OXFORD INSTRUMENTS

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Cypher Instrument Family User Guide

Including beta (complete, reviewed) chapters. Including draft (nearly complete, not reviewed) chapters.

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Asylum Research

an Oxford Instruments company

Contents

Introduction

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

AR Software Version It is assumed that AR Software version 13 or later is installed on your system. To download the latest software, please register at our support site: `http://support.asylumresearch.com`.

Getting Help For additional help with your Asylum Research instrument, including software support, refer to: https://afm.oxinst.com/Support-US

Updates to the Manual Bundled with the software updates.

Send Feedback Send e-mail to: sba.manuals@oxinst.com (clickable link) and mention which version of the user guide you are using and what chapter and section on which you are commenting.

Part I

System Overview & Powering Up

Who is this part for? After the Cypher SPM has been installed in your lab and you (or someone in your facility) have completed the initial training, this part of the user guide will review the main parts of the instrument and software. Instrument power up is also covered.

Part Contents

1. Safety Precautions

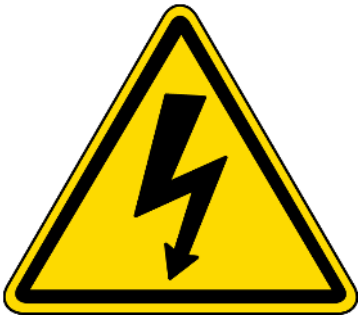
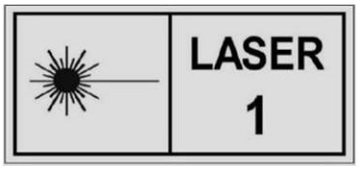
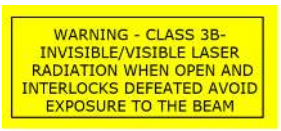
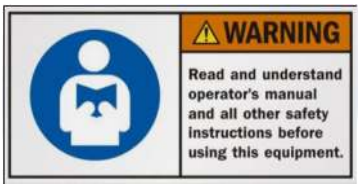
CHAPTER REV. 2423, DATED 08/14/2021, 18:50. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

During normal use, the Cypher SPM poses no harm to the operator. Nevertheless, there are potential dangers in and around the instrument. Please read this section carefully to understand where the potential dangers lie before attempting to use the Cypher.

1.1. Safety Label Explanations

Specific examples of each warning label on the instrument are covered in the next section.

Description	Warning Label
Pinch Point Avoid placing hands in these areas. In case of unexpected motor movement, injury could result.	
High Voltage Voltages of up to 165VDC can be present on these connectors. Always turn off the ARC2 controller power (see Figure 3.2 on page 25) before connector or disconnecting cables.	
Laser Product The Cypher AFM is a Class 1 laser product during normal operation. It is safe to view with the unprotected eye under all circumstances of regular use.	
Laser Warning Harmful levels of laser power do occur inside the protective housing. **Do not disassemble or defeat interlocks.**	 
Laser Shut Off Different types of laser modules can be purchased for the Cypher. Before exchanging a laser module, one must first turn off power to the laser.	
Manual Label Before using the AFM, one must understand all potential safety hazards. Read the entire safety section found in this manual.	

1.1.1. Additional Labels

The following additional labels appear on the Cypher Systems:

Identification Labels

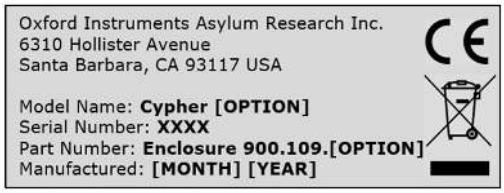


Figure 1.1.: Example of an identification label.

The serial number identification labels for each major component are located on:

- side of the Enclosure
- back of the Scanner
- back of the View Module
- bottom of the Head
- front of the Chassis

1.2. Motor Safety

The Cypher SPM contains six motors which direct the laser beam and move the objective lens and cantilever holder into position. All the motors are highly geared and can generate powerful torques that could seriously pinch a finger or possibly break a bone. The most important moving parts to be aware of, from a safety perspective, are the cantilever holder and the objective lens, along with its carriage. Always keep your hands clear of the Cypher when performing any software or manually controlled motor moves.

1.2.1. Avoiding unsafe situations

- Familiarize yourself with potential pinch points. Figure 1.2 on page 6 indicates the areas of concern: directly under the objective lens and cantilever holder, at the edges of the scanner module, and both above and below the two cross-roller bearing stages.
- It is advisable to always leave the optics cover in place. Removing it will expose additional areas where one could get pinched.
- Operate motors only with the enclosure door closed.

1.2.2. How to stop the motors

In case of an emergency, the motors can be stopped in one of the following ways:

- The motor control knob at the lower front of the Cypher can be used at any time to override computer commanded **objective lens** or **cantilever holder** motor moves. Turning the knob will immediately stop any current motor moves and will transfer control of both the cantilever holder and objective motion to the knob. Note that turning the knob always moves BOTH the cantilever holder and the objective lens simultaneously. *This means that if you were to have your finger pinched between the cantilever holder and the objective lens, the best way to release your finger would be to use the software to motor the objective lens upwards while leaving the cantilever holder stationary.*

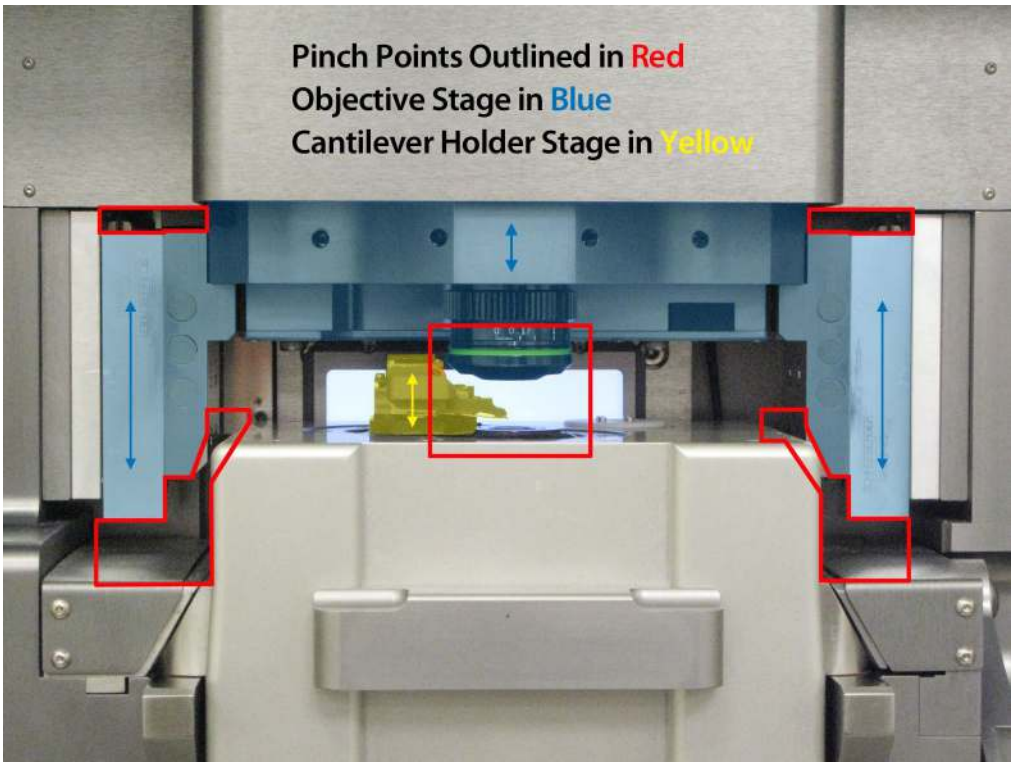


Figure 1.2.: Areas to avoid particularly when motors are driving the objective lens or cantilever holder. Tinted parts can move as shown by the arrows.

- Press and HOLD DOWN the 'Esc' key on the PC keyboard. This will always stop any automated motor moves.
- Turn off the ARC2 controller. Though this is not advised since it will not allow you to use the knob or software to control the motors and manually move them to a safer position.
- Unplug the power brick, which is connected to the Cypher. This power brick supply feeds the motors. As was the case with turning off the controller, cutting power will stop the motors but will not give you any quick method for moving the motors to a safe position.
- If the power is cut and you must move the objective, you can reach inside the enclosure and feel for a wheel at the lower left-hand side, near the rear of the Cypher chassis. This wheel turns the motor manually. Ten turns are required for every millimeter of objective motion. Turn clockwise to move the objective DOWN and counterclockwise to move the objective UP.

1.3. Light Source Safety

Caution	Use of controls, adjustments, or performance procedures other than those specified herein may result in hazardous invisible laser energy exposure!

1.3.1. Non-visible Laser Diode or Super Luminescent Diode Light

The Cypher SPM contains a laser diode (LD) or super luminescent diode (SLD) light source. Superluminescent diodes are like lasers but have a shorter coherence length. As of the writing of this manual, all Cypher light

sources have an output of several mW around 850 nm, which is non-visible. From a safety perspective, LDs and SLDs can be regarded as identical, and the terms will be used interchangeably.

The Cypher laser is sufficiently well shielded that the Cypher SPM qualifies as IEC Class 1 laser product that complies with 21 CFR 1040.10 and 1040.11, except for deviations pursuant to Laser Notice No. 50, dated June 2007. Complies with IEC/EN 60825-1, 2014-05 Ed. 3 and IEC/EN 60825-1:2007-03 Ed. 2.0. In layman's terms, this means the Cypher SPM is in the same class as a home DVD player and in a safer class than a laser pointer. When used as prescribed, there is no danger of exposure. Nonetheless, it is still good to have an understanding of the laser in the instrument and the safety features.

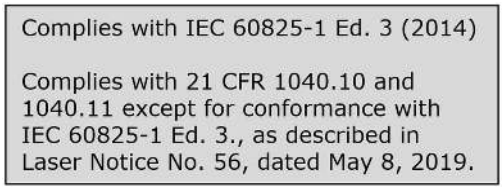


Figure 1.3.: Laser Compliance Label. A class 1 laser product is safe under all conditions of normal use.

1.3.2. The Detection Laser Optical Path

Understanding the laser optical path is the best way to reduce the possibility of harmful exposure to non-visible light, which may cause eye damage. Figure 1.4 on page 8 shows a simplified picture of the laser optical path. The light originates inside the removable laser module, then reflects via a mirror (called the "hot mirror" because it reflects infrared light while transmitting visible light) into the objective. The only place a person can be exposed to the light during normal operation is where the light exits the objective. As you will see in the next section, there are numerous interlocks to make the possibility of exposure very low.

Still, here are a few things you should avoid:

- The IR light source is always on, regardless of whether the enclosure door is open or closed. In some cases, the laser is turned off by software, or it can be turned off by rotating the key on the ARC2 controller.
- While unlikely, it is possible to place a small mirror below the objective and reflect the light in the direction of the user. Even then the beam spreads rapidly and is not powerful enough to classify the instrument differently. Nevertheless, you should be aware that non-visible light is coming out of the objective.
- Never remove the objective lens or you may void your warranty. It can also expose you to a collimated invisible laser beam.
- Never change or remove the laser module without first turning off the laser power key on the ARC2 controller. Never look directly into a plugged-in laser module (Figure 1.5 on page 9).

1.3.3. blueDrive Laser Information

Cypher S and ES systems can optionally be equipped with blueDrive. All Cypher VRS models are equipped with blueDrive. See Chapter 35 on page 406 for background information. BlueDrive employs a 405 nm blue Laser which follows the same path as the IR light source described in the previous section. Because of the intensity of the light, the blue laser can only be on when the enclosure door is closed.

There is a blue LED on the front of the blueDrive filter cubes (see Figure 35.1 on page 406). This LED should only be illuminated when the enclosure door is closed. If you ever see this LED illuminated when the door is open, please contact Customer Service immediately and stop using the instrument.

While nearly all of the blue laser beam never leaves the vertical path in Figure 1.4 on page 8, there is small chance some fraction of light could be directed toward the AFM operator. To prevent this, the glass on the enclosure is yellow, blocking all 405nm light.

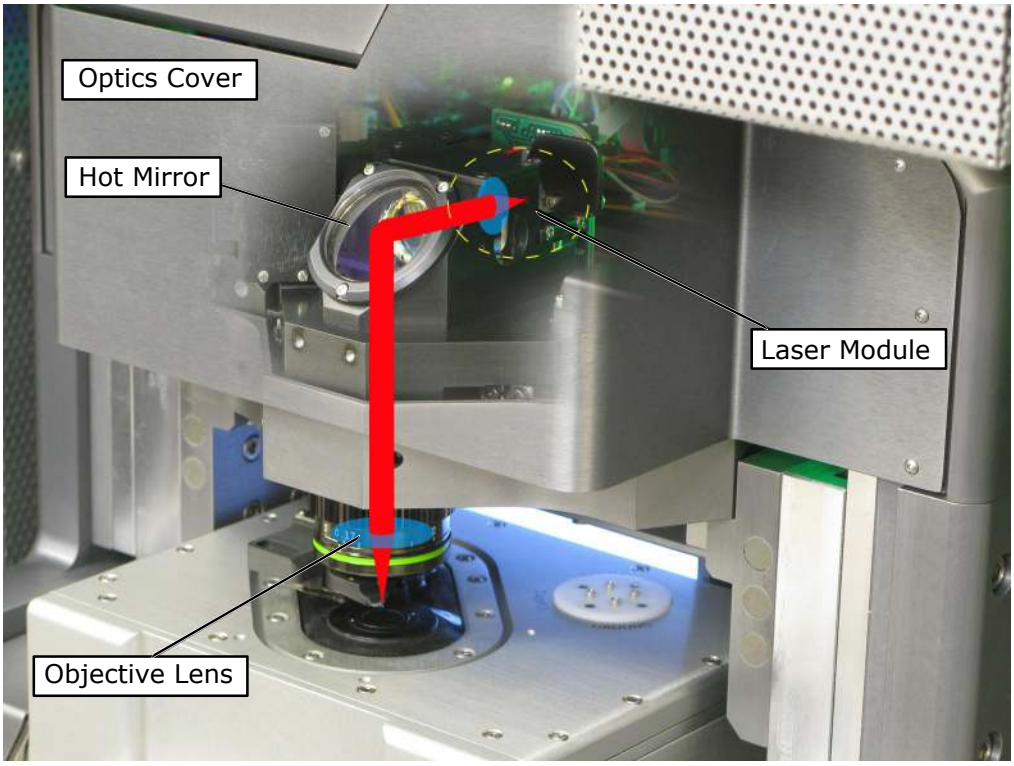


Figure 1.4.: Simplified laser path. Removing the optics cover exposes the hot mirror and laser module. The non-visible laser light originates in the laser module and comes out as a collimated beam. The collimated beam is reflected off of the hot mirror and into the objective lens, which focuses it onto the cantilever. During normal operation, the only place a person can be exposed to the light is where the light exits the objective.

1.3.4. Laser Switches and interlocks

Cypher has various switches and interlocks which control the state of the lasers. Understanding their function is a important part of using Cypher safely. There are three interlocks which control the lasers on Cypher:

1. The door interlock, which turns off the blueDrive laser when the enclosure door is opened.
2. The blueDrive filter cube interlock, which turns off the blueDrive laser when the filter cubes are removed. This typically has no effect as the door interlock will have already turned off the laser, but in cases where this interlock has been tampered with and overridden, the filter cube interlock offers extra protection.
3. The source module interlock. In case you not turn off the ARC2 power when exchanging source modules (see Chapter 34 on page 398), a magnetic interlock cuts power to the IR laser or SLD in the source module before it is fully removed from its cradle.

The following examples include some scenarios where the various interlocks play a role.

Figure 1.5.: Light source module removed from the Cypher AFM. During the removal process, it is possible to tamper with safety overrides in such a way that collimated light shines out of the module. As a safety precaution, never look directly into the module while its power cable is attached! (Power cable is not shown in this image.)

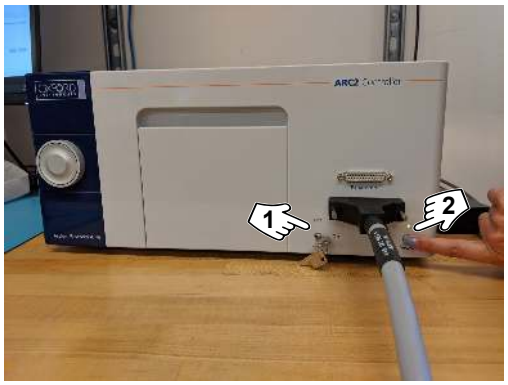
1.

Turning Lasers Off:

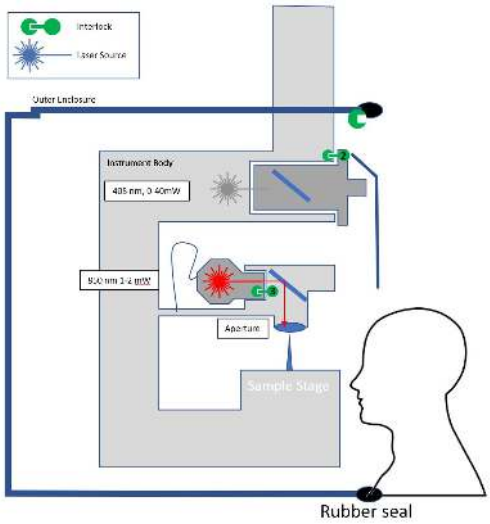
- If there is ever any reason to turn lasers off, do the following:

  1. Turn the laser key on the ARC2 controller to the OFF position. This will leave all other systems operating.

  2. Turn off the power to the ARC2 controller. This will cut power to all lasers, and many other systems as well.



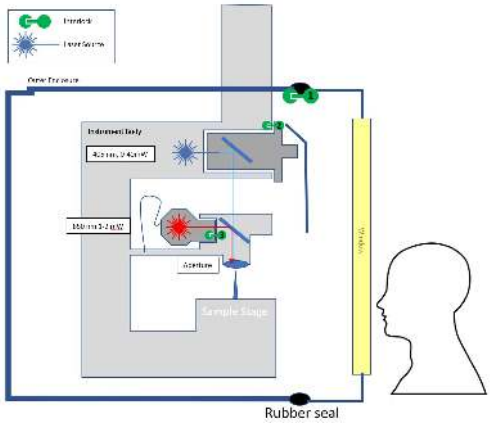
2.



SAFE: Normal operation with the door open

- With the door open, Interlock 1 turns off the blueDrive excitation laser.
- The IR detection laser is always on but is still safe to view with the unprotected eye.
- Typically, nearly all of the light travels down to the sample and immediately back up into the Cypher optics.

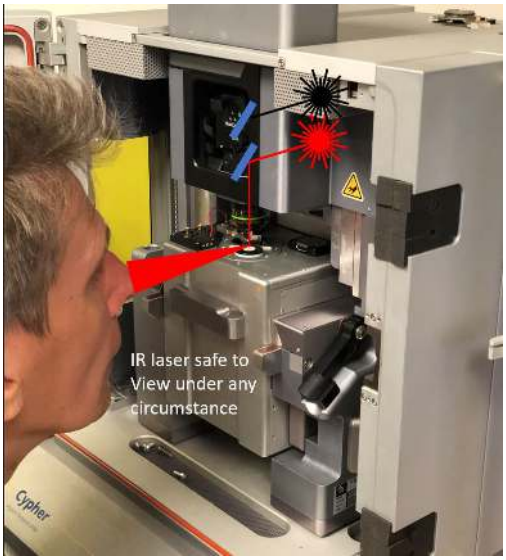
3.

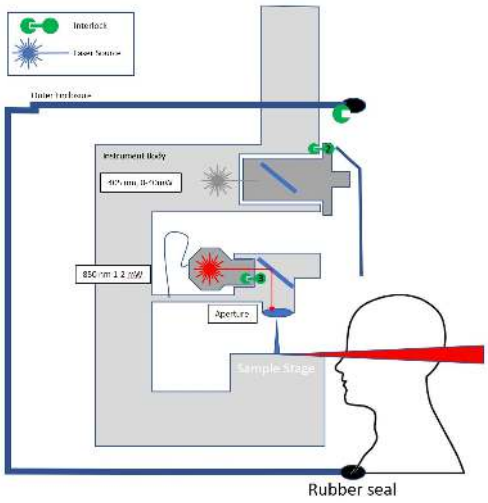


SAFE: Normal operation with the door closed

- With the door closed, Interlock 1 allows the blueDrive laser to activate.
- Typically, nearly all of the light travels down to the sample and immediately back up into the Cypher optics.
- Any additional blue light is absorbed by the yellow filter on the door.

4.

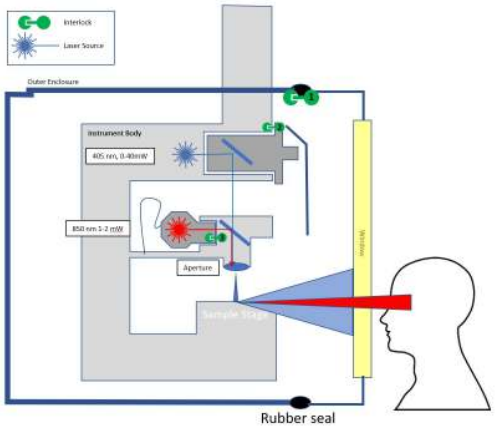




SAFE: Sample with reflective edge, door open

- With the door open, Interlock 1 turns off the blueDrive excitation laser.
- In rare cases, a sample might have a reflective beveled edge.
- The Cypher IR detection laser power is low enough to be safely viewed with unprotected eye, even in this worst-case scenario.

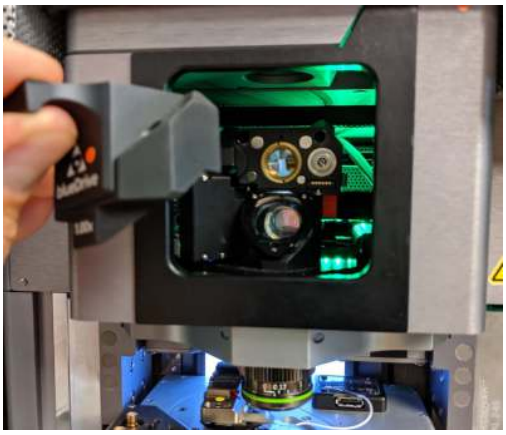
5.

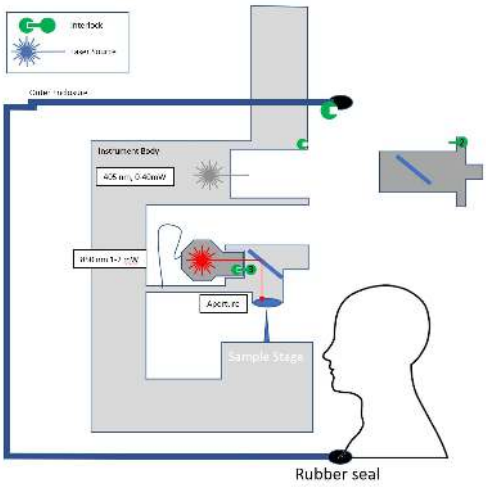


SAFE: Sample with reflective edge, door closed

- With the door closed, Interlock 1 allows the blueDrive laser to activate.
- In rare cases, a sample might have a reflective beveled edge.
- The Cypher IR detection laser is still safe to view.
- The Cypher blueDrive excitation laser light is fully blocked by the yellow filter on the window. It is safe to view the sample without protective eyewear.

6.

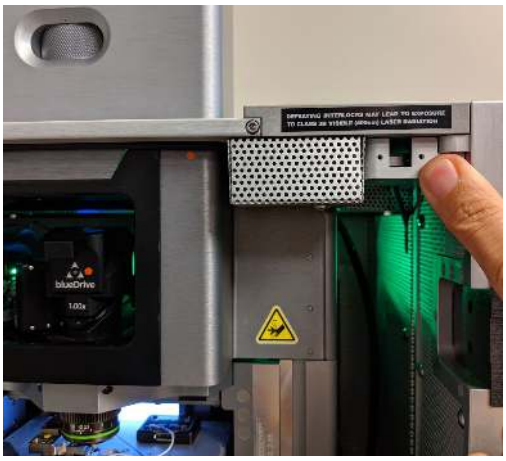


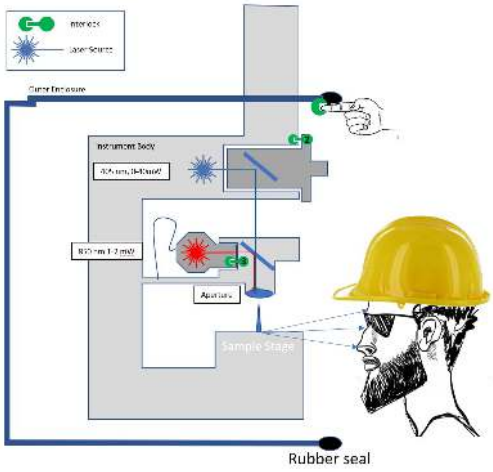


SAFE: Removing blueDrive filter cubes

- As soon as a blueDrive filter cube is removed, gold plated pins (Interlock 2) break contact with the cube. The blueDrive laser turns off.
- Since the cube is only accessible when the door is open, Interlock 2 is additional protection.
- In any case, it is safe to look into the lenses behind the filter cube.

7.

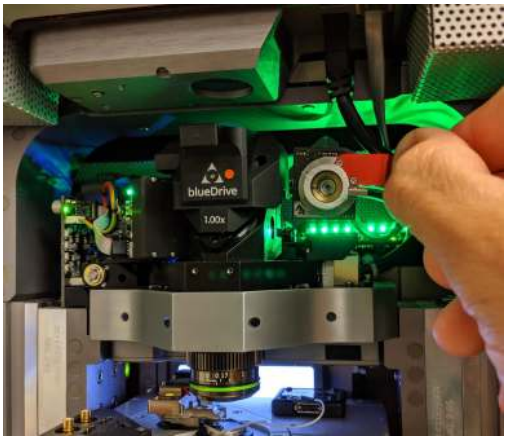


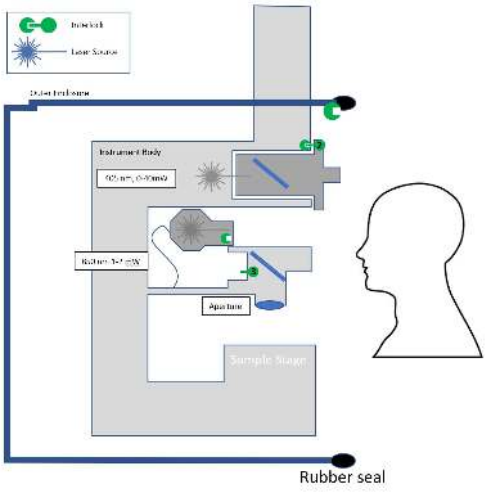


<u>UNSAFE</u>: Overriding the door interlock

- The door interlock switch should not be depressed.
- When depressed, the blueDrive laser can be ON while the door is open.
- You may be exposed to class 3B 405nm radiation, up to 20mW. This can lead to permanent eye damage.

8.





SAFE: Removing blueDrive filter cubes

- Removing the laser source module is described in Section 34.2 on page 400.
- This operation should only be performed with the system power turned off. In case this is forgotten, Interlock 3 turns off the IR detection laser before it is removed from the cradle.
- It is safe to look into the lens of the source module.

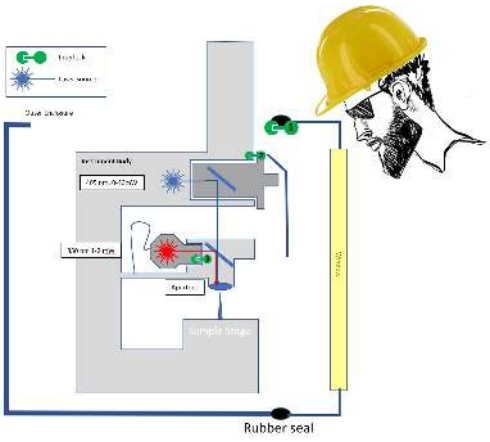
Note There is also a red LED (not shown as lit in the photo above, as it should be) that indicates if the IR laser is on.

Note The photo above shows the system power turned on, only for demonstration purposes. The system power must be off when performing this procedure.

9.

UNSAFE: Disassembling the enclosure:

- Do not disassemble the enclosure! This is intended only for Oxford Instruments service personnel.
- The door interlock does not protect against removal of enclosure parts.
- There are no user-maintainable components that can be accessed by tampering with the enclosure.



1.4. Power Supply Safety and Thermal Management

1.4.1. High Voltage

The voltages inside the Cypher SPM are as dangerous as those present in a standard wall socket; therefore, you should respect all of the components under the instrument covers as you would a wall socket. Never touch anything or insert anything conductive under the instrument covers! Also, the piezoelectric actuators in the scanner have a

large capacitance and can hold charge for many minutes after the scanner is disconnected from any power supply. *For this reason, never touch the scanner connector, unless it has been unplugged for at least 10 minutes!*

Warning!	The Cypher SPM and ARC2 controller contain internal voltages up to 165VDC, 0.5A. Use caution when handling system pieces to avoid electrical injury as these voltages may be lethal!

1.4.2. Fuses

Adhere to the fuse ratings appropriate to the main supply voltage listed on the backside of the ARC2 controller. Not following the recommended ratings may cause the instrument to overheat or sustain damage!

1.4.3. Overheating

Keep the backside of the ARC2 clear. Cool air is drawn into the heat sinks on the back of the ARC2 controller and two fans exhaust warm air from the same place. Obstructing any part of the ARC2 back will cause power supplies and electronics to overheat!

Keep the top of the Cypher SPM backpack clear of items. The backpack is passively cooled and requires all the heat fins on the side and top be in open air. Don't place paper or notebooks on top of the backpack!

1.5. Instrument Specifications

1.5.1. Weight

The Cypher instrument is made of many heavy metal parts. Be prepared to have two people on hand whenever lifting of the empty enclosure is required. If you are thinking of lifting or moving the instrument (even a few inches) you MUST first contact Asylum Research, or your instrument will be damaged, and you will experience downtime and incur costs!

Note Even the scanner module is quite heavy. Be prepared to support its weight as you pull it from the support rails.

1.5.2. General Specifications

Cypher is a product of Asylum Research, an Oxford Instruments Company, and is located at 6310 Hollister Ave, Goleta, CA 93111.

The Cypher SPM is designed to be run inside a laboratory setting. This means that, ideally, the room is clean and quiet, with temperature and humidity regulated. Under no circumstances should a system be set up outdoors!

1.5.3. Voltage Ratings

The Cypher SPM will run on 100-240V, but it is factory configured to operate at only one voltage. Contact Asylum Research if you need to have your input voltage changed between 50-60Hz and a maximum of 400W. This information can be found on the backside of the ARC2 controller. Also see Chapter 3 on page 24 for more information about the ARC2 controller.

User input and output connection ratings.

- For inputs on the ARC2 controller, see Chapter 3 on page 24.
- For inputs on the back of Cypher (backpack) see Chapter 4 on page 29.

2. System Overview

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

2.1. Basic Cypher SPM Hardware

Before starting the tutorial, the user should be familiar with the names and functionality of each of the Cypher components. Don't worry if you don't understand everything in this section, as the main goal is to just get familiar with the basic purpose of each component. Figure 2.1 on page 17 shows a typical setup for the Cypher SPM. The top-level components are the computer, the ARC2 controller, and the microscope itself.

Computer The **Computer** is the primary interface for controlling the **Microscope**; its main communication is via a USB1.1 connection to the **ARC2** AND via USB 2.0 directly from the computer to the **Backpack**. See Section 38.2 on page 442 for recommended USB ports to use.

ARC2 The **ARC2 (Asylum Research Controller 2)** is what is colloquially referred to as "the controller". It houses power supplies and the necessary electronics for controlling the scan motion and acquiring image data from the microscope. To learn more about the **ARC2** and its functions, please refer to Chapter 3 on page 24.

Microscope The **Microscope** itself, where the actual imaging takes place, is the *core* of the AFM system. Although the computer, controller, and microscope all comprise the Cypher, the microscope itself will often be referred to as the Cypher.

The microscope is comprised of five basic components (see Figure 2.2 on page 18): enclosure, chassis, camera, scanner, and backpack. The enclosure and chassis are common to all versions of the Cypher, though the enclosure and backpacks are different for different versions. In contrast, the scanner, the backpack, and camera are designed to be modular and easily interchanged by the user.

Enclosure The primary function of the **Enclosure** is to isolate the imaging portion of the microscope from acoustic noise such as talking or music. Acoustic noise can cause the mechanical components holding the sample to move, thereby showing up as noise in the microscope images. The secondary role of the **Enclosure** is to provide a local environment for the microscope itself, in which the temperature can be controlled. Keeping the microscope at a constant temperature is important for maintaining long-term control of the relative position between the cantilever and the sample. The air temperature controller (ATC) is a Cypher option that can be used to maintain the temperature inside the enclosure. To learn more about the **Enclosure** and its options, please refer to Part V on page 389.

Chassis The **Chassis** is the central structural unit supporting the scanner, camera, and head. While the scanner and camera are modular units designed to be interchanged by the user, the **Head** is just a sub-assembly of the

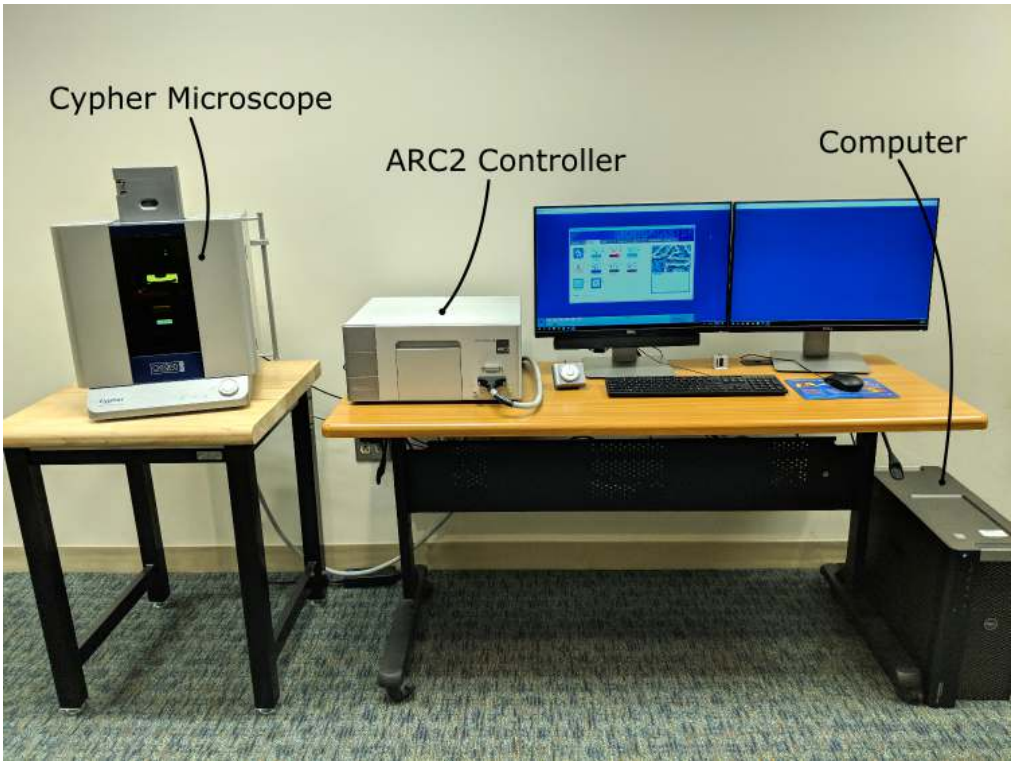
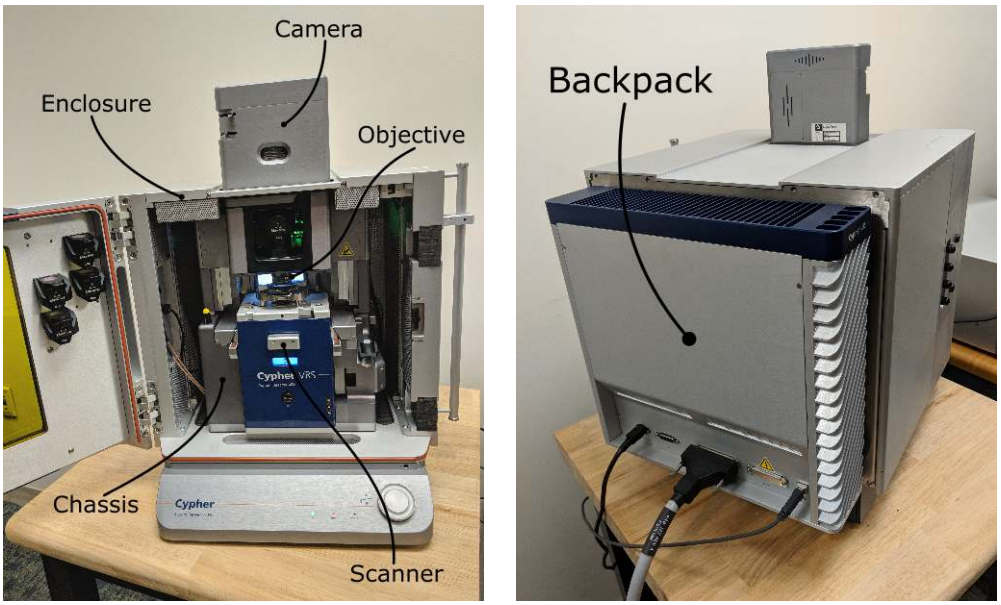


Figure 2.1.: Ideally, the Cypher SPM is set up as shown, with the controller and computer on one table, and the microscope on its own table. The air temperature controller (ATC) is not shown here. Please see Chapter 36 on page 418 for more information.

chassis and is permanently attached to the **Chassis**. The **Head** is responsible for the detection of the cantilever deflection and has integrated motors that allow the user to automatically position the laser spot onto the cantilever. The **objective lens** attached to the head has two important functions: it focuses the laser light onto the cantilever and works with the **camera** to create an optical view of the sample. To learn more about the chassis and its options, please refer to Part V on page 389.

Camera The **camera** (also called the "**view module**") is a user-changeable module that provides a top-down optical view of the cantilever and sample. It is comprised of a tube lens, Koehler illumination with an LED source, and a digital camera. The camera module uses the objective lens in the head to create the optical view. The standard camera module has a bright field reflected light topology and has a 690µm by 920µm field of view with sub-micron resolution. Depending on the application, the view module can be swapped by the user in about 10 minutes but requires Allen wrenches to complete.

Scanner The primary function of the **Scanner** is to move the sample relative to the cantilever during imaging and other measurements such as force curves. There are various scanner modules which excel at various tasks. The Cypher scanners are "sample scanners", which means that relative to the room that the microscope is sitting in, the cantilever is stationary, and the sample moves. The scanner is a modular unit that can be interchanged by the user, depending on the application. The scanner modules are based on a flexure design that uses piezoelectric stacks to move the sample up to 30µm in XY and 5µm in Z. The secondary function of the scanner is to provide motorized course positioning of the cantilever relative to the sample in the Z-axis. The **cantilever holder** is a component of the scanner that physically holds the cantilever during imaging. There are different cantilever holders for air and liquid operations, and there are also application specific holders for techniques like scanning tunneling microscopy (STM), see Chapter 13 on page 132. Each scanner type has its own family of cantilever holders and other accessories. The available scanner modules are:



(a) Front View. In this image the enclosure door is open, and the scanner is partially pulled out.

(b) Rear View

Figure 2.2.: Cypher parts basic nomenclature

- The **Standard** scanner, described in Part II on page 37.
- The **Environmental** scanner, described Part III on page 195.
- The **Video-Rate** scanner, described Part IV on page 337

Backpack The **Backpack** is located on the backside of the **Enclosure**. It houses a very powerful set of digital and analog electronics that greatly extend the functionality of the **ARC2** controller. Just like the **ARC2**, the **Backpack** has ADCs, DACs, BNC connections, and a CrossPoint switch. To learn more about the **Backpack** and its functions, please refer to Chapter 4 on page 29. .

Q Why is there both a Backpack and a Controller? Isn't the Backpack redundant since there is already a controller?

A In a typical AFM design, most of the electronics housed in the Backpack would be located in the Controller. The Backpack, however, moves these electronics closer to the microscope; Cypher is able to achieve such low noise levels in part because of the proximity between some of its electronics and the actual microscope. Keeping these low noise electronics external to the Enclosure balances noise performance with the management of the heat generated by electronics.

Q AFM or SPM? What is the difference?

A AFM stands for Atomic Force Microscope. It scans a cantilever over a sample to generate an image. SPM stands for Scanning Probe Microscope. It is the more general, all-encompassing term, which also includes techniques that image using non-cantilever probes, such as sharp metal needles (Scanning Tunneling Microscopy), optical fibers (NSOM), or tiny hollow glass tubes (SICM). Since Cypher is capable of both AFM and STM, it is classified as an SPM. You may see Cypher referred to in the context of an AFM when its AFM-like functions are being described.

2.2. Parts List

This list includes the contents of the accessory kit which accompanies Cypher. Asylum Inventory Number 900.110.1. These parts accompany the AFM irrespective of the type of scanners you purchased.

Itm	Part #	Item Description	Qty	Picture
1	080.122	15mm AFM Specimen Disc. Also available from Ted Pella, part number 16218.	50	
2	290.101	2A Tweezer, SA Tapered Round Blunt, Standard Grade.	1	
3	290.102	7 Tweezer, SA Curves Sharp, Standard Grade.	2	
4	290.103	3A Tweezer, Extra Fine Sharp, Standard Grade.	1	
5	290.139	Hex Driver, 1/16” Small Handle.	2	
6	312.003	Renishaw Encoder Readhead Spacer (0.8mm).	1	
7	803. OLY. AC 55 TS	Olympus Cantilevers, Model AC 55 TS.	5	
8	803. OLY. BL- AC 40 TS	Olympus Biolevers (Mini): Model BL - AC40TS.	10	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
9	804. NW. ARROW - UHF AUD	Nanoworld Cantilevers, Model: ARROW UHFAuD	5	
10	900.237	AR calibration Grating - Steel Puck Mounted.	1	
11	1-72 x 3/16" SHCS SS	1-72 x 3/16" screw, spares. Fastens the cantilever holder onto the standard scanner (Step 6 on page 44) and also fits the cantilever holder changing stations.	5	
The scale in the photos is in cm and mm.				

2.3. The Igor Pro Software Environment

The Asylum Research software is primarily written within the programming environment of the commercially available software package Igor Pro, which is developed by WaveMetrics. Igor Pro itself has nothing to do with scanning probe microscopes. Rather it is a standalone program that has extensive scientific graphing, data analysis, image processing, and macro programming capabilities.

Tip The "Volume I - Getting Started" manual found on the WaveMetrics website (www.wavemetrics.com) takes two to three hours to complete and is an excellent way to learn about the basic graphing and analysis functionality of Igor Pro. Although it is not necessary to complete the Igor Pro portion of the "Getting Started" manual at this time, it is a highly recommended part of all new user training.

When you launch the software, you open an Igor Pro "Experiment" in which extra software specific to the operation of the AFM has been loaded. An Igor pro experiment is the file that saves the state of Igor Pro.

Refer to the screen shot in Figure 2.3 on page 21 as we introduce the various controls and data displays. We'll go clockwise from the upper left. Note that if you are viewing this file on a computer, you can zoom in on the screen shot for further scrutiny.

Master Panel Upper left-hand window (Ctrl + 5). It has five tabs with controls and data displays for:

Image AFM imaging, see Chapter 7 on page 42.

Thermal Cantilever thermal spectroscopy.

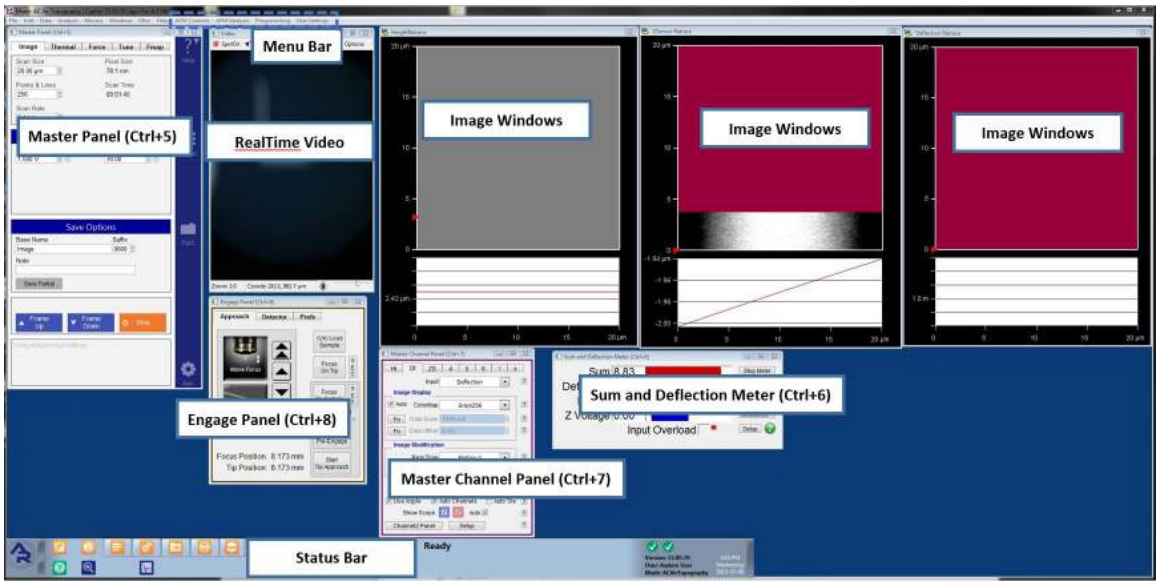


Figure 2.3.: Typical start-up screen for the Asylum Research SPM Software. A few image panels have been cut off in this figure and can usually be found across the second monitor of your system.

Force Cantilever force versus distance curves.

Tune Cantilever vibrational tuning, see Section 7.3.2 on page 57.

Fmap Maps of force versus distance curves.

Master Channel Panel (Ctrl + 7) During imaging, multiple data streams, such as height, cantilever amplitude, and phase, return from the AFM to the computer. This panel contains information about those data streams and allows for some real-time scaling and processing.

Igor Command Window (Ctrl + J) Not shown. The Igor Command window has two parts: the history, and the command line. On occasion, items executed by clicking software buttons generate output and print it to the history. It is not a bad idea to keep tabs on what is being printed into the history, especially if you are tracking down software bugs. Advanced users will use the command line to directly execute a variety of tasks. If you followed the Igor tutorial recommended in the Tip on page 20, you know how to use the command line. The Igor Command Window always has the name of your current experiment, which you can also see at the top of the border of the software window.

Sum and Deflection Meter (Ctrl + 6) This is a real-time display of various channels such as cantilever sum and deflection, piezo voltage, amplitude, and phase.

Engage Panel (Ctrl + 8) This panel controls the entire process of the cantilever approach to the sample. Its three tabs control:

Approach Motorized approach of cantilever and microscope objective toward the sample.

Detector Centering of reflected light beam (laser or SLD) onto the optical detector.

Prefs Preferences for the engage process, such as approach speed and approach step size.

Real Time Image Display This is an example of an image window, in this case displaying the individual lines of the sample topography as the cantilever moves left to right over the sample. There is usually one such window per active tab in the 'Master Channel Panel' (Lower left window). The amplitude and phase data windows are to the right of this clipped screen shot. While this panel is primarily a data display, right-clicking with the mouse can activate various commands such as 'Zoom' and 'Translate'. The white area at the bottom of this window shows a real-time oscilloscope view of the most recent line of image data.

Scope Graph This oscilloscope view shows a graph of the most current scan line. Both trace and retrace can be selected on the 'Master Channel Panel'.

Q Oops! I accidentally closed one of the control panel windows. How do I get it back?

A You can reactivate the panels via *AFM Controls* in the top menu bar.

A few other items of note are:

Menu Bar Along the top of the screen. There are many more controls which can be invoked by items in the menu bar. Menu items to the left are typically standard Igor Pro items, with some Asylum Research functionality. Items to the right of "help" are exclusively SPM-related. In particular, the *AFM Controls* menu item is a complete list of all real-time controls, and the *AFM Analysis* menu item is a complete list of all offline controls.

Status Bar Along the bottom of the screen. Icon controls relate to the status of connected instrument components. The low-level software version is also displayed.

We won't dwell on the purpose of all of these controls but will proceed with the general process of imaging a sample. This will necessarily cover the most pertinent software controls.

Tip Note that nearly each individual item in the software control panels has a small question mark button next to it. You can click this button to read the relevant parts of the software help file.

2.4. Preparing Coarse Samples

When preparing samples for use on the Cypher family of AFM systems, it is highly recommended to mount the sample (using epoxy or silver paste) on a steel puck for handling, installation, and storage. When installing the sample for an experiment, the puck is placed on the sample stage and is then held down magnetically by a built-in magnet in the stage.

The four direction arrows on the Video panel can be used to move the sample to a region of interest. The moving speed can be changed by adjusting the 'Strength' on the *Engage Panel > Prefs* tab, as shown in the figure below.

Coarse sample positioning is accomplished using stick-slip motion by applying a waveform to the X or Y piezo to move the sample in one direction.

To move the sample in the +Y direction:

1. First, the Y Piezo moves in the +Y direction with a small acceleration. The puck also moves in the +Y direction driven by static friction. This is the "Stick".
2. Then the Y Piezo stops with a large reverse acceleration and retracts in the -Y direction. The static friction is not enough to hold the puck as the stage moves in the -Y direction, and thus the puck slips on the sample stage. This is the "Slip".
3. This process is repeated continuously to translate the sample puck across the stage. Here is a link to a PI animation for better understanding of this phenomenon: https://www.youtube.com/embed/zVNYxsudul0?rel=0

Tip

Sometimes you may find that it is difficult to move the sample puck. Here are a few troubleshooting steps that should correct the problem:

1. The most common reason is that there is some contamination on the back of the sample puck or on the surface of the sample stage, which leads to strong adhesion and no slipping. For example, sometimes we stick the puck on double-sided tape, and after removing it, there is actually some residual glue on the back. The solution is to carefully wipe the back of the puck and the surface of the sample stage with a small amount of alcohol.
2. For the Cypher ES, if the sample stage is replaced (for example removing the ambient stage and installing the heating stage), make sure that the installed stage is fixed securely, and the screws tightened (finger tight is sufficient).
3. If the sample is not glued to a puck, and is placed directly on the sample stage, the sample may not stick easily and cannot be moved stably due to low friction. Under these conditions, it is recommended NOT to use too large a Strength value to move the sample. A smaller Strength (such as 1~2) may work better.
4. If you have cleaned the bottom side of the puck and the sample stage and the sample still will not move, you can increase the "Sample Motion Strength" parameter in the Video Panel "PREFS" window. The default value is 5, but if your sample doesn't move, you can increase this value until it does. We recommend increasing the strength value in small increments (+2) until you can see the sample moving in the video panel. If you increase to the maximum value (30) and the sample is still not moving, please revert to the default "strength" parameter of 5 and then install a standard sample (such as the calibration grating) and see if it moves correctly. If not, please contact support@AsylumResearch.com.

3. ARC2 SPM Controller

CHAPTER REV. 1970, DATED 10/06/2017, 17:13. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

Figure 3.1.: The ARC2 Controller with Hamster extended and BNC connector hatch open.

3.1. Parts List

The following table lists all the parts in your accessory kit. It is useful as a visual table of contents and may include links directing you to the specific uses of each part. When ordering parts, please refer to the part numbers in the second column.

Itm	Part #	Item Description	Qty	Picture
1	249.012	Laser Relay Plug. Laser Remote Safety Plug - 2.1 MM. Must be plugged into the back of the controller or the AFM's laser(s) will not work. Contact customer support to wire this plug to a laboratory laser safety interlock.	1	
2	657.001	Laser Relay Key. Key Switch w/Keys. Used to turn on the AFM's laser(s) before it can work..	1	

The scale in the photos is in cm and mm.

Figure 3.2.: Front view of the ACR2

3.2. Connectors

Back (see 3.3):

- AC line input (100-240V depending on factory configuration, 50-60 HZ, 400W max)
- AC output (150W) connected to the external power supply for the backpack controller
- USB 1.1 to be connected to the computer
- Laser interlock jack (contact Asylum Support on its use)

Front:

- a large connection to the SPM
- a smaller DB25 expansion connection for various Asylum Research accessories, such as the digital interface modules discussed below

3.3. User Input / Output Connections

Various BNC connectors are located behind the door on the front of the ARC controller. See Figure 3.1 on page 24.

The front of the ARC2 controller is equipped with 15 BNC connections (all various analog functions, rated +/- 10V), a 3.5mm audio jack,

3.4. Fuses

The controller is protected by a number of fuses on the main input power feed. If your system is not working properly after a power surge or a similar electrical event in your lab, you may wish to check the fuses. If you see that one or more of the fuses is damaged, replace with a like-rated part. All fuses are "Slow Blow" or "Time Lag" type. Please note the current rating on the fuse itself or on the label on the back of the controller.

To remove the fuse for inspection, use a screwdriver or the edge of a coin to rotate the slot on the inside of the voltage selector 1/4 turn counterclockwise. Please consult the back of your controller to find the specific fuse information. It can vary from instrument to instrument, depending on when it was manufactured.

For newer models of ARC2 controller the voltage selectors are no longer present (voltage is set at the factory but can still be changed by a field service technician) and fuse holders are clearly marked on the back of the controller.

Figure 3.3.: Back view of the ARC2

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3.5. System Diagram

3.6. Low Level Signal Access

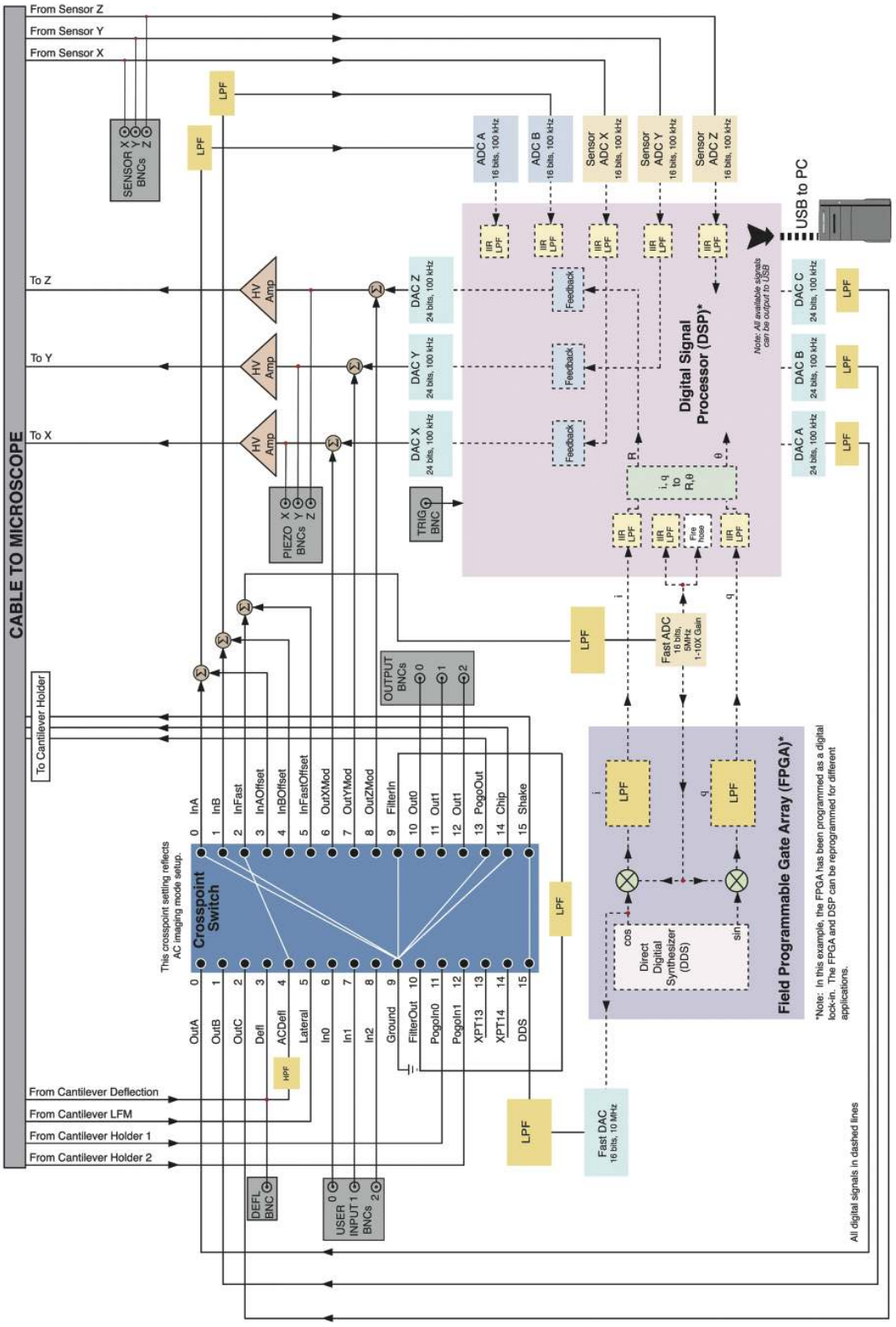


Figure 3.4.: System Diagram including the ARC2 CrossPoint Switch.

(a) Digital Access Module. **(b)** Extended Digital Interface Module.

Figure 3.5.: Options which allow access to low level ARCs signals.

4. Backpack SPM Controller

CHAPTER REV. 1970, DATED 10/06/2017, 17:13. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

Figure 4.1.: A Front view of the Backpack Controller.

Figure 4.2.: Side view of Backpack Controller showing BNC connections.

4.1. Input and Output connections and ratings

4.1.1. User IO

There are three sets of BNC user input/output connections on the Backpack (see Figure 4.2 on page 30):

Attention	Power down the system before connecting or disconnecting BNC cables to prevent unexpected system behavior.

- 5 Analog Input (rated +/-10V),
- 5 Analog Output (rated +/-10V)
- 5 Digital I/O (rated 0-5V)
- One headphone jack for use with standard audio headphones during system troubleshooting.

4.1.2. Rear backpack connections

WARNING!	Power down the system before connecting or disconnecting cables to prevent system damage and electrical shock hazards.

Rear backpack connections include:

- Large cable to the ARC2 controller
- Computer USB 2.0
- Motor power (included with system), the other end of which may plug into an AC wall outlet or the AC output on the ARC2 controller
- Cypher Air Temperature Controller (optional)
- Expansion port (intended for approved Asylum Research accessories only). This port has high voltage (165VDC) behind it and should only be removed by trained service personnel.

4.2. System Diagram

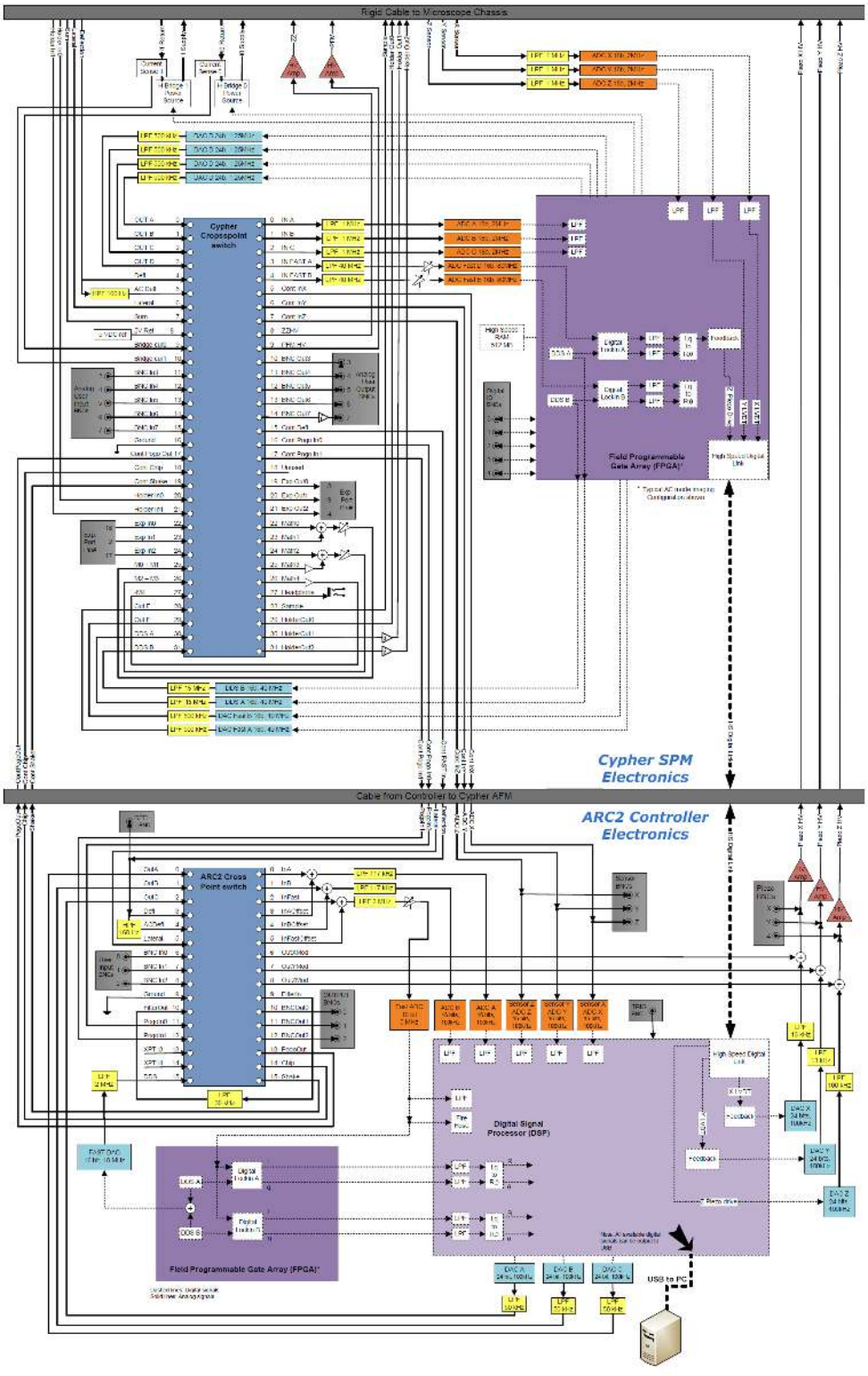


Figure 4.3.: System Diagram including the ARC2 CrossPoint Switch.

4.3. CrossPoint

The left two columns in the **Crosspoint Panel** are for all signals passing through the CrossPoint in the Backpack. The right single-column is for all the signals passing through the CrossPoint in the ARC2.

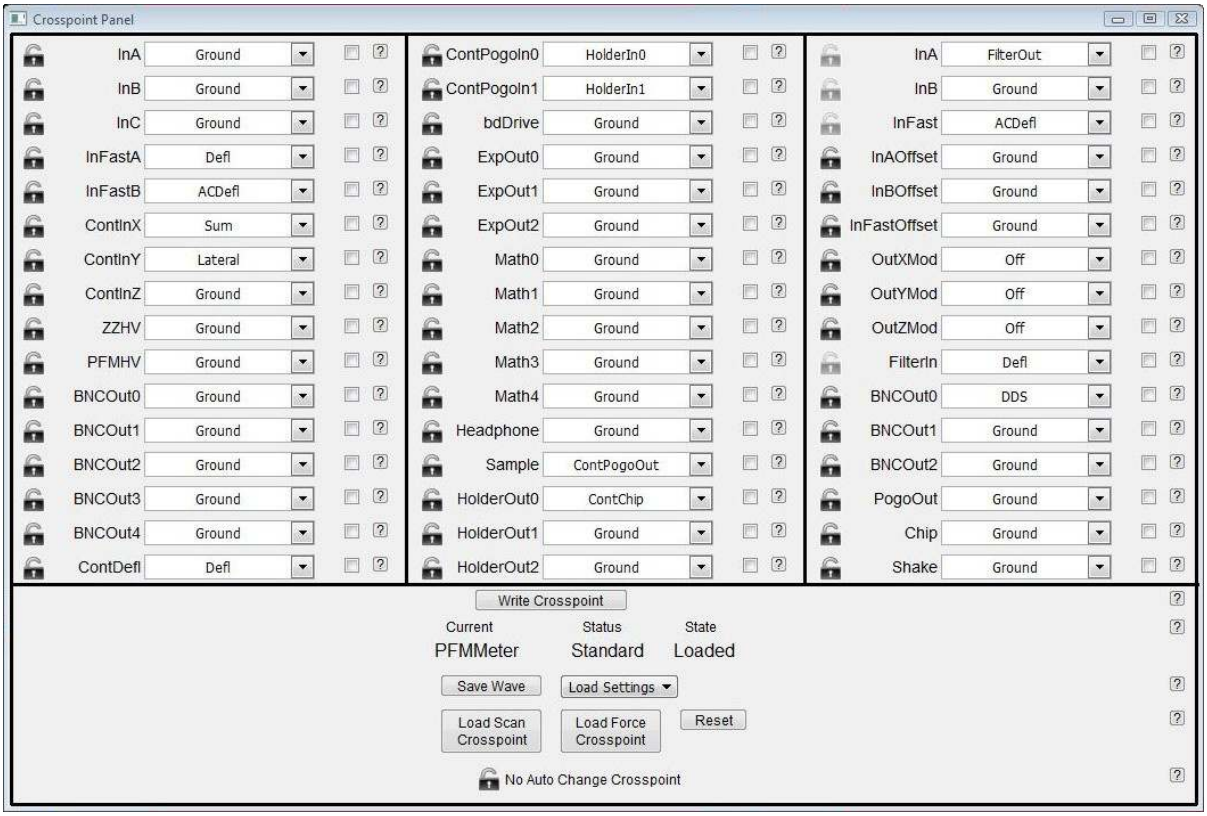


Figure 4.4.: Backpack & ARC2 CrossPoint set up for standard PFM mode.

4.3.1. Decoding the CrossPoint

The CrossPoint and signal routing in general can become a bit more complicated on the system when the Backpack is present. In this section, we "decode" some of the names and aliases you will encounter in the various columns and rows.

Left Column Outputs [Backpack]

InA, InB, InC 18-bit 2 MHz ADCs. Signals on these paths will be accessible by reading "cypher.input.a", "cypher.input.b", and "cypher.input.c", respectively.

InFastA, InFastB, InFastC 16-bit 80 MHz ADCs. Signals are accessible by reading "cypher.infasta" and "cypher.infastb".

ContInX, ContInY, ContInZ Connections to the ARC2 that were originally used for reading the LVDT (sensor) signals. Now the LVDTs are read by the Backpack and these channels can be reused.

ZZHV, PFMHV Connected to the inputs of the optional (non-standard) high voltage ampltifiers in the in Backpack. ZZHV is unipolar (-10V to +150V) [and, so far, unused.] PFMHV is bipolar (±150V) and used for applications like PFM.

BNCOut0, BNCOut1, BNCOut2, BNCOut3, BNCOut4 Output BNCs on the Backpack.

ContDefl Connection to the Deflection input of the ARC2. Signals routed here can be accessed by selecting "Deflection" in the right column, which is the ARC2 CrossPoint. This connection path is served by a coaxial cable in the Main Microscope Cable.

Center Column Outputs [Backpack]

ContPogoIn0, ContPogoIn1Signal Signal paths from the Backpack to the ARC2. Signals routed through here can be accessed by selecting "PogoIn0" or "PogoIn1" in the right column, which is the ARC2 CrossPoint.

bdDrive Drive voltage for controlling blueDrive output. In older versions of the SPM software, this is called "Unused".

ExpOut0, ExpOut1, ExpOut2 Outputs to the labeled Expansion port on the rear of the Backpack.

Math0, Math1, Math2, Math3, Math4 Connections to the "math" circuits.

Headphone Connection to the headphone jack on the Backpack.

Sample Signal path to the top of the Cypher Scanner for sample bias.

HolderOut0 Connection to cantilever holder. Normally used for tip bias. No buffer, lower noise.

HolderOut1 Connection to cantilever holder. Normally used for the shake piezo. Has a 100 mA current buffer.

HolderOut2 Connection to cantilever holder. Has a 100 mA current buffer.

Right Column Outputs [ARC2]

InA, InB 16-bit 100 kHz ADCs. Signals on these paths will be accessible by reading "arc.input.a" and "arc.input.b", respectively.

InFast 16-bit 5 MHz ADC. This channel has an additional LPF and is piped back into the DSP for the digital lock-in. The digital lock-in is used to calculate the Phase and Amplitude signals. This output is also piped into the "Fire Hose" (Thermal), as well as the USB banks. It is accessible by reading "arc.input.fast".

InAOffset, InBOffset, InFastOffset These channel signals are added to the InA, InB, or InFast data lines as, hence, offsets.

OutXMod, OutYMod, OutZMod These channel signals are added to the voltage applied to the X, Y, or Z piezos. Gain was originally set to 15x. After May 2012, gain is 1x for lower noise performance.

FilterIn Input to a 36 kHz LFP.

BNCOut0, BNCOut1, BNCOut2 Output BNCs on the front of the ARC2.

PogoOut Connection from the ARC2 to the Backpack CrossPoint input "ContPogoOut".

Chip Connection from the ARC2 to the Backpack CrossPoint input "ContChip".

Shake Connection from the ARC2 to the Backpack CrossPoint input "ContShake".

Right & Center Column Inputs [Backpack]

Off Completely disconnected; high impedence.

Ground Analog ground (0 Volts); quieter than the "Off" input signal.

OutA, OutB, OutC, OutD 24-bit 1.25 MHz DACs. Write to "cypher.output.a", "cypher.output.b","cypher.output.c", and "cypher.output.d", respectively.

Defl The PD signal of the cantilever vertical deflection.

ACDefl The PD signal of the cantilever vertical deflection passed through a 160 Hz high-pass filter.

Sum The signal from the PD indicating the total amount of the light hitting it.

5VRef A 5 Volt reference signal.

BridgeCur0, BridgeCur1 A voltage signal proportional to the current passing through the 2 H-bridges in the Backpack.

BNCIn0, BNCIn1, BNCIn2, BNCIn3, BNCIn4 Input BNCs on the Backpack.

ContPogoOut Signal connection from ARC2 "PogoOut" signal to the Backpack CrossPoint. "PogoOut" output is in right column.

ContChip Signal connection from ARC2 "Chip" signal to the Backpack CrossPoint. "Chip" output is in right column.

ContShake Signal connection from ARC2 "Shake" signal to the Backpack CrossPoint. "Shake" output is in right column.

HolderIn0, HolderIn1 Signal connections from the Cantilever Holder to the Backpack CrossPoint.

ExpIn0, ExpIn1, ExpIn2 Signal connections from the labeled Expansion port on the rear of the Backpack to the Backpack CrossPoint.

M0+M1 A signal that is the sum of the Math0 (M0) and Math1 (M1) outputs.

M2-M3 A signal that is the difference between the Math 2 (M2) and Math3 (M3) outputs.

-M4 A signal that is inverted of the Math4 (M4) output.

OutE, OutF 16-bit 40 MHz DACs. Write to "cypher.output.e" and "cypher.output.f".

DDSA, DDSB The direct digital synthesizer (DDS) signals on the Backpack. They make sine waves.

Right Column Inputs [ARC2]

Off Completely disconnected; high impedence.

Ground Analog ground (0 Volts); quieter than the "Off" input signal.

InA, InB, InC 24-bit 100 kHz DACs. Write to "arc.output.a", "arc.output.b", and "arc.output.c", respectively.

DefI Signal connection from Backpack CrossPoint output "ContDefl" to ARC2 CrossPoint.

ACDefI Signal connection from Backpack CrossPoint output "ContDefl" to ARC2 CrossPoint through a 160 Hz high-pass filter.

Lateral The PD signal of the cantilever horizontal deflection. Not connected in the Backpack.

BNCIn0, BNCIn1, BNCIn2 Input BNCs on the front of ARC2. Originally these were 10 kΩ input impedance. After Jan 2010, the input impedance is >20 MΩ.

FilterOut Output of the36 kHz LFP. See "FilterIn" signal.

PogoIn0, PogoIn1 Signal connections from the Backpack CrossPoint outputs "ContPogoIn0" and "ContPogoIn1", respectively.

XPT13, XPT14 Currently unused.

DDS The direct digital synthesizer (DDS) signal on the ARC2. It makes sine waves (for AC Mode, Dual-AC, etc).

5. System Power Up

CHAPTER REV. 2422, DATED 08/10/2021, 18:16. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Before you start the following tutorials, the system must be properly powered up. This section walks you through the process.

Before you start: We assume that you understand all aspects of running the Cypher safely (see Chapter 1 on page 3).

1. **Power up:.**

   - Boot up the computer software.
   - Turn on the Cypher SPM by depressing the power switch on the front of the ARC2 controller.

   Note If everything is working correctly, two different green LEDs will be illuminated. The first LED is located above the power switch on the ARC2, and the second LED (labeled “power”) is located at the front of the Cypher enclosure.

2. Double-check that the laser key on the ARC2 controller is in the ON position. The red LED labeled “laser” at the front of the Cypher enclosure should be illuminated. Note that the Igor software needs to be open for the red LED to be illuminated on the front of the Cypher
3. Locate the shortcut to the software on the desktop and double-click the icon to start the software.
4. Wait while the software initializes.

Warning	Make sure your fingers are clear from any pinch points before homing the motors (See Figure 1.2 on page 6). You can abort the homing process at any time by pressing and holding down the ‘Esc’ key located at the top left corner of the keyboard. If you abort the homing process before it is finished, you will not be able operate the motors.

5.

- Once the software has finished initializing, you will get a prompt asking if you would like to home the engage motors.
- If necessary, slide the scanner all the way into the chassis. Close the microscope enclosure door. For safety reasons, the motors cannot home unless the door remains closed during the process.
- Click 'Yes'. You will hear motors moving during the homing process, which takes about 20 seconds.

6. If you are new to the Cypher AFM system, please take the tutorial that is appropriate for your scanner:

- For the Standard scanner see: Chapter 7 on page 42.
- For the Environmental Scanner see: Chapter 18 on page 214.

Part II

Standard Scanner (S)

Who is this part for? After the Cypher S SPM has been installed in your lab, and you (or someone in your facility) have completed the initial training, this part of the user guide will be the principal reference for operating the instrument. Although written with the novice user in mind, experienced SPM users should complete the basic imaging tutorial at least once before attempting to use this instrument.

Part Contents

6. Standard Scanner Overview

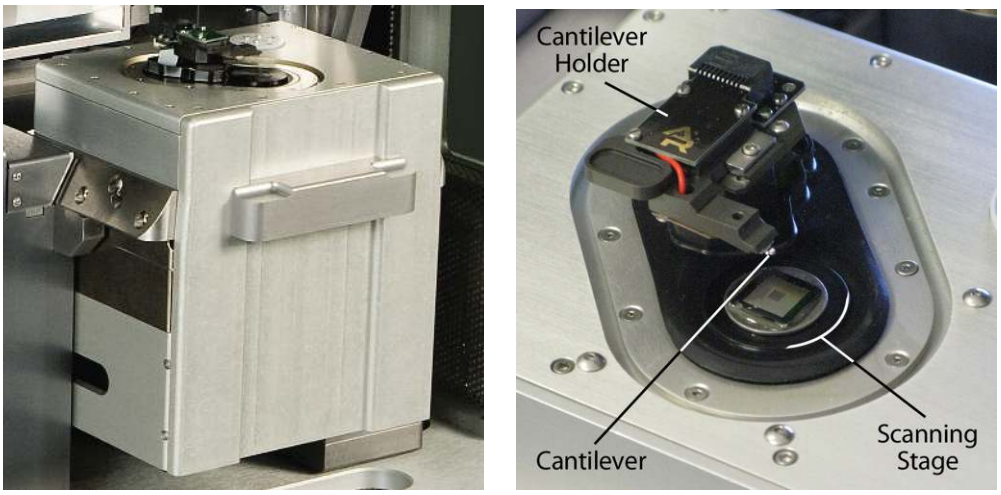
CHAPTER REV. 2342, DATED 04/08/2021, 19:08. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

The module dubbed “the scanner” contains the entire mechanics of the AFM except for the optical means for detecting cantilever deflection. This includes:

- actuators and sensors for closed loop XY scanning of the sample
- an actuator and sensor for the sample Z motion
- a cantilever holder and mechanical means for engaging the cantilever with the sample surface

Since Cypher’s scanners are whole AFMs unto themselves, each comes with its own dedicated collection accessories, such as cantilever holders and sample stages. In other words, a cantilever holder for one scanner usually does not fit onto a different scanner. Also, an expert user of one model of scanner will not necessarily know anything about operating another model.

This part of the user guide describes in many chapters the use of the Standard Scanner and its many accessories. Once the scanner is exchanged for another, as described in Chapter 32 on page 391, a different part of the user guide will need to be consulted. Typically, the first user of a new scanner will need to be trained by Asylum Research personnel.



(a) Standard Scanner. **(b)** Names of the basic components.

Figure 6.1.: The Standard Scanner.

Figure 6.1 on page 40 shows the standard scanner partially withdrawn from the rest of the AFM. The standard scanner is included with the “Cypher S” AFM but can also be purchased separately. The Standard scanner is designed primarily for imaging in ambient conditions, either in air or in a liquid droplet. The optical access to the sample and cantilever is superior to other Cypher scanner models.

Many Standard scanner cantilever holders allow for a variety of imaging modes. See Chapter 8 on page 71 for more information.

The scanner itself comes in regular and high voltage models. Figure 6.2 on page 41 shows the magnetic high-voltage contact and specialized cantilever holder with high-voltage connection to the tip. This arrangement is typically use for PFM techniques. This topic is covered in depth in *Applications Guide, Chapter: PFM Using DART* and *Applications Guide, Chapter: Single Frequency PFM*.

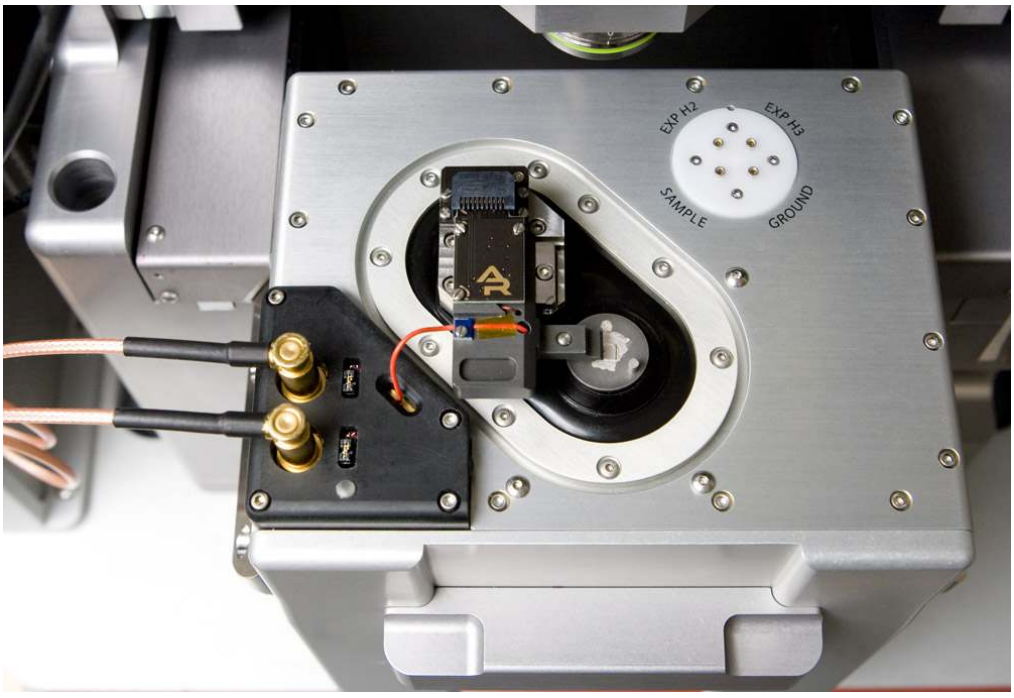


Figure 6.2.: Detailed view of the high-voltage option.

7. Tutorial: AC Mode Imaging in Air with the Standard Scanner

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

This tutorial provides a quick path to learning the basic operation of the Cypher SPM equipped with the Standard scanner. If you own the Environmental scanner, please follow the tutorial in Chapter 18 on page 214. The tutorial contains a set of steps that provides a new user with a basic understanding of AFM operation how to obtain an AC mode topography image in air.

All new users should complete and understand this “AC Mode Imaging in Air” tutorial before attempting any imaging.

The Cypher is a research grade instrument and improper use of the instrument can cause both damage to the instrument and injury to the user. This tutorial takes approximately 3 hours.

Before you start:

- You should understand the aspects of running this system safely: (Chapter 1 on page 3.)
- You should be familiar with the basic names of the hardware components and software controls (Chapter 2 on page 16).
- You should have powered up the Cypher and launched the software (Chapter 5 on page 35).

7.1. Required Materials

This tutorial is designed to be performed, not merely read. You will learn the most if you operate the instrument yourself, with an experienced user watching and providing advice.

It is necessary to gather a few items prior to beginning the tutorial, including:

1. Cantilevers: You will need an AC160TS cantilever, which is manufactured by Olympus. The AC160TS, with a spring constant of ~42N/m and a resonance frequency of ~300kHz, is a workhorse for AC mode imaging in air. Every Cypher ships with a package of AC160s, but if these cantilevers are unavailable, any cantilever with a similar spring constant and resonance frequency should work fine.
2. Sample: The tutorial will use the Asylum Research calibration grating that ships with every system (Asylum Part# 290.237).
3. Tweezers: It is preferable to use tweezers with curved tips (for example, Asylum Part# 290.102).
4. Wrench: A 1/16" ball head wrench (for example, Asylum Part# 290.139) is required.
5. SPM: This tutorial is designed for a Cypher equipped with the Standard scanner and a large spot SLD or Laser Module (See Chapter 34 on page 398).

7.2. Loading the Cantilever and Sample

This section covers sample and cantilever loading, as well as the course approach of the cantilever tip toward the sample.

1. **Raise the cantilever holder:**

   - Rotate the 'Engage Control Knob' on the Cypher *clockwise* and hold it until the cantilever holder is far from the sample or is at its upper limit of travel.

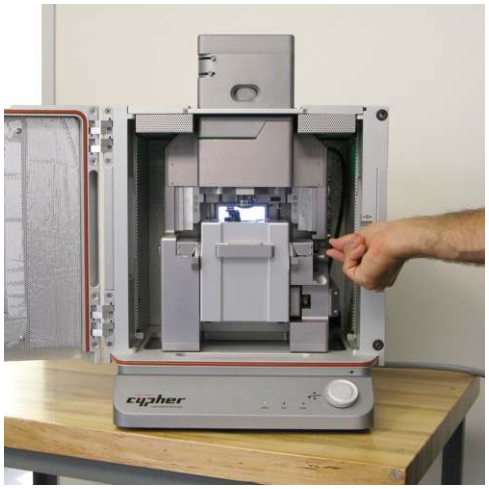
   Note Although it is not required, for safety reasons we recommend making motor moves with the door closed. Beware of pinch points (Figure 1.2 on page 6).

2. **Open enclosure:**

   - Lift the door latch and open the enclosure door.

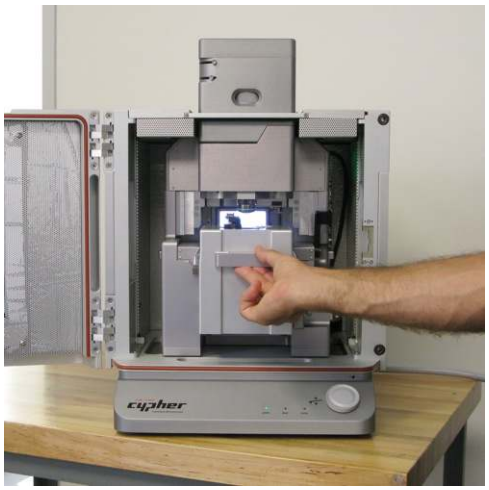
3. **Unlock scanner:**

   - Lift the lever to the right of the scanner.



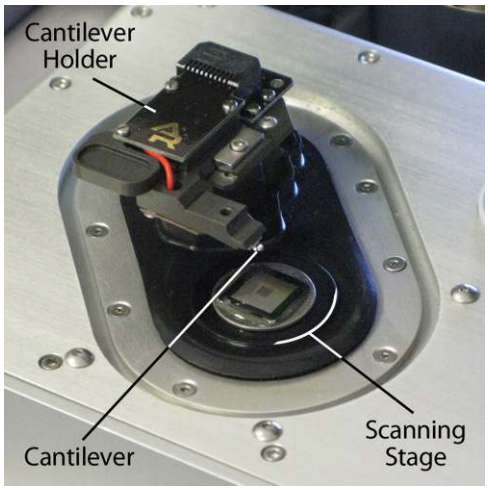
4. **Pull the scanner out:**

   - Pull the scanner forward gently and stop when it is about halfway out. If you continue pulling the scanner, at some point you will feel resistance and should pull no further.



5. **Familiarize yourself with the sample area:**

   - While it may look solid, the scanner stage moves the sample in the X, Y, and Z directions imperceptibly up to 40μm.

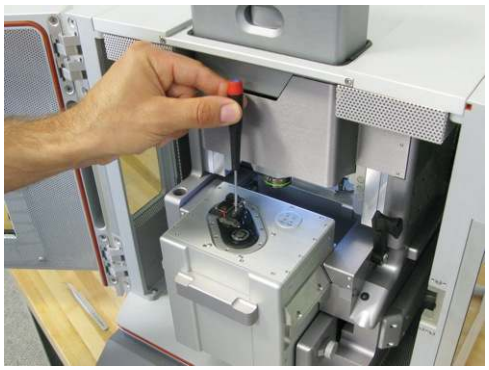


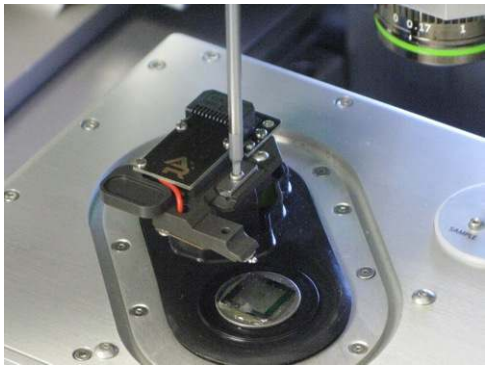
6.

Release the cantilever holder:

- Loosen the screw clamping the cantilever holder. One turn *counterclockwise* should be enough.
- Replace the tool in its storage place (hole in the chassis to the left of the scanner).

Note The chassis hole is included on all Cyphers, and only the S scanner has the holder locking screw





7.

Remove the cantilever holder:

- Hold by the tab with the circular recess and pull straight out towards you.

8.

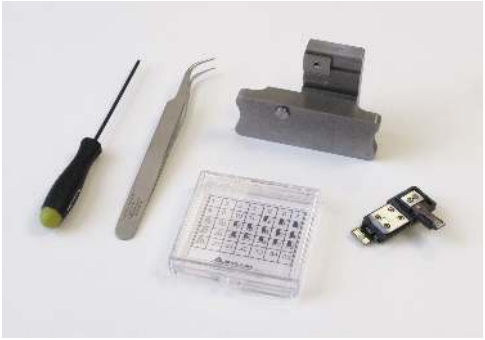
Select AC mode cantilever holder:

- Identify the cantilever holder. This demo requires the standard "AC Air" holder, Asylum Part# 901.705.

Note To learn more about cantilever holders for the standard scanner, please refer to Chapter 8 on page 71.

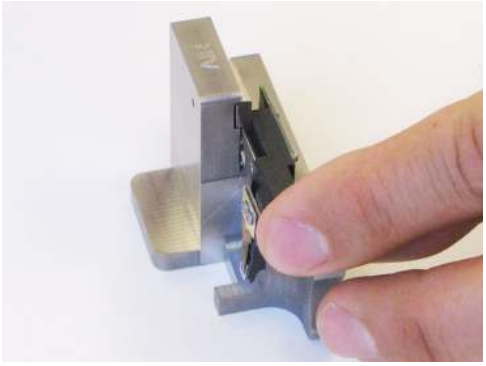
9. **Prepare cantilever mounting workspace:**

- Set out your changing station, tweezers, and cantilevers on a clean, well-lighted surface. Make sure that the changing station is labeled “Air”. (There is also a “Droplet” changing station for the droplet holder.)
- A low-power binocular dissection stereoscope with light source can be useful for some of the following steps.
- Cleaning the tweezer tips with alcohol improves the handling of the cantilevers.



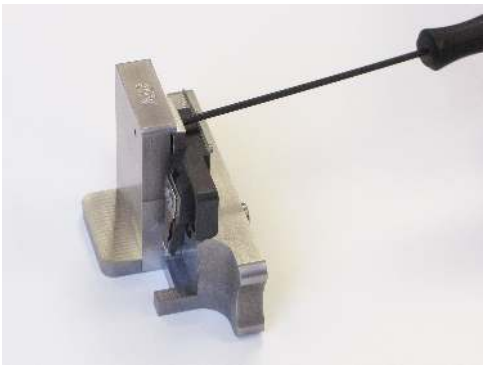
10. **Mount the cantilever holder in the changing station:**

- Carefully insert the cantilever holder as shown. The V-shaped piece of metal on the back of the holder slides into the dovetail joint on the changing station. The cantilever should be pointing down.
- If the cantilever holder does not slide in easily, loosen the screw on the clamping mechanism.



11. **Tighten the clamp:**

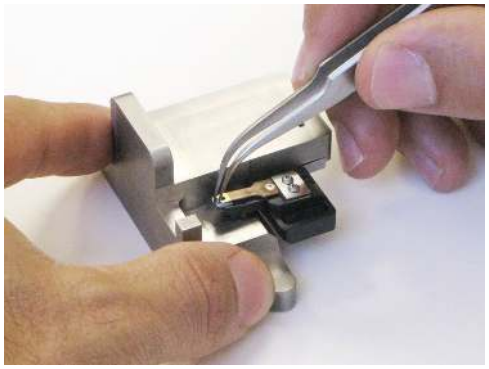
- Once the cantilever holder is fully inserted, use the ball head wrench to gently tighten the clamp.



12.

Remove the old cantilever:

- Position the changing station, as shown, on a flat hard surface.
- Take the tweezers in your dominant hand.
- Press down on the station with your other hand, as shown. This depresses a button on the bottom of the station which drives a pin up under the cantilever retaining clip.
- Remove the cantilever and release pressure on the station.
- Inspect the cantilever area for tiny silicon grit and blow clean with compressed air if necessary.



13.

Select new cantilever:

- Select a new cantilever and pick it up with tweezers.
- Caution: Close the box! Ruining $1k of levers by putting your hand on an open box is not unheard of.

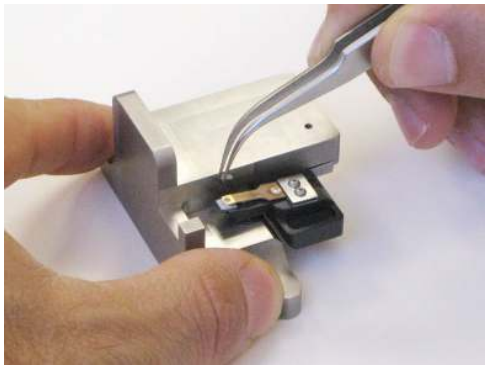
Note If your lab saves some old cantilevers, consider practicing with a “dummy” cantilever.

Tip Some find it useful to first lay the chip down on a non-sticky surface and re-grip it before continuing.

14.

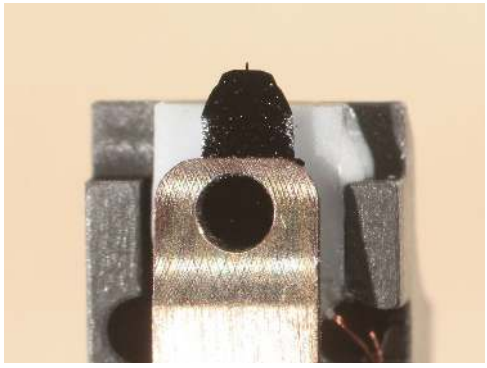
Load new cantilever:

- Place and center the cantilever in the holder (also see photo in next step for alignment).
- A good technique is to release pressure on the changing station while still gripping the cantilever chip with tweezers. This prevents misalignment caused by the cantilever chip sticking to the tweezers.



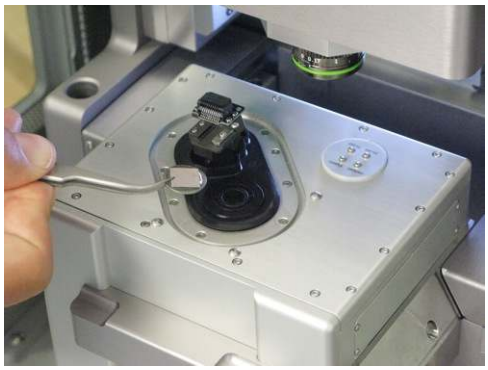
15. **Check cantilever alignment:**

- A properly aligned cantilever seen from above is shown at right.
- It helps to do this at least once under a binocular stereo microscope.



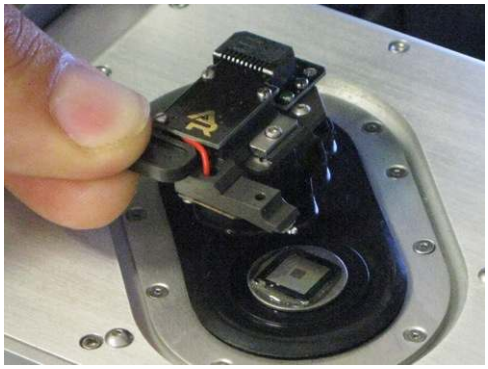
16. **Prepare scanner and load sample:**

- Leave the cantilever holder in the changing station for now.
- Remove any sample that may be present on the scanner.
- Wipe the scanner stage (defined in Step 5 on page 44) clean with a soft cloth. Any dust or grit will prevent the sample disk from being properly seated.
- Place the Asylum Research calibration grating onto the scanner stage. It will attach magnetically.



17. **Insert cantilever holder into scanner:**

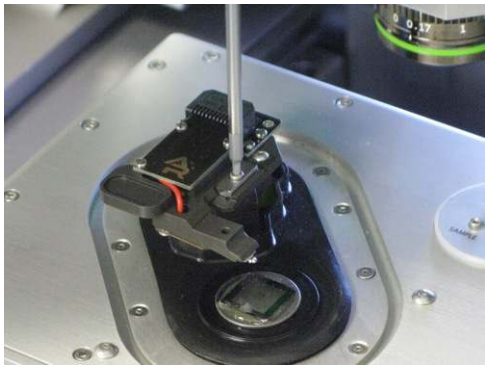
- Remove the cantilever holder from the changing station.
- Insert the cantilever holder into the scanner. Pay attention that the metal dovetail engages properly.
- If it will not go in, loosen the screw by half a turn (see Step 6 on page 44).

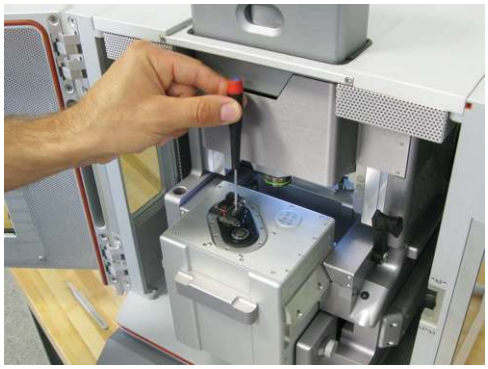


18.

Tighten cantilever holder:

- Use the ball headed wrench to *gently tighten* the screw that clamps the cantilever holder.
- Caution: Do not use your whole hand! Be gentle!

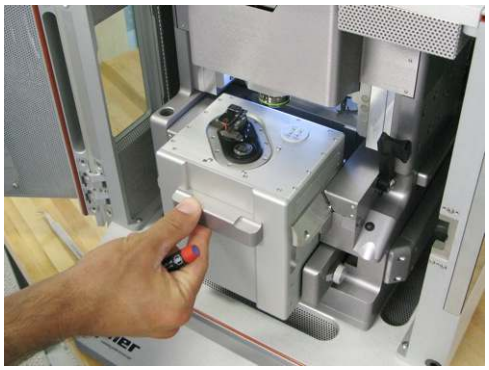




19.

Slide scanner into chassis, lock down:

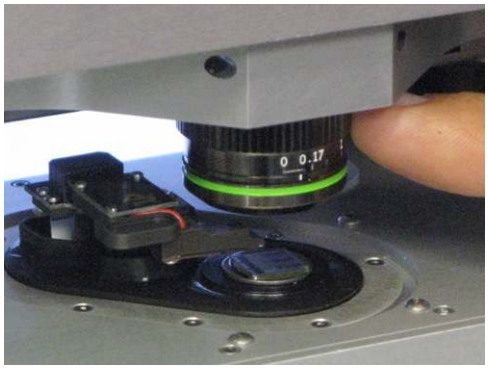
- Gently slide the scanner back into the chassis.
- Push the lever at the right of the scanner downward to secure the scanner in place.



20.

Check correction collar:

- Check that the green correction collar on the objective is set to zero (this cantilever holder has no glass window through which the light must focus).

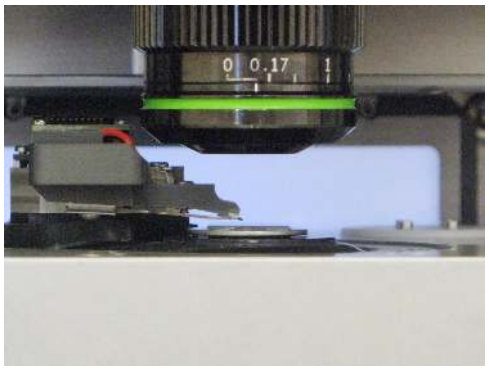


21. **Close enclosure door:**

- *Gently* close the door and latch it.

22. **Motor cantilever toward sample:**

- Place your eyes level with the cantilever and sample so that you can clearly see the gap between cantilever and sample.
- Slowly turn the 'Engage Control Knob' on the AFM enclosure *counterclockwise*. This will lower the cantilever holder and objective toward the sample. The more you turn, the faster it goes.
- Close the gap between tip and sample to about 1 millimeter. There is no harm in playing it safe and stopping a little farther away. It will only cause the automated engage to take a little longer.



Warning: Nothing but your attentiveness will prevent the cantilever holder from crashing into the sample. If you crash the cantilever holder you may cause *SERIOUS* damage to your cantilever holder and scanner.

23. This concludes the manual interaction with Cypher. We next turn our attention to the computer.

7.3. Engaging the Cantilever on the Sample

7.3.1. Bringing the Cantilever Close to the Sample

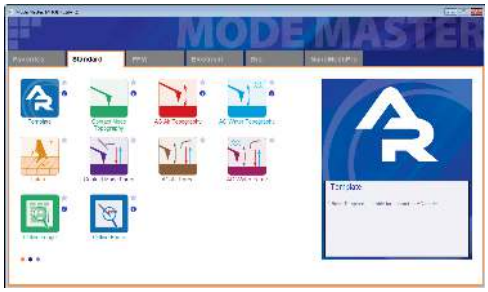
Before you start this section, you should have done the following:

- started up the software (Chapter 5 on page 35)
- homed the motors (See Step 5 on page 35)
- positioned the cantilever about 1mm above the sample (Section 7.2 on page 43)

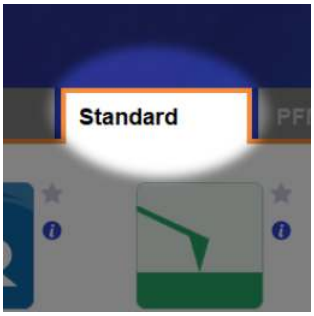
1.

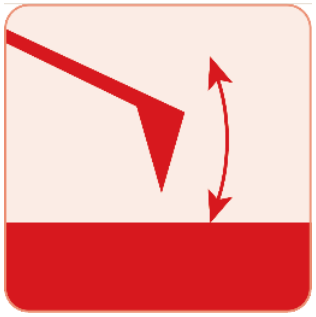
The Mode Master window:

- The software should now be showing the Mode Master window.
- If not, click the Mode Master button at the bottom of the screen: .



2.





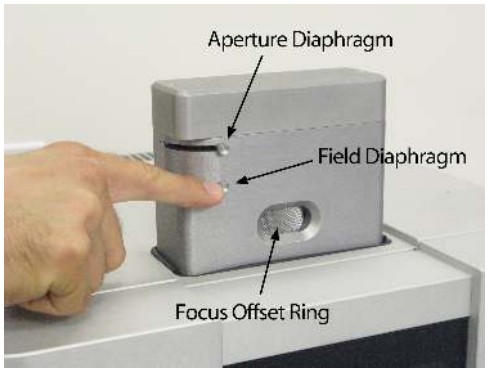
Select Mode:

- Select *Standard tab > AC Air Topography*
- The screen will now rearrange and present all the controls necessary for this type of AFM imaging.

3.

Prepare the view module:

- Turn the **focus offset ring** until the white dot is centered on the alignment mark. There will be a gentle click when this occurs.
- Fully open the **field and aperture diaphragms** by pushing both levers toward the front as shown.



4.

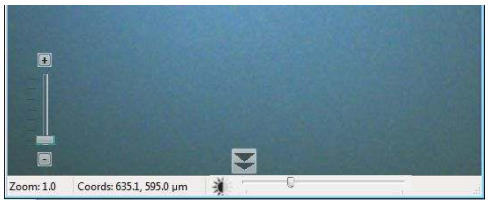
Open video window (if necessary):

- In the software, click on the camera icon on the bottom status bar.
- This will open the Video window (or highlight it in case it was already open), which displays an optical view of the cantilever and sample.

5.

Setting video zoom and illumination:

- IMPORTANT: Slide the vertical slider (at the lower-left corner of the video window) all the way to the bottom. "Zoom 1.0" will be indicated just below.
- Turn up the illumination by moving the slider (at the bottom of the video window) to the right a quarter or third of its full range.



6. Familiarize yourself with the Approach tab on the Engage Panel as described next in Step 7 on page 52. Caution: *Failure to understand the Approach controls may lead to serious damage to the Cypher*.

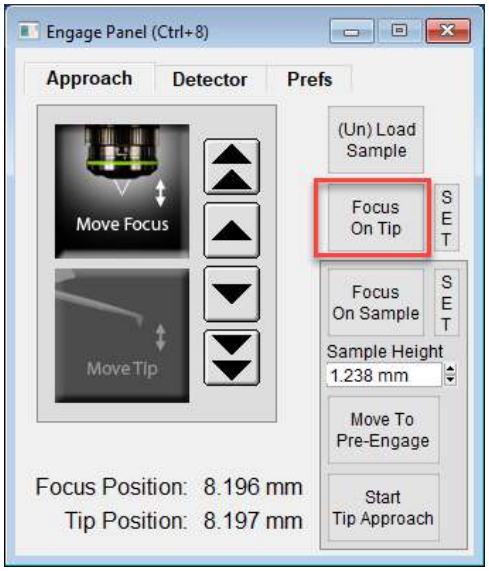
7.

Go to last known Tip Focus:

- On the Engage panel, hit 'Focus on Tip'. This will move the objective lens to the last known position where the cantilever was in focus.

Notes

- Since the 'Focus on Tip' button only moves the objective to the *last known tip focus* and does not actually perform an auto-focus, the cantilever will most likely not be perfectly in focus after the motors are finished moving. (Cantilever chips have varying thicknesses and how the cantilever chip gets positioned in the holder affects the sample position.)
- Do not be alarmed if the cantilever is not at all visible. It most likely means that when you placed the cantilever chip in the holder, you put it in a place outside the ~1mm field of view of the objective. This is addressed in the next step.
- If you hit the 'Focus on Tip' button and nothing happens (e.g., the motors do not move), it just means that the objective is already at the tip focus point. Note that after the motors are homed, the objective is moved automatically to the tip focus point.



8.

Locate cantilever in image:

- The goal of this step is just to get the cantilever into the field of view. Use the four arrows at the top left of the Video window to look for the edge of the cantilever chip and/or the cantilever. As mentioned in the previous step, most likely the cantilever will not be perfectly in focus.
- If you are oriented such that you are sitting directly in front of the Cypher microscope, hitting the left arrow will move the objective to your left, while hitting the top arrow will move the objective away from you.
- *If you see nothing at all in the field of view, most likely the cantilever chip is located to the left of the field of view.*
- Click the *left arrow* to move the objective towards the left and look for the cantilever chip edge.

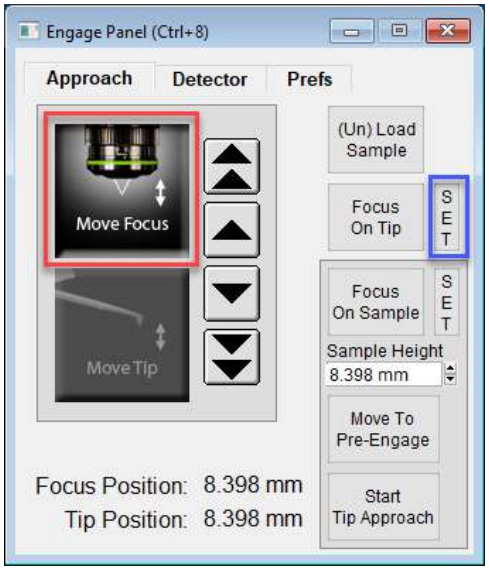
See Figure 7.1 on page 58 for example images.

9.

Set the tip focus position:

- Under the 'Approach' tab on the 'Engage Panel' select the 'Move Focus' large picture button (see red box at right).
- Use the on-screen arrow buttons until cantilever is in focus. Single arrows are slow; double arrows are fast.
- Click 'Set' next to 'Focus on Tip' button (see blue box at right).

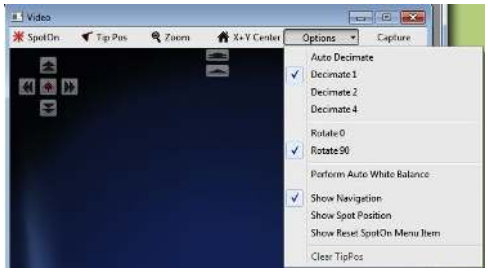
Important The cantilever is at an 11° angle, and the whole lever cannot be in focus at once. Bring the end of the cantilever closest to the tip in focus.



10.

Optional image enhancement and zoom, particularly useful for small cantilevers:

- If you want to see the image with more resolution, select *Decimate 1* from the *Options* pull-down menu. This brings all the pixels down from the video camera but slows the screen update rate.
- To the left of the *Options* menu is a 'Zoom' button. This button, once clicked, will change the cursor into a magnifying glass. Click on the cantilever to get an enlarged view.
- Both of these items may improve your ability to focus from the previous step. If you do refocus, be sure to click 'Set' next to the 'Focus on Tip' button.

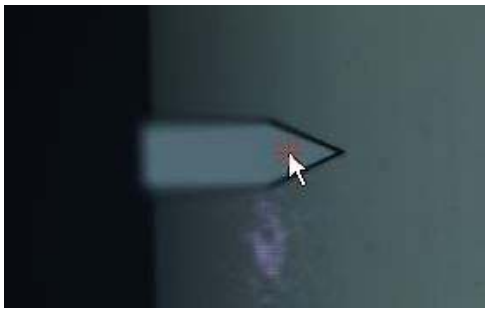


11.

Center laser spot on cantilever:

- Click the 'Spot On' button at the top left of the Video window. The mouse pointer acquires some small red lines.
- Then click the center of the cantilever (see figure at right).

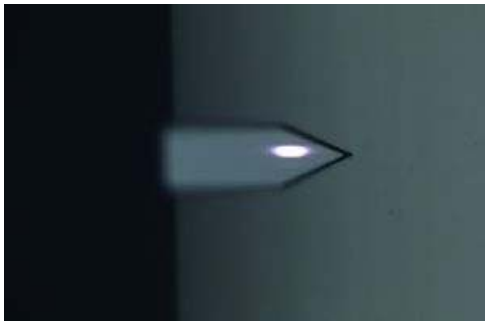
Note Alternately, right-click on the center of the cantilever and then select the 'Spot On' option.



12.

Observe spot on lever:

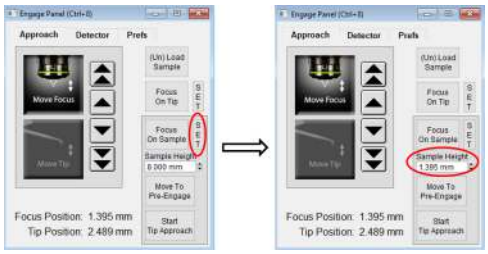
- The motors inside Cypher now move to bring the laser spot where you clicked.
- The spot position does not need to be perfect here, only roughly centered on the cantilever to produce a decent reflected beam (measured by the Sum signal in the Sum and Deflection Panel).
- If needed, the spot position will be fine-tuned in a later step.



13.

Locate sample surface optically:

- Under the 'Approach' tab on the 'Engage Panel' use the down-arrow keys to motor the microscope objective down toward the sample, until it comes in focus (see image in the next step). Use the up-arrows if you overshoot.
- Single arrows are slow, double arrows fast.
- Once in focus, click the 'SET' button next to the 'Focus on Sample' button. Note that the sample height value updates.

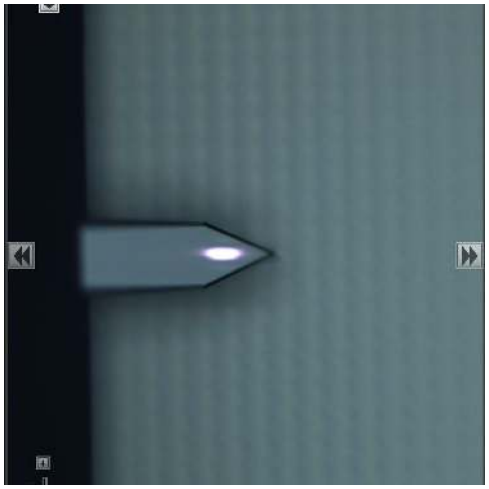


14.

Observe sample surface optically:

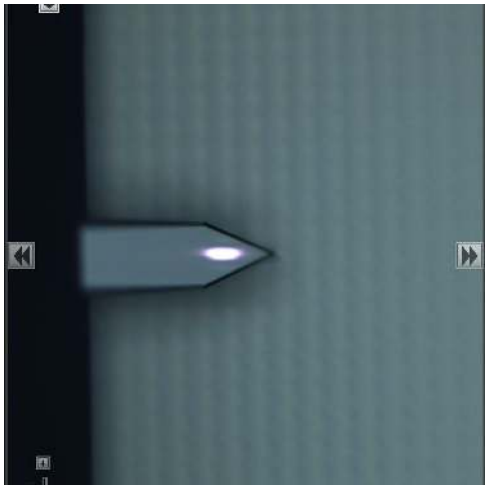
- Features may be hard to see at zoom = 1 and auto decimation turned on. For instance, in the image to the right, decimation = 1 and zoom = 2. See Step 10 on page 53 for more information.

Fun Now that you have stored tip and sample position, you can repeatedly click the buttons 'Focus on Sample' and 'Focus on Tip' to go back and forth between tip and sample.



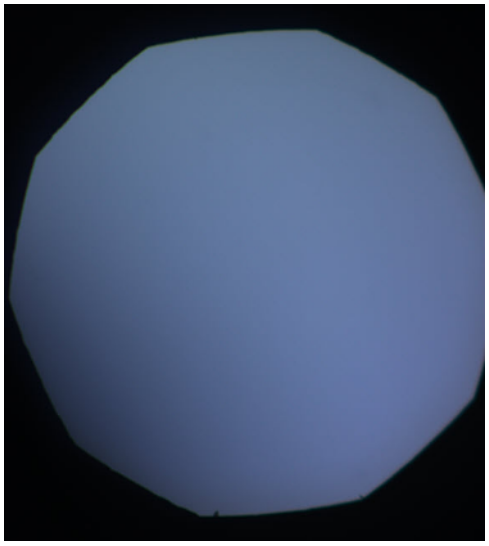
Question I cannot seem to focus on the surface of my rough sample.

Answer The objective can only motor down a few millimeters before it meets up with the cantilever. If you did not manually move the cantilever close enough, you will never get a focused image of the sample (such as the one at right). In this case, click the 'Focus on Tip' button in the Engage panel and repeat Step 22 on page 50.



Question I cannot seem to focus on the surface of my smooth sample.

Answer Perfectly reflecting samples may not offer enough features to allow the focus to be determined. In this case, move the 'Field Diaphragm' lever (marked with an "F") on the view system until you see a dark circle on the screen. As you motor down, this circle becomes sharper. When the ring is in focus, so is the sample.

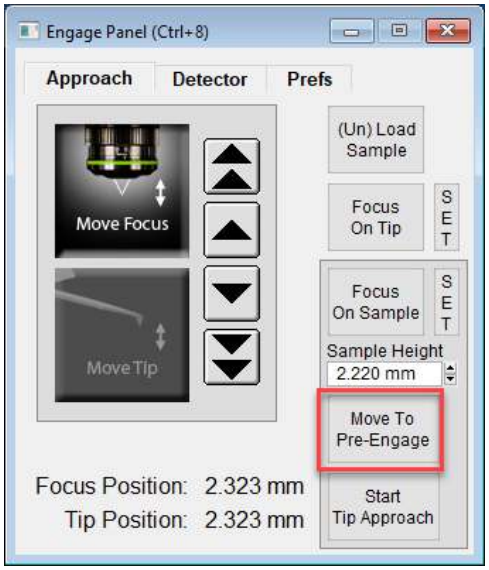


15.

Prepare to land the tip:

- Click the 'Move to Pre-Engage' button. The motors automatically bring the tip to 50µm from the surface.

WARNING If you set a bad sample height and/or tip position, you may ram your cantilever into the sample and break it. A firmware safety feature immediately cuts motor power when the optical detector fails to measure reflected light from the broken lever. This prevents the cantilever holder from ramming the sample.

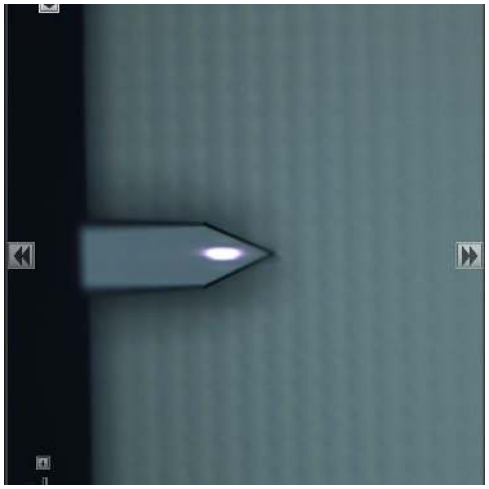


16.

Fine tune spot position:

- At this point, you should adjust the spot position so that it is near the end of the cantilever using the controls in the upper left-hand corner of the Video panel.
- Note that a decrease in Sum Signal indicates that light is spilling off the cantilever. The latter is undesirable and should be avoided.

HINT For fine positioning, hold down the 'SHIFT' key on your keyboard while clicking the onscreen buttons which move the cantilever under the laser spot (See Step 8 on page 52).



17.

Center the laser on the photo detector:

- Click the red button at the center of the Video window motion controls and watch the Deflection Signal go to zero. This action causes small motors to steer the laser beam to the optimum position on Cypher's photo detector.

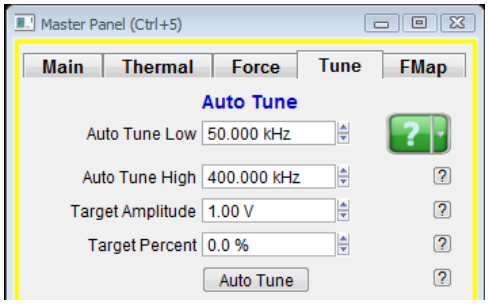
7.3.2. Tuning the Cantilever and Setting Scan Parameters

Since this tutorial focuses on AC imaging, we will proceed to tune the cantilever.

1.

Initiate cantilever tune:

- Select the 'Tune 'tab in the Master Panel.
- Set the four Auto Tune parameters (Low, High, Amplitude, Percent), as shown at right.
- Click the 'Auto Tune' button.

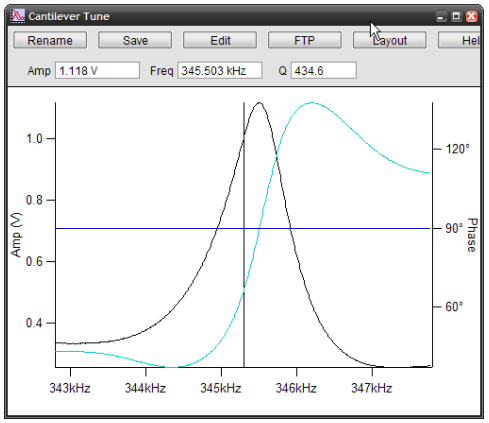


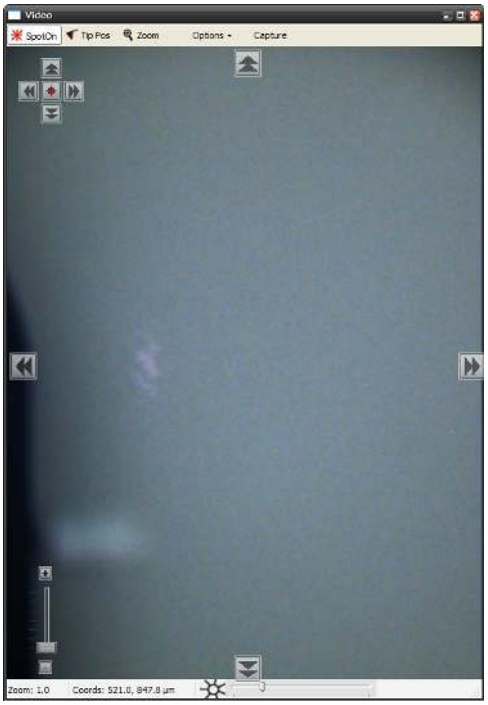
2.

Observe tune result:

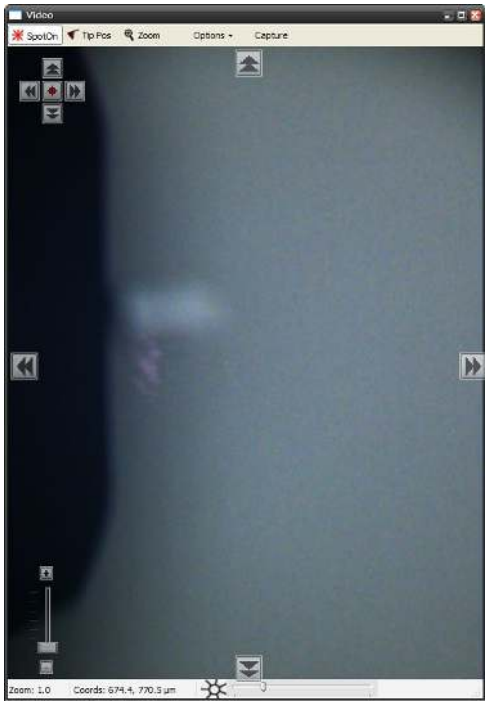
- A graph will pop up with the tune result.
- The resonance curve should peak at around 300kHz.
- The relevant results are automatically stored. After inspecting that the amplitude and phase curves look "clean", you can close the graph.

HINT Cleaner tunes can be obtained by blowing the cantilever holder with clean compressed air prior to loading cantilever to get rid of any leftover silicon/glass debris.

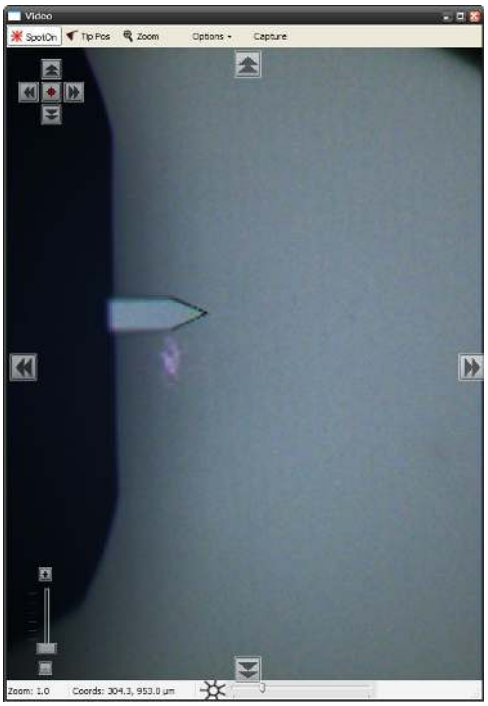




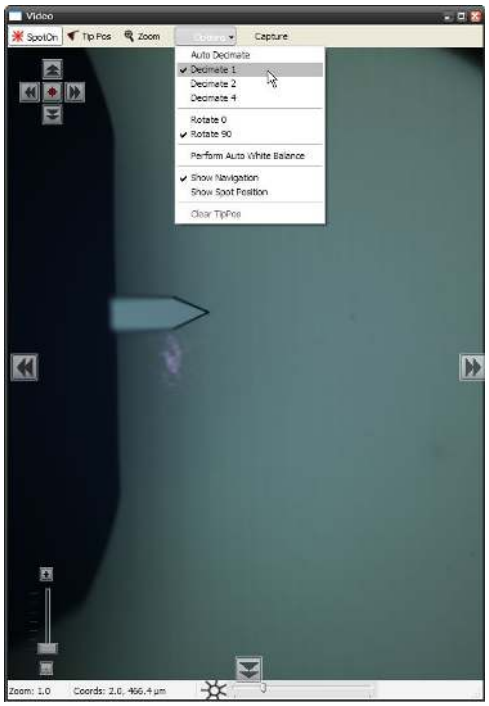
(a) First view of new cantilever.



(b) Cantilever found and roughly centered on screen. Step 8 on page 52.

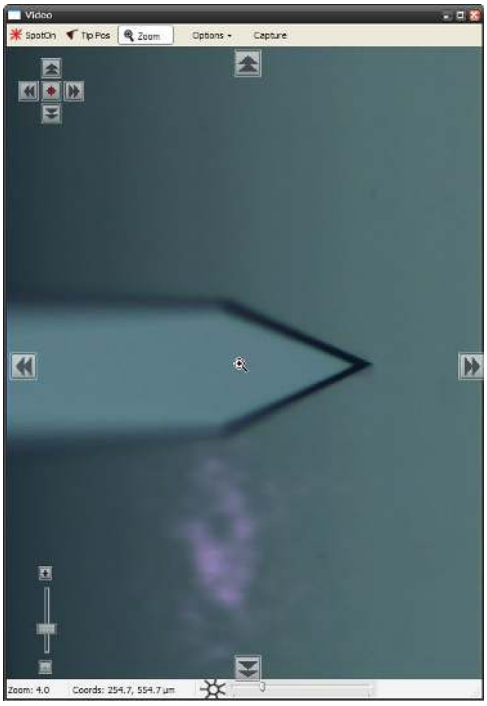


(c) Cantilever in focus. See Step 9 on page 53.

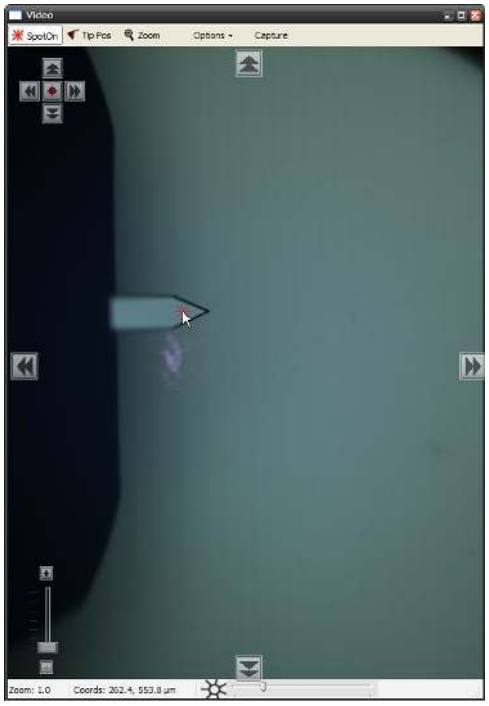


(d) Optimize Resolution. See Step 10 on page 53.

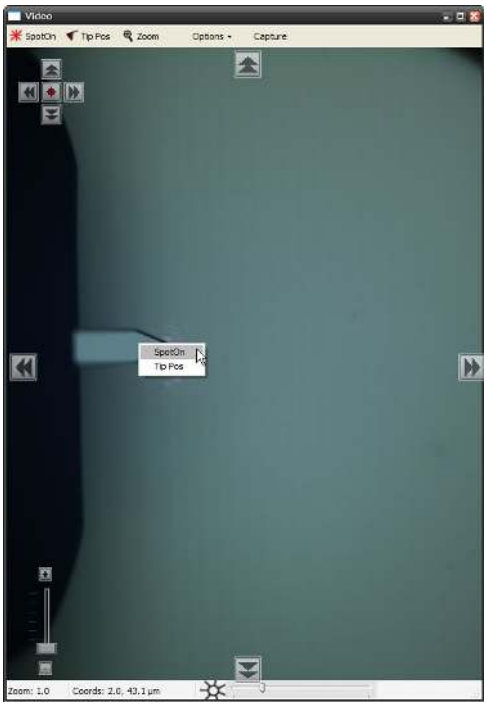
Figure 7.1.: Finding the cantilever and optimizing the video.



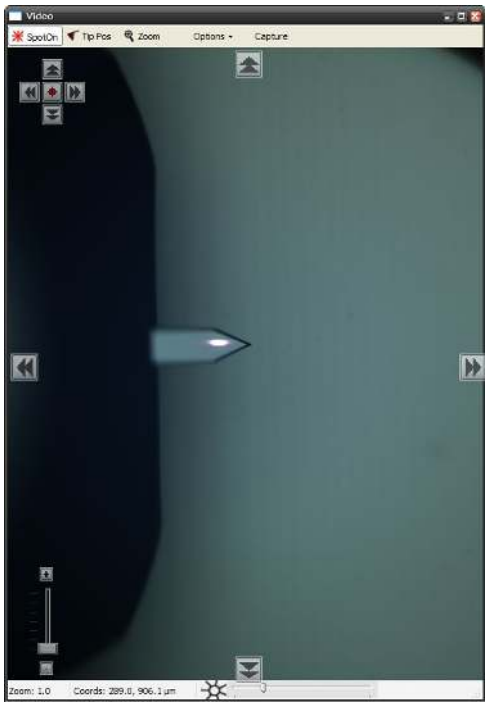
(a) Cantilever Zoom. See Step 10 on page 53.



(b) Laser Spot ON. See Step 11 on page 54.



(c) Spot On by right-clicking. See Step 11 on page 54.



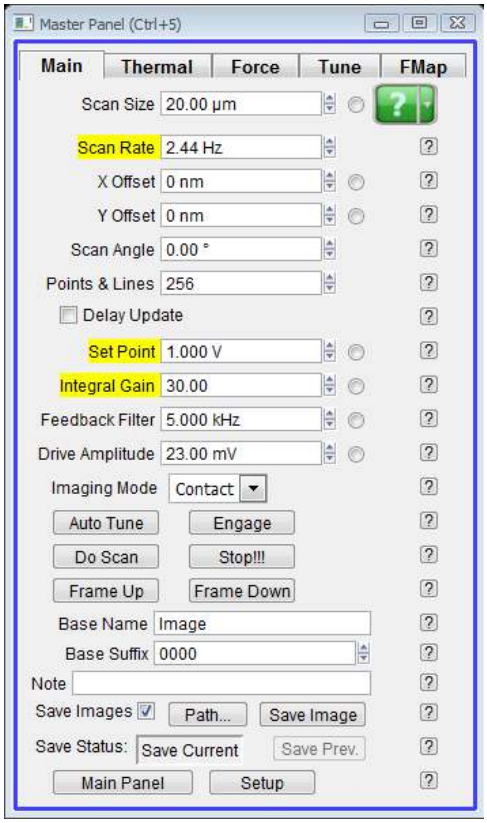
(d) Laser spot on the lever. See Step 12 on page 54.

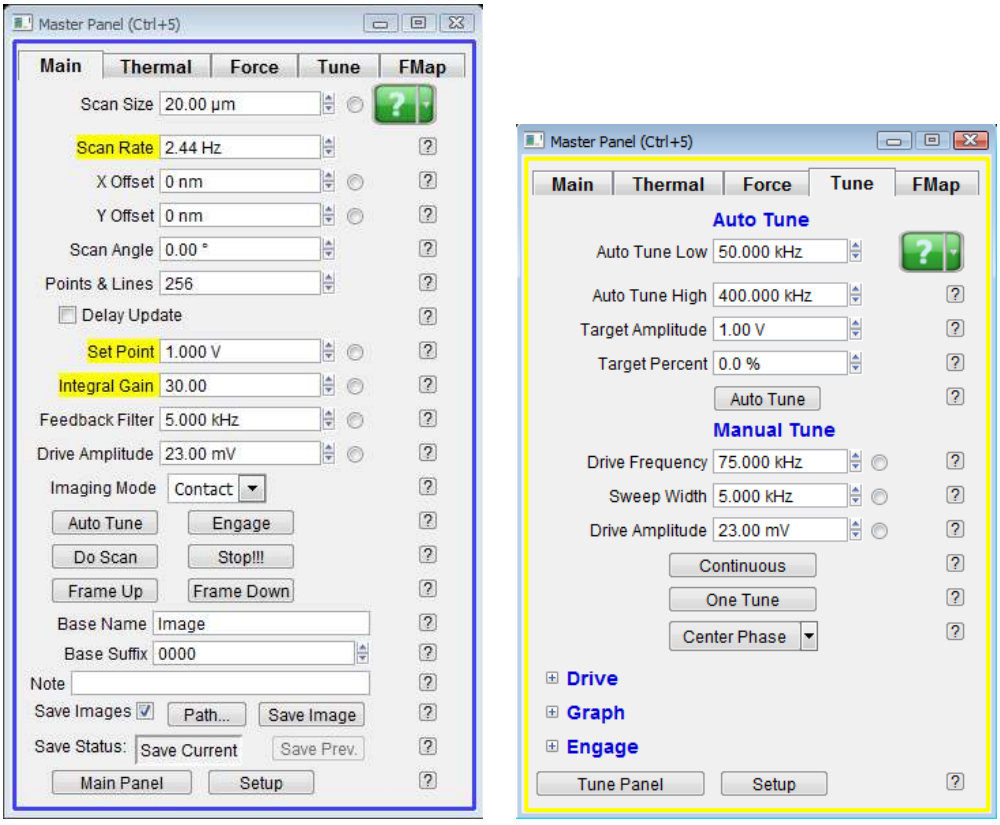
Figure 7.2.: Various methods for aligning the laser spot onto the cantilever.

3.

Set scan parameters:

- Select the 'Main' tab on the Master Panel .
- Set the Set Point value (the second item highlighted in yellow in Figure 7.4a on page 61) to 700mV .
- Make sure that all the other values and check boxes on your 'Main' tab panel are the same as 7.4a.





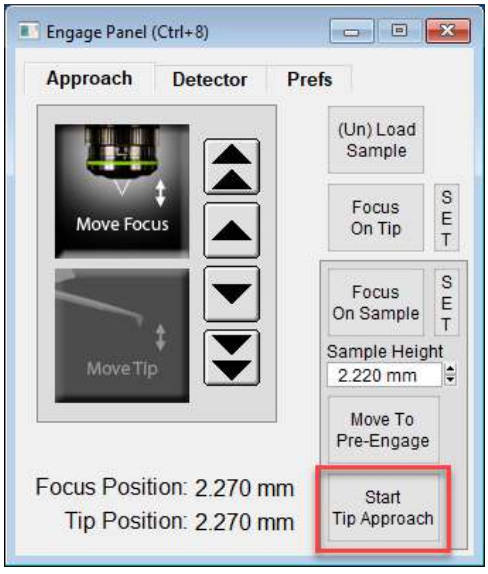
(a) Master Panel, Main tab. **(b)** Master Panel, Tune tab.

Figure 7.4.: Master Panel: Main and Tune tabs.

7.3.3. Landing the Tip

The preceding sections have left the tip vibrating at about 50 microns above the sample surface. It's time to land.

1. **Start final tip approach:**
   - Locate the Engage Panel.
   - Click the 'Start Tip Approach' button.



2.

Wait for tip to reach sample:

- For the next minute or so, Cypher systematically moves the tip closer to the sample until the set point is reached.
- You can cancel the approach at any time by pressing and HOLDING the 'Esc' key on your computer keyboard.
- When the process is complete, the computer will beep, and the tip will be left at about half the Z range (about 3 microns) off the sample surface.

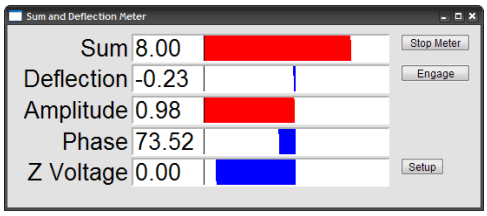
Q What's going on during the tip approach?

A Cypher executes a series of repeated steps. First the scanner fully extends the sample toward the tip while monitoring the cantilever amplitude. If the amplitude reaches the set point, the process stops. If not, the sample is fully retracted again, and motors move the cantilever down by one extension length. The process is repeated until the sample is close enough to the vibrating cantilever to reduce its amplitude to the set point. One final time, the sample is fully retracted, and the cantilever is motored down just enough so that when the sample is brought back up it will trip the tip amplitude set point at half the scanner's vertical extension range.

3.

Meter check:

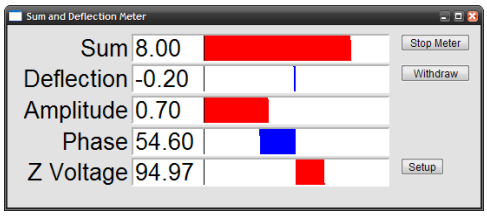
- Locate the Sum and Deflection Meter panel (Ctrl + 6).
- The values should be similar to the image at right and are typical for a cantilever that is a few microns off the sample surface.



4.

Engage:

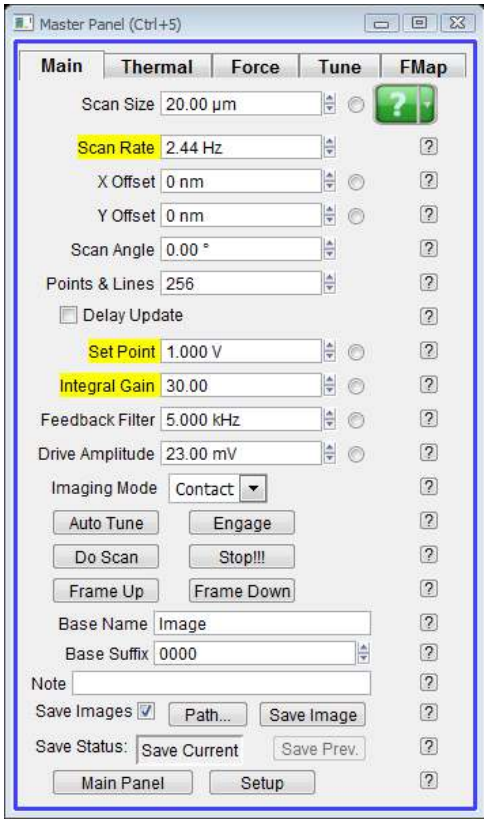
- Click the 'Engage' button on the Sum and Deflection Meter panel.
- The scanner will extend the sample into contact with the tip, and the Sum and Deflection Meter panel will look like the image at right.



Congratulations The tip is on the sample surface.

Question How do I know if my tip is firmly engaged on the sample surface?

Answer Type in a slightly lower set point (such as 650mV). If the Z voltage in the Sum and Deflection Meter panel does not change noticeably (e.g., more than a Volt), the tip will be firmly on the surface.



Question Why does the sample look out of focus when the tip is on the surface? How do I fix this?

Answer The laser and video image both pass through the same microscope objective. While performing AFM, the objective must remain focused on the back of the cantilever to keep the laser focused. Since the sample sits one tip height farther away, it will not be in focus. The fix is extra optics just before the video camera. Adjust the focus ring (at the center in the photo on the right) on the view system until the sample is in focus. Of course, the cantilever and laser spot will now appear blurred in the video image.

Note When it comes to focusing on the next cantilever (see Step 9 on page 53), you must be sure to set the focus adjustment back to zero, as in Step 3 on page 51. Cypher includes a sensor to ensure that this occurs, and the software will warn you to zero the focus offset if necessary.

7.4. Imaging

This section gets you scanning and tracking the surface.

7.4.1. Set-Up and Initial Parameter Selection

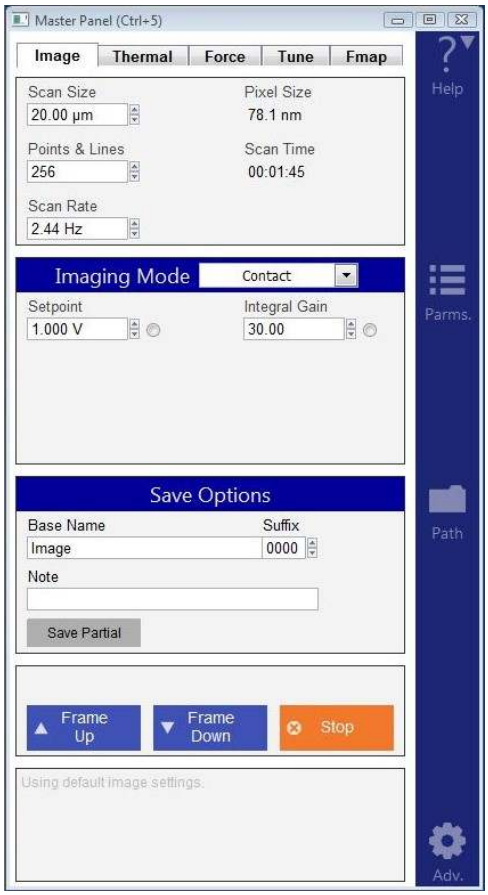
Based on the previous section, it is assumed that:

- The cantilever tip is on the surface or was just disengaged from the surface.
- The laser is aligned on the cantilever, and the photo detector difference (deflection) signal has been zeroed.

1.

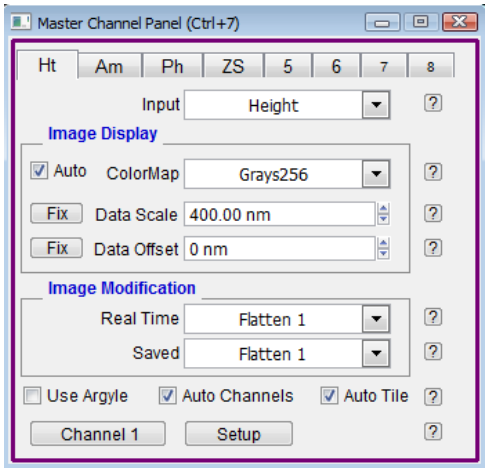
Set up Master Panel:

- In the 'Image' tab of the Master Panel, confirm that *AC Mode* is selected from the Imaging Mode pull-down menu.
- Integral gain (I): 24 to 30.
- Scan rate: 2.44 Hz.
- Scan angle, resolution (scan Points & Lines), and image size is up to you. These can be found on the Parms. tab (to the right the right of Imaging Mode).



2. **Image channel selection:**

- Go to the Master Channel Panel
- Select the leftmost tab and confirm the default setting of 'Height' under the *Input* pull-down menu.
- On the next two tabs, Am and Ph, do the same for 'Amplitude' and 'Phase'.
- On the fourth tab, ZS, do the same for 'Zsensor'.



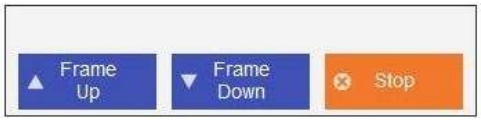
Note While not necessary, it's a good habit to activate the Z sensor channel when imaging, especially when sample features are larger than a few hundred nanometers; the LVDT sensors are more linear than the piezo actuators, and thus it is a more precise Z measurement.

3. Images are saved to disk automatically at the end of every image by leaving the 'Save Images' check box selected (lower left-hand corner of the Master Panel in Step 1 on page 64).

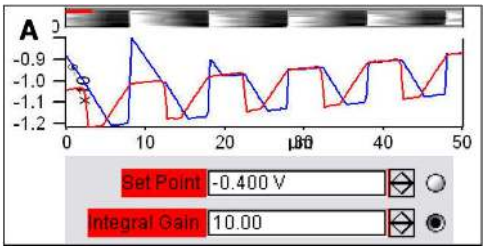
7.4.2. Start Imaging and Parameter Tuning

1. **Start Imaging:**

- Click the 'Frame Up' button on the 'Image' tab of the Master Panel; imaging will begin after a moment. Scan initiation first moves the tip to the starting point of the image, then lowers the tip onto the surface, and then begins an endless series of image scans. The red cursor to the left of each image window indicates the scan line/location of the tip.



Tip To enhance contrast on the image display, click, hold, and drag a box around the area of interest. Then right-click to select fix scale.

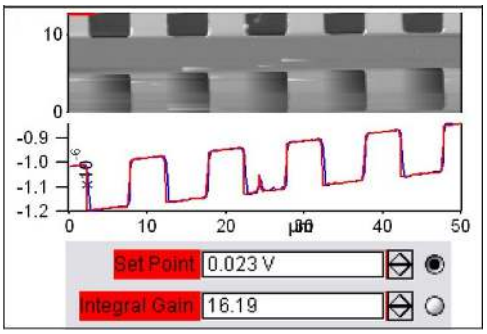


2. **Determining image quality:**

- Start the learning process on a sample with a known topography, like the Asylum Research Calibration Grating being used in this tutorial.
- Look at the 'Scope Trace' below the image. This graph represents the most recent line of the image. Blue indicates the tip moving left to right (a.k.a. **trace**) and Red indicates tip returning from right to left (a.k.a. **retrace**).

On most samples with relatively slowly changing features, trace and retrace should look the same. In other words, the landscape should look the same if you are flying the exact same route one way or the reverse. The image above shows the two as being quite different; this is an indication that imaging parameters need to be adjusted.

Nomenclature	In the previous image, the tip is not following the surface. As the blue trace shows (left to right), the tip seems to climb up out of the pits of the calibration grating quite nicely (the left edge of each pit is quite sharp), but then it descends back into the next pit along a relatively gentle slope. During this descent, the tip actually "flies through the air" while it is completely un-deflected, a bit like a hang glider running off a cliff. The lateral motion of the tip simply marches on as dictated by the XY scan pattern. The feedback control algorithm is simply not aggressive enough to bring the tip back down to the bottom of the pit. Such behavior is commonly called **parachuting** or **poor tracking**.
Hamster	The next steps go into details of strategies for tuning parameters in the main panel. Use the arrow clickers (to right of variable fields) to adjust parameters, rather than typing the values in. Alternatively, you can fine-tune the parameters using the 'Hamster' wheel on the front of the controller. Any parameter with a radio button next to it can be changed during a scan when it is activated (looks like black/ green dot in circle) with the 'Hamster'. The Hamster gives "digital control with analog feel". On the MFP-3D AFM controller, the toggle switch to the left of the 'Hamster' allows you to toggle between radio buttons in the panel. On the ARC2 SPM controller, the outer 'Hamster' ring performs this function. This tactile experience lets you concentrate on the image while tuning parameters.



Adjusting Set-Point, i-gain, and scan speed:

3.

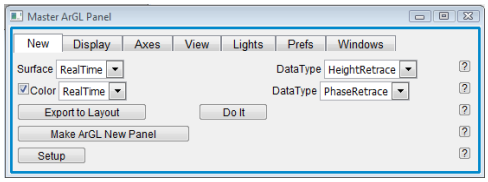
- The goal is to get trace and retrace to fall on top of each other (as shown above).
- Increase the 'Integral Gain' or *i-gain* parameter (second highlighted item in Step 1 on page 64) and see what happens. The match between trace and retrace should improve. The feedback becomes more aggressive and the slope with which the tip "parachutes" down into the pits should become steeper.
- Keep increasing the 'Integral Gain' until suddenly the trace and retrace start to oscillate wildly, a phenomenon called "ringing". The feedback loop is now unstable, and the i-gain needs to be lowered a bit.
- If increasing the i-gain does not seem to help, try to increase the 'Drive Amplitude' a bit. This will cause the cantilever to oscillate with greater amplitude.
- Lowering the 'Set Point' can also improve tracking.
- Decreasing the 'Scan Rate' control will also improve tip tracking.

Note The overall goal is usually to make a good compromise between imaging speed and tip tracking.

View Data in Real Time 3D:

4.

- Open the Master ArGL Panel by selecting from the menu bar *AFM Analysis > 3D Surface Plots.*
- From the 'Surface' pull-down select 'Real time'
- From the 'Data Type' pull-down to the right of it, select the desired channel in that image (usually *Height* or *Zsensor*).
- Click the 'Do It' button.
- You can click, hold, and drag the 3D data to change the view.



Moving the Sample Between Scans:

- Sometimes, it is desirable to move to another point of interest after some scans have been taken. For features within 12.5 microns, use the 'X Offset' and 'Y Offset' fields. Note that a negative is to the left or below the initial area.
- For features that are further away, use the arrows toward the edges of the Video Panel. The single arrows are slow, and the double arrows are fast. It is also possible to click and hold the double arrows for faster, continuous movement. See Figure 7.5 on page 70 for an example of sample movement.

Note These buttons move the sample rather than the cantilever, and so the laser and objective stay in alignment. Be sure to avoid accidentally moving the tip and remember that the smaller arrow buttons in the upper left hand corner of the Video Panel are set to the cantilever rather than to the sample.

Q When I make changes to scanning parameters, when do those changes take effect in the scanned image?

A Most parameters in the main tab of the main panel (See 1) update as soon as you make a change. Note that changing points, lines, or scan rate, will take effect next frame.

If you check the 'Delay Update' box just above the 'Setpoint' parameter, any changes you make to parameters above that box will only update next frame. Until the image is complete, the changed variables are highlighted in blue.

You can always force a new image by clicking the 'Frame Up' or 'Frame Down' buttons. A nice way to see the effect of changing imaging parameters can be as follows:

- Check the 'Delay Update' box, as described above.
- Click 'Frame Up' and collect a dozen scan lines. Observe the image quality.
- Make some changes to the scan parameters (number of points, rate, gains, setpoint).
- Click 'Frame Up' again.
- Observe as the exact same scan region is "painted over" with new data taken with your new parameter choices.

7.4.3. Image Refinement

To learn more about using the Asylum Research SPM software to refine your imaging parameters, please refer to *Applications Guide, Chapter: AC Mode Imaging in Air* and also *MFP-3D User Guide, Chapter: Tutorial: AC Mode Imaging in Air*. Also consider watching this introductory video: AC Mode Imaging in Air (requires an internet connection).

7.5. Stopping Imaging

- **General Procedure for Stopping Imaging:**
  - Once a scan has begun, a 'Continuous' button on the Master Panel will appear and can be changed to 'Single Scan'.
  - Clicking on 'Single Scan' will cause scanning to cease once the current scan has completed.

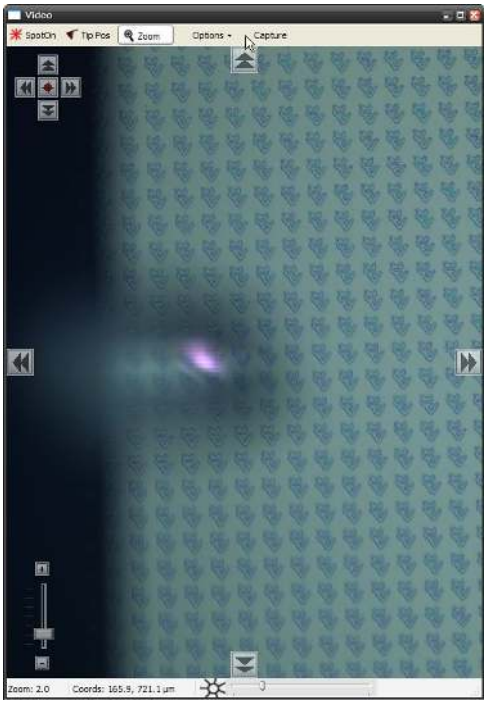
Note 'Last Scan' will change to 'Undo Last' if clicked. Clicking Undo Last will cause the software to keep imaging with current parameters.

- **Emergency Stopping Procedures:**
  - Clicking the 'Stop' button on the Master Panel will halt the scanning mid-image; this is a fairly abrupt way of halting scanning and should only be used if there is a problem. For instance, it would be appropriate to use this button if the cantilever were gouging holes in the sample.
  - Measures such as closing the software, turning off the controller, or unplugging the microscope will stop scanning, but are not recommended except in extreme circumstances because of complications and the risk of tip, sample, or hardware damage.

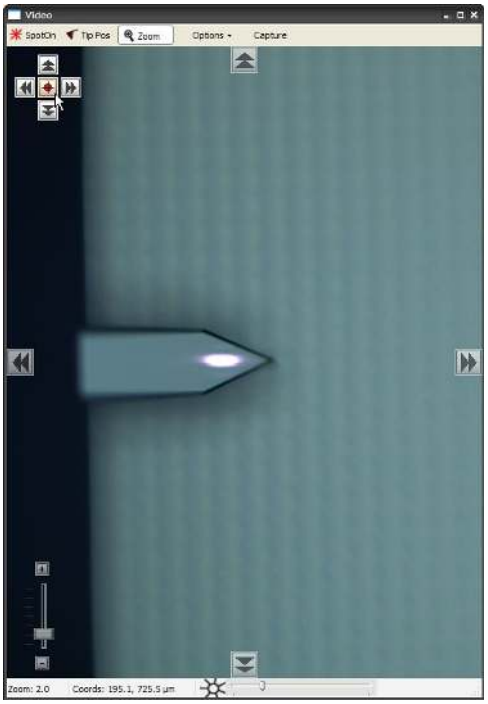
7.6. Shutting the System Down

The following procedure should be used if the Cypher will not be used for some time, for instance, at the end of the workday.

1. Once you are done imaging, save your data to a desired directory. Close Igor and shut down the computer.
2. The tip will disengage automatically when imaging stops, but for added safety, motor the tip away from the sample. You may want to remove the sample at this point.
3. Turn off the laser key on the controller.
4. Power off the controller.

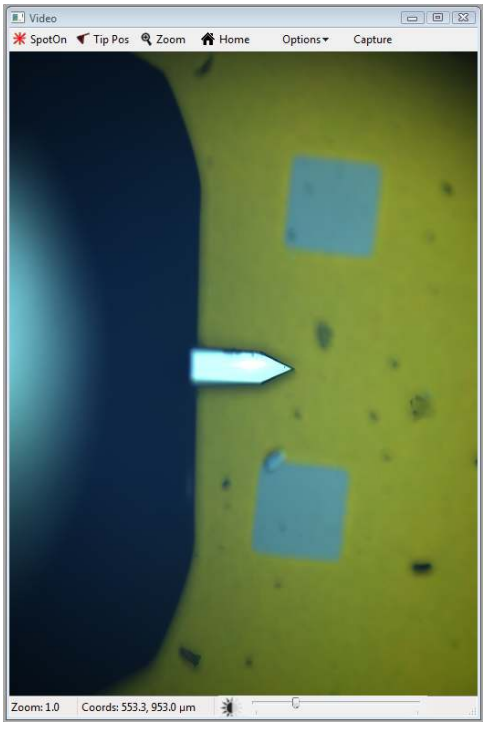


(a) Offset.

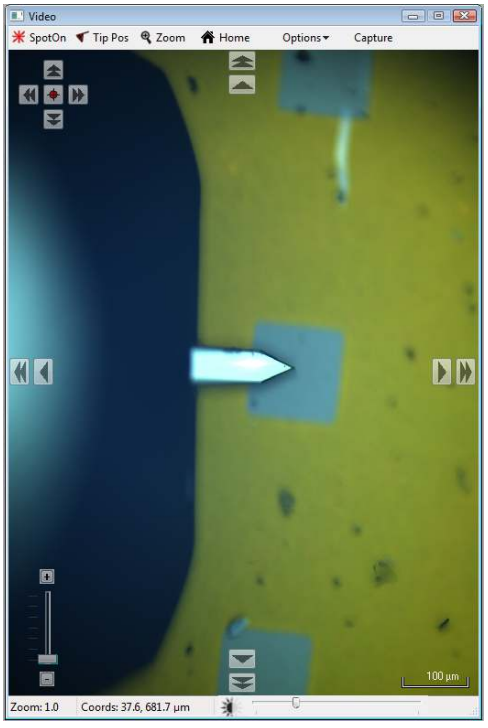


(b) The focus offset is centered, and the optical image is confocal with the focused laser spot.

Figure 7.3.: By turning the focus offset knob, it is possible to focus on the optical image on the sample while the laser stays focused on the cantilever.



(a) Before the move.



(b) After the move.

Figure 7.5.: Moving a sample after a scan.

8. Cantilever Holder Guide

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

Depending on your specific imaging application, the appropriate cantilever holder must be used. This chapter serves as a guide to available options for the Standard Scanner and to help you identify the types of cantilever holders you may already own.

All of the available cantilever holders have many features in common:

- All have a circuit board that allows the system to identify the type of cantilever holder and to activate the appropriate software control panels.
- Nearly all have a piezoelectric actuator and also allow AC mode and contact mode imaging.
- Nearly all have the ability to apply DC and AC voltage to the cantilever.

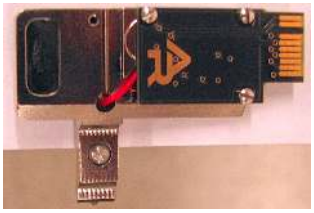
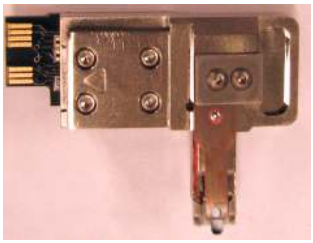
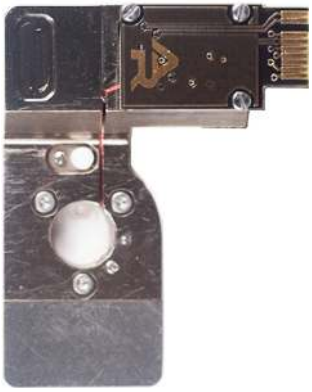
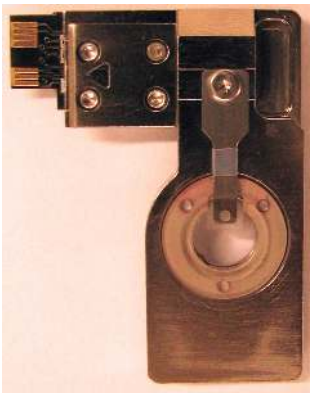
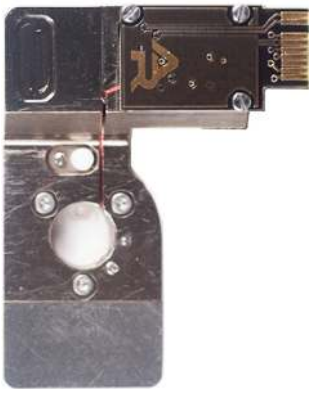
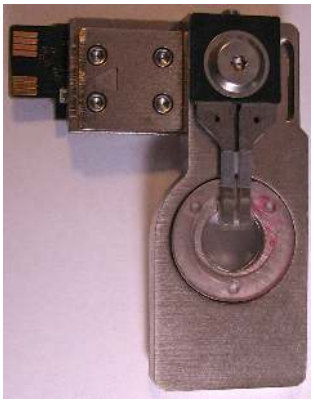
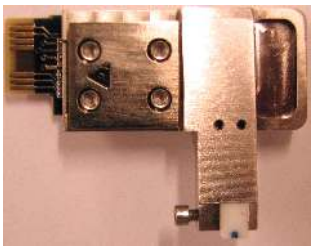
Many more contain specific electronics allowing for current measurement, application of high voltage to the tip, and more.

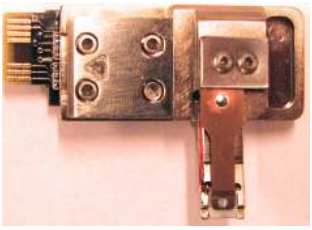
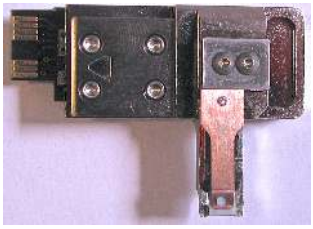
Be Careful!	Cantilever holders are the most delicate components of the AFM. Treat it like you might treat your great grandfather's pocket watch. Never drop it! Remember that even the most basic cantilever holder costs thousands of dollars to replace.

8.1. Identifying Cantilever Holders

8.1.1. Visual Guide of Cantilever Holders

Please use this table to identify your cantilever holders and to find the relevant sections which describe them.

Part #	Holder Description	Front Photo	Back Photo
901.705	**Air** For most contact and AC mode Imaging. Its use is well-described in this tutorial: Section 7.2 on page 43. Fits in the Air Changing Station. For use in air only.		
901.730	**Droplet*** For fluid imaging in a droplet. See Chapter 9 on page 74. Fits in the Droplet Changing Station. For use in air or liquid.		
901.740	**iDrive*** For Electromagnetically Driven imaging, in air and droplets. See Chapter 10 on page 94. Fits in the Droplet Changing Station. For use in air or liquid.		
901.727	**STM** Scanning Tunneling Microscopy. See Chapter 13 on page 132. Fits in the Air Changing Station. For use in air or liquid.		

Part #	Holder Description	Front Photo	Back Photo
901.73x	**ORCA** Conductive AFM with a single current range. See Chapter 12 on page 121. Fits in the Air Changing Station. For use in air only.		
901.708	**Dual Gain ORCA** Conductive AFM with two simultaneous current ranges. See Chapter 12 on page 121. Fits in the Air Changing Station. For use in air or liquid.		

8.1.2. Electronic Identification of Cantilever Holders

1. Attach the cantilever holder to the Cypher Scanner. (See Step 17 on page 48).
2. From the main menu bar in the software, select *Programming > Cantilever Holder and Sample Panel*.
3. At the bottom left of this panel, click the 'Check Holder' button, and the type of cantilever holder will be highlighted.

8.2. Cantilever Holder Changing Stations

Part #	Item Description	Picture
901.715	Air Cantilever Holder Changing Station. Used with Cypher cantilever holders that look like the Standard Air Cantilever Holder.	
901.716	Air Cantilever Holder Changing Station. Used with Cypher cantilever holders that look like the Standard Droplet Cantilever Holder.	

9. Fluid Imaging in a Droplet

CHAPTER REV. 2423, DATED 08/14/2021, 18:50. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

This chapter explains the use of the droplet cantilever holder designed for use with the Cypher Scanner. In this design, the sample is such that the scanning area is submerged in a small volume of water (typically around 100uL) encapsulating both the scanning area and the cantilever. The water environment is maintained by the meniscus bridge formed between the sample substrate and the underside of the glass window of the droplet holder.

Caution: Liquids other than water are not recommended. Volatile solvents may fill the Cypher enclosure with damaging or harmful vapors. The membrane is made of silicone and was not designed for a high level of chemical resistance.

The cantilever holder can be used for contact mode and AC mode imaging in fluid. It has a built-in piezoelectric actuator for driving cantilevers at resonance. Please refer to Chapter 10 on page 94 for specifics of iDrive imaging only.

9.1. Nomenclature

For droplet cantilever holder nomenclature, refer to Figure 9.1 on page 75.

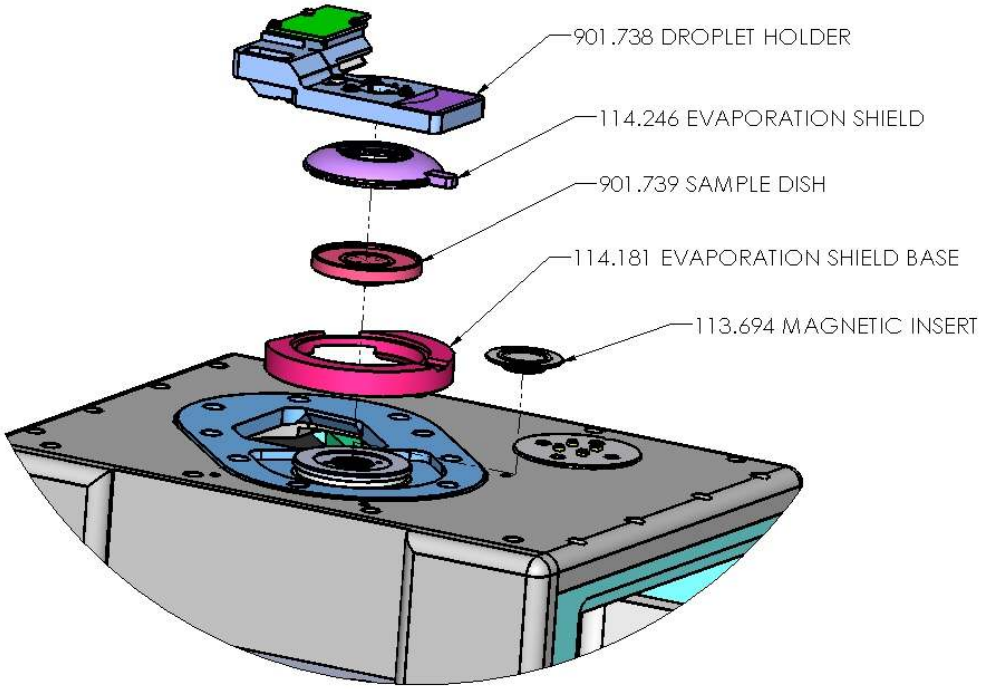
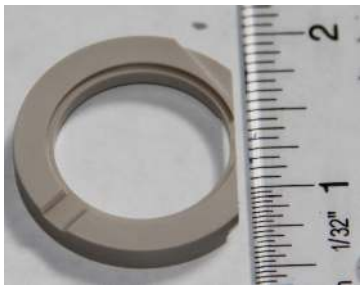
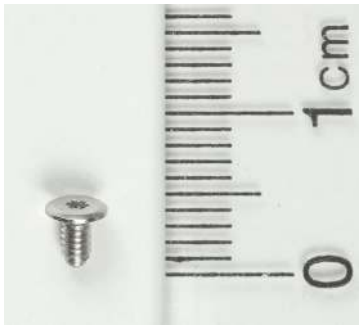
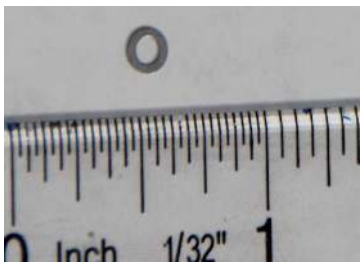
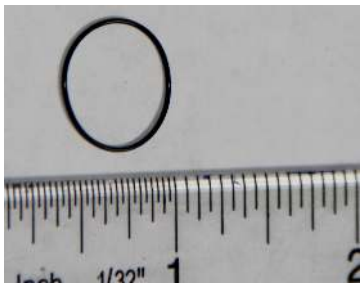


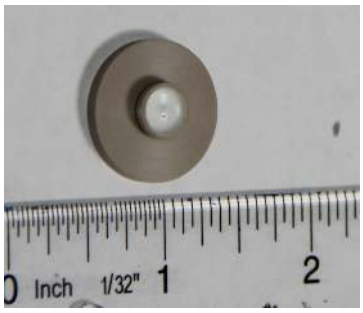
Figure 9.1.: Droplet Cantilever Holder nomenclature.

9.2. Parts List

Asylum Inventory Number 901.738.1

Itm	Part #	Item Description	Qty	Picture
1	004. SETS <#80 x 0.063> CUP	0-80 x 1/16” set screw, cup point.	6	

Itm	Part #	Item Description	Qty	Picture
2	114.181	Ring, Gasket Base	1	
3	114.246	Shield, Low Profile Evaporation.	3	
4	222.070	Socket Head Cap Screw, 0-80 X 7/64”	12	
5	222.072	Screw, M2 X 4, Stainless.	5	
6	222.094	Washer, 0.157” x 0.096” x 0.010” 17-7 stainless steel.	5	
7	230.035	O-ring, 0.551” x 0.022”, 60 Durometer FKM.	2	

Itm	Part #	Item Description	Qty	Picture
8	290.111	0.050": Wiha Allen Driver 263	1	
9	290.136	Short arm hex key, 0.028".	1	
10	290.144	T5 2.5MM Torx Driver.	1	
11	901.738	Cypher Droplet Holder Assembly, V2.	1	
12	901.739	Small Diameter Droplet Holder Cup Assembly.	1	

9.3. Preparing for Imaging

Before you start: We assume that:

- you understand the aspects of running this system safely: (Chapter 1 on page 3.)
- you are familiar with the basic names of the hardware components and software controls (Chapter 2 on page 16.)
- you have powered up the Cypher and launched the software: (Chapter 5 on page 35.)
- you are comfortable with AC Mode Imaging in Air, as instructed by the tutorial: (Chapter 7 on page 42).

9.3.1. Mounting the Sample Dish

The sample dish was originally integral to the evaporation control in an earlier droplet holder design where an evaporation shield attached to the droplet holder. This scheme was difficult to use, so the evaporation control

components were redesigned as is now described. The sample dish is now only used to catch fluid overflow.

Fluid scanning experiments can be carried out with or without the use of the sample dish, since in either case the fluid should be confined between the glass of the droplet holder and the sample. The dish is not intended to be used as a reservoir for liquids. To install the sample dish, you remove the magnetic insert in the scanner cap and thread the dish into the scanner.

1.

Remove the magnetic insert:

- Use a tool like the point of a pair of tweezers to fit into one of the holes in the insert.
- Push the insert counterclockwise to loosen the threads.
- Remove the insert and store in a safe place.

2.

Install the sample dish

- Thread the dish into the scanner cap and *gently* tighten.

9.3.2. Mounting the Cantilever

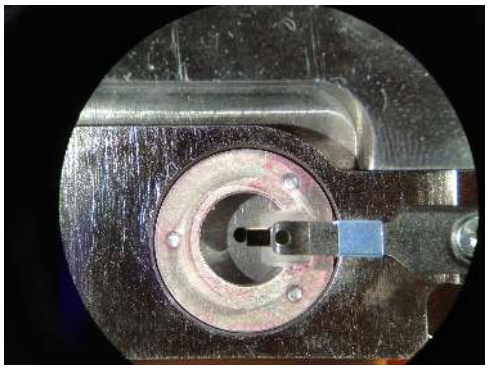
This cantilever holder requires the 901.716 droplet changing station (see Figure 9.2a on page 79).

Warning Using the wrong changing station will not work and may damage your cantilever holder!

Once you have located the changing station, the procedure is the same as you are already probably familiar with from AC mode imaging in Air. If you are not familiar with this, you should seriously consider following the tutorial in Chapter 7 on page 42 at least once. Herein, the specifics of mounting cantilevers is described in Step 9 on page 45 through Step 14 on page 47.

When finished your aligned cantilever should look like Figure 9.2b on page 79.

(a) Droplet Cantilever Holder Changing Station. Notice the markings.



(b) Properly centered cantilever in the Droplet Cantilever Holder.

Figure 9.2.

9.3.3. Using the Evaporation Shield

Since the volume of liquid is small, evaporation will limit the experiment time to about 30 minutes. It is possible to extend the experiment without disengaging the tip by adding liquid into the gap between the sample and the droplet holder from the side by using a pipette.

The droplet holder is supplied with a set of parts which will allow you to build a semi enclosed chamber to help reduce the rate of evaporation. With the evaporation control in place, the typical time of the experiment can be extended about three times compared to scanning without them. Basically, the evaporation shield surrounds the scanning area while contacting the underside of the droplet holder window.

The current design of the evaporation base is sized to work with or without the sample dish using a sheet of mica or a glass cover slip mounted to a steel puck. Thicker bases can be provided if your typical specimen thickness prevents the shield from contacting the holder.

1. **Install the evaporation shield base:**

   - Place the base into the recess around the sample stage.

   Note The top of the base has a lip where the evaporation shield fits.

2.

Install the evaporation shield

- Place your sample onto the scanner.
- Place the evaporation shield on the base.
- Add a drop (approx. 100uL) of liquid to submerge the sample.

Note: The tab on the shield makes a nice handle that allows you to manipulate it into position. Use tweezers to fit the bottom edge of the shield into the groove on the base.

9.3.4. Sample Mounting

Typically, a sample is mounted directly to a steel AFM puck as you would for air imaging. The sample should be large enough to allow a drop of liquid to be placed on it. If the specimen is a material which requires a substrate, a piece of mica or a 15mm glass cover slip should be epoxied to the steel puck.

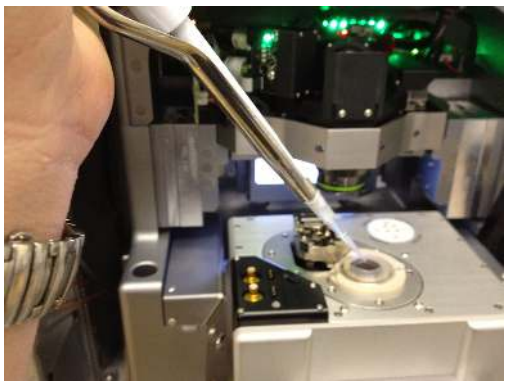
9.3.5. Installing the Cantilever Holder

1. Install the appropriate cantilever for your experiment.

2.

Immerse the sample:

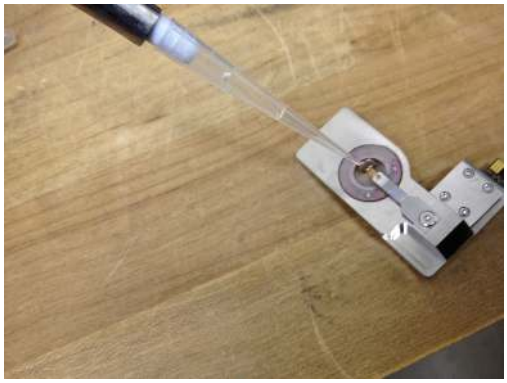
- Add a drop of liquid (approx. 100uL) onto the sample surface.
- A laboratory pipette is recommended to deliver the liquid.



3.

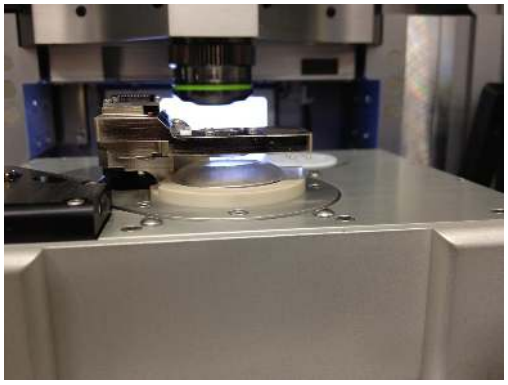
Wet the cantilever:

- Add a small drop of liquid to the window of the droplet holder to submerge the cantilever.
- This prevents bubbles and unwanted bending of very soft levers.



4. **Mount the cantilever holder:**

   - Fit the droplet holder into the dovetail socket on the scanner as you would for the air cantilever holder (see Step 17 on page 48).
   - If necessary, use the coarse approach wheel on the front of the enclosure to raise the cantilever holder pillar high enough to clear the evaporation shield if it's installed.



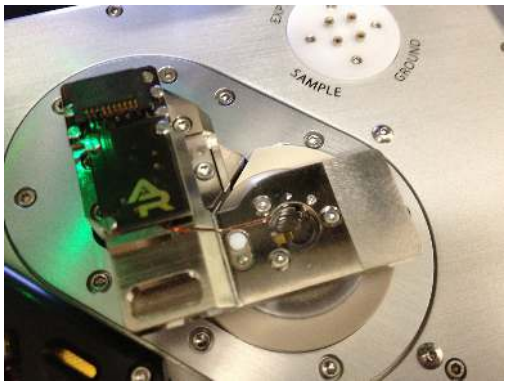
5. Secure the droplet holder to the engage pillar by tightening the dovetail clamp. Remember to hold the driver tool only with your fingertips and gently tighten the screw.

9.3.6. Engaging

9.3.6.1. Pre-engage adjustments

1. **Coarse Engage:**

   - Pull the scanner forward.
   - Using the control wheel on the instrument base, slowly lower the holder toward the sample.
   - Look down through the glass window and watch for the moment it contacts the liquid.
   - You will notice the drop on the window will disappear, and the view through the glass becomes slightly darkened. Stop lowering the holder when this happens.



2. Push the scanner into the chassis and close the scanner clamp on the chassis.

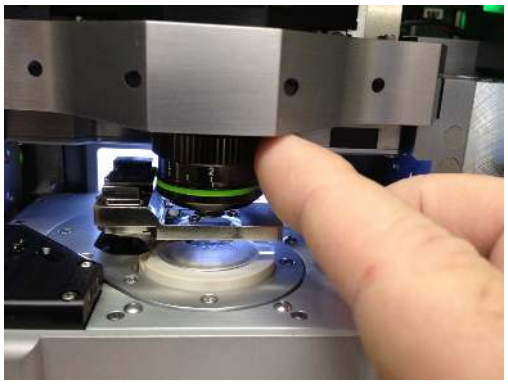
Warning The droplet holder is designed to work only in fluids. Do not try to engage the tip in air. The software automatically compensates for the refractive index of water. Focusing on the tip and sample in air will cause the actual distances to be incorrect, and the cantilever will crash into the sample. This feature can be disabled, but for general usage please only focus the optics through water.

9.3.6.2. Focus on the cantilever

To focus on the cantilever:

1. **Adjust objective focus ring:**

   - Move the focus offset ring on the objective to the 2mm position. This is necessary in order to compensate for the change in focal depth of the objective focusing through the glass window and liquid.

   Note Moving the focus offset ring to 2mm is important to correctly focus the instrument's optics. The system requires correctly knowing the tip and sample focus in order to avoid the tip crashing into the sample, as well as for proper deflection detection.

2. Focus on the cantilever as you would normally do for air imaging, outlined in more detail in 7.3. We assume that you are familiar with this tutorial and will only cover the main points briefly.
3. Set the cantilever focus position.
4. Use 'Spot On' to move the cantilever under the AFM light spot.
5. Zero the deflection voltage.

Note On occasion, an air bubble may get trapped between the glass window and the cantilever. If this has happened, raise the droplet holder out of the liquid and lower it back into coarse position over the sample. If the bubble is still there you may need to remove the droplet holder, suck off any liquid on the window and reapply a fresh drop to the cantilever area.

9.3.6.3. Focus on the sample

To focus on the sample:

1. Lower the objective until features on the sample surface come into focus.
2. Set the sample focus position.
3. Click on the 'Move to Pre-Engage' button.
4. Make any adjustments to the AFM spot or the deflection voltage before engaging the tip.

Using the Field Diaphragm to focus on transparent samples

In cases where there is nothing to focus on, because the specimen is featureless and the substrate is transparent, you can focus on the edge of field diaphragm which typically comes into focus about 30um above the actual sample surface.

Being familiar with this method takes a little practice but once you know what visual ques to look for, it becomes relatively easy.

1.

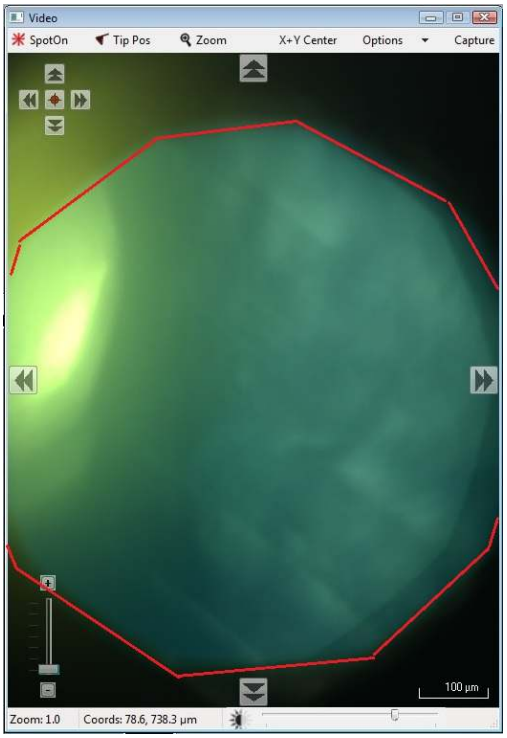
Adjust the aperture diaphragm:

- Adjust the Aperture Diaphragm lever (labeled “A”) on the View Module to reduce the illumination by about 90%.
- In the software, increase the illumination brightness to compensate for the reduction of light. This will help increase the image contrast, and, in many cases, this is enough to see fine surface details.

2.

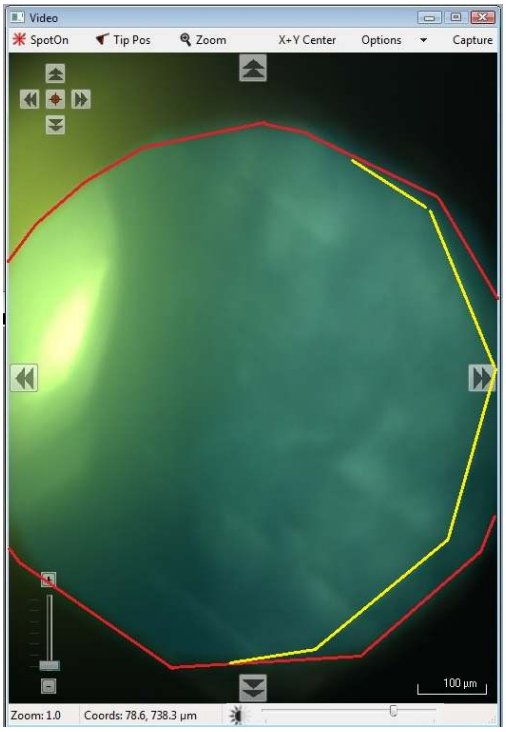
Adjust the aperture diaphragm:

- Adjust the Field Diaphragm lever (labeled “F”) on the View Module until the edge of the aperture comes into view in the video image.



3. **Lower the objective:**

   - Lower the objective while watching for the surface to come into focus.
   - As you lower the objective, you will first see the edge of the field diaphragm come into focus.
   - Once the field diaphragm is in focus, slowly continue to lower the objective. Look for subtle structures, such as the edge of a layer of mica or small bits of debris. This most likely is the sample surface.

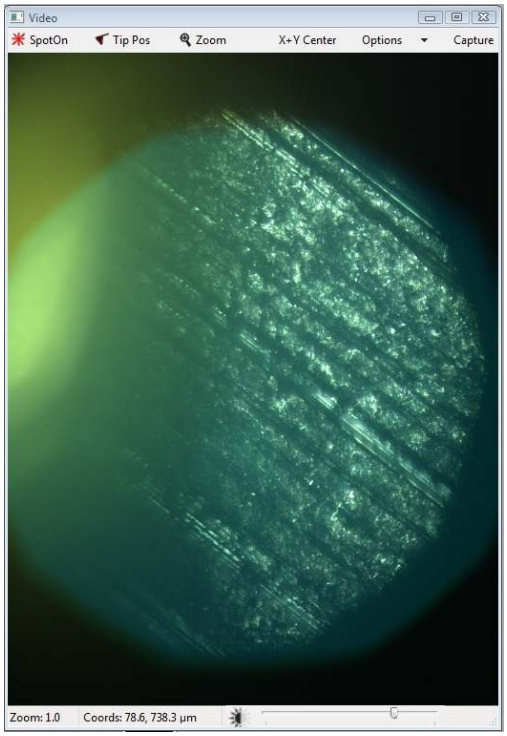


4. One way to confirm this is to note the focus position distance, located just below the arrow buttons. Raise the objective back up to focus on the field diaphragm and note how much the focus distance has changed. Typically, the sample focus distance is about 30um below the focus distance of the field diaphragm.

   Note You may see that the edge of the field diaphragm is shifted off center. This is due to a small amount of misalignment of the illumination path in the view module. In many cases, this can help you distinguish when the edge of the field diaphragm is in focus.

5. **Going too far:**

   - If you cannot confirm you are focused on the surface, slowly continue to lower the objective until you see lots of coarse looking features. These features are typically scratches on the steel puck you have mounted beneath the substrate. If you see this type of structure, you have focused below the sample surface and need to raise the objective.
   - In this case, slowly raise the objective until you see one of the following:
     - 1st - a feature on the sample surface
     - 2nd - the edge of the field diaphragm
     - 3rd - the cantilever



6. If you have raised the objective focus all the way up to the level of the cantilever, then lower the objective back down to focus on the field diaphragm and set the sample focus there. You will be approximately 30um higher than the actual sample. The result of this is a slightly longer time for the system to engage the tip.
7. Once the tip and sample (Field Diaphragm) focus have been set, click on the 'Move to Pre-Engage' button and make any small adjustments to the AFM spot position or deflection voltage prior to engaging the tip.

9.4. Imaging with the Droplet Holder

9.4.1. AC Mode Tuning Specifics

The technique of AC mode imaging in fluid relies on the motion of the piezoelectric actuator in the droplet holder to be sent to the cantilever through the fluid. This indirect or "acoustic" drive of the cantilever is greatly affected by the volume of fluid, the stiffness of the cantilever, and the frequency of the drive signal.

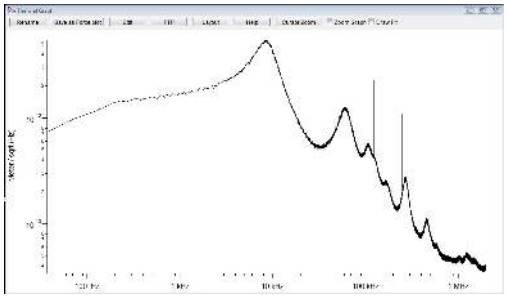
In most cases, it is not possible to simply auto tune the cantilever at its resonance. Manually tuning the drive signal is the preferred method. To know where to tune, you typically find the amplitude peak by first measuring the thermal resonance of the lever. Once the thermal resonance is found, you can overlay the thermal spectrum on the tune plot. As you drive the piezo in the droplet holder will see several peaks in the amplitude plot as the drive frequency is swept. The peak you choose is typically the highest peak inside or near the thermal peak.

Once an amplitude peak is selected and the engage routine initiated it is not uncommon for the system to false engage as the driving forces on the cantilever change. It is therefore common to retune the system as the tip gets closer to the surface.

A typical tuning session goes something like this:

1. **Capture a thermal plot:**

   - Collect the thermal signature of the cantilever. For more information on capturing thermal spectra please read *Applications Guide, Chapter: Thermals*.
   - In this example, the cantilever used is an Olympus TR400PSA which has a nominal air resonance of about 40KHz and a spring constant of .1nN/nM.
   - In water, the thermal resonance is about 7KHz.

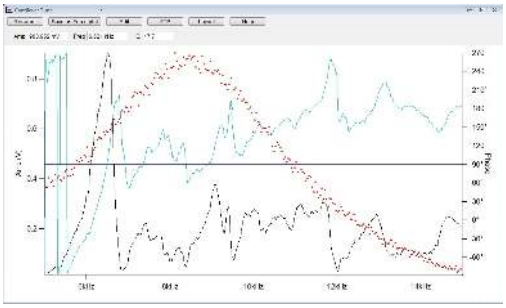


2. **Manually tune the cantilever:**

   - In the manual tune, parameters set the drive frequency to the approximate frequency of the cantilever's thermal resonance.
     - Set the Sweep Width to 10KHz.
     - Set the Drive Amplitude to 1-2v.
   - Click the 'Continuous' (tune) button and sweep the drive frequency.

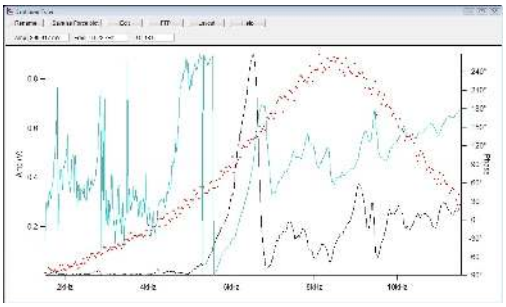
3. **Select an amplitude peak:**

- Select the Append Thermal check box to overlay the thermal data onto the amplitude plot.
- Look for a peak inside the thermal signature. Generally, the peak with the highest amplitude is the one to try. The peak should have a smooth rise in amplitude and have stable output as the frequency is swept.
- The peak near 6Khz is good, although the lower amplitude peak at 9KHz would also work.



4. **Set the drive frequency and calibrate the phase signal:**

- Move the mouse cursor to the apex of the amplitude peak, right-click and select *Set Drive Frequency*.
- The software will center the pot on the peak.
- Click the 'Center Phase' button in the Tune panel to adjust the phase signal to the center of its range.



5. Click the 'Stop' button in the Tune panel when the system is tuned.

9.4.2. Imaging Specifics

9.4.2.1. Engaging in fluid in AC mode

As the tip is being lowered to the surface during the engage routine, the Cypher is doing a series of triggered-force curves looking for the free amplitude to equal the setpoint voltage. Once the free amplitude is seen as equal to the setpoint voltage, the system stops the approach and is considered to have found the surface. This works well, but in fluid there are several things that can trigger a false engagement, such as:

- Feedback Filter - The default frequency response of the feedback filter is 5KHz. Since the resonance of the cantilever in this example is around 6.5KHz, the instrument is allowed to see frequencies too close to the oscillating frequency of the lever. This will cause the software to detect the alternating movement of the cantilever as the amplitude is changing and trigger a false engagement. Lowering the feedback filter value to around 2KHz will avoid this. Using stiffer cantilevers with a higher natural resonance does not need this adjustment.
- Hydrodynamic drag - The abrupt drop in the cantilever holder pillar during a motor step can cause a jump in the deflection signal. This is caused by the drag of the liquid bending a low-spring constant cantilever. Lowering the Feedback Filter to around 2KHz can help to reduce this effect. Stiffer cantilevers do not show this problem.
- Amplitude changes due to the peak shifting frequency - As the probe is lowered to the surface, the amount of liquid between the glass in the droplet holder and the sample surface can change the coupling of the

drive signal into the cantilever. This may excite the cantilever at a different frequency, so a previously tuned cantilever may not be in tune anymore. If the instrument triggers an engagement, you may want to go back to the Tune panel and do a single tune to see the amplitude response and retune as needed.

Check for a real tip engage by clicking on the 'Engage' button in the Sum and Deflection Meter panel. Reduce the setpoint voltage in the Master Controls panel and watch the behavior of the Z control voltage. If by lowering the setpoint voltage you see the Z voltage move all the way to 150volts, then the system has false-engaged, and you should check the tuning of the lever and adjust as needed. If you see the Z control voltage move to a value and stop, then you most likely have correctly engaged. You can begin scanning.

Tip

One useful approach is to monitor the deflection signal. Normally, the deflection signal is not shown since the feedback signal is the Amplitude. Monitoring the deflection signal is helpful because, in some cases, the deflection will jump up as though the tip is has engaged in contact mode when the amplitude is falling. If this happens, it indicates that the amplitude signal may be the result of deflections from the droplet holder components themselves resonating or the cantilever bending in a way that produces angular motion of the optical spot and not the result of the cantilever flexing at the tip end. If you see the deflection signal changing as though it's engaging in contact mode, most likely you should retune the system and try driving the lever at a different frequency (choosing a different peak). This behavior is the result of using low-spring constant cantilevers. Stiffer levers typically do not do this.
To display the deflection signal, click the 'Setup' button in the Sum and Deflection meter panel. Change the deflection from "Auto" to "Show".

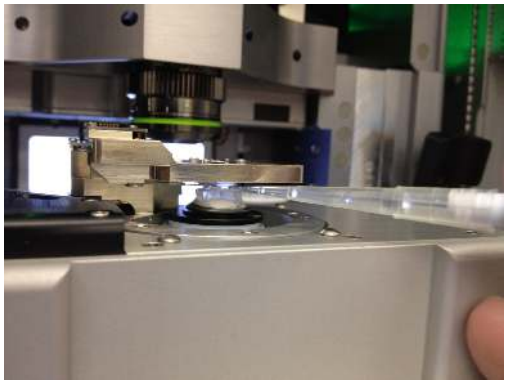
Tip

Do a force curve and monitor the amplitude signal. The amplitude signal should show an abrupt drop to 0 volts just before tip contact is made. Performing a force curve is the equivalent to seeing the conditions of the last engage cycle during the tip approach.

1.

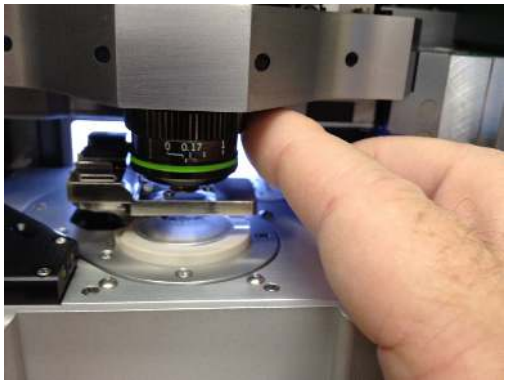
Adding additional fluid during scanning:

- During the experiment, you may find that the tip develops a tendency to float off the surface. This may be due to evaporation causing a loss of fluid volume, which directly affects the AC drive oscillating the cantilever. If you suspect this has happened, use a pipette to add additional fluid to the tip/sample area and retune the system.



2. **After scanning:**

   - After you are finished scanning, move the focus offset ring on the objective back to 0mm.



9.5. Removal and Storage

9.5.1. Removing the Dish

To remove the dish:

1. Unscrew the sample dish from the scanner.
2. Thread the standard scanner magnetic insert into the scanner sample stage.
3. Use the point of a pair of tweezers to tighten the insert.

Please see Section 9.3.1 on page 77for more detailed information.

9.5.2. Storage

Always clean the cantilever holder before storage. If it is particularly dirty, disassemble it before cleaning. Please see Section 10.2 on page 99 for more detailed information. When clean and dry, store the cantilever holder and the other parts and tools in its designated kit box.

9.6. Cleaning and Repair

In daily use, the droplet holder can be cleaned by rinsing the exposed surfaces of the glass window and cantilever clips with clean deionized water. Following the rinse, the holder can be dried using low-pressure compressed air or by blotting with a soft tissue.

For thorough cleaning, the droplet holder must be disassembled. Only the parts exposed to the sample liquid should be cleaned. The cantilever holder body and associated electronics should be kept dry.

The cantilever holder clip, window assembly, and evaporation control components can be cleaned by soaking in ethanol. Sonication of the parts can also be performed. Rinse the parts in clean deionized water. Dry the parts with either low-pressure compressed air or a soft tissue before reassembling the holder.

9.6.1. Disassembly

The following steps guide you through removing various components for cleaning, as well as reassembling, the holder afterward.

Before you disassemble the droplet holder, take the time to familiarize yourself with the way it is assembled.

The key components are:

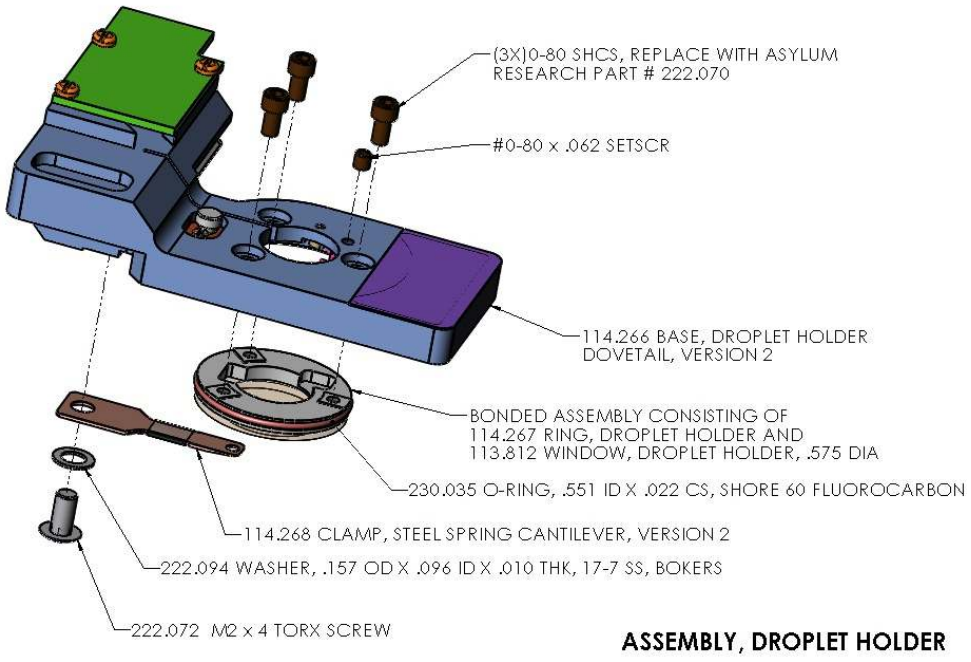


Figure 9.3.: Droplet Cantilever Holder Assembly Overview

- Cantilever clip and associated mounting hardware
- Droplet holder window assembly and associated mounting hardware

As you disassemble the holder, take note that the screws for attaching the window assembly are a specific length. Reassembling the window with the longer screws can result in damage to the glass by either cracking or causing it to become detached from the metal mounting ring.

- Use only 0-80 x 7/64 Socket Head Cap Screws to attach the window assembly.
- Use only 0-80 x 1/16 Cup Point Socket Set Screws for the piezo preload screw.

Due to wear and tear of use, the droplet holder accessory kit comes with replacement screws. Please contact Asylum Research for additional hardware if proper replacements cannot be obtained locally.

1.

Required tools and fasteners:

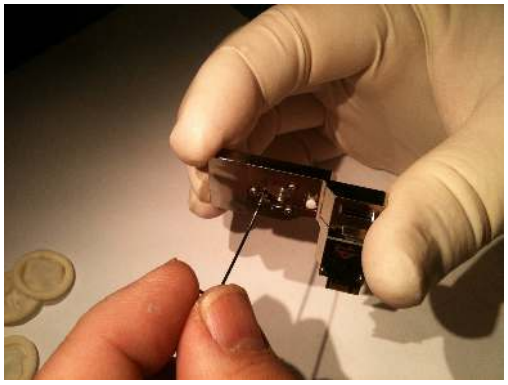
- 0.050” hex driver or Allen wrench for the 0-80 x 7/64” socket head screws to attach the window assembly
- T5 x 40 Torx driver for removing the cantilever clip
- 0.028” hex driver or Allen wrench for the 0-80 x 1/16 Cup Point Socket Set Screws for the piezo preload screw

Warning Using fasteners other than those specified will damage your equipment.

2. **Loosen the piezo preload screw:**

Tools 0.028” hex driver or Allen wrench

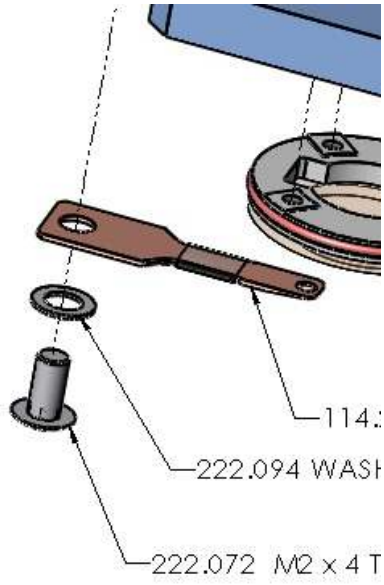
- Loosen the piezo preload setscrew one turn.



3. **Remove the spring clip:**

Tools T5 x 40 Torx driver

- Remove the screw securing the spring clip to the droplet holder body.
- Remove the clip from the droplet holder body.
- Set the parts aside for cleaning.

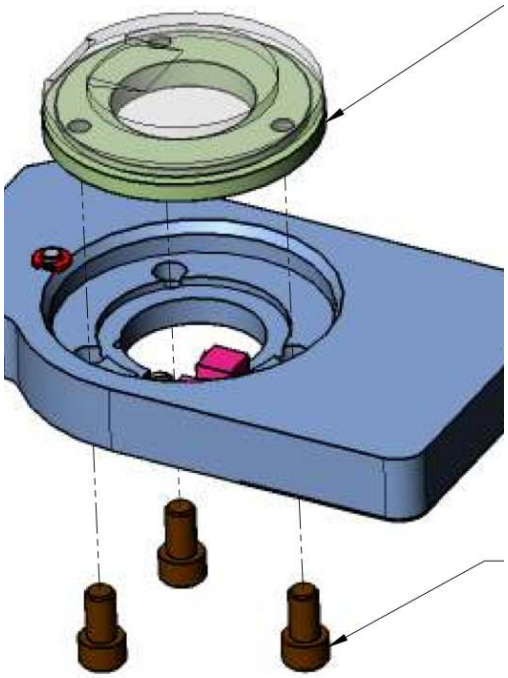


4. **Remove the window assembly**

Tools 0.050” hex driver or Allen wrench

- Remove the three (3) screws holding the glass insert into the holder body.
- Remove the window assembly.
- Use a Q-Tip[a] to gently push the window out of the droplet holder body

Caution: Push gently on the top side of the window. Be careful not to push on the piezo actuator (pink block in illustration).



[a] Typically a wooden or rolled paper stick tipped with cotton. Commonly used for cleaning your ears. Also called “cotton swab”.

9.6.2. Cleaning

The cantilever holder clip, window assembly, and O-ring can be cleaned by soaking in ethanol. Sonication may result in weakening the glue bond of the adhesive used to attach the window to its mounting plate, so limited amounts of sonication (less than 15 minutes) of the parts is recommended. Rinse the parts in clean deionized water. Dry the parts with either low-pressure compressed air or a soft tissue before reassembling the holder.

The rest of the holder parts can be cleaned with a cotton swab and ethanol. Avoid areas with electrical wiring or circuit boards. If you are unsure about having gotten the wrong bits wet, dry the parts for a while (perhaps under the warmth of a desk lamp). Dry the parts with low-pressure compressed air, in any case.

9.6.3. Reassembly

Reassembling the holder clip and window assembly includes:

1. Fit the O-ring into the groove on the window mounting plate. (Spare O-rings are supplied in the accessory kit for the droplet holder, and more can be obtained from Asylum Research, if necessary.)
2. Place the window in the holder aligned so that the ramp in the glass points toward the hole for mounting the cantilever spring clip. The O-ring around the edge of the window mounting ring will prevent the window from fitting directly into the holder body.
3. Use a finger to gently push the window into the holder body. As you push on the window, be aware that the O-ring will need to compress in the recess of the holder body. For this to happen, it may be necessary to use a small tool like the point of a pair of tweezers to help guide the O-ring to fit.

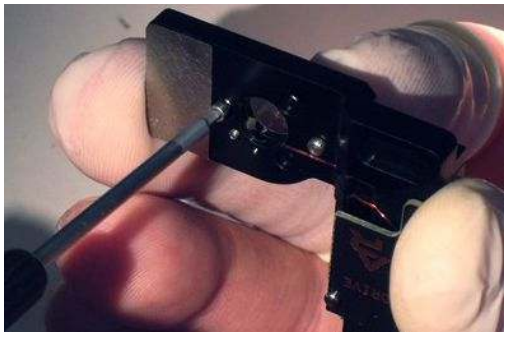
Note: There is a small, recessed area in the metal ring where the piezo actuator fits. Be careful not to hit the piezo or twist the window into position.

1. **Secure the window assembly**

Tools 0.050” hex driver or Allen wrench.

- Using a finger to hold the window in place, thread the three 0-80x7/64” socket head screws to the holder.
- Once all three screws are started, gently tighten them with uniform pressure.

Note Do not over-tighten the screws. A small amount of torque is all that is required.



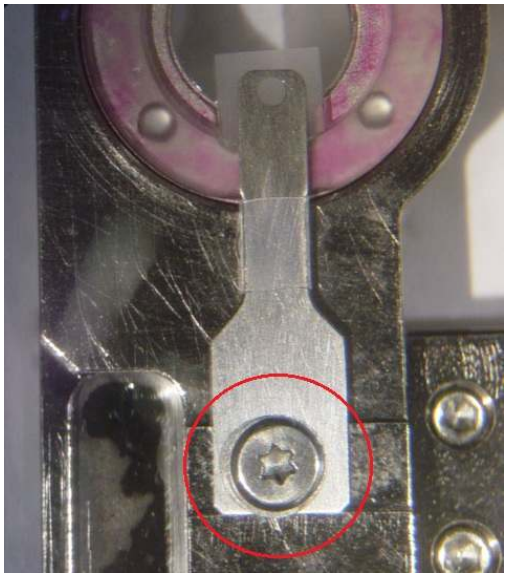
2. **Install the cantilever clip**

Tools 0.050” hex driver or Allen wrench.

- Lay the cantilever holder body circuit board side down.
- Place the clip on the holder body with the taper on the clip facing away from the window.

Note The end of the clip is tapered to provide clearance between the underside of the clip and the sample surface. Be sure the flat side is against the glass and the taper is away from the glass.

- Secure the clip to the holder with the Torx screw and washer. The clip may want to rotate as you tighten the screw. Use a pair of tweezers to hold the clip in the center of the ramp while you tighten the screw.



9.6.4. Adjusting Piezo Preload

When first disassembling the droplet holder for cleaning, the preload screw was loosened. Doing this allows you to readjust the compression on the piezo element properly after it is reassembled. This is recommended since the amount of compression is very small and the piezo position may change when you remove and reinstall the glass window.

1. Install the droplet holder into the scanner.
2. Lock the clamp on the scanner to secure the droplet holder.

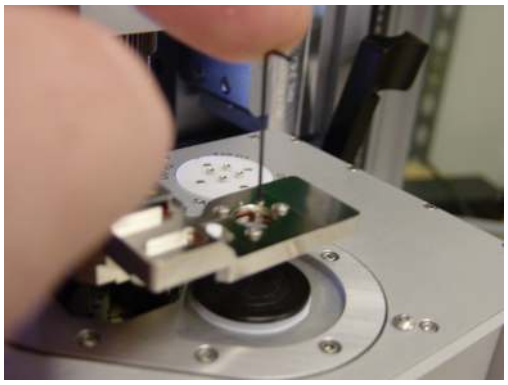
3. **Activate the tune sweep:**

- In the AR SPM Software, select the 'Tune' tab of the Master Panel.
- Under Manual Tune, set the parameters as shown at right.
- Click the 'Continuous' (tune) button.

4. **Adjust the piezo compression:**

Tools 0.028" hex driver or Allen wrench

- Listen for a small chirping sound coming from the droplet holder.
- Gently tighten the preload setscrew until the chirping sound becomes abruptly louder. This is the point where the set screw has compressed the piezo into the back of the window assembly. Once this happens the preload is set.



9.6.4.1. Finishing up

1. Back to the software, under Manual Tune, click the 'Stop' tune button.
2. Remove the cantilever holder and store it or put it in a cantilever and start imaging.

10. iDrive Imaging

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

This section explains the use of the iDrive version of the Cypher Droplet Cantilever Holder. In addition to the standard Droplet Cantilever Holder's functionality, the iDrive version has the ability to drive a small AC current through special iDrive compatible cantilevers. It also contains a small magnet, the field from which causes a torque on the current flowing through an iDrive cantilever causing it mechanically oscillate. This allows for an AC mode imaging experience in liquid superior to that achieved with standard acoustically driven AC Mode imaging.

Before you start

- We assume you understand the aspects of running this system safely: (Chapter 1 on page 3.)
- You are familiar with the basic names of the hardware components and software controls (Chapter 2 on page 16.)
- You have powered up the Cypher and launched the software: (Chapter 5 on page 35.)
- You are comfortable with AC Mode Imaging in Air, as instructed by the tutorial: (Chapter 7 on page 42.)
- You have mastered fluid imaging in a droplet: (Chapter 9 on page 74.)

Review The iDrive cantilever is based on the Droplet Holder covered in Chapter 10 on page 94. Please read this chapter for general use of the cantilever holder and the basics of using it for contact mode and AC imaging in liquid drops.

10.1. Nomenclature

For iDrive Droplet Holder nomenclature, see figure Figure 10.1 on page 95.

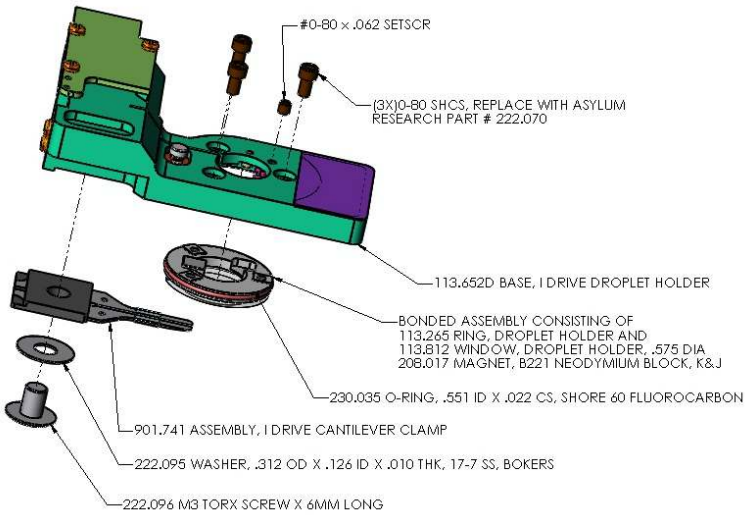


Figure 10.1.: iDrive Droplet Holder nomenclature

10.1.1. Specific iDrive Droplet Holder Differences

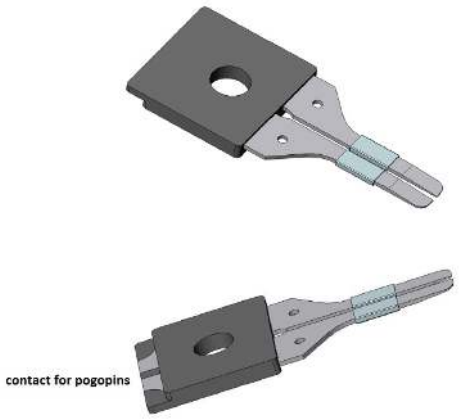
10.1.1.1. The cantilever clip assembly

The spring clip that holds the cantilever in the droplet holder is an assembly of two thin clips molded into a plastic block which together are the same basic shape as the single clip found on the standard droplet holder. In addition to clamping the cantilever, the split clip design is used as pair of electrical contacts to send the AC drive signal through an iDrive style cantilever. Inspecting the design of the iDrive holder will show that there are two gold spring clips (Pogo pins) that contact the back of the clips. These pins carry the AC drive signal from the droplet holder's circuit board.

Figure At right are top and bottom views of the split clip assembly.

Notes

- The exposed area of the clips are the contacts for the pogo pins.
- The step along the molded section is used for keying the clip into the droplet holder body.
- The bands of Teflon act as a hydrophobic barrier.
- Like the standard droplet holder clip, the bottom of the clips are tapered to provide sample clearance.

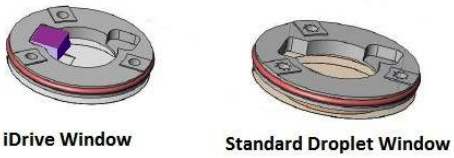


10.1.1.2. The window assembly

The window assembly used in the iDrive droplet holder differs only in that there is a magnet bonded to the top side of the glass window just above the cantilever.

Figure At right is a view of both window assemblies for comparison.

Note Due to limited space in the design of the droplet holders, the windows are not intended to be interchangeable. However, the standard window will fit into the body of the iDrive holder, but the window from the iDrive holder will not fit in the standard droplet holder body.



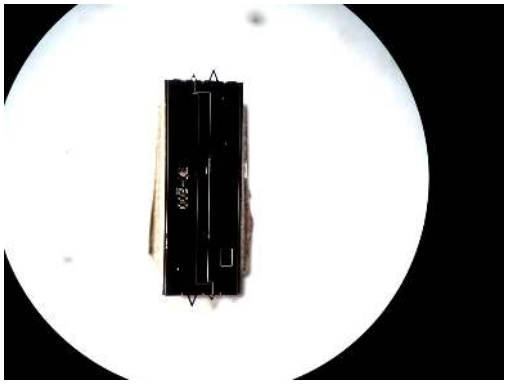
10.1.1.3. Installing an iDrive cantilever

Installing an iDrive style cantilever is basically the same process as a standard cantilever. The difference is that you need to pay close attention to the placement of the cantilever chip so that the split in the contact area on the chip is between the split in the cantilever clip. This will create a circuit so that AC current flows up through one clip through the cantilever and returns through the other clip.

Figure At right is a view of the surface of an iDrive style cantilever.

Notes

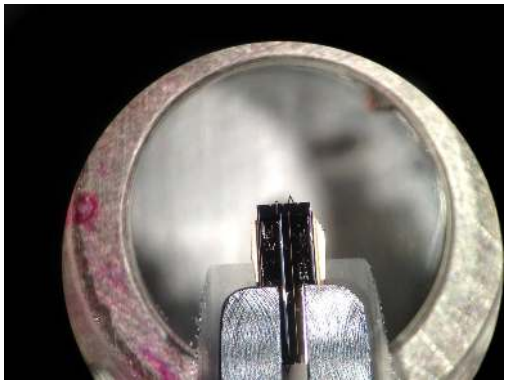
- The entire surface of the cantilever is coated with a layer of gold.
- The insulating lines are etched in surface to create to contact pads.
- Each of the outer pads are connected to one leg of the smaller cantilever.
- The center area is isolated and is not associated with the cantilever's function.
- The typical resistance between the electrodes is 10 Ohms with both of the small cantilevers intact.
- It is okay to scan with both levers intact. Breaking off the unused small lever will simply raise the resistance of the conducting path but generally doesn't improve performance.



1. **Install an iDrive cantilever into the droplet holder:**

   - Align the chip under the electrodes so that only one cantilever clip contacts one of the contact pads.
   - Use an Ohm meter to check the resistance between the cantilever holder spring clips.

   Note The center, narrower electrode is isolated so it's okay to allow one of the clips to touch it.



10.1.2. Preparing for Imaging

Since this cantilever holder is nearly identical mechanically to the Droplet Cantilever Holder, please refer to Chapter 10 on page 94 for details on the following functions:

- mounting the sample and the sample dish
- using the evaporation shield
- installing the cantilever holder in the scanner
- contact mode or acoustic AC mode imaging specifics
- removal and storage

Continue reading only for the specifics of iDrive imaging, cleaning, and assembly instructions.

Tip For contact mode or acoustic AC mode imaging, there is no need to use special iDrive cantilevers. You can still use any standard cantilever for this type of imaging, just as you would with the standard Droplet Holder. Only use special iDrive cantilevers if you actually intend to use this method of exciting the cantilever.

10.1.3. iDrive AC Mode Tuning Specifics

To tune the iDrive AC Mode:

1. With an iDrive cantilever installed, align the laser onto the lever, and take a thermal measurement.
2. Perform the same steps to manually tune the drive signal around the frequency range of the thermal peak, as you would do for acoustic AC mode imaging.

Feature: Activating the tune for iDrive cantilevers

The software automatically scans the cantilever holder socket and identifies the type of holder you are using. In the Tune tab, the check box labeled iDrive is automatically checked if an iDrive droplet holder is detected. In this case, the drive frequency is routed to the cantilever clip instead of the piezoelectric actuator.

- Uncheck the iDrive check box to deselect the iDrive signal and send the drive frequency back to the piezo for acoustic AC imaging.

Figure iDrive Tune

At right is an example of a magnetically driven iDrive cantilever.

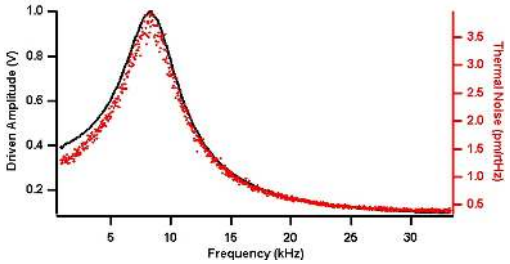
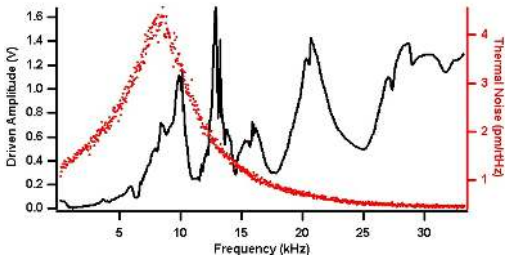


Figure Acoustic AC tune

At right is the same iDrive cantilever acoustically tuned using the piezo actuator.



10.1.4. Imaging Specifics

Once the cantilever is tuned and you initiate the engage routine, you may notice the free amplitude slowly decreases as the tip gets closer. This is due to the interacting of the steel sample puck interfering with the magnetic field lines emitted by magnet in the iDrive holder. As you see this begin to happen, you may want to increase the drive amplitude in the main controls tab. Generally, a few "UP" clicks while the tip is approaching is all that's needed.

As a point of reference, a free amplitude of around 500mv may require 2-5v of drive. This is not a problem but simply a point to note as you learn to operate the system with these types of probes.

Another thing to note is that the volume of liquid has little affect over the amplitude response of the cantilever with iDrive, since the cantilever is driven magnetically and not by the acoustic pressure waves transmitted through the fluid.

After an imaging session has completed, clean the cantilever holder before storage. If it is particularly dirty, disassemble it before cleaning. Please see 10.2 for more detailed information. When clean and dry, store the cantilever holder, along with the other parts and tools, in its designated kit box.

10.2. Cleaning and Repair

In daily use, the iDrive cantilever holder can be cleaned by rinsing the exposed surfaces of the glass window and cantilever clips with clean deionized water. Following the rinse, the holder can be dried using low-pressure compressed air or by blotting with a soft tissue.

For more stringent cleaning, the iDrive cantilever holder must be disassembled. Only the parts exposed to the sample liquid should be cleaned. The cantilever holder body and associated electronics should be kept dry.

The cantilever holder clip, window assembly, mounting hardware, and evaporation control parts can be cleaned by soaking in ethanol. Sonication of the parts can also be performed. Rinse the parts in clean deionized water. Dry the parts with either low-pressure compressed air or a soft tissue before reassembling the holder.

10.2.1. Disassembly

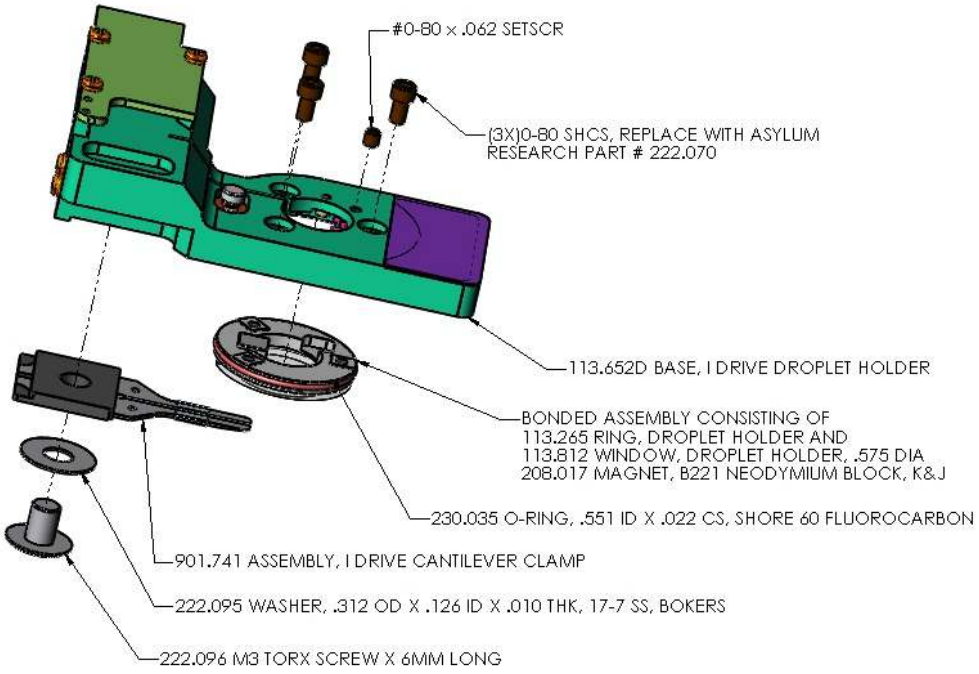


Figure 10.2.: Droplet Cantilever Holder Assembly exploded view

With the exception of the cantilever spring clip and the addition of a magnet to the window assembly, the iDrive Droplet holder is mechanically identical to the standard Droplet Holder. Please refer to the cleaning and repair section for the standard Droplet Holder Section 9.6 on page 88 for more detailed information.

Summary of steps to disassemble and clean the holder:

1. Remove the cantilever clip.
2. Loosen the preload set screw above the piezo actuator.
3. Remove the three screws retaining the window.
4. Gently push the window out of the holder body.
5. Clean the parts.

10.2.2. Reassembly

Summary of steps to reassemble the iDrive holder:

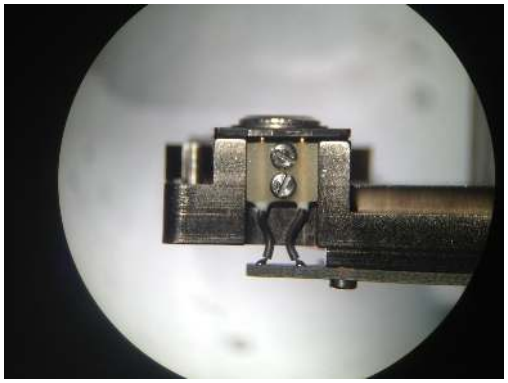
1. Install the window assembly.
2. Install the cantilever clip assembly.
3. Set the preload on the piezo for acoustic AC imaging.

Attention You may ask why the acoustic AC mode piezo is necessary when the iDrive system is available as an AC drive for the cantilever. Practically speaking, it's useful to switch back and forth between acoustically driving the cantilever and using iDrive. Even if you don't see the need, the next person using the cantilever holder might, so it's a good idea to perform the final piezo preload steps.

Tip Pogo pin usage.

The pogo pins are spring loaded and carry the signal to the cantilever clip. Be careful not to bend them as you reinstall the cantilever clip assembly.

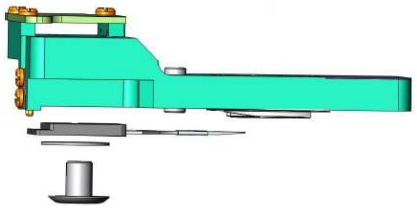
- Start by placing the cantilever clip in place.
- Loosely thread the retaining screw. Don't forget the washer.
- Use tweezers to help keep the clip from rotating until the step on the back of the assembly mates with the step that is machined into the holder body.



Tip Aligning the cantilever clip on the body.

The cantilever holder body and the clip assembly have a step that engages to help align the clip straight. Please note the following:

- The step in the plastic of the clip assembly can be crushed if you tighten the retaining screw with the clip improperly aligned.
- If the step becomes damaged residual plastic may be pushed over the pogo pin area and prevent the clip from touching the pins.
- Take time to familiarize yourself with the parts.
- Take your time when reassembling the holder



10.3. Older Models

There has been one significant redesign to both the standard and iDrive droplet holder. The design addresses:

- the complexity of disassembling and reassembling the holders after cleaning
- improvements in sealing the window from fluid leaks
- ease of use of the evaporation shield

If you have one of these versions of the droplet holders, please refer to this section for cleaning and maintenance.

Note	These revision holders are no longer made. There is an ongoing campaign to replace all of these holders free of charge. If you have already received a replacement droplet holder and you experience a failure of this design, we cannot support it. If you have not yet received a replacement droplet holder and are experiencing a failure, please contact Asylum Research for assistance.

10.3.1. Cleaning and Repair

In daily use, the iDrive cantilever holder can be cleaned by rinsing the exposed surfaces of the glass window and cantilever clips with clean deionized water. Following the rinse, the holder can be dried using low-pressure compressed air or by blotting with a soft tissue.

For stringent cleaning, the iDrive cantilever holder must be disassembled. Only the parts exposed to the sample liquid should be cleaned. The cantilever holder body and associated electronics should be kept dry.

The cantilever holder clips, insulator plates, window assembly, and evaporation skirt can be cleaned by soaking in ethanol. Sonication of the parts can also be performed. Rinse the parts in clean deionized water. Dry the parts with either low-pressure compressed air or a soft tissue before reassembling the holder. Please see 10.3.1.1.

10.3.1.1. Disassembly

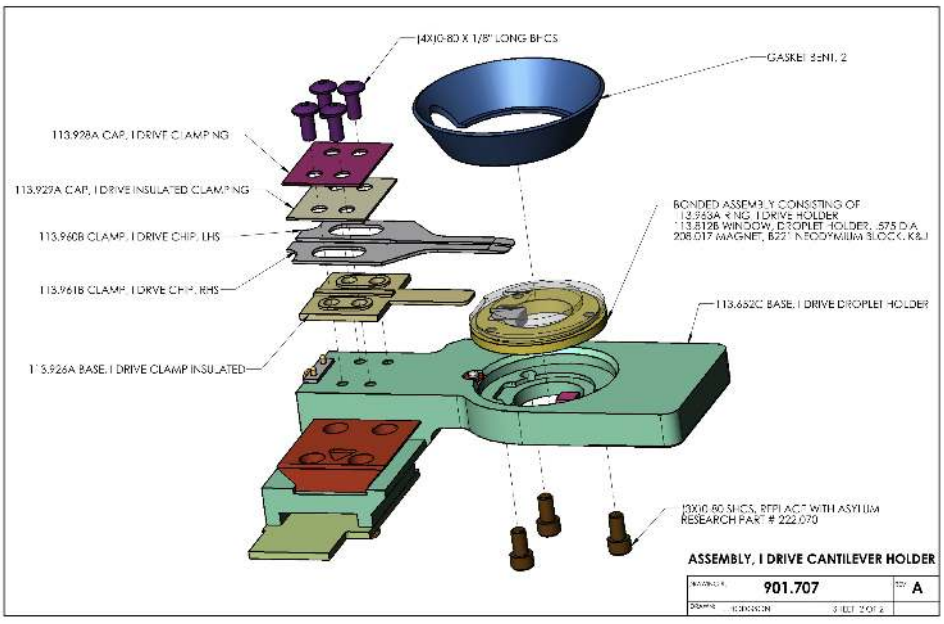


Figure 10.3.: Droplet Cantilever Holder Assembly Overview

The following steps will guide you through removing various components for cleaning as well as reassembling the holder afterward.

Before you disassemble the droplet holder, take the time to familiarize yourself with the way it is assembled.

The key components are:

- cantilever clip and the associated mounting (insulating) plates
- droplet holder window assembly
- piezo actuator for performing AC mode

As you disassemble the holder, take note that the screws for attaching the window assembly are shorter than the screws holding the cantilever clips. Reassembling the window with the longer screws can result in damage to the glass by either cracking or causing it to become detached from the metal mounting ring.

- Use only 0-80 x 7/64” Socket Head Cap Screws to attach the window assembly.
- Use only 0-80 x 1/8” Button Head Cap Screws to attach the cantilever holder clips.
- Use only 0-80 x 1/16” Cup Point Socket Set Screws for the piezo preload screw.

Due to wear and tear of use, the droplet holder accessory kit comes with replacement screws. Please contact Asylum Research or your local Asylum distributor for additional hardware if proper replacements cannot be obtained locally.

1.

Required tools and fasteners:

- 0.050” hex driver or Allen wrench for the 0-80 x 7/64” socket head screws, used to attach the window assembly
- 0.035” hex driver or Allen wrench for the 0-80 x 1/8” button head screws, used to attach the cantilever holder clip.
- 0.028” hex driver or Allen wrench for the 0-80 x 1/16” Cup Point Socket Set Screws, used for the piezo preload screw

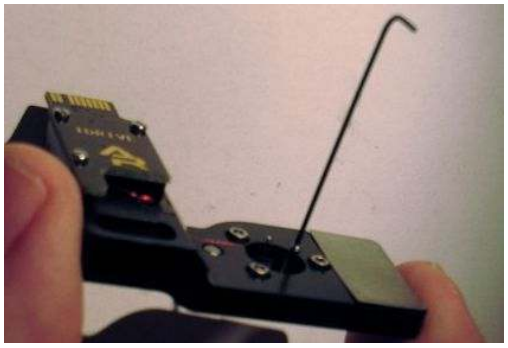
Warning Using any other fasteners than those specified will damage your equipment!

2.

Loosen the piezo preload screw:

Tools 0.028” hex driver or Allen wrench

- Loosen the piezo preload setscrew one turn.

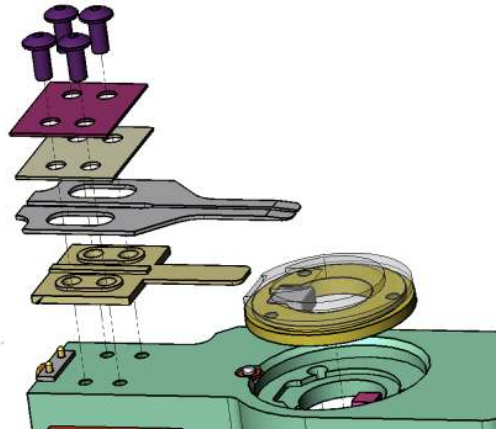


3.

Remove the spring clip:

Tools 0.035” hex driver or Allen wrench

- Remove the screws securing the spring clips to the droplet holder body.
- Remove the clip and the spacer plates from the droplet holder body.
- Set the parts aside for cleaning.

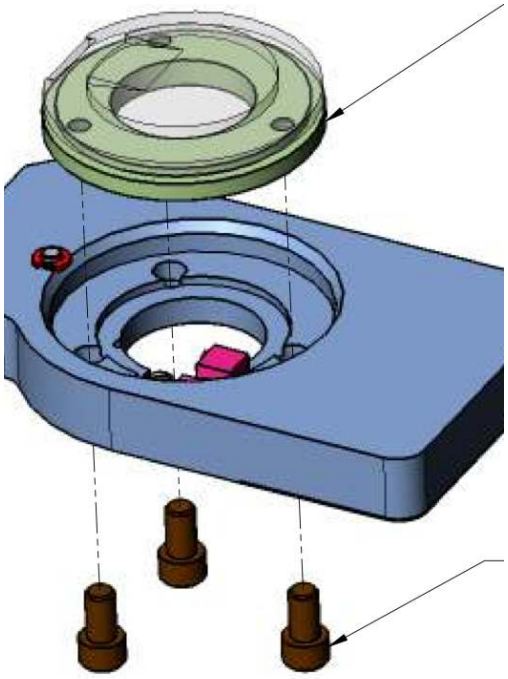


4.

Remove the window assembly

Tools 0.050” hex driver or Allen wrench

- Remove the three screws holding the glass insert into the holder body.
- Remove the window assembly.
- Separate the silicone evaporation skirt if installed.



10.3.1.2. Cleaning

The cantilever holder clips, spacer plates, window assembly, and evaporation skirt can be cleaned by soaking in ethanol. Sonication of the parts can also be performed. Rinse the parts in clean de-ionized water. Dry the parts with either low-pressure compressed air or a soft tissue before reassembling the holder.

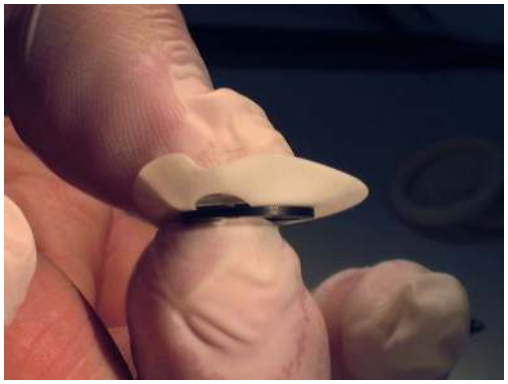
The rest of the holder parts can be cleaned with a cotton swab and ethanol. Avoid areas with electrical wiring or circuit boards. If you are unsure about having gotten the wrong bits wet, dry the parts (perhaps under the warmth of a desk lamp) for a while. Dry the parts with low pressure compressed air in any case.

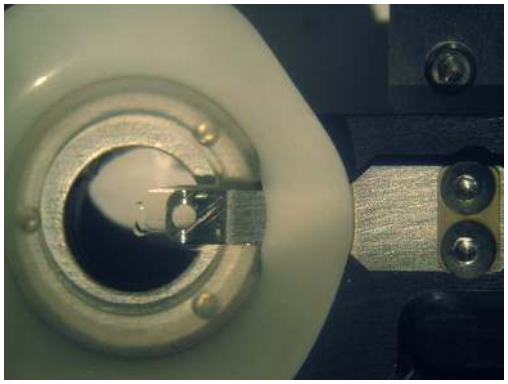
10.3.1.3. Reassembly

1. **Optional: Install the evaporation skirt:**

   - Stretch the evaporation skirt around the edge of the window. The edge of the window has a small groove where the skirt fits.
   - Align the cutout in the skirt with the cantilever pocket. The cutout is made to allow a hole for the cantilever clip to fit through the skirt.

   Note The evaporation skirt is an optional part and not required for normal use. If you decide not to use this part, please disregard the steps where reference to the skirt is mentioned.





2. **Position the window assembly:**

   Tools 0.050” hex driver or Allen wrench

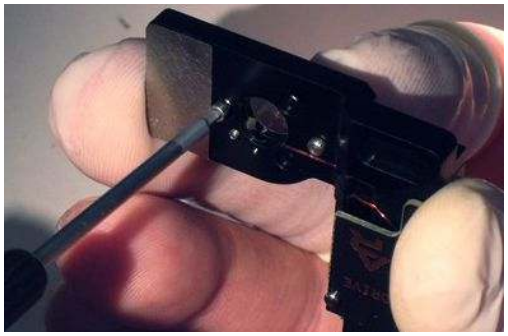
   - Place the window in the holder and use a finger to gently press the window into position.

3. **Secure the window assembly:**

   Tools 0.050” hex driver or Allen wrench

   - Secure the window to the holder using three 0-80 x 7/64 Socket Head Cap Screws.

   Note Do not overtighten the screws. A small amount of torque is all that is required.

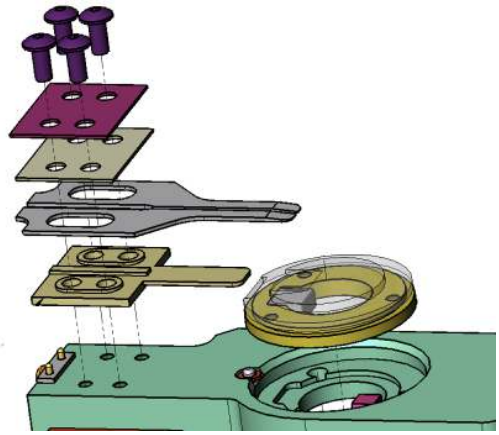


4.

Install the cantilever clip:

Tools 0.035” hex driver or Allen wrench

- Lay the cantilever holder body circuit board side down.
- Using tweezers, place the bottom spacer, clip, and top spacers. Pay attention to the raised features on the bottom spacer. They must face up to mate with the clips.
- The top-most spacer is metal (purple in the drawing). The one below that is plastic. Caution: Don’t reverse the order. The clips must be sandwiched between plastic, or the iDrive current will be shorted before it reaches the cantilever.



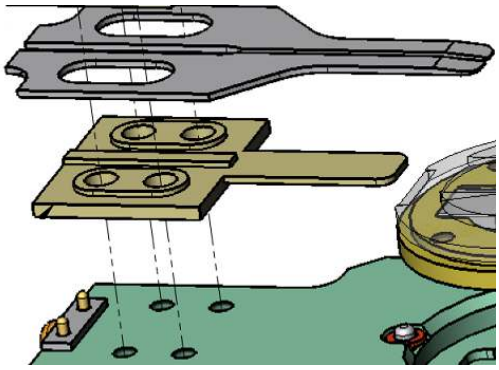
Note The tips of the clips are tapered. Be sure the flat side is against the glass.

- If using the evaporation shield, maneuver the clips through the hole in the shield.
- Thread in the 0-80x1/8 button head screws by only a few turns.

5.

Settle the parts together:

- Adjust the clips so that they seat over the raised portions of the lower insulator. As you shift the position of the clips, they will locate around the raised areas on the lower insulator. When this happens, the clips will feel looser in the stack-up of the assembly.
- Continue to gently tighten the screws and readjusting the clip position until the gap between the parts is gone. Do not tighten the screws yet.

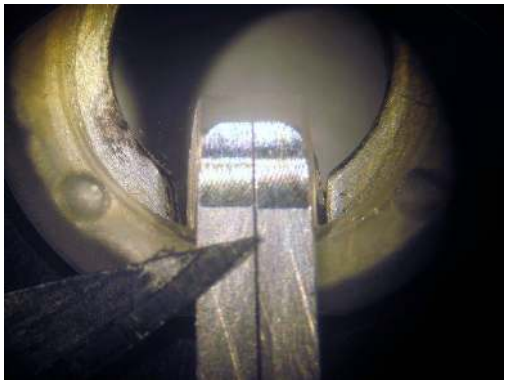


6.

Adjust clips, tighten screws:

Optional Tools Stereoscope, scalpel, or razor blade

- Inspect the two clips at the end where the cantilever is held. The two clips should not touch. Adjust the clips if necessary. The point of a sharp razor or scalpel works well for this step. A stereoscope helps to see the details.
- Gently tighten the screws. Do not over tighten the screws. A small amount of torque is all that is required. Use only your fingertips on the hex driver tool.

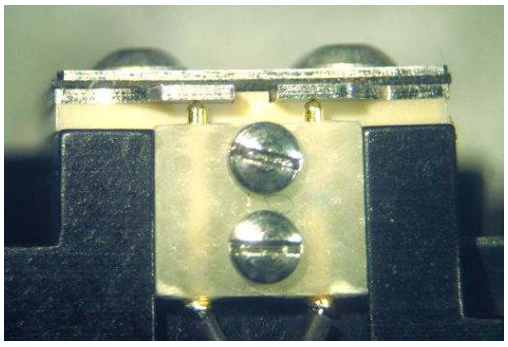


7.

Optional final inspection:

Optional Tools Ohm meter

- Where the clips are widest, measure the resistance between the two clips. It should be infinite (open circuit). If it is finite, then the clips are touching, and you should loosen the four button-head screws and repeat the previous step.
- The photo at right shows a view from behind where you should see a stack-up (from top to bottom) of screw heads, metal plate, plastic plate, clips, thicker plastic plate, and then the aluminum cantilever holder. Note the two gold-coated spring-loaded pogo pins that must make contact with the clips for the iDrive system to function properly.



10.3.1.4. Adjusting Piezo Preload

When first disassembling the droplet holder for cleaning, the preload screw was loosened. Doing this allows you to readjust the compression on the piezo element properly after it is reassembled. This is recommended since the amount of compression is very small and the piezo position may change when you remove and reinstall the glass window.

1.

Install the cantilever holder:

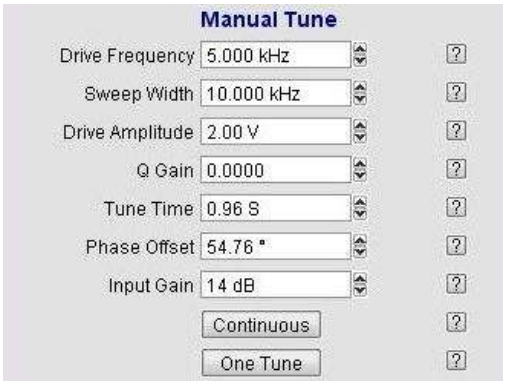
Tools 0.050” hex driver or Allen wrench

- Take the assembled cantilever holder, without cantilever installed, to the Cypher SPM.
- Insert the cantilever holder into the scanner.
- Finger-tighten the screw which clamps it down.
- No need to do any motoring up or down. Move to the next step.

2.

Activate the tune sweep

- In the AR SPM Software, select the Tune tab of the Master Panel.
- Under Manual Tune, set the parameters as shown at right. Note the phase offset is not important, and sweep time of 1s is fine, too.
- Uncheck the *iDrive* control check box, or the piezo will not receive any drive signal.
- Click the ‘Continuous’ (tune) button.

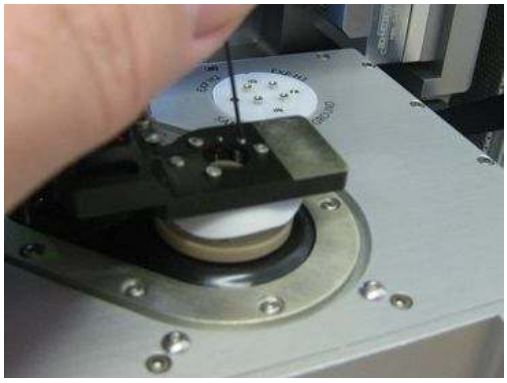


3.

Adjust the piezo compression:

Tools 0.028” hex driver or Allen wrench

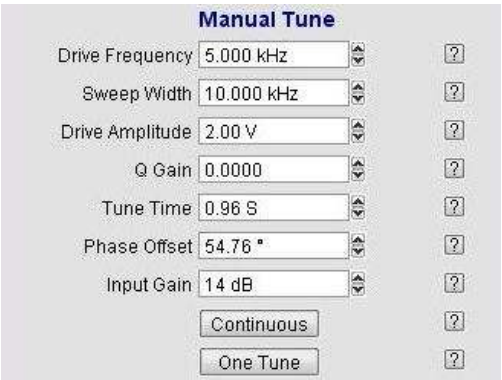
- Listen for a small chirping sound coming from the droplet holder.
- Gently tighten the preload setscrew until the chirping sound becomes abruptly louder. This is the point where the set screw has compressed the piezo into the back of the window assembly. Once this happens the preload is set.



4.

Finishing up:

- Back to the software, under Manual Tune, click the 'One Tune' button to stop the chirping.
- Remove the cantilever holder and store it or put in a cantilever and start imaging.



You may ask why the acoustic AC mode piezo is necessary when the iDrive system is available as an AC drive for the cantilever. Practically speaking, it's quite useful to switch back and forth between acoustically driving the cantilever and using iDrive. Even if you don't see the need, the next person using the cantilever holder might, so it's a good idea to perform the final piezo preload steps above.

11. Scanning Capacitance Microscopy (SCM)

CHAPTER REV. 2258, DATED 07/29/2020, 12:12. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

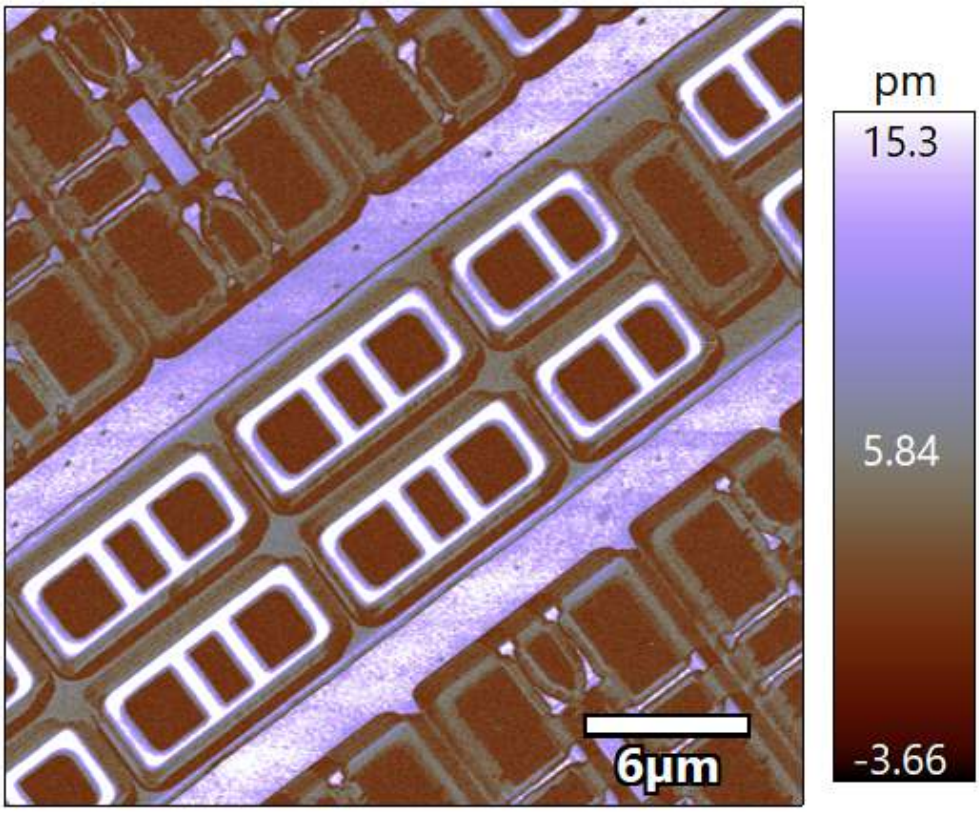


Figure 11.1.: 3D Rendition of SRAM Sample Imaged in SCM Mode

11.1. Compatibility and Prerequisites

The Scanning Capacitance Microscopy (SCM) accessory is compatible with the Cypher S and Jupiter XR AFMs.

11.2. Overview and Specifications

The SCM was designed to enable Failure Analysis engineers to probe the devices at the sub-micron scale and identify issues both during device fabrication and failure analysis. The unique capabilities of this SCM accessory include higher sensitivity, faster imaging, higher resolution, and direct measurement of capacitance instead of only differential capacitance (dC/dV). The sensitivity of the circuit also allows the probing of metals and insulators, along with non-linear materials outside the class of traditional semiconductor devices—including those that do not form a native oxide.

This accessory requires a SCM probe holder, SCM expansion module (for Jupiter XR only), SCM RF module, and probe impedance matching cables.

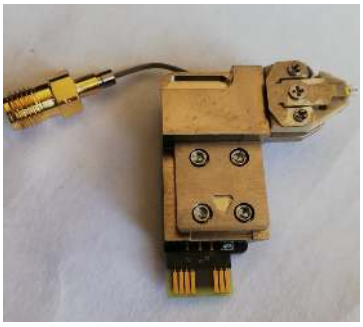
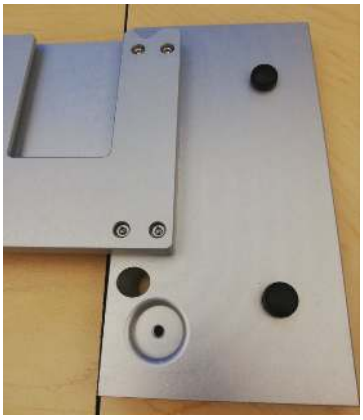
11.3. Parts List

The following list includes all parts in your accessory kit. The table is useful as a visual table of contents and may contain links directing you to the specific uses of each part. When ordering parts, please refer to the part numbers in the second column.

Itm	Part #	Item Description	Qty	Picture
1	001.SHCS<#1-72X.250>SST	1-72 X ¼" SHCS S/S. (Spares for 902.804)	4	
2	222.226	Cross Recessed FHMS, M1.6 X 3MM. Attaches RF connector to cantilever holder.	2	
3	222.227	Cross Recessed FHMS, M1.4 X 2MM	4	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
4	222.230	Aluminum Thumb Screw. (Spares for 902.804)	2	
5	290.106	#00 Wiha Phillips Screwdriver, 261 PH 00 X 40. For small Phillips screws.	1	
6	290.183	2.5MM Allen Wrench. For 3mm Allen screws. Used to remove the RF electronics box from its mounting bracket.	1	
7	290.174	#0 Phillips Wiha Screwdriver 261 PH 0 x 50	1	
8	448.146	STM Wire Assembly	3	
9	448.223	Cypher SCM Backshell Cable Assembly	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
10	448.224	SCM Coax Cable. Used with silicon probes coated in a conducting material, like the MFM-R2 probes included in this kit.	1	
11	448.225	SCM Coax Cable. Used with solid metal probes like the 25Pt300B included in this kit.	1	
12	805. ASYMFM. HM-R2	Asylum Research High Moment MFM-R2 Cantilevers	5	
13	808.RMN. 25PT300B	Rocky Mountain Nanotechnology Cantilevers, Model 25Pt300B	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
14	901.978	Cypher SCM Cantilever Holder.	1	
15	902.803	SCM RF Electronics Box	1	
16	902.804	Cypher SMA Connector Mount	1	
17	902.805	Enclosure Left Side Door	1	
The scale in the photos is in cm and mm.				

11.4. SCM Setup

11.4.1. Sample Preparation

The sample for SCM measurement needs to be flat and free of contamination. If starting with a semiconductor device, several rounds of polishing and cleaning are necessary. Additionally, a thin layer of oxide (a few tens of

nanometers) is needed on top of the surface by either depositing or chemical reaction. A recommended way to prepare most semiconductor samples is to heat them on a hot plate up to 200° C for 30 minutes, and then irradiate them under UV light for 10 min. [V. V. Zavyalov, J. S. McMurray, S. D. Stirling, C. C. Williams, and H. Smith, J. Vac. Sci. Technol. B 18, 549 (2000).]. Samples can also be hydrogen terminated with dilute HF acid; however, extreme care and special training and equipment are needed to use HF acid safely and effectively.

11.4.2. Hardware Setup

The SCM measurement is based on contact-mode imaging with modulation voltage applied to the sample. A specific SCM probe holder is necessary, and the probe choices include solid platinum probes (Rocky Mountain Nanotech), other coated silicon probes (SCM-PIC, AsyMFMMHM), or conductive diamond probes.

The SCM module is shipped with two SMA coaxial cables, one with red shrink wrap and one with black. The red cable is to be used with silicon probes coated in a conducting material, and the black cable is to be used with solid metal probes. Using the incorrect cable will result in a large shift in the tune frequency and dramatically reduced sensitivity.

11.4.3. Cantilever RF Tune

After setting up the hardware, the operator runs a quick cantilever RF tune, using the latest V17 Igor software, to check that the probe is loaded properly the hardware is working. In the tutorial, the cantilever RF tune is shown in on the SCM control panel with a broad resonance near 1.8 GHz. The minimum value is within the green region, indicating that the impedance matching is working. The software also automatically calculates the optimal RF detection frequency. In this case, at 1.7687 GHz. There is yet no signal in the dC/dV channel because the tip is still far away from the surface.

The setup is then ready to engage the tip on the surface and start scanning. Imaging parameters including sample bias are set to default values; however, the user can change them anytime during imaging.

11.5. Tutorial: Cypher SCM (Scanning Capacitance Microscopy)

11.5.1. Installation

1. Install the latest Igor software for the SCM.

2.

Install the SCM RF module box:

- Turn off the controller.
- Insert the large plug into the D-sub connector on the backpack.

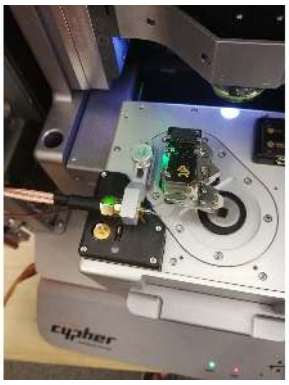
  Note You may need to remove the safety cover from the plug.

  Caution There are high voltage connectors on this plug–make sure the controller is turned off!
- Plug the small plug into the connector on the RF electronics box

3.

Configure the hardware:

- Hang the box on the side of the enclosure.
- Choose the cable that corresponds to the probes you will use for imaging. The cable with the red shrink wrap is for coated Si probes, and the cable with the black shrink wrap is for RMN solid Pt probes.
- Route the cable to the scanner top. For newer hardware, route the cable through the side port.
- Connect the SMA cable to the RF electronics box. The end of the cable that has color-coded shrink wrap connects to the side of the probe holder.

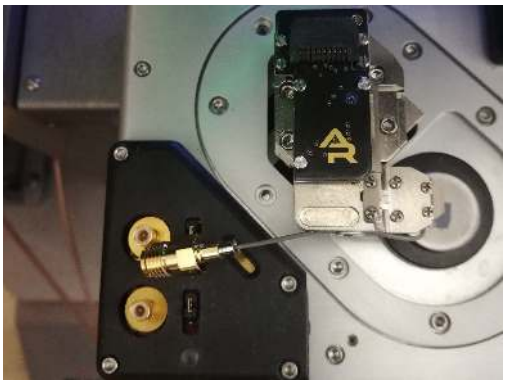


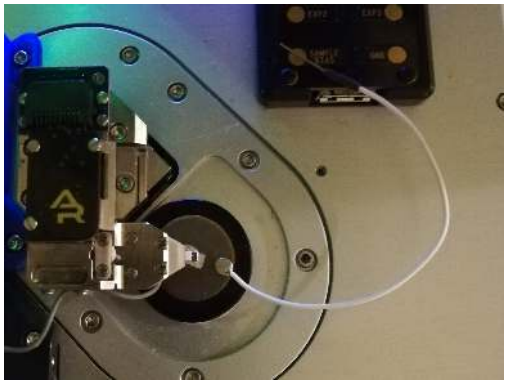
4.

Load a probe in the holder:

- Insert the probe holder into the probe changing station
- *Carefully* insert the probe into the holder. Be sure the probe is all the way to the back of the holder pocket. This will ensure good electrical contact between the clip and the probe.

5.

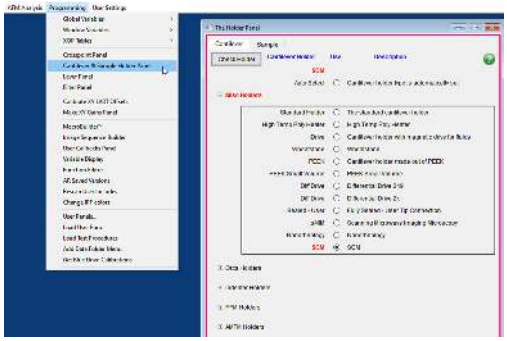




Connect the SMA cable to the probe holder:

- Place the female SMA connector attached to the probe holder into the strain relief bracket attached to the scanner top.
- If you have a 5 lb-in (0.57Nm) torque wrench, use that to connect the SMA connector. Otherwise, tighten the SMA finger- tight.

6.

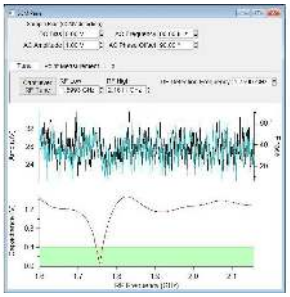


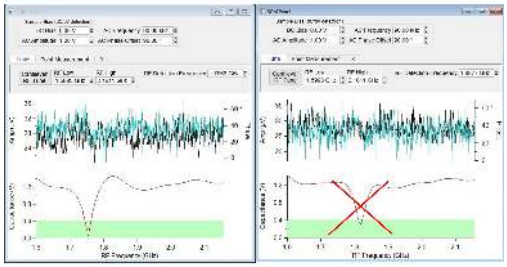
Configure software:

- Launch the Igor software. On the ModeMaster panel, click the 'Electrical' tab, then click *SCM Contact*.

Note If the tile for *SCM Contact* does not appear, you need to manually select the SCM holder in the Cantilever & Sample Holder Panel. To do this, under Programming > Cantilever & Sample Holder Panel, select 'SCM' (under Misc Holders). You should now be able to select *SCM Contact*.

7.

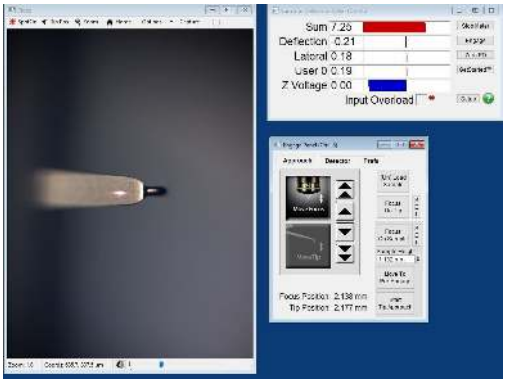


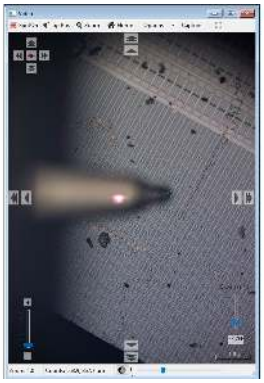


Radio Frequency Tune:

- On the SCM panel, click the button labeled 'Cantilever RF Tune'. This will sweep the RF circuit through its frequency range and find the point of maximum slop. The frequency will automatically be set based on the tune.
- The image above-right shows a good tune (left) and a bad tune (right) for a Rocky Mountain Nanotech solid Pt probe. If the tune resembles the image on the right, remove the probe holder, adjust the position of the probe in the holder to be all the way to the back of the stop in the pocket, and ensure the probe is centered

8.





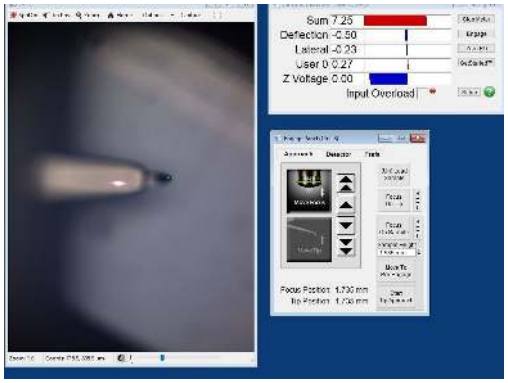
Align the laser and find the surface:

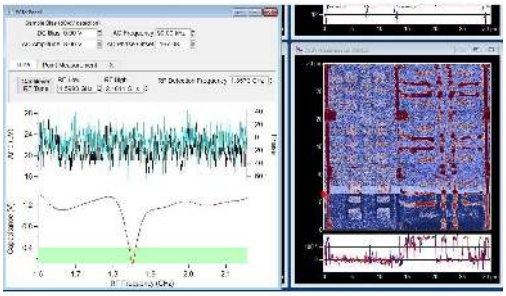
- Locate the cantilever in the camera window. Focus on the back of the cantilever and click 'Set' (to the right of the button marked 'Focus On Tip').
- Click 'Spot On' (in the upper-left corner of the video window). The cursor will change to a red dot; click on the cantilever near the probe tip.
- Optional: Right-click the window at the location of the tip apex and click 'Tip Position'. This will place a marker at the tip position as a reference.
- Focus the objective down toward the surface using the single down-arrow button on the Engage Panel. The sample surface should come into focus. Once the surface is in clear focus, click the 'Set' button (next to the 'Focus On Sample' button).

9.

Approach and image:

- Once at pre-engage, click 'Start Tip Approach'.
- When the approach is finished, the system notifies you with a chime. Click frame-up or frame-down to begin collecting the SCM data. Channel scales and colors can be modified on the Master Channel panel.
- Adjust the dC/dV drive amplitude to get a clear image of the variation in dopant concentrations and types on the surface.
- Adjust the Phase Offset on the SCM panel to remove "digital wrapping" of the phase signal. This will be apparent when the phase is clipped off scale-up or scale-down and wraps around to the opposite limit of the scale.





10.

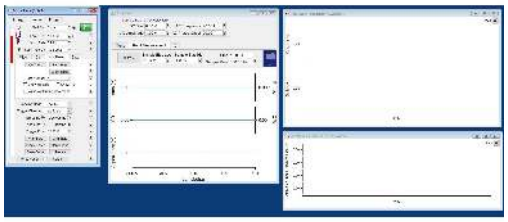
Optional: Decouple camera optics

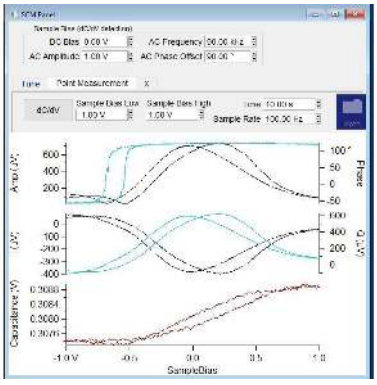
- It is possible to decouple the camera objective from the laser optics to better visualize the surface without disrupting the optical lever system.
- When the system has successfully approached the surface, adjust the knurled knob on the camera module on the top of the enclosure. Moving the knob to the left will focus the camera down toward the probe tip apex.

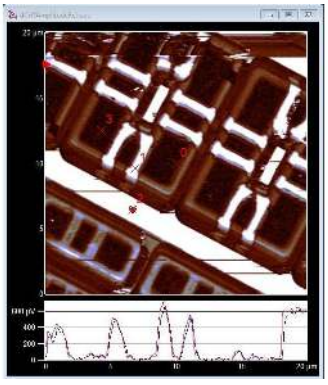
11.

Point measurements: dC/dV vs Voltage

- To perform point spectroscopy, click the 'Force' tab on the Master Panel. Click the 'Point Measurement' tab on the SCM Panel.
- Click the 'Go There' tab on the 'Force' tab of the Master Panel. Click 'Pick Point' and move the cursor on the image channels to select points on the sample surface for point measurements.
- Cycle through the selected points and click the 'Go There' button to move the probe tip to the set points.
- Click 'Engage' on the Sum and Deflection Meter panel. Set the sweep voltage limits and the sweep time.
- Click the 'dC/dV' button on the SCM Panel. This will sweep the voltage and collect the spectroscopic data.







11.5.2. Scanning

When devices are installed and set up, you can start scanning.

11.6. Cleaning and Care

Refer to the parts list for the materials from which the SCM is made. All parts used can be gently wiped with isopropanol and tissue paper. Do not immerse the probe holder or the cables in liquids. SCM is meant to be performed in air (gas) environment.

If you need replacement parts, contact your local Asylum Research office or distributor, using the parts list as a guide.

12. Conductive AFM (ORCA)

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

This chapter explains the use of the ORCA cantilever holder. In practical terms, the ORCA cantilever holder is simply a standard air cantilever holder with the addition of a current-to-voltage converting amplifier.

Basic AC and Contact mode imaging can be performed with the ORCA holder. One major difference in its construction, however, is the use of the electrical connection to the cantilever spring clip. The cantilever clip is used as a connection to the input of the current amplifier rather than a connection to a bias voltage source. Because of this difference, the ORCA holder will not work for measurement techniques where the tip needs to be biased.

Note EFM (Electric Force Microscopy), Surface Potential - SKPM (Kelvin Probe Microscopy), PFM (Piezoelectric Force Microscopy) imaging techniques require the use of the standard air cantilever holder.

12.1. Parts List

The following items are included in the ORCA cantilever holder kit. These accessories are included in both the single gain and dual gain versions of the holder.

Itm	Part #	Item Description	Qty	Picture
1	901.730 901.708	ORCA Holder 2nA/V Dual Gain ORCA 1uA/1nA/V For other available versions see 12.2.	1	
2	ASTELEC-01	10 pack of conductive levers. Used for the measurements described in this section.	1	
3	823.009	HOPG sample. Used as a conductive AFM test sample. See Section 12.3.2 on page 127.	1	
4	448.079	Sample bias wire assembly. Connects sample to voltage source on top of the scanner. See Step 1 on page 127.	6	
5	208.05	Samarium Cobalt Magnet, 0.07” D X 0.104” L. Used to connect the bias wire to the sample. See Section 12.3.2 on page 127.	6	

Itm	Part #	Item Description	Qty	Picture
6	290.160	Leitsilber Conductive Paint, 0.5 Oz. Used to conductively glue the sample to an AFM disc. See Section 12.3.2 on page 127.	1	
7	448.082	Cypher ORCA 500M Resistor Assembly. A 500M Ohm Test Resistor. See Section 12.5 on page 128.	1	
8	448.081	Cypher ORCA 1M Resistor Assembly. A 1M Ohm Test Resistor (Dual Gain ORCA Only). See Section 12.5 on page 128.	1	

12.2. The ORCA Amplifier

There are a variety of ORCA cantilever holders each based on either a single or dual amplification design. The design type and amplification gain are labeled on the top of the holder. Like all the Cypher cantilever holders, a built-in circuit in the holder allows the software to automatically sense the type of holder and configure the system accordingly.

The amplification range of the ORCA amplifier is expressed by its sensitivity. Basically, the ability to produce a voltage output from a certain current flow into the tip. In terms of the full range of the ORCA amplifier, the output is +/-10v so multiplying the sensitivity by +/-10 will tell you the full range.

The ORCA amplifier incorporates the use of a trans-impedance amplifier which converts the input current from the tip to an output voltage. The input potential of the amp is referenced to ground, so the tip is essentially held at 0v potential. During the measurement, the sample can be biased between +/-10v using a voltage source provided by the Cypher electronics.

Each ORCA cantilever holder has a fixed gain(s) to provide the highest current measurement range while considering the lowest noise. The following ORCA holders are currently available. Custom holders can be configured on request.

Part number	Sensitivity	Current Range	Typical noise 1-1KHz
901.730	2nA/V	+/-20nA	1.5pA
901.737	0.2nA/V	+/-2nA	750fA
901.708	1nA/V	+/-10nA	3pA
	1uA/V	+/-10uA	75pA

12.2.1. Single Gain

Here is a conceptual block diagram of the single gain ORCA amplifier. The sample is biased from a voltage source within the Cypher electronics. The feedback resistor R1 sets the amplifier's sensitivity. The output signal representing tip/sample current flow can be monitored by enabling the 'Current' channel in the master channel control panel. See Figure 12.1 on page 124.

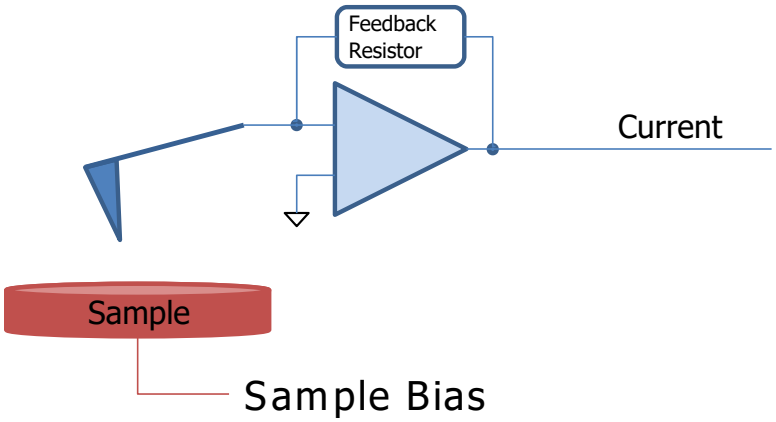


Figure 12.1.: Single Gain ORCA

12.2.2. Dual Gain

A conceptual diagram of the dual gain ORCA amplifier shows the initial current to voltage converter stage feeding the input of a second gain stage to create an additional output signal. In the case of this design the more sensitive signal comes from the second stage and is monitored as Current from the master channel panel like the single gain ORCA holder.

The output of the current to voltage amplifier's first stage has a lower gain (more total current range) signal is monitored as Current 2 from the master channel panel.

Having a dual gain design is useful in that it expands the dynamic range of your measurement capability but at a sacrifice of some increased noise at small current levels. In many cases, the sample you may wish to measure may have widely different regions of conductivity where the current may be too large for the range of the more sensitive stage but suitable for the lower gain stage where more current can be measured. In this case, it is common to see the 'Current' signal (high gain stage) saturate while the Current 2 signal show a measurable current flow. See Figure 12.2 on page 124.

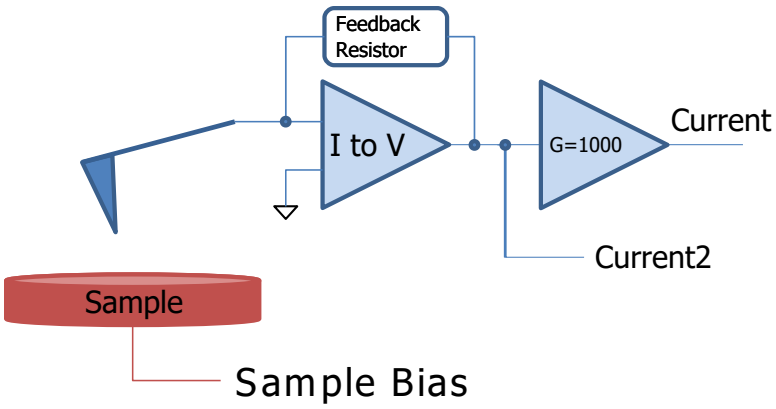


Figure 12.2.: Dual Gain ORCA

12.3. Preparing for Imaging

12.3.1. Zeroing the ORCA Current and Sample Bias signals

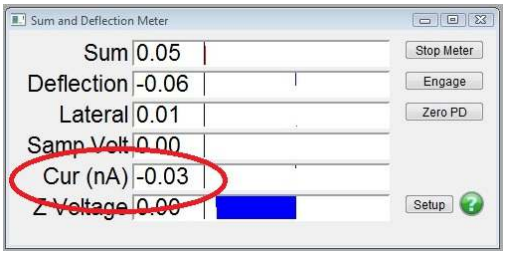
The signal path through the Cypher can pass through many stages of signal conditioning. Each particular circuit in the signal path can introduce a voltage offset which when added together can skew the zero point of your measurement. The following adjustments should be made to your system prior to imaging.

12.3.1.1. Zeroing the ORCA current signal

1. Launch the Cypher software, if not already running.
2. Select *Contact Mode* as the 'Imaging Mode'.
3. **Install the ORCA holder into the scanner's tip engage pillar:**
   - The software will automatically add the ORCA current and sample voltage to the items shown in the Sum and Deflection Meter panel.
   - Push the scanner into the chassis and close the enclosure door.

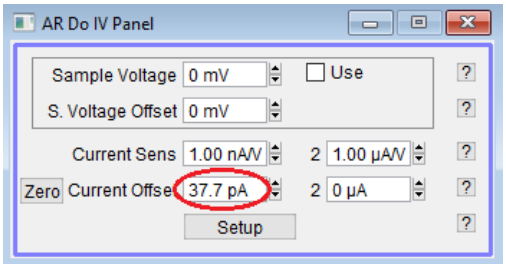
   Note The ORCA current amplifier is sensitive to RF and other emitted signals, such as florescent lighting.
   - Note the current being registered in the 'Cur' display. In this example, the offset current is around -30pA.

   Note If the Sum and Deflection Meter Panel does not update, try adding 'Current' as one of the data channels in the Master Channel panel, and then reselect *Contact Mode* as the 'Imaging Mode'.



4. **Open the Do IV control panel:**
   - Go AFM Controls to locate and open the AR Do IV Panel.
   - Locate the 'Current Offset' parameter at the bottom of the window.

   Note If you are using a Dual Gain ORCA holder, the Current 2 Offset and Sens will be active.

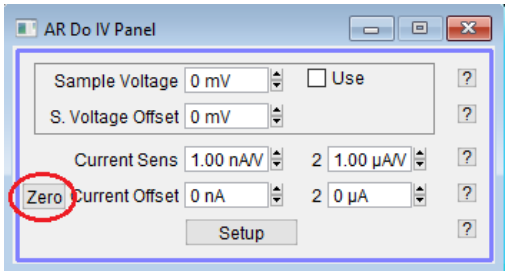


5. **Press the 'Zero' button to zero the current:**

   - The software will add the appropriate offset from the current data to make the Current 0A.
   - Verify that the current is zeroed.

   Notes

   - This is only a software offset. The actual electrical offset in the instrument is still present.
   - If the Zero button is not present in your version of the software, zero the offset current by typing the amount of current shown in the Cur value in the Sum and Deflection Meter Panel.

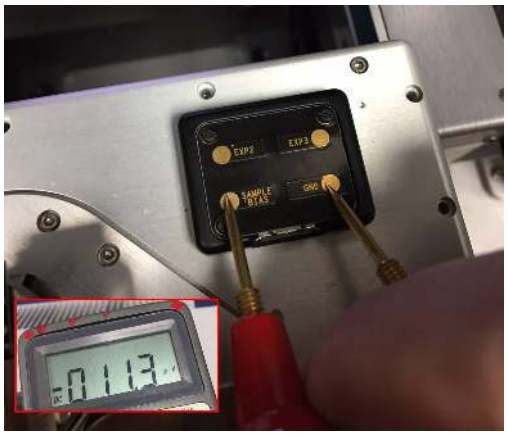


12.3.1.2. Zeroing the Sample Bias

1. **Measure the Sample Bias voltage on the scanner's terminal block.**

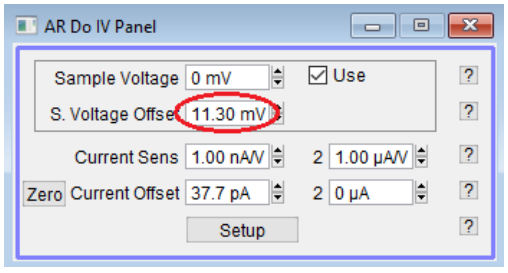
   - Check the 'Use' check box next to the Sample Voltage parameter in the AR Do IV Panel.

   Note In this example, the measured offset Bias voltage is -11.3mV



2. **Enter amount of offset voltage needed**

   - Enter amount of offset voltage needed in the 'S. Voltage Offset' control parameter.
   - Use the opposite sign of the voltage measured on your voltmeter to negate the actual voltage.
   - Make sure that the 'Sample Voltage' parameter is set to 0 volts when making this adjustment.



3. Change the 'Sample Voltage' parameter as needed and verify that the corresponding voltage appears on the Sample pin on the scanner's terminal block.

12.3.2. Preparing the Sample

Sample preparation varies, but basically the goal is to provide an electrical path between the sample bias and the surface of your sample. In addition to the electrical connection, care should be taken to mount the sample mechanically to a sample puck as you would with any sample.

The ORCA kit comes with a practice sample of graphite (HOPG) mounted to a steel puck. A small magnet is attached to the sample puck to provide an easy way for the bias lead to attach.

The magnetic connection method is convenient but is not necessary. A bias lead of your own design can be mounted directly to the sample puck and used as long as the end of the lead is able to fit into the sample voltage socket on the scanner's terminal block. Also, be certain to use wire that is flexible enough to not impede normal scanning.

The following steps describe how this sample was prepared.

1. Use a small amount of 5-minute epoxy to attach the HOPG to the sample puck.
2. Place a magnet onto the puck.
3. Cover the sides of the sample and the entire magnet with silver paint.

Attention	The silver paint is not an adhesive. It will not provide good attachment of the sample to the sample puck. Use the paint only to make an electrical connection from the sample to the bias voltage lead.

12.3.2.1. Install the sample on the scanner and connect the bias lead

1. **Place the sample on the scanner stage and connect the bias lead:**

   - Position the sample so that the magnet is on the right side of the scanner to prevent interference with the cantilever holder.
   - Attach one end of the bias wire to the magnetic contact on the scanner top.
   - Place the other end of the lead on the magnet. The lead is magnetic and will stick to the magnet when it's close enough.

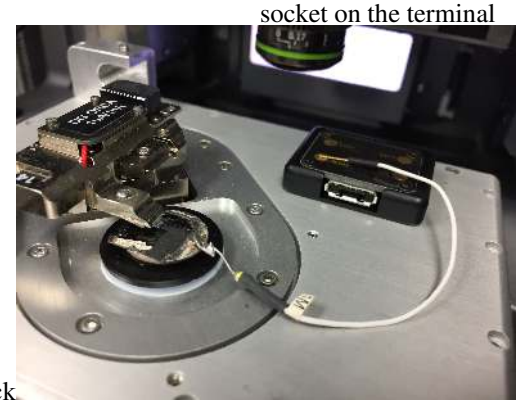
   Note The scanner cap is hard, anodized aluminum and will insulate the sample puck so that bias voltages up to +/-10v can be directly connected without additional insulation.

2. **Adding resistance in the bias voltage path**

   - In cases where your sample is highly conductive, you may want to add a known resistance to keep the ORCA amplifier from saturating. An example of this is the HOPG sample provided in the kit.
   - Substitute the Bias lead with a bias lead including a resistor.

   Note The ORCA holder kit includes a 500Meg. Ohm test resistor. If you are planning to scan the HOPG sample in practice using the ORCA holder, use the test resistor instead of the bias lead.

   Plug on end of a bias voltage lead into the sample socket on the terminal block.



12.3.3. Mounting the Cantilever

Mounting a cantilever is the same procedure as is used in all other AFM applications, with the exception of lever type. Conducting AFM (ORCA) requires a conductive path between the tip and the cantilever spring clip. The ORCA kit includes a sample pack of 10 Electrilevers. Additional levers can be purchased from Asylum Research.

If you are not familiar with basic AFM operating practices, please review the basic operating tutorials section at the beginning of this guide.

12.4. Imaging with the ORCA

For information about imaging with the ORCA, please refer to *Applications Guide, Chapter: Conductive AFM*.

12.5. Testing the ORCA Amplifier

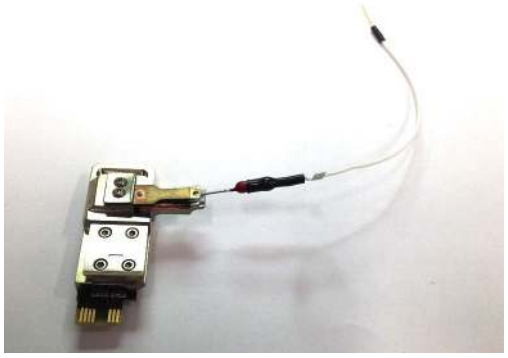
The ORCA cantilever holder kit includes an appropriately sized resistor to test the measurement range of the ORCA amplifier. Testing the ORCA is fairly straight forward. Basically, the test resistor in installed between the sample bias and the cantilever clip. An I/V ramp is plotted, and the correct current flow through the resistor should be observed.

1. **Install the test resistor under the clip on the ORCA holder**

   - Hold the ORCA holder upside down in your hand and use a fingernail to press on the button on the top side of the holder to open the cantilever clip.
   - Slide the resistor lead under the clip.
   - Release the button to clamp onto the resistor lead.

   Notes

   - Using the changing stand also works, but you may find that getting the resistor installed and removing the holder from the stand can be a bit tricky. Using your fingers as described works well.
   - The cantilever holder body is conductive. Position the lead under the clip so that is does not touch the holder body. Basically, take care not to insert the resistor lead too far under the clip or have it off center.
   - It is not harmful if the resistor shorts to ground. The current from the sample bias through the resistor will not be measured by the holder.

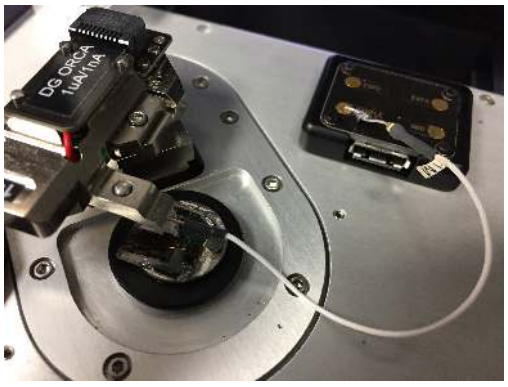


2. **Option 1: Install the cantilever holder and connect the test resistor to sample bias.**

   - Insert the ORCA holder into the scanner's engage pillar.
   - Plug the lead from the test resistor into the sample bias socket on the scanner's terminal block.
   - Slide the scanner into the chassis and close the enclosure door.

   Notes

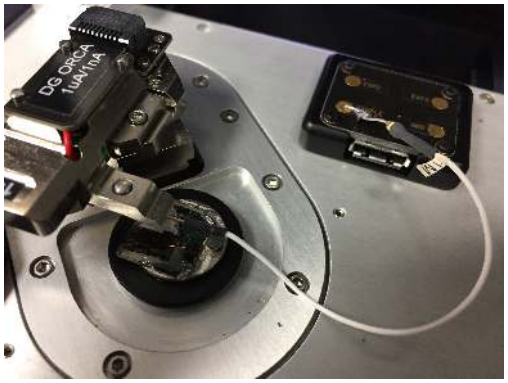
   - You may wish to double-check the lead under the cantilever clip to ensure it is not shorted to the holder body.
   - The enclosure acts like a Faraday shield, which will help reduce outside electrical noise.



3.

Option 2: Install the cantilever holder and bias sample directly

- As is noted here and in the Applications manual, having a resistor is beneficial as in a closed circuit, as the current will be much higher than normal. A resistor will help dissipate issues.
- Insert the ORCA holder into the scanner's engage pillar.
- Connect the bias wire to the scanner's terminal block, either by plugging the wire in or with a magnet, then connect the other end to sample.
- Slide the scanner into the chassis and close the enclosure door.



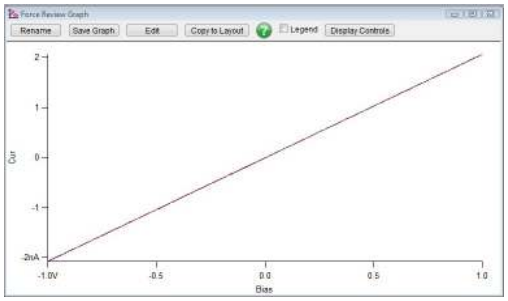
Notes

- You may wish to double-check the lead under the cantilever clip to ensure it is not shorted to the holder body.
- The enclosure acts like a Faraday shield which will help reduce outside electrical noise.

4.

Perform an I/V plot and confirm the correct current flow:

- Go to 'AFM Controls > Do IV' panel and open the I/V voltage controls.
- Press the 'Do I/V' button to perform an I/V curve.
- Confirm the current flow is the correct amount based on the test resistor value.
- Confirm the I/V plot is linear with 0 current flow coinciding with 0 volts of bias voltage.
- If the current is not flowing through 0, then recheck the current and voltage offsets are set correctly.



Note The 500M Ohm resistor should allow 2nA of current to flow for 1V of bias voltage.

12.5.0.1. Testing the first gain stage of a Dual Gain ORCA Amplifier

Testing the final output of a Dual Gain ORCA amplifier is done in the same manner as testing the Single Gain ORCA amplifier. Since the two gain stages are in series, the first gain stage is automatically checked by default.

You can verify that both the Current and Current 2 data channels are active in the software by monitoring both signals when doing an IV plot. When using the 500M Ohm test resistor, you will notice the limits of resolution

and noise in the Current 2 signal as compared to the Current signal. For more information, see Figure 12.3 on page 131.

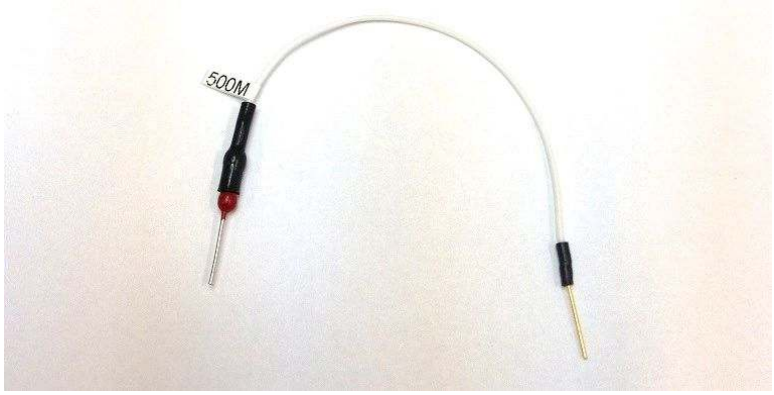


Figure 12.3.: 2nA current flow through a Dual Gain ORCA

The Dual Gain ORCA accessory kit includes a second 1M Ohm test resistor which is more suitable for the current ranges of the primary gain stage.

1. **Test the first gain stage of the Dual Gain ORCA Amplifier:**

   - Install the 1M Ohm test resistor.
   - Do an IV plot.
   - Monitor both the Current and Current 2 data channels.
   - Verify that the 1Meg Ohm resistor produces 1uA for 1 volt of sample bias.

   Note Notice the behavior of the Current signal as it saturates from too much current flow through the circuit. This test is not harmful to the ORCA amplifier. It is mainly a way of demonstrating the behavior of the circuit when the final gain stage is saturated.

13. Scanning Tunneling Microscopy (STM)

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

13.1. Introduction and Preparation

This is a fairly basic set of instructions on STM imaging with Cypher. In the future, we will provide an STM tutorial chapter which focuses on imaging graphite with atomic resolution.

This chapter assumes you are familiar with AFM techniques on Cypher. You should have first completed the tutorial in Chapter 7 on page 42 at least once.

13.2. Required Equipment

- Cypher Standard Scanner
- handheld digital voltmeter
- Cypher STM tip holder (see Figure 13.1 on page 133)
- STM tips
- jumper wire for applying bias to conducting sample
- some tiny magnets and some tweezers and a few tools

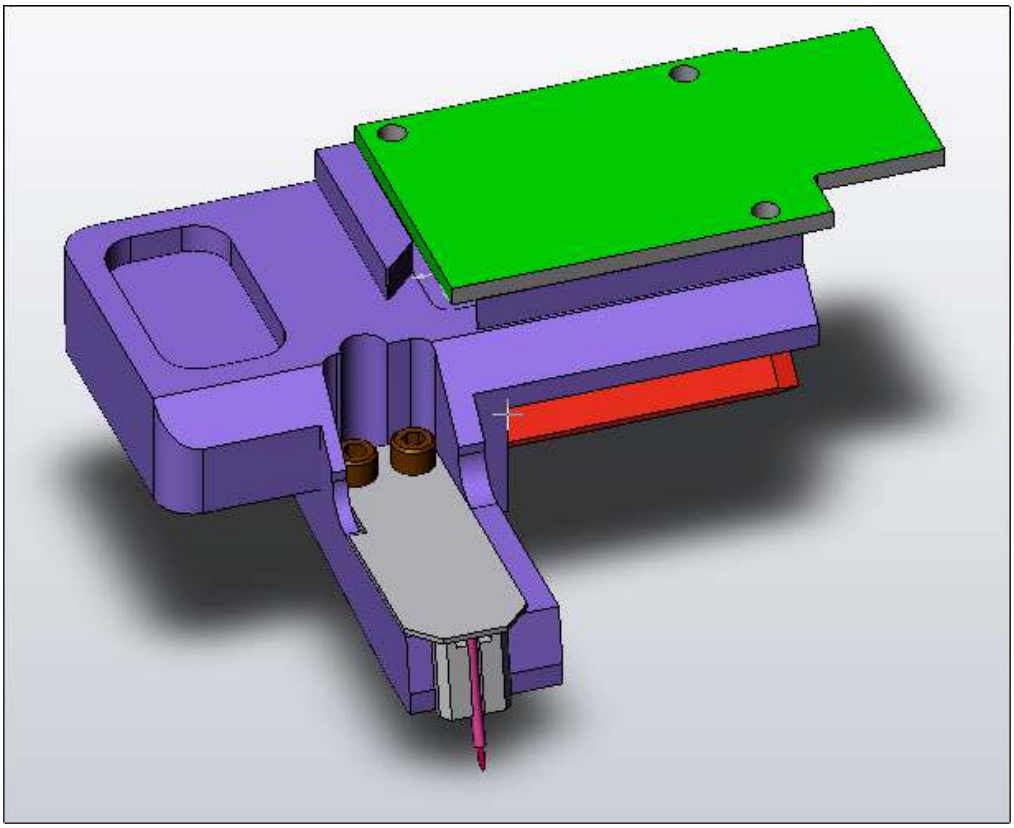


Figure 13.1.: Cypher STM tip holder. Note the probe tip sticking from the small tube.

13.3. Preparing an STM sample

1. Place your sample on a steel AFM disc. It's assumed the sample is conducting and has a relatively flat bottom.
2. Put some small dots of silver paint around the perimeter of the sample. Let the paint dry in a warm place, such as under a desk lamp.
3. Place the sample on the scanner.
4. Place a small magnet next to the sample. (The STM kit includes 5 magnets.)
5. Plug the bias wire into the 'Sample' socket on the scanner's terminal block.
6. Affix the other end of the wire to the magnet next to the sample. You can also attach the wire directly to the sample puck.

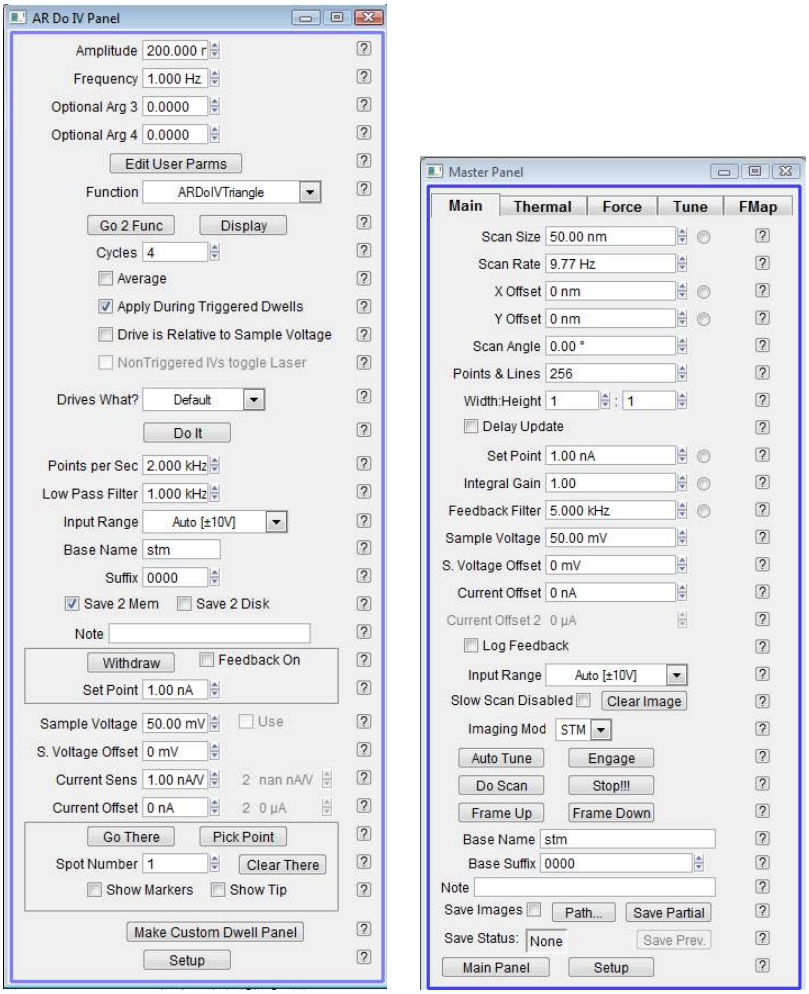
The surface onto which you place the magnetic AFM disc is black anodized aluminum. The anodization acts as an insulator which means the sample is only electrically connected to the attached bias wire. If you ever see any metal through the black surface (a possibility due to excessive wear, nicks, or abuse), the sample bias may not work properly. If that is the case, a thin insulating layer, such as a thin sheet of mica, can be placed under the sample disc.

Additional magnets and bias leads can be purchased separately if this mounting method is preferred.

Alternately, simply bonding a small length of wire directly to the sample puck with solder or silver epoxy works well. In many cases, fixing the sample and bias voltage connections are sample-specific. The sample socket on the terminal block is sized to accept the diameter of a standard 1/4-watt resistor lead or similar diameter wire for making your own bias leads.

13.4. Load the Tip

1. Locate your box of STM probe tips or see Section 13.10 on page 140 for information about making your own.
2. With tweezers, insert the STM probe into the holder. Note that the probes are straight and not curved; this is intentional. The tube in the STM holder is bent with a slight curve. This bend will cause the straight probe wire to fit tightly and reduce potential of drift due to the effects of stress in the wire. Push the probe wire into the tube until the end of the wire begins to extend out of the top of the tube. Don't touch the tip at any time!
3. Install the sample on the scanner.
4. Attach bias voltage lead from sample to 'Sample' socket on scanner's terminal block.
5. Insert the tip holder into the scanner and secure it with the 0.050" hex driver tool.



(a) DO IV Panel with highlights for STM

(b) Master Panel Main Tab set up for STM imaging

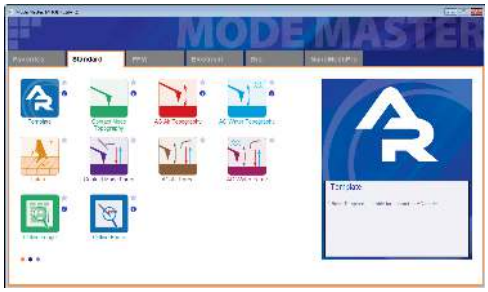
Figure 13.2.: Some relevant control panels you will encounter during STM operation.

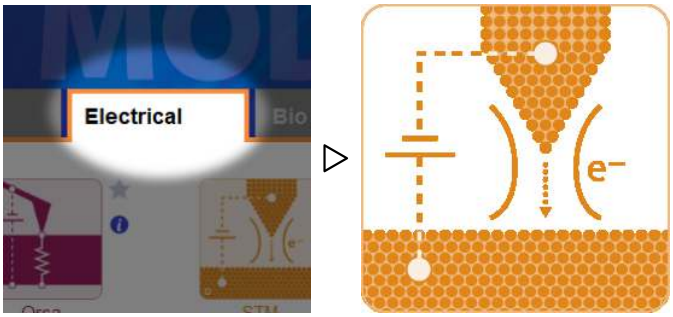
13.5. Zero Various Offsets

NOTE The specific values used on the screen shots in the following steps are often simply examples of what you might encounter. Read the instructions carefully and record your own values where necessary. The parameters used in this procedure are for the Graphite test sample provided.

1. **The Mode Master window:**

   - The software should now be showing the Mode Master window.
   - If not, click the 'Mode Master' button at the bottom of the screen: .



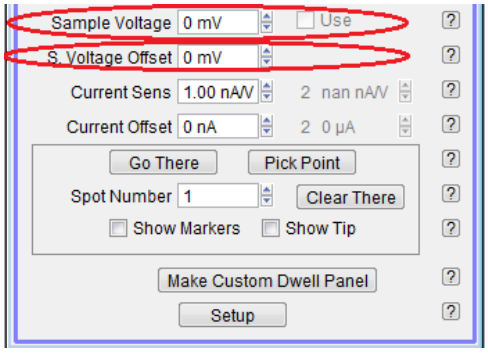
2. 

   Select Mode:

   - Select *Electrical tab > STM*
   - The screen then presents all the controls necessary for this type of AFM imaging.

3. **Set surface voltages**

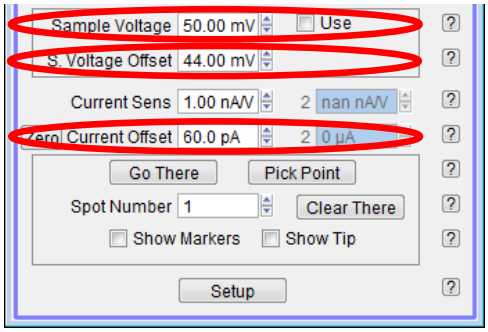
   - On the Do IV Panel, set both the Sample Voltage and the bias offset to "0V".



4. Use a hand-held digital voltmeter to measure the voltage between the Sample and Ground pins on the scanner's terminal block.

5. **Enter Bias Offset**

   - Enter the measured voltage for the sample bias offset, but the opposite sign to zero the offset voltage.
   - Enter “100mV” for the ‘Sample Voltage’ and click Enter.

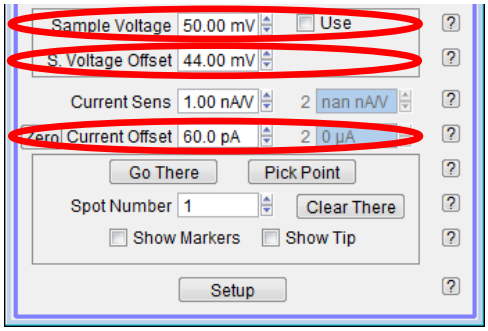


6. Use your digital voltmeter to measure the voltage between the Sample and Ground pins on the scanner terminal block again and verify that it also reads “100mV”. Try this a few more times for some additional surface voltage values to make sure the offset bias is doing its job.

7. When done, set the surface voltage back to “0V”.

8. **Enter Current Offset**

   - Go back to the AR Do IV panel.
   - Click on the zero button to the left of current offset. The current should now read “0” in the Sum and Deflection Meter.



13.6. Setup to Engage

NOTE The optics in the view module were intended for an AFM cantilever. Due to the tip position pointing down below the probe wire and focus distance of an STM probe from the objective being relatively long, it is necessary to bypass the normal AFM alignment process and simply bring the tip down manually close to the surface and then click the ‘Engage’ button.

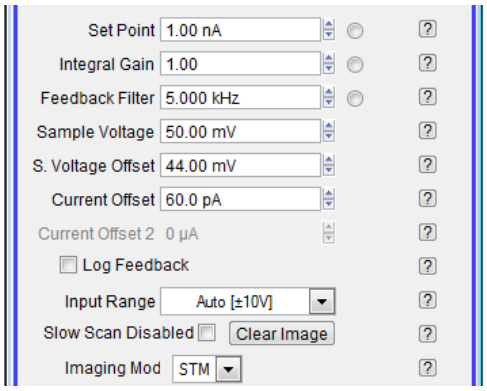
Use the wheel on the enclosure to move the tip to the sample. Get the tip to the desired engage distance of about 0.5 to 1 mm above the sample. Use the tip and the reflection of the tip in the sample surface as a guide to bring the probe close.

13.7. Set Scan Parameters

1. Go back to the Master Panel, Main Tab (see Figure 13.2b on page 134). You can enter your scan size and rate as in the figure and modify it once you are scanning.

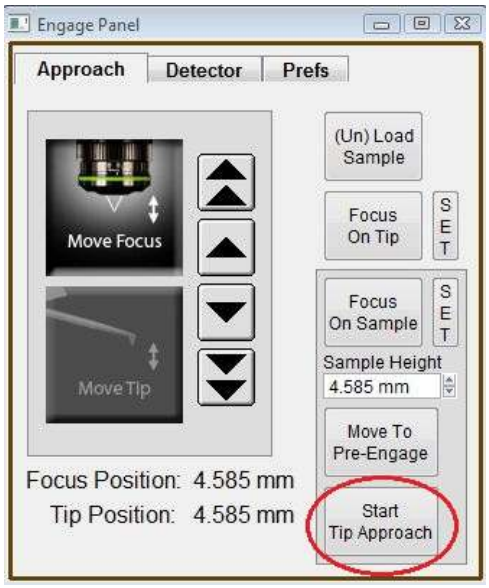
2. **Enter Feedback Parameters**

   - Set the surface voltage to the desired bias voltage for the sample. For Highly Oriented Pyrolytic Graphite (HOPG), use "~50mv".
   - Set the *Set Point* voltage to the desired tunneling current. Use "~1nA" for HOPG.
   - Set the Feedback Filter to 10KHz.
   - Set the *Integral Gain* to ~0.5 or 1.0. The STM feedback uses much less gain than typical AFM due to the use of 'Log Feedback'.



3. **Start Tip Approach**

   - In the Engage Panel, click the 'Start Tip Approach' button. Because the sample surface was not optically located, as we do with a typical AFM approach, this process may take a little longer than you are used to from AFM. In that case, Cypher will first rapidly motor-down to about 50 µm above the surface (measured from the optical image focus position) and then start its slower final engage process (for more information see the Q&A box on page 62). In the case of STM, the slower engage process starts from where you manually motored the tip.



4. Once on the surface, you can perform scans as you are used to with AFM.

13.8. STM IV Curves

The process of doing an STM IV curve utilizes a triggered force curve where the system will perform the following:

- trigger off of the Current channel
- dwell at the surface using the Z position sensor to hold the tip at a constant Z height above the surface
- ramp the bias voltage using the ramp functions in the Do IV panel

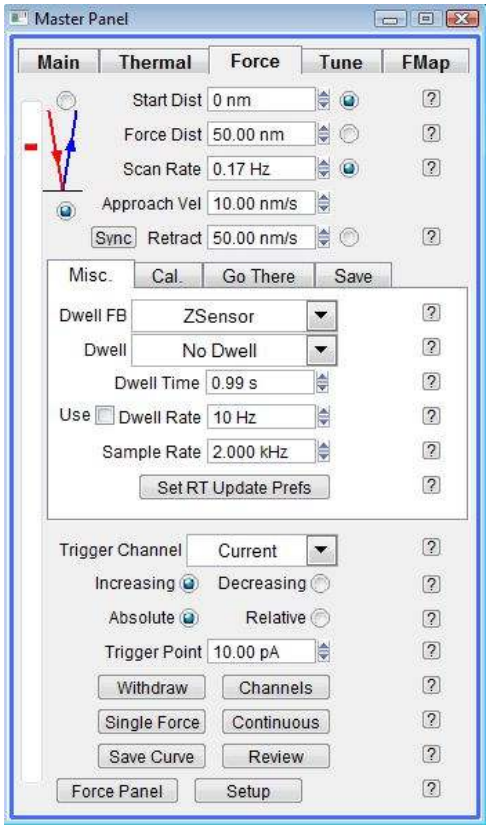
13.9. Set IV Parameters

1. The *Feedback Filter* must be reduced to 1kHz in the *Master Panel*. Typically for scanning, the feedback filter is between 5kHz and 10kHz, but for IV measurements when triggering on currents as low as 10pA (in a quiet lab), high frequency signals must be filtered.

2.

Trigger Channel Current

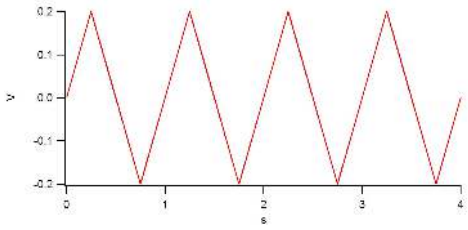
- Click on the *Force* tab and set the desired trigger point. The trigger point is the setpoint current where the system establishes the tip height during IV measurements. The lowest trigger current possible is ~10pA, any current lower than this will be close to the noise limit and cause the system to false engage.
- To reduce the approach velocity, the *Force Dist.* is set to 50nm and *Scan Rate* ~0.2Hz. This is necessary to avoid the tip moving beyond the height when the feedback loop clamps the Z-position. A sudden change in tip speed can cause the tip to overshoot.



3.

AR Do IV Panel

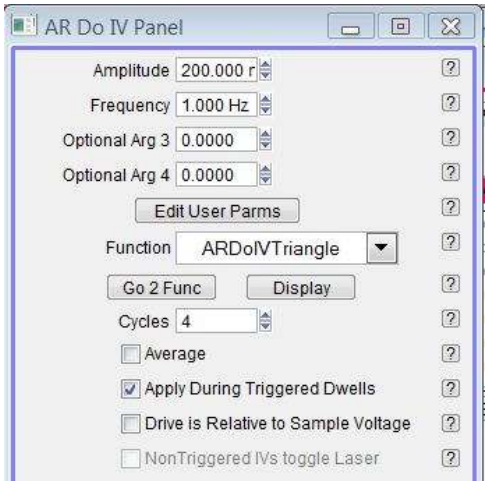
- Click on the 'Display' button in the AR Do IV Panel to display the bias ramping waveform.



4.

Drive Graph

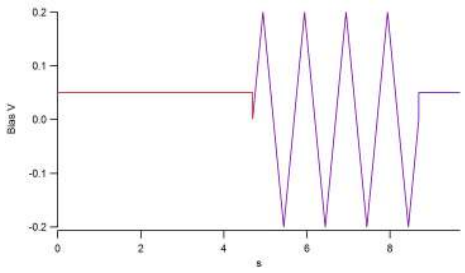
- Adjust the *Amplitude*, *Frequency*, *Optional Arg 3* (Phase Offset), *Optional Arg 4* (Voltage Offset) parameters to make the wanted ramping waveform.



5.

Drive Graph

- This is the result of the ramp during IV measurements. Notice that the voltage before and after the ramp is not 0v. The voltage used for the triggered current (in this case, 50mV) is kept before and after the ramp.

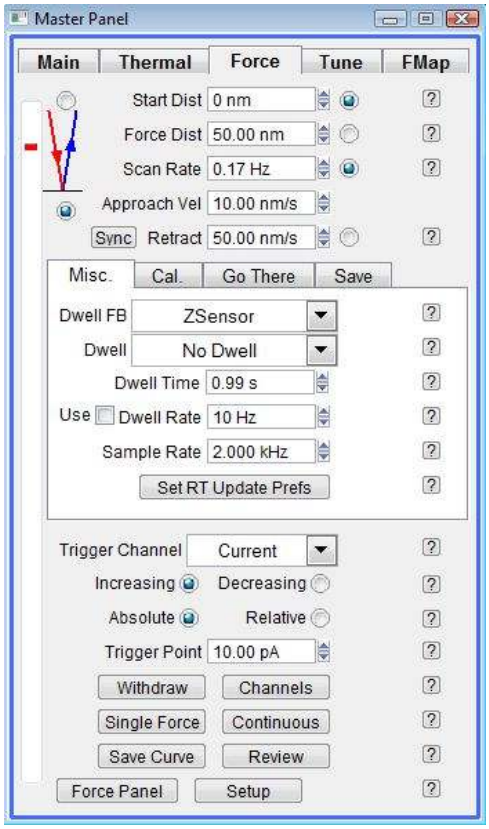


6.

Finding the Surface There are two ways to bring the tip close to the surface for IV curves:

a) Click 'Engage' in the Master Panel. When the tip is on the surface, hold the shift button and left-click mouse button on the vertical bar for the z-range piezo. This will set the force range to be close to the surface. Make sure the Integral Gain is low enough such that the tip does not oscillate.

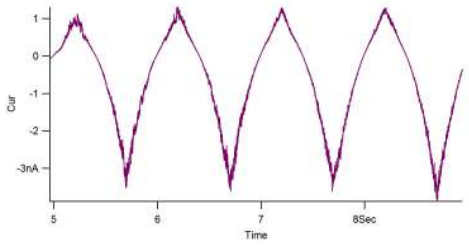
a) The other method is to change the *Force Distance* to "300nm", and then click 'Single Force' (50nm force distance will take too long). Make sure to switch the *Force Distance* back to "50nm" when making measurements.



7.

Single Force

- Click the 'Single Force' button to perform individual IV measurements.
- This executes a triggered force curve and dwell on the surface using the Z sensor while running the ramp waveform from the Do IV Panel.

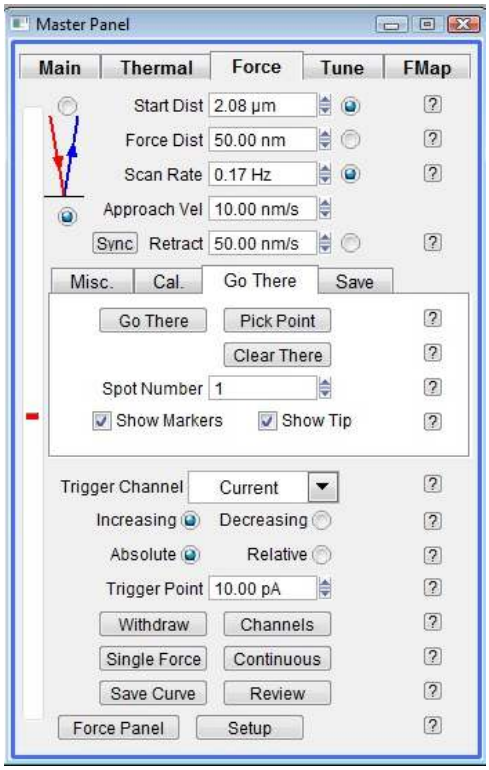


8.

Point and Shoot Tip positioning

- Engage and image the sample surface.
- Click 'Stop' or 'Withdraw' to pull the tip away from the surface.
- Select 'Show Markers' and 'Show Tip'.
- Select *Sights* on the image to do a *Force Curve*, and then click *Pick Point*.

- Click the 'Single Force' button at each point, and then click the 'Go There' button (both buttons are located on the *Go There* tab) to move for each numerical location.



Note	A 10pA trigger current works only if the system is acoustically well isolated. If the trigger point is reached before the tip is on the surface, increase the trigger current greater than 10pA. Try 20pA or 50pA.

Note	If the magnitude of the current increases with time during the cycles, this is probably due to thermal drift. If the temperature of the sample is still equilibrating, there could be expansion of the sample which will increase the tunneling current.

13.10. STM probes

The Cypher STM kit comes supplied with 20 mechanically formed (i.e. carefully clipped with super sharp wire cutters) probes. Additional probes can be purchased from Asylum Research. If you wish to make your own probes, the material and dimensions for making the supplied probes are:

- Material: 80%/20% Platinum Iridium. Wire should be drawn straight. Wire cut from a roll has a small radius and may not hold tightly into the tube on the STM holder. The tube is bent with a large radius. This is intentional to help reduce drift due to the stress of bending the probe wire upon insertion into the holder.
- Wire size: 0.01" diameter (0.25mm) , cut approximately .2" (5mm) long.

Contact Asylum Research about additional tools and techniques required to make the proper cuts.

Attention	Longer probes can be used but may introduce image distortion from drift due to the length.

Attention	The approximate range of the camera focus is about 3mm below the underside of the tip holder tube. Tips that extend below 3mm will not allow the sample to be viewed.

13.11. Troubleshooting

13.11.1. Testing the STM holder

A 500M Ω resistor is supplied in the STM accessories kit. The resistor is soldered to a short length of wire terminated by some Pt Ir probe wire.

To test the holder:

1. Insert the platinum wire into the tip tube.
2. Plug the resistor into the Sample socket in the terminal block.
3. Set the surface bias to “1V”. Note the measured current. It should be 2nA (1/500e6 Ω).
4. Use the Test panel (email Support@AsylumResearch.com for details on loading this software).
5. Use the Noise tab to measure the current noise of your holder. The typical noise should be ~8mV (~8pA) Adev from 1hz-1kHz, with little perceivable periodic noise in the spectrum.

13.11.2. The Current2 Signal

The initial current to voltage conversion takes place in the first stage of a two-channel op amp. The first gain stage, labeled Current2 in the data channels, has an output sensitivity of 20nA/V. In most cases, this signal is not suitable for feedback but can be monitored as well as the final 1nA/V final stage if desired. The reason for this signal is derived from the design of the STM amplifier that is the same basic circuit as the ORCA - CAFM cantilever holder.

14. Fast Force Mapping

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Chapter Contents

Fast Force Mapping (FFM) is a deflection (sub-cantilever resonance frequency) technique that allows you to acquire data (topography, adhesion, mechanical, electrical properties, etc.) on a very wide range of materials. In contrast to AC Mode or Contact Mode, the principle behind FFM is based on force/deflection feedback force curves performed at rates much higher than traditional force curve maps. Force curves are acquired at frequencies ranging from 10 Hz up to 300 Hz (for MFP-3D Infinity AFMs only) and up to 1000 Hz (for all Cypher AFMs) while a feedback loop is using the maximum force detected (deflection) for each force curve. Each force curve is recorded and analyzed to extract topography information and mechanical properties of the sample. Additional signals, such as electrical conductivity, can be measured to obtain supplementary information about the sample (through use of the ORCA cantilever holder). The main advantage of the FFM technique is the much faster data acquisition, compared to standard force mapping, while still capturing all the information provided by force curves.

A sinusoidal driving voltage applied to the Z-actuator is used to oscillate the probe at frequencies ranging from 10 Hz to 300 Hz (Infinity) and 1000 Hz (Cypher). For each force curve, the maximum deflection signal, read on the photodetector, is used to calculate the Z-height measured by the Z-sensor. Maximum force (Max Force) is calculated in real-time and is used as error signal for Z feedback loop. While the probe is driven (sinusoidal wave) in Z, the sample is moving underneath it in a raster pattern in the XY plane. The resolution of the image is determined by the number of points in each scan line.

The software continuously digitizes Deflection, Z-Sensor, Z-Voltage, and Current (or Current2) signals at 2MHz (Cypher) or 500kHz (Infinity).

Each force curve is analyzed to provide sample topography and its Young's modulus (based on a chosen model and parameters). If a bias is applied to the sample and a conductive tip is used, ORCA measurements can be performed at the same time.

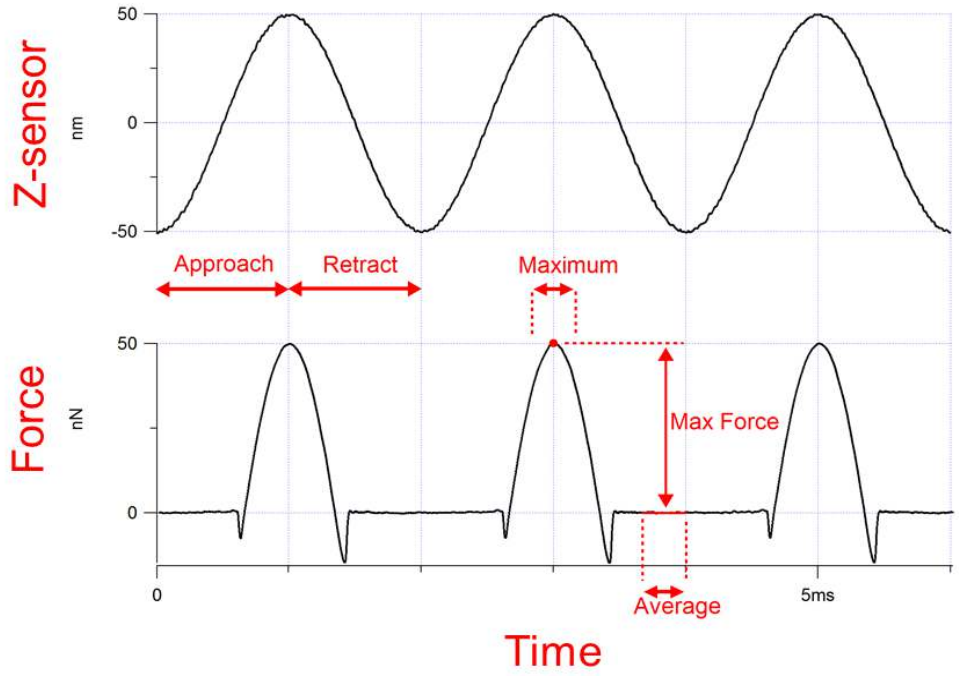


Figure 14.1.: Plot of cantilever oscillation measured by the Z-sensor (excited by driving voltage) (top) and the resulting force (bottom) when the tip is pushing on a hard surface. The 'Max Force' arrow indicates how the setpoint force is calculated for each force curve.

14.1. Required for Fast Force Mapping

Fast Force Mapping is an optional imaging technique and requires a software license to be activated. Software version 16 or higher is required to run FFM mode.

14.2. Fast Force Mapping in Contact Mode

Fast Force Mapping in Contact mode is a technique based on contact-mode; therefore, you need to choose a probe with a spring constant matching the stiffness of the sample.

Items used in this demonstration include:

- Polystyrene/Polypropylene thin film sample
- AC 200 Olympus probe with ~150 kHz resonance and 10 N/m spring constant

14.2.1. FFM automatic experimental setup: GetStarted

Automatic imaging setup: GetStarted

1.
- In the Mode Master menu, select *NanomechPro*, and then select *Fast Force Map*.
- FFM mode automatically starts in the GetStarted configuration.
- Follow the instructions to set up your experiment.
- You will be asked to set initial imaging parameters (look for descriptions below):
  - scan size
  - scan points & lines
  - approximate sample roughness
  - Z-rate
  - setpoint
  - force distance
  - sample stiffness
- Some of the above parameters are adjusted automatically depending on your input.

Specific parameters for FFM: GetStarted routine

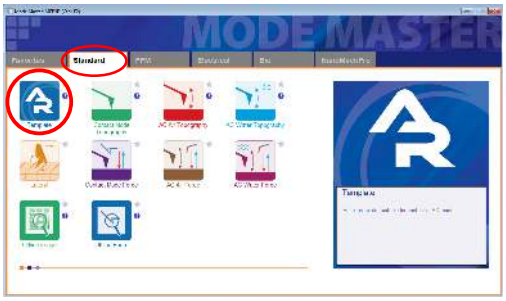
2.

- Scan Points & Lines
  - The Scan Points & Lines parameters determine the number of scan points (pixels) in each scan line and the number of lines in the scanned area. In contrast to other techniques, during FFM, the number of force curves corresponds exactly to the number of pixels in the image. For comparison, during AC mode, the tip oscillates at a much higher frequency than the number of pixels. Therefore, for each pixel, it is the average value of the amplitude of oscillations that is measured by the feedback loop. To improve the resolution of images, these two parameters can be increased, however the acquisition time will increase at the same time.
    - Acquisition time = (lines x points per line x 2.5)/ Z rate
- Sample Roughness
  - The Sample Roughness parameter is used to determine the force distance. It should be chosen to roughly represent the height difference, the lowest and highest features on the sample.
- Z-Rate
  - Z-rate is the ramp rate at which the force curves will be performed, and it is how fast the Z-actuator is moving up and down above the surface. This parameter can be increased until you see instability in the force curve (the instability arises when the deflection is changing rapidly and the setpoint cannot be met on all curves). On Infinity, the maximum Z-rate is 300 Hz. On Cypher (S or ES), the maximum Z-rate is 1000 Hz.
- Setpoint
  - The Setpoint parameter is the setpoint value used for feedback. Maximal force is calculated in real-time and used as an error signal for the Z-feedback loop. When the setpoint value is reached, the lever is pulled away from the surface. The setpoint value is displayed in units of Newton and Volt (they are related by the cantilever calibration values InvOLS and k). The Newton value provides information about the amount of normal force applied on the sample. The Volt value can be compared with the deflection value displayed on the Sum and Deflection Meter panel. For example, before engaging on the surface, the deflection should read 0 Volt and the setpoint should be set to a value (Volt or Newton) greater than 0. The setpoint value is also used to obtain an image of the surface topography. For each pixel, the software measures the Z height at which the setpoint value was reached. The Z height for each pixel is then used to map the topography of the surface.
- Force Distance
  - Force Distance is the distance the piezo will travel during the force curve (extend or retract portion). This distance should match (or exceed) the topography variations of the sample.
- Sample Stiffness

14.2.2. FFM experimental setup by the user

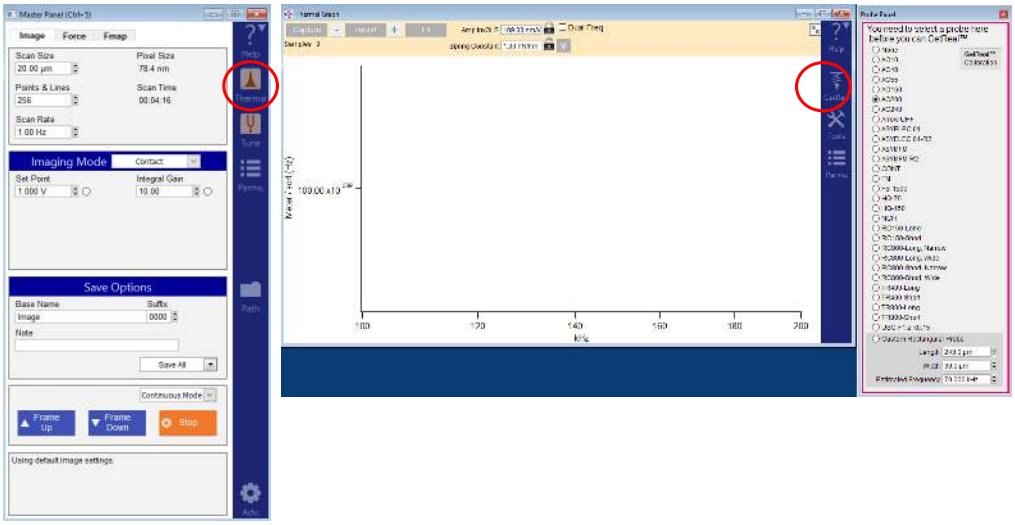
1. **Mount probe and start software:**

   - Place the probe in the cantilever holder and mount it in the instrument.
   - In the Mode Master menu, select *Standard* and then *Template*.



2. **Align laser:**

   - Open the Video Panel.
   - Use the arrows in the Video Panel to move the laser spot onto the cantilever and maximize the SUM signal.

3. 

   Calibrate probe:

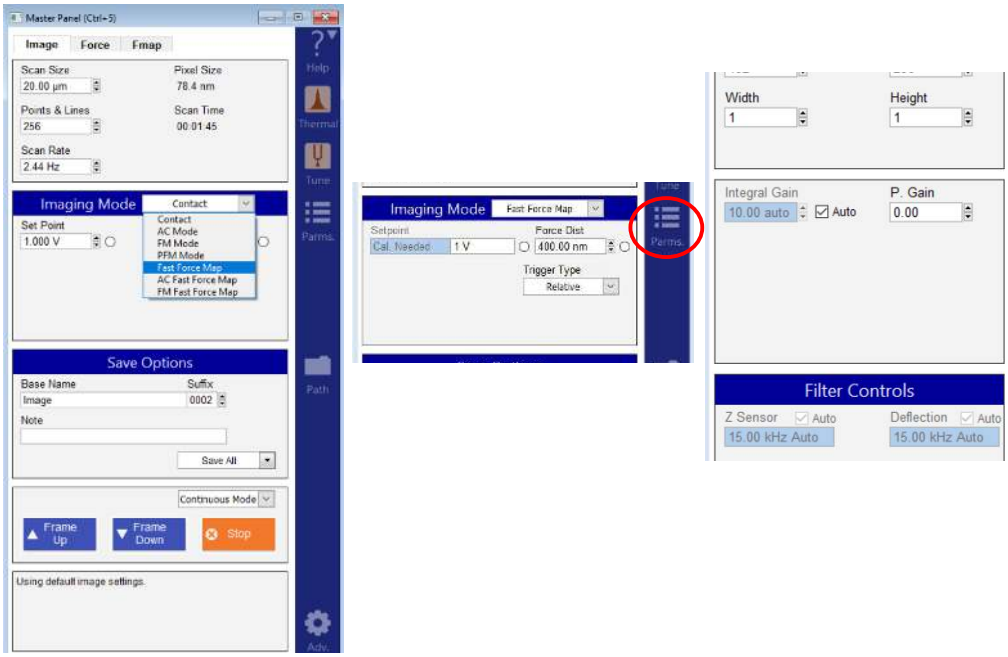
   - Click the ‘Thermal’ icon in the Master Panel. The thermal graph appears.
   - Click the ‘GetReal’ icon which will open the Probe Panel.
   - Select the probe you are using for the experiment.
   - Click the ‘GetReal Calibration’ button.
   - Once the calibration has completed, the Amp InvOLS and Spring Constant values at the top part of the thermal graph are updated.

4.

Approach the sample surface:

- In Master Panel (see screenshot in previous step):
  - Imaging Mode: Contact
  - Set Point: 0.2 V (for this particular probe and sample)
- In the Engage Panel (see screenshot at right):
  - Set the tip position while focused on the tip.
  - Set the sample position while focused on the sample.
  - Click 'Start Tip Approach'.

5.



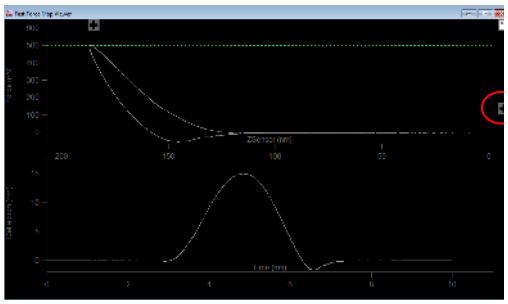
Switch to FFM mode and start imaging:

- In Master Panel, switch from *Contact Mode* to *Fast Force Map*.
- The Master Panel now displays parameters related to FFM, as follows:
  - Set Point is now displayed in units of Newtons and in Volts: set it to 10 nN
  - Force Distance: 200 nm
  - Trigger Type: Relative
- Set the other parameters in the Master Panel as follows:
  - Scan Size: 5 µm
  - Scan Points & Pines: 256
  - Z-rate: 500 Hz
- The Gains are located in the Parms panel (rightmost).
- Click 'Engage' in the Sum and Deflection Meter panel.

6.

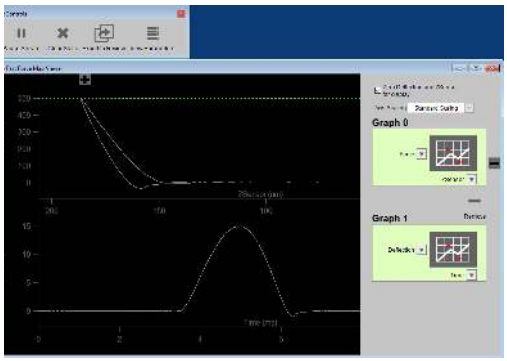
Engage:

- As the tip engages on the surface, the Fast Force Map Viewer appears.
- Display choices are available by clicking the "plus" sign (as shown at right).



7. **Real-time force curves:**

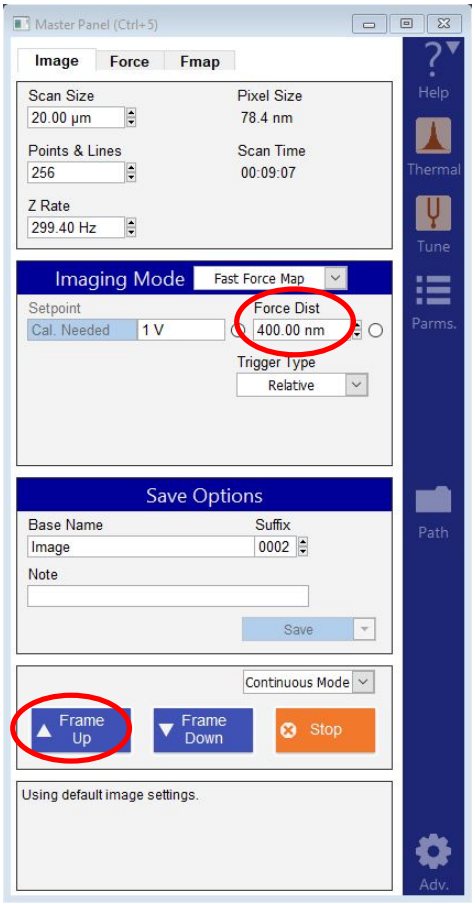
- The Fast Force Map Viewer can show up to two channels.
- Each graph has several options for each axis, in this example:
  - Graph 0 is Force versus Z-sensor.
  - Graph 1 is Deflection versus time.
- The green line on the top graph indicates the setpoint.
- The top controls allow for superposition of several already acquired curves over the real-time curve.

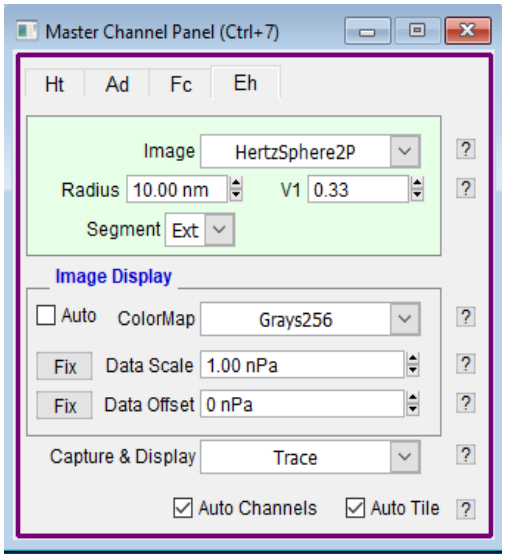


8.

Imaging parameters:

- Four Channels can be collected simultaneously during FFM imaging, including:
  - Ht - Height
  - Ad - Adhesion
  - Fc - Max Force
  - Eh - Calculated modulus channel. Model (here Hertz) and tip radius must be adjusted prior to imaging.
- Adjust the force distance (Force Dist.) and setpoint.
- When the imaging parameters are optimized, and the force curves look good in the FFM viewer, click 'Frame UP' to start imaging.





14.3. Fast Force Mapping in AC mode

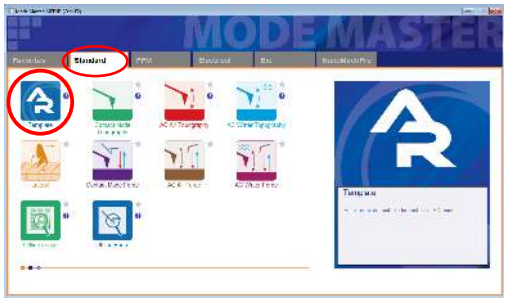
The following instructions are for Fast Force Mapping in AC mode (intermittent contact mode or tapping mode) and are intended for imaging a flat sample in liquid.

1. **Prepare:**

   - You will need the following items:
     - Fresh calcite sample
     - ArrowUHF AuD probe
   - Clean cantilever holder that will be used for imaging (either liquid holder or perfusion holder).
   - Make sure that the software version is 16 or higher and that the FFM mode is enabled.
   - Use blueDrive for cantilever drive and place the 0.1x filter cube in the laser's path.

2. **Mount probe and start software:**

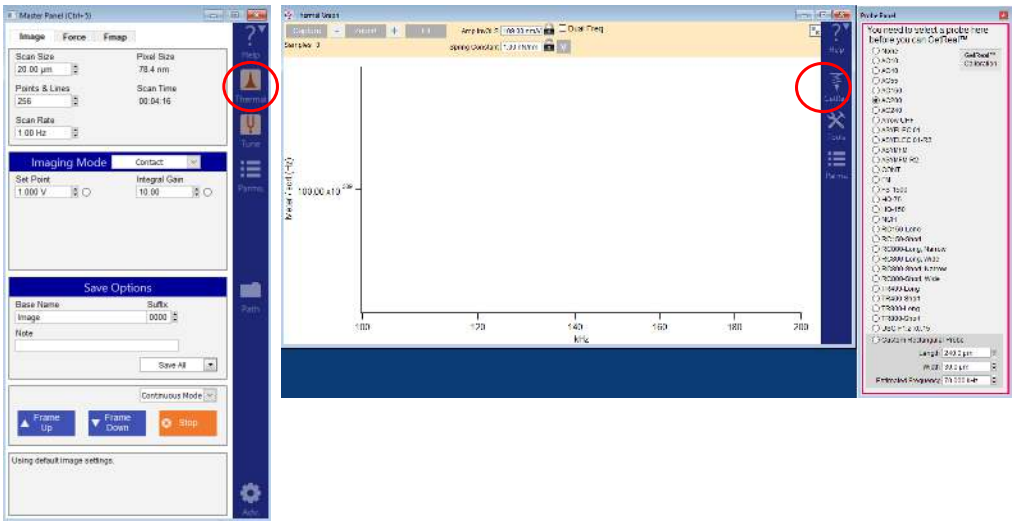
   - Place the probe in the cantilever holder and mount the holder on the scanner.
   - In the Mode Master menu, select *Standard* and then *Template*.



3. **Align laser:**

   - Open the Video Panel.
   - Use the arrows in the Video Panel to move the red laser spot onto the cantilever. Try to maximize the SUM signal.

4.

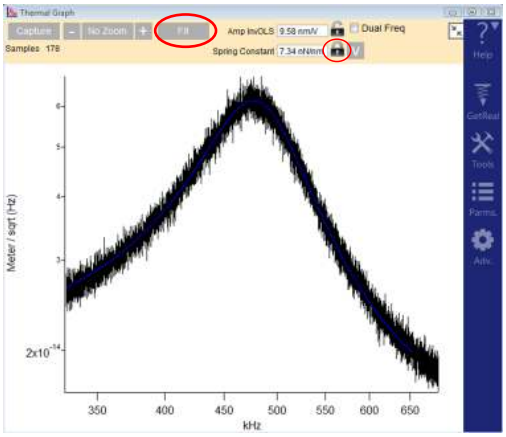


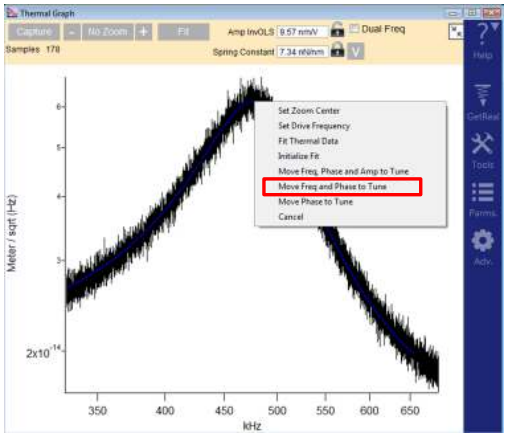
Calibrate Probe (in air):

- Click the 'Thermal' icon in the Master Panel
  - thermal graph will appear
- Click the 'GetReal' icon which opens the Probe Panel.
- Choose the probe you want to use for the experiment.
- Click 'GetReal Calibration'.
- Once the calibration is completed, the Amp InvOLS and Spring Constant values at the top part of the thermal graph are updated.

5. Remove the cantilever holder and place the freshly cleaved calcite sample on the scanner.
6. Add a drop of water to the sample.
7. Put a drop of water on the probe and place the cantilever holder back on the scanner.
8. While looking at the distance between the probe and the sample, approach the sample to the tip so that both water drops join, and the sample and lever are both in water environment.
9. Refocus and realign the laser on the cantilever (now in liquid) - Sum should be ~ 6V.

10.

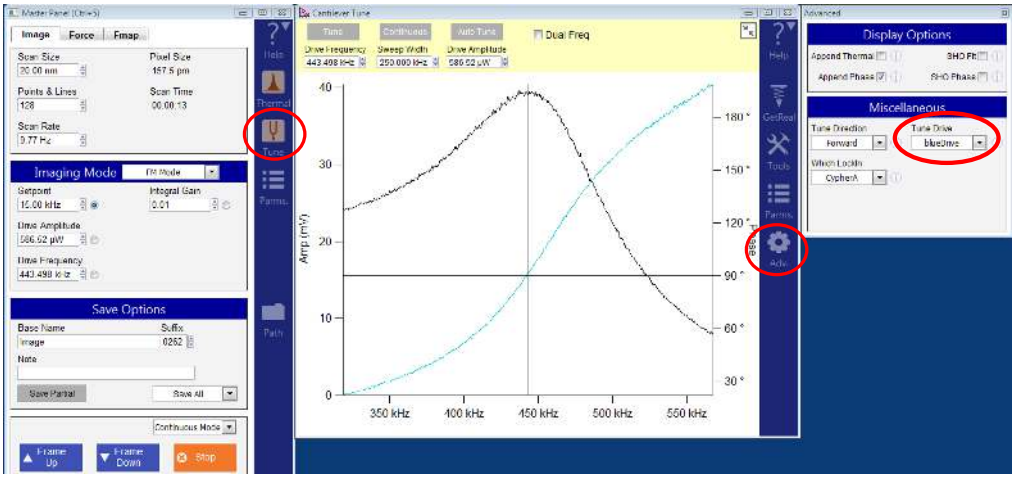




Capture a thermal of the cantilever in liquid:

- Make sure that the padlock besides Spring Constant is locked.
- Refit the thermal data to obtain the updated (water) InvOLS value.
- Transfer the frequency of thermal peak to the tune panel as follows:
  - Right-click on the peak of the Thermal Graph and select *Move Freq and Phase to Tune*.

11.

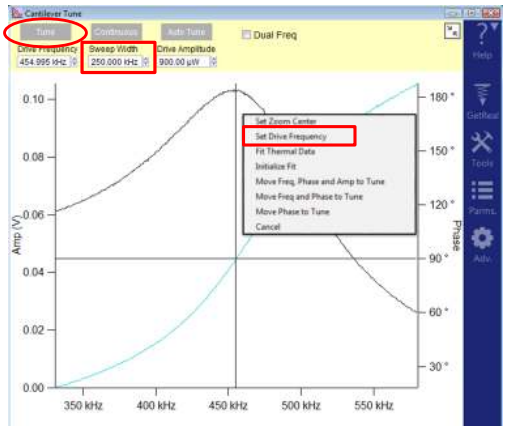


Turn on blueDrive photothermal excitation:

- In the Tune graph, click the 'Adv.' (gear) icon
- In the Advanced panel, select *blueDrive* from the Tune Drive drop-down menu.

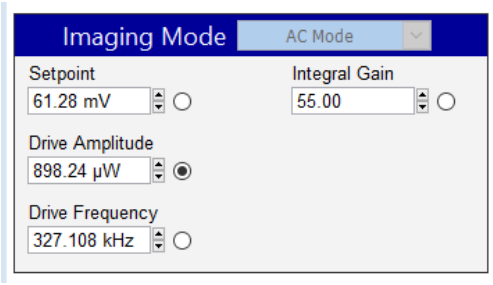
12. **Set up Tune panel:**

- Set the 'Sweep Width' to "250 kHz".
- Click the 'Tune' button.
- When the tune is done, right-click on the peak to select *Set Drive Frequency*.
- Move the blue laser spot around the base of the cantilever to maximize the amplitude.
- Adjust the 'Drive Amplitude' (in the Master Panel) until the amplitude reaches ~ 80 mV (visible on the graph and in the Sum and Deflection Panel).



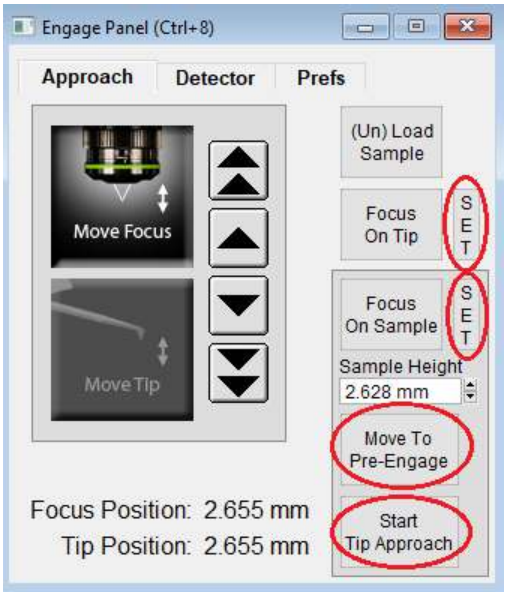
13. **Master Panel settings:**

- Set the 'Setpoint' to ~ 60 mV.
- Set the Integral Gain to "55".



14. **Engage Panel: Approach tip:**

- While focused on the tip set the Tip Position by pressing the 'SET' button next to the 'Focus On Tip' button.
- While focused on the sample, set the sample Focus Position by pressing the 'SET' button next to the 'Focus On Sample' button. This will update the Sample Height value.
- Click the 'Move to Pre-Engage' button.
- Click the 'Start Tip Approach' button.



15. Once the tip is in piezo range of the sample, capture another Thermal Tune with Z-piezo set at "0V" (sample is 2-3 um from surface).

16. Lock padlock besides spring constant on Thermal Graph to recalculate Amplitude InvOLS.

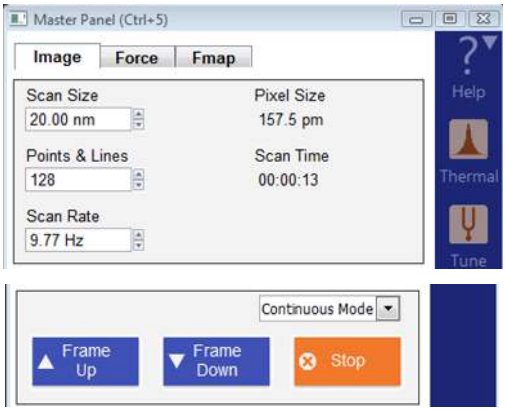
17. Right-click on the peak in Thermal Graph to select *Move Freq and Phase to Tune*.

18. When the tune is complete, right-click on the peak and center phase.

19.

Set imaging parameters on the Master Panel:

- Set the Scan Size to “20 nm”.
- Set the Scan Rate to “10 Hz”.
- Start imaging by pressing either ‘Frame Up’ or ‘Frame Down’.



20. Start imaging as follows:

a) Decrease the free amplitude (by decreasing ‘Drive Amplitude’ in the Master Panel).

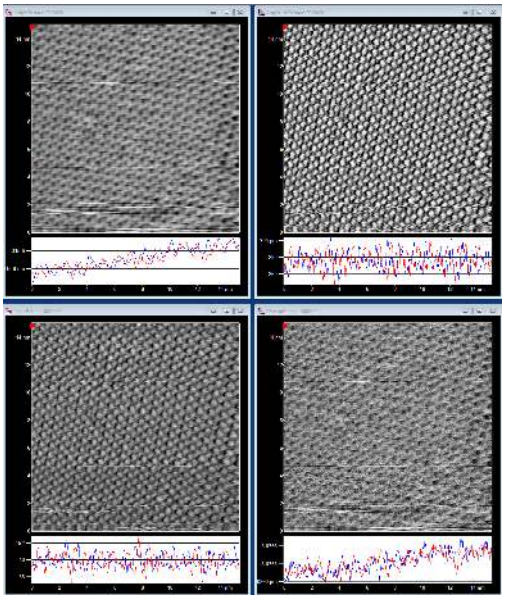
b) Decrease the ‘Setpoint’ until Trace and Retrace overlap each other.

c) Repeat a and b several times to image with the lowest amplitude possible.

21.

Imaging

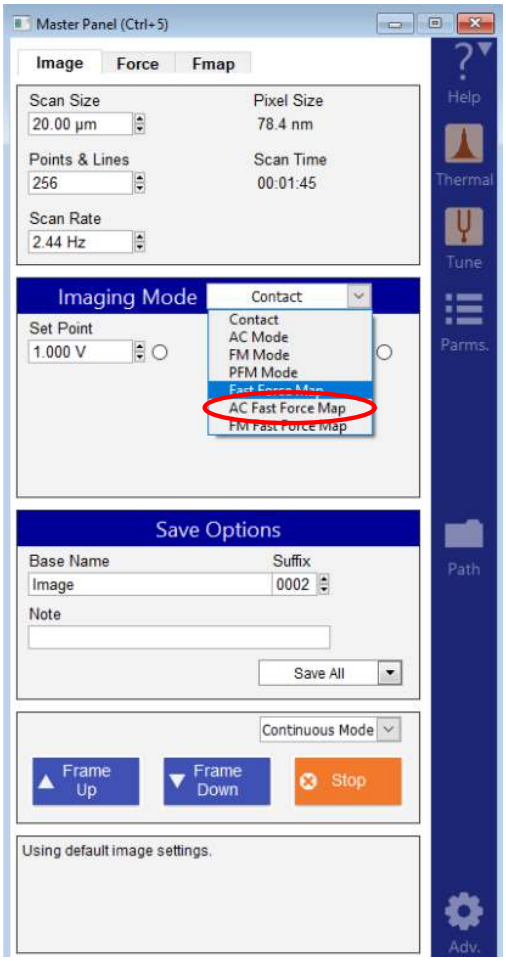
- Acquire a 20 nm image and resize the scan to “10 nm”.
- Channels that are acquired include:
  - Height
  - Amplitude
  - Phase
  - Z-Sensor
- Once the imaging looks optimized, stop the scan, and switch to *AC FFM* mode.



22.

Imaging mode AC FFM:

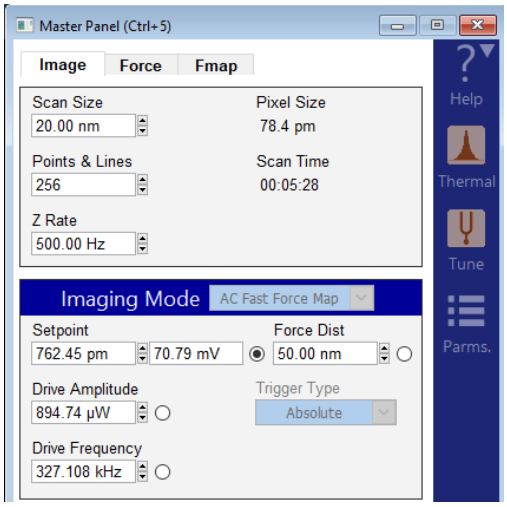
- Switch to *AC Fast Force Maps* from the Imaging Mode drop-down menu in Master Panel.



23.

Imaging Parameters

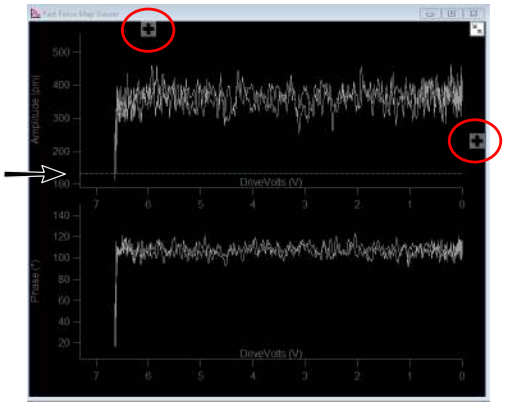
- The Master Panel now display parameters related to AC FFM mode, as follows:
  - Setpoint is now shown as units of meters and in Volts. Set it to “70 mV”.
  - Force Distance: Set to 50 nm
  - Trigger Type: Absolute
- Set the other parameters in the Master Panel, as follows:
  - Scan size: 20 nm
  - Scan Points & Lines: 256
  - Z-rate: 500 Hz
- The Gains are located in the Parms panel. Set to “10”.
- Click ‘Engage’ in the Sum and Deflection Panel.



24.

Engaging:

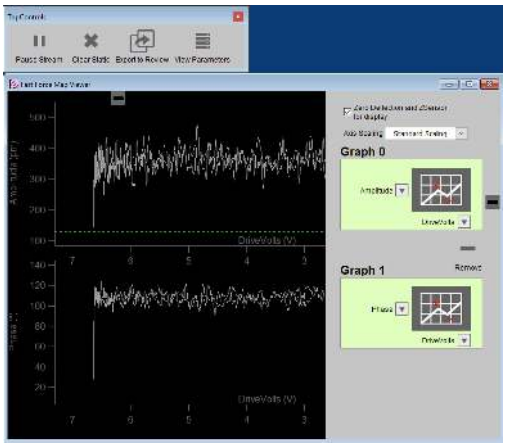
- As the tip engages on the surface, the Fast Force Map Viewer appears.
- Display choices are available by clicking the “plus” sign, as shown at right.
- The arrow in the top graph indicates the Setpoint value.



25.

Real-time force curves:

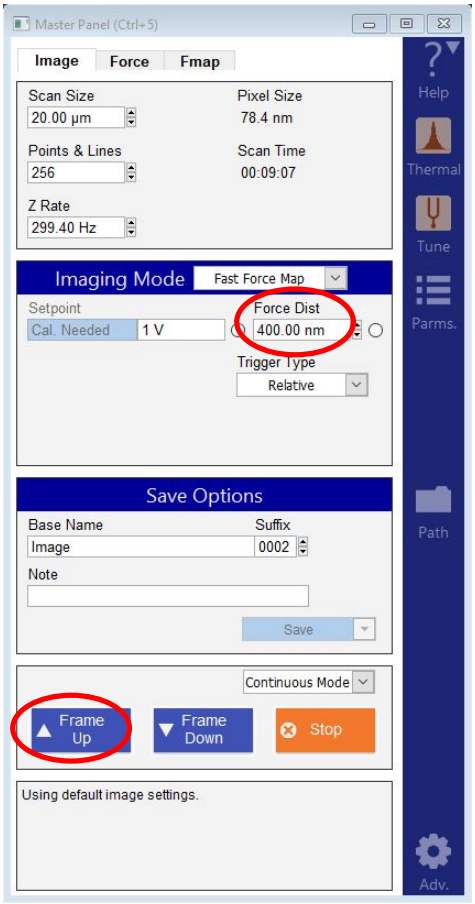
- The Fast Force Map Viewer can display up to two channels (graphs).
- Each graph has several options for each axis. You select the appropriate axes for the imaging mode. For AC FFM, choose:
  - Graph 0 (top): Amplitude (pm) vs DriveVolts (V)
  - Graph 1 (bottom): Phase (°) vs DriveVolts (V)
- The top controls allow for the superposition of several already acquired curves over the real-time curve.

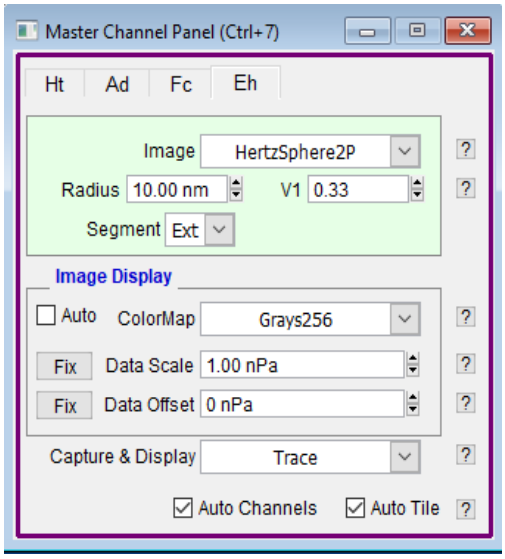


26.

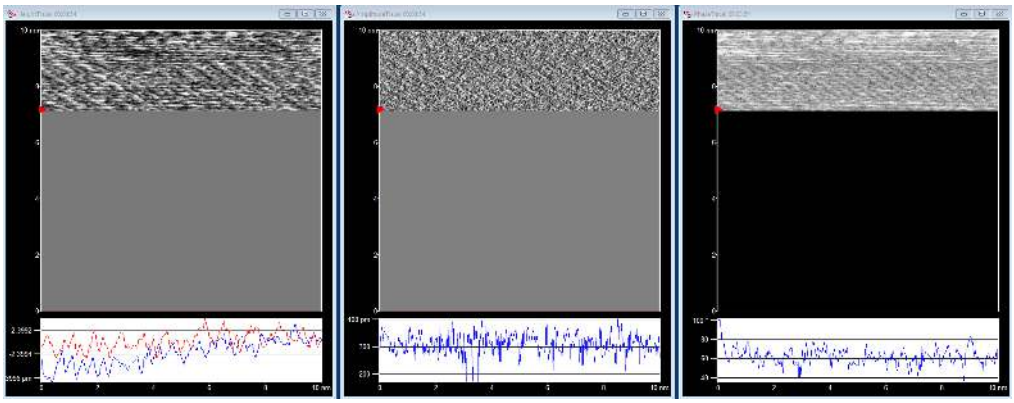
Imaging parameters:

- Adjust the force distance and setpoint until the curves in the FFM viewer look good.
- When the imaging parameters are optimized, click 'Frame UP' on the Master Panel to start imaging.





27.



Imaging data collection:

- Data is collected in three channels during AC FFM:
  - Height
  - Amplitude
  - Phase

14.4. Fast Force Mapping in FM mode

The following instructions are for Fast Force Mapping in Frequency Modulation (FM) mode and are intended for imaging a flat sample in liquid. A freshly cleaved calcite sample and clean deionized water are used for this demonstration.

You are guided through the following steps:

1. Calibration of a probe.
2. Acquisition of an AC mode image. This step allows you to determine if the probe is sharp and if the area of interest is flat/clean.
3. Acquisition of AC force curves. This step allows you to approach the sample to a defined distance.
4. Acquisition of FM curves. This step allows you to check FM gains.
5. Acquisition of FM FFM images and force curves.

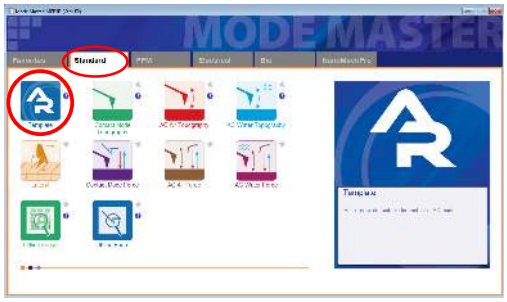
14.4.1. Preparation and Calibration

1. **Prepare sample, holder, and software:**

   - You will need:
     - Fresh calcite sample
     - Arrow UHF AuD probe
   - Clean the cantilever holder that will be used for imaging (either liquid holder or perfusion holder).
   - Make sure that the software version is 16 or higher and that the FFM mode is enabled.
   - Use blueDrive for the cantilever drive and place the 0.1x filter cube in the laser's path.

2. **Start software:**

   - Place the probe in the cantilever holder and mount the holder on the scanner.
   - In Mode Master menu, select *Standard* and then *Template*.

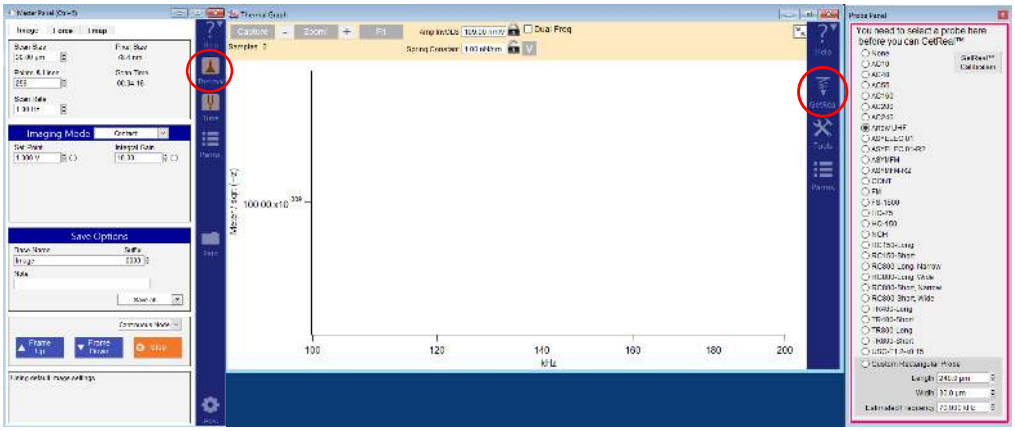


3.

Align Laser

- Open the Video Panel.
- Use the arrows in the Video Panel to move the red laser spot onto the cantilever. Try to maximize the SUM signal but keep the laser spot at the end of the lever (see image at right).

4.



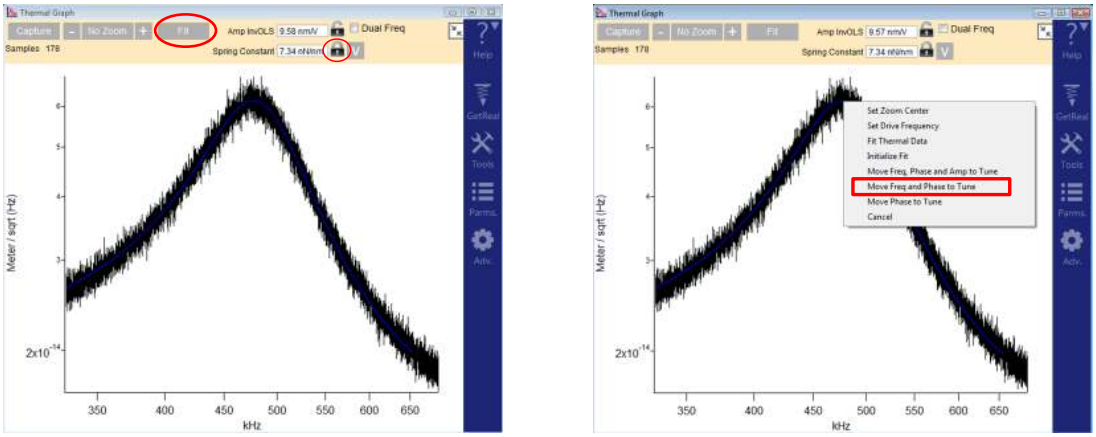
Calibrate Probe (in air)

- Click the 'Thermal' icon in the Master Panel. The thermal graph appears.
- Click the 'GetReal' icon which opens the Probe Panel.
- Choose the probe that is used for the experiment. For this experiment, select 'Arrow UHF'.
- Click 'GetReal Calibration'.
- Once the calibration is completed, the Amp InvOLS and Spring Constant values at the top part of the thermal graph will be updated

5. Remove the cantilever holder and place a freshly cleaved calcite sample on the scanner.
6. Add a drop of water onto the sample.
7. Add a drop of water onto the probe and place the cantilever holder back on the scanner.
8. While looking at the distance between the probe and the sample, approach the sample to the tip so that both water drops join.

9. Refocus and realign the laser on the cantilever (now in liquid). The Sum should be ~ 6V.

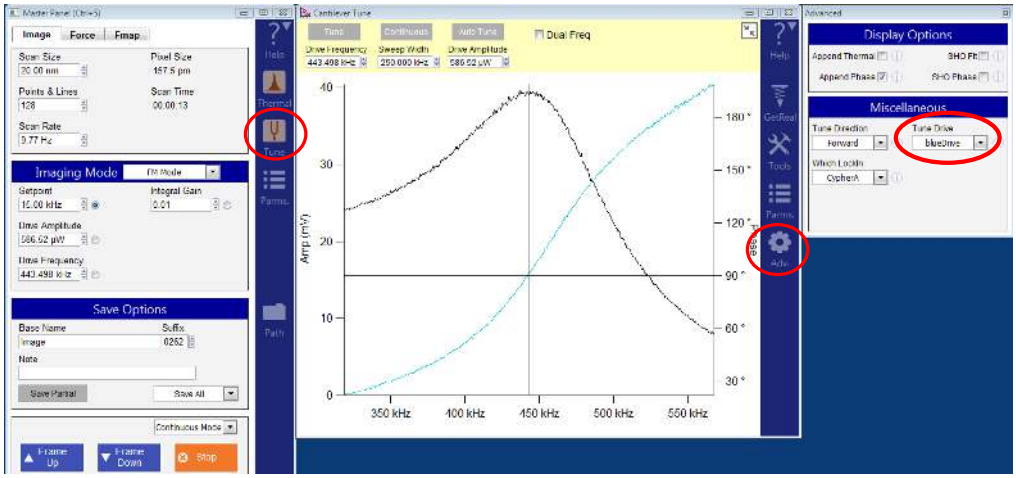
10.



Capture a thermal of the cantilever in liquid:

- Make sure that the padlock besides Spring Constant is locked.
- Refit the thermal data to obtain the updated (water) InvOLS value.
- Transfer the frequency of the thermal peak to the tune panel as follows:
  - Right-click on the peak of the Thermal Graph and select *Move Freq and Phase to Tune*.

11.



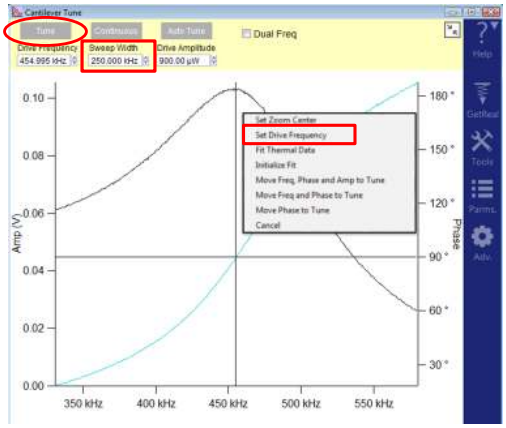
Turn on blueDrive photothermal excitation

- In the Tune graph, click the 'Adv.' icon.
- In the Advanced panel, select *blueDrive* from the drop-down menu of Tune Drive (under Miscellaneous).
- Use the blue arrows in the Video panel (top right side) to move the blue laser spot to the base of the cantilever.

12.

Tune panel

- Set the Sweep Width to “250 kHz”.
- Click the ‘Tune’ button.
- When tune is done, right-click on the peak and select *Set Drive Frequency*.
- Move the blue laser spot around the base of the cantilever to maximize the amplitude (look at the amplitude value in the Sum and Deflection panel).
- Adjust the drive amplitude until the tune amplitude reaches ~ 80 mV (visible on the graph and in the Sum and Deflection Panel).



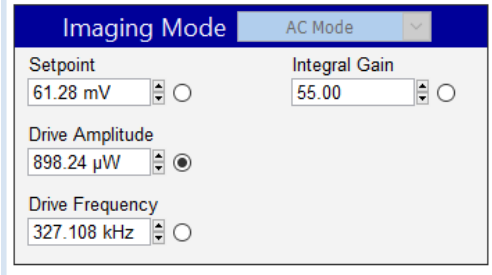
14.4.2. Imaging in AC mode

Acquire an AC image of the calcite sample to check tip sharpness and to define the area of interest.

1.

Master Panel settings:

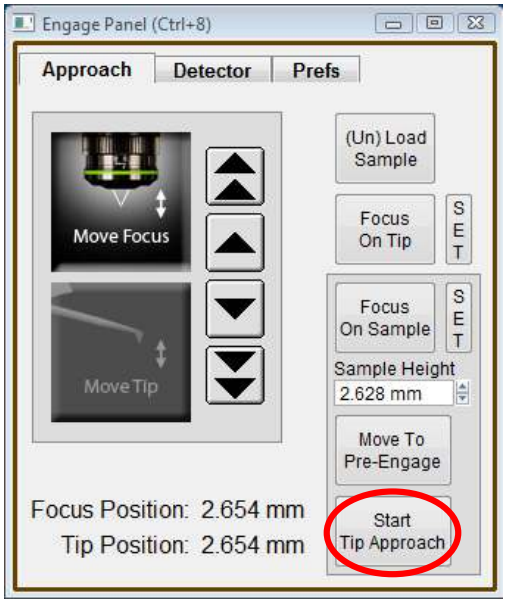
- Set the Setpoint to ~ 60 mV.
- Set the Integral Gain to “55”.



2.

Video and Engage Panel Settings:

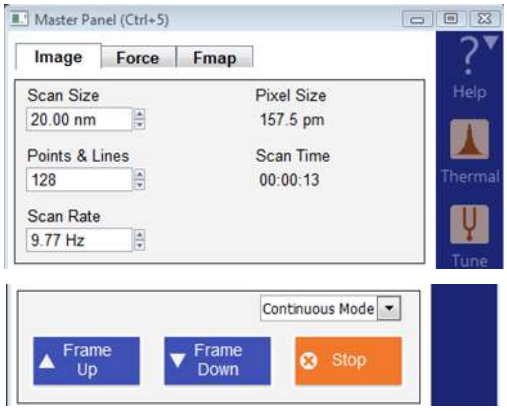
- Click the ‘SET’ button (beside Focus On Tip) while focused on the tip.
- Click the ‘SET’ button (beside Focus On Sample) while focused on the sample.
- Click the ‘Move to Pre-Engage’ button (tip is now 50 µm from surface).
- Click the ‘Start Tip Approach’ button.



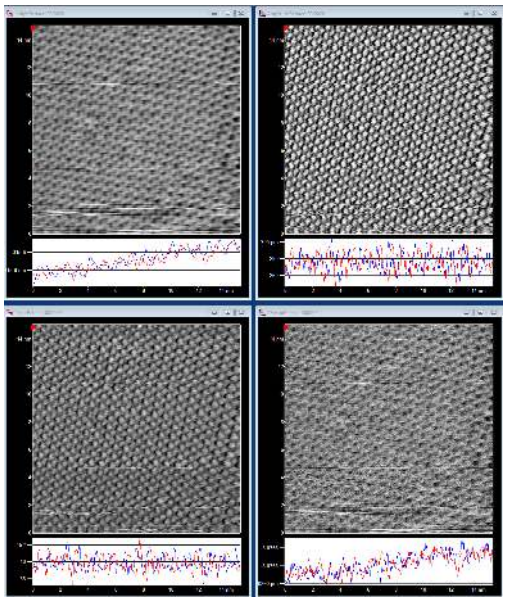
3. Once the tip is in piezo range of the sample, capture another Thermal Tune with Z-piezo at 0V (tip is 2-3 µm from surface).

4. In Thermal Graph panel, keep the padlock beside Spring Constant locked and recalculate Amplitude InvOLS.

5. Right-click on the peak in Thermal Graph to select *Move Freq and Phase to Tune*.
6. When the tune is complete, right-click on the peak and center phase.
7. **Imaging parameters on Master panel:**
   - Set the Scan Size to “20 nm”.
   - Set the Scan Rate to “10 Hz”.
   - Start imaging by clicking the ‘Frame Down’ button.



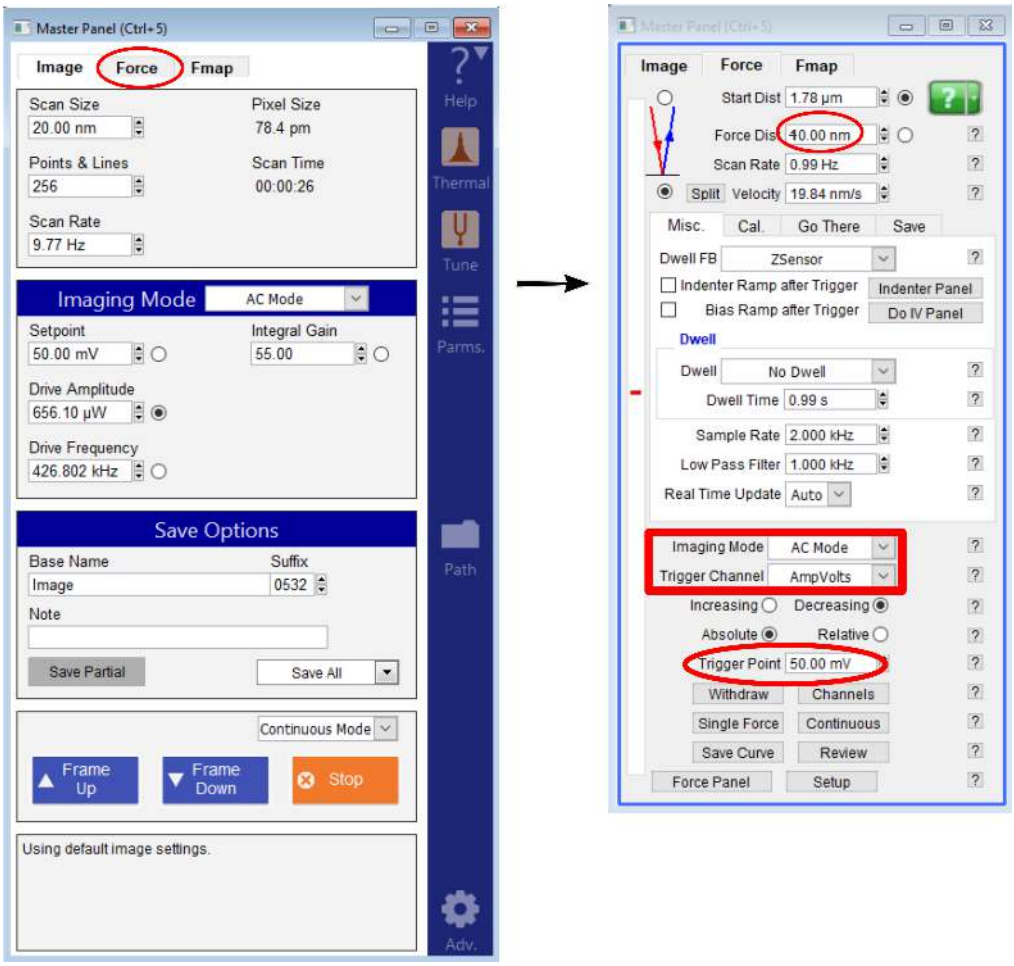
8. Start imaging and optimize imaging parameters, as follows:
   a) Decrease the free amplitude (by decreasing drive amplitude in the Master Panel).
   b) Decrease the setpoint until trace and retrace overlap each other.
   c) Repeat a and b several times to image with the lowest amplitude possible.
9. **Imaging in AC mode:**
   - Acquire a 20 nm image and re-size the scan to 10 nm
   - Channels that are acquired include:
     - Height
     - Amplitude
     - Phase
     - ZSensor
   - Once the imaging looks optimized, stop (click the ‘Stop’ button) the scan and switch to the Force tab.



14.4.3. AC force distance curves

You will acquire several AC force curves to bring the tip within 50 nm of the surface and retune the cantilever.

1.



Force Tab

- In the Master Panel, switch from AC Mode imaging to AC Mode force curves.
- The Force tab now displays parameters related to AC Mode force curves.
- Set these parameters as follows:
  - Set the Setpoint to about 60% of the amplitude (for example, if the amplitude is 80 mV, set the setpoint to 50 mV).
  - Begin with force distance (‘Force Dist.’) set to ~ 500 nm. Force distance is the distance between the tip and sample after the curve has been acquired.
  - Set the ‘Trigger Channel’ to AmpVolts, Decreasing, and Absolute.

2.

AC Force Curves:

- Acquire a single force curve.
- Click 'Continuous' and, as the curves are being acquired, gradually decrease the force distance down to 50 nm.
- Acquire ~20 curves with 50 nm force distance and click Stop curves.
- The Z-voltage should remain between 70 and 150 volts.

Re-tune 50 nm away from the surface

- Click 'Tune' on the Tune Graph.
- Right-click on the peak of the graph to select *Set Drive Frequency*.
- Right-click on the graph again and select *Center Phase*.
  - Z-voltage should still be at the same value as when the force curve was acquired (meaning that the tip is ~ 50 nm away from the sample).

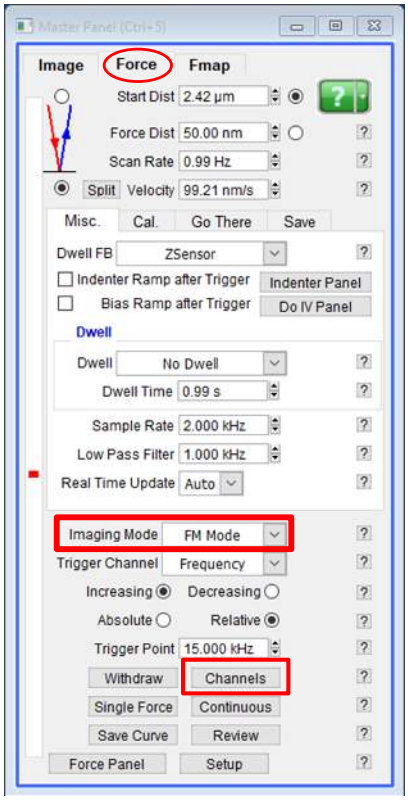
14.4.4. FM force distance curves

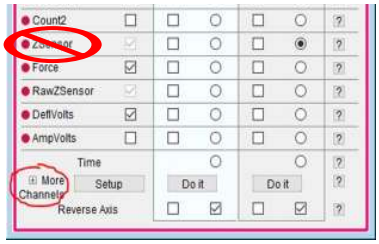
The following steps allow you to calculate FM gains and check if the setpoint is appropriate.

1.

Master Panel settings:

- Choose *FM Mode* for Imaging Mode.
- Click the 'Channels' button in the Force tab and select the following items:
  - Frequency
  - Dissipation
  - Amplitude
  - Phase
  - Make sure to plot versus DRIVE (not ZSensor) for short curve (tens of nm) as Drive is a quieter signal than ZSensor (which has 50 pm of noise in a1 kHz bandwidth)
- Select the Trigger Channel: *Frequency*, the select Increasing and Relative, as shown at right.
- Set the Trigger Point: 5 kHz is a good start

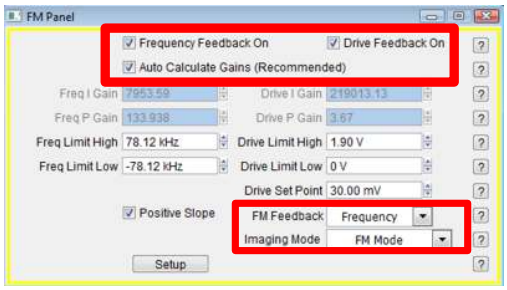


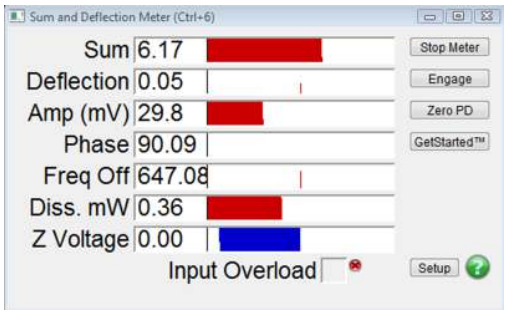


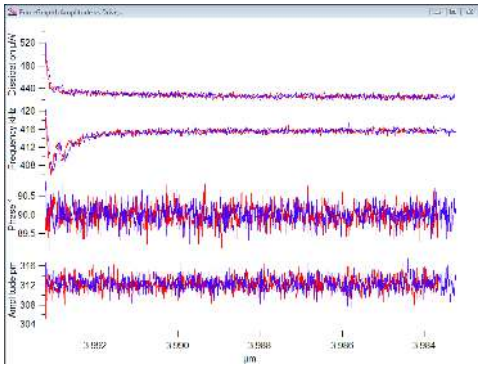
2.

FM Panel and Sum and Defection Meter Panel settings:

- Open the FM Panel: *AFM Controls > Other > FM Panel*
- Uncheck and check the 'Auto Calculate Gains' checkbox. The values below should update.
- Set the 'Drive Set Point' to the required amplitude as follows:
  - Start with 3 Angstroms: 3A = 0.3nm. For example, 0.3nm / 10.25nm/V InvOLS = 0.0292V = 29.3mV.
- Activate Feedback Loops by checking the:
  - 'Fequency Feedback On' check box
  - 'Drive Feedback On' check box
- When the frequency and drive feedbacks are ON, the following values appear in the Sum and Deflection Meter panel:
  - Amp (mV) = Drive SetPoint Value
  - Phase = 90
  - Freq Off = frequency offset compared to the value of drive frequency from tune, here 647 Hz
  - Diss mW = power (in mW) needed to keep the Drive Set Point Value at 30 mV
- Click Single curve to acquire a FM force curve. If the FM gains are correct, force curve should appear on the graph.



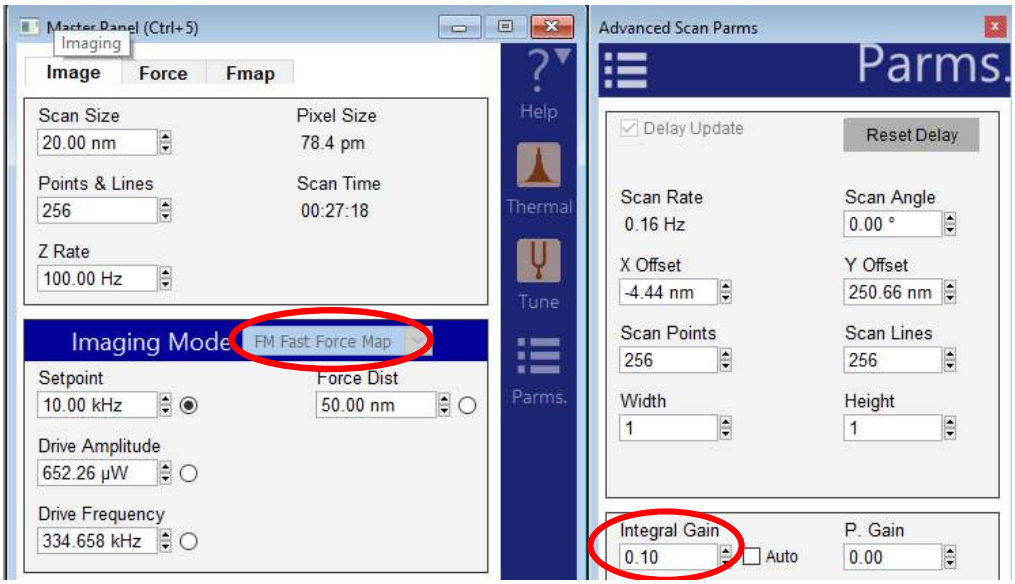




14.4.5. Fast Force Mapping in FM

These steps describe how to perform FFM in FM.

1.



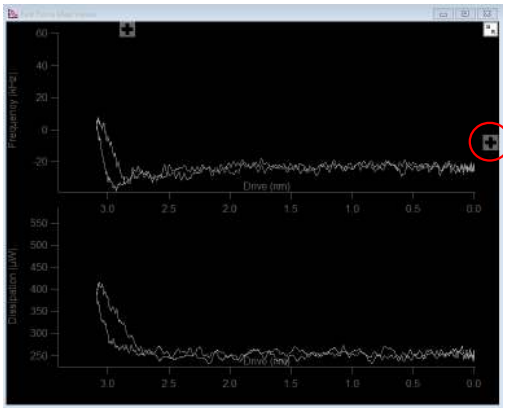
Imaging Parameters:

- In Master Panel, switch from *FM Mode* to *FM Fast Force Map*.
- The Master Panel now displays parameters related to FM FFM mode as follows:
  - 'Setpoint' is now displayed in units of Hertz (if frequency feedback is selected in the FM Panel) or watts (if dissipation feedback is selected in the FM Panel).
  - Set the force distance to "50 nm".
- Set the other parameters in the Master Panel as follows:
  - Scan Size: 20 nm
  - Scan Points & Lines: 256
  - Z-rate: 100 Hz
  - Z-voltage should still be between 70 and 150 V.
- Click 'Engage' in the Sum and Deflection Meter panel, where FM FFM settings should now be reflected.
- Set the Integral Gain, located in the Parms panel, to "0.1".

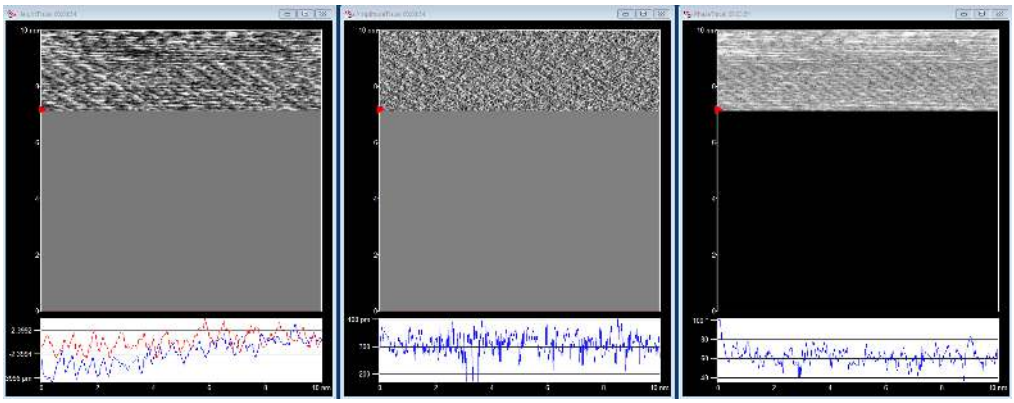
2.

Real-time force curves

- The Fast Force Map Viewer can display up to two channels. (To add a graph, click the plus sign on the right side of the viewer, as shown at right.)
- Each graph has several options for each axis. You define the appropriate axes for the imaging mode. For FM FFM, choose:
  - Graph 0 (top): Frequency (Hz) vs DriveVolts (V)
  - Graph 1 (bottom): Dissipation (µW) vs DriveVolts (V)



3.



Imaging

- Data is collected in the following three channels during FM FFM imaging
  - Height
  - Frequency
  - Dissipation

15. High Voltage Techniques

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

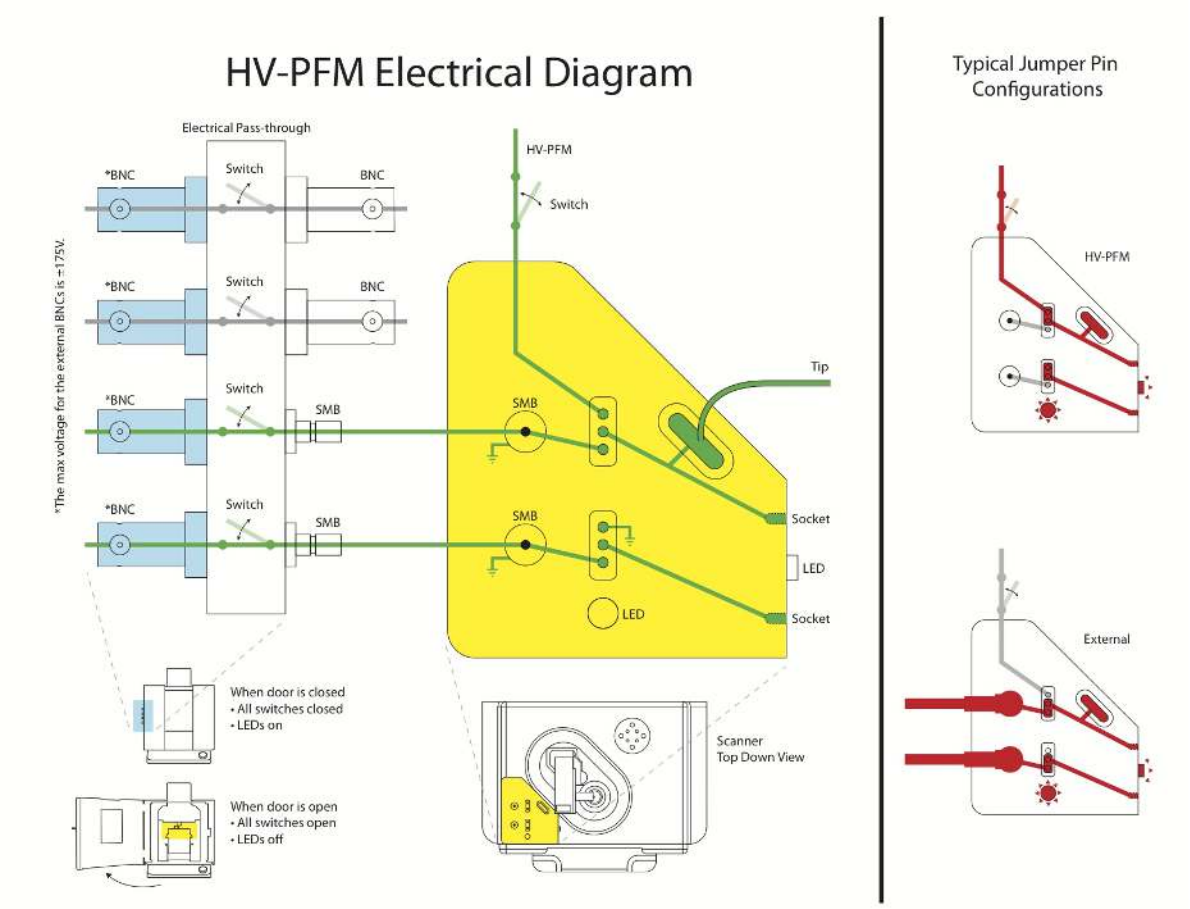


Figure 15.1.: The Cypher High Voltage Option

16. Contact Resonance (CR)

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

16.1. Gluing a Cantilever to the CR Cantilever Holder

16.1.1. Overview

The series of figures below outlines the steps for gluing a cantilever to the Cypher ™ Contact Resonance Cantilever Holder. For many applications this is not necessary; however, it will result in the cleanest, frequency independent (flat) drive function.

Figure 1. Top view of the Contact Resonance Cantilever Holder for Cypher.

Figure 2. We recommend using "Double Bubble" fast setting epoxy. Make sure you have everything ready before you mix it and make sure you take enough time, ~45-60 seconds, stirring it to insure it is well mixed. Also make sure to use the entire package to insure proper proportions.

Figure 3. Remove the spring clip from the cantilever holder for gluing. If you have attached it, return it to the Contact Resonance Cantilever Holder kit box for safe keeping.

Figure 4. Use a sharp tool (for example one of the toothpicks included in the kit) with a very small amount of the epoxy on the end. This shows the target amount of glue. It should be much smaller than the size of the cantilever chip.

Figure 5. Position the chip on the glue with the cantilever overhanging about 1mm. Allow it to cure for a full five minutes.

Figure 6. Close-up of final glued cantilever chip.

Figure 7. Comparison of the ~900kHz contact resonance of an AC160 in contact with a metal surface. The blue curve was measured by driving the contact resonance with the sample actuator (with a drive amplitude of 1,000mV) while the red curve was measured using the HF Cantilever holder (drive amplitude 15mV).

16.2. CR Imaging in DART Mode

In dual-amplitude resonance tracking (DART) mode, you can detect and track the contact resonance frequency as the tip scans across the sample. The frequency images created by DART can be analyzed to obtain quantitative modulus maps. Here we will focus on the first step of this process, acquiring contact resonance frequency images with DART.

16.2.1. Dart Mode with Sample Actuator

16.2.1.1. Required Hardware

- Sample actuator
- To be compatible with the sample actuator, the top of the scanner must be fitted with a plug. (section 16.1.2, image 3)
- Depending on the scanner version, it may need a compatibility board replacement.

16.2.1.2. Required Software

- Igor Software version 14 or later is recommended, but version 13 can also be used.
  - In ModeMaster, select the NanoMechPro tab then click 'DART CR' as shown below:

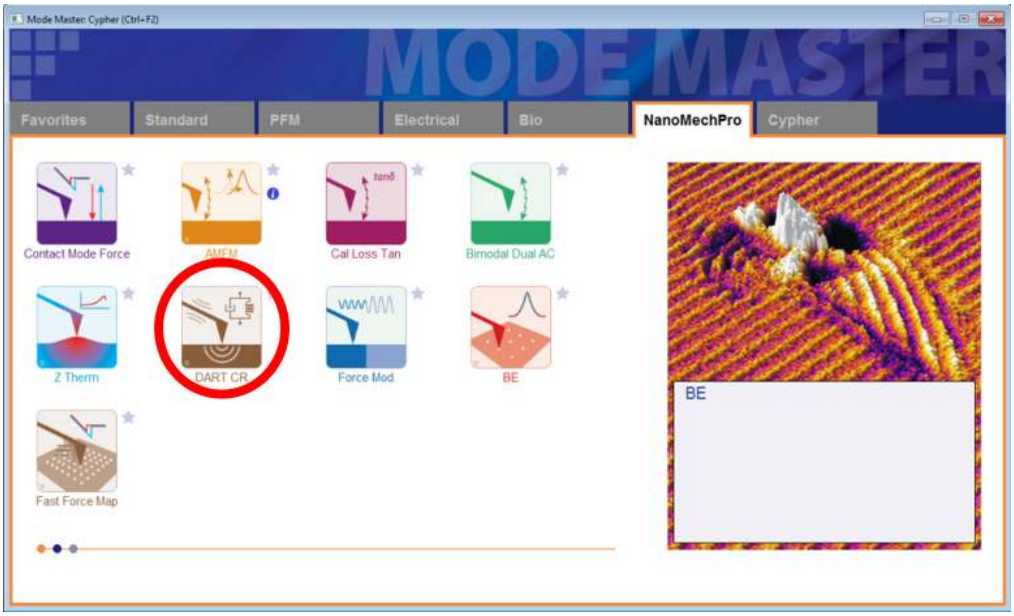


Figure 16.1.: ModeMaster DART Panel selection

– A new DART Panel is displayed, as shown below.

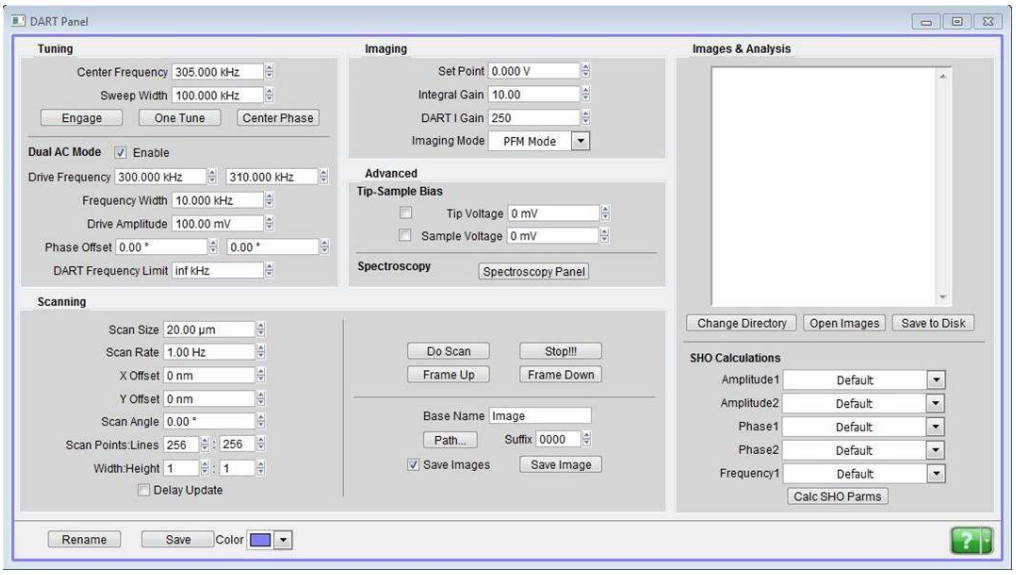


Figure 16.2.: DART Panel

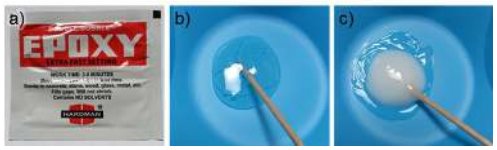
16.2.1.3. Setup for Sample Actuator

1. **Make sure sample is cut to a low profile:**

- To fit under the cantilever holder, make sure the sample is cut to a low profile. The clearance between the sample and cantilever is limited to couple of millimeters due to the significant height of the sample actuator.

2. **Mix the glue:**

- It is best to use stiff, solvent free glue that can be easily removed after the experiment. We recommend Double Bubble fast-setting epoxy (a).
- Make sure you have everything ready before you mix the glue, and make sure you thoroughly mix it for ~45-60 seconds: it will turn from clear (b) to white (c).



3.

Glue sample directly to the sample actuator:

- Use a sharp tool (a paper clip in this example) to place a very small amount of the epoxy directly on the actuator (d-e).
- Press the sample down on the corners to make sure the glue has spread uniformly under the sample (f).
- Before the epoxy hardens, you can gently slide the sample around to position it at the center of the actuator.

Note Having the sample well-centered will result in a clean contact drive spectrum, and hence, good force modulation or contact resonance imaging.

4.

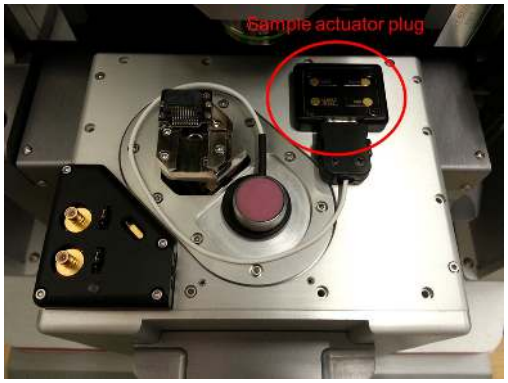
To remove the sample after an experiment:

- Use a sharp blade to lift off the glue. Place the blade as parallel as possible to the surface and slowly cleave off the glue with the sample (g-h).
- After the sample is off the actuator, gently remove any excess glue (i) before gluing another sample.

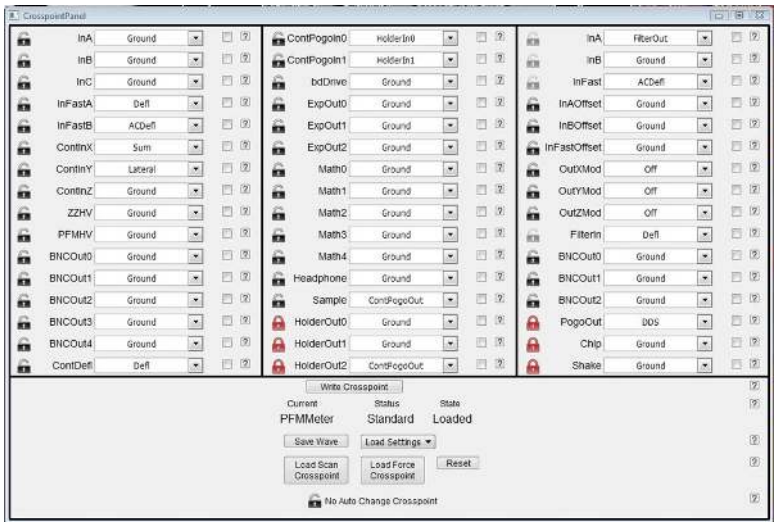
5.

Plug the sample actuator into the scanner

- Plug the sample actuator into the scanner, as shown at right.



6.



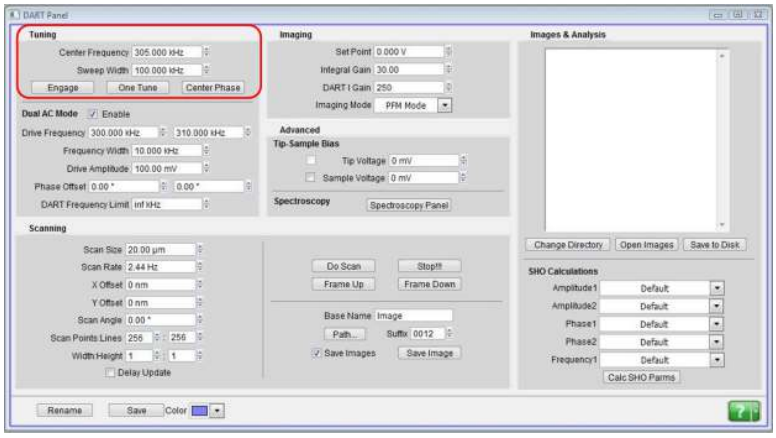
Rescan bus after connecting everything:

- If you are using software version 14.01.91 or higher, refer to section **D: Calibrate and Tune.**
- If you are using software version 13.10.93 or higher:
  - Go to the Programming > Cantilever & Sample Holder Panel in the Holder panel,
  - Select the 'Sample' tab
  - Check the 'CRM' checkbox.
- If you are using earlier software versions, do the following:
  - Go to Programming > Crosspoint Panel.
  - Set the Crosspoint Panel fields as shown at right.
  - Click 'Write Crosspoint'.

16.2.1.4. Calibrate and Tune

1. Use a standard cantilever holder, then choose and mount a cantilever.
   - For example, an Olympus AC240 cantilever includes:
     - Resonance frequency in air is 70 kHz
     - Contact resonance frequency is ~3-5 times resonance frequency (~300 kHz)
     - Spring constant ~ 2 N/m
2. Calibrate the cantilever (use GetReal) to determine:
   - InvOLS
   - Spring constant
   - Free Resonant Frequency (of the 1st and 2nd mode)
   - Q-factor (of the 1st and 2nd mode)
3. Approach and engage on the surface of the sample with a typical deflection setpoint that would be used to image that particular sample. For example, for a silicon surface, use a Set Point of 1 V (~160 nN) to start.

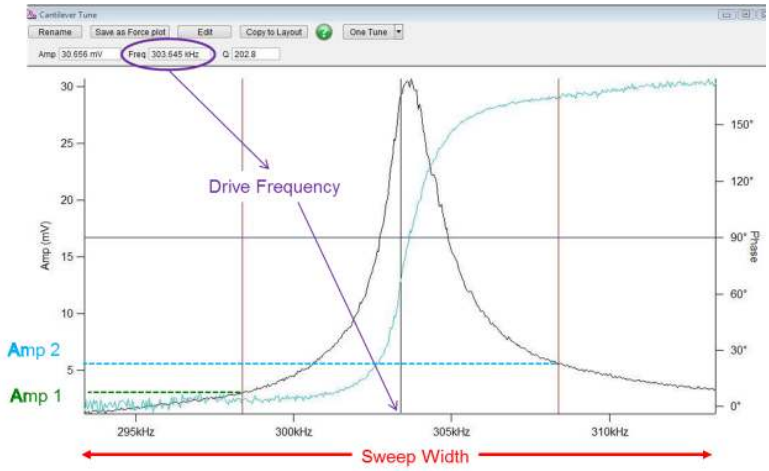
4.



Determine the CR frequency:

- Once within range of the surface, use the Tuning section of the DART Panel to determine the contact resonance frequency, as follows:
  - 'Center Frequency' is the contact frequency of the lever. For an AC240 lever, the value should be around 300 kHz.
  - An initial 'Sweep Width' of 100 kHz is a good start.

5.



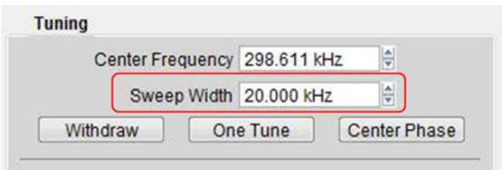
Start tuning:

- Click 'One Tune'.
- The tip will engage on the surface, and the actuator will oscillate the sample while the optical detection system reads the cantilever oscillation signal.
- The tune plot should look something like the one at right with a contact resonance of about 300 kHz.

6.

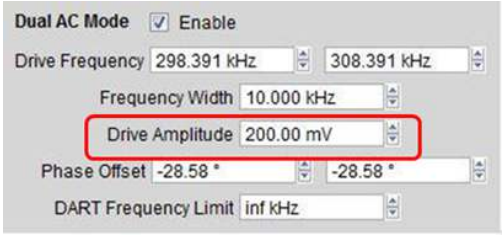
Set the peak frequency:

- Right-click on the peak and set as the *Center Drive Frequency*.
- Reduce the 'Sweep Width' to about 20 mV. This setting will zoom in the contact resonance peak.



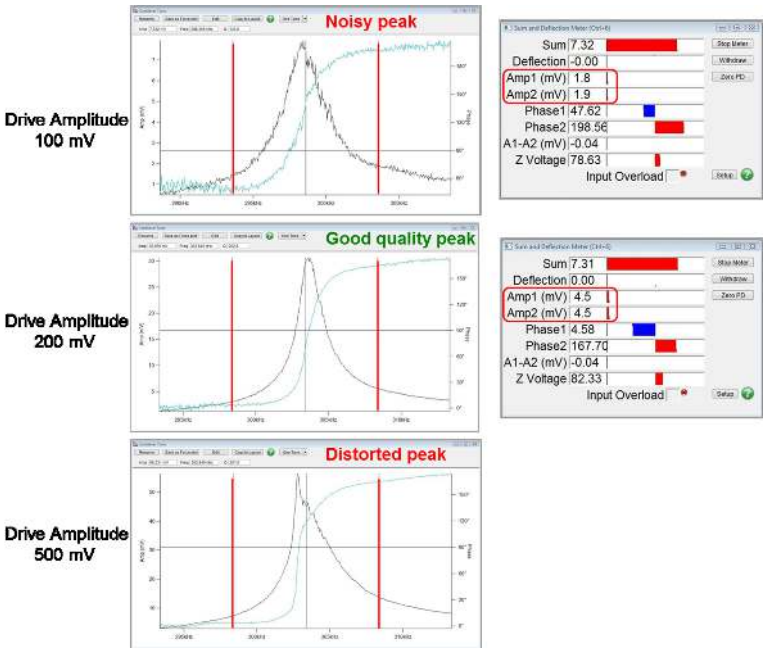
7. **Change the drive amplitude:**

- Change the Drive Amplitude and watch what happens to Amp1 and Amp2 values (visible in the Sum and Deflection Meter panel).



Note Amp1 and Amp2 are the two shoulder frequencies indicated with red lines on the graph.

8.



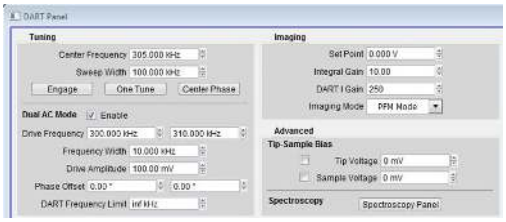
Examples of different 'Drive Amplitude' settings:

- Amp1 and Amp2 should be well above the noise level (> 2 mV). In the example at right, 'Amp1' and 'Amp2' are ~4.5 mV at drive amplitude of 200 mV.
- If you set the drive too high, e.g., 500 mV, the peak starts to be distorted.

Note The stiffness of the tip-sample contact in CR-FM is usually a very non-linear function of the indentation depth. Because of this, if the cantilever amplitude is too large, the tip-sample interaction may vary significantly over a single oscillation cycle and lead to "distorted" tunes. For some, these nonlinear tunes are an active research area, for routine CR-FM imaging, they should be avoided.

9. **Explore the tune parameters:**

   - Once you have acquired a contact spectrum, practice by adjusting the following parameters:
     - Drive Frequency
     - Sweep Width
     - Drive Amplitude
     - Set Point
   - For example, increasing the Set Point should increase the contact resonance frequency. When you increase the Set Point (deflection), we are increasing the static force on the tip and thus the tip-sample contact area. Because the frequency is proportional to the contact area, it should increase as you increase the force.

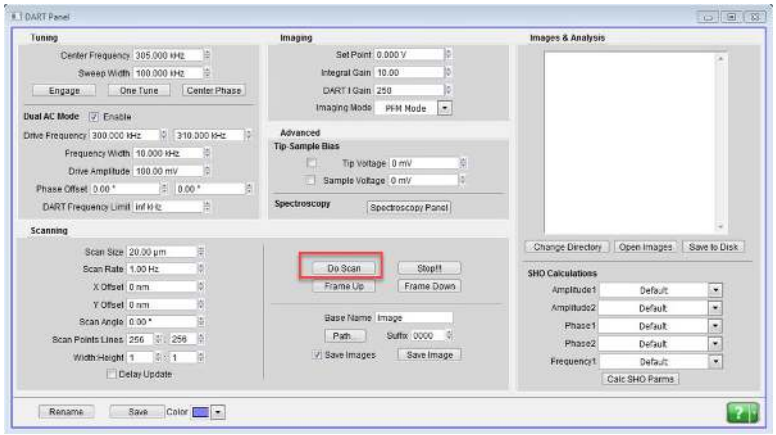


10. Once the peak in the Cantilever Tune Panel looks nice (symmetric, low noise), check the Scanning settings on the Dart Panel:

    - Scan Size
    - Scan Rate - Start slowly with 1Hz.
    - Scan Angle - Set the scan angle to 90 degrees.

11. You can now start imaging.

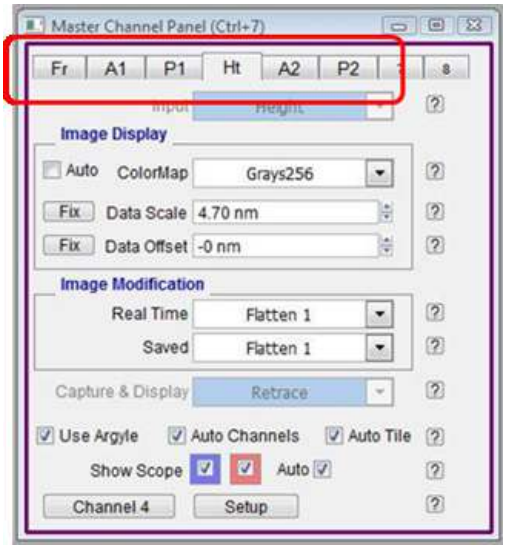
16.2.1.5. Imaging

1. 

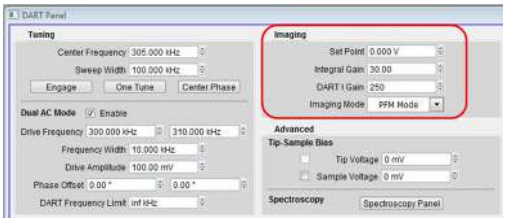
   To start imaging, on the Dart Panel:

   - Start imaging by clicking ‘Do Scan’.

2. **Check the Master Channel Panel (Ctrl+7):** Six data channels (shown as tabs at right) should be acquired:
   - Frequency (Fr)
   - Amplitude1 (A1)
   - Phase1 (P1)
   - Height (Ht)
   - Amplitude2 (A2)
   - Phase2 (P2)



3. **Adjust Integral Gain:** In the Imaging section of the DART Panel, adjust 'Integral Gain' as usual to obtain a nice height image (trace/retrace overlap).

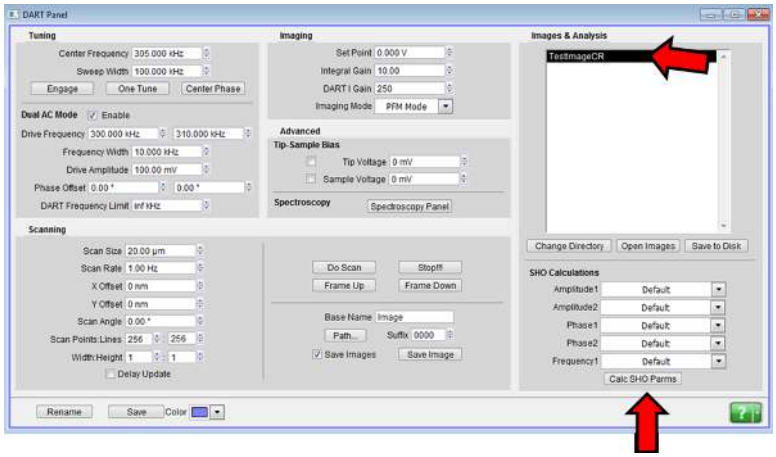


4. Start adjusting the DART 'Integral Gain' and watch the frequency channel for improvement in tracking, i.e., trace/retrace overlap, no ringing.
5. The deflection 'Set Point' can be increased to improve tip contact with the surface. Frequency will shift due to increase in contact area, so withdraw and retune when changing this parameter.
6. To compare modulus values of different samples:
   a) Calibrate the cantilever that will be used for imaging.
   b) Acquire images on a sample with known modulus (take note of all the settings).
   c) Acquire images on unknown samples with the same settings as used on the reference (known modulus) sample.
   d) Use the Viscoelastic Panel to calculate the modulus values and compare the results.

16.2.1.6. Analysis: Converting CR images into E' and E"

1. Acquire Information needed to perform the analysis includes Free Resonant Frequency of the cantilever and Free Q factor of the cantilever.
2. During imaging, save data for the following settings:
   - Frequency
   - Amplitude 1
   - Amplitude 2
   - Phase 1
   - Phase 2
3. Acquire images on reference and unknown samples, and then save the data.

4.



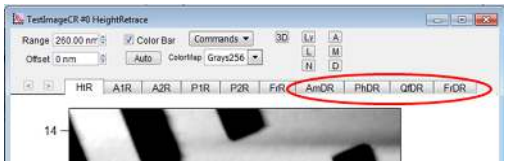
Open a sample image:

- Open an image of a sample with a known modulus by using the 'Images & Analysis' window in the DART Panel.

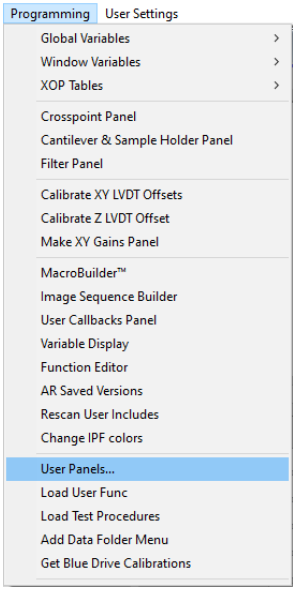
5.

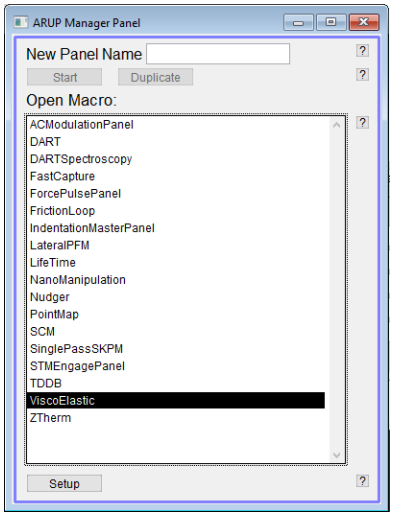
Calculate SHO parameters:

- Click the 'Calc SHO Parms' button (see screenshot in previous step).
- Additional panel tabs should appear in the image, as shown at right.



6.



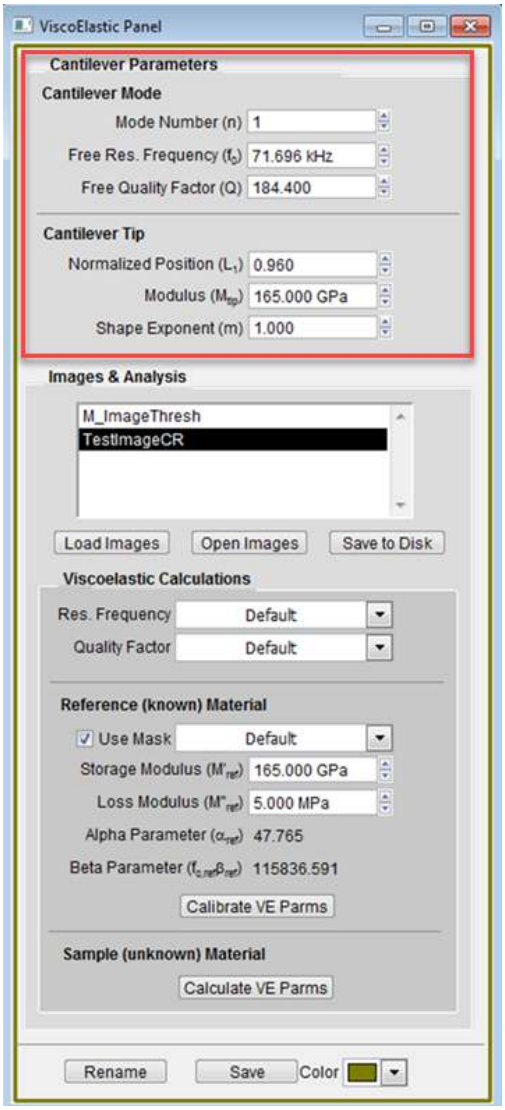


Open the ViscoElastic Panel:

- Open the ViscoElastic Panel by selecting User Panels in the Programming drop-down (located on top menu)
- On the ARUP Manager Panel, select *ViscoElastic*.

7. **Complete the Cantilever Parameters section in the panel, including:**

   - 'Mode Number (n)' is the cantilever mode used for imaging.
   - 'Free Res Frequency (f0)' is the free resonant frequency of the cantilever at the mode that was used for imaging.
   - 'Free Quality Factor (Q)' is the Q factor of the cantilever obtained at the frequency measured in b.
   - 'Normalized Position (L1)' is the position of the tip in relation to cantilever length.
   - 'Modulus M (tip)' is the tip material modulus.
   - 'Shape Exponent (m)' is the exponent used to descry the shape of the tip, including:
     - Punch = 1.0
     - Sphere = 1.5
     - Cone = 2.0

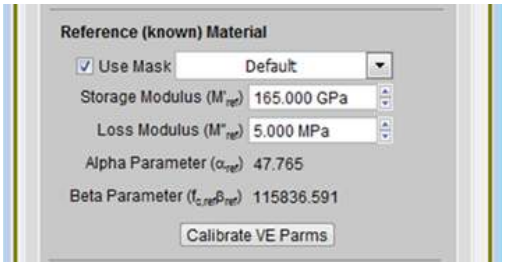


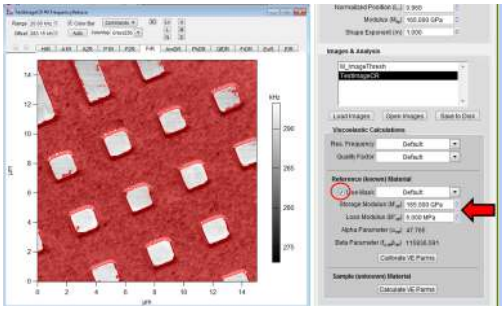
8. Highlight the image to analyze in the *Images & Analysis* section of the panel.

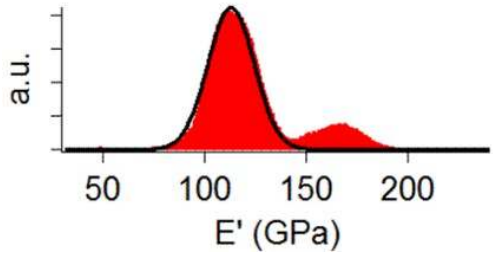
9. In the *Viscoelastic Calculations* section, leave the settings at "Default".

10. **Enter modulus values and calibrate VE parameters:**

    - In the *Reference (known) Material** section, input values for the 'Storage Modulus' and 'Loss Modulus'.
    - Click 'Calibrate VE Parms'.
    - New panels appear in the reference image, including EsR (E storage Retrace) and ElR (E loss Retrace).







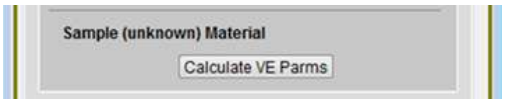
***Note** If the sample imaged is composed of two or more materials, and the modulus of one of them is known, mask function can be used to calculate modulus values of the other materials in relation to the known one. If this is the case:

- Check the box beside 'Use Mask'.
- When left to "Default", the mask of the active channel is used (in the example at right, top, the mask in the Frequency Retrace channel is used). Alternatively, you can also choose (from the dropdown menu) which channel should be used for the calculation.
- After masking the image, click 'Calibrate VE Parms'.
- The EsR and ElR panels appear in the image. The moduli, both unmasked and masked regions of the image, are automatically calculated, and the respective values can be obtained from a histogram of the image.

If sample is unknown:

11.

- Redo steps 1-6.
- Click 'Calculate VE Parms' to calculate the storage and loss moduli of the unknown sample.



16.2.1.7. Transfer function of the CR FM actuator

Most analyses of dynamic AFM response start out with the assumption of a simple harmonic oscillator being driven by a perfect, frequency-independent actuator. Unfortunately, this actuator rarely exists. Most actuators, especially including those that involve piezoelectric crystals are very frequency dependent. The amplitude and the phase of the driving force can change rapidly over just a few kilohertz in frequency. While this is a particularly challenging issue for CR-FM, it is ubiquitous for many kinds of AC or dynamic AFM techniques.

In the case of CR-FM measurements, resonances in the AFM or even in the sample itself, depending on the geometry and mounting details, can distort or overwhelm the actual cantilever resonance. An experiment that was designed to show this is sketched out in 16.3. A rectangular Si chip was epoxied with a small dab of glue to the top of the sample actuator. The glue was limited to only be under the center of the chip, leaving the "wings" free. Contact resonance tunes were then made at three positions: directly over the center of the glue and the chip (red), halfway between the center and the edge (green), and nearly at the edge of the chip (blue). The contact tunes were made with the cantilever deflection used as the feedback error signal, exactly as is done during CR-FM imaging. Using this approach, we expect that any variations in the contact resonance are due to variations in either the local sample elasticity or the contact area due to sample roughness, or both.

The tune in Position 1(red) is centered over the Si slide, and the glue underneath shows a symmetric, single peak at ~1.25MHz. Careful inspection of the trace does show some slightly non-ideal curvature; but, in general, it looks

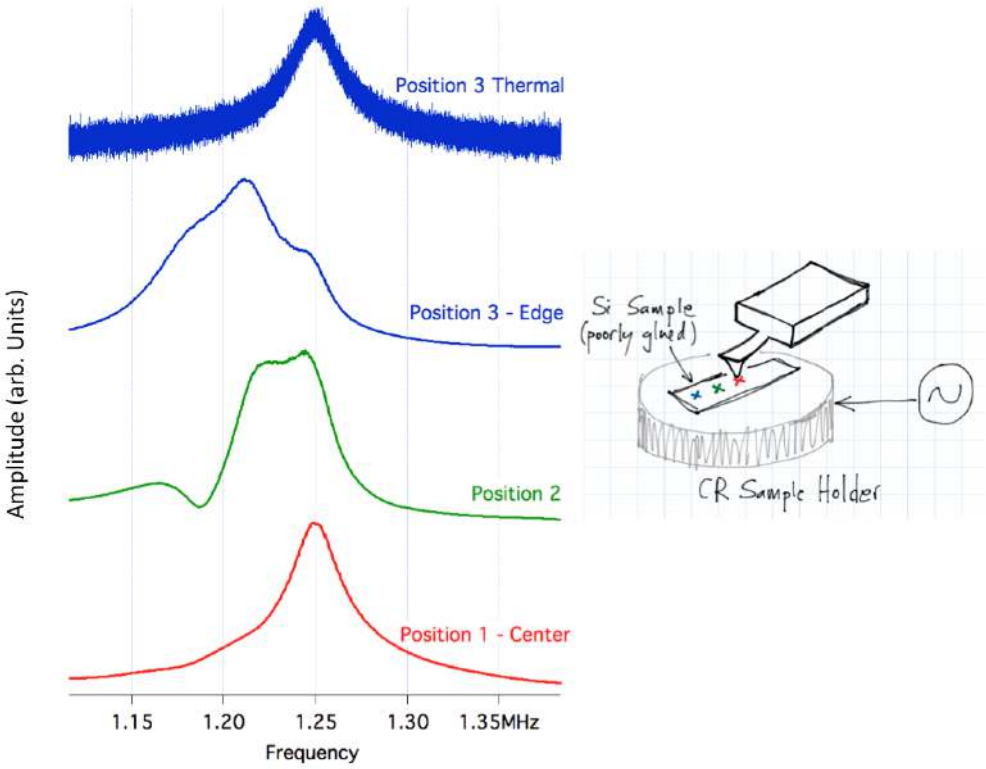


Figure 16.3.: Experiment with Si chip position results

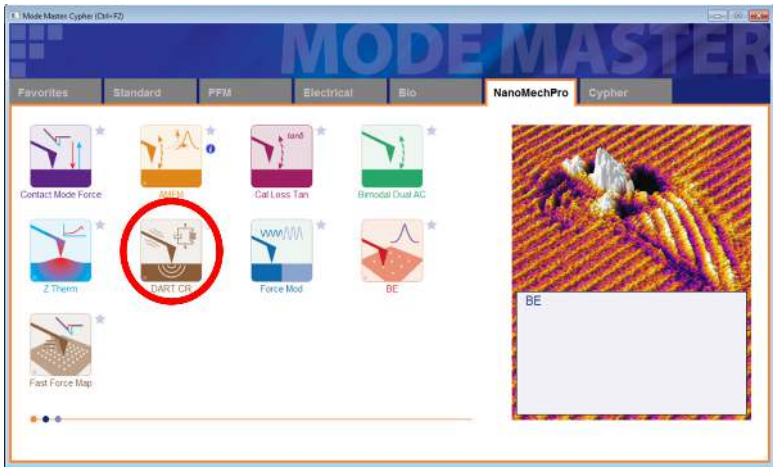
quite ideal. The tune at Position 2 (green), halfway in between the center and the edge of the Si chip, appears quite different. It is not obvious where the resonance might be. The contact tune at position 3 (blue), near the edge of the Si chip, shows a resonance peak at ~1.22MHz, roughly 30kHz lower than the center value measured at Position 1. The final curve shown in the figure, however, has a very different implication. The Brownian motion of the cantilever is expected to be roughly frequency independent. When the spectrum of that motion is plotted (blue, top trace), we recover the original ~1.25MHz resonance. This is strong evidence that a sample resonance (perhaps a "flapping mode") due to the faulty mounting scheme we used, in turn provides a frequency dependent drive to the sample that varies strongly enough to mask the actual cantilever resonance itself. Inspection of the Position 3 (blue) tune in fact shows a small shoulder on the peak that may well be the cantilever resonance at 1.25MHz. The takeaway lesson from this is that you can cause artifacts in CR-FM measurements through faulty sample mounting. If your CR tune looks asymmetric or "bumpy", it is a good idea to have a look at the contact thermal just to make sure that your CR sample holder response gives the same values as the Brownian-driven response.

16.2.2. Dart Mode with blueDrive

16.2.2.1. Setup for blueDrive

1. Use an air cantilever holder.
2. Use the most recent software version (version 17, as of May 2021).

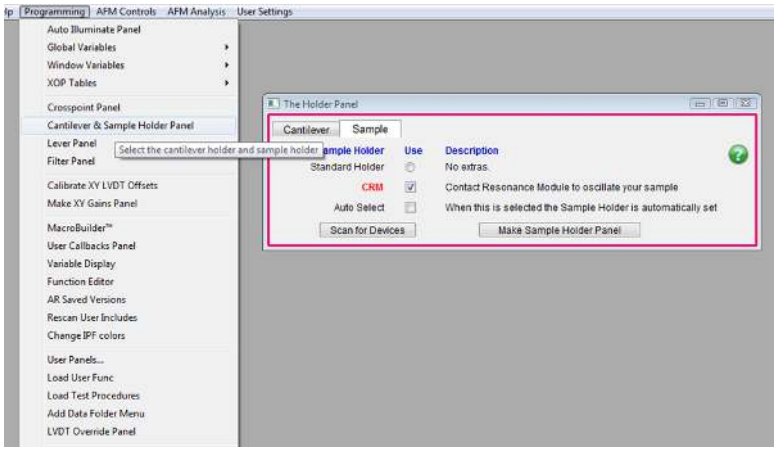
3.



Select DART CR in the software:

- In ModeMaster, select the NanoMechPro tab, then click ‘DART CR’.

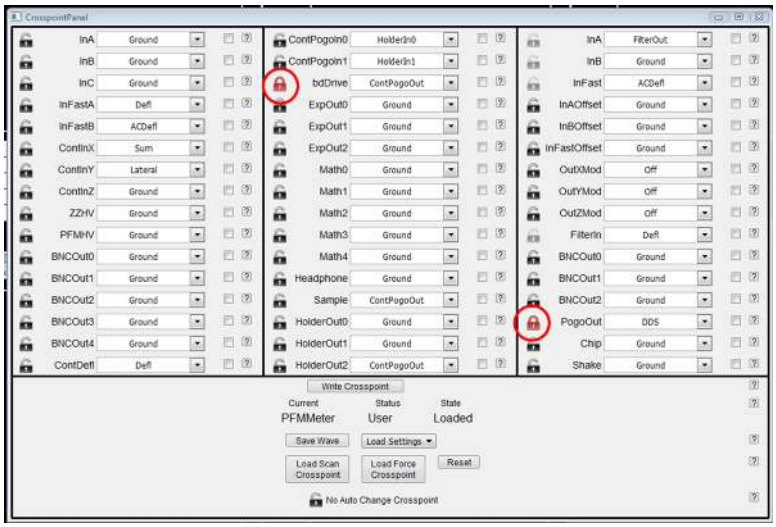
4.



Select DART CR in the software:

- On the Programming tab, select *Cantilever & Sample Holder Panel*.
- On the Holder Panel, select the Sample tab and check the ‘CRM’ (Contact Resonance Module) checkbox.

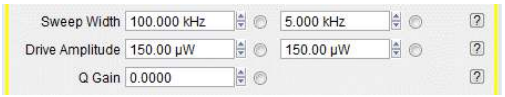
5.



Configure Crosspoint settings:

- On the Programming tab, select *Crosspoint Panel*.
- To turn blueDrive ON, configure Crosspoint as follows:
  - bdDrive = ContPogoOut
  - PogoOut = DDS
- Lock the settings by clicking on the padlock beside the above settings.
- Click the 'Write Crosspoint' button.

6.



Configure the Drive Amplitude settings:

- Open the Master Panel.
- Select the Tune tab and set 'Drive' to blueDrive.
- Focus on the tip.
- blueDrive should be ON, and the laser spot visible in the video.
- 'Drive Amplitude' units should be in WATTS.
- Set the 'Drive Amplitude' with the same value for both frequencies, e.g.,150 W.

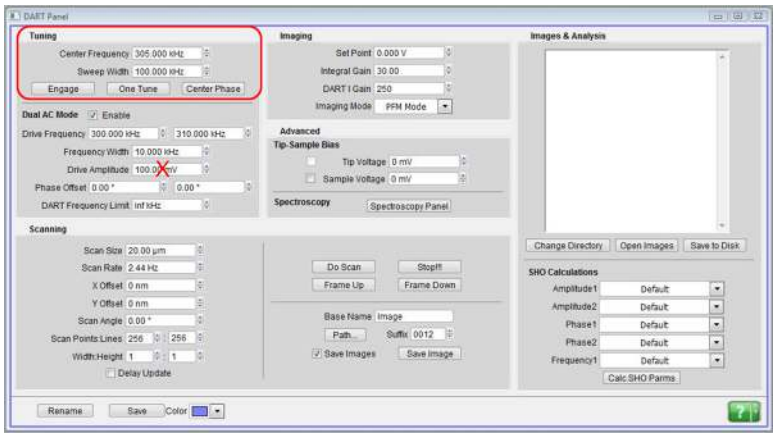
16.2.2.2. Approach, Calibrate, and Tune

1. You should have a standard cantilever holder mounted with a cantilever. For example, an Olympus AC240 cantilever (gold-coated probes are preferred for blueDrive operation) should include:
   - Resonance frequency in air is 70 kHz.
   - Contact resonance frequency is ~3-5 times resonance frequency (~300 kHz).
   - Spring constant is ~ 2 N/m.
2. Approach the sample until you are ~ 2 mm above the surface.
3. Calibrate the cantilever (you can use GetReal) to determine:

- InvOLS
- Spring constant
- Free resonant frequency
- Q factor

4. Approach the tip and engage on the surface of the sample with a deflection setpoint typically used to perform contact imaging with that particular sample. For example, for a silicon surface, use a Set Point of 1 V (~160 nN) to start.

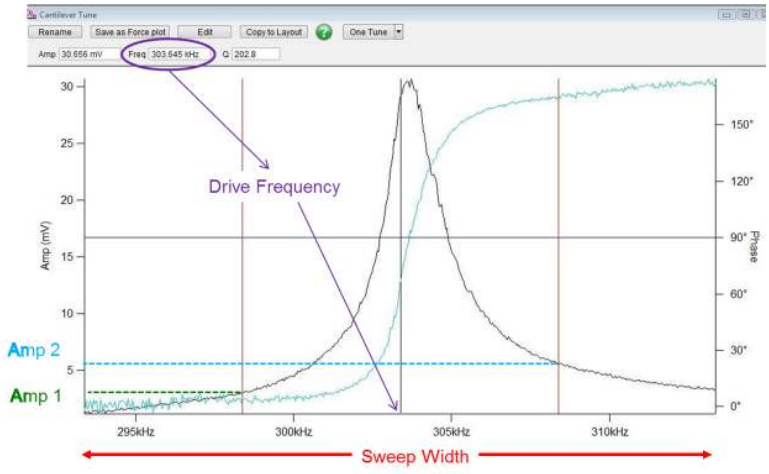
5.



Determine the CR frequency:

- Once within range of the surface, configure the Tuning section in the DART Panel to determine the contact resonance frequency:
  - 'Center Frequency' is the contact frequency of the lever. For an AC240 lever, the value should be around 300 kHz.
  - 'Initial Sweep Width' of 100 kHz is a good start.
  - Set 'Frequency Width' to 10 kHz.

6.



Plot the tune:

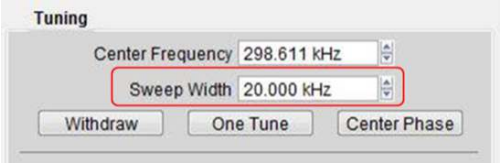
- Click 'One Tune'. The tip will engage on the surface, and the blueDrive will oscillate the lever while the optical detection system reads the oscillation signal.
- The tune plot should look something like the one shown above with a contact resonance of about 300 kHz.

Note The AFM automatically brings the tip into contact when you perform a manual tune. However, the tip is not withdrawn at the end of the tune and stays in contact. To prevent excessive tip wear, you may want to manually withdraw the tip after tunes.

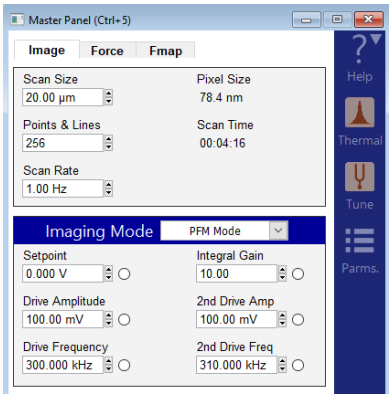
7.

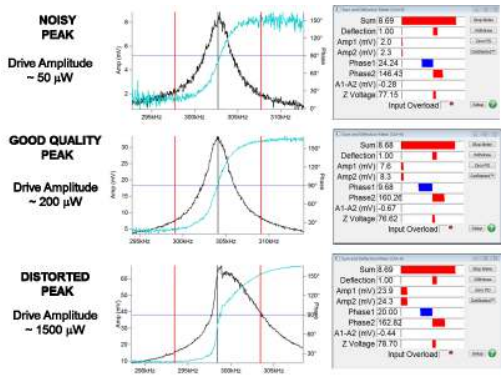
Set the peak frequency:

- Right-click on the peak and set as the *Center Drive Frequency*.
- Click the 'Center Phase' button.
- Reduce the 'Sweep Width' to about 20 kHz. This setting will zoom in the contact resonance peak.
- Click 'One Tune' again.



8.





Change Drive Amplitude:

- Start changing the Drive Amplitude.

  Caution Only use the 'Drive Amplitude' in the Master Panel (units should be mW).
- Watch what happens to Amp1 and Amp2 values, visible in the Sum and Deflection Meter panel. Amp1 and Amp2 are the two shoulder frequencies indicated with red lines on the graph.
- Amp1 and Amp2 should be well above the noise level (> 2 mV). In the example above, Amp1 and Amp2 are ~8 mV at drive amplitude of 200 W.
- If you set the drive too high, e.g., 1500 W the peak starts to be distorted.

Note The stiffness of the tip-sample contact in CR-FM is usually a very non-linear function of the indentation depth. Because of this, if the cantilever amplitude is too large, the tip-sample interaction may vary significantly over a single oscillation cycle and lead to "distorted" tunes. For some, these nonlinear tunes are an active research area; for routine CR-FM imaging, they should be avoided.

9.

Explore the Tune parameters:

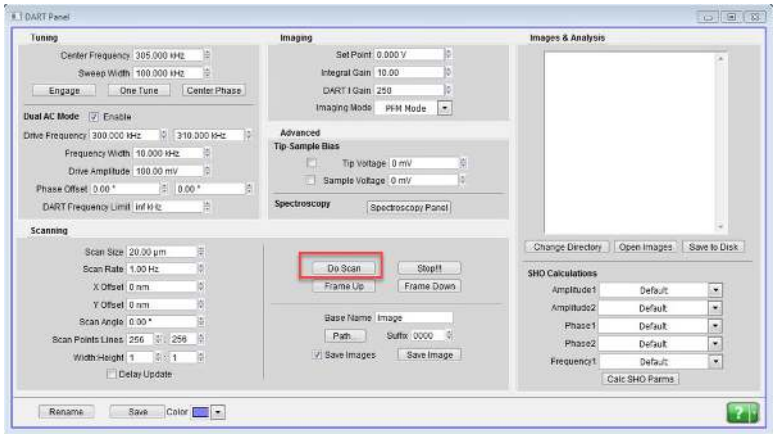
- Once you have acquired a contact spectrum, practice by adjusting the following parameters: Sweep Width, Drive Amplitude, and Set Point.
- For example, increasing the set point should increase the contact resonance frequency. When we increase the set point (deflection), we are increasing the static force on the tip and thus the tip-sample contact area. Because the frequency is proportional to the contact area, it should increase as you increase the force.

10. Once the peak in the Cantilever Tune Panel looks nice (symmetric, low noise), check the *Scanning* settings on the DART Panel:

- Scan Size
- Scan rate - Start slowly with 1Hz.
- Scan angle - Set the scan angle to 90 degrees.

11. You are now ready to start imaging.

16.2.2.3. Imaging

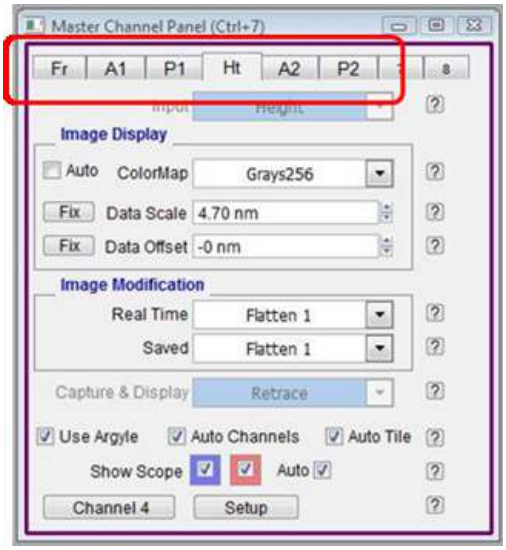
1. 

   To start imaging, on the Dart Panel:

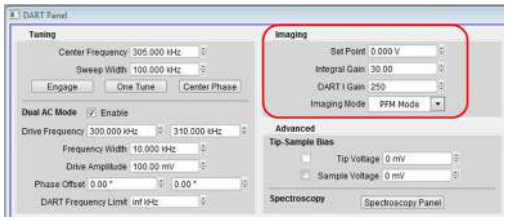
   - Click ‘Do Scan’.

2. **Check the Master Channel Panel (Ctrl+7):** Six data channels (shown as tabs at right) should be acquired:

   - Frequency (Fr)
   - Amplitude1 (A1)
   - Phase1 (P1)
   - Height (Ht)
   - Amplitude2 (A2)
   - Phase2 (P2)

3. **Adjust Integral Gain:** In the Imaging section of the DART Panel, adjust ‘Integral Gain’ as usual to obtain a nice height image (trace/retrace overlap).

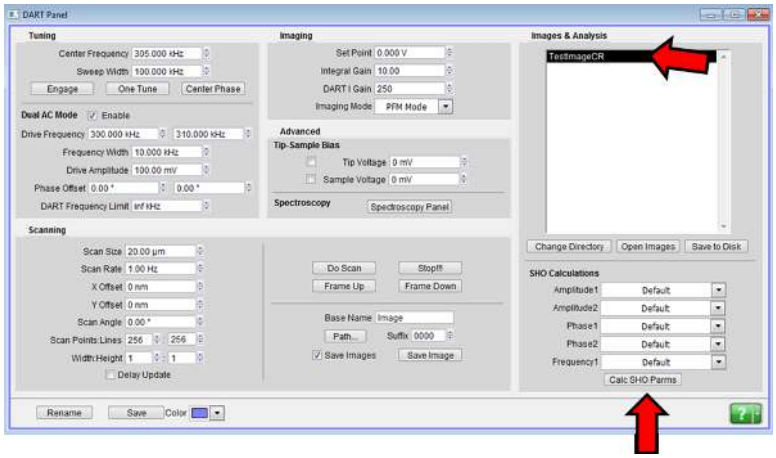
4. Start adjusting the DART ‘Integral Gain’ and watch the frequency channel for improvement in tracking, i.e., trace/retrace overlap, no ringing.
5. The deflection ‘Set Point’ can be increased to improve tip contact with the surface. Frequency will shift due to increase in contact area, so withdraw and retune when changing this parameter.
6. To compare modulus values of different samples:
   a) Calibrate the cantilever that will be used for imaging.

b) Acquire images on a sample with known modulus (take note of all the settings).

c) Acquire images on unknown samples with the same settings as used on the reference (known modulus) sample.

d) Use the Viscoelastic Panel to calculate the modulus values and compare the results.

16.2.2.4. Analysis: Converting CR images into E' and E"

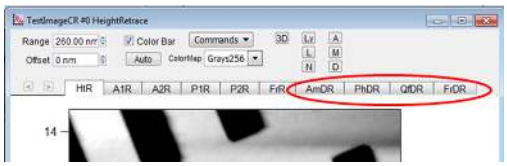
1. Information needed to perform the analysis includes Free Resonant Frequency of the cantilever and Free Q factor of the cantilever.
2. During imaging, save data for the following settings:
   - Frequency
   - Amplitude 1
   - Amplitude 2
   - Phase 1
   - Phase 2
3. Acquire images on reference and unknown samples and save the data.
4. 

   Open a sample image:

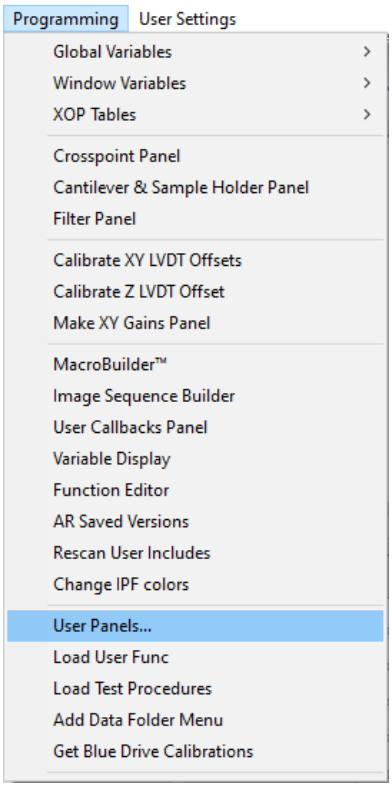
   - Open an image of a sample with a known modulus by using the 'Images & Analysis'

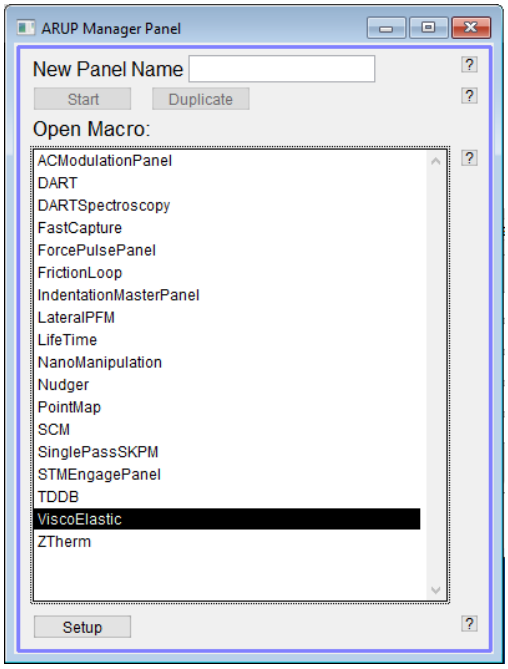
5. **Calculate SHO parameters:**

   - Click the 'Calc SHO Parms' button (see screenshot in previous step).
   - Additional panel tabs should appear in the image, as shown at right.



6.



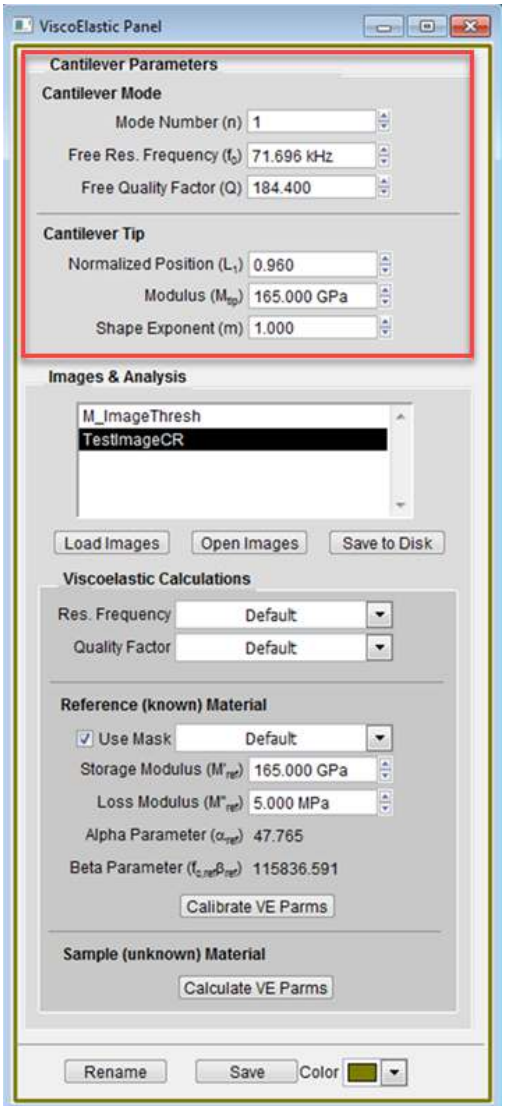


Open the ViscoElastic Panel:

- Open the ViscoElastic Panel by selecting *User Panels* in the Programming drop-down (located on top menu).
- On the ARUP Manager Panel, select *ViscoElastic*.

7. **Fill in the Cantilever Parameters section values in the panel:**

   - 'Mode Number (n)' is the cantilever mode used for imaging.
   - 'Free Res Frequency (f0)' is the free resonant frequency of the cantilever at the mode that was used for imaging
   - 'Free Quality Factor (Q)' is the Q factor of the cantilever obtained at the frequency measured in b
   - 'Normalized Position (L1)' is the position of the tip in relation to cantilever length
   - 'Modulus M (tip)' is the tip material modulus
   - 'Shape Exponent (m)' is the exponent used to descried the shape of the tip:
     - Punch = 1.0
     - Sphere = 1.5
     - Cone = 2.0

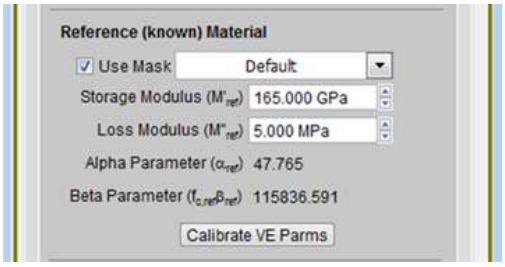


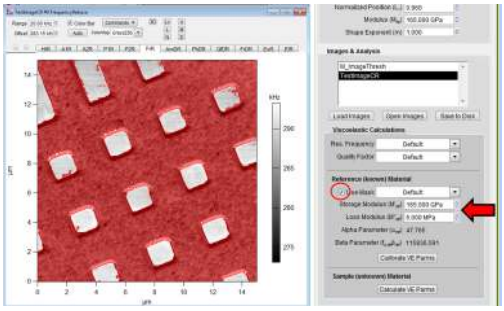
8. Highlight the image to analyze in the *Images & Analysis* section of the panel.

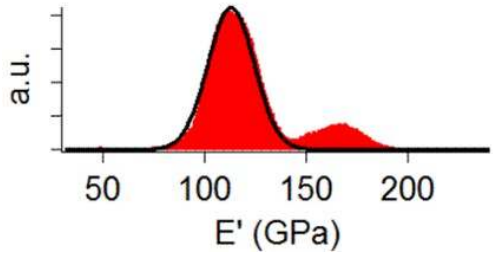
9. In the *Viscoelastic Calculations* section, leave the settings at "Default".

10. **Enter modulus values and calibrate VE parameters:**

    - In the *Reference (known) Material** section, input values for the 'Storage Modulus' and 'Loss Modulus'.
    - Click 'Calibrate VE Parms'.
    - New panels appear in the reference image, including EsR (E storage Retrace) and ElR (E loss Retrace).







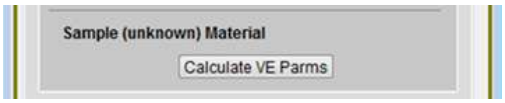
***Note** If the sample imaged is composed of two or more materials, and the modulus of one of them is known, mask function can be used to calculate modulus values of the other materials in relation to the known one. If this is the case:

- Check the box beside 'Use Mask'.
- When left to "Default", the mask of the active channel is used (in the example at right, top, the mask in the Frequency Retrace channel is used). Alternatively, you can also choose (from the dropdown menu) which channel should be used for the calculation.
- After masking the image, click 'Calibrate VE Parms'.
- The EsR and ElR panels appear in the image. The moduli both unmasked and masked regions of the image are automatically calculated, and the respective values can be obtained from a histogram of the image.

If sample is unknown:

11.

- Redo steps 1-6.
- Click'Calculate VE Parms' to calculate the storage and loss moduli of the unknown sample.



Part III

Environmental Scanner (ES)

Who is this part for? After the Cypher ES Environmental AFM has been installed in your lab, and you (or someone in your facility) have completed the initial training, this part of the user guide will be the principal reference for operating the instrument. Although written with the novice user in mind, experienced SPM users should complete the basic imaging tutorial at least once before attempting to use this instrument.

Part Contents

17. Environmental Scanner Overview

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

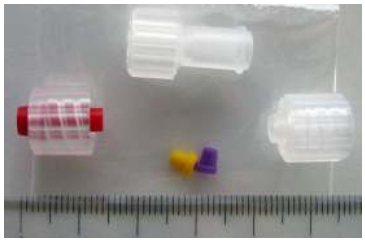
Chapter Contents

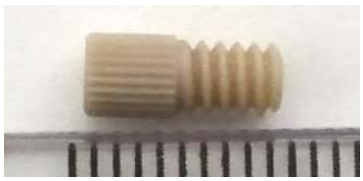
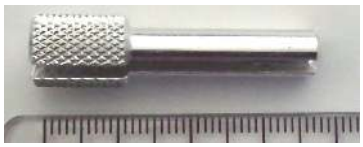
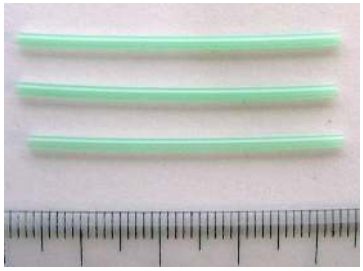
The Environmental Scanner is included with the Cypher model ES AFM. Please review the parts lists below as well as the basic imaging tutorial in Chapter 18 on page 214.

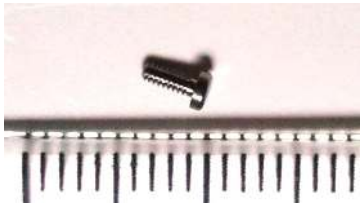
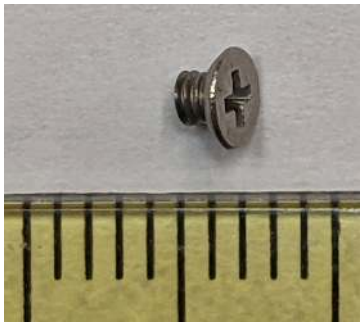
17.1. Parts list

The following table describes all the tools and other items included with your Environmental Scanner. Please refer to the part number when seeking support or ordering replacements.

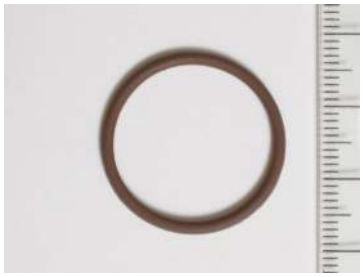
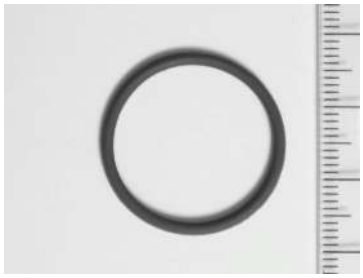
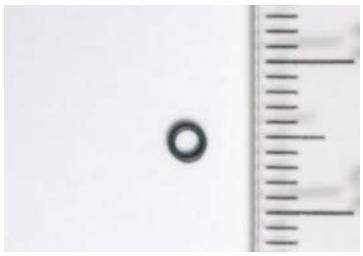
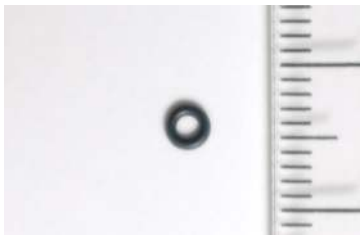
Itm	Part #	Item Description	Qty	Picture
1*	290.147	Scalpel Handle. Used to attach liquid perfusion tubing to the cantilever holder. Also useful when trimming thicker tubing for gas exchange.	1	
2*	290.148	No. 15 scalpel blade. Used to attach liquid perfusion tubing to the cantilever holder.	10	
3	290.130	1/16” Hex Driver. Used to remove the cantilever holder (see Step 6 on page 216) and chamber bodies (see Step 2 on page 253).	1	
4	290.106	#00 Phillips Screwdriver. Used to replace the cantilever. See Step 12 on page 218.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
5	290.163	5/64” (2mm) Hex Driver. Used to remove the cell body from the scanner. See Step 1 on page 253.	1	
6*	080.165	1cc HSW Norm-Ject Syringe. Typically used to perfuse fluid.	4	
7*	080.010	5cc syringe, HSW. Typically used for fluid or gas perfusion.	4	
8	231.006	PFA Tubing, 1/16” OD, 0.040” ID. 5 ft package. Special large bore tubing for gas perfusion. IDEX part number 1503.	2	
9*	231.028	FEP Tubing, 1/32” OD, .016” ID. 5 ft package. Used for fluid perfusion. IDEX part number 1692. Note, all 4 sections are in one container.	4	
10	231.008	Luer Right fitting 1/16”. Used for connecting tubing to syringes. IDEX number P-837.	4	
The scale in the photos is in cm and mm.				

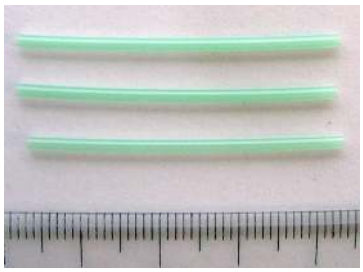
Itm	Part #	Item Description	Qty	Picture
11	232.015	Fittings, 1/16". Used to connect 1/16" tubing to the cell chamber wall. See Step 4 on page 253. IDEX part number M660. Use with tool 290.164.	4	
12	114.800	Extender Tool Fitting Wrench. Use to tighten Fittings 230.015. See Step 1 on page 262. IDEX part number N-290. DO NOT use on the cell body fittings: Step 4 on page 253.	1	
13	232.016	1/16" Ferrules. Each ferrule requires a metal and a plastic part. Used with 1/16" fittings. IDEX part number M660.	6	
14	114.721	Fitting Compression Fixture. Required to attach fitting ferrules to 1/16" tubing.	1	
15	290.165	Platypus Tweezers. EMS part 78317 style 2AZ. Useful for removing standard sample pucks from the sample chamber. See Step 19 on page 220.	1	
16*	231.019	1/16" to 1/32" tubing reducer sleeve. Used to connect 1/32" OD fluid perfusion tubing to standard syringes. IDEX part F-247X	4	
17	1-72 x 0.25 SHCS SS	1-72 x 1/4" long screw. Spare screws used to lock down the cantilever holder. See Step 2 on page 247.	10	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
18	114.576	Stage locking screw with integrated ball end. Spare screws used to lock down the sample stage. Step 8 on page 256. Be careful, these are very expensive screws.	2	
19	2-56 x 0.125 SHCS SS	2-56 x 1/8” long screws. Spare screws used to lock down the cell chamber body. See Step 2 on page 253.	12	
20	00-90 x 1/8” Pan Head SS	00-90 x 1/8” screw. Spare screws used to fasten the sample stage membrane to the cell body. See Step 4 on page 247.	12	
21	230.040	FKM O-ring, 1.5mm x 0.5mm, 75A Durometer. Now obsolete, use 230.039 instead.	0	
22	230.039	FKM O-ring, 1.5mm x 0.7mm, 75A Durometer. Used to seal the cantilever clip. Custom size, only available from Asylum Research. Equivalent FFKM part is 230.050.	18	
23	114.738	Modified (shortened) Flat Head Phillips M2 screw, Stainless steel. Spare screws to hold down the cantilever clip.	3	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
24	222.077	1/16” dowel pin. Used to restore slightly compressed fitting parts.	2	
25	290.168	1.8mm slotted screwdriver. Used to fasten the sample stage membrane to the cell body.	1	
26	232.017	1/8” NPT X 6mm tubing connector. Used to connect 6mm tubing to gas flow rotameter.	1	
27	232.018	1/8” X 1/4” union. Used to connect 1/4” tubing to gas flow rotameter.	1	
28	xxx.xxx	1/8” OD X 1/16” ID vinyl tubing. Used to connect to the gas flow rotameter.	6 ft	
29	114.801	Thermal pad, spare. Adheres to the back of the environmental scanner. Use as a replacement in case the original is damaged.	1	
30	114.820	Cantilever holder cleaning cup. Used to rinse and clean the cantilever holders while not causing damage to the circuit board.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
31	230.044	O-ring, 0.75"ID X 1"OD Viton, Durometer 75A. Equivalent FFKM part is 230.038. Standard, AS568-020 size, can also be purchased from other vendors. Sits around cantilever holder perimeter.	2?	
32	230.038	O-ring, 0.75"ID X 1"OD Kalrez 6375, Durometer 75A. Equivalent FKM part is 230.044. Standard, AS568-020 size, can also be purchased from other vendors. Sits around cantilever holder perimeter. Stored in separate box; see Figure 17.2 on page 213.	1	
33	230.050	O-ring, 0.022" C/S X 0.063” ID x 0.107” OD, FFKM, 75A Durometer. Obsolete, now use 230.051.	0	
34	230.051	O-ring, 0.032" C/S X 0.062” ID x 0.126” OD, FFKM, 75A Durometer. Custom size, only available from Asylum Research. Equivalent FKM part is 230.039. Used to seal the cantilever holder clip. Stored in separate box, see Figure 17.2 on page 213. For use,	3	
35	448.140	Electrical Sample Puck Assembly. Used to electrically bias or ground a sample. Includes Puck Bias Lead wire (448.139) and Modified 000-120 screw (114.853).	3	
36	901.778	ES tubing kit. Pre-made tubing with fittings and ferrules for making connections between the cell side ports and manifold. See 21.	2	
The scale in the photos is in cm and mm.				

* These items are likely to be used only for fluid perfusion experiments which require a perfusion capable cantilever holder and a fluid compatible sample stage.

Itm	Part #	Item Description	Qty	Picture
6*	080.165	1cc HSW Norm-Ject Syringe. Typically used to perfuse fluid.	4	
7*	080.010	5cc syringe, HSW. Typically used for fluid or gas perfusion.	4	
9*	231.028	FEP Tubing, 1/32” OD, .016” ID. 5 ft package. Used for fluid perfusion. IDEX part number 1692. Note: All 4 sections are in one container.	4	
16*	231.019	1/16” to 1/32” tubing reducer sleeve. Used to connect 1/32” OD fluid perfusion tubing to standard syringes. IDEX part F-247X	4	
The scale in the photos is in cm and mm.				

17.2. Terminology

Information referring to the terms in Figure 17.1 on page 205:

- **Cantilever Holders:** Chapter 19 on page 224
- Cell Bodies: Section 20.2 on page 232
- Sample Stages: Section 20.3 on page 243
- Scanner Swapping: Chapter 32 on page 391

17.3. Cypher ES Quick Reminder List

In this section, we provide a list of pitfalls to avoid during use of the Cypher ES. The target audience of this section is fairly specific: it is written for those users who are experienced Cypher S users but have had only a

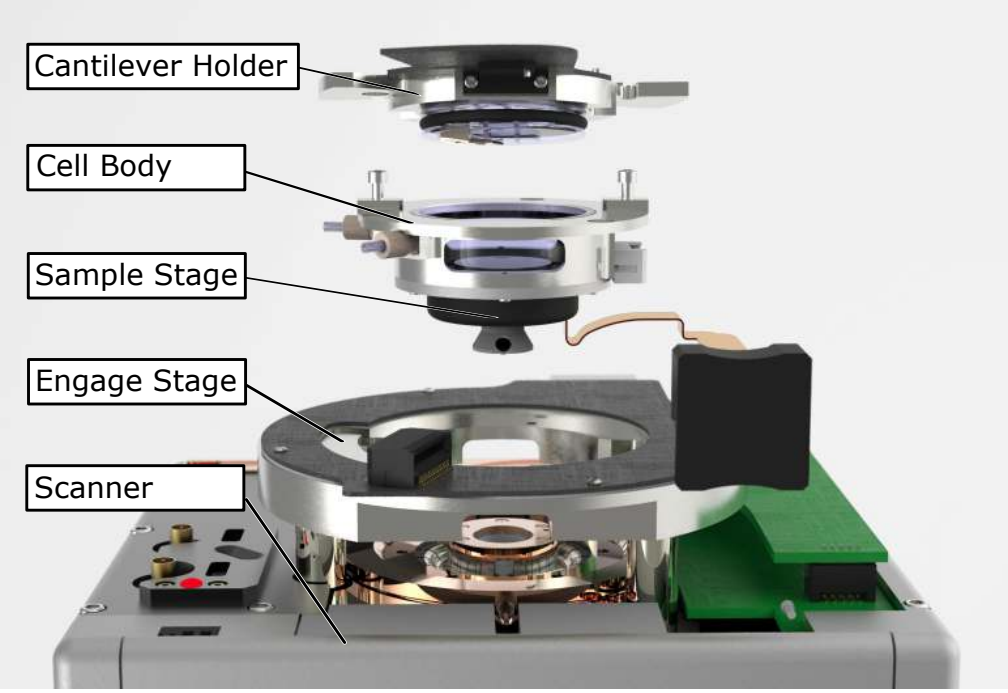


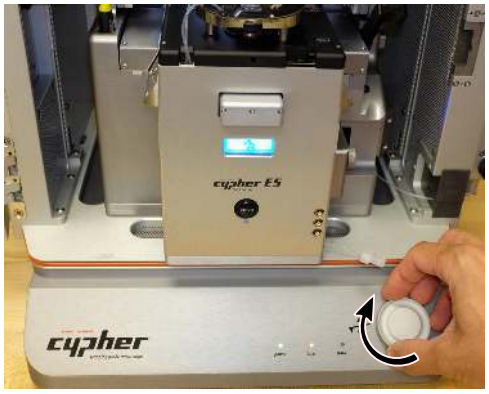
Figure 17.1.: Cypher Environmental Scanner Basic Parts

basic training on the Cypher ES. This list then allows the regular Cypher S user a quick way to remind themselves of important warnings before using the Cypher ES. Note that this reminder list assumes the user has already received basic training on the ES. New users can safely skim this section and then return to it after they have had a better understanding of ES operation.

17.3.1. Reminders for imaging

1. **Fully raise the engage stage before loading the cantilever holder!**

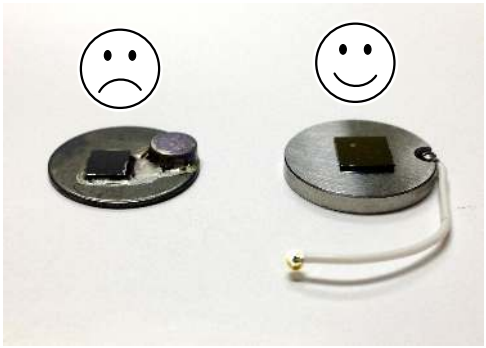
   - Compared to the Cypher S scanner, it is more difficult on the ES scanner to see if there is enough clearance for safely loading the cantilever holder.
   - The stage needs to be high enough so that when you mount the cantilever holder, it does not come in contact with the sample.
   - Rotate the 'Engage Control Knob' on the Cypher *clockwise* and hold until the Engage stage is at its upper limit of travel. Alternatively, press the '(Un)/Load Sample Button' prior to mounting the cantilever holder. The upper position is safe for all samples other than those that are too thick to be run in the ES.



Warning: Pay attention! Failure to check for clearance may cause serious damage to your cantilever holder and/or sample stage.

2. **Never load "non-flat" samples into the ES scanner!**

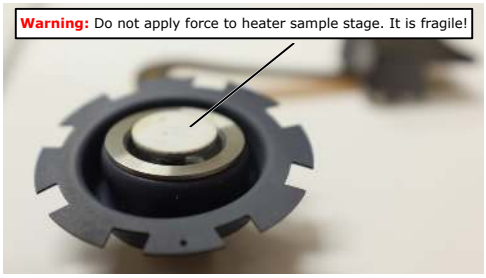
- Unlike the S scanner, the ES scanner cannot accept "non-flat" samples.
- *A "non-flat" sample is any sample in which the region to be scanned is not the tallest feature on the sample puck.* For example, in the image at right, the sample on the left has a magnet glued to the puck that is actually taller than the sample itself.
- Given the low profile of the ES cantilever holders, it would not be possible to engage on the sample shown on the left as the magnet would collide the cantilever holder before the cantilever engaged on the sample.
- For electrical measurements, use the low-profile electrical sample puck (as shown on the right side, at right) provided in the accessory kit.



Warning: Pay attention! Attempting to start a tip approach on a "non-flat" sample may cause serious damage to your cantilever holder and/or sample stage.

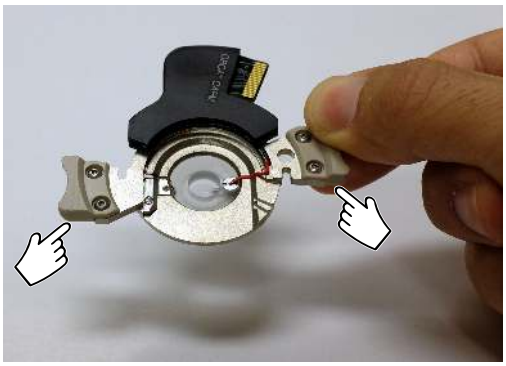
3. **Never touch the Heater sample stage with your tweezers!**

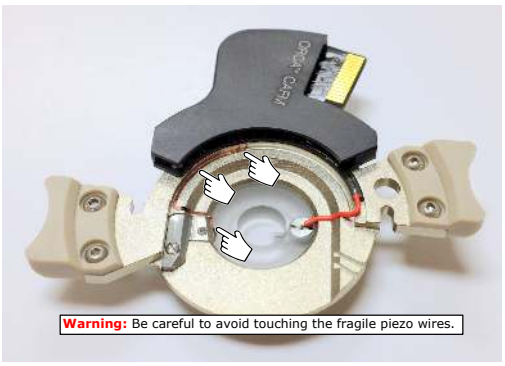
- The Heater sample stage is extremely fragile. To achieve high temperatures while still maintaining low-drift performance, the Heater Sample Stage is constructed with fragile ceramics.
- When loading and unloading the samples, take care to never push directly on the sample stage with your tweezers.
- When adjusting the lateral position of the sample, use minimal force. Pushing on the sample with too much force may crack the heater.



Warning: Pay attention! If you are careless when loading/unloading the Heater sample stage you will break it.

4.





Hold the cantilever holder only by its handles!

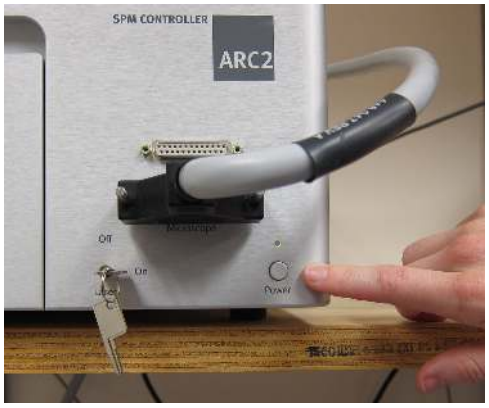
- Hold the cantilever holder only by its two plastic handles (above, left image).
- Especially avoid contacting the wires running to the tapping piezo (above, right image).
- Be careful not to scrape the piezo wires when tightening the right side cantilever holder locking screw.

Warning: Pay attention! The piezo wiring is very fragile. Be careful not to touch it.

5.

Always power off the microscope *before* exchanging scanners!

- Before exchanging scanners, remember to first motor the objective to its upper limit of travel.
- Then, *power off the controller* before disconnecting the scanner.
- Exchange scanners and then power the controller back on after the new scanner has been reconnected.



Warning: Pay attention! On older Cyphers, exchanging the scanner while the system is powered up can damage the backpack electronics.

6. **Make sure the scanner is fully seated against the chassis before locking in place!**

- Unlike the S scanner, which has a hard stop on the back of the scanner, the ES scanner has a spring-loaded stop that needs to be fully engaged.
- When inserting the ES scanner into the chassis, push the scanner until you feel the spring on the back of the scanner engage. Fully depress the spring until you feel the hard stop.
- After fully engaging the spring-loaded stop at the back of the scanner, lock the scanner in place.

Note Proper seating of the scanner is most important when using the cooling capabilities of the Cooler-Heater sample stage. During normal operation it is not particularly important.

7. **Take care to properly set the correction collar on the objective.**

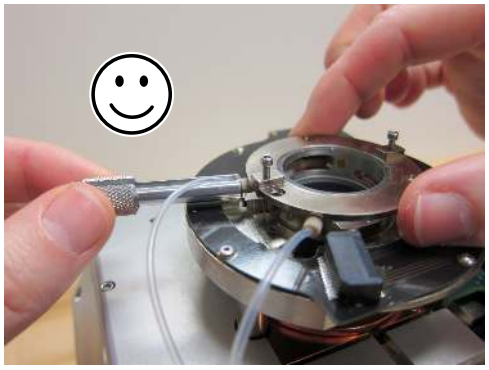
- The ES scanner uses different correction collar settings than the S scanner.
- The ES scanner uses 1.5 for gas operation and 2.0 for liquid operation.

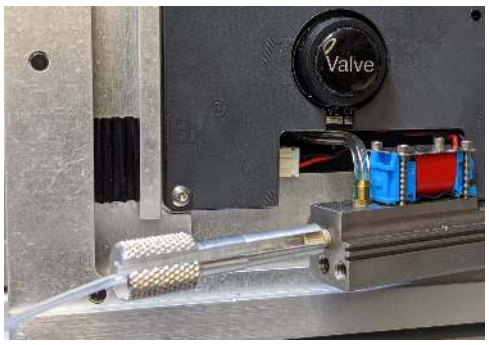
Note Incorrectly setting the correction collar will degrade the optical image quality. In addition, it will cause slight errors in the XY calibration of the scanner.

17.3.2. Reminders for cell exchange and handling

1. **Use the fitting wrench to attach gas lines to the cell body:**

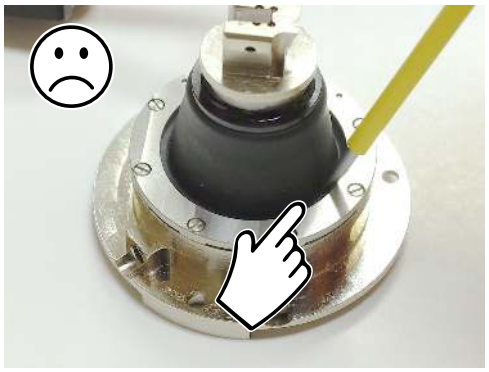
   - The fitting wrench (114.800) supplied in the accessory kit may now be used with the cell bodies (see top image at right).
   - The fitting wrench may also be used with the fitting fixture (114.721), also supplied in the accessory kit, or the manifold on the front of the scanner. See bottom right image.





2. **Be careful not to poke the sample stage diaphragms!**

   - Be careful never to poke the sample stage diaphragms with tweezers or screwdrivers, as this may cause a hole in the diaphragm.
   - Some versions of the sample stages have permanently attached diaphragms. These stages cannot be repaired if their diaphragms are damaged.



Warning: Pay attention! Don't poke a hole in your sample diaphragm by being careless.

3.

Be careful when reattaching the cantilever clip to not crack the glass!

- Anytime the cantilever clip has been completely removed (for cleaning), care must be taken when reattaching the clip.
- Do not tighten the screw until you are sure that the clip is aligned correctly and has dropped into the pocket in the glass body.

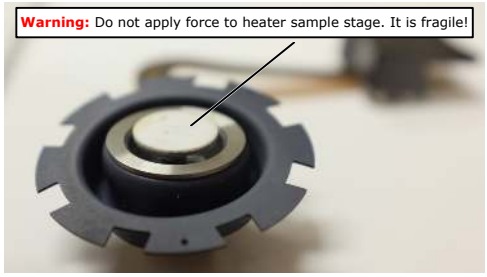
Warning: Pay attention! Failure to align the cantilever clip with the pocket before tightening the clip will break the cantilever holder glass.

4.

Use extra care when loading the heater sample stage!

- The heater sample stage is extremely fragile. Only use minimal force when seating the sample stage dovetail against the scanner.
- If you use your finger to push the stage down into the scanner, you should only need enough pressure to extend the diaphragm. Any more pressure will break the heater. If it is taking more pressure to push the dovetail into the scanner, simply push the dovetail over (with a wrench from the bottom of the sample stage) until it drops into the scanner.
- It is a good idea to wear gloves when doing this so that you minimize finger grease getting cooked onto the heater stage.

Warning: Pay attention! If you apply too much force to the heater stage, you will break it.



5.

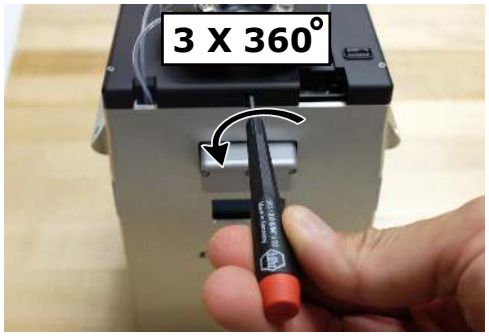
Remember to cut off extra tubing before connecting gas lines to the cell body!

- For Cypher ES that shipped after 2014, ideally there should be the extra length of tubing that extends past the ferrule. This allows for a better fit with the updated parts that include an O-ring. You can use the extra bit of tubing to fit with O-ring in current design. (See photo, top right.)
- If you are using a Cypher ES that shipped before 2014, it is likely that you need to cut off the extra length of tubing that extends past the end of the ferrule on the gas lines.
  - Cut the tubing so that is flush with the ferrule. You only need to do this for the end of the line that connects to the cell body. (See photo at bottom right.)
  - Failure to cut off the tubing will not break anything, but it will make it much more difficult to get a good seal between the gas line and the cell body.

6.

When unlocking the sample stage from the scanner you need only 3 turns.

- To unlock the sample stage from the scanner, turn the locking screw counterclockwise three turns.
- Turning the locking screw more than three turns may cause the screw to come all the way out.
- If the screw comes all the way out, you may need to lean the scanner forward until the screw slides out to a place where you can put it back on the wrench.

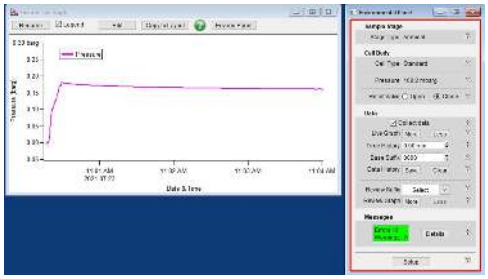


7. **Don't apply more than 200mbar pressure to the cell.**

   - When checking for leaks in the cell, it is useful to apply pressure to the chamber by using a syringe.
   - Don't pressurize the chamber over 200mbar, as higher pressures may damage the diaphragm.
   - Make sure the Relief Valve is closed when pressurizing.

8. **View Environmental Panel and Live Graph**

   - Chamber pressure can be seen in the Environmental panel under AFM controls in Igor.



17.4. Chemical Compatibility

The ES scanner is equipped with FKM (Viton equivalent) O-rings in the factory. For cases where Viton is chemically attacked, please switch to the included FFKM (Kalrez equivalent) O-ring. These rings can be found in a small box, shown in 17.2.

When you no longer require these O-rings, please store them back in the case. FFKM Costs between 10 and 100 times more than FKM, so do not misplace them.

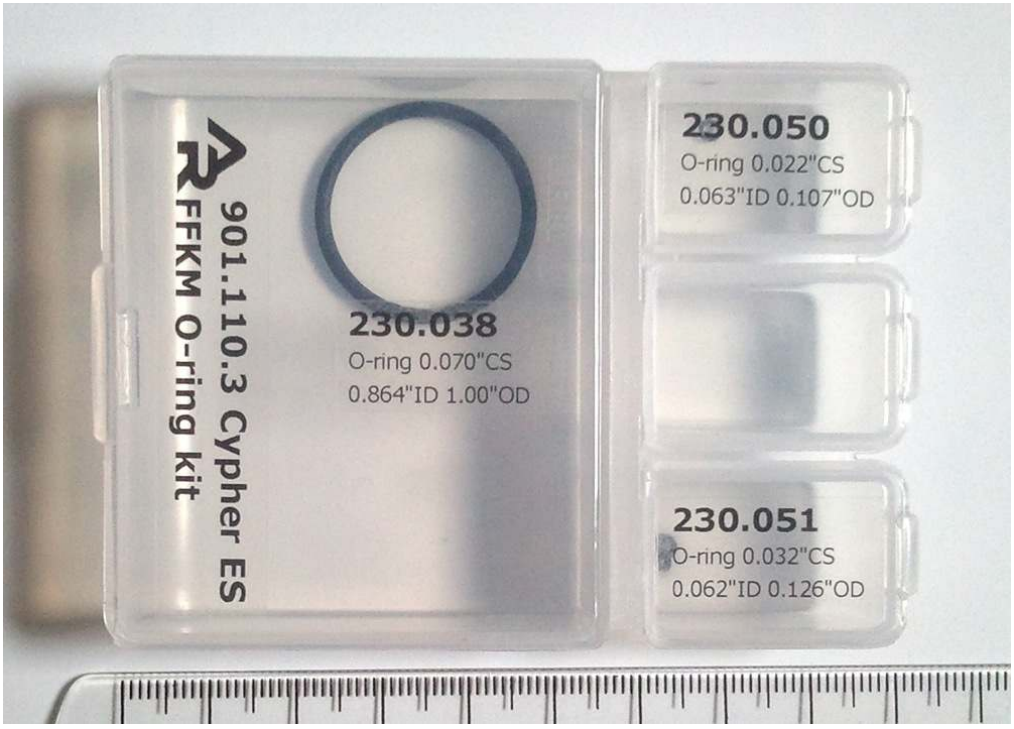


Figure 17.2.: The FFKM O-ring Kit, 901.110.3.

18. Tutorial: AC Mode Imaging in Air with the Environmental Scanner

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

This tutorial provides a quick path to learning the basic operation of the Cypher ES Environmental AFM. If you own the standard scanner, please follow the tutorial in Chapter 7 on page 42. The tutorial contains a set of steps that will teach a new user with a basic understanding of AFM operation how to obtain an AC mode topography image in air.

> *All new users should complete and understand this "AC Mode Imaging in Air" tutorial before attempting any imaging.*

The Cypher is a research grade instrument and improper use of the instrument can cause damage to the instrument and/or injury to the user. This tutorial will take approximately 3 hours.

Before you start:

- We assume you understand the aspects of running this system safely. (See Chapter 1 on page 3.)
- You are familiar with the basic names of the hardware components and software controls. (See Chapter 2 on page 16.)
- You have powered up the Cypher and launched the software. (See Chapter 5 on page 35.)

18.1. Required Materials

This tutorial is designed to be performed hands-on, not merely read. If possible, take the tutorial under the supervision of an experienced user (though have them mostly sit back, or you will not learn as much as you would by yourself).

It will be necessary to gather a few items prior to beginning the tutorial:

- Cantilevers: You will need an AC160TS cantilever, manufactured by Olympus. The AC160TS has a spring constant of ~42N/m and a resonance frequency of ~300kHz and is a workhorse for AC mode imaging in air. Every Cypher ships with a package of AC160s, but if these cantilevers are unavailable, any cantilever with a similar spring constant and resonance frequency should work fine.
- Sample: The tutorial will use the Asylum Research calibration grating that ships with every system (Asylum Part# 290.237).
- Tweezers, preferably with curved tip (for example, Asylum Part# 290.102)
- Tweezers, "platypus style" (Asylum Part# 290.165)

- 1/16” ball head wrench (for example, Asylum Part# 290.139)
- Cypher equipped with the Environmental Scanner and a large spot SLD or Laser Module (See Chapter 34 on page 398.)

18.2. Loading the Cantilever and Sample

This section covers sample and cantilever loading, as well as the coarse approach of the cantilever tip toward the sample.

1. **Raise the cantilever holder:**

   - Rotate the ‘Engage Control Knob’ on the Cypher *clockwise* and hold until the cantilever holder is far from the sample or is at its upper limit of travel.

   Note Although it is not required, for safety reasons we recommend making motor moves with the door closed. Beware of pinch points (Figure 1.2 on page 6).

   Warning: Pay attention! If you turn the knob the wrong way (counterclockwise), you will *lower* the cantilever holder instead of raising it. When you lower the cantilever holder, you can crash the cantilever holder into the sample and cause serious damage to the scanner.

2. **Open enclosure:**

   - Lift the door latch and open the enclosure door.

3.

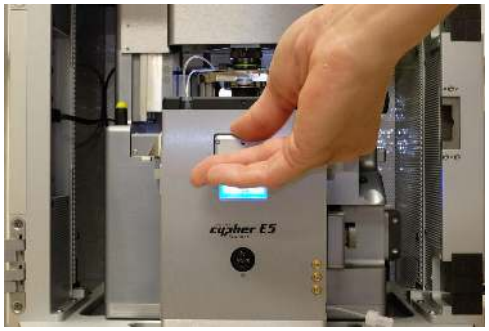
Unlock scanner:

- Lift the lever located to the right of the scanner.

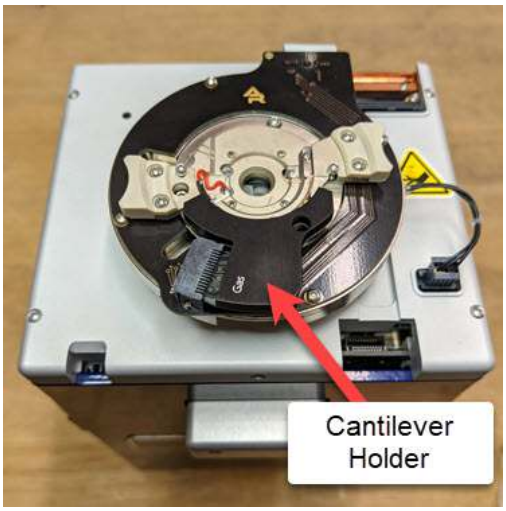
4.

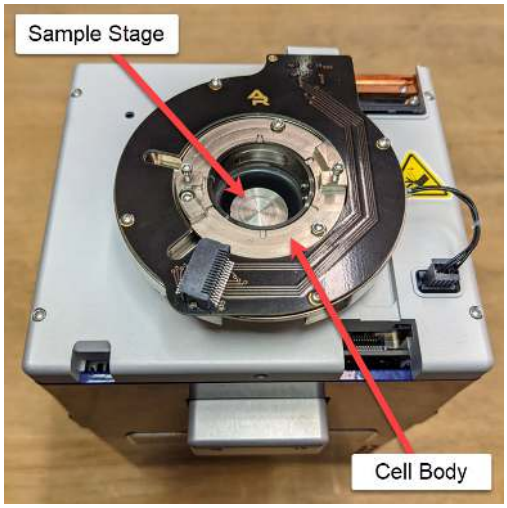
Pull the scanner forward:

- Pull the scanner forward gently and stop when it is about halfway out. As you pull the scanner out, at some point you will feel resistance and should pull no farther.



5.



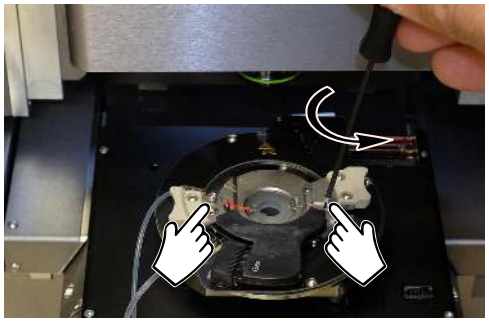


Familiarize yourself with the sample area:

- While it may look solid, the scanner sample stage moves the sample in X, Y, and Z imperceptibly up to 40μm.

6. **Release the cantilever holder:**

- Locate the tool with yellow tip in the chassis to the left of the scanner.
- Use the tool to loosen the two screws clamping the cantilever holder. One turn *counterclockwise* should be enough (do not completely unthread screws).
- Replace the tool.



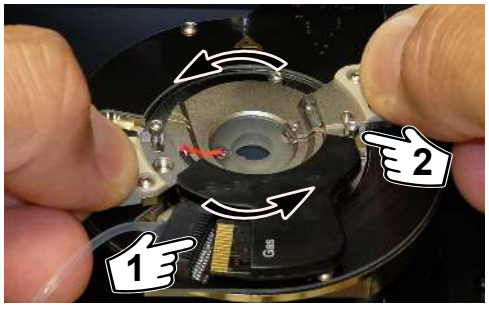
7. **Rotate the cantilever holder:**

- Place your fingers on the two side cantilever holder handles.
- Rotate *counterclockwise* a few degrees.

In the image at right:

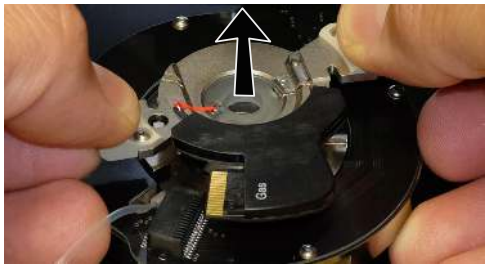
1 The cantilever holder circuit board comes out of its mating connector. Stop when the board has cleared the connector.

2 Notice the screws come away from the cutouts in the cantilever holder.



8. **Remove the cantilever holder:**

- Once the board has cleared the connector, *carefully* wiggle the cantilever holder up and out.
- The resistance you feel as you remove the cantilever holder comes is the cantilever holder O-ring sliding out from the cell body. Once the O-ring clears the cell body, be ready for the resistance to drop suddenly.
- **Caution:** *Keep a firm grip on both handles* to keep from flinging the cantilever holder across the room!



9. **Set aside the cantilever holder:**

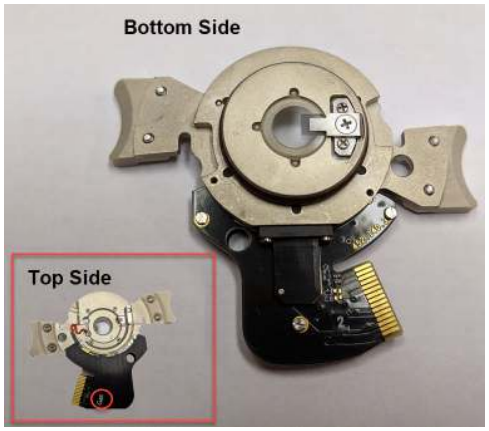
- Set the cantilever holder on its handles with the cantilever facing up so that you do not crush the cantilever.

10.

Locate your cantilever holder:

- Identify the appropriate cantilever holder. This tutorial requires the standard Gas cantilever holder, Asylum Part# 901.758.

Note To learn more about cantilever holders for the Environmental Scanner, please refer to Chapter 19 on page 224.



11.

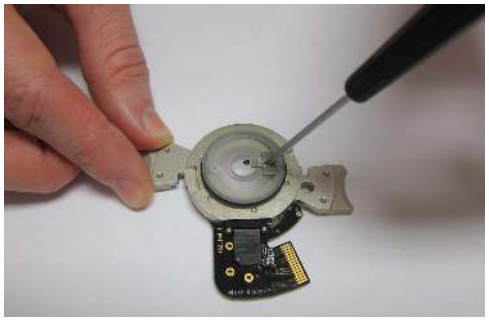
Prepare cantilever mounting workspace:

- You will need the following items:
  - Tweezer, curved (Asylum Part #290.102)
  - 300 Philips Screwdriver (Asylum Part #290.106)
  - Box of AC160TS cantilevers
  - Gas cantilever holder
- A low-power binocular dissection stereoscope with light source can be useful for some of the following steps.
- Clean the tweezer tips with alcohol to improve the handling of the cantilevers.

12.

Loosen the cantilever clip:

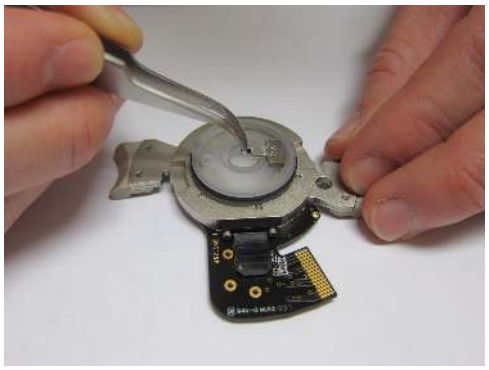
- Unscrew the clamping screws by one half turn.
- Do not unthread the screw completely. If you accidentally do, please refer to the cantilever holder chapter (8).



13.

Remove the old cantilever:

- If a cantilever has already been loaded, use tweezers to remove it from the cantilever holder.



14.

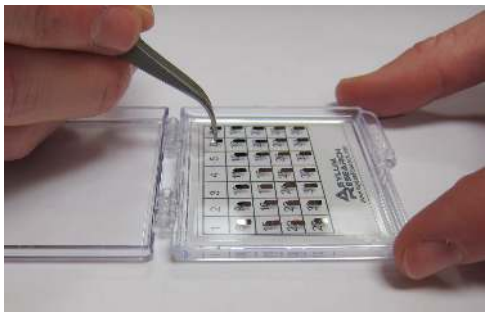
Select new cantilever:

- Use tweezers to pick up the new cantilever.

Caution: Close the box! Ruining $1k of levers by putting your hand on an open box is not unheard of.

Note If your lab saves old cantilevers, consider practicing with a "dummy" cantilever.

Tip You may find it useful to first lay the chip down on a non-sticky surface and re-grip it before continuing.

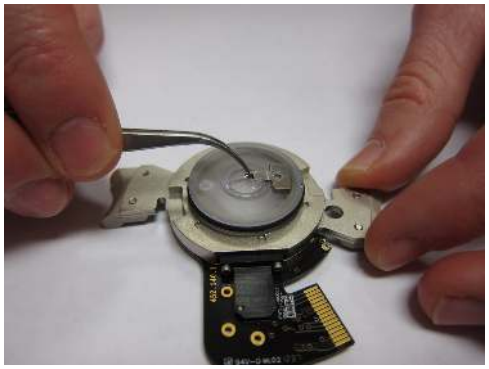


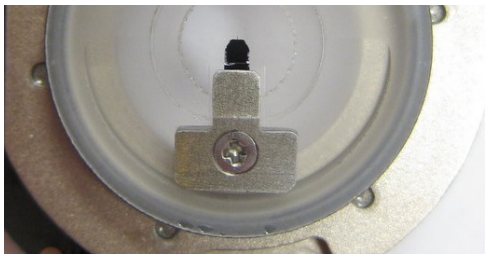
15.

Load new cantilever:

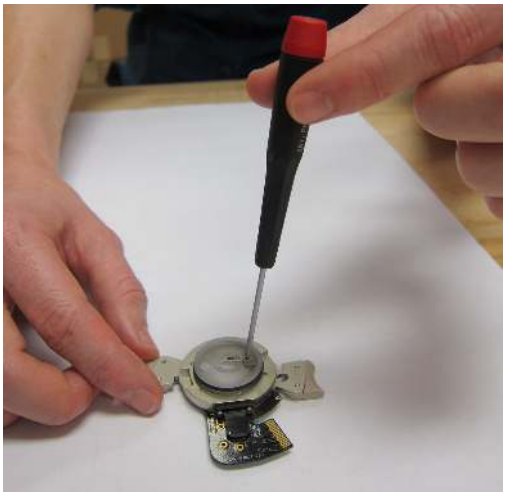
- Use tweezers to slide the new cantilever under the clip. You can "push" or "pull" the cantilever into place as you find comfortable.
- Position the cantilever so that the tip is approximately centered in the cantilever holder window.

Note You may nudge the cantilever chip from the side to align it, but do NOT nudge the end of the chip, as you risk damaging the cantilever. It helps to do this at least once under a binocular stereo microscope.





16.



Secure the new cantilever:

- Using *fingertip pressure only*, lightly tighten the screw securing the cantilever to the cantilever holder.
- The clip should be just tight enough to secure the cantilever chip when it is pushed from the side.

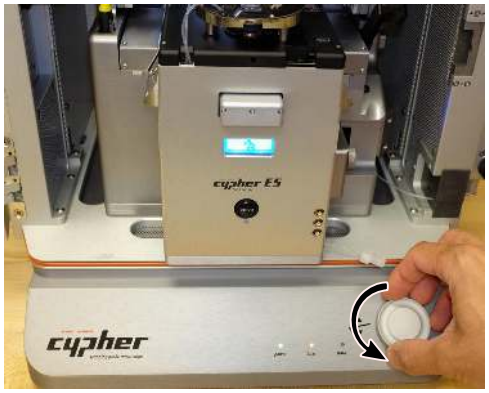
17.

Prepare scanner to remove old sample:

- Leave the cantilever holder on your workspace for now.

Note To load or unload a sample, you will need to lower the coarse engage stage so that you can access the sample stage.

- Rotate the Engage Control Knob *counterclockwise* until it reaches its lower limit of travel.



18.

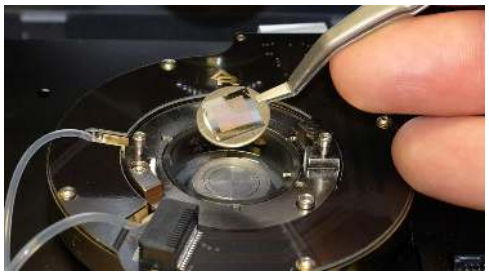
Remove old sample:

- Use “Platypus” tweezers (Asylum Part # 114.721) to remove the old sample from the sample stage.
- Wipe the sample stage clean with a cloth or use compressed air. Any dust or grit will prevent the sample disc from seating properly.

19.

Replace with new sample:

- Use “Platypus” tweezers (Asylum Part # 290.165) to place new sample on sample stage. For this tutorial, use the Asylum Research calibration grating sample, part # 900.237. It will attach magnetically.

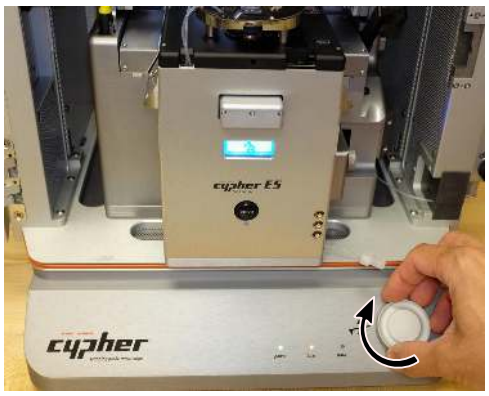


20.

Prepare scanner to load cantilever holder:

- RAISE THE COARSE ENGAGE STAGE by turning the Engage Control Knob *clockwise.* Raise the stage until it reaches its upper limit of travel.

Warning: Before loading the cantilever holder, raise the coarse engage stage. If you do not raise the coarse engage stage, you will crash the cantilever into the sample and ruin your cantilever, sample, and possibly even the cantilever holder!



21.

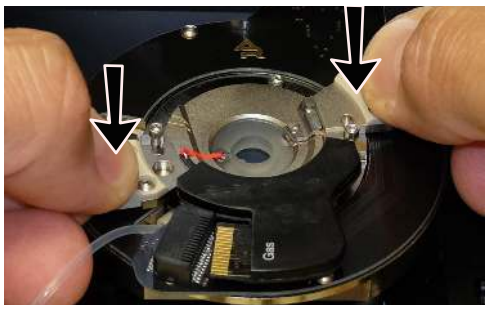
Place cantilever holder:

- Check to make sure that the coarse engage stage is raised to its highest position.
- Position the cantilever holder in the cell body, as shown in the image at right. The cantilever holder should sit level and be rotationally aligned so that the cantilever holder board is almost touching its mating connector.

22.

Press the cantilever holder down:

- Before starting this process, it is important that the cantilever holder is sitting level with respect to the top of the cell body.
- With fingers on both handles of the cantilever holder, use firm pressure to wiggle the cantilever down into the cell body. The O-ring will offer some resistance. When the cantilever holder is firmly seated, you will feel a hard stop as metal parts make contact.

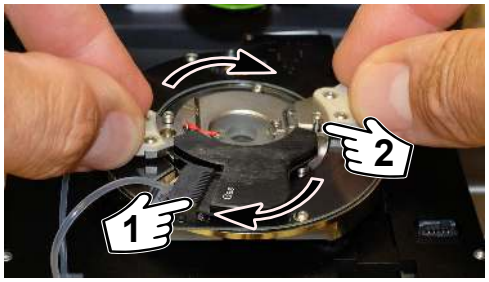


23.

Rotate: Rotate the cantilever holder *clockwise* until you feel a hard stop.

1 The cantilever holder contacts slip into the mating connector.

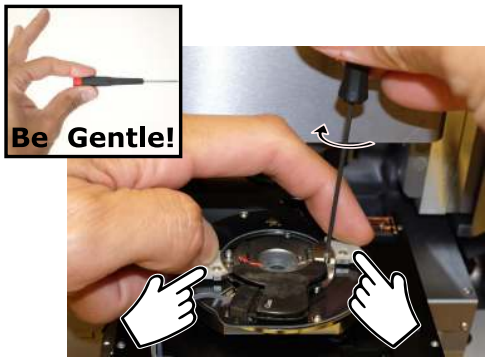
2 Notice the screws cause the hard stop as they slip into the matching metal cutouts.



24.

Tighten the screws:

- Place pressure on the cantilever holder handles as shown. Make sure it's firmly seated.
- Finger-tighten one of the screws and ***stop as soon as you feel any resistance***.
- Do the same to the other screw.
- Release the pressure on the handles and tighten both screws a tiny bit more.
- At all times, only hold the screwdriver with your fingertips.



25.

Slide scanner into chassis:

- Slide the scanner back into the chassis. Use firm pressure until you feel a hard stop. Before you feel a hard stop you will feel a spring-like resistance. You are making thermal contact between the scanner and the chassis. This is necessary for best performance.
- Maintain pressure on scanner and press the lever at the right downward to lock the scanner into place.

26.

Check correction collar:

- Check that the green correction collar on the objective is set properly. For the Gas cantilever holder, set the correction collar to 1.5.

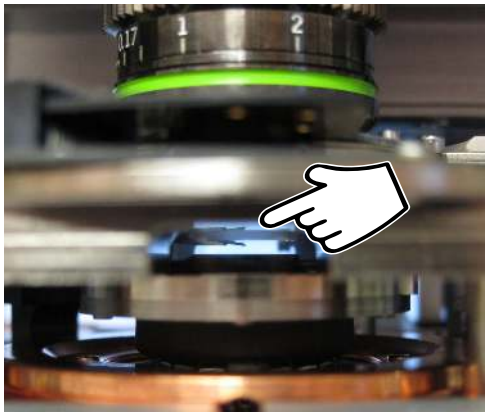
27. **Close enclosure door:**

- *Gently* close the door and latch it.

28. **Motor cantilever toward sample:**

- Place your eyes level with the cantilever and sample, so you can clearly see the gap between cantilever and sample.
- Slowly turn the 'Engage Control Knob' on the AFM enclosure *counterclockwise*. This lowers the cantilever holder and objective toward the sample. The more you turn, the faster the stage moves.
- Close the gap between tip and sample to about 1 millimeter.

Warning: Nothing but your attentiveness will prevent the cantilever holder from crashing into the sample. If you crash the cantilever holder you may cause **serious** damage to your cantilever holder and scanner.



29. This concludes the manual interaction with Cypher. We next turn our attention to the computer. Please jump to Section 7.3 on page 50.

19. Cantilever Holder Guide

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

Depending on your specific imaging application, the appropriate cantilever holder must be used. This chapter serves as a guide to the available options and to help you identify the types of cantilever holders you may already own.

All available cantilever holders have many things in common:

- All have a circuit board which allows the system to identify the type of cantilever holder and to activate the appropriate software control panels.
- All have a piezoelectric actuator and also allow AC mode and contact mode imaging.
- Nearly all have the ability to apply a voltage to the cantilever.

Many more contain specific electronics allowing for current measurement, application of high voltage to the tip, and more.

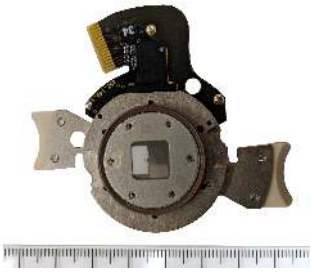
Caution	Cantilever holders are the most delicate components of the AFM. Treat it like you might treat your great grandfather's pocket watch. Never drop it! Remember that even the most basic cantilever holder costs thousands of dollars to replace.

19.1. Identifying Cantilever Holders

19.1.1. Visual Guide of Cantilever Holders

Please use this table to identify your cantilever holders and find the relevant sections which describe them.

Part #	Holder Description	Top Photo	Bottom Photo
901.758	**Gas** For most contact and AC mode Imaging.		
901.770	**Liquid** For fluid imaging in a droplet.		
901.745	**Perfusion** For fluid imaging in a droplet with flow.		
901.767	**ORCA** Conductive AFM with a single current range, 2nA/V.		
901.771	**High Voltage** Typically used for High Voltage PFM Imaging.		

Part #	Holder Description	Top Photo	Bottom Photo
901.777	**STM** Scanning Tunneling Microscopy.		

19.1.2. Electronic Identification of Cantilever Holders

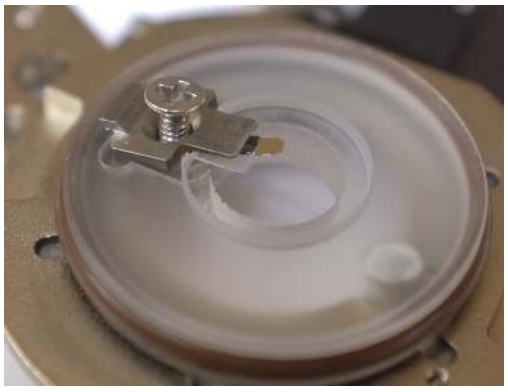
1. Attach the cantilever holder to the Cypher Scanner. (See 7.2.)
2. From the main menu bar in the software, select *Programming > Cantilever Holder and Sample Panel*.
3. At the bottom left of this panel, click the 'Check Holder' button, and the type of cantilever holder will be highlighted.

19.2. Disassembly

Only the cantilever clip can be removed from the cantilever holder. All other parts should not be removed or serviced by the user.

19.2.1. Removing the Clip

1. **Remove the screw:**
   - Using a small Phillips screwdriver, unscrew the screw completely.



2. **The clip:**
   - The clip should come off as shown at right.
   - Spare screws and O-rings can be found in the parts kit (see Section 17.1 on page 198).

19.2.2. Attaching the clip

1.

Cantilever holder:

- The cantilever holder should be sitting ready without a clip, as shown at right.

2.

Prepare the clip:

- Put the screw, clip, and O-ring together, as shown at right.
- Screw only a few turns into the threaded hole in the glass of the cantilever holder.
- ***Do not tighten yet!***

3.

Check clip rotation:

- Rotate the clip so it does not overhang any of the glass cutout's edges.
- Press down on the clip so it settles down into the cutout area of the glass.

4.

Tighten the screw:

- Using a small Phillips screwdriver, tighten the screw until you feel some resistance. This will be the O-ring compressing.
- If the clip still tends to turn around during tightening of the screw, consider holding the clip pressed down (right photo) so that it seats properly in the cutout feature.

19.3. Cleaning

Only the glass parts of the cantilever holder should come into contact with fluid during cleaning. To prevent fluid from touching the circuit components, please use the Cleaning Cup as shown below.

1.

Locate parts:

- Locate the Cleaning Cup (114.820).
- Select the cantilever holder you want to clean.
- Optionally, remove the screw and clip (see Section 19.2.1 on page 226).

2.

Attach holder to cup:

- Make sure the screws are loose enough.
- Fit and rotate the holder onto the cup, the same procedure as fitting the holder to the AFM (see Step 22 on page 221).
- Tighten the screws lightly.

Note: A perfusion cantilever holder should have plugged fluid ports before proceeding to the next step.

3. **Add cleaning fluid:**

   - Pour a small amount of solvent or water into the cup. We recommend ethyl or isopropyl alcohol or deionized water.

   Caution Acetone and Methylene Chloride and other aggressive solvents should not be used since they can attack epoxy which is exposed when the cantilever holder clip is removed.

4. **Clean:**

   - Use a soft swab to clean the surface of the cantilever holder.
   - Rinse and repeat as desired.

5. Blow-dry with compressed, filtered air.
6. Remove the holder from the cup.
7. Blow-dry around the large O-ring, some solvent may have become trapped in the outer perimeter O-ring groove.

19.4. Storage

After cleaning, store the cantilever holder in the membrane box in which it originally shipped.

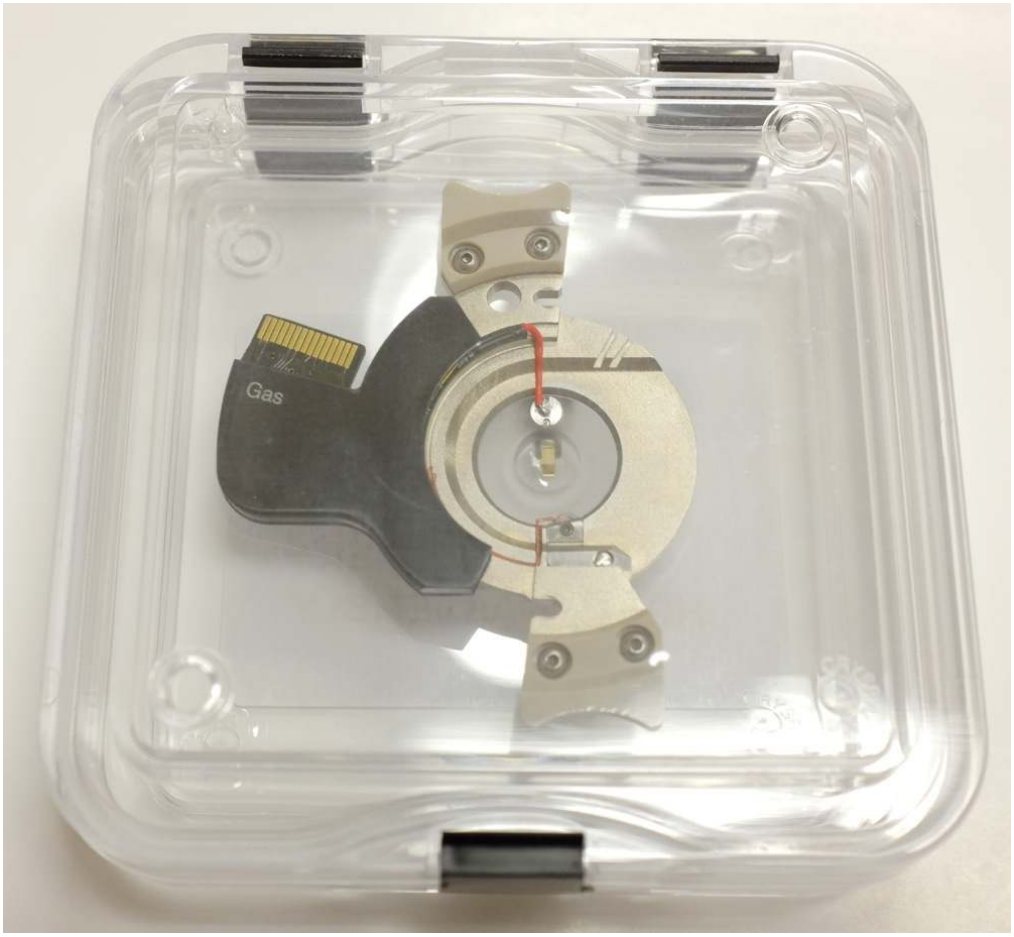


Figure 19.1.: A cantilever holder properly stored in its box.

20. Cell Body and Sample Stage Guide

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

20.1. Cell Body and Sample Stage Guide Overview

Sample stages and cell bodies must always be attached to a sample stage for the AFM to function properly.

The sealed environment around the sample and cantilever is formed by three components:

- Cantilever holder, which forms the lid
- Cell body, which forms the sides
- Sample stage, which forms the bottom

(a) A Perfusion Cantilever Holder, Fluid Cell Body, and Ambient Sample Stage, disassembled.

(b) A fully sealed assembly.

Figure 20.1.: Ambient Stage

The various cell bodies are described in Section 20.2 on page 232, the cantilever holders in Chapter 19 on page 224, and the sample stages in Section 20.3 on page 243.

The connection between cell body and sample stage is always the same mechanism and is described in Section 20.4 on page 246.

The process of attaching the gas lines to the side of the cell body is described in Section 20.5.4 on page 254.

20.2. Cell Body Guide

20.2.1. Identifying Cell Bodies

Please use this table to identify your cantilever holders and find the relevant sections which describe them.

Part #	Holder Description	Front Photo	Back Photo
901.746	**Gas** This cell is meant to be used primarily for gas environments as it has electrical feedthroughs. However, it can also be used in a droplet environment.		
901.760	**Fluid** This cell is the same as the Gas cell, but it does not have electrical feedthroughs.		

20.2.2. Gas Cell

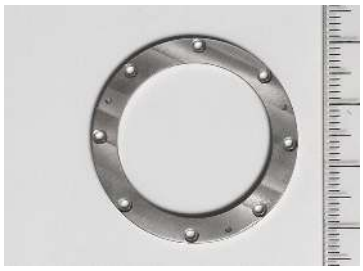
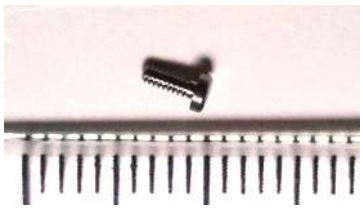
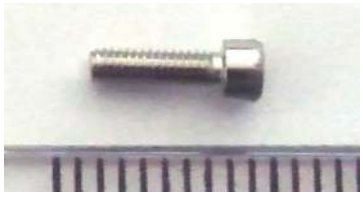
The Gas cell has three magnetic contacts which can be used to route electrical signals to the sample.

(a) Electrical Connector

(b) Magnetic Contacts

Figure 20.2.: Gas Cell Body

20.2.2.1. Glass Cell Parts List

Itm	Part #	Item Description	Qty	Picture
1	901.746	Gas Cell Body.	1	
2	114.916	Boot Clamp Ring, 8 Bolt Pattern. Connects to the bottom of the cell to form the seal between the cell body and the sample stage membrane. See Step 3 on page 249.	1	
3	00-90 x 1/8" Pan Head SS	00-90 x 1/8" screw. Spare screws used to fasten the sample stage membrane to the cell body. See Step 4 on page 247.	8	
4	1-72 x 0.25 SHCS SS	1-72 x 1/4" long screw. Used to lock down the cantilever holder.	2	
5	448.137	Gas Cell Cable. Cable to connect to magnet contacts. See Section 20.2.2.3 on page 235.	1	
The scale in the photos is in cm and mm.				

20.2.2.2. Specifications

Exposed Materials:

Cell wall: Borosilicate Glass.

Contacts: Nicked or Gold Plated.

Other: Epoxy.

Electrial: Current and voltage ratings for those contacts.

Cleaning The whole cell body can be immersed or sonicated in ethyl or isopropyl alcohol or water. Do not use acetone or methylene chloride or other aggressive solvents as it will attack the epoxy between the glass and metal parts.

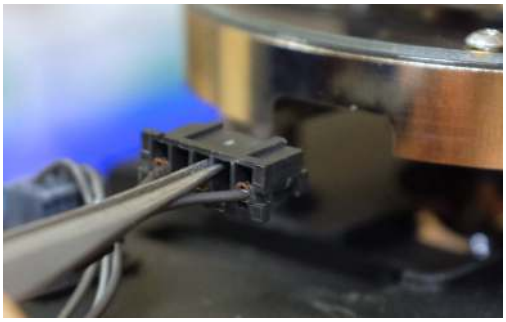
Liquid use Safe for use with most liquids being used in a droplet, but we recommend the fluid cell body for use with liquids.

20.2.2.3. Gas Cell Cable Installation

1. Raise the engage stage fully (see Step 1 on page 215).
2. Unlock the scanner and pull it all the way forward. This allows enough access to plug in the cable. You may find it more comfortable to take the scanner out of the AFM completely. To do so, turn off the system power and follow instructions here: Section 32.1 on page 391.
3. **Locate Cable:**
   - Locate the Gas Cell Cable (448.137).

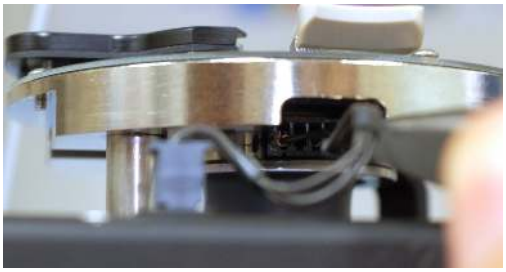
4. **Orient the cable:**
   - The cable will plug in with the holes facing toward the top of the scanner.

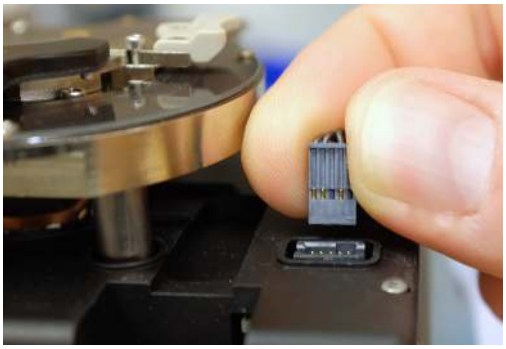
5. **Grip the cable with tweezers:**
   - Using sharp straight tipped tweezers, grip the connector as shown.



6. **Insert the connector:**

   - Carefully insert the connector as shown.



7. 

Insert the smaller connector:

- Insert the smaller connector as shown on the left.
- The final cable position is shown on the right.

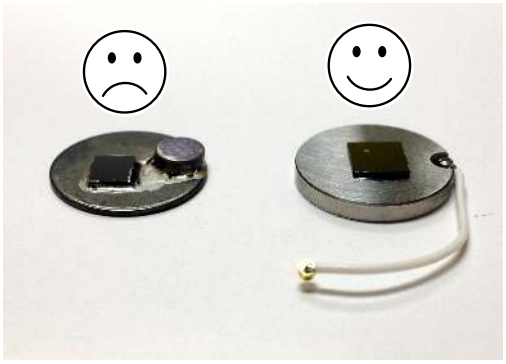
20.2.2.4. Applying Sample Bias or Ground

1. **Sample mounting:**

   - Mount your sample onto the Electrical Sample Puck Assembly (448.140) using silver paint or some other conductive adhesive.

2. **WARNING: Never load "non-flat" samples into the ES scanner.**

- Unlike the S scanner, the ES scanner cannot accept "non-flat" samples.
- A "non-flat" sample is any sample in which the region to be scanned is not the tallest feature on the sample puck. For example, the sample shown on the left has a magnet glued to the puck that is taller than the sample.
- Given the low profile of the ES cantilever holders, it would not be possible to engage on the sample shown on the left since the magnet would hit the cantilever holder before the cantilever engaged on the sample.
- For electrical measurements, use the low-profile electrical sample puck (as shown on the right) provided in the accessory kit.

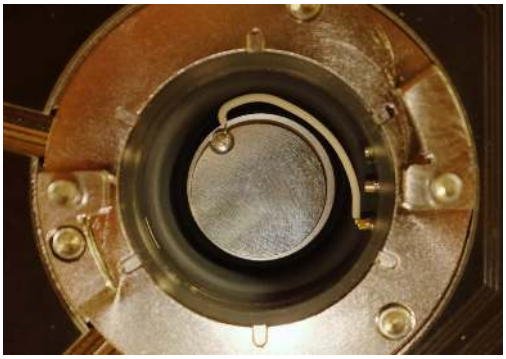


Warning: Pay attention! Attempting to start a tip approach on a "non-flat" sample may cause **serious** damage to your cantilever holder and/or sample stage.

3. **Sample BIAS Connection:**

- Insert the sample puck as shown.
- Use blunt-tipped tweezers to connect the wire, as shown at right, to the FRONT connection. This applies a sample bias.

Note Lower the cell body to get more sample access (see Step 17 on page 220).

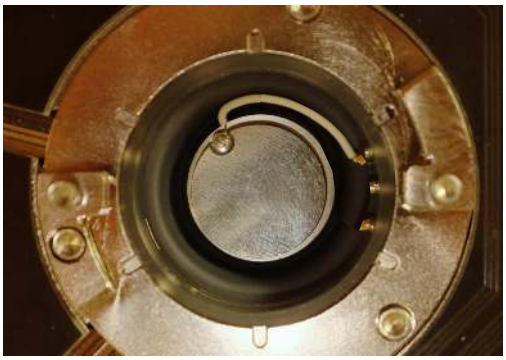


4. **Sample GROUND Connection:**

- Insert the sample puck, as shown at right.
- Use blunt-tipped tweezers to connect the wire, as shown, to the MIDDLE connection. This grounds the sample.

Note Lower the cell body to get more sample access (see Step 17 on page 220).

5.



Sample User Connection:

- The middle SMB connector on the front of the scanner connects directly to the third magnet, as shown in the photo above on the left.

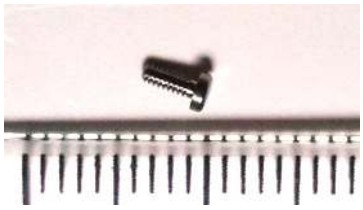
Note This is for when using the standard gas cell cable (448.137). There are other cables which assign the magnet connections differently.

Note If you don't have a connector, then use the Crosspoint Switch Panel to route one of the BNC inputs from the side of the backpack to the "sample" line. You won't get an ohmic connection, because the input passes through a buffer, then through the crosspoint switch, and then through another buffer. In that case, the input range is like all the others: +/-10 V.

20.2.3. Fluid Cell Body

20.2.3.1. Parts list

Itm	Part #	Item Description	Qty	Picture
1	901.760	Fluid Cell Body.	1	
2	114.916	Boot Clamp Ring, 8 Bolt Pattern. Connects to the bottom of the cell to form the seal between the cell body and the sample stage membrane. See Step 3 on page 249.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
3	00-90 x 1/8" Pan Head SS	00-90 x 1/8" screw. Spare screws used to fasten the sample stage membrane to the cell body. See Step 4 on page 247.	8	
4	1-72 x 0.25 SHCS SS	1-72 x 1/4" long screw. Used to lock down the cantilever holder.	2	
The scale in the photos is in cm and mm.				

20.2.3.2. Specifications

Exposed Materials:

Cell wall: Borosilicate Glass.

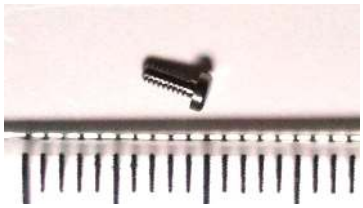
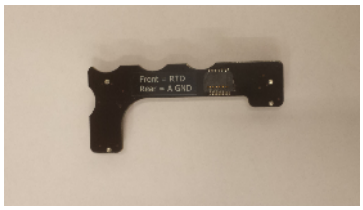
Cleaning The whole cell body can be immersed or sonicated in ethyl, isopropyl alcohol, or water. Caution: Do not use acetone or methylene chloride or other aggressive solvents as it will attack the epoxy between the glass and metal parts!

Liquid Use Safe for use with any liquid compatible with borosilicate glass.

20.2.4. Humidity Cell

20.2.4.1. Parts List

Itm	Part #	Item Description	Qty	Picture
1		Humidity Cell Body.	1	
The scale in the photos is in cm and mm.				

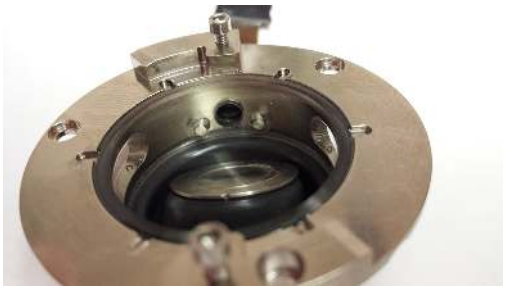
Itm	Part #	Item Description	Qty	Picture
2	114.916	Boot Clamp Ring, 8 Bolt Pattern. Connects to the bottom of the cell to form the seal between the cell body and the sample stage membrane. See Step 3 on page 249.	1	
3	00-90 x 1/8" Pan Head SS	00-90 x 1/8" screw. Spare screws used to fasten the sample stage membrane to the cell body. See Step 4 on page 247.	8	
4	1-72 x 0.25 SHCS SS	1-72 x 1/4" long screw. Used to lock down the cantilever holder.	2	
5	458.257.1	Applications Board. Used to connect the Humidity sensor to the scanner electronics.	1	
The scale in the photos is in cm and mm.				

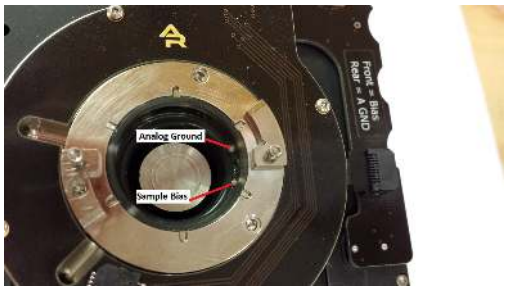
20.2.4.2. Overview

1. **Humidity Cell assembly:**

   - The Humidity Cell assembly consists of the cell body and an interconnect board called an "applications board".

2.





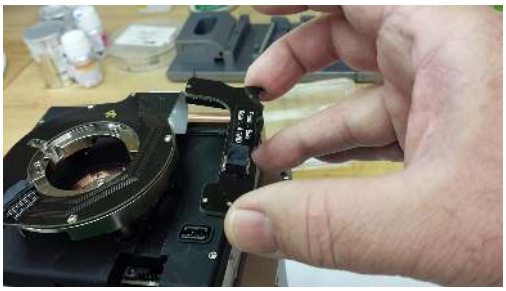
Sensor and electrical connections:

- The humidity sensor is located behind the center hole in the side of the cell's interior.
- The two electrodes located on either side of the sensor opening provide electrical connections to analog ground and sample bias. Note that the stock applications board is configured this way.
- Other connections can be provided if your experiment requires something different. Please contact Asylum Research for more information.

20.2.4.3. Installation

1. Use the coarse adjust wheel on the enclosure to raise the scanner's tip stage to the top of its range.
2. Remove the cell/stage assembly that is currently installed in the scanner. (See 20.5.)
3. **Install the applications board:**

   - Place the applications board over the connections on the top right side of the Environmental Scanner.



4. **Seat the board:**

   - Gently push the board onto the connectors.

5. Fit the cell body into the scanner and secure it to the engage stage ring with 3, 2-56 x 1/8" screws.
6. Secure the stage base into the scanner. Note that the humidity cell is delivered with an Ambient Stage already installed. The humidity cell body is compatible with all the Environmental Scanner Stages. If your experiment requires temperature control, the ambient stage can be substituted for one of the active stages.

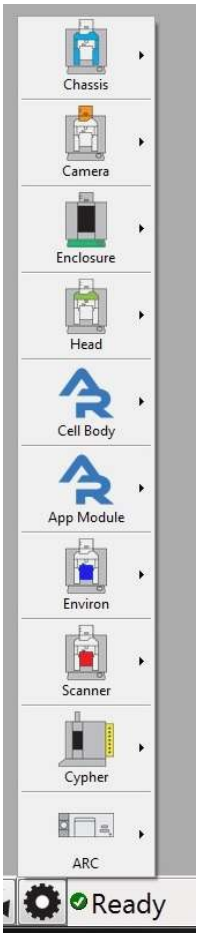
7. **Connect the “pigtail” cable from the humidity cell body to the applications board:**

   - Gently push the plug on the cable into the connector on the board.

8. **Rescan the smart start bus:**

   - Rescan the smart start bus to allow the software to detect the addition of the applications board and the cell body.
   - Verify that the devices are now present in the device list.

Note You may need to update the version of the software to version 14.16.136 or higher.



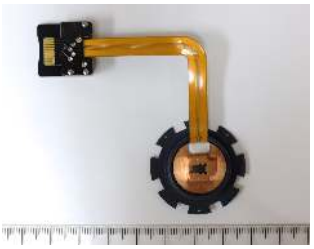
9. **Record the humidity:**

   - The humidity and temperature data from the humidity sensor should now appear in the Environmental controls panel.

20.3. Sample Stage Guide

20.3.1. Identifying Sample Stages

Please use this table to identify your sample stages and find the relevant sections which describe them.

Part #	Holder Description	Front Photo	Back Photo
901.761	**Ambient** For imaging at ambient temperatures. Safe for gas and fluid operation.		
901.747	**Heater** For imaging at ambient temperatures up to 250C. Safe for gas operation only.		
901.748	**Cooler Heater** For imaging at 0C to 120C. Safe for gas and fluid operation.		

20.3.2. Ambient Sample Stage

The ambient sample stage is the first choice for imaging at room temperature.

20.3.2.1. Parts list

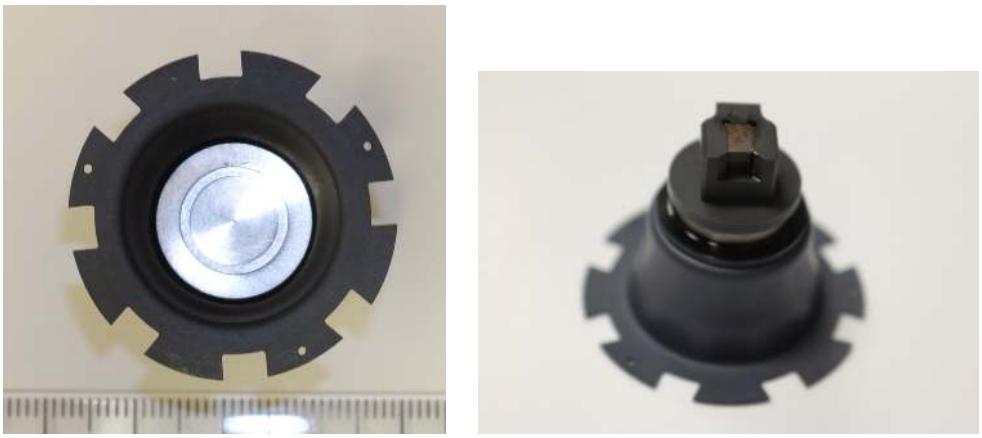
There are no associated parts. The stage comes by itself as part number 901.761.

20.3.2.2. Specifications

Exposed Materials:

Membrane: FFKM.

Stage surface: 316 Stainless Steel.



(a) Top Surface **(b)** Bottom View

Figure 20.3.: Ambient Stage

Sample Hold down: Embedded magnets.

Electrical: Stage surface is not grounded (floating) and sufficiently isolated to be safe for use with high voltage applications.

Cleaning: The whole stage can be immersed or sonicated in ethyl or isopropyl alcohol or water.

Liquid use: Safe for use with samples in liquid droplets.

20.3.3. Heater Sample Stage

Figure 20.4.: Heater Stage

The ambient sample stage is the first choice for imaging at room temperature

20.3.3.1. Parts list

There are no associated parts. The stage comes by itself and has part number 901.747.

20.3.3.2. Specifications

Exposed Materials

Membrane: FFKM.

Stage surface: Alumina Ceramic.

Other surfaces: Stainless steel, Epoxy.

Sample Hold down: Embedded magnets.

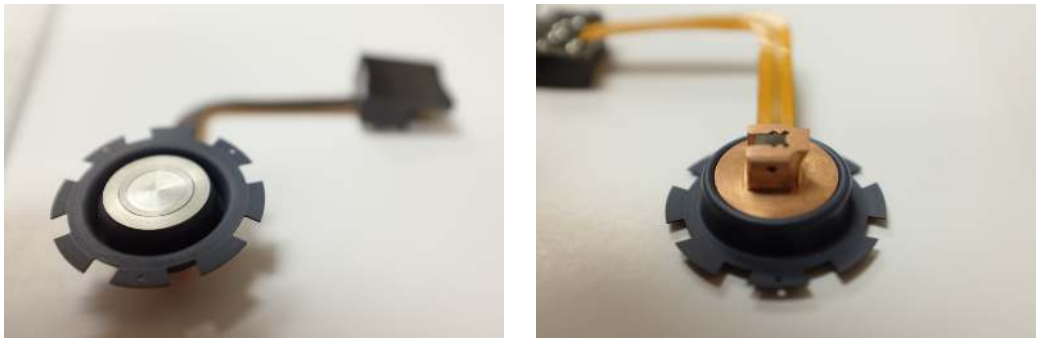
Electrical: Stage surface is not grounded (floating) and sufficiently isolated to be safe for use with high voltage applications.

Cleaning: Only wipe the sample stage with a swab dampened with alcohol.

Temperature range: Ambient to 250CºC as measured by a sensor embedded several 0.5mm below the surface on which the sample sits.

Liquid use: Not for use with any kind of liquid. Only use in air or with inert gas purge.

20.3.4. Cooler Heater Sample Stage



(a) Top Surface **(b)** Bottom View

Figure 20.5.: Cooler Heater Stage

The ambient sample stage is the first choice for imaging at room temperature

20.3.4.1. Parts list

There are no associated parts. The stage comes by itself as part number 901.748.

20.3.4.2. Specifications

Exposed Materials:

Membrane: FFKM.

Stage surface: 316 Stainless Steel.

Sample Hold down: Embedded magnets.

Electrical: Stage surface is not grounded (floating) and sufficiently isolated to be safe for use with high voltage applications.

Cleaning: When mounted to a CES cell body, it can be filled with nearly any solvent compatible with FFKM and Stainless steel and quartz. When disassembled, wipe with a solvent soaked swab or cloth, but keep liquids away from the back of the device and the circuit board connector.

Temperature range: 0ºC to 120ºC as measured by a sensor embedded several mm below the surface on which the sample sits.

Liquid use: Safe for use with samples in liquid droplets.

20.4. Tutorial: Disassembling the Sample Stage from the Cell Body

This tutorial goes through the steps of removing one sample stage from a cell body and replacing it with another. This tutorial starts with the combination of a gas cell body attached to an ambient stage and replaces that ambient stage with a heater stage.

Your cell body and sample stage may differ, but the way the two attach is universal.

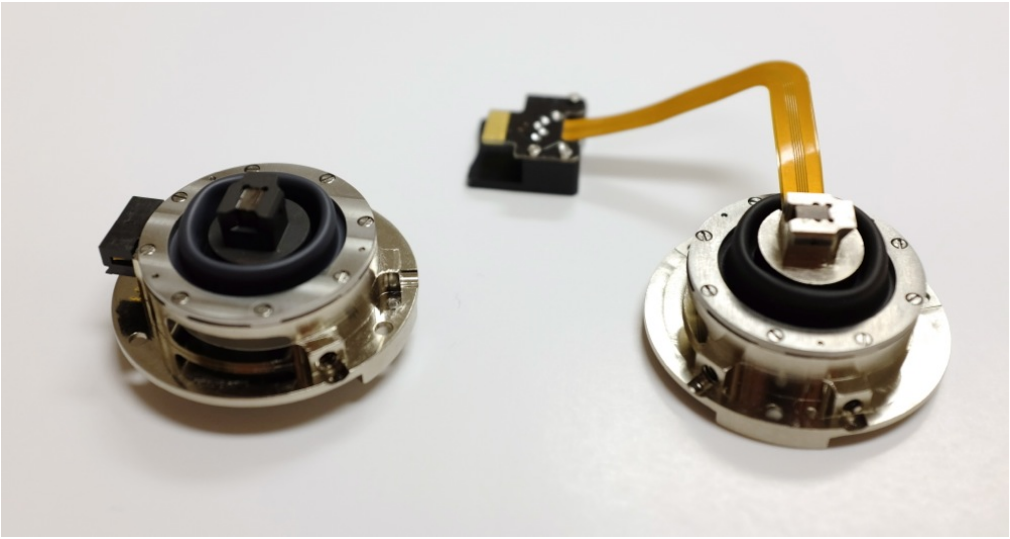
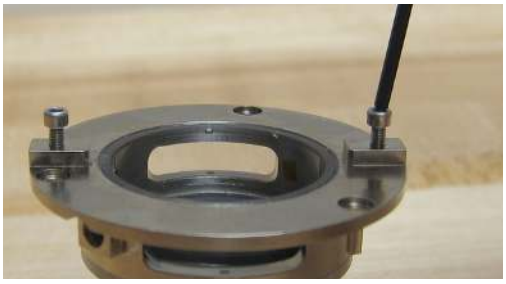


Figure 20.6.: Ambient stage / fluid cell body combination on the left. Heater stage / gas cell body combination on the right.

20.4.1. Separate Sample Stage and Chamber

1. Locate the 1.7mm slotted screwdriver and a pair of curved tweezers.

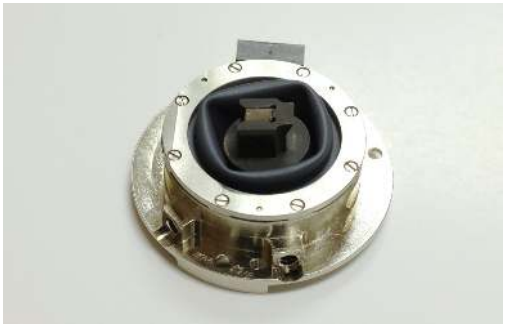
2.



Remove screws:

- Remove the two cantilever locking screws as shown and lay them aside.

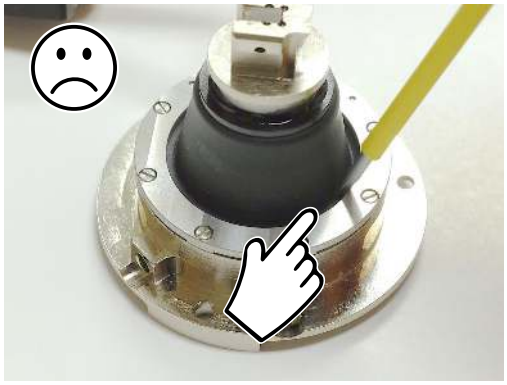
3.



Extend the membrane:

- Place the assembly as shown.
- Pull on the sample stage bottom and extend the membrane as shown.

4.



Loosen all the screws:

- Using a 1.7mm flat tipped screwdriver, loosen all the screws.

Note Be careful not to slip and possibly puncture the membrane with the screwdriver.

5. **Remove the ring:**

   - Remove the retaining ring and set it and the screws aside.

6. **Remove the sample stage:**

   - Lift off the sample stage.

7. **Finished:**

   - Your parts should now be as shown at right.
   - Store the sample stage as discussed in Section 20.6 on page 257.

20.4.2. Attach Cell Body and Sample Stage

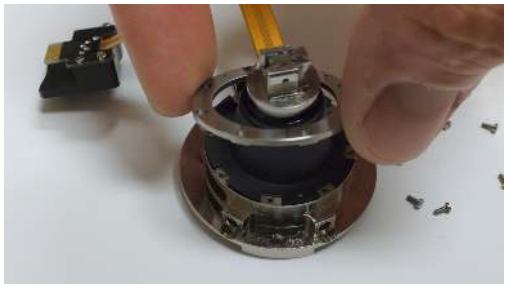
1. **Prepare parts:**

   - Sample stage, shown on the left
   - Cell body, shown in the middle
   - Membrane clamping ring and screws to the right. More screws (00-90 X 1/8”) can be found in the Environmental Scanner accessory kit (see Section 17.1 on page 198).

2. **Place the stage:**

   - Note the three small holes in the “petals” on the diaphragm perimeter.
   - Align those holes with the three pins on the bottom of the cell. The parts can only go together in one way.
   - Assist the diaphragm so the pins go through the holes.

3.



Place the ring:

- Place the ring, as shown in the photos above.
- The ring has three small holes that line up with the pins.
- Seat the ring flush against the diaphragm.

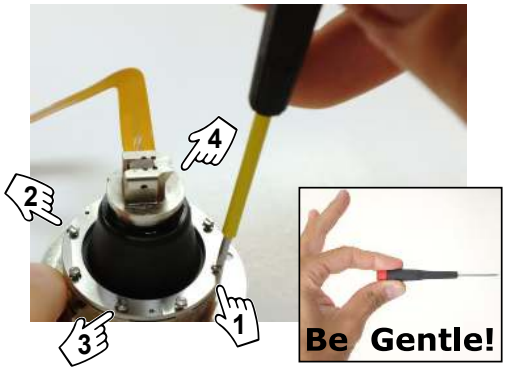
NOTE: The ring has a smooth side and a side with raised metal features. When the ring is properly placed, the smooth side is showing, and the raised features face the diaphragm.

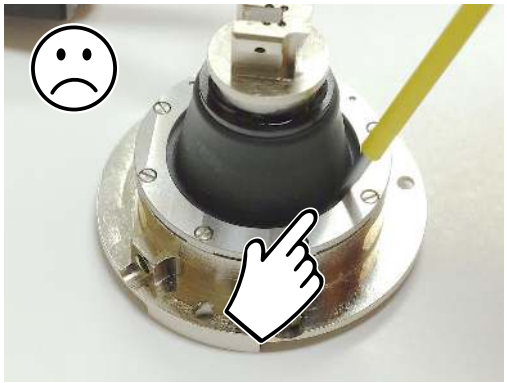
4.

Place the screws:

- As shown, place all the screws in the holes.
- Double-check to make sure the ring is not upside down!

5.





Tighten the screws:

- Using a 1.7mm flat tipped screwdriver, *gently* tighten all the screws.
- First tighten them in the pattern shown for the first four. When all 8 screws are snug, go around once more and tighten firmly while holding the tool only with fingertips to prevent over-tightening.

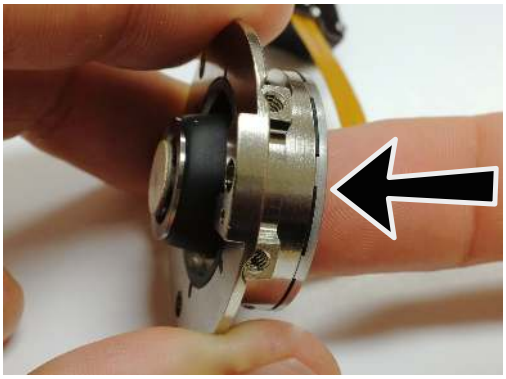
Caution Be careful not to slip and possibly puncture the membrane with the screwdriver!

6. **Finished:**

   - Your parts should now appear as shown to the right.

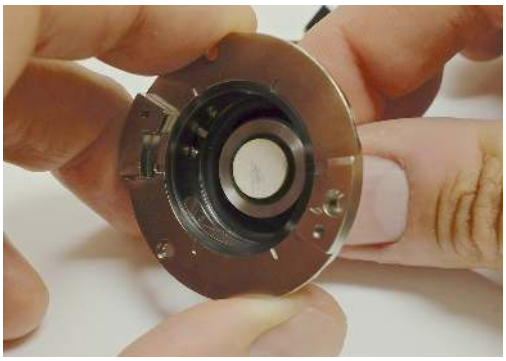
7. **Preforming the diaphragm:**

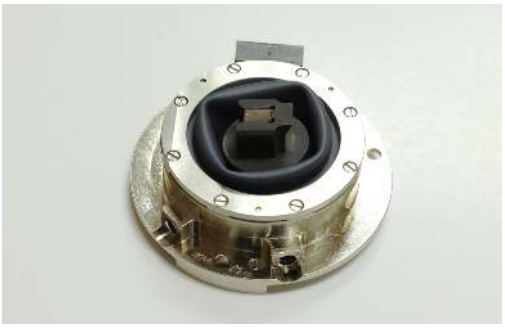
   - Gently press the sample stage as shown so the diaphragm pops through to the other side.



8. **Press back:**

   - Press the stage back a little until you can grab it from the back side.

9. 



Finish preforming:

- Pulling from the back of the sample stage, move the stage back and forth until the membrane is formed as shown on the right.

At this point the stage is ready to be mounted on the Scanner (see Section 20.5.4 on page 254) or stored away it

in its storage container (see Section 20.6 on page 257).

20.5. Tutorial: Exchanging the Sample Stage and Cell Body

This tutorial provides a quick path to learning the basics of changing cell bodies (see Chapter 20 on page 231 for options) and sample stages (see Section 20.3 on page 243 for options).

The Cypher is a research grade instrument and improper use of the instrument can cause damage to the instrument and/or injury to the user.

Before you start:

- We assume you understand the aspects of running this system safely (see Chapter 1 on page 3).
- You are familiar with the basic names of the hardware components and software controls (see Chapter 2 on page 16).

This tutorial makes the rather arbitrary choice of starting with an environmental scanner equipped with an ambient stage/ fluid cell body and replacing that with a heater stage / gas cell body combination (See Figure 20.7 on page 251). Depending on what you have available, please make the necessary substitutions.

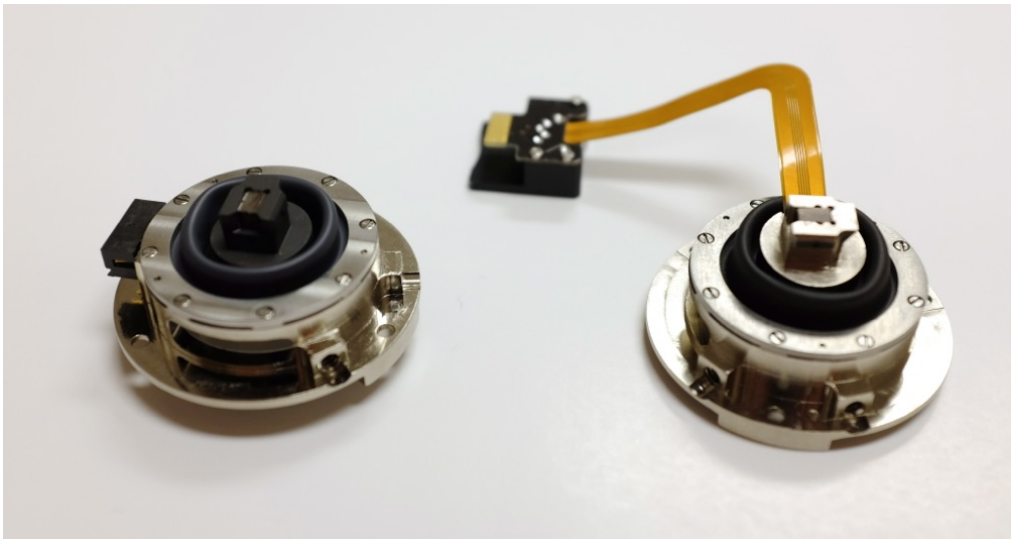


Figure 20.7.: Ambient stage / fluid cell body combination on the left. Heater stage / Gas cell body combination on the right.

20.5.1. Required Materials

This tutorial is designed to be performed, not merely read. If possible, take the tutorial under the supervision of an experienced user (though have them sit mostly back, or you will not learn as much as you would by yourself).

1. **Prepare your materials:** It will be necessary to gather a few items prior to beginning the tutorial:

   - Tweezers, preferably with curved tip (for example, Asylum Part #290.102)
   - 1/16” Hex Driver (Asylum Part #290.130)
   - 5/64” (2mm) Hex Driver
   - Sample stage already attached to a cell body

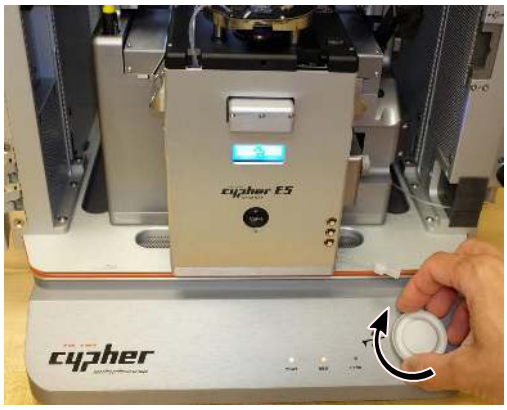
20.5.2. Prepare the scanner

1. **Remove the cantilever holder:**

   - Remove the cantilever holder and place it, cantilever tip facing UP, to the side. See Step 6 on page 216 and the following few steps.

2. **Prepare scanner for Removal.**

   - Raise the coarse Engage stage by turning the Engage Control knob *clockwise.* Raise the stage until it reaches its upper limit of travel. This prepares the engage mechanism for a later step of installing a heated temperature stage.



3. **Remove the scanner:**

   - Turn off the ARC2 controller power and remove the scanner from the Cypher Chassis (see Section 32.1 on page 391 for more information). Place it on a well-lighted and clean work surface.

20.5.3. Remove the Sample Stage and Chamber

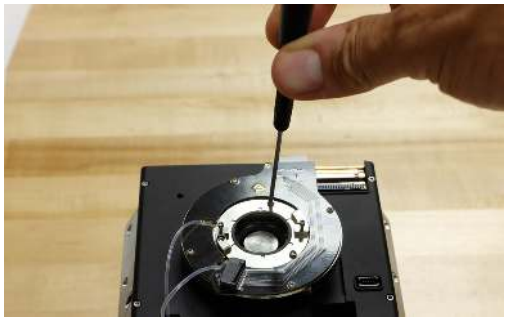
1. **Loosen the sample stage clamping screw:**

   - Insert the 5/64” Hex Driver into the screw hole at the front of the scanner.
   - Turn the Hex Driver *counterclockwise* at most **three** full rotations to loosen sufficiently.

   Note Turning the locking screw (114.576) more than three turns may cause the screw to come all the way out. If this occurs, you may need to lean the scanner forward until the screw slides out to a place where you can put it back on the wrench.

2. **Loosen the cell body screws:**

   - Use the 5/64” Hex Driver to loosen the screws at the top of the cell body.
   - Set the screws aside.
   - Extra screws are available in your kit. Replace old screws with Item 20, 2-56 x 1/8” long screws (Asylum Part #SHCS SS).



3. **Pull the cell body partway out:**

   - Grasping the cell blocks, pull up on the cell body until the diaphragm is fully extended.

4. **Loosen fittings of gas in/out lines:** In this step, you will remove the gas in/out lines. Notice that the gas lines are sealed by a small O-ring (230.039) which may or may not come out when you pull out the gas lines.

   - Grasp the cell body with your dominant hand.
   - With the opposite hand, use ONLY YOUR BARE FINGERS to loosen the fitting by turning it *counterclockwise* to loosen it.
   - Detach both gas lines.

5. Check the gas lines to see if small O-rings are stuck to the end. Usually, the rings will stay inside the cell, but in case they are stuck to the gas lines, please unstick them and place aside.

6. **Lift out the sample stage:**

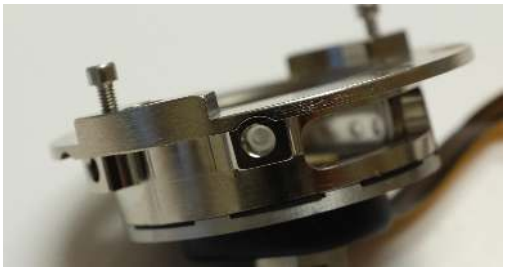
   - Lift the sample stage out of the cell body.
   - If you encounter resistance, you may need to further loosen the sample stage clamping screw (see Step 1 on page 253).

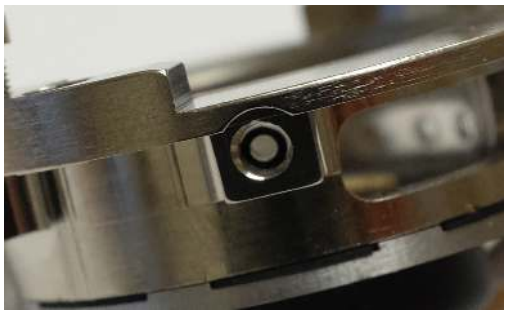
7. Store the sample stage / cell body combination it its proper storage container. See Section 20.6.1 on page 257.

20.5.4. Mount the New Stage/Cell Combination

1. **Check the membrane shape:**

   - Make sure the rubber membrane on the sample stage is properly formed as shown in the photo.
   - If necessary, see Step 7 on page 250 on how to form the membrane.

2. 



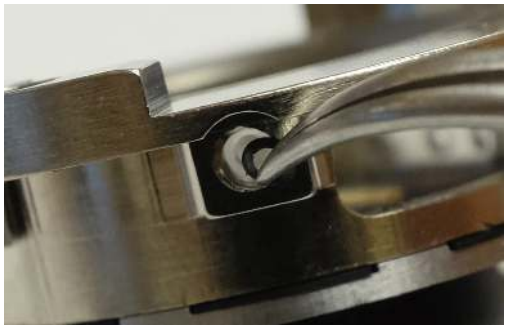
Check for O-rings:

- The cell body on the left has the O-ring (230.039) missing.
- The cell body on the right has a properly seated O-ring.

3.

Place O-rings if necessary:

- If no O-ring (230.039) is present, place it in the bottom of the port, up against the glass.
- If the O-ring is not properly centered, remove it (preferably with a sharp wooden stick) and replace it.
- If you are gentle with the cell, the O-rings should stay in place during the following steps.



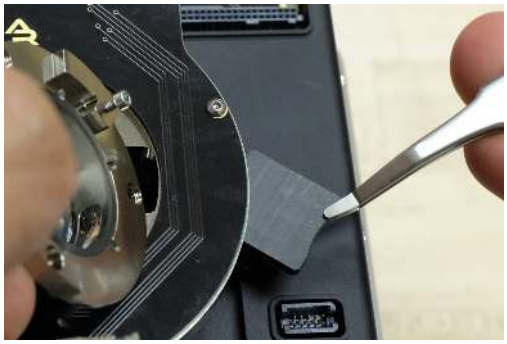
4.

Prepare to route the cable:

Note This step requires that the engage stage was fully raised before the scanner was unplugged.

- Hold the stage/cell as shown and tuck the cable connector under the engage ring using the “platypus” tweezers.

5.



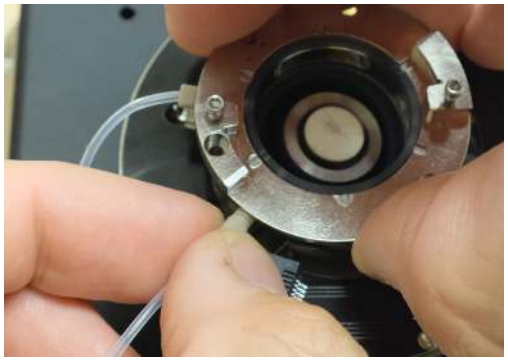
Guide the heater cable under the ring:

- As shown, guide the connector under the ring.
- Leave it sitting loose on the top of the scanner.

6.

Attach gas lines:

- Using the fitting tool (or your fingers) to tighten the screws, attach the gas lines to the cell body as shown.
- Tighten until snug, don’t overdo it.



7. **Seat the sample stage:**

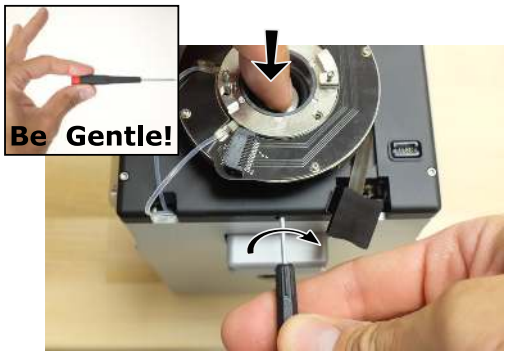
- Lower the dovetail connection at the bottom of the stage into the receiving hole on top of the scanner.
- Press lightly onto the top of the sample stage until the sample stage sinks down into the hole.
- Check that the cell body is seated on the scanner engage ring.

Note A properly seated sample stage is centered in the cell body.

BE *GENTLE*!

8. **Tighten sample stage:**

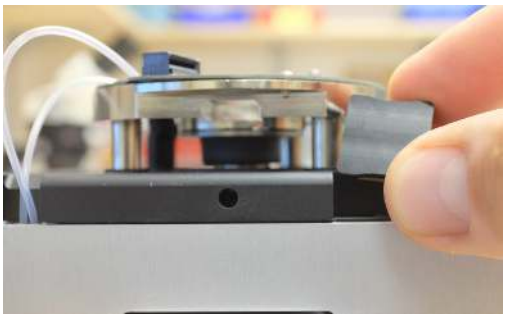
- Press down on the sample stage to keep it flush against the scanner.
- Tighten the screw using the 5/64” driver. This will take about three turns before you feel resistance.
- Tighten snug, only using your fingertips to handle the tool.

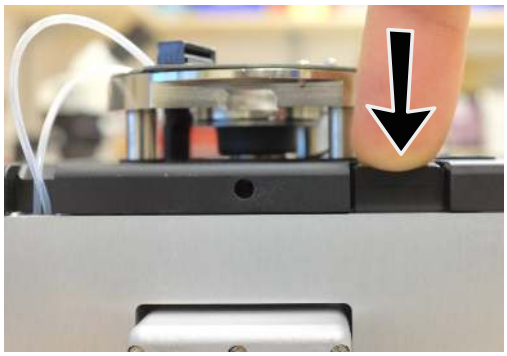


9. **Secure the cell body:**

- Locate three 2-56 X 1/8” screws and the 1/16” hex driver tool.
- Fasten the screws as shown, using only your fingertips to hold the tool. This will prevent over tightening.

10.





Connect the heater cable:

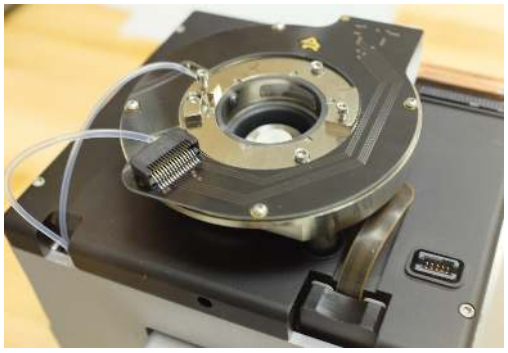
- As shown, insert the heater cable connector.
- Push it flush.

11.

Final checks:

- Your scanner should now look like the photo to the right.

CHECK The sample stage top surface sits quite deep as shown, below the glass sidewalls of the cell body. View the stage through the windows on the sides of the cell and make sure you cannot see it. If the sample stage was not seated properly, you will crush it when you go to insert the cantilever holder.



20.5.5. Replace the Scanner

1. Refer to 32.3.
2. When pressing the scanner against the back of the chassis, you should sense a hard stop of metal touching against metal. This requires a little extra pressure to compress some springs behind the copper plate at the back of the scanner. Not pressing the scanner all the way into the chassis may hamper thermal performance when using cooling or heating stages.

20.6. Storage

20.6.1. Storing Cell Bodies

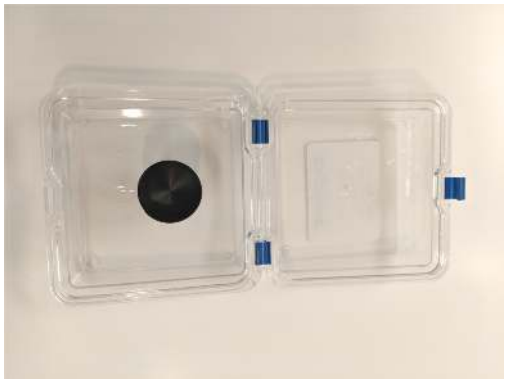
Once a sample stage has been removed from a cell body (See 20.4.1), the cell body should be stored in the membrane container in which it shipped. See Figure 20.8 on page 259.

For cell bodies were recommend storing the membrane clamping ring attached to the cell body with its eight screws and placing any other associated accessories such as cables with the cell in the box. (See Storing sample stage / cell body combinations.)

Since cell bodies must always be used while attached to a sample stage, we recommend storing them together when possible. For this purpose, the cell body is shipped in a somewhat larger container and includes a plastic cap which is used when storing cell bodies and samples stages together in the box. This works as follows:

1. **Locate the box:**

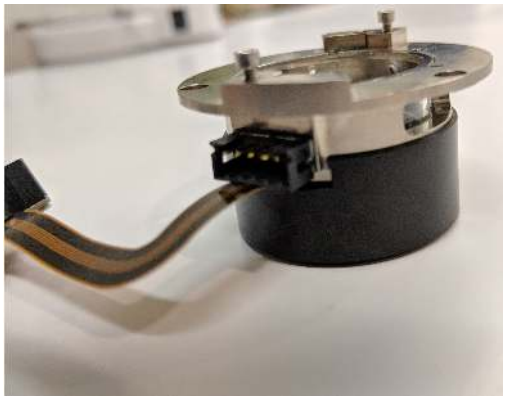
   - Locate the cell body storage container.
   - It should include the storage cup.



2. **Place the cap:**

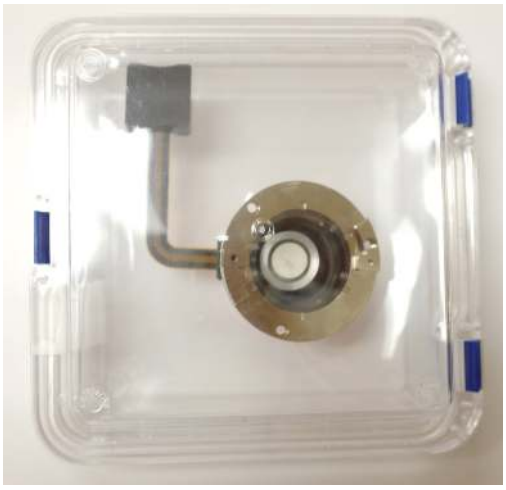
   - Open the box and remove the cup.
   - Place the cap on the cell body as shown.

   Note The cutout on the cup should be placed where the cable exits the heater stage and where the electrical vias are located.



3. **Close the box:**

   - Close the box and latch it.
   - Inspect the other side to confirm that the diaphragm is not overly deformed.



20.6.2. Storing Disassembled Cell Bodies

Once a sample stage has been removed from a cell body (See 20.4.1), it should be stored in the membrane container in which it shipped. See Figure 20.8 on page 259.

(a) Heater Stage **(b)** Gas Cell Body **(c)** Passive Stage

Figure 20.8.: Various stages and cell bodies stored in their containers.

21. Gas Handling and Leak Testing

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

21.1. Gas Handling Overview

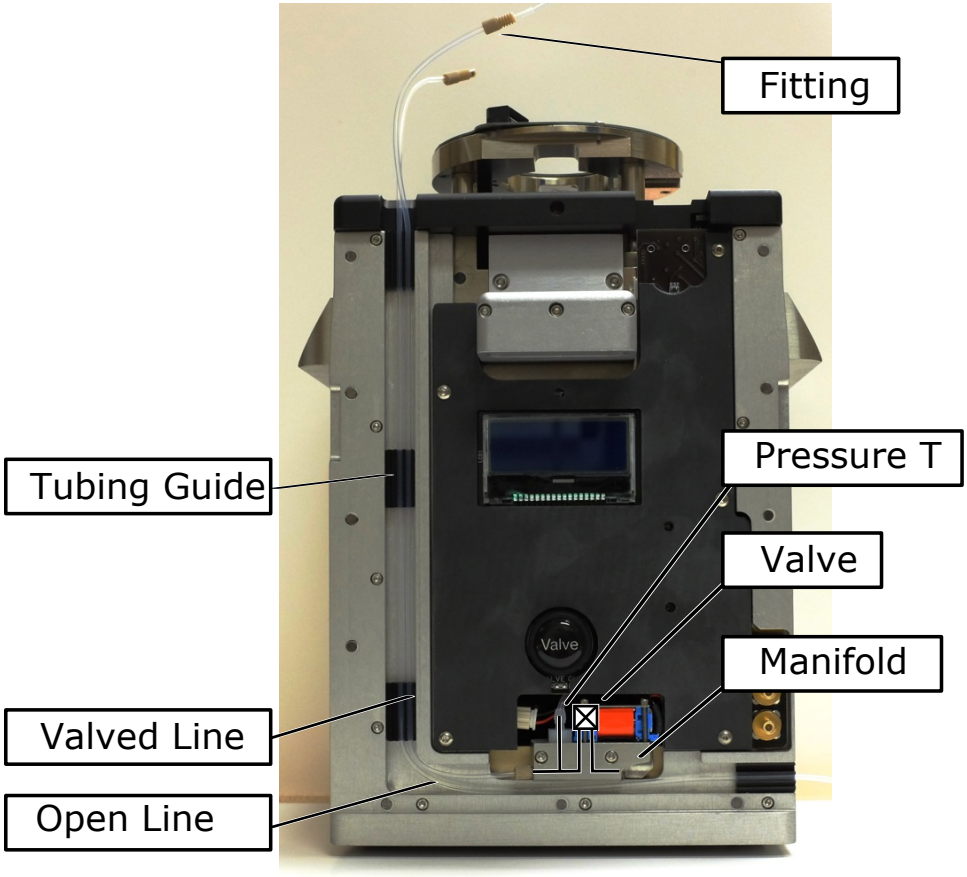


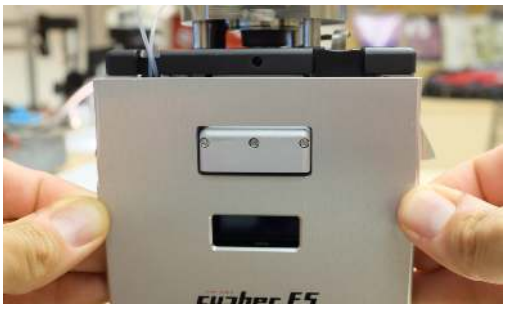
Figure 21.1.: Gas Handling Overview

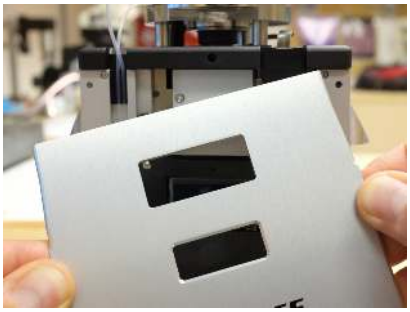
Figure 21.1 on page 261 shows the front of the scanner with the cover removed (see 21.2.1 to accomplish this). The two gas lines attached to the sample cell body are routed via tubing guides to the bottom left of the scanner. One of the lines branches off to a pressure sensor and then passes through a computer-controlled valve. Depending on the application, this valve may be open or closed, and it may be manually, or computer controlled.

21.2. Manifold Connections

21.2.1. Scanner Faceplate Removal

1.





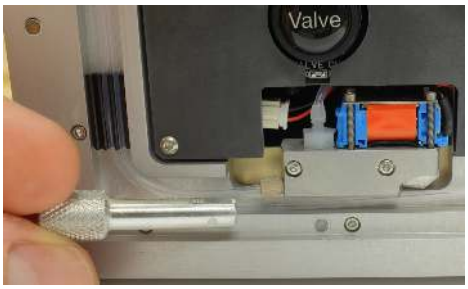
Remove the scanner cover:

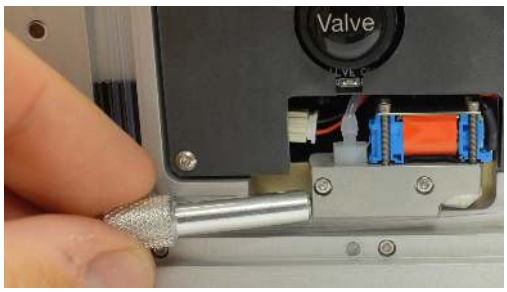
- Grip the cover as shown above.
- Pull forward. The cover is attached magnetically.

21.2.2. Manifold cell-side connection

Process for removing the tubing connected on the cell-side of the valve manifold.

1.





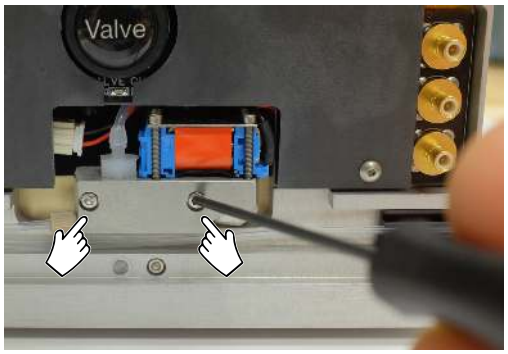
Remove the fitting using the tool:

- Place the tool on the fitting. The slot in the tool slides over the tubing.
- Unscrew until the fitting comes loose. An O-ring should stay attached to the tubing; if not, you may need to retrieve it from the port with tweezers.
- If it feels there is not enough room to complete this step, please continue to the next step.

2.

Remove the manifold (optional):

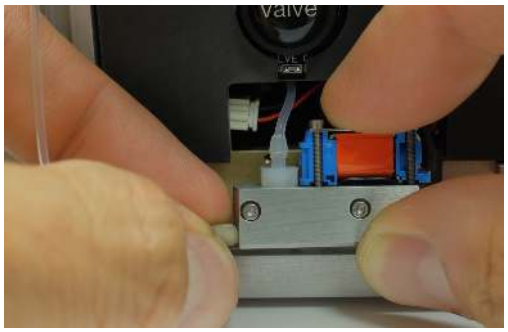
- ONLY IF there was not enough room to complete the last step, remove the two screws shown in the photo.



3.

Remove fittings while holding manifold:

- Pull the manifold forward a little. Pay attention not to apply tension to the tubing or the wires.
- Remove the fitting using fingers or the tool shown in previous steps.
- When the tubing has been replaced, reverse attach the manifold again with its screws.

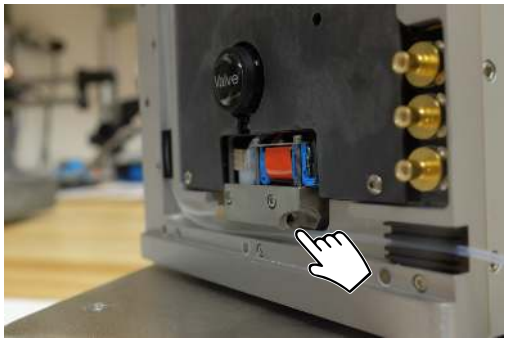


21.2.3. Manifold lab-side connection

1.

Locate the manifold port:

- The lab-side port of the valve manifold is shown in the photo.



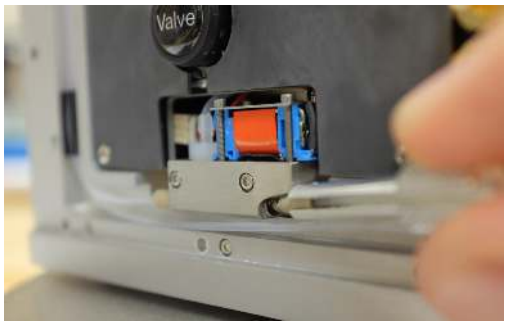
2.

Prepare tubing:

- Attach the tubing with fitting to the fitting tool as shown.
- Note that the tubing should have a small bit extending beyond the ferrule. This space holds the O-ring (230.039)

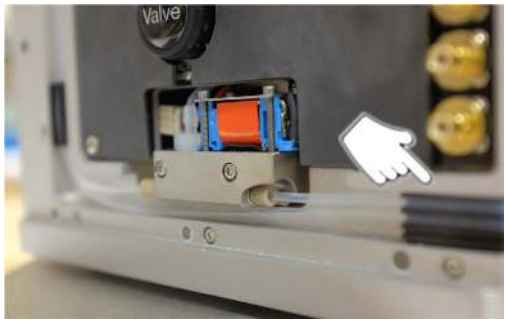
3. **Tighten fitting:**

   - Tighten the fitting as shown.
   - Do not over tighten. Holding the tool with fingertips only should prevent any damage to the fitting.



4. **Guide the tubing:**

   - Press the tubing into the guides. Note that the tubing is not perfectly round, and it may fit better if it is twisted a bit.



21.3. Gas Perfusion Imaging

21.4. Attaching Threaded fittings to tubing

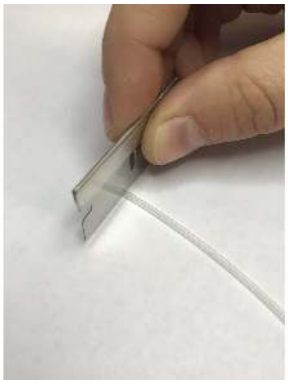
For leak testing of the ES gas cell and/or for gas perfusion imaging, tubing must be connectorized and fitted into the side ports of the ES cell body. This section gives step-by-step instructions on how to connectorize and insert this tubing. The process will require a sharp blade, attention to the specific orientation of parts at critical steps, and possibly a stereoscope to verify the correct orientation of small parts (e.g., the SS ring).

The following list of supplies can be found in the CES Accessories Kit 900.100.3:

- 231.006 Tubing
- 232.015 Fitting Nut
- 232.016 PEEK Ferrule
- 232.016 SS Ring
- 114.721 Fitting Fixture (Trim Tubing Block Chamber)
- 114.800 Fitting Wrench

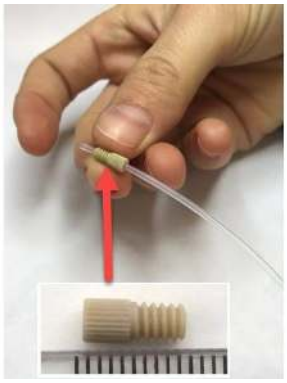
1. **Cut the tubing:**

   - Use a sharp, new razorblade to cut off the end of the tubing to the length needed for your setup.
   - Make sure that you have a clean cut that does not squish or squeeze the end of the tube.



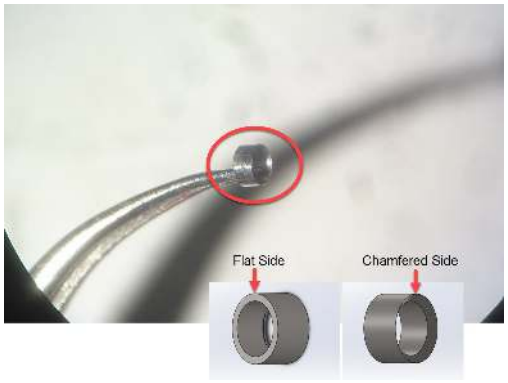
2. **Put the fitting nut on the tube:**

   - Put Fitting Nut (232.015) on the tube.
   - Orient the nut so that the threads of the nut are on the same side of the tube end you are connectorizing.



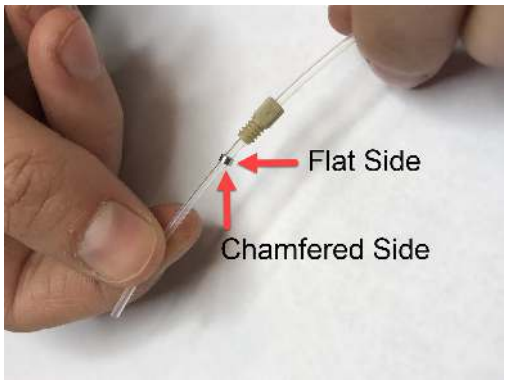
3. **Inspect the SS ring:**

   - Inspect the SS Ring (232.016) under microscope.
   - The ring is asymmetrical: side 1 is flat; side 2 is chamfered. Determine which end is flat and which end is chamfered.



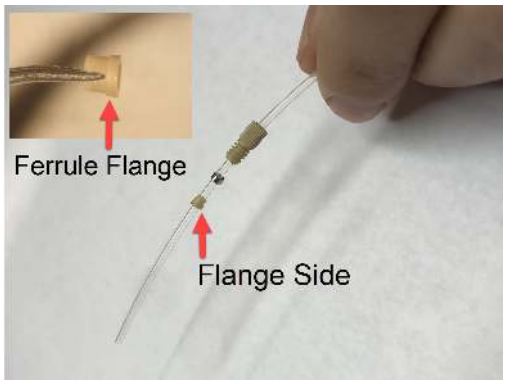
4. **Put ring on tube:**

   - Put the ring onto the tube such that the FLAT end is pointed towards the threads of the Fitting Nut, and the CHAMFERED end is pointed towards the tube end.



5. **Put PEEK Ferrule on tube:**

- Put the PEEK Ferrule (232.016) on the tube.
- Orient the ferrule so that its flange is pointed towards the tube end.



6. **Put tube into Fitting Fixture:**

- Put the tube into Fitting Fixture (114.721) such that a small length of tubing is overhanging the other side
- Slide PEEK Ferrule + SS ring along tubing until both are out of view inside the hole of the Fixture.

7. **Screw fitting nut into Fitting Fixture:**

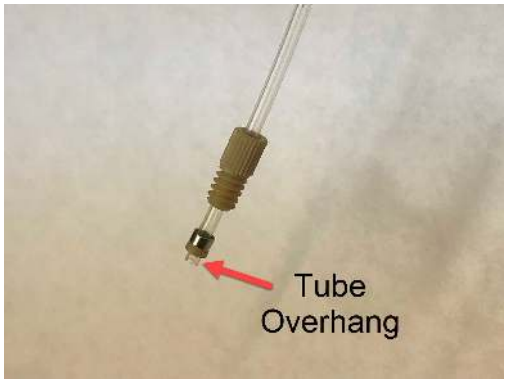
- Butt the threaded end of the Fitting Nut up against the Fitting Fixture.
- While holding the overhanging tube in place, screw the Fitting Nut into the Fixture until it runs out of travel.

8. **Remove the overhanging piece of tubing:**

- Use a sharp razor blade to remove the overhanging piece of tubing.

9. **Pull tubing out from Fixture:**

- Unscrew the Fitting Nut from the Fitting Fixture.
- Pull the tubing out from the Fixture and verify the following:
  - The end of the tube is not squished.
  - The SS ring is now fitted over the PEEK Ferrule close to the Ferrule's flange.
  - A small amount of overhanging tube remains.



10. **Push tubing into Cell Body:**

- To connect tubing to Cell Body, first slide the Fitting Nut away from the tube end.
- Push the tubing into the Cell Body's threaded hole so that the short piece of overhanging tube fits inside the O-ring (you will feel the tube snugly pop in place).

11. **Gently tighten the Fitting Nut:**

- *Gently* tighten the Fitting Nut into the Cell Body's threaded hole either by hand or using the Fitting Wrench (114.800).

22. Tutorial: Fluid Imaging

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

22.1. Tutorial: Fluid Imaging in a Droplet

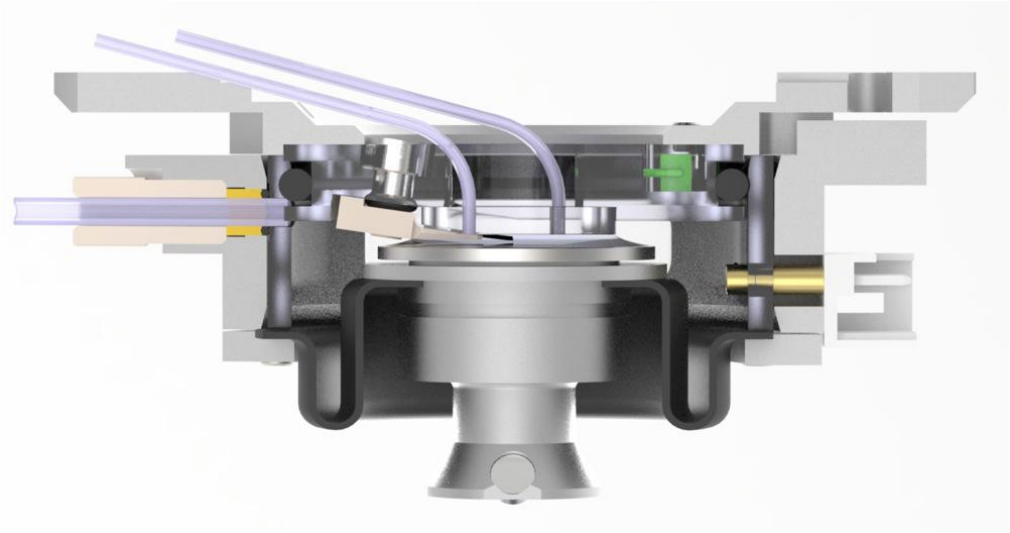


Figure 22.1.: Diagram of the fluid droplet while engaged on a sample.

22.2. Tutorial: Fluid Imaging with Perfusion

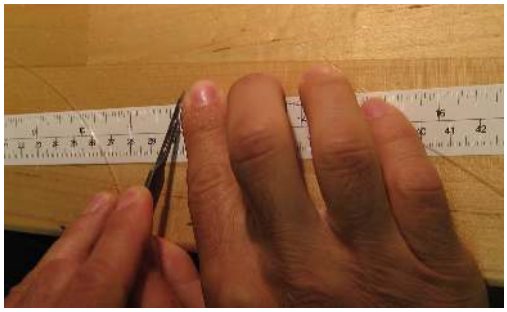
This tutorial walks you through the simplest form of perfusion imaging, using two 1cc syringes to successively inject and withdraw small amount of fluid.

22.2.1. Prepare the tubing

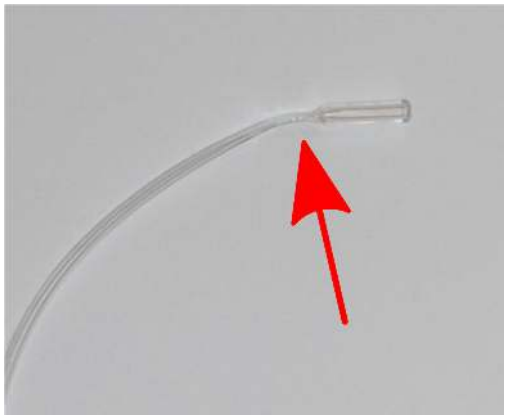
1.

Prepare 1/32" tubing:

- Think ahead of how much you need to move your reservoirs.
- Allow for enough tubing so the syringes can comfortably be placed outside the AFM enclosure.



2.



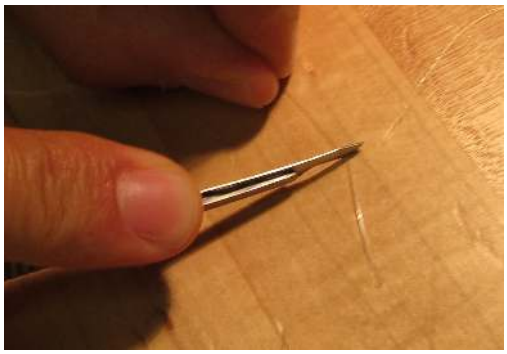
Stretch tubing:

- Thin out one end of each piece of tubing. If no pliers are around, just pinch between your fingernails.

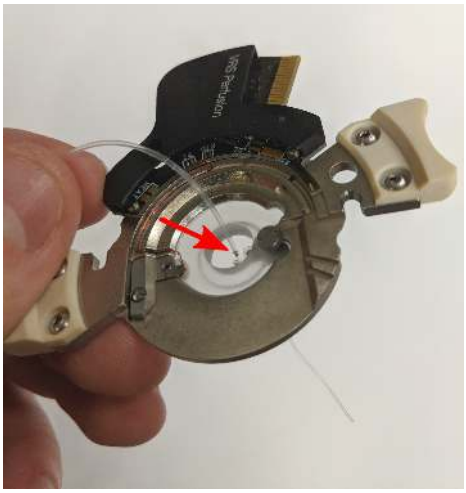
3.

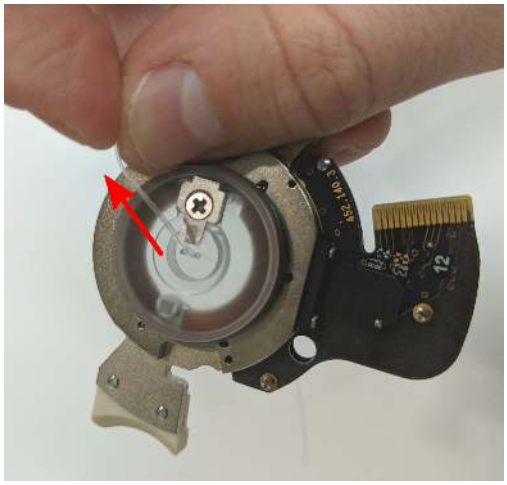
Trim tubing:

- With a fresh #15 scalpel blade, trim the tubing in the thin section. Leave as much thin tubing as possible.



4.





Insert the tubing:

- From the outside in, thread in thinned tubing.
- From the inside, pull it through until a few cm of unstretched tubing comes through the hole.

5.

Trim off excess tubing:

- Using a sharp #15 scalpel blade, trim the excess tubing flush with the glass.
- Repeat the process for the adjacent perfusion port and tubing.

6.

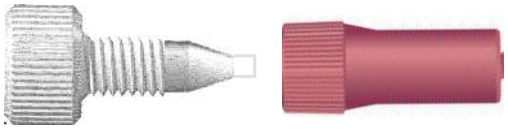
Finished:

- The cantilever holder should look as in the above photos.

22.2.2. Prepare for syringe attachment

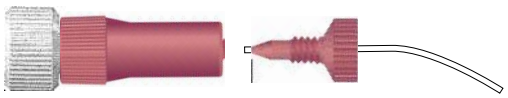
1. **Gauge Plug:**

   - Finger-tighten the white Gauge Plug into the larger end of the Adapter.



2. **Insert the tubing:**

   - Thread the 1/32” tubing through the small fitting. Leave a small amount sticking out of the end.
   - Thread the fitting into the adapter. Before it is tight, slide the tubing as far as it will go into the fitting. It will come to a stop against the white Gauge Plug.
   - Finger-tighten the fitting fully. Tug on the tubing to make sure it stays in place.
   - Remove the white Gauge Plug and store it for later use.



3. **Attach the luer adapter:**

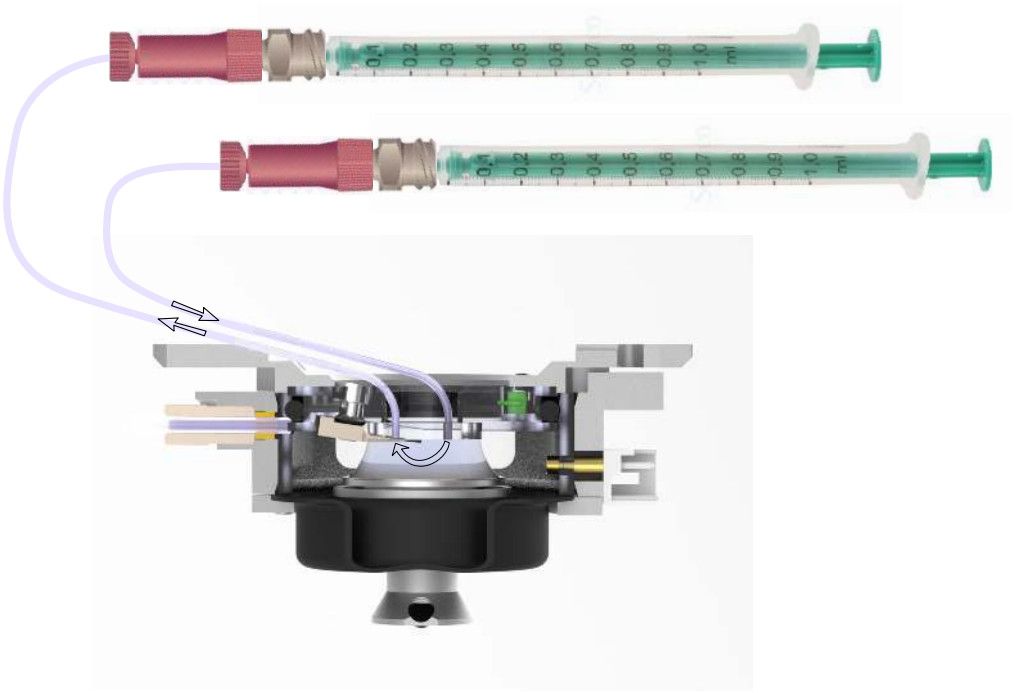
   - Finger-tighten the luer adapter as shown.

4. **Attach the syringe:**

   - For a non-luer lock syringe, press and twist the syringe firmly into the fitting.
   - For a luer locking syringe, twist the syringe into place.

22.2.3. Prepare the cantilever holder for imaging

1.



Attach syringes:

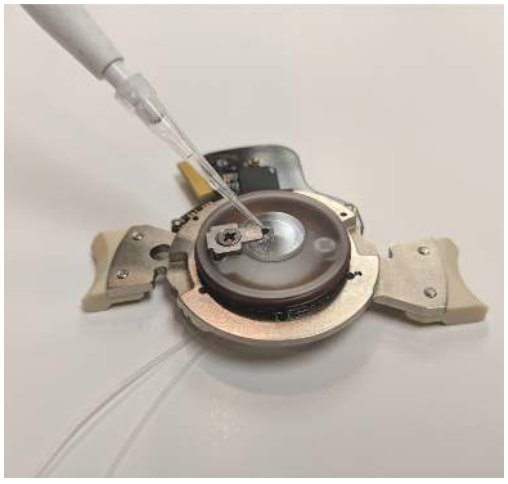
- At this point you should have two syringes attached as shown.
- The top syringe, connected to the port closest to the cantilever holder, should be filled with a very small amount of fluid, perhaps 0.1cc.
- The bottom syringe, connected to the port furthest from the cantilever, should be filled with fluid.

Note It is helpful to mark the syringes with IN and OUT, using little flags of adhesive tape. Halfway through the perfusion process it will be difficult to tell which is which.

2.

Prime the tubing:

- Push bubbles out of the tubing for both IN and OUT syringes.
- Suck up any excess fluid. In the photo at right, a laboratory pipette is being used.
- Leave a small droplet on the cantilever.



22.2.4. Prepare the sample

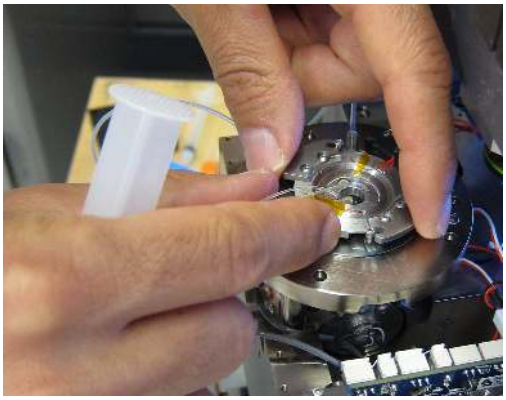
To prepare the sample:

1. Put the sample in the chamber.
2. Put about 100 uL of fluid on the sample.
3. Motor UP to a level of a few mm below where the lever will crash.

22.2.5. Seal the chamber

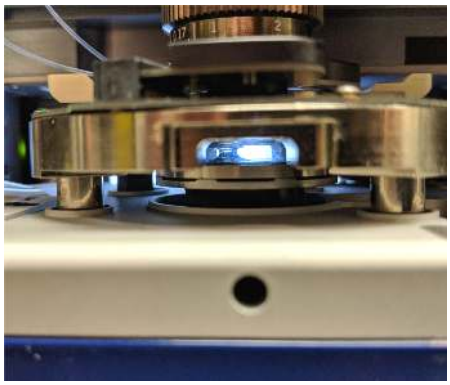
Install the cantilever holder:

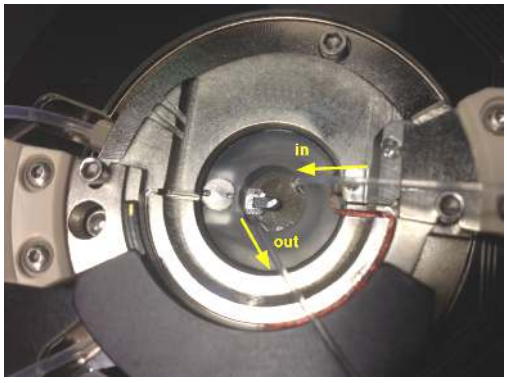
- As described in Section 18.2 on page 215, install the cantilever holder onto the chamber.



22.2.6. Imaging during perfusion

1.





Motor down:

- The valve on the front of the scanner should still be in the **OPEN** position.
- With the scanner still pulled out Motor Down manually.
- Inspect Droplet as it necks down. Don't **CRASH**!
- Stop before reaching the surface.

Note A safe way to go about this is to engage first without any liquid and store the sample position in the software.

2. **Go to pre-engage height:**
   - With all valves as they were, follow the usual process of going to pre-engage height.
   - We'll assume imaging will commence after fluid is flowing.

3. **Close gas valve:**
   - While still at pre-engage heigh, close Gas Valve (button on scanner front).

4. Image as usual.

22.2.7. Perfusion

To perfuse fluid:

1. Depress the syringe marked "IN" by a modest amount: ~30 micro liters. This will cause the droplet to swell slightly.
2. Pull the plunger back on the syringe marked OUT by an equal amount.
3. Repeat the process a few times if desired.

22.2.8. Disassembly

Remove the cantilever holder:

1. Open Gas Valve (C).
2. Motor Up (E).
3. Remove the cantilever holder.
4. If all went well, there should be no fluid in the membrane gutter, and there should be about 150 microliters of fluid on the sample.

22.3. Perfusion Flow Characteristics

This section includes a perfusion experiment with two glass syringes: one filled with water, and the other with dye and water. The two are connected to a valve, and about 1 foot of 1/32" tubing runs between the valve and the cell. Another foot of tubing runs from the cell to a balance to measure flow rate.

In perfusion experiment A, the flow is switched at about 1 minute. Flow rate ~1.3 uL / second.

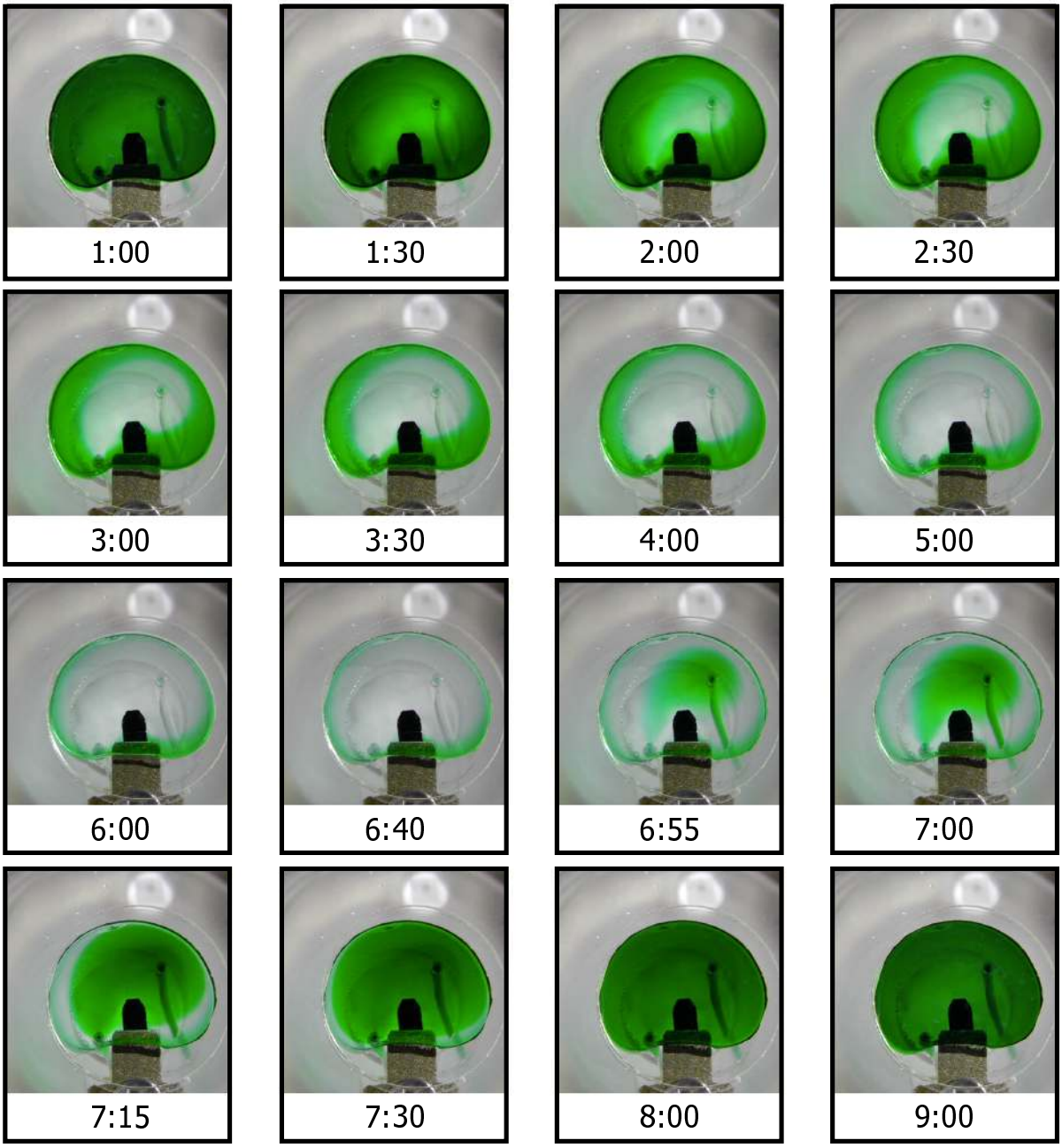


Figure 22.2.: Perfusion Experiment A

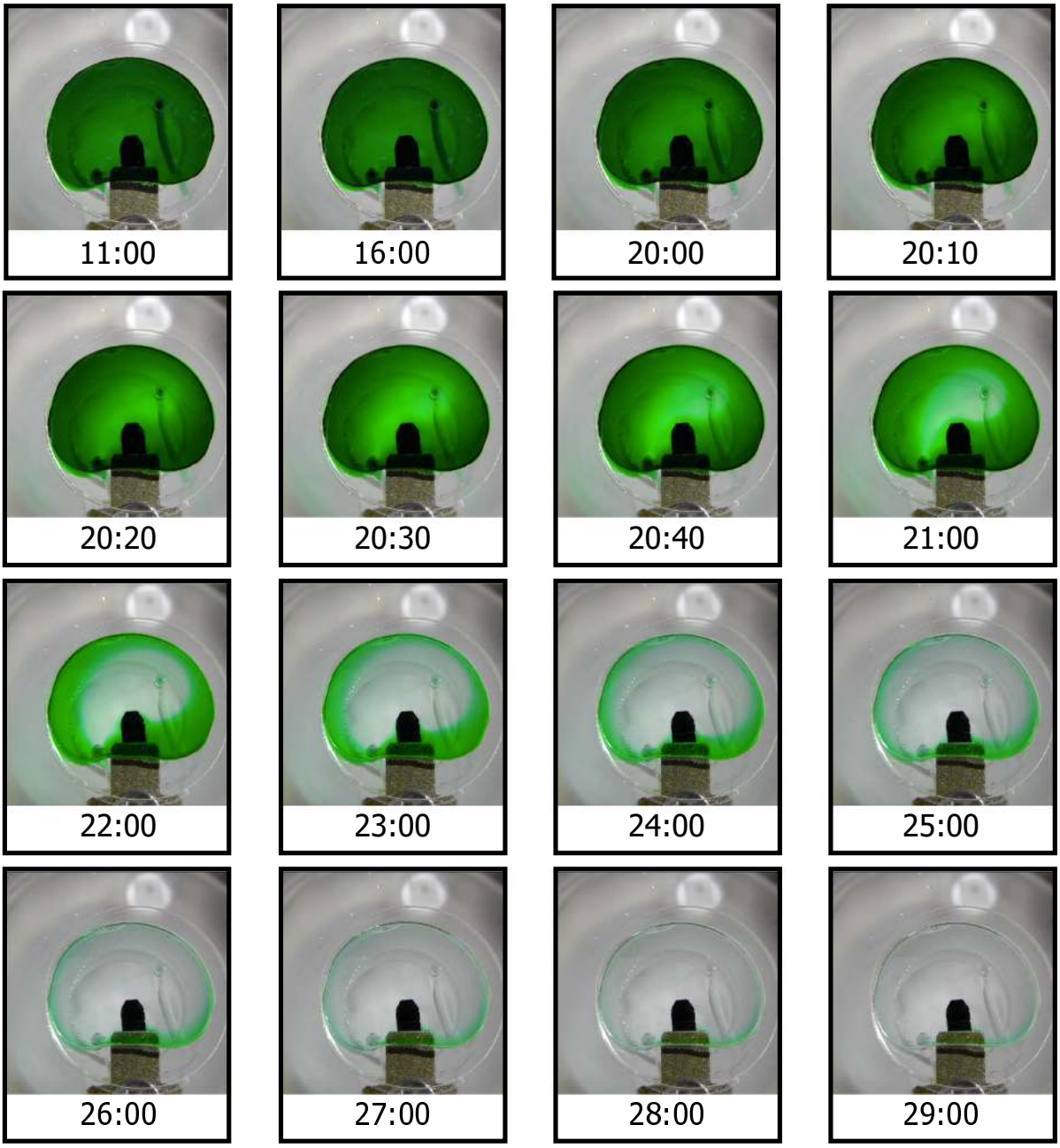


Figure 22.3.: Perfusion Experiment B

23. Tutorial: FM Mode Imaging in Fluid

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

Note All new users should complete and understand the "AC Mode Imaging in Air" (7) tutorial before attempting any imaging.

FM Mode imaging is fairly advanced, and you should first be quite proficient at AC mode imaging in air and fluid before proceeding.

This tutorial should be performed on a running Cypher, as opposed to merely read, and if possible, should be done under the supervision of a user already familiar with the operation of the Cypher.

Before you start:

- We assume you understand the aspects of running this system safely: (Chapter 1 on page 3)
- You are familiar with the basic names of the hardware components and software controls: (Chapter 2 on page 16)
- You have powered up the Cypher and launched the latest version 16 software: (Chapter 5 on page 35)

23.1. Required Materials

Prior to beginning the tutorial, gather the following items:

1. Cypher instrument, equipped with blueDrive photothermal excitation
2. Probes: You will need FS1500 AuD probe, which has a cantilever resonant frequency between 800 kHz and 2000 kHz, and spring constant is between 1.5 N/m and 10 N/m.
3. Samples: Freshly cleaved calcite and clean filtered DI water

23.2. Cleaving and Preparing the Calcite Sample

Prepare a fresh and clean calcite surface.

23.3. Prepare hardware

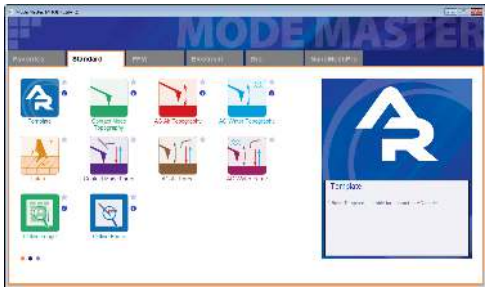
To prepare hardware for FM Mode Imaging in Fluid:

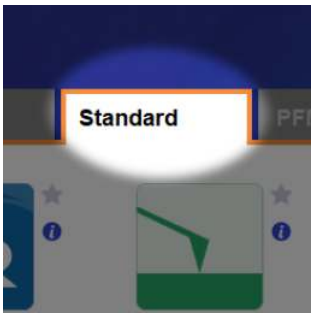
- Place the lowest possible blueDrive filter cube in the laser path. For ArrowUHF probe, start by using 0.1x or 0.03x filter cube.
- Clean a cantilever holder that will be used for imaging (either a liquid or a perfusion cantilever holder).
- Load a cantilever into the holder.

23.4. Calibration and Approach

1. **The Mode Master window:**

   - With the software launched, the Mode Master window should be displayed.
   - If not, click the 'Mode Master' button at the bottom of the screen:  .



2. 

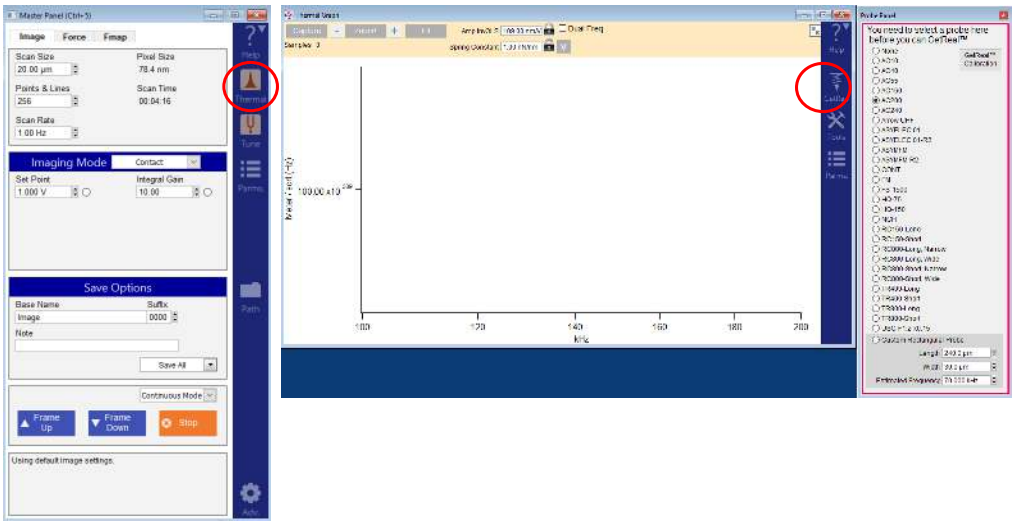
   Select Mode:

   - Select *Standard tab > Template*
   - The screen will rearrange and make available all controls necessary for this type of AFM imaging.

3. **Align Laser**

   - Open the Video Panel.
   - Use the arrows in the Video Panel to move the laser spot onto the cantilever and maximize the SUM signal.

4.



Calibrate Probe

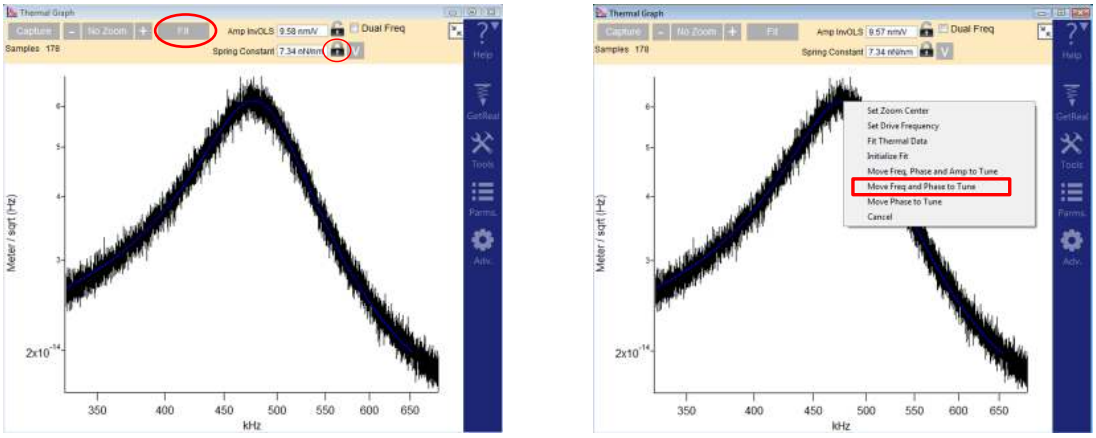
- Click the 'Thermal' icon in the Master Panel. The thermal graph appears.
- Click the 'GetReal' icon, which opens a Probe Panel.
- Select the probe you are using for the experiment.
- Click 'GetReal Calibration'.
- Once the calibration is completed, the Amp InvOLS and Spring Constant values at the top part of the thermal graph will be updated.

5. Remove the cantilever holder and place the freshly cleaved calcite sample on the scanner.
6. Add a drop of water to the sample.
7. Put a drop of water on the probe and place the cantilever holder back on the scanner.
8. While looking at the distance between the probe and the sample, approach the sample to the tip so that both water drops join, and the sample and lever are both in water environment.

23.5. Software setup and liquid imaging in AC mode

1. Realign the laser on the cantilever (now in liquid). Sum should be ~ 6V.

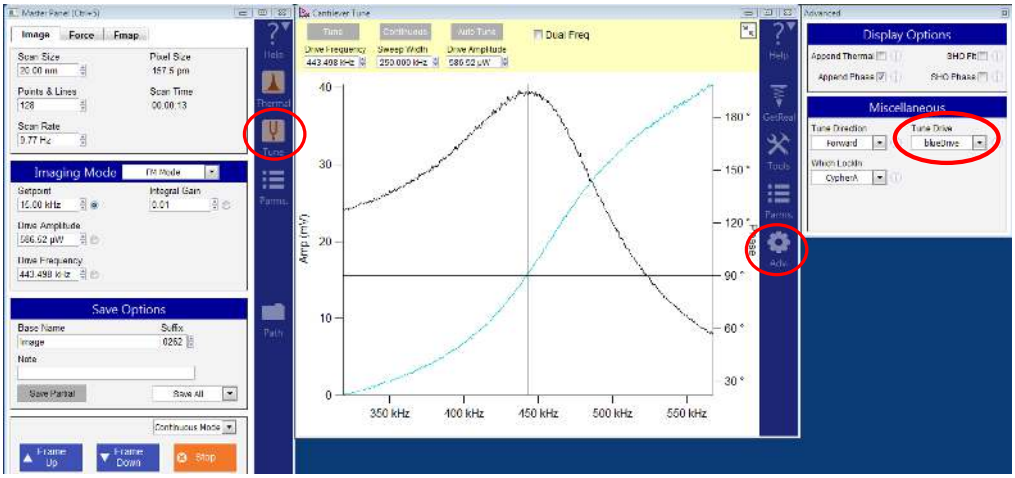
2.



Capture a thermal of the cantilever in liquid:

- Make sure the padlock beside Spring Constant is locked.
- Refit the thermal data to obtain the updated (water) InvOLS value.
- Transfer the frequency of thermal peak to the tune panel:
  - Right-click on the peak of the Thermal Graph and select *Move Freq and Phase to Tune*.

3.

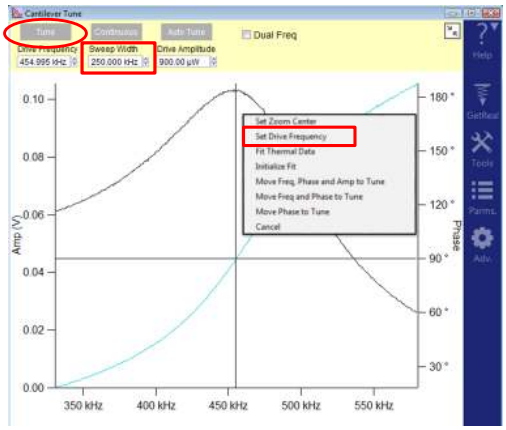


Turn on blueDrive photothermal excitation:

- In the Tune graph, click the 'Adv.' (gear) icon.
- In the Advanced panel, select *blueDrive* from the dropdown menu of Tune Drive.

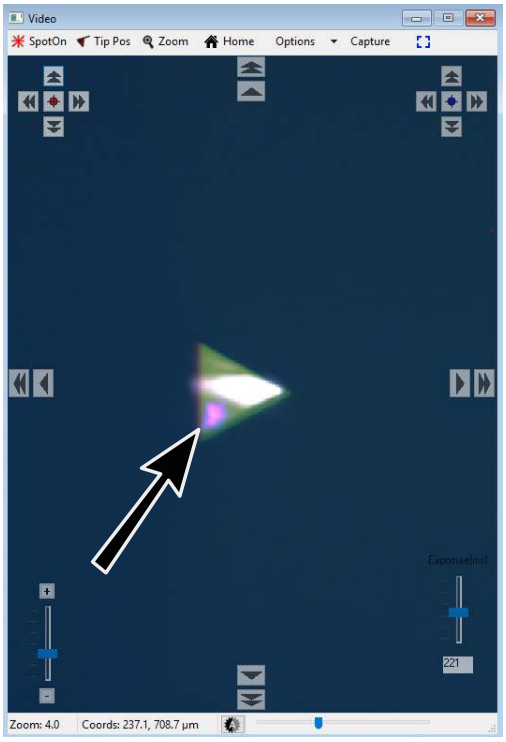
4. **Tune the cantilever:**

- Set the 'Sweep Width' to 250 kHz.
- Click 'Tune'.
- When the tune is done, right-click on the peak and select *Set Drive Frequency*.
- Move the blue laser spot around the base of the cantilever to maximize the amplitude (see note below).
- Adjust the drive amplitude (in the Master Panel) until the amplitude of the tune reaches ~ 50 mV (visible on the graph and in the Sum and Deflection panel).
- Set the setpoint (in the Master Panel) to ~ 40 mV.



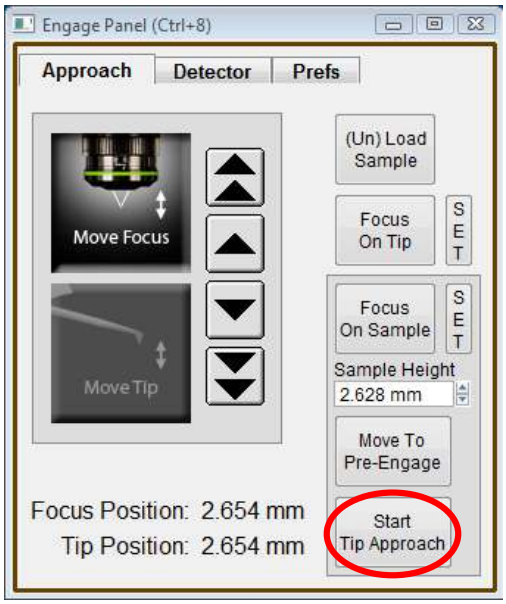
Tip Move the blueDrive spot around to maximize amplitude.

Note It is possible to attain a higher oscillation amplitude by moving the blueDrive spot to the side, as shown in the image at right.



5. **Engage Panel:**

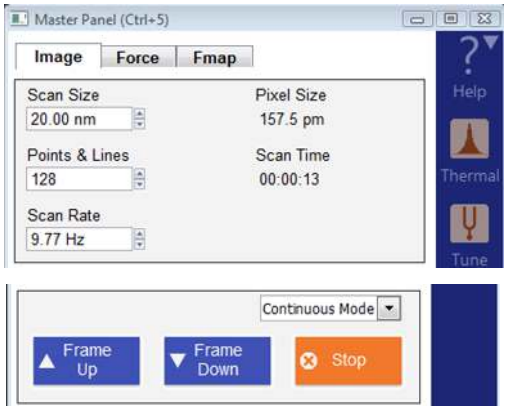
   - Set the tip position while focused on the tip.
   - Set the sample position while focused on the sample.
   - Click 'Start Tip Approach'.



6. Once the tip is in piezo range of the sample, capture another Thermal Tune with Z-piezo at 0V (sample is 2-3 um from surface).
7. Lock the padlock beside spring constant on the Thermal Graph to recalculate Amplitude InvOLS.
8. Right-click the peak in Thermal Graph and select *Move Freq and Phase to Tune*.
9. When the tune is complete, verify that that the phase is set to 90 degrees.

10. **Imaging parameters - Master panel:**

    - Set the Scan Size to "20 nm".
    - Set the Scan Rate to "10 Hz".
    - Set the gain to ~60.
    - Start imaging by pressing 'Frame Down'.



11. Start imaging:

    a) Decrease the free amplitude (by decreasing drive amplitude in the Master Panel).

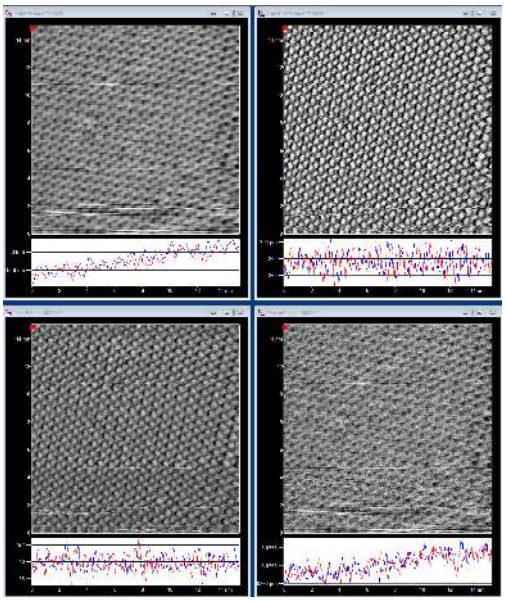
    b) Decrease the setpoint until trace and retrace overlap each other.

    c) Repeat a and b several times to image with the lowest amplitude possible.

12.

Imaging:

- Acquire a 20 nm image and resize the scan to 10 nm.
- Once the imaging looks optimized, stop the scan and switch to FM mode.

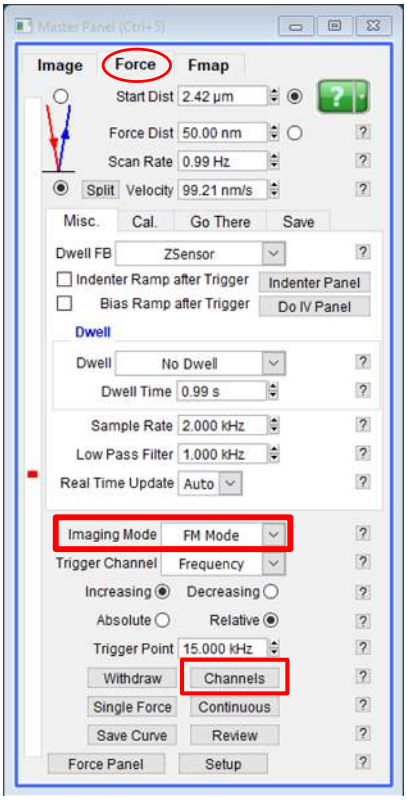


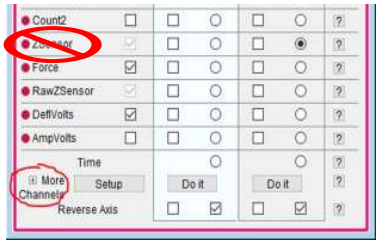
23.6. FM curves

1. In Master Panel, go to the Force tab.

2. **Select the FM imaging mode and parameters:**

   - Choose *FM Mode* for Imaging mode.
   - Click the 'Channels' button and select the following:
     - Frequency
     - Dissipation
     - Amplitude
     - Phase
     - Make sure to plot versus DRIVE (not ZSensor) for short curve (tens of nm), as Drive is a quieter signal than ZSensor (which has 50 pm of noise in a1 kHz bandwidth).
   - Select *Frequency* for Trigger Channel and select the following:
     - Increasing
     - Relative
   - Trigger Point: 15 kHz is a good start

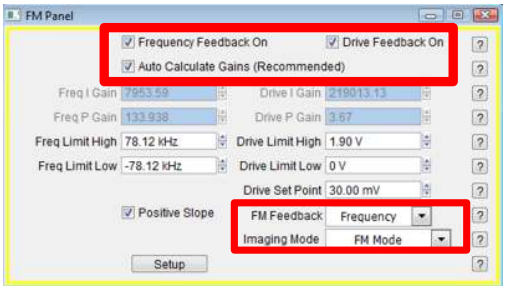


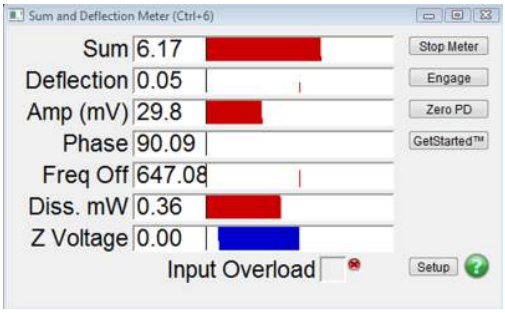


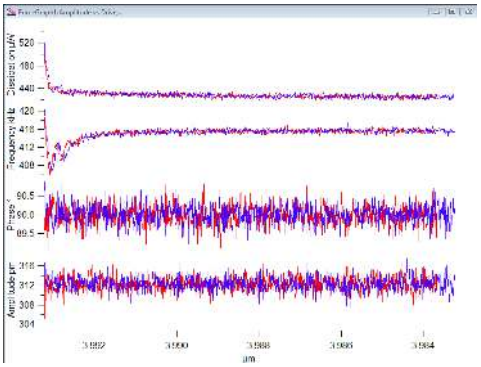
3.

Locate the FM Panel (AFM Controls > Other > FM Panel)

- Uncheck and check the 'Auto Calculate Gains'.
  - The values should update.
  - The gains can be adjusted by the user.
- Set the 'Drive Set Point' to required amplitude as follows:
  - Start with 3 Angstroms: 3A = 0.3nm.
  - For example: 0.3nm / 10.25nm/V InvOLS = 0.0292V = 29.3mV
- Activate Feedback Loops by checking the boxes beside the following:
  - FM feedback ON
  - Drive Feedback ON
- When the Frequency and Drive Feedbacks are ON, the following values appear in the SUM and Deflection Meter panel:
  - Amp (mV) = Drive SetPoint Value
  - Phase = 90
  - Freq Off = frequency offset compared to the value of drive frequency from tune, here 647 Hz
  - Diss mW = power (in mW) needed to keep the Drive Set Point Value at 30 mV



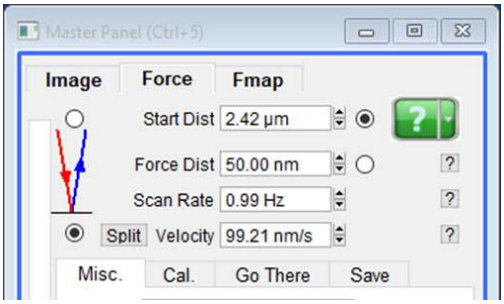


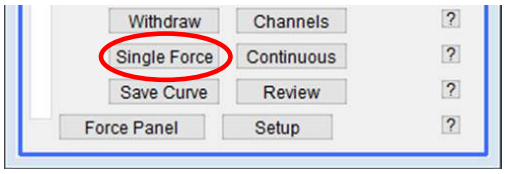


4.

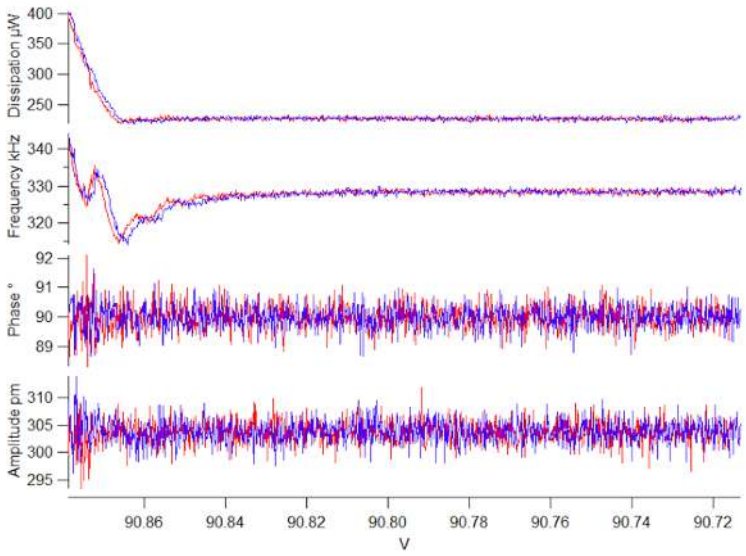
Curve parameters - Master Panel; Force Tab:

- Begin with the force distance set to ~100 nm.
- Set the scan rate to 1 Hz.
- Acquire a force curve by clicking 'Single Force'.
- Retune the cantilever to obtain the cantilever frequency close to the surface.





5.



Optimize parameters for FM curves:

- FM curves should resemble the curves shown in the image above.
- Dissipation, Frequency, Phase, and Amplitude are plotted against Drive.
- Click 'Continuous' and gradually decrease the force distance to 5 nm.
- Adjust the FM gains in the FM Panel as follows:
  - Uncheck the 'Auto Calculate Gains' and adjust the values until extension/retraction curves overlap and no ringing is observed.

6.

FM curves: absolute frequency trigger *versus* relative frequency trigger

- When *absolute trigger* is used, the frequency at the beginning of the curve is not taken into account. The trigger is reached when the frequency value equals the trigger value added to the drive frequency (from the tune).
  - For example, if the drive frequency is 75 kHz and the absolute trigger value is set to 1 kHz, the trigger will be reached at 76 kHz.

-OR-

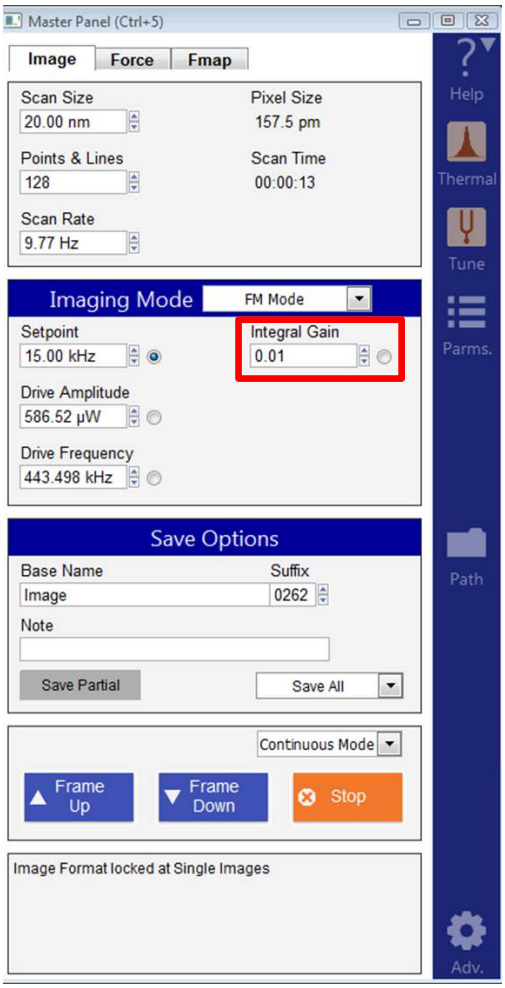
- When *relative trigger* is used, it is the frequency at the beginning of the curve to which the value of trigger is added. Therefore, if the drive frequency differs from the frequency at the beginning of the curve, it's the frequency at the beginning of the curve that will be used.
  - For example, if the drive frequency is 75 kHz and the relative trigger is 1 kHz but the frequency at the beginning of the curve is 75.2 kHz, the trigger will be reached at 76.2 kHz.

23.7. FM imaging

1. We recommend that you go through sections AC mode imaging and FM force in this guide prior to FM imaging. Doing so allows you to calibrate the cantilever, approach the sample, and determine the FM gains for optimal imaging.

2. **In Master Panel - Image tab:**

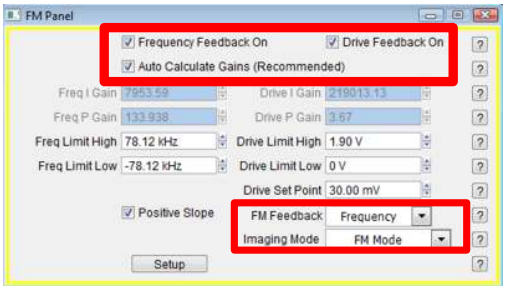
   - Select *FM Mode* as Imaging Mode.
   - Set Integral Gain to "0.01", when using blueDrive.
   - Set the Setpoint to 10-15 kHz, or whatever value was used during FM curves.

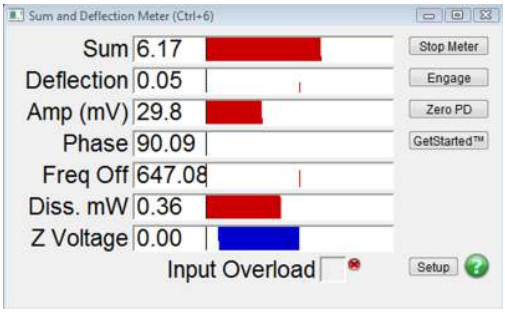


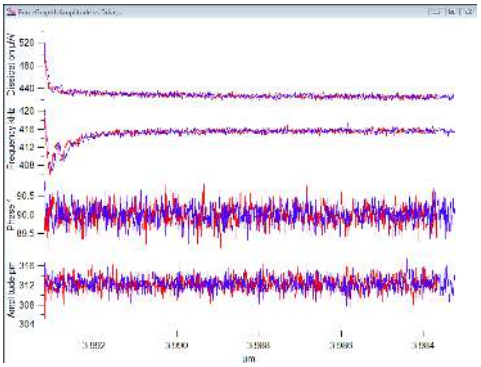
3.

On the FM Panel:

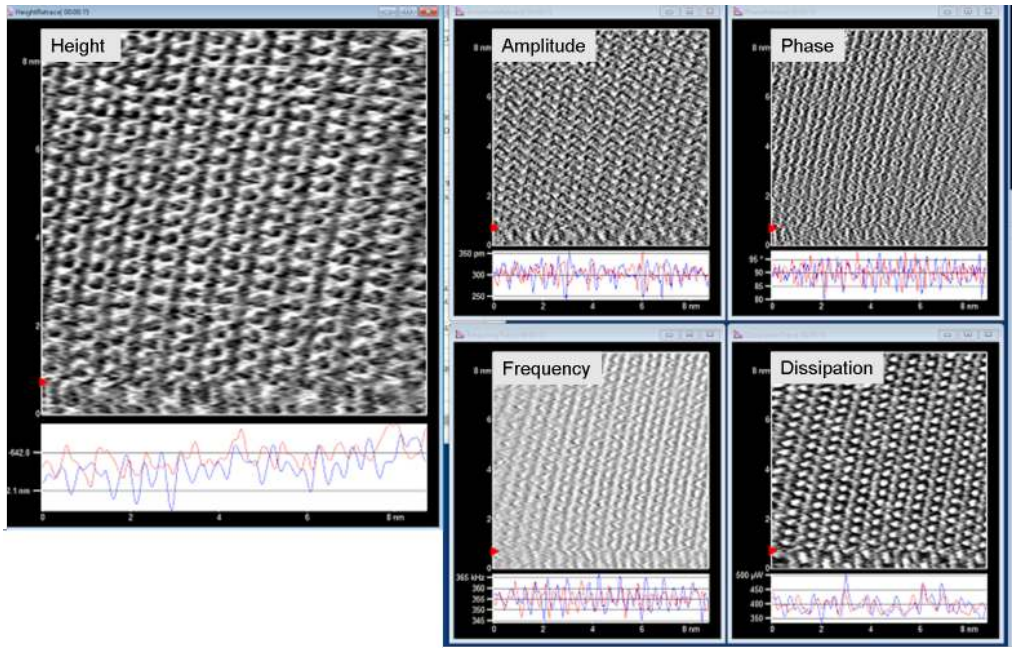
- Set the Drive Set Point to required amplitude as follows:
  - Start with 3 Angstroms: 3A = 0.3nm
  - For example: 0.3nm / 10.25nm/V InvOLS = 0.0292V = 29.3mV
- Activate Feedback Loops by checking the boxes beside the following:
  - FM feedback ON
  - Drive Feedback ON
- When the frequency and drive feedbacks are ON, the following values appear in the SUM and Deflection Meter panel:
  - Amp (mV) = Drive SetPoint Value
  - Phase = 90
  - Freq Off = frequency offset compared to the value of drive frequency from tune, here 647 Hz
  - Diss mW = power (in mW) needed to keep the Drive Set Point Value at 30 mV or lower







4.



Start imaging:

- On the Master Panel, click ‘Frame Down’.
- Height, Phase, Amplitude, Frequency and Dissipation channels should appear.
- Adjust the following variables to optimize images:
  - Drive Setpoint value
  - Setpoint
  - FM gains

24. Glovebox Protocols

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

Cypher AFMs offer superb imaging performance inside of inert gas glovebox systems. While this solution offers the ultimate protection of the sample and cantilever from ambient conditions, it is not always practical to sequester an AFM inside of a glovebox for long periods of time. A decent alternative strategy is to assemble the cell inside of a glovebox and then carry this assembly to the AFM which is located elsewhere. This chapter suggests a protocol for cell assembly inside of a glovebox.

While it is possible to leave the cell sealed during imaging, we suspect that oxygen and water vapor will find their way into the cell over the course of minutes and hours, driving the conditions inside of the cell away from the ~1ppm levels inside of the glovebox where the cell was first assembled. Therefore, this protocol suggests the attachment of an inert gas purge line as soon as the cell is mounted on the AFM.

Note Imaging electrically insulating samples under conditions of very low water vapor can lead to unwanted forces on the cantilever due to static electric charge. With the whole AFM inside the glovebox, one can often mitigate these effects with devices (such as a static master) which locally ionize the air around the sample. Inside the sealed cell of the Cypher ES, it may prove challenging to introduce static control devices.

24.1. Preparations

This section prepares you for imaging under inert gas purge conditions. This process leaves the AFM ready to accept the sealed cell as it comes out of the glovebox.

24.1.1. Prerequisites

Preparation requirements:

- A Cypher ES outfitted with a rotameter device for regulating inert gas flow
- Familiarity with basic imaging using the Cypher ES, described in Chapter 18 on page 214
- Cypher ES with standard gas cantilever holder (901.758, See Section 19.1.1 on page 225.)
- The sample stage can be ambient, heated, or cooled. For this tutorial, we chose the ambient sample mount (see Section 20.3.2 on page 243).

Steps in preparation:

1. Start with a Cypher ES, fully prepared for imaging with gas perfusion. See Section 21.3 on page 264 for a tutorial on that subject.
2. Load a test sample and cantilever identical to what you plan on loading inside the glovebox.
3. Start imaging under the conditions (speed, scan size, etc.) you eventually plan to use on the sample mounted and sealed inside the glovebox.
4. Observe image quality while you experiment with the inert gas flow and note the maximum flow before imaging noise becomes noticeable.
5. Turn off the gas flow.
6. Stop imaging and disengage the tip.
7. Remove the cantilever holder.
8. Remove the entire sample cell from the AFM. For a tutorial on this subject see: Section 20.5 on page 251.

24.1.2. Preparing the cell and gas lines

We will completely bypass the gas valve and pressure manifold for this demonstration.

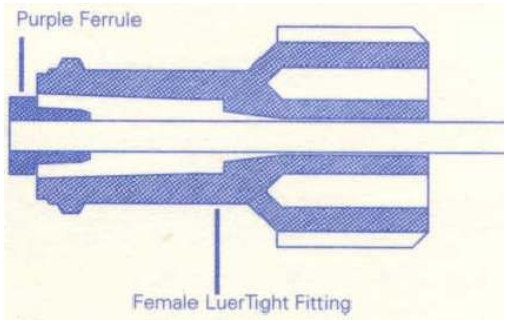
1. Start by removing and storing the gas lines that were already present on your Cypher AFM. See Section 21.2 on page 262.
2. Store the tubing sections in a safe place.
3. **Locate tubing and fittings:** Consult the Cypher ES accessory parts list (Section 17.1 on page 198) and find the following items:
   - 231.006 1/16” O.D. Tubing
   - Luer Fittings: 231.008
   - 5cc Syringe: 080.010
   - Threaded fittings: 232.015
   - Scalpel knife with blade: 290.147 and 290.148
   - Ferrules: 232.016
   - Extender Tool Fitting Wrench: 114.800
   - Fitting Compression Fixture: 114.721
4. **Locate a three-way valve:**
   - The luer-type valve, as shown at right, is provided by Asylum

5. Cut two sections of tubing long enough to reach from the scanner cell to the outside of the ES enclosure, perhaps half a meter.

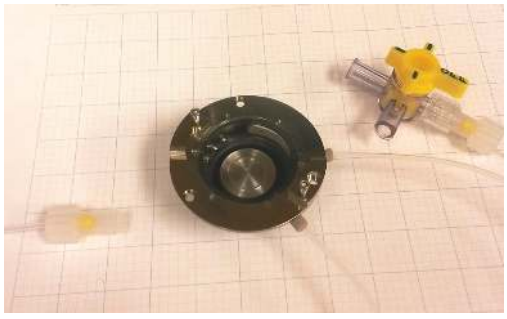
6. At one end of each of these pieces of tubing, secure a threaded fitting and a ferrule. See Section 21.4 on page 264 for instructions.
7. At the other end of each piece of tubing, attach a female Luer fitting, as shown in the following steps.
8. **Locate parts:**
   - 231.008 Luer Tight Fittings, which includes all the parts shown on the right.

9. **Attach purple ferrule:**
   - Slide the female Luer Tight fitting and purple ferrule, in that order, over the tubing.

Note Make sure to keep the end of the tubing flush with the base of the ferrule, as shown in the diagram.



10. **Seat the fitting:**
    - Attach the red Luer Tight seating tool (has a piece of red plastic embedded into it) to the female Luer Tight fitting by screwing it firmly onto the female Luer Tight body. This will seat the ferrule into the Luer fitting.
    - Remove the seating tool and store for later use.
11. Attach the two tubes to the two side ports of the cell. See Step 3 on page 254 and the following few steps.
12. **Attach valve:**
    - Attach a valve to one of the two tubing ends, as shown at right.



24.1.3. Seal the cell

1. Read ahead through this section and collect all the tools, screws, tweezers, cantilever holders, etc. you will need to place inside the glovebox.

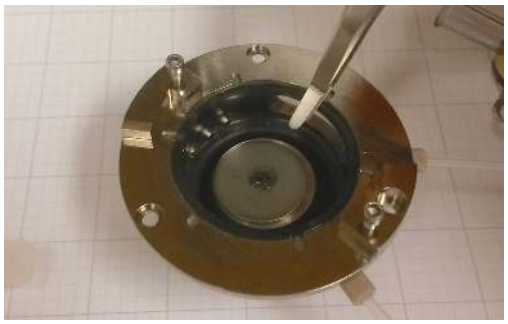
2. **Inspect the cantilever holder:**

   - Some models of cantilever have a hole at the indicated location, and some do not. It depends on the date on which the holder was designed and the amount of circuitry on the circuit board.
   - The third hole is optional, and the instructions below will give you the option of using the third hole.

3. Place all these items, the cell body with tubing and syringe attached, and the cantilever holder into the glovebox via the vacuum load lock. Evacuating the lock will not harm the cell since it has not yet been sealed.

4. **Place the sample:**

   - With the sample mount in the position shown at right, place the sample.



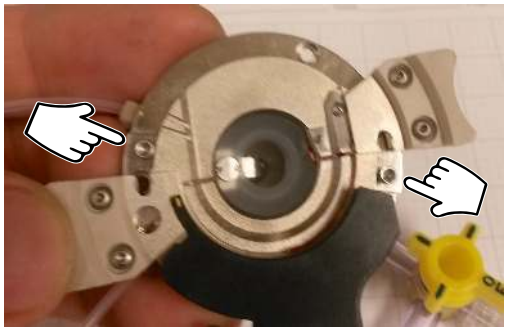
5. Load the cantilever into the cantilever holder and set that aside.

6. **Extend the membrane:**

   - Pull down on the bottom of the sample stage to fully extend the rubber membrane, carefully, as shown at right.

7. **Place the cantilever holder:**

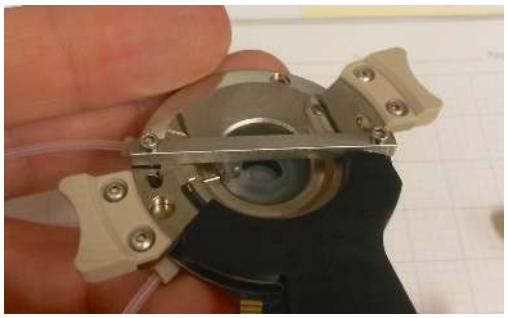
   - Remove the two screws indicated and set them aside. You will need to take them out of the glovebox along with the cell later.
   - Place the cantilever holder in the position shown.
   - It should not be rotated clockwise, as typically is during use while imaging.



8. **Attach the bar:**

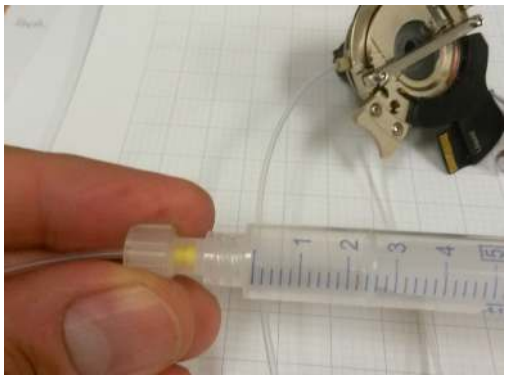
- Using two longer screws, attach the metal bar as shown.

Note Tighten the screws very *gently* and keep the bar from tilting. Tightly securing only one screw might cause the bar to tilt and tightening the other screw will cause the bar to press down too hard on the cantilever holder, possibly causing irreversible damage.



9. **Seal the tubes:**

- Set the plunger of a 5CC syringe around the 2CC position.
- Attach the syringe to one of the tubes.
- Make sure the valve on the other tube is in the closed position.



10. **Close the valve:**

- Make sure the valve is in the OFF position, if not already done.
- The cell is now fully sealed on all sides.

11. **Take the assembled cell out of the glovebox:**

- Place the cell, and everything attached, into the load lock and close it.
- DO NOT EVACUATE. There is no need for evacuation when taking things back into the ambient atmosphere.
- Open the door on the ambient side and take the cell to the AFM.

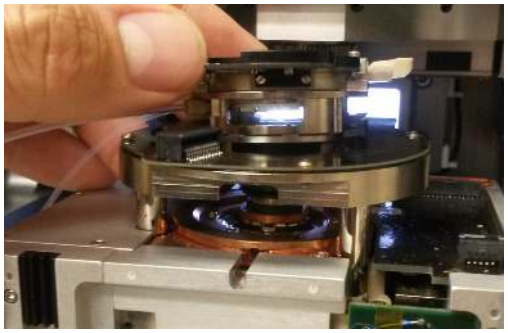
Note Some customers like to first place their cell and tubing inside of a jar, like the desiccator shown on the right. This gives you even more time and peace of mind when transporting the cell back to the AFM.

24.1.4. Mount the sealed cell onto the AFM

Chop Chop	The faster you work through the following steps, the smaller the chance of impurities diffusing into the sealed cell. The membrane is made of Kalrez and is quite impervious to water and oxygen. Nevertheless, it is best to get pure dry gas flowing through the cell as quickly as possible.

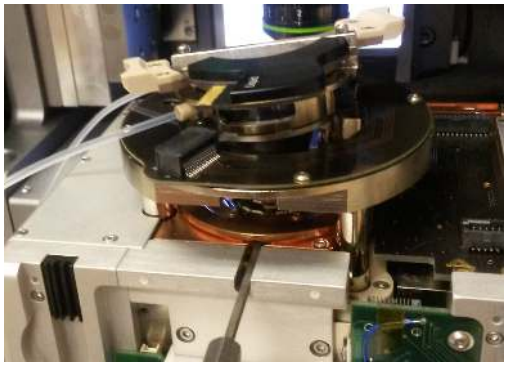
1. **Place the cell onto the scanner:**

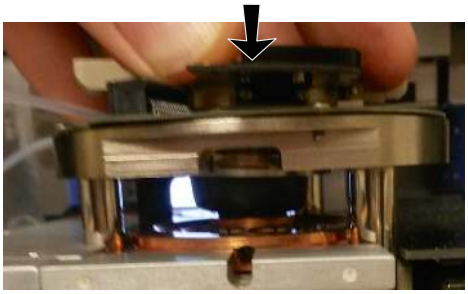
   - Seat the foot of the cell onto the receptacle located on top of the scanner.
   - The trapped air inside the cell will prevent the tip from colliding with the sample.



2. **Lock the foot:**

   - Lock the foot of the tool, as shown at right. Do not overtighten. This is discussed in more detail in Step 8 on page 256.



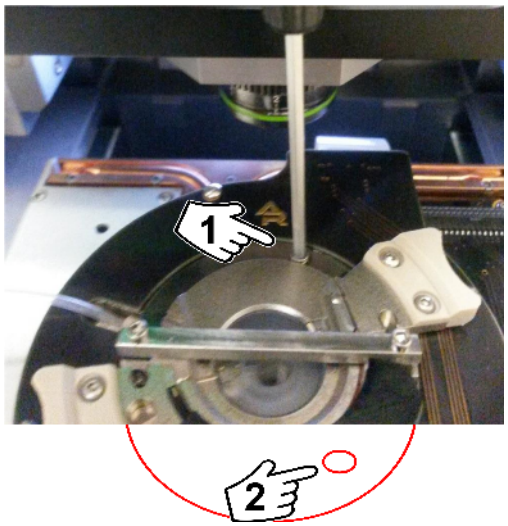
3. 

   Press down and seat the cell body:

   - Press down on the sample stage, as shown above on the left. You will see the membrane bulge a bit under the building pressure. This pressure will assist the proper forming of the membrane.
   - During this process, retract on the syringe to relieve the pressure as the cell lowers.
   - About 3CC of gas should be removed to properly seat the cell body. A little overpressure is better than a slight vacuum in terms of keeping contaminants out of the cell.

4. **Secure sample chamber to scanner with two screws:**

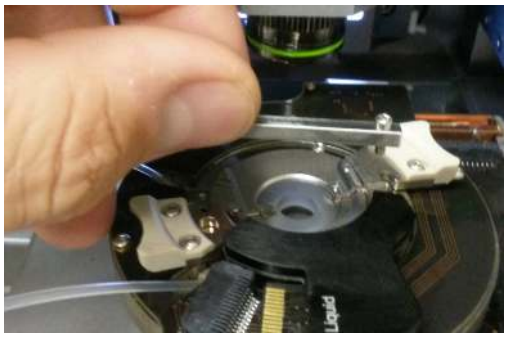
   - Secure the sample chamber with screw number 1. Do not overtighten.
   - If your cantilever holder has a hole in the position marked (2), secure that screw also. As mentioned before, some equipment does not grant access to (2). In that case, skip this screw. It does not affect performance.



5. **Remove the bar:**

   - Unscrew the bar and remove it, as shown at right.
   - Ensure the fastening screws are in place but not screwed all the way down, as they will be used to secure the cantilever holder in the next step.

Caution During these steps, the cantilever holder could now be pulled loose, exposing your sample.



6. **Rotate the cantilever holder into position and secure:**

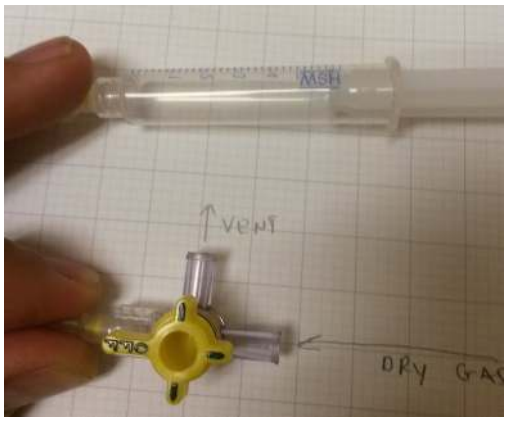
   - While pressing down to maintain the O-ring seal, rotate the holder clockwise so that the circuit board seats with its connector and the screws line up with their grooves on the holder.
   - Tighten screws.

7. **Put the final screw into place securing the cell body to the scanner:**

   - With the holder in place and fastened to the sample chamber, tighten the third and final screw that fastens the sample chamber to the scanner.

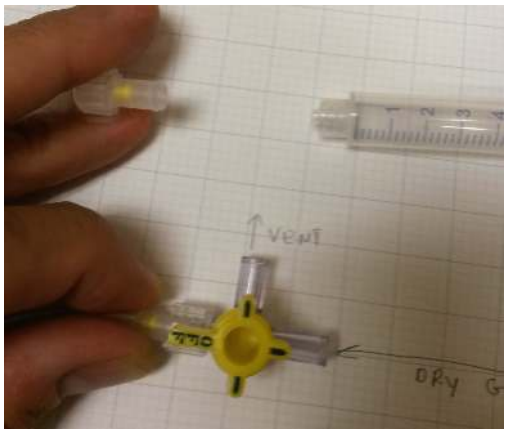
8. **Attach the gas line to the valve, and turn on the gas flow to purge the interior of the valve:**

   - Gas flow rate is chosen by you.
   - Gas will flow to atmosphere.



9. **Remove the syringe from the tubing at one end:**

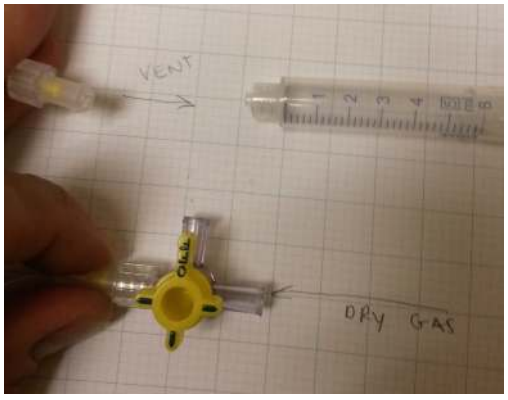
   - This very briefly breaks the seal to atmosphere, but there is NOT sufficient time to contaminate the sample chamber, and the next step creates gas flow that continuously purges atmosphere.



10. **Turn the valve clockwise to divert gas flow through the sample chamber:**

    - The gas will now flow into the sample chamber and out of the tube where the syringe was just removed.

Caution If there are organic or toxic fumes contained in the sample chamber, the vent line should be routed to a fume hood.



25. High Voltage

CHAPTER REV. 2423, DATED 08/14/2021, 18:50. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

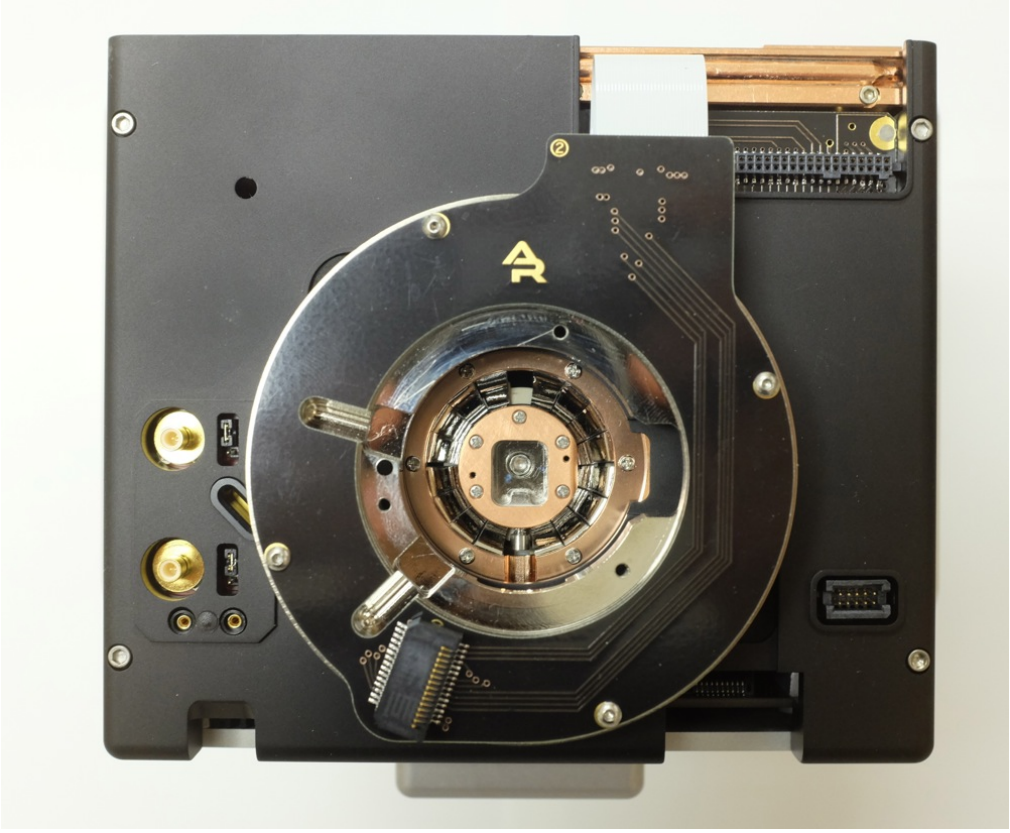


Figure 25.1.: High Voltage Scanner seen from above.

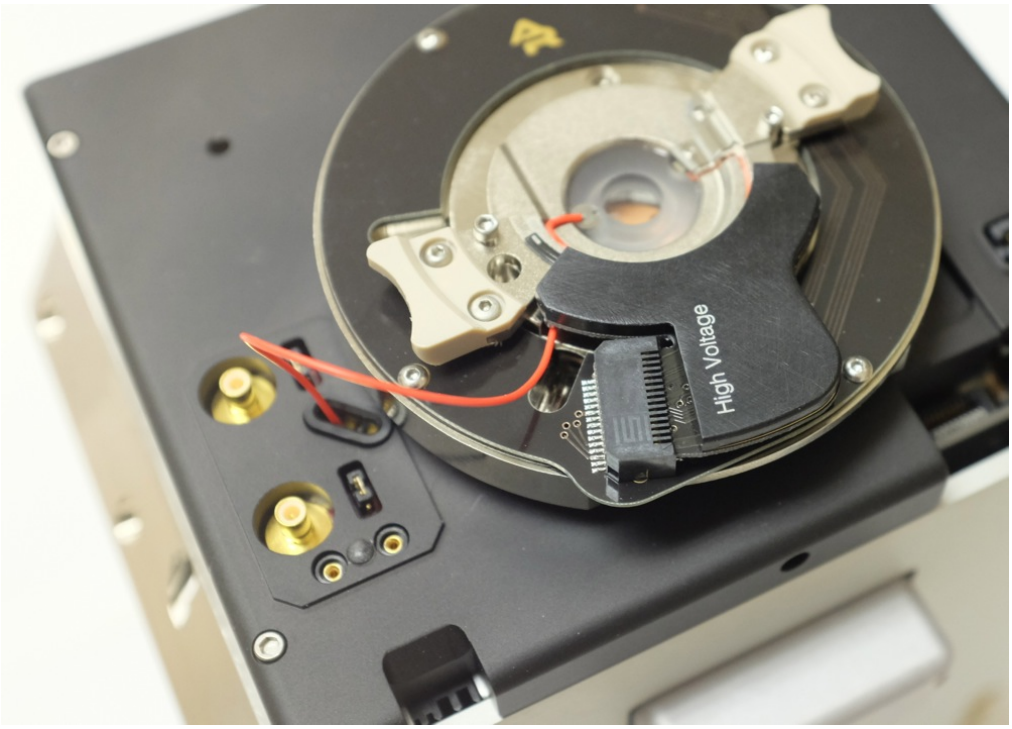


Figure 25.2.: High Voltage Scanner with High Voltage cantilever holder and its fly wire connected to the high voltage contact.

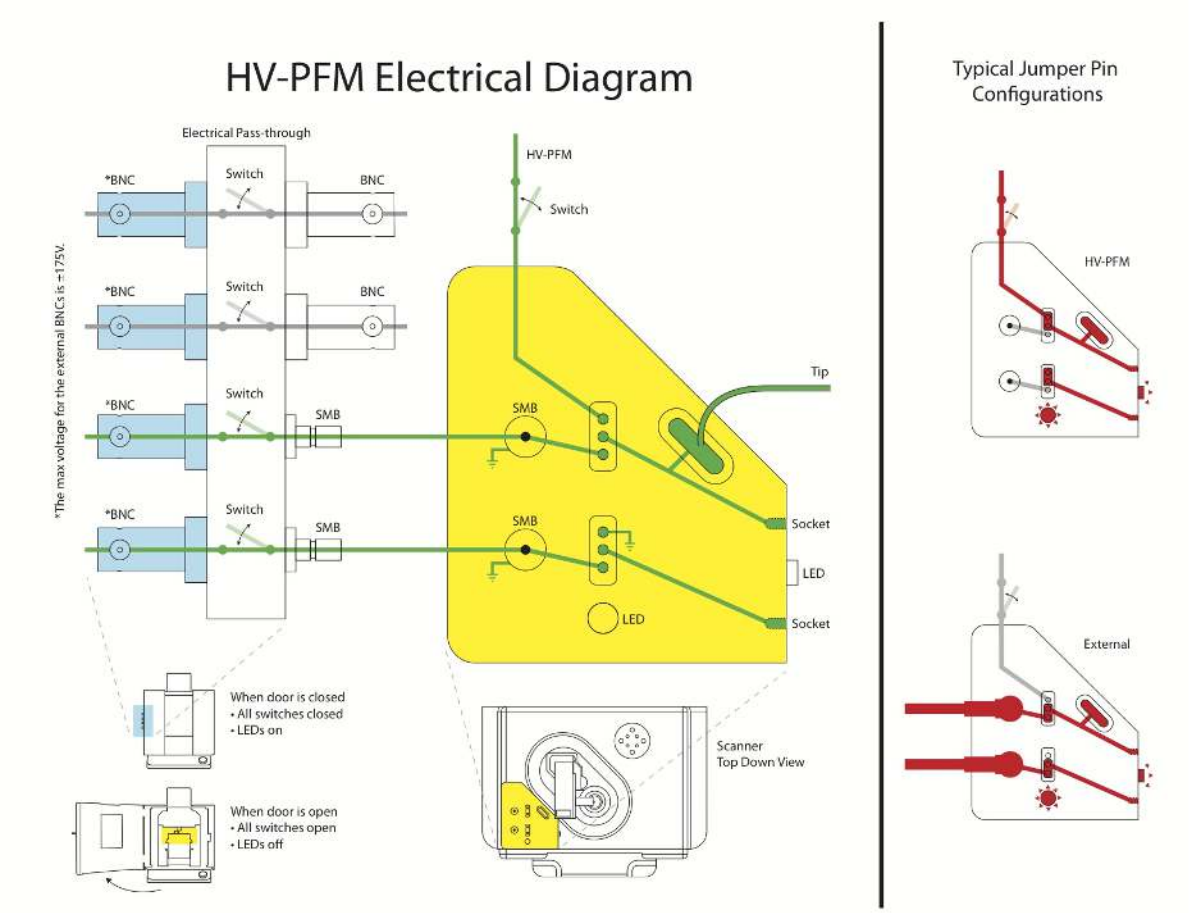


Figure 25.3.: The Cypher High Voltage Option

26. Electrochemistry Cell

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

26.1. Requirements and Prerequisites

Electrochemical AFM is considered an advanced technique. You should have mastered the following basic techniques before attempting to use the EC AFM accessory:

- Familiarity with the Cypher ES system and AFM imaging of a basic sample in air, covered in Chapter 17 on page 198 and Chapter 18 on page 214
- Mastery of basic imaging in fluids, covered in Chapter 22 on page 268

It is also assumed that you have the following items:

- Cypher ES AFM
- blueDrive photothermal excitation equipped, to image in tapping mode

26.2. Overview

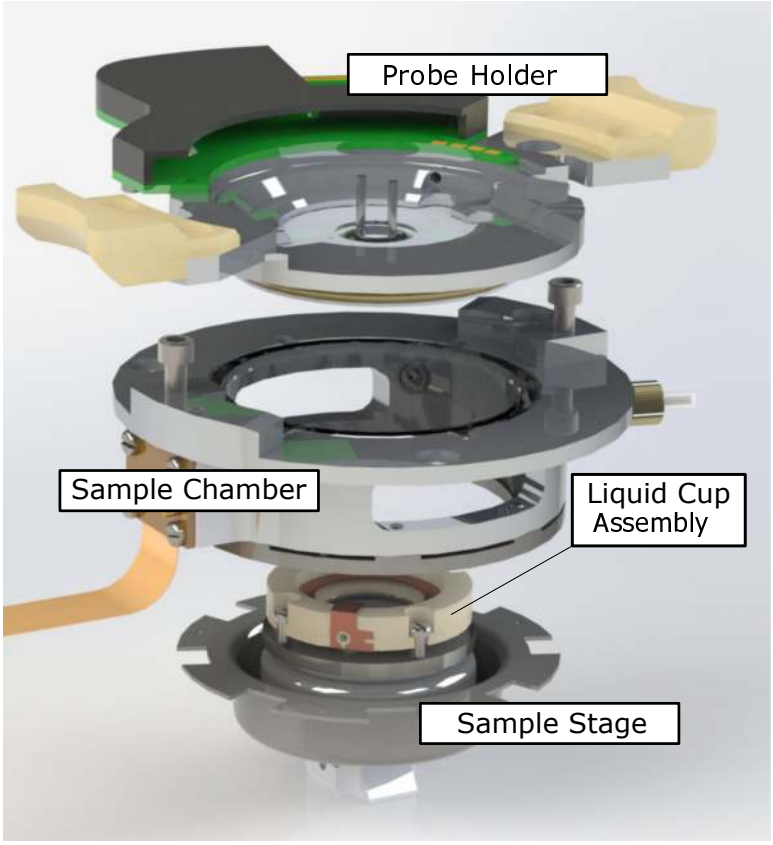


Figure 26.1.: The four major components of the Cypher ES electrochemistry cell

The Electrochemistry (EC) Cell for the Asylum Research Cypher ES Atomic Force Microscope (AFM) has been designed to perform EC-AFM with the Cypher ES. It enables study of, for example, deposition, oxidation, corrosion, and mass transfer of metals and other materials. Simultaneously, the nanoscale topographical changes

induced by these electrochemical reactions can be precisely monitored with the AFM. Please note: This manual does not cover any hardware or applications where voltage or current are applied or measured through the probe tip.

Electrochemical experiments with the Cypher ES EC Cell are conducted in a standard three-electrode configuration with the sample as the working electrode (WE), a geometrically concentric counter electrode (CE), and a reference electrode (RE) that enters the cell through the probe holder. Virtually infinite combinations of materials may be used for these electrodes. The cell can be operated in a sealed configuration, and the electrolyte is contained in a liquid containment cup that both seals to the sample to define the working electrode area and provides support for the counter electrode in solution. All components contacting the electrolyte solution are chemically inert.

The connection between an external potentiostat and the electrodes is established through an EC-specific electric-blue-colored circuit board and related connector cables. This circuit board interfaces with the top of the Cypher ES Scanner, and it is conveniently labeled with the orientation of the magnetic working (sample), ground, and counter electrode contacts inside the Sample Chamber.

The probe holder for the Cypher ES EC Cell is provided either with or without perfusion capability and is designed so that the cantilever may be lowered to the sample surface in the electrolyte in the liquid containment cup. The probe clip is made of inert PEEK or PPS and holds the probe in place by friction for use in tapping or contact mode. For tapping mode, note that there is not a shake piezo, so the cantilever must be photothermally excited with Cypher's blueDrive laser.

26.2.1. List of Abbreviations Used In This Chapter

AFM Atomic force microscope/microscopy

CE Counter electrode

EC Electrochemistry/electrochemical

FEP Fluorinated ethylene-propylene

FKM Fluoroelastomer (equivalent to Viton®)

FFKM Perfluoroelastomer (P-Rex®, equivalent to Kalrez®)

PCB Printed circuit board

PEEK Polyetheretherketone

PFA Perfluoroalkoxy alkane

PH Probe holder

PPS Polyphenylenesulfide (Ryton®)

PTFE Polytetrafluoroethylene (Teflon®)

RE Reference electrode

WE Working electrode (sample)

26.2.2. Quick Start Guide

The following order of operations is suggested to optimize your workflow in setting up and using the Cypher ES EC Cell. Additional details related to these steps may be found in 26.6:

1. Turn on Cypher, start software, calibrate position and blueDrive motors. For best performance, allow the instrument to equilibrate for a few hours.

2. Clean all parts as needed and make solutions. The cleaning cylinder (114.820) may be useful for this. See Table 26.1 on page 311.
3. Assemble Standard Probe Holder Subassembly (901.933) without probe (see Section 26.3.2 on page 306 for more information).
    a) Install RE through top port (1 mm diameter) in probe holder. If atmospheric control is needed, ensure that the RE is fully sealed into the top port, as described in Section 26.7 on page 323, to prevent inward diffusion of external gases.
    b) Install probe clip on probe holder; ensure clip strap is aligned with the bevel on the optical window so that when the probe is mounted the cantilever will be centered on the optical window (see Figure 26.3 on page 307 for rendering).
    c) If using the Perfusion Probe Holder Subassembly (901.939), ensure perfusion tubes are set up. See Section 26.6.1 on page 321 for more information on installing these tubes.
4. Install the Enclosure Bulkhead Subassembly (901.937) and connect its SMB cables to the lower-right front connections on the Cypher ES Scanner inside the enclosure. **Top**: WE. **Middle**: RE. **Bottom**: CE. Related images may be found in Figure 26.7 on page 309.
5. Affix the Generic Potentiostat Cable Subassembly (448.172) cables to the Bulkhead subassembly, and connect your potentiostat to the free ends of these. Note: Specifically for CH Instruments potentiostats, we offer a Potentiostat Cable Subassembly (Table 26.1 on page 315) that plugs directly into the back of the potentiostat from the enclosure.
6. Install the EC AppMod Printed Circuit Board (PCB) Subassembly (458.293.1) circuit board on top of the scanner, ensuring that the ribbon cable from the Sample Chamber seats into the receiving port on the PCB. See Figure 26.9 on page 310 for picture and additional description.
7. Test electrical connections from the Potentiostat Cable leads to the sample chamber magnets with a multimeter to ensure proper signal pathways.
8. Mount the WE (sample) in the Liquid Cup Subassembly (901.934), as shown in Figure 26.5 on page 308.
    a) Affix CE and its magnetic jumper wire to the liquid cup with a lateral screw (00-90 x 3/32" or shorter).
    b) Locate four screws (00-90) of equal length to mount your sample, and thread one of the screws 1-2 turns into base plate.
    c) Locate needed number of shims of preferred material to support WE.
    d) Locate O-ring of preferred material (Viton or FFKM) for sealing the liquid cup to the WE.
    e) Place the liquid cup on the base plate so that the previously inserted screw (b) seats into the side of the cup. Thread a second screw of same length 1-2 turns into base plate in an adjacent screw groove in the liquid cup. This fastens the cup to the plate loosely, leaving room for the sample to be inserted between. (Note that the assembly can also be cleaned in this state.)
    f) Locate another jumper wire and, if possible, solder it to the conductive surface of the WE.
    g) Place O-ring on sample/WE, and with tweezers slide the sample/O-ring into the cup-plate assembly from steps (e-f). Ensure that the O-ring aligns with the recessed edge in the bottom of the liquid cup.
    h) Insert shims (generic plastic sheet of any type is sufficient) below the sample substrate if wanted. This has two effects: making the sample effectively thicker, and electrically insulating the sample from the base plate (important for fully conductive samples).
    i) Thread the remaining two screws 1-2 turns into their holes in the base plate via grooves in the liquid cup.
    j) If soldering was not used in (f), create electrical contact to the WE by inserting a free jumper wire lead between the liquid cup and the WE surface (screws are not fully tightened yet).

k) Evenly tighten all screws, simultaneously compressing the O-ring to seal the WE and clamping the jumper wire to the WE surface.

l) Ensure the sample is properly aligned and test electrical connections with a multimeter to ensure that no electrical leads are shorted.

9. Place the EC Cell Liquid Cup in the Sample Chamber Subassembly (901.935) and, with tweezers, contact magnetic jumper wires from the Liquid Cup to the inner wall contacts of the Sample Chamber. The WE contact is closest to the front, while the CE contact is furthest back, and the middle contact is ground.
10. Mount probe of choice on the probe holder. Further detail may be found in Section 26.6.4 on page 322.
11. Optionally expose liquid cup assembly and mounted probe/cantilever to UV irradiation for a few minutes to decompose any remaining organics.
12. Add 200-300 µL electrolyte to liquid cup and a small droplet of electrolyte to the probe to prevent bubble formation.

    Optionally, you may perform a "calibration" EC experiment (such as cyclic voltammetry with 5 mM ferro-/ferricyanide redox couple in 100 mM KCl) to confirm electrical continuity and electrochemical performance. Specifically for this experiment with ferro-/ferricyanide, the blueDrive laser should not be used because its 405 nm wavelength is strongly absorbed by the molecules and can result in photochemical transformations; this is only used as an electrochemical test.
13. Affix the probe holder with mounted probe to the sample chamber; tighten screws to finger tightness.
14. Lower probe until electrolyte wicks onto probe, clip, RE, and laser window. If using the perfusion probe holder, you can optionally perfuse electrolyte into/out of the liquid cup.
15. Ensure objective is set to 2 for liquid imaging.
16. Tune cantilever, approach surface, and start imaging with EC-AFM. Note that if the tip is far from the surface, approaching may require several iterations of maximizing the objective's z-position followed by lowering the tip and repeating. Sometimes, this also makes the deflection laser become misaligned with the cantilever which is simply fixed by realigning it to maximize the sum signal. (Please refer to Cypher ES manual for additional information regarding any of these steps.)

26.3. EC Cell Kit (901.800) Description and Parts List

The EC Cell is an accessory for the Cypher ES scanner and may be purchased separately and immediately integrated with the system. **Part number 901.800 specifies this entire accessory kit, consisting of all of the parts listed in** Section 26.3.9 on page 310.

Note The EC Cell is **not** compatible with the Cypher S scanner.

26.3.1. Items Included in the EC Cell Kit

- Probe Holder, standard (901.933) or with perfusion (901.939)
- Liquid Cup subassembly (901.934), with base plate for mounting sample/WE
- Sample Chamber subassembly (901.935)
- Remaining accessories, including:
  - Probe clips in PEEK (116.086) and PPS (116.129)
  - EC AppMod Circuit Board, light blue (458.293.1)

- Electrical connections bulkhead assembly (901.937) for facilitating use of an external potentiostat of the user's choice and routing electrical signals out of the instrument enclosure
- Reference electrode wire in Teflon (PFA)-coated Ag, with the option to use any other type of metal wire as the RE
- Concentric counter electrode in Cu, with the option to use other types of metal or a simple loop of wire as the CE (116.070)
- Tubing, O-rings, connectors, PTFE tape, and various fittings
- Essential tools, such as screwdriver, tweezers, scalpel

Note that all of these items are thoroughly specified in Section 26.3.9 on page 310.

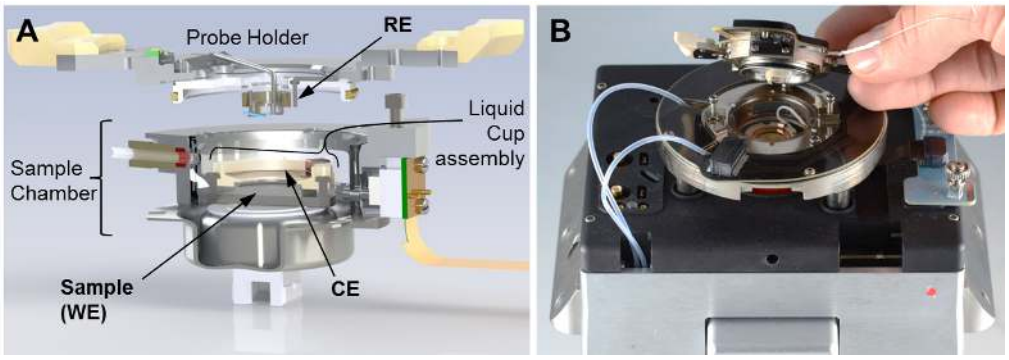


Figure 26.2.: The EC Cell. (A) Exploded cutaway schematic rendering of the major EC Cell components. (B) Photograph of the EC Cell in place inside the Cypher ES scanner. Securing the probe holder completes assembly and completes the 3-electrode circuit in the electrolyte (see Section 26.6 on page 321 for full assembly instructions).

26.3.2. Standard Probe Holder Subassembly (901.933)

The probe holder comes in two flavors: regular, and perfusion. The "moving parts" of this component that you will modify/exchange/replace are:

- Probe
- Probe clip
- RE wire

Any brand or type of probe may be used; however, keep in mind that no current or bias can be applied to the tip in the Cypher ES EC Cell. The probe clip uses spring force and friction to hold the probe in place (see Figure 26.3 on page 307). It has been designed to be easy to manipulate and easy to clean without sacrificing imaging performance in contact or tapping mode (please see Section 26.8 on page 324 for cleaning instructions).

26.3.3. Perfusion Probe Holder Subassembly (901.939)

The perfusion probe holder is identical to the standard probe holder, save for the features that provide perfusion capability. Namely, the optical window that supports the probe has two offset holes (~0.029" diameter) bored in it, and FEP tubes (~0.031" diameter) have been inserted in these. The hole closer to the cantilever is intended as the inlet in order to maximize solvent flow at the tip-surface locus. Please see Figure 26.4 on page 307 for further detail.

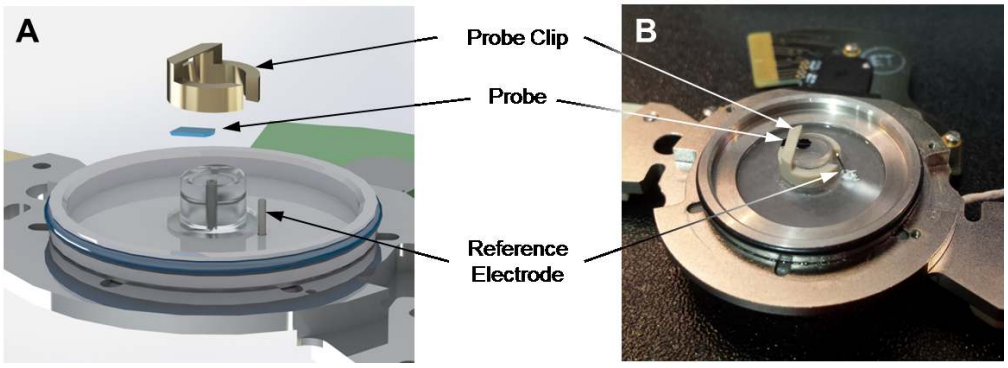


Figure 26.3.: Standard Probe Holder. (A) Schematic upside-down view of the probe holder. (B) Upside-down view photo of the assembled probe holder showing probe, probe clip, and reference electrode in recommended orientation.

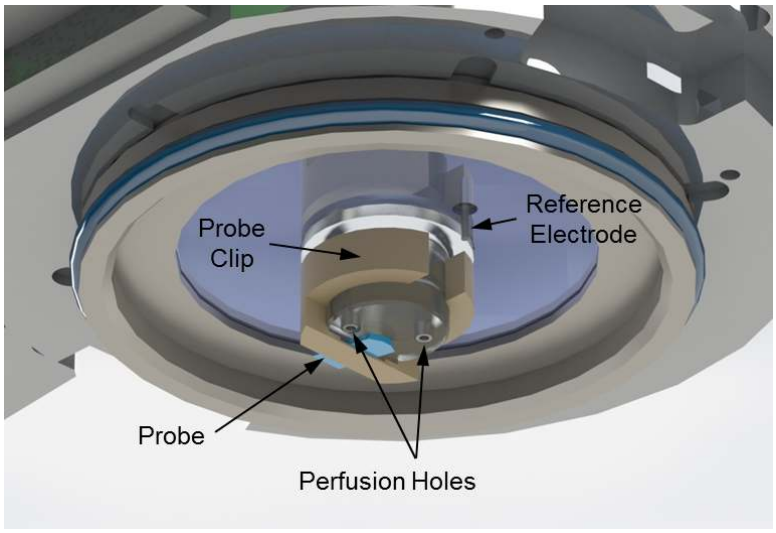


Figure 26.4.: Bottom view rendering of the fully assembled Perfusion Probe Holder (901.939)

26.3.4. Liquid Cup Subassembly (901.934)

The liquid cup subassembly may be seen in Figure 26.5 on page 308. It is used to simultaneously:

- Contain the electrolyte for EC
- Support the CE
- Support the WE (sample) for imaging
- Seal the WE (sample), providing a consistent surface area defined by the O-ring

A working volume of electrolyte inside the cup of 225 µL fills the liquid cup full to the brim with the tip engaged. Any volume in the range 150-300 µL will work well, and larger volumes in that range aid the user in visually confirming that the liquid wicks onto the probe and probe holder. The standard CE is a concentric annulus made from Cu or Pt with a bent protrusion for electrical contact; please keep in mind that a loop of any metal wire will also work. The liquid cup accommodates samples (WEs) that are 0.9–1.5 cm in circular diameter or square edge, with a range of >0–5 mm thickness. Users typically find square samples to be more convenient due to the ease of contacting the jumper wire to a corner of the sample. A conductive WE sample surface should have a non-conducting substrate or otherwise be insulated from the base plate (e.g., with plastic shims). Contact between the sample and the potentiostat WE lead is achieved by either soldering a free jumper wire to an exposed sample edge/corner or clamping a free jumper wire lead lightly between the liquid cup and the sample (as shown in

Figure 26.5 on page 308). Two gas perfusion ports in the sample chamber (see C in Figure 26.9 on page 310 for the entry points of the tubes into the Sample Chamber) allow for atmospheric exchange and control around the electrolyte (gas blanket).

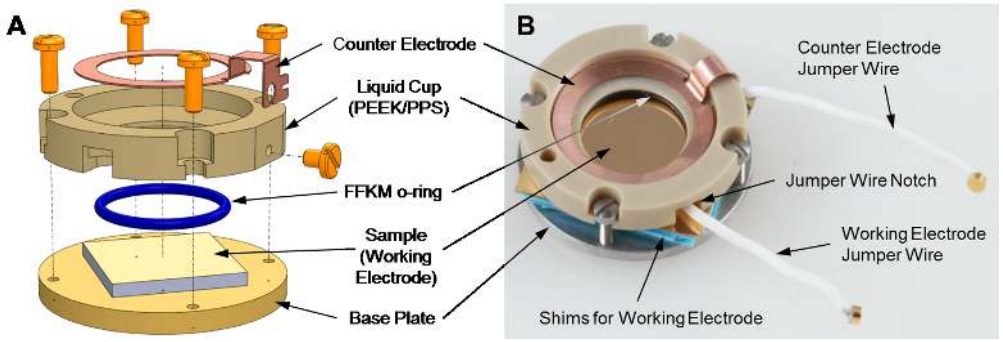


Figure 26.5.: EC Cell Liquid Cup Subassembly. (A) Exploded schematic of the liquid cup. (B) Photograph of the assembled Liquid Cup including sample shims and WE and CE jumper wires. For scale, O-ring inner diameter is 8.4 mm, and base plate diameter is 18.5 mm. Notice the blue plastic shims in use, as well as the notch that is provided in the liquid cup to aid in physically clamping a jumper wire to the sample surface.

26.3.5. Sample Chamber Subassembly (901.935)

Typically, the sample chamber subassembly comes pre-installed in the Cypher ES scanner. It is worth pointing out that a few small changes have been made to the design so that it is compatible with the design of the EC Cell, as well as previously offered functions. First, the metal contacts that feed through the glass cylinder enclosure are now made of nickel, which is rust-proof and yet still ferromagnetic for making contact to the WE and CE jumper wires. Second, the permanent ribbon cable replaces the former removable black 3-wire cable that used to exit the sample chamber. This ribbon cable connects directly to a female adapter on the light blue EC AppMod PCB (458.293.1) that routes WE, CE, and RE (and eventually a second WE for SECM and EC-STM type applications) through the scanner to the external potentiostat.

Figure 26.6.: Sample Chamber Subassembly.

26.3.5.1. Sample Chamber Electrical Connections

Please refer to Figure 26.9 on page 310 for the magnetic contact pin location assignments inside the sample chamber that are depicted on the blue AppMod PCB.

26.3.6. Enclosure Bulkhead Sub-assembly (901.937)

The enclosure bulkhead subassembly allows electrical signals to pass from the potentiostat to the scanner to the EC Cell through the Cypher ES enclosure. As can be seen in Figure 26.7 on page 309, the SMB ports at the bottom right front of the Cypher ES Scanner are labeled for Working (Top), Reference (Middle), and Counter (Bottom) electrodes. These labels correspond to the same labels in the EC Cell and the AppMod PCB.

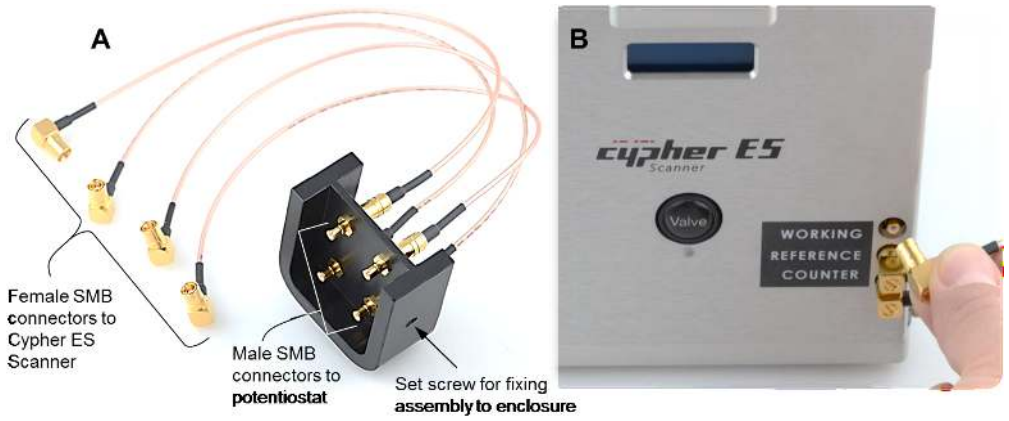


Figure 26.7.: (A) Enclosure bulkhead subassembly depicting SMB type connectors. (B) SMB connections and labeling corresponding to the electrical path to the pins inside the sample chamber.

26.3.7. Generic Potentiostat Cable Sub-assembly (448.169)

The generic potentiostat cable subassembly is comprised of coaxial cables with SMB connectors. It interfaces with the SMB barrel connectors on the outside of the bulkhead assembly (901.937), allowing you to connect an external potentiostat to the EC Cell while the Cypher enclosure door is sealed (see Figure 26.8 on page 309).

Figure 26.8.: Generic 5-lead potentiostat cable showing SMB connectors (to attach to bulkhead assembly) and free contacts for alligator clips.

26.3.8. EC AppMod Printed Circuit Board (PCB) Subassembly (458.293.1)

The EC AppMod PCB subassembly is a light blue circuit board that plugs into the top of the Cypher ES scanner. It has a port for connecting the ribbon cable from the sample chamber (901.935) that routes the electrical signals

out of the EC Cell. This part is necessary for routing electrochemical signals through the provided hardware. Note that the labels for the magnetic pins are printed on this board for reference.

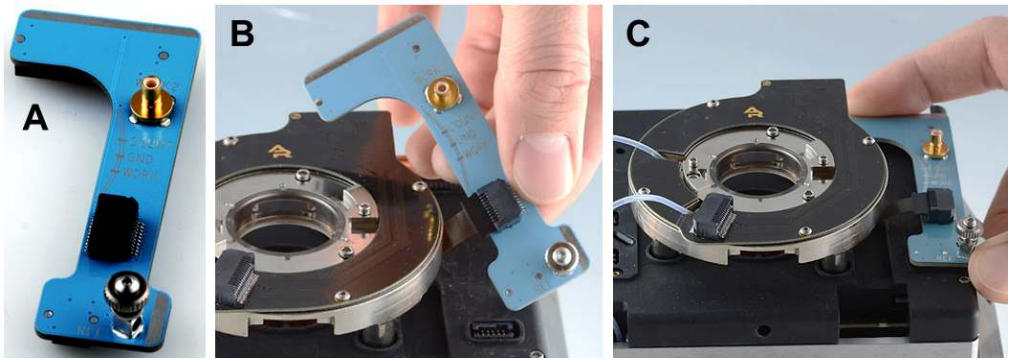


Figure 26.9.: EC AppMod PCB. (A) Circuit board as an independent part. Note the labels "COUNT," "GND," and "WORK" that correspond to CE, ground, and WE for the nickel pins on the inside of the sample chamber. The RE contact is the knurled silver knob, allowing for the RE wire to exit the top of the probe holder and be clamped for connection to the potentiostat. (B) View of the sample chamber ribbon cable installed into the port on the circuit board. (C) View of the proper installation site of the circuit board, which is easily connected with fingers.

26.3.9. EC Cell Full Parts List

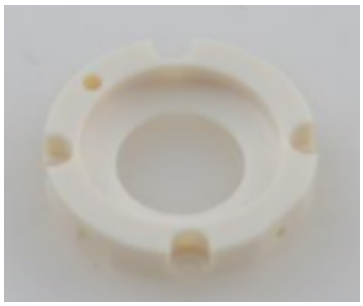
Below is a list of all components contained in the EC Cell kit (901.800). Please always refer to the relevant six-digit (###.###) Asylum Research part numbers during support calls or when buying replacements. Note that only the following inert components come into contact with the electrolyte: glass, glass-filled PEEK, PPS, and FKM or FFKM.

The part numbers for the major components of the EC Cell kit include:

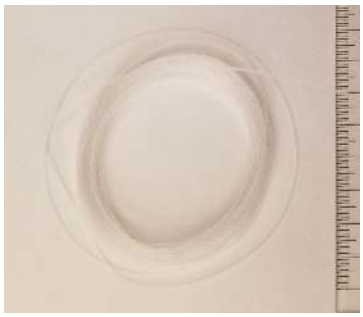
- 901.800 (Entire Accessory Kit)
- 901.933 (Standard Probe holder) or 901.939 (Perfusion Probe Holder)
- 901.934 (Liquid Cup)
- 901.935 (EC Sample Chamber)

Itm	Part #	Item Description	Qty	Picture
1	n/a	Screws and fittings in Meiho box	1	
2	080.165	Syringes, Norm-Ject, 1 ml, tuberculin	5	
The scale in the photos is in cm and mm.				

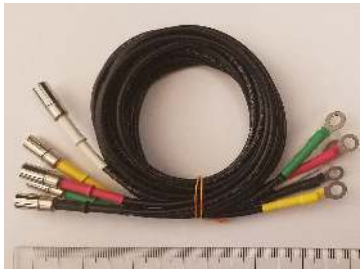
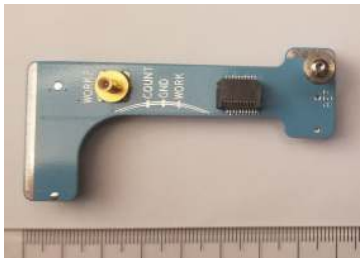
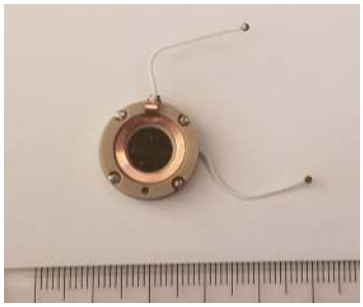
Itm	Part #	Item Description	Qty	Picture
3	010.106	PFA-coated silver wire for reference electrode; OD 0.010" uncoated, 0.013" coated	12”	
4	010.107	PFA-coated silver wire for reference electrode; OD 0.025" uncoated, 0.030" coated	12”	
5	114.820	Probe holder cleaning cup. Used to rinse and clean the probe holders while not causing damage to the circuit board.	1	
6	116.046	Base Plate, EC Liquid Cup (included in liquid cup assembly)	1	
The scale in the photos is in cm and mm.				

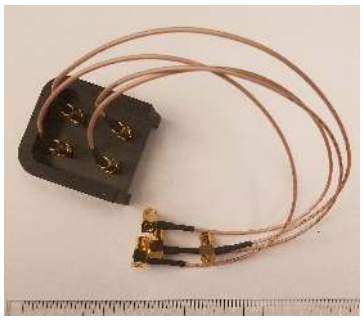
Itm	Part #	Item Description	Qty	Picture
7	116.070	Counter electrode, Copper ring	3	
8	116.071	Cup, EC Liquid, PEEK; included in liquid cup assembly	1	
9	116.086	Probe clip, PEEK (cantilever clamp); 7 mm OD; included in Probe Holder assembly	1	
10	116.119	Cup, EC Liquid, PPS	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
11	116.129	Probe clip, PPS (cantilever clamp); 7 mm OD	1	
12	116.173	Counter electrode, Platinum ring	1	
13	116.281	Gold Plated mica disc; 0.5" diameter	1	
14	230.059	O-ring; 0.930" ID, 0.040" CS; Viton (FKM); for Probe Holder seal to Sample Chamber	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
15	230.060	O-ring; 0.332" ID, 0.031" CS; P-Rex (FFKM) durometer 75; For Liquid Cup seal to Sample/WE	2	
16	230.061	O-ring; 0.332" ID, 0.031" CS; Viton (FKM) durometer 70; For Liquid Cup seal to Sample/WE	5	
17	231.028.1	Tubing, FEP, natural, 0.016" ID, cut	20’	
18	232.019	Female Luer to 10-32 Male	1	
19	232.020	Adapter, 1/16" to 1/32"	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
20	279.160	Tape, TaegaSeal PTFE 3/8" wide	1	
21	290.103	3C Tweezer, Extra Fine Sharp, Standard Grade.	1	
22	290.109	Leitsilber Conductive Paint. Used for mounting samples. Can be purchased from Asylum Research or directly from Ted Pella (16035).	1	
23	290.110	WIHA Screwdriver, Flat Tip 260 1.5 X 40.	1	
24	290.147	Scalpel Handle. Used to attach liquid perfusion tubing to the cantilever holder. Also useful when trimming thicker tubing for gas exchange.	1	
25	290.148	No. 15 scalpel blade. Used to attach liquid perfusion tubing to the cantilever holder.	10	
26	448.169	Potentiostat Cable; CH Instruments specific Cypher EC Cell.		
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
27	448.171	4 Jumper wire and magnet assembly for EC Cell contacts	18	
28	448.172	Cypher EC Cell generic potentiostat cable	1	
29	458.293.1	Cypher EC AppMod PCB Assembly	1	
30	901.934	Liquid Cup Assembly, PEEK	1	
31	901.933	Electrochemistry Probe holder assembly, Standard	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
32	901.935	Cypher ES Electrochemistry Chamber Assembly; includes three 2-56 x 1/8" SHCS S/S	1	
33	901.937	Cypher enclosure bulkhead assembly	1	
34	901.939	Electrochemistry Probe holder assembly, Perfusion	1	
The scale in the photos is in cm and mm.				

26.4. Chemical Compatibility

Descriptions of the materials comprising the three major components of the Cypher ES EC Cell are provided below. Please refer above to the Full Parts List for more information about these EC Cell parts.

The Cypher ES EC Cell probe holder (901.933 or 901.939) comes into direct contact with the electrolyte during normal operation and is comprised of the following items:

- Monolithic fused silica (quartz) downtube probe support and optical window
- Glass-filled PEEK and PPS probe clips (both provided)
- Reference electrode of your choosing (Ag wire provided), sealed as appropriate
- **For perfusion probe holder only**: PTFE tubes that enter through the probe holder for liquid perfusion/exchange in the liquid cup

The Cypher ES EC Cell liquid cup (901.934) comes into direct contact with the electrolyte and is comprised of the following items:

- Glass-filled PEEK and PPS (both provided)
- O-ring seal in FFKM or FKM (both provided)
- Copper or Platinum concentric CE (both provided), or a wire/sheet of metal of your choosing
- Sample (WE) of your choosing

The Cypher ES EC Cell sample chamber (901.935) does not come into direct contact with the electrolyte during normal operation, and is comprised of:

- Fused silica inner wall (makes contact with chamber atmosphere)
- Rust-proof magnetic nickel contacts for WE, CE, and ground connections (make contact with chamber atmosphere). Optional: Ground contact can be permanently substituted for a humidity sensor (must be submitted as a Special Request prior to ordering).
- PTFE tubes, for gas perfusion/exchange in the Sample Chamber
- FFKM bellows, for emergency liquid containment and mechanically adaptive atmosphere isolation
- Stainless steel chassis

Below is a table of the compatibility levels between the materials used in the EC cell and some common chemicals. Depending on your sample, electrode, electrolyte, and any additional materials used for sample mounting, this list may not be comprehensive. Please use additional resources to look up the chemical compatibility of more specialized materials such as one of the following:

- https://www.coleparmer.ca/Chemical-Resistance
- https://www.burkert.com/en/content/download/9318/334992/file/COM_Chemical_Resistance_Chart.pdf

If you have any doubt about chemical compatibility, we recommend that you soak a piece of material in your electrolyte solution and notice any change in the material aspect (e.g., use the AFM to image the surface, or measure the dimensions before and after to measure dissolution or swelling). As a key example, PEEK will swell when in prolonged contact with concentrated sulfuric acid. Please contact Asylum Research with any additional inquiries about chemical compatibility.

26.4.1. Chemical Compatibility Table

Compatibility Legend details include:

- 1. **Excellent**: prolonged high performance; negligible corrosion/discoloration/swelling
- 2. **Good**: minor effect; very slight corrosion/discoloration/swelling; elevated temperatures increase effect
- 3. **Fair**: moderate effect; discouraged for continuous use; softening, loss of strength, swelling may occur
- 4. **Poor**: severe effect; not recommended for ANY use
- Blank. **Information not available**: not found or not tested

Chemical	PEEK	PPS	FKM	FFKM	PTFE	Quartz*	Copper	Platinum
Acetaldehyde	1	1	4	4	1	1		
Acetate solvents	1	1	4	1/2	1		1	
Acetic Acid 20%	1	1	2	2	1		2	1
Acetic Acid 80%	1	1	3	3	1	1	2	1
1: Excellent 2:Good 3:Fair 4:Poor Blank:Unknown.								

Chemical	PEEK	PPS	FKM	FFKM	PTFE	Quartz*	Copper	Platinum
Acetic Acid, Glacial		1	4	3	1	1	2	1
Acetic Anhydride		1	4	2/3	1		2	
Acetone	1	1	4	1	1		1	
Alcohols, Amyl	1	1	1	1	1	1	1	
Alcohols, Benzyl	1	1	1	1	1		2	
Alcohols, Butyl	1	1	1	1	1		1	
Alcohols, Ethyl	1	1	1	1	1		1	
Alcohols, Hexyl			3		1			
Alcohols, Isobutyl	1	1	1	1	1			
Alcohols, Isopropyl	1	1	1	1	1		2	
Alcohols, Methyl	1	1	3	1	1		2	
Alcohols, Octyl			2				1	
Alcohols, Propyl	1	1	1	1	1		1	
Amines	1	2	4	1 (MeNH2: 4)	1			
Ammonia	2	1	4	3	1		4	
Aromatic hydrocarbons	1	2	1	1	1			
Aqua Regia (80% HCl / 20% HNO3)	4	4	2	1	1	1	4	4
Benzaldehyde	1	1	4	1	1		2	
Benzene	1	1	4	1	1		2	
Benzene Sulfonic Acid	4	1	1	1	1	1		
Bromine	4	4	1	1	1			3
Butyl Amine	1	4	4	1	1			
Carbolic Acid (Phenol)	4	1	1	1	1		4	
Carbon Disulfide (aka Bisulfide)	4	1	1	1	1			
Carbon Tetrachloride (dry)	1	2	1	2	1			
Chlorine (dry)	1	4	1	2	1		1	4
Chlorine, Anhydrous Liquid	4	4	1	2	1			4
Chlorine Water	4	4	1	1	1		4	4
Chlorobenzene	1	4	4	1	2			
Chlorosulfonic Acid	1	4	4	1	1			
Chromic Acid 10%	4	1	2	1	1		4	
Chromic Acid 30%	1	1	1	1	1		4	
Chromic Acid 50%	4	1	1	1	1		4	
Chlorox (Chlorine Bleach, Sodium hypochlorite)	2	4	1	1	1			1
Diethyl Ether	1	1	4	1	1		1	
Diethylamine	1	3	4	1	4		1	
Dimethyl Sulfoxide (DMSO)	3	1		1	1			
Ethanolamine		2	4	1	1			
Ethylene Diamine	1	1	2	2	1		4	
Ethylene Oxide	1	4	4	3	1		4	
Ferric salts	1	1	1	1	1		4	
Fluorine	4	4	3	2	4		3	
Fluosilicic Acid		4	2	1	1			
Formaldehyde <40%	2	2	2	1	1		2	
Formic Acid	3	1	3	2	1		3	
1: Excellent 2:Good 3:Fair 4:Poor Blank:Unknown.								

Chemical	PEEK	PPS	FKM	FFKM	PTFE	Quartz*	Copper	Platinum
Furfural		1			1		1	
Glycerol (Glycerin)	1	1	1	1	1			
Gold Monocyanide					4			3
Hexane	1	1	1	1	1		1	
Hydraulic Oil (Petro)	1	4	1	1	1		1	
Hydrobromic Acid, 20-100%	4	1	1	1	1		4	3
Hydrochloric Acid, 20-100%	1	4	1	1	1		4	1
Hydrofluoric Acid, 20%	4	1	1	1	1	4	2	1
Hydrofluoric Acid, 50%	4	1	2	1	1	4	2	1
Hydrofluoric Acid, 75%	4	2	2	1	1	4	2	1
Hydrofluoric Acid, 100%	4	4	2	1	1	4	2	1
Hydrogen Peroxide, <30%	1	3	1	1	1		4	
Iodine	3	4	2	1	1		4	1
Iodoform					3		2	
Lye: KOH Potassium Hydroxide	2	1			1	4	2	
Lye: NaOH Sodium Hydroxide	2	1			1	4	2	
Magnesium Chloride	2	1	1	1	1		1	
Magnesium Hydroxide	1	1			1		2	
Morpholine		3	2	2	1	1		
Nitrating Acid (<15% HNO3)		3			1	1		
Nitrating Acid (>15% H2SO4)		4			1	1		
Nitric Acid, 5-10%	1	2	1	1	1	1	4	
Nitric Acid, 20%	1	3	1	1	1	1	4	
Nitric Acid, 50%	3	3	1	1	1	1	4	
Nitric Acid (Concentrated)	3	3	1	1	1	1	4	
Oxalic Acid	1	1			1	1		
Petrolatum	1				3	1		
Phenol (Carbolic Acid)	4	1	3	1	1	1	4	
Phosphoric Acid	1	1	1	1	1	2	4	2
Potassium Hydroxide (Caustic Potash)	1	3	4	1	1	4	2	2
Sodium Hydroxide, 20-80%	1	1	1	1	1	1	2	2
Sulfides	1	1	2	1	1		4	1
Sulfuric Acid, <10%	1	1	1	1	1	1	3	1
Sulfuric Acid, 10-75%	3	1	1	1	1	1	4	1
Sulfuric Acid, 75-100%	4	1	2	1	1	1	4	1
Sulfuric Acid (cold concentrated)	4	1	1	1	1	1	4	1
Sulfuric Acid (hot concentrated)	4	4	1	1	1	1	4	2
Water, Deionized	1	1	1	1	1	2	2	1
1: Excellent 2:Good 3:Fair 4:Poor Blank:Unknown.								

26.5. Fluid Volume Guidelines

Do not fill the entire sample chamber with liquid as this will cause electrical shorting between contacts, or worse (e.g., electrochemical corrosion of the instrument). The liquid cup can be operated successfully with liquid volumes in the range 150-300 µL. Based on experience and excluded volume calculations, a volume of 225 µL will

completely fill the liquid cup with a tip engaged; however, capillary forces will also cause some liquid to wick up onto the probe holder glass and electrodes, so you may find 300 μL more intuitive to work with.

26.6. EC Cell Assembly

This section provides additional detail for the following tasks:

- Installation of perfusion lines (for perfusion probe holder only)
- Installation of the EC AppMod PCB
- Connecting SMB cables for routing electrical signals from the sample chamber out of the Cypher enclosure to an external potentiostat
- Connecting a potentiostat
- The process of mounting electrodes, AFM probe, and sample (WE)

IMPORTANT: In order for the EC Cell to function properly, all of the electrical connections must be robust and correct, and the electrodes must contact the electrolyte solution.

26.6.1. Installing and Using Liquid Perfusion Lines (For Perfusion Probe Holder Only)

The process of installing and using the perfusion lines is fully analogous to the same procedure for the non-EC droplet imaging perfusion probe holder found in Section 22.2 on page 269. One subtle difference is that the distance between the perfusion line ports and probe are all much smaller for the EC probe holder, as can be seen in Figure 26.10 on page 321, but this does not affect the procedure.

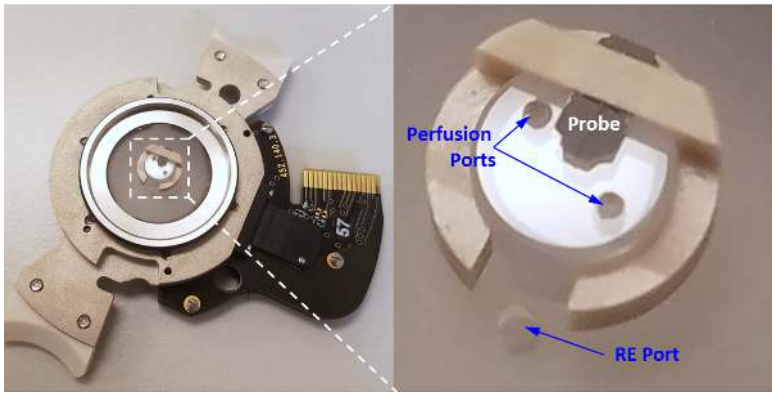


Figure 26.10.: Cypher ES EC Perfusion Probe holder. (Left) Full probe holder. (Right) Zoomed in view of the optical window showing clip, probe, and perfusion ports. Note the position of perfusion tube ports relative to the probe chip and RE port. For scale, the optical window is 5 mm in diameter.

26.6.2. Installing the EC AppMod PCB

The EC AppMod PCB, shown in Figure 26.9 on page 310, must be plugged into the top of the scanner in order to use the EC Cell in its designed configuration. In order to do this, first plug the ribbon cable extending from the sample chamber into the socket on the PCB, and then with two fingers, press the circuit board into its corresponding sockets in the top of the scanner. Please refer to the same figure for visual cues. Note that the PCB may be left installed on the scanner without interfering with normal operation (e.g., if standard imaging techniques are desired without electrochemical control). It is also possible to use the EC Cell concurrently with the Heater or HeaterCooler stages that both plug into a different socket on the top of the scanner.

26.6.3. Connecting SMB and Potentiostat Cables

On the bottom right of the ES Scanner, there are three male SMB connections for use with the EC Cell that correspond to the working, reference, and counter electrodes. These receive female SMB connectors, as can be seen in Figure 26.7 on page 309, that connect to the bulkhead feedthrough (the astute user will note that there are four SMB leads in this figure, which belies the future intent to enable tip bias for techniques such as SECM and EC-STM). Then, the potentiostat cable (Figure 26.8 on page 309) is connected to the male SMB leads on the outside of the bulkhead feedthrough, with free ends now available for further electrical connections. The free ends may be clipped with alligator clips from the potentiostat, completing the connection between the potentiostat and the electrical contacts in the sample chamber. It is now important to ensure that these signal lines are properly routed from the scanner through the Cypher enclosure to the potentiostat.

One qualitative check for this is to color coordinate the leads and ensure that the same colors propagate from the potentiostat to the scanner, but we note that potentiostats vary a bit between brands as to how the electrical leads are labeled for working, reference, and counter electrodes. (For CH Instruments, the WE is green, RE is white, and CE is red, so we have chosen these as our standards.)

A multimeter set to resistance measurements should now be used to test electrical continuity of the system. If the leads are routed appropriately, the user should obtain resistances <10 ohm between the potentiostat alligator clips and the corresponding lead (WE and CE are magnetic pins inside the sample chamber, while the RE is the knurled knob affixed atop the EC AppMod PCB).

26.6.4. Mounting Electrodes and AFM Probe

Recall that the WE and CE are connected to the liquid cup and base plate, while the RE enters through the probe holder independently of the WE and CE. Through many trials, and as described in Section 26.2.2 on page 303, it has become clear that it is easiest to first install the RE on the probe holder, then mount the CE to the liquid cup, mount the WE/sample to the liquid cup, and finally mount the probe to the probe holder. This order of operations may be modified, but serves to:

- Minimize opportunities for inadvertent contamination of the sample via accidental contact with gloves, tweezers, etc.
- Minimize the amount of time required to mount the sample, which may be of concern for certain time-sensitive experiments
- Minimize risk of damaging or contaminating the probe

26.6.4.1. Mounting Reference Electrode (RE)

The RE enters the top of the probe holder through a 1 mm diameter hole that accommodates a wide variety of reference electrode options. A wire of any diameter less than 1 mm may be inserted through the hole and optionally sealed in place with Teflon tape or epoxy (depending on the permanence of a given experimental setup), as seen in Figure 26.11 on page 323. Additionally, a 1 mm OD, leak-free, PEEK-based, fritted Ag/AgCl (saturated KCl) reference electrode constructed by Innovative Instruments is compatible and is shown in Figure 26.11 on page 323. It is stable in all experimental conditions that the EC Cell components withstand (see Section 26.4 on page 317 for chemical compatibility of PEEK). If the system requires full hermetic seal, the RE should be sealed into the RE port with epoxy or equivalent to prevent gas exchange.

26.6.4.2. Mounting Counter Electrode (CE)

The CE is mounted inside the liquid cup, as shown in Figure 26.5 on page 308, and can be either a ring electrode as provided (see Table 26.1 on page 312 or Table 26.1 on page 313) or simply a piece of wire of any type bent into

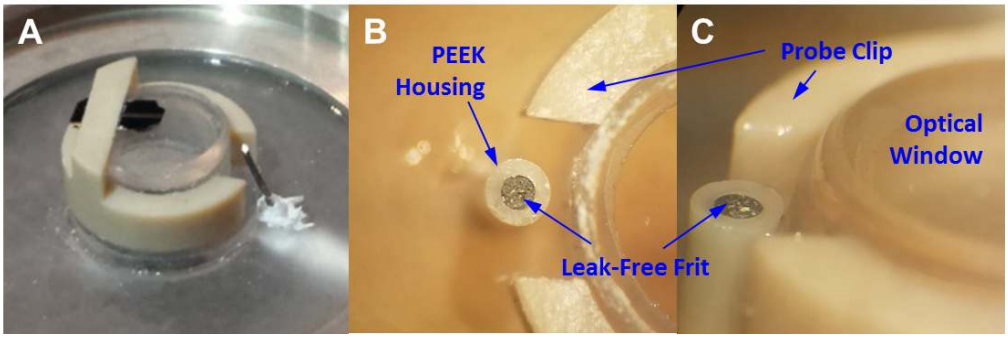


Figure 26.11.: (A) Probe holder with mounted probe showing an Ag RE wire sealed in place with Teflon tape. (B) Top-down view of 1 mm OD leak-Free PEEK fritted Ag/AgCl (sat. KCl) RE manufactured by Innovative Instruments inserted through RE port. (C) Zoomed side view of 1 mm OD leak-Free PEEK fritted Ag/AgCl (sat. KCl) RE manufactured by Innovative Instruments inserted through RE port.

an appropriate ring. The CE is fastened to the liquid cup with a lateral 00-90 screw (3/32" or shorter) to which a magnetic jumper wire is also fastened, providing electrical connection to the contact in the sample chamber. Care should be taken to ensure that the CE is as flush as possible with the liquid cup surface to avoid introduction of mechanical noise or errant electrical contact with other components.

26.6.4.3. Mounting Working Electrode (WE, sample)

As described in the order of operations in Section 26.2.2 on page 303, it is advised that the sample be mounted after the RE and CE to minimize the risk of accidental WE contamination or damage. The WE is mounted to the liquid cup with an O-ring seal, insulating shims (optional), and four screws. If possible, solder a jumper wire that extends laterally from your sample prior to mounting.

26.6.4.4. Mounting Probe

The probe is mounted by lightly raising the probe clip from the optical window using tweezers, sliding a probe under the strap of the probe clip, and pushing the clip back into its fully seated position. Note that this holds the probe in place with spring friction force of the clip upon the glass tube, and there are no screws or fasteners involved. This requires very little force to accomplish.

26.6.5. Testing Electrical Connections

Similar to the description in Section 26.6.3 on page 322, the full electrical connections from potentiostat to mounted CE, WE, and RE should now be tested. The simplest way to do this is to attach sharp contacts to your multimeter and touch the CE, WE, or RE with one contact while simultaneously contacting the same connection at the potentiostat alligator clip. Continuous connections provide resistance of <10 ohm, while non-continuous connections provide overload resistance.

26.7. Sealing the EC Cell Sample Chamber

The EC Cell probe holders are designed to function the same way as standard probe holders for the Cypher ES, and they should therefore be able to maintain an internal inert atmosphere inside the sample chamber without

having to be inside a glovebox. This has proved to be of high utility for some customers desiring to conserve laboratory space.

One of the key spots that the sample chamber may allow diffusion of gas is through the RE port (see Figure 26.10 on page 321 and Figure 26.11 on page 323 for reference). This is a hole in the top glass of the EC probe holder, where the RE lead enters. Depending on the type of RE used, this hole will need to be sealed with something like epoxy surrounding the RE. Note that 5-minute epoxy is sufficient, and also compliant enough that it can be removed if the electrode needs to be replaced.

Another spot that the sample chamber may allow gas diffusion is through the gas tube and perfusion tube seals. The gas tube fittings may be seen in Figure 21.1 on page 261, and are the connections that seal into the sample chamber. The perfusion lines are installed into the Perfusion Probe holder and that process is outlined in Section 26.6.1 on page 321; if these are not fully seated into the perfusion ports, gas may leak.

26.7.1. Outside of a Glovebox: Maintaining Strict Inert Gas Atmosphere

Once the above considerations have been accounted for, the procedure outlined in Chapter 24 on page 290may be followed in order to generate and maintain a temporary inert atmosphere for imaging of sensitive samples outside the glovebox. Additional comments are provided below. It is worth mentioning that the probe holder O-ring for the EC Cell probe holder (see Table 26.1 on page 313) has different cross sectional dimension than other designs of the Cypher ES probe holders, making it easier to damage the O-ring if lots of force is applied. Care should be taken during the glovebox procedure to avoid such damage.

For experiments beyond ~1-hour, continuous flow of inert gas will likely be needed because the sample chamber depressurizes at < 1 mbar/min. The incoming gas can be provided an extremely low flow rate. One useful trick is to bubble the incoming gas through the same solvent as is used for the electrolyte solution so that the incoming gas stream is saturated with solvent and therefore won't slowly evaporate the electrolyte. The idea is that while the EC chamber remains sealed, the gas input line is purged on low flow of your inert gas (Ar, N2, etc.) for at least 15 minutes (sufficiently long to displace the volume many times over, depending on flow rate) by having the 3-way valve turned to allow gas flow to atmosphere (step 1). Then, without breaking the flow of gas, the valve is turned 90 degrees, so the gas stream is redirected to the chamber (step 2), and the pressure regulating syringe is simultaneously removed (step 3). This outlet stream that now flows out where the syringe was will contain solvent vapor and should be directed to a fume hood if needed. This process is also discussed in Section 24.1.4 on page 295.

26.8. Cleaning and Storage

Components of the EC Cell are designed for chemical inertness and may therefore be cleaned with a variety of solvents of different composition and polarity. Please refer to Section 26.4.1 on page 318 for considerations of the proper solvent for your needs.

Warning	The probe holder should never be fully immersed in solution, nor sonicated, due to its electrical contacts and external glue bonds. Instead, please use the probe holder cleaning cup as described in Table 26.1 on page 311.

26.9. Troubleshooting

26.9.1. General EC-AFM Considerations

26.9.1.1. Incorrect Electrical Connections

Poor electrical contacts between the elements of the EC Cell, the external potentiostat, and/or the controller may cause malfunctions. If a problem arises that may stem from the electrical wiring, electrodes, and connectors, please check all electrical connections (a multimeter set on resistance mode should show <10 ohms between connected elements). Connections may be found between the following:

- The controller and scanner
- The potentiostat and the bulkhead feedthrough on the Cypher enclosure
- The bulkhead feedthrough and the base of the scanner
- The scanner and the EC AppMod PCB
- The EC AppMod PCB and the sample chamber cable
- The EC AppMod PCB and the RE wire
- The Sample Chamber magnetic contacts and the jumper wires to the liquid cup
- The jumper wires and the sample (WE) or CE
- The electrolyte solution and the RE, WE, and CE

26.9.1.2. Chemical Reactivity of EC Cell Components

Please refer to 26.4 to verify that your experimental conditions are compatible with the EC Cell materials in use. For example, very caustic chemicals such as HF or concentrated NaOH will slowly etch the quartz window of the probe holder, and concentrated H2SO4 is not compatible with the PEEK liquid cup and probe clip (so the PPS versions should be used). Generally, solutions in the range 1 < pH < 13 (i.e., 0.1 M strong acid to 0.1 M strong base) will not cause problems. Whenever possible, the concentrations of reactants in the electrolyte should be kept low, and special attention should be paid for concentrations above 0.2 M. For proprietary solutions (e.g. ionic liquids, deep eutectic solvents, etc.), for which it is less likely to find documentation of material reactivity, one may place a small drop of the solution on a part of the liquid cup outside of the liquid containment area and monitor for changes. **We also sell a chemical compatibility test kit (Asylum P/N 901.813)**. Please call Asylum Research if you are uncertain about the chemical compatibility of the EC Cell and your electrolyte or would like to purchase this test kit.

26.9.1.3. Leaks in the EC Cell: Predicting and Identifying

Check that the O-ring is level and properly sealed against the sample surface and that the jumper wire is not pinched between the sample and the liquid cup. Verify that the sample has no cracks or divots that could let electrolyte flow out of the liquid cup. If these precautions fail to prevent leaks, please take into consideration the composition of your electrolyte: Low surface tension of the electrolyte will contribute to its ability to flow out, so eliminating surfactants (for example) from the solution will help. If such surfactants are essential, we recommend coating the O-ring with Dow Corning High Vacuum Grease, provided this is compatible with your solvent (refer to product information at www.dowcorning.com).

26.9.1.4. Colloidal Electrolyte Solutions (such as Bacterial Suspensions)

Colloidal components present in an electrolyte may diffuse and/or block either the reflected laser beam for measuring cantilever deflection or the blueDrive laser for driving cantilever oscillations. Colloidal particles can adhere to the cantilever, tip, optical window, liquid cup, sample (WE), or counter/reference electrodes, thus modifying the optical path, tip-sample interactions, effective concentrations, or electrochemical signals.

Even for electrolytes that are completely chemically compatible with the EC Cell, high concentrations of solute/analyte can promote surface crystallization on the cantilever, tip, optical window, liquid cup, sample (WE), or counter/reference electrodes and cause similar problems as above.

26.9.1.5. blueDrive Laser Electrolyte Absorption

The output wavelength of the blueDrive laser is 405 nm. If the electrolyte solution or solute has a strong absorption band at this wavelength, unwanted photoinduced reactions such as polymerization, crosslinking, lysis, oxidation, deposition, etc., may occur. It is recommended that an absorbance spectrum of the electrolyte solution be obtained prior to use in the EC Cell, as this aids in troubleshooting photoinduced effects.

26.9.2. EC-Specific Considerations

26.9.2.1. Unexpected or Transient Peaks in Electrochemical Signals

Trace contaminants adhered to the EC Cell components or mixed into the electrolyte solution may be one predominant contributor to errant signals in electrochemical experiments due to their chemical or surface interactions. The cleanliness of not just the liquid cup, but all electrodes, tubes, connectors, tweezers, etc., that contact the electrolyte during preparation will affect the purity of your electrolyte and resulting experimentally detected currents.

Additionally, external mechanical vibration (e.g., construction, music, slamming doors, etc.) may cause anomalous signals during electrochemical measurements due to shaking of the sample (perturbs the electrochemical interface) or vibration of the electrical contacts (may cause intermittent breaks in electrical continuity—especially for magnetic contacts—or intermittent unwanted contact between the electrodes in the electrolyte).

For some experiments where material is electrochemically deposited on the sample/WE surface, and in particular if the deposit is conductive, an electrical pathway may be formed between the sample and the counter electrode, causing large transient spikes in the current and/or saturating the current signal. If cleanliness and mechanical vibration have been ruled out, it may be worth removing the probe holder and visually inspecting the liquid cup.

All electrodes should be making electrical contact with the electrolyte in the EC Cell, but not with each other. Sometimes the electrolyte volume will decrease over time due to evaporation from heat or current running through the solution, causing an electrode to lose contact with the solution. One technique for mitigating this is to add a small volume of the solvent to the Cypher ES sample chamber bellows to help saturate the atmosphere inside the sample chamber; if gas is being flowed through the sample chamber, a pre-stage where the gas is bubbled through the solvent will help saturate the gas entering the cell and slow the evaporation of the solution.

Check that the reference and/or counter electrodes have not been covered by solids, oxidized, or changed properties significantly during the electrochemical reaction. In the case of these occurrences, try cleaning or refurbishing or replacing these electrodes. Platinum is well cleaned in an oxygen-free hydrogen flame, or by dipping in Piranha solution (3:1 H2SO4:H2O2) until clean. Storing Pt in a 10% HNO3 solution keeps it contaminant free. Ag/AgCl reference electrodes can often be refurbished by replacing the internal electrolyte (saturated KCl) as well as replacing the AgCl wire with a fresh wire (Ag wire in chlorine bleach or biased in KCl may do the trick); the frit can often be refurbished by heating to 80°C in dilute H2O2 to remove organic contaminants from its porous microstructure.

26.9.2.2. Electrochemical Topography not Imaged with Probe

Sometimes the electrochemical experiment will indicate that reductive deposition, oxidative stripping, or otherwise redox-mediated conformational changes are occurring on the electrode surface, and yet the probe does not detect these topographical changes where it is scanning. Some but not all of the effects that may contribute are as follows:

- The reduced/oxidized species deposited on the surface are so loosely bound that the lateral force of the tip sweeps them out of the scan area.
- The reaction is occurring in a location on the sample away from the tip. This could occur due to a weak electrolyte (<0.1 M) creating a strong or non-uniform electric field, or if the counter electrode is mounted in an asymmetric position around the exposed area of the sample/WE. Another possibility is that the Probe Holder and/or probe and/or Probe Clip are shielding the diffusion of the electroactive species at the site of imaging and preventing the reaction from occurring there; this may be evaluated by raising the probe several microns from the surface, running the same electrochemical experiment (while also monitoring the surface with the optical microscope), and re-engaging the tip and imaging to observe if the surface changed.
- The signal detected electrochemically is not correlated to the sample/WE. The assembly should be checked for shorted connections and cleanliness.

27. Scanning Tunneling Microscopy

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

27.1. Introduction

This chapter discusses the use of the Scanning Tunneling Microscopy (STM) tip holder with the Cypher ES Scanner. The chapter assumes that you are familiar with operating the Cypher ES using basic AFM scanning techniques.

27.2. Required Equipment

Equipment required for using the STM tip holder with the Cypher ES Scanner:

- Cypher ES

- Cypher ES STM tip holder
- Cypher ES stage equipped with a Gas Cell body
- STM probes - A box of Platinum Iridium STM probes is included in the accessories kit.
- Appropriate STM sample - A HOPG sample is included in the accessories kit.
- ES electrical sample pucks - Supplied in the accessories kit.
- Conducting paint - Supplied in the accessories kit.
- Voltmeter
- Tweezers

27.3. About the Cypher ES STM Tip Holder

The ES version of the STM tip holder is designed around the same concept as the ES AFM tip holders. The closed cell of the ES is sealed by a Viton O-ring surrounding the edge of the holder. The tip holder tube is sealed to the glass window with epoxy. The tip tube is sealed at the top of the tube with RTV silicone glue.

Warning	The tip holder tube is sealed with a drop of RTV silicone glue at the top. Inserting probes more than .2” (5mm) into the tube will push the silicone glue “plug” out of the tube and cause a gas leak. The seal is repairable by the user and may be ignored if the user is not concerned about a sealed sample chamber.

The ES STM holder is currently designed as an air only holder. Although STM experiments are possible, they have not been tried on this design. The main risk of scanning in liquids is a fluid leak through the top side of the tip holder tube due to capillary forces drawing fluid into the tube and causing a leak to the top of the holder. Liquid scanning requires that the bottom of the tip holder tube be sealed so that the inside of the tube is not contaminated causing poor electrical contact to the STM probe. Please contact Asylum Research if your experiment involves STM scanning in fluids.

Warning	The tip holder tube is sealed with a drop of RTV silicone glue at the top. Inserting probes more than .2” (5mm) into the tube will push the silicone glue “plug” out of the tube and cause a leak.

The tip tube is connected to a current to voltage converting preamplifier built into the tip holder body assembly. Currently, the sensitivity of the amplifier is 1nA/V meaning that 1nA of current flow into the tip generates 1v of output signal from the amplifier circuit. The total detectable current range is 10nA, where the absolute current is 10nA, and the sign of the flow is determined by the polarity of the bias voltage applied.

Warning	The electrical connection between the tip tube and the preamp circuit is made using small gauge magnet wire. The wire is delicate and, if broken, is difficult to repair. Please be careful when using sharp tools or other objects around the top of the STM holder near the tip wire.

Due to the inherent design of the Cypher’s video system, which is optimized for AFM use, some of the features are not possible to perform as an STM, so they have been deactivated. Please see the section on using the video system for detailed information. The engage sequence is primarily affected where the tip and sample focus cannot be determined. The tip must be lowered to the surface by eye and then the engage routine is initiated.

27.4. Using the Video System

The video system on the Cypher ES that is equipped with an STM holder is different from AFM.

- The 'Set' button for saving the tip focus location has been deactivated. The view of the tip is from the top-down, so it is not possible to see the actual end of the tip.
- The 'Set' button for saving the Sample Focus position has been deactivated in software. It is possible to move the focusing objective to view the sample surface prior to engaging as long as the tip length is less than 3mm from the bottom side of the glass window.
- The 'Move to Pre-engage' button has been deactivated. Since the Tip and Sample locations cannot be stored, it is not possible to quickly move to a close distance prior to engage.

27.5. ES Cell Electrical Connections

The sample bias in the STM is generated by the system and is located on the front magnet inside the gas cell body by way of an interconnect cable. See Section 20.2.2 on page 233 for more information.

27.6. Preparing an STM Sample

An STM sample must be electrically conductive between the sample puck and the top surface to be scanned. The sample may first be fixed to an ES electrical sample puck with a drop of 5-minute epoxy. Silver paint is used to create an electrical connection from the puck to the sample surface. Bias voltage is made to the sample by way of a wire lead magnetically attached to the front most magnet in the gas cell body. See Section 20.2.2.4 on page 236 for more information.

Steps for preparing an STM sample are as follows:

1. Mount your sample to an ES electrical sample puck.
2. Use silver paint to connect the sample puck to the top side of the sample.
3. Install the sample into the ES fitted with a Gas cell body. Make sure that the gas cell cable is installed.
4. Use tweezers to connect the bias lead from the sample puck to the front magnet in the gas cell body.

27.7. Loading and Preparing an STM Tip to Engage

27.7.1. Loading an STM tip

The tip holder tube is slightly curved and is designed to be used with STM tips made from straight wire. The reason for this is to reduce lateral drift in the system caused by stress in the tip wire if it is bent during installing. Inserting the tip wire into the curved tube allows the tip to be secured and also make good electrical connection without the stress of bending the wire.

1. Use tweezers to grab an STM probe from the probe box. Be sure not to touch the end of the probe with the tweezers, otherwise the probe tip will be damaged.
2. Insert the probe into the tip tube.
3. Gently push the probe into the tube until you feel resistance.
4. Continue to push the probe into the tube until about 2mm of the probe wire is protruding from the tube.

Attention	The STM probes supplied in the accessory kit are cut to .2" (5mm). This is a sufficient length to allow the tip to extend about 2mm from the tip holder tube while being short enough to not extend out the top of the tube which will compromise the gas tight seal of the tip holder.

27.7.2. Preparing the STM to engage

The optics in the View module were intended for use with an AFM cantilever. Due to the tip position pointing down below the STM probe wire, it is necessary to bypass the normal AFM alignment process and simply bring the tip down manually close to the surface, and then click the 'Engage' button.

1. Use the coarse adjust wheel, on the front of the Cypher, to raise the cell high enough to allow the STM probe to clear the sample surface.
2. Install the STM tip holder onto the ES and secure it in place with the two locking screws.
3. Use the wheel on the enclosure to move the tip to the sample. Get the tip to the desired engage distance of about 0.5 to 1 mm above the sample. Use the tip and the reflection of the tip in the sample surface as a guide to bring the probe close.

27.8. Zeroing Electrical Offsets in the System

Due to multiple circuits in the signal path, it is necessary to adjust the zero points of the system for both the STM current amplifier and the sample bias. Each Cypher system is different and should be characterized as part of the initial system setup. Once the offsets are known, the offset values typically do not change over time, and so this is a one time adjustment to your system.

Hint	The software will save the offsets in the experiment but not carry them over if a new experiment is started. You might want to record the current and sample bias offsets once they have been determined.

27.8.1. Zeroing the STM current amplifier

Zeroing the current amplifier signal is mainly necessary to normalize the current signal to 0A for the feedback signal during scanning. Zeroing the offset does not modify the electrical offset present in the Current signal path.

1. Install the ES STM tip holder into the scanner. It is not necessary to install an STM probe.
2. If the system does not automatically detect the holder type, use Mode Master to select *STM* operation or select *STM* mode in the Main tab of the Master Controls panel.
3. Monitor the current signal in the Sum and Deflection Meter panel.
4. Note the value being displayed in the Current signal.
5. Enter the value only with the opposite sign into the *Current Offset* menu item on the Main tab of the Master Controls window.
6. You may also use the *Zero* button next to the current offset menu item in the AR Do IV control panel.

7. Once the offset is entered, you should see that the Current signal has changed to 0A in the Sum and Deflection Meter panel.

Note A typical offset current is around +/- 200pA

27.8.2. Zeroing the Sample Bias voltage

Zeroing the sample bias ensures that the voltage you are applying matches the voltage in the sample bias menu item. The S bias offset adjustment is added to the Sample Bias menu item so the electronics is actually adjusted.

1. Install the ES STM holder into the scanner and allow the software to detect it. The operating mode should change to STM mode but if it doesn't, use the Mode Master buttons to select *STM* or change the mode to *STM* in the Main tab in the Master Controls window.
2. Once the software is set to STM mode, remove the ES STM holder.
3. Set the *Sample Bias* to 0V in the Main tab of the Master Controls window.
4. Set the *S Bias Offset* to 0V.
5. Use a voltmeter to measure the sample bias located on the front most magnet inside the gas cell body. Use the gold ground shell of one of the three SMB connectors on the front of the ES as the ground reference for the voltmeter.
6. Enter the voltage measured on the meter with the opposite sign into the *S. Bias Offset* menu item.
7. Remeasure the bias voltage on the magnet. It should now be 0v.
8. Enter a voltage in the *Sample Bias* menu item and confirm that the correct voltage appears on the magnet.

Note A typical offset voltage is around +/- 50mv.

27.9. Basic Scanning Parameters

27.9.1. Atomic Scale Scanning on HOPG

The following parameters are used as starting values to get atomic resolution imaging using the supplied HOPG sample and the supplied PtIr STM probes:

- Scan Size: 10nm
- Scan Rate: 20Hz
- Resolution: 512 lines x 512 pixels
- Integral Gain: .5 to 1
- Setpoint current: 1nA
- Sample Bias: 20-50mv
- Feedback bandwidth: 2-5KHz

- Scan mode: Hybrid. This parameter may be hidden in the Main scan controls tab. Click the 'Setup' button to expand the tab to see all the settings.

Step to get atomic resolution imaging using the supplied HOPG sample:

1. Cleave the surface of the HOPG sample by using a strip of adhesive tape. Stick the tape to the surface and then pull it off. A thin layer of graphite will peel off the surface and reveal a clean sample. The layers of the graphite may often tear and leave small flaps of material pointing up. Try another cleave and possibly adjust the direction you peel the tape to create a smooth surface.
2. Install the sample and connect the bias lead to the front magnet the cell body. Don't forget the interconnect cable from the cell to the scanner.
3. Install a probe and lower the tip to the surface.
4. Set the initial scan parameters and engage the tip.
5. Begin scanning.

Once the tip is engaged and scanning, pay attention to the current signal in the sum and deflection meter panel. It should match the setpoint current and be stable. Also monitor the current signal. initially the lattice may appear distorted due to the system settling. The settling time may be immediate or take several minutes, typically from 5-30 minutes.

If the current signal appears to be noisy or low quality, don't give up. The tip may be passing through a bad location on the sample. The tip may also be alternately tunneling from several places, as the actual end of the probe cannot be that well defined. One trick we use is to "clean up" the tip by abruptly crashing it into the surface. To do this, *gently* give a sharp tap on the View module with a small tool like your tweezers. The mechanical disruption to the system often knocks off neighboring tips on the end of the probe and makes them less likely to be in the tunneling distance to the surface.

Adjusting the integral gain helps increase the tracking of the height signal, while also showing good resolution in the current signal. Raising the gain too high will reduce the current signal, which is normal but may end up causing the feedback loop to oscillate and may blunt the tip.

Depending on the quality of the current signal, you may want to adjust the feedback bandwidth to filter out higher frequencies or increase it for better tracking.

27.9.2. Larger scan sizes on HOPG

The same scanning parameters are typically used to scan the HOPG sample at a larger scan area like 1-5um, with the exception of the scan rate.

- Scan rates for larger scan sizes are typically .5 to 2Hz. The tip velocity increases with scan size, so you need to allow time for the feedback loop to track the sample surface.
- For optimizing the image, try increasing the integral gain and/or then the bias voltage. Raising the gain will increase the feedback adjustment rate to the Z-piezo, while increasing the bias voltage makes the tunneling current easier to achieve. Though spatial resolution may be reduced, the effect will be negligible.

27.10. STM Probes

The Cypher ES STM kit is supplied with 20 mechanically formed (i.e., carefully clipped with super sharp wire cutters) probes. Additional probes can be purchased from Asylum Research.

If you wish to make your own probes, the material and dimensions for making the supplied probes include:

- Material: 80%/20% Platinum Iridium. Wire should be drawn straight. Wire cut from a roll has a small radius and may not hold tightly into the tube on the STM holder. The tube is bent with a large radius. This is intentional to help reduce drift due to the stress of bending the probe wire upon insertion into the holder.
- Wire size: 0.01" diameter (0.25mm), cut approximately .2" (5mm) long.
- Contact Asylum Research about further tools and techniques required to make the proper cuts.

Attention	Longer probes can be used but may introduce image distortion from drift due to the length.

Attention	The approximate range of the camera focus is about 3mm below the underside of the tip holder tube. Tips that extend below 3mm will not allow the sample to be viewed.

27.11. STM Holder Testing and Maintenance

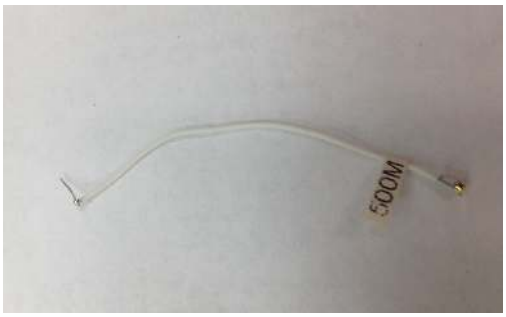
27.11.1. Testing

27.11.1.1. Using the test resistor

A 500M Ω resistor is supplied in the STM accessories kit. The resistor is soldered to a short length of wire terminated by Pt Ir probe wire. The other end of the resistor is soldered to a magnet which allow connection to the sample bias terminal in the gas cell body.

To use the test resistor:

- Insert the platinum wire into the tip tube.
- Connect the magnet on the test resistor to the front magnet in the gas cell body.
- Install the STM tip holder into the ES, while making sure not to pinch the test resistor lead in between the cell body and the O ring on the STM tip holder.
- The software should detect the STM holder and change the operating mode to STM. If this does not happen, use Mode Master to change to STM or change the operating mode to STM in the main tab of the Master control window.
- Set the surface bias to "1V".
- Note the measured current on the Sum and Deflection Meter panel. It should be 2nA (1/500e6 Ω).



Note You can also use the Do IV controls to ramp the bias voltage and observe the current relationship of 2nA per 1V applied bias.

27.11.1.2. Testing the STM preamp noise level

To test the STM preamp noise level:

1. Install the ES STM holder into the scanner.
2. Push the scanner into the chassis and close the enclosure door to shield the scanner from stray electrical interference.
3. In the upper command bar, select *Programming > Load Test Procedures*. The word “testing” will be added to the command bar and the test controls window will appear.
4. Click the Noise tab.
5. Current is not typically a choice in the noise panel so type the word ’current’ into the source field in channel 1. The software will automatically add the current channel as a data choice.
6. Set the Resolution to “1Hz”.
7. Set the filter cutoff to “1KHz”.
8. Go to *Programming > Filter* panel and deselect the ’1 pole’ filter in the feedback channel.
9. Click the ‘Start’ button to start recording the STM current noise.
10. The typical noise should be ~1mV (~1pA) Adev from 1hz-1kHz with little perceivable periodic noise in the spectrum.

Hint	You can change the units of the noise measurement by selecting *Custom* in the Sensitivity display item. Enter “A” for the units and “1e-9” for the scale. The sensitivity should change to 1nA/V.

27.11.2. Maintenance

27.11.2.1. Cleaning the STM tip tube

Normal operation does not require that the tip tube be cleaned. If for some reason debris has gotten into the tip tube, the ES STM accessories kit is supplied with a length of 0.01” diameter tungsten wire. The wire is very stiff and will allow you to push the wire through the tube to force out the obstruction through the top of the tube.

Warning	Be aware that the top end of the tube is sealed with a small drop of silicone glue, so this seal will also be removed.

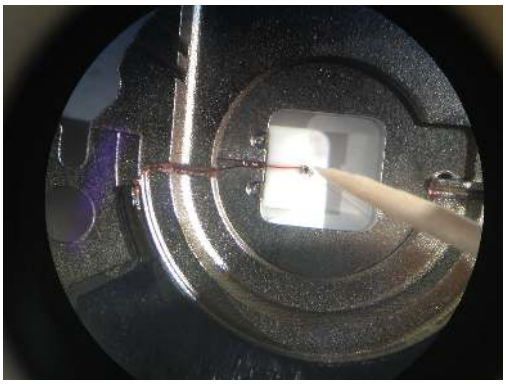
Warning	Be very careful of the tip wire soldered to the top of the tip tube. It is very fragile and can break if hit. Also, please observe caution when handling sharp tools or objects around the top of the STM holder assembly.

27.11.2.2. Sealing the tip tube

The tip tube is sealed with a small drop of silicone glue. If for some reason you wish to reseal the tube, use RTV silicone glue that is thick enough not to flow into the tube. In particular, we use Dow Corning 3545 RTV silicone glue.

To seal the tip tube:

1. View the top of the ES STM tip holder under an optical microscope.
2. Collect a small amount of glue onto the end of a wire or small tool, such as the point of a broken wooden cotton swab.
3. Touch the glue to the top of the tube and deposit a small amount on the tube.
4. Allow glue to dry.



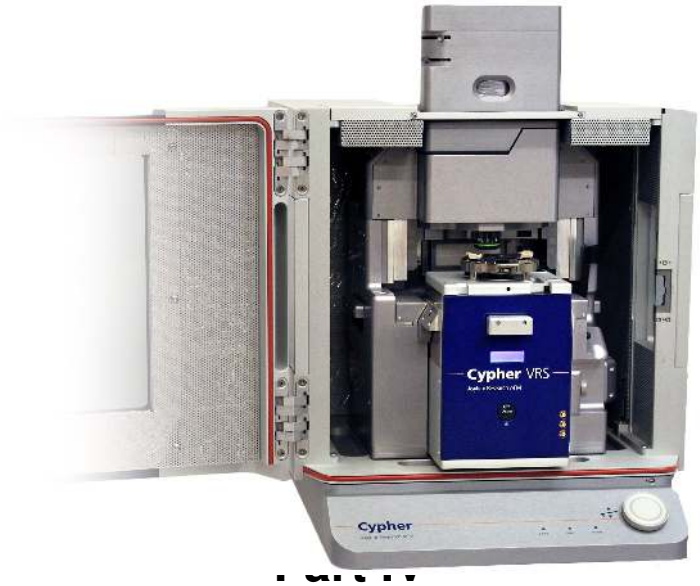
Caution Be very careful of the tip wire soldered to the top of the tip tube! It is very fragile and can break if hit. Also, please be careful when handling sharp tools or objects around the top of the STM holder assembly.

27.11.2.3. Replacing the bias lead on the electrical sample pucks

The wire lead on the electrical pucks can fatigue and break during use. The ES STM Tip Holder accessory kit is supplied with 6 replacement puck bias leads. Additional replacement wires can be purchased from Asylum.

To replace the bias lead on electrical sample pucks:

1. Use a screwdriver to loosen the screw on the puck.
2. Remove the small 1/2 washer from the broken lead that may be trapped under the head of the screw.
3. Slip the new lead under the screw.
4. *Gently* tighten the screw to capture the new lead. It is not necessary to tighten the screw very tight. Light pressure is all that is necessary.



Video Rate Scanner (VRS)

Who is this part for? After the Cypher VRS AFM has been installed in your lab and you (or someone in your facility) have completed the initial training, this part of the user guide will be the principal reference for operating the instrument. Although written with the novice user in mind, experienced SPM users should complete the basic imaging tutorial at least once before attempting to use this instrument.

Part Contents

28. Cypher VRS1250 Overview

CHAPTER REV. 2427, DATED 08/22/2021, 18:55. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

28.1. Cypher VRS AFM

The Cypher VRS is the third AFM in the Cypher family, where VRS stands for "video rate scanner." It is based on the platform of the Environmental Scanner (ES) where the sample is placed in a sealed cell.

The Cypher VRS1250 has a dedicated VRS sample stage, as well as updated electronics in the Backpack, Controller, and Scanner. Cypher VRS is compatible with all of the existing ES sample stages, thereby allowing imaging in all modes already available with the Cypher ES.

The original "Classic" Cypher VRS was first released in 2017 and allowed for a maximum scan rate of 625 lines per second in both tapping and contact modes. In 2021, the VRS1250 was released in which the new maximum scan rate is 1250 lines per second, and the new maximum frame rate is 45 FPS. Both stages can be used with the scanner and have unique resistor IDs that the MFP3D software can identify in order to set up the appropriate scan/frame rates for each stage.

28.1.1. VRS Hardware

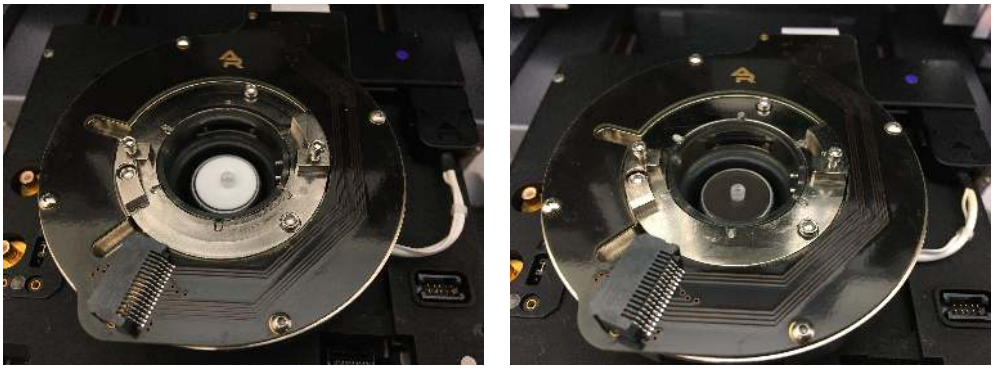
There are 3 unique hardware components for both the VRS Classic and the VRS1250:

1. **VRS Sample Stage**. The VRS sample stages are visibly distinct – they have a 15mm in diameter shiny sapphire sample disk surface. For the new VRS1250 this surface is white; whereas, for the VRS Classic stage the surface is black (see Figure 28.1 on page 340). They are also different from all existing ES sample stages in that they contain an additional Z-actuator in the center, beneath the sapphire surface. This actuator, solely used for high-speed imaging, is not sensored and has a Z range ~2 μm. These stages also have a connector containing high voltage that is plugged into the Scanner via the VRS Application Module.
2. **VRS Application Module**. This module (see Figure 28.2 on page 340) is a connector that is installed into the port located on the top right rear of the VRS Scanner, as seen in Figure 28.3 on page 341. This module provides high voltage to the VRS Stages. It is important to note that there is a specific orientation in which the cable plugs into the module – make sure they are properly lined up. Typically, this module always remains connected to the Scanner; however, when it is required to be removed or reattached, the ARC2 controller MUST be powered OFF when doing so.
3. **VRS Perfusion Cantilever Holder**. With the VRS stages, the standard gas and liquid cantilever holders can be used just like with the Cypher ES. However, for high-speed imaging experiments specifically using liquid

perfusion a new VRS Perfusion Cantilever Holder was developed to accommodate the reduced sample size requirement. Specifically, the inlet and outlet ports sit very closely on either side of the cantilever chip so that they are within the smaller liquid droplet. Pictures of the standard and VRS liquid perfusions can be found in Figure 28.4 on page 341.

(a) VRS1250 Stage

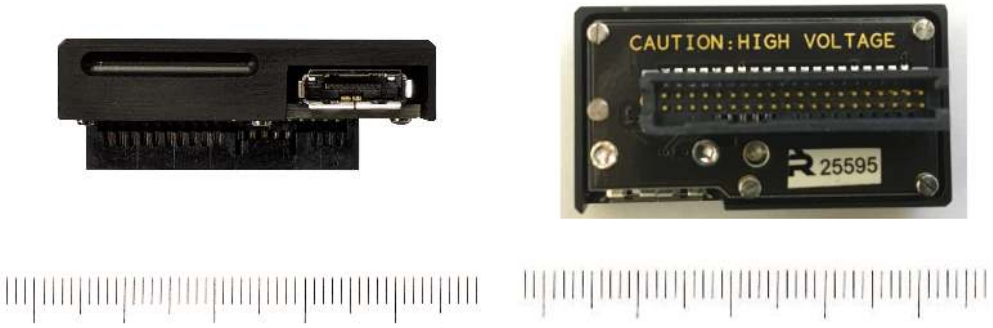
(b) VRS Classic Stage



(c) VRS1250 Stage on Scanner

(d) RS Classic Stage on Scanner

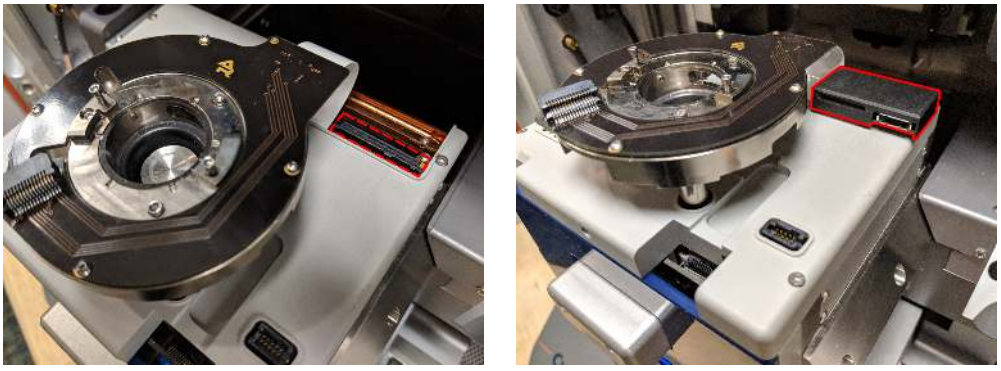
Figure 28.1.: VRS Sample Stages: a, b) mounted in the cell body; c,d) view from the top of the VRS scanner with the VRS stage plugged into the high voltage connector located on the top right-side of the scanner.



(a) Front Connector

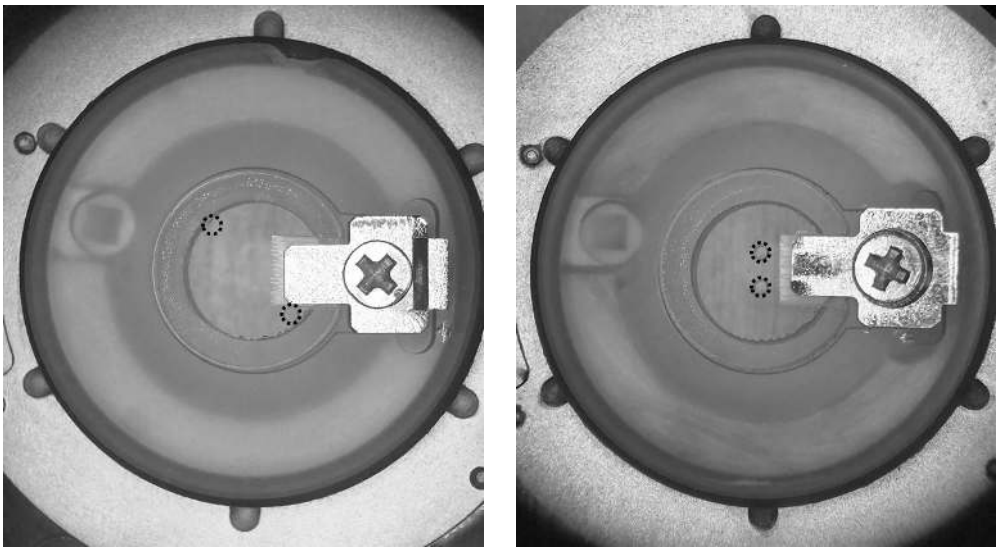
(b) Bottom Connector

Figure 28.2.: VRS Application Module



(a) Empty Top-Right Port **(b)** VRS Application Module Attached

Figure 28.3.: VRS Application Module Installation



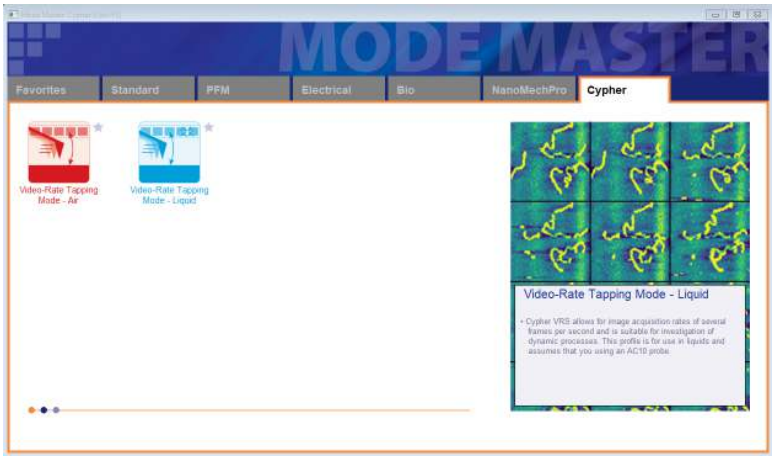
(a) Standard perfusion holder **(b)** VRS perfusion holder

Figure 28.4.: Liquid Perfusion Holders: a) standard holder with inlet/outlet ports far apart, optimized for a 15 mm diameter sample, b) VRS perfusion with inlet/outlet ports placed directly next to cantilever chip to accommodate the smaller 3 mm diameter sample size.

28.1.2. VRS1250 Software

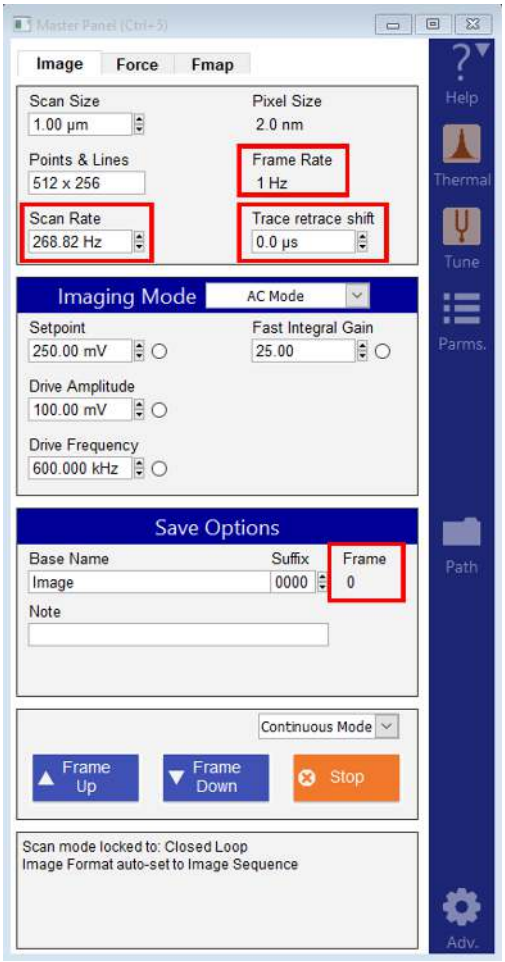
Currently, Cypher VRS1250 can only be used with software version 18. VRS "Classic" can be used with version 16 or greater, but we recommend that you upgrade to version 18. With video rate imaging, the data is now acquired in a new file format, Asylum Research Image Sequence with the file extension being ARIS. Each ARIS file consists of x number of frames. By consolidating all frames within a single file, post-processing steps such as flattening can be applied to all the frames simultaneously. Furthermore, movies can be easily created using all or a user-defined set of frames from the ARIS files.

The VRS1250 software has several new parameters added to several of the existing panels.



Mode Master window:

- To properly set up both the hardware and software, begin by opening the Mode Master window.
- Select the Cypher tab.
- Select either the *Video-Rate Tapping Mode – Air* or *Video-Rate Tapping Mode – Liquid* template depending on whether you will image in air or liquid.



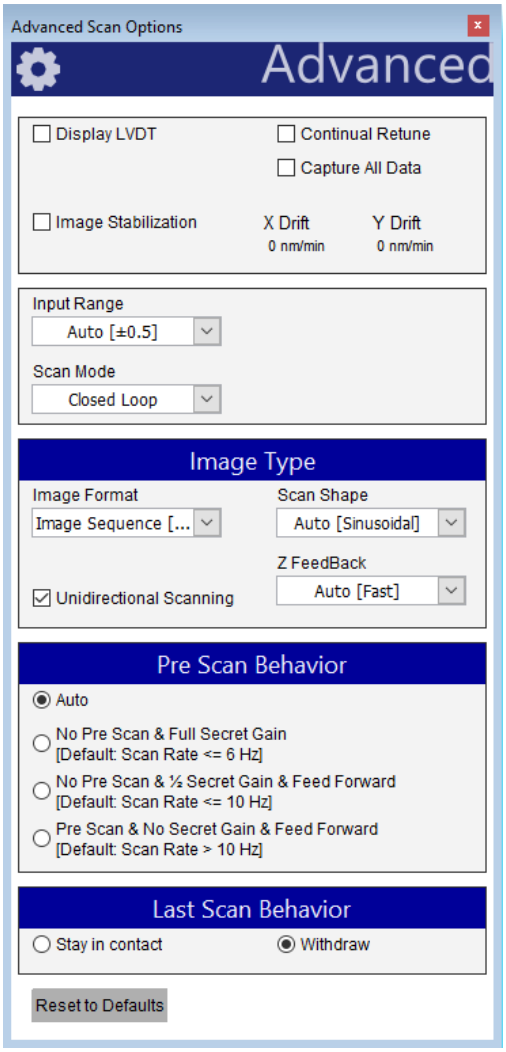
Master Panel, Image tab:

- *Scan Rate* indicates how many lines are scanned per second.
- *Frame Rate* indicates how many frames are acquired per second.
  - Frame Rate = Scan Rate/ [number of lines in the image + # Return + # Skip lines]

    For example: Frame Rate = 268.82 Hz/[256 + 6 +2] = 1 (actual 0.97) Hz
- *Trace retrace shift* realigns the Trace and Retrace lines
- "Frame" indicates the number of frames in an individual ARIS file.

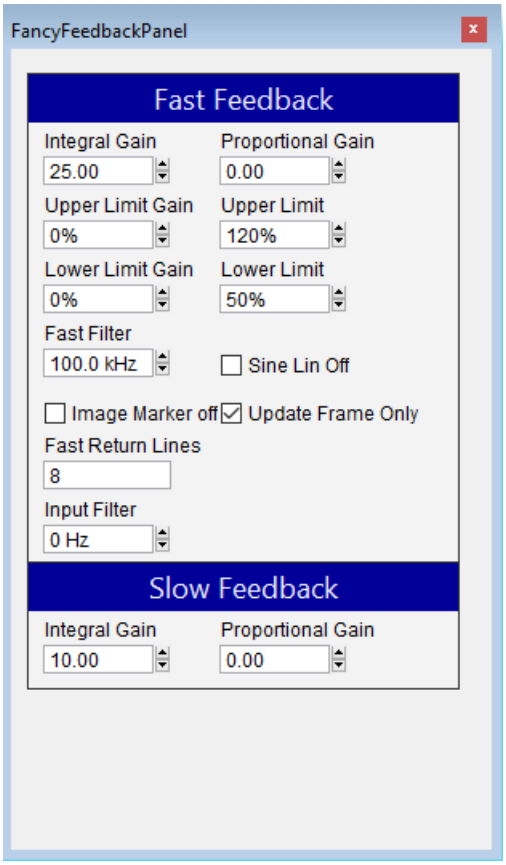
Advanced Scan Options panel:

- *Image Format:*
  - By default, the software automatically changes the image file format from ibws (slow scan rates) to ARIS (higher scan rates).
  - ARIS files are “image sequence” files that contain user-selected frames within a single file.
- *Scan Shape:*
  - Scan shape can be triangular or sinusoidal.
  - Sinusoidal scan shape is automatically set at scan rates of 101 Hz and higher.
- *Z Feedback* should always be set to “Auto”.
- *Unidirectional Scanning:*
  - Images are acquired line-by-line in one direction only (top to bottom or bottom to top).
  - When the scan shape is set to sinusoidal, scanning is automatically set to unidirectional.



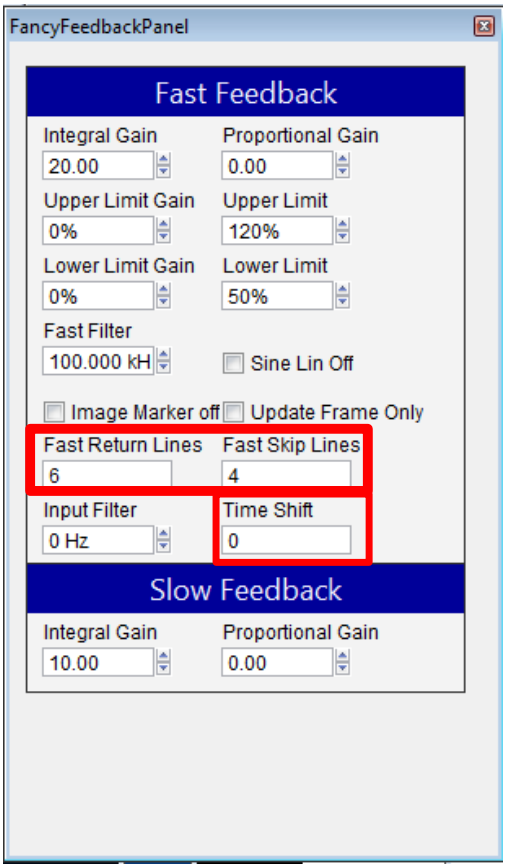
Fancy Feedback Panel:

- *Image Marker Off:*
  - During imaging, the red marker on the left side of the image shows the location of the trace and retrace that appears in the scope.
  - When scanning fast, the marker can be distracting, so you can hide it by checking this box.
- *Update Frame Only:*
  - Instead of updating the image line by line, it can be updated every frame.
  - This setting is automatically set when the scan rate reaches 100 Hz.
  - The software automatically places the red marker on the real-time window but you can set the scope line by right-clicking on the line and selecting *Set Scope Line*.



Fancy Feedback Panel, continued:

- *Fast Return Lines:*
  - During unidirectional scanning, once an image is acquired, you need to move to the beginning of the next image.
  - *Fast Return Lines* is the number of lines that are used to perform this movement.
- *Fast Skip Lines* is the number of lines that are skipped (are not shown) in the image.
- *Time Shift* is a scanner specific value in milliseconds.
  - When left at 0, a lag between trace and retrace may be visible in the scope (it is scanner dependent).
  - This value is calculated and automatically applied in the software, though you can adjust it.



29. Sample Preparation and Cantilever Selection

CHAPTER REV. 2427, DATED 08/22/2021, 18:55. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

29.1. Sample Preparation

As mentioned in Section 28.1.1 on page 339, the fast Z-actuator is located inside the VRS sample stage, beneath the polished sapphire surface. As a result, the sample must be placed directly on top of the stage surface. Depending on whether you will image in liquid or air, different preparations are required.

29.1.1. Imaging in Liquid

When working in liquid, the sample needs to be mounted some distance away from the surface of the VRS stage to prevent the excitement of resonances in the cantilever chip when the sample is moving fast in the Z direction. For this reason, we attach the substrate (mica, silicon, HOPG, coverslips, etc.) to a sapphire post, 3mm in diameter and approximately 2.5mm in height and glue this assembly directly to the VRS stage. Pre-mounted posts with mica are included in the VRS kit. Additional packs can be purchased from Asylum Research, part # 939.059 (see Figure 29.1 on page 346). You can also make your own mica posts (see Section 29.2 on page 348 for detailed instructions).

(a) 3mm in diameter mica disk **(b)** Box of pre-mounted mica posts

Figure 29.1.: Pictures of sapphire post with 3mm in diameter mica disk (left) and a box of pre-mounted mica posts, part # 939.059 (right)

The post, with attached mica, is glued directly to the polished sapphire surface of the VRS stage using 5-minute epoxy, Devcon part # 14250 (see Figure 29.2 on page 347).

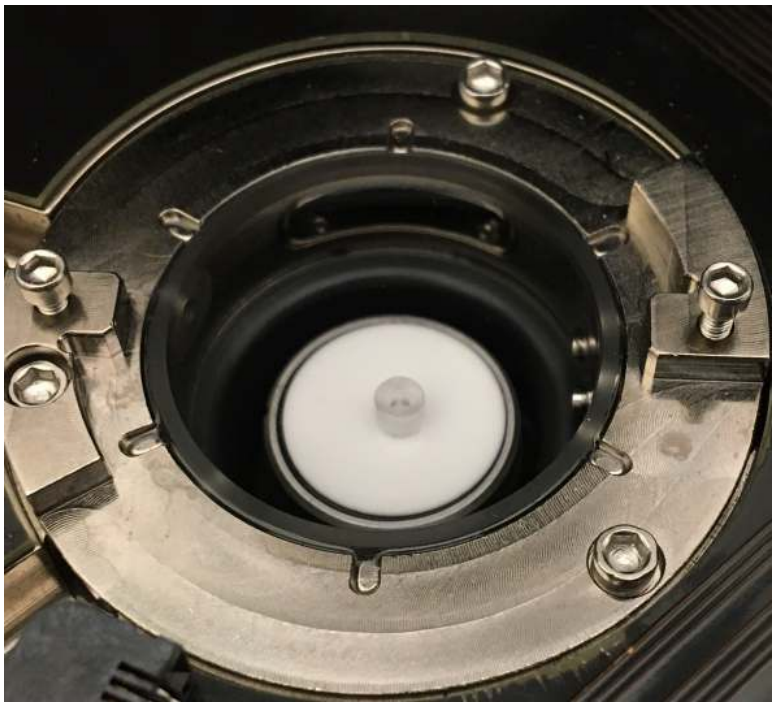


Figure 29.2.: Mica post glued directly to VRS1250 stage

For the same reason mentioned above, it is important to engage the cantilever and image near the left edge of the mica surface. Figure 29.3 on page 348 demonstrates the correct placement of the cantilever chip with respect to the mica sapphire surface.

29.1.2. Imaging in Air

When performing VRS experiments in air, there are no major restrictions on sample size or shape, and requirements are similar to those for convention scanning with the Cypher ES, with a few exceptions. Although the sample (mica, silicon, HOPG, coverslips, etc.) can be mounted on a metal puck, we recommend that it be secured directly to the sapphire stage using epoxy or Red Sticky Wax (Universal Photonics # SS-66). This ensures a strong attachment so that at higher scan rates the sample remains stationary.

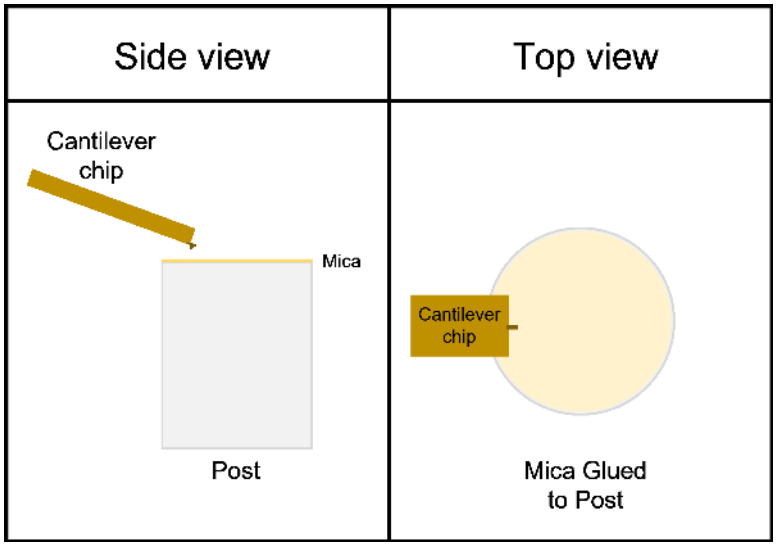


Figure 29.3.: Placement of the cantilever post above the mica sapphire post, side, and top views

Figure 29.4.: Silicon chip mounted directly to stage using red sticky wax

29.2. Mica Post Preparation

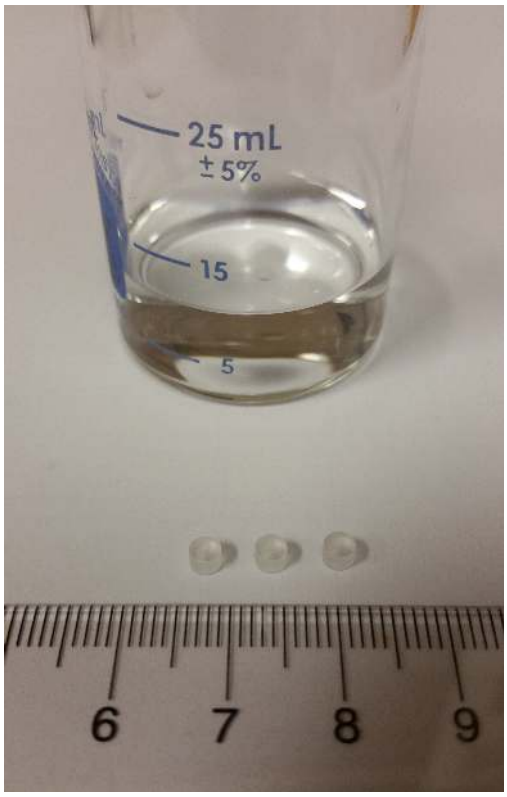
To prepare your own mica posts, you first need to purchase sapphire posts, Asylum Research's part # 569.029, and 3mm mica disks. We recommend using V1 grade mica, which can be purchased from SPI (or equivalent vendor) as either a 20-pack, part # 01900-CA, or 100-pack, part # 01900-MB.

Glue the mica disks to the sapphire post using an inert adhesive such as EPON 1004F (1st choice) or 5-minute epoxy, Devcon #14250 (2nd choice). It is important that all components (posts, mica, etc.) are clean and free of dust. A minimal amount of glue should be used so that the mica can be easily cleaved. Too much glue may spread to the edge of the disk preventing the mica from being cleaved.

For substrates other than mica such as HOPG, SPI part # 425HP, replace the mica with HOPG in the procedure outlined below.

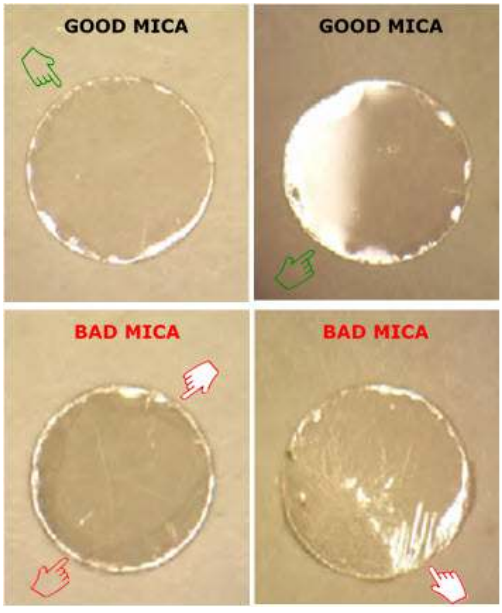
1. **Clean the sapphire posts:**

   - Sonicate the posts in pure HPLC grade (or equivalent) isopropyl alcohol for 10 minutes.
   - Rinse the posts with pure water (Millepore or equivalent) several times.
   - Place the posts on a Kim Wipe or membrane filter and let them air dry.



2. **Qualify the mica disks:**

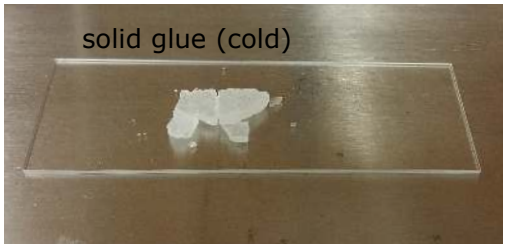
   - The edges of the mica disk must be sharp and as flat as possible (top two images).
   - Make sure at least one edge of the mica is sharp as you will position this edge on the left side where the probe will approach the surface.
   - Flaky and delaminated mica will result in a difficult or impossible approach and/or unstable imaging (bottom two images).
   - If you purchased mica disks from a vendor (e.g., SPI), and they are broken or damaged, then contact the vendor immediately for replacements.

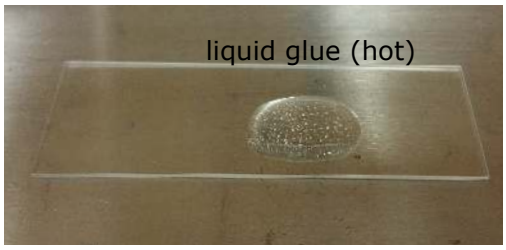


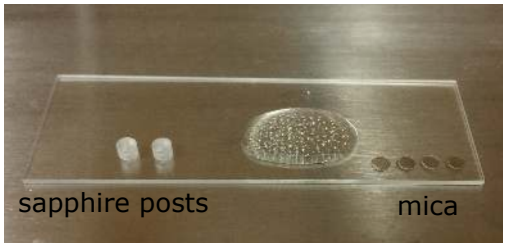
3.

Prepare EPON1004F epoxy:

- Place some EPON1004F glue crystals on a glass slide and heat it up on a hot plate.
- Place several clean sapphire posts on a glass slide and heat them up beside the glue.
  - Orient the sapphire disks so that the flat surface is facing up, the side with the crevice should face down.
- Finally, place several pieces of good, clean mica disks on the glass slide.
- Since the sapphire post and mica disks are so small and challenging to handle, it is highly recommended that you place the hot plate and glass slide with epoxy, posts, and mica disks under a stereomicroscope.



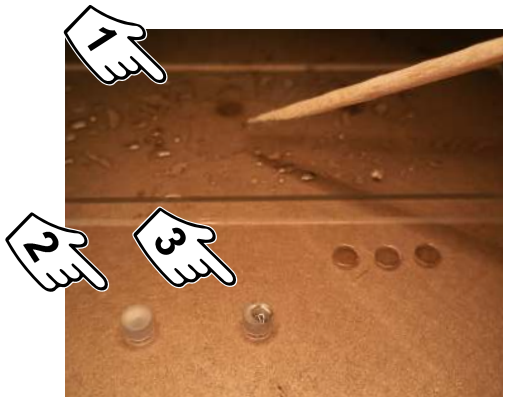


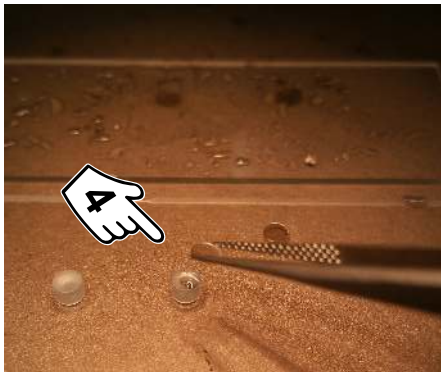


4.

Attaching Mica disks to sapphire posts:

- Step 1: Wait for glue to melt (~140 degrees C).
- Step 2: Keep the sapphire posts on the hotplate so that they are at the same temperature as the glue.
- Step 3: Put a drop of glue onto the post using a wooden applicator. Use the smallest amount possible in order to prevent the glue from spilling over the edge.
- Step 4: Place a mica disk on top of the glue and let the glue spread under the mica. Try to avoid pressing the mica down onto the sapphire post as it might float off the surface! However, if the mica doesn't sit flat, you might have to gently push it into the sapphire surface.

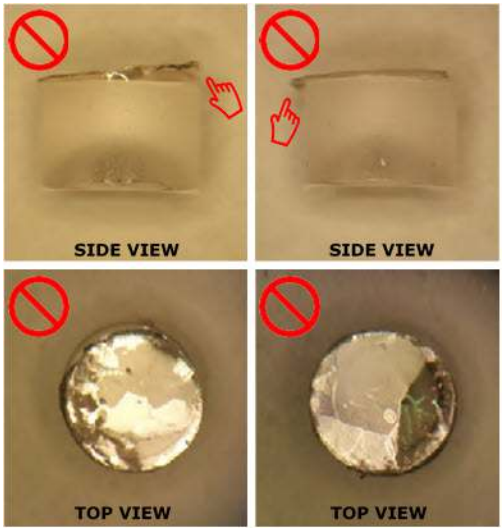




5.

Examine the prepared mica posts:

- Carefully examine the mica posts to ensure that the mica is flush.
- If too much glue was applied, the sample will be tilted (top left image).
- If the mica disk is improperly positioned, it will overhang on the sapphire post.
- Uneven or flaky mica is easy to see under the microscope and should be avoided. Poor quality mica typically results in poor cleavage, sample attachment, and overall imaging.



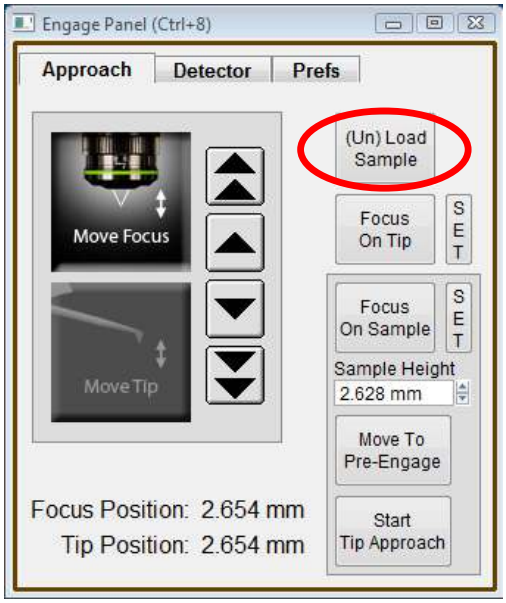
29.3. Mica Post Replacement

When there is no more mica on the sapphire post to cleave, you need to remove the used post and glue a new (and clean) pre-mounted mica post.

1.

Unload the sample:

- Stop imaging by clicking 'STOP' on the Image tab of the Master Panel.
- Move the sample away from the tip by clicking the '(Un)Load Sample' button on the Engage Panel.
- Remove the cantilever holder from the scanner.



2.

Turn the controller OFF:

- Turn the controller OFF by pressing the 'Power' button.
- Green LED light will turn OFF.

Note It is important to turn the controller off when unplugging/re-connecting the VRS stage from the high voltage applications module/connector.

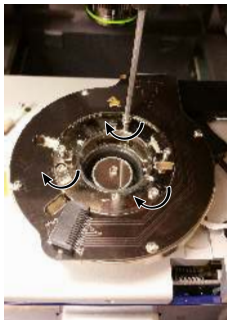
3.

Unplug the VRS stage:

- *Gently* pull on the connector to unplug the VRS stage.

4. **Remove the VRS stage from the scanner:**

- Unscrew the three (3) screws on top of the stage, turning counterclockwise.
- Unlock the VRS sample stage from the scanner by turning the set screw counterclockwise three full turns.
- *Slowly* remove the cell body from the scanner.

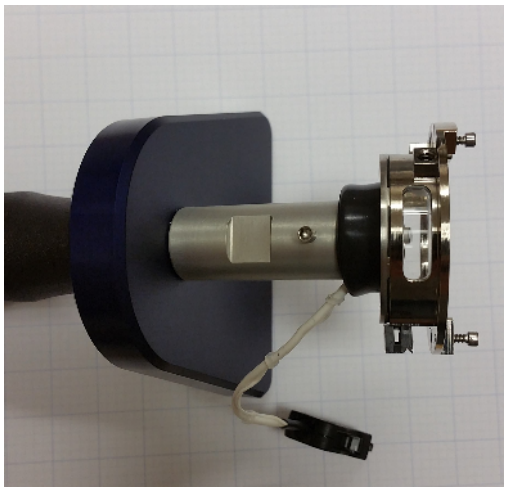


5. **Locate the VRS sample stage holder:**

- The holder accommodates the dovetail of the VRS stage, similar to what is on the scanner.
- It also has the same set screw lock-in mechanism to secure the stage.

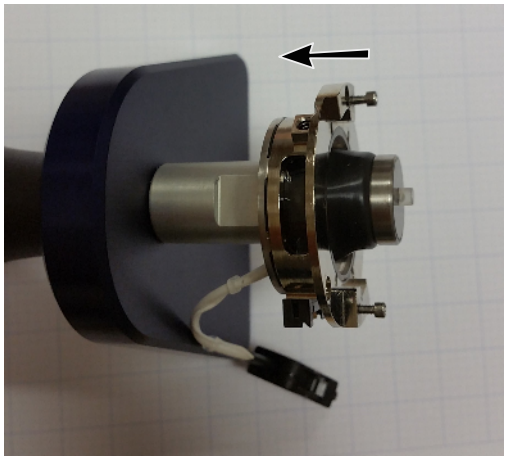
6. **Place the VRS stage on the holder:**

- Place the stage on the holder.
- *Gently* tighten the screw so that the stage is firmly attached to the holder.



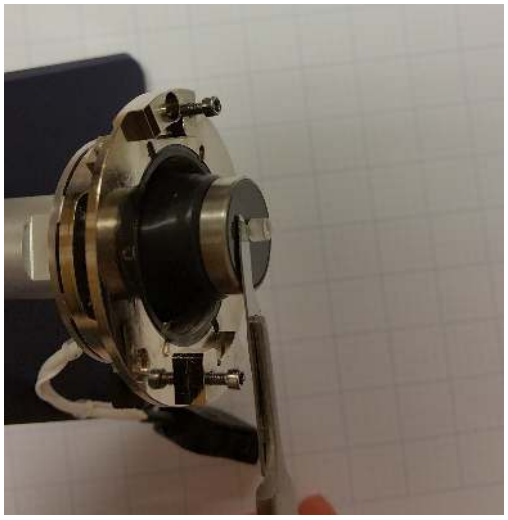
7. **Expose the sample stage:**

- Pull the cell body away from the sample stage.



8. **Cleave off the sapphire post:**

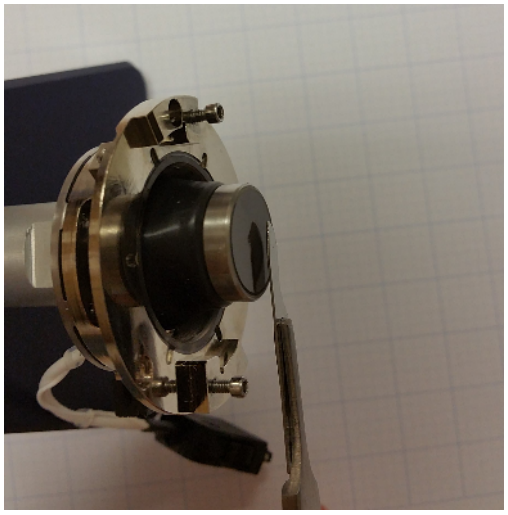
- Using a scalpel or a blade, place the blade parallel to the sample stage and apply pressure.
- The post should pop off.



9. **Clean off residual glue:**

- Scrape any residual glue off the sample stage.

  Note The sample stage is made of sapphire so do not worry about scratching it.
- Clean the surface using a Q-tip moistened with isopropyl alcohol or ethanol.
- Remove the stage from the sample stage holder.



10. **Refold the cell body membrane:**

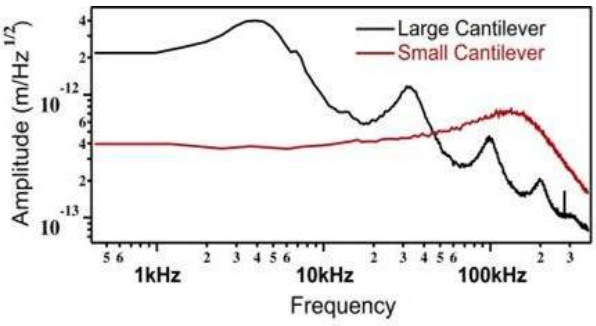
- Grab onto the bottom/dovetail of the VRS stage.
- Pull on the dovetail and extend the cell body membrane. Move it back and forth until the membrane folds into place and looks like the image at right.

11. **Reinstall the VRS sample stage onto the scanner:**

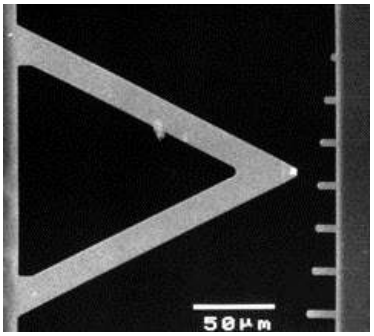
- Lock the stage in place by turning the set screw clockwise in the front of the scanner (finger tight).
- Tighten the three (3) screws on top of the cell by turning them clockwise.
- Plug the VRS sample stage to the applications module/connector on the top, right-back of the scanner.
- Turn the controller ON.
- Follow the steps in the Chapter 30 on page 356 (Cypher VRS Tutorial) to glue a new mica post to the VRS stage.

29.4. Cantilever Selection

Regardless of whether you are imaging in air or liquid, in order to achieve video rate speeds it is necessary to use small cantilevers. Small cantilevers have higher resonance frequencies, which is one of many criteria that allow for higher scan rates (see Figure 29.5 on page 355).



(a) Thermal tunes (left) of large cantilever (black line) vs. small cantilever (red line)



(b) SEM image (right) comparing sizes of small cantilevers relative to a large cantilever

Figure 29.5.: Thermal tunes and SEM image comparing a large cantilever with a small cantilever

Cantilever response (τ) is related to the resonant frequency (f_0) and quality factor (Q) of the cantilever: ($\tau \sim \pi Q/f_0$)

The higher the resonance the faster the response. Similarly, the lower the Q, the faster the cantilever response. In liquid, in spite having a lower resonance frequency the Q is significantly lower, so the response time is higher compared to air.

Typical cantilevers for liquid and air imaging are listed in the table below. To achieve the fastest scan rates/frame rates, select the smallest cantilevers. It is also important to point out that in order to use these smaller cantilevers, the small spot laser module is required.

	Model #	Cantilever Length (µm)	Cantilever Resonance (in air)
Liquid	USC-F0.3-k0.3	20	300
	USC-F1.2-k0.15	7	1200
	USC-F1.5-k0.6	7	1500
	AC10DS	10	1500
Air	FS1500/FS1500Aud	35	2000
	USC-F1.2-k7.3	20	1200
	USC-F2-k3	20	2000
	USC-F5-k30	10	5000

30. Cypher VRS1250 Tutorial

CHAPTER REV. 2427, DATED 08/22/2021, 18:55. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

This tutorial will guide the user though the steps necessary to image a sample in liquid. As an example, we will use DNA immobilized on a mica post. A more thorough description of how to image DNA in liquid can be found in Chapter 31 on page 378.

Prior to starting this tutorial, please read the Environmental Scanner section of the manual, Part III on page 195.

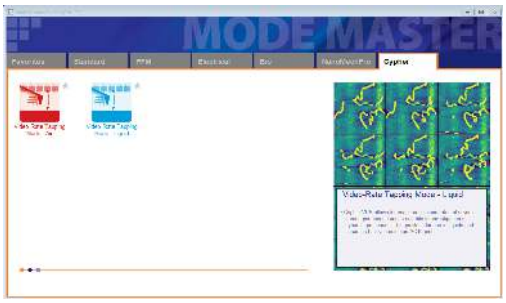
30.1. Mode Master Templates for VRS1250

The Mode Master window:

- When the software opens, the Mode Master window appears. By default, the Standard tab is displayed.
- If not, click the 'Mode Master' button at the bottom of the screen

Select Mode:

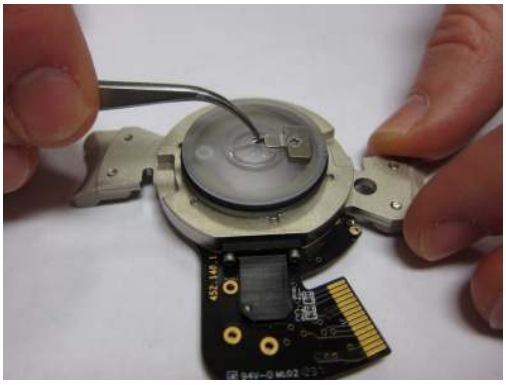
- For VRS1250 operation, click on the last tab, “Cypher”.
- There are two options: “Video-Rate Tapping Mode – Air” and “Video-Rate Tapping Mode – Liquid”. Select the appropriate template depending on whether you are imaging in air or liquid.
- For liquid imaging in this tutorial, we will select “Video-Rate Tapping Mode – Liquid”.

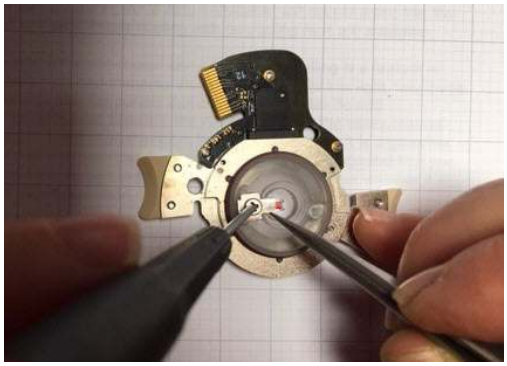


30.2. Loading the Cantilever

The cantilever chip is held in place by a metal clamp. More detailed, step-by-step instructions can be found in Section 18.2 on page 215.

1.

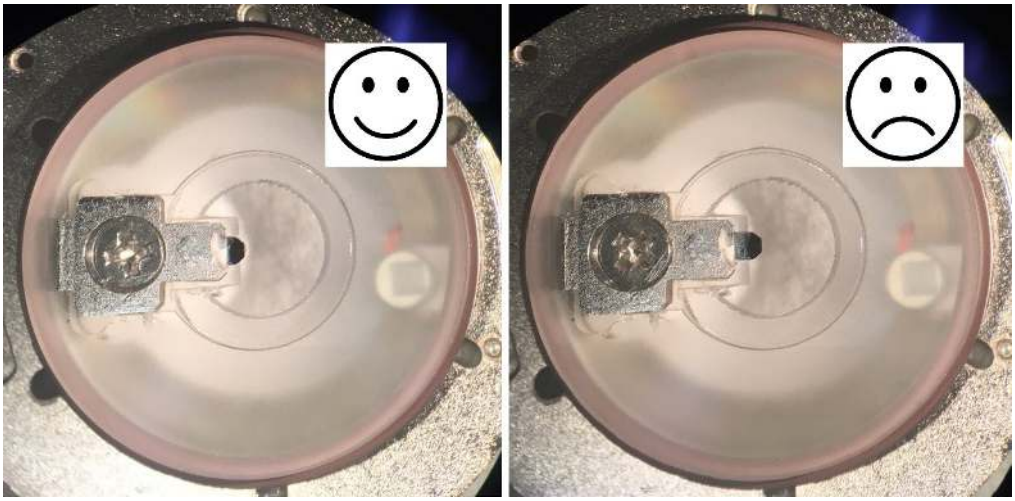




Mount a cantilever:

- Use the Liquid Cantilever holder. (The perfusion holder can also be used if you plan to flow a solution to the sample. Installing a cantilever chip is identical for both cantilever holders.)
- Choose a short probe. (Nanoworld’s USC-F1.2-k0.15 works well for this experiment. Olympus’ AC10DS can also be used; however, they have been recently discontinued so availability is limited.)
- Place the cantilever chip all the way in under the clamp until it reaches a physical stop.
- It is important that the metal clamp is centered in the pocket. If the clamp rotates, use a pair of tweezers at the edge of the clamp to secure it in position while tightening the screw.
- *Gently* tighten the clamp. The clamp should be tight enough to secure the chip when it is nudged from either side.

2.



Check tip position:

- To achieve VRS speeds, it is important that a larger portion of the chip is under the clamp. As a result, the chip is pushed significantly more than normal.
- On the left, the cantilever probe is properly positioned.
- On the right side, the probe is pulled out too far and should be repositioned further under the clamp.

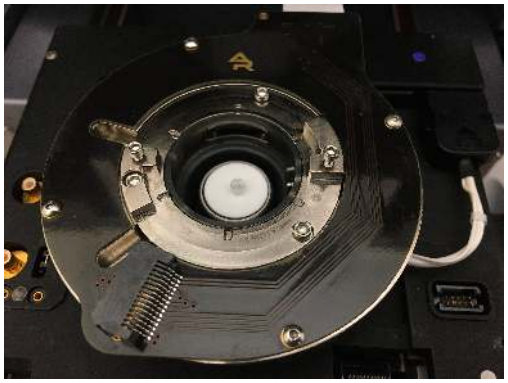
30.3. Mounting Mica Posts on the VRS stage

1.

Glue the mica post to the VRS stage:

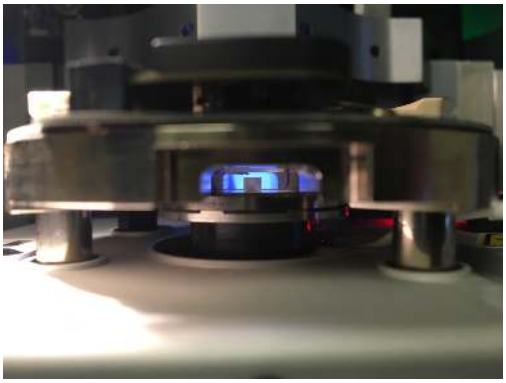
- Prepare 5-minute epoxy by mixing the two components vigorously for at least 1 minute.
- Using a Q-Tip, dab a small droplet of epoxy onto the center of the stage.
- Using tweezers, place the post in the epoxy and gently push it down, securing it firmly and flush with the stage surface.

Note It is helpful to grab the post and move it in a circular pattern to properly seat it onto the stage.



Warning	It is crucial for the probe to be aligned near the edge of mica post! If the probe is not near the edge, you will not be able to image at the highest scan rates.

2.



Align the Post with the Cantilever:

- It is important to do this step before the epoxy completely cures so you only have a few minutes to complete this step.
- RAISE THE COARSE ENGAGE STAGE by turning the Engage Control knob CLOCKWISE. Raise the stage until it reaches its upper limit of travel.
- Carefully place the cantilever holder mounted with a cantilever on the scanner. Although you are at the upper limit, check to ensure that the tip/cantilever holder doesn't crash into the mica post.
- First, look through the cell body window from the front of the scanner so that you can see the position of the cantilever relative to the post (top). If it helps and there is sufficient distance, slowly lower the cantilever using the Engage Control knob until the cantilever is several millimeters above the post.
- Next, look through the top glass window of the cantilever holder to check the front-to-back alignment of the cantilever relative to the post (middle).
- If the chip is not centered (front-to-back) and is not near the edge of the mica post, as seen in the bottom schematic, take the cantilever holder off, realign the post, place the holder back on the scanner, and recheck for the alignment.
- Repeat as needed until the cantilever is properly centered (front-to-back) and near the left edge of the mica post.
- Let the epoxy cure for approximately 5-10 minutes.

30.4. Sample Preparation

1.

DNA deposition on the mica:

- Cleave a layer of mica with adhesive tape to expose a fresh mica surface.
- Place ~15 µL of 1 µg/ml DNA solution onto mica.
  - For DNA to attach to the negatively-charged mica surface, it is important to dilute the DNA in a buffer containing divalent cations such as $NiCl_2$ or $MgCl_2$. For this experiment the DNA was prepared in 5mM $NiCl_2$ (plus 40mM HEPES, pH 6.6).
- Incubate the DNA for 5-10 minutes.
- Rinse the sample by removing and adding a total of ~45-90 µL of buffer or pure water (~ 3-6 pipets worth).

Note To prevent any contamination, keep the liquid on top of the mica so that it does not touch the sides of the glass post or the VRS stage.

2.

Pre-wet the cantilever and install the cantilever holder:

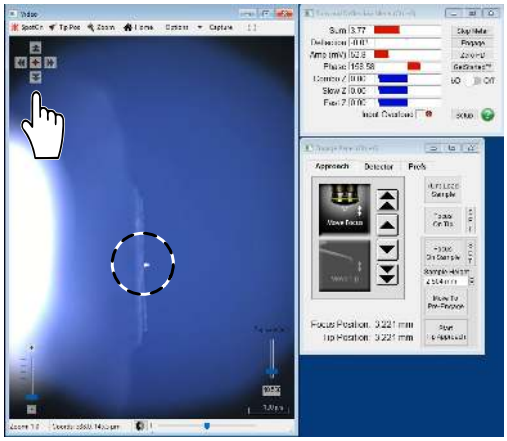
- Add a droplet of liquid (buffer or pure water) to the cantilever.
- Place the cantilever holder in the cell body.
- As the cantilever holder is lowered, the droplet on the cantilever will merge with the sample droplet.
- Firmly seat the holder in the cell body before tightening the two screws to secure it into position.
- If not already done, set the correction collar on the objective to “2”.

30.5. Align the Laser and Focus on the Sample

1.

Align the laser:

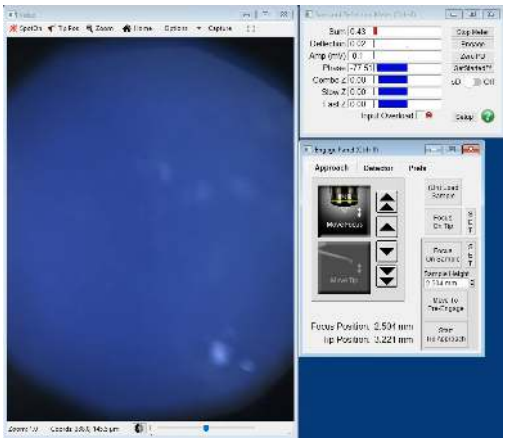
- Use the controls of the Engage Panel to locate and bring the cantilever into focus.
- Click ‘SET’.
- In the Video window, place the cursor on the cantilever and right-click to select *SpotOn*.
- The Sum should be between 3-6 V.
- For smaller steps during alignment, hold the Shift key when pressing laser controls in the Video window.
  - USC-F1.2-k0.15 and AC10DS cantilevers are 10 µm long and might be difficult to locate the first time.



2.

Focus on the sample:

- Use the controls of the Engage Panel to locate and bring the sample into focus.
- Typically for mica, it is difficult to see any features on the surface, unless there is a crack or defect in the mica.
- To overcome this challenge:
  - First decrease the aperture diaphragm to reduce the amount of light reaching the surface.
  - Second, decrease the field diaphragm until you can see the edge of the aperture in the video.
- Adjust the focus until the aperture is in focus (typically ~ 30um from the surface). For safety purposes, use this as your sample height.
- Once the surface has been located, click 'SET'.

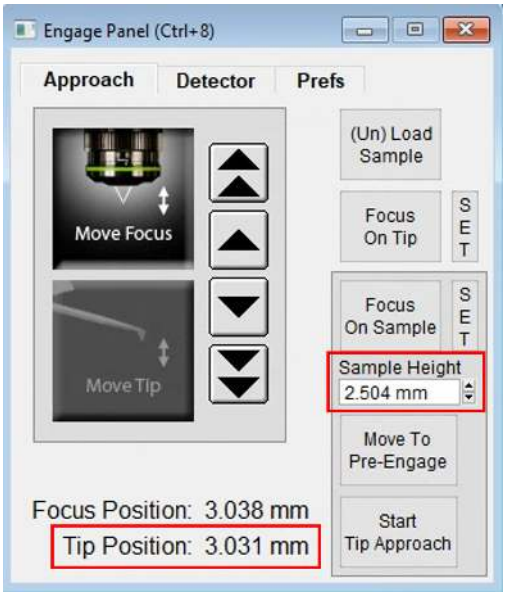


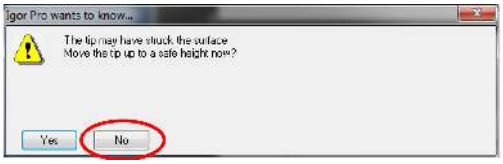
30.6. Move to the Pre-Engage Position

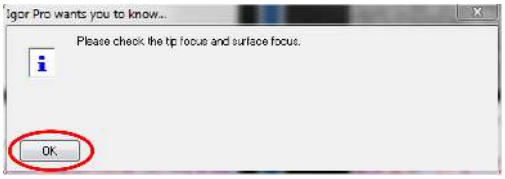
1.

Bring the sample close to the probe:

- In most cases, the tip is significantly above the sample surface. In this case, the tip is only ~ 500um above the surface. But, in many cases, it can be over 1mm from the surface.
- You can click the 'Move to Pre-Engage' button, but you will most likely encounter a couple of warnings alerting you that the tip may have struck the surface and that you should check the tip and sample focuses, as shown below. This is not usually the case. In reality, the laser spot moved off the cantilever, causing the SUM to drop.
- Click 'No' when prompted to "Move the tip up to a safe height now?", as shown below.



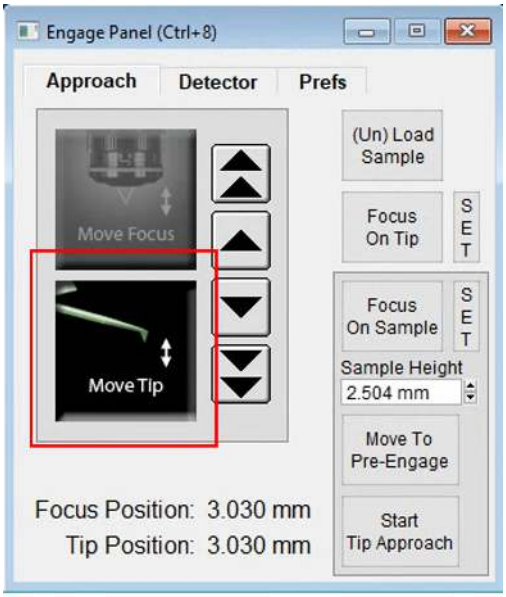


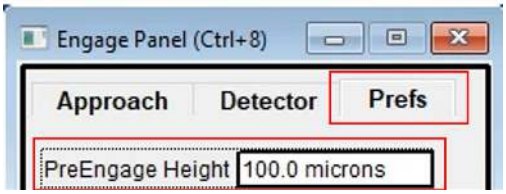


2.

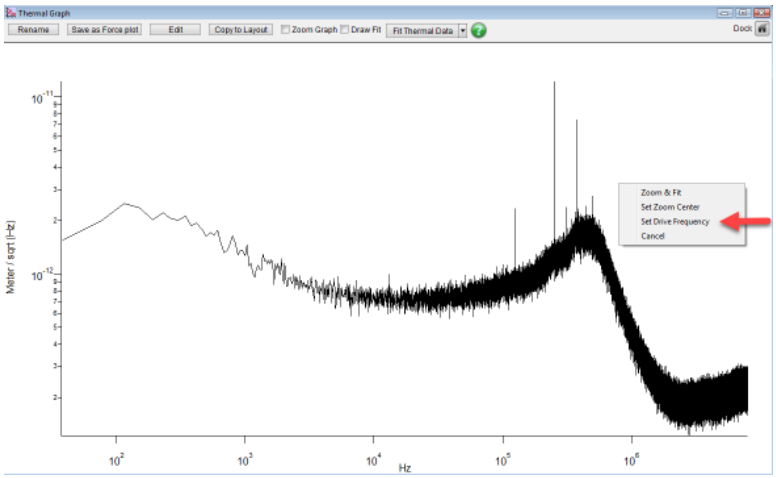
Move the sample using the motors:

- Instead of using the 'Pre-Engage' button, select the 'Move Tip' box, and slowly move the sample closer to the tip, ideally 100-200um away from the tip.
- Realign the red laser on the cantilever tip to maximize the SUM.
- Now that the cantilever is much closer, and the laser will most likely not fall off the cantilever during the Z movement, click the 'Move to Pre-Engage' button.
- If crashing the tip is a concern, increase the 'Pre-Engage Height' by entering "100.0 microns", located in the Prefs tab as shown at right. The default is 50 microns.





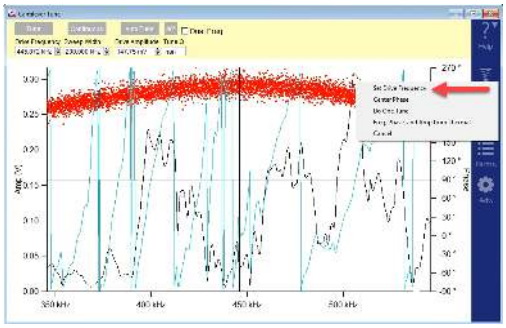
30.7. Tune the Cantilever

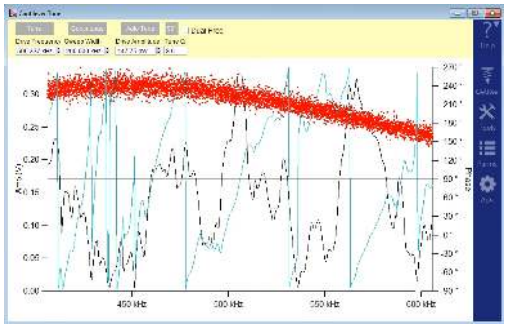
1. 

Capture a thermal: For either the USF-F1.2-k0.15 or AC10DS probes.

- In the Master Panel, click the 'Thermal' icon.
- Click 'Capture Thermal Data'.
- Acquire ~ 250 samples.
- Click 'Stop Capture Data'.
- In the Thermal Graph, place the cursor over the resonance peak.
- Right-click and select *Set Drive Frequency*.
  - For both the USC-F1.2-k0.15 and the AC10DS cantilever, the frequency should be 400-600kHz in liquid.
- If using the USC-F1.2-k0.15 cantilever, continue to step 2.
- If using the AC10DS cantilever, skip step 2 and follow steps 3 and 4.

2.



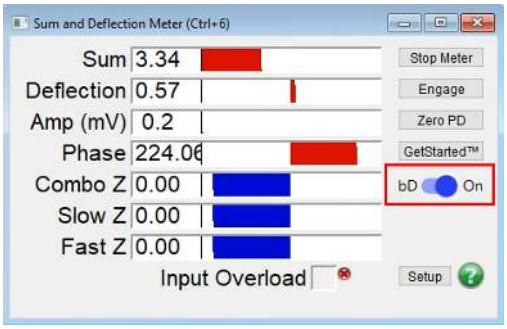


Manual tune the USC-F1.2-k0.15 probe using piezo drive:

Note These steps also apply to the slightly stiffer USC-F1.5-k0.6 probe.

- For the USC-F1.2-k0.15 probe, use piezo drive to excite the cantilever.
- In the Master Panel, click the 'Tune' icon.
- 'Drive Frequency' was pre-set from the Thermal Tune and is currently at ~450 kHz. (This may or may not correspond to an actual driven peak.)
- Set 'Sweep Width' to 200 kHz.
- Click 'One Tune' (top).
- Look for the largest peak under the Thermal, right-click on it to select *Set Drive Frequency* (bottom).
- Adjust the 'Drive Amplitude' until the Free Amplitude is 500 mV.

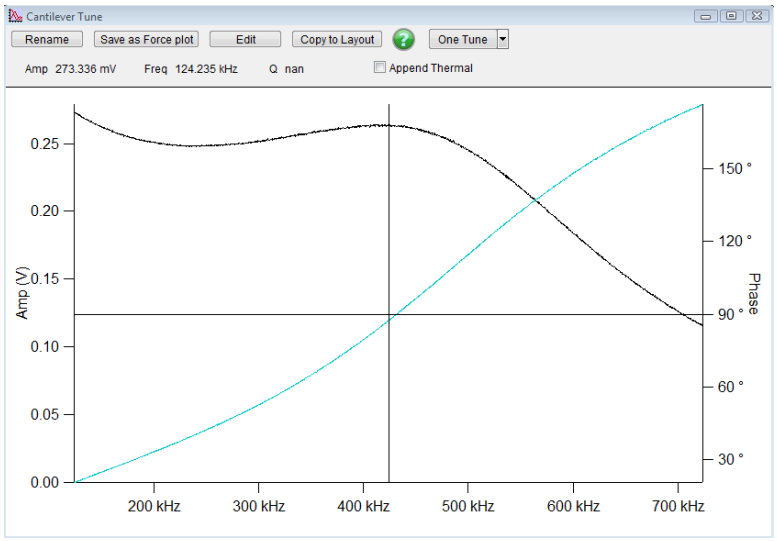
3.



Turn ON and align the blueDrive laser on the AC10DS probe:

- Since the AC10DS probe has a complete gold coating along the reflective side of the cantilever, blueDrive can be used to drive the cantilever.
- Place the 0.1x filter cube in the laser path.
- In the Sum and Deflection Meter panel, turn on blueDrive by moving the slider from the left to the right; the button turns blue when blueDrive is enabled, and the unit the Drive Amplitude parameter should now be in Watts.
- Controls for blueDrive alignment should now be visible in the top right corner of the Video window.
- Place the cursor on the cantilever, right-click and select *Blue SpotON* to align the blue laser on the cantilever.
- Move the blue laser around the base of the cantilever to maximize Amp value visible in the Sum and Deflection Meter panel.

4.

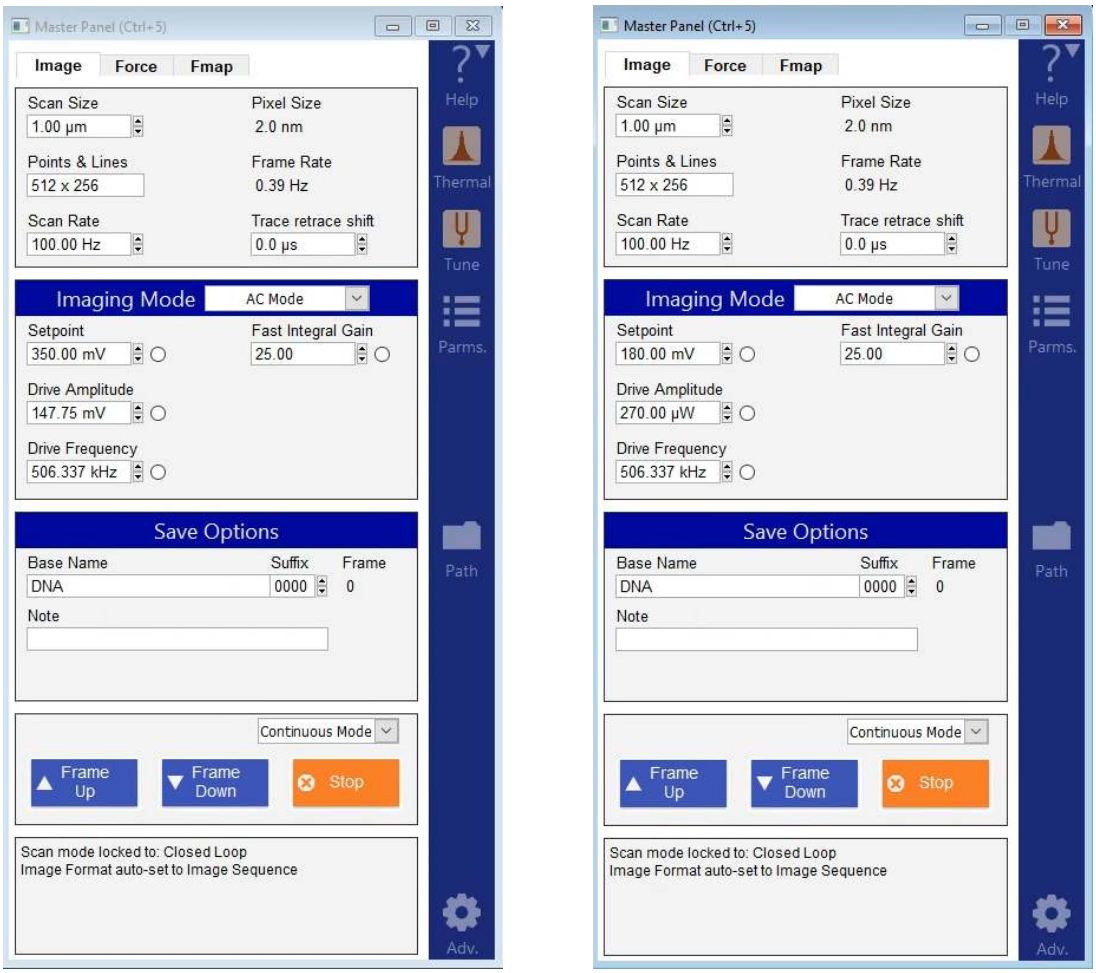


Manual tune the SC-F1.2-k0.15 probe using blueDrive:

- In Master Panel, select the 'Tune' icon.
- 'Drive Frequency' should be set to "500 kHz" (from Thermal Data).
- Set 'Sweep Width' to "400 kHz".
- Click 'One Tune'.
- The Tune Graph should show a clean broad peak at ~500 kHz.
- Adjust 'Drive Amplitude' until Amp on the Sum and Deflection Meter panel indicates 250 mV, typically the maximum value for this blueDrive filter cube.

30.8. Approaching the Sample

1.



Approach parameters: In the Master Panel, on the Image tab, set the parameters to:

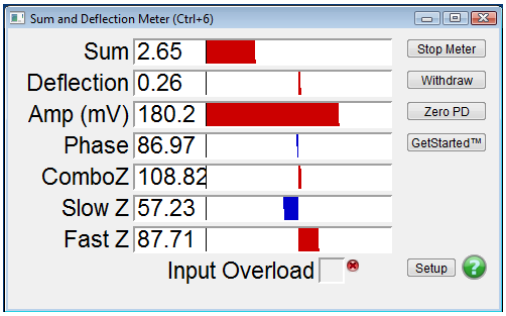
- Scan Size: 1 um
- Points & Lines: 512 x 256
- Scan Rate: 100 Hz
- Fast Integral Gain: 25
- Set Point:
  - Top left: USC-F1.2-k0.15: 350 mV (Free – Amplitude = 400 mV)
  - Top right: AC10DS: 180 mV (Free Amplitude = – 250 mV)

2.

Approach tip:

- During the approach, the standard Z piezo inside the Scanner (i.e., the "Slow" Z) is used to move the sample towards the tip.
- Since the cantilever is small and in liquid, the approach parameters under "Prefs" have been optimized to minimize fluctuations in the free amplitude, resulting in a stable approach:
  - Step size: 3µm
  - Engage approach speed: slower

3.

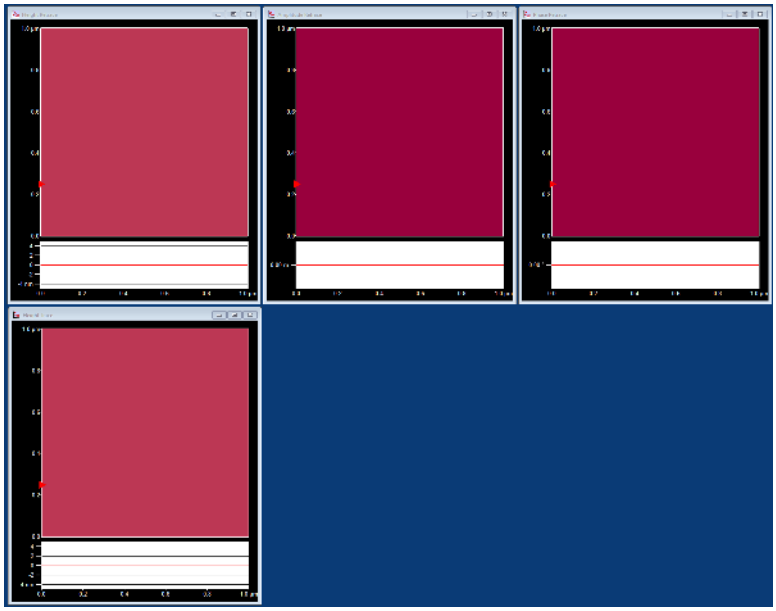


Engage:

- When the Set Point is reached, click 'Engage'.
  - Initially, the Combo Z/ Slow Z start to extend but quickly withdraw. Next the Fast Z extends and locks at 80V. Combo Z/Slow Z extend again until the surface/setpoint is reached. At this point the feedback is transferred to the Fast Z.
  - Adjusting the setpoint will affect the Fast Z position only.
- Using the AC10DS as an example, if the tip is at the surface, the Sum and Deflection Meter will read:
  - Amp (mV): 180
  - Combo Z, Slow Z, and Fast Z should be somewhere in the middle of their ranges.
- Decrease the setpoint until the Fast Z hits a hard stop – the tip is now in good contact with the surface.
- If either Combo Z, Slow Z, or Fast Z are out of range, withdraw and do this:
  - Realign laser.
  - Set 'Drive Amplitude' to "250 mV".
  - Click 'Start Tip Approach'.
- When the Set Point is reached, click 'Engage' and decrease the setpoint as above to determine if the tip has made contact with the surface.

30.9. Imaging Settings

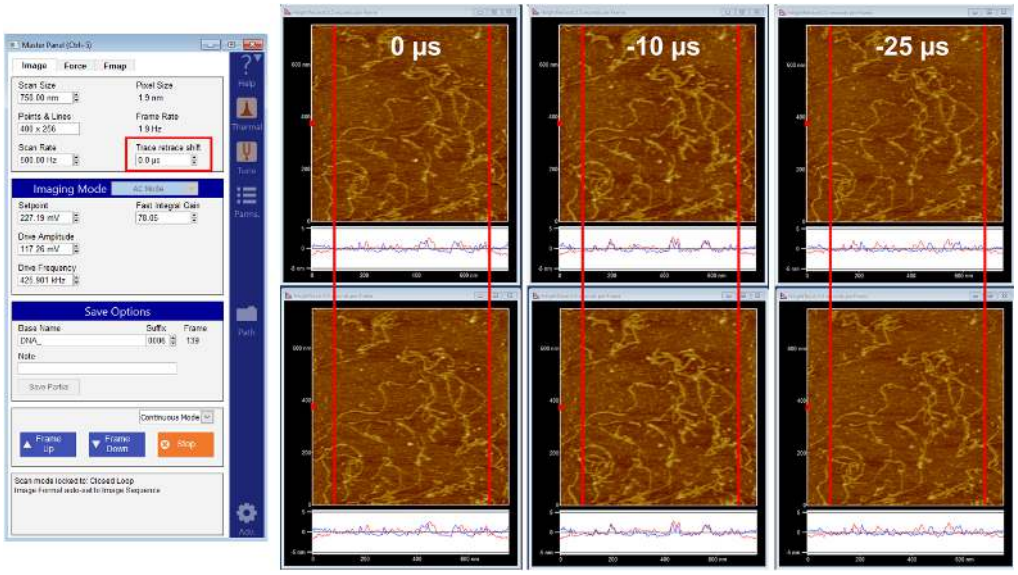
1.



Start Scanning:

- The VRS Mode Master templates include both the Height Retrace and Height Trace channels. Since there is a new adjustment at the highest scan rates that align the same feature in both of these channels, ideally these two channels should be arranged one above the other.
- Click ‘Frame Up’ or ‘Frame Down’.
- Adjust ‘Setpoint’ until trace and retrace overlap.
- Increase ‘Fast Integral Gain’ to improve image quality:
  - If the mica post has been well immobilized on the VRS sample stage, the Fast Integral Gain can be increased greater than the default value of 25.
  - If the Fast Integral Gain value is low (20-30) and ringing is visible, consider remounting your sample.

2.



Adjust the 'Trace retrace shift' parameter:

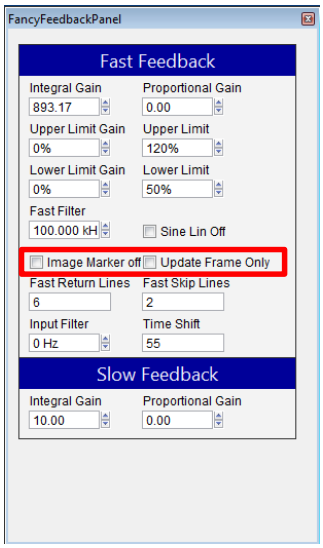
- With sinusoidal scanning (scan rates > 100 Hz), the tip movement is not constant across the scan line.
  - The tip moves faster at the edges, so features look sharper and somewhat compressed.
  - The tip moves slower in the middle, so features are not as sharp and slightly stretched.
- To compensate, increasing/decreasing this parameter aligns the features so that they appear identical in the two traces.
  - At 500 Hz, the DNA molecules in the Retrace and Trace channels do not correctly align. Two red vertical lines have been drawn to help see this.
  - With -10us 'Trace retrace shift', the molecules are properly aligned. The Trace and Retrace lines in the Scope Trace are also perfectly overlapping.
- With -25us, this value is too aggressive, and the molecules are again no long aligned.

3.

Adjust Scan Rate and Scan Lines:

- To increase the Frame Rate, do one or both of the following:
  - Increase the Scan Rate (Maximum = 1250 Hz).
  - Decrease the number of Scan Lines (Minimum = 16).

4.



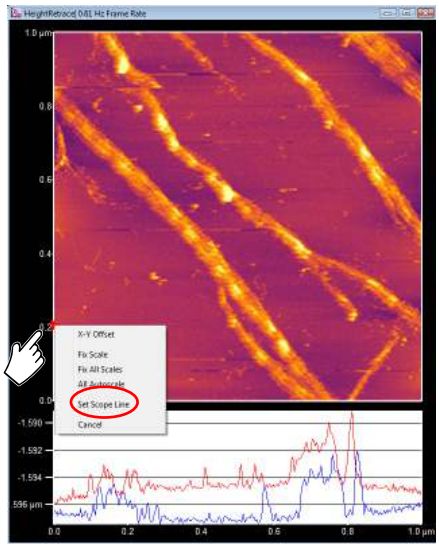


Image and Scope update:

- Images are updated line by line up to 1 Hz frame rate.
- When imaging at a Frame Rate of 1 Hz and higher, images are automatically set to update every frame.
- Red Image Marker on the left side of the image moves continuously to show where the tip is scanning.
- When images are updated every frame, the position of the Image Marker is static and can be set by the user as follows:
  - Right-click on the position where the marker should be placed.
  - Set Scope Line.

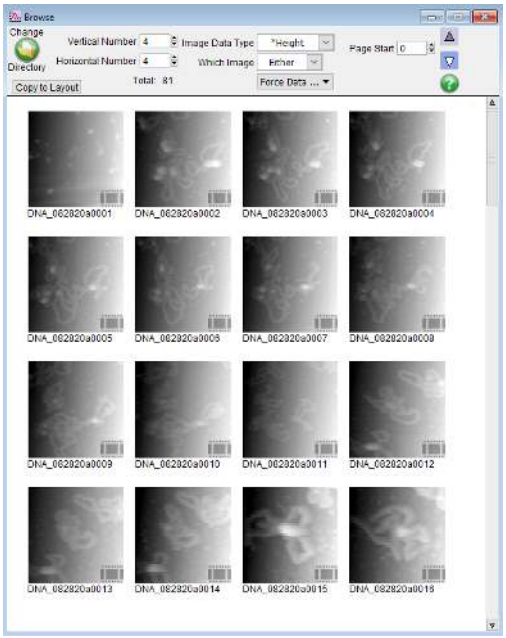
30.10. Data Analysis

Data is acquired in **A**sylum **R**esearch **I**mage **S**equence format (file extension ARIS). Each file contains all the individual frames that were acquired for that ARIS file. ARIS files can be opened directly from the data browser.

1.

Opening Saved Files:

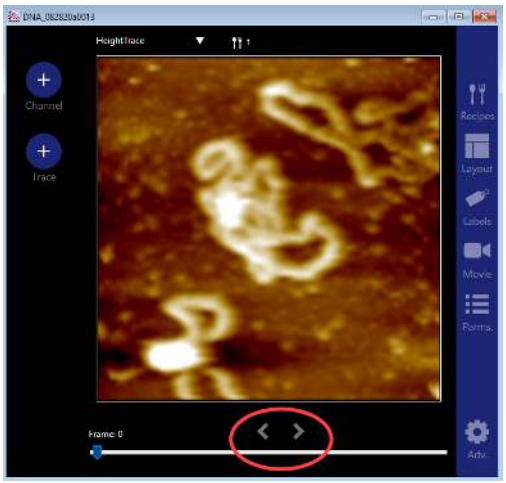
- From AFM Analysis, select *Browse Saved Data*.
- Browser window should open with thumbnails of all the saved files.
- To open a file, simply double-click on a thumbnail that has a filmstrip on it.



2.

Offline Movie Viewer:

- The file opens at the first frame.
- You can step through the frames by clicking on the arrows below the image (slow).
- You can also grab the bar in the slider to move it to a specific region/frame; or click on a particular region of the slider, and the software will automatically go there.

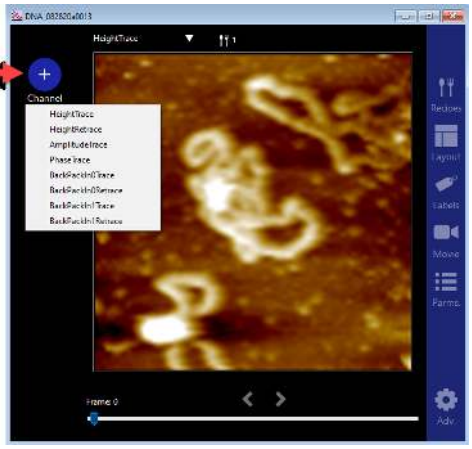


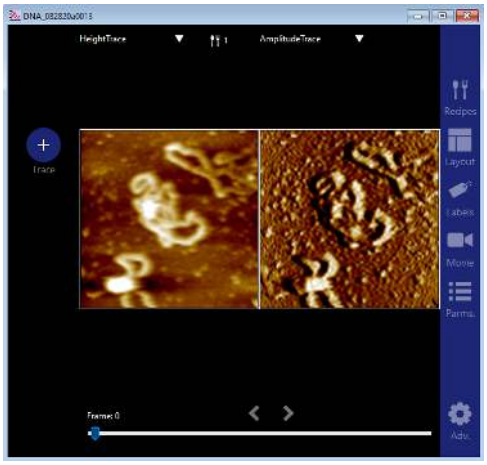
3.

Adjusting Data Scale:

- By clicking anywhere within the frame, a hidden section with options appears.
- This section contains the color map, data scale range, and data offset.
- There is also an option, called “Auto”, which when selected tells the software to automatically adjust the data range and scale for each frame. (This can be useful if your sample is growing or receding over the course of the image frames.)
- To hide this section, click anywhere outside of the image frame.

4.

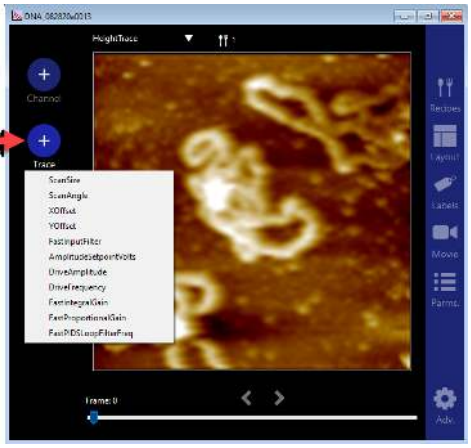


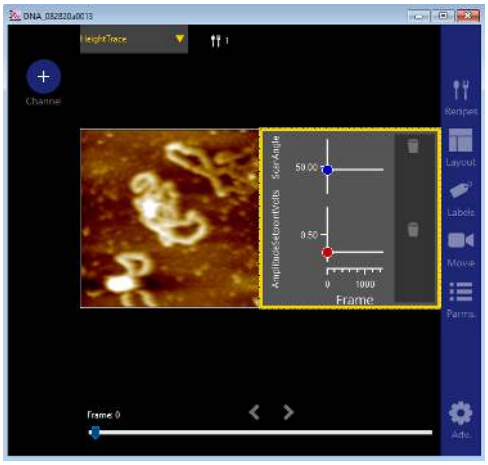


Channel button:

- This button allows you to add channels that were acquired during imaging.
- In this case, it is the Amplitude image; other channels, including Phase, are available.
- To close one of the channels, click anywhere within the image frame to display the hidden section, and then click the Trash icon located on the far upper-right corner.

5.

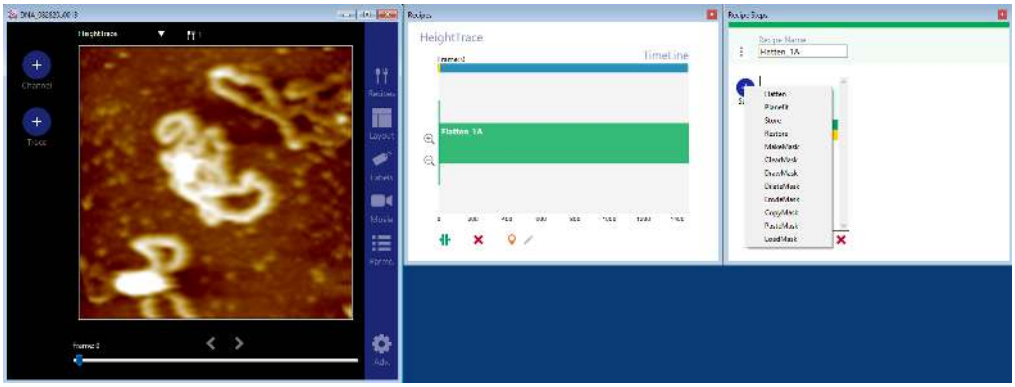




Trace button:

- This button allows you to add supplemental information collected during imaging.
- If collected, choices include the following:
  - Scan Size
  - X and Y offsets
  - Filter inputs
  - Feedback Filter Bandwidth
  - Amplitude Setpoint Volts
  - Drive Amplitude
  - Drive Frequency
  - Fast Integral Gain
- A maximum of three choices can be displayed at a time.
- To hide the additional information, click on the 'Trash' icon located on the far upper-right corner.

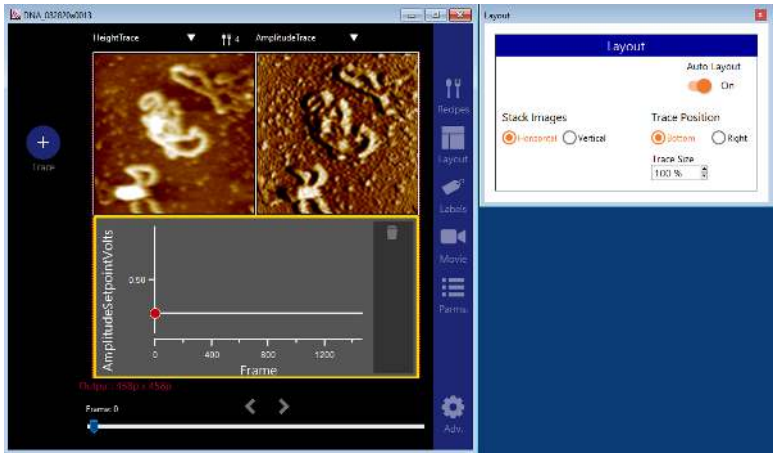
6.



Recipes:

- Recipes allow for batch modification of frames.
- Recipes include the following functions that can be used together:
  - Flatten
  - Planefit
  - Store
  - Restore
  - Mask (includes several options, such as MakeMask, ClearMask, DrawMask, DilateMask, ErodeMask, CopyMask, PasteMask, LoadMask)
- You can choose to apply a recipe with a given number of modification functions on either all or chosen frames.
- Recipes can be saved (and later loaded) in the software.

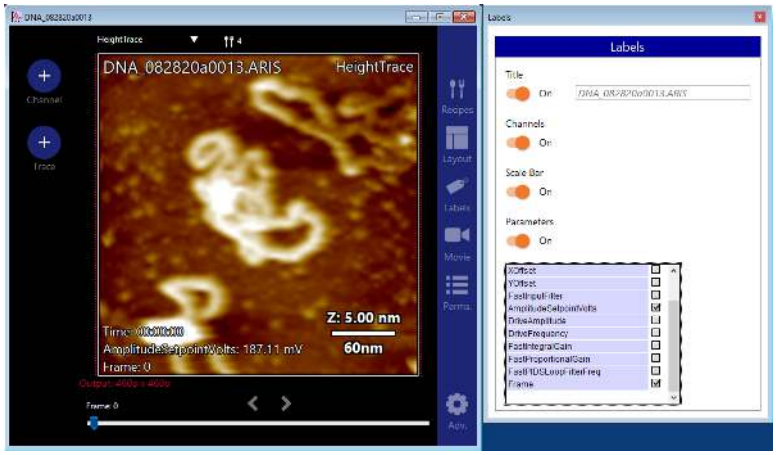
7.



Layout:

- Layout lets you choose the display of the two or three (maximum) channels that are being viewed.
- By default, (when Auto Layout is set to ‘On”), images are Horizontally Stacked. Traces are either on the right (if there are only 2 items selected) or on the bottom (when 3 items are selected).

8.



Labels:

- Labels allow you to add the following to the image:
  - Title (image filename)
  - Channels (HeightTrace, HeightRetrace, AmplitudeTrace, etc.)
  - Scale Bar (Z and XY)
  - Parameters, some included are:
    - Time
    - Scan Size
    - Scan Angle
    - AmplitudeSetPointVolts
    - Frame

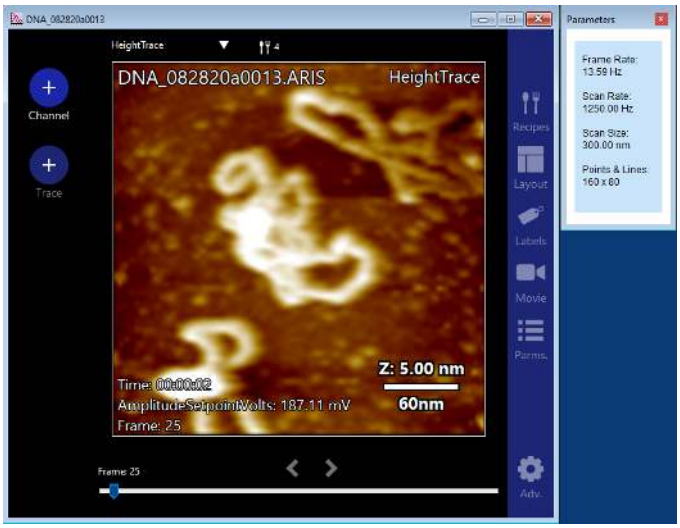
Movie:

9.

- Capturing numerous frames into one ARIS file allows you to easily create a movie of all the frames.
- There are several important options when making a movie, including:
  - Which frame to start and end with. For example, you may want to exclude the first few frames if the setpoint wasn't optimized, and, as a result, the tip wasn't tracking the surface.
  - Which frames to skip. There might be scans where the tip got stuck onto the surface.
  - Playback Rate/ Total Time. These two parameters are somewhat tied together. Movies can be played back at Realtime (1X), faster, or slower. If there are a lot of frames, you may want to increase the Playback Rate which will automatically decrease the Total Time).

    Note If you increase the Playback Rate too much, you may get a warning that the Playback rate is too high, and not all data frames will be included.
- Other options include:
  - Movie Size ("Size To Window" is the default; however, other options are available in the drop-down menu.)
  - Output type (mp4 or wmv)

10.



Parameters:

- "Parms." is short for Parameters.
- When selected, parameters relating to the movie appear beside the image window, including:
  - Frame Rate
  - Scan Rate
  - Scan Size
  - Points & Lines

11.

Advanced:

- "Adv." is short for Advanced.
- Popout Frame:
  - Extracts a frame out of the ARIS file.
  - This frame can be processed and saved as an .ibw file using the List Panel. Use 'Save to Disk' or 'Save As to Disk'.
- Export Graphic saves the frame as a graphics .png file

31. Cypher VRS Experimental Protocols

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

31.1. Imaging DNA in Liquid

The goal of this experiment is to image individual DNA molecules immobilized onto a mica surface. Depending on the buffer composition, DNA molecules can be loosely bound or well-attached to the mica. For experiments looking at the interaction of molecules (i.e., proteins) with DNA, loosely bound DNA is preferred since it allows the DNA to interact with molecules floating in the buffer. If you are mainly interested in imaging DNA by itself (as in this experiment), then firmly attaching the DNA to the mica surface is ideal.

Background Information: Commercially available DNA is readily available and can be purchased from several vendors, such as Sigma (Lambda Digest, catalog # D9780) or New England Biolabs (puc19 Plasmid DNA, catalog # N3041). Mica is the ideal substrate for DNA since it is atomically flat and has an overall negative charge in liquid. Although the DNA is also negatively charged, the presence of divalent cations, such as Ni2+, in the buffer bridges the two negatively charged species resulting in a firm attachment of DNA molecules onto the mica surface.

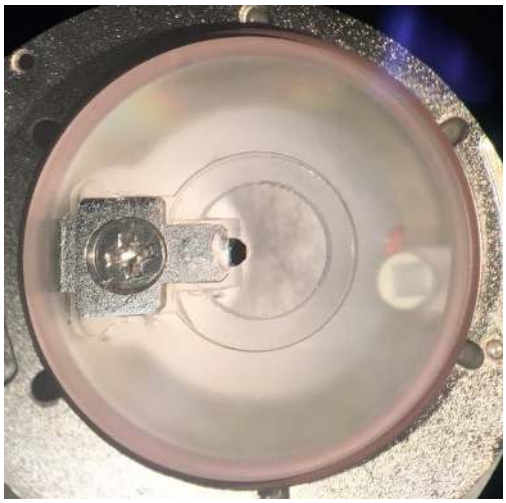
Materials Required:

- 40 mM HEPES/ 5 mM $NiCl_2$ buffer pH ~ 6.6
- DNA solution, 1 μg/ml diluted in the $NiCl_2$ buffer
- USC-F1.5-k0.6 (k = 0.6 N/m) or USC-1.2-k0.15 (k = 0.15 N/m)
- ES/VRS liquid holder
- Mica posts (Refer to Section 29.2 on page 348 for instructions on how to prepare mica posts.)
- Pure water (Millepore grade or equivalent)

31.1.1. Methods

1. **Place cantilever in holder:**

   - Place a USC cantilever in the holder.
   - Make sure that the chip is sitting as far as possible into the pocket.

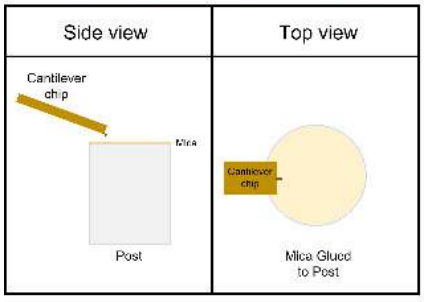


2. **Glue a post to the VRS stage:** Glue a fresh, clean mica post to the sapphire surface of the VRS stage using Devcon 5-minute epoxy.

   - The mica surface can be cleaned before and/or after it has been glued to the stage by gently rinsing with pure IPA or ethanol. Blow dry with air.

     Note Using a cleaning duster, such as Dust Off Compressed Gas Disposable Cleaning Duster, is sufficient to dry the surface.

   - Refer to 29.2 for detailed instructions on how to glue the mica post.
     - Make sure to align the mica post relative to the cantilever chip so that the tip will engage near the left edge of the mica surface.



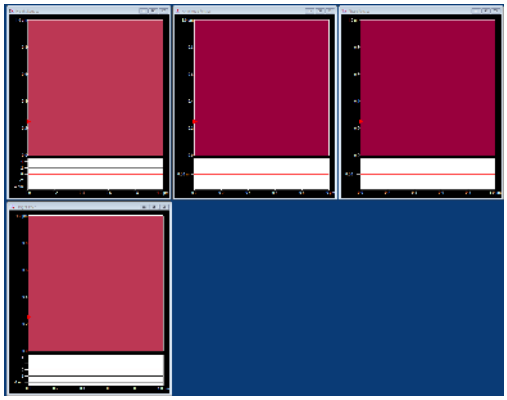
3. **Deposit DNA solution to mica surface:** Deposit 15 µl of the DNA solution (1 µg/ml) to the cleaved mica surface.

- As stock DNA solution from the vendor is typically very concentrated (>500 µg/ml), serial dilute from the concentrated solution to ensure that the 1_2g/ml solution is consistent.
- We recommend first preparing stock solution #1 at 100ug/ml, then stock solution #2 at 5_2g/ml, and then finally the imaging solution of 1ug/ml.

4. **Incubate the DNA solution:**

- Incubate the DNA solution for 5-10 minutes.
- Since the volume is quite small, cover the cell body to prevent evaporation. The lid of a petri dish works very well.

5.



Open the Igor software:

- While waiting for the DNA to attach, open the Igor software.
- Load the "Video-Rate Tapping Mode – Liquid" Mode Master template
- Arrange the real-time windows so that Height Retrace and Height Trace are aligned vertically.

6. **Rinse with buffer or pure water:**

- Try to avoid spilling the liquid over the edge of the sapphire post and onto the VRS stage as this may contaminate your sample.
- Using a pipet, first remove ~ ½ of the liquid, then deposit an equal amount of pure water. Gently rinse by pipetting off and then depositing the water back onto the sample. Do this a couple of times before removing ~1/2 of the liquid. Add more water and repeat this step 2-3 more times.

7. **Pre-wet the cantilever:**

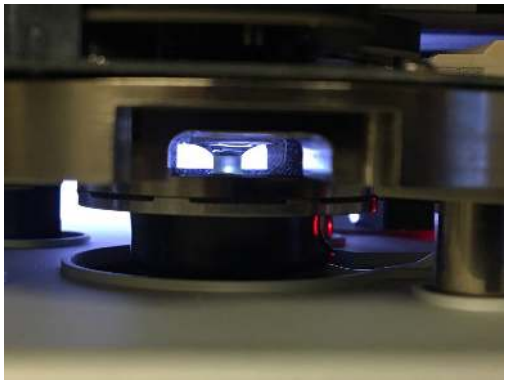
- Add a drop (~15µl) of buffer/water to the cantilever chip.
- Make sure not to touch the cantilever with the pipet tip as you may break it.

8. **Place the holder onto the VRS stage:**

- Make sure that the coarse engage stage is raised to its highest position so that the sample is at its bottom-most position.
- Secure the holder by tightening the two clamping screws.

9. **Bring the sample close to the cantilever:**

- Look through the cell body window
- If the droplet on the cantilever has not yet merged with the droplet on the sample, then lower the cantilever down until the two droplets merge using the Engage Control knob.
- Try to keep the liquid on the mica post to prevent any contamination that might be on either the edge of the post or on the sapphire stage surface.



10. **Approach the cantilever towards the surface:**

- Align the laser.
- Move to Pre-Engage position.
- Tune the USC cantilever using piezo drive:
  - Use a target Free Amplitude = 400 mV
  - Use an Amplitude Setpoint = 350 mV
- Enter the following values for these parameters.
  - Scan Size = 1-2 μm
  - Fast Integral Gain = 25 (default)
  - Points & Lines = 512 x256
  - Scan Rate = 100 Hz (Frame rate = 0.39 FPS)

For more detailed instructions for each step, review Section 30.5 on page 360 through Section 30.8 on page 367 in the Chapter 30 on page 356.

11. **Scan the surface:**

- Once engaged onto the surface, start scanning the surface at the slow rate of 100 Hz.
- Verify that the tip is on the surface and did not false engage by clicking the 'Engage' button.
- Once the Fast Z voltage is extended on the surface, adjust the Setpoint to confirm that the Fast Z doesn't change. If it increases, continue to decrease the setpoint until the Fast Z stops moving.

12. **Optimize the imaging parameters:**

- Increase the 'Fast Integral Gain' and decrease 'Amplitude Setpoint'.

13.

Scan Angle:

- The default scan angle is 45 degrees. This can still be changed, but there are limits: only 10-80 degrees and 110-170 degrees.

14.

Trace/Retrace phase shift:

- There is a new parameter unique to VRS1250.
- At scan rates greater than 100Hz, the scan shape changes from Triangular to Sinusoidal.
- With Sinusoidal scanning, the scan movement is faster at the edges, so the features look slightly compressed and sharper. In the center of the scan, the movement is slower, so the features look blurrier and more stretched. This difference becomes more pronounced at the highest scan rates. As a result, the trace and retrace stop overlapping. To correct for the mismatch and minimize distortion, adjust the T/R phase shift value until the trace and retrace are overlapping. Make sure to select a molecule that isn't moving.

15.

Increase the frame rate:

- Increase the frame rate by either increasing the Scan Rate and/or decreasing the Line Rate (i.e., # of lines). As the scan rate increases, there are limits to the scan size and points per line.
- Refer to the table at right for details.

Scan Rate (lines/s, Hz)	Max Scan Size (µm)	Max Points per Line
641	2	312
658	2	304
694	1	288
735	1	272
758	1	264
781	0.75	256
833	0.75	240
893	0.75	224
926	0.75	26
962	0.75	208
1042	0.5	192
1136	0.5	176
1250	0.5	160

16.

Image Processing:

- There is a new parameter unique to VRS1250.
- At scan rates greater than 100Hz, the scan shape changes from Triangular to Sinusoidal.
- With Sinusoidal scanning, the scan movement is faster at the edges, so the features look slightly compressed and sharper. In the center of the scan, the movement is slower, so the features look blurrier and more stretched. This difference becomes more pronounced at the highest scan rates. As a result, the trace and retrace stop overlapping. To correct for the mismatch and minimize distortion, adjust the T/R phase shift value until the trace and retrace are overlapping. Make sure to select a molecule that isn't moving.

31.2. Imaging Collagen with Perfusion

The goal of this experiment is to observe the dynamic process of collagen molecules assembling into fibrils onto a mica surface. The experiment begins by imaging clean/cleaved mica in collagen buffer (pH 10). Once imaging is stable and the surface is visibly clean, a small amount of solution containing concentrated collagen molecules (pH ~ 4) is flowed through the perfusion line to the sample surface. Since the total volume of perfused solution is small, imaging can continue, capturing the assembly of collagen molecules into fibrils in real-time. Final acquisition time is approximately 1 frame/sec.

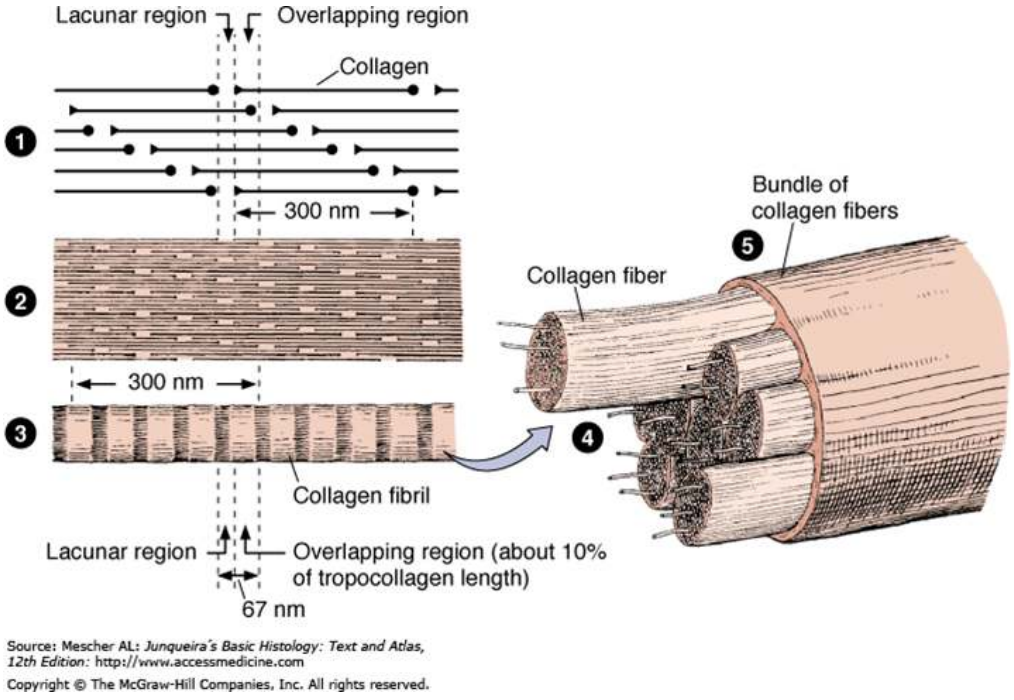


Figure 31.1.: Structure of collagen

Background Information: Collagen molecules, approximately 300nm long and 1.5 nm thick are composed of three subunits: two (2) alpha1 chains and one (1) alpha2 chain. Three of these propeptide molecules (left-handed helices) first assemble into a triple right-handed helix called procollagen which are further processed into a tropocollagen by the removal of their "loose ends" (step 1 of Figure 31.1). This quaternary structure is stabilized by hydrogen bonds and hydrophobic interactions.

Further assembly of collagen (tropocollagen) results in:

- Fibrils, polymers of tropocollagen with diameters between 20-90 nm and several microns in length. These fibrils have striations (banding) with a periodicity of ~67 nm (Figure 31.1, part 3)
- Fibers, bundles of fibrils (Figure 31.1, part 4)
- Bundles, aggregates of fibers (Figure 31.1, part 5)

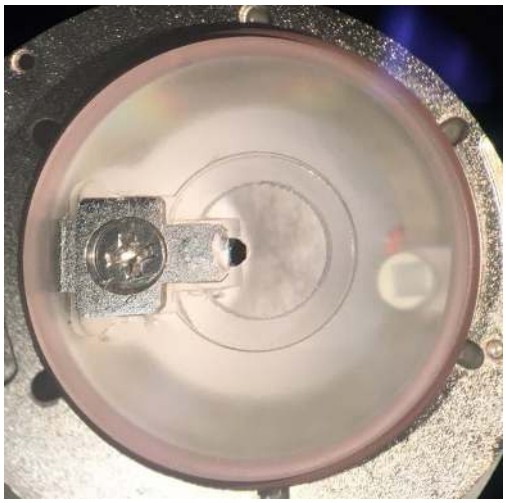
Materials Required:

- 300 mM potassium chloride/ 10 mM sodium phosphate dibasic buffer pH: 10
- Concentrated collagen solution (3100 ppm = 3100 mg/mL)
- AC10 probes
- VRS perfusion holder
- Tubing and tools to set up the perfusion holder (refer to Section 3 on page 385 for perfusion setup)
- Plastic syringes (Henke Sass Wolf 1mL NORM-JECT Luer fit)
- Mica posts (Refer to Section 29.2 on page 348 for instructions on how to prepare mica posts.)

31.2.1. Methods

1. **Place an AC10DS cantilever in the holder:**

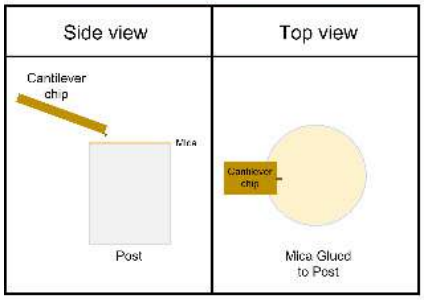
   - Make sure that the chip is sitting as far as possible into the pocket.



2. **Glue post to the VRS stage:**

   - Glue a fresh, clean mica post to the sapphire surface of the VRS stage using Devcon 5-minute epoxy.
   - The mica surface can be cleaned before and/or after it has been glued to the stage by gently rinsing with pure IPA or ethanol. Blow dry with air.

     Note Using a cleaning duster, such as Dust Off Compressed Gas Disposable Cleaning Duster, is sufficient to dry the surface.
   - Refer to Section 29.2 on page 348 for detailed instructions on how to glue the mica post.
     - Make sure to align the mica post relative to the cantilever chip so that the tip will engage near the left edge of the mica surface.



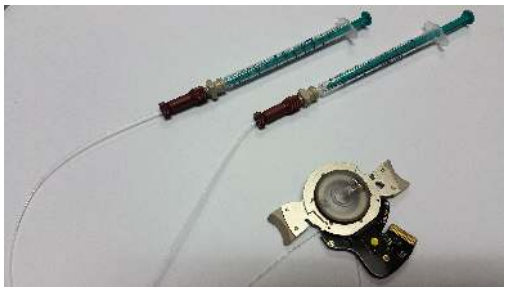
3. **Preparing the perfusion holder:**

- Wash and dry the holder. Similar to the mica post, the holder can be rinsed with IPA or ethanol then blown dry with air.
- Follow the detailed instructions outlined in Section 22.2.1 on page 269 to attach the tubing to the holder. To briefly summarize:
  - After cutting a fixed length of tubing, thread the stretched end through the holes in the perfusion holder.
  - Pull the tubing until tight; it has now formed a complete seal.
  - Trim excess tubing on the front of the cantilever holder.
  - Make sure the tubing is well connected by *gently* pulling on it.

4. **Connect the perfusion lines to the syringes:**

- Clean the syringe adapters by sonicating them in pure IPA or ethanol for ~10 minutes. Set them out on a KimWipe to allow them to fully dry.

  Note This step can be done well in advance, and the cleaned adapters can be stored in a sealed container.
- Follow the detailed instructions outlined in Section 22.2.2 on page 271 to attach the syringe adapters to the other end of the tubing that is connected to the holder.
- One syringe will be used to inject buffer or collagen. You may want to label this “input line” to easily distinguish it from the waste or output line.
- The other syringe will be used to remove solution from the tip-sample area and can also be labeled “output line”.



5. **Deposit collagen buffer onto the mica surface:**

- Pipet ~ 10µl of buffer directly onto the mica.

6. **Prime the tubing:**

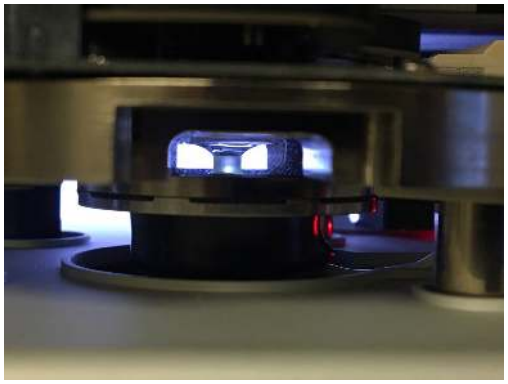
- Fill one syringe with the collagen buffer and connect it to the syringe adapter.
- Push the solution through until you see it come out near the cantilever.
- Continue pushing until all bubbles are out of the tubing.
- Pull excess liquid until only a small amount of buffer is surrounding the cantilever.

7. **Place the holder onto the VRS stage:**

- Make sure that the coarse engage stage is raised to its highest position so that the sample is at its bottom-most position.
- Secure the holder by tightening the two clamping screws.

8. **Bring the sample close to the cantilever:**

- Look through the cell body window.
- If the droplet on the cantilever has not yet merged with the droplet on the sample, then lower the cantilever down until the two droplets merge using the Engage Control knob.
- Try to keep the liquid on the mica post to prevent any contamination that might be on either the edge of the post or on the sapphire stage surface.



9. **Approach the cantilever towards the surface:**

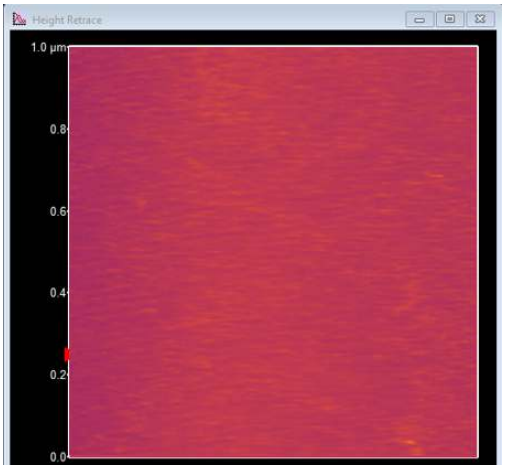
- Align the laser.
- Move to Pre-Engage position.
- Tune the cantilever using blueDrive.
  - Use a target Free Amplitude = 250 mV
  - Use an Amplitude Setpoint = 180 mV
- Enter the following values for these parameters:
  - Scan Size = 1-2 μm
  - Fast Integral Gain = 25 (default)
  - Points & Lines = 512 x256
  - Scan Rate = 100 Hz (Frame rate = 0.39 FPS)

For more detailed instructions for each step, review sections 30.5 through 30.8 in Chapter 30 on page 356.

10.

Start scanning the mica surface:

- Once engaged onto the surface, start scanning the mica surface.
- The surface should be clean and flat.
- If there are any features, it is probably contamination. If this is the case, consider re-cleaving the mica.



11.

Prepare the collagen solution:

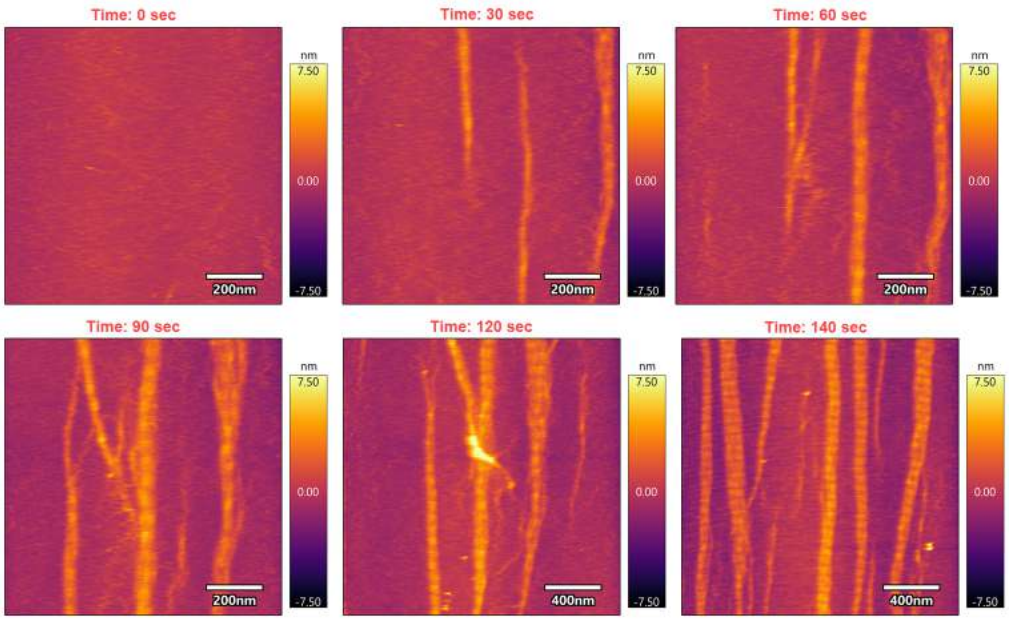
- Prepare the collagen solution (31 mg/ml).
  - Add 4 µl of the concentrated collagen solution (3100 mg/ml) to 360 µl collagen buffer in a 1.5ml Eppendorf tube.
  - Transfer the diluted collagen solution to a 1 mL syringe.

12.

Introduce the collagen solution:

- Introduce the collagen solution
- Stop scanning. This is optional since swapping syringes can be done while scanning.
- Exchange the pure buffer syringe with the collagen syringe (INPUT line).
  - Make sure that no bubbles are introduced when swapping syringes by adding extra liquid to the syringe adapter and pushing liquid to the tip of the syringe.
- Start scanning again if scanning was stopped.
- Slowly add collagen molecules by pushing on the INPUT syringe.
- At the same time, slowly remove liquid using the OUTPUT syringe to maintain a constant volume of liquid on the sample.
  - A syringe pump could be used for this step.

13.



Imaging the assembly of collagen fibrils:

- Continue scanning until collagen molecules aggregating on the mica surface begin to assemble into fibrils.
- Adjust the setpoint so that the fibrils are not being perturbed and dislodged from the mica surface by the AFM tip.
- Over the course of three minutes, distinct collagen fibrils are visible on the mica surface.

Note The first five images have a scan size of 1 m; the sixth image has a scan size of 2 um.

14.

Image Processing:

- Once the data have been acquired, follow the steps outlined in Section 30.10 on page 371 to process the .ARIS files and create movies.

Part V

Chassis & Enclosure

Who is this part for? This part covers general topics relating to the “frame” of the instrument, such as scanner exchange, laser module exchange, and air temperature control.

Part Contents

32. Tutorial: Scanner Exchange

CHAPTER REV. 2424, DATED 08/17/2021, 18:58. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

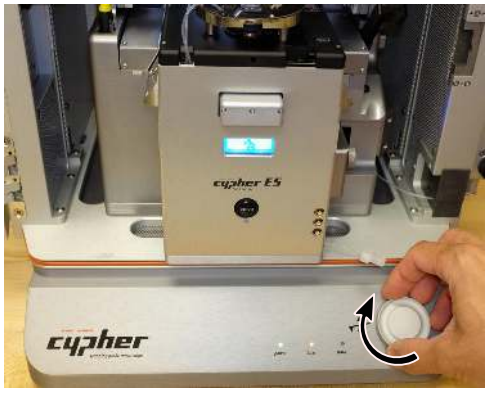
The Cypher AFM can be purchased with various scanner modules. This tutorial describes how to safely swap from one scanner to another.

32.1. Removing the scanner

The following photos show the removal of the Environmental Scanner. The steps are the same for any other model.

1. **Raise the coarse engage stage:** It's likely you will need to change a cell body after scanner removal, so it's best to raise the stage now to make those later actions possible.

   - Rotate the Engage Control Knob on the Cypher ***clockwise*** and hold it until the cantilever is at its highest position.



2. **Open the enclosure:**

   - Lift the door latch and open the enclosure door.

3. **Power OFF the ARC2:**

   - Turn the ARC2 off before proceeding.
   - Press the 'Power' button as shown and verify the green light is OFF.

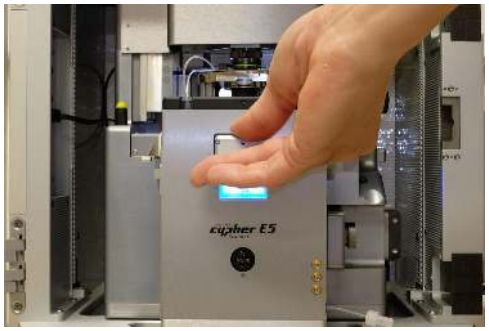
   Note The light behind the display on the scanner front will not turn off because it is powered by a different power supply. It's OK to unplug the scanner with this power supply active.

4. **Unlock scanner:**

   - Lift the lever to the right of the scanner.

5. **Begin to pull the scanner out:**

   - Pull the scanner out about halfway.



6. Clear out a place to set the scanner down once you remove it. We recommend that you first place the scanner right in front of the AFM system, and then grip the scanner again to move it elsewhere. If the scanner is dropped, it might become irreparably damaged.

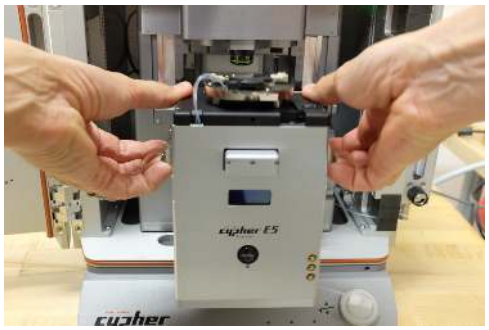
7. **Detach the scanner from the chassis:**

- Make sure the light behind the scanner is off. This confirms the controller was turned off.
- The light behind the display on the front of the scanner will remain on. This is OK. It is powered by an alternate power supply.
- Turn the knob on the right side of the scanner ***counterclockwise*** to unscrew.
- The knob will disengage and retract back towards the chassis. If the knob does not disengage readily, make sure the scanner body is pulled out sufficiently from the chassis.

8. **Remove scanner:**

- Use two hands to grasp both sides of the scanner and remove. Place your fingers under the two dovetail rails, as shown in the photo at right.

  Caution Use care because the scanner is heavy!
- Preferably place the scanner right in front of the AFM, and then pick it up again to move it to its storage location.



32.2. Scanner Storage

The scanner should be stored under basic laboratory conditions, preferably in a locked cabinet or drawer where it will not collect dust or be accidentally knocked over or “borrowed”. At the very minimum, place it on a shelf and cover it with a soft cloth.

32.3. Replacing the Scanner

1. **Verify that the ARC2 is off:**

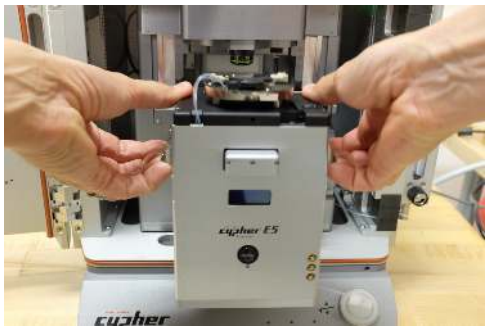
   - Double-check that the ARC2 power light is indeed off. If not, turn off the controller.

2. Clear a space in front of the AFM.
3. Set the scanner down in front of the AFM.
4. Swing the scanner connector to the side so it does not block the scanner's insertion.
5. Make sure the locking lever on the chassis is in the RAISED position.

6. **Set the scanner on the rails:**

   - Use two hands to grasp both sides of the scanner and remove. Place your fingers under the two dovetail rails as shown in the photo.

     Caution Use care because the scanner is heavy!
   - Lift the scanner as shown and set it on the rails.
   - Push it in far enough so it sits stably on the rails.

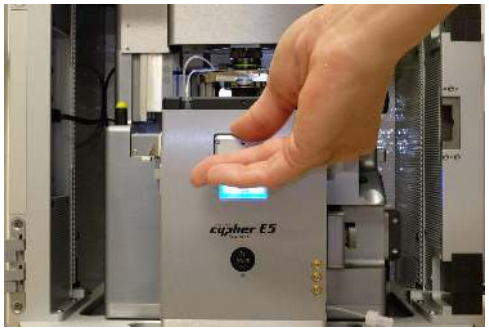


7. **Secure the scanner connector:**

   - Press the connector into its mating socket.
   - Turn the knob on the right side of the scanner ***clockwise*** until firmly tight.

8. **Push the scanner in:**

   - Push the scanner all the way into the chassis until it connects with the chassis.



9. **Lock the scanner:**

   - Continue to apply pressure against the scanner.
   - Lower the lever to the right of the scanner to lock it into place.

10. **Turn the AC2 back on:**

    - Press the 'Power' button as shown. The light must remain green.

11. Sometime during the following steps, you may be asked to re-home the motors. Please do so when the software requests it.

12. When the process completes, click the  (gear) icon to display the popup list of attached hardware including the scanner.

33. Optical System

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

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33.1. Overview

The Cypher AFM has an excellent optical system. A high-quality microscope objective sits at the heart of this system. It affords an excellent optical view of the sample but also guides laser beams for the cantilever detection system.

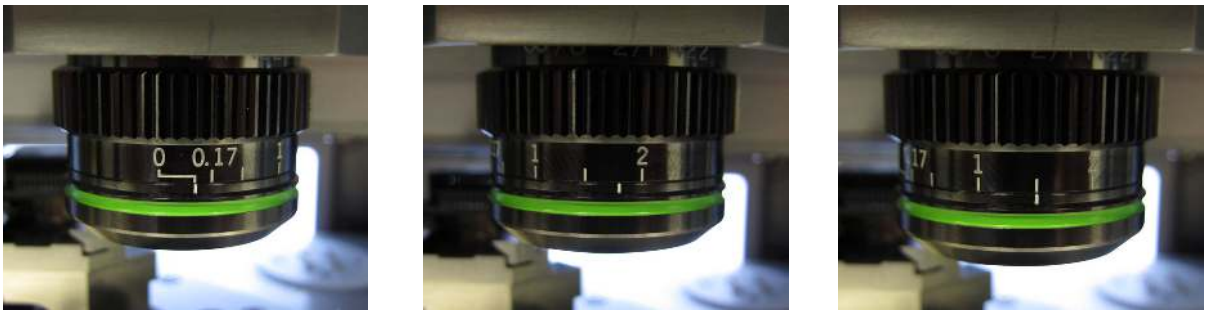
A camera and white light source are embedded in the "view system" which protrudes from the top of the cypher enclosure.

33.2. Microscope Objective

The objective is not user replaceable. The magnification is fixed. Only digital zooming is possible within the software.

Depending on the imaging conditions (air, fluid) a correction collar on the objective must be adjusted. The tutorials in this user guide will always indicate the correct setting. An overview of these various settings is given in the table below.

Collar setting	Cypher S Cantilever Holders	Cypher ES Cantilever Holders
0	Any "Air Only" cantilever holder.	Never.
1.5	Any "Droplet" cantilever used *without* liquid.	Any cantilever holder used with gas.
2	Any "Droplet" cantilever holder used *with* water.	Any cantilever holder used with water.



(a) Zero **(b)** 2 **(c)** 1.5

Figure 33.1.: Some examples of correction collar settings

33.3. View System

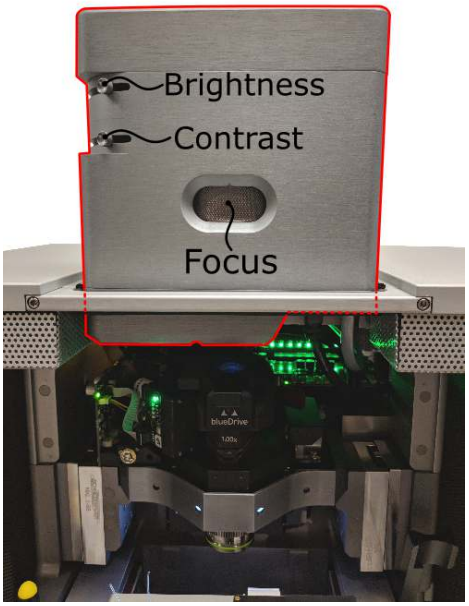


Figure 33.2.: Front view of Cypher view system and controls

The Cypher view system protrudes from the top of the AFM enclosure. It contains a software controlled white light source and camera. It also has three user controls. Two levers for adjusting image brightness and contrast, and a focus ring.

The focus ring is typically set at its detent position, in which case the optical view in the camera is focused in the same plane as the laser spot used to detect cantilever motion, which usually brings the back of the cantilever into focus. In most cases a focused view of the sample, and not the back of the cantilever is desirable. During imaging, use the focus ring on the view system to bring the sample into focus.

During the engage process, the ring should be returned to the neutral (detented) position. A sensor will inform the software if this is not the case, and you will be warned before engaging to adjust the view system focus if necessary.

34. Laser Source Modules

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

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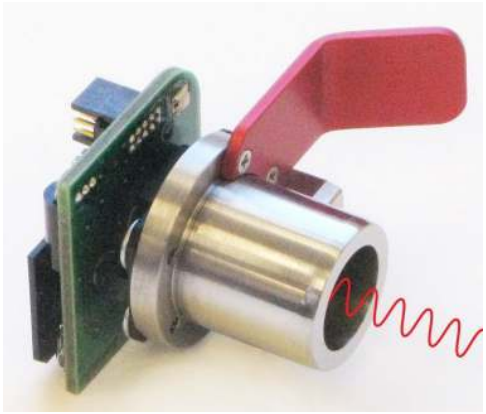


The following instructions describe exchanging laser source module assembly in the Cypher AFM. The Laser Source Module can be exchanged without disassembly through the front of the instrument with a little familiarity of the process. For clarity, the instructions describe exchanging the Laser Source Module by disassembling the enclosure followed by the process performed through the front of the instrument.

34.1. Types of Laser Source Modules and How to Identify Them

The Cypher can be equipped with interchangeable light sources. Some of the nomenclature:

Laser Diodes: Best all-around choice for AFM imaging. Lower noise for imaging techniques, but with some interference effects which can lead to background oscillations when performing force curve measurements.

Super Luminescent Diodes: Also known as SLDs. Best all-around choice for force curve measurements. Slightly higher noise than the laser diode sources, but remarkably lower oscillating background for force curve work.

Small Spot: 3µm by 9µm spot size. Mandatory for cantilevers smaller than the large spot, to prevent light from spilling over the sides.

Standard Spot: 10µm by 30µm spot size. Preferable for larger (traditional) cantilevers since a spot that fully fills the cantilever leads to lower imaging noise..

Part #	Item Description	Picture
901.601	SLD Source Module, standard spot size.	
901.602	SLD Source Module, small spot size.	
901.603	Laser Diode Source Module, standard spot size.	
901.604	Laser Diode Source Module, small spot size.	

34.2. Tutorial: Laser Source Module Exchange

As noted at the beginning of this procedure, it is possible to exchange the Laser Source Module without removing the top of the enclosure. It is highly recommended that you first familiarize yourself with the system by removing the top cover once so that you are certain of the location of the components involved.

1. Remove your sample from the scanner.

2. **Lower the cantilever holder:**

   - Rotate the Engage Control Knob on Cypher *counterclockwise* to lower the tip to a close distance ~1mm) above the top of the scanner. This allows easy access to the top of the head assembly where the Laser Source Module is located.

   Note Although it is not required, for safety reasons we recommend making motor moves with the door closed. Beware of pinch points (Figure 1.2 on page 6)!

3. Turn the ARC2 controller power off. This shuts off most of the Cypher's power. It is not necessary to disconnect the motor power supply when exchanging the Laser Source Module assembly.

4. **Remove optics cover:**

   - Grip the cover at its lower edge and *gently* pull forward. It is attached magnetically and detaches off smoothly.

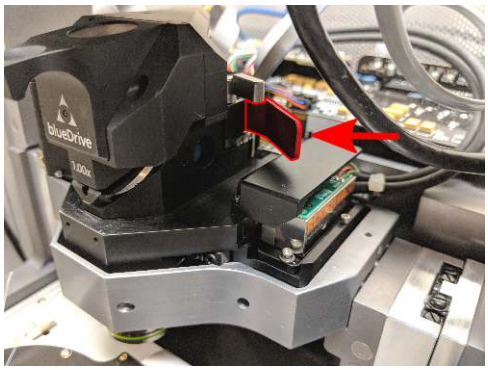
   Note This exposes the head assembly and allows access to the Laser Source Module from the front.

5. Locate the Laser Source Module container for the one you are removing now. You will need to store it properly as soon as it is removed from the SPM.

6. Locate the Laser Source Module (hopefully in its container) which you will be installing.

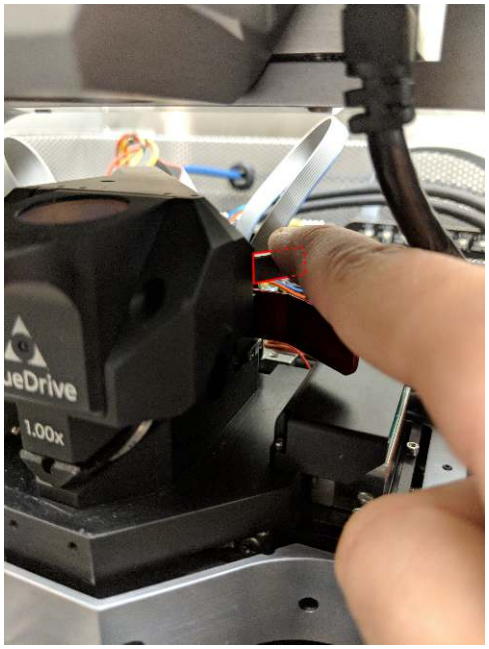
7. **Locate the Laser Source Module:**

   - Look in the area where you just removed the optics cover.
   - The Laser Source Module is easily identified by its red handle.
   - Above that, note the metallic silver cross-shaped clamping knob.



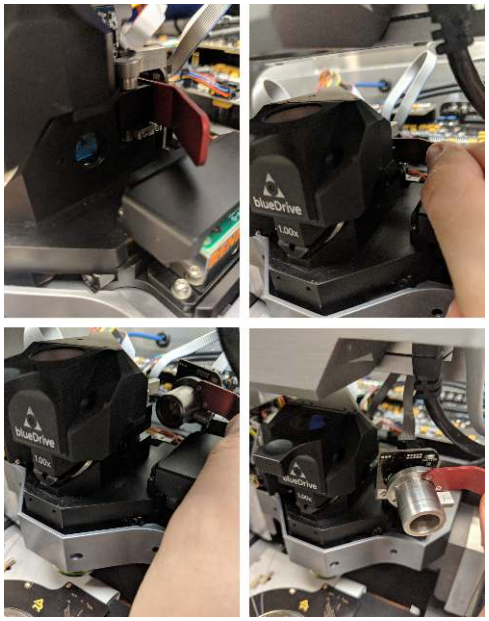
8. **Loosen the clamp:**

   - Turn the clamping knob about one-half turn *counterclockwise* or until it feels loose.



9. **Pull the Laser Source Module out:**

   - Touch a metal part of the SPM instrument to ground yourself.
   - Grip the red handle on the Laser Source Module.
   - *Gently* push straight back. The Laser Source Module should slide out very smoothly. If it does not, further loosen the knob.
   - Once the tube clears its cradle, move it to the right and pull it forward and out.



10. Unplug the power cable.

11. **Store the Laser Source Module immediately:**

    - Store in a safe place. The Laser Source Module is fragile and can be damaged or knocked out of focus if it is dropped.

12. Connect the new Laser Source Module to the power cable.
13. Install the Laser Source Module (reversing the removal process) into the head, making sure that the tube is fully seated. The tab on the left side of the tube will key the rotation of the tube in the head.
14. Tighten the clamping knob to secure the Laser Source Module.
15. Replace the optics cover (See Step 4 on page 400)
16. Turn theARC2 power back on.
17. Power the system back up and align the laser spot on the cantilever. If you are unsure of this process, perform the following steps:

    a) Power up the system as described in Chapter 5 on page 35.

    b) Follow the "AC Mode in Air tutorial" (Chapter 7 on page 42) up to Step 12 on page 54.

    c) If the 'Spot On' process does not perfectly center the laser, then please see Section 34.3 on page 402.

34.3. SpotOn Calibration

A small amount of misalignment in the light spot position is normal after the Laser Source Module is exchanged. Great care is taken at the factory to ensure that the "spot on" calibration is correct, but due to subtle position shifts in the relationship of the optical components, the light may settle in a new location. Once the Laser Source Module is installed and any corrections are made to the calibration, the spot accuracy of the SpotOn routine is very repeatable. If the SpotOn software routine appears to misalign the light on the back of the cantilever, it may be for either of the following reasons:

- The Laser Source Module you installed may be clamped in a different position from when it was tested at the factory. With the Laser Source Module powered on, loosen and jiggle the Laser Source Module to allow it to reseat in the head. Retighten the clamp. If the light moves back to the same (unwanted) location repeatedly, then consider recalibration.
- If the Laser Source Module was purchased at a later time from when the system was delivered, or it was exchanged due to a repair, then the SpotOn parameters were calibrated on a different Cypher and small system-to-system differences make the SpotOn calibration parameters in the Laser Source Module incorrect for your system.

34.3.1. Readjusting the SpotOn calibration.

The SpotOn software routine relies on two parameters stored in the Laser Source Module calibration information block (info block). These parameters are set at the factory but may need to be changed if the Laser Source Module is exchanged.

If you find that the SpotOn position is still off after reseating the Laser Source Module and allowing it to warm up, perform the following steps:

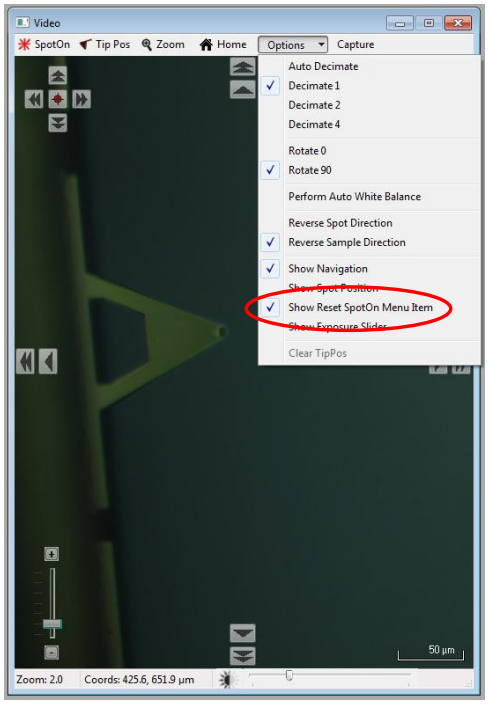
1. Allow the Laser Source Module to come to operating temperature.

   a) Subtle changes in the relative position of the optical emitter and focusing lenses inside the Laser Source Module can occur during warm up after installation. Allow an hour for the Laser Source Module to come to thermal equilibrium with the system. You may find that the error in the SpotOn positioning will reduce.

   b) If the Cypher is left powered-off for an extended amount of time (i.e., overnight or for a day), you may find that the SpotOn position may be off. Allow ample time for the system to reach thermal equilibrium before deciding to make adjustments.

2. **Prepare to reset:**

   - Select *Show Reset SpotOn Menu Item* from the 'Options' pull-down menu of the Video panel.



3.

Reset the spot position:

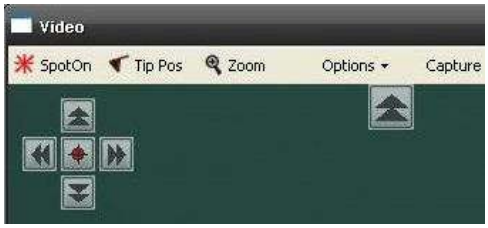
- Zoom in at least once.
- Point the mouse cursor to the center of the laser spot. Right-click and select *Reset SpotOn Position*.
- The new XY spot centers will be stored in the Laser Source Module's info block.

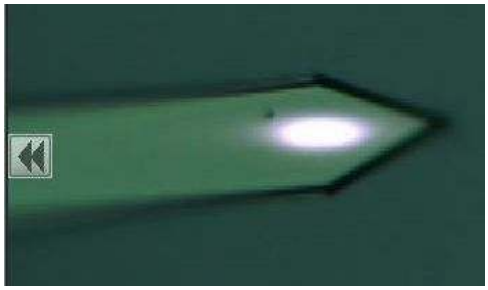
Note The photo shows a laser spot which did not quite center onto the cantilever holder, hence the need for the recalibration.

4.

Test SpotOn:

- Use the arrow markers at the top of the Video panel to steer the spot off the cantilever.
- Click the 'SpotOn' button, located on the top menu of the Video panel.
- Click the center of the cantilever and confirm that SpotOn is properly calibrated.





From now on, this light module should be properly calibrated for use (and re-use) in your Cypher SPM, since each Laser Source Module has a small memory element which stores the calibration values.

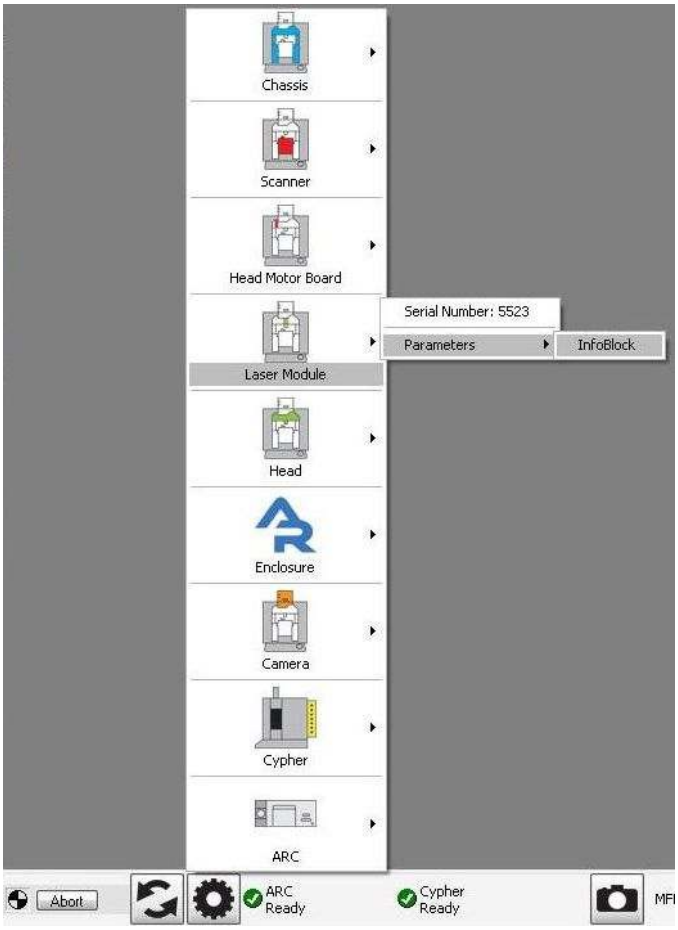


Figure 34.1.: To open the Laser Source Module InfoBlock, click on the “gear” icon at the bottom of the screen, hover the mouse over the Laser Module tile to Parameter and InfoBlock, and only then release the mouse button.

35. blueDrive™ Photothermal Excitation

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

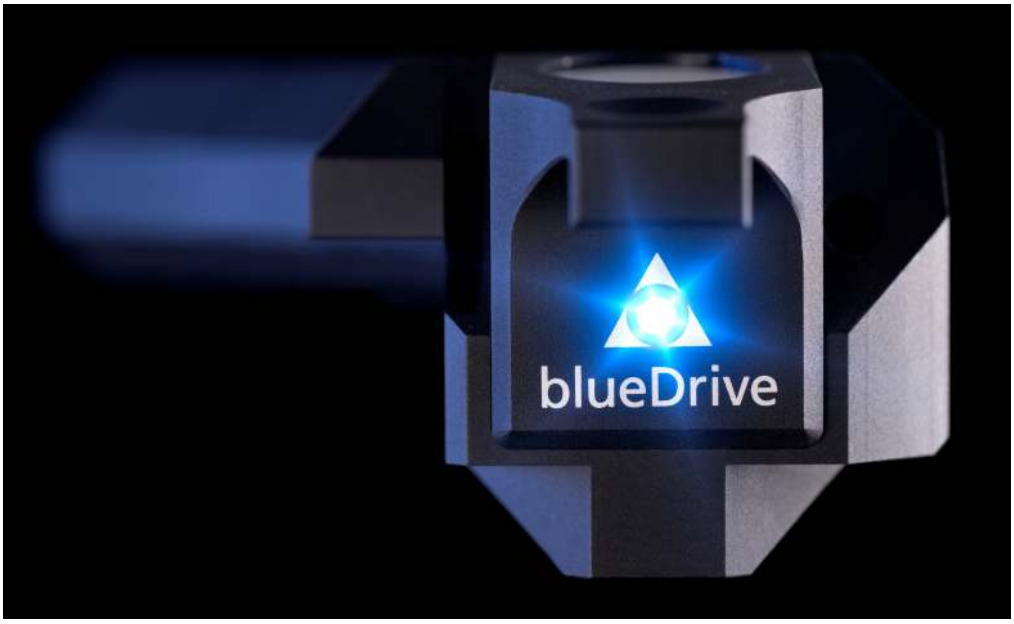


Figure 35.1.: blueDrive optomechanical unit that mounts onto the Cypher Head.

35.1. Overview

blueDrive™ is an optional accessory mounted onto the Cypher Chassis and focuses a blue laser spot onto the cantilever. The blue laser provides an alternative drive mechanism to the standard piezo acoustic drive mechanism used to excite the cantilever (a.k.a. piezo drive).

35.1.1. The principle of photothermal excitation

In addition to the infrared detection laser (850 nm), a blue laser (405 nm) is focused on the base of the cantilever, causing photothermal excitation. The local heating of the cantilever leads to a thermal stress that changes the cantilever deflection proportionally to laser power. High frequency modulation of the laser power up to 8 MHz allows the excitation of cantilevers of all sizes, as well as excitation of higher eigenmodes.

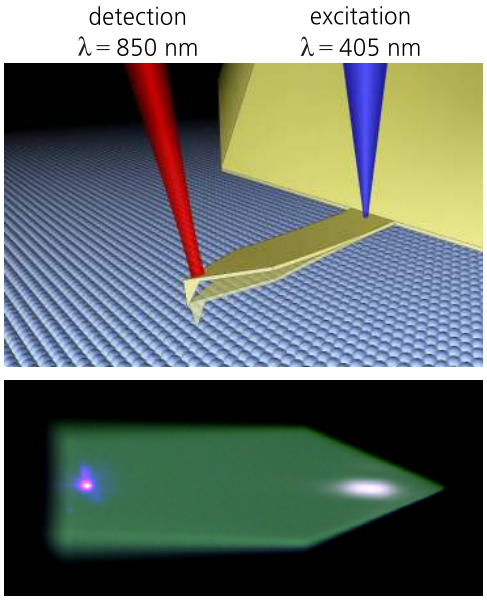


Figure 35.2.: Schematic of photothermal excitation

The benefits of such a direct drive mechanism are similar to those of magnetic actuation (iDrive) but with the advantage of being applicable to cantilevers of all shapes and sizes. Photothermal excitation allows quantitative imaging in all environments, and the benefits are especially noteworthy in liquids, where blueDrive enables stable imaging for hours. The only drawback is that cantilevers **may** require a gold coating to achieve high amplitudes with photothermal excitation.

For more detailed information about the benefits of blueDrive, refer to the following webinar:

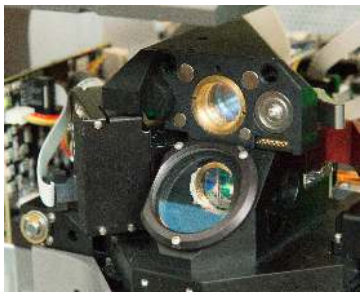
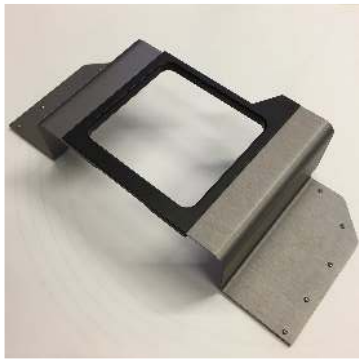
"AFM Imaging and Nanomechanics with New blueDrive™{} Photothermal Excitation"

35.1.2. How much light power do you need?

As shown in the 35.3 below, there are five (5) Filter Cubes that can be selected. The purpose of the Filter Cubes is to attenuate the blue light power to a desired level. Depending on the cantilever shape, the coating, or the imaging environment, up to 10 mW of blue light might be necessary to drive the cantilever substantially, or as little as 0.1 mW may be enough. Choosing the right Filter Cube ensures that the right amount of blue light power reaches the cantilever in order to avoid having excess blue light hitting the cantilever. This also minimizes cantilever heating and maximizes the performance of blueDrive.

Figure 35.3.: blueDrive unit with 5 Filter Cubes for setting the power level of the blue laser

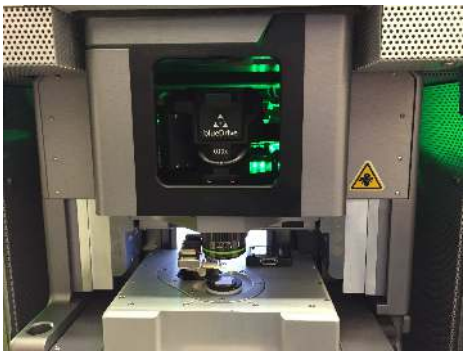
35.2. Parts List

Itm	Part #	Item Description	Qty	Picture
1	934.001	The main blueDrive optomechanical unit comes pre-mounted onto the Cypher head	1	
2	900.266.01-05	Filter Cubes (900.266.01-05), labelled by their gain factor: 0.01x, 0.03x, 0.10x, 0.30x, or 1.00x	5	
3	934.005	blueDrive Accessory Kit 290.169 Hex Key, 2mm 900.249 Gold sample 934.006 Opal Diffuser	1	
4	901.803	blueDrive Optics Cover	1	

35.3. Switching Filter Cubes

35.3.1. blueDrive Optics Cover (optional)

The blueDrive Optics Cover (901.803) magnetically attaches to the View Module bridge, as shown in the photograph at right. It serves an aesthetic purpose, and therefore is not required for proper functioning of the Cypher system.



35.3.2. Changing Filter Cubes

The standard blueDrive package comes with five (5) Filter Cubes (900.266.01-05), labelled by their gain factor: 0.01x, 0.03x, 0.10x, 0.30x, or 1.00x. The spare Filter Cubes dock on the Cypher door when they are not in use, as shown in Figure 35.4 on page 410.

Figure 35.4.: Spare Filter Cube docked on the Cypher door.

Changing Filter Cubes is easy. Simply grip the Filter Cube mounted to the blueDrive unit, as shown below, then pull on the Filter Cube directly away from the blueDrive unit towards the front of the Cypher, as shown by the red arrow.

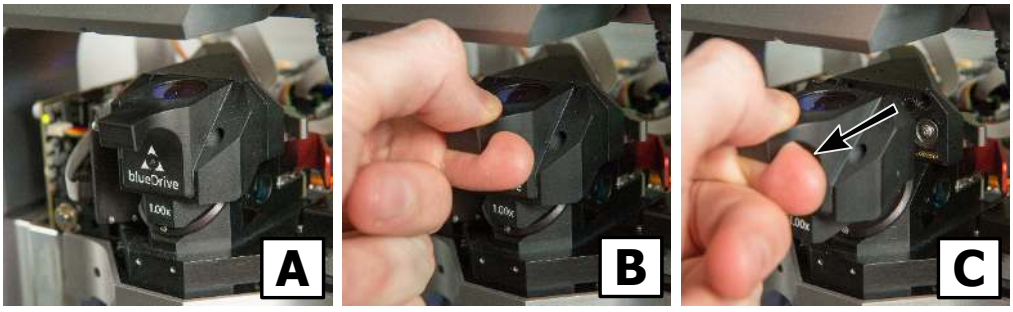


Figure 35.5.: Remove the Filter Cube by pulling directly towards the front of the Cypher.

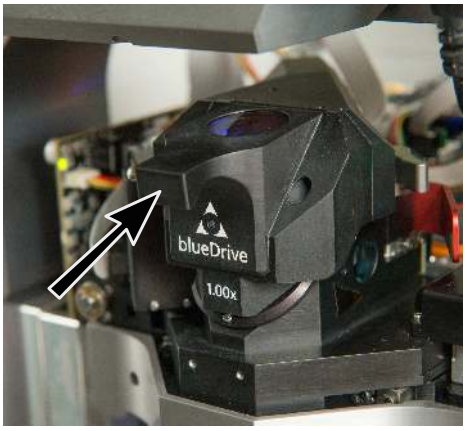
Caution DO NOT apply torque on the Filter Cube!

To install a new Filter Cube:

1. Locate the Filter Cube in proximity to its final position.
2. Make sure that the gage pins line up with the holes on the blueDrive unit.
3. Once the Filter Cube is close to being in place, let go of the Filter Cube and let the magnetic force position the Filter Cube appropriately.
4. Igor software automatically recognizes the Filter Cube and scales drive amplitude (in mW) accordingly.

Tip Ensuring high accuracy blue spot positioning:

In some cases, the locating pins may have some friction that prevents proper indexing of the Filter Cube (e.g.: if some dust gets into the indexing holes). This may lead to a laser spot positioning error of several tens of microns.
In order to ensure high accuracy positioning of the blue spot between changing Filter Cubes, apply a small force with your finger onto the Filter Cube after installation. As shown to the right, the force should be slightly upwards to makes sure the Filter Cube indexes properly.



35.4. Cantilever and Filter Cube Selection

35.4.1. Choosing the right cantilever

As a general rule, gold-coated cantilevers have the best photothermal response and lead to the highest amplitudes for the least amount of blue light power. We recommend the use of gold-coated cantilevers to achieve the largest amplitudes with blueDrive.

However, other cantilevers may be used, especially in applications which require small amplitudes. The best way to determine if a cantilever will work with blueDrive is to simply test it out by acquiring a cantilever tune with blueDrive and noting the maximum attainable amplitude.

35.4.2. Choosing the right Filter Cube

The maximum cantilever oscillation amplitude that is attainable for a particular cantilever scale with the Filter Cube gain factor (0.01x, 0.03x, 0.10x, 0.30x, or 1.00x). For example, if a 0.10x Filter Cube results in a maximum amplitude of 100 nm on some cantilever, it is expected that a 0.30x Filter Cube will result in a maximum amplitude of 300 nm. However, some cantilevers are very photothermally sensitive and provide large amplitudes, such as 100 nm, even with the lowest Filter Cube (0.01x). Using a 1.00x Filter Cube on such a sensitive cantilever could cause overheating and permanent damage to the lever!

It is always best to start with a low Filter Cube when testing a new type of cantilever. If the desired amplitude is not attained with the 0.01x Filter Cube, you can scale the Filter Cube up to attain the desired amplitude. Once the right Filter Cube has be defined for a particular cantilever, all cantilevers with the same model number are expected to achieve similar amplitudes (within a factor of ~2), such that the Filter Cube test can be avoided in future experiments.

Importantly, blueDrive has the best performance when the lowest Filter Cube required to attain the desired amplitude is used. For example, it is better to use the 0.30x Filter Cube to drive the cantilever with 2 mW of drive amplitude, rather than using the 1.00x Filter Cube with 2 mW of drive amplitude. Both of these Filter Cubes allow a drive amplitude of 2 mW; however, the 0.30x Filter Cube has only 3 mW of DC light power at the cantilever, as opposed to the 1.00x Filter Cube which has 10 mW of DC light power. In other words, the same cantilever amplitude will be attained in both scenarios but using the 1.00x Filter Cube will lead to roughly 3x more heating of the cantilever.

Filter Cube gain factor	DC power @ cantilever	Max (AC) drive amp.	Min (AC) drive amp.
1.00x	10 mW	9 mW	1 mW
0.30x	3 mW	2.7 mW	0.3 mW
0.10x	1 mW	0.9 mW	0.1 mW
0.03x	0.3 mW	0.27 mW	0.03 mW
0.01x	0.1 mW	0.09 mW	0 mW

Table 35.2.: Blue light power output for blueDrive as a function of Filter Cube

The Minimum (AC) drive amplitude, fixed in the software, is there to remind you to switch the Filter Cube to a lower value when requiring lower cantilever amplitudes, rather than simply turning down the drive amplitude electronically via the Tune Panel in Igor.

The 35.6 below explains why it is better to change Filter Cubes rather than significantly lowering the drive amplitude in the Tune Panel. The DC power of the blue light at the cantilever is fixed, according to Table 35.2 on page 412. Meanwhile, the drive amplitude, which is the AC power modulation of the blue light at the drive frequency, can be adjusted continuously on Tune Panel, as with conventional piezo drive. It is the AC power, not the DC power, which determines the oscillation amplitude of the cantilever.

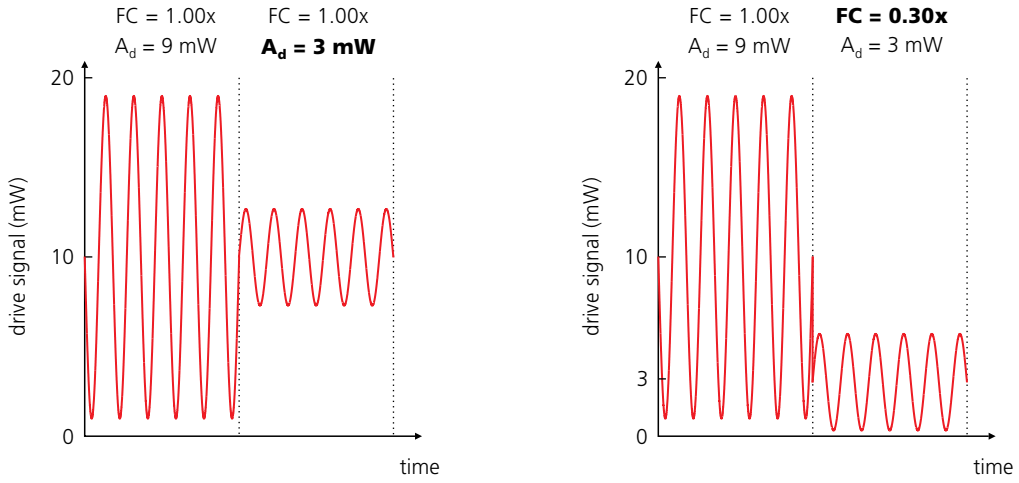


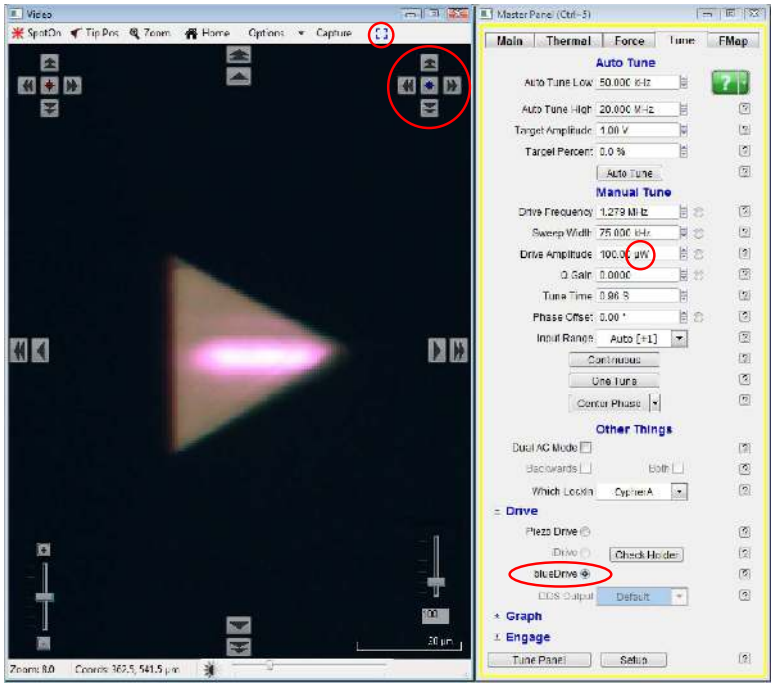
Figure 35.6.: The AC and DC power of the blue light are illustrated with respect to (left) changing the amplitude on the Tune Panel versus (right) changing the Filter Cube (FC), which is the preferred option because it lowers the amount of blue light shining on the cantilever.

35.5. Software interface

35.5.1. Software requirements

blueDrive requires version 13 or above of the AFM software. The latest version can be downloaded here: https://support.asylumresearch.com/forum/content.php?4-Software

35.5.2. Turning on blueDrive



To turn on blueDrive:

- Select the “blueDrive” option on the Tune Panel, as an alternative to the conventional “piezo drive” (see screenshot above).
- In blueDrive mode, the drive amplitude changes from units of “V” to units of “mW” to remind you that the cantilever is driven by blue light power.
- Also note that there are new View Module controls that appear in the Video window when blueDrive is selected. These are used in the following section.

Tip Reducing sample exposure to blue light:

1. When blueDrive is turned on, the blue light spot **may** be directed at the sample inadvertently. If sample exposure to blue light is potentially problematic, it is advisable to retract the cantilever from the sample as far as possible before turning blueDrive on. To avoid unnecessary exposure of the sample, only when the blue spot is positioned at the base of the lever should the cantilever be approached towards the surface.

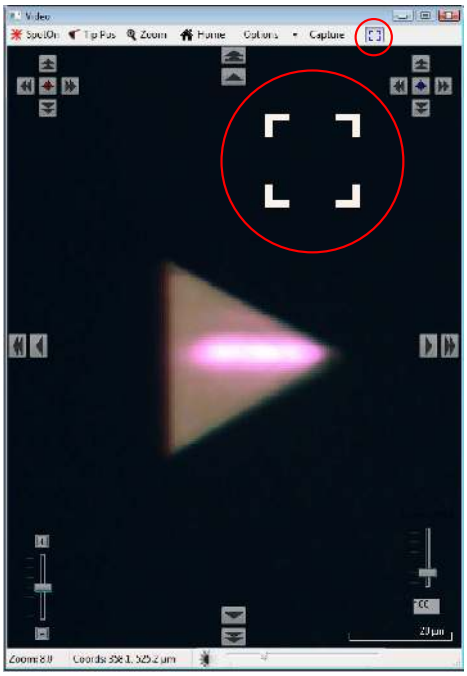
35.5.3. Positioning the blue spot

The blue spot may be positioned in several ways using the new controls that appear on the View window once ‘blueDrive’ is selected on the Tune Panel (shown in previous section).

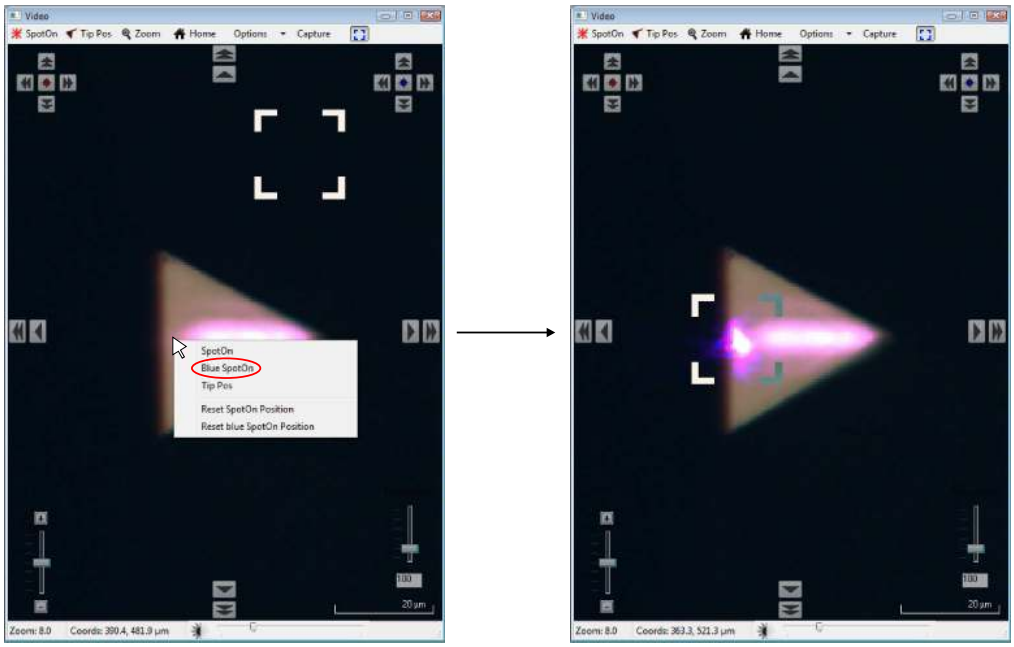
1.

The blue box can be toggled.

- When enabled, the blue box displays the blue spot location on the View window, as shown at right.
- If the blue spot is not incident on a reflective surface, such as the cantilever, it may be impossible to see the blue spot. However, the blue box indicates the position of the blue light spot, known to Igor because of a photodetector inside the blueDrive unit which measures the location of the blue spot **before** it reaches the cantilever and sample.

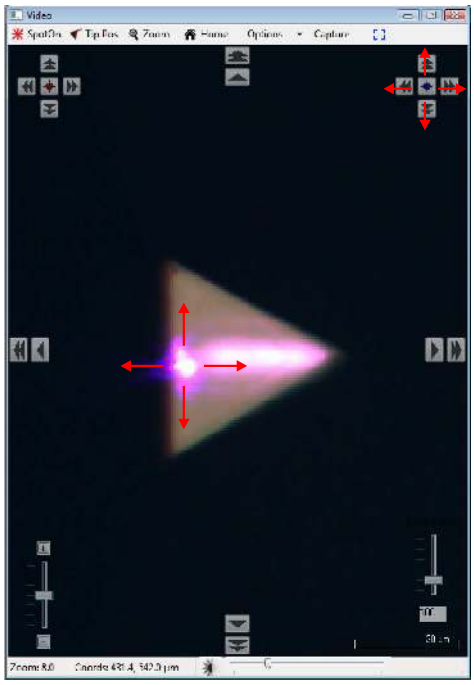


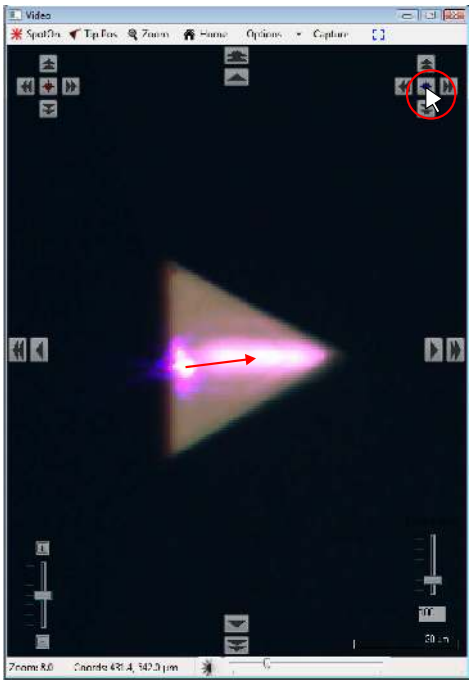
2.



Using *Blue SpotOn*: Right-click on a particular location of the View window and then select *Blue SpotOn* to automatically move the blue spot to that location.

3.



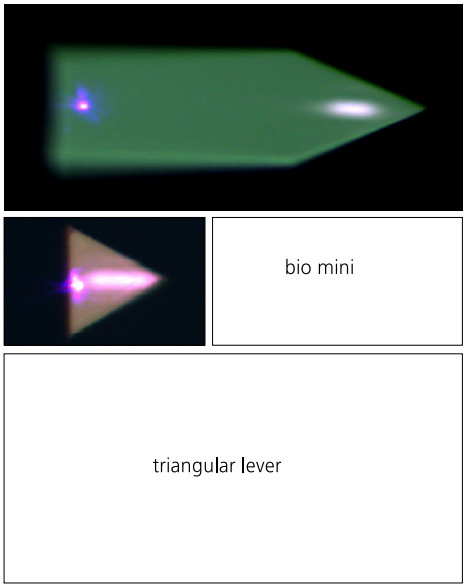


Using the arrows:

- The blue arrows on the top/right of the View window can be used to move the blue spot position in all directions. If enabled, the blue box follows the location of the moving blue spot, as shown above left.
- Clicking on the center circle in between all four arrows commands the blue light to move on top of the infrared detection light spot, as shown above right.

35.5.4. Acquiring a cantilever tune

- The optimal location for the blue spot for best photothermal performance is the base of the cantilever, along the axis of the cantilever. An example of the optimal location for several levers is shown at right.
- For hollow triangular cantilevers, the blue spot should be placed at the base of either of the cantilever legs.



Although it is not necessary to fine-tune the blue spot location, it may be beneficial in certain cases to move the blue spot with the arrows near the cantilever base in order to find the position with the maximum amplitude.

35.5.5. Operating on the thermal resonance

The true resonance of the cantilever is best determined from a fit to the thermal spectrum of the cantilever. This ensures that the cantilever is being driven at its true resonance, where the phase response is 90 degrees. For ultimate accuracy, the thermal should be acquired with the blue light positioned where it will reside during imaging.

Note that it is not actually necessary to acquire a cantilever tune before imaging. Taking a thermal and fitting it to set the drive frequency to the cantilever resonance may suffice. That being said, cantilever tunes are very useful in determining whether everything is functioning appropriately.

36. Air Temperature Controller (ATC)

CHAPTER REV. 2438, DATED 09/05/2021, 18:28. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

36.1. Overview

This chapter covers the installation and operation of the Cypher ATC (Air Temperature Controller).

If the temperature of your laboratory varies by more than a few tenths of a degree over time (as most labs do), your SPM images may be suffering from distortion due to differential thermal expansion and contraction of the entire instrument. The Cypher ATC can improve the situation by gently forcing filtered temperature-controlled air through the AFM enclosure.

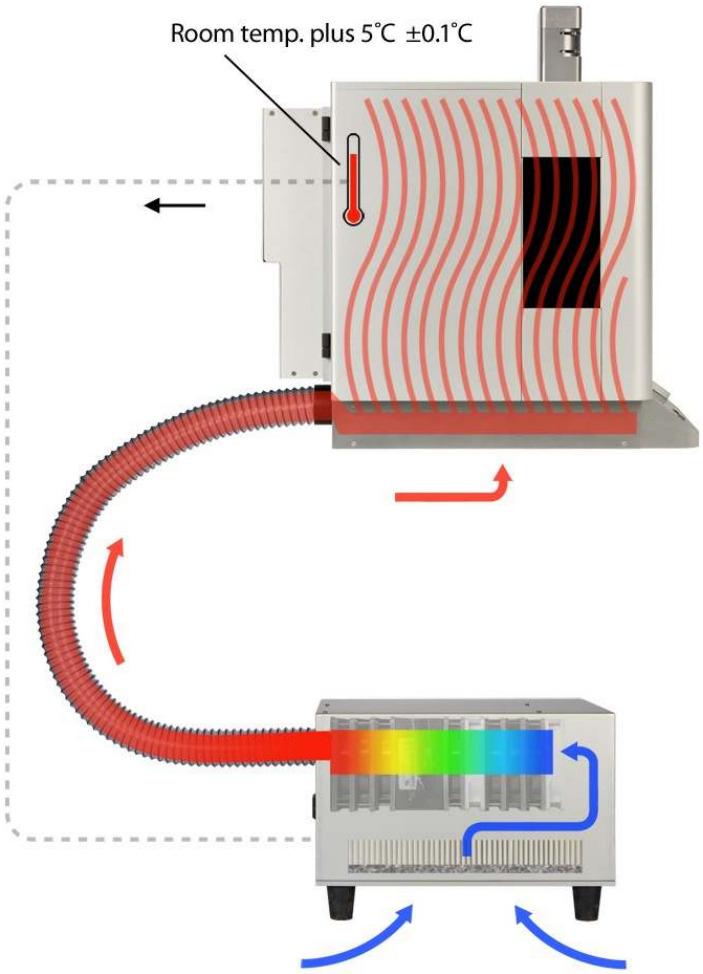


Figure 36.1.: Basic Diagram of ATC airflow. Cool lab air enters via a HEPA filter at the bottom of the Cypher ATC, passes through fans and heater coils, and travels via corrugated hose to the Cypher enclosure. Temperature signals travel from the Cypher back to the ATC (dotted line). Air exits the Cypher from the bottom rear.

The Cypher ATC is a box containing a heater/fan subassembly and an electronics board for controlling the amount of heat generated and the volume of air flowing into the Cypher SPM enclosure. The interior temperature of the Cypher enclosure is regulated by a software driven feedback loop that controls the ATC system's heater to maintain the temperature of a thermal sensor located on the back of the Cypher SPM chassis.

The temperature in typical labs will vary by a few degrees during the course of the day, often oscillating due to the turning on and off of air conditioning. The ATC will counter these temperature swings by adding more heat to counteract these temperature swings. Since the ATC can only add heat, the temperature setpoint must be set above the maximum temperature swing of the laboratory, typically 2-5 C above the average room temperature.

The ATC is not designed as an environmental controller to drive the working temperature of the sample.

36.1.1. Good Practices

To get the most out of your Air Temperature Controller, please follow these guidelines (even if you don't own an ATC, they will improve your imaging stability):

- A laboratory with decent temperature control will always lead to improved imaging stability. Typically, a windowless room with air conditioning operating at all times is preferred.

- Do not place your Cypher AFM in strong air currents, such as from an air conditioning vent. If you have no choice, fashion some sort of air deflector panel.
- It can take between 6 and 12 hours for all the metal and components of the Cypher AFM to thermally stabilize. Therefore, the best approach is to always leave the Cypher AFM turned on and the ATC regulating the instrument temperature. If you wish, you can turn off the laser at night to extend its lifetime but note that there may still be a period of thermal drift even from turning on the laser.
- Keep the enclosure door closed as much as possible. Only open it briefly when exchanging samples or cantilevers.
- Keep critical items, such as spare cantilever holders, somewhere inside the Cypher enclosure. This will ensure all parts are at the same temperature. Some people also leave the cantilever holder changing station (see Step 9 on page 45) inside the enclosure to prevent the cantilever holder from cooling down during tip exchange. Work quickly when replacing tips!

36.2. Parts List

Itm	Part #	Item Description	Qty	Picture
1	901.901.1	ATC Unit.	1	
2	330.002	Air Hose.	1	
3	113.407	Hose Fitting.	1	
4	279.066	Hose Clamp.	1	

Itm	Part #	Item Description	Qty	Picture
5	409.002	AC power Cable.	1	
6	449.025	ATC Control Cable.	1	
7	448.088	Auxiliary Temperature Sensor.	1	
8	290.118	5/64” Hex wrench.	1	

36.3. Hardware Setup

This section describes how to set up the air temperature controller hardware.

36.3.1. Power requirements

The ATC has a fixed power input for use with either 100/120VAC or 220/240VAC. In all cases, the power should be single-phase power where the main supply has a load and neutral line in addition to an earth ground.

During normal ATC operation, the voltage on the load line is “chopped” with a circuit to supply pulses of current to the heater coils in order to vary the amount of heat generated. The return path of the current from the heater flows to the neutral line, which is essentially at 0V relative to earth ground. The chopping circuit is only on the load line and cannot operate correctly with two-phase power.

The ATC has built-in sensors on both the load and neutral lines to monitor their condition. If the sensor on the neutral line detects a voltage higher than 10VAC, the system will detect a fault state and the software will display an error message indicating that two-phase power is present.

Please consult your facilities personnel to establish whether or not you have the proper supply voltage in your lab.

36.3.2. Connect the ATC air hose

The hose feeding the ATC air connects to a fitting on the lower half of the enclosure. If the ATC was purchased with the Cypher, the fitting will be attached. If the ATC was a separate purchase after the Cypher, the fitting will need to be attached using the following instructions.

1. Shut the ARC2 controller power off.

2. **Disconnect Cypher cables:**

   - Motor Power
   - Main controller cable
   - USB cable
   - Fire wire cable

   Note For ease of installation only, if you have unobstructed access to the back of the Cypher, you may choose to leave the cables connected.

3. Position the Cypher so you have access to the back of the enclosure.

4. **Remove cover:** The air inlets and outlets need to be uncovered as follows:

   - Locate the 5/64" hex wrench.
   - Remove the six (6) screws holding the cover onto the enclosure.
   - Remove the cover and save with the other Cypher accessories.

5. **Install the air inlet fitting:**

   - Insert the fitting into the hole in the enclosure. The end with the short flange goes in the hole.
   - Secure the fitting onto the enclosure using four (4) of the screws you removed with the cover.

6. **Install the air hose:**

   - Adjust the hose clamp so it fits loosely over the hose.
   - Slide the hose clamp onto the hose.
   - Push the air hose over the fitting on the enclosure.
   - Tighten the hose clamp to secure the hose to the enclosure.

7. **Connect the air hose to the ATC:**

   - Place the ATC on the floor below the table under the Cypher.
   - Move the Cypher back to its normal location.
   - Route the air hose over the edge of the table and down to the ATC.
   - Push the hose into the exhaust hole on the back of the ATC:
     - Start by guiding the end of the spiral wire inside the hose into the hole.
     - *Gently* push the hose and allow the wire to go into the hole on the ATC until about 1 to 1-1/2 turns of the wire is inside the ATC.

Note If your instrument position does not allow you to have the ATC on the floor, you may need to install the ATC next to the Cypher. Be aware that the ATC has two small fans which could induce vibration into the system.

36.3.3. Connect the ATC

This section explains how to connect cables and temperature sensor to the ATC.

1. **Connect the ATC control cable:**

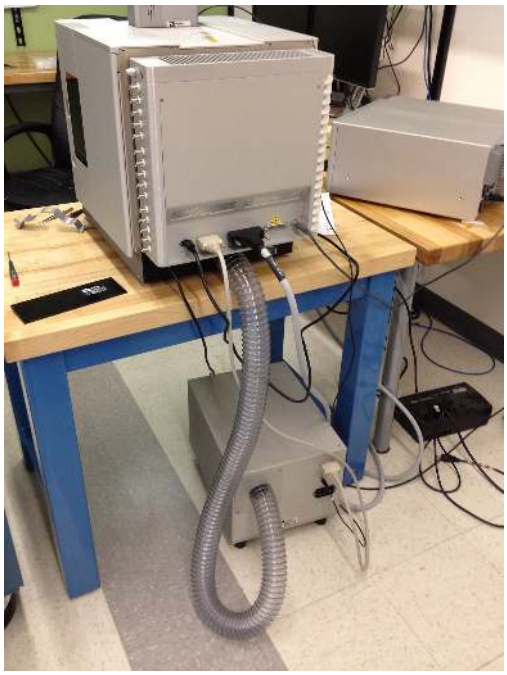
   - Connect the cable to the connection labeled "ATC" on the backpack.
   - Route the cable over the table and down to the ATC unit.
   - Connect the ATC Control cable to the ATC.

2. Connect the AC power cord to the ATC and plug it into wall power.

3. **Reconnect the Cypher cables:** Reconnect the following:

   - Motor Power
   - Main controller cable
   - USB cable
   - Fire wire cable

   The final result is shown in the photo at right.



4. **Plug in remote temperature sensor:**

   - As shown at right, connect the auxiliary temperature sensor.
   - Locate the sensing end in a place where it is sampling the room temperature. Choose a location away from warm instrument enclosures or plumes of warm instrument exhaust air.

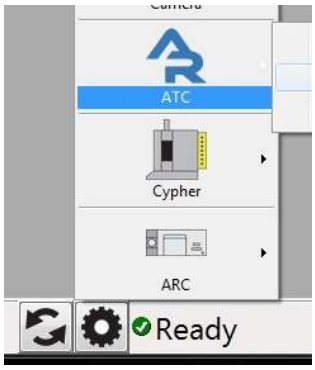
36.3.4. Power-up and Software Initialization

This section describes how to power-up the ATC and ARC2 and initialize the software.

1. Turn the ATC power on.
2. Turn the ARC2 controller on.
3. Restart the AR SPM software and home the Cypher motors.

4. **Check connectivity:**

   - In the status bar of the software, click the 'Settings' (gear) icon and confirm that the ATC is in the list of attached instruments. (If it is not, check all of the cable connections, and then click the 'Refresh' icon (two curved arrows) followed by clicking the 'Settings' (gear) again.)



36.4. Operation

With the ATC connected and the software running, from the Main Menu Bar, select *AFM controls* then *ATC* to open the ATC Control Panel. The ATC Panel is broken into sections of controls grouped by function. Like all the other control panels in the software, a detailed explanation for each menu item can be seen by clicking the '?' (question mark) button to the right of the item of interest.

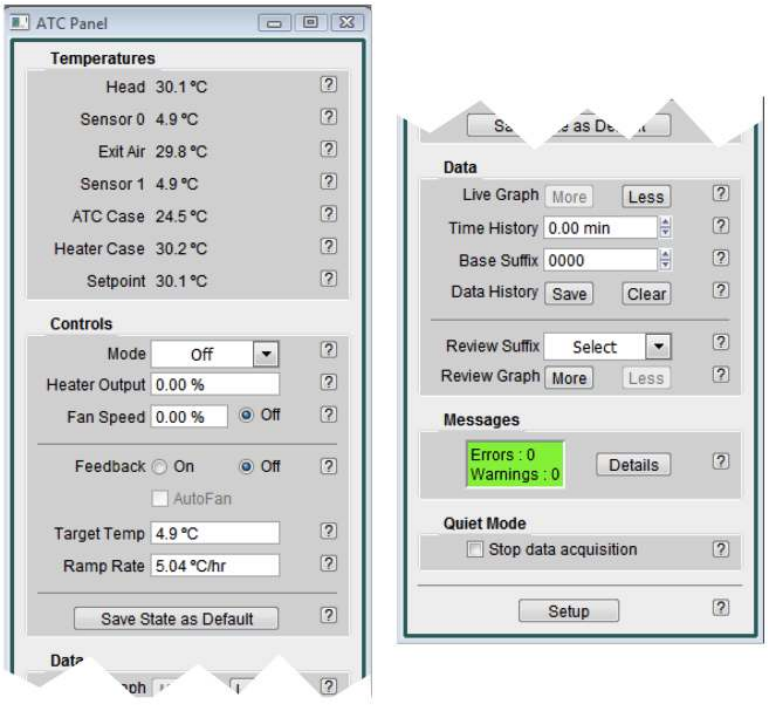


Figure 36.2.: Typical view of the ATC control panel.

The ATC control panel (Figure 36.2 on page 425) allows you to control every aspect of ATC operation and to adjust the conditions for both heating and air flow. Two basic mode settings are as follows:

Manual Temperature control feedback operates based on values typed into the ATC panel. When the AR SPM software is exited or restarted, temperature control quits. This mode is typically used for the first few days when optimal parameters are being determined. Once these parameters are saved into ATC firmware, Auto mode will likely become the typical ATC setting.

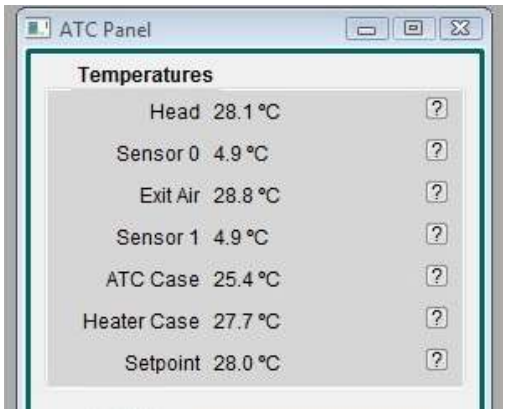
Auto The ATC operates autonomously based on values stored in its firmware. It will continue operating as long as the ARC2 controller is turned on, regardless of what the software is doing. This will likely be the typical mode of ATC operation.

The following subsections include additional information about controls and sensor as well as operation.

36.4.1. Manual Control

1. **Note the room temperature:**

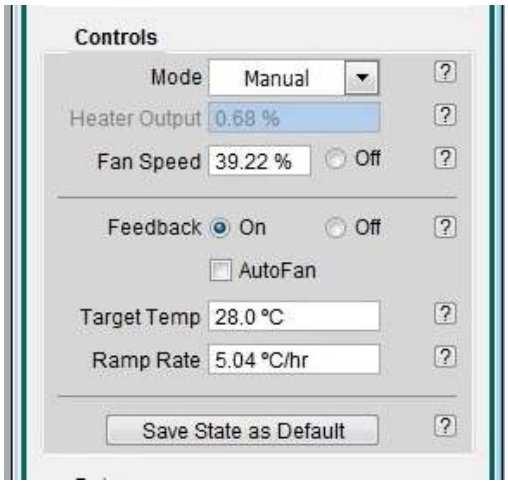
   - From the ATC Panel, note the 'sensor 0' temperature. This is the room temperature based the external sensor connected in Step 4 on page 424.



2. **Choosing the temperature setpoint:**

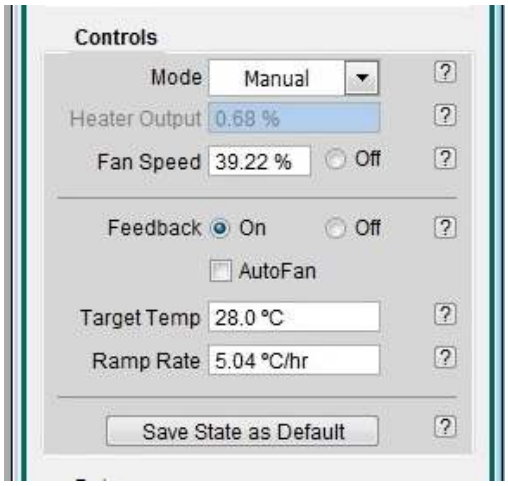
   - Make sure 'Feedback' is set to *Off*.
   - Make sure the 'Mode' is set to *Manual*.
   - Set the 'Target Temp' to ~3 degrees C above the room temperature recorded in the previous step.
   - Check that the Ramp Rate is 5C/sec and change if needed.

   Note The 'Target Temp' is the temperature setpoint for the ATC temperature feedback control.



3. **Set the fan speed:**

   - Start with a 'Fan Speed' of *100%*.
   - If you find imaging artifacts related to the fan, consider lowering the speed.

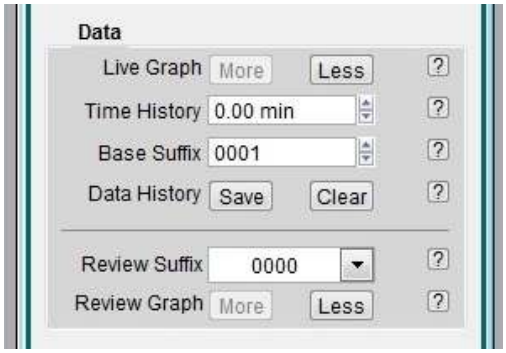


4.

Display datalogging: It is important to be aware of how well the Temperature Feedback is operating. You should monitor the temperature data history as follows:

- Display the live graph:
  - Click on the 'More' button once to display the graph.
  - Click on the 'More' button multiple times to add sensors to the data plotted.
- For more information on datalogging, please see Section 36.7 on page 430.

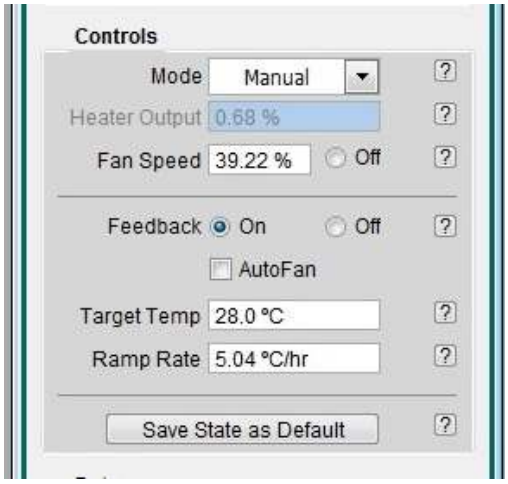
Note Even if you do not display the data, it is still logged for later inspection.



5.

Turn the feedback on:

- Click the Feedback control *On*.
- Over the next hours, observe the heater power variation and the head temperature converging on the temperature setpoint.
- A properly operating system will apply between 5% and 15% heater power to keep the head temperature within 0.1C of the setpoint.



36.4.2. Further Description of ATC Controls

Heater Output This controls the amount of power provided to the heating coils. The power can be adjusted to 50% power. Typically, the amount of power required to maintain temperature stability is under 5%. Although not recommended, you can use this control to briefly send a burst of hot air into the enclosure to accelerate initial warm-up time.

Fan Speed Has a range of 0% to 100% and is variable from 39-100%. In cases where you are scanning near atomically flat surfaces, you may see periodic noise from the fans. Reducing the fan speed will eliminate this but be aware that you need to circulate air through the Cypher in order to maintain thermal stability. In most cases, reducing the speed to 39% for a few hours will not be a problem, as long as the lab temperature is sufficiently below the Target Temperature. Noise levels from the fans at 39% show negligible to no effect on the Cypher's performance.

Feedback On/Off. Enables the feedback loop which controls the heater power to maintain a constant head sensor temperature.

Target Temp The final temperature to be maintained by the feedback loop.

Ramp Rate The rate at which the ATC adjusts the current Setpoint Temperature to achieve the Target Temp.

Note The Setpoint Temperature is located in the Temperatures display area. This is a transition temperature based on the ramp rate parameter changing the feedback loop's current operating point to the Target Temp.

36.4.3. Explanation of Temperature Sensors

There are three temperature sensors located inside the ATC that are primarily used to monitor the operating temperature of the unit while it is being used.

ATC Case This sensor is attached to the inside wall of the ATC box. It is used to monitor the incoming air from the room, which is essentially the room temperature.

Heater Case This is a sensor mounted directly on the portion of the heater/fan assembly containing the heating coils. Typically used for diagnostic purposes in case of overheating.

Exit Air This sensor is mounted directly in the path of the outgoing air just inside the hole where the air hose is connected to the ATC. Used for diagnostic purposes.

Head Temperature This sensor is located on the back of the Cypher Chassis about one inch above the rear air vent in the enclosure floor. This sensor is used by the ATC as the primary feedback source. Its location allows the sensor to be shielded from abrupt thermal changes when the door is opened.

Sensor 0 Input connection on the outside of the ATC unit. For auxiliary use.

Sensor 1 Second input connection on the outside of the ATC unit. For auxiliary use.

The ATC is shipped with one remote temperature sensor. This sensor can be used as the feedback sensor or to simply monitor some specific place in your setup, or, as suggested above, to monitor room temperature.

The ATC Case sensor is a reasonable second choice indicator of the lab temperature if you are using the auxiliary sensor (see Step 4 on page 424) for another purpose.

36.4.4. Automatic Operation

After a few days of data logging, you will have characterized the temperature swings in your laboratory and will have chosen an ATC temperature setpoint, which is a few degrees above the maximum daily room temperature.

Tip	Your goal is to run the Cypher AFM as cool as possible, as this minimizes thermal shock to the instrument when the door is opened. Ideally, you choose this setpoint so that the ATC is producing heat at about the 5% level when the room temperature is maximum.

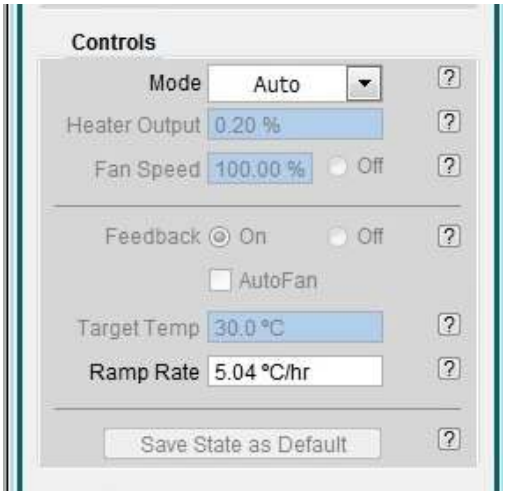
Now is a good time to store the values of the ATC panel in the firmware of the ATC unit itself with the following step:

- Under the "Controls" section of the ATC Panel, click the 'Save State as Default' button.

To set the ATC to Automatic mode:

Setting the mode to Auto:

- From the 'Mode' pulldown in the ATC Panel, select *Auto*.
- Notice that some values are "grayed out" as they are now controlled by values stored in the ATC firmware.



During operation, the ATC feedback is maintained as long as the power to the ARC2 controller and ATC unit are left on. Exiting the software will not interrupt the ATC operation.

36.5. Enclosure Door Function

When the enclosure door is open, the ATC cannot realistically attempt to control the temperature inside the Cypher enclosure. Therefore, the ATC senses the opening of the door and temporarily freezes the feedback process but keeps the fans running and also keeps the air heater power at the same level as when the door opened.

Once the door is closed again (we urge you do this quickly, see Section 36.1.1 on page 419), the air keeps blowing for about a minute, and then the feedback automatically resumes. You will see the LEDs on the front of the ATC change colors while this is happening. For more information on the LEDs, continue on to the next section.

36.6. ATC Front Panel LEDs

The LEDs on the front of the ATC unit provide a basic visual cue regarding the condition of the system.

Summary	If all three lights are green, the ATC is functioning properly and attempting to control the temperature inside the Cypher enclosure. For any other combination of colors, please read further.

ATC temperature control OFF: The ATC power is ON (green light), the Cypher enclosure door is closed (green light), and the ATC Feedback is OFF.

- This is a normal setting until the feedback is activated in the software.

Normal Operation, ATC ON, and controlling temperature. The ATC is ON, the door is closed, and the ATC Feedback is ON (green light).

- Normal setting where the system should be.

Note If the ATC was just activated, the lights will not indicate if stable Feedback has been reached.

ATC Feedback ON, but door open: The ATC is ON, the door is OPEN, and the ATC Feedback loop is in STANDBY mode (yellow light).

- This is a typical condition when the Cypher door is opened during use.
- When the door is opened, the Feedback loop is paused, and the software holds the fan and heater power steady. Feedback resumes a few minutes after the door is closed again.

Caution Be aware that the longer you keep the door open, the more unstable the interior will become due to heat loss. It is good practice to open the enclosure door only long enough to remove the cantilever holder or exchange samples in the instrument.

Note Close the door when exchanging the tip in the cantilever holder or when you are prepping a sample.

Door just closed, but Feedback still on hold: The ATC power is ON, the door is CLOSED, and the ATC Feedback is in STANDBY mode (yellow light).

- The is a typical condition when the Cypher door is opened and closed.
- The Feedback loop stays in STANDBY mode for an additional 30 seconds and then reactivates automatically, turning all three lights to green again.

36.7. Data Logging

The data logging area allows you to monitor the temperature sensors, as well as the fan speed and heater power, over time. You already encountered this in 4, and we will follow up here with more detail.

Please refer to the Data section of the ATC panel (see Figure 36.2 on page 425) for the following controls.

Live graph Click the 'More' button once to display the graph. Click the 'More' button multiple times to add sensors to the data plotted.

Data History Click the 'Save' button to capture the history of the data.

- The data history is saved with the experiment.
- To review the data, click the pull-down arrow attached to the *Review Suffix* menu item. Select the particular data set you wish to review and click the 'More' button to load the data into a graph.

Clear Erases the data in the graph and restarts the data acquisition. Be sure to click the 'Save' button first, or this data will be lost!

Note Remember to click the '?' (question mark) button to the right of any menu item for a description of its function.

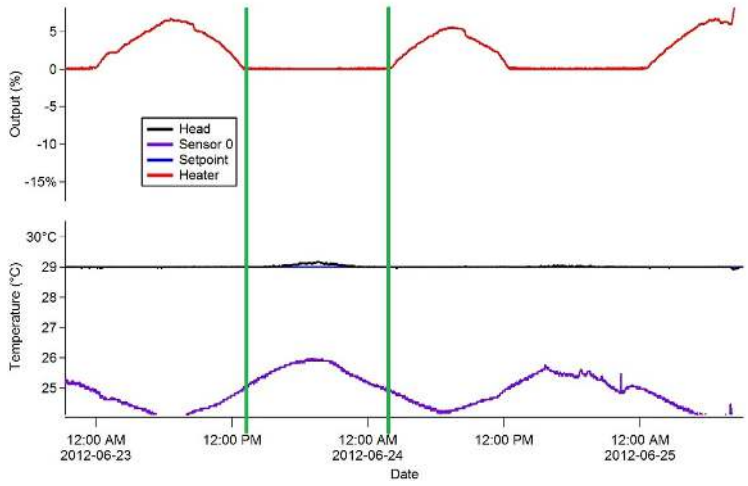


Figure 36.3.: Sample Data Log with ATC unit under feedback set for 29 degrees C.

Some examples of logged data are shown in Figure 36.3 on page 431. This particular data log shows an ATC unit under Feedback set for 29 degrees C. The fan was set to 100%

Notice The data between the green lines. The Feedback loop has shut the heater power off. The Cypher was already warmed up, so all that needed to happen was for the fan to cool the head sensor down. At this point, the ambient temperature is rising high enough so that the fans cannot cool the enclosure temperature down to maintain 29 degrees. This is indicated by the head temperature increasing above the Target Temperature.

Note This is an example where the Target Temperature should be raised a degree to allow the ATC to regulate the enclosure air temperature better. Ideally, the target temperature is set so that the ATC never applies 0% power. This means that when your lab is at its maximum daily temperature swing, that the heater is still able to control temperature.

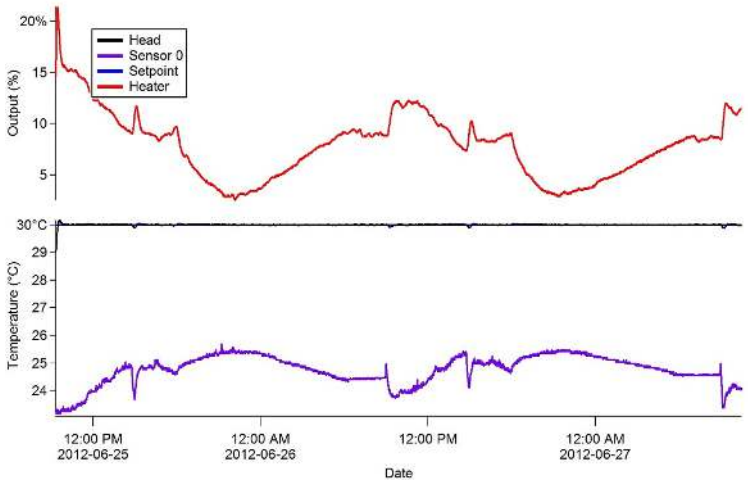


Figure 36.4.: Sample Data Log with the Target Temperature set to 30 degrees C.

This is an example of the ATC running with the Target Temperature set to 30 degrees C.

Notice

- The Head temperature is now equal to the target temperature.
- The Heater power is between 2 and 15%. The ATC fan is still at 100% so air flow is constant.

Note This is an example where the Target temperature is set high enough above the room temperature to allow the Head temperature to remain constant.

36.8. Using the Remote Temperature Sensor

36.8.1. Inserting the Sensor Inside the Cypher Enclosure

Step 4 on page 424 already suggests using the remote sensor for measuring room temperature, but there are other uses. The Remote Temperature Sensor can be used to monitor the temperature of a particular location inside the Cypher enclosure and can even be used as a feedback source for the ATC.

To insert the sensor indie the Cypher enclosure:

1. Turn off power to the ARC2 controller and the ATC box.

2.

Connecting the sensor:

- Plug the remote temperature sensor into either Sensor input connector on the back of the ATC unit.
- The temperature is displayed on the corresponding sensor channel in the ATC control window.
- Hold the sensor between your fingers and watch the temperature rise as a test.
- Log the sensor data in the data logger as an additional channel.

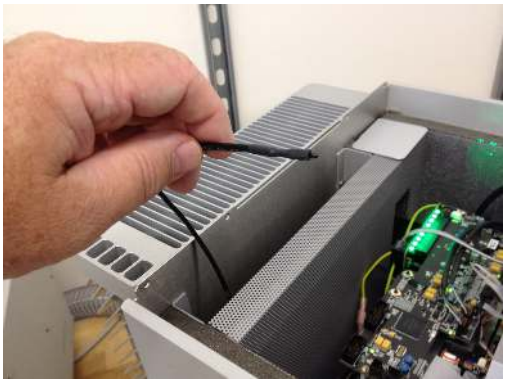
Note Additional sensors can be purchased from Asylum Research.

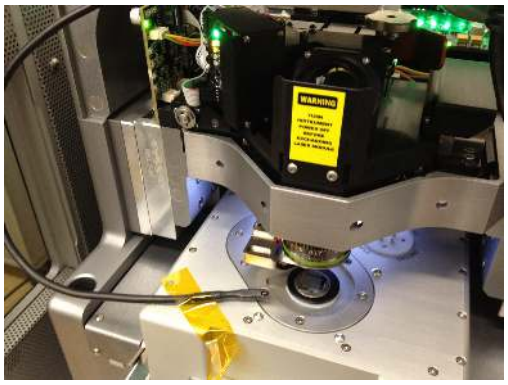
3. Remove the top of your Cypher enclosure by following these instructions: **??** on page **??**.

4.

Insert Sensor inside the Cypher enclosure:

- Insert the sensor into the exhaust port on the back of the enclosure.
- Pull the sensor cable up through the space in the back of the enclosure.
- Locate and secure the sensor in the position you wish to monitor.





5. Replace the top of the enclosure.
6. Turn the ARC2 and ATC power back on.
7. You can now monitor the sensor from the ATC Panel or its data logging function.

36.8.2. Using the Remote Sensor for Temperature Feedback Control

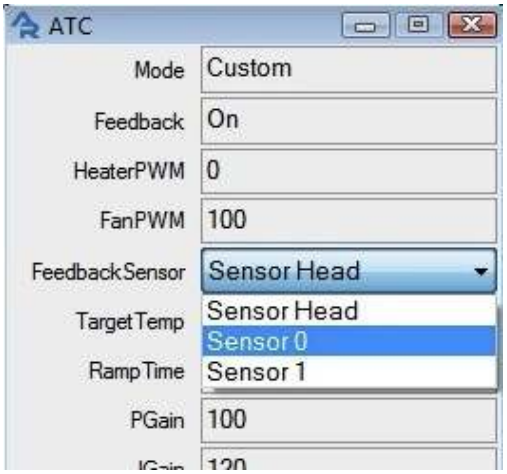
1. **Open the ATC default parameter window:**

   - Click the 'Device List' button (gear icon).
   - Select *ATC > Parameters > Default*.

2. **Select the Feedback Sensor:**

   - Select the sensor input channel to use from the 'Feedback Sensor' drop-down menu.
   - Click the 'Write' button to set the change.

Note This only changes the feedback sensor temporarily. The software defaults to *Head Sensor* when you exit the software.

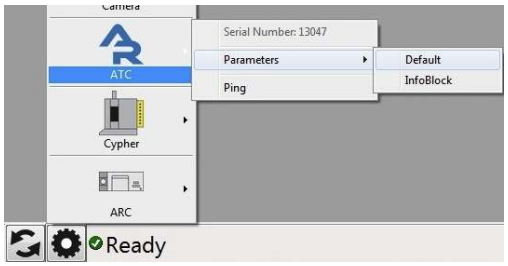


36.8.3. Permanently Assigning Feedback Control to the Remote Sensor

Note Permanently assigning feedback control to the remote sensor is not advised in a multi-user facility or for long term use. If you feel strongly that the location of the standard head feedback sensor should be different, it can be relocated. The sensor is on a 12" cable and can be moved, or a replacement sensor can be purchased and substituted, leaving the original one in place. Please contact Asylum Research for additional information.

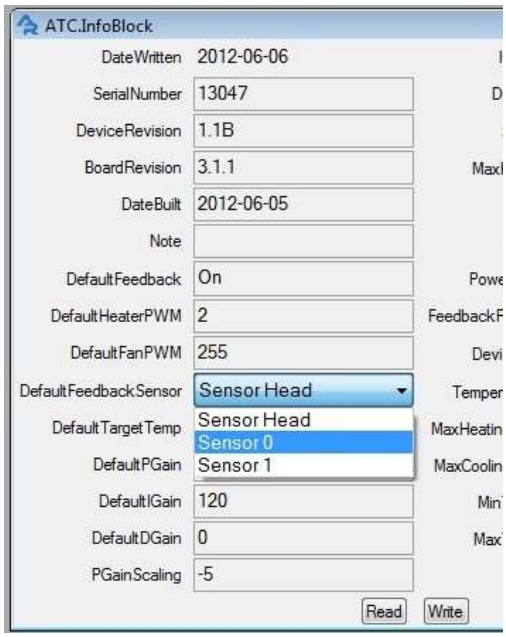
1. **Open the ATC default parameter window:**

   - Click the 'Device List' button (gear icon).
   - Select *ATC > Parameters > InfoBlock*.



2. **Permanently select the remote feedback sensor:**

   - Select the sensor input channel you want to use from the 'Feedback Sensor' drop-down menu.
   - Click the 'Write' button to save the change.
   - Rescan the Smart Start Bus by clicking the "blue spiral" button, or close and reopen the software.



36.9. Error Messages

36.9.1. ATC Messages

The **messages** area on the ATC control panel (see Figure 36.2 on page 425) shows the current operating state of the ATC. The window is typically green, indicating normal operation with no errors. If an error occurs, the message box turns red. Clicking the 'Details' button displays a description of the error in a pop-up window.

36.9.2. Critical Error Messages

Any time a critical error is generated, the ATC will go into a state where all heating capability is disabled. A critical error requires that a service technician reset the system. In the event of a critical error, please contact Asylum Research for assistance. A report log is generated by the system computer which can be sent to us to help diagnose the reason for the error. Go to open: my documents\asylumresearch\devices\ATC. In the ATC folder, you will see a file called ERRORDUMP.PXP. If there is more than one error dump file, please send us the newest one along with a brief description of any events that may be helpful in troubleshooting the problem.

36.9.2.1. Overheating

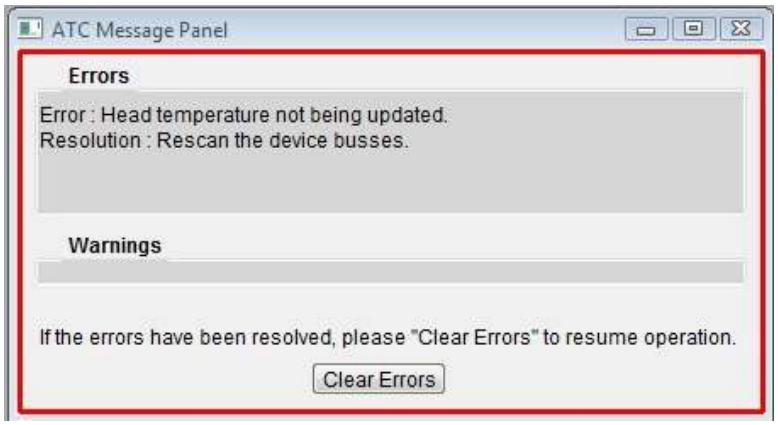
This error is the result of the ATC overheating internally, which has caused one of the two thermal switches to trip. An audible high-pitched alarm will also sound. Possible reasons for this error happening include:

- The heater power is set too high for too long in Manual mode. This causes the heater case to overheat and trip its safety switch. To avoid this, do NOT drive the heater power at maximum power for more than a minute or keep the heater case temperature below 50C.
- The exit air is too hot, causing the thermal switch in the exit air to trip. It is possible to run the heater at maximum power with the fan set to 100% for a longer period of time. This will keep the heater case cool but will generate too much hot air and overheat the heater hose. The exit air switch trips at about 70C.
- The door on the enclosure is not latched completely. If the door is left ajar, it may be closed enough to keep the door detection switch closed. This essentially allows the air to escape and forces the ATC to ramp up the heat to try to compensate for the heat loss.
- The ATC air hose has fallen off or is disconnected. Same situation as above. The interior of the Cypher enclosure is cooling down while the ATC continues to heat up in trying to compensate.

36.9.3. Noncritical Error Messages

Noncritical error messages indicate a problem with the setup and can be cleared after the fault is corrected. After the error is cleared, the message box turns green, and full function of the ATC is restored.

36.9.3.1. Head Temperature Not Updating

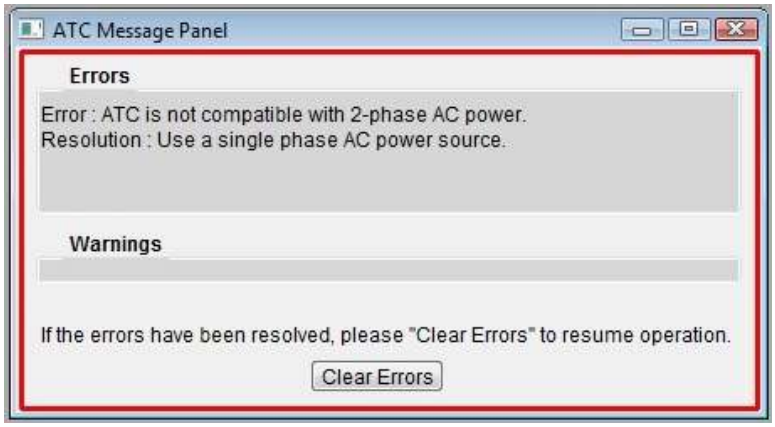


This error is caused by the Feedback Sensor (Head Sensor) not being read. The possible causes can be as follows:

- The ATC power was shut off for more than a minute while the software was running.
- A change was made to the ATC's InfoBlock programming.
- The software was started with the ATC off, and then the ATC power was turned on.

Note Click on the blue spiral button to the left of the device list. This will rescan the smart start bus. Alternately, you can power cycle the ARC2 controller to force a smart start rescan. Finally, you can exit and reenter the software.

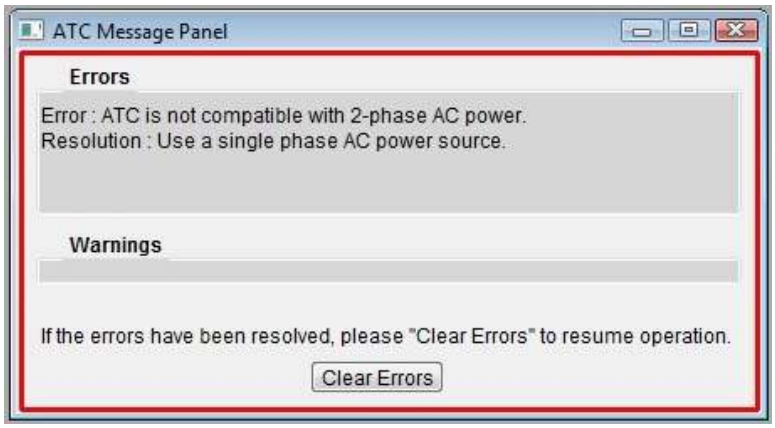
36.9.3.2. Reverse AC Power Polarity



This error is caused by faulty wiring in the wall receptacle. This usually happens only on new installations, or if you've relocated the instrument to a new location. Reverse plug on non-polarized receptacles.

Note Contact your facilities personnel to confirm correct wiring exists. Once repaired, you can clear the error.

36.9.3.3. 2 Phase AC Power



The ATC requires single phase power. This error is usually caused by the wrong type of wall power, or one of the following reasons:

- The earth ground connection may be faulty.
- There is current flowing in the neutral power line, which may indicate a disconnected neutral line, and the return path is through the earth ground connection.

Note Contact your facilities personnel to confirm correct wiring exists. Once repaired, you can clear the error.

36.10. Alarms

The ATC has two thermal switches that will trip an audible alarm when their respective temperature is reached. The alarm is an indication that an unsafe temperature has been reached inside the ATC. This condition will generate a critical error to display in the status box and cause the ATC to go into a disabled state until the error is cleared by a factory service technician.

In the event that the alarm sounds, and the ATC does not shut the heater power off, an additional thermal switch on the main AC power will activate turning the unit completely off. This switch will reset after the ATC has cooled down but will reactivate if the unit continues to overheat.

Part VI

Setup, Shipping, Maintenance, and Troubleshooting

Who is this part for? Every new user should read the safety section at least once. If you need to move your AFM or ship it to Asylum Research for any reason, please consult this chapter. Beyond that, this portion of the manual will probably not see much day-to-day use.

Part Contents

37. Shipping or Moving

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Warning	If you are contemplating moving or relocating your Cypher AFM, please contact your nearest Asylum Reseach office or distributor or send an e-mail to support@asylumresearch.com. The instrument can be damaged if moved improperly or taken apart incorrectly. Opening the enclosure may also lead to unsafe situations, as the enclosure protects the user from potentially harmful electronics and laser radiation!

38. Troubleshooting and Maintenance

CHAPTER REV. 2425, DATED 08/19/2021, 18:32. USER GUIDE REV. 2438, DATED 09/05/2021, 18:28.

Chapter Contents

38.1. Maintenance

Cypher is a maintenance-free instrument. The exterior housing can be cleaned with a damp cloth using a nonaggressive solvent, such as isopropyl alcohol.

There are no user-maintainable or user-serviceable parts inside the instrument.

If necessary, fuses in the AFM controller can be replaced as needed. (See Chapter 3 on page 24.)

38.2. Using a USB drive Causes Cypher Communication Loss

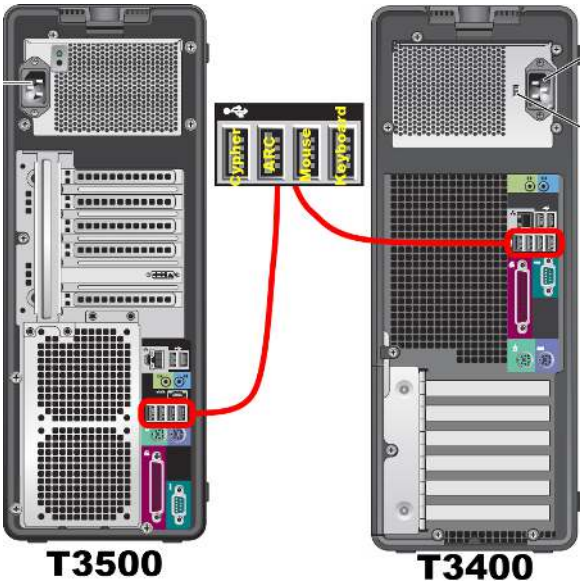
38.2.1. Problem Summary

If a USB memory stick or portable USB drive is plugged into the front panel of the computer, it can cause all sorts of communication errors to show up in the Igor software log window. Remember to check for this before reporting any communication errors.

38.2.2. Workarounds

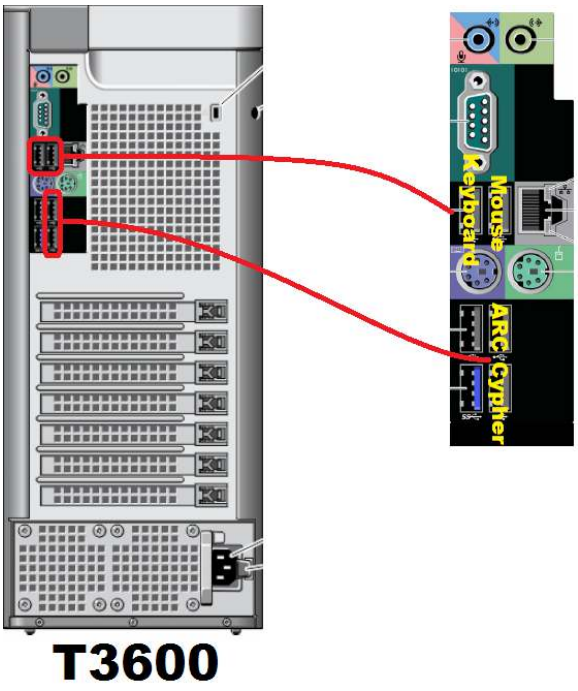
38.2.2.1. Dell T3400 and T3500

- Plug Cypher, ARC, mouse, and keyboard into the **bottom USB ports**, as shown in the picture below.
- USB 2.0 devices like USB drives, printers, and LCD monitor hubs may now safely be plugged into the remaining non-circled ports, including the two USB ports on the front of the computer.



38.2.2.2. Dell T3600

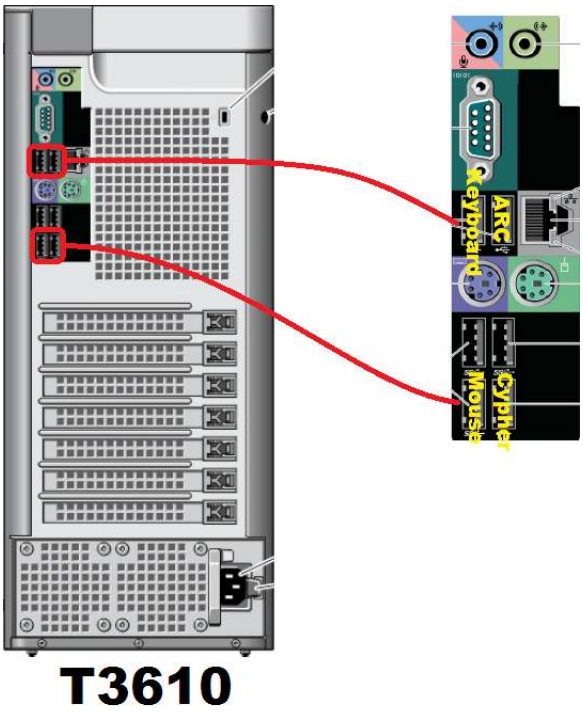
- Plug Cypher and ARC into the **bottom-right USB ports**, as shown in the picture below. Do NOT plug Cypher or ARC into the USB 3.0 ports (marked SS for SuperSpeed).
- Plug mouse and keyboard into the **top USB ports**, as shown below.
- USB 2.0 and 3.0 devices like USB drives, printers, and LCD monitor hubs may now safely be plugged into the remaining non-circled ports, including the four USB ports on the front of the computer.



38.2.2.3. Dell T3610

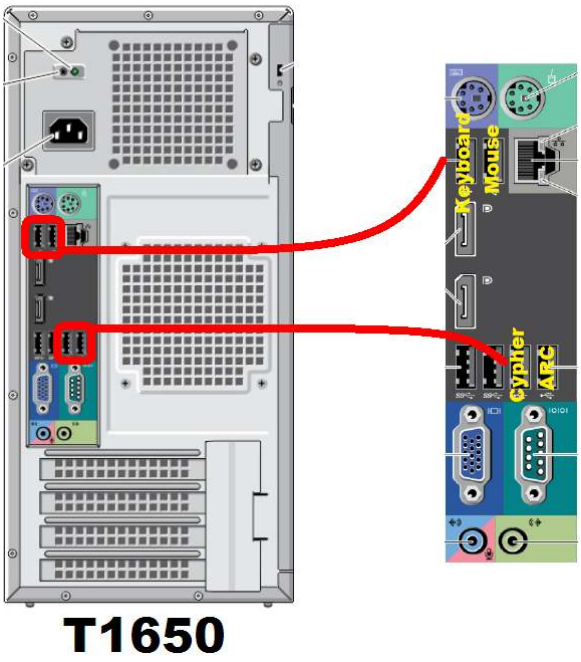
- Plug ARC into the **top-right USB port**, as shown in the picture below.

- Plug Cypher into the **bottom-right USB port**, as shown below.
- Do not plug Cypher or ARC into the USB 3.0 ports (marked SS for SuperSpeed).
- Plug keyboard into the **top-left USB port**, as shown below.
- Plug mouse into the **bottom-left USB port**, as shown below.
- USB 2.0 and 3.0 devices like USB drives and printers and LCD monitor hubs may now safely be plugged into the remaining non-circled ports, including the four USB ports on the front of the computer.



38.2.2.4. Dell T1650

- Plug Cypher and ARC into the ***bottom-right USB ports*** as shown in the picture below.
- Plug mouse and keyboard into the ***top USB ports*** as shown below.
- USB 2.0 devices like USB drives and printers and LCD monitor hubs may now safely be plugged into the remaining non-circled ports, including the four USB ports on the front of the computer.



38.2.2.5. Computers that do not have two USB 2.0 host controllers

Buy another USB 2.0 host controller on a card. Plug Cypher, and only Cypher, into it.

Part VII

Bibliography, Glossary, and Index

Part Contents

PART CONTENTS

Bibliography

Cited Scientific References

Cited Asylum Research Documents

Applications Guide, Chapter: AC Mode Imaging in Air.

Applications Guide, Chapter: Conductive AFM.

Applications Guide, Chapter: PFM Using DART.

Applications Guide, Chapter: Single Frequency PFM.

Applications Guide, Chapter: Thermals.

MFP-3D User Guide, Chapter: Tutorial: AC Mode Imaging in Air.

Index

About the index:

- Page numbers are preceded by the user guide part. The two are separated by a dash.
- **Bold** printed page numbers are references to the definition of terms.
- Other page numbers indicate the use of a term.