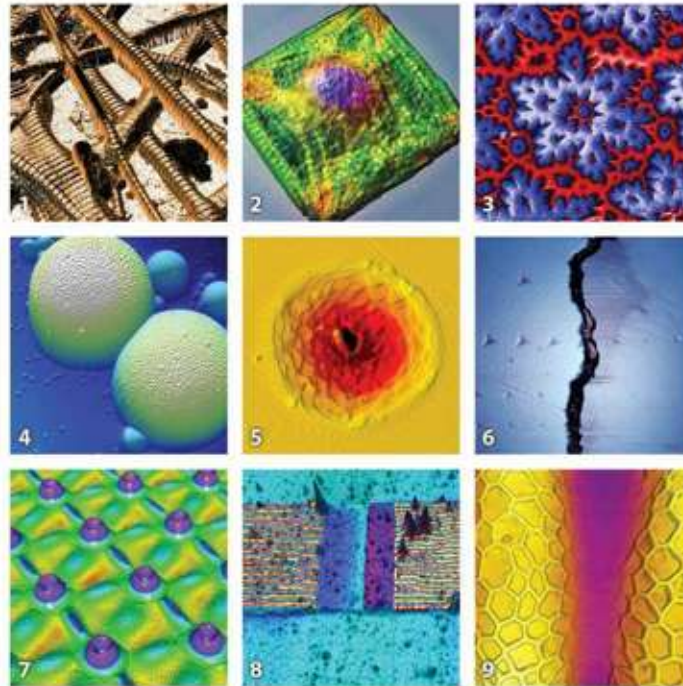


MFP-3D Manual

Version 04_08



Cover Image Descriptions

- 1. Type I Collagen** – Second resonance mode phase (Dual AC Mode) data overlaid on the topography. 4 μ m scan.
- 2. Patterned Cardiac Myocyte** – Epifluorescence overlaid on AFM topography, 92 μ m cell.
- 3. Single Crystal Garnet Thin Film** – 72 μ m scan. Sample courtesy of Mitsubishi Gas & Chemical.
- 4. PMMA Latex Spheres** – Aggregate of colloidosomes formed from the flash-curing of methacrylate emulsion droplets that are stabilized with 400nm PMMA latex spheres. 50 μ m scan. Image courtesy of J. Benkoski.
- 5. Laser Ablation Crater in Sapphire** – Crater in a sapphire substrate hit with a femtosecond laser pulse, followed by annealing to develop the step structure. Steps on the surface are 3 angstrom atomic steps. A small chunk was blown out of the center of the crater. Image courtesy of S. MacLaren (sample courtesy Fumiya Watanabe, UIUC), University of Illinois at Urbana-Champaign.
- 6. Dentin-Enamel** – Indentation on dentin (left of crack) and enamel (right). Each horizontal line of indents performed at same force both left and right of the crack. 70 μ m scan. Sample courtesy of D. Wagner and S. Cohen, Weizmann Institute of Science.
- 7. Periodic Structure on a Fe/Al Thin Film** – Periodic structure on a Fe(30nm)/Al(120nm) thin metallic film on glass substrate produced by Laser Interference Metallurgy. The sample was irradiated with an interference pattern produced with three laser beams. Because planar laser waves are applied, this structuring can proceed for the macroscopic diameters of the laser beam and consequently it allows a fast functionalization of surfaces with dimensions of technical relevance. Image courtesy of A. Lasagni, Department of Materials Science, Functional Materials, Saarland University, Germany.
- 8. Iomega Zip Drive** – Iomega Zip 1GB drive write head. The MFM phase signal was overlaid on top of the topography, 20 μ m scan.
- 9. Pollen Grain** – AFM Z sensor image, AC in air, 55 μ m scan.

Installation and Operation Manual

Version 04_08

4/8/2008

Table of Contents

| | |
|--|----|
| Chapter 1 MFP-3D™ Introduction..... | 15 |
| 1.1 Overview | 15 |
| 1.2 MFP-3D™ System Overview | 15 |
| 1.3 MFP-3D Stand-Alone (MFP-3D-SA™) | 15 |
| 1.4 Technical Support | 19 |
| 1.5 Software Update Policy..... | 21 |
| 1.6 Manual Distribution Policy | 22 |
| 1.7 Manual Update Policy | 22 |
| Chapter 2 MFP-3D™ Safety | 24 |
| 2.1 Overview | 24 |
| 2.2 Safety Requirements | 24 |
| 2.3 Safety Precautions | 25 |
| 2.4 Light Source Safety | 25 |
| 2.5 Power Supply Safety | 27 |
| 2.6 Labels | 27 |
| Chapter 3 Facilities | 28 |
| 3.1 Overview | 28 |
| 3.2 Power | 28 |
| 3.3 Footprint | 28 |
| 3.4 Weight | 30 |
| 3.5 Location..... | 31 |
| 3.6 Inputs..... | 32 |
| Chapter 4 Installation..... | 33 |
| 4.1 Overview | 33 |
| 4.2 Choose a Location | 33 |
| 4.3 Install the Components..... | 33 |
| 4.4 Install the Workstation | 37 |
| 4.5 Install the Controller | 38 |
| 4.6 Install the Base | 43 |
| 4.7 Install the Head | 46 |
| 4.8 Power-Up | 47 |
| 4.9 Power Connectors | 48 |
| 4.10 Initiate the Software | 51 |
| Chapter 5 Set-Up & Operation..... | 53 |
| 5.1 Overview | 53 |

| | |
|--|------------|
| 5.2 Components of the MFP-3D™ | 53 |
| 5.3 Cantilever Loading | 65 |
| 5.4 Subassembly Controls | 69 |
| 5.5 Aligning the Light Source and Zeroing Deflection..... | 75 |
| 5.6 Cantilever Selection | 79 |
| 5.7 Using the Controller | 87 |
| 5.8 Signal Accessibility | 91 |
| 5.9 Data Channels | 95 |
| 5.10 Imaging Feedback | 102 |
| 5.11 Imaging Basics: What Is Scanning?..... | 107 |
| 5.12 Using the Computer | 113 |
| 5.13 Using the Software | 116 |
| 5.14 Powering-Up the System..... | 117 |
| 5.15 Thermal Power Spectral Density (PSD)..... | 118 |
| | |
| Chapter 6 AC Mode Imaging in Air | 121 |
| 6.1 Overview | 121 |
| 6.2 Cantilever Selection and Loading and Light Source Alignment. | 126 |
| 6.3 Setting-Up the Software | 127 |
| 6.4 Tune the Cantilever | 129 |
| 6.5 Sample Preparation and Loading | 131 |
| 6.6 Software and Hardware Initialization | 134 |
| 6.7 Engaging | 135 |
| 6.8 Start Scanning | 139 |
| 6.9 Saving Your Images | 141 |
| 6.10 Advanced Topics: Phase and Digital Q Control | 142 |
| | |
| Chapter 7 Contact Mode Imaging in Air | 157 |
| 7.1 Overview | 157 |
| 7.2 Theory | 157 |
| 7.3 Operation: Setting Up the Software | 160 |
| 7.4 Troubleshooting: Contact Mode in Air | 170 |
| 7.5 Lateral Force Microscopy | 172 |
| | |
| Chapter 8 AC Mode Imaging in Liquid | 176 |
| 8.1 Overview | 176 |
| 8.2 Theory | 176 |
| 8.3 General Procedure Using the Water Droplet Technique..... | 177 |
| 8.4 General Procedure Using the Closed Fluid Cell | 189 |
| 8.5 Recipes for Imaging Samples..... | 191 |
| 8.6 Sample Experiment: Atomic Force Microscopy Imaging of Bacteriorhodopsin | 192 |

| | |
|---|-----|
| 8.7 Recommendations and Troubleshooting for AC Mode Imaging in Liquid | 205 |
|---|-----|

Chapter 9 Contact Mode Imaging in Fluid 208

| | |
|--|-----|
| 9.1 Overview | 208 |
| 9.2 General Procedure | 209 |
| 9.3 Operation: Setting-Up the Software | 209 |
| 9.4 Engage | 209 |
| 9.5 Optimizing Parameters | 209 |
| 9.6 Measuring Vertical Forces | 210 |
| 9.7 Troubleshooting and Maintenance | 211 |
| 9.8 Advanced Topics | 212 |
| 9.9 References | 218 |

Chapter 10 Force Plots 220

| | |
|-------------------------------------|-----|
| 10.1 Overview | 220 |
| 10.2 Theory: Getting Oriented | 220 |
| 10.3 Contact Mode Force Plots | 227 |
| 10.4 AC Mode Force Plots | 231 |
| 10.5 Advanced Topics | 234 |

Chapter 11 Lithography 236

| | |
|--|-----|
| 11.1 Overview | 236 |
| 11.2 Theory and Getting Oriented | 236 |
| 11.3 Operation: Setting-Up the Software (MicroAngelo) | 238 |
| 11.4 Operation: Setting-Up the Software for Litho Groups | 240 |
| 11.5 Operation: Setting-Up the Software for Litho Step | 241 |
| 11.6 Advanced Topics | 242 |

Chapter 12 Macros and Savant Writing 246

| | |
|--|-----|
| 12.1 Overview | 246 |
| 12.2 Creating Functions in Igor | 246 |
| 12.3 Function Basics | 246 |
| 12.4 Flow Control Templates | 248 |
| 12.5 Waves: Part I | 249 |
| 12.6 Dealing With Data Folders | 250 |
| 12.7 Waves Part II: Back to Waves and Igor Functions | 251 |
| 12.8 Strings and Variables | 253 |
| 12.9 Callbacks: Functions and Commands | 254 |
| 12.10 Syntax Issues and the Exception character \ | 255 |
| 12.11 Events | 256 |

| | |
|--|-----|
| 12.12 Time Scaling and MFP-3D™ Programming..... | 258 |
| 12.13 Useful Commands for Programming with the MFP-3D™ | 261 |

List of Figures

Figure 1.1 MFP-3D-SA™ base model 16

Figure 1.2 MFP-3D-BIO™ 18

Figure 1.3 MFP-3D-CF™ 19

Figure 2.1 Light Source Warning label 26

Figure 3.1 Schematic of MFP-3D-SA™ (dimensions are in inches) 29

Figure 3.2 Schematic of MFP-3D-BIO™ (dimensions are in inches) 30

Figure 4.1 Recommended System Footprint 34

Figure 4.2 Workstation Layout..... 38

Figure 4.3 Controller Placement..... 39

Figure 4.4 Controller Connections - Back 40

Figure 4.5 Controller Connections - Front 40

Figure 4.6 Light Source Remote Plug (Shown greater than actual size for detail.) 41

Figure 4.7 Fuses on back of MFP-3D™ Controller (Bipolar controllers and High Voltage Bipolar controllers have four fuses)..... 42

Figure 4.8 Stand-Alone (SA) Base 44

Figure 4.9 Unpark the MFP-3D™ 45

Figure 4.10 MFP-3D™ Standard Head 46

Figure 4.11 Head Installation and Connections 47

| | |
|--|---------|
| Figure 4.12 A Power Connections – Back of the Computer | B Power |
| Connections – Back of the Controller | 49 |

Figure 4.13 Controller Power 50

Figure 4.14 Computer Power 51

Figure 4.15 MFP-3D™ Icon 52

| | |
|---|----|
| Figure 5.1 Schematic of the Standard Head with OptoPort Options | 54 |
| Figure 5.2 Standard Head, side view | 55 |
| Figure 5.3 Schematic of the Nanopositioning System (NPS™) | 56 |
| Figure 5.4 Piezo Hysteresis Loops | 57 |
| Figure 5.5 Piezo Creep | 58 |
| Figure 5.6 Optical lever detection components | 60 |
| Figure 5.7 Amplification of cantilever deflection (not to scale) | 61 |
| Figure 5.8 Segmented photodetector quadrants | 62 |
| Figure 5.9 Deflection and corresponding spot positions on photodiode | 63 |
| Figure 5.10 Cantilever Holder Stand | 65 |
| Figure 5.11 Expose the Spring Clip | 66 |
| Figure 5.12 Prepare the Cantilever Holder. Figure A Remove from Head. Figure B Place in Holder | 67 |
| Figure 5.13 Inserting a Cantilever | 68 |
| Figure 5.14 MFP-3D™ Head | 69 |
| Figure 5.15 Optical Detector Adjustments (PD, LDY and LDX) | 70 |
| Figure 5.16 Photo Diode (PD) | 71 |
| Figure 5.17 Bottom View Stand-Alone Base | 72 |
| Figure 5.18 Sample X and Y Position | 73 |
| Figure 5.19 Focus on the Cantilever | 75 |
| Figure 5.20 Sum and Deflection Meter Window | 76 |
| Figure 5.21 Bottom View Camera X and Y Position | 83 |
| Figure 5.22 Adjustable BIO Base Plate | 85 |
| Figure 5.23 Optical Axis Alignment X and Y | 86 |

| | |
|---|-----|
| Figure 5.24 Sample X and Y Position | 87 |
| Figure 5.25 MFP-3D™ Controller Front | 88 |
| Figure 5.26 Controller Rear..... | 90 |
| Figure 5.27 Schematic of the Crosspoint Panel..... | 94 |
| Figure 5.28 Contact Mode No Feedback..... | 103 |
| Figure 5.29 Contact Mode Feedback..... | 104 |
| Figure 5.30 AC Mode Deflection Signal and Cantilever Motion | 105 |
| Figure 5.31 AC Mode No Feedback..... | 105 |
| Figure 5.32 AC Mode Feedback | 106 |
| Figure 5.33 Fast Axis X Piezo Drive (at 0° scan angle). Y-axis of graph is piezo voltage; X-axis is time. The rounded caps (blue regions) are called the turn-around, where the fast axis changes direction. Data from the turn-around is not typically saved, though it is possible to access it for special applications. | 108 |
| Figure 5.34 Slow Axis of Raster Scan. Y-axis is Y piezo voltage (scan angle 0°); X-axis is time in image. Axis limits are zoomed in to show the same region as in Figure. The blue sections denote turn-around regions..... | 109 |
| Figure 5.35 X-Y Motion of the Tip across surface in raster imaging. The X-axis is the fast axis drive, while the Y-axis is the slow axis drive. Less than 3 scan lines are shown to better illustrate the concept. The blue sections denote turn-around regions. | 110 |
| Figure 5.36 Slow Axis of Ortho Scan. In image, Y-axis is Y piezo voltage (scan angle 0°); X-axis is time. Axis limits are zoomed in to show the same region as in preceding Figure (Slow Axis of Raster Scan). The blue sections denote turn-around regions. Approximately 7 scan lines are shown here, each comprising two steps (one for Trace and one for Retrace). | 111 |
| Figure 5.37 Motion of the Tip Across Surface in Ortho Imaging. The X-axis is the fast axis drive, while the Y-axis is the slow axis drive. Three scan lines are shown. The blue sections denote turn-around regions. | 112 |
| Figure 5.38 Drift Correction Parameters, Master Panel | 113 |
| Figure 5.39 Computer Tower Front..... | 114 |

| | |
|--|-----|
| Figure 5.40 Computer Tower Rear..... | 115 |
| Figure 5.41 Video Port Connections. The older “Barney” version is shown on the left and the new connectors on the right..... | 116 |
| Figure 5.42 Power-Up the System..... | 117 |
| Figure 5.43 Master Panel, Thermal | 119 |
| Figure 5.44 Thermal PSD Graph in Air | 120 |
| Figure 6.1 Basic Principle of AC Mode | 122 |
| Figure 6.2 AmpDef. The amplitude, A, and peak-to-peak amplitude, Ap-p, for a sinusoidal signal..... | 123 |
| Figure 6.3 Setting the Imaging Mode in the Main Tab, located in the Master Panel | 127 |
| Figure 6.4 Data Channels in the Master Channel Panel | 128 |
| Figure 6.5 Master Panel, Tune | 130 |
| Figure 6.6 Sample Positioning | 132 |
| Figure 6.7 Sum and Deflection Window* | 133 |
| Figure 6.8 MFP-3D™ Icon | 134 |
| Figure 6.9 Master Panel, Main | 136 |
| Figure 6.10 Tune Tab, located in the Master Panel..... | 137 |
| Figure 6.11 Cantilever Tune Window. Typical tune for the Olympus AC160 cantilever | 138 |
| Figure 6.12 SUM and Deflection Meter; the Z voltage is at its maximum, the Z piezo is fully extended..... | 139 |
| Figure 6.13 Sum and Deflection Meter; Z voltage is centered..... | 139 |
| Figure 6.14 Initiating the Scan and Saving Images | 140 |
| Figure 6.15 In and Out Of Phase. A, the red curve, represents the reference wave. B, the black curve, is “in phase” ($\theta = 0^\circ$) with the reference while the blue curve, C, is “out of phase” ($\theta = 180^\circ$)..... | 143 |

| | |
|--|-----|
| Figure 6.16 Lagging Response Curve. | 145 |
| Figure 6.17 SHO Diagram. Simple Harmonic Oscillator model. A mass, m , is attached to an oscillating base through a spring with compliance, k , and a dashpot with dissipation, b . The mass responds with an amplitude and phase that depends on the driving frequency..... | 146 |
| Figure 6.18 Resonance Curves. Calculated response curves of the cantilever amplitude and phase using equations SHO Amp and SHO phase | 149 |
| Figure 6.19 Attractive and Repulsive Regimes | 150 |
| Figure 6.20 Don Quixote. Height and phase image of a Don Quixote lithography on a polycarbonate surface. Sharp surfaces increase the likelihood of repulsive interactions. This gives a large negative phase contrast over the etched ridges in the polycarbonate sample..... | 151 |
| Figure 6.21 Q-Control. Comparison of resonance frequencies with and without Q-control of four cantilevers | 152 |
| Figure 6.22 SEBS Polymer sample, with and without Q-control..... | 153 |
| Figure 6.23 Response Time versus Q..... | 153 |
| Figure 7.1 Saving Options in the Master Panel | 161 |
| Figure 7.2 Imaging Mode in Master Panel | 162 |
| Figure 7.3 Data Channels in Master Channel Panel | 162 |
| Figure 7.4 Primary Scan Parameters, located in the Master Panel..... | 164 |
| Figure 7.5 Initiating the Scan, Master Panel | 167 |
| Figure 7.6 Secondary Scan Parameters | 168 |
| Figure 7.7 Blurry Topography Image..... | 170 |
| Figure 7.8 “Triple” DNA Molecules | 171 |
| Figure 7.9 Cross-Section: Theoretical Lateral Scan Line, for a theoretical topography. The low friction areas on the topography are marked in red | 173 |
| Figure 8.1 Typical Sample Mounting..... | 177 |

| | |
|---|-----|
| Figure 8.2 Typical sample mountings: A: Gold deposits on a glass slide. B: Mica disc glued to a glass slide. C: HOPG attached to a glass slide..... | 178 |
| Figure 8.3 Close view of how to pre-wet the cantilever with a pipet before immersing it in liquid | 181 |
| Figure 8.4 Master panel, Thermal tab | 182 |
| Figure 8.5 Typical thermal graph obtained in liquid with a 60 μ m Biolever. Note the broad peak at about 8 kHz, corresponding to the resonance frequency of the cantilever in an aqueous buffer | 183 |
| Figure 8.6 Frequency tune obtained in liquid with a 60 μ m Biolever..... | 184 |
| Figure 8.7 R Filter Value | 185 |
| Figure 8.8 Master Panel Main Tab | 188 |
| Figure 8.9 Cantilever Tune of the short TR800 cantilever in fluid | 196 |
| Figure 8.10 Purple Membrane Patch, 1.6 μ m scan size | 197 |
| Figure 8.11 Close-up of Purple Membrane Patch (extracellular surface), 300nm scan size..... | 198 |
| Figure 8.12 Close-up of the extracellular surface of the membrane patch, revealing the bacteriorhodopsin trimers, 100nm scan size..... | 199 |
| Figure 8.13 Close-up of the cytoplasmic surface of the membrane patch, revealing the bacteriorhodopsin trimers, 100nm scan size..... | 199 |
| Figure 8.14 Images seen in Figure 8.12 and Figure 8.13 after 3rd order flattening only (100nm scans) | 201 |
| Figure 8.15 Images seen in Figure 8.12 and Figure 8.13 after 3rd order flattening and Gaussian filtering (100nm scans) | 202 |
| Figure 8.16 Images seen in Figure 8.12 and Figure 8.13 after 3rd order flattening, Gaussian filtering and FFT (100nm scans) | 203 |
| Figure 8.17 Cross-section of the patch seen in Figure 8.9 | 204 |
| Figure 8.18 Dimension of individual trimer..... | 204 |
| Figure 8.19 This side view shows how to lift the MFP-3D™ head to straighten bent cantilevers or tap it to remove air bubbles. This procedure can also be | |

| | |
|--|-----|
| used to eliminate bubbles stuck on the cantilever: tap the front leg a few times on the stage and check optically for the bubbles. | 207 |
| Figure 9.1 Recommended Parameters for Cell Imaging | 215 |
| Figure 9.2 Simultaneous optical phase contrast image (top left), deflection error (top right) and rendered topography (bottom) of a live Fibroblast Cell | 216 |
| Figure 9.3 Fixed Aplysia Bag Cell Neuron. Height image of the leading edge of a growth cone acquired in Contact mode, 13 μ m scan. Section analysis of filopodia reveals that the tips are taller and wider than the shafts. Gryzwa et. al. (2006), Journal of Neurobiology. Courtesy of G. Lee, Purdue University. | 217 |
| Figure 9.4 Simultaneous optical phase and contact mode topography of a Living Cardiac Myocyte. Image Courtesy of Dr. Nicholas Geisse and Prof. Kevin Kit Parker, Harvard University School of Engineering and Applied Sciences. | 218 |
| Figure 10.1 Regions of a Force Curve..... | 224 |
| Figure 10.2 Setting-Up the Trigger Point | 226 |
| Figure 10.3 Saving Force Plots | 227 |
| Figure 10.4 Start and Force Distances..... | 228 |
| Figure 10.5 Force Graph..... | 229 |
| Figure 10.6 Master Panel, Thermal | 230 |
| Figure 10.7 Anatomy of an Amplitude Plot | 232 |
| Figure 10.8 Calibrating the Amplitude InvOLS | 233 |
| Figure 11.1 Master Litho Panel | 239 |
| Figure 11.2 Pair of Carbon Nanotubes | 244 |
| Figure 11.3 Pair of Carbon Nanotubes, with spiral path drawn | 245 |

Chapter 1 MFP-3D™ Introduction

1.1 Overview

This chapter includes an introduction to each of the MFP-3D™ systems. It also provides the resources available to our customers, whether it is by phone to our corporate office, by email, or through our Forum Support, our On Site Web-Based Support, or our worldwide offices and representatives.

1.2 MFP-3D™ System Overview

The MFP-3D™ standard control system components include the following: two monitors; a computer and controller; the MFP head; the MFP scanner; and a choice of base(s). The available base models include: the MFP-3D-SA™ (Stand-Alone) and the MFP-3D-BIO™ (Inverted Optical).

1.3 MFP-3D Stand-Alone (MFP-3D-SA™)

The MFP-3D-SA™ (Stand-Alone) uses a square bottom base to house the optics configuration. This base (see [Figure 1.1](#) on [page 16](#)) may be configured with top view, bottom view or dual view optics. The SA base is our multi-purpose model—it is more appropriate for opaque samples and is less sensitive to vibrational noise than the MFP-3D-BIO™.

Top View

The top view base allows illumination and view of the cantilever and sample through a 10X objective. The base contains a port for inserting fiber guide illumination and two C-mounts with differing magnifications (1300 and 300 micron field of view for 1/3" CCD cameras).

Bottom View

The bottom view base includes a micrometer-driven, crossed-roller stage for mechanical alignment of the optical axis with the cantilever tip and sample. The base uses an inverted optical 10X, 0.25 NA infinity-corrected objective (other infinity-corrected objectives may be used) and provides two separate magnifications (210 x 270 μ m and 630 x 810 μ m fields of view).

Dual View

The dual view base contains all the features of both the top and the bottom view bases. The dual view base allows the operator to easily switch between the two views. This base includes a two-dimensional sub-micron mechanical stage for positioning the sample, as well as an inverted video microscope with two magnifications for viewing the sample and tip. [Figure 1.1](#) on [page 16](#) shows the MFP-3D-SA™ base model.



Figure 1.1 MFP-3D-SA™ base model

MFP-3D Inverted Optical (MFP-3D-BIO™)

The MFP-3D-BIO™ integrates the atomic force microscope with an inverted optical microscope. By combining the two technologies, optical data (e.g. functional labeling) can be fused with both quantitative force and topography data. The sample stage of the commercial microscope is replaced with a more rigid mechanical stage called the MFP-3D-BIO™ baseplate, which allows for sub-micron positioning of the AFM tip and sample relative to the optical field of view. The MFP-3D-BIO™ includes TopViewIO functionality, a camera that enables simple alignment of the SLD spot on the cantilever and allows positioning of the cantilever over a specific feature on the sample. Compatible models are the Olympus IX51, IX71, IX81; Nikon TE2000S, TE2000U; Zeiss Axiovert 200, 200M, Axio Observer (with extended focusing drive option for 13 mm focus travel). Partial compatibility is available for Olympus IX50/70 and Nikon TE200. Optional accessories include the PHIL phase contrast illuminator for Ph1 and Ph2 illumination of cells and other aqueous samples, and the IRBF infrared blocking filter, custom-designed to block the SLD light from sensitive fluorescence detectors. [Figure 1.2](#) shows the MFP-3D-BIO™ on a Nikon TE200 microscope.

MFP-3D Integrated with Confocal (MFP-3D-CF™)

The MFP-3D-CF™ (see [Figure 1.3](#)) builds on the MFP-3D-BIO™, adding compatibility and integration with confocal optical microscopy. It includes integration components, labor, and support. Transmitted light detection may be optionally included. Available for Olympus FV1000, Nikon C1, Zeiss LSM 510. Contact Asylum Research Sales for further details.



Figure 1.2 MFP-3D-BIO™



Figure 1.3 MFP-3D-CF™

1.4 Technical Support

Asylum Research is dedicated to providing technical support to all customers worldwide. Several options are available and are listed below. This information can also be found on our website by either clicking 'Support' on our homepage at <http://www.AsylumResearch.com> or by entering <http://www.AsylumResearch.com/Support/support.shtml>.

Asylum Research Corporate Office

Phone: 805-696-6466 or 1-888-472-2795

Fax: 805-696-6444

Web: <http://www.asylumresearch.com/>

Mail: 6310 Hollister Ave, Santa Barbara, CA 93117

FAQ: <http://www.asylumresearch.com/Support/FAQ3D.shtml>

Forum: <http://www.asylumforum.com/forum/index.php?>

Forum

This is a good resource for software downloads and "how to" (application) type questions. Bug reports should go through email. You need to register to read the posts. Registering with a generic type email address, such as Gmail, slows things down, so it would be a good idea to send an email to support letting us know, so we don't think you are some spammer.

Email

Please refrain from sending multiple unrelated questions in a single email. We tend to lose track of which questions have been answered, and which have not, as it will require multiple people here to answer those questions.

Phone

This is the best option if you are completely lost, and don't know where to begin, but please try and tell the receptionist if your questions regard software, hardware, or applications (how to questions), whatever is more of a problem for you. If it is hardware, please tell them which piece of hardware; tips, bioheater, closed fluid cell, etc. This will help us get you in touch with the person that is best suited to helping you. For bugs, it is best if your MFP3D computer is connected to the internet and if you can call us from the instrument. We have some third party software that with your permission, allows us to both connect to a server and then we can view your screen.

In order to help us help you in the fastest way possible, please try to classify your problem as best you can:

Software Bugs

This form of support works best by email.

Send us the MFP3D version number, the 6 digit number in the title bar of Igor. It is often followed by +3 or 4 digits for stable versions. Also please attach any ibw files or pxp file that shows us the problem / helps us to reproduce the problem. Please refrain from sending us screen shots unless you can't find any other way to show us the problem. Video and Argyle (3D surface rendering) problems, the video card make, model and driver version is also very helpful for us.

Software requests

Please send your requests via email or through our Forum. It will help us answer your questions faster if you define what you want to do as best you can. Some ball park numbers, ranges of values you want to try, are a great way to help us figure out how best to answer your questions.

Hardware and Applications Problems

You may seek support via email or phone (and through our Forum regarding Applications questions.) As in software requests, it is helpful to have a good description of your hardware issue or application questions. Please describe the problem to the best of your ability. Please include a phone number where you can be reached.

Worldwide Distributors

Asylum Research has representatives and offices worldwide, including Australia, China, Japan, Korea, Singapore and Europe. For the most current list, please refer to our website at <http://www.AsylumResearch.com/About/About.shtml#D>

1.5 Software Update Policy

The MFP-3D™ software is constantly being updated and improved with new features — software upgrades are free. To get software updates you must first understand version numbers. There are multiple components to the MFP3D software. Primarily there is Igor pro and the MFP3D software.

Igor pro is written by a separate company, Wavemetrics (<http://www.Wavemetrics.com>). Their software has a standard 4 digit version number. The string of that version number is displayed in the title bar of Igor when it starts up. The number can be obtained from the help -> about Igor pro menu. Often times when the last digit is a 0, the version is a release version, considered more stable than the beta versions. For example 6.0.2.0 would be displayed as 6.02 in the title bar, is a release version, where as 6.0.2.3 would display as 6.02 beta 3, and is a semi-stable testing version. Occasionally Wavemetrics will release updated release versions, such as 6.0.2.4, which is displayed as 6.02A. The fact that the title bar has a letter in it tell you this is an updated release version, and is more stable than 6.02.

The MFP3D software that controls the instrument runs in Igor. The version number of the MFP3D software is also in the title bar of Igor, and is a 6 digit time stamp of when it was built YYMMDD. Such as 071030, being from October 30 2007. When we build "stable" code, we will append a + 4 digit number, which is MMDD since the branch was made with the unstable code. Such as 071011+830

is a stable installer with the base code from January 11 2007 with 8 months and 30 days worth of bug fixes.

Updates

There is often a range of Igor versions that will run a given MFP3D version. It is very possible that upgrading Igor will cause the MFP3D software to stop working, as Wavemetrics has changed the behavior of some of their functions. Or vice versa, upgrading the MFP3D software will require you to update Igor, as we now use some new feature. When downloading the MFP3D software there will most likely be a recommend and required version of Igor to have listed on the forum. Updates for Igor pro can be obtained from the Wavemetrics site, which can be launched direction from the Help -> Updates for Igor menu.

MFP3D software. Download links are posted to the forum, you need to register on the forum to read and download these links (see section 1.4). They are broken into 2 threads, stable and unstable, where the last post in each of those threads is our latest version. We recommend you go to the forum User control panel (User CP), and change your edit options so that thread display option is set to linear-newest first. This way the first post listed is the most recent version of the MFP3D software.

ADVANCED NOTES: When dealing with unstable installers it is often useful to do so in a copy of Igor so as to not impact the other users of the instrument. Details of working with Igor copies are posted on the forum, FAQ, and in some future section of this manual.

1.6 Manual Distribution Policy

With each MFP-3D™ system, you receive a PDF (Adobe Acrobat) version on CD. You may print a copy from the CD for each user at your facility. Periodically, we may revise this manual. At that time, we will mail you a CD with a PDF version of the revised manual—no printed copies of revised manuals will be provided automatically. If you want to purchase additional copies of the manual (\$50 each), please contact Asylum Research.

1.7 Manual Update Policy

Please note that the software controlling the MFP-3D™ is constantly changing. The behavior described in this manual may or may not be the same as in your version of the software. This manual is only accurate for the version specified. To determine which version you are running, go to the Help -> MFP Version menu

from the MFP-3D™ software. To update your MFP-3D™ software, please see the section on our [Software Update Policy](#), above.





Chapter 2 MFP-3D™ Safety



2.1 Overview

This chapter provides important safety information. Please read this chapter before installing or operating the MFP-3D™ system.

2.2 Safety Requirements

Table 2.1 Safety Symbols Key

| Symbol | Symbol Definition | |
|---|-------------------|--|
|  | WARNING: | <p>This symbol identifies conditions or practices that could result in damage to the equipment or other property, and in extreme cases, possible personal injury.</p> <p>A potential risk exists if the operating instructions are not followed.</p> |
|  | WARNING: | <p>This symbol identifies conditions or practices that involve potential electric shock hazards.</p> <p>To avoid risk of injury from electric shock, avoid contact with energized electrical circuits.</p> |
|  | WARNING: | <p>This symbol identifies a thermal hazard. Touching could result in skin burns upon contact.</p> <p>To reduce the risk of injury from a hot component, allow the surface to cool before touching.</p> |
|  | WARNING: | <p>These symbols identify a mechanical crushing or pinch point hazard. Moving parts can result in serious injury to hands or fingers.</p> <p>To avoid risk of bodily injury, keep away from moving parts.</p> |

| | | |
|---|-----------------|---|
|  | <p>WARNING:</p> | <p>This symbol identifies a heavy object. Improper lifting can cause muscle strain or back injury.</p> <p>To reduce the risk of personal injury or damage to the equipment, observe local occupational health and safety requirements and guidelines.</p> |
|  | <p>WARNING:</p> | <p>This symbol indicates the presence of a sharp edge or object that can cause cuts or other bodily injury.</p> <p>To prevent cuts or other bodily injury, do not contact sharp edge or object.</p> |

2.3 Safety Precautions

| | |
|-------------------------------|---|
| <p><u>WARNING:</u></p> | <p>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</p> |
| <p><u>CAUTION:</u></p> | <p>Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.</p> |

2.4 Light Source Safety

MFP-3D™'s 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.



Figure 2.1 Light Source Warning label

WARNING: Viewing the laser output with certain optical instruments (eye loupes, magnifiers, and microscopes) may pose an eye hazard.

The output under the specified measurement criteria could be up to 0.78 mW at 850 nm.

Laser Safety Precautions

Do not tamper with the following safety measures. Tampering with the following may result in eye injury.

Tilt Switch

When the MFP-3D™ head is tilted by 30° or more from its position during scanning the light source switches off.

Light Source Remote Jack

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. Refer to [page 41](#) for **Light Source Remote Jack** installation.

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.

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.

2.5 Power Supply Safety

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 [Chapter 1](#).

WARNING: Connecting a computer or MFP-3D™ controller configured to run using low-voltage power to a high-voltage power source is likely to cause serious damage to the equipment and will void the warranty.

ATTENTION: 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.

2.6 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: Do not attempt to open the MFP-3D™ head or the Stand-Alone base. Doing so will negate the product warranty.

Chapter 3 Facilities

3.1 Overview

This chapter includes the facilities requirements for each of the designed MFP-3D™ systems.

3.2 Power

600W

100, 120, 220 & 240 V single-phase

50 or 60 Hz

3.3 Footprint

Line drawings and footprints for both the MFP-3D-SA™ models and the MFP-3D-BIO™ models are included in this section.

MFP-3D-SA™

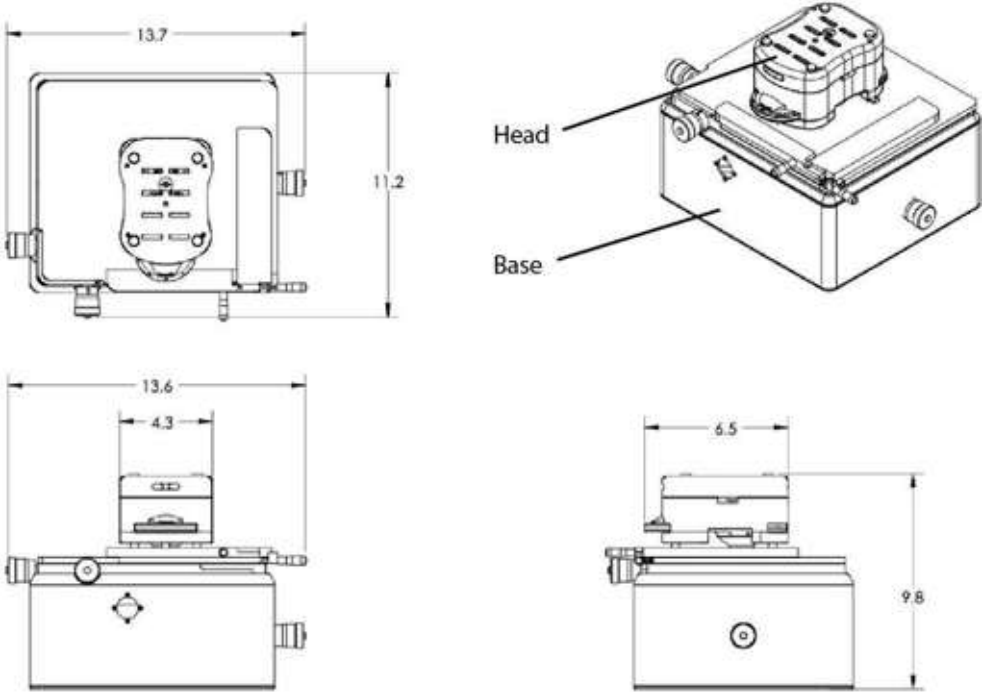


Figure 3.1 Schematic of MFP-3D-SA™ (dimensions are in inches)

MFP-3D-BIO™

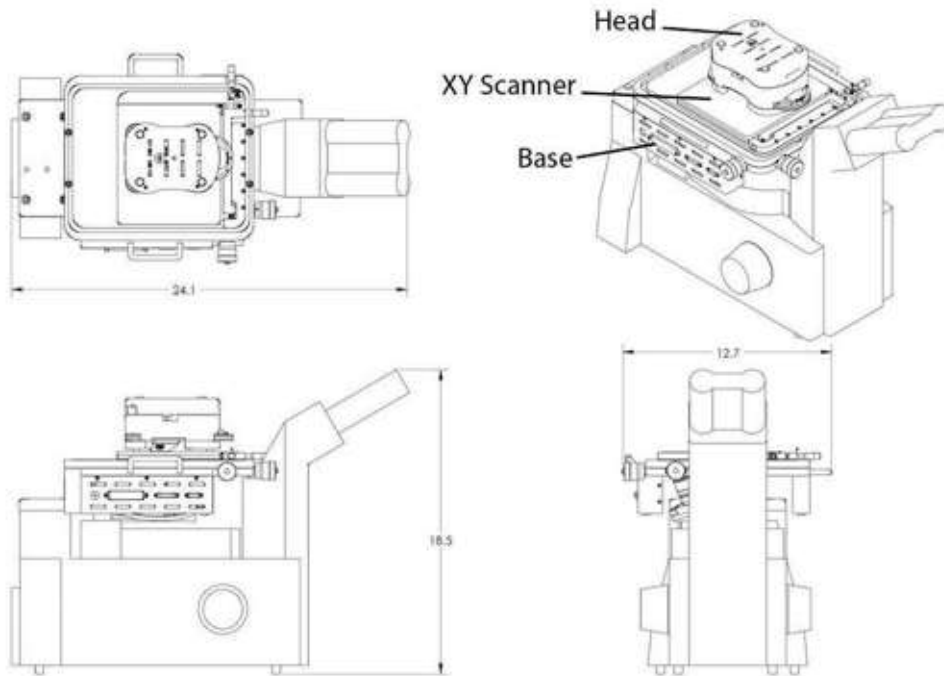


Figure 3.2 Schematic of MFP-3D-BIO™ (dimensions are in inches)

3.4 Weight

Table 3.1

| Components | Lbs | Kgs |
|-----------------------|-----|------|
| Controller | 45 | 20.4 |
| Computer | 45 | 20.4 |
| Monitor | 25 | 11.3 |
| Scanner | 4 | 1.8 |
| Head | 9 | 4.1 |
| SA Base – Bottom View | 40 | 88.2 |

| | | |
|---------------------------|------|-------|
| SA Base – Top View | 32 | 13.5 |
| SA Base – Dual View | 37 | 16.8 |
| Oly Base | 21 | 9.5 |
| Nikon TE2000 | 15 | 6.8 |
| Nikon TE200 | 17 | 7.7 |
| Zeiss Base | 17 | 7.7 |
| Heater Controller | 17 | 7.7 |
| Optional Isolation Tables | | |
| Herzan TS-130 | 50 | 22.7 |
| Herzan TS-150 | 75 | 34 |
| Minus K 50BM-4C | 60 | 27.2 |
| Minus K 90BM-3C | 75 | 34 |
| Optional Acoustic Hoods | | |
| Herzan AEK 2002 | 700 | 317.5 |
| Herzan 5004 | 1300 | 589.7 |

3.5 Location

Because an AFM is highly sensitive to noise, it is best to keep the MFP-3D™ based on a concrete slab ground floor. Keep away from noisy instruments and heavy traffic areas or devices such as pumps and A/C units. Also keep the MFP-3D™ away from airflows such as heating or air conditioning vents or constantly opening doors.

Range of Environmental Conditions

The MFP-3D™ is *only* designed for use under the following environmental conditions:

Indoors

Altitudes up to 2000 m

Temperatures in the range between 5°C and 40°C

Maximum relative humidity of 80% for temperatures up to 31°C, and then decreasing linearly to 50% relative humidity at 40°C

Mains supply voltage fluctuations up to + 10 % of the nominal voltage

Transient over-voltages up to 1kV on the Mains supply

Pollution degree II, in accordance with International Electrotechnical Commission (IEC) standards

3.6 Inputs

No vacuum or compressed air is needed to run either the MFP-3D™ or the vibration isolation system.

Chapter 4 Installation

4.1 Overview

This chapter describes the unpacking, installation and power-up instructions for the system. Please read the instructions thoroughly before attempting installation to ensure a proper and timely result.

4.2 Choose a Location

The MFP-3D™ is sensitive to environmental factors, especially vibration and noise. (See [Chapter 3](#) for environmental and facilities requirements.) When selecting a location for your MFP-3D™, consider the following system environmental needs:

Consistent Temperature Range

Choose a location with a consistent temperature. Locations near doors, windows, vents, etc. will vary in temperature and cause noise and drift in the system.

Minimal Vibration

Vibration and noise skew your results; therefore a location with minimal vibration is ideal. Vibrations below ~200 Hz are easily transmitted through tables. We recommend using a vibration isolation table for the scanner and head assemblies. If a vibration isolation table is not available, make sure the scanner and head assemblies are on a table separate from all other subassemblies or other systems.

Vibrations in the acoustic range ~100 Hz - 20 kHz are easily transmitted through the air to the cantilever. If your lab is noisy, we recommend placing the MFP-3D™ head and scanner assembly beneath an acoustic cover.

Consistent Power Levels

Inconsistent power levels affect your MFP-3D™ measurements. We suggest using a surge suppressor or a UPS device for all three MFP-3D™ power connections.

4.3 Install the Components

Verify, unpack and place each subassembly (workstation, controller, base and head) as described. A recommended footprint is provided below.



Figure 4.1 Recommended System Footprint

Verify Components

The MFP-3D™ components ship in a number of boxes depending upon the MFP-3D™ system shipped. Upon receiving the MFP-3D™ locate the shipping lists and verify that all system components are included in the shipment. Below is a general packing list.

Table 4.1 General Parts List

| Accessories | |
|-------------|----------------|
| Qty | Item |
| 1 | 2A Tweezer, SA |
| 1 | 7 Tweezer, SA |

| | |
|---|------------------------------|
| 1 | 3C Tweezer |
| 1 | IGOR Pro 4 Software |
| 1 | IGOR Pro 4 Manual Set |
| 1 | Head Phones |
| 1 | IR Sensing Card |
| 1 | Calibration Grating |
| 1 | Oly Cantilever; AC160TS-10* |
| 1 | Oly Cantilever; AC240TS-10* |
| 1 | Oly Cantilever; TR400PSA-10* |
| 1 | Oly Cantilever; Biolever-24* |

* Not included in European systems.

| MFP-3D™ Stand-Alone Base | |
|---------------------------------|--|
| Qty | Item |
| 1 | 3D Stand-Alone Base |
| 1 | SA Driver Assembly |
| 3 | Extension Spring; 0.180" OD x 0.630" L |
| 1 | Extension Spring; 0.240" OD x 1.250" L |
| 1 | FO Light Guide 1/4" OD x 36" |

ATTENTION: If you believe components are missing, contact Asylum Research using the contact information provided in [Chapter 1](#).

MFP-3D™ Inverted Optical (IO) Base

See your original packing list for an itemized list for your model specific BIO base.

Computer and Monitors

Table 4.2

| Qty | Item |
|---------------------|---|
| 1 | Dell PC configured for MFP-3D™ |
| 1 | Dell Optoplex Documentation |
| 1 | System Test Results |
| 1 | Keyboard |
| 1 | Mouse |
| 1 | Dell Optoplex Software |
| 1 | Power Cord - Standard 3 pin |
| 1 | Video Cable - Composite |
| 1 | ATI Graphics Card Input Cable (Purple Barney) |
| 2 | Monitors with power cord cabling |
| Controller | |
| Qty | Item |
| 1 | MFP-3D™ Controller |
| 1 | 3D Controller Cable - V1 |
| 1 | Keys / Key Switch (2) |
| 1 | Laser Remote Safety Plug - 2.1mm |
| 1 | Power Cord - Standard 3 pin |
| 1 | 6-foot USB Cable - Black |
| MFP-3D™ Head | |
| Qty | Item |

| | |
|----------------|---------------------------------------|
| 1 | MFP-3D™ Head (w/ & w/o optical port) |
| 1 | MFP-3D™ Fluid Cell Assembly w/ Piezo |
| 1 | 3D Fluid Cell Stand |
| Scanner | |
| Qty | Item |
| 1 | Scanner |
| 2 | Magnet Holder Assembly - Glass Slide\ |
| 1 | X-Y Driver Assembly (L-Plate) |

4.4 Install the Workstation

| | |
|--------------------------|--|
| <u>NOTE:</u> | If your system is equipped with an acoustic hood, make sure to direct all cables through the hood cable clamp, and secure them. Loose cables may create vibration and noise. |
| <u>ATTENTION:</u> | We recommend using a workspace that allows the workstation and microscope to reside on separate surfaces so as to minimize imaging noise. |

1. Place the following in the area provided (See [Figure 4.2](#)) for your workstation.

Computer tower

Monitors (2)

Mouse

Keyboard



Figure 4.2 Workstation Layout

2. Make the following workstation connections

Monitor 1 to computer CPU (#1)

Monitor 2 to computer CPU (#2)

Keyboard to computer CPU (#3)

Mouse to Computer CPU (#4)

- 3.** Locate the small, recessed switch indicating the power input on the computer. Verify that the number shown matches your local AC line voltage.

4.5 Install the Controller

ATTENTION: We recommend a workspace that allows the controller and computer to reside on a separate surface from the microscope, so as to minimize imaging noise.



Figure 4.3 Controller Placement

1. Place the controller near the computer tower (within 4.5-feet or 1.3-meters) and within range of the base (3-feet or 0.9-meters).
2. Make the following controller connections (See [Figure 4.4](#) and [Figure 4.5](#))

USB: controller to tower (#1)

3D Controller Cable: controller to Base #2



Figure 4.4 Controller Connections - Back



Figure 4.5 Controller Connections - Front

3. Insert either the light source remote jack or the light source remote plug to enable the light source (See [Figure 4.6](#) and [Figure 4.4](#))



Figure 4.6 Light Source Remote Plug (Shown greater than actual size for detail.)

ATTENTION: The light source remote plug shorts the conductors to enable the light source. The light source remote jack allows you to connect an external circuit to disable the light source under specific conditions.

Configuring and Replacing Fuses for the MFP-3D™ Controller

The MFP-3D™ Controller fuses can be configured to allow the MFP-3D™ controller to use AC input voltages of 100, 120, 220 and 240 volts. The MFP-3D™ controller fuses are initially configured for the standard power used in your country.

WARNING: It is important that you unplug the MFP-3D™ controller power cord before changing voltage selections or changing fuses.

To change the controller AC line voltage selection:

1. Unplug the MFP-3D™ Controller power cord
2. Determine the appropriate settings for your input voltage. See [Figure 4.7](#) and the diagram on the back of the MFP-3D™ Controller show a table of fuse settings for all supported voltages.
3. Use a flathead screwdriver to remove all three fuses. To remove a fuse, push in with the screwdriver and turn it 1/4 of a turn counter-clockwise.

4. Use a coin to set all three fuse locations to the correct settings. Point the arrow to the setting for your input voltage.
5. Reinstall all three fuses using a flathead screwdriver.

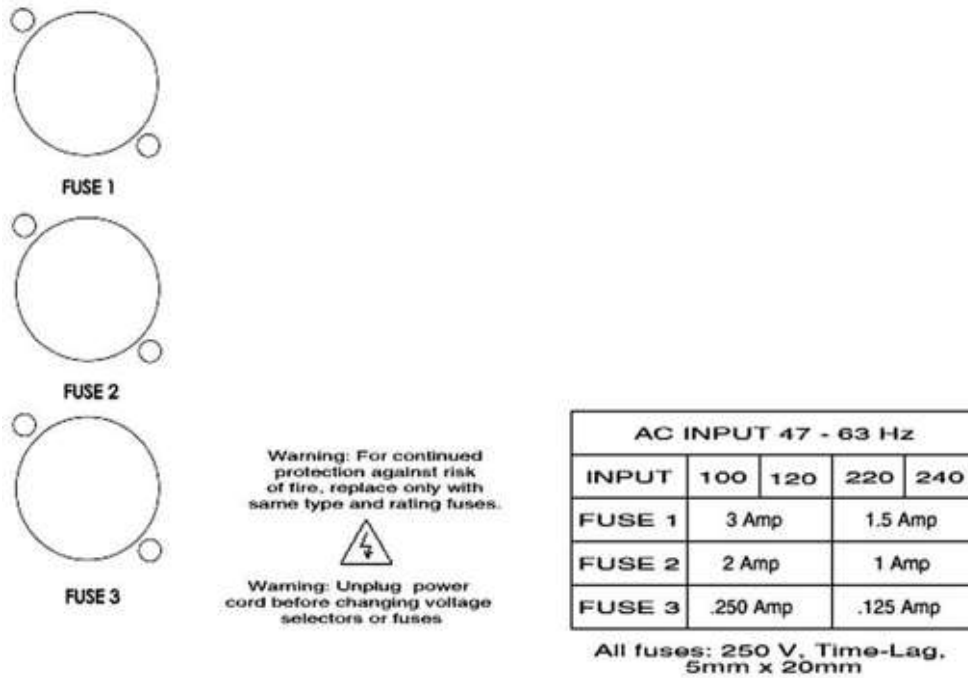


Figure 4.7 Fuses on back of MFP-3D™ Controller (Bipolar controllers and High Voltage Bipolar controllers have four fuses)

To replace a fuse:

1. Unplug the MFP-3D™ Controller power cord.
2. Use a flathead screwdriver to remove a fuse. Push in with the screwdriver and turn it 1/4 of a turn counter-clockwise.
3. Make sure that the fuse setting is still correct for your input voltage.
4. Use the same screwdriver to replace the fuse with a new one of the same type and rating.

4.6 Install the Base

The MFP-3D™ is available with either a stand-alone (SA) base or an inverted optical (IO) base. Installation differs slightly between the two bases. Please identify the correct installation directions for your base model.

Install the Stand-Alone Base (MFP-3D-SA™ only)

1. Place the SA base on the identified scanner and head workspace (See [Figure 4.8](#)).

| |
|--|
| <p><u>ATTENTION:</u> We recommend preparing a workspace for the head and scanner separate from the controller and computer in order to avoid image noise caused by vibrations from the controller and computer.</p> |
|--|



Figure 4.8 Stand-Alone (SA) Base

2. Unpark the video camera by turning the microscope focus knob counter-clockwise 5 to 10 turns (See [Figure 4.9](#)).

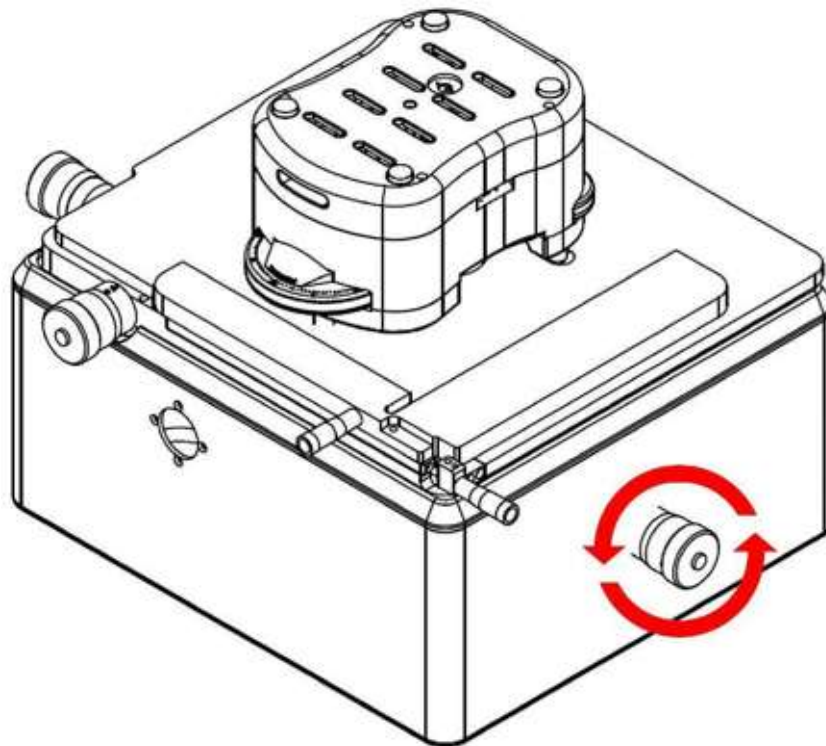


Figure 4.9 Unpark the MFP-3D™

- ATTENTION:** The stand-alone base ships in a parked position to avoid video camera damage. You must adjust the microscope focus knob, but do not adjust any other knobs as this will damage the video camera and void the warranty.
- ATTENTION:** After unpacking, do not turn the base over or on its side. Doing so may misalign the video camera.
- ATTENTION:** If the base is moved or shipped, be certain to repark it before taking either action.

3. Place the filter tube from the filter light into the filter port.

4.7 Install the Head

The MFP-3D™ standard configuration ships with an OptoPort™ . If you have ordered any optional optics, the head will look like [Figure 4.10](#).

Contact Asylum Research for optical support notes if you have ordered optional optics.



Figure 4.10 MFP-3D™ Standard Head

1. Connect the head cable to the scanner (See [Figure 4.11](#)).
2. Position the legs of the head over the corresponding indentations on the base (See [Figure 4.11](#)) and place the head.

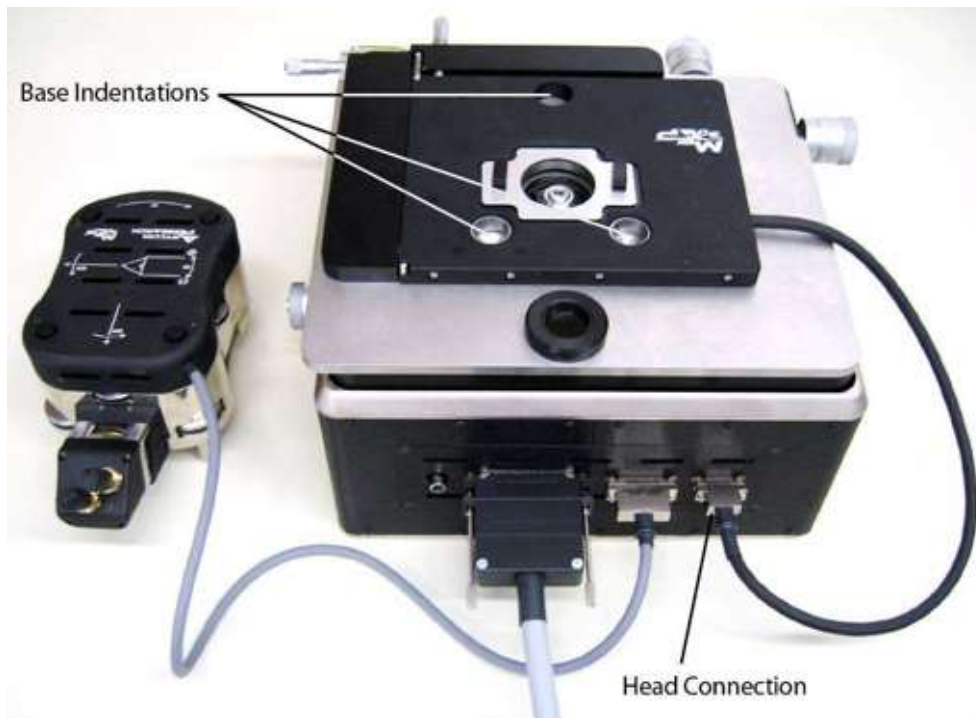


Figure 4.11 Head Installation and Connections

3. Connect the cable from the head to the base (See [Figure 4.11](#)).

4.8 Power-Up

Verify Assembly Connections

Verify the following connections have been made before proceeding with power-up.

Computer (See [Install the Workstation on page 37](#))

1. Monitor 1 to tower
2. Monitor 2 to tower
3. Keyboard to tower
4. Mouse to tower

Controller (See [Install the Controller on page 38](#))

1. USB: controller to tower
2. Video: controller to tower
3. Controller: controller to base

Base (See [Install the Base on page 43](#))

1. Scanner: scanner to base
2. Video: base to tower (optional)

Head (See [Install the Head on page 46](#))

1. Piezo: piezo to head
2. Head: head to base

4.9 Power Connectors

ATTENTION: The MFP-3D™ controller fuses are initially configured for the standard power used in your country. See [Chapter 2](#) for information regarding configuring or replacing fuses.

ATTENTION: If there is a problem with the power configuration, please contact Asylum Research using the contact information (See [Chapter 1](#)).

1. Connect the following system power cords (See [Figure 4.12](#)):

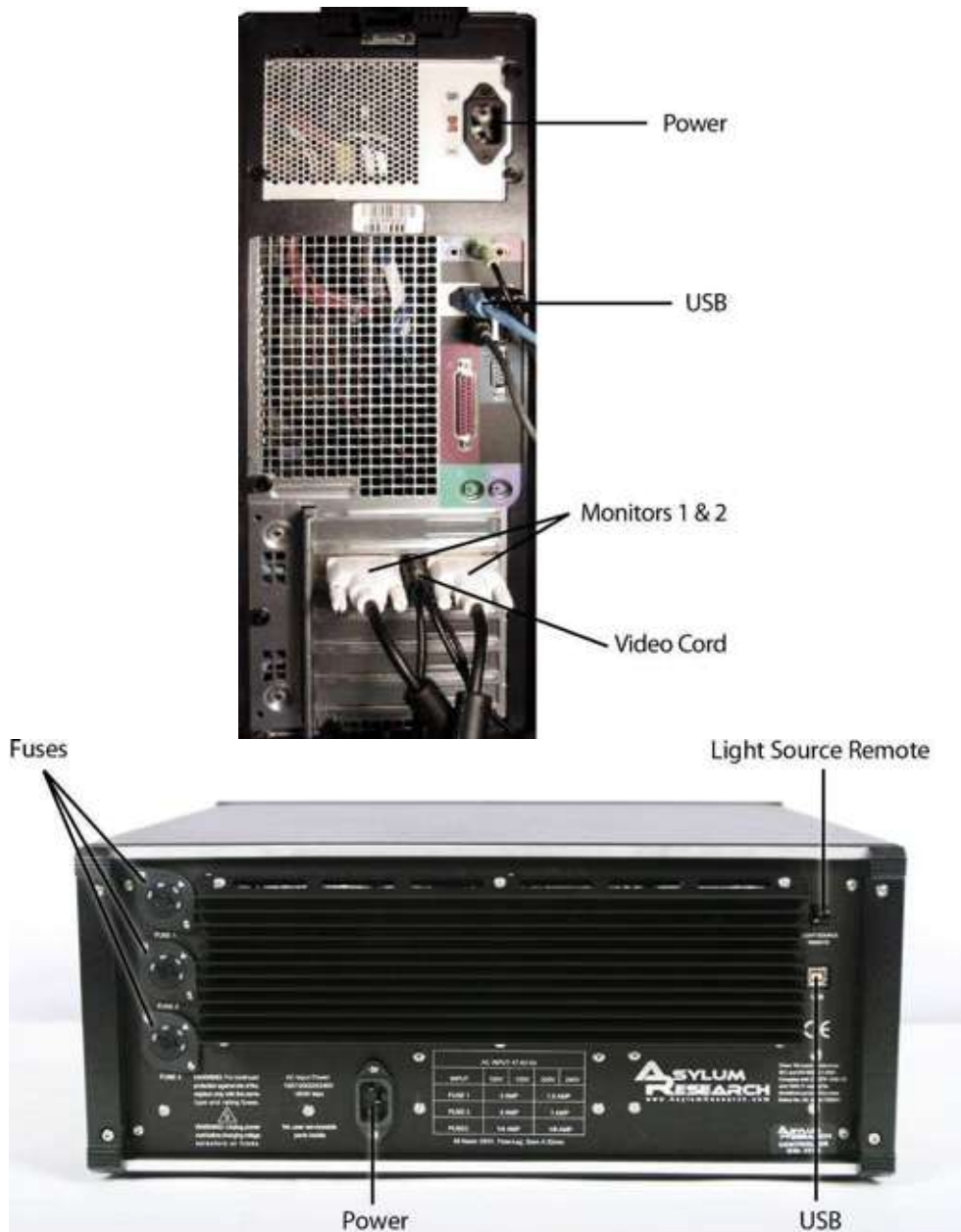


Figure 4.12 A Power Connections – Back of the Computer B Power Connections – Back of the Controller

ATTENTION: 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.

2. Power on the controller by toggling the controller POWER switch to the 'on' position (See [Figure 4.13](#)).
3. Locate the laser safety plug and insert it into the back of the controller.
4. Place the light source key in the controller, and turn the key a quarter turn to enable the light source.



Figure 4.13 Controller Power

5. Power on the monitors and the computer by pressing the front POWER buttons (See [Figure 4.14](#)).
6. Power-up the computer by pressing the front POWER switch.



Figure 4.14 Computer Power

4.10 Initiate the Software

1. Double-click on the MFP-3D™ software icon to open the imaging software (See [Figure 4.15](#)).



Figure 4.15 MFP-3D™ Icon

When the software opens, the status bar on the bottom of the screen indicates the system's current status. If all connections are properly connected and all subassemblies are powered-up, the status bar reads <MFP-3D™ READY>.

2. Verify the system is powered-up correctly, as indicated by the bottom status bar.

| |
|---|
| <u>NOTE:</u> You are now ready to begin imaging. |
|---|

Chapter 5 Set-Up & Operation

5.1 Overview

This chapter describes the operation of individual system components and the steps required for general set-up your MFP-3D™ system. This chapter also offers a summary of the basic concepts necessary for working with atomic force microscopy, paying particular attention to introducing the various data channels available to the user.

The steps described in this chapter to set-up and run the MFP-3D™ are general to any experiment. Please keep in mind that there are many different imaging and data acquisition modes. The specific setup and operation details will vary for these different modes. For some common modes, please refer to the following four chapters. Make sure to read the instructions thoroughly before attempting operation of the instrument.

5.2 Components of the MFP-3D™

Figure 5.1 and Figure 5.2, show a schematic view of the standard MFP-3D™ head, from both the front (Figure 5.1) and the side (Figure 5.2). The function of each part of the MFP-3D™ head is explained in the following sections.

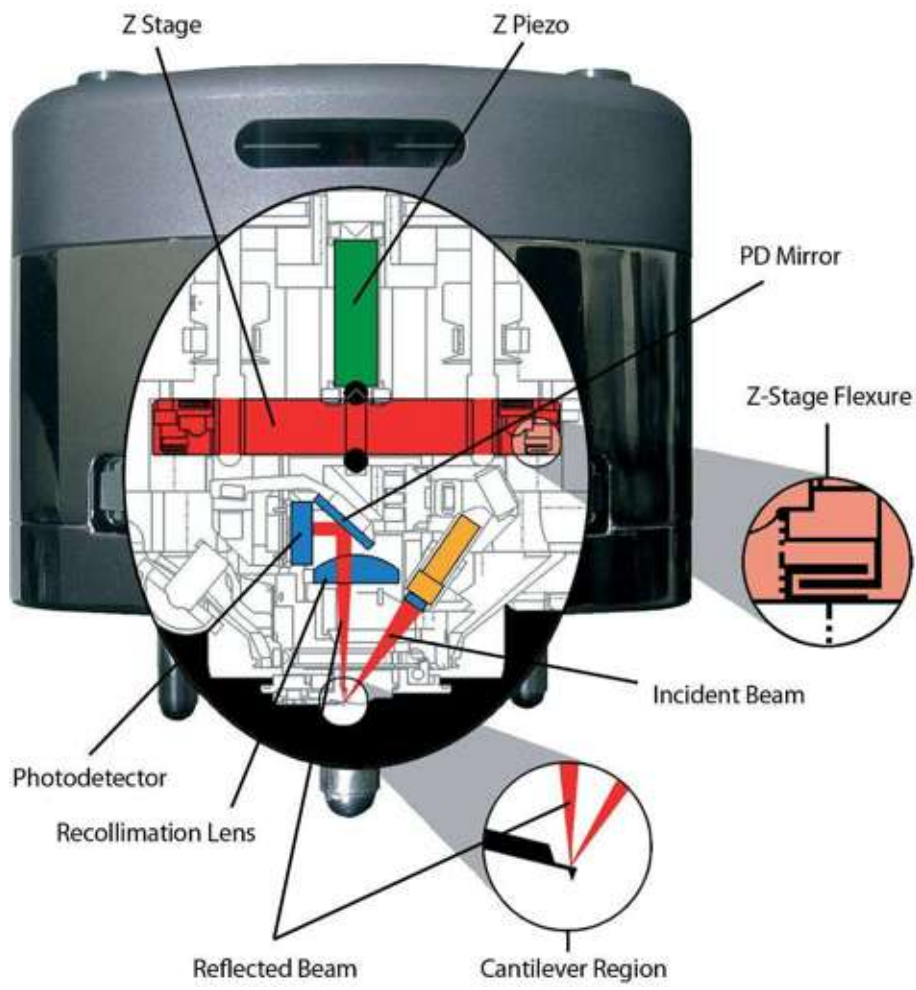


Figure 5.1 Schematic of the Standard Head with OptoPort Options

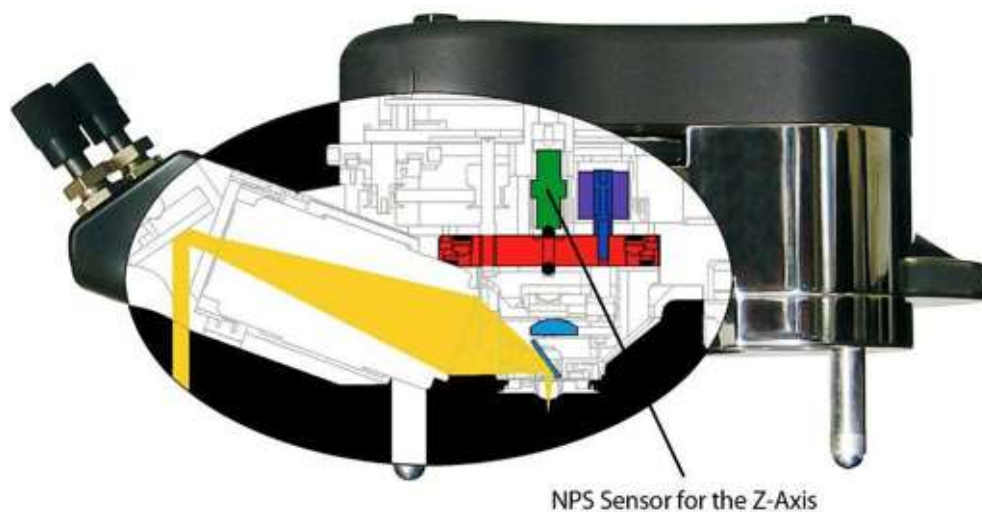


Figure 5.2 Standard Head, side view

Moving on the Nanoscale: Piezo Actuators and the Nanopositioning System (NPS™)

Piezos are the most common actuators for AFM scanners. The piezoelectric effect is the property of certain materials to generate a voltage in response to an applied mechanical stress. This property is used to light your camp stove – you turn a knob, which compresses a piezo, which generates a voltage and a spark to ignite the gas. The effect is reversible — piezoelectric crystals change shape in response to an applied electric field. In AFMs, a piezo is a device that expands (or contracts) in a desired direction when a voltage is applied to it. The MFP-3D™ uses piezos to move the sample in x and y, to move the cantilever in z, and to oscillate the cantilever for AC mode.

Piezos have a number of properties that limit the accuracy of their displacements. When a voltage is applied to move the piezo to a specific location, it will not move to that exact spot - there is error in the resulting location. In open loop scanning, this error directly affects the accuracy of the x and y dimensions of features within the AFM image. In closed loop scanning on the MFP-3D™, proprietary position sensors we call Nano-Positioning System (NPS™) sensors measure the relative position of the sample and cantilever in three dimensions. The x-y position measurement is then used by the controller to move the sample to the intended location, greatly improving the accuracy of the x and y scanning and measurements. The MFP-3D™ is designed to run in closed loop for all general operation. Although not commonly recommended, the user can turn off the feedback and run in open loop (in the Main panel select ‘Set-up’, then set Scan

Mode to 'Open Loop'). Open loop may be appropriate for running a home built AFM with the MFP-3D™ controller.

Each of the three axes has a piezo stack that changes its length in response to a voltage. The motion of the piezos is somewhat unpredictable, especially for metrology purposes, with effects such as creep, and hysteresis (See [Figure 5.4 on page 57](#) through [Figure 5.8 on page 62](#)). To correct for these issues, the MFP-3D also has Nano Positioning System (NPS™) sensors for each axis. The proprietary NPS sensor has its roots in well known Linear Variable Differential Transformers (LVDTs). At Asylum Research, we made a number of significant improvements in the performance of these devices, making them ideal sensors for nanoscale navigational purposes. The NPS sensors have a voltage range of ± 10 V; however we typically adjust the sensitivity so that the full range of piezo motion corresponds to about ± 7 Volts. This allows for changes in the piezo zero position and range changes – as normally occurs throughout a piezo stack's lifetime.

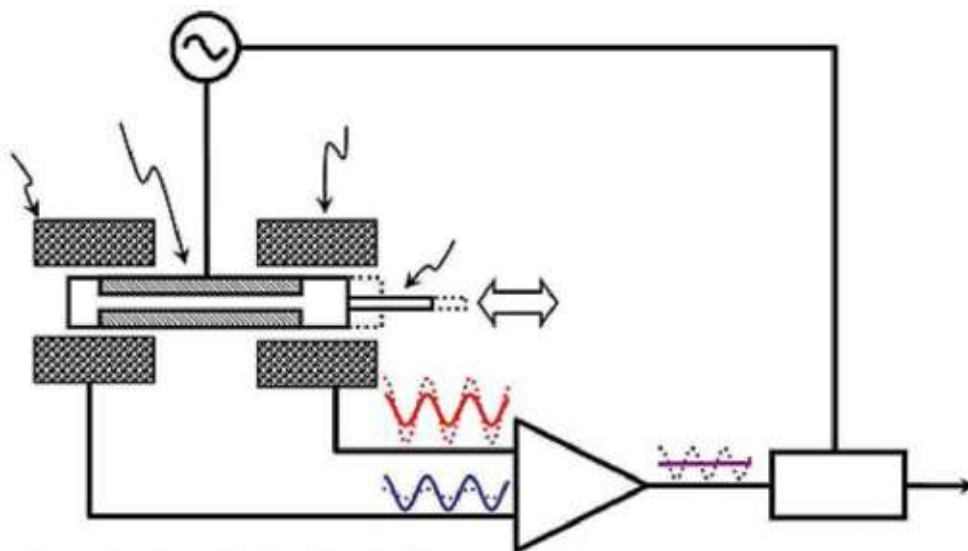


Figure 5.3 Schematic of the Nanopositioning System (NPS™)

When the piezo moves its axis, the core moves relative to the secondaries. This changes the signal we detect from the (NPS™), which changes linearly with displacement.

What this means is that, as the piezo is moved by ramping with a linear voltage vs. time, the NPS™ tells you where it went.

Large and Small Hysteresis Loops for a Piezo Actuator

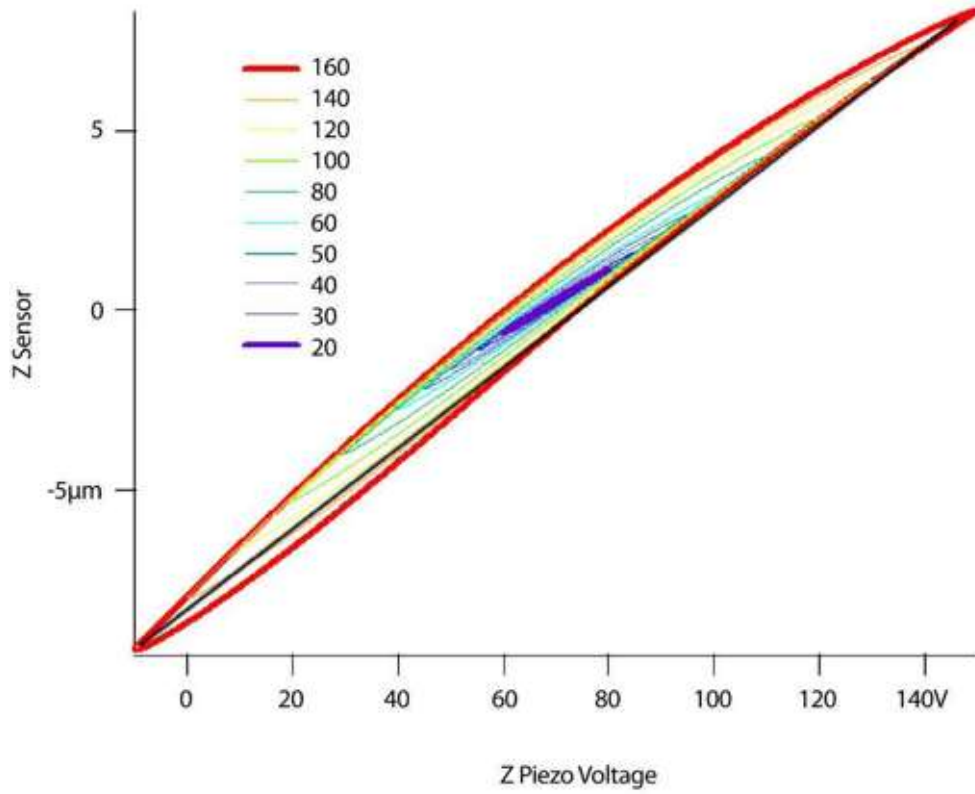


Figure 5.4 Piezo Hysteresis Loops

In the above plot, we apply a linear voltage ramp to the piezo. This provides a non-linear piezo displacement. The black line in the upper plot is just a best-fit line to demonstrate the non-linearity of the piezo displacement. This effect becomes more dramatic as the voltage range becomes greater.

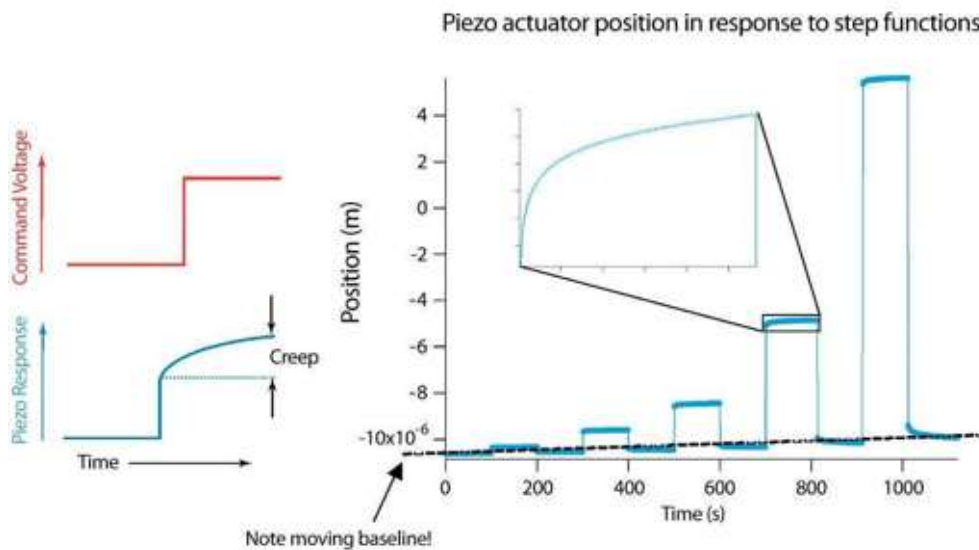


Figure 5.5 Piezo Creep

This figure shows the measured piezo response to different voltage steps. After a step to a new voltage, the actual piezo position “creeps” over time (see inset). Typically, this creep is proportional to the size of the step. An additional complication is that the piezo doesn’t return to the same position after the voltage returns to zero (see the dashed line).

For more details on NPS™ and piezo voltages, see Force Plot [Terminology](#) on page 221.

Z Piezo Stage

The piezoelectric actuators in the Z scanning stage move the light source/cantilever assembly up and down along the z-axis in response to a command voltage. The typical piezo stack in the MFP-3D z-axis expands 16 microns over its operating range. This is also the range of the z-axis flexure. For the majority of imaging applications, this command voltage originates from a feedback loop that is tasked with maintaining some cantilever parameter, such as deflection at a constant value. For applications such as force curves, the command voltage may simply ramp the light source/cantilever assembly up and down.

Z NPS

There are two basic components of the z-axis NPS. They are the NPS sensors discussed above and an optional feedback loop which uses the sensor measurements to control the z-position of the optical/cantilever assembly.

The MFP-3D™ uses the Z NPS sensor to measure the distance the Z piezo causes the light source/cantilever assembly to move up and down. This distance cannot simply be calculated based on the input voltage sent to the Z piezo because of the hysteresis and non-linearity of the piezo throughout the movement range.

The second part of the NPS system is a feedback loop. In the case of the z-axis, this feedback may be optional. For example, as discussed above, in imaging applications the sample position is controlled by a separate feedback loop keeps a cantilever property like deflection or amplitude constant. In this case, the NPS sensor can be used to provide a quantitative measure of the sample height, but it is not used in any feedback loop per se. A counter example is a closed-loop force curve. In this case, it is important that the cantilever is ramped in a very well defined path relative to the sample surface. Since piezos are unpredictable, the NPS sensor measurements can be used in a feedback loop to control the cantilever trajectory.

X-Y Piezo Stage

As with the z-stage, the x-y flexure stage of the MFP-3D is actuated with piezo stacks. The X-Y stacks are different from the z-axis stack in that they have a longer range and they use flexure mechanics to amplify their motion. Thus, while the average x-y piezo stack expands roughly 35 microns over the full operating voltage, the mechanical amplification means that the sample stage can move over 100 microns.

X-Y NPS

As with the z-axis, there are two components to the NPS – the actuators and the feedback control.

The MFP-3D xy stage uses piezo actuators to scan the sample in the x-y plane. Because the NPS sensors have low positional noise, scanning is almost always performed in closed loop. This means that the NPS sensor information is passed on to the controller, which in turn uses this information to force the xy scanner to move to the desired position. For very low noise scanning or for situations using a scanner without position sensors (for example, in a home built SPM) this closed loop control can be turned off and simple voltage ramps will be output to the piezo actuators.

Optical Lever Detection

This section explains the concepts and components used in the optical lever cantilever sensing mechanism employed in the MFP-3D™.

Overview

Optical lever detection is the most common method used in AFMs to sense the force on the cantilever. Optical techniques are used to measure the bending of a flexible cantilever as it responds to mechanical interactions with the surface, electrostatic forces, etc. In the MFP-3D™, an SLD (super luminescent diode) shines onto the back of the reflective cantilever which is mounted at roughly eleven degrees with respect to the sample plane. A lens focuses the light beam from the SLD at the lever. The light reflects off the cantilever and up to a recollimation lens and mirror to the position sensitive detector, a segmented photodiode.

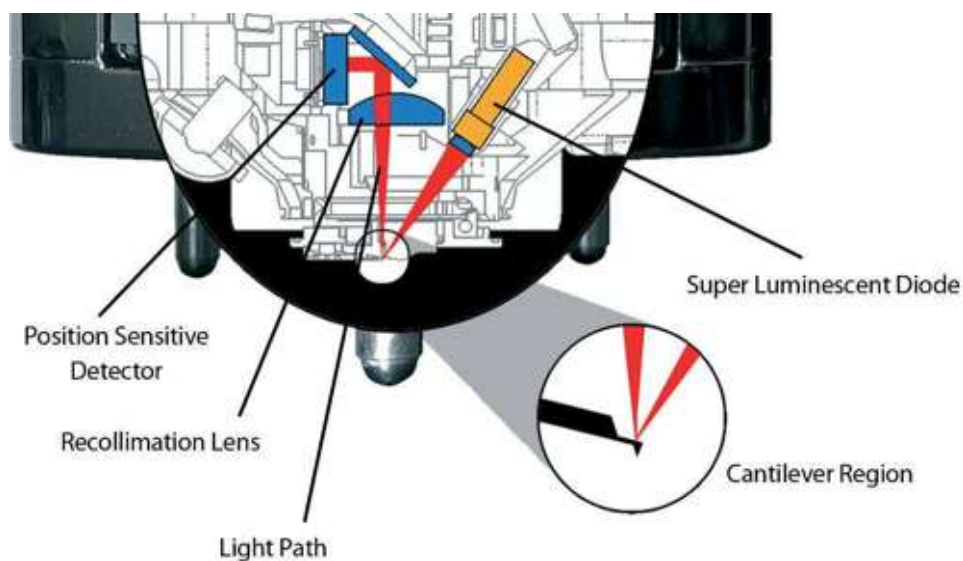


Figure 5.6 Optical lever detection components

The position of the reflected beam on the photodiode is determined by the angle of the deflected cantilever. Imagine holding a large metal ruler in your hand with a laser pointer shining on it from above. If you want to move the reflected laser spot up and down a wall across the room from you, it is easy to do with only a small motion of your hand. By moving only one end of the ruler to change its angle you get a large amplification of distance on the wall, translating the spot along the wall a much larger distance than you moved the ruler. If however you keep the angle of the ruler constant and just move the whole ruler up or down, you get no amplification of distance (for every 1 cm you move the ruler upward, the spot moves up the wall 1 cm). In the AFM the cantilever bends rather than tilts and the reflected beam bounces up to a mirror before going off to the photodiode (“wall”), but the idea is the same.

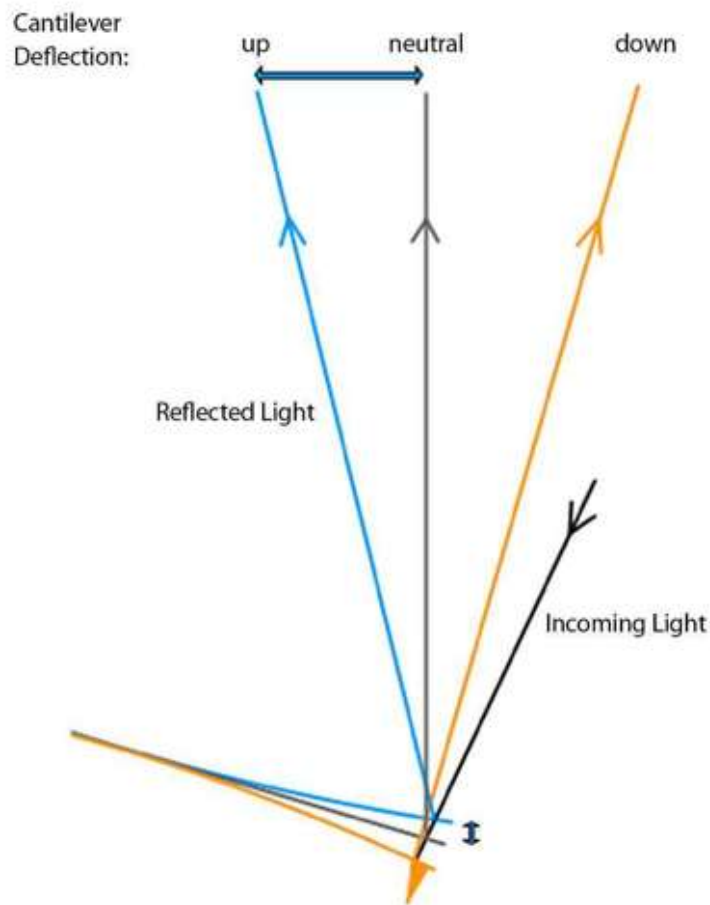


Figure 5.7 Amplification of cantilever deflection (not to scale)

This basic concept of an optical lever is what allows an AFM to detect sub-nanometer changes in the cantilever deflection using standard optoelectronic components. The photodiode does not need sub-nanometer precision because the deflection of the cantilever has been amplified to a larger scale.

Photodiode and Deflection

The photodiode is segmented into four quadrants. A voltage is generated from each quadrant that is proportional to the amount of light hitting it.

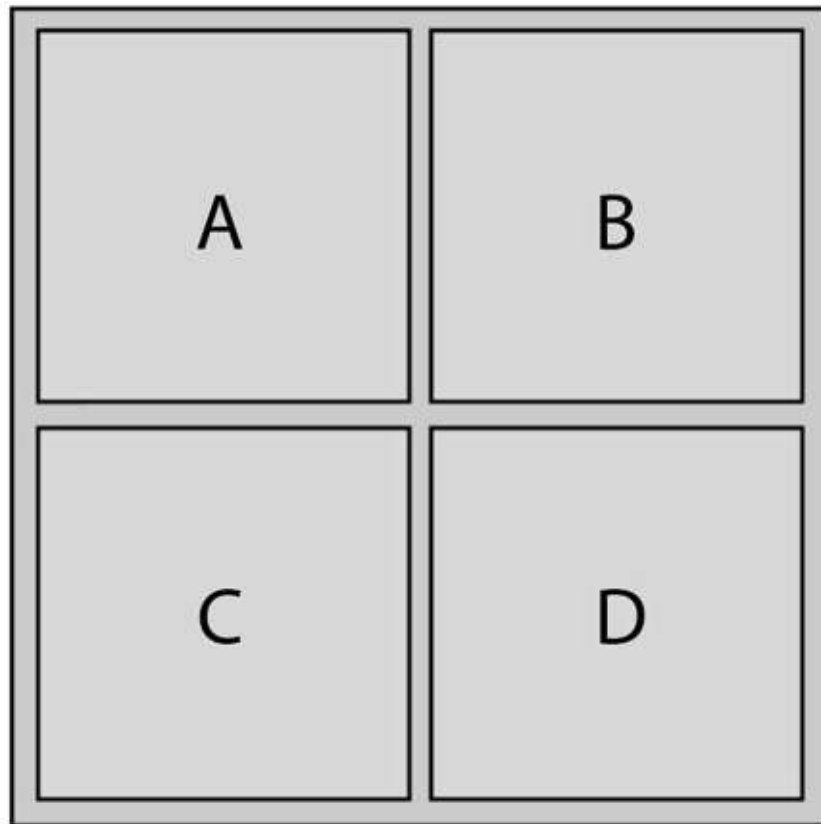


Figure 5.8 Segmented photodetector quadrants

The deflection signal is the difference between the voltages generated in the top and bottom halves of the detector.

$$\text{Deflection} = V_{\text{top}} - V_{\text{bottom}} = (V_A + V_B) - (V_C + V_D)$$

The lateral signal is the difference between the voltages generated in the left and right halves of the detector.

$$\text{Lateral} = V_{\text{left}} - V_{\text{right}} = (V_A + V_C) - (V_B + V_D)$$

Typically the light source is aligned so the spot hits the center of the photodiode when the cantilever is undeflected (far from the surface). When the cantilever

experiences an overall repulsive force, as when it pushes down onto a hard surface, it deflects upward and the spot moves up on the detector. More light hits the top half than the bottom half of the photodiode, so $V_{\text{top}} > V_{\text{bottom}}$ and the deflection is positive. When the cantilever experiences an overall attractive force, as when it pulls away from an adhesive surface, it deflects downward and the spot moves down on the detector. More light hits the bottom than top, so $V_{\text{top}} < V_{\text{bottom}}$ and the deflection is negative.

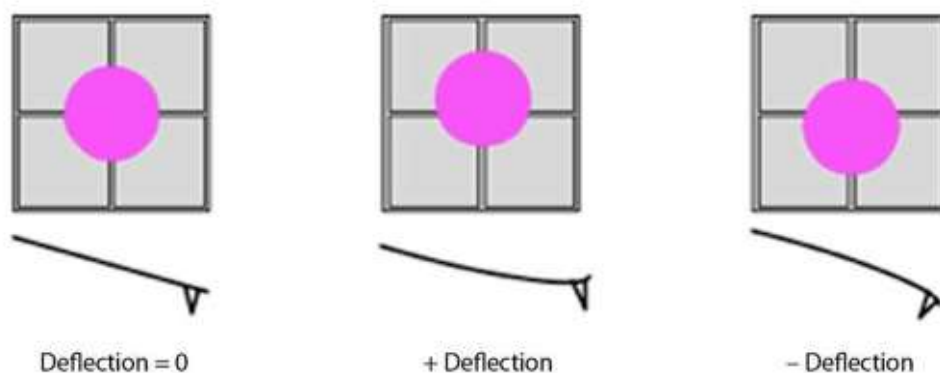


Figure 5.9 Deflection and corresponding spot positions on photodiode

InvOLS

How much signal is generated in the photodiode for a given amount of cantilever deflection is called the optical lever sensitivity (OLS). The more the spot moves at the detector, the more signal is generated and the higher the OLS. Some factors affecting the OLS are constant such as the geometry of the optical elements and the sensitivity of the photodiode. Other factors, such as the laser alignment on the cantilever and the style (beam or V shaped) and length of the cantilever, typically change from one experiment to the next. In the MFP-3D™ software the inverse of the optical lever sensitivity (InvOLS) is used for convenience. The InvOLS converts the cantilever deflection from volts to meters. Displaying the deflection in meters is most commonly done in force curve experiments and is required to determine the force on the cantilever ($F = -k * \text{Deflection}$, where k is the spring constant). The InvOLS for a given cantilever and laser alignment is determined by performing a force curve on a hard surface (for details see [InvOLS](#) on page 229) A typical InvOLS for an Olympus TR800 (100 μm , $k=0.61 \text{ nN/nm}$) or equivalent cantilever is 20 – 35 nm/V .

Note that the InvOLS is not needed for taking height data. The height feedback loop keeps the cantilever at a constant deflection, so it is the calibrated motion of the Z piezo (or sensor) that scales the height data.

Light Source

A low coherence light source emits light at approximately 860 nm. The light shines on the back of the cantilever at an angle of 11° perpendicular to the cantilever axis. Adjusting the LDX and LDY knobs positions the angle of the light source to allow it to be reflected off of different cantilevers.

Cantilever

The cantilever has a sharp tip that interacts with the sample. This is the working end of the AFM. The interactions between the tip and the surface change the deflection of the lever, which in turn is measured by the reflected light detected by the segmented photodiode.

Photodetector Mirror

This mirror directs the light reflected off the back of the cantilever toward the segmented photodiode. When you zero the deflection with the photodetector (PD) knob, you are adjusting the angle of this mirror.

Segmented Photodiode

This detector is segmented into four segments, made up of a 2x2 matrix. The segments are often referred to as A, B, C, and D with A and B being the left and right top-most segments respectively, and C and D being the left and right bottom-most segments respectively. The difference between the upper two segments (A+B) and the lower two segments (C+D) gives the deflection signal, while the difference between the left two segments (A+C) and the right two segments (B+D) gives the lateral signal. For more details on this, please see the section on [Photodiode and Deflection](#) on [page 61](#).

Light Source Adjustment (LDY and LDX)

The LDY and LDX knobs (See components #2 and 4 in [Figure 5](#) on [page 70](#)) align the light source with the cantilever by tilting the light source emitter. LDY aligns the light source perpendicular to the axis of the lever (Y axis). LDX aligns the light source parallel to the cantilever axis (X axis). The cantilever drawing on the top of the head references the direction of the LDX and LDY axis.

5.3 Cantilever Loading

The MFP-3D™ may be used with a variety of cantilevers. Your specific application and sample determine which type of cantilever you should use.

ATTENTION: Store cantilevers at room temperature. Temperature differences between the cantilever and the surrounding air may cause problems with collected data.

The MFP-3D™ allows the operator to manually load a cantilever. A cantilever holder ships with the system to allow easy cantilever installation. You may either use the shipped cantilever holder stand or the cantilever holder stand built into the bottom of the head (see [Cantilever Holder Stand Cantilever Loading](#))

Cantilever Holder Stand Cantilever Loading



Figure 5.10 Cantilever Holder Stand

NOTE: The setscrew presses against the backside of the spring clip to free the cantilever

1. Remove the head from the base and turn the head upside-down so that the spring clip is exposed (See [Figure 5.11](#)).



Figure 5.11 Expose the Spring Clip

NOTE: Cantilevers point from left to right.

NOTE: There is also a back-up cantilever holder stand built into the bottom of the head.

2. Remove the spring clip assembly from the head by pressing the lever holding it in place, and seat it in the cantilever holder (See [Figure 5.12](#)).

NOTE:

When seating the spring clip assembly, seat the forward two points first. Level the assembly, and then seat the back point. Do not force the assembly while seating, or seat it from the back forward.



Figure 5.12 Prepare the Cantilever Holder. Figure A Remove from Head. Figure B Place in Holder

3. Remove any remaining cantilevers using a pair of tweezers. The spring clip should be released while the cantilever holder is latched into the stand. (See [Figure 5.13](#)).

NOTE:

If the experiment requires ultra clean fluid, refer to the allowed cleaning procedures in [Maintenance](#) on page [211](#).

4. Using tweezers, select a new cantilever. Adjust its orientation so the tip side faces up.
5. Using tweezers slide the new cantilever - tip side up - beneath the spring clip.



Figure 5.13 Inserting a Cantilever

6. Remove the cantilever holder from the stand by releasing the latch. This holds the cantilevers in place, as the spring clip is allowed to push against the cantilever chip. Insert the cantilever holder back into the head.
7. Using a stereoscope or a magnifying glass, verify that the tip is well seated and aligned in the pocket. If the tip is not aligned, return the assembly to the cantilever stand and realign.

| |
|---|
| <u>NOTE:</u> Continue to Cantilever Focus. |
|---|

5.4 Subassembly Controls

Using the MFP-3D™ Head

The MFP-3D™ is a sensed optical lever with diffraction-limited optics and a low coherence light source. Its design reduces interference artifacts to a negligible amount. The NPS™ sensed Z axis provides precise measurements of the cantilever position for accurate force and topography measurements. An optional Extended Head allows for a $28\mu\text{m}$ Z range. The MFP-3D™ head supports a number of adjustment knobs which allow the user to adjust the diodes, cameras, etc. manually (See [Figure 5.14](#)).



Figure 5.14 MFP-3D™ Head

Vertical Coarse Adjustment (UP)

The large knobs (See #1, 3 and 5 in [Figure 5.14](#)) on the MFP-3D™ head raise and lower the cantilever (and the entire MFP-3D™ head) in distance to the sample. For best operation, use all three knobs in order to keep the MFP-3D™ head level while rising or lowering.

Turn the knobs clockwise to raise the cantilever.

Turn the knobs counter-clockwise to lower the cantilever.

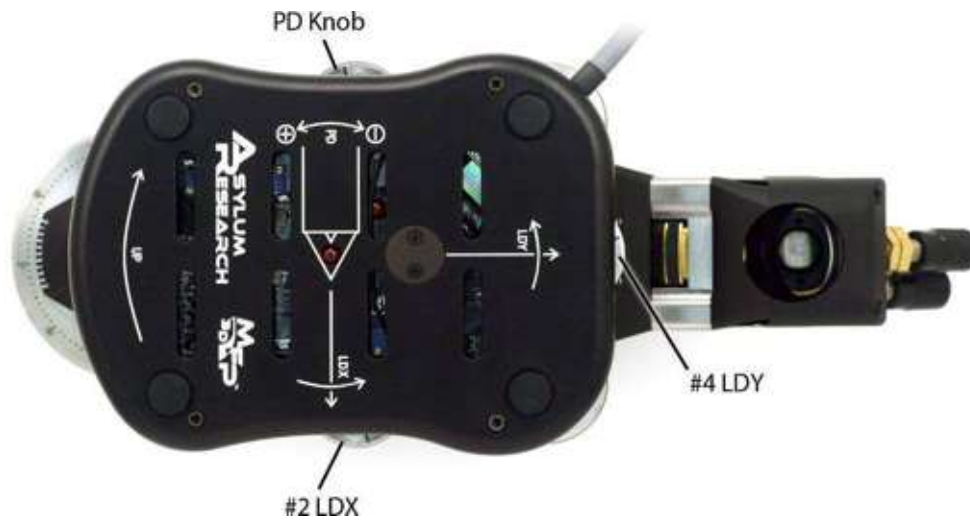


Figure 5.15 Optical Detector Adjustments (PD, LDY and LDX)

NOTE: The following directions explain operation from the perspective behind the lever, facing the PD knob (See [Figure 5.](#)).

- Turn LDY clockwise to move the light source spot to the standard side (left side) of the chip
- Turn LDY counter-clockwise to move the light source spot to the right side of the chip
- Turn LDX clockwise to move the light source spot toward the chip
- Turn LDX counter-clockwise to move the light source spot away from the chip
- The PD knob increases and decreases the deflection in order to bring it to zero deflection (See [Figure 5.16](#)). Moving this knob tilts the mirror that directs light reflected off the cantilever at the photodiode. The photodiode is most linear at 0. For best results, you should calibrate in volts at approximately 0 deflection as you collect your data.

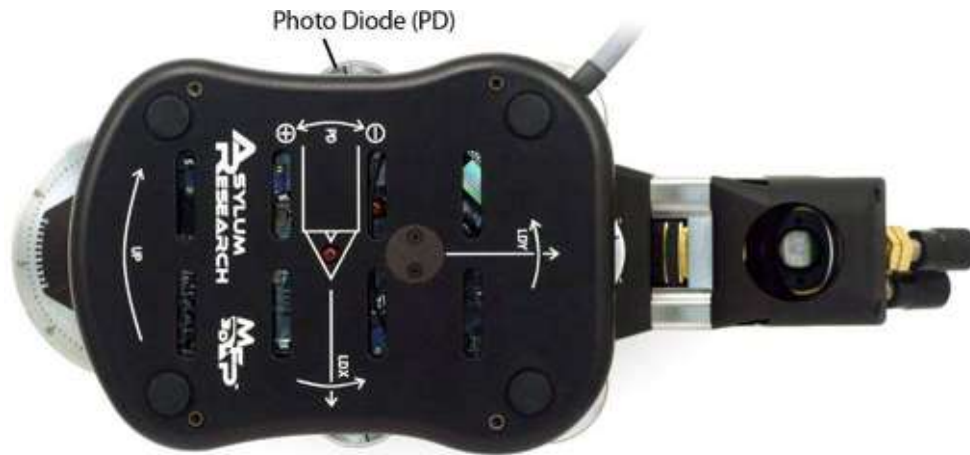


Figure 5.16 Photo Diode (PD)

Turn PD clockwise to decrease the deflection

Turn PD counter-clockwise to increase the deflection

Using the Bottom View Stand-Alone Base

The stand-alone base is a one-assembly system, which includes cameras and positioning ability within the base (See [Figure 5.17](#)). You may adjust the sample, camera and parking positions by using the following controls.



Figure 5.17 Bottom View Stand-Alone Base

ATTENTION: When the base is parked turn **ONLY** the microscope focus knob to avoid video camera damage.

The sample X and Y positioning knobs (See [Figure 5.18](#)) adjust the sample location beneath the cantilever tip. The X knob positions the sample along the X-axis (left to right). The Y knob positions the sample along the Y-axis (front to back).



Figure 5.18 Sample X and Y Position

Turn X clockwise to move the sample to the left

Turn X counter-clockwise to move the sample to the right

Turn Y clockwise to move the sample backward

Turn Y counter-clockwise to move the sample forward

Built-In Cantilever Holder Stand Cantilever Loading

The built-in stand does not have a latch to hold the cantilever holder down. You must press down gently so that the setscrew releases the cantilever from the spring clip.

NOTE: Not all setscrews on the built-in stand have properly adjusted heights. You may have to adjust the height of your setscrew to match your cantilever holder.

1. Place the cantilever holder in the built-in cantilever stand in the same orientation as it is mounted in the head.
2. While pressing down on the cantilever holder, change the tips as is described in Steps 3-through 5 (in [Cantilever Holder Stand Cantilever Loading](#) on page 65)
3. Using a stereoscope or a magnifying glass, verify that the tip is well seated and aligned in the pocket. If the tip is not aligned, realign.

NOTE: Continue to Cantilever Focus.

4. Click the video button on the Igor status bar to open the Video window (See [Figure 5.19](#)).

NOTE: The video button is just to the right to the AR symbol

5. Focus on the cantilever you wish to use employing the following:

If using a top-view system, focus using the thumb wheel on the head.

If using a bottom-view or dual-view system, focus using the FOCUS knob on the SA base or the inverted optical microscope on the IO base.

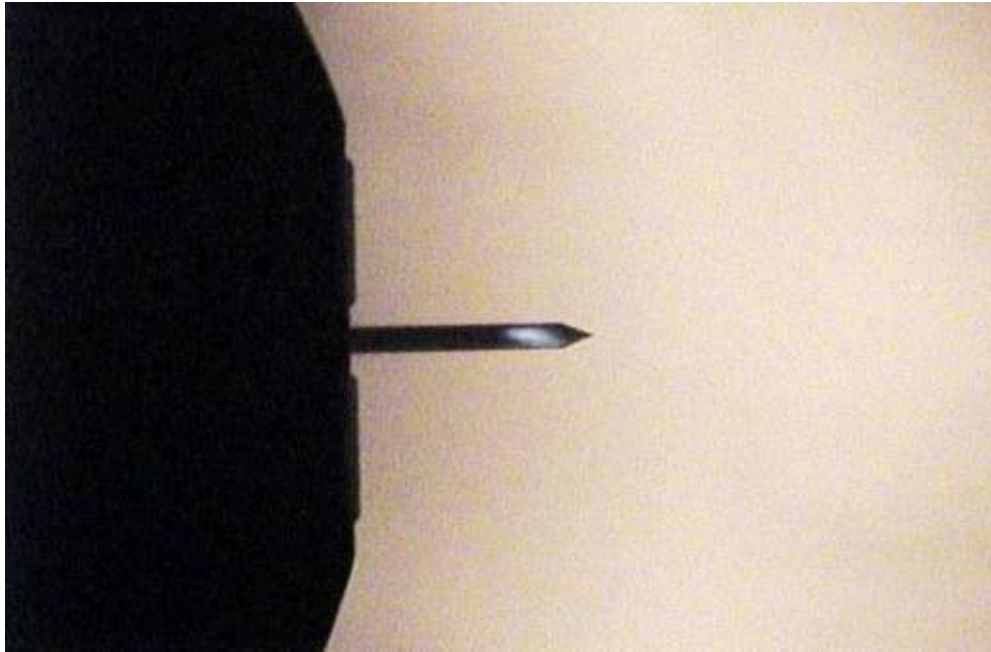


Figure 5.19 Focus on the Cantilever

5.5 Aligning the Light Source and Zeroing Deflection

After inserting the cantilever, you must align the light source that detects the cantilever position, and adjust the angle of the photo detector mirror to zero the deflection. (See [Optical Lever Detection](#) on [page 59](#) for background on this topic). There are two basic methods to align the light source on the cantilever: the video method, which is generally easier for new users; and the infrared, or IR, card method, which is quicker for more experienced users.

Regardless of your method, use the Sum and Deflection Meter window (MFP Controls / Sum and Deflection Meter) while completing alignment (See [Figure 5.20](#)). This shows you the real time status of the important optical detector parameters.

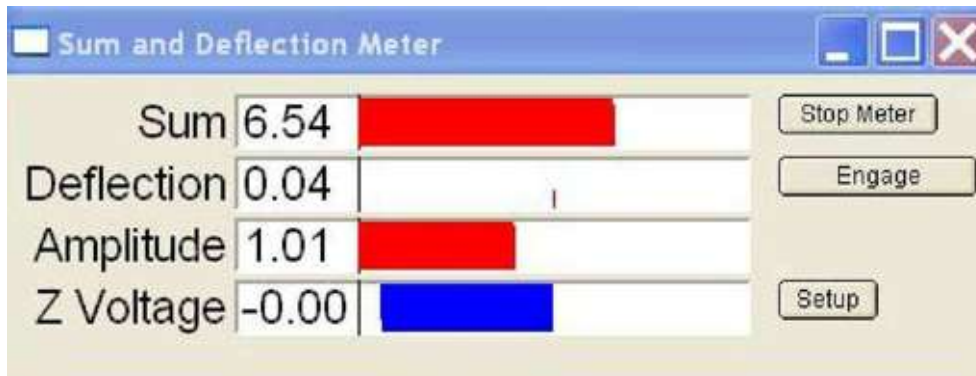


Figure 5.20 Sum and Deflection Meter Window

When aligning the light source, the Deflection measures how well the reflected light is centered on the photo detector in the Z direction. Deflection is measured in volts and is computed as the difference between the light on the top half (A+B) and the bottom half (C+D) of the photo detector.

NOTE: See [Figure 5](#) on [page 70](#) for a diagram of the MFP-3D™ head that shows the optical detector adjustment knobs.

Video Method of Light Source Alignment

In this method we will use the video camera to find the light source on the cantilever.

Open the Sum and Deflection Meter (See [Figure 5.20](#)). MFP Controls -> Sum and Deflection Meter menu or (Ctrl+6).

The Sum is the total amount of light collected by the photo detector in volts. It is generally a good measure of how well the light source is aligned on the cantilever. In air, the sum should be in the 6 to 7 range; in water, its range should be about 8 to 9.

ATTENTION: If the maximum Sum decreases over several days or weeks, or if the maximum Sum is less than 4 volts in air, you may have a damaged light source. Contact Asylum Research Support.

Align the Light Source Using the Video Window

1. If the Sum and Deflection Meter is not already open, select MFP Controls/ Sum and Deflection Meter (See [Figure 5.20](#)).
2. If the Video window is not already open, click the TV icon on the Igor Pro bottom status bar.
3. In the Video window, find the scattered light.

NOTE: The actual location of the light spot to the cantilever is slightly to the left of the light seen in the Video window. This difference occurs because the light hits the cantilever at an 11° angle.

4. Turn the LDX and LDY knobs on the MFP head to align the light seen in the Video window.
5. Adjust the knobs to maximize the Sum value in the Sum and Deflection Meter.
6. Align the light source in the middle of the cantilever in the vertical (LDY) direction.

NOTE: Turning the LDY knob clockwise moves the spot in the Video window toward the left of the image. This corresponds to the front end of the MFP-3D™ head because the camera resides beneath the cantilever (when using a bottom-view system).

7. Turn the LDX knob counter-clockwise to move the spot down and off the cantilever. Verify the Sum drops close to zero.
8. Turn the LDX knob clockwise to move the spot and maximize the Sum value.

NOTE: Positioning the spot as close to the end of the cantilever as possible while maximizing the Sum typically gives the best signal response.

9. Turn the PD (photo detector) knob on the MFP head to zero the Deflection in the Sum and Deflection Meter.

NOTE: The PD knob changes the angle of the photo detector mirror. This then changes the location of the spot hitting the photodetector.

IR Card Method of Light Source Alignment

| |
|--|
| <u>NOTE:</u> Using an infrared card to align the light source is a faster but more advanced method. |
|--|

This section describes how to align the super luminescent diode (SLD) onto your cantilever by using your Infrared (IR) card, and assumes you have your IR card in place and your cantilever loaded.

1. Align LDY so that it is in the middle of the travel. This is most easily done by judging by eye where the knob is in its range of travel.
2. Move LDX counter-clockwise all the way to the end of its travel.
3. Move LDX clockwise so that the spot is moving towards the chip. At the start, the spot will not be visible on the IR card as the tip holder is clipping it. As you move the spot closer to the chip, it will become visible again. Continue moving towards the chip until the spot disappears.
4. When the spot blinks out you have hit the chip, so move LDX slightly counter-clockwise until you can see the entire unobstructed spot.
5. Move LDY along the edge of the chip, watching for a shadow in the spot on the IR card.
It is these shadows in the spot that are generally due to the cantilevers. If you move LDY too far in either direction, the spot will be obstructed by the edge of the optical part of the tip holder, in which case, you should move back towards the mid-range.
6. While moving LDY back and forth slightly, watch the sum voltage. The sum should drop to near zero, rise to some higher voltage (by a few volts at least) and then drop back down to near zero again as you move the spot across the lever. If the sum does not behave in that manner, what you're seeing is probably not a lever, so keep searching.
7. Once you have found your lever, it is a matter of getting the spot to the end of the lever. Right now it should be near the base, near where it connects to the chip.
8. If you have a straight-legged lever, you should be able to move LDX counter-clockwise, while watching the sum voltage. When the sum voltage drops, you should be fairly close to the end of the lever.
9. If it is a V-legged lever, you will need to move both LDX and LDY. To do this, walk the spot up one of the legs of the lever by alternating moving it away from the chip (LDX), and then moving it back towards the lever again (LDY.) To do this, you should first move LDX counter-clockwise a little ($< \frac{1}{4}$ turn), so that the spot moves away from the chip.

The sum will drop at that point, so you'll need to move LDY back and forth to determine which direction to move it to increase the sum to where it was. Then switch to moving LDX counter-clockwise again, by another small amount. Now move LDY in the same direction that you moved it before, to increase the sum again. Repeat this procedure until you cannot get the sum voltage to increase by moving LDY back and forth a small amount. This is the point at which the spot is located just past the end of the lever. Now you can move LDX slightly clockwise, and again try to get your sum back with LDY (again only move a small amount).

10. Now that you are near the end of the cantilever, adjust LDX and LDY to increase your sum such that you have a signal of approximately > 5 volts (a general guideline).
11. Adjust the photo detector knob so that the deflection signal is within range, +/- 10 volts.
12. Fine-tune LDX and LDY to maximize the sum.
13. Do a thermal PSD to determine on which lever you've landed. Use the resonance frequency it finds and compare that to the manufacturer's values. (Note: don't trust the spring constant value from the thermal PSD, as you have not calibrated InvOLS yet).
14. If you are not on the lever you wanted, then you will have to determine which direction you need to travel to get to that lever. One trick to doing that if you are on a relatively short lever is to simply use LDY to move to another lever (Remember to watch for shadows in the IR card and also to watch the sum voltage). If you are on a relatively long lever, you will have to move LDX clockwise so that the spot is closer to the chip before trying to jump to the next lever. How much you move it depends on the length scales you are dealing with, but it is generally a < 1/4 turn. At that point, start over at step 7 again.

5.6 Cantilever Selection

Each time you use the MFP-3D™, replace the cantilever. Using old cantilevers may contaminate your sample, because bending upon contact, and/or create noise in the image. The money you might save from reusing cantilevers will never come close to the cost of time spent dealing with flawed data. It is also a good idea to store your cantilevers at room temperature. Cantilevers bend as they change temperature, and you will have to wait for your lever to equilibrate before collecting good data unless they are stored at an appropriate temperature.

Cantilevers Types

Diving Board Cantilevers

Also called straight-legged or rectangular cantilevers, these levers have only one leg. Historically, they were used primarily for AC mode, but more and more contact mode diving boards are now being produced. There are more methods for calibrating a diving board lever, because the mathematics are easier with them than with V-legged levers. This is particularly true for calculating lateral spring constants. Also, it is slightly easier to align the laser onto a diving board lever than it is to align it onto another type of lever.

V-Legged Cantilevers

Also called triangular, these levers have two legs forming a V-shape. V-legged levers are often less expensive than diving board levers. They are almost always made for use in contact mode with a hard Si₃N₄ tip; however, this type of lever will work for AC mode in liquid for some situations.

Silicon Cantilevers

Pure silicon is a hard but brittle material. Cantilevers made from it are generally quite thick, partly due to difficulties in controlling the thickness when working with silicon, and partly due to the need to make the levers durable. Thin silicon levers are quite easy to break. Generally this material is used to make AC mode diving board levers.

Silicon Nitride (Si₃N₄)

A hard material that can be made quite thin and flexible, Silicon Nitride is generally used to make contact mode cantilevers. Historically the problem with this type of lever has been bluntness, but advances have been made recently to sharpen their tips. These advances generally increase the cost of these cantilevers.

Cantilever Coatings [Tip Side]

Levers today are available in a variety of coatings, each designed for specific types of experiments, such as conductive coatings for electrical experiments, or Cr/Au coatings for functionalization.

Cantilever Guide

For specific product information on cantilevers, please see the Asylum Research Cantilever Guide, located under the Products category at:

<http://www.AsylumResearch.com>

You may also download the Asylum Research Cantilever Price Guide in a .pdf format for a complete list of all cantilever models, available quantities, and pricing. The price guide may be downloaded from the above referenced link.

Please see section [5.3 Cantilever Loading](#) on [page 65](#) for more specific instructions on cantilever positioning in the holder.

There are three basic questions you need to ask yourself when choosing the cantilever for your experiment. In order of importance:

1. Will the lever be able to provide the data I am seeking?

Will it be able to respond quickly enough for the conditions I'll be studying? Is the spring constant low enough to provide enough signal, but high enough so that the expected forces are not saturated?

2. How easy will it be to use this lever?

Time wasted trying to use a specific lever is very important to factor into your decision. How easy is it to calibrate the lever? How durable is the tip?

3. How expensive are the cantilevers?

Using expensive tips will undoubtedly give you the urge to try and reuse them. You need to fight that urge as much as possible, for all the reasons listed in section [5.3 Cantilever Loading](#) on [page 65](#).

To answer these questions, you first need to identify the primary data type you are concerned with obtaining. Then, you need to pinpoint in which mediums you are able to work (air, water, etc.).

The following information on cantilever selection is sorted into general considerations, based on the data to be collected.

Contact Mode

Obtaining just the height data of a hard surface is relatively easy. Your primary concern is the durability of the tip; consequently nearly any Si₃N₄ cantilever will work well. You also need to pick a tip that is sharp enough to see the features you are looking for, but those are about your only concerns.

AC Mode [Air]

In air, a high frequency, high quality factor, or Q value cantilever is usually the right choice. These levers have a high spring constant, and are generally quite sharp. Another consideration is how fast you want to scan. The fundamental response time of a cantilever is roughly the resonance frequency times the quality factor (Q). You want this to be as fast as, or faster than, the sampling frequency on the fast axis (Scan Points * Scan Rate). That is,

$$\text{Resonance Frequency} * Q > \text{Scan Points} * \text{Scan Rate.}$$

This is not generally a problem, because the resonance frequencies are typically 3-4 orders of magnitude larger than the Scan Rate.

AC Mode [Liquid]

Acoustic (piezo) drive

Choosing correctly is more difficult in this mode. The mechanics of how the cantilever is driven in liquid are a bit more complicated, since the liquid is being driven as well, and that in turn drives a number of other components. As a result, it can be much more difficult to find the correct peak to use, because you may see quite a few false peaks in your tune. On the bright side, the energy required to remove the tip from the surface is less in liquid than it is in air, because of the lack of a capillary condensation between the tip and the surface. Consequently you can use softer levers in fluid.

iDrive

By driving an oscillating current through the legs of a cantilever in the presence of a magnetic field, it is possible to cause it to oscillate. This method has several advantages for fluid imaging including the ability to automatically tune the cantilever and to use Q-control in fluid. Please contact Asylum Research for the iDrive support note which has more details on this new technique.

Height Data

First determine if you want to work in AC mode or contact mode. As a rule of thumb, if your sample is soft, choose AC mode; if it is a hard sample, use contact mode.

Deflection [Force] Data

For Deflection Force data, you should almost always choose a soft Si₃N₄ lever. You need to have a rough idea of the range of force you want to measure. You want a spring constant and InvOLS that will mean you get a few volts of signal, but keep the signal within the ± 10 V range.

Amplitude and Phase Data

Here you want to have a good high frequency, high Q cantilever. The same considerations for AC mode height data in air apply here. If you need to work in

liquid, then you will need to try some different levers to find one that works well for your conditions.

Lateral [Force] Data

You have the same concerns here as you do for contact mode imaging: choosing a durable tip (once again Si₃N₄ tips are a good choice) that is also sharp enough to see the features of interest to you. You will also once again want to have a lateral spring constant low enough to get a decent signal, but high enough to keep that signal in the ± 10 V range. This last criterion makes tip selection fairly difficult, since lateral spring constants are not widely calibrated, and as a result it is difficult to get this information. As a general rule, the lower the vertical spring constant, the lower the lateral spring constant. Yet another consideration is whether or not you need to get quantitative values for friction; if you do, you'll need to calibrate the lateral spring constant of the cantilever. As mentioned earlier, this is much easier to do with diving board cantilevers.

Bottom View Camera X and Y Position

The camera X and Y knobs (See [Figure 5.21](#)) adjust the location of the video camera under the cantilever and sample. The X knob positions the camera along the X-axis (left to right). The Y knob positions the camera along the Y-axis (front to back).



Figure 5.21 Bottom View Camera X and Y Position

Turn X clockwise to move the camera to the right

Turn X counter-clockwise to move the camera to the left

Turn Y clockwise to move the camera towards the back

Turn Y counter-clockwise to move the camera forward

Microscope Focus and Parking

The microscope focus and parking knob (See [Figure 5.21](#)) adjust the video microscope to focus on either the sample or the cantilever. Turning this knob moves the objective of the camera along the Z-axis (up and down). This knob also parks and unparks the video camera. To park the camera, turn this knob, camera align X and Y, and then turn the focus knob as far clockwise as possible.

Turn the focus knob clockwise to move the camera up

Turn the focus knob counter-clockwise to move the camera down

Using the BIO Base Plate

There are two types of MFP-3D-BIO™ base plates available: the static stage and the adjustable stage. To move the sample with the static stage, you must manually move the sample. To move the sample with the adjustable stage, you may position the sample using the adjustment knobs (See [Figure 5.22](#) and [Figure 5.24](#)).



Figure 5.22 Adjustable BIO Base Plate

Optical Axis Alignment X and Y

The optical axis alignment knobs (See [Figure 5.22](#)) position the MFP-3D™ cantilever within the microscope view. The X knob adjusts the cantilever along the X-axis (left to right). The Y knob adjusts the cantilever along the Y-axis (front to back).

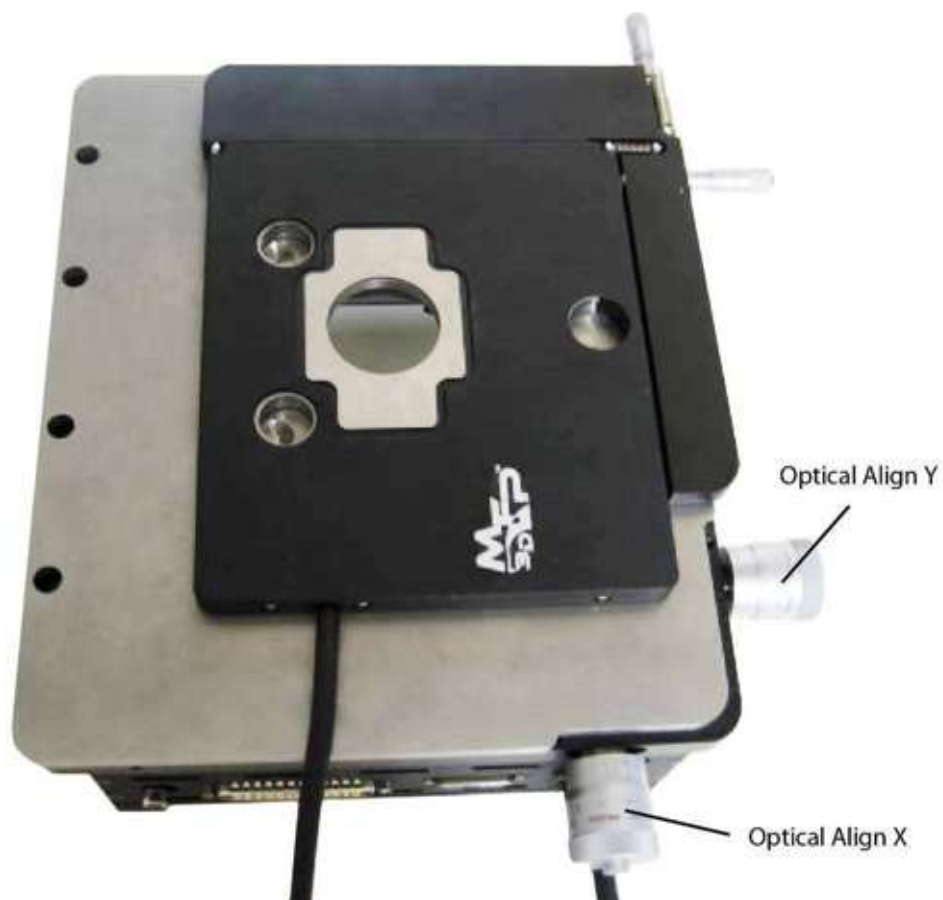


Figure 5.23 Optical Axis Alignment X and Y

- Turn X clockwise to move the cantilever to the right
- Turn X counter-clockwise to move the cantilever to the left
- Turn Y clockwise to move the cantilever towards the back
- Turn Y counter-clockwise to move the cantilever forward

Sample X and Y Position

The sample X and Y position knobs (See [Figure 5.24](#)) move the sample beneath the cantilever tip. The X knob adjusts the sample along the X-axis (left to right). The Y knob adjusts the sample along the Y-axis (front to back).

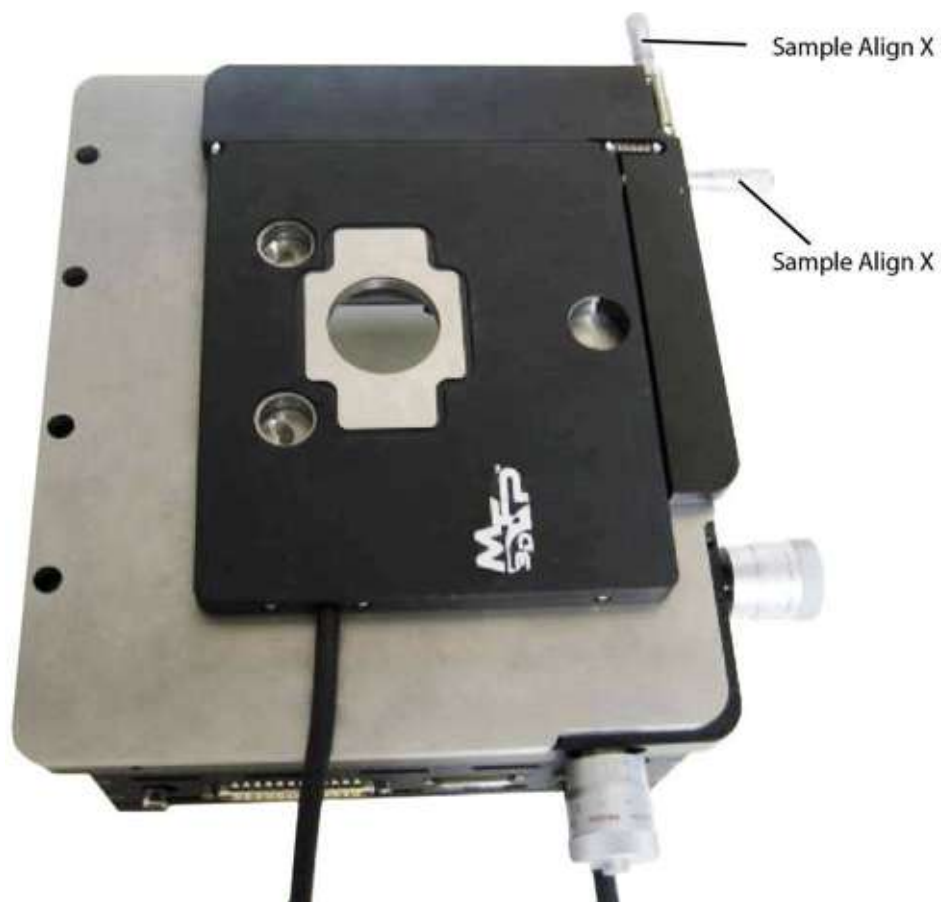


Figure 5.24 Sample X and Y Position

Turn X clockwise to move the sample to the right

Turn X counter-clockwise to move the sample to the left

Turn Y clockwise to move the sample backward

Turn Y counter-clockwise to move the sample forward

5.7 Using the Controller

The MFP-3D™ controller (See [Figure 5.25](#)) converts commands from the computer into the signals necessary to run the AFM. The controller switches, knobs, etc. are detailed below for operational use.



Figure 5.25 MFP-3D™ Controller Front

Functions of Controller Parts

MFP-3D™ Port

The MFP-3D™ port (See [Figure 5.25](#)) is the main connector between the controller and the stand-alone base or IO base plate.

Audio Out

The audio output jack allows the user to plug headphones into the controller to listen to the cantilever deflection signal. Listening to the signal allows the operator to diagnose problems more quickly and effectively.

WARNING: If you plug speakers into this connector, you may cause audio feedback. The cantilever is sensitive to acoustic signals, which in turn will drive the speakers, which will in turn drive the cantilever.

Volume

The volume knob controls the volume of the audio out signal. Turn clockwise to increase volume. Turn counter-clockwise to decrease volume.

Function

The FUNCTION switch is a software-programmable switch; it allows the operator to change its function. The FUNCTION switch on the Main tab of the Master panel controls the radio buttons to the right of the controls. The radio button selected determines what the hamster controls. On the Force tab of the Master panel, FUNCTION down allows a force pull; FUNCTION up switches between the force distance and the start distance, controlled by the hamster.

Hamster

The hamster knob provides fine control over the item selected with the function switch (either the Start distance or the Pull distance, as previously described). The hamster knob is a software-programmable knob; its function may be changed if desired. Radio buttons select the parameter controlled by the hamster. Radio buttons may be selected with the mouse or with the Function key (See [Function](#) in Section 5.7 on page 89).

Power

The controller power is controlled by the power switch

Signal Access

The signal BNC connectors allow the operator to input and output various signals.

Light Source Lock

The light source lock enables or disables the light source, depending upon its position.

Locked (key is in a vertical position): the light source is off.

Unlocked (key is in a horizontal position): the light source is on.

| |
|--|
| <p><u>ATTENTION:</u> The light source is ‘on’ only if: the power is on; the head is not tilted beyond 30°; the light source is unlocked; the remote jack circuit is closed (shorted). When the light source is on, the red LED on the head illuminates.</p> |
|--|



Figure 5.26 Controller Rear

Light Source Remote

The light source remote jack allows operators to configure the MFP-3D™ light source to disable automatically under certain conditions. A light source remote plug is provided that shorts the connectors in this jack, enabling the light source.

USB

The USB port allows the controller to communicate with the computer. A cable connects from this controller port to a USB port on the computer tower.

Video

The video port allows the base to communicate video to the computer. A cable connects from this base port to the video port on the back of the computer tower.

Controller

The controller to base port allows the controller to communicate with the scanner and head assembly. A cable connects from this controller port to the controller port on the base.

Fuses 1-3

The fuses are initially configured for the standard power used in your country. However, these fuses can be configured to allow the MFP-3D™ controller to use AC input voltages of 100, 120, 220, and 240 volts.

AC Input Power

The input power port allows the operator to connect the controller to a power source.

5.8 Signal Accessibility

There are three ways of interacting with the many data channels of the MFP-3D: through the crosspoint switch, the front panel BNC connectors, and/or through low level software commands. [Table 5.1](#), lists all of these data channels and summarizes where to gain access to them.

Table 5.1 Data Channel List

| | Data Signal | Accessibility | | | Signal Origin | Signal Destination |
|-------------------------------|-------------|---------------|------|----------------|-------------------|--------------------|
| | | BNC | Xpnt | Software | | |
| AFM Positional Control | X Piezo | x | | x | 24bit 100kHz DAC | Piezo |
| | Y Piezo | | | | | |
| | Z Piezo | | | | | |
| | X Sensor | x | | x | Sensor output | 16bit,100kHz ADC1 |
| | Y Sensor | | | | | |
| | Z Sensor | | | | | |
| User DACs and ADCs | OutA | | x | x | 24bit 100kHz DAC2 | Input of Xpnt |
| | OutB | | | | | |
| | OutC | | | | | |
| | InA | x | x | Output of Xpnt | 16bit,100kHz ADC3 | |
| | InB | | | | | |
| Fast DAC and ADC | DDS | | x | x | 16bit, 10MHz DAC4 | Input of Xpnt |

| | | | | | | |
|----------------------------------|---------------------|---|---|---|--------------------------------|-------------------------------------|
| | InFast | | | | Output of Xpnt | 16bit, 5MHz ADC5 |
| Photodetector signals | Defl | x | x | | Photodetector output | Input of Xpnt |
| | ACDefl ⁶ | | | | | |
| | Lateral | | | | | |
| Lock-in Signals | Amplitude | | | x | Calculated from ACDefl and DDS | None |
| | Phase | | | | | |
| | I | | | | | |
| | q | | | | | |
| Cantilever holder signals | PogoInO | | x | | Cantilever holder | Input of Xpnt |
| | PogoInI | | | | | |
| | PogoOut | | x | | Output of Xpnt | PogoOut screw |
| | Chip | | | | | Cantilever spring clip |
| | Shake | | | | | Cantilever shake piezo |
| Offset and Mod | InAOffset | | x | | Output of Xpnt | Summing junction with named channel |
| | InBOffset | | | | | |
| | InFastOffset | | | | | |
| | OutXMod | | x | | Output of Xpnt | Summing junction with named channel |
| | OutYMod | | | | | |
| | OutZMod | | | | | |
| User Inputs and Outputs | UserIn0 | x | x | | Input BNC | Input of Xpnt |
| | UserIn1 | | | | | |
| | UserIn2 | | | | | |
| | UserOut0 | x | x | | Output of Xpnt | Output BNC |
| | UserOut1 | | | | | |

| | | | | | | |
|----------------------|-----------|--|---|--|-------------------|---------------|
| | UserOut2 | | | | | |
| Miscellaneous | Ground | | x | | Electrical ground | Input of Xpnt |
| | FilterOut | | | | FilterIn | Input of Xpnt |
| | FilterIn | | | | Output of Xpnt | FilterOut |

1 Digital IIR Filter, typically 1kHz for X and Y and 2kHz for Z.

2 52kHz 1 pole LPF for A and B. 78Hz 1 pole LPF for C.

3 117kHz 1 pole LPF and Digital IIR Filter, typically 2kHz

4 2MHz 6 pole LPF

5 2MHz 6 pole LPF and Digital IIR Filter, typically 1.5kHz

6 160Hz HPF

Crosspoint Switch

The Crosspoint switch allows the user to make links between various data channels entirely through a software interface. These links can be treated as actual “hardware” connections for all intents and purposes. In this way, custom experimental set-ups in the MFP-3D are facilitated by allowing various data channels to be connected together at will.

The crosspoint switch plays the digital role of the switchboard operators back in the early days of the telephone. Any of the 16 inputs on the crosspoint (Xpnt) switch can be connected to any of the 16 outputs. This also provides one of the easiest ways to customize the MFP-3D™ to your specific experiment.

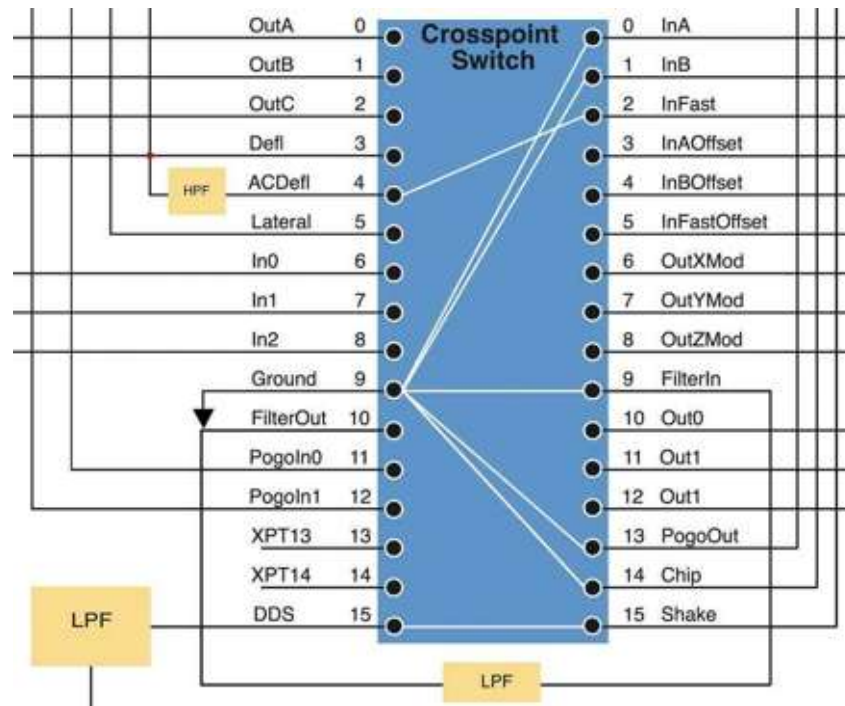


Figure 5.27 Schematic of the Crosspoint Panel

The majority of data channels in the MFP-3D must be routed through the Crosspoint switch, which can be located under the Programming menu. Some of these data channels are inputs while the others are outputs to the Crosspoint. Henceforth, these shall be referred to respectively as Xpnt Inputs and Xpnt Outputs.

As a general rule, any data channel whose terminus is a Xpnt Input must be connected to one whose origin is a Xpnt Output. The converse of this statement is also true.

Front Panel BNC Connectors

Many signals can be accessed from their corresponding BNC, located on the front panel of the controller. The signals on these BNCs range within +/-10V limits and have input impedances of 10kΩ.

These include all of the AFM positional control signals – namely the X, Y, and Z Piezo and Sensor signals. For obvious safety reasons, not to mention voltage limitations, only the low voltage versions of the Piezo voltage signals are accessible.

Also included are three UserIn and three UserOut BNCs. Note that when used in conjunction with the Crosspoint switch, any Xpnt Input can also be monitored on a UserOut BNC.

Lastly, the raw cantilever deflection signal, Defl, can also be accessed. Note that with proper filtering, the DC and AC deflection can be extracted from this signal.

Software Commands

Every data signal must ultimately be either written by a DAC or read by an ADC in the controller. Low-level software commands allow one to do just this by referencing the appropriate parameter name. The commands used to write a value or read a value are as follows:

`td_wv("ParameterName")`

`td_rv("ParameterName")`

Where ParameterName can be any of the following:

Piezo signals: X%Output, Y%Output, and Z%Output

Sensor signals: X%Input, Y%Input, and Z%Input

UserOutputs DACs: A%Output, B%Output, C%Output

UserInputs ADCs: A%Input, B%Input

DDS: Amp%DDS, Freq%DDS, (and others)

Fast ADC: Fast%Input

Lockin signals: R%Input, Theta%Input, I%Input, and Q%Input

5.9 Data Channels

During normal operation of the MFP-3D, signals are continually being routed and re-routed, written and read. All of this takes place automatically in the background and can be as transparent to the user as s/he wants it to be.

The following sections group all of the data channels involved in the system according to their function. The data channel's origin and destination as well as its primary functions are described. In addition, any limitations of the actual voltage signal are detailed. Please refer to [Table 5.1 on page 91](#) for a list of the data channels and their aliases.

These sections will be particularly useful for those interested in reconfiguring the MFP-3D for custom experimental set-ups. But it will also be useful to those who simply want to gain a better understanding of the AFM system's normal operation.

AFM Positional Control

Positional control is implemented in each of the three axes of the MFP-3D through an actuator and a sensor, both of which require specific data signals.

More specifically, voltage signals are sent out to the piezo actuator on each axis, and read back from the corresponding NPS™ sensor. With the ability to both actuate movement *and* sense how far it actually moved, the controller “closes the feedback loop” for precise motion control.

Actuators: Piezo X, Piezo Y, and Piezo Z

Each of the three piezo data channels originate from a dedicated 24-bit 100 kHz DAC as a low voltage signal (ranging from -0.66V to +10V). These low voltage signals are amplified by a factor of 15X at high voltage amplifiers. These high voltage signals (ranging from -10V to +150V) are then sent out to the appropriate piezos.

These data signals are used while imaging to move the scanner sample stage in X and Y and raise or lower the cantilever tip in Z in response to topography. All three data signals are also used while taking force curves with Z ramping up and down and X and Y maintaining a specific position.

Sensors (LVDTs): Sensor X, Sensor Y, and Sensor Z

The X, Y, and Z sensor signals originate as the voltage output from the corresponding LVDT sensor. The ranges of these sensor output signals are typically well within their maximum +/-10V bounds (i.e. approximately -7V to +7V).

Each of these voltage signals is sent back to the controller and read on a dedicated 16-bit 100 kHz ADC. They are then digitally filtered with a Digital IIR LPF filter which can be set from the Filter Panel. Typically, the filters for the X and Y Sensors are set to 1 kHz and 2 kHz for the Z Sensor to eliminate higher frequency noise.

User DACs and ADCs

The ability to both read and write data signals is integral to most custom experiments. These data channels allow the user to do exactly that. Three User DAC channels and three User ADC channels are provided, enabling the user to directly communicate with the MFP-3D AFM.

User DACs: OutA, OutB, and OutC

Each of the three User DAC channels originates at a 24-bit 100kHz User DAC and terminates at a Xpnt Input. Each channel's User DAC can generate signals ranging anywhere in magnitude within the +/-10V limits. The OutA and OutB data channels both pass through a 52 kHz LPF, which caps their signal's highest possible frequency to approximately this value; their signal's lowest possible frequency is DC. The OutC data channel is filtered to a mere 78Hz LPF thereby rejecting higher frequency noise components and making it the best channel for a low noise DC voltage source.

While the OutA and OutB data channels are not often utilized by the software, the OutC data channel is occasionally used for applying DC biases (e.g. to the cantilever tip as in EFM).

User ADCs: InA and InB

Both of these data channels originate at Xpnt Outputs and terminate at 16-bit 100 kHz User ADCs. Signals on either of these channels can vary in magnitude within the +/-10V limits. Although signals on both of these channels are filtered with a 117 kHz LPF, the highest possible frequency that can be correctly digitized is set by the Nyquist criterion at half of the User ADCs' 100 kHz sampling frequency. Thus signals on these data channels can range from DC up to approximately 50 kHz. Both are then digitally filtered typically at 2 kHz with an adjustable Digital IIR LPF that can be set from the Filter Panel.

The InA and InB data channels are used for collecting auxiliary signals during basic imaging (e.g. Lateral, UserIn0, etc). Alternatively, they can be used to read most of the AFM's signals of interest.

Fast DAC and ADC

Of all the DACs and ADCs present in the MFP-3D, there is one of each that run substantially faster than the rest. These are normally used in AC mode to drive and detect the cantilever oscillations.

DDS

The DDS data channel originates at a 16-bit 10MHz (and hence, "Fast") DAC and terminates at a Xpnt Input. The magnitude of the DDS signal can vary within the +/-10V limits. The frequency can range from DC up to approximately 2MHz – the corner frequency of the LPF that filters signals on the DDS data channel.

Typically, the DDS data channel is routed to drive the shake piezo, which in turn oscillates the cantilever in AC mode.

InFast

The InFast data channel originates at a Xpnt Output and terminates at a 16-bit 5MHz (and hence, “Fast”) ADC. The InFast ADC can digitize signals within the +/-10V limits. The InFast data channel’s signal passes through a 2MHz LPF, which caps its highest possible frequency to approximately this value; the signal’s lowest possible frequency is DC. The signal on this data channel is then further filtered by an adjustable Digital IIR filter, typically set to 1.5 kHz.

Typically the InFast data channel is used to digitize the cantilever’s deflection signal in AC mode.

Photodetector Signals

Everything that we can know about any given tip-sample interaction is contained within the signals carried by the photodetector data channels. Specifically, these interactions include cantilever deflections and perturbations in the cantilever’s oscillation. Furthermore, the cantilever’s DC and AC deflection are used as the error signals of the Z feedback loop while imaging.

Lateral

The Lateral data channel originates at the photodetector and ends as a Xpnt Input. The signal is calculated as the difference between the voltage outputs of the left and right halves of the quad photodetector and is bounded within +/-10V limits. The photodetector’s bandwidth is approximately 2MHz, which defines the highest detectable signal frequency.

Most commonly used in Lateral Force Microscopy (LFM), the Lateral data channel carries the Lateral signal, a measure of the torsional loading applied to the cantilever tip from frictional forces.

Defl

The Defl data channel originates at the photodetector and terminates as a Xpnt Input.

The signal is calculated as the difference between the top and bottom halves of the quad photodetector and is bounded within +/-10V limits.

While the Defl signal initially contains frequency content up to the photodetector bandwidth, this signal is most often used as a measure of the cantilever’s static or DC deflection. Consequently, this signal is often routed via a 36 kHz LPF (low pass filter) through the Crosspoint switch.

This LPF version of the Defl signal is called DeflVolts, and is used as the error signal of the Z closed loop feedback while imaging in contact mode. Since this

signal is proportional to the cantilever's actual deflection and hence force, it is commonly plotted in one form or another as the vertical axis in force curves.

AC Deflection

The AC Deflection channel originates at the photodetector and terminates as a Xpnt Input. The signal is identical to the Defl signal except that it is sent through a 160Hz HPF. This decouples any DC or low frequency components of the cantilever such as static deflections or thermal drift. The highest frequency that can be detected is limited by the bandwidth of the photodetector – 2MHz. It is also bound within +/-10V limits.

AC Deflection is most commonly used as the error signal for the closed loop feedback in Z while imaging in AC mode. This signal's amplitude is proportional to the physical amplitude of the cantilever's oscillation. Furthermore, this signal contains information about the cantilever's phase which corresponds to tip-sample interaction.

Lock-In Signals

These signals are obtained by the controller's digital lock-in. The lock-in essentially compares the signal on the InFast data channel with the signal on the DDS data channel. Using the cantilever's original drive signal as reference, the lock-in can extract information about the cantilever's oscillation.

i and q

These signals are derived from the deflection signal as it passes through the lock-in. They represent the "in-phase" (i) and "quadrature" (q) components of the deflection data. They are filtered by an adjustable Digital IIR filter which is typically set to 1.5 kHz.

Amplitude and Phase

These signals can be extracted from i and q through a rectangular to polar conversion. Both of these signals are commonly plotted as image channels while imaging in AC mode. The conversion equations for moving from polar to rectangular are:

$$i = A \cos \phi$$

$$q = A \sin \phi,$$

And from rectangular to polar are:

$$A = \sqrt{i^2 + q^2}$$

$$\varphi = \tan^{-1} \frac{q}{i}.$$

Cantilever Holder Signals

There are a few data channels going to and coming from the cantilever holder. These may be used to apply a bias the tip or sample, or to drive the cantilever's oscillations. Additionally, there are some auxiliary data channels that can be used for custom set-ups.

To the Cantilever Holder: Shake, PogoOut, and Chip

Each of the data channels to the cantilever holder originate as Xpnt Outputs and terminate at specific points within the cantilever holder itself. The signals on these channels are not filtered and are bounded within +/-10V limits.

The terminus of the Shake data channel is the cantilever drive piezo, which is used in AC mode to oscillate the cantilever. The PogoOut data channel ends at a flathead screw on the cantilever holder board which can be used as a contact point from which to locally route a voltage signal. And the Chip data channel leads to the metal spring clip of the cantilever holder allowing one to send a DC or AC bias to a conductive cantilever.

From the Cantilever Holder: PogoIn0 and PogoIn1

There are two data channels that originate from the cantilever holder (PogoIn0 and PogoIn1) and terminate as Xpnt Inputs. The signals on these channels are not filtered and are bounded within +/-10V limits.

For the most part, PogoIn0 and PogoIn1 are auxiliary user channels and are unused in basic AFM operation. The PogoIn0 data channel is occasionally used when additional information needs to be passed back to the controller from the cantilever holder.

Offset and Modify

Each of these data channels can be used to specifically modify/offset another channel by adding a DC and/or AC voltage signal.

Offsets: InAOffset, InBOffset, and InFastOffset

These data channels originate as individual Xpnt Outputs and terminate at a summing junction with the intended data channel that they are meant to offset. The intended data channels are the three ADC data channels, InA, InB, and InFast. The signals on these channels are not filtered and are bounded within +/-10V limits.

Mod: XMod, YMod, and ZMod

Just like the Offset data channels, these originate as individual Xpnt Outputs and terminate at a summing junction with the intended data channel that they modify. The intended data channels are the three low voltage piezo data channels, Piezo X, Piezo Y, and Piezo Z. The signals on these channels are not filtered and are bounded within +/-10V limits.

User Outputs and Inputs

The User Output channels essentially allow a user to route an internal signal to one of the external BNCs for access, whereas the User Input channels allow the user to input an external voltage signal into the controller.

User Outputs: UserOutput0, UserOutput1, and UserOutput2

Each of the UserOut data channels originate as a Xpnt Output and terminate at a BNC on the front panel. The signals on these channels are not filtered and are bounded within +/-10V limits.

These data channels allow any Xpnt Input to be accessed from one of the User Output BNCs when it is appropriately routed through the Crosspoint switch.

User Inputs: UserIn0, UserIn1, and UserIn2

All of the UserIn data channels originate from a BNC on the front panel and terminate as a Xpnt Input. The signals on these channels are not filtered and are bounded within +/-10V limits.

These data channels allow signals to be sent into the controller to the Crosspoint switch where they can then be routed elsewhere. The signal can originate from an external source (e.g. function generator), or an internal source. In addition to internal signals that are already available as a front panel BNC, any Xpnt Input signal can be routed to a UserOut BNC, which can in turn be sent back into the controller through a UserIn channel.

Miscellaneous

This section contains the remaining data signals, which could not be classified under any of the categories above, but are quite important nonetheless.

Ground

The Ground data channel originates at electronic board ground and terminates as a Xpnt Input. This signal is most often used to “ground out” other data channels that are not being used.

Filter: FilterOut and FilterIn

A quick glance at the Crosspoint schematic reveals that these “two” data channels are, in actuality, one and the same. This unique data channel originates at the Xpnt Output FilterIn and terminates at the Xpnt Input FilterOut. The signal on this data channel passes through a 36 kHz LPF.

This Filter channel is most commonly used as an anti-aliasing filter for the Deflection signal in contact mode.

Real-Time Image [Graph]

Graph that displays the image data as it is collected. Real-Time images are created when a data channel is selected. (See [Select Your Data Channels](#) on [page 162](#)). Up to five channels may be opened simultaneously (for example: height, lateral, deflection...) You can choose individually whether to display and whether to save None, Trace, Retrace, or Both for each Channel.

The Real-Time Image is always displayed with the Fast Scan Direction horizontal and the Slow Scan Direction vertical. At 0° Scan Angle, this corresponds to the physical X and Y axes of the scanner, and the orientation of the Real Time Image will agree with the Video window. At 90° Scan Angle, the bottom of the Real Time Image corresponds to the left side of the TopView video window.

Scope

If selected, the Scope graph appears below the Real-Time Image graph for each Channel. The Scope graph displays the data from the last completed scan line. Trace data is displayed in blue and Retrace is displayed in red.

5.10 Imaging Feedback

A feedback loop takes information from the output of a process and sends it back to the input to better guide the process. An every day example is a heater with a thermostat control. A sensor measures the room temperature and turns the heater

on or off depending on whether the room is colder or hotter than the desired temperature set point.

For both contact and AC mode, a feedback loop on the Z piezo provides the height data for the image. The position of the Z piezo is adjusted to maintain either a deflection or amplitude set point.

Contact Feedback

As explained in the [Optical Lever Detection](#) on [page 59](#), the deflection signal corresponds to the angle of the cantilever. If the cantilever is scanned over a sample without feedback, the height of the lever is not adjusted and the angle of the lever changes as the tip moves up and down over surface features of varying heights. The deflection signal thus varies according to the surface features. This is not a desirable imaging method for numerous reasons - calibrating the deflection signal (from volts to nanometers) must be done frequently because it is different for each lever and light source alignment, the z range of the sample that can be accommodated is very small, and the force between tip and sample varies with the surface height.

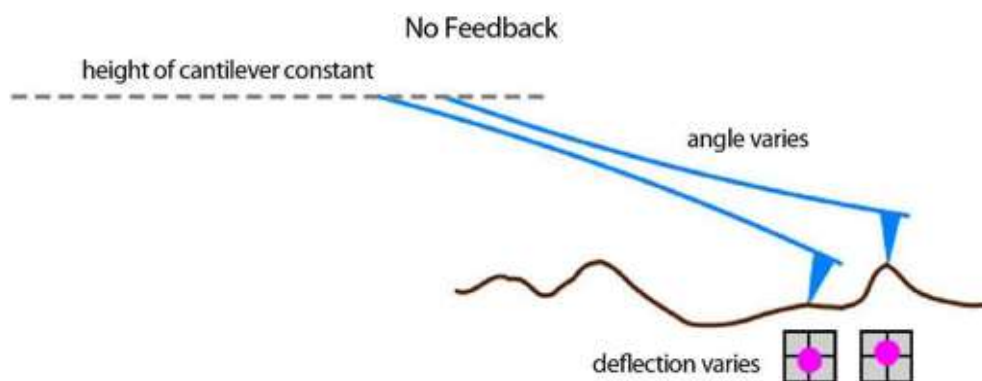


Figure 5.28 Contact Mode No Feedback

The height of the cantilever is controlled by the Z piezo. As the tip scans over the surface with contact feedback on, the height of the lever is adjusted up or down as needed to keep the deflection constant at a level chosen by the user (the set point). To keep the angle of the lever constant, the Z piezo has to move the cantilever so it traces out the contour of the sample surface - maintaining a constant separation between the back end of the lever and the point of the sample in contact with the tip. The calibrated Z piezo position is recorded as the height data. With contact

feedback the calibration rarely needs updating, the z range is large, and the force between tip and sample is constant (a higher set point is more force).

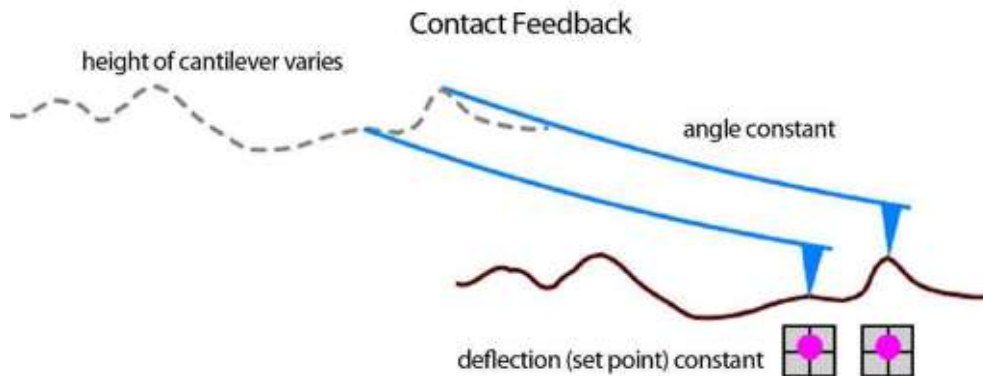


Figure 5.29 Contact Mode Feedback

The Z piezo is moved by adjusting the value of the voltage applied to it. If the deflection signal is higher than the set point, the Z voltage is decreased which moves the tip away from the surface. If the tip is in hard contact with the surface, this will decrease the deflection signal. If the deflection signal is less than the set point, then the feedback loop will increase the Z voltage, which moves the piezo closer to the surface. If the tip is already in hard contact with the surface, then increasing the Z voltage will increase the deflection signal. If the tip is not in contact with the surface, then increasing the Z voltage will get the tip closer to the surface.

AC Mode Feedback

The basic principles for AC mode feedback are the same as those for contact except the deflection is no longer a DC signal. In AC mode the cantilever is shaken at its resonant frequency by a small piezo in the cantilever holder. The angle of the cantilever changes throughout its oscillation causing the deflection signal to oscillate as well. The amplitude of the AC deflection signal characterizes the cantilever's motion.

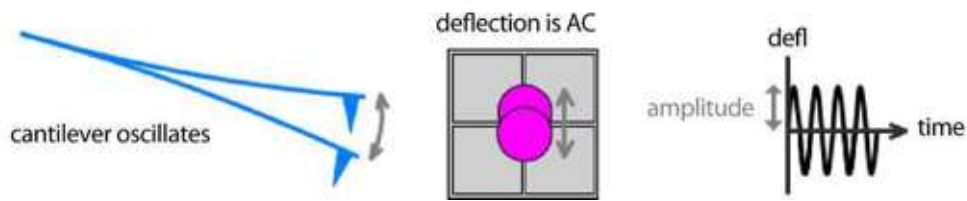


Figure 5.30 AC Mode Deflection Signal and Cantilever Motion

If a cantilever in AC mode were scanned over a sample without feedback, the amplitude would vary as the tip moved over surface features of different heights. The amplitude would get smaller over taller features as the average separation between lever and surface decreased. This is not a desirable imaging method for similar reasons as mentioned for contact without feedback (calibration is unique for each lever and alignment, limited z range, variable force).

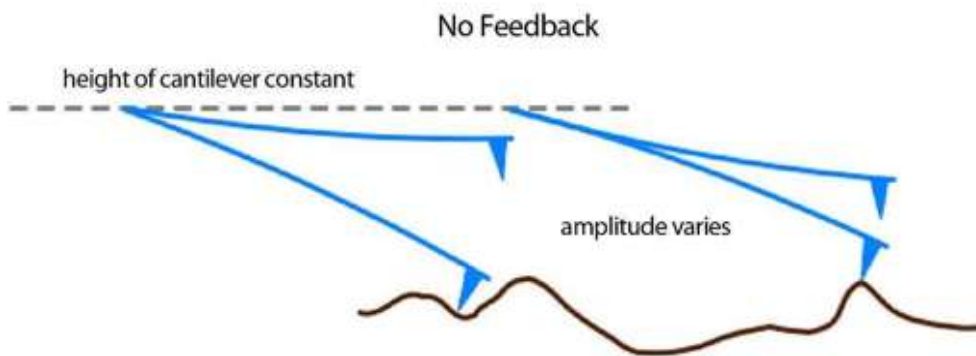


Figure 5.31 AC Mode No Feedback

As the tip scans over the surface with AC feedback on, the height of the lever is adjusted to keep the amplitude constant at the set point value. To maintain a constant average separation between the lever and the point of the sample under the tip, the Z piezo moves the cantilever so it traces the contour of the sample surface. The calibrated Z piezo position is recorded as the height data. A smaller amplitude set point means more force between tip and sample.

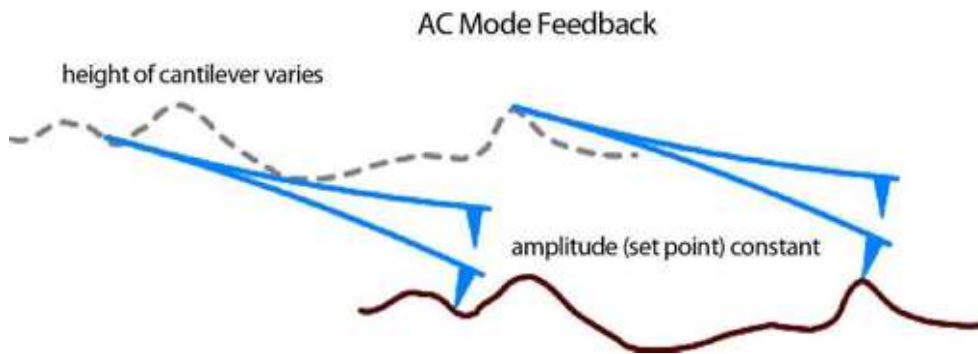


Figure 5.32 AC Mode Feedback

If the amplitude becomes smaller than the set point, the Z voltage is decreased which moves the tip away from the surface allowing the amplitude to increase. If the amplitude becomes greater than the set point, feedback will increase the Z voltage moving the piezo closer to the surface and decreasing the amplitude.

Feedback Gains (PIS)

The difference between the height feedback signal (deflection or amplitude) and the set point is called the error. When there is error the feedback adjusts the Z voltage to minimize that error by moving the piezo in the direction that brings the signal closer to the set point. How much the Z voltage is adjusted for a given amount of error is determined by the gains of the feedback loop. The higher the gains are, the larger the response.

There are 3 gains used in the feedback loops throughout the MFP-3D – Proportional, Integral, and Secret gains. Secret is a double integral (the name is an homage to the double secret probation in Animal House).

$$\text{Feedback Output} = P_{\text{gain}} * \text{error} + I_{\text{gain}} * \int (\text{error}) dt + S_{\text{gain}} * \int \int (\text{error}) dt dt *$$

For the height feedback loop only the proportional and integral gains are used. It is very important to the quality of the height data that the gains are set appropriately. If the gains are too low the Z voltage will not be adjusted enough, the error will be significant, and the height data will be a poor representation of the sample topography. If the gains are too high then the feedback will be too reactive and over-adjust the Z voltage causing the Z piezo (and height data) to oscillate. The piezo will overshoot its optimal position then turn around and overshoot it again in the opposite direction, etc. See the Contact or AC Mode Operation chapters for instructions on setting those gains.

The secret gain is not shown by default but can be accessed on the Main panel by selecting the Setup button. Secret gain is not intended to be used with the height feedback and should be set to zero. Secret gain is important in the X and Y feedback loops, which are set at the factory and should not be adjusted by the user.

5.11 Imaging Basics: What Is Scanning?

Overview

Imaging is scanning the tip of the cantilever across the surface. Topography is determined by detecting where the tip is in its Z trajectory as the X-Y position of the tip is moved along the surface. The scanning axes are divided into the fast scan axis and slow scan axis. These are different than the X and Y axes of the scanner, in that the scan angle is applied to fast and slow scans to determine what the drive is for the two axes of the scanner. However in the display of the data, we display the fast scan direction as horizontal and the slow scan direction as vertical in the Real-Time Image display. For this section, we will consider only 0° and 180° scan angles so that the fast scan direction is the same as the physical X-axis of the scanner and the slow scan direction is the physical Y-axis of the scanner.

* The gains entered in the software by the user have various scaling factors in the underlying code.

You should be aware that for 90° and 270° scan angles the slow axis is the X-axis of the scanner, and the fast axis is the Y-axis of the scanner.

Fast Scan Direction

This is what is shown on the X-axis of the Real-Time graph. It is the X-axis of the scanner when scanning at 0° (or 180°). It is the motion of the scanner that is performed at the frequency of the scan rate. The tip is moved by Scan Size (the size of the image) to collect trace data, and then back, to collect retrace data. This is repeated as many times as is required to collect the number of Scan Lines that has been requested.

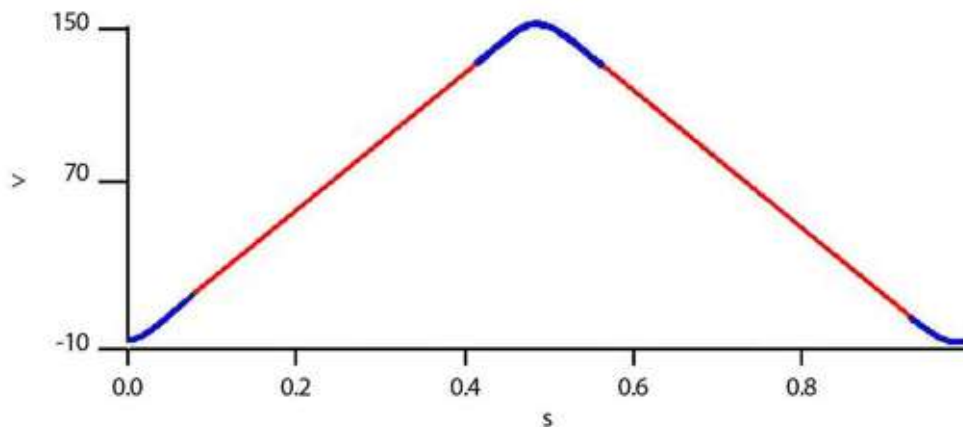


Figure 5.33 Fast Axis X Piezo Drive (at 0° scan angle). Y-axis of graph is piezo voltage; X-axis is time. The rounded caps (blue regions) are called the turn-around, where the fast axis changes direction. Data from the turn-around is not typically saved, though it is possible to access it for special applications.

Slow Scan Direction

This is displayed vertically on the Real-Time graph. It is the Y-axis of the scanner when scanning at 0° (or 180°). The scanner advances in the slow scan direction to access each successive scan line. Every time the fast scan direction completes a line, the slow scan is at a different position. The Slow Scan Direction is handled differently in Raster scanning and Ortho scanning.

Raster Scanning

Raster scanning is an older method for scanning the tip across the surface, and is fairly straightforward to implement using analog circuits. In a raster scan, the

slow axis is simply changing linearly with time (Figure 5.34), so that even as the tip is moving along the fast axis, it is moving in the slow axis.

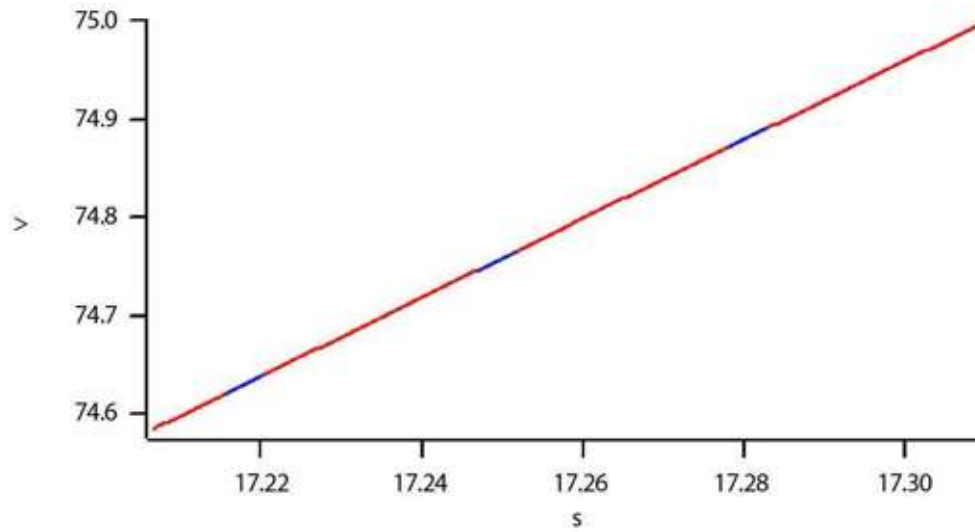


Figure 5.34 Slow Axis of Raster Scan. Y-axis is Y piezo voltage (scan angle 0°); X-axis is time in image. Axis limits are zoomed in to show the same region as in Figure. The blue sections denote turn-around regions.

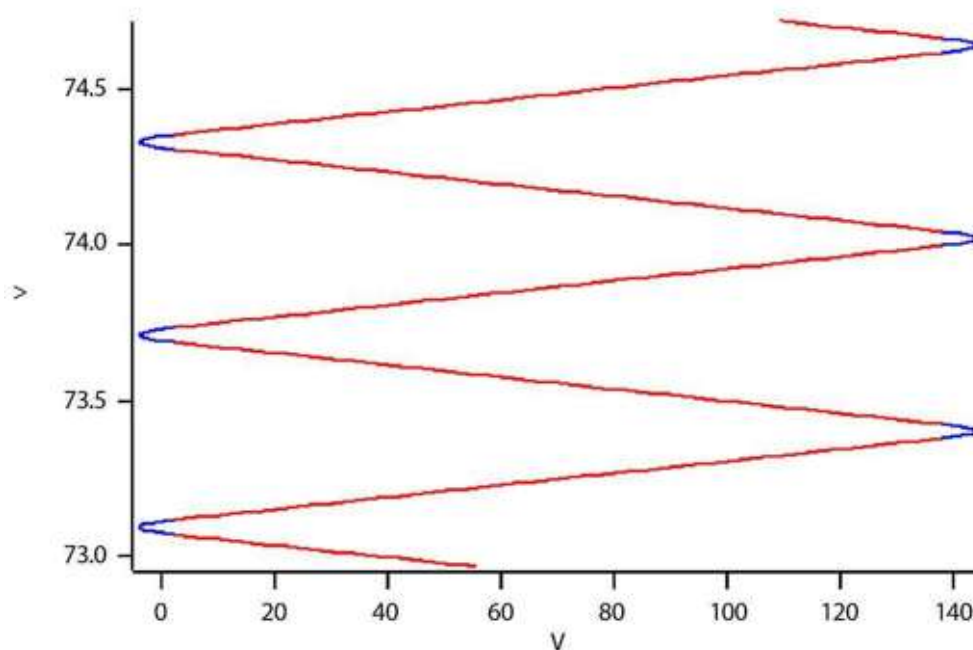


Figure 5.35 X-Y Motion of the Tip across surface in raster imaging. The X-axis is the fast axis drive, while the Y-axis is the slow axis drive. Less than 3 scan lines are shown to better illustrate the concept. The blue sections denote turn-around regions.

There is an issue with raster scanning with the way the images are displayed. The positions of the pixels in the image do not exactly match the position of the tip where that pixel was collected on the surface (except for the middle column of pixels). This is because the rows of image lines are displayed as if they were all at the same slow axis position, but in reality that position is changing as the fast scan is being collected (see [Figure 5.35](#)). This effect is at most $2 * \text{ScanSize}/\text{ScanLines}$, which is generally small enough to ignore. However, there are certain situations in which this effect becomes important, such as drift rate calculations. This effect also causes a slight rotation between Trace and Retrace images; in [Figure 5.35](#), note that the set of scan lines for Trace is at a different angle than that for Retrace.

Ortho Scanning

Ortho scanning is also a method of scanning the tip across the surface, but has not been widely implemented. In this method, the slow axis only moves during the turn-around of the fast axis, where data is not being collected. Then while the data is being collected, (where the fast scan is changing linearly), the slow axis is held constant. This mode eliminates the pixel position problems discussed (above) in raster scanning. Ortho mode does not work well for open loop scanning, since

piezos have an additional slow response, and would actually continue to creep while the fast scan is collecting data (See [Moving on the Nanoscale: Piezo Actuators and the Nanopositioning System \(NPS™\)](#) on page 55 for more details on creep). For closed loop systems however, ortho scanning works quite well.

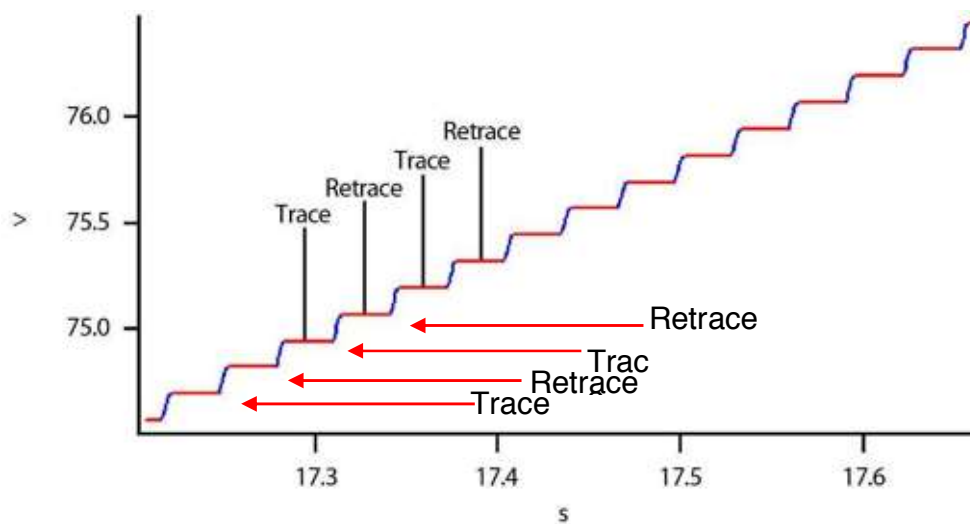


Figure 5.36 Slow Axis of Ortho Scan. In image, Y-axis is Y piezo voltage (scan angle 0°); X-axis is time. Axis limits are zoomed in to show the same region as in preceding Figure (Slow Axis of Raster Scan). The blue sections denote turn-around regions. Approximately 7 scan lines are shown here, each comprising two steps (one for Trace and one for Retrace).

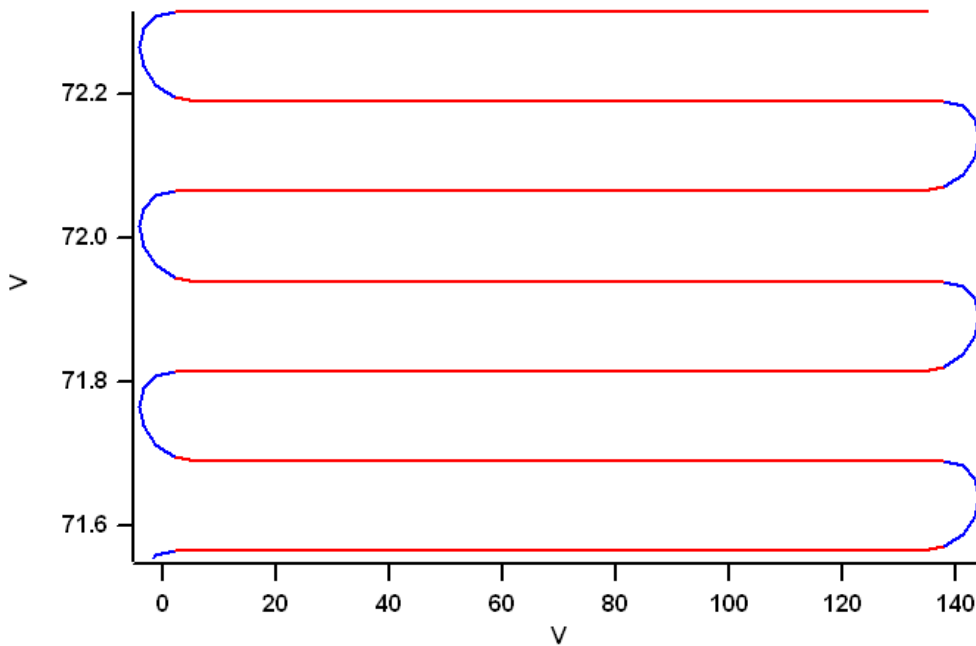


Figure 5.37 Motion of the Tip Across Surface in Ortho Imaging. The X-axis is the fast axis drive, while the Y-axis is the slow axis drive. Three scan lines are shown. The blue sections denote turn-around regions.

Note that in Ortho scanning, the tip does not pass over the same point on the sample in Trace and Retrace. Instead, the Trace and Retrace images are shifted by one-half of a pixel in the Slow Scan direction. This is usually negligible, but may be noticed on samples with steep features and images with a small number of lines.

In the current version of the MFP-3D™ software both raster and ortho scanning can be done. When imaging with less than 800 lines, ortho mode is used. For scans larger than 800 lines, ortho scanning would require too much data storage in the Controller; therefore, raster scanning is used. This limitation may be removed in future versions of the MFP-3D controller.

Drift Correction

Due to temperature changes in the room containing the instrument, the components of the microscope can change with respect to each other. In addition to careful mechanical design, software can be used to combat this problem. In the MFP-3D, the software can calculate the offset between two images, and then use

that drift rate to enter a new X and Y offset automatically. Furthermore, since this calculation can be made on the fly, the drift rates can be continuously updated.

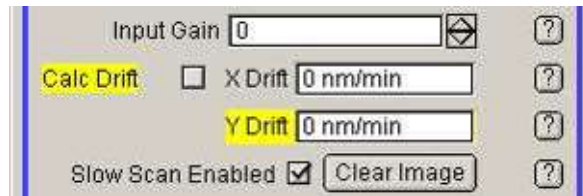


Figure 5.38 Drift Correction Parameters, Master Panel

To use this feature, first click on 'Set-Up' in the Master Panel and select to show the 'Drift' controls (these controls are hidden by default). To use them, just click on the 'Calc Drift' checkbox. Once that is checked, after two real-time images have been collected, the X drift and Y drift values will have changed and the software will update the X and Y offsets for you. It accomplishes that by taking the cross correlation of the FFTs of the two previous images. This feature does not calculate scan angles, so only use it if you are working at 0° . This feature will not correct for image compression or expansion caused by drift during image collection. Since the drift happens as the image is collected, it will alter how features appear as it drifts along the slow scan axis. Specifically, if the sample is drifting along with the slow scan axis, all the features will be compressed in the Y axis of the image and if the sample is drifting *against* the slow scan axis, then all the features will appear elongated in the Y axis of the image. This assumes the drift rate is linear with time however, which is not always the case.

5.12 Using the Computer

The MFP-3D™ computer (See [Figure 5.39](#)) communicates with the controller, head and scanner and runs the MFP-3D™ software designed to gather and analyze data. The computer ports, drives, etc. below are detailed for operational use.

Functions of the Computer Parts



Figure 5.39 Computer Tower Front

Compact Disc

The compact disc drive allows the operator to insert compact discs to transfer information to the computer.

Power On/Off

The power on/off switch located on the front of the computer allows the operator to turn on the computer by depressing the button. When the computer is powered on, the front LED illuminates.

ATTENTION: The computer configuration is constantly being updated and will most likely NOT be the shown configuration.

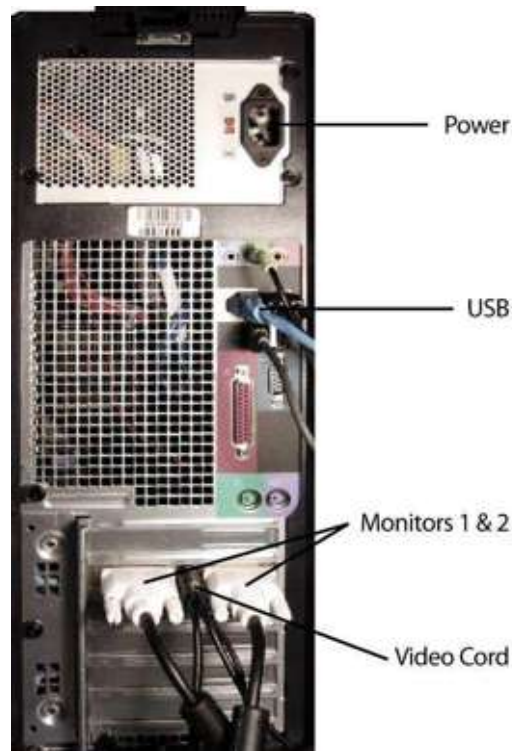


Figure 5.40 Computer Tower Rear

Input Power

The input power port allows the operator to connect the computer to a power source.

USB

The USB port allows the computer to communicate with the controller. A cable connects from this computer port to a USB port on the back of the controller.

Video

The video port allows the computer to communicate with the imaging equipment in the scanner and head assembly. This connection changes the most between systems. Early systems require a purple "Barney" connector to convert the RCA signal from the base to the computer input (See [Figure 5.41](#), left). Newer versions (See [Figure 5.41](#), right) allow the RCA signal from the base to connect directly to the computer. For additional information on these connectors, contact support@asylumresearch.com.



Figure 5.41 Video Port Connections. The older “Barney” version is shown on the left and the new connectors on the right

Monitor #1 and #2

The primary monitor connects with the digital (white) connector to the top-most video card. The secondary monitor connects with either the digital (white) or the analog (blue) connectors to the next available video slot. Older systems have 2 video cards with a total of 3 video slots. Newer systems have only one video card with 2 monitor slots.

5.13 Using the Software

The MFP-3D™ includes a flexible, easy-to-use software interface based on Igor Pro from Wavemetrics. This interface gives the user control over data acquisition and analysis. It is also possible to "tune" the interface to the user's desires.

The software also includes online help, which describes each of the main controls. The operator may enable the help by clicking on a '?' beside a control.

5.14 Powering-Up the System



Figure 5.42 Power-Up the System

1. Press the power button on the front of the computer (See [Figure 5.42](#)).
2. Press the power button on each of the monitors.
3. When you are prompted for a user name, type MFP-3D™ and leave the password field blank.
4. Toggle the controller power switch to the on position.
5. Verify the laser safety lock is plugged in, and the light source key is in the horizontal (unlocked) position .
6. Verify the following LEDs (indicating power) are lit:

Monitor #1 (front)

Monitor #2 (front)

Computer (front)

Controller (front)

Light Source (top of head)

#1 Power

#4 Power

#5 Light Source

#2 Power

5.15 Thermal Power Spectral Density (PSD)

Measure the Thermal Power Spectral Density (PSD) to determine the resonant frequency of the cantilever and to confirm that the cantilever and light source are aligned, which indicates the system is functioning properly.

| |
|--|
| NOTE: Each '?' button to the right of a control opens a help file describing its purpose and use. |
|--|

1. In the Master Panel, click the 'Thermal' tab to bring it to the front of the window (See [Figure 5.43](#)).
2. Using the drop-down menu, choose your desired resolution.

| |
|---|
| NOTE: Default resolution is 7. For the best resolution (but at a slower pace), choose 1. |
|---|

3. In the Master Panel, click 'Do Thermal.'

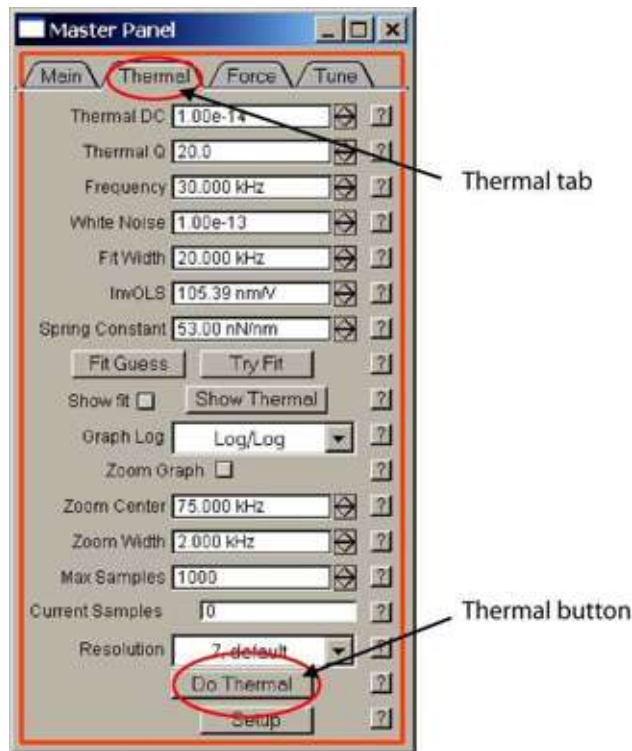


Figure 5.43 Master Panel, Thermal

As the system runs the Thermal PSD, it collects the deflection data using Fourier transform. The results are plotted as the deflection amplitude in $\text{m}/\sqrt{\text{Hz}}$ vs. the frequency in Hz.

4. After a few samples, you will notice the graph does not change much between them. Click 'Stop Thermal'.

NOTE: This may take a few tries because the software is busy collecting data. If there is no clear peak, the light source is not aligned with the cantilever, or the cantilever may be touching the surface.

5. Select 'Thermal Graph' from the main menu.
6. Select 'Graph/ Show Info'
7. In the Thermal Graph window, select and drag a cursor to the peak of the resonance to find the necessary values.

NOTE: You must place the cursor to verify that the current values are being used.

8. Check the Zoom Graph check box to zoom in on the peak (See [Figure 5.44](#)). You can also zoom in on the peak using Zoom Center and Zoom Width.

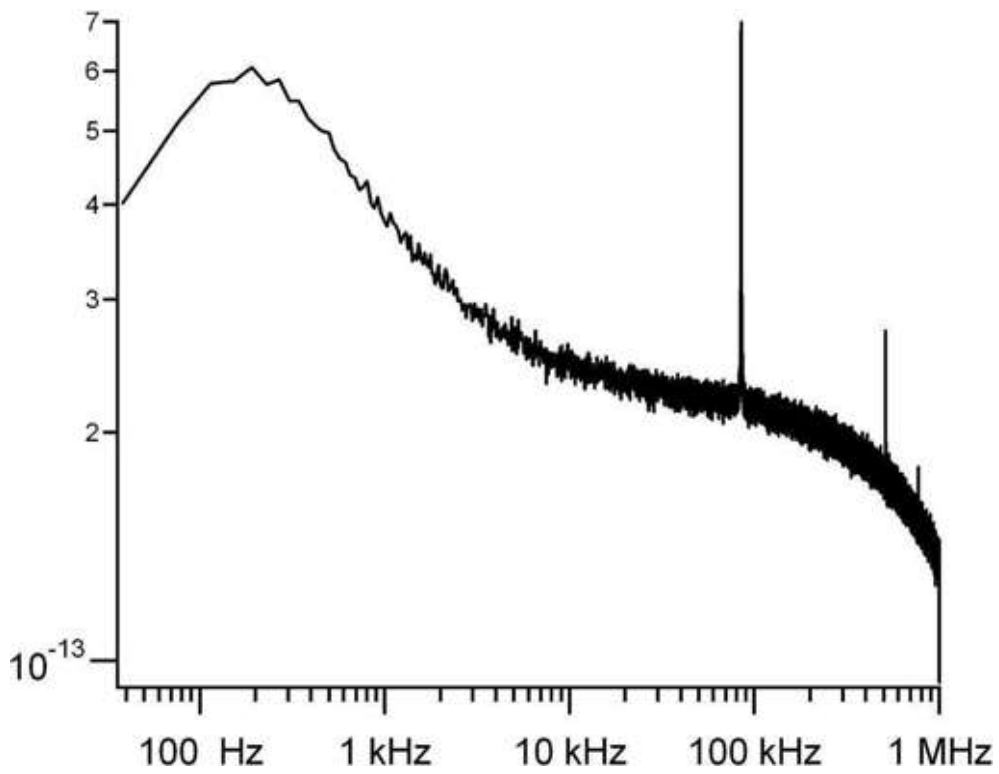


Figure 5.44 Thermal PSD Graph in Air

Chapter 6 AC Mode Imaging in Air

6.1 Overview

This chapter introduces the user to the basic set-up and operation for imaging in air in AC mode. As it is the first chapter to discuss the specifics of AC mode operation, it begins with the background information, proceeds to detailed instructions, and ends with frequently asked questions.

Theory

In AC mode atomic force microscopy, a cantilever is attached to an actuator, commonly a piezo. We will call this actuator the “shake” piezo. In response to voltage input, the shake piezo converts this electrical signal into an oscillatory motion that drives the base of the cantilever, causing it to oscillate in turn. Generally, the cantilever is excited near or below its fundamental oscillation frequency and in this frequency range, the Simple Harmonic Oscillation (SHO) model is a reasonable approximation of the cantilever motion.

In AC-mode the vibration amplitude will typically decrease when the tip is brought closer to the surface. The feedback loop of the MFP tries to hold the vibration amplitude constant by adjusting the Z piezo position.

Unlike contact mode imaging, the oscillating cantilever intermittently interacts with the surface, which helps reduce lateral (frictional) forces. This minimizes sample damage on soft samples (biological samples, polymers) and reduces tip wear on hard samples. Various other AFM modes like phase imaging, MFM, EFM, Kelvin Probe-Mode, ... are derived from AC mode imaging. These modes usually detect small changes in the oscillatory behavior of the cantilever by measuring small phase- or frequency variations (See [Cantilever Selection and Loading](#) on [page 126](#) for explanation of phase). The mechanical response of the cantilever can provide the user with useful information about the sample. Some information about the sample surface, such as the topography is relatively easy to extract. Other properties such as the elastic and dissipative moduli or adhesive properties can have less obvious effects on the cantilever motion.

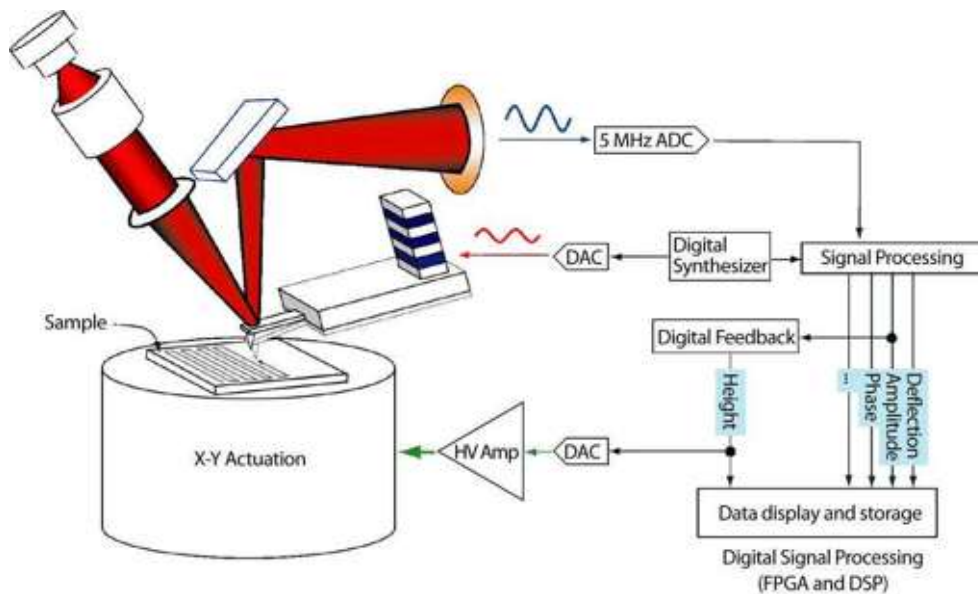


Figure 6.1 Basic Principle of AC Mode

The position detector converts the oscillating optical signal into a voltage that is in turn digitized into a number by the analog to digital converter. These deflection values are used by the FPGA and DSP to digitally calculate the amplitude, phase and other properties of the cantilever motion.

AC mode images are typically taken to measure the topography of a soft surface as the lateral interaction between the tip and the surface are greatly diminished as compared to contact mode imaging. AC mode imaging in liquid has similar advantages (See [Chapter 8](#) on [page 176](#)).

Prerequisites

It is advised that you review the following sections preparatory to beginning work:

[Moving on the Nanoscale: Piezo Actuators and the Nanopositioning System \(NPS™\)](#)page 55

Imaging BasicsSection 5.11 on page 107

Imaging FeedbackSection 117156064.0 on 102

General Set-Up and OperationChapter 5 on page 53

Cantilever Selection.....Section 5.6 on page 79*

Cantilever LoadingSection 5.3 on page 65*

*** Pay particular attention to these sections**

Tuning SectionSection 6.4 on page 129

Engaging SectionSection 6.7 on page 135

AC Mode Imaging in FluidChapter 8 on page 176

Terminology

Amplitude

This is the size of the oscillating signal. There are a number of common definitions for this value. The amplitude (A) is compared to the peak-to-peak amplitude (A_{p-p}) as seen in Figure 6.2. For a signal symmetric about mean, as is the case for a sine wave, the amplitude is $\frac{1}{2}$ of the magnitude of the peak-to-peak amplitude.

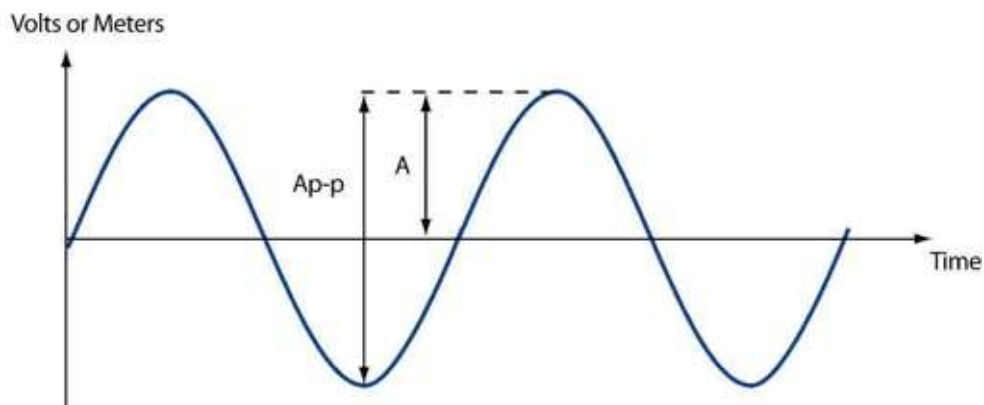


Figure 6.2 AmpDef. The amplitude, A , and peak-to-peak amplitude, A_{p-p} , for a sinusoidal signal

Another common definition of the amplitude is the rms or “root mean squared” amplitude. In units of meters, it is defined as:

Equation 6-1

$$A_{rms} = \sqrt{\sum_i z_i^2}$$

where z_i is the deflection of the cantilever. In this definition, any constant offset is included in the amplitude. Typically, we are interested in just the oscillating part of the signal, not in the constant offset. To make this calculation, we can simply subtract the constant value z_{const} inside the squared term:

Equation 6-2

$$A_{rms} = \sqrt{\sum_i (z_{const} - z_i)^2}.$$

This definition is usually referred to as the “standard deviation” but in the AFM world, it is commonly still called the rms amplitude.

One important note for the rms amplitude is that for a sinusoidal wave, the rms amplitude and amplitude are related by $A_{rms} = A / \sqrt{2}$. Equation 6-2 can also be used to show this relationship. However, this is not true in general. For a noisy sinusoidal wave, or for a square wave, for example, this relationship does not hold.

The amplitude of an oscillating signal can be in various units. On the MFP-3D™ this value is typically displayed in volts. To convert from voltage amplitude to meters, simply multiply the voltage amplitude by the AC InvOLS. For example, $A_{rms} (meters) = InvOLS \cdot A_{rms} (volts)$ or $A (meters) = InvOLS \cdot A (volts)$.

NOTE: Unless you have calibrated the InvOLS for the cantilever you are using, you cannot be confident of the value displayed in nm. See [InvOLS](#) on page 229 for the steps required to calibrate InvOLS.

Height

As with contact mode imaging, this is the voltage applied to the Z piezo in order to maintain a cantilever parameter (typically the amplitude) equal to the set point. The data are multiplied by -1, so that higher Z voltages (where Z piezo is further extended) will appear as lower points on the surface. This channel is scaled from voltage to distance with the ZPiezoSens ([Moving on the Nanoscale: Piezo Actuators and the Nanopositioning System \(NPS™\)](#) on page 55). This means that the height data collected will be uncertain to some degree, due to the hysteresis of a piezo response. Generally speaking, this means that height features of < 500nm are fairly accurate, but for height features larger than that, the Z sensor should be used. The noise on the Z sensor channel is small compared to the size of the features being viewed at this level. It is also important to know that the piezo response becomes more non-linear at the limits of its travel. Consequently, it is good practice to keep the Z voltage within 50-90 V if using the height channel.

Phase

The mechanical response of the cantilever can provide the user with information about the mechanical properties of the sample. One of the cantilever parameters available is the phase. The phase is simply a time lag (or lead) between the response and the drive sine waves, expressed in angular units (degrees or radians).

Free Amplitude (A_{free})

This is the amplitude of the cantilever oscillations when it is not damped by the surface, i.e. when it is away from the surface. All discussions of the set point are relative to free amplitude and it is set when you do your tune. If your lever is above the surface, then the amplitude displayed on the sum and deflection meter is your free amplitude.

Drive Frequency

This is the frequency of the drive wave to the cantilever piezo. Typically, the AutoTune software will select a resonance frequency that provides the largest response (i.e. the greatest free amplitude for any given drive signal). In air, this value corresponds to the natural resonance of the cantilever. In fluid, this value may or may not be the natural cantilever resonance since there is coupling between the liquid and the cantilever holder (or other microscope components).

Drive Amplitude

This is the amplitude of the drive signal that goes to the piezo in order to drive the cantilever for predetermined free amplitude like the cantilever amplitude; it is in units of volts but doesn't have any straightforward connection with the size of the signal from the optical detector. As the drive amplitude goes up, so should the cantilever amplitude. The drive amplitude will vary a great deal between air and fluid imaging. For example, silicon cantilevers in air could have a drive amplitude of tens of millivolts that gives a response amplitude of 1 volt from the optical detector. On the other hand, silicon nitride cantilever in fluid could require a drive amplitude of several volts to give an amplitude response of 1 volt.

Target Amplitude

This is the amplitude (in volts) that you would like to get out of the cantilever. When auto tuning the cantilever the Target amplitude will be reached by adjusting the Drive amplitude. When far from the surface, the Free amplitude equals the Target amplitude.

Auto Tune Low

This is the lower limit of the frequency range when auto tuning the cantilever.

Auto Tune High

This is the upper limit of the frequency range when auto tuning the cantilever.

Target Percent

This is the percent offset applied to the Drive frequency, typically set to a negative value (i.e. -5%) to ensure a gentler engage onto the surface.

Set Point

This is the amplitude value that the Z feedback loop will maintain as it scans the surface under the tip. It needs to be less than the value of the free amplitude, A_{free} . This means that the z feedback loop will move the piezo towards the surface, until the amplitude is damped by the surface to the value of the set point. Based upon the hardness of the surface, the adhesion, or other mechanical properties, the user must choose the set point before engaging the tip on the surface.

6.2 Cantilever Selection and Loading and Light Source Alignment.

Cantilever Selection and Loading

For AC imaging in air, it is preferable to use a silicon cantilever, such as the Olympus AC160 or AC240. For specific product information on silicon cantilevers, please see the Asylum Research Cantilever Guide, located under the Products category at: <http://www.asylumresearch.com/>

You may also download the Asylum Research Cantilever Price Guide in PDF format for a complete list of all cantilever models, available quantities, and pricing. The price guide may be downloaded from the same link referenced.

Please see [Cantilever Selection](#) on [page 79](#) and [Cantilever Loading](#) on [page 65](#) for more specific instructions on cantilever positioning in the holder.

Light Source Alignment on the Cantilever

The light source can be positioned onto the cantilever using either the IR card or the video. Refer to [Section 5.5](#) on [page 75](#), for more specific instructions.

6.3 Setting-Up the Software

Set Your Imaging Mode

When the MFP software is first started, the Imaging Mode, located in the Master Panel, automatically defaults to 'AC Mode.' (See [Figure 6.3](#)) Verify that the Imaging Mode has been properly set, especially if previously imaging in Contact mode.

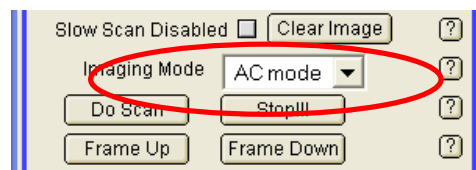


Figure 6.3 Setting the Imaging Mode in the Main Tab, located in the Master Panel

Select Your Data Channels

The default input channels are Height, Amplitude, and Phase ([Figure 6.4](#)). These are the typical channels that are collected when imaging in AC Mode. If your sample has features >500nm, it is also recommended to collect the Z sensor data. When imaging normally you have the capability to collect up to five data channels but special imaging modes (NAP mode, Dual AC, etc) can allow many more.

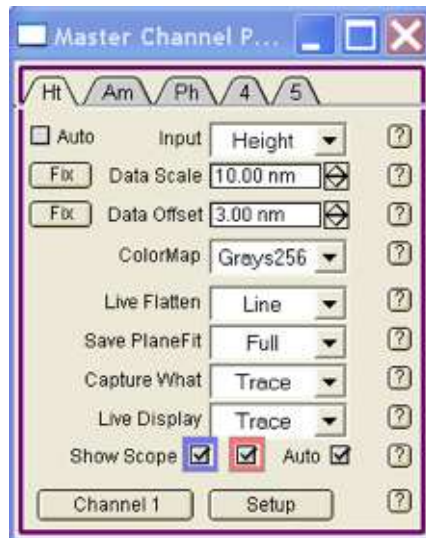


Figure 6.4 Data Channels in the Master Channel Panel

Set Primary Scan Parameters

Depending upon your sample, enter value for scan size and scan rate. The scan rate for many samples is typically 1Hz but you may find that for samples with larger features or for softer samples, a lower scan rate (0.25 – 0.75 Hz) may improve tracking and/or prevent damage to the sample.

When imaging a new sample, it is recommended to zero the X and Y offsets. Also, unless you need to scan your sample in a specific direction, keep the scan angle set to 0 degrees.

The Scan Points and Scan Lines can remain at 256 (default values) until the user prefers a higher resolution scan: the current range (as of October 2006) for Scan Lines is from 8 to 4096, in 8 line steps, and for Scan Points from 32 to 4096, in 32 point steps. Take note that selecting a greater number of pixels will generally increase the time required to capture an image.

Set Secondary Scan Parameters

Select a Set Point value less than the Free Amplitude (A_{free}). Typically, this value is approximately 80% of the Free Amplitude (at least for soft samples). For example, for a soft sample, choose a free amplitude equal to 1V and a set point equal to 0.8V (800mV).

Next enter values for the gains. Start with an Integral Gain of 10 and a Proportional Gain of 0: Both values are the default settings. During imaging, you

can increase the gain values until you notice ringing. At that point, you should back off a bit: This will help optimize the tracking and yield a better reproduction of the sample topography.

6.4 Tune the Cantilever

Once the light source has been properly aligned onto the cantilever and the head has been placed onto the stage, lower the head until the cantilever is 1-2 millimeters (mm) above the sample surface. Tune the cantilever by clicking on the Tune tab, which is the last tab in the Master panel. (See [Figure 6.5](#))

Tuning

When working in this mode the MFP-3D™ system must be tuned to the cantilever you are using. To use Tune, you must set the low and high frequencies to create a range for your lever.

1. In the Master Panel, click the Tune tab (See [Figure 6.5](#)).
2. Select your target amplitude, and enter the target percent. If you want to drive the lever at a frequency less than the resonant frequency, enter a negative number.

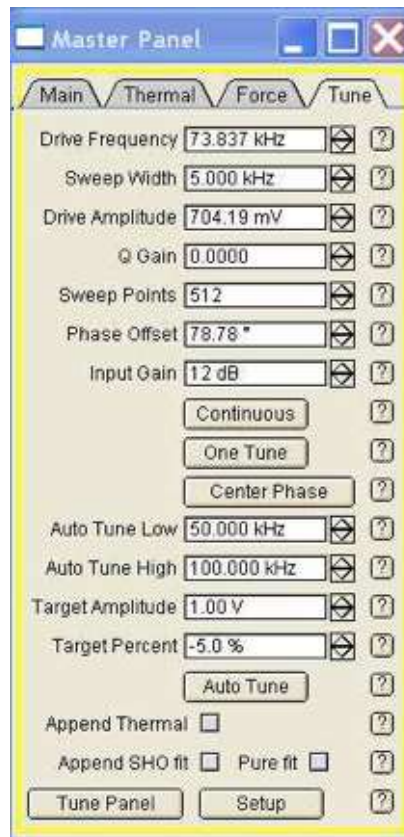


Figure 6.5 Master Panel, Tune

3. Take the resonance frequency obtained from the Thermal PSD, and enter 30kHz higher than that value for the Auto Tune High (See Figure 6.5).
4. Take the resonance frequency obtained from the Thermal PSD, and enter 30kHz lower for the Auto Tune Low (See Figure 6.5).
5. Enter the Target Amplitude (See Figure 6.5).
6. The target percent offsets the drive frequency relative to the resonance frequency. Entering a target amplitude of 1V and a target percentage of -5% will drive the tapping piezo to get 1.05 V of amplitude at resonance, and will drive the tip at a frequency less (due to the sign of the target percentage) than the resonance, resulting in 1 V of amplitude. For more details click on the ? buttons to the right of the controls on the Tune panel.
7. Click 'AutoTune'
8. Confirm that the phase goes to 90° at the resonance frequency.

NOTE: If you wish to bring the plots together, click the 'Append Thermal' box.

6.5 Sample Preparation and Loading

The MFP-3D™ systems give a bottom-up view of the sample and cantilever through a transparent sample support such as a microscope slide, Petri dish, or cover slip. In most cases, it is best to perform force measurements under fluid. In air, most samples are covered with a thin layer of condensed water and other contaminants. As a result, the flexible cantilever can be damaged when the tip encounters the high surface tension due to these contaminants. Submerging the cantilever in liquid greatly reduces the effects of these high forces.

However, working in fluid is an advanced function. You should practice and feel comfortable working in air before moving to the more difficult experiment of working in fluid. Provided are instructions for working in both air and liquid. For more details on the procedures, please see the chapters specific to each mode.

Load the Sample in Air

1. Turn the thumb wheel clockwise to raise the cantilever. Remove the head from the system and set it aside.
2. Place the sample slide on the MFP-3D™ and center the slide. The first step for positioning the sample is to properly load it on to the scanner sample plate. This typically makes use of the small magnets to clamp your sample (on a glass microscope slide or one of a large variety of specialized holders) (See [Figure 6.6](#)). Following this rough positioning step, you can use the built in micrometers to adjust both the xy position of the sample and the xy position of the viewing optics.

A For bottom-view systems:

Turn the large knobs to adjust the optics position.

Turn the small knobs to adjust the scanner position.

B For top-view systems:

Turn the small knobs to adjust the sample position.

If necessary, move the mirror using the X and Y head knobs. This moves the field of view and allows you to re-center the image.

3. Place the magnets (2) to secure the slide to the system.
4. Replace the head, verifying the legs are seated in the base indentures as follows:

Hold up the front end and place the back legs first.

Watch the clearance between the tip and surface while slowly lowering the front end.

If it appears that the tip will hit the surface before the front leg reaches the base, extend the front leg.

5. Using a bubble level, verify that the head is level to the scanner and sample.
6. If using a bottom view system, your sample is transparent, and the video is not enabled, select 'Window/Video' to open the video window to use while positioning the sample.



Figure 6.6 Sample Positioning

7. In the Master Panel, click the 'Main' tab. Enter the set point.

In AC mode, enter a set point slightly less than the amplitude

In contact mode, enter a set point higher than the deflection

| | |
|--------------|---|
| NOTE: | Some cantilevers will experience drift in their deflection value for a few minutes after loading. You may need to correct for this by |
|--------------|---|

re-zeroing the deflection using the PD knob. If the drift persists, contact support@asylumresearch.com.

8. In the Sum and Deflection window, click 'Simple Engage' (See [Figure 6.7](#) on page 133).

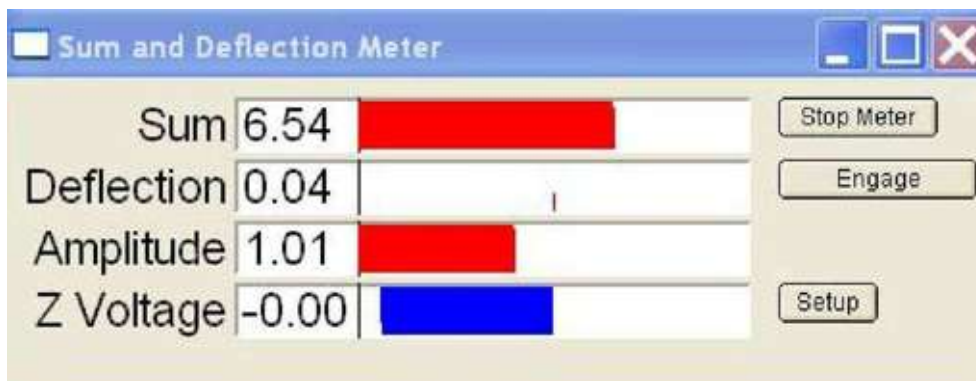


Figure 6.7 Sum and Deflection Window*

9. Turn the head thumb wheel counter-clockwise to lower the cantilever to the surface. Continue until you hear an audible beep, or the amplitude changes from the pre-engage amplitude to the setpoint amplitude. Continue until the deflection changes from 0 to the set point deflection.

ATTENTION: If the Sum drops more than 50%, you have crashed the tip. Time to replace it and try again!

NOTE: The system beeps when the feedback determines the Z voltage at <130V, which communicates the tip is engaged.

10. Continue to lower the head until the Z voltage is 70V (at center range).
11. Lower the set point until the hard stop or until the Z voltage does not change much.

NOTE: One method for testing how well the cantilever is engaged is to type in small variations in the setpoint. If you observe large changes in voltage it probably means that you have a “false

engage.” This occurs when the air damping rather than the sample damping has reduced the cantilever amplitude to the setpoint.

12. At the hard stop, turn the front head knob counter-clockwise until the Z voltage is $\sim 70V$.

6.6 Software and Hardware Initialization

Initiate the Software

1. Double-click the MFP-3D™ software icon.



Figure 6.8 MFP-3D™ Icon

2. Using the Master Panel tabs, select Imaging Mode, Contact Mode or AC Mode.
3. Select the channels you wish to view from the five available channels in the Master Channel Panel.

Initiate the Hardware

6.7 Engaging

After loading the sample, entering the default values and tuning the system, you are ready to engage.

As the system starts scanning, it opens the channels you selected (See Section 6.3, [Select Your Data Channels on page127](#))

1. In the Master Panel, click 'Do Scan'

| | |
|---------------------|---|
| <u>NOTE:</u> | 'Do Scan' moves to the top or the bottom of the frame (whichever is closer) and then begins the rendering. You can also use 'Frame Up' or "Frame Down' to begin the rendering either at the bottom or the top of the image. |
|---------------------|---|

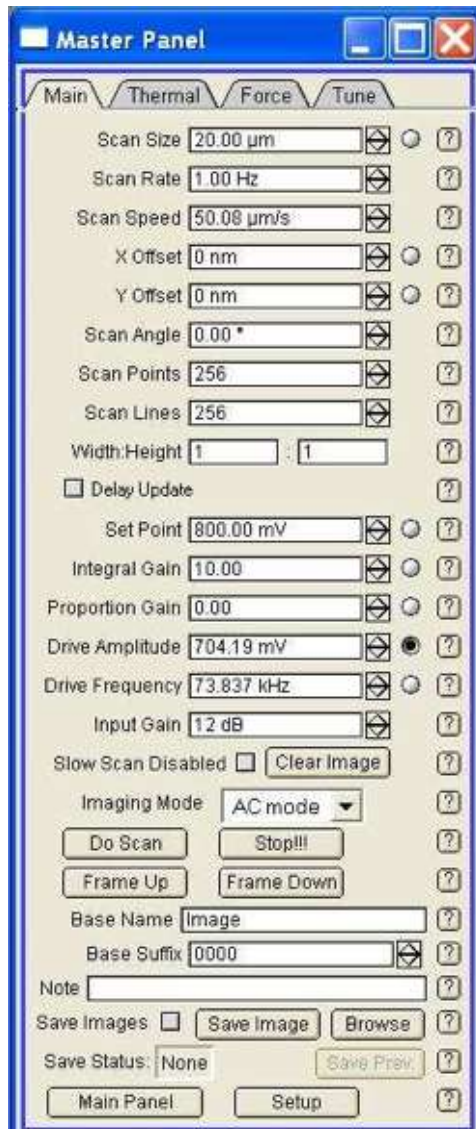


Figure 6.9 Master Panel, Main

2. As the system engages, verify that the channels you selected above.
If a picture does not come up in one of the windows, return to the Master Channel Panel and re-enter your channels.
3. You may adjust the values as the system scans and the changes will reflect immediately (except for the angle, points and line and scan rate).

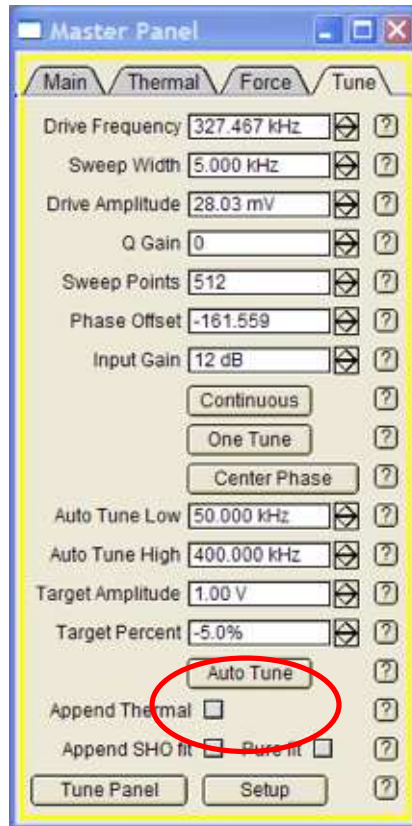


Figure 6.10 Tune Tab, located in the Master Panel

For AC Mode imaging in air, the user can auto tune the cantilever. First, verify that the resonant frequency of your cantilever is between the Auto Tune Low and Auto Tune High parameters. The default values for these parameters are 50KHz and 400 KHz respectively. For example, for Olympus AC160s, the resonant frequency is approximately 325 KHz. Since this value falls between the default settings, the user doesn't need to change the parameter values.

Second, verify that the Target (Free) Amplitude is set to the desired value. The default value is 1V and this is typically the preferred setting.

Finally, click on the 'Auto Tune' button. The 'Cantilever Tune' window will appear and the software will look for the cantilever resonance frequency between the Low and High Tune values. Once it has located the peak, you will hear a beep and the curve will be displayed with the phase centered at 90 degrees (See [Figure 6.11](#)) The Drive amplitude will also be pre-selected to a voltage value that is necessary to reach the Target amplitude. Please note that the Amplitude value

located in the ‘Sum and Deflection Meter’ panel is the same as the Target (Free) Amplitude in the Tune panel once the user has autotuned the cantilever and the tip is away from the sample surface.

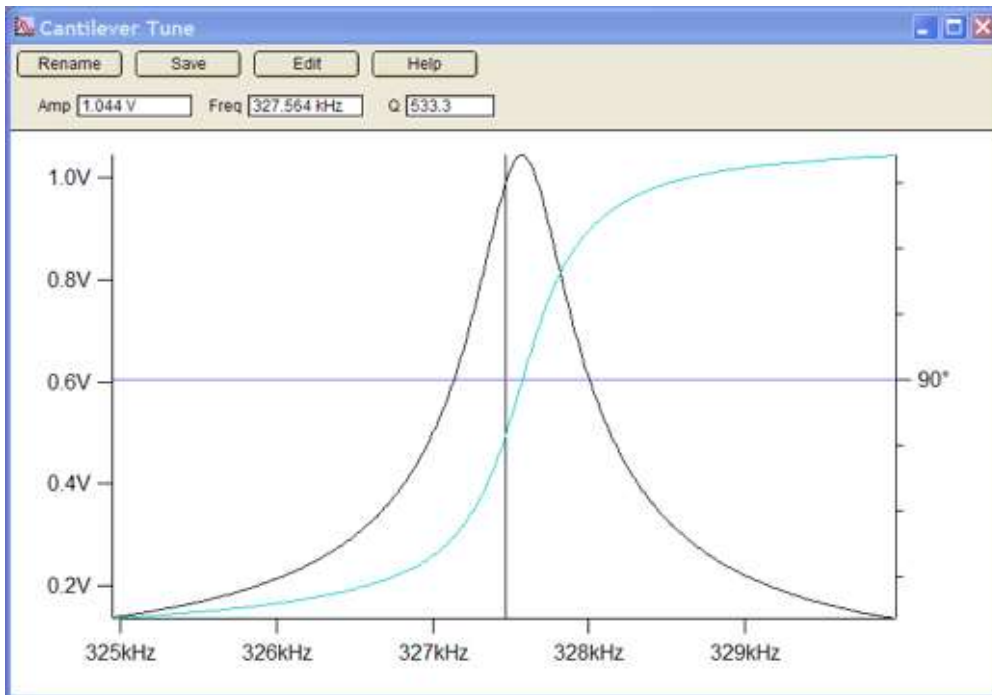


Figure 6.11 Cantilever Tune Window. Typical tune for the Olympus AC160 cantilever

Approach the Surface

Begin your engage process by clicking the ‘Simple Engage’ button located in the ‘Sum and Deflection Meter’ panel, to turn on the feedback loop (Figure 6.7 on page 133) You should see the red bar graph at its maximum value of 150V as the Z feedback loop attempts to match the chosen set point by extending the Z-piezo voltage towards the surface. In other words, turning on the feedback loop tells the MFP-3D™ controller to use the Z-piezo to decrease the amplitude to reach the set point. At first, since the head is far above the surface, the piezo does its best by extending completely to its maximum value of 150 Volts. There should be little or no effect on the amplitude.

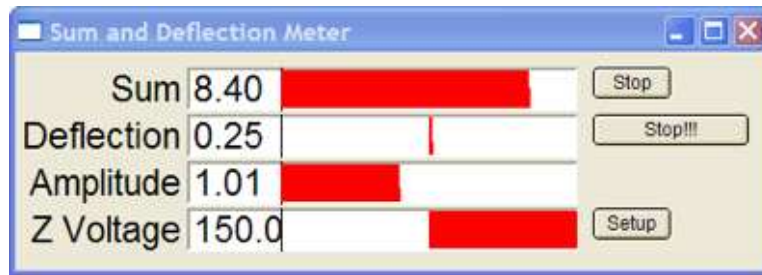


Figure 6.12 SUM and Deflection Meter; the Z voltage is at its maximum, the Z piezo is fully extended.

Finally, lower the head by turning the large front thumbwheel counter-clockwise. As the tip begins to approach the surface, you will notice the amplitude (in the Sum and Deflection Meter panel) decreasing. When the amplitude reaches the chosen set point, the computer will beep an alert that the tip is on the sample surface. The Z-piezo voltage indicator slightly decreases from its maximum value of 150V.

Continue turning the thumbwheel (and lowering the head) further until this value is about 70 V (See [Figure 6.13](#)). At this value, the Z-piezo voltage bar should be in the middle, which allows for the greatest range of motion in both the positive and negative directions.

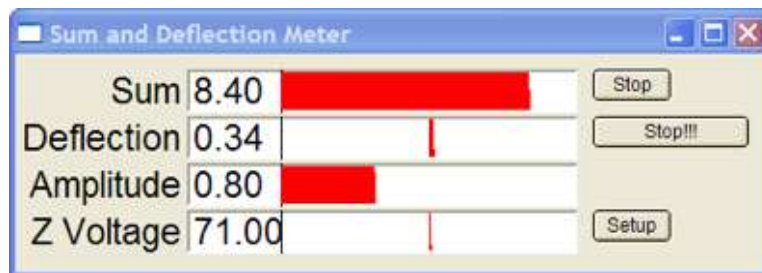


Figure 6.13 Sum and Deflection Meter; Z voltage is centered

6.8 Start Scanning

Once your tip is on the sample surface and you have entered the desired primary scan parameters (Section 6.3, [Set Primary Scan Parameters](#) on [page 128](#)) begin imaging by clicking on the 'Do Scan' button ([Figure 6.9](#) on [page 136](#)). You can

also specifically select 'Frame Up' or 'Frame Down' if you prefer, to begin from either the bottom or the top of the scan image.

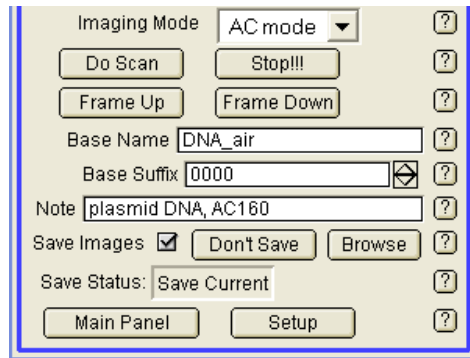


Figure 6.14 Initiating the Scan and Saving Images

Optimize Scan Parameters

When first initiating a scan, the image quality may not be optimal. Improve the image by changing the parameters, typically in the following order:

Setpoint

It is important to play with this parameter a lot. If the setpoint is too low, the tip may be pushing too hard on the surface. If the setpoint is too high, the tip may not be tracking the surface. One way to ensure that the setpoint corresponds to the lighter force is to increase the setpoint until the tip comes to the surface. The image will look blurry and the cross-section graph will show that the trace and retrace curves do not overlap. Then you can decrease the setpoint until the image looks fine on the screen again. At that point, you know that you are most likely imaging with the smallest possible vertical force.

Gains

Bear in mind that the Integral gain, or IG, is usually more effective. Gains are important because they are responsible for the Z response of the cantilever. For example, if the gains are too low, the tip will not track the surface very well (as can be observed at the cross-section graph at the bottom of the height image, when the trace and retrace curves do not overlap). Maximize the gain value until you see some ripples on the amplitude image. Then lower the gain just enough to see the ripples disappear.

Start with the Proportional Gain, or PG, at zero. Many users leave the PG at zero for convenience. In some cases it is possible to get enhanced performance with a nonzero value of the PG, typically less than 1/10 of the IG value.

Drive Amplitude

The drive amplitude parameter can be treated like the setpoint. If you think you are not tracking the surface very well, try to increase the drive amplitude. Sometimes it just works better then decreasing the set point, especially if the tip is sticky to the surface. Increasing the drive amplitude increases the vertical oscillation amplitude of the cantilever. Conversely, you can decrease the drive amplitude if you think the tip may be pushing too hard on the surface.

Scan Rate

A good starting value for the Scan Rate is 1 Hz. As you go to large scan sizes you should decrease the scan rate to maintain good tracking. For very soft and large-featured samples, you may need to go more slowly. If you have selected a large number of points per line (> 512) then you may need to scan slowly to achieve good feedback. A few samples, such as gels with sticky surfaces, may need to be scanned faster than 1 Hz.

Scan Angle

This parameter is less crucial, but users need to think about it when the image is not satisfactory. For example, if there is some friction on the surface, changing the scan angle may help, as the bending of the cantilever will change. Also, if there a pattern of long features running parallel to the axis of the cantilever, it is highly recommended to scan at a 90° angle, so that the tip encounters the features perpendicularly.

Tip geometry also interacts with Scan Angle. Cantilevers type AC240 and AC160 from Olympus have a very steep leading edge. If this collides with a steep slope on the sample, you may observe a sawtooth instability. This is minimized by scanning at 90°.

Of the five parameters listed however, the first two: Setpoint and Gains, are the most important for imaging.

6.9 Saving Your Images

By using the Master Channel Panel, you can choose what data you want to save and display. Typical channels for AC mode are:

1. Height

2. Amplitude
3. Phase
4. Z sensor, for surfaces with tall or deep features
5. To save the pre-selected channels, give your files a 'Base Name.' (See [Figure 6.9](#) on [page 136](#)) The software will automatically assign the Base Suffix, typically beginning with '0000.' Click on the 'Save Images' box and select a directory in which to save your files: When the current image is being saved 'Save Current' will appear next to the 'Save Status' parameter.

6.10 Advanced Topics: Phase and Digital Q Control

Phase

Overview

The phase is basically the time lag between the response of the cantilever and the drive sine waves sent to the shake piezo. The phase of a signal only makes sense in terms of a reference to another oscillating signal. In [Figure 6.15](#) when the signals match up (black sinusoid), they are said to be "in phase", with phase difference of 0° or 0 radians. If the signal differs by half a period (compared to the reference signal blue sinusoid), they are "out of phase", with a phase difference of 180° or π radians. If the response curve crosses zero after the drive or reference curve, we say that the phase of the response curve is "lagging" the drive. If it is ahead of the drive, we would say it is "leading" the drive. The response of the cantilever generally lags the drive, so unless otherwise specified, phase shifts are always "phase lags".

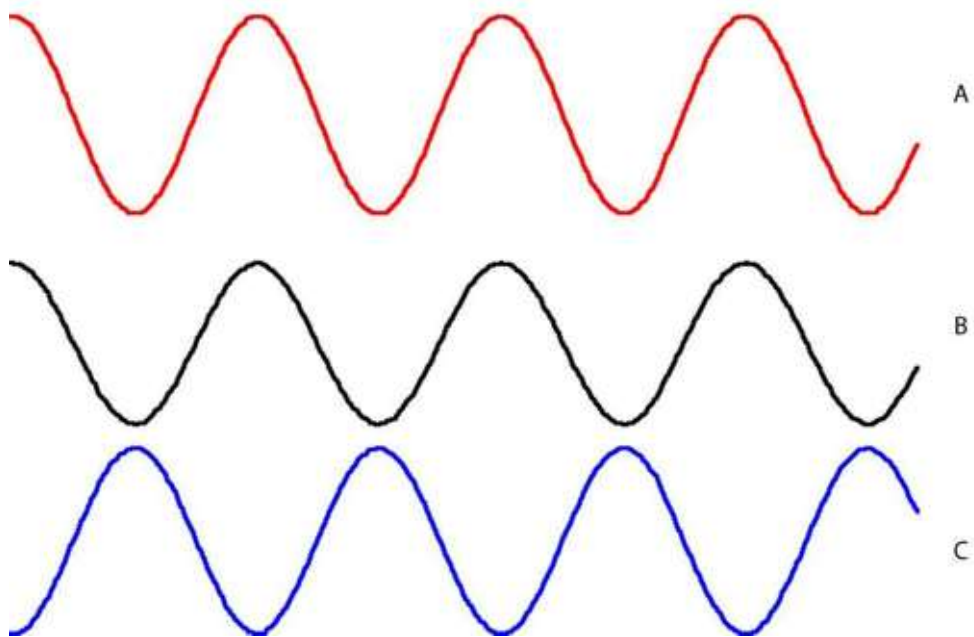


Figure 6.15 In and Out Of Phase. A, the red curve, represents the reference wave. B, the black curve, is “in phase” ($\theta = 0^\circ$) with the reference while the blue curve, C, is “out of phase” ($\theta = 180^\circ$)

Quantifying the Phase Shift

The shake piezo is driven with an oscillating electrical signal

Equation 6-3

$$V_d = A_d \sin(2\pi ft)$$

in which A_d is the amplitude of the drive and f is the drive frequency.

The position detector generates a voltage representing the oscillating light in the form

Equation 6-4

$$V_{resp} = A_{resp} \sin(2\pi ft + \theta)$$

where A_{resp} is the amplitude, f is the frequency and θ is the phase. In this expression it is measured in radians. In AFM applications, it is commonly expressed in degrees:

Equation 6-5

$$\theta_{deg} = (180 / \pi) \theta_{rad}$$

The period of an oscillator (the time to complete a single cycle) is the reciprocal of the frequency $T=1/f$.

In this example, even though the drive (V_d) and the response (V_{resp}) have the same frequency, they differ by a phase shift (θ) that appears as an argument in the sine function.

Note that the phase is periodic, that is to say that the sine wave of a signal with frequency F and phase 0° is indistinguishable from the sine wave of a signal with frequency F and phase $\pm 360^\circ$ or $\pm 2\pi$ radians. The second signal corresponds to a one period phase shift (+ = lead; - = lag).

Because the oscillations occur as a function of time, the phase shift can also be described in terms of a time lead or lag. The relationship between the phase angle (in radians) is given by

Equation 6-6

$$\theta = 2\pi \left(\frac{\Delta t}{T} \right) \text{radians}$$

or in degrees by

Equation 6-7

$$\theta = 360\left(\frac{\Delta t}{T}\right)^\circ$$

In the example plotted in **Figure 6.16**, a reference wave is plotted in solid black and a response is plotted as a dashed blue curve. The frequency of each sine wave is $f = 74\text{kHz}$, corresponding to a period of $T = 1/7400\text{Hz} = 13.51 \mu\text{sec}$. The response wave is shifted in time by $\Delta t = 1.13 \mu\text{sec}$. Using the expressions above, this corresponds to a phase shift of $\Delta\theta \approx 30^\circ = \pi/6$ radians. Because the response curve crosses zero after the drive or reference curve, the phase of the response curve is “lagging” the drive.

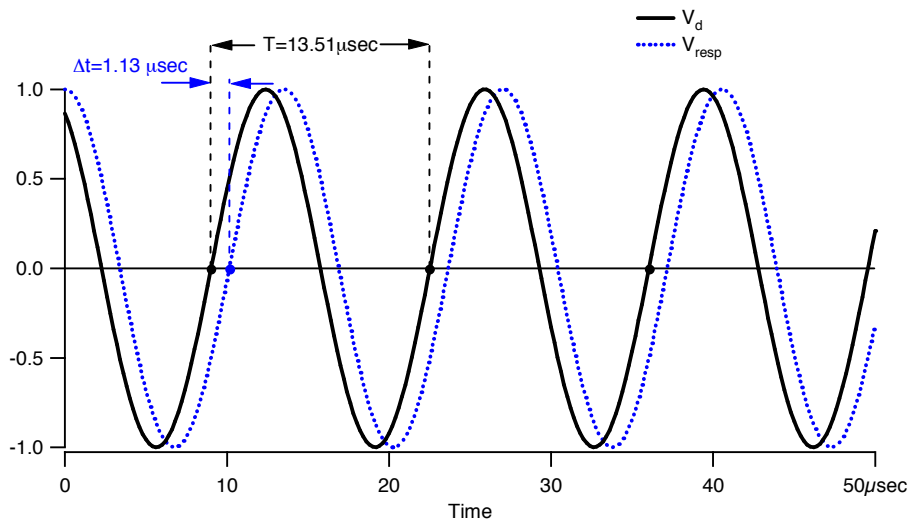


Figure 6.16 Lagging Response Curve.

The two sine curves share the same frequency. The blue curve, representing the position detector’s voltage conversion of the cantilever’s oscillation, lags in time by Δt or $1.13 \mu\text{-seconds}$. This corresponds to a phase shift of $\Delta\theta \approx 30^\circ = \pi/6$ radians.

Typically, cantilever motion is simplified into a one-dimensional problem. In this simplified case, the equation of motion for the transverse (perpendicular to the specimen surface) position w of the cantilever is a fourth order, time dependent partial differential equation given by

Equation 6-8

$$\text{(Cant diff eq)} \quad EI \frac{\partial^4 w(x,t)}{\partial x^4} + \mu \frac{\partial^2 w(x,t)}{\partial t^2} = F_d(x,t)$$

Where $w(x, t)$ is the transverse displacement of the cantilever beam, E is the elastic or Young's modulus of the cantilever, I is the moment of inertia, μ is the mass per unit length and $F_d(x,t)$ is the force acting on the cantilever per unit length.

Solutions to this equation are not trivial and a considerably simpler approach is to approximate the extended cantilever as a simple point mass on a spring. This Simple Harmonic Oscillator (SHO) model provides useful insight into the behavior of cantilever dynamics, at least through the first oscillation mode. [Figure 6.17](#) shows a schematic of a driven SHO. In this figure, the base supports a mass, m , with a spring, k . Motion is damped, indicated in the figure with a dashpot, b .

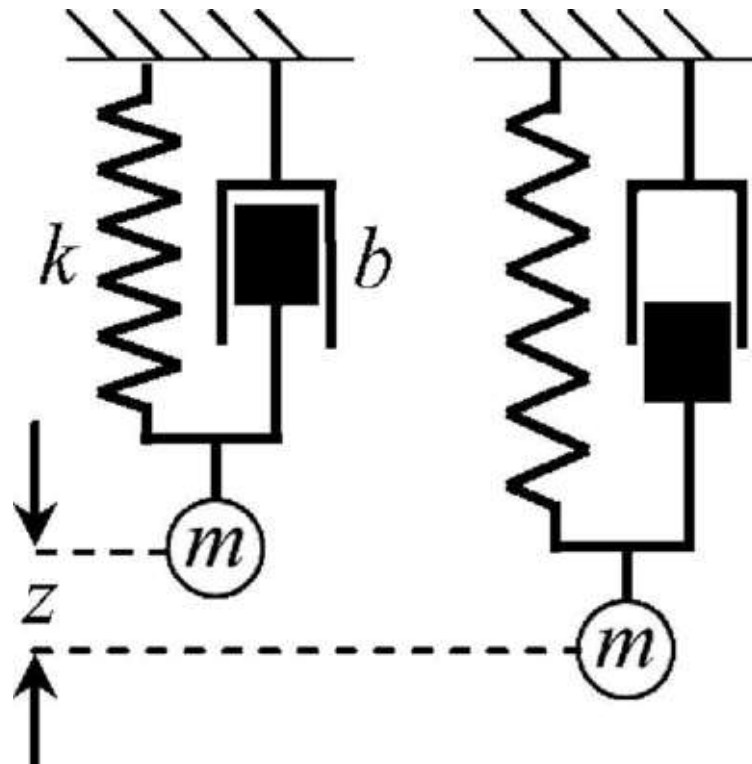


Figure 6.17 SHO Diagram. Simple Harmonic Oscillator model. A mass, m , is attached to an oscillating base through a spring with compliance, k , and a dashpot with dissipation, b . The mass responds with an amplitude and phase that depends on the driving frequency

Generally, the cantilever in an AFM operated in air or vacuum is excited near or below its fundamental oscillation frequency and in this frequency range, the SHO model is a reasonable approximation. Also, AC operation of the instrument allows the use of a variety of AC techniques to extract the signal from the noise and moves the measurement away from the ubiquitous $1/f$, or low frequency noise, present in all practical experimental environments. Operation in fluid is more complicated and will be covered in Chapters 8 and 9.

Conservative interactions, primarily through the force gradient, shift the resonant frequency of the cantilever. This shift in resonance can be measured in a number of ways, some of which will be described below. In addition, information on non-conservative interactions between the oscillating tip and sample can be extracted.

From an elementary physics text[†], the differential equation for the motion is that of the more familiar damped harmonic oscillator expression

Equation 6-9

$$\text{(SHO diff eq)} \quad m\ddot{z} = F_{drive} - kz - b\dot{z} + F_{tip}(z),$$

where $z = w(L, t)$ if the tip of the cantilever is at position $x = L$, k is the spring constant, $F_{tip}(z)$ is the force acting between the tip and sample, m is the effective mass of the cantilever, ω_0 is the angular resonant frequency, and b is the viscous damping. For the case when $F_{tip}(z)$ is independent of z or constant, then the zero point is shifted and the oscillatory motion is identical to the oscillator without $F_{tip}(z)$, i.e., just like a mass and spring system on a frictionless horizontal surface and when suspended in a gravitational field. If, however, the force gradient, $dF_{tip}(z)/dz$, is non-zero then we have an effective spring constant given by

Equation 6-10

$$\text{(keff eq)} \quad k_{eff} = k - dF_{tip}(z)/dz.$$

[†] Vibrations and Waves. A.P. French. W.W. Norton & Company, New York.

If we drive the system with an external force $F_{drive} = kA_{drive} \cos\omega t$, then eq. (SHO diff eq) has a steady state solution of the form

Equation 6-11

$$\text{(SHO SS Solution)} \quad z = A(\omega) \cos[\omega t + \varphi(\omega)]$$

where the amplitude $A(\omega)$ and the phase $\varphi(\omega)$ are given by the expressions

Equation 6-12

$$\text{(SHO Amp)} \quad A(\omega) = \frac{A_{drive} \omega_0^2}{\left[(\omega_0^2 - \omega^2)^2 + \frac{\omega_0^2 \omega^2}{Q^2} \right]}$$

and

Equation 6-13

$$\text{(SHO phase)} \quad \tan \varphi(\omega) = Q(\omega / \omega_0 - \omega_0 / \omega).$$

In these expressions ω_0 is the resonant frequency determined by the spring constant and mass, $\omega_0^2 = k/m$, and $Q = m\omega_0/b$ is the quality factor.

As the expression for the resonant frequency shows, attractive interactions lower the resonant frequency while repulsive interactions raise the resonant frequency.

The expressions for $A(\omega)$ and $\varphi(\omega)$ are plotted in the vicinity of the resonant frequency in [Figure 6.18](#). This figure also shows the effect of the cantilever interacting with both attractive and repulsive force gradients. These small force gradients shift the amplitude and phase curves, as shown by the dashed lines. These dashed curves are the origin of some of the terminology common in AC AFM imaging. For a repulsive interaction, the phase shift is negative and for an attractive interaction the phase shift is positive. In most real experimental situations, there will be both attractive and repulsive interactions between the tip and the sample. The sign of the phase shift allows us to determine which force is dominating. However, it does not tell us anything about the absolute magnitude of those forces, just which one dominates.

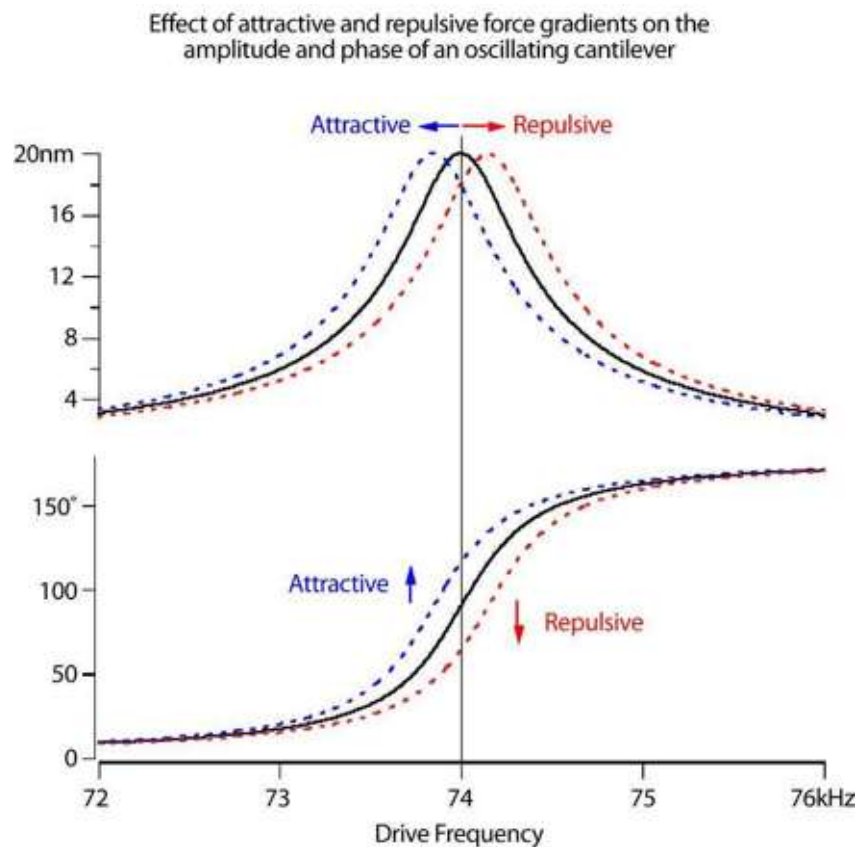


Figure 6.18 Resonance Curves. Calculated response curves of the cantilever amplitude and phase using equations SHO Amp and SHO phase

By varying operating parameters of the AFM, it is possible to control whether the cantilever operates in attractive or repulsive mode. Figure 6.19 shows how phase and dissipation responds as a function of amplitude and Z position. By changing the amplitude and z-position of the cantilever, the cantilever will either experience overall attractive interactions (positive phase shifts) or repulsive interactions (negative phase shifts). In Figure 6.20 of Don Quixote, the operating parameters have been adjusted so that the cantilever switches between attractive mode (positive phase shifts) over the flat regions of the sample to repulsive mode (negative phase shifts) over the hilly regions made by the scratch lithography. The sections made of the height (red) and phase (blue) illustrate the correlation. Even though there are large changes in phase between the regions, we expect that they are due only to topographic effects; the polycarbonate material in both locations should be the same.

Amplitude, Phase and Dissipation vs. Z Position
 AC-240 Cantilever above freshly cleaved mica in air

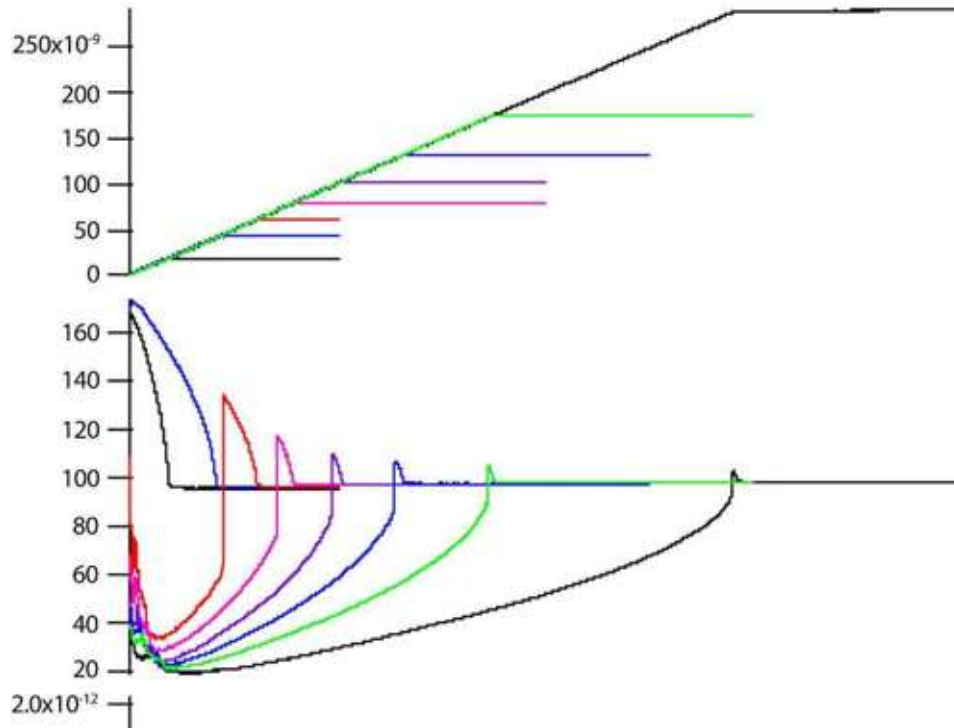


Figure 6.19 Attractive and Repulsive Regimes

Figure 6.19 shows the effects of amplitude on the phase shifts. At small amplitudes, the interactions are predominantly attractive. As the amplitude increases, repulsive interactions become more likely.

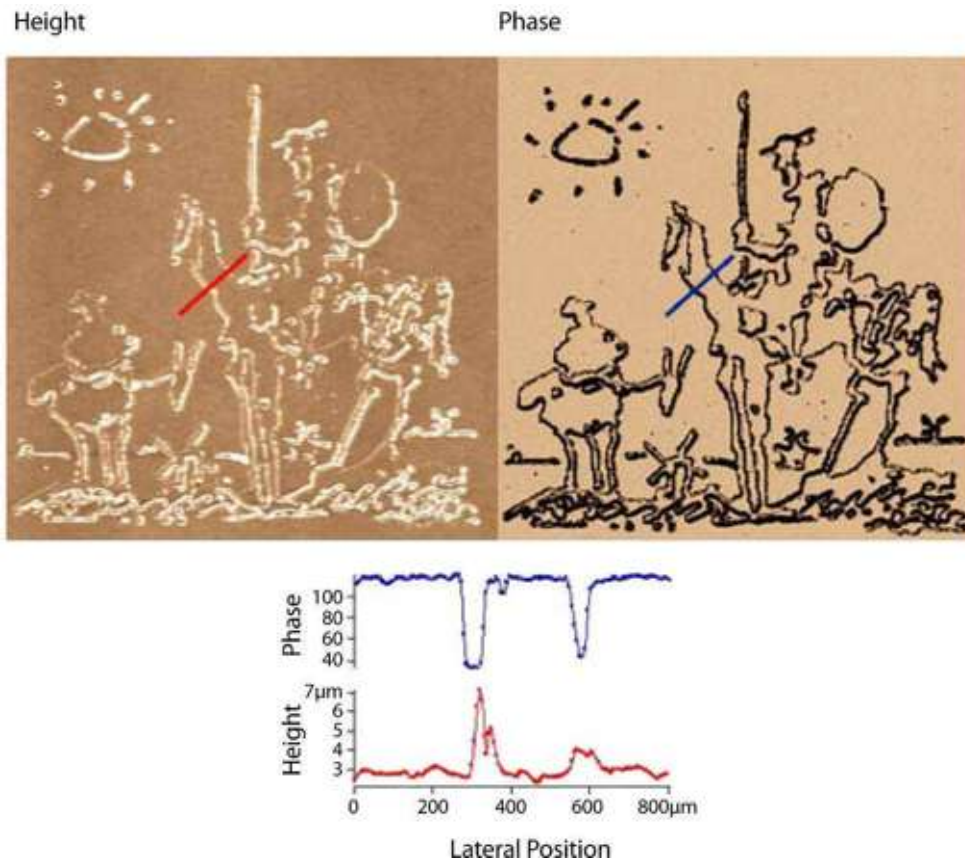


Figure 6.20 Don Quixote. Height and phase image of a Don Quixote lithography on a polycarbonate surface. Sharp surfaces increase the likelihood of repulsive interactions. This gives a large negative phase contrast over the etched ridges in the polycarbonate sample.

Damping has a direct effect on the response speed of the cantilever sensor. In addition to the steady state solution SHO SS Solution (Equation 6-11), there is a transient solution to the equation of motion given by

Equation 6-14

$$(\text{SHO Trans Solution}) \quad z_t = A_t e^{-\omega_0 t / 2Q} \sin(\omega_0 t + \varphi_t).$$

One main feature of this equation is it reaches $1/e$ of its initial amplitude after $2Q$ cycles. Put another way, this transient has a relaxation time $\tau = 2Q/\omega_0$. This implies

a higher Q requires the AFM to scan more slowly for the motion to relax into the steady state solution. For typical MFM cantilevers operated in ambient conditions the Q is ~ 150 and with a resonant frequency of ~ 70 kHz, the time constant is $\tau \sim 0.7$ ms. This is, in general, sufficiently small to raster at a scan line frequency of 1 Hz or so. This acquisition speed translates into a time of roughly 9 minutes for a 256×256 pixels image. If the same cantilever was operated in a vacuum environment, however, its Q would approach something like 104, and the time constant would become $2 \sim 45$ ms, increasing time to acquire a 256×256 image to something on the order of 5.5 hours.

Figure 6.21 shows improved phase contrast of a polymer sample with the implementation of Q-control. However, for samples with greater height features, as with the calibration grating seen in Figure 6.22 higher Q results in poor tracking due to a slower response time.

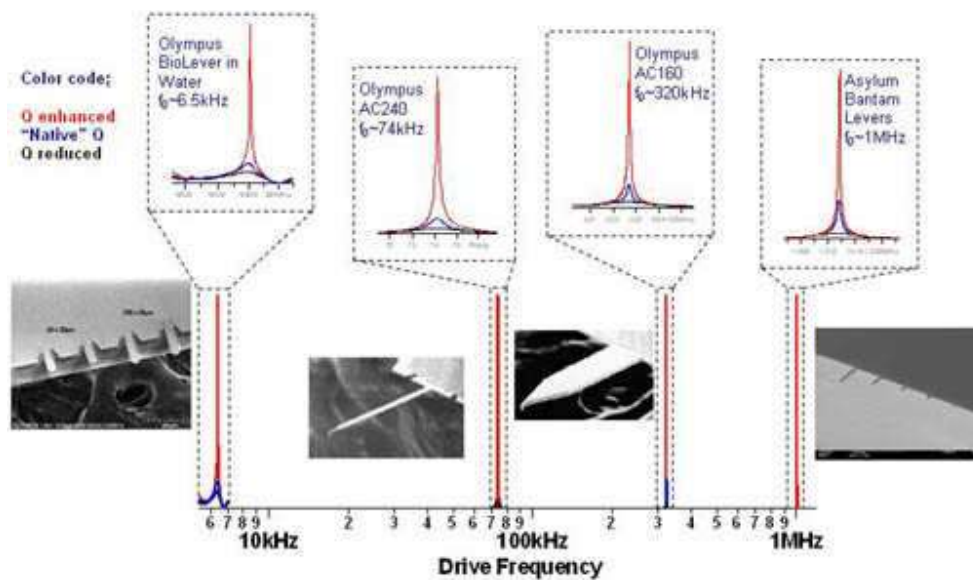


Figure 6.21 Q-Control. Comparison of resonance frequencies with and without Q-control of four cantilevers

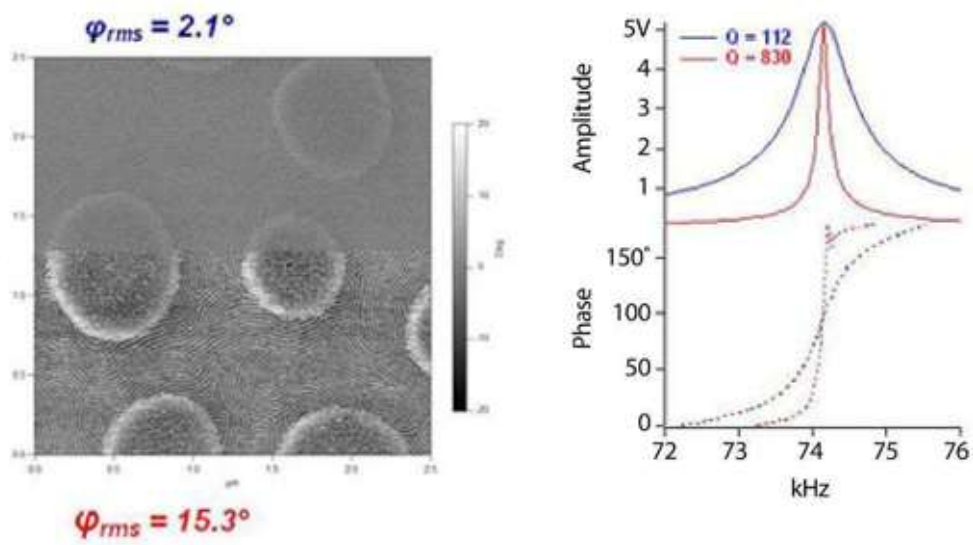


Figure 6.22 SEBS Polymer sample, with and without Q-control

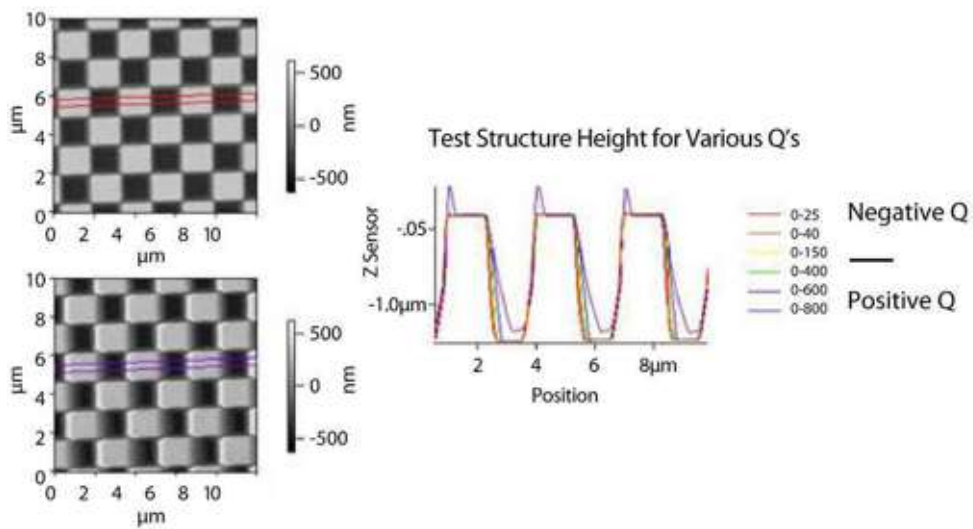


Figure 6.23 Response Time versus Q.

Digital Q Control

The quality factor, known as “Q,” is a measurement of the rate at which energy is dissipated from a resonance system. Important to AC mode imaging, “Q” is associated with resonance in that everything has a resonance frequency, and each resonance has its associated “Q” measurement. Therefore the response of a cantilever can be modified by adjusting the Q gain parameter, located in the Tune tab of the Master panel.

With Q-control, the phase of the cantilever oscillation is monitored and used as a feedback signal on the drive amplitude of the oscillation piezo. In this, it is similar to Force Modulation AFM, or FM-AFM. Q-control uses the oscillation actuator to simulate an increase or decrease in the damping forces acting on the cantilever. This changes the effective time constant of the cantilever, allowing faster response but lower sensitivity, or slower response and higher sensitivity. In other words, Q-control allows one to change the natural averaging time of the cantilever. On the MFP-3D™, Q-control is accomplished with digital computations inside the controller. Positive gains will increase the Q-value of the cantilever, while negative gains will decrease it. For most cantilevers, there is a practical limit of roughly 10x for either increasing or decreasing the Q. In [Figure 6.21](#) four flavors of cantilevers are being controlled, ranging from a very soft, ~6.6kHz resonant frequency Biolever in fluid up to a very high frequency, ~1MHz small cantilever in air.

Q-Control allows imaging to be optimized for various applications, giving increased sensitivity for certain measurements. Some of the advantages of imaging with Q Control are:

Flexibility, low noise and frequency independence due to the all-digital operation

Longer stays in the attractive (or repulsive) mode, even with a sharp tip and/or large amplitudes

Minimized sample and tip damage through reduction of transients

Less traumatic imaging for soft samples

Digital Q Control is now standard on all MFP-3D™ systems, and is also available as a simple software upgrade for MFP-3D™ owners. For more details, contact: support@AsylumResearch.com.

AC mode Frequently Asked Questions

Q: Are the degrees in Phase Imaging REAL or derived in some other way? How could one find out either way?

A: With the MFP-3D™ controller, the approach taken is to eliminate all analog signal processing and their associated errors and non-linearities. A very high speed ADC digitizes the deflection signal directly, and feeds the bytes right

into a digital down converter, where they are digitally mixed and filtered by the I&Q reference pair. The advantage here is that after the ADC conversion, everything in the signal chain is computed purely digitally, so the magnitude and phase quantities are essentially perfect.

Q: Do we get an increase or decrease in the amplitude with attractive mode imaging?

A: In the following discussion, the "free amplitude" refers to the amplitude right before the tip starts interacting with the surface. This assumes that you have approached close enough that all the squeeze film damping has already taken place.

The following favor attractive mode:

- 1. Smaller free amplitudes**

Starting with a free amplitude of 1 V will make the attractive operating regime bigger than starting with a free amplitude of 5 V.

- 2. Setpoints close to the free amplitude**

For example if we start with a free amplitude of 5 V. A setpoint of 4.5 V is more likely to be in attractive mode than a setpoint of 3V.

- 3. Higher Quality factors**

This generally isn't in your control unless you use active damping.

- 4. Bigger attractive forces**

This also often isn't within your control unless you can control the potential between the tip and the sample. You will notice that it is easier to get attractive mode when the tip wears (bigger tip = bigger attractive forces).

Q: When we have a stronger attraction between sample and tip, do we get an increase or decrease in the height image?

A: The thing to remember is that in attractive mode, the tip turns around before it ever touches the surface. Imagine the feedback loop is maintaining an amplitude of 10 nm (20 nm peak to peak). If you are in attractive mode, the center of the tip motion may be 12 nm above the surface. In repulsive mode, it would be 10 nm. This means that the surface imaged in attractive mode looks higher.

Q: Is the height image an overlay of the "real" height and the attractive force between tip and sample?

A: This is roughly true if your sample is flat and the attractive forces don't vary over the surface (a rare thing). For rough surfaces, you get more attractive forces when the tip is in a valley (surface appears raised) and less when the tip is on a hill so running in attractive mode should decrease the apparent roughness.

Q: Is it possible that the attractive force is a mixture of electrostatic, hydrophobic and Van der Waals interactions?

A: Absolutely, although in air, the meniscus is usually the biggest player by 10X or more.

Q: Does a dark phase signal mean a softer surface or lower AC mode forces?

A: You need to be careful about interpreting dark phase as being soft. A better criterion is that the areas where the phase is closest to 90 degrees are the most dissipative. This is because at constant amplitude, it is the sine that indicates dissipation. The sine peaks at 90 degrees and is symmetric around 90 degrees ($\sin(100) = \sin(80)$).

On most samples (especially hard ones) there are two distinct operating regimes:

1. Attractive

This is where the average phase value is greater than 90. In this case, dark areas are higher dissipation, which often translates to softer in polymer samples. In general, the following makes it more likely that you are in attractive mode:

Small amplitudes

High quality factors for the lever

Big tips (bigger attractive forces)

Set points close to the free amplitude

Weaker levers (An Olympus AC240 cantilever, $k \sim 2\text{N/m}$ is more likely to be attractive than an AC160 with $k \sim 40\text{N/m}$)

2. Repulsive

This is where the average phase is less than 90 degrees. In this case, light areas are higher dissipation (closer to 90) and probably softer. The opposite of the above list applies.

Note that on many samples you often get huge (say 20 degrees or bigger) phase contrast when you are bouncing back and forth between the two modes. In this case interpretation is much more difficult.

Chapter 7 Contact Mode Imaging in Air

7.1 Overview

This chapter introduces the basic set-up and operation applicable to all contact mode imaging, and then proceeds to apply those specifically to contact mode imaging in air.

7.2 Theory

Contact mode is predominately used to measure the topography of hard surfaces. It is also useful for measuring the frictional forces between the tip and the surface in order to determine differences in nanotribological properties of the sample. Because the lateral forces between the tip and the surface are relatively high, it is inadvisable to use this technique on soft samples since the data collected may be an inaccurate representation of the topography. Sample damage may occur. Biological samples, such as isolated proteins or DNA molecules, are difficult to image in contact mode. Also, this mode is not good for samples with movable surface features, such as nanoparticles or Nanotubes.

Getting Oriented

Contact (DC) Mode is the technique in which the cantilever tip is held in constant contact with the surface. As previously described, the MFP-3D™ head contains the optical detection system and Z piezo, which is used to monitor and control the deflection of the cantilever. The scanner moves the sample in X and Y under the tip of the cantilever in a raster scan pattern. Each axis also has a position sensor (NPS™) to detect the exact position of the piezo, providing the MFP-3D™ with its closed loop system. A surface topography map is then created by detecting the Z position of the cantilever for each X-Y position on the surface. More precisely, the topographic map is generated by applying the Z piezo voltage required to maintain a constant deflection of the cantilever, then measuring the Z piezo voltage and/or the Z sensor voltage.

Contact mode is typically an easier mode to learn and operate than AC, or non-contact mode, particularly for new users, because the Z feedback loop is more straightforward than the feedback loop for AC mode. There are also fewer parameters to adjust and no need to tune the cantilever.

Prerequisites

It is assumed that you have worked through the following sections:

| | |
|--|---------------------------------|
| Moving on the Nanoscale: Piezo Actuators and the Nanopositioning System (NPS™) | Section 5.2 on page 55 |
| Imaging Basics | Section 5.11 on page 107 |
| Imaging Feedback | Section 117156064.0 on page 102 |
| Engaging | Section 6.7 on page 135 |

Terminology

Some of the data channels and terms specific to contact mode imaging are listed and described below.

Deflection

This data channel is the difference of voltages between the upper two segments and the lower two segments of the segmented photodiode (See also [Section 5.2 Optical Lever Detection on page 59](#)). It gives the vertical deflection of the cantilever, which is close to the setpoint value chosen by the user. The difference between the deflection before engagement on the surface (also called free deflection) and the deflection after engagement is related to the force acting on the surface. In practice, the deflection before engagement is often chosen to be close to zero. The setpoint is then chosen, depending mainly on the hardness or adhesion of the sample surface. The higher the difference between the free deflection and the setpoint, the higher the tip will push on the surface. The deflection channel will then tell you the error signal of the feedback, since it is the feedback channel. In other words, if a feedback loop could be perfect, there would be no difference between the setpoint value and the actual deflection of the cantilever and the image would look perfectly flat. Features in the deflection image will tell you where the feedback did not track well during a scan. i.e. where the deflection of the cantilever did not match the setpoint value. The Deflection image often has a “shaded” appearance similar to a view of the sample lit from one side.

Lateral

This is the lateral deflection of the cantilever (or torsion of the cantilever) when scanning perpendicularly to its long axis. It is given by the difference between the left two segments and the right two segments of the photodetector. (See also [Section 5.2 Optical Lever Detection on page 59](#)). It is typically used to determine the frictional forces acting on the cantilever. Also note that this channel’s reading

only makes sense if scanning at 90° or 270° i.e. perpendicular to the long axis of the cantilever.

| | |
|--------------|--|
| NOTE: | If you want quantitative values, you need to turn off “Save Plane fit” for this channel. It is also a good idea to turn off “Real-Time Flatten” when viewing this channel. Both of these parameters are located in the ‘Master Channel Panel.’ |
|--------------|--|

Height

This channel plots the voltage applied to the Z piezo in order to maintain the deflection set point. If the tip is too far from the surface, the deflection of the cantilever is smaller than the setpoint, and the feedback will bring the cantilever down by applying a voltage to the Z piezo. The Z piezo will then extend. Inversely, if the tip is pushing too hard on the surface, the deflection of the cantilever will be too high compared to the setpoint. The feedback loop will then apply a voltage to the Z piezo to retract it. The data is multiplied by -1, so that higher Z voltages (where Z piezo is further extended) will appear as lower points on the surface. It is also very important to realize that this image is scaled from voltage to distance with the linear scale factor ZPiezoSens (See [Section 5.2 Piezos vs. LVDTs on page 53](#)). Since piezo materials do **not** respond linearly to voltage, the height data collected will not be accurate for sample features > 0.1 μm. In those cases, the Z sensor signal should be used. Also, the ZPiezoSens is calibrated at a Z voltage of 70 V. At the limits of travel, this scale factor is different due to piezo nonlinearity. Consequently, it is good practice to keep the Z voltage within 50-90 V if using the height channel.

In general, the Z Sensor signal will present a more accurate and quantitative measurement of the sample surface. However, the Height signal has a higher Z resolution (lower noise floor) and will show a smoother view of the sample surface, especially for small features such as DNA, proteins, and lipid bilayers.

Z Sensor

This is the Z LVDT signal. LVDTs are position sensors physically attached to the piezo stacks. The Z LVDT signal corresponds to the real vertical travel of the tip over the surface of the sample, and therefore, corresponds to the real topography of the surface. For small features (roughly <100 nm) it gives approximately the same information as the height channel if the Z voltage is kept between 50-90 V. Typically the Z sensor signal channel has higher noise than the Height signal (specified at 300 pm instead of 60 pm) but is free from artifacts that may appear in the Height image. Unless disk space is a limiting factor, it is often wise to capture both Channels.

Set Point

This is a parameter chosen by the user that will correspond to the deflection of the cantilever during the scan. It is the deflection value that the Z feedback loop will maintain as it scans the surface under the tip. The higher the setpoint, the higher the vertical force applied by the tip to the sample. It does not directly state the force maintained between the surface and the tip, since the set point is an absolute value, and the force is a relative value. To get an approximation of the force maintained by the Z feedback loop, subtract the Set Point from the off-surface deflection value. Then multiply by the cantilever spring constant using (Hooke's Law) $F=k \times d$, where k is the cantilever spring constant and d is the deflection of the cantilever. In some cases this must be increased by the tip-sample adhesion force.

Integral Gain

Integral Gain (IG) is used to maintain the deflection value as closely as possible to the setpoint value. (See also [Section 5.10 Imaging Feedback](#) on [page 102](#)) The IG is essential to tracking the surface. (It's easy to remember that, if you call the 'I' gain the 'important' gain.) Increase the IG while watching the Deflection Real-Time Image and Scope for oscillations. The oscillations are usually high-frequency, with a period of a few pixels. When you see them appear, decrease the IG until the oscillations stop. See [Section 6.3 Set Secondary Scan Parameters](#) on [page 128](#) for details.

Proportional Gain

Proportional gain (PG) assists the IG in the Z feedback loop to maintain the deflection equal to the setpoint. (See also [Section 5.10 Imaging Feedback](#) on [page 102](#)). It provides a minor improvement on some samples, but the PG can safely be left at zero for convenience.

7.3 Operation: Setting Up the Software

This section will cover the steps involved to set up the software in order to image a sample in contact mode in air. A grating is recommended as an easy practice sample. It is assumed that you have already reviewed Chapter 6; also, that you have loaded a soft cantilever, secured a hard sample to the sample plate using the magnets, and lowered the head so that the tip is fairly close to the surface. You can skip Section 6.4 since we are not going to drive the cantilever.

Select Saving Options

Located at the bottom of the Master Panel are the parameters required to save your data to a pre-selected directory (See [Figure 7.1](#)).

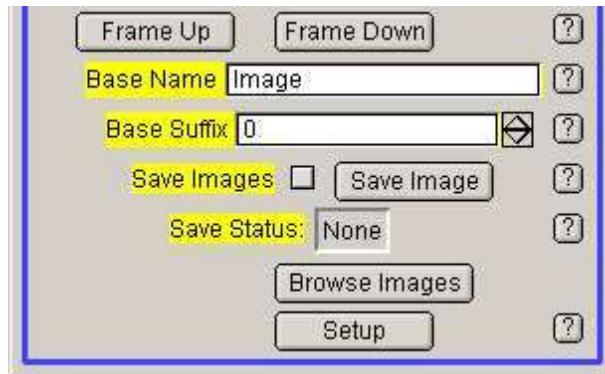


Figure 7.1 Saving Options in the Master Panel

1. Select or Create a Folder

If you want to save your data, select the 'Save Images' check box. The software will then ask in a new window where you want to save the image. You can either browse your hard drive to select an existing directory or you can create a new folder one level down from the existing directory.

2. Name Your Data

What do you want to call the data? This is what you need to enter in the 'BaseName' field. Picking logical names now will help to differentiate your data later when you return to it. For example, inserting the date in the file name is an easy way to sort the data and experiments.

3. Base Suffix

The 'Base Suffix' doesn't generally require an entry; it's the number of the data file. So in the example above, it would save Image0000.ibw first, then Image0001.ibw, and so on. If you reuse an old BaseName, you will need to make sure that the suffix will not conflict with any of your previously saved data. The MFP-3D™ will overwrite your old data if there is a conflict.

4. Save Your Data

When the software is first opened, the user must click on either the 'Save Images' box or the 'Save Image' button. The 'Save Status' parameter tells you whether your images are being saved:

'None' No images are being saved. This is the default setting when opening a new experiment in the software.

'Save Image' The current image is being saved.

‘Save Next’ If a primary scan parameter is changed, the scan status changes from ‘Save Image’ (if you have opted to save your data) to ‘Save Next’. **This means that the software will not save the current image.** You can force the software to save the current image by clicking on the ‘Save Image’ button. This changes the scan status from ‘Save Next’ to ‘Save Anyway’ but keep in mind that you have changed a vital parameter and the data may be unusable.

Set Your Imaging Mode

Set the ‘Imaging Mode’ to ‘Contact’ (See [Figure 7.2](#)).



Figure 7.2 Imaging Mode in Master Panel

Select Your Data Channels

Prior to scanning, or even initiating the manual approach, you must select all of the data channels you wish to acquire. This is done in the ‘Master Channel Panel’ (See [Figure 7.3](#))

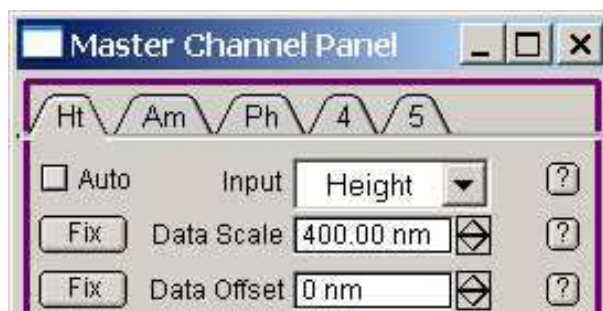


Figure 7.3 Data Channels in Master Channel Panel

Each tab at the top of the panel represents a separate data bank called a channel. Each data bank may be used to record a defined signal chosen by the user. Up to

five channels can be open simultaneously and all the corresponding signals will be saved within one image. The software starts up in AC mode by default, so height, amplitude and phase are turned on when you start. Since we are doing contact mode work, amplitude and phase should be turned off before you proceed. Leave the first tab set to 'Height.' Click on the second tab, where the pop-up menu is set to 'Amplitude' and change that to 'Deflection' (to see the error signal). For the third channel, which is automatically set to 'Phase', either turn it off or set it to 'Lateral.' The other two tabs are labeled 4 and 5, which means that they are already turned off, and no further adjustments are necessary for contact mode imaging. However you can choose to assign these channels to other types of parameters for more advanced techniques such as electrical measurements. You may also want to set data channel to Z-sensor if you are imaging tall (>100 nm) features.

Set Primary Scan Parameters

Primary scan parameters are those parameters that are typically not changed during a scan that you wish to save. If you change them, the image would be difficult to interpret. For example: if the lower 20% of your image is collected with a scan size of 5 nm, and the upper 80% of the image is collected with a scan size of 90 μm , the resulting image will be distorted. To prevent this type of situation, whenever one of the primary scan parameters is changed *during* a scan, the software will wait until the end of the next image to save the image. However, you can force the software to save your image if necessary. Click on 'Save Next.' The text will change into 'Save Current' and your image will be saved. Note that changes to the primary scan parameters often require fine-tuning of the secondary scan parameters. As a result, it is often easier to set-up the primary scan parameters first, and then adjust the secondary scan parameters.

Your primary scan parameters are:

Scan Size

Scan Rate

Scan Speed

X Offset

Y Offset

Scan Angle

Scan Points

Scan Lines

Height: Width Ratio

Top Line

Bottom Line

Please review [Figure 7.4 Primary Scan Parameters, located in the Master Panel](#) for the specific position of each parameter.

NOTE: Some of the controls listed here are advanced features, and are hidden by default. To see them, click on the set-up button at the bottom of the main panel.

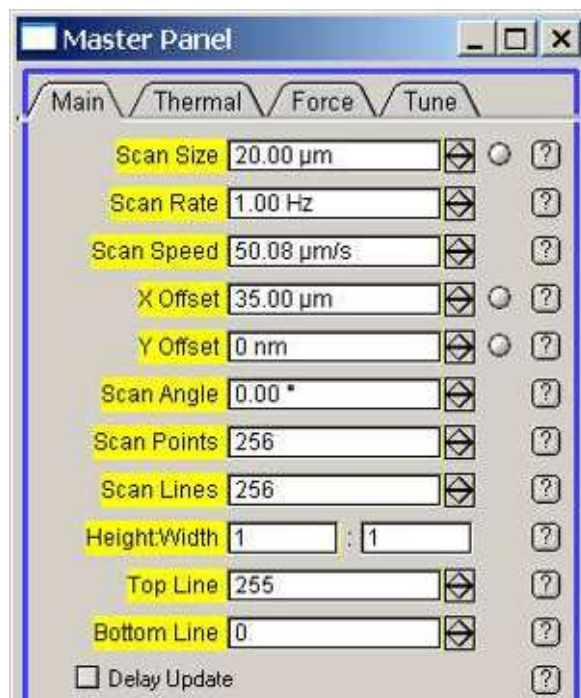


Figure 7.4 Primary Scan Parameters, located in the Master Panel

NOTE: You can click on the "?" buttons to the right of the controls to have the software bring up a 'Help' topic specific to that control.

Scan Size

This is the size of your image in metric units. The engagement process does not initiate the scan. The tip will start scanning the area only after you choose to do so, by clicking on 'Start' (the scan will begin at the position where the tip stopped) ; 'Frame Up' (to start the scan from the bottom) or 'Frame Down' (to start the scan from the top).

Scan Rate

This is the frequency at which scan lines will be completed. For example, 1 Hz means that the tip will complete a trace and retrace path in one second; 2 Hz means a complete trace and retrace path will take 0.5 seconds, and so on. The total image will take 'Scan Lines / Scan Rate' seconds to complete. Low scan rates may increase the problems from thermal drift whereas high scan rates can make it difficult for the Z feedback loop to track the surface. Note that the 'Scan Speed' parameter is essentially the same as the 'Scan Rate' control, but just gives different units.

Remember that if you change the scan rate during a scan, the speed will not be updated immediately but when a new image begins.

NOTE: At the top of each image the time remaining for scanning the area is displayed.

X Offset

This function offsets the scanner along the X axis. As the offset increases the maximum 'Scan Size' that you can use decreases. But by starting at zero, you have the full range of the scanner with which to work. See the 'Show Box' button at the bottom of the Main Panel for a good illustration of this: Once you have the 'Scan Box' showing, you can enter different values of the X and Y offsets to see how they change the maximum 'Scan Size' you can enter.

Y Offset

This function offsets the scanner along the Y axis. Again, begin at zero for the full range of the scan size.

Scan Angle

This is the angle between the X axis of the scanning tip with the X axis of the scanner itself. If you are collecting lateral data, you should enter 90° : the X axis is perpendicular to the axis of the cantilever, which allows you to scan the X axis while simultaneously twisting the lever, and thereby getting lateral data. If you are not collecting lateral data, it is generally better to run at 0°. However, depending on the sample and the cantilever interactions, it can be useful to scan at different angles. For example, if there is some adhesion on the surface, or more generally, if the cantilever bends along its long axis during the scan (this can sometimes be seen optically) you may choose another angle. In practice, if trace and retrace curves do not overlap even after optimizing the gains, try to change the scan angle and see if the image improves.

Scan Points

This is the number of data points collected in each line. 512 pixels per image is the most common setting at which to start. Scan points up to 4096 may be entered, but at scan points greater than 512, the scan rate will be slow. At scan points of less than 256, the quality of the image's resolution may be compromised (as there are too many nm per pixel).

Scan Lines

This is the number of lines collected per image, and therefore defines how long it will take to collect the image, as well as playing a role in its resolution. The time to collect the image will be the 'Scan Lines / Scan Rate' seconds, so increasing this value will cost a fair amount of time (See also 'Scan Rate' on page 141). Decreasing the 'Scan Lines' however, decreases the resolution of the image. The range from 128 to 256 will give a decent resolution without taking too long to collect. Once you like the way the secondary parameters are working, you can increase the number of lines per image (again, 512 is a commonly chosen number) and you will collect a nice image. Changes to the 'Scan Line' parameter do not alter the secondary scan parameters at all. Changing the number of points or lines carelessly will disengage the tip from the surface and stop the scan.

Height: Width

These two controls define the ratio of the image height to the image width. This function is useful when the user does not need a square image but would prefer a rectangular one. It can save time to not scan the entire square, especially if only part of the area being scanned is of interest to the user.

NOTE:

Height: Width is different than the number of pixels; it is the distance between the two axes. It is an advanced feature, so for the time being leave it at 1:1. For more details on using this control, click on the "?" buttons to the right of the control.

Top Line

This is an advanced feature (hidden by default), and should not be adjusted yet (leave it hidden). For more details on using this control, click on the "?" buttons to the right of the controls.

Bottom Line

This is another advanced feature (hidden by default), and should also not be adjusted yet (leave it hidden). For more details on using this control, click on the "?" buttons to the right of the controls.

Save Status

See Section 7.3. [Select Saving Options](#) on page 160, #4, Save Your Data

Start Scanning

Once you have engaged upon the surface, and have entered the primary scan parameters, you may begin imaging by simply clicking on one of the buttons ‘Do Scan’, ‘Frame Up’ or ‘Frame Down’ (See [Figure 7.5](#) on page 167.) If you are not familiar with the engage process, please review [Section 6.7 Engaging](#) on page 135.

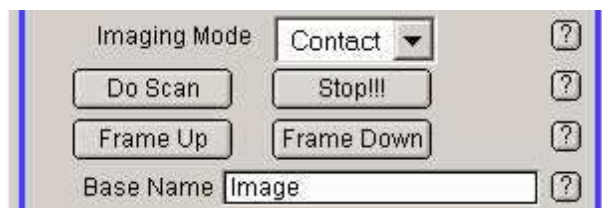


Figure 7.5 Initiating the Scan, Master Panel

Set Secondary Scan Parameters

These parameters are used to optimize the image quality as you scan. The changes to these parameters are saved in the wave note file on the hard drive, along with the scan line at which they were changed. Changing one of the secondary scan parameters does not alter the ‘Save Status.’ Secondary scan parameters are also often dependent on the values of the primary scan parameters. If you change a primary scan parameter, you often need to reevaluate you secondary scan parameters.

Your secondary scan parameters are:

Set Point

Integral Gain

Proportion Gain

Secret Gain

Drive Amplitude

Drive Frequency

Input Gain

For contact mode imaging however, mainly Set Point and Integral Gain are relevant.

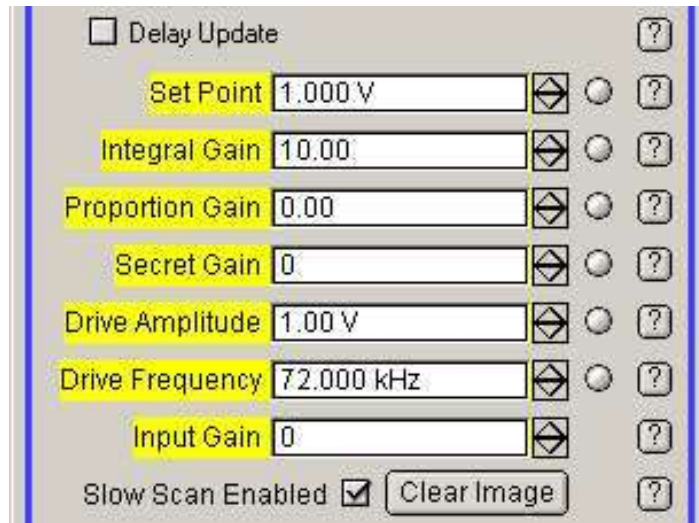


Figure 7.6 Secondary Scan Parameters

Set Point

This is the deflection value that the Z feedback loop is trying to maintain. For contact mode work it needs to be higher than the deflection value away from the surface (as read from the sum and deflection meter). The higher the Set Point value, the harder the tip is pressed against the surface. Ideally the tip should be pressed against the surface hard enough that there is solid contact, but not so hard that the tip or surface is damaged. Generally 1 V higher than the pre-engage deflection is a good starting point. Of course, this depends on the spring constant, the InvOLS value, the cantilever, and the surface.

Integral Gain (I Gain)

Now that the tip is in hard contact with the surface the gain needs to be adjusted. Increase the value until you see high frequency noise in the height image scope (indicated as the piezo starts ringing). This means that the gains are too high, and the Z piezo is overreacting to the error signal. Decrease the I Gain until it stops ringing. This number is the highest usable gain, which also means that this I Gain will track the surface the best. Note that the highest usable gain depends on a number of parameters, in particular the scan rate and the set point. If either of those parameters is changed, it is generally a good idea to reevaluate your integral gain as well. Another factor to monitor is how well the trace and retrace lines overlap each other in the scope traces. There is always going to be some offset between them (they are different regions of the surface), but they should be fairly close.

Proportional Gain (P Gain)

This gain is less of a factor in the MFP-3D™ system. You may want to change it after the integral gain has been fine tuned, and if so, it is done in the same way. The I Gain should always be tuned first, since it has more of an effect on the feedback loop. If you don't notice much difference as you change the P gain, then leave it as is.

Drive Amplitude

Drive Amplitude is not applicable to contact mode imaging since the cantilever is not oscillated as it is in AC mode; it does not change anything on the controller when in contact mode.

Drive Frequency

This again, is a parameter used for AC mode and has no effect on contact imaging.

Input Gain

This is a more advanced feature and should be left at zero for contact mode imaging. For more help on this parameter, click on the "?" button to the right of the control.

7.4 Troubleshooting: Contact Mode in Air

Blurry Image

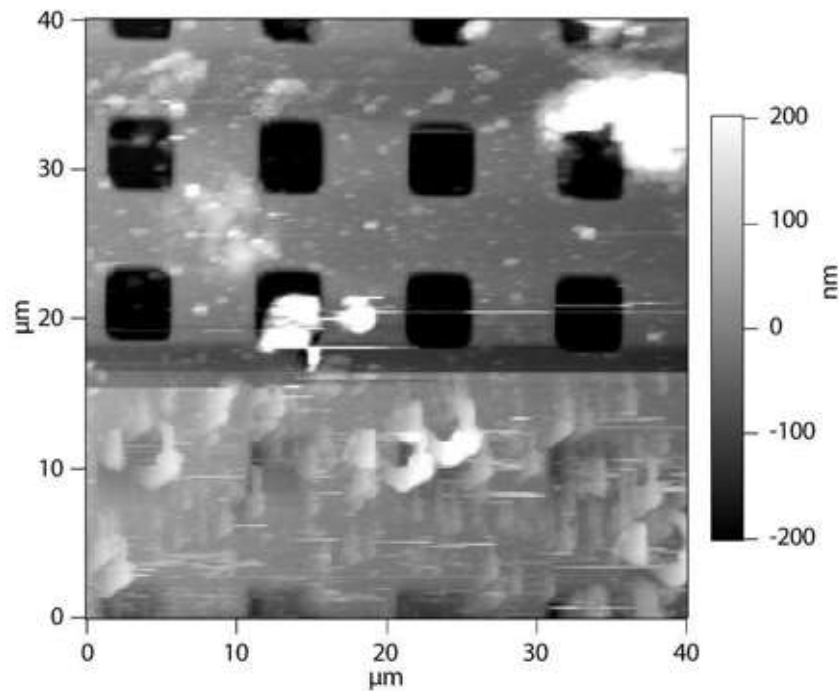


Figure 7.7 Blurry Topography Image

Symptom: You are scanning an image, and in spite of the feedback tracking the surface notably well, the features that you expect to be sharp are blurry --- particularly if the images collected to that point were sharp, and all of a sudden they become blurry.

Problem: The tip is dirty / blunt.

Solution: Switch to a new tip and discard the old one. Avoid reusing an old tip.

In [Figure 7.7 Blurry Topography Image](#), the top of the image is fairly clear but quite dirty. As illustrated, when the tip reached the bottom of the image it had picked up a large piece of dirt from the surface, which caused the image to blur.

This is an extreme case, in that the dirt particle was so large it actually prevented the tip from entering the grating pits.

Double / Triple Images

Symptom: While scanning an image, every feature looks as if it is on the surface twice, with the duplicate images quite close to each other, or possibly even overlapping. It looks a lot like you are seeing double.

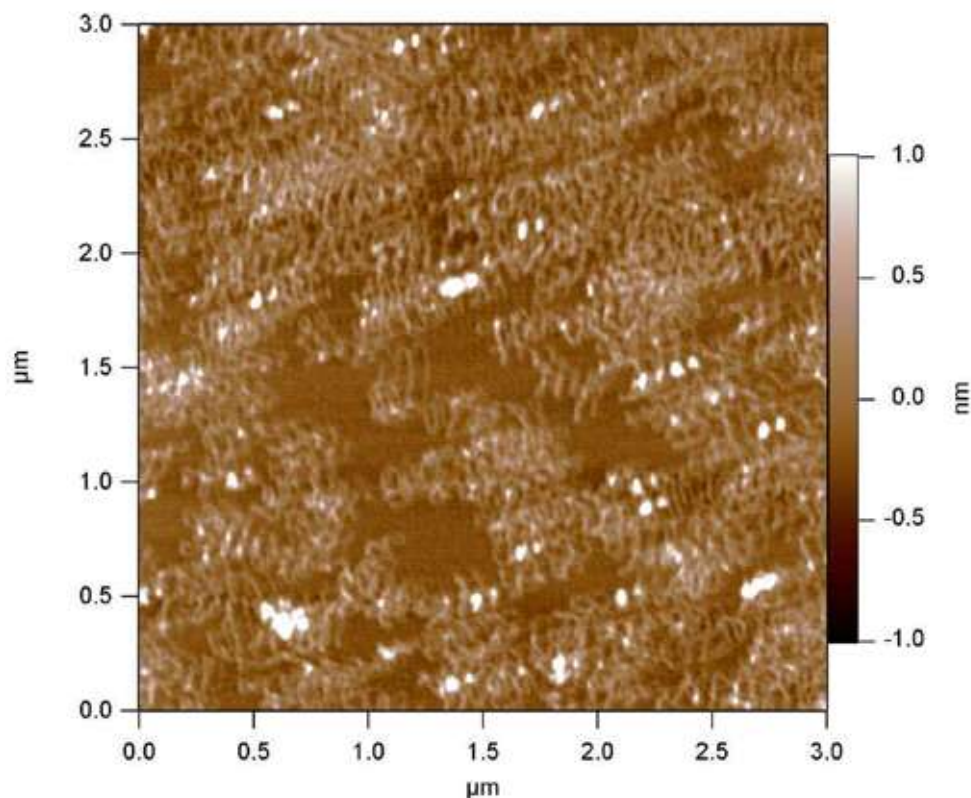


Figure 7.8 “Triple” DNA Molecules

Problem: There are actually two (or more) areas on the tip that are in contact with the surface (This means your tip is cracked, or dirty). **Figure 7.8 “Triple” DNA Molecules** is an image of DNA molecules in which each molecule is seen three times. The sample also appears extremely concentrated, even though it is not.

Solution: Switch to a new tip and discard the old one. Avoid reusing an old tip.

Digging a Pit?

Symptom: Every time the scan size is increased from a previously scanned image, large lumps of material are lined up along left and right edges.

Problem: Your set point is too high for your soft sample; the tip is operating in "dynamic plowing mode."

Solution: Lower the set point.

Poor Surface Tracking

Symptom: Pinpointing a good gain to track the surface seems impossible.

Problem: There are a variety of reasons this can happen. The most likely is that the feedback is not responding fast enough to keep up with the movement of the tip, or you are not totally engaged on the surface. The cause is most likely a combination of the cantilever chosen, your gains and your setpoint.

Solution: First try increasing the setpoint a bit (you may not be totally engaged on the surface). If that doesn't work, try decreasing the 'Scan Rate.' Also, decreasing the 'Scan Size' (if possible) can help. If none of these options help, try a different type of lever with that sample. (See [Section 5.6 Cantilever Selection](#) on [page 79](#)).

7.5 Lateral Force Microscopy

Lateral Force Microscopy, also referred to as LFM.

Anatomy of Lateral Forces

To better understand the meaning of lateral forces, we will start with a virtual example displayed in [Figure 7.9](#).

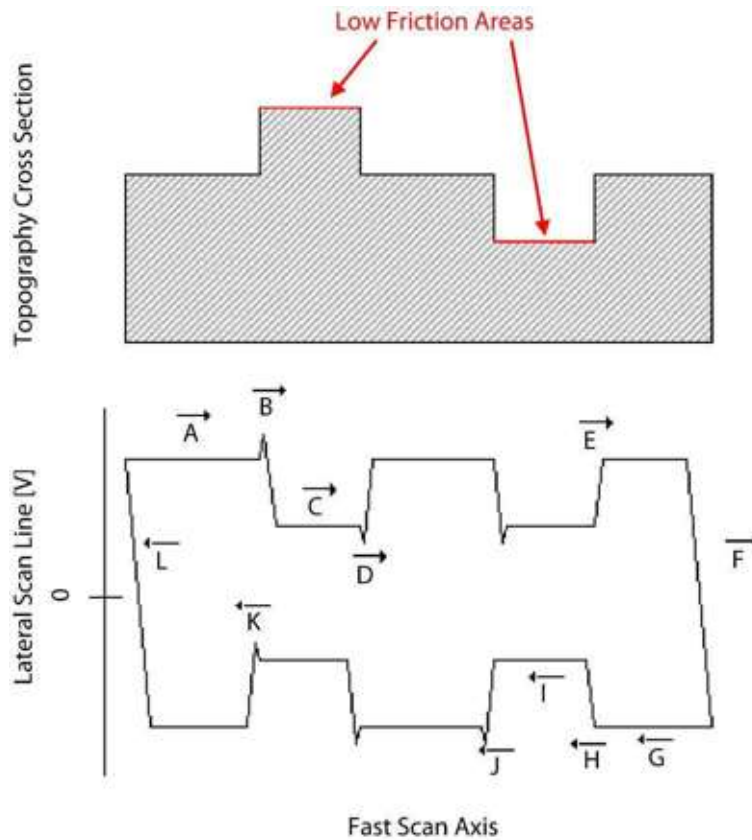


Figure 7.9 Cross-Section: Theoretical Lateral Scan Line, for a theoretical topography. The low friction areas on the topography are marked in red

The various regions of the lateral scan line in [Figure 7.9](#) are described as the following:

- A** Lateral trace data, the tip is moving from left to right over the surface on a high friction region.
- B** The tip trips over the height feature and a spike up in the friction is seen.
- C** The tip makes it to the top of the height feature, which has a low friction area on it, so the lateral deflection is lessened.
- D** Think of this as the tip "freefalling" off of the height feature. As it freefalls, there is nothing keeping the tip bending laterally, and the lateral signal heads towards zero. Notice that there is another region similar to this, before the tip reaches E.

- E** The tip would trip over the height feature it now hits, but it is difficult to see that since it is moving from a low friction area to a high friction area.
- F** The turnaround from trace to retrace. This region is typically used to calibrate the LFM InvOLS. To do it, take the inverse slope to get the distance per volt of lateral photodiode signal. The idea behind this is that during the turnaround the tip is not moving laterally: all of the lateral motion of the scanner is going into bending the cantilever laterally. So while the turnaround occurs, factoring the change in lateral photodiode voltage and assuming that the tip is not moving, the amount the scanner has moved is the amount that the lever has been laterally bent. Therefore, taking this slope will give you the lateral sensitivity of the photodiode.
- G** The start of the retrace data, the tip is moving right to left across the surface.
- H** Another free-fall area. This time however you don't see any spikes in the friction as the tip is moving from a high friction to a low friction area.
- I** Tip is happy to scan along the low friction area. Notice that friction on the scope mode is measured as how far the signal is from zero. So -5 is a higher friction than -2 for the retrace data. Another way to look at the scope trace is the further the trace and retrace are separated from each other, the higher the friction is.
- J** The tip trips over the step from the pit to the high friction area, and just as in Section B, the tip trips over the height feature and a spike up in the friction is seen.
- K** The tip free-falls again at this point, so that the friction heads towards zero as it did in Section D. Once again, as the tip free falls, there is nothing keeping it bending laterally, and the lateral signal heads towards zero.
- L** The tip switches from retrace to trace, reversing the process as described in Section F.

Interpretation of the Data

As demonstrated above, topography features show up in the lateral data and that makes it difficult to interpret the data. How do we get correct for that? A careful look at the theoretical lateral data shows that the artifacts go in the same direction for trace and retrace data. Where the tip trips over an edge going in one direction, it will fall off the edge going the other. This means that the artifact spikes in the lateral data are either pointed up or down, regardless of the direction in which the tip moved over them. However the *true* friction data has *opposite signs* for trace

and retrace. Therefore one trick to get the true friction data is to subtract the retrace image from the trace image. Doing that subtracts the artifact spikes, and leaves only the frictional data. Of course, this will not give perfect information: the magnitude of the artifact spikes in each direction is not equal. It can however, help to reduce lateral force artifacts.

"Zeroing" Lateral

On the standard MFP-3D™ system to zero the lateral signal, apply an offset voltage to the ADC that is reading the lateral data. This will not, however, offset the range of values in which you can read. Zeroing the lateral data this way does make the offline part easier though, since zero means there is no lateral force: just bear in mind the reduced range.

To zero lateral force:

1. Download <http://www.rasy.com/Files/LFMrocks.zip>
2. Unpack the zip file
3. Open the ipf file in the MFP-3D™ software
4. Hit 'Ctrl + Shift +W' (to hide and compile the procedure)
5. Go to the Macro Menu and select 'ZeroLateral'

Chapter 8 AC Mode Imaging in Liquid

8.1 Overview

Two options for imaging in liquid with the MFP-3D™ will be discussed in this chapter: using a liquid droplet and using a closed fluid cell.

A user will decide which option is the most appropriate depending upon whether they will be statically imaging the sample or exchanging fluid through a sealed chamber.

8.2 Theory

AC mode in fluid is frequently used to observe structures, predominately biological in nature, in their native fluid 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.

Prerequisites

It is advisable that you review the following chapters in their entirety preparatory to beginning work in AC Mode in Fluid:

General Set-Up and Operation.....[Chapter 5 on page 53](#)

A C Mode in Air[Chapter 6 on page 121](#)

NOTE:

These guidelines assume that the user is familiar with the MFP-3D™ and knows how to image samples in air. Only topics specifically related to working in liquid conditions are being described.

Terminology

(See [Figure 8.1](#))

Before describing the procedures, some definitions would be useful:

Sample

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

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.

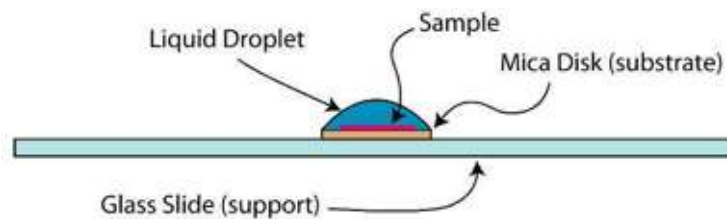


Figure 8.1 Typical Sample Mounting

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 is not exhaustive:

6. Use soapy water and gently rub the holder with your finger, preferably wearing gloves. Make sure not to scratch the glass window through which the light beam travels. Rinse copiously with deionized water.
7. Follow the same procedure as in #1 (above), followed by two additional steps: a rinse with 70% ethanol in water, and a final rinse with deionized water.
8. Immerse the top of the holder (the Kel-f part) in ethanol and sonicate for 2 minutes.
9. Use a gentle plasma treatment: 30s at 50W and 0.15 torr.

Note that cantilevers are difficult to clean. You can try ozone treatment for 1 min, or plasma treatment, as mentioned above. Some authors are also using piranha solutions.

8.3 General Procedure Using the Water Droplet Technique

This set-up is simple but does not prevent evaporation of the liquid.

Prepare the Substrate for the Sample

A sample may be immobilized for imaging on either an atomically-flat substrate such as 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. The substrate may be attached on a glass slide or its equivalent (See [Figure 8.2](#)).

Petri dishes, either a Corning 60mm polystyrene dish, catalog #430166, or WPI 50mm glass-bottom dish, catalog #500863, may be used but require a modified sample plate. Additional accessories such as the coverslip sample holder and sunken sample holder are available specifically for the MFP-3D-BIO™ where the user requires the use of higher NA objectives.

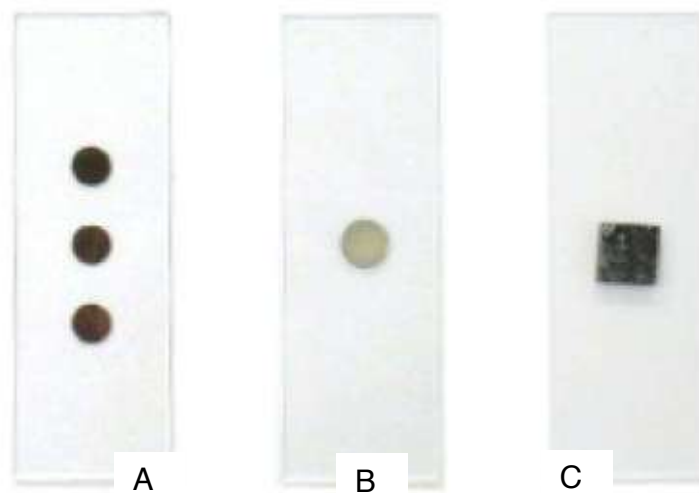


Figure 8.2 Typical sample mountings: A: Gold deposits on a glass slide. B: Mica disc glued to a glass slide. C: HOPG attached to a glass slide

If using mica as a substrate, a “hole punch” (for example: Precision Brand, #40110 or McMaster-Carr’s 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 “glue” that does not dissolve in water) to immobilize the substrate on the slide. Double-sided tape is not recommended as it

may create some drift when it expands. To avoid the liquid from spreading on the support (which could contaminate the sample if the support is not clean) a hydrophobic film may be deposited around the substrate. PAP pens are, for example, very convenient for this purpose, as well as Teflon tape. An alternative is to use PTFE printed slides that are available from Electron Microscopy Sciences. They have a wide selection to choose from, but #63419-12 is the most appropriate for our system.

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.

Choose and Insert a Cantilever Chip

Choose and insert the cantilever chip in the holder. Select a low spring constant cantilever, such as those made of silicon nitride. For example, silicon nitride cantilevers, like the short, 60 μ m long Biolever from Olympus, are a good choice for AC mode in liquid. Long silicon levers with a low spring constant (on the order of a few N/m) may be used as well.

Position the Light Beam

It is easier to position the light beam on the cantilever in air before the cantilever is placed into the fluid. The infrared card may be used to see the spot and focus it on the cantilever. Alternately, the CCD camera in the AFM base may also be used. 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. The SUM signal obtained on silicon nitride cantilevers, despite their gold coating, is typically lower than on silicon cantilevers (between 3-6).

Place the Support on the Scanner

Put the sample/substrate/support onto the scanner and hold it in place with the magnets. 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.

Lower 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, 60 μ m long Biolever it is recommended that the user angle the head so that the right rear head leg is slightly lower than the other two legs. This will ensure the small cantilever will be the first to contact the surface, not the

cantilever chip or longer Biolever.) For other cantilevers, such as the Olympus TR400 or TR800, the head should be more or less level.

Distance Between Cantilever and Substrate Unknown

If a new sample with different thickness is mounted onto the AFM, or if the user is switching from a silicon (thinner) to a silicon nitride (thicker) cantilever, it is advisable to perform the following steps in air to avoid crashing the tip into the surface.

Use an extra substrate support (i.e. mica disc glued onto a glass slide) with no liquid on the substrate for this step. Lower the head in air so that the tip is about 1 mm above the surface. Then remove the head and the substrate/support. Proceed to Section 8.3.7 Wet the Cantilever.

Distance Between Cantilever and Substrate Known

If the cantilever is at a known distance (i.e. the user has just put a new cantilever into the cantilever holder and the sample is the same), then proceed to Section 8.3.7 Wet the Cantilever.

Wet the Cantilever

(See [Figure 8.3](#))

This step is also optional, but highly recommended since it seems to reduce the risk of creating air bubbles on the cantilever, and also facilitates filling the space between the substrate and the bottom of the cantilever holder with liquid. To wet the cantilever, using a pipette, put 2 or 3 drops of liquid on the cantilever chip while tilting the AFM head. Without tilting the AFM head, the liquid drop could fall on the side of the quartz, but when the head is tilted back, gravity will bring the liquid drop to the lower point, which is the cantilever.



Figure 8.3 Close view of how to pre-wet the cantilever with a pipet before immersing it in liquid

Place the AFM Head on the Stage or Base

Make sure the sample/substrate is totally immersed in liquid, as well as the cantilever chip. This is important as sometimes the liquid will not fill the whole space between the cantilever chip and the holder, and the beam of light will not reach the photodetector.

Realigning the Light Beam

Once the cantilever has been fully immersed in fluid, the SUM signal will disappear. This is due to the fact that in the presence of liquid, the optical path is different; therefore, the light beam on the cantilever needs to be refocused. To do that, turn the knob corresponding to the motion of the light in the X direction on the AFM head clockwise. Notice that the SUM signal should be increasing 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 camera once you are in liquid.

NOTE: For AC Mode in Liquid Troubleshooting topics see [Section 8.7](#), [page 205](#)

Thermal Tune

A thermal tune looks at the spontaneous vibrations of the cantilever while it is excited by the Brownian motion of either the air or water molecules in its vicinity. Cantilevers have different vibrational “modes” which correspond to different vibrational shapes. In a guitar string, for example, the modes are harmonics. Unlike the vibrational modes of a guitar string, higher order frequencies are not harmonics (f_0 , $2f_0$, $3f_0$, etc...) of the fundamental. Ideal diving-board-shaped cantilevers for example, have modes at f_0 , $6.19f_0$, $17.5f_0$, etc. So, for the Biolever example ([Figure 8.5](#) on [page 183](#)), if $f_0 = 8 \text{ kHz}$ then there should be a second mode at $8 * 6.19 = 49.52 \text{ kHz}$.

In the Master panel, select the ‘Thermal’ tab (See [Figure 8.4](#)). Then click on ‘Do Thermal’.

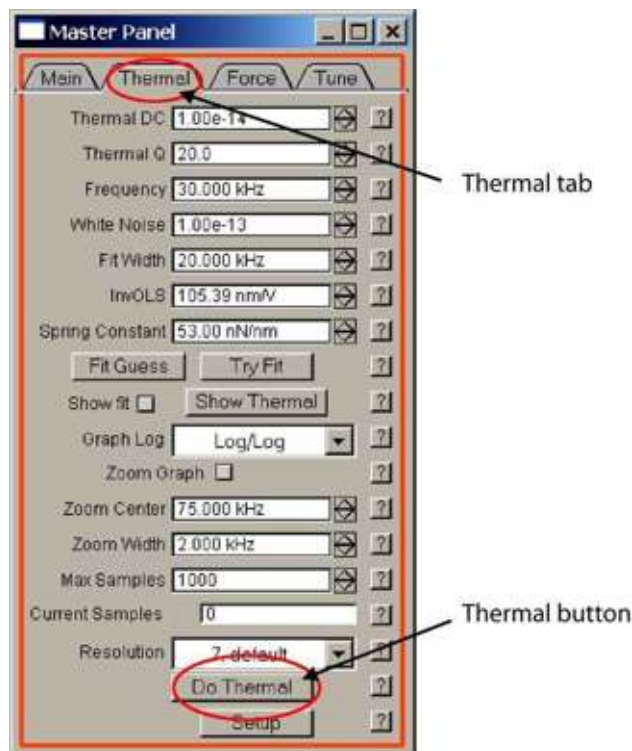


Figure 8.4 Master panel, Thermal tab

There should be a broad peak around the resonance frequency of the cantilever (See [Figure 8.5](#)). 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 60 μm long Biolever from Olympus, the peak is at about 8 kHz (See [Figure 8.5](#)).

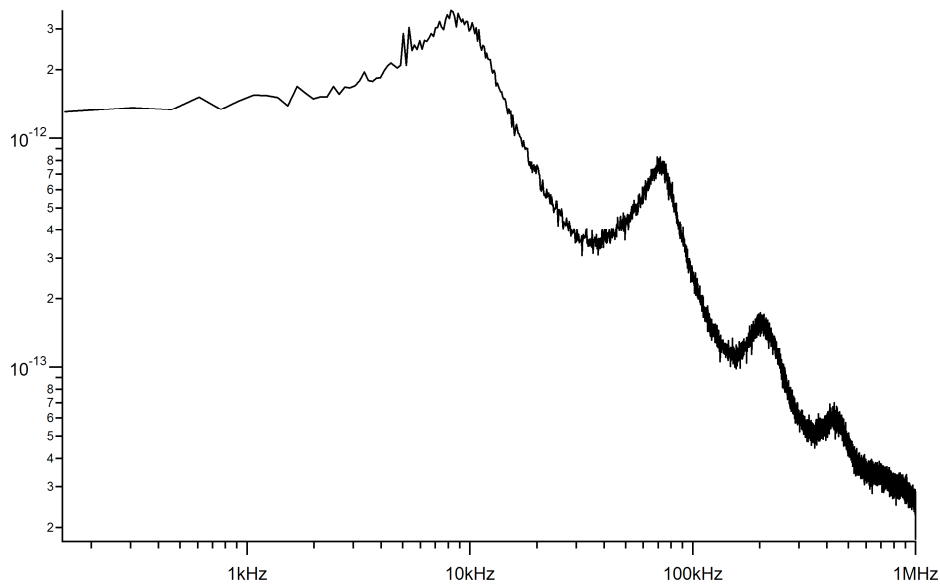


Figure 8.5 Typical thermal graph obtained in liquid with a 60 μm Biolever. Note the broad peak at about 8 kHz, corresponding to the resonance frequency of the cantilever in an aqueous buffer

With the MFP-3D™ software, the thermal peak can be appended to the cantilever tune, which helps the user to select an adequate oscillation frequency of the cantilever.

Cantilever Tune

In the cantilever tune mode, a piezo drives the cantilever at different frequencies and the cantilever's response is displayed. This will help in choosing the frequency that will be used. In air, only the cantilever vibrates, and in a frequency tune, only the resonance frequency and harmonics appear. In liquid however, there is a coupling between the liquid and the cantilever, and possibly the cantilever holder and other components as well. This creates multiple peaks and the result can be uncertainty about which peak to use. Most of the time, users choose a frequency that corresponds to the resonance frequency of the cantilever in liquid. Sometimes users will choose very different values like 60 kHz for a cantilever

with a resonance frequency of 8 kHz, just because there is a nice peak in the tune. For these guidelines however, we will use the more commonly chosen resonance frequency of the cantilever.

In the Master panel, select 'Tune.' At the bottom of the Tune panel, click the box "Append Thermal.' If a resonance frequency of about 8 kHz is expected (as would appear for the short Biolever, for example) then choose a 'Drive Frequency' of 8 kHz at the top of the Tune panel. Then choose a 'Sweep Width' of 20 to 30 kHz. (Note that it is also possible to explore more frequencies by choosing other values, like a drive frequency of 50 kHz and a sweep width of 100 kHz). Then click on 'One Tune.' (Figure 8.6 on page 184 is an example of a tune.) The shapes and positions of the peaks vary greatly from cantilever to cantilever; consequently the aspect of the tune can change a lot.

NOTE: There is now an alternate method available. A new feature in the MFP-3D™ software allows you to zoom in on the thermal peak, and it automatically selects both the Drive Frequency and the Sweep Width in the 'Tune' tab. Then the user would only need to fine-tune their selection by picking a specific peak.

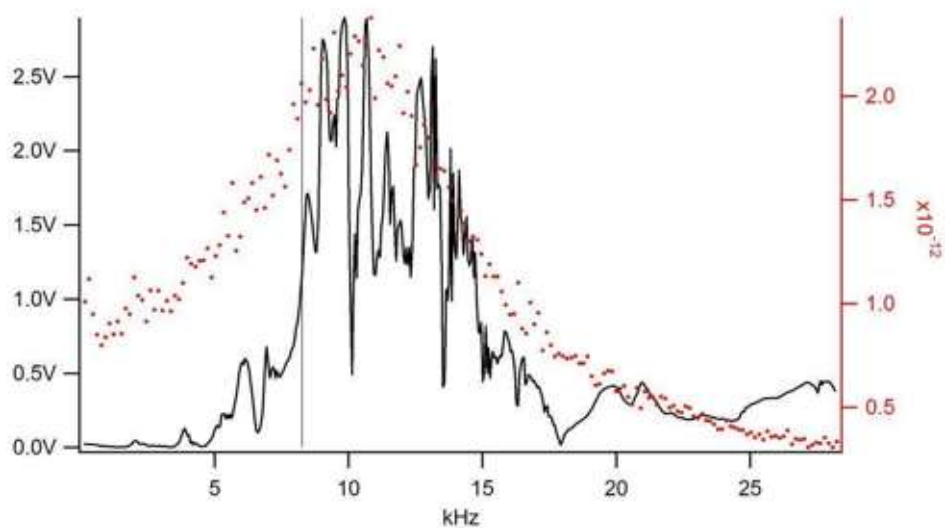


Figure 8.6 Frequency tune obtained in liquid with a 60 μm Biolever

For the 60 μm Biolever, choose a frequency of 7 kHz or 5 kHz. These are empirical values that usually work well. But depending on the cantilevers and their geometries, (they are difficult to manufacture consistently), other values may

be chosen depending on the aspect of the frequency tune. We also recommend that once the peak that will be used is identified, a frequency on the left side of that peak is actually chosen.

Change the R Filter Value

At the top of the screen, in the menu of topics select 'Programming,' then select 'Make Filter Panel'. A list of filters will appear. Change the value of the 'R' filter to 500 Hz. See [Figure 8.7](#). 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.

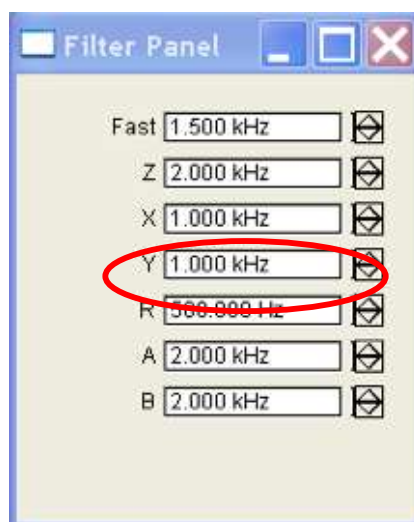


Figure 8.7 R Filter Value

NOTE: _____ If you are using any version of the software released after version 050811, you no longer need to adjust the R filter value.

Summary - Loading the Sample in Liquid

1. Prepare a buffer or other fluid solution as appropriate for your sample.
2. Verify that the fluid is at room temperature.

NOTE: _____ Temperature differences between the fluid and the tip are likely to cause problems in tuning the system and collecting the data.

3. Align the light source on the cantilever before adding the liquid (See [Section 5.5](#)).

NOTE: It is easier to adjust the alignment of the light source to the fluid than it is to align the light source from scratch once the fluid has been added.

4. Verify the legs of the head are adjusted so the tip is within a millimeter of the surface.

NOTE: The cantilever must be close to the surface in order to complete Step 6.

5. Place the head upside down. Use a syringe to wet both the cantilever and the sample.

ATTENTION: Wet the cantilever gently. We recommend placing a drop of solution next to the cantilever chip, and using the syringe tip to gently wet the cantilever with that drop. If water gets into the head, immediately power it down by turning off the controller.

The cantilevers are extremely flexible; subjecting them to strong, sudden surface tension force can bend or break them. Injecting solution directly between the cantilever and sample with the head in position on the base or base plate is likely to damage the cantilevers.

ATTENTION: Be careful not to allow any fluid to enter the head when the head is resting on its back. If any fluid does get into the head, set the head right side up and remove the cantilever holder. Dry any visible fluid and let the head dry out. Extra care should be taken the next time you use the head to ensure it is working properly.

6. When both the cantilever and the sample are wet, place the head over the sample and connect the drops of fluid.
7. Visually check the fluid between the tip and the surface, and verify that there is a solid meniscus between the tip and the surface that covers the cantilever well.
8. If you have a transparent sample, use the bottom view optics to ensure that no air bubbles have been trapped on the cantilevers.

9. Adjust the light source to maximize the Sum and zero the Deflection. Turn LDX about a 1/4 turn clockwise.

NOTE: Generally a quarter turn is all that is needed to adjust the light source to work in water. Depending on the index of refraction of the fluid you are using, the amount you need to turn LDX may differ. This step is much easier if you have a transparent sample and can use the bottom view optics to align the light source.

10. Repeat Section 6.11 (Thermal PSD). Note that the same effects that altered the Thermal PSD will alter the cantilever tune.

NOTE: The Thermal PSD graph appears much different in liquid than in air because the fundamental resonance and higher modes of the cantilever are shifted to lower frequencies in liquid. This occurs due to the increased damping and because some of the liquid acts as carried mass increasing the effective mass of the cantilever.

Choose Imaging Parameters

The values below are only suggestions and may be optimized later with some user experience. (See [Figure 8.8](#) on page 188)

Return to the Master panel and review and adjust each parameter as follows:

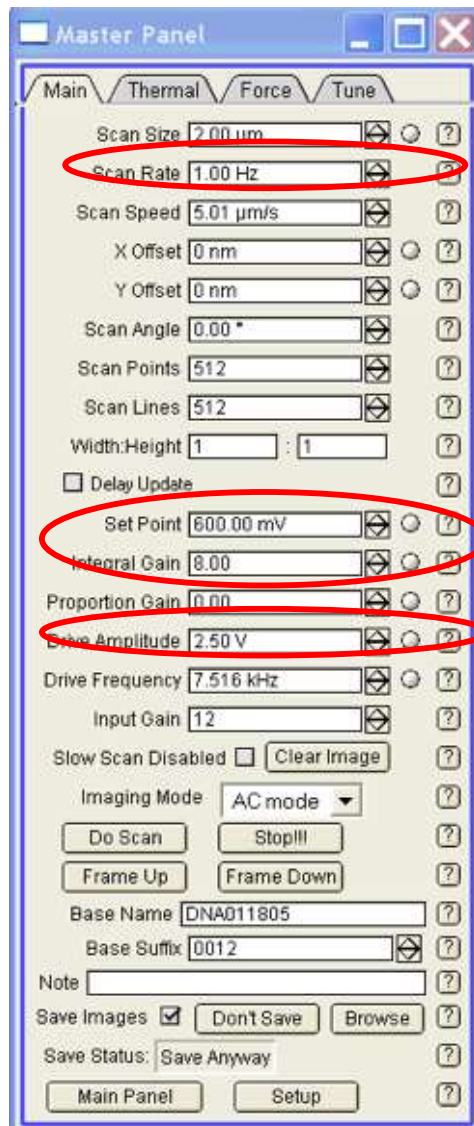


Figure 8.8 Master Panel Main Tab

Drive Amplitude

This value is unimportant itself but it will determine the free amplitude of oscillation, which is very important. The value of the free amplitude, called 'Amplitude' in the Sum and Deflection Meter panel, is cantilever and sample dependent. 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 is sometimes better to use higher values.

Set Point

The setpoint has to be smaller than the free amplitude, typically at 80% of the free amplitude.

In order to be gentle when imaging soft samples, it is preferable to use a set point value close to the free amplitude. For example, when imaging DNA in liquid with a free amplitude of 0.8V, the setpoint may be 0.6V.

Scan Rates

The typical scan rate in liquid is 1Hz. Some samples may require lower speeds, like living cells for example, which can require 0.2 Hz.

Integral Gain

Start with a value of 10 and optimize the gain constantly while imaging. Typically, 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 air.

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 6.7 Engaging](#) on [page 135](#). In the Sum and Deflection Meter panel, click on the 'Simple Engage' button. Carefully lower the head until you hear the audible engage notification and the Z voltage is centered at approximately 70V.

Using the Trace/Retrace graph as a guide, decrease the setpoint until the tip is tracking the surface well. Adjust the Integral Gain and Scan Rate as needed.

8.4 General Procedure Using the Closed Fluid Cell

The Closed Fluid Cell allows for both fluid exchange and environmental control. There are separate instructions on how to use it, which come on a CD-ROM with your fluid cell. Please see the fluid-cell-specific instructions for how to operate

this piece of equipment. You can attach any substrate at the bottom of the fluid cell. The imaging parameters are the same as with the glass slide option.

Below is a recommended protocol on how to use it:

Assemble the Fluid Chamber

Please refer to the CD-ROM you received with your fluid cell for the specifics on its assembly.

Prepare the Sample

There is no difference in the sample preparation between the water droplet technique and that for the closed fluid cell.

Prepare the Substrate

Attach the substrate (mica, glass cover slip...) on the round glass slide that fits the bottom of the fluid cell. Depending on the size of the cover slip, Asylum Research also provides plastic adapters to hold the cover slips (for 12 and 25 mm cover slips). The cover slips may be held on the adapters using vacuum grease.

Set-Up the AFM

Insert the cantilever, focus the light on it and approach the tip close to the surface in air.

At this point, don't put the sample inside the fluid cell with the fluid cell empty until you feel comfortable with the process.

Insert the Sample

When you feel comfortable with the procedure without the sample in place, you are ready to insert the sample into the fluid cell. The rest of the procedure is the same as above; please see [pages 179 to 185](#).

8.5 Recipes for Imaging Samples

Imaging DNA in Liquid

Buffer and DNA Solutions

DNA is usually prepared on mica. Although both DNA and mica are negatively charged, the presence of a divalent cation in the buffer, usually Ni^{2+} or Mg^{2+} , acts as a bridge between the two negative species.

Make a pure solution of DNA in the following buffer:

1. Unmodified Mica

40mM HEPES-HCl

10mM NiCl_2

pH 6.5 to 6.8

As an alternative, DNA can be prepared in a TRIS/EDTA buffer and deposited onto silanized-mica. This might be preferred by researchers who do not want Ni^{2+} in the buffer, specifically for DNA-protein interaction experiments.

2. Silanized – Mica

10mM Tris/HCl

1mM EDTA

pH 6.5 to 6.8

The DNA concentration should range between 1 and 10 $\text{ng}/\mu\text{l}$, depending on the size of the DNA molecule to be imaged and the preferred density of the same (i.e. imaging vs. pulling).

DNA Sample Preparation

To attach DNA onto mica, first cleave the mica surface with a piece of tape: apply the tape to the surface and pull it. A flat shiny area should now be visible, free of any cracks. Incubate about 50 μl of DNA solution on the mica for 15 minutes. This is an approximate volume for a disc of mica about 0.5cm in diameter. The liquid should make a meniscus above the surface. There is no need to rinse. The sample is ready.

AFM Imaging

Use AC mode, with a silicon nitride cantilever (Biolever 60 μm long for example). Typical scan rate is 1 Hz, free amplitude of oscillation about 0.8 V and setpoint of 0.6V.

8.6 Sample Experiment: Atomic Force Microscopy Imaging of Bacteriorhodopsin

Introduction

High-resolution images of 2D membrane protein crystals (such as, porin OmpF, hexagonally packed intermediate (HPI) layer, aquaporin-1 (AQP1), Ø29 bacteriophage connectors, and bacteriorhodopsin) have been acquired frequently with the atomic force microscope. Due to the tight packing of the crystal structure in proteins, it is feasible to get nanometer lateral and sub-nanometer vertical resolutions at small scan sizes. These samples are quite challenging and test the capability (particularly, the low noise performance) of the AFM, especially when closed loop XYZ is enabled.

Bacteriorhodopsin is a light-driven proton pump, which accounts for approximately 75% of the inner cellular membrane of *Halobacterium salinarum*, known as the Purple Membrane. The remaining 25% of the membrane is composed of lipids. When the extracted purple membrane patches are incubated on mica substrate, the deposition of the purple membrane is non-specific. Therefore, it is possible to observe both the cytoplasmic and extracellular faces of the membrane surface.

The salt composition of the imaging buffer is crucial in obtaining high-resolution images. Under unfavorable salt conditions (i.e. low potassium chloride, or KCl) the long-range electrostatic double-layer (repulsive) force predominates and prevents the tip from approaching the sample surface. This results in poor structural resolution of the bacteriorhodopsin trimers. Additionally, a higher salt concentration functions to rigidify the membrane so that cantilevers with higher spring constants may be used.

In addition to acquiring high-resolution images of the bacteriorhodopsin trimers, reversible conformation changes of these trimers can be observed by adjusting the applied force of the cantilever onto the cytoplasmic face of the membrane surface. At a higher force (approximately 300pN), the trimers appear as distinct “donuts” consisting of 6 protrusions. At lower forces (<200pN), there is an increase in the height of the trimers and 3 more prominent protrusions become visible.

This document provides step-by-step instructions to:

Prepare a 50µg/ml imaging solution of bacteriorhodopsin

Set-up and operate the AFM

Analyze the images offline

Bacteriorhodopsin Solution Preparation

Bacteriorhodopsin from *Halobacterium salinarum* is now commercially available through Sigma-Aldrich as a lyophilized powder, catalog # B0184. Prepare the dilution buffer containing Tris/KCl, pH 8 (Table 8.1) and dissolve the lyophilized powder in a stock solution of 1mg/ml. A final solution of 50µg/ml is used for imaging.

Supplies:

Plastic storage container, 50ml volume

Microcentrifuge tubes, 1.5ml volume

Balance (plus weigh paper)

Pipet and tips

pH meter

Table 8.1 Chemicals Required to Prepare the Dilution Buffer

| Chemicals Required | Sigma Product # | Formula Weight (g/mol) | Molarity (mol/l) | Quantity Needed for 50ml (g) |
|---------------------------|------------------------|-------------------------------|-------------------------|-------------------------------------|
| Tris/Trizma base | T4661 (crystals) | 121.13 | 10mM | 0.061 |
| Kcl | 208000 (crystals) | 74.56 | 150mM | 0.559 |
| Purified Water | NA | NA | NA | 50 ml |

Tris/KCl Buffer Preparation

Prepare 50ml of the Tris/KCl buffer by combining 0.061g Tris and 0.559g KCl in a sterile container. These quantities have been calculated using the following equation:

Equation 8-1

$$\text{Weight (g)} = \text{Formula Weight (g/mol)} \times \text{Molarity (mol/l)} \times \text{Volume (l)}$$

Add 45ml of purified water to the container. Swirl the solution until the chemicals have dissolved.

Decrease the pH of the solution to 8 by adding HCl, dropwise, to the solution. Add additional purified water until the solution has a final volume of 50ml. Store in the refrigerator (4°C). This buffer should last several months.

Bacteriorhodopsin Preparation

A Stock Solution (1mg/ml):

1. Prepare a stock solution of 1mg/ml by adding 1ml of Tris/KCl directly to the Sigma vial.
2. Mix well, until all of the purple-colored lyophilized powder has dissolved.
3. Separate the solution into aliquots and freeze (-20°C). These solutions should last for several years in the freezer.

B Imaging Solution (50µg/ml):

1. Prepare an imaging solution of 50µg/ml solution by first sonicating one aliquot of the 1mg/ml solution for approximately 3 minutes.
2. Prepare 1ml (=1000µl) of a 50µg/ml solution of bacteriorhodopsin by combining 50µl of the stock solution to 950µl of Tris/KCl buffer in a microcentrifuge tube.
3. Store the solution in the refrigerator (4°C). It should last several months in the refrigerator.

AFM Imaging

Bacteriorhodopsin is imaged in fluid in either contact mode or AC mode. This protocol will provide instructions to image bacteriorhodopsin using Olympus probes, model # TR800.

Supplies:

Mica, 8-12mm diameter circle glued to a cover glass

Pipet and tips

Clock or timer

Scotch tape

A Standard (Fisherbrand catalog # 12-550A) or Teflon-coated (EM Sciences catalog # 63415-12) 25mm x 75mm x 1mm coverglass can be used.

Sample Preparation

Prepare a freshly cleaved surface of mica by pressing a piece of Scotch tape onto the mica and peeling the tape away, exposing a clean surface. Repeat this process, as necessary, until a clean layer of mica, free of cracks, is exposed.

Deposit first 10 μ l of the Tris/KCl buffer solution onto the freshly cleaved mica surface. Then add 10 μ l of the 50 μ g/ml bacteriorhodopsin solution.

Incubate for ten minutes.

Lightly rinse the sample using 100 μ l of buffer.

Add an additional 100 μ l of buffer to mica. Use the buffer solution to maintain a constant volume while imaging.

The sample is now ready to be mounted onto the sample holder for analysis.

Cantilever Preparation

To acquire consistent high-resolution images of bacteriorhodopsin, use the short Olympus v-shaped cantilever, model # TR800. The spring constant of this cantilever is approximately 0.57N/m.

Position an Olympus TR800 cantilever into the cantilever holder.

Break off the large cantilever. Add a drop of buffer to the cantilever to prevent air bubbles from forming under the cantilever once you place it in fluid.

Position the head onto the stage. Re-adjust the position of the SLD to optimize the SUM signal. Zero the deflection, if necessary.

Do a thermal tune; then do a regular tune. Select a peak to the left of the thermal tune. The selected drive frequency should be around 20-30 kHz ([Figure 8.9](#))

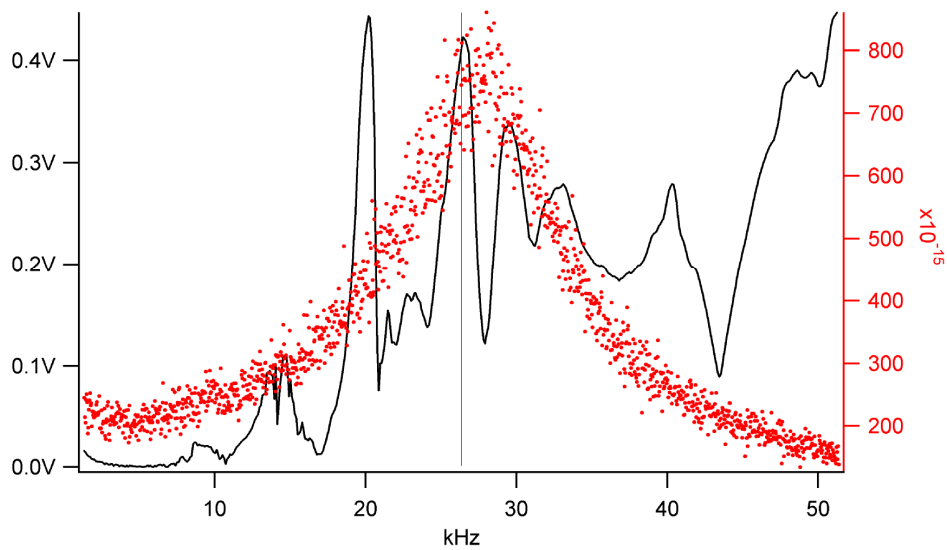


Figure 8.9 Cantilever Tune of the short TR800 cantilever in fluid

Once you are engaged on the sample surface, you may need to adjust the drive frequency so that you are still “on” the peak. This generally applies if you have tuned the cantilever a significant distance above the surface.

Imaging Parameters

1. Adjust the drive amplitude to get a couple hundred mVs of free amplitude.
2. Select an initial scan size of 5-10 μ m to locate a flat purple membrane patch. (Figure 8.10)

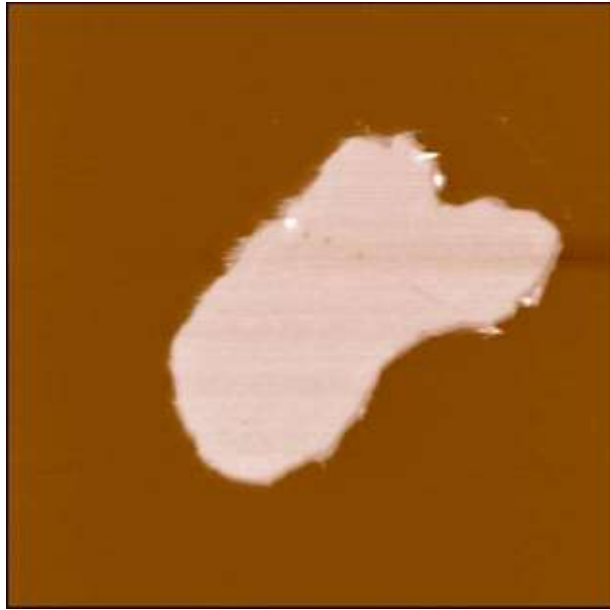


Figure 8.10 Purple Membrane Patch, 1.6 μ m scan size

3. Once a patch has been located, zoom in to visualize the crystal lattice. The largest scan size at which it is possible to begin seeing the lattice structure is approximately 500nm. The lattice is quite distinct in a 300nm scan (Figure 8.11).

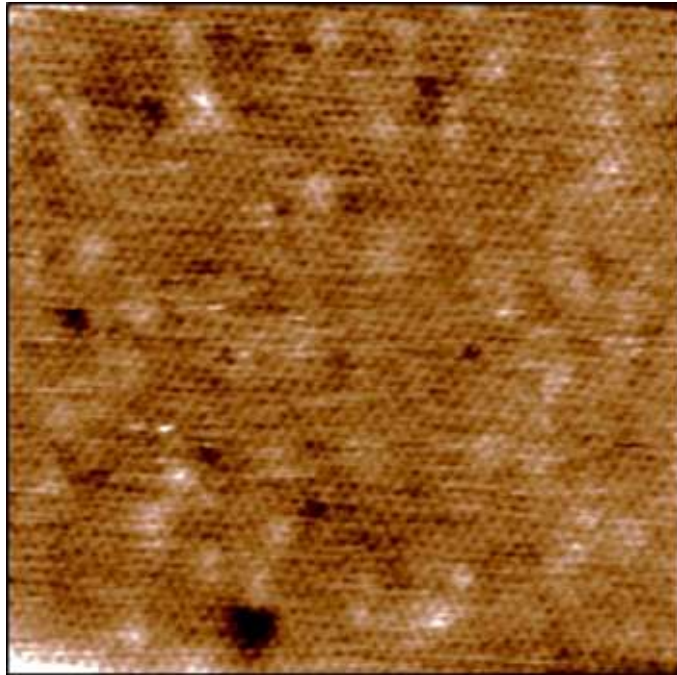


Figure 8.11 Close-up of Purple Membrane Patch (extracellular surface), 300nm scan size

4. As you zoom in on the crystal structure, decrease both the drive amplitude and the setpoint so that the final setpoint value is in the tens of mV. To minimize the force exerted onto the sample, first decrease the drive amplitude until the tip is no longer tracking the surface, and then reduce the setpoint until the tip begins to track the surface again.
5. Increase the scan rate so that the final scan rate is between 4-6 Hz.
6. Increase the Integral Gain to a value of 10-30, the highest value possible without seeing noise in the image.
7. Continue to zoom in to capture a 100nm scan (Figure 8.12 and Figure 8.13.) The trimer structure of the cytoplasmic face as seen in Figure 8.13 is consistent with previous data published where the applied force is low, typically under 200pN.

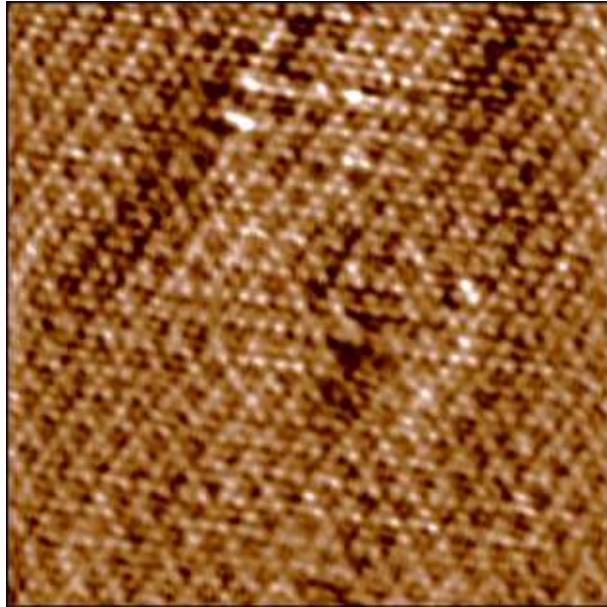


Figure 8.12 Close-up of the extracellular surface of the membrane patch, revealing the bacteriorhodopsin trimers, 100nm scan size

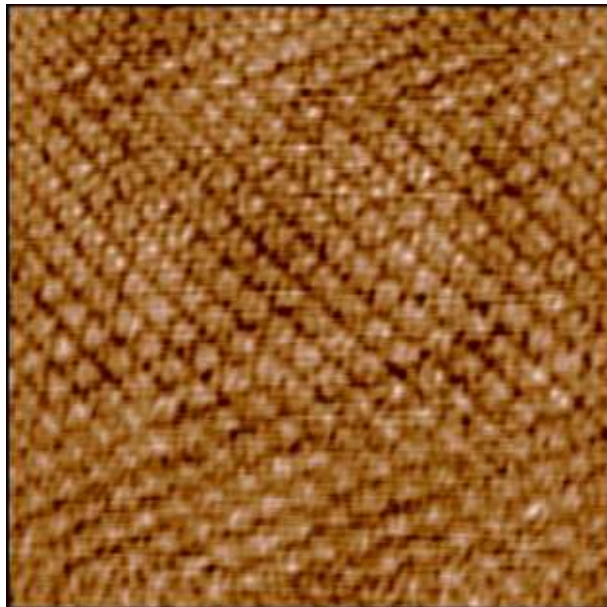


Figure 8.13 Close-up of the cytoplasmic surface of the membrane patch, revealing the bacteriorhodopsin trimers, 100nm scan size

General Notes

1. Typically for AC imaging in fluid, the R-value, located in the software under 'Programming-Make Filter Panel,' is set to 500Hz. Since the scan rate is set to a much higher rate than is normally used (up to 6Hz), a higher rate of 1.0 kHz is needed to avoid oscillations in the image.
2. For optimal imaging, the MFP-3D™ should be placed in a hood with its door closed.

AFM Offline Analysis

Data Filtering

In order to clearly observe the lattice structure on the extracellular or cytoplasmic surfaces, as seen in [Figure 8.12](#) and [Figure 8.13](#) a series of offline filtering steps must be done to the original image to enhance the trimer structure:

1. Flatten: 3rd order. Trimers are visible but not extremely clear ([Figure 8.14](#)).
 - a) Extracellular
 - b) Cytoplasmic

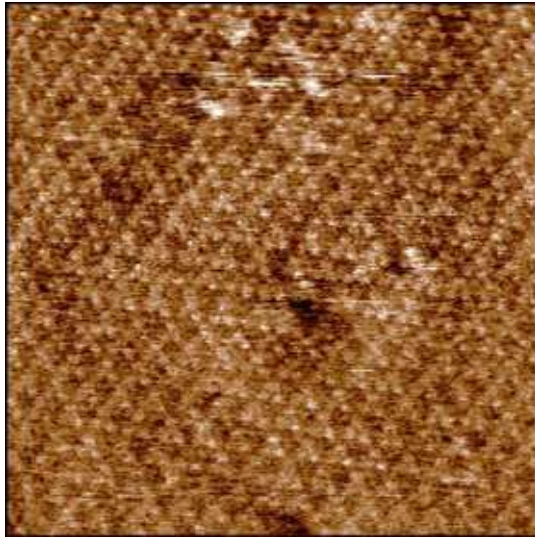


Figure 8.12

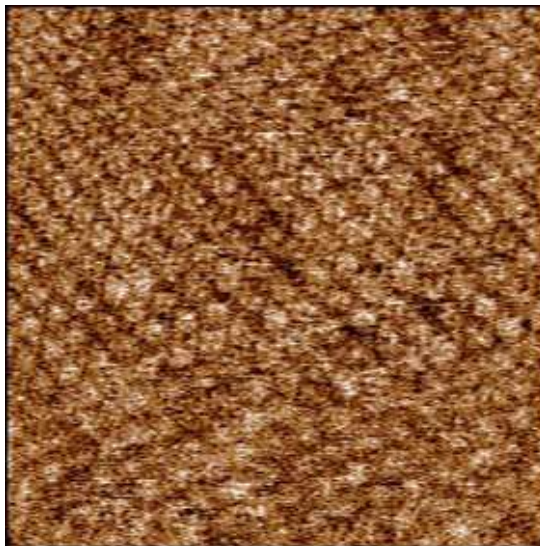


Figure 8.13

Figure 8.14 Images seen in [Figure 8.12](#) and [Figure 8.13](#) after 3rd order flattening only (100nm scans)

2. Gaussian filter: Size = 3. The edges are smoothed out ([Figure 8.15](#)).

- a) Extracellular
- b) Cytoplasmic

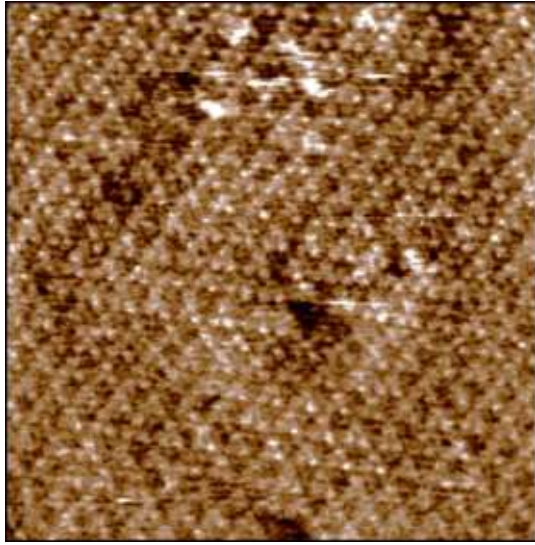


Figure 8.12

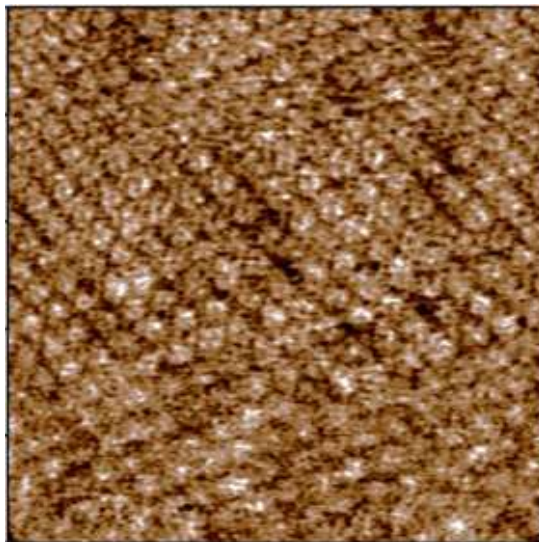


Figure 8.13

Figure 8.15 Images seen in [Figure 8.12](#) and [Figure 8.13](#) after 3rd order flattening and Gaussian filtering (100nm scans)

3. FFT: Using the “Exclude Points” function, noise not representing true topography is removed.

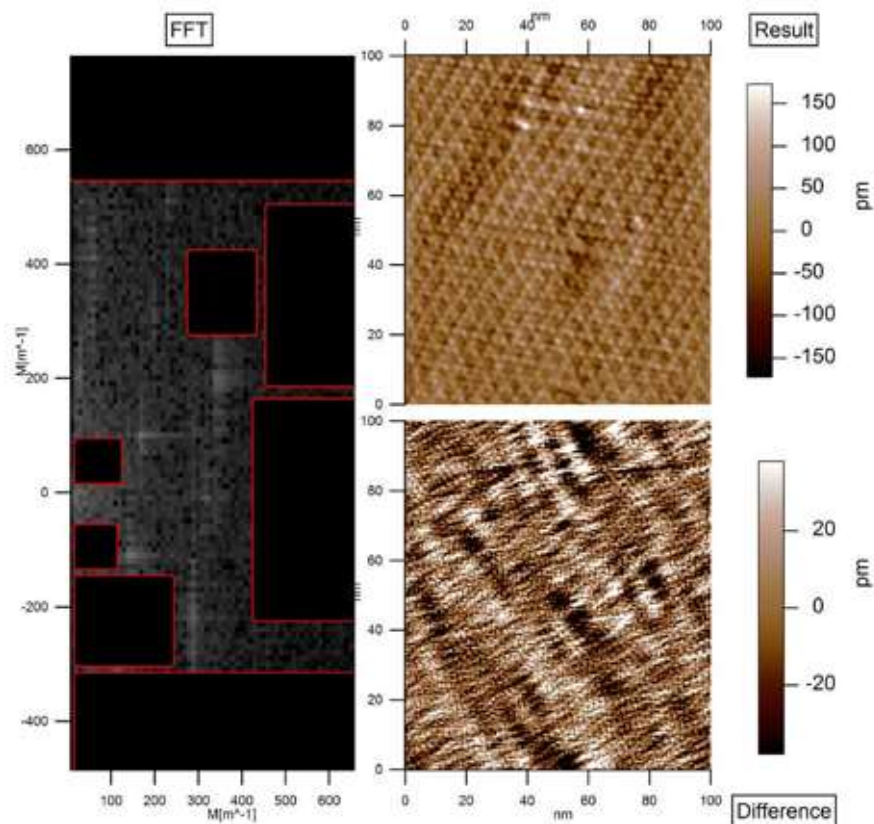


Figure 8.16 Images seen in [Figure 8.12](#) and [Figure 8.13](#) after 3rd order flattening, Gaussian filtering and FFT (100nm scans)

Height Analysis

The offline analysis software allows you to accurately measure the height of a membrane patch ([Figure 8.17](#)) and the width of an individual trimer ([Figure 8.18](#)). Existing data in the literature has shown that this height ranges from 5.2 nm to 11 nm depending on the buffer solution and sample substrate, and ranges from 5.82 \pm 0.2 nm with the specific salt conditions that were used for imaging (i.e. 10mM Tris-HCl, 150mM KCl, and pH 8).¹

For the dimension of the trimer, the measured width is 6.07 nm. Again this is consistent with the published value of 6.2 nm.²

a) Image

b) Section analysis, $dY = 5.81$ nm

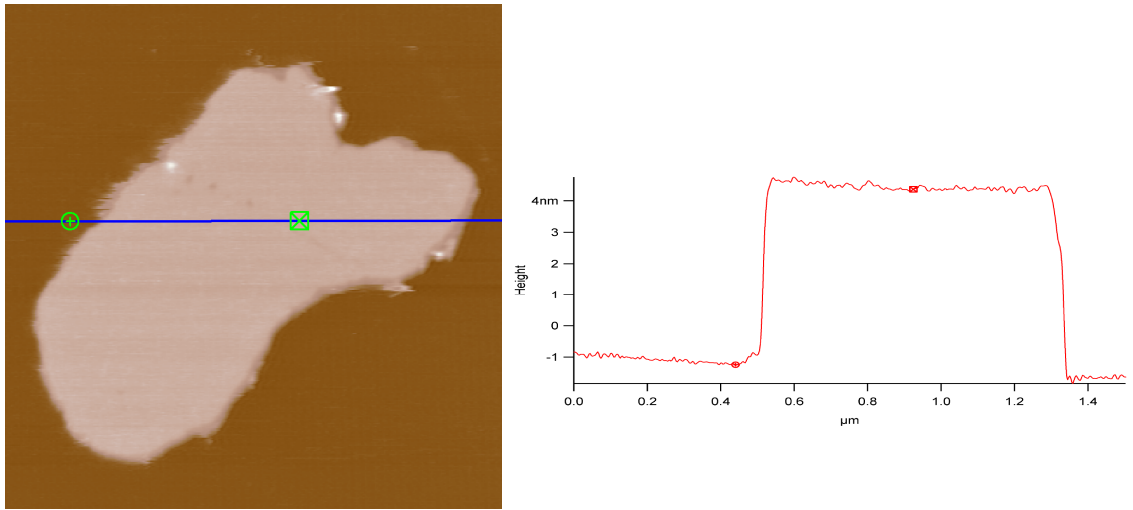


Figure 8.17 Cross-section of the patch seen in Figure 8.9

a) Close-up of trimers

b) Section analysis, $dX = 6.07$ nm

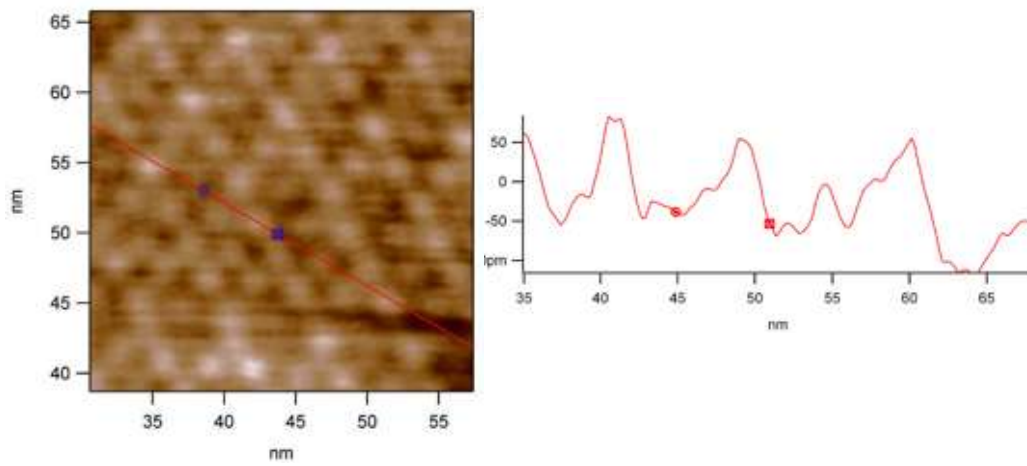


Figure 8.18 Dimension of individual trimer

Conclusion

These high resolution images of both the extracellular and cytoplasmic faces of the purple membrane obtained on the MFP-3D™ AFM support the argument that this system has low noise performance, even in closed loop XYZ. In addition, the software's extensive offline analysis options allow the user to process their data and measure key dimensions of their sample for presentation and publication purposes.

References

1. Müller, D. J., Amrein, M. and Engel, A. (1997). "Adsorption of biological molecules to a solid support for scanning probe microscopy." J. Struct. Biol. 119(2): 172-188.
2. Müller, D. J., Schoenenberger, C. A., Schabert, F. and Engel, A. (1997). "Structural changes in native membrane proteins monitored at subnanometer resolution with the atomic force microscope: a review." J. Struct. Biol. 119(2): 139-157.

8.7 Recommendations and Troubleshooting for AC Mode Imaging in Liquid

Recommendations for Successful AC Mode Imaging in Liquid

Introduction to AC Mode Imaging Cells in Liquid

Cells can be imaged in either contact mode or AC mode. AC mode is a little bit more complicated at first, but it may ultimately provide more details of the membrane. With the MFP-3D™ cells can be imaged directly in the Petri dishes where they have been cultured. They can also be imaged in the fluid cell or the BioHeater. If doing so, it is best to grow the cells on a round glass cover slip (regular: Fisher catalog #12-545-10225CIR; cell growth: 12-545-86 25mm circular). The glass cover slip can also be attached with epoxy or vacuum grease onto a glass slide. If available, use the round pieces of glass provided with the closed fluid cell.

The biggest challenge is to grow cells that are attached well to their substrate. If the cells are not adherent enough and are being removed by the AFM tip while scanning, some typical procedures may be used. You may need to use a biological adhesive such as poly-lysine (Poly-L-Lysine or Poly-D-Lysine), collagen, laminin or fibronectin. Additionally, 2% FBS (instead of 10%) may be used to promote

cell flattening. Some cells are also more difficult to image than others due to their size. If they are too tall, even if the Z range of the scanner is large enough, the cell membranes are moving underneath the tip, making the cells difficult to image.

Recommended Parameter Values and Conditions

Scan Rate

Imaging should usually be done very slowly, to minimize feedback errors that can damage the membranes (0.2-0.3 Hz).

Setpoint

Use small amplitudes (free amplitude of 0.3 V for example) or small deflections in contact mode ($\Delta V=0.5$ for example).

Cantilevers

Choose cantilevers with a low spring constant. As with Scan rate (above), small errors in the feedback can stress the membranes. Very sharp tips are not always the best since they apply large localized forces that can disrupt fragile membranes.

Sterile Environment

It is important to keep the cells free of contamination so that they can be imaged multiple times. When working with the MFP-3D™, the fluid cell can be sterilized in an autoclave. Directing UV light onto the tips might also decontaminate them from organic components.

Imaging Proteins in Liquid

Proteins are predominately imaged in AC mode. An exception is 2D protein crystal arrays that can withstand the lateral forces exerted by the cantilever in contact mode. Similar to other biological samples, it is critical to attach the molecules to the substrate well. To attach protein to negatively charged mica, it is recommended to adjust the buffer pH below the isoelectric point (pI) of the protein; this renders the protein to have an overall positive charge.

An alternative is to deposit proteins onto positively charged silanized-mica (which is a positively charged surface). This allows the protein to be imaged under more physiological conditions. Sample preparation varies a great deal depending on the protein. Typical protein concentrations range from 1-100 μ g/ml. Incubation times will also vary from several minutes (i.e. 10 minutes) to as long as 24 hours.

Troubleshooting AC Mode in Fluid

No SUM Signal/Bent Cantilever

Silicon nitride cantilevers tend to bend in liquid because they are very floppy. If that happens, the sum signal on the photodetector may not be seen. First verify that the cantilever is bent using the CCD camera. If it is bent, lift the front of the AFM head about 1cm to pull the cantilever off the liquid (See [Figure 8.19](#)). Then bring the head back down, and immerse the cantilever in liquid again. Repeat this process until the cantilever stays straight in the liquid.



Figure 8.19 This side view shows how to lift the MFP-3D™ head to straighten bent cantilevers or tap it to remove air bubbles. This procedure can also be used to eliminate bubbles stuck on the cantilever: tap the front leg a few times on the stage and check optically for the bubbles.

Air Bubbles on the Cantilever

If the cantilever is not bent and there is no sum signal, it could be that there is an air bubble on the cantilever. One way to get rid of bubbles is to gently tap the front of MFP-3D™ head 3 or 4 times ([Figure 8.19](#)). If this technique doesn't work, the best way to get rid of a bubble is to remove the head, remove the cantilever holder, carefully dry it with a wipe (being careful not to touch the cantilever!); add a few drops to wet the cantilever and then try again to place the head over the surface to immerse the cantilever.

Chapter 9 Contact Mode Imaging in Fluid

9.1 Overview

In this chapter, we will describe how to image samples in contact mode in fluid. Working in contact mode in liquid is similar to working in contact mode in air, except for the sample prep. In this chapter, we will only emphasize the differences between these two kinds of operation.

Introduction

Contact mode imaging in liquid is less frequently used than other imaging techniques. Samples that need to be imaged in liquid are typically biological samples, and generally samples of that type are imaged in AC mode. However, some samples, like living cells for example, may be imaged in either mode. Some users might prefer to use contact mode rather than AC mode in order to obtain high resolution when imaging samples such as bacteriorhodopsin membranes or lipid bilayers.

Working in liquid is, in general, also a good way to get rid of adhesion forces between the tip and the sample: many times, this is the effect that the user is seeking. This absence of adhesion explains the use of soft cantilevers in contact mode imaging in liquid (the same type of cantilevers used for contact mode in air or AC mode in liquid).

Prerequisites

The user is assumed to be comfortable working with contact mode in air and all functions described in the previous chapters before beginning to work with contact mode in liquid. In [Section 6.5 on page 131](#) a detailed description on how to load a sample for liquid imaging can be found. If the user needs to learn how to perform contact mode, guidelines are provided in [Chapter 7](#) and [Chapter 9](#).

| | |
|--|---|
| Set-Up & Operation | Chapter 5 on page 53 |
| Sample Preparation and Loading | Section 6.5 on page 131 |
| Contact Mode Imaging in Air | Chapter 7 on page 157 |

9.2 General Procedure

The steps in the general procedure should be followed in the order in which they are presented.

1. Prepare your sample (see [Section 8.3 on page 177](#))
2. Choose and install your cantilever in the cantilever holder. Typically, cantilevers with small spring constants are required. Values usually range roughly from 0.02 N/m to 0.2 N/m. As a first approximation, the softer the sample is, the softer the cantilever should be. But this choice will become more empirical as the user learns over multiple experiments how to image his specific sample.
3. Load the sample onto the sample stage. This part of the procedure has been extensively described in chapter 8 AC Mode Imaging in Liquid, [Section 8.3 on page 177](#).
4. Set-up the imaging parameters in the software (see [Section 9.3 on page 209](#)).
5. Engage the tip on the surface.
6. Optimize your image (see [Section 9.5 on page 209](#)).

9.3 Operation: Setting-Up the Software

Select 'Contact Mode' in the main panel. Set all the parameters exactly as you would set them for imaging in air. Just keep in mind that if you are imaging soft samples, it is probably better to choose a set point close to the initial deflection value (before engagement). It is advisable to choose zero as this initial deflection value, since the photodetector is more linear at the center.

9.4 Engage

Before approaching the tip toward the surface, place a drop of liquid (buffer or medium corresponding to the sample) on the tip. For detailed explanations, please refer to [Section 8.3 on page 189 in Chapter 8](#). The rest of the engagement procedure is exactly the same as it is for contact mode imaging in air.

9.5 Optimizing Parameters.

The main parameters that need to be adjusted during the scan are the **integral gain** and the **set point**.

Increase the integral gain until the tip is ‘ringing’ (you will see stripes on the image) and then decrease the integral gain until the image looks best.

Adjust the set point so that the tip is tracking the surface correctly: you should see no white stripes when it is scanning prominent features on the surface, and trace and retrace curves at the bottom of the image should overlap.

These signs of proper set point adjustment are true unless you are looking at big features: in that case, because of piezo hysteresis, the two curves (trace and retrace) corresponding to the height image will not completely overlap. In those cases, you will need to look at the Z sensor signal, which gives you the real topography of the surface. You can minimize the vertical force by decreasing the set point until the tip comes off the surface (this is easily recognizable on the trace-retrace graph: the two curves don’t overlap and obviously don’t follow the contour of the surface). Then increase the set point again, until a good image is obtained on the screen (and the trace and retrace curves overlap).

To optimize your image you can also vary the **scan rate** (usually up to 2Hz) or the **scan angle** and see how the image is affected.

9.6 Measuring Vertical Forces

First, image your sample and calculate the absolute value of the difference between the deflection before engagement (most of the time it will be zero) and the deflection when you image. To calculate the vertical force you are applying on the surface, you first need to take a force curve on a hard surface. By measuring the slope of curve when the cantilever is deflecting on the surface (repulsive part of the curve) you will know how much the tip is moving in nm for a given voltage signal coming from the photodiode. To learn how to measure this slope, please refer to [Section 10.3](#) on [page 229](#) in. Using the conversion factor you have obtained while making your force curve (i.e. the value of the slope), you will know the deflection of the cantilever in nm. If you calibrate your cantilever (see [InvOLS](#) and [Spring Constant](#) on pages [229](#) and [229](#)) then you can calculate the force using the simple Hook’s law:

Equation 9-1

$$F = k \times x$$

where k is the spring constant of cantilever in N/m and x is the deflection of the cantilever in nm.

Note that this type of calculation is valid only when the tip is not deforming the surface. The interpretation of force curves is much more complicated if the tip is actually inducing some deformation of the surface.

9.7 Troubleshooting and Maintenance

Troubleshooting

False engagement is the most common issue: during the engagement procedure, when the deflection decreases and meets the set point value, the tip should be engaged on the surface. However, sometimes the deflection is still drifting after you have stopped manually approaching the tip toward the surface. This is usually the sign of false engagement, when the tip is not tracking the surface. If you are working with a closed fluid cell, make sure the cantilever holder is not touching any part of the fluid cell. It is also possible that the chip of the cantilever is touching the surface before the cantilever itself makes contact. In that case, slightly lower one leg of the MFP-3D™ head: lower the corner of the head corresponding to the corner of the chip where the cantilever is located. It is also not uncommon that some bubbles attach themselves to the cantilever or the chip and prevent the tip from engaging. In that case you can try to gently ‘tap’ the AFM head as explained in [Section 8.7](#) on [page 207](#) in Chapter 15.

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 is not exhaustive:

1. Use soapy water and gently rub the holder with your finger, preferably wearing gloves. Make sure not to scratch the glass window through which the light beam travels. Rinse copiously with deionized water.
2. Follow the same procedure as in #1 (above), followed by two additional steps: a rinse with 70% ethanol in water, and a final rinse with deionized water.
3. Immerse the top of the holder (the Kel-f part) in ethanol and sonicate for 2 minutes.
4. Use a gentle plasma treatment: 30s at 50W and 0.15 torr.

Note that cantilevers are difficult to clean. You can try ozone treatment for 1 min, or plasma treatment, as mentioned above. Some authors are also using piranha solutions.

9.8 Advanced Topics

Imaging Living Cells in Contact Mode

Surprisingly, mammalian cells in culture can be imaged in contact mode as easily as they are in AC mode. It even seems easier sometimes, depending on the cells being imaged.

Below are guidelines on how to image living cells in culture.

Prepare the cell culture as explained in [Chapter 8](#) on AC Mode Imaging in Fluid and place the Petri dish on the sample plate.

Choose a soft cantilever and insert it into the holder. Place the holder in the AFM head and focus the light beam on it.

‘Scan Speed’ is the most important parameter to set. It is vital to be as gentle as possible when beginning the procedure, and choosing a low scan speed will assist in that. Choose a value of 0.3 Hz to begin. If the cells are easy to image, you can try later to increase this scan speed.

Place a drop of liquid on the cantilever and lower the head so that the cantilever is totally immersed.

CAUTION: Make sure there is not too much liquid inside the Petri dish: the medium should not flow over the bottom of the cantilever holder. For example, when using a plastic Petri dish of 6 mm in diameter, the amount of liquid should be 2 to 3 ml.

Zero the deflection of the cantilever and choose a negative set point, one that is very close to zero. A typical set point value is $-0.5V$.

Lower the head and watch the deflection.

When the tip encounters the surface, the cantilever deflects and the deflection increases, until it reaches the chosen set point. The system will beep to alert you that the tip is engaged on the surface.

Start scanning, optimizing your image by using mainly the ‘Set Point’ and the ‘Integral Gain’. Other parameters may also be used, such as the ‘Scan Rate’ and ‘Scan Angle’.

[Figure 9.3](#) on page [217](#) is an image of neuronal growth cones imaged in contact mode in liquid. Filopodia tips are clearly visible.

Sample Experiment: Imaging Cells Using Contact Mode AFM

Introduction

Living or fixed cells are frequently imaged with an atomic force microscope in either Contact Mode or AC mode. Both techniques can provide useful information on cellular structure. Typically, AC mode, which gently taps the surface, reveals features at the cell surface or just below the cell membrane. Contact Mode, on the other hand, applies more pressure onto the surface so that subcellular features such as the cytoskeleton are more prominent. Another advantage to Contact Mode is that the applied force onto the surface can be calculated after calibrating the cantilever's spring constant.

Most adherent cell lines can be imaged using the AFM. Either the MFP-3D-SA (Stand Alone system) or MFP-3D-BIO (Inverted Optical Microscope system) can be used. The MFP-3D-BIO is preferred since using the inverted optical microscope facilitates cell location and cantilever positioning over a desired cell. Researchers can also acquire additional data using optical techniques such as fluorescence so that a more complete understanding of cell structure and function can be attained. However using a Dual View MFP-3D-SA with the knobs set to "Bottom View" and "Top Illumination" allows you to observe quite clearly transparent samples such as cells.

Cell Sample Preparation

Sample preparation can be minimal, especially when imaging adherent living cells in culture medium. Cell can be grown on either coverslips to be used with the Closed Fluid Cell or BioHeater or in plastic (polystyrene) or glass bottom Petri dishes to be used with the standard or heated Petri dish plate. In order to have a number of available cells to image, cells can be grown to approximately 70-80% confluency but less is also acceptable if isolated cells are desirable.

One crucial requirement to successful imaging is cell attachment to the substrate. There are some cell lines that naturally attach well to glass (coverslips) or plastic (Petri dishes) which make sample preparation quite easy. However, other cells may require biological adhesives such as poly-D-lysine (preferred over poly-L-lysine since it is not susceptible to proteolysis), collagen, laminin, or fibronectin. It may be best to first prepare your cells as usual and try to image them. Then if the cells are easily dislodged by the cantilever the use of one of the above-mentioned adhesives can be used.

AFM Imaging: Set-Up/Parameters

It is recommended that prior to placing the cells on the AFM stage, the AFM has already been set up: 1) a new cantilever has been placed into the holder, 2) the light source has been aligned onto the cantilever and 3) the parameters in the software have been adjusted for cell imaging. This minimizes the time the cells are outside of the incubator without being imaged.

It is important to use cantilevers that have very low spring constants, typically less than 0.15N/m. Longer cantilevers, such as the 200 μ m long TR400s/RC800s or the 100 μ m long Biolevers, usually have such low spring constants.

Typical parameters are listed in [Figure 9.1](#). In order to apply the minimal amount of force, begin with a Deflection value of -0.5V and a Deflection Setpoint Value of 0V. Adjust the Integral Gain so that the highest value possible is selected before noise/ringing appears in the image. Another critical requirement is using a slow scan rate, typically between 0.25 up to 0.5 Hz depending upon the scan size. Since it takes a long time to acquire one image using the slow scan rates, acquiring images at a lower resolution (256/256) is recommended until a region of interest has been located and all of the parameters have been optimized.

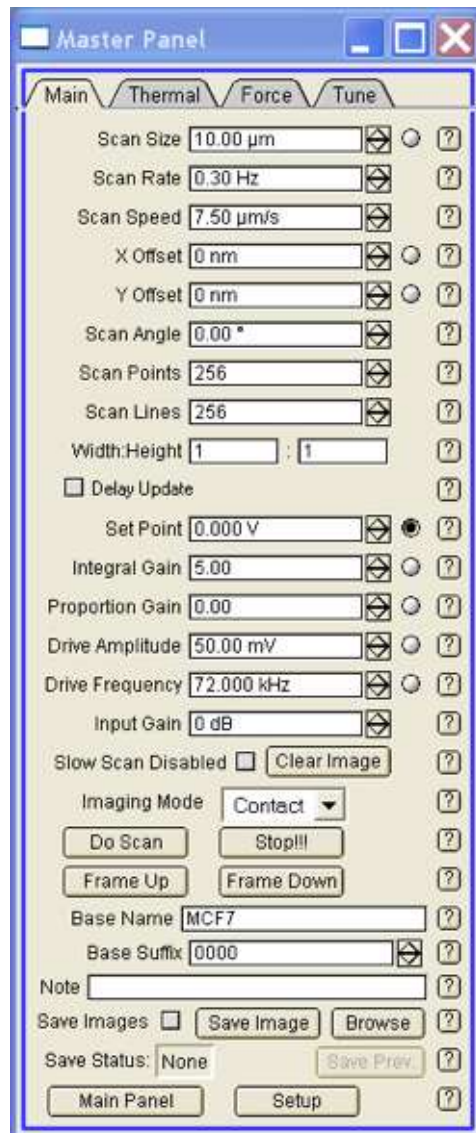


Figure 9.1 Recommended Parameters for Cell Imaging

Images

Typical AFM images of cells can be seen in [Figure 9.2](#), [Figure 9.3](#), and [Figure 9.4](#) (all below). [Figure 9.2](#) is of a living fibroblast cell (Cell line LL24) grown on a poly-L-lysine coated glass slide and imaged in contact mode in a PBS solution using an Olympus 200 μm long v-shaped TR400PSA cantilever (spring constant = 0.02N/m). The scan size is 27 μm . The top left image shows an optical phase contrast image of the cantilever scanning over the cell. The top right image is the

deflection (error) signal while the large image on the bottom is an Argyle rendered image of the sample topography.

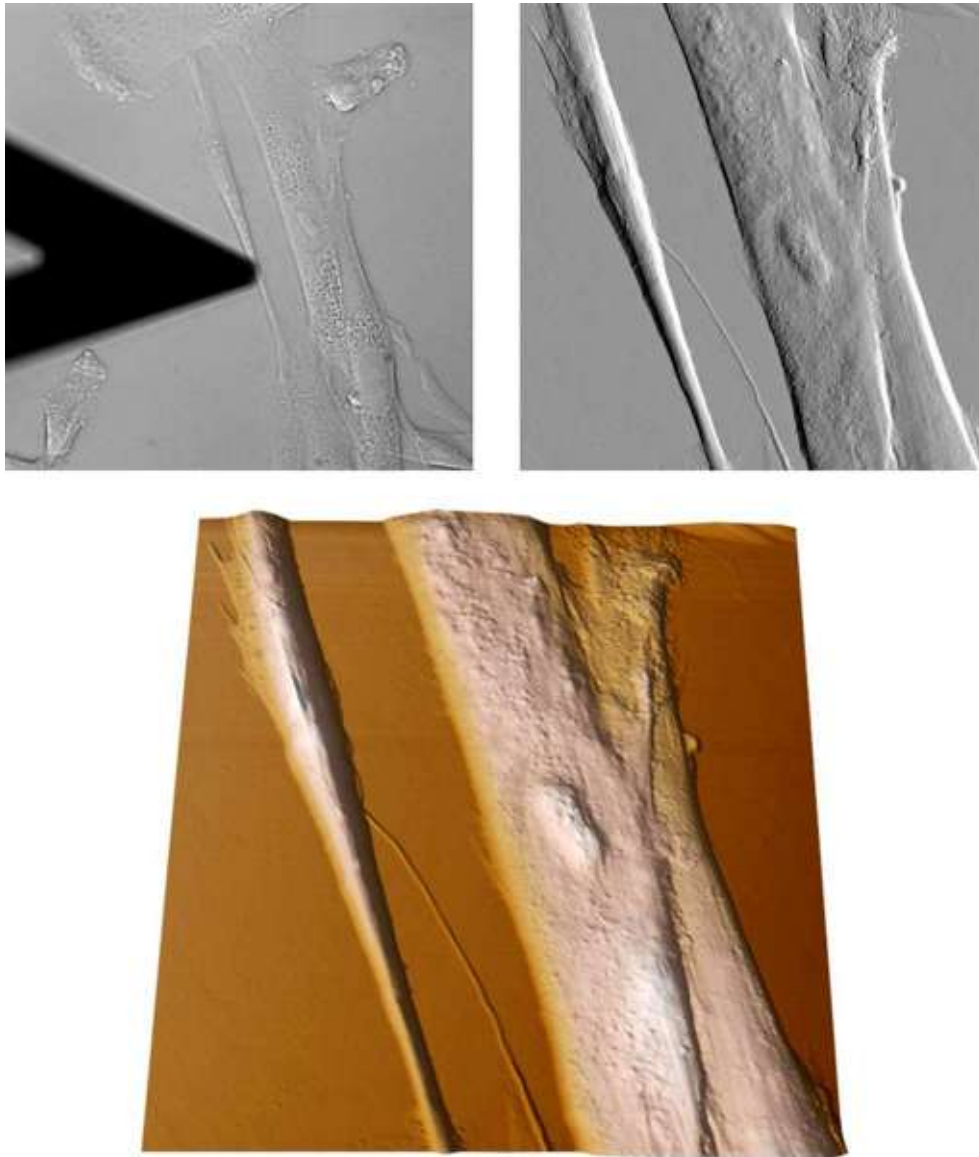


Figure 9.2 Simultaneous optical phase contrast image (top left), deflection error (top right) and rendered topography (bottom) of a live Fibroblast Cell

1. Fibroblast cells: 3 images. The brightfield image is cropped; it is not the same size as the AFM image but closer than the original.

Fixed fibroblast cells (MRC-5 cell line) acquired in contact mode using the 200 μm long Olympus TR400PB cantilever. The cells were grown in a Falcon petri dish and fixed with 4% glutaraldehyde. They were imaged with the new Petri Dish Holder Accessory on the MFP-3D-BIO. Brightfield illumination was initially used to locate cells of interest and accurate position the cantilever over the cells (a). AFM Deflection image (b) and 3D rendered topographic image using Argyle (c). 70 μm AFM scan size.

2. Cancer cells: description applies to both.

Live breast adenocarcinoma cells (MCF7 cell line) acquired in culture medium using AC mode. The image is of a fairly continuous monolayer of cells. The cell margins and cytoskeletal networks are clearly visible. 65 μm scan size.

3. Bio class fibroblast cell.

Live fibroblast cell imaged in culture medium using AC mode. 65 μm scan size. Image acquired during the Bio Class (May 2007) by J. Albuschies (ETH Switzerland) and M. Sorci (RPI).

Figure 9.3 reveals the detailed structure of the filopodia of the neuronal growth cones from the sea hare, *Aplysia*. The cells were plated on poly-L-lysine coated coverslips, fixed with formaldehyde and imaged in artificial sea water (ASW) using an Olympus 200 μm long v-shaped TR800PSA cantilever (spring constant = 0.15N/m). The scan size is $\sim 14\mu\text{m}$.

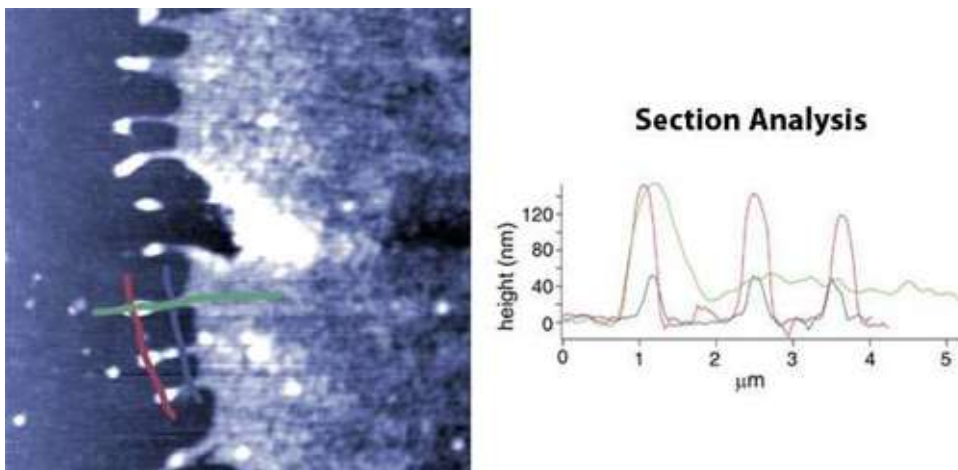


Figure 9.3 Fixed *Aplysia* Bag Cell Neuron. Height image of the leading edge of a growth cone acquired in Contact mode, 13 μm scan. Section analysis of filopodia reveals that the tips are taller and wider than the shafts. Gryzwa et. al. (2006), Journal of Neurobiology. Courtesy of G. Lee, Purdue University.

Figure 9.4 is a contact mode image of a living cardiac myocyte. A neonatal rat ventricular myocyte was harvested and plated onto a layer of polydimethylsiloxane (PDMS) coated over a glass coverslip. The myocyte was imaged using a 40X phase-contrast objective and the MFP-3D PHIL accessory (optical image). The cell was scanned in contact mode with a silicon nitride cantilever. At various points during the scan, the heart muscle cell began beating; causing jumps in some of the height scan lines across the image. AFM data shown is the Z-Sensor image which has had a zero-order flatten applied to it.

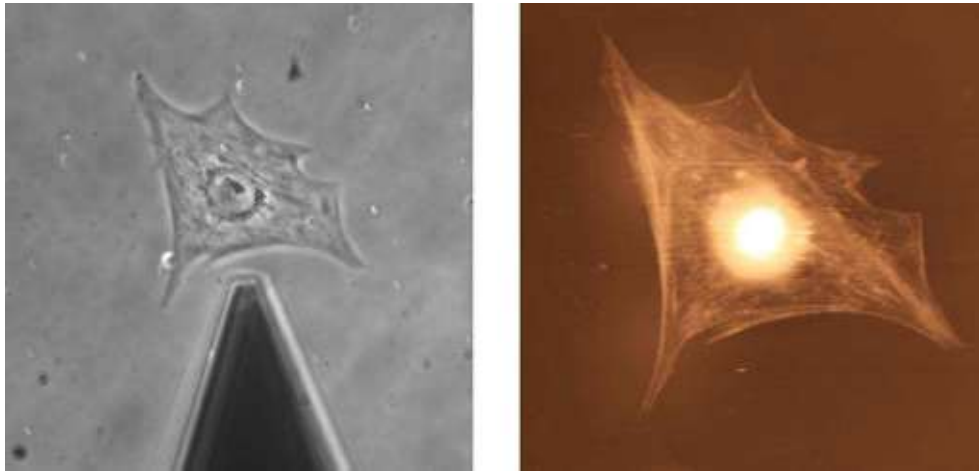


Figure 9.4 Simultaneous optical phase and contact mode topography of a Living Cardiac Myocyte. Image Courtesy of Dr. Nicholas Geisse and Prof. Kevin Kit Parker, Harvard University School of Engineering and Applied Sciences.

9.9 References

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Pesen, D. and J.H. Hoh. (2005) "Micromechanical Architecture of the Endothelial Cell Cortex: Biophys. J. 88: 670-679.

Source Figure 9.1: High- resolution analysis of neuronal growth cone morphology by comparative atomic force and optical microscopy. E.L. Grzywa, A.C. Lee, G.U. Lee, D. Suter. Journal of Neurobiology, In Press. Reproduced with permission from the authors and Journal of Neurobiology.

Chapter 10 Force Plots

10.1 Overview

This chapter introduces force plots and explains both what they are used for and how to work with them.

10.2 Theory: Getting Oriented

Prerequisites

It is assumed that you have worked through the following sections:

Optical Lever Detection [Section 5.2 on page 59](#)

Engaging [Section 6.7 on page 135](#)

Moving on the Nanoscale: Piezo Actuators and the Nanopositioning System (NPS™) [Section 5.2 on page 55](#)

Actuation and Navigation [Section 5.2 on page 55](#)

What Is a Force Plot?

A force plot (also referred to as a force curve) is the act of collecting data as the Z piezo is ramped towards the surface and away from it. The term originated from the era when deflection data (which can be converted to units of force See: [Terminology in Section 10.2 on page 221](#)), was the primary data collected. With the MFP-3D™, there is no need to collect deflection data, but the operation is still referred to as a force plot. In this section, force curves are discussed from the point of view of collecting deflection data, as that comprises the majority of the work being done with force plots.

Force plots are often used to measure the interaction forces between the tip and the surface. This is done by pushing the tip against the surface, and then separating the tip and surface. From this data, a number of things can be studied, such as adhesion, indentation and polymer elasticity. It is advised to review the sections on Data Channels, [Section 5.9 on page 95](#), and Optical Lever Detection, [Section 5.2 on page 59](#), to understand exactly what the deflection data is.

Terminology

It is not necessary that you read this section in order with the other sections. It is here to provide a reference glossary of the force plot terminology.

Extend (also called Approach).

This is the part of the force curve where the tip is approaching the surface. In other words, the Z piezo voltage and the Z LVDT signal are becoming more positive.

Dwell

This is the section of the force curve where the Z piezo voltage is maintained at a constant measurement for a period of time. This can be done either between the change from extend to retract, or after the retract section of the force curve. Note that the controls for this section of the force curve are hidden by default; to view those controls, go to the Force tab of the Master Panel and click on 'Set-Up'. See the "?" button to the right of the controls on the Force tab for more details.

Retract

This is the section of the curve where the tip is pulled away from the surface. In other words, the Z piezo voltage and the Z LVDT signal are becoming more negative.

Reverse Pull

This is a force plot that switches the order of extend and retract. By default, the tip first extends, and then retracts. A reverse pull first retracts and then extends. The idea is to leave the tip in contact with the surface, and then do a reverse pull that separates the tip and surface. This way the time between pulls is also the time the tip is in contact, which allows the tip to remain in contact for a much longer period of time. This behavior is controlled by the radio buttons to the upper left of the Force Panel (Click on any of the Help buttons near the top of the panel to get to the "Forward / Reverse Pull" Help Topic in the MFP-3D™ Software Help Menu).

Drive and Drive V

This is the name given to the Z piezo voltage. Drive is measured in units of distance while DriveV is measured in units of voltage applied to the Z piezo. The conversion between the two is done with the calibration constant ZPiezoSens. See [Table 10.1](#).

Raw RawV, and RawLVDT

This is the Z LVDT signal. Raw and RawLVDT are synonymous and have units of distance. RawV is the original voltage unit of the signal. The conversion between them is done with the calibration constant ZLVDTsens. See [Table 10.1](#).

LVDT and LVDTVolts

This is where some confusion may occur. In the force software, we refer to a polynomial fit to the LVDT signal as LVDT. We take the Raw data and fit it to a 7th order polynomial over the regions where the Z piezo voltage was linearly ramped. Then that 7th order polynomial is taken and is called it the LVDT signal. This removes the noise from the signal, but leaves the low frequency data you are probably more interested in obtaining. LVDTVolts is simply stated in units of Raw Voltage, while LVDT is stated in units of distance, just like Raw and RawV. See also: [Table 10.1](#).

Tip-Sample Separation

When working with force plots, it is often useful to plot the data in units of distance between the tip and the surface. To do this, you subtract the tip deflection from the Piezo position. The real-time software will not calculate this for you, but the offline software will. For more detail on this subject, contact the Asylum Research software support staff at support@asylumresearch.com.

DeflV, Defl and Force

These three data types are from the same deflection data, which comes from the segmented photodiode detecting the deflection of the cantilever. The original signal is collected in units of volts (DeflV), and is then converted to units of distance (Defl) with the InvOLS value (See Optical Lever Detection in [Section 5.2](#) on [page 59](#)). Finally, the Defl data is scaled by the spring constant to get the Force data in units of Newtons.

Getting Oriented

Understanding the coordinate system may be somewhat difficult. This is partly due to the difference between the Z Piezo Stage and the Z , so be sure to review [Section 5.2](#) before attempting to do so. Also the differences between the data types that are most often used as the X-axis (Drive, Raw, LVDT and tip-sample separation) may cause more confusion, so be sure to read about those terms in [Section 10.2, Terminology](#) on [page 221](#). And finally read through Open Loop vs. Closed Loop Scanning in [Section 5.2](#) on [page 53](#). Force curves can be acquired in both open and closed loop modes.

Open loop force curves are generated by telling the controller to drive the piezo actuator with a predetermined voltage, usually a ramp. The ramps are entered as distances (Drive) units, based on the ZPiezoSens. What that means is that the distances entered are only estimates because the piezo behaves non-linearly. Then as the piezo is driven through a voltage ramp, we collect the LVDT signal (RawV), and convert that to a distance (Raw) with the ZLVDTsens. This way we know accurately where the piezo went. Then for most of the displays we fit a 7th order polynomial to the Raw signal and use that fit (LVDT). Remember that each of these signals have the same orientation: positive is towards the surface, negative is away from it. See [Table 10.1](#), which also summarizes the differences between the channels.

Table 10.1

| | Range | Zero Means | Conversion | Also used for: |
|--------|---|--|---|-------------------------------|
| DriveV | -10, +150 V | Zero volts on the z piezo | None | Piezo Voltage |
| Drive | -500 nm, +12 μm (roughly) | Zero volts on the z piezo | DriveV*ZPiezo Sens | Start Dist, Force Dist |
| RawV | -6, +6 V (roughly) | LVDT is in middle of range (you have close to 70 V on Z piezo) | None | Mostly hidden |
| Raw | -7 μm , +7 μm (roughly) | See above | Raw*ZLVDT sens | Real-Time and offline display |
| LVDT | -7 μm , +7 μm (roughly) | See above | 7 th order polynomial fit to Raw | Real-Time and offline display |

Once again, each of these signals is more positive the closer it is to the surface, and more negative the further away it is. Zero does not however indicate the surface level.

Closed Loop Force Plots

For more information on this topic, please contact support@asylumresearch.com.

Anatomy of a Force Plot

What can a force plot tell you? Here is a generalized break-down of the various regions of a force curve and what information they offer:

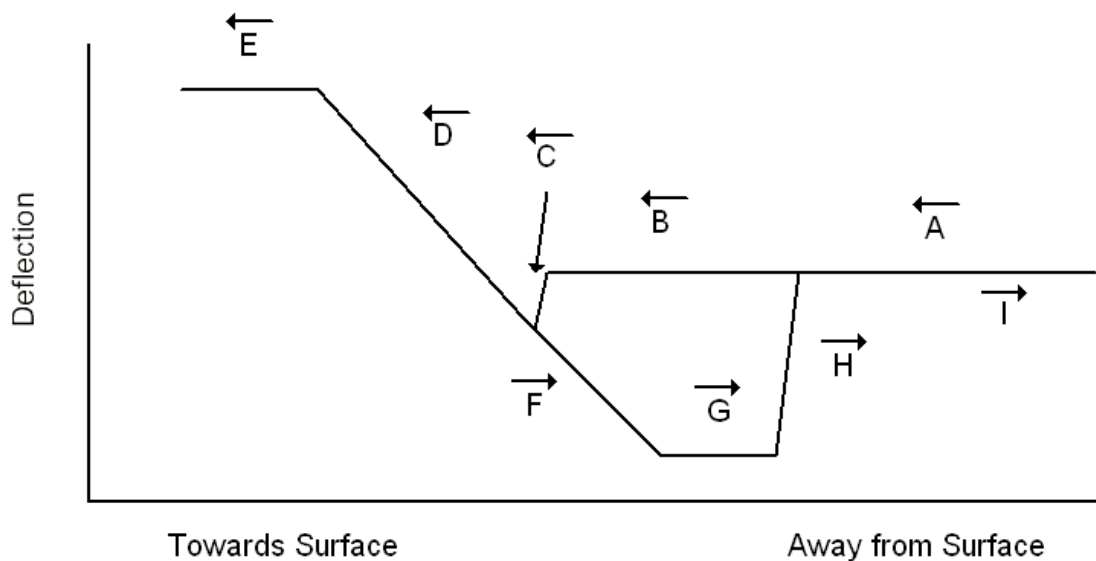


Figure 10.1 Regions of a Force Curve

- A** Away from the surface, the tip starts moving towards the surface. This is generally considered the zero force region where the tip is far enough away from the surface so that there is no appreciable force acting on it. Of course the distance from the surface depends upon the user, but generally a micron or two is enough distance to minimize the force. This does not mean that the deflection value is zero; it means that the rest of the values of the deflection are relative to this data.
- B** The tip is getting fairly close to the surface and may exhibit long-range forces. If there is a gradual curve down, then there is undoubtedly some long-range attractive force at work, such as electrostatic forces in the air. If there is a gradual curve up, then there is a long-range repulsive force, such as a polymer brush or double layer in liquids.

- C** The tip jumps to contact the surface. The gradient of the short-range attractive forces become larger than the spring constant, and the tip gets sucked into contact with the surface. If there were a weak attractive force between the tip and surface, and/or if you had a larger spring constant, you may not see this step.
- D** The tip is in contact with the surface. This region may provide you with indentation data. By knowing the movement of the piezo and the deflection, you can then determine how much less the tip was deflected than the piezo was moved. This is how much the tip has indented the surface.
- E** The photodiode has saturated (we called it ‘railed’). You have pushed so far that the tip has deflected to the point where the photodiode is reading +10 V. It’s a good idea to display the DeflVolts data at this point, just to be sure. If you do see this region, then it is advisable to decrease the distance you push against the surface.
- F** The tip is retracting, but it is stuck against the surface due to the adhesion, so that the lever is deflected downward.
- G** The tip is held very strongly against the surface, and as you pull it away it is deflected so much that the photodiode is railed at -10 V. (See [Figure 10.1](#) region E). If you see this you need to either decrease the adhesion between the tip and surface, or increase the spring constant by picking a different lever. If you are working in air, then you probably have capillary adhesion between the tip and surface. But you will not have capillary condensation if you put the tip-surface system in water, as that greatly decreases the adhesion.
- H** The energy stored in the lever is enough to pull it off the surface and as a result, the tip jumps free of the surface. If you can see region G, you’ll be able to discern how much force this jump requires (at least an estimate of it), but doing so is quite a bit more work. If you are concerned with getting the adhesion force from region H, then getting rid of region G will make that much simpler.
- I** The end of the force curve, where the tip returns to "zero force." This region may contain additional downward deflections and jumps back to zero, which are most likely polymer-stretching events. These occur when polymer chains attach to the tip after it has jumped away from the surface (See [Figure 10.1](#) region H).

What Is a Triggered Force Plot?

The trigger function prevents the tip from pushing too hard on the surface. The user will use a “trigger point” which is a value of the deflection, or force, or other parameter of choice. As the tip approaches the surface, when the deflection (or

other parameter) reaches the trigger point, the tip is retracted away from the surface. In other words, hitting the trigger point will only change direction from extended to retracted, or if you are doing a reverse pull, change it from retrace to extend. If it hits the trigger point before starting the force plot, then it will not do a pull at all, and will print out in the history as "triggered at start." If it never reaches the trigger point in the force curve, it will act the same way as a simple force plot. If it does reach the trigger point, it will change directions there, and the resulting force plot will have fewer points than was originally suggested by the software because it took less time to complete the pull. The piezo velocity is maintained as constant (technically as the rate of change on the piezo voltage), but the scan rate will be greater than entered if it triggers.

How Do You Set-Up the Trigger Point?

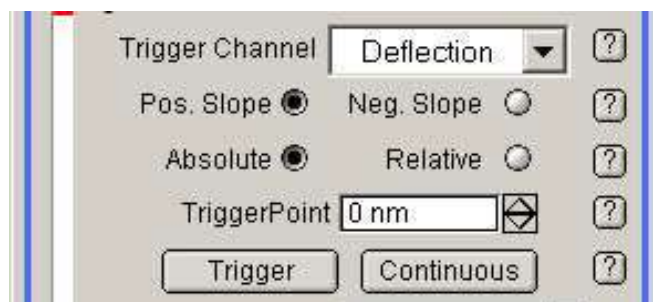


Figure 10.2 Setting-Up the Trigger Point

Here you see five rows of controls. The top one selects the channel from which you want to trigger; as an example, let's pick DeflVolts for simplicity. In the next row, positive and negative slope are only used for absolute triggers (located in the third row). If you pick an absolute trigger, and you know that the trigger point is higher than the current state of the channel, then select positive slope. If your trigger point is less than the current state of the channel (for example, amplitude), then pick negative slope. Absolute triggers are fairly straightforward; they will trigger if that channel reaches that level. Relative triggers take the trigger point entered and calculate the absolute trigger based on the state of the channel when the trigger button is clicked. For example, if you have the photo detector, or PD, knob set so that the deflection volts is +3, and you want to trigger at +6 volts, you can either enter 6V for a positive slope absolute DeflVolt trigger, or +3 for a relative DeflVolt trigger. (Remember that the slope is ignored for the relative triggers.) The Trigger and Continuous buttons start the triggered pull, or start doing multiple triggered pulls. See the help buttons (they appear as question marks) on the Force Panel for more up-to-date details.

10.3 Contact Mode Force Plots

Here we will set-up the software to run a simple contact mode force plot in air. It is assumed that you have read [Chapter 7](#) so that you have a flimsy cantilever and a hard sample loaded, and the tip is fairly close to the surface.

Saving Force Data

Check the data output settings.

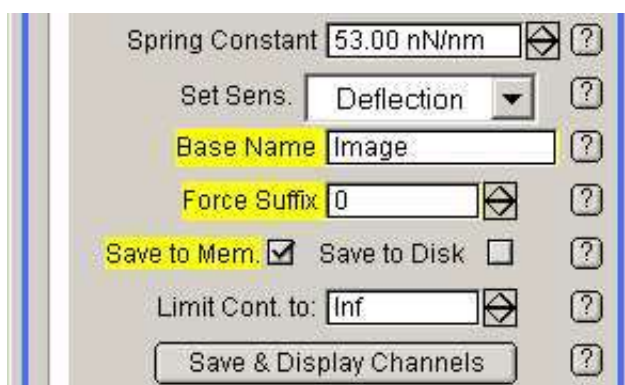


Figure 10.3 Saving Force Plots

There are two options for saving Force Plots. You can either save them to memory or to disk. Saving them to memory, which is the default setting, puts them in the Igor experiment. This means that if you save the experiment (File > "Save Experiment As" menu), then all the force plots in the experiment at that time will be saved in the packed experiment file (pxp) you just saved. This also means that you must be careful that you do not modify them in ways you do not want them modified. In the offline force, there are 'Undo' and 'Restore' options. 'Undo' however will only turn back as far as the last change made. So if, for example, you change the InvOLS, and then change the spring constant, the software will NOT remember the original value of the InvOLS (only the last change can be undone). The 'Restore' options restore to the hard drive copy, but if you save your data directly to memory, the software will not know where to locate it (even if you simultaneously save to disk). It can only do a restore if the data was loaded from the hard drive. However you can save all the waves that are in memory to the hard drive with the offline code. The MFP-3D™ software maintains the disk copy as a reference, and the memory copy is used as the working version to which you may make any changes you wish.

The other things to consider here are the BaseName and the suffix. The BaseName is the name of the files that will be created. Since there is no note in the real-time software, this is probably the best bet for keeping the data organized. The Force Suffix is simply a number that will be incremented to keep each file name unique. The trick to working with the Force Suffix is knowing that if you go back to an old BaseName, it will reset the suffix to 0, which can overwrite your old data. It is imperative therefore, to maintain a naming protocol and avoid overwriting stored data files.

Set the Start and Force Distances

The next step is to set-up the Start and Force distances on the Force panel.

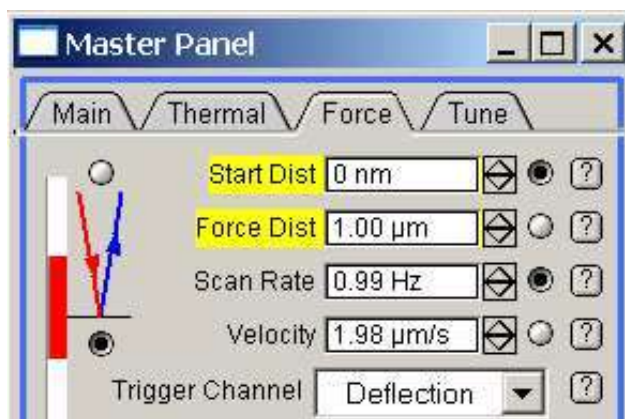


Figure 10.4 Start and Force Distances

There is a little trick to this as well. The red bar to the left is the display bar for the Z piezo. The white region represents the entire piezo range, so that the bottom of the bar is towards the surface, +150V, and the top is away from the surface at -10 V. The red part of the bar is the region over which the force plot will be done: the top of this region is called "start distance." So the force plot will start at the top of the red region, and turn around at the bottom of the red region. As you have already done a simple engage you should know the Z voltage at the sample surface (from the Sum and deflection meter). Most of the time simply eyeballing the display bar is enough to get fairly close to the surface.

Now everything is set in place and you are ready to click on 'Single Force' to do your force curve.

InvOLS

Now that you have done a force curve on a hard surface, let's go through the steps required to calibrate the InvOLS. If you are unsure what InvOLS is, go back and review [Section 5.2](#) (on [page 59](#)) on the optical lever detection method.

1. Bring up your real-time Deflection vs. Raw graph.
2. Hit 'Ctrl + I' to show the Igor cursors.
3. Place the round (A) cursor on the trace in the region where the tip is in contact with the surface, and place the square (B) cursor on that trace as well. Make sure that both cursors are in the same region (either in Extend or in Retract). The easiest way to do that is to push the left or right arrow key and check to see that both cursors move in the same direction. If they move in different directions, then they are in different regions and need to be adjusted until they occupy the same region.

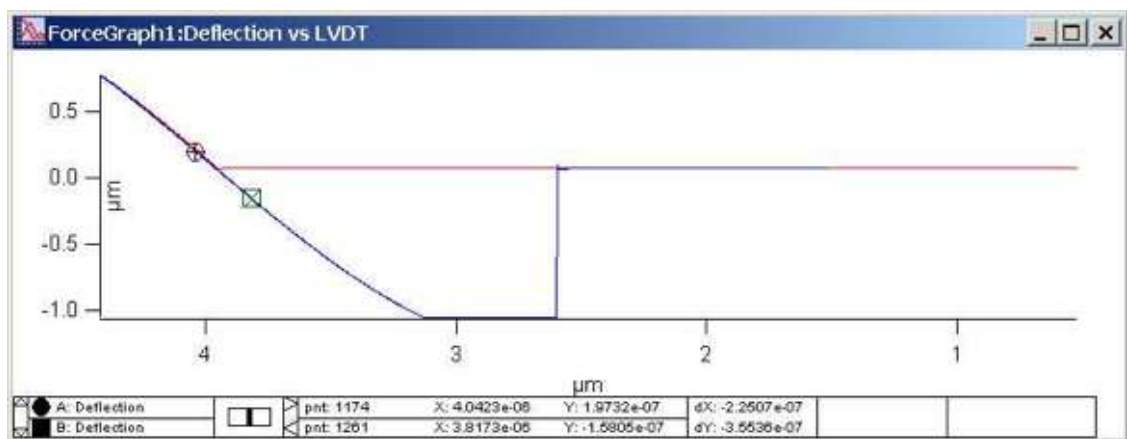


Figure 10.5 Force Graph

4. Once you have the cursors correctly situated on the graph, click on the 'Set Sens' pop-up and select 'Deflection.' That fits the region between the cursors to a straight line and uses it as the InvOLS. Once you are satisfied with that fit, right click on the black line between the cursors and select 'Remove Fit_Deflection.'

Spring Constant

With InvOLS calculated, you can calibrate the spring constant. It is generally a good idea to move the tip away from the surface while you do this (a turn or two up with the front wheel should be sufficient).

Thermal Power Spectral Density (PSD)

Measure the Thermal Power Spectral Density (PSD) to determine the resonant frequency of the cantilever and to confirm that the cantilever and light source are aligned, which indicates the system is functioning properly.

NOTE: Each '?' button to the right of a control opens a Help file describing its purpose and use.

1. In the Master Panel, click the Thermal tab to bring it to the front of the window (See: [Figure 10.6 Master Panel, Thermal](#))
2. Using the drop-down menu, choose your desired resolution.

NOTE: Default resolution is 7. For best (but slower) resolution choose 1.

3. In the Master Panel, click DO THERMAL.

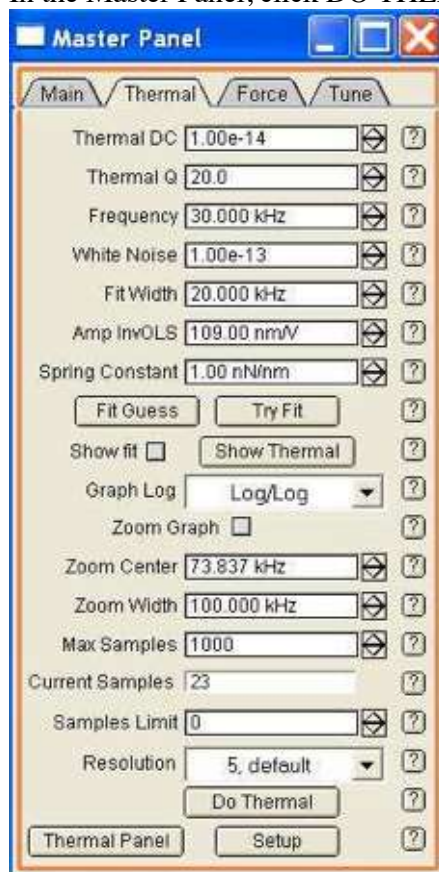


Figure 10.6 Master Panel, Thermal

As the system runs the Thermal PSD, it collects the deflection data using Fourier transform. The results are plotted as the deflection amplitude in $\text{m}/\sqrt{\text{Hz}}$ vs. the frequency in Hz.

4. After a few tens of samples you will notice the graph does not change much. Click 'STOP THERMAL.' (See: [Figure 10.6](#))

| | |
|--------------|--|
| NOTE: | This may take a few tries because the software is busy collecting data. If there is no clear peak, the light source is not aligned with the cantilever, or the cantilever may be touching the surface. |
|--------------|--|

Next you need to identify which peak is from the first harmonic of the cantilever. The very narrow peaks are most likely electronic noise spikes. As a general rule of thumb, it is safest to choose the lowest frequency strong peak and one that has some appreciable width to it. It is also useful to refer to your cantilever manufacturer's specifications to get a ballpark range of the frequencies to consider.

Once you have chosen a peak, the next step is to zoom in on that peak. Type the frequency of the chosen peak (a rough value of it, there is no need to be exact) in the Frequency Center. Note that this is actually the drive frequency, so if you have done a cantilever tune it is probably very close. In the Frequency Center, enter a value that will allow you to see more than the width of the peak. Then click 'Zoom Graph.' This zooms the axis center to the frequency center and sets the X-axis range to be the same as the Frequency width. Now that the graph is focused on a range, click on 'Fit Guess.' This takes the data that is shown in the current data limits and gives a starting estimate for the fit. This is NOT a fit: it merely sets the initial conditions for the fit, and by so doing, gives the curve fitting routine a much better chance to converge. If the blue fit line (the 'Show Fit' check box should now be selected) looks fairly close, then click on 'Try Fit.' That does the actual fitting. And it also uses the fitting parameters to calculate the spring constant. If the 'Guess From Fit' estimate looks really off, either try to adjust the Frequency Center and frequency width to show only the peak of interest and then hit 'Fit Guess' again, or enter new fit parameters manually and get the initial estimate closer to the data.

10.4 AC Mode Force Plots

Overview

Force plots in AC mode are fairly similar to those done in contact mode. There is no feedback, so the only real difference is that the tip piezo is driven. The only

thing you need to do differently for force plots in AC mode is that you do need to perform (see [Tuning](#) on [page 129](#).)

Anatomy of an Amplitude Plot

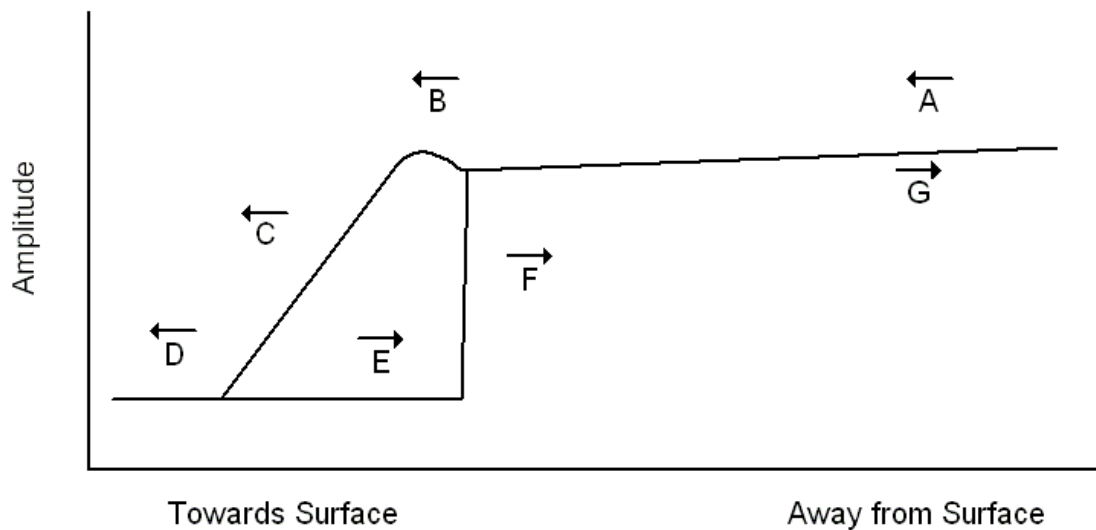


Figure 10.7 Anatomy of an Amplitude Plot

- A** Moving toward the surface, there is often a gradual decrease in the amplitude due to long-range damping from the air and surface (squeeze film).
- B** This little bump is not always as apparent as it is shown here because the short-range attractive interaction between the tip and the surface will decrease the resonant frequency of the cantilever. If you are driving the tip at a frequency that is less than the resonance of the lever (away from the surface), then as you get close to the surface, this decrease in the resonance will mean that you are driving it closer to resonance. That in turn means that the amplitude is increasing. If you are driving the lever higher than the resonance, region B will show a gradual decrease in amplitude.
- C** A linear decrease in amplitude with separation, caused by short-range repulsive tip-surface interactions. This region is used to calibrate the Amp InvOLS, (See Section 10.4 Calibrate Amplitude InvOLS on page

233.) Also, this region is generally where you would look to pick a setpoint for AC mode imaging.

- D** You have pushed the tip far enough against the surface so that it is stuck there, and is therefore not oscillating anymore.
- E** As you switch directions due to the adhesion of the tip and surface, the tip will remain stuck to the surface for a while.
- F** Once enough energy is stored in the lever to overcome the adhesion energy, it jumps free of the surface, and starts to oscillate freely again.
- G** The lever continues on its return path to its point of origin.

Calibrate Amplitude InvOLS

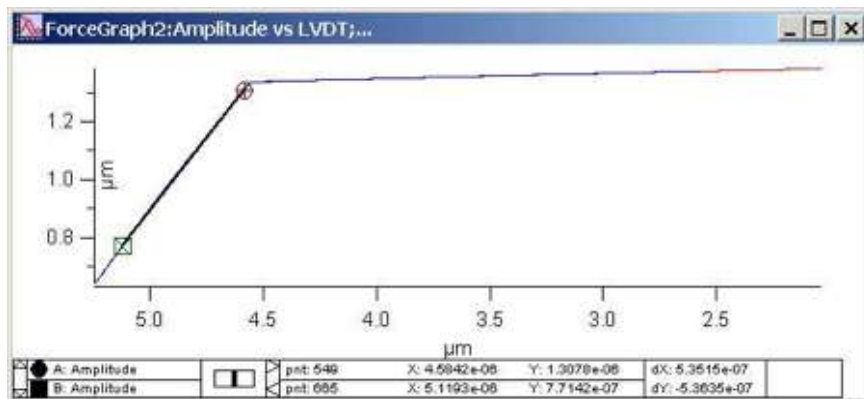


Figure 10.8 Calibrating the Amplitude InvOLS

Calibrating the Amplitude InvOLS uses essentially the same process as described in [Section 10.3](#) on [page 229](#), except that cursors are placed on the Real-Time Amplitude vs. LVDT graph.

To recap the process:

1. Bring up the Real-Time Amplitude vs. LVDT graph
2. Place the two cursors on the decrease in the amplitude as it approaches the surface.
3. Make sure they are both in the same region (either Extend or Retract)
4. Select 'Amplitude' from the Set Sens pop-up on the Force tab of the Master Panel.

5. When done, right click on the black line and select 'Remove Fit_Amplitude.'

10.5 Advanced Topics

Go There: Picking Where You Want To Do A Force Plot

One of the most flexible tools of the Force Plot controls is the 'Go There' button. This powerful button allows you to pick in which X-Y position on the surface you want to do a force plot.

Assuming you have taken an image, either in contact mode ([Chapter 7 on page 157](#) or [Chapter 9 on page 208](#)) or AC mode ([Chapter 6 on page 121](#) or [Chapter 8 on page 176](#)), from there.

1. Click on the 'Show Tip Location' (Optional but advised.)
2. Click on 'Pick Point.'
3. Now your topmost real-time image graph has a cursor on it so that you can move it to the position where you want to take the force plot.
4. Click on 'That's It': This sets that point in memory. You can return to this point at any time simply by entering the spot number in the 'Spot Number Set Variable' control. Note that zero is the reserved spot number for the center of the image.
5. Select 'Numbered Markers' from the spot display. (Also optional but advised.)
6. Click on 'Go There' and if Step 1 was done, a little red dot will show you that the X-Y scanner has moved so that the tip is now over the spot you marked.
7. Do your force plot before thermal drift moves things out of alignment.

The X-Y Spot locations you set are BaseName dependant. When you change to a new BaseName, you will need to reset your spot locations. If you return to an old BaseName, you start with whatever Spots you had set for that BaseName.

If you want to copy a set of Spots from an existing BaseName to a new one, here is how to do it:

1. Go to the Data Folders menu and select 'RealForce'
2. Type in:

Duplicate/O OldBaseNameSpotX, NewBaseNameSpotX

Duplicate/O OldBaseNameSpotY, NewBaseNameSpotY

Where OldBaseName is the BaseName that has the spots you want to copy, and NewBaseName is the New BaseName you just entered into the BaseName control and to which you want to copy the Spots.

3. Turn the Spot Display off and then back on again to get the software to correctly update the plot .

Force Maverick: Custom Force Plots

Force Maverick allows you to program custom force plots. You should be well versed in the standard force plots before working with Force Maverick (FMV). FMV breaks force plots down into drive and feedback events, so that you can set-up a force plot to drive towards the surface, turn on a feedback loop, and then when some condition is met, drive or do another feedback, and so on. If your force plot can be accomplished with the standard force controls, it is advisable to use those. Note that there is a 200 ms delay between events during the Maverick pull, while the next event is set-up.

For help on Force Maverick, refer to the Igor Help files or please contact Support@AsylumResearch.com.

Hamster Pulls: Real-Time Control of the Z Piezo

The MFP-3D™ has a user function that allows control of the position of the Z piezo with the hamster knob. This can be an easy and intuitive way of doing custom force plots. To get more information on this, please contact Support@AsylumResearch.com.

Force Mapping

There is a user function available from Asylum Research that sets up a grid of points within which to perform force plots. To get more information on this, please contact Support@AsylumResearch.com.

Chapter 11 Lithography

11.1 Overview

This chapter provides a definition of lithography as well as introducing the user to its specialized software, and the means of using it.

11.2 Theory and Getting Oriented

Prerequisites

It is assumed that the following sections have been read and understood::

Imaging Basics [Section 5.11 on page 107](#)

It is not required, but for advanced use a working knowledge of the crosspoint switch is very useful. To review the material on the crosspoint switch, see [Section 5.8 on page 93](#).

What is Lithography?

Lithography is the movement of the tip in XY, while at the same time maintaining a Z feedback loop. Through a variety of possible mechanisms this XY movement alters the surface. Examples include dynamic plowing, where the tip is used to gouge out a surface; biasing, where the tip is used to oxidize and/or etch the surface; and dip pen, where a layer of molecules are deposited wherever the tip has passed. With these mechanisms, it is possible to create nanoscale drawings or specific structures on your surfaces.

Terminology

It is not necessary that this section is read in order with the other sections. It is here to provide a reference glossary of the lithography terminology.

Litho Sections

Litho sections (also referred to as segments) are individual paths drawn on an image. The tip is held at a constant setpoint for the duration of the drawing of this path. The entire lithography pattern will most likely be comprised of numerous Litho sections.

Litho Groups

This is a collection of a number of Sections. The system will maintain the same setpoint only during the Sections, and will switch to the "Normal Setpoint" between them. The overall pattern drawn on the image can be comprised of numerous groups. The major difference between litho sections and litho groups is that groups can be manipulated with the Litho Groups tab (See [Section 11.4](#) on [page 240](#)).

Litho Step

This function allows the user to build topography grids to ramp the tip on the surface. It is intended that the setpoint or contact time would be changed between the points. The litho step is chiefly intended as a tool to determine at which setpoint and/or contact time an individual experimental set-up will best work. See [Section 11.5](#) on [page 241](#).)

Litho Setpoint

The setpoint for the Z feedback (either Deflection or Amplitude) that will be maintained during the Litho section(s).

Normal Setpoint

The setpoint for the Z feedback (either Deflection or Amplitude) that will be maintained between the Litho sections.

GDS

Graphic Data System, referred to as GDS, is a binary file format (technically GDS II) that is commonly used in technical drawings (much like AutoCAD files). There are import routines to allow GDS II files to be imported into the Lithography Groups (See [Section 11.4](#) on [page 240](#))

Getting Oriented

The easiest form of lithography to master is the so-called dynamic plowing method, where the tip is used to gouge out a path along the surface. Probably the easiest sample on which to do this is polycarbonate. The surface of polycarbonate can be polished to a fairly smooth surface by heating the sample above the glass transition temperature.

11.3 Operation: Setting-Up the Software (MicroAngelo)

MicroAngelo is designed to allow freehand drawing. Presently the software allows contact mode dynamic plowing, as well as contact mode and AC mode DC bias to the tip (AC mode refers to the Z feedback). Additional modes can be added if the need arises; to request the addition of other modes please contact Support@AsylumResearch.com for more details.

Collect the Image

Before drawing a lithography pattern, an image of the surface should first be collected to serve as a background along which to draw. This is not required, only recommended. It is necessary however, to at least begin with an image, so that the real-time image is the correct size. This image does not need to be collected in the same mode in which the lithography is done; it is possible, for example, to take an AC mode image and do contact mode lithography on it.

Draw the Path on the Image

Make sure that the image to be used in the lithography is topmost, and then bring up the Lithography Panel (from the MFP Controls -> Lithograph Panel menu). Click on 'Draw Path.'

Click on the real-time image and draw the path. Click after each section is drawn. Specifically, first drag the mouse around the image to mark the path, and then let up the mouse. At that point, it is necessary to click once somewhere in the image to finalize that section. (Once finalized, the section turns blue.) Then proceed to drag the mouse around the surface to define a second section, a third, etc. The first mode that the section is in (when it is red) is actually Igor's Edit mode. This means it is possible to click on the little square red markers and drag around the various points. This is not recommended however, as it destroys the uniformity of the point density, which means in turn, that the tip velocity is not going to be uniform. The software is designed to give the best possible uniform point spacing, but any interpolation that is done slightly changes the original path drawn (hard angles will be made softer).

Once all the sections have been drawn, return to the Lithograph panel and click 'Stop Draw.'

Enter the Setpoints

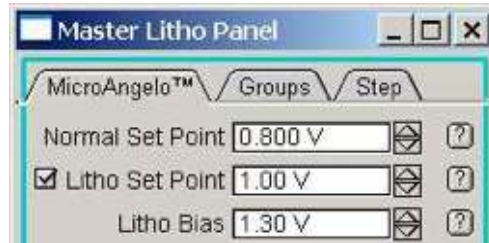


Figure 11.1 Master Litho Panel

Normal setpoint is the setpoint between sections.

Litho Setpoint is the setpoint during the sections.

NOTE: If working in AC mode lithography, make sure a cantilever tune was done before you begin. (See [Section 6.4](#) on [page 129](#)).

To work in voltage modes, the tip is biased during the lithography sections. The default choice is the Normal Setpoint during the lithography sections, since the writing is being done with the bias (Litho bias setvar). If a different feedback setpoint is wanted in voltage mode, just check the box to the left of the Litho Setpoint control (See [Figure 11.1](#) and also [Section 11.6](#) on [page 242](#).)

ADVANCED NOTE: An entire wave may be specified as the setpoint. In this way, the setpoint can be changed during lithography sections. To specify a wave as a setpoint, check the 'Setpoint Wave' checkbox. The interface allows for some simple functions to calculate setpoint waves.

Enter Velocity and Scan Time

Enter the maximum velocity the tip should be allowed to travel. This will not be a constant velocity across the surface, since the point spacing is not constant for the section drawn, but it will generally be fairly close to constant. Consequently the estimated time is an under-estimation, as is the time between sections. If the desired amount of time the process can take is entered, Igor will automatically calculate the Maximum Velocity.

Run the Lithography Pattern

Everything is now in place to run the lithography pattern. Click on 'Do It' on the MicroAngelo tab of the Lithography panel. To save deflection and height data during the lithography, select the 'Save Data' checkbox. For more details, click on the "?" button to the right of the 'Save Data' checkbox.

11.4 Operation: Setting-Up the Software for Litho Groups

Introduction

As stated in 11.2.3, litho groups are a collection of litho sections. They are intended to be treated as a single entity (hence the name). The controls on this panel are chiefly intended for replicating and positioning the various groups.

Importing and Creating Groups

There are three ways to import a litho group:

1. Use the 'Make Group' button on the MicroAngelo tab, which converts the drawn sections into a single group. Then click on the 'Save Group' button, which will ask for a name for the new group.
2. Import a picture (tiff, bmp, etc.) with the 'Load Picture' button on the Groups tab.
3. Import a GDS II file with the 'Load GDS' button on the Groups tab.

Displaying Groups

In order to use a group, it must first be positioned where it is wanted. The first step is to select the desired group on the Groups tab of the Lithography panel. Then click on 'Display Groups.' This displays the group as a set of red traces on the real-time images. Now it can be rotated, shifted, and scaled with the use of the various sliders on the Groups tab. Once it is in place, it must be set. This is done with the 'Add Group' button on the Groups tab. Once that has been done, the set of traces should change to blue. This step may be repeated as many times as desired; there is no limit to the number of groups within the image.

Running Groups

At this point in the process, litho groups work in exactly the same way as litho sections. Please refer to [Section 11.3 on page 239](#) for these instructions.

11.5 Operation: Setting-Up the Software for Litho Step

Introduction

This function allows the user to build topography grids to ramp the tip on the surface.

Setting Number of Gridpoints

After the image has been taken, or at least scaled to the real-time image as described in [Section 11.3 on page 238](#), the number of grid points to do must be decided. Enter the number of X and Y points desired in 'X Count' and 'Y Count' on the Step tab of the Litho panel. At this point, the grid may be appended to see how it will look on the image.

Setting the Time and Voltage Parameters

Once the number of gridpoints has been decided, the time and voltage parameters should be set. To set the time first click 'Edit Time' then click 'Update Time'. Either use the controls on the Step tab to calculate times, or enter them manually in the table.

Setting the voltage is done in essentially the same way as for the time wave. Its meaning depends on what litho mode is in operation: if it is a voltage mode, where bias will be applied to the tip, then the voltage wave is the bias applied to the tip. If a dynamic plowing mode is in use, then the voltage is the setpoint for the Z feedback loop. The most important thing to remember about step litho voltage is that it will be ignored if the check box to the left of the Volt Start is NOT selected. If that checkbox is not selected, then the Litho setpoint is used for either the Z feedback or the tip bias. If it is selected, then whatever data is in the wave is used. Note that each grid point corresponds to a data point in the time and voltage wave, with point 0 starting from the lower left corner. Point 1 is the next point in the first row. So if you have 2 points in X and 10 points in Y, then point 0 is the lower left, point 1 is the lower right, point 2 is the second row, left side, and so on. See also [Section 11.6 on page 242](#).

Check the Normal Setpoint

Once time and voltage have been set, double check the normal setpoint to make sure that it is a consistent number. Set the normal setpoint to an amount that will withdraw the tip from the surface. Doing that will ensure that the tip is not interfering with the surface between steps.

Engage

All parameters have been set, and it is now time to proceed to the actual lithography. Just click 'Do It'.

Review the Result

Typically the step litho is done before imaging, to see which set of conditions created the best spot.

ADVANCED NOTE: To see which grid point is at which time and/or voltage, it is possible to quickly change the way that Igor displays it.

From the command line (with the Real-time image of interest in the window):

```
SetDataFolder(GetDF("Litho"))
```

To view the time:

```
ModifyGraph  
textMarker(LithoStepY)={LithoTimewave,"default",0,0,  
5,0.00,0.00}
```

To view the voltage:

```
ModifyGraph  
textMarker(LithoStepY)={LithoVolts,"default",0,0,5,0  
.00,0.00}
```

To return to the original view:

```
ModifyGraph textMarker(LithoStepY)=0
```

11.6 Advanced Topics

Electrical Lithography

In this mode, a bias is applied to the tip during the writing of the segments. Turn this on by setting the lithography mode on the MicroAngelo tab to 'Contact Voltage' or 'AC Voltage'. One possible point of confusion here is that AC voltage in this instance does not refer to alternating current voltage, but rather, the AC here refers to the Z feedback loop, where it will be maintaining an amplitude setpoint while applying a DC bias to the tip. The contact voltage mode will maintain a deflection setpoint on the Z feedback loop while maintaining a DC bias on the tip. The code is written to apply C% output bias to the tip. The user C DAC has a 72 Hz low pass filter on it, so it should be a low noise DC power supply. (Note that "C %" and "user C DAC" are synonymous.) To apply an

external bias to the tip, please contact Support@AsylumResearch.com for details on the specific head in use.

More detail on how to run in these modes is described in Section 11.3. Remember that when the litho mode is set to a voltage mode, then by default the litho setpoint is NOT used. The Z feedback will maintain the normal setpoint during the write as well as between segments. During the write, Litho bias will be applied to the tip. For a different Z feedback setpoint during the write, check the small checkbox to the left of the Litho setpoint. That will ensure the Z feedback to maintain the litho setpoint during the write, and the normal setpoint between segments, as well as applying the litho bias during the write.

NanoManipulation

NanoManipulation is the act of moving nanoparticles across a surface. This can easily be done with the MFP-3D™ by using its proprietary lithography software. Nanomanipulation is often accomplished by drawing a spiral pattern on the surface, in such a way as to make a number of grazing passes at an object. It is much easier to move objects by brushing them across the surface, rather than trying to plow them in a direction with the tip. The directional arrows on the lithography pattern are useful tools for doing this since the direction moved along the path will alter how the particle moves.

An example of setting-up to move a carbon nanotube is given.

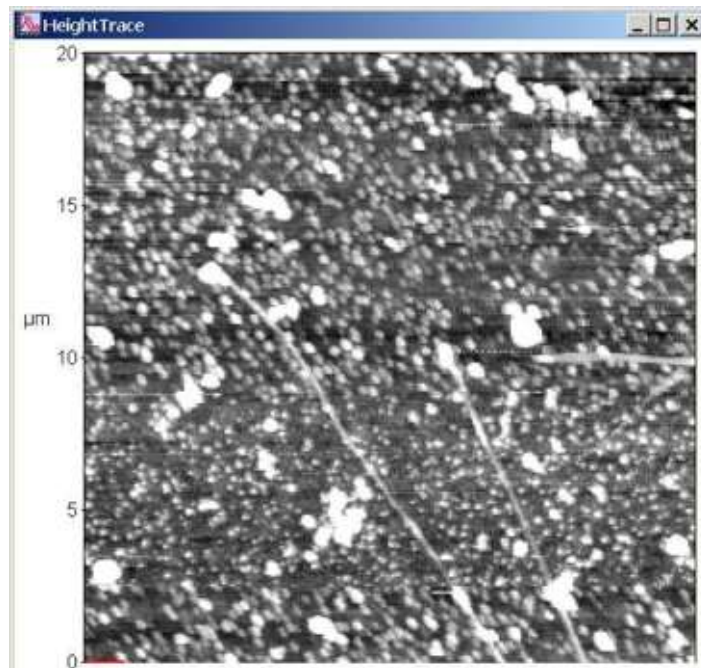


Figure 11.2 Pair of Carbon Nanotubes

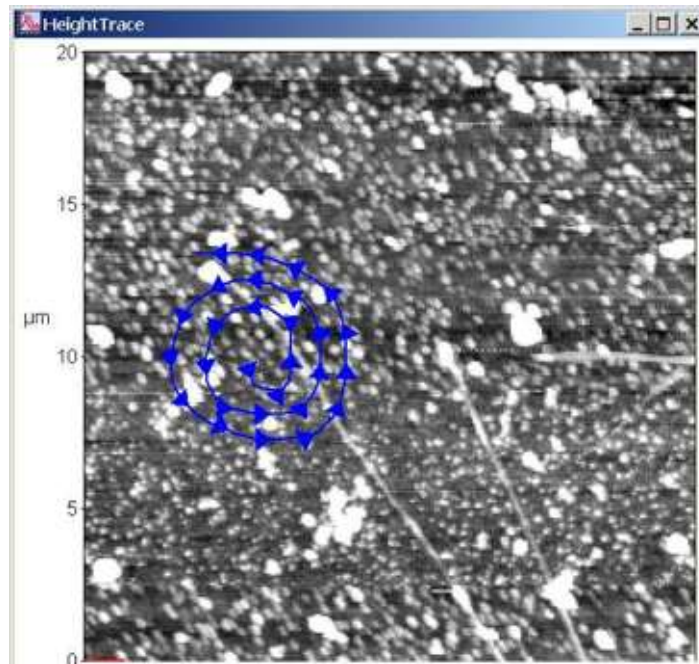


Figure 11.3 Pair of Carbon Nanotubes, with spiral path drawn

With directional arrows turned on, a spiral path is drawn. In this way it is possible to determine that the nanotube should move up and to the right on this image.

Save Lithography Data

It is possible to save deflection and Z sensor data during lithography. The waves are stored in the root:SavedLitho: folder, which is easily accessed from the Data Folders menu. They are saved according to the BaseName.

Chapter 12 Macros and Savant Writing

12.1 Overview

This chapter is intended to give advanced MFP-3D™ users the tools they need to do basic programming with ARMacro.

What Is a Macro?

A macro is a single computer instruction that stands for a sequence of instructions: it simply repeats a set of controls with no intelligence. If macros are combined with simple data transfer, wave manipulation, and basic programming logic, their utility can be greatly expanded. To establish a reasonable set of vocabulary, macros will be referred to as anything created with the AR Programming Panel. Functions are still just Functions, but a Function that requires a macro to be run and loaded will henceforth be called a Savant Function. It is assumed that a user has thoroughly read and understood both the IgorPro Getting Started book, and the 3D Programming Start in the Asylum Research (AR) Help files.

12.2 Creating Functions in Igor

Functions are the backbone of Igor programming, as mentioned in the 3D Programming Start in the Help menu. Functions are generally located in procedure files, either in the default procedure file that loads with an MFP-3D™ template or separately. Often, if trying to write a short Function for one time use, it is simpler to type Ctrl+M to bring up the default procedure window, and write the Function there. If the Function will be saved for future use however, it will either need to be written in a separate procedure window, or copied to one when it is written and saved separately. To create a new procedure window, go to Windows-->New-->Procedure and then begin writing Functions.

12.3 Function Basics

One of the biggest stumbling blocks of programming in Igor is knowing what tools are available to use. It is all in the Help files, but finding it can be tough. This primer includes many basic commands and tools found useful when learning to program. It also references the Help files from the commands themselves when appropriate.

QUICK REMINDER: The Help for any command in Igor can be found by highlighting the command, right clicking on it, and clicking the 'Help for...' selection. Similarly with Functions, highlight and right click on them, and Igor will present the 'Go to...' the Function option. These shortcuts should be used frequently as they are invaluable.

The primary use of a Function is to run compiled code, including creating Strings and Variables, executing operations, stepping through a series of instructions, and making logical decisions. In other words, it is the primary component of the Igor program. A Function can be as simple as:

```
Function SayHiOrBye(HiOrBye)
    String HiOrBye
    if (StringMatch(HiOrBye,"Hi"))
        Print "Hi to you as well"
    elseif (StringMatch(HiOrBye,"Bye"))
        Print "Bye, then"
    else
        //Unknown input
        Print "greeting unknown"
    endif
End //SayHiOrBye
```

Notice the double forward slash in the last line of code. This is noted as a comment in Igor: the compiler will ignore anything preceded by two slashes. Notice that the Function expects an input. In this case it expects a String, which must be entered in quotes. Igor expects a declaration of any Function parameters IMMEDIATELY after the Function is named. That is why the String HiOrBye is declared immediately after the Function statement. If this is not done correctly, a difficult to interpret error message will be the result at the compile stage. Something like "(whatever you put instead of the parameter) is not a parameter". This is an extremely common error, and is very irritating. Here is the result of running this Function at the command line with three inputs:

- SayHiOrBye("hi")
Hi to you as well
- SayHiOrBye("bye")
Bye, then
- SayHiOrBye("blah")
greeting unknown

A detailed description of Functions [referred to as User Defined Functions] in Igor can be found in the programming manual, Volume IV, p. 34.

Obviously this is a simple case, but the logic is seldom very different, simply more complicated. Now let's move on to some data handling protocols.

12.4 Flow Control Templates

Igor conveniently provides Flow Control templates for novice programmers. To begin, open a procedure window and look at the bottom left corner of the window. There will be a drop-down menu button called 'Templates'. This button has a complete list of commands, Functions, and flow control templates and is an invaluable tool to provide the jump-start needed to begin coding.

Two of the templates are of particular use when calling real-time macros, as they tend to compartmentalize the code; as a result, things that normally take longer to process will not cause a problem with timing. These flow control protocols are `StrSwitch-Case-EndSwitch` and the `Switch-case-EndSwitch`. See also `Switch Statements`

Here is an example. If I write the following:

```
Function GetThermalFreq()
    Variable Freq

    RunARMacro("DoAThermal") //imaginary Macro that
    does a thermal tune from the
    master control panel      //thermal panel on the

    Freq = GV("ThermalQ")      //This function
    looks in the global Variable table for a Variable
    this in the text of the help file //called thermalQ. More on

    Print "The Thermal Q is:", Freq

End //GetThermalFreq
```

At first glance, it looks as if this program will run a macro called 'GetThermalFreq,' and then afterwards, get the thermalQ from the resulting thermal tune. What will really happen however, is that the system will start the thermal tune, and then immediately move on to the next line, which gets the value from the Master Global Variable table. But because the thermal tune takes quite some time to execute, the value that is recovered from the table will be whatever value was there before the thermal tune executed, creating considerable confusion. This case is particularly heinous because you will get a reasonable value, so the program will not fail, but that reasonable value is incorrect. These are generally among the most difficult problems to track down and correct.

There are several solutions to this, but only one will be shown here: how to use a case flow control structure to make this work. Instead of the Function above, write:


```

Function GetThermalFreq2(CaseToggle)
    String CaseToggle
    CaseToggle = "DoAThermal"

    Variable Freq
    StrSwitch(CaseToggle) // string switch
    case "FirstDoAThermal": // execute if
case matches expression
        RunARMacro("DoAThermal")
        CaseToggle = "NextGetTheThermalQ"
        break //
exit from switch

    case "NextGetTheThermalQ": // execute
if case matches expression
        Freq = GV("ThermalQ")
        break

    EndSwitch
End // GetThermalFreq2

```

Now the Function will execute correctly: it will run through first case first, giving the Function time to complete the thermal, no matter how long the macro needs to finish the thermal tune.

12.5 Waves: Part I

This section is by no means meant to give an exhaustive description of waves in Igor. Rather, it aims to give a basic understanding of what these waves are and how to use them for some basic Function programming, specifically for use with Macros. However, this could surely be used to other ends as well.

Waves are the backbone of Igor. They are arrays that can be scaled, redimensioned, multiplied, compared, and manipulated in any of thousands of ways. For the purposes of this discussion, consider a wave to be a list of indexed numbers or Strings. Most often, they are a convenient place to put data, or to create a list of Variables that will be used in Functions. To create a wave, use the 'Make' command. To view or edit a wave, use the 'Edit' command.

Note first that some of the parameters that are fed to 'Make' are listed in the Help file in brackets []. These are optional, and include flags (the first set of options that are listed with a slash /), and optional parameters. The flags make commands more specific; the optional parameters simply add to the command. This is the basic structure of the Igor Help files. It is advisable to be familiar with the structure and syntax of these Help files, as they will be frequently seen. The Help

file for 'Edit' can be brought up by typing 'Edit' in the command line, highlighting it, then right clicking on it and selecting 'Help for...' — Try that now.

Another important aspect of waves is that they always reside in a specific data folder. It is therefore important to digress a bit and discuss how to deal with Data Folders before returning to the vital facts about waves needed for programming basic Igor Functions.

NOTE: The following topic breaks the logical flow in the discussion of waves to discuss data folders. If this subject is already familiar, skip to Waves II: Back to Waves and Igor Functions

12.6 Dealing With Data Folders

Waves may be made in either the command window or within a Function. Waves exist in data folders, and there can be multiple waves with the same name in different data folders. This is often a major point of confusion when writing Igor Functions. When an Igor experiment is open, all the Variables and waves Igor is using are kept in the memory (Ram or the Windows Swap file), and structured with the data browser. It is possible to see what data folder is currently in use by clicking Data-->Data Browser. The red arrow shows the current data folder. A nice shortcut to use to change from one data folder to the next is to hold the Alt key, and left mouse click to the left of the desired data folder. Try it.

One trick to dealing with the data folders in Igor successfully is to work in one data folder for the entire Function. To do this, open the current data folder and either make a new one or move to the root, proceed to execute the Function, and then go back to the original data folder. This can either be done in a shell Function, or from within the Function that is running. Here are some examples:

The object of the first example is to feed the Function a parameter that will be the name of a data folder the Function will create to work in. Any waves created in the Function will exist in this experiment data folder, and when the Function is complete, the Function will set the data folder back to the original data folder.

```
Function CreateFolderExample(exptID,  
PasstoAnotherFunction)  
    String exptID  
    Variable PasstoAnotherFunction  
    String SavedDataFolder = GetDataFolder(1)  
    NewDataFolder/S /O $"root:"+exptID  
    RunAnotherFunction(PasstoAnotherFunction)  
    SetDataFolder SavedDataFolder  
End //CreateFolderExample
```

Notice that this Function does nothing except create a new data folder, Run a Function, and pass a single parameter to that called Function. The String `exptID` is the name of a new folder that will be created in the root data folder. The `/S` flag tells the system to set that data folder as active after it is created. The `/O` flag overwrites the old folder if the user chooses the same name. This nested Function structure, or shell Function structure, is a nice way to compartmentalize code. Calling other Functions from within shells helps with both organization and troubleshooting, and also helps to interface the code with panels.

A factor of note in this Function is the use of the `$`. This is a subtle but essential part of basic Igor programming. This character —`$`— tells Igor to look at the String value contained by the String `exptID` rather than the characters `exptID`. If the `$` were not used, Igor would have created a new data folder with the name `exptID` in its root, rather than creating a directory with the String the user typed in when he/she called the Function.

See also: `NewDataFolderGetDataFolder`

`SetDataFolder`

Here is a simpler example:

```
Function CreateFolderExample2(PasstoAnotherFunction)
    Variable PasstoAnotherFunction
    String SavedDataFolder = GetDataFolder(1)

    //Store the current data folder
    SetDataFolder root:
        RunAnotherFunction(PasstoAnotherFunction)
    SetDataFolder SavedDataFolder
End //CreateFolderExampleFolder2
```

Here the current data folder is stored, and changed to root for the Function, then called another Function (again passing on a parameter), and finally set the system to the original data folder.

Of course it is possible to ignore this instruction and work in whatever data folder happens to open when the Function begins running, but if waves are being used, doing so will cause unexpected problems, especially if the system is performing other work while running these Functions. It is therefore strongly recommended that some sort of folder management in Functions is put into use.

12.7 Waves Part II: Back to Waves and Igor Functions

Now that the subject of how to manage data folders has been covered, the focus returns to waves. As stated earlier, waves are simply a series of numbers or

Strings that are indexed. They can be created either at the command line or from within Functions. It is a logical assumption that those reading this do so with the intent to couple Functions with macros (i.e. create Savant Functions), and thereby make this Functional from experiment to experiment, and from day to day. It is therefore logical to write Functions so that the waves are created from within the Function, or at least so that the Function prompts the user for the path to the wave they have created for the particular experiment they are running. If a Function is written that will nearly always use the same wave, or for the exclusive use of one person, so that the code can be modified to account for changes to the waves then these waves may be created in a Function that acts as a shell, and the called Functions will simply reference the waves created in the shell Function. Here is an example of this:

```
Function WaveFeederFunction(exptID,MaxIndex)
    String exptID
    Variable MaxIndex
    Variable Index = 0
    String SavedDataFolder = GetDataFolder(1)
    NewDataFolder/S/O $"root:"+exptID

    Make/O/N=(MaxIndex) YCoordWave //Here we
make a wave to
                                //feed from within
this Function
    YCoordWave[Index,MaxIndex] = P*5
    Make/O/N=(MaxIndex) XCoordWave //This wave
will be defined in the inner Function,
                                //but is created here
    RunAFunctionThatNeedsThesewaves() //here is
where the shell Function
                                //calls the inner
Function
    SetDataFolder SavedDataFolder
End //WaveFeederFunction
Function RunAFunctionThatNeedsThesewaves()
    //Making the inner Function, where
                                //waves will be
manipulated
    wave YCoordWave //the wave
reference tells this Function
                                //to look for a wave
                                //that already exists
in the current data folder.
    wave XCoordWave

    XCoordwave = P
    Edit/K=1 XCoordwave, YCoordwave
    //brings up a chart with the waves in it
End //RunSomeFunctionThatNeedsThesewaves
```

Copy and paste this into a procedure and run it. It will bring up a table with the two waves in it. Notice that in the first Function, the one that calls the inner Function, the waves are created with a 'Make' command, whereas in the second Function, the waves are simply referenced using the 'Wave' command. 'Wave' commands will only work if the wave exists in the active data folder. That is why the first Function sets the data folder to a specific folder, then creates the waves, then calls the inner Function.

12.8 Strings and Variables

Along with waves, it is necessary to know some basics about Variables and Strings (See Variable and String). To put it simply, Variables are numbers and Strings are letters. The simplest use of these is local. Local Variables and Strings are declared within a Function, used therein, and then forgotten when the Function has run. To declare Variables locally, simply use the Variable command:
`Variable xyz`

This will create a Variable within the Function called xyz. Strings are just as simple; just use the String command: `String myString`

There are tags that can be added to these, and the initial value of the String or Variable can be added with a simple equal sign:
`Variable xyz =12.5`
`String myString = "mine"`

These statements will both create and set an initial value for the Variable xyz and the String myString.

Along with local Variables and Strings, there are global counterparts. Global Variables and Strings are kept in the Igor memory space and can be accessed from anywhere, regardless of which data folder happens to be in use. They are generally more difficult to program with, and so should be avoided if what is needed can be done with local Variables and Strings. To create a global Variable or String, simply append the /G flag to the Variable or String declaration:
`Variable/G Gxyz`

`String/G myString = "mine"`

These are now global, and can be referenced from within any Function using the SVAR and NVAR commands. If the above global Variables have been created,, then they be can referenced locally with:

`NVAR Gxyz`
`SVAR myString`

These can now be used in the local Function. An example of the creation of a global Variable and its local reference is shown in two of the examples below.

Remember that when a Function is told to expect a Variable or parameter, it takes the following form:

```
Function EggSample (myString,xyz)
    String myString
    Variable xyz
End //EggSample
```

Here, myString and the Variable xyz are called parameters, and must be declared immediately after the Function statement. Another fact of parameters is that they must be declared as local Variables immediately after the Function statement. If they need to be set equal to a global Variable, then it is necessary to make the global Variable immediately after you have made your parameter declaration. A good example of this is shown below in the function CallbackExample. In this case, the String was made global because local variables will have been forgotten after the function has finished running. Since the Callback will be running later, that information needed to be stored in a global String.

12.9 Callbacks: Functions and Commands

Another important tool in programming in Igor for the MFP-3D™ is the use of Callback Functions and commands. Callbacks can be used for something as simple as notifying the user or programmer that a routine has completed, or as complicated as calling on one of various Functions, depending on the output of another Function.

To put it simply, a Callback is something that runs when the Function using the Callback is finished with what it is doing. For those readers out there familiar with the 'Execute' command, this operates similarly to using the command 'Execute/P/Q.' With these flags, Igor will process the Function or command immediately if Igor is not busy. If Igor is busy, then it will cue the Callback until Igor is free to process it.

Here is an example of a Callback at its simplest (Reference: ARU_Callback):

```
Function CallbackExample(Message)
    String Message
    String SavedDataFolder = GetDataFolder(1)
    SetDataFolder root:
    String/G GMessage = Message
    // template: ARU_Callback(number,milli
seconds,Callback)
    ARU_Callback(0,5000,"Print root:Gmessage")

    SetDataFolder SavedDataFolder
End //CallbackExample
```

Paste this into a procedure window, compile it, and run it. The command 'ARU_Callback' will simply wait the amount of seconds fed in the second parameter (in this case 5), then execute the Callback. This is quite a simple case, but demonstrates how Callbacks can be used. One thing to note is that the above Function uses a String/G declaration instead of the typical String declaration without the /G flag. This is because the command 'ARU_Callback()' expects a global String or Variable. And that is because using a Callback is similar to using an Execute Function, in which Igor executes the command or Function as if at the command line, where global Strings and Variables are needed.

The next example is more or less the same as the example above, but with a slight variation, and is intended to show how a Callback can call a Function rather than a simple command.

```
Function CallbackExample2(Message)
    String Message

    String SavedDataFolder = GetDataFolder(1)
    SetDataFolder root:
    String/G Gmessage2 = Message

    ARU_Callback(0,5000,"callbackPrintFunction(root:Gmessage2)")
    SetDataFolder SavedDataFolder
End //CallbackExample2
Function CallbackPrintFunction(Message)
    String Message

    Print Message

End //CallbackPrintFunction
```

Notice that the Function Callback Function2 calls a Function that Prints, rather than executing the command from within the code. Compartmentalizing code like this can be quite helpful in more complicated Functions.

12.10 Syntax Issues and the Exception character \

This is a good time to address an annoyance of programming in Igor, but one that is necessary. To create a Callback that called the Function 'CasesForNonContactCalc' it might seem tempting to enter the following:

```
//Callback =
"CasesForNoNontactCalc(width,Length,EstimatedFreq)"
```

If it were written that way, it would compile, but would not execute properly, because Igor is expecting a long String. In this case, the parameters entered would confuse Igor, since Igor actually wants to enter the String that has those

parameters, rather than the values in the parameters themselves. This means to the following should be written instead:

```
//Callback =  
"CasesForNonContactCalc("+num2str(width)+", "+num2str  
(Length)+", "+num2str(EstimatedFreq)+", "\"NextDoSader\  
")"
```

This is a bit clearer if it is written:

```
//Callback = "CasesForNonContactCalc("  
//Callback += num2str(width)  
//Callback += ", "  
//Callback += num2str(Length)
```

These break down the steps to create the Callback statement...annoying perhaps, but clear.

```
//Callback += ", "  
compared to the way above where all the steps are combined  
//Callback += num2str(EstimatedFreq)  
//Callback += ", "  
//Callback += "\"NextDoSader\  
//Callback += ")"
```

Notice that this Callback uses the ‘+=’ characters and the backslash ‘\’. The ‘+=’ characters together tell Igor to add anything that follows to the String that precedes the ‘+=.’

The backslash ‘\’ is the exception character. It tells Igor to ignore the next character from a programming perspective. So instead of ending the quotes in the second to last line before ‘”\”NextDoSader\”’, Igor will treat the quote as a String. This is the only way to pass quotes to Igor when they are contained in a String.

12.11 Events

Like callbacks, events are an Asylum Research contribution to Igor programming. See ‘Events’ under Parameter Names. Scroll down to ‘Events’ to read the description. Events live in the controller as parameters. As such, it is necessary to have a controller that is connected (and turned on) to play with them. The purpose of events is rather simple, and once some time is spent using them, their syntax and use will be more obvious. Events are used to trigger real-time processes at a time that is convenient for a user at the command line or in a function. Think of it this way: to set-up a wave on one of the DACs that will produce a voltage at the cantilever that is sinusoidal, another wave must be set up on one of the ADCs to read the resulting deflection of the cantilever. In order for this to work smoothly,

both the outwave and the inwave must start up at the same time. This is done with Events.

Now would be a good time to read the explanation of outwaves at: [Extra Help for td_xSetOutWave](#).

Events themselves will be contained within the 'td_ function' that will end up using them. When an event is called in a function, it will be listed as 'eventString' in the template:

```
td_xSetOutwave(whichBank, eventString,  
channelString, wave, interpolation)
```

When this wording is seen, the DSP (Digital Signal Processor) is expecting two entries for the event string. The first tells the controller what to do when the event is first set. The second tells the controller what to do when the function is finished executing. Here is a layman's version of how this actually works in the DSP:

1. The DSP checks 'Events' periodically, likely once per DSP cycle
2. User triggers event to set by specifying the first event string as "0%event@controller" which will later be written as 'set' in the code, and setting the second event to 'never'.
3. The next time the DSP cycles through the 'Events,' it looks at what function is associated with the event, and executes the function.
4. Event is still set as specified above, so each time the DSP cycles through the event it will continue to do nothing.
5. When the wave has finished running, the DSP code sets the event to the second event string in the function, in this case, 'never.'
6. The next time the DSP cycles through and checks the events, it sees that the event is set to 'never' and does not execute the function that is associated with the event string.

This may be more information than is strictly necessary, but it is offered as a means to help understand just what happens. Here is another example:

```
#pragma rtGlobals=1      // use modern global  
access method.  
Function DoIV(range)  
    variable range  
  
    td_StopInWaveBank(-1)  
    td_WriteString("0%event", "clear")  
  
    make/o/n = 8192 voltagewave CurrentWave  
  
    CurrentWave = 0
```

```

        VoltageWave = -range + p/8192 * (2*range)

        td_xSetInWave(2,
"0%event@controller,never", "A%Input",
CurrentWave,"IVCallback()",20)

        td_xSetOutWave(0,"0%event@controller,never",
"A%Output", VoltageWave, -20)

        td_writeValue("A%Output", -range)
Sleep/T 20

        td_writestring("0%event", "once")

End //DoIV

Function IVCallback()

    WAVE CurrentWave

    SetScale d 0,0,"A", CurrentWave
CurrentWave/= -500e6

End // IVCallback

```

Notice that both the 'xSetOutWave' and 'xSetInWave' commands have two calls to the controller. In this case, the entire parameter string is entered, but "0,0" or "0%event,0%event" for "0%event@controller,0%event@controller" could just as easily be substituted. In the procedure, the outwaves will be set up, and then executed when the function reaches the event writestring.

12.12 Time Scaling and MFP-3D™ Programming

A common goal in programming with Igor for the MFP-3D™ is to send a wave out over one of the DACs within a set amount of time. Because of the way that DACs and ADCs sample data, this task is often confusing, as the user must keep track of the number of points in the wave, the sampling rate of the DAC or ADC, and the amount of decimation or interpolation that is done with the wave as well.

The scan time depends on the number of points in the wave created, and on the sampling rate of the DACs or ADCs. Fortunately, the sampling rate for the ADCs

and DACs is always the same— 100kHz. Before trying to grasp the concepts that will be presented here, it would be helpful to review the SetScale command, as it gives a good description of what is being explained. The essence is that each wave has a certain number of points that are indexed by number, and the user must decide how much of some other variable each of these points equals. For example, if there are 256 points in a wave, and each wave is to represent 10 seconds, that means that the start point is zero seconds, and the end point is 10 seconds, which corresponds to a start point of point number 0 in the wave and an end point of point number 256 in the wave, which in turn means every point will equal 0.256 seconds. The nice feature of programming for the MFP-3D™ is that when td_ commands are used, the X scaling is set up automatically, so many of the issues that would normally roadblock a new programmer become transparent.

The simplest way to solve this problem is to set up a variable called a scan time or a scan rate, depending on what is needed for the particular application. A scan time is the time it will take for a wave that has been set on a DAC or ADC to execute; the scan rate will be useful to run a wave continuously and cycle it at a certain rate. It is possible to switch from one to the other by simply setting the scan rate to 1/ScanTime. Scan rate depends on two DSP parameters called decimation and interpolation which in turn depend on whether a DAC (decimation) or an ADC (interpolation) is being used. Interpolation and Decimation are discussed briefly in Extra help for td_xSetOutWave, td_xSetInWavePair, and td_xSetOutWavePair. While all this information is quite useful and necessary for advanced use, for simple use, the following relation may be used:

Decimation or Interpolation = Round [(1e5 × desired scan time)/number of points in wave]

To use a scan rate, set the desired scan time to 1/(scan rate)

Here is an example that uses this to create a pulse that is to be sent out onto user DAC B (OutB) and an imaginary response into ADC B (InB):

```

Function Pulse(wait, Pulsewidth, PulseHeight)
//wait will pause for a short period of time before
initiating the pulse
    variable Pulsewidth, PulseHeight, wait

    variable ScanTime = 2*wait+Pulsewidth //
set the total time the wave will take to execute
with 2 times the wait for

    //wobble room
    variable Decimation
    variable Point1, Point2, ActualWaitTime

```

```

        td_StopOutWaveBank(0) //clears any waves
already in the bank we will use
        td_StopInWaveBank(0) //clears inwave bank
        td_writestring("0%event@controller",
"clear") //clears the event string

        make/O/N=(2^8) ThePulse PulseResponse

        Decimation =
round(1e5*Scantime/dimsize(ThePulse,0)) //This is
the programming version of the equation in the text
        ActualwaitTime =
dimsize(ThePulse,0)*Decimation/1e5 // this converts
the desired wait time to one that will fit the math
        wait = (ActualwaitTime-Pulsewidth)/2

        td_xsetinwave(0, "0", "b%Input",
PulseResponse,"ImaginaryCallback()",Decimation)
//set up the waves. but the
        td_xsetoutwave(0,"0", "b%Output",
ThePulse, Decimation) //y values are
zero, this is only to get

        //the x scaling correct

        Point1=x2pnt(ThePulse,wait)
//This converts the xscaled values returned by
the controller
        Point2=x2pnt(ThePulse, wait+Pulsewidth)
//into points to make the math better

        ThePulse[0,Point1]=0
//pulse is zero until it reaches point one
        ThePulse[Point1,Point2] = PulseHeight
//now pulse is equal to pulse height
        ThePulse[Point2,] = 0
//back to zero. Notice if the second point is
left
//blank Igor
defaults to the end.

        td_xsetoutwave(0,"0", "b%Output",
ThePulse, Decimation) //now that we have the y
values we need to resend the wave.

td_writestring("0%event@controller","once") //set
the event to set the wave in motion

        End //Pulse

```

This is a good example of how to set-up a timed wave that will execute once on one of the DACs. Notice in the `td_xsetoutwave` and `td_xsetinwave` commands that there is only one event. This is typical of non-repeating waves. If the intent is to create a repeating wave, two events should generally be used. This tells the DSP that the waveform will be repeating, and it will then adjust the scaling appropriately so that the wave is not made discontinuous by extra or missing data points. The `td_xSetInWave` will then look like:

```
td_xsetoutwave(0,"0%event,0%event", "b%Output",  
ThePulse, Decimation).
```

So if `ThePulse` was to be a repeating wave, the second event would be set to "set."

12.13 Useful Commands for Programming with the MFP-3D™

To program macros in Igor, some Functions written by Asylum Research (AR) are needed, and also a few that are an inherent part of Igor. This section will provide an explanation of the AR written Functions, and links to the Igor Functions that may be useful.

RunARMacro

```
RunARMacro(MacroName,[Callback])
```

This Function will call a macro if it is loaded into the memory. Instructions on how to load the macros into memory can be found in the `ARMacro Help MFP-3D™ AR Macro Panel`. Notice that this Function has a `Callback`. Executing it will run the loaded macro, then when that is finished, will execute the `Callback`. Be sure to read the `Help (above) on Callbacks`.

ARExecuteControl(ControlName,GraphStr,VarNum,VarStr)

This Function will use a piece of the `ARMacro` code to enter a value into a control panel within the `MFP-3D™` code. This is a crucial Function for the user who wishes to do something other than simply execute a series of macros. This is the Function that allows values to be entered into the control panels to enable `Smart Macro` programming. A good structure to use is to have a shell that either prompts the user for values or creates `Variables` that will be fed into another Function that will then execute a series of `ARExecuteControl` commands. The Function containing the `ARExecuteControl` lines will in turn call another Function that calls the macros and analyzes the results. If the name of the `Variable` to be entered is not known, do the following:

1. In Igor, select the panel that contains the blank into which the value is normally entered
2. Click Ctrl+T to bring up the Tools options on the panel
3. Click on the lower of the two buttons on the upper left corner to go into Drawing mode
4. Next, double click on the box where the value to be changed is normally entered
5. The names of the controls are standardized so that the panel number and the name of the Variable may be grabbed:
ThermalDCSetVar_1

This is from the Thermal tab on the main Control Panel. SetVar_1 states that this is a Variable on tab one of the panel. The underscore1 means tab one, and var means Variable. The Variable name is ThermalDC.

As an example, let's say that we want to enter the number contained by the Variable 'zoom' into the zoom width box on the thermal tab of the master Control Panel. Using the Ctrl+T steps listed above, we find that the name of the box is SweepWidthSetVar_1, so we enter the following into the ARExecuteControl command.

```
ARExecuteControl ("SweepwidthSetVar_1", "MasterPanel",
Zoom, num2str(Zoom))
```

Look at the template:

```
ARExecuteControl (ControlName, GraphStr, VarNum, VarStr)
```

Notice the last two parameters, VarNum and VarStr in particular: these are used by the AR procedures to allow the changing of more than just Variables. They modify the String version of the entry so that units can be understood, tabs can be changed, buttons can be pressed, and pop- up menus can be facilitated. Here is a brief rundown on their uses in specific cases:

| Type of Entry | NumVar | VarStr |
|---------------|----------------------------|---|
| Variables | Variable or number | String version so units can be understood |
| Strings | Ignored | String to be entered |
| Check boxes | 0 for uncheck, 1 for check | Ignored |

| | | |
|---------|----------------------------|---------|
| Buttons | Any number- Use 0 for ease | Ignored |
|---------|----------------------------|---------|

At the end of this section there is an Igor procedure file that illustrates many of the points - Use "" for ease discussed here.

Get Value (GV)

This capitalizes on the structure used in Igor to keep track of all the Variables needed to run the MFP-3D™. The Variables are stored as waves, and can be viewed by clicking on the menu: Programming-->Global Variables--> and any of the selections that follow. The GV command allows the user to grab any Variable in these waves by simply calling the GV("") Function and inserting the name of the Variable to retrieve. If the name of the Variable for which a description is needed is not known, see the description on how to find Variable names under the ARExecuteControl topic.

Other Functions

Here are some other Functions found useful in programming. They are links to the Help files on each of these.

DoWindow

Prompt

DoPrompt

num2str

x2pnt

Escape Characters in Strings

*note there is also a brief explanation of this under Dealing With Data Folders.

Example of a Savant procedure

The following example shows a complete working Savant Procedure that calls a macro called DoAThermal. The entire procedure finds the optical lever sensitivity, the spring constant as determined by the Sader Function, then prints them in the history. In order for a user to run it, they must have the macro called 'DoAThermal' loaded using the AR Macro panel.

To run it, copy it into a new procedure window, load the DoAThermal macro, and run the AutoInvolvs Function. Note that some of the lines may not have survived the Copy/Paste from the procedure to here in the Help file and back to the procedure, so a couple of the longest lines may need to be cleaned up a bit before you can proceed.

```

Function AutoInvol(CantileverType)
    String CantileverType

    //gets the cantilever type from the user and
    feeds the parameters from the
    //cantilever to
    //NonContactKCalc

    Variable Length, width, EstimatedFreq
//cantilever dimensions
    Variable Zoom
    Variable Fitwidth //Zoom width for the
fit guess. This has to be bigger
//for higher spring
constant cantilevers

    Print ""

    //the If switch will put information about our
    cantilever into some variables
    //then if we get to the end of the function
    //we will use that information to calculate the
    Invol without touching the surface.

    if (StringMatch(CantileverType,"AC240"))
        Length = 240e-6 // units are meters
        width = 30e-6
        EstimatedFreq = 65e3
        Zoom = 100e3 //units are in Hz

    elseif (StringMatch(CantileverType,"AC160"))
        Length = 160e-6 //meters
        width = 30e-6
        EstimatedFreq = 300e3 //Units are Hz
        Zoom = 150e3 //these units are in Hz ---
need a wider Zoom since there is more
//variation in the freq of
ac160s

    elseif
(StringMatch(CantileverType,"Biolever60"))
        Length = 60e-6 //meters
        width = 30e-6
        EstimatedFreq = 37e3 //Units are Hz
        Zoom = 74e3 //these units are in Hz

    elseif
(StringMatch(CantileverType,"Biolever100"))
        Length = 100e-6 //meters
        width = 30e-6
        EstimatedFreq = 13e3 //Units are Hz

```



```

        Zoom = 26e3 //these units are in Hz
elseif (StringMatch(CantileverType,"NCH"))
    Length = 125e-6 //meters
    width = 30e-6
    EstimatedFreq = 320e3 //Units are Hz
    Zoom = 150e3 //these units are in Hz

elseif (StringMatch(CantileverType,"NCL"))
    Length = 225e-6 //meters
    width = 38e-6
    EstimatedFreq = 190e3 //Units are Hz
    Zoom = 60e3 //these units are in Hz

elseif (StringMatch(CantileverType,"FM"))
    Length = 225e-6 //meters
    width = 28e-6
    EstimatedFreq = 75e3 //Units are Hz
    Zoom = 60e3 //these units are in Hz

elseif (StringMatch(CantileverType,"ArrowNC"))
    Length = 160e-6 //meters
    width = 345e-6
    EstimatedFreq = 285e3 //Units are Hz
    Zoom = 90e3 //these units are in Hz

elseif (StringMatch(CantileverType,"ArrowFM"))
    Length = 240e-6 //meters
    width = 35e-6
    EstimatedFreq = 75e3 //Units are Hz
    Zoom = 70e3 //these units are in Hz

elseif (StringMatch(CantileverType,"Silex1"))
    Length = 40e-6 //meters
    width = 5e-6
    EstimatedFreq = 70e3 //Units are Hz
    Zoom = 135e3 //these units are in Hz

elseif (StringMatch(CantileverType,"Silex2"))
    Length = 15e-6 //meters
    width = 5e-6
    EstimatedFreq = 350e3 //Units are Hz
    Zoom = 200e3 //these units are in Hz

elseif (StringMatch(CantileverType,"Silex3"))
    Length = 12e-6 //meters
    width = 5e-6
    EstimatedFreq = 550e3 //Units are Hz
    Zoom = 200e3 //these units are in Hz

else

```

```

        Print "unknown cantilever"

        Dump()          //dumps you and Prints an
error message with some guidance on cantilevers
        return(0)      //leave before we try to
calculate things.
    endif

    //Finally, use the information.

    Fitwidth = Zoom
    NonContactKCalc(width, Length, EstimatedFreq,
Zoom, Fitwidth)

End //AutoInvol

Function NonContactKCalc(width, Length, EstimatedFreq,
Zoom, Fitwidth)
    Variable Length, width, EstimatedFreq, Zoom,
Fitwidth

    //this will be a shell that calls the different
cases for the NonContactKCalc
    // it also enters the values needed into the
various control panels

    String SavedDataFolder = GetDataFolder(1)

    //Store the current data folder
(SavedDataFolder): grabs

    //the current data folder and assigns it to a
//String so that it can be restored
//after each macro or function is executed.
//This could probably be made into a function.

    ARExecuteControl("DriveFrequencySetVar_1", "Mast
erPanel", EstimatedFreq, num2str(EstimatedFreq))
    ARExecuteControl("SweepwidthSetVar_1", "MasterPa
nel", Zoom, num2str(Zoom))
    ARExecuteControl("FitwidthSetVar_1", "MasterPane
l", Fitwidth, num2str(Fitwidth))

    SetDataFolder root:

```

```

        //CasesForNonContactCalc(width, Length,
EstimatedFreq, "FirstDoAThermal")
        Execute/P/Q
"CasesForNonContactCalc("+num2str(width)+", "+num2str
(Length)+", "+num2str(EstimatedFreq)+", \"FirstDoAther
mal\")"

```

```

        SetDataFolder SavedDataFolder

```

```

End //NonContactKCalc

```

```

Function CasesForNonContactCalc(width, Length,
EstimatedFreq, CycleCase)

```

```

    Variable Length, width, EstimatedFreq
    String CycleCase

```

```

    //this will cycle thru the cases to first get
the thermal tune done,
    //then call the ksader Function

```

```

    Variable freak //resonance frequency from
thermal

```

```

    Variable kyoo // q factor from thermal
    Variable KaySader
    Variable Involsguess
    Variable Involscorrect
    Variable KayThermalTune

```

```

    StrSwitch(CycleCase) // String switch
        case "FirstDoAThermal": // execute
if case matches expression

```

```

        String callback =
"CasesForNonContactCalc("+num2str(width)+", "+num2str
(Length)+", "+num2str(EstimatedFreq)+", \"NextDoSader\
")"

```

```

        //callback =
"CasesForNonContactCalc(width,Length,\"NextDoSader\"
)" -- this was the first try. obviously doesn't work

```

```

        //callback =
"CasesForNonContactCalc("
        //callback += num2str(width)
        //callback += ", "
        //callback += num2str(Length)

```

```

        //these break down the steps to create the
callback statement...pain in the ass, but clear

```

```

        //Callback += ","
        //compared to the way above where all the
steps are combined
        //Callback += num2str(EstimatedFreq)
        //Callback += ","
        //Callback += "\"NextDoSader\""
        //Callback += ")"

        //Print Callback

        RunARMacro("DoAThermal",Callback=Callback)

        //Print "made it this far first case"

        break //
exit from switch

        case "NextDoSader": // execute if
case matches expression
        freak = GV("ThermalFrequency")
        kyoo = GV("ThermalQ")
        Invo1sGuess = GV("Invo1s")
        KayThermalTune = GV("SpringConstant")

        //Print "made it this far second
case"

        KaySader =
kSader(width,Length,kyoo,freak)

        Invo1sCorrect =
Invo1sGuess*(sqrt(KayThermalTune/KaySader))

        Print "Invo1sGuess= ", Invo1sGuess,
"m/V"
        Print "thermalQ= ", kyoo
        Print "thermal frequency= ", freak,
"Hz"
        Print "estimated frequency= ",
EstimatedFreq, "Hz"
        Print "spring constant by Sader
method, k = ",KaySader, " N/m"

        Print "the inVOLS is: ",
Invo1sCorrect, "m/V"

        Print ""

        ARExecuteControl("Invo1sSetVar_2","MasterPanel")

```

```

,InvoIsCorrect,num2str(InvoIsCorrect))
        break
        default:                                //
optional default expression
        //executed
        Print "something screwed up"
        // when no case matches
        EndSwitch
End //CasesForNonContactCalc

Function Dump()

Print "please enter a known cantilever and start the
function again"
    Print "known cantilevers are AC240, AC160, NCH,
NCL, FM, ArrowNC, ArrowFM, Biolever100, Biolever60
and Silex levers."
    Print "a description of each of these can be
found on our website
http://www.rasy.com/Products/Levers/LeverGuide.shtml
"
    Print "note that coated cantilevers are
referred to by their uncoated counterpart's
designation for this function"
    Print ""

```

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