Environmental Control Atomic Force Microscope (AFM) Cypher ES Standard Operating Procedure

1. Introduction

Basic information: Environmental Controlled Atomic Force Microscope (AFM) Cypher Es main unit (including main body, optical system, ARC2 controller, ES scanner, computer and operating software); electrical performance testing module, high voltage PFM module, blueDrive photo-thermal excitation module, scanning tunneling imaging module, and electrochemical liquid cell module.

Key Technical Parameters:

Scanning mode: Sample scanning mode
Maximum scanning range in XY direction: 30x30 μm
Maximum scanning range in Z direction: 5 μm
System closed-loop noise level in XY direction: 60 pm
System open-loop noise level in XY direction: 8 pm
System closed-loop noise level in Z direction: 15 pm
Environmental control system: Integrated environmental control scanner, can be sealed
Leak-proof pressure sensor: Available
Gas control: Sealed, gas control can be performed
Temperature control: RT - 250℃, can be heated in a sealed environment
Automation operation: Automated laser position and detector zeroing
Fast scanning function: The fastest scanning speed is 128 Hz, and one scan can be completed within 1 second. Conventional scanning speed is 20 Hz.

The main components of the environmental control AFM Cypher ES are as follows, as shown in Figure 1.

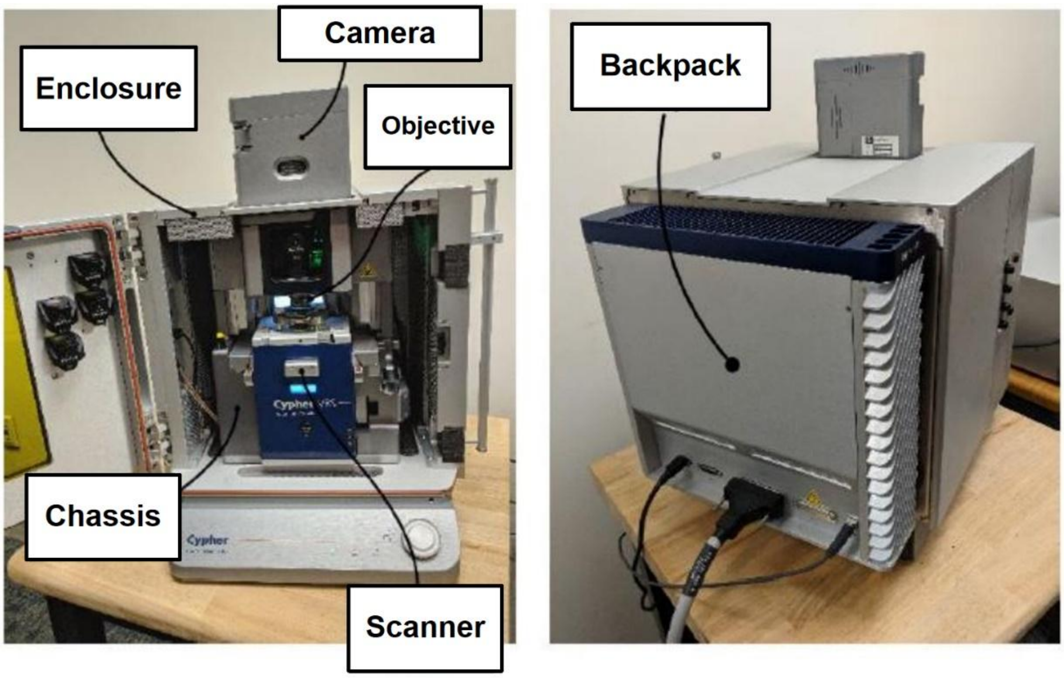


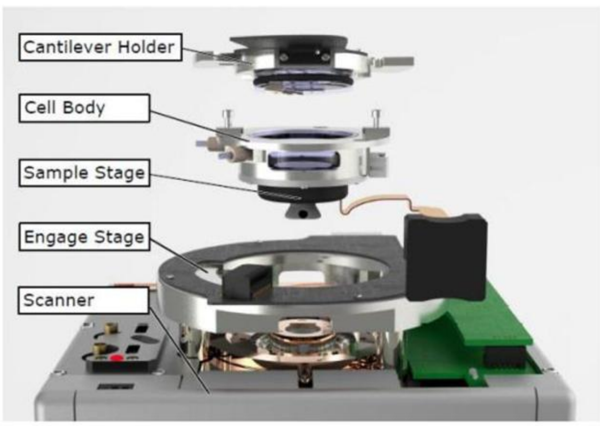
Figure 1. Schematic diagram of the environmental-controlled AFM Cypher ES system

Controller: Controls the XYZ three-axis movement and collects data, etc.

Scanner: Holds the sample and the probe, with the holder placed. During scanning, the XYZ three axes are controlled by piezoelectric ceramics for their movement. The scanning range is 30 μm for the XY axis and 5 μm for the Z axis;

Backpack: Acts as the second controller, containing various electrical components such as ADC/DAC. It is a key factor for the Cypher series to achieve low noise levels and fast scanning speeds.

Scanner:



The Cypher Es series AFM not only features fast scanning speed, high resolution and low noise level, but also has an environmental control function. As shown in the left picture, its sealed sample chamber can be used to control the gas environment inside the chamber. It can also be used in combination with various sample stages with temperature control functions.

To maintain the airtightness of the chamber, please be careful not to scratch the black rubber at the bottom of the sample stage with sharp objects such as forceps.

Figure 2. Schematic diagram of the environmental control sample chamber

2. Sample preparation

The test samples are required to be clean, with a flat surface, and the height variation should not exceed 5 μm. The maximum diameter should be less than 15 mm, and the height should be less than 7 mm.

3. Probe preparation

Prepare the appropriate probes based on your own samples. If the sample is of significant importance or if the test results are to be used for publication in an article, it is recommended to use new probes.

4. System examination

Before the test, please complete the following checks:

1) Before the test, make sure that the Power light of the Controller is on and the Laser key is in the "Off" position.
2) Before inserting the needle, select the correct holder and check if there are any abnormalities on the holder, such as cracks or dirtiness. If any problems are found, inform the instrument supervisor immediately.

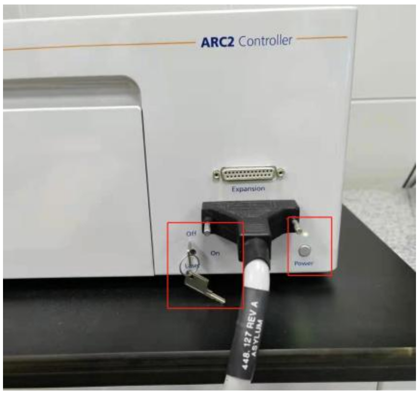


Figure 3

5. Sample preparation of AFM imaging

Use double-sided tape, conductive silver glue and AB glue to stick the sample onto the sample holder. Please place the area to be tested as close as possible to the center of the sample holder. Before sample preparation, please check if the sample holder is clean. Make sure both the front and back sides are smooth and free of residue. Otherwise, the sample will not be able to move.

Warning: 1) During sample preparation, ensure that the area to be scanned is at the highest point of the sample. Do not place objects higher than the sample on the sample holder as shown in Figure 6-4. Only one sample should be placed on one sample holder.

2) The sample preparation tools for AFM, such as the sample holder, tweezers, and screwdriver, are public laboratory items. Please use them with care and do not take them away.
3) Before and after the test, use lint-free paper dipped in alcohol to carefully clean both sides of the sample holder. Take away or handle your own sample and do not keep the public sample holder occupied all the time.

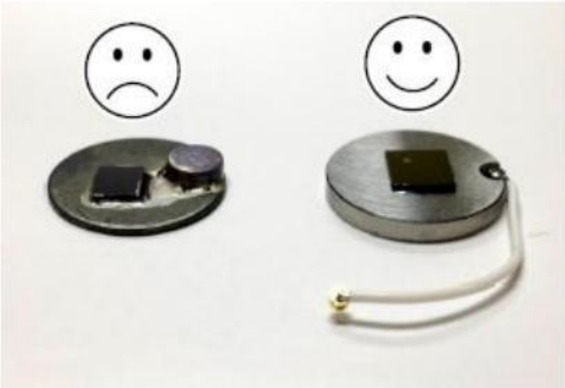


Figure 4

6. Probe insertion

Use the special probe tweezers to hold the middle part of the probe chip, carefully place it under the pressure plate of the holder, approximately at the middle position of the chip, and slightly tighten the fixing screws of the pressure plate.

Warning: 1) Before inserting the needle, use a nitrogen gun or an ear aspirator to thoroughly clean the pressure plate and glass part of the holder, ensuring that there are no chip fragments remaining. Otherwise, when tightening the screws, it may damage the glass.
2) When fixing the screws, just slightly tighten the fixing probe. Do not apply excessive force to avoid causing the pressure plate to deform and curl up, which will lead to an increasing force on the glass and result in damage.
3) When holding the holder, simply remove the plastic parts on both sides of the holder. Pay special attention not to touch the copper wire and chips on the surface of the holder with any sharp objects. Otherwise, it may cause the holder to be damaged and unable to be recognized, and the user's research group will be required to compensate.
4) Be sure to hold the holder firmly. If it accidentally falls and causes damage to the glass or the holder fails to be recognized, the user's research group will be required to compensate.

7. Sample insertion

1) Open the door of the soundproof cover of the AFM host, lift the black handle on the right side of the Scanner, and gently pull the Scanner out.

Note: Do not pull the Scanner more than half of its side width away, that is, at the middle screw position, to prevent it from slipping out of the soundproof cover.

2) Use the special tweezers to place the sample on the sample platform. First, confirm that there is enough distance between the sample and the holder. Do not bump the probe during the installation of the holder. As shown in Figure 6-5, align the two holes on the holder with the two screws on the Scanner, gently press down the holder to press the rubber O-ring on the holder tightly, enhancing the sealing of the cavity, and then rotate the holder clockwise to insert the golden chip into the slot. Tighten the two screws separately with a screwdriver.

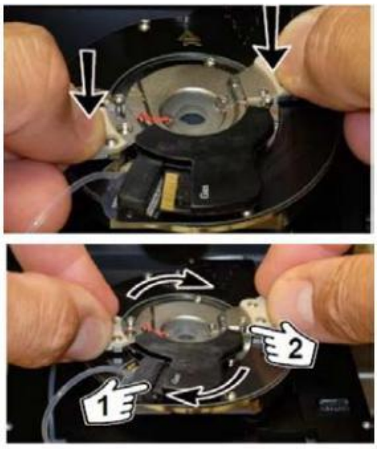


Figure 5

Warning: During the installation process of the holder, if you accidentally bump into the probe, it will not only damage the probe but also potentially cause damage to the holder. Please make sure to check!

3) Push the Scanner back to its original position, pull down the black handle on its right side, and confirm that the focusing adjustment ring of the objective lens (Figure 6-6) is at position 1.5. Then close the soundproof cover.
4) Assess the distance between the probe and sample with naked eye, then slowly rotate the knob on the AFM host until the probe descended and is 2-3mm above the sample.

Warning: Fast rotation of the knob accelerates the descending prob. The rotations should be slight to avoid collision of the prob and sample, which can damage the holder and Scanner.

Caution: If the prob does not move when the knob is rotated, you should start the software, initialize the sample platform, detach the holder and click Initialize Motor in the Engage panel. This will be elaborated below.

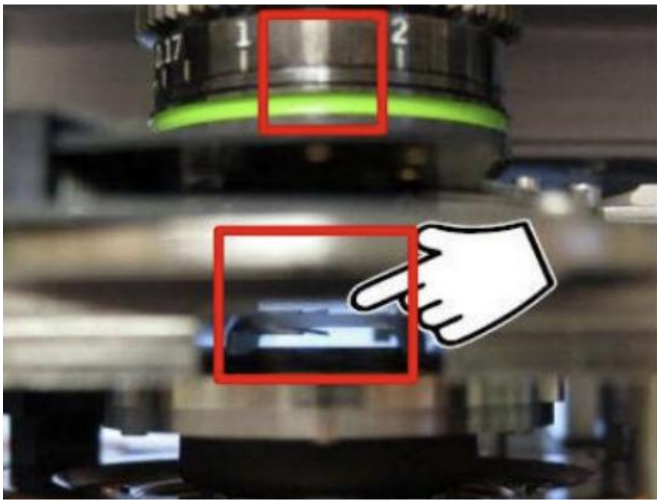
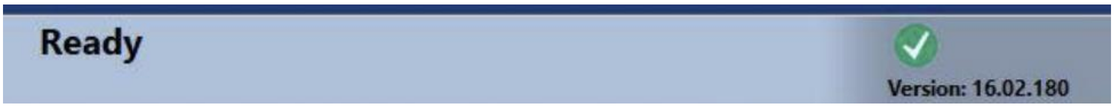


Figure 6

8. Software operation

   1) Input account and password to log in, then go to the desktop. Double click to open the software "AR16" , turn on the switch for the Laser, turning the switch from "off" to "on" (No particular order for these two steps). Wait for the progress bar to show "Ready".



   2) Choose the testing mode.

      A Mode Master window will pop up in the software. Choose "AC Air Topography", and enter the software environment of Tap Mode.

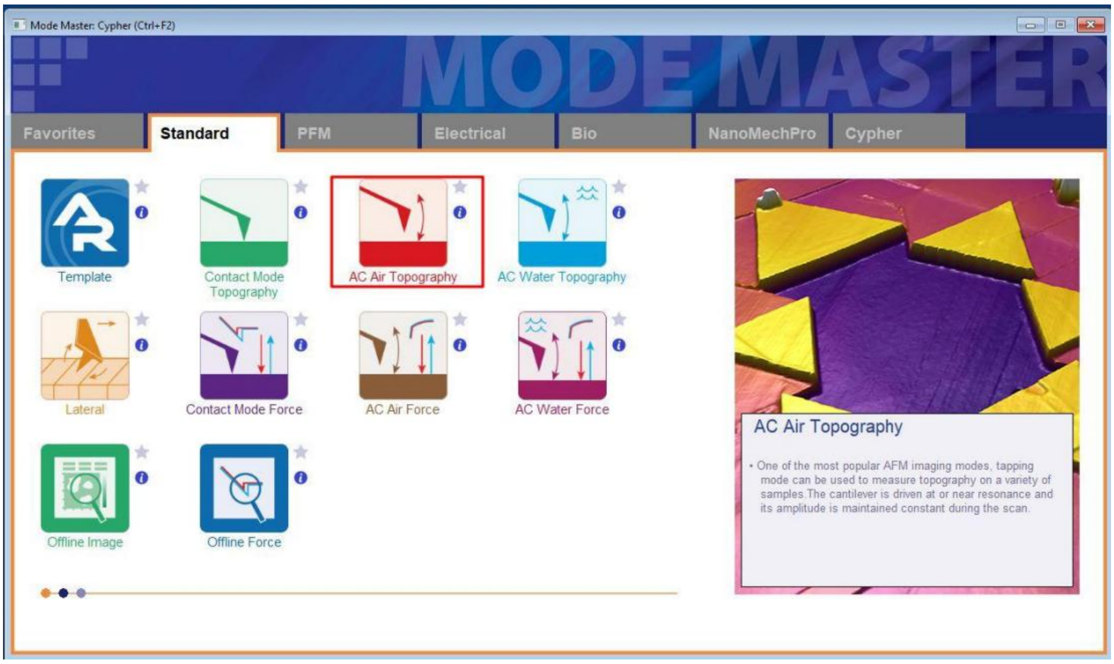


Figure 7

3) Initialize the Sample Motor Platform

Upon each controller restart, the system will automatically prompt for initialization in the Engage Panel. Click the "Initialize Motor" button (see Figure 8).

(Note: This step is not required if the controller has not been restarted.)

Figure 8

4) Find the probe.

In the "Video" window, use the "Home" button at the top and the highlighted yellow CCD button in the upper left corner to locate the probe. After clicking the Home button, observe the Video window; you should roughly see the contour of the cantilever or be able to determine the direction of the cantilever within the current field of view. Then, in the "Engage Panel" window, adjust the objective lens focus up and down. (Please note: you must select "Move Focus" to adjust the objective.) The single arrow is for slow focusing, and the double arrow is for fast focusing. After determining the focal plane of the tip, click the "SET" button next to "Focus On Tip".

5) Get laser

Right-click on the tip and select "Spot On". The laser spot should then appear in the corresponding position. Use the highlighted yellow arrows in the upper left corner to adjust the laser's position. Hold down the Shift key for fine adjustment. Note the principles for laser adjustment: 1. Position the spot as close to the tip as possible; 2. In the "Sum and Deflection Meter" window, the "Sum" value should be as high as possible, preferably above 3; 3. Ensure there is no light leakage.

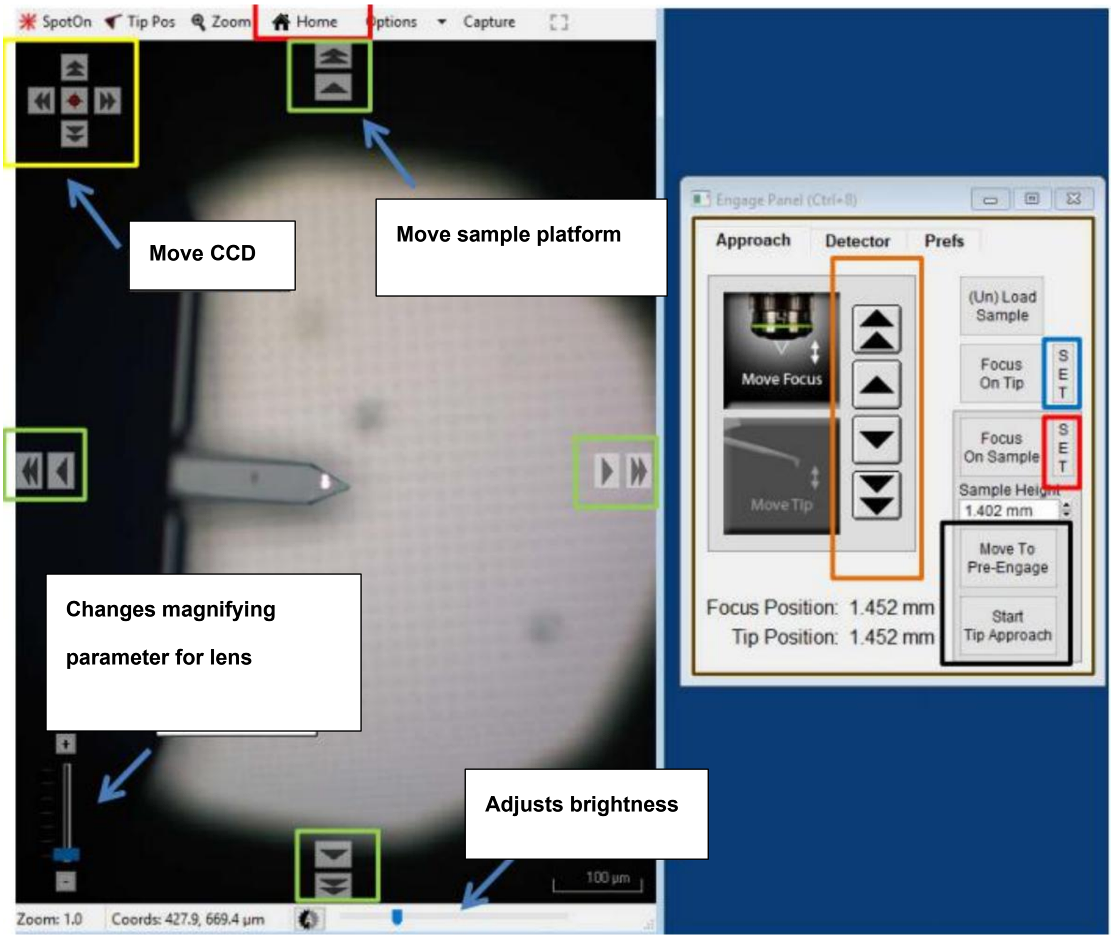


Figure 9

6) Find sample

Continue to lower the focus until it is on the sample surface, then click the SET button on the right side of "Focus on Sample". The Sample Height value will then be refreshed.

7) Click the "Move To Pre-Engage" button highlighted in Figure 9. This will position the probe 50 μm above the sample surface. Observe whether the laser spot has shifted and make fine adjustments to its position if necessary. Warning: If the previously set probe position and sample height are incorrect, this step is highly likely to cause the probe to crash into the sample, resulting in damage! Therefore, after setting the two focal planes, carefully check the settings to prevent probe damage, or worse, the holder colliding with the sample and causing more severe equipment damage!

8) Click "Zero PD" in the "Sum and Deflection Meter" window to zero the Deflection value (it is acceptable if it is close to zero).

9) Click the "Tune" button in the right-hand column of the "Master Panel" window to perform the Peak Finding/Tune procedure. This process finds the resonance frequency of the probe in use and sets the Free Amplitude value in air (Target Amplitude).

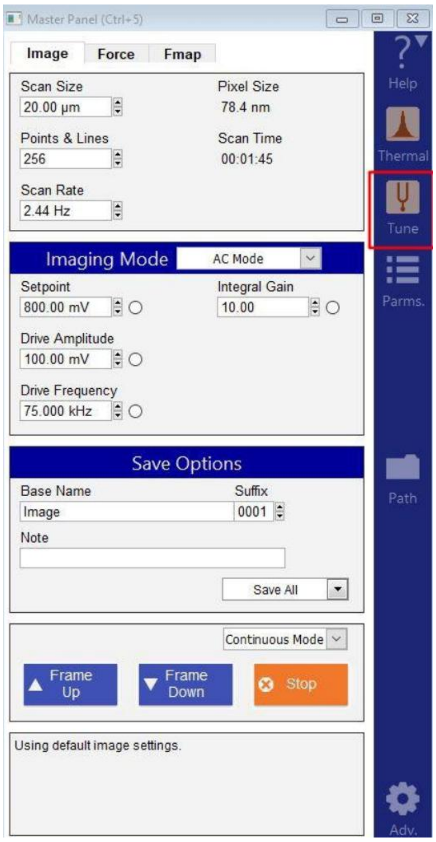


Figure 10

The "Cantilever Tune" window will pop up. Click the "Auto Tune" button.

Note: Based on the frequency range indicated on the probe package, you can manually modify the Auto Tune Low/High values by clicking the "Parms." button on the right side of this window to set the device's peak search range.

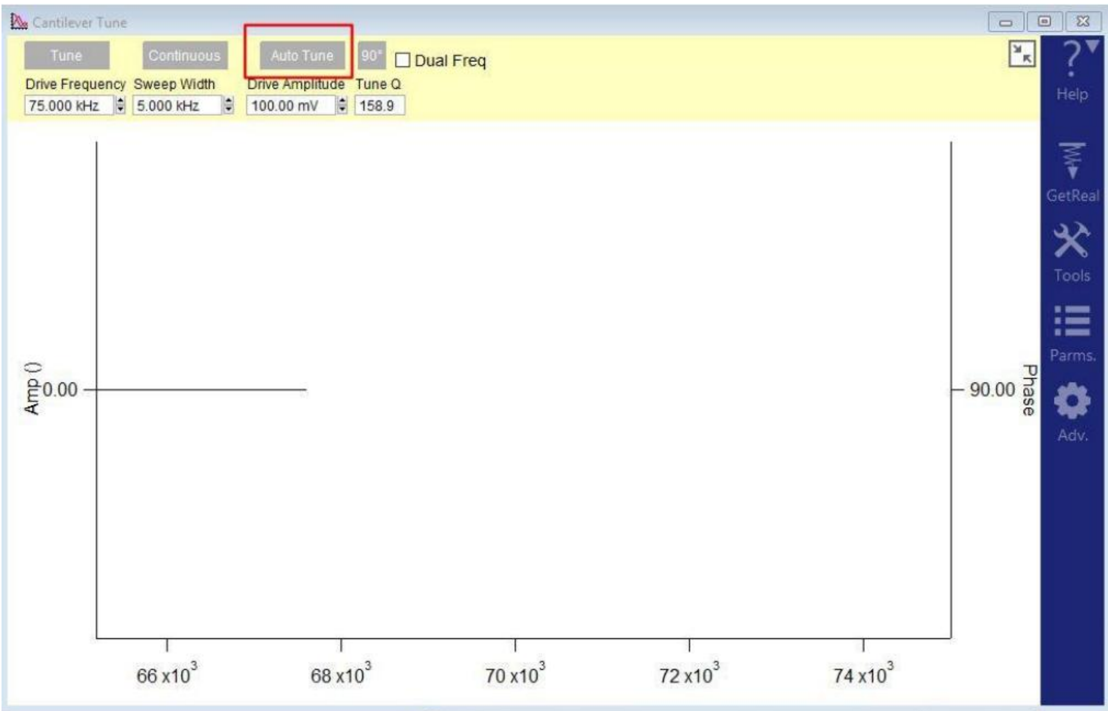


Figure 11

Wait until the peak finding results are observed. The relevant parameters obtained from peak finding (Drive Amplitude and Drive Frequency) will be saved automatically, after which this window can be closed.

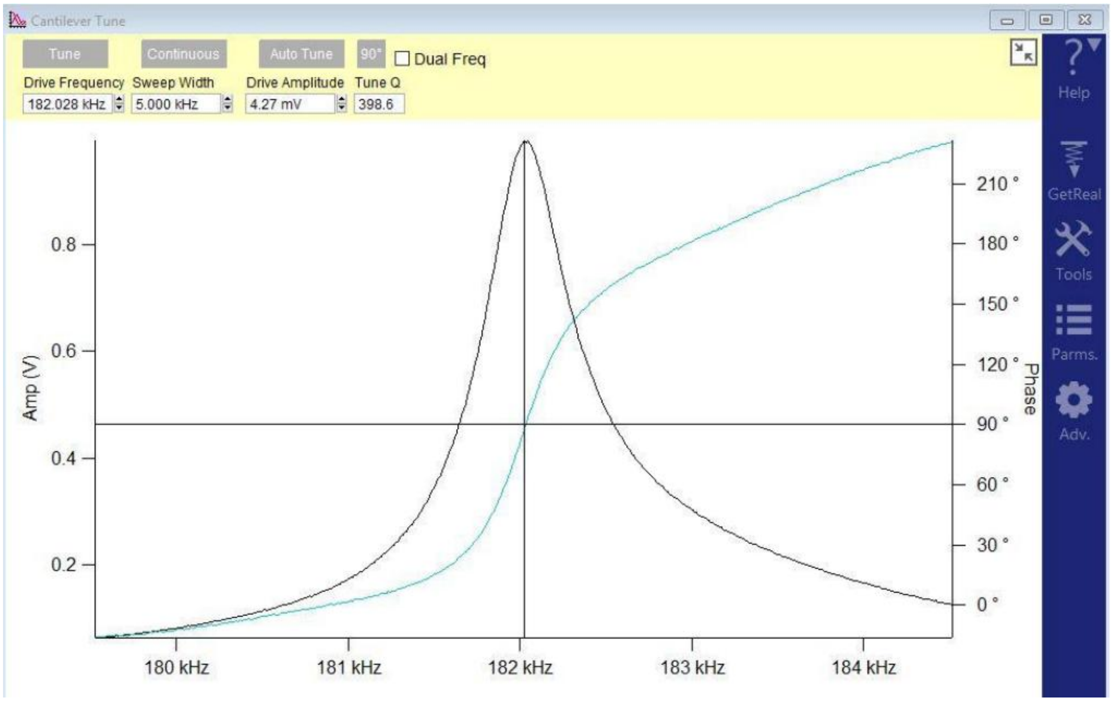


Figure 12

10) Click the "Start Tip Approach" button in the "Engage Panel" window to begin the automatic tip approach. A warning tone will sound upon successful engagement.

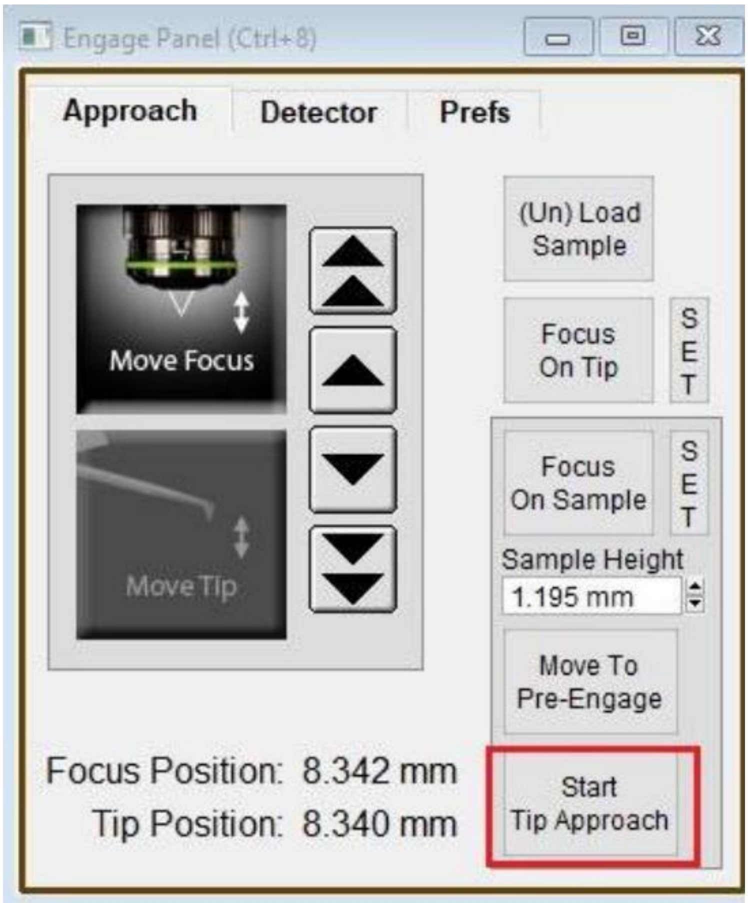


Figure 13

11) Set and optimize the parameters for scanning

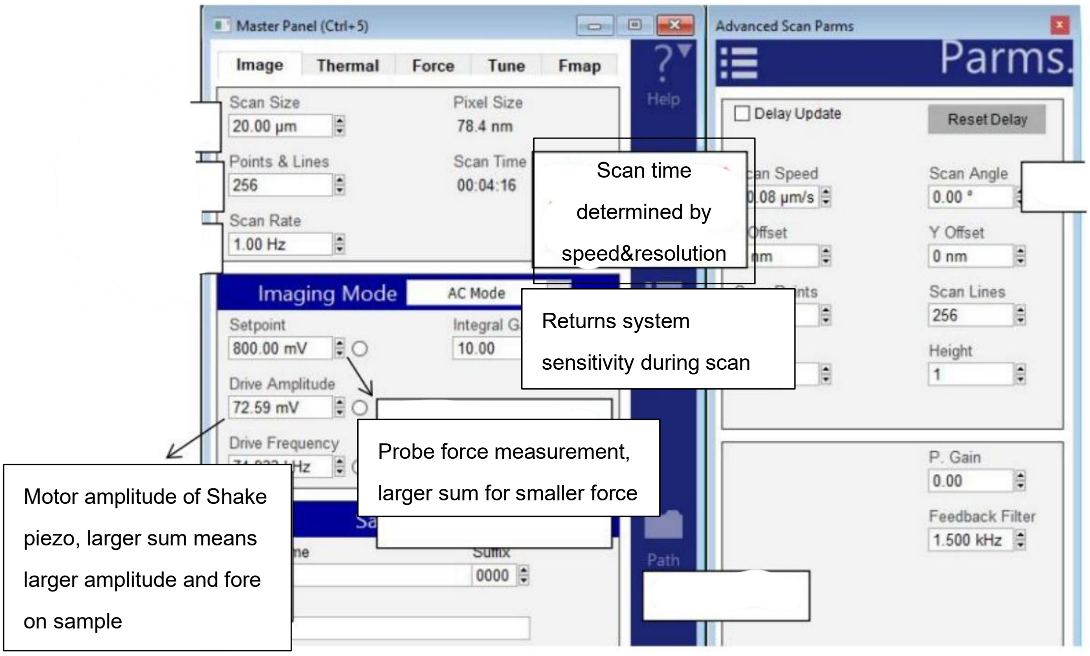


Figure 14

"Scan Size": Defines the lateral dimensions of the scanned image area. The maximum value for the Cypher ES is 30 µm.

"Points & Lines": Specifies the image resolution as (number of points per line) × (number of lines). Please use a multiple of 256.

"Scan Rate": The scanning speed. The default is 2.44 Hz, and it can generally be set up to 40 Hz. For softer samples, a slower scan rate is recommended.

— The above three parameters should be set according to the actual experimental requirements. You can also click "Parms." in the right-hand column to access the "Advanced Scan Parms" interface for more detailed settings based on experimental needs.

"Setpoint": The default is 800 mV. It is recommended to start between 700-800 mV and adjust during scanning. In tapping mode, the ratio of the Setpoint to the cantilever's Free Amplitude (i.e., the Target Amplitude set during the Auto Tune step) reflects the force applied by the probe during scanning. Therefore, the Setpoint value must be set lower than the Free Amplitude value. However, since the Free Amplitude is typically set to 1 V during Auto Tune, it can be approximately understood that a lower Setpoint value applies greater force from the probe, and vice versa. To protect the probe, during scanning, it is recommended to set this value as high as possible while maintaining clear image quality.

"Integral Gain": It is recommended to start at 30 and adjust during scanning. The I Gain value reflects the sensitivity of the system's feedback. To obtain high-quality images, it is recommended to increase this value as much as possible during scanning to improve sensitivity, until just before noise appears in the image.

"Drive Amplitude": This indicates the drive amplitude of the piezoelectric ceramic. If reducing the Setpoint does not yield satisfactory results during scanning, increasing this value will amplify the probe's oscillation. It is recommended to increase the Drive Amplitude and Setpoint in parallel to protect the probe.

— The above three parameters can be adjusted in real time during scanning. They should be tuned based on the alignment of the red and blue lines beneath the Height image.

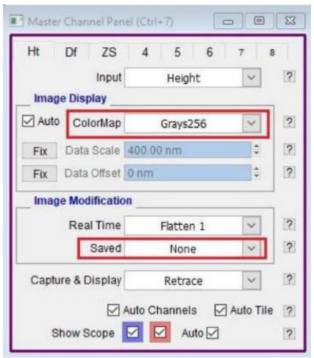


Figure 15

In the top section of the "Master Channel Panel" interface or within the "Input" dropdown menu, select the desired image data channel (Height/Amplitude/Phase/ZSensor) for configuration. "ColorMap": allows selection of image coloration; within its dropdown menu, click "Setup" to access additional color options. In Image Modification, under the "Saved" options, set Height to "None" to preserve the raw data.

12) connection between parameter adjustments and physical consequences remains clear. In the "Master Panel" interface, click "Frame Up" or "Frame Down" to quickly display the various images. Then, finely adjust the Set Point, Integral Gain, and Scan Rate based on the alignment of the red and blue lines beneath the Height Image, making real-time adjustments to obtain the desired high-quality image results. You may also select "Stop" at any time to halt the scanning.

Note: In tapping mode, the AFM detects changes in the cantilever's amplitude. As the probe moves downward, the cantilever's amplitude attenuates accordingly. Engagement is considered successful when the amplitude decays from the Free Amplitude to the Setpoint. Therefore, it can be understood that increasing the Drive Amplitude (resulting in a larger Free Amplitude) or decreasing the Setpoint will both cause the probe to move further downward, meaning the distance between the probe and the sample becomes smaller.

Tips:

1. Before engaging the probe, use the Video interface to preliminarily identify the desired sample location and area for measurement.
2. After starting, check the Z Voltage value in the "Sum and Deflection Meter" interface. A value in the mid-range, around 70-80, is recommended. If it remains at the maximum value (150), it indicates the previous engagement was unsuccessful. Stop the scan ("Stop"), decrease the Setpoint, re-engage the probe, and start again. If it remains at the minimum value (-10), it indicates a problem with the previous surface focus. Stop the scan ("Stop"), click "Move To Pre-Engage", increase the Setpoint, re-engage the probe, and start again.
3. If you need to change the scan area during scanning, stop the scan ("Stop") first, then click "Pre-Engage" before moving the sample stage. Never move the probe directly, as this may damage it!

13) Parameter Adjustment During Scanning

During scanning, observe the red and blue lines in the Height Retrace window. If the red and blue lines completely overlap, it indicates the current scan parameters are set appropriately and no further adjustment is needed. If the lines do not overlap, try the following steps in sequence:

Increase the probe force by decreasing the Setpoint or increasing the Drive Amplitude.

Increase the feedback system sensitivity by increasing the Integral Gain value. Note: Excessively high gain values can cause system oscillation, manifesting as obvious wavy noise in the image.

Reduce the Scan Rate.

If the above steps still fail to make the red and blue lines coincide, consider whether the selected probe is appropriate or if the current probe is contaminated or worn, and try replacing it with a new one.

14) Regarding Attractive and Repulsive Mode Scanning

In tapping mode, the Phase value indicates the type of force between the probe and the sample. A Phase value less than 90° corresponds to repulsive mode interaction, while a Phase value greater than 90° corresponds to attractive mode interaction.

It is generally accepted that attractive mode scanning is less invasive, providing better protection for both the probe and the sample, as the probe does not make "true contact" with the surface. However, this often comes at the cost of reduced image resolution. Conversely, repulsive mode scanning typically yields clearer images but can cause some degree of wear to both the probe and the sample. The choice between attractive or repulsive mode scanning depends on the specific sample properties.

It is crucial to avoid mode hopping, where the probe intermittently switches between attractive and repulsive regimes, characterized by the Phase value oscillating around 90°. The following section explains how to maintain the probe in the more commonly used repulsive mode during scanning.

The ability to achieve and maintain repulsive mode depends on both the probe and the sample characteristics:

Samples that are more adhesive, softer, or have significant electrostatic forces can make it difficult to enter repulsive mode.

Stiffer probes (higher spring constant, k) more readily enter repulsive mode.

Sharper probes more readily enter repulsive mode. Therefore, worn or contaminated probes often struggle to achieve stable repulsive mode operation.

~ Entering Repulsive Mode by Parameter Adjustment During Scanning

Increasing the Drive Amplitude, which consequently increases the probe's free amplitude, makes it easier to enter repulsive mode. Similarly, setting a smaller Setpoint also facilitates entering repulsive mode.

It is important to note that, as discussed previously, the effective force exerted by the probe during scanning is determined by the ratio of the Setpoint to the Free Amplitude. Therefore, when adjusting these two parameters, this ratio should be maintained within an approximate range of 50% to 80%. This is achieved by gradually increasing the Setpoint while simultaneously increasing the Drive Amplitude until the Phase stabilizes within a range below 90°.

5.Data processing and Saving

1) Data Saving.

After completing a scan, the software will automatically save the data. By default, data is saved in a folder named with the current date. It is recommended to select the required data and copy it to your project folder. Storage path: Desktop/AsylumResearch Data/p-[Principal Investigator Name]/[Username]/[Date] (a new folder is created each day).

2) Opening Data

Method 1: Click AFM Analysis in the menu bar, then select Browse Save Data. In the pop-up AR Load Path panel, click the Browse button and select the folder containing the data. The Browse panel will display the files within the selected folder. To change the directory, click the Change Directory button within the Browse panel.

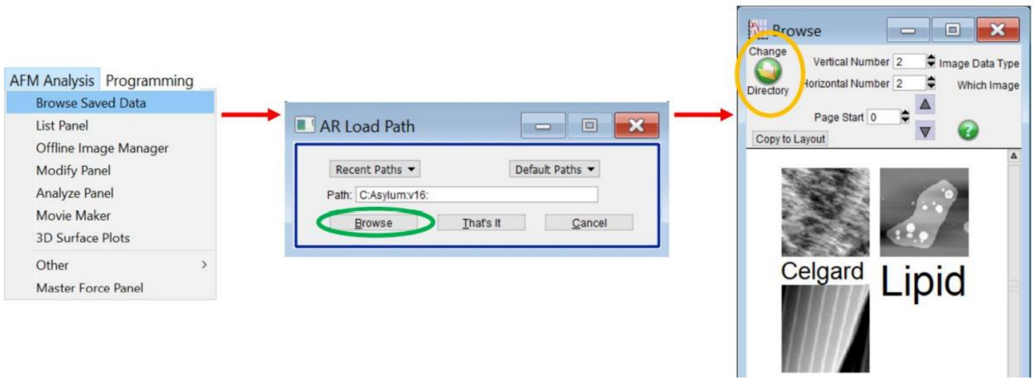


Figure 16

Method 2: In the Browse panel, double-click the icon to open the data. To open an existing .ibw data file directly, first open the AFM software and then drag the .ibw file into it. It is best not to open the data by double-clicking the .ibw file.

3) Data post-processing.

Click the M button above the image to open the Modify panel.

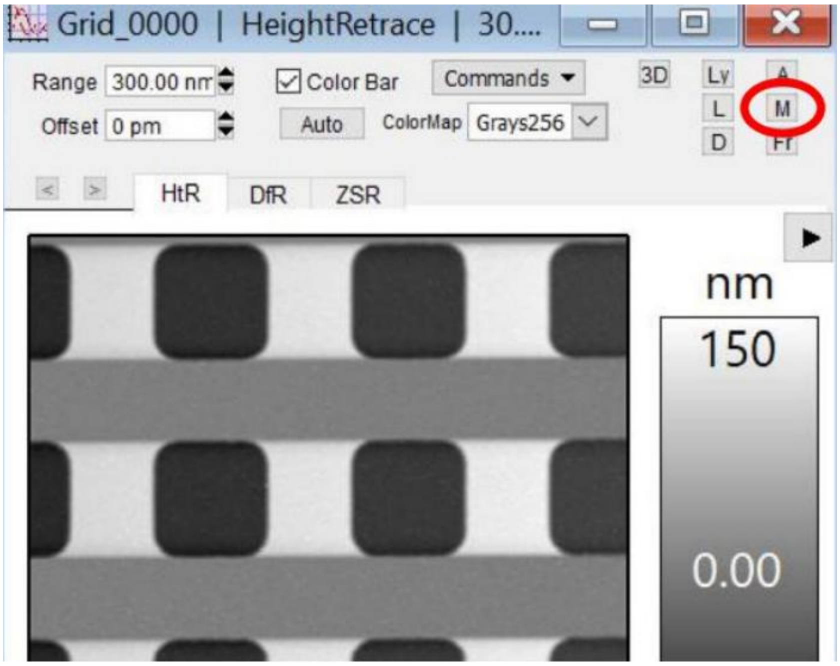


Figure 17

The most frequently used functions in the Modify panel are Flatten and Planefit. They are used to level the topography images (the Height map and the Z Sensor map). Flatten and Planefit are generally not applied to images other than topography maps.

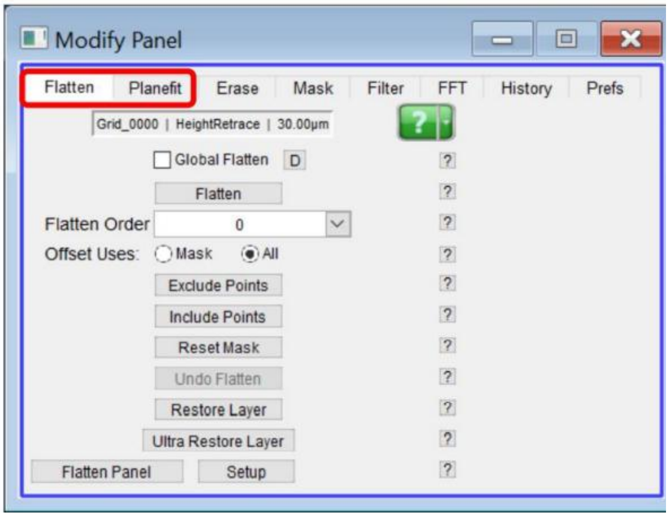


Figure 18

Planefit is used to correct the overall tilt of a topography image and eliminate its inclination. Select the image and channel to be processed, choose a Planefit Order of 1, and click XY (indicating the Planefit is applied in both the X and Y directions of the image). A 1st-order fit and Histrogram Planefit are the most commonly used. A 0th-order fit only removes the offset and is less useful. A 2nd-order fit may alter the shape and should be used with caution. Please select the appropriate option based on the resulting image's effectiveness in tilt elimination.

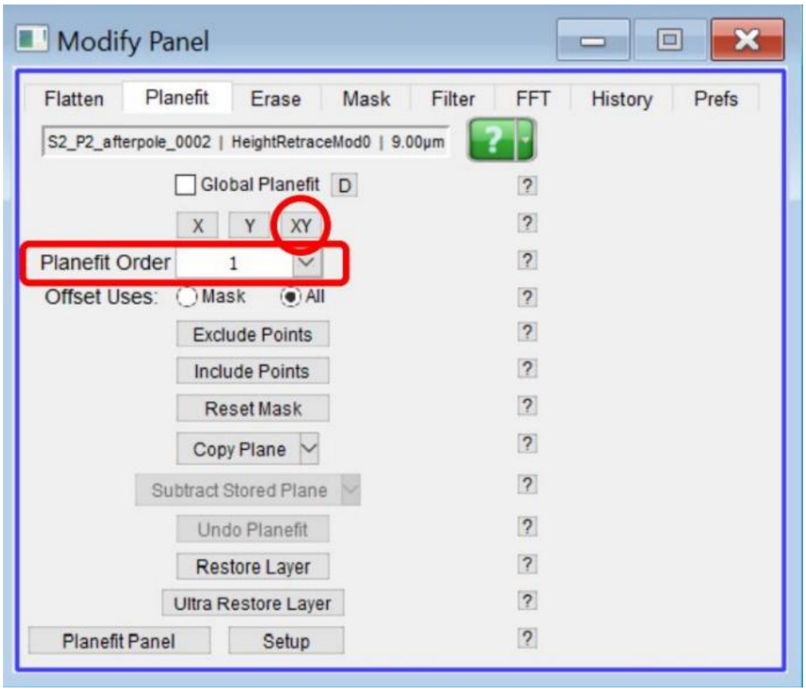


Figure 19

Flatten aligns each individual AFM scan line. It performs line-by-line leveling and alignment, similar to applying a Planefit to each line separately. Select the image and channel to be processed, choose an appropriate Flatten Order, and click Flatten. If the result is unsatisfactory, click Undo Flatten. A 1st-order fit and Histrogram Flatten are the most commonly used. A 0th-order Flatten can align the lines and is also sometimes applied. A 2nd-order fit may alter the shape and should be used with caution.

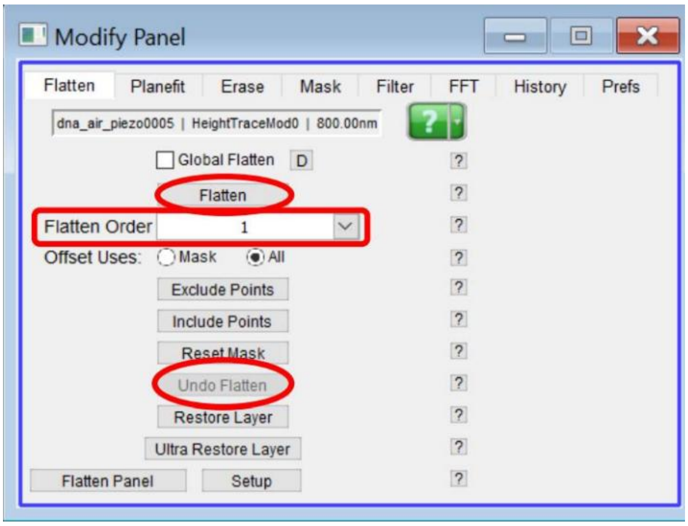


Figure 20

All the aforementioned image processing operations (Flatten, Planefit) only affect the data in memory; the data saved on the hard disk remains unchanged. When the software is restarted and the data is reopened, it will appear in its original, unprocessed state. To save the processed image, you can either select "Save

Image" from the Commands dropdown menu (which overwrites the original data) or choose "Save As..." (which saves the data under a new filename).

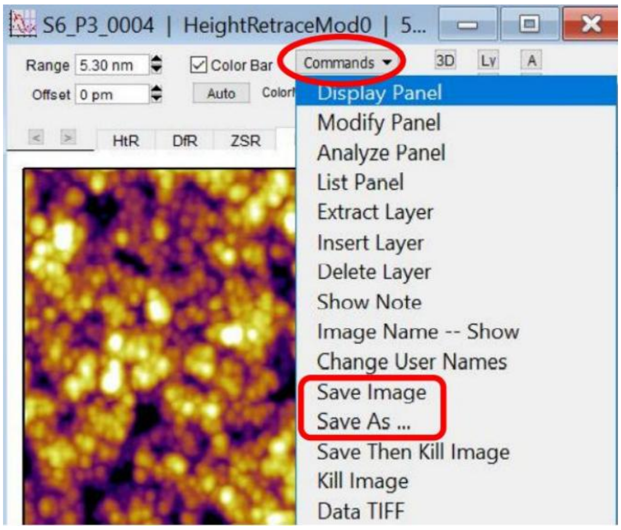


Figure 21

After performing post-processing (Flatten, Planefit) on an image, the original data can be restored by clicking either "Restore Layer" or "UltraRestore Layer", so there is no need to worry about being unable to revert after saving changes.

4) Image color and contrast adjustment:

The color map can be selected from the ColorMap dropdown menu within the data. Clicking "Auto" will automatically adjust the color display, while "Range / Offset" can be used to manually adjust the color representation.

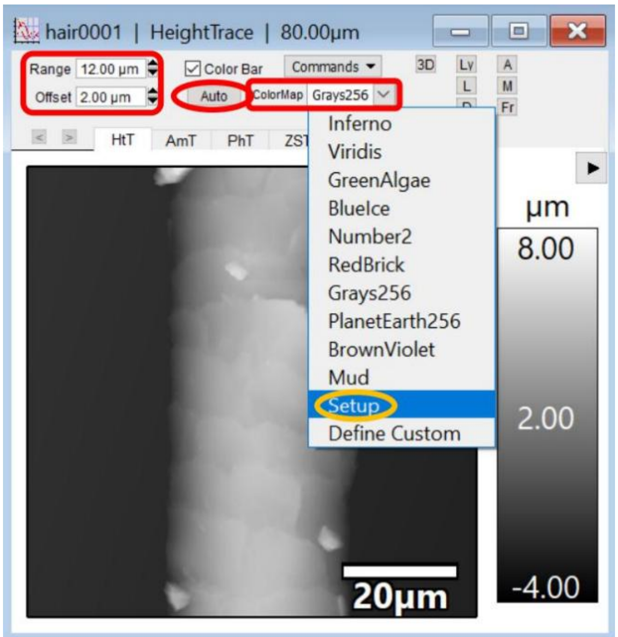


Figure 22

5) Exporting images or data.

Select the target image, navigate to the menu bar and select File → Save Graphics. In the Format options, choose the desired export format (TIFF format is generally recommended). Click Do It, then specify the export file name and path.

Click the Ly button above the image to copy the image to the Layout (which functions similarly to a clipboard). In addition to images, elements such as cross-sectional lines, histograms, and Masks can also be saved to the Layout. The Layout can be exported as an image or a PDF report via the menu bar by selecting File → Save Graphics or Print Layout. Elements within the Layout can also be copied and pasted directly into Office software.

An AFM image is fundamentally a data table, and the underlying data can be exported. From the Commands dropdown menu on the data image, select ASCII Export to save the data in TXT format.

For topography images (Height and Z Sensor maps), the software can generate 3D representations. Click the 3D button above the image to create the 3D view. Use the left mouse button to rotate the model and the right mouse button to adjust the lighting angle. 3D images cannot be exported using the Save Graphics function; instead, use Edit → Export Graphics from the menu bar to export them.

6) Data Analysis.

The data analysis functions are primarily located in the Analyze panel. Click the A button above the image to open the Analyze panel.

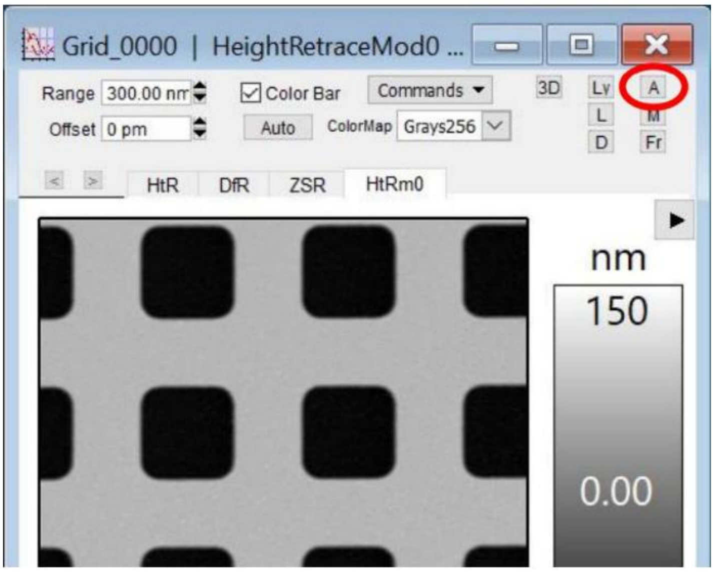


Figure 23

Commonly used data analysis tools include Roughness (for statistical values like roughness) and Section (for cross-sectional analysis). The Roughness page provides statistical information for the topography image. Sdev [Rq] and Adev [Ra] are commonly used to describe roughness; Max/Min: the maximum/minimum values among the data points; Avg: the average value of the data points; Area: the surface area of the sample; Area%: the percentage by which the sample's surface area exceeds the scanned area; Volume: the sample volume, calculated relative to the Z=0 plane.

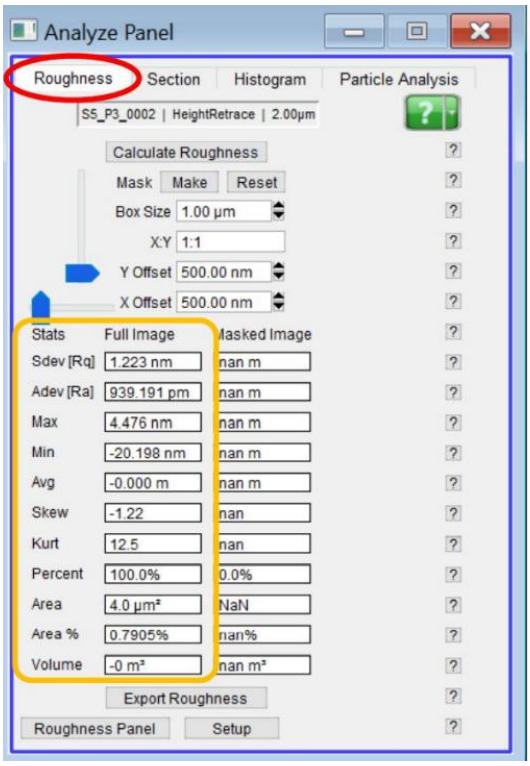


Figure 24

Section is used to extract data along a line drawn across the image. For topography images, this functions like taking a cross-sectional profile. Click Draw to plot a line on the image, which will generate the cross-sectional profile. Drag the two cursors to adjust the line's position; click Clear to remove the section line. There are two Modes: Line (straight) and Free Hand (freeform trajectory). In Line mode, checking the Full box makes the section line span the entire image. Also in Line mode, checking the Angle box and entering a value constrains the line to a specific angle. Increasing the Width broadens the section line, providing an averaging effect that can yield smoother data.

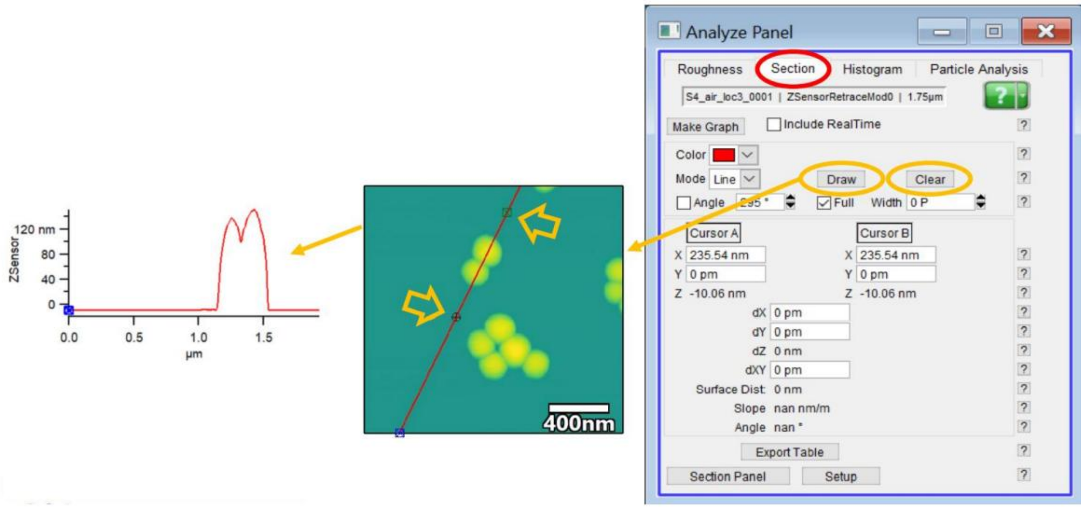


Figure 25

Below the section line window is the Cursor toolbar. The Cursor toolbar can be hidden or displayed using the keyboard shortcut Ctrl+I. By dragging the Cursor onto the curve with the mouse, the X and Y values of that point can be obtained. Using two Cursors, the differences in their X and Y values can also be determined. Clicking Edit in the section line window will bring up a data table, which can be saved as a CSV file or copied to other software.

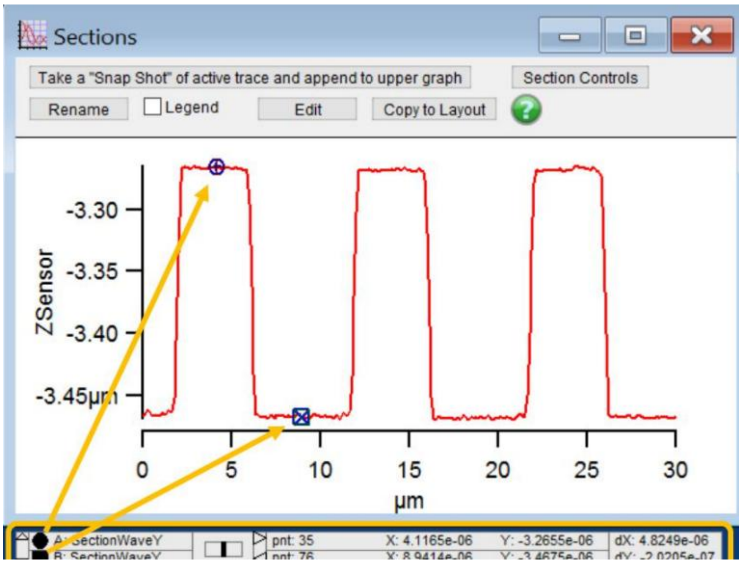


Figure 26

6. End Of Experiment

After scanning is complete, click (Un)Load Sample in the Engage Panel to retract the probe. Remove the probe and the sample, turn off the laser, and close the software. Exit the large instrument system, restore the workspace and sample preparation area, and record the usage in the experiment logbook. Do not shut down the controller or the computer.