image of the microstructure sample with too low vibration amplitude. The line graphs show a cross section of the images above at the position indicated by the arrow. The topography image is smeared out and the topography line graph shows a too small slope. The reason therefore can be found in the amplitude signal. The peaks are larger; this means that the correction to the amplitude to the Set point value is not as quick as in *Figure 27: Large Amplitude*. Due to the small vibration amplitude when the tip needs more time from the moment where it lost the contact to the surface to the moment it gains contact again. During this time the topography is uncertain and the tip is vibrating at the free vibration amplitude. Increasing the vibration amplitude or decreasing the scan speed will increase the quality again.

When scanning in dynamic mode the amplitude image should be mostly constant indicating that the feedback loop reacts fast enough on changes in the topography of the sample as ssen in *Figure 27 : Large Amplitude*. Only at sharp features on the surface the amplitude might deviate.
When scanning a sample with too low vibrational amplitude the amplitude image will not correspond with features in the topography because the feedback loop is not able to react properly to the large changes in the amplitude when coming across a high feature on the sample surface (*Figure 28: Small Amplitude*).

Task 3

Task

Chip structure

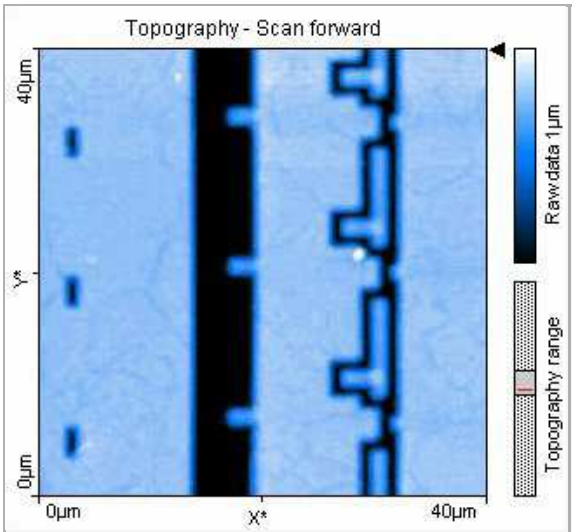


Figure 29: The SCA Chip Structure. AFM image taken at the center of the chip sample. The depth of the trenches is approximately 1.6 µm.

Image acquisition

1. Set a large scan range, somewhere between 10 and 80 µm. The chip structure can be clearly seen at this size.
2. Approach the reflective part at the center of the sample. This is the section that contains the most interesting structures of the chip. Note the well-ordered, repeating pattern. The height of the structures (or rather: the depth of the trench) is approximately 1.6 µm.

The characterization of chips, also known as Integrated Circuits (ICs), is an important application of AFM technology. The dimensions of the structures in these circuits are decreasing rapidly, and no other tool is able to characterize these dimensions without destroying the sample. This particular chip is a Switched Capacitor Array (SCA) chip. SCA chips are custom-made silicon chips which can sample an analog input signal at high speed (in this chip up to 950 GHz), but can then be read out at lower speeds.

Carbon nanotubes

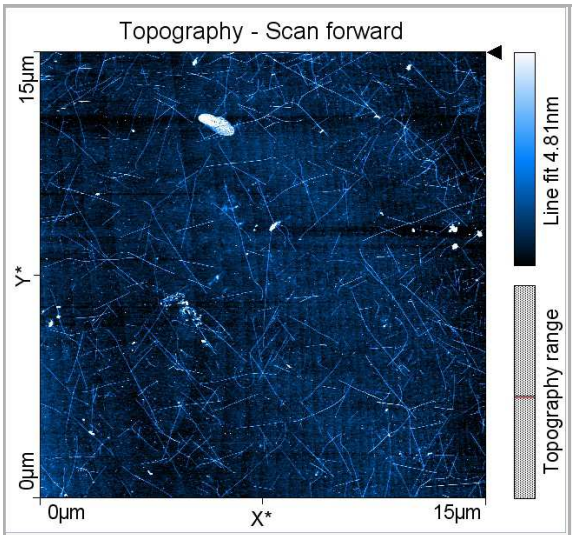


Figure 30: Nanotube Image in static mode. Carbon nanotubes can be seen lying on the silicon surface. Set point (2nN).

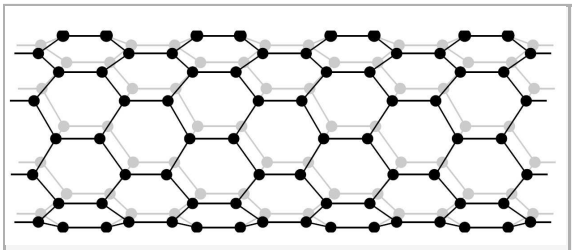


Figure 31: Nanotube molecular structure.

Image acquisition

1. Set a small scan range (2 µm or less).
2. Take a scan.
3. Optimize scanning parameters
4. Zoom out by taking a scan at a relatively large scan range (~15 µm).
5. Identify an area of interest.
6. Zoom back in.

A carbon nanotube is, as the name suggests, a tiny cylinder composed of carbon atoms. More specifically, it is a lattice of graphitic carbon rolled into a tube. *Figure 31: Nanotube Molecular Structure*shows an example of the molecular structure of a carbon nanotube. The ends of the tube are not capped, but it is possible to seal a nanotube at both ends with a fullerene. A fullerene is similar to a nanotube in molecular structure, but it is spherical rather than cylindrical.

The bonds that hold nanotubes together are entirely sp2 bonds, as in graphite. These bonds are stronger than the chemical bonds of diamonds, making nanotubes very durable. Nanotubes naturally align themselves into bundles held together by Van der Waals forces.

Robert-Bosch-Breite 10
D - 37079 Göttingen
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107
info@phywe.de
www.phywe.com

Microstructure

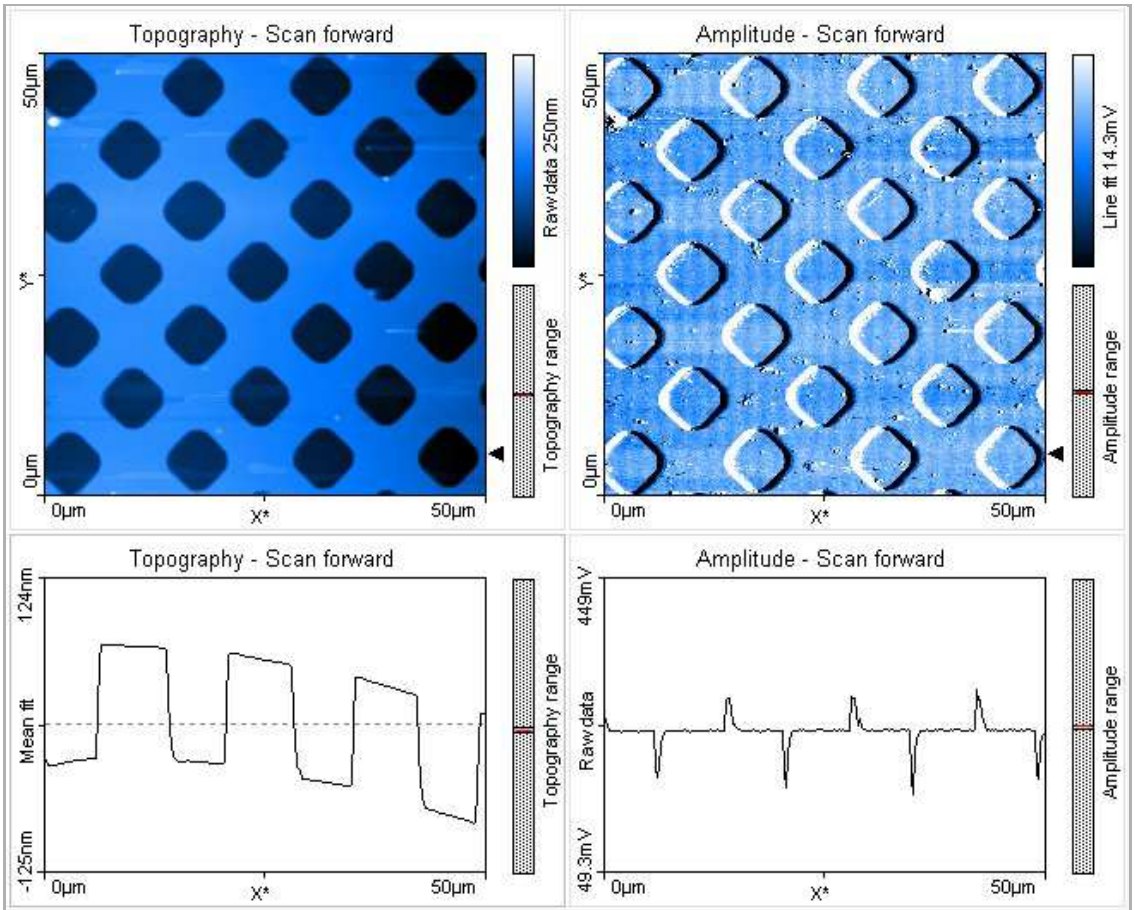


Figure 32: Microstructure measured in dynamic mode. Topography and amplitude are shown. The line graphs show a cross section of the images above at the position indicated by the arrow at the right of the scans respectively.

Image acquisition

1. Find a clean spot on the sample
2. Approach the sample
3. Start the measurement
4. Adjust PID gains
5. Find the optimum vibration amplitude

The microstructure sample consists of a structured silicon dioxide layer on silicon. This sample is in general quite easy to measure and there are not any special settings to be considered. However due to the abrasive characteristics of the oxide layer, the tip quality decreases quite fast compared to usual tip wear.
Due to the sharp steps, this sample is very sensitive to the settings of the feedback loop and vibration amplitude in dynamic mode.

CD stamper

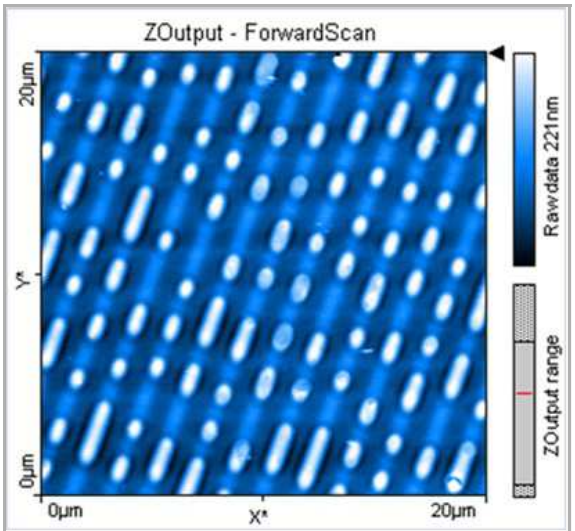


Figure 33: 20-µm Image of CD Stamper. Note that the curva-ture of the tracks is not discernible at this scan size.

Image acquisition

1. Set a large scan range, approximately 50 µm. At this size, you can see ma bumps, and it is even possible to make out the curvature of the rows (trac Each bump is approximately 200 nm high.
2. Practice zooming in on individual bumps. This sample is good for practicin zooming in on individual surface features, as bumps are visible at a variet scan sizes.
3. Take an image of well-ordered bumps at least 5 or 6 tracks wide. Try to ge image similar to Figure 33: 20-µm Image of CD Stamper, which is suitable measuring the bump length (Figure 34: Bump length). Furthermore, you ca determine the track distance if interested.

The size of CD and DVD structures must be very well-defined, and this requirem well served by the measurement evaluation tools in AFM software, which is demonstrated in this measurement.

The CD stamper sample contains a piece of the master copy of a CD. This is the original that creates the imprint in the pressed CD that you listen to. A CD has s indentations, called pits, whereas the stamper has bumps in the corresponding places.

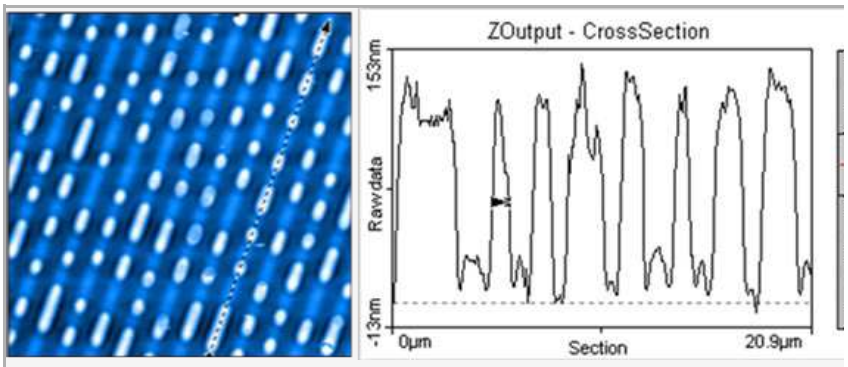


Figure 34: Bump length. Using the Measure Length tool in the track direction.

Glass beads

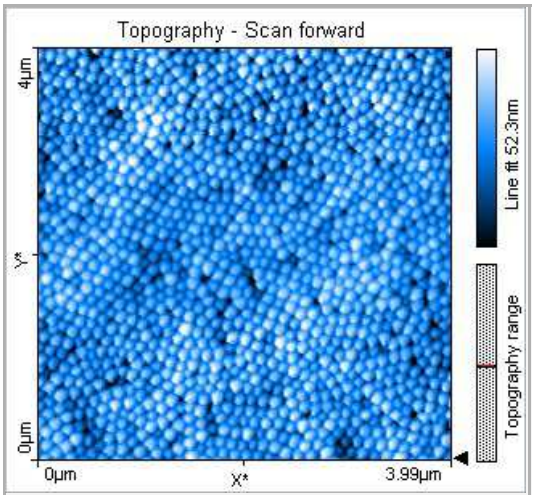


Figure 35: Well Ordered Beads in static mode. The center part of a spot of bead solution. Some sections have a crys-talline structure while others are less ordered.

Approaching the Sample

This sample is measured in static mode using a suitable cantilever. Furthermore, it is one of the more difficult to approach, as it is non-metallic, and not very reflective. If you can see the cantilever's shadow or reflection, you can use it to judge the distance. If you find it difficult to recognise the cantilever's reflection, then slightly move the sample holder: the structures on the sample will move, but the reflection will stay in the same place. If you cannot see the cantilever's reflection, perform a very slow coarse approach while judging the distance on the focal plane of the side view as follows:

- When the tip is on the sample, the focal plane crosses the sample at the tip position.
- When the tip is further away, the focal plane crosses the sample more behindthe cantilever.

Image acquisition

1. Start with a low force set point for best results. Applying too much force may move some of the beads around and create wide horizontal stripes across the image.

If you get stripes in your image:

1. Lift the tip, and then
2. Bring it back into contact.

If the tip is simply dirty, you can remove the dirt by:

1. Retracting the tip
2. Re-extending it again.

If there are still stripes in your image, the problem may be that the region where you are scanning does not have perfectly fixed beads. In a region of more ordered beads, the beads will stay in place. Therefore:

- Move to another region on the sample.

1. Set the scan range to 1 µm.

Since the beads are approximately 120 nm in diameter, you should be able to see about 10 of them across the image. If your image shows islands of beads surrounded by very flat areas:

- Move to a region of better ordered beads.

In general, the region with the best ordering is close to the center of the spot on the slide. *Figure 35*shows a well-ordered region near the center of the spot.

Staphylococcus aureus

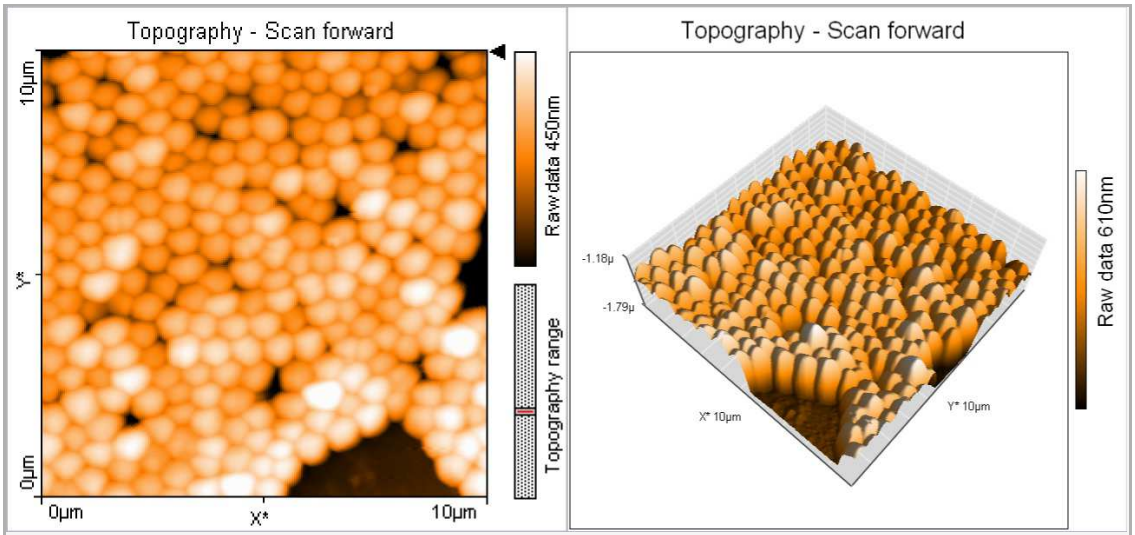


Figure 36: Staphylococcus Bacteria Images in dynamic mode. (Left) Topography with a scan range of 10 µm. Some regions appear to have almost crystalline structure, while other regions contain unordered gaps. The variations in color brightness of the individual bacteria correspond to the variations in height of the bacteria. (Right) 3D view of the left scan.

Image acquisition

The glass slide is only slightly reflective, so it can be difficult to judge the tip-sample distance for the approach. If you can see the cantilever's shadow or reflection, you can use it to judge the distance. You can try to make the reflection more visible by moving the sample holder slightly.

If you cannot see the cantilever's reflection, perform a very slow coarse approach while judging the distance on the focal plane of the side view as follows:

- When the tip is on the sample, the focal plane crosses the sample at the tip position.
- When the tip is further away, the focal plane crosses the sample more behind the cantilever.

The bacteria have been fixed to the glass slide with a burning process. The process leaves a mark where the bacteria have been burned, which makes it possible to locate the parts of the slide that are covered with bacteria.

The individual bacteria are approximately 0.7 µm in diameter, so it is possible to make out several bacteria in a relatively large scan range. This sample has regions with a very high concentration of bacteria as well as some with lower concentrations and some bare spots. The left image in *Figure 36*shows a 20-µm scan region densely packed with bacteria. It should be easy to zoom in on a much smaller scan region where the bacteria are still very concentrated.
The height scale of 450 nm is small considering that the free bacteria are spherical with an approximate diameter of 0.7µm. It is likely that the process which fixes the bacteria to the slide results in flattening them as well.

Furthermore, 3D representation of the data is possible by right-clicking on a graph and select “chart type” >> “3D View” (*Figure 36*, right), making the scan more descriptive.

Human skin

The human skin sample is an example of a soft biological sample. Although the measurement of this sample is possible in static mode, soft biological samples are likely to get damaged if measured in static mode. Therefore measurement in dynamic mode is recommended using a suitable cantilever. In contrast to the other samples in the sample kit, the macroscopic position of the AFM tip on the sample determines what kind of structures you will see. Thus, this sample is a good sample to practice coarse positioning of the sample using the included video camera. Moreover the skin is hardly visible (see *Figure 37: Skin Overview)*

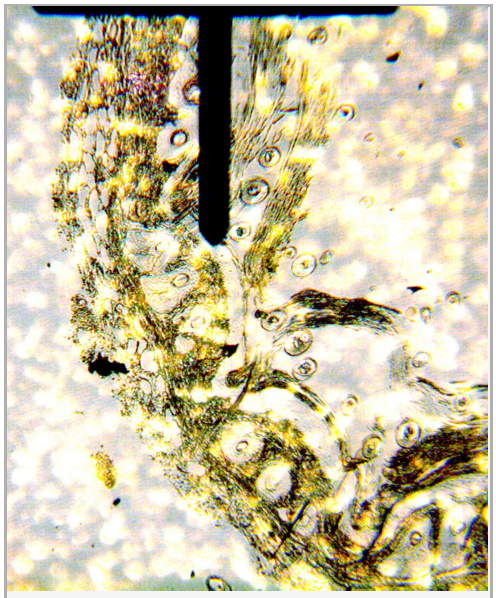


Figure 37: Skin Overview. The image is an overview of the skin cross section sample recorded by the included video camera. The different layers are visible as well as the AFM cantilever.

Image acquisition

The fact that the skin specimen has many different layers is also important in scanning, since not all of the layers will be visible in one scan range.The best strategy to see all of the structures within the skin sample is:

1. Choose a large scan range (50–100 µm)
2. Begin at one side of the skin cross section
3. Take an image
4. Retract to a safe position
5. Move slowly across the sample

The different areas of the skin cross-section can be seen in *Figure 38: Skin cross-section overview*.

The skin sample as other soft samples will get damaged by the sharp AFM tip scratched across them as the case in static mode. In contrast, the force applied in dynamic mode is much smaller and the tip is not scratched across the surface. Therefore, dynamic mode is preferable for these kind of samples.

The kinds of layers shown in *Figure 38*show different structures depending on their depth in the skin:
(A) shows the outer layers of dead epithelial skin cells. The outermost layer on the right side in the image is already beginning to flake off and is much less dense than the inner layers depicted on the left. Continuing to move in the same direction across the skin will unveil deeper and deeper layers of skin.
(B) contains multiple layers of skin. The image shows the “living” epithelial layer of skin. The structures at the bottom right hand corner of the image are the beginning of yet another layer of skin. This region is the one which contains collagen, the primary protein responsible for binding tissues within the skin.
(C) shows a hair follicle and the structure within it. This is the hole through which actual hair would have grown. Hair follicles are quite large compared to the other structures in skin, so an entire follicle may not fit into one scan range.
(D) shows the collagen layer of the skin section. The widely varying height of the collagen bundles makes it difficult to resolve detail on the structure of the collagen. The collagen cross sections appear circular when they run through the skin cross section, but they may not always be exactly perpendicular to the cross section of skin.

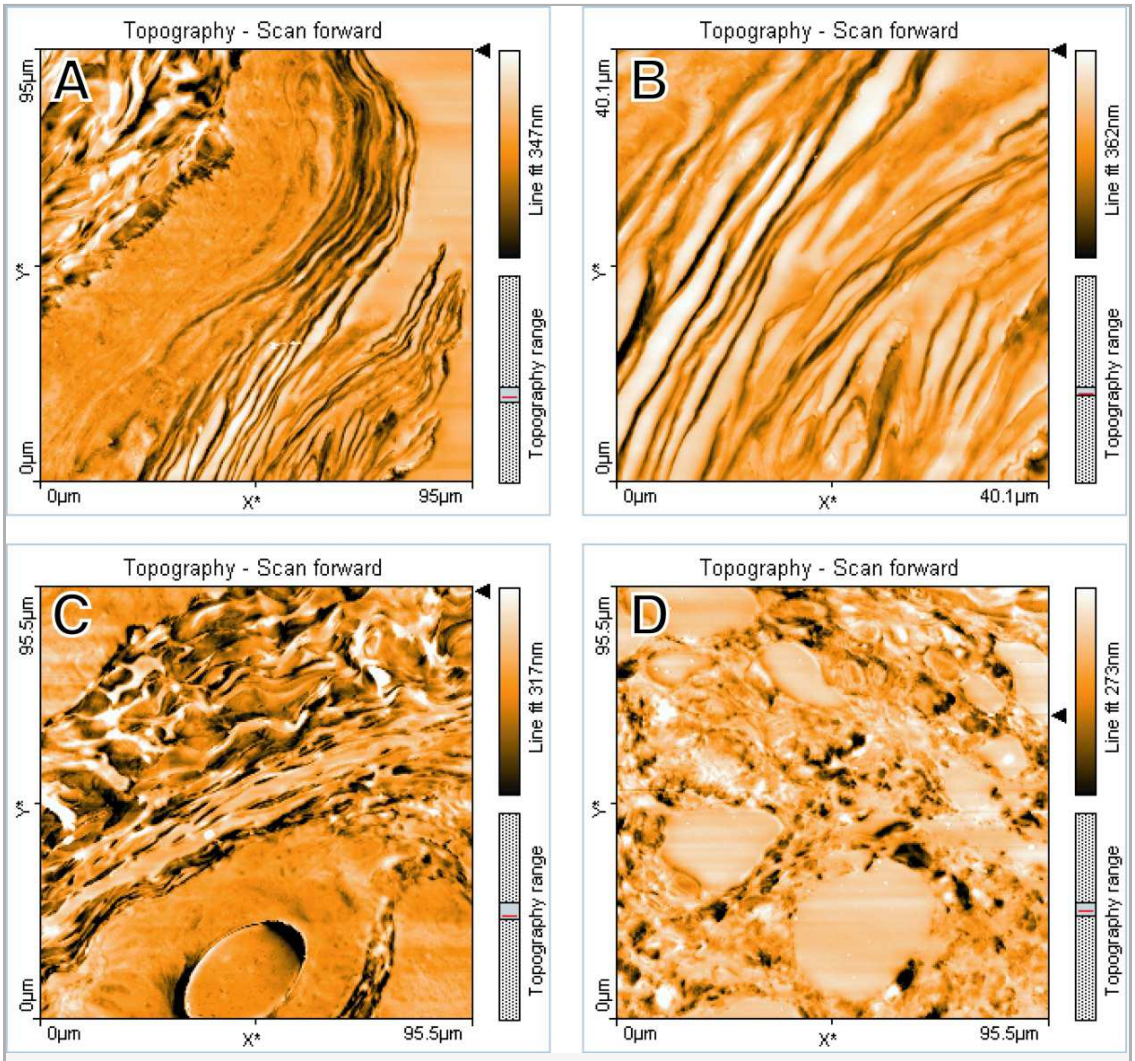


Figure 38: Skin cross-section overview. (A) Outer layers of dead epithelial skin. (B) Epithelial layer of skin. (C) Hair follicle. (D) Collagen layer.