Molecular Imaging

Training Manual

10 Years of Discovery and Innovation

Molecular Imaging

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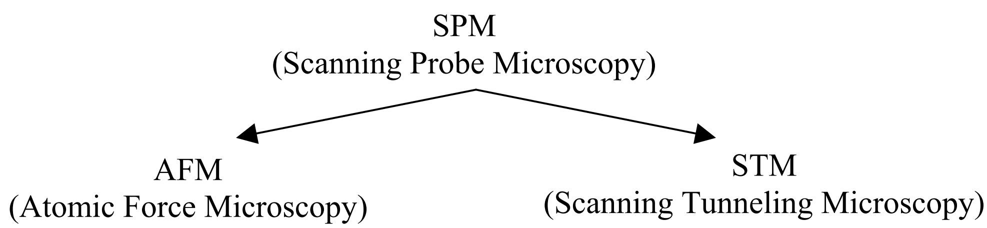
1. Introduction

- Scanning Probe Microscopy (SPM): SPM consists of a family of microscopes where a sharp probe (tip) is scanned across a sample (tip-scanning system) or a sample is scanned across a tip (sample-scanning system) at nano-scale distances to detect interactions between the tip and the sample.
- The SPM system can be divided into two major types of microscopies as shown below:



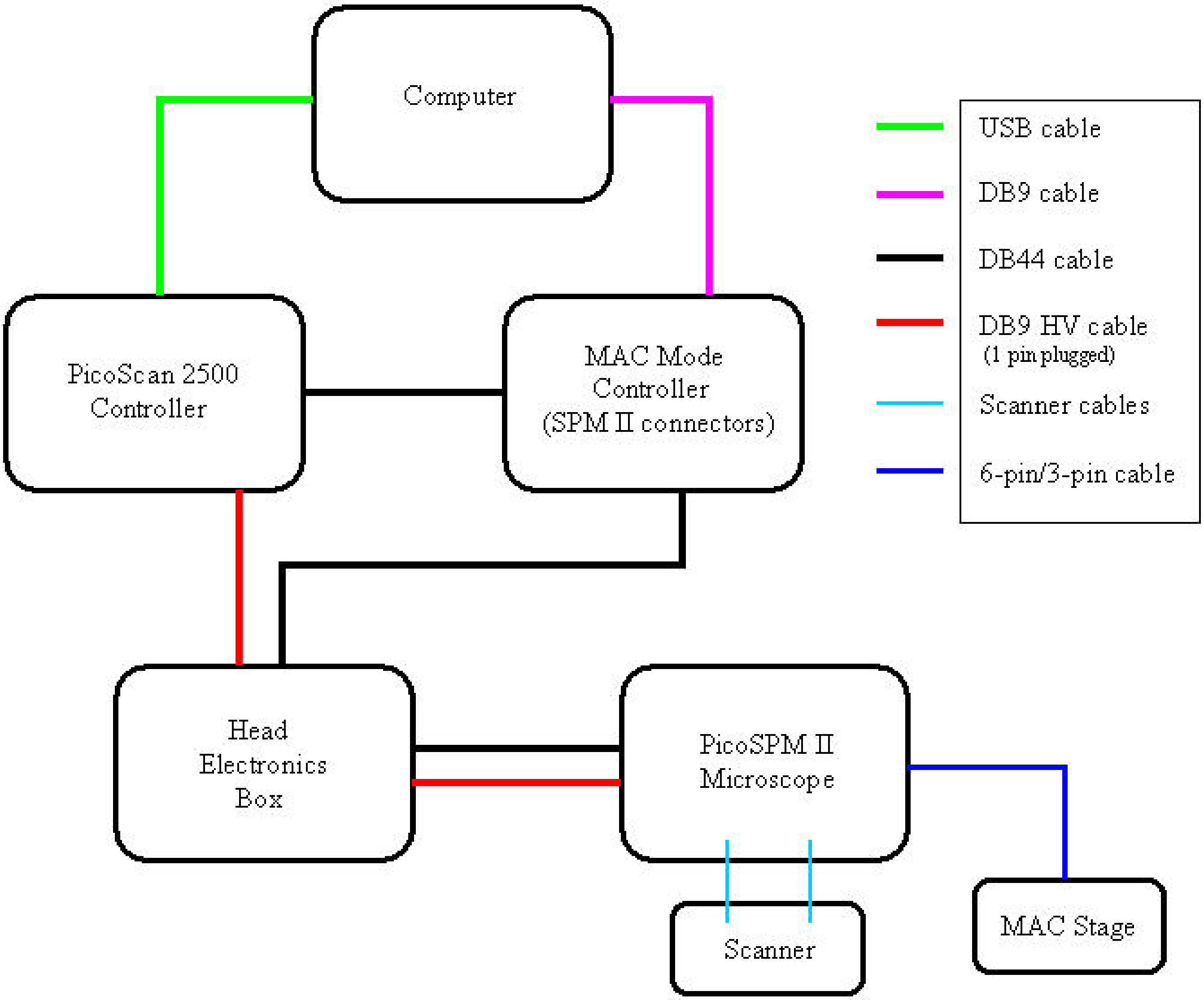
- The following table shows brief comparison of AFM and STM. [1]

Microscope	AFM	STM
Detected interaction	Force	Current
Tip material	Si or Si3N4	Pt/Ir or W wire
Sample	Conductive and insulating	Only conductive

- Molecular Imaging system is capable of working in both AFM and STM configuration as required.
- A generic SPM system has following three basic components: 1) microscope 2) controller electronics and 3) computer.
- A typical MI system is shown in the following connection diagram.

[1] For comparison of SPM with other microscopy techniques such as TEM, SEM, please visit http://www.molec.com/what_is_afm.html.

Connection Diagram for a PicoPlus system with a MAC Mode controller[2]



[2] The connection diagrams for PicoLE and PicoSPM systems vary and can be found in the respective manuals. Different operating modes may require adding/eliminating different system components not shown above.

- A short description of the function of each component in the system is given below:

1. Computer: Runs the Molecular Imaging SPM system through PicoScan software. The two monitors are used either to operate the system or to display the results as desired.
2. PicoScan Controller: Contains the analog electronics as well as the necessary ADC and DAC boards to generate and process various signals such as servo and scanning voltages etc.
3. AC Mode Controller: Generates either AAC or MAC mode drive signal depending on the mode of operation.
4. Head Electronics (Pico LE or Pico Plus): Processes and communicates the signals between the microscope and the controller depending on the configuration of the system (AFM or STM).
5. Microscope: Consists of piezoelectric scanner used for imaging as well as the probe, photo detector (required only for AFM) and sample stage.

- The three basic operational configurations viz. STM, Contact mode AFM and AC mode AFM are explained below in some detail.
- Molecular Imaging system is capable of working in many other modes including Current Sensing AFM (CSAFM), Pulsed Force AFM (PFM, Force Modulation AFM (FMM), Electrostatic Force Microscopy (EFM) and Magnetic Force Microscopy (MFM).[3]
- All of these modes can be operated under desired environmental conditions using Molecular Imaging accessories such as liquid cell, PicoApex chamber and glove box.[4]

[3] For more details on these modes please visit http://www.molec.com/apps_imagingmodes.html.
[4] For more information on the accessories, http://www.molec.com/products_accessories.html.

Scanning Tunneling Microscopy (STM)

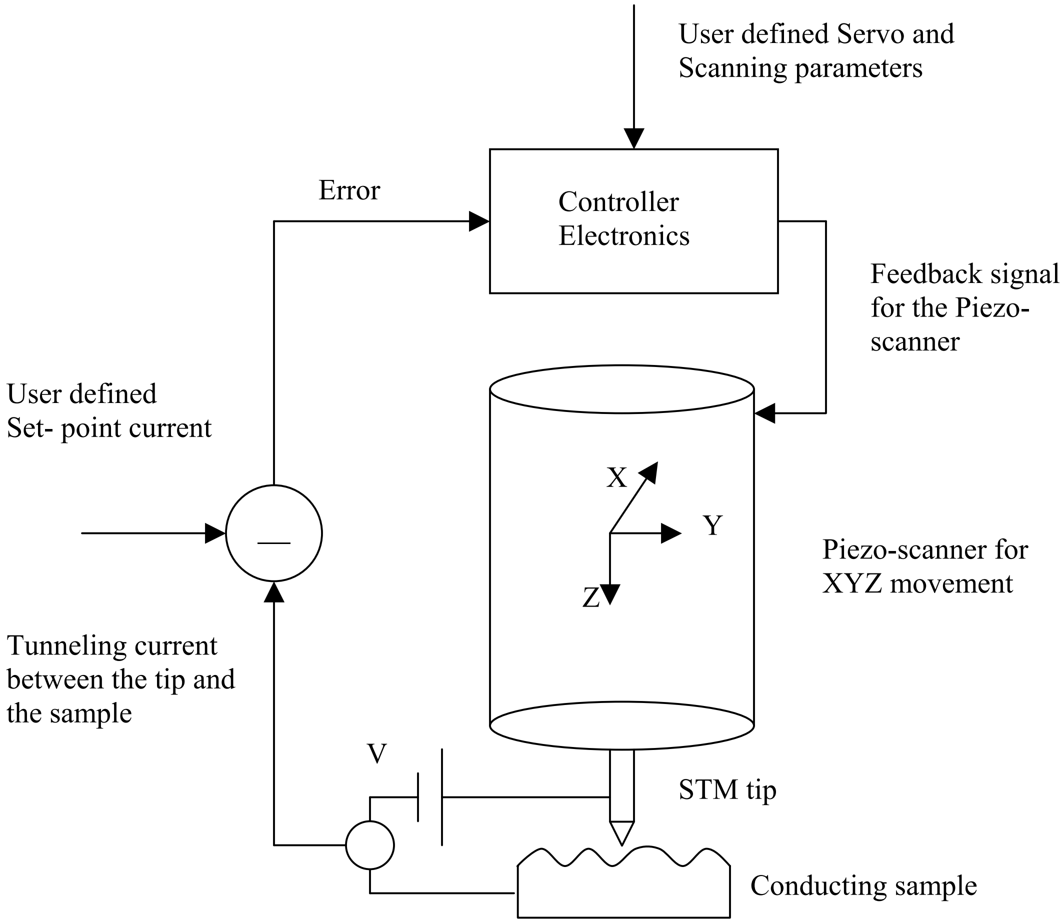


Fig. 1.1 Schematic of an STM system

- Principle: Tunneling current between a conductive tip and sample varies exponentially as the distance between them.
- If a bias V is applied between a conductive tip and sample separated by a distance d, then the tunneling current I flowing between them is given by,

  $$I = Ve^{-cd} \ldots (1)$$

  Where c=constant.
- The tunneling tip is moved up and down (Z) by the piezoelectric scanner with the help of the feedback as it is raster scanned across the surface (X, Y) to maintain a constant set-point tunneling current.
- The Z position of the tip at each data point in the X-Y plane produces the topographic image of the surface.

Contact Mode AFM

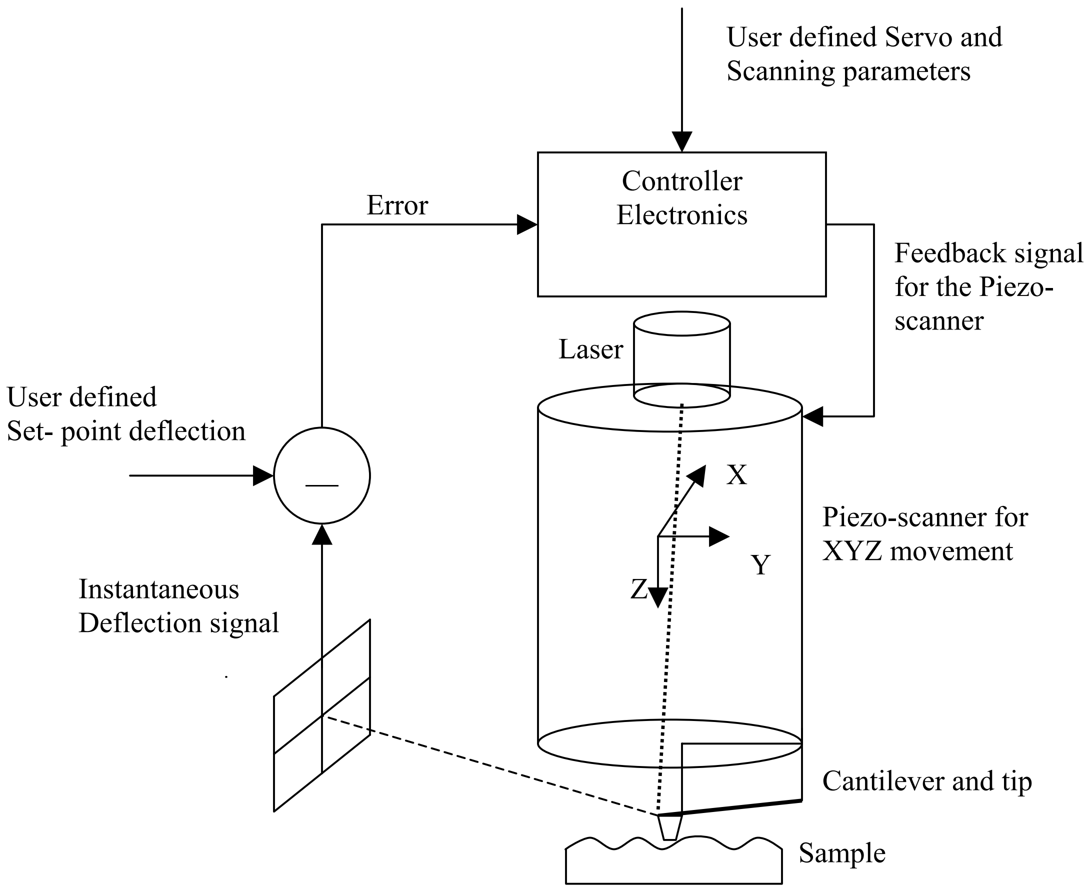


Fig. 1.2 Schematic of a contact mode AFM system

- Principle: Deflection of the cantilever as it is raster scanned across the sample by a piezoelectric scanner is monitored by bouncing a laser beam off it onto a photodetector.
- If x is the deflection of the cantilever with a spring constant K, the force acting on it can be given by Hooke's law,

$$F= -Kx\ldots(2).$$

- The fixed end of the cantilever is moved up and down (Z) by the piezoelectric scanner with the help of the feedback as it is raster scanned across the surface (X, Y) to maintain a constant setpoint deflection and hence a constant force.
- The Z position of the tip at each data point in the X-Y plane produces the topographic image of the surface.

Acoustic AC (AAC) Mode AFM[5]

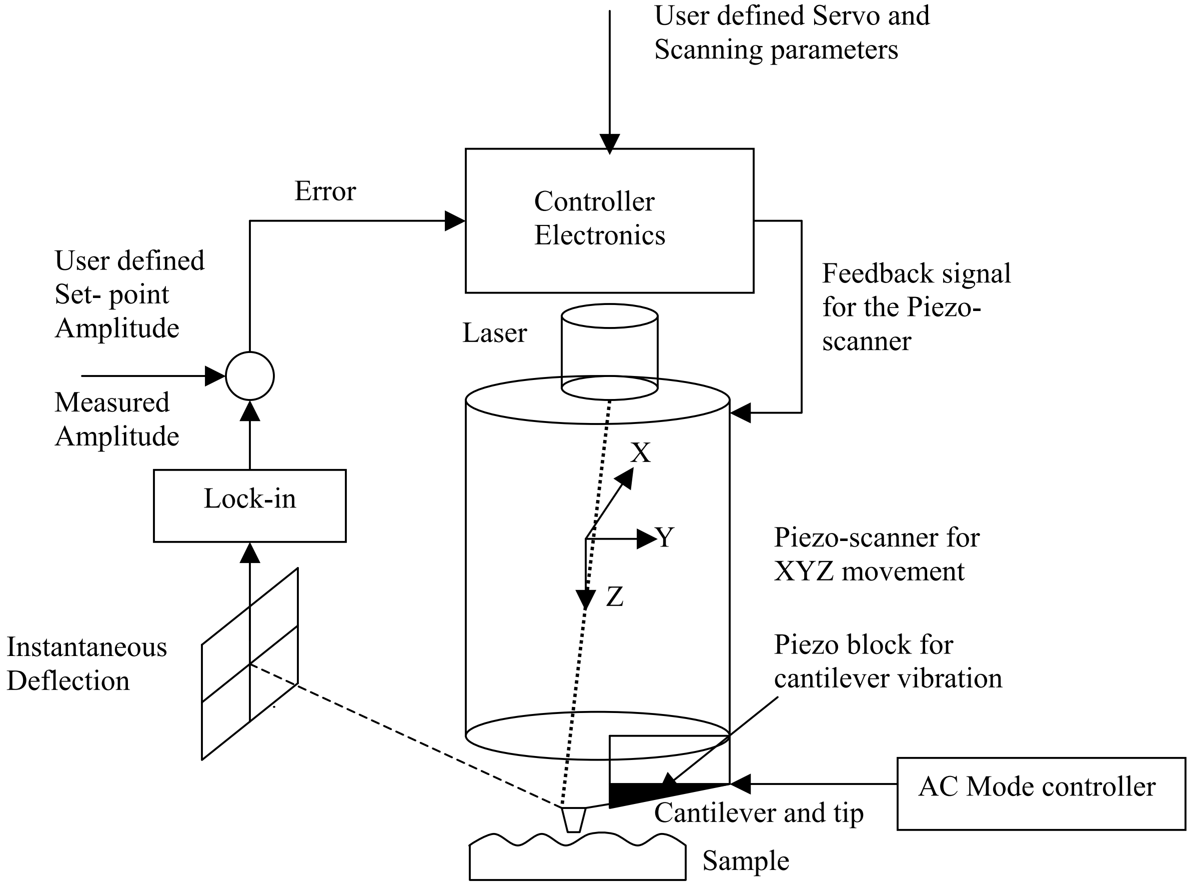


Fig. 1.3 Schematic of an Acoustic AC system

- Principle: Amplitude of a vibrating cantilever as it is raster scanned across the sample by a piezoelectric scanner is monitored by bouncing a laser beam off it onto a photodetector.
- The cantilever is vibrated at its resonance frequency by a piezoelectric block on which the cantilever sits.
- If a cantilever has mass m with a spring constant K, its resonant frequency *w* is given by[6],

$$W = \sqrt{(K/m)} \ldots (3).$$

- The fixed end of the cantilever is moved up and down (Z) by the piezoelectric scanner with the help of the feedback as it is raster scanned across the surface (X, Y) to maintain constant setpoint amplitude and hence a constant force derivative.
- The Z position of the tip at each data point in the X-Y plane produces the topographic image of the surface.

[5] Acoustic AC Mode and Magnetic AC Mode can be operated in intermittent contact or non-contact mode.
[6] The mathematical treatment associated with AC mode is described in 'Advanced Theory' section of the ' AC Mode' chapter of the User's Manual.

Magnetic AC (MAC) Mode AFM[5]

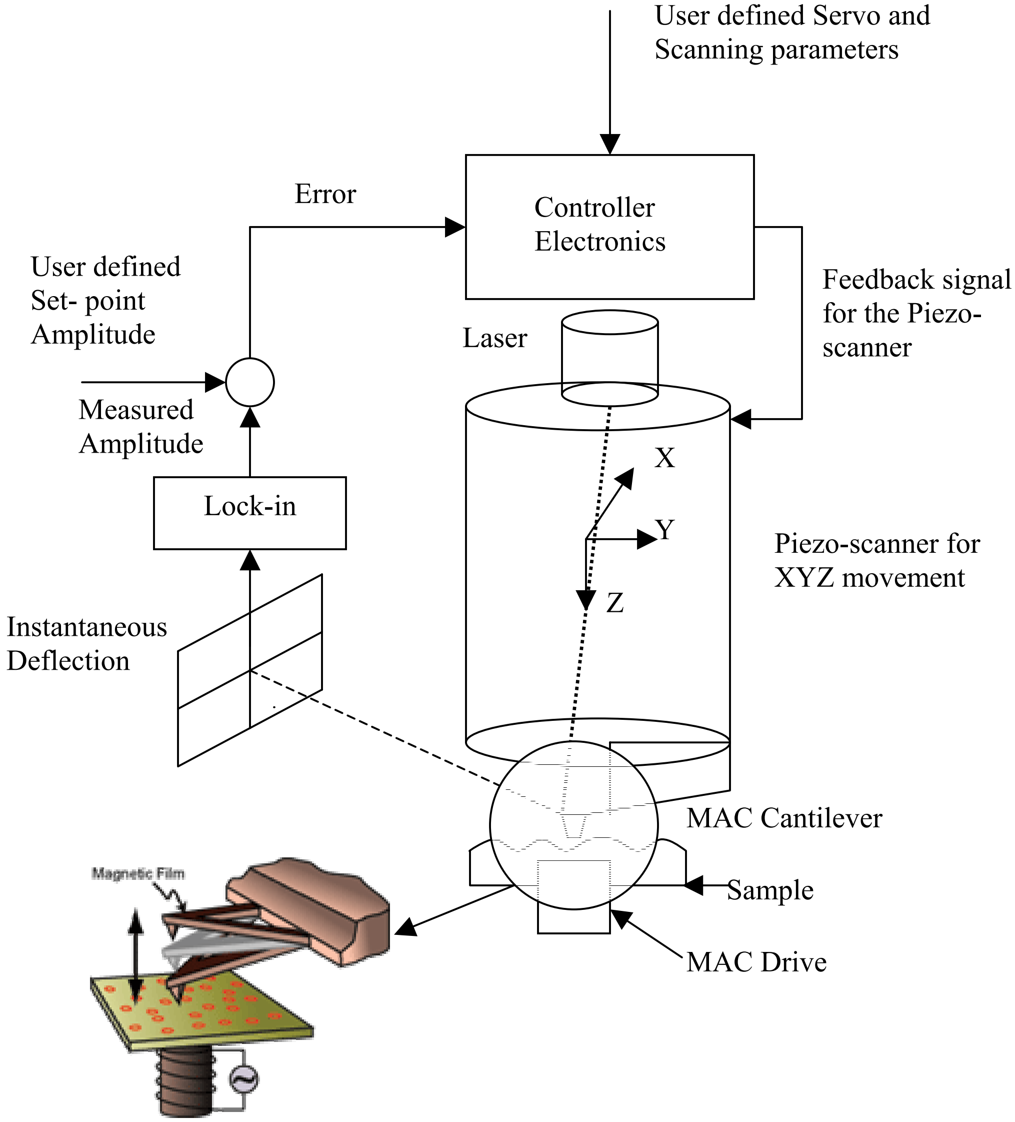


Fig. 1.4 Schematic of a MAC mode system

- Principle: Amplitude of a vibrating cantilever as it is raster scanned across the sample by a piezoelectric scanner is monitored by bouncing a laser beam off it onto a photodetector. This is same as that for AAC.
- The MAC cantilever[7] (a magnetically coated cantilever) is vibrated off or at its resonance frequency by a magnetic coil, which is either under the sample (as shown above) or above the cantilever (Top MAC mode).
- The method of topographic image formation is also same as that for AAC.

- The MAC mode is better than AAC mode in terms of dissipation of energy and getting a clean frequency sweep while imaging in fluid.
- Users are encouraged to know more about the advantages of MAC mode by visiting http://www.molec.com/apps_imagingmodes_macmode.html and the references therein.

[7] MAC mode cantilevers (MAC levers) are solely supplied by MI.

2. Piezo Basics

- MI Pico systems are tip-scanning systems (also called top-down systems) in which the cantilever sits on a scanner for raster scanning across the stationary sample. The scanner is made of piezoceramic materials usually having base material as lead zirconium titanate.
- When an electric field is applied to piezoelectric materials they elongate or contract depending on the direction of the field. It is illustrated in Fig. 2.1 below:

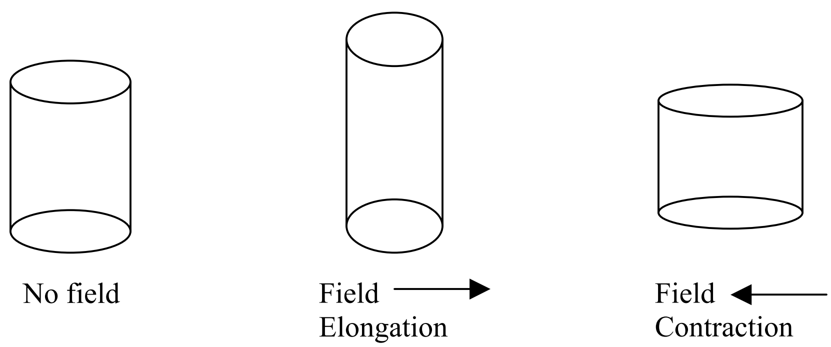


Fig. 2.1 Principle of piezo movement

- Z-motion of the tip (using feedback or servo) is achieved by simple elongation or contraction of the piezo element in the scanner. X-Y raster scanning is achieved by applying alternating voltages to opposite piezo elements in the scanner so that one element elongates and the other contracts.
- While raster scanning, the tip collects the data moving along a line in the direction called fast-scan direction (usually X). After scanning the line back and forth, it moves one line along the slow-scan direction (usually Y) to reach to the next line. The process is repeated to achieve the raster scan.
- If the piezoelectric materials were to be perfect, the following waveform (Fig. 2.2) would produce the above mentioned raster process.

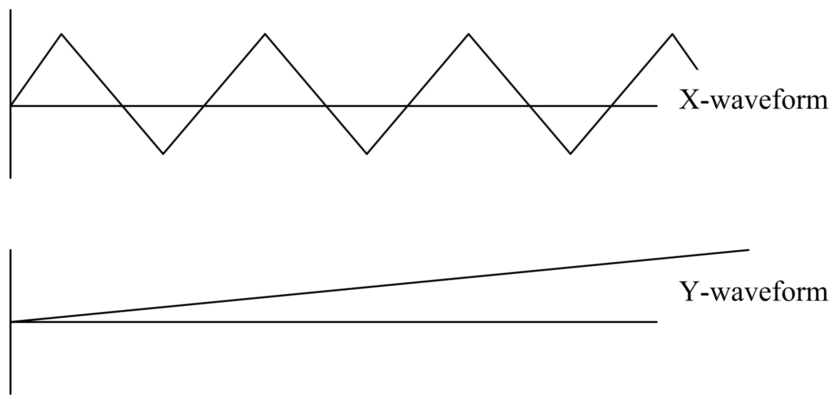


Fig. 2.2 Voltage waveforms applied to the piezo scanner

- Piezoelectric materials inherently exhibit non-ideal properties such as non-linearity, hysterisis, bow, creep, aging, cross-coupling etc. The effects of some of these on the scanner behavior are explained below. Though they are explained separately for simplicity, they may not be independent of each other.
- Non-linearity causes the piezo response to the triangular waveform to change as shown in Fig. 2.3 below.

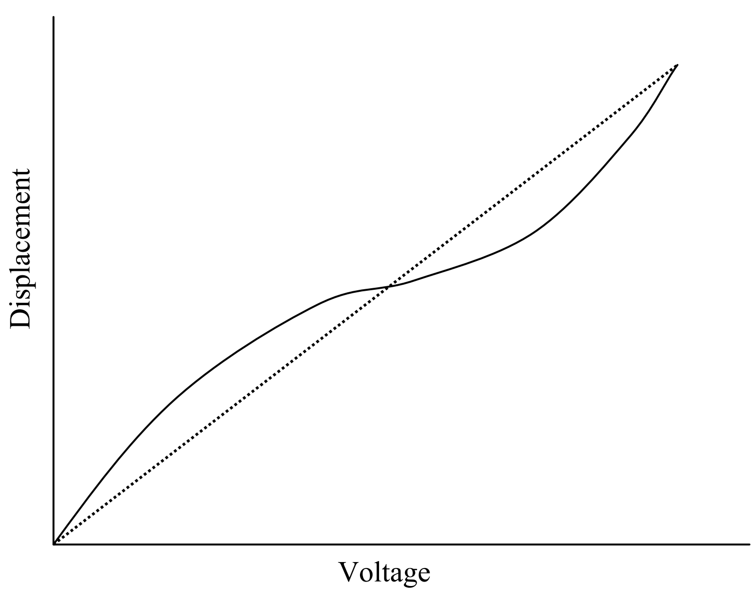


Fig. 2.3 Displacement vs. voltage plot for piezo material

- As a result of this, a standard grating imaged with a non-linear scanner looks as shown in Fig. 2.4 below. The size of the pits is not uniform across the scan range.

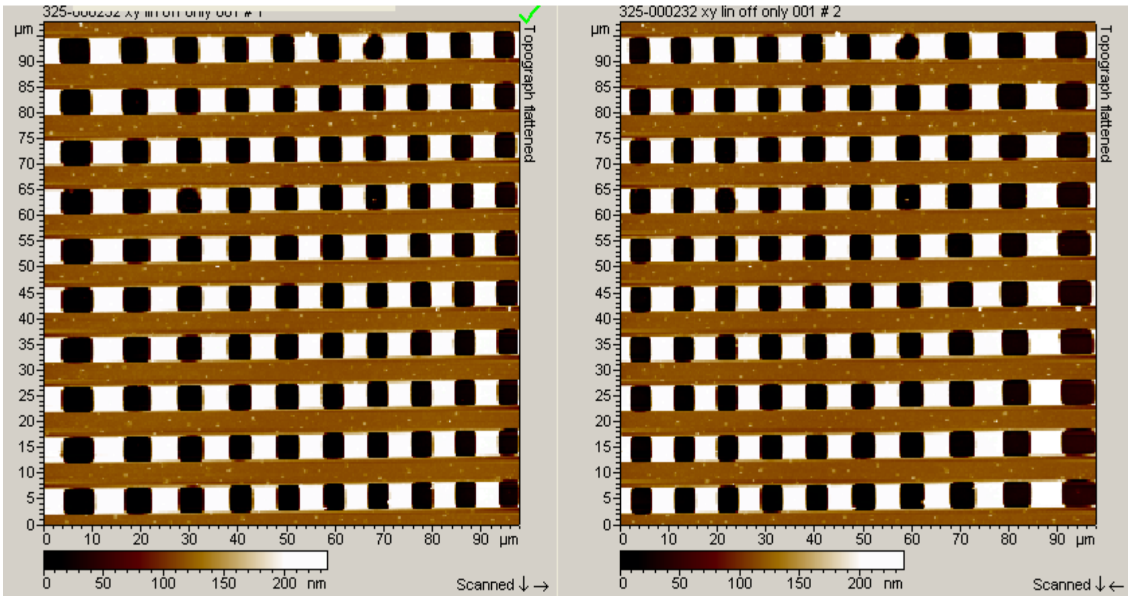


Fig. 2.4 Trace and retrace topography images of a standard grating showing effect of non-linearity in a scanner

- Hysterisis is the effect in which the curve showing the extension of the scanner with the applied field in one direction does not match the contraction of the scanner with the applied field in the other direction. The effect of hysterisis is shown in the Fig. 2.5 below where the scan cross-section in one direction (called trace) has an offset to the scan cross-section in the other direction (called retrace).

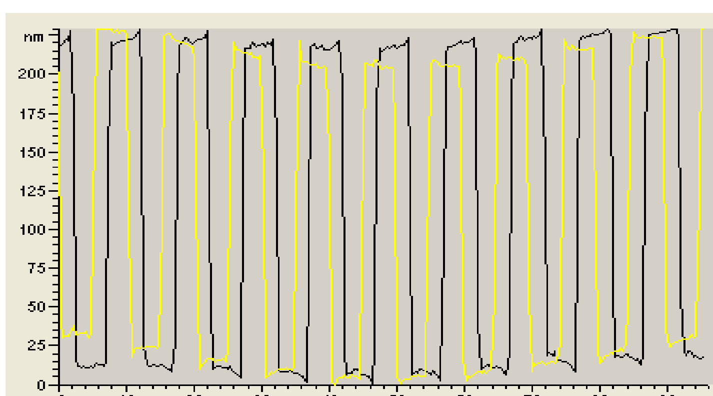
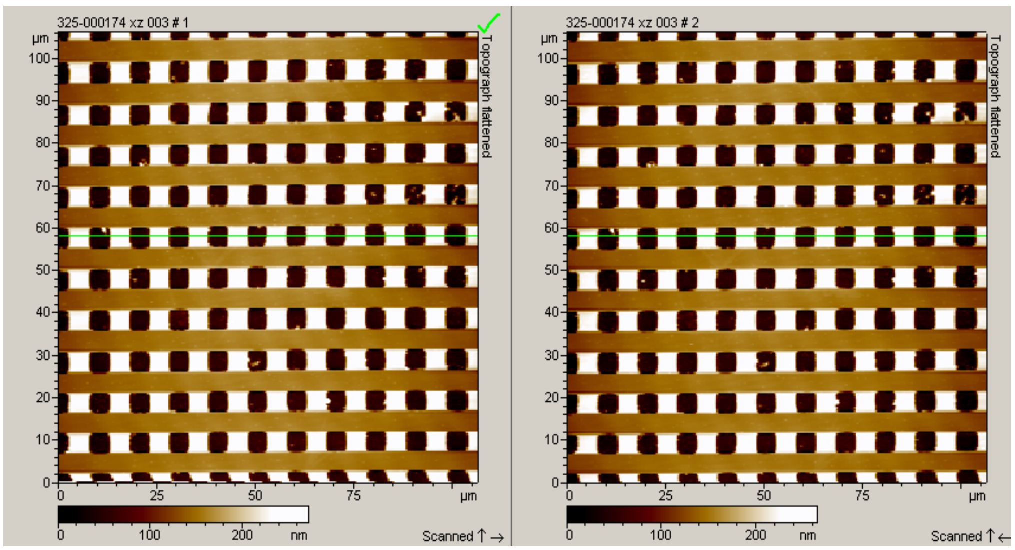
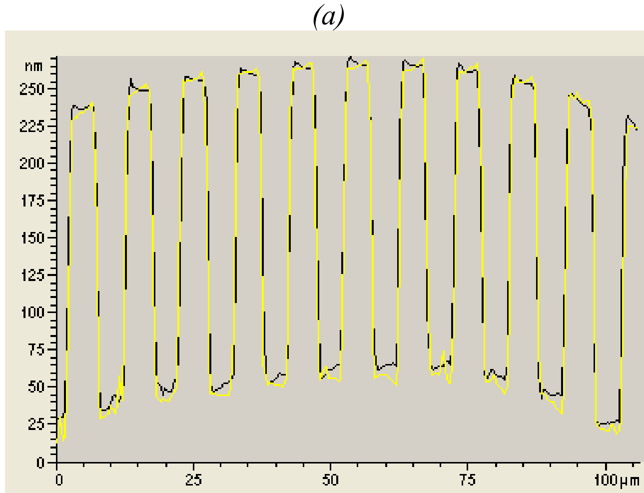


Fig. 2.5 Cross-section of a trace and a retrace showing hysterisis in a scanner

- The scanner calibration procedure takes care of the above effects. After the calibration, the image of the standard grating looks as shown in Fig. 2.6 below. The size of the pits is uniform across the scan range and the trace (shown with black color in the cross-section) and the retrace (shown with yellow color in the cross-section) overlap.[8]



(a)

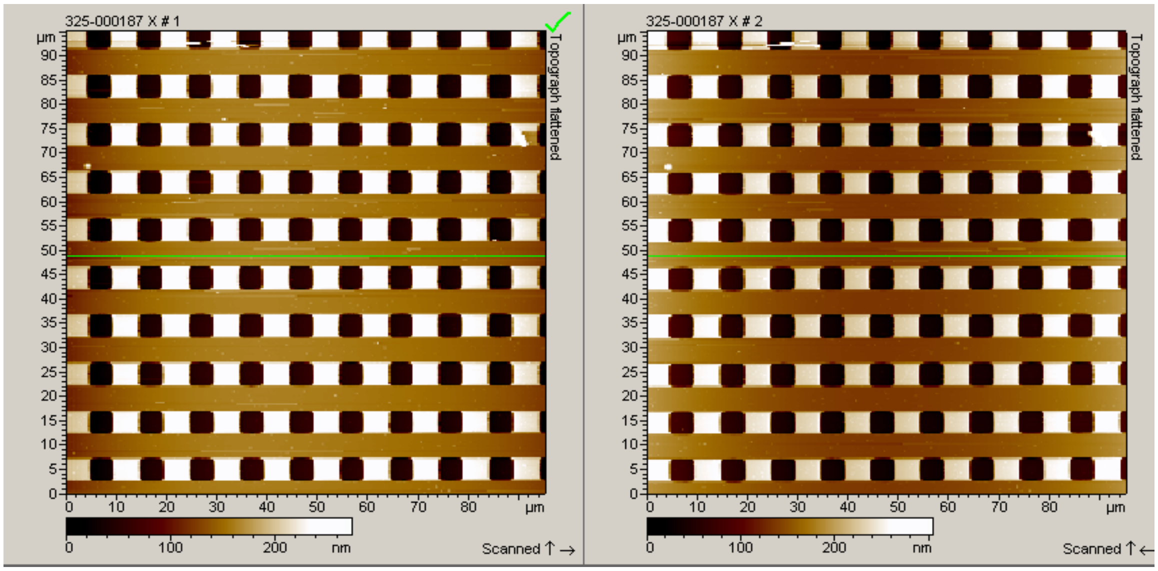


(b)

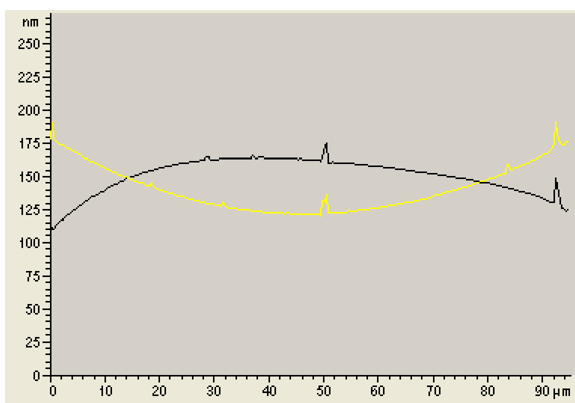
Fig. 2.6 Images showing trace and retrace topography (a) and cross-section (b) of a standard grating after scanner calibration

[8] All MI scanners are calibrated in the factory. The user has to copy the correct calibration file in the hardware folder of the Pico Scan.

- Bow: the scanner is fixed at one end with the tip attached at the free end. While raster scanning, the free end of the scanner moves in an arc (instead of a flat line) over the full range of the scanner. The adverse effect on the imaging is called bow and is shown in Fig. 2.7 below.



(a)



(b)

Fig. 2.7 A cross-section profiles (b) taken across a topography image (a) showing bow in a scanner

- Some of the bowing effect can be taken care of by the software during the image processing as shown in Fig. 2.8 below. The bow was removed by rendering the data in one buffer (shown yellow) at a different order than for the other buffer (shown black). However there is always the danger of introducing artifacts in the image if the correct order[9] of rendering is not chosen.

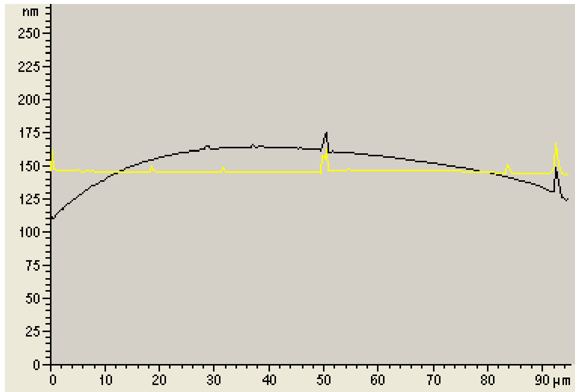


Fig. 2.8 Cross-section profiles showing removal of bow (yellow line) after image processing

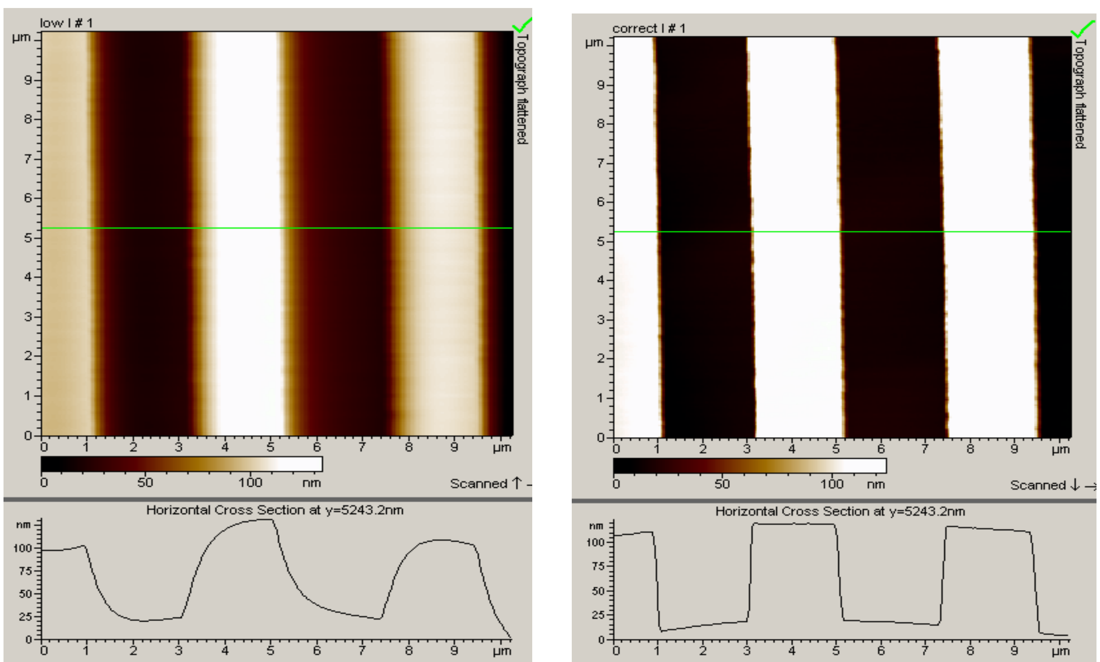
- Cross coupling is the effect in which movement of the scanner along one axis (usually X or Y) causes unwanted movement along the other axis (Z). The tube scanners are more susceptible to geometric cross coupling because a single four-quadrant tube provides motion in all the three axes. *Patented scanners by Molecular Imaging use two separate sets of plates for X-Y movement while the Z movement is provided by a tube. This configuration helps to reduce the cross coupling between different axes.*
- Aging is the time-dependent effect in which the sensitivity (extension per unit of field) of the scanner decreases (approx. exponentially) over time. A large amount of decrease takes place during first few hours. So MI scanners are exercised or burned in the factory for sufficient time before they are calibrated. The aging rate is very slow (depending on the use) after this, which makes the calibration viable over a long period. However for accurate imaging a scanner may need multiple calibrations over time.
- Creep is the effect that causes a delayed or sluggish displacement of the scanner when a sudden voltage offset is applied to the scanner. Due to the creep, the scanner achieves only a fraction (usually 98% or more) of its displacement instantaneously after the offset. The rest of the displacement takes place very

[9] For more information on the order of rendering please refer section 5: ‘Image Processing’.

slowly over a period ranging from 10 to 100 seconds. Creep appears as elongation of the feature in the direction of the offset because of this slow movement. This can lead to difficulties while performing functions such as zooming in on a feature or relocating the scan center, which require offset.

3. Servo/Feedback control

- The quality of the image is dependent on many inter-related scanning parameters and conditions. User needs to optimize the scanning parameters to obtain images with desired quality.
- Some of the frequently needed parameters are located under 'Servo Control' and 'Scan and Approach Control' menu.
- Servo gains integral 'I' and proportional 'P' determine how the scanner piezo responds to the servo signal (tunneling current / deflection / amplitude for STM / Contact AFM / AC mode AFM respectively).
- If the gain settings are too low, the scanner will not react to topographical changes quickly and the image will be blurred.



(a) *(b)*

Fig. 3.1 Images showing the effect of integral gain (I) on topography (top) and cross-section (bottom): low gain (a) and correct gain (b)

- If the integral gain is too high, the scanner will overshoot and as a result oscillate. This can be seen in Fig. 3.2 (the oscillations can be observed in horizontal cross-section plot in real time).

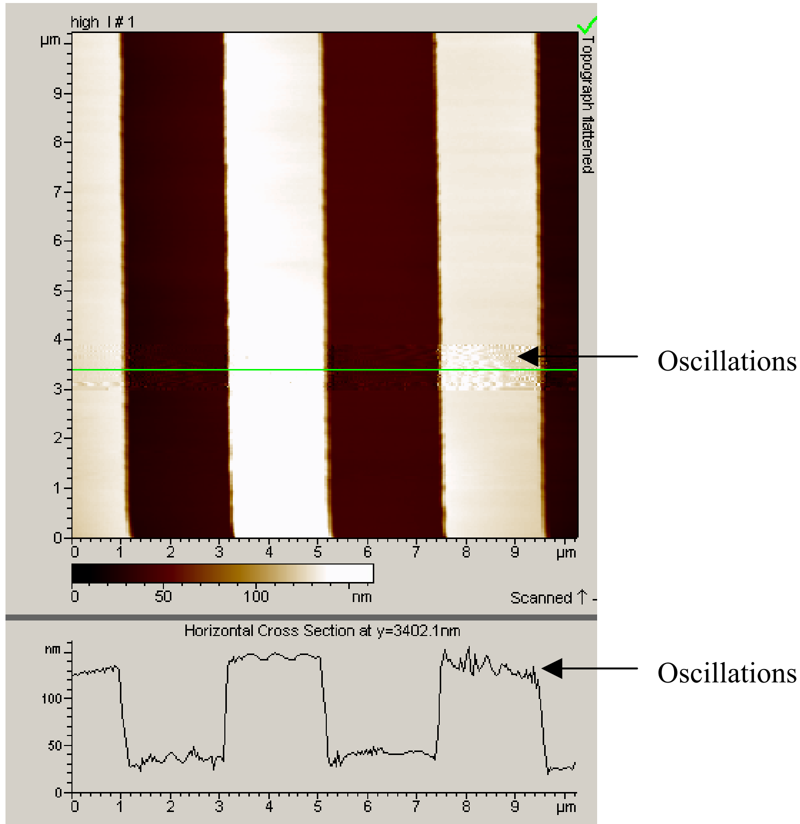
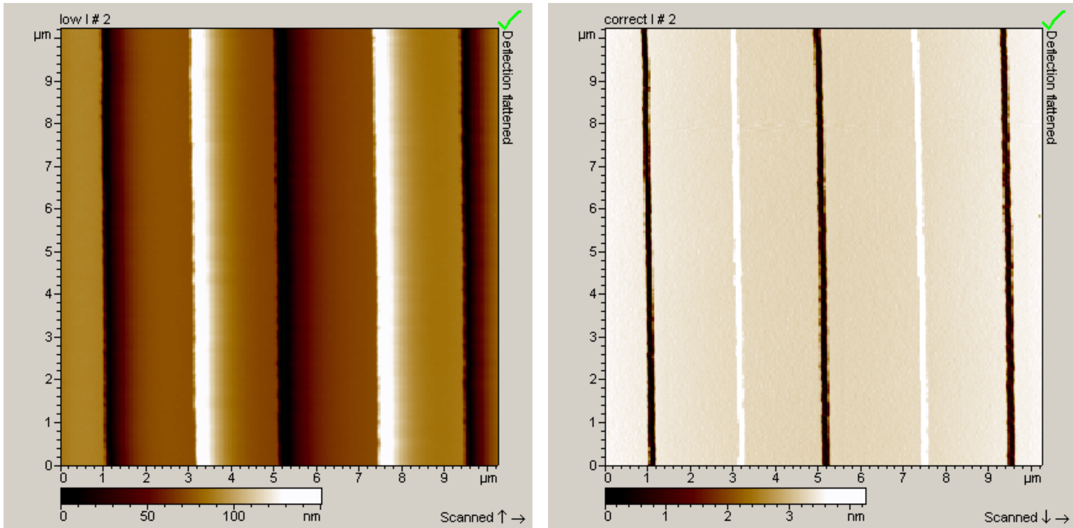


Fig. 3.2 Images showing the effect of high integral gain (I) on topography (top) and cross-section (bottom)

- I should be adjusted such that contrast and detail of topographic image is as high as desirable while keeping the feedback stable (no oscillations).
- Increasing I helps to keep the servo signal as close to set point as possible minimizing error signal (tunneling current / deflection / amplitude for STM / Contact AFM / AC mode AFM respectively).
- Thus higher I values increase the quality of topographic image but may change the quality of error signal image. This can be seen in Fig. 3.3 below.



(a) *(b)*

Fig. 3.3 Error signal images showing the effect of changing the integral gain (I): low gain (a), high gain (b)

- An optimal (not high-not low) value of I may be needed to get good images in both topographic and error signal channels
- Proportional part of the servo gain acts on the instantaneous error signal (no averaging effect provided by ‘I’) and thus affects only the high frequency portion of the signal. So the servo is more sensitive to I than P. ‘I’ is optimized first and then P is adjusted as necessary to increase image quality.
- Servo range gives the maximum displacement range of the Z piezo. Reducing the servo range increases the vertical resolution. This is useful for imaging very smooth samples. However care should be taken so as not to reduce the servo range less than the size of the highest feature within the scan range.
- Setpoint determines how well the tip tracks the surface and how much interaction is present between the tip and the sample. Setpoint should be adjusted such that this interaction would be minimum while allowing the tip to track the surface as accurately as possible. Increased interaction (e.g. force in AFM) may bring undesired changes in the sample while decreased interaction may make imaging impossible.

- Scan speed or tip velocity determines how much time is available for the servo to react to changes in the sample-tip interaction. It is dependent on two parameters: scan rate and scan size.
- Scan rate determines the number of trace / retrace the tip performs per second and is given in lines per second (Hz). A choice of scan rate depends on the scan size and the height of the topographical features. A simple rule is: tall features need more servo tracking time hence need slower scan rate and vice versa.
- In general, the scan rate in liquid could be slower than the scan rate in air.
- Increasing scan rate with the same scan size or increasing the scan size with the same scan rate increases tip velocity. This reduces the reaction time for servo and may result in poor tracking of surface. If increased scan speed (or tip velocity) is needed, higher servo gains should be used to achieve faster servo action.
- Other 'servo' and 'scan' controls include servo offset, scan origin (offset) and rotation, scan tilt (correction), overscan etc. More information on these can be found in PicoScan User's Manual.
- A particular set of scanning parameters (servo gains, setpoint, scan rate etc.) may be ideal for one kind of setup (scanning mode, type of tip, sample, environment etc.), but may become unsuitable if one or more of the conditions are changed. Thus a user may have to come up with a new set of ideal scanning parameters each time for a new application.

4. Probes

- SPM probes consist of cantilevers for AFM and tips for STM. AFM cantilevers are fabricated from silicon or silicon nitride and are integrated with a sharp tip at the end. STM tips can be made from Pt/Ir or W by mechanical cutting or electrochemical etching.
- Some probes are coated with reflecting materials such as Au or Al. For MAC mode imaging, probes are coated with a magnetic material. For ECSTM, coated STM tips are used to minimize leakage current.
- Most common shapes of the cantilevers are triangular or V and rectangular. Tips are tetrahedral, pyramidal or conical in shape.
- Quality of the image depends on the properties such as spring constant and resonant frequency of the cantilever and sharpness of the tip (defined by radius of curvature and sidewall angles).
- The spring constants of AFM cantilevers vary from a fraction of N/m (soft) to tens of N/m (hard). The frequencies range from tens of kHz to hundreds of kHz. The tip radius is usually less than 10 nm.
- Soft cantilevers are usually used for contact mode imaging and hard cantilevers for AC mode imaging. However, selection of the cantilever depends not only on the imaging mode but on the type of the sample also.
- The SPM image results from the convolution of tip shape with the sample topography. A general rule of thumb is: the smaller feature convolves (or images) the larger feature. If a feature is sharper than the tip, the image represents the shape of the tip and not vice versa. Fig. 4.1 below shows an example of a tip (larger in size) imaged by carbon nanotubes (smaller in size).

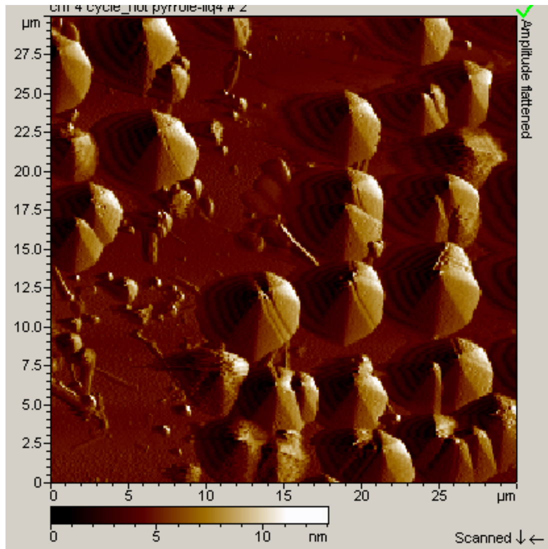


Fig. 4.1 Image showing artifacts due to tip-shape imaging

- Therefore, a sharp tip has a more lateral (XY) resolution than the blunt tip. The blunt tip can result from a defect in fabrication process, accumulation of debris or wear during imaging.
- STM has a greater lateral resolution than AFM. This is due to the fact that unlike AFM, tunneling interaction is exponentially dependent on tip-sample separation. This results in STM tip consisting of a single atom while AFM tip consists of a few atoms. Fig. 4.2 shows the tip and sample (white circles) and interaction areas (represented by black circles) in STM (left) and AFM (right) respectively.

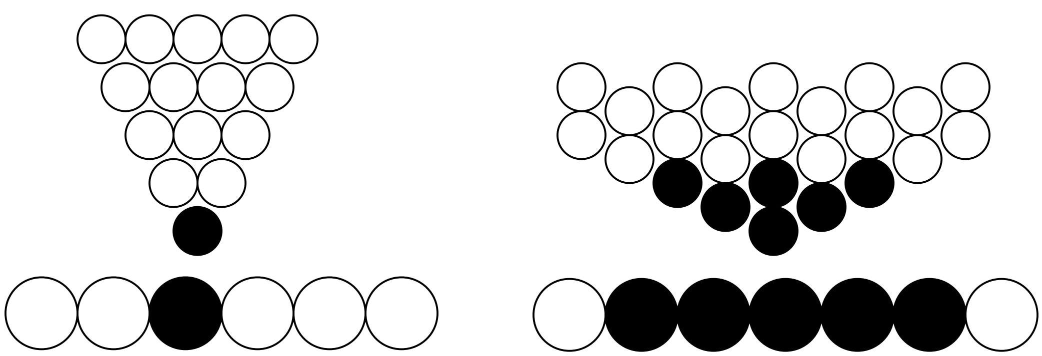
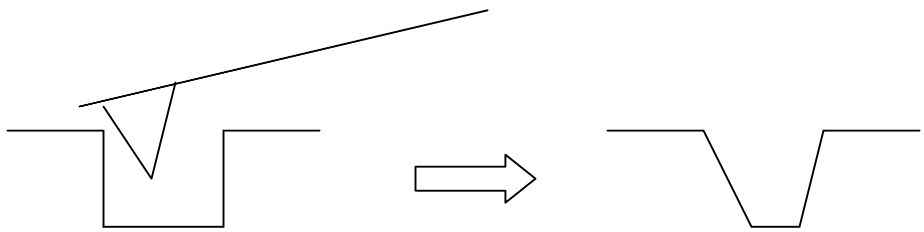


Fig. 4.2 Schematic of tip-sample interaction areas in STM (left) and AFM (right)

- The ability to image a deep trench or a step in AFM is limited by the sidewall angles of the tip. Here the rule of thumb is: the tip is unable to image surface features with sidewall angles greater than the sidewall angles of the tip. In such a case, the image shows the sidewall angles of the tip and not the feature.



A trench with vertical sidewalls　　　　　Image of the trench with tip sidewalls

Fig. 4.3 Schematic showing the effect of tip sidewall angles on imaging

- There may be a similar artifact introduced because of a dirty tip and is illustrated schematically in Fig. 4.4 below.

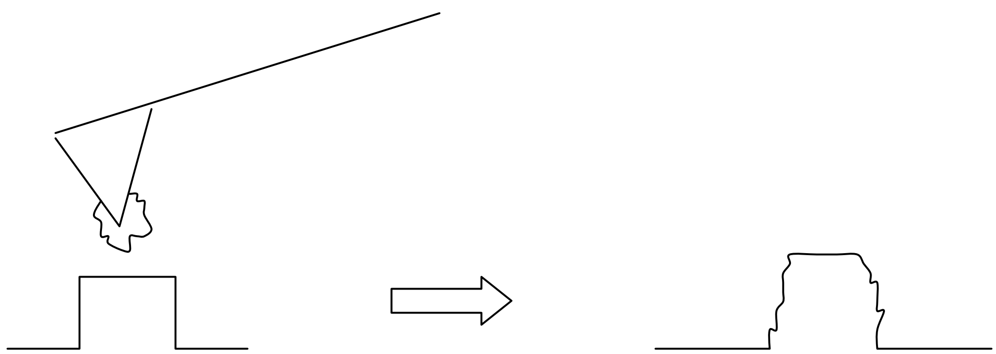


Fig. 4.4 Schematic showing the effect of a dirty tip on imaging

The image in Fig. 4.5 shows how a dirty tip introduces artifacts in the image. Here the ferritin molecules are imaged and appear mushroom-shaped instead of round.

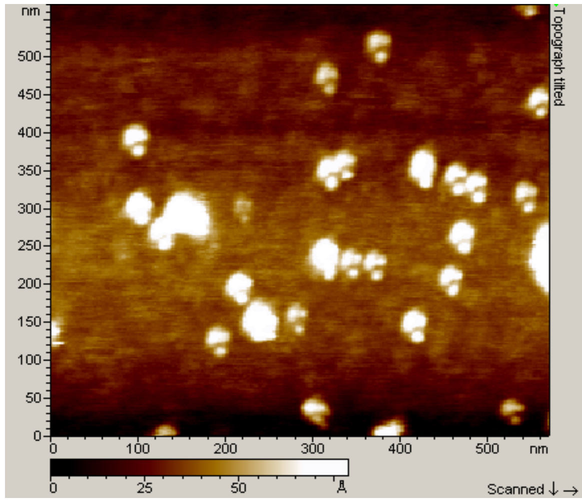


Fig. 4.5 Image showing the effect of a dirty tip on imaging

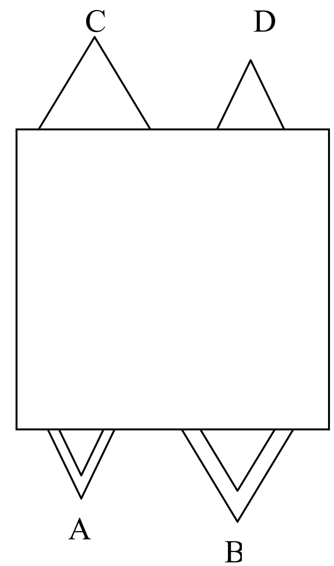
- Z resolution of AFM is virtually independent of the tip and depends on the vertical resolution of the scanner. Multipurpose small and large scanners with a Molecular Imaging system have vertical resolution of 0.2 Å and 0.7 Å respectively.

Type of cantilever	Contact				AAC	
	Silicon Nitride Probe				NCH	NCL
	A	B	C	D		
Shape	T	T	T	T	R	R
Length (μm)	115	196	115	196	120	220
Width (μm)	25	41	17	23		
Thickness (μm)	0.6	0.6	0.6	0.6	3.5	6.5
Resonant Frequency (kHz)	57	20	56	18	250	160
Force constant (N/m)	0.58	0.12	0.32	0.06	21	31

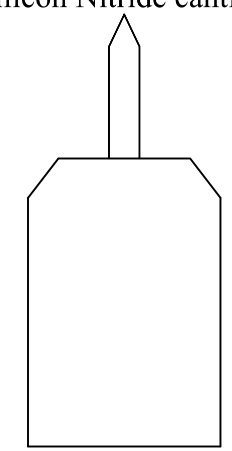
R= Rectangular, T=Triangular, all values in the table are typical values, for more detail see manufacturers' specifications.

- Molecular Imaging offers Etched STM tips.
  All tips are Pt (.8)Ir (.2) wire, .25mm diameter.
  They are available as coated or uncoated tips
  For detailed information, please visit
  http://www.molec.com/products_consumables.html#STM

*Figures are not to scale

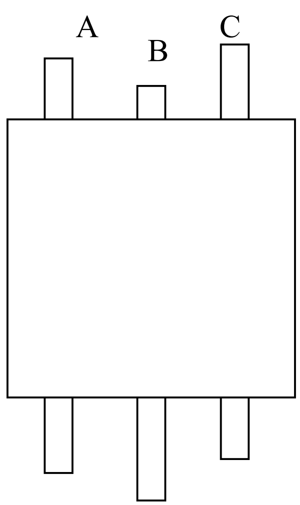


Silicon Nitride cantilever

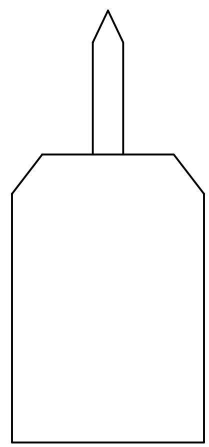


NCH or NCL cantilever

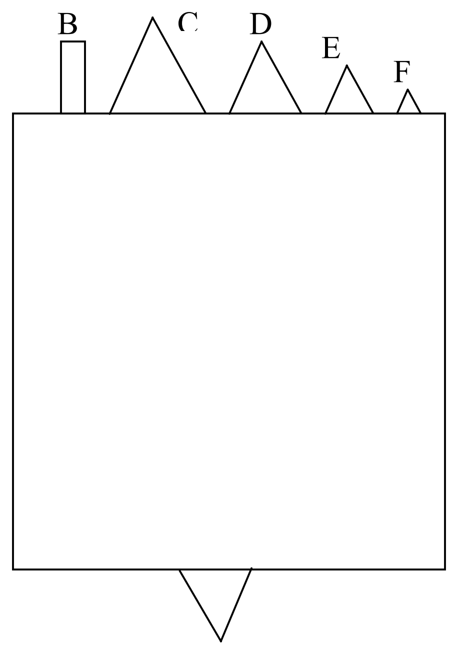
Type of cantilever	MAC								
	Type I			Type II	Type IV				
	A	B	C		B	C	D	E	F
Shape	R	R	R	R	R	T	T	T	T
Length (µm)	110	90	130	225	200	320	220	140	85
Width (µm)	35	35	35	28	20	22	22	18	18
Thickness (µm)	1	1	1	3	0.6	0.6	0.6	0.6	0.6
Resonant Frequency (kHz)	105	155	75	75	15	7	15	38	120
Force constant (N/m)	0.95	1.75	0.6	2.8	0.02	0.01	0.03	0.10	0.50



MAC Lever Type I



MAC Lever Type II



MAC Lever Type IV

*Figures are not to scale
*MAC levers are susceptible to environmental degradation. Please refer to the specification sheet that comes with the MAC levers for more information
*MAC lever type II can also be used as Force Modulation Levers

5. Image Processing

- PicoScan provides many real-time and off-line image analysis capabilities. These can be used either as a tool for data analysis and/or to make the image more presentable.
- This section describes some of the frequently used tools either from Data Rendering window (real-time or off-line) or from Filter Toolbox window (off-line). Filter Toolbox has many additional features and the description can be found in PicoScan User's Manual.
- In general, it is a good practice to modify the image as little as possible. However, if needed, user should understand how the filters change the image and how it affects the measurements.
- The image data can be rendered as Raw, Flattened or Tilted.
- Raw image rendering simply shows the data as it comes in. There is no correction for the slope of the sample. In Fig. 5.1 below this slope is noted in the Y direction. This slope is due to the fact that the sample is not perfectly leveled with respect to the scanner.

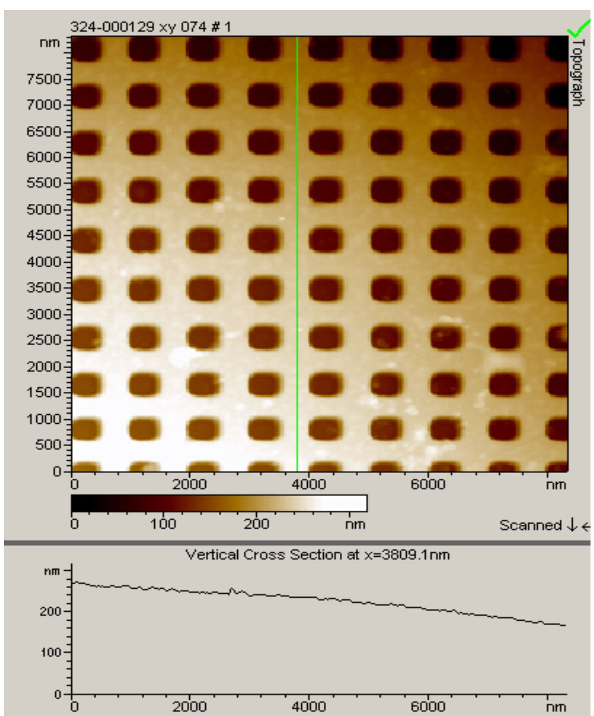


Fig. 5.1 Topography (top) and cross-section (bottom) images of a standard grating with raw image rendering

- The flatten algorithm for image rendering sets the average to be equal for each line on the X-axis as displayed. This is done by applying a polynomial fit to the data, which is then subtracted from the raw data, and the rendered data is then displayed in the image buffer. This means that lines where the data is flat will be adjusted so they are displayed in the center of the data. This can be seen in Fig. 5.2 below. Notice how all of the data is centered in the Z display range.

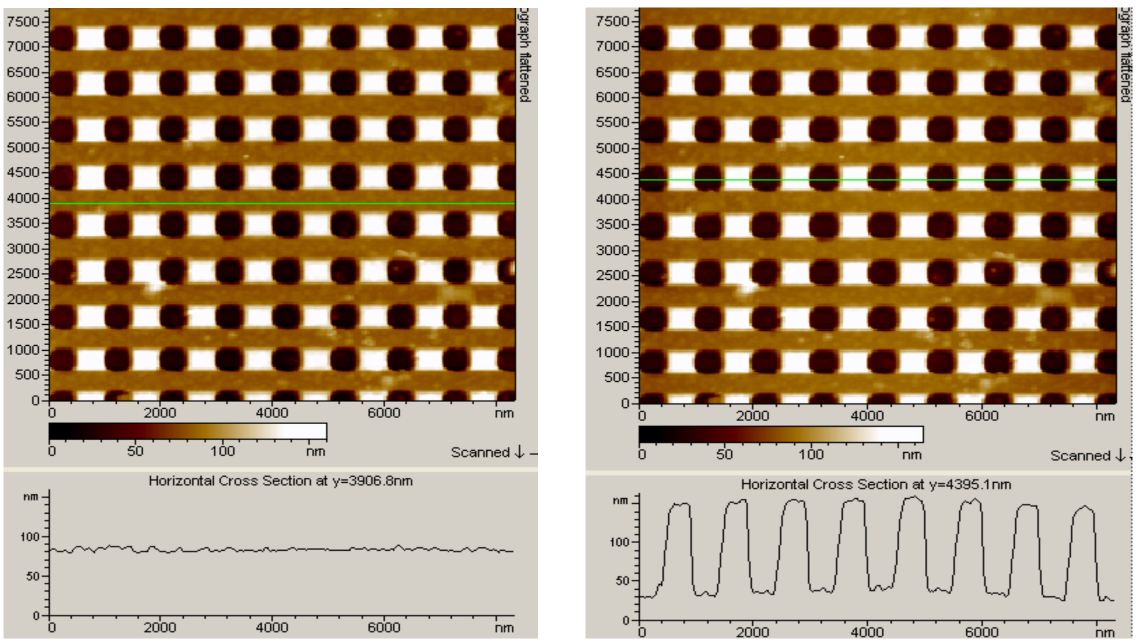


Fig. 5.2 Topography (top) and cross-section (bottom) images of a standard grating with flattened image rendering

- The polynomial fits to the data are:
  1st order: linear fit - the highest degree of the polynomial fit in x and z is 1.
  2nd order: quadratic fit - the highest degree of the polynomial fit in x and z is 2 and so on….
- Since the flatten filter removes the Z offset between each scan line along Y direction, it can introduce an offset in the data for regions that should appear as having the same height. This can be seen in Fig. 5.3. The average of this data is still set about the middle of the Z display range in the same place that it was set in the previous figure. However it has the effect of introducing an offset in the height of regions as shown by brown and white colors. Thus, in order to make accurate cross sectional measurements after applying the flatten

filter, the features must be aligned along X.

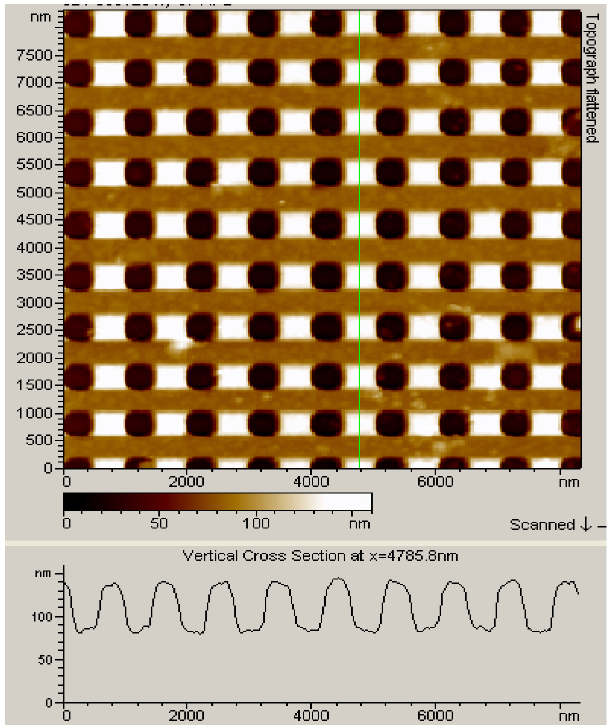
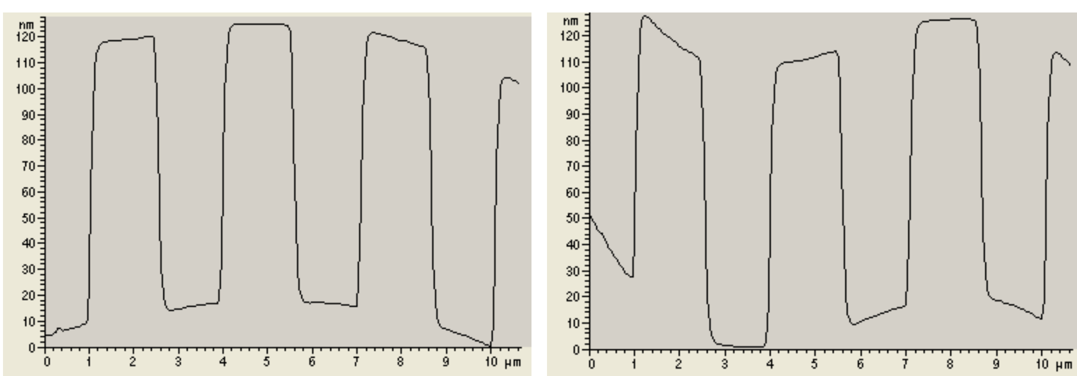


Fig. 5.3 Topographic (top) and cross-section (bottom) images of a standard grating showing the artifact introduced by flattened rendering

- When flattening data the order selected may also introduce artifacts into the image, based on the order of the fitting that is done to the data. This can be seen in Fig. 5.4 showing the cross section image below.



1st order flatten　　　　3rd order flatten

Fig. 5.4 Cross-section images showing the artifact introduced due to incorrect order of flattening

- Tilted filter fits a surface to the raw image surface data (two dimensional). Depending on the order selected a polynomial surface is generated from the raw data. E.g. 1st order: 1st degree surface (or plane) fit where the highest degree of a variable in x, y, or z is 1. 2nd order: 2nd degree surface (or parabolic surface) fit where the highest degree of a variable in x, y, or z is 2 and so on.
- Thus when the tilted algorithm is used to display the data, instead of offsetting each line so that the average height is the same; each line will have the slope corrected and each line will be adjusted down or up by an amount that varies as a polynomial based on the order selected. This can be seen in Fig. 5.5 below. Note how the artifact introduced by the flatten rendering and shown in Fig. 5.3 is absent in this case.

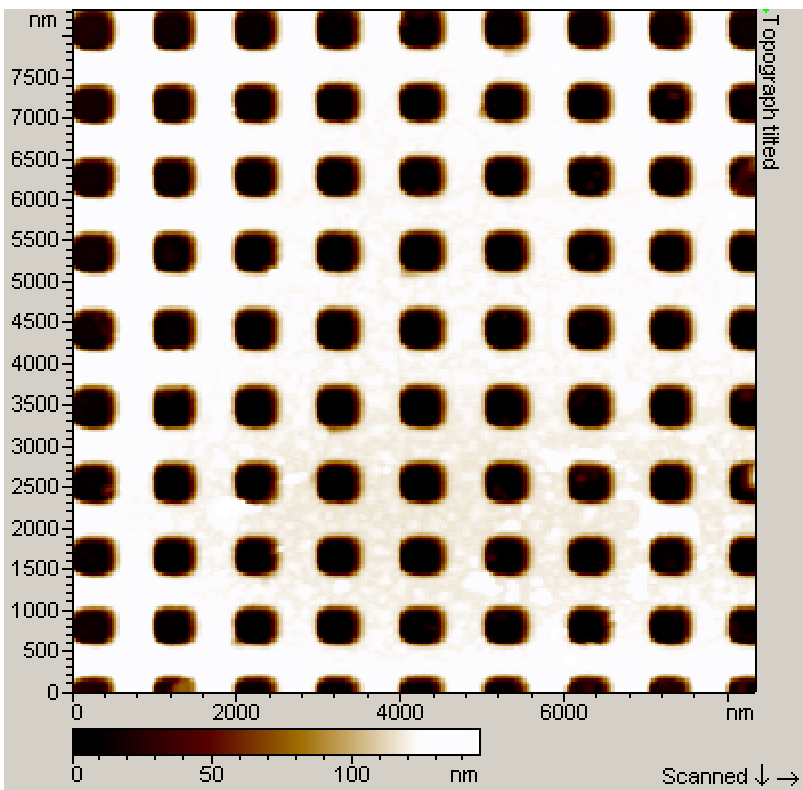


Fig. 5.5 Topography image of a standard grating showing the effect of tilted image rendering

- The surface fitting coefficients for the surface fit can be generated automatically by the software or by selecting ‘included’ and ‘excluded’ regions in the image manually. For details please refer to the PicoScan manual. One limitation to the tilted data is it is difficult to calculate the correct coefficients while scanning. So this type of rendering is usually better when the image has been already fully acquired.
- Data rendering window has ‘Signal Levels’ tab, which can be used to adjust the Z range of the data displayed and to optimize the distribution of signal levels in the image. The color scale can be edited through the ‘Color’ menu. These features are useful to improve the quality of the image display.
- Other tools and filters provided in the ‘Filter toolbox’ menu include FFT, arithmetic operations on buffers and matrix operations. Please refer to PicoScan manual for detail.

6. Spectroscopy in AFM

- AFM can be used to study a material property at a single x, y point on the sample surface through the measurement of spectroscopy curves.
- In contact mode, a force-distance (f-d) curve is such a tool. It is a plot of the vertical force that the AFM tip applies to the surface (proportional to the deflection of the cantilever) as a function of the Z position of the scanner.
- The following figures represent a sequence of AFM cantilever movement in air (Fig. 6.1) and in water (Fig. 6.2) during a cycle of a force-distance measurement.

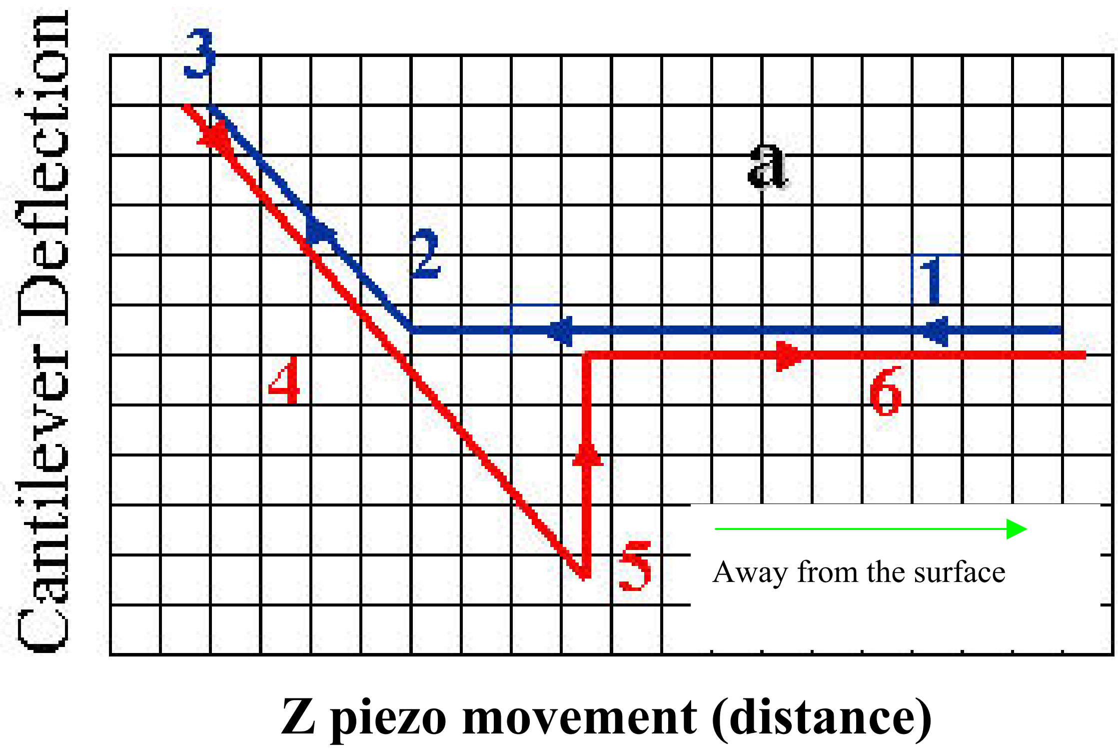


Fig. 6.1 Schematic f-d curve in air illustrating different cantilever positions during a f-d curve cycle

- The blue and the red colors represent approach and retraction portion of the complete cycle respectively.
    1. At the right side of the curve, the scanner is retracted up to the end of its user-defined range. The cantilever is not deflected since the tip is not touching the surface (*position 1*).

2. As the scanner begins to extend, the cantilever remains not deflected (*between positions 1 and 2*) until it gets very close to the surface and the tip starts to feel the attractive force. As a result the tip snaps into the surface (*position 2*) and the cantilever suddenly bends slightly towards the surface.
3. During further extension of the scanner, the cantilever bends almost linearly away from the surface due to the repulsive force (*between positions 2 and 3*). Following a full extension up to the end of its user defined range (*position 3*); the scanner begins to retract and pulls the tip away from the surface.
4. Assuming no scanner hysterisis, the cantilever deflection retraces the same linear curve (*between positions 3 and 4*).
5. The thin layer of water present on many surfaces in air exerts a very strong attractive capillary force and holds the tip in contact with the surface as the scanner pulls away from the surface. Thus the cantilever now bends strongly towards the surface (*between positions 4 and 5*).
6. The scanner has to retract beyond position 2 before the tip can snap out of the surface (*position 5*). As the scanner continues to retract the cantilever returns to its original unbent status (*position 6*).

- In liquid, since the large capillary force is isotropic the typical shape of the force-distance curve becomes the one shown in Figure b and the total force that the tip exerts on the sample can be reduced.

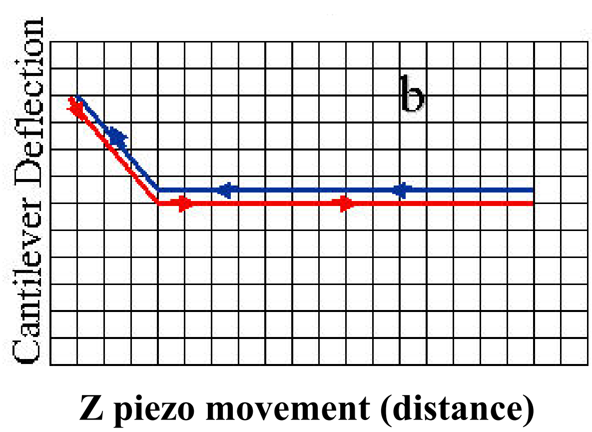


Fig. 6.2 Schematic f-d curve in a fluid showing the absence of strong adhesion

- The hysterisis in the open-loop scanner changes the f-d curve such that the approach and retrace portions do not overlap. This can be seen in Fig. 6.3 below.

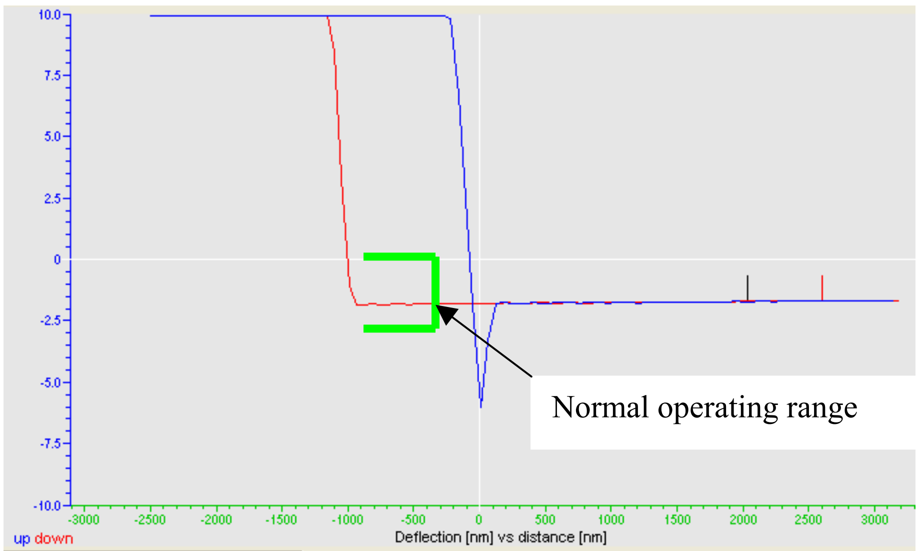


Fig. 6.3 F-d curve showing 'Normal operating range'

- F-d curve can be used to 1) check whether the tip has actually reached the surface or not 2) select the appropriate setpoint if the tip has reached the surface 3) measuring the force between the tip and the sample.
- The f-d curve in Fig. 6.4 below indicates that the tip does not deflect even when the z-piezo is extended all the way up to the end of its user defined full range.

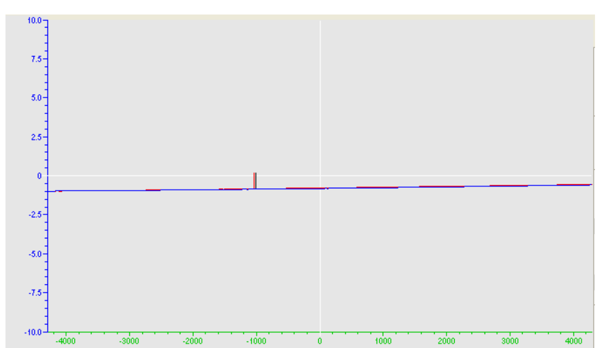


Fig. 6.4 F-d curve showing the absence of cantilever deflection

If the tip has not reached the surface even though the system thinks the setpoint has been reached, the f-d curve may look like the one shown by Fig. 6.5 which shows an instance of a false engage where the flat line (or non-contact portion) of the f-d curve lies above 0 on Y-axis (which is also the setpoint). Usually in this case the Z-piezo voltage is railed to -200V and error signal reading on the microscope and oscilloscope window in the software is positive.

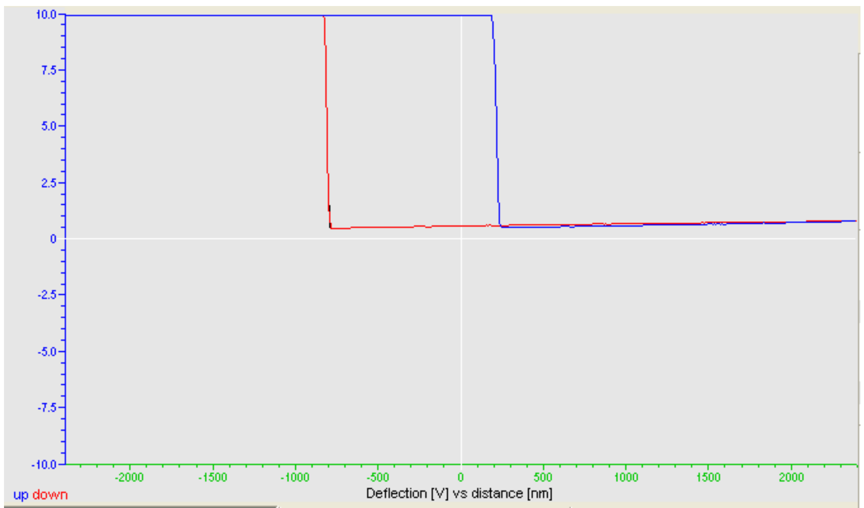


Fig. 6.5 F-d curve showing an instance of a false engage

- In AC mode AFM spectroscopy an amplitude-distance (a-d) curve replaces the f-d curve. A typical a-d curve is shown in Fig. 6.6 below. The amplitude above the setpoint (zero) line is almost constant and can be seen as a flat line. Once the tip interacts with the surface the amplitude drops and forms a shoulder as is evident below.

Fig. 6.6 Amp-distance curve showing 'Normal operating range'

- Fig. 6.7 shows a case where the cantilever does not interact with the surface even when the piezo is extended up to the end of its user-defined range. The a-d curve in this case is just a sloping straight line and the shoulder indicating the reduction of amplitude is absent.

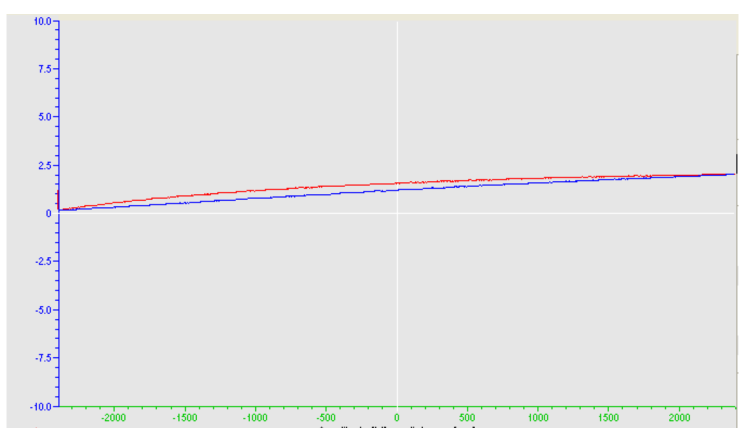


Fig. 6.7 Amp-distance curve showing an instance when there is no amplitude reduction for the full length of the sweep

- A-d curve can also be used to check for a false engage and to obtain a good operating setpoint. Fig. 6.8 is an instance of a false engage where the sloping line portion of the a-d curve and the shoulder lie below 0 on Y-axis (which is also the setpoint). Usually in this case the Z-piezo voltage is railed to -200V and the error signal reading on the microscope and oscilloscope window in the software is positive.

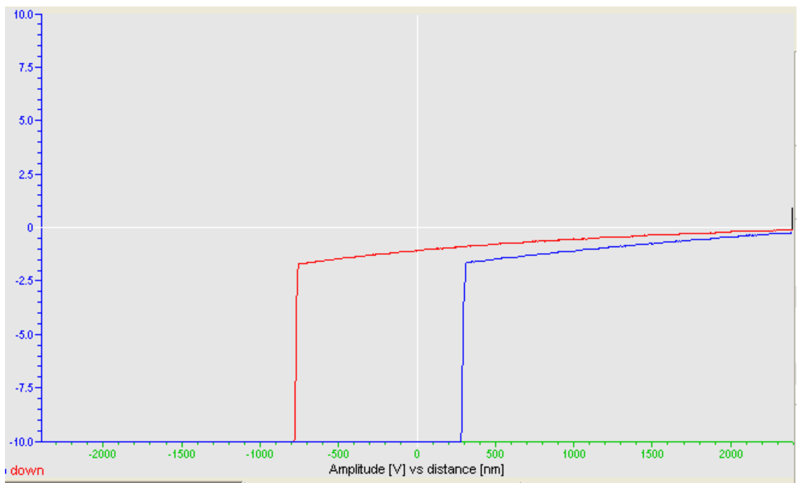


Fig. 6.8 Amp-distance curve showing an instance of a false engage

- Changing the setpoint moves both the f-d curve and a-d curves along the Y-axis (up and down). The setpoint should be adjusted such that a) for a f-d curve the point where the cantilever starts deflecting is below 0 on Y-axis (which also is the setpoint) and is situated in the 'Normal operating range' (see Fig. 6.3). The more this point is below the setpoint line, the more the cantilever bends and hence contacts the surface harder. b) For an a-d curve the shoulder is above 0 on Y-axis (which also is the setpoint) and there should be sufficient reduction in amplitude between the shoulder and the setpoint line as shown by the 'Normal operating range' (see Fig. 6.8). The more the shoulder lies above the setpoint line, the more the amplitude reduces and hence the cantilever taps the surface harder.
- When running the f-d curve or a-d curve, it is always a good idea to limit the interaction of the cantilever with the surface. This is done by using 'Limit' option

(e.g. 2 V for a f-d curve and –2 V for an a-d curve) in the SPS window of PicoScan. This way the chances of the tip or the sample getting damaged during the sweep are minimized. In this case the f-d curve and the a-d curve look like as those shown in Fig. 6.9.

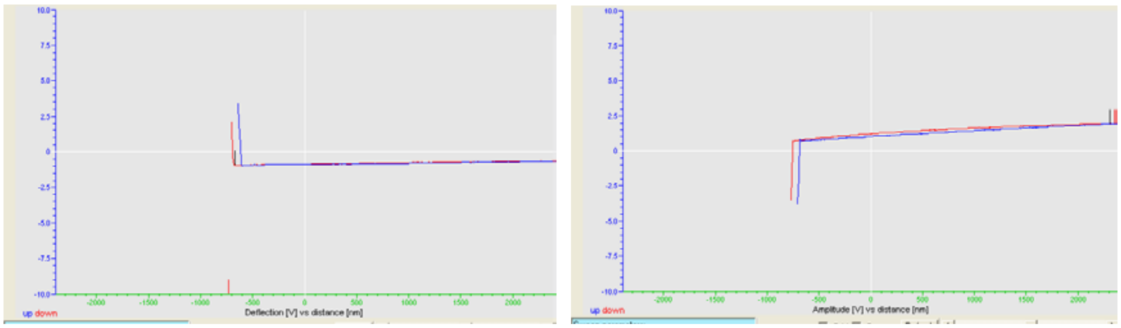


Fig. 6.9 Examples of f-d sweep (left) and amp-distance sweep (right) when the 'limit' option is turned on.