

Atomic Force Microscopy on the WITec Alpha 3000 Microscope



Introduction

In atomic force microscopy (AFM), an ultra sharp tip on the end of a long cantilever is passed over the surface of the sample. A laser beam is bounced off the back of the tip and onto a four quadrant detector. The movement of the laser spot on the detector is used to measure the deflection of the cantilever by features on the surface of the sample. This allows for the measurement of very small features down to the nanometer scale. Additionally, phase images in tapping mode can be used to visualize regions of different materials on the surface.

Appropriate Samples and Sample Prep

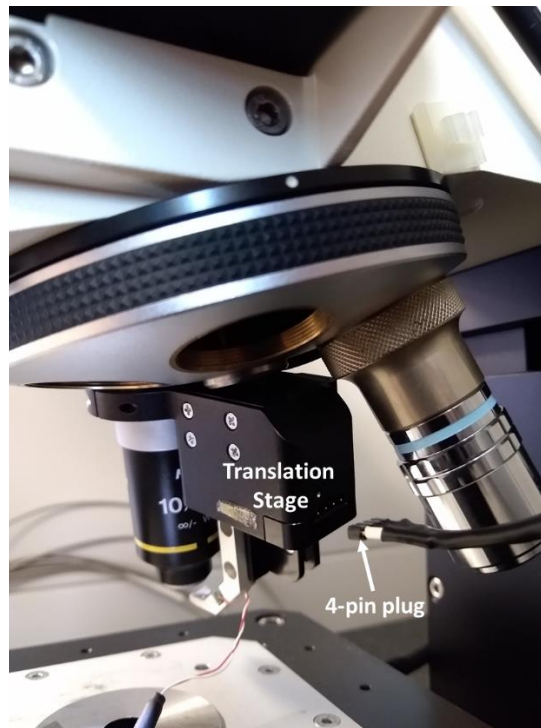
You can attempt to image anything that can fit on the stage. Samples must be flat since the microscope cannot measure features taller than a few microns. **Samples must be nonmagnetic**, since there is a strong magnet on the bottom of the cantilever holder that gets very close to the sample surface. The maximum area that can be imaged with the microscope is $20\mu\text{m}$ by $20\mu\text{m}$.

Stuff to Buy

Description	Vendor	Part no.
Magnetic washers for mounting probes	McMaster-Carr	91166A190
Adhesive tabs	Ted Pella	16079
10mm mica disks	Ted Pella	50
Tweezers for handling probes	Ted Pella	5374-NM

Getting Started

1. Turn on the power strip to the left of the cabinet to turn on the microscope.
2. Open the cabinet using the foot pedal.
3. Rotate the objective mount so that you can plug the 4-pin connector into the small translation stage behind the 10x objective. Make sure the white pin on the plug lines up with the white dot on the stage (see pic below).



4. Rotate the 10x objective into place.
5. Put your sample on the stage.

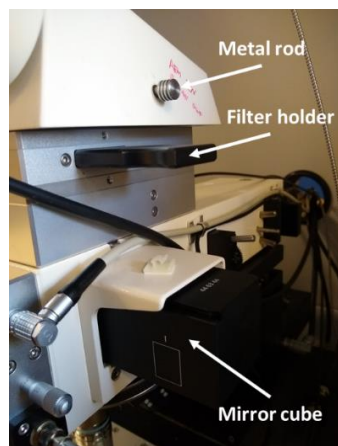


Warning: Your sample must be nonmagnetic so that it does not attract the magnet on the bottom of the cantilever holder. If the sample and holder collide, the cantilever can be broken!

6. Log in to the PC and start *WITec Control 1.60* on the desktop.
 - a. Username: labuser
 - b. Password: Sample123!

Preparation for Imaging

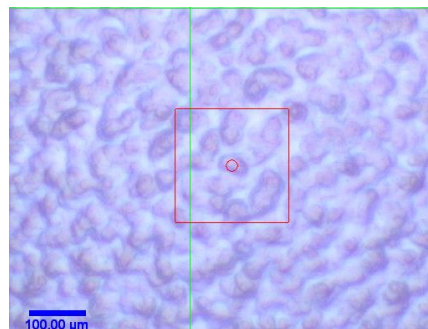
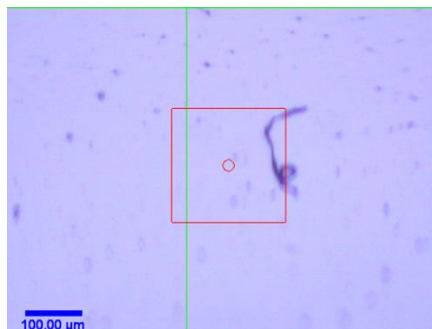
1. On the menu bar click *Configurations* → *AFM* → *AFM AC*
2. To see an image of the sample on the monitor, you need to adjust the metal rod, mirror cube, and filter holder from the right side of the microscope (see pic below).
 - a. The metal rod has two positions: in and out. To view the sample on the monitor it must be pushed in.
 - b. The mirror cube has three positions labeled I, II, and III. While inserting the cube from the right side of the microscope, the first position you land on is I followed by II then III. To view the sample on the monitor, the cube must be in position I, which puts a dichroic mirror in place to illuminate the sample with white light.
 - c. The filter holder has two positions, 1 and 2. When inserting the filter holder from the right side of the microscope, you will first land on position 1 which does not contain a filter. This is the position you want to be in while viewing your sample on the monitor. If you push the filter holder in further it will move to position two, which contains the laser line filter. If this filter is in place the image on the monitor will be very dark and have a blue/purple color.



3. Use the remote control to raise the microscope objective
 - a. Repeatedly press one of the arrows on the remote control until *RC: Microscope Z* shows below the menu bar



- b. Rotate the knob on the remote control all the way counterclockwise, then rotate it clockwise to the desired speed. Speed setting can be viewed in the *Graphic Control* window.
 - c. Press and hold *+Z* button on the remote control to raise the microscope objective
4. In the *Control* window, expand the settings for *Illumination*. Enter a value for *Illumination* (0 to 100) and turn the illumination on. You should see a white spot on the surface of your sample. If you do not see a spot even at high settings, the mirror cube probably needs to be moved to position I
5. Use the remote control with a speed around 100 to lower the microscope objective. You will need to reduce the illumination intensity as you lower the objective. As you come down the first thing you will see is the worm. Next you will see the clouds. Then your sample surface will come into focus. Use a lower speed and adjust the illumination to get a good focus on your sample.

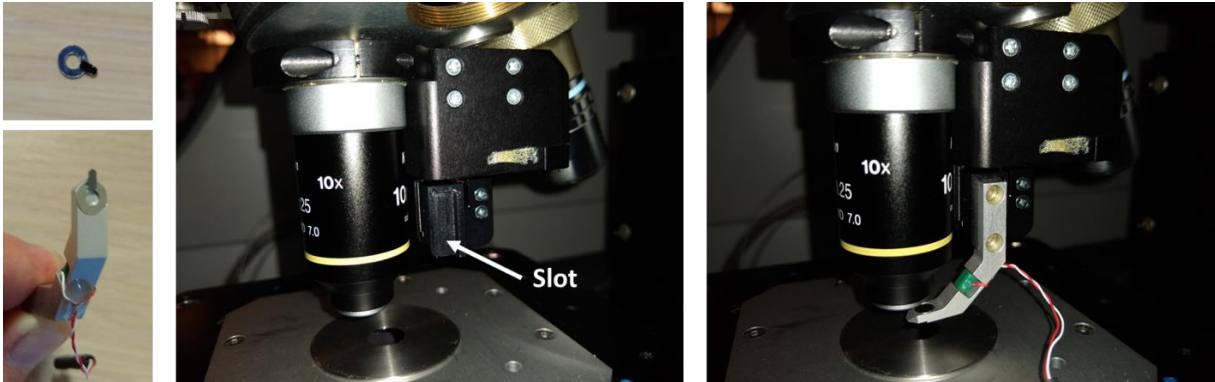


6. Use the micrometers on the sample stage to find the area of the sample that you want to image. Place your target under the red circle in the center of the image.
7. In the *Control* window, expand the settings for *Microscope Z Control*. Click *Set Zero* to set the *Microscope Z* value in the *Status* window to zero.
8. Use the remote control to raise the objective back up until you see the worm.

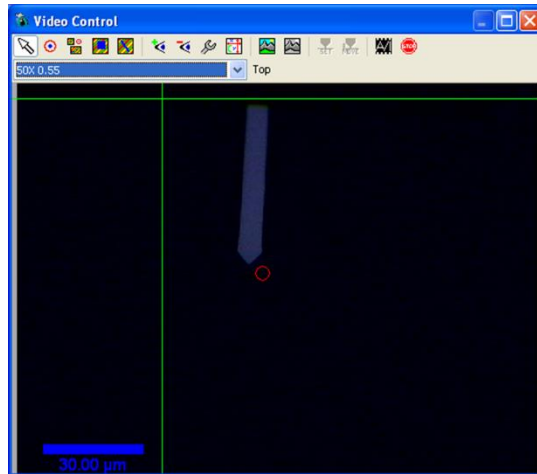
9. Glue your probe to a washer using a small amount of nail polish as shown below. Put the washer on the bottom of the cantilever holder as shown, then put the cantilever holder into the slot behind the 10x objective as shown.



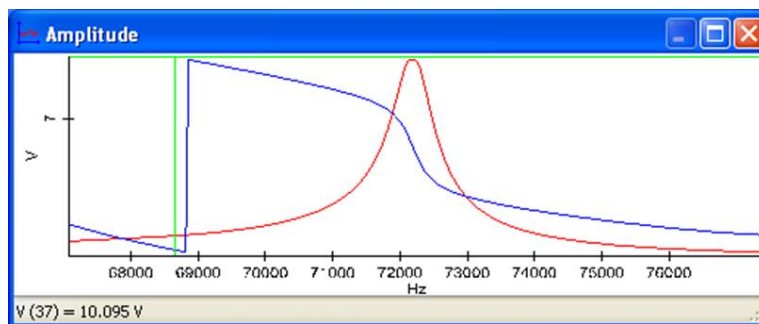
Warning: Grasp the cantilever holder tightly when inserting it into the magnetic slot behind the 10x objective. Failure to do so could result in the holder leaving your fingers and snapping into the slot, potentially breaking the cantilever.



10. Connect the two-wire connector coming from the cantilever holder to the mating connector laying on the right side of the microscope.
11. Physically move the translation stage behind the 10x objective with your hands until the cantilever is visible on the video screen.
12. Use the remote control on the *Cantilever Position* setting to bring the cantilever into focus and center the tip on the video screen.
13. Use the remote control to lower the objective until it is about 180 μ m above the surface as indicated in the *Status* window.
14. In the *Control* window, expand the setting under *Adjustment* and click *Start Adjustment*. Follow the instructions given in the *Message* window. After each step, click *Next Step* in the *Control* window.
 - a. The first step involves positioning the cantilever tip underneath the laser beam. The invisible laser spot is a little bit up and to the left of the center of the video screen near the red circle. Use the remote control to sweep the cantilever left and right (x direction) until you see the sum signal maximize in the *Quadrant* window. Then move the cantilever up (+y direction) until the sum signal starts to decrease. Move the cantilever down (-y direction) just until the sum signal is maximized. The laser is now focused on the cantilever just above the tip.



- b. Step two involves centering the reflected laser beam on the four quadrant detector. Use the T-B (top to bottom) and L-R (left to right) knobs on the front of the microscope to zero the *T-B* and *L-R* voltages shown in the *Status* window. This will also move the red dot to the center of the *Quadrant* window.
- c. Step three is determining the drive amplitude and frequency for the cantilever. For a *Driving Amplitude*, enter 0.5 V. In the *Control* window, under *Frequency Sweep*, enter the *Initial Frequency* and *Final Frequency* corresponding to a range suitable for your probe, then click *Next Step*. View the tuning results to verify that they are satisfactory. If the frequency peak flat-tops, click *Repeat Last Step* and use a lower value for *Driving Amplitude*.





- d. In step four confirm the *Driving Amplitude* determined in the previous step.
 - e. In step five enter the *Setpoint* as 80% of the suggested value.
 - f. In steps six and seven, enter 5V for proportional gain and integral gain.
15. In the *Control* window, under *Microscope Z Control*, click *Start Approach*.

Acquiring Images

1. In the *Control* window, under *Image Scan*:
 - a. Under *Geometry*:
 - i. Enter the size of the area to be scanned and the Time/Line (scan rate).
 - ii. Set *Gamma* to 90.
 - b. Under *Scan Details* → *Scan Mode*, select *Single* to acquire a single image, or *Continuous* to rescan the same area continuously.
 - c. Enter other parameters such as points per line, number of lines, and Time/Line (scan rate).
 - d. Also enter a *Sample Name* and *Number* under *Data Labeling*.
2. In the *Control* window → *Image Scan*, click *Start Scan* to begin data acquisition. Completed images will appear in the *Project Manager* window.
3. Image quality can be adjusted by varying the setpoint, proportional gain and integral gain under *Feedback Settings* as well as *Time/Line* under *Image Scan*.


Data Analysis

The *Project Manager* window shows all of the data files associated with a project. This includes raw data acquired with the microscope as well as processed data. Images are represented by . Graph data such as cross sections are shown as graph files represented by . You can double click any file in the *Project Manager* window to open it.

1. Exporting data:
 - a. To export raw image data, right click the image file in the *Project Manager* window and select *Export*
 - b. To export any open image as a picture, right click the image and select *Export* → *Bitmap to File*.
 - c. To export a picture of a cross section graph, right click the graph and select *Export* → *Bitmap to File*
 - d. To export the raw data (XY pairs) of a cross section graph, right click the spectrum and select *Export* → *ASCII to File*

2. Flattening topography data using N-order surface subtraction:

- a. In the *Project Manager* window, drag a topography image to *Image N-Order Surface*

Subtraction  in the *Drop Actions* window.

- b. In the *Image Tools* window, select either *Draw Point*, *Draw Square*, or *Draw Circle*



- c. In the *Image Surface Subtraction (DrawField)* window, while holding down the shift key, either highlight the features in your image that ARE NOT part of the substrate/background, or if it is easier, highlight large areas of substrate/background.

- d. If you highlighted background instead of features in the previous step, click *Invert draw field*




in the *Image Tools* window.

- e. In the *Image Surface Subtraction* window, change the *Order* and click back in the *Image Surface Subtraction (Preview)* window to observe the effect. When you are satisfied with the results, click *Extract* to create a new image in the *Project Manager* window.

- f. Close the *Image Surface Subtraction* window.

3. Measure heights, depths, and distances between features using cross sections:

- a. In the *Project Manager* window, drag a topography image to *Image Cross Section*  in the *Drop Actions* window.

- b. In the *Image Cross Section* window, check *Listen Both*, and check *Auto* next to *Number of Sampling Points*.

- c. In the *Image Tools* window, click *Mark Line* 

- d. In the *Image Cross Section* image window, click and drag to draw a line in the image where you want to create a cross section. You will see the cross section data appear in the *Image Cross Section* graph window.

- e. If you want to save the image with the cross section line shown, right click it and select *Export* → *Bitmap to File*

- f. Click *Extract All* in the *Image Cross Section* window to create a new graph file in the *Project Manager* window.

g. Close the *Image Cross Section* window.

When you are finished

1. If scanning, in the *Control Window* under *Image Scan* click *Stop*.
2. Under *Microscope Z Control*, click *Retract Tip* to raise the tip away from the surface.
3. Use the remote control to move the tip further away from the sample surface.
4. Disconnect the two wire connector from the cantilever holder and remove the cantilever holder from the microscope.
5. Unplug the four pin connector from the translation stage behind the 10x objective.
6. Remove your sample from the stage.
7. Remove your probe from the cantilever holder and put the cantilever holder back inside the cabinet.
8. Close the *WITec Control* software. There is a wait step that must pass before the software closes.



Warning: Do not skip the wait period or you risk damaging the spectral camera!

9. Close the cabinet using the foot pedal.
10. After the waiting period has passed and the software is fully closed, turn off the power strip to the left of the cabinet.
11. Log out of the PC.