MFP 3D User Guide

Including beta (complete, reviewed) chapters.

Version 13, Revision: A-1715

Dated 10/28/2013

Asylum Research

an Oxford Instruments company

Contents

Introduction

The MFP-3D Atomic Force Microscope (SPM) manual comes in volumes. To date these volumes are:

Part I *Basic Operation Guide and Tutorials.* This part has detailed step by step tutorials on how to image basic sample topography in air and fluid. If you do not know which part of the user guide is for you, this is probably it.

Part II *Advanced Imaging Hardware.* This part covers the use of advanced hardware accessories such as fluid cells and sample heaters.

Part III *System Safety, Specifications, Set-Up, and Relocation.* This part covers placement and set-up of the instrument, essential safety information, and shipping/packing instructions.

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 There are many ways to get help with your Asylum Research instrument, and it is always free:

- Join the support site and download software, current manuals, and ask questions in our user forum. http://support.asylumresearch.com. Note that all Asylum scientists are forum members and frequent contributors.
- E-mail us at support@asylumresearch.com.
- Call your local office or distributor.
- Call us at +1-805-696-6466. During US west coast business hours you will get a human being to speak with. After hours you still have a good chance of catching one of our scientists. Within the US you can call our toll free number if you wish (1-888-472-2795).
- If necessary we can initiate a remote session and have one of our scientists operate your AFM over the internet.

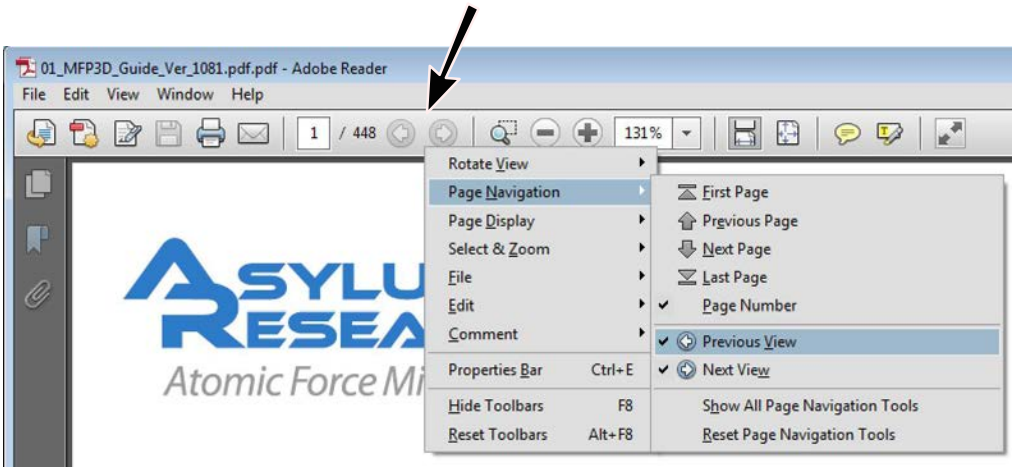
Updates to the Manual Bundled with the software updates.

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 your commenting on.

PDF reader setup

This document makes frequent use of links from one section to another. This reduces repetition of material, but does require some flipping back and forth. To make this process easier, please make the following adjustments to your pdf reader.

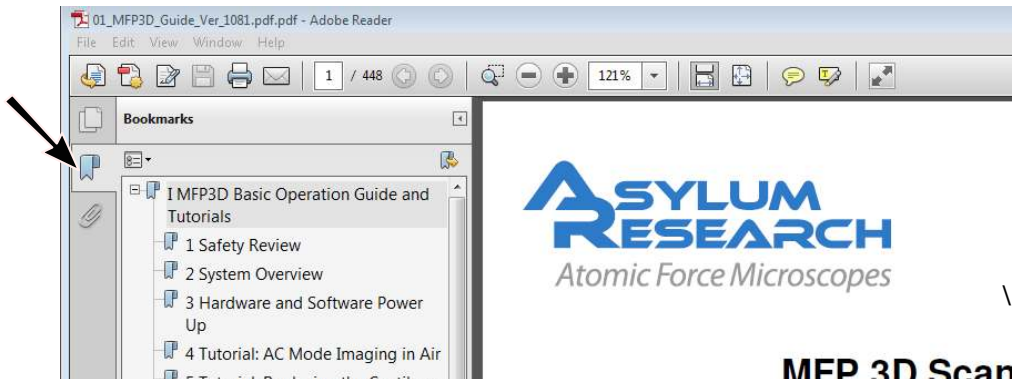
Adobe Reader (PC or Mac)

1. 

   Add Page Navigation:

   - Right click on the toolbar.
   - From the pop-up menu, select *Page Navigation*.
   - From the sub pop-up menu select *Previous View* and *Next View*.
   - This will place two buttons (see the arrow in the figure above) in the toolbar which function like the *Back* and *Forward* buttons on any web browser.

2. When reading a section in the manual which refers to another page, click on the link to jump to another page. When done reading, click the *Previous View arrow* in the toolbar to go back to the original page.

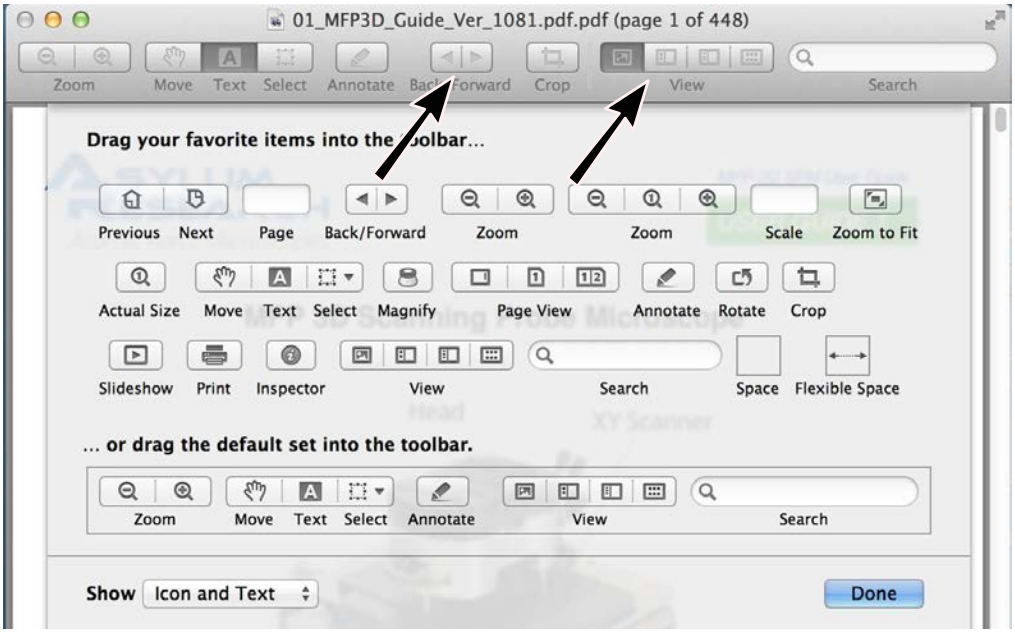
3. 

   Bring up the table of contents:

   - Click the icon indicated by the arrow in the figure above.
   - Keep the table of contents at the left of the screen to navigate the manual.

4. Adobe Reader should remember these changes the next time the program is opened.

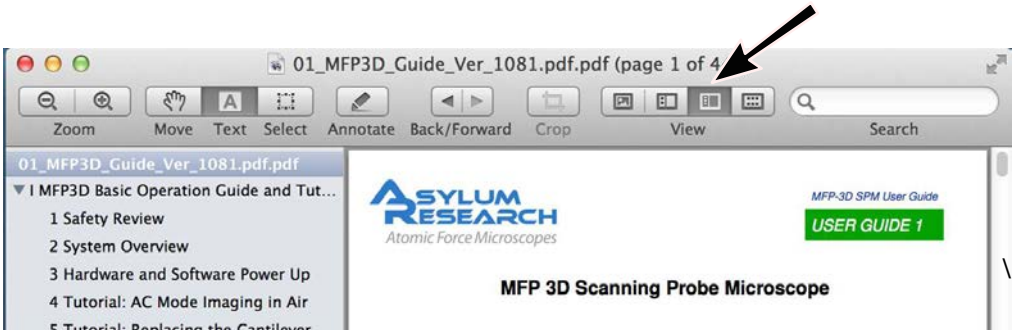
Preview (Mac)

1. 

Add Back/Forward and View buttons:

- Right click on the toolbar.
- Drag the *Back/Forward* and *View* tools into the toolbar.
- Click *Done*.

2. When reading a section in the manual which refers to another page, click on the link to jump to another page. When done reading, click the *Back Arrow* in the toolbar to go back to the original page.Appication

3. 

Bring up the table of contents:

- Click the icon indicated by the arrow in the figure above.
- Keep the table of contents at the left of the screen to navigate the manual.

4. The Preview Application should remember these changes the next time the program is opened.

Part I

MFP3D Basic Operation Guide and Tutorials

Part I: Who is it for? After the MFP-3D AFM has been professionally installed in your lab, and you or someone in your facility has completed the initial training (at the time of the installation), this volume will be the principal reference for operating the instrument. Although this volume is written with the novice user in mind, experienced users should complete the basic imaging tutorial at least once before attempting to use the MFP-3D.

Part Contents

1. Safety Review

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

It is recommended that everyone who uses the MFP-3D AFM read Chapter 26 on page 402 at least once. As a reminder, here are the important points:

- The AFM head contains a laser-like SLD light source. The light is in the near infrared so you cannot see it with your eye, and your blink reflex will not protect you. The beam is diverging, so it is only intense enough if viewed though an appropriate magnifier (an unlikely event) or if your eye is brought very close to the location of the cantilever (also an unlikely event). The head contains tilt switches which turn the light source off if it is not standing upright on its legs. So in general, the light is a relatively weak and diverging beam which is only on when the head is in a level position, with the beam shining downward.
- The AFM's XYZ piezos operate on voltages up to 165 V_{DC}, and with sufficient current to be harmful to human life. Note that the cables connecting the controller to the base and the head and scanner to the base carry these voltages. Do not pinch or cut these cables. Turn off the controller before disconnecting any of these cables. It is safest to plug them all in and then turn on the controller. Also do not remove any covers from the controller or other instrument components while the controller is turned on or plugged into an AC outlet. Dangerous voltages are exposed with the covers removed.
- Respect the weight of the AFM head as you handle it, the original model heads weighs 14 pounds, and can be difficult to lift with one hand. Get a good grip on the head with both hands when removing it or carrying it around. If dropped on your hand or foot (even from a small height) it could cause injury.

In a nutshell, this is an industrial/scientific research grade instrument. While it is quite safe, even compared to an electric drill or home cooking stove, there are a few possible dangers of which the user should always be aware.

2. System Overview

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

This section will establish names for the various parts of your AFM system. The system should have been set up, connected, and tested by an Asylum Research scientist. For more information on troubleshooting any connections, please refer to Chapter 9 on page 78.

2.1. Basic MFP-3D SA Hardware

2.1.1. Major Hardware Components

The MFP-3D Stand Alone (SA) AFM system and its various components are shown in Figure 2.1 on page 6, and explained briefly (in alphabetical order) below:

Acoustic Enclosure Also called "the hood". A heavy steel chamber with special acoustic damping materials and vibration isolating pads under the legs. Keeps lab noise and vibrations from affecting high resolution AFM images. Also shields the instrument from air currents which degrade imaging stability. The typical lab will require an acoustic enclosure to get the best performance out of the MFP-3D AFM.

AFM The parts sitting inside the enclosure are typically referred to as the AFM. The main components are, from top to bottom (also see Figure 2.2b on page 7):

Head The AFM component which holds the cantilever chip and contains the optical lever detection system, and electronics and the vertical (Z) motion actuator and sensor. In short, it moves the cantilever vertically as the sample moves laterally beneath it. It also contains optics for illuminating and optically imaging the sample and cantilever from above.

Scanner The AFM component which holds the sample and scans it laterally in X and Y beneath the tip. It contains piezoelectric actuators, flexure based translations stages, and high resolution position sensors.

Base The metal plate on which the head and scanner sit. It typically contains a rudimentary optical microscope with CCD cameras and sample illumination controls for optically viewing the sample and cantilever. It also contains critical signal conditioning electronics for the scanner and acts as the electronic hub for connecting the controller to the scanner and head and it routes CCD output to the computer.

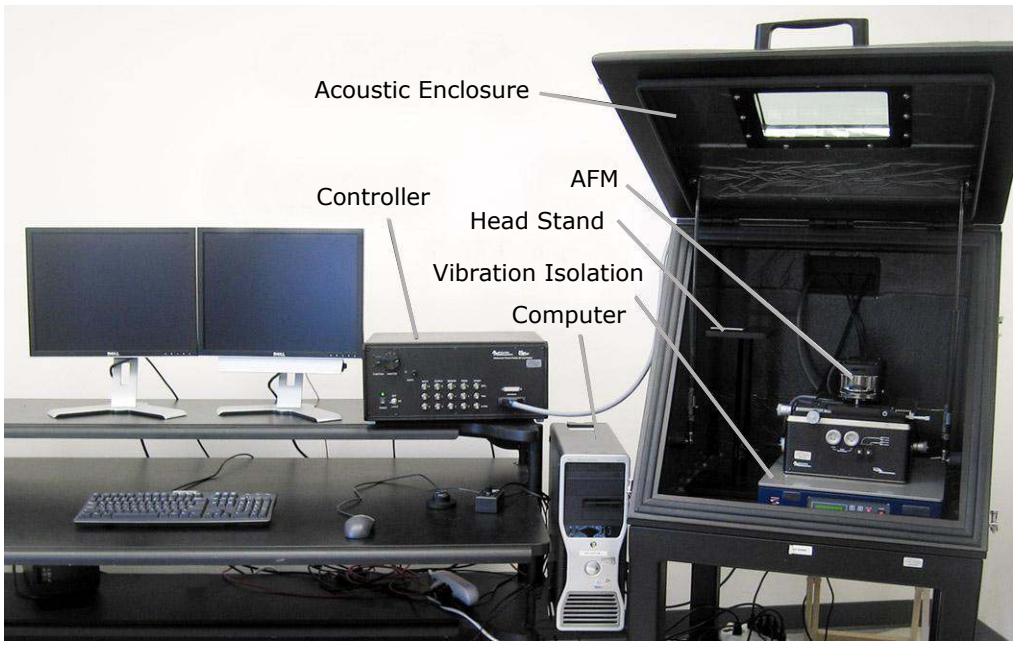


Figure 2.1.: Ideally the MFP-3D AFM with Stand Alone Base is set up as shown, with the controller and computer on one table and the AFM inside an acoustic enclosure. Note that newer systems operate with the ARC2 controller (see **??** on page **??**).

Computer The computer is the primary interface for controlling the microscope and its main communication is via a USB1.1 connection to the ARC2.

Controller The controller houses power supplies and the necessary electronics for controlling the scan motion and acquiring image data from the microscope. Your MFP-3D AFM system will be equipped with a black MFP-3D AFM controller or the newer ARC2 SPM controller (see Figure 2.2a on page 7). Either will work identically with the AFM and Software discussed in this user guide.

Fiber Lite Halogen dimming light source with fiber optic light guide. Connects to the base and illuminates the sample for optical viewing.

Head Stand Platform attached to the side of the acoustic enclosure. A place to store the head when loading samples or changing cantilevers. The rear legs of the head should be placed in the two holes at the back of the head stand.

Vibration Isolation Typically an active vibration isolation table and occasionally a passive vibration isolation platform. In most labs this is essential equipment for high resolution imaging.

Q SA or "Stand Alone", what does that mean?

A "Stand Alone" refers to the optical microscope on which the AFM head and scanner rest. In this case it is a compact, purpose-built microscope also referred to as the "Stand Alone Base". The other option is a third party (Olympus, Nikon, Zeiss) inverted optical (IO) microscope with a custom Asylum Research Base plate mounted on top to support the AFM head and scanner. That custom base plate is typically called the IO base (IO = Inverted Optical). So the IO Base is designed to go with an IO microscope while the Stand Alone base, well, stands alone.

(a) Newer MFP-3D systems ship with the ARC2 controller

(b) Basic AFM Components

Figure 2.2.: Some Instrument Details and Names.

2.1.2. AFM Head Controls

The controls on the AFM head are as follows, in alphabetical order (See Figure 2.3 on page 8):

Front leg thumb wheel Clockwise motion lengthens the leg and raises the cantilever and head away from the sample. Also changes the level (pitch) of the head.

LDX thumb wheel Moves the laser spot along the length of the cantilever. Counterclockwise rotation moves the spot from the cantilever base to the tip, as indicated by the graphic on top of the head.

LDY thumb wheel Moves the laser spot perpendicular to the length of the cantilever. Counterclockwise rotation moves the spot as indicated by the graphic on top of the head.

PD thumb wheel Centers the reflected beam on the head's photo diode detector. This is also known as "zero-ing the cantilever deflection".

Rear leg thumb wheels Clockwise motion lengthens each leg and raises the cantilever and head away from the sample. Can change the level (roll) of the head.

Top view objective focus wheel Focuses the top view optical image of the cantilever or sample.

XY mirror movement Translates the top view optical image of the cantilever or sample.

2.1.3. AFM Base Controls and Components

The controls and features on SA base are as follows (See Figure 2.4 on page 9):

Aperture Diaphragm A knurled metal ring around the entry point of the optical fiber illumination. Controls the amount of light that enters the base. Used for adjusting contrast and light intensity.

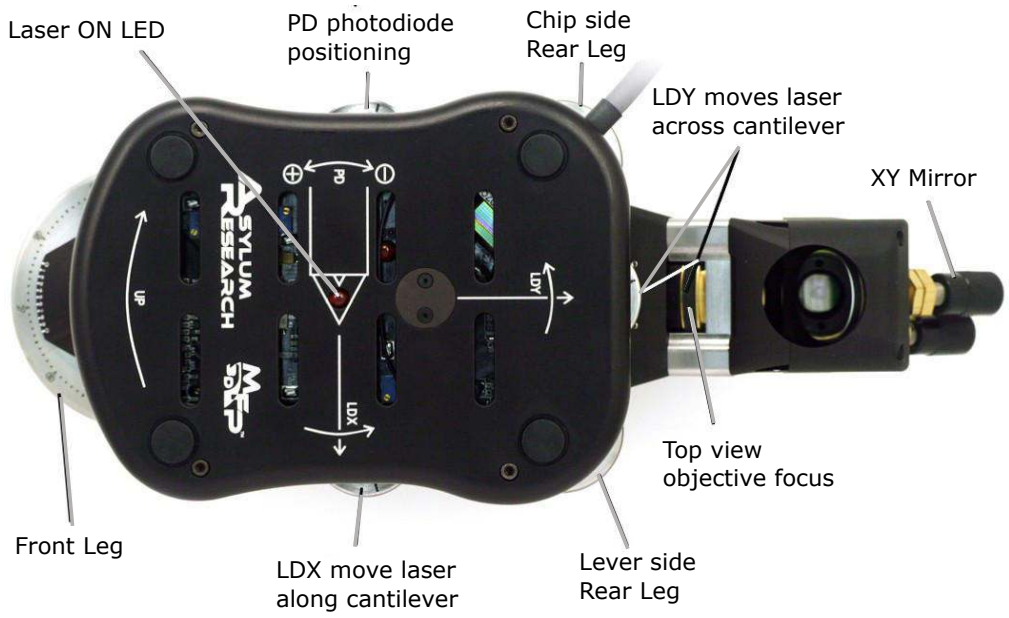


Figure 2.3.: Top view of the AFM head with named controls. Note the cantilever shape in the middle (same orientation as the real cantilever) and the LED at the tip of this lever. The red LED lights up when the laser is on (but not necessarily focused on the tip).

Base Plate Top surface of the SA base on which the head and scanner sit.

Bottom and Top View Field Diaphragm Diaphragms used to enhance contrast on the top and bottom view images.

Bottom View Focus A large knob which focuses the bottom view objective.

Camera Selector Directs the image from the top or bottom view objectives to the CCD camera built into the base.

Driver Bar Pushes the scanner for XY sample alignment.

Illumination Selector Directs illumination to the top or bottom of the sample. For instance, top view on an opaque sample will require top illumination, but a transparent sample will require bottom view illumination.

Leg Hole Holes in the scanner through which the legs on the head can rest on the base plate.

Magnetic Sample Clamp Magnets (part number 910.004) provided with your system for holding microscope slides to the scanner top plate.

Optical Align X and Y Controls the movement of the entire base plate relative to the base and the bottom view microscope objective. A way to translate the cantilever and sample in the field of view of the bottom view camera.

Optics Window Directs light and images between the base and head. The top view objective is only half a microscope. The other half resides in the base.

Sample Typically mounted on a standard microscope slide.

Sample Align X and Y Controls the lateral movement of the entire scanner. Used to move the sample relative to the cantilever, to select a place to image.

Figure 2.4.: Controls on the SA Base. Missing from view are (on the left) the aperture diaphragm which is a knurled ring around the entry point of the fiber optic illumination and (on the right) a large knob for focusing the bottom view objective.

Scanner Electromechanical scanning stage which moves the sample laterally in X and Y beneath the head and cantilever.

Scanner Top Plate Moving part of the scanner onto which samples are placed.

3. Hardware and Software Power Up

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

Before you start any of the following tutorials, the system must be properly started up.

Before you start: We assume you understand the aspects of running this system safely: (Chapter 26 on page 402)

1. Turn on and enable the active vibration isolation platform under your AFM if you have it.
2. In no particular order, boot up the computer and turn on the MFP-3D AFM by depressing the power switch on the front of the controller (ARC2 or MFP-3D model). When all is working properly a green light should show on the face of the controller.
3. Double check that the laser key on the controller is in the ON position. Then double check that the red LED light on top of the AFM head is on.
4. Locate the shortcut to the software on the desktop, and double click on the icon to start the software.

System check:

5.
   - During initialization the software will not be responsive. Look at the bottom status bar for the initializing message.

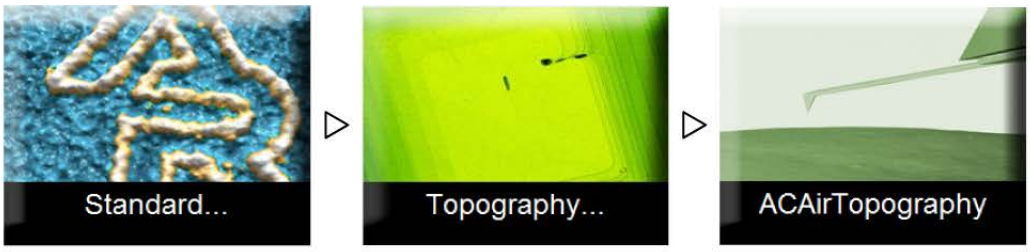
   - When the software is done the status bar will say it is ready.

6. **System check:**

- At the bottom of the software you will find the status bar.
- These areas will be described in more details in Section 3.1.2 on page 13.
- Location 1, 2 and 5 should read Ready
- If some components are reported missing, check their connections. Once you have the cables secured and powered, click on the rescan bus (3). If that does not solve the problem, please contact support.

7. **The Mode Master window:**

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

8. 

Select Mode:

- Select *Standard* ▷ *Topography* ▷ *ACAirTopography*
- The screen will now re-arrange and present all the controls necessary for this type of AFM imaging.

3.1. 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 stand alone 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 launched the software you opened 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.

Figure 3.1.: Typical start up screen for the Asylum Research SPM Software, after the mode master panel has been closed. A few image panels have been left off the right of the screen, which usually extends across the second monitor of your system.

Refer to the screen shot in Figure 3.1 on page 12 as we introduce the various controls and data displays for the often used AC Mode imaging technique. We'll go clockwise from the upper left. (Note that if you are viewing this file on a computer, you can zoom into the screen shot for a closer look.)

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

Main AFM imaging, see Chapter 4 on page 15.

Thermal Cantilever thermal spectroscopy see the ARApplicationsGuide.pdf, Section on spring constant calibration.

Force Cantilever force vs distance curves.

Tune Cantilever resonance tuning, see Section 4.7 on page 33.

Fmap Maps of force vs 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) The Igor Command window has two parts: the history and the command line. On occasion items executed by clicking software buttons will generate some output here. Power users will type commands at the command line to accomplish a variety of advanced tasks. If you followed the Igor getting started recommended in the Tip on page 12 you will know all about this window.

Sum and Deflection Meter (Ctrl + 6) Also called the S&D Meter. A real time display of various data such as cantilever deflection, amplitude, piezo voltage, and various other user definable channels. Also contains buttons for engaging and withdrawing the AFM tip.

Image Windows For each active channel on the Master Channel Panel, one image will appear on the screen. They balloon to proper size as soon as scanning starts. The windows display in real time, line by line, the sample topography (height), phase, amplitude, voltage, or any other measured quantity, acquired as the sample is scanned. There is usually one such window per active tab in the Master Channel Panel (Lower left hand window). While these windows are primarily a data displays, right clicking with the mouse can activate various commands such as zoom and translate. A white area at the bottom of this window shows you a real time "oscilloscope view" of the most recent line of image data, which is very useful when tuning imaging feedback parameters.

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

A You can re-activate the panels via *AFM Controls* in the menu bar.

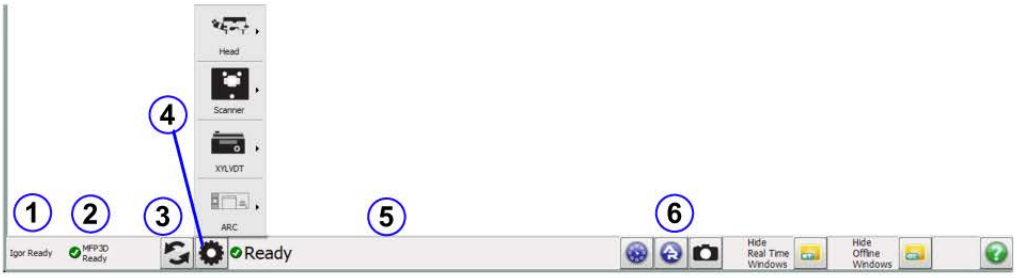
Q How do I get the mode master panel to appear again?

A Click on the on:  button near the bottom of the screen. When you select a new mode, the appropriate windows will appear and the SPM controller will re-configure itself accordingly.

3.1.1. 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 the help menu are exclusively AFM related.

3.1.2. 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.

While we do not want to get bogged down with the details of all of these controls, it is good to have a basic grasp.

1 Igor Pro Status If Igor is ready to accept a software request from you, it will say Ready. If it is busy calculating it will show an Abort Button and a rotating quartered circle.

2 System Status If you are missing hardware, or there is a critical error, it shows up here.

3 Rescan Smarstart Bus Click when adding new components to the system (heaters, different cantilever holders, etc.).

4 Device Manager Click on this gear icon to see what components are communicating with the controller. Furthermore, individual information (temperature, serial number, etc.) on each component can be accessed by clicking the triangular button to the right of each component icon.

5 Current OperationDisplays what operation the system is currently preforming, thermals, scanning, etc. Some actions have progress bars that show up here, and additional warnings can show up here as well (hard drive filling up).

6 Buttons

 Mode Master

 Master Panel

 Video Window

Hides / Shows Real Time Windows

Hides / Shows Offline Windows

 Help Browser

Realtime? Offline?

These terms have been around for a long time in image processing and acquisition system. Offline has nothing to do with network connection.

Realtime Window Displayed data that are in the process of being acquired.

Offline Window Displayed data from a saved file.

4. Tutorial: AC Mode Imaging in Air

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

This tutorial provides a quick path to learning the basic operation of the MFP-3D AFM. The tutorial lists a set of steps that will teach a new user, with a minimal to basic understanding of AFM operation, how to

- Load a probe into the cantilever holder
- Place the head onto the stage
- Align the spot onto the cantilever
- Select a cantilever drive frequency
- Engage the tip on a standard sample

Before you start:

- This tutorial is designed to be performed, not merely read. If possible, it is advised take the tutorial under the supervision of an experienced user.
- We assume you understand the aspects of running this system safely: (Chapter 26 on page 402.)
- You are familiar with the basic names of the hardware components and software controls (Chapter 2 on page 5.)
- You have powered up the System and Launched the Software: (Chapter 3 on page 10.)

Note The MFP-3D 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 the first time.

Note	There are many ways to skin a cat. There are also many ways to operate the MFP-3D AFM. These instructions assume that the AFM was left in some unknown state of alignment by a previous user. Once you have gone through the process a few times, you will find that some steps can be skipped or re-arranged to best suit your style. In Chapter 5 on page 47 there is a reduced set of steps which are applicable to changing a cantilever on a system which you have already aligned for your sample and needs.

4.1. Required Materials

- Cantilevers: You will need to collect several AC160TS or AC240TS cantilevers, which are manufactured by Olympus. The AC160TS has a spring constant of ~42N/m and a resonant frequency of ~300 kHz. The AC240TS has a spring constant of ~2N/m and a resonant frequency of ~70 kHz. Both are used for AC mode imaging in air. Every AFM ships with a package of AC160s and AC240s, but if these cantilevers are unavailable any cantilever with a similar spring constant and resonant frequency should work fine see `http://www.asylumresearch.com/ProbeStore/TYPE?Entry=JustLooking#AC%20MODE%20(AIR)`.
- Sample: The tutorial will use the Asylum Research calibration grating that ships with every system (part number 900.237). It is mounted on a standard glass microscope cover slide.
- Sharp tipped tweezers (some people prefer straight tips, others prefer curved).
- Small Phillips (+) screwdriver.

4.2. Loading the Cantilever

4.2.1. Nomenclature

Please refer to Figure 4.1 on page 17 for the names of all the parts in the cantilever holder. For overall AFM system nomenclature, please refer to Chapter 2 on page 5.

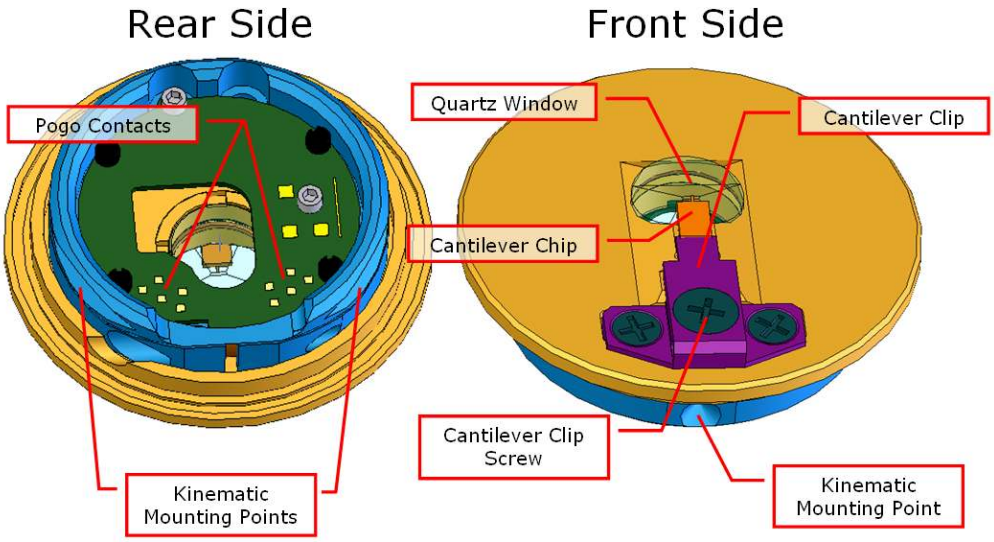


Figure 4.1.: Standard cantilever holder nomenclature

4.2.2. Instructions: Preparing the Holder

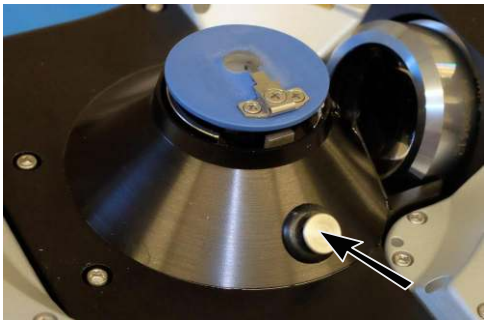
Note on Color	The cantilever holder in the following photos is translucent (Kel-F). Yours may be Blue (PEEK) or Tan (PEEK). The procedure is the same, regardless of color.

1. **Prepare cantilever mounting work area:**

- Set out your cantilever changing stand, tweezers, and box of cantilevers on a clear work surface, preferably close to the AFM so you can easily wheel your chair over.
- A low power binocular dissection microscope with light source is recommended.

2. **Remove the cantilever holder from the head:**

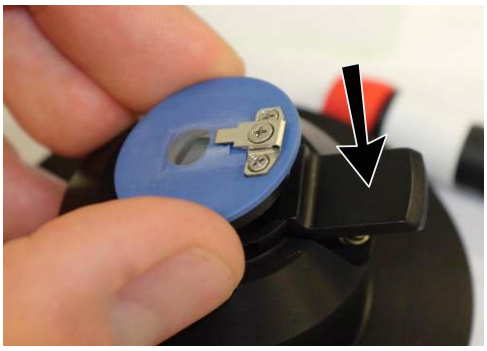
- Place the AFM head upside down (usually on the metal platform or "head stand" next to the AFM.)
- Depress the button on the head.
- Gently lift the cantilever holder straight up out of the head and carry it to your cantilever changing area.



Note Older model AFMs may have a "rubber dome" covering this button.

3. **Mount the cantilever holder:**

- Orient the cantilever holder with the clip's screws towards the lever on the stand.
- Press the lever on the stand down, as shown.
- At the same time, angle the cantilever holder into the stand. Two fixed balls in the stand, opposite the lever, must match up with the two matching kinematic mounting points on the cantilever holder.
- Lower the cantilever holder so the final kinematic mounting point lines up with the ball on the stand's lever, then release the lever.
- Inspect that the cantilever holder sits flat in the stand and that all the balls sit properly in the mounting points.



4. **Prepare your tweezers:**

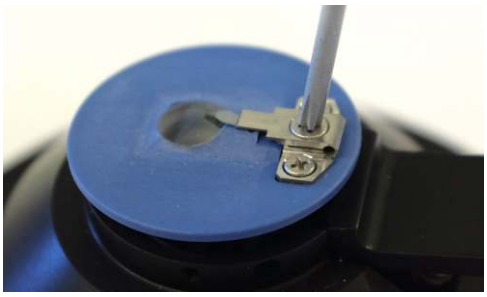
- Locate tweezers with straight sharp tips. This technique does not work well with curved tweezers.

Good Habit Cleaning the tweezer tips with alcohol will prevent cantilevers from sticking.

5.

Remove the used cantilever:

- Loosen the middle screw on the clip about one turn, just to the point of freeing the cantilever.
- Remove the old cantilever with tweezers.



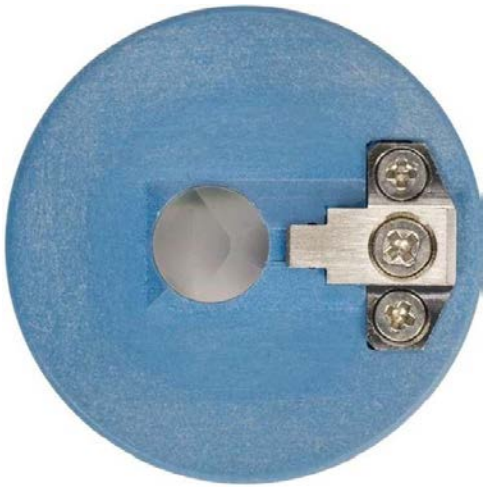
Good Habit Blow the area under the cantilever clip clean with *clean* compressed air. Bits of silicon and other debris can lead to a poorly seated cantilever and poor quality AC mode images.

Note The following steps suggest a certain method of inserting the cantilever. A quick poll at Asylum Research showed that nearly every veteran AFM operator has their own way of doing this. Please see Section 4.10.1 on page 39 for more insights. Also ask your lab mates, or practice yourself with some old cantilever chips.

6.

Prepare for cantilever mounting:

- Orient the cantilever holder in front of you as shown.
- Open the box of levers with the tips pointing to the left.
- With the tweezers in your right hand, pick up a lever. Some find it works best to hold the tweezers perpendicular to the tabletop when picking up the lever.



Note If you are left handed, you may want to reverse this process. The tweezer is usually best held in your dominant hand.

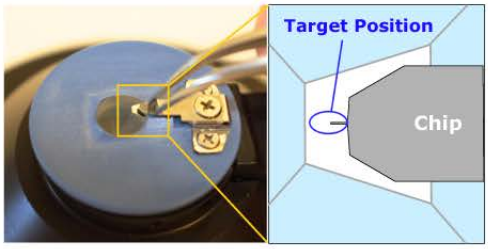
Note Close the cantilever box now. The longer you wait, the more likely you will one day put your hand into a box of 30 pristine cantilevers.

7. Close the cantilever box now. The longer you wait, the more likely you will put your hand in it later and break 30 tips at once.

8. **Insert the new cantilever:**

- With tweezers, slide the cantilever chip under the clip. Resting your wrist on the table helps keep it stable.
- Position the cantilever as shown in the clear trapezoidal shaped quartz optical window. You can do this by letting go of the chip and nudging it around with the tweezer tips.

Note Do not push the cantilever chip too far back. This can cause misalignment. We'll check for this in a few steps.

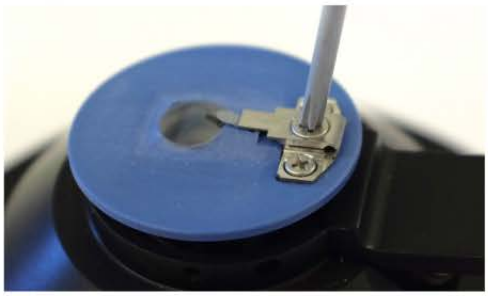


Note The quartz window is resilient to scratching from tweezers, so do not be paranoid.

9. **Tighten the screw:**

- Gently tighten the clip's screw.
- The chip should not be able to move if nudged with the tweezers. Firmly mounted probes will perform best during AC mode imaging.

Note Do not over tighten the clamp on the cantilever holder - this can crush the chip, strip the screw threads and / or result in an excessively bent clamp.

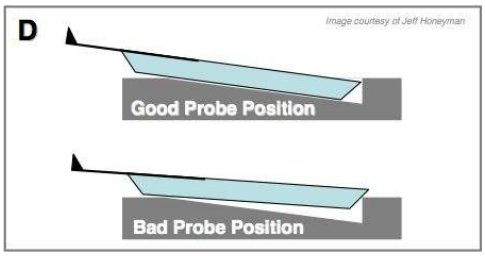


10. Close the cantilever box if you did not already do so.

4.2.3. Mounting the Cantilever Holder

Inspect the cantilever "seating":

1.
- Hold the changing stand so you can look from the side between the cantilever clip (a low power microscope or a jeweler's loupe, 10× is recommended).
- Check that the cantilever chip is parallel to the mounting surface and glass prism facet.
- If the probe is mounted too far back, it causes an improper angle, and you will not be able to align the laser when the cantilever has been loaded into the AFM. (See Section 2 on page 29 for an example of improper alignment). Correct the position by loosening the screw and moving the cantilever forward in the pocket. Tighten the screw and inspect the "seating" again.

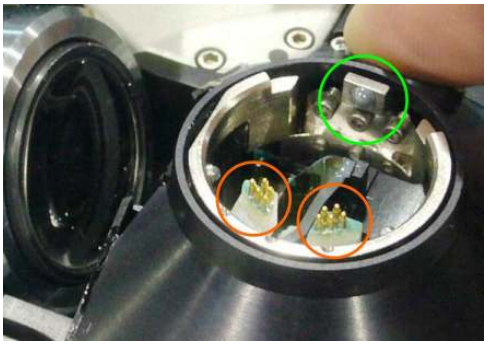


Install the cantilever holder on the head:

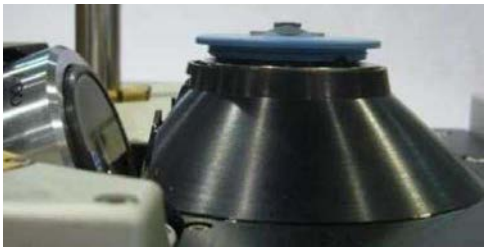
2.
- Remove the cantilever holder from the stand by depressing the lever. Take the holder over to the head.
- Insert the cantilever holder into the head. This action is similar to using the changing stand (See Step 3 on page 18) except the rubber dome on the head replaces the lever on the stand.

Warning

- Unlike the changing stand, the AFM head has a number of spring loaded electrical contacts called “pogo pins.”
- Take care not to touch these contacts with the cantilever holder during installation.
- While it is not likely that the contacts will bend or fail, some of them do carry low voltages which could short out via the cantilever holder. To prevent this, current cantilever holders have a black non conductive coating on the rear side metal parts. Be more cautious if your cantilever holder is older with uncoated metal parts.



3. **Inspect mounting of the cantilever holder:**
   - The cantilever holder should sit perfectly level with the top and bottom planes of the head.



Tips & Tricks Please see Section 4.10.1 on page 39.

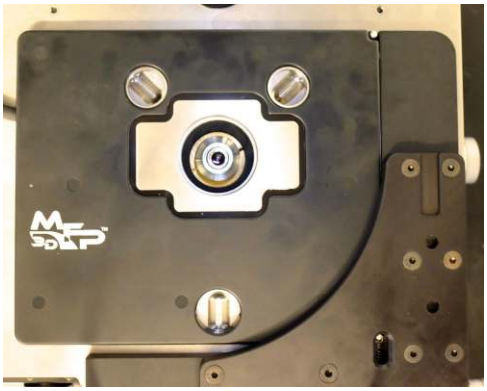
4.3. Head and Sample Placement

Refer to Section 2.1.2 on page 7 and Section 2.1.3 on page 7 to look up the names of various head and base controls.

4.3.1. Instructions: Head and Sample Placement

1. **Prepare the scanner:**

   - Remove any sample that may be on the scanner.
   - Looking from above, turn the XY sample alignment micrometers (thin knobs) until the divots or grooves in the base plate are aligned over the leg holes in the scanner.



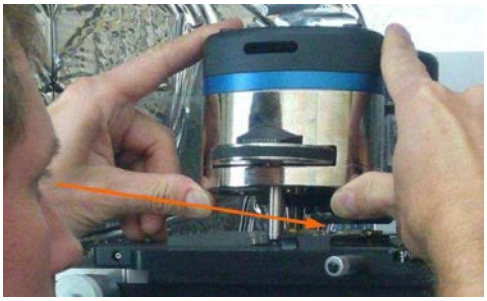
Note Base plates prior to 2009 usually only have divots for the rear legs. Current model base plates have one groove for each leg.

2. **Place the head on the base:**

   - Firmly grip the head and place it on the base. Each leg goes through one of the leg holes in the scanner.
   - Since no sample is present there is no worry about crashing the cantilever into anything.

3. **Adjust the legs on the head:**

- Look for clearance between the scanner top plate and cantilever.
- Turn the thumb wheels that control the legs to create sufficient space for the sample. Seen from above, clockwise motion raises the head; see the arrow on the top of the head for guidance.
- The goal is to have at least 1mm of clearance for the cantilever when the head is first set down over the actual sample. Be conservative on your first attempts. If you are worried, wheel the legs all the way up.
- In the process try to end up with the head reasonably level.



Tip A white piece of paper taped to the back of the hood makes for a good background against which to see the cantilever holder.

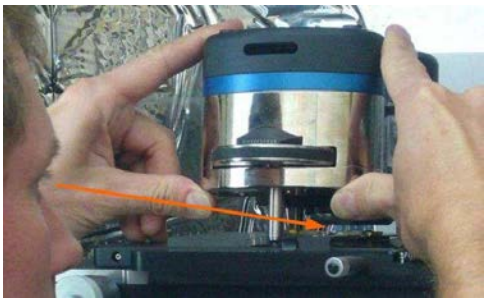
4. Remove the head from the base and place it to the side (preferably on the head stand).

5. **Place the sample on the scanner:**

- Locate the microscope mounted calibration grating (part number 900.237) that shipped with your AFM.
- Wipe the scanner top plate clean of any particles.
- Place the microscope slide on the scanner top plate, center it over the hole.
- Place the magnetic sample clamps as shown.

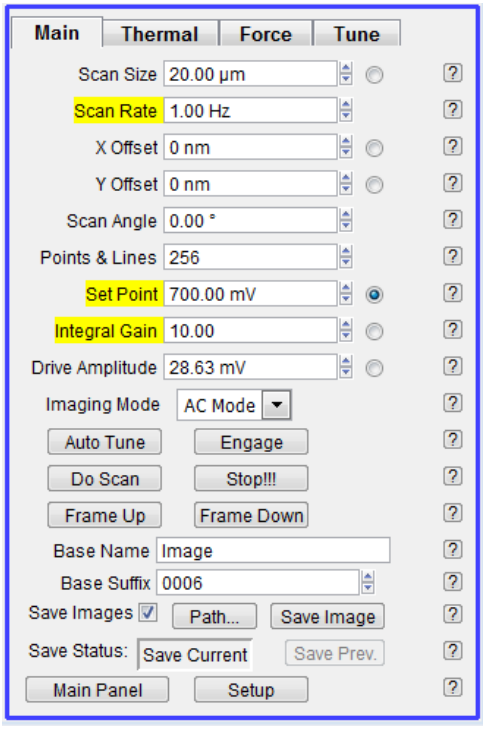
6. **Visually lower the head over the sample:**

   - Place the head on the base. As shown on the right, place the rear legs first, and then gently touch down the front leg while looking for clearance between tip and sample.
   - If it looks like the tip might crash, remove the head, place it on the head stand, extend the legs a little, and try again. Be conservative.
   - Once the head is standing on the base, adjust the legs to lower the head so there is about a millimeter of clearance between tip and sample.

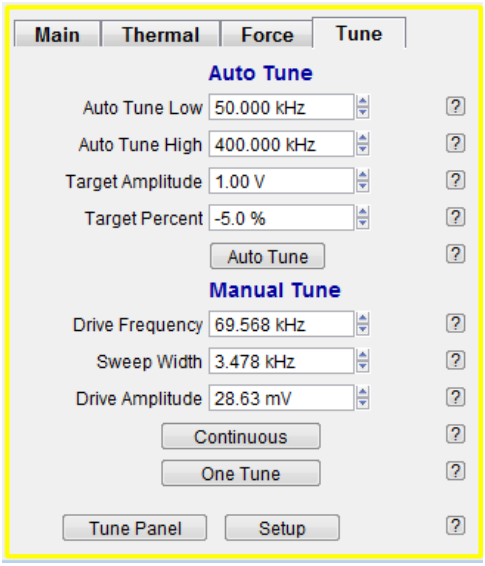


4.4. Software Prep

1. If you have not done so already, open the software as shown in Chapter 3 on page 10.



(a) Master Panel, Main Tab.



(b) Master Panel, Tune Tab.

Figure 4.2.: Main tab and Tune tab of the Master Panel

4.5. Head and Base Optics Alignment

Refer to 2.1.2 and Section 2.1.3 on page 7 to look up the names of various head and base knobs.

Note While the majority of AFM systems sold by Asylum Research have top view optical capability, there are some systems in the field which do not. If your head or base are lacking the top view focus adjustment and XY mirror adjustments in Figure 2.3 on page 8, please skip this section.

1. 

Open the live video window:

- Turn on the computer, and open the Asylum Research AFM software.
- In the software, click the *Camera* icon on the bottom status bar.

-OR-

- From the menu bar at the top, select *AFM Controls* ▷ *Other* ▷ *Live Video*.
- The video window has its own menu bar. Check that
  - *Source* ▷ *Composite (For SA Systems)*
  - *Source* ▷ *SVideo (For IO Systems)*

2. **Adjust the base optical path:**

- Rotate the rightmost field diaphragm knob fully counterclockwise. This illuminates a maximum area around the cantilever.
- Pull out both illumination and camera controls to route light and viewing to the top of the sample.
- Turn the knurled ring around the entry point of the fiber optic bundle (at the left of the base) fully counterclockwise to admit maximum light into the base.

Note Once you have an image of the cantilever you can adjust the diaphragms for better contrast.

3. **Turn on the Fiber Lite:**

- Turn on the Fiber Lite.
- Turn the tethered dimmer control to 50%.
- The Live Video window should become brighter and there should be light visible on the sample and the cantilever.

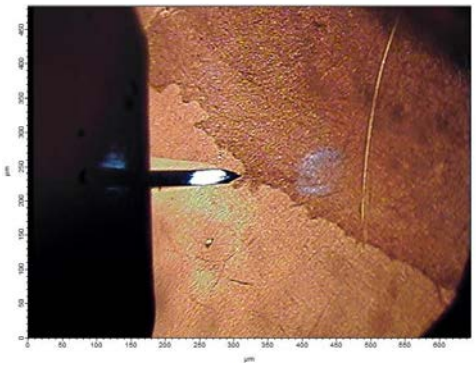
4. **Adjust Top View focus and mirror:**

- Adjust the focus ring on the "tail" of the head.
- Adjust the two thumbscrews at the end of the "tail" to *center* the cantilever in the on-screen video image.

Note Typically the adjustments should be minor since the cantilever is always in nearly the same place on the cantilever holder. You may have to hunt a bit for a good image.

5. **Improve image contrast:**

- Adjust the top view field diaphragm on the base and observe. Smaller aperture typically produces better contrast. It is common to leave some of the diaphragm showing in the image.
- Adjust the aperture diaphragm (knurled ring at the fiber lite entry point on the base).
- Adjust the intensity on the Fiber Lite if necessary.



Tips & Tricks Please see Section 4.10.2 on page 41.

4.6. Aligning the Laser

With a good video view of the cantilever in the live video window, we can proceed with aligning the spot onto the cantilever. Refer to 2.1.2 and Section 2.1.3 on page 7 to look up the names of various head and base knobs.

Note When a "laser" is mentioned in the text, it actually refers to a super luminescent diode (SLD, emits at ~860 nm). The SLD is a cousin of the solid state laser, but different in that it produces fewer artifacts during imaging and force curves.

Easy does it!

- When adjusting any of the head's thumb wheels, if you feel resistance turning them, DO NOT FORCE IT. It is probably at the end of its travel. If you over torque, it becomes very difficult to reverse direction, OR the belt that is attached to the knobs to turn the pivot points on the optical assembly can get irreversibly damaged, and it will have to be repaired at the factory.
- Use a gentle touch when adjusting the LDX, LDY & PD knobs.

1. **Turn on the laser:**
   - Turn the laser (SLD) key on the controller to the ON position. Check that the LED on top of the head is ON.

   Note With the ARC2 Controller, if the Igor software is closed the laser will be off.

   Note If the system will not be in normal use for long durations (greater than several days), we recommend that the SLD be turned to the OFF position to preserve its lifetime. For day to day use the laser is better left ON. Each time the laser is turned on or off, the head must thermally equilibrate for several hours, which can lead to unwanted drift during imaging.

Adjust the fiber light intensity:

- Turn down the power of the Fiber Lite to zero and then *just slightly* back up again to the point where a decent image of the cantilever appears again.

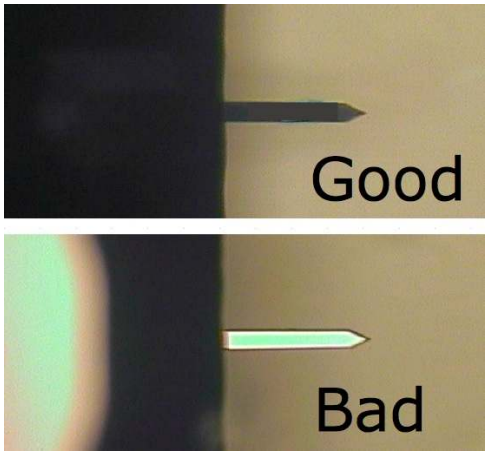
2. **Note** You may also see blue spots, which are either the laser spot itself or its reflections. The brightness of the image on the screen may not change much as you turn the fiber lite control. This is due to the auto gain control in the camera which makes up for the changes in brightness. What you will notice is an apparent change in the brightness of the laser spots. The key is to allow just enough light into the camera so there is a good balance between fiber light intensity and laser light intensity.

Tip Inspect cantilever seating:

You can judge the angle of the cantilever by looking at the reflected white light.

Dark = Good: A dark outline of the cantilever and chip, as seen in the top image, indicate good chip alignment.

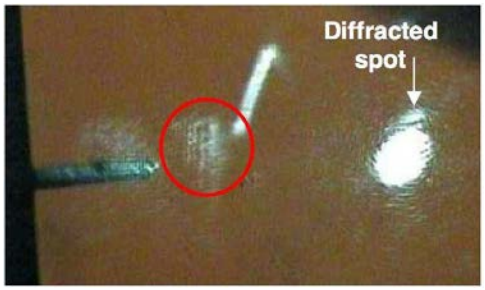
Bright = Bad: If you see a bright cantilever AND chip, as seen in the lower image, you have an issue with an improperly seated cantilever (See Step 1 on page 21)



Practice moving the laser spot

- Observe the live video screen while doing the following:
- Rotate LDY clockwise (CW) until it reaches the end of its range and you feel some resistance. Then, while counting the number of turns, go completely counterclockwise (CCW) to the other end. Now go CW again, but half the counted number of turns. The laser is now near the center of its Y range.
- Turn LDX CCW until the end of its range, which will put the spot beyond the right end of the screen.
- You may see some spots moving around on the live video window. Get a feel for how they move while you rotate the wheels.

3.



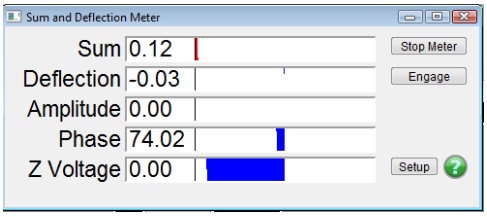
Note

Due to multiple reflections from various optical components inside the AFM head, there are often multiple spots visible on screen. The brightest spot is not always the correct one, and may be a “phantom” reflection. In this tutorial, chances are the sample is still quite far away from the cantilever, and there is no way to actually see any spot that is not on the cantilever. Please follow the steps below as a recipe for finding the right spot.

4.

Sum and Deflection meter

- Locate the sum and deflection meter (Ctrl + 6).
- Make sure the button to the right of the SUM signal says *stop meter*. If the button says *start meter*, click on it so the meter starts running.
- The SUM signal displays the total laser light reflected from the cantilever. It should have a near zero value based on the laser position from the previous step.
- The SUM signal will be the test for seeing if the real spot was moved onto the cantilever.



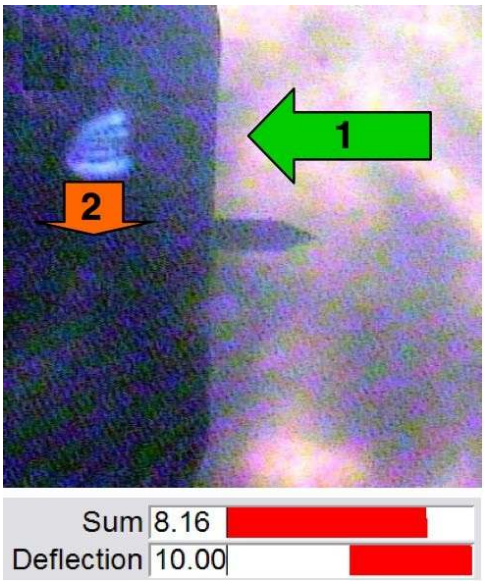
5. **Steer the laser spot onto the cantilever chip (substrate).**

(1) Rotate LDX clockwise while observing the SUM meter. Most likely the SUM will go up above 6 when reflected off the chip. It is at the same angle as the cantilever and perfectly angled to reflect light back into the detector.

Note There is a small chance that you will actually hit the lever. If you see this happen on the video screen, you can skip the next two steps.

- You should see a spot on the substrate. If not, lower the Fiber Lite intensity a bit.
- Move LDX some more to see the spot moving.

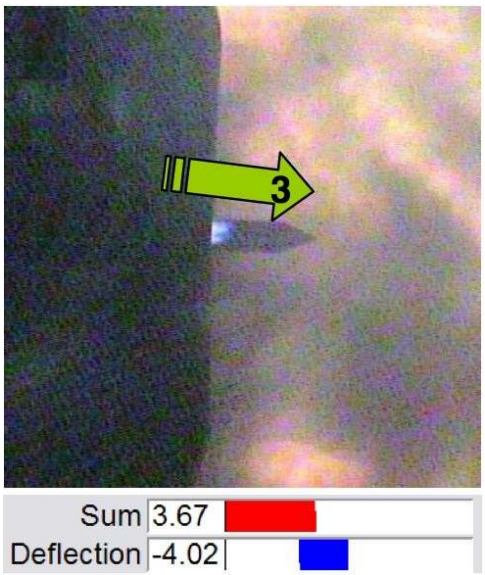
(2) Now move LDY until the spot is in line with the long axis of the cantilever. The SUM should remain high.



6. **Steer the laser spot on the cantilever**

(3) Turn LDX counterclockwise to move the spot out along the base of the cantilever and to the tip.

- You will see the sum signal go very low and the spot disappear from view for a moment as the beam traverses the sloped area of the substrate.
- Adjust LDY as necessary to keep the spot centered on the cantilever.
- The SUM should end up a bit lower than it was in the last step.

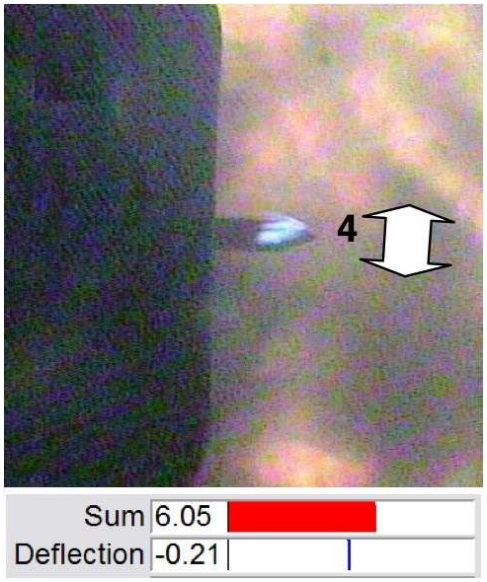


7.

Adjust LDY and maximize SUM

(4) Turn LDY back and forth to observe the SUM fluctuating and the spot going on and off of the lever.

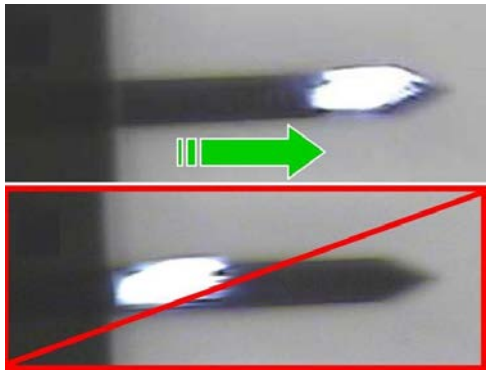
- Leave LDY where the SUM is highest.
- The spot should now be near the end of the lever, centered, and ready for AC tuning.



8.

View your final alignment

- From the live video window menu bar, select *Magnify.*
- This will zoom in on the center portion of the video window.
- Fine tune your final laser spot position near the end of the lever if necessary. Maximize the SUM while moving LDY.



9.

Zero the deflection

- Note the deflection level in the Sum and Deflection meter.
- Use the PD thumb wheel on the head to zero the deflection. If the display indicates blue (negative) then rotate the thumb wheel counterclockwise. Go clockwise if it is red.
- This action steers the reflected beam so it is centered on the photo detector.

Tips & Tricks Please see Section 4.10.3 on page 42.

4.7. Tuning the Cantilever

The tutorial steps given are with an AC160TS. The stiffer, higher resonance frequency of the AC160TS cantilever makes it easier to track the large hard features of the calibration grid. However, similar results can be obtained with the AC240TS, which is the tip of choice for biological and soft samples, or samples that do not have large changes in topography.

1. **Initiate cantilever tune:**

   - Select the Tune tab in the master panel (Figure 4.2b on page 25).
   - Set the four auto tune parameters (*Auto Tune Low*, *Auto Tune High*, *Target Amplitude*, *Target Percent*) as shown to the right.
   - Click the *Auto Tune* Button.

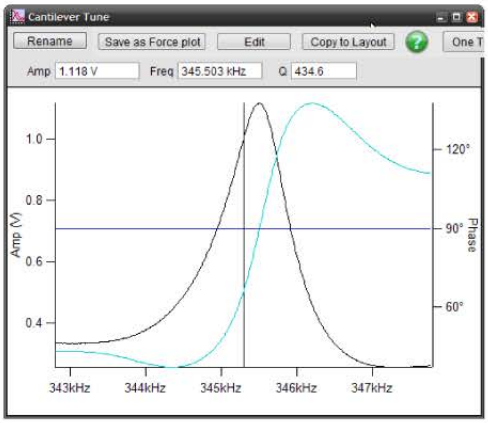
   -5%: Amount the drive frequency will be below the maximum. This helps ensure that the tip will remain in repulsive mode when engaged.

   50 kHz and 400 kHz: The default *Auto Tune Low* and *High* values accommodating most common commercially available AC mode cantilevers (in air).

2. **Observe tune result:**

   - A graph will pop up with the tune result.
   - The resonance curve should peak around 200-400 kHz.
   - The relevant parameters, *Drive Frequency*, *Drive Amplitude* and *Phase offset*, are automatically set.
   - After inspecting and confirming that the the amplitude and phase curves look good, you can close the graph.

   Note For the AC240TS, the resonance curve should peak around 70 kHz.

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

Tips & Tricks Please see Section 4.10.4 on page 43.

4.8. Landing the Tip

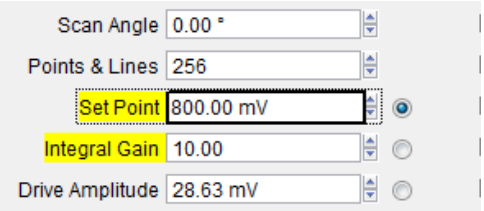
Hard vs. Soft

The following steps are for the “hard engage”. This is an easy-to-master and perfectly acceptable method of engaging the tip on the surface. In cases where a super sharp tip is required, we recommend you master the somewhat more elaborate “Soft Engage” method, described in Section 4.10.6 on page 44.

1. **Set the feedback setpoint for hard engage:**

   Note: For better tip preservation, consider the more elaborate Soft Engage Section 4.10.6 on page 44.

   - Set the *Setpoint* value to 80% of the free air amplitude. Typically the amplitude away from the surface is 1 volt, after a standard autotune, which would call for an initial setpoint of 800 mV.
   - All the other parameters should already be set from tuning the cantilever.



2. Click the *Engage* button in Sum & Deflection Meter window. The Z Piezo meter will extend red all the way to the right of the meter (150V).

3. **Wheel down until ’beep’:**

   - Slowly turn the front thumb wheel counterclockwise to lower the head towards the surface.
   - Observe the amplitude and slow the wheeling when the amplitude starts to decrease. The amplitude decreases before contact due to air damping between lever and surface.
   - When the computer beeps stop, the tip has contacted the surface.

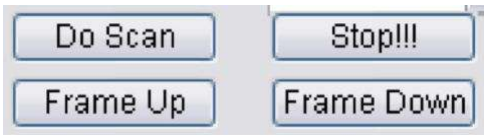
4. Move the front thumb wheel until the Z voltage registers about 70V (in the blue). This brings the Z piezo to its midpoint.
5. Hit the ‘Withdraw’ button on the *Sum and Deflection Panel*. This will save the tip from jostling during the next step.

6. Close the acoustic enclosure. At this point manual interaction with the instrument is at an end. The tip is still above the surface and the vibrations of closing the hood will not damage it.

Tips & Tricks Please see Section 4.10.5 on page 44.

4.9. Start Imaging

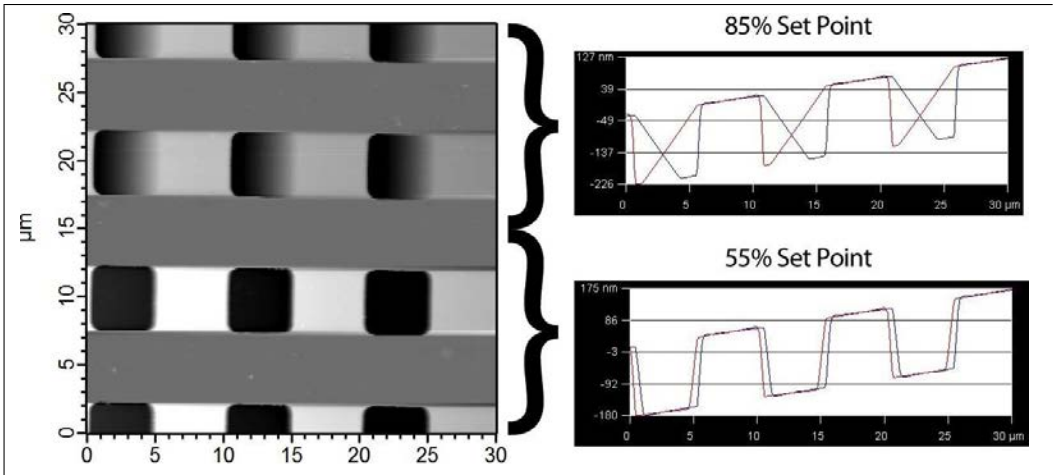
1. **Start the scan:**
   - Click the *Do Scan* button (or Frame Up or Frame Down) on the *Main Tab* in the *Master Panel*. The tip will begin scanning from the top or bottom of scan area. The red marker to the left of each image window indicates the current scan line.



2. Look at the Height Channel image (ignore amplitude and phase for the moment) and locate the blue and red scope traces beneath it. These are the last collected trace and retrace scan lines. The tracking should look nearly identical to the retrace. For the calibration grating sample, they should look like a square wave. To achieve this, some adjustment of the parameters is usually needed.

   Note Wait until the tip is actually scanning over some pits in the calibration grating. There is not much to image on the plateaus in between the pits. This is a good time to turn off the slow axis, see Section 4.10.7 on page 45.

3.

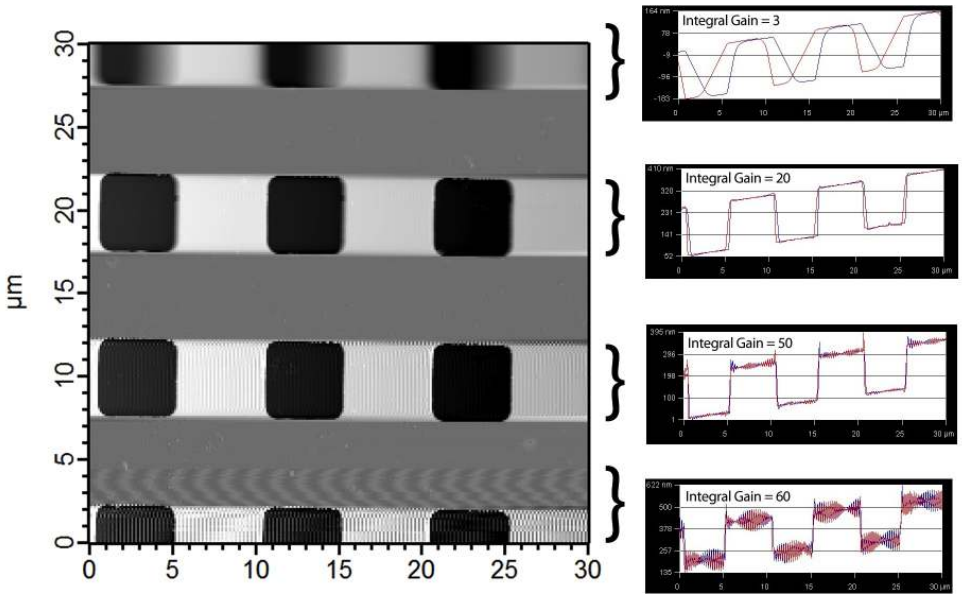


Setpoint Voltage

- The *Setpoint Voltage* generally needs adjustment:
- Decrease the *Setpoint Voltage* value to increase the force applied to the sample, and to improve the tracking.
- Higher *Setpoint* voltages may help preserve the tip apex, but may not allow proper tracking of the surface.
- For this example, use a *Setpoint* between 600-750mV.
- Practically speaking, amplitude set points from 20-75% of the free air amplitude will put you in repulsive mode, which tend to track well, but may wear down your tip.

Advanced Note	Adjusting the initial setpoint while scanning as described here is more to illustrate things. Typically experienced users do this during the engage process, where you engage on the surface, then decrease the setpoint until the Z does not move much.

4.



Integral gain adjustment:

- Increase the *integral gain* to improve the tracking, but at a certain level the signal will start to ring.
- Decrease gain until ringing ceases in trace and retrace.

Note There is a slight offset between the trace and retrace lines in the graph. Good tracking means that they should have similar shapes, not necessarily perfect superposition.

Note When adjusting *integral gain*, another channel to look at is the amplitude image. Feedback oscillations are easily detected in the amplitude image because it is an image of the feedback loop error.

5.

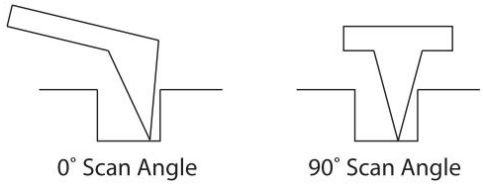
Scan rate adjustment:

- If tracking still seems to be an issue, try lowering the *Scan Rate*. Slowing the *Scan Rate* down will help the feedback keep up with the image features.
- The Scan Rate cannot be updated during imaging like other imaging parameters. You must click *Frame Up* or *Frame Down* buttons to initiate the newly entered scan rate.

Note Too slow of a scan rate can introduce image artifacts due to thermal drift.

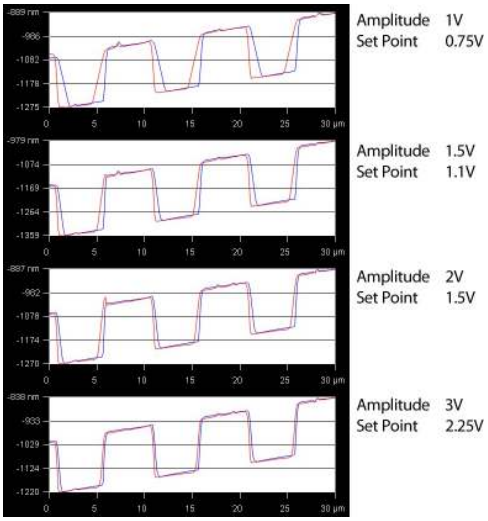
6. **Scan angle adjustment:**

Note For the Olympus AC160 and AC240 cantilevers, the tip will be symmetric or asymmetric based on the scan angles shown on the right. For a better representation of side walls 0º scan angle should be used. However, tracking could be difficult due to the steep edge of the tip. Better tracking could be achieved with a 90º scan angle. This is one example; refer to cantilever documentation for different tips.



7. **Drive amplitude adjustment:**

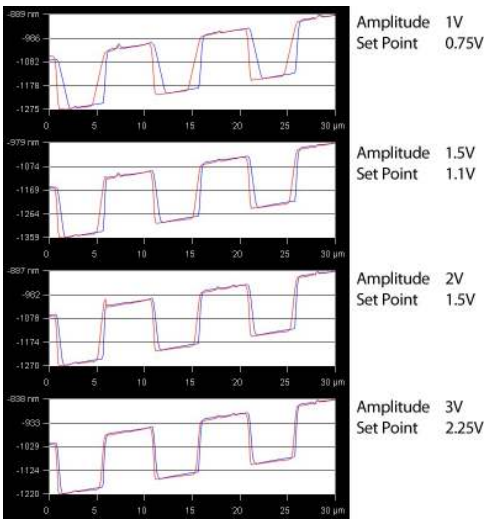
- The *Drive Amplitude* can be adjusted to increase the amount of drive to the shake piezo which increases the amplitude of the cantilever.
- Try different combinations of Free Air Amplitude (tip far away from surface) and *setpoint*. For educational purposes, the *setpoint* was kept at 75% and the *integral gain* at 10 to show the advantage of hitting the sample harder with bigger amplitudes to get better tracking.
- To change the amplitude, click *Stop* and either click *Auto Tune* with the new *Target Amplitude* or increase the *Drive Amplitude* manually.



8.

Tracking improvement:

- When the tip is tapping harder, the cantilever becomes more responsive to changes in height, which improves tracking.
- Bigger amplitudes can help the tip stay in repulsive mode. Please refer to *Applications Guide, Chapter: AC Mode: Theory* for more information.
- When imaging sticky samples, larger amplitudes help the tip escape the forces of the sample.



Note You could also simply increase the *Drive Amplitude* however, be aware of the setpoint. It is often good to maintain a constant setpoint ratio, the ratio of the setpoint to the free air amplitude.

Tips & Tricks Please see Section 4.10.7 on page 45.

4.10. AC Mode Imaging Tutorial Tips & Tricks

4.10.1. Loading the Cantilever Tips & Tricks

If everything went according to plan, then this section can be skipped (or only read once to learn a few tips). What follows is not strictly part of the necessary instructions.

Q What's the harm in forcefully tightening the screw which clamps the cantilever?

A Over tightening can strip screw threads or result in excessive bending on the tongue of the clip, which can cause issues with obtaining suitable deflections with cantilevers of unusual thicknesses.

Q Which Cantilevers can I use with the MFP-3D?

A The MFP-3D cantilever holder accepts virtually all brands of commercially available probes.

Q What's the best way to clean the cantilever holder?

A The safest way is to use a cotton swab (Q-tip) and some alcohol. For more thorough cleaning instructions, please consult Chapter 11 on page 94.

Tip Fitting unusually thick cantilever chips under the clip:

When switching from silicon cantilevers (with thin silicon chips) to silicon nitride levers (with thick glass chips) you may notice that the cantilever holder clip does not open enough to let the lever slide in. Here's the remedy:

- Unscrew the center screw an extra turn or two. Do not screw it all the way out since it will prevent you from bending the clip too far in the next step.
- Use tweezers or a small flat-head screwdriver to GENTLY pry up on the tongue near the base. In our experience it is best to start lightly, try for fit, and repeat a bit more forcefully until the clip stays open high enough to fit the thicker substrate.

Tip There is evidence that static charges can create such large fields at the tip of a lever that they are dulled just by handling from corona discharge. Consider wearing a grounding wrist strap and using a grounded work surface if your work requires the sharpest tips possible.

Some tips on successfully handling cantilevers.

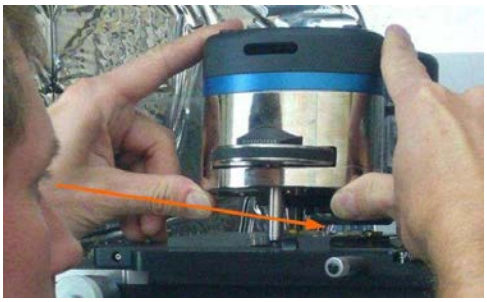
Tips

- Use good tweezers. The cost of good tweezers is easily justifiable considering the cost of cantilevers. Some people swear by the curved tweezers; some prefer the straight ones. Remove any sticky residue by cleaning the tips of the tweezers with an alcohol soaked lint free cloth.
- Keep your box of cantilevers close to the cantilever holder when loading the holder. It is nice to do most of the work with the wrist.
- Rest as much of your arm on the table as possible for stability. For added stability you can use your non-dominant hand to stabilize the hand holding the tweezers.
- Roll cantilevers off of the sticky gel instead of trying to lift them straight up. Roll to the side to protect the levers themselves.
- Grab the substrates a little forward (toward the cantilever) of the middle. If you pick up the back side first, you run the risk of breaking the lever.
- Set the cantilever down in an intermediate location to allow you to get a new grip before loading it into the holder. This is not always necessary, but it is nice to have a place available for an emergency landing if needed. You may also take this opportunity to close the box of unused levers.
- Practice with dead cantilevers. Keep your used levers and practice moving them from one side of a box to another, trying to maintain an orderly pattern.

4.10.2. Head and Base Optics Alignment Tips & Tricks

Tip Using the live video window when placing the head:

If your head mirror and focus are already aligned when you change samples, it can be useful keep an eye on the live video as you put the head in place. By doing this, you can view whether or not the lever will crash while placing the head.



Tip Getting more consistent head placement:

Each time the head is placed into the divots on the base, it does not land in exactly the same place. It may even settle a little over time, leading to unwanted movement of the cantilever during imaging.
We have found that pressing down on the head while applying a counterclockwise rotational force at the same time greatly improves absolute placement position. This will also prevent most settling effects.

Tip Streamlined head placement process:

Experienced users at Asylum Research will skip a few of the above steps:

- Eyeball the sample thickness and guess at the leg lengths and adjust with the head removed from the scanner.
- Place the sample on the scanner.
- Place the head on the scanner while propping a thumb between the head and scanner, holding the tip about 1mm above the sample, while wheeling down the front leg until it touches down

4.10.3. Aligning the Laser Tips & Tricks

Tip	The correct laser spot will show some elongation along the length of the cantilever. If a spot without this elongation is seen, it is a "phantom" spot and will give no Sum voltage in the S&D meter even though there appears to be a spot on the end of the cantilever.

Tip	In some cases the correct spot may appear to be off the cantilever while there is a large SUM signal. This is usually due to poor focusing. Adjust the head's top view objective focus wheel to correct.

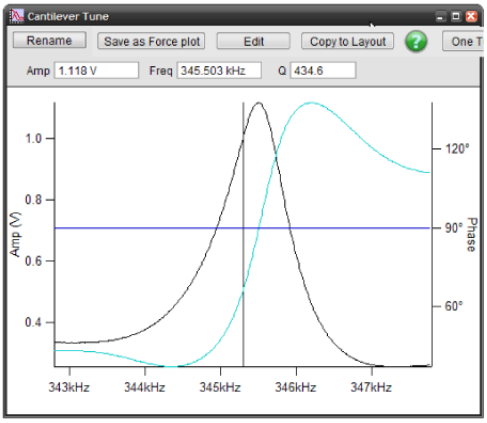
Tip	If there is any question if the spot is aligned on the cantilever or the probe substrate, there is a very easy way to tell - do a thermal tune! (See[a]) If the SLD spot is on the chip, there will not be any resonant peak (with significant amplitude or Q) in the thermal. This is especially useful for systems without top view optics.

[a] *Applications Guide, Chapter: Thermals.*.

4.10.4. Tuning the Cantilever Tips & Tricks

Tip How to Save Tune plots?

- Click *Save as Force Plot*. The saved tune can then be reviewed in the Master Force Panel.
- Click *Rename*. The graph is saved in a separate window. Subsequent tunes can be overlaid on top of each other if they are given the same name or saved in a new graph when given a new name.
- Click *FTP*. This saves the experiment on the computer in a Temp Folder. This allows you to upload the file to the Asylum Research FTP site for discussion with Asylum Research technical staff.
- Click *Layout*. This appends the graph to a layout.



Tip Drive Amplitude too high or too low?

If the drive amplitude is greater than ~1V or you do not see a clean tune, then the coupling to the tip may not be sufficient.

- Loosen and re-tighten the screw or move the chip. Mostly likely the probe chip was seated improperly under the clamp.

If using high aspect-ratio tips, there is a chance the Q (Quality factor) of the cantilever will be so high that the drive amplitude will be very low, and a message will come up during the Auto Tune that says it is having difficulty completing the Auto Tune. There are a few things you can do to get around this:

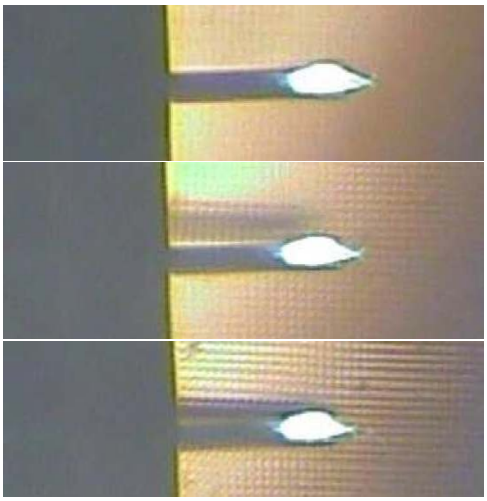
- Manually increase the Drive Amplitude in the *Tune* tab, click *One Tune* and it should work.
- Click *Center Phase* if using *One Tune*.
- Use Negative Q Gain. See *Applications Guide, Chapter: AC Mode Imaging in Air* for more information.

4.10.5. Landing the Tip Tips & Tricks

Tip If the sample is reflective, the shadow of the cantilever can be seen in the top view CCD camera image as it approaches the surface of a calibration grating.

Images (from top to bottom):

- No cantilever shadow is visible because the tip is too far away from the surface.
- Moving tip towards the surface, cantilever shadow appears, surface coming into focus.
- Moving tip closer to the surface, the cantilever almost eclipses the shadow. The surface is in focus.



4.10.6. Hard and Soft Engage Techniques

The soft engage technique is an advanced method that provides the softest possible engaging of the tip. With this method you can preserve even the sharpest, most fragile of tips, which will provide you with the sharpest of images. This method is more advanced, but with some practice can be done fairly quickly.

4.10.6.1. Hard engage method

1. Click 'Engage' to fully extend the Z piezo.
2. Wheel down the large thumbwheel.
3. Slow down when the amplitude starts to decrease. The cantilever is starting to respond to long range tip sample forces.
4. Wheel down very slowly until the beep.
5. Wheel down slowly until the Z voltage is 70V.
6. Decrease your setpoint a click or 2 (down arrow to the right of setpoint control), make sure Z does not move much. This confirms that the tip is firmly on the surface.

4.10.6.2. Soft Engage Method

1. Enter a setpoint of 85% of the free air amplitude, click 'Engage' to fully extend the Z piezo.
2. Wheel down the large thumbwheel.

3. When the cantilever starts to feel long range forces, the amplitude decreases (as the thumbwheel turns) until the setpoint is reached. At that point the feedback will start retracting the piezo (software beeps) as the head continues downward.
4. Stop wheeling when the Z voltage indicator approaches 30V.
5. Gradually decrease the setpoint and watch the Z voltage increase in response as the tip extends toward the surface. Stop decreasing the setpoint when the Z voltage stops responding (the tip has reached the sample surface and you are done) **- OR -** when the Z voltage exceeds 100V. A more responsive phase signal is another indication that the tip is on the surface.
6. If the Z piezo kept extending past a value of 100V, start wheeling down the head and go back to step 4.

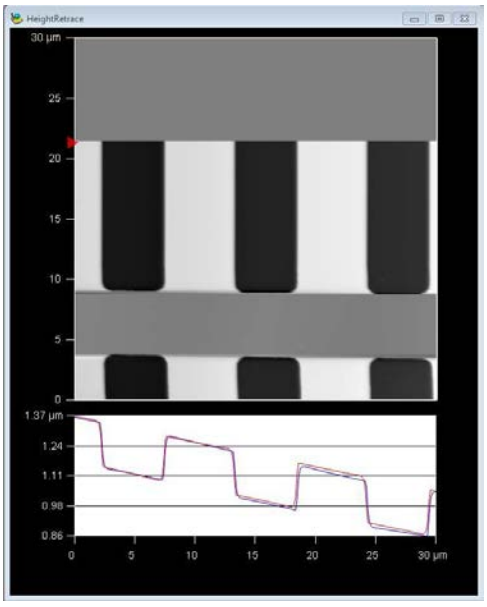
It is uncommon to repeat steps 4 and 5 more than once. If so, you may want to consider using a lower initial setpoint relative to the free amplitude.

If you end up with a good engage, but with Z outside 70±20V, see Section 9.2.2 on page 79 for a softer centering of Z.

4.10.7. Start Imaging Tips & Tricks

Tip Adjusting scan parameters with *slow scan disabled*:

When the *Slow Scan Disabled* checkbox (on the main tab of the master panel) is checked, the tip scans the same line repeatedly. This is easier for comparison when optimizing parameters as the features will be the same from scan line to scan line.



- Increase the integral gain until oscillations are seen in the trace and retrace lines for the height channel.
- Reduce *Integral Gain* until oscillations disappear.
- Adjust *Setpoint* and 'Drive Amplitude' until good tracking occurs.
- 'Scan Rate' and *Scan Angle* can be adjusted to further improve image quality.
- Un-check it once you have your imaging parameters where you want them.

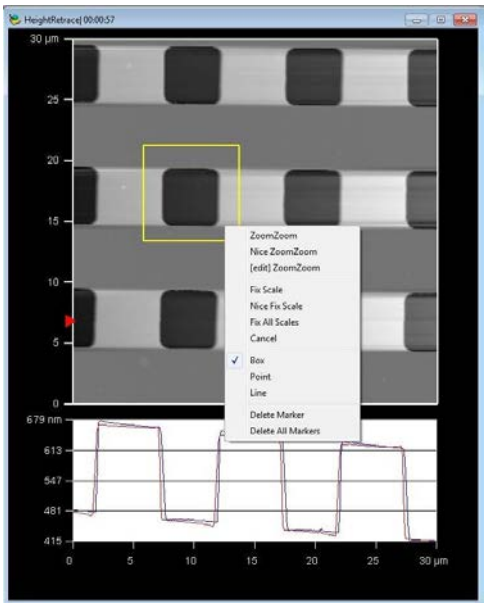
Tip Delay Update:

- The *Delay Update* check box allows the user to change the parameters during a scan. The changes you make will take effect at the beginning of the next frame.
- During the period before the update is performed, the parameters changed will be highlighted in a light blue color.

Tip Zoom:

This will stop the current image and restart imaging a smaller selection.

- To scan a selected area, drag a box.
- Right click and select *ZoomZoom* to scan the selected area, *Nice ZoomZoom* to zoom an area to the nearest rounded number, or *[edit] ZoomZoom* to type an exact scan area.



Tip Proportional Gain

- Adjusting the *Proportional Gain* does not generally improve the imaging - this is because the frequency range of the scanner is below the range where the proportional gain can contribute.
- For most samples, keep *Proportional Gain* at 0. The exception to this rule is when very large or sharp topography features are present, and the feedback loop needs to react very quickly to the sample surface.

5. Tutorial: Replacing the Cantilever

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

Before you start:

- We assume you have followed the tutorial in Chapter 4 on page 15, which finishes with AFM images being collected. This tutorial picks up at that point.

The previous tutorial assumed that you found the instrument in a completely unknown state and included many alignment steps which do not apply to a typical situation, such as changing a cantilever and resuming scanning on the same sample. Scanner alignment, optical alignment, and even most scanning parameters should already be ready for imaging in most cases.

This tutorial assumes the starting point where the last tutorial ended up: imaging the calibration grating sample in AC mode. When finished, it will leave you at the same point again, scanning the same sample, but with a fresh cantilever.

1. Halt the scan by clicking the Stop!!! button in the Main tab of the Main Panel. This will retract the tip from the surface and stop the XY scanning of the sample.
2. Open the acoustic enclosure.
3. **Wheel up one full turn:**
   - Wheel the front leg up one full revolution clockwise to raise the cantilever about 100 µm above the sample surface.

4. **Remove the cantilever holder from the head:**

   - Place the AFM head upside down (usually on the metal platform or "head stand" next to the AFM.)
   - Depress the rubber ball on the head.
   - Gently lift the cantilever holder straight up out of the head and carry it to your cantilever changing area.

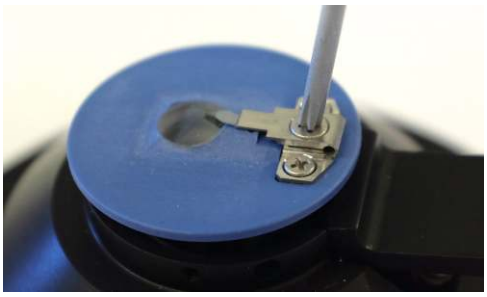
5. **Mount the cantilever holder:**

   - Orient the cantilever holder with the clip's screws towards the lever on the stand.
   - Press the lever on the stand as shown.
   - At the same time, angle the cantilever holder into the stand. Two fixed balls in the stand, opposite the lever, must match up with the two matching kinematic mounting points on the cantilever holder.
   - Lower the cantilever holder so the final kinematic mounting point lines up with the ball on the stand's lever, then release the lever.
   - Inspect that the cantilever holder sits flat in the stand and that all the balls sit properly in the mounting points.

6. **Remove the used cantilever:**

- Loosen the middle screw about one turn, just to the point of freeing the cantilever.
- Remove the old cantilever with tweezers.

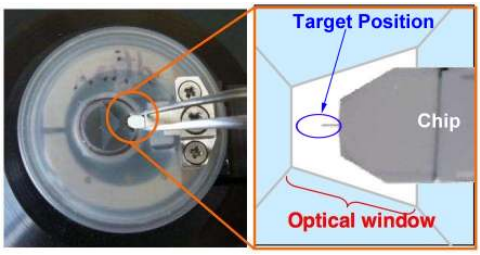
Good Habit Blow the area under the cantilever clip clean with *clean* compressed gas. Bits of silicon and other debris can lead to a poorly seated cantilever and sub-optimal AC mode images.



7. **Insert the new cantilever:**

- With tweezers, slide the cantilever chip under the clip.
- Center the cantilever tip (approximately) in the clear trapezoidal shaped quartz optical window.

Note Do not push the cantilever chip too far back. This can cause misalignment. We'll check for this in a few steps.

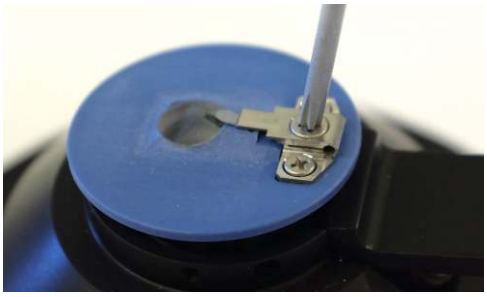


Note The cantilever holder was designed to be resilient, so do not worry about scratching it with tweezers.

8. **Tighten the screw:** Gently tighten the clip's screw.

- The chip should not be able to move if nudged with the tweezers. Firmly mounted chips will perform best during AC mode imaging.

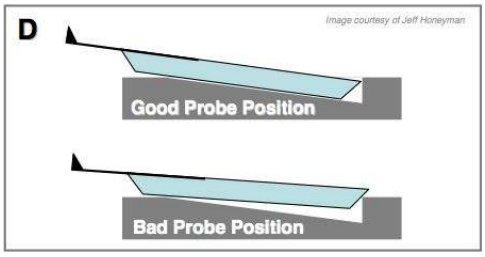
Note Do not over tighten the clamp on the cantilever holder - this can crush the chip, strip the screw threads and / or result in an excessively bent clamp.



9.

Inspect the cantilever seating:

- Hold the changing stand so you can look from the side between the cantilever clip (a low power microscope or a jeweler's loupe, 10× is recommended).
- Check that the cantilever chip is parallel to the mounting surface and glass prism facet.
- If the probe is mounted too far back, it causes an improper angle, and you will not be able to align the laser when the cantilever has been loaded into the AFM. (See Section 2 on page 29 for an example of improper alignment). Correct the position by loosening the screw and moving the cantilever forward in the pocket. Tighten the screw and inspect the "seating" again.



10.

Install the cantilever holder on the head:

- Remove the cantilever holder from the stand by depressing the lever. Take the holder over to the head.
- Insert the cantilever holder into the head. This action is similar to using the changing stand (See Step 3 on page 18) except the rubber dome on the head replaces the lever on the stand.

- Pulling or jiggling the cantilever holder should result in no motion.

11.

Place the head on the base:

- Firmly grip the head and place it on the base. Each leg goes through one of the leg holes in the scanner.
- Since the sample is the same as when the head was removed and the front leg was wheeled up to a safe height, there is no worry of crashing the tip.
- Place a hand on the head and press down while slightly rotating counter clockwise. This helps ensure consistent seating of the head on the base.

12.

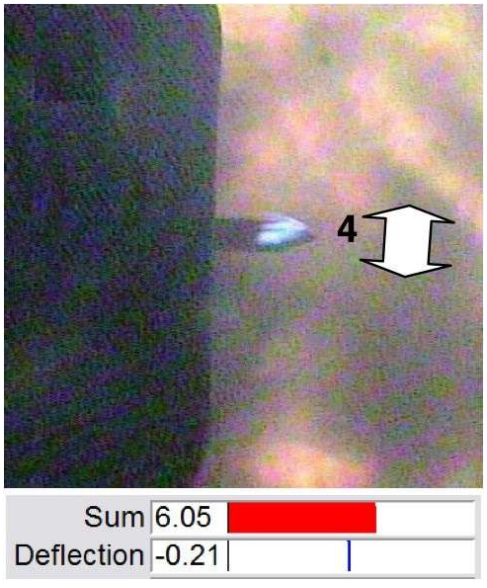
Open the live video window:

- In the software, click the Camera icon at the lower left hand side of the window
- If necessary turn on the Fiber Lite and turn the XY mirror adjustments on the “tail” of the AFM head. The next step requires a visual of the cantilever.

13.

Center the laser on the cantilever:

- Using the LDX and LDY controls, center the laser on the cantilever holder.
- A high Sum signal assures that there is not a phantom spot on the lever.



14. **Zero the deflection**

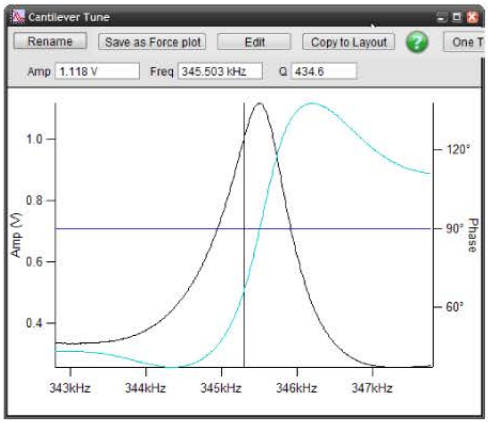
- Use the PD thumb wheel on the head to zero the deflection.

15. **Initiate cantilever tune:**

- Select the Tune tab in the master panel (Figure 4.2b on page 25).
- Set the four auto tune parameters (*Auto Tune Low*, *Auto Tune High*, *Target Amplitude*, *Target Percent*) as shown to the right.
- Click the *Auto Tune* Button.

16. **Observe tune result:**

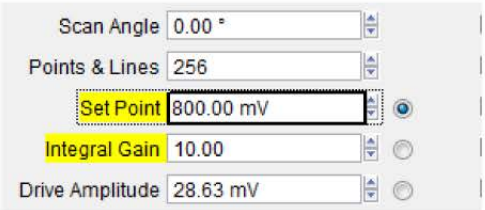
- A graph will pop up with the tune result.
- The resonance curve should peak around 300kHz for an AC160. For an AC240, the curve will peak around 70kHz.
- The relevant results are automatically stored. After inspecting the amplitude and phase curves to confirm that they look “clean”, you can close the graph.



17. **Set the feedback set point for hard engage:**

Note: For better tip preservation, consider the more elaborate Soft Engage: Section 4.10.6 on page 44.

- Set the *Set Point* value to *800mV* for typical cantilevers such as AC160TS or AC240TS, the first item highlighted in yellow to the right.
- All the other parameters should still be fine from the scanning before replacing the cantilever.



18. Click the *Engage* button in Sum & Deflection Meter window. The Z Piezo meter will extend

red all the way to the left of the meter (150V).

19. **Wheel down until beep:**

- Slowly turn the front thumb wheel counterclockwise to lower the head towards the surface.
- Observe the amplitude and slow the wheeling when it starts to decrease by a few percent. (Amplitude decreases before contact due to air damping between lever and surface.)
- If your computer speakers are on, then you can listen for a beep to know when you have contacted the surface. Otherwise you will have to keep one eye on the Z voltage to see when it moves back from +150.

Note This is the hard engage. For the soft engage please see Section 4.10.6 on page 44.

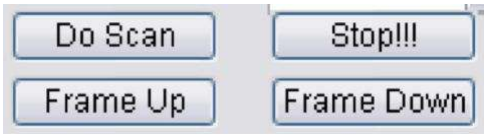
20. Move the front thumb wheel down until the Z voltage registers about 70V (in the blue). This brings the Z piezo to its midpoint.

21. Hit the 'Withdraw' button on the *Sum and Deflection Panel*. This will save the tip from jostling during the next step.

22. Close the acoustic enclosure. At this point manual interaction with the instrument is at an end. The tip is still above the surface and the vibrations of closing the hood will not damage it.

23.

- Click the *Do Scan* button (OR *Frame Up* or *Frame Down*) on the Main Tab in the Master Panel. The tip will begin scanning from the top or bottom of scan area.



6. Tutorial: Contact Mode Imaging in Air

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

Contact Mode AFM, also known as constant force mode, is one of the more commonly used imaging modes in AFM. It is often used in imaging hard materials, in some electrical techniques, and in imaging biological materials - e.g. cells under low setpoint force and scan range.

6.1. Required Materials

- Cantilevers: Any standard contact cantilever will work. A list of contact cantilevers we offer can be found here: Contact Cantilevers.
- Sample: The tutorial will use the Asylum Research calibration grating that ships with every system (part number 900.237). It is mounted on a standard glass microscope cover slide.
- Sharp Tipped Tweezers (some people prefer straight tips, others prefer curved).
- Small Phillips (+) screwdriver.

6.2. Instrument Setup

Several of the initial steps have already been explained in the AC Mode Imaging Tutorial chapter. Click the links contained in the steps below to refresh your memory on these processes.

1. For loading the cantilever, see Section 4.2 on page 16.
2. For mounting the cantilever holder, see Section 4.2.3 on page 21.
3. For head and sample placement, see Section 4.3 on page 22.
4. For aligning the laser, see Section 4.6 on page 28.

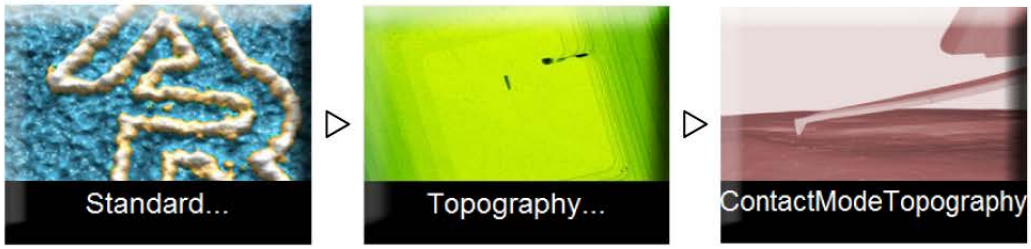
6.3. Software Prep

1. Start up the Asylum Research AFM software.

2. **Select Contact Mode Imaging:**

   - The software should now be showing the Mode Master home window.
   - If not, click the 'Mode Master' button at the bottom of the screen:  .
   - In this example we will start with contact topography (see below).

   Note For imaging cells, a more appropriate template can be found by clicking on *Bio*, then on *ContactCell*.

3. 

   Select:

   - Select *Standard* ▷ *Topography* ▷ *ContactModeTopography*
   - The screen will now re-arrange and present all the controls necessary for this type of AFM imaging.

6.4. Tip Engagement

1. With a properly loaded cantilever, adjust the Photodetector (PD) to 0 volts or a slightly negative value. The slight negative free air deflection will place the SLD spot in the middle of the PD (where the range is more linear) when the tip is engaged.
2. To conceptually aid the selection of a Setpoint voltage, a qualitative depiction of cantilever deflection vs. tip-substrate distance is shown in Figure 6.1 on page 56. In contact mode the AFM will try to maintain the measured deflection equal to the setpoint. The greater the setpoint voltage is (above the free air deflection voltage), the greater the force applied by the cantilever to the sample. To know exactly how much force is being applied during imaging, the cantilever spring constant must be determined. This will be not be necessary for this tutorial.
3. Choose a setpoint voltage that is more positive than the free air deflection value in the SUM and Deflection (S&D) meter. In Figure 6.2 on page 56, the free air deflection is -0.20 V:

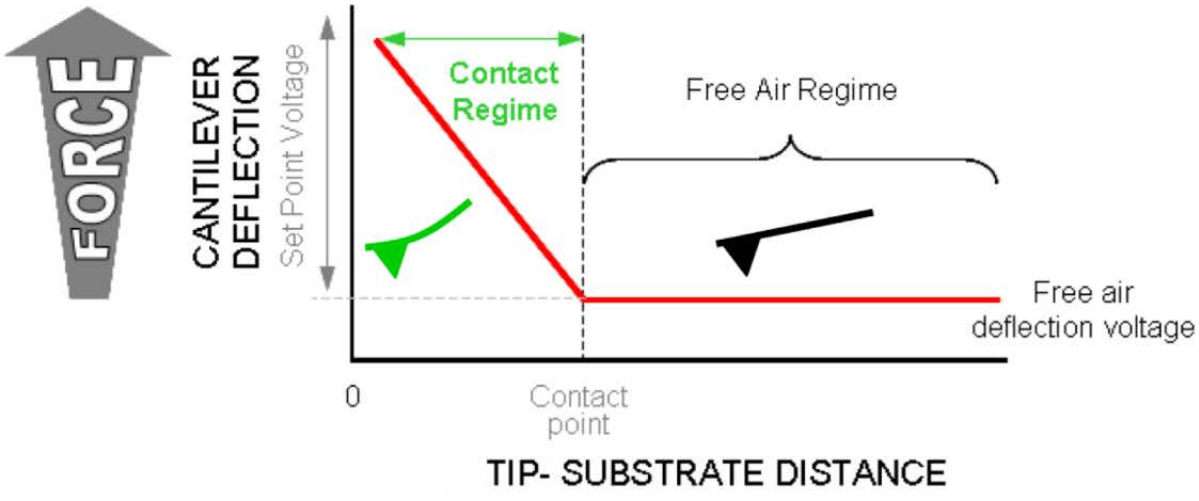


Figure 6.1.: Deflection vs. Distance

therefore a setpoint voltage of -0.19V or more is needed to engage the tip. Setpoint voltages just positive of the free air deflection mean the tip will apply low force to the sample or will false engage, while setpoint voltages much more positive than the free air deflection voltage mean the tip will apply greater force to the sample. A .5V - 1V setpoint should be suitable for this tutorial. So you would want a setpoint of about .3V to .8V in this case.

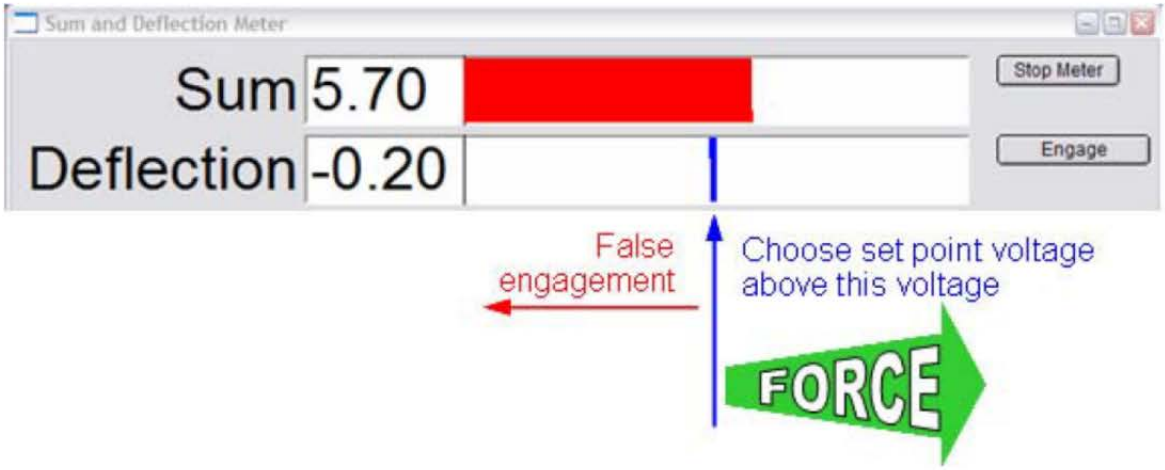


Figure 6.2.: SUM and Deflection Window

4. Click 'Engage' in the Sum and Deflection window. Assuming the cantilever is high enough above the sample, the Z voltage should go all the way to 150V. The Z feedback loop is trying to maintain the setpoint by changing the voltage to the Z actuator. The more voltage the feedback loop applies to the Z actuator causing it to extend the higher deflection value it expects. Since we are too high above the sample, the deflection never changes towards the setpoint and the Z voltage increase to its maximum value.
5. Slowly turn the thumbwheel counter-clockwise, propelling the cantilever towards the surface

at a rate of several microns per second. When the cantilever comes into contact with the sample the deflection should very quickly hit the desired setpoint. You will hear a chime sound from the computer when this happens. You will also notice the Z voltage decreasing. The more you wheel down the more the Z actuator needs to retract to maintain the setpoint and thus the Z voltage decreases. It is good practice to turn the thumbwheel until you have Z voltage around 70V. Centering the Z voltage ensures you have ample room on either while imaging. As you are imaging, peaks will requires a lower Z voltage, and valleys will require a higher Z voltage.

6. At this point hit the *withdraw* button, so the cantilever is not in contact with the sample. You may then close the acoustic hood. If you do this while engaged you risk ruining or dulling your cantilever.

Note If you have the volume up on the transducer (front of controller, headphones plugged in), you can hear a frequency change as engagement occurs- it will sound like static and will change to a dampened sound when in contact.

6.4.1. Gentle Engagement:

The gentle engage method is more time consuming, and generally not required. Contact mode is typically fairly rough on the cantilever, and most of the time you don't care if the tip gets blunted in this process. If you do care about preserving the tip sharpness in contact mode, you should first consider doing the soft engage in AC mode, and then switching to contact once you have found the surface, seeSection 4.10.6 on page 44. If AC mode is problematic for you, you can try this adapted contact mode soft engage:

1. A simple gentle engage is to select a Setpoint slightly greater than the free air deflection, as described previously.

2.
   - An even more gentle engagement occurs by wheeling down 10 µm, then clicking *Engage*.
   - If the tip does not engage, click the *Withdraw* button, wheel down another 10 µm, and repeat.
   - Notice that each graduation on the front thumbwheel is 1 µm.

3. Once feedback has been activated, continue to lower the head with the front thumbwheel to ~ 70 V (i.e. no color in Z-Piezo meter). This indicates that the piezo is in the middle of its Z- range (~ 7.5 µm standard head; ~ 20 µm extended head).

4. At this point the tip is engaged and just sitting on the surface- it does not begin rastering until you tell it to do so. You can now begin imaging or determine the Spring Constant, see[1].

5. If the Z-piezo voltage is railed all the way blue (-10 V) upon clicking simple engagement, the piezo is fully retracted because it thinks it has crashed. This indicates a false engagement,

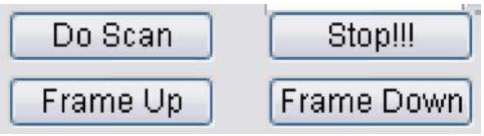
[1] *Applications Guide, Chapter: Thermals.*.

and adjustment must be made to the Setpoint voltage to allow extension of the piezo. This often occurs when imaging in fluids or in high humidity, or with too low a Setpoint voltage (i.e. low force).

6. Check if the acoustic hood door hatch is still open. If it is, follow these steps to preserve the tip's apex while shutting the door:
   - Click on the *Withdraw* button.
   - Close the hood door.
   - Click on the *Engage* button.
   - Proceed with scanning.

6.5. Start Imaging

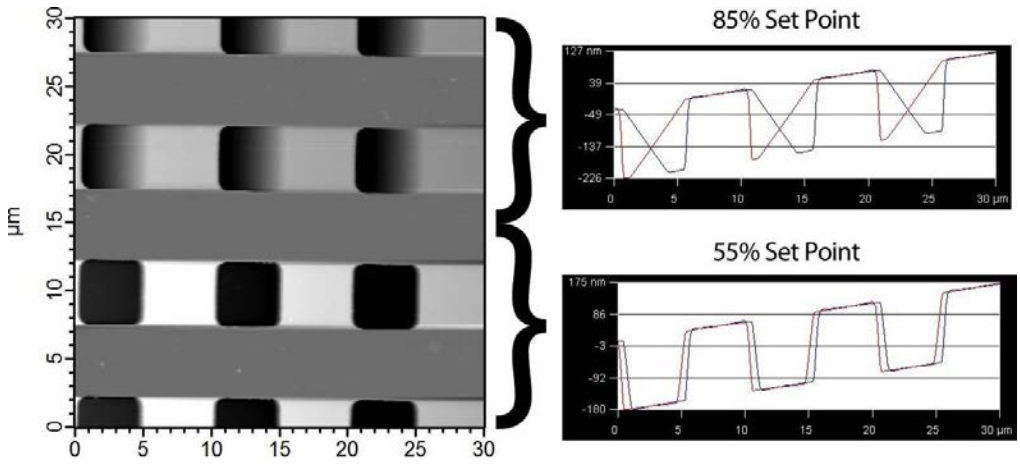
1. **Start the scan:**
   - Click the *Do Scan* button (or Frame Up or Frame Down) on the *Main Tab* in the *Master Panel*. The tip will begin scanning from the top or bottom of scan area. The red marker to the left of each image window indicates the current scan line.



2. Look at the Height Channel image and locate the blue and red scope traces beneath it. These are the last collected trace and retrace scan lines. The tracking should look nearly identical to the retrace. For the calibration grating sample, they should look like a square wave. To achieve this, some adjustment of the parameters is usually needed.

   Note Wait until the tip is actually scanning over some pits in the calibration grating. There is not much to image on the plateaus in between the pits. This is a good time to turn off the slow axis, see Section 4.10.7 on page 45.

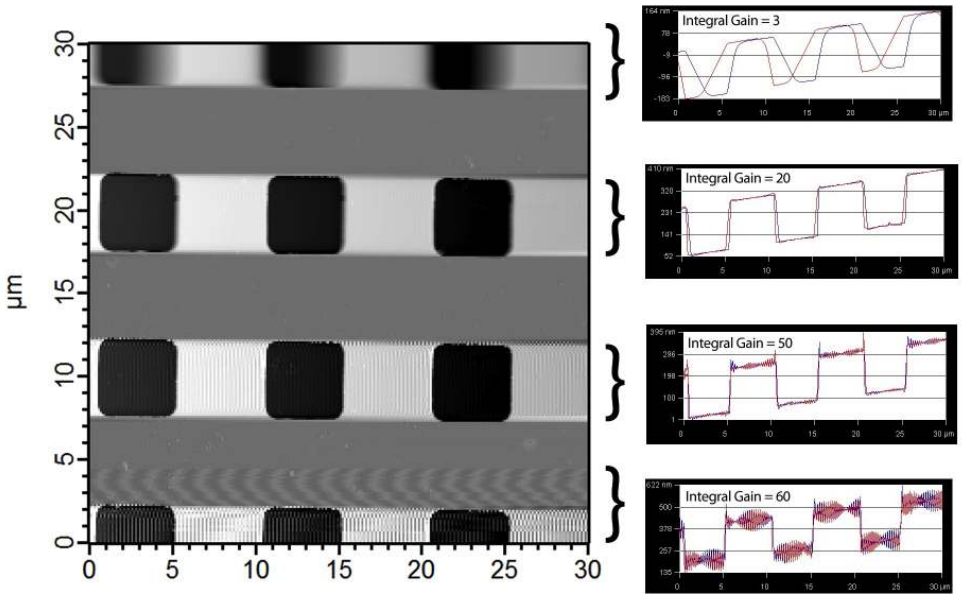
3.



Setpoint Voltage The *Setpoint Voltage* generally needs adjustment:

- Increase the *Setpoint Voltage* value to increase the force applied to the sample, and to improve the tracking.
- Lower *Setpoint* voltages may help preserve the tip apex, but may not allow proper tracking of the surface.
- For this example, use a *Setpoint* between .5V - 1V.

4.



Integral gain adjustment:

- Increase the *integral gain* to improve the tracking, but at a certain level the signal will start to ring.
- Decrease gain until ringing ceases in trace and retrace.

Note There is a slight offset between the trace and retrace lines in the graph. Good tracking means that they should have similar shapes, not necessarily perfect superposition.

Note When adjusting *integral gain*, another channel to look at is the deflection image. Feedback oscillations are easily detected in the deflection image because it is an image of the feedback loop error.

5.

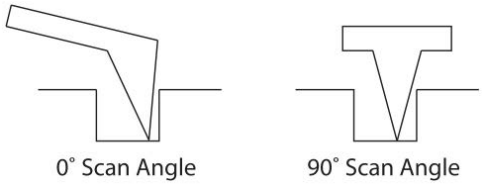
Scan rate adjustment:

- If tracking still seems to be an issue, try lowering the *Scan Rate*. Slowing the *Scan Rate* down will help the feedback keep up with the image features.
- The Scan Rate cannot be updated during imaging like other imaging parameters. You must click *Frame Up* or *Frame Down* buttons to initiate the newly entered scan rate.

Note Too slow of a scan rate can introduce image artifacts due to thermal drift.

6. **Scan angle adjustment:**

Note For the Olympus AC160 and AC240 cantilevers, the tip will be symmetric or asymmetric based on the scan angles shown on the right. For a better representation of side walls 0º scan angle should be used. However, tracking could be difficult due to the steep edge of the tip. Better tracking could be achieved with a 90º scan angle. This is one example; refer to cantilever documentation for different tips.



7. Working with Fluids around the MFP-3D AFM

CHAPTER REV. 1714, DATED 10/25/2013, 20:36. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

The MFP-3D AFM was designed to work with fluids.

- All cantilever holder has a built in O-ring sealed window. See Figure 11.2 on page 102.
- Many accessories, such as the closed fluid cell, are specifically designed for use with fluids.
- A variety of cell culture dishes can be used for imaging in fluids. See Chapter 22 on page 291.

However, the AFM is not "waterproof". It cannot withstand large spills and it is your responsibility to understand its limitations.

Chapter 22 on page 291 shows the areas of the scanner where fluids should not be allowed to enter. Take care to avoid spills in this area.

7.1. General Fluid Imaging

- We recommend the use of a a basic fluid imaging accessory, such as the Fluid Cell Lite, described fully in Chapter 13 on page 145. This will contain a small amount of fluid in a dish, and it will protect the cantilever holder by means of an elastomer membrane.
- For cases where perfusion is required, we recommend at least a full Closed Fluid Cell system, which can be sealed shut to allow small amounts of overpressure and an overflow tube. ALWAYS test your sealed cell for leaks before placing it on the AFM, as described in Section 14.7 on page 183.
- When refilling dishes with pipettes, do not drop any fluid around the perimeter of the scanner. See 7.1.
- Always have some absorbent paper towels at hand to immediately blot up any fluids that spill onto the scanner.
- When lifting the AFM head after imaging in fluids, be very careful not to have any drops fall onto the scanner.

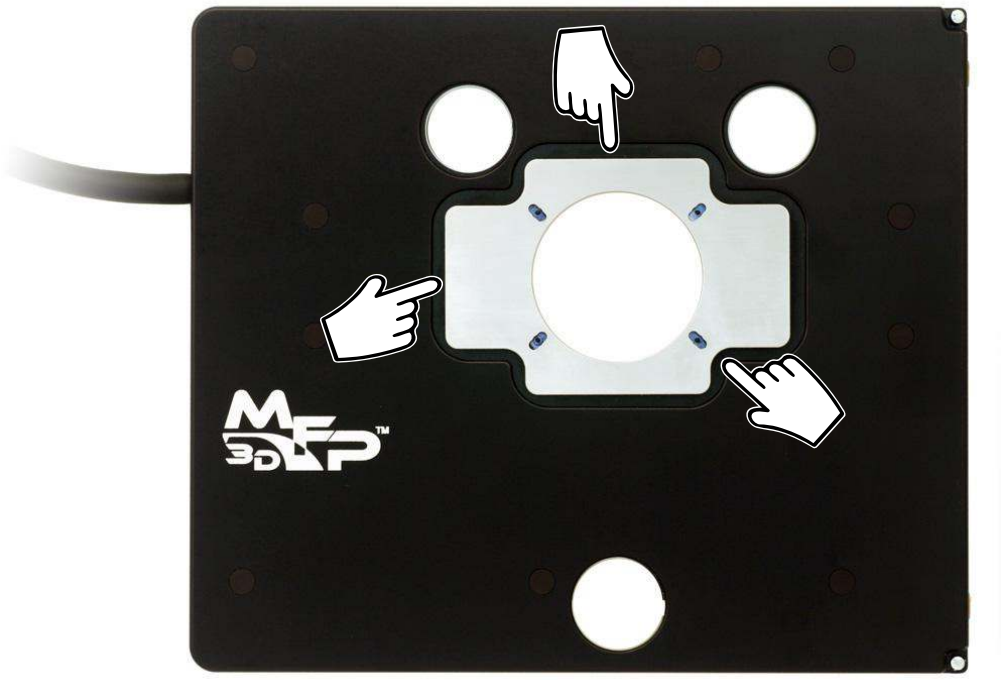
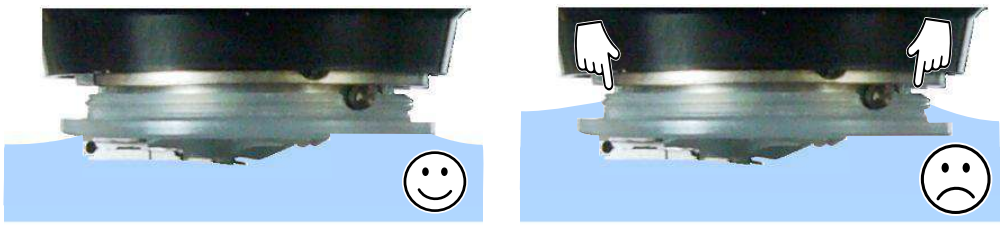


Figure 7.1.: Prevent fluid from entering the gap indicated in the photo.

7.2. Imaging in a Small Droplet

Warning	Whenever possible, attach a membrane to your cantilever holder. The process is described in Section 13.4.3 on page 153.

Some advanced users of our instruments prefer to image in small liquid droplets with just the bare cantilever holder. While we don't recommend it, be sure to pay very close attention to the fluid level. If it flows behind the holder, the fluid will come into contact with the electrical contacts inside the AFM head and can cause serious damage.



(a) Reasonable fluid level

(b) Dangerous fluid level. Liquid is about to enter the indicated areas.

Figure 7.2.: Flooding of the cantilever holder can be a serious problem. Keep an eye on fluid levels, or better yet, image only with a membrane attached to the cantilever holder.

8. Tutorial: AC Mode Imaging in a Liquid Droplet

CHAPTER REV. 1714, DATED 10/25/2013, 20:36. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

AC mode in liquid is frequently used to observe structures, predominately biological in nature, in their native environments. AC mode is often the preferred technique over contact mode due to the reduction of the vertical (applied) forces and the elimination of the lateral forces.

This tutorial focuses on the simplest experimental setup, i.e. a small droplet of liquid sitting on a microscope slide. Once you have mastered this tutorial, you can move on to more advanced accessories, such as:

Fluid Cell Lite: For general purpose imaging with an evaporation shield and thin glass coverslips. See Chapter 13 on page 145.

Closed Fluid Cell: Similar to the Fluid Cell Lite, but with the ability to seal and allow general liquid exchange under slight pressure. See Chapter 14 on page 160.

Bioheater: Also for general purpose imaging, but with an immersion heating element. See Chapter 17 on page 209.

Petri Dish Holder: For imaging in a variety of cell culture dishes. See Chapter 22 on page 291.

Petri Dish Heater: Similar to the Petri Dish Holder, but with heated temperature control ability. See Chapter 23 on page 301.

Cooler Heater: For imaging in drops or small volumes with cooling or heating, but without bottom view access. See Chapter 20 on page 258.

MicroFlow Cantilever Holder: For use with all of the above sample types, plus the ability to exchange 50 µL liquid volumes near the AFM tip. See Section 11.6 on page 116.

Tip	If you prefer imaging in liquid, consider the iDrive cantilever holder (see Section 11.7.2 on page 135). It excites the cantilever magnetically and does not suffer from the "forest of peaks" phenomenon. Autotune (see Section 4.7 on page 33) immediately works in liquids and Q-control also works quite well. For more information also read *Applications Guide, Chapter: iDrive Imaging*.

8.1. Prerequisites

This is a somewhat advanced imaging mode. You must be familiar with the AC Mode imaging in air tutorial (Chapter 4 on page 15). In the following sections much detail will be skipped and basic AFM imaging proficiency is assumed.

8.2. Terminology

Sample Material to be imaged (molecules, cells, etc.)

Substrate Surface on which the sample is immobilized — typically mica, graphite, gold, glass, etc. The substrate is attached to the support.

Support The support (generally a glass slide) is mounted on the AFM.

An example of typical mounted sample is shown in 8.1.

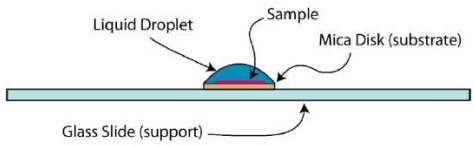


Figure 8.1.: Sample mounting examples.

8.3. Sample Prep

Common sample substrates include mica, graphite (Highly Ordered Pyrolytic Graphite or HOPG), silicon or gold, or on a rougher substrate such as glass if the sample is larger, such as living cells.

Warning Do not spill liquids on your AFM head or scanner, as this can lead to damage. READ the following before you proceed:
Chapter 7 on page 62.

1. The substrate are preferably attached to the bottom of a spill proof dish, such as the Fluid Cell Lite, described in Chapter 13 on page 145. While we don't recommend it, it can also be attached to a standard glass microscope slide, but this greatly increases the risk of spills and damage to your instrument.

2. **Prepare the substrate**

   - If using mica as a substrate, a "hole punch" (for example: Precision Brand 40110 or McMaster Carr Shim - stock or Gasket/Washer Punch sets, #s 3472A11, 3430A12, 3430A15) may be used to quickly make the discs. It is inadvisable to use scissors to cut the mica as that may induce the crystal layers to separate, which will in turn allow liquid to leak through the layers and peel off the top layers during imaging. Alternately, it is possible to buy pre - made mica discs (from Ted Pella Inc. for example).
   - Use a small drop of epoxy (or any adhesive that does not dissolve in water) to immobilize the substrate on the slide. Double-sided tape should be avoided, it commonly caused large scale drift problems when used to attach substrates to the support.

3. **Prepare the sample**

   - Immobilize the sample on the substrate. Typically this step involves depositing a drop of the sample onto the substrate and letting it incubate for a period of time. Set the sample aside if incubation is required.

4. **Prepare the solution**

   - Things work better if the solution is at room temperature, there will be less thermal equilibration then if the solution came out of the refrigerator.

8.4. Prepare the AFM Head (Dry Run)

This process is very similar to that of AC mode imaging in air:

- Loading the cantilever holder (Section 4.2 on page 16).
- Mounting the cantilever (Section 4.2.3 on page 21).
- Head and sample placement (Section 4.3 on page 22).

Since that has been covered in detail, the remainder of this section will only cover the major steps:

1. **Choose and insert a cantilever chip:**

   - Insert the probe into the holder.
   - Select a low spring constant cantilever. For example, silicon nitride cantilevers, like the short, 40 µm Biolever Mini (BLAC40TS) from Olympus, are a good choice for AC mode in liquid. Please visit our probe store where you can browse by application type if you are in need of cantilevers: http://www.AsylumResearch.com/ProbeStore

2. **Place the support on the scanner:**

   - Put the dry sample mounted in the fluid cell, or on its substrate, onto the scanner and hold it in place with the magnets.

3. **Place the AFM head:**

   - Before lowering the head, make sure that its legs have been raised sufficiently to prevent crashing the cantilever (or even the cantilever holder) onto the surface.
   - If using the shorter, 40 or 60 µm Biolever it is recommended that the user angle the head so that lever side rear leg is slightly lower than the other two legs. This will ensure that the small cantilever will be the first thing to contact the surface, instead of the cantilever chip or the longer Biolever.
   - For other cantilevers, such as the Olympus TR400 or TR800, the head should be more or less level.

4. Adjust the legs so that the cantilever is about a millimeter above the surface.

5. **Position the laser spot:**

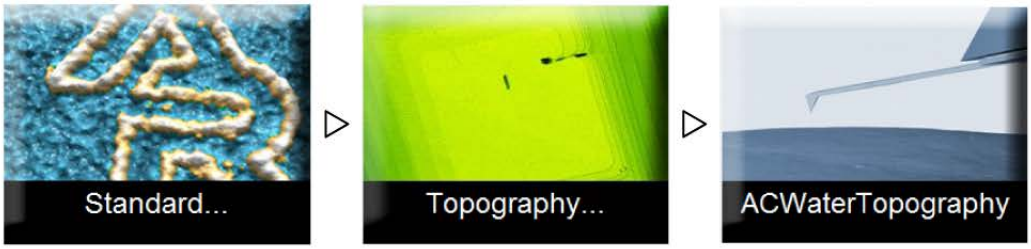
   - It is easier to position the light beam on the cantilever in air, before the cantilever is placed in the liquid.
   - If the MFP - 3D - BIO ™ is being used, a CCD camera may be plugged into the inverted optical microscope, or you can use the standard camera attached to the Top View IO pillar.
   - In any case, once a good video image of the lever is obtained, put the laser spot on the lever.
   - The SUM signal obtained on silicon nitride cantilevers, despite their gold coating, is typically lower than on silicon cantilevers (between 3 and 6 volts).

8.5. Software Prep

1. Start up the software as described in Chapter 3 on page 10.

2. **Select your mode:**

   - The software should now be showing the mode master window.
   - If not, click the Mode Master button at the bottom of the screen: .
   - In this example we will start with AC water topography (see below).

3. 

   Select:

   - Select *Standard* ▷ *Topography* ▷ *ACWaterTopography*
   - The screen will now re-arrange and present all the controls necessary for this type of AFM imaging.

8.6. Place head above the wet sample

1. **Place the sample on the scanner.**

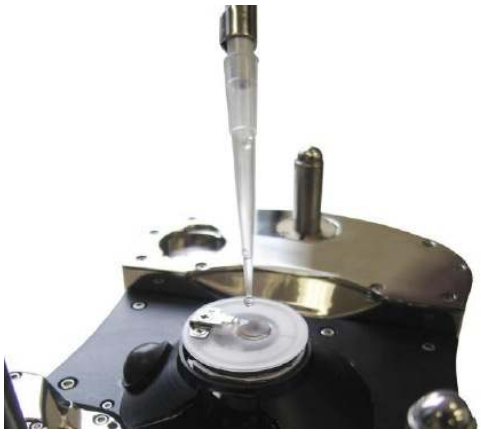
   - Now that everything is aligned, remove the head and dummy sample.
   - Place the actual sample which may have already been wet and incubating. If it is still dry, add enough water to make a drop about 1cm in diameter (see **??** on page **??**). In the case of a mica disc substrate, surface tension should keep the drop from spilling over (see **??** on page **??**)

   Note Make sure the support does not touch the black part of the scanner since only the central silver part scans in the X and Y directions.

   Note As stated previously, be sure to angle the head accordingly if using the 40 or 60µm Biolever Step 3 on page 67.

2. **Wet the Cantilever**

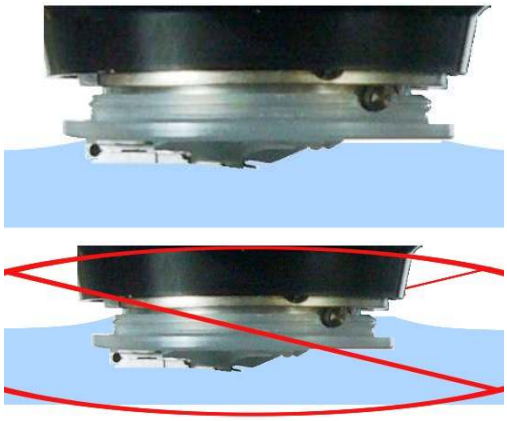
   - To wet the cantilever using a pipette, put 2 or 3 drops of liquid between the lever and the cantilever holder. You can place one drop at the corner of the chip, and pull it slowly and gently towards the lever to pull the drop under the lever. Don't flood the head, just a few drops, liquid in the head electronics is, really bad.
   - When liquid gets into the head, immediately power it down by turning off the controller. Set the head right side up and remove the cantilever holder. Dry any visible liquid and let the head dry out. Extra care should be taken the next time you use the head to ensure it is working properly.

   Note This step is optional, but highly recommended since it reduces the risk of creating air bubbles on the cantilever, and fills the space between the substrate and the bottom of the cantilever holder with liquid.

Place the AFM head on the stage or base

- Make sure the sample, substrate and cantilever chip are totally immersed in liquid. This is important, since the liquid will not always fill the whole space between the cantilever chip and the holder. If this happens, the the laser beam will not reach the photodetector.



3. **Note** We recommend using the Fluid Cell Lite and the included membrane which protects the cantilever holder. See Section 13.4.3 on page 153.

   Note If not using a fluid cell or membrane, beware of using too much liquid, as it should never spill around the cantilever holder or onto the electronics behind it. We recommend the use of a membrane to protect the cantilever holder from damage. See Section 13.4.3 on page 153.

Realigning the Laser Beam Once the cantilever has been fully immersed in liquid, the SUM signal will disappear. This is because the optical path is different due to the change in the index of refraction. The light beam needs to be redirected to the lever to regain the signal.

4.
   - Turn LDX clockwise. The SUM signal should increase to its maximum value.
   - Typically, the value will be lower than it was in air. Typical values for silicon nitride cantilevers in liquid are between 3 and 4V.
   - You may also want to refocus the video view once you are in liquid.

Trouble? For AC Mode in Liquid Troubleshooting topics see Section 8.10 on page 75.

8.7. Cantilever Tune

Tuning in liquid is more difficult than in air. There is coupling between the liquid and the cantilever, and possibly the cantilever holder and other components as well. This creates multiple peaks (a "forest of peaks") which makes choosing the correct peak difficult.

For more details on how to tune a cantilever from a thermal, see *Applications Guide, Chapter: Thermals*.

Here we will go through a quick guide on how to work with the Biolevers 150 in water.

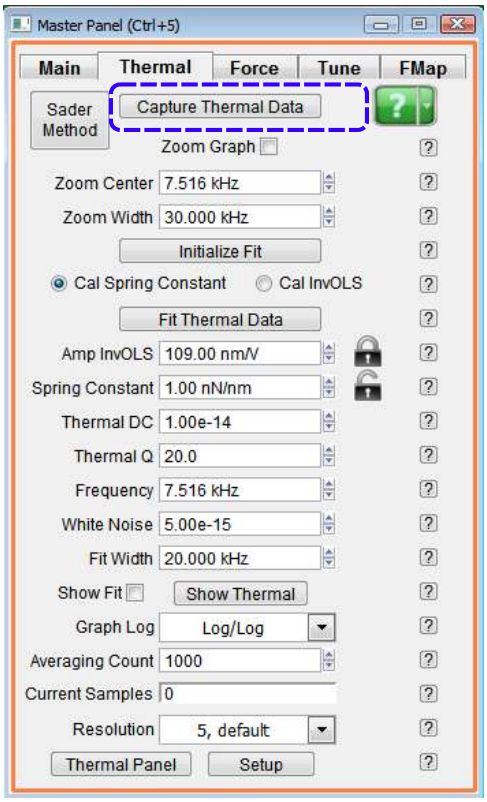


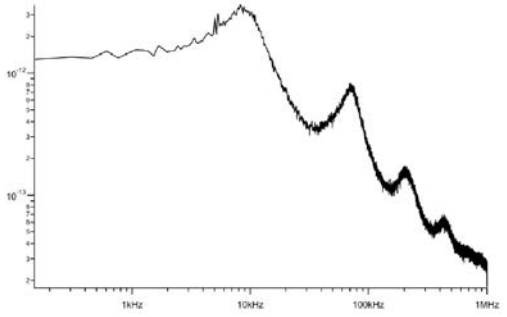
Figure 8.2.: Master panel, Thermal tab

1. **Do a thermal:**

   - In the *Master panel* (Ctrl + 5), select the Thermal tab (See Figure 8.2 on page 71) .
   - Then click on *Do Thermal* (Ctrl + 2).

2. **The resulting spectrum:**

   - There should be a broad peak around the resonance frequency of the cantilever.

   Note If the value of the resonance frequency in liquid is unknown, it is (very approximately) 1/3 of its value in air. For example, when using a 60µm long Biolever from Olympus, has a nominal air resonance of 37 kHz, the peak in water is at about 8 kHz.

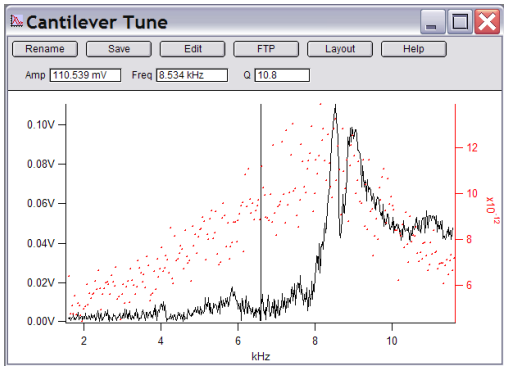
3. Zoom in on the peak, and fit it. If the fit is decent, right click on the thermal and select Move Freq and Phase to the tune.

4. **Set up the tune:**

   - In the Master panel, select the 'Tune' tab.
   - Near the bottom, expand graph, turn on 'Append Thermal'.
   - Then, set the 'Sweep Width' to 20 or 30 kHz.

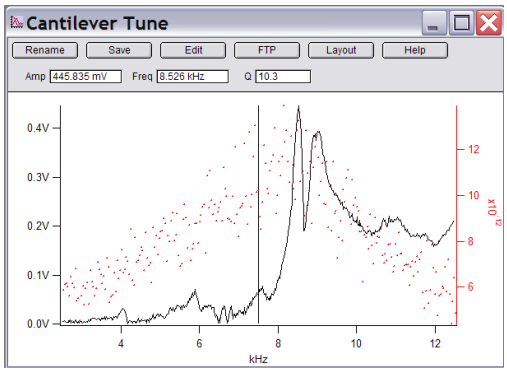
5. **Click on 'One Tune' (Ctrl + 4)**

   Note The the "forest of" peaks vary greatly from experiment to experiment.



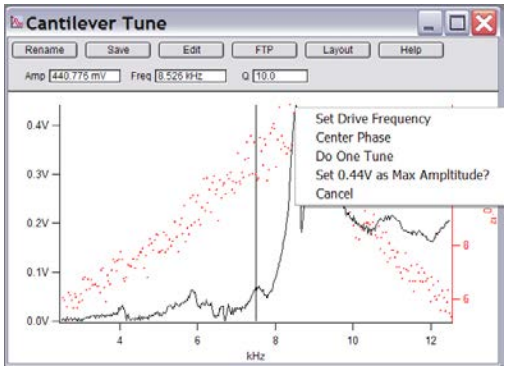
6. **Try a higher drive amplitude:**

   - If the signal noise is low, you can increase the *Drive Amplitude*, and click *One Tune* (Ctrl + 4) again.



7. **Select a peak:**

   - Choose the most prominent peak that overlaps the thermal. Right click just left of that peak.
   - Choose 'Set Drive Frequency As' .



8. Click the 'Center Phase' button . This will center the phase at 90°on resonance. This allows you to monitor whether the tip is in the attractive or repulsive regime. However, this does not hold as true in liquid (using shake piezo drive) as it does in air.

Tip

If you prefer imaging in liquid, consider the iDrive cantilever holder (see Section 11.7.2 on page 135). It excites the cantilever magnetically and does not suffer from the "forest of peaks" phenomenon. Autotune (see Section 4.7 on page 33) immediately works in liquids and Q-control also works quite well. For more information also read *Applications Guide, Chapter: iDrive Imaging*.

8.7.0.1. Change the R Filter Value

1. Change the value of the Feedback filter on the main tab (may need to use setup to show the control) to 500 Hz.

This digital filter is applied to the AC signal coming from the photodetector. It has to be at a different value when working at these relatively low frequencies. In contrast, the R value should be 1500Hz (1.5 kHz) for AC mode in air.

8.8. Choose Imaging Parameters

The values below are only suggestions and may be optimized later with some user experience. (See Figure 8.3 on page 74) Return to the Master panel and review and adjust each parameter as follows:

8.8.1. Drive Amplitude

This parameter determines the free amplitude of oscillation. When imaging most biological samples in liquid, smaller oscillation amplitudes are better. Typical small values are 0.7 to 0.9V. For harder surfaces like biomaterials (polymers, ceramics, metals) a free amplitude of 2V can be used. If the sample is sticky, it may be better to use higher values.

8.8.2. Setpoint

The setpoint has to be smaller than the free amplitude - typically about 80% of the free amplitude. In order to be gentle when imaging soft samples, it is preferable to use a setpoint value close to the free amplitude. For example, when imaging DNA in liquid with a free amplitude of 0.8V, the setpoint should be 0.6V.

8.8.3. Scan Rates

The typical scan rate in liquid is 1Hz, although some samples may require lower speeds. For example, living cells can require 0.2 Hz.

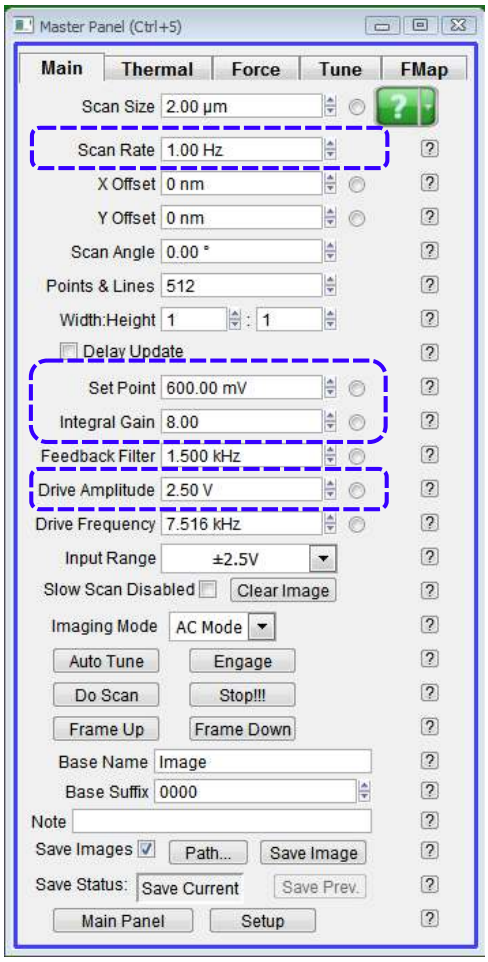


Figure 8.3.: Master Panel Main Tab

8.8.4. Integral Gain

Start with a value of 10 and optimize the gain constantly while imaging. Typically, you should increase the value until noise in the amplitude is visible, then reduce the value until the noise disappears. This value is ideal for optimal tracking of the sample. Note that Proportional Gain is less effective and can be set to 0V most of the time. Drive frequency was already chosen when the cantilever was tuned. The other parameters should be similar to those for AC mode in airChapter 4 on page 15.

8.9. Engage

The engagement procedure is the same as in air. Below is a brief reminder of the engagement process. For more detail on engagement, please see Section Section 4.8 on page 34 for the so-called “Hard Engage.” Better yet, see Section 4.10.6 on page 44 for the “Soft Engage”, which is much gentler on your tip.

1. In the Sum and Deflection Meter panel, click on the ‘Simple Engage’ button.

2. Carefully lower the head until you hear the audible engage notification and the Z voltage is centered at approximately 70V.
3. Using the Trace/Retrace graph as a guide, decrease the set-point until the tip is tracking the surface well.
4. Adjust the Integral Gain and Scan Rate as needed.

8.10. Troubleshooting

8.10.1. Bubbles

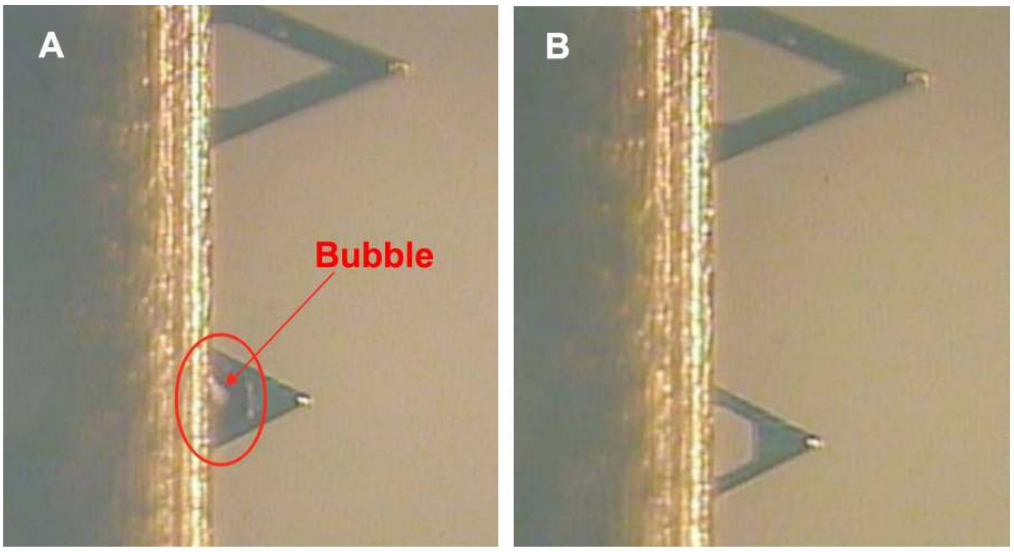
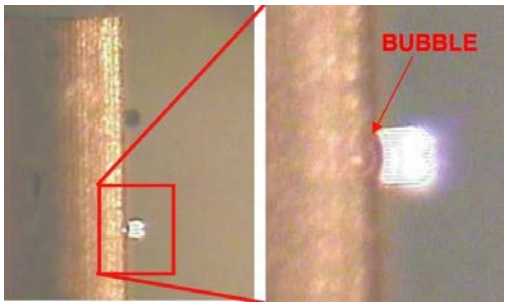


Figure 8.4.: (A) bubble trapped between the legs of a triangular cantilever, and (B) view with the bubble absent.

An air bubble may get trapped somewhere by the cantilever, which will eventually compromise the deflection signal because the bubble may migrate. It is best to get rid of these bubbles before imaging.

Observe a bubble:

1.
- On a triangular lever the bubble may look as it does in Figure 8.4 on page 75.
- Very floppy cantilevers may be deflected due to the surface tension from a bubble. This can angle the lever so it reflects a lot of illumination, as seen in the image to the right.

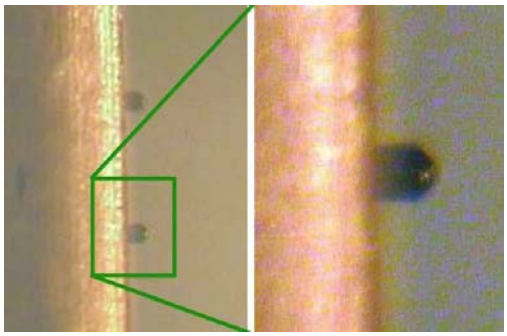


2. **Lift the head:**

   - Gently lift the front of the head up, pivoting on back legs, such that the cantilever holder comes out of the liquid.
   - Plunge it back into the liquid.
   - If necessary repeat a few times.

3. **Observe to see that the bubble is gone:**

   - A dark lever indicates that there is no longer a bubble.



4. If the bubble is not removed after trying multiple times, lift the head up off the stage, place on its side and dispense some imaging liquid to remove the bubble with the force of the liquid under gravity.
5. Re-wet the tip and place back on stage. Try again.

8.11. Maintenance

At the end of your experiment, remove the cantilever (it is advisable to discard it) from the holder and clean the holder. There are multiples ways of cleaning the cantilever holder: the following list describes only some of the available methods.

- Use soapy water and gently rub the holder with your finger, preferably while wearing gloves. Make sure not to scratch the glass window through which the light beam travels. Rinse copiously with deionized water.
  - Optionally, you can followed that by two additional steps: a rinse with 70% ethanol in water, and a final rinse with deionized water.
- If more cleaning is required, partial disassembly of the cantilever holder is required. See Section 11.2.4.1 on page 101.

8.12. Troubleshooting

8.12.1. Difficulties with Soft Engage

The soft engage is difficult in air, in liquid is can be even more difficult.

You should be proficient with soft engage in air (see Section 4.10.6 on page 44), before you attempt to engage with the soft engage in liquid. But some additional things to consider when doing a soft engage in liquid.

1. As you thumbwheel down, you will notice that the Amplitude value in the S&D Meter is **increasing** instead of the expected decrease. This is (believed to occur) because of the liquid being compressed between the tip and sample from the oscillating shake piezo in the cantilever holder, effectively imparting a larger Free amplitude onto the cantilever as it approaches the surface.
2. Use the Hamster wheel to occasionally decrease the Drive amplitude to maintain the proper setpoint ratio (i.e., ~95% of the free air amplitude).
3. Slowly decrease the setpoint voltage with the Hamster Wheel such that the tip 'hard' engages on the surface.
4. Move piezo into middle of Z range (~70 V).
5. Start scanning.

8.12.2. Tuning imaging parameters in liquid:

Tuning the imaging parameters in liquid can also be a little trickier than in air. We suggest exercising patience, especially when imaging soft biological samples because this should be done at low scan rates (< 0.5Hz), increasing acquisition times.

Although calibration grids are also great for learning how to image, it is NOT a good idea to put water onto the provided 10 μm calibration grid, they are never the same after that.

NOTE: Depending on the sample, obtaining really good tracking is usually NOT a frequent occurrence when imaging in liquid. When you get something that looks real, go with it.

It is also possible to image at very low Drive Amplitude and setpoint voltages. The problem is that you may not be able to engage with low amplitudes. The trick to this is once the tip is engaged and imaging, slowly step down the setpoint and Drive Amplitudes iteratively. It can take a while, but the results can be very good - lower tip oscillations mean less sample perturbation (especially with cells and other bags of water).

Additionally, the Drive Frequency can be adjusted a small amount (using the Hamster wheel works well for this) until the better imaging is obtained.

9. Troubleshooting

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

In no particular order a variety of trouble shooting advice related to things discussed in this manual.

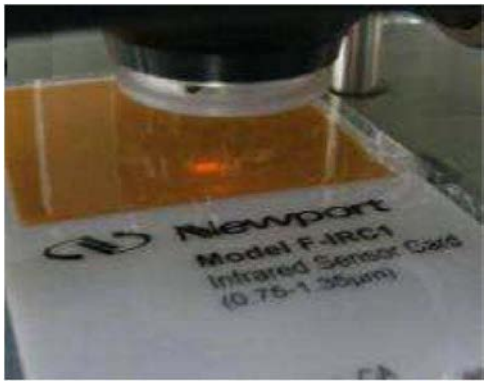
9.1. Laser alignment without top view optics

For the AFM heads without top view optics, or the situations where one may want to set up the cantilever without having to place the head on the base, there are a few options.

The simplest method is to look only at the SUM signal. Since the general position of the cantilever and chip are always the same in the field of view, one can quite successfully imagine its position as seen in Steps 3 through 7, starting on page 18.

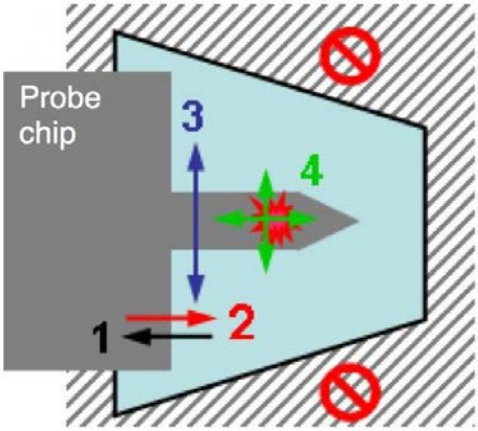
A simple tool called an IR card can greatly reduce the guesswork. Note that as of June 2009, IR cards are no longer shipped with our AFM systems, but they are still available for those who request them. The IR card needs to be charged up in daylight or fluorescent light. Any IR beam that then falls on the card will manifest itself as a red dot. If the dot sits in one place for too long, it will fade unless the card is moved a little, or the card is charged up again.

1. **Set up the head and IR card**
   - Place the head (with cantilever holder and cantilever) on the head stand next to the base.
   - Slide the IR card under the head as shown.



2. **Move the laser onto the lever**

   - The figure to the right shows the motions in numerical order and where you should imagine the spot to be.
   - Observe the red spot on the card and associate it blinking in and out of view with your mental picture
   - Look at the SUM meter on the computer and maximize it when you think you are the end of the lever.



3. A successful positioning will result in a red spot on the IR card which looks split since its center is obscured by the cantilever. A slight movement of LDY away from the lever should reduce the SUM to near zero and make the spot on the IR card light up brightly again.

9.2. Centering the Z Piezo Range After Contact with the Surface

Ideally the Z voltage as read in the Sum and Deflection voltage falls in the middle of its range. The piezo response is most linear in the center of the range, and the LVDT signal has the lowest noise. Being in the center of the range also gives an equal amount of vertical travel for a potentially unknown sample topography and mechanical and thermal instrument settling.

As mentioned earlier, if the voltage upon contact falls in the range of 50-90V, then it is probably not worth making any adjustments. If it falls outside of this range, you have two options:

9.2.1. Brute Force Z Centering

1. While monitoring the Z voltage on the Sum and Deflection Meter, gently turn the front thumb wheel on the head. The tick marks on the wheel correspond to about 1 micron of Z motion. Turn the wheel until the Z voltage is centered in its range.

Note This can damage your tip. The feedback loop reacts to an error signal, and as you are approaching the sample, it is accumulating error of one sign. Once you reach the surface, you need to exceed the setpoint, to produce an error signal of the opposite sign.

9.2.2. Gentle Z Centering

1. Note the current Z Voltage, and subtract this number from 70V. Example: If the voltage reads 30V, your answer is 70V-30V = 40V.
2. Divide this number by 10V/µm (the standard Z range is about 15 µm spread over about 150V). In our example we get +4 µm . This means we need to raise the center of the cantilever holder by 4 µm to land the Z voltage at 70V.

3. You could also run this from the command line (Ctrl + J), Print (70-td_RV("Height"))/10, which will print out the number of µm you need to move, the sign will tell you if it is up or down.

4. **Locate the Large Thumb Wheel Markings:**

   - Locate the markings on the large thumb wheel on the head. On older AFMs it will look as to the right. On newer models the tick marks on the black portion have been removed.
   - Locate any tick mark you like on the black portion. Ticks on the wheel correspond to approximately 1 µm of Z travel at the cantilever.

5. Click the *Withdraw* Button on the Sum and Deflection Meter Panel. This pulls the tip from the sample.
6. Rotate the large thumb wheel by the appropriate amount based on what you calculated in the earlier steps. For our example we rotate clockwise so that 4 tick marks on the silver wheel pass one of the tick marks on the black part.
7. Click the *Engage* button on the Sum and Deflection Meter Panel. Check to see that the Z voltage is now around 70V.

Since the tip was withdrawn from the surface before the front leg of the AFM was adjusted, this method avoids laterally raking the tip across the sample surface.

9.3. Various issues

No significant Deflection voltage, or very low Sum voltages: Typically either the cantilever is not seated in the pocket properly, or some debris may be acting as a fulcrum, moving the orientation of the lever out of the proper plane.

- The probe may be bad (i.e., has a bend/sag that does not allow the SLD beam to bounce off of it properly).
- There may be no cantilever on the probe (verify in CCD camera; or perform Thermal Tune). The signal may be coming from reflection off the probe chip.

Values of NaN in S&D Meter: This means not a number. What may have occurred is a slight grounding issue at the cable plug interfaces. Sometimes the grounding clamps may need a better bite into the outer ground frame of the plug. Try checking the connection.

Reducing abuse on the head cable

- Be careful not to torque/twist the cable to the head: always follow the same rotation path that you took the head off with. The head cable should only experience 180 ° of rotation in its regular on stage, off stage cycling. Continual twisting of the head can:
- Break down the head cable over time.

Part II

Advanced Imaging Hardware

Part II: Who is it for? Once you have become familiar with basic imaging, as described in the tutorials in I, this manual will guide you through the many advanced accessories which the MFP-3D offers. For example, to collect images on samples heated above 200° C, a Polymer Heater and Environmental Controller are required. Use of such accessories will be described in this manual.

Part Contents

10. Chemical Compatibility Guide

CHAPTER REV. 1630, DATED 09/05/2013, 09:08. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

The question of chemical compatibility and the MFP-3D is often posed.

We can tell you the materials that are wetted by the solvent (or gas). However, because there are thousands of solvents, we cannot provide a comprehensive list of compatible and incompatible solvents. It will also depend on the criterion for compatibility: short term exposure or long term? Damage to the fluid cell or contamination of the experiment? You will need to educate yourself about compatibility to be certain of a good experimental result. We will update the information in this chapter whenever we learn of someone pushing the envelope with solvents and chemicals.

Contact support@asylumresearch.com with further questions.

10.1. Internet Resources

A good web site for compatibility information is

- Cole Parmer

Since PEEK polymer is central to much of our design, please refer to the following websites for detailed information on compatible and incompatible solvents for various temperature ranges:

- flaretek.com
- Zeus

Dupont Elastomer Guides are in the form of a piece of software or a web interface, and require a username and password you can request form Dupont. If you cannot manage, please contact Asylum Research and we can run the programs for you.

- Dupont Elastomer Chemical Resistance Guide
- Dupont Kalrez Application Guide

More on Elastomers:

- O-rings west, select the Fluid Compatibility Guide

10.2. Conduct your own tests

If your chemicals are simply not to be found in any resources, or the description is vague, you can always do an experiment. Take one of the materials in question, like an O-ring, and soak one of the spare parts in your chemical. Measure the dimensions beforehand, and after an hour, or a day. If there is nothing visible and minimal swelling of a few percent, then the materials are compatible with the chemical in question. When it comes to PEEK, you can use something non critical like the 111.725 tool, of which you may have multiple copies. Contact us if you need some extra bit of material to test with.

10.3. Your Strategy for Determining Chemical Compatibility

The typical construction for a sealed sample environment consists of four main components:

- The Cantilever
- Cantilever Holder
- Sealing Membrane
- Sample Cell

Many of the above items are discussed in detail in various other places of this manual. For more detailed information on materials of construction, consult the spare parts list typically found at the beginning of each chapter. Below we summarize most of the information in one place.

10.4. Materials of Construction

10.4.1. Common Cantilevers

Silicon cantilevers

- Silicon: the chip, lever, and tip itself.
- Aluminum: reflective coating (in some cases, e.g. AC240TS cantilevers from Olympus)
- Platinum: (in some cases, e.g. conductive coating on AC240TM).
- Check with your cantilever vendor about other possible coatings.

Silicon nitride cantilevers

- Glass (borosilicate/Pyrex): the chip that is used for handling the cantilever
- Silicon nitride Si_3N_4: the thin film of the cantilever itself. Since this is covered by a native oxide layer, its chemical resistance should be similar to SiO_2.
- Chromium/Gold or Titanium/Gold: the reflective layer is a few nanometers of either chromium or titanium covered by many nanometers of gold. A reflective layer is required on silicon nitride cantilevers.
- Silicon: in some cases the tip is made of silicon. Check with your cantilever manufacturer about the specifics.

Solid diamond cantilevers

- Solid ultra nano crystalline diamond: tip and lever, solid.
- Silicon: typically the chip substrate material.

Solid platinum cantilevers

- Solid platinum: probe tip and lever, formed from a single platinum wire.
- Conductive epoxy: used to attach the lever to the substrate.
- Gold patterned alumina: chip substrate.

10.4.2. Cantilever Holders

Standard MFP-3D Style Cantilever holders

These look similar to the holder covered in Section 11.2 on page 99.

- Medical Grade Glass filled PEEK: the body of the cantilever holder.
- Stainless steel #301: the spring clip (111.511, magnetic).
- Stainless steel : the screws for the spring clip (non magnetic).
- Stainless steel #303: Press fit treaded holes/electrical feedthrough.
- FKM** (Generic Viton): the O-ring (230.011) that secures the fused silica prism. Note: An FFKM (Kalrez equivalent) O-ring is available. See Section 24.4.2 on page 323.

Cousins of the standard cantilever holder that are made of the same materials are the ones that look the same, such as the ORCA and iDrive cantilever holders. For a full list see Section 11.1.1 on page 95. Note that some of the split clip cantilever holders have a pure PEEK screw and another small PEEK device around the screw.

Kel-F MFP-3D Style Cantilever holders

Same as Standard MFP-3D cantilever holder but with translucent a Kel-F cantilever holder body. This legacy part was replaced with the glass filled PEEK version in early 2008. It is likely you may have one depending on the age of your system. They are still available per special request. Kel-F has superior chemical resistance rivaling PTFE (Teflon), but is much softer than glass filled peek and has poorer dimensional stability. Kel-F has superior optical properties for sensitive fluorescence microscopy experiments.

- Kel-F: the body of the cantilever holder (111.561).

PEEK MFP-3D style cantilever holders

These look similar to the holders covered in Section 11.4 on page 108.

- Polyetheretherketone (PEEK): cantilever holder clip and screw
- Medical Grade Glass filled PEEK: cantilever holder body (blue or beige)
- Fused silica (SiO_2): prism (111.683) allowing for cantilever optical access.
- FKM** (Generic Viton): the O-ring (230.011) that secures the fused silica prism. Note: An FFKM (Kalrez equivalent) O-ring is available. See Section 24.4.2 on page 323.

10.4.3. Membranes

These are used to seal sample cells as described in the following sections.

- FKM**: 100% FKM** (generic Viton equivalent, black membrane).
- Silicone: 100% silicone rubber translucent membrane.
- FFKM: a generic equivalent of Dupont Kalrez. See Section 24.4.2 on page 323.

10.4.4. Sample Cells

Closed Fluid Cell and Fluid Cell Lite:

See Chapter 14 on page 160.

- Polyetheretherketone (PEEK): the body of the fluid cell
- FKM** (Generic Viton Equivalent): the bottom O-ring, the O-rings for the ports, and the membrane that seals the cell (also supplied in silicone).
- Silicone Rubber: fluid cell membrane (also supplied in FKM)
- Teflon PFA: the plugs for the larger tubing ports
- Stainless steel, 18-8: the plugs for the smaller tubing ports (also available in PFA Teflon)
- Float glass: the standard 35 mm diameter x 1 mm thickness sample plates. Glass made by Glaverbel. Fused silica available by special request.

Bioheater

See Chapter 17 on page 209.

Same as the closed fluid cell +

- PTFE: heating element coating.
- Surgical Stainless Steel 316: temperature sensor housing.

Polymer heater

See Chapter 18 on page 224.

Only for use with inert gases:

- Polyetheretherketone (PEEK): the body of the polymer heater
- FKM** (Generic Viton): O-rings for the ports, and the membrane that seals the cell (also supplied in silicone)
- Teflon PFA: the plugs for the tubing ports
- Glass ceramic: support disc for heated sample stage
- Tungsten alloy: Heated sample stage
- Stainless steel: sample hold down clips and screws

Cooler heater

See Chapter 20 on page 258.

For use with inert gases or liquid drop experiments.

- Black anodized aluminum: the body of the polymer heater
- Polyetheretherketone (PEEK): the plastic surrounding the cooled center sample stage
- FKM** (Generic Viton): the O-rings for the ports, and the membrane that seals the cell (also supplied in silicone)
- Teflon PFA: the plugs for the tubing ports
- Tungsten alloy: the heated sample stage

Humidity sensing cell

See Chapter 19 on page 239.

Only for use with inert gases.

- Polyetheretherketone (PEEK): the body of the humidity sending cell
- FKM** (Generic Viton): the bottom O-ring, the O-rings for the ports, and the membrane that seals the cell (also supplied in silicone)
- Teflon PFA : the plugs for the larger tubing ports

- Stainless steel, 18-8: the plugs for the smaller tubing ports (Also available in PFA Teflon)
- Float glass: the standard 35 mm diameter x 1 mm thickness sample plates. Glass made by Glaverbel. Fused silica available per special request.
- Honeywell HIH 4000 humidity sensor
- Delrin and gold coated sockets: the sensor connector, which is **not** O-ring sealed and can be exposed to the gases in the cell
- Teflon: the dish to replace glass bottom when using salt solutions to regulate humidity

10.4.5. Notes

FKM** : Membranes and O-rings are made of a Type 1 FKM flouropolymer composed of vinylidene fluoride (VDF) and hexafluoropropylene (HFP) di-block copolymers. This is similar to Dupont Viton® type A.

10.5. Comments on pH

10.5.1. Low pH (Acidic)

Stainless steel in contact with fluids will often be a limiting factor, especially if there are chloride ions in solution. If your experiment can tolerate transition metal ions, you will be able to work at lower pH since it will leach transition metals before you see any physical damage to the standard cantilever holder metal retaining clip and screws.

The Stainless steel clip is available in Beryllium copper, but still affixes with stainless steel screws into holes made of stainless steel.

Glass sample disks will suffer at low pH and can be replaced by a more resistant material, since the fluid cell will hold any disk that has a 35 mm diameter and is 1 mm thick at the edge (it can be thicker in the center). Most gases will be OK except for halogens, ammonia, and anhydrous acids.

10.5.2. High pH (Basic)

Silicon cantilevers will etch. In most liquids you will not be able to use cantilevers with an aluminum reflective coating; even in neutral buffer the chloride ions attack it. However, you can use uncoated or gold-coated silicon cantilevers.

Viton seals will limit work at high pH.

10.6. Comments on Solvents

Solvents will often cause swelling of elastomers, and not necessarily outright degradation. For instance, placing an FKM O-ring or membrane into a beaker of acetone will immediately make it writhe and curl up like a potato chip. Eventually it will flatten out, swollen to over twice its original size. In this state the material easily tears, but when left to dry out it will return to its original shape

and regain its original strength. You can always test your materials by soaking them in the solvent in question, AFTER you have consulted the internet resources to at least avoid outright destruction of the parts.

10.7. Tips and Tricks

To eliminate the stainless steel, you could remove the spring clip and attach the cantilever to the cantilever holder with vacuum grease or opt for the all peek cantilever holder.

In some cases PEEK will be attacked while PPS will not. Please contact Asylum Research about parts which can be made of PPS, and in some cases are already stocked based on previous custom requests.

11. Cantilever Holder Guide

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

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 the available cantilever holders have many things in common:

- All of inert materials such as PEEK, Stainless Steel, Quartz, and fluoroelastomer seals.
- All but a few are usable in liquid and gas and can be outfitted with a membrane for use as part of a sealed imaging environment.
- All have a circuit board which allows the system to identify the type of cantilever holder and activate the appropriate software control panels.
- Nearly all have a piezoelectric actuator and 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, injecting small volumes of fluid, and more.

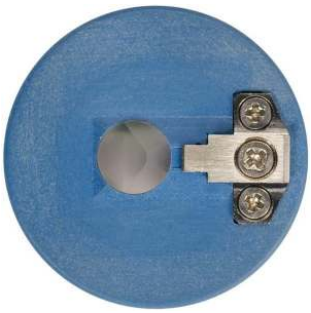
Be Careful	Cantilever holders are the most delicate components of the AFM. Treat them like you might treat your great grandfather's pocket watch. Never drop it. Remember that even the most basic cantilever holder costs thousands of dollars to replace.

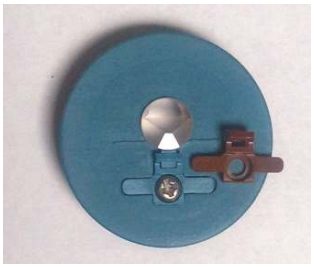
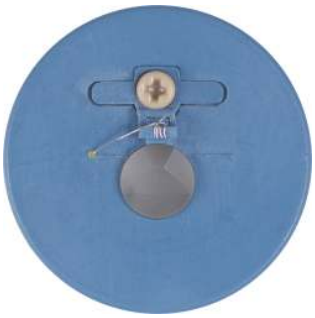
11.1. Identifying Cantilever Holders

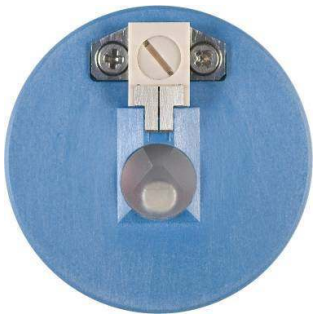
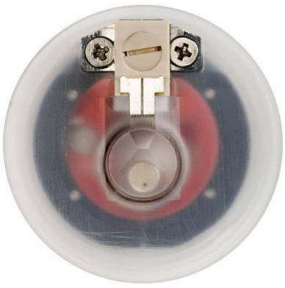
11.1.1. Visual Guide of Cantilever Holders

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

Color	A note on color: Most of the cantilever holders are made of glass filled PEEK. In many instances this PEEK has a blue color to identify it as traceable medical grade PEEK. In some cases the blue tinted version is not available at the time of manufacturing and the same material in the natural beige PEEK color is substituted. Do not worry if the photos in this chapter do not reflect the exact color of your cantilever holder.

Part #	Item Description	Front Photo	Back Photo
908.021	**Standard** Note metal cantilever clip and "V1.5" on back. See Section 11.2 on page 99.		
908.033	**Standard, Kel-F Version for Fluorescence Studies** Note "V1.5" on back. See Section 11.2.5 on page 108.		
908.036	**ORCA (Conductive AFM)** Note "V1.5" on back and circuit components. See Section 21.3.2 on page 284.		
908.045	**Dual Gain Orca** Note "DG" and amplifier chip on the back. See Section 21.3.2 on page 284.		
908.037	**PEEK** Note the PEEK clip and v1.5 on backside of circuit board. See Section 11.4 on page 108.		
The scale in the photos is in cm and mm.			

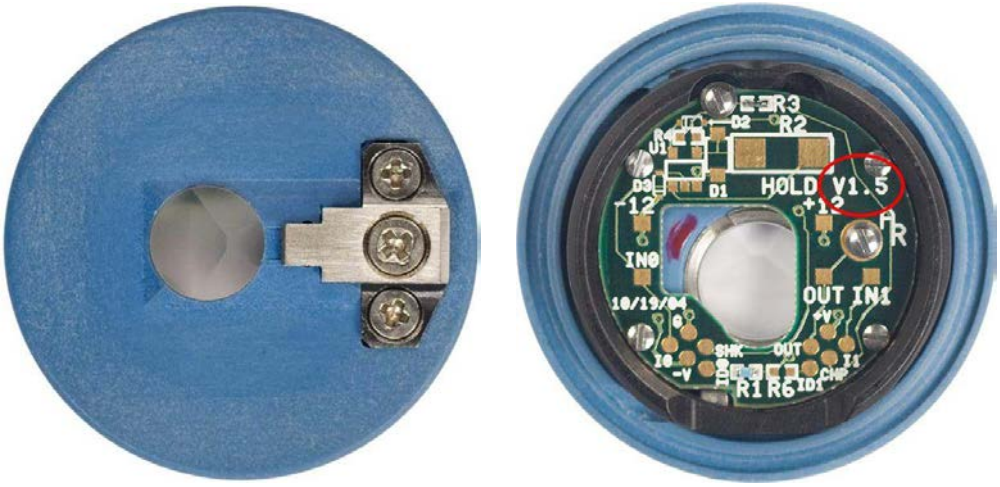
Part #	Item Description	Front Photo	Back Photo
908.085	**High Temperature PEEK** Note v1.5 on backside of circuit board. See Section 11.5 on page 114. The specialized brown clip has part number 114.550.		
	PPS Note v1.5 on backside of circuit board. Pure PPS version of PEEK cantilever holder.		
908.072	**MicroFlow** Note two plateaus with holes. See Section 11.6 on page 116.		
908.064 1M1G	**PEEK ORCA Dual Gain** Note “DG” and amplifier chip on the back. See Section 11.7 on page 128.		
The scale in the photos is in cm and mm.			

Part #	Item Description	Front Photo	Back Photo
900.325	**iDrive** Note “I” on back, magnet inside glass prism, and split cantilever clip. See Section 11.7.2 on page 135.		
same..	**iDrive for Fluorescence Studies** Same as above but with Kel-F Body. Note “I” on back ,magnet inside glass prism, and split cantilever clip. See Section 11.7.3 on page 135.		
The scale in the photos is in cm and mm.			

11.1.2. Electronic Identification of Cantilever Holders

1. Attach the cantilever holder to the MFP-3D Head. (See Step 2 on page 21).
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.

11.2. Standard Cantilever Holder



(a) Front side. **(b)** Back side and identifying features.

Figure 11.1.: Standard Cantilever holder.

11.2.1. Overview

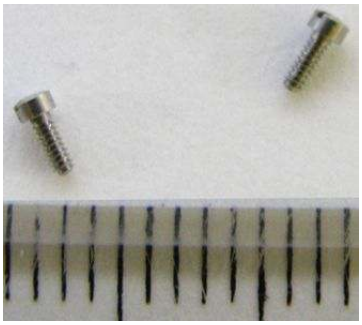
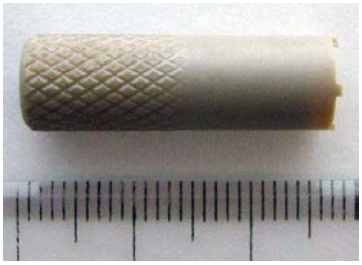
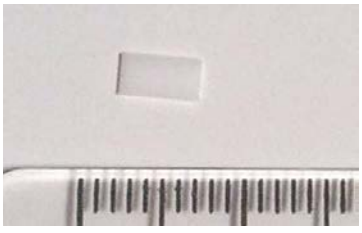
The standard cantilever holder ships with every MFP-3D system and is the workhorse for basic imaging modes such as:

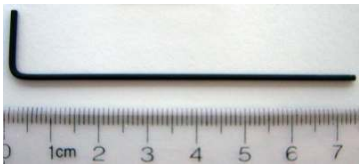
- Contact Mode Imaging
- AC Mode Imaging
- Kelvin Probe Force Microscopy
- Force Curves, AC and DC

It is made of inert materials and can be used in air or liquid and can be part of a sealed environment such as the closed fluid cell, polymer heater, and other related accessories.

11.2.2. Standard Cantilever Holder Kit Parts List

The cantilever holder comes with a small kit of spare parts and tools:

Itm	Part #	Item Description	Qty	Picture
1	001.FILL #000 - 120 X 0.094 SST	000-120 X 3/32” Fillister Head Stainless Steel Screws. Item A in Figure 11.2 on page 102. Connects electrical tip bias voltage from the cantilever holder circuit board to the clip. Use screwdriver 290.110. A few are included since these screws, while standard, can be tricky to find outside the USA.	2	
2	111.725	Fluid Cell Ring Tool. Used to adjust retaining retaining ring C in Figure 11.2 on page 102. Used only during disassembly.	1	
3	111.737	Modified 0-80 Screw. Used to attach the cantilever clip to the body. Note that these screws have been machined to a nonstandard length. You must only use this Asylum part number. Item J in Figure 11.2 on page 102.	4	
4	111.738	Modified 1-72 Screw. Tighten the cantilever under the clip. Note that these screws have been machined to a nonstandard length. You must only use this Asylum part number. Item H in Figure 11.2 on page 102.	5	
5	112.495.02	Coupling Pad 0.015”. Ensures the mechanical contact between the AC mode shake piezo and the cantilever holder. Without this pad, AC mode imaging is not possible. Item F in Figure 11.2 on page 102.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
6	230.011	O-Ring, 0.244” ID X 0.016”CS, Viton, 55 Durometer. Item E in Figure 11.2 on page 102.	5	
7	290.106	#00 Phillips WIHA Screwdriver 261 PH 00 X 40. Used on Items J and J in Used for tightening item A in Figure 11.2 on page 102. Used on a regular basis when inserting and removing cantilevers.	1	
8	290.110	WIHA Screwdriver, Flat Tip 260 1,5 X 40. Used on item A in Figure 11.2 on page 102. Used only during disassembly.	1	
9	290.116	.050” Ball End Allen Wrench. Used when disassembling the cantilever holder for cleaning. See Section 11.2.4.1 on page 101.	1	
The scale in the photos is in cm and mm.				

11.2.3. Cantilever Holder Component Parts

Please refer to 11.2 for an “exploded” view of the cantilever holder.

11.2.4. Thorough Cleaning

In some cases the cantilever holder must be thoroughly cleaned or sterilized in an autoclave. The back side circuit board prevents the whole device from being immersed so some disassembly is required.

11.2.4.1. Disassembly

Tip Please refer to Figure 11.2 on page 102 for part names, numbers, and letter references.

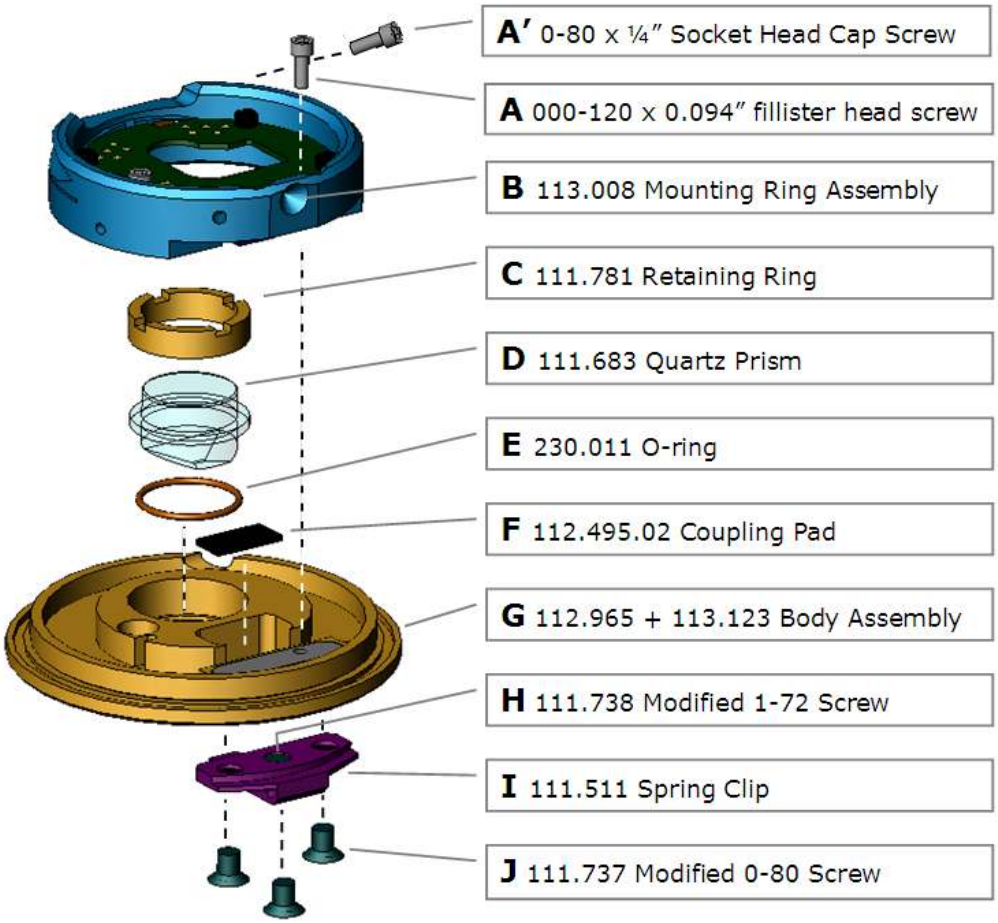
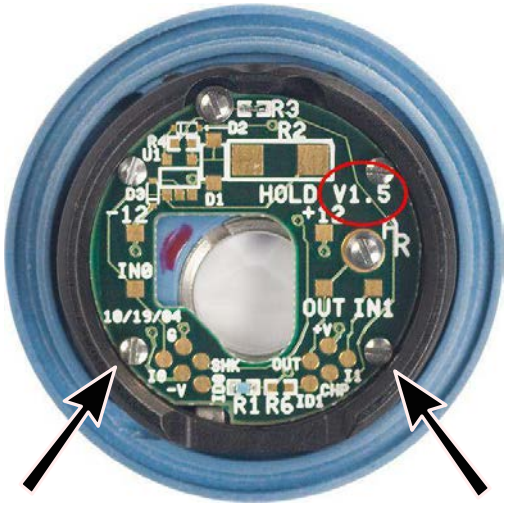


Figure 11.2.: Standard cantilever holder, exploded.

Note The photos in these instructions were taken of the Kel-F cantilever holder. Your holder is likely made of beige or blue PEEK. This does not affect the instructions.

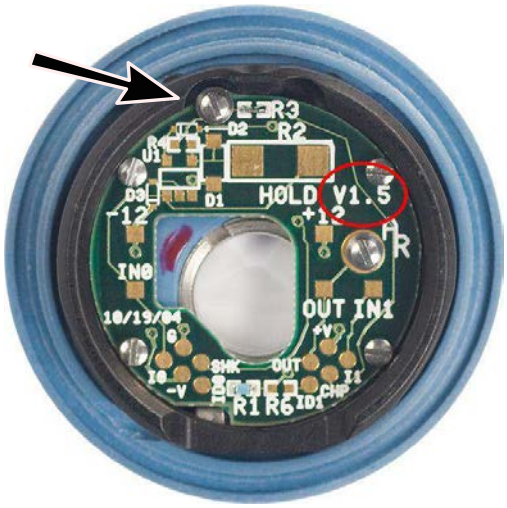
1. **Loosen screws:**

   - Using the 290.110 flat tip screwdriver, loosen the indicated board mounting screws by 1/2 to 1 turn.



2. **Remove the bias screw:**

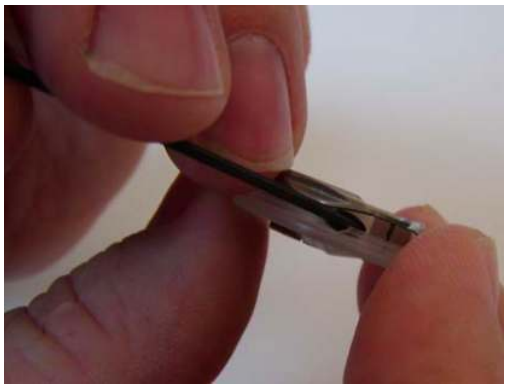
   - Completely remove the indicated bias screw.
   - Keep it somewhere safe. It is required for re-assembly.



3. **Loosen tension screw:**

   - Using a 0.050” hex wrench, loosen the tension screw on the side of the ring.

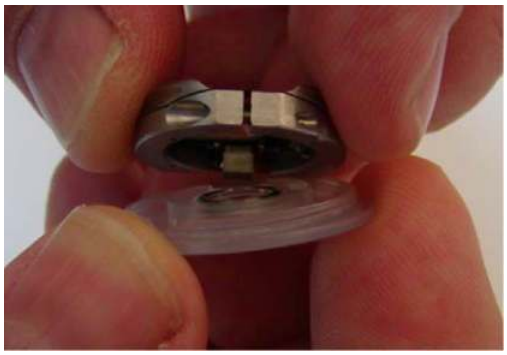
   Note: Rotate counter clockwise 1/2 – 1 turn as needed.



4. **Separate parts:**

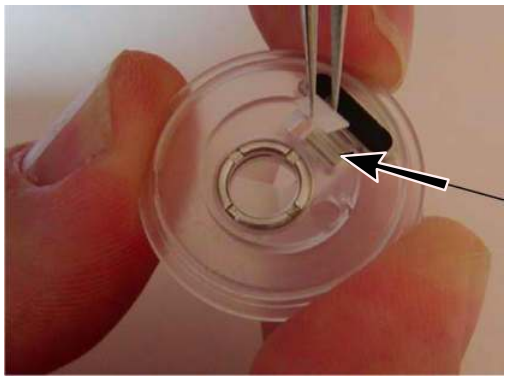
   - Separate the plastic body from the mounting ring by gently pulling the pieces apart.

   Note: Beware that a small white plastic bit may fall out. Keep it for re-assembly.



5. **Remove coupling pad:**

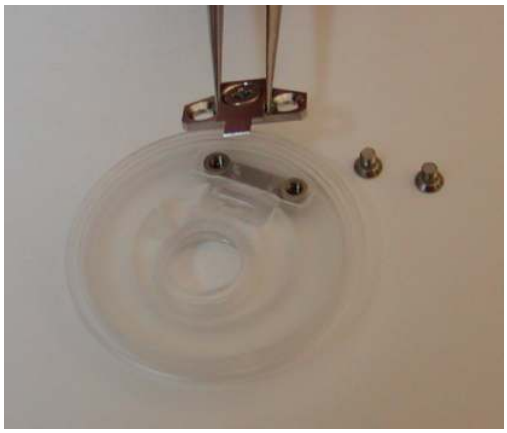
   - If it did not fall out already, use tweezers to remove the drive coupling pad from the drive piezo pocket.



6. **Remove the clip:**

   - Unscrew the two cantilever clip mounting screws and remove the cantilever clip.

   Note: The metal part which remains is press fit into the plastic of the cantilever holder. It cannot be removed.

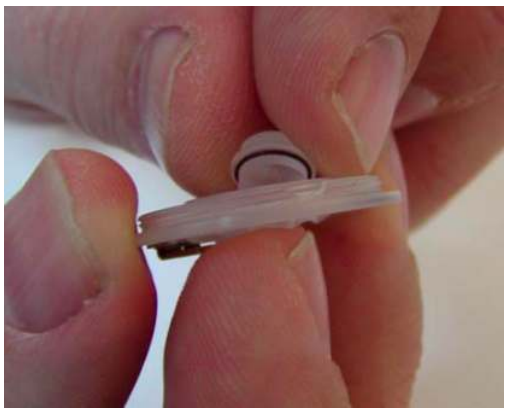


Stop! At this point you can rinse, sonicate, or autoclave the plastic/glass part of the cantilever holder. If this is sufficient for your cleaning needs, please skip ahead to Step 4 on page 106 for re-assembly instructions. Only proceed with further disassembly if you require it.

7. **Loosen the prism retaining ring:**
   - Line up the 4 tabs on the rim of the supplied tool with the 4 notches in the retaining ring.
   - Unscrew and remove the ring nut from the plastic body.

8. **Remove the prism:**
   - From the top side of the cantilever holder (clip side), push the glass insert through with your finger.

   Note: The prism is a somewhat delicate and very expensive precision ground component. Complete this action directly above a soft cloth in case the prism falls. If the prism does not dislodge easily, please do not force it or you may break the glass ridge which seals against the O-ring. Try rotating the prism to work it loose.



9. Remove the o-ring with tweezers.

The holder is now completely disassembled and ready for rigorous cleaning by means of sonication in typical solvents (alcohol) or autoclaving.

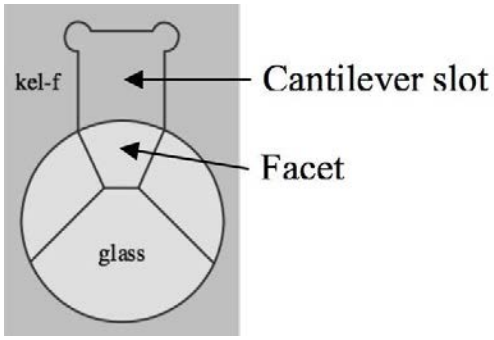
11.2.4.2. Re-assembly

1. Put the o-ring onto the glass insert. The o-ring seats against the glass lip toward the faceted side of the insert. Note that the standard O-ring (230.011, made of brown FKM material), slips over the prism easily. The optional FFKM O-ring (230.049, see Section 24.4.2 on page 323 for more information) is a bit undersized and needs to be stretched into place.

2. **Replace the prism:**

   - Push the prism into the body from the underside of the cantilever holder.
   - Place the retaining ring in the groove around the glass insert. Use the tool to thread the ring into the cantilever holder body but do not fully tighten it.
   - Rotate the glass insert with your fingers to align the small facet of the glass with the cantilever slot in the plastic body.
   - Screw the ring down a little more. Note that this will also rotate the prism out of alignment again.
   - Realign the facet and slot, then tighten the ring a little more. It is an iterative process of aligning the facet and tightening the ring until both are accomplished.

   Attention! Only tighten the ring nut until just snug. Do not over-tighten.



3. **Attach the clip:**

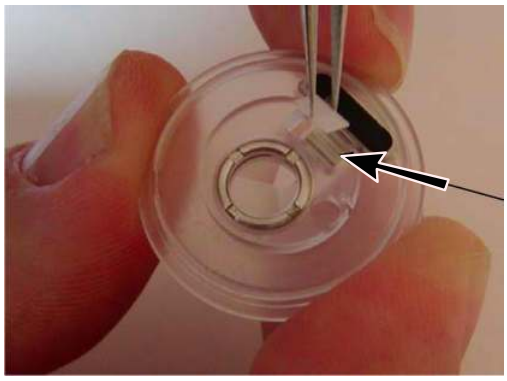
   - Put the cantilever clip back on.

   Attention! Do not over-tighten the two cantilever clip mounting screws; this can create small cracks in the plastic body that may cause fluid leaks.

4. Clean the underside of the glass insert from the bottom side of the cantilever holder using camera lens paper and alcohol. Cotton swabs and tissues such as Kimwipes are too coarse and can scratch the glass surface and its anti reflective coating.

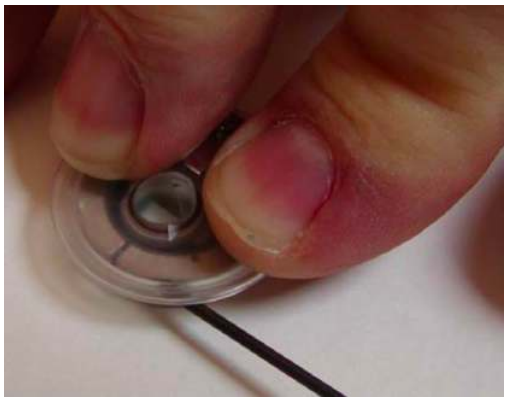
5. **Insert the coupling pad:**

   - Drop the drive coupling pad into the pocket in the plastic body.



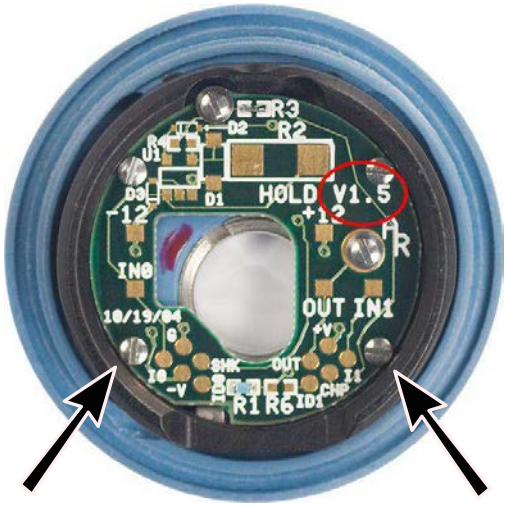
6. **Re-install the mounting ring:**

   - Place the mounting ring onto the body, lining up the drive piezo cube with the pocket in the plastic body.
   - Place the cantilever holder onto a flat working surface so that the plastic is facing upward.
   - Place a finger on each side of the body and press downward (to compress the drive coupling pad) while using the hex wrench to tighten the tension screw.

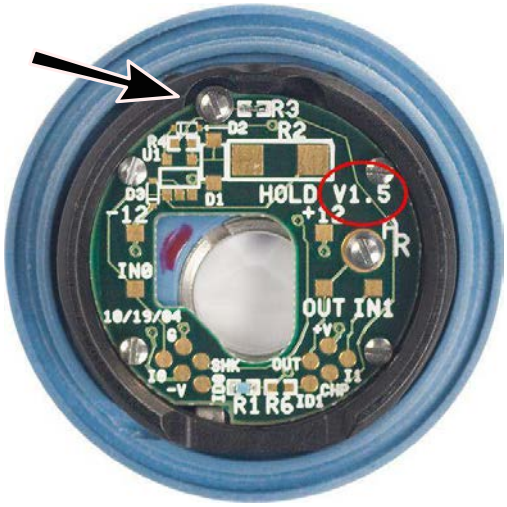


7. **Tighten screws:**

   - Gently tighten the 2 board mounting screws.



8. **Install the bias screw:**
   - Install the tip bias screw until just snug.
   - Do not over-tighten; this can crush the circuit board. Note that the tip bias screw serves no mechanical purpose, only electrical.



9. Clean the top of the glass insert from the upper side of the cantilever holder to remove any fingerprints from the reassembly. Use lens paper and alcohol.

11.2.5. Kel-F Version for Fluorescence Studies

When performing concurrent AFM and Fluorescence microscopy studies, the cantilever holder must be made of a substance which does to fluoresce itself. Kel-F is such a material. Beware that Kel-F is much softer than the glass filled PEEK material typically used in MFP-3D cantilever holders. Place your cantilevers with care and be careful not to scratch the Kel-F surfaces with tweezers or other tools. Be particularly careful to avoid cross threading of the membrane retaining ring. Save your Kel-F cantilever holder only for experiments where it is absolutely required and revert back to the standard holder (made of tougher PEEK) when possible.

In all other aspects, the Kel-F cantilever holder is used in exactly the same way as the standard cantilever holder.

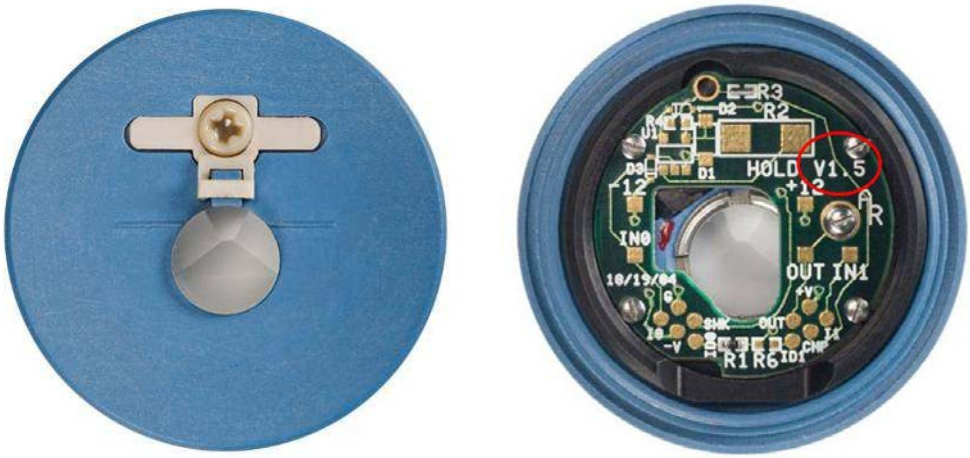
11.3. ORCA Cantilever holder

Please see Chapter 21 on page 279 for a complete description.

11.4. PEEK Cantilever Holder

Asylum Part Number 908.037.

The PEEK cantilever holder is so-called because the majority of the materials which are in contact with fluid (during fluid imaging) are made of PEEK polymer. This includes the spring clip which holds down the cantilever and the screw which tensions the clip. The cantilever holder is particularly useful for fluid imaging experiments which cannot tolerate contact with the steel parts of the standard cantilever holder. The PEEK cantilever holder is also useful when imaging at very high temperatures with the polymer heater. The reasons for this are discussed in Section 18.7.2 on page 234.



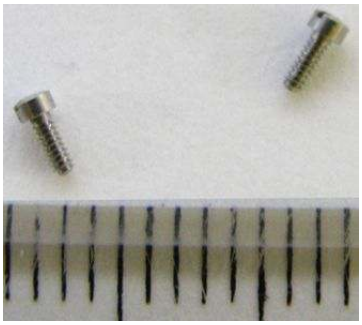
(a) Front side. **(b)** Back side and identifying features.

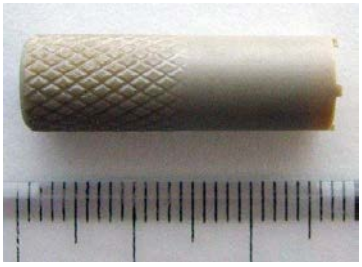
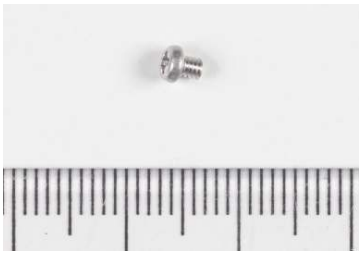
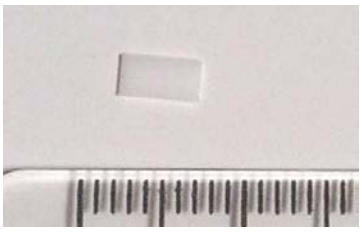
Figure 11.3.: PEEK Cantilever holder.

Note that the cantilever holder ships with a stainless steel screw holding down the cantilever clip. Only in situations where the stainless steel may contaminate the sample (such as in some electrochemistry experiments) switch over the the pure peek screw. The PEEK screw is soft, so some care has to be taken not to damage the head of the screw. Because of this, when this specialized screw is not completely necessary it is easier to work with the steel one.

Always use the small green screwdriver to tighten the screws. This will minimize the damage to the PEEK screw head, but will also make stripping out the plastic threads of the PEEK cantilever holder body less likely. Be sure to only tighten either screw as much as necessary to get a proper AC mode tune, even when using the proper screwdriver.

11.4.1. PEEK Cantilever Holder Kit Parts List

Itm	Part #	Item Description	Qty	Picture
1	001.FILL <#000-120X .125 000-120>	000-120 X 1/8” Fillister Head Stainless Steel Screws. Item A in Figure 11.4 on page 112. Use screwdriver 290.110. A few are included since these screws can be tricky to find outside the USA.	8	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
2	111.725	Fluid Cell Ring Tool. Use to adjust retaining ring. Use only during disassembly.	1	
3	112.454	Modified M2 PEEK phillips screw. Holds down the cantilever clip.	4	
4	112.921	Modified M2 SS phillips screw. Holds down the cantilever clip.	2	
5	112.495.01	Coupling Pad 0.010”. Ensures mechanical contact between the AC mode shake piezo and the cantilever holder. Without this pad, AC mode imaging is not possible. Item F in Figure 11.4 on page 112.	5	
6	113.143	PEEK holder screwdriver. Its special tip is designed to minimize stripping of the PEEK screws 112.454. A spare is included since a regular phillips screwdriver cannot be used interchangeably.	2	
7	230.011	O-Ring, 0.244” ID X 0.016”CS, Viton, 55 Durometer. Item E in Figure 11.4 on page 112.	3	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
8	290.110	WIHA Screwdriver, Flat Tip 260 1,5 X 40. Used on item A in Figure 11.4 on page 112. Used only during disassembly.	1	
9	290.146	Blue Screwball Case Opener. See Section 13.4.3 on page 153.	1	
10	908.037	Peek cantilever holder assembly	1	
The scale in the photos is in cm and mm.				

11.4.2. Cantilever Holder Component Parts

11.4.3. Thorough Cleaning

In some cases the cantilever holder must be thoroughly cleaned or sterilized in an autoclave. The back side circuit board prevents the whole device from being immersed so some disassembly is required.

11.4.3.1. Moderate Disassembly

Please refer to Figure 11.4 on page 112.

1. Using a small slotted screwdriver, remove the four tiny screws (A) on the back of the cantilever holder. Note that these screws also hold the circuit board onto the ring (B), so be careful when you separate the ring from the peek body (G).
2. Be sure to collect the loose coupling pad (F)
3. Fully Remove screw (J) and the clip (I).
4. The body (G) with prism still installed can be cleaned, sonicated, or autoclaved, along with the screw (I) and clip (J).

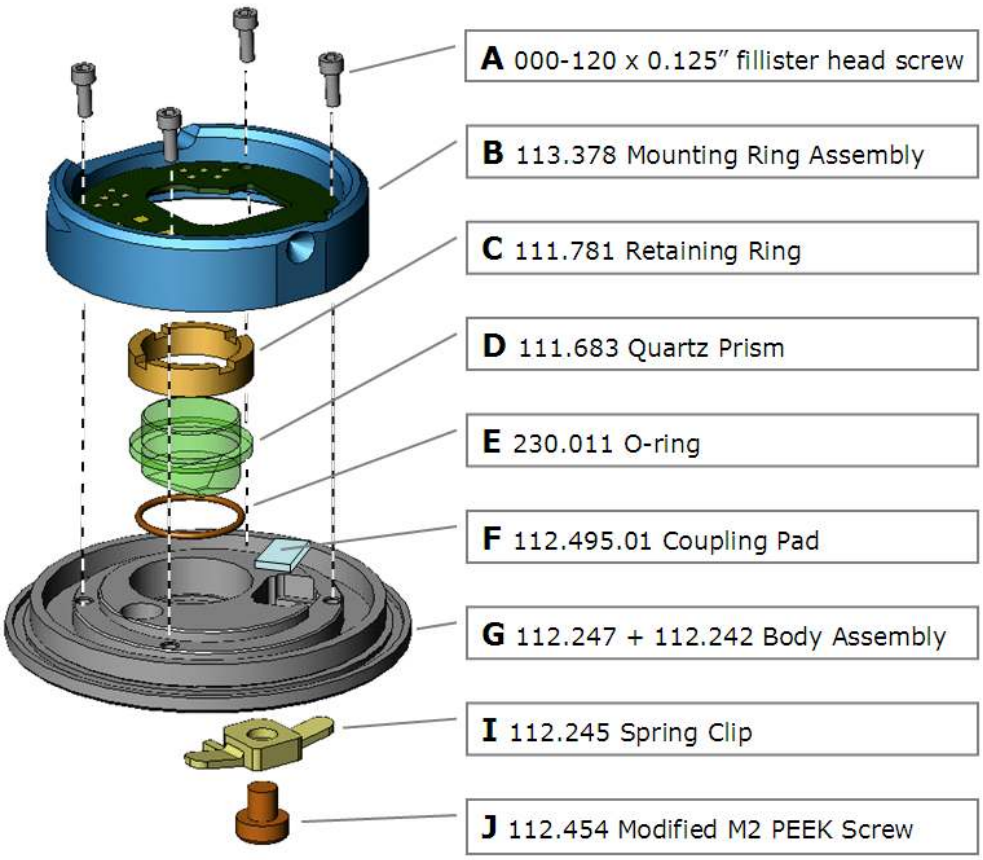


Figure 11.4.: PEEK Cantilever Holder Exploded.

11.4.3.2. Prism and Membrane

The process of replacing the prism is identical to that for the standard cantilever holder (See Step 7 on page 104). Likewise for attaching the membrane (See Section 13.4.3 on page 153). Note that since there are very few protrusions (and none of them are very strong), the blue ball is required to successfully tighten the membrane enough for it to seal properly.

Warning	The Quartz window makes up much of the cost of the cantilever holder. It is a precision ground custom quartz piece with polished surfaces and custom coatings. When it is removed from the cantilever holder, its exposed edges can break if not treated with care.

11.4.3.3. Reassembly

1. See the first few steps of Section 11.2.4.2 on page 105 about re-inserting the prism.
2. Insert the coupling pad (F) into the body (G).
3. Place the ring (B) onto the body and insert the screws (A).

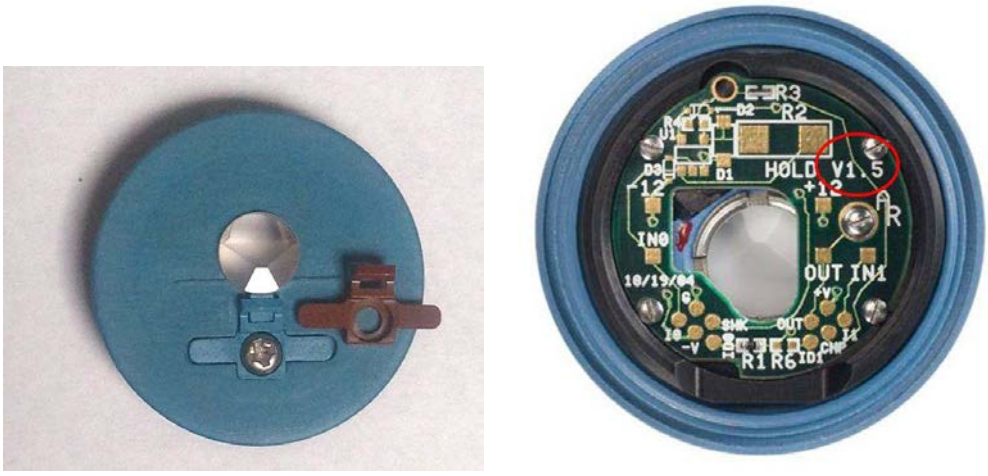
4. Gently fasten the screws working from screw to screw in a circular pattern. DO NOT over tighten. The tiny screws secure into very small metal pieces which are fit to the PEEK body. Too much force can dislodge the metal inserts.
5. Replace the clip (I) with its screw (J).
6. Clean the glass piece using alcohol and lens paper.

11.4.4. Mounting the Cantilever

This is fairly self explanatory. When the screw (J) is loosened, the "wings" of the clip (I) push it away from the cantilever holder body. This will allow for the old lever to be removed and a new one to be slipped in its place. This cantilever holder has no "pocket", so it is up to you to decide where to place the chip.

Note: If the cantilever chip is not far enough forward (away from the screw) then the plastic "strap" that holds down the chip may hit the sample first.

11.5. High Temperature PEEK cantilever holder



(a) Front side.

(b) Back side and identifying features.

Figure 11.5.: High Temperature PEEK Cantilever holder.

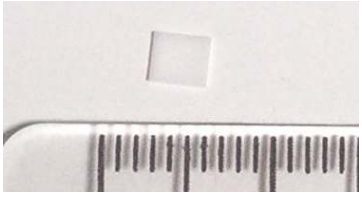
Asylum Part number 908.085 and is required for use with the Polymer Heater Plus (See Section 18.12 on page 237). Identical to the PEEK cantilever holder (see Section 11.4 on page 108) except that the clip which holds down the cantilever is made of an ultra high temperature brown plastic, part number 114.550.

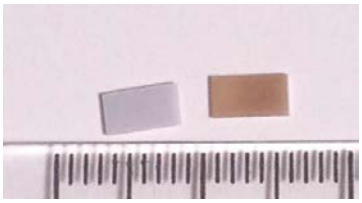
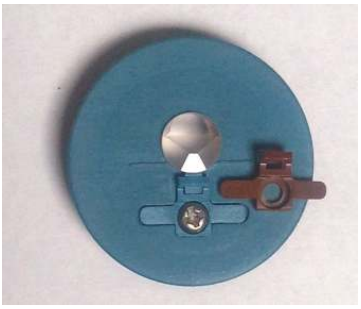
This clip is recommended for use up to 400C, whereas the standard PEEK clip (112.245) is only recommended for use up to 300C.

When used in the range from 300C to 400C, please operate with a sealed cell and purge with inert gas. See Section 18.7 on page 233, otherwise the oxygen in the atmosphere will cause the cantilever holder body to discolor after prolonged use.

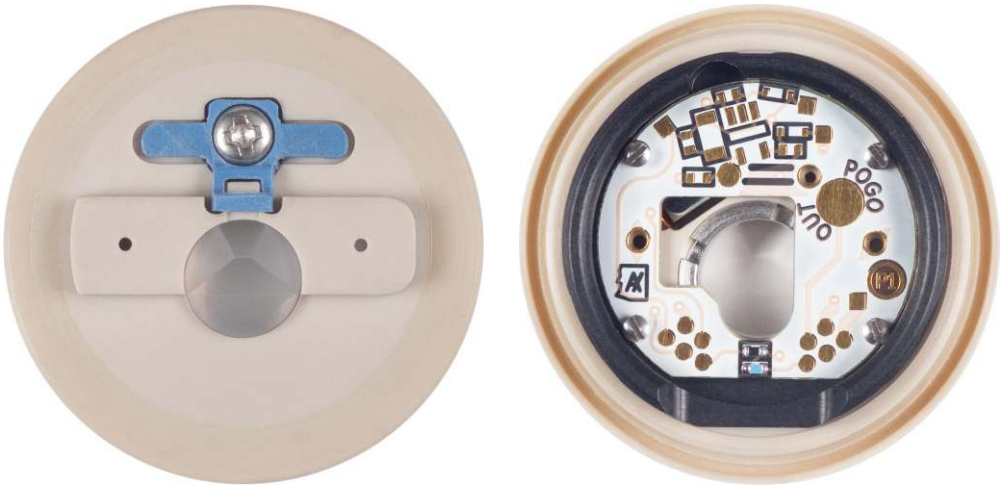
11.5.1. Parts List

This cantilever holder comes in the kit 908.085.1. The spare parts and tools in this kit are identical to the standard PEEK cantilever holder (see Section 11.4.1 on page 109) with a few added parts shown below.

Itm	Part #	Item Description	Qty	Picture
1	112.495.01	Coupling Pad 0.010". Ensures mechanical contact between the AC mode shake piezo and the cantilever holder. Without this pad, AC mode imaging is not possible. Item F in Figure 11.4 on page 112.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
2	112.495.03	Coupling Pad 0.020”. Ensures mechanical contact between the AC mode shake piezo and the cantilever holder. Without this pad, AC mode imaging is not possible. Item F in Figure 11.4 on page 112.	5	
3	908.085	High Temperature Peek cantilever holder assembly	1	
The scale in the photos is in cm and mm.				

11.6. MicroFlow Cantilever Holder



(a) Front side. **(b)** Back side and identifying features.

Figure 11.6.: MicroFlow cantilever holder

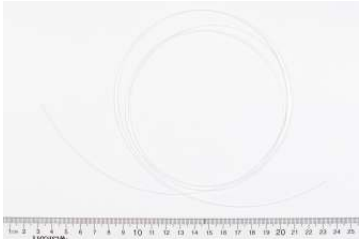
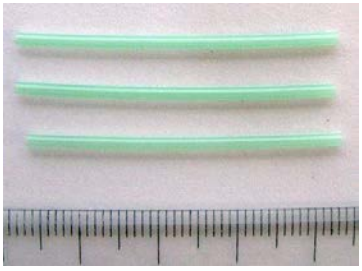
This cantilever holder allows for the attachment of two small fluoropolymer tubes which can deliver and extract fluid very close to the cantilever. When sitting over a planar sample, surface tension defines a fluid drop with a volume of approximately 50 micro liters. Fluid can be withdrawn and added to allow for a fairly quantitative exchange with minimal mixing between fresh and existing fluid.

The design is based on the PEEK cantilever holder described in Section 11.4 on page 108. Please refer to that section for instructions on disassembly, cleaning, and membrane mounting.

11.6.1. Parts List

Includes the parts of the standard PEEK cantilever holder (See Section 11.4.1 on page 109) plus the following items:

Itm	Part #	Item Description	Qty	Picture
1	908.072	MFP-3D MicroFlow Cantilever Holder	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
2	231.028	Tubing, FEP, natural, 0.016" ID, 0.032” OD.	5ft	
3	290.148	Number 15 scalpel blade. Attaches to handle 290.147. Use to trim Teflon tape during sample mounting.	10	
4	290.147	Scalpel Knife Handle. Can also be ordered from McMaster Carr (Part# 36325A63). Use to trim Teflon tape during sample mounting.	1	
5	231.019	Tubing Sleeve, Green, 1/16”x.033”x1.55”. IDEX Upchurch #F-247x.	3	
6	80.165	Syringe, HSW Norm-Ject, 1cc.	4	
7	231.008	Luertight Fitting. Use to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
The scale in the photos is in cm and mm.				

11.6.2. Tubing Installation

When you first receive your MicroFlow cantilever holder, the tubing will need to be attached as described in this section. For parts numbers and pictures, please refer to Section 11.6.1 on page 116.

Warning	If you intend to use a sealing membrane in your experiment, we recommend that you attach the membrane to the cantilever holder before inserting the tubing. Please refer to Section 13.4.3 on page 153 for instructions on attaching the membrane.

1. **Locate the cantilever holder:**

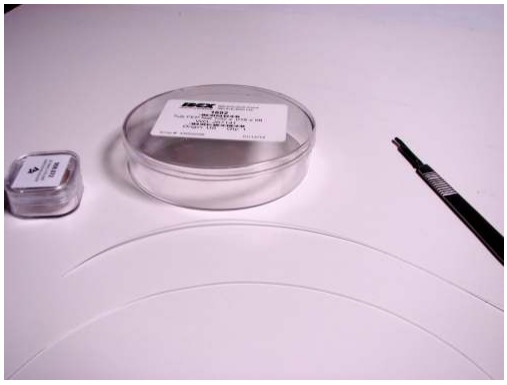
- Here it is shown with a steel screw. Your kit also includes PEEK screws, which may be of use depending on your chemical environment.
- For disassembly and cleaning purposes, this cantilever holder is identical to the standard PEEK cantilever holder. Please see Section 11.4 on page 108 for more information.

2. **Locate tubing and knife:**

- Carefully assemble a #15 blade into the scalpel handle blade.

WARNING: EXTREMELY SHARP!

- Take the 231.028 FEP tubing and cut it in half.

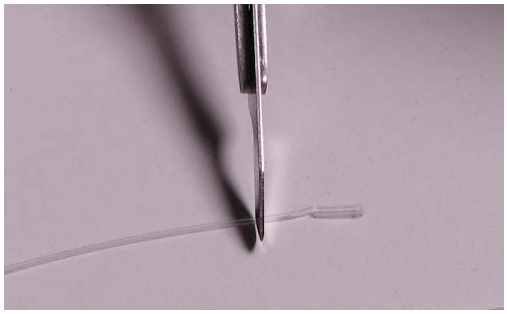


3. **Stretch the tubing:**

- Firmly hold one piece of tubing about 3cm from the end.
- Pinch the very tip of the tubing between your thumbnail and forefinger (or use some small pliers to grab the end).
- Stretch the tubing to two or three times its original length. It should thin down to about half of its former diameter.
- Repeat this for one end of the other piece of tubing.

4. **Trim tubing:**

- Trim off the unstretched end with the knife. Cut against a hard surface.

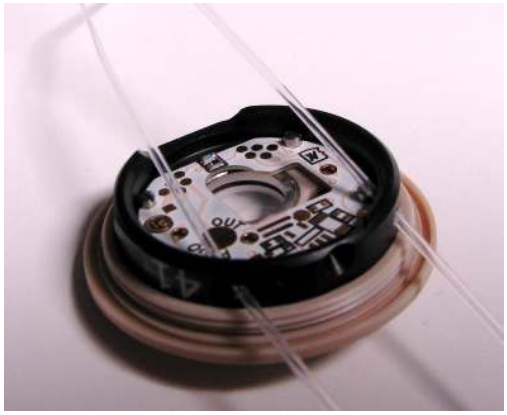


5. **Thread tubing through outer ring:**

- Locate two small holes in the metal ring on the back of the cantilever holder.
- Thread the thin ends of the tubing until they get snugly stuck in the holes. Note that the holes are slightly undersized.

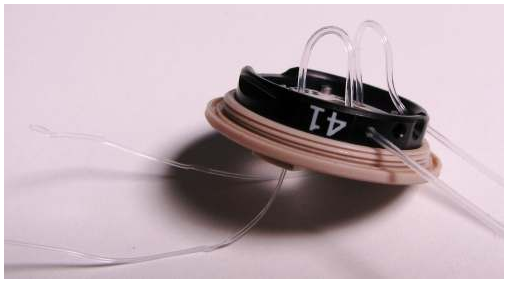
6. **Advance tubing through the ring:**

   - Pull harder on the tubing until about 3 to 4 cm are pulled through.
   - Repeat for the second piece of tubing.



7. **Thread tubing into cantilever holder body:**

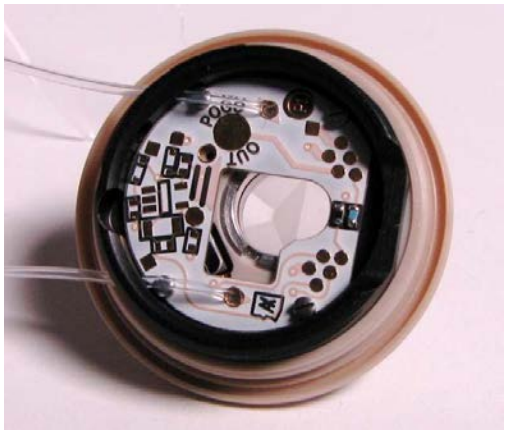
   - Thread each piece of tubing through the holes in the circuit board and out the front of the cantilever holder.
   - Pull lightly until snug.



8. **Pull tubing taut:**

   - Pull on each tubing end with more force.
   - Stop pulling when the loops of tubing near the circuit board lie flat.

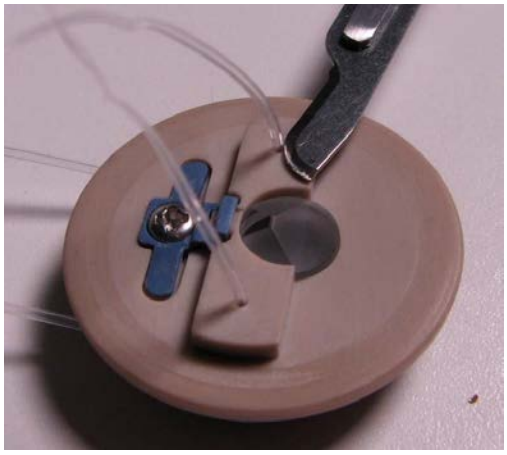
Note Do not overdo it. The tubing should not become pinched by the small screws on the cantilever holder circuit board. The tubing should also not kink where it goes through the circuit board.



9. **Trim excess tubing:**

   - Hold the #15 scalpel blade parallel to the cantilever holder and trim the tubing as shown.

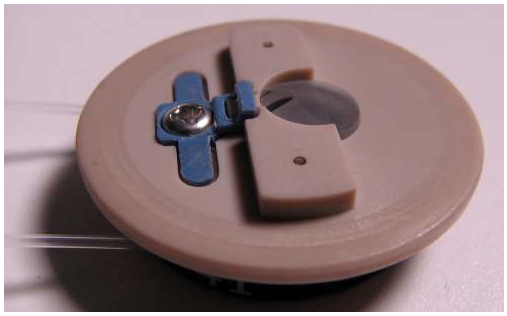
Note Only angle the blade enough to make a flush cut. Do not angle so much that plastic is removed from the cantilever holder body itself.



10.

Inspect the final result:

- As shown, the tubing should be cut flush with the cantilever holder body.
- You have now achieved a perfect seal with very inert materials, without O-rings or any other complicated fittings and clamping mechanisms.
- The tubing will stay in place until you choose to remove it. It will not slip out accidentally, and you can easily leave filled syringes dangling without fear of breaking the seal.



11.

Mark the tubing:

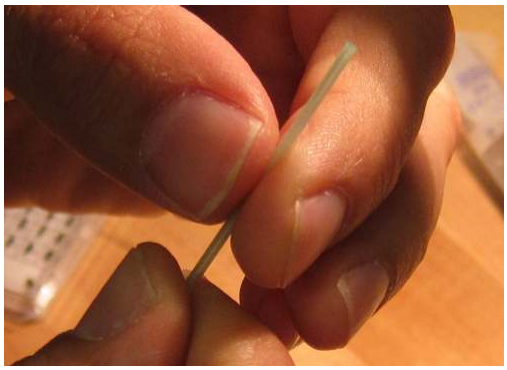
- The fluid will be injected from the tube in the plateau with the rounded corners. See 11.6 for a more detailed view.
- Follow the tubing which leads to this rounded plateau and attach a tape flag and write “IN” on it.
- This will be the tube through which fluid will be injected.

11.6.3. Attach Syringes to the Tubing

1.

Sleeve the tubing:

- Locate two of the green bits of plastic tubing (231.019).
- Sleeve a piece onto the end of one of the tubes attached to the cantilever holder
- If it is loose, you’ll need to hold it in place so the ends of the inner and outer tubing stay flush.



2. Follow the instructions in Section 14.4.3 on page 172. In this case you’ll be inserting the green tubing into the fittings.
3. Once the fittings are adjusted to the appropriate tightness, you should be able to tug gently

on the thin tubing and it should not come loose from the fitting. If it does come loose, you will need to twist things together more firmly in the previous step.

4. Repeat this process for the second tube.
5. Any Luer tipped syringe can be attached to the tubing.

11.6.4. Preparing for Imaging

1. The cantilever holder is mounted the same way as the standard PEEK cantilever holder. Please refer to Section 11.4.4 on page 113 for detailed instructions.
2. **Mount the cantilever holder to the head:**
   - This works the same as any of the cantilever holders. For instance, see 2.
   - The tubing should not touch the black metal parts of the AFM head, though some very gentle contact typically does not cause problems.

3. Prepare your AFM scanner and sample. While careful users may get away with using a glass slide, we recommend using at least the Fluid Cell Lite, Closed Fluid Cell, or a small petri dish as long as fluids are involved. Whichever surface you are using, prepare the legs of the AFM so the head is in a position to engage the cantilever with only a few turns of the front wheel on the head. We recommend you try this with a dry dish or cell and, if you wish, an old dull cantilever. Please refer toSection 4.3 on page 22 for techniques on getting the head adjusted properly.
4. Prepare the head to be very level from front to back. Because of the plastic plateaus on this cantilever holder, even a modest front to back head tilt will cause the outer edges of the plateaus to hit the sample before the cantilever.
5. Using a mock sample without any fluid, engage on the surface in AC mode. If you encounter a sudden large change in deflection before the cantilever has clearly engaged in AC mode, then it is likely that the head was not sufficiently level and that one of the outer plateau edges hit the sample before the cantilever. Please try to better level the head and perform the test again.
6. Please review the tutorial on imaging in a fluid droplet (Chapter 8 on page 64). The volume of fluid under this cantilever holder is typically a small drop, so please prepare your samples accordingly.
7. Have the head connected to the AFM and sitting upside down, next to the scanner.
8. Fill one syringe with the fluid you wish to flow over the sample and attach it to the IN tube going to the cantilever holder.
9. Fill another syringe with about 0.2cc of the same fluid and attach it to the OUT tube.
10. Prime the “OUT” tube by pushing fluid from the out syringe into the tube. Stop when you see a tiny droplet appear on the cantilever holder. In case the droplet gets too large, be ready

to mop it up with a paper towel or lab wipe. Pulling excess fluid away works best when there are no bubbles in the tubing.

11. Prime the "IN" tube in the same way. Again, bubbles are best flushed out before starting the experiment.
12. Pre wet the sample and cantilever as is customary with liquid drop imaging.
13. Put head down as with regular droplet imaging, assuming the head was prepped to be nearly engaged, or go ahead and fully engage to start imaging.

11.6.5. Viewing the Action

To see the fluid flowing past the imaging area, it is very helpful to get a bottom view of the entire cantilever holder and surrounding area. This is a much larger area of view than any microscope objective will ever give, so this section details a trick to obtain the bottom view.

First, rotate the objective turret to a position where there is no objective at all and remove the cover in that position of the turret. If there are no open spaces, remove one of the objectives. Consider placing a glass slide over the hole to prevent any dust or fluid from falling into the hole.

The type of high end microscope sold with the MFP-3D-BIO often has phase contrast imaging capability, including a Bertrand lens assembly. Rotate the Bertrand lens into the optical path and adjust its focus until you see a sharp image of the bottom of the cantilever holder.

Some illumination will be required to view from the bottom; we suggest a gooseneck fiber light, a small LED headlamp, an LED book light, or anything else you can fit under the IO microscope's sample stage.

Note that this image can only be viewed through the eyepiece, unless you have an eyepiece camera.

Microscopes which do not have a built in Bertrand lens can do the same thing with an eyepiece telescope (sometimes called a phase centering telescope) which is a special focusable eyepiece that takes the place of one of the regular eyepieces on the IO microscope.

This process of visualization will not work for the MFP-3D SA (Stand Alone Base). However, it is possible to use this cantilever holder with the SA version by simply monitoring the amount of fluid injected and withdrawn very carefully.

11.6.6. Fluid Exchange

The water in these images was colored with dye to make it more visible.

Note: The photos below were taken with a prototype which may look slightly different than your model of the cantilever holder.

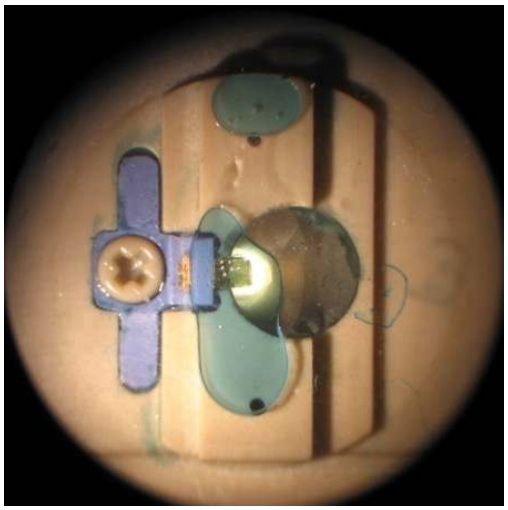
A few words about volume:

- We recommend using a syringe with a maximum volume of 1cc, like the ones included with this kit. With a syringe of this volume it is easy to control volume changes of about 10 micrometers or more. The included disposable syringes are made by Henke Sass Wolf. You can find more durable ones from vendors like Hamilton.

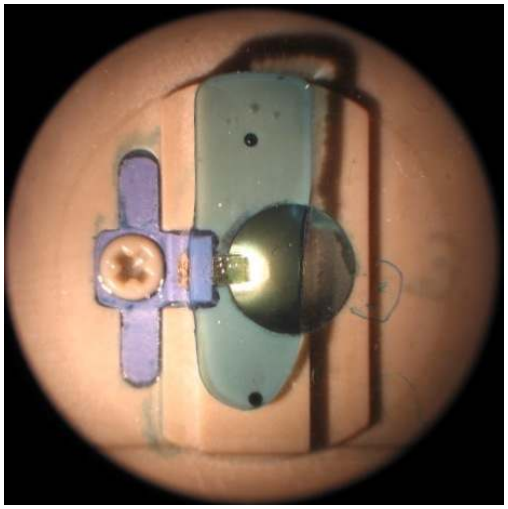
- The tubing used with this cantilever holder can hold about 10 micrometers of fluid for every 8 cm of tubing. So, even with a comfortable length of tubing around 40cm, there is only a dead volume of 50 micrometers.
- The total amount of fluid typically under the cantilever holder is 30 to 50 micrometers.
- Based on the above figures we do not recommend syringes with volumes less than a few hundred micrometers.

1. **Start filling:**
   - Note the position of the “IN” syringe.
   - While observing through the microscope, start pushing in fluid.
   - A drop will start to form from the bottom port in the photo.

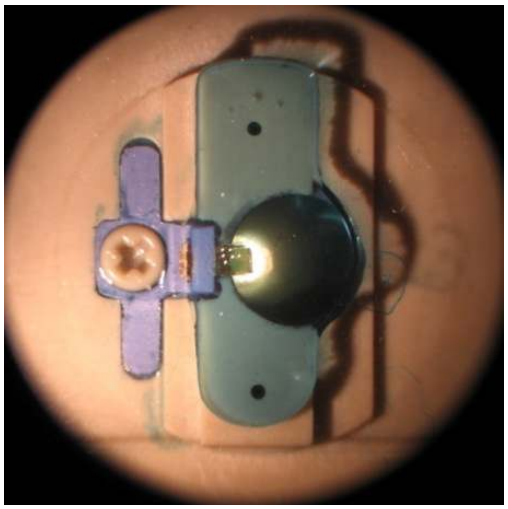
   Note In our case a bit of fluid from a previous experiment is still visible at the top of the photo. Your situation may look different depending on how large a fluid droplet you start imaging with.



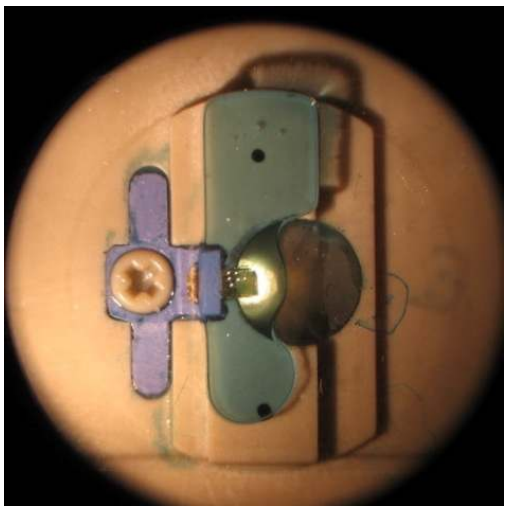
2. **Continue filling:**
   - Surface tension will pull fluid toward the exit plateau.
   - Once that plateau is full, fluid will start to bulge out over the glass prism.
   - Now is a good time to note the syringe position and get a feel for how much fluid was required for a complete fill.



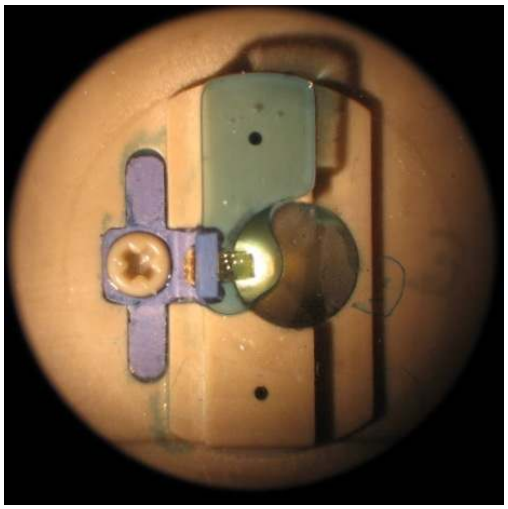
3. **Slight overfill:**
   - Overfilling will force fluid over the rest of the prism and beyond.



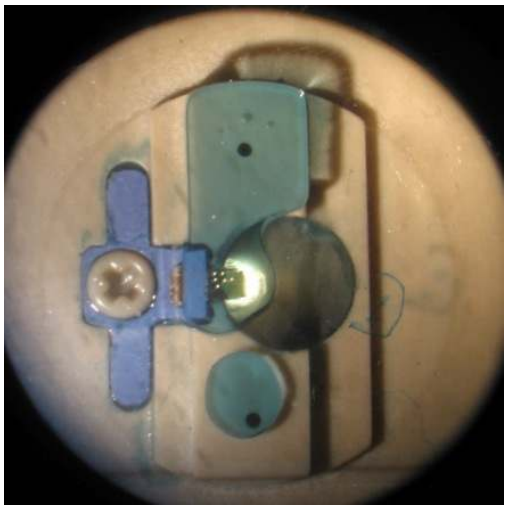
4. **Begin fluid extraction:**
   - Start to pull on the “OUT” syringe.
   - Surface tension will first drain excess fluid from the prism.
   - The lower “entrance” plateau will be the next area to drain.



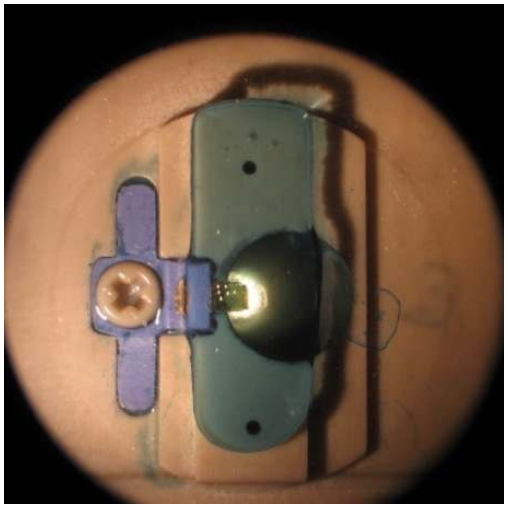
5. **Stop extraction:**
   - Stop extracting before the fluid pulls clear of the cantilever. The area being imaged should stay wet.



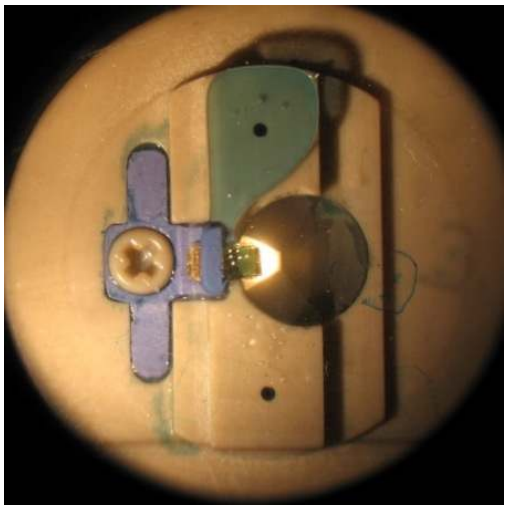
6. **Resume filling:**
   - A second round of filling will start as a droplet from the inlet port.

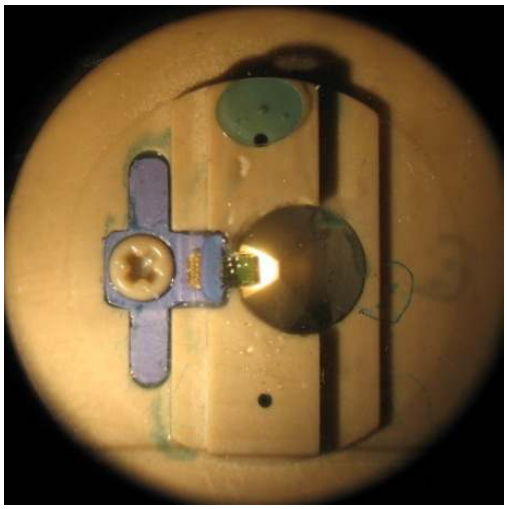


7. **Nearly overfilled:**
   - Continuing to fill until a nearly overfilled state is reached.
   - Note syringe positions to get a feel for how much syringe movement is needed for proper filling and extraction. After some trials it will be possible to move the fluid with confidence without having to look through the microscope.



8. **Too much extraction:**
   - In this case too much fluid was extracted. The cantilever has become dry.



9.	**Complete extraction:** • A small droplet of fluid cannot be extracted and the “OUT” syringe starts to pull in air. • Once there are bubbles in the extraction syringe, it becomes more difficult to start and stop the extraction flow.	

11.6.7. Sample mounting

We recommend using the fluid cell lite (Chapter 13 on page 145) with a membrane attached to the cantilever holder (Section 13.4.3 on page 153) and slightly sealed using the magnetic ring method (Section 19.7.2.3 on page 254) to prevent evaporation.

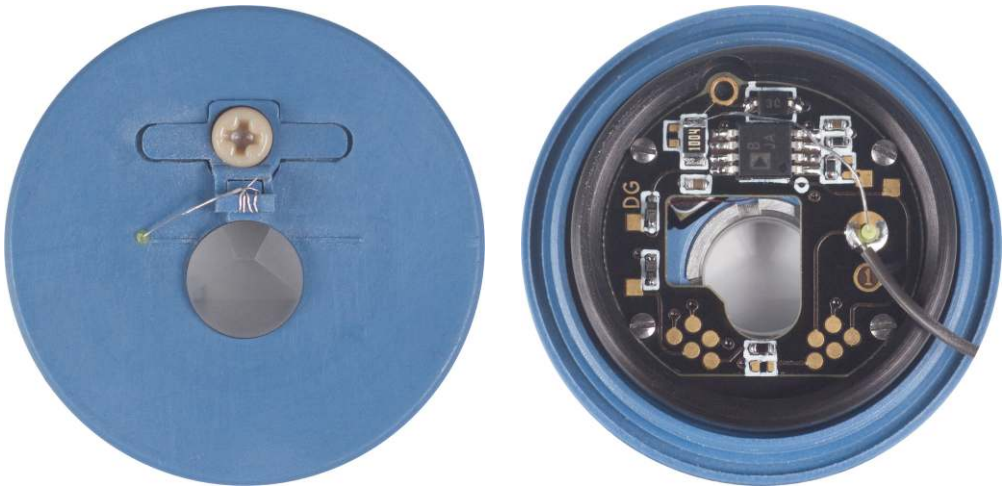
If a flat mica substrate is required, it should be alt least 0.75” in diameter.

A thin bottom cell culture dish may also be a good sample support. See Section 22.6.1 on page 297.

11.7. PEEK Orca Cantilever Holder

This variation of the Peek cantilever holder (see Section 11.4 on page 108) includes the current measuring capabilities of the ORCA cantilever holder (see Section 21.3 on page 283). Since the PEEK cantilever holder is made entirely of electrically insulating materials, the PEEK ORCA cantilever holder includes a mechanical modification in the form of a liquid sealed wire which connects the circuit board and the cantilever holder.

The design is based on the PEEK cantilever holder described in Section 11.4 on page 108. Please refer to that section for instructions on disassembly, cleaning, and membrane mounting.



(a) Front side.

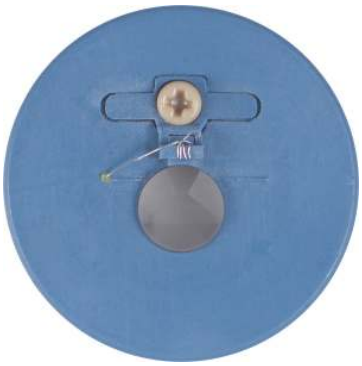
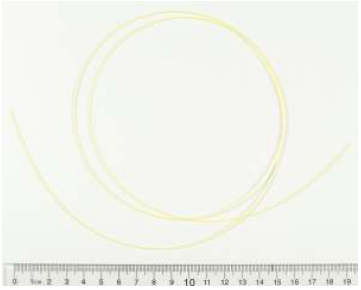
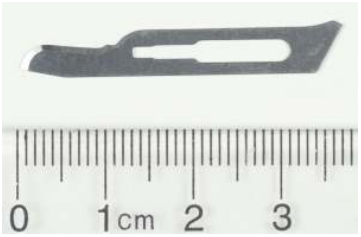
(b) Back side and identifying features for the Dual Gain model.

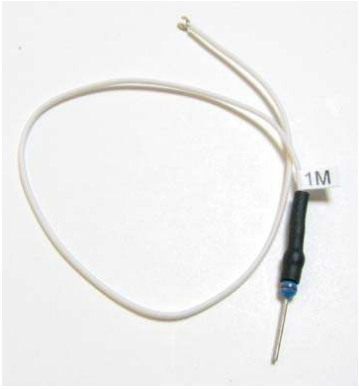
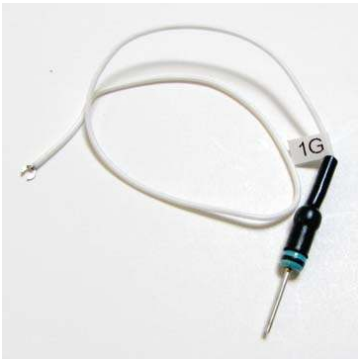
Figure 11.7.: PEEK ORCA Cantilever holder (Dual Gain).

11.7.1. Parts List

This specific list is for the Dual gain, 1M + 1G version, asylum part number 908.065. Your version may differ slightly depending on the current range.

Includes the parts of the standard PEEK cantilever holder (See Section 11.4.1 on page 109) plus the following items:

Itm	Part #	Item Description	Qty	Picture
1	908.064	Peek ORCA cantilever holder	1	
2	080.154	PTFE coated Platinum Wire, .005" OD conductor/.007" OD with insulation	30”	
3	231.025	Tubing, FEP, natural, 0.007" ID, 0.032” OD.	5ft	
3	290.148	Number 15 scalpel blade. Attaches to handle 290.147. Use to trim Teflon tape during sample mounting.	10	
4	290.147	Scalpel Knife Handle. Can also be ordered from McMaster Carr (Part# 36325A63). Use to trim Teflon tape during sample mounting.	1	
5	290.149	Butane torch with fuel cell.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
5	448.053	1 M test resistor assembly	1	
5	448.054	1 G test resistor assembly	1	
The scale in the photos is in cm and mm.				

11.7.2. Wire Feedthrough Replacement Instructions

Instructions follow on how to service or replace this wire feedthrough. Due to lack of space for proper o-rings and fittings, the process is somewhat unorthodox, but when done properly it works well.

1. **Locate equipment**

- If you have one, a binocular dissection stereoscope. It will improve your experience with these instructions.
- A few inches of Teflon 1/32 OD, 0.007" ID Upchurch tubing (Asylum Research Part Number 231.025, Upchurch Part Number 1687).
- Teflon coated platinum medwire PT5T wire (0.007"OD). This can be purchased from Sigmund Cohn (http://www.sigmundcohn.com/pdf/EN/medwire.pdf).

2.

Remove the old wire and tubing

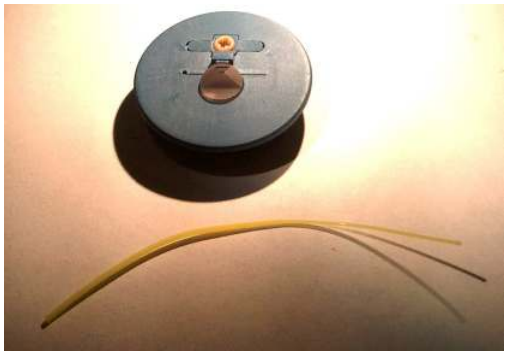
- Without scratching the cantilever holder and wire feedthrough hole, remove the existing platinum wire and yellow tubing.
- The best chance is probably had by pulling the tubing out from the circuit board side of the cantilever holder using tweezers. It may help to knot the platinum wire on the other side (if enough remains) and use it to pull the tubing through.

Note If you need to use a tool (like a pin) to push the tubing out, make sure not to damage the hole or it may not seal properly for the next use.

3.

Prepare the tubing

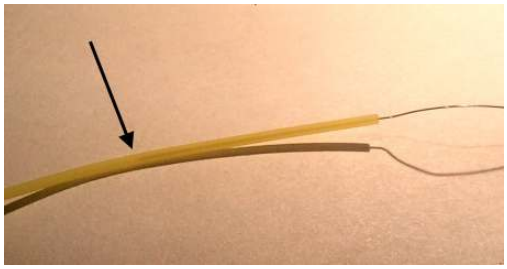
- Using a fresh razor or scalpel blade, cut a few inches of yellow 1/32 OD, 0.007" ID Teflon tubing.
- Wrap about an inch of it around a wooden swab stick once and pull hard to draw a section out to a thinner OD. You may need needle nose pliers to grip the slippery tubing.
- On the right you can see the thinner section bending up off of the page.
- Then, with your sharp blade, trim the thicker section to about an inch long.



4.

Insert wire into tubing

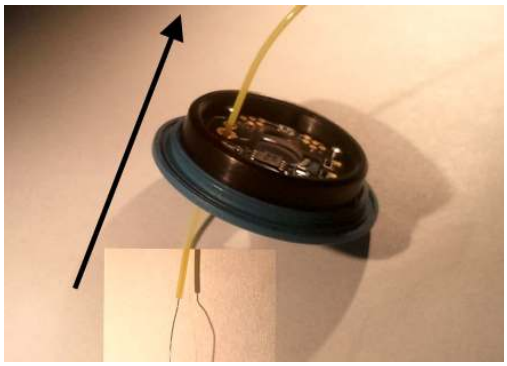
- Take a section of platinum medwire (PT5T wire 0.007"OD). This wire is quite expensive, so be conservative and use only a few inches.
- Insert the platinum wire into the cleanly cut unstretched part of the tubing, all the way up to the stretched end where it will get stuck.
- The wire is soft, so do it carefully. If the wire kinks, you may not be able to straighten it enough to perform this operation. Be very gentle with the spool of wire.
- On the right the arrow indicates where the wire end can be seen inside the tubing.



5.

Pull the tubing through the hole

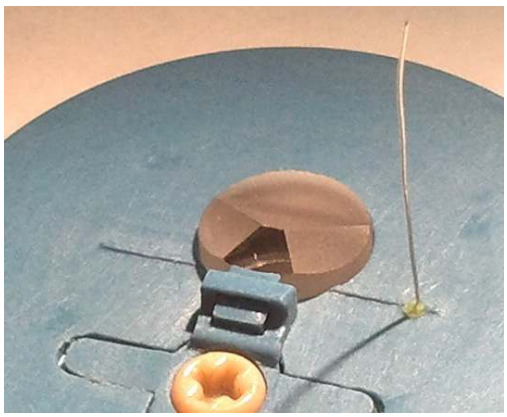
- Stick the stretched end of the yellow tubing through the hole in the cantilever holder.
- Insert as shown to the right, putting the thin end into the non-circuit board side of the cantilever holder.
- Pull until you feel resistance and keep pulling the unstretched section of tubing with wire inside clear through the hole.



6.

Pull tubing flush

- Gently pull the tubing until only a tiny bit protrudes from the front of the cantilever holder.
- Note that on the back side the wire inside the tubing should have cleared the circuit board by over half an inch.



7.

Trim yellow tubing

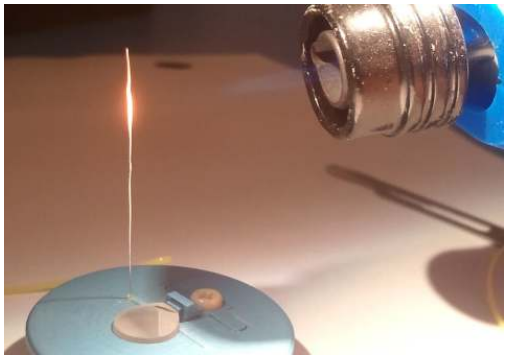
- Using a scalpel blade, cut very carefully around the tubing perimeter at a level a bit above the circuit board.
- The goal is to sever only the yellow tubing without nicking the platinum wire, or even the Teflon coating on the platinum wire.
- Leave a little more yellow showing so that when you need to remove the yellow tubing at some future time, there is enough to grab onto with tweezers or pliers.

Note Use the stereoscope for this procedure if possible.

8.

Stripping the insulation

- Strip the insulation from the top 5-10 mm of the platinum wire as shown.
- Since platinum has a high melting temperature and does not oxidize when hot, a butane torch is a great way to burn off insulation without damaging the wire.

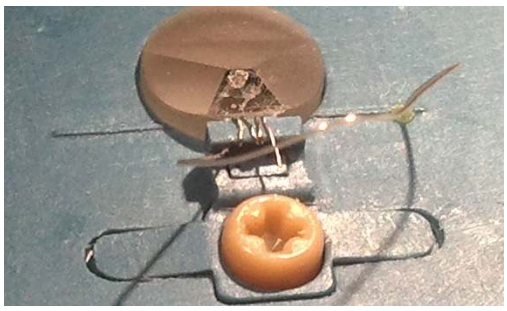


Note If you are not using platinum wire, you may have to think of another way to strip the insulation since another type of metal wire may not be able to take the heat.

9.

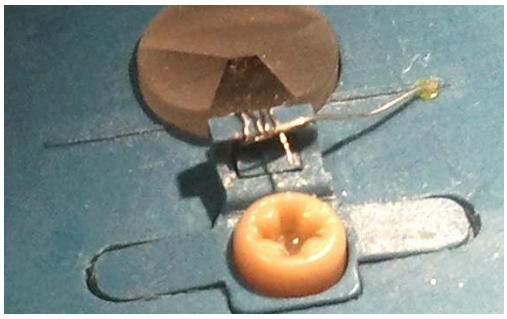
Attach the stripped wire

- Using tweezers wrap the stripped portion of the wire around the cantilever clip.
- Alternatively, leave only a very short portion of uninsulated wire and position it under the clip when mounting the cantilever.



10. **Tension the wire**

- Gently pull the wire from behind; take up the slack until it is almost or just barely taut.



Note The following instructions depict a dual gain orca cantilever holder (see Section 21.3.2 on page 284) . If you have the standard (single gain) version (see Section 21.3.1 on page 283), please contact Asylum Research for soldering instructions.

11. **Prepare wire for soldering**

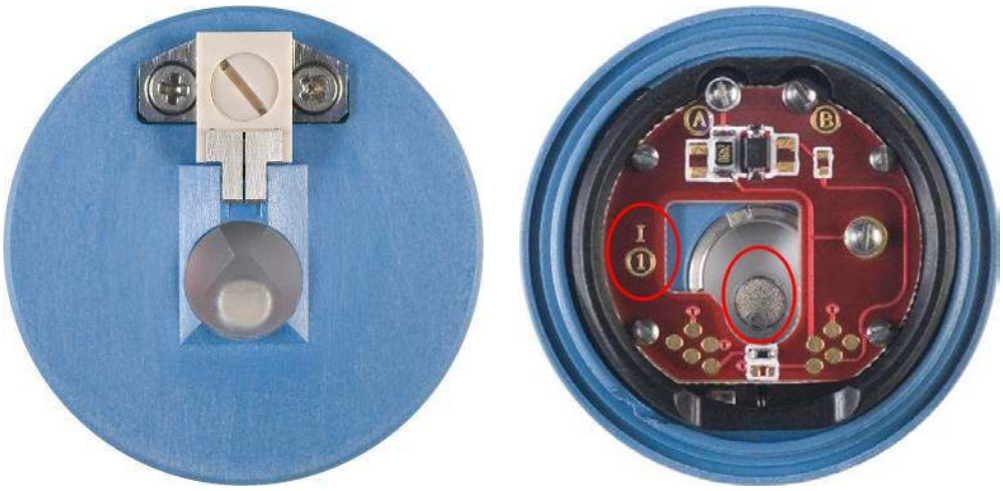
- Position the wire as shown so it reaches the indicated point.
- Trim the wire as shown.

12. **Solder wires**

- Solder the platinum wire as shown by the rightmost arrow.
- Solder the bias wire as shown by the leftmost arrow.

At this point we assume you have read about how to use the PEEK cantilever holder (for instance, how to mount cantilevers) in Section 11.4 on page 108 and about how to use the ORCA cantilever holders in Section 21.3 on page 283.

iDrive Cantilever Holder



(a) Front side.

(b) Back side and identifying features. Note the magnet embedded inside the glass.

Figure 11.8.: iDrive Cantilever holder.

The iDrive cantilever holder has a special clip which has been split in two. Both sides of the clip are part of an electrical circuit which can apply different voltages to the two halves of the clip. When using special cantilevers (see *Applications Guide, Chapter: iDrive Imaging*), one can drive small currents through the arms of the lever. A very strong magnet situated near the cantilever turns this current into a force exerted on the cantilever. This allows for very clean AC mode tunes and relatively effortless AC mode imaging in fluids.

11.7.3. iDrive Cantilever Holder for Fluorescence Studies

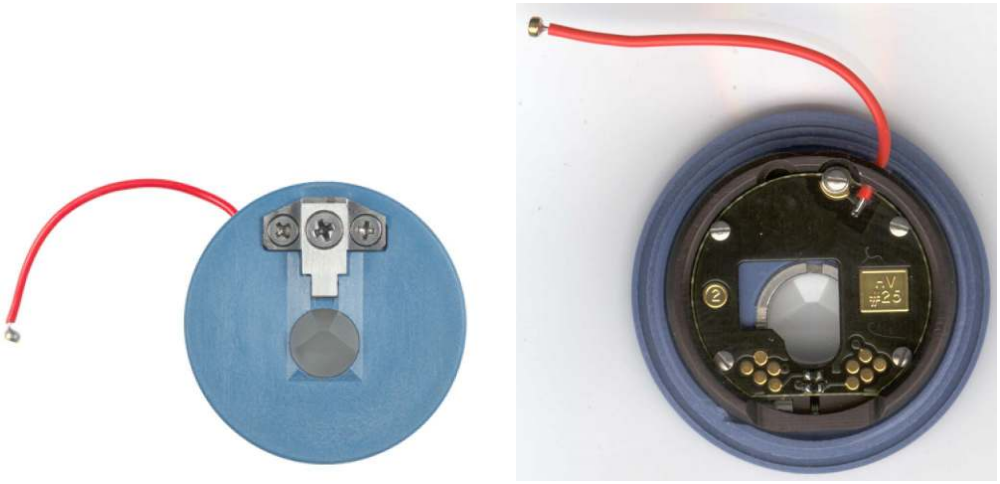
Same the standard iDrive holder only with a Kel-F body which is not as mechanically durable, but creates less background fluorescence.

(a) Front side. **(b)** Back side and identifying features.

Figure 11.9.: iDrive Fluorescence Cantilever holder.

11.8. High Voltage Cantilever Holder

For more information, please see **??**. This is a necessary component of an MFP 3D AFM capably of applying high AC of DC voltages to the cantilever.



(a) Front side. **(b)** Back side and identifying features.

Figure 11.10.: High Voltage Cantilever holder.

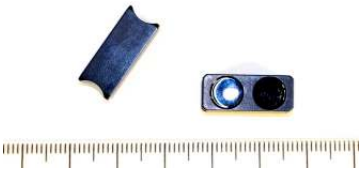
12. Sample Mounts

CHAPTER REV. 1592, DATED 08/30/2013, 17:15. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

12.1. Basic Sample Mounts

Note All of these sample mounts are held down onto the scanner with magnets, as shown in Figure 2.4 on page 9 and Step 5 on page 24.

Itm	Part #	Item Description	Picture
1	910.004	Magnet Hold Assembly for use with glass microscope slide (like 504.005). Note: sold SEPARATELY and not as a set. See Step 5 on page 24.	
2	504.005	25mm X 75mm microscope slide. Can be bought just about anywhere, for instance: http://www.tedpella.com/histo_html/s Secure to the scanner using magnets 910.004.	
The scale in the photos is in cm and mm.			

Itm	Part #	Item Description	Picture
3	080.122	15mm AFM Specimen Disc. Also available from Ted Pella, part number 16218. Hold in place using 900.146.	
4	080.105	12mm AFM Specimen Disc. Also available from Ted Pella, part number 16208. Hold in place using 900.146.	
5	900.150	Electro Contact Sample Holder: A mount which is used with the ORCA conductive AFM imaging module.	
6	900.143	Phillips SEM Mount Holder, 12mm: A mount which adapts a 12mm Phillips style SEM mount. The Phillips mount is held in with a spring load and can be mounted with SEM tweezers. Secure to the scanner using magnets 910.004.	
7	900.144	JEOL SEM Mount Holder, 10mm: A mount which adapts a 10mm JEOL style SEM mount. The JEOL mount is held in with a spring load and can be mounted with SEM tweezers. Secure to the scanner using magnets 910.004.	
8	900.145	Hitachi SEM Mount Holder, 15mm: A mount which adapts a 15mm Hitachi style SEM mount. The Hitachi mount is held in with a 4mm screw and can be mounted with SEM tweezers. Secure to the scanner using magnets 910.004.	
The scale in the photos is in cm and mm.			

Itm	Part #	Item Description	Picture
9	111.479	Cover Slip Holder: A mount which allows you to use three sizes of cover slips with the Asylum MFP-3D. Holds 25mm round, 22mm square, and 50mm x 25mm rectangular cover slips. Use vacuum grease or beeswax to seal the slip. Secure to the scanner using magnets 910.004.	
10	900.148	Generic Sample Holder: A mount with metal spring clips which allows the installation of many unusually sized samples. Spring clips have three length combinations. Secure to the scanner using magnets 910.004.	
11	900.146	Sample Puck Holder: A mount which allows you to use industry standard 12 and 15mm round metal “pucks”. The mount has an embedded magnet which allows for quick and easy installation. Secure to the scanner using magnets 910.004.	
12	900.184	Vertical Sample Clamp for Cypher and MFP-3D AFMs: This sample clamp allows sectioned, micro-cryotomed and other samples to be firmly held for a variety of AFM measurements. Compatible with the Cypher AFM and the sample puck holder on the MFP-3D AFM. Requires Sample Puck Holder (Model# 900.146) for MFP usage. For samples 0-2mm thick.	
The scale in the photos is in cm and mm.			

12.2. Vacuum Chuck

The Vacuum Chuck is a sample holder option for the MFP-3D that allows flat samples to be held without any mechanical contacts to the top surface or adhesives on the bottom surface. The Vacuum

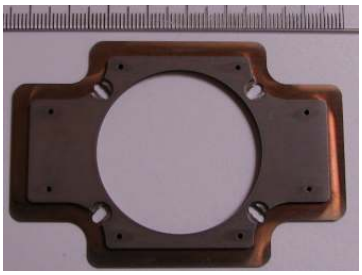
Chuck has three rings to which vacuum can be routed to accommodate 3" or 4" wafers, or sample sizes between 0.8" and 3". Not all regions of 3" or 4" wafers are accessible by the tip for imaging.

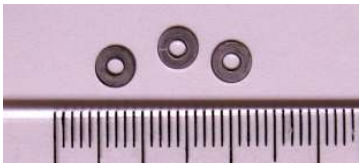
Be Gentle!	Any scratches or other damage to the polished top surface of the vacuum chuck may prevent it from properly sealing against your sample. Treat it with care and store it in a box or other place where nothing can bump into the surface.

Figure 12.1.: Vacuum Chuck System.

12.2.1. Parts list

(Preliminary, incomplete)

Itm	Part#	Item Description	Qty	Picture
1	112.041	VFM Scanner cover plate. Allows the VFM to be attached to the AFM scanner. See Section 25.5 on page 374.	1	
The scale in the photos is in cm and mm.				

Itm	Part#	Item Description	Qty	Picture
2	290.102	Tweezer, Curved Sharp, Standard Grade. Used to hold tiny screws and washers when swapping scanner top plates.	1	
3	290.114	Screwdriver, Slotted, 3.0 mm Width. Wiha 260 3,0 X 50. Used to mount the Probe Station to the scanner top plate 112.041.	1	
4	SHCS 0-80 X 5/16" SS	0-80 X 5/16” socket head cap screws, stainless steel. Spares for attaching the scanner top plate 112.041. Use with a #0 washer (next item in the list). See Section 25.5 on page 374.	8	
5	#0 FLAT WASHER MS801	#0 stainless steel washer. Spares for attaching the scanner top plate 112.041 (see Section 25.5 on page 374). Use with the previous item.	8	
6	290.111	0.050”: Wiha Allen Driver 263 1,3 – 0.05” X 40. For most socket head screws on the VFM2. Typically used to remove the pole pieces. See Section 25.9.2 on page 387.	1	
The scale in the photos is in cm and mm.				

12.2.2. Attaching to the AFM scanner

This accessory attached to the scanner in the same way as the variable field module, VFM.

1. Locate the scanner top plate, screws, washers, and tools shown in the parts list above.
2. Please see Section 25.5 on page 374 about swapping your current plate for the 112.041 model.
3. The four freely rotating retaining screws in of the vacuum chuck key into the four slots on the 112.041 scanner top plate. Use the slotted screwdriver to first align the keys of the screws with the slots.
4. Place the vacuum chuck onto the scanner. Once all four screws fully seat into the slots, rotate each screw by a quarter turn.
5. Now you can magnetically attach the sample plate and any of the probe tools.

12.2.3. Attaching to the AFM scanner

This accessory mounts to a special scanner to plate (112.041). Please see Section 25.5 on page 374 about swapping your current plate for the 112.041 model.

12.2.4. Operation

1. Plug in the pump in and turn it on.
2. Place your sample on the chuck.
3. Use the toggle valves to connect vacuum to one or more of the grooves.
4. Read the gauge to see that vacuum is being maintained. A groove should always be completely covered for vacuum to be maintained.

12.3. Probe Station

The Probe Station attaches to the MFP-3D scanner and allows easy electrical probing of sample properties, electrical biasing, and other measurements while the sample is being scanned with the AFM. A variety of electrical connections can be made and combined with various imaging modes.

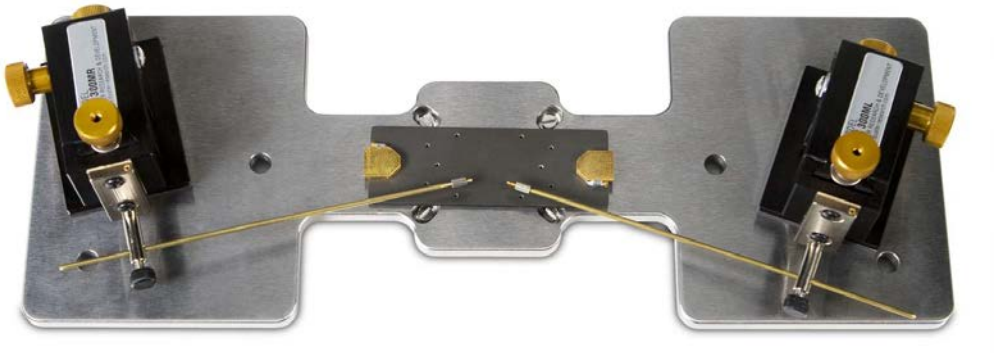
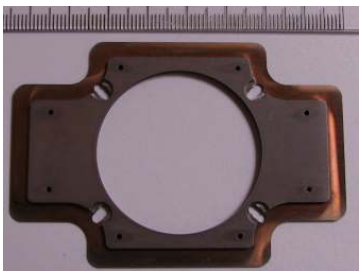


Figure 12.2.: Probe Station Adapter Plate

12.3.1. Parts list

(Preliminary, incomplete)

Itm	Part#	Item Description	Qty	Picture
1	112.041	VFM Scanner cover plate. Allows the VFM to be attached to the AFM scanner. See Section 25.5 on page 374.	1	
2	290.102	Tweezer, Curved Sharp, Standard Grade. Used to hold tiny screws and washers when swapping scanner top plates.	1	
The scale in the photos is in cm and mm.				

Itm	Part#	Item Description	Qty	Picture
3	290.114	Screwdriver, Slotted, 3.0 mm Width. Wiha 260 3,0 X 50. Used to mount the Probe Station to the scanner top plate 112.041.	1	
4	SHCS 0-80 X 5/16" SS	0-80 X 5/16” socket head cap screws, stainless steel. Spares for attaching the scanner top plate 112.041. Use with a #0 washer (next item in he list). See Section 25.5 on page 374.	8	
5	#0 FLAT WASHER MS801	Number 0 stainless steel washer, Spares for attaching the scanner top plate 112.041 (see Section 25.5 on page 374). Use with a the previous item.	8	
6	290.111	0.050”: Wiha Allen Driver 263 1,3 – 0.05” X 40. For most socket head screws on the VFM2. Typically used to remove the pole pieces. See Section 25.9.2 on page 387.	1	
The scale in the photos is in cm and mm.				

12.3.2. Attaching to the AFM scanner

This accessory attached to the scanner in the same way as the variable field module, VFM.

1. Locate the scanner top plate, screws, washers, and tools shown in the parts list above.
2. Please see Section 25.5 on page 374 about swapping your current plate for the 112.041 model.
3. The four freely rotating retaining screws in the probe station plate key into the four slots on the 112.041 scanner top plate. Use the slotted screwdriver to first align the keys of the screws with the slots.
4. Place the probe station plate onto the scanner. Once all four screws fully seat into the slots, rotate each screw by a quarter turn.
5. Now you can magnetically attach the sample plate and any of the probe tools.

13. Fluid Cell Lite

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

13.1. Prerequisites

It's assumed that you are familiar with:

- General operation of the AFM, covered in Chapter 4 on page 15.
- Fluid Imaging in a droplet, covered in Chapter 8 on page 64.
- Working with fluids around the AFM, covered in Chapter 7 on page 62.

Warning Even small fluid spills around an AFM can lead to costly repairs. Educate yourself on how to work safely with fluids. Be sure to read Chapter 7 on page 62.

13.2. Overview and Specifications

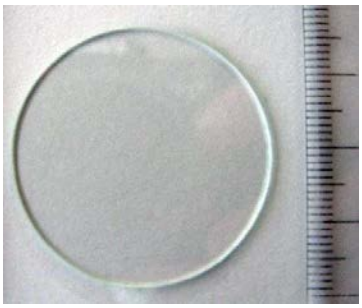
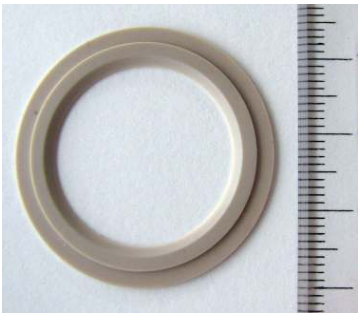
The Fluid Cell Lite is the simplest of the environmental accessories. It is used for basic liquid imaging where optical access from the bottom side of the sample is possible. The cell itself consists

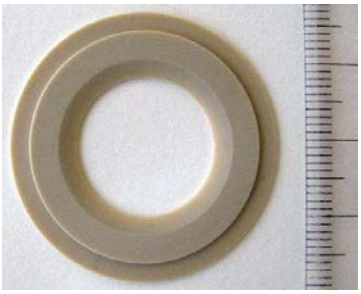
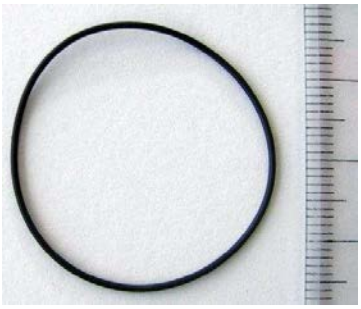
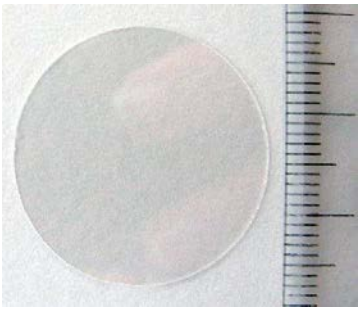
Figure 13.1.: Fluid Cell Lite body, top view.

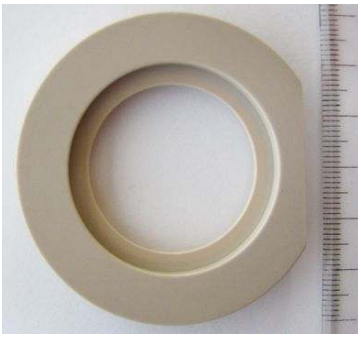
of a peek dish with a removable glass bottom. Figure Figure 13.2 on page 150 outlines the main parts of this dish.

Warning	While imaging in fluid is considered routine with the MFP-3D, one should always be *extremely* cautious about spilling fluid on or around the MFP-3D AFM. Please read Section Chapter 7 on page 62 to familiarize yourself with the dos and don'ts of imaging in fluids.

13.3. Parts list

Itm	Part #	Item Description	Qty	Picture
1	111.420	Closed Cell Bottom Clamp. The stainless steel retaining ring which holds the glass bottom or the cover slip holders against the closed fluid cell. See Section 13.4.1.1 on page 149, Section 14.12.2 on page 190 and subsequent sections through page 190.	1	
2	111.425	35mm X 1mm glass disc, custom made out of Glaverbel float glass by Asylum Research. See Section 13.4.1.1 on page 149 for insertion instructions.	5	
3	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153.	2	
4	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
5	112.789	Clamp, 25mm cover slip top. Used with 112.790 to sandwich a 25mm glass cover slip and then insert as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
6	112.790	Clamp, 25mm cover slip bottom. Used with 112.789 to sandwich a 25mm glass cover slip and then insert as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
7	230.015	O-ring, 1.228”ID X 0.032”CS, Viton, 75 Durometer. This FKM O-ring creates a seal between the sample disc 111.425 and the fluid cell body. See Section 13.4.1.1 on page 149.	5	
8	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
9	504.002	25mm Cover Slip. #1 thickness, can be purchased from almost any microscopy vendor, or from Asylum Research. Must be used with 112.789 and 112.790. See Section 14.12.2 on page 190.	10	
10	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
11	939.010	Portless Fluid Dish. Made of PEEK plastic. For use where fluid ports and pressurized operation are not important. The best choice for any initial investigation. Simple to clean, easy to assemble. See Chapter 13 on page 145.	1	
The scale in the photos is in cm and mm.				

13.4. Tutorial: Fluid Cell Lite

13.4.1. Assembly

13.4.1.1. Inserting the Fluid Cell glass bottom

Part numbers are noted in parentheses.

1. **Locate parts:**

939.008	Spanner wrench
111.420	Bottom retaining ring
111.903	Assembly base (shown, but not used in this tutorial)
111.425	1mm x 35mm glass bottom
230.015	Bottom O-ring
230.012	Top O-ring
939.010	Fluid Cell Lite Body

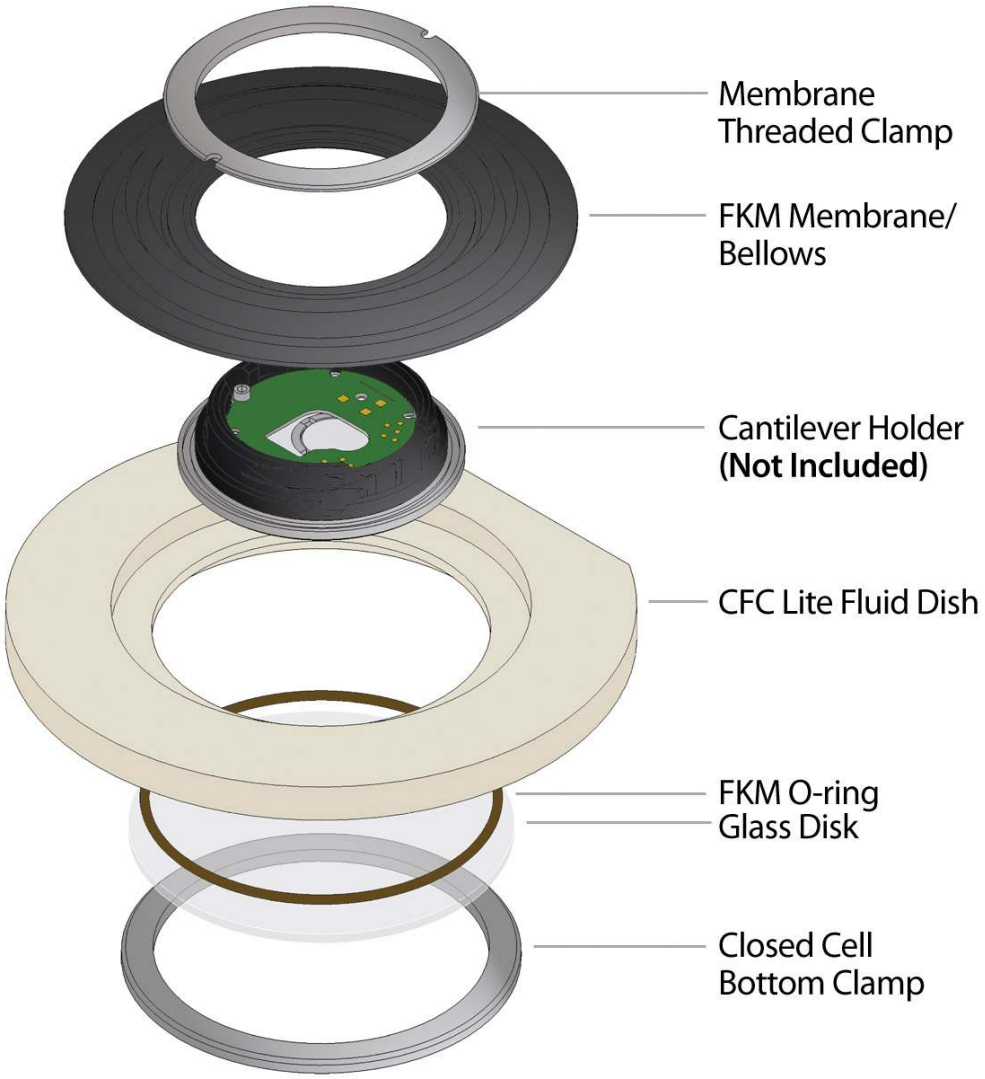
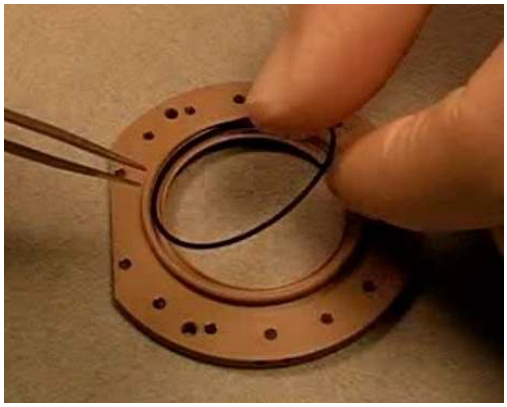


Figure 13.2.: Fluid Cell Lite components. SHOULD INCLUDE PART NUMBERS!!!!

Place the Bottom O-ring

- Take the bottom O-ring (230.015) and place it in the groove on the bottom of the Fluid Dish.



2. **Note** The O-ring (230.015) will shrink a little over time, so you will likely need to stretch it out with your fingers.

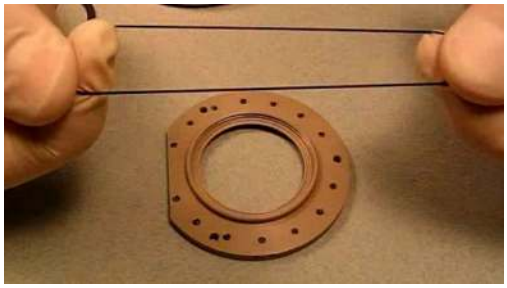
- If the ring fits, skip to step Step 4 on page 151.
- If the ring is too small, move to the next step to enlarge the ring.

3.

Stretching the bottom O-ring

- Stretch the O-ring a bit and try fitting it again (previous step) and repeat the process by stretching up doubling the ring size.

Note If you overstretch the ring, it will shrink back in a few minutes, or you can try again with a spare.



4.

Join Retaining Ring and Spanner Wrench

- Set the spanner wrench upright with the narrow end on the table.
- Match the indentations in the retaining ring (111.420) to the pins on the spanner wrench.
- Place the ring on the wrench. They will hold together magnetically.

5.

Place Glass Bottom against Cell Body

- Inspect the O-ring to be sure that it fits perfectly in the groove.
- Place the glass bottom onto the cell body.

6. **Secure glass with retaining ring:**

   - Place the wrench/spanner ring on top of the glass and hold it all as shown in the photo.
   - Rotate the wrench a few turns counterclockwise (loosening). You should feel a click when the threads properly align. Stop at the click.
   - Tighten clockwise. It should turn easily at first, then finish with moderate force. The O-ring should be fully compressed and the glass bottom needs to be firmly against the plastic. If the glass bottom is only "floating" on the O-ring, you will likely experience thermal drift in your images.

   Note The initial threading process should be smooth. If not, you may have crossed threads - this can cause damage the peek cell body.

7. Your Fluid Cell is now ready for sample mounting.

13.4.2. Sample mounting

We discuss a few choices for sample mounting below. Please do not feel limited by these choices. For additional support, do not hesitate to contact Asylum Research. Please see the Introduction on page iv for support options.

Epoxy A sample in the form of a small chip of silicon or glass can be mounted with a few small dots of five minute epoxy around the sample perimeter. Place the sample on the glass, then apply mixed epoxy with a toothpick. While one can put epoxy directly under the sample, it may lead to thermal drift while imaging. Since prolonged exposure to water can soften the epoxy, consider another epoxy which is advertised as being water resistant (2 ton clear epoxy from Devcon is one option). The choices of epoxy are almost endless, so we will not discuss them further here.

Epon An epoxy resin which is melted by heating to about 80C. Epon is famous in the surface science community for very low contamination of water. Epon can be bought from or contact support@asylumresearch.com and we can arrange to sell you some from our stock.

Magnets In case you are worried about chemical contamination or need work with volatile organic solvents as your imaging medium, magnets can be useful to hold down the sample. One Asylum Research customer has successfully used the following:

1. Punch an annulus from a thin sheet of magnetic stainless steel. The outer diameter should be small enough to fit inside the bottom of the fluid cell and the inside diameter should be a bit smaller than the size of your sample.
2. Cut a similar annulus from a sheet of a plastic magnetic sheet (refrigerator magnet)
3. Epoxy the refrigerator magnet to the bottom of the cell's glass bottom.
4. Place the sample inside the cell and place the magnetic stainless steel annulus on top. It will hold the sample in place, and only the stainless steel will contact your sample and liquid.

On the Glass Samples such as cells or thin films can be grown or placed directly on the glass bottoms. Additional discs may be purchased from Asylum Research. See Section 13.3 on page 146 for the appropriate part number.

Custom Bottom You may also want to build your own cell bottom with sample mounting options to your liking. In this case, the dimensions are 35mm in diameter and 1mm thick. The cell bottom only needs to be 1mm thick where the retaining ring holds it in place. Note that if you want to use the supplied spanner wrench (part number 939.008 in the list in Section 13.3 on page 146) you will need to make sure the cell bottom does not interfere with its use.

13.4.2.1. Inserting 25mm cover slips

Please refer to Section 14.12.2 on page 190 for details on the process of mounting a 25mm circular cover slip in the place of the 1mm thick glass bottom. Note that the instructions refer to an assembly base (111.903) which is not included with the Fluid Cell Lite kit. It was omitted to reduce the cost of the kit. It can be purchased separately from Asylum Research, but it is not necessary for successful assembly of the Fluid Cell. Simply placing the spanner wrench near the edge of a table so you can grip it with thumb and forefinger achieves about the same effect as placing it in the assembly base.

13.4.3. Attaching the Membrane to the Cantilever Holder

These steps will require a lot of finger contact with the cantilever holder. We recommend the use of latex or nitrile rubber gloves. Powder free gloves are best, but if you cannot find those, washing off the powder with soap and water after putting on the gloves is a good alternative. If you do not have gloves, you will probably want to swab the cantilever holder with alcohol when you are finished. Inspect for fingerprints on the front and back of the glass window in the cantilever holder.

1. **Locate parts:** Refer to Section 13.3 on page 146.

112.491	O-ring Membrane Treaded Clamp / Retaining Ring.
112.256	Closed Cell Bellows / Membrane. Black Viton or Clear Silicone.
939.008	Spanner Wrench
290.146	Blue Ball

   A cantilever holder of your choice. See Chapter 11 on page 94 for cantilever holder options.

NOTE: Old versions of retaining ring did not have an O-ring groove. Make sure you are using the ring which does indeed have the O-ring groove.

2. **Prepare the spanner wrench:**

   - Place the retaining ring onto the narrow end of the spanner wrench. Small magnets in the wrench hold the ring in place.

3.

Familiarization with threading:
Advanced users may choose to skip this step.

- Hold the cantilever holder in one hand and the retaining ring and wrench in the other.
- Thread the ring on the back side (circuit board side) of the cantilever holder. This step is to get a feel for the threading process. The ring should thread all the way on without much resistance.

Note If there is any resistance, do not apply more force. Back off and turn the wrench a full turn counter clockwise to make sure ring seats properly onto the threads. This will give you a feel for the process and you may skip this step in the future.

- Unscrew the retaining ring again and leave it magnetically attached to the spanner wrench.

4.

Place the membrane:

- Place the membrane on the circuit board side of the cantilever holder. A built in O-ring in the membrane will seat into a groove on the cantilever holder plastic body.

Note The bellowed ridges on the cantilever holder should face in the direction of the cantilever circuit board.

5. **Tighten the retaining ring:**

   - Place the ring against the cantilever holder and turn counter clockwise (loosening) until you feel a slight click indicating the threads are properly lined up.
   - Turn clockwise until the ring is tight.

6.

Final tightening:

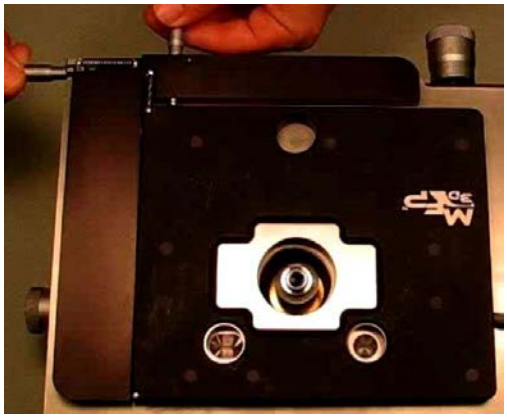
- You can use the blue ball to press down on the cantilever holder while you tighten the ring with the spanner wrench.
- If your cantilever holder has a metal clip, you can also put a fingernail against the side of the clip to prevent the cantilever holder from turning.

7. **Load the cantilever:**

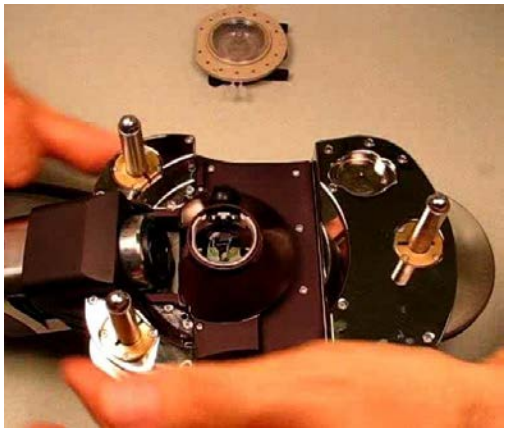
   - Follow the directions in Section 4.2 on page 16.
   - Set the assembled parts aside.

13.4.4. Preparing Head and Scanner

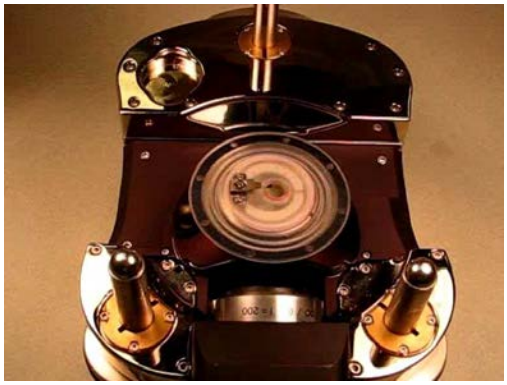
1. **Center the scanner:**
   - Using the scanner's micrometers, center the divot or groove like features (depending on the vintage of your MFP-3D AFM) in the three small holes in the scanner.
   - The bottom view objective (if your model of AFM has one) should be centered in large hole in the scanner.



2. **Raise the legs:**
   - Depending on who last used the AFM and the thickness of your sample, it is good practice to raise the legs a bit so as not to crash the cantilever when first placing the head.



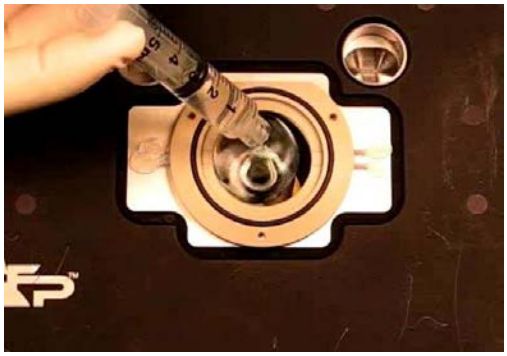
3. **Attach the cantilever holder:**
   - Depress the rubber button on the AFM head and attach the cantilever holder. For more information see Step 2 on page 21.



4. **Adjust the legs for proper clearance:**
   - Without any fluid cell on the scanner, place the head down over the scanner and look in between the cantilever holder and scanner for clearance. See Step 3 on page 23 for more detailed instructions.
   - The top of the glass in the fluid cell sits slightly below the top of the scanner. If you can see about 1 mm of clearance between your cantilever and the top of the scanner then the legs are at a good height for later engagement on the bottom of the cell. Add a little more to adjust for a finite sample thickness.

13.4.5. Filling the Cell and Imaging

1. **Place and fill the cell:**
   - Place the cell on the scanner as shown.
   - Add a few cc of fluid to the cell.



2. Prepare your cantilever for fluid imaging. Wet the cantilever with a bit of fluid as described in Step 2 on page 69.

3. **Place the head:**
   - Place the head as shown.
   - Lower slowly and keep an eye out for excess displaced fluid. Do not hesitate to back off, take out some fluid, and start over again.

4. **Collect data:**
   - Image as you usually would in fluid. For more guidelines see Chapter 8 on page 64.

13.4.6. After Imaging

1. **Break the seal:**
   - At the point indicated in the photo, using something long and flat (perhaps the tips of flat tweezers), lift the edge of the membrane to break the seal.

2. **Lift the head:**
   - Gently lift the head, making sure the cell does not lift up due to suction of the membrane.

3. Rest the head on its side and place something under the head to catch any liquid drops.

13.5. Cleaning and Care

Refer to the parts list (Section 13.3 on page 146) for the materials of which the fluid cell is made. All the parts used during imaging can be cleaned with solvents such as alcohol. Parts can also be autoclaved.

Avoid the use of acetone. The rubber parts will not do well when exposed to acetone.

Please store your fluid cell in its designated case. If you own multiple accessories that are similar to this one, it is best to keep all the parts where they belong and not get things mixed up.

If you need replacement parts, contact your local Asylum Research office or distributor and use the parts list (Section 13.3 on page 146) as a guide.

14. Closed Fluid Cell

CHAPTER REV. 1710, DATED 10/23/2013, 21:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

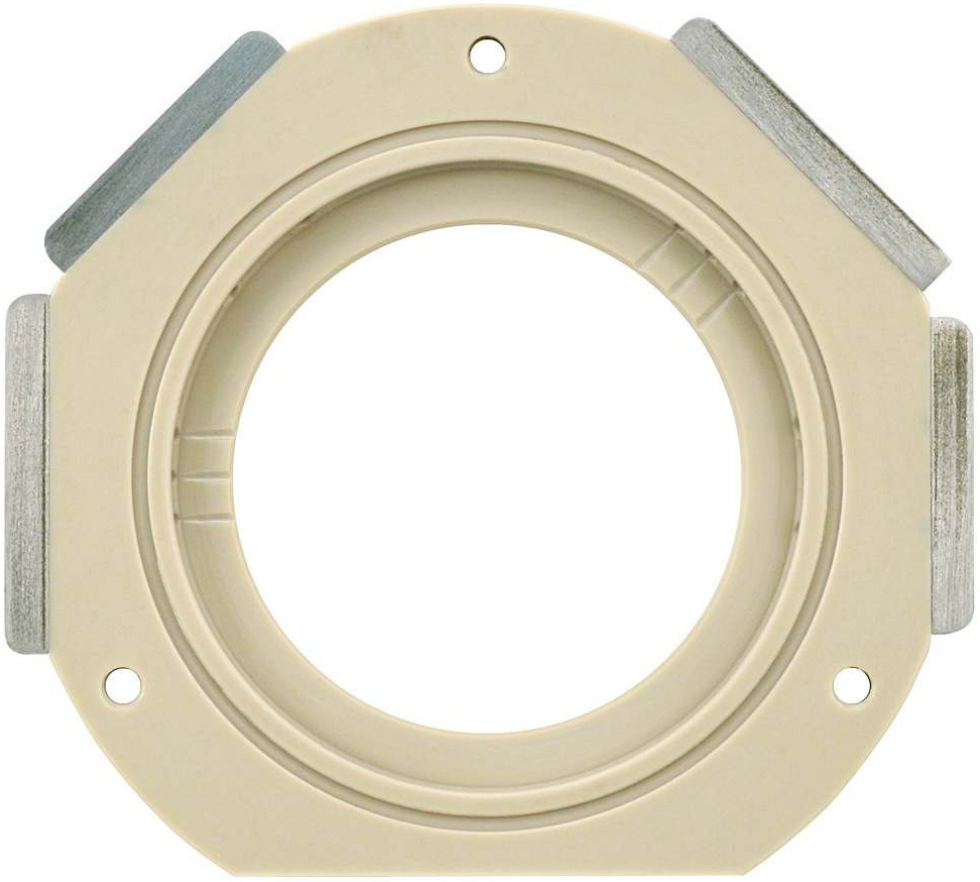


Figure 14.1.: Top view of the Closed Fluid Cell Body with port clamps installed.

14.1. Prerequisites

It's assumed that you are familiar with:

- General operation of the AFM, covered in Chapter 4 on page 15.
- Fluid Imaging in a droplet, covered in Chapter 8 on page 64.
- Working with fluids around the AFM, covered in 7.

Warning	Even small fluid spills around an AFM can lead to costly repairs. Educate yourself on how to work safely with fluids. Read Chapter 7 on page 62.

14.2. When and when not to use

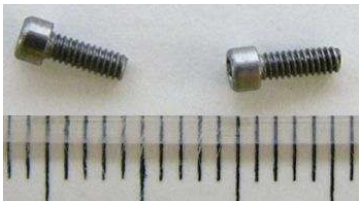
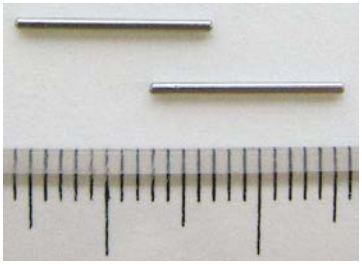
The Closed Fluid Cell is an advanced version of the Fluid Cell Lite (described in Chapter 13 on page 145). It adds a variety of O-ring sealed ports for injecting fluids or routing sensors and wires. The Closed Fluid Cell also includes the necessary hardware for making a completely sealed sample chamber, which can be fluid or gas tight up to several pounds per square inch.

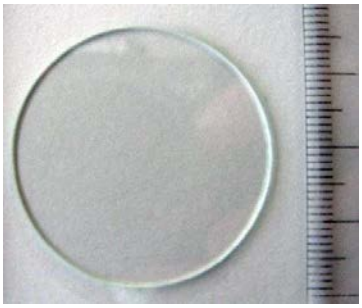
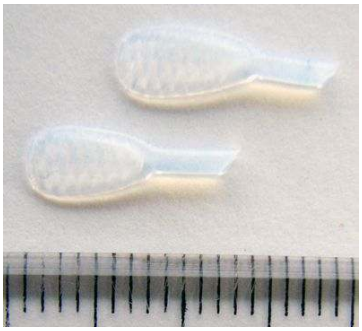
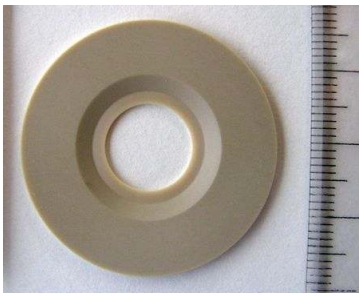
While all of these features may be necessary for some experiments to succeed, many will only require a simple dish to hold some liquid. In this case, just use the simple fluid cell (939.010) provided in every closed fluid cell kit.

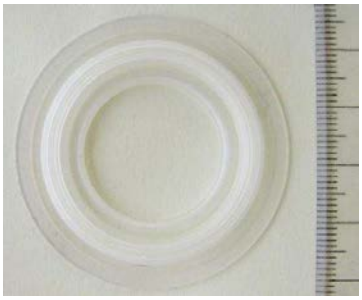
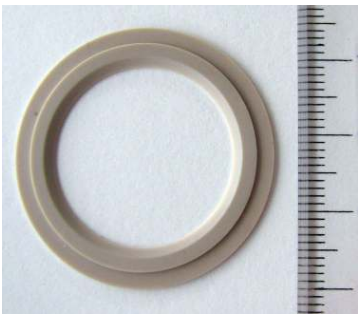
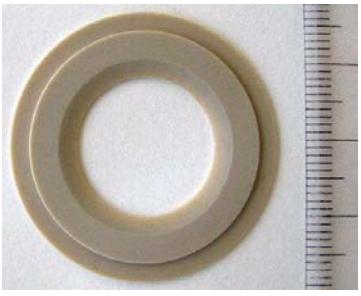
Attention	We strongly encourage beginning users to master the Fluid Cell Lite before trying a full blown experiment with fluid exchange in a sealed cell. Please read Chapter 13 on page 145 carefully before proceeding.

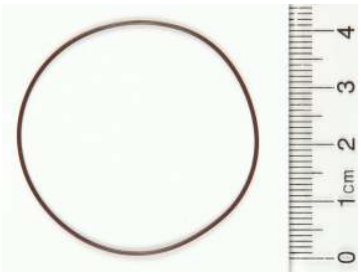
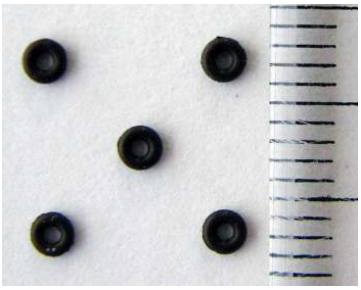
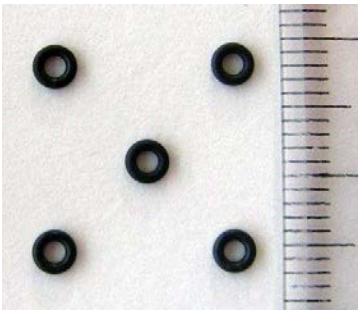
14.3. Parts List

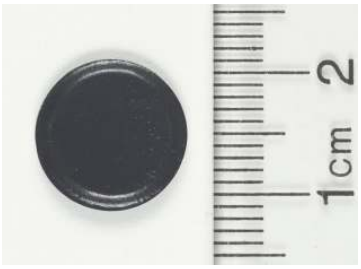
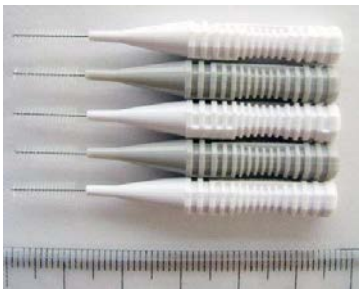
The following lists all the parts in your accessory kit. The table is useful as a visual table of contents with links directing you to the specific uses of each part. When ordering parts, please refer to the part numbers in the second column.

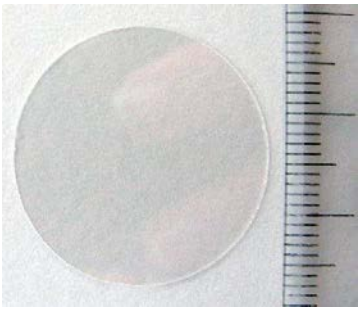
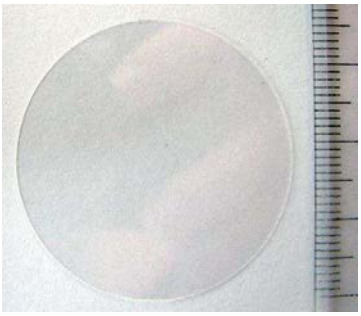
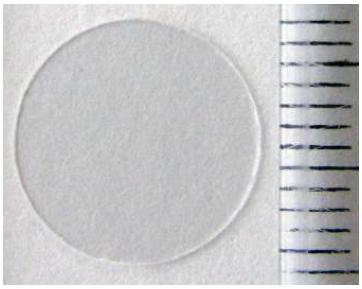
Itm	Part #	Item Description	Qty	Picture
1	001.SHCS <#0-80X.188> SST	0-80 X 3/16” Stainless Steel Socket Head Cap Screw. Connects 111.886 and 102.204 O-ring clamps to the fluid cell body. See Section 14.4.2 on page 172.	8	
2	080.010	5 ml Syringe. We prefer this Norm-Ject model from Henke Sass Wolf (HSW) since it does not contain any rubber and contamination is minimal. See Section 14.4.3 on page 172 on how to attach the necessary tubing.	2	
3	005.DOWL <.031X.500> SST	1/32” diameter X ½” 18-8 stainless steel dowel pin. Used with Super Mini O-ring Triple Clamp (Part 112.430) to plug the fluid cell’s small diameter sealed feed- through ports. See Section 14.4.1 on page 169. Also consider using 0.035" diameter PTFE cord from McMaster Carr (Part #84935K36).	12	
The scale in the photos is in cm and mm.				

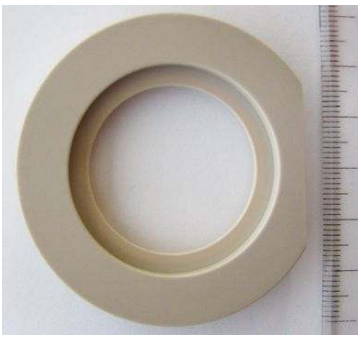
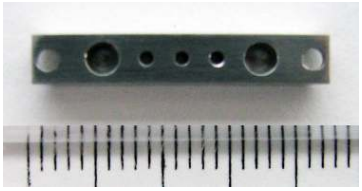
Itm	Part #	Item Description	Qty	Picture
4	111.425	35mm X 1mm glass disc. Custom made by Asylum Research out of Glaverbel float glass. See Section 13.4.1.1 on page 149.	5	
5	111.903	CFC Bio Assembly Base. Used to hold the spanner wrench (939.008) during various closed cell assembly steps. (*e.g.* Step 1 on page 192)	1	
6	111.924	1/16” OD Port Plug. PTFE (generic Teflon) plugs for blocking unused in/outlets on the closed fluid cell. Also consider using PTFE cord 0.062" diam. (McMaster Carr Part Number 84935K48.) See Section 14.4.1 on page 169.	10	
7	111.925	CFC 12mm Cover Slip Holder. This PEEK plastic holder makes it possible to mount 12 mm diameter coverslips in the closed fluid cell. Please see Section 14.12.3 on page 194.	1	
The scale in the photos is in cm and mm.				

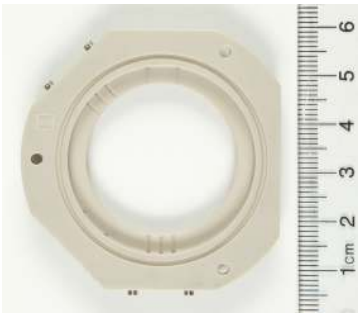
Itm	Part #	Item Description	Qty	Picture
8	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153 .	2	
9	112.256.02	Closed Cell Bellows, 30 Durometer Silicone rubber. See Section 13.4.3 on page 153.	2	
10	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
11	112.789	Clamp, 25mm cover slip top. Used with 112.790 to sandwich a 25mm glass cover slip and then inserts as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
12	112.790	Clamp, 25mm cover slip bottom. Used with 112.789 to sandwich a 25mm glass cover slip and then inserts as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
13	230.011	O-ring, 0.244”ID X 0.016”CS, Viton, 55 Durometer. Seals the cantilever holder quartz prism. See Step 7 on page 104.	3	
14	230.012	O-ring, 1.595”ID X 0.030”CS, Viton, 55 Durometer. This FKM O-ring makes the seal between the bellows membranes and the sealed cell body. See Section 14.4.5 on page 173.	5	
15	230.015	O-ring, 1.228”ID X 0.032”CS, Viton, 75 Durometer. This FKM O-ring makes the seal between the sample disc 111.425 and the fluid cell body. See Section 13.4.1.1 on page 149.	5	
16	230.016	O-ring, 0.031”ID X 0.028”CS, Viton, 75 Durometer. FKM O-rings that seal around the six 0.036" fluid ports in the Fluid Cell. See Section 14.4.2 on page 172.	15	
17	230.018	O-ring, 0.062”ID X 0.032”CS, Viton, 70 Durometer. FKM O-rings that seal around the four 1/16" fluid ports. See Section 14.4.1 on page 169.	15	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
18	231.006	Tubing, PFA, 1/16"OD X .040"ID. This PFA tubing makes it possible to introduce and remove fluid or gas from the closed fluid cell. Order from Asylum or purchase directly from Upchurch Scientific (Part #1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	5 ft	
19	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16" OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
20	270.020	0.500" diameter x 0.060" thick Rubber Bumper. Goes under part 111.903.	3	
21	290.103	3C Tweezer – Extra Fine Sharp – Standard Grade. For placing samples, tiny o-rings (e.g. 230.018) , and small screws.	1	
22	290.111	0.050": Wiha Allen Driver 263 1,3 – 0.05" X 40. For all socket head screws. Used, for example, for sealing fluid ports.	1	
23	290.113	Brush, 1/16 Cleaning. Small enough to clean deposits from inside the fluid ports.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
24	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
25	504.002	25mm Cover Slip. #1 thickness, can be purchased from almost any microscopy vendor, or from Asylum Research. Must be used with 112.789 and 112.790. See Section 14.12.2 on page 190.	10	
26	504.003	Cover slip, 35mm round. #1 thickness This 35 mm coverslip is 0.17 mm thick. To suppress vibrations and allow proper sealing, these coverslips should be backed by 111.925. See Section 14.12.1 on page 190.	10	
27	504.004	Cover Slip, 12mm. #1 thickness, can be purchased in almost any microscopy catalog, or from Asylum Research. Must be used with 111.925. See Section 14.12.3 on page 194.	10	
28	939.007	Membrane Clamp, DISCONTINUED. Replaced by 939.015. Can be retrofitted with kit 939.014. See **??** on page **??**.		
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
29	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
30	939.010	Portless Fluid Dish. Made of PEEK plastic. For use where fluid ports and pressurized operation are not of importance. The best choice for any initial investigation. Simple to clean, easy to assemble. See Chapter 13 on page 145.	1	
31	939.015	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173. **Warning**: Not following the instructions and not leak testing before use will seriously damage your AFM from fluid spills.	1	
32	112.204	Fluid Port Clamp Bar. Stainless Steel. Compresses and seals two mini O-rings (230.018). Fastens with 0-80 X 3/16” screws. Part of 1/16” fluid ports. See Section 14.4.2 on page 172.	2	
33	112.430	Closed Fluid Cell Triple Clamp. Fluid Port Clamp Bar. Stainless Steel. Compresses and seals three mini O-rings (230.016). Fastens with 0-80 X 3/16” screws. Part of 1/16” fluid ports. See Section 14.4.2 on page 172.	2	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
34	928.005	Closed Fluid Cell Body. Made of PEEK plastic. Due to its 10 fluid ports, this is the ideal choice for advanced experiments where a pressure tight seal or flow through are necessary. See Section 14.4 on page 169.	1	
35	111.420	Closed Cell Bottom Clamp. The stainless steel retaining ring which holds the glass bottom or the cover slip holders against the closed fluid cell. See Section 13.4.1.1 on page 149 and following sections through page 190.	1	
The scale in the photos is in cm and mm.				

14.4. Tutorial: Closed Fluid Cell Sealed Operation

Warning	Quite often it is sufficient to use the included portless open fluid dish as described in Chapter 13 on page 145. You will use the closed fluid cell only if you need to work with a fully sealed cell, or if you require attached tubing.
For Beginners	If it is your first time, consider completing this tutorial without fluid. Just leave one port open to air and go through the process of imaging in air in a sealed cell. This way if something goes wrong, you will avoid a spill.

14.4.1. Sealing the large ports

For this tutorial we'll attach two tubes. One to add fluid with a syringe, and one to let out the excess gas or fluid while engaging.

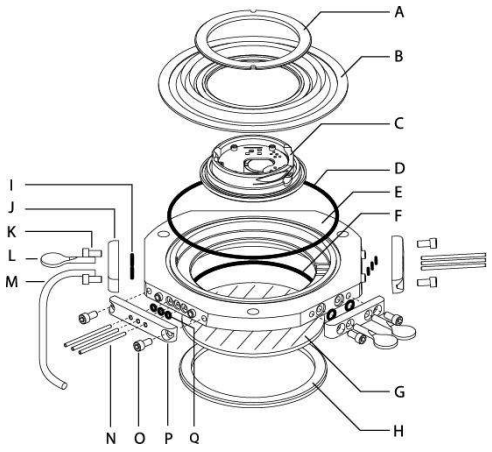
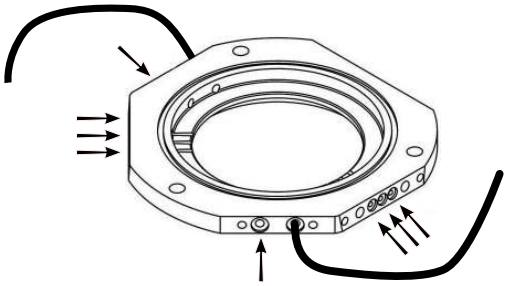


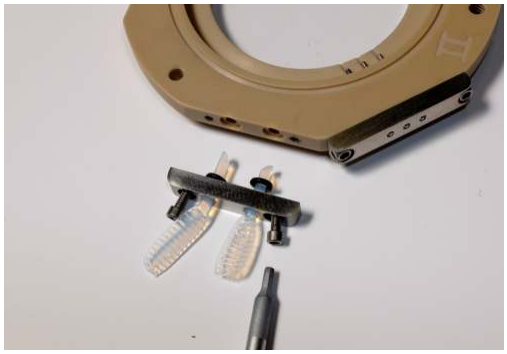
Figure 14.2.: Complete Parts breakdown of the Closed Fluid Cell.

	Part Number	Description
A	112.491	Cantilever holder retaining ring
B	112.256	Closed Fluid Cell FKM Membrane
C		Cantilever Holder
D	230.012	Closed Fluid Cell Top O-ring
E	928.005	Closed Fluid Cell Body Assembly
F	230.015	Closed Fluid Cell Bottom O-ring
G	111.425	35mm X 1mm Glass Sample Disc
H	111.420	Closed Fluid Cell Bottom Retaining Ring
I	230.018	Closed Fluid Cell Mini O-ring
J	111.886	Closed Fluid Cell Mini O-ring Clamp
K		0-80 X 3/16” SHCS S/S
L	111.924	Closed Fluid Cell Teflon Plugs 1/16”
M	231.006	1/16” OD 1mm ID FEP tubing
N	222.018	1/32” X 5/8” Dowel Pin Plug
O		See K
P	112.430	Closed Fluid Cell Triple Clamp
Q	230.016	Closed Fluid Cell Super Mini O-ring

1. **Port plugging overview:**
   - The ports indicated with arrows are to be plugged.
   - The other two ports will have tubing attached.



2. **Get plugs and O-rings into place:**
   - Place each plug into the hole on the clamp.
   - While doing so, thread an O-ring onto the plug.
   - The result should be as shown to the right. It is important the the plug protrude into the PEEK cell body, or it will not seal properly.



Note The photo demonstrates with two port plugs. The method for inserting tubing is the same.

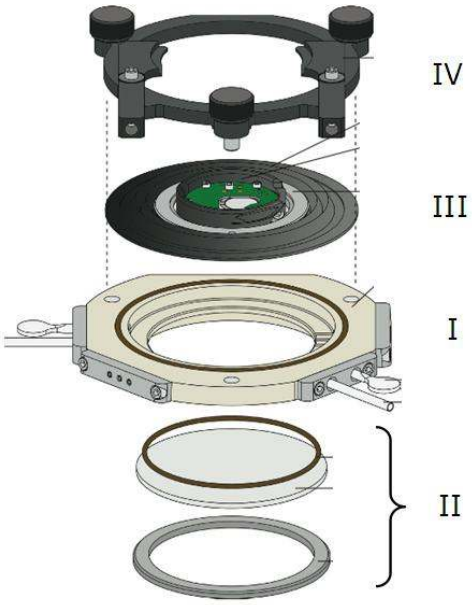


Figure 14.3.: Basic Overview of Assembly Steps

3. **Attach the clamp to the cell body:**
   - Screw the clamp and plugs to the cell body.
   - Alternate between the screws while tightening. Do not fully tighten one side with the other screw very loose.
   - Gently tighten the clamp until it is flush. Do not over tighten and strip out the threads.

   Note Make sure the plugs stick a little bit into the cell body. They will only properly seal if they are fully inserted through the O-rings.

4. If your fluid cell does not already have the tubes attached, please locate parts I, J, and K using Figure 14.2 on page 170.
5. Cut two pieces of the supplied tubing (231.006), each about 12 inches (30 cm) long. Use a fresh razor blade to get a good clean cut at the end.
6. Use the same method as described above for the port plugs.
7. As a final inspection, make sure all port plugs are inserted as far as they can possibly go and give the tubing a gentle pull to make sure it stays in place.

For more information on the fluid ports and the tubing used, please see Section 14.6 on page 182.

14.4.2. Sealing the small ports

For this tutorial we will plug the six smaller fluid cell ports. If they are already plugged, please skip to the next section.

1. Gather up items N, O, P and Q shown in Figure 14.2 on page 170.
2. Follow the same method as described for the larger ports in 14.4.1. The only difference is that you will be using steels pins instead of plastic plugs.

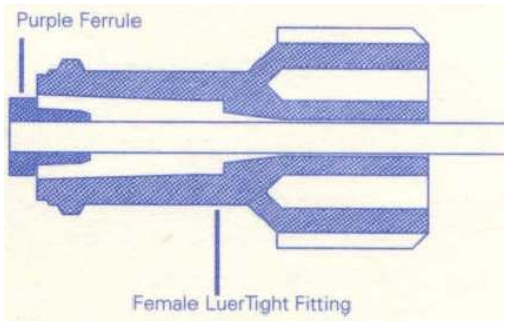
14.4.3. Attaching Tubing to a Syringe

Choose one of the two pieces of tubing from the previous section and attach a syringe to it by following the instructions below.

1. **Locate parts:**
   - 231.008 Luer Tight Fittings.
   - 231.006 1/16" OD Teflon tubing.
   - Any Luer Lock syringe, such as the supplied 080.010.

2. **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.



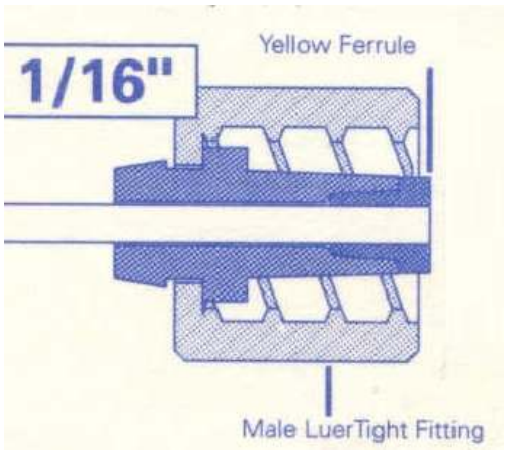
3. **Seat the fitting:**
   - Attach the red Luer Tight seating tool to the female Luer Tight fitting by screwing it firmly onto the female Luer Tight body. This will seat the ferrule into the luer fitting.
   - Remove the seating tool and store for later use.

4. **Attach syringe:**
   - You are ready to attach the syringe to the tubing. However, in this tutorial, we'll wait until a later step.

5. **Mating connection:**

Note The male luer fitting is not necessary to connect to a syringe, but it can be used to splice to another piece of tubing. Instructions follow.

- Slide the male Luer Tight fitting and yellow ferrule, in that order, over the tubing.
- With the ferrule facing down, firmly press the fitting and ferrule combination down onto a hard surface to seat the ferrule and allow it to grip the tubing properly.
- Go ahead and attach this to the outlet tube. This will come in handy later during leak testing.



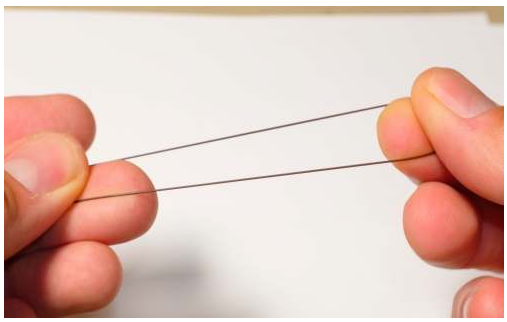
14.4.4. Prepare the Cantilever Holder

1. Attach a membrane to the cantilever holder. Please follow the instructions here: Section 13.4.3 on page 153.
2. If you did not do so already, load a cantilever into the holder. See instructions here: Section 4.2 on page 16.

14.4.5. Sealing the Fluid Cell

1. **Stretch the O-ring**

- Find the largest thin O-ring (230.012) and stretch it out a bit.



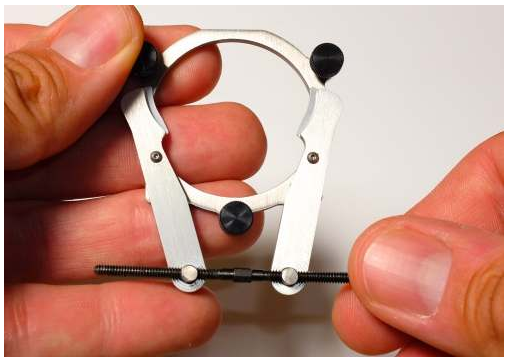
2. **Place the O-ring:**

   - Place the O-ring in the groove on top of the fluid cell body. Stretch it some more if it will not lie flat.

   Note An over stretched O-ring will shrink back to size over time. If you cannot wait, exchange with a spare O-ring.

3. **Open the jaws:**

   - Turn the knob on the clamp until the jaws are fully opened.



4. **Orient parts:**

   - Place the cantilever holder and clamp as shown. Clamp legs point to 9 o'clock. Cantilever tip points to 12 o'clock.
   - The clamp is placed with its three screws facing up.

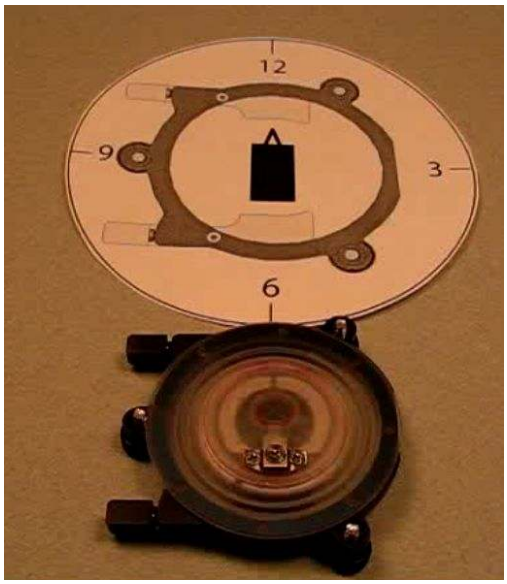
   Note The clamp and membrane in this photo are older models than you might have.

5.

Join clamp and membrane:

- Place the cantilever holder and membrane on top of the clamp as shown.

Note The clamp and membrane in this photo are older models than you might have.

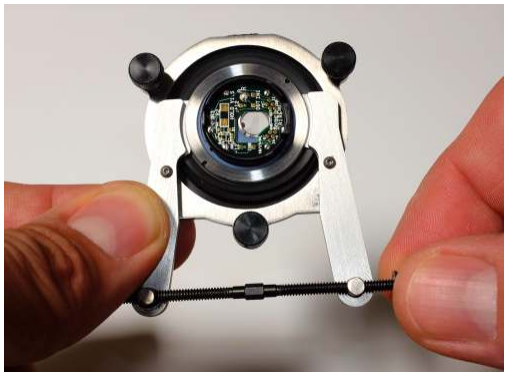


6.

Engage the jaws of the clamp:

- While holding the membrane and cantilever holder against the clamp, close the jaws by turning the knob.
- The thin part of the jaws must go under the lip of the stainless steel ring on the back of the cantilever holder.

Note Be careful that the jaws do not "bite" a hole into the membrane. If you keep tightening the screw, a membrane can be punctured if pinched.

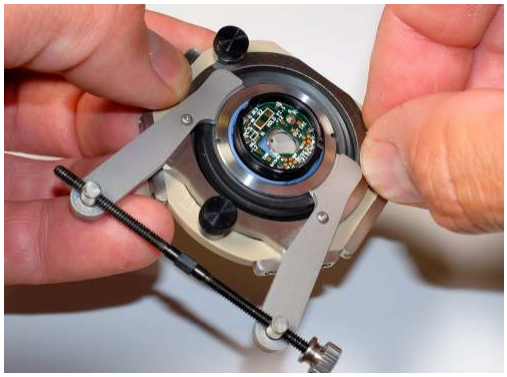


7.

Attach the clamp to the cell body:

Note In the case of fluid imaging it may help to wet the cantilever a bit to prevent bubble problems. See Step 2 on page 69.

- Attach the clamp to the cell body.
- If the flat side of the cell body is facing away from you, the paddles should be facing toward you.
- First tighten the three screws very loosely, taking up the slack in the threads.
- Then go around again and tighten the screws firmly. This process ensures equal pressure on the entire O-ring and a good seal.



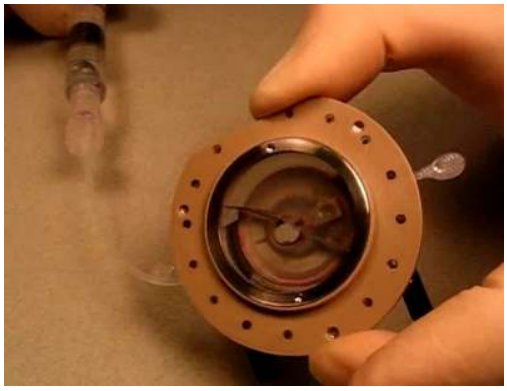
14.4.6. Filling the Fluid Cell

If you are working with liquids (and not, for instance, dry gases) then attach a syringe of fluid to the inlet tube and fill the cell.

1.

Fill the cell:

- Orient the cell so that the inlet port is low and the exhaust tube is high.
- Slowly inject the fluid and observe.
- When fluid spills from the outlet tube, stop. Try to arrange it so no bubbles get trapped in the cell.



2.

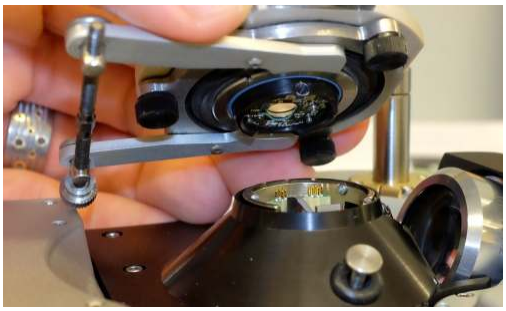
Check for leaks:

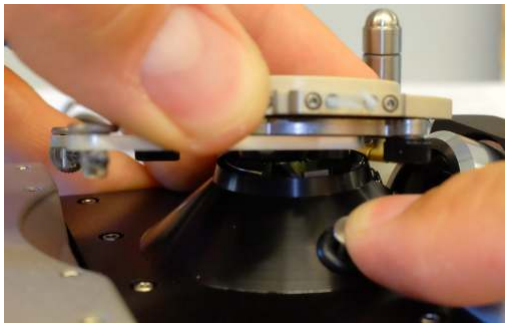
- Remove the syringe and attach the inlet tube to the outlet tube. The system is now fully sealed.
- Turn the knob on the clamp to release the paddles. The Cantilever holder can now move somewhat freely. Be careful not to crash your cantilever.
- Press down with a few pounds of force on the cantilever holder. This creates a few PSI of pressure inside the cell.
- Look for leaks. If any are found, disassemble and reassemble the parts in question.
- When done, re-engage the clamp's jaws under the cantilever holder.
- Unhook the two tubes, and attach the syringe back to one of them.

Note For more information on leak checking, please read Section 14.7 on page 183. **A few minutes of prevention can save you thousands of dollars in repairs and several days of instrument downtime.**

14.4.7. Mounting the Cell to the AFM Head

1.





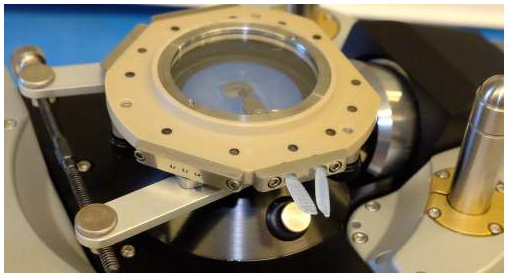
Attach the assembly to the AFM head:

- Arrange the AFM head and the cell as shown on the left.
- Then press down the button and mount the whole assembly just as you wold a cantilever holder by itself.

2.

Inspect the AFM head:

- The cell should be sitting level.
- Wiggle the cell a bit and make sure it stays secure.
- Fully extend the legs .



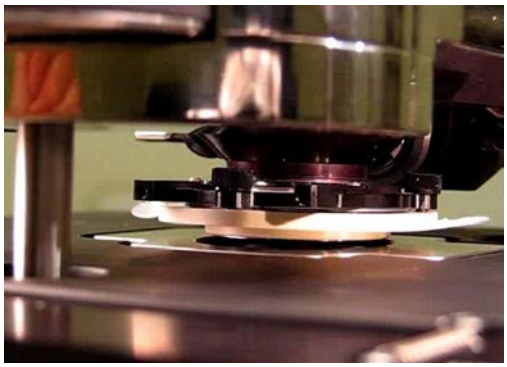
14.4.8. Placing the Head

1. Center the scanner. See Step 1 on page 157.

2. **Place the AFM head over the scanner:**
   - Rear legs first, then front leg.

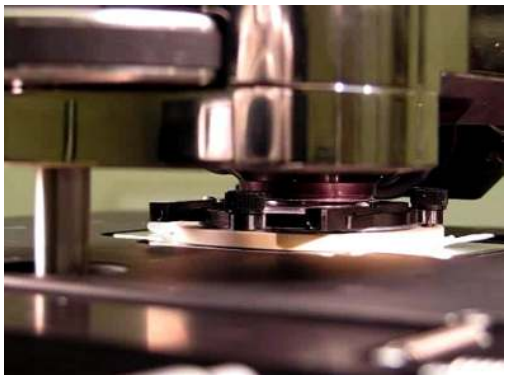
   Note The clamp and cell in the following few photos are older models.

3. **Start lowering the legs:**
   - Slowly lower the legs until the cell sits horizontally above the scanner.
   - Carefully continue to lower until the cell just touches the scanner.



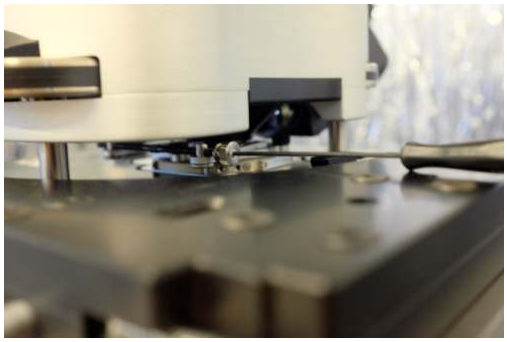
4. **Prepare to release the jaws:**
   - Inspect that the cell body is just barely resting on the scanner.

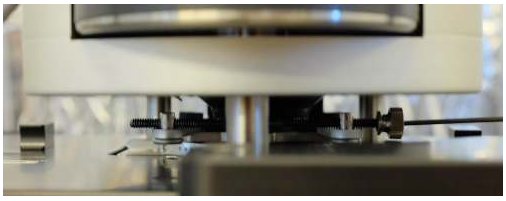
   Note It is useful to measure the distance between the scanner and the head with a ruler and take note of this measurement. At a later stage we'll need to return to this height to gracefully remove the head.



14.4.9. Engaging on the Surface

1. **Release the jaws:**
   - Inspect that the cell body is just resting on the scanner.
   - Position a 3/32” allen driver as shown.



2. 


   Release the paddles:
   - Keep an eye on the clamp as in the photos.
   - Turn the driver clockwise until it looks like the photo on the right.

3. Align the top view optics to get a view of the cantilever, then get the laser spot on the lever and engage as usual. For more details, see Chapter 4 on page 15 and Chapter 8 on page 64.

Do not forget!	Be sure to plug one of the tubes with a syringe, and place a cup under the end of the second tube to catch the displaced fluid. It should drip out as you lower the AFM head to engage.

4. As you lower down to engage, you can alternately turn the front and rear legs to keep the cantilever in the center of the video picture. This indicates that you are making a vertical descent.
5. Use the video image to anticipate the surface of the sample, but also keep an eye on the AC mode amplitude. Engage as you learned in the AC Mode Imaging tutorial.

14.4.10. Imaging

The process of imaging is no different from what has already been described in the imaging tutorial chapters.

Note that coarse positioning (see the micrometers called Sample Align X and Y as in Section 2.1.3 on page 7) will be limited to only a few mm before the pull on the membrane is too much.

14.4.11. Liquid Flow through

If you set the cell up from the description up to this point, there are two tubes attached, one of which is plugged with a syringe. The safest method is to disconnect the syringe, pull out the plunger, and then to attach it again to act as a reservoir. Hang this reservoir up above the AFM head and allow gravity to flow fluid through. You can observe the rate by seeing how fast the catch basin fills up. If you want to get quantitative, you can place the catch dish on a milligram laboratory balance. During setup, be careful not to allow any bubbles to get into the tubing, or they will end up in your cell as a gas pocket. Also keep in mind that every bubble stuck in the tubing will require a bit of extra pressure to dislodge it. A train of small bubbles can make the tubing seem clogged.

It's also possible to simply put pressure on plunger of the attached syringe. Pay extra close attention when doing this, since the cell will start to leak at any pressure higher than 3 psi (~ 1/5 of an atmosphere). Syringe pumps can be useful, but dangerous, since they do not have a sensor for building pressure.

Note that the liquid pushed into the side ports does not necessarily travel efficiently to the center of the cell where the sample is. It will mostly choose the path of least resistance, which is to travel around the outer perimeter of the cell. If you are concerned about delivering fluid very close to the cantilever, consider the MicroFlow cantilever holder, described in Section 11.6 on page 116. In that description it is used for imaging in a small liquid drop. It can just as well be used in a fully filled fluid cell. One can then either flow in one port and out the other, or one can introduce liquid into both ports and let the fluid drain from a fluid port on the perimeter of the fluid cell.

14.4.12. Lifting the AFM Head

If you do not care if the cantilever and sample run into each other after imaging, then you can simply slowly lift the AFM head. Since the jaws are not holding the cantilever holder in place, the fluid cell body will flop around since it is only held in place by the bellows membrane. Since this method is a bit crude, a more predictable approach is described here.

1. Stop imaging and withdraw the tip from the surface.
2. If you do not mind air being pulled into the cell when withdrawing, then proceed. If you want the cell to fill with liquid upon withdrawal, then set up a fluid reservoir from which the outlet tube can draw in fluid.
3. Slowly wheel up the legs (use the trick of keeping the cantilever in the center of the screen) until you have reached the height where the jaws of the clamp can be closed to hold the cantilever holder in place. If you took down this height in Step 4 on page 178, then please use your notes to get as close to this height as you can.
4. Using the 3/32" allen tool, slowly close the jaws. Look very carefully if the jaws are heading under the lip on the ring at the back of the cantilever holder. If the head is too low, and you keep closing the jaws, they can cut through the membrane wall.
5. Once the jaws are properly gripping the cantilever holder, you can lift the AFM head and the fluid cell will not flop around.

14.4.13. Cantilever or Sample Exchange

This is a suggested method for changing the sample or the cantilever without a full disassembly of the clamp:

1. Drain the cell of fluid by drawing it out with the syringe.
2. With the AFM head on its back, use the spanner wrench tool to unscrew the retaining ring which holds in the cell bottom. You will need to hold the edge of the cell body to absorb some of the torque from the tool.
3. Once the ring is off, close off the exhaust tube (this can be done with a second syringe) and pump a little air into the cell using either syringe. The cell bottom should pop loose.
4. Remove the cell bottom and change the sample if necessary. You also have access to the cantilever at this point, but it may be awkward to hold a new cantilever and properly insert it under the clip. Of course it is also possible to remove the clamp from the cell body and gain access to the cantilever that way.
5. When you are done, replace the cell bottom, refill the cell, and repeat imaging as described earlier in this chapter.

14.5. Gas flow through

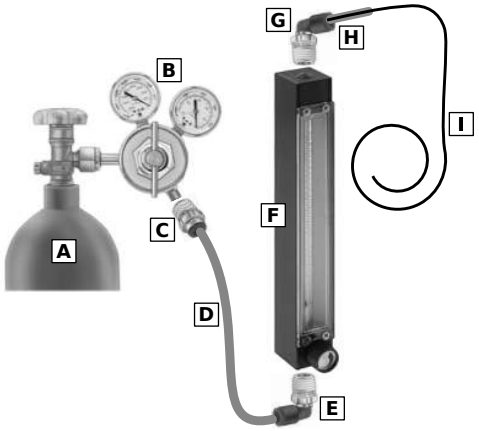


Figure 14.4.: Typical gas flow system. M denotes McMaster Carr part number, A indicates Asylum Research Part Number.

	Part Number	Description
A		Gas Supply.
B		Regulator, depends on gas supply.
C	M 5779K115**	3/8" NPT** X 1/8" push to fit fitting.
D	M 5114K11	1/8" OD 1/16" ID Tygon tubing.
E	M 5779K143	1/8" NPT X 1/8" push to fit fitting.
F	M 4112K431	Rotameter, 0-100 cc/min.
G		Same as E.
H		Small sleeve of tubing D.
I	A 231.006	1/16" OD 1mm ID PFA tubing.

** This part will differ depending on your regulator.

In some cases one wants to flow an inert or dry gas around the sample. We recommend a setup like the one in Figure 14.4 on page 181. For customers in the USA we give some suggested part numbers from the McMaster Carr supply house, but there are many other suppliers who carry similar products. Many institutional buildings may already have a built in inert gas supply. You will want to regulate the supply pressure down to about 5 PSI (~ 1/3 atm). Item C is only a suggestion, its threaded fitting will depend on the style of your gas pressure regulator. If you have trouble finding these parts in your region, please contact support@asylumresearch.com and we can send

you a quote for the parts that will fit your gas supply regulator. Note that the short piece of tubing H makes a seal and simultaneous reduction from the Tygon tubing to the 1/16" OD tubing that fits the fluid cell ports. This could also be done with a more expensive compression fitting like those from Swagelok.

Once the setup is complete, a good gas flow rate to target is 1cc/second, or 60cc/minute. Look at how the gas flow affects your images and find a flow that meets your flow through and imaging needs.

14.5.1. Inert Environment Sample Mounting

Some of our customers have successfully imaged samples under inert gas flow using the following protocol involving a laboratory grade inert gas glovebox:

1. Bring the entire closed fluid cell with clamp, membrane, cantilever holder, and sample into the glovebox via its airlock.
2. Also bring a small vacuum desiccator into the box.
3. Mount the sample and cantilever and fully assemble the cell inside the glovebox.
4. Attach two lengths of tubing to two of the fluid ports. One of the tubes has a three way valve on it (Hamilton or Upchurch).
5. Place everything inside the desiccator and evacuate it.
6. Bring the desiccator out via the glovebox airlock and carry it over to the AFM lab.
7. Prepare the AFM.
8. Purge the desiccator with dry inert gas and open it.
9. Immediately attach an inert gas flow to the three way valve. Purge the valve and turn it to connect the gas flow to the fluid cell. Excess gas runs out of the cell via the exhaust tube.
10. Mount the closed fluid cell to the AFM head. Engage and image as usual.

If this does not meet your inert imaging needs, please contact Asylum Research about placing your AFM inside of an inert gas glovebox.

14.6. Tubing Port Additional Information

The Closed Fluid Cell allows up to 10 tubes, wires, or probes to be attached. Compressed O-rings seal the tubes into place. Four tubing ports can accept tubes of up to 1/16" outer diameter. Please refer to Figure 14.5 on page 183 for the port locations. Note that the larger ports enter the fluid cell body parallel to the sample plane while the small ports enter at a downward angle of 3.5 degrees. Due to this angle, a 20 gauge hypodermic needle will angle underneath the cantilever holder and can be brought very close to the tip of the cantilever.

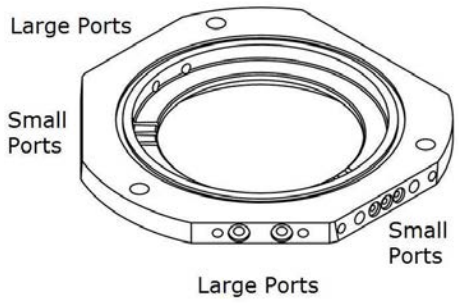


Figure 14.5.: 4 Large Fluid ports accept 1/16" OD tubing and 6 small ports accept 20 gauge hypodermic tubing or needles (0.036" OD).

14.6.1. Recommended Tubing

For the larger tubing ports any tubing with an outer diameter of 1/16" can be used. We particularly recommend Upchurch 1503 PFA tubing (Asylum part number 231.006) since it has a larger inner diameter (0.040") than the typical tubing (0.032"). This larger inner diameter will allow for liquids and gases to flow more easily. Remember that the viscous drag of slow laminar flow in a tube goes up with diameter to the fourth power. The Upchurch 1503 tubing therefore has nearly 2.5 times less drag (for a given length of tubing) for liquids than the typical 1/32" ID tubing. Since the O-ring seals compress around the tubing, somewhat smaller sizes of tubing, such as 1.5mm are also acceptable.

For the small tubing ports a 20 gauge (0.036" OD) syringe needle is a perfect fit. Since the O-ring seals will compress to accept somewhat smaller diameter tubing, 1/32" OD tubing (Upchurch 1569 PEEK) is also an acceptable choice.

If you cannot find appropriate tubing from your local supplier, Asylum Research sells the above mentioned Upchurch products.

14.7. Preventing Leaks

When working with liquid filled pressurized fluid cells (Closed Fluid Cell and Bioheater), liquid leaks are always a possibility if the equipment is not properly prepared or operated. It is important to understand that fluid leaking into the MFP-3D scanner and head can lead to catastrophic failures very quickly. Electrical short circuits in the head can even damage the MFP-3D controller. Anyone who wants to work with a pressurized fluid cell should read this section thoroughly. Please also read Chapter 7 on page 62 to familiarize yourself with the business of working with fluids around the MFP-3D AFM.

14.7.1. Rules of Thumb for Leak Free Operation

First, a few rules of thumb which will help you protect your AFM and keep it in proper working order.

- Keep the membrane clamp (see **??** on page **??**) stored away when not in use. This prevents those not instructed about its proper use from trying to use it and potentially causing harm by untrained users.

- Ask yourself if you truly need to operate a sealed sample cell. For many experiments, the Fluid Cell Lite (described in Chapter 13 on page 145) with the membrane operating as an evaporation barrier does the job.
- Always practice your flow through experiments away from the AFM before doing any real imaging on the AFM. If there are leaks, you will be glad to have spotted them during a practice run.
- Always fully extend the head's legs when placing a head with sealed and filled cell onto the scanner. If the cell hits the scanner first, the full weight of the head will rest on the fluid cell. If the paddles on the membrane clamping assembly (Step 4 on page 178) are released, high fluid pressures and fluid leaks are often the result.
- Always have some tubing connected to at least one of the fluid ports. This tubing must be open on one end so that fluid can freely flow out when pressure builds. Only use the supplied tubing which has an unusually large 1 mm inner diameter to help relieve pressure quickly. Make the tubing as short as possible to facilitate fluid flow. Asylum recommends this tubing be no longer than 2 feet (or 0.5 meters). Use tubing with a large ID. See Section 14.6.1 on page 183 and read why.
- Lower the AFM legs SLOWLY when approaching your first engage. The cantilever holder lowering down into the fluid cell is like a piston driving up pressure inside the cell. Give the fluid a chance to escape through the tubing mentioned above. A sufficiently slow rate is achieved by always keeping your finger or thumb on the front leg wheel while you are turning it. Do not flick the wheel hard enough so that it keeps spinning by itself.
- When performing a flow through experiment, consider PULLING the fluid through with a syringe pump, instead of pushing. If there is a leak, it will draw in air, not force fluid out.
- When pushing fluid through, ALWAYS use a pressure reservoir hanging no higher than 2 meters above the fluid cell. AVOID the use of a positive displacement pump such as a syringe pump to push fluid through the cell, since it can drive pressures to very high levels.

14.7.2. Tips for Leak Free Assembly

When assembling the fluid cell for the first time, or when using new parts or O-rings, it is always advised to test its limits before attaching it to the AFM. **Every new user of the fluid cell should follow these steps at least once**:

Refer to Figure 14.2 on page 170 for the letters in the text below.

- Follow the tutorial on sealed cell assembly (Section 14.4 on page 169) up through Section 14.4.6 on page 176 where the cell is sealed and filled and has two tubes attached. DO NOT put in a sample or cantilever for this exercise in leak free assembly.
- Connect the two pieces of tubing attached to the cell together. The cell is now completely sealed with no exit path for the fluid.
- RELEASE THE PADDLES on the clamp.
- Place the cell on a piece of paper towel and gently press down gently on the cantilever holder. This will drive up the pressure inside the cell. Inspect the cell for leaks.

Figure 14.6.: Maximum pressure test by placing a weight onto a fully sealed closed fluid cell. Note the picture shows the Bioheater variation of the Closed Fluid Cell. Observe that the jaws on the clamp are OPEN to perform this test. Otherwise the clamp would support the weight of the bottle, and not the fluid inside.

- To simulate the maximum rated pressure (about 2 meters of water) fill a 2 liter soda bottle between 3/4 full and completely full with water and screw on its cap. See Figure 14.6 on page 185. Invert the bottle and rest its cap on the cantilever holder. Inspect for leaks.
- The typical causes for leaks are O-rings that:
  - Are not properly seated in their grooves. If this is the case, reseat them.
  - Have slight surface imperfections. Replace suspect item with a spare O-ring.
  - Store your fluid cell with the fluid port clamps a little loose. This allows the small O-rings to relax and make a stronger seal the next time the cell is used. It's also possible to use "relaxed" spare O-rings for your experiments. The compressed O-rings will relax again for later use.
  - Fluid plugs or tubing not inserted all the way through the O-ring into the body of the cell. Simply insert deeper. It helps to cut tubing at a 45 degree angle.

Once you are confident that the fluid cell is leak free, continue to follow the instructions. Remain vigilant. Keep an eye out for leaks and keep and eye on new users of the fluid cell. Some users take the precaution of putting some food wrap over the scanner to prevent small spills from entering the scanner.

Do not hesitate to contact Asylum Research if you are unsure about using your closed fluid cell or bioheater accessory.

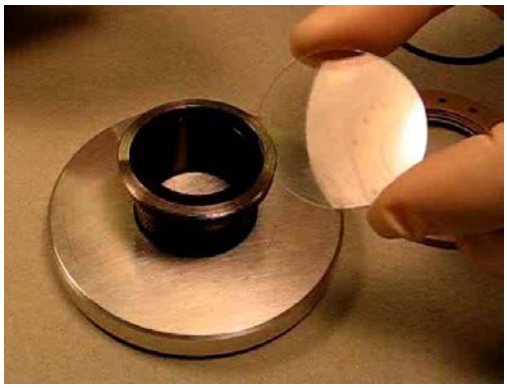
One should always leak test the closed fluid cell when it is used in in a new configuration of tubing inlets and outlets. A few minutes spent on leak testing may save you thousands of dollars in repairs and weeks of AFM downtime.

14.8. Alternate Methods of Cell Bottom Assembly

In some cases the cell bottom must be kept horizontal during assembly, for instance if a thin layer of water must remain on the glass. In this case, follow the steps below.

1. **Join Retaining Ring and Spanner Wrench**
   - Set the spanner wrench upright with the narrow end on the base.
   - Match the indentations in the retaining ring (111.420) to the pins on the spanner wrench.
   - Place the ring on the wrench. They will hold together magnetically.

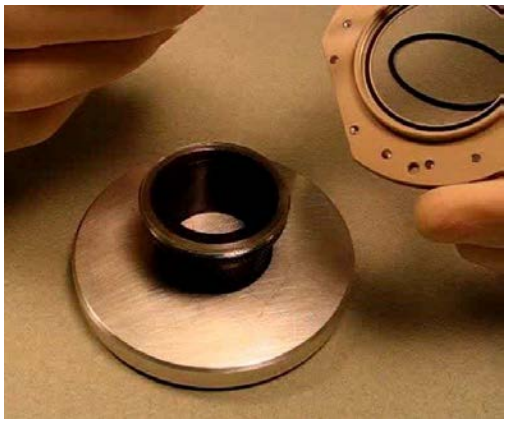
2. **Place the glass**
   - Place the glass sample disc (cell bottom) onto the retaining ring.



3.

Bring parts together

- Fetch the fluid cell body with O-ring inserted.
- Make sure you can hold it upside down without the ring falling out.
- Place the fluid cell body on top of the retaining ring.

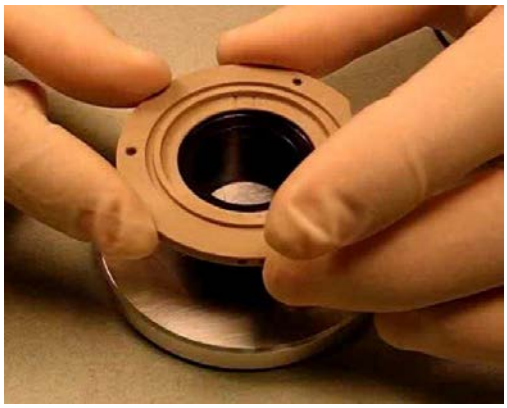


4.

Screw parts together

- First rotate the fluid cell body counter clockwise (loosening) until you can feel the threads click. This means the threads are aligned and it prevents cross threading and damage to the cell body.
- Rotate clockwise until firmly tight.

Note If the bottom is not tightened fully, the cell bottom will be supported by the O-ring and may cause unstable AFM images due to thermal drift. Only when the O-ring is properly compressed will the glass bottom rest on the more stable hard plastic of the cell body.



It is also possible to add the cell bottom at the very last point, just before engaging.

1. With the cell body already completely attached to the AFM head, lay the head on its side. Tubing with a filled syringe should already be attached. A second tube, located near the top of the cell, must be attached to allow displaced air or fluid to exit.
2. Remove the cell bottom using the spanner wrench.
3. If necessary, replace the cell bottom O-ring.
4. Quickly insert your glass bottom disc with sample.
5. Tighten the retaining ring.
6. Inject fluid into the cell and watch the liquid level rise against the window.
7. Once a little fluid comes out of the other tube, you are done and ready to place the head onto the AFM and start the engage process.

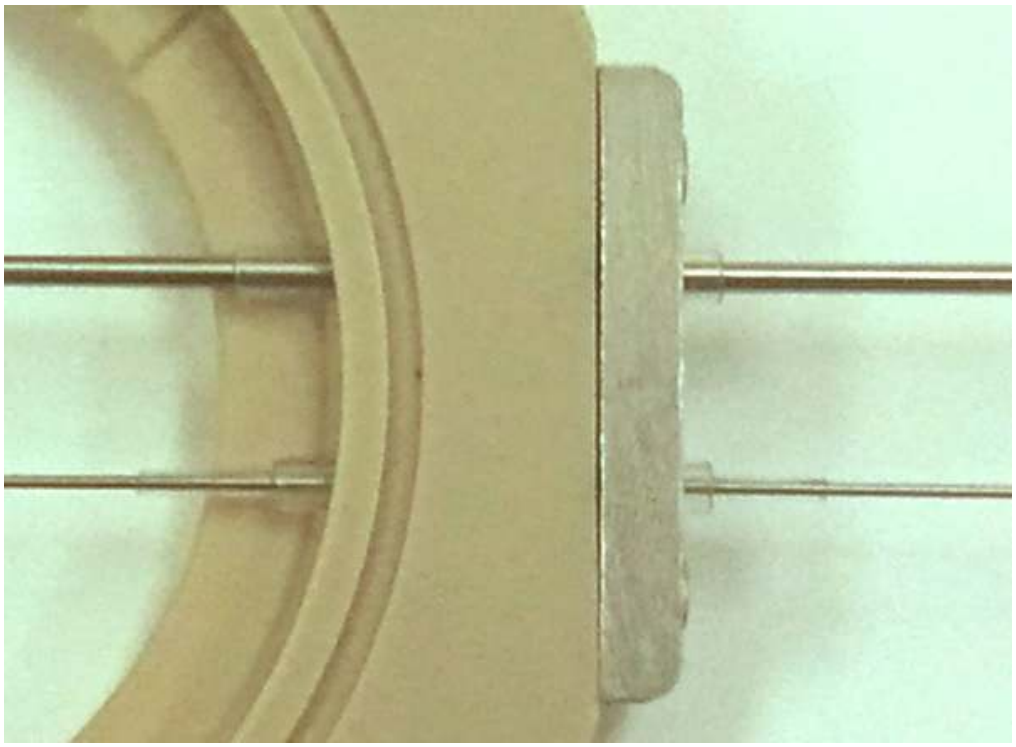


Figure 14.7.: Top: A piece o 230.006 tubing sleeved around a 1.0mm diameter wire. Bottom, a piece of 231.019 tubing sleeved around a piece of 231.028 tubing sleeved around a 0.4mm diameter wire.

14.9. Wire Feedthroughs

In some cases it is desirable to insert wires into the cell, and to keep those wires insulated from the metal parts of the closed fluid cell, and to ensure a liquid or gas tight seal is maintained. This is easily achieved by sleeving an appropriately snug fitting piece of tubing around the wire, and fitting that tubing through one of the CFC's fluid ports. For example, a short section of the 231.006 tubing included with your kit can be sleeved around a wire with a diameter ranging from 1.0 to 0.8mm. For very thin wires it is sometimes easier to use a double tubing sleeve. See Figure 14.7 on page 188 for an example photo.

Asylum Research stocks a few types of tubing as outlined in the chart below. Typically one picks the tubing that closely fits the wire. Then insert it into the fluid port. The wire should move back and forth freely. After tightening the fluid port clamp (see Section 14.4.1 on page 169), the wire should become stuck, and only move when pulled on with considerable force. Generally if the wire is stuck, a good seal has formed. To be absolute certain, perform a leak test a described in Section 14.7 on page 183.

14.10. Cleaning and Care

Refer to the parts list (Section 15.3 on page 197) for information about the materials of which the fluid cell is made. All of the parts used during imaging can be cleaned with solvents such as alcohol. Parts can also be autoclaved.

Tubing Asylum Part Number	Tubing OD	Tubing ID	Wire Range
231.006*	1/16” (1.6 mm)	0.040” (1.0 mm)	0.040” - 0.032” (1.0 - 0.8 mm)
231.019	1/16” (1.6 mm)	0.032” (0.8 mm)	0.031” - 0.024” (0.8 - 0.6 mm)
231.012	1/16” (1.6 mm)	0.019” (0.48 mm)	0.019” - 0.018” (0.48 - 0.45 mm)
231.028	1/32” (0.8 mm)	0.016” (0.41 mm)	0.016” - 0.013” (0.41 - 0.33 mm)

Table 14.2.: Some PFA and FEP tubing varieties stocked by Asylum Research and the wire diameter that can be successfully sealed using the closed fluid cell. Asylum also stocks 1/32” tubings for smaller wires down to 0.1mm. * Included in your Closed Fluid Cell kit.

Avoid the use of acetone. The rubber parts will not do well when exposed to acetone.

Please store your fluid cell in its designated case. If you own multiple accessories that are similar to this one, it is best to keep all the parts where they belong and not get things mixed up.

If you need replacement parts, contact your local office or distributor and use the parts list in this chapter as a guide.

14.11. Storage

After cleaning, store your fluid cell and accessories in the supplied kit box.

Loosen the fluid port clamps a little so the small O-rings can relax back to their original shape. This will promote tighter fluid port seals the next time the cell is used in a sealed configuration.

14.12. Thin Glass Fluid Cell Bottoms

Only when you bottom view optics require a thin 0.17mm glass support should you proceed to the sections below. Otherwise, please use the standard 1mm thick glass bottom, described in detail in Section 13.4.1.1 on page 149.

The CFC offers 12, 25, and 35mm coverslip bottoms. The following table describes the pros and cons of each. Further sections described how to use the different types.

Size (mm)	Liquid Seal Integrity	Imaging Performance	Comments
35	Excellent (O-ring)	Good??	For beginning users. Only available from Asylum Research. Too large for some experiments.
25	Good (grease and clamped)	Good	Good all around choice for the experienced user. Readily Available.
12	Tolerable (grease adhesion only)	Best??	Experienced Users only. Only choose this unless the other options will not suffice. Most prone to leaking.

14.12.1. Installing 35mm Coverslips

Warning	35mm coverslips will not seal unless you back them up with spacer 111.925. Failure to do so will lead to massive leaking of the cell and possible damage to your instrument.

Asylum stocks a 35mm diameter coverslip. This can be used in the same way as the regular 35mm x 1mm thick glass bottom (see Section 13.4.1.1 on page 149), but it must be backed by spacer 111.925 (See Section 14.12.3 on page 194). The spacer serves two purposes:

- It builds up the thickness of the 35mm coverslip to roughly 1mm which allows the retaining ring at the bottom of the fluid cell to properly compress the bottom O-ring.
- It provides some rigid support to the otherwise flimsy coverslip which can lead to very noisy AFM images and cantilever engage problems. Adding a little vacuum grease between the support and the "dry" side of the coverslip will further improve AFM performance.

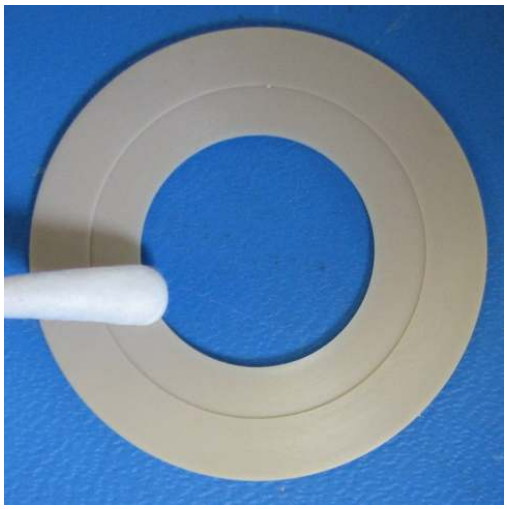
14.12.2. Inserting 25mm cover slips

When using the MFP-3D AFM on an inverted optical microscope, a glass bottom thinner than 1mm is required when performing concurrent AFM and optical microscopy with high magnification objectives. Your kit includes parts which can clamp a standard 25mm circular #1 cover slip (0.13 - 0.16mm thick). Instructions on their use follows:

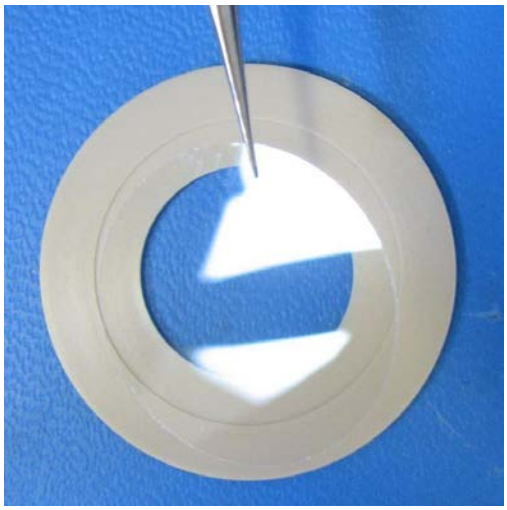
1. Locate the 25mm cover slip top (112.789, Step 4 on page 190) and bottom (112.790, Step 6 on page 191) clamp parts.
2. Depending on your kit, you may have to supply your own cover slips, or you may find a few (504.002) to get started with. Cover slips are available from any scientific supplier. Asylum Research can supply them, but your price will be much better from an outside vendor.
3. Locate some silicone grease. We recommend Dow Corning High Vacuum Grease[1] as we have found that it does not inhibit live cell growth in biological samples. The grease is not included in our kits.

[1] For many years this product was designated as Dow Corning 976 or 976V High Vacuum Grease but the name has now been streamlined to just "Dow Corning High Vacuum Grease". You can be sure although there has been a name simplification, the product formulation itself has remained unchanged. Any specifications calling for Dow Corning 976 or Dow Corning 976V can be satisfied with Dow Corning High Vacuum Grease.

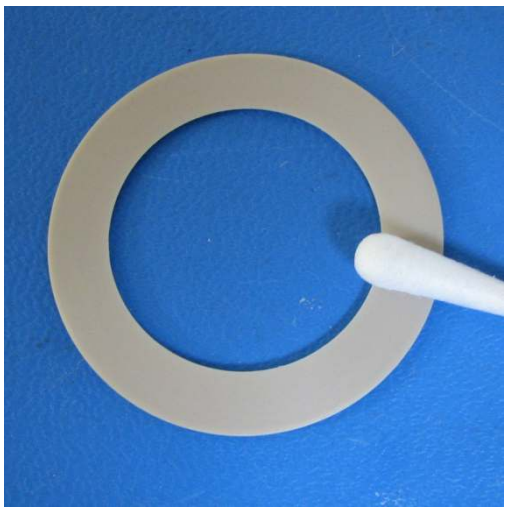
4. **Apply grease:**
   - Apply a thin layer of grease to the bottom piece (112.790).



5. **Place glass:**
   - Place the cover slip (504.002) onto the greased part.



6. **Grease second part:**
   - Grease the smooth side of the top part (112.789).



7. **Stack parts:**
   - Place the top onto the bottom and press the two pieces together.

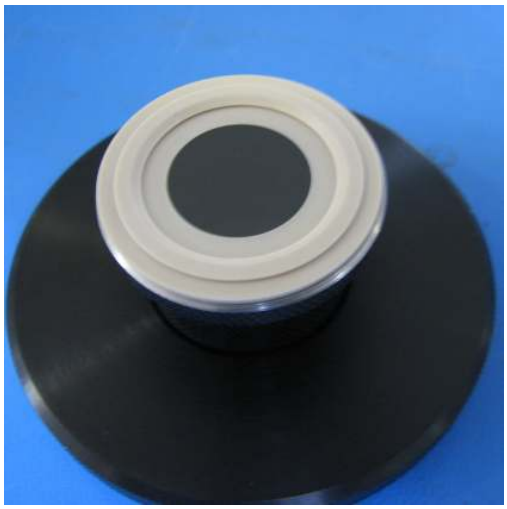
At this point one can follow the steps for inserting the glass bottom into the fluid cell as described in Section 13.4.1.1 on page 149, only using the mounted cover slip in the place of the standard 35mm cover slip. You may also continue with the following directions which may be of use when the cover slip must be held level.

1. **Locate parts:**
   - Spanner wrench (939.008)
   - Assembly base (111.903)
   - Bottom clamp retaining ring (111.420)
   - Body of your fluid cell (will vary depending on your model).

2. **Prepare retaining ring:**
   - Place the spanner wrench on the base.
   - Place the ring on top of the spanner.

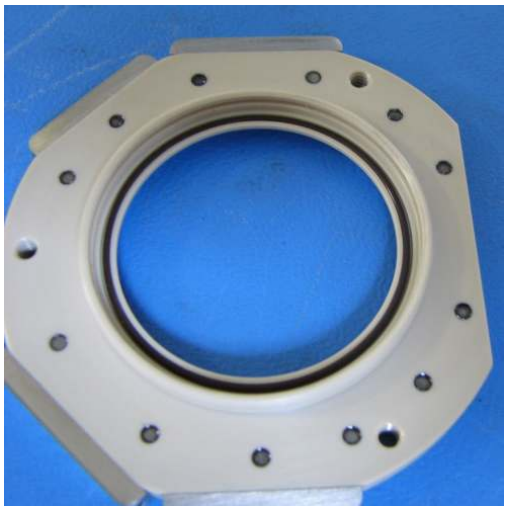
3. **Place the glass assembly**

   - Place the plastic and glass sandwich created from Step 7 on page 191 onto the retaining ring.



4. **Locate cell body:**

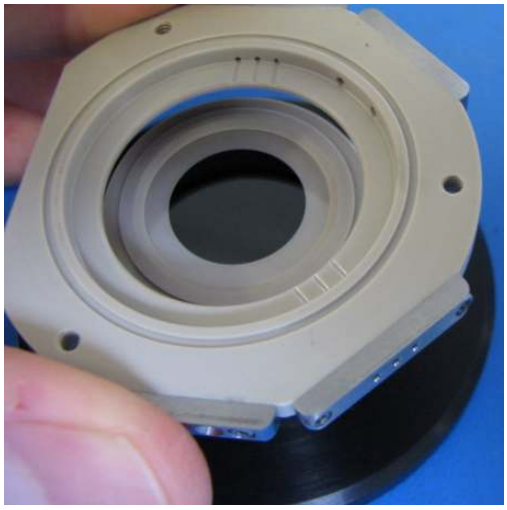
   - Locate your fluid cell body with O-ring in place.
   - On placing the O-ring, see Step 3 on page 150.



5. **Thread parts together:**

   - Place the fluid cell body on top of the stack prepared in the previous steps.
   - Rotate the cell body clockwise and thread the cell body onto the retaining ring.

   Note To prevent cross threading and damage to the fluid cell body, please see Section 13.4.1.1 on page 149.



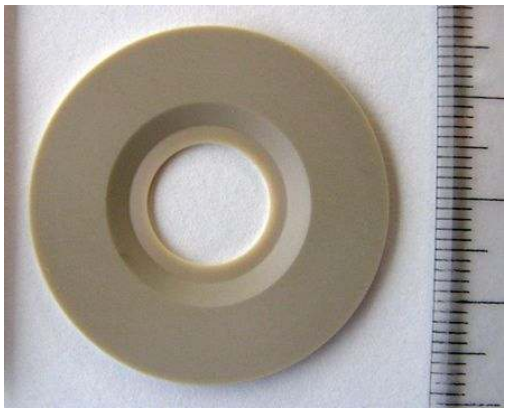
Warning	Since the cover slips are only sealed with grease and not with compressed O-rings, the fluid cell with cover slip adapter is not suitable for sealed use under pressure. If you have the need for fluid flow through when using a cover slip adapter you may do so at your own risk. We recommend you use a slightly elevated vessel as a fluid source and that you always test your experiment away from the AFM. Only proceed to the AFM when you are confident that there will be no leaks.

14.12.3. Installing 12mm cover slips

Warning	**Note:** Not Recommended for completely sealed cell operation. Since the 12mm coverslip is not clamped between two supports (like the 25mm version) this is the least secure of the coverslip mounting options. Be especially careful if your cell is completely sealed. A sudden raising of the AFM head may create enough suction that the slip may be pulled loosed from its support and the cell's fluid contents will be dumped onto the optical microscope below. If you must operate the cell fully sealed, consider the 35mm coverslip option (see Section 14.12.1 on page 190).

This is a very similar process to the previous section except that you will use 12mm coverslips (504.004, but also available from many microscopy suppliers) and a single backing support (111.925).

1. **Locate parts**
   - Locate the support (111.925) and turn it **upside down** as shown in the image shown to the right.
   - You should see a small groove which will accept the coverslip.
   - Locate the 12mm coverslip (504.004).



2. Apply vacuum grease sparingly to this groove.
3. Place the 12mm coverslip onto the grease layer (sample side facing up) and press down gently around the edges so the grease wets every part of the glass and forms a seal around the entire perimeter.
4. Continue as in Step 1 on page 192.

Be sure to always have at least one fluid port connected to an open tube if using this adapter with a fully sealed cell. Be very careful when raising the head, since this will create some suction which may dislodge the glass and cause fluid to spill on your optical microscope. Raise the head VERY slowly.

14.13. Electrical Connections to the Sample

When performing conductive AFM (See Chapter 21 on page 279) experiments that require an inert gas atmosphere, the Closed Fluid Cell can be used. Note the Electrical Closed Cell described in Chapter 15 on page 196 gives more flexibility than the closed fluid cell, but if you already own a closed fluid cell and don't need to so such experiments frequently, then we suggest the Environmental Cell Electrical Connectivity Kit . This kit has everything required to make electrical connections to the sample.

14.13.1. Electrical Connection Tutorial

This tutorial sets up a sample in the closed fluid cell, ready for ORCA conductive AFM imaging.

1. Please refer to Table 21.2 on page 290 and select the necessary parts from the kit.
2. Prepare the closed fluid cell with the 1mm thick glass bottom, as shown in Section 14.12.3 on page 194.
3. Quite likely, inert gas purge lines will be attached, as described in 14.5.
4. Take a magnet from the kit (208.020) and, using a sticky dot (249.033) attach it to the outside of the glass cell bottom. Place the magnet in the center of the glass.
5. Place your sample on an AFM disc with a socket (939.031) using the silver paint from your ORCA kit (290.160). Usually only a tiny bit of paint at the corners of the sample is required. Let the paint dry for a while.
6. Insert the bias wire into one of the unused fluid ports.
7. Place the mounted sample in the center of the cell. The magnet on the outside of the cell will keep the sample in place.
8. Plug the wire end from the bias wire into the socket on the sample.
9. At this point the process is just as if you were using the Electrical Closed Cell. Please continue at Step 7 on page 202.

If you only want to apply a sample bias or ground from some external piece of equipment, simply use one of the Jumper Wires. See Section 15.5 on page 203.

15. Electrical Closed Cell

CHAPTER REV. 1658, DATED 10/07/2013, 20:47. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

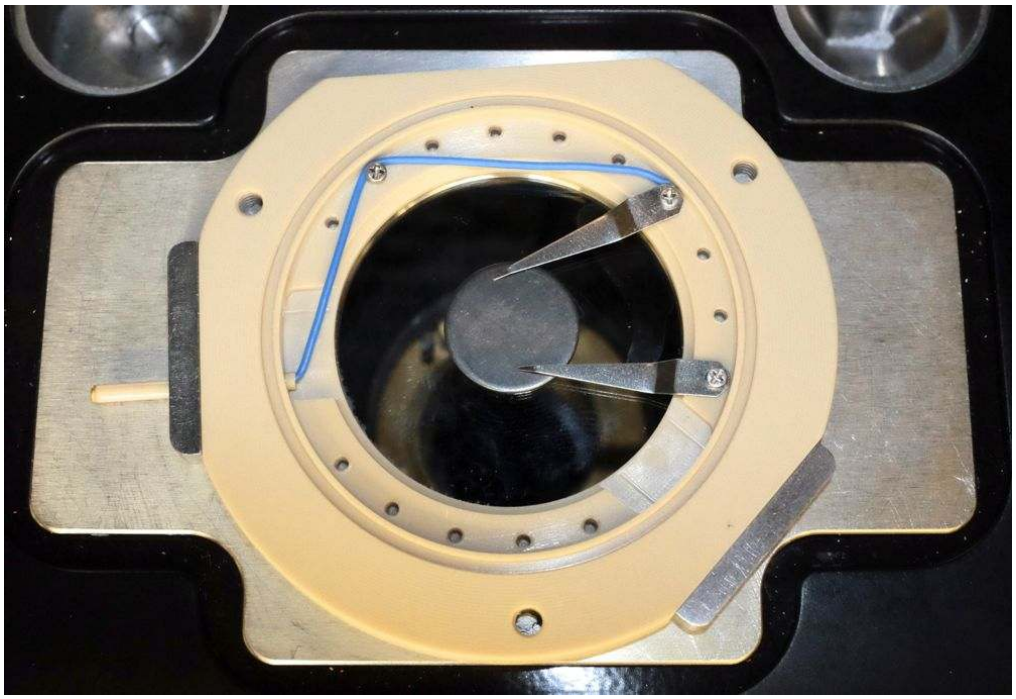


Figure 15.1.: The electrical closed cell in a typical configuration.

15.1. Prerequisites

It's assumed that you are familiar with:

- General operation of the AFM, covered in Chapter 4 on page 15.
- Working with fluids around the AFM, covered in Chapter 7 on page 62.

Warning Even small fluid spills around an AFM can lead to costly repairs. Educate yourself on how to work safely with fluids. Read Chapter 7 on page 62.

15.2. When and when not to use

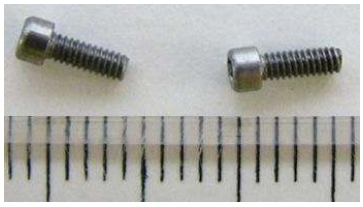
This version of the Closed Fluid Cell is intended to be used with inert gases. The sample is placed at the bottom of the cell and held in place with metal clips. These clips can be attached to wires which lead to the outside of the cell, typically to the ORCA cantilever holder (See Chapter 21 on page 279), used for conductive AFM experiments.

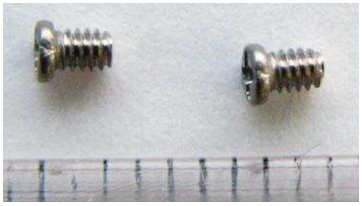
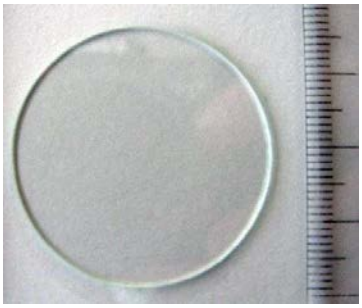
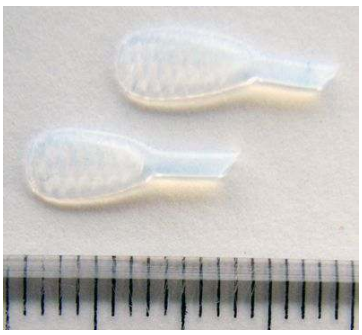
While this cell can be used with liquids, it was not the intended purpose, primarily since electrical methods in liquids are not commonplace. For liquid use we recommend the Closed Fluid Cell (See Chapter 15 on page 196) or the Fluid Cell Lite (See 13).

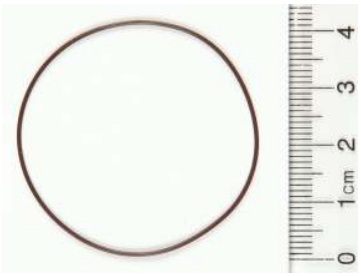
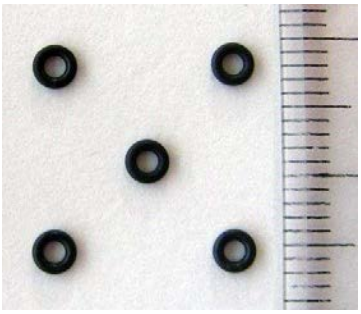
15.3. Parts List

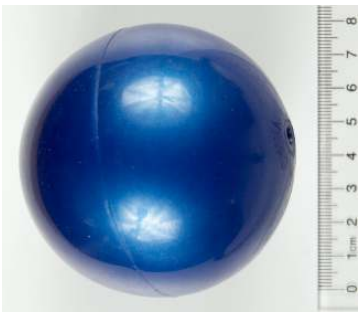
The Asylum Part number for the entire kit with cell is 900.264.

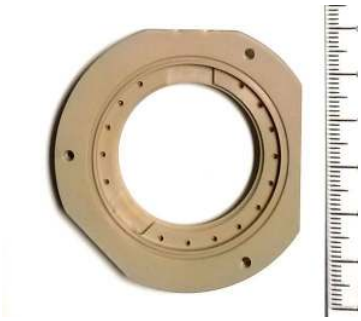
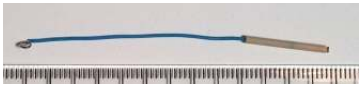
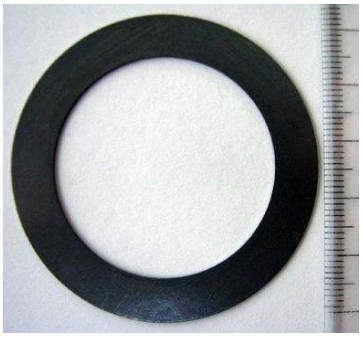
The following lists all the parts in your accessory kit. The table is useful as a visual table of contents with links directing you to the specific uses of each part. When ordering parts, please refer to the part numbers in the second column.

Itm	Part #	Item Description	Qty	Picture
1	001.SHCS <#0-80X.188> SST	0-80 X 3/16” Stainless Steel Socket Head Cap Screw. Connects 111.886 and 102.204 O-ring clamps to the fluid cell body. See Section 14.4.2 on page 172.	8+4*	
2	112.240	Mini Sample Holder Clip. Used for holding down samples. Stainless steel. Attach with screws 222.021. See Step 3 on page 202.	6	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
3	222.021	Screw, Pan Head Phillips, M1.4 X 2. 2mm long Stainless steel metric miniature screw for holding down clips 111.240. See Step 3 on page 202.	12	
4	111.425	35mm X 1mm glass disc. Custom made by Asylum Research out of Glaverbel float glass. See Section 13.4.1.1 on page 149.	5+1*	
5	111.924	1/16” OD Port Plug. PTFE (generic Teflon) plugs for blocking unused in/outlets on the closed fluid cell. Also consider using PTFE cord 0.062" diam. (McMaster Carr Part Number 84935K48.) See Section 14.4.1 on page 169.	10	
6	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153 .	2	
7	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
8	230.012	O-ring, 1.595”ID X 0.030”CS, Viton, 55 Durometer. This FKM O-ring makes the seal between the bellows membranes and the sealed cell body. See Section 14.4.5 on page 173.	5	
9	230.015	O-ring, 1.228”ID X 0.032”CS, Viton, 75 Durometer. This FKM O-ring makes the seal between the sample disc 111.425 and the fluid cell body. See Section 13.4.1.1 on page 149.	5+1*	
10	230.018	O-ring, 0.062”ID X 0.032”CS, Viton, 70 Durometer. FKM O-rings that seal around the four 1/16" fluid ports. See Section 14.4.1 on page 169.	15+4*	
11	231.006	Tubing, PFA, 1/16”OD X .040”ID. This PFA tubing makes it possible to introduce and remove gas from the closed cell. Order from Asylum or purchase directly from Upchurch Scientific (Part #1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	5 ft	
12	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the closed cell inlets. See Section 14.4.3 on page 172.	2	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
13	290.103	3C Tweezer – Extra Fine Sharp – Standard Grade. For placing samples, tiny o-rings (e.g. 230.018) , and small screws.	1	
14	290.111	0.050": Wiha Allen Driver 263 1,3 – 0.05" X 40. For all socket head screws. Used, for example, for sealing fluid ports. See Section 14.4.1 on page 169.	1	
15	290.106	#00 Phillips WIHA Screwdriver 261 PH 00X40. For small Phillips screws 222.021. See Step 3 on page 202.	1	
16	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
17	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
18	939.044	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173.	1	
19	112.204	Fluid Port Clamp Bar. Stainless Steel. Compresses and seals two mini O-rings (230.018). Fastens with 0-80 X 3/16" screws. Part of 1/16" fluid ports. See Section 14.4.2 on page 172.	2*	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
20	928.010	Closed Fluid Cell Body with Clips. Made of PEEK plastic.	1*	
21	939.049	Electrical Closed Cell Jumper Wire. See Section 15.5 on page 203.	4	
22	448.141	Electrical Closed Cell Bias Wire. See Step 3 on page 202.	4	
23	111.420	Closed Cell Bottom Clamp. The stainless steel retaining ring which holds the glass bottom or the cover slip holders against the closed fluid cell. See Section 13.4.1.1 on page 149 and following sections through page 190.	1*	
24	207.004	Steel Shim, 0.020” Thick x 1-½” ID x 2-1/8” OD. Used to moderately seal the cell. See Section 19.7.2.3 on page 254.	2	
The scale in the photos is in cm and mm.				

* These items will already be assembled onto the cell in your kit.

15.4. Tutorial: Electrical Closed Cell and ORCA (Conductive AFM)

1. The glass bottom of the cell assembles as described in Section 13.4.1.1 on page 149.
2. For ORCA, conductive AFM, only one electrical feedthrough is needed. Please locate one Electrical Closed Cell Bias Wire (448.141) and three port plugs (11.924). If you need to make more electrical connections, the kit contains more bias wires.

3.

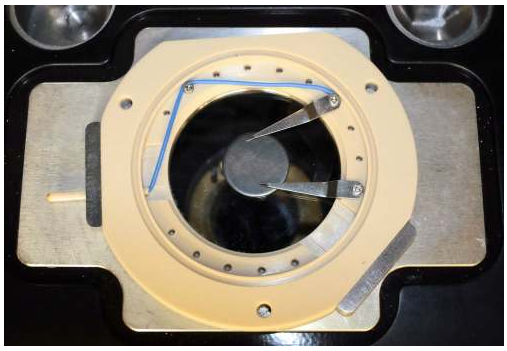
Attach clips and wire:

1

- Using screws 222.021, attach one or more clips (111.240) in the positions shown.
- Once clip should have wire 448.141 fastened under it.

2

- Feed the peek tube through the side port as shown. It's usually easiest to completely remove the clamp and O-ring first, then put the tube through the hole, then put the O-ring on the tube, and then replace the clamp.
- Be sure to put a plug or tubing into the other port at the same time.



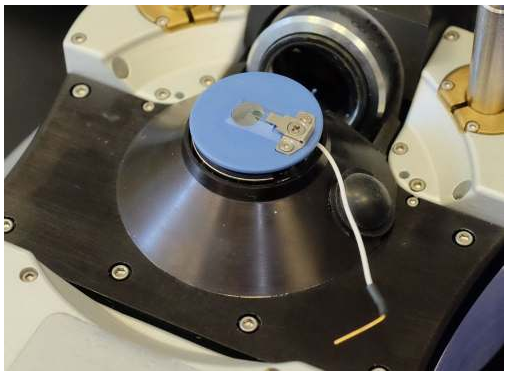
3

- Use a screw to keep the wire off to the side as shown.

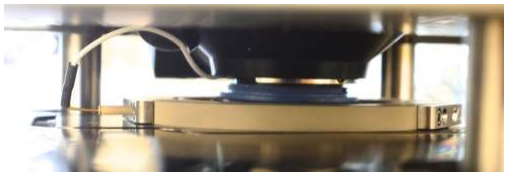
WARNING: This screw should not be tightened down to crush the wire. Only thread it a few turns so it acts like a pin to route the wire only.

4. Once the clips are in place, you can pivot them aside a bit and place your sample under the clips. Lift the clips with tweezers.
5. It may be necessary to bend the clips down a bit to give them enough spring force to hold down your sample. This may require the removal and replacement of the glass cell bottom. See Section 13.4.1.1 on page 149.
6. Attach any other gas inlet and outlet tubes and seal any remaining ports.

7. **Prepare the ORCA cantilever holder:**
   - Prepare the ORCA cantilever holder as described in Section 21.3.4 on page 285.
   - Tough not strictly necessary, it's useful to bend the wire end as shown in the photo.



8. Since it is likely you will be operating the cell in a fully sealed configuration, attach the membrane to the cantilever holder as shown in Section 13.4.3 on page 153. Still, before operating in a sealed configuration, we recommend you try to engage on a test sample with the cell open. Make sure that the image looks good and that no clips are rubbing against the cantilever holder. Read ahead a few steps and familiarize yourself with the process.
9. Mount a conductive cantilever and seal the cell using the membrane clamp, as shown in 14.4.5.
10. Then mount the combined cell and cantilever holder to the AFM head as shown in Section 14.4.7 on page 177.
11. Before placing the head on the AFM, plug the wire coming from the cantilever holder into the socket protruding from the fluid cell port.
12. **Place the head:**
    - Follow instructions in 14.4.8 to place the head onto the scanner.
    - Photo on the right shows the final result, with the membrane and clamp left off, to remove clutter in the photo.



13. See the chapter on conductive AFM for advice on imaging: Chapter 21 on page 279.

15.5. External Sample Bias

To attach an external sample bias source, use the supplied 939.049 wires. Clip or solder the wire of your choice to one end and plug the other end into the bias wire 448.141,

15.6. Inert Gas Purge

See Section 14.5 on page 181.

15.7. Semi sealed operation

When purging with inert gas, it's not always necessary to have the cell sealed with a clamp. The kit includes a metal ring which allows for a simply magnetic seal. This technique is descried in Section 19.7.2.3 on page 254.

16. The Environmental Controller

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

16.1. Overview

Figure 16.1.: The Environmental Controller. Older models are black, newer ones silver gray. The function and performance is the same.

The Environmental Controller is a low noise bidirectional power supply which "speaks" the smart-start protocol with which the various components of the Asylum Research MFP-3D AFM communicate with each other. The Controller has a built in microprocessor and memory so it can perform temperature control and data acquisition tasks autonomously.

16.2. Parts List

Itm	Part #	Item Description	Qty	Picture
1	900.054	Environmental Controller	1	
1	448.023	DB25 cable. Modified with interrupted outer shield.	1	
1	449.002	AC Power Cord, USA style.	1	
The scale in the photos is in cm and mm.				

16.3. Placement

Under large current loads, (sample stages set to very high or low temperatures) the AC transformer might vibrate slightly. To prevent those vibrations from affecting AFM images, it is wise not to place the environmental controller on top of the AFM's acoustic enclosure. Placing it on top of the AFM controller is a good alternative (See Figure 16.2 on page 207).

16.4. Connection

The controller connects to the MFP-3D or ARC2 AFM controllers via a DB25 cable. It also receives AC power for the occasionally large current demand of some of the heating and cooling accessories. Various heated and cooled sample stages can plug into the Environmental Controller. These accessories have some "intelligence" and the AFM system will automatically identify which items are plugged in.

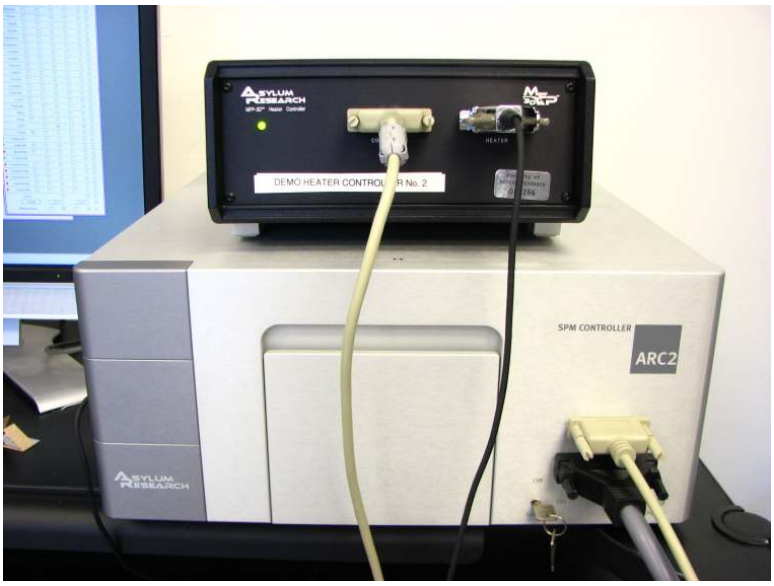


Figure 16.2.: Environmental Controller connected to an ARC2 AFM Controller with a DB25 cable with interrupted shield (see gray sleeve close to one end). A temperature stage is plugged into the Environmental Controller.

16.5. Daisy Chaining

When multiple controllers are used (such as a combination of the Environmental Controller and the Coolant Pump for the Cooler heater, the controllers can be "daisy chained" together with DB 25 cables. Note that the Heater Controller should preferably be the last in the chain, and it should preferably be connected with an Asylum Research DB25 cable with a clamp on the cable. Under this clamp the shield around the cable has been cut to prevent ground loops between the ARC2 controller and the Environmental Controller. See Figure 16.3 on page 208.

16.6. Swapping Temperature Stages

While it is generally not harmful to plug and unplug temperature stages when the Environmental Controller is powered up, it is still wise to fully power the unit down first.

1. Turn off the AC power switch.
2. Unplug the DB25 cable connecting it to the AFM controller (or turn off the AFM controller). Since the unit's circuits are powered by DC power from the AFM controller, unplugging the DB25 cable is important to fully power down the unit.

16.7. Maintenance

The unit is generally maintenance free.

A single AC fuse can be replaced (see the Environmental Controller's back panel for the fuse requirements in your country) when the unit is completely unplugged.

(a) 448.023 cable, DB25, modified with interrupted outer shield.

(b) 449.011 DB25 cable, shield in tact.

Figure 16.3.: Two styles of cables.

The AC input voltage can be selected between 120 and 220 Volts. Unless you are transporting it between countries, this setting will already have been made at the factory. Switching voltages also requires a change in fuses. Running the unit on the wrong voltage setting will damage it.

17. Bioheater

CHAPTER REV. 1710, DATED 10/23/2013, 21:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

17.1. Overview

The Bioheater is exactly a closed fluid cell with an added heating element and temperature sensor. The heater is only to be used for fluid immersion and over a relatively modest temperature range.

This accessory requires the environmental controller (Chapter 16 on page 205).

17.1.1. Is this the Right Heater for your Experiments?

- The bioheater was specifically designed for experiments that require fully sealed operation with bottom view access. If you do not require sealing, consider the alternative Petri Dish heater discussed in Chapter 23 on page 301. It's generally easier to use and maintain than the Bioheater.
- If no bottom view is required, a larger temperature range can be reached using the Cooler Heater discussed in 20.

Both of these alternatives can be combined with fluid exchange when using the MicroFlow cantilever holder described in Section 11.6 on page 116.

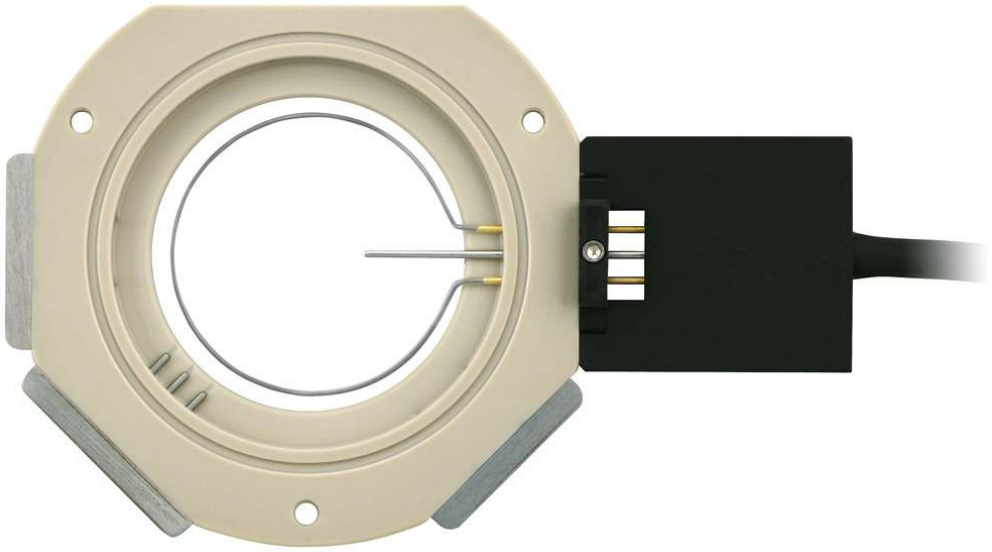


Figure 17.1.: Top View of assembled bioheater.

17.2. Prerequisites

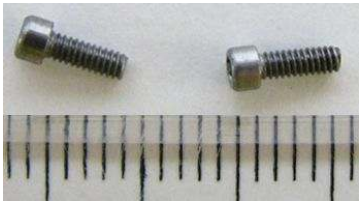
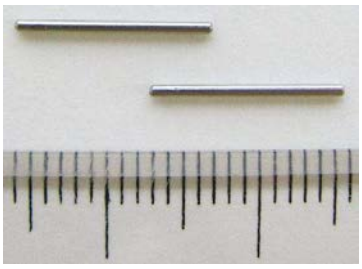
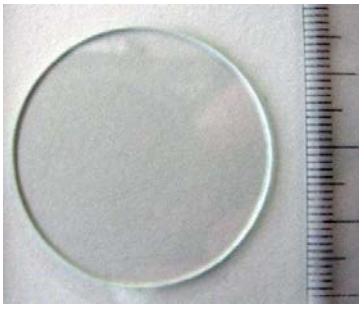
This accessory is a variant of the closed fluid cell discussed in Chapter 14 on page 160. You must be completely familiar with the closed fluid cell before you embark on this chapter. Here we will only discuss specific issues relating to fluid heating.

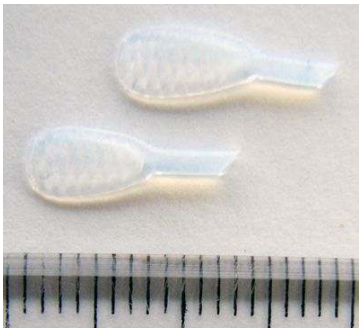
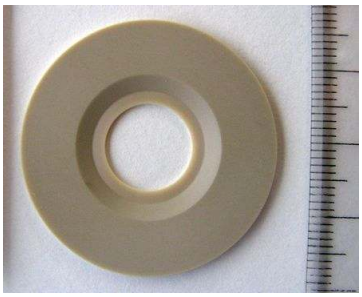
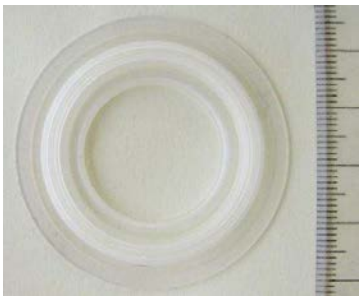
It's ALSO assumed that you are familiar with:

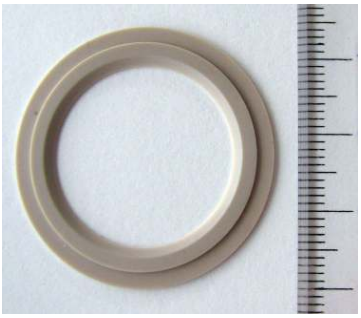
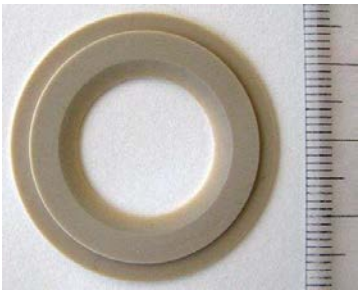
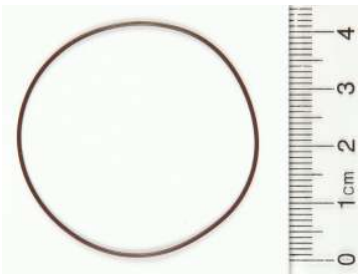
- General operation of the AFM, covered in Chapter 4 on page 15.
- Fluid Imaging in a droplet, covered in Chapter 8 on page 64.
- Working with fluids around the AFM, covered in 7.

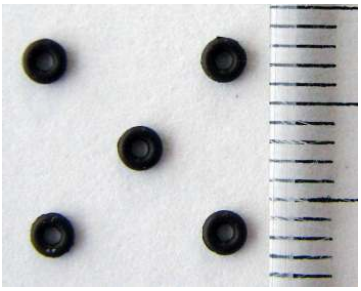
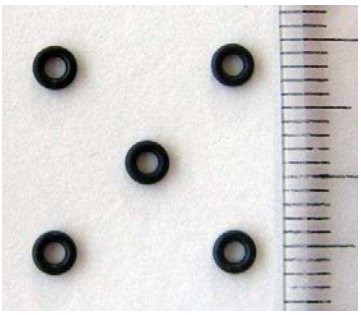
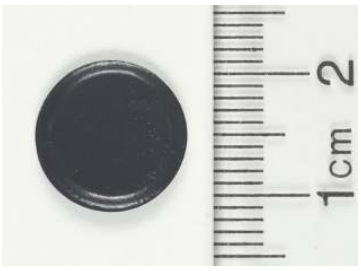
Warning	Even small fluid spills around an AFM can lead to costly repairs. Educate yourself on how to work safely with fluids. Read Chapter 7 on page 62.

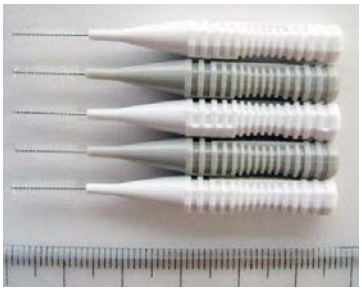
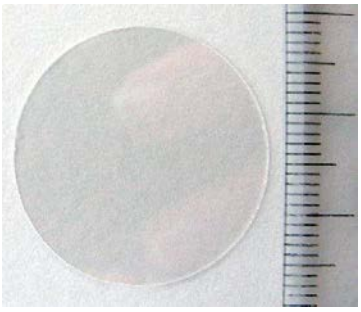
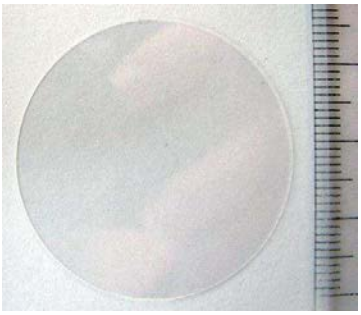
17.3. Parts List

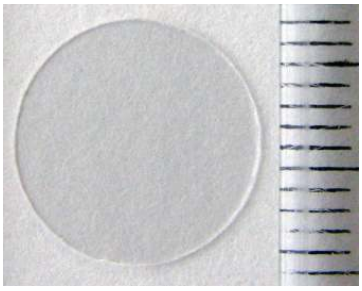
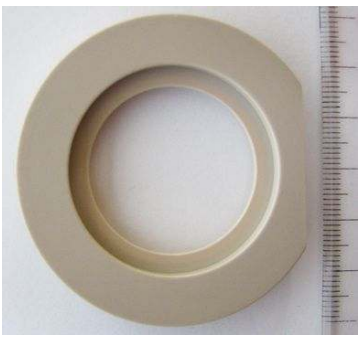
Itm	Part #	Item Description	Qty	Picture
1	001.SHCS <#0-80X.188> SST	0-80 X 3/16” Stainless Steel Socket Head Cap Screw. Connects 111.886 and 102.204 O-ring clamps to the fluid cell body. See Section 14.4.2 on page 172.	8	
2	080.010	5 ml Syringe. We prefer this Norm-Ject model from Henke Sass Wolf (HSW) since it does not contain any rubber and contaminates fluids minimally. See Section 14.4.3 on page 172 on how to attach tubing.	2	
3	005.DOWL <.031X.500> SST	1/32” diameter X ½” 18-8 stainless steel dowel pin. Used with Super Mini O-ring Triple Clamp part (112.430) to plug fluid cell small diameter sealed feed- through ports. See Section 14.4.1 on page 169. Also consider using 0.035" diameter PTFE cord from McMaster Carr Part # 84935K36.	12	
4	111.425	35mm X 1mm glass disc. Custom made by Asylum, and is made out of Glaverbel float glass. See Section 13.4.1.1 on page 149.	5	
5	111.903	CFC Bio Assembly Base. Used to hold the spanner wrench 939.008 during various closed cell assembly steps. (*e.g.* Step 1 on page 192)	1	
The scale in the photos is in cm and mm.				

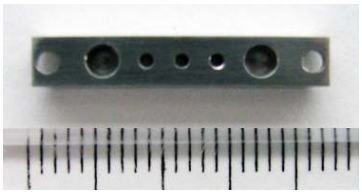
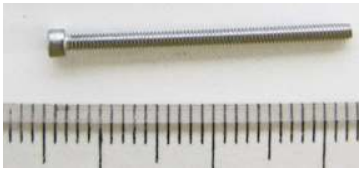
Itm	Part #	Item Description	Qty	Picture
6	111.924	1/16” OD Port Plug. PTFE plugs for blocking unused in/outlets on the closed fluid cell. Also consider using PTFE cord 0.062" diam. (McMaster Carr Part Number 84935K48.) See Section 14.4.1 on page 169.	10	
7	111.925	CFC 12mm Cover Slip Holder. This PEEK plastic holder makes it possible to mount 12 mm diameter coverslips in the closed fluid cell. Please see Section 14.12.3 on page 194.	1	
8	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153.	2	
9	112.256.02	Closed Cell Bellows, 30 Durometer Silicone rubber. See Section 13.4.3 on page 153.	2	
10	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
The scale in the photos is in cm and mm.				

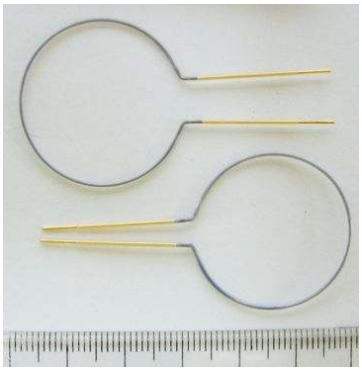
Itm	Part #	Item Description	Qty	Picture
11	112.789	Clamp, 25mm cover slip top. Used with 112.790 to sandwich a 25mm glass cover slip and then inserts as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
12	112.790	Clamp, 25mm covers slip bottom. Used with 112.789 to sandwich a 25mm glass cover slip and then inserts as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
13	230.011	O-ring, 0.244”ID X 0.016”CS, Viton, 55 Durometer. Seals the cantilever holder quartz prism. See Step 7 on page 104.	3	
14	230.012	O-ring, 1.595”ID X 0.030”CS, Viton, 55 Durometer. This FKM O-ring makes the seal between the bellows membranes and the sealed cell body. See Section 14.4.5 on page 173.	5	
15	230.015	O-ring, 1.228”ID X 0.032”CS, Viton, 75 Durometer. This FKM O-ring makes the seal between the sample disc 111.425 and the fluid cell body. See Section 13.4.1.1 on page 149.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
16	230.016	O-ring, 0.031”ID X 0.028”CS, Viton, 75 Durometer. FKM O-rings that seal around the six 0.036" fluid ports. In the Fluid cell. See Section 14.4.2 on page 172.	15	
17	230.018	O-ring, 0.062”ID X 0.032”CS, Viton, 70 Durometer. FKM O-rings that seal around the four 1/16" fluid ports. See Section 14.4.1 on page 169.	15	
18	231.006	Tubing, PFA, 1/16”OD X .040”ID. This PFA tubing makes it possible to introduce and remove fluid or gas from the closed fluid cell. Order from Asylum or purchase directly from Upchurch Scientific (p/n 1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	5 ft	
19	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
20	270.020	0.500” Diameter X 0.060” thick Rubber Bumper. Goes under part 111.903.	3	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
21	290.103	3C Tweezer – Extra Fine Sharp – Standard Grade. For placing samples, tiny o-rings (e.g. 230.018) , and small screws.	1	
22	290.111	0.050": Wiha Allen Driver 263 1,3 – 0.05" X 40. For all socket head screws, for instance used for sealing fluid ports.	1	
23	290.113	Brush, 1/16 Cleaning. Small enough to clean deposits from inside the fluid ports.	5	
24	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
25	504.002	25mm Cover Slip. #1 thickness, can be purchased in almost any microscopy vendor, or from Asylum Research. Must be used with 112.789 and 112.790. See Section 14.12.2 on page 190.	10	
26	504.003	Cover slip, 35mm round. #1 thickness This 35 mm coverslip is 0.17 mm thick. To suppress vibrations and allow sealing these coverslips should be backed by either holder 111.893 or 111.925. See Section 14.12.1 on page 190.	10	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
27	504.004	Cover Slip, 12mm. #1 thickness, can be purchased in almost any microscopy catalog, or from Asylum Research. Must be used with 111.925. See Section 14.12.3 on page 194.	10	
28	939.007	Membrane Clamp, DISCONTINUED. Replaced by 939.015.		
29	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
30	939.010	Portless Fluid Dish. Made of PEEK plastic. For use where fluid ports and pressurized operation are not of importance. The best choice for any initial investigation. Simple to clean, easy to assemble. See Chapter 13 on page 145.	1	
31	939.015	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173. **Warning**: Not following the instructions and not leak testing before use will seriously damage your AFM from fluid spills.	1	
32	112.204	Fluid Port Clamp Bar. Stainless Steel. Compresses and seals two mini O-rings 230.018. Fastens with 0-80 X 3/16” screws. Part of 1/16” fluid ports. See Section 14.4.2 on page 172.	2	
The scale in the photos is in cm and mm.				

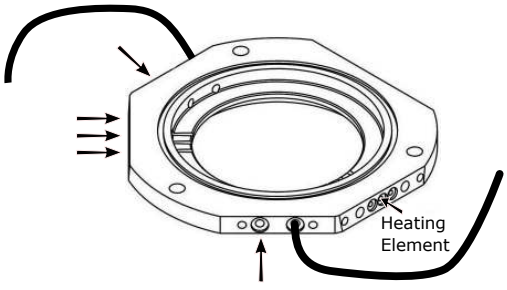
Itm	Part #	Item Description	Qty	Picture
33	112.430	Closed Fluid Cell Triple Clamp. Fluid Port Clamp Bar. Stainless Steel. Compresses and seals three mini O-rings 230.016. Fastens with 0-80 X 3/16" screws. Part of 1/16" fluid ports. See Section 14.4.2 on page 172.	2	
34	928.005	Closed Fluid Cell Body. Made of PEEK plastic. Due to its 10 fluid ports, the choice for advanced experiments where a pressure tight seal or flow through are necessary. Typically a good choice for advanced experiments. See Section 14.4 on page 169.	1	
35	111.420	Closed Cell Bottom Clamp. The stainless steel retaining ring which holds the glass bottom or the cover slip holders against the closed fluid cell. See Section 13.4.1.1 on page 149 and following sections through page 190.	1	
36	001.SHCS #0-80 X 1.00 SS	0-80 X 1" long Socket Head Cap Screw, Stainless Steel. Connects thermistor assembly to Bio-Heater body. Use any comparable 0-80 screw to replace. See Section 17.4 on page 218.	1	
37	112.305	Bioheater Heating Element Clamp. Screws into 112.351 and holds the heating element in place when the thermistor is being inserted and fastened. See Section 17.4 on page 218.	1	
38	112.351	Bioheater Heating Element O-ring Block. Used to compress small O-rings around heating element and thermistor sensor. Also accepts heating element clamp. See Section 17.4 on page 218.	1	
The scale in the photos is in cm and mm.				

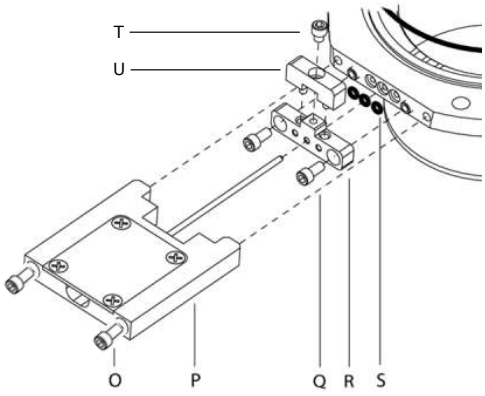
Itm	Part #	Item Description	Qty	Picture
39	0-80 x 0.125 BHCS SS	0-80 X 1/8” screw which threads into 112.351. Section 17.4 on page 218.		
40	928.002	Bioheater Wire Heater Assembly. PTFE Teflon-coated metal. See Section 17.4 on page 218.	1	
41	928.003	Bioheater Thermistor Assembly. Calibrated temperature sensor. Do not immerse the black part in liquid. Tip is NON-passivated 316 stainless steel. The tip is electrically floating (not grounded). See Section 17.4 on page 218.	1	
The scale in the photos is in cm and mm.				

17.4. Assembly

1. **General assembly:**

   - The assembly and use are the same as the Closed Fluid Cell procedure described in Section 14.4 on page 169.
   - Except you leave the three small ports, shown on the right, unplugged. Here we’ll insert the heating element.



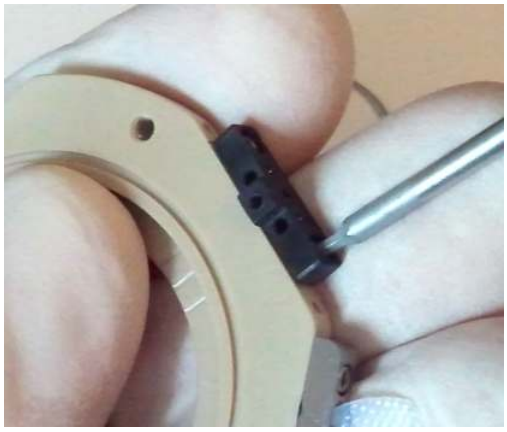


	Part Number	Description
O		0-80 X 1” SS Socket Head Screw
P	928.003	Bioheater Thermistor Assembly
Q		0-80 X 3/16” SS Socket Head Screw
R	112.351	Heating Element O-ring Clamp
S	230.016	Tiny O-ring
T		0-80 X 3/32” Socket Head Screw
U	112.305	Bioheater Heating Element Clamp

Figure 17.2.: Breakdown of Bioheater specific parts. For the rest of the parts, refer to Figure 14.2 on page 170.

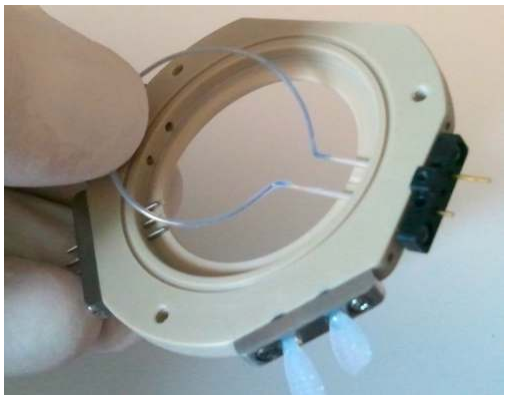
2. **Place O-rings:**
   - Place three of the tiny 230.016 O-rings into the matching grooves.

3. **Attach the O-ring clamp:**
   - Using an 0.050” allen drive, attach the Heating Element O-ring Clamp (112.351) with the 0-80 X 3/16” screws.
   - Stop tightening when you feel resistance. Go tighter and you will damage the metal inserts.
   - The block should remain a little loose, this is by design. It will be compressed in a later step.



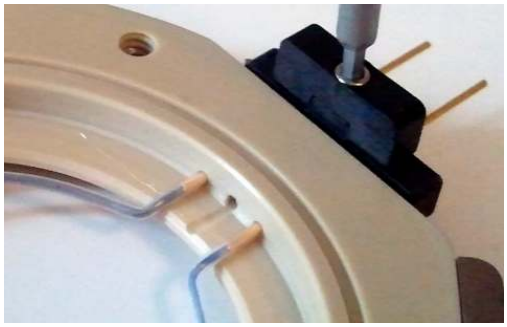
4. **Insert the heating element:**

   - Orient the heating element so that it steps down toward the glass once it is inserted.
   - CAREFULLY insert the ends from inside the cell. Do not nick the insulation or it will become difficult to insert.
   - Push the element forward, through the clamp and the O-rings, until the ring is centered as shown in Figure 14.1 on page 161.



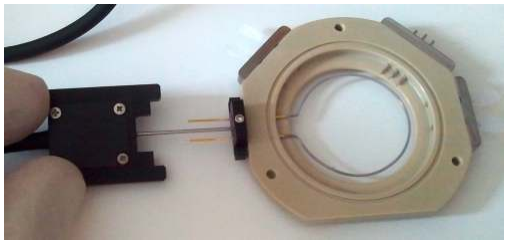
5. **Clamp the heating element:**

   - Using the 0-80 X 1/8” screw to attach the heating element clamp (112.351) as shown.
   - Do not over tighten, you are threading a steel screw into a small plastic part. It only needs to be snug.



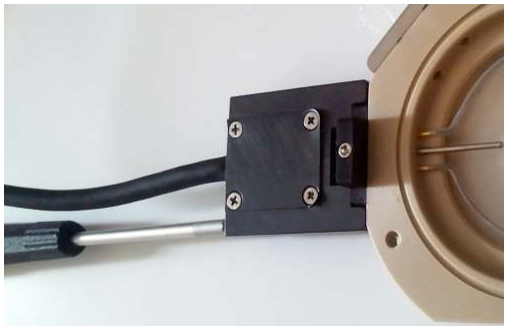
6. **Insert the sensor:**

   - Guide the sensor plug through the O-ring.
   - Then be careful to line up the heating element legs to fit into the sockets on the plug.



7. **Tighten the connector screws:**

   - Tighten the screws, again only snug enough to compress all the parts against the body of the cell. Do not overdo it, since you are threading metal screws into a PEEK plastic body.



8. Before using the cell on the AFM you should perform a leak test as outlined in Section 14.7 on page 183.

17.5. Disassembly

Simply follow the assembly steps in reverse.

17.6. Testing the Bioheater

17.6.1. General Environmental Accessory Preparations

1. Connect the Environmental Controller (EC) to the AFM controller as shown in Figure 16.2 on page 207 and plug the EC AC power cord.
2. Be sure the software is up and running as described in Chapter 3 on page 10, including the selection of AC mode imaging.
3. Plug the bioheater into the EC front panel.
4. Set the heater stage on the desk in front of you.
5. Turn on the power switch in the back of the EC.
6. In the AFM software, rescan the smart start bus (see item two in Step 6 on page 10).
7. Click the "gear" shown in the same figure to see that the heater controller and the bioheater sample stage (also called a T-stage) show up in the list of attached and recognized accessories.
8. A heater control panel should appear on the screen. If it does not, you can always bring it to the front by selecting *AFM Controls* ▷ *Heater Panel*.
9. Hit the More button a few times next to "live graph" in the "data" section of the panel. This will start a time history graph of the sensor temperature and heater power setting.

17.6.2. Bioheater Specific Testing

1. Touch your finger lightly on the sensor to see that the temperature rise is recorded.
2. **Fill with water:**
   - Pour some water into the top of the cell so the heating element is immersed. Fill it right to the brim.

Warning	NEVER run the bioheater without fluid. The water is the only link between the temperature sensor and the heating element. Placing the heater in feedback without water means the heating element will get very hot without the temperature sensor ever registering a change to the feedback algorithm.

3. Enter a “Target Temp” setpoint of 45C.
4. Enter a ramp rate of 3C/min.
5. Set the Mode to On.
6. Set the feedback to On.
7. Watch the temperature rise on the history graph. When it is warm you can feel the hot water with your finger. See Figure 17.3 on page 222 for the temperature history you can expect to see.
8. You can play around changing the setpoint and eventually turn off the heater.

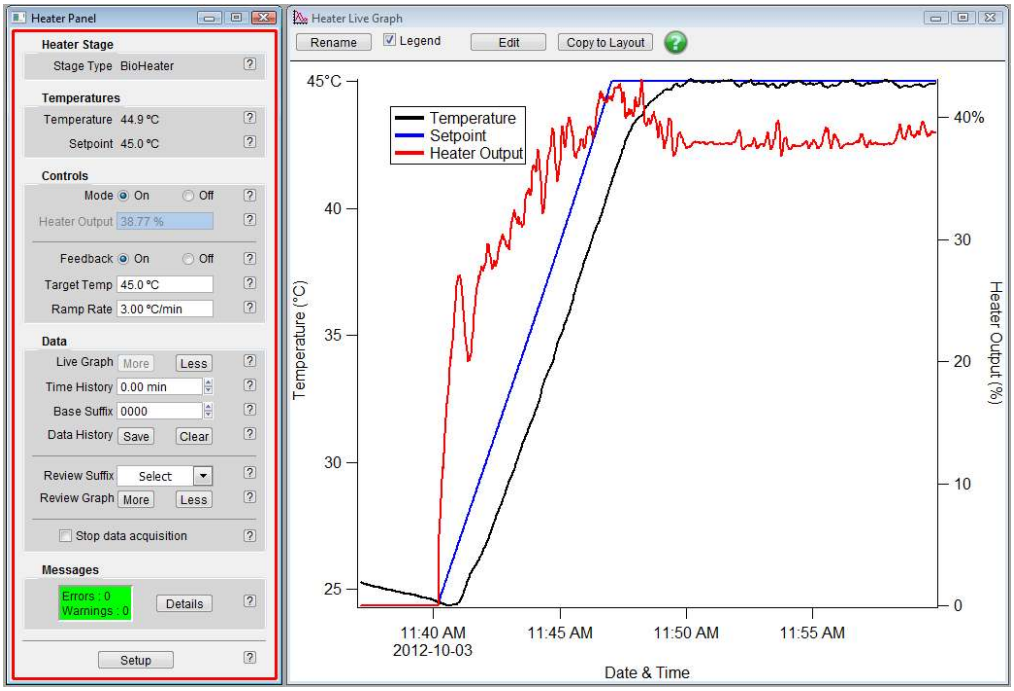


Figure 17.3.: Test of the Bioheater. What you might expect to see. Note that the temperature control will be less noisy if the cell is covered. You’ll discover this when using it inside the AFM.

17.7. Imaging with the Bioheater

This is basically the same as using the fluid cell lite (see 13) if you do not require a fully sealed setup, or like the closed fluid cell (see 14). Note that your bioheater kit includes as a subset everything from the fluid cell lite and closed fluid cell kits.

17.8. Cleaning and Care

Refer to the parts list (Section 17.3 on page 210) about the materials of which the fluid cell is made. All the parts used during imaging can be cleaned with solvents such as alcohol. Parts can also be autoclaved.

If you remove the sensor plug from the rest of the assembled cell body (heating element can remain attached) then you can sonicate of autoclave the assembled cell.

The sensor assembly should not be fully immersed or autoclaved. The entire tip of the sensor can be cleaned with alcohol or dipped in boiling water. In the case of the boiling water, consider wrapping the rest of the connector with parafilm, so that water will not condense inside the contact that connect to the heater wire.

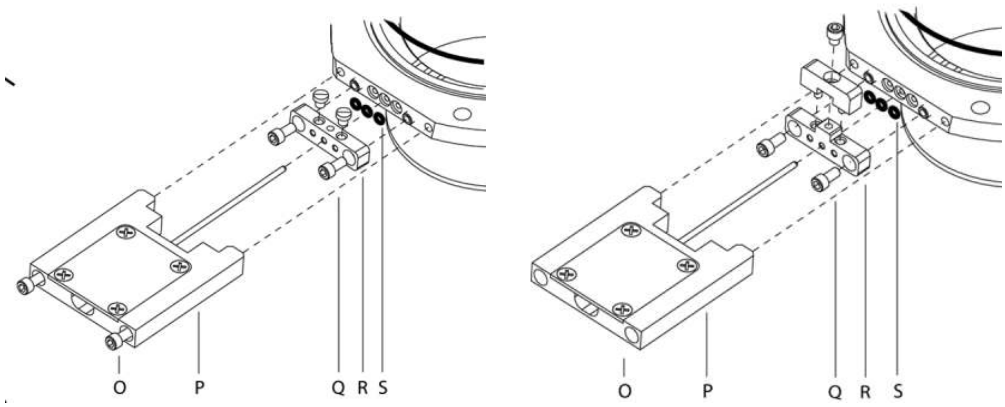
Avoid the use of acetone. The rubber parts will not do well when exposed to acetone.

Please store your bioheater in its designated case. If you own multiple accessories that are similar to this one, it is best to keep all the parts where they belong and not get things mixed up.

If you need replacement parts, contact your local office or distributor and use the parts list in this chapter as a guide.

17.9. Older models

The main difference with the very early models of the bioheater are in the clamp which holds the heating element. The original model had nylon screws which tended to break through the insulation and short out on a metal clamp. Since then, plastic parts have been included which only press down on the heating element without a twisting motion. The new parts are also electrically insulating. See Figure 17.4 on page 223 for a visual comparison.



(a) Old Style **(b)** New Style

Figure 17.4.: Bioheater heating element clamping mechanisms.

17.10. High Voltage

DO NOT USE the bioheater with any of our accessories that allow for high voltage tip bias. High voltage and fluids typically are not safe to use together. Doing so might cause harm to you or your equipment.

18. Polymer Heater

CHAPTER REV. 1710, DATED 10/23/2013, 21:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

18.1. Overview

The polymer heater was designed to heat small samples in air (or inert gas) to elevated temperatures of 300C above room temperature, while keeping thermal drift and unwanted heating of the AFM to a minimum.

This accessory requires the environmental controller (Chapter 16 on page 205).

Figure 18.1.: Top View of the polymer heater.

18.2. Prerequisites

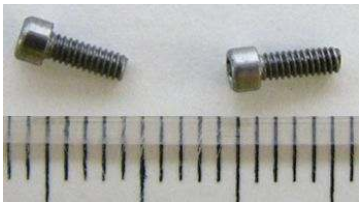
This accessory is a variant of the closed fluid cell discussed in Chapter 14 on page 160. You should be familiar with the closed fluid cell before you embark on this chapter.

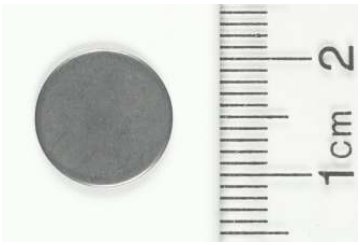
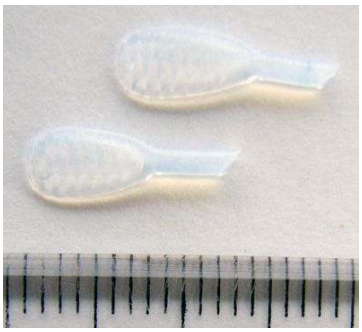
It's ALSO assumed that you are familiar with:

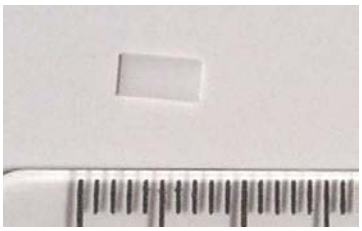
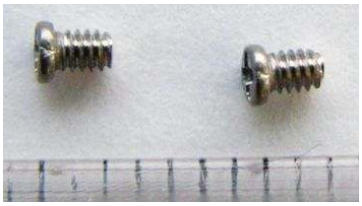
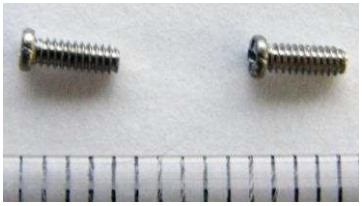
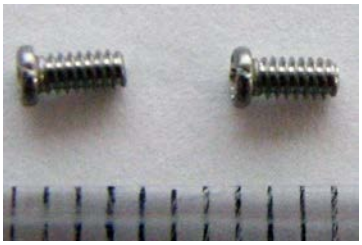
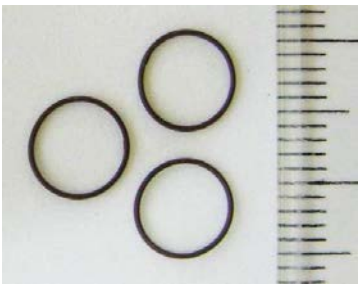
- General operation of the AFM, covered in Chapter 4 on page 15.

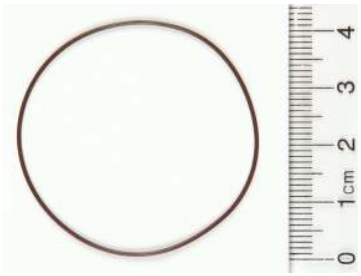
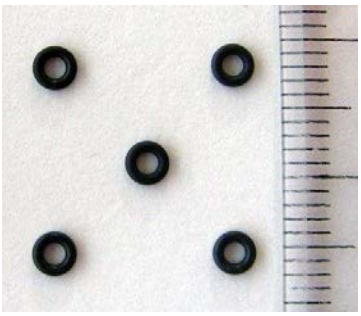
Warning	DO NOT use this accessory with liquids. It was designed for use with gases only.

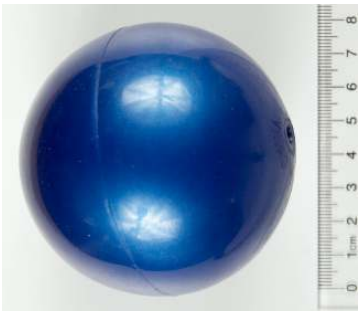
18.3. Parts List

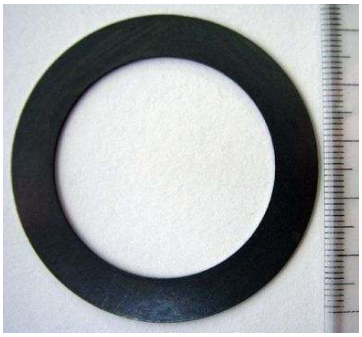
Itm	Part #	Item Description	Qty	Picture
1	001.SHCS <#0-80X.188> SST	0-80 X 3/16" Stainless Steel Socket Head Cap Screw. Connects 111.886 and 102.204 O-ring clamps to the fluid cell body. See Section 14.4.2 on page 172.	8	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
2	080.105	12 mm AFM Specimen Disc. Magnetic Disc which is permanently attached to the Sample. Can be ordered from Asylum Research or Ted Pella (16208). See Section 18.6 on page 232 about sample mounting.	10	
3	111.924	1/16” OD Port Plug. PTFE plugs for blocking unused in/outlets on the closed fluid cell. Also consider using PTFE cord 0.062" Diameter (McMaster Carr Part Number 84935K48.) See Section 14.4.1 on page 169.	10	
4	111.240	Mini Sample Holder Clip. Used for holding down samples in the polymer heater. Stainless steel. Use screws 222.021 to affix to polymer heater. See Section 18.6 on page 232.	4	
5	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153.	2	
6	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
7	112.495.02	Coupling Pad, .015". Only for very old clear plastic cantilever holders. Polymer coupling pads to be inserted inside of the cantilever holder for AC mode imaging at high temperatures. SeeStep 5 on page 104.	5	
8	222.021	Screw, Pan Head Phillips, M1.4 X 2. 2mm long Stainless steel metric miniature screw for mounting thin samples directly to the heater stage. Also used for mounting spring clips 111.240 to the polymer heater body. See Section 18.6 on page 232.	12	
9	222.022	Screw, Pan Head Phillips, M1.4 X 3. 3mm long Stainless steel metric miniature screw for mounting medium thickness samples directly to the heater stage. NOT USED for mounting spring clips 111.240 to the polymer heater body. See Section 18.6 on page 232.	6	
10	222.023	Screw, Pan Head Phillips, M1.4 X 4. 4mm long Stainless steel metric miniature screw for mounting thicker samples directly to the heater stage. NOT USED for mounting spring clips 111.240 to the polymer heater body. See Section 18.6 on page 232.	6	
11	230.011	O-ring, 0.244"ID X 0.016"CS, Viton, 55 Durometer. Seals the cantilever holder quartz prism. See Step 7 on page 104.	3	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
12	230.012	O-ring, 1.595”ID X 0.030”CS, Viton, 55 Durometer. This FKM O-ring makes the seal between the bellows membranes and the sealed cell body. See Section 14.4.5 on page 173.	5	
13	230.018	O-ring, 0.062”ID X 0.032”CS, Viton, 70 Durometer. FKM O-rings that seal around the four 1/16" fluid ports. See Section 14.4.1 on page 169.	15	
14	231.006	Tubing, PFA, 1/16”OD X .040”ID. This PFA tubing makes it possible to introduce and remove fluid or gas from the closed fluid cell. Order from Asylum or purchase directly from Upchurch Scientific (p/n 1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	5 ft	
15	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
16	290.103	3C Tweezer – Extra Fine Sharp – Standard Grade. For placing samples, tiny o-rings (e.g. 230.018) , and small screws.	1	
17	290.106	#00 Phillips WIHA Screwdriver 261 PH 00X40. For small Phillips screws (222.021, 022, and 023) in the polymer heater.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
18	290.109	Leitsilber Conductive Paint. Used for mounting samples (See Section 18.6 on page 232). Can be purchased from Asylum Research or directly from Ted Pella (16035).	1	
19	290.111	0.050": WiHA Allen Driver 263 1,3 – 0.05" X 40. For all socket head screws, for instance used for sealing fluid ports.	1	
20	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
21	939.007	Membrane Clamp, DISCONTINUED. Replaced by 939.015.	0	
22	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
23	939.015	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173. **Warning**: Not following the instructions and not leak testing before use will seriously damage your AFM from fluid spills.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
24	112.204	Fluid Port Clamp Bar. Stainless Steel. Compresses and seals two mini O-rings 230.018. Fastens with 0-80 X 3/16” screws. Part of 1/16” fluid ports. See Section 14.4.2 on page 172.	2	
25	900.155	Heated metal sample stage embedded in ceramic and plastic. See Chapter 18 on page 224.	1	
26	908.038	Peek Cantilever Holder Kit. This cantilever holder is well suited to high temperature studies. See Section 18.7 on page 233 and Section 11.4 on page 108.	1	
27	207.004	Steel Shim, 0.020” Thick x 1-½” ID x 2-1/8” OD. Used to moderately seal the polymer heater cell. See Section 18.7 on page 233.	2	
The scale in the photos is in cm and mm.				

18.4. Assembly Instructions

There is not much to disassemble from the polymer heater. Only the port clamps around the perimeter of the cell should be removed. This is identical to the process described for the closed fluid cell in 14.4.1.

Do not remove any other screws or items from the polymer heater. There are no serviceable parts inside.

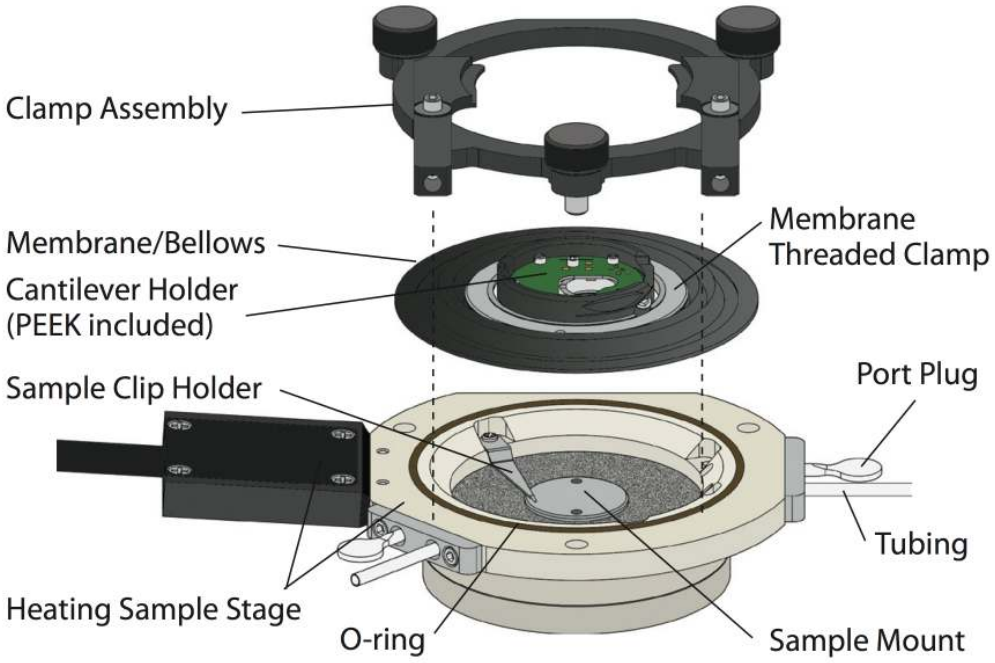


Figure 18.2.: A nice overview of the polymer heater and its parts.

18.5. Testing the Polymer Heater

1. Follow the general setup steps in Section 17.6.1 on page 221.
2. Enter a “Target Temp” setpoint of 150 ºC.
3. Enter a ramp rate of 50 ºC/min.
4. Set the Mode to “On”.
5. Set the feedback to “On”.

Warning	The polymer heater can get hot enough to cause serious burns. Even the black ceramic material around the perimeter of the device will get hot enough to cause injury. The plastic parts can get hot to the touch, but are always safe to handle and will not cause burns.

6. Watch the temperature rise on the history graph. An example of what you might see is in Figure 18.3 on page 232.
7. You can play around changing the setpoint and eventually turn off the heater.
8. Turn the mode to “Off”, and observe the temperature graph until the heater is below 50 ºC. This will take at least five minutes.
9. Now it is safe to handle without getting burnt and safe to store.

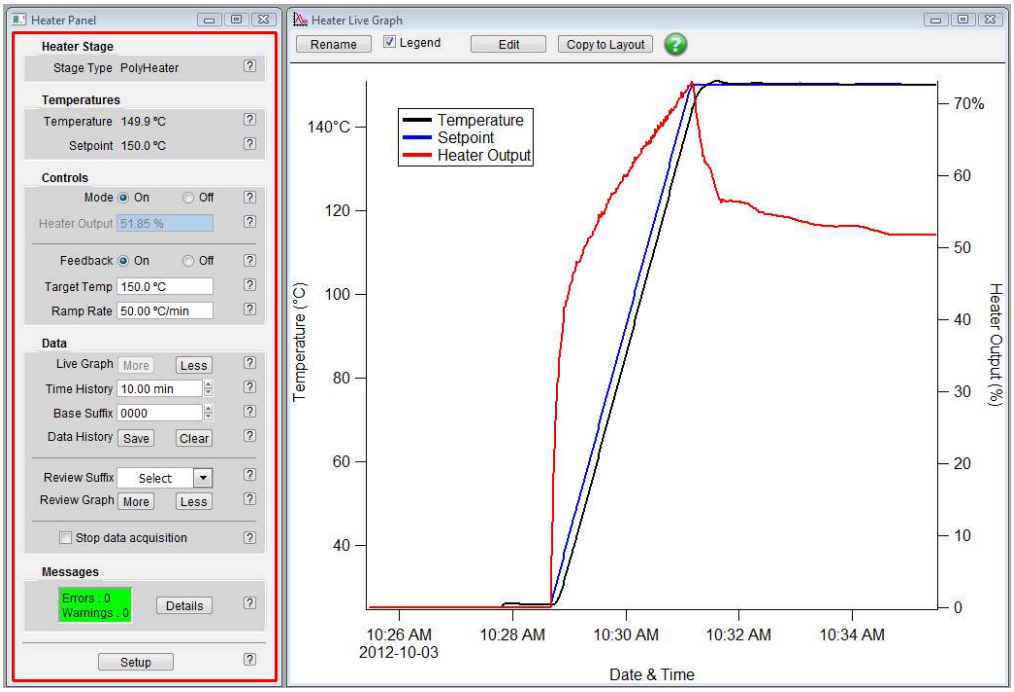
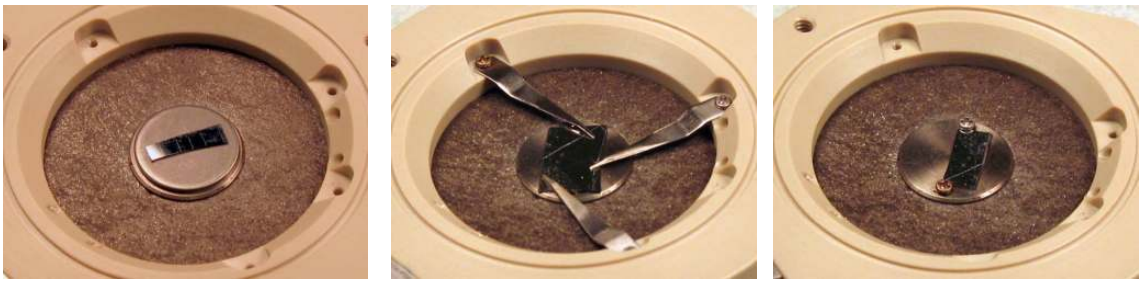


Figure 18.3.: Test of the polymer heater. What you might expect to see.



(a) Magnetic Disc **(b)** Clips **(c)** Screws

Figure 18.4.: Some ways to mount a sample.

18.6. Sample Mounting Techniques

18.6.1. Silver Paint

It's common to use a bit of silver paint to attach a small sample to an AFM disc, which in turn magnetically attaches to the polymer heater.

For very small samples we often use a tiny bit of silver paint to attach the sample directly to the metal stage at the center of the heater. Be careful not to spill the paint onto the black ceramic perimeter. It may not be possible to completely clean the paint from there.

After imaging, one can easily pop the sample off with some tweezers and clean the sample stage with a cotton swab and some solvent.

18.6.2. Clips

Some hold down clips are supplied (111.240) that attach to the four cutouts in the plastic perimeter of the polymer heater. You can see one clip in place here: Figure 18.2 on page 231. IMPORTANT: only use the shortest screws in your kit (222.021) to attach the clips. Do not over tighten, since you may strip out the delicate threads in the plastic body.

Note that the clips are magnetic: Beware. When you loosen the screws they can be pulled toward the center of the sample, possibly damaging the surface of the sample. See Figure 18.4 on page 232.

18.6.3. Screws

Three lengths of tiny screws are supplied to help you attach small samples directly to the heated center of the polymer heater. We've used tiny washers under each screw to clamp a sample or we have also taken a large washer and drilled to small holes in it (the holes are exactly 10mm apart) to old down a sample. Contact us if you need more help with sample mounting. See Figure 18.4 on page 232.

18.6.4. Epoxy

A few good choices are Cotronics Duralco™ 4703 (good to 370 ºC) or Cotronics Duralco™ 124 or Resbond 989FS. Use these adhesives only to attach a sample to a magnetic AFM disc since it may be difficult to remove these adhesives from your polymer heater. See Figure 18.4 on page 232.

18.7. High Temperature Imaging

Imaging at high temperatures does not differ much from imaging at room temperature. For large temperatures you will experience more thermal drift than usual. You may "run out of" Z range as the sample might approach or retreat by more than the full range of the Z actuator. You will have to experiment.

18.7.1. Inert gas flow

For samples that might otherwise combust (react with ambient oxygen) it is common to flow inert gas (Nitrogen or Argon) through a sealed polymer heater. Learn how to do this by reading two sections elsewhere:

- To learn how to run our fluid cell related accessories in a fully sealed configuration see Section 14.4 on page 169. Note that you should ignore any steps that talk about filling with fluid! Only learn about how to seal the cell and attach tubing. It may also be sufficient to seal the holder less rigorously, as described in Section 19.7.2.3 on page 254.
- To set up an inert gas flow system see Section 14.5 on page 181.

18.7.2. Condensation and AC Mode Tuning Issues

When imaging some materials at high enough temperature, sometimes the smaller polymer chains start to evaporate. They will typically condense on the nearest object colder than the sample. When using the standard cantilever holder (see Section 11.2 on page 99) this will be the cantilever and the metal clip which clamps it down. Over time, the condensed droplets will add enough mass to the cantilever to shift its resonant frequency away from the AC mode imaging fixed driving frequency. Eventually the amplitude of the cantilever will be less than the setpoint and the cantilever will float away from the surface, making imaging impossible. For such cases it is recommended to use the PEEK cantilever holder which is made entirely of thermally insulating PEEK polymer (see Section 11.4 on page 108). In this case the cantilever will heat up to nearly the same temperature as the sample and the condensation problem is either entirely eliminated or at least greatly reduced.

18.8. Thermal Drift

When the temperature setpoint is changed, the materials inside the polymer heater will thermally expand and contract. Asylum Research has tried to minimize these effects though careful choices of materials and design geometry. Nevertheless, images taken while the temperature is changing will experience thermal drift. Looking at Figure 18.3 on page 232 you can get an idea of the period during which thermal drift will be worst. Certainly wile the temperature is changing, expect some thermal drift effects. Note that once the temperature setpoint is reached, the heater power does not immediately reach a steady state. It slowly tapers off during the next 5-10 minutes. During this period the low thermal conductivity materials surrounding the sample are slowly heating up. You can also expect thermal drift effects during this period.

18.9. Cleaning and Care

The Polymer heater was not designed to be disassembled for thorough cleaning. Typically one should rub the metal and black ceramic surfaces with a cotton swab moist (not soaking!) with alcohol.

Note that the heated metal surface is made of a hard tungsten alloy and can be gently scraped with steel objects like tweezer tips or curved blades without worrying about scratching the surface. The black ceramic material around the heater, on the other hand, is not very hard and should NOT be scraped with metal objects.

Please store your polymer heater in its designated case. If you own multiple accessories that are similar to this one, it is best to keep all the parts where they belong and not get things mixed up.

If you need replacement parts, contact your local office or distributor and use the parts list in this chapter as a guide.

18.10. Heated Humidification Option

Based on the requests of some customers to perform measurements in a controlled humidity environment at elevated temperatures, we developed a simple add-on for the polymer heater. Fig-

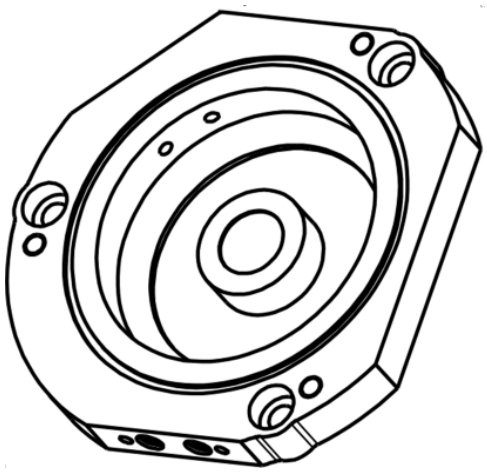


Figure 18.5.: Optional Polymer Heater Humidity Attachment, Asylum part number 112.854.

ure 18.5 on page 235 shows a drawing of the part. It functions exactly like the salt solution reservoir shipped with the humidity sensing cell described in Chapter 19 on page 239. This accessory is intended for the advanced user who does not mind to some tinkering.

18.10.1. Parts list

Itm	Part #	Item Description	Qty	Picture
1	113.854	Polymer Heater Fluid Insert. Designed to surround the sample with a heated salt solution to control relative humidity.	1	

18.10.2. Instructions

Put a very small dot of thermal grease on top of the heated surface of the polymer heater to promote thermal contact. Simply bolt the fluid reservoir attachment to the top of the polymer heater. Transfer the port clamps and O-rings from the polymer heater to the humidity attachment. Then follow most of the descriptions in Chapter 19 on page 239. The idea behind this attachment is to heat the sample AND the salt solution to the same temperature. This will create a legitimate constant relative humidity environment around the sample. Any setup where the sample is hotter or colder than the salt solution will create difference in RH value which will be difficult to quantify. To keep this attachment design simple, we opted to leave out the humidity sensor.

To double check the RH values we suggest buying multiple humidity sensors (the same Honeywell HIH-4000-003 sensors used in our humidity sensing cell can be bought from supply houses like Digikey or Newark Electronics for under $40, or contact Asylum Research to buy some). Supply 5V and ground to two pins and read out a voltage from the third pin which can be converted to RH

from the supplied piece of paper with calibration constants. Place the sensor in the place of the sample and run a sealed humidity experiment at an elevated temperature to confirm the values in Table 19.2 on page 250. The pins on the sensor can be cut short and soldered to some wires which can be routed out through the ports.

If you want to confirm the temperature of the sample, contact Asylum Research and we will send you a few small temperature sensors which can also be put in place of the sample. It's likely that the sensor in the polymer heater itself will register a somewhat higher temperature than the sample since it is not located optimally close to the sample.

Do not exceed 80 ºC. Note that the entire aluminum attachment will get hot, so do not touch the metal if you do decide to go to temperatures above 50 ºC.

18.11. Electrical Connections to the Sample

When performing conductive AFM (See Chapter 21 on page 279) experiments that require sample heating, we suggest the the Environmental Cell Electrical Connectivity Kit . This kit has everything required to make electrical connections to the sample.

18.11.1. Electrical Connection Tutorial

This tutorial sets up a sample in the polymer heater, ready for ORCA conductive AFM imaging.

1. Please refer to Table 21.2 on page 290 and select the necessary parts from the kit.
2. In case inert gas purge lines are required, please see 14.5.
3. **Attach the bias wire:**
   - Follow the directions in 3, but look at the photo to the right on which port to choose.

4. Take a mica disc from the kit and place it on top of the polymer heater.
5. Place the sample no top of the mica disc and hold everything down with one, or preferably two, clips. This disc insulates the sample electrically from the metal below. If you worry about thermal conductivity, place a tiny bit of silver paint under the mica and also under the sample, or or a tiny dollop of thermal grease. Consider using http://www.arcticsilver.com, readily available in computer shops or on the internet.
6. At this point the process is just as if you were using the Electrical Closed Cell. Please continue at Step 7 on page 202.

If you only want to apply a sample bias or ground from some external piece of equipment, simply use one of the Jumper Wires. See Section 15.5 on page 203.

18.12. Polymer Heater Plus

Asylum Research part number 900.265. Except for the label on the cable, this polymer heater will look identical to the standard version. You may notice a blue tint to the metal surface on which the sample is placed. This is caused by operating the heater at 400C. The following table shows the differences.

	Polymer Heater	Polymer Heater Plus
Max Temp	300ºC	400ºC
Temp Resolution	higher	lower
Cantilever Holder	PEEK (see Section 11.4 on page 108)	PEEK High Temp (see Section 11.5 on page 114)
Max heating rate	120ºC/min	20ºC/min

18.12.1. High temperature cantilever holder

Warning When imaging in air above 300C for prolonged periods of time, we have noticed some discoloration of the PEEK cantilever holder body. We suspect the PEEK holder is slowly reacting with the oxygen in the air. To prevent this we strongly recommend imaging in pure Argon or Nitrogen gas. Please see Section 18.7 on page 233 for more information.

18.12.2. Instructions for use

There are no special instructions for us, only a few points of interest.

- The plastic parts of the polymer heater running above 300C get fairly hot. It won't cause burns, but it will feel hot to the touch. Handle with care.
- The metal parts of the scanner which support the heater will also get warm. This may lead to some unwanted thermal drift.
- Since the larger temperature range is digitized by the same number of bits in the heater controller, the temperature setpoint resolution is reduced by approximately 25%.
- When imaging in AC mode at high temperatures, you may notice that the voltage drive required to sustain a given amplitude increases. At some point, even a maximum drive voltage will not achieve a reasonable cantilever oscillation amplitude. In this case we have had success with disassembly the cantilever holder and adding an additional coupling pad. See item 112.245 in Figure 11.4 on page 112. Take the holder apart by removing the four screws, and simply drop in an extra pad from your kit, and carefully put the holder back together and attempt imaging again.
- Temperature ramp rates are lower since the heating element has to compete with more energy diffusing out of heater into the lab. Lower temperature rise rates are the result.
- The magnet inside the heated surface of the polymer heater may weaken over time if it spends a lot of time above 400C. This is an unavoidable side effect of high temperature heating.

- Note that the temperature readout in our software is only an accurate representation of the internal temperature of the metal on which you place your sample. The actual surface temperature of your sample will depend on sample material, geometry, gas surrounding the sample, etc. If you require a publishable surface temperature, you will need to supply your own surface temperature sensor and measurement equipment, applicable to your particular situation. Asylum research can assist you in selecting a suitable third party solution if necessary.

Some notes from one of our applications scientists imaging at temperatures close to 400C:

- I was able to image in AC mode at higher temperatures with the high temperature clip.
- I used 1 0.01" teflon coupling pad together with one 0.005" piece that was cut in half. If I used a full piece the electronic board would not tighten properly. I think the holder expands and reduces the piezo coupling.
- At 85% heater power, a drive Amplitude of 800mV and gave 1V cantilever Amplitude
- At 90% heater power, a drive Amplitude of 2.17V gave 1V cantilever Amplitude. Imaged for 60 minutes in AC mode
- Sample was a steel puck, cantilever was Olympus AC160.
- See Figure 18.6 on page 238 for some sample images at very high temperatures.

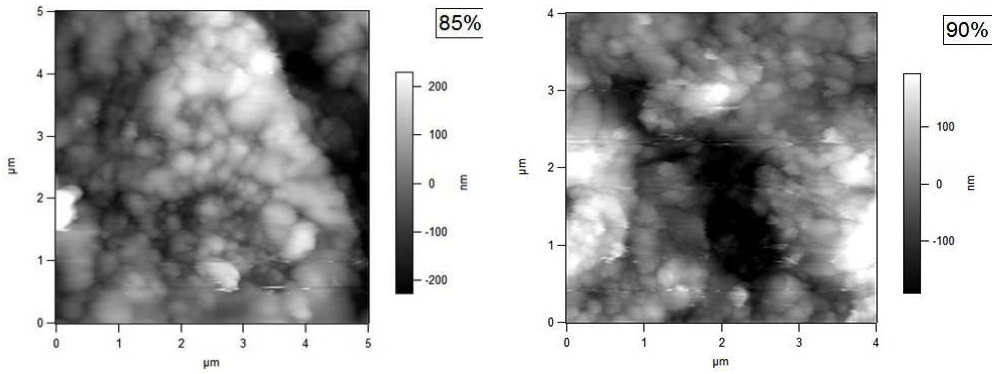


Figure 18.6.: Sample images taken in AC mode at temperatures approaching 400C.

19. Humidity Sensing Cell

CHAPTER REV. 1710, DATED 10/23/2013, 21:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

19.1. Overview

The Humidity Sensing Cell (HSC) is a modified closed fluid cell with a special port for a humidity sensor.

This accessory requires the environmental controller (16).

19.2. Prerequisites

This accessory is a variant of the closed fluid cell discussed in Chapter 14 on page 160. You should be familiar with the closed fluid cell before you embark on this chapter. Here we will only discuss specific issues relating to measuring and controlling humidity around your sample.

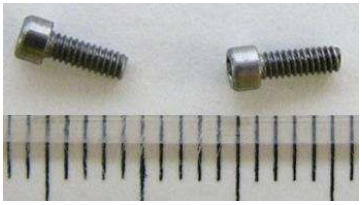
It's ALSO assumed that you are familiar with:

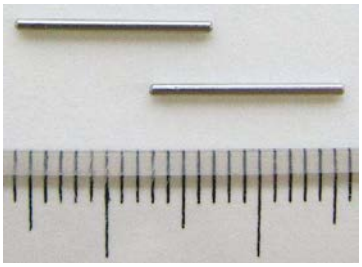
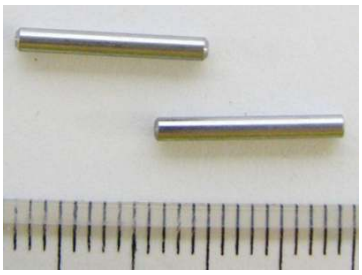
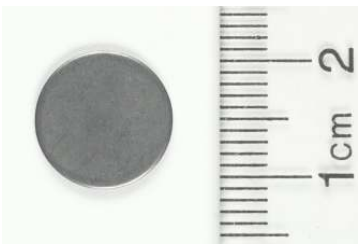
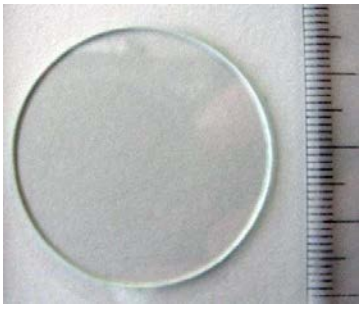
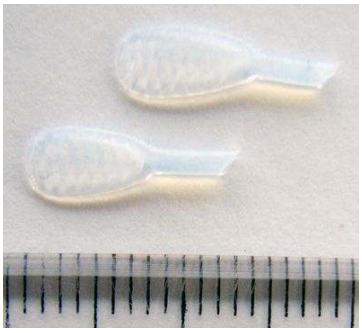
- General operation of the AFM, covered in Chapter 4 on page 15.

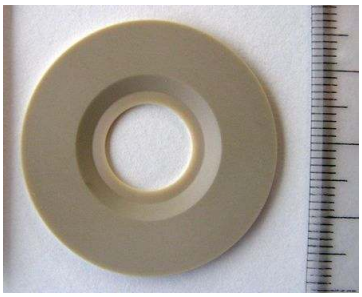
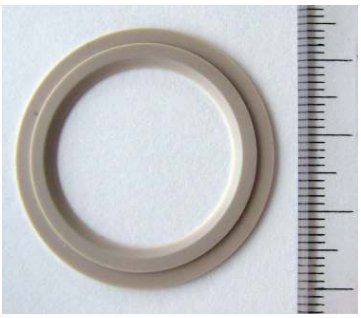
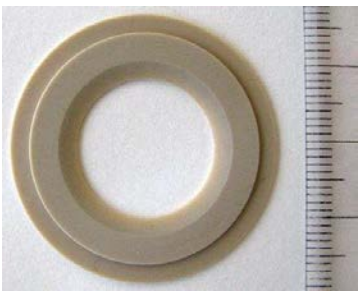
Figure 19.1.: Top View of assembled Humidity Sensing Cell.

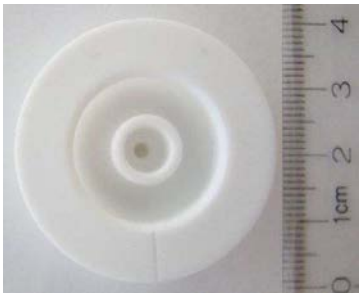
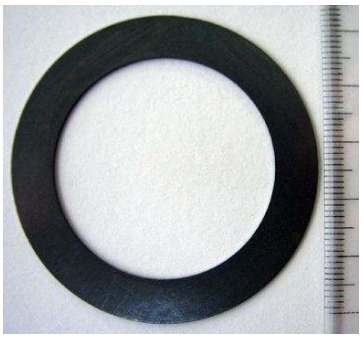
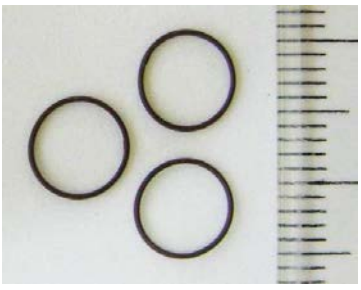
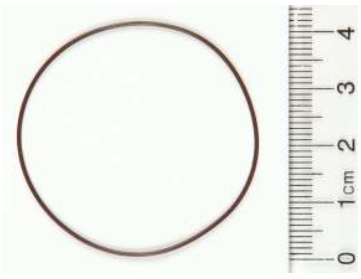
Warning	DO NOT fill this accessory with enough fluid to allow it to reach the sensor. Fluid is never meant to cover the sample or the cantilever. It should always stay in the “moat”, part 113.425.

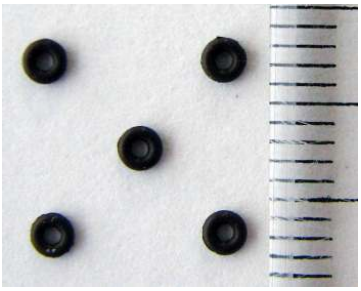
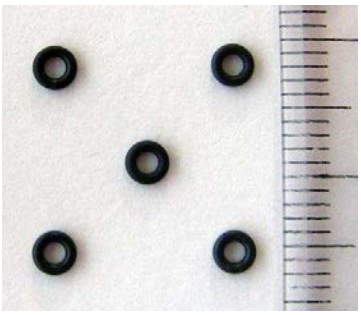
19.3. Parts List

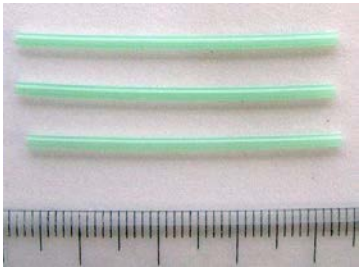
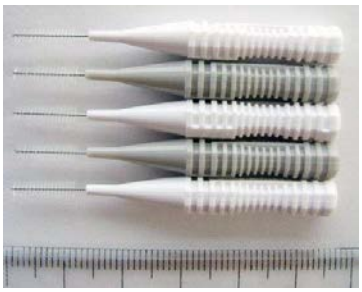
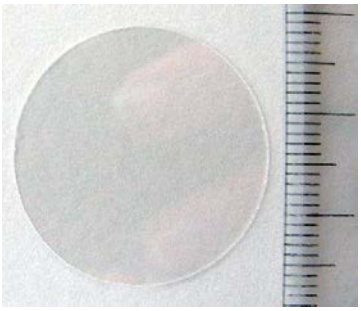
Itm	Part #	Item Description	Qty	Picture
1	001.SHCS <#0-80 X 0.188> SST	0-80 X 3/16” Stainless Steel Socket Head Cap Screw. Connects 111.886 and 102.204 O-ring clamps to the fluid cell body. See Section 14.4.2 on page 172.	8	
2	080.010	5 mL Syringe. We prefer this Norm-Ject model from Henke Sass Wolf (HSW) since it does not contain any rubber, and contaminates fluids minimally. See Section 14.4.3 on page 172 on how to attach tubing.	2	
The scale in the photos is in cm and mm.				

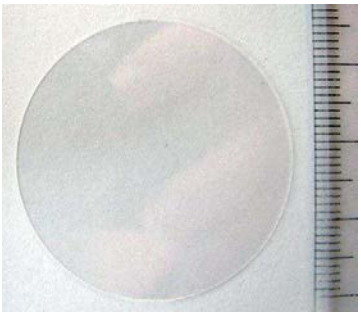
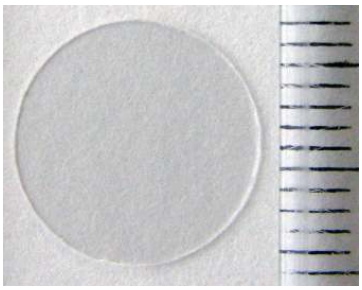
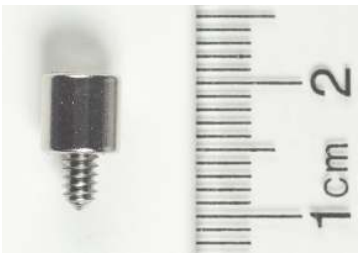
Itm	Part #	Item Description	Qty	Picture
3	005.DOWL <.031 X 0.500> SST	1/32” diameter x ½” 18-8 stainless steel dowel pin. Used with Super Mini O-ring Triple Clamp part (112.430) to plug fluid cell small diameter sealed feed- through ports. See Section 14.4.1 on page 169. Also consider using 0.035" diameter PTFE cord from McMaster Carr (Part # 84935K36).	12	
3	005.DOWL <.063X.500 >SST	1/16” OD X ½” Dowel Pin. Used for plugging the four larger fluid ports. See Section 19.7.2.1 on page 250.	6	
4	080.105	12 mm AFM Specimen Disc. Used for holding samples when using the salt solution reservoir 113.425.	10	
5	111.425	35mm x 1mm glass disc. Custom made by Asylum, and is made out of Glaverbel float glass. See Section 13.4.1.1 on page 149.	5	
6	111.924	1/16” OD Port Plug. PTFE plugs for blocking unused in/outlets on the closed fluid cell. Also consider using PTFE cord 0.062" Diameter (McMaster Carr Part Number 84935K48.) See Section 14.4.1 on page 169.	10	
The scale in the photos is in cm and mm.				

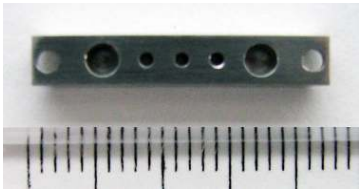
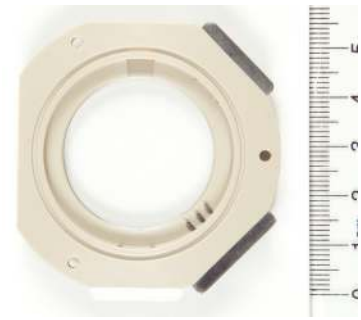
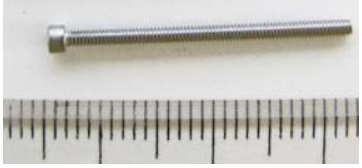
Itm	Part #	Item Description	Qty	Picture
7	111.925	CFC 12mm Cover Slip Holder. This PEEK plastic holder makes it possible to mount 12 mm diameter coverslips in the closed fluid cell. Please see Section 14.12.3 on page 194.	1	
8	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153.	2	
9	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
10	112.789	Clamp, 25mm cover slip top. Used with 112.790 to sandwich a 25mm glass cover slip and then inserts as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
11	112.790	Clamp, 25mm covers slip bottom. Used with 112.789 to sandwich a 25mm glass cover slip and then inserts as a fluid cell bottom. See Section 14.12.2 on page 190.	1	
The scale in the photos is in cm and mm.				

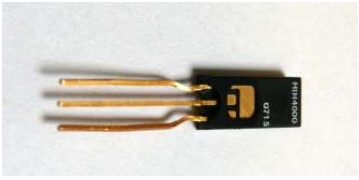
Itm	Part #	Item Description	Qty	Picture
12	113.425	Humidity Cell Reservoir. Used in conjunction with 928.009 to hold samples above a constant humidity salt solution. See Section 19.7.2.1 on page 250.	1	
13	207.004	Steel Shim, 0.020” Thick x 1-½” ID x 2-1/8” OD. Used to moderately seal the humidity cell. See Section 19.7.2.3 on page 254.	2	
14	230.011	O-ring, 0.244”ID x 0.016”CS, Viton, 55 Durometer. Seals the cantilever holder quartz prism. See Step 7 on page 104.	3	
15	230.012	O-ring, 1.595”ID x 0.030”CS, Viton, 55 Durometer. This FKM O-ring makes the seal between the bellows membranes and the sealed cell body. See Section 14.4.5 on page 173.	5	
16	230.015	O-ring, 1.228”ID x 0.032”CS, Viton, 75 Durometer. This FKM O-ring makes the seal between the sample disc 111.425 and the fluid cell body. See Section 13.4.1.1 on page 149.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
17	230.016	O-ring, 0.031”ID x 0.028”CS, Viton, 75 Durometer. FKM O-rings that seal around the six 0.036" fluid ports in the Fluid cell. See Section 14.4.2 on page 172.	15	
18	230.018	O-ring, 0.062”ID x 0.032”CS, Viton, 70 Durometer. FKM O-rings that seal around the four 1/16" fluid ports. See Section 14.4.1 on page 169.	15	
19	231.006	Tubing, PFA, 1/16”OD x 0.040”ID. This PFA tubing makes it possible to introduce and remove fluid or gas from the closed fluid cell. Order from Asylum or purchase directly from Upchurch Scientific (p/n 1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	5 ft	
20	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
21	231.018	Tubing, Orange Peek, 1/32” x 0.020” x 5FT. Used to inject fluid into the salt reservoir 113.425. See Section 19.7.2.2 on page 252.	6in	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
22	231.019	Tubing Sleeve, green. 1/16” OD x 1/32” ID x 1.55”. Used to connect Orange Peek tubing to Syringe. See Section 19.7.2.2 on page 252.	3	
23	290.103	3C Tweezer – Extra Fine Sharp – Standard Grade. For placing samples, tiny o-rings (e.g. 230.018) , and small screws.	1	
24	290.111	0.050”: Wiha Allen Driver 263 – 0.05” X 40. For all socket head screws, for instance used for sealing fluid ports.	1	
25	290.113	Brush, 1/16” Cleaning. Small enough to clean deposits from inside the fluid ports.	5	
26	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
27	504.002	25mm Cover Slip. #1 thickness, can be purchased in almost any microscopy vendor, or from Asylum Research. Must be used with 112.789 and 112.790. See Section 14.12.2 on page 190.	10	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
28	504.003	Cover slip, 35mm diameter. #1 thickness. This 35 mm coverslip is 0.17 mm thick. To suppress vibrations and allow sealing these coverslips should be backed by either holder 111.893 or 111.925. See Section 14.12.1 on page 190.	10	
29	504.004	Cover Slip, 12mm diameter. #1 thickness, can be purchased in almost any microscopy catalog, or from Asylum Research. Must be used with 111.925. See Section 14.12.3 on page 194.	10	
30	928.009	Humidity Sensor Magnet Assembly. Inserts into the salt reservoir 113.425 to hold AFM sample discs (080.105). See Section 19.7.2.1 on page 250.	1	
31	939.007	Membrane Clamp, DISCONTINUED. Replaced by 939.015.	0	
32	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
33	939.015	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173. **Warning**: Not following the instructions and not leak testing before use will seriously damage your AFM from fluid spills.	1	
34	112.204	Fluid Port Clamp Bar. Stainless Steel. Compresses and seals two mini O-rings (230.018). Fastens with 0-80 X 3/16" screws. Part of 1/16" fluid ports. See Section 14.4.2 on page 172.	2	
35	112.430	Closed Fluid Cell Triple Clamp. Fluid Port Clamp Bar. Stainless Steel. Compresses and seals three mini O-rings (230.016). Fastens with 0-80 X 3/16" screws. Part of 1/16" fluid ports. See Section 14.4.2 on page 172.	2	
36	928.006	Humidity Cell Body. Made of PEEK plastic. NOTE: part has 2 metal inserts, 7 O-ring sealed tubing ports, and one slotted port for a humidity sensor. See Section 19.7.2.1 on page 250 for assembly instructions. Note that photo shows metal parts not included with 928.006.	1	
37	111.420	Closed Cell Bottom Clamp. The stainless steel retaining ring which holds the glass bottom or the cover slip holders against the closed fluid cell. See Section 13.4.1.1 on page 149 and following sections through page 190.	1	
38	001.SHCS #0-80 X 1.00 SS	0-80 X 1" long Socket Head Cap Screw, Stainless Steel. Connects humidity sensor plug a to the PEEK body.	2	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
39	302.009	Honeywell HIH-4000-003 humidity sensor. Includes calibration data. Can be purchased from Asylum Research or separately from vendors like Newark Electronics or Digikey (480-2906-ND). See Section 19.5 on page 248.	1	
The scale in the photos is in cm and mm.				

19.4. Assembly

Assembly of the HSC is mostly the same as the assembly of closed fluid cell, described in 14.4.1.

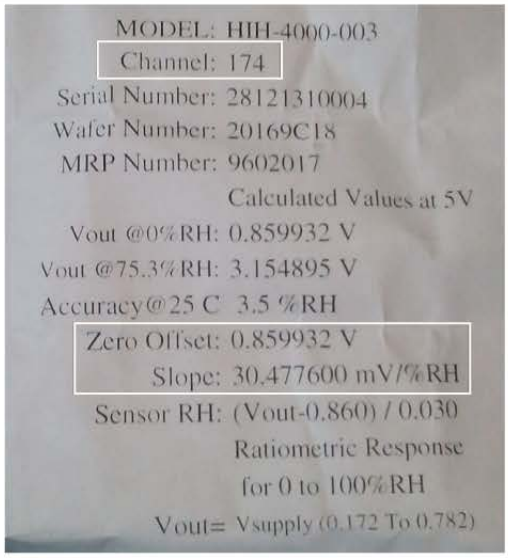
19.5. Replacing the Humidity Sensor

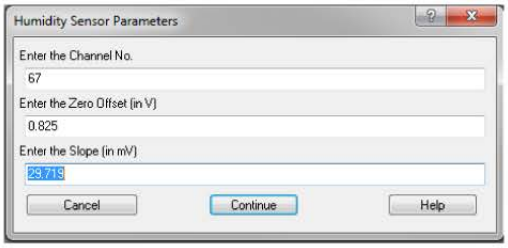
The humidity sensor is quite robust and should not need much care. However, there may be reasons to occasionally replace it. For specs on the sensor, simply Google "HIH-4000" and you will find datasheets on the internet. Asylum also has them stored internally. Contact us if you need datasheets sent to you.

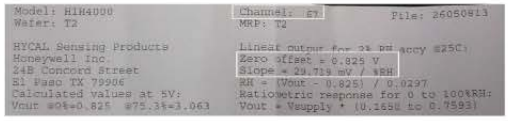
1. Unplug the Humidity Sensing Cell (HSC) from the Environmental controller.
2. Unscrew the two screws at the back of the black connector attached to the HSC.
3. Gently pull the connector away from the cell body. It is a bit of a tight squeeze to get the sensor out.
4. Unplug the old sensor.
5. Insert the new sensor. Take care to get the orientation correct, and to not touch the sensor surface. There is a sticker on the connector to guide you.
6. Don't lose the small piece of paper in the box, it contains the calibration coefficients that need to be entered shortly.
7. Attach everything back to the cell body and be gentle with the screws taking care not to strip the threads out of the plastic cell body.
8. Turn on the cell (See Section 19.7.2.4 on page 255 for more information).
9. In the humidity panel, click the 'Update Sensor Calib.' button. See Update Sensor Calib. See Figure 19.2 on page 256.
10. When asked, click 'Yes' to proceed and enter in the new infoblock items.

11. **Enter the coefficients:**

    - Two varieties of calibration sheets are shown as examples.
    - Find the circled items and type them into the software as shown.
    - Click on 'Continue'.







12. In the AFM software, rescan the smart start bus (see item two in Step 6 on page 10) and the software should be indicating the correct humidity.

19.6. Specifications

The sensors are typically specified to an accuracy of 2 or 3.5%, depending on what is stated on your specific calibration sheet. The sensors are made by Honeywell, model HIH-4000-3. Please conduct an internet search on this part number to find the data sheet for more information on this sensor. The manufacturer may change the specifications over time.

19.7. Controlling Humidity

19.7.1. Moist gas flow

We have tried, with some success, to control humidity with a variation of the inert gas flow system described in Section 14.5 on page 181. Take a second rotameter and split the gas supply so each rotameter can create its own inert gas flow. Then pass one of those flows through a container filled with wet foam (or better yet, one of the high humidity salt solutions like potassium sulphate). Then join the most and dry gas lines together again and feed them into the Humidity Sensing Cell. The result is workable, but one has to continually adjust the knobs to keep the humidity at a constant value. One could work with automated proportional valves, but we did not go that far. We found that most experiments require only a single humidity level maintained for a long time. For that we use the salt solutions discussed in the next section.

19.7.2. Salt Solution Humidity Control Tutorial

Concentrated salt solutions (preferably a salt slurry) confined in a sealed volume will control the relative humidity of the trapped air in that volume. See Table 19.2 on page 250 for an example of how various choices of salts can target RH values from ~10% to ~99%. Also note the relative insensitivity to temperature for most of the listed salts. Based on this principle, we have developed a special bottom piece for the humidity cell (or the closed fluid cell for that matter) which allows the sample to be surrounded by a moat of salt. This tutorial will outline how to fill the cell with one salt solution and then swap out the solution for another.

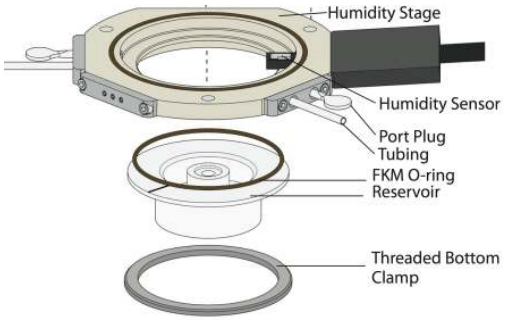
Salt Type / T (ºC)	0	5	10	15	20	25	30	35	40	50	60
Potassium Sulphate	99	98	98	98	98	97	97	97	96	96	
Potassium Nitrate	96	96	96	95	95	94	92	91	89	85	
Potassium Chloride	89	88	87	86	85	84	84	83	82	81	80
Ammonium Sulphate	82	82	82	82	81	81	81	80	80	79	
Sodium Chloride	76	76	76	76	75	75	75	75	75	74	75
Sodium Nitrate					65	64	63	62	61		
Ammonium Nitrate			75	70	67	64	60	53			
Sodium Dichromate	61	59	57	57	55	54	53	51	50	49	47
Magnesium Nitrate	60	59	57	56	54	53	51	50	48	45	
Potassium carbonate	43	43	43	43	43	43	43				
Magnesium Chloride	34	34	33	33	33	33	32	32	32	31	29
Potassium Acetate			23	23	23	23	22				
Lithium Chloride	11	11	11	11	11	11	11	11	11	11	11
Potassium Hydroxide		14	12	11	9	8	7	7	6	6	5

Table 19.2.: Relative Humidity as a function of temperature for various concentrated aqueous salt solutions. Taken from http://www.kayelaby.npl.co.uk/general_physics/2_1/2_1_4.html. Please refer to the link for references relating to the data in the table.

19.7.2.1. Cell Assembly

1. **Assemble the cell:**

   - If not already done, seal all but one of the larger Port Plugs on the cell body. See Section 14.4.1 on page 169 and Section 14.4.2 on page 172.
   - The humidity sensor should be in place. If not, see 19.5 on how to do that.
   - Your aim is to have the top assembly shown to the right, with all the ports filled except for the center small port.



2. **Install magnetic insert:**

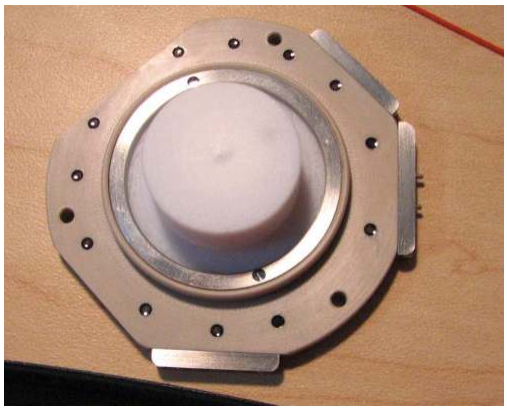
   - Locate the reservoir (113.425) and thread in the magnetic insert (928.009)
   - Adjust the insert to sit just below the top of the reservoir. The sample should only be attracted by the magnet, but should not rest on it.

Oops! The photos below do not show the humidity sensor attached. Please ignore this fact. We'll try to take some new photos soon with the sensor in place.

3. **Attach the bottom gutter:**

   - Using spanner 939.008 and retaining ring 111.420, clamp the cup 113.425 and O-ring 230.015 and against the cell body.

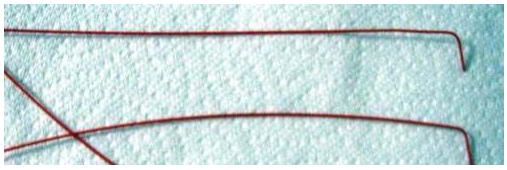
Note There is a groove in the flange at the top of the cup. This indicates the deepest point. Rotate the cup until this groove lines up with the middle of the three small fluid ports.



4. If you are only interested in maintaining one humidity value, you can plug any remaining open ports, place the humidity cell on the scanner, fill the reservoir with the desired salt solution, place your sample, and skip ahead to Section 19.7.2.3 on page 254. If you want to have the option of changing humidity during an experiment, then please keep following the next steps.

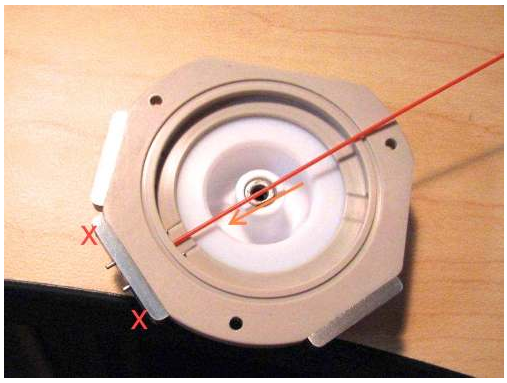
5. **Locate the snorkel tube:**

   - Locate the hard orange tubing (231.019). It should be pre cut and have a bend in the end.
   - If not, please contact Asylum Research to instruct you on bending it with the use of a heat gun or open flame.



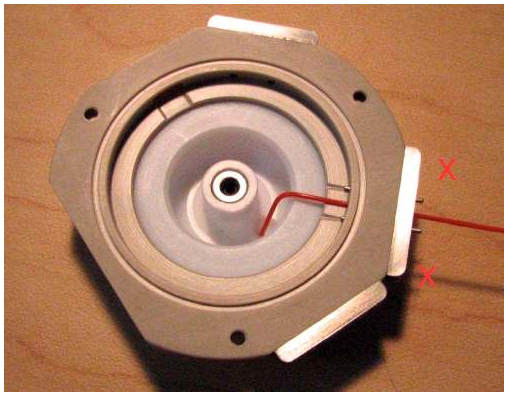
6. **Start threading the tubing:**

- Loosen the screws marked (X) in the picture.
- Stick the tubing into the middle of the small ports.
- Push the tubing out of the cell, through the hole.



7. **Finalize the tubing position:**

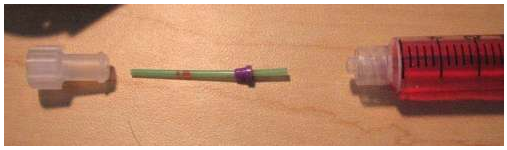
- Slide the tubing until the tube has its end at the deepest point of the reservoir.
- Tighten the two marked screws (X)

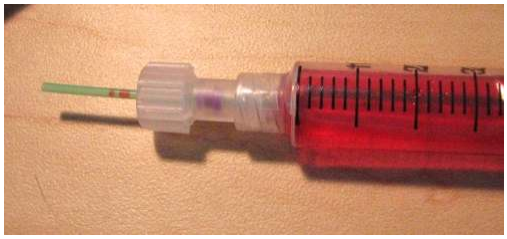


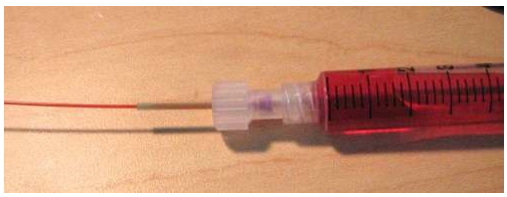
19.7.2.2. Filling the Cell

1. **Prepare the syringe:**

- Locate the luertite fittings (231.008), green tubing (231.018) and a syringe (080.010).
- As shown, thread the purple ferrule onto the green tubing.
- Place the green tubing into the syringe and LIGHTLY thread the luer fitting as shown.
- Place the orange tubing inside the green tubing and make sure it passes well beyond the purple ferrule.
- Firmly tighten the luer fitting to the syringe.
- Gently tug on the orange tubing. It should be gripped by the fitting. If not, tighten the luer fitting with more force.

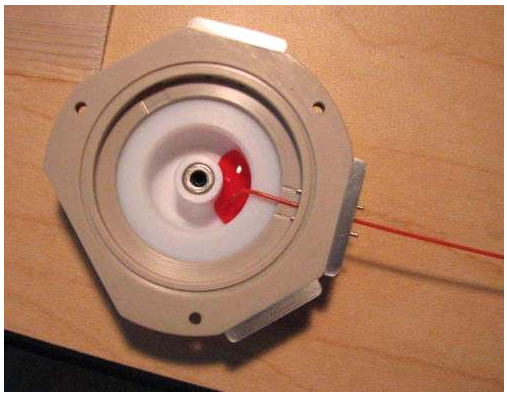




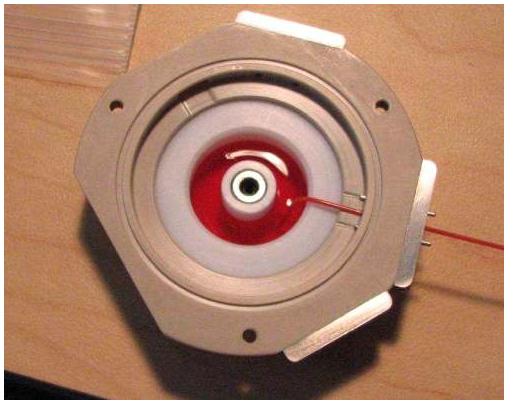


Note: the fluid in the syringe has red dye added to it for dramatic effect.

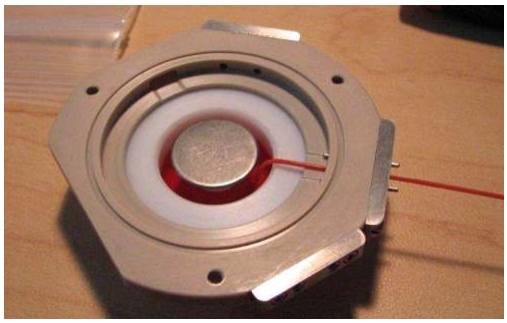
2. **Test solution transfer:**
   - Note the plunger position on the syringe.
   - Start transferring fluid.



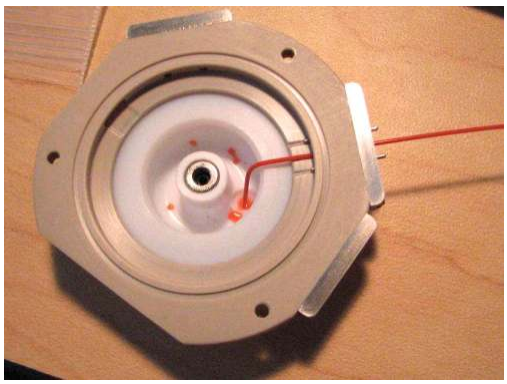
3. **Measure the transferred volume:**
   - Stop the fluid transfer when the liquid reconnects and fills the full circumference of the reservoir.
   - Record the plunger position and remember it as the volume required to fill the reservoir. Later you will do this “blind” with the AFM engaged on the sample.



4. **Test AFM disc placement:**
   - Place a test sample and make sure it does not touch the liquid. Adjust the volume if necessary and record that change.



5. **Withdraw the fluid:**
   - Pull back on the syringe slowly and see how the liquid leaves the cell.
   - Practice a few times to learn what keeps the fluid from breaking into multiple pieces. Minimize the remaining drops.



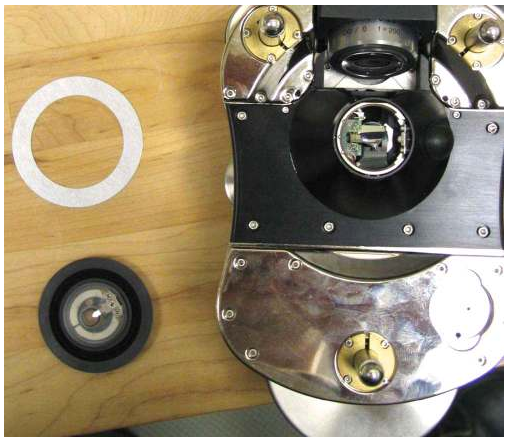
6. Move the the cell over to the AFM and place your sample on it. You can start out by filling up the reservoir without the AFM head in place. If needed, you can pull out the fluid, and put in a different fluid all while the AFM is operating.

19.7.2.3. Magnetic Sealing of the Cell

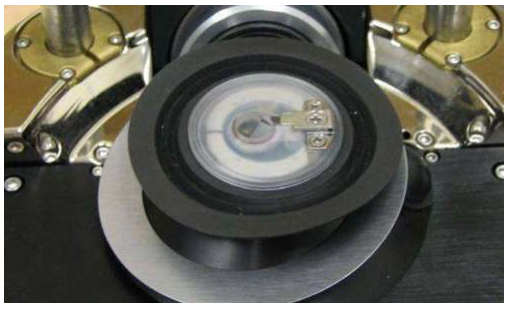
This is an alternative to the somewhat cumbersome business of a cell sealed with a clamped membrane, as described in Section 14.4 on page 169. While there is nothing wrong with this method, it may be overkill when a more modest seal is sufficient for many experiments.

1. **Locate parts:**
   - Locate the metal ring (207.004).
   - Attach a black FKM membrane to a cantilever holder (see Section 13.4.3 on page 153).
   - Insert a cantilever.
   - Have the AFM head sitting ready on its back.

   Note The black membrane is preferred since light falling on the humidity sensor can affect its accuracy.



2. **Load up the AFM head:**
   - First place the metal ring as shown.
   - Then attach the cantilever holder.



3. **Properly seat the magnetic ring:**
   - Set the head up on its legs.
   - Usually it lands improperly as shown by the top photo.
   - Move the ring a bit with your fingers until it seats properly as in the lower image.
   - Adjust the AFM head's legs a bit for safe placement over the sample.

4. **Place the head and engage:**
   - At some point during the engage process the magnets embedded in the perimeter of the cell body will pull the metal ring down.
   - The cell is now sealed and the RH values inside the cell will start to approach the desired value from Table 19.2 on page 250.

Caution! **DO NOT** suddenly lift up the AFM head. The combination of suction and magnetic clamping will likely cause the cell to fly up, which will break the probe and make a salty mess all over your AFM. **FIRST break the seal** by prying a flat tool between the cell body and the membrane. **ONLY THEN lift the head**.

19.7.2.4. Monitor the Humidity

1. Connect the Environmental Controller (EC) to the AFM controller as shown in Figure 16.2 on page 207 and plug the EC AC power cord.
2. Be sure the software is up and running as described in Chapter 3 on page 10, including the selection of AC mode imaging.
3. Plug the Humidity Sensing Cell into the EC front panel.
4. Turn on the power switch on the back of the EC.
5. In the AFM software, rescan the smart start bus (see item two in Step 6 on page 10).
6. Click the "gear" shown in the same figure to see that the environmental controller and the Humidity Sensing Cell sample stage (also called a T-stage) show up in the list of attached and recognized accessories.
7. A humidity control panel (see Figure 19.2 on page 256) should appear on the screen. If it does not, you can always bring it to the front by selecting *AFM Controls* ▷ *Humidity Panel* .
8. Hit the "More" button a few times in the history section to start a time history graph of the sensor. The panel operates very much like any of the environmental controls. Consult the (?) buttons next to each item for more detailed help.
9. Depending on the salt that you chose, you should see a constant value of RH at the level you chose from 19.2.

19.8. Electrical Connections to the Sample

When performing conductive AFM (See Chapter 21 on page 279) experiments that require a humid atmosphere, the Humidity Sensing Cell can be used with the Environmental Cell Electrical Connectivity Kit . This kit has everything required to make electrical connections to the sample.

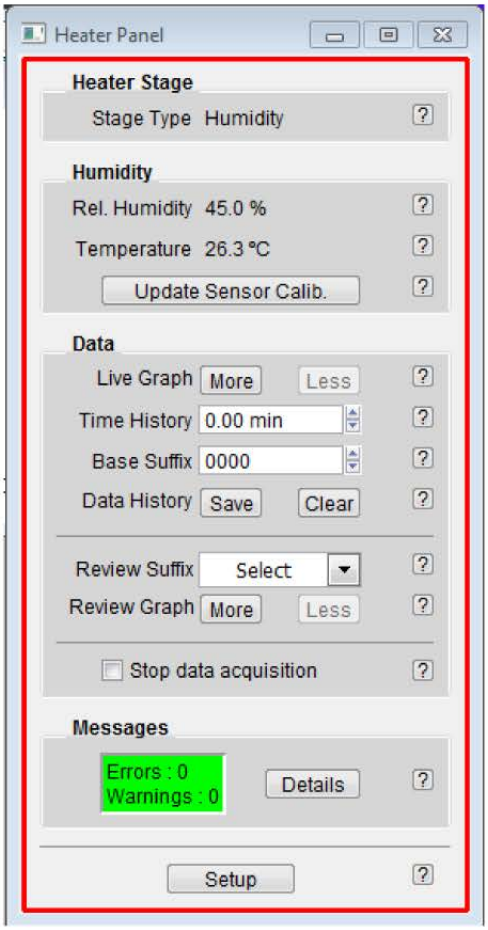


Figure 19.2.: Humidity Control Panel.

19.8.1. Electrical Connection Tutorial

This tutorial sets up a sample in the Humidity Sensing Cell, ready for ORCA conductive AFM imaging.

1. Please refer to Table 21.2 on page 290 and select the necessary parts from the kit.
2. If you are using the humidity sensing cell with a glass bottom, please mimic the protocol for the closed fluid cell: Section 14.13 on page 195. If you are using the "salt cup" with your humidity cell, please continue to the following steps.
3. Place your sample on an AFM disc with a socket (939.031) using the silver paint from your ORCA kit (290.160). Usually only a tiny bit of paint at the corners of the sample is required. Let the paint dry for a while.
4. Insert the bias wire into one of the unused fluid ports on the cell's perimeter.
5. Place the mounted sample in the center of the cell.
6. Plug the wire end from the bias wire into the socket on the sample.
7. At this point the process is just as if you were using the Electrical Closed Cell. Please continue at Step 7 on page 202.

If you only want to apply a sample bias or ground from some external piece of equipment, simply use one of the Jumper Wires. See Section 15.5 on page 203.

20. Cooler Heater

CHAPTER REV. 1710, DATED 10/23/2013, 21:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

20.1. Overview

The cooler heater was designed to cool and heat small samples in air (or inert gas) or liquid drops from below freezing to above boiling. To go to temperatures significantly below freezing, the included coolant pump must be attached.

Figure 20.1.: Top View of the polymer heater.

20.2. Prerequisites

This accessory is a variant of the closed fluid cell discussed in Chapter 14 on page 160. You should be familiar with the closed fluid cell before you embark on this chapter.

It is ALSO assumed that you are familiar with:

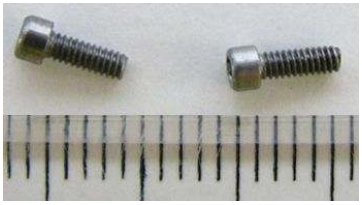
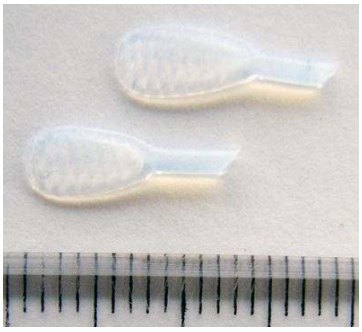
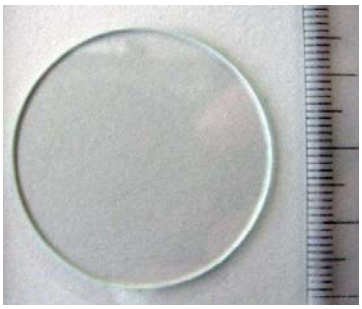
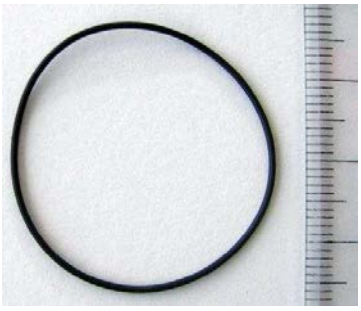
- General operation of the AFM, covered in Chapter 4 on page 15.

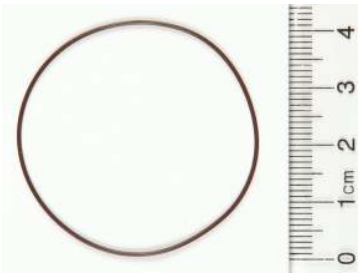
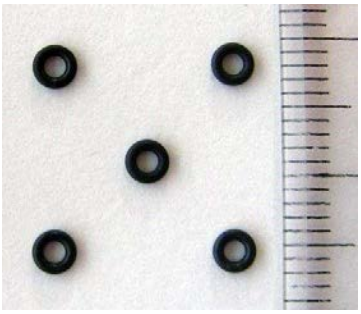
Warning	DO NOT use this accessory with large amounts of fluid. It was designed for use with gases or small amounts of fluid.

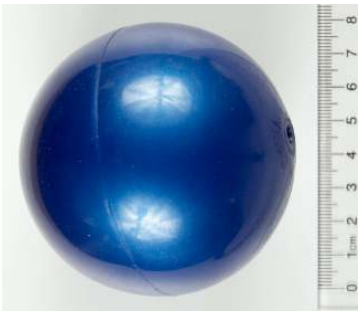
20.3. Parts List

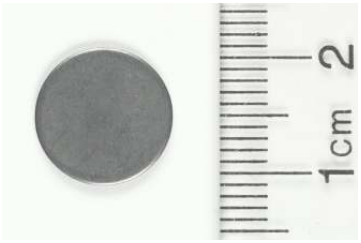
Note Many of the items in the following parts lists are included in the Cooler Heater Accessory Kit (900.172).

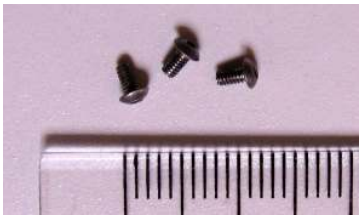
Note The following table will refer to other chapters with regard to components like tubing clamps and O-ring seals that are common across a variety of sample stages.

Itm	Part #	Item Description	Qty	Picture
1	001.SHCS <#0-80X.188> SST	0-80 X 3/16” Stainless Steel Socket Head Cap Screw. Connects 111.886 and 102.204 O-ring clamps to the fluid cell body. See Section 14.4.2 on page 172.	8	
2	111.924	1/16” OD Port Plug. PTFE plugs for blocking unused in/outlets on the closed fluid cell. Also consider using PTFE cord 0.062" diameter (McMaster Carr Part Number 84935K48.) See Section 14.4.1 on page 169.	10	
3	111.425	35mm X 1mm glass disc. Custom made by Asylum, and is made out of Glaverbel float glass. See Section 13.4.1.1 on page 149.	5	
4	230.015	O-ring, 1.228”ID X 0.032”CS, Viton, 75 Durometer. This FKM O-ring makes the seal between the sample disc 111.425 and the fluid cell body. See Section 13.4.1.1 on page 149.	5	
5	111.420	Closed Cell Bottom Clamp. The stainless steel retaining ring which holds the glass bottom or the cover slip holders against the closed fluid cell. See Section 13.4.1.1 on page 149 and following sections through page 190.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
6	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153.	2	
7	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
8	230.011	O-ring, 0.244”ID X 0.016”CS, Viton, 55 Durometer. Seals the cantilever holder quartz prism. See Section 11.2.4.1 on page 101.	3	
9	230.012	O-ring, 1.595”ID X 0.030”CS, Viton, 55 Durometer. This FKM O-ring makes the seal between the bellows membranes and the sealed cell body. See Section 14.4.5 on page 173.	5	
10	230.018	O-ring, 0.062”ID X 0.032”CS, Viton, 70 Durometer. FKM O-rings that seal around the four 1/16" fluid ports. See Section 14.4.1 on page 169.	15	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
11	231.006	Tubing, PFA, 1/16"OD X .040"ID. This PFA tubing makes it possible to introduce and remove fluid or gas from the closed fluid cell. Order from Asylum or purchase directly from Upchurch Scientific (p/n 1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	5 ft	
12	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16" OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
13	290.103	3C Tweezer – Extra Fine Sharp – Standard Grade. For placing samples, tiny o-rings (e.g. 230.018) , and small screws.	1	
14	290.111	0.050": Wiha Allen Driver 263 1,3 – 0.05" X 40. For all socket head screws, for instance used for sealing fluid ports.	1	
15	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
16	939.007	Membrane Clamp, DISCONTINUED. Replaced by 939.015.		
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
17	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
18	939.015	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173. **Warning**: Not following the instructions and not leak testing before use will seriously damage your AFM from fluid spills.	1	
19	112.204	Fluid Port Clamp Bar. Stainless Steel. Compresses and seals two mini O-rings 230.018. Fastens with 0-80 X 3/16” screws. Part of 1/16” fluid ports. See Section 14.4.2 on page 172.	2	
20	080.105	12mm AFM Disc.	10	
21	900.170	Cooler Heater Sample Stage.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
22	900.171	Coolant Pump. See Step 8 on page 267 about adding coolant. Also see Figure 20.8 on page 278.	1	
23	290.129	Coolant. Can be used undiluted. Protects the cooler heater from internal corrosion. See Step 8 on page 267.	1	
24	0-80 X 1/8 BHCS SS	0-80 X 1/8" Button Head Cap Screws, SS. Used to attach items to the three threaded holes in the cooler heater body.	12	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
25	449.011	Cable CB25M-DB25F, 2 meters. Shield in tact.	1	
The scale in the photos is in cm and mm.				

20.4. Product Overview

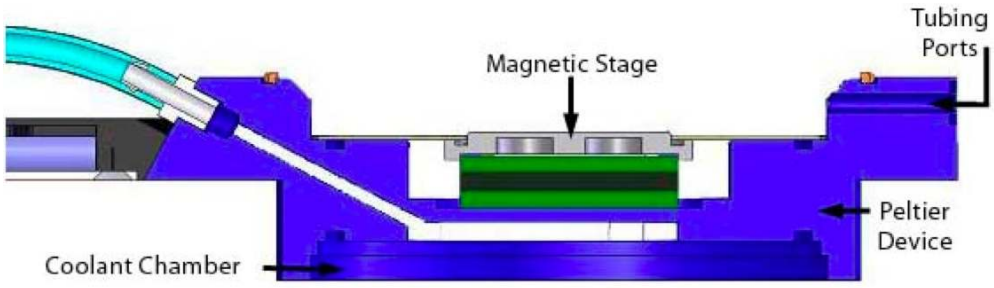
The CoolerHeater stage is another variation of our family of closed environmental chambers. Like the Bioheater, High Temperature Heating stage, Petri Dish Heating stage, and Humidity stage, the CoolerHeater stage is driven through the Environmental Controller (EC). The CoolerHeater stage utilizes a Peltier thermoelectric device to achieve an operating temperature range of –20º to +120ºC. A flow-through liquid cooling system is supplied to allow the stage to reach temperatures below ambient conditions. Although the system is primarily designed to operate in liquid and air environments above 0ºC and non-liquid below 0ºC, it is also possible to configure the stage to operate in a closed cell configuration where specific gas environments are required regardless of operating temperature.

Like the other environmental systems, there is an integrated software control panel that allows for control of temperature as well as coolant flow rate.

20.5. The Heater Cooler Stage

Some key points of interest in the stage are:

- Samples are typically mounted to a magnetic steel puck. A supply of pucks comes in the accessory kit.



- **Side view cross section**
  - The side view cross section shows that only the center part of the cooler heater is temperature controlled. The entire perimeter of the sample chamber is thermally connected to the hot side of the Peltier element.
  - Fluid operation is therefore only recommended for a small liquid drop which does not spill over to the "blue" areas, which are not temperature controlled. If you are having trouble containing the liquid, consider the small volume fluid insert described in Section 20.14 on page 273.

20.6. Setup

20.6.1. For -20 to +120C operation:

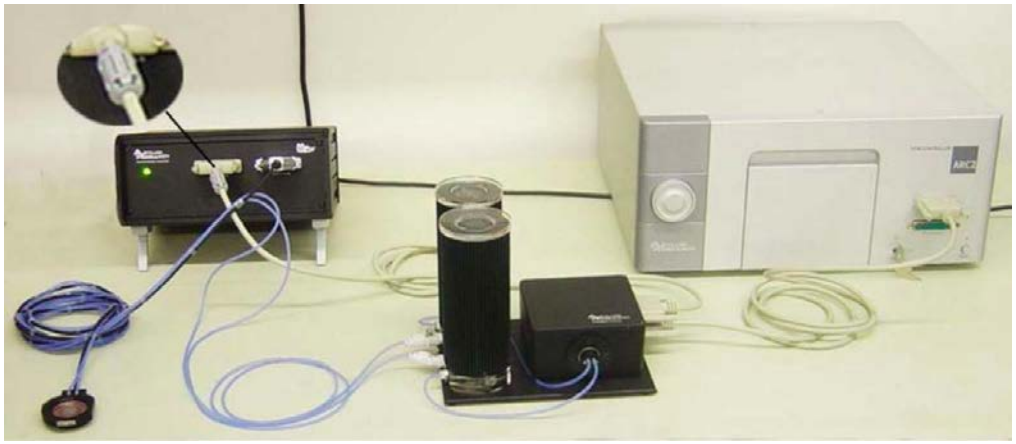


Figure 20.2.: Full setup with coolant pump, for -20 to +120C operation.

This configuration allows for –20º to +120ºC temperature range.

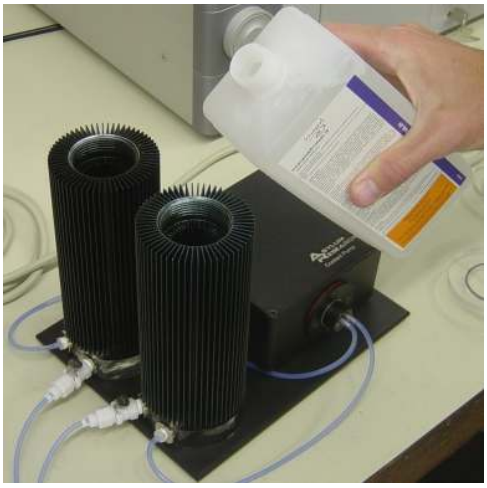
1. Use the 25-25 pin signal cable (449.011) provided with the CoolerHeater system to connect between the MFP3D controller's expansion port and the Coolant Pump housing.
2. Use the 25-25 pin signal cable (448.023, with gray sleeve) provided with the Environmental controller (see Chapter 16 on page 205) to connect the Coolant Pump assembly.

   Note: The 25-25 pin signal cable with the gray sleeve attached is only intended to be connected to the Environmental controller. This cable isolates the ground connection of the

environmental controller from the rest of the instrument in order to prevent electrical ground loops.

3. Connect the CoolerHeater stage to the Environmental Controller.
4. Connect the two coolant hoses from the CoolerHeater stage assembly to the Coolant Pump assembly. There is no specific flow direction through the coolant housing; therefore, the connections of the two hoses to the pump are not important.
5. See Figure 20.2 on page 266 for a photo of the fully connected setup.
6. Connect the Environmental Controller to wall power with the supplied power cord.
7. **Open the pump reservoirs:**
   - Remove the tops from the cooling towers.

8. **Add coolant:**
   - Fill each tower between 1/2 and 3/4 full of coolant.
   - Tightly screw the caps back onto the reservoirs.



20.6.2. Setup for -20º to +120ºC

It is also possible to run the CoolerHeater without the coolant pump in circuit. There is no benefit to this configuration other than simplification of the amount of hardware in your system. Configuring the system in this way is the same as the full system connections with the exception of eliminating the coolant hoses and the coolant pump. See Figure 20.3 on page 268 for a diagram of this option.

Note that the 25-25 pin signal cable (448.023, gray sleeve attached) for the environmental controller should still be used to connect the environmental controller to the MFP controller's expansion port.

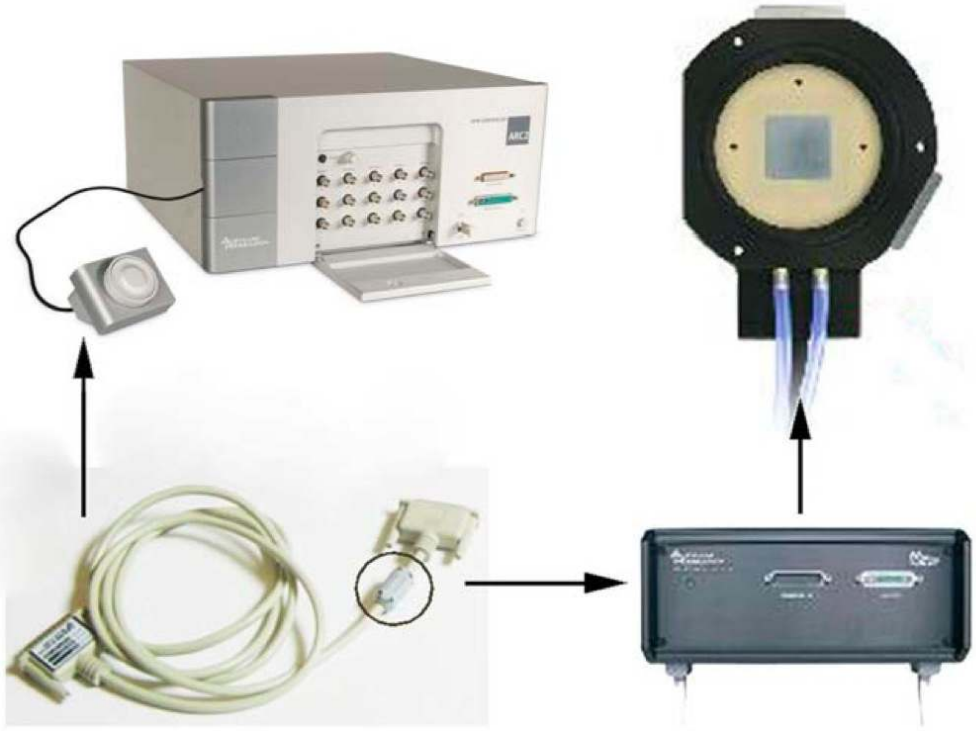


Figure 20.3.: Setup without the coolant pump, for 0 to +120C operation.

20.6.3. Further setup

Once the basic connections are made place the CoolerHeater stage on the scanner. The stage has magnets embedded into the bottom of the stage body that will hold it to the scanner. Place the stage on the scanner so the cable and coolant hoses are routed to exit toward the left. Like with all closed fluid cells, the flat area on the cell body aligns with the rear of the system.

The MPF-3D normally lives in an acoustic enclosure (hood). All the cables are then routed through the cable clamp but this causes issues when changing the setup with regard to the cables and hoses going to the CoolerHeater stage. It is acceptable to build the stage into the cable clamp (and if so then watch out for pinched hoses) but it works best if you have the hoses and cable come out through the gap between the door and the hood. If this happens, you need to make sure that you adjust the hood latches a little bit to allow room for the hoses otherwise they will get pinched and stop the fluid from circulating.

20.7. Testing the Cooler Heater

1. Follow the general setup steps in Section 17.6.1 on page 221.
2. Enter a setpoint of 5°C.
3. Enter a ramp rate of 50°C/min.
4. Set the mode to On.
5. Set the feedback to On.

6. Watch the temperature fall on the history graph. When it is cold you should see condensation on the stage.
7. Turn the pump on by setting “pump speed” to 50%.
8. Lower the temperature setpoint to -10°C.
9. Frost should form on the stage. See Figure 20.4 on page 269 for an example of what your temperature history might look like.
10. Turn the pump off, the mode to off to conclude the test.

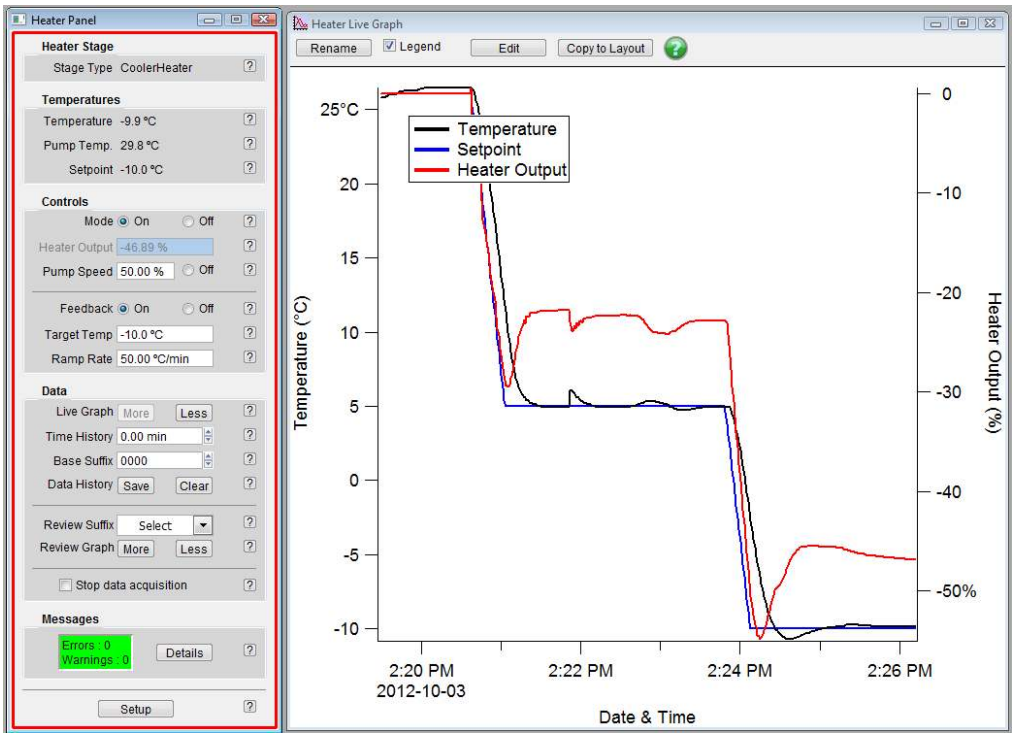


Figure 20.4.: Test of the Bioheater. What you might expect to see. Note that the temperature control will be less noisy if the cell is covered. You’ll discover this when using it inside the AFM.

20.8. Sample mounting

The standard method is to glue the sample to a magnetic AFM disc, some of which are supplied with your system. A small dab of a basic epoxy (like 5 minute epoxy) should be sufficient. Be sure to not use double sided tape or glue tabs. For a few more ideas on sample mounting please see Section 18.6 on page 232.

The sample stage is made of a very hard tungsten based alloy, and should withstand scratching from tweezers or other steel tools.

20.9. Operation

Simply operate the temperature controls as you did on the tabletop test in Section 20.7 on page 268. To minimize thermal drift effects, consider using the image stabilization software feature described in *Hoods and Isolation User Guide, Chapter: Software Options for Image Stabilization*.

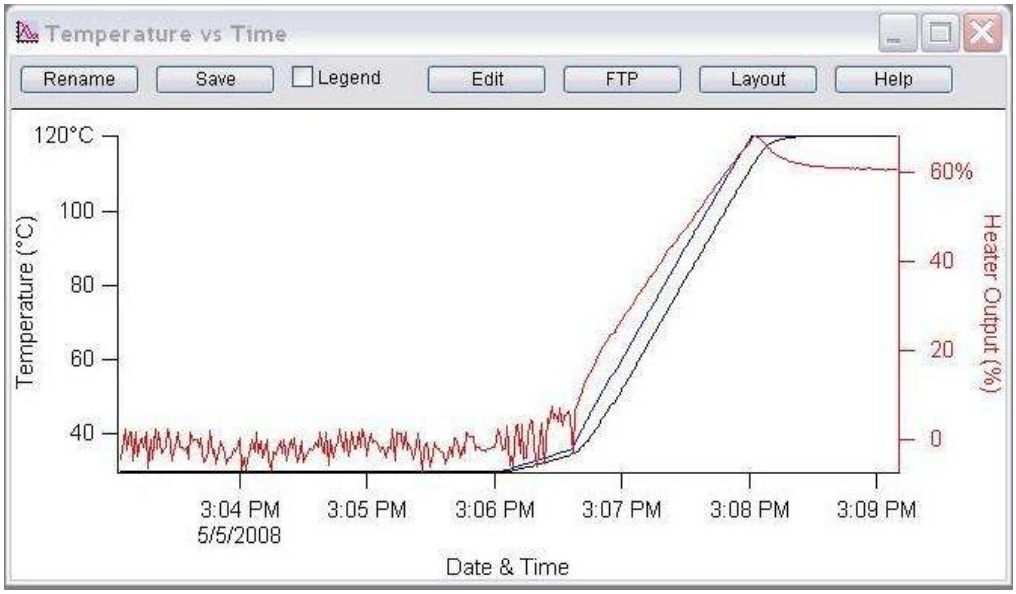


Figure 20.5.: The latter part of this graphs shows the heater power as the temperature ramps up to 120C.

20.9.1. Heating

When heating it is a good habit to note the heater power percentage as the system is heating. Typically heating the stage to 120ºC will result in the heating power running at around 60% (see Figure 20.5 on page 270).

20.9.2. Cooling

The Heater Output percentage is an important indicator that should be noted during cooling experiments. The typical range of the heater output should be kept within +/-85%. In cooling experiments, the Peltier device draws current thus generating heat. When temperatures become lower than 0ºC, the system's coolant pump is needed to circulate fluid in order to draw off excess heat from the sample stage. As the set temperature becomes closer to the limit of -20ºC, the Peltier device requires increasing current flow to reduce temperature. This in turn creates more heat which then requires greater heat transfer into the coolant. This effect is most noticeable between -15ºC and -20ºC. It is important to monitor the Heater Output percentage when temperature feedback is used during experiments below 0ºC to ensure that the heat removal from the sample stage is adequate to prevent the power output from going beyond -85%. If the stabilized cooling temperature is reached but the heater power climbs during the experiment or requires more than -85%

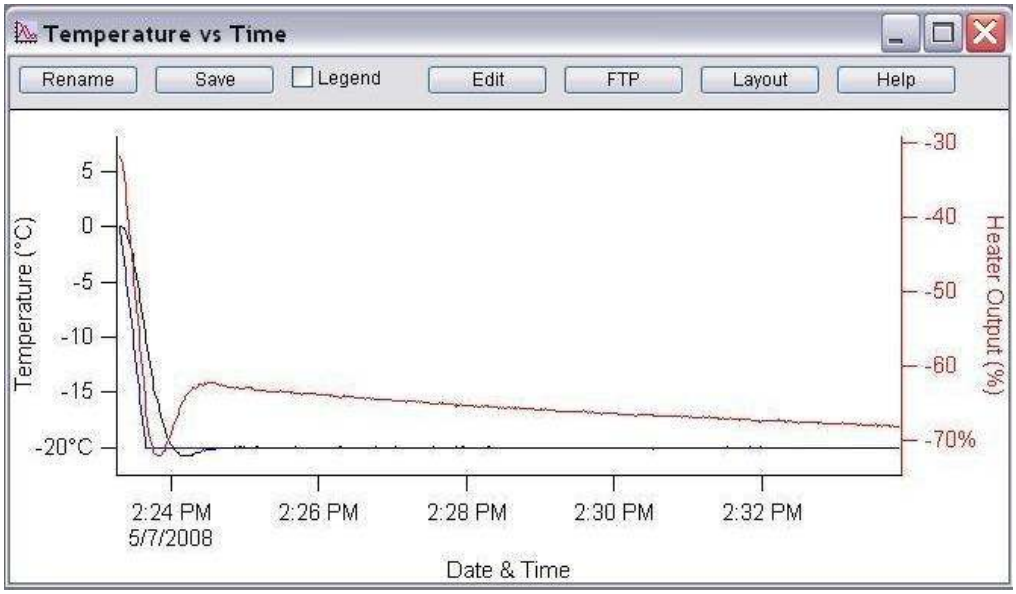


Figure 20.6.: The latter part of this graphs shows the heater power as the temperature ramps down to -20C. Note the steady increase in cooler power due to the body of the cooler heater warming up and the Peltier has to work harder to maintain the temperature setpoint.

to maintain temperature, the surrounding ambient temperature must be lowered or the coolant temperature must be lowered. The plot below shows the heater power slowly increasing in order to maintain -20ºC. The plot was taken with the coolant pump flowing at a rate of 600. We have found that -20ºC can be maintained with a heater output of ~-85% given a lab temperature of 21ºC.

20.9.3. Inert Gas Purge

For a typical laboratory the relative humidity will be between 40 and 70 percent, or a dew point range between 10 and 20°C. I other words, below 10 or 20°C water will start to condense onto the sample in a typical lab. Below 10°C the condensation will become significant and below 0°C it will turn into frost. Purging with dry gas will alleviate most of these problems. Please follow the directions for the closed fluid cell on dry gas purging in Section 14.5 on page 181.

20.9.4. Operating the coolant pump

The speed of the cooling pump is controlled through the “pump speed” control in the heater panel. Typically run it at as low a speed as you can while cooling. It’s not required when heating. If you find you cannot reach your cold temperature setpoint, try turning up the pump speed. If that’s still not enough, resort to Section 20.12 on page 272.

20.10. About Peltier Devices

Peltier devices work by creating a thermal difference between two heating plates. As one or the other plates are heated, the thermal difference between them causes the heating or cooling of the

sample stage. An indication of activity of the Peltier device can be seen by monitoring the direction of the heating current seen in the heating power output in the Heater control panel. Positive current flow results in heating (red box next to the Heater On/Off status). Negative current flow cools the stage indicated by the heater status box changing to Blue. Monitoring the heater output percentage is especially important during cooling experiments where the sample is held below 0ºC. This is explained further in the cooling operation section. The first step in learning the limits of the system, as well as becoming familiar with the controls, is to operate the instrument without a sample or scanning experiment being performed. Due to thermal loss from the surrounding air, you should cover the top of the cell while you are operating the cell controls. You can use a business card or an adhesive note to cover the top of the cell.

20.11. Finding the lower limit of the cooler in your lab

A good way to test the cooling limit of the CoolerHeater system in your lab is to set the Feedback to Off and set the Heater Output to –85%. This will set the heater power to its lower operating limit without allowing the system to go into feedback runaway.

1. Place the CoolerHeater stage on the MFP-3D scanner.
2. Place a cover over the stage to keep the surrounding air from affecting the operating temperature.
3. Set the Feedback control to Off.
4. Set the Heater Power to –85%.
5. Monitor the Current Temperature parameter with the coolant pump off. Allow adequate time for the temperature to stabilize. You should see the temperature stabilize within 10-20 minutes.
6. Turn the coolant pump on and monitor the Current Temperature. The coolant pump operates at a good flow rate when set to around 500-600. Setting the pump to its maximum range of 700 will not remove heat much faster so it is typically not necessary or desired, due the possibility of the motor stalling. You should see the current temperature drop to between –15º and –20ºC. Allow adequate time to stabilize. Usually within 20 minutes you will see the lower limit of the system's cooling range.

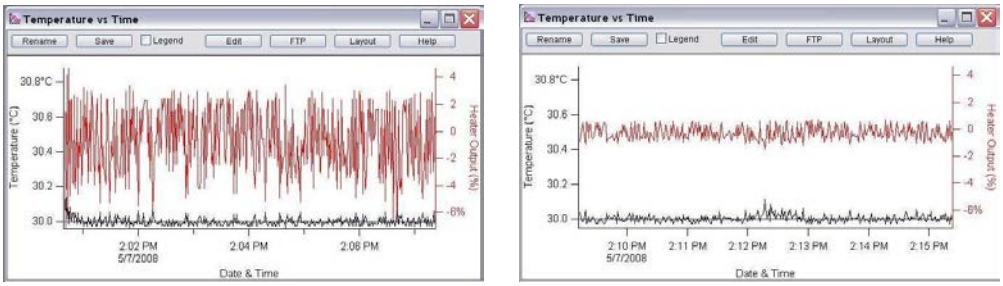
20.12. Cooling below -20C

The coolant reservoirs can be detached from the pump by removing a screw from the bottom. With extra tubing (please contact Asylum Research and we will send you some, or purchase some generic silicone tubing with ID 1/16" and OD 1/8", or metric ID ~1.5mm, OD ~ 3.0mm) the container between the pump outlet and the cooling cell (there is a arrow on the pump head which indicates the direction) can be placed in an ice bath, or in a bath circulator filled with cold temperature controlled antifreeze fluid. At Asylum we have reached below -35C using this method.

20.13. Operating the CoolerHeater near Room Temperature

The CoolerHeater stage has a window of operation near room temperature where very little heater power may be needed to maintain the setpoint temperature. By maintaining the temperature close to ambient with temperature feedback enabled, the heater power may end up switching on and off or alternate between positive and negative power conditions. We have seen this condition cause scan line noise in the AFM image. Typically the noise appears as line-by-line variations or abrupt height changes in a single scan line. The temperature plot below shows this condition as it relates to the heater power while maintaining a temperature of 30ºC. Notice the heater power fluctuates a total of 8% and is switching between positive and negative states. See Figure 20.7a on page 273 for an example.

You may try to either turn the coolant pump on or off, or even place one of the coolant reservoirs in ice water to shift the base temperature of the cooler heater and force it either into a cooling only or heating only state to dodge this issue, or read on about the low noise checkbox.



(a) Normal Feedback **(b)** Low Noise Feedback

Figure 20.7.: Controlling Temperature near room temperature with and without the low nose option.

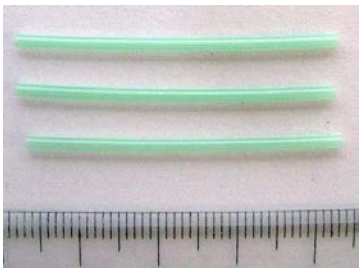
20.13.1. Low noise checkbox

In software release versions newer than 070111+0830 there is a checkbox available which reduces the feedback correction gain used to maintain the current temperature. Reducing the feedback gain allows the thermal stage to operate at temperatures closer to ambient with lower switching noise from the heater power output. A trade off for using this feature is that the feedback response is slower and will regulate the temperature with a lower tolerance. You may wish to disable the low noise feature when working outside of the noisy operating region of the Peltier device for better temperature control. Notice in Figure 20.7 on page 273 below that the heater power deviates 2% while switching between positive and negative states.

20.14. Fluid Insert Accessory (900.207)

Working with a small liquid drop which is held by surface tension between the sample and cantilever holder is always preferred. Filling the Cooler Heater full of liquid will create a thermal short circuit between the cooled (or heated) central sample stage portion and the metal parts on the perimeter. In response to the need for the whole Cooler Heater to be filled with fluid, we developed a chemically inert Teflon insert which forces the liquid primarily to the center of the Cooler Heater.

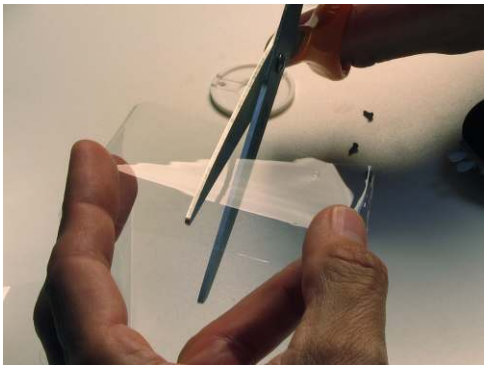
20.14.1. Parts List

Itm	Part #	Item Description	Qty	Picture
1	001.BHCS <#0-80X.188> SST	0-80 X 3/16” Stainless Steel Button Head Cap Screw. Connects 113.751 small volume insert to the Cooler Heater. See Step 3 on page 276.	6	
2	080.010	5 ml Syringe. We prefer this Norm-Ject model from Henke Sass Wolf (HSW) since it does not contain any rubber and contaminates fluids minimally. See Section 14.4.3 on page 172 on how to attach tubing.	2	
3	113.751	Cooler Heater small volume fluid insert. Made of pure PTFE. See Step 2 on page 275.	1	
4	207.011	Gold plated 10mm AFM specimen disc. Used for superior corrosion resistance for sample mounting. Can also be purchased from TedPella.com, part number 16207-G.	2	
5	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172. Can also be bought from idex-hs.com (part number P837).	2	
6	231.019	Tubing Sleeve, green. 1/16” OD X 1/32” ID X 1.55”. Used to connect thin Teflon tubing to the Cooler Heater, and also to the syringes. (see Step 6 on page 276) Can also be bought from idex-hs.com (part number F-247x).	6	

Itm	Part #	Item Description	Qty	Picture
7	231.028	Tubing, FEP (Teflon), Natural, 0.016" ID, 1/32” OD. Must be used with 231.018 sleeving. See Step 6 on page 276.	5 ft	
8	279.091	FEP Fluoropolymer tape, 0.001"THK, Roll. See Step 1 on page 275.	50 ft	
9	290.104	0.035”: Wiha Allen Driver. For the screws (BHCS 0-80X1/8" SS,) that hold down the teflon insert (113.751). See Step 3 on page 276.	1	

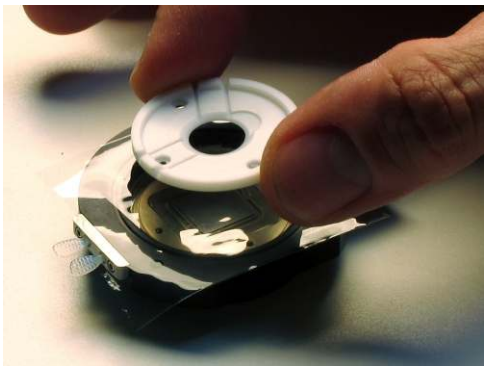
20.14.2. Assembly Instructions

1. **Cut Foil:**
   - With scissors or a sharp knife, cut a 2” piece of the FEP foil (231.028).



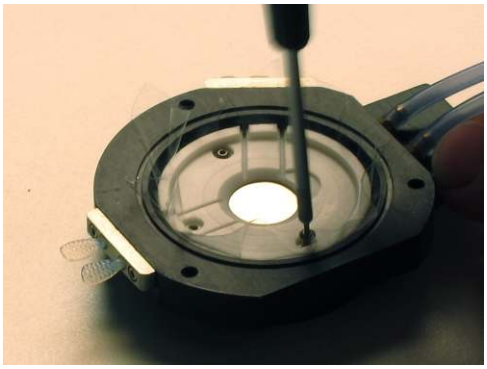
2. **Place Parts on the Cooler Heater:**

- Place the foil over the cooler heater.
- Place the teflon insert (113.751) on top, aligning the three screw holes.
- Press down firmly on the insert, allowing the foil to deform.



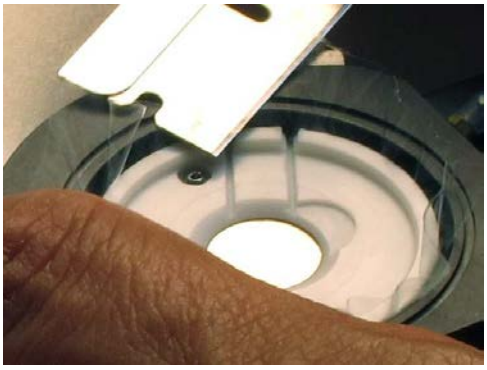
3. **Fasten Insert:**

- Using the 0.035” hex driver (290.104), attach the insert with three of the supplied screws (001.BHCS <#0-80X.188> SST).
- You can use the tip if the tool to first punch through the foil, but usually the screw itself will do the job.
- Tighten only until you start to feel resistance. Do not over tighten.



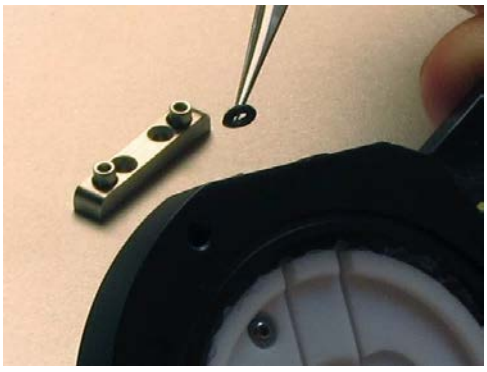
4. **Trim Excess Foil:**

- Preferably using a fresh blade or scalpel, trim the excess foil so that it is flush with the rim of the cooler heater.
- Be careful not to nick or cut the cooler heater or the insert (or your fingers for that matter).



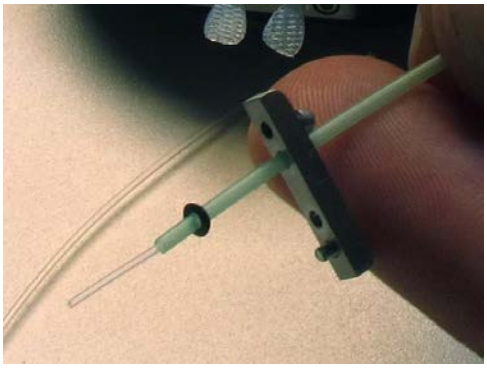
5. **Remove Fluid Port Clamp:**

- Skip to step Step 8 on page 277 if you will not require fluid exchange.
- Remove one of the fluid port clamps (112.204) using the 0.050” hex driver (290.111).
- In case they stick, remove one of he O-rings (230.018) using tweezers.



6. **Assemble Tubing:**

- Cut enough of the thin 1/32” OD tubing (231.028) to connect to a syringe.
- Thread a green tubing sleeve (231.019) over the tubing.
- Thread on the fluid port clamp (112.204) and O-ring (230.018) as shown.

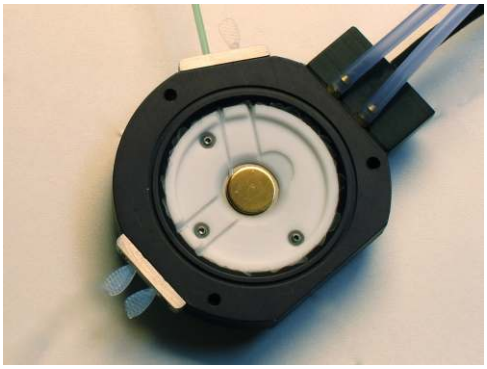


7. **Re-assemble the Clamp:**

- Put the clamp back onto the Cooler Heater.
- Put a port plug (111.924) in any remaining open ports, or attach other pieces of tubing.
- Wait until the next step to fully tighten the screws.

8. **Place Sample:**

- Place your sample, preferably mounted to a gold coated AFM disc (207.011).
- If applicable, adjust the tubing so it reaches the disc, and then press it down into the groove.
- Now tighten the screws on the fluid port clamp.



20.15. Electrical Connections to the Sample

When performing conductive AFM (See Chapter 21 on page 279) experiments that require sample cooling and heating, the Cooler Heater can be used with the Environmental Cell Electrical Connectivity Kit . This kit has everything required to make electrical connections to the sample.

20.15.1. Electrical Connection Tutorial

This tutorial sets up a sample in the Humidity Sensing Cell, ready for ORCA conductive AFM imaging.

1. Please refer to Table 21.2 on page 290 and select the necessary parts from the kit.
2. Place your sample on an AFM disc with a socket (939.031) using the silver paint from your ORCA kit (290.160). Usually only a tiny bit of paint at the corners of the sample is required. Let the paint dry for a while.
3. Insert the bias wire into one of the unused fluid ports on the cell's perimeter. Choose a port which allow the ORCA cantilever's bias wire to connect, toward the left of the instrument.
4. Take a mica disc from the kit and place it on top of the polymer heater.
5. Place the sample no top of the mica disc and it will be held down magnetically. The mica disc insulates the sample electrically from the metal below. If you worry about thermal conductivity, place a tiny bit of silver paint under the mica and also under the sample, or a tiny dollop of thermal grease. Consider using http://www.arcticsilver.com, readily available in computer shops or on the internet.
6. At this point the process is just as if you were using the Electrical Closed Cell. Please continue at Step 7 on page 202.

If you only want to apply a sample bias or ground from some external piece of equipment, simply use one of the Jumper Wires. See Section 15.5 on page 203.

20.16. A Little History

At some point in 2011 the a supplier discontinued the coolant pump reservoirs. We replaced them. See20.8 for examples of the two styles. Both perform the same.

(a) Old Style **(b)** New Style

Figure 20.8.: Coolant Pumps.

21. Conductive AFM (ORCA) Hardware

CHAPTER REV. 1671, DATED 10/16/2013, 22:18. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

This chapter describes the hardware required to perform conductive AFM with the MFP-3D AFM. Conductive AFM Theory and SPM software instructions are described in another manual: *Applications Guide, Chapter: Conductive AFM*.

21.1. Prerequisites

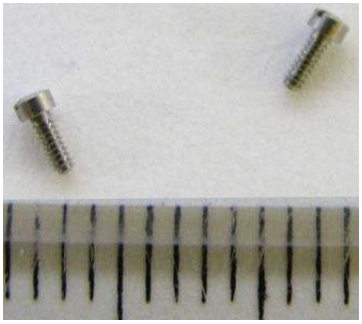
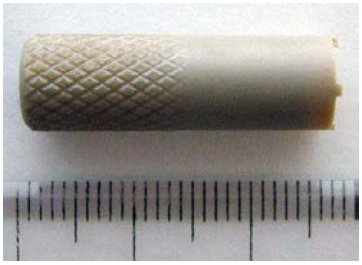
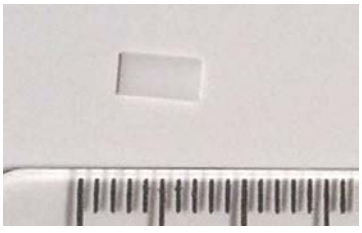
Conductive AFM Imaging is a fairly advanced technique. It is assumed that you are proficient at:

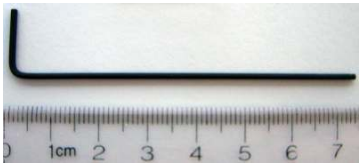
- Basic AFM Safety (Chapter 26 on page 402).
- AC Mode Imaging in Air (Chapter 4 on page 15).
- Contact Mode Imaging, described in another manual: *Applications Guide, Chapter: Contact Mode Imaging*.

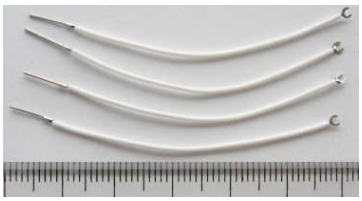
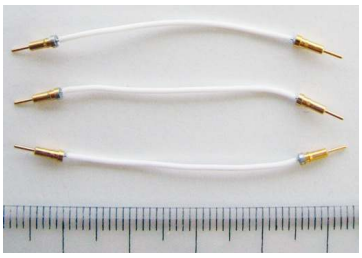
21.2. Parts List

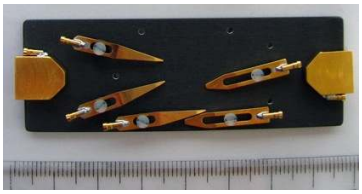
The following list contains all of the parts in your accessory kit. The table is useful as a visual table of contents with links directing you to the specific uses of each part. When ordering parts, please refer to the part numbers in the second column.

This kit has asylum part number 900.231.

Itm	Part #	Item Description	Qty	Picture
1	001.FILL #000 - 120 X 0.094 SST	000-120 X 3/32” Fillister Head Stainless Steel Screws. Item A in Figure 11.2 on page 102. Use with screwdriver 290.110. A few are included since these screws, while standard, can be tricky to find outside the USA.	2	
2	111.725	Fluid Cell Ring Tool. Used to adjust retaining retaining ring C in Figure 11.2 on page 102. Used only during disassembly.	1	
3	111.737	Modified 0-80 Screw. Used to attach the cantilever clip to the body. Note that these screws have been machined to a nonstandard length. You must only use this Asylum part number. Item J in Figure 11.2 on page 102.	4	
4	111.738	Modified 1-72 Screw. Tighten the cantilever under the clip. Note that these screws have been machined to a nonstandard length. You must only use this Asylum part number. Item H in Figure 11.2 on page 102.	5	
5	112.495.02	Coupling Pad 0.015”. Ensures the mechanical contact between the AC mode shake piezo and the cantilever holder. Without this pad, AC mode imaging is not possible. Item F in Figure 11.2 on page 102.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
6	208.06	SCMagnet .17” Diameter X .200” L Unpainted		
7	230.011	O-Ring, 0.244” ID X 0.016”CS, Viton, 55 Durometer. Item E in Figure 11.2 on page 102.	5	
8	290.106	#00 Phillips WIHA Screwdriver 261 PH 00 X 40. Used for tightening item A in Figure 11.2 on page 102. Used on a regular basis when inserting and removing cantilevers.	1	
9	290.110	WIHA Screwdriver, Flat Tip 260 1,5 X 40. Used on item A in Figure 11.2 on page 102. Used only during disassembly.	1	
10	290.116	0.050” Ball End Allen Wrench. Used when disassembling the cantilever holder for cleaning. See Step 4 on page 106.	1	
11	290.160	Leitsilber Conductive Paint, 0.5 Oz. For conductively mounting samples to an AFM disc.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
12	439.028	Micro Alligator Clip. See Section 21.3.5 on page 286.	1	
13	448.017	Orca Wire Assembly. See Section 21.3.4 on page 285.	1	
14	448.xxx	ORCA test resistor. Find your model of ORCA cantilever holder in Section 21.3.1 on page 283 or Section 21.3.2 on page 284. See Section 21.3.5 on page 286 on how to us this resistor.	1	
15	448.021	1.5” ORCA sample holder wire assembly. See Section 21.4 on page 287.	1	
16	803.OLY. AC240 TM	Olympus Electri-Levers, Model AC240TM.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
17	900.150	ORCA (Electro Contact) Sample Holder. See Section 21.4 on page 287.	1	
The scale in the photos is in cm and mm.				

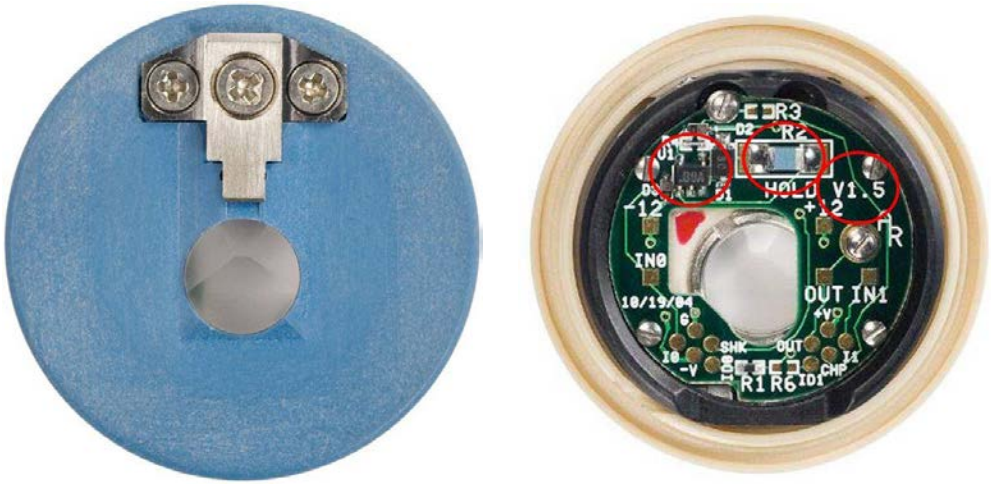
21.3. ORCA (Conductive AFM) Cantilever Holders

The ORCA conductive AFM cantilever holder can, via a small tether wire, apply a software controlled voltage to an electrically isolated sample. The on board electronics hold the tip at virtual ground and measure the current flow between tip and sample using a low noise transimpedance amplifier (current to voltage converter) built into the cantilever holder.

The ORCA cantilever holder is identical to the standard cantilever holder (Section 11.2 on page 99) in nearly all respects. It can be used for contact mode and AC mode imaging, as well as in fluid (when not performing current measurements). It is disassembled and cleaned in the same way. However, since the metal clip is part of the current measurements system and sits at virtual ground, it cannot be used for techniques which require the application of a DC tip bias voltage (like KPFM, see *Applications Guide, Chapter: Scanning Kelvin Probe Microscopy (SKPM)*).

The orca cantilever holder comes in various varieties described in the next few sections.

21.3.1. Standard ORCA Cantilever Holder



(a) Front side.

(b) Back side and identifying features. The circled components are only present on the ORCA cantilever holder, and not on the standard cantilever holder.

Figure 21.1.: Standard or Single Gain ORCA Cantilever Holder.

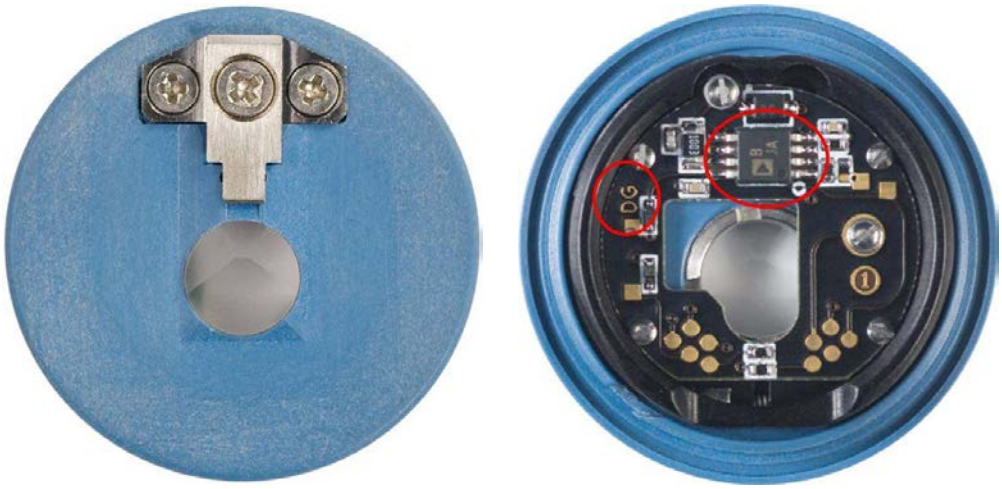
The standard (Single Gain) ORCA cantilever holder can be purchased in a variety of preconfigured current ranges. At the time of purchase you will need to select the one appropriate for your experimental requirements.

- The ID is the number printed on the side of the black ring on the circuit board side of the cantilever holder.
- Test resistors are provided for testing the operation of the transimpedance amplifier.

Part #	Gain R	ID	Sensitivity	Current Range	Typical Noise (1kHz BW)	Test Resistor (AR part number)
908.027	500kΩ (5e5)	6	2µA/V	±20 µA	1nA	5 MΩ (448.030)
908.028	5MΩ (5e6)	7	200nA/V	±2 µA	75pA	5 MΩ (448.030)
908.029	50MΩ (5e7)	8	20nA/V	±200nA	3pA	500 MΩ (448.018)
908.036	500MΩ(5e8)	3	2nA/V	±20nA	1pA	500 MΩ (448.018)
908.030	5GΩ (5e9)	9	200pA/V	±2nA	0.5pA	1 GΩ (448.054)

21.3.2. Dual Gain ORCA Cantilever Holder

The Dual Gain ORCA cantilever holder is almost like owning two standard ORCA cantilever holders in one package. It has a current to voltage converter for the low gain stage followed by a gain of 1000 for the high gain stage. Both outputs are available simultaneously, though usually only one is meaningful 5Mat any given time. This does come at the expense of some added noise in the high gain measurements.



(a) Front side. **(b)** Back side and identifying features.

Figure 21.2.: Dual Gain ORCA Cantilever Holder.

- The ID is the number printed on the side of the black ring on the circuit board side of the cantilever holder.
- Test resistors are provided for testing the operation of the transimpedance amplifier.

Part #	Gain R	ID	Sensitivity	Current Range	Typical Noise (1kHz BW)	Test Resistor (AR part number)
908.xxx	1kΩ	29	1mA/V	±10mA	150nA	
	x1000		1µA/V	±10µA	1.5nA	
908.067	20kΩ	40	50µA/V	±500µA	7.5nA	
	x1000		50nA/V	±500nA	75pA	
908.045	100kΩ	22	10µA/V	±100µA	1.5nA	100 kΩ (448.052)
	x1000		10nA/V	±100nA	15pA	100 MΩ (448.051)
908.051	1MΩ	14	1µA/V	±10µA	150pA	1 MΩ (448.053)
	x1000		1nA/V	±10nA	4pA	1 GΩ (448.054)

21.3.3. Identifying your ORCA holder

You can visually identify your type of cantilever holder from the previous sections and by looking for an ID number on the cantilever holder ring and look it up in the tables.

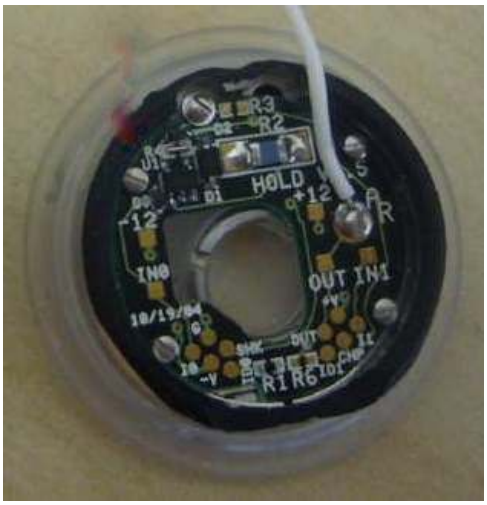
If you have a functioning MFP-3D nearby, you can simply attach the cantilever holder to the AFM head (see Step 2 on page 21) and use the software to identify the type from the *programming* ▷ *Cantilever and Sample Holder panel* menu. Every MFP-3D cantilever holder type has a unique electronic ID.

21.3.4. Attaching the Bias Wire

Skip this section if a white wire is already attached to your ORCA cantilever holder. Typically there is no reason to remove it between imaging sessions.

1. Place the ORCA holder with the circuit board facing up.
2. Locate the small slotted screwdriver (part 290.110) and a spare bias wire (448.017).
3. **Attach the bias wire:**
   - Loosen by a few turns the screw which in the photo has the bias wire leading to it.
   - Position the C shaped ring on the wire under the screw and tighten the screw until snug.

   Note The screw at the top must be present since it connects the circuit to the metal clip on the other side of the cantilever holder. The other four screws only keep the circuit board attached to the cantilever holder.



21.3.5. Testing the ORCA holder

For a multi user facility, it is a good idea to test the ORCA holder before use each time a new experiment is attempted. For a single group system, this is probably not necessary. For older ORCA holders that do not have painted rings on the metal part of the holder (see Figure 21.3 on page 286), caution should be used in loading the holder into the head. If the holder is rotated so that the wrong pogo pins are shorted, the op amp can be damaged and will have to be replaced. Holders with painted rings will not have this problem.

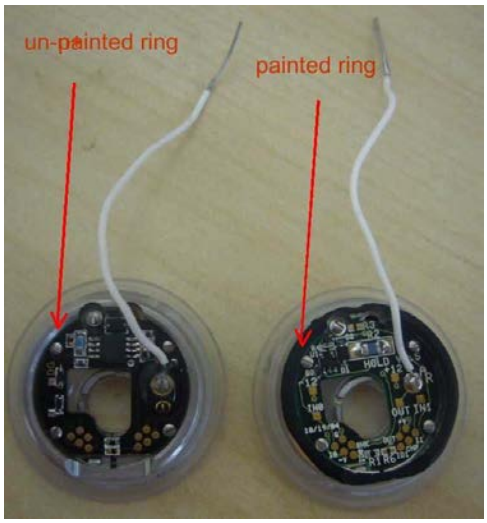


Figure 21.3.: For ORCA holders without the painted ring, please use caution in loading them into the head, as the metal ring can short the op-amp.

To test the holder:

1. See Figure 21.4 on page 287. Locate the test resistor in the ORCA kit (see the tables above for details) , and connect it between the cantilever holder and the bias wire. A convenient way to do this is to put the wire end of the resistor through the small loop in the cantilever clip. Use the alligator clip included with the ORCA kit to connect the bias wire to the resistor. Make sure the clip does not short out on the head. One way to be sure this does not happen is to slide a piece of paper between the clip and the head.
2. Note that for the ORCA holder with sensitivity 1nA/V you will need a 500kΩ resistor. For the dual gain, we typically use a 10MΩ, as that will test both gain stages, or consult the tables above for using different resistors for different current ranges.
3. Once the resistor is in place, open the software. From the menu bar, select *AFM 3D controls ▷ DoIV panel*.
4. Set the amplitude to 1 V, frequency to 1 Hz. Set the function to 'ARDoIVTriangle', and click 'display'. This will show the waveform that will be used to test the resistor.
5. Click 'do it'.
6. Display the curve.
7. The curve should be linear, and the slope should equal the resistance of the test resistor.

A more complete description of current-voltage curves is given below in the I-V curve section in the *Applications Guide, Chapter: Conductive AFM*.

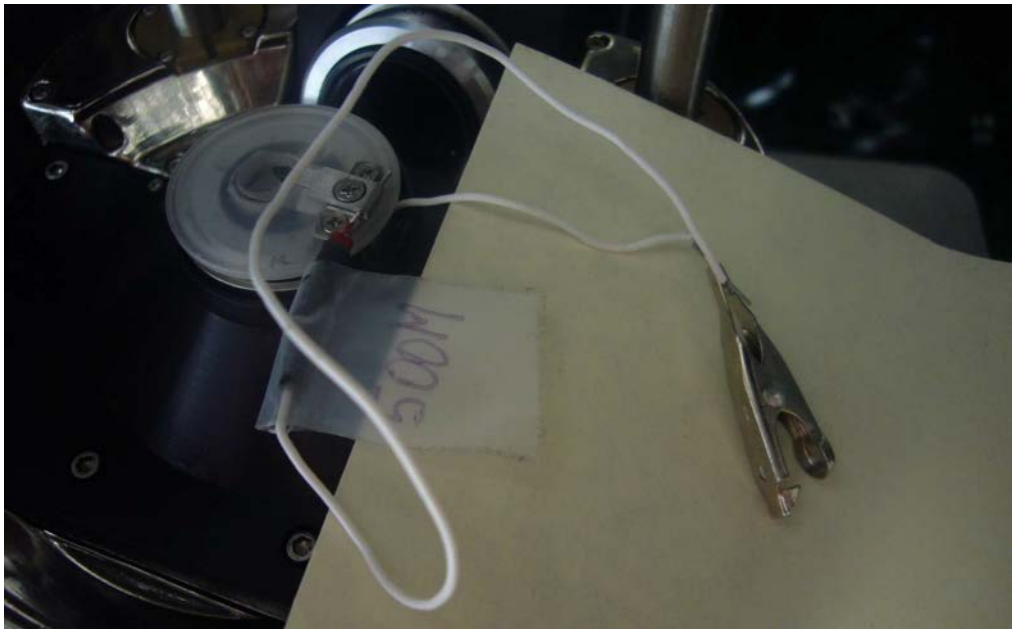
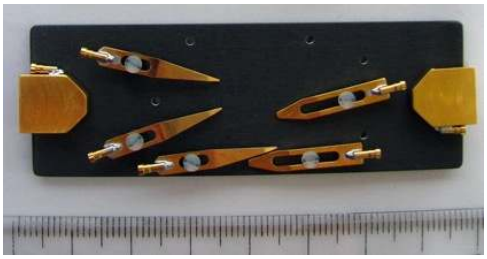


Figure 21.4.: Connect the test resistor in between the bias wire and the probe clip as shown

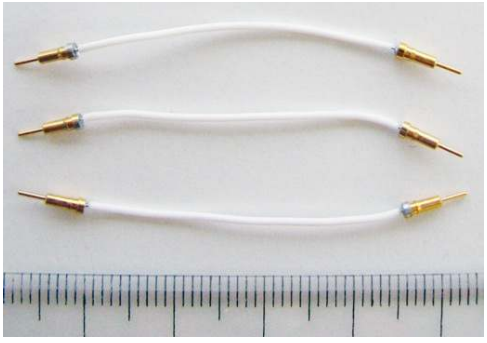
21.4. Sample mounting

Samples must be less than a few cm in diameter and preferably less than 1mm thick.

1. **Mount the sample:**
   - Locate the sample mount (900.150). It is made of aluminum with an anodized non conducting surface. Remove and store any excess clips and plastic screws.
   - Use one of the supplied clips to clamp your sample against the plate. The tip of the clip must make contact with the conducting top surface of the sample.



2. **Connect the clip to the magnet:**
   - Locate a jumper wire (448.021) to connect the end of the clip to one of the magnetic gold plated blocks.



3. Place the whole sample mount on the MFP-3D scanner plate. See Figure 21.5 on page 288

for an example a mounted sample with the jumper wire attached.

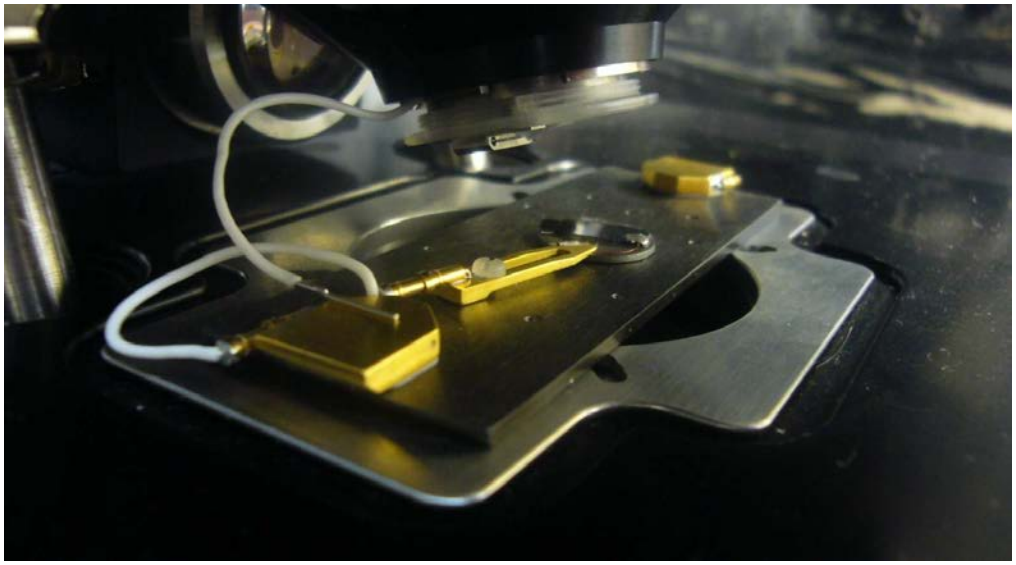


Figure 21.5.: Connect the bias wire that drops from the cantilever holder down to the magnet on the ORCA sample holder magnet as shown.

21.5. Engaging the tip on the sample

1. Adjust the legs of the MFP-3D head so that the tip will sit at least a few millimeters above the sample when the head is placed over the sample.
2. Check that the bias wire sticking out of the cantilever holder is facing toward the rubber button which releases the cantilever holder.
3. When the head is placed over the sample, the end of the bias wire should magnetically attach to the gold plated magnetic block at the edge of the sample mount. See Figure 21.5 on page 288.
4. The bias wire now applies voltage via the metal block, to the clamp, to the sample. When the tip (held at virtual ground) touches the sample, current flows from the surface of the sample into the tip and is measured by the amplifier on the cantilever holder circuit board.

Warning	The black anodization on the sample mount must remain pristine to prevent electrical connections between it and the the top of the scanner. Please use it carefully and store it safely when it is not in use.

21.6. Conductive AFM Imaging

The specifics of conductive AFM imaging are describe in another manual: *Applications Guide, Chapter: Conductive AFM*.

21.7. Environmental Control

For conductive imaging in room temperature inert gas atmospheres, please consider the closed cell specifically designed for that purpose (See Chapter 15 on page 196). For making a flow through gas handling system, please see Section 14.5 on page 181.

In case you require temperature or humidity control, or simply already own a closed fluid cell and would like to use it as a sealed sample chamber with electrical connections, then the best approach is our MFP-3D Environmental Cell Electrical Connectivity Kit. This kit contains everything necessary to make electrical connections to samples mounted inside the

- Closed Fluid Cell. See Section 14.13 on page 195 on how to use the Electrical Connectivity Kit with this closed cell.
- Polymer Heater. See Section 18.11 on page 236 on how to use the Electrical Connectivity Kit with this closed cell.
- Cooler Heater. See Section 19.8 on page 255 on how to use the Electrical Connectivity Kit with this closed cell.
- Humidity Cell. See Section 20.15 on page 277 on how to use the Electrical Connectivity Kit with this closed cell.

Table 21.2 on page 290 shows exactly which components of this kit are necessary for each of the listed accessories.

Note If you don't own all the the closed cells listed above, there will be a few items in the kit which you will not need to use. Table 21.2 on page 290 explains the details.

Itm	Part #	Description	Qty	Picture	CFC	HumC	PHeat	CoolHt
1	448.141	Electrical Closed Cell Bias Wire. Used to make connections inside the polymer heater.	2		-	-	✓	-
2		15mm mica disc. Also available from Ted Pella, part number 50-15. Used to insulted a conducting sample.	4		-	-	✓	✓
3		Closed Fluid Cell Bias Wire. Used to make a connection to socketed AFM discs (939.031) inside of a sealed cell.	2		✓	✓	-	✓
4	939.031	AFM Disc with socket. Used to support conducting samples in closed cells.	2		✓	✓	-	✓
5	208.020	Magnets. Used to secure socketed AFM discs (939.031) in closed cells with glass bottoms.	4		✓	✓	-	-
6	249.033	Sticky dots. Used to adhere magnets 208.020 under closed cells with glass bottoms.	15		✓	✓	-	-
7	939.049	Electrical Closed Cell Jumper Wire. Used to connect bias wires like 448.141 to external equipment.	2		✓	✓	✓	✓

Table 21.2.: Contents of the Environmental Cell Electrical Connectivity kit. A "✓" indicates that the item is used with an a particular cell, and a "-" indicates that it is not.

22. Petri Dish Holder

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

(a) Top view **(b)** With magnetic clamp

Figure 22.1.: Top (left) and side (right) views of the Petri Dish Holder. The photo on the right also shows the magnetic clamp holding down a glass bottom Petri dish.

22.1. Overview

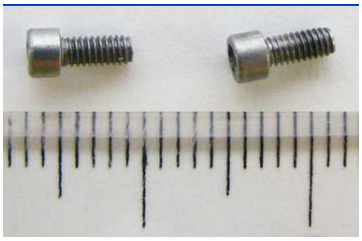
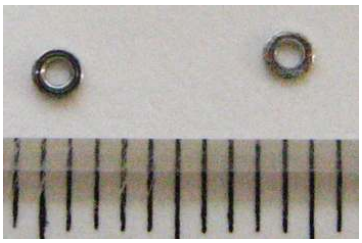
The Petri Dish Holder is, as of March 2008, included with all MFP-3D-BIO systems (Figure 22.1 on page 291). It was specifically designed for researchers who prefer to grow and image cells in culture dishes. The key advantages, as compared to the assembly of our Closed Fluid Cell / BioHeater, are minimal handling of the cells and reduced evaporation rate. The Petri Dish Holder is still compatible with standard microscope slides.

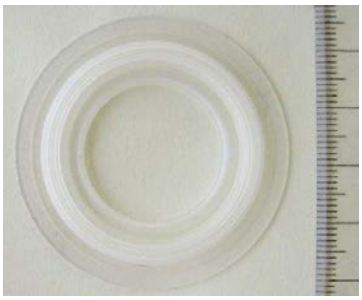
The hole diameter of the sample plate is 16mm. It is large enough for use with all high numerical aperture (NA) objectives, such as a 100x oil immersion lens when using cover slips or dishes with 0.17mm glass bottoms. Due to the hole size, many Asylum Research accessories including the Fluid Cell Lite, Closed Fluid Cell, BioHeater, Polymer Heater, Humidity Sensor, Cooler Heater and Variable Field Module will not fit in the sample plate and require that the original, larger hole plate be re-installed on the scanner.

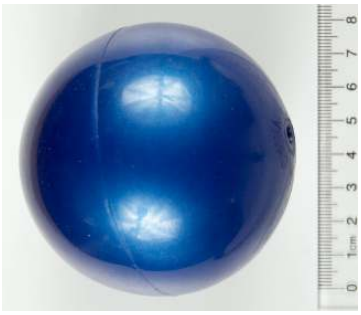
The sample plate includes three grooves (two large and one small) which were designed to accommodate some of the more commonly used Petri dishes (refer to Section 22.6.1 on page 297). The Petri dishes are secured to the sample plate using a magnetic clamp. There is currently only one dish clamp available for the low profile Petri dishes (dish height is 9mm or less). Dishes can also be held in place with grease.

22.2. Parts List

Petri Dish Holder Kit (900.168)

Itm	Part #	Item Description	Qty	Picture
1	001. SHCS <#0-80 X .313> SST	0-80 X 5/16” SHCS S/S. Spare screws for the standard scanner top plate (See Section 22.4 on page 295). Use with 0.050” allen driver.	8	
2	001. SHCS <#1-72 .188> SST	1-72 X 3/16” SHCS S/S. Screws (and spares) for the Dish Holder scanner top plate (See Section 22.4 on page 295). Use with 1/16” allen driver.	16	
3	003. WSHR <#0> FLAT	#0 Flat Washer MS801. Spare washers for scanner top plate (See Section 22.4 on page 295).	16	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
4	080.113	WPI Fluorodish #5040. Sample dish with thin glass bottom.	1	
5	080.115	Falcon 50 X 9MM Dish #351006. Sample dish with plastic bottom.	1	
6	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153 .	2	
7	112.256.02	Closed Cell Bellows, 30 Durometer Silicone rubber. See Section 13.4.3 on page 153.	2	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
8	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
9	290.111	0.050”: Wiha Allen Driver 263 1,3 – 0.05” X 40. Used to remove 0-80 screws on standard scanner top plate (See Section 22.4 on page 295).	1	
10	290.114	Wiha Screwdriver, Slotted, 3.0mm Width 260 3,0 X 50. For adjusting the magnets on the dish clamp. (See Section 22.5 on page 296).	1	
11	290.130	1/16” Wiha Hex (Allen) Driver. Used for 1-72 screws on Dish Holder scanner top plate. (See Section 22.4 on page 295).	1	
12	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
13	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
14	939.012	Magnetic Dish Clamp Assembly. See Section 22.5 on page 296.	1	
15	112.499	Petri Dish Holder scanner top plate.	1	
The scale in the photos is in cm and mm.				

22.3. Hardware Requirements

For new MFP-3D-BIO systems, the Petri Dish Holder Accessory Kit is included with the shipment (but the plate is not installed on the scanner). If you will be imaging samples (i.e. cells) in culture dishes on a regular basis then your installer can swap out the sample plate for you. If you prefer to not have the Petri dish holder plate installed, your installer can at least show you how to do so or you can follow the step by step instructions outlined below.

22.4. Installation and Operation

To replace the existing scanner top plate with a dish heater or holder, please follow the nearly identical instructions for the variable field module: Section 25.5 on page 374. This will also include some software checks on scanner hysteresis.

NOTE: The dish holder and heater use a different screw length from the standard and VFM scanner top plates. Please store the LONGER screws used with the regular top plate and use the SHORTER screws with the dish holder or heater scanner top plates. Also use a different tool for the 1-72 screws.

Top Plate	Screw	Washer	Tool
Standard	0-80 X 5/16” SHCS S/S	#00	0.050” Allen driver (290.111).
Dish Holder or Heater	1-72 X 3/16” SHCS S/S	#00	1/16” Allen driver (290.130).

22.4.1. Some Uncommon Variations:

Current versions of the Petri dish Holder (shipped after Q1 2008) and Heater require 1-72 x 3/16” SHCS S/S screws to attach the plate to the scanner. Older versions (shipped through Q1 2008)

require 0-80 x 3/16” SHCS S/S screws. The two plate versions are visually discernible; the current version has three circular grooves on top while the older plate has only two.

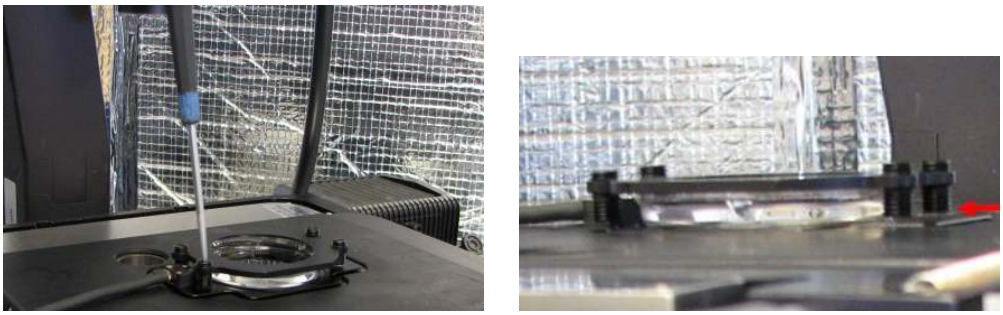
For the high clearance scanner which is required for the Olympus IX81 and the Nikon Ti-series, only four screws can be used. Material on the underside of the scanner where the four center holes are located has been removed and what is left of the holes has been filled with epoxy.

22.5. Petri Dish Clamp

Figure 22.2.: Schematic diagram of complete assembly, in this particular case with the Petri Dish Heater stage.

Place the magnetic clamp on top of the Petri dish to secure it to the plate. If necessary, adjust the position of the magnets on the clamp using a slotted screwdriver so that the bottom of the magnet is 1-2 mm above the surface of the plate. The magnets should not touch the plate (Figure 22.3 on page 297).

The Petri Dish Holder and Heater kits include one ring shaped magnetic clamp (939.012) which is suitable for the low profile dishes. To secure the taller dishes, i.e. the Corning 60mm and 35mm dishes, there are two options. The easiest (and least intrusive) option is to use vacuum grease (such as Dow Corning’s High Vacuum Grease available from SPI). Gently smear the grease across the plate, making sure to avoid the center hole so that the grease does not come into contact with the objectives. You also do not need to use immersion oil. A second option is to use small magnets



(a) Magnet Adjustment **(b)** Side View

Figure 22.3.: Left: Adjusting the clamp screw depending on the height of the Petri dish. Right: Side view of the clamp on a low profile Petri dish showing a 1-2mm space between the magnet and the Petri Heater plate red arrow)

with an inert coating such as gold-plated NdFeB magnetic cubes (~3mm wide). Once the magnets have been sterilized, they can be strategically positioned inside the dish, preferably 3-4 magnets towards the very edge of the dish. The magnets should only be used for short-term experiments, i.e. one day or less.

22.6. Compatible and Incompatible Petri Dishes

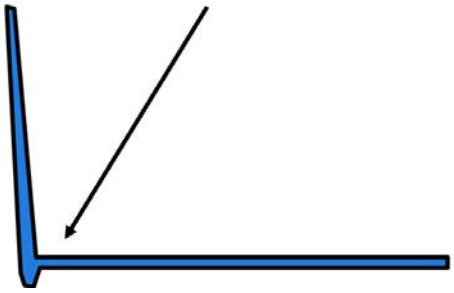


Figure 22.4.: Schematic diagram of side view of Petri dish with lip

22.6.1. Compatible Petri Dishes

Both the Petri Dish Holder and Heater are compatible with some of the more commonly used Petri dishes (Table 22.2 on page 298). Most (if not all) polystyrene dishes are manufactured with a “lip” or ridge on the underside of the dish (Figure 22.4 on page 297). Unfortunately, the thickness of this lip varies between Petri dish models and manufacturers. In order to image cells without any noise issues, the bottom of the Petri dish must be flush with the sample plate (i.e. must completely touch the metal surface).

We have incorporated three grooves in the Petri Dish Holder and Heater to accommodate the lips for some Petri dishes. The maximum height of this lip is 0.020” or 0.5mm. Both glass-bottom dishes listed in Table 22.2 on page 298 have a smooth bottom (without any lip) so they do not have any compatibility issues with the Petri Dish Holder or Heater.

Manufacturer	Model	Notes
Willco Wells*	GWSt-5030	Glass-bottom dish: Sterile, untreated, 50 x 7mm, 30mm diameter cover glass, 0.17mm thick glass well
	GWSt-5040	Glass-bottom dish: Sterile, untreated, 50 x 7mm, 40mm diameter cover slip, 0.17mm thick glass well
WPI	FD5040-100	Glass-bottom dish: Fluorodish: sterile, untreated, clear wall, 50 x 7mm, 35mm diameter cover slip, 0.17mm thick glass well
	FD-5040B-100	Glass-bottom dish: Fluorodish: sterile, untreated, black wall, 50 x 7mm, 35mm diameter cover slip, 0.17mm thick glass well
BD Falcon	351006	Polystyrene dish: Sterile, tight-fit lid, 50 x 9mm
Corning	430166	Polystyrene dish: Sterile, cell-culture treated, 60 x 15mm
	430165	Polystyrene dish: Sterile, cell-culture treated, 35 x 10mm

*available in the USA through BioScience Tools.

Table 22.2.: List of compatible cell culture dishes.

22.6.2. Incompatible Petri Dishes

Although we haven't tested all of the available Petri dishes, we have begun to compile a list of confirmed incompatible Petri dishes (Table 22.3 on page 298).

Manufacturer	Model	Notes
Mattek	P50G...	Glass slide is mounted on the underside of the Petri dish; therefore, the cantilever holder touches to the bottom of the plastic dish before the tip comes into contact with the glass substrate (and sample).
Nunc	150288	The lip on the bottom of the dish exceeds the depth of the groove of the Petri Dish Holder. The bottom of the dish doesn't touch the plate surface and can vibrate, possibly leading to imaging noise.

Table 22.3.: List of **in**compatible cell culture dishes.

22.7. Suggested Imaging Protocol

1. Select one of the approved Petri dishes listed in Table 22.2 on page 298.

2. Adjust the height of the AFM head to avoid crashing the cantilever into the sample surface. This can be done most easily with a spare dish without any fluid. Position the cantilever a few millimeters above the surface. Remove the spare dish.

3. **Deposit oil on the plate:** For the best vibrational performance

   - Dispense a swirl of immersion oil on the plate.
   - Place the dish with fluid/sample (i.e. dish with cultured cells) on the plate.
   - Push it firmly into the plate and gently twist the dish.
   - Observe the immersion oil spread evenly underneath the Petri dish.

   NOTE: Be careful not to use an excessive amount of immersion oil. You SHOULD NOT get oil on the XY scanner as it may flow inside the scanner and cause damage.

4. Place the magnetic clamp on top of the Petri dish to secure it to the plate. If necessary, adjust the position of the magnets on the clamp using a slotted screwdriver so that the bottom of the magnet is approximately 1-2 mm above the surface of the plate (Figure 22.3 on page 297).

   NOTE: Remember that the magnets should not touch the plate.

5. To prevent fluid damage to the head and reduce evaporation, attach the membrane to the cantilever holder by tightening the threaded clamp to the holder using the spanner wrench assembly (Section 13.4.3 on page 153).

6. Don't forget to add a drop of fluid to the cantilever chip to prevent air bubbles. Use the same technique as when imaging in a droplet of fluid (Chapter 8 on page 64).

7. Place the AFM head on top of the XY scanner and gently lower the cantilever into the fluid and towards the surface.

Warning	DO NOT USE more than 2-3ml of fluid in the Petri dish. An excessive amount of fluid will cause it to overflow and damage either the head or scanner or both.

8. Check the head's position with respect to the magnetic clamp and dish. Make sure that no part of the head (i.e. thumb knob, objective, and nose cone) is touching. This is typically not an issue with the low profile dishes (dish height of 9mm or less) but becomes more of a concern with the high profile dishes (dish height of ~15mm).

9. Follow standard fluid imaging procedures outlined in Chapter 8 on page 64.

22.8. Membrane Extensions

When “hunting” for an area of interest in the dish by moving the XY micrometers on the scanner, it may be the case that the membrane will slump into the dish. To prevent this, we make available two sizes of PEEK film which can be attached to the membrane. This gives the membrane a larger outer diameter and also a smooth surface to slide on the edge of the dish or the magnetic clamp. Two sizes are available. The smaller one is best suited for use with the magnetic clamp and the larger one can be better for dishes which are stuck to the dish holder with vacuum grease.

These items may be included in dish holder kits in the future, but if you do not have one, please contact Asylum Research to purchase them.

Itm	Part #	Item Description	Qty	Picture
1	939.041	Large Diameter Evaporation Shield Assembly. PEEK annuli with self stick Acrylic adhesive. Silicone membrane.*	1	
2	939.042	Small Diameter Evaporation Shield Assembly. PEEK annuli with self stick Acrylic adhesive. Silicone membrane.*	1	

* Assemblies made with Viton membranes can be made by special request.

23. Petri Dish Heater

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

23.1. Overview

(a) Top view **(b)** Bottom View

Figure 23.1.: Top (left) and side (right) views of the Petri Dish Holder. The photo on the right also shows the magnetic clamp holding down a glass bottom Petri dish.

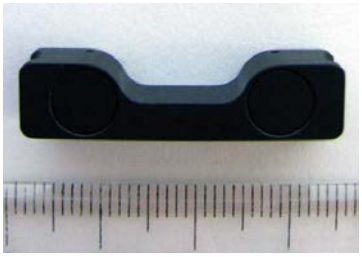
The Petri Heater is an optional accessory specifically designed to maintain biological samples at physiologically relevant temperatures, *i.e.* 37ºC. It is identical to the Petri Dish Holder in terms of opening size and sample compatibility but differs in that it includes a heating element to control the temperature of the sample plate from ambient to 45ºC. The Petri Dish Heater requires the use of the Environmental Controller (Chapter 16 on page 205).

MUST READ! Please read the entire chapter on the Petri Dish holder before you continue: Chapter 22 on page 291.
What follows in this chapter only covers the operation of the heating element.

The temperature across the bottom of a Petri dish will vary some but typically the temperature of the dish above the hole is 0.5ºC and 0.1ºC lower than the perimeter for polystyrene and glass bottom dishes, respectively.

23.2. Parts List

Nearly identical to the Petri Dish Holder Kit (900.169) parts list (See 22.2) , but with a different scanner top plate and a set of special magnets.

Itm	Part #	Item Description	Qty	Picture
		parts 1-14 are identical to the Dish Holder parts list: See 22.2.		
15	939.013	Petri Heater Magnet Holder Assembly. Used to clamp standard microscope slides onto the dish heater (See Section 23.6 on page 305).	1	
16	900.167	Dish heater assembly. Cable and connector not shown.	1	
The scale in the photos is in cm and mm.				

23.3. Software Requirements

AR SPM software version 070111 +0400 or later is required to recognize and run the Petri Dish Heater. In general, it is recommended to use Igor 6 and download the latest version of MFP3D software to benefit from the latest features and bug fixes. See on page iv on getting the latest software.

23.4. Petri Dish Heater Installation and Software Heater Controls

1. Install the Dish Heater to the scanner (Section 22.4 on page 295).
2. Plug in the Environmental Controller, but leave the AC power switch off.
3. Plug the Petri Dish Heater plate cable into the Environmental Controller.
4. Plug the 25-25p interconnect cable from the Environmental Controller into the ARC2 controller (which may or may not be turned on). Make sure that the cable with a gray sleeve

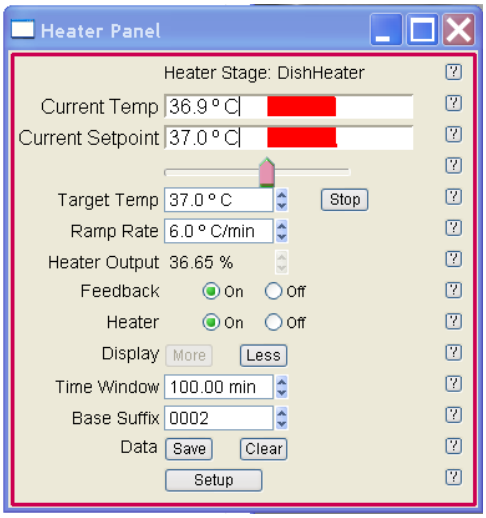


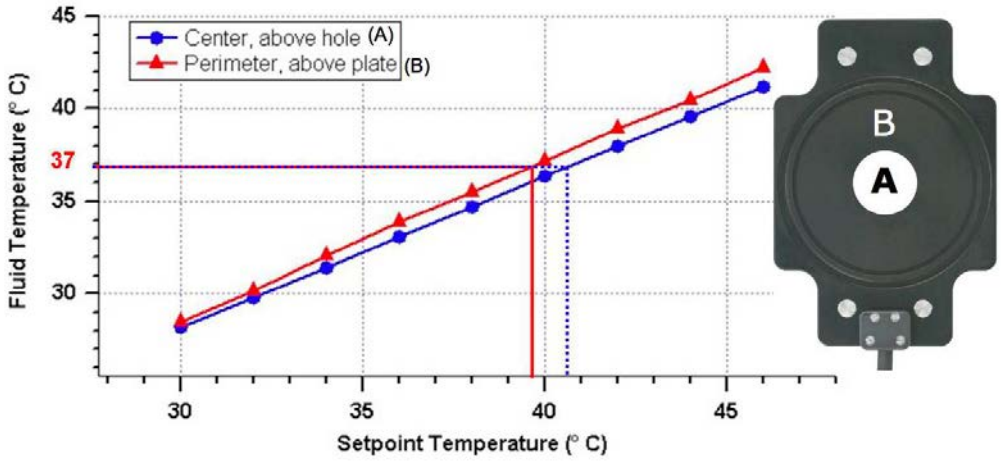
Figure 23.2.: Heater Panel window

attached is used since it isolates the ground connection of the environmental controller from the rest of the instrument (See Section 16.4 on page 206).

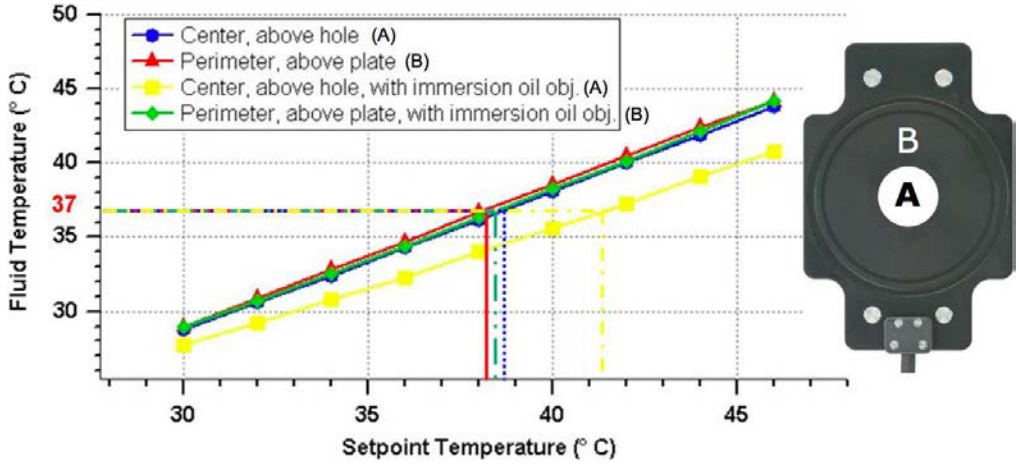
5. Turn on the Environmental Controller.
6. Open the AR SPM software. It should automatically recognize the new hardware and display the Heater Panel window (Figure 23.2 on page 303). If the Heater Panel window doesn't appear, then rescan the BUS (Item 2 in **??** on page **??**) or turn the ARC2 controller OFF then ON.
7. With a fluid-filled Petri dish placed on the Petri Dish Heater plate, turn on the heater by selecting "On" for both the Feedback and Heater parameters. To minimize evaporation place the head (with evaporation membrane) on the XY scanner or keep the lid on the dish. If the head is placed on the scanner, use the Petri dish clamp to secure the dish.
8. Enter the desired values for "Target Temp" (i.e. sample temperature 37.0 ºC is typical for mammalian cells) and "Ramp Rate" (i.e. how quickly the sample should reach the target temperature; 6.0 ºC/min is typical). It is important to keep in mind that the temperature sensor is located inside the metal plate under the dish. The fluid temperature is therefore lower than what the sensor reports and the difference is dependent on how well the heat is coupled into the bottom of the dish. Adjust the "Target Temp" accordingly to achieve the desired fluid temperature. This is discussed in more detail in the next section.

23.5. Temperature Gradients

Figure 23.3 on page 304 shows the relationship between Fluid Temperature vs. Controller Setpoint (i.e. Target Temp) for both the Falcon polystyrene (catalog # 351006) and WPI glass bottom (catalog # FD5040-100) Petri dishes. The fluid temperature was recorded using an insulated thermocouple and controller from Omega Engineering. Similar setups were used, except for the volume of water added to each dish, 3ml for the Falcon dish and 5ml for the WPI dish (minimum amount needed to completely cover the bottom of the dishes).



(a) Falcon polystyrene dish, cat. # 351006.



(b) WPI glass bottom dish, cat. # FD5040-100.

Figure 23.3.: Fluid temperature as a function of controller setpoint, i.e. Target Temp.

A swirl of immersion oil was placed on the sample plate before adding the dish and securing it with the dish clamp.

To minimize evaporation the Petri dish lid and a sheet of parafilm was placed on top of the dish clamp. The parafilm sheet was necessary since the thermocouple probe prevented the lid from sitting flush on top of the clamp. For the WPI dish, fluid temperature was also recorded with a 60x oil immersion objective touching the cover glass (referred to in the graph as "with immersion oil obj.") since this may be a common experimental scenario.

Several factors affected the difference between the fluid temperature and the setpoint, including the type of dish (polystyrene vs. glass) and whether the immersion oil objective (with immersion oil) was touching the cover glass. To briefly summarize the results:

- There was greater temperature uniformity for the glass bottom dish compared to the polystyrene dish.
- The fluid temperature above the plate was higher than the fluid temperature above the center hole for both dish types.

- The immersion oil objective acted as a heat sink and the temperature difference was most drastic above the center hole. If this is a concern, you can consider purchasing a third party objective heater, though Asylum Research cannot vouch if this will add electrical or vibrational noise to degrade AFM image quality.

So when selecting a setpoint based on fluid temperature it is important to keep in mind the variability across the dish bottom. To determine the exact value it is recommended that you measure the fluid temperature on your system using a temperature sensor (thermocouple) and some sort of data logger.

NOTE: Since the temperature feedback sensor is located INSIDE the Dish Heater plate, the fluid temperature will be lower than the value that you enter for the "Target Temp" in the Heater Panel.

To open the Temperature vs. Time Graph (Figure 23.4 on page 305), click on the "More" button next to "Display". Click on this button a couple times to bring up both Y axes: Temperature (on the left) and Heater Output (on the right). You will see a quick increase in the fluid temperature with virtually no overshoot (Figure 23.4 on page 305). During the initial heating, monitor the Heater Output to ensure that it doesn't reach its maximum output. Typically the heater output shouldn't exceed 60% when heating to 45ºC.

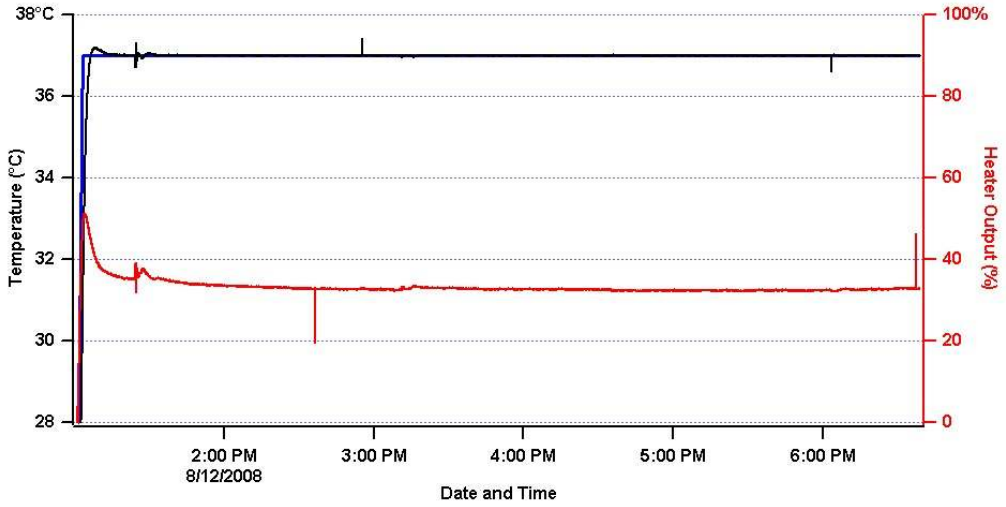


Figure 23.4.: Temperature vs time graph.

23.6. Using Standard Microscope Slides

The Dish Heater can accommodate a standard microscope slide. This is not likely to be used when heating, but is handy when someone wants to look at a slide mounted sample when not having to remove the heater plate from the scanner. Special magnetic clamps (939.013, see the parts list above) are required to line up with the magnetic inserts in the dish heater.

Note that a microscope slide cannot sit completely centered on the Dish Heater plate due to the heater wiring connector (an unavoidable design constraint).

23.7. Live Cell Imaging at Elevated Temperature (37 ºC)

This section assumes you are familiar with:

- General operation of the Dish Heater as previously described in this chapter.
- Imaging in cell culture dishes as outlined in Section 22.7 on page 298.
- AC mode imaging in air. (See Chapter 4 on page 15).
- AC mode imaging in fluid. (See Chapter 8 on page 64).

NOTE: The key to successful live cell imaging is to minimize the time that the dish of cells is "sitting" on the heater plate without being imaged. While it is sitting unattended on the plate, please leave it covered at all times to minimize evaporation and temperature gradients. It is important to have the AFM completely setup and "ready to go" once the cells are placed on the sample plate.

1. Grow cells in one of the approved Petri dishes listed above in the "Compatible Petri Dish" section using normal culturing protocol. Keep them in the incubator until the system and heater control have been set up.
2. Using a dummy empty dish, adjust the height of the AFM head and the relative position of the cantilever to the surface.
3. Ideally, to keep all cells "happy" and to avoid overheating the peripheral cells which will cause them to detach and contaminate the cantilever, adjust the "Target Temp" based on the warmest area of the dish (i.e. perimeter). As mentioned before, the temperature sensor is located within the plate so the actual fluid temperature will be slightly lower than the setpoint temperature. Temperature uniformity across the Petri dish in contact with the metal plate is 0.10 ºC or better. The center hole will be approximately 0.5ºC cooler than the dish perimeter. So, if you want the cells at the perimeter to be close to 37ºC, enter 39ºC for the "Target Temp". Refer to Figure 23.3 on page 304 depending on your exact combination of dish, temperature, and objective use.
4. After adjusting the head height with respect to the sample "dry", remove the head and add 2-3ml of pure water to the spare Petri dish. Add a drop of fluid to the cantilever chip then place the head back onto the stage, completely submerging the cantilever. Enter 39 ºC for the "Target Temp". Enable the heater by clicking "On" for both the Heater and Feedback. Allow the temperature to stabilize, approximately 10 minutes.
5. Begin capturing the Temperature data by clicking on the "More" button next to Display.
6. While the fluid temperature is stabilizing, align the laser on the back of the cantilever. Zero the deflection. You will notice that the deflection will continue to drift until the fluid temperature is stable. During this period you can also tune the cantilever if you will be imaging in AC mode.
7. Once the deflection has stopped fluctuating, you are now ready to take the cells out of the incubator.
8. Remove the cells from the incubator and quickly exchange the dishes on the Petri Heater plate (Figure 23.5 on page 307).

   NOTE: Use no more than 2-3mL of solution in the Petri dishes to prevent overflow and damage to the head and/or scanner. Remember to use immersion oil on the Petri dish plate to minimize vibration and therefore noise in your image.

9. Place the magnetic clamp on the dish, add a drop of culture medium to the cantilever chip, place the head on the scanner and gently lower the cantilever into the fluid. Check to make sure that your SUM value is unchanged. Wait a few minutes to ensure that the fluid temperature is stable. Zero the deflection if necessary.
10. Proceed with standard fluid imaging techniques.

Figure 23.5.: Picture of Petri dish with clamp sitting on the scanner. Open access to the sample when the head has been removed.

24. EC Cell

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

24.1. Overview

The Electrochemistry cell for the Asylum Research MFP-3D AFM allows for in situ electrochemical experiments with connections for up to five different electrodes. The Cell is attached via a single ribbon cable to a junction box which in turn accepts connections from a potentiostat. A variety of sample mounts are available. This chapter describes the assembly and use of the EC Cell.

24.2. Prerequisites

Imaging while performing electrochemical measurements is a fairly advanced technique. It is assumed that you are proficient at:

- Basic AFM Safety (Chapter 26 on page 402).
- AC Mode Imaging in Air (Chapter 4 on page 15).
- AC Mode Imaging in Fluid (Chapter 8 on page 64).
- General practices of using fluids with an AFM instrument and know how to avoid damage to the instrument (Chapter 7 on page 62).
- Basic use of the MFP-3D Fluid Cell and cantilever holder sealing membrane (Chapter 14 on page 160).

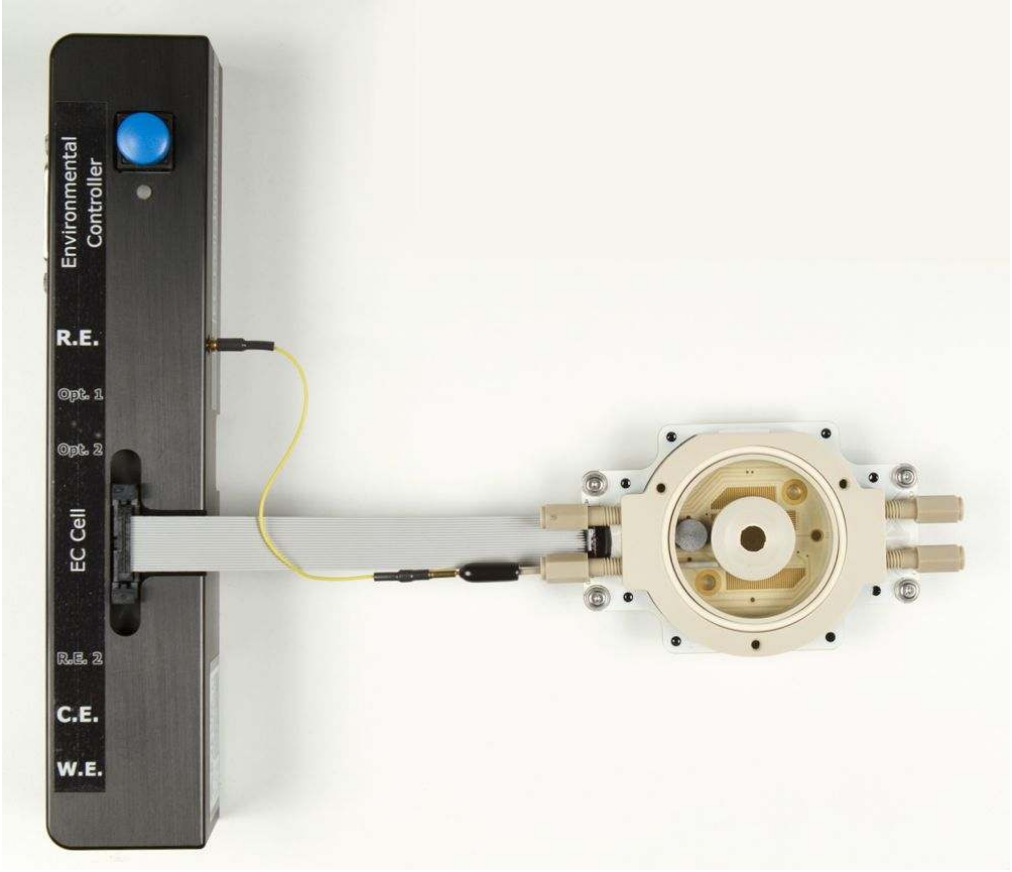


Figure 24.1.: EC Cell Fully Assembled

- Using your potentiostat for electrochemical measurements and depositions. (See your potentiostat manual).

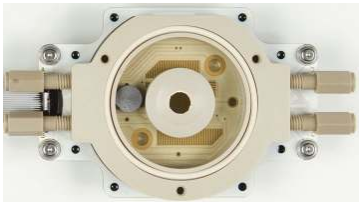
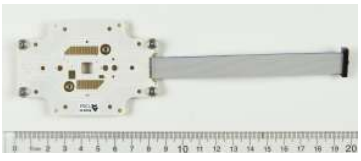
24.3. Parts List

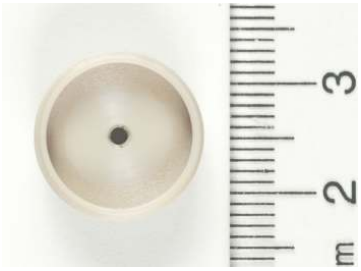
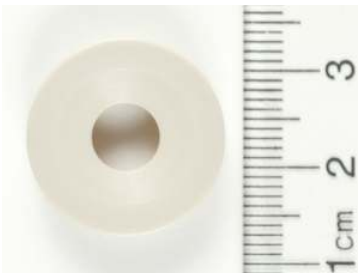
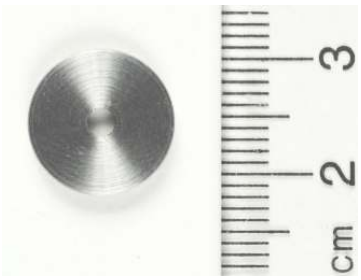
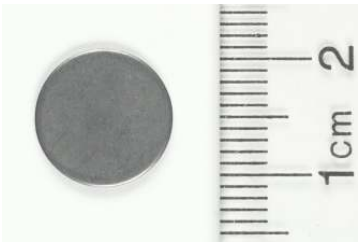
Your Rosetta stone to the parts of the EC cell. This table lists them all, with photos and links to the relevant parts of the documentation which describe how to use them. All parts and assemblies have six digit Asylum Research part numbers. If you ever see such a number in the text and do not know what it refers to, go to the top of this document and run a search for that number and you'll find it in the list.

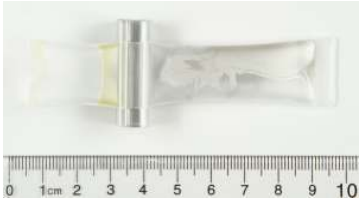
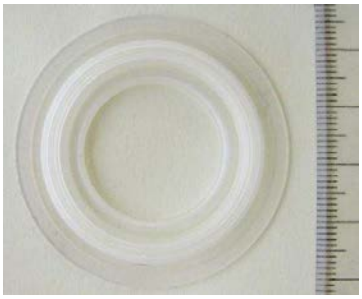
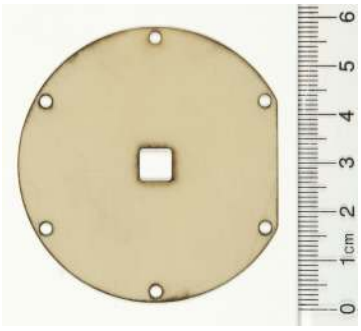
Always refer to the relevant Asylum Research part numbers during support calls or when buying replacements.

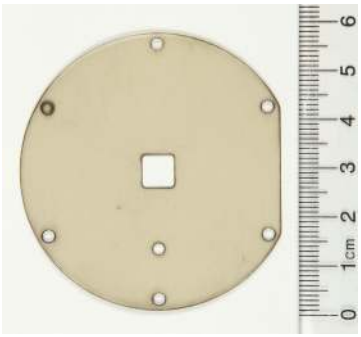
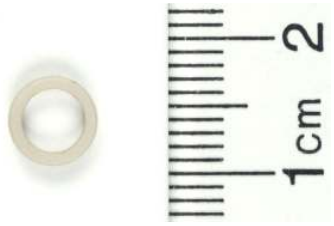
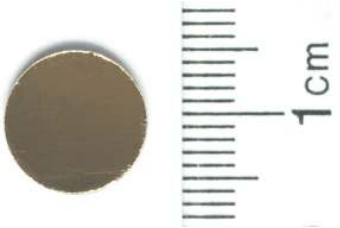
The part number for the entire parts kit is 900.141.

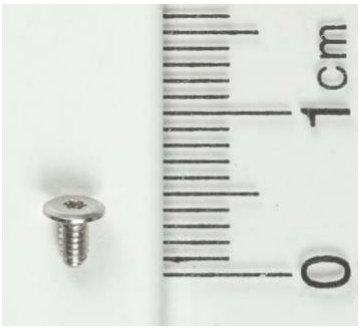
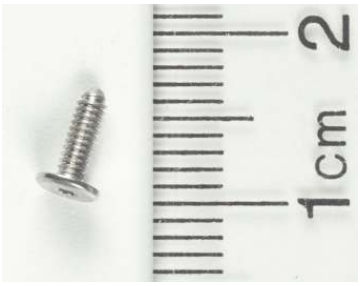
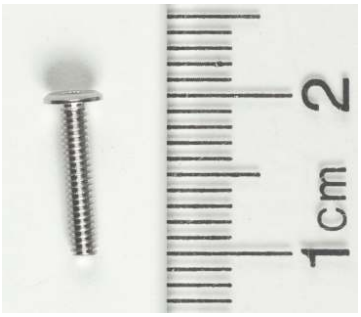
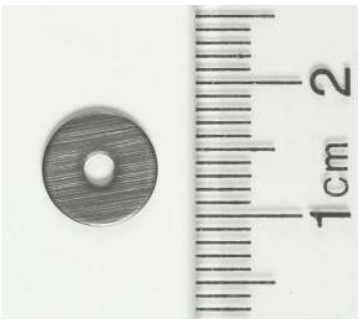
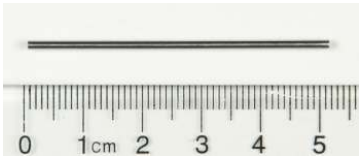
Figure 24.2.: Most of the very small parts are stored in plastic boxes. Please make use of these boxes and keep the parts organized so you can find them based on part numbers referred to in the manual.

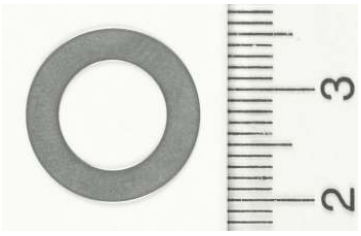
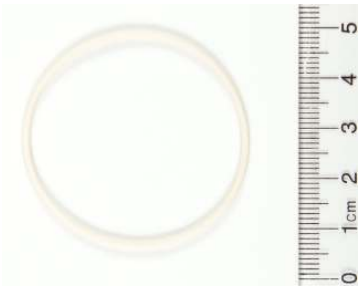
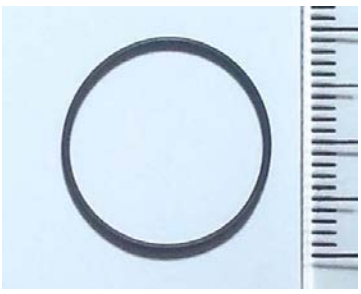
Itm	Part #	Item Description	Qty	Picture
1	939.	EC Assembled as shipped. Items which are used to build up this configuration will follow in this table.	1	
2	113.752	EC Cell Body. Main component of the EC Cell. See Section 24.6 on page 326 about its use.	1	
3	458.190.2	EC Cell Board Assembly. Main component of the EC Cell. See Section 24.6 on page 326 about its use.	1	
The scale in the photos is in cm and mm.				

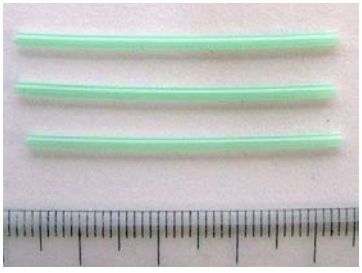
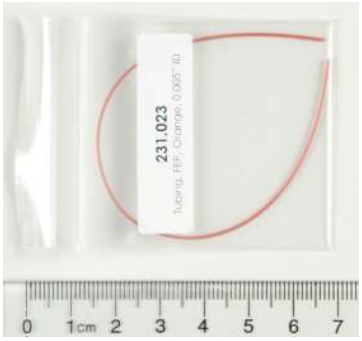
Itm	Part #	Item Description	Qty	Picture
4	114.189	EC Thin Film Sample Mount Cup. See Section 24.7.1 on page 327.	1	
5	114.190	EC Thin Film Sample Mount Nut. See Section 24.7.1 on page 327.	1	
6	114.191	EC Thin Film Sample Mount Disc. See Section 24.7.1 on page 327.	1	
7	002.HNUT <M2>	M2 Nuts S/S. Used to secure electrodes to the EC cell circuit board bottom. See Section 24.8.1 on page 336 and Section 24.8.2.1 on page 337. Use Nut driver tool 290.143.	6	
8	080.010	5 ml Syringe. We prefer this Norm-Ject model from Henke Sass Wolf (HSW) since it does not contain any rubber and contaminates fluids minimally. See Section 14.4.3 on page 172 on how to attach tubing.	2	
9	080.105	12mm AFM Specimen Disc. Can be used with the thin film sample mount. See Section 24.7.1.3 on page 334.	5	
The scale in the photos is in cm and mm.				

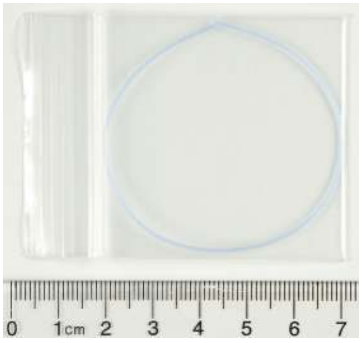
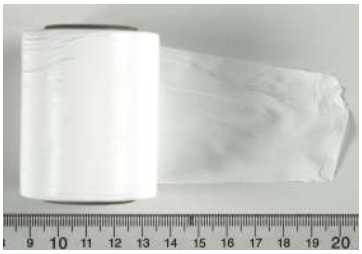
Itm	Part #	Item Description	Qty	Picture
10	080.108	Bipak Tra-Duct Epoxy -2902. Silver, conductive, can be used for various sample mounting tasks.	3	
11	112.256.01	Closed Cell Bellows, Viton. 50 durometer black FKM fluoroelastomer. See Section 13.4.3 on page 153.	2	
12	112.256.02	Closed Cell Bellows, 30 Durometer Silicone rubber. See Section 13.4.3 on page 153.	2	
13	112.491	O-Ring Membrane Threaded Clamp. Stainless Steel cantilever holder retaining ring with O-ring groove. See Section 13.4.3 on page 153.	1	
14	114.067	PEEK Film, EC CELL No Holes. Main component of the EC Cell. See Section 24.6 on page 326 about its use.	2	
The scale in the photos is in cm and mm.				

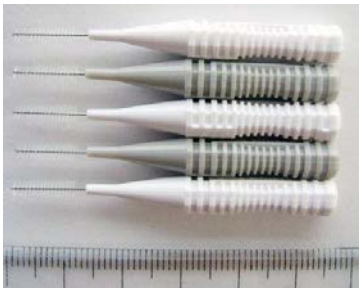
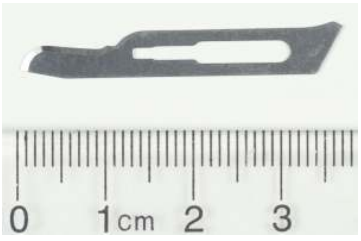
Itm	Part #	Item Description	Qty	Picture
15	114.095	PEEK Film, EC CELL One Hole. Typically used with Carbon (Section 24.8.2.1 on page 337) or Platinum (Section 24.8.2.2 on page 338) counter electrodes.	2	
16	114.164	EC Cell Sample Stub Spanner Tool for use with a future sample holder. Currently of no use and may not have been included in your kit pending later shipment.	1	
17	939.026	EC Cell Sample Mount Cleaning Tool. Main plastic body has part number 114.165. See Section 24.16 on page 354	1	
18	114.243	EC Cell Gasket Cutting Spacer.	3	
19	939.028	Gold Plated Mica Disc on Magnetic AFM Puck assembly. Mostly for demo purposes to have at least a few samples on hand for instructional purposes. They have been pre mounted on AFM specimen discs. (See Section 24.7.1.3 on page 334 for that process.)	3	
The scale in the photos is in cm and mm.				

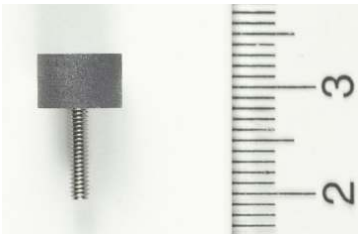
Itm	Part #	Item Description	Qty	Picture
20	222.072	Screw, M2 X 4, Stainless. Used exclusively for the four screw terminals at the corners of the circuit board. See Section 24.8.2.3 on page 340.	4	
21	222.073	Screw, M2 X 6, Stainless. Used for basic cell assembly when not using the heater accessory. See Section 24.6 on page 326.	12	
22	222.074	Screw, M2 X 10, Stainless. Used for cell assembly when using the heater accessory (See Section 24.15 on page 352). In that case these replace the 6mm screws above. Also use at the center of sample mounts such as in Step 2 on page 327.	12	
23	222.075	#2 Washer, 3/32”ID X 11/32”OD. Used at the bottom of the cell when attaching sample mounts (working electrodes) to the EC Cell. See Section 24.8.1 on page 336.	5	
24	222.077	Dowel Pin, 1/16" X 2". Used to open up fittings (232.006) after they have become too compressed to easily accept tubing. See Section 24.9.2 on page 345.	3	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
25	222.090	Shim, 0.010” thick, 3/8” ID, 5/8” OD. Used as spacers to ensure proper fit for thin samples in the thin film sample mount. See Step 5 on page 329.	6	
26	230.026	O-ring, AS568B-031, 75 Durometer. Black FKM (Viton), 1-3/4” ID x 1-7/8” OD. Used to seal between EC cell body and bottom film. See Step 3 on page 326. Equivalent FFKM part is 230.054.	5	
27	230.027	O-ring, FKM (Viton), 1/32W" x 1/8"OD. Used to seal counter electrodes. Custom size only available from Asylum. See Section 24.8.2.1 on page 337 and Section 24.8.2.2 on page 338.	10	
28	230.031	O-ring, 1 5/8"ID x 1 3/4"OD FKM (Viton). Standard AS568-030 size, can also be purchased from other vendors. Sits on top of EC Cell to seal against the membrane. For use, see Section 24.13.1 on page 351. Equivalent FFKM part is 230.053.	3	
29	230.052	O-ring, .030 C/S X .690 ID X .750 OD, FKM, 75A Durometer. Custom size, only available from Asylum Research. Equivalent FFKM part is 230.047. Used to seal the thin film sample mount against the bottom of the EC Cell. See 24.8.1.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
30	231.006	Tubing, PFA, 1/16”OD X 0.040”ID. This PFA tubing makes it possible to introduce and remove fluid or gas from the closed fluid cell. Order from Asylum or purchase directly from Upchurch Scientific (p/n 1503). See Section 14.6.1 on page 183 why it is important to use only this tubing.	1	
31	231.008	Luertight Fitting. Used to connect a Luer fitted syringe to the 1/16” OD tubing, which in turn connects to the fluid cell inlets. See Section 14.4.3 on page 172.	2	
32	231.012	Tubing, ETFE .062”OD x 0.020”ID. Used as a sealing sleeve for fixing 0.5mm OD wire in the fluid ports. See Section 24.8.2.3 on page 340.	12	
33	231.019	Tubing Sleeve, Green, 1/16”x.033”x1.55”. Upchurch #F-247x. Used as a sealing sleeve for fixing metal wire in the fluid ports. See Section 24.8.2.3 on page 340.	3	
34	231.023	1/32”x.005”ID Tubing - Orange FEP. Upchurch #1685. Used as a sealing sleeve for fixing very thin metal wire in the fluid ports. See Section 24.8.2.3 on page 340.	6	
The scale in the photos is in cm and mm.				

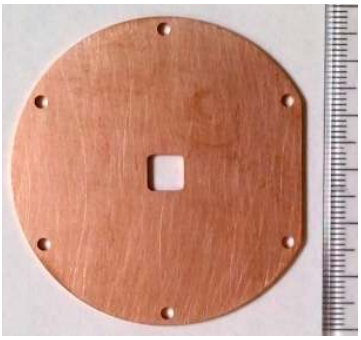
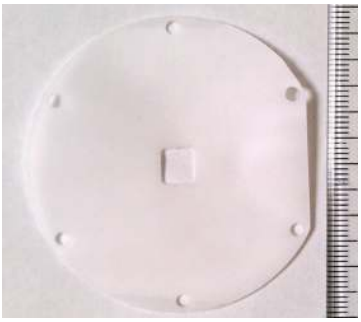
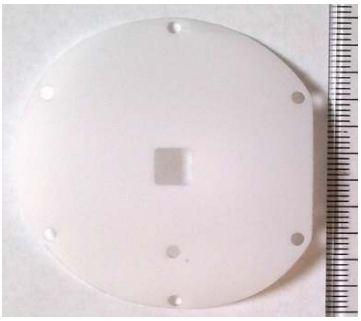
Itm	Part #	Item Description	Qty	Picture
35	231.027	1/32”x.009”ID Tubing - Light Blue FEP. Upchurch #1689. Used as a sealing sleeve for fixing metal wire in the fluid ports. See Section 24.8.2.3 on page 340.	6	
36	231.028	1/32”x.016”ID Tubing - Clear FEP. Upchurch #1692. Used as a sealing sleeve for fixing metal wire in the fluid ports. See Section 24.8.2.3 on page 340.	6	
37	231.028	1/16” OD PTFE Teflon solid cord. Used for sealing unused fluid ports. See Section 24.9.1 on page 344.	12	
38	232.006	Fitting, 10-32 Fingertight PEEK. Used to seal the fluid ports. See Section 24.9 on page 344 and Section 24.6 on page 326.	2	
39	279.084	Expanded PTFE tape. Can also be ordered from McMaster Carr (Part# 6802K16). Used for sealing samples in the thin film sample mount. See Section 24.7.1.1 on page 331 and **??** on page **??**.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
40	290.109	Leitsilber Conductive Paint. Used for mounting samples (See Section 18.6 on page 232). Can be purchased from Asylum Research or directly from Ted Pella (16035). See Section 24.7.1.3 on page 335.	1	
41	290.113	Brush, 1/16 Cleaning. Small enough to clean deposits from inside the fluid ports.	5	
42	290.143	2mm Nut Driver. Used to tighten the M2 nut that holds down the working electrode. See Section 24.8.1 on page 336.	1	
43	290.144	T5 2.5MM Torx Driver. Used to fasten the various Torx screws used all over the EC Cell.	1	
44	290.146	Screwball Case Opener. Used to fasten membranes to the cantilever holder. See Section 13.4.3 on page 153.	1	
45	290.147	Scalpel Knife Handle. Can also be ordered from McMaster Carr (Part# 36325A63). Used to trim Teflon tape during sample mounting. See ?? on page ??.	1	
46	290.148	Number 15 scalpel blade. Attaches to handle 290.147. Used to trim Teflon tape during sample mounting. See ?? on page ??.	10	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
47	300.007	Reference Electrode, 48mm X 1/16"OD. Sealed PEEK body. See Section 24.8.3.1 on page 341.	1	
48	900.200	EC Cell Junction Box. Connection hub between EC Cell and Potentiostat. See Section 24.10 on page 345.		
49	908.038	Peek Cantilever Holder Kit. All chemically inert parts. See Section 11.4 on page 108 and Section 24.13 on page 351.		
50	939.008	Spanner wrench Assembly. Used to attach membranes to cantilever holders (See Section 13.4.3 on page 153) and to secure bottom pieces into closed cells (See Section 13.4.1.1 on page 149).	1	
51	939.015	Membrane Clamp Assembly. Used when operating flowing fluids through sample cells. See Section 14.4.5 on page 173. **Warning**: Not following the instructions and not leak testing before use will seriously damage your AFM from fluid spills.	1	
52	939.023	EC Cell Reference Electrode Jumper Wire. Connects the reference electrode 300.007 to the junction box. See Section 24.8.3.1 on page 341.	1	
53	939.024	EC Cell Carbon Counter Electrode. Graphite, 200 micron particle size, less than 50ppm ash content. See Section 24.8.2.1 on page 337.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
54	113.758	EC Cell Bottom Cup. Protects the electrical contacts on the bottom of the cell. See Section 24.8.2.1 on page 337.	1	
55	939.029	Gasket Punch Tool. Used to punch thin teflon gaskets for use with the thin film sample mount. See Section 24.7.1.1 on page 331.	1	
56	114.290	Gasket punch pad. The required backing for the punch tool. See Section 24.7.1.1 on page 331.	1	
57	448.130	Leak Detection Circuit Cable. Supplies voltage to the interdigitated electrodes on the EC cell which cause a beep when a leak occurs. See	1	
58	290.162	Narrow Stem Pipette for easily transferring fluids in and out of the EC cell.	2	
The scale in the photos is in cm and mm.				

24.3.1. Optional parts:

Itm	Part #	Item Description	Qty	Picture
59	114.504	Copper Film No Holes. This can be configured as a very large counter electrode. See Section 24.6 on page 326 about its use.	2	
60	114.584	PTFE Film, No Holes. A more chemically robust version of the PEEK film. See Section 24.6 on page 326 about its use.	2	
61	114.585	PTFE Film, One Hole. A more chemically robust version of the PEEK film. Typically used with Carbon (Section 24.8.2.1 on page 337) or Platinum (Section 24.8.2.2 on page 338) counter electrodes.	2	
62	114.583	EC Cell Body, PPS. EC Cell Body. Main component of the EC Cell. See Section 24.6 on page 326 about its use. PPS is more resistant to sulfuric acid than the standard PEEK cell body.	2	
The scale in the photos is in cm and mm.				

24.4. Chemical Compatibility

The materials from which the EC-Cell parts are made are listed in the preceding parts lists. Depending on your particular configuration of electrodes and sample mounting technique, please use internet resources to look up the chemical compatibility of those parts. Please see Chapter 10 on page 87 for some good starting points for your search. If you are unsure, you can always contact our support (See on page iv).

To be absolutely certain, you can always take a spare PEEK fitting or a spare O-ring and immerse it in the chemical in question. Before you immerse it, carefully measure the part with a digital caliper or micrometer and note down the dimensions. Then soak for some period of time and measure the part again. If there is no swelling, then the parts are safe to use. If there is some swelling, or severe degradation, then you may need to opt for parts made of some other materials, some of which we do offer. You can always contact us to discuss custom parts made of other materials that would suit your needs.

24.4.1. O-rings and Membrane

The standard O-rings and membrane included with the EC Cell are made of FKM (A generic version of Dupont Viton). FKM is the best all round choice for a cost effective elastomer, but it does have it's limitations. In the cases where FKM degrades, Asylum Research offers an FFKM O-ring Kit (generic version of Dupont Kalrez). This rubber like materials has near Teflon like properties but has a VERY high cost associated with it. For instance, at the time this was written, the O-ring that seals the bottom of the EC cell costs about a third of a dollar when made of Dupont Viton (FKM), but 300 TIMES more when made of Dupont Kalrez (FFKM). Hence we decided to make the FFKM parts a separate kit, to be purchased only by those who require it.

24.4.2. FFKM O-ring Kit

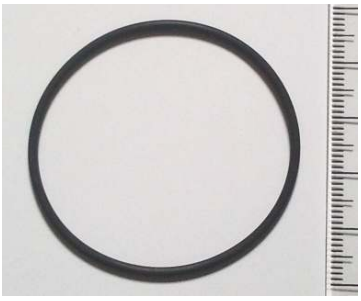
Figure 24.3.: EC Cell FFKM O-ring Kit, Asylum Part Number 939.047

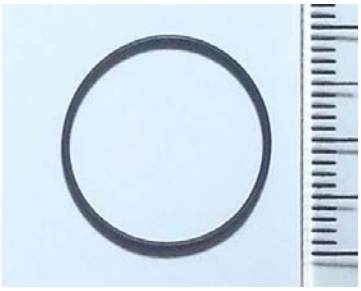
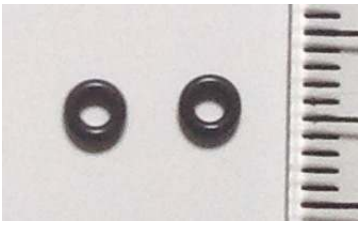
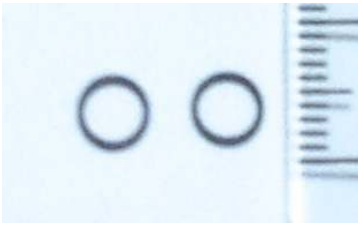
Asylum Part Number 939.047

Note that some of the O-rings are standard AS568 sizes and we order those “off the shelf” and they are made by Dupont, of Kalrez 6375 FFKM material. Non standard O-rings and membranes are custom made by Asylum Research of a competing FFKM compound.

Attention

FFKM can cost between 10 and 100 times more than FKM, but the two materials look nearly the same. Carefully keep your FFKM parts stored in its kit box when not in use. If you are ever unsure if a part is made of FKM or FFKM, drip a little acetone on the part. FKM will swell VIOLENTLY and FFKM will do nothing at all. The FKM parts will recover again if left to dry out for a day.

Itm	Part #	Item Description	Qty	Picture
1	112.256.03	Closed Cell Bellows. 75A durometer black FFKM fluoroelastomer. Equivalent FKM part is 112.256.01. See Section 13.4.3 on page 153.	1	
2	230.053	O-ring, 1 5/8"ID X 1 3/4"OD Kalrez 6375, Durometer 75A. Equivalent FKM part is 230.031. Standard AS568-030 size, can also be purchased from other vendors. Sits on top of EC Cell to seal against the membrane. For use, see Section 24.13.1 on page 351.	1	
3	230.054	O-ring, 1 3/4" X 7/8", Kalrez 6375, Durometer 75A. Equivalent FKM part is 230.026. Standard AS568-031 size, can also be purchased from other vendors. Used to seal between EC cell body and bottom film. See Step 3 on page 326.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
4	230.047	O-ring, .030 C/S X .690 ID X .750 OD, FFKM, 75A Durometer. Custom size, only available from Asylum Research. Equivalent FKM part is 230.052. Used to seal the thin film sample mount against the bottom of the EC Cell. See 24.8.1.	2	
5	230.048	O-ring, 0.040" C/S X 0.070” ID x 0.150” OD, FFKM, 75A Durometer. Custom size, only available from Asylum Research. Equivalent FKM part is 230.027. Used to seal counter electrodes. Custom size only available from Asylum. See Section 24.8.2.1 on page 337 and Section 24.8.2.2 on page 338.	2	
6	230.049	O-ring, 0.020" C/S X 0.170” ID x 0.210” OD, FFKM, 75A Durometer. Custom size, only available from Asylum Research. Equivalent FKM part is 230.011. Used to seal “cantilini” prisms in the cantilever holder. Item E in Figure 11.2 on page 102. NOTE: this O-ring needs to be stretched over the prism during installation.	2	
The scale in the photos is in cm and mm.				

24.5. Quick Start Guide

The EC cell offers a lot of different sample mounting and electrode options. This section will cover the basic steps involved and will refer to other sections for details.

1. Assemble the basic parts of the cell.
2. Mount your sample to create the EC working electrode.
3. Select and install your counter electrode.
4. Install the reference electrode.
5. Perform some EC cell tests on the bench top away from the AFM.

6. Mount the EC cell on the AFM.
7. Perform EC experiments while imaging.

24.6. Basic Assembly

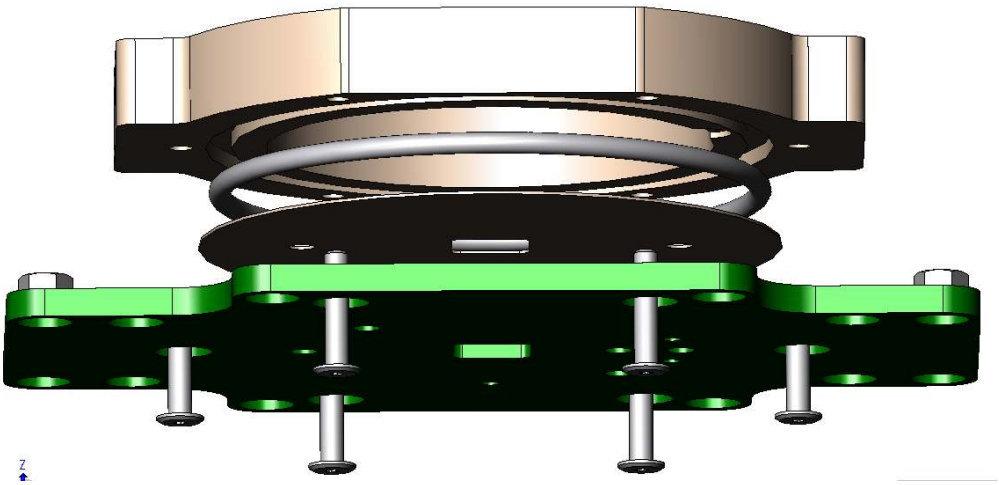


Figure 24.4.: Basic Cell Assembly

Basic Cell Buildup:

1. From top to bottom the parts shown in Figure 24.4 on page 326 are:
   - 1 EA 113.752 EC Cell Body
   - 1 EA 230.026 FKM O-ring
   - 1 EA 114.095 Peek Film Bottom Disc, with one circular hole for a bottom mounted counter electrode
   - 1 EA 458.190.2 EC Cell Board Assembly
   - 6 EA 222.073 6mm long screws, Torx head
   - 1 EA 290.144 Torx Driver Tool
   - 4 EA 232.006 PEEK fluid port fittings
2. Place the PEEK film disc onto the circuit board. Line up the square holes in both. If need be you can use the square bottom of one of the sample mounts (*e.g.* 114.189) to keep things aligned.

   Note the Orientation If the cable extends to the left, the flat side of the cell should be facing away from you, as shown in Figure 24.1 on page 310.
3. Insert the O-ring into the cell body and place on top of the disc.

4. Insert the six M2 by 6mm screws and thread in nearly tight. Note that there is a small screw length scale printed on the back of the circuit board where you can identify the screw length accurately.
5. Inspect the square hole alignment once more.
6. Lay the whole thing on the table, bolt heads facing up, and tighten them all to where they are flush, but apply no excess torque.
7. In a star pattern, tighten the screws a little one by one, going around a few times before they are all tight. If you tighten one screw too much before the others, then the cell will end up askew and may leak.

24.7. Sample Mounting

This section discusses how your sample is attached to the working electrode. Please see Section 24.8.1 on page 336 about mounting the electrode into the EC Cell.

24.7.1. Thin Film Sample Mount

This sample mount will accommodate thin samples up to a few mm in thickness. But circular and square chips of material can be mounted, on conducting or insulating substrates. In the case of these instructions the sample is a thin gold coated mica disc (114.242) mounted with silver paint on a standard 12mm steel AFM specimen disc (080.105). For more instructions on sample preparation, please see Section 24.7.1.3 on page 334.

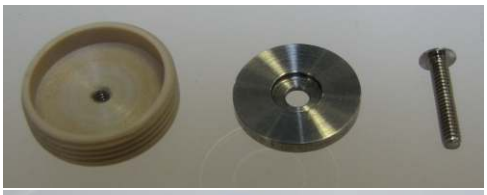
A note on cleanliness. While we did not wear gloves during the following procedure, you may choose to do so. Also, the whole mounted sample can be cleaned by sonication or immersion in fluids as described in Section 24.16 on page 354.

1. **Prepare Sample Stand**
   - Locate the EC Cell Sample Mount Cleaning tool (939.026).
   - Take it apart and remove the plastic cylinder and place it on the table as shown.

2.

Prepare Sample Mount Cup

- Locate the Thin Film Sample Mount Cup (114.189) and Disc (114.191) and some shims (222.090) and an M2x10 Torx Screw.
- Depending on the sample thickness you should first place a few of the the shims in the cup. Exactly how many shims will become evident in Step 10 on page 330, so some trial and error may be required when switching to a new sample thickness.
- Then place the metal disc as shown.
- Assemble as shown and fasten the screw tightly using the Torx driver. It may help to use the cleaning tool (Section 24.16 on page 354) to hold the cup while tightening.



3.

Align and Place Sample Cup

- Aligning the marks on the bottom of the sample cup and the top of the cylindrical stand.
- Place the Sample Cup on the stand.

4. **Optionally Apply Thread Tape**

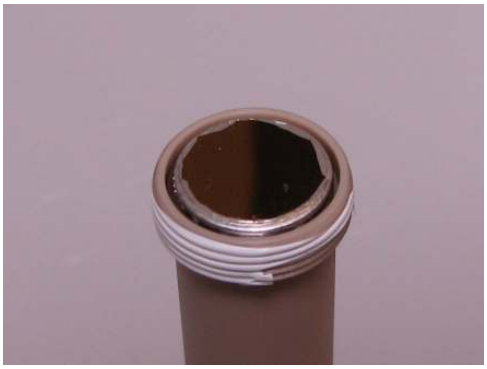
- Cut a narrow ribbon of Teflon membrane, just wide enough to cover the threads and just long enough to cover the circumference one or two times.
- Wrap it around the threads in a clockwise direction, while stretching. Ideally the Teflon only covers the threads.

Note To prevent the sample cup from moving during this process it may be beneficial to temporarily attach the threaded rod and nut as described in Section 24.16 on page 354.

Note In an older version of the sample mount this tape performed (poorly) as a liquid seal. In the latest design, an O-ring forms the seal.

5. **Place the Mounted Sample**

- Place the mounted sample (see Section 24.7.1.3 on page 334 for mounting instructions) on top of the cup.



Note The sample must stick slightly above the edge of the cup. If it does not, please put a few spacer shims (222.090) underneath.

6. **Place Gasket**

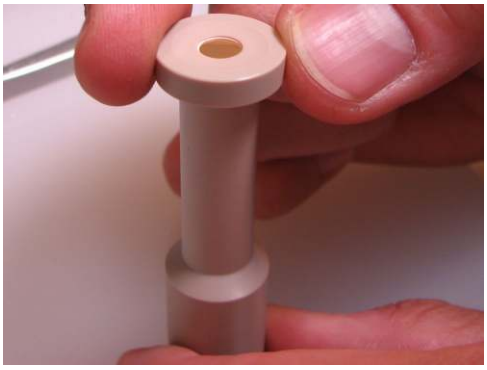
- Place an annular piece of the gasketing material centered on top of the sample.

Note See Section 24.7.1.1 on page 331 on making the gaskets.

7. **Tighten the Nut**

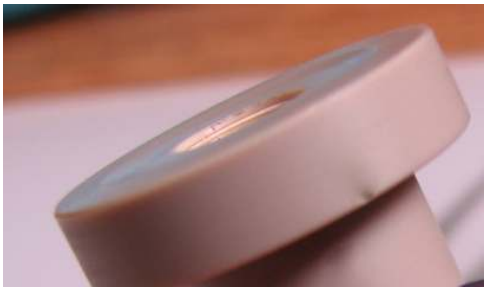
- Locate the Sample Mount Nut (114.190) and tighten it until you start to feel a slight increase in resistance.
- The hole in the nut should be flush with the top of the sample.

How much? See the next step on how much to tighten.



8. **Inspect compression:**

- The top of the slopes slightly inward. Tighten the nut to the point where the top of the nut deforms to nearly flat, as shown in the picture.



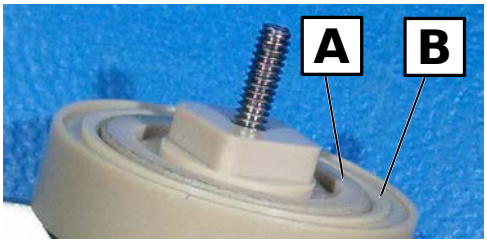
9. **Inspect the Seal (only for beginners)**

- Only follow this step as you are learning the correct tension. The point is to compress the teflon seal enough so it looks like that in the photo.
- Too little tension and the seal will not become translucent. Too much and you might break through it and damage the nut.
- After a few tries you will get the feel for the proper tightening of the nut and you will skip this step.

For more examples see Figure 24.7 on page 336.

10. **Inspect back side alignment:**

- Surface A should be flush with surface B for the O-ring which will sit in the groove next to B to compress properly.
- If the sample is too thin, B will advance Beyond A. In that case the O-ring will seal properly, but the sample may drift during imaging since the sample is best supported by surface A.
- One can adjust the sample thickness by using



11. **Final Result**

- Photo at right shows the sealed sample.
- 24.8.1 explains how to mount this assembly in the EC Cell as a whole.

At this point one can do various things with the mounted sample:

- Mount the Sample on the Cleaning Tool for ultrasonic cleaning (See Section 24.16 on page 354).
- Use the same tool for electrochemical characterization of the sample in a traditional EC setup (like a small beaker). See Section 24.16.3 on page 355.
- Mount the sample as a working electrode in the EC Cell for AFM studies (See Section 24.8.1 on page 336).

24.7.1.1. Punching Teflon Gaskets

The Teflon gaskets mentioned in the previous section can be punched using the supplied punching tool (939.029) and punching pad (114.290). You will need to supply a hammer.

Warning

The gasket punch is a precision tool. Please use only a follows:

- Do not drop the punch.
- Only use the punch for cutting Teflon gaskets.
- Store it in a safe place.
- Always use the backing pad to punch against.

1.

Locate Gasket and Punch

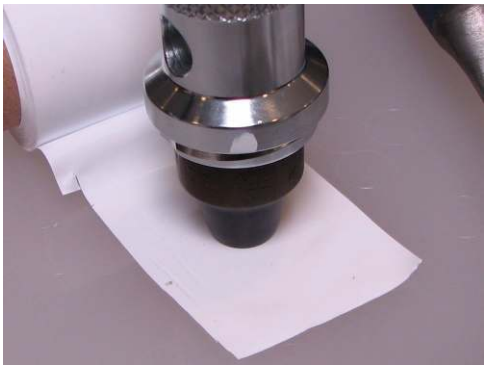
- Locate the PTFE Tape (279.084), Punch Tool (939.029) and Punch Pad (114.290).
- You will need to supply a hammer.

Note: The tool is heavy and round and can roll from the table. If it falls on the cutting edge it may require replacement parts.

2.

Prepare to Punch

- Roll out some tape onto the punch pad.
- Gently set the punch down onto the tape.



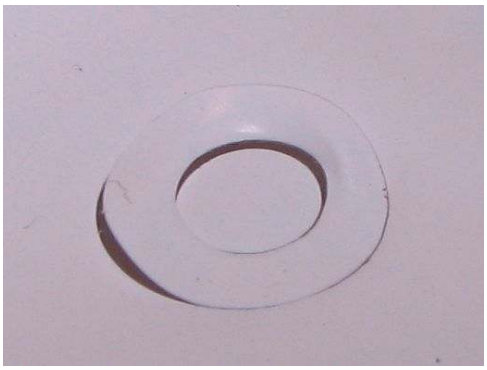
3. **BANG!**

- Gently press the punch down onto the tape and keep holding it during the hammer blow..
- Gently hit the hammer, raising it only a few inches above the punch tool.

Note: It's better to start very gently and try again if the punch does not go all the way through the tape. Excessive hammer force will lead to poor gasket quality and a damaged punch pad (and maybe even a damaged punch tool).

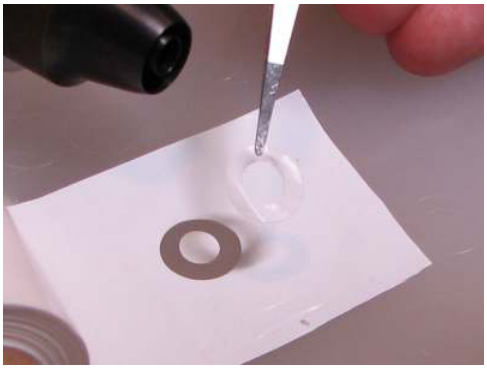
4. **The Result**

- Proper punch force will leave a nicely cut gasket behind.
- If the gasket stays inside the punch, remove the (black) outer punch cutter by rotating it counter clockwise.



5. **Gasket complete:**

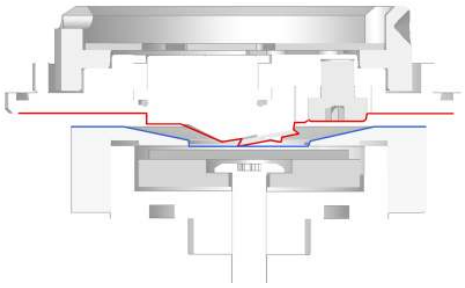
- Carefully transport the gasket with flat tipped tweezers.



24.7.1.2. Imaging with the Thin Film Sample Mount

There is relatively little clearance between the thin film sample mount and the PEEK cantilever holder. Please see 24.5b to get an idea on how to engage the surface. If the AFM head and scanner are slightly misaligned, a part of the cantilever holder may come into contact with the sample holder before the tip reaches the sample surface. When approaching the sample surface in AC mode (see Section 4.8 on page 34), the cantilever deflection as seen in the Sum and Deflection meter (see **??** on page **??**) should remain close to zero. A sudden change in the Sum Signal is a good indication that the cantilever holder is hitting the sample holder and that head and scanner need to be realigned.

(a) Cantilever holder clearance when using the thin film sample mount. Note that for optimal clearance the cantilever does not land in the center of the visible part of the sample.



(b) Cantilever holder clearance when using the thin film sample mount. Space is tight. In reality the nut which seals against the sample bends a bit making the gently sloped sides closer to horizontal, creating a bit more space than what is shown in this CAD drawing.

Figure 24.5.

24.7.1.3. Sample Preparation

Maximum Sample Dimensions:

- **Circular:** 0.520” in diameter (13.2 mm)
- **Square:** 0.365” on a side (9.3 mm)
- **Thickness:** Approximately 2 mm.

Minimum Sample Dimensions:

- **Circular:** 0.4” in diameter (10 mm)
- **Square:** 0.34” on a side (8.5 mm)
- **Thickness:** As long as very thin samples are backed by an AFM specimen disc, the only limit is that the sample can be handled and glued to an AFM disc without becoming torn or damaged.

Note that the minimum and maximum square sample sizes are very close. This is a competition between the largest square that can fit inside the sample cup and the business of requiring that the edges of the square stay away from the sealing surfaces of the nut. You can see in Figure 24.7c on page 336 that for a square which fits perfectly into the sample cup, the nut seals right up to the edges of the sample.

If you can cut the corners off of your square sample then it will be a lot more comfortable to mount. Also, if you have a thin square sample glued to a 12mm AFM specimen disc and spaced up a bit so the sample extends above the rim of the cup, you can get away with perhaps another mm or two (meaning you can mount a 1cm by 1cm sample that way). To be more precise, a sample of nearly 0.6” or 15mm (circular diameter) or a square of 0.41” (10.5mm) on a side.

Figure 24.6.: Typical square sample of ITO on glass substrate. Note the dabs of silver paint at the corners which electrically connect the sample top surface to a metal disc carrier.

Flat Solid Metal Samples If a solid metal sample is sufficiently flat, the sample can simply be dropped into the sample cup and the process of applying the seal (Section 24.7.1 on page 327) can be started. If the seal does not seem to be compressing uniformly around the perimeter of the sample, the sample may not be flat enough to use with this sample mount.

Metal Foil Samples Metal foil samples should be glued to a 12mm AFM specimen disc (080.105) with silver paint (290.109) or silver epoxy(080.108). Then the process of applying the seal can start (Section 24.7.1 on page 327).

Thin Films on Insulating Substrates Assuming the substrate is brittle (glass or silicon), it should be cut into a 9mm by 9mm square, or a slightly larger square with the corners cut off. The square should be placed centered on a 12mm AFM specimen disc (080.105) and glued down with 5 minute epoxy. A small amount of silver paint (290.109) should be used to paint over the corners of the sample. This connects the top conducting film of the sample to the metal AFM disc carrier. Once the paint has thoroughly dried, the process of applying the seal can start (Section 24.7.1 on page 327).

Figure 24.6 on page 335 shows a mounted piece of Indium Tin Oxide on glass. Figure 24.7 on page 336 shows various levels of Teflon membrane compression during the process of determining the proper nut compression (a process starting at Step 7 on page 329).

In a similar fashion, one can punch out discs of gold coated mica (best diameter is slightly smaller than 12mm) and then use 5 minute epoxy to glue them to a 12mm AFM disc (080.105). Then

carefully paint around the perimeter with silver paint (290.109). The paint should stay away from the area that is to be sealed. See **??** on page **??** for a photo of a proper final result.

(a) Slightly under compressed (but still OK)

(b) Prefect compression

(c) Over compressed

Figure 24.7.: Image (a) shows a Teflon film which was slightly under compressed (too white). Image (b) shows a Teflon film which was perfectly compressed (becoming translucent). Image (c) shows a Teflon film which was overcompensated and the corners of the square sample are starting to bear part of the load of the nut, which has deformed to come into contact with the outer reaches of the sample.

Other Types of Samples We have created a few types of sample holders for specific customer needs. Read more about these in Section 24.18.1 on page 357.

If you want to make your own sample mounts, there are some drawings available in Section 24.17 on page 355.

24.8. Mounting the Electrodes

24.8.1. Mounting the Thin Film Sample Mount as a Working Electrode

This section discusses how to attach the sample mounts to the EC Cell. It is assumed you have:

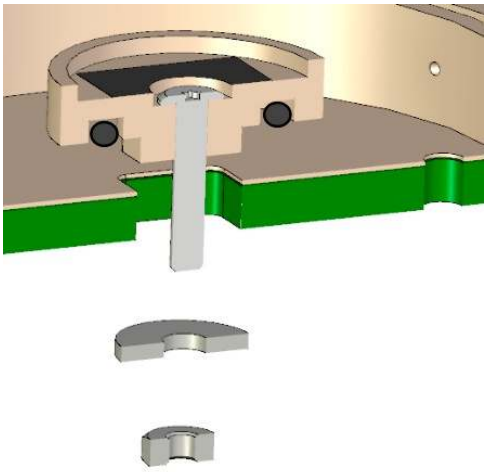
- Attached your sample to the sample mount (see Section 24.7 on page 327).
- You have assembled the EC cell (see Section 24.6 on page 326).

1. **Locate your Parts**
   - 1 M2 Nut (002.HNUT<M2>)
   - 1 M2 Nut Driver (290.143)
   - 1 Washer (222.075)
   - 1 O-ring (230.052, or 230.047 if you have FFKM O-rings) for the thin film sample mount.

2. **Place the O-ring:**
   - Place the O-ring as shown.

3. **Attach the electrode:**
   - Place the mount in the EC cell.
   - Add washer and tighten nut on top of it until the mount is flush with the cell bottom and the O-ring is fully compressed.

   Note: The image is just of a representative electrode, similar to the thin film sample mount.



The nut makes contact with the circuit board which then automatically routes the connection to the junction box via the flat cable.

24.8.2. Counter Electrodes

There are a variety of counter electrode options for the EC Cell.

24.8.2.1. Carbon Counter Electrode

This electrode is a small carbon cylinder with a stainless steel threaded rod bonded in place with silver epoxy. When installed with the O-ring, neither steel nor epoxy will contact the electrolyte. Originally this electrode was made of a soft carbon with <50ppm ash content. This part can be identified in that the O-ring very snugly sunk into a narrow groove. The second generation electrode is made of a harder and purer form of carbon with <5ppm ash content. The main metallic impurities are: Fe <1.0ppm, Si <0.75 ppm, V <1.0ppm, Ti < 0.3ppm, Al <0.35ppm, Ni <0.2 ppm, Cu <0.15ppm. Porosity is <0.8 microns. The second generation electrode is easily recognized by an O-ring groove which varies in width.

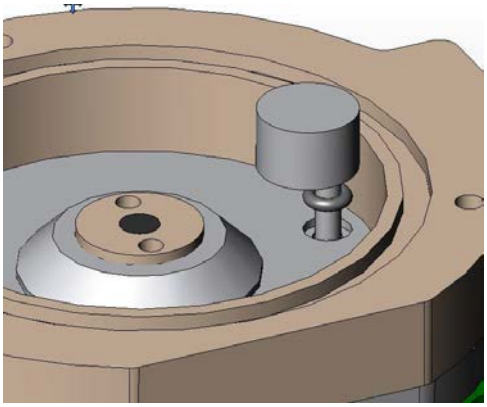
Note: Ash content is a term for everything that remains after a carbon sample has been fully combusted, i.e. minerals some fraction of which are metals.

An O-ring seals the electrode against the peek and keeps fluid from touching the metal rod. To assemble:

1. **Locate your Parts**
   - 1 M2 Nut (002.HNUT<M2>)
   - 1 M2 Nut Driver (290.143)
   - 1 O-ring 1/16” ID, 1/8” OD (230.027)

2. **Attach the Electrode**
   - Place the O-ring on the threaded shaft.
   - Place the shaft through the hole in the cell bottom.
   - Tighten until the electrode is flush with the cell bottom and the O-ring is fully compressed.

   Note Do not overdo it on the tension. The graphite is somewhat fragile.

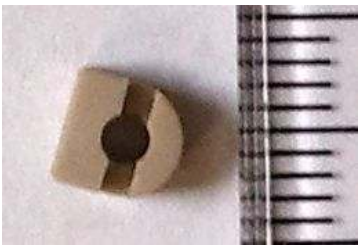


Note that with the second generation electrode, the O-ring groove varies in width so that the O-ring can be more easily removed. Only pry the O-ring loose with a soft toothpick and be careful not so scratch the soft graphite surface against which the O-ring must seal.

24.8.2.2. Platinum Ring Counter Electrode

This is an optional item which can be ordered as the following kit. Use of all these parts is described further down in this section.

Itm	Part #	Item Description	Qty	Picture
1	113.766	EC Cell Platinum Ring Counter Electrode.	1	
The scale in the photos is in cm and mm.				

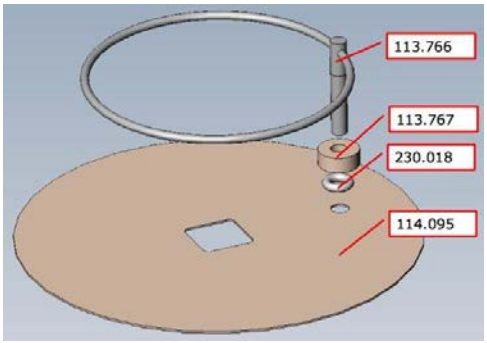
Itm	Part #	Item Description	Qty	Picture
2	113.767	EC Cell O-ring spacer.	1	
3	230.027	O-ring, FKM (Viton) , 1/32W" x 1/8"OD. Used to seal counter electrodes. For spares, please use the ones from your EC cell Kit.	10	
4	002.HNUT <M2>	M2 Nuts S/S. Used to secure electrodes to the EC cell circuit board bottom. For spares, please use the ones from your EC cell Kit.	6	
The scale in the photos is in cm and mm.				

This electrode is made of 90% platinum, 10% Iridium, laser welded into shape. The electrode can be easily cleaned by complete immersion in strong acids. It connects to the EC Cell as follows:

1. **Locate your Parts**
   - 1 M2 Nut (002.HNUT<M2>)
   - 1 M2 Nut Driver (290.143)
   - 1 O-ring 1/16” ID, 1/8” OD (230.027)
   - 1 PEEK spacer (113.767)
   - 1 Platinum Electrode (113.766)

2. **Attach the Electrode**

   - Build up your EC Cell (See Section 24.6 on page 326) with the PEEK film shown to the right.
   - Place the PEEK spacer and O-ring onto the shaft as shown.
   - Place the shaft through the hole in the cell bottom.
   - Tighten until the electrode is flush with the cell bottom and the O-ring is fully compressed.



Note Do not overdo it on the tension. The ring can bend if stressed too much.

This electrode is not standard equipment - it can be bought from Asylum Research for an extra charge, due to the high price of platinum. Please inquire about assembly number 939.020 which will include all the parts described in this section.

24.8.2.3. Arbitrary Wire Counter Electrode

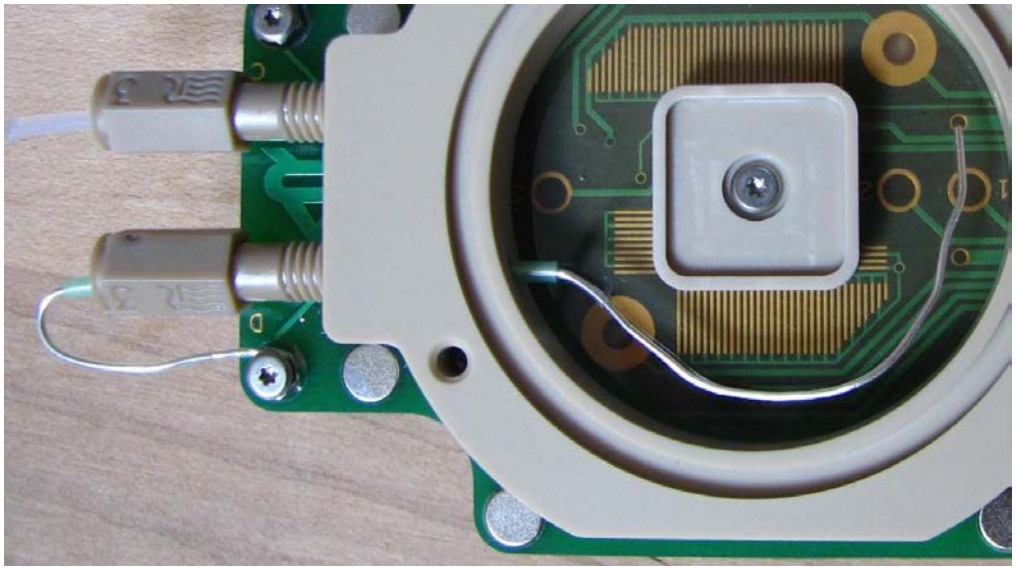


Figure 24.8.: A silver wire passed through a fluid port fitting using a green Teflon sleeve. Wire is connected to screw terminal D.

It is also possible to feed wires in through the fluid ports. Note that of the four EC Cell fluid ports, only two go straight through and are suitable for wire feed through.

The fluid port ferrules themselves are 1/16” ID but will seal around wires slightly smaller than that also. A straight wire that has properly sealed will not move when pulled on.

In order to also seal around thinner wires we have included a variety of tubing segments with different IDs. These can be used as compliant sleeving around wires. For instance, the green tubing segments have an ID of 1/32". Inside of that you may be able to fit your wire of choice. For even smaller wires, we have included more bits of colored tubing with an OD of 1/32" and a variety of IDs. The 1/32" OD tubing fits perfectly inside the green tubing sleeves. Finally, the long tubing intended for filling the cell with fluid has an ID of 1mm (or 0.040") exactly.

If none of these tubing combinations are able to firmly grip your wires, there is the option of using epoxy to adhere a wire into one of the extra PEEK fittings. These only cost a few dollars each and more spares can be ordered from Asylum Research. For Epoxy we recommend products from Reltekllc.com. Bondit 45 is available from McMasterCarr and is a good choice since it adheres to PEEK and has good chemical resistance. Other products from McMasterCarr, like B4811 and B755, are also suitable and have excellent resistance to caustic and acidic environments. They can be ordered directly from Reltek.

Once your wire is properly inserted, it can be attached to one of the four screw terminals on the corners of the circuit board. In Section 24.10.3 on page 348 you can learn how to route those signals to various connectors on the junction box.

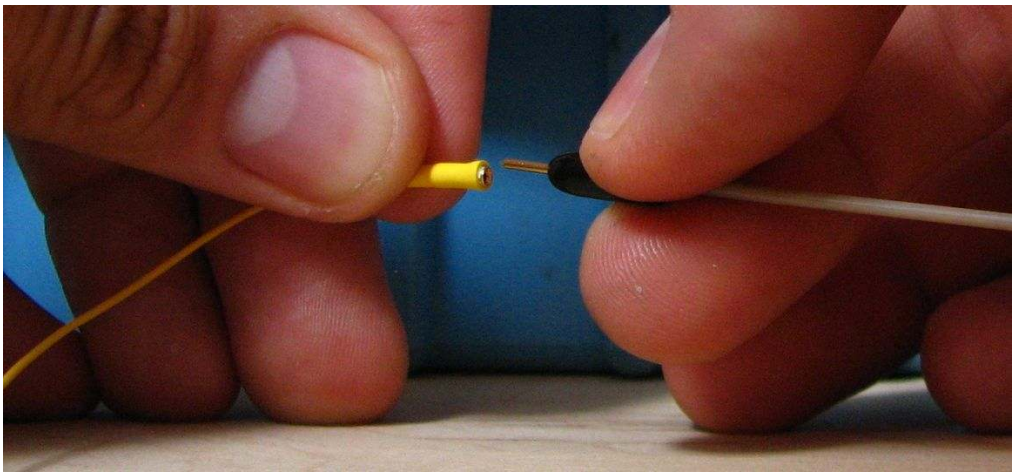
24.8.3. Reference Electrodes

24.8.3.1. Fluid Filled Ag-AgCl Reference Electrode

Warning	While the measurement end of these electrodes is quite robust, the gold connector pin is quite fragile. Please be very gentle when connecting it.

1. **Prepare the reference electrode:**
   - Remove the small black cap and store it carefully. A small amount of water inside this cap keeps the electrode working properly.

2.



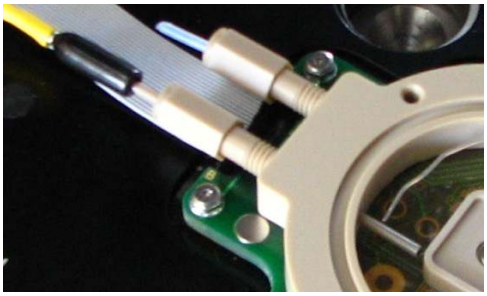
Connecting the wire

- First connect the wire to the reference electrode, hold the parts as shown.

3.

Insert the Electrode into the Cell

- Loosen the fluid port fitting.
- Sleeve the fitting onto the electrode.
- Insert the combo into the cell.
- Adjust the position and tighten firmly.



4.

Connect to the Junction Box

- Place the EC Cell onto the scanner.
- connect the Reference Electrode wire to the Junction Box. There is only one place to plug a wire on the side of the box facing the AFM.

Asylum Research supplies an Ag-AgCl fluid filled (3.4 M KCl) reference electrode which can be inserted through one of the two fluid ports. The Electrode has a PEEK barrel, a proprietary inert frit, and requires no maintenance. In other words, it cannot be opened and it never needs to be filled. It can be stored dry and may only required soaking in de-ionized water before use.

Here is some basic information on the electrode from the manufacturer:

> Our new Leak-Free junction series, LF, enables you to perform your experiments in organic solvents, perchlorate and silver salts solutions, or Hydrofluoric acids without

being worried about clogging or degradation! It can also be used for long term experiments without the worry that the filling electrolyte be diluted or run out. This Leaking-Free reference electrode uses our newly developed conductive junction (patent pending). The filling electrolyte is confined to the barrel and will not leak at all (zero leakage). The Junction has very high conductivity with resistance under 10 K-Ohm. It has exceptional mechanical stability, zero swelling, resistance to organic solvents, and robust. The junction potential is independent of the sample nature or ionic strength. The electrode body is constructed from PEEK for superior chemical resistance. The filling electrolyte (3.4 M KCl) does not leak through the junction which prevents sample contamination with chloride and potassium ions. This means no clogging and no need for double junction. Since the electrode construction does not involve any glass, it can be used in Hydrofluoric Acid solutions.

A leaflet with general information is included with your electrode. The electrode is manufactured by 2in.com. It is of a custom diameter and length made specifically for Asylum Research, which is based on their LF2 electrode. Technical questions about the electrode can best be directed at 2in.com, or contact Asylum Research and we can contact them for you.

When you are finished using the reference electrode, please follow the storage instructions (see) to keep it functioning properly.

24.9. Putting on the Bottom Cup

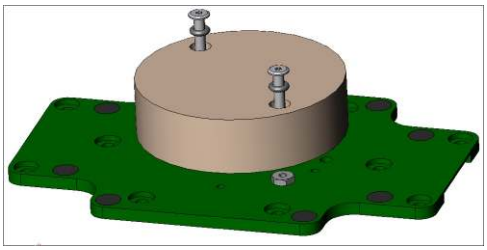
A small peek cup can be attached to the bottom of the EC cell. While the cell can operate without it, the cup does help center the cell on the AFM scanner. The cup also prevents the exposed electrical elements on the bottom of the cell from shorting out against the scanner, and is able to catch a small amount of fluid in case there is a leak. The metal screws which attach the cup are electrically connected to the leak detection circuit of the EC cell. You can read more about that in Section 24.11 on page 349.

1. **Locate your Parts**
   - 1 EA 113.758 PEEK Cup.
   - 2 EA O-ring 1/16” ID, 1/8” OD (230.027)
   - 2 EA 222.074 10mm long screws, Torx head.
   - 290.144 Torx Driver Tool.

2.

Attach the Cup

- Thread the O-rings onto the screws.
- Place the cup onto the bottom of the cell.
- Stick in one screw and lift the edge of the cup to get the screw engaged into the threads.
- Tighten the first screw slightly, then align the cup to put in the second screw.
- Tighten both screws until snug.



Note Do not overdo it on the tension. The Ring threaded bosses on the circuit board could tear off if overtightened.

Fluid exchange

The EC Cell has four 1/16” ID fluid ports which will accept any 1/16” OD tubing. At Asylum Research we like Upchurch 1609 PFA tubing (Asylum part number 231.006) since it has a nice 1mm ID which is larger than the 1/32” ID found on typical tubing. This allows better fluid flow and is also handy as sleeving for making tight fitting connections around 1mm OD objects.

All four fluid ports can be used for fluid exchange, but two were designed for fluids specifically since they lead directly to the bottom of the cell and allow for easy withdrawal of all the fluid in the cell.

24.9.1. Connecting all the Fluid Ports

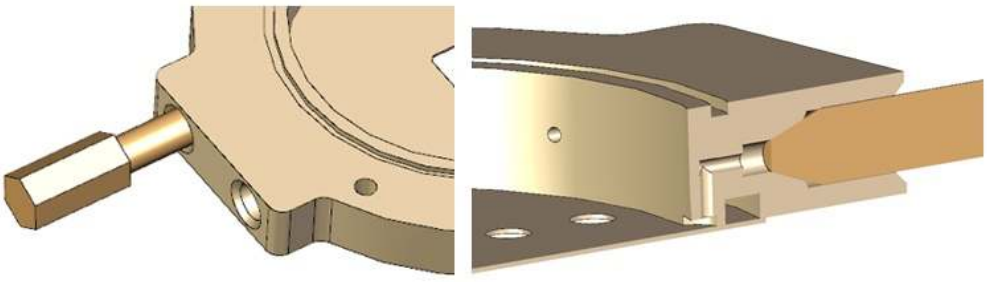


Figure 24.9.: A view of a fluid port fitting on the left. The cutaway view on the right shows the straight through port in the back (for fluid or wire feedthrough) and a cutaway of the fluid only port, designed to drain the fluid from the bottom of the cell.

For a closeup of the EC Cell fluid ports see Figure 24.9 on page 344.

In a typical setup, one of the straight through ports holds the fluid filled reference electrode (Section 24.8.3.1 on page 341) and the other straight through port holds tubing (231.006) connected to a syringe (080.010) using the Luertight Fittings (231.008).

The remaining unused fluid ports must always be plugged with a piece of 1/16” OD Teflon cord. (Asylum part number 249.034).

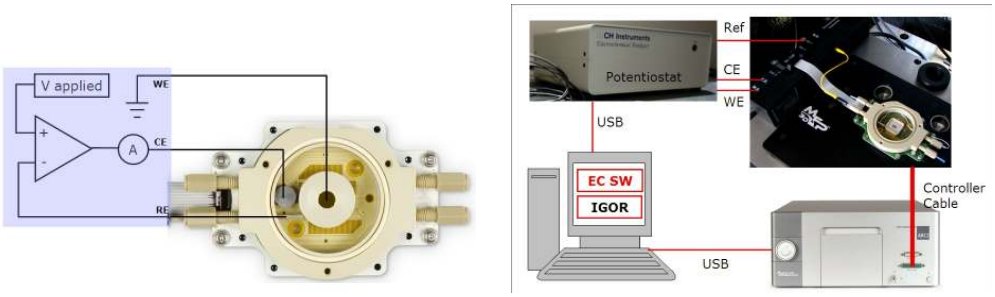
24.9.2. Crimped Fittings

Since the PEEK fittings on the fluid cell are conical in design, tightening them firmly only once can lead to a permanent reduction in the tip size. This, in turn, can make it difficult to fully insert tubing for fluid exchange. To widen the fitting back to the original size, we have included some 2” long steel dowel pins (222.077) which can be pushed through the fittings. To do this, completely unscrew the fittings, insert the pin into the blunt end of the fitting and press down on a hard surface (like a tabletop) to force the pin all the way through the conical point of the fitting. After this procedure the tubing should be able to pass through again.

24.9.3. Other Uses for Fluid Ports

Please see Section 24.8.2.3 on page 340 on using fluid ports for feeding through wires of various diameters.

24.10. Connecting to a Potentiostat



(a) Basic connection to be made to the idealized potentiostat, shown diagrammatically.

(b) The basic connection setup. The potentiostat and the AFM run independent softwares on the PC.

Figure 24.10.

The EC Cell is built on a thick circuit board which routes all the electrode connections to a large junction box via a single flat cable. This can be seen in Figure 24.10b on page 345.

Any cables running to the potentiostat or other external equipment are plugged into the junction box. The box (See Figure 24.11 on page 346), which acts as a strain relief by isolating all the heavy potentiostat cables from the EC cell, is attached to the AFM base with strong magnets.

The Junction Box has a choice of standard Banana or 2mm pin connectors. If your potentiostat has a different type of connector, please contact Asylum Research and we can suggest a solution to make your connections.

The overall connection layout between AFM, potentiostat, and computer is diagrammed in Figure 24.10b on page 345.

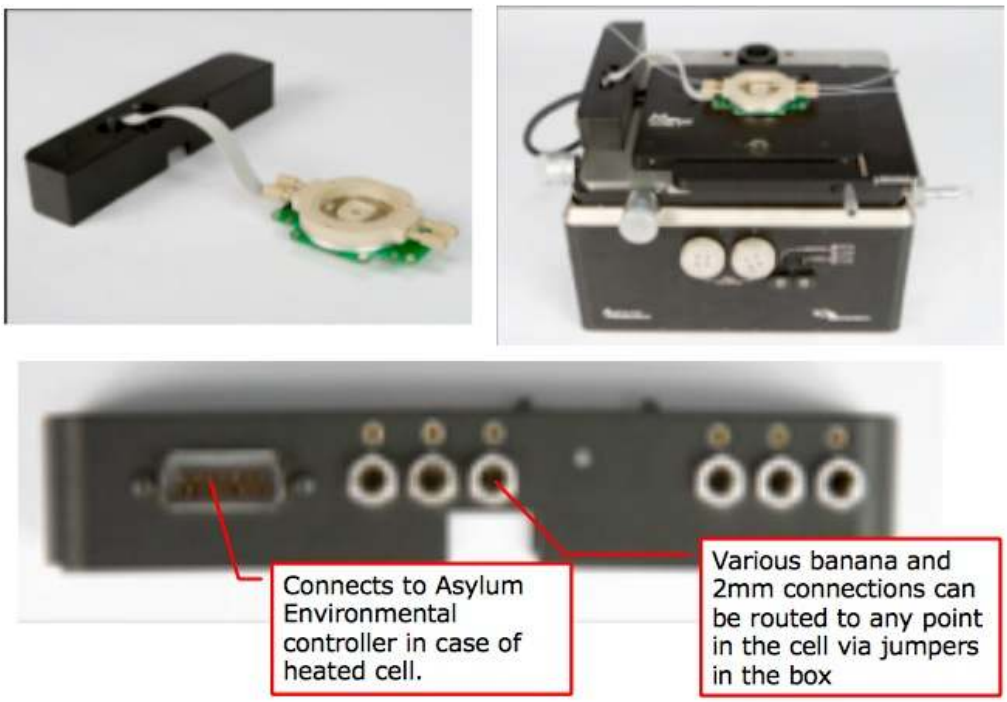


Figure 24.11.: The EC Cell plugged into the Junction Box, and the Junction Box and EC Cell on the MFP-3D AFM. Finally, a close-up of the Junction Box Connections.

Figure 24.12.: Typical potentiostat connection scheme.

Since there are 6 connections on the patch box and eight connections on the circuit board, it was decided to put some selection switches inside the patch box so that the connector marked CE (Counter Electrode) can be routed to nearly any point on the circuit board. Some additional (and often unused) electrode connections on the patch box can be routed likewise. The Working Electrode is the only one exclusively routed to the center (sample) of the EC Cell. Finally there is a place to directly patch through a reference electrode. It is also possible to attach the reference electrode to one of the corner terminals and route the signal via the ribbon cable.

To accommodate particular types of electrode configuration, it is necessary to set the dip switches inside the patch box once (or infrequently).

Lets take Figure 24.10b on page 345 as an example case.The sample is set up in the center of the cell (Working Electrode, WE), with a fluid filled reference electrode (RE) in one of the side ports, and a handmade wire counter electrode (CE) coming in from the other side port. The potentiostat is connected as shown in Figure 24.12 on page 346. We would like to route these signals to the WE, CE, and RE connectors on the junction box.

From the bottom of the junction box, you can see five banks of dip switches. Figure 24.13 on

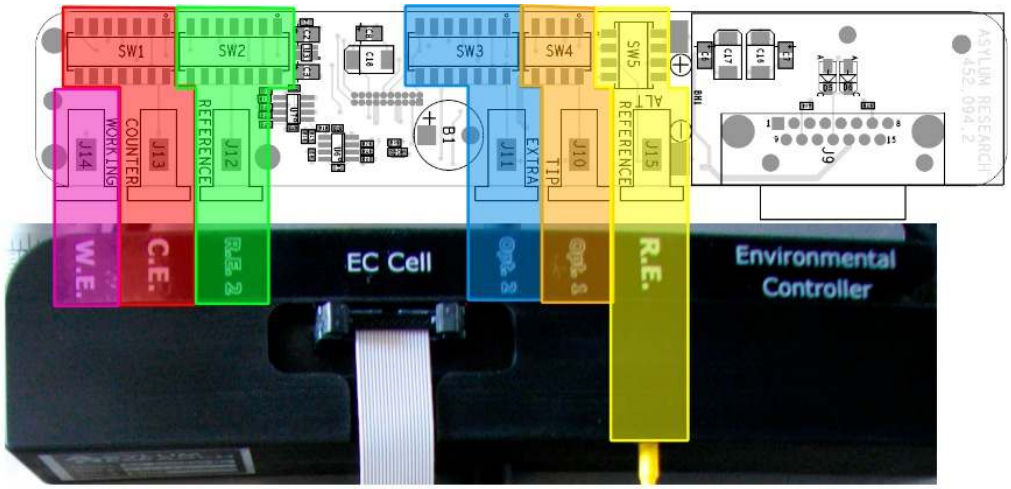


Figure 24.13.: Dip switches and circuit board seen from the bottom of the patch box. This graphic indicates the corresponding connector for each switch on the outside of the Junction Box.

page 347 details which bank of switches is associated with which connector on the outside of the Junction Box. Since the working electrode is always in the center, it has no dip switches. The next two electrodes, C.E. (Counter Electrode), R.E. 2 (Reference Electrode 2), Opt. 2 (Optional electrode 2) have banks of seven switches. The number is seven since the EC Cell has seven electrode positions (four on the corners of the circuit board and three under the cell interior). By setting the switches appropriately, either of these connections can also be routed to all seven positions in the EC cell. Electrode Opt. 1 has only four switches and can be routed only to the four corners of the cell. Finally there is the R.E. (Reference Electrode) connection. This design allows a reference electrode to be routed straight from a wire coming out of the EC cell to the potentiostat, as shown in Figure 24.10b on page 345. Of course one could also attach the same wire to one of the four screw terminals of the cell and route the signal out to the Junction box ribbon cable. In any case, there are a variety of options to explore.

Warning	On any dip switch bank, only one switch should be ON and all others should be OFF. Setting more than one switch to ON for a single bank can cause short circuits or other undesirable effects.

24.10.1. Routing the Working Electrode

As mentioned before, the working electrode at the center of the EC cell is always routed to the W.E. connector on the Junction Box. There is nothing to set or adjust.

24.10.2. Routing the Reference Electrode

If you are using the Asylum Research fluid filled reference electrode (for more details see Section 24.8.3.1 on page 341) then you will likely want to plug it straight into the junction box. In that case the junction box dip switch bank number 5 needs to be configured with position 3 to ON and all other switches to off. See Figure 24.14 on page 348.

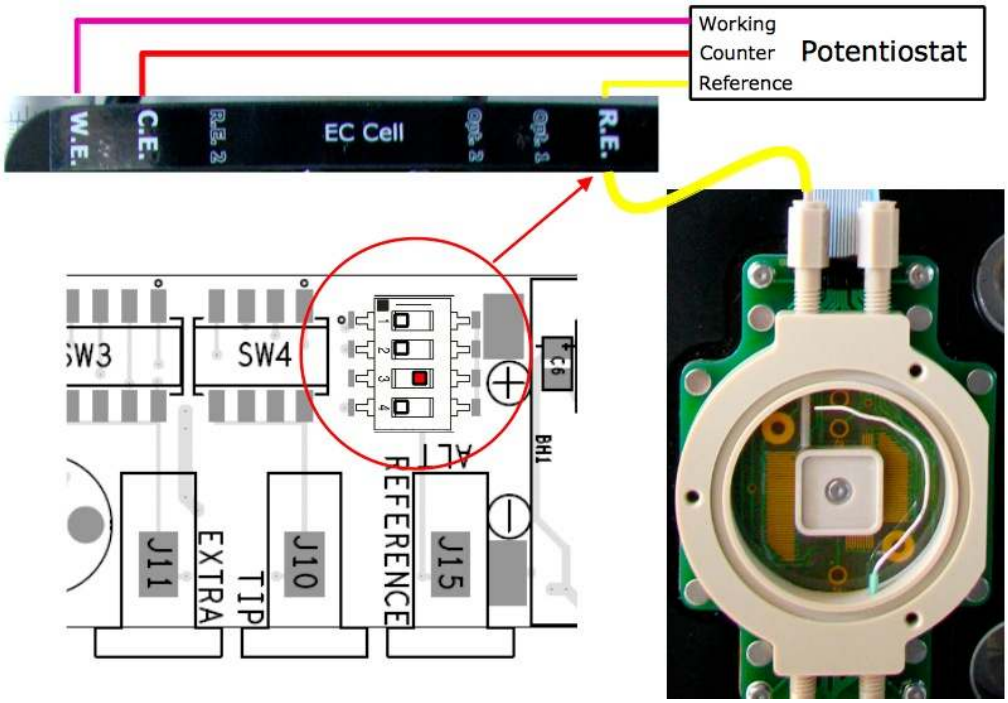


Figure 24.14.: The fluid filled reference electrode Connected to the Junction Box and dip switch 5,3 set to ON to connect to the potentiostat reference electrode.

You may also choose to connect the wire to one of the four screw terminals and have the signal go to the junction box via the EC Cell's ribbon cable. Then you will want to use the R.E.2 connector on the junction box and keep reading on how to properly set its dip switches.

24.10.3. Routing the Counter Electrode

Dip switch Bank 1 will connect the C.E. connector on the Junction box to any of the seven connections on the EC Cell. Figure 24.15 on page 349 gives a good overview of the setup.

In that figure, the counter electrode is a wire inserted through one of the fluid ports. The end of the wire is clamped under screw terminal D. The figure shows that position 7 on switch 1 should be set to ON. All other switches of that bank should be set to OFF. As noted before, never turn on more than one switch per bank, or you may short your working and counter electrodes together inside the junction box.

24.10.4. Other electrodes

The Junction Box allows for up to three additional electrodes. Figure 24.16 on page 350 shows how to connect the additional junction box connection to the various terminals on the EC Cell. As mentioned before, be careful how you set the switches. It is possible to short two or more junction box connections together and it is possible to connect two or more junction box connectors to a single terminal on the EC cell. We left the design very open but one needs to be proactive about keeping the connections unique.

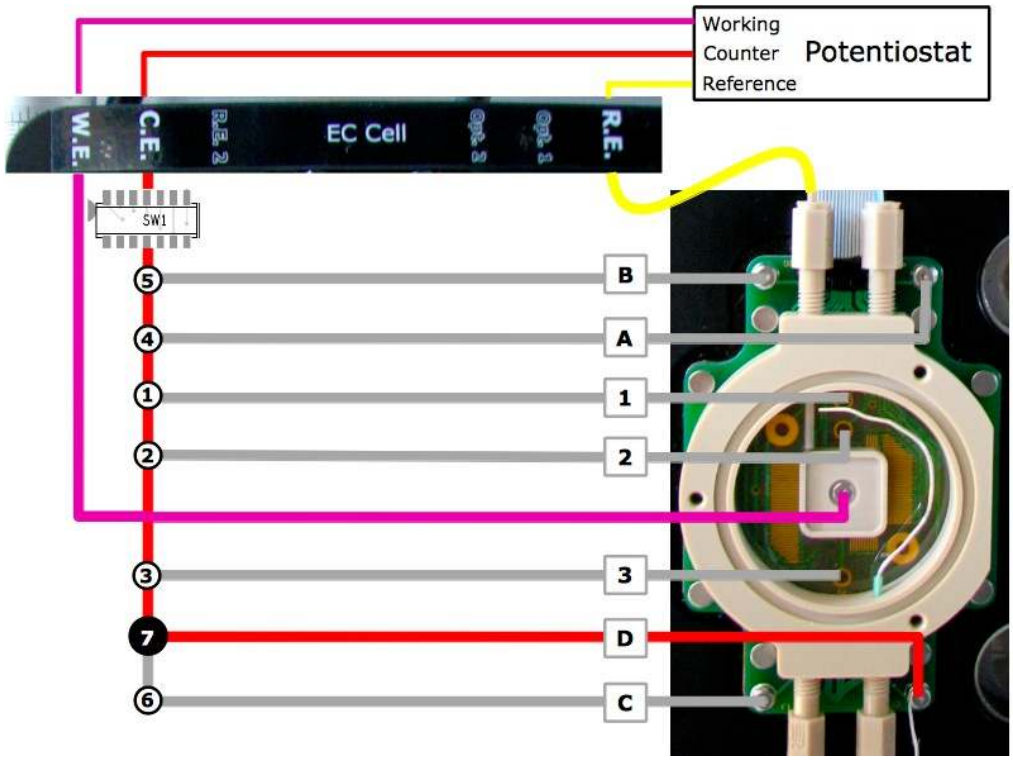


Figure 24.15.: Dip Switch 1, position 7 is set to ON so that corner terminal D (Connected to a metal wire counter electrode) is routed to the C.E. connector on the Junction Box.

Prevent Short Circuits	A good rule of thumb is to never have more than one switch per bank in the ON position and to leave all switches associate with unused junction box connectors in the OFF position.

24.10.5. Junction Box Circuit Schematic

For a more detailed circuit diagram of the junction box circuit board see Figure 24.17 on page 351.

24.11. Detecting Fluid Leaks

There are several inter-digitated electrode patterns on the top and bottom of the EC cell circuit board. If fluid bridges the gap between these electrodes, a circuit inside the junction box will beep.

In order to beep, the box needs to be powered. This can be done by connecting the supplied USB cord between a USB port on the computer and the junction box (see Figure 24.18 on page 352). If you are using a heated EC cell, the box can be powered by connecting the heater controller to the junction box and the AFM controller. Either one of these methods will supply the 5V signal required to sound the beeper.

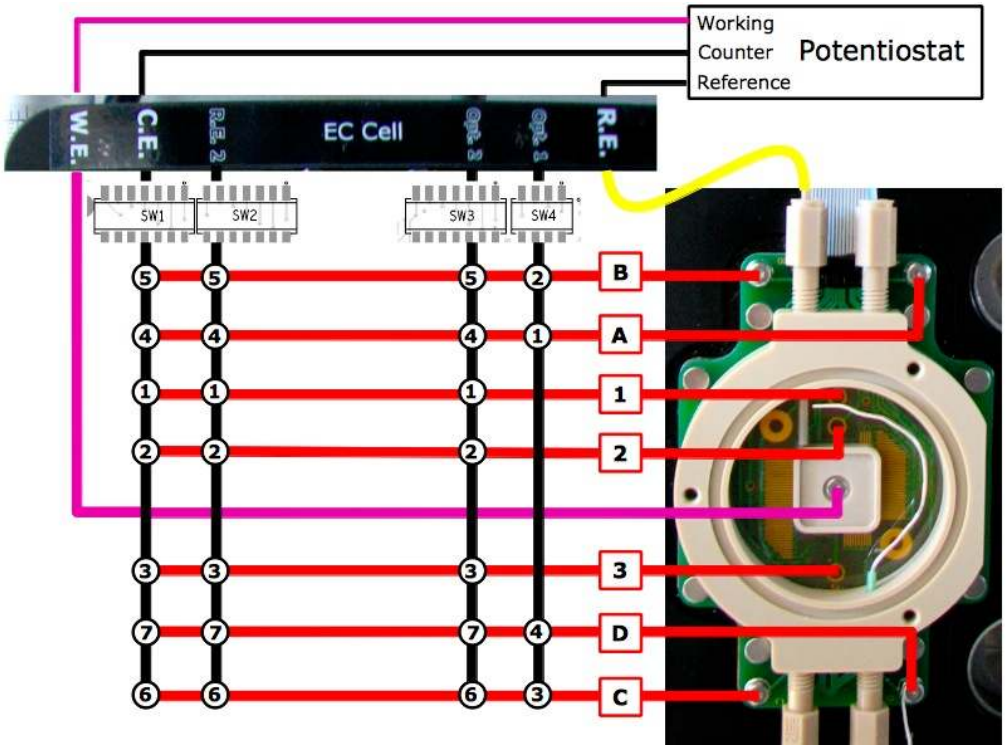


Figure 24.16.: Graphic which shows exactly how each dip switch connects the Junction Box connectors to the terminals on the EC Cell.

All but the very first models of the junction box have a **blue button** on the top. When the button is pressed it triggers the audible leak alarm. Get into the habit of pressing the button before filling the cell just to make sure everything is properly plugged in.

Note that the screws on the bottom cup (Section 24.9 on page 343) are also part of the beeper circuit. If the bottom cup ever fills up with a thin layer of liquid that touches both the screws, you will be alerted.

Warning	Be aware that when your acoustic hood is closed, it is pretty hard to hear anything happening inside, like the beep. Still, we felt it was better to have some alarm than none at all.

24.12. General guidelines

Warning	If you do not fully understand how to operate a fluid cell accessory in a sealed manner, you will likely cause serious damage your AFM and incur delays and expenses associated with repairs. Please read the necessary sections (Chapter 7 on page 62 is a good start) and practice everything, at least once, away from the AFM.

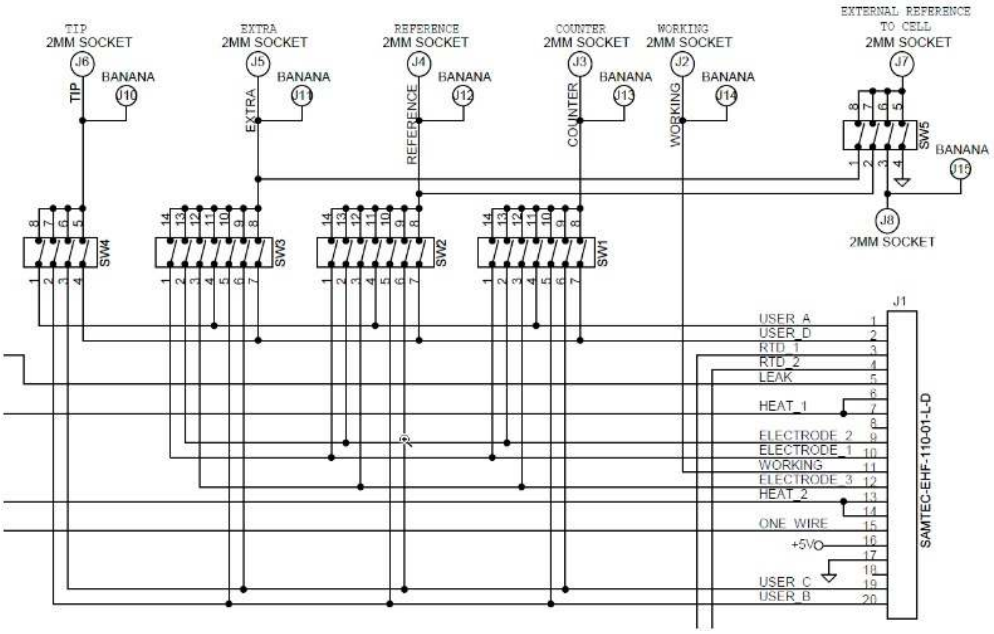


Figure 24.17.: The circuit diagram of the Junction Box.

24.12.1. Cell Volume

24.13. Fluid Imaging with the EC Cell

Imaging with the EC Cell is typically done with the all PEEK cantilever holder described in Section 11.4 on page 108, included with your EC Cell kit. Note that this cantilever holder is typically shipped with a stainless steel screw which comes into contact with the electrolyte solution. To avoid possible contamination from this screw, consider the more inert PEEK screw included with the cantilever holder kit.

24.13.1. Sealed Operation

This requires O-ring 230.031 in the groove on top of the EC Cell. Follow the procedure described for sealed operation of the Closed Fluid Cell, in Section 14.4.5 on page 173.

Practice sealing the cell with plain water before moving on to potentially corrosive electrolytes. Successfully operating any of the Asylum Research sealed imaging accessories takes practice!

24.14. Cleaning and Storage

24.14.1. Reference Electrode Storage

The reference electrode (described in Section 24.8.3.1 on page 341) needs to be stored in water to keep functioning properly.

Figure 24.18.: Cable 448.130 supplies 5V to the junction box leak detection circuit. Plug one end into a free USB port on your PC, the other end into the junction box as shown.

1. Take the small back cap (that should have been stored in Step 1 on page 341) and squeeze it tightly while is immersed in some de-ionized water, then release. This will fill the cap with water.
2. Place the cap on the end of the electrode. A few drops of water might escape.
3. Store the electrode in its baggie and keep inside the EC cell kit box.

If the reference electrode is left dry, it can be revived by soaking in de-ionized water overnight , according to the manufacturer's instructions.

24.14.2. EC Cell cleaning

For a quick cleaning one can simply take the cell with all of its electrodes attached and properly sealed, and withdraw the electrolyte to refill with pure water (in the case of an aqueous electrolyte) repeatedly. One can also take the cell to the sink and fill with water from above, then pour it out for rinsing. Beware of any water that makes its way over the edge, since once it wicks between the thin bottom film of the cell, it can get stuck there and set off the spill alarm or allow for unwanted current to flow between the counter and working electrode connections.

Serious cleaning is best done by completely disassembling all the parts of the cell and cleaning them individually, followed by complete re-assembly.

24.15. Heating

An optional heated stage (939.021) is available for the EC cell. It can heat the solvent inside the EC cell to 60ºC. We have managed to make it go higher than this, so please contact Asylum Research for instruction if you need a little more range.

24.15.1. Parts List

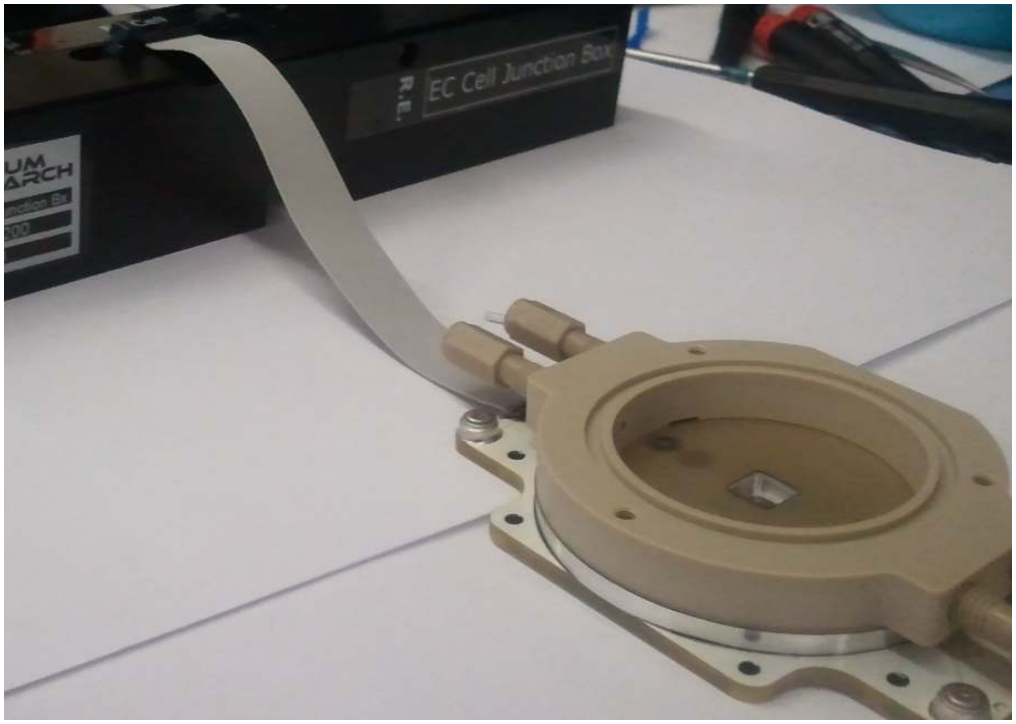
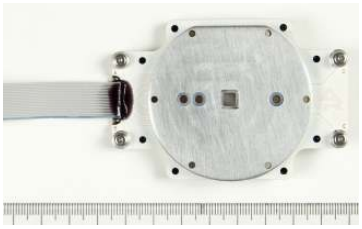


Figure 24.19.: EC Cell assembled with the heater stage installed.

Itm	Part #	Item Description	Qty	Picture
1	939.021	EC Heated Stage. Replaces the circuit board.	1	
2	448.073	EC Cell Heater Cable. Connects the junction box to the Environmental Controller.	1	
The scale in the photos is in cm and mm.				

24.15.2. Assembly

1. Remove the screws which join the EC cell body to the white circuit board. Store the screws and the circuit board.
2. Locate 6 of the 10mm (222.074) longer version of the same screws.
3. Re-assemble the EC cell, but this time using the circuit board with the aluminum heater attached. The basic process is described in Section 24.6 on page 326. The final assembly should look like Figure 24.19 on page 353.
4. If you mount your sample, you may also need to use a longer screw in your sample mount assembly.

24.16. Uses of the Cleaning Tool

Figure 24.20.: EC Cell Sample Mount Cleaning Tool (939.026). Main plastic body has part number 114.165

Once your sample is mounted (See Section 24.7 on page 327), it is nicely contained in a small PEEK carrier. We included a tool to hold the sample, and the carrier if need be, while cleaning.

Figure 24.20 on page 354 shows the assembled tool. It consists of a PEEK plastic body, a thumb nut, and a threaded shaft. Look closely at the shaft and you will notice one end has a threaded hole in it. This hole accepts the metal screw coming out of the back a sample mount.

24.16.1. Attaching a sample to the cleaning tool

1. Have your mounted sample ready.
2. Find its matching O-ring and insert in the groove.
3. Completely disassemble the three parts of the cleaning tool: body, threaded shaft, and thumb nut.
4. Thread the mounted sample into the hole of the large threaded shaft.
5. Insert the resulting combination into the **wide** end of the plastic tool body. Do not forget the O-ring.
6. Tighten it all together with the thumb nut.

24.16.2. Cleaning a Mounted Sample

Now that the sample is attached to the cleaning tool, you can easily immerse it into an ultrasonic cleaner while holding one end. This prevents the sample from moving around and keeps fluids from entering via the bottom of the mount.

24.16.3. Traditional EC Experiments

You may want to immerse your sample in a more traditional EC reaction environment, like a stirred beaker or other glass vessel. This can be done with the cleaning tool. The narrower end of the cleaning tool is the same diameter as standard chemistry lab ring stands and clamps. An alligator clip can be used to connect to the metal threads extending beyond the thumb nut of the tool. You can also clamp a wire under the thumb nut if necessary.

When the thumb nut is tight, the O-ring seals all the metal parts to make your sample the working electrode in the reaction.

24.17. DIY (Do it Yourself)

Every sample will have its own superior mounting method. We have included a few drawings of our sample mounts so you can design and machine your own sample mounts for best results. You can also contact Asylum Research for help with making a new type of sample mount.

24.17.1. Sample Mounts

24.17.1.1. Sample Mount Dimensions

Figure 24.21 on page 356 gives the dimensions of a basic sample mount, especially the square key. This particular mount would be used with a standard AS568B-021 US O-ring. Of course, you could design for any suitable O-ring available in your region.

24.17.2. O-Rings

The EC Cell was designed with off the shelf O-rings. FKM (Viton Equivalent) was chosen because of its chemical resistance/inertness and reasonable cost. If more inertness or chemical resistance are required, one can purchase a higher quality material from any USA vendor. If you are in a foreign country, Asylum Research can help you with this matter. Some material suggestions are Kalrez or Chemraz (very expensive, but almost as good as Teflon), Teflon (likely too stiff and not tested for leak tightness, but you could try), or FEP encapsulated Rubber (a reasonably priced choice with more resilience than Teflon). Fluorosilicone is another material to consider.

The O-ring sizes you will require can be found in Table 24.6 on page 355.

Use	OD	ID	Real OD	Real ID	Size Code	Asylum #
Counter Electrodes	1/8”	1/16”	0.150”	0.070”	AS568A-001-1/2	230.027
Thin Film Sample Mount	11mm	9mm	11mm	9mm		230.033
Cell/Membrane	1 3/4"	1 5/8"	1.614”	1.754”	AS568B-030	230.031
Cell/Bottom	1-7/8”	1-3/4”	1.739”	1.879”	AS568B-031	230.026

Table 24.6.: O-rings used in the EC Cell. To order a replacement with a different material, give your vendor the size code and you will get the correct size. Asylum part numbers only refer to O-rings made of FKM (Viton equivalent).

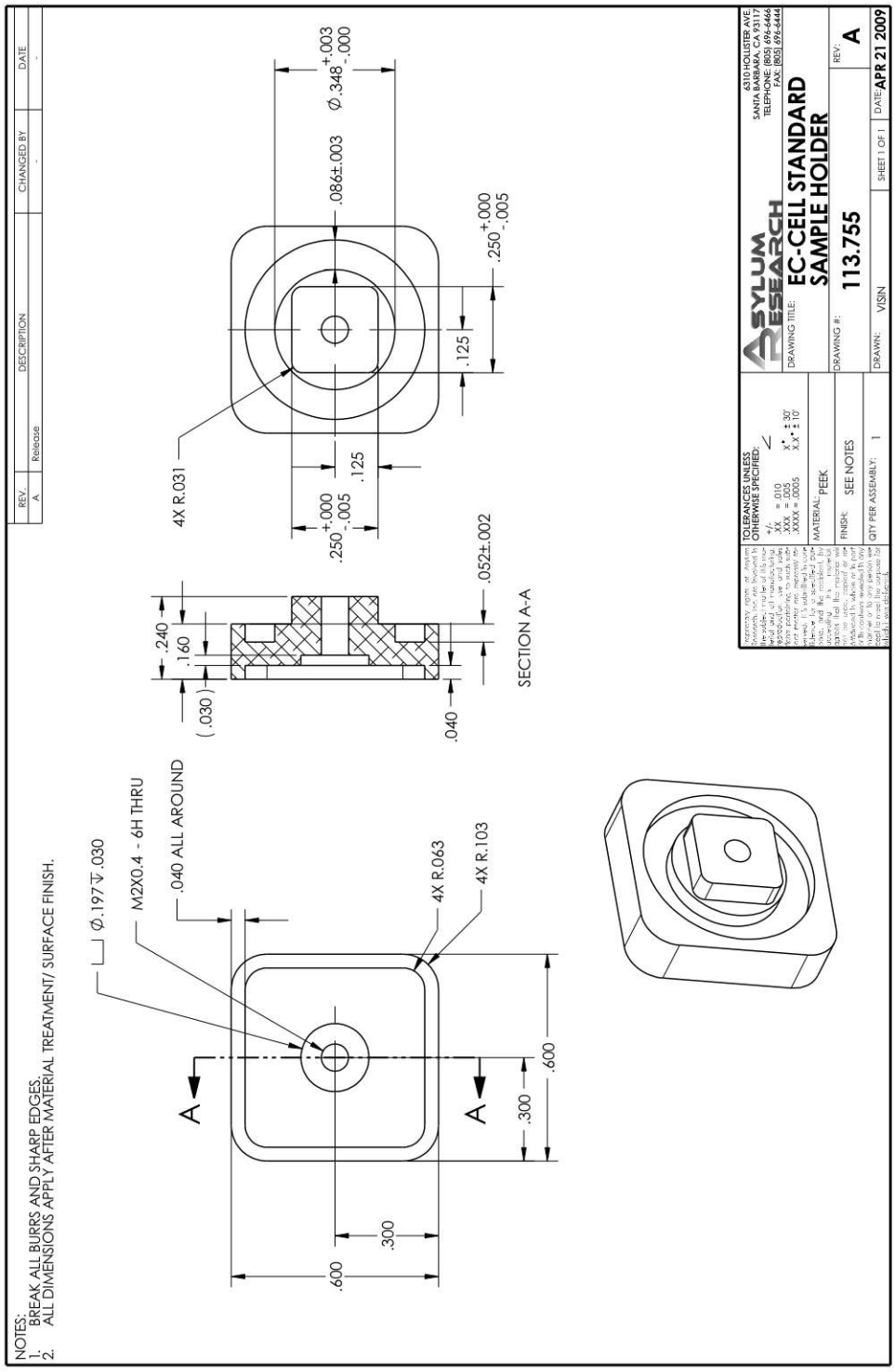


Figure 24.21.: Gives you all the necessary dimensions to duplicate this part or to design your own variation.

Note

24.6 lists part 230.027 (used for sealing some counter electrodes and the bottom cup) as having OD 1/8" and ID 1/16", but those are not accurate representations of the true OD and ID but an American industry standard for denoting O-ring size which is actually a little larger. To add to the confusion, Asylum carries a custom made O-ring (230.018) which is truly 1/8"OD and 1/16" ID. This O-Ring is used for sealing tubing entering a number of our closed fluid cell ports. For instance, see item I in Figure 14.2 on page 170.

24.18. Miscellaneous

24.18.1. Legacy Sample Mounts

During the R&D process, to address very specific customer needs, we designed some other variations on sample mounting. We will briefly cover these in case they seem of interest. Please contact Asylum Research if you want to learn more.

24.18.1.1. Epoxy Potting

This basic sample mount is for samples which are typically conducting throughout and are irregular in shape. While cumbersome, the epoxy potting method is in some cases the only way to properly mount a sample. Electrical contact will be made on the back of the sample. For making front side contact we recommend different styles of sample holders such as those described in Section 24.7.1 on page 327 and Section 24.18.1.2 on page 358.

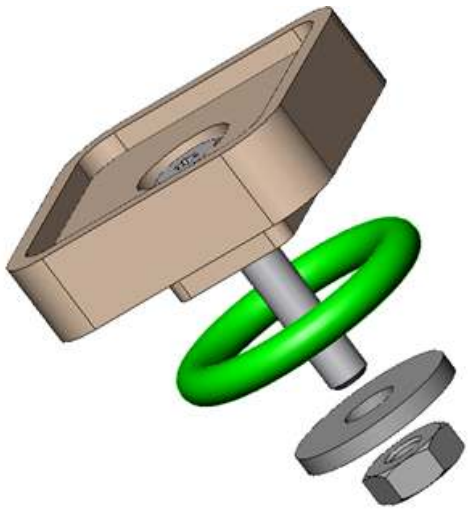


Figure 24.22.: Potting sample mount 939.016.

1. Locate 939.016, which is the sample mount shown in 24.22.

It is recommended to disassemble all the parts and sonicate them in a clean solvent like alcohol before building up the sample.

1. Insert the M2x10 screw (222.074) and tightly screw it in with the 290.144 Torx driver.
2. Cut your sample so it will easily fit in the sample mount.
3. Mix some silver epoxy. You may already have your laboratory favorite. If you do not have any, three packets of Henkel Hysol Tra-Duct 2092 are included in your kit (080.108).
4. Put a small amount of silver epoxy on top of the screw head and place the sample on top. If you are using Tra-Duct 2902, let it cure 24 hours at room temperature, two hours at 65ºC, or one hour at 110ºC.
5. Prepare some potting epoxy, like Henkel Loctite E-60NC Hysol (080.084 epoxy and 290.145 dispenser), or use your own favorite.
6. Pour potting epoxy around the sample to cover up the edges and keep the electrolyte from contacting the backside of the sample.
7. Let cure. For the NC60 epoxy this will be 24 hours at room temperature or less time (a few hours) at temperatures up to 93ºC. Do not exceed the maximum temperature.

Note that this sample mount can also be made with different dimensions. If you have access to a local machine shop, you can have them make parts for you. For drawings, please refer to Section 24.17 on page 355.

24.18.1.2. Parafilm Sample Mount

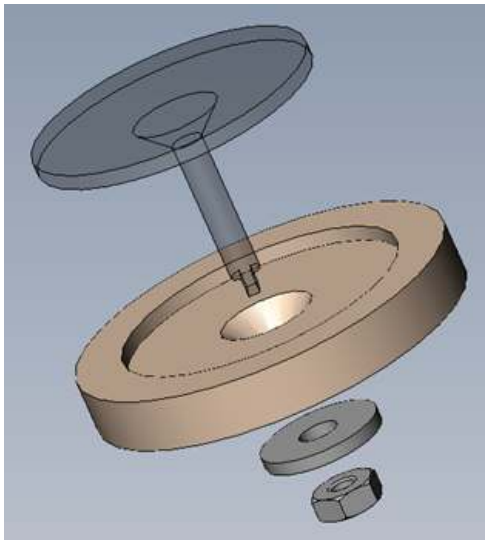


Figure 24.23.: Experimental Parafilm sample mount 939.018.

This sample mount is experimental and can be purchased by special request from Asylum Research. Note that fluid comes into contact with Parafilm, which can be acceptable in some cases.

1. Locate 939.018, which is the sample mount shown in Figure 24.23 on page 358.
2. It is recommended to disassemble all the parts and sonicate them in a clean solvent like alcohol before building up the sample.
3. Screw the metal part (113.761) into the PEEK part (113.761).

4. Place the sample on top of the metal disc and put some silver paint on the corners. This will hold the sample down and will also connect the top surface to the metal, in case the substrate is not conducting.
5. Locate some standard Parafilm M, commonly found in chemistry labs where it is used to seal beakers and bottles.
6. Heat the sample up to about 85ºC. An oven is ideal, but a desk lamp light bulb placed near the sample will usually suffice.
7. Cut a piece of Parafilm (a little larger than the sample holder) and punch a small circular hole in the middle.
8. Place the film onto the pre-heated sample with the hole centered and let it sit in the heat for a few minutes.
9. The Parafilm will slump over the sample and adhere to it very well. It will also adhere to the peek surrounding the metal of the sample holder.
10. Finally, let it all cool down and use a blade to trim off the excess Parafilm. You may have to play around with this recipe a little for the best results.

If Parafilm is not acceptable in terms of contaminating the solution, ask Asylum Research about a modified sample mount with a higher PEEK lip which is flush with the top of the sample. This will depend on the sample thickness. Glue the sample down to the metal support and then place an annular inert sticky backed film on top of the sample. One suggestion is Kapton tape (McMaster Carr part number 7648A715), which can be ordered with a 25 micron thick layer of either silicone or acrylic adhesive. Only the adhesive near the edges will be in contact with the electrolyte. Other film choices are adhesive backed FEP (McMaster Carr part number 5805T11) or acrylic adhesive backed PEEK film (McMaster Carr part number 4671T51).

Figure 24.24.: Parafilm M.

Parafilm Properties

Hydrochloric Acid Concentrated (12N): No apparent effect in 24 hours. Dilute (6N): No apparent effect in 24 hours.

Sulphuric Acid Concentrated (36N): No apparent effect in 24 hours. Dilute (6N): No apparent effect in 24 hours. Fuming: No Effect expected short term.

Nitric Acid Concentrated (16N): No apparent effect in 24 hours. Dilute (6N): No apparent effect in 24 hours. Fuming: No Effect expected short term.

Sodium Hydroxide Concentrated 22%: No apparent effect in 24 hours.

Ammonium Hydroxide Concentrated (28% NH3): : No apparent effect in 24 hours.

Potassium Permanganate 5%: No apparent effect except permanent black brown coloration in 18 hours. 0.1%: Same as above except slightly less color.

Iodine Solution 0.1 N: No effect except staining brown in 18 hours.

Salt (NaCl) solution 20%: No apparent effect in 24 hours.

Carbon tetrachloride Will start to dissolve Parafilm M on contact.

Ethyl alcohol, 95% No apparent effect except some face whitening in 24 hours.

Isopropyl alcohol, 99% No apparent effect in 24 hours.

Acetonitrile, 99+% No apparent effect after 24 hours.

Acetone Parafilm M is not stable in or around acetone as it will swell and to some degree lose its dimensional integrity. But even though acetone will not dissolve Parafilm M, after 24 hours of immersion in acetone, about 1.3% by weight of the components of Parafilm M will have been extracted into the liquid. Surprisingly, even after this amount of material is extracted, Parafilm M still remains at least to some degree, functional as a covering material. However, our best recommendation is to not use Parafilm M in the presence of acetone.

Hydrofluoric acid We would urge caution with the use of Parafilm M® in the presence of HF, liquid or vapor. We do not have any specific information on how Parafilm M® will respond to HF vapor. But we would expect that it would provide some level of protection to rubber and other elastomeric seals, since it would be less reactive than the seals they would be protecting. However having said that, our concern would be that at the low level of 1 ppm there may be some solubility in the Parafilm since it is fairly amorphous.

25. Variable Field Module 2 (VFM)

CHAPTER REV. 1580, DATED 08/30/2013, 06:11. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

25.1. Overview

Note	This chapter was written on the second generation VFM2 model. For a discussion about differences of the previous VFM model, please see 25.13.

25.1.1. Prerequisites

Imaging with applied magnetic fields is a fairly advanced technique. It is assumed that you are proficient in:

- Basic AFM Safety (Chapter 26 on page 402).
- AC Mode Imaging in Air (Chapter 4 on page 15).
- Magnetic Force Microscopy, which is described in *Applications Guide, Chapter: Magnetic Force Microscopy (MFM)*.

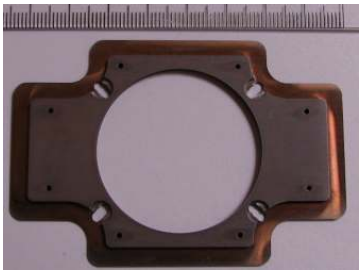
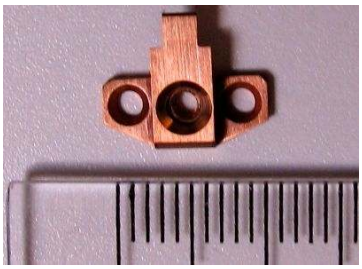
25.2. Parts List

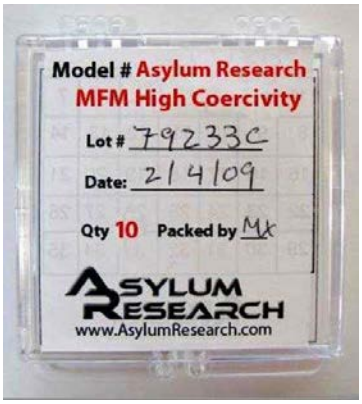
This table lists all relevant parts, with photos and links to the relevant parts of the documentation which describe how to use them. All parts and assemblies have six digit Asylum Research part numbers. If you ever see such a number in the text and do not know what it refers to, go to the top of this document and run a search for that number and you will find it in the list.

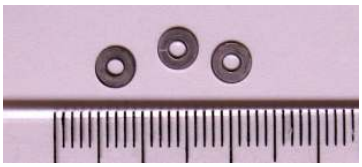
Before you begin, please check that you have the following components and tools. If you are missing anything or have questions about obtaining consumables, please contact Asylum Research for assistance.

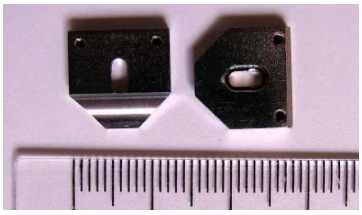
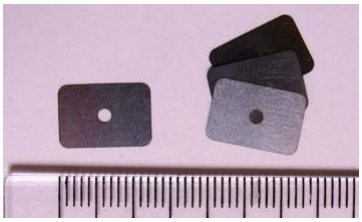
Note Many of the items below are part of the VFM2 accessory kit (Asylum Research Part #900.244).

Itm	Part #	Item Description	Qty	Picture
1	111.737	Modified 0-80 Screw. Used to attach the cantilever clip to the body. Note that these screws have been machined to a nonstandard length. You must only use this Asylum part number. Item J in Figure 11.2 on page 102.	2	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
2	111.738	Modified 1-72 Screw. Used to tighten the cantilever under the clip. Note that these screws have been machined to a nonstandard length. You must only use this Asylum part number. Item H in Figure 11.2 on page 102.	1	
3	112.041	VFM Scanner cover plate. Allows the VFM to be attached to the AFM scanner. See Section 25.5 on page 374.	1	
4	112.966	Screw down spring clip, non magnetic Beryllium Copper. See Step 8 on page 372.	1	
5	249.033	Adhesive tab sheet. You may order more from Ted Pella, part 16079. Can be used to stick flat samples on top of the VFM pole pieces. See Section 25.9.6 on page 393.	2	
6	290.102	Tweezer, Curved Sharp, Standard Grade. Typically used for handling samples and the small screws supplied with the VFM.	1	
7	290.114	Screwdriver, Slotted, 3.0 mm Width. Wiha 260 3,0 X 50. Used to mount the VFM to the scanner. See Step 5 on page 369.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
8	805. ASYMFM. HC	Asylum Research high coercivity MFM cantilever. A useful cantilever for MFM imaging. See Section 25.3 on page 367.	10	
9	805. ASYMFM. SMPL	Asylum Research MFM sample pack. 5 EA of STD, HC, HM, LC, LM cantilevers, 25 in all. A useful collection of cantilevers for MFM imaging. See Section 25.3 on page 367.	1	
10	908.026	Leg extender assembly for most current model MFP3D AFMs. See Step 6 on page 370. * The correct leg extenders will be shipped to you based on your AFM serial number.	1*	
11	908.052	Kinematic leg extender assembly for most MFP3D AFMs prior to Dec. 2008. See Step 6 on page 370. * The correct leg extenders will be shipped to you based on your AFM serial number.	1*	
12	916.016 + 112.014	Objective removal tool and dummy objective. Used to remove the bottom view objective from the scanner. Its use is described in Step 3 on page 369.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
13	449.011	Cable CB25M-DB25F, 2 meters, unmodified. See Section 25.4.1 on page 372.	1	
14	BHCS 0-80 X 1/8" SS	0-80 X 1/8” button head cap screws, stainless steel. Use 0.035”: Allen Driver (290.104). Used to fasten the clips (112.959) or the ground wire (448.114 and see Step 3 on page 395).	8	
15	SHCS 0-80 X 1/4" SS	0-80 X 5/32” socket head cap screws, stainless steel. For holding down the pole pieces with few or no shims. See Section 25.9.2 on page 387.	8	
16	SHCS 0-80 X 3/16" SS	0-80 X 3/16” socket head cap screws, stainless steel. For holding down the pole pieces when using many shims. See Section 25.9.2 on page 387.	8	
17	SHCS 0-80 X 5/16" SS	0-80 X 5/16” socket head cap screws, stainless steel. Spares for attaching the scanner top plate 112.041. Use with a #0 washer (next item in the list). See Section 25.5 on page 374.	8	
18	#0 FLAT WASHER MS801	Number 0 stainless steel washer, Spares for attaching the scanner top plate 112.041 (see Section 25.5 on page 374). Use with the previous item.	8	
19	112.959	Beryllium Copper Clip. Used with BHCS 0-80X1/8" SS screws. See Section 25.9.6 on page 393.	5	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
20	113.672	Low profile pole pieces. Attach with SHCS 0-80X5/32" SS. See Section 25.9.2 on page 387. Note, not compatible with earlier VFM stages. See Section 25.13 on page 396.	2	
21	114.250	High profile pole pieces. Obsolete, but they were included in a few VFM2 models.	2	FIGURE NEEDED
22	290.111	0.050”: Wiha Allen Driver 263 1,3 – 0.05” X 40. For most socket head screws on the VFM2. Typically used to remove the pole pieces. See Section 25.9.2 on page 387.	1	
23	290.104	0.035”: Wiha Allen Driver. For the screws (BHCS 0-80X1/8" SS) that fasten the clips. See Section 25.9.6 on page 393. (112.959) or the ground wire (448.114 and see Step 3 on page 395).	1	
24	114.645	0.003” (75 µm) thick shim stock. Used for spacing the pole pieces when using large flat samples. See Section 25.9.2 on page 387.	30	
25	900.056.1	VFM2 controller box. Connects between the AFM controller and the VFM2 sample stage. See Section 25.4.1 on page 372.	1	
The scale in the photos is in cm and mm.				

Itm	Part #	Item Description	Qty	Picture
26	900.251	VFM2 Sample Stage - the subject of this chapter.	1	
27	279.061	Objective Storage Case. Used to store the bottom view microscope objective after removal with tool 916.016 as described in Step 3 on page 369.	1	
The scale in the photos is in cm and mm.				

25.2.1. Temperature Sticker

The VFM2 contains magnetic materials which can lose their "potency" if exposed to temperatures above 80°C. Please inspect the sticker attached to the VFM cable. If the sticker has turned black indicating temperatures in excess of 80°C, please test the ability of your VFM to reach its specified maximum field (see Section 25.9.4 on page 389). Note that the VFM2 should never be stored or transported in such a way that the temperature exceeds 80°C. While it is not likely, the interior of a car or shipping container can reach such temperatures in sunny weather. Please contact Asylum Research Support if your VFM stage appears to have been exposed to high temperatures or does not meet its maximum field specification.

25.3. AFM and MFM in an Applied Magnetic Field

A powerful tool for understanding the behavior of a magnetic sample is an applied field. Applying a field may prove useful in applications such as imaging the domain reversal behavior of a ferromagnetic thin film, studying magnetic field dependent resistance in sensor devices, or imaging magnetic particles that have been used as biological tags. The bibliography at the end of this note gives a partial list of references where researchers have used an applied field in magnetic force microscopy (MFM) studies.

MFM imaging in an applied magnetic field can actually complicate the interpretation of your images significantly. In addition to the sample behavior being field dependent, the magnetic state of the MFM tip can also change in an applied field. One way to simplify interpretation in an applied field is to use a tip with a coercivity (or switching field) that is very different from that of the sample, either much lower or much higher. Then, when the contrast is changing, one can be relatively confident of the origin of the change. For this reason, super paramagnetic tips or very high coercivity tips can be useful for applied field imaging. For applied field MFM work, we generally recommend starting with high coercivity MFM cantilevers such as the type ASYMFMHC. These cantilevers have coercivities greater than 5,000 G and therefore will not be re-magnetized by use with the VFM. For your specific application, do not hesitate to contact Asylum Research if you would like further information.

25.3.1. How it works

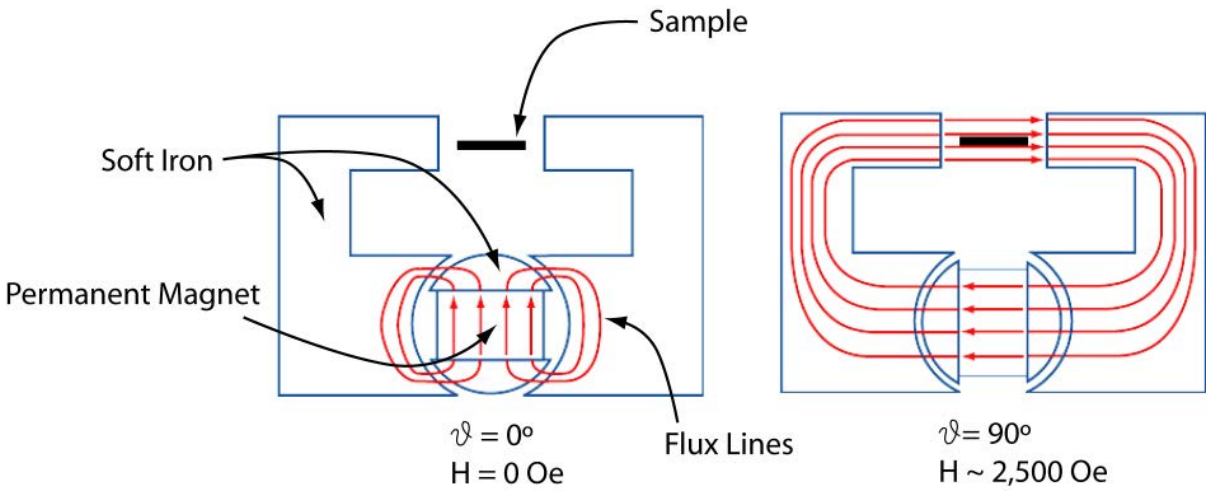


Figure 25.1.: How it works: A rare earth permanent magnet is at the heart of the VFM. The strength and sign of the magnetic field applied to the sample depends on the rotation angle of the magnet. When the magnet is at 0° or 180°, the magnetic flux is shunted away from the sample by the soft iron armature and pole pieces. As the magnet rotates, more and more flux is channeled instead through the sample. At 90° and 270°, the field magnitude is maximized.

The Asylum Research Variable Field Magnet (VFM) module relies on a rare-earth permanent magnet to apply a field to the sample. By rotating the magnet, different amounts of magnetic flux can be channeled through the sample. Referring to Figure 25.1 on page 368, the flux through the sample is maximized when the magnet is oriented at 90° and 270° and minimized when the magnet is oriented at 0° and 180°. By using permanent magnets we avoid using electromagnetic coils that can require significant current to maintain a large magnetic field. This current inevitably leads to unwanted joule or resistive heating. Heating can degrade the performance of the AFM as well as change the physics of the sample being studied. A motor controls the magnet rotation. Motion of the motor is controlled through menu settings in a control panel within the MFP-3D/Igor Pro software interface, detailed below. The software uses the signal from a magnetically sensitive sensor embedded close to the sample mounting area to control the desired field strength of the VFM stage to within ± 1 G. The software feedback control can also be disabled to allow the user to manually control the VFM field strength.

25.4. Installing the Hardware

1. Lower the bottom view objective as far as it will go. Use the fat micrometer on the lower right hand side of the base (See Figure 2.4 on page 9).

2. **Determine if the objective retracted enough**

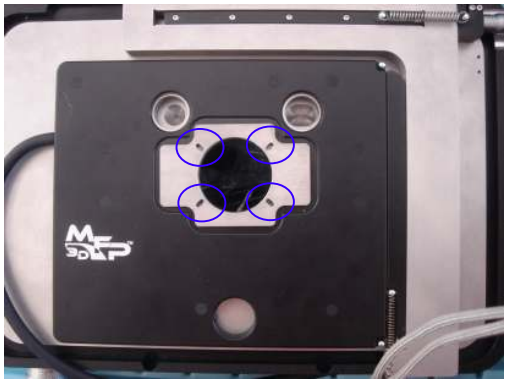
- Determine if the objective retracted completely below the top surface of the base. If you are not sure, remove the scanner (Step 2 on page 374) and place something flat over the objective hole to see if it sunk completely below the surface.
- IF the objective is below the surface, THEN skip the next step and go to step 4
- IF the objective still protrudes above the plate for some reason, THEN follow the next step: objective removal.

3. **Remove the bottom view objective (if needed)**

- Locate the objective removal tool and cover (), and the objective storage case (279.061).
- Using only finger pressure, use the tool to unscrew the objective and place it in the storage case.
- Use the tool to place the cover over the threaded hole in the AFM base. Only tighten very gently.
- Replace the scanner if necessary (Step 10 on page 376).

4. **Install the VFM scanner top plate (if needed)**

- If it is not already installed, replace the scanner top plate with the one supplied with your VFM (112.041). It has four oblong slots that are not present on the standard model.
- Please read Section 25.5 on page 374 for directions on this process.



5.

Mount the VFM onto the Scanner

- Using screwdriver 290.114, **carefully** align the slots of the four screws with the white lines on the VFM.
- Place the VFM onto the scanner in the orientation shown to the right. If necessary, adjust the screws slightly so they properly engage the slots in the scanner plate.
- Rotate all screws by 90° to lock the VFM down.

6.

Determine the need for leg extenders

- If the three legs on your AFM head do not have a groove just above their spherical tips, then you need to extend the length of the legs being used. Skip to the next step and install the supplied leg extenders.
- If there are grooves on the legs, these indicate a newer model of AFM head with longer travel on the legs. If your sample is less than 1mm thick, then you will not require leg extenders. To be sure this is the case, please follow the procedure below.
  - Extend all three legs to their full extent and place the AFM head (with cantilever holder in place) over the mounted VFM.
  - First seat the rear legs, then carefully lower the seat to the front leg while looking at the clearance above the VFM.
  - There should be a few millimeters of remaining space. Judge whether or not this space is enough to accommodate your sample thickness. Typically there should be a few millimeters of clearance and you should not have to install leg extenders. A bit more detail follows:
  - The clearance above your sample may fall into three categories:
    * If the sample surface sticks up higher than the highest possible position of the AFM head, installation of leg extenders will be required. Skip to the next step for instructions. Note that samples extending above the VFM pole faces are not very well suited due to field gradients. Please read more on optimal sample mounting in section Section 25.9 on page 387.
    * If there are a few millimeters of clearance, you cannot use leg extenders. Once you install them you may find that the head will not be able to go low enough to reach the sample. Do not install the leg extenders if this is the case.
    * There is a small window (about a 1mm range) of overlap where you can engage a sample either without leg extenders but with the legs fully extended, or with leg extenders with the legs fully retracted. We recommend that you do not use the extenders if it is not required.

7. **Install the leg extenders (if needed)**

- Slide the leg extensions over the ends of the head legs.
- If one leg extender is shorter than the others, place it on the front leg.
- Push the extensions on the head legs until the balls on leg ends fit into the cupped surface inside the extension.
- Secure the extensions to the legs by gently tightening the two hex screws on each extender.

8. **Replace the cantilever holder spring clip**

- Using a small Phillips screwdriver, remove the two outer screws.
- Lift the clip off and store in a safe place.
- With the same screws, affix the new Beryllium Copper clip (112.966). Do not over tighten the screws. Spare screws are in your kit (111.737).
- If the center screw is not yet present, install it now (item 111.738).

Note The center screw is larger than the outer screws. Do not mix them up.

The standard spring clip that holds the cantilever is slightly magnetic and leads to unwanted field gradients and poor magnetic field sensor readings. We have provided a nonmagnetic clip made of beryllium copper that can be substituted when using the VFM.

When done with the VFM experiments, we recommend that you replace the original steel clip. The BeCu clip may corrode if it is used in fluid

25.4.1. Installation – Electrical connections

While not strictly necessary, it is good practice to turn off your AFM controller before making these connections.

The Variable Field Magnet (VFM) connects to its interface box that is in turn connected to the expansion port of the MFP 3D controller using the supplied DB25 cable (449.011). The connections are illustrated in Figure 25.2 on page 373

Figure 25.2.: TheVFM sample stage plugs into the 9-pin connector on the VFM controller box. This in turn plugs into the 25-pin expansion connector on the front of the MFP-3D controller. This photo shows the older MFP-3D controller. The newer ARC2 controller can be used as well.

We typically leave the controller box outside of the acoustic enclosure and simply let the black cable between the VFM and the controller box hang from the front of the enclosure. The cable can be safely pinched between the sealing gasket of the hood's front door.

When the controller is turned on, a bright blue light should shine from the small circuit board between the pole pieces.

It is acceptable to have the cable between the VFM and its controller box hanging out of the front of an acoustic enclosure. The cable is thin enough to be pinched between the enclosure door seal. You may also route the cable through the proper cable clamps, but it is not strictly necessary, and probably only advisable for cases where the VFM stage is used a lot.

25.5. Changing the Scanner Cover Plate

This section assumes the VFM scanner top plate is not installed. As described before (See Step 4 on page 369), it has four oblong slots which the VFM latches onto. If your lab never deals with samples in fluid, it is perfectly fine to leave this plate attached indefinitely. Otherwise, fluid can leak through the holes and bypass the scanner liquid shield, so it is recommended to replace the original scanner plate when you are finished with the VFM experiments.

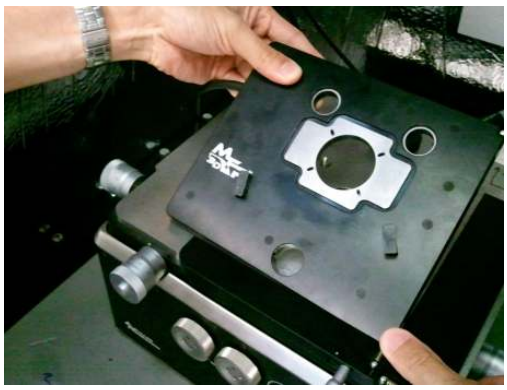
If the VFM was delivered separately, the scanner's sample stage plate needs to be replaced.

The following instructions only apply to customers who have the latest version of MFP-3D scanners, which began shipping on February 1st, 2006. They are easily distinguishable from the older version, in that they provide access to the four additional screws around the center hole. For older models, contact Customer Service at 1-888-472-2795 for assistance (Figure 5).

Note: The four additional holes to mount the VFM also create four additional locations for fluid to leak into the scanner. Please remove and replace the original sample stage if the VFM is not going to be used for extended periods of time.

1. Before changing anything, measure the hysteresis for both X and Y (See Section 25.5.1 on page 376). Make note of the results, preferably with screen captures or saving of graphs.
2. **Remove the scanner**

   - To release tension on the scanner spring, translate the scanner as far forward as possible.
   - Unplug the scanner cable from the base of the microscope.
   - Gently tilt the scanner upward from the AFM base (held down by magnets) and disconnect the retaining spring that holds it to the base. Hold your finger on the spring to keep it from jumping away.

   
3. Lay the scanner down on a clean flat surface.

4.

Remove the scanner top plate

- Use the 0.050” Allen tool (290.111), to remove the eight screws (0-80 X 5/16") and washers (#0) from the scanner bottom. Be careful not to lose the screws and washers. If necessary, spares are included with your kit. Use tweezers to handle the parts.
- It is best to completely remove all the screws to be certain that none are still holding onto the plate.
- Lift the scanner up, leaving the scanner top plate behind. If the plate sticks, turn the scanner over and remove the plate with your fingers.

Note Scanners delivered prior to 2008 may be missing access holes for the outer screws. If this is the case, please contact the Asylum Research support department about obtaining a new cover plate with the access holes.

5.

Clean the water shield

- Clean the water shield and exposed stage parts with a cotton swab and alcohol as needed. Some residue from prior spills may be present in labs that perform imaging in liquid.
- The photo to the right shows a clean scanner and liquid shield (black plastic ridge adjacent to visible metal cross).

6.

Prepare the new scanner top plate

- Place the new scanner top plate upside down on your working surface.
- Place scanner on top of the plate (bottom of scanner facing upward) and slide it around to visually center the 8 screw holes.

7. **Install the screws and washers**
   - Partially thread in all 8 screws.
   - Center the screw heads as best you can.
   - Gently tighten all the screws in a “star pattern” alternating screws that are diametrically opposed.

   Note Be careful not to over torque screws when tightening.

8. Replace the scanner on the AFM base and plug it in. At this point, it is not necessary to attach the spring to the driver bar since this process may require a few iterations.

9. **Measure the XY hysteresis**
   - Follow Section 25.5.1 on page 376.
   - If any of the three hysteresis values are within 5% of the previously measured values, you are done and can proceed to the next step.
   - If any of the new values exceed the old ones by 5%, the stage plate is likely rubbing the water shield. In this case, remove the scanner and loosen the eight screws until the top plate slides freely.
   - Attempt to center the plate again and tighten screws as instructed in the previous steps.
   - Repeat the XY hysteresis measurement again until you pass the 5% test. Feel free to call Asylum Research if you are having trouble.

10. **Replace the scanner**
    - Place the scanner on the AFM base in the same tilted manner that was used when removing it.
    - Reconnect the spring between the pin on the scanner and the pin on the micrometer driver bar.
    - Tilt the scanner back gently until it lies flat and move the micrometers until the grooves (or divots, depending on the vintage of your MFP-3D) in the baseplate are centered over the three holes in the scanner.
    - Plug the scanner cable connector back into the AFM base.

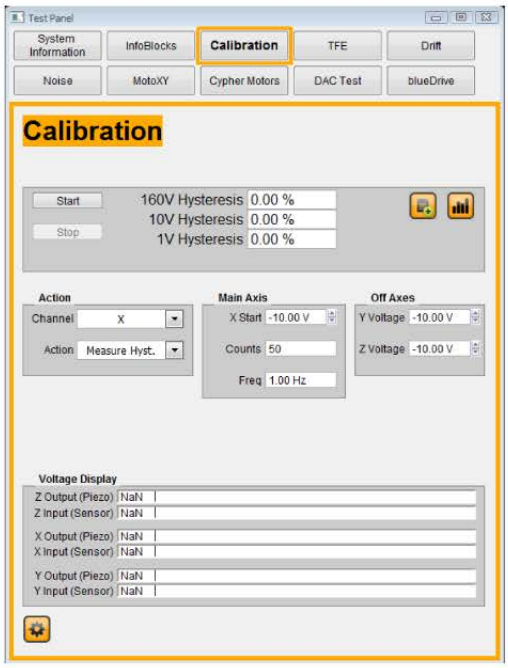
25.5.1. XY Scanner Hysteresis

This procedure measures the hysteresis of the XY scanner (piezos and mechanical assembly). The measurement is done off the surface; no tip or sample is required. In fact, the AFM head does not need to sit on the scanner at all. The X and Y piezos are exercised in open loop (no feedback from sensors) through three voltage ranges and the hysteresis for each is calculated from the corresponding sensor data.

1. Load the test procedures by selecting *Programming* ▷ *Load test procedures* from the main menu bar.. The word *Testing* will appear on main menu bar.

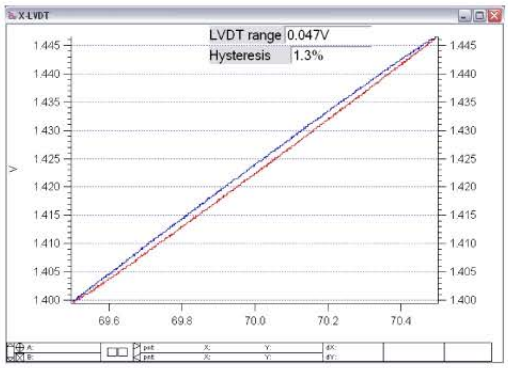
2. **Bring up the Hysteresis Measurement Panel**

   - Select the 'Calibration tab'.
   - Set Channel to X-axis.
   - Set Action to Measure Hysteresis.
   - Set Frequency to 1 Hz.
   - Set Cycles to 50.



3. **Collect the X axis hysteresis plot**

   - Click Start. The piezo cycles 50 times through each of three voltage ranges: 160, 10, and 1 V.
   - A graph of the sensor (LVDT) signal vs. piezo drive voltage appears.
   - As the piezo begins to move, its range increases. Wait for the LVDT range to stabilize (about 50 cycles), and then click Start again. The measurement is complete when the Stop button changes to Start.
   - Write down hysteresis values for the three voltages.



4. Change the Channel to Y-axis and repeat the previous step.

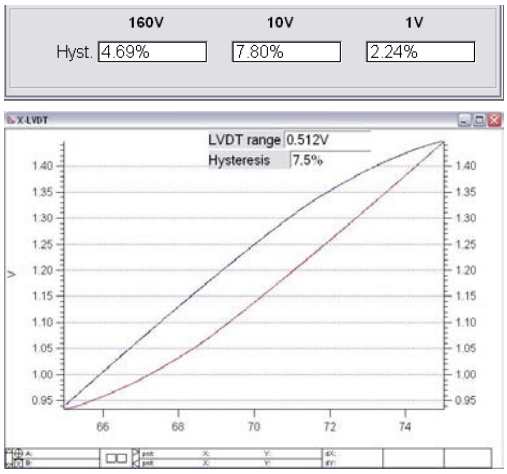
5.

Evaluate Hysteresis Results

Good Acceptable hysteresis for X and Y should be less than or equal to 5% for 160 V, 4% for 10 V, and 3% for 1 V. It is typical for Y to have a slightly larger hysteresis than X due to the greater mass the Y scanner moves.

Bad It is more common for the 10 and 1 volt ranges to fail when there is rubbing. Bad values and a bad graph are shown to the right.

If the hysteresis is above spec, the moving component of the scanner (stage top plate) is likely rubbing against the housing or has some other mechanical interference.



25.6. Software Tutorial

This tutorial can be performed with the VFM sitting on the bench top, as long as it is connected as shown in Figure 25.2 on page 373.

Turn your controller back on or rescan the smart start bus (see Step 6 on page 10).

The control panel for the VFM (Figure 25.3 on page 379) will automatically appear once the software is started, as long as it is connected to the MFP3D controller. The panel can also be displayed by selecting *AFM controls* ▷ *VFM panel* from the main menu bar.

A complete description of each control parameter in the panel can be found by clicking on the box with the question mark next to the parameter of interest.

This tutorial gives instruction for a few rudimentary tasks to demonstrate the controls.

1. The panel should start out as it appears in Figure 25.3 on page 379, with mode and feedback turned off. Your field may be reading a different value than that in the figure, depending on the rotor position of the VFM when it was last used.
2. Make sure the pole pieces are 3mm apart for the following examples to function. Use the supplied 0.050” allen driver (290.111) to move the pole pieces. Also see Section 25.9.4 on page 389.
3. Click the More button next to “Live Graph” three times. This will bring up a realtime record of field, motor speed, and field setpoint.
4. Set the ramp rate to 3000 G/m, target field to 2000 G, and Error Tolerance to 5.0.
5. Set Feedback to On and Mode to On. The motor should start turning and the values on the graph will change. Wait until the field settles down to 2000G.
6. Set Feedback to Off and Mode to Off.
7. Click the Clear button next to “Data History” to clear the graph.

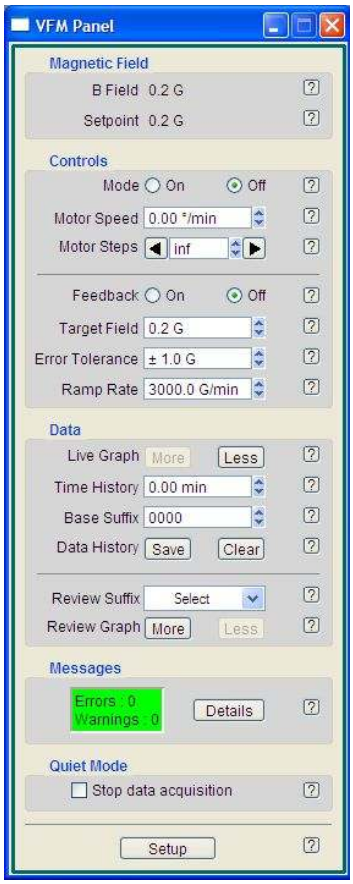
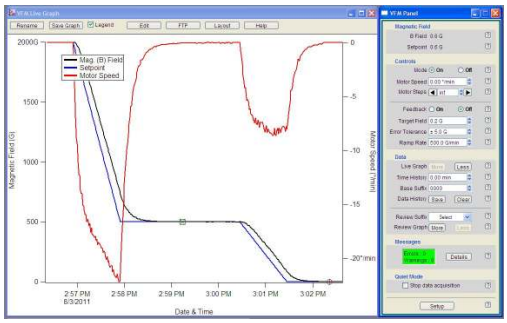


Figure 25.3.: The VFM panel. A complete description of each control parameter in the panel can be found by clicking on the box with the question mark next to the parameter of interest.

8.

Controlled Field Ramp

- Set the ramp rate to 2000 G/m.
- Set the Target Field to 500 G.
- Click 'Feedback On' and 'Mode On'.
- The motor will execute a controlled field reduction and gently stop at 500G. Once it reaches the target value within the set error tolerance, it will turn the feedback off and the motor speed to zero.

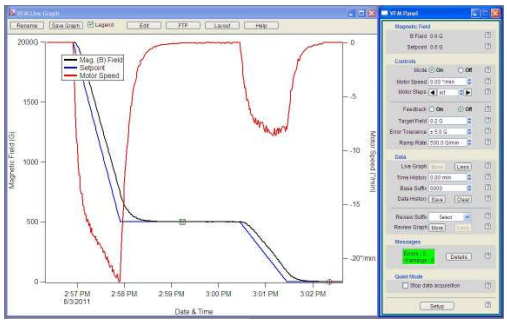


Note If the error tolerance is too narrow, the motor will never stop adjusting and may cause imaging noise. Always turn the mode to off before imaging if you are uncertain.

9.

Controlled Field Ramp, continued

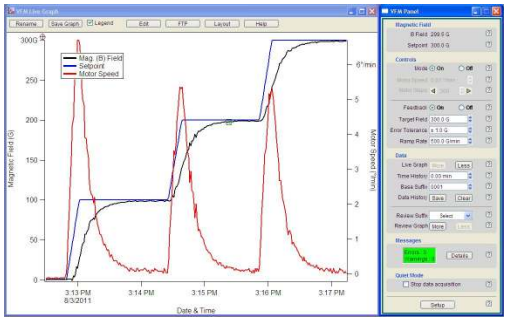
- Set the ramp rate to 500 G/m.
- Set the Target Field to 0 G.
- Click Feedback On and Mode On.
- The motor will execute a slower ramp down to zero.



10.

Changing the field by 100 G steps

- Set the Target Field to 100 G.
- Set Feedback On and Mode On.
- Observe and repeat at 100G increments to produce the graph to the right.



Note A real ramp never quite executes. The feedback control parameters are set conservatively. If faster settling times are needed, please contact Asylum Research, or read on for more suggestions.

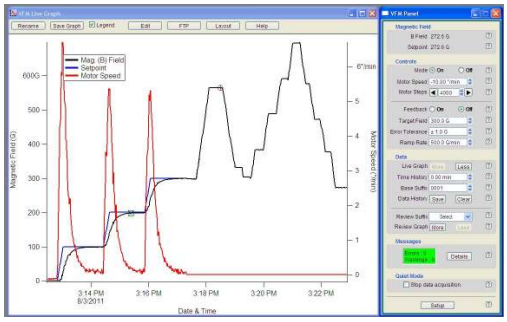
11.

Changing the field by motor steps

- Set Feedback to OFF.
- Set Motor speed to 10 °/minute.
- Enter 10,000 steps.
- Click on the RIGHT arrow next to Motor Steps.

The motor turns on immediately, executes its steps, and stops. The graph shows the field history.

- Click on the LEFT arrow next to Motor Steps.

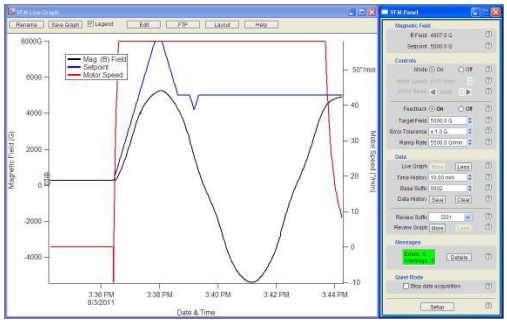


Note The field stops short of where it was before. This is due to backlash in the motor's gearbox. We had continued with a number of 4000 step positive increments followed by negative 4000 step increments. The settling time is faster but the benefits of feedback control, like backlash elimination, are compromised.

Setpoint out of range

- Set Ramp Rate to 5500G.
- Set Target Field to 8000G.
- Turn Mode and Feedback to On.

12. For a while the motor tries to keep up with the rising setpoint, but eventually the VFM2 reaches the maximum field for the given 3mm pole face separation. In any case, we chose a setpoint too high and the motor will keep spinning forever trying to reach it.



Note The setpoint was reduced to a reachable 5000G after the field had reached its maximum. The VFM can only operate in the positively sloped region of field vs motor direction. Even though it might reach its setpoint, it will complete one more full rotation until it is back in the positively sloped area before slowing the motor to land at 5000G.

25.7. Field Gradients

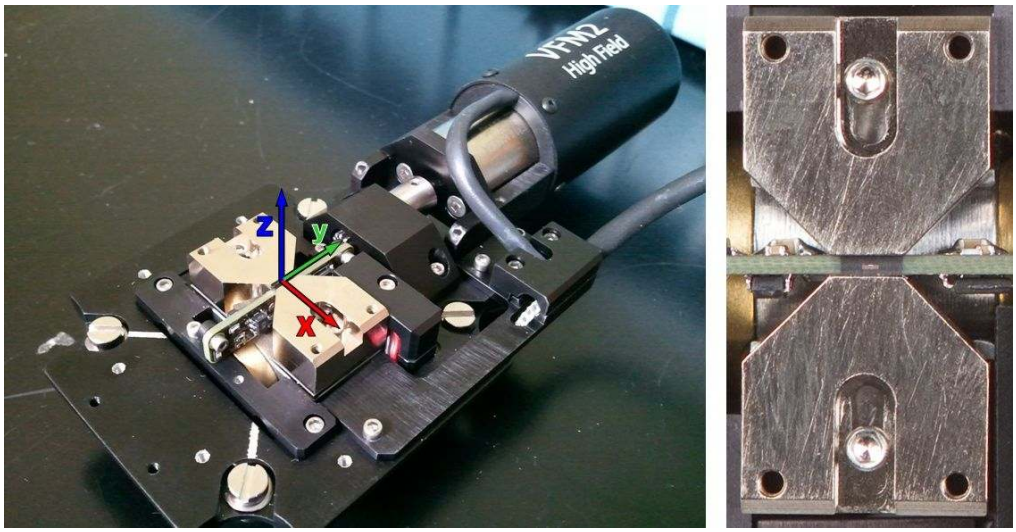


Figure 25.4.: Coordinate Axes defined and the field orientation. Positive sensor values are defined as B field components along the positive X-axis. To the right, a close up view of the preferred origin for imaging, which lies directly over the field sensor, highlighted as a tiny white line between the pole faces.

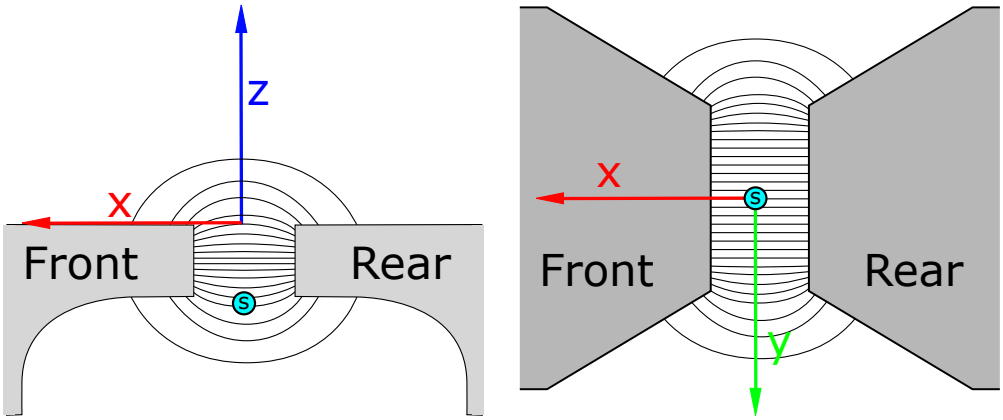
VFM2 vs VFM	This discussion largely focuses on the magnetic sensor of the VFM2. The original VFM had a larger sensor which was located much farther away from the sample. Please see Section 25.13 on page 396 for further discussion about their differences.

Proper sample mounting is only possible with some understanding of the magnetic field gradients produced by the VFM. In an ideal world, the field would be very uniform and the magnetic field sensor would be only few microns long, separated from the imaging region by only a few microns. In reality, the sensor is hundreds of microns on a side (with an active measuring area of 70 by 70 microns) and is embedded in a package which adds at least several hundred more microns to its size, and the sample thickness will likely add hundreds more microns. As shown later in more detail, the field can change by 1000's of Gauss over this distance.

To overcome this problem of not being able to put the sensor directly in the same place as the imaged surface of the sample, we take advantage of the symmetry of the field. With proper sample and pole face placement, the relative locations of sensor, sample, and pole faces can maximize the likelihood that the sensor is registering a meaningful field.

Figure 25.4 on page 381 defines the coordinate system of the VFM. It also shows the exact position of the magnetic sensing element, aligned with the bottom edge of the pole pieces. The origin of the coordinate axes has been placed in the plane of the tops of the pole pieces and directly above the center of the sensing element. This is the optimal position for the sample's top surface.

Figure 25.1 on page 368 shows how the VFM routes flux from a permanent magnet to the sample. This cartoon shows the field lines as perfectly parallel and uniform and the sample not accessible for imaging. A more realistic depiction can be seen in Figure 25.5 on page 382. The scale of the pole faces is 1.5mm tall and 3mm wide.



(a) View from +y to the origin. **(b)** View down from +z to the origin.

Figure 25.5.: Cartoons of the approximate fields between the pole faces. On the left, a side-view section, on the right a top down view. "s" denotes the position of the field sensor's active element. The origin was chosen at the optimal point for imaging. The central position of this point will maximize the field along x and minimize the other components. The origin also best mirrors the sensor position so the field measurement is as accurate as possible.

Some observations:

- The field decreases in strength away from the area between the pole faces (we'll quantify this shortly).
- At x=y=0 the field is horizontal (parallel to x), which is also the only component the sensor can measure. Moving away from the origin along the x axis (and to a lesser extent along y) the field vector will gain other components and rotate away from its direction at the origin.
- The sensor is placed so that it measures a field which is a mirror image of the field at the origin. Asylum Research individually aligns the sensor position on each VFM so that it is aligned with the bottom edge of the pole faces.

Some conclusions:

- Place the area of the sample to be imaged as close to the origin as possible and it will enjoy:
  - "Horizontal" applied field.
  - Fields which are very close to what the sensor measures.

These observations do not change with larger pole face separations; only the field values and the field gradients will reduce in size.

25.7.1. Vertical Field Variation

To take a more quantitative look at the field profiles and gradients, we mounted a magnetic field sensor on an XYZ translation stage and made various measurements of the field along the X, Y, and Z axes. Note that the sensor was oriented to measure only the field component parallel to the X axis, or B_x.

Figure 25.6 on page 384 shows B_x for various separations of the pole faces on a VFM2 set to maximum field strength. Lets dissect a few of the curves. The yellow (top) curve with triangle markers shows data gathered with the pole faces as close as possible, touching the sensor circuit board between them. At Z=0, B_x is slightly more than 1 Tesla. Note that when this VFM is placed on the scanner, the field drops a bit. This experiment was performed on the bench top. Going down into the shaded area (indicates the sensor is going between the pole faces) the field rises, and then falls again as the sensor emerges from between the pole faces. The symmetry of the field caused us to choose the sensor position at Z=-1.5mm, which does a respectable job of matching B_x at Z=0.

Going up from Z=0, the field falls quickly. Only 500 microns above the pole faces, it has dropped to a little over 6000 G. The implication is that if you are studying a sample which is 500 microns thick and set it flat on top of the pole faces, your maximum field can only be 6000 Gauss. You will have to cut the sample smaller so it can fit between the 1mm separated pole pieces to get to closer to the VFM's maximum field.

Lets look at the brown curve with circular markers (one up from the bottom). The magnet orientation was left in the same position, but the pole faces were separated from 1mm to 3mm. The maximum B_x at Z=0 has dropped to ~4000 G, but the field gradients are also significantly less. B_x at Z=0.5 is now ~3000 G. Smaller gradients tend to make for better measurements. To conclude, it is always best to separate the pole faces as far as possible depending on your maximum field requirements.

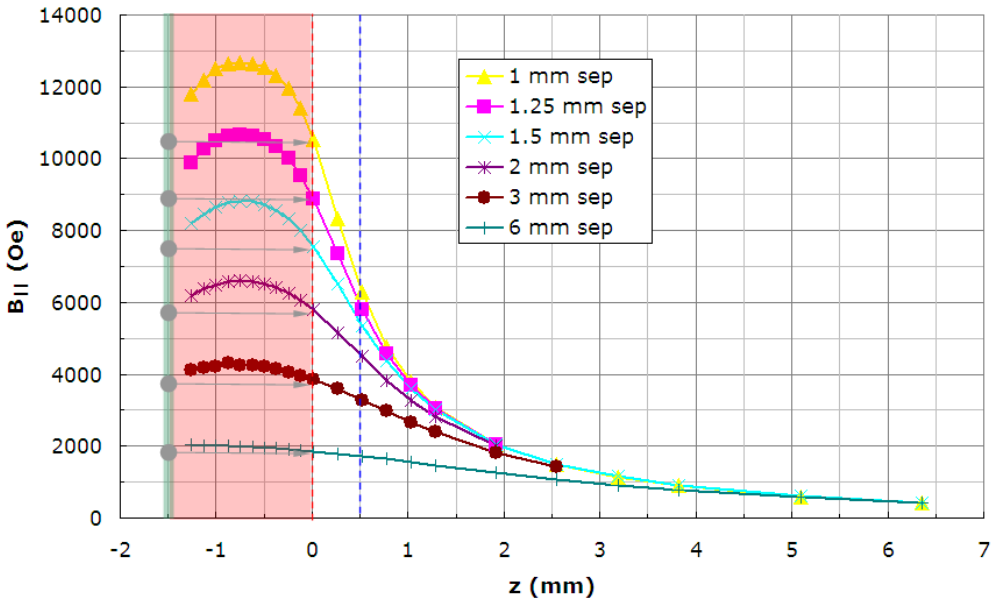


Figure 25.6.: Magnetic field component parallel to the x-axis as a function z position above the pole faces in a VFM2 set to maximum field strength. Different curves represent different pole face separations (see legend). The shaded area is the volume between the pole faces. The red dashed line (z=0) indicates the preferred plane for a sample surface to be imaged. The blue line (z=0.5) indicates a sample which is 0.5mm thick. The broad green line indicates the position of the Hall sensor attached to each VFM2. The gray arrows connect the field measured by the sensor to the field experienced by a sample mounted flush with the pole pieces.

25.7.2. Fields vs pole piece separation and vertical gradients

Figure 25.7 on page 385 summarizes some key information from the family of curves in Figure 25.6 on page 384. For various pole face separations it shows:

- The maximum B_x at Z=0, i.e a sample clamped between the pole faces so its top surface is flush with the tops of the pole pieces. Use this curve to determine the pole face separation needed, based on your maximum field requirements. Always choose the largest separation possible.
- The maximum B_x at Z=0.5mm, i.e a larger sample, 0.5mm thick, sitting on top of the pole faces.
- The approximate field gradients at Z=0.

25.7.3. Lateral Field Gradients

B_x variations along X and Y in the plane of the pole faces (Z=0) were also measured for a pole face separation of 3mm. The results are shown in Figure 25.8 on page 386.

The main observation is the nearly zero gradients in B_x as long as the imaging region is within 0.25mm from X=Y=Z=0.

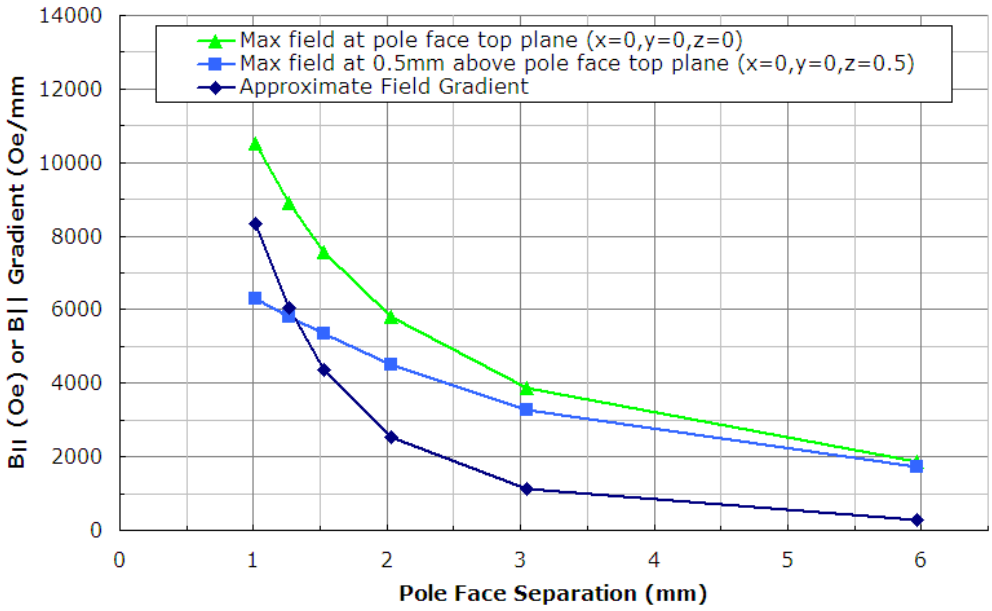


Figure 25.7.: Maximum field along the x-axis measured at the origin (sample in plane with pole face tops) and 0.5mm above (0.5mm thick sample resting on pole face tops). Also, the approximate field gradients at each point for Z=0.

25.8. Things to keep in mind

25.8.1. Samples' effect on the field

A magnetic sample can be a bridge for magnetic field lines from one pole piece to the other. The sensor placement at the "mirror image" of the sample assumes the field is symmetric at the upper and lower edges of the pole faces. Placing a piece of magnetic material (i.e. the sample) will break that symmetry and will direct flux through the sample and away from the sensor. Luckily the effect is negligible for most samples.

A suitable test is to set the field at a relatively high value (we chose 5000 G with an approximately 2mm pole face spacing) and to monitor the sensor reading for a minute or so. Then place the sample on top of or in between the pole pieces. If the field changes measurably, then the field experienced by the sample will no longer equal the field measured by the sensor. Here are the results for four materials:

Sample	Effect
8mm wide piece of video tape	no measurable effect
8 x 8mm piece of hard drive platter	no measurable effect
4mm wide strip of 1 micron thick amorphous cobalt alloy	5 G (0.1%) drop in measured field
5mm wide strip of 0.25mm thick magnetic steel	100 G (2%) drop in measured field

In the last case, it is not clear that the 2% drop at the sensor implies a 2% increase at the sample, but at least it gives some cause for further investigation. The best course of action is probably to substantially reduce the size of the sample and repeat the test until the presence of the sample has little or no effect on the sensor reading.

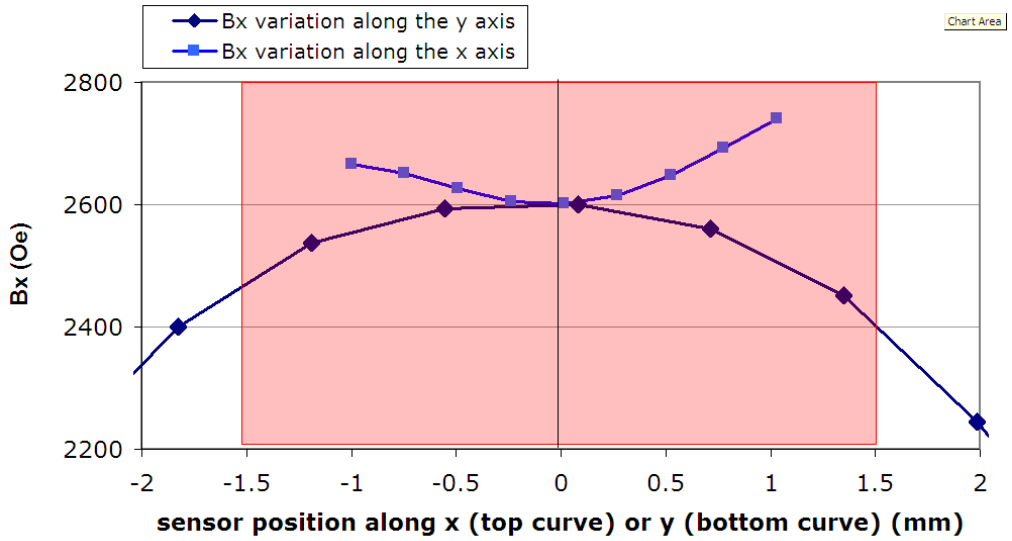


Figure 25.8.: Variation of the x component of the magnetic field along the x and y axes (with z=0). Pole faces were separated by ~3mm. x-axis data cannot extend the full 3mm range due to the sensor plastic enclosure hitting the pole faces. The pole faces are also 3mm wide andF so the red area indicates measurements taken with the sensor in the gap between the faces.

A good indication that there is going to be an effect is when the sample is noticeably attracted to the gap between the pole pieces. This can best be felt with the VFM set to a high field value.

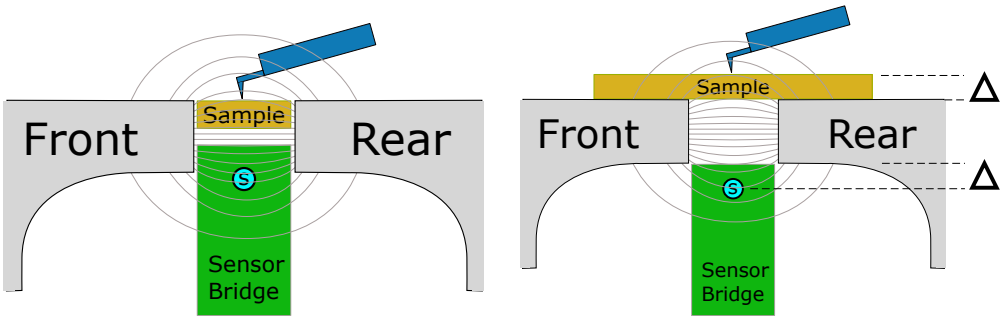
25.8.2. Scanner's effect on the field

If you set the VFM to its maximum field and then place it on the scanner, you may notice a drop of about 10%. This is due to magnetic field lines under the VFM "short circuiting" via the magnetic steel inside the scanner. If you must have more field than the reduced maximum, contact Asylum Research to fashion a spacer to mount the VFM a little higher on top of the scanner.

Also beware that if a sample is very near some maximum field it should not exceed, be sure to reduce the field before removing the VFM from the scanner, or there may be a sudden 10% jolt in increased field.

25.8.3. Temperature Effects

The strength of the permanent magnets used in the VFM vary with temperature. While the effect will be relatively small, you may notice that the sensor reads a lower field value once the AFM warms up the inside of its acoustic enclosure. Also, for long term measurement, it is possible that there will be a small field variation due to daily temperature changes of the lab. Most labs with proper air conditioning should not notice this effect.



(a) Side view of a small sample between the pole faces.

(b) Side view of a large flat sample on top of the pole faces. The sensor reads true when the spaces marked with Delta are equal.

Figure 25.9.: Side views showing a small sample mounted between the pole faces with its top surface flush with the top of the pole faces. This allows for the maximum field. In sub figure B, a larger flat sample sitting on top of the pole faces is shown. The max field is less and the distance from the top edge of the pole faces to the top of the sample needs to equal the distance from the bottom edge of the pole faces to the sensor (The two Delta spaces).

25.9. Sample mounting

As described in Section 25.7 on page 381 (if you did not read this, you should, in order to correctly interpret the sensor readings) the magnetic field sensors sit below the sample at a position where the field strength mirrors that experienced by the sample.

25.9.1. Small Samples (best)

As shipped from the factory, the pole pieces are configured for a sample which can be mounted as shown in Figure 25.9a on page 387. The sample should be thin enough to fit on top of the sensor bridge without rising above the top surface of the pole pieces.

To achieve the highest possible field, the sample should be narrow enough to allow the pole pieces to slide all the way together and touch the sensor bridge. The sample may have to be clamped between the pole faces to keep its top surface flush with the tops of the pole pieces. A few small dots of five minute epoxy or some wax may come in handy when mounting the sample.

Note that keeping the sample small has other advantages, as described in Section 25.8 on page 385

WARNING	The sensor bridge is somewhat fragile. It is made of epoxy and fiberglass. Do not cover it with paint, glue, or other contaminants. While you can clean it with a mild solvent such as isopropyl alcohol, but you should NOT scrape it with sharp objects such as tweezers, scribes, or scalpels.

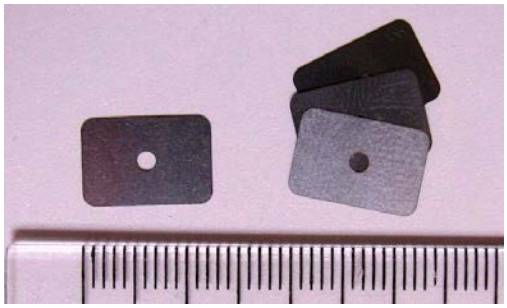
25.9.2. Larger Thin Samples (good)

Often a sample will be in the form of a thin film on a wafer substrate. If very high fields are not necessary, or it it is not possible to dice the sample into the preferred 1mm wide strip, then one can

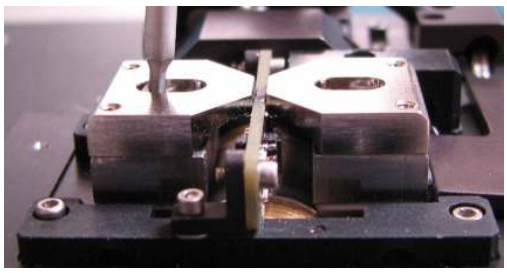
proceed by mounting the sample on top of the pole faces as shown in Figure 25.9b on page 387. See the steps below to mount the sample and properly adjust the field sensor:

1. Measure the sample thickness with an accuracy of 25 µm (0.001 inches).
2. Divide the thickness in microns by 75 and round to the nearest integer. For instance, a 300 µm silicon wafer results in 4, a 500 µm wafer gives 7. This is the number of shims (per side) you will need in step 4.
3. If the VFM was not already at a very low field, please see Section 25.6 on page 378 and set the VFM to zero field. This will make it easier to remove the pole faces without suddenly snapping together and possibly damaging the sensor bridge.
4. **Gather shims:**
   - Choose twice that number of shims calculated in step 2. (114.645).
   - Divide the shims into two equal stacks.

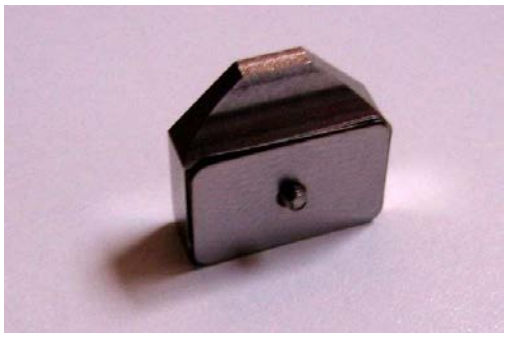
   Note These shims were cut from 75 µm thick magnetic stainless steel.



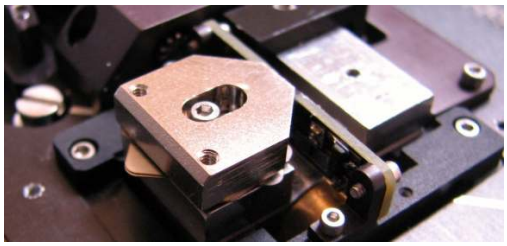
5. **Remove the pole pieces:**
   - Using the 0.050” allen driver, remove both pole pieces and set aside.



6. **Place the shims under the pole piece:**
   - Align the shims under the pole piece.
   - Use the screw to keep the shims centered as shown.



7. **Replace the pole pieces:**
   - Place the pole piece back on the VFM, while keeping the shims against the pole piece.
   - Tighten the screw with a few turns.
   - Nudge the shims to rotate them flush. The photo on the right shows shims in need of adjustment.
   - Position the pole face at the desired distance from the sensor bridge.
   - Tighten the screw until it is snug, taking care not to misalign anything.



8. Repeat this process for the other pole face, making sure the two pieces are equidistant from the sensor bridge.

NOTE	For thick samples, you may need so many shims (we allow for up to 1.5mm total) that the screws which hold the pole pieces down will no longer reach. In that case, your kit contains some longer screws (see the parts list). Store the other screws in the parts kit. They are easily obtained in the US, but cannot be found internationally.

25.9.3. Large thick samples (discouraged)

Samples thicker than a few mm should be cut down to a smaller size in order for the VFM to work properly. Contact Asylum Research and we can help you solve your sample mounting problems.

Note that bulk magnetic samples thicker than even a fraction of a millimeter will distort the field so the sensor reading is no longer reliable. Please see Section 25.8 on page 385 to determine if your sample is affecting the sensor reading.

25.9.4. Checking the Maximum Field

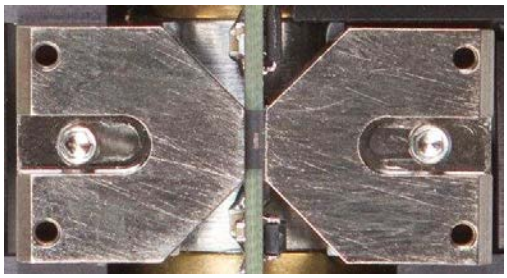
Note The pole pieces on the original VFM should not be separated. For more discussion, see Section 25.13 on page 396.

As discussed at length in Section 25.7 on page 381, unwanted field gradients are reduced by separating the pole pieces. Please follow these steps to accomplish this.

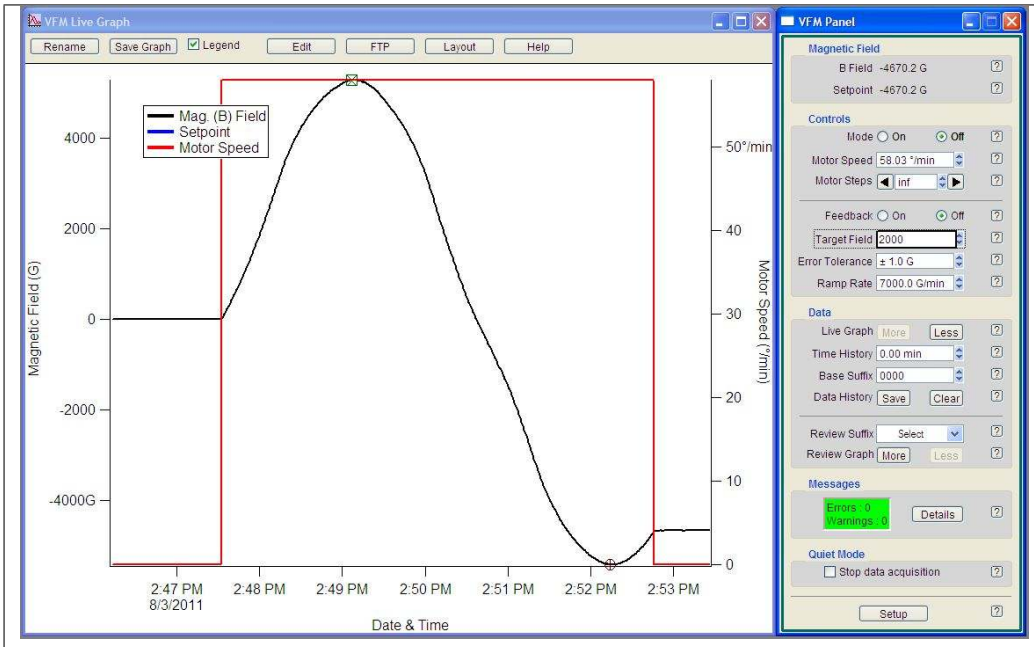
1. Determine the maximum field your experiments will require. This should be done with the VFM mounted in the AFM since the AFM reduces the field a little. See Section 25.8 on page 385.

2. **Start the pole pieces in a close position:**

   - Loosen the two screws shown to the right using the 0.050” Allen tool.
   - Move the pole pieces so they are touching the sensor bridge.



Note If there are enough shims raising up the pieces, then manually adjust them so that seen from above, it looks like the photo.

3. 

Explore the VFM field extremes

- Set the feedback and mode to off.
- Set the motor speed to 100. This is beyond the max and it will automatically change to the maximum allowed value instead.
- Set the motor steps to “inf” it is not already so.
- Set the mode to on.
- Next to Live Graph click “more” a few times.
- Next to data history click “clear”
- Observe the field sensor values until the maximum and minimum field have been reached.
- Set the mode to off, preferably when the field is still at maximum or minimum.

4. If the maximum field reached is more than what you need, we advise that you separate the

pole pieces a little to reduce field gradients and the error in the sensor reading. Remember, if the vertical sample position is off by even 50 µm when operating in the strongest field (smallest pole face separation), you may have absolute error in field reading by hundreds of Gauss.

Note Moving pole pieces at high field levels can be somewhat challenging since the pole pieces strongly attract each other. One may have to rotate to a lower value first, move the pole pieces, and then go back to see what the maximum has changed to. Remember that a pole piece snapping against the sensor bridge can cause serious damage.

The goal is to get the maximum separation while still achieving the required fields. As mentioned before, this minimizes gradients and maximizes the likelihood that the field sensor will give an accurate reading of the field your sample will experience.

25.9.5. Centering the Sample

As explained in Section 25.7 on page 381, the sensor reading is best represented at a point directly above the sensor, in the plane formed by the tops of the pole pieces. Please follow these steps to center the VFM with respect to the cantilever tip.

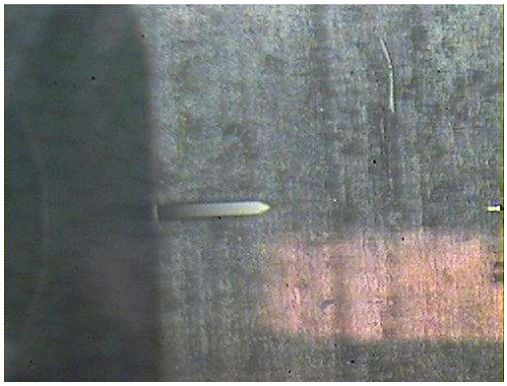
1. Follow the hardware installation instructions in Section 25.4 on page 368. Have the legs extended to the point where the cantilever clip is a few millimeters above the top of the VFM pole pieces.
2. With the 0.050" allen tool, remove both pole pieces and set them aside. Note that it helps to have the field set to a low value or the pieces will snap together when the screws are loosened.
3. Put a cantilever (this can be an old one) in the cantilever holder and place the head on the AFM base.
4. Align the head optics until you have a clear and centered image of the cantilever. See Section 4.5 on page 26 for more information. Try to have the head relatively level.
5. Press down on the head and exert force with a clockwise twisting motion. This seats the head firmly in the grooves. If you repeat this same twisting pressure each time, you will greatly improve your odds at placing the head so the cantilever ends up in the same area.
6. **Coarse center the VFM sample stage**

   - Using the sample align-x and y micrometers (see Figure 2.4 on page 9) center the cantilever as best as you can above the center of the sensor circuit board.

   Note The small white dash was added to the image to indicate the top down view of the sensor's active area.

7. **Lower the head**

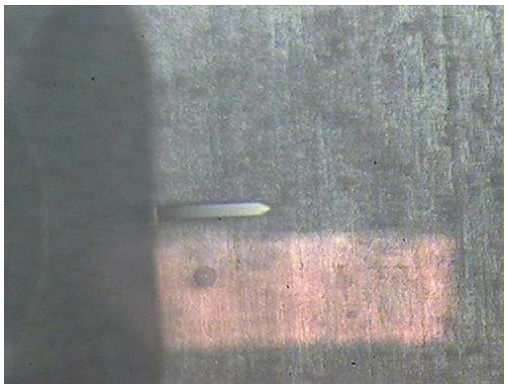
- While looking at the video image of the cantilever, lower the AFM head by moving all three legs.
- Notice that as you lower only one leg, the tip will move from the center of the video image. Adjust the other two legs to bring the image back to center. The image is centered when the head is lowering without tilting.
- Once you start to see a blurry image of the sensor circuit board, start hunting around with the sample align XY micrometers until you see something like the picture to the right.



Note Do not go down too far or the lever will crash. Using an old lever is recommended for beginners.

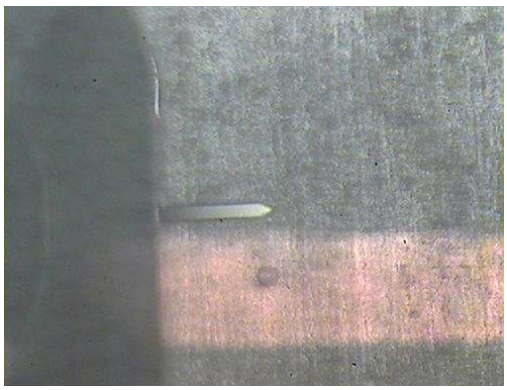
8. **Explore the width of the sensor.**

- Using the x-align micrometer, explore the left and right edges of the light colored area. This area is a metal tab which holds the magnetic field sensor. The sensor is about as wide as the length of the cantilever and mounted on the back side of the tab, as seen from the front of the AFM.



9. **Center the tip over the sensor**

- Finally, move align-x and y until the tip is at the upper edge of the metal tab and centered between the left and right edges. At this point you have found X=Y=0 and the tip is directly over the active area of the magnetic field sensor.



10. **Raise the head without tilting.**
    - Place a marker on the computer screen directly over the tip of the cantilever in the video image. This can be the mouse pointer, a piece of tape, or the corner of a post-it note.
    - Raise the head a few millimeters using all three legs, keeping the cantilever tip centered on your marker. This means the head is raising straight up and the tip is starting at X=Y=0.

11. **Mount the sample, replace the head for imaging**
    - Once you have a few mm of clearance, remove the head, replace the pole pieces, and mount your sample.
    - Replace the head using the usual twisting pressure. Hopefully the cantilever is still near the marker on your screen.
    - Lower the head while keeping the tip centered on your marker. Once you are close to engaging the sample, you can remove the marker and proceed with regular imaging.
    - This was our best attempt at keeping X=Y=0 directly above the magnetic field sensor, for the most accurate field readings of the area that will be imaged.

25.9.6. Flat Sample Mounting

Preferably use the clips (112.959) and short button head screws and the 0.035" allen driver (290.104) supplied with the kit.

If the sample is too small for the clips to reach it, use the sticky tabs (113.672). Note that the sticky tabs may cause some thermal drift and you must take the thickness of the glue into account when determining the number of shims to use when raising up the pole pieces (see 25.9.2).

25.10. Imaging

There are no particular requirements with regard to imaging. Most often one will perform Magnetic Force Microscopy (see *Applications Guide, Chapter: Magnetic Force Microscopy (MFM)*), but sometimes one might also perform Piezo Force Microscopy (See *Applications Guide, Chapter: PFM Using DART* or *Applications Guide, Chapter: Single Frequency PFM*). PFM might be done with the optional VFM High Voltage Kit: Section 25.12 on page 394.

25.11. Storage

- If you replaced the objective with the dummy, your lab mates will be thankful if you replace the objective again. See Step 3 on page 369.

- If others in your lab work with fluids around the AFM, we recommend that you remove the VFM compatible metal plate(112.041) from the top of the scanner and put the original one back. Please follow the steps in Section 25.5 on page 374.
  - Also if you installed the BeCu clip on the cantilever holder, consider replacing it with the original and storing the BeCu clip with the VFM. See Step 8 on page 372.
- Put everything back into the VFM storage box and attach the stage to the controller box. Store the cable with the VFM.

25.12. High Voltage Kit

Note The high voltage kit is only compatible with the VFM2, it is not compatible with the original VFM. You would need to contact Asylum Research to inquire about upgrading an existing VFM to a VFM2 to operate with the High Voltage Kit.

25.12.1. Parts list

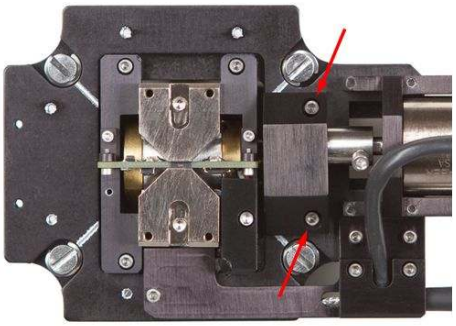
Itm	Part #	Item Description	Qty	Picture
1	448.114	Ground wire to connect between high voltage contact and pole piece. Two spares and two pins in case you want to make your own wire to attach directly to your sample.	3	
2	900.255	High Voltage Contact for the VFM2	1	
3	SHCS 0-80 X 5/16" SS	0-80 x 5/16” SCHS SS screws for attaching the HV contact assembly to the VFM2 stage.	12	

25.12.2. Installation

1. **Remove the gear cover**

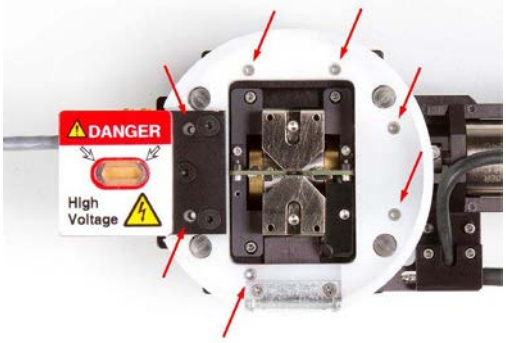
   - Use the 0.050” allen driver (290.111) and remove the two screws indicated.
   - Remove the cover and screws, and store them in your VFM kit.

   Note Replace this cover when you remove the high voltage contact. If it is not replaced and a bit of hard sample falls between the gears, it can seriously damage the VFM’s motor.



2. **Attach the high voltage contact**

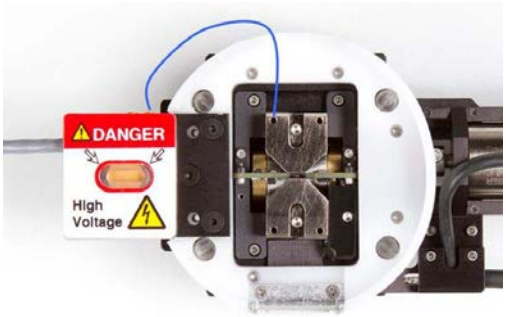
   - Position the contact module as shown.
   - Using 0-80 X 5/16” screws and the same allen tool as above, put in at least four screws, or all 7 if you wish to be more thorough.



3. **Connect the ground wire**

   - Take wire 448.114 and plug the gold end into the socket.
   - Using the small button head screws (BHCS 0-80X1/8" SS) and 0.035” allen tool 290.104, attach to the pole piece as shown.

   Note Both pole pieces are electrically isolated from the rest of the VFM and AFM. The ground wire is the only reference the sample has to ground.



General sample mounting is described in Section 25.9 on page 387. Depending on your application you may need to make certain that the sample has (or does not have) a good electrical connection to the grounded pole pieces. We have supplied a few extra ground wires and pins in case you want to arrange for a wire directly to your sample.

Please refer to **??** on page **??** for instruction on connecting to the high voltage amplifier and information on high voltage imaging techniques.

Note The HV220 and VFM controller boxes both need to be connected to the AFM controller (ARC2 or MFP-3D). This is accomplished by "daisy chaining" the controller boxes together. First connect one to the AFM controller and then connect the second to the output of the first. The order of HV220 and VFM controller does not matter.

25.13. VFM vs VFM2

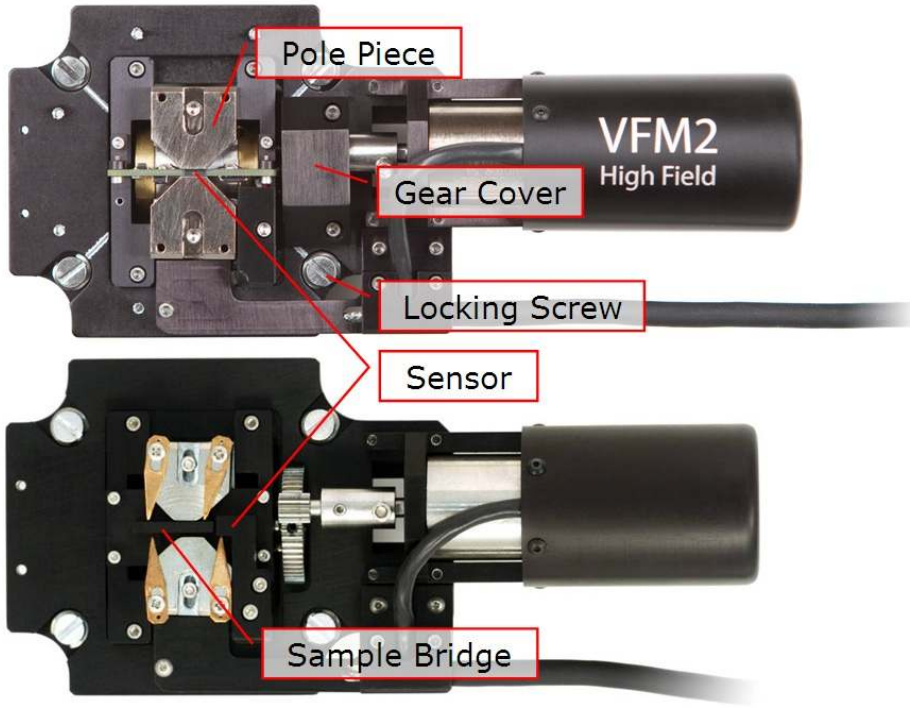


Figure 25.10.: Some of the basic components of the VFM defined. Also a side by side comparison of the samples stages of the VFM and the VFM2-Tesla. The original VFM can be distinguished by the lacking text on the motor cover, no gear cover, and no small sensor circuit board between the pole pieces.

25.13.1. Basic Differences

	VFM	VFM2-Tesla	Notes
Max Field	<=2500G	>9000G	
Sensor Position	Off to the side	Under Sample	VFM2 sensor makes a much better estimate of the field.
Sensor Range	<=2500G	>10% over max field	VFM2 sensor has more headroom and better linearity.
Sensor Resolution	~1G	~0.5G	VFM2 has an improved 16 bit sensor ADC.
Movable Poles	Yes*	Yes	*Moving the poles from the closest spacing renders the sensor calibration incorrect.
High Voltage	No	Yes	VFM2 high voltage accessory attaches to the Samuel stage. Not available for the VFM for safety reasons.
Field Gradients	Strong	Weaker-Stronger	Typically field gradients go up as pole pieces are brought closer. See Section 25.7 on page 381.

The original VFM had its sensor located away from the sample (See Figure 25.11 on page 398). At the time this was due to a lack of sensors small enough to fit under the sample. The VFM was calibrated once with a larger calibrated sensor between the pole pieces and this value was tabulated against the VFM's built in sensor. As long as the orientation of the pole pieces stayed fixed as they were during calibration, the remote sensor gave a reasonably accurate value of the field. This had a number of requirements:

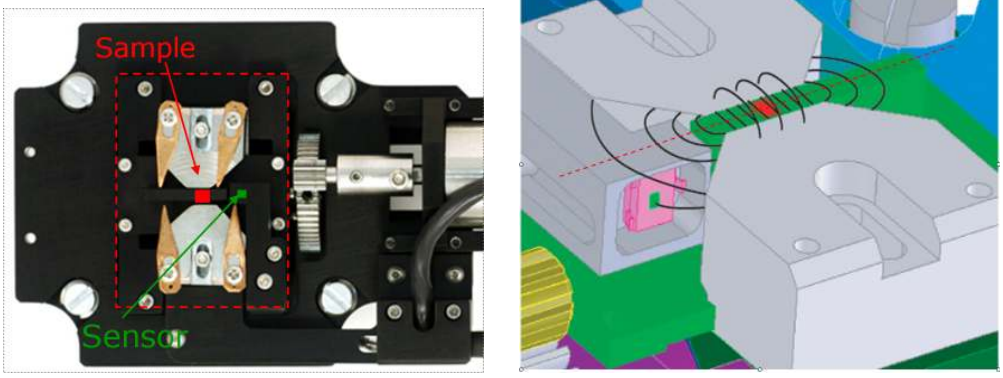
- The pole pieces could never be moved.
- The sample had to fit between the pole pieces so that the sample top surface was flush with the top of the pole pieces.
- If the sample had any substantial volume and was ferromagnetic, the subsequent field distortion was enough to render the sensor calibration ineffective.

The only advantage to the old setup was the absence of a sensor bridge directly below the sample. One could remove the otherwise inert bridge between the pole pieces and put a rather thick sample in between, as long as it was no more than 1.5mm wide.

The VFM2 improves this situation by placing the sensor much closer to the sample, in a place where the field has a higher degree of symmetry, allowing the use of shims (see Section 25.9.2 on page 387) to keep the sensor reading very close the field values near the area being imaged.

The VFM2 also allows the pole pieces to be moved. As long as they are placed symmetrically on either side of the sensor, measurements will remain valid.

Please contact Asylum Research if you would like a quote to upgrade your original VFM to an VFM2.



(a) Sample and sensor positions for the original VFM.

(b) Artistic rendition of the field lines in the original VFM. Sample position is red, sensor position is green.

Figure 25.11.: The original VFM had the sensor positioned far away from the sample.

25.13.2. VFM2 evolution

The pole pieces of the VFM2 have evolved somewhat until they settled at the design shown in this document. Initially they were taller and the metal parts of the VFM itself were lower. The shims can still be applied to this situation. For reasons related to manufacturing processes, the final design of the pole faces. A few VFM2s with these thicker pole pieces were released into the field.

25.14. Scientific References

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Part III

Safety, Specs, Setup, and Shipping

Part III: Who is it 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 manual. Beyond that, this part of the manual will probably not see much day to day use.

Part Contents

26. MFP-3D Safety

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Chapter Contents

26.1. Light Source Safety

26.1.1. The MFP-3D Super Luminescent Diode

The MFP-3D head uses a super luminescent diode and is classified as an IEC class 1M laser product that complies with 21 CFR 1040.10 and 1040.11, except for deviations pursuant to Laser Notice No. 50, dated 24 June 2007. Complies with IEC/EN 60825-1, Ed.2:2007.

Attention	Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Figure 26.1.: Light Source Warning label

The output under the specified measurement criteria could be up to 0.78 mW at 850 nm.

26.1.2. Laser Safety Precautions

Do not tamper with the following safety measures. Tampering with the following may result in eye injury.

26.1.2.1. Tilt Switch

When the MFP-3D head is tilted by 30° or more from its position during scanning the light source switches off.

26.1.2.2. Light Source Remote Jack

The light source remote jack is located on the back of the controller, in an upper corner. When connected, this jack allows the MFP-3D light source to automatically shut off under specific conditions (i.e. foot/hand switch). If you do not intend to use this jack as an automatic shutoff circuit, the light source remote plug, provided with the MFP-3D, shorts these two conductors, which enables the light source.

26.1.2.3. Light Source Lock Switch

The key on the front of the MFP-3D controller allows the operator to turn the light source on and off by turning the lock vertically to power on and horizontally to power off.

Warning	Viewing the laser output with certain optical instruments (eye loupes, magnifiers, and microscopes) may pose an eye hazard. If you have an Inverted optical system, please make sure that the infrared cutoff filters are in place in the binocular eye pieces and in the filter slot beneath the turret.

26.1.2.4. Shutter

Some commercial inverted optical microscopes—such as the Olympus IX Series —have a shutter that allows the operator to choose to exclude all light from the optical path.

26.2. Power Supply Safety

Warning	The MFP-3D system uses high voltages and currents of up to 165V, 0.5A. Use caution when handling system pieces to avoid electrical injury

Both the MFP-3D controller and the computer provided with your MFP-3D are configured for the standard power used in your location. However, you should check the configuration before connecting the computer and MFP-3D controller to a power source. If there is a problem with the power configuration, please contact Asylum Research using the information provided in on page iv

26.3. Labels

The following labels appear on the MFP-3D controller:

Identification Label The serial number identification label is located on the back of the MFP-3D controller at the bottom center or at the top right as seen from behind on the Arc2 Controller. You may need to remove the stored hamster cover to see it.

Warning	Connecting a computer or MFP-3D controller configured to run using low-voltage power (100-130V) to a high-voltage power source (200-240V) is likely to cause serious damage to the equipment and will void the warranty.

Warning	Power cables are supplied by Asylum Research for MFP-3D installations in the USA. For installations in other locations, your distributor may supply the power cables. If no power cables are supplied, obtain grounded power cables that fit the power connectors on the computer and monitor and your power outlets.

Warning	Do not attempt to open the MFP-3D head or the Stand-Alone base. Doing so will negate the product warranty.

27. Installation

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

Currently the AFM should only be installed by personnel certified by Asylum Research and its affiliates. Even if it is relocated within your laboratory, we cannot guarantee it will meet published specifications unless we are involved. By all means call Asylum Research if you have any questions. Support calls are always free and chances are we can direct you through the process of installation and testing of specs over the phone.

28. Shipping

CHAPTER REV. 1659, DATED 10/07/2013, 22:54. USER GUIDE REV. 1715, DATED 10/28/2013, 12:18.

SAVE YOUR CARTONS!	You must use the official cartons and foam inserts to ship your equipment back to Asylum Research for repairs. We can provide replacements, but you will be charged for materials, shipping, and handling.

Shipping should be done in the original cartons. If you cannot find them or did not save them, please contact Asylum Research and we can supply you with new cartons (for a fee). Also note that there are some parts of the instrument (such as movable optics in the AFM head and floating components in vibration isolation stages) which must be locked down in preparation for shipping or else instrument damage may result. Please contact Asylum Research and we will help you.

Part IV

Bibliography, Glossary, and Index

Bibliography

Cited Scientific References

Cited Asylum Research Documents

Applications Guide, Chapter: AC Mode Imaging in Air.

Applications Guide, Chapter: AC Mode: Theory.

Applications Guide, Chapter: Conductive AFM.

Applications Guide, Chapter: Contact Mode Imaging.

Applications Guide, Chapter: iDrive Imaging.

Applications Guide, Chapter: Magnetic Force Microscopy (MFM)., Placeholder

Applications Guide, Chapter: PFM Using DART.

Applications Guide, Chapter: Scanning Kelvin Probe Microscopy (SKPM).

Applications Guide, Chapter: Single Frequency PFM.

Applications Guide, Chapter: Thermals.

Hoods and Isolation User Guide, Chapter: Software Options for Image Stabilization.