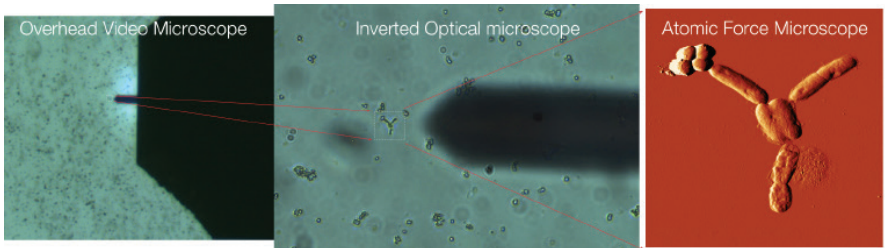


AFM WORKSHOP™

LS-AFM Users Guide V 1.1



Definitions and Symbols

The following terms and symbols are used in this document and also appear on the product where safety-related issues occur.

General Warning or Caution



The exclamation symbol may appear in warning and caution tables in this document. This symbol designates an area where personal injury or damage to the equipment is possible.

European Union CE Mark



The presence of the CE Mark on AFM Workshop equipment means that it has been designed, tested and certified as complying with all applicable European Union (CE) regulations and recommendations.

Warnings/Cautions/Notes

The following are definitions of the warnings, cautions and notes that may be used in this manual to call attention to important information regarding personal safety, safety and preservation of the equipment, or important tips.

WARNING

Situation has the potential to cause bodily harm or death.

CAUTION

Situation has the potential to cause damage to property or equipment.

NOTE

Additional information the user or operator should consider.

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1. Introduction to Atomic Force Microscopy

In an AFM (atomic force microscope), a probe is scanned over a surface and the motion of the probe is monitored to create a three-dimensional image of the surface. These unique instruments are capable of measuring high-resolution images in both ambient air and liquids, with surface features of only a few nanometers in size.

The three-dimensional motion of the sample (or probe) is generated by piezoelectric ceramics. These sensitive ceramics allow motions as small as a fraction of a nanometer. Typically, the sample (or probe) is moved in a raster pattern as the probe glides across the surface.

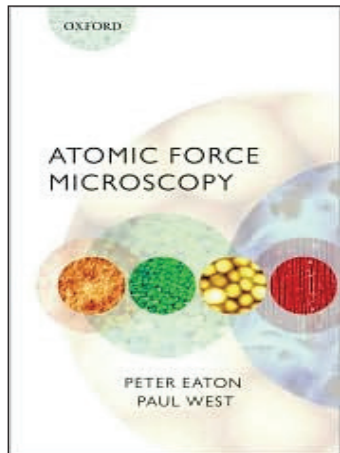
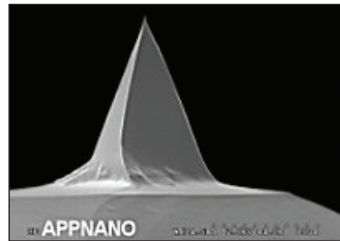
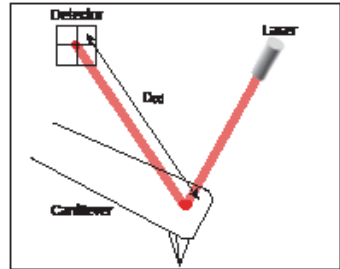
A light lever sensor is used for controlling the force of the probe on the surface while the sample is scanned. The light lever reflects a laser beam off the surface of a cantilever into a photo-detector. As the probe interacts with a surface, the cantilever deflects, and this motion is sensed by the photo-detector.

With this light lever, forces as small as a pico-newton are possible. With such small forces, very small probes may be used. With micro-machining methods, probes can have diameters of only a few nanometers.

The light lever can be made more sensitive by vibrating the cantilever with a small piezoelectric ceramic and modulating the light. When the vibrating probe interacts with the surface, the amplitude of vibration may be monitored and used to control the probe's force on the surface.

Modern atomic force microscopes include not only a probe and piezoelectric scanner, but additional hardware for bringing the probe rapidly into the proximity of a surface. A video optical microscope is very helpful for operating an AFM. The video microscope helps with aligning the light lever and probe approach, and for finding features for scanning.

For an in-depth description of AFM instrumentation, we recommend the book *Atomic Force Microscopy* by Peter Eaton and Paul West. This book provides a complete theoretical, as well as practical explanation for the design and application of AFMs.



2. Introduction to the LS-AFM

When fully assembled, the LS-AFM comprises four subunits. They are the control computer, the Ebox, the stage, and the video optical microscope.

2.1 Computer

The control computer is a standard IBM/PC-type computer with a Microsoft Windows operating system. There are two programs required to operate the LS-AFM: the AFM control software and the software for the color video camera.



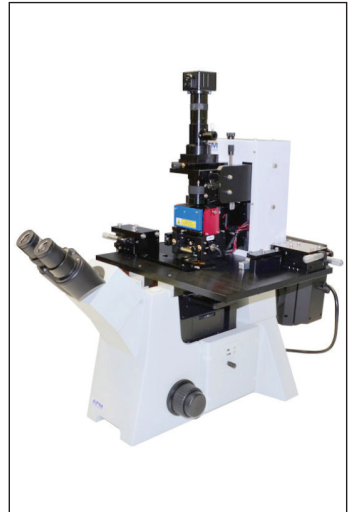
Standard control computer

2.2 Stage

Samples are held and scanned on the AFM stage. On the upright inside the stage is a linear translator which moves both the light lever force sensor and the piezoelectric scanner in a vertical direction. The stage also includes a base plate fitted with precision XY translators.

Optimal images are measured with the AFM stage if it is in a vibration- and acoustic-free environment. If necessary, a vibration and acoustic isolation system should be used. Appendix A provides more information on the best location for the AFM stage.

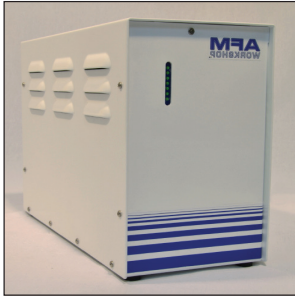
On the back cover of the stage is a modes connector. Signals required for implementing additional modes such as conductive AFM, STM, and EFM are provided. Additional information on the cable configuration is provided in Appendix A.



LS-AFM stage combined with the video microscope. The key components in the stage are the light lever force sensor, piezoelectric scanner, XY positioner and Z motor.

2.3 Ebox

The Ebox sends and receives signals from the computer through a single USB cable. Electronic signals are then sent to the stage through a 60-pin ribbon cable. Additionally, a grounding cable is connected between the stage and the Ebox. All cables are connected at the rear of the Ebox. Besides the cable to the stage, there is a plug for an auxiliary 50-pin cable that gives access to the Ebox's internal electronic signals.



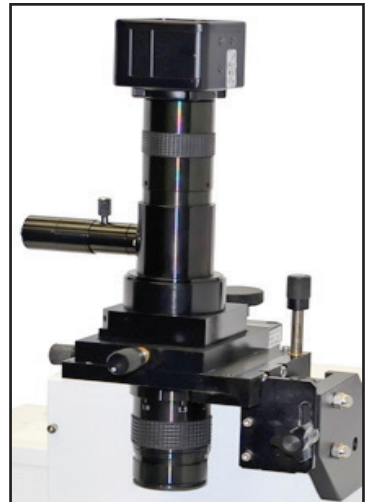
Front and back views of the LS-AFM Ebox showing indicator lights and connectors for ribbon cables and USB

2.4.1. Top View Video Optical Microscope

Aligning the light lever force sensor is greatly facilitated by the aid of the video optical microscope. The video microscope can help locate features on a surface for scanning. With the aid of the video microscope, tip approach can be undertaken much more rapidly. Images from the video microscope are displayed on the computer's video monitor.

2.4.2. Inverted Optical Microscope

The inverted optical microscope, below the AFM, is used for high resolution optical viewing of transparent samples.



3. Software

The TT-2 AFM includes three separate software modules in the AFM Installation files: AFM Workshop Acquisition Software, Video Microscope Software, and Gwyddion Image Analysis Software. This installation guide is targeted towards a computer running Windows OS.

3.1 Installation Procedure



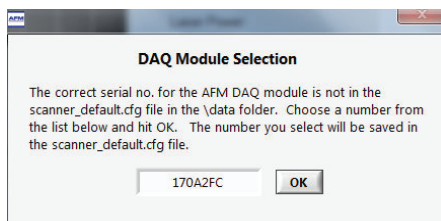
NOTE: If the target computer has a previous version of LabVIEW installed, it is imperative that it first be completely removed.

Locate the provided AFM installation files for the USB camera, Gwyddion, and AFM software and follow the prompts. The user will be asked to input his/her scanner size, so that the setup procedure will copy the files that correlate with that scanner size onto the desktop. Reboot when directed. All instructions can be found in the readme file.



NOTE: A Windows Security pop-up will appear when installing the Video Microscope driver that states that Windows cannot verify the publisher of the driver software. Choose the option to “Install this driver software anyway.”

Upon initializing the AFM-View software, the user will be asked to confirm the serial number of the National Instruments DAQ card in the Ebox. The software will display this pop-up every time the user plugs in a different Ebox.



3.1.1 Gwyddion Image Analysis Software

Gwyddion is open-source software and the latest version of this image-analysis software is available on the Internet at: <http://gwyddion.net/>. The functions of the Gwyddion image analysis software are:

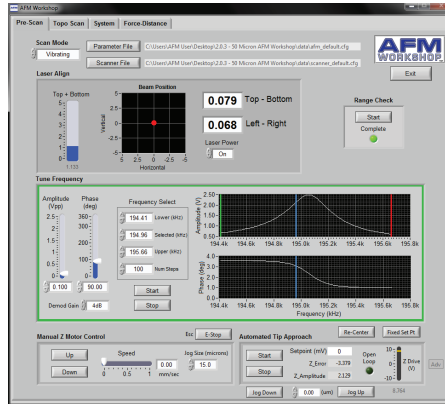
1. Processes such as leveling, deglitching, and smoothing which alter the images.
2. Display functions which change how the data is viewed, including 2-D, 3-D, light shading, and color mapping.
3. Analysis options that are used for obtaining measurements from images, such as line profiling and histogram analysis.

3.2 AFM-View Software

Once launched, the AFM-View software has four screens that can be viewed by pressing the tabs at the top right-hand side of the screen. The first tab is for the Pre-Scan window (section 3.2.1.) and the second tab is for the Scan window (section 3.2.2.). These two windows are all that are needed for measuring AFM images. The third tab labeled System (section 3.2.3.) is used for several other functions, such as measuring the Z noise floor and XYZ scanner calibration. There is a fourth tab that, when activated, permits force-distance curve measurements (section 3.2.4.).

3.2.1 Pre-Scan Window

The Pre-Scan window contains all of the functions that require adjustment before an image is measured. In this window, when a function is being used it appears within a green frame. When the green frame is activated, no other functions can be performed at this time.



3.2.1.1 Files

Two files are required to operate the AFM-View software. The Parameter File contains parameters that are used to operate the microscope. The Scanner File contains calibration parameters for a specific scanner. Upon launching the AFM-View software, default files are loaded into the software. Changing the files used by the program is possible with the File buttons. These files may be edited to change parameters with a text editor such as Notepad. Appendix B lists the contents of both the configuration and scanner files.

Parameter File	C:\Users\AFM User\Desktop\2.0.3 - 50 Micron AFM Workshop\data\afm_default.cfg
Scanner File	C:\Users\AFM User\Desktop\2.0.3 - 50 Micron AFM Workshop\data\scanner_default.cfg

3.2.1.2 Modes

There are two scanning modes: Vibrating mode and Non-Vibrating (contact) mode. The modes are selected with the Scan Mode button. When Vibrating mode is selected, the frequency sweep window is activated. When Non-Vibrating mode is selected, the frequency sweep window is deactivated.

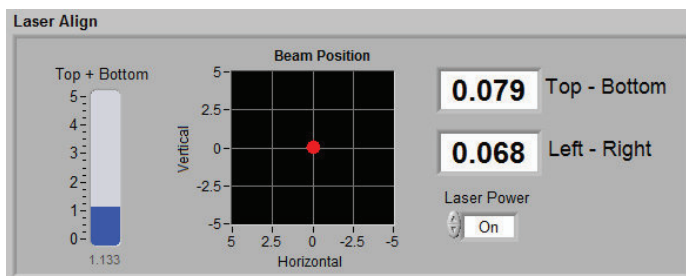


3.2.1.3 Laser Align

The position of the laser on the four-quadrant photo-detector is presented numerically and visually in the Laser Align window. These two indicators are both updated in real time and are used for aligning the light lever. There is a switch for turning the laser On and Off. This switch will automatically turn the laser off after the system has been inactive for a certain length of time.



NOTE: After the TT-2 AFM light lever is set up for the first time (see Appendix A.6), the thumb screws used for laser alignment should not need to be turned more than a few turns.



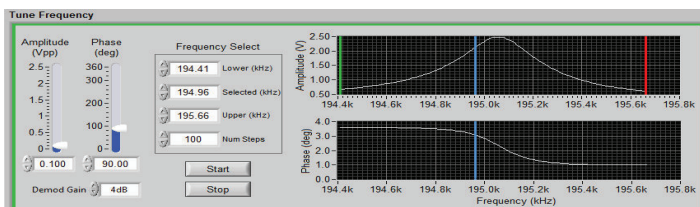
3.2.1.4 Tune Frequency

The Tune Frequency window is used for selecting the optimal conditions for producing vibrating-mode images. There are two oscilloscope windows: the upper window shows the amplitude of the probe's vibration, while the lower window shows the phase between the drive frequency and the measured frequency. The variable controls are:

1. *Amplitude* and *Phase* sliders: Adjusting the amplitude scroll bar will alter the driving amplitude of the probe's vibration. Adjusting the phase scroll bar alters the phase degree. For optimal scanning, the amplitude should not exceed 2.5V or the resonance will become saturated (see section 5.4).
2. *Lower*, *Selected*, and *Upper Frequency*: The lower, selected, and upper frequencies correspond to green, blue, and red vertical bars in the oscilloscope windows, respectively.

When trying to find the resonance peak of a new probe, it is advised to set the lower and upper frequencies to the boundaries of the frequency of the probe itself, which should be located on the probe box. The selected frequency should fall just to the left of the resonance peak and on the steepest part of the slope of the phase curve, owing to attractive and repulsive forces at work on the cantilever's oscillation.

3. **Start and Stop:** These buttons can be used to start and stop frequency sweeps.
4. **Demod Gain:** This function adjusts the gain used when driving the tickler piezo. 4dB is a good starting value for a reference sample, but should be altered in conjunction with the drive amplitude with different probes/samples to maintain a resonance peak under 2.5V.

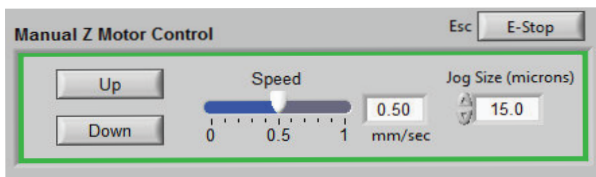


3.2.1.5 Manual Z Motor Control

The Z motor, which raises and lowers the light lever, is activated with this window. The speed of this motion can be controlled with the speed slider. When the Up and Down buttons are held down the motor slews. The motor is jogged by touching the Up and Down buttons. The jog size can also be controlled. The E-Stop button is the emergency stop function, and can be accessed via the Esc button.

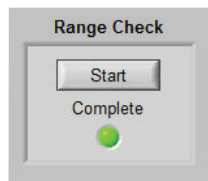


CAUTION: Always visually monitor the position of the Z motor. The Z motor can drive the cantilever into the sample stage and damage both the probe holder and the scanner.



3.2.1.6 Range Check

The usable range of the piezoelectric scanner is measured with the Range Check option. When started, the scanner is moved in a square pattern, which can be readily observed with the video optical microscope. This function must be completed before beginning a scan.

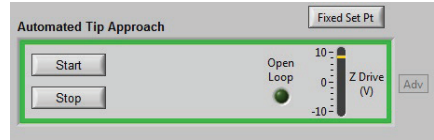




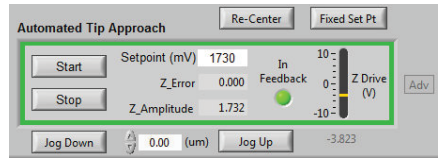
CAUTION: Before activating the Range Check function, make sure that the probe is moved away from the sample surface. If the probe is too close to the surface, the probe may be damaged.

3.2.1.7 Automated Tip Approach

The Automated Tip Approach button starts the process of the probe moving toward the surface. A woodpecker motion is used, which results in noticeable clicking sounds from the microscope stage. This sound is from two sources: the stepper motor being energized and the Z piezoelectric ceramic being retracted. When the system is engaged in feedback, the Open Loop button will light up and say "In Feedback."

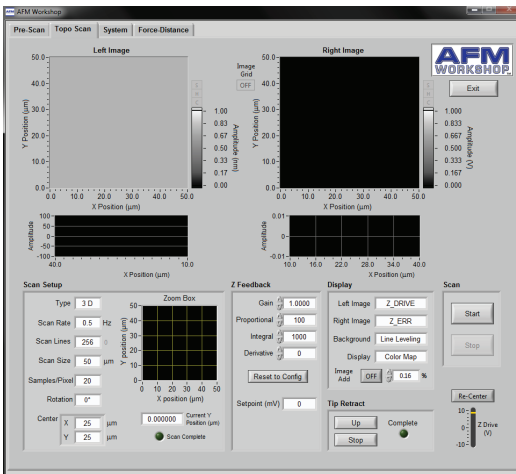


A small Adv button on the far right of the Z Drive range allows advanced users to manually manipulate tip approach, by altering the setpoint and jogging the Z motor. This can achieve a lighter feedback for high resolution scanning. See section 6 for more information on high resolution scanning.



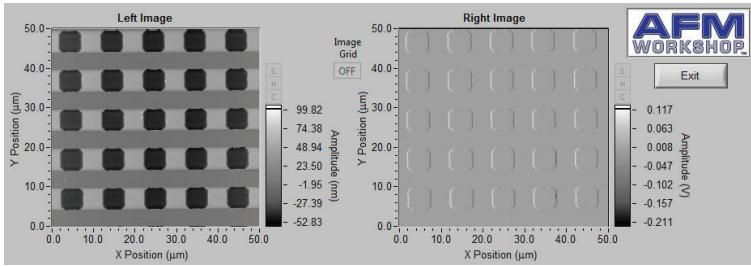
3.2.2 Topo Scan Window

After the Pre-Scan functions are ready, the Topo Scan window is used for measuring AFM images.

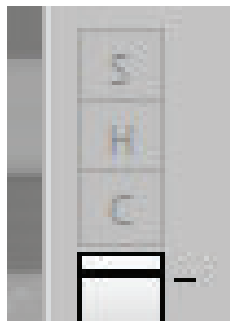


3.2.2.1 Image Window

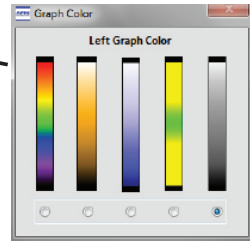
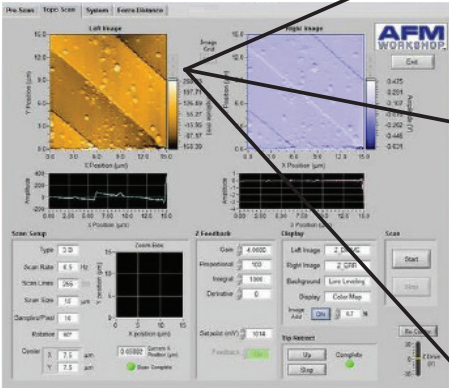
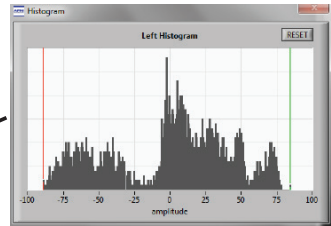
Two images are displayed simultaneously while scanning. The type of images and their appearances are selected in the Display menu window. As the images are displayed, there is a constant normalization of the data so that the images appear in full scale.



SHC buttons on the right side of the Image Windows allow users to open the scans in a larger window and adjust the color scheme and histogram in real time. Each of the windows is opened by clicking on *S*, *H*, or *C*; the window can be closed by re-clicking on the *S*, *H*, or *C*.

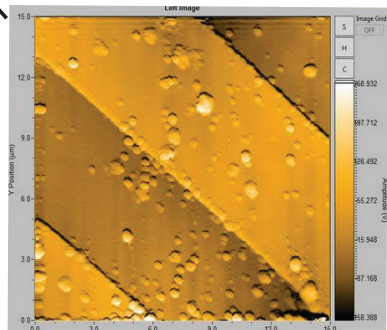


The histogram (H) can be adjusted by moving the red and green vertical bars.



The C button allows the user to choose between a number of color schemes.

The S button opens a larger version of the image in a new window.



3.2.2.2 Oscilloscope Window

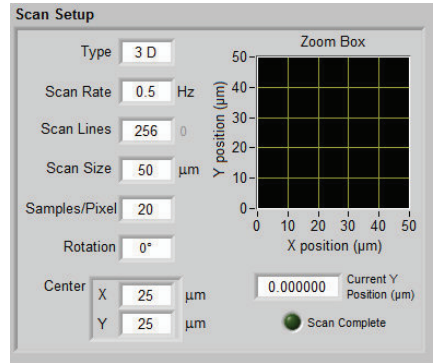
A Z vs. X position plot of the probe as it scans across a surface is plotted in two screens. The data plotted is selected in the Display window.



3.2.2.3 Scan Setup

All of the parameters required for scanning are presented in the Scan Setup window. Once scanning is started, these parameters may not be changed. The parameters are:

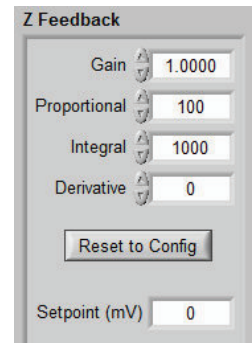
1. **Type:** The scan can be either 2-D, which scans just the x-axis, or 3-D, which scans both the x- and y-axis.
2. **Scan Rate:** The rate in Hertz that the probe is scanned across the surface (max 2 Hz).
3. **Scan Lines:** The number of lines in the y-axis and the number of pixels measured in the x-axis.
4. **Scan Size:** The range of the scan, from 0.1 micron to either 15 or 50 microns (depending on scanner size).
5. **Samples/Pixel:** The number of data points signal averaged per pixel while a scan is being taken.
6. **Rotation:** A scan can be completed with 0° or 90° rotation.
7. **Center:** The center of the scan in x and y coordinates.
8. **Current Y/X Position:** The position of the probe along the y-axis as a scan is taken at 0°, along the x-axis for a 90° scan.
9. **Scan Complete:** This indicator turns green when a scan is completed.
10. **Zoom Box:** This shows the position and size of the scan in a grid. The scan area must be within the zoom box area.



3.2.2.4 Z Feedback

All of the parameters for maintaining feedback are provided here. The parameters are:

1. **Gain:** Scale factor for the feedback control signal.
2. **Proportional:** Scale factor for the proportional term in the PID feedback controller.
3. **Integral:** Scale factor for the integral term in the PID feedback controller.



4. *Derivative*: Scale factor for the derivative term in the PID feedback controller.
5. *Setpoint*: Parameter that controls the magnitude of interaction between the probe and surface.

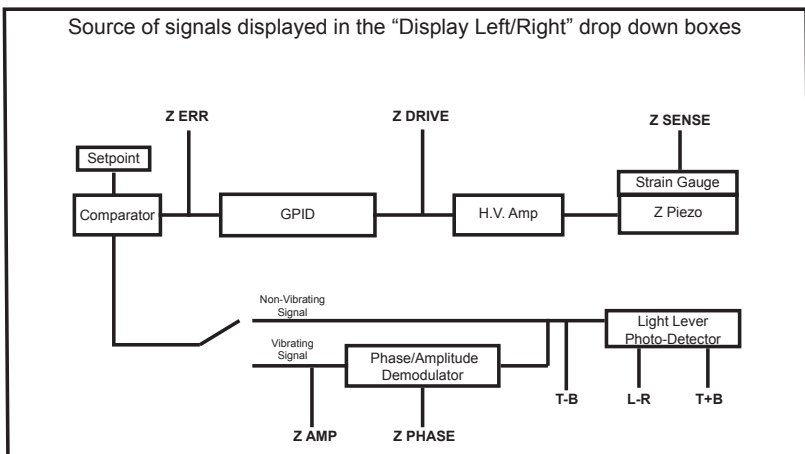
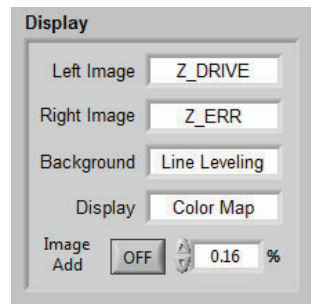


CAUTION: If the Z Feedback parameters are too high, the Z ceramic can begin to oscillate and potentially cause damage to the ceramic.

3.2.2.5 Display

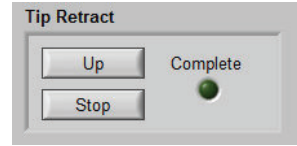
There are several options for changing the appearance of the images displayed in the Scan window. They are:

1. *Left Image*: The source of the image displayed in the left image window.
2. *Right Image*: The source of the image displayed in the right image window.
3. *Background*: When Line Leveling is selected, the background is subtracted from the image one line at a time.
4. *Display*: Two types of displays may be selected: Color Map and Light Shaded.
5. *Image Add*: This function allows the user to overlay the left and right images while scanning. The opacity may be adjusted by the % value.



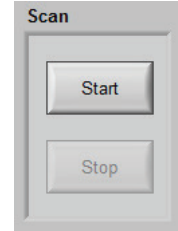
3.2.2.6 Tip Retract

After scanning a sample, this function is used to move the tip away from the surface. The Tip Retract function should always be used to assure that the probe does not get damaged after scanning.



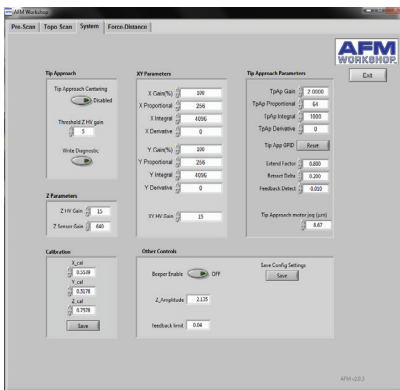
3.2.2.7 Scan

These buttons allow the user to start and stop scans.

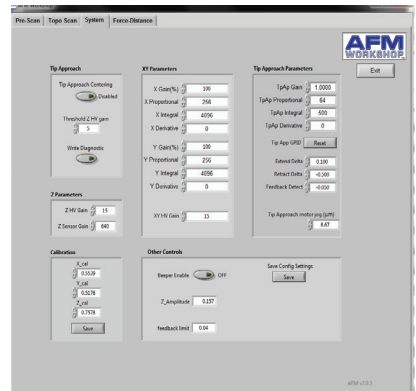


3.2.3 System Window

The system tab has several parameters that control the functionality of the TT-2 AFM. These parameters should not be changed without a detailed understanding of the function that is being modified. Incorrect use of the system functions can damage the TT-2 AFM.



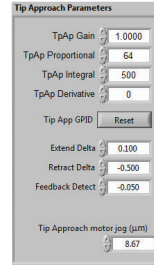
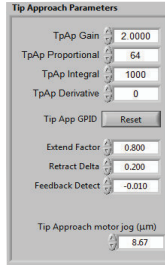
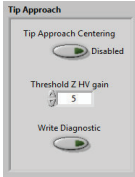
System tab in vibrating mode



System tab in non-vibrating mode

3.2.3.1 Tip Approach

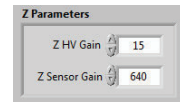
This sub-window controls the step used for the stepper motor in tip approach. These functions are very useful for high resolution imaging (see section 6). The Tip Approach motor jog value automatically adjusts to changes in the Z HV Gain (reduces by a factor of 1/3). When the advanced tip approach options are not selected on the Pre-Scan tab, the Tip Approach motor jog function moves to the Other Controls sub-window.



Vibrating mode values Non-Vibrating mode values

3.2.3.2 Z Parameters

The high-voltage amplifier that drives the Z ceramic in the XYZ scanner has 15 gain settings. This function allows reduction of the Z gain of the high-voltage amplifier. Reducing the gain reduces the dynamic range of the ceramic, which also reduces the noise floor of the instrument.



NOTE: A new high voltage gain value is entered into the system when tip approach is being undertaken. Without enabling tip approach, the Z setting will not change.

3.2.3.3 XYZ Calibration

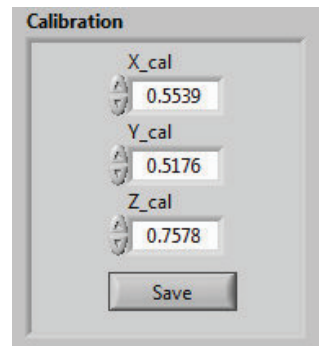
The calibration values in this sub-window can be adjusted when scanning a standard reference sample. Appendix C explains how to calibrate the TT-2 AFM in detail.



NOTE: Changing the calibration values will change the scale on images measured with the TT-2 AFM. Only change the values when calibrating with a reference sample.

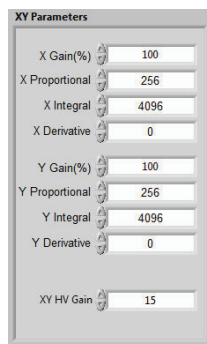


NOTE: The Save button will turn green when pushed. However, the new values will not be saved in the Scanner configuration file until the Exit button is pushed in the Topo Scan window. The user must have administrator rights in order to save the calibration values.



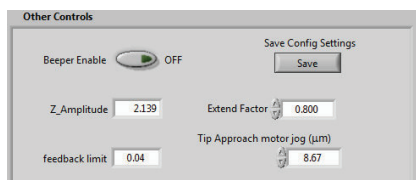
3.2.3.4 XY Parameters

Adjusting these parameters will alter the voltages applied to the X and Y ceramics, which can be used for measuring and reducing Z noise in images (see Appendix D for instructions on how to complete a Noise Floor). XY HV Gain allows gain reduction of the X and Y high-voltage amplifiers, similar to Z HV Gain. Reducing the X and Y GPID settings will decrease strain gauge activity. This will affect linearization, but will also reduce the noise floor of the system.

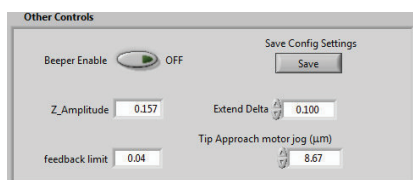


3.2.3.5 Other Controls

1. **Beeper:** Enables a beeper that notifies an operator that a scan is completed with an audible tone. (Speakers must be enabled)
2. **Amplitude Value:** Displays the current Z amplitude used in Tip Approach. This value should correspond with the Selected Frequency on the Pre Scan tab.
3. **Feedback Limit:** This function controls the feedback LED that turns green when Tip Approach has achieved feedback. If the feedback light is flickering when the tip is in feedback, increase this value by 0.01V.
4. **Extend Factor:** This function determines the percent decrease of vibrational amplitude of the probe in vibrating mode. The typical range for extend factor is 0.7-0.95, with 0.95 creating a 5% reduction in vibrational amplitude for high resolution scanning.
5. **Extend Delta:** This function determines the amount of probe deflection on the surface in non-vibrating mode. Extend delta is typically 0.1, meaning the Top-Bottom signal will deflect by 0.1V before feedback is initiated.
6. **Tip Approach motor jog:** This function will appear in the Other Controls sub-window when the advanced tip approach options are not visible.



Other Controls for vibrating mode

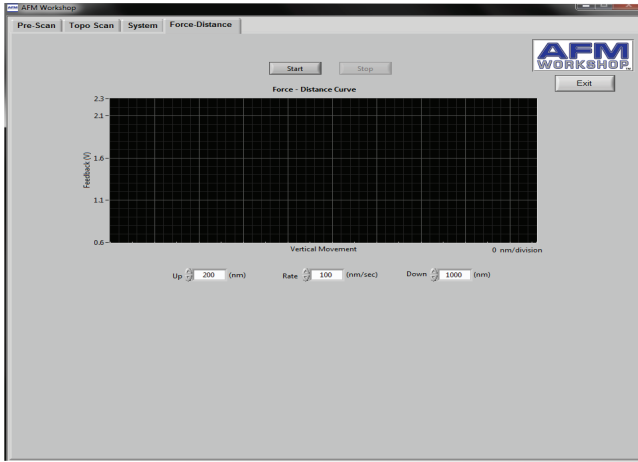


Other Controls for non-vibrating mode

3.2.4 Force/Distance Curves

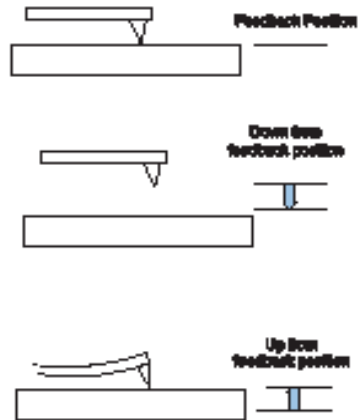
Force/distance curves are created by measuring the deflection of the cantilever as the sample is moved towards and then away from the probe at the end of the cantilever. The shape of a F/D curve depends primarily on the cantilever stiffness and the thickness of a surface contamination layer. For information about how to analyze F/D curves, see Appendix I.

Measurement of F/D curves is found on the Force-Distance tab.



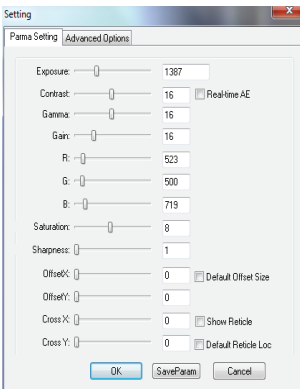
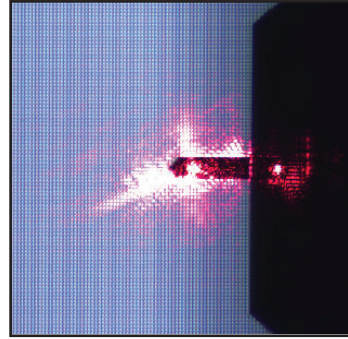
The AFM must be in feedback in non-vibrating mode before a F/D curve is measured. There are three important parameters that must be set before the F/D curve is measured. They are:

1. **Down:** The distance in nanometers that the sample will be moved away from the probe when the F/D curve measurement is initiated.
2. **Up:** The distance in nanometers that the sample will be moved up from the feedback position during the F/D curve. During this part of the curve, the probe is touching the sample surface.
3. **Rate:** The rate or velocity of the sample as it is moved towards and away from the probe.

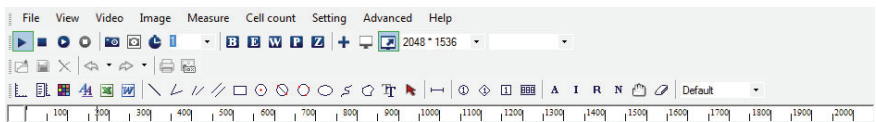


3.3 Video-View Software

The video optical microscope software allows real time visualization of the probe and surface. The software also allows for real time capture of both images and videos. Operation of the video software requires substantial computer processing speed; when computers with slower processing speeds are used, the image can often change contrast. The change in contrast is triggered by other programs using the computer's processing capacity. Below is a brief description of the function found in the software package. It is recommended to optimize the parameters for each type of sample. Before operating the video camera, it must be properly aligned; see Appendix A.



Many of the video camera's adjustments can be accessed by selecting the P for parameter settings. These adjustments include exposure, contrast, gamma, gain, and offset in X and Y. The software also allows users to measure features on the screen, cell count, and export their screen captures to Excel or Word. See the tool bar below.



The quality of the image viewed with the video microscope depends directly on the reflectivity of the sample being viewed. Therefore, it is not possible to give instructions for optimizing the video microscope images. It is recommended that operators experiment to find the best parameters.

4. Measuring Images With the LS-AFM

Once the LS-AFM is assembled, users can expect to take a few hours to learn how to measure images. It is very important to learn how to:

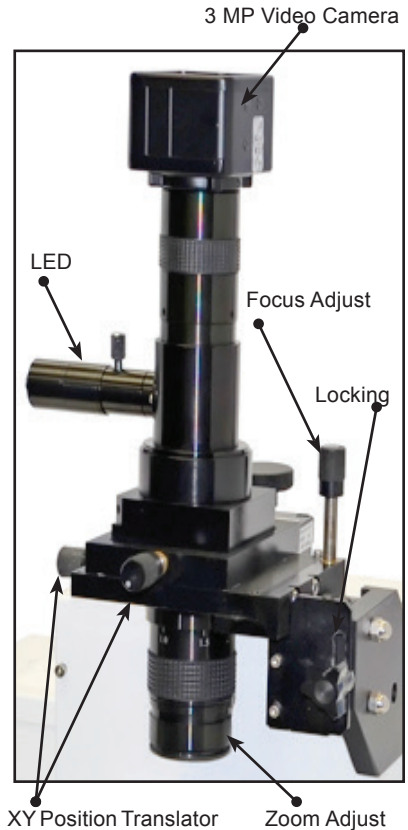
1. Operate the video microscope.
2. Prepare samples.
3. Exchange probes.
4. Align the light lever force sensor.
5. Perform video-microscope-assisted tip approach.

Once these techniques have been mastered, measuring images is relatively simple.

4.1 Operating the Video Microscope

Operating the video microscope is essential for efficient operation of the LS-AFM. It allows for proper viewing of the sample, assists in manual tip approach, and is critical for laser and photo-detector alignment. It is advised to practice operating the video microscope before attempting to replace probes or measure images. The components of the video microscope are:

1. *XY Position Translator*: Just above the focus adjust are XY position translators used for centering the probe in the video microscope window.
2. *Zoom Adjust*: The zoom lens allows for viewing of a surface at high or low magnification. The light intensity must be adjusted when using the zoom lens.
3. *Light Intensity Adjust*: It is often critical to adjust the intensity of the light on a sample to get the best image. (not shown)
4. *Focus Adjust*: The user can manually alter the microscope's focus with these z-motion adjustment knob.
5. *Locking Mechanism*: The user should lock the optics in place using this knob.



NOTE: If the operation of the video optical microscope does not seem familiar, please review the contents of Appendix A, section 2.

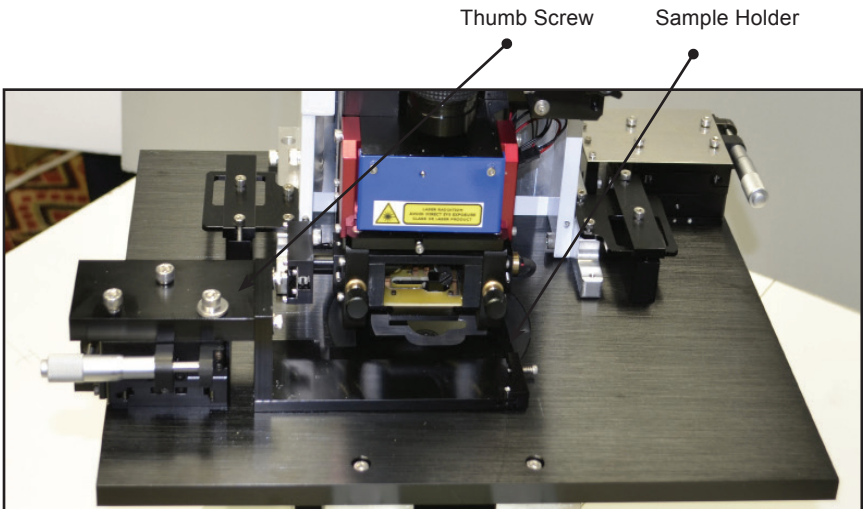
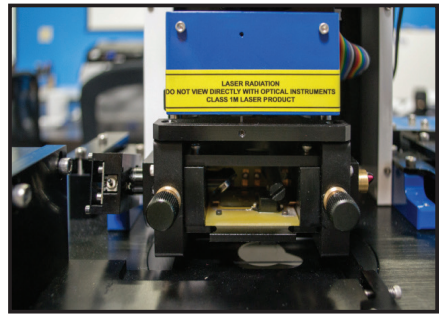
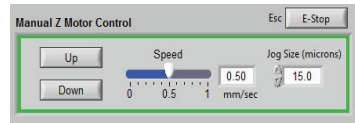
4.2 Preparing Samples

Samples can be prepared on glass slides or on petri dishes. It is critical that the samples are properly adhered to the slides/dishes, to avoid additional vibrations.

4.2.1 Exchanging Samples

Follow the steps below to successfully exchange samples with the LS-AFM.

1. Raise the Z motor until the probe is at least $\frac{1}{4}$ " from the sample's surface.
2. Remove the probe holder (see section 4.3).
3. Remove the thumb screw on the sample XY positioning stage, and rotate the sample holder away from the AFM.
4. Place either the glass slide or the petri dish in the sample holder.
5. Rotate the sample holder back under the AFM stage and replace the thumb screw.
6. Align the sample using the video optics (see section 4.1).
7. Replace the probe holder with the probe tip facing the sample.



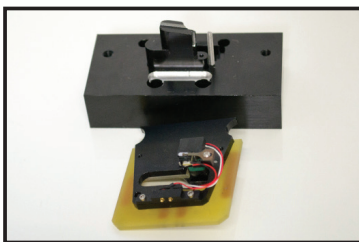
4.3 Exchanging Probes

When a tip becomes damaged or broken, these steps can be followed to exchange probes quickly and efficiently.

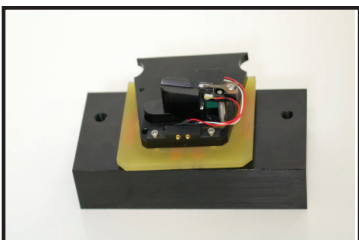
1. Raise the Z motor until the probe is at least $\frac{1}{4}$ " from the sample's surface.



NOTE: Make sure the new probe is close at hand and facing in the same orientation as the probe exchange tool.

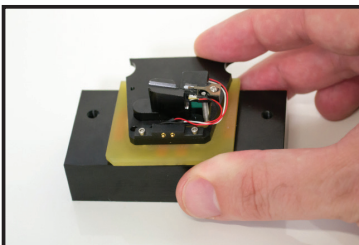


2. Remove the probe holder from the light lever.
3. Place the probe holder on the probe exchange tool with the spring facing up.
4. Push down on the probe holder, such that the spring releases the probe. Tap the probe exchange tool lightly on a flat surface at a 30° angle until the probe slides down through the narrow channel of the exchange tool. Gently dispose of the old probe with tweezers.



CAUTION: The exchange tool will not prevent expulsion of the probe if the tapping angle is too extreme.

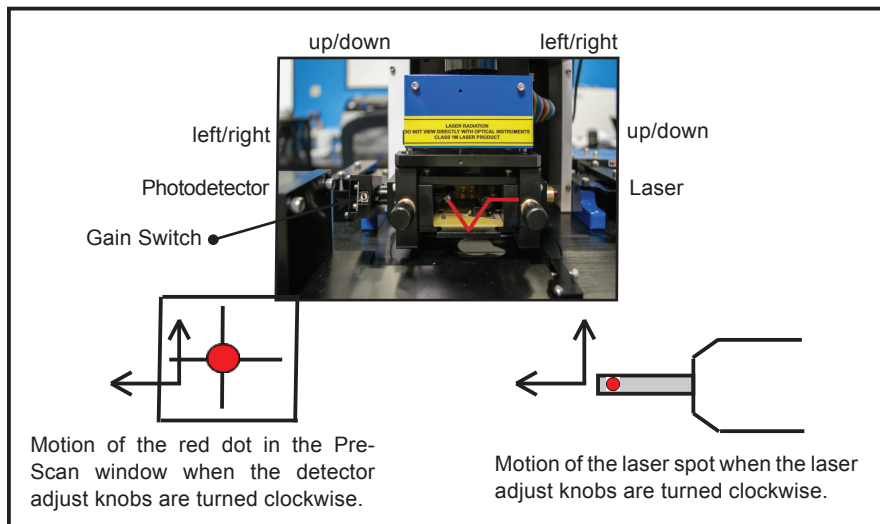
5. Using the tips of very sharp tweezers, pick up the new probe and, while pressing down on the probe holder, gently slide the probe into place. Take care not to touch the cantilever with the tweezers.
6. Once the new probe is positioned correctly, place the probe holder back into the microscope.



NOTE: If the probe holder does not sit completely level with the probe exchange mechanism, it is possible to adjust to the screws on the bottom of the exchange tool to achieve the desired height.

4.4 Aligning the Light Lever Force Sensor

Aligning the light lever force sensor is greatly facilitated by understanding the optical path of the light in the sensor. The following figure shows the light path and the effect of turning the adjustment knobs on the light lever.

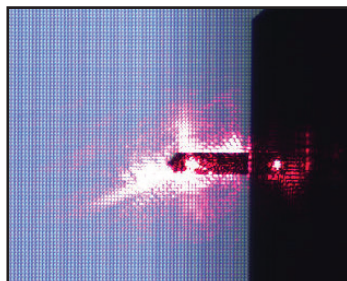
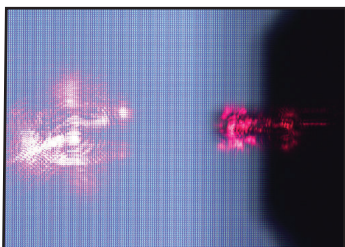


4.4.1 Tips for Aligning the Light Lever Force Sensor

1. Bringing the cantilever within a millimeter of the sample's surface can help the user see the laser on the sample.
2. If the cantilever does not align within a few minutes, try another cantilever.
3. After replacing a cantilever, the laser and photo-detector adjustments are usually less than a single turn.
4. The adjustment screws have backlash; sometimes the motion is not perfectly linear.
5. The detector has two modes: high and low gain. High gain allows more laser light through the detector than low gain. Low gain is commonly used for tip scanning systems.
6. When in vibrating mode, if the resonance peak appears inverted at the top, reduce the amplitude slider. If further adjustments are necessary, switch the detector gain from high to low.
7. If the Beam Position is not aligned within a few turns of the adjustment knobs, the photo-detector can be manually adjusted by loosening the set screws holding the detector down and repositioning the detector so that the Top + Bottom signal is maximized while the Beam Position is mostly centered.

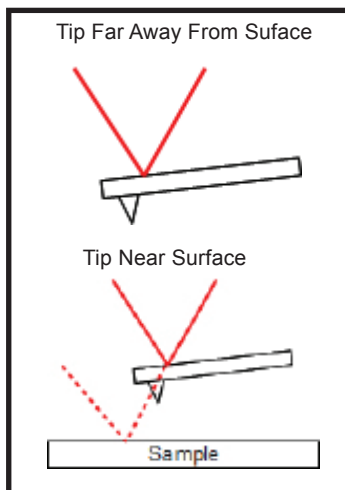
4.5 Optically-Assisted Tip Approach

Tip approach with the LS-AFM is achieved using a woodpecker algorithm activated in the Pre-Scan window. To save time during tip approach, move the probe as close to the surface as possible before activating the Automatic Tip Approach function. The video microscope can prove very helpful for manual probe approach. Below are two images of a probe as it moves toward a standard reference sample surface.



4.5.1 Perform optically assisted tip approach

1. Focus the video microscope on the sample surface.
2. Carefully lower the Z motor with the Manual Z Motor Control, or manually with the motor knob, until the cantilever is visible in the video microscope image. Make sure the Jog Size has been set to a low step size and the Z motor speed is set to 0.00 mm/sec in order to avoid crashing the probe into the sample.
3. Slowly jog the probe down until it is only slightly out of focus.
4. As the probe approaches the surface, a second laser spot will appear on the surface if the sample is reflective. Once the second laser spot merges with the spot on the cantilever, the tip is flush with the surface. This effect is useful for judging the distance from the probe to the sample.



CAUTION: If the tip touches the surface before the feedback is activated, the tip may be damaged or even broken.

4.6 Contact Mode Imaging

These instructions assume the AFM-View software is properly loaded and working, and that the material presented in section 4 has been reviewed.

4.6.1 Pre-Scan Tab

1. Follow the instructions in section 4.2. and place a sample in the instrument.
2. Follow the instructions in section 4.3. and place a contact mode probe in the instrument. See Appendix E for probe recommendations.
3. Focus the video optical microscope on the AFM probe.
4. Follow the instructions in section 4.4. to align the laser on the probe. Make sure the Top – Bottom signal is between 0.4 and 0.8 for contact-mode imaging.
5. Select Non-Vibrating in the Scan Mode window.
6. Focus the video microscope on the sample surface.
7. If this is the first scan since opening the AFM-View software, click on *Start* in the Range Check window.



NOTE: The probe must NOT be in contact with the sample surface during Range Check. The piezoelectric ceramics will move to measure the usable range, which may cause damage to the tip.

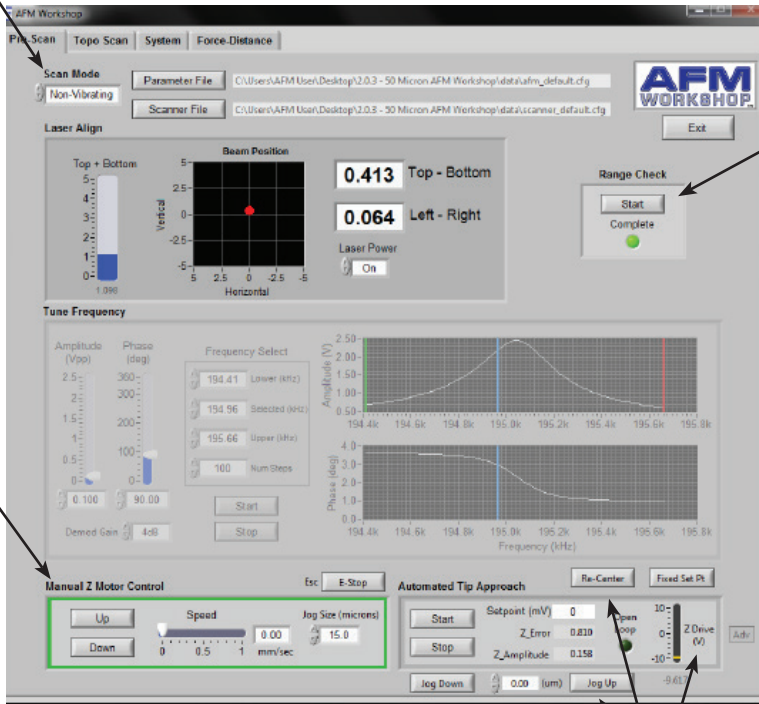
8. Use the Manual Z Motor Control to move the light lever towards the sample (see section 4.5.).
9. Initiate Automated Tip Approach. Wait for the green light to indicate that feedback was achieved.
10. If the Z Drive indicator is above the center of its range, click the Adv button and use the Re-Center function. If the Re-Center function does not initiate a new tip approach, increase the setpoint value by 100mV.
11. If the Z Drive indicator is below the center of its range, click the Adv button and jog the motor up by small increments until the indicator is at the center of the Z range.
12. Once true feedback has been achieved, open the Topo Scan tab at the top of the AFM Workshop window.

5

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10,11



4.6.2 Topo Scan Tab

1. Select scan parameters such as Scan Size, Scan Rate and the number of lines. Samples may be scanned at 0° or 90° by adjusting the Rotation parameter.



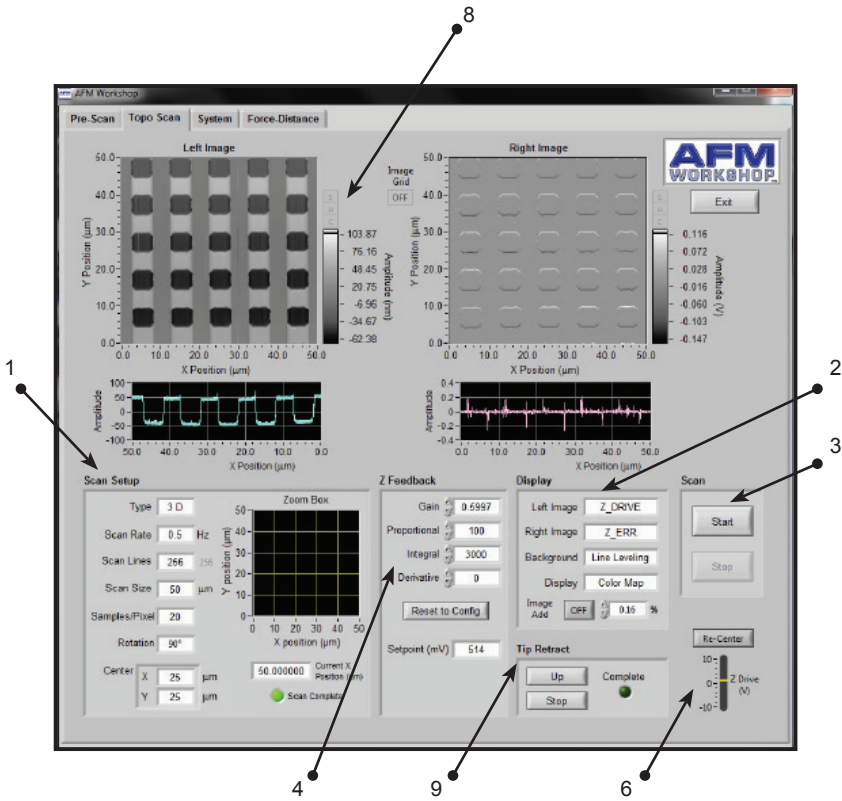
NOTE: A scan rate of 0.5 Hz and 256 scan lines will achieve a basic low resolution scan.

2. Select the desired images to be displayed in the Display window. The Display window also allows for control of leveling, as well as the ability to transpose both the Left and Right Images with the Image Add button. Some display options for contact mode scanning include:
 - Z-Drive, the change in voltage through the z-piezoelectric ceramic, shows the sample's topography. It is common to set Z-Drive to the Left Image.
 - Z-Error, the error signal, is used to help optimize the Z Feedback parameters. When initially setting up a scan, it is common to use Z-ERR as the Right Image.
 - L-R, lateral force, detects cantilever torsion as the tip applies constant force along the surface. This mode is unique to contact mode scanning, and can only be properly used when scanning at 90°.
3. Begin a scan by pressing the Start button in the Scan window.



NOTE: When the GPID parameters are optimized, the Z piezoelectric ceramic will more closely follow the probe tip as it scans the surface. When tip movement is minimized the Z error signal is decreased. **Increasing** the setpoint by small increments (50-100 mV) sharpens the image by pushing the tip harder on the surface.

4. While scanning, optimize the GPID and setpoint parameters (see Appendix G).
5. Once the parameters are optimized, stop the scan by pressing the Stop button.
6. Before beginning a new scan, check to see if Z Drive is still near the center of its range. If not, see sections 4.6.10 - 11.
7. Begin a new scan. When the scan is completed, the light turns green.
8. If desired, the user can open a larger window of both the Left and Right Images by selecting the S button to the right of the scan window. The H and C buttons allow the user to alter the histogram and color scheme of the scans, accordingly.
9. When finished scanning, press Tip Retract. This function lightly releases the probe from the sample surface.



4.7 Vibrating Mode Imaging

These instructions assume the AFM-View software is properly loaded and working, and that the material presented in section 4 has been reviewed.

4.7.1 Pre-Scan Tab

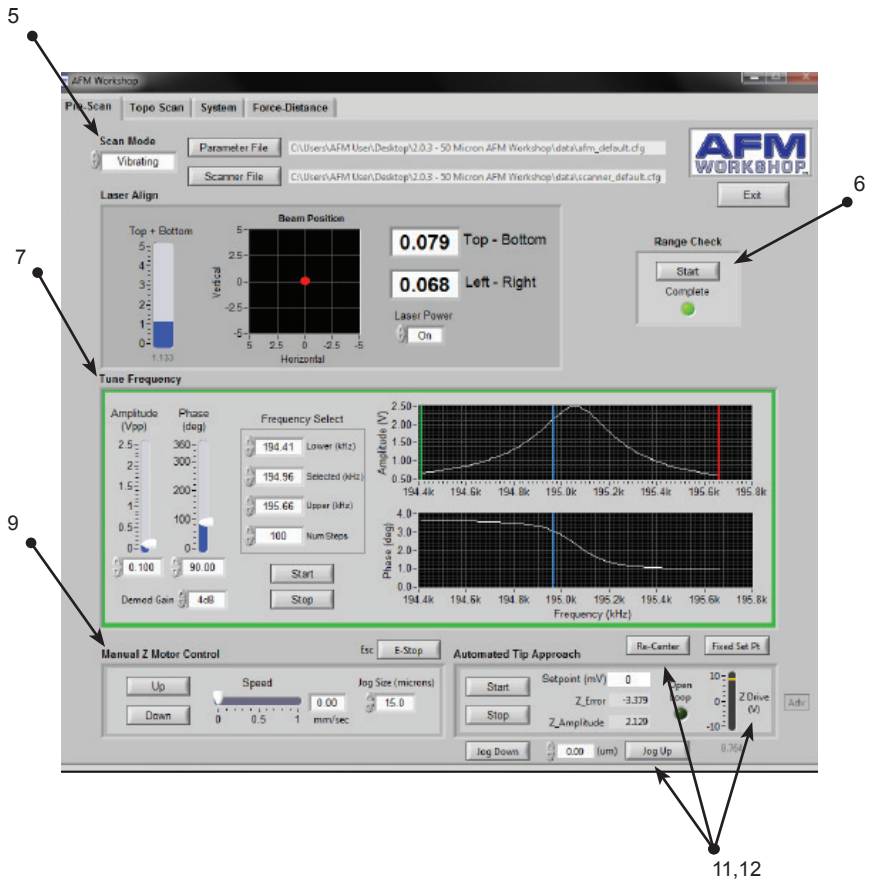
1. Follow the instructions in section 4.2. and place a sample in the instrument.
2. Follow the instructions in section 4.3. and place a vibrating mode probe in the instrument. See Appendix E for probe recommendations.
3. Focus the video optical microscope on the AFM probe.
4. Follow the instructions in section 4.4. to align the laser on the probe. Make sure the Top – Bottom signal is close to 0.0 for vibrating mode imaging.
5. Select Vibrating in the Scan Mode window.



NOTE: The probe must NOT be in contact with the sample surface during Range Check. The piezoelectric ceramics will move to measure the usable range, which may cause damage to the tip.

6. If this is the first scan since opening the AFM-View software, initiate the Range Check function.
7. Select the frequency under the Tune Frequency window.
 - a. Set the Lower limit to the lowest frequency on the probe box.
 - b. Set the Higher limit to the highest frequency on the probe box.
 - c. Set the Selected value to any frequency between the two limits. If the selected frequency exceeds either limit, the software will automatically change the values.
 - d. Set the number of steps (50-100) and the Demod Gain (4dB).
 - e. Set the setpoint in the Automated Tip Approach box to 3000mV to fully retract the z ceramic, so it does not interfere with the frequency sweep.
 - f. Scan the frequency.
 - g. Move the green and red cursors toward the resonance peak to decrease the range, and place the blue cursor corresponding with the selected frequency 80% up the left side of the resonance peak.
 - h. Repeat steps (iv) and (v) until a resonance peak no greater than 2.5V is observed. If the amplitude of the resonance peak exceeds 2.5V, adjust the driving amplitude of vibration by altering the Amplitude scroll bar.
 - i. For phase mode imaging, the resonance peak must correspond with the slope of the phase curve. To alter the phase degree, adjust the Phase scroll bar accordingly.
8. Focus the video microscope on the sample surface.
9. Use the Manual Z Motor Control to move the light lever towards the sample (see section 4.5.).
10. Initiate Automated Tip Approach. Wait for the green light to indicate that feedback was achieved.

11. If the Z Drive indicator is above the center of its range, click the Adv button and use the Re-Center function. If the Re-Center function does not initiate a new tip approach, increase the setpoint value by 100mV.
12. If the Z Drive indicator is below the center of its range, click the Adv button and jog the motor up by small increments until the indicator is at the center of the Z range.
13. Once true feedback has been achieved, open the Topo Scan tab at the top of the AFM Workshop window.



4.7.2 Topo Scan Tab

1. Select scan parameters such as Scan Size, Scan Rate and the number of lines. Samples may be scanned at 0° or 90° by adjusting the Rotation parameter.



NOTE: A scan rate of 0.5 Hz and 256 scan lines will achieve a basic low resolution scan.

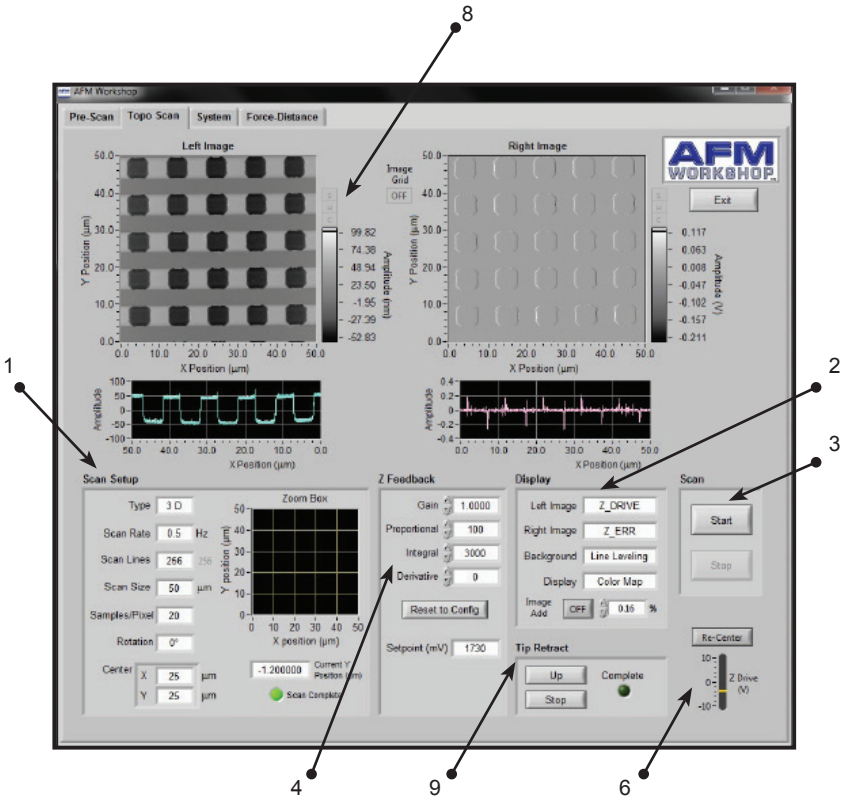
2. Select the desired images to be displayed in the Display window. The Display window also allows for control of line leveling, as well as the ability to transpose both the Left and Right Images with the Image Add button. Some display options for vibrating mode scanning include:
 - Z-Drive, the change in voltage through the z-piezoelectric ceramic, shows the sample's topography. It is common to set Z-Drive to the Left Image.
 - Z-Error, the error signal, is used to help optimize the Z Feedback parameters. When initially setting up a scan, it is common to use Z-ERR as the Right Image.
 - Z-Phase, phase imaging mode, uses the differences in the probe's oscillation to form image contrast. The phase shifts when the probe comes into contact with areas of the sample surface with differing stiffness, adhesion, etc. Thus phase imaging mode is optimal for composite samples, those with varying surface densities and characteristics.
3. Begin a scan by pressing the Start button in the Scan window.
4. While scanning, optimize the GPID and setpoint parameters (instructions in Appendix G).



NOTE: When the GPID parameters are optimized, the Z piezoelectric ceramic will more closely follow the probe tip as it scans the surface. When tip movement is minimized the Z error signal is decreased.

Decreasing the setpoint by small increments (50-100 mV) sharpens the image by pushing the tip harder on the surface.

5. Once the parameters are optimized, stop the scan by pressing the Stop button.
6. Before beginning a new scan, check to see if Z Drive is still at the center of its range. If not, see sections 4.6.10 - 11.
7. Begin a new scan. When the scan is completed, the light turns green.
8. If desired, the user can open a larger window of both the Left and Right Images by selecting the S button to the right of the scan window. The H and C buttons allow the user to alter the histogram and color scheme of the scans, accordingly.
9. When finished scanning, press Tip Retract. This function lightly releases the probe from the sample surface.

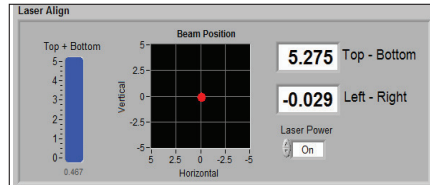


5. Troubleshooting – What Can Go Wrong When Measuring Images

Although the operation of the LS-AFM is quite simple, some problems can occur while imaging samples. The following sections detail some of the most common problems that may occur. It is important to keep these potential problems in mind when operating the LS-AFM.

5.1 Saturation of the Photo-Detector

Highly-reflective cantilevers can sometimes pass too much laser light to the photo-detector. When this occurs, the light lever force sensor will not work properly. When there is too much light, the “Top + Bottom” signal in the “Laser Align” window displays a value greater than 3. This problem can often be solved by flipping the gain switch on the photo-detector down to low gain (see section 4.4 for the location of the gain switch).



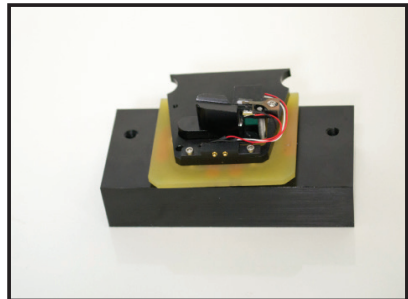
5.2 Sample Not Mounted Correctly

It is essential that the sample is rigidly mounted on either a glass slide or petri dish. If the sample is not mounted rigidly, it can be difficult to get into feedback, and images appear to have excessive vibrations.



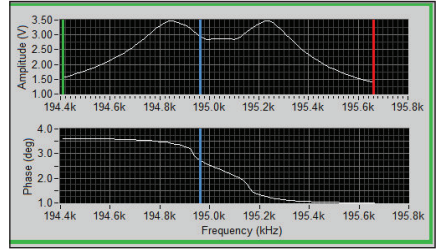
5.3 Probe Not Seated Correctly

If the probe is seated correctly, it will be in essentially the same place before and after the probe is removed from the AFM. If it is too difficult to align the light lever after a probe exchange, examine the probe holder closely to make sure that the probe is seated correctly. Improper probe alignment may cause resonance saturation.



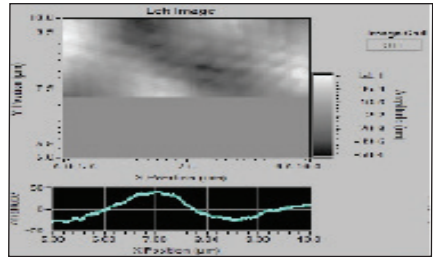
5.4 Resonance Saturated

When scanning, the frequency of the cantilever produces a double-peaked resonance, as shown on the right. Decrease the driving amplitude of the probe by lowering the Amplitude scroll bar until the resonance peak sits below 2.5V. If the resonance is still saturated, the probe may have to be re-seated.



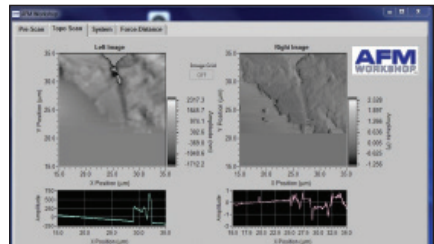
5.7 False Feedback

In vibrating mode, the probe can go into false feedback, resulting in wispy, featureless images. False feedback is achieved when the probe is caught up in the surface contamination layer. This can be corrected by activating the tip approach function a second or third time, as well as by utilizing the advanced tip approach functions to jog the tip toward with surface (see section 6).



5.5 Scanner at End of Z Range

When the Z ceramic expands or retracts to the end of its range while scanning, the image will suddenly lose contrast and appear flat. To solve this problem, redo tip approach so that the Z ceramic is in the center of its range (see section 4.6. j-k).

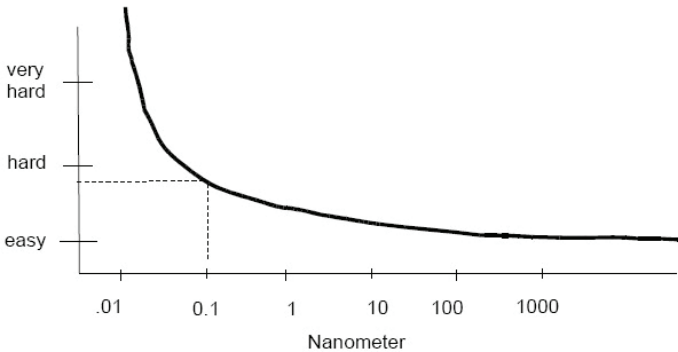


5.6 Defective Probe

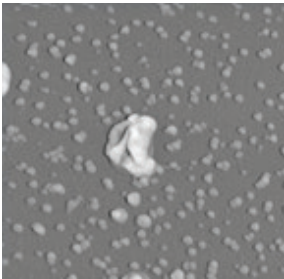
In the event that none of the above problems exist and it is not possible to get an image, the probe may be defective.

6. High Resolution Imaging

High resolution images are measurable with the LS-AFM. However, as with any AFM, obtaining those images requires that the AFM be optimally set up and that the operator knows the critical parameters for measuring high resolution images. The table below illustrates the difficulty in measuring images as a function of the desired dimensions.



Examples of high resolution images include:



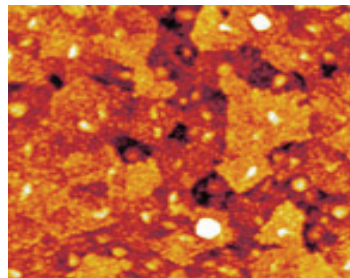
(500x500nm) 1-3 nm Test Particles



1.2x1.2µm Tobacco Mosaic Virus



0.3nm Si Terraces



2x2µm Indium Tin Oxide

There are three critical requirements for measuring high resolution images with the LS-AFM:

- 1) The instrument must not be influenced by structural and acoustic vibrations.

For high resolution imaging, the AFM must be placed in an environment with minimal vibrations. Typically, both a vibration table as well as an acoustic enclosure is required. Often measurements must be made in the middle of the night when there are minimal structural vibrations. Measuring the noise floor of the AFM establishes the Z resolution of the AFM. Measuring images of structures with 0.3 nm in vertical dimension requires a Z noise floor below 0.1 nm. See Appendix D to learn how to measure the noise floor of the LS-AFM.

- 2) The Electronics Gain must be properly set up.

The scanner used for the measurements must be capable of resolving the desired features. The resolution of the scanner depends on the electronic noise in the control electronics and the mechanical gain of the piezoelectric ceramics in the scanner.

In the LS-AFM the electronic noise can be reduced by reducing the Gain on the high voltage amplifiers that drive the scanner piezoelectric ceramics. The LS-AFM scanner has an XY mechanical gain of 3:1. The LS-AFM probe holder can have either an 8µm Z piezoelectric ceramic or a 17µm Z piezoelectric ceramic. The theoretical resolution of both probe holders can be seen below.

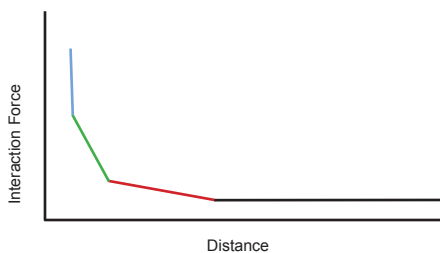
Theoretical resolution for LS-AFM				
Z Gain	17µm Z Ceramic		8µm Z Ceramic	
	Z Range (nm)	Z Noise (nm)	Z Range (nm)	Z Noise (nm)
15	17000	0.85	8500	0.43
14	15867	0.79	7937	0.41
13	14733	0.74	7371	0.39
12	13600	0.68	6804	0.36
11	12467	0.62	6237	0.33
12	11333	0.57	5670	0.30
9	10200	0.51	5103	0.27
8	9067	0.45	4536	0.24
7	7933	0.40	3969	0.21
6	6800	0.34	2402	0.18
5	5667	0.28	2835	0.15
4	4533	0.23	2268	0.12
3	3400	0.17	1791	0.09
2	2267	0.11	1135	0.06
1	1133	0.06	567	0.03

As lower Z high voltage gains are used, the maximum Z range decreases. Therefore, the step size of the Z motor must be reduced when lower Z gains are used. This will assure that the probe does not crash into the sample surface on tip approach. The software automatically adjusts the tip approach motor jog for changes in the Z HV Gain.

3) Tip approach must be made such that the tip is not broken.

Preserving a sharp tip for imaging requires making a perfect tip approach. This means that the probe must not crash into the surface and break the tip off. The best method for preserving the tip in probe approach is to bring the tip to the surface and engage in a “false feedback.” Then, very gently bring the tip into feedback such that the tip is interacting with the surface.

The following graph shows the interaction forces of the probe with the surface:



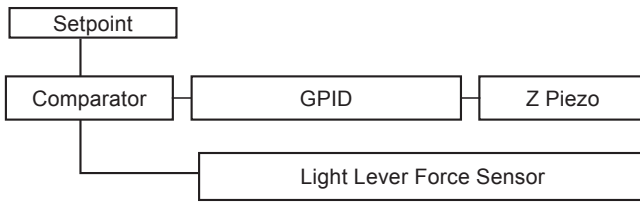
There are 4 regions of interaction forces between the tip and surface:

- No interaction force
- Acoustic interaction forces
- Interaction of the tip with the surface contamination
- Direct interaction between the probe and the surface.

These interaction forces will cause the vibrating cantilever to decrease in its amplitude as it passes through each of these interaction regimes.

The method for getting into perfect feedback is to stop the tip approach in the green regime, and then slowly bring the probe into the direct interaction regime. This is achieved by setting the feedback threshold low such that the tip approach stops when the probe is interacting with the surface contamination (Extend Factor < 0.9). Then the probe can be slowly jogged towards the surface using the advanced tip approach functions. The user will know when the tip has entered the direct interaction regime when the yellow Z Drive slider no longer moves as the sample is pulled toward the tip via small setpoint changes.

The AFM control software is designed to make a careful probe approach under manual control. This is achieved with the “Advanced Tip Approach” window under the Pre-Scan tab in conjunction with the “Tip Approach Parameters” window under the System tab.



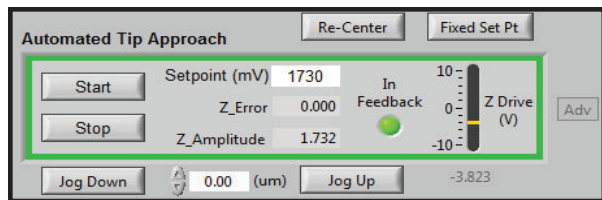
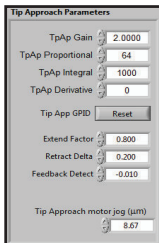
Tip Approach Sequence in Vibrating Mode

Step	Description	Result	Tip Approach Parameter
1	Measure vibration amplitude of the probe off the surface.	Amplitude used for initial tip approach. Setpoint is raised and lowered around this value which causes the sample to move up and down.	Z Amplitude value in the Automated Tip Approach Window
2	Raise setpoint.	Z piezo extends toward the sample.	Extend Factor
3	Check for feedback.	If in feedback, end tip approach.	Feedback Detect
4	Lower setpoint.	Z piezo retracts away from the sample.	Retract Delta
5	Activate stepper motor.	Moves probe toward the sample.	Tip Approach Motor Jog
6	Repeat step 2-5.	Feedback achieved with full Z Drive range.	

It is advantageous to move the probe toward the sample at a slow rate to avoid crashing the probe into the surface. The rate at which the Z piezo, and subsequently the probe, moves up and down depends on:

- The magnitude of the initial “Z Amplitude”
- Tip approach GPID parameters
- Extend Factor
- Retract Delta

The initial value for the setpoint when feedback is established is the “Z Amplitude” multiplied by the “Extend Factor.”



Appendix A: Setting Up the LS-AFM

A.1 Selecting a Location

Structural vibrations from a building's floor and acoustic vibrations can greatly reduce the resolution of an AFM. Ideally, the AFM would be placed in a vibration-free environment, but this is typically not possible. A few tips:

- Set the AFM up in the basement or lower floor near a bearing wall.
- Avoid setting the AFM near fans or other sources of wind turbulence.
- Avoid locations where there are elevators, a lot of foot traffic, or freeways.
- If you must place the AFM in an undesirable location, consider purchasing a vibration table and/or acoustic enclosure.

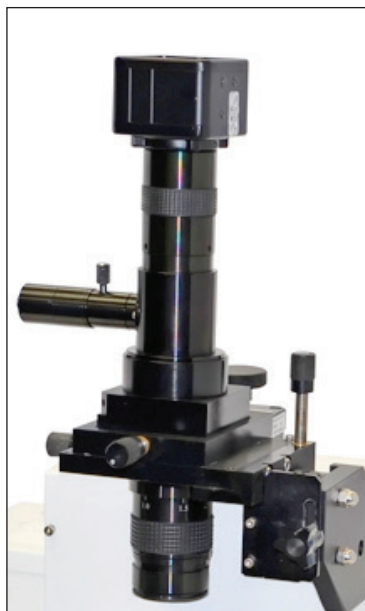
A simple test to see if a location has a problem is to fill a coffee cup with coffee and set it in the potential location. If there are ripples in the coffee's surface, there are going to be problems.

A.2 Setting Up the Optical Microscopes

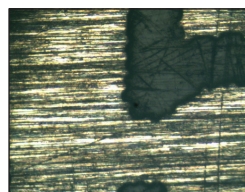
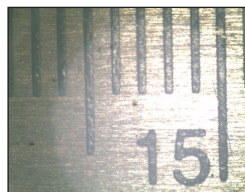
A.2.1. Top View Optic

The video optical microscope is essential for efficient operation of the LS-AFM. After setting up the microscope, we recommend spending some time learning to operate it by viewing some samples.

1. Assemble the Video Microscope
 - a. Remove the dust covers on the zoom tube and video camera, and screw the video camera into the zoom tube.
 - b. Insert the microscope collar (spacer) on the zoom tube.
 - c. Slowly lower the zoom tube into the XY translators.
 - d. Attach the LED and USB cables.
 - e. Test the locking knob and XYZ translators for fluid movement.



2. Test the microscope and verify its operation.
 - a. Launch the Video View software (see section 3.3).
 - b. Turn on the light and adjust the intensity to its mid-range.
 - c. Adjust the height of the zoom tube so that the bottom of the tube is 115 mm from the surface of the table.
 - d. Place a coin or other metal object under the objective. The light should be visible on the object.
 - e. On the zoom tube, turn the zoom adjustment counter clockwise all the way, thus reducing the magnification.
 - f. At this time, an image should be visible on the computer screen; use the focus adjust on the video microscope to sharpen the image.
 - g. Increase the magnification with the zoom tube adjuster and increase the light intensity as needed.



A.2.2. Inverted Optical Microscope

The inverted microscope for the LS-AFM can come from two sources:

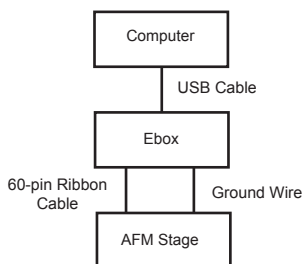
1. Another Manufacturer - Follow the manufacturer instructions for setting up the inverted microscope. Remove the manufacturer's sample plate and secure the LS-AFM sample plate to the inverted microscope stage.
2. AFMWorkshop Inverted Microscope - Follow the instructions listed in Appendix J for setting up the inverted microscope. After the inverted microscope is prepared, remove the sample plate and mount the LS-AFM microscope stage. The sample plate is held



After the inverted microscope is properly set up, place an appropriate sample into the microscope and assure that the video microscope is working properly.

A.3 Connecting the LS-AFM to the Cables

1. Connect the USB cable between the computer and the Ebox.
2. Connect the 60-pin ribbon cable between the Ebox and the LS-AFM stage.
3. Connect the grounding wire between the Ebox and the LS-AFM stage.



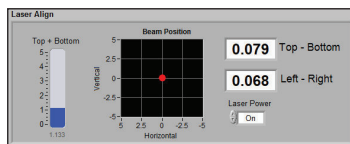
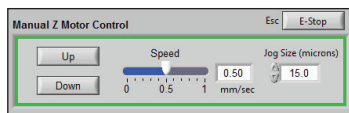
WARNING: Failure to connect the ground wire can cause damage to the stage and/or Ebox electronic circuits.



WARNING: Always turn off the master power to the Ebox before plugging or unplugging any of the cables on the LS-AFM.

A.4 System Check

1. Turn on the computer and the Ebox.
2. Launch the AFM View software.
3. Using the Manual Z Motor Control in the Pre-Scan tab, move the Z motor a few steps.
4. If the Z motor does not respond and the Top-Bottom numbers in the Laser Align section of the Pre-Scan tab are not flickering, the Ebox is not communicating with the computer. In this case, cycle power to the Ebox and restart the AFM View software.

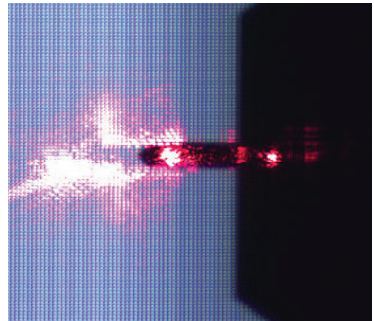
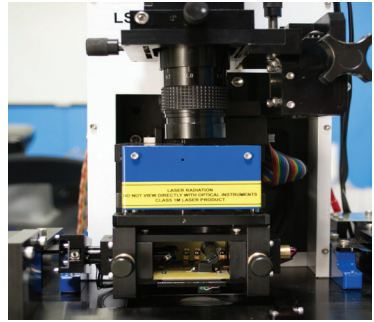


A.5 Aligning the Video Microscopes to the Cantilever

A.5.1. Aligning the Top View Optic

1. Raise the zoom tube on the optical microscope so that the scanner and light lever can fit under the bottom of the zoom tube.
2. Move the micrometers on the video microscope XY translators to their central position. Move the micrometers on the LS-AFM stage to their central position.

3. Align the zoom tube such that the hole in the scanner top plate is directly below the zoom tube optical objective.
4. Adjust the height of the zoom tube so that the bottom of the tube is about 115 mm from the top of the AFM sample holder. Move the stage by hand and adjust the optical focus.
5. Place a probe in the probe holder (see section 4.3) and place the probe holder into the microscope.
6. With the Manual Z Motor Control, move the probe such that it is about $\frac{1}{2}$ in. above the sample plate.
7. With the focus adjustment knob, raise the zoom tube approximately $\frac{1}{2}$ in.
8. Slowly move the stage by hand until the probe is viewed in the video microscope window. As required, adjust the focus, the lighting, and the position of the stage until the probe is in the center of the optical view.



A.5.2. Aligning the Inverted Microscope with the Cantilever

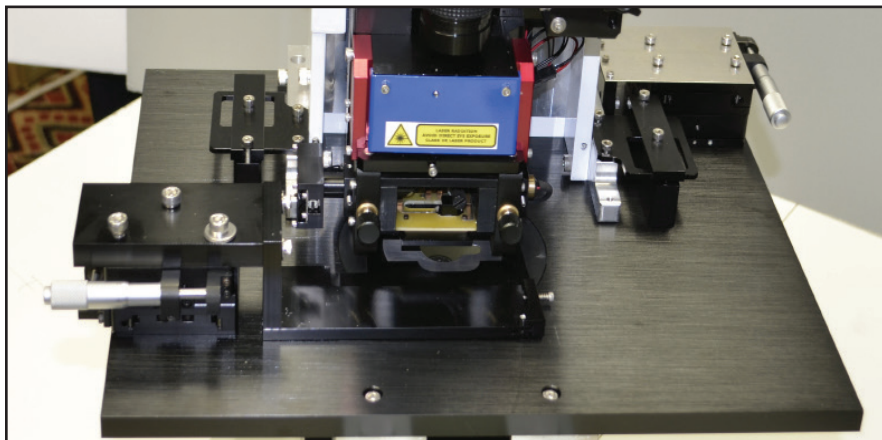
Viewing the probe through the inverted optical microscope requires moving the the entire AFM stage until the probe is directly over the optical axis of the inverted microscope. This alignment should be made with no sample in the microscope sample holder.

1. Using the Manual Z Motor Control, lower the probe such that it is at the focal plane of the inverted optical microscope.
2. Select the lowest magnification available in the inverted microscope turret.
3. Using the micrometer screws on the back right-hand side of the stage, move the LS-AFM stage until the probe is visible in the center of the inverted microscope image.



NOTE: The micrometers will move the LS-AFM stage in one direction only. To move in the opposite direction, the user must manually push the stage.

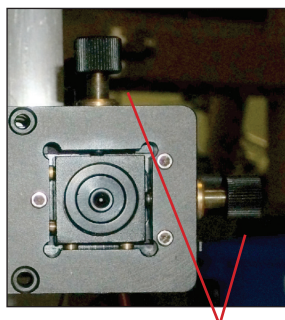
4. Tighten the lock down screws located on the left and right side of the LS-AFM stage. See the picture below.



A.6 First Alignment of the Light Lever

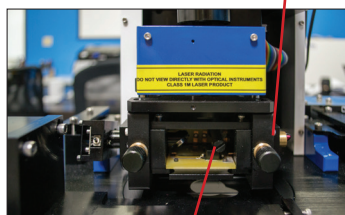
This procedure assumes that the light lever was correctly aligned at the factory or by an authorized agent.

1. Place a sample in the sample holder, and slide the sample holder under the light lever.
2. Place the probe holder in the light lever sensor with a contact or vibrating mode probe installed.
3. Adjust the optical microscope until the probe/substrate can be viewed on screen.
4. Center the laser and detector translation mechanisms with thumb screws.
5. Turn on the laser.
6. Use a flathead screwdriver to rotate the mirror until the laser spot is on the back side of the cantilever.



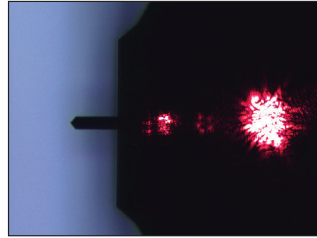
Translation thumb screws

Set screw holding laser

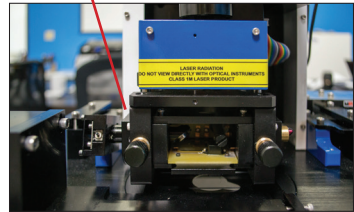


Rotating mirror mechanism

7. Lower the Z motor until the probe is just above the sample.
8. Rotate the mirror to move the laser light until it is visible on the probe.
9. Loosen the set screws that are holding the laser.
10. Slide the laser in or out until it is focused.
11. Tighten the set screws holding the laser in place.
12. Move the laser spot out to edge of the cantilever.
13. Release the set screws that hold the photo-detector in place.
14. Move the photo-detector in and out until the T-B signal is zero. Tighten the set screws.
15. Move the L-R signal to zero by adjusting the detector translation thumb screws.

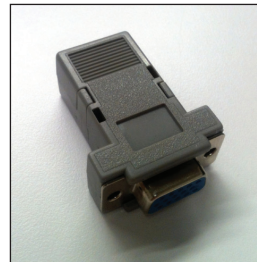


Set screw holding photo-detector



A.7 Grounding the LS-AFM

There are several grounding options for the LS-AFM. The green ground wire should be used to connect the stage with the Ebox. The probe and sample are grounded by a grounding connector, which should be attached to the Modes/IO connection on the back of the stage. A wire can be added to the ground connector to receive signals from the probe.



Appendix B: LS-AFM Files

The LS-AFM software has three separate files that it uses. The first is a parameter file which stores all of the parameters used for operating the microscope. The second is a scanner file, which is unique for each scanner and has range and calibration information. Finally, data files contain images measured by the microscope.

Parameter File	C:\Users\AFM User\Desktop\2.0.3 - 50 Micron AFM Workshop\data\afm_default.cfg
Scanner File	C:\Users\AFM User\Desktop\2.0.3 - 50 Micron AFM Workshop\data\scanner_default.cfg

B.1 Parameter Files

When the AFM-View software is launched, the default parameter file is used to set all of the parameters that are displayed on the screen. A new parameter file may be created from the default parameter file and stored with a different name. The new parameter file can be loaded in the Pre-Scan window. Below is a list of all of the functions in the parameter file.

```
***** = Scan NoVib | Proportional = 120           points_per_bin = 25
*      LS-AFM          * = Scan NoVib | Integral = 3000         Demod Gain = 4
*      AFM Workshop   * = Scan NoVib | Derivative = 0          Laser Control = 1
*      Atomic Force Microscope * = Scan Vib | Gain = 1462           Rotation = 0
*      Configuration File * = Scan Vib | Proportional = 200       Feedback_Limit = 0.040
*      Jan. 4, 2013    * = Scan Vib | Integral = 2000
*      ***** = Scan Vib | Derivative = 0
*
* [Display]
* Left Image = 1
* Right Image = 2
* Background = 1
* Display = 0
* Left Pallet = 4
* Right Pallet = 4
* Image Add % = 0.16
* Image Add Sw = 0
*
***** = [Tip App values]
* [Program]
* Testdata_path = "Test Data"
* Testdata_file_ext = ".wsf"
* Diagnostic_path = "Diagnostic"
* Tip_Scan = "0"
* Debug_flag = "0"
* Show Adv = "1"
* Scan_complete_wav = "Windows XP Shutdown.wav"
*
* [Tip Retract values]
* [Scan Mode]
* Scan Mode = 0
*
* [System Control]
* T-B_OFF = 0
* XYZ_relay_wait_time = 1.0
* Scan Rate limits = 2,2,2,2,2
* Samples/Pixel limits = 25,25,25,25,25
*
* [Z Piezo Settings]
* Z HV Gain = 7
* Z Sensor Gain = 640
*
* [XY Piezo Settings]
* XY Acceleration = 900
* XY Velocity = 0
* XY HV Gain = 15
* X Sensor Gain = 5
* Y Sensor Gain = 5
*
* [Scanning]
* scan type = 1
* state = 0
* First_flag = 0
* new_line_scan_data = 0
* Current Vertical Position (um) = 21.465
* line_index = 103
* num_lines = 256
* <_A (um) = 2.500
* <_B (um) = 17.500
* r_A (um) = 27.500
* r_B (um) = 12.500
* line_delta = 0.06
* scan_rate (Hz) = 0.40
* size (um) = 15.000
*
* [Motor]
* Drive (%) = 50
* Acceleration (microsteps/sec^2) = 1000
* Velocity (microsteps/sec) = 1034
* Position = 0
* Jog Size (um) = 1.0
*
* [Tune Frequency]
* Amplitude (V) = 0.050
* Phase (deg) = 90.00
* Lower Freq (Hz) = 175865
* Upper Freq (Hz) = 177429
* Selected Freq (Hz) = 176648
* Num Steps = 50
* Tune period (ms) = 500
*
* [Z Piezo Control]
* Tip Approach NoVib | Gain = 2048
* Tip Approach NoVib | Proportional = 64
* Tip Approach NoVib | Integral = 500
* Tip Approach NoVib | Derivative = 0
* Tip Approach Vib | Gain = 1024
* Tip Approach Vib | Proportional = 64
* Tip Approach Vib | Integral = 1000
* Tip Approach Vib | Derivative = 0
* Scan NoVib | Gain = 2561
```

B.2 Scanner Files

The scanner file has the calibration and range information used by the AFM-View software. The contents of the file are:

```
***** =
*           LS-AFM |           * =
*           AFM Workshop       * =
*           Atomic Force Microscope * =
*           Scanner File       * =
*           Jan. 4, 2013       * =
*                               * =
***** =

[System Information]
System_Serial_No. = 0
Firmware_Revision = 0
DAQ_Serial_No. = "193D3C0"

[Range Check]
Max X = -1970405377.0
Max Y = -1966473217.0
Min X = 1646133249.0
Min Y = 1650065409.0
Margin (%) = 3.0

[Cal Size]
Cal Size (um) = 40.0
Zoom Box Increment = 3
Range Span (counts) = 3625713666
Z_Range (um) = 8

[Calibration]
X_cal = 0.6442
Y_cal = 0.7116
Z_cal = 0.7124

[XY Positioning]
X Scale = 1
Y Scale = 1
X Offset = 0
Y Offset = 0

[XY Piezo Control (GUI)]
X_Gain(%) = 0.00000000
Y_Gain(%) = 0.00000000
X_Proportional = 256
Y_Proportional = 256
X_Integral = 2096
Y_Integral = 2096
X_Derivative = 0
Y_Derivative = 0
```

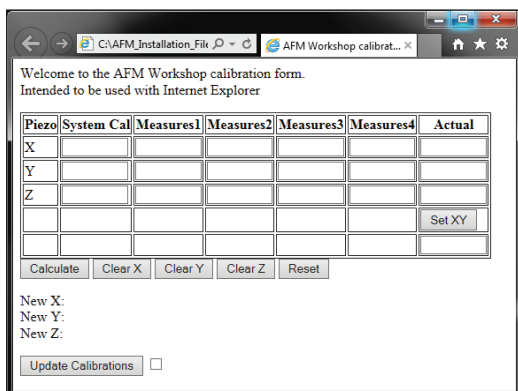
B.3 Data Files


LS-AFM data files are stored in a text format. At the top of the data file are the parameters used for the scan.

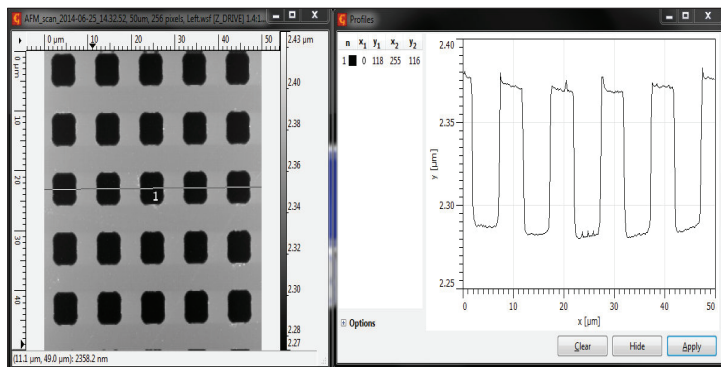
Appendix C: Calibration

Calibration of the LS-AFM requires scanning a standard sample that has known X,Y and Z dimensions. If a sample with squares is used, the pitch in X and Y should be around 10 μm . The height of the features in Z should be around 100 nm.

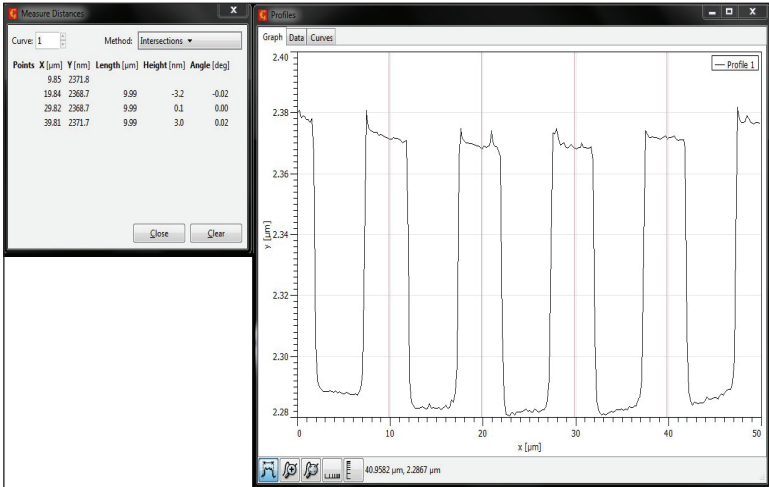
1. Scan a standard sample with known dimensions in X,Y and Z in both X (0°) and Y (90°).
2. Open the AFM Calibration software. Make sure to “allow blocked content.”



3. Insert the current calibration values, located on the System tab, under “System Cal” in the calibration software.
4. Insert the known values for the calibration standard in the “Actual” column in the calibration software.
5. To calibrate X, open the completed 0° scan in Gwyddion and level the image.
6. Extract a profile  over the nicest row of squares, in this case, and click “Apply.”



- Using the “Measure Distances” feature on the bottom left-hand corner of the extracted profile, create vertical lines that bisect each square at approximately the same point.



- Insert the values in the “Length” column into each X Measures blanks in the calibration software.

Welcome to the AFM Workshop calibration form.
Intended to be used with Internet Explorer

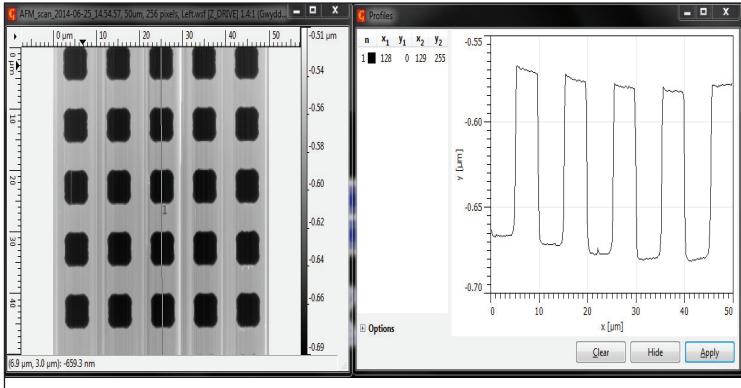
Piezo	System Cal	Measures1	Measures2	Measures3	Measures4	Actual
X	.65	9.99	9.99	9.99		10
Y	.65					10
Z	.7					100
						Set XY

Calculate Clear X Clear Y Clear Z Reset

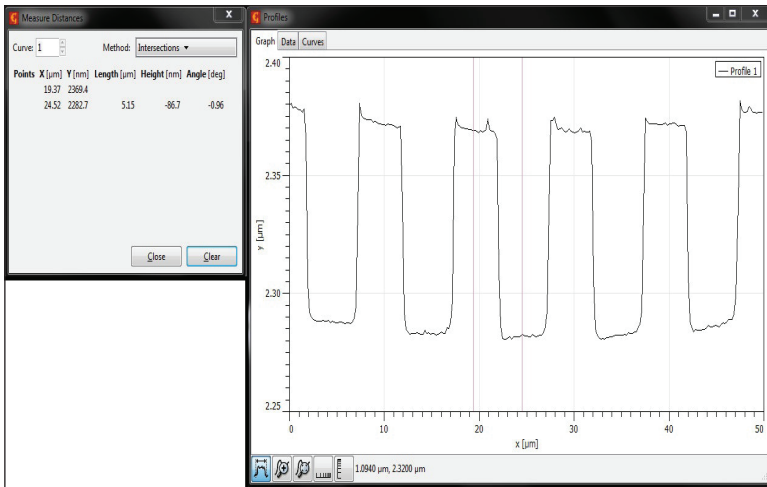
New X:
New Y:
New Z:

Update Calibrations

- Repeat steps 5 - 8 with the completed 90° scan to obtain Y Measures values. Make sure to extract a profile along the best looking column of squares in the Y-direction.



- To calibrate Z, the user may either use the 0° or 90° scan. Extract a profile, and measure distances from the top of each square to the bottom of each pit. Enter the Height values in the Z Measures blanks in the calibration software.



11. Once the calibration software is completely filled out, select “Calculate” to obtain the new calibration values. Enter these values in the System tab and click “Save.”

Welcome to the AFM Workshop calibration form.
Intended to be used with Internet Explorer

Piezo	System Cal	Measures1	Measures2	Measures3	Measures4	Actual
X	.65	9.99	9.99	9.99		10
Y	.65	9.96	10.03	10.25		10
Z	.7	86.7	88.3	86.3	85	100
						Set XY

Calculate Clear X Clear Y Clear Z Reset

New X: 0.6493493493493494
New Y: 0.6551587301587302
New Z: 0.8085475021657522

Update Calibrations

Calibration

X_cal
0.5539

Y_cal
0.5176

Z_cal
0.7578

Save

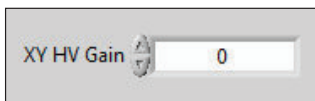


NOTE: To obtain the most accurate calibration values for the scanner, the user may have to complete multiple calibration iterations.

Appendix D: Noise Floor

To measure the noise floor, it is necessary to scan the sample with no voltage applied to the X and Y piezoelectric ceramics. The AFM-View software can measure the structural noise of the system when the tip is in feedback with the surface.

1. Make sure the tip is a sizeable distance from the surface. Initiate the Tip Retract function, if necessary.
2. Under the System tab in XY Parameters, change the value of the XY HV Gain to zero. This shuts off the voltage to both the X and Y ceramics.



3. Under the Topo Scan tab, set the scan parameters for noise measurements:
 - Scan Size = 0.5 μm
 - Scan Rate = 1 Hz
 - Scan Lines = 512
 - X,Y Center = (2, 2)
 - Left, Right Image = Z-Drive, Z-Error
4. Using the Manual Z Motor Control, move the probe toward the sample until the probe begins to come into focus.
5. Initiate Automated Tip Approach.
6. Begin a scan. It is not necessary to complete a whole scans; 50 scan lines is plenty.
7. To analyze the noise floor measurement, open the completed Z-Drive scan in Gwyddion.

8. Crop the data, if necessary.

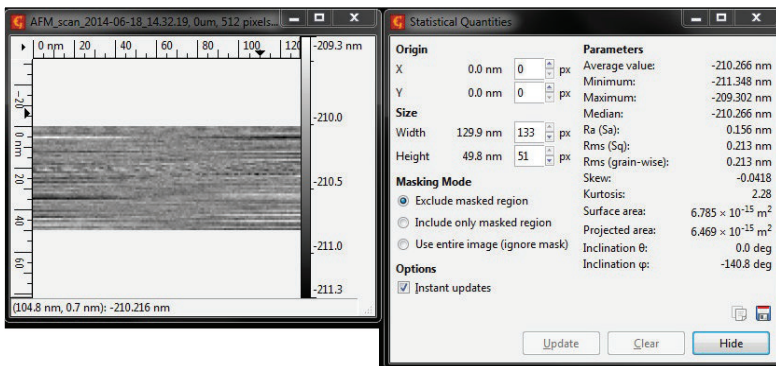
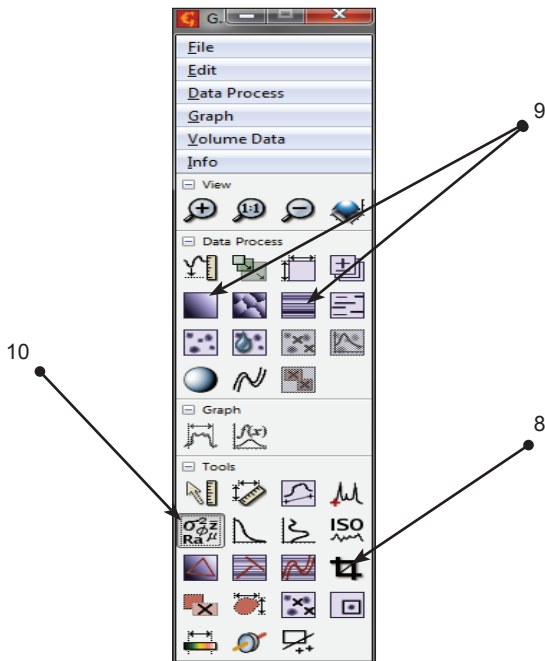


9. Level the image by pressing the “Level data by mean plane subtraction” button and the “Correct lines by matching height median” button.



10. Find the noise value with the “Statistical Quantities” button, under Ra (Sa) for surface roughness.





Noise can be reduced by lowering the Z HV Gain in the System tab. When the Z gain is lowered, a new tip approach must be initiated because the Z ceramic's dynamic range has been reduced.



CAUTION: Increasing the Z HV Gain will increase the Z ceramic's dynamic range, and will expand the Z ceramic (and probe) toward the sample. Make sure the tip is removed from the surface before increasing Z HV Gain.

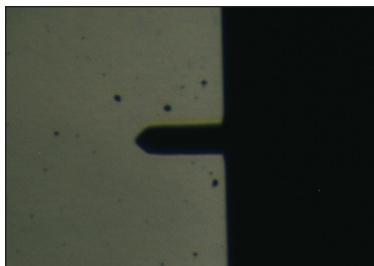
Appendix E: Probes

There are two major types of probes, vibrating mode and non-vibrating mode probes. Each of these probes may have a coating, which makes them more reflective.

1. Vibrating Mode (VM) Probes

Vibrating mode probes are in the shape of a rectangle. Typical VM probe specifications:

Resonance Frequency	150 – 350 kHz
Length	125 – 225 micron
Width	30 – 40 micron
Thickness	0.5 – 1 micron
Force Constant	30-50 N/M

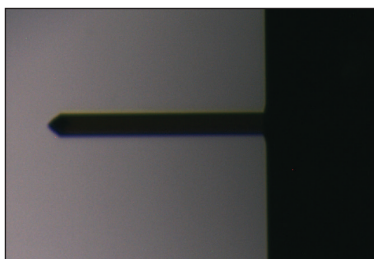


TM190-A or ACLA probes from Applied Nanostructures recommended.

2. Non-Vibrating Mode (NVM) Probes

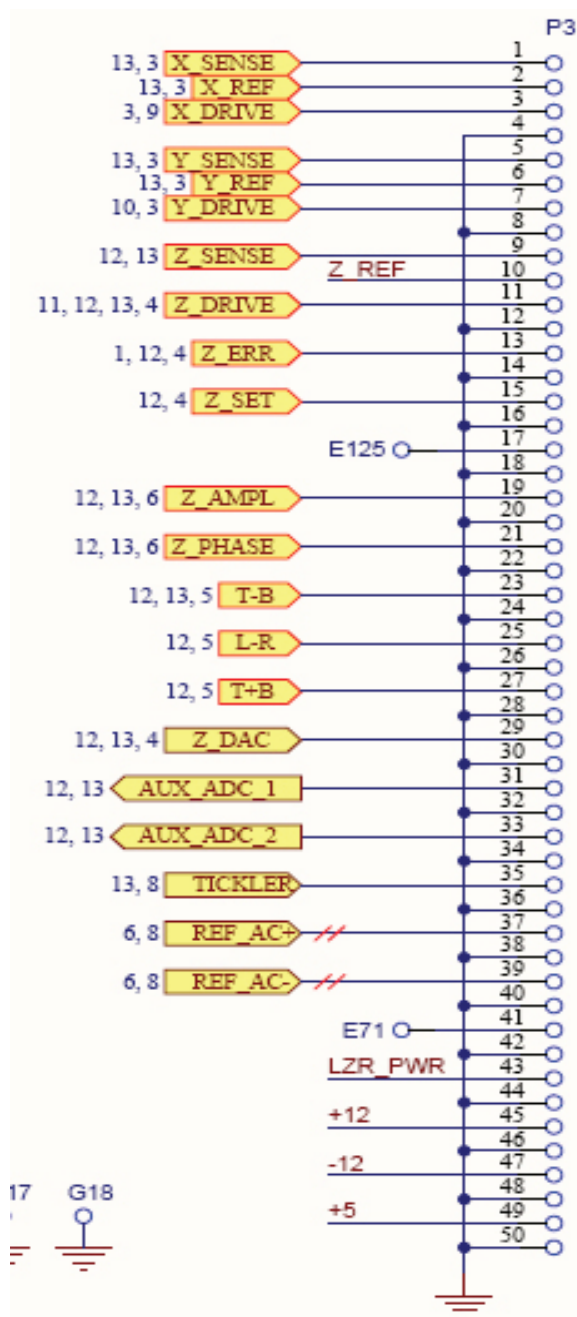
Non-vibrating mode probes can be in the shape of a V or rectangular. Typical NVM probe specifications are:

Resonance Frequency	150 – 350 kHz
Length	225 – 450 micron
Width	30 – 40 micron
Thickness	0.1 - 0.5 micron
Force Constant	0.1 – 0.5 N/M



CM28-A probes from Applied Nanostructures recommended.

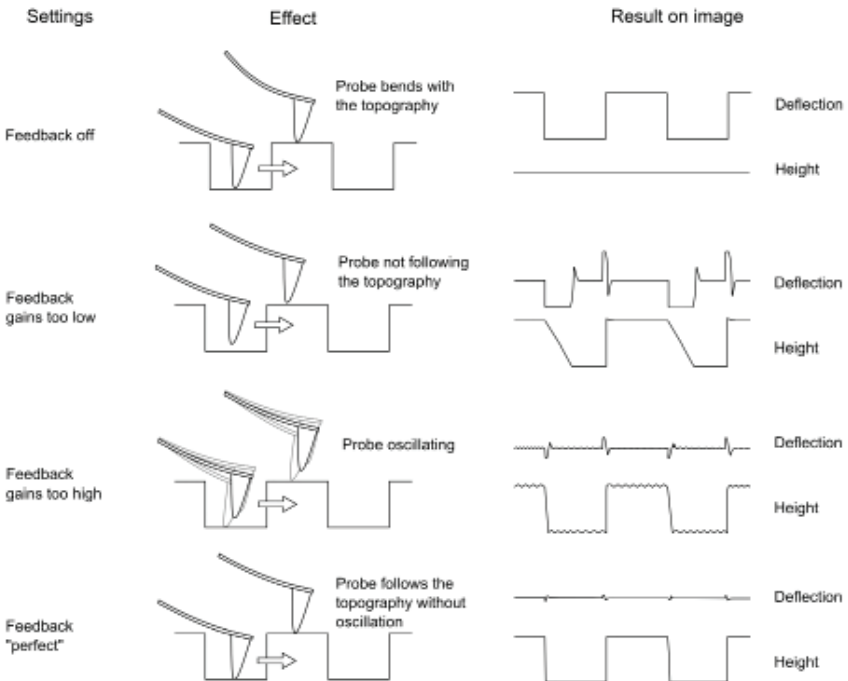
F.2 Ebox and Modes Pin Assignments



Appendix G: Optimizing GPID Parameters

The Z feedback scan parameters, such as the setpoint voltage and the GPID parameters, are to be adjusted as the line scan is being made. The goal for adjusting these parameters is to have the probe perfectly track the surface. The probe is tracking the surface when the Z error image has a minimal signal. Establishing the optimal conditions requires practice and some intuition.

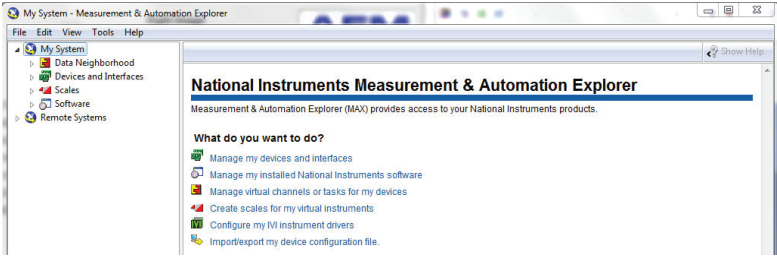
When first learning to operate an AFM, it is helpful to scan a reference sample in 2D and adjust the GPID settings to see the effect on the Z voltage and the Z error signal, as shown in the figure below.



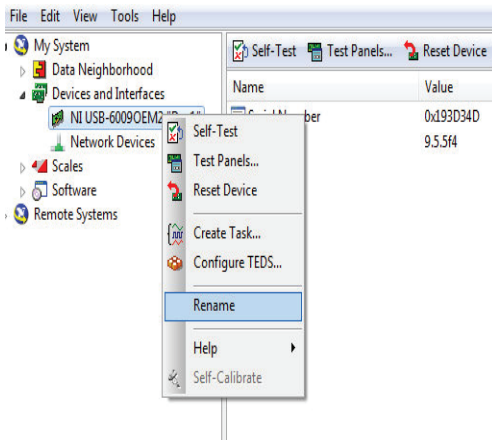
Appendix H: Renaming National Instruments DAQ Device

The following procedure will allow the user to reset the National Instruments DAQ card registry with the device number corresponding with the user's Ebox. This may be useful if the user's AFM-View Software does not initialize.

Open the NI MAX: Measurement and Automation Explorer software as follows:

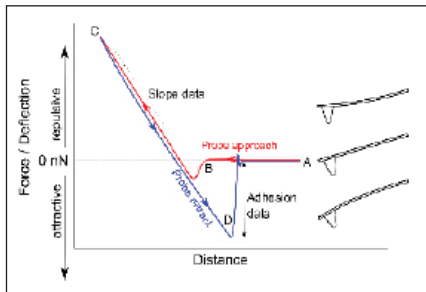


- a. Expand Devices and Interfaces under My System on the left scroll tab.
- b. Making sure the correct Ebox USB is plugged into the computer, delete any devices that are not green. There should only be one green device, corresponding with the DAQ card in the user's Ebox.
- c. Rename the only remaining green device as Dev1. This can be accomplished by right-clicking on the device and selecting Rename.

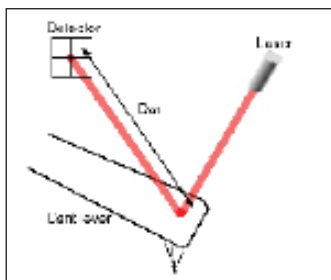


Appendix I - Analyzing Force/Distance Curves

A force/distance curve is a graph that shows the amount a cantilever tip bends as it is systematically pushed into a sample. The probe may be lowered onto the surface, or the surface may be raised into the probe.



What is actually measured is the deflection of the cantilever, or the Top - Bottom (T-B) signal of the photo-detector.



The deflection of the cantilever can be converted to Force using Hook's law:

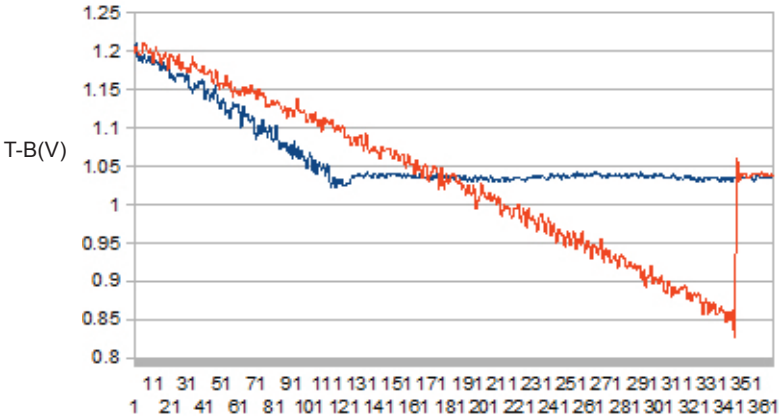
$$\text{Force} = -K * d$$

K = force constant

d = deflection of the cantilever

In a light lever AFM, the user must convert the T-B signal into the cantilever deflection. This is achieved by measuring the change in the T-B signal as the sample is directly pushing against the probe. This is labeled slope data in the first figure above. The force constant for the cantilever is supplied by the manufacturer.

An AFM force/distance curve is illustrated in the figure below. The data is measured using a TM190-A probe from Applied Nanostructures on a hard silicon surface. Data was analyzed using an industry standard spreadsheet program. The blue line shows the curve measured as the sample is moved towards the probe, and the red line is the curve measured as the sample is pulled away from the probe.



Blue – Sample Up
 Red – Sample Down

The spring constant for TM190-A probes is 40 N/m. Thus, to calculate the adhesion force from the data in the figure above:

$$F_{ad} = ((1.03 - .85 \text{ v}) / (.05 \text{ v/nm})) * 40 \text{ nN/nm} = 7.2 \text{ nN}$$

Appendix J: Inverted Optical Microscope

J.1 Precautions When Packing/Unpacking the Microscope

J.1.1 Release the Transport Lock of the Revolving Nosepiece

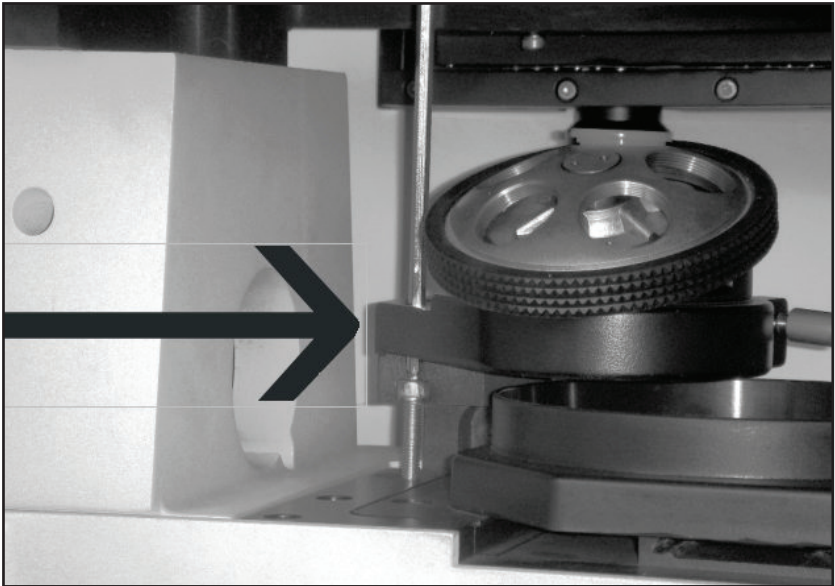
1. Loosen the screw of the clamping rod using the screwdriver provided with the inverted microscope.
2. Rotate the coarse and fine adjustment knobs in the direction of the arrow and remove the clamping rod. Retain the clamping rod and screw because they will be used again the next time the microscope is transported.



CAUTION: Never attempt to rotate the coarse or fine adjustment knob without removing the clamping rod. Otherwise, the focusing mechanism may be damaged.

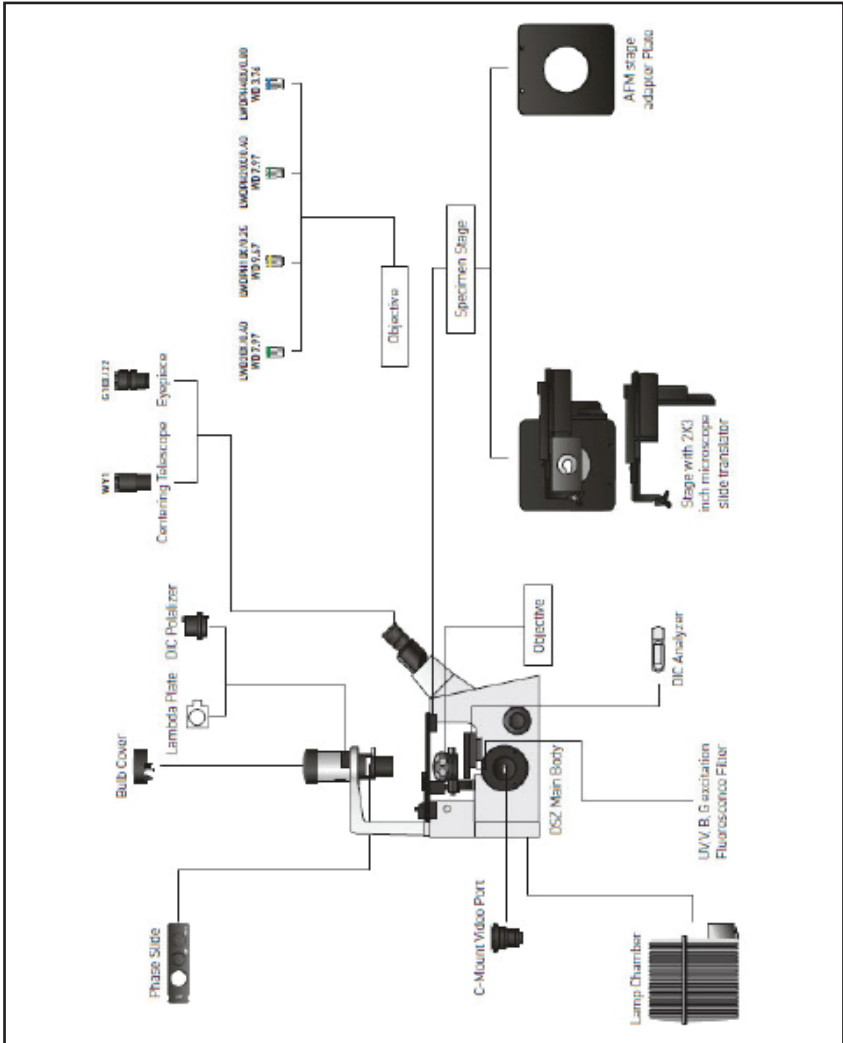
J.1.2 Stage

Before transporting the stage, fix the flexible knobs with pieces of adhesive tape so that they will not move.



J.2 Module Nomenclature

This diagram illustrates all of the components provided with the AFMWorkshop inverted microscope. Items required for fluorescence microscopy are not shown here but are provided.



J.3 Preparing the Inverted Microscope for Use

Please refer to the diagram on page 63 for the names of the following items.

J.3.1 Attaching the Eyepieces

1. Remove the eyepiece dust caps.
2. Insert the G10X eyepiece into the eyepiece sleeve.

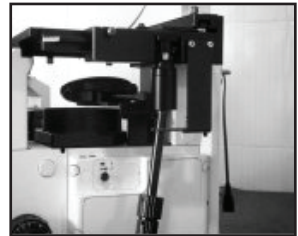


J.3.2 Attaching the Objectives

1. Remove the stage center plate.
2. Attach the objectives to the revolving nosepiece through the hole by the left-hand side of the stage plate.
3. Attach the objectives in such a manner that the magnification increases from lower to higher powers in the clockwise direction.



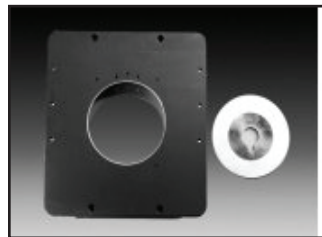
NOTE: In the inverted microscope, the front lens of the objective faces upward, and is therefore more exposed to contamination than the top view optic.



J.3.3 Attaching the Fixator of the Universal Handwheel

This is an optional piece, which may be attached according to user demand.

Join the fixator to the stage and to the connecting rod with hexagonal screws.



J.3.4 Attaching the Stage

The stage is designed to be very thin so that the objective will not make contact with the stage when the revolving nosepiece is rotated.



CAUTION: Do not subject the stage to impact or excessive force, as this may deform it.



1. Gently place the stage on the microscope frame by aligning the stage mounting holes with the threaded holes on the frame.
2. Insert the provided screws into the mounting holes and tighten.

J.3.5 Attaching the Holder for 76mm * 76mm slice

This part is used for the observation of the 76mm * 76mm slice.

1. Open the spring clip of the rectangular stage via the flexible right handle.
2. Slide the holder into position.
3. Be sure that the rectangular groove is facing upward.
4. Gently loosen the spring clip, and place the slice in the holder.



NOTE: Cables and cords are vulnerable when bent or twisted. Never subject them to excessive force.

J.3.6 Connecting the Cables

1. Turn the main power supply switch to "O" (OFF).
2. Connect the plug of the lamp housing or illumination column to the connector.
3. Connect the manual controller.





NOTE: Make sure that the power supply is properly grounded.

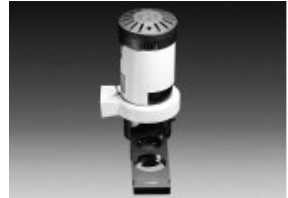


CAUTION: Make sure the cables do not come into contact with the lamp housing, to avoid melting and shock hazards.

J.3.7 Attaching the Phase Contrast Optical Slider

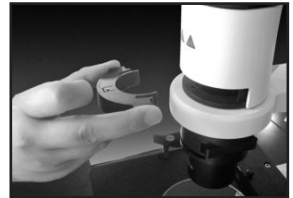
This is an optional part for the observation of phase contrast. The phase contrast slide should be matched with the objectives when the inverted microscope is in use. The phase contrast slide option can be removed when no relevant observation is conducted.

1. Attach the phase contrast slider to the illumination column
2. Face the slider's indication surface upward. Make sure that the finger hook position is on the right.



J.3.8 Attaching the Filters

1. Select the appropriate filter.
2. Hold the filter by its edge, to avoid leaving fingerprints on the filter.
3. While holding the mounting lever of the filter holder, insert the filter.

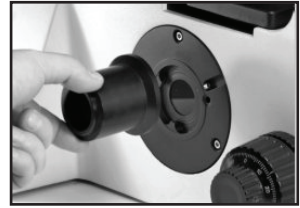


NOTE: After the illumination has been ignited, the filter will be very hot. Allow the filter holder and filters to cool before replacing them.

4. Engage each filter in the light path by moving the filter holder in the direction of the arrow.

J.3.9 Attaching the Video Microscope

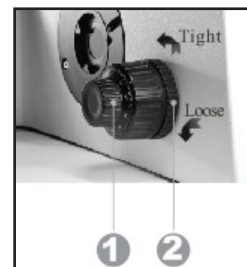
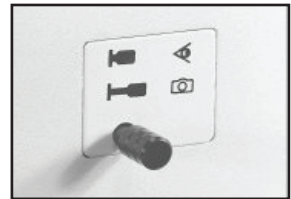
1. Loosen the clamping screw on the side port, and remove the dust cap.
2. Connect the video camera to the adaptor provided.
3. Insert the video camera and adaptor into the side port.



J.4. Operation of the Inverted Microscope

J.4.1 Transmitted Light Brightfield Observation Procedure

1. Set the main power switch to on (I) and adjust the intensity of illumination.
2. Make sure the light intensity control knob is in the MIN (minimum intensity) position.
3. Rotate the light intensity control knob to MAX (maximum intensity) to increase the intensity and illumination brightness.
4. When the microscope system will not be in use for a prolonged period of time, turn the main power switch to Off (O).
5. Disengage the filter from the light path.
6. Select the light path with the light path selector lever. The lever allows the user to switch between eyepiece observation and video camera viewing.
7. Place a specimen on the stage.
8. Engage the 10X objective in the light path.



9. Bring the specimen into focus.
 - a. Rotate the coarse or fine adjustment knob to raise and lower the objective, bringing the specimen into approximate focus.



NOTE: If the objective cannot be easily focused, or lowers on its own accord, the coarse adjustment knob tension is set too low. In this case, turn the rotation tension adjustment ring to increase tension.



NOTE: Locking the pre-focusing lever limits the upper position of coarse adjustment, preventing collision between the specimen and objective.

- b. Adjust the brightness
- c. Adjust the interpupillary distance.



While looking through the eyepieces, adjust the binocular vision until the left and right fields of view coincide completely.

- d. Adjust the diopter.



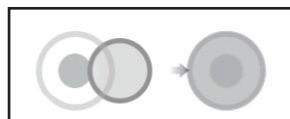
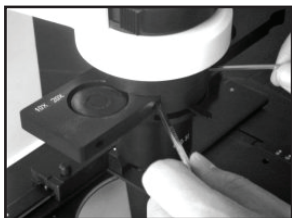
While looking through the left eyepiece, rotate the diopter adjustment ring on the left eyepiece to bring the specimen into focus.

While looking through the right eyepiece, adjust the coarse/ fine adjustment knobs to bring the specimen into focus.

- e. Adjust the aperture iris.
- f. Insert the filters.
- g. Begin observation.

J.4.3 Phase Contrast Observation Procedure

1. Set the main power switch to On.
2. Disengage the filter from the light path.
3. Select the light path with the light path selector lever.
4. Attach the phase contrast slider.
5. Engage the objective matched with the phase contrast slider in the light path.
6. Disengage the 10X eyepiece and insert the WY1 central telescope.
7. Adjust the aperture iris.
8. Adjust the phase contrast.



Using the optical element centering knobs, turn the two centering screws of the phase contrast slider so that the ring slit image overlaps with the phase plate of the objective.

9. Disengage the WY1 central telescope and insert the 10X eyepiece.
10. Bring the specimen into focus.
11. Adjust the brightness.
12. Adjust the interpupillary distance and diopter.
13. Engage the appropriate objective and bring the specimen into focus.
14. Adjust the aperture iris and field iris diaphragms.
15. Engage the required filters.
16. Adjust the brightness.
17. Begin observation.

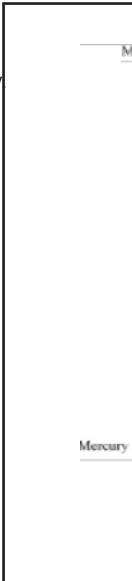
J.4.4 Fluorescence Observation Procedure

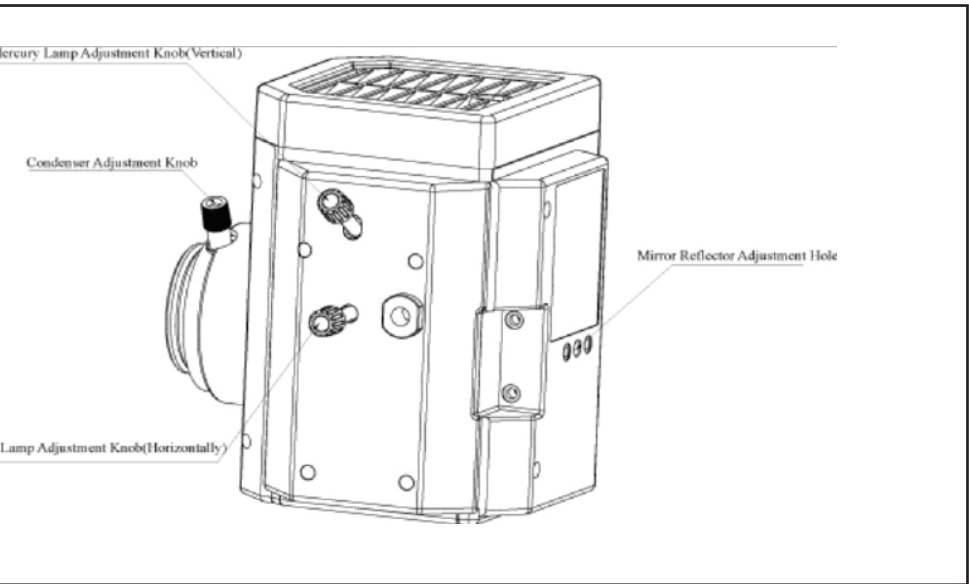
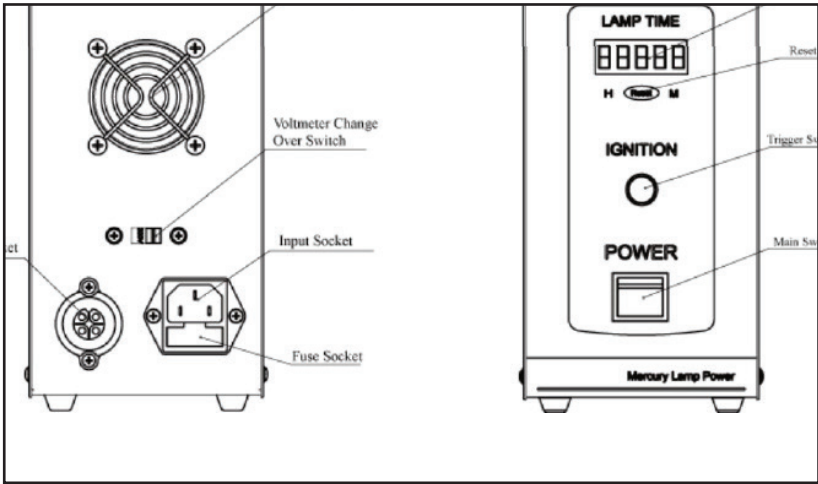


NOTE: Confirm supply voltage conforms to the following requirements before inserting the mercury lamp plug into the socket:

Input Voltage AC: 220V/110V±15% at 50HZ

1. Turn on the mercury lamp. It may take more than 10 minutes to reach the maximum luminous efficiency.
2. Engage a 10X objective in the light path.
3. Place the specimen on the stage.
4. Rotate the X-axis and Y-axis knob to move the specimen to a desired position.
5. Rotate the fluorescence module turret to select the right dichroic mirror unit in the light path.
6. Adjust the coarse/fine adjustment knob to make the image clear.
7. Adjust the mercury lamp center.
8. Rotate the condenser axial adjustment knob to focus the light.
9. Adjust the mercury lamp to center on the viewing field.
10. Rotate the condenser axial adjustment knob to illuminate the viewing field evenly.





J.5 Troubleshooting Guide

If problems occur, please review the following list and take remedial action as indicated. If you cannot solve the problem after checking the entire list, please contact your local representative for assistance.

Problem	Cause	Remedy
1. Optical System		
a) The bulb does not light.	Power cord of the power supply unit is unplugged.	Plug in the power cord.
	Main switch of the power supply unit is not ON.	Switch the main power switch to "I"(on).
	The bulb is burnt out.	Replace the bulb
b) The bulb lights but the field of view is dark.	The voltage is too low	Increase light intensity.
	Revolving <u>nosepiece</u> is not in a click position.	Make sure that the <u>revolving nosepiece</u> clicks properly into place.
	Light path selector knob is set for the side port light path.	Set the knob to the binocular eyepiece light path position.
	Too many filters are used.	Reduce the filters to the minimum required.
	The stage central plate is engaged in the optical path.	Move the stage and place the specimen again.
c) Field of view is obscured or not evenly illuminated.	Light path selector knob is set to an intermediate position.	Set the light path selector button to a click position according to the purpose.
	Revolving <u>nosepiece</u> is in an intermediate position.	Engage the revolving <u>nosepiece</u> at a click stop.
	A filter is stopped in an intermediate position.	Set the filter at the appropriate position.
	The frost filter is not engaged.	Engage the frosted glass.
d) The image glares.	Aperture iris diaphragm is stopped down too far.	Adjust the aperture iris diaphragm.
f) Visibility of the image is poor: Image is not sharp. Contrast is poor. Details are poorly visible.	The front lens of the objective is dirty.	Clean the objective.
	Inappropriate slide or cover glass thickness.	Change the slide for one with suitable thickness.
	The optical parts are covered with dust.	Clean them all.
	Ring slit and phase plate are not centered.	Center them correctly.

Problem	Cause	Remedy
f.) Visibility of the image is poor: Image is not sharp. Contrast is poor. Details are poorly visible.	Poor contrast during observation.	Replace the plastic culture vessel with a glass vessel.
g) The image is blurred.	Objective is engaged incorrectly in the light path.	Make sure that revolving <u>nosepiece</u> clicks into place correctly.
	Specimen is tilted with respect to the stage.	Place the specimen correctly on the stage and secure it with the specimen holder.
h) Field of one eye does not match that of the other.	The <u>interpupillary</u> distance is incorrect.	Adjust the <u>interpupillary</u> distance.
	Incorrect diopter adjustment.	Adjust the diopter.
i) The coarse/fine adjustment knobs will not rotate easily or at all.	The transport lock is not released	Remove the transport lock.
	The rotation tension adjusting ring is too tight.	Loosen it moderately.
	The <u>pre-focusing</u> lever is locked.	Unlock it.
j) The revolving <u>nosepiece</u> lowers by its own weight or <u>defocusing</u> occurs due to slipping of fine adjustment.	The tension adjustment ring is too loose.	Tighten the ring optimally.
The coarse focus adjustment cannot move the objective above a certain level.	The <u>pre-focusing</u> lever limits the lower limit.	Unlock the <u>pre-focusing</u> lever.

J.6 Replacing the Halogen Bulb



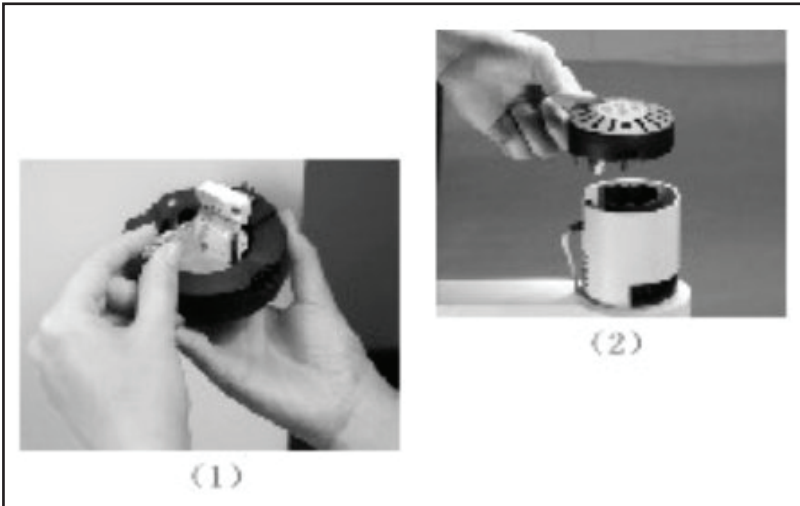
NOTE: The halogen bulb, lamp socket and surrounding area will be extremely hot during and right after use.

1. Set the main switch to Off. Disconnect the power cord from the wall outlet. Allow the old bulb and lamp socket to cool before removing the bulb.
2. Remove the lamp socket and old bulb as shown.



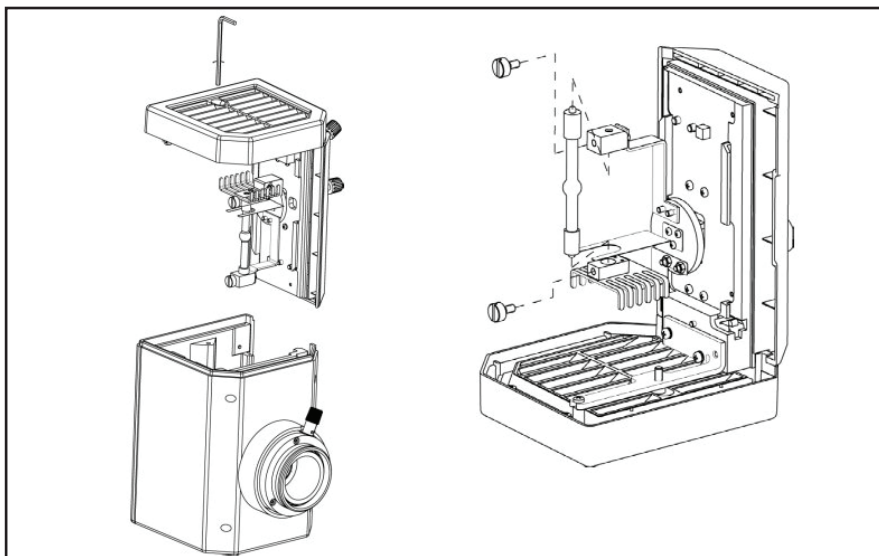
NOTE: Do not touch the new halogen bulb directly. If the bulb is contaminated with fingerprints, etc., wipe carefully with a soft cloth to prevent cracking of the bulb when it is heated.

3. Hold the new halogen bulb with gloves or a piece of gauze.
4. Align the bulb pins carefully into the pin holes on the lamp socket.
5. Push in gently. If an excessive force is applied or the bulb is twisted, the bulb may be damaged.
6. To reattach the lamp socket, insert the plug into the socket and push the guide pins gently into the guide holes.



J.7 Replacement of the Fluorescence Mercury Lamp

1. Turn off the power of fluorescence.
2. Loosen the screws on the mercury lamp box.
3. Take the lamp holder out slowly.
4. Loosen the two screws on the mercury lamp, and exchange the old lamp with the new.
5. Tighten the two screws on the new mercury lamp, and put the unit back in the holder,



J.8 Maintenance of the Inverted Microscope



NOTE: Do not attempt to disassemble any part of the microscope.



NOTE: Do not use organic solvents on the objective extremity, as these will deform the electrically insulated section of the extremity. Do not use organic solvents on plated or painted surfaces.

1. Clean the oil immersion objective after every use.
2. Clean all glass components by wiping gently with **gauze**. To remove fingerprints or oil smudges, wipe with **gauze** slightly moistened with **a mixture of ether (75%) and alcohol (25%)**.
3. Clean the painted and plated surfaces with neutral detergent.
4. Clean plastic surfaces with clear water.
5. When not using the microscope, set the main switch to Off, confirm that the lamp housing is cool, and cover the microscope with the provided dust cover.