PicoSPM User's Manual

Version 2.4

Molecular Imaging

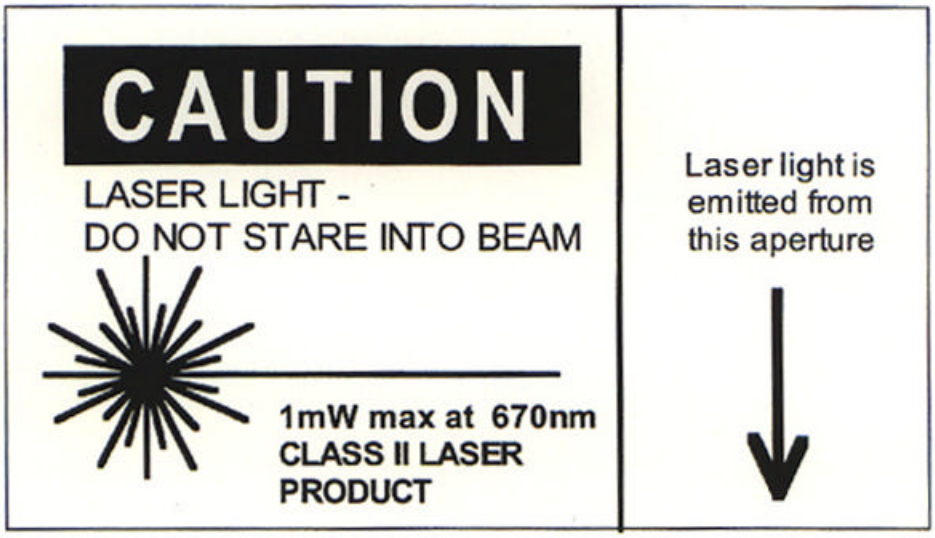
CAUTION – LASER SAFETY INFORMATION

READ THIS SECTION FIRST!

The PicoSPM is designed to be used with a class II diode laser with an output of up to 1 mW of visible radiation at 670 nm. The aperture in the AFM scanning head is labeled with the logotype (shown below). DO NOT stare directly into the laser beam. To ensure safe operation, the scanner must be operated and maintained in accordance with the instructions included with the laser. The laser must only be powered by a controller that includes an on/off switch, such as the PicoScan controller. DO NOT attempt to make any adjustments to the laser, the laser's electronics, or optics. If laser malfunction is suspected, immediately return the scanner back to MOLECULAR IMAGING for repair or replacement; there are no user-serviceable parts.

WARNING! *Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous light exposure. Furthermore, the use of optical instruments with this product may increase eye hazard.*

In accordance with federal FDA requirements, the following laser precaution is affixed to the scanner:



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INTRODUCTION

SCANNING PROBE MICROSCOPY

The advent of high-resolution scanning probe microscopy (SPM) occurred in the early 1980's with the invention of the scanning tunneling microscope (STM) by Binnig and Rohrer. The STM scans a very sharp conducting tip over a conducting surface, holding the two just a few atomic diameters apart, so that electric current can flow between the two by means of quantum mechanical tunneling. The rapid decay of tunnel current gives the STM remarkable sensitivity to small features on the surface, so that atomic resolution is easily attained by moving the tip over the surface in a raster scan and measuring the height changes needed to maintain a constant tunneling current. In 1986, Binnig and Quate introduced the atomic force microscope (AFM), a scanning probe microscope for insulating surfaces. The key point was that a macroscopic spring and two atoms bonded together have about the same stiffness, therefore sweeping a weak spring (or cantilever) across a surface will not displace the atoms significantly. In the AFM, small deflections of the cantilever are sensed optically as the cantilever is raster scanned over the surface of a sample. Since there are many short range and long-range interactions between atoms, true atomic resolution can be harder to obtain with AFM, but it is possible in many systems.

Many variants of the original STM and AFM have been developed, and they all work in a similar manner. A sharp sensor is moved back and forth in the plane of the sample (the X and Y plane) while its height (the Z range) is controlled using a sensor signal sensitive to an interaction between the probe and the surface. A map of this control signal as a function of position (X-Y) forms an image of the surface properties. An excellent fundamental exposition of STM (and some AFM) is to be found in Julian Chen's *Introduction to Scanning Tunneling Microscopy*, Oxford University Press, 1993.

THE PICOSPM™ SYSTEM

The early work in SPM was carried out using elaborate instruments mounted in an ultrahigh vacuum (UHV) chamber, allowing cleaning and precise control of the surface under investigation. However, one of the great virtues of SPM is the ability to operate in non-vacuum environments. Much work has been carried out in ambient conditions, but it is hindered by the difficulty of controlling or characterizing surfaces. The PicoSPM system is designed for studying controlled interfaces outside an UHV. It is the first SPM of its kind: The scanning probe (STM or AFM) can be used in a hermetically-sealed environmental chamber. The probe protrudes into the hermetically-sealed chamber through a flexible seal. The sample is then placed on a magnetically-held stage, which is easily exchanged and cleaned. The sample stage is also equipped with a simple liquid cell; specially designed so high-quality electrochemical and biological experiments can be carried out *in-situ* in the microscope chamber. The environmental chamber is connected to a gas-flow system so the atmosphere can also be controlled. In addition, provisions are made for fluid lines that can be used to exchange the solution during imaging. The scanner (STM or AFM) is a small cylindrical plug-in module. The scanning techniques (the scan range capabilities) are changed by simply exchanging a module. Within these conditions,

the microscopes are capable of atomic resolution, nearly drift-free operation, and clean environmental and electrochemical control. To maximize the microscope's capabilities, familiarity with the microscope and the recommended procedures is required. Therefore, it is in your best interest, and the interest of your experiments to carefully read and understand this manual.

Molecular Imaging also supplies an entire range of ancillary products – from insulated tips and various cantilevers (MAClevers), to isolation chambers and video access – all designed to support high-quality studies at controlled interfaces. Furthermore, Molecular Imaging has on hand a large and diverse staff of application scientists to advise customers in various chemistry, electrochemistry, materials sciences, physics, and biological microscopy techniques. Our scientist are continually developing new applications, pushing the envelope of what SPM can really do for you.

ENVIRONMENTAL AND ELECTROCHEMICAL CONTROL

Electrochemistry has often been regarded as difficult or arcane, in part because of the lack (until now) of atomic resolution microscopy, and because of the need for elaborate procedures for experiments. The PicoSPM system removes many of these obstacles. Electrochemistry offers all the advantages of environmental control, coupled with the enormous power and control of electric potential differences across interfaces. It has been called "surface science with a joystick." We will outline some of these key concepts in this manual.

ENVIRONMENTAL CONTROL

Before considering the complications of electrochemical control, we will describe the first step, which is control of the environment. By this, we mean imaging outside of the UHV, but still in a controlled environment. Environmental control offers important advantages over ambient conditions. Inevitably, a surface prepared outside of UHV is contaminated, most often with hydrocarbons and water molecules. These do not appear in SPM images because these molecules diffuse freely on many surfaces (they do not stay in one place long enough to form an image). Although they are not imaged, they can have an important effect on friction (in AFM) or on the stability of molecules that are weakly bound to the surface. For most applications, the investigator is interested in molecules and atoms that react chemically with the surface. In this case, retarding spurious reactions with reactive contaminants in the laboratory environment is adequate (UHV is not required). For such experiments, the PicoSPM can often be placed in ambient conditions with further contamination being prevented by running clean argon (Ar) or nitrogen (N_2) through the environmental chamber.

Another degree of control comes from covering the sample with water or some other clean, inert fluid. If conditions are such that convective stirring of the fluid is unlikely, then this fluid layer protects the sample by inhibiting transfer of contamination onto the surface (because diffusion is generally a very slow process). It is, of course, an advantage if the fluid is one of direct relevance to the problem under study (such as water in the case of biopolymer sample, or electrolytes in electrochemical studies).

In other cases, fluids can serve to minimize adhesion between a scanning AFM tip and a sample. The use of propanol for this purpose is an example, but the use of any immersion fluid generally reduces friction. This is because the major source of unwanted interaction is the capillary attraction owing to a naturally occurring thin film of water on the sample surface. Complete immersion eliminates this effect. Another approach is to reduce the naturally occurring water layer to atomic dimensions, and this is easily accomplished by operating in a flow of dry gas.

ELECTROCHEMICAL CONTROL

A potential difference exists across the interface between a metal in contact with an electrolyte, as a direct consequence of the change in charge density between the electrons in the metal, and the ions in the solution. The science of electrochemistry is based on quantifying and controlling this potential difference. It is this potential difference, commonly referred to as the surface potential, that drives adsorption, desorption, and electron transfer reactions (of which the rusting process is a prime example). The amount of this potential is limited by the breakdown of the electrolyte. In water it can be a volt or so, while in some nonaqueous electrolytes many volts are possible. No comparable control is possible in the case of a surface in UHV. For example, to change a surface potential by a volt using temperature would require going from room temperature to 12,000 K.

Two electrodes must be inserted into the electrolyte in order to measure a potential, and therein lies the problem: A total voltage is measured, but is composed of two unknown potential drops (one across each metal-electrolyte interface). The reference electrode was invented to solve this problem. This is a chemically reactive electrode maintained in equilibrium with the ions (in solution) that are oxidized and reduced at its surface. In equilibrium, when just as many ions are being oxidized as reduced, the electrode is pinned at a potential halfway between that of oxidation and reduction. Here, the word potential has the physical meaning of the work done in removing or adding an electron from a position at rest in vacuum. This situation holds only as long as the concentration of reactants at the electrode surface does not change, a condition established by ensuring that a negligible current flows through the reference electrode. The choice of the right reference for a given experiment is a tricky one. Books are dedicated to the subject of electrochemistry (e.g. *Reference Electrodes*, D.J.G. Ives and G.J. Janz (eds.), Academic Press, New York, 1961). Nonetheless, a simple wire made of a reactive metal (like silver) will often suffice. This is discussed further in the Appendix.

Control of the potential drop across the sample (working electrode) is carried out by using a three-electrode cell. A servo-control, based on an operation amplifier, is used to establish a desired potential difference between the working electrode and the reference electrode by driving the potential of a third (counter) electrode. The controller is called a pontentiostat, of which the PicoStat is an example. It is specially adapted for scanning probe microscopy.

The potential of the working electrode is specified as a voltage difference with respect to the type of reference that is used. For example, hydrogen and protons at normal concentrations on a platinum surface form the normal hydrogen electrode (NHE). A second electrode, held at zero volts on the NHE scale would be at a potential of about minus 5 volts, with respect to the vacuum and the potential of a surface maintained in this way, would be specified as 0 V vs. NHE.

A thorough description of electrochemical methods is to be found in *Electrochemical Methods, Fundamentals and Applications*, by A.J. Hard and L.R. Faulkner, Wiley, New York, 1980. A straightforward and very readable introduction is *Electrodics: Modern ideas concerning electrode reactions*, by Henry H. Bauer, Thieme, Stuttgart, 1972.

TABLE OF CONTENTS

Chapter 1: SPECIFICATIONS

PICOSTM

Atomic Range Scanner:

Dimensions:	3.3cm (D) 4.32cm (H)
Weight:	113 g
Noise:	Vertical, less than 0.06 Å RMS Horizontal, less than 0.6 Å RMS
Range:	Vertical, 0.7 µm Horizontal, 1 µm
Sensitivity:	0.1V/nA, 1V/nA, and 10 V/nA

Small Range Scanner:

Dimensions:	3.3cm (D) 5.08cm (H)
Weight:	141 g
Noise:	Vertical, less than 0.1 Å RMS Horizontal, less than 1 Å RMS
Range:	Vertical, 1.6 µm Horizontal, 10 µm
Sensitivity:	0.1V/nA, 1V/nA, and 10 V/nA

Medium Range Scanner

Dimensions:	3.3 cm (D) 7.62 cm (H)
Weight:	170 g
Noise:	Vertical, less than 0.5 Å RMS Horizontal, less than 5 Å RMS
Range:	Vertical, 7 µm Horizontal, 50 µm
Sensitivity:	0.1V/nA, 1V/nA, and 10V/nA

PICOAFM

The PicoAFM scanner features laser tracking for top-down scanning with a bow-correcting method of scan to minimize the distortions usually associated with top-down scanners.

Atomic Range Scanner

Dimensions:	3.3 cm (D) 6.35 cm (H)
Weight:	113 g
Noise:	Vertical, less than 0.06 Å RMS Horizontal, less than 0.6 Å RMS
Range:	Vertical, 0.7 µm Horizontal, 1 µm

Small Range Scanner

Dimensions:	3.3 cm (D)	7.62 cm (H)
Weight:	141 g	
Noise:	Vertical, less than 0.1 Å RMS Horizontal, less than 1 Å RMS	
Range:	Vertical, 2 µm Horizontal, 6 µm	

Medium Range Scanner:

Dimensions:	3.3 cm (D)	8.89 cm (L)
Weight:	170 g	
Noise:	Vertical, less than 0.5 Å RMS Horizontal, less than 5 Å RMS	
Range:	Vertical, 7 µm Horizontal, 30 µm	

PICOSTAT

Modes of operation:

1. Under external control using PicoScan or other software
2. Under external control, user supplied signals
3. Front panel controls

Potential control range:	± 4.5 V
Galvanostat control:	± 6 mA (± 100 mA optional)
Current display:	10 µA to 100 mA full scale, 10 nA sensitivity
Voltage Display	10 V full scale, 0.3 mV sensitivity

Conductive AFM cantilever can be biased

AC MODE AFM

Operating frequency range: DC to 250 kHz
Typical operating frequencies in air: 90 to 120 kHz
Typical operating frequencies in liquid: 5 to 30 kHz
Oscillation amplitude in fluid: Max. 30 nm peak-to-peak, typical 5 nm.
Noise < 0.1 nm RMS

TEMPERATURE CONTROLLERS

Temperature controller accuracy:
Model 48-800: 0.1 °C
Model 48-801: 0.025 °C

MICROSCOPE BODY

The DPM display associated with STM, and the sum and difference signals in AFM are automatically switched.

Dimensions:	10.2 cm (D)	24 cm (H)
Weight:	4.5 kg (w/ standard environmental chamber)	

ENVIRONMENTAL CHAMBER

STANDARD

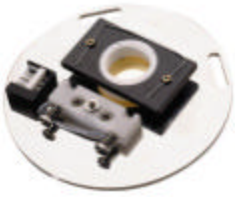
Material:	Pyrex construction	
Dimensions:	Diameter: 10.2 cm	Volume: 543 mL

PICOAPEX

Material:	Pyrex construction	
Dimensions:	Diameter: 15.0 cm	Volume: 921 mL

LIQUID CELL

Material:	100% Teflon
Volume:	Large: 700 µL
	Small: 150 µL



SAMPLE STAGE

Stainless steel disk with a three-electrode mount and liquid cell clamp. MAC plates come equipped with MAC solenoid and connector underneath.

Dimensions:	6.4 cm (D)	0.16 cm thick
Translation:	4 mm x 4 mm	
Sample size:	Max. standard sample size: 2.5 cm x 3.8 cm	

Temperature Stages:

- High-Temperature Stage: ambient to 200 °C
- One-Stage Peltier Stage: -5 °C to 40 °C
- Three-Stage Peltier Stage: -30 °C to ambient

Chapter 2: BEFORE YOU USE THE SYSTEM

SAFETY CONSIDERATIONS

The AFM scanner system contains a class II laser. Up to 1 mW at 670 nm is emitted from the laser housing. This manual also describes alignment procedures that require the beam be projected onto a screen. The beam reflected from the cantilever is projected onto a frosted Lucite screen for viewing safety. Follow the alignment procedures described carefully. Never stare into the beam directly. Irreversible eye damage will occur if the beam is viewed directly for more than ¼ second. Protective eyewear is recommended.

There are no user-serviceable parts inside the AFM scanner module. If you encounter any problems, return it immediately to Molecular Imaging for service. The laser will not operate unless the unit is plugged into the microscope controller and the controller is switched on. The unit is designed to operate with the PicoScan controller, as well as other controllers on the market – as long as they have an on/off switch and a warning lamp to show that power is applied to the unit. Do not operate the AFM scanner with any controller that lacks an on/off switch and indicator lamp.

The ribbon cable to the unit provides the high voltage necessary for scanning. If the cable becomes worn or broken it will pose a risk of electric shock and/or fire. Inspect the cable prior to every use of the microscope to ensure it is not hazardous. In the event of any defects, return the cable to Molecular Imaging for replacement.

UNPACKING

DO NOT unpack any of the boxes of the equipment if the system is scheduled for future installation by a Molecular Imaging representative. The unpacking of any items by a person other than a Molecular Imaging representative in such case may result in the loss of warranty coverage on these items.

Otherwise, when unpacking any equipment it is advantageous to check for any hidden damages as a result of shipping. A loose part inside the controller could have profound consequences on the performance and reliability of the equipment. The best way to detect any possible damage due to shipping is to gently turn the pieces upside down and listen for any rattling noises, which would indicate loose parts inside. Should you detect anything unusual please contact Molecular Imaging and **DO NOT** use the equipment under any circumstances.

Unpack all the components and retain all packing materials and shipping containers for your future shipping and storage needs. The equipment must be placed upright and on a hard surface. **DO NOT** obstruct the ventilation slots in any way, as this could have a catastrophic consequence on the equipment. Finally, make sure all equipment is placed far enough away from any solutions or moisture that could result in damage.

LIST OF ALL COMPONENTS

The parts list below is for a complete system with all available options. Your parts list may differ, depending on the options you have purchased.

- GENERAL

    1. PicoSPM Microscope
    2. PicoScan Controller
    3. Microscope Stand and Stage
    4. Environmental Chamber (optional)
    5. PicoIC (optional)
    6. DB25 and patch cables
    7. Power cables

- EC PACKAGE

    1. PicoStat
    2. Electrode and EC Cable Set
    3. Gold Substrates (small or large)
    4. Microreference electrode and salt bridge (optional)

- STM PACKAGE

    1. STM Scanner (atomic, medium, or small)
    2. Sample Plate and EC Cable
    3. Small Gold Substrate
    4. STM Tips (10 coated and 10 etched)

- AFM PACKAGE

    1. AFM Scanner (atomic, small, medium, or large)
    2. Sample Plate
    3. Large Gold Substrate
    4. AFM Contact Cantilevers (10)
    5. Photo Detector
    6. Cantilever Holder
    7. Lucite Alignment Block

- AC MODE PACKAGE

    1. AC Mode Controller
    2. MAC Mode Plate or top-down MAC Cantilever Holder
    3. Acoustic AC Module
    4. MACLevers (10)
    5. Acoustic AC Cantilevers (10)

- TEMPERATURE PACKAGE

    1. Temperature Controller (0.1K or 0.025K accuracy)
    2. Heating Stage
    3. Heating and Cooling (1x Peltier) Stage (optional)
    4. Cooling (3x Peltier) Stage (optional)
    5. Hot and Cold MAC (1x Peltier) Stage (optional)
    6. Hot MAC Stage (optional)

7. Water Cooling Tube (optional)
8. Hot Ring Liquid Cell (optional)

- CURRENT SENSING AFM PACKAGE

1. Current Sensing Cantilever Holder
2. Current Sensing Preamp Module
3. Current Sensing Cables
4. Pt-coated Conductive Cantilevers (5)

- ENVIRONMENTAL PACKAGE

1. Standard Glass Chamber (optional)
2. PicoAPEX (optional)
3. Flow-through Liquid Cell System (optional)
4. Glove Box (optional)

- MISCELLANEOUS OPTIONS

1. HOPG Sample
2. Mica Sample
3. Gold Substrates (large or small)
4. Calibration Grating
5. Liquid Cell Kit (large or small)
6. Alignment Plate
7. Beak Pusher for Cantilever Holder
8. Tool Set
9. Eye Loupe

CHOOSING A MIRCROSCOPE LOCATION

When used properly the PicoSPM is capable of remarkable performance in noisy conditions. This is due, in part, to the additional acoustic isolation provided by the environmental chamber. For noisy environments, we strongly recommend the PicoIC™, a desktop isolation chamber containing a suspension system for the microscope. Temperature stability is also an important factor; therefore, for optimal stability, the ambient temperature should not change by more than 0.5º C per hour.

The microscope should be situated in a temperature-controlled room away from windows, doors, and vents. The effect of small local temperature fluctuations is greatly reduced by placing the microscope in a large enclosure (such as the PicoIC). In most cases, sources of mechanical noise and thermal change are least in basement environments. However, since the PicoSPM system is engineered to minimize the difficulty of sample handling, we recommend placing the microscope close to the sample preparation area, and making adequate changes necessary to ensure the environment is conducive. Often, using the PicoIC will suffice. During shows, in which there is a lot of noise, the PicoSPM has produced atomic resolution routinely. Using the PicoIC, the microscope will operate on any convenient sturdy surface.

INSTALLING CALIBRATION FILES

Your microscope scanner comes with a calibration file supplied on a 1.44MB diskette. This calibration file contains a list of parameters that were measured for each piezo scanner at the factory. These files are read by PicoScan, as well as other controllers (NanoScope). The PicoScan program already contains two generic files, one for AFM and the other for STM. However, because they are simply "generic" calibration files, the parameters will not give a true representation of the sample. If you purchase a complete PicoScan system with a scanner, the calibration file will be installed for you at the factory. If you have since purchased additional scanners after purchasing PicoScan, a calibration file will need to be installed. To do this follow the standard Windows procedures to install the calibration file into the PicoScan folder. For all NanoScope users, the files are automatically read when placed in the C:\SPM\EQUIP directory.

Once the calibration files have been installed you are then able to easily access them in the program. Depending on whether the use is for STM or AFM the file numbers will appear in the program matching the serial number located on each scanner head. Be sure to verify the correct calibration file has been selected for the chosen scanner prior to any scanning. It can be very frustrating when in the middle of imaging or compiling data you realize the wrong calibration file was chosen for the scanner used.

CALIBRATING YOUR SCANNER

In order to maintain the scanner's accuracy while imaging, it is important to periodically calibrate the scanner. A scanner should be calibrated every 3 to 6 months, depending on the usage and care taken with the scanner. PicoScan allows you to calibrate your scanner when necessary.

PROCEDURE FOR MANUAL XY CALIBRATION

1. Scan in a L to R pattern only on an XY grating (available from Molecular Imaging). Verify the X and Y orientations of the grating are the same as the X and Y axes of the PZT. Set the scan rate to 3 lines/sec. The number of data lines should be 256 X 256. Set the X and Y offset to zero. For a small scanner, Å/V = 170, $Å/V^2 = 0$ for both X and Y.
2. If the initial images are distorted (grating spacing on left is larger than the right), adjust $Å/V^2$ of X until the left and right gratings are equally spaced.
3. Adjust Å/V of X until the spacing is equal to the grating standard. If Å/V is significantly different from 170 (the original setting), repeat step (2).
4. Increase $Å/V^2$ of Y until the top and bottom gratings are equally spaced.
5. Adjust Å/V of Y until the spacing is equal to the grating standard. If Å/V is significantly different from 170 (the original setting), repeat step (4).

PROCEDURE FOR AUTOMATIC XY CALIBRATION

PicoScan ships with a calibration script that simplifies the XY calibration process. See the PicoScan manual for more details.

PROCEDURE FOR Z CALIBRATION

1. Scan in a L to R pattern only on a Z grating (available from Molecular Imaging). The grating should have a step size close to the Z range you expect your sample to have.
2. Adjust the sample plate until the scanned image is not tilted by withdrawing the sample, turning the coarse adjust screws as needed, approaching, and scanning again. Use Tilt mode (in the Data Rendering window in PicoScan) to level the data if you cannot perfectly level the grating.
3. Use the manual approach functions to adjust the height of the grating until Vz, the Z piezo voltage, is close to 0 (displayed on the oscilloscope in PicoScan), and then scan again.
4. Adjust Å/V of Z until the spacing between plateau and valley is equal to the grating standard.

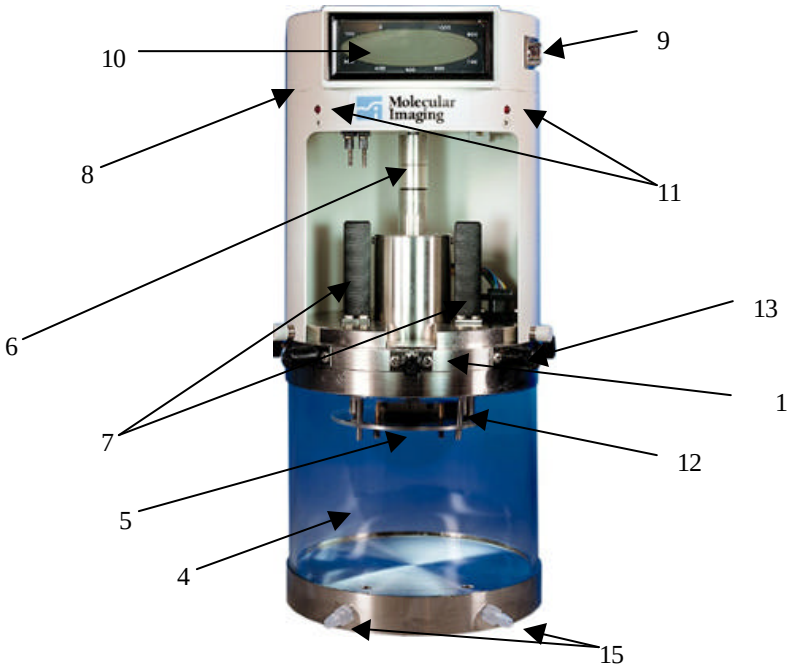
NOTES

- Generally, Z travels over a much smaller range than X and Y. As such, a second-order correction is not necessary. However, for best results, you should image in the same Vz range you used for calibration.

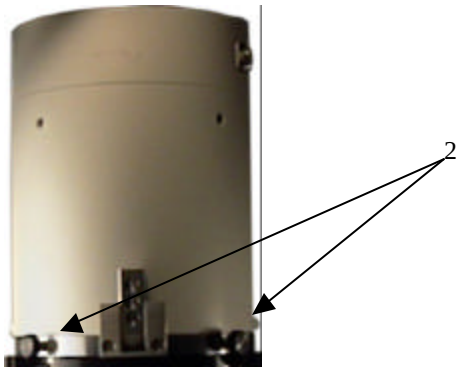
Chapter 3: SPM COMPONENTS

OVERVIEW OF PICOSPM

The purpose of this chapter is to provide a brief overview of the PicoSPM system. Refer to later chapters for specific applications and descriptions for STM and AFM operation.



The microscope in the above figure contains an STM scanner. An STM or AFM scanner module will rest inside the opening of the **Microscope Body (1)**.



After placing the scanner into the body, it is locked into place by the two **Locking Screws (2)** situated on the back of the microscope body.

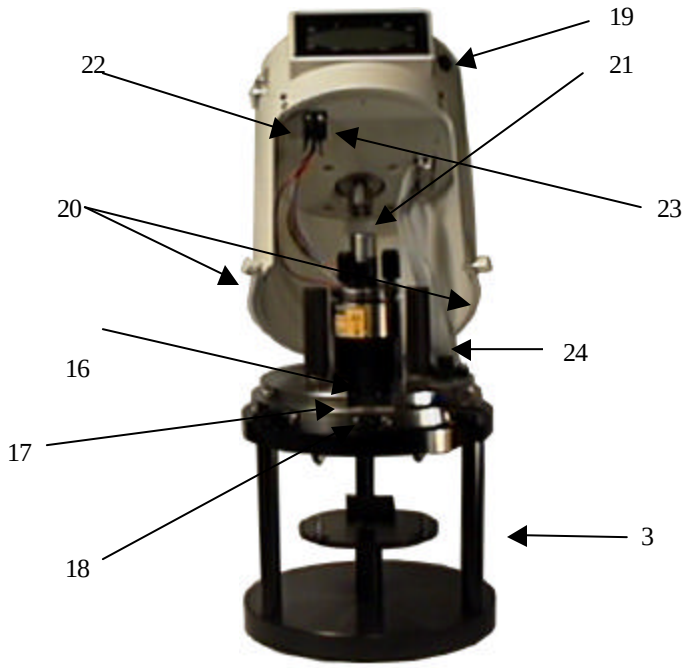
The microscope can rest on either an **Environmental Chamber (4)** as in the above picture, or on a **Stand (3)** (below). The **Sample Plate (5)** attaches to the underneath of the microscope via magnets on the **Motor Screw (6)**, and the **Coarse Screws (7)**. The vertical height distance between the tip and the sample can be manually adjusted with these screws; however, the final adjustment is made by the controller turning the motor screw to engage the tip with the sample. All electronic circuits are enclosed within the **Head (8)** of the microscope. The controller connects to the microscope head at the 25 pin **Head Connector Site (9).**

The electrical connection between the controller and the microscope is displayed in the **Head Panel Display (10)**. The head panel display displays the A – B (top – bottom) and C – D (left – right) signals used in AFM and LFM,[1] and the tip current converted to volts for display and computation purposes in STM.

Below the head panel display are the **Contact Warning Lamps (11),** used to show when the sample translation mechanisms contact the stage. After adjustment, the sample **Translation Mechanisms (12)** are backed off - by turning the **Translation Screws (13)** – and the contact warning lamps will turn off, so that there are no residual strains on the sample stage.

When using the **Environmental Chamber (4)**, gas flows through the chamber by means of 1/8th inch id tubing connected to the **Gas Connector Nipples (15)**.

For AFM use, the AFM **Photodiode Detector (16)** is placed into the groove of the microscope body and connected at the **Base (17).** The A – B signals are adjusted by the detector **Adjustment Screw (18)**. Turning the screw clockwise moves the detector away from the scanner, while turning the screw counter clockwise moves the detector towards the scanner. The C – D signals are adjusted by sliding or rotating the photo detector from left to right. The C – D signal can be displayed on the Head Panel Display by depressing the **LFM Adjustment Switch (19)**.



[1] The actual equations are (top – bottom) / (total intensity) and (left – right) / (total intensity). See **Chapter 5** for more details.

The **Head Retaining Pins (20)** allow the SPM head to be tilted back from the body when depressed.

Motor coupling is via the **Magnetic Motor Clutch (21)**.

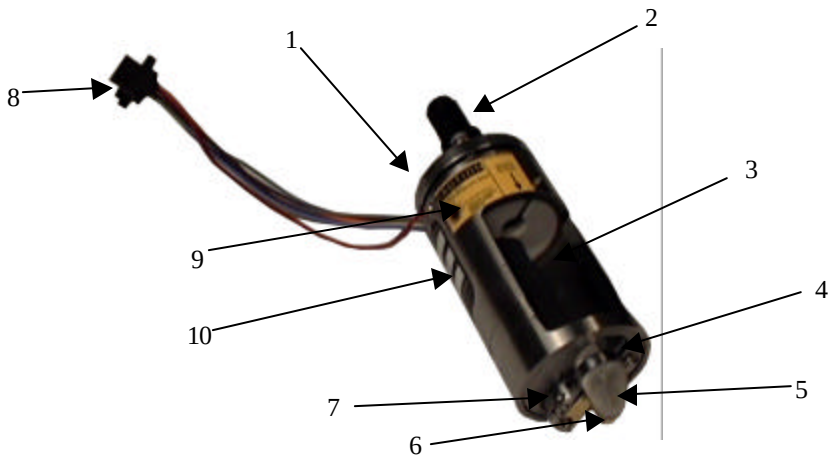
The **AFM D Port (22)**.

The **STM D Port (23)**.

Connection of **Photo Detector Response Cable** to microscope head **(24).**

OVERVIEW OF SCANNERS

AFM SCANNER MODULE



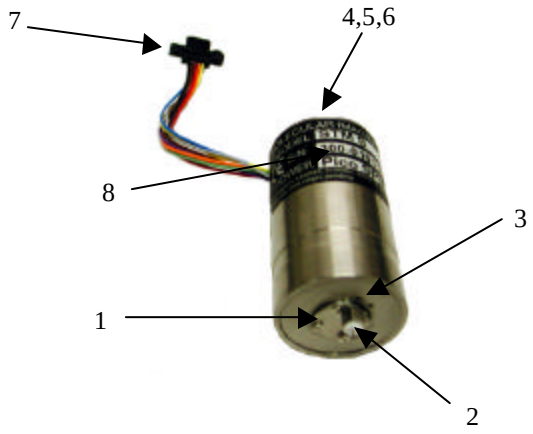
The AFM scanner is housed in a protective **Scanner Body (1)**. On top of the body are mounted the **Laser Tilting Assembly Screws (2)**. This assembly can rock the laser in two orthogonal directions. The right screw adjusts the laser in the X direction, while the left screw adjusts the laser in the Y direction.

The laser beam passes down alongside the **Scanning Tube (3)**, through the tracking lens, the **Sample Chamber Window (4)**, and the **Cantilever Glass Rod (5)**, and finally onto the back of the **Cantilever (6)**. The cantilever assembly attaches magnetically to the **Endcap (7)**.

The AFM scanner is connected to the microscope head by means of the **Miniature D Connector (8)**. This connector plugs into a keyed receptacle – on the left – and the function of the microscope (STM and AFM) is automatically switched when the appropriate head is connected.

The **Laser Warning Label (9)** is located on the front of the AFM scanner**,** while the **Scanner Identification Label (10)** is located on the back of the scanner body. This contains the scanner's identification and should match the calibration file found in your controller.

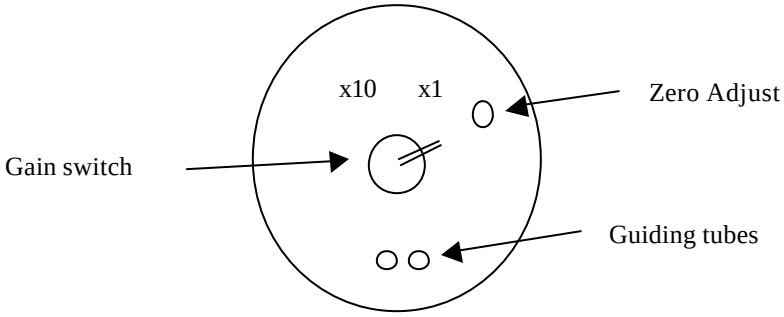
STM SCANNER MODULE



The diameter of the STM scanner is identical to the AFM, this allows the two to be easily interchanged. The Scanner ends with an **STM End Cap (1)**, on which the **Tip Holding Assembly (2)** is mounted.

The extender is sealed off by a thin disk, through which the **Fluid Line Guide Tubes (3)** protrude. Fluid line guide tubes are used to place fluid lines into the liquid cell from the bottom while having tubes extend from the top **(4)** for fluid insertion.

Also at the top of the scanner are the **Zero Adjust (5)** and the **Gain Select Switch (6)**.



STM Scanner Top View

The STM scanner is connected to the microscope head by means of the **Miniature D Connector (7).** This connector plugs into a keyed receptacle – on the right – and the function of the microscope (STM and AFM) is automatically switched when the appropriate head is connected.

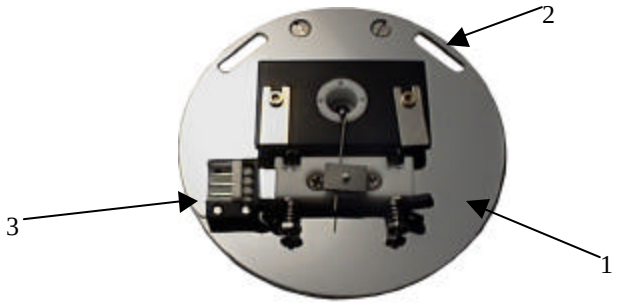
The STM scanner has a **Scanner Identification Label (8)** located on the scanner body. This contains the scanner's identification and should match the calibration file found in your controller.

See also: **Chapter 5** and **Chapter 7** for additional information.

Chapter 4: SAMPLE PLATE AND LIQUID CELL

SAMPLE PLATE

The sample plate and liquid cell are used in both AFM and STM operations, with some sample plates equipped for temperature control and MAC mode – contact Molecular Imaging for additional information. The purpose of the sample plate is to hold the sample and offer translation abilities of those greater than the scanner piezo.



The stage consists of a disk shaped **Sample Plate (1),** which is suspended from the ends of the adjustment screws and the motor screw by magnetic support.

The **Translation Slots (2)** engage the translation pegs, which adjust the stage in the X and Y plane. These pegs are adjusted via the translation screws on the microscope body. Indication for mechanical contact is given by the contact warning lamps as previously described.

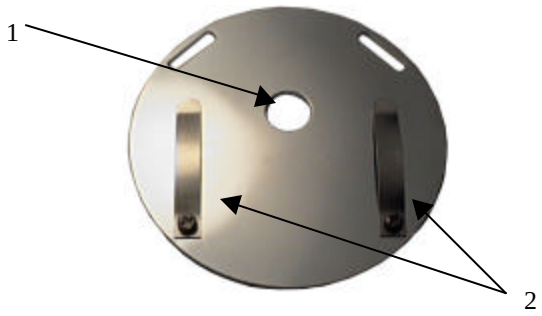
Connections for the sample ground and for electrochemistry are via the **Sample Stage Connector (3)**. The first pin is for the counter electrode, the second is for the working electrode, and the third is for the reference electrode.

NOTES

- The liquid plate shown above has a liquid cell in place with an electrode attached.

SAMPLE ALIGNMENT PLATE

The sample alignment plate is a very useful tool in SPM imaging. It is HIGHLY recommended you use the sample alignment plate prior to attaching the sample plate. It may seem time consuming, however, it can save more time in the long run. The purpose of the sample plate is to gauge the distance between the sample and tip; therefore, you can save the frustration of crashing a tip when you place your actual sample. Also, if all adjustments are made prior to loading the real sample, then waiting time for the microscope to engage and drift to settle is limited. This is particularly important when the lifetime of the sample is limited.



The sample alignment plate is the same diameter as the sample plate. The sample alignment plate has a **Center Hole (1)**. The purpose of this is to ensure the tip doesn't crash into the plate when adjustments are being made.

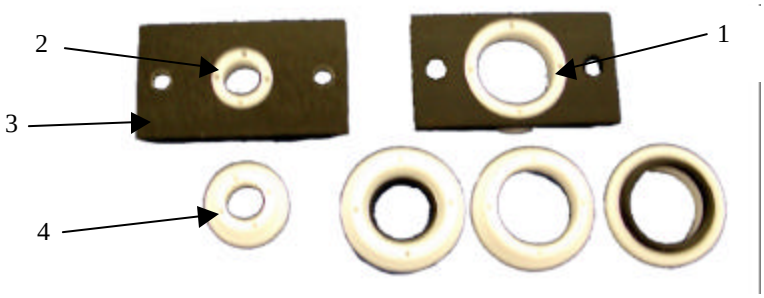
The alignment plate also consists of **Sample Clamps (2**) to hold down a glass plate or piece of mica. In some instances you will want to obtain a dummy sample to represent the Z height of your real sample.

NOTES

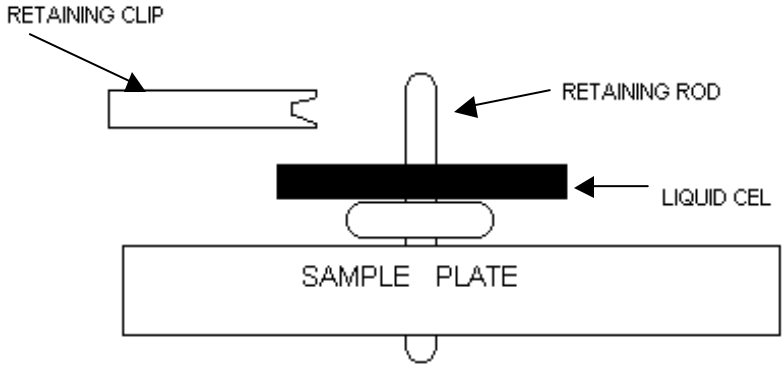
- The alignment plate is essential when using the liquid cell because the tip-sample gap is not visible.
- A sample of the same thickness as that to be used in an experiment is mounted on this plate. Adjustments of the microscope should be carried out with the tip over the safe area above the hole.
- The microscope will be ready for automatic engagement after adjustments have been made, this will limit the approach time considerably if the plate is within a minimal distance of the tips.
- It is often desirable to run the microscope in air (on the stand) instead of on the environmental chamber for ease of adjustment.

LIQUID CELL

The liquid cell is an important component of both AFM and STM imaging. It allows for experiments to be imaged in-situ, offering better control under more realistic environments.



The liquid cell comes in two sizes. The **Large Cell (1)** is 12.5mm inside diameter and the **Small Cell (2)** is 6.5mm inside diameter. The larger cell may be used for both STM and AFM, while the smaller cell will only accommodate the STM probe.



The liquid cell consists of the **Cell Clamping Plate (3)** and the Teflon **Fluid Cell Ring (4).** The ring is inserted into the cell clamping plate, the ring is pushed against a glass plate, mica, or some other substrate, and then the **Retaining Clips** are inserted.

NOTES

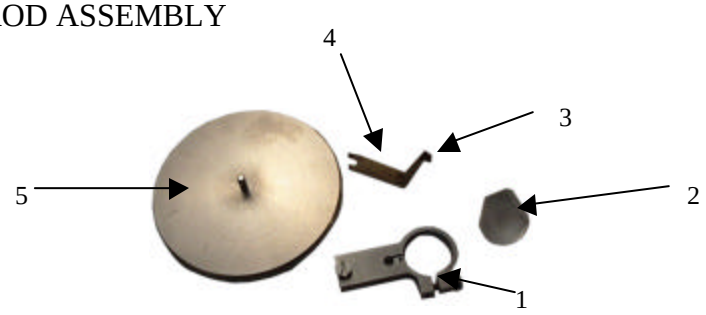
1. It is important that the liquid cell lies flat on the sample plate, to prevent the cell from leaking.
2. The retaining rods on the sample plate can be adjusted for various tensions by using a flat head screwdriver.
3. If using a hot or cold MAC mode plate, be certain not to place a sample directly onto the plate, because the ferrite core could react in solution or when in contact with the sample.
4. Liquid cells are also available for fluid lines to be attached directly.
5. The liquid cell has four small holes. The purpose of these holes is to allow the pogo contact pins to be inserted for simple electrical contact.

See also: **Chapter 3, Chapter 5, Chapter 7,** and **Chapter 8** for additional information on **sample plates**, **liquid cells**, and e**lectrodes** for electrochemistry.

Chapter 5: ATOMIC FORCE MICROSCOPY – AFM

Because AFM is dependent upon the deflection of the laser beam off of the cantilever, it essential to understand the glass rod assembly, how to clean the assembly for applications, and how to mount the cantilever onto the glass rod assembly. Because the cantilever and glass rod assembly are a vital aspect of AFM, we start this chapter here.

GLASS ROD ASSEMBLY



The assembly consists of a magnetic stainless steel **Body (1)** which is held onto the end of the scanner by a magnet.

The body holds the cantilever **Mounting Glass Rod (2)**.

The cantilever is held in the proper position by the **Beak (3)** on the **Cantilever Retaining Clip (4)**.

The **Beak Pusher (5)** is used to push the beak upward when mounting the cantilever.

The glass rod assembly is cut from fused quartz. The shape of the rod is designed with a number of important considerations in mind. The angled groove cut into the top of the rod, beneath the beak, helps position the cantilever at the appropriate angle for the incident laser beam. The height of the rod is calculated to hold the cantilever within the focal plane of the beam tracking crystal. The angled sides of the glass rod are to minimize the volume of the rod without interfering with the laser beam.

The magnetic-stainless steel body holds the rod and cantilever retaining clip via two screws. It has a small hole in the middle through which the beak pusher protrudes to lift the beak for cantilever placement. The cantilever retaining clip then firmly holds down the cantilever chip on the glass rod. The clip is gold plated to minimize contamination in solutions.

CLEANING THE GLASS ROD ASSEMBLY

Cleaning the glass rod assembly is an optional procedure; however, is recommended for in-situ experiments. This prevents the gold plated clip and the glass rod assembly from cross-contamination. For most experiments, simply rinsing the glass rod assembly in an appropriate pure solvent or sonicating, followed by rinsing in pure water, and air-drying or drying with a lint-free towel will suffice. However, the glass rod assembly can be taken apart if a very thorough cleaning is deemed necessary.

PROCEDURE

1. Loosen screw **1** with a small flat blade screwdriver and screw **2** with a 1/16th inch hex wrench. The glass rod and cantilever retaining clip can now be removed.
2. The glass rod, gold-coated clip, and stainless-steel body, can now be washed in dilute acidic solution. You may wish to sonicate the entire glass rod assembly for a period in a solvent to remove all lipids as well.
3. Rinse thoroughly – if holding with tweezers, always allow the water to flow toward the tweezers – with pure distilled water.
4. Allow the parts to completely dry in air, gas, or with a lint-free towel.

ASSEMBLY

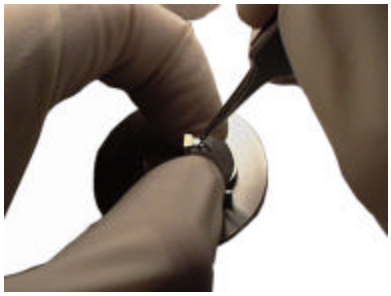
1. Be sure that the glass rod is positioned so the chip-holding groove is lined up directly beneath the beak.
2. Also, make sure the bottom of the glass rod is flush with the rod holder.
3. Tighten the two screws. **CAUTION: Do not over tighten the glass rod screw, it can cause the rod to crack.**

MOUNTING CANTILEVERS

The cantilever chip mounts into the assembly between the retaining clip beak and the glass rod. A small, angled groove cut in the glass rod snugly holds the cantilever in position. The clip is extended upward by pressing the assembly against the beak pusher.

PROCEDURE

1. Working on a hard flat surface, place the glass rod assembly onto the beak pusher.
2. Grasp a cantilever chip with the tweezers. CAUTION: Be careful not to grasp the cantilever chip too tightly or it could 'pop' out or break.



3. While grasping the cantilever with tweezers in one hand, gently press down the rod assembly onto the beak pusher. This will raise the beak allowing you to place the cantilever underneath the beak on the glass rod.
4. Carefully orient the chip into the angled groove, then release the pressure on the beak pusher, allowing the beak to hold the chip.

NOTES

- Make sure the chip is slid all the way back under the beak. If the chip is off at an angle or not all the way back, it can be difficult or even impossible to align the laser onto the cantilever.
- Sometimes the beak sticks in the extended position. If this occurs, very gently press down on the beak, being careful not to knock off your cantilever chip.
- If there doesn't seem to be enough pressure to hold the cantilever in place (if you can easily slide the chip from side to side after you placed it under the beak), you can unscrew the first screw and make a slight bend in the cantilever retaining clip near the screw.
- We all make mistakes: Make sure the cantilever is facing in the right direction – AWAY FROM THE BEAK.

ATTACHING THE GLASS ROD TO THE SCANNER

PROCEDURE

1. After placing the scanner into the microscope body, tighten down the locking screws to hold the scanner in place.
2. Be certain the head of the microscope is shut, take the microscope off the stand, and snap the cantilever into position in the slot on the bottom of the scanner. A magnet holds the assembly in place.
3. The assembly fits precisely into position.

NOTES

- The glass rod assembly can be locked in position for some applications by a hex screw located on the side.
- After placing the assembly in position, while the laser is still off, make sure everything looks stable, so that the cantilever and glass rod will not move easily.

INSERTING THE AFM SCANNER MODULE

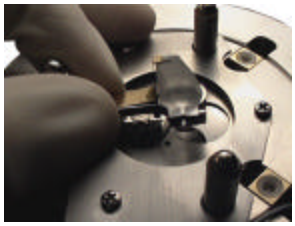
When the both the scanner and microscope head are plugged in, the laser will turn on. DO NOT stare directly into the laser beam. Be cautious when inserting the AFM scanner, be sure the microscope is unplugged from the controller prior to insertion.

PROCEDURE

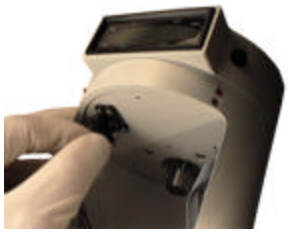
1. To open the microscope head, gently press in the head retaining pins and tilt the head backwards.
2. Place the scanner into the center of the microscope head's base plate; it should fit snugly.

3. Orient the scanner so the opening faces the front of the microscope.

4. Tighten the locking screws located on the back of the microscope. This will hold the scanner in place.
5. Mount the glass rod onto the end of the scanner. Notice the glass rod mounts magnetically via interlocking grooves. OPTIONAL: The chip holder can be locked into place by tightening the small set screw at the end of the scanner – this feature is not available for all scanners.



6. Plug the scanner's miniature D connector (male) into the left female miniature D connector on the microscope head.



LASER SAFETY

When the scanner assembly is plugged into the microscope body and power is applied to the unit, a beam of up to 1mW of 670 nm laser light is emitted from the laser module down the scanner tube. When a reflecting cantilever is in place, the beam is reflected back out of the opening in the front of the scanner. Use caution to ensure that you do not stare into this beam. The opening in the AFM scanner module is labeled with the caution logotype printed on the first page of this manual, warning you not to stare into the beam. The unit is not to be used with any power source that does not include an on/off power warning light and a power switch.

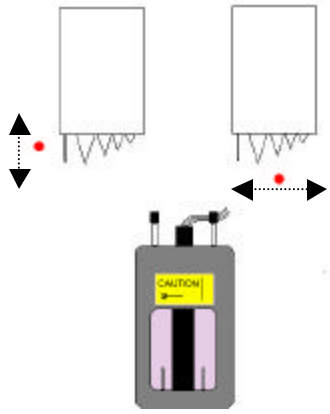
CAUTION: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous laser light exposure.

ALIGNING THE LASER BEAM

This section will discuss how to align the laser beam on the cantilever. To align the laser with success, it is important to understand how the AFM scanner operates and what to look for during alignment. Briefly, once the laser is aligned on the cantilever, the incident laser beam will shine through the scanner module, through the glass rod, and onto the cantilever. The beam then reflects off the back of the cantilever at an

angle through the glass rod and into a photo-diode detector. A slight deflection of the cantilever changes the angle of the laser beam and it's reflection into the photo detector. This is how the microscope detects a sample's topography.

The left laser alignment screw on the AFM scanner translates the laser up and down on the Y plane. Meanwhile, the right alignment laser screw on the scanner translates the laser beam left to right along the X plane.

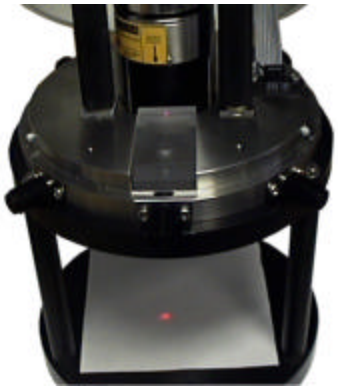


During alignment you will use a sheet of paper to monitor the intensity and shape of the transmitted beam – that portion that does not hit the cantilever or chip – and you will also use the Lucite block to monitor the intensity and shape of the reflected beam. DO NOT STARE DIRECTLY INTO THE TRANSMITTED OR REFLECTED LASER BEAM.

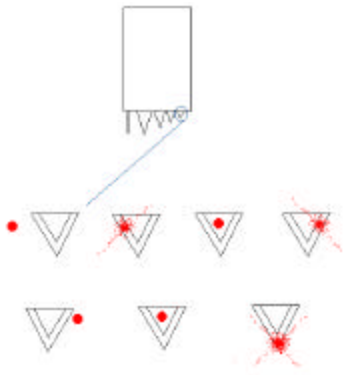
PROCEDURE

1. Open the top of the microscope by pressing the head retaining pins on the back of the microscope.
2. If the microscope is on the stand, place a small white piece of paper to be used for a screen. Alternatively, let the microscope lie on a table as shown below.

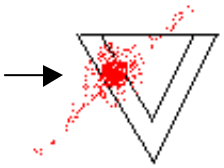
3. Place the Lucite block into the detector groove. It attaches by a magnet – you can see the magnet in the block. Be sure the magnet end is away from the scanner.
4. Connect the DB25 cable to the microscope. If the controller is turned on, the laser beam will automatically appear.
5. Using the laser tilting assembly screws, adjust the position of the beam until you find a clear spot either on the screen or Lucite block.



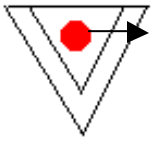
6. With the left screw, move the spot forward, down the Y plane. When it suddenly disappears, you know you have reached the front edge of the cantilever chip. Slowly back the laser off of the chip and stop when the laser beam spot reappears.
7. Now turn the right screw counterclockwise until the laser spot disappears. You have now reached the far extreme edge of the chip. Slowly back the laser off of the edge and stop turning when the laser beam spot reappears.
8. You are now ready to find the individual cantilever.



9. To do this, turn the right assembly screw clockwise very slowly. Follow the spot along the X axis on the screen. You know you have hit the cantilever leg when you see the diffraction of the laser beam on both the screen and Lucite block.

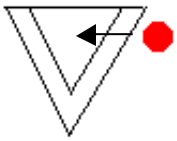


10. Continue to move the spot in the same direction. You should see the spot reappear. The laser beam is now situated in between both legs of the cantilever.

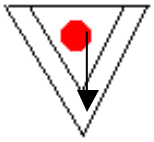


11. If you proceed with moving the laser beam in the same direction you will see the spot disappear, this indicates you have hit the other cantilever leg and will see diffraction of the laser beam.

12. If you see the laser spot disappear then appear, this indicates you have passed over the final cantilever leg and are now past the cantilever.



13. Move the laser alignment screw clockwise so the laser beam spot is in between both cantilever legs again.



14. Find the cantilever center and then move the laser to the tip of the cantilever by turning the left assembly screw. The spot on the screen should be almost completely obscured by the cantilever.

15. The final spot on the Lucite block should look more like an X. This pattern will occur because the laser beam is directly on the tip of the cantilever. You still, however, should see a strong reflection signal on the Lucite block, indicating you are positioned correctly and your cantilever is good.
16. Finally, position the spot in the center of the Lucite block by loosening the scanner and rotating it until the laser beam spot is in the middle of the Lucite block. There is a line running vertically down the Lucite block to serve as a guide. Retighten the scanner screws to lock the scanner in place.

NOTES

- REMEMBER: Unless the beam is hitting the cantilever or the cantilever chip, a clear spot will be seen on the paper underneath the head.
- Because of the tracking lens, motion in the perpendicular direction will appear on the screen to be in the opposite direction to the motion of the spot on the chip (because the focus is on the chip).
- Be careful not to move the spot so far that it leaves the tracking lens. Position the beam near the center.

- When the spot is extinguished rapidly twice in succession as it is swept parallel to the edge of the chip, it is clearly passing the legs of one of the cantilevers.

PLACING THE SAMPLE

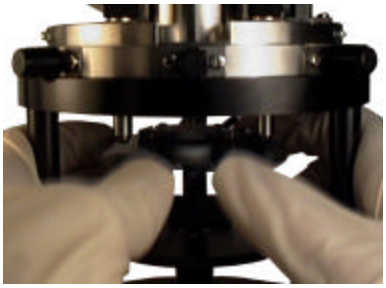
For AFM you can use the sample alignment plate, however, there are no real advantages when imaging in air. If, however, you will be using a liquid cell you might want to consider using the samp le alignment plate, since the sample is hidden by the liquid cell. In any event, the procedures for placing the sample plate and the sample alignment plate are identical.

There are three screws that contact the sample plate. The front two are the coarse adjustment screws, and the back screw is the motor screw. All three can be adjusted manually; however, only the back motor screw turns when the controller's approach command is chosen.

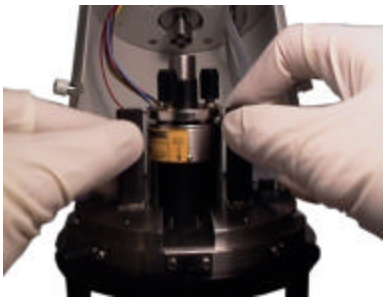
PROCEDURE

Without a liquid cell:

1. Prepare and load your sample as desired.
2. Leave the Lucite block in position.
3. Place the sample plate in position. The plate will "clip" into place as the magnets hold the plate to the adjustment pegs.



4. Adjust the coarse screws (front screws) first to reach a position close to your cantilever tip. To bring the sample plate closer to the cantilevers rotate the coarse adjustment screws clockwise. As the final position is reached, move the screws slightly past the final position and then back the screw off a little. This helps to reduce sample drift.



5. Now adjust the back motor screw to bring the sample within 0.5mm of the cantilever tips. Turn the motor screw counterclockwise to raise the sample plate towards the cantilever.

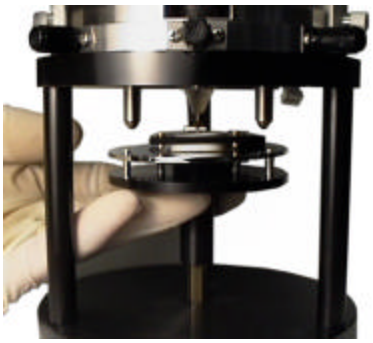
PROCEDURE

Using a liquid cell:

1. Prepare your sample and attach the liquid cell as previously discussed.
2. Leave the Lucite block in position.
3. Using a sample alignment plate, place the sample plate into position on the adjustment pegs and motor screw.
4. Adjust the screws as described above (4 and 5) leaving a space of 0.5mm between the sample alignment plate and the cantilevers.
5. Remove the sample alignment plate and replace it with the sample plate in the same position.
6. As you place the sample plate with the liquid cell into position, the position of the laser on the Lucite block will change (due to the refractive index of the solution). You will need to ever so slightly adjust the laser alignment screws to re-align the laser on the cantilevers.

NOTES

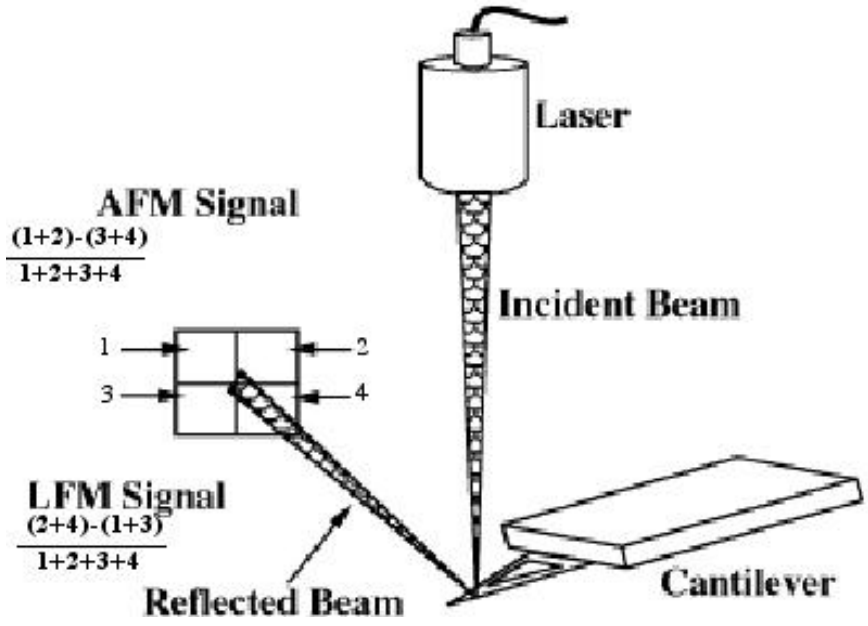
- When using the sample alignment plate (recommended) you can see the reflection of the cantilever tip in the sample plate as you are raising it towards the cantilevers.
- Since the laser was previously aligned on the cantilever, you will be able to determine if you crashed the tip while placing the sample on the plate by observing a single laser dot appear on the Lucite block instead of the standard diffraction caused by the laser hitting the cantilever.
- A flashlight might shed some light as to the exact distance between the sample plate and cantilevers.
- The sample plate needs to be level on the X axis; however, you may want the Y axis to be tilted slightly. If the back portion of the sample plate (in contact with the motor screw) is slightly lower than the front portion of the sample plate (in contact with the coarse adjustment screws), this is okay. Because, as the motor screw is turning to engage the sample (bringing the back of the sample plate up), the sample plate will completely level off when the force set point is reached.
- The cantilever tip should be a distance of 0.5 mm to 0.25 mm from the sample.
- A sample alignment platform is provided to aid you in loading the sample; however, this should only be used after the sample alignment plate was used to determine the distance between the sample plate and cantilever. Simply place the sample plate on the alignment platform, grasping the alignment platform, gently pull the platform and sample plate up to the coarse adjustment screws and the motor screw.



- Some people find the sample alignment platform difficult to use. If you do not use it, we recommend you remove it from the base of the microscope stand. This adds additional room for placing the sample plate.
- Loading the sample plate with a liquid cell for AFM imaging can be difficult due to the size of the glass rod, but with persistence, patience, and practice you will soon be doing it with ease. BE CAREFUL: when loading the sample plate with liquid cell do not hit the cantilever on the cell. Also, be certain that the glass rod is not touching the liquid cell once engaged, this could lead to excessive drift.
- The laser position on the Lucite block (when using the liquid cell) will move forward – toward the user – on the block.

ALIGNING THE PHOTODIODE DETECTOR

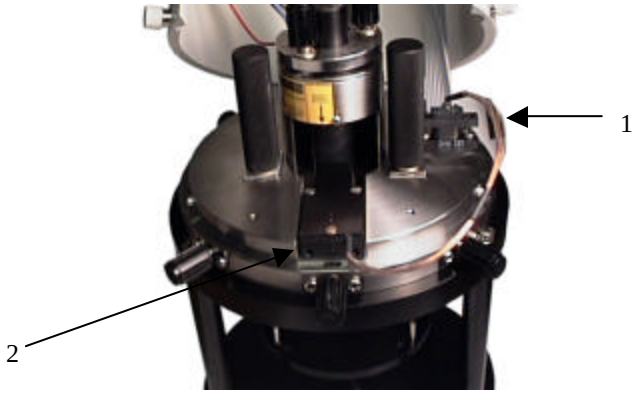
You will need to align the detector for getting the AFM topography, deflection and lateral force (Lateral Force Mode or LFM) images. This is a fairly straightforward process. Normal contact AFM measures the three-dimensional topography of a sample surface with angstrom resolution, while LFM measures the lateral or frictional forces exerted by a probe on the sample surface.



In normal AFM mode the optical detection system senses the vertical deflection of the cantilever as features on the sample surface are encountered. The basic idea is to position the photodiode detector such that the reflected laser beam is centered top to bottom on the detector (see AFM Signal in the schematics). The LFM mode uses additional photo detectors and electronics to measure twisting, or torsional deflection, of the cantilever. Thus the detector is positioned such that the reflected laser beam is centered left to right on the detector (see LFM Signal in the schematics).

PHOTODIODE DETECTOR

There are no user serviceable parts on the photodiode detector. If you encounter any problems, please return it to Molecular Imaging immediately.



The photodiode detector consists of four quadrants. The top and bottom quadrants (A and B) are used in AFM imaging, while the two side quadrants (C and D) are used in LFM imaging. The detector has a 9 pin female cord and plug, which connects the detector to inside bottom of the microscope **(1)**. Three small magnets are mounted on the detector to hold it in place on the microscope, and the detector pin keys it into the slot on the microscope **(2)**.

MICROSCOPE LCD DISPLAY

The microscope LCD display for AFM and LFM displays the signals that are picked up from the photo detector. This display is also known as the Head Panel Display. The graded oval bar around the display shows the total power signal. This indicates how strong the reflective beam signal is. The numeric display in the center, displays

the beams position as detected on the photodiode detector; this is better known as the A – B signal. The LFM adjustment switch (little black button to the side of the LCD) indicates the lateral position of the beam; this is also known as the C – D signal.

ADJUSTING FOR AFM

PROCEDURE

1. Unplug the ribbon cable from the microscope and replace the Lucite screen with the detector facing downward. The detector plugs into the miniature D connector on the right of the microscope body's base. Power up the microscope.
2. Manually slide the detector forward and backward to maximize the signal and to be sure the detector is detecting the deflected laser beam. You should notice the power bar increasing and decreasing and the A – B number value changing from + 9.00 to – 9.00.
3. Position the detector to obtain the maximum power signal possible. You may also try loosening the scanner and rotating it a little to try to increase the power bar signal; however, if the laser beam was positioned in the middle of the Lucite block earlier, you will probably not need to do this. When the largest signal has been obtained, lock the scanner in place and close the microscope.
4. Now adjust the fine adjustment screw located in front. This will move the photo detector forward or backward and will change the A – B signal. If the signal (A – B) is a positive number, move the adjustment screw counterclockwise. If the signal is negative, move the adjustment screw clockwise. Once these adjustments have been made there should be little drift.

NOTES

- Make sure the detector pin is in the slot on the microscope base.
- Another step to help maximize the power signal even more after adjusting the photo detector is to slightly adjust the laser tilting screws. Barely rotate one screw and then the other to see if you can maximize the power; however, be careful not to move the laser onto the chip or onto a leg of the cantilever. This is apparent by a significant jump in the power bar display.
- While the LFM reading need not be zeroed, you can increase the signal strength by rotating the detector and/or scanner until the LFM reading is about 5 or less.

ADJUSTING FOR LFM

If you plan on using the friction option while scanning it is essential you adjust the photo detector so that the detector picks up the signal of the laser deflection reliably. The momentary LFM push-button switch is located next to the Head Panel Display. During lateral force measurements, the system continues to feedback, keeping the cantilever at a constant deflection to maintain a constant loading force on the cantilever, while torsional data is displayed and recorded. Rotating the scan angle parameter dramatically changes the effect of torsional forces on the twisting of the cantilever. Therefore the key to obtaining good lateral force data is the proper selection of angle.

Selecting friction in the software will cause the lateral force signal to be displayed on the data monitor. The PicoScan software manual contains more information regarding LFM and the friction option. Operationally, the LFM is very similar to standard AFM, but there are some notable differences in the microscope hardware.

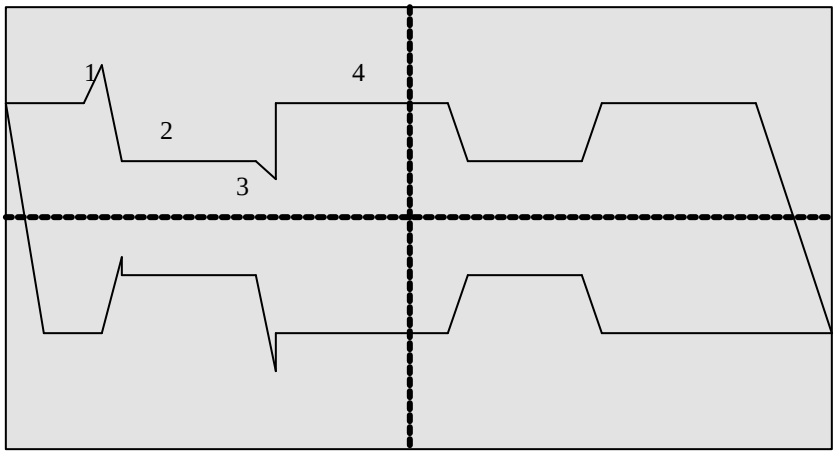
PROCEDURE

1. After adjusting the A – B (forward and backward) and sum signal, push the LFM button and move the detector from side-to-side until the head panel reading (C - D) is 0.00 (or as close to as possible).
2. Release the LFM button to check the A – B reading. Normally the A-B reading has changed and you need to adjust the fine adjustment screw located in front to get the desired A-B reading back.
3. Repeat 1 and 2 until both A – B and C - D readings are desirable.
4. Now follow the procedure outlined in the controller's manual for engaging the sample. After engaging, open an additional buffer window and select the **Friction** option in the program.

NOTES

- The pin on the underneath of the photodetector keys into the sliding slot on the microscope base. This permits the detector to move from left-to-right (for LFM) while maintaining its position set for the A – B signal.
- If you still are unable to obtain a reading around 0.00 for the LFM by moving the photo detector, you may want to loosen the scanner retaining screws and rotate the scanner slightly back and forth.
- Lateral force on the cantilever is caused by both friction and the cantilever touching the edges of sample surface features; therefore, it is important to verify that the data on the friction channel is a result of friction between the cantilever and the sample. Refer to your controller's manual for more information regarding friction; however, in general, the trace and retrace lines will coincide vertically if there is no friction, and the vertical separation between trace and retrace lines will increase as the friction increases.
- Rotating the scan angle can maximize separation between trace and retrace – increasing friction. This occurs because friction force is measured by the amount of cantilever twisting.

EXAMPLES OF FRICTIONAL DATA



This graph depicts the ideal traces on-scope for a sample with low friction areas where one is a flat bump (possibly due to some low friction material sticking to a high friction surface). Notice on the trace that as the probe strikes the edge of the bump (1), the lateral force increases momentarily and then decreases as the tip begins scanning across the low friction bump (2). At the other edge of the bump, the lateral

force may decrease as the probe comes off the bump (3), and then will increase as the probe scans across the high friction surface (4). On the retrace, notice the sign of the frictional force will change, but the shape will remain the same. The lateral forces due to topography shift to the other side of the bump and also change sign.

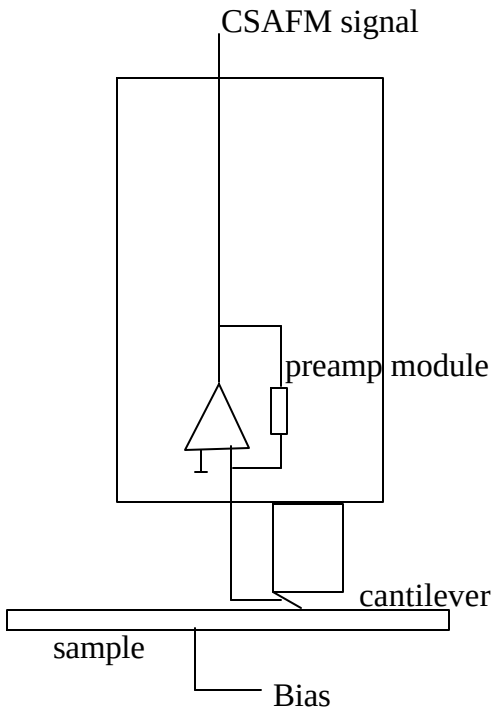
Chapter 6: CURRENT SENSING AFM

Current Sensing AFM (CSAFM™) utilizes a conducting AFM cantilever and operates in contact AFM mode. It simultaneously probes the conductivity and topography of a sample surface. By applying a voltage bias between the sample and conducting cantilever, a current flow is generated. This current is used to construct a spatially resolved conductivity image.

Typical operational range of the current depends strongly on the sample conductivity and its surface conditions. It varies from sample to sample. The default sensitivity of CSAFM is set to 1nA/V. Its operational range is from a few pA to 10 nA. Other ranges can be obtained by changing the preamp module with one that has a different sensitivity.

Surface contamination (especially a moisture layer on the sample surface) has been a major obstacle in preventing CSAFM from getting good and clear images. We strongly recommend the operation of CSAFM in a controlled environment, such as with a PicoAPEX chamber.

A block schematic for CSAFM is shown below.



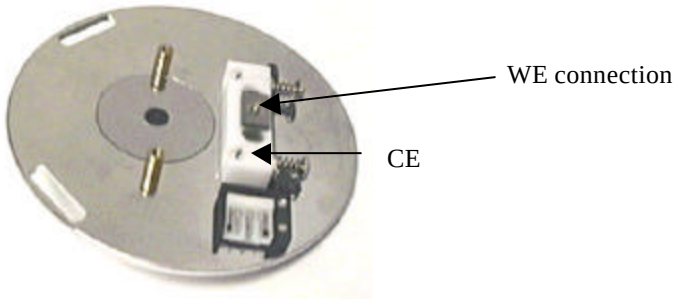
The bias voltage for CSAFM is applied through the working electrode (WE)[1] to the sample. The conducting cantilever is kept at virtual ground.

[1] For NanoScope™ controller, bias is applied to CE instead.

CAUTION: The CSAFM cable for PicoScan™ (Molecular Imaging) or NanoScope™ (Digital Instrument) provided by Molecular Imaging MUST be used for CSAFM measurement.

SETTING UP THE SAMPLE FOR CSAFM MEASUREMENT

1. Affix the sample to the stage using appropriate hardware.

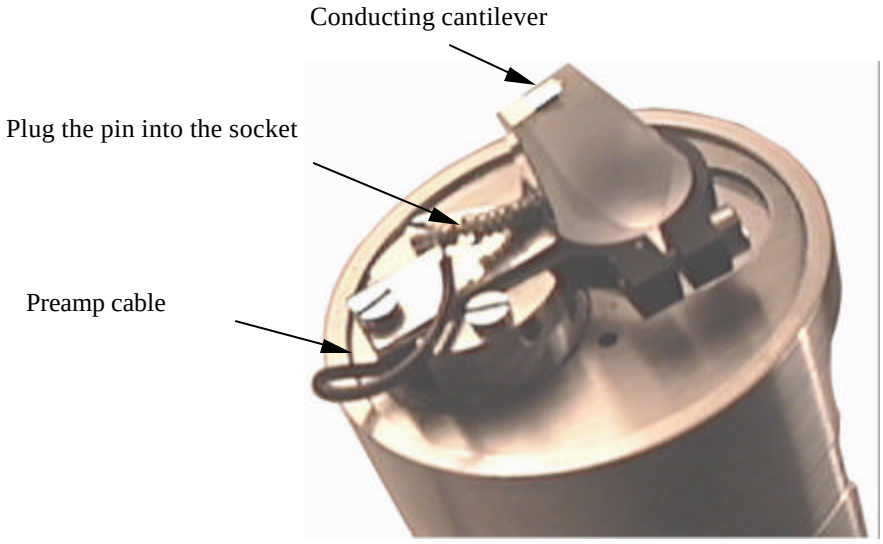


2. Using a conductive wire, connect sample to WE[2] connection.
3. Check the continuity between the WE contact and sample to make sure a proper connection is achieved.

4. Connect the sample stage to the microscope by using the 3-pin EC cable.
5. Check the potential between the WE contact and ground (microscope body) with a voltmeter, it should be the same as the bias voltage[3] set in the software.

[2] For NanoScope™ controller, Use CE instead.
[3] Check the section of setting CSAFM with controller

SETTING UP CSAFM SCANNER



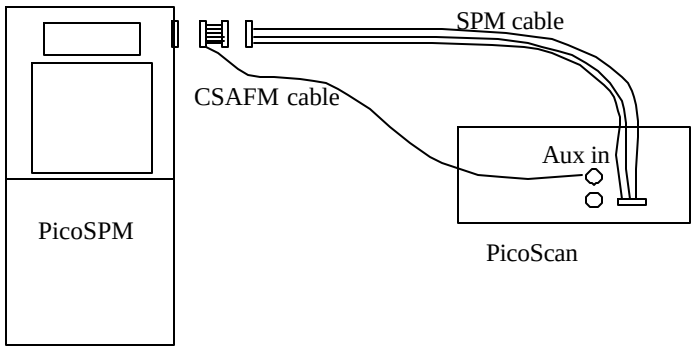
The CSAFM scanner has two 9-pin connectors. One of the connectors is for AFM operation (8 wires); the other is for the current sensing function (3 wires).

1. Plug both 9-pin connectors into the microscope: the 8-wire connector into AFM socket (left), the 3-wire connector into STM socket (right).
2. Follow the procedure described in Chapter 5: AFM to set up the AFM operation.

SETTING UP CSAFM WITH CONTROLLER

Using CSAFM with PicoScan™

1. Plug the CSAFM cable for PicoScan between the microscope and controller as shown below.



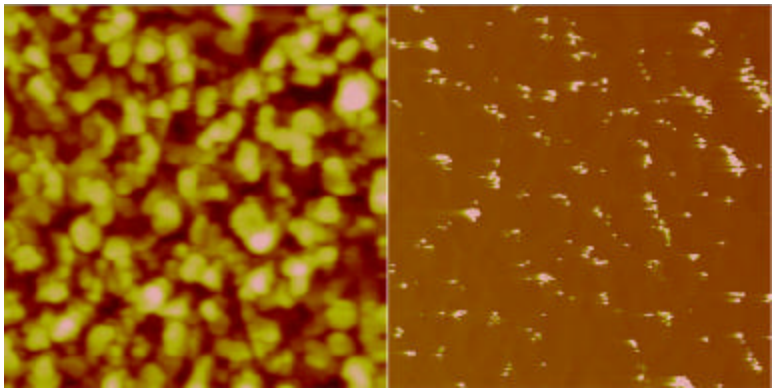
2. Follow the procedure described in the PicoScan manual. Activate Topography, Deflection, and AUX in BNC into 3 windows.
3. Activate the AFM IV spectroscopy window. The bias for CSAFM is applied through Voltage (V). Depending on the conductivity of a sample, the optimized bias may vary. It is a good practice to run an AFM IV spectroscopy first and use the IV plot as a guidance in selecting a good Voltage. Start with a small Voltage value and gradually increase it to optimize the CSAFM image contrast. For a typical Cu film, start with a bias voltage of 0.1V. A good check is to reverse the bias (e.g. change 0.1V to –0.1V to see if the image contrast reverses).

Using CSAFM with NanoScope

1. Use the special DB37 to DB25 CSAFM cable for Nanoscope controller.
2. CSAFM signal is fed through AUX B channel.
3. Bias is applied to the sample WE via. Analog 1.

IMAGING WITH CSAFM

The cantilever tip is kept at virtual ground at all times and bias is applied to the sample. The current signal is shown as positive when sample surface is biased negatively. The CSAFM image shows highly conductive regions as high features. The amplitude of the current signal obtained from CSAFM is strongly dependent on the condition of the cantilever tip and sample surface as well as the force applied to the surface. The following image is a CSAFM image of Cu film on mica substrate. The current image is displayed in unit of Volts; it can be converted by using the CSAFM module sensitivity times the value of the voltage measured.



Scan size = 7.57 µm x 7.57 µm
Topographic image on the left with z range=108 nm,
CSAFM image on the right with z range = 0.271 Volt.

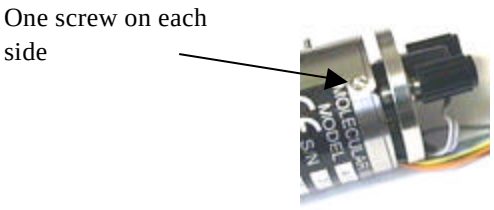
TROUBLESHOOTING AND THINGS TO AVOID WITH CSAFM

1. CSAFM images become unstable when the surface of the sample is not clean. Most of the time by allowing CSAFM to scan the surface for a while, it will clean up the image.
2. For some conductive samples the CSAFM image will show no contrast because the current may have reached saturation. Thus it is better to start with a very small Voltage and gradually increase it until a desired image is obtained. For highly conductive metallic samples even zero voltage in the software may give reasonable current image due to some small voltage offset because of possible voltage drops throughout the system.
3. Use lowest possible force during imaging to reduce the wear of the metal coating at the very end of the conductive tip.
4. Be sure to insert the cantilever onto the holder carefully to avoid damage of the conducting film coated on it.
5. The cantilever clamp (which holds the cantilever in place) is isolated electrically from the black holder piece. Place the holder carefully on the end of the scanner. There will be no CSAFM signal if the clamp is shorted to the holder.
6. It is normal for the CSAFM signal channel to have a large noise level without a sample plate placed onto the microscope. This noise will be significantly reduced when the sample plate is in place. This shields the CSAFM probe.

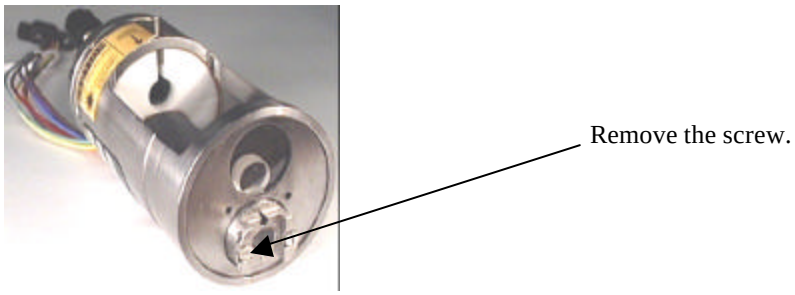
CHANGING CSAFM PREAMP MODULES

A different sensitivity of the preamp module is available as an option. The preamp module is fastened at the end of the scanner by two screws and it can be exchanged easily.

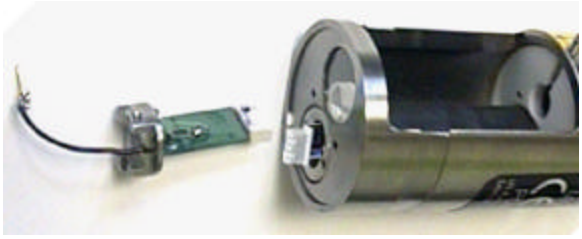
1. Remove the two screws on the topside of the scanner as shown. It allows the 3-wire cable to be moved freely.



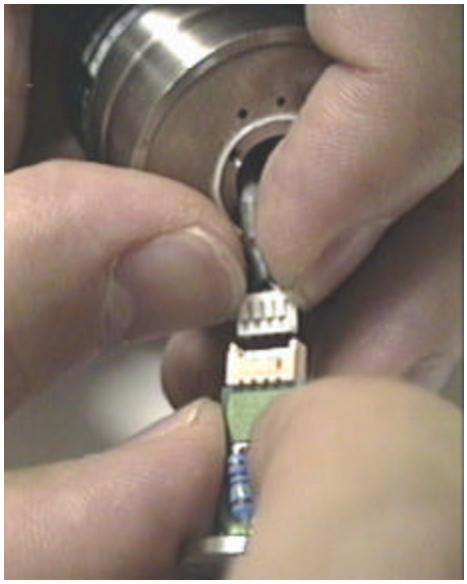
2. Remove the two screws on the bottom of the scanner as shown.



3. Carefully remove the module.

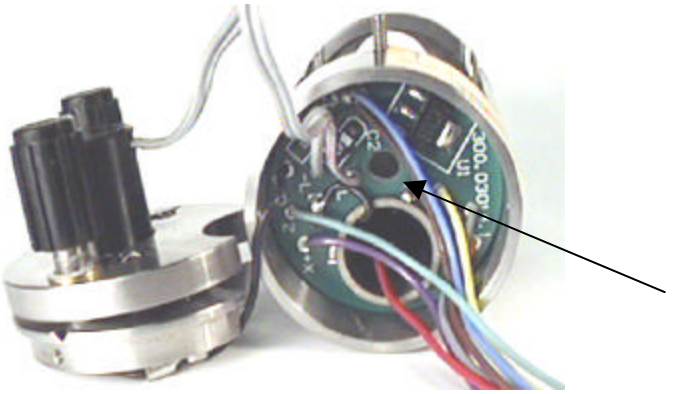


4. Hold the module firmly and unplug it from the 4-pin connector.



5. Replace it with the new module, and plug it back in.
6. Carefully place it back into the scanner and fasten the two screws at the bottom of the scanner.
7. Adjust the 3-wire cable by pulling it out a little.
8. Fasten the two screws on the topside of the scanner.

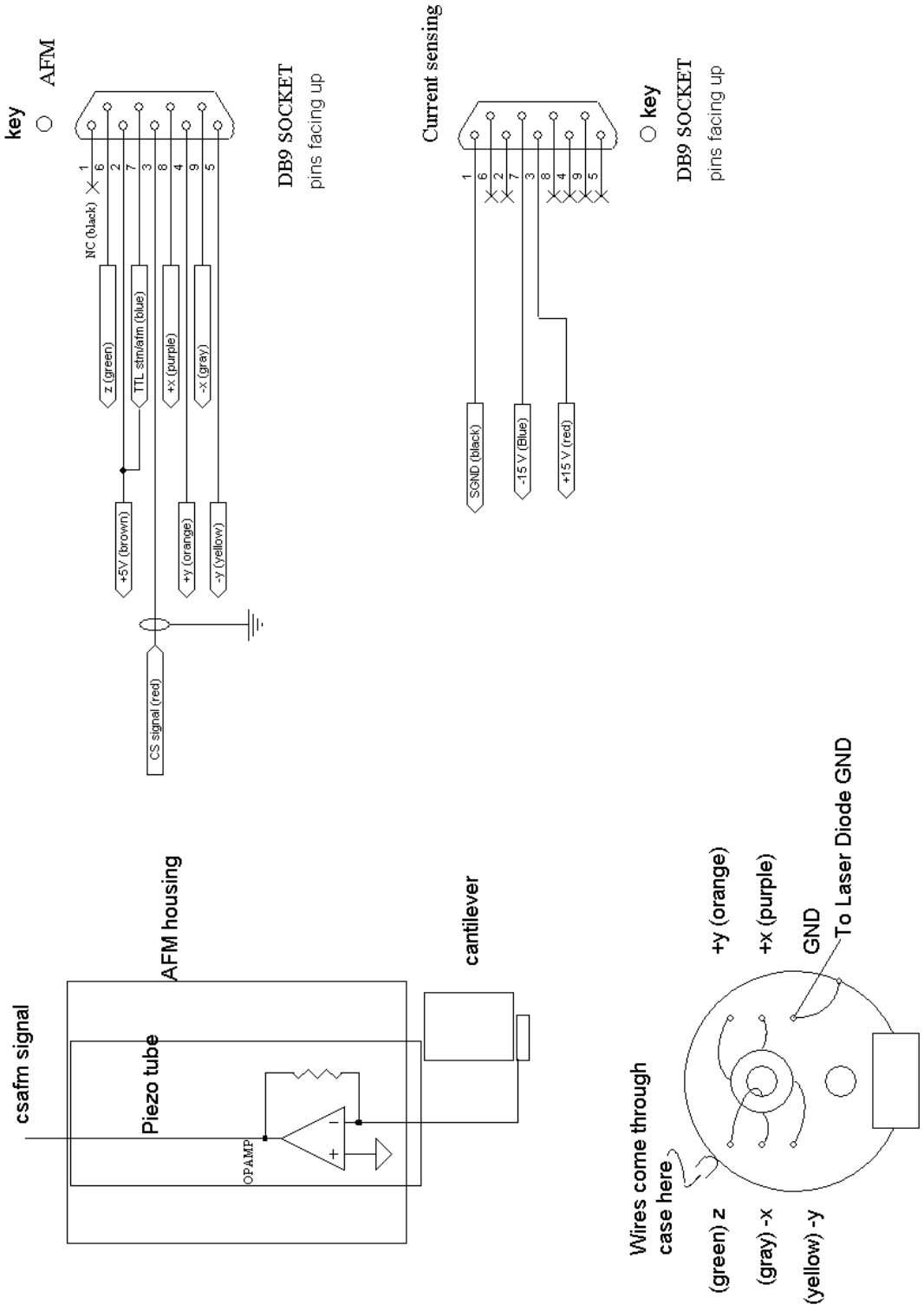
CAUTION: Make sure wires do not block the laser path.



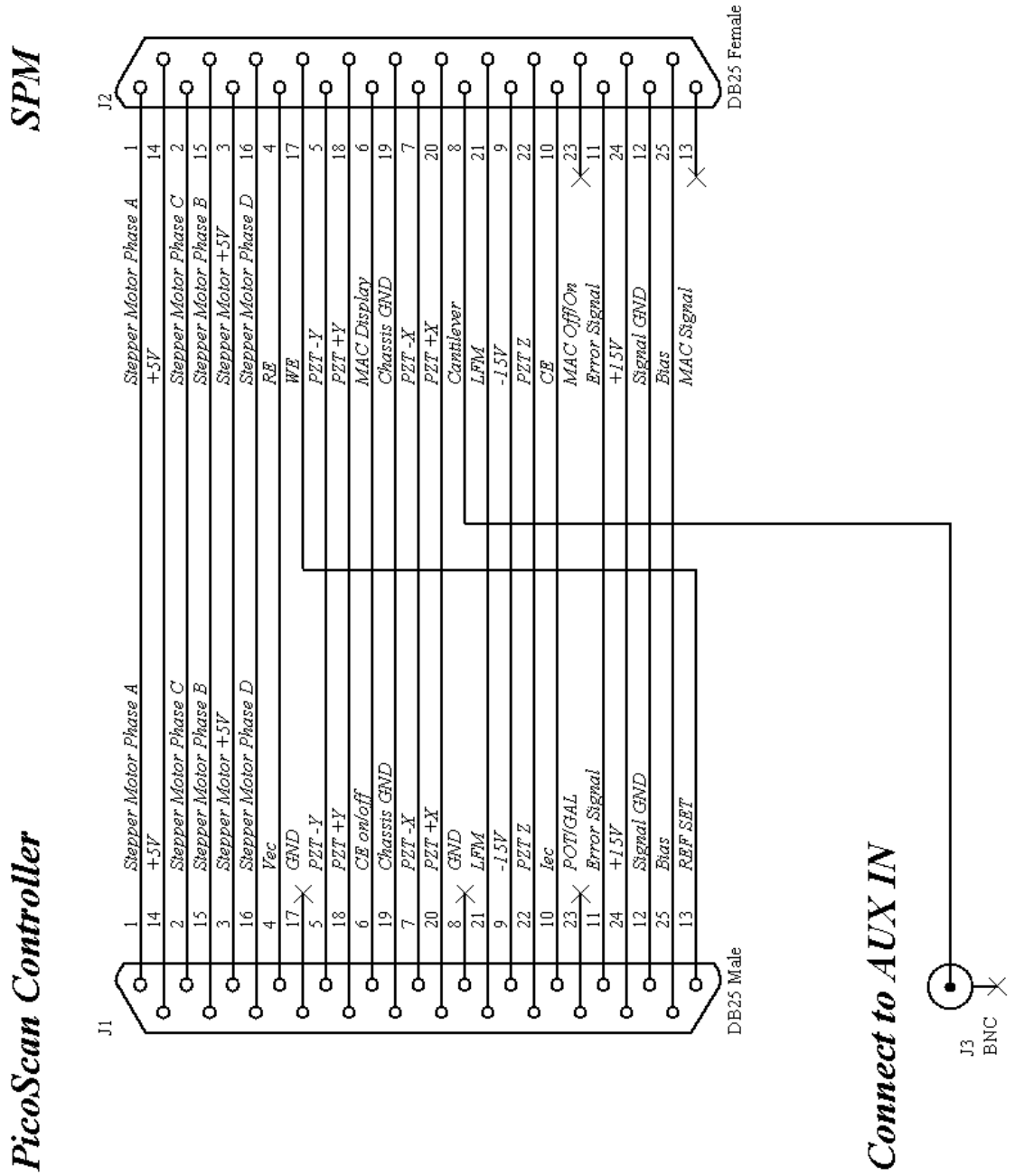
Keep the laser path clear.

SCHEMATICS

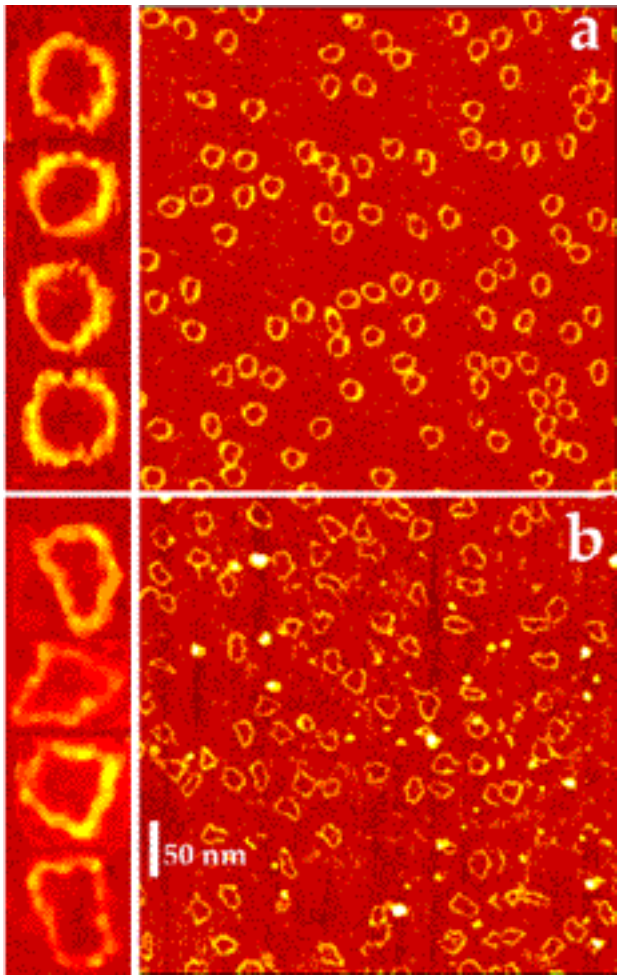
CSAFM wire schematic



Connection cable schematic for PicoStat



Chapter 7: AC MODE AFM



DNA KINKING in MAC Mode: Taken from NATURE April 10, 1997

The PicoSPM AC Mode controller greatly expands the potential uses of the PicoSPM system. It allows the user to induce oscillations in the cantilever acoustically or magnetically, facilitating the imaging of various "soft" samples with precision, as seen in the above picture.

CONNECTIONS

POWER CORD CONNECTION

Insert the power cord supplied with your AC Mode package into the back of the AC Mode controller. Do not power on the controller at this time.

PICOSPM CONNECTION

Connect the male end of one of the DB25 cables to the top, **MICROSCOPE**, connection on the AC Mode controller. Be sure the cable fits snugly and is not loose; if the connection is loose this may cause serious problems while imaging.

After connecting the cable to the AC Mode controller, connect the other end into the connector on the PicoSPM.

SERIAL PORT CONNECTION

For most users, the AC Mode controller will be controlled using a standard RS-232 serial link. Connect to the computer COM1, COM2, or COM3. The default is COM1.

PICOSCAN CONTROLLER CONNECTION

Connect the female end of the other DB25 cable to the bottom connection labeled **CONTROLLER** on the AC Mode controller. Be sure the cable fits snugly and that it is not loose, this too can result in serious malfunctions while imaging.

After connecting the cable to the AC Mode controller, connect the other end of the DB25 cable to the **MICROSCOPE** connection on your PicoScan™ controller.

MAC MODE CABLE CONNECTION

Connect the BNC end of the MAC Mode cable to the back of the MAC Mode controller labeled **MAC** (**CURRENT** or **DRIVE** on older MAC Mode controllers). It is this connection that will ultimately drive the solenoid under the MAC Mode sample stage, creating the magnetic field to drive the cantilever. The 6-pin connector on the other end attaches to the MAC Mode sample stage.

ACOUSTIC AC CABLE CONNECTION

Attach the BNC end of the Acoustic AC drive cable to the back of the AC mode controller labeled **AAC** on the AC Mode controller (**VOLTAGE** on older models supporting Acoustic AC Mode or **DRIVE** on an Acoustic AC controller). The 3-pin connector on the other end attaches to the Acoustic Module in the AFM Scanner.

It is not possible to do both MAC Mode and Acoustic AC imaging at the same time.

INSTRUCTIONS FOR USING AC MODE ELECTROCHEMISTRY

If a PicoStat® is also used it must be placed between the PicoScan controller and the AC Mode controller. This makes the order of connection PicoScan, PicoStat, AC Mode controller, and then PicoSPM.

THE AC MODE CONTROLLER

The AC Mode controller is the driving force behind AC Mode. It is here that the drive signal is produced and controlled. After preparing and mounting the sample it is now time to turn our attention to the AC Mode controller. Note: the first part of this section will discuss the MAC Mode controller. For the Acoustic AC controller the back panel only has 4 BNC connectors. DRIVE is described as the AAC connector under the back panel section following. The other signals are AMPLITUDE, PHASE, and INPUT. All are described in the following section.

AC Mode controller front panel

FRONT PANEL

Select one of the following four signals to monitor: **OUT**, **DRV**, **S.P.** (Setpoint), **IN**.

OUT
This is the amplitude of the cantilever motion from the PicoSPM. This is in volts but can be converted to distance as follows:

$$Amplitude(nm) = -\left[\frac{DisplayVoltage(V)}{20xInputGain} x4.3xSensitivity(\frac{nm}{V})\right]$$

Equation 1.

Where InputGain is the Input Gain setting in the AC Mode controls.
Sensitivity is the PicoScan controller pre-amp conversion coefficient. This is calibrated in PicoScan by taking the inverse of the Force vs. Distance contact region slope. Note that you should recalibrate the sensitivity each time you use a different cantilever. See the PicoScan manual for more information.

When using the data displayed in the PicoScan controller software, adjust for Force Setpoint, and distance would be as follows:

$$Amplitude(nm) = -\left[\frac{(DisplayVoltage(V) + 4.3xS.P.(V))}{20xInputGain} xSensitivity(\frac{nm}{V})\right]$$

Equation 2.

Where S.P. = Force Setpoint

DRV
This is the RMS value of the drive signal in mA. This is actually the RMS value of the drive signal going to the coil on the MAC Mode sample stage. In Acoustic AC Mode it is the RMS value of the drive voltage. The conversion factor for mA to V is as follows:

$$drive(mA) * 0.1\frac{V}{mA} = drive(V)$$

Equation 3.

S.P.
When selected, this will display the Force Setpoint voltage.

IN
The RMS value of the raw input to the lock-in amplifier.

AC MODE
Switches between intermittent contact and contact mode. With **AC MODE** on the following occur:

1. The microscope is sent a signal to switch to AC Mode.
2. The AC Mode controller replaces the deflection signal with amplitude for the signal sent to the controller.
3. The AC Mode controller replaces friction signal with phase for the signal sent to the controller.

MONITOR
This is the buffered output of the signal displayed on the meter.

DRIVE OFF/ON SWITCH
This turns the current and voltage drives on and off. This must be switched to on for AC Mode to work.

BACK PANEL

MAC Mode controller back panel

INPUT
This is the input to the lock-in amplifier. In AC Mode this is the raw amplitude signal from the PicoSPM.

MAC
Drive output for MAC Mode. Connect to the MAC Mode cable that plugs into the MAC Mode sample stage. Could be labeled **CURRENT** or **DRIVE** on older versions of MAC Mode.

AAC
Drive output for Acoustic AC. Connect to the Acoustic AC cable that plugs into the Acoustic AC module in the AFM Scanner. Could be labeled **VOLTAGE** on older AC Modes and is labeled **DRIVE** on Acoustic AC controllers. Some older MAC Modes DO NOT support Acoustic AC.

X
Outputs a DC voltage that represents the amount of the output signal that is in phase with the drive signal.

Y
Outputs a DC voltage that represents the amount of the output signal that is 90° out of phase with the drive signal.

AMPLITUDE
Amplitude of the center frequency component of the PicoSPM input signal. Same as front panel **OUT**.

$$Amplitude(V) = \sqrt{X(V)^2 + Y(V)^2}$$

Equation 4.

PHASE
Phase shift of the center frequency component of the input signal. The conversion factor is 9 degrees per volt. (Note: for hand held terminal versions the conversion factor is 18 degrees per volt.)

$$Phase = \tan^{-1}\frac{Y}{X}$$

Equation 5.

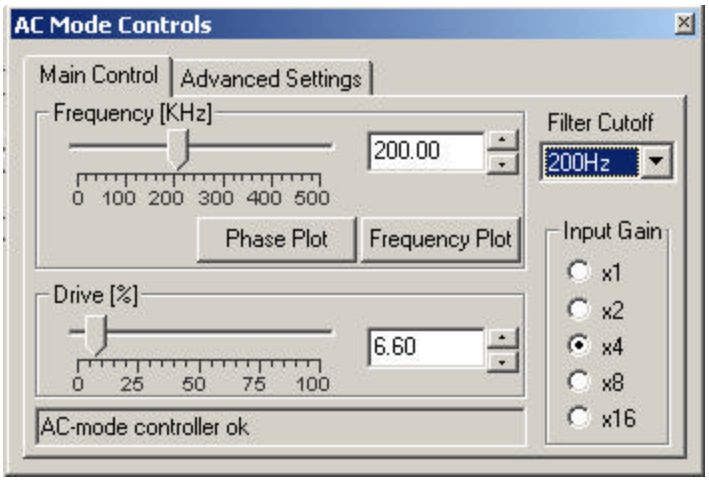
DRIVE IN
This is for an auxiliary input signal to be summed into the MAC Mode drive signal.

AC MODE SOFTWARE CONTROLS

SOFTWARE INSTALLATION

The software for the AC Mode controllers comes standard in PicoScan version 4.19 and later. To run the AC Mode controls in the PicoScan software simply click the AC Mode controller supported check box under the edit options and calibration window start-up options. Then click on the AC Mode button on the main menu.

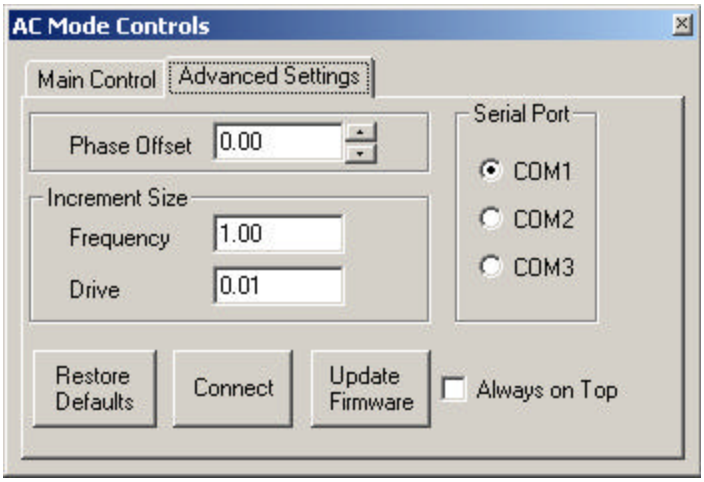
The controls shown below are from PicoScan version 4.19 software.



AC Mode software controls Main Control window

MAIN CONTROLS

- Frequency [KHz] – the frequency of the drive signal. Adjusted by using the slider bar or the edit controls. Currently 5KHz – 500KHz.
- Phase Plot – opens the Phase vs. Frequency spectroscopy window.
- Frequency Plot – opens the Amplitude vs. Frequency spectroscopy window. Use this spectroscopy window to "tune" your cantilever.
- Drive % – the magnitude of the drive signal as a percentage of the maximum possible. Adjusted by using the slider bar or the edit controls.
- Filter Cutoff – sets the cutoff frequency for the lock-in amplifier. Adjusted by using the pull down menu.
- Input Gain – sets the gain for the input signal into the AC Mode controller's lock-in amplifier.



AC Mode software controls Advanced Settings

ADVANCED SETTINGS

- Phase Offset – allows the Phase signal to be adjusted about zero for best imaging results. Adjusted by using the edit controls.

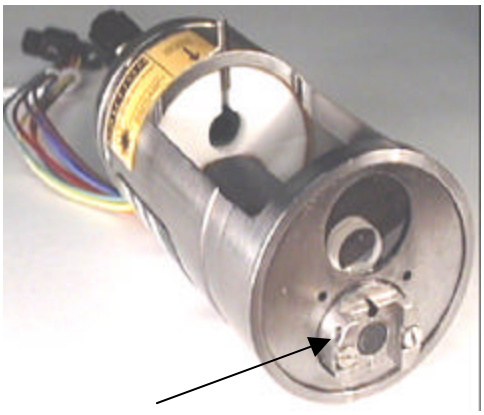
- Increment Size – allows the step size of the Frequency and Drive signals to be determined by the user. Adjusted by using the edit controls. Increment size is used by the Frequency and Drive controls arrow buttons.
- Serial Port – allows the serial port to be chosen by the user. Default is COM1.
- Restore Defaults – sets all settings back to the factory default.
- Connect – the software goes out and searches for the AC Mode controller. Used when the AC Mode controller is not detected at software start.
- Update Firmware – will reprogram the AC Mode controller firmware with the latest version. Follow the instructions given carefully.
- Always on Top – keeps the AC Mode controls on top of the desktop at all times when selected.

SELECTING AND MOUNTING A CANTILEVER

Choice of an appropriate AFM cantilever is critical to the successful operation of AC Mode AFM. Typically cantilevers with spring constant on the order of 1N/m or higher are used. Most vendors will specify force constant and resonance frequency of their cantilevers; make note of them. The actual values of a cantilever's force constant and resonant frequency typically vary by 10-15% of the published values.

MOUNTING THE CANTILEVER

In AC AFM, mounting of the cantilever is the same as that of contact AFM. For AAC AFM, there is a notable exception to the standard procedure – since an acoustic signal is being excited through the holder, it is also important that the two small set screws (shown below) be tightened to provide a more rigid connection. This is done to minimize the likelihood that stray vibrations will couple into the detector and degrade resolution.



Set screw at the end of a scanner.

ALIGNING AND TUNING THE CANTILEVER

As previously noted, the AC Mode provides the best signal to noise response when the cantilever is oscillated at or near it's resonance frequency. It is, therefore, important to become skilled in properly aligning the cantilever and identifying the cantilever resonance frequency.

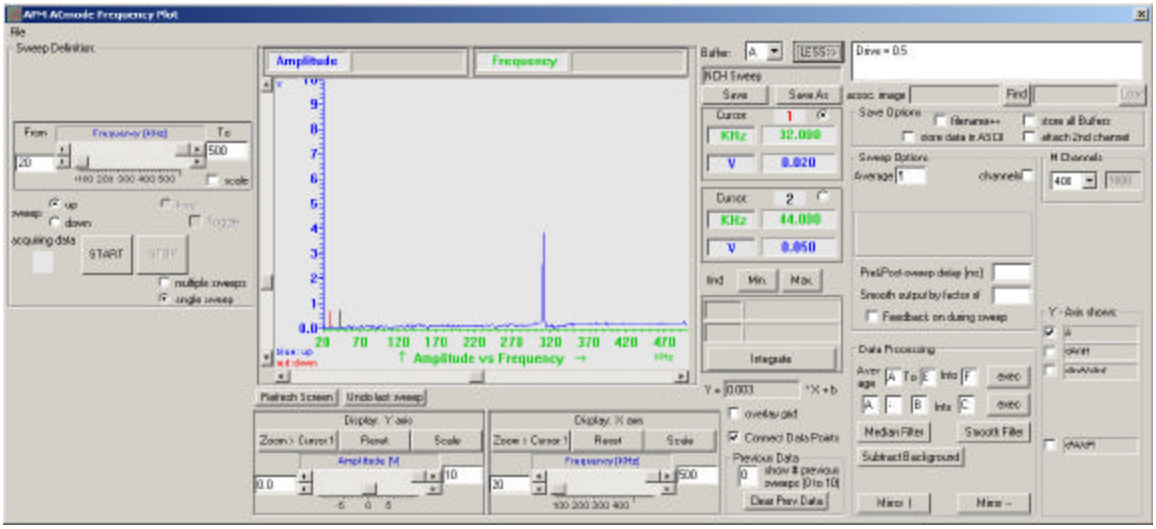
SYSTEM SETUP

- Attach all cables as described in earlier in this chapter.
- The AC Mode electronics must be switched on prior to operation, the power switch can be found on the rear panel alongside the power cable.
- On the AC Mode controller, ensure that the "AC Mode" and "Drive" switches are turned off. ("AC Mode" is off when the LED is not lit).
- In the PicoScan controller software adjust the Force Setpoint to zero. (The control can be found in the Scan Control window of the PicoScan software, for operation on other controllers, refer to your manual.)
- Using the appropriate AC Mode equipment, set up the microscope as you would for a standard AFM experiment and align the laser on the apex of the cantilever. This procedure is described in Chapter 5.
- Align the detector in the microscope such that both signals on the head of the microscope (with and without the LFM button depressed) are close to zero. This procedure is described in detail in Chapter 5.

TUNING THE CANTILEVER

"Tuning" is a commonly used term referring to the systematic location of the cantilever's natural resonance frequency and the selection of an appropriate driving signal to establish an appropriate oscillation amplitude.

- In the software, open the AC Mode controls in PicoScan version 4.19. For other controllers using software that runs in an operating system other than non-modern Windows see the end of this chapter for instructions on using the Hand Held Terminal.
- The AC Mode Controls software interface is shown above. Set Input Gain (upper right of control window) to x1. Set Drive to 5%. Set the filter cutoff to 5 kHz. Click on the Frequency Plot button. In MAC Mode ensure that the MAC Mode sample stage is positioned such that the ferrite core is directly under the cantilever and as close as possible without interfering with the free oscillation of the cantilever.
- Select an appropriate frequency sweep range for your cantilever. Select sweep up and single sweep and the #channels (samples) found by pushing the More button. The resolution is determined by the frequency range divided by #channels.



AFM AC Mode Frequency Plot

- On the AC Mode controller, turn the Drive switch to ON. This switches both the current and voltage drives on in the AC Mode controller.
- Press the "Start" button in the AFM AC Mode Frequency Plot window. The software will perform a simple sampling procedure measuring the oscillation amplitude of the cantilever while stepping the frequency from Start Frequency to End Frequency. The sweep will be displayed on the window.
- The resonance response curve, also called the tuning curve, should show a single, sharp peak that stands several times taller than the baseline noise. The height of the peak can be changed by adjusting the Drive(%) on the AC Mode Controls and the frequency axis can be changed using the From/To Frequency controls in the spectroscopy window. Using these controls and, if necessary, decreasing the Step Size, locate the frequency at which the curve reaches its maximum value. This is the natural resonance frequency of the cantilever. Set the frequency at which you will image to be on the steep slope on the low side of the resonance frequency. This will give you the best image quality, especially if the resonant frequency decreases when you engage the sample, which sometimes happens.
- The resonant frequency can be found by placing a cursor on the peak using the left mouse button. The frequency and amplitude will be displayed to the right of the image. Once the resonance frequency is determined, enter your desired frequency value into the Frequency control in the AC Mode control window. Beneath the display on the AC Mode controller, press the OUT switch (a green LED indicates it is on). The display will now show the amplitude of oscillation.
- Note: the oscillation amplitude is negated such that a decrease in amplitude corresponds to an increase in value, making it respond similarly to the detector signal in contact AFM. In other words, amplitude is proportional to the absolute value of the OUT signal.
- Turn AC Mode on by pressing the AC Mode button on the front of the AC Mode controller (the light above the button will come on indicating that the controller is in AC Mode). The absolute value of the number displayed on Head Panel Display is now proportional to the absolute value of the amplitude minus the setpoint. In other words, the AC Mode controller converts the amplitude of the cantilever into a DC voltage that is analogous to deflection. Adjust the Drive control in the software until the OUT signal on the AC electronics displays roughly –8.0 V. In Acoustic AC Mode if you haven't already placed the sample on the PicoSPM do so now. In the PicoScan software adjust the Force Setpoint

such that the Head Panel Display reads roughly –3.0 (Note: do not adjust the detector position at this stage in the procedure). You are now ready to engage the microscope and image.
- For softer samples the setpoint will need to be adjusted as necessary to minimize the tip-sample interactions. If the difference between the setpoint and free amplitudes is too great there is a potential for degrading the sample quality.

OBTAINING AN IMAGE WITH AC MODE

- Approach the probe to the sample surface in the same way as in contact mode AFM and start to image after the probe is in range.
- It is important to note that the controller approaches the tip to the sample until the amplitude of the cantilever in volts has been decreased to the Force Setpoint voltage.
- Using the force-distance curve to adjust the set point is a good way to optimize imaging conditions. The amplitude is represented by a negative voltage, thus free amplitude is at the bottom of the plot and zero amplitude is at the top. Under normal conditions you should adjust the force set point to about 50% of free amplitude. Again with softer samples this value may need to be adjusted to a higher percentage of the free amplitude.
- Typically in AC Mode AFM, it is desirable to use lower gain settings (I,P = 0.3, 0.3) and a slower scan rate (2-3 Hz).

ADDITIONAL TIPS FOR ACOUSTIC AC IMAGING

If you cannot seem to get a good image in Acoustic AC mode, these tips may help you:

- Place the microscope in a PicoIC with the door closed. Make sure that there are no significant sources of noise in the area.
- Separate all relevant cables so that their magnetic fields will not interact to produce noise. Also keep the cables away from any other power cords or electric devices.
- Handling the microscope can cause thermal instabilities in the system. Try leaving the microscope alone for at least an hour after setting it up to allow the system to reach thermal equilibrium before imaging.
- If the piezo is drifting in the Z direction, try restarting the software and/or controller.
- If you cannot find a good resonance peak with the frequency sweep, or if the cantilever drifts away from the laser beam, try gluing or otherwise securely fastening the cantilever to the cantilever holder.

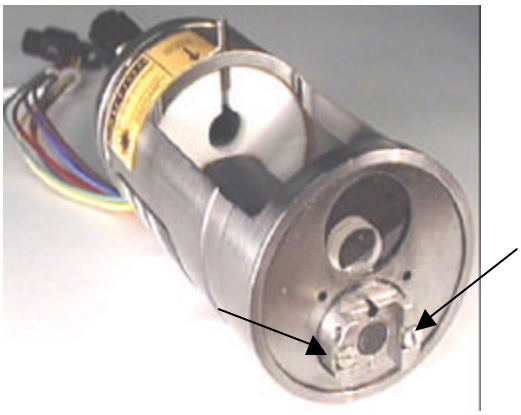
Refer to Appendix F for an in-depth analysis of AC mode.

AFM SCANNER MODIFICATION FOR ACOUSTIC AC MODE

Upgrading an AFM scanner to AAC scanner is straightforward. (Note: Only the AFM scanner with removable end cap as shown in figure 7 can be upgraded to do Acoustic AC AFM. Also note: This modification only needs to be done once and may have already been done on your scanner. If you have any questions, contact Molecular Imaging before proceeding.)

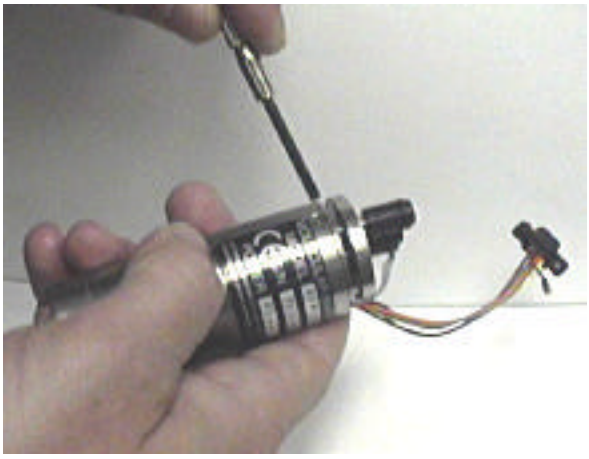
SCANNER MODIFICATION

Unscrew the two small screws on the end cap of the scanner and remove it. The end cap may be held in place by silicone sealant so a little effort may be needed to remove it.



The end cap fastened by two small screws

Unscrew the two screws on the topside of the scanner to remove the laser mount top. The serial number label may be covering the screws. Just peel back the corner to get access if necessary.



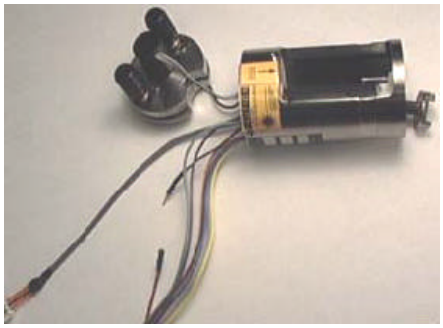
Remove the laser mount top

Carefully insert the 3-pin connector on the AAC module into the scanner from the bottom as shown below.

AAC module

Insert the 3-pin connector into the scanner

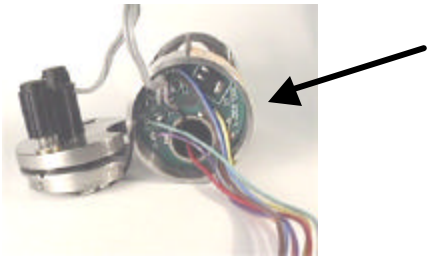
Feed the wires through the hole to the other end of the scanner.



Feed the wire through

Replace the end cap on the bottom of the scanner and fasten the two screws.

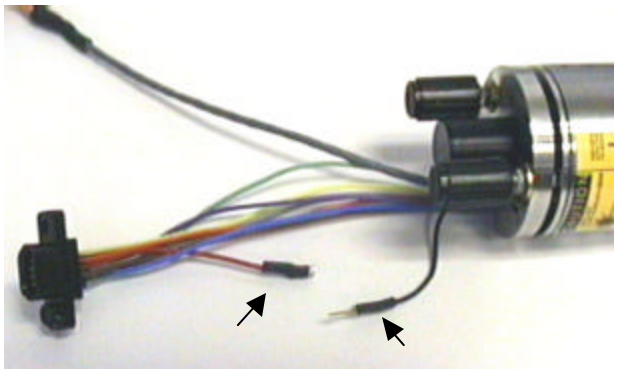
Replace the laser mount top, and make sure that wires do not block the hole for the laser path. Also be careful to bring the wires out through the opening in the top and not pinch any of them.



Hole for laser path

Fasten the two screws on the side to secure the laser mount top in place.

Plug the pin into the socket as shown below.



Pin and socket for AAC scanner

TOPMAC

The TopMAC works by relaying the field from a coil via a ferrite rod down to the cantilever. In this way, a magnetic field is generated close to the cantilever while leaving space for the position-sensing laser beam. The arrangement is illustrated below in Figure 1A. It permits operation of MACMode AFM on an inverted optical microscope or with any of MI's optional heating/cooling sample stages.

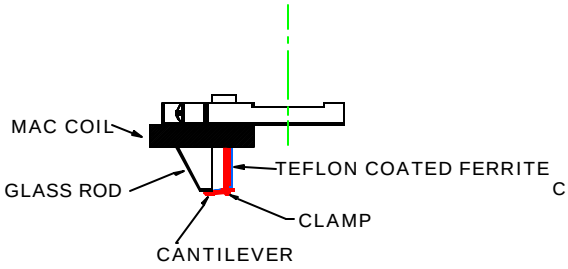


Figure 1A: Layout of top-down MacMode Cantilever holder

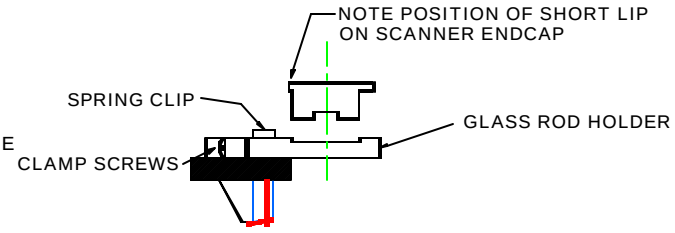


Figure 1B: Position of new cantilever holder clamp

MOUNTING THE CANTILEVER

By pressing down the cantilever holder, the cantilever can be placed underneath the clamp (unlike the regular cantilever holder, there is no cantilever mounting fixture needed for TopMAC). Place the cantilever so that the edge of the cantilever substrate protrudes a little bit from the ferrite.

INSTALLATION

The spring clip for the cantilever clamp requires some extra clearance. The scanner endcap must be replaced by the new endcap supplied with the TopMAC. See figure 1B. To do this, you must have one of the more recent scanners with a removable endcap.
Note that the new endcap for TopMAC does not have a magnet. The cantilever holder is retained with the set screws only. (This is very important. Magnetic

components placed near the driver coil will respond acoustically giving a noisy "tapping-mode" like response. Also, note that the clamp screws used on the glass rod holder should be electrically non-conductive.)

LASER ALIGNMENT

The holder is designed to operate with the laser beam just grazing the edge of the glass. The alignment is a little different from the usual cantilever assembly and some care is required to get this right.

DRIVING

Make sure that the driving cable is connected to the MACmode box (to the output current drive). The procedure for driving the cantilever is the same for bottom-MAC and TopMAC.
Note: The size of the coil has been reduced (relative to the bottom-Mac driver). However, it has been redesigned electrically so that higher current drive is available. The existing Mac box will drive it all the way to 140 mA, permitting somewhat greater amplitudes than the bottom Mac. To check for correct installation, be sure to measure an amplitude-frequency response curve in liquid, making sure that the expected smooth response is obtained.

PLACING THE SAMPLE STAGE

Mounting the sample stage is done as described for contact mode AFM or MACmode AFM. In contrast to the bottom-MAC, the maximizing of the MAC signal is not influenced by the position of the sample stage anymore (in TopMAC operation, there's an increased flexibility regarding the positioning of the sample stage).
Please note: Due to the fact that the cantilever holder clamp is thicker than the clamp of the regular holder, it is very important that the sample stage is lower in the back than in the front (slightly tilted even after approach).

HANDLING

The TopMAC cantilever holder is more fragile than the regular holder. Especially the wires need extra attention and can be easily "ripped apart".

CLEANING

Hold over a small petri dish of clean water and rinse the end carefully. Do not wash the coil.

HAND HELD TERMINAL

Note: The hand held terminal is only used for systems with a controller running on a non-modern Windows operating system or a controller other than PicoScan.

HAND HELD TERMINAL CONNECTION

Connect the hand held terminal into the back of the AC Mode controller. Make sure the connection is secure.

The MAC Mode hand held terminal is used to control the driving current and frequency signal supplied to the solenoid on the MAC Mode sample stage. It contains a main menu and three sub-menus. Normally the main menu is the menu, which appears and is most commonly used. When the AC Mode controller is first turned on, the parameter menu appears. The parameters are user defined and are saved at their last setting.

FUNCTION KEYS

- F1 – Save the current settings
- F2 – Go to the previous line
- F3 – Go to the next line
- F4 – Increment a value down
- F5 – Increment a value up
- CTRL F4 – Reset to the default settings
- CTRL F3 – Go to the next page of the setup menu, usually not needed
- CTRL F2 – Go to the previous page of the setup menu, usually not needed

PARAMETER MENU

- CENTER FREQUENCY – This is the frequency of the drive signal.
- GAIN SELECT – Gain for the AC Mode input signal.
- CURRENT DRIVER – Amplitude of the drive signal. Increasing the drive linearly increases the drive on the cantilever.
- CENTER FREQ +/- -- This determines the step size for increasing/decreasing the frequency.
- CURRENT DRV +/- -- This determines the step size for increasing/decreasing the current driver.

Note: Frequency sweeps are done by manually stepping through the appropriate frequency range and watching the output on the AC Mode controller.

DEFAULT SETTINGS

Following is a list of the default settings for the AC Mode hand held controller. To access these settings press CTRL then F2.

AC Mode (V3.2c) MAIN

- CENTER FREQUENCY – 25 KHz
- GAIN SELECT – 4
- CENTER FREQ +/- - 1000 KHz
- CURRNET DRV +/- - 001.0 mA

AC Mode (V3.2c) SETUP 1

- ACTIVE LPF CO – OFF
- ACTIVE LPF ATTN – 24 dB
- MAGNITUDE GAIN – 4095
- PHASE GAIN – 4095
- MUX A/B – OUTO/90
- GAIN/OFFSET +/- - 0500

AC Mode (V3.2c) SETUP 2

- INPUT SELECT – FLT & GN
- OUT A SELECT – MAG
- OUT B SELECT – PHASE
- OUT A POLARITY – NORMAL
- OUT B POLARITY – NORMAL
- PHASE OFFSET – 0000

AC Mode (V3.2c) SETUP 3

- LPCO > 21 KHz – 10K
- LPA > 21 KHz – 24 dB
- LPCO 11-21 KHz – 5 K
- LPA 11-21 KHz – 24 dB
- LPCO < 11 KHz – 2 K
- LPA < 11 KHz – 24 dB

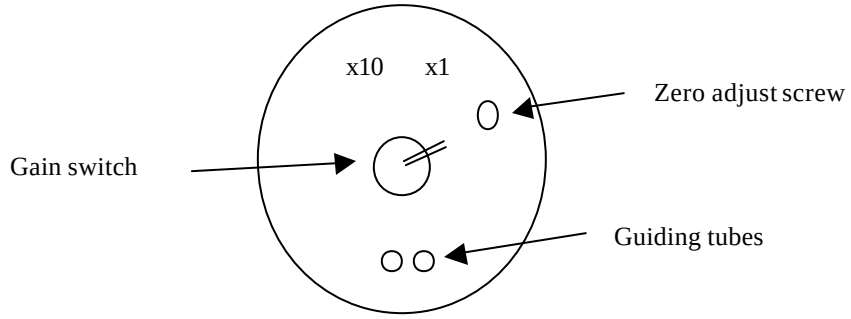
Chapter 8: SCANNING TUNNELING MICROSCOPY – STM

STM SCANNER

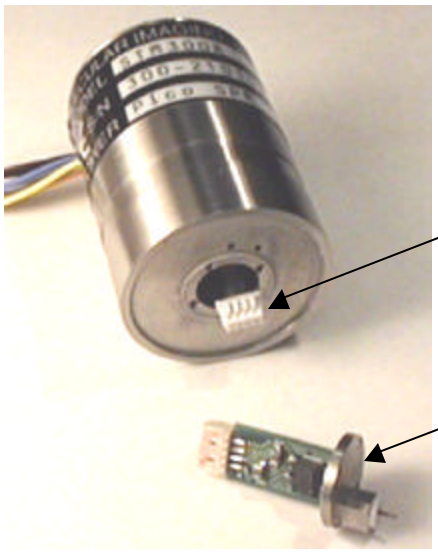
When you receive your STM scanner, you will need to select and calibrate the current to voltage conversion for the scanner. The gain settings of the PicoSTM may be altered by means of a Gain switch on the scanner module. The input to the control is a signal that varies between plus and minus 10 volts. For optimum operation, the setpoint current should produce a signal of 1 volt. For example, if operating at 50 pA, the most sensitive (10 V/ nA) range should be used. The output signal from the head would then be 0.5 V. This signal is displayed on the head panel meter, which shows the voltage out of the head. The polarity reflects the tip current. The controller's software sets the sample b ias, so a negative bias in the software will give a positive reading on the head meter.

ADJUSTING THE GAINS ON THE SCANNER

The STM scanner has two amplifier stages: preamp stage and gain stage.
Gain stage setting:
Top view of an STM scanner is shown below. The mini-switch to the right gives a gain of 1, and to the left gives gain of 10.



The **preamp stage** consists of a preamp board module. It can be replaced with boards with different sensitivity settings.



The standard scanner has the module with 10nA/V sensitivity. The optional module has a sensitivity of 1nA/V. They are color coded as following:
Red = 10nA/V
Blue = 1nA/V
To change these modules, please follow the procedure described below.

PROCEDURE

1. Remove the four screws on the tip holder piece.
2. Pull out the pre-amp module carefully.
3. Unplug the module from the cable, which has a 4-pin connector at the end.
4. Replace the module and plug it back onto the 4-pin connector.
5. Tighten the module back onto the scanner.

CAUTION: *Do not pull the cable while unplugging the module. Hold the 4-pin connector at the end of the cable firmly, and then unplug the module.*

How to calculate final sensitivity:
Final sensitivity (nA/V) = pre-amp sensitivity (nA/V) / gain.

For example:
pre-amp = 10 nA/V, gain stage setting = 10
final sensitivity = 1nA/V

Pre-amp / **Gain**	**10 nA/V** 3dB Bandwidth: **1.6 kHz**	**1 nA/V** 3dB Bandwidth: **160 kHz**
x1	Sensitivity: **10 nA/V** Lowest Imaging Current: **30 pA**	Sensitivity: **1 nA/V** Lowest Imaging Current: **3 pA**
x10	Sensitivity: **1 nA/V** Lowest Imaging Current: **3 pA**	Sensitivity: **0.1 nA/V** Lowest Imaging Current: **0.4 pA**

NOTES

- The zero must be reset after changing the gains.

- The current range of the scanner is ± (final sensitivity × 10 V), e.g., a sensitivity of 1 nA/V gives a current range of ± 10 nA.
- The chosen final sensitivity must be entered into the software. Select the Calibration Options and insert the appropriate sensitivity according to the above equations and table into the Pre-Amplifier Conversion Coefficient box.

The head must be zeroed after gains are changed. To do this, bring up the signal on the controller. Having previously calibrated the current sensitivity, carefully adjust the zero adjust screw (shown in figure) with a small screwdriver until the output on the tunneling scope in the program reaches zero. This adjustment is easier with the PicoScan program because of the oscilloscope available.

LOW CURRENT STM OPERATION

Speed and sensitivity are, in theory, mutually exclusive requirements in the current-to-voltage amplifiers used in STM. In practice, however, the dominant limitation is pickup of stray signals (such as high-voltage scanning ramps). The proprietary circuitry and careful layout of the PicoSTM has eliminated this problem. The PicoSTM has a bandwidth of 30 kHz, permitting scanning at up to 100 Hz (line frequency with 265 pixels) for high signal levels on relatively flat surfaces. The RMS current noise is close to the theoretical limit of 7 pA in this bandwidth. Therefore, slower scanning under feedback control permits operation at substantially lower currents. Using the PicoScan program controller and reasonably clean substrates, the PicoSTM has been operated down to sub-pA. This performance can be obtained in electrolytes if the highest grade of STM tip is used.

Low current operation is essential for application such as STM imaging of weakly adsorbed organic molecules[1], imaging via surface conduction in thin water films[2], or measurements of conductance over a wide range of currents[3]. On the other hand, the head amplifier should have an adequate bandwidth to permit scanning at speeds that approach the resonance frequency of the piezoelectric element so that rapid imaging is possible at high currents. Thus, it would seem that different head amplifiers must be used for high and low current applications; however, the performance of the amplifier is usually limited by DC and AC coupling of the high voltages used for scanning. If this problem is solved, low noise levels and reasonable bandwidths are attainable. Furthermore, when used in a feedback system with a controller, the STM will operate at currents substantially below the RMS noise level of the front-end amplifier. This is because of the signal averaging effect of the integrator. Scanning rates have to be reduced substantially as the noise-floor of the complete system is approached, but this is facilitated by the low drift of the PicoSTM. Thus, the one scanning head can be used for imaging at up to 30 kHz bandwidth and for imaging at pA current levels.

For ultra low-current imaging, the following steps should be taken.

PROCEDURE

1. The switch on the scanner needs to be set to 10 nA/V for low current imaging (gain switch at x10 with a 1 nA/V preamp module).
2. Prior to loading the sample stage, adjust the scanner to have the offset nulled. One way to do this is to use an oscilloscope window in the Picoscan software to monitor the tunneling current (in Nanoscope software select Head Offset and Head Leakage).

If the reading on the oscilloscope reads null offset, and does not on the software, then this indicates the controller has on offset at its ADC. Contact the controller manufacturer if this is significant.

3. If a large leakage offset cannot be nulled by adjustment, clean up the tip holder area with methanol and let it dry completely.
4. After loading the sample stage, check the offset again. It should not have increased. In many cases the attachment of the sample stage helps to stabilize the tip current reading and make it easier to null the offset because of the shielding by the stage.
5. If imaging in solution make sure that the leakage is lower than the setpoint current. If using extremely low leakage STM tips, make sure they are relatively fresh, for they can degrade over time.
6. Engage the microscope at a setpoint current of 50 to 100 pA, scan size approximately 100 nm, and a bias voltage of 50 mV.
7. After engaging, gradually lower the current to the desired value, and then lower the gain and the scan rate accordingly to optimize image quality. For imaging at sub-pA, the scan speed used can be around 1 line/s.

INSERTING THE STM TIP

The microscope is designed for operation with a 0.25 mm diameter wire. STM tips are available from Molecular Imaging and are recommended for all Molecular Imaging STM scanners.

PROCEDURE

1. With the tweezers, grasp the STM tip firmly. If using insulated tips be careful not to hold the insulated section of the tip too tightly or you could damage the insulated portion resulting in current leakage.
2. Trim the butt end section of the tip so at least 6.5 mm will protrude out of the scanner when using the ambient cell, and 8 mm will protrude when using the liquid cell.
3. Now place the tip into the holder.
4. Be certain that the tip was held in the tip holder firmly; you can feel it by pushing the tip gently with the tweezers. If not, you may pull the tip out to an extent and bend the wire against the holder wall, and then reinsert the tip into the holder.
5. The tip should stand straight up.

NOTES

- If the tip is bent prior to insertion, it usually causes noticeable drift for some time, and you should allow the drift to settle if it affects your imaging.
- If the tip is not standing straight up, and if it is bent so it crosses the centerline connecting the two adjustment screws, the microscope will never engage.

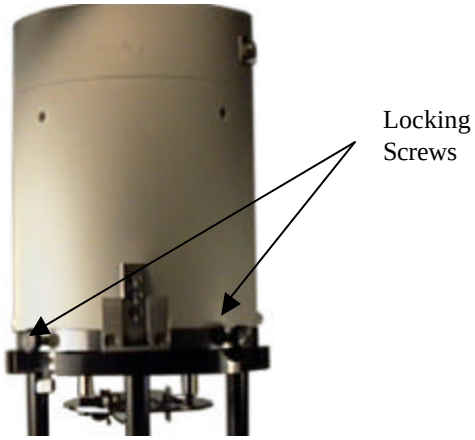
INSERTING THE STM SCANNER

After all adjustments have been made, you will need to insert the STM scanner into the microscope body.

PROCEDURE

1. To open the microscope head, gently press in the head retaining pins and tilt the head backwards.
2. Place the scanner into the center of the microscope head's base plate; it should fit snugly.

3. Orient the scanner so the STM tip holder faces the front of the microscope.
4. Tighten the locking screws located on the back of the microscope. This will hold the scanner in place.



5. Plug the scanner's miniature D connector into the right miniature D connection on the microscope body. The connector is keyed so it will not fit into the left socket (for AFM).

PLACING THE SAMPLE

For STM it is highly recommended that the sample alignment plate be used to pre-align the microscope. This is absolutely essential when using a liquid cell because the tip-sample gap is not visible. If the microscope adjustments are made prior to loading the real sample, waiting time for the microscope drift to settle is minimized. This is particularly important when the lifetime of the sample is limited. The dummy sample can be any material chosen so that it is the same thickness as the real sample (within 0.1 mm). If the real sample can be handled prior to use, there is still some advantage to using the dummy sample plate first (even if the liquid cell is not to be used). This is because a hole in the alignment plate allows for greater freedom in manipulating the STM tip in its holder.

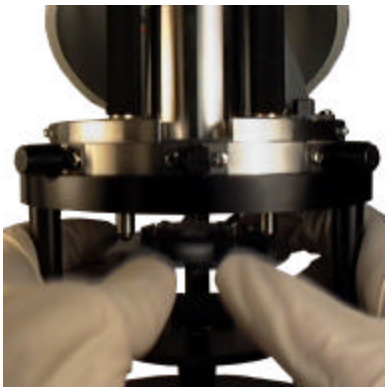
There are three screws that contact the sample plate. The front two are the coarse adjustment screws, and the back screw is the motor screw. All three can be adjusted manually; however, only the back motor screw turns when the controller's approach command is chosen.

LOADING THE DUMMY SAMPLE

It is important, when loading the dummy sample, that the dummy sample thickness is the same thickness as the real sample. Therefore, it is important to use a micrometer to measure the thickness.

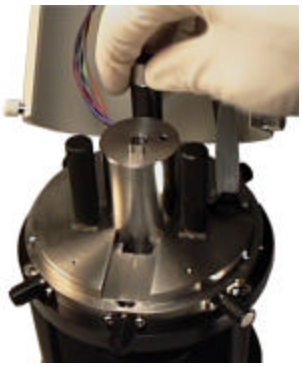
PROCEDURE

1. Measure the thickness of the real sample and use a dummy sample to represent the real sample's thickness.
2. The dummy sample should be positioned so that one edge is just over (but not blocking) the hole in the alignment plate – this ensures you will not crash the tip.
3. Before placing the sample plate into position, make sure the tip will be adjacent the dummy sample, but over the hole in the sample alignment plate.
4. Place the sample plate into position. The plate will "clip" into place as the magnets hold the plate to the adjustment pegs.



5. Adjust the stage so the tip is directly over the dummy sample.
6. Adjust the coarse screws (front screws) first to reach a position close to your STM tip. To bring the sample plate closer to the tip, rotate the coarse adjustment screws clockwise. As the final position is reached, move the screws slightly past the final position and then back the screw off a little. This helps to reduce drift.

7. Now adjust the back motor screw to bring the sample within 0.5 mm of the tip. Turn the motor screw counterclockwise to raise the sample plate towards the tip. Again, follow the same procedure listed in step 6 to reduce drift.



PLACING THE REAL SAMPLE

The sample may be secured to the sample plate by several methods. An ambient plate may be used to secure the sample, a liquid cell may be used for operations in solutions, or the sample may be simply attached to a glass plate. Choose the method

that best fits your scanning application needs. All the sample stages except heating and cooling stages have a glass plate glued on the sample plate to provide insulation from the metal plate that is grounded. Therefore, when using heating and cooling stages with a conductive sample for electrochemical experiments, the sample should not be in electrical contact with the sample plate or it will be grounded out. In this case, put an insulating material between the sample and the sample plate.

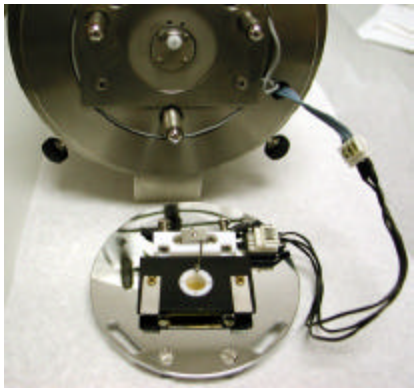
PROCEDURE

1. Place the sample onto the glass plate.

2. Place the ambient or liquid cell onto the sample plate.

3. Push the L-shaped pogo contact into the hole in the wall of the Teflon cell nearest to the electrode clamping assembly on the sample stage. Push up the rectangular nut on the electrode clamping assembly and place the end of the pogo under the nut.

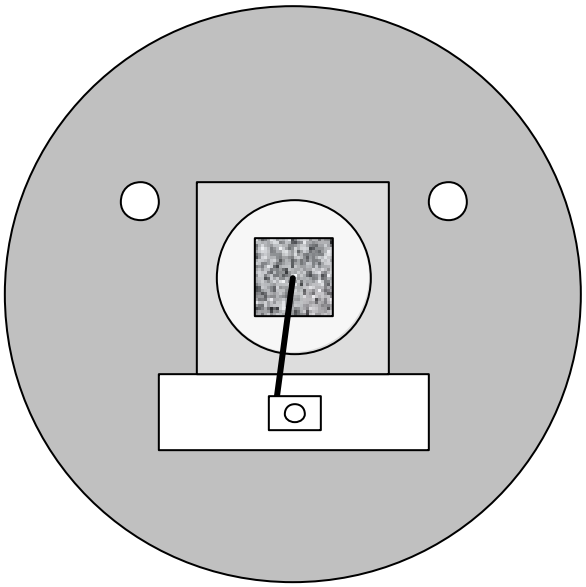
4. Plug the STM sample plate connection cable (from the microscope) firmly into the sample stage connector.



5. Now place the sample plate with the real sample onto the microscope as detailed in the previous section.

ALTERNATIVE SAMPLE PLACEMENT

For very small or odd shaped samples you do not need to use the sample clamping plate. Instead, the sample may be attached to a small metal disk with double-sided tape, glue, or silver paint. The disk is securely held by the magnet on the sample plate. The electrical contact is made from the rectangular nut on the contact mount to the sample by a pogo or spring contact that can be custom-made. The example is shown by the schematic below.



Chapter 9: SETTING UP STM/AFM WITH LIQUID CELL FOR ELECTROCHEMISTRY

Once you have experienced imaging under ambient conditions, it is time to try using the liquid cell. Because of the complexity of imaging with a liquid cell in electrochemistry, we have decided to include a special section on doing electrochemical work. If you are, however, not doing electrochemical work, proceed through this section while ignoring references to the potentiostat and electrodes. When you are ready to try clean electrochemistry, follow the procedures very carefully.

CLEANING AND PREPARING CELLS AND ELECTRODES

Two different liquid cells are supplied by Molecular Imaging. The larger cell is 12.5 mm inside diameter while the smaller cell is 6.5 mm inside diameter. The larger cell may be used for both STM and AFM while the smaller one will only accommodate the STM tip. The sample surface must be very flat and its size larger than the diameter of the cells to avoid liquid leakage.

The Teflon insert should be removed from the liquid cell plate and cleaned. There are many methods for cleaning; however, we have only listed a few.

NON-CRITICAL APPLICATIONS

PROCEDURE

1. Sonicate the liquid cell in laboratory detergent.
2. Rinse in 18 MΩ·cm water – rinse towards the tweezers.
3. Rinse in methanol.
4. Blow dry under Ar or N_2 gas.

CRITICAL APPLICATIONS

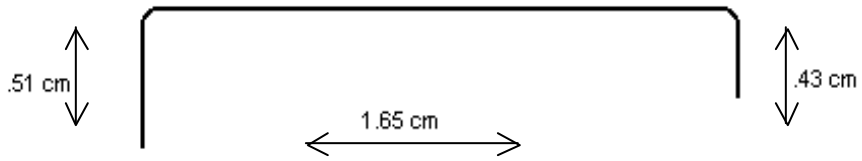
PROCEDURE

1. It is important that all glassware be thoroughly cleaned, or it will be of no value to clean apparatus; therefore, it is important to first follow these procedures for all glassware to be used.
2. Soak overnight in a solution of 70% concentrated sulfuric acid and 30% hydrogen peroxide (of 30% v/v concentration). USE EXTREME CAUTION HANDLING THIS SOLUTION, IT IS EXTREMELY CORROSIVE AND A STRONG OXIDIZING AGENT.
3. Rinse thoroughly at least four times in 18 MΩ·cm water.
4. Boil for one hour in 18 MΩ·cm water, changing the water every 15 minutes.
5. Instead of step four you can rinse overnight in 18 MΩ·cm water.
6. Rinse two more times in 18 MΩ·cm water.
7. Dry under Ar or N_2 gas.

ELECTRODES

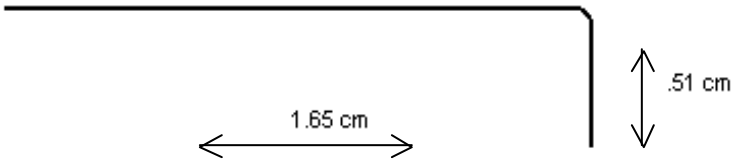
COUNTER AND REFERENCE ELECTRODES

You can purchase prepared electrodes from Molecular Imaging, or you may bend wires using the approximate dimensions:



WORKING ELECTRODE

This is the center electrode used in electrochemistry and has a different shape because of where it's situated on the sample plate. This electrode doesn't require special cleaning because it is not in contact with the sample inside the liquid cell.



SUBSTRATE PREPARATION

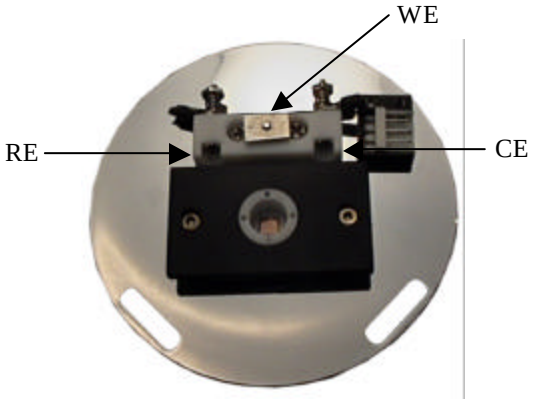
If you use gold substrates, you must hydrogen flame anneal them prior to imaging. For more information on annealing substrates refer to Appendix C. Gold substrates are available from Molecular Imaging, but they still must be annealed prior to use.

ASSEMBLING AND LOADING THE LIQUID CELL

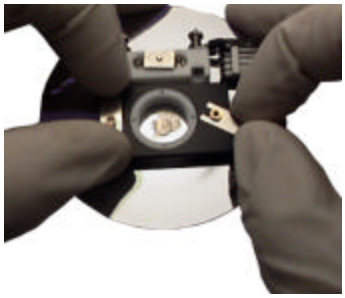
As previously discussed, you my use either the large or small Teflon cells. We do recommend you carry out the assembly procedure in a laminar flow hood or other clean environment.

PROCEDURE

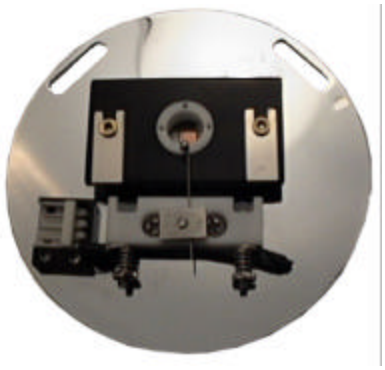
1. Push the top of the liquid cell plate onto the Teflon cell. Orient the cell with at least one of the working electrode contact holes near 'top dead center.'
2. Place a clean substrate onto the sample plate and push the cell onto the spring loaded pins on the sample plate.



3. Pushing down, expose the pin slots (now pushing through the liquid cell plate) and insert the cell clamps.

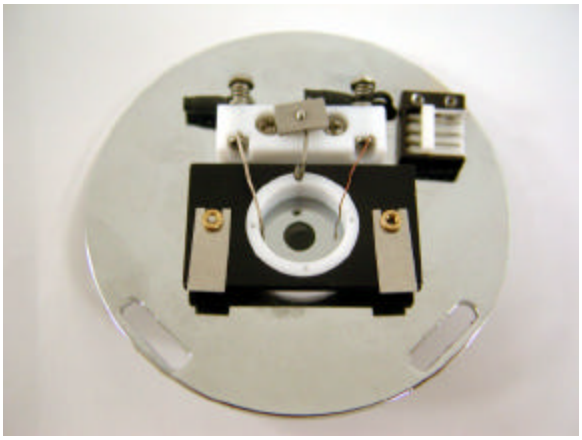


4. Push the L-shaped working electrode contact into the hole in the wall of the Teflon cell nearest to the electrode clamping assembly on the sample plate.



5. Push up the working electrode clamp and place the end of the wire under the nut, letting the clamp spring back to clamp the electrode.

6. Test that contact is established by inserting a wire into one of the three remaining holes in the Teflon cell and checking for continuity with the multimeter.
7. If you do not need to carry out experiments under potential control, you can fill the cell and load it onto the microscope. Otherwise, proceed to load the reference and counter electrodes.
8. Place the reference electrode into the left clamp. Make sure the electrode reaches over the liquid cell (to establish a good contact with the electrolyte solution) but does not touch the substrate.
9. Place the counter electrode in the right clamp. Again, making sure the electrode reaches over the liquid cell (to establish a good contact with the electrolyte solution) but does not touch the substrate.

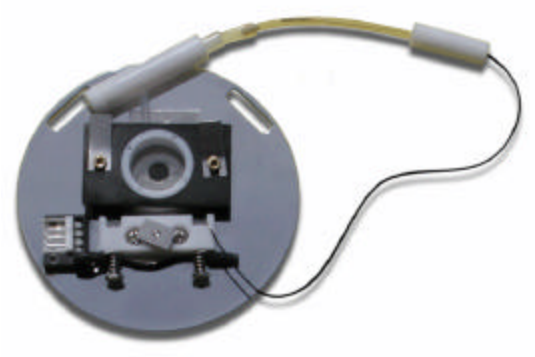


10. Check that both the reference and counter electrodes are making contact with the electrode clamping assembly on the sample plate with a multimeter.
11. Double check that the wires will clear the STM or AFM tip and will not touch any part of the underneath of the microscope.
12. Load the sample plate as previously outlined.

SETTING UP THE MICROREFERENCE ELECTRODE WITH SALT BRIDGE

The T-shape salt bridge should be filled with the same electrolyte you use for EC experiment in the liquid cell. When filling the salt bridge you need to make sure that it is completely bubble free because any bubble inside the bridge will cut off the electrical conductivity to the reference electrode. Then the end with ceramic frit of the salt bridge should be inserted into the side hole of the liquid cell (this is a cell specially designed for the use with the salt bridge). The open end of the salt

bridge is for the insertion of the Ag/AgCl microreference electrode which itself should be filled with the supplied 3M KCl solution (the instruction should come within the box containing the microreference electrode). Finally the wire end of the electrode should connect to the RE contact on the sample stage. See the picture below.



NOTES

- The dimensions of the electrodes may need to be adjusted for different applications.
- Be sure to check for leakage of the insulated STM tip. The current should read very close to zero as it touches the solution.
- Don't forget to plug in the lead from the microscope into the sample plate.
- Before turning on the counter electrode, the resting potential needs to be checked. Verify REF is set to MSR and with the CE off, read the measured open-circuit voltage (resting potential), and then set the REF switch to SET. Adjust the voltage to agree with the resting potential.

See also: **Chapter 7** and **Chapter 4** for additional information on sample plate and liquid cells.

TROUBLESHOOTING

The most frequently encountered problem is leakage from around the bottom of the cell. It is generally more of a problem with solvents that "wet" the substrate well (like methanol). This causes leakage current and very erratic imaging. Carefully inspect for signs of leakage. The bottom of the cell can be cleaned off with a very fine-grained abrasive paper from time to time. Follow the cell-cleaning procedure described. Pressure on the cell can always be increased by tightening the sample plate screws. Also, with certain solvents, or with samples of certain uneven surface, it may be impossible to tighten the spring clamps down hard enough to prevent leakage. In these cases, it is recommended you use an O-ring cell instead. This will improve the seal. Contact Molecular Imaging for O-ring cell options.

Chapter 10: USING THE PICOSTAT

The Molecular Imaging PicoStat is a potentiostat/galvanostat used for SPM imaging while the sample is held under electrochemical control. The automatic mode of the PicoStat provides full compatibility for the use of the PicoSPM microscope with controllers such as Molecular Imaging PicoScan and the Digital Instruments NanoScope III or NanoScope E. The manual mode allows electrochemical investigations to be performed without special hardware or software by using the built-in set voltage and output display. The PicoStat receives the power necessary to operate from the microscope controller.

The PicoStat has three working modes controlled by switches on the back of the PicoStat. One mode is fully under the control of the controller's software. In the second mode, the cell potential may be set directly from the front panel. In the third mode, the unit may be controlled by a signal applied to the back of the panel via an external source. Furthermore, external inputs and outputs are also available for auxiliary functions such as capacitance measurements and spectroscopy.

FRONT PANEL CONTROLS

DIGITAL PANEL METERS

The **POTENTIAL** digital panel meter displays either the set or the measured potential of the working electrode as sensed by the reference electrode in Volts.

The **CURRENT** digital panel meter displays the value of the current flowing through the working electrode in volts. The rotary switch directly below this display determines the scale factor of the displayed value. The equation is:

Display (V) = Current (μA) · Scale Factor (μA/V)

1 = 1 μA/V
10 = 10 μA/V
100 = 100 μA/V
1K = 1,000 μA/V or 1mA of the working electrode current
10K = 10,000 μA/V or 10mA of the working electrode current

FRONT PANEL CONTROLS

DISPLAY – The DISPLAY switch determines whether the set (**SET**) or the measured (**MSR**) potential of the working electrode (WE) is displayed on the potential DPM. The **SET** gives you the voltage that is being supplied. The **MSR** is the actual measured voltage difference between the working and reference electrodes.

POT. ADJUST – The potential adjust multi-turn knob allows the user to adjust the set potential of WE in manual mode. This control has no effect in automatic mode.

PROBE – The probe switch determines whether the PicoStat is configured for **STM** or **AFM**.

CE – The switch determines whether the counter electrode (CE) is **ON** or **OFF**.

REAR PANEL CONTROLS

DB25 CONNECTIONS

CONTROLLER – The controller DB25 connector provides the interface between the PicoStat and microscope controller.

SPM – The SPM DB25 connector provides the interface between the PicoStat and the PicoSPM microscope.

WARNING! Drive voltages for the scanner as high as ± 220 Volts are present in these connectors. Although the voltage is essentially DC and current-limited, it can cause serious injury. For safe operation, do not apply power to the controller unless all cable connections between the controller, the PicoStat, and the microscope are secure.

SWITHCES

MODE – The switch determines whether the PicoStat operates as a potentiostat (**POT**) or a galvanostat (**GAL**) in the manual mode. This switch has no effect in the automatic mode.

CANTILEVER – If this switch is set to **WE** (or **ON** on older versions), the cantilever is biased. If the switch is set to **EXT** (or **OFF** on older versions) the cantilever is electrically isolated. If electrochemistry is not being used, setting to **WE** will ground the cantilever. This is very useful since charge can build up on the cantilever when working in ambient/gaseous environments.

CONTROL – The switch determines whether the PicoStat operates in an automatic mode (**AUTO**) under direct control of the controller hardware and software, or in a manual mode (**MAN**) where the user is responsible for setting values and recording results from the front panel displays.

REF SRC – The reference source switch determines whether the Reference Electrode (RE) – the reference source – is internal (**INT**, set by the front panel **POT. ADJUST**) or external (**EXT**, set by the signal connected to the **REF SET** BNC connector) in the manual mode. This switch has no effect in automatic mode.

BNC JACKS

VEC – The electrochemical voltage output BNC jack provides a low impedance voltage output that is identical to the voltage displayed in the front panel **PONTENTIAL** display.

CANTILEVER – This BNC connector is connected directly to the cantilever without any circuitry or buffer. When the **CANTILEVER** switch is set to **EXT**, this BNC can be used to apply a voltage externally between the cantilever and the sample (if the cantilever is conductive).

BIAS MOD (older versions only – replaced by **CANTILEVER** in the current version) – The bias modulation signal BNC connector is a high impedance (1MΩ) voltage input for applying an AC modulation signal to the electrochemical cell bias in **STM** mode. This input has no effect in **AFM** mode.

REF SET – The reference set BNC jack is a high impedance (1MΩ) voltage input for setting the RE potential with an external voltage source in the manual (**MAN**) mode. This switch has no effect in the automatic (**AUTO**) mode.

IEC – The electrochemical current output BNC jack provides a low impedance voltage output that is identical to the voltage displayed in the front panel **CURRENT** display.

REF MOD – The reference modulation BNC jack is a high impedance (1MΩ) voltage input for applying an AC modulation signal to the RE in the manual mode. This input has no effect in the automatic mode.

SETTING UP THE PICOSTAT

The PicoStat should be connected with DB25 cables in between the controller and the microscope and is powered by the controller. For Digital Instruments controllers you will need to use a DB25 to DB37 conversion cable. The connector pinouts of the DB25 cable are listed below. When the PicoStat is not being used, the microscope is connected directly to the controller with a Patch cable and DB25 cable, and the electrochemistry pins are disabled.

	CONTROLLER CONNECTION	**SPM CONNECTION**
1	Phase A	Phase A
2	Phase C	Phase C
3	Stepper Motor + 5 V	Stepper Motor + 5 V
4	VEC (aux D)	RE
5	- Y	- Y
6	CE on/off	NC
7	- X	- X
8	NC	Cantilever
9	- 15 V	- 15 V
10	IEC	CE
11	Signal	Signal
12	GND (signal)	GND (signal)
13	Ref Set (S/A)	NC
14	+ 5 V	+ 5 V
15	Phase B	Phase B
16	Phase D	Phase D
17	NC	WE
18	+ Y	+ y
19	GND (chassis)	GND (chassis)
20	+ X	+ x
21	LFM	LFM
22	Z	Z
23	Pot/Gal TTL	NC
24	+ 15 V	+ 15 V
25	Bias / Force Setpoint	Bias / Force Setpoint

Plug the controller to the **CONTROLLER** port and the microscope to the **SPM** port on the back of the PicoStat using the DB25 cables. Make sure that the **CE** (counter electrode) switch is turned off, and the **PROBE** switch is set to the mode (AFM or STM) that you are using.

If you are using AFM, you have the option of biasing the cantilever (if it is conductive). If you image under electrochemical control, you should leave the switch to **EXT**. When electrochemistry is not being used (the **CE** is off), putting the switch is to **WE** can help to make tip engaging easier if charge has built up on the cantilever in ambient/gaseous environment.

USING THE PICOSTAT FOR ELECTROCHEMISTRY

AUTOMATIC MODE

Before setting up the sample and the liquid cell for electrochemical (EC) SPM, make sure that all the parameters in EC software and the PicoStat switches are set correctly. Using time to do this after the sample has been prepared can lead to poor results due to degradation and contamination of the sample.

First, verify that the PicoStat is properly and securely plugged into the controller. On the front panel, set the **CE** switch to off, set the **REF** (reference electrode) switch to **MSR** (measure) and set the **PROBE** switch to **AFM** or **STM**. On the rear panel, make sure that the **CONTROL** switch is set to **AUTO** and **REF SRC** to **INT**. This allows automatic control from the controller, which is set by the software. In the software select Potentiostat or Galvanostat for the right mode you are going to use.

Set up and align the microscope, and set up the sample and EC cell as described previously in this manual. Make sure that all the electrodes are submerged in the fluid. You now need to have the software and PicoStat agree on units.

For NanoScope software, after you have selected the microscope environment you are using (ECSTM for example), go to the **Microscope** menu item and select **Calibrate** and then **Detector**. The **Aux. A sens.** (μA/V) should agree with the setting at which the current meter is set on the front of PicoStat. The meter should be set at a value so as not to max it out (around 8) but high enough that you get 2 or 3 significant digits in the reading, that is somewhere between 0.80 and 8.00. The **Aux A polarity** should be "reversed" and the units should be μA. If the unit is not μA, go to **Aux. A Units** and type in μA. The "μ" key is the tilde (~), or you can simply type "u."

For PicoScan software, open the AFM Potentiostat/Galvanostat window. Click "MORE" and select one of the current scale values under Gain that corresponds to the same scale value under **CURRENT** on the PicoStat.

To enable the electrode potential control, switch the **CE** to ON. In the NanoScope EC software, select **Cell** On. In PicoScan software, click **EC** ON. The electrochemical control is enabled only when both the PicoStat switch (**CE**) and the software 'switch' (**Cell** or **EC**) are set to ON. For detailed instruction on how to carry out various functions of ECSPM please refer to the controller software manuals.

MANUAL MODE

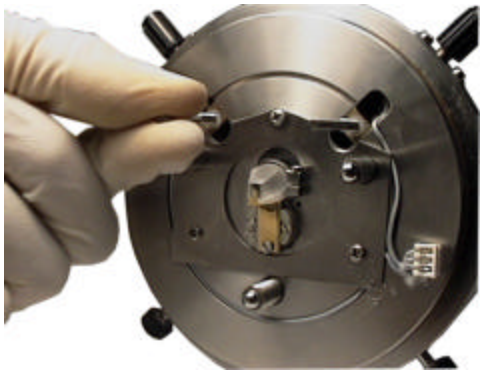
If you choose not to use software-controlled EC, or if we do not support EC on your controller (as for NanoScope II), you can use the front panel manual control. Switch the **CONTROL** switch on the rear panel to **MAN** (manual) mode. Doing this enables the **REF SRC** switch on the rear panel, which should be set to **INT** (internal). The potential adjust knob allows you to set the potential across the RE and WE. The **DISPLAY** switch allows you to set the voltage without measuring it, e.g. before the **CE** is turned on. Once the **CE** is turned on, the cell will immediately react to the set voltage. Switching over to **MSR** allows you to monitor the measure voltage.

Alternatively an external device can be used to control the signal supplied to the electrochemistry cell. To enable this feature, the **CONTROL** switch on the rear must be set to **MAN** (manual) and the **REF SRC** switch on the back must be set to **EXT** (external).

There are five BNC connectors on the rear of the PicoStat for external and manual control. The **MAN** on the back panel may be a bit misleading when using external control, for you could conceivably automate the external device.

Chapter 11: FREESTANDING OPERATION

Freestanding operation mode (FSOM) can be used to image larger samples, or samples that require special setup which do not fit into the standard sample stage. In this mode, the microscope is placed onto a larger, flat, hard surface, which can be a sample surface itself, or a platform with the sample placed on it.



AFM

PROCEDURE

1. You must remove the two front translation pegs. To do this, turn them counter-clockwise. It is now possible for the microscope to rest on the three sample plate supports, the two coarse adjustment screws and the motor screw.
2. Using the dummy sample plate, adjust the height of the microscope with respect to the sample, by using a dummy sample of similar height. This is to ensure the real sample is not higher than the surface on which the microscope will be resting.
3. Follow all procedures, as previously discussed, to mount the cantilevers and operate the AFM scanner and microscope.

STM

PROCEDURE

1. Make sure the sample is electrically isolated from the microscope body and connect the sample to the working electrode. If a large sample is being used and the microscope is sitting on the sample, use the special “microscope pad” to isolate the microscope from the sample.
2. Remove the two front translation pegs. To do this, turn them counter-clockwise. It is now possible for the microscope to rest on the three sample plate supports, the two coarse adjustment screws and the motor screw.
3. Using the dummy sample plate, adjust the height of the microscope with respect to the sample, by using a dummy sample of similar height. This is to ensure the real sample is not higher than the surface on which the microscope will be resting.
4. Position the STM tip so the gap is less than 0.5 mm, remove the plate, and set the microscope onto the surface.
5. Follow all procedures, as previously discussed, to mount the STM tip and operate the microscope.

Chapter 12: ENVIRONMENTAL CONTROL

STANDARD GLASS CHAMBER

The standard glass environmental chamber is provided with two nipples for 1/8th inch id tubing. One may be used for filling the chamber with gas and the other for venting the chamber. The venting line is provided in the event the chamber is filled with a vapor that cannot be safely vented into the lab. In this situation, the venting line should be placed into a fume hood. An example would be saturation of an inert gas with an organic solvent when non-aqueous electrochemistry is carried out.

One important use of the chamber is for displacing oxygen from solutions used for electrochemistry. We have obtained good results by first bubbling an inert gas (nitrogen or argon) through the solution to be placed into the liquid cell, and then setting the environmental chamber up with a steady flow-through rate of 1 to 2 SCFH. When the microscope and environmental chamber are placed in the PicoIC™ isolation chamber both nipples can be connected by tubes to inlet and outlet ports on the PicoIC™ feed-thru panel. The PicoIC™ is equipped with a needle valve and gas flow gauge suitable for this type of operation.

Even if control of the atmosphere is not important, the environmental chamber offers excellent acoustic isolation and protects the microscope from drafts.

PICOAPEX™

The PicoAPEX™ replaces the standard glass environmental chamber when electronic or liquid feed-through to the sample plate is required or when it is used in conjunction with the CleanLoad™ glove box for setting up samples under controlled gas environment. The necessary connections for the MAC and temperature stages are provided with the devices and can easily be screwed into 8 ports of the PicoAPEX™. There are three parts to the PicoAPEX™: Glass chamber, stand, and flange.

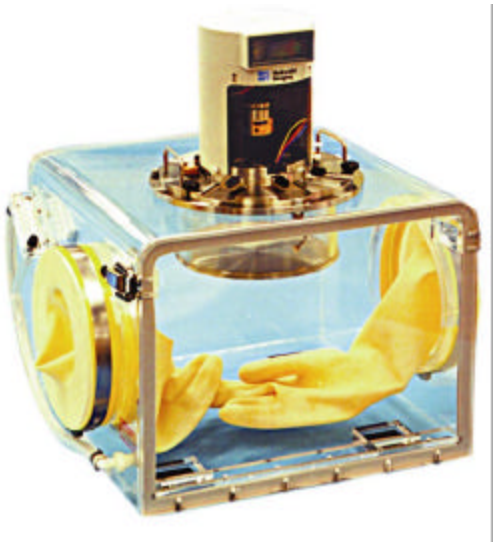
PROCEDURE

1. Place the flange of the PicoAPEX™ into the black stand. The legs of the black stand are not equally spaced and the largest gap indicates the front of the black stand. The glass chamber will only fit through the front of the stand. The large groove indicates the front of the flange.
2. Make the connections to the feed through on both sides of the flange.
3. Connect the cooling tubes and electronics to the sample plate.
4. Place the microscope onto the flange.
5. Proceed with the usual sample mounting routine.
6. Bring the glass chamber through the front two legs of the black stand and mount it up on the flange. It is held up magnetically, but must be pressed on since there is an O-ring seal.

NOTES

- Make certain there are no wires or tubes pinched between the flange and the glass chamber before pressing together.
- Use PicoAPEX™ to avoid water condensation while cooling and evaporation while heating by flowing through dry inert gas.

CLEANLOAD™ GLOVE BOX



The CleanLoad™ glove box is designed for work with sensitive samples the environment of which needs to be controlled at all times from the sample mount to imaging stages. The flange of the PicoAPEX™ has to be used with the glove box. The unique design of the CleanLoad™ allows full access to the microscope while the

sample can be loaded and imaged under a controlled atmosphere. It offers "gas-in" and "gas-out" ports and flow meter for the control of gasses. Used with the entire PicoAPEX™ chamber the CleanLoad™ permits the loaded microscope to be uncoupled from the glove box with the sample under the control of a separate gas purging system. The microscope may then be transferred to a low-noise environment (such as the PicoIC™) for higher resolution imaging under continuously controlled gas atmosphere.

PROCEDURE

1. Set up the microscope as usual (such as the scanner, tip, the laser alignment, etc.).
2. Find a piece of dummy sample that has the same thickness as the real sample and carry out the sample-tip distance adjustment so that when the real sample is mounted onto the microscope in the glove box later on it won't crash the tip (for it is difficult to do this adjustment inside the glove box due to the viewing difficulty).
3. Place the flange of the PicoAPEX ™ on the glove box and fix it with the three screws. Place the microscope (without the sample stage attached) and press it downward to ensure the tight O-ring seal. Feed through all the cables for temperature and/or MAC stages if applicable. Connect the gas tubes to the "gas-in" (from the gas cylinder) and "gas-out" ports.
4. Open the front hinged door of the glove box. Put the real sample (must be inside a sealed container), sample stage, liquid cell / solution or electrodes (if applicable), all the necessary tools for later use, and the glass chamber of the PicoAPEX™ in the glove box.
5. Close the hinged front door and begin to purge the gas. Allow enough time for purging.
6. Mount the sample, liquid cell and electrodes (if needed), plug the connector to the sample stage (for MAC or temperature operation), all just as you would do for conventional procedures but now operate with the gloves.
7. You may need to slightly readjust the laser spot and detector position from the outside. Then engage the tip and start scan.
8. Low resolution imaging can simply be carried out with the microscope sitting on the top of the glove box. The resolution can be improved if the glove box is placed on an anti-vibration table. For higher resolution low noise imaging, connect a separate gas purging line via the extra ports on the PicoAPEX™ flange, attach and seal the glass chamber to the flange, remove the three fixing screws and lift the whole PicoAPEX™ chamber out of the glove box and transfer it into the PicoIC™ chamber.

LIQUID FLOW LINES

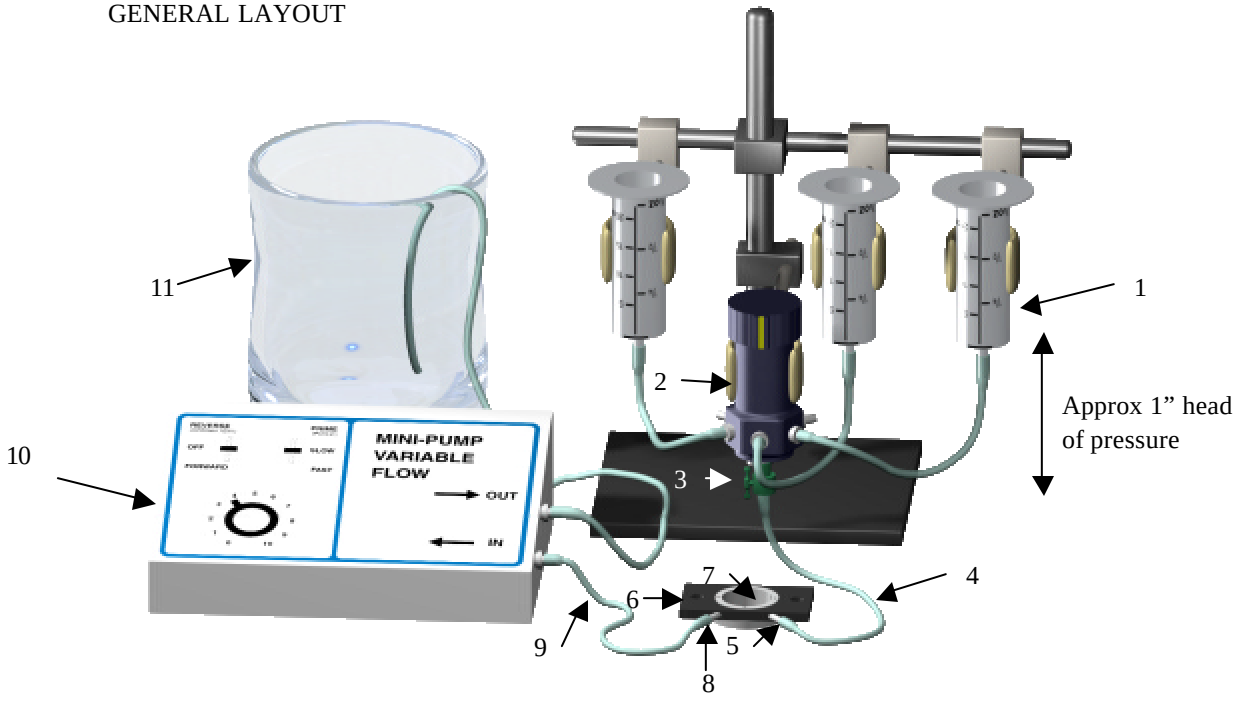
Two hypodermic tubes pass through the scanning heads down to the region of the STM tip or AFM cantilevers. These are used to guide Teflon liquid lines down into the liquid cell. When not in use, they may be plugged with Delrin stoppers to minimize leaks from the environmental chamber. To use a simple flow-through arrangement, cut two lengths of 0.045" od (or less) Teflon tube long enough to pass from the liquid cell up through the scanner to the point where the fluid input and output will be controlled (by a syringe). If cleanliness is important, the tubes should be cleaned with acid and rinsed. The tubes are pushed into the hypodermic tubes on the scanner head so that they will sit in the liquid cell 1mm or so from the sample surface. They should not contact the sample cell because this is a possible source of drift.

The simplest arrangement is to remove a small amount of liquid from one line using a hypodermic syringe, and replenish the fluid with the second line. For continuous flow, two syringes may be coupled back-to-back. For very gentle addition of fluid, we have left a drop of fluid in one line at the end near the cell, sealed the line, and then heated it so the air in the line expands the tubing and expels the liquid drop into the fluid cell. In this case, we have found it possible to add liquid while scanning at atomic resolution. In other cases where the solvent evaporates fast, continuous addition of the solvent to the liquid cell can be performed with one line using a syringe by pushing the liquid slowly and gently.

FLOW-THROUGH CELL SYSTEM

The flow-through cell system consists of a gravity-feed system that operates at about 1mL/minute. The fluid is removed by a peristaltic pump, operated somewhat faster than the inflow, so that the level is always maintained at the height of the output tube.

GENERAL LAYOUT



Syringe body (1) is the fluid reservoir. Up to 6 syringes can be connected to the 6 port **stream selection valve (2)**. There is an **on/off tap (3)**. The system uses 0.8mm id **silicone tube (4)**. Two **Teflon tubes** of 1mm od **(5 and 8)** are fed through **cell clamping plate (6)** and into the **sample cell (7)**. The 0.8mm id **silicone tube (9)** couples up to 1/16" id silicone tube for connection to the **peristaltic pump (10)**. Output is collected in a **beaker (11)**. The bottom of the syringe should be about 1 inch above the level of the sample plate to achieve about 0.5mL/minute with the syringe nearly empty and the tubing system described here. Flow is faster with a full syringe.

The reservoirs are the bodies of large syringes with Luer connectors (e.g., Beckton Dickenson Luer Lok, 60cc). They are arranged in a 'menorah' on a lab stand and

should all be filled to the same level so the flow rates don't change on switching. They are connected via female Luer to barb adapters and a length of 0.8mm id silicone tubing to a screw-in male Luer adapter, threaded for use with stream selection valve. This adapter is screwed into the stream selection valve. A male Luer in the bottom of the stream selection valve is connected to a tap with a Luer fitting. A Luer to barb fitting is used to connect 40 inches of the 0.8mm id silicone tubing to feed the fluid to the cell. One end of a 2" long Teflon tubing section (1mm od) is pushed into the 0.8 mm id silicone tubing. The other end of the 1mm od Teflon is inserted into the cell clamping plate. The end pokes through into the cell. The tubing should be cut at a very sharp angle for easier insertion into the clamping plate.

If the PicoAPEX ™ is used, the tube should be fed through a brass fitting with a tight fit hole for the od of the silicone tubing (2.4mm).

A similar arrangement of 1mm Teflon tubing and 0.8mm id silicone tubing is used to stick into the cell for drainage: note that the level of the drainage tube end determines the fluid level in the cell.

The tube is fed out (via an adapter for a PicoAPEX™ if needed) and coupled to a larger bore silicone tube with a double barb connector. This is connected to the barb on the 3/32" pump tube used in the peristaltic pump. This gives about the right pumping rate for drainage at a slow speed setting (switch on slow, pump speed about 1/3 way up). The output is connected to a drainage collector (e.g., a beaker).

PARTS LIST

FLT-CP1	Modified cell clamping plate.
FLT-FT1	Modified PicoAPEX™ plugs: 2 per unit
FLT-TB1	Silicone tubing, 0.8mm id, 0.8mm wall. Need 2.5m per unit.
FLT-FL1	Female Luer to barb connector. Need 13 per unit.
FLT-V1	Luer-fitted tap. Need 1 per unit.
FLT-V2	6 Port Stream Selection Valve. Need 1 per unit.
FLT-ML1	Tefzel threaded male Luer adapters for valve. Need 7 per unit.
FLT-TB2	Silicone tubing, 1/16" id, 3/16" od. Need 10 ft per unit.
FLT-CP1	Barbed 3/32" coupler. Need 1 per unit.
FLT-TB3	1mm od Teflon tubing, 22 gauge Teflon tube. Need 4" per unit.
FLT-PP1	Variable flow peristaltic pump. Need 1 per unit.
FLT-SY1	Sterile syringes with Luer-Lok tip, 60cc. Need 6 per unit.

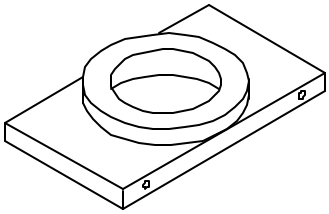
ASSEMBLY INSTRUCTION

(a) Assemble reservoirs: Cut 6 six inch lengths of 0.8mm id silicone tubing (FLT-TB1). Insert a barb to Luer adapter at each end (FLT-FL1). Take the bodies of 6 syringes (FLT-SY1) and attach the 6 inch tubes.

(b) Assemble flow valve: Screw threaded male Luer adapters (FLT-ML1) into each of the six input ports and the output port (on bottom) of stream valve (FLT-V2). Insert tap (FLT-V1) onto bottom Luer. Cut a 40" length of 0.8mm id silicone tubing (FLT-TB1) and fit a Luer to barb connector (FLT-FL1) at one end. Connect this to the bottom of the tap.

(c) Assemble the pump: Select the 3/32" tubing (third from smallest) and assemble the pump (FLT-PP1) according to the instructions. Push an 8 ft length of the 1/16" id silicone tubing (FLT-TB2) onto the INPUT side (marked on pump). Push a 2ft length onto the output side. Use a coupler (FLT-CP1) to join the input tube to a 15" length of the 0.8mm id silicone tube (FLT-TB1).

(d) Assemble the fluid cell: Cut two 2inch lengths of the Teflon tubing (FLT-TB3). Push them into the cell clamping plate (FLT-CP1) so they just protrude into the liquid cell:

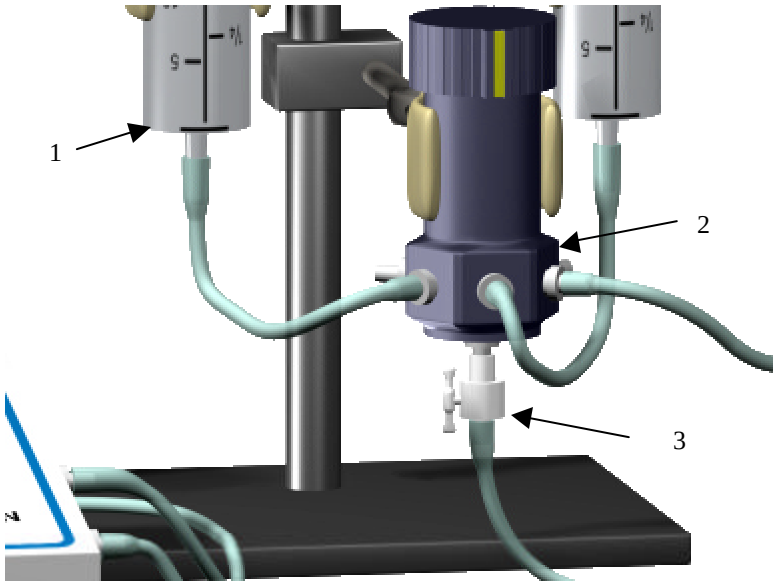


1 mm OD TEFLON TUBING CUT AT SHARP ANGLE FOR INSERTION IN CELL.

The above schematic shows the 1mm tubing pushed through cell clamping plate. The level of the drain tube will be the level of the top of the liquid

FINAL ASSEMBLY AND OPERATION

Set up the reservoirs and stream selection valve next to the PicoIC™. Use a lab stand to hold the reservoirs above the selection valve:



The 'menorah': up to six reservoirs (1) are connected to the input side of the stream selection valve (2). Output is controlled by the tap (3). As long as the tap is below the selection valve, unneeded inputs can be left blank. Selecting them will merely run air into the system.

Couple the 0.8mm id tube to the 1mm od Teflon tube by pushing 0.5" of the Teflon tube into the silicone tube. The tight fit will hold.

Fill the reservoirs with clean water, pull out the Teflon tubes and flush the whole system.

Assemble the sample stage for an experiment. Push the Teflon tubes in to the desired depth (use clean tubes for each experiment). Reconnect the Teflon and silicone tubes.

Note: pass the inlet and outlet tubes through openings in the PicoIC™ and PicoAPEX™ prior to connecting the Teflon and silicone tubes. Use the modified PicoAPEX™ Plugs (FLT-FT1) if a sealed chamber is desired.

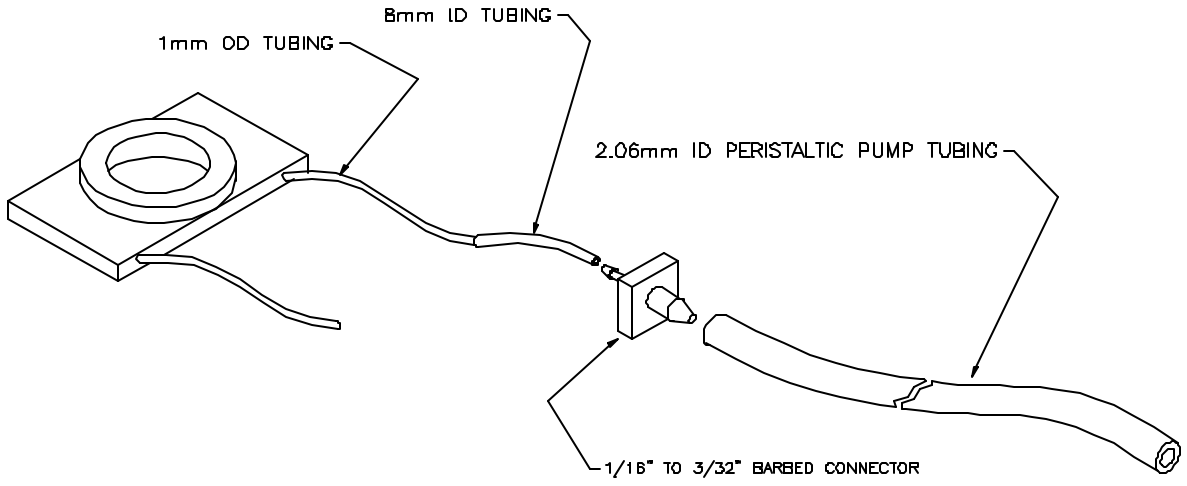
Fill each reservoir to the same level with the desired reagent. Start the drainage pump at the slow setting on the switch, but turned up all the way. Run a dummy or test reagent into the cell and back off the pump speed until the cell is just kept level. Practice imaging like this.

For an experiment, top off all reservoirs to the same level before switching. Note that it is important to have the microscope and reagents *at the same temperature to avoid drift!*
Resolution does not appear to be compromised at flow rates of a few mL/minute.

TWO-CHANNEL "MASTERFLEX" PUMP CONNECTION

The option of the two-channel MASTERFLEX pump allows greater flexibility. One channel can be used for pumping liquid into the cell, while the second channel is used to evacuate the cell. Since both channels operate at the same flow rate, no adjustment in syringe height is needed, as it is with the gravity feed setup.

Various diameter tubing can be used with the pump, allowing flow rate from 0.002 - 1.30 mL/min. The 2.06mm id tubing has been shipped with your pump, which will allow flow rates from 0.15 - .88 mL/min. The diagram below shows one possible connection scheme. This can be duplicated for both channels if needed. The .8mm id tube is used to adapt the tiny 1mm od tubing up to a size that can be mated to the barbed connector. Any convenient length can be used.



NOTE

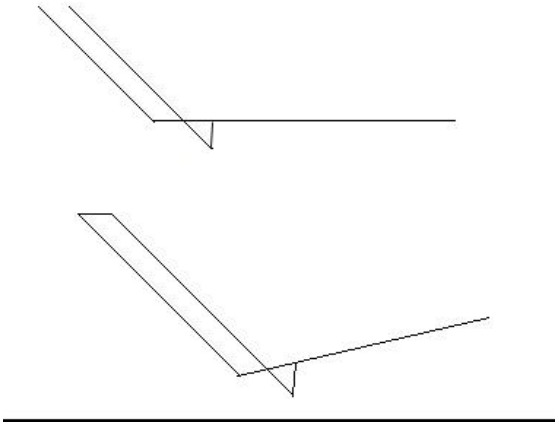
- It is critical to maintain the whole system (from the reservoirs, the tubing to the liquid cell) at a constant temperature in order to avoid any imaging drift due to the liquid temperature change.
- Always keep an eye on the liquid level in the cell to make sure that the rate of in-flow and out-flow are kept equal and constant.

Chapter 13: TEMPERATURE CONTROL

The Molecular Imaging temperature controlled sample stage adds another aspect to SPM studies. They can now be done while now maintaining physiological temperatures, melting two-dimensional crystals, or while imaging any temperature-sensitive surface. The instruction assumes some experience with the standard sample plate and electrochemistry cell. If you have no prior experience simply refer back to the appropriate sections of this manual.

CHOOSING CANTILEVERS

Temperature changes during AFM experiments present a special problem when using gold-coated cantilevers. Cantilevers coated on one side will bend due to differential thermal expansion. These cantilevers are still useful, but the stepper motor and set point (or detector position for extreme values) may have to be adjusted each time there are temperature changes. Because the temperature influences the cantilever, even when it is far from the surface, the force is not easily calculated without observing a force-distance curve. Silicon cantilevers are not coated and therefore will not bend. This makes silicon cantilevers essential when imaging while the temperature is being changed (as discussed in the following sections).



Force-distance curves at and above room temperature. Bending due to thermal expansion causes deflection before the cantilever contacts the surface. The direction and amount of slope depends upon the temperature and cantilever.

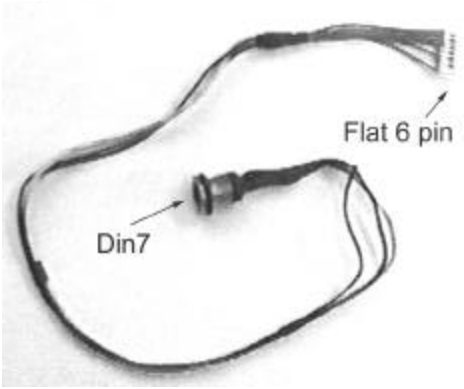
HIGH TEMPERATURE STAGE

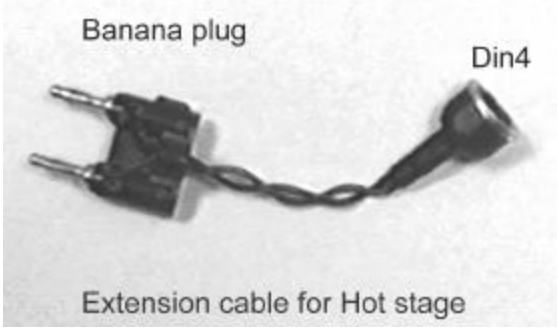
The high temperature stage has a temperature range from ambient 200 °C. Specifications for the temperature controller are given in the Lakeshore Temperature Controller User's Manual. Please limit the ramping rate of the temperature to less than 10 degrees per minute to avoid permanent damage of the stage.

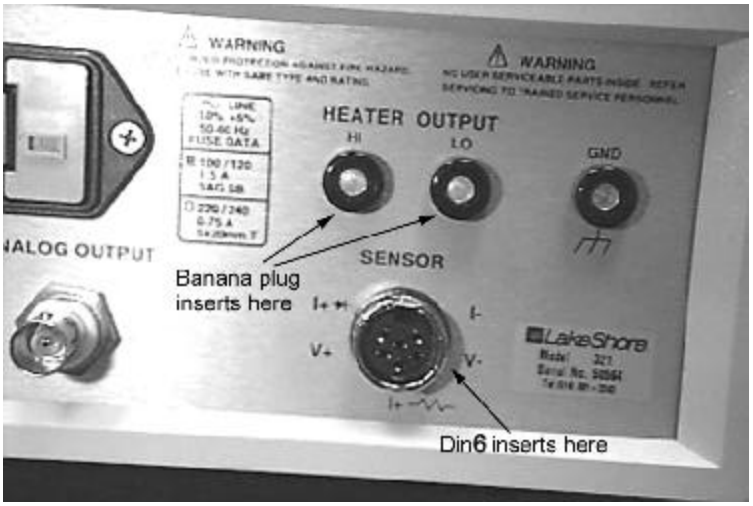
CONNECTION

The following three cables are supplied with the heating stage package as shown

- One long cable (round Din 7 plug connector to Din 6 and Din4 round connectors)
- One short flexible cable (round Din 7 socket to flat 6-pin female connector)
- One extension cable (round Din4 to Banana plug)







PROCEDURE

1. Connect the extension cable with Din4 (female) to the Din4 (male) on the long cable.
2. Plug the round Din 6 connector from the long cable into the sensor input on the controller back panel as shown below.
3. Insert the red and black banana jacks into the Hi and Lo Heater outputs respectively on the controller back panel.
4. Connect the round Din 7 plug from the opposite end of the long cable into the Din 7 socket of the shorter cable.
5. Plug the flat 6-pin female connector from the short cable into the 6-pin male connector on the bottom of the hot stage.
6. Connect the other cables the same as operating the microscope without the temperature control stages.

NOTES

- Set up the microscope and align the tip-sample distance as previously described. Mount the sample using the air cell or liquid cell. Try not to use double-sided tape to fix the sample on the heating stage if possible because during the heating the glue may soften or melt, causing large sample drift.
- Every sample is different. In general keep the sample thin and avoid solvents that evaporate quickly when the heating stage is used.
- Use uncoated cantilevers when it is undesirable to have the cantilevers bend during heating experiments. Silicon cantilevers, which do not have a metallic coating, are available through Molecular Imaging.

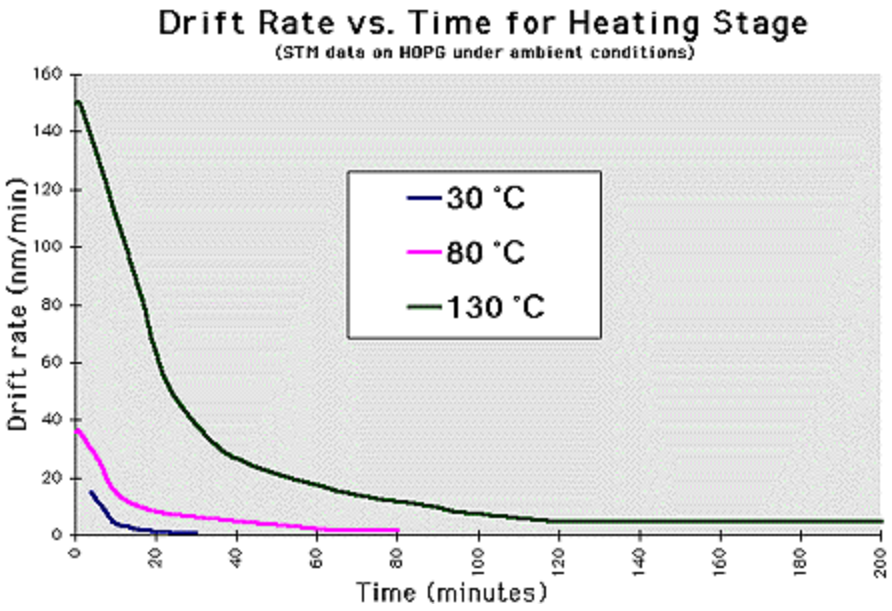
USING THE LAKESHORE CONTROLLER WITH THE HIGH TEMPERATURE STAGE

PROCEDURE

1. Turn the Lakeshore controller on, and the Heater should be initially Off.
2. Press Auto-Tune and then the up or down arrow keys until the display reads Tune: Manual.
3. Enter the proportional, integral, and differential gains. For example to set the proportional gain press P then entering a number followed by Enter. The numbers 20, 20 and 100 for P, I and D respectively work well in factory tests.
4. Press Set Point, and enter a temperature slightly lower than room temperature.
5. Set the ramp rate to no more than 10 degrees per minute (typically 5).
6. Press Heater Low or Heater High (Lakeshore controller model 330 also has Heater Medium setting). Use Low for desired temperatures lower than 20 °C above room temperature, and Medium or High for other desired temperatures; however, these settings are sample-dependent.
7. Enter the desired final temperature and press Enter.
8. Once the temperature has stabilized, engage the microscope as previously described and start scanning. If the temperature has not stabilized, the signal will continue to drift due to thermal expansion. The following diagram shows a set of data for several temperature jumps from room temperature 23 °C, zero on the time axis corresponds to the time the sample reached the setpoint temperature.

WARNING

- Setting the rate too high will damage the heater permanently.



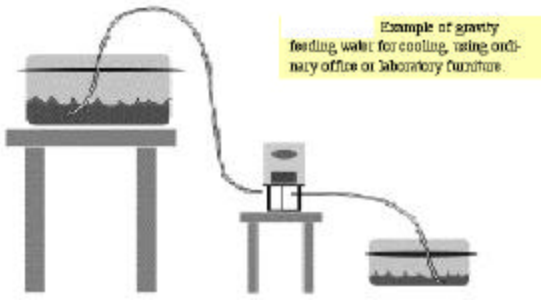
PELTIER STAGE

The 1x Peltier Stage has a temperature range of –5 °C to 40 °C (assuming room temperature is 23 °C), and 3x Peltier Stage has a temperature range of –30 °C to room temperature. The temperature range for a specific experiment will depend upon the thermal load of the sample. The current booster should be used with the Peltier stage and the water cooling must be used when lowering the temperature. The current booster includes a safety device that shuts off the power to the Peltier cooler if the reverse side of the Peltier becomes excessively hot, for example, if the water should stop flowing. The maximum power rating of the Peltier stage cannot be exceeded by the supplied current booster.

WATER COOLING FOR THE PELTIER STAGE

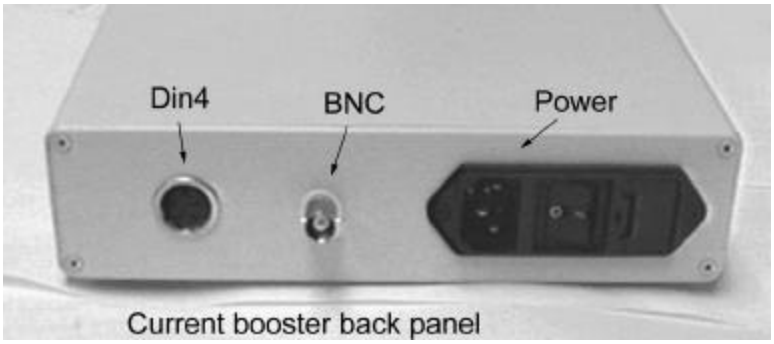
When a sample is cooled using the Peltier stage the opposite side of the device becomes hot. The hot side of the device is water cooled to decrease the minimum sample temperature, reduce power requirements to prevent overheating of the device. Vibrations from the water cooling are minimized by using gravity feeding rather than mechanical pumping. Two reservoirs are provided with the Peltier stage. One is used as a source and the other is a receptacle to store the water for recycling. A three foot height difference gives a minimum temperature of –25 °C or approximately 50 °C below room temperature. Some ice cubes can be added into the reservoir to increase the water cooling efficiency and give a minimum temperature of –30 °C. A diagram showing how to connect the tubing to the reservoirs is given.

Warning: The water cooling connections should be made and tested for leaks before the electronics are connected.



THE CURRENT BOOSTER

The front and back panels of the current booster are shown below respectively. The LED indicator will light up after the power is on. The power range controls the amount of power output into the Peltier stage. The more power, the colder (or warmer in the case of 1X Peltier for heating) the stage will get. The overheat indicator will be on when the back of the Peltier stage is overheated (it often happens when the cooling water is off) and the current booster will turn off the current supply to the Peltier stage. It automatically recovers when the backside of the stage cools off.

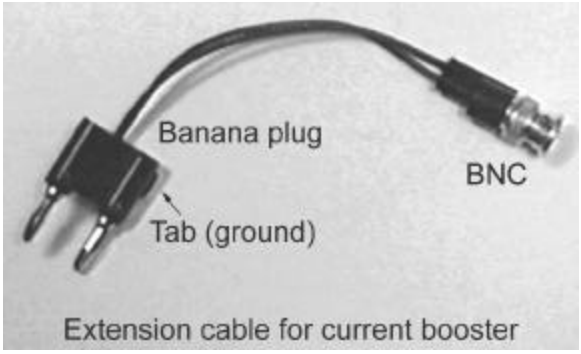


Current booster back panel

There are three cables supplied with the Peltier stage,

- One long cable (round Din 7 plug connector to Din 6 and Din 4 round connectors, same as the one for heating stage)
- One short flexible cable (round Din 7 socket to flat 6-pin female connector, same as the one for heating stage)
- One BNC to double banana plug cable as shown below.

In addition there are two tubes for water cooling.



CONNECTION

1. Connect the round Din 7 plug from the opposite end of the long cable into the Din 7 socket of the shorter cable.
2. Take the long cable and plug the male Din 4 to the female Din4 on the back panel of the power booster, and insert the round 6-pin male connector into the sensor input Din 6 female connector on the Lakeshore controller back panel.
3. Take the BNC to the double banana plug cable and plug the BNC end into the power booster. Plug the double banana plug into the Lakeshore controller with the tabbed end in the LO. (see the schematic below)
4. Insert the flat 6-pin female connector of the short flexible cable into the 6 pin male connector on the back of the sample stage.
5. Other connections are the same as operating the microscope without the temperature control stages.

USING THE LAKESHORE CONTROLLER AND CURRENT BOOSTER WITH THE PELTIER TEMPERATURE STAGE

PROCEDURE

1. Make sure the Range adjust knob is turned to the minimum setting (counterclockwise all the way).
2. Turn on the Lakeshore controller and current booster.
3. Press Auto-Tune then press the Up or Down arrow keys until the display reads Tune: Manual.
4. Enter the proportional, integral, and differential gain. For example, to set the proportional gain press P and then enter a number followed by pressing Enter. The number 12, 12, and 5 respectively work well in previous tests.
5. Press Set Point, enter the desired final temperature and press Enter.
6. Turn the range adjust to maximum, so the current booster gets full power.

7. Press Heater Low for Model 321 or Medium for model 330 Lakeshore controller.
8. Once the temperature has stabilized, engage and scan the sample as usual. If the temperature has not stabilized, the microscope will continue to drift due to thermal expansion.

WARNING

- Never use Heater High with the Peltier stage.

TIPS AND TRICKS

- Make sure there is good thermal contact between the sample and the sample plate. Mount the sample using the air cell or liquid cell even for ambient imaging. Adding an extra layer such as the double-sided tape for sample mount will reduce the thermal conductivity between the sample and the temperature stage.
- Do not forget to set the Z range to the full scale in the software before beginning scanning and changing the temperature.
- It is possible to ramp the temperature while imaging. This depends upon the difference between the initial and final temperature, the thermal expansion coefficient of the sample, the ramp rate, and the type of cantilever (for AFM). Using slow ramps, typically less than 1 °C per minute, may prevent the user from having to withdraw due to limited Z range.
- Remember: every sample will react differently under temperature control. A thin piece of graphite makes a good test sample. For example, it is possible to image a piece of graphite less than 1mm thick while ramping the temperature at less than 1 °C /min from 25 °C to 45 °C using a small STM scanner. When the AFM medium scanner is used, however, it is possible to ramp the temperature at 5 °C /min from room temperature to over 100 °C and still maintains to image the same area of a sample surface.
- Temperature fluctuations due to excessive gains will cause the surface to appear wavy.
- Make connections to the sample and electrochemistry cell as needed (electrode connections) when combining the electrochemical and temperature controls
- The use the environmental chamber is recommended because generally it can help to keep the temperature steady. The PicoAPEX™ chamber can be used to control the sample environment when, for examples, the water condensation during the cooling for ambient imaging and liquid evaporation for liquid imaging during heating become a problem.

Chapter 14: TROUBLESHOOTING

Imaging either in STM or AFM is fairly easy but may take some time to master; however, the rewards are great when you become an accomplished imager. Listed below are a few troubleshooting ideas that might assist you while imaging. If you still are unable to obtain the quality image that you are looking for please feel free to contact any one of our support staff or application scientists at Molecular Imaging, and they will be more than eager to assist you.

NOISY OR STREAKY IMAGES

1. Chances are you have a bad tip. Replace the tip and try again. The cost of trying a new tip is neither worth the time nor frustration of not having your image turn out as expected.
2. A dirty sample is a possibility. Take care in preparation and ensuring your sample is as clean as possible.
3. Did you align the laser on the cantilever correctly? See Chapter 5 for additional information for aligning the laser on the cantilever.
4. If you are imaging a biological sample in solution the tip could have picked up part of the sample (usually occurs when working with a soft sample. You may try to knock it off the tip by keeping imaging for a while or performing a force-distance spectroscopy. If this does not help, change the cantilever.
5. Try restricting the Z value on your controller's software.
6. The integral and proportional gains set by the controller's software may be to high. Try reducing them.
7. STM leakage current is higher than normal: you need to minimize it by adjusting the Zero Adjust screw on the top of the scanner (see Chapter 6). A poorly insulated STM tip may give high tip leakage current in liquid, you have to replace the tip.
8. The microscope may pick up acoustic or electrical noise from certain environments. Place the microscope in the PicoIC™ with the door closed.
9. Old bungee cords in the PicoIC™ may be fully stretched, or the suspended block is touching something in the PicoIC™; either would make poor vibration isolation.

EXCESSIVE DRIFT

1. The key elements attributed to drift are creep in the coarse adjustment screws and creep in the STM tip wire. Follow the procedure in the manual for STM and AFM alignments (chapters 5 and 6).
2. If using AFM, be certain the glass rod is not touching any part of the sample plate, and if using a liquid cell, be certain the glass rod assembly is not touching any part of the liquid cell.
3. Make certain the sample plate is level in the X and Y planes.
4. Make certain all connections are correctly in place.

5. If using a coated STM tip, make certain the tip holder is not contaminated with the coating such as the wax.
6. Grease and oil from fingers can cause drift. Be careful not to touch the tip, cantilever, glass rod, alignment or motor pegs, liquid cell, or the sample plate with your hands.

LASER ALIGNMENT

This could be the most common problem with AFM imaging. However, do not be alarmed, for in the beginning this causes trouble for all. In fact, even seasoned AFM users will occasionally be unable to find the cantilevers or align the laser beam. REMEMBER: Aligning the laser beam on the end of the cantilever is crucial for high quality, accurate images, so take your time and make certain the laser is properly aligned on the end of the cantilever.

1. Refer to Chapter 5 on how to align the laser on the cantilevers. Pay particular attention to the advice offered in the NOTES section as well.
2. If you cannot seem to find the cantilever chip at all while aligning the laser, remove the glass rod and verify the cantilever chip is not off-center in the beak holder.
3. When using a liquid cell, when you place the sample plate and you lose your spot, follow the adjustment procedures outlined in Chapter 5 for liquid cell use. However, it is a common mistake when placing the liquid cell to hit the edge of the liquid cell either on the beak, shifting the entire cantilever chip, or hitting the cantilever itself and breaking it off. Be careful when placing the sample plate to avoid these common mistakes.

See also: **Chapter 5**, specifically, the section on aligning the laser beam.

APPENDIX A

QUASI REFERENCE ELECTRODES

Considerable simplification arises when a simple wire surface can be used as a reference electrode in the SPM liquid cell. It is extraordinarily difficult to prevent residual contamination from the ions needed to operate many of the standard reference electrodes. There are no simple rules to guide the choice of ions and wires used to establish a reference. Silver wire appears to be a good choice in many situations. Its reproducibility is often enhanced by oxidizing the wire in a solution of the electrolyte to be used in the experiment. The stability, reproducibility, and calibration all have to be established by suitable experiments.

STABILITY

We recommend running cyclic voltammetry in the conditions to be used in the SPM experiment using the appropriate liquid cell. Check that features in the voltammogram do not move noticeably over the lifetime of a typical experiment.

REPORDUCIBILITY

This appears to be the major drawback of quasi reference electrodes. Their potential against a standard can vary considerably from wire to wire. It is essential to calibrate each wire before and after each experiment.

CALIBRATION

This is, in principle, a simple procedure. Measure the potential difference between the quasi reference and a standard reference electrode using, for example, the mV measuring input on a good pH meter while both are immersed in the electrolyte solution being used in the experiment. However, contamination of the quasi reference by , for exa mple, chloride, can cause the calibration to drift. So, it is important to establish conditions that are as clean as those used in the SPM cell. We have found it very useful to use a salt bridge to slow diffusion of chloride from a standard Ag/AgCl/KCl reference. Two containers are used, one for each electrode. Each contains the electrolyte of interest, but the one containing the reference is contaminated. The two are coupled by an inverted U-tube containing the salt for which both anion and cation have equal mobilities (minimizing potential drop across the bridge). The ends of the bridge are sealed with plugs that slow diffusion of electrolyte across the bridge.

MAKING A SALT BRIDGE

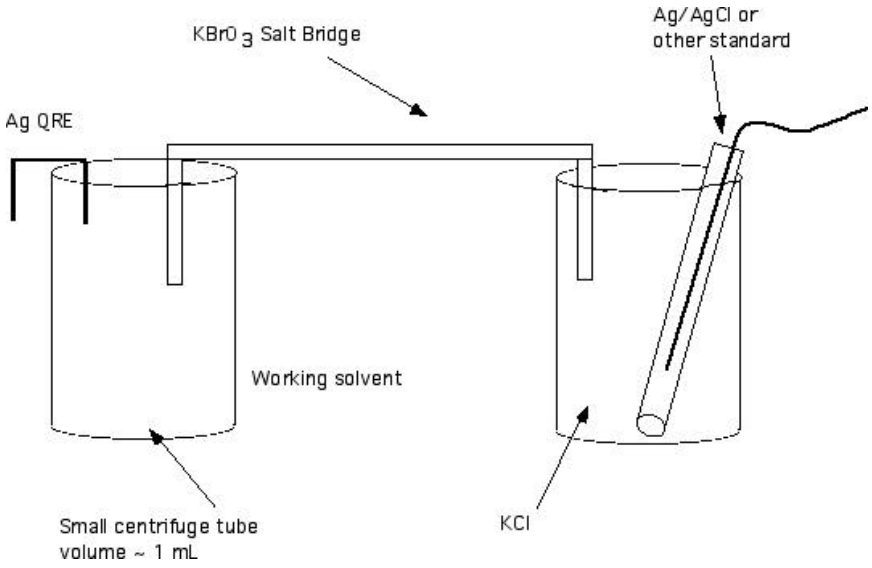
EQUIPMENT

- Pyrex Tube (inner diameter is approximately 3 mm)
- 100 mM $KBrO_3$
- Filter paper
- Bunsen burner

PROCEDURE

1. Heat the tube over the burner and bend the ends so that you have a C-shaped tube.
2. Fill the tube with the 100 mM $KBrO_3$ and soak the filter paper in the same solution.
3. Stuff the ends of the tube with the pieces of the filter paper, making sure that there is no air left in the tube.
4. The bridge is placed between the working solution and the KCl solution.
5. Be sure to add water to the KCl solution as it evaporates.

After using the salt bridge, rinse the salt bridge thoroughly to remove any Cl. Store the bridge in the 100 mM $KBrO_3$ solution in a refrigerator covered (~ 5 °C). Make sure the reference end is stored in the reference solution. When reusing the salt bridge, be sure to rinse it thoroughly to prevent $KBrO_3$ contamination and replace the working solution each time.



APPENDIX B

STM NOISE AND BANDWIDTH: THEORETICAL CONSIDERATIONS

The sensitivity of the instrument is primarily limited by the front-end current-to-voltage converter.

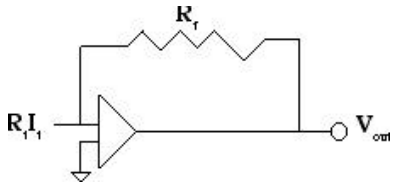


FIGURE B1: Schematic of current to voltage converter for STM

The tunnel junction (resistance R) shown as a source of current I, with stray capacitance C_t which feeds a virtual ground at the input of an operational amplifier. The feedback resistor R_f, converts the input current to a voltage V_{out}, given by $I_t R_f$. For operation at low currents, the junction resistance R_t, can generally be ignored.

If all else is perfect, then the dominant source of noise is thermal (Johnson noise) generated by thermal fluctuations in the resistor R_f. The RMS voltage fluctuations are given by

$$E_{RMS} = \sqrt{4kT\Delta f R_f} \qquad 1$$

Which is equivalent to a current noise of

$$I_{RMS} = \sqrt{\frac{4kT\Delta f}{R_f}} \qquad 2$$

Where I is the bandwidth of the system and k is Boltzmann's constant (1.38 X 10 –23 J/K). Thus, the signal to noise is increased by using a larger value of conversion resistor. Unfortunately, this also leads to a reduced bandwidth as the response of the circuit shown in Figure B1 which is reduced by 3 dB at a frequency of

$$f = \frac{1}{2pR_fC_t} \qquad 3$$

It is very difficult to reduce stray capacitance below a pF while bandwidths above a few tens of kHz are required if the head-amplifier is not to introduce unwanted phase shifts as the speed of operation approaches some fraction of the mechanical resonance frequency of the microscope. The resonance lies, at best, in the 50 to 100 kHz frequency region. With careful design, C can be reduced to 0.5pF, so with a bandwidth of 30 kHz, Eqn. 3 shows that R_f must be no more than 10 MΩ. Eqn. 2 then yields an RMS current noise of 7 pA.

The effective signal bandwidth is actually limited by the low pass characteristics of the proportional-integral (PI) controller. At slow scan speeds and low integral gain settings required for successful imaging at pA level, the effective bandwidth of the control loop (and thereby the error signal) is certainly less than a few kHz.

PRACTICAL CONSIDERATIONS

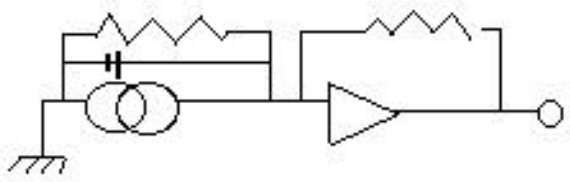
Noise comes from many other sources, some of which are listed below:

- Pick-up of scanning signals from the PZT
- Movement of molecules in the tunnel gap
- Pick-up and microphony on the cable that connects the head to the controller
- Digitization error

The first is difficult to overcome because the tip is mounted on the scanning assembly, to which several hundred volts of scanning signal are applied. The layout and electronic circuitry of the PicoSTM have been designed to eliminate this problem. The second is property of the sample and cannot be changed. However, it points to the difficulty of imaging 'dirty' samples in ambient conditions at very small currents. Indeed, the noise level is one very good test of sample cleanliness: If there is no significant increase in noise when the tip is put into tunneling, scanning a small, flat area, then one may conclude that the sample is reasonably clean. The electrochemical capabilities of the PiocSTM permit such clean operation.

Pick-up and microphony on the cables can easily generate mV signals, equivalent to nA currents with a 10 MΩ conversion resistor. Digitization error can also be significant. If a 10 V signal is digitized to 16-bit accuracy, the resulting noise corresponds to 15 pA with a 10 MΩ conversion resistor. Thus, the signal should be boosted at the head as much as possible.

One way to do this is to increase the gain of the converter using a "T" in the feedback, as shown:



The frequency response is still given by Eqn. 3, but the output is now boosted by the ratio of R_1/R_2. This is still not adequate for the most sensitive operation where, in order to overcome cable noise, pA signals should generate tens of millivolts. This would require $R_1/R_2 > 1000$ with consequent amplification of the drift and noise associated with the operational amplifier. Thus, a further stage of programmable amplification is used following the current to voltage converter.

APPENDIX C

HYDROGEN FLAME ANNEALING SUBSTRATES

MI gold substrates are produced by epitaxially growing high purity gold on green mica in a high vacuum. The resulting gold surface is ~ 2000 Å thick and composed of flat Au (111) terraces up to 280000 nm^2. Hydrogen flame annealing of MI's epitaxially grown gold substrates is extremely important for clean electrochemical work. Hydrogen flame annealing produces contaminant free reconstructed Au (111) surfaces.

SAETY CONSIDERATIONS

Hydrogen is a flammable gas that burns with oxygen to form water. The flame is bright and colorless. Make sure that all of the safety requirements are met before starting.

EQUIPMENT

- Pressurized H_2 gas Cylinder
- Single stage high purity flow regulator
- Anti-flashback arrester
- Square quartz plate (approx. 5 cm X 0.5 cm thick)
- Small quartz piece (approx. 1 cm^2)
- Goggles or safety glasses
- Torch with a quartz tube narrowed to a fine tip with id less than 0.25 mm
- Teflon tubing
- Mask
- Gloves
- Tweezers

***SAFETY*: BEFORE STARTING MAKE SURE…**

- The regulator you use for the hydrogen cylinder meets the requirements for flammable gasses.
- The anti-backflash arrester is attached to the regulator.
- All flammable materials have been removed from your workspace.
- The work area is well vented.
- You have adequate goggles.

PROCEDURE

1. You must have a clean environment to proceed.
2. Place the quartz plate on the counter, with the gold substrate on top. Make sure the "MI," that was scratched on the back of the substrate, is facing down.
3. Place the small quartz piece on the edge of the substrate to hold it in place.
4. Adjust the flow of the regulator to allow just enough hydrogen to pass to make a faint audible sound.
5. Turn off all the lights (the darker the room, the better).
6. Light the torch and adjust the flow of gas so that the flame is about 4 cm long.

7. Gently heat the quartz plate around the substrate to assure even heating. When water no longer condenses, it has been heated enough.
8. Bring the tip of the flame to the substrate at about a 30° angle.
9. Sweep back and forth (at approx. 1 Hz freq) for 30 to 60 seconds, keeping the small flame spot on the film a dim orange. If your room is not dark you will not see this glow and could burn your substrate, thus it is essential the room be very dark.
10. DO NOT OVERHEAT the film.
11. You will see the substrate bend as it is heated and then return to its original flat shape as it cools.
12. Blow out the flame and turn off the gas.
13. Let the substrate cool.
14. Remove it with tweezers.

APPENDIX D

PicoIC

The block the microscope sits on weighs nearly 40 pounds and moves erratically if any attempt is made to move the whole enclosure with the stage in place. Always detach the stage and move it separately.

The PicoIC comes packed in two assemblies. One is the external box with the feed thru panel and the other is the microscope stage with bungee cord suspension. To mount the stage, place a protective pad into the base of the box. This will keep the bolts in the bottom of the stage from tearing the acoustic padding on the bottom of the chamber. Use an old phone book and a cushion so that the stage is at the right height from the bottom of the case. After suspension, the stage should hang with a gap of 4 to 5 inches between the stage bottom and the foam in the PicoIC.

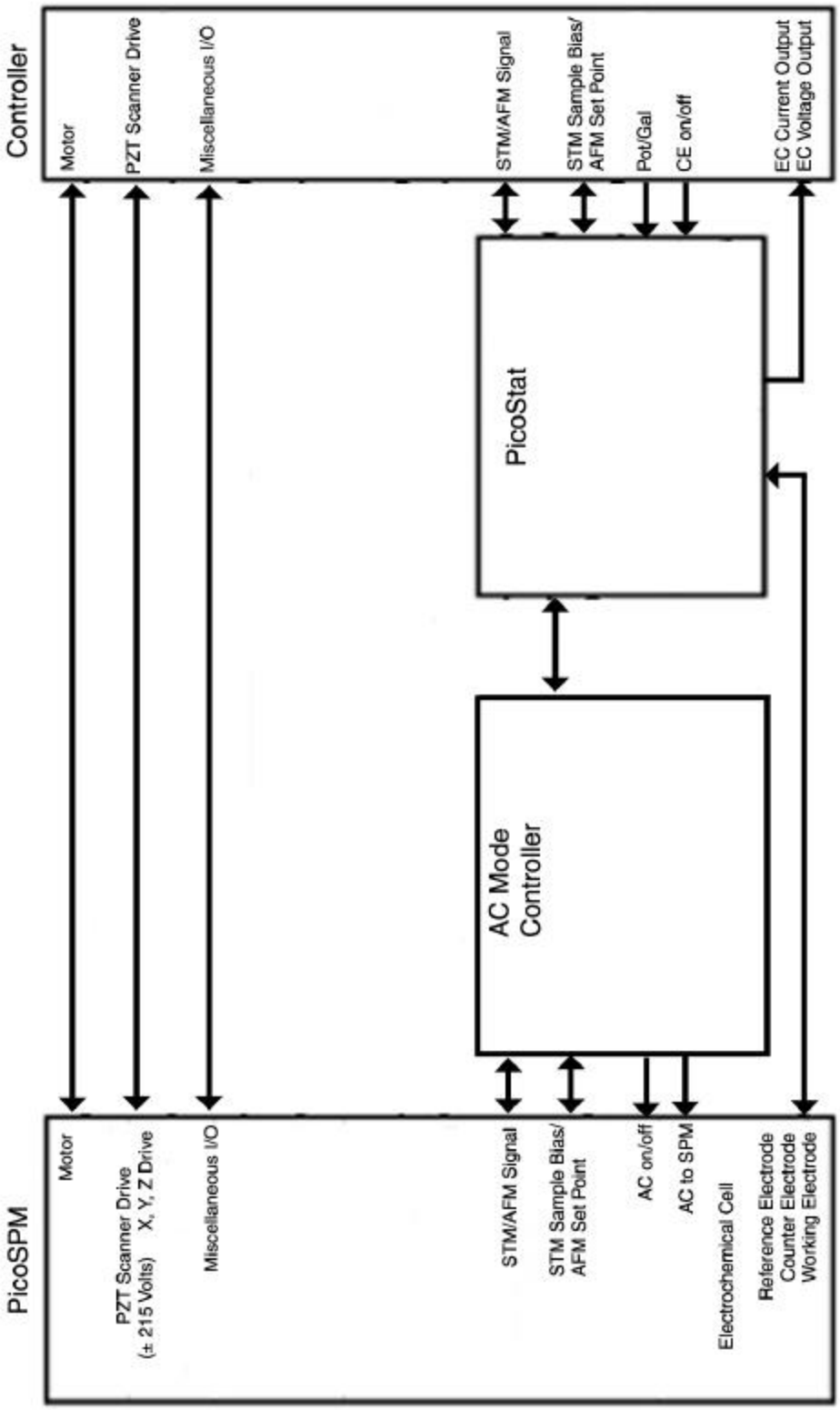
Be certain the PicoIC is in the desired location, near the controller. Place the stage on the phone book and hook the bungee cables into place. The fixed hook goes into the eyebolt on the stage, while the adjustable hook goes into the eyebolt in the PicoIC ceiling. Adjust the cords until the stage is the proper distance from the bottom.

When placing the microscope on the stage, makes sure it is centered so it will not slide. You may want to increase adhesion by placing a pad onto the stage. A mouse pad is ideal.

The bungee cords will stretch and slip with time, so adjust them accordingly. After time, you will want to inspect the cords for wear. If you need to replace cords, feel free to contact Molecular Imaging or you may replace them with similar ¼ inch cords. These cords have been chosen for their spring constant, so replace worn cords with similar bungee cords.

On the side of the PicoIC is the feed thru panel. It is equipped with 1/8 inch Tygon tubing in which tubes can be connected for inlet or outlet purposes. The quick disconnect is removed by pushing on the steel tab and pulling the outer fitting outwards. The connection to the inner fitting is the “gas-in” port, which flows through the needle valve on the panel. Avoid getting liquid into the system as this can damage the flow regulation valve. Finally, the panel also has a thermometer attached for temperature regulation.

APPENDIX E


Controller
Motor
PZT Scanner Drive
Miscellaneous I/O
STM/AFM Signal
STM Sample Bias/
AFM Set Point
Pot/Gal
CE on/off
EC Current Output
EC Voltage Output
PicoStat
AC Mode
Controller
PicoSPM
Motor
PZT Scanner Drive
(± 215 Volts) X, Y, Z Drive
Miscellaneous I/O
STM/AFM Signal
STM Sample Bias/
AFM Set Point
AC on/off
AC to SPM
Electrochemical Cell
Reference Electrode
Counter Electrode
Working Electrode

SYSTEM SCHEMATICS

A complete system is composed of the PicoSPM microscope, the controller, and optional PicoStat or an AC Mode Controller. Please consult the controller documentation for details of its configuration.

GENERAL CONFIGURATION

In a system that does not use the PicoStat, the controller is connected directly to the PicoSPM by the DB25 cable *with a short Patch cable*. In a system that includes the PicoStat or the AC Mode, the controller is connected first to the PicoStat or the AC Mode and then to the PicoSPM with the DB25 cable (*the Patch cable is not required*). If an application requires AC mode imaging under electrochemical (EC) control, both PicoStat and AC Mode are needed and should be connected in the sequence shown above. Many of the signals between the microscope and the controller are passed directly through the PicoStat or AC Mode without modification. The PicoStat and/or AC Mode can always be connected in between the microscope and controller for ambient STM/AFM that does not require EC control or AC mode. The electrical power for the PicoStat, AC Mode, and microscope is provided by the controller.

APPENDIX F

The following document was written by Chad Rigetti concerning Acoustic Mode ACAFM. Most of the concepts also apply to MAC Mode. The document contains a wealth of information on the finer points of AC Mode imaging. It is presented as-is on the following pages.

Optimizing Acoustic Mode Image Quality with Molecular Imaging Corp.'s PicoSPM

Introduction

The PicoSPM is an all-purpose scanning probe microscope. Its capabilities include acoustic mode atomic force microscopy (AFM), MacMode AFM, and scanning tunneling microscopy (STM). While this paper is intended to be a guide to acoustic mode imaging in air, much of the information will be applicable in MacMode as well. No prior knowledge of AFM has been assumed, so the paper can act as a self-contained guide for new users.

Physics of Acoustic Mode Atomic Force Microscopy

When playing a guitar, we strum the strings to create standing mechanical waves, which in turn produce acoustic waves. Acoustic excitation of a cantilever works in the opposite way – acoustic waves are used to induce mechanical vibrations. We can flex the cantilever up and down by setting the drive frequency to one of the cantilever's *bending modes* (which we call its resonant frequency). In the most general case, the amplitude and phase of the induced oscillations as a function of drive frequency are described by (1) and (2),

$$A(\omega) = \frac{F_0 / m}{\left[\left(\omega_o^2 - \omega^2\right)^2 + \frac{\omega_o^{\,2}\omega^2}{Q^2}\right]^{1/2}} \qquad (1)$$

$$\tan\varphi = \frac{2\omega_o\omega}{Q\left(\omega_o^2 - \omega^2\right)} \qquad (2)$$

where ω_o is the cantilever's resonant frequency, ω is the drive frequency, F_o is the drive amplitude and m is the effective mass of the cantilever. Q is the *quality factor*; it is a measure of the peak's sharpness, as well as the energy dissipated per oscillation. These equations describe the free oscillation of a cantilever (though there are other factors that must be included in a rigorous analysis, such as the bending of the cantilever holder and the chip itself[1]).

Acoustic mode imaging relies on an interaction between the oscillating cantilever/tip and the sample surface. The interaction introduces a net force on the cantilever according to the Lennard-Jones force curve. Depending on the mean tip-sample separation, the net force on the cantilever over one cycle of motion can be either attractive or repulsive. The interaction force is illustrated in figure 1. Incorporating the effects of this force on the cantilever's oscillation is non-trivial, primarily due to its non-linearity over the course of a single oscillation. Indeed, for large amplitudes (10 – 100 nm), where the tip spends time in both the attractive and repulsive regimes, the system dynamics are not amenable

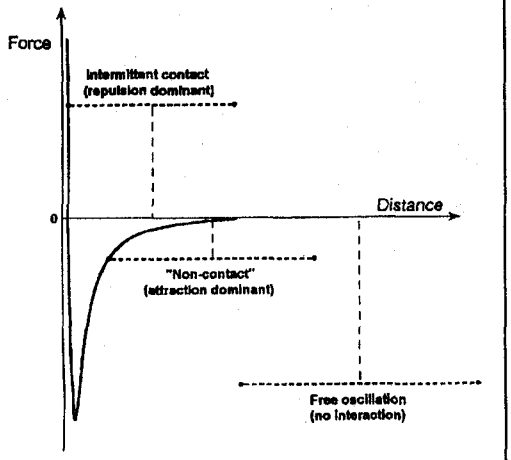


Fig. 1. Schematic illustration of three distance-dependent regimes of interaction between an oscillating cantilever/tip and a sample surface. Dashed horizontal line segments denote distance intervals explored by the tip in each regime, at three values of mean distance (vertical dashed lines). The interaction regimes are labeled free oscillation (no interaction, bottom), non-contact (attraction dominant, middle), and intermittent contact (repulsion dominant, top).

to analytical techniques[2]. However, the system can be solved numerically by first writing the differential equation of motion as:

$$m\ddot{z} + c\dot{z} + kz + F(z) = F_o \sin \omega t \qquad (3)$$

where m is the effective cantilever/tip mass, c is the damping constant, k is the cantilever's spring constant, F_o is the drive amplitude and ω is the drive frequency. The $F(z)$ term is the non-linear force of interaction with the sample surface[3].

The behavior of the system is revealed by the solutions to this equation. If the drive frequency is set at ω_o, the free resonance of the cantilever/tip, we will see the amplitude and phase behavior described in b) of the figure below. Note the small jump in amplitude (on the linear portion of the graph) as the mean tip-sample separation is reduced. This is accompanied by a corresponding phase discontinuity; it marks the transition from net attraction (non-contact) to net repulsion (intermittent contact). As the separation is reduced further, a second discontinuity denotes a return to net attraction.

If the drive frequency is set below ω_o, these jumps in amplitude will not appear. There will, however, be a sharp peak at the top of the linear portion, as seen in part a). In the case that the drive is higher than ω_o, we see a combination of these two behaviors, as shown in c).

This behavior also implies a shift in the resonance peak as a result of the tip-sample interaction. In the attractive regime, the location of the resonance is shifted *downward* relative to the free resonance, while in the repulsive regime it is shifted *upward.* At the interface between the two regimes, the tip begins 'tapping' the surface at the peak of its motion. Ostensibly, this should lead to a *decerease* in amplitude, because the interaction is surely dissipative. A more thorough examination, however, reveals that this tapping causes an abrupt shift in the location and width of the resonant peak, and thus gives rise to the phase and amplitude discontinuities discussed above. The amplitude actually *increases* because the net effect of the shift is to move the resonance *nearer* to the drive frequency.

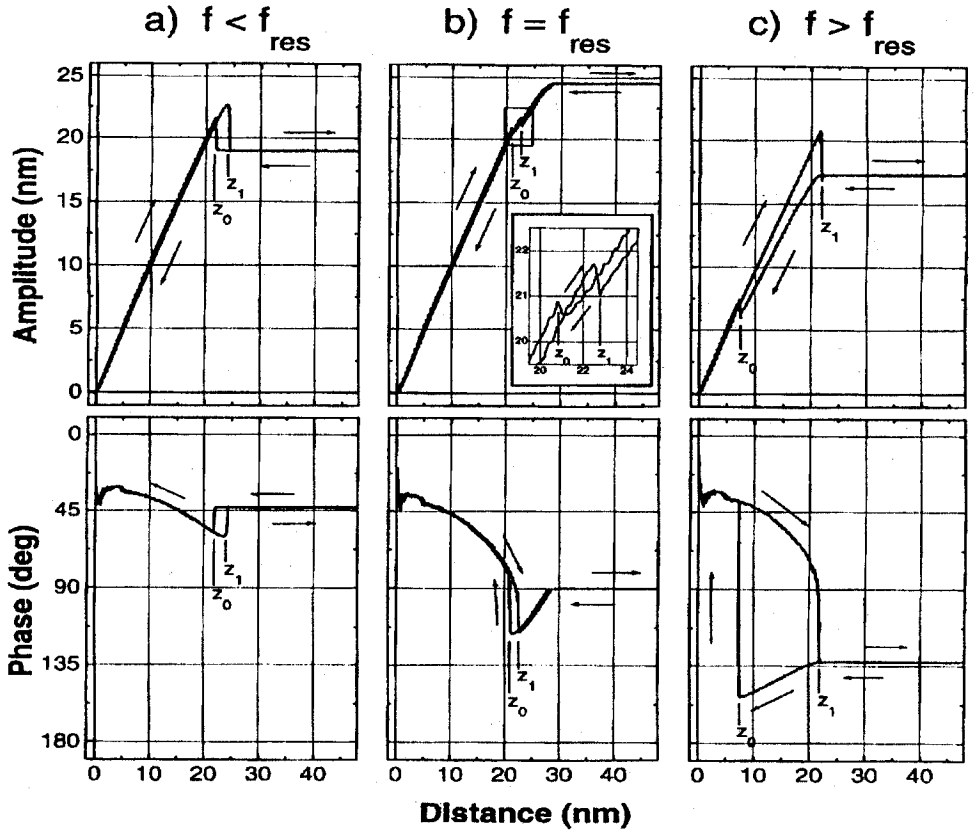
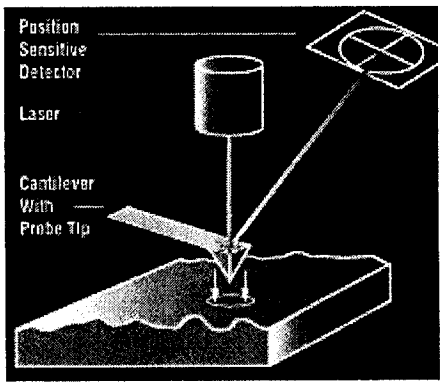


Fig. 1. Experimental DFS curves as obtained by measuring the oscillation amplitude and phase shift as a function of the average tip–sample distance. The dynamic system was driven (a) below ($f = 299.45$ kHz), (b) at ($f = 299.95$ kHz), and (c) above ($f = 300.45$ kHz) its resonance frequency f_{res}. The DFS curves were probed by a silicon tip on a silicon wafer.

Why do we care about all this? Because if we know precisely how an oscillating cantilever is affected by the forces arising from the sample surface, then we can translate the cantilever's dynamics into information about the surface itself. That, essentially, is the general principle of atomic force microscopy.



Exactly how the information is translated is very complex. The first step is to gather the required data about the cantilever's position and amplitude. The amplitude is measured by reflecting a laser off the end of the cantilever to a *segmented photodiode.* A schematic is shown at left. The photodiode is sensitive to the position at which the laser beam is incident. An oscillating cantilever will result in an ac output (though it is ultimately converted to a voltage for the servo) that contains the amplitude information. Now, we know from figure 4 that the amplitude is a function of tip-sample separation. So the instrument uses a servomechanism to maintain a *constant* cantilever amplitude by moderating this z-distance. The z-modulation is

effected by a piezoelectric column. As the tip is scanned over the surface (also effected by the piezoelectric column), the voltage applied to the z-piezo as a function of position provides the raw topographical data. The cantilever's phase relative to the drive is also contained in the signal from the photodiode, which allows the simultaneous formation of a phase image. The phase data can be interpreted as a measure of *energy dissipation* in the tip-sample interaction[4].

It is also typical to save the cantilever amplitude data itself. Even though the servo attempts to maintain constant amplitude, it can do so perfectly only in an idealized situation. The amplitude data is essentially the *error signal*, or the error in the topography image.

Keep in mind that all of this signal processing requires some serious circuitry. This is very much a trivialized overview of an extremely complex electronic process.

Imaging

1) Preparing The Microscope
Begin by opening the Picoscan software. Under *View*, open the following windows: *Approach, Input/Verror Signal Monitor, Buffers/Images, Scan Control* and *Tools 2D/3D.* Make sure the *Setpoint* in the *Scan Control* window is set at zero. Also ensure that the *Drive* and *MAC* are switched off on the Control Box that sits to the right of the monitor. The microscope itself should be sitting in three parts: the system and mount, the scanner, and the sample plate. Begin by mounting a cantilever. The cantilever-holder is easily removed from the head by loosening the two hex screws that hold it in place. Using very fine tweezers, gently grasp a 'chip' by its sides, near the end with the cantilever. (To loosen it from the adhesive, it is easiest to twist the entire casing and hold the chip still.) Press down on the lever plate to open the holder, and slip the chip into place. Let the plate clamp down onto the chip before you release it to make sure it remains aligned correctly. Replace the chip mount in the scanner and gingerly tighten the clamping screws.

2) Aligning The Laser
This can be tedious. It is absolutely necessary to have the chip mounted correctly. Place the scanner, tip down, in the hole in the mount, and tighten it in place with the two horizontal screws on the outside of the plate. Plug it in to the appropriate receptor on the inside roof of the scope. The laser will come on. By looking down through the lens, you will be able to see the edge of the chip and the cantilever. Adjust the laser position by twisting the two knobs on the top of the scanner. The laser must be as near to the end of the cantilever as possible in order to obtain a quality signal. Your primary tool in finding the cantilever is the diffraction pattern displayed on the white paper on the base of the scope. The correct position will produce a very distinct pattern, which you will quickly learn to recognize.

Once you find the pattern place the small crystal in the slot on the front of the microscope. This allows you to see the reflected laser beam. Fine adjust the laser

position until the brightness of the laser spot in the crystal is maximized. Now remove the crystal and replace it with the segmented photodiode that's dangling on the right side of the microscope. Place the photodiode directly where the laser spot was seen in the crystal, then gently move it until the total signal is near its maximum (as displayed by the circular plot on the microscope). Now press the button on the top left side of the microscope housing. This changes the displayed voltage from (top – bottom) to (left – right). Holding this button, gently move the photodiode until the reading is as near to zero as possible; less than 0.5 is acceptable. Make sure the total signal is still maximal. Now let go of the button. Turn the knob that sticks out just below the photodiode until the voltage reading, (top – bottom), is near zero. It is very sensitive, but try to get it as close as possible.

3) The Resonance Peak

Your now ready to measure the cantilever's resonant frequency. Plug in the drive cable to the scanner, and switch on the *Drive* and *MAC* on the control box. A green light will come on on the *MAC* button. Click on the blue *MAC Mode* icon in the tool bar. A window will open that allows you to control the drive output and frequency. If you are using an ncl, set the drive to about 3%; for nch, use around 10%. Next, determine the resonant frequency by clicking on the *frequency plot* tab. The *Spectroscopy* window will open up to an Amplitude vs. Frequency plot. Set the sweep range, and press start.

Low-frequency cantilevers have resonant frequencies in the 160kHz range, while high-frequency cantilevers' are around 330kHz. If you *do not* see a characteristic peak in the appropriate region, there are two possibilities. First, if the signal shows a significant response at *other* frequencies, then the laser is probably too far down on the cantilever, or even on the chip, and you are measuring the response of the chip and chip-holder, *not* the cantilever. If this is the case, remove the photodiode and adjust the laser's position on the cantilever. This ought to correct the problem. If it does not, then it is likely that the chip is inadequately clamped, and secondary resonances are overwhelming the cantilever's response. You may then have to reset the tip in the clasp.

The second possibility is that the signal shows no response at all over the frequency sweep. In this case, the laser light incident on the photodiode has not been reflected off the chip or cantilever at all, but rather of some part of the microscope's structure. Adjust the laser position and start over.

An appropriate frequency sweep should be similar to that shown in figure 3. Once a suitable response has been obtained, note the frequency of max response by clicking on that point on the graph. A marker will be placed there, and its position (in volts and kHz)

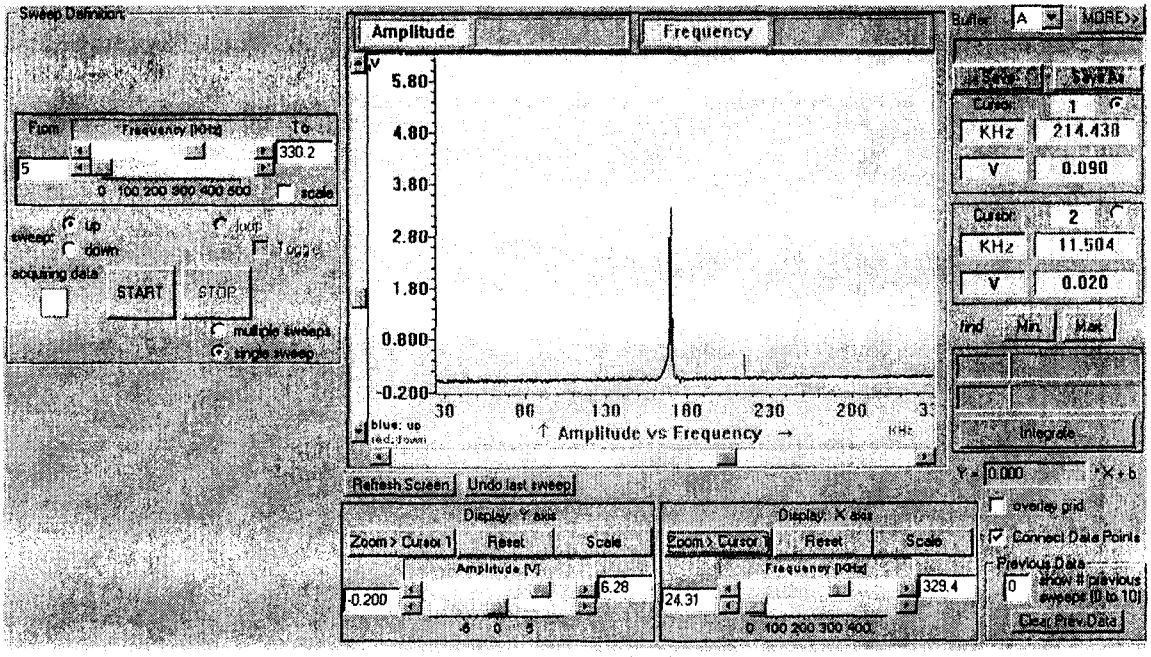


Figure 3. Frequency sweep for Non-Contact Low Frequency cantilever. Drive is 0.25%

will be displayed on the right side of the spectroscopy window. Now, return to the small *AC Mode* window. Set the drive to the resonant frequency you just measured. The voltage displayed on the control box will indicate the cantilever's amplitude (it can be converted using 20.25nm/volt). Now fine adjust the drive frequency until it's at the exact maximum of the response curve. Next, set the drive output so that the free-oscillating amplitude is around 10-15nm, or 0.5v. (This is just to get started. You will find that smaller amplitudes allow better sensitivity, but of you set it too low the signal becomes overwhelmed by noise. Use your discretion.)

4) Mounting The Sample Plate

Put a small piece of two-sided tape on the sample plate. Place the sample on the tape. Ensure that the sample, once adhered, is flat. The sample plate is held in place below the scanner/tip by three magnetized posts. The height of the front two posts is controlled manually by rotating the two knobs at left and right of the scanner. It is important to note that *these two posts are threaded* ***counter****-clockwise,* opposite to convention. The third post (rearmost) provides fine-adjusting ability, either manually when the top of the microscope is open, or with the step-motor when it is closed. The rear post is threaded in the conventional way. After ensuring that the posts are sufficiently low to allow the sample plate to be positioned without contacting the cantilever, gingerly snap the sample plate onto the magnetized posts. Angle it slightly upward so it becomes even as the rear post raises the sample plate to engage the tip. Close the top of the microscope.

5) Engaging The Tip

With the sample plate in place, place the microscope carefully on the suspension plateau in the isolation chamber. Now look at the voltage reading on the microscope's display. If everything has been done correctly, the reading will be negative, between –2 and –9

volts, depending on the amplitude you chose (more negative for larger amplitude). (This is the voltage signal derived from the photodiode, and ultimately represents the cantilever's amplitude; it serves as the input for the servomechanism.) Now, in the *Scan Control* window, lower the setpoint until it is equal to the microscope's voltage reading. (Remember, the setpoint better have been at zero before now! Otherwise you better re-zero the photodiode reading – that means go back to step 2.) The setpoint voltage allows you to control the photodiode output (in dc) when the tip is at the desired height above the sample. Basically, it measures the magnitude of the tip-sample interaction. The more negative the setpoint is, the weaker the interaction required to engage the tip. The reason we set the setpoint equal to the scope's voltage reading right now is so it thinks its engaged at the height when the interaction is just beginning. We will then fine-adjust it from there.

Once you set it correctly, just press *Approach* in the *Approach Window*. Make sure the speed of approach, adjustable on the dial graph in the same window, is below 20. The step motor will then begin raising the sample plate until the (top-bottom) signal is zero. This may take a few minutes. It is best to close the door to the isolation chamber at this point, and let any vibrations in the scope die away. Also, if you have just been handling the microscope, it may be best to take a break *before* you approach. This allows thermal instabilities and mechanical vibrations to exhaust themselves. If you have the time, give the system an hour or two.

When the setpoint has been reached, a window will pop up on the screen that requires you to click *okay* to continue. This window often emerges below all the other open

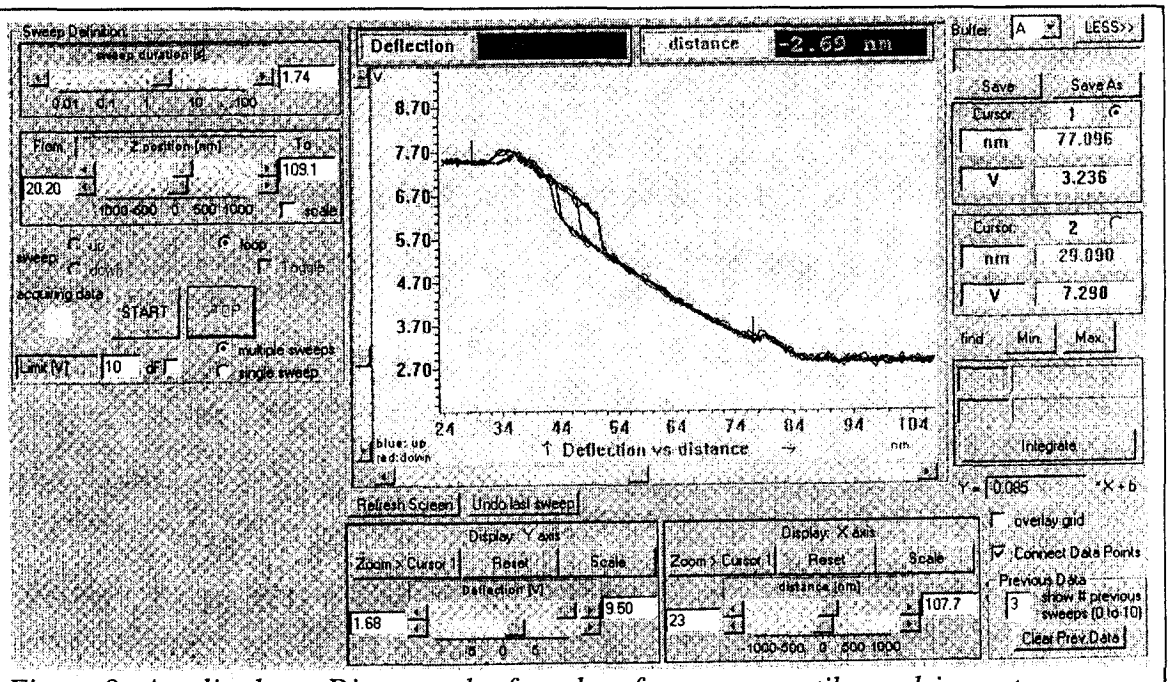


Figure 8. Amplitude vs. Distance plot for a low-frequency cantilever driven at resonance.

windows, so you may have to drag them around to find it. Now click on the *Spectroscopy* tab on the top right toolbar. Go to *Amplitude vs. Distance* and click start. The microscope will begin moving the tip up and down using the z-piezo. The display shows the cantilever's amplitude as a function of height above the surface. This is when you will see the behavior described in figure 1 a), b) and c). Since we're driving at resonance, it should look like b). The entire spectroscopy window for the case of resonance drive is shown in figure 4. Note the very distinctive "kinks" on the linear portion of the graph that characterize resonance driving.

If the plot looks more like a) or c), then the drive is obviously not exactly at the top of the resonance peak. Adjust it by again clicking on the *MAC Mode* icon to open the drive control window.

Once you observe the correct behavior, all you need to do is fine-adjust the setpoint. Do this by using the *up* and *down* arrows next to the setpoint reading. As you adjust it, the entire amplitude vs. distance curve will shift up or down. Now, as we have learned, the topographic image is based on the servomechanism maintaining the cantilever's amplitude constant, while the phase image is based on subtle phase shift as the resonance frequency shifts with tip-sample separation. So clearly, *stable imaging necessitates that we avoid the discontinuities in these plots.* This means *stay away from the kinks.* It is best to adjust the setpoint until the zero-voltage line intersects the plot when it's just

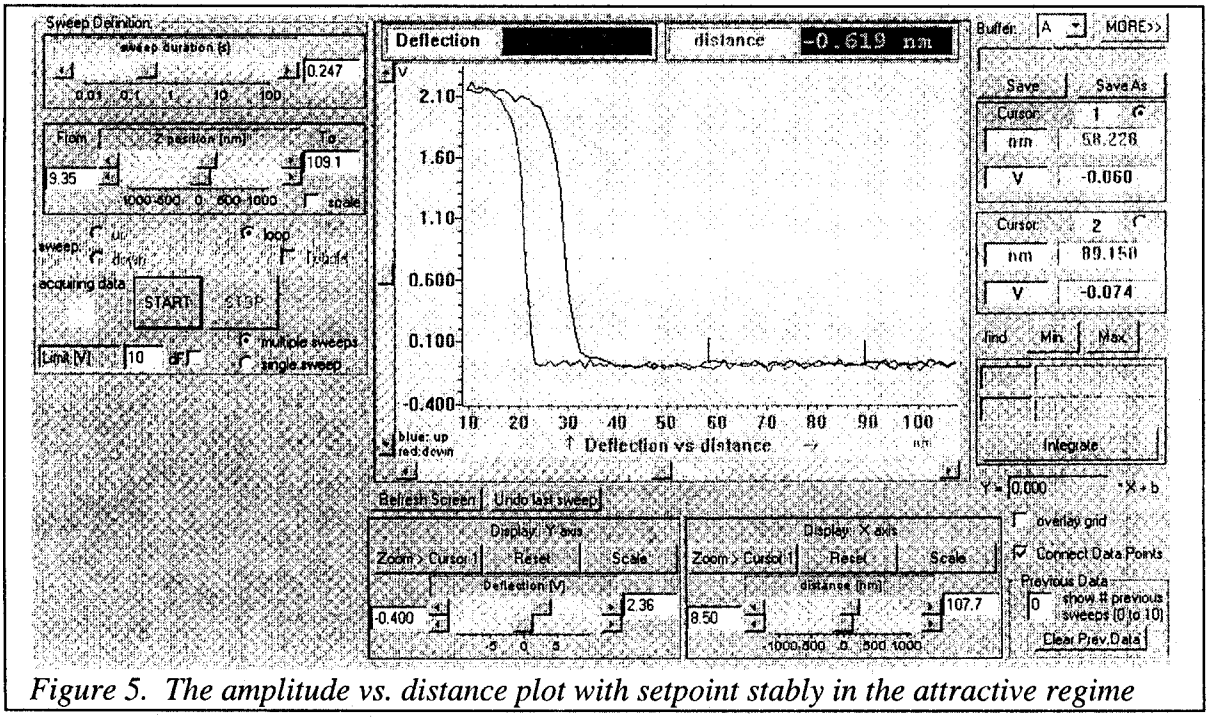


Figure 5. The amplitude vs. distance plot with setpoint stably in the attractive regime

starting to curve upwards. The correct setpoint adjustment is shown in figure 5. As a general rule, set the zero-line as low on the curve as possible such that it is still above the noise that appears on the flat portion.

6) Scanning

You're now ready to start a scan. Press *Stop* on the amplitude vs. distance plot and hide the window. You should usually open three data buffers, *Topograph, Phase* and *Amplitude.* Start a scan by pressing the green *Start* button in the scan control window. It will take a minute for the scan to appear. Now you will have to play with the various settings – servo range, servo offset, gain (I and P) - in scan control to optimize the quality of the image. Scan speed usually needs to be kept in the 1.3Hz range.

Troubleshooting

Be prepared for some time-consuming fidgeting. The microscope is neither robust nor ergonomic. However, if you have patience, you will get quality images. Here are some of the things likely to trouble you.

-The amplitude vs. distance curve is unstable and overwhelmed with noise

This is common. Make sure the microscope is in the isolation chamber and the door is closed. If there is a lot of traffic in the lab, with the door opening and closing, youmay just wan to come back later and try again. Also, be wary of ambient acoustic noise. Specifically, the NANOpure water distillation system on the west wall in the lab can get very noisy, and it will usually show up on the amplitude curve. If it's noisy, come back later. Also, give the scope time to thermally equilibrate. This will often clean up the curve.

Another thing to check is the level of the sample plate. If it is markedly uneven, the interaction between the tip and sample becomes much more complicated, and a very irregular amplitude vs distance curve results (such as spikes and severe hysteresis). If you see this behavior, it may just be that the plate is uneven when the tip is engaged.

-The phase picture is nothing but noise.

As we learned, the phase changes discontinuously as net repulsion takes over. If the cantilever is close to this transition point, the phase will continually jump from the top to the bottom of the discontinuity, overwhelming any subtle phase contrast that carries information about the surface. However, from experience, the phase *and* topograph pictures cannot both be optimized simultaneously. So depending on the nature of the information you seek, choose one and optimize it. (I am referring here to the settings in *scan control,* but **not** the setpoint. By definition, stability in one image implies stability in the others. In fact, we can use this fact, along with the phase contrast, as a very powerful tool to determine whether the cantilever is stably in the attractive regime or not. To quantify the stability, click on the *cross section* icon in the *Buffers/Images* window, then click on the phase image. Below the picture there will appear a cross section view of the phase. If the cantilever isn't stable the cross-section will be overwhelmed by spikes. If it's stable, then the cross section will be relatively flat.)

-The amplitude vs. distance curve keeps moving to the right when I try to fine-adjust the setpoint.

There is an unfortunate transient instability in the z-piezo. When it's present, there appears to be a severe hysteresis that shifts the entire plot to the right every sweep. There are a few things you can try (though I guarantee none of them). First, try shutting down the software for a moment. Sometimes this is sufficient to regain stability. If that doesn't work, try unplugging the microscope for a minute, then reconnecting it. Finally, you can turn the entire setup off, then restart it. If none of these works, try living with it for a while, because it sometimes goes away on its own. The problem is being addressed on a fundamental level.

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[1] S.M. Lindsay, *The Scanning Probe Microscope In Biology, 305-307 (1999).*
[2] B. Anczykowski, D. Kruger, K.L. Babcock, and H. Fuchs, *Ultramicroscopy* ***66****, 251-259 (1996).*
[3] Ibid.
[4] G Haugstad and R. R. Jones, *Ultramicroscopy,* ***76****, 77-86 (1999).*

INDEX

M

N

O

P

Q

R

S

T

V

W

Z