

Atomic Force Microscopy (AFM) Topographic Imaging of sample

Lab Manual

M.Sc Physics (Sem IV)
Nanoscience Lab

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1 Objective

To use atomic force microscopy (AFM) to produce 2-d and 3-d topographical images of a given sample and use them to analyse the surface features and perform various measurements of length, angle, roughness, etc.

2 Materials and Apparatus used

Atomic force Microscope (Nanosurf Easyscan 2 AFM), Reference sample.

3 Theory

3.1 Introduction

Atomic force microscope (AFM) is an extremely versatile and powerful high resolution (typically in nanometer range) microscope, that can provide detailed scans revealing the nanoscale topographical features of sample. The AFM can operate in environments from ultra-high vacuum to fluids, and therefore cuts across all disciplines from physics and chemistry to biology and materials science.

3.2 What kind of things can AFM do?[1]

Atomic force microscopy (AFM) can be used to perform various kinds of operations like imaging, force-distance spectroscopy and surface manipulation (lithography).

Imaging means to perform a 2d or 3d topographical scan of the surface. This can then be used to obtain various measurements of the surface features.

Force-distance spectroscopy is used to get information like the surface elasticity/stiffness, information about the interaction between the surface and the probe, etc.

AFM probe can also be used to deliberately modify the surface features by pressing the tip/probe aggressively to the sample. It can be used to actually write or manipulate features on the sample.

3.3 Working Principle[2]

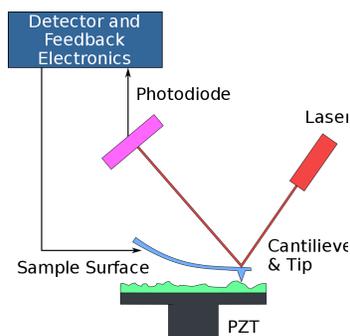


Figure 1: AFM block diagram(Credit: https://en.wikipedia.org/wiki/Atomic_force_microscopy)

The most important part of AFM is the cantilever-tip assembly that interacts with the sample. This assembly is also commonly referred to as the probe. The AFM probe raster scans the sample. The cantilever bends up and down depending on the surface features of the sample. The up/down motion of the tip as it scans along the surface is monitored through the beam deflection method. The beam deflection method consists of a laser that is reflected off the back end of the cantilever and directed towards a position sensitive detector that tracks the vertical and lateral motion of the probe. The deflection sensitivity of these detectors has to be calibrated in terms of how many nanometers of motion correspond to a unit of voltage measured on the detector.

The atomic interaction between the tip and the surface is shown below.

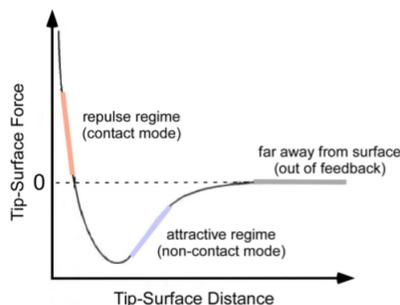


Figure 2: Atomic interactions curve(Credit: http://www.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf)

It is evident that at very small tip-sample distances (a few angstroms) a very strong repulsive force appears between the tip and sample atoms. It's origin is the so-called exchange interactions due to the overlap of the electronic orbitals at atomic distances. When this repulsive force is predominant, the tip and sample are considered to be in contact. As the distance between the tip and sample increases further we have the attraction (van der Waals) regime. It's origin is a polarization interaction between atoms: an instantaneous polarization of an atom induces a polarization in nearby atoms and therefore an attractive interaction.

The probe can also be mounted into a holder with a shaker piezo. The shaker piezo provides the ability to oscillate the probe at a wide range of frequencies (typically 100 Hz to 2 MHz) enabling dynamic modes of operation in the AFM. The dynamic modes of operation can be performed either in resonant modes (where operation is at or near the resonance frequency of the cantilever) or off-resonance modes (where operation is at a frequency usually far below the cantilevers resonance frequency).

3.4 AFM components

3.4.1 Cantilever-Tip Assembly (Probe)[2]

The cantilever is usually of rectangular or triangular geometry of micrometer dimensions. The cantilever consists of a very sharp tip (typical radius of curvature at the end for commercial tips is 5-10 nm) that hangs off the bottom of a long and narrow cantilever. As mentioned previously, the cantilever/tip assembly is also referred to as the probe.

AFM cantilevers are typically made of either silicon or silicon nitride, where silicon nitride is used for softer cantilevers with lower spring constants. The spring constant (k) of the cantilever is determined by it's dimensions using the following formula:

$$k = \frac{Ewt^3}{4L^3}$$

where w =cantilever width; t =cantilever thickness; L =cantilever length and E =Youngs modulus of the cantilever material. Nominal spring constant values are typically provided by the vendor when buying the probes, but there can be significant variation in the actual values.

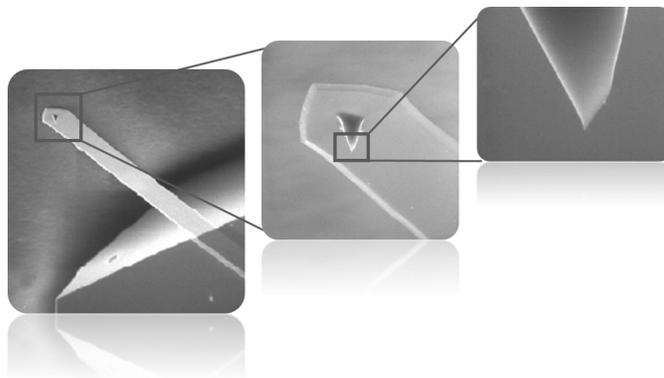


Figure 3: Cantilever SEM image(Credit: <https://www.nanosurf.com/en/support/afm-modes#lithography>)

3.4.2 Raster scan[1]

AFM raster scans the sample by either moving the sample below the tip or by moving the tip above the sample. The latter is more popular. The movement is achieved by a piezoelectric material, which expands and contracts proportionally to an applied voltage. Whether they elongate or contract depends upon the polarity of the voltage applied. Traditionally the tip or sample is mounted on a 'tripod' of three piezo crystals, with each responsible for scanning in the x,y and z directions. Later tube scanners were incorporated into AFMs. The tube scanner can move the sample in the x, y, and z directions using a single tube piezo with a single interior contact and four external contacts.

3.4.3 Cantilever deflection measurement[1][5]

The most common method for cantilever-deflection measurements is the beam-deflection method. In this method a laser light is reflected-off the back of the cantilever, and is collected by a position-sensitive photodiode (PSPD) that consists of two closely spaced photodiodes, whose output signal is collected by a differential amplifier. Deflection of the cantilever results in one photodiode collecting more light than the other photodiode, producing an output signal (the difference between the photodiode signals normalized by their sum), which is proportional to the deflection of the cantilever. The sensitivity of the beam-deflection method is very high. A longer beam path increases the motion of the reflected spot on the photodiodes, but also widens the spot by the same amount due to diffraction, so that the same amount of optical power is moved from one photodiode to the other.

Nowadays, quad PSPDs are used that have four photodiode quadrants, and can also measure the lateral deflection of the cantilever.

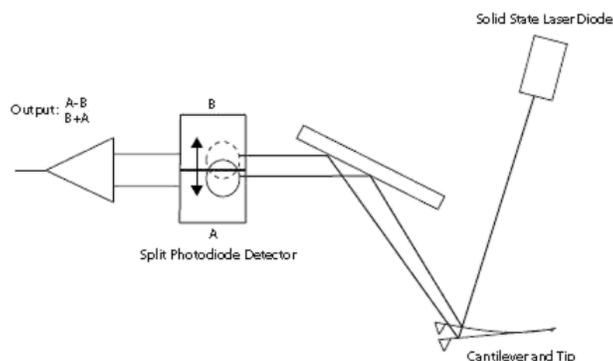


Figure 4: Schematic of beam-deflection method

Credit: Creepin475 (https://commons.wikimedia.org/wiki/File:AFM_beamdetection.png)

3.4.4 Feedback

A feedback loop is employed to maintain a constant deflection for constant force mode. The Z-controller feedback loops moves the cantilever back to the initial deflection. As the probe scans a feature on the sample, the cantilever gets deflected which changes the position of the laser spot on the PSPD. The Z-controller feedback loop then moves the cantilever along z-axis to bring the spot back to it's initial position. Similarly for other modes like tapping/ non-contact mode a set amplitude is maintained. The value of deflection/amplitude to be maintained is called the setpoint.

3.5 Operation modes

AFM typically operates in either Contact mode (static mode), Non-contact mode and Tapping mode(dynamic force mode).

In **contact mode**[6], the tip is in perpetual contact with the sample. The tip is attached to the end of a cantilever with a low spring constant, lower than the effective spring constant holding the atoms of most solid samples together which is on the order of $1 - 10nN/nm$

Further there are two imaging methods of contact modes: constant force mode and constant height mode.

In **constant force mode**, the force on the cantilever is kept constant by keeping the deflection of the cantilever constant. Contact force works in the repulsive region therefore the cantilever bends away from the sample causing it to have some initial deflection. As the scanner gently traces the tip across the sample (or the sample under the tip), the contact force causes the cantilever to bend and the deflection to change. The deflection can be kept constant by employing a feedback

loop to bring the cantilever back to the initial deflection.

In **constant height mode**, the distance between the sample and the cantilever is kept constant. As the scanner gently traces the tip across the sample (or the sample under the tip), the contact force causes the cantilever to bend and the Z-feedback loop works to maintain a constant cantilever deflection.

Constant force mode is more popular than the **constant height mode** as the forces are controlled in contrast to constant height where forces large enough to break the tip could develop.

Non-contact mode[6] refers to the modes that make use of an oscillating cantilever. A stiff cantilever is oscillated in the attractive regime, meaning that the tip is quite close to the sample, but not touching it (hence, non-contact). The forces between the tip and sample are quite low, on the order of pN (10^{-12} N). The scanning is done by measuring changes to the resonant frequency or amplitude of the cantilever as the interaction between the tip and sample dampens the oscillation.

In **Tapping mode**[7][8], also known as **intermittent-contact mode**, the most commonly used of all AFM modes, maps topography by lightly tapping the surface with an oscillating probe tip. The cantilever's oscillation amplitude changes with sample surface topography, and the topography image is obtained by monitoring these changes and closing the z feedback loop to minimize them. Very stiff cantilevers are typically used, as tips can get stuck in the water contamination layer. Tapping mode imaging is implemented in ambient air by oscillating the cantilever assembly at or near the cantilever's resonant frequency using a piezoelectric crystal.

3.5.1 Contact mode vs. Non-Contact mode vs. Tapping mode [6] [7]

Contact mode imaging is heavily influenced by frictional and adhesive forces, and can damage samples and distort image data. This also causes the cantilever tips to wear out quickly. Only hard surfaces that won't get damaged by the tip are can be imaged in this mode.

Non-contact imaging generally provides low resolution and can also be hampered by the contaminant (e.g., water) layer which can interfere with oscillation. The cantilever tips don't wear out quickly in this mode.

Tapping Mode imaging (right) takes advantages of the two above. It eliminates frictional forces by intermittently contacting the surface and oscillating with sufficient amplitude to prevent the tip from being trapped by adhesive meniscus forces from the contaminant layer. With the Tapping mode technique, the very soft and fragile samples can be imaged successfully.

3.6 AFM force-distance spectroscopy [1] [2]

Another major application of AFM (besides imaging) as mentioned earlier is force-distance spectroscopy, the direct measurement of tip-sample interaction forces as a function of the gap between the tip and sample (the result of this measurement is called a force-distance curve). In this, the AFM tip is first made to approach the sample from non-interaction region through the attractive regime all the way to the repulsive regime and the force is measured. Then the probe is retracted and the forces are measured again.

A typical force curve is shown below

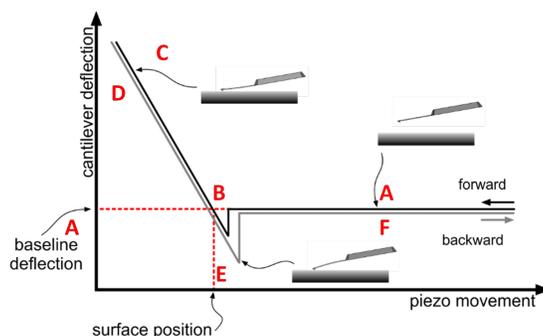


Figure 5: AFM force-distance curve Credit: <https://www.nanosurf.com/en/support/afm-modes#lithography>

The force curve above is divided into different segments where the black line from A-C refers to the tip approaching the surface and D-F (gray line) is for the tip retracting from the surface. The gray line has been given an artificial offset for

illustrative purposes.

A. Cantilever is approaching the surface. But is far enough to not feel any force.

B. Snap-in point: the cantilever suddenly snaps into contact with the sample. This snap-in is due to tip-surface interactions.

C. Repulsive portion: the repulsive forces come into play and bend the tip upwards upon further movement of the z-piezo. This section is referred to as the net-repulsive portion. It is interesting to note here that the cantilever deflection and hence the force are proportional to the z-distance. This curve is actually used to convert the volts produces in PSPD to nanometers.

D. Repulsive portion on withdrawal: the tip is now unbending while being withdrawn from the surface.

E. Pull-out: the tip gets stuck in an adhesive dip before it is able to emerge from the adhesion at the interface.

F. The cantilever has returned to its unperturbed state while the z-piezo further increases the tip sample distance.

Problems with the technique include no direct measurement of the tip-sample separation and the common need for low-stiffness cantilevers, which tend to 'snap' to the surface.

Force curves can be mined for various mechanical properties of the sample including adhesion, stiffness (modulus), and indentation depth (how much the tip penetrates into the sample at a given load).

3.7 Lithography/ Manipulation[1][2]

AFM tip can be used to perform deliberate damage to the surface to manipulate some features. This process is known as lithography. This is typically done in static mode, the cantilever/probe can carve out patterns or structures on surfaces through an aggressive interaction between the tip and sample configured with a high deflection setpoint. In terms of manipulation, the probe can be used to cut or move around structures.

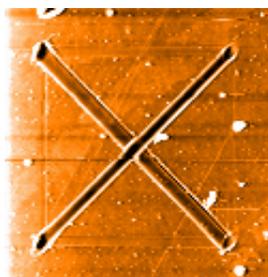


Figure 6: Lithography-carving an X(Credit: <https://www.nanosurf.com/en/support/afm-modes#lithography>)

3.8 Advantages[1]

AFM has several advantages over the scanning electron microscope (SEM). Where SEM provides only a 2d image, AFM provides a 3d surface profile. In addition, samples viewed by AFM do not require any special treatments (such as metal/carbon coatings) that would irreversibly change or damage the sample, and does not typically suffer from charging artifacts in the final image. AFM doesn't need an expensive vacuum environment for proper operation, and can work in ambient air or even fluids. This is advantageous as this lets one study biological macromolecules and even living organisms. AFM also has a higher resolution than SEM.

3.9 Disadvantages[1]

Although extremely versatile, AFM does have some limitations. The scan speed of AFM is pretty slow when compared to SEM.

The scan image size of AFM is also drastically smaller ($\approx 150\mu\text{m} \times 150\mu\text{m}$) as compared to SEM (order of mm).

As with any other imaging technique, there is the possibility of image artifacts, which could be induced by an unsuitable tip, a poor operating environment, etc. These image artifacts are unavoidable; however, their occurrence and effect on results can be reduced through various methods. Artifacts resulting from a too-coarse tip can be caused for example by inappropriate handling or collisions with the sample by either scanning too fast or having an unreasonably rough surface, causing actual wearing of the tip.

Due to the nature of AFM probes, they cannot normally measure steep wall type features.

3.10 Applications[1]

The AFM has been applied to problems in a wide range of disciplines of the natural sciences, including solid-state physics, semiconductor science and technology, molecular engineering, polymer chemistry and physics, surface chemistry, molecular biology, cell biology, and medicine.

Applications in the field of solid state physics include (a) the identification of atoms at a surface, (b) the evaluation of interactions between a specific atom and its neighboring atoms, and (c) the study of changes in physical properties arising from changes in an atomic arrangement through atomic manipulation.

In molecular biology, AFM can be used to study the structure and mechanical properties of protein complexes and assemblies. For example, AFM has been used to image microtubules and measure their stiffness.

In cellular biology, AFM can be used to attempt to distinguish cancer cells and normal cells based on a hardness of cells, and to evaluate interactions between a specific cell and its neighboring cells in a competitive culture system. AFM can also be used to indent cells, to study how they regulate the stiffness or shape of the cell membrane or wall.

4 Technical specification of Nanosurf Easyscan 2 AFM



Figure 7: Nanosurf Easyscan 2 AFM

(Credit: <https://physics.appstate.edu/students/facilities/appnano-microscopy-laboratories/instruments/nanosurf-easyscan-ii-afm>)

4.1 Operation settings [10]

Measurement environment= Air
 Operating mode= Static Force
 Cantilever type= CONTR
 Head type= EZ2-AFM
 Scan head= 10-09-436.hed
 Laser working point= 0.0%
 Deflection offset= 0.0%
 Software ver.= 3.5.0.38
 Firmware ver.= 3.5.0.8
 Scan speed: Up to 60 ms/line at 128 data points/line

4.2 Cantilever Type [3]

The following are some of the technical specification of the cantilever used:

Cantilever type: CONTR (Contact mode- Reflex coating)
 Spring constant: $0.2N/m$
 Length: $450\mu m$
 Mean Width: $50\mu m$
 Thickness: $2.0\mu m$

Resonance Frequency air: $13k Hz$

Coating Description (Aluminum Reflex Coating)

The aluminum reflex coating consists of a 30 nm thick aluminum layer deposited on the detector side of the cantilever which enhances the reflectance of the laser beam by a factor of 2.5. Furthermore it prevents light from interfering within the cantilever.

As the coating is almost stress-free the bending of the cantilever due to stress is less than 2 degrees.

4.3 Feedback Control Settings

Setpoint= 20nN

P-Gain= 10000

I-Gain= 1000

D-Gain= 0

Tip Voltage= 0 V

Feedback Mode= Free Running

Feedback algo.= Adaptive PID

Error range= 20 V

5 Reference Calibration Sample [4]

Sample: Grid HS-100 MG

This is a SiO₂ (Silicon oxide) on Silicon sample.

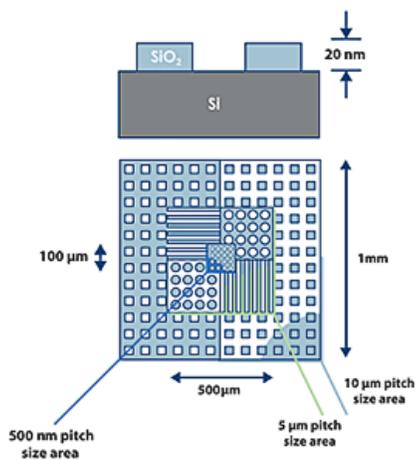


Figure 8: Calibration Sample schematic

(Credit:

http://www.tedpella.com/calibration_html/AFM_SPM_Calibration.ntm#HS20MG)



Figure 9: Sample kit

6 Procedure

1. Take the reference sample outside the storage unit carefully.
2. Place the sample on the stage very carefully so as not to break the tip.
3. Open the 'Nanosurf Easyscan 2 software on the PC'.
4. Wait for the controller to come online.
5. Advance the tip towards the sample in automatic mode.

6. Select imaging area settings, and the PID loop gains.
7. Perform a scan.
8. Save the scanned image and perform measurements using the 'Analysis' tab.

7 Observations

The following is the $50\mu\text{m}$ scan of the reference calibration sample(HS-100 MG) used.

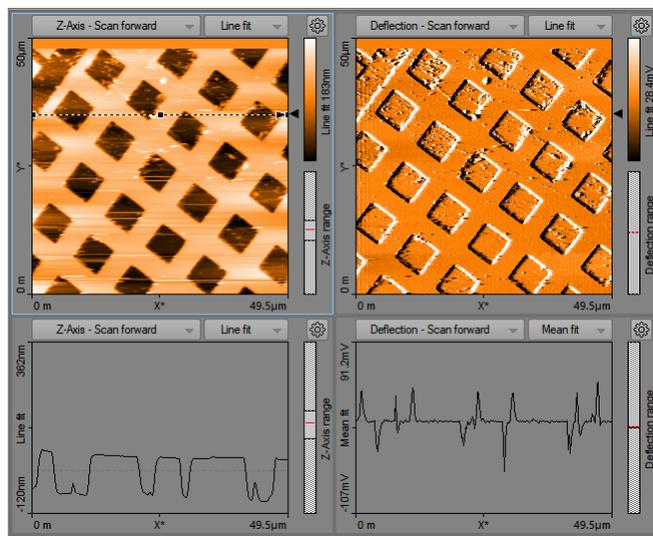


Figure 10: $50\mu\text{m}$ scan of the sample in the square well region.

The image shows color maps(depths indicated by change in hue) and line charts. The left color and line charts are the Z-axis forward scans while the right color and line charts are the deflections scans. Notice how the color scans are inverse of each other. The deflection scans are obtained by measuring the voltage developed in the PSPD and hence the scales are in mV . These are then converted to Z-axis scans by calibrating the voltage to the distance between the cantilever and the sample.

The following is the 3-d chart version of the above scans:

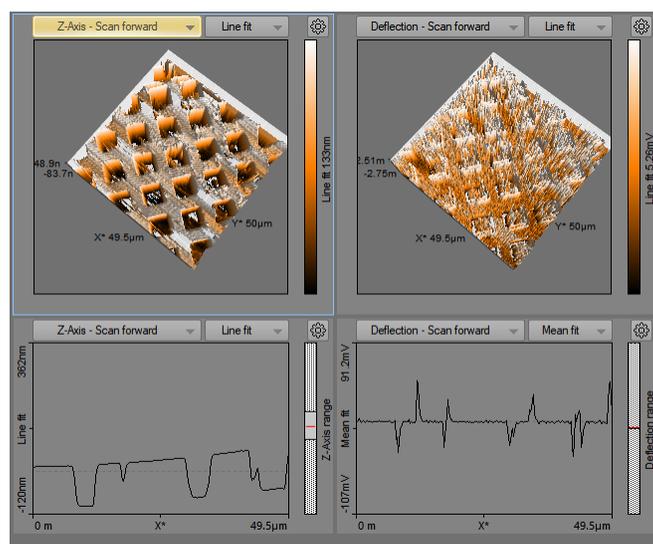


Figure 11: $50\mu\text{m}$ 3d profile of the sample in the square well region.

Nanosurf Easyscan 2 allowed us to make various measurement of the surface features like angle, distance between two

parallel lines, length b/w two points, line roughness, area roughness etc. We made measurements on the $50\mu\text{m}$ scanned area as well as some zoomed in features. We used the cut out area tool to zoom into an area to make precise measurements.

The measurements are shown below:

7.1 Length measurement

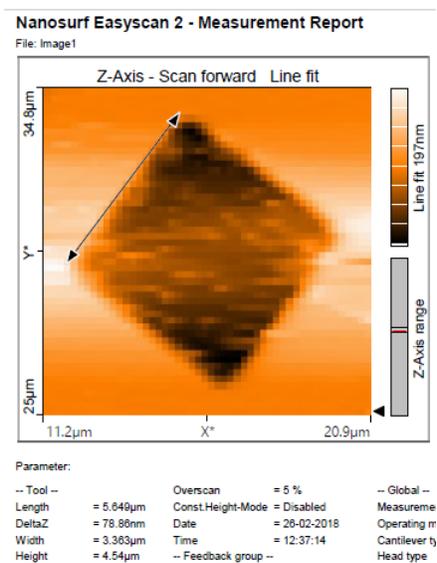


Figure 12: Length measurement in a zoomed in portion

7.2 Angle measurement

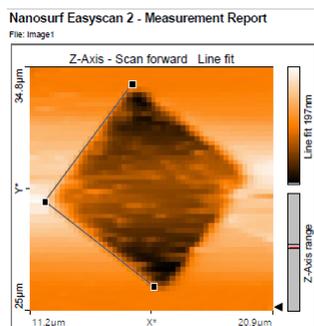


Figure 13: Measured angle 92°

7.3 Distance measurement in a zoomed in area

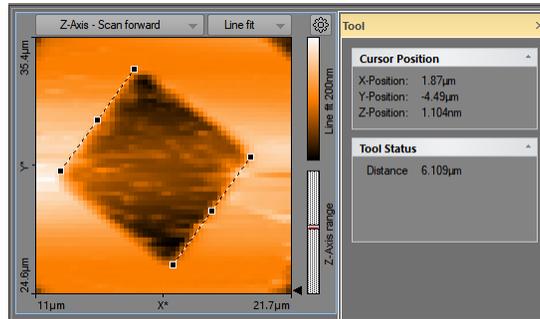


Figure 14: Distance measurement in a zoomed in area

7.4 Period verification of squares

In this measurement we verified the period of the squares. We already knew this to be $10\mu m$.

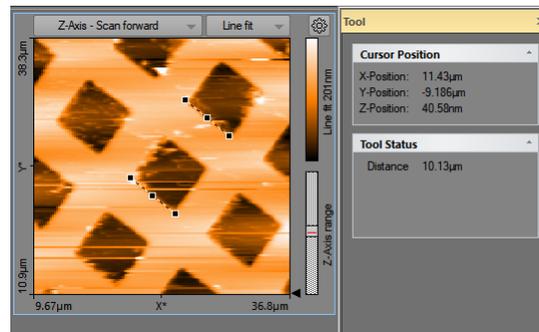


Figure 15: Period verification

7.5 Background Correction

Removes the effect of an ill-aligned scan plane.

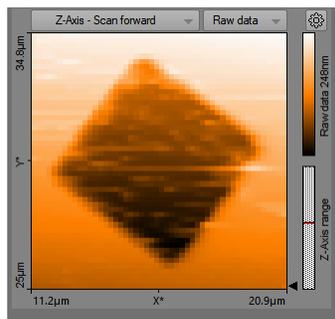


Figure 16: Background correction

7.6 Line roughness

Line roughness parameters were calculated by choosing a particular scan line. The parameters are given by:
Average roughness:

$$R_a = \frac{1}{N} \sum_{l=0}^{N-1} |z(x_l)|$$

Mean roughness:

$$R_m = \frac{1}{N} \sum_{l=0}^{N-1} z(x_l)$$

Root mean square roughness:

$$R_q = \sqrt{\frac{1}{N} \sum_{l=0}^{N-1} (z(x_l))^2}$$

Peak height:

$$R_p = \text{highest value}$$

Valley depth:

$$R_v = \text{lowest value}$$

Peak-Valley Height:

$$R_y = R_p - R_v$$

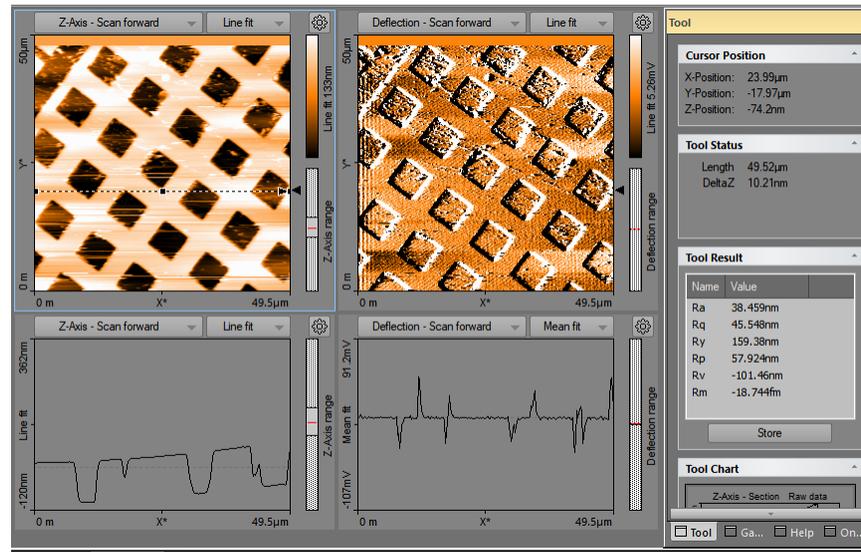


Figure 17: Line roughness

7.7 Area roughness

Area roughness parameters are calculated by selecting an area.

The parameters are defined in a similar fashion as the line roughness parameters.

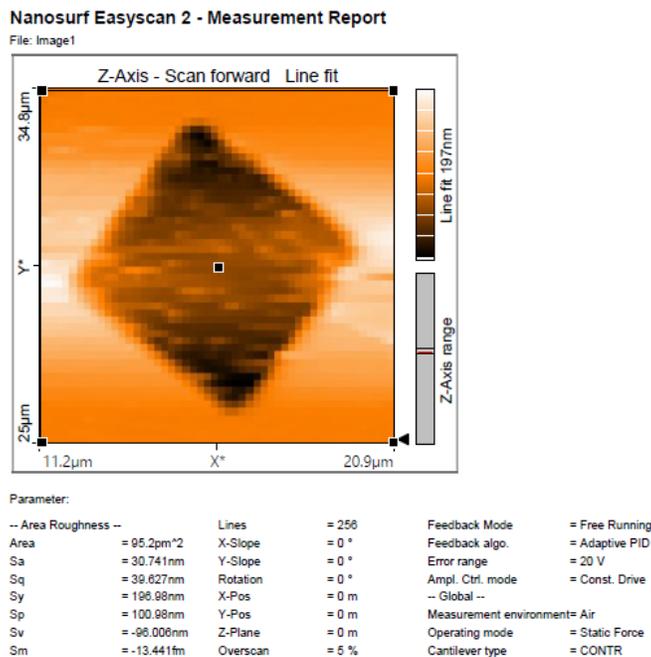


Figure 18: Area roughness of a zoomed in portion

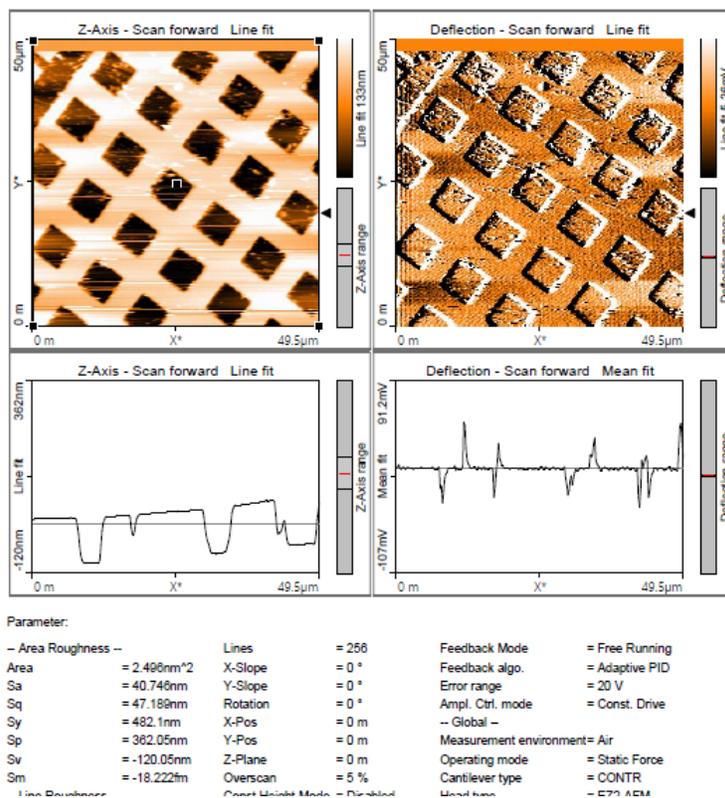


Figure 19: Area roughness of the complete image

8 Results and Discussion

The features of reference sample were imaged and their characteristics were verified.

However, we didn't take a lot of precautions while performing these measurements which led to a lot of noise in the data,

and hence the scans weren't perfect. Our measurements could have been improved in a variety of ways.

8.1 Improving measurement quality[10]

We didn't take care of any interfering signals while performing the measurements. Measurements could be improved by removing any sources of interference. Interference can be of three types:

- **Mechanical:** from machines or heavy transformers in direct vicinity (e.g. pumps). Make sure no pumps or heavy machinery is being operated in the vicinity.
- **Electrical:** from electronic items in the vicinity of the tip/sample emitting electromagnetic radiation. Make sure there are no loudspeaker, cathode ray tubes or other em field sources near the AFM instrument.
- **Light:** stray light entering the microscope. Infrared and other light sources can influence the cantilever deflection detection system.

This problem is especially severe when measuring in the Static Force mode. Try the following in order to reduce the influence of infrared light sources:

- (i) Turn off the light.
- (ii) Shield the instrument from external light sources.

8.2 Adjusting the measurement plane

We didn't make sure that the measurement plane was completely aligned or not. This could cause significant differences in the scanned image. Ideally the sample surface and the XY-scanner should be parallel to each other. The inclination between the sample and the scanner is undesirable for several reasons:

- (i) It makes it difficult to see small details on the sample surface, because the Average, Plane fit, or higher order filters cannot be used properly.
- (ii) The Z-Controller functions less accurately, because it continuously has to compensate for the sample slope. The XY-plane of the scanner is aligned with the sample plane using the three leveling screws on the Scan Head. This alignment can, however, not easily be performed once the automatic approach has been done, as this would damage the tip.

8.3 Tip quality

We didn't use a new tip for this measurement. Rather we just performed the scanning with the existing tip already in place in the holder. This tip could have been worn out by the previous users. This could explain the shabby nature of the scan caused by the tip artifacts. A good tip quality is essential for high quality images and high resolution. When the image quality deteriorates dramatically during a previously good measurement, the tip has most probably picked up some material from the sample. As a result, the image in the color map charts consist of uncorrelated lines (as in our case) or the image appear blurred.

8.4 Scan rate and Feedback loop optimization [9] [2]

The scan speed also affects the quality of the scanned image. The scan speed should be such that the tip must be able to follow the surface while scanning, which has two preconditions: :

- (i) The scan mechanics must be able to position the tip fast enough.
- (ii) The sensor must be able to deliver the information from the surface fast enough.

This results in the following rules:

- (i) The scan speed of the speed optimized AFM is limited by the response time of the sensor.
- (ii) For similar image quality, a higher scan speed requires more information per time unit from the surface and therefore more interaction between tip and sample is needed. This may cause streaks appear on the trailing edge of surface features. Streaks are an indication of the tip not tracking the surface properly.

One should also keep the following points in mind while setting the feedback control gains.

Control of the feedback loop is done through the proportion-integral-derivative control, often referred to as the PID gains. These different gains refer to differences in how the feedback loop adjusts to deviations from the setpoint value, the error signal. For AFM operation, the integral gain is most important and can have a most dramatic effect on the image quality. The proportional gain might provide slight improvement after optimization of the integral gain. The derivative gain is mainly for samples with tall edges. If gains are set too low, the PID loop will not be able to keep the setpoint accurately. If the gains are chosen too high the result will be electrical noise in the image from interference from the feedback.

The other parameters that are important in feedback are the scan rate and the setpoint. If the scan rate is too fast, the PID loop will not have sufficient time to adjust the feedback parameter to its setpoint value and the height calculated from

the z piezo movement will deviate from the true topography at slopes and near edges. Very slow scan rates are typically not an issue for the PID loop, but result in long acquisition times that can pose their own challenges such as thermal drift. Optimization of the PID gains and the scan rate are necessary in order to optimize feedback loops. The setpoint affects the interaction force or impulse between probe and sample. A setpoint close to the parameter value out of contact feedback is most gentle for the sample, but tends to slow down the feedback.

References

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- [9] Scan speeds
<http://dme-spm.com/geschw.html>
- [10] Nanosurf manual
Nanosurf FlexAFM Operating instructions