MultiMode PicoForce
NanoScope Software v 6, 7

004-101-000 (standard)
004-101-100 (cleanroom)

Document Revision History: MultiMode PicoForce

Revision	Date	Section(s) Affected	Reference	Approval
F	01-26-2011, 02-04-2011	Re-branded		Ruth Wishen-grad, K. Madler
E	15-Aug-2007			Vinson Kelley
D	4-June-04	Chapters 4, 5, 6, 8, 9, 11, 13		Vinson Kelley
C	19-May-03	Chapters 6,8,9		Vinson Kelley
B	02-Oct-02	Chapters 3, 5, 6, 7, 8		Vinson Kelley
A	31-Jul-02	Initial Release	0430	Alan Rice

Product Names:
NanoScope®
MultiMode™
Dimension™
BioScope™
Atomic Force Profiler™ (AFP™)
Dektak®

Software Modes:
TappingMode™
Tapping™
TappingMode+™
LiftMode™
AutoTune™
TurboScan™
Fast HSG™
PhaseImaging™
DekMap 2™
HyperScan™
StepFinder™
SoftScan™

Hardware Designs:
TrakScan™
StiffStage™

Hardware Options:
TipX®
Signal Access Module™ and SAM™
Extender™
TipView™
Interleave™
LookAhead™
Quadrex™

Software Options:
NanoScript™
Navigator™
FeatureFind™

Miscellaneous:
NanoProbe®

Table of Contents

MultiMode PicoForce Manual

List of Figures

Chapter 1   Introduction

1.1   Overview

The MultiMode PicoForce (see Figure 1.1a) is an instrument designed for the ultra-sensitive measurement of very small forces. The measurable range of forces is approximately between 1 piconewton (pN) and 10 millinewtons, an extraordinary ten orders of magnitude. This range of forces includes, at the high end, the forces required to deform advanced materials (polymers, composites, ceramics) at the nano- and micro-meter scale. The middle of the range includes important forces such as those required to bend biological membranes and fundamental intermolecular interactions such as electrostatic and Van der Waals forces. Some of the most interesting possibilities, however, exist within the lower 10-500 piconewton range of the instrument. This range encompasses such exciting and important forces as those that hold proteins together (see Figure 1.1b), those that bind antibodies to their corresponding antigens, those that attract drugs to their targets, and a host of other forces in the biological world. The analysis of forces in this lower range has been dubbed *force spectroscopy* by Dr. Hermann Gaub, a leader in this field of research.

Figure 1.1a   PicoForce Control Module, PicoAngler and MultiMode Scanning Probe Microscope (SPM)
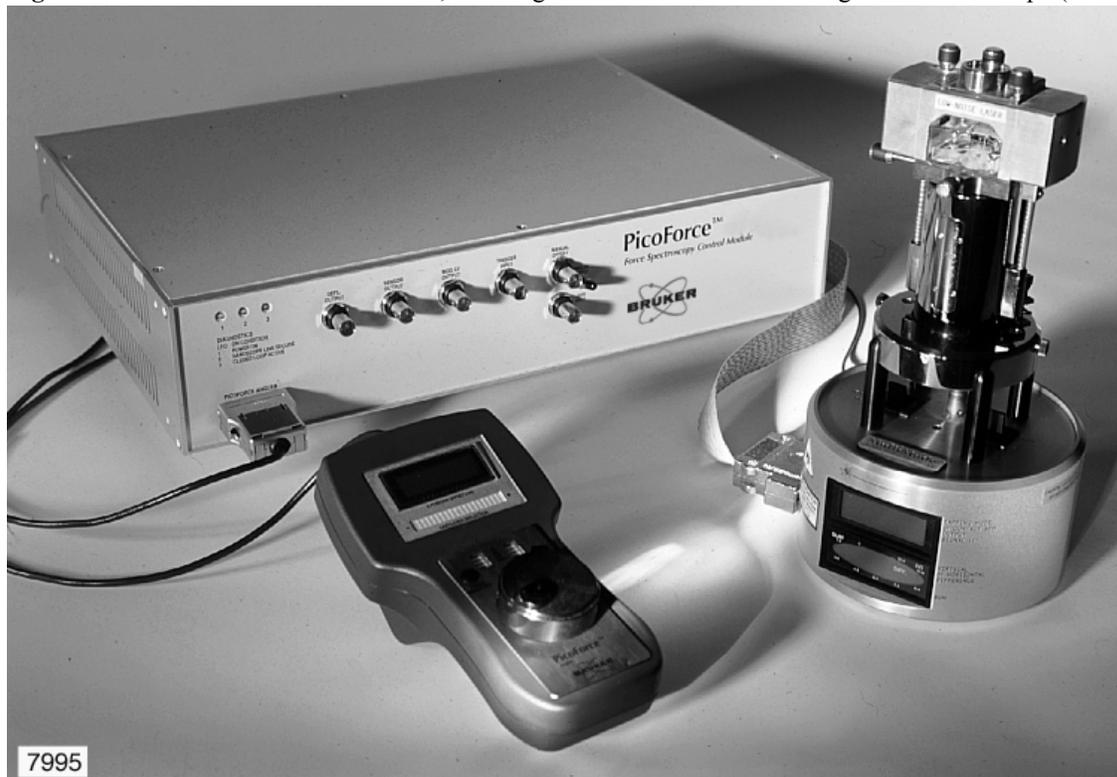
Figure 1.1b MultiMode PicoForce Stretching a Molecule

Unfolding a Protein
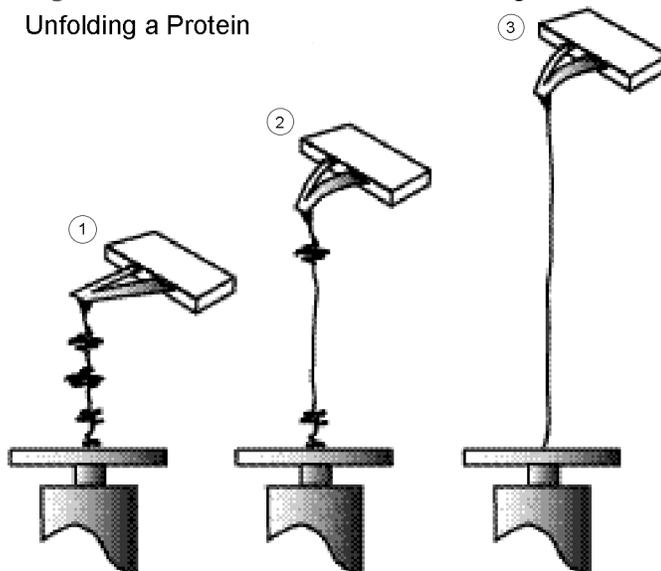
The MultiMode PicoForce achieves this incredible sensitivity and range by sensing the deflection of a micro-machined cantilever with an optical beam detector. The cantilevers can be manufactured with various spring constants, starting at less than 10pN/nm. This means that a force of ten piconewtons at the end of the cantilever bends the lever one nanometer from its resting position. Deflections in this range are easily quantifiable with the optical lever detection method (see Figure 1.1c). The cantilever includes an ultra-sharp (<30nm radius) tip at its end that interacts either directly with the sample or via some chemical functionalization. This technology is very similar to that employed in conventional Atomic Force Microscopes (AFMs) and more generally in Scanning Probe Microscopes (SPMs). In fact, researchers have used existing SPM/AFM systems for much of the work published so far in the field of force spectroscopy. However, for various reasons, the existing commercial SPM/AFM hardware is not well suited to these measurements. The MultiMode PicoForce addresses these deficiencies and hence provides the ideal instrument with which to investigate piconewton scale interactions.

Figure 1.1c The Optical Lever Detection Method
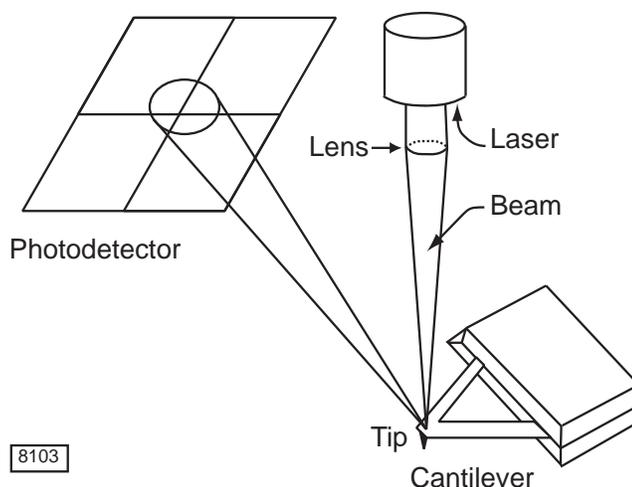
The forces measured may be either attractive (pulling the tip down) or repulsive (pushing the tip up). These forces may occur as the tip-sample distance is increased or decreased. The ability to precisely and reproducibly control tip-sample separation is one key feature of the MultiMode PicoForce. In brief, the tip-sample separation is controlled with a piezoelectric device with 20 microns (µm) of range. Piezoelectric devices are manufactured from materials that expand and contract with variation of the voltage applied across them. Since the response of piezoelectric devices is not inherently linear or constant, a sensor is included for measuring the actual displacement in the Z-axis with a precision better than 0.5nm. The capacitive sensor design takes advantage of the fact that the capacitance between two parallel plates varies inversely with separation distance. The sensor is included in an ultra-fast feedback loop that ensures that the Z-axis moves the desired distance at the specified speed.

Tip-sample separation and data acquisition are controlled with a custom software graphical user interface (GUI) written in the Microsoft Windows environment. This GUI enables researchers to measure forces in response to automated control of tip-sample separation, to measure forces associated with complex user-scripted tip-sample separation profiles, and to measure forces encountered while controlling the tip-sample separation manually with a novel, tactile-feedback device, the PicoAngler.

The PicoAngler includes a knob that controls the tip-sample separation with four levels of sensitivity. The knob incorporates a variable torque device that amplifies the tip-sample force by a variable gain of roughly ten billion. This extremely high gain on the real-time tactile-feedback device allows researchers to actually feel the forces involved as proteins unfold and antibodies unbind. In addition to its usefulness in mapping out these forces, the PicoAngler provides researchers with a truly new (patent pending), unique, and exciting perspective on their measurements.

In addition to controlling tip-sample separation, the MultiMode PicoForce allows the tip to be moved in the X-Y plane relative to the sample with a range greater than 40µm square. This allows precise positioning of the tip for force measurements and also allows the MultiMode PicoForce to be used for standard SPM/AFM imaging modes. It is the very first commercial instrument in its class to include both precise force measurement capabilities and X-Y scanning capabilities.

These capabilities can be brought to bear on a variety of samples in the fields of biology, biophysics, physics, materials science, and chemistry. Samples can be investigated in their appropriate, native environments, including controlled gas atmospheres, biological buffers and other liquids.

1.2   Scope of this Document

This *MultiMode PicoForce Manual* details the use of the MultiMode PicoForce Option on an SPM. The MultiMode may be operated by a NanoScope IIIa (NSIIIa) Controller in conjunction with an Extender Electronics Module or Quadrex, or operated by a NanoScope IV (NSIV) or NanoScope V Controller (with Quadrex built-in). Refer to *Support Note 322, Quadrex* for discussion of the Quadrex module. Refer to the *NanoScope IV* or *V Controller Manual* for discussion of Quadrex features for SPMs operated by NanoScope IV or V Controllers. Quadrex capabilities are identical for phase-equipped SPMs, regardless of which controller is used.

For specifics of your SPM, refer to its accompanying *MultiMode Scanning Probe Microscope Instruction Manual*. For coverage of all software commands and details of the various modes of operation (Realtime, Offline, Image, Scope, Force, etc.), refer to the *Command Reference Manual* and/or the *NanoScope Software 7 User Guide*. Similarly, add-on modules, sensors, and even areas of investigation (such as electrochemistry) are introduced in Support Notes and Application Notes. Programmable aspects of NanoScope software applicable even to SPM systems without robotic sample loading are described in the *Automation Supplement*. The *Scanning Probe Microscopy Training Notebook* is a compact introduction for getting started on MultiMode and Dimension SPMs.

1.3   Conventions

- In the interest of clarity, certain nomenclature is preferred. An SPM *probe* is comprised of a *tip* affixed to a *cantilever* mounted on a *substrate*, which is inserted in a *probe holder*.

- Three font styles distinguish among contexts. For example:
  Window or Menu Item > **BUTTON OR PARAMETER NAME** is set to **VALUE**.

- A *phase-equipped* SPM implies that an SPM has the required modification to perform PhaseImaging in any of the following configurations:

  - NSIIIa Controller and Quadrex module

  - NSIIIa Controller and Extender Electronics Module

  - NSIV Controller

  - NSV Controller

  A phase-equipped SPM may also be referred to as *extended,* or *equipped for Quadrex,* or *equipped for Extender Electronics.*

- In pre-Version 6 NanoScope software, the *Control Monitor* is generally used for providing access to control parameters and the *Display Monitor* for displaying scanned images or other results (e.g., sweep curves).

- Software buttons are labelled with options available. For instance, if the Strip Chart Controls **START** button has been clicked, then the system is in Strip Chart Controls Mode and the same button is now labelled **STOP**, the remaining option available: to exit the mode.

List of Tables

Chapter 2　　Safety

This section summarizes safety precautions to observe when installing and operating a Picoforce spectroscopy-capable MultiMode SPM. Additional cautions need to be observed when operating the MultiMode SPM, a component of MultiMode PicoForce; refer to the *MultiMode Scanning Probe Microscopy Instruction Manual*.

	WARNING:	Use MultiMode PicoForce equipment only as specified in this manual and as specified in any documentation associated with its components. Any use of the equipment in an unspecified manner is strongly discouraged and may result in damage or injury as cautioned by signed warnings in this chapter and throughout the documentation.

Table 2.0a Safety Symbols Key

Symbol	Definition
	This symbol identifies conditions or practices that could result in damage to the equipment or other property, and in extreme cases, possible personal injury.
	Ce symbole indique des conditions d'emploi ou des actions pouvant endommager les équipements ou accessoires, et qui, dans les cas extrêmes, peuvent conduire à des dommages corporels.
	Dieses Symbol beschreibt Zustände oder Handlungen die das Gerät oder andere Gegenstände beschädigen können und in Extremfällen zu Verletzungen führen können.
	This symbol identifies conditions or practices that involve potential electric shock hazard.
	Ce symbole indique des conditions d'emploi ou des actions comportant un risque de choc électrique.
	Dieses Symbol beschreibt Zustaende oder Handlungen die einen elekrischen Schock verursachen koennen.
	This symbol identifies a laser hazard. Exposure could result in eye damage.
	Ce symbole indique un risque lié à un laser. Une exposition à ce laser peut entraîner des blessures aux yeux.
	Dieses Symbol bedeutet "Gefährliche Laserstrahlung". Laserstrahlung kann zu Beschädigung der Augen führen.

WARNING: Service and adjustments should be performed only by qualified personnel who are aware of the hazards involved.

AVERTISSEMENT: Tout entretien ou réparation doit être effectué par des personnes qualifiées et conscientes des dangers qui peuvent y être associés.

WARNUNG: Service- und Einstellarbeiten sollten nur von qualifizierten Personen, die sich der auftretenden Gefahren bewußt sind, durchgeführt werden.

	WARNING:	Follow company and government safety regulations. Keep unauthorized personnel out of the area when working on equipment.
	AVERTISSEMENT:	Il est impératif de suivre les prérogatives imposées tant au niveau gouvernmental qu'au niveau des entreprises. Les personnes non autorisées ne peuvent rester près du système lorsque celui-ci fonctionne.
	WARNUNG:	Befolgen Sie die gesetzlichen Sicherheitsbestimmungen Ihres Landes. Halten Sie nicht authorisierte Personen während des Betriebs vom Gerät fern.

	WARNING:	Voltages supplied to and within certain areas of the system are potentially dangerous and can cause injury to personnel. Power-down everything and unplug from sources of power before doing ANY electrical servicing. (Bruker personnel, only).
	AVERTISSEMENT:	Les tensions utilisées dans le système sont potentiellement dangereuses et peuvent blesser les utilisateurs. Avant toute intervention électrique, ne pas oublier de débrancher le système. (Réservé au personnel de Bruker seulement).
	WARNUNG:	Die elektrischen Spannungen, die dem System zugeführt werden, sowie Spannungen im System selbst sind potentiell gefährlich und können zu Verletzungen von Personen führen. Bevor elektrische Servicearbeiten irgendwelcher Art durchgeführt werden ist das System auszuschalten und vom Netz zu trennen. (Nur Bruker Personal).

	DANGER:	Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous laser light exposure. The use of optical instruments with this product increases eye hazard.
	DANGER:	Toute utilisation, ou étalonnage ou essai de modification, autre que ci-dessous décrits, peut entraîner une exposition dangereuse à la lumière du laser. L'utilisation de systèmes optiques avec ce produit peut entraîner un danger pour les yeux.
	GEFAHR:	Die falsche Verwendung dieses Gerätes mit nicht in diesem Handbuch beschriebenen Vorgehensweisen kann gefährliche Laserstrahlung freisetzen. Optische Instrumente, die zusammen mit diesem Produkt verwendet werden, können evtl. Augenschäden hervorrufen und verstärken.

Chapter 3   Installation: PicoForce on a New MultiMode System

This chapter includes a list of MultiMode PicoForce system components, including those that comprise a MultiMode system (see **System Components**, page 11).

> **Note:** If you are not sure if you want to upgrade an existing MultiMode system, see **Chapter 4, Installation: Upgrade to PicoForce**, for detail as to which components require upgrade.

3.1   Unpacking MultiMode PicoForce System Components

A MultiMode PicoForce system is typically shipped in more than one crate.

| | **CAUTION:** | Do not store the equipment outside, even in a dry weather location. |

The PicoForce-specific components are packed together in one crate, see Figure 3.1a. Carefully unpack the components from their crates.

| | **CAUTION:** | Handle sensitive electronics with care. Avoid dropping or bumping the NanoScope Controller and PicoForce Control Module, especially the PicoForce scanner, and especially outside the shipping crate(s). |

Provide the proper environmental conditions (see Table 3.1a) for system operation and storage.

Table 3.1a   Environmental Requirements

Equipment Use	Condition	Requirement
Operation	Temperature	15ºC (59ºF) to 35ºC (95ºF)
"	Relative Humidity	45% to 80%, non-condensing
Storage	Temperature	10ºC (50ºF) to 50ºC (122ºF)
"	Relative Humidity	35% to 90%, non-condensing

> ⚠️ **CAUTION:** Do not obstruct the ventilation slots of either the NanoScope controller or the PicoForce Control Module.

Figure 3.1a Packaged PicoForce Option Components: a) Top Layer, b) Bottom Layer

---
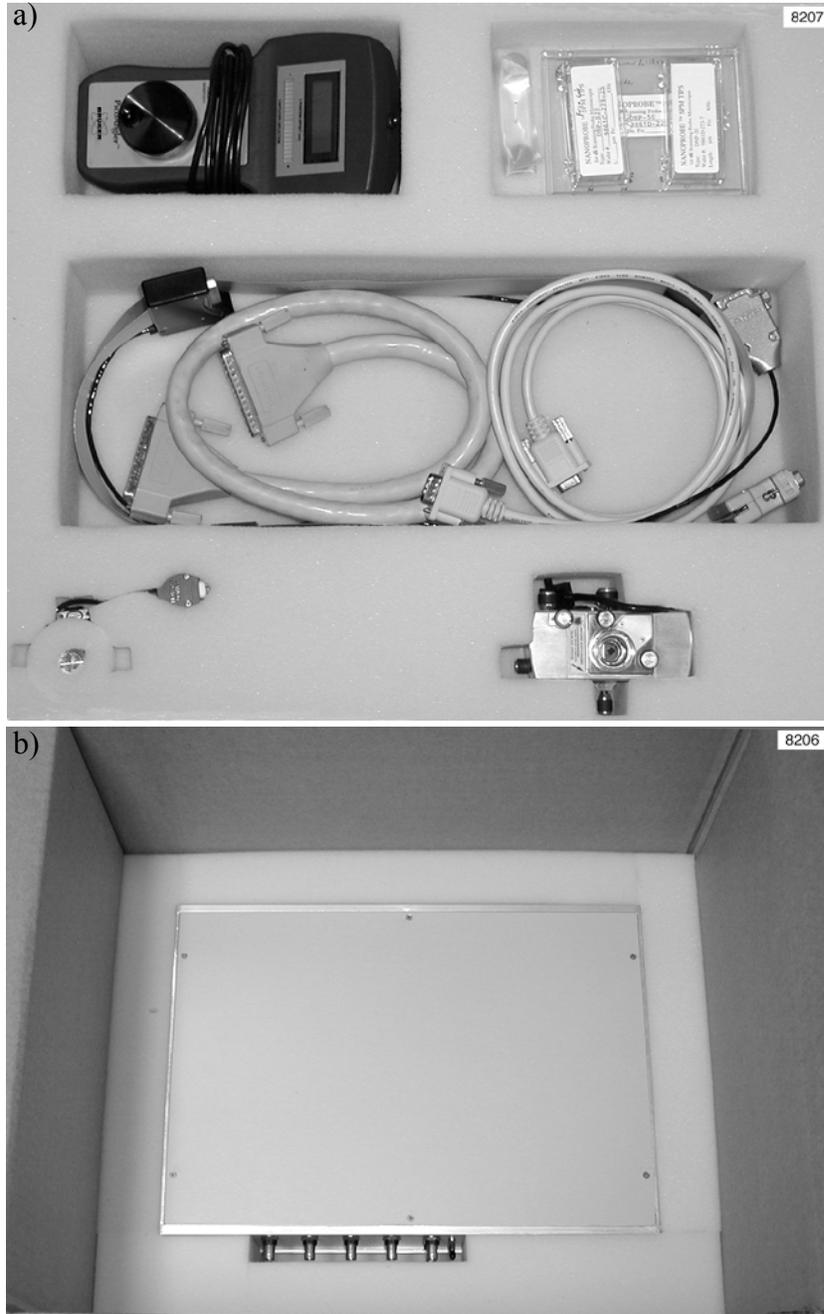
3.2   System Components

A MultiMode PicoForce system can be purchased as an upgrade to the MultiMode SPM; its minimum component list:

- Low Noise MultiMode SPM head (see Figure 3.2a), a required upgrade

Figure 3.2a   A MultiMode SPM Head

- MultiMode SPM scanner stabilizing screw

- MultiMode SPM scanner parameter files (hardcopy, softcopy if sold with computer)

  Note: Find calibrated scanner parameter files in the hard disk EQUIP directory.

- Scanner calibration reference samples: XYZ, 3μm×3μm, 180nm vertical

- *Calibration Standard Application Note*

- A closed-loop Z-axis "PF" vertical engage scanner (see Figure 3.2b)

- Storage box for PicoForce scanner

- PicoForce Probes Model MLCT-AUNM

Figure 3.2b   PicoForce Closed Loop Z MultiMode "PF" Vertical Engage Scanner

- A PicoForce Control Module

- A PicoAngler with cable to the PicoForce Control Module

- Cantilever Holder for Scanning in Fluid (TappingMode). Also referred to as a tapping fluid cell, model MTFML

- A compound cable between the MultiMode base and PicoForce Control Module (see Figure 3.2f, cable "A")

- A cable between the PicoForce Force Spectroscopy Control Module and Extender, or Quadrex, Module with 37 pin connectors (see Figure 3.2f, cable "B")

- A 9-pin serial cable between the PicoForce Control Module and the computer (see Figure 3.2f, cable "D")

- PicoForce Sensor Module Jumper (only used with NanoScope IV Controller or Quadrex)

- PicoForce Passband gain Dongle (not included with the NanoScope V Controller)

- Picoforce Deflection Gain Dongle (not included with the NanoScope V Controller)

- This *MultiMode PicoForce Manual*.

- *PicoForce Thermal Tune Procedure Note*

Additional compatible options that may be purchased with a MultiMode PicoForce system:

- Enhanced optical top viewing system, either: optical microscope (OM), OMV (video, see Figure 3.2c), CCD only, or CCD plus OM

- vibration isolation with tripod (TRV1) or table (VT-102)

The MultiMode PicoForce Option for the MultiMode requires a NanoScope IIIa Controller and either an Extender Electronics Module or a Quadrex module, a NanoScope IV or V Controller (with Quadrex built-in). The Extender and Quadrex monitor cantilever vibration phase with respect to the driving force of the tapping oscillator in TappingMode, enabling PhaseImaging.

See the *MultiMode Scanning Probe Microscope Instruction Manual* for system components included with the MultiMode SPM.

Figure 3.2c   The MulitMode PicoForce System with Optional OMV Video Optics

7990

3.2.1   Hardware Configuration

One recommended system layout is shown in Figure 3.2c. Refer to the installation instructions in the *MultiMode SPM Instruction Manual* as required in addition to the following configuration and cabling guidelines. Once you have the computer and controller cabled (including cable E), make the PicoForce Option connections with cables A, B, C and D as shown in Figure 3.2f, selecting the configuration option based on whether the system includes an NSIIIa Controller with the Extender Electronics Module (option 2), an NSIIIa Controller with Quadrex (option 3), an NSIV or NSV Controller with built-in Quadrex (option 1). Cable A has a 37 pin and a 15 pin connector at the PicoForce Force Spectroscopy Control Module end (see Figure 3.2e) and a 25 pin connector with a smaller embedded 9 pin connector (to the scanner) at the MultiMode end (see Figure 3.2d); cables B and C are identical and have 37 pin connectors at both ends. Cable D is a 9 conductor serial port

cable. Cable E is a 25 conductor parallel port cable. The labels on the mating connectors on the instruments are listed in Table 3.2a (option 1), Table 3.2b (option 2) and Table 3.2c (option 3).

> **Note:** Additional cables from the computer to other peripherals are not shown here. These are configured by Bruker personnel during installation and are associated with specific serial port assignments.

Figure 3.2d   Cable A Between the MultiMode SPM and the PicoForce Control Module
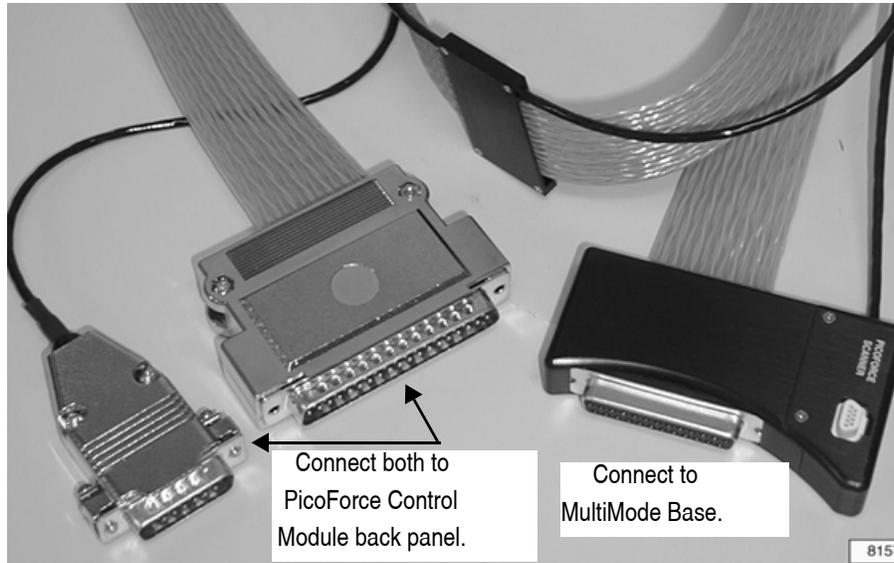
Figure 3.2e   Connectors on the Back Panel of the PicoForce Control Module
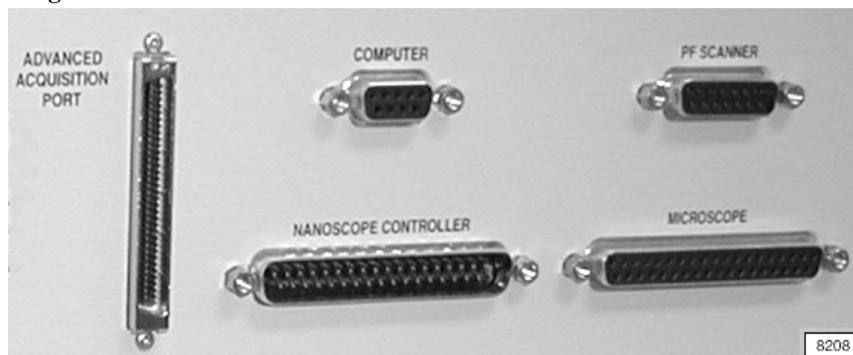
Figure 3.2f MultiMode PicoForce System Interconnection Options
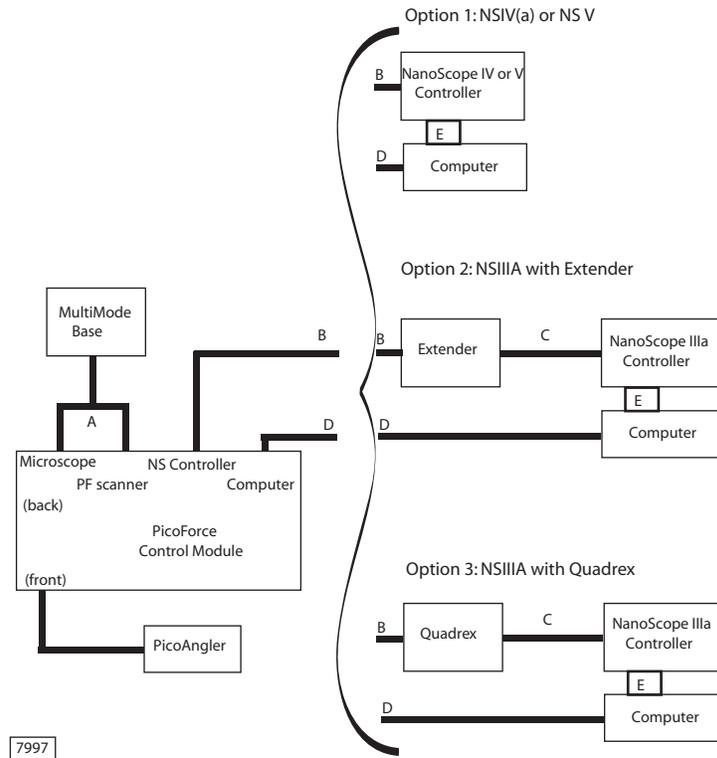
Table 3.2a Option 1: PicoForce Cabling with NSIV(a) or NSV

Cable	Location	Connector Label
A (25 pin)	MultiMode SPM	unlabeled (on the base of the SPM)
A (37 pin)	PicoForce Control Module	Microscope
A (15 pin)	PicoForce Control Module	PF Scanner
B (37 pin)	PicoForce Control Module	Nanoscope Controller
B	NSIV	To Microscope (back of NSIV)
D (9 pin)	Computer	A COM port
D	PicoForce Control Module	Computer
E (25 pin)	NSIV	To Computer (back, middle)
E	Computer	To Nanoscope Controller

Table 3.2b   Option 2: PicoForce Cabling with NSIIIa and Extender

Cable	Location	Connector Label
A (25 pin)	MultiMode SPM	unlabeled (on the base of the SPM)
A (37 pin)	PicoForce Control Module	Microscope
A (15 pin)	PicoForce Control Module	PF Scanner
B (37 pin)	PicoForce Control Module	Nanoscope Controller
B	Extender	To Microscope
C (37 pin)	Extender	To NanoScope
C	NSIIIa	unlabeled (front of NSIIIa)
D (9 pin)	Computer	A COM port
D	PicoForce Control Module	Computer
E (25 pin)	NSIIIa	To Computer (back, top, right)
E	Computer	To Nanoscope Controller

Table 3.2c   Option 3: PicoForce Cabling with NSIIIa and Quadrex

Cable	Location	Connector Label
A (25 pin)	MultiMode SPM	unlabeled (on the base of the SPM)
A (37 pin)	PicoForce Control Module	Microscope
A (15 pin)	PicoForce Control Module	PF Scanner
B (37 pin)	PicoForce Control Module	Nanoscope Controller
B	Quadrex	To Microscope
C (37 pin)	Quadrex	To NanoScope
C	NSIIIa	unlabeled (front of NSIIIa)
D (9 pin)	Computer	A COM port
D	PicoForce Control Module	Computer
E (25 pin)	NSIIIa	To Computer (back, top, right)
E	Computer	To Nanoscope Controller

MultiMode PicoForce requires a terminated connector, the PicoForce Sensor Module Jumper (a "dongle") with Quadrex-capable NS IIIa or NSIV(a) systems. When operating the PicoForce Option, connect the Sensor Module Jumper (see Figure 3.2g) to the 15-pin **SENSOR MOD.** connector (see Figure 3.2h) on the back of the NSIV(a) Controller (which includes Quadrex) or to the **APPLICATIONS MODULES** connector (see Figure 3.2i) on the back of the Quadrex Module (used with an NSIIIA Controller).

> **Note:**   PicoForce will operate without the jumper. However, the Z sensor data channel will be disconnected.

Figure 3.2g   The PicoForce Sensor Module Jumper

Figure 3.2h   Close-Up of Connectors, Back of the NanoScope IV Controller
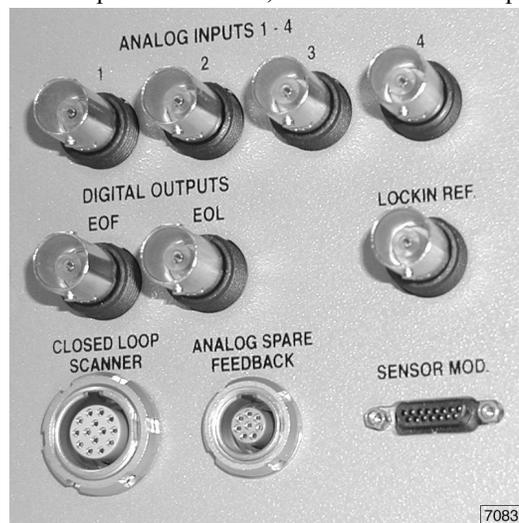
Figure 3.2i   Close-Up of Connectors, Back of the Quadrex Module

3.3   Important: Powering Sequence

When the unpowered hardware is configured:

1.   Attach their power cords to the computer and NanoScope controller, then connect these to appropriate power outlets.

Note: Ensure that the NanoScope Controller, PicoForce Controller and computer are powered by the same outlet to avoid ground loops. Ground loops have been shown to increase noise.

2. Attach the power cord to the connector at the left of the back panel of the PicoForce Control Module (see Figure 5.2a).

3. Verify the available power matches the ratings specified on the PicoForce Control Module back panel (see Table 13.2b) and the fuses installed (see Table 13.2a).

4. Connect the other end of the same power cord to a wall outlet. Always supply and remove power from components in the correct order.

Note: If the computer is turned off, the NanoScope controller must also be unpowered.

⚠	**CAUTION:**	Never supply power to the NanoScope Controller and PicoForce Control Module unless the computer is already running.

Power on sequence: 1) computer
2) NanoScope controller
3) PicoForce Control Module

Power off sequence: 1) PicoForce Control Module
2) NanoScope controller
3) computer

Proceed to **Chapter 5, Hardware Orientation**, for an introduction to the PicoForce Control Module.

Chapter 4   Installation: Upgrade to PicoForce

4.1   Existing System Component Upgrades

An existing MultiMode SPM system may be upgraded to include the PicoForce Option (see **System Components,** page 11, for both MultiMode and PicoForce-specific components).

> **Note:**   Contact Bruker (either your local representative or 805 967 1400) for assistance evaluating an existing MultiMode SPM system for upgrade to MultiMode PicoForce and/or for coordination of the installation.

The flow chart in Figure 4.1a outlines the steps to convert whatever components are available into a full MultiMode PicoForce system. The one-step upgrade of a MultiMode SPM from a system including a pre-NSIIIa Controller to a full MultiMode PicoForce appears across the top of the flow chart, In general, components that may require upgrade appear in decision diamonds, mostly along the left of the chart; the individual upgrade solutions appear in boxes, mostly along the right of the chart. Each element is discussed below.

- MultiMode PicoForce requires an SPM controller with phase measurement capability. There are three possibilities:

  ▶ an NSV Controller (which incorporates the Quadrex module),

  ▶ an NSIV(a) Controller (which incorporates the Quadrex module),

  ▶ an NSIIIa Controller with an Extender Electronics Module, or

  ▶ an NSIIIa Controller with a Quadrex module.

  > **Note:**   Select Quadrex over Extender phase signal handling if your applications include quantitative PhaseImaging of cantilever oscillation phase angles with respect to the driving frequency.

- MultiMode PicoForce uses an additional analog-to-digital converter (ADC) compared to standard MultiMode. The PicoForce Option "ADC5 Upgrade" (see Figure 4.1a) to MultiMode systems based on the NSIIIa Controller adds an ADC either via the ADC5 product (providing three more ADCs in all) or by installation of a single integrated circuit. The NSIV Controller includes all features of both the NSIIIa Controller with the ADC5 Upgrade, so does not require an additional ADC5 Upgrade.

  > **Note:**   Refer to *Support Note 255, A/D Upgrade* for details of the ADC5 Upgrade and its installation procedure. Adding the one ADC integrated circuit is also easy. Either installation is typically performed by Bruker personnel.

Figure 4.1a MultiMode PicoForce System Configuration Flow Chart
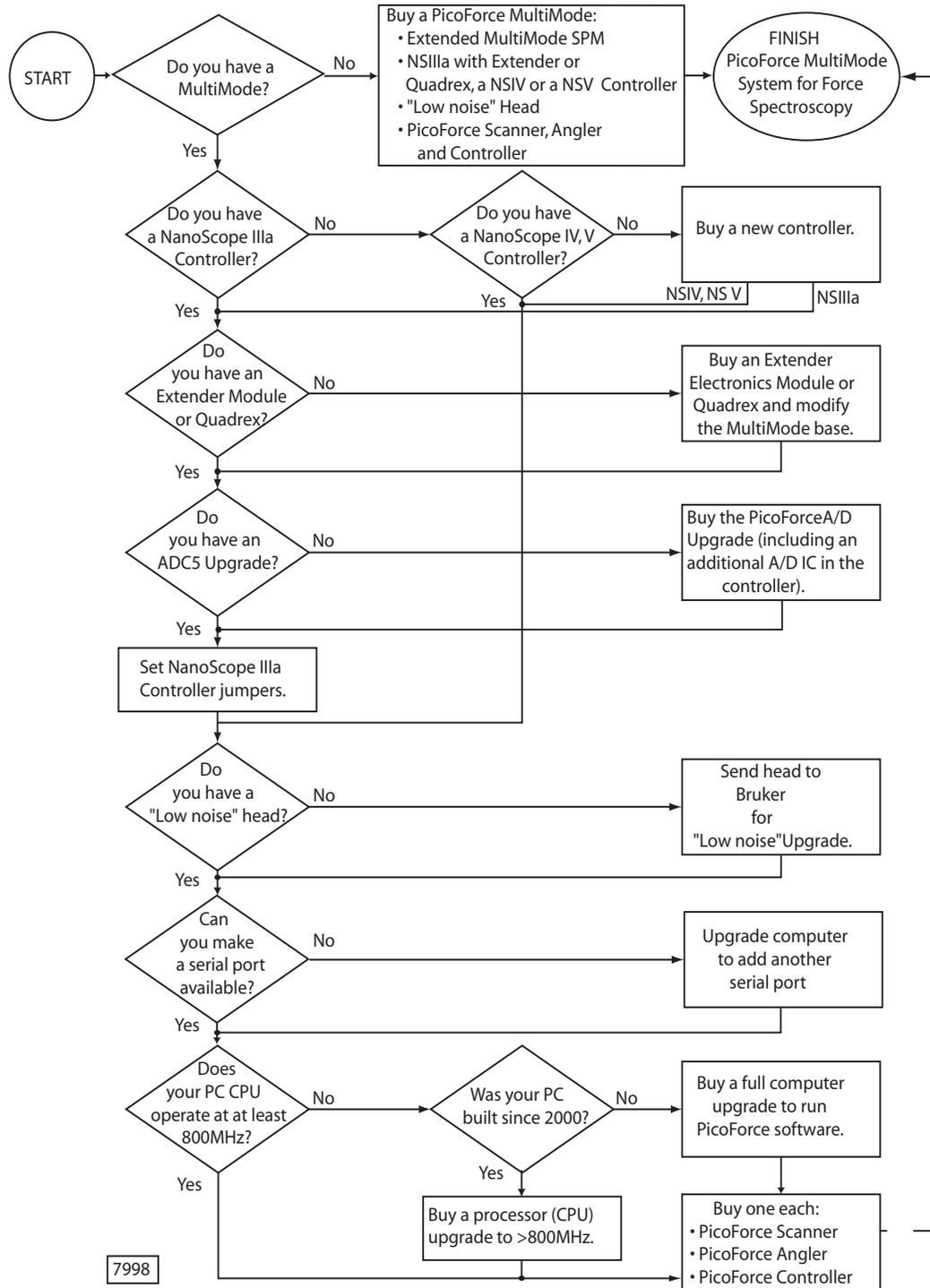
- The laser light source used for optical lever photodetection (see Figure 1.1c) in MultiMode PicoForce systems generates visible red light like the laser used in standard MultiMode SPMs. The superior force resolution of MultiMode PicoForce derives from specified light emission characteristics of the laser and the geometric arrangement of components of the optical lever. Together, these enhancements define the low noise MultiMode head used in the PicoForce Option.

- The PicoAngler interface requires an additional serial port for MultiMode PicoForce compared to standard MultiMode SPM operation.

- The combination of NanoScope software version 6 or 7, the data rate requirements for cantilever deflection signal sampling, PicoAngler interaction and realtime display result in the need for a minimum of 1700MHz computer clock speed in a MultiMode PicoForce-compatible computer. Bruker recommends a minimum of 512MB of RAM.

- The PicoForce Scanner is optimized for push/pull applications orthogonal to the sample plane and includes an embedded Z-axis sensor for closed loop control in that axis. The PicoForce Scanner can also be used for imaging.

> **Note:** For the highest resolution imaging applications without the need for pushing or pulling, use an alternative scanner to the PicoForce "PF" scanner.

4.2 Installation of Upgraded Components

Contact Bruker (either your local representative or 805 967 1400) for assistance evaluating an existing MultiMode SPM system for upgrade to MultiMode PicoForce and/or for coordination of the installation. Coordination of the upgrade of components can speed the process of assembling a MultiMode PicoForce system from a MultiMode SPM. Upgradable components are listed by upgrade procedure in Table 4.2a.

Table 4.2a MultiMode PicoForce Component Upgrade Processes

Owner Installs (or Bruker Assists): External Cabling Only	Owner Installs (or Bruker Assists): Including Internal Modifications	Owner Sends Item; Bruker Upgrades & Returns It
MultiMode SPM	—	Low noise head
NSIIIa, NSIV(a) or NSV Controller	—	—
Extender/Quadrex module (with NSIIIa only)	ADC5 Upgrade, including jumpers (NSIIIa only)	—
MultiMode PicoForce-compatible computer	Additional computer serial port	—
PicoForce Scanner	—	—
PicoAngler	—	—
PicoForce Control Module	—	—

Reference the documentation, including installation procedures, provided with major MultiMode PicoForce components:

- MultiMode SPM—*MultiMode Scanning Probe Microscope Instruction Manual*

    Note: Conversion between PicoForce and other scanners is described under **Unpack the System** within **Chapter 3**, **Setup & Installation** of the *MultiMode Scanning Probe Microscope Instruction Manual.*

- NanoScope IV(a) Controller—*NanoScope IV(a) Controller Manual*

- NanoScope V Controller—*NanoScope V Controller Manual*

    Note: There is no corresponding manual for the NanoScope IIIa Controller.

- Extender Electronics Module—*Support Note 205, Hardware and Software Installation— Extender Electronics Module: MultiMode AFM*

- Quadrex—*Support Note 322, Quadrex PhaseImaging*

- ADC5 Upgrade—*Support Note 255, ADC5 Installation and Operation*

- NanoScope Version 6 Software—*NanoScope Software 6.0 User Guide*

- NanoScope Version 7 Software—*NanoScope Software 7.0 User Guide*

Cabling interconnections among MultiMode PicoForce components is the same for new and upgraded systems (see **Hardware Configuration, page 13**).

	CAUTION:	Make sure you review **Important: Powering Sequence, page 17**, before powering your upgraded MultiMode PicoForce system.

Chapter 5   Hardware Orientation

The most complete introduction to each component of a MultiMode PicoForce system appears in the directly associated documentation. Refer to the manuals listed at the end of Chapter 4 for details of MultiMode SPM, the NanoScope IV and V Controllers, the Extender Electronics Module, Quadrex and Versions 6 and 7 of NanoScope software. The PicoForce-specific components are introduced in this manual:

- the PicoForce scanner (see **MultiMode PicoForce Specifications,** page 136, for its essential characteristics)

The PicoForce scanner scans over up to 40µm along both the X and Y axes and incorporates a Z-axis capacitive sensor for closed loop positioning within 0.5nm RMS over 20µm of travel toward and away from the sample stage.

Figure 5.0a   PicoForce Scanner

- the PicoAngler (see **PicoAngler Mode,** page 68)

The PicoAngler provides the most unconstrained method of applying force to a sample through the probe. The PicoAngler is discussed following the presentation of more constrained force application techniques: pre-programmed basic and scripted force curves.

- the PicoForce Force Spectroscopy Control Module (see the next section).

5.1   The PicoForce Control Module Front Panel

The PicoForce Force Spectroscopy Control Module, shown in Figure 5.1a, provides a switch between (Pico)**ANGLER OUTPUT** and **USER INPUT**, as well as labelled BNC connectors as described below.

> **Note:**   The PicoForce Control Module front panel BNC connectors are not required for MultiMode PicoForce operation. They are included to provide additional access to "internal" signals that would otherwise require a Signal Access Module to observe.

Figure 5.1a   The PicoForce Control Module Front Panel
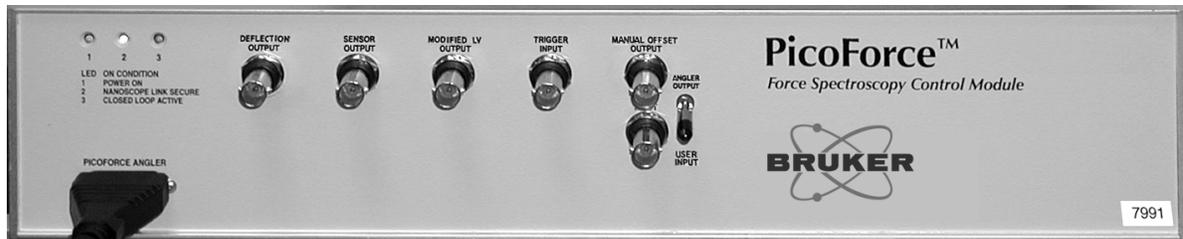
When illuminated, the three lights in the upper left of the PicoForce Control Module front panel indicate, from left to right,

1.   PicoForce Control Module power on,

2.   communication established: PicoForce Control Module and NanoScope controller, and

3.   the PicoForce scanner is operating under Z-axis closed loop control.

The signal specifications for the PicoForce Control Module BNC connectors are described below and first summarized in Table 5.1a.

⚠	WARNING: Because the ±220V piezoelectric element drive signals are routed from the NanoScope controller through the PicoForce Control Module and on to the MultiMode, precautions must be observed to contain equipment damage and avoid injury in the unlikely event of a failure resulting in a high voltage appearing on the signal conductor of an external BNC terminal of the PicoForce Control Module. Therefore:
	
	        Only make connections to equipment with no accessible ungrounded surfaces.
	        Only make connections to equipment with signal-to-ground insulation rated for at least 450V.
	Only make connections to equipment designed for the intended use and with electrical termination that constrains the conveyed signal within the limits set in Table 5.1a. For instance, a 10V analog output introduced to an oscilloscope through an input impedance of 1kΩ limits current to 10mA.

Table 5.1a   Connections to PicoForce Control Module Front Panel External Terminals

Connector Label	BNC Signal Type	Voltage Range	Current Limit
DEFLECTION OUTPUT	Analog output	±10V	10mA
SENSOR OUTPUT	Analog output	±10V	10mA
MODIFIED LV OUTPUT	Analog output	±12V	10mA
TRIGGER INPUT	TTL input	0-5V	n/a
MANUAL OFFSET OUTPUT	Analog output	±10V	10mA
MANUAL OFFSET INPUT	Analog input, 1M¾ impedance	±10V	10mA

Description of the individual signals accessible on the PicoForce Control Module front panel:

- **DEFLECTION OUTPUT**—is the electrical representation of the bending of the cantilever. The "vertical" photodetector output signal from top quadrants A (left) and B (right) and bottom quadrants C (left) and D (right), combine (Figure 5.1b) in the voltage [(A+B)-(C+D)]/[A+B+C+D], representing the degree of deflection of the optical lever. The deflection signal range is approximately ±10V and is proportional to the cantilever bending. Its initial value while the cantilever is off the sample surface is set using the vertical deflection adjustment knob on the MultiMode head as described in the *MultiMode SPM Manual*. Deflection values greater than this non-contact value indicate that the tip is deflecting upwards (pressing on the surface). Conversely, lesser values

indicate that the tip is bending down (pulling on the sample). See **Realtime Analysis,** page 77 for more information on how this voltage signal can be converted to units of distance and force.

Figure 5.1b   Optical Lever Monitoring by Segmented Photodetection
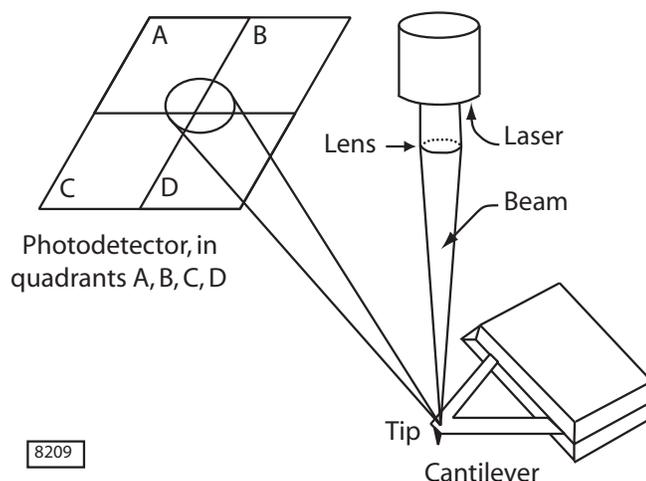
*   **SENSOR OUTPUT**—is the closed loop sensor signal which is proportional to the Z-position of the PicoForce scanner. The sensor generates the closed loop feedback signal, so is the essential monitoring component of the Z-axis control loop. The sensor output signal ranges over ±10V and is linearly proportional to the Z-position of the scanner, where 10V indicates that the scanner is fully extended (the tip-sample distance is minimized) and -10V indicates the scanner is fully retracted (the tip-sample distance is maximized).

    Note: During calibration before shipment, **Z SENSOR SENSITIVITY** is set to -1000nm/ V. The parameter should not be changed from this value.

*   **MODIFIED LV** (Low Voltage) **OUTPUT**—is the Z-axis position drive signal. The "low voltage" (LV) signal, which is amplified to become the "high voltage" (HV) signal that drives the scanner piezoelectric Z-actuator, may be modified from either of two external sources, the PicoAngler or an external input, but not both simultaneously. (This signal is also modified internally as part of engaging and withdrawing the tip with respect to the sample.) When the Control Module switch is set to **ANGLER OUTPUT**, adjustment of the Z-position knob on the PicoAngler modifies the LV signal. When the Control Module switch is set to **USER INPUT**, the signal input to the **MANUAL OFFSET INPUT** BNC is similarly added to the LV signal. See **External Input Mode,** page 73, to use this input.

*   **TRIGGER INPUT**—can be used to synchronize a force measurement with an external signal. A pulse applied to the **TRIGGER INPUT** BNC initiates a force measurement through the software. See **External Input Mode,** page 73 for more information on this function.

- **MANUAL OFFSET OUTPUT**—the signal produced by adjusting the PicoAngler knob and which is added to the internal LV Z-axis signal when the Control Module switch is set to **ANGLER OUTPUT**.

- **MANUAL OFFSET INPUT**—available, when the Control Module switch is set to **USER INPUT**, for external input of a signal to add to the internal LV signal in the same way PicoAngler adjustments do.

- **ANGLER OUTPUT**—the Control Module switch position for PicoAngler control of the sample platform position.

- **USER INPUT**—the Control Module switch position for externally supplying a signal to control the sample platform position.

5.2   The PicoForce Control Module Back Panel

The PicoForce Force Spectroscopy Control Module back panel is shown in Figure 5.2a. Input AC power requirements and fuse ratings are specified in tables along the bottom, left of center, of the back panel and reproduced in **Troubleshooting: General,** page 117.

Figure 5.2a   The PicoForce Control Module Back Panel
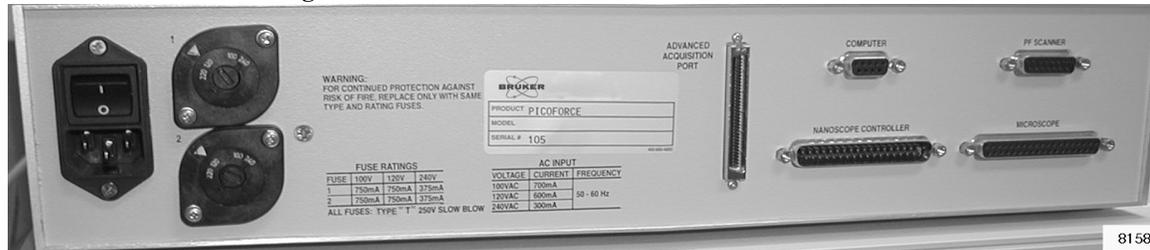
> **Note:**   The **POWER** on/off switch is integral to the power cord connector at the left of the back panel.

> **CAUTION:**   MultiMode PicoForce supports three alternative power supply voltages. Verify that the selected option is visible where the power cord attaches and that the fuses installed are consistent with the voltage available: either 100, 120 or 240VAC

> **Note:**   The Advanced Acquisition Port (center of back panel) is used for the High Sampling Rate Option (see **Advanced Data Acquisition Port Pin Identification,** page 30).

The four multi-conductor connectors at the right of the back panel link the PicoForce Control Module to the computer, the PicoForce scanner, the NanoScope controller and the MultiMode SPM. The system connections and distinguishing characteristics of the different cables are detailed in **Hardware Configuration,** page 13.

5.3   Using the Advanced Acquisition Port

> **Note:**   NanoScope III, IIIA and IV controllers acquire data at a maximum rate of 60kHz. For most MultiMode PicoForce applications that is sufficient and this section can be skipped.

If you need a higher sampling rate, the MultiMode PicoForce Advanced Data Acquisition Port (see Figure 5.2a) is provided for convenient optional interfacing with the National Instruments® (NI) brand of data acquisition (DAQ) boards. This option is useful if you desire faster data capture rates or a custom software interface. The interface has been tested by Bruker with the NI 6052E DAQ board, which is a 16-bit eight input (differential mode), 2 output, 333kHz sampling model. However, many of the NI DAQ boards share the same interface connector pinout but with varying speeds, numbers of inputs/outputs and other features. Please contact National Instruments for details on their DAQ card capabilities and application information.

> **Note:** Bruker does not sell, warrant, or support any NI product. As such, Bruker cannot provide hardware, software, or technical support for this feature beyond our own circuitry which provides and accepts signals to/from the NI DAQ board. Although Bruker has made every effort to protect the interface against short circuits and other electrical faults, the user must accept all responsibility for damage to Bruker or other equipment resulting from misuse of this interface.

Alternatively, the NanoScope V Controller provides much higher sampling rates.

5.3.1   Connector Pinout

The Advanced Data Acquisition Port is a standard 68 pin connector used on many NI DAQ products. Please consult NI on the availability of cables to connect your NI DAQ board to the Advanced Data Acquisition Port.

Signals are configured as follows:

Inputs to a National Instruments DAQ Card

The inputs should be configured as differential inputs on the NI card, which means that each input has an independent ground reference.

Table 5.3a   Advanced Data Acquisition Port Pins Used to Input to NI DAQ Cards

Pin #	NI Signal	PicoForce Signal
68	ACH0	Raw Deflection
34	ACH8	Raw Deflection ground reference
33	ACH1	Filtered Deflection (see note below)
66	ACH9	Filtered Deflection ground reference
65	ACH2	Knob/User Offset
31	ACH10	Knob/User Offset ground reference
60	ACH5	Modified low voltage Z (LVZ)
26	ACH13	Modified LVZ ground reference
57	ACH7	Z Sensor
23	ACH15	Sensor ground reference

> **Note:** The filtered deflection signal is filtered by an 8th order Butterworth response switched capacitor lowpass filter. The corner frequency of this filter is programed by a clock on Pin 1 of the NI connector (FREQ_OUT). The clock frequency to corner frequency is set at a ratio of 50:1 (i.e.- 5MHz clock results

in 100kHz lowpass filter, 2MHz clock results in 40kHz low pass filter). Allowable clock frequencies are 1-5MHz, which gives a tunable filter range of 20-100kHz. The output of this filter is not valid if no clock is present.

Outputs from a National Instruments DAQ Card

Table 5.3b   Advanced Data Acquisition Port Pins Used to Output from NI DAQ Cards

Pin #	NI Signal	PicoForce Signal
22	DAC0OUT	Adds NI offset to the existing Modified LVZ (Modified LVZ= LVZ (from NS controller) + Knob/User Offset + NI offset)
55	AOGND	NI offset ground reference

The Advanced Data Acquisition Port pin assignments appear in Figure 5.3a.

Figure 5.3a   Advanced Data Acquisition Port Pin Identification

8277   Pins Protruding in Connector on Back Panel of PicoForce Control Module
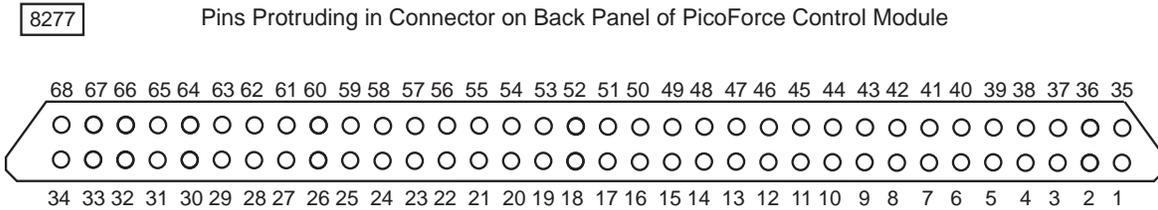
Chapter 6   Software Orientation

PicoForce is among the first Bruker products available exclusively in Version 6 of NanoScope software. Version 6 features a new look and a re-organization of SPM control parameters and options. Version 7 is an evolution of Version 6 and is required to support the NanoScope V Controller.

> **Note:** For a more detailed introduction to the Version 6 or 7 NanoScope software environment, refer to the *NanoScope Software 6 User Guide* or the *NanoScope Software 7 User Guide*.

6.1   Network Connection

MultiMode PicoForce is shipped with a network port in the computer, but not enabled. This avoids a warning message if you wish to first run the system without connecting to a network. To enable the network card, perform the following *before starting NanoScope software*:

1. Click desktop icon **MY COMPUTER** to open the **My Computer** panel (see Figure 6.1a).

2. Click the **CONTROL PANEL** icon to open the **Control Panel**.

3. Click the **SYSTEM** icon to open the **System Properties** panel.

4. Select the **Hardware Profiles** tab. Click **PROPERTIES** to open the **Original Configuration Properties** panel.

5. Select the **Network** tab in the **Original Configuration Properties** panel.

6. Remove the check from **NETWORK-DISABLED HARDWARE PROFILES** box.

7. Close (click the X in the upper right corner of the panel) the **Original Configuration Properties** panel, the **System Properties** panel, the **Control Panel**, then the **My Computer** panel.

> **Note:** Bruker does not supply network services (such as Internet access). Consult a local provider to make use of the computer communications capabilities now enabled in your MultiMode PicoForce system.

Figure 6.1a Enabling the Computer Network Card
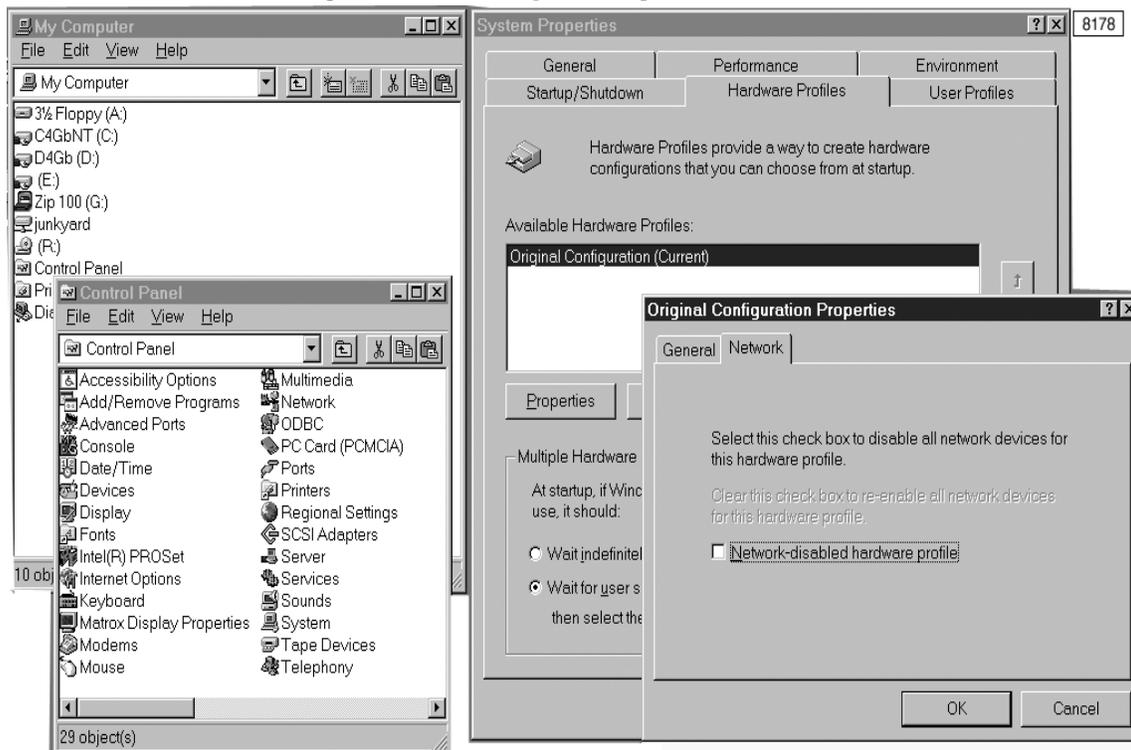
6.2   PicoForce Software Configuration

Double-click the desktop NanoScope icon, shown in Figure 6.2a, to run NanoScope software.

Figure 6.2a   NanoScope Icons, version 6 (left), version 7 (right)
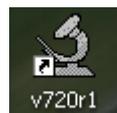
Initially, the software is configured for MultiMode PicoForce and the following section can be skipped. To verify or change your system configuration:

1. Click **Tools** > **SELECT MICROSCOPE** to open the **Microscope Select** panel (see Figure 6.2b).

2. Click the **EDIT** button in the **Microscope Select** panel (see Figure 6.2b) to open the **Equipment** panel (see Figure 6.2c).

3. Verify or enter a distinguishing name for your SPM system under **DESCRIPTION**. The name entered will be added to the **MICROSCOPE SELECT** list when the **Equipment** panel is closed.

4. Select **V** (for NSV), **IV** (for NSIV) or **IIIA** (for NSIIIa) for **CONTROLLER** in the **Equipment** panel.

Figure 6.2b   The **Microscope Select** Panel
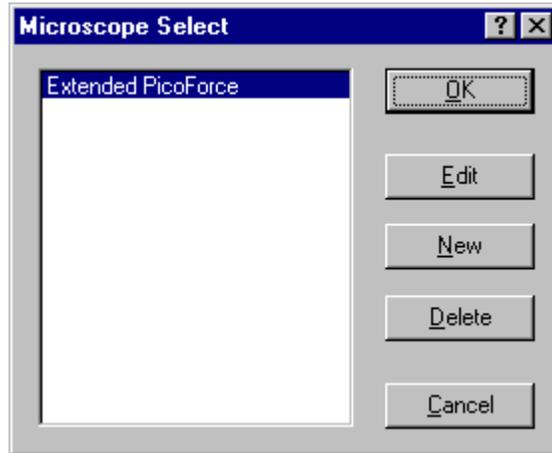
5. Select **PICOFORCE** for **MICROSCOPE** in the **Equipment** panel (see Figure 6.2c). Select **BASIC** or **QUADREX** (if installed) for the **EXTENDER** of your NSIIIA system. (**EXTENDER** is not an option if the **NSIV** or **NSV CONTROLLER** is selected.)

Figure 6.2c   System Component Designation

6. Select **NONE** for **SENSOR** for PicoForce operation. The remaining parameter assignments shown in Figure 6.2c are representative; appropriate values appear initially and do not need adjustment.

Note: The PicoForce Control Module-to-computer serial communication cable (see Figure 3.2f, page 15, cable "D") is assigned to computer port COM1 in this Manual. If you choose an alternative port, be consistent in hardware and software configurations.

7. Click the SERIAL... button of the **Equipment** panel to open the **Serial Port Configuration** panel (see Figure 6.2d). Select COM 1 then press the EDIT button to open the **Edit Port Setup** panel (see Figure 6.2e). Verify or select PICOFORCE from the drop-down menu for COM 1 EQUIPMENT TYPE. The other parameters in the panel should be set as they appear in Figure 6.2e. Click **OK** to close the **Edit Port Setup** panel. Click DONE to close the **Serial Port Configuration** panel. Click **OK** to close the **Equipment** panel. Click **OK** to close the **Microscope Select** panel.

Figure 6.2d Serial Port Configuration Panel
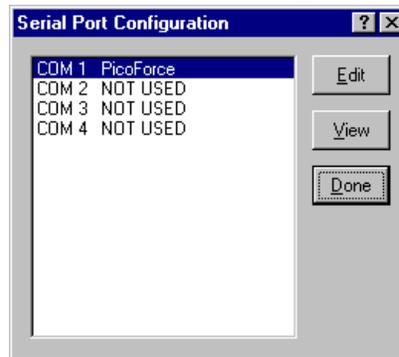
Figure 6.2e The Edit Port Setup Panel
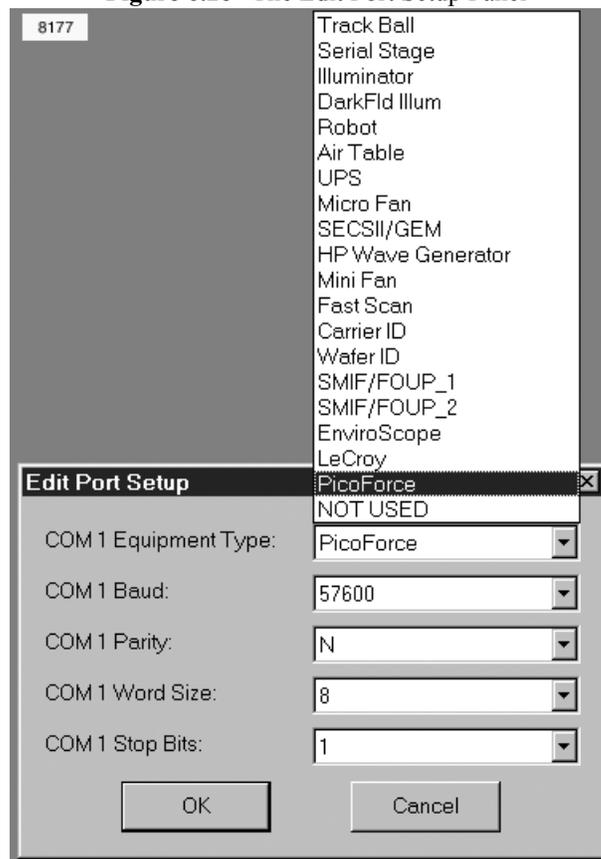
6.3 NanoScope Software Versions 6 and 7

Refer to the *NanoScope Software Version 6* or *Version 7 User Guides* for use information regarding NanoScope software.

Chapter 7   Pulling: Theory and Principles

7.1   Context: Conventional Force Plots

Conventional force plots are simply a plot of cantilever deflection on the Y-axis versus Z-piezo position on the X-axis. They normally include two traces, an approach curve and a retract curve. On a hard surface in air, a curve similar to that shown in Figure 7.1a is commonly obtained. The tip-sample distance decreases as the curve goes from right to left (arrow one). Very close to the surface, the tip snaps into contact with the surface due to short range attractive forces (arrow two). As the piezo continues to extend, the tip is pressed harder into the surface and the cantilever deflection increases. The piezo then reverses direction and begins to increase the tip-sample distance, thus decreasing the cantilever deflection. However, instead of immediately returning to its non-contact value, the deflection commonly continues to decrease. This indicates that the tip is "stuck" to the surface in the thin layer of water and other debris. As the piezo continues to retract, the cantilever strains against this force until it finally breaks free and returns to its non-contact value (arrow 4). Force plots obtained during single-molecule force spectroscopy experiments are similar except that they are conducted in fluid, which greatly reduces the snap-into-contact distance (arrow 2), and the attractive force of the retract portion is due to a molecule being stretched between the tip and the sample surface.

Figure 7.1a   A Traditional Force Plot
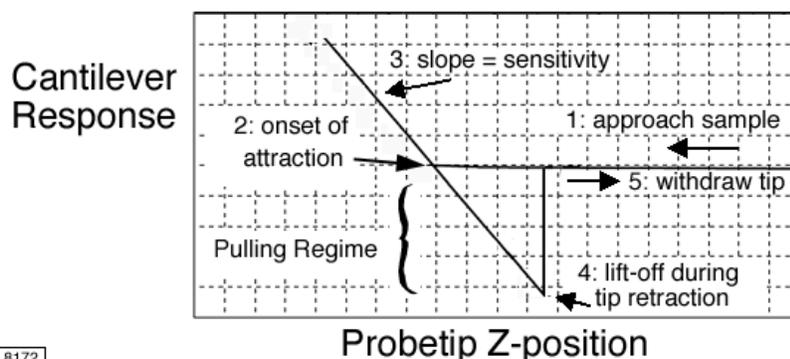
More generally, a *force plot* can be any graph of cantilever response (i.e.- deflection, TappingMode Amplitude, TappingMode Deflection, etc.) vs. some value indicative of the Z-axis position (calculated Z position, Z sensor value, tip-sample separation, etc.). These other options will be considered in more detail in other sections.

7.2   Sensitivity Enhancements for Force Measurement

Turning a MultiMode into a MultiMode PicoForce capable of resolving piconewton forces requires enhanced force plot data collection and interpretation techniques. The three primary issues are: measuring the cantilever deflection, plotting the forces against an accurate position axis, and converting the deflection to units of force.

The most important factor in improving force measurement performance is to ensure that the instrument is *thermally-limited*. This phrase refers to the thermal motion, or "noise," of the cantilever itself. Any cantilever will vibrate at its resonant frequency due to thermal energy alone. Any measurement of cantilever deflection will have this noise superimposed on the rest of the data, provided that the measurement bandwidth includes the resonant frequency. The magnitude of this noise varies with the spring constant of the cantilever and with temperature, but typically is less than 0.5nm for most typical cantilevers. A common, and at first intuitively appealing, misconception for those new to the field is that you should always be able to measure smaller forces by simply using a softer cantilever, the idea being that a given force will produce a larger deflection on the softer cantilever, thus being easier to measure. The problem with this idea is that thermal noise increases with decreasing spring constant. Thus one rapidly approaches a point of diminishing returns when using extremely soft cantilevers. There have been experimental attempts with some success at either actively damping this noise or moving the thermal noise to a frequency higher than the measurement bandwidth so that softer cantilevers can be used. However, just achieving thermally-limited operation is sufficient for most force measurements.

To reach thermally-limited operation, the instrument must not add noise to the deflection measurement or to the actual cantilever oscillations. Therefore the MultiMode PicoForce uses a low noise AFM head that greatly reduces laser interference effects that can occur using a standard MultiMode AFM head. The standard MultiMode AFM head contains a red laser diode for measuring the cantilever deflection with the optical lever technique. This standard head was developed for normal imaging modes in which the cantilever-surface separation is typically very small and does not change very much. Because of these conditions laser interference is rarely a problem when using the standard AFM head for imaging. However, during the measurement of a force plot, the cantilever-sample separation changes from almost zero to up to several microns. These conditions make the optical lever path much more subject to laser interference. This leads to periodic noise on the force plots, which can interfere with force measurements on the piconewton scale. The low-noise head incorporates a low-coherence length laser diode. The result is an optical lever path that is free of laser interference noise.

> **Note:**   Spurious laser interference is periodic with amplitude maxima (or minima) at one half of the coherent light wavelength. To spread apart these occurrences of concentrated noise in the light signal, a longer wavelength laser is an option. To substitute an infrared (IR) laser for the low-noise MultiMode PicoForce red laser, contact Bruker. However, because infrared light is invisible to the unaided eye, laser alignment is more complicated and greater care must be taken to avoid eye damage with an IR laser.

In addition to eliminating detection noise, the MultiMode PicoForce avoids having noise coupled into the cantilever oscillations. Additional oscillations can result if, for instance, the sample position is vibrating and these motions couple to the cantilever through the intervening fluid. The MultiMode PicoForce uses a low-noise capacitive sensor integrated into a carefully designed and

tuned feedback loop to control the sample Z-axis position. This design results in a system with low noise in both sample position and cantilever deflection.

The closed-loop Z-axis also accomplishes the second goal of plotting the forces against an accurate position axis. Most SPMs, including the standard MultiMode, plot forces versus a calculated Z-position based on the anticipated response of the Z-piezo to a given voltage. However, undesirable piezo properties such as creep, hysteresis, and nonlinearity can make this position axis inaccurate, especially for irregular, non-cyclic force plots. The low-noise Z-axis sensor and closed loop Z-axis control ensure that the Z-position is both known and at the exact position specified by software.

The final issue, converting the deflection to units of force, is somewhat more complicated. It requires calibrating the spring constant of the force-measuring cantilever so that one can convert nanometers of deflection to piconewtons of force. This calibration is discussed at length in **Spring Constant Calibration,** next. The PicoForce package includes the ability to calibrate the spring constant with the thermal noise method. However, alternative strategies are included below so you can appreciate the complexity of the issue and perhaps try other techniques on your own.

7.3   Spring Constant Calibration

While the MultiMode cantilever moves in response to forces on it, the measurement made by *optical lever*—bouncing a laser off the top of the cantilever and into a photodetector—is of the cantilever free end position as a function of time. When cantilever deflection is sufficiently small, as is the case in most Contact Mode and TappingMode applications, the relation of the force on the tip to the cantilever free end position is elastic, that is, linear, given by Hooke's Law applied to a spring:

$$F = -k \times h$$

where F = the force on the tip in newtons,
      h = vertical displacement of the free end of the cantilever toward or away from the sample in meters, and
      k = the proportionality constant, known as the *spring constant*, in newtons/meter (or equivalently, and more typically in nanoscale work, in piconewtons/picometer).

Given the spring constant of a cantilever, measured cantilever deflections are converted into inferred tip/sample forces. Therefore, the first step in making accurate force measurements is the determination of the spring constant of the cantilever to be used.

> **Note:** The spring constant is a property of the cantilever. For instance, if determined in air, the spring constant applies to the same cantilever in water or vacuum.

Four methods to deduce a cantilever spring constant are described. Arguably trading off some accuracy for negligible time and effort, MultiMode PicoForce automates the thermal tune method.

1.   estimation from representative geometry,

2.   comparison with and without added mass,

3.   thermal tuning, and

4.   by hydrodynamic model.

A complete discussion of this topic can be found in Bruker Application Note 94, "Practical Advice on the Determination of Cantilever Spring Constatnts," available at http://bruker.com/library. Additional information may be found in "Cantilever spring constant calibration using laser Doppler vibrometry," Benjamin Ohler, Rev. Sci. Instrum., **78**, 063701 (2007).

7.3.1   Estimation from Cantilever Geometry

This first method is quick, but suffers from uncertainty because typically the specific cantilever geometry is not measured and may vary in production. Instead, a representative of the appropriate family of cantilevers is used. Each cantilever family is distinguished by a *tip factor*, b in newton-seconds^3/meter, and the spring constant for a cantilever in the family is derived from the measured resonant frequency, f in seconds^{-1}, of the free cantilever in air by:

$$k = b \times f^3$$

The assumptions that go into this relation are that cantilevers within a family agree in material properties and in geometry, with the exception of cantilever thickness, which varies within the family. Typical average tip factor values by cantilever class are provided in Table 7.3a.

Table 7.3a   Typical Tip Factor Values

Cantilever Class	Representative Cantilever Families	Tip Factor
V-shaped, Contact Mode	NP, DNP, OTR, ORC	7×10^{-15} newton-seconds^3/meter
Single beam, TappingMode	ESP, FESP, LTESPW, MESP, RTESPA, TESP, FIBLADE, FISPIKE, IBMSC	4×10^{-15}
Single beam, indentation	DNISP	2×10^{-12}

Identify your probe cantilever type, measure its resonant frequency (click the **CANTILEVER TUNE** icon (shown)), and calculate its spring constant using the above formula. See the Bruker website, **www.bruker.com**, for more information about available probes.

> **Note:**   For an explicit expression for the spring constant of a single beam cantilever with rectangular cross section in terms of its geometry (other than cantilever thickness), material properties and resonant frequency (cubed, as noted above) see the end of **Adding Mass to the Cantilever,** next.

7.3.2   Adding Mass to the Cantilever

The second method of spring constant determination contrasts with the first in requiring careful work that is rewarded with a more accurate value. Measure the resonant frequency before and after adding a known mass to the cantilever and the resonant frequency shift specifies the cantilever spring constant.

The method is derived here for a model appropriate to TappingMode probes, a single beam cantilever of rectangular cross-section, whose spring constant is given by.

$$k = \frac{Et^3w}{4L^3}$$

where E = the elastic modulus (Young's Modulus),
    t = the cantilever thickness
    w = the cantilever width,
    L = the cantilever length.

The resonant frequency of such a beam is:

$$f = \frac{\omega}{2\pi} = \frac{1}{2\pi}\sqrt{\frac{k}{M + m^*}}$$

where m^* = the effective mass (Ý 0.24m, for beam mass, m = ρtwL, ρ = the cantilever density),
    M = the known mass added to the cantilever free end.

Re-arranging the last equation, added mass, M, varies linearly with $(2\frac{1}{4}f)^{-2}$: M = k$(2\frac{1}{4}f)^{-2}$ - m^*. As a linear relation, comparing the resonant frequency with one added mass to that without any is sufficient to solve for both the spring constant and the effective mass of the cantilever:

$$k = (2\pi)^2\frac{M}{(1/f_1^2) - (1/f_0^2)} \qquad\qquad m^* = M\frac{f_1^2}{f_0^2 - f_1^2}$$

The first solution of the simultaneous equations provides the spring constant, given the added mass and the two resonant frequencies, with added mass, f_1 and without, f_0. The accuracy of the spring constant determination depends primarily on the uncertainty in the added mass as the resonant frequencies typically may be measured to higher precision.

Various methods have been used to affix particles to cantilevers. Three such procedures, developed by researchers using Bruker NanoScopes, are detailed in *Support Note 226B, Attaching Particles to AFM Cantilevers*. By using a cantilever before calibrating it, one can avoid the step of removing the added mass to perform an experiment with a calibrated cantilever.

Substituting the first equation of this section, the expression for spring constant in terms of cantilever geometry and material properties, k(E, t, w, L), into the second equation of this section, the expression for cantilever resonant frequency in terms of spring constant and mass components, f(k, M, m^*), the latter equation, f(E, t, w, L, M, m^*), now reflects geometric characteristics. Re-arranging the new equation as an expression of cantilever thickness in terms of the other variables,

now including resonant frequency, that is, as $t(E, w, L, M, m^*, f)$, the re-arranged equation is substituted for thickness back in the first equation to demonstrate that spring constant is proportional to resonant frequency cubed, independently of cantilever thickness:

$$k = 2\pi^3 L^3 w f_0^3 \sqrt{\rho^3 / E}$$

For further discussion of this derivation, refer to *A Nondestructive Method for Determining the Spring Constant of Cantilevers for Scanning Force Microscopy*, by J. P. Cleveland, et. al., in Review of Scientific Instruments, 64 (2), February 1993, page 403.

7.3.3   Measuring Thermal Noise

The third method of spring constant calibration is often preferred because it is neither as demanding and time-consuming as attaching a particle (method two) nor has as large an associated uncertainty as relying on a representative model (method one). The measurement data consists of a time interval of the deflection signal in Contact Mode (i.e., with no driving oscillation applied electronically) at thermal equilibrium, while the cantilever is suspended, away from any solid surface. Brownian motion of surrounding molecules (e.g., air) impart random impulses to the cantilever during the sampling. The resulting function of time is Fourier transformed to obtain its Power Spectral Density (PSD) in the frequency domain. Integrating the area under the resonant peak in the spectrum yields the power associated with the resonance.

Expressing the dynamics of the cantilever as a harmonic oscillator by the total system energy (the Hamiltonian), the average value of the kinetic and potential energy terms are both

$$\frac{1}{2} k_B T$$

according to the Equipartition Theorem, where T is the temperature in kelvins and k_B is Boltzmann's constant $= 1.3805 \times 10^{-23}$ joules/kelvin. In particular, for the potential energy,

$$\langle \frac{1}{2} m \omega_0^2 z^2 \rangle = \frac{1}{2} k_B T$$

where $\omega_0 = Đ(k/m)$ is the resonant angular frequency, m is the effective mass, z is the displacement of the free end of the cantilever and the "angle" brackets indicate average value over time. Simplifying, the temperature and average displacement determine the cantilever spring constant.

$$k = (k_B T) / \langle z^2 \rangle = (k_B T) / P$$

The original displacement time-series is Fourier transformed to segregate other, broadband, noise contributions from the narrowband thermal noise around resonance. By integrating the area under the resonance in the PSD, while excluding both the noise floor and the "shoulders" to either side of the resonance peak, only the power, P, of the thermal cantilever fluctuations is included. The latter is equal to the mean square of the time-series data. For further discussion of this derivation, refer to *Calibration of Atomic-Force Microscope Tips*, by Jeffrey L. Hutter and John Bechhoefer, in Review of Scientific Instruments, 64(7), July 1993, page 1868.

The MultiMode PicoForce automates the thermal tune calculations. Ten seconds of sampling is sufficient to define the PSD with 25Hz frequency resolution.

The accuracy of the thermal method of cantilever spring constant determination depends not only on the effective isolation of nonthermal noise contributions to the PSD, but also on the accuracy of the PSD and on its magnitude. The Nyquist Sampling Theorem guarantees Fourier Transform accuracy for frequencies up to one half the sampling rate. The standard NanoScope III or IV controller samples the deflection signal every 16.5μs, corresponding to a 64kHz sampling rate. Therefore, cantilever resonances with contributions from frequencies above 32kHz can produce distorted PSDs. This particularly constrains using the method for small cantilevers in air. For this reason a High Sampling Rate Option is available for MultiMode PicoForce systems (see **Using the Advanced Acquisition Port,** page 28). Most biologically interesting samples are best preserved in a fluid environment where cantilever resonant frequencies are considerably lower than in air.

Because the magnitude of the PSD is proportional to the average cantilever displacement, higher spring constant (stiffer) cantilevers produce a smaller signal to analyze. For instance, a spring constant of 0.05N/m produces cantilever displacements of approximately 0.3nm at room temperature. This is representative of the probes with the smallest spring constants in Table 7.3a: SiN, NP, NP-STT, DNP, DNP-S (0.01-0.6N/m), and OTR4 (0.02-0.08N/m) among V-shaped cantilevers, and ESP (0.02-0.1N/m) among single-beam cantilevers.

7.3.4   Rectangular Cantilever, Hydrodynamic Model

By comparing resonance in vacuum to in fluid for a rectangular cantilever and introducing the hydrodynamic function, $\Gamma(\omega)$, which depends on the fluid Reynolds number, cantilever spring constant is computed from easily obtained resonance characteristics and the less variable of probe dimensions:

$$k \; = \; 0.1906\rho_f w^2 L Q_f \Gamma_i(\omega_f)\omega_f^2$$

where w and L are defined as before (see page 41), ρ_f = density of the fluid (e.g., 1.18kg/m^3 for air),

Q_f = the cantilever resonance quality factor in fluid,

ω_f = cantilever resonant frequency in the fluid,

Γ_i, the imaginary component of the hydrodynamic function, $\Gamma(\omega)$, where

$$\Gamma(\omega) \; = \; \Omega(\omega)\left[1 + \frac{4iK_1((-i)\sqrt{iRe})}{\sqrt{iRe}K_0((-i)\sqrt{iRe})}\right]$$

and $\Omega(\omega) = \Omega_r(\omega) + i\Omega_i(\omega)$, where,

$$\Omega_r(\omega) = \frac{0.91324 - 0.48274\tau + 0.46842\tau^2 - 0.12886\tau^3 + 0.044055\tau^4 - 0.0035117\tau^5 - 0.00069085\tau^6}{1 - 0.56964\tau + 0.4869\tau^2 - 0.13444t^3 + 0.045155\tau^4 - 0.0035862\tau^5 + 0.00069085\tau^6}$$

and

$$\Omega_i(\omega) = \frac{-0.024134 - 0.029256\tau + 0.016294\tau^2 - 0.00010961\tau^3 + 0.6458 \times 10^{-4}\tau^4 - 0.00004451\tau^5}{1 - 0.59702\tau + 0.55182\tau^2 - 0.18357\tau^3 + 0.079156\tau^4 - 0.014369\tau^5 + 0.0028361\tau^6}$$

where $\tau = \log_{10}(Re)$, and the Reynolds number, $Re = \rho_f \omega w^2/(4\eta)$ for fluid viscosity η. K_0 and K_1 are modified Bessel functions of the third degree (refer to a source for mathematical functions).

The simplicity of data acquisition and computation (once the equations are set up!) are balanced by a few limitations to the method:

- the leading numerical term (0.1906) assumes a high aspect ratio rectangular cantilever with $L/w > 5$

- $Q_f \gg 1$; a high quality resonance is required: air is a suitable fluid, water is not.

For a derivation of this method, refer to *Calibration of Rectangular Atomic Force Microscope Cantilevers*, John E. Sader, James W. M. Chon and Paul Mulvaney, Review of Scientific Instruments, Vol. 70 (10), October 1999.

7.4   Single Molecule Force Spectroscopy

One key application for which the MultiMode PicoForce is intended is single molecule force spectroscopy. This field encompasses a variety of applications, but can be broadly grouped into two categories: intra-molecular forces (the forces that maintain the folded domain structure of proteins) and inter-molecular forces (the binding force between an antibody and its antigen or between cell receptor molecules and their targets). Although somewhat different in their experimental setup, both categories require piconewton precision because they involve forces on the single molecule scale.

A typical intermolecular binding experiment involves binding one of the molecules to the tip and the corresponding molecules with which it interacts to the substrate surface (see **Tip and Sample Preparation,** page 47). The tip is then brought to the substrate surface before being retracted. If a binding event occurs during the brief period at the surface, a negative deflection results on the retract portion of the force plot. Care must be taken in interpreting the magnitude of the force since it is quite likely for there to be multiple molecular pairs interacting. Commonly the experiment is repeated many times and the unbinding force plotted in a histogram. Often a pattern will emerge where the unbinding force is always some integer multiple of some smaller force, which is presumably the magnitude of a single binding event.

Single molecule pulling experiments on intra-molecular forces are conducted quite similarly. However, in these cases the tip is used without modification and the molecule of interest is simply

allowed to bind to a flat substrate such as mica or gold-coated glass. As before, the tip is brought down to the surface. Commonly it is allowed to sit on the surface with a force of ~1nN for a second or so before being retracted. If a molecule binds to the tip in that time, a negative deflection pattern will appear on the retract portion. Sometimes no binding occurs and sometimes several molecules are stretched at once. But often a single, reproducible "fingerprint" emerges such as that shown below in Figure 7.4a. Here, each one of the spikes in the retract portion represents the unfolding of a single domain in the protein.

Figure 7.4a Force Exerted Unfolding a Titin Molecule

8199
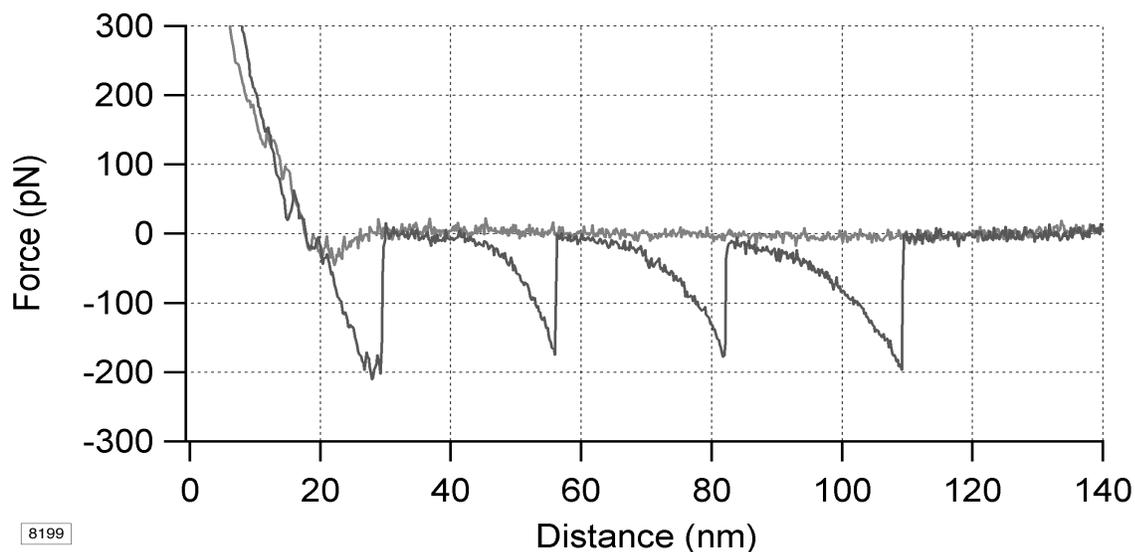
Chapter 8   Pulling: Operation

8.1   Pulling Modes

MultiMode PicoForce features four force measurement modes, one of which has its own dedicated controls and data display, while the other three share a common data display interface better suited to the modes in which they are obtained.

- Basic Force Mode—Standard approach/retract force plots (see **Basic Force Mode,**)

- Scripted Force Mode—More flexible interface allowing numerous user-defined segments to the force curve (see **Scripting Mode,**)

- PicoAngler Mode—Realtime, manual control of force measurement with the PicoAngler force-feedback knob (see **PicoAngler Mode,**)

- Manual External Input Mode—Control of tip-sample separation with an external voltage signal (see **External Input Mode,**)

Tip and sample preparation issues are the same for all force measurement modes.

> **Note:** To display cantilever deflection data as force in piconewtons, a value must have been entered for **DEFLECTION SENS** (see **Realtime Analysis,**) and a value must have been entered for **Force Tab** > **SPRING CONSTANT** either manually, or by activating thermal tune.

8.2   Tip and Sample Preparation

The MultiMode PicoForce takes little time to be ready for measurements once the system is set up. Whereas some samples, such as many polymers, require negligible preparation to be pulled, many interesting specimens must be prepared and stored appropriately until placed on the SPM sample stage. The tip also may be pre-conditioned for certain types of experiments.

8.2.1   Biological Samples

In general, biological samples must be prepared and measured in conditions closely approximating their native environment in order for the results to be meaningful. This generally means conducting the experiment in a fluid buffer of specified ionic composition and pH and, sometimes, temperature. Refer to *Support Note 331*, *Biological Sample Preparation* for more information on typical preparation

techniques for cells, proteins, and DNA. The TappingMode fluid cell probe holder (model MTFML), Figure 8.2a, is used for fluid samples.

For single molecule force spectroscopy experiments, the molecule of interest is typically adsorbed on a hard, flat substrate such as mica or gold-coated glass. Many proteins will adsorb to gold due to the sulfur-containing cysteine groups that they contain. The gold surfaces are commonly gold sputtered, or evaporated onto glass or a polished silicon wafer.

Figure 8.2a   MutiMode (TappingMode, Force Modulation) AFM Fluid Cell
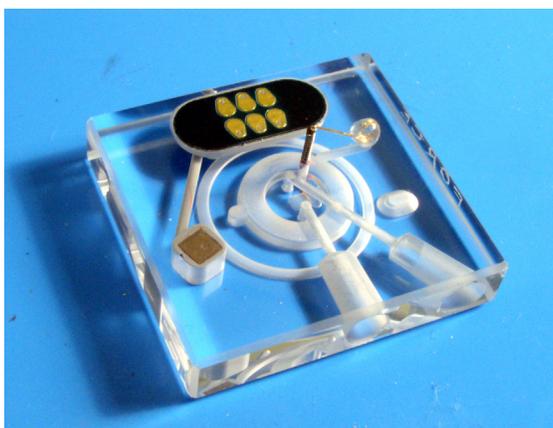
8.2.2   Functionalizing the Tip

In certain force spectroscopy and imaging techniques it is desirable to attach molecules to the surface of the tip, generally with the intent that these molecules preferentially interact with other molecules on the sample substrate. This sort of tip modification is commonly referred to as *functionalizing the tip*. There have been several methods reported in the literature for attachment, including physical adsorption of proteins and covalent attachment via silane or gold-thiol chemistry.

Note that it is generally not important to attempt to confine the functionalization to the tip itself. In practice, the entire cantilever chip substrate is treated at once. When the measurements are actually made, the cantilever and substrate chip are very far from the surface on the scale of the tip and the interaction, so whether they are coated is irrelevant.

Depending on the exact functionalization used, the tip may or may not be able to be reused. Tips with a very delicate attachment or very low surface concentration of molecules may be rendered inactive after a single force measurement. Other tips are more robust and can be used to measure many binding events. If you experience a rapid loss of activity, try making your force measurement first, taking care to only touch the surface once. For this first measurement, use **REALTIME** > **STEP MOTOR...** to lower the tip (using **TIP DOWN**) and take a force curve, successively lowering the tip by the **SPM STEP SIZE** which is set in the **STEP MOTOR...** panel.

8.3   Starting a PicoForce Session

The following procedure initiates a PicoForce session, once the sample and probe are in place:

1. Double click the Version 6 NanoScope icon, shown in Figure 8.3a, to start the SPM software.

    Figure 8.3a   NanoScope Icons, version 6 (left), version 7 (right)
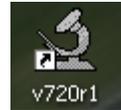
2. Click the **REALTIME** icon (a microscope, shown) or click **REALTIME > START REALTIME** to open a new workspace. The **Scan View** opens with the workspace.

    Note: To reduce setup time, restore and reuse a previously defined workspace (see your *NanoScope Software User Guide*).

    Note: MultiMode PicoForce ships with a PicoForce workspace to get you started with some common settings.

3. Confirm that the software is properly configured for PicoForce (see **PicoForce Software Configuration,** page 32).

4. Engage normally on the surface in Contact Mode (refer to your *MultiMode Scanning Probe Microscope Instruction Manual* and the *Command Reference Manual* for details).

5. Select a Channel 1 **DATA TYPE** from the following options:

    a. **HEIGHT**—traditional Contact Mode image signal: height signal generated from voltage sent to Z-piezo in response to the deflection setpoint feedback.

    b. **DEFLECTION**—the deflection setpoint feedback loop error signal is near zero when the feedback is well-tuned, so this data type is most useful to verify that the gains are well-tuned, and for observing very small height features.

        Note: **FRICTION**, or lateral deflection, is another option in Force Mode. There are other options in other modes.

6. Select a Channel 2 **DATA TYPE** from the following options:

    a. **Z SENSOR**—the Z-position of the sample platform, the sensor represents the actual Z-position.

    b. **Low Voltage Z**—the height signal sent to the Z-piezoelectric actuator (before amplification), which should correspond well with the height signal in closed loop, but not be quite as accurate as the Z sensor in defining probe position vertically.

> **Note:** This data channel can be configured with a jumper inside the PicoForce Controller. Recent PicoForce Controllers (~2006 — present) are configured to pass through the "In1D" data line, which disconnects this signal. This is necessary to support some modes including STM and TRmode. The Z Sensor data type than Modified LV Z. You may consult Bruker for details about changing this configuration.

> **Note:** Channel 3 and its plot are constructed from Channel 1 and Channel 2 signals. Channel 3 in Force Mode displays a force plot, with some representation of cantilever response on the vertical axis and a representation of tip and/or sample Z-displacement on the horizontal axis. To display a force plot where Z-displacement is represented by the tip/sample separation, see **Plotting Deflection versus Tip/Sample Separation,** page 58.

7. Click **ACQUIRE > PICOFORCE** or, from the workspace view, right click the Real Time icon and select **PICOFORCE**.

8. Select **PICOFORCE** from the list of view options.

> **Note:** Spring constant calibration by the thermal tune method is a natural first step once in Force Mode; see **Determine Cantilever Spring Constant by Thermal Tune,** page 77.

9. If desired, select **Workspace** > **SAVE AS**, giving your workspace a name to save it.

8.4   Basic Force Mode

MultiMode PicoForce displays two constituent curves along with their resultant force plot. Cantilever response (deflection in Contact Mode, amplitude in TappingMode) and sample Z-position are each shown as a function of time (**DATA TYPE**s **DEFLECTION** and **Z SENSOR,** respectively) as well as combined as a force versus Z-position plot (see Figure 8.4a).

The Force Mode Tab includes seven buttons (whose functions are discussed in greater detail in the *Command Reference Manual Software Version 5.12 Revision B* and the *NanoScope Software Version 6* or *7 User Guide*):

SINGLE
Perform one force plot extend/retract cycle.

CONTINUOUS
Begin performing force plot extend/retract cycles in succession.

STOP
Quit performing force plot extend/retract cycles after completing the current one.

CAPTURE
Turn on the recording system for saving a force plot.

WITHDRAW
Immediately disengage the tip from the sample.

ZERO SETPOINT

Sets the deflection setpoint equal to the non-contact deflection value in order to correctly represent the force with the force feedback feature of the PicoAngler.

DEFL(ection). SENS(itivity).

Define the conversion factor from cantilever deflection signal voltage to nanometers of cantilever displacement (in nm/V) using data in the contact region of a force plot.

> **Note:** The PicoAngler, which may be used for fully manual force plot generation, is also useful with basic force plot generation, which is otherwise automated (see **Using the PicoAngler in Conjunction with Basic Force Plots,** page 72).

Figure 8.4a   Force Mode: Channels Tab Featuring Inverted Channel 2 Z-Axis
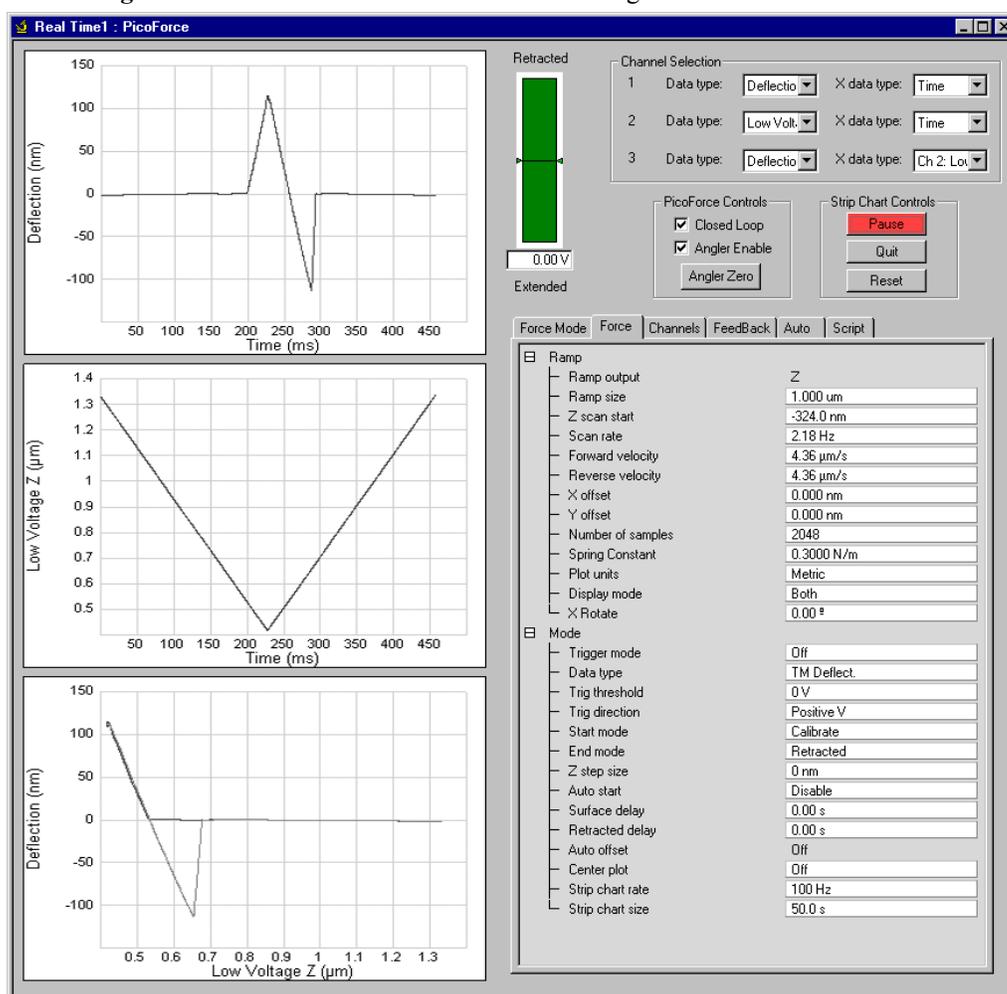
- The bottom plot in Figure 8.4a shows a force plot in its traditional orientation: with cantilever deflection away from the sample increasing upwards on the graph and tip/sample Z-separation increasing to the right. This results from setting **Channel 2** > **PLOT INVERT** to **INVERT**. Channel 1 (the vertical axis of the plot) may be inverted as well and in the same manner.

• The Z-Center indicator in the upper middle of the Force Mode panel, between the words "Retracted" and "Extended," includes a reading of the voltage applied to the Z-piezo. However, any contribution to this voltage from the PicoAngler is not included in this number. The Z-Center indicator, useful for assessing tip/sample separation in many SPM applications, should be ignored when the PicoAngler is active.

• Right click in any displayed graph to bring up a functions menu (see Figure 8.4c). Click **COLOR** and select one of up to five curves, either of the two marker pairs, or one of four other graphic elements, and a color box appears for you to specify its color. Click **FILTER** to customize the display of a graph (not its underlying data set). For instance, a strip chart recording might consist of hundreds of thousands of data points while its display is only 512 pixels across. **Filter** > **Type** > **NONE** results in all data being displayed, effectively plotting multiple vertical axis (y-)values at each horizontal axis (x-)value. Select **4K**, **8K**, **16K** or **32K Points** to limit the display to 4, 8, 16 or 32 times 1024 points. The **MEAN**, **MAXIMUM** or **MINIMUM** y-value per x-value is plotted based on **Filter** > **Type**.

• See Figure 13.3a and the accompanying notes for discussion of the **SCALE** option.

• The **X TRANSLATE** and **Y TRANSLATE** options allow you to redefine the X- and Y-axis zero points of any graph by simply adding or subtracting an offset value. To use, drag a vertical (for **X TRANSLATE**) or horizontal marker (for **Y TRANSLATE**) from either the left or right graph edge (for **X TRANSLATE**) or the top or bottom (for **Y TRANSLATE**) of any graph to the value you wish to redefine as zero. Right-click on the graph and click **X** (or **Y**) **TRANSLATE** in the pop-up menu. The position of the marker becomes the new zero point for the axis (see Figure 8.4b). To go back, right-click to activate the pop-up menu and de-select **X** (or **Y**) **TRANSLATE**. You must also de-select the option before you can change the offset from one you set previously.

Figure 8.4b   X Translate Example
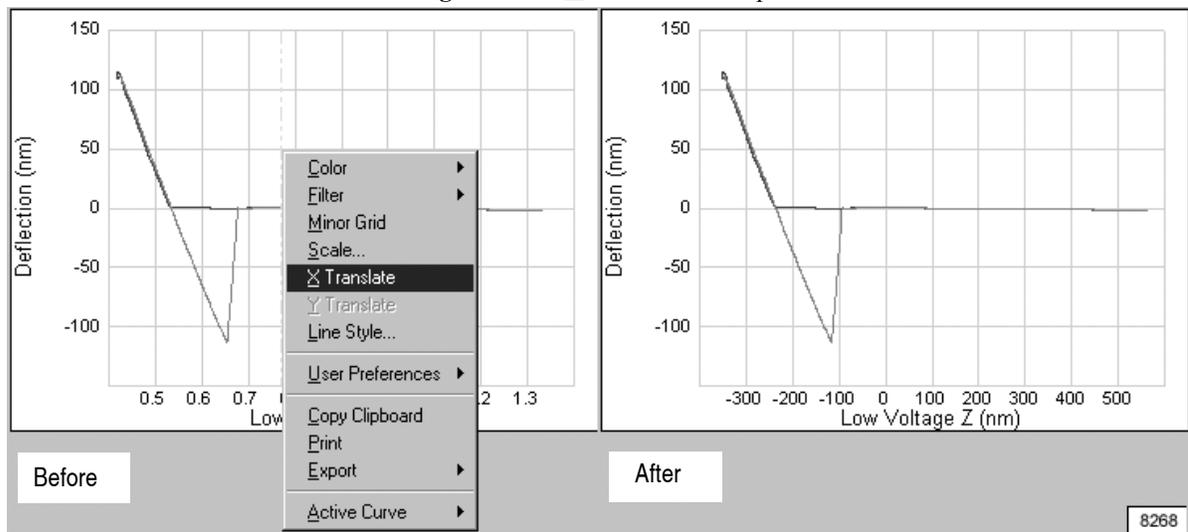
The Parameter Controls shown in Figure 8.4a are a subset selected to be the most frequently used in Force Mode. Parameters appearing under the Force Mode Tab are duplicated from their standard locations in the other tabs; changed values are updated in both places simultaneously.

The definitions of most parameters appearing in the Force Mode tabs are given in the *Command Reference Manual Software Version 5.12 Revision B* and the *NanoScope Software Version 6* or *7 User Guide* under Force Mode (Force Tab, see Figure 8.4f) and under Panels Menu (Channels, see Figure 8.4f; Feedback, and Other Tabs, see Figure 8.4g).

> **Note:** The Z-axis signal is sampled at 30kHz by NanoScope IIIa and IV(a) controllers while NanoScope V controllers sample the Z-axis at 40kHz. The amount of averaging inherent in a reported sample value depends on the SCAN RATE, its partition according to FORWARD VELOCITY and REVERSE VELOCITY, and the NUMBER OF SAMPLES selected. To calculate the number of digitized data points averaged to yield one (Z-position, deflection) force sample, the time interval of a half cycle (either extending or retracting the tip) is multiplied by 30kHz and divided by the NUMBER OF SAMPLES.

Figure 8.4c   Functions Menu: Right Click in Any Displayed Graph
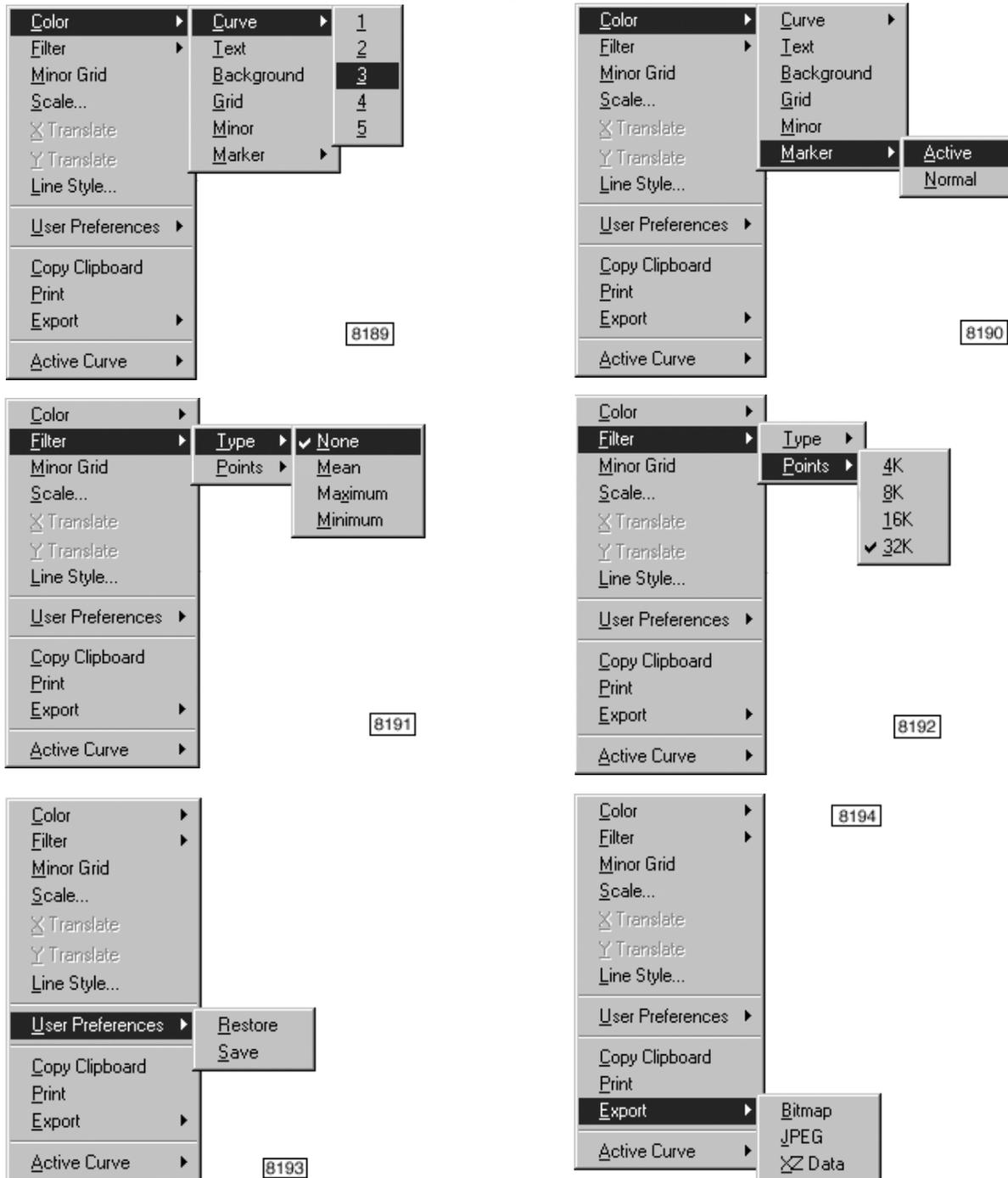
- The display preferences selected in the graph functions menu (Figure 8.4c) are saved by selecting **User Preferences** > SAVE. Saved graph style preferences may be applied to a new graph by selecting **User Preferences** > RESTORE.

- An individual graph may be exported by selecting for **Export** either BITMAP (*.bmp format), **JPEG** (*.jpg format). or **XZDATA** (tab-delimited text).

- To zoom in on any plot, hold down the **CONTROL** keyboard key, position the cursor in the graph and drag open the box that appears. When released, the area in the box is magnified to fill the plot area. Repeat as needed. Click the magnifying glass icon in the bottom left corner of the plot or double-click on the plot to restore the original magnification (see Figure 8.4d).

Figure 8.4d   Zoom In on a Force Plot
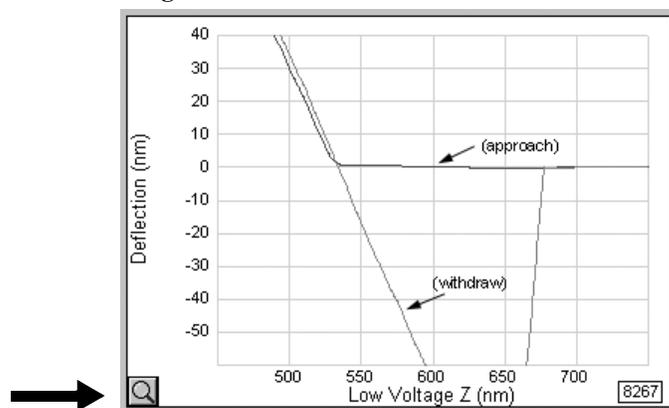
- In Force Mode, the Classic Controls **Other** panel is eliminated and its parameters distributed among the remaining tabs.

The leftmost tab in Force Mode is labeled Force Mode. Right click in the Force Mode tab to open the **Select Custom Control Parameters** panel (see Figure 8.4e) to select up to twelve parameters to display in the Force Mode Tab. For a detailed procedure for the same operation applied to the Main Tab in Scan View see the *NanoScope Software Version 6* or *7 User Guide*.

Figure 8.4e Selection (Left) and Display (Right) of Force Mode Tab Parameters
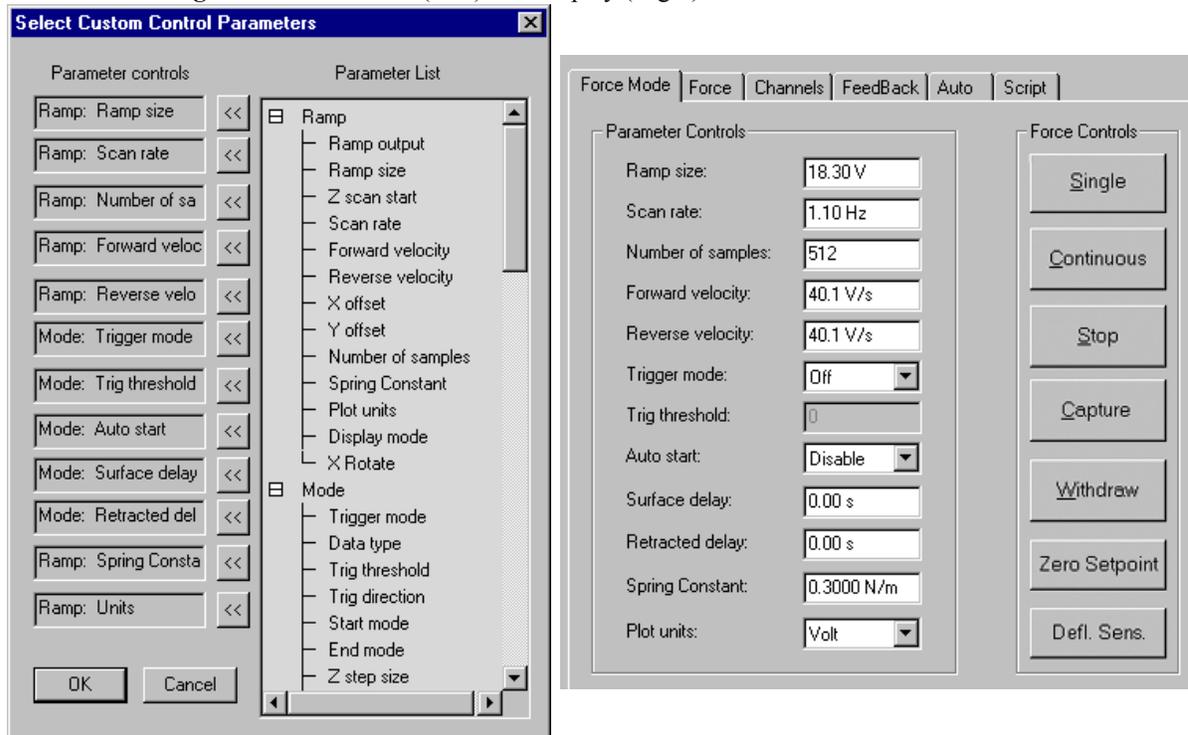
The leftmost tab in each mode contains parameters selected from other tabs, either in the same or another mode. The remaining tabs in each mode contain pre-selected parameters. The Force and Channels Tab parameters are shown in Figure 8.4f, the Feedback Tab parameters in Figure 8.4g and the Script Tab parameters in Figure 8.5b.

Figure 8.4f Force Mode: Force and Channels Tabs
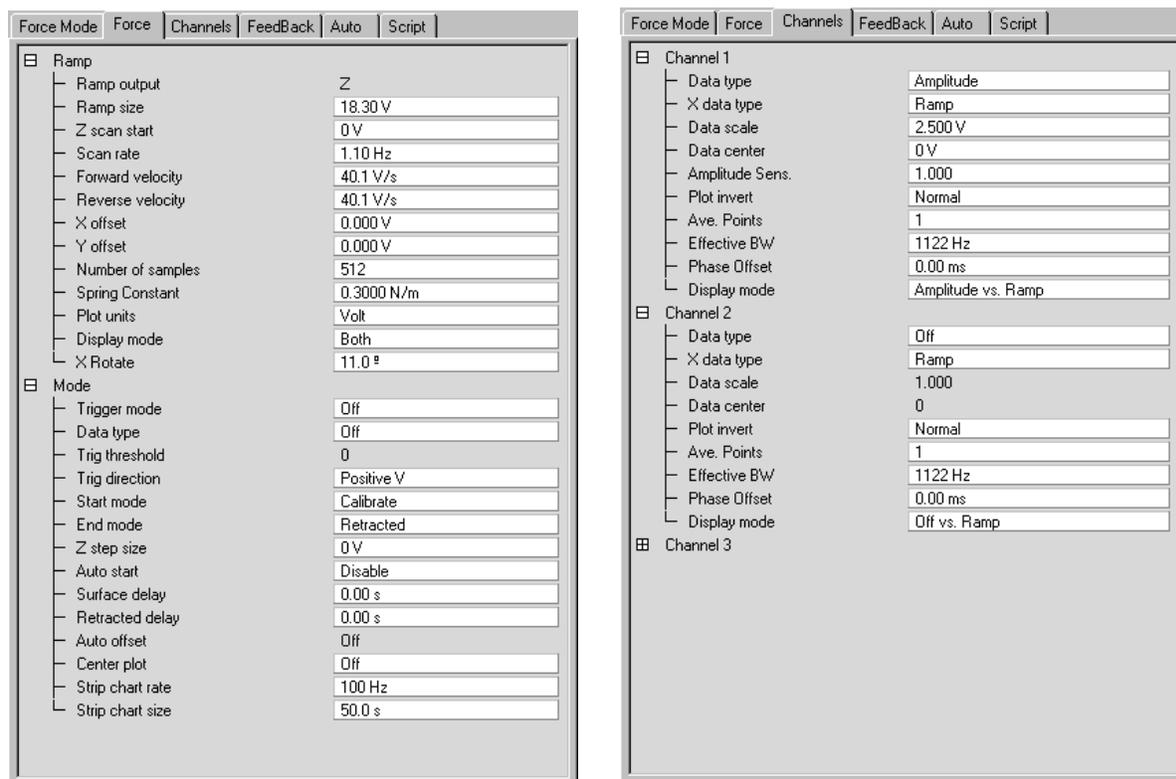
In the Channels Tab, **AVE. POINTS** designates the number of consecutive data channel samples to be averaged *in the display*. No averaging occurs if the value of **AVE. POINTS** is **1**. For a larger **AVERAGE COUNT** value, the data is low-pass filtered by averaging.

Channels Tab > **EFFECTIVE BW** is a display (you cannot input a value to it) representing the sampling frequency with display averaging taken into account:

$$\text{EFFECTIVE BW} = 2 \times (\text{NUMBER OF SAMPLES}) \times (\text{SCAN RATE}) / (\text{AVE. POINTS}).$$

Note: In averaging consecutive samples in an input datastream, real, unaveraged, data is appended to the output datastream if there are less than the designated number of samples per average.

Note: A complete discussion of the effect of measurement bandwidth can be found in Bruker Application Note 104, "Force Resolution in Force Spectroscopy Experiments: Thermal Noise and Bandwidth," available at http://bruker.com/library.

Especially at high pulling speeds, you may see an offset between the tip/sample contact region during extension and retraction, whether displaying a force plot with Channel 2 **DATA TYPE** set to **Z SENSOR** or to **LOW VOLTAGE Z**. This apparent hysteresis is actually an artifact caused by an electronics delay and is easily removed. Adjust Channel 1 **PHASE OFFSET** to shift vertical axis coordinates and Channel 2 **PHASE OFFSET** to shift horizontal axis coordinates until the two contact regions overlay.

Note: The numerical values of the two **PHASE OFFSET** parameters can be ignored; set them to whatever values minimize the apparent hysteresis. Particularly when **FORWARD VELOCITY** and **REVERSE VELOCITY** are unequal, the same numerical value for each **PHASE OFFSET** may not correspond to an identical displacement on each axis.

Figure 8.4g   Force Mode: Feedback and Auto Tabs
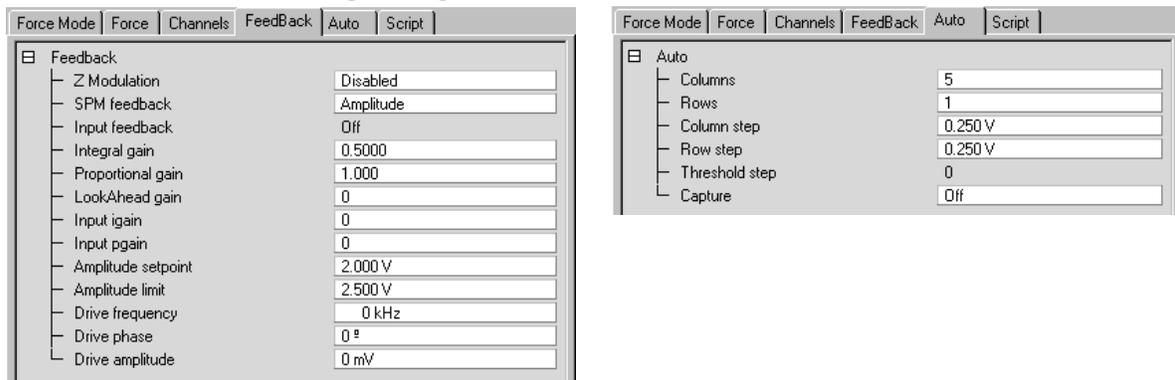
Note: If you have the Bruker vision board option installed and, while performing force plots rapidly, **Scan View** is open with **VIDEO** selected to display in the Vision Window. If force plot execution is slow, select **SCOPE** or **LINE PLOT**, rather than **VIDEO**, to disable the video display and restore speed.

8.4.1   Plotting Deflection versus Tip/Sample Separation

You may convert the Z-piezoelectric actuator signal that moves the sample stage vertically along with the cantilever deflection signal into a single representation of tip/sample separation and choose to display force curves as cantilever deflection versus this separation. In Basic Force Mode, select for the Channel 3 **DATA TYPE**: **DEFLECTION VS. SEPARATION** (see Figure 8.4h).

Note: The contact region of a Deflection vs. Separation force plot is typically vertical because the tip is constrained to press into the sample surface at a point. If the surface deforms under pressure from the tip, the contact region of the force plot deviates from vertical.

Figure 8.4h A Deflection vs. Separation Force Plot
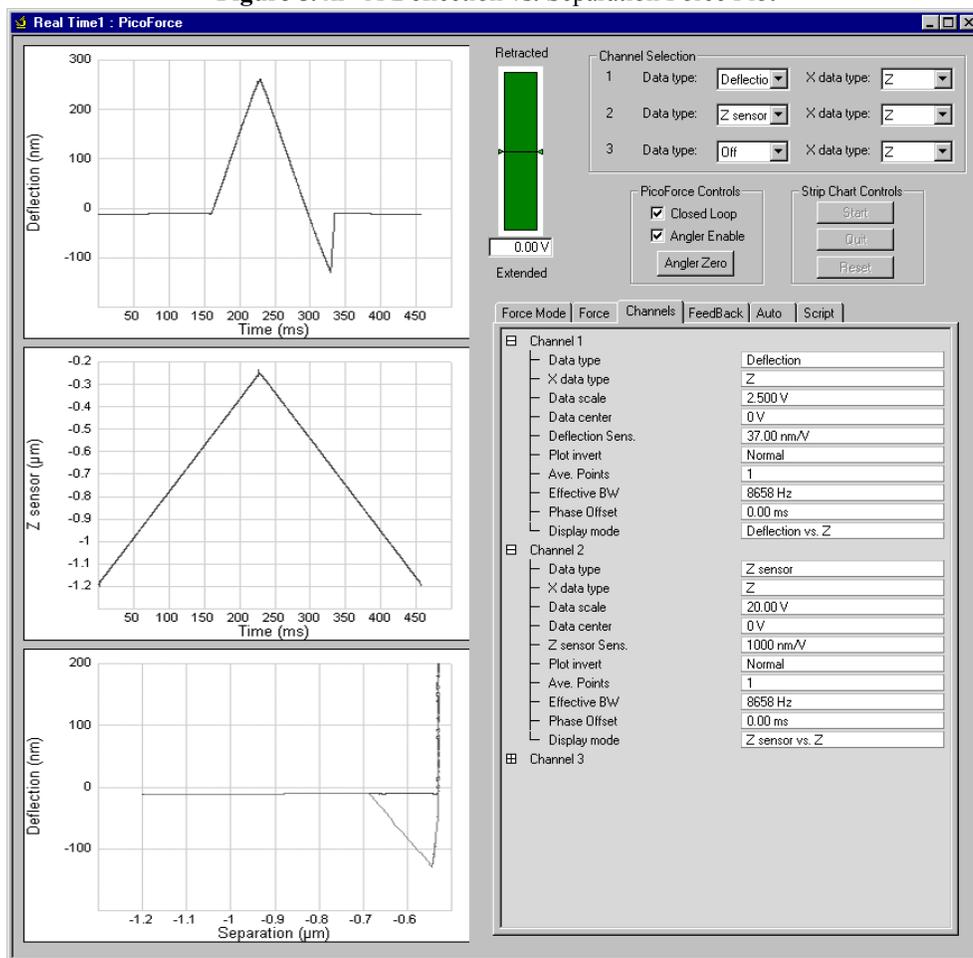
8.5   Strip Chart Display

Strip Chart Controls are provided in Force Mode (see Figure 8.5a) to acquire and display
DEFLECTION and **Z SENSOR** data over extended time intervals:

START/PAUSE
       Toggle switch to initiate, or pause, respectively, (Z-position, deflection) data point
acquisition.

QUIT
        Clears the data and returns to ramp interface.

RESET
       Discard acquired (Z-position, deflection) data points. Next acquisition starts a new chart.

Two strip chart parameters are included in the Force Tab (see Figure 8.4f):

STRIP CHART RATE
       Frequency of (Z-position, deflection) data point acquisition.

Range: 20kHz. Typical value: 1kHz.

STRIP CHART SIZE
       Time interval over which (Z-position, deflection) data points are displayed.

Typical value: tens of seconds.

Note: Although the strip chart collects and displays data over the time interval
defined by clicking **START**, then later, **STOP**, this data is not saved for
subsequent use until the **CAPTURE** icon (shown) is clicked. If **CAPTURE** is
clicked while strip chart data is being taken, what is saved begins at the start of
the chart (sooner than the icon is clicked).

Figure 8.5a   The Strip Chart Display
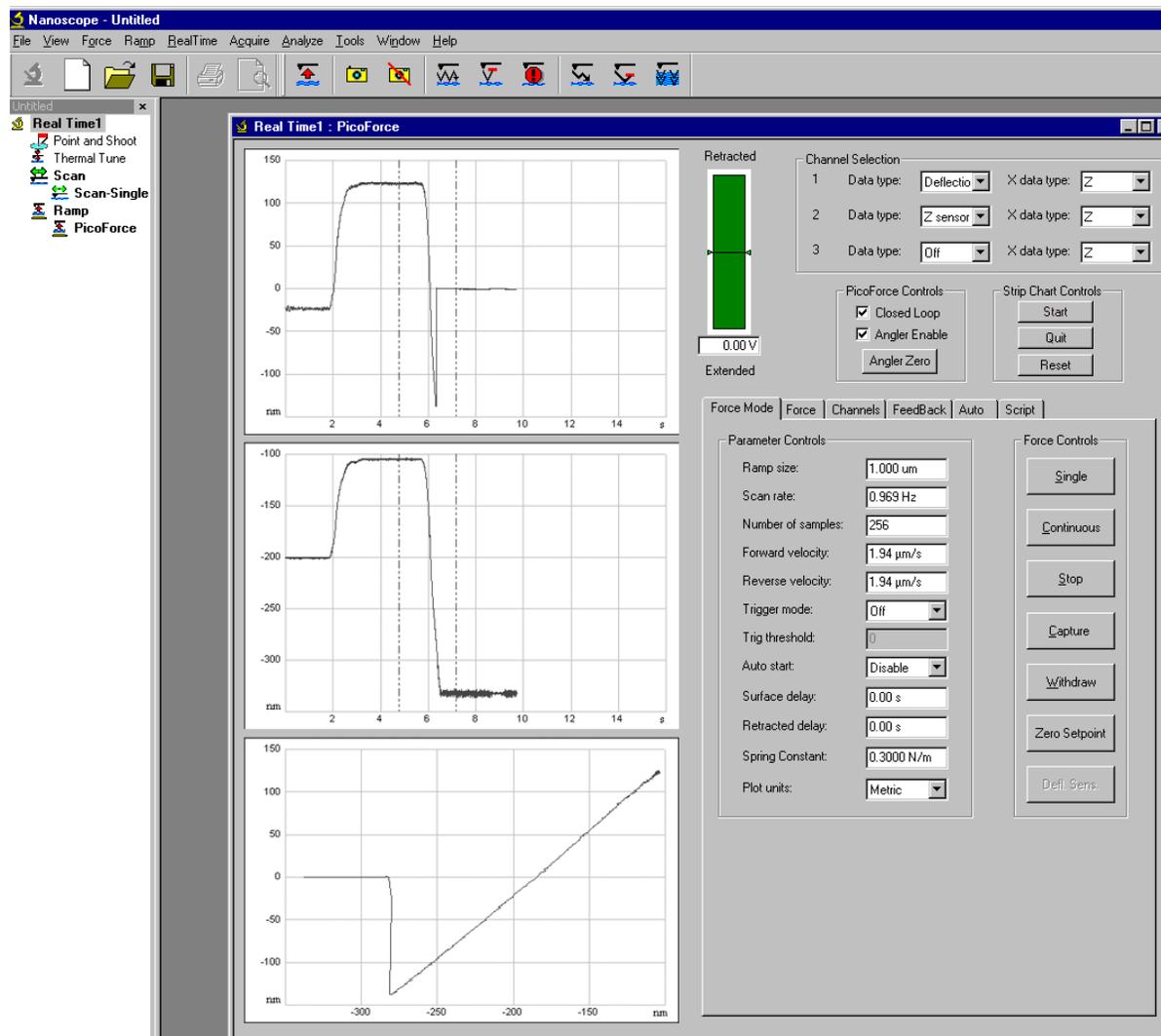
8.5.1   Scripting Mode

Scripting is provided to develop complex pulling sequences for unfolding large molecules. A feature is included, **FETCH MOLECULE**, that enable you to "catch" a single molecule between the tip and the surface. You can unfold and refold by ramping Z or you can hold the molecule under constant force or subject it to a defined ramp force. Scripting mode runs in real time and uses parameters set in other **Real Time** tabs. If you experience difficulty setting a parameter in **Script**, check parameter settings in **Real Time Scan-Single**.

> **Note:**   The Script Tab parameters (see Figure 8.5b) for Z-axis pulling are not to be confused with NanoScript, a C/C++ programming language-like aid to in-XY-

plane lithography operations using an SPM. Refer to the *Nanolithography* manual or contact Bruker for information about NanoScript.

Figure 8.5b Force Mode: Script Tab
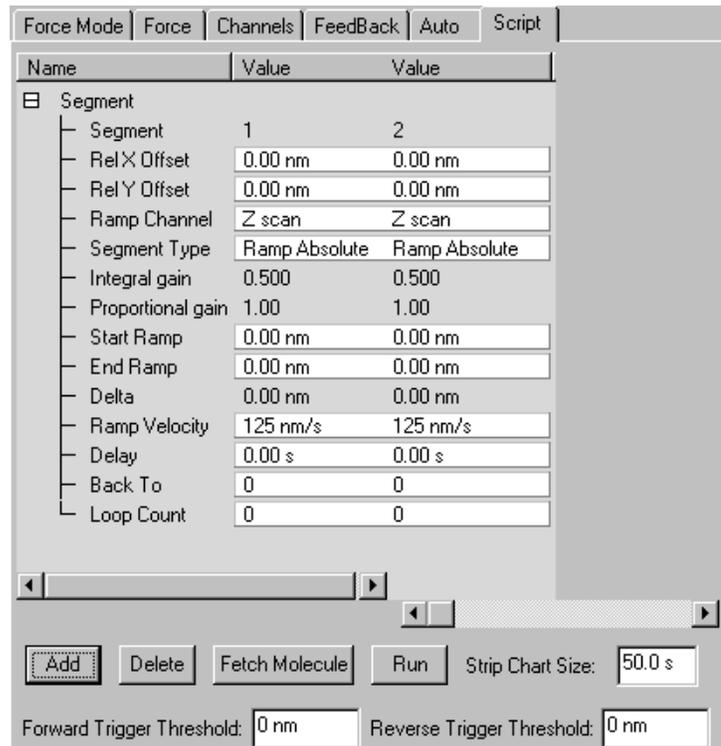
Script Tab parameter definitions:

SEGMENT

The numerical order of the script.

Range and Settings: `1 - 100`.

REL X OFFSET

X offset relative to the current position. **REL X OFFSET** is applied before the ramping portion of a segment.

REL Y OFFSET

Y offset relative to the current position. **REL Y OFFSET** is applied before the ramping portion of a segment.

RAMP CHANNEL

The parameter being ramped.

Range and Settings: `Z SCAN` or `SETPOINT`.

> **Note:** When **RAMP CHANNEL** = **SETPOINT**, the deflection signal that is displayed is actually **DEFLECTION** minus **SETPOINT**. Consequently while **RAMP SETPOINT** is running, the **DEFLECTION** is zero. Figure 8.4f shows **INPUT 3** selected rather than **DEFLECTION**, The **DEFLECTION** signal (before setpoint subtraction) was patched from the front panel of the PicoForce Controller to the Aux Input 3 BNC connector on the back of the NanoScope IV Controller.

SEGMENT TYPE

The type of segment.

Range and Settings: **RAMP ABSOLUTE** or **RAMP RELATIVE**.

INTEGRAL GAIN

User settable for **RAMP CHANNEL** = **SETPOINT**.

PROPORTIONAL GAIN

User settable for **RAMP CHANNEL** = **SETPOINT**.

START RAMP

The initial sample platform **Z SCAN** position or **SETPOINT** voltage at the beginning of a segment.

Range and Settings: **±10MM**.

END RAMP

The final sample platform **Z SCAN** position or **SETPOINT** voltage at the conclusion of a segment.

Range and Settings: **±10MM**.

DELTA

User settable for **SEGMENT TYPE** = **RAMP RELATIVE**.

RAMP VEL.

Speed at which the tip is moved along the Z-axis during a **RAMP CHANNEL** = **Z SCAN** script segment or the rate at which the Setpoint is ramped in a **RAMP CHANNEL** = **SETPOINT** segment.

Range and Settings: **1NM/s - 200MM/s**.

DELAY

Causes the sample platform position to remain unchanged for a time interval following the motion induced by the current script segment. If the preceding **SEGMENT** was a **SETPOINT** ramp, **FEEDBACK** remains on the ending deflection value during the **DELAY**.

Range and Settings: **0 - 32,000** seconds.

BACK TO

A looping mechanism for scripting which causes script execution to continue, not at the subsequent segment, but at an earlier segment whose **SEGMENT** value matches the current segment **BACK TO** value. After executing the earlier segment, *its* subsequent segment is executed. Eventually, the segment with the active **BACK TO** is reached again. Branching back is performed **LOOP COUNT** times successively; thereafter the segment **BACK TO** has no effect until the script is run again.

Range and Settings: `0 - N-1`, where the current **SEGMENT** value is `N`.

> **Note:**   Loops can not be nested.

LOOP COUNT

The **BACK TO** loop counter: designates the number of times to branch back to an earlier segment upon reaching the segment with a nonzero **LOOP COUNT** value.

> **Note:**   If **COUNT** = `0` in a segment, then **GO TO** in that segment has no effect, regardless of its value.

Range and Settings: `0 - 32,000`.

Script button definitions (see Figure 8.5b, bottom:

ADD

Causes the current settings of the Script Tab to define a new segment which is added to the end of the script.

DELETE

Prompts for a range of **SEGMENT**s to be removed. Subsequent **SEGMENT** values are decremented to maintain segment ordering.

RUN

Execute the current script of sequential segment tip motions.

FETCH MOLECULE

FETCH MOLECULE enables the user to go to the surface, grab a molecule and put Z into feedback at that force. **FETCH MOLECULE** moves to the **FORWARD TRIGGER THRESHOLD**, retracts to the **REVERSE TRIGGER THRESHOLD** and then goes into feedback to maintain the setpoint. An example of **FETCH MOLECULE** is shown below.

1. In the **Script** tab, input a **FORWARD** and **REVERSE TRIGGER THRESHOLD**. You may want to take a force curve to facilitate setting these parameters. See Figure 8.5c.

2. Click **FETCH MOLECULE**. This will stop, if running, the Strip Chart, and run the tip toward the sample surface unit the **FORWARD TRIGGER THRESHOLD** is reached. The tip will then retract until it reaches the **REVERSE TRIGGER THRESHOLD**. If it doesn't find the **REVERSE TRIGGER THRESHOLD** (an adhesion), **FETCH MOLECULE** will keep trying until it does.

3. Once **FETCH MOLECULE** succeeds in catching a molecule, **FETCH** turns on feedback at the **REVERSE TRIGGER THRESHOLD** and starts a Strip Chart. It t captures the successful "fetch event" as a regular force curve file, shown in Figure 8.5d.

4. Clicking **RUN** will then run a **Script**, shown in Figure 8.5c. The results of this Script are shown in Figure 8.5e. Segment 1 holds the molecule for 15 seconds at constant force. Segment 2 retracts by 1um at 499nm/sec. Segment 3 returns to the start position.

> **Note:**   If you wish to hold a molecule under constant tension, choose **RAMP RELATIVE** with **DELTA** = `0` and a **DELAY** for as long as you wish to hold the molecule.

Figure 8.5c   Fetch Start
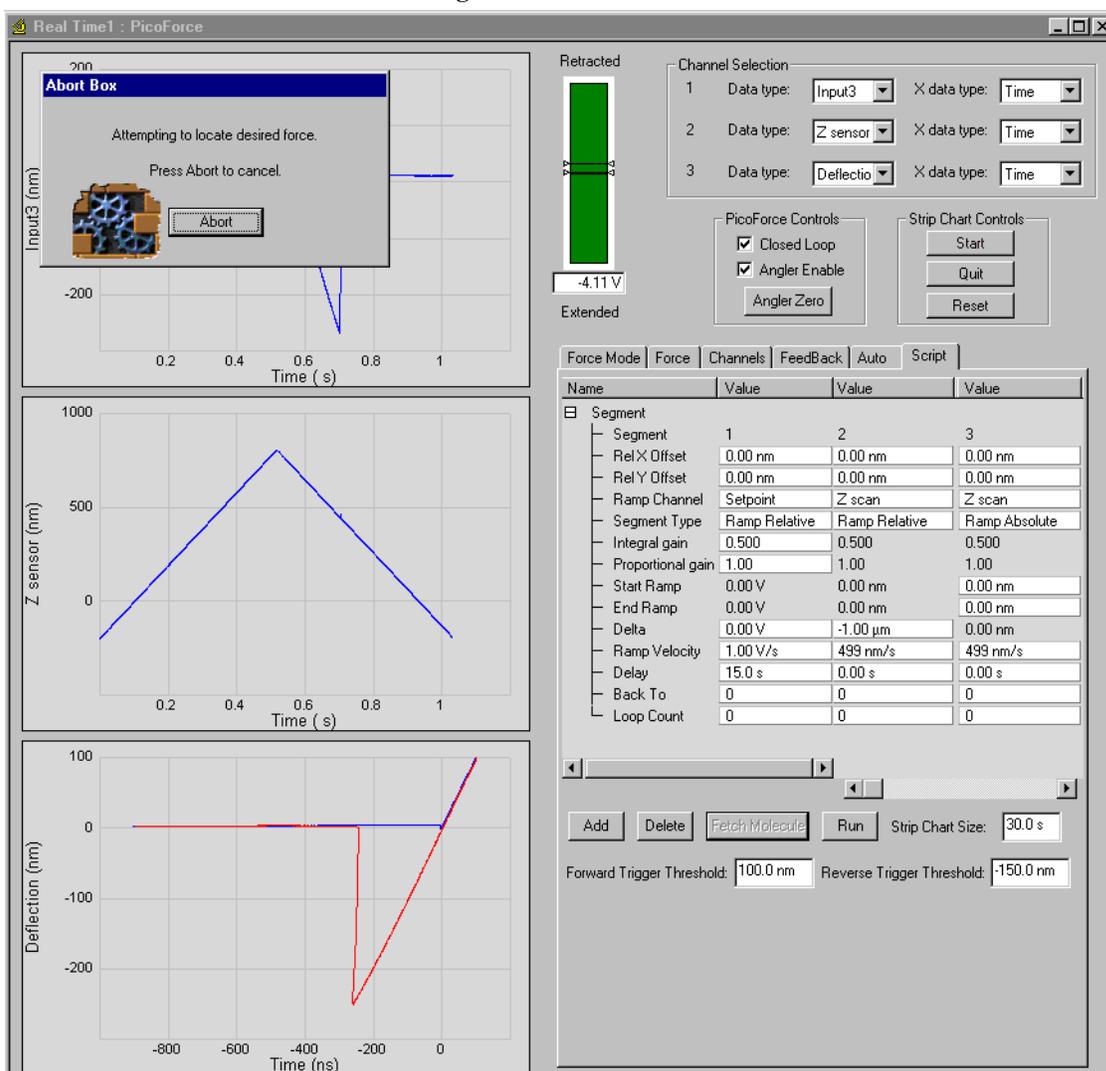
Figure 8.5d   Fetch Molecule Strip Chart
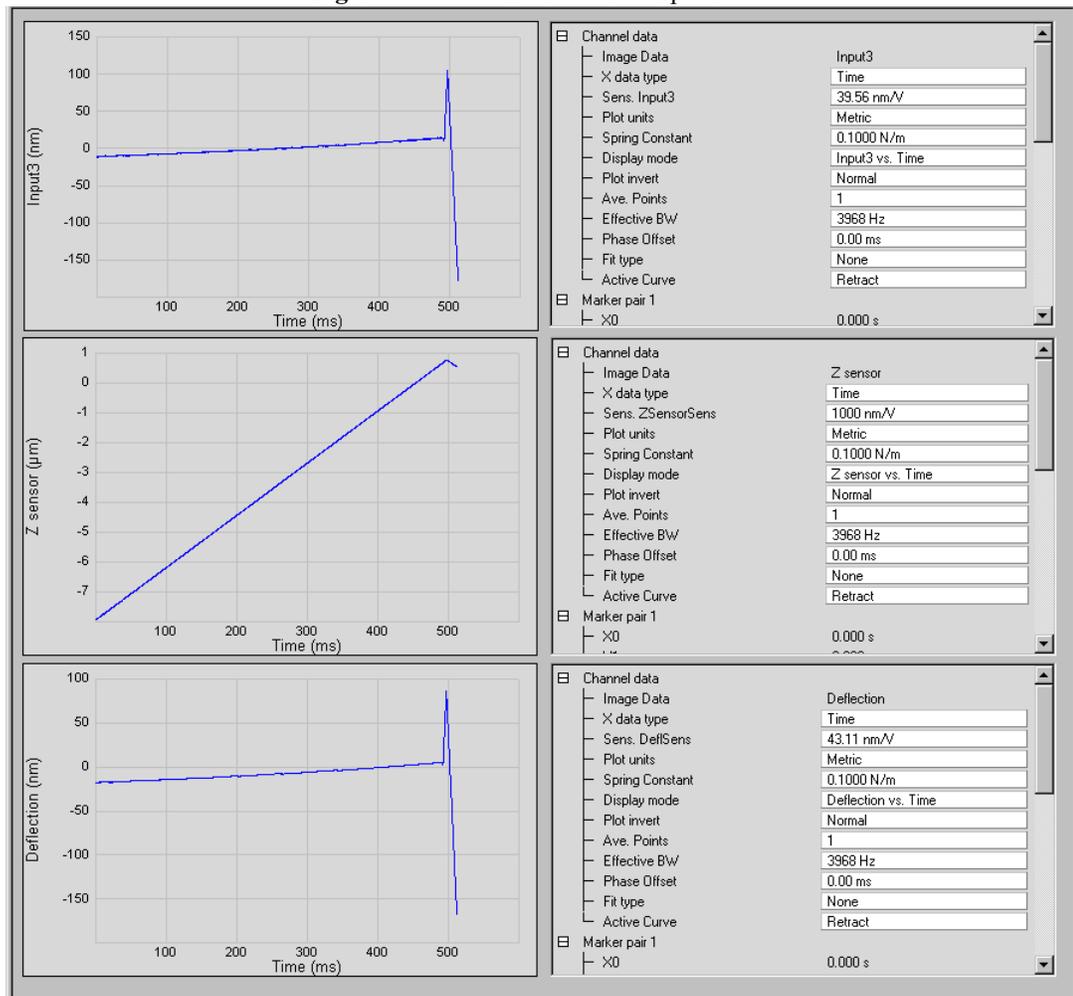
Figure 8.5e   Fetch Script
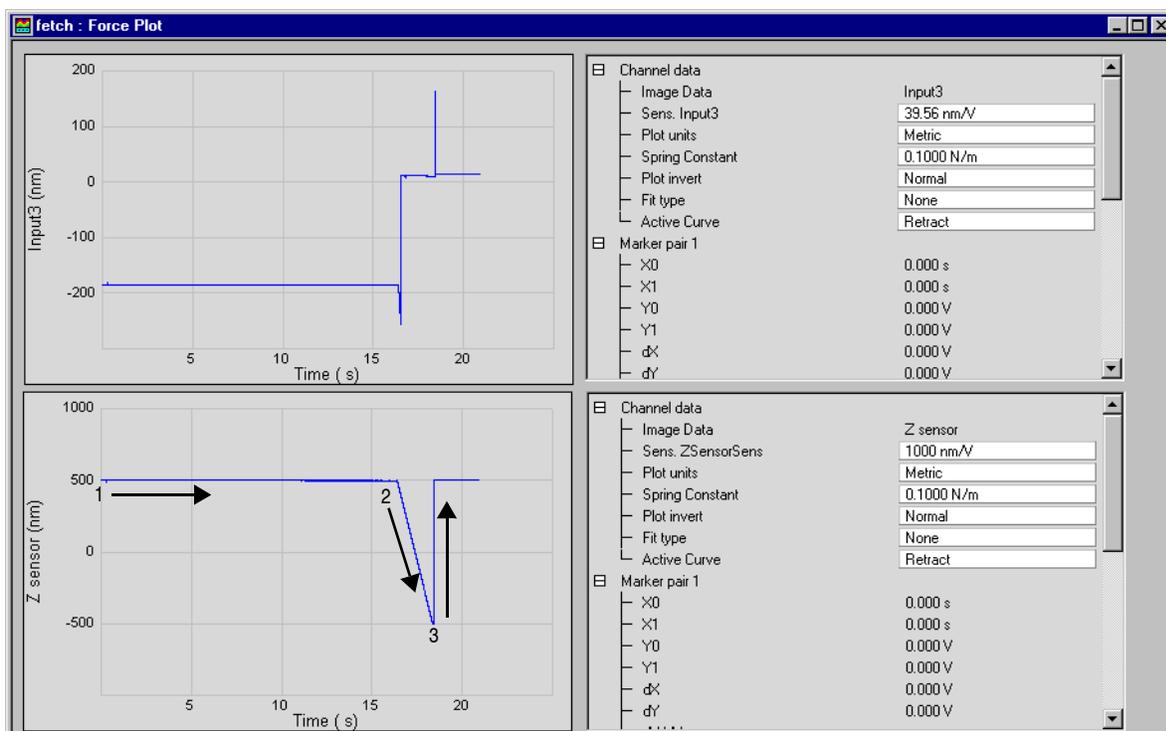
A script example is shown in Figure 8.5f. Segment 1 ramps **Z** by **1.5ᴍᴍ** at a velocity of **200ɴᴍ/s**. Segment 2 ramps the setpoint up by **0.5V** at a velocity of **100ᴍV/s**. Segment 3 ramps the setpoint down by **-4.0V** at a velocity of **10V/s** and holds it for **10.0ꜱᴇᴄ**. Segment 4 ramps **Z** by **1.0ᴍᴍ** at a velocity of **2.0ᴍᴍ/s**. Segment 5 returns to the starting position.

Figure 8.5f Script Example
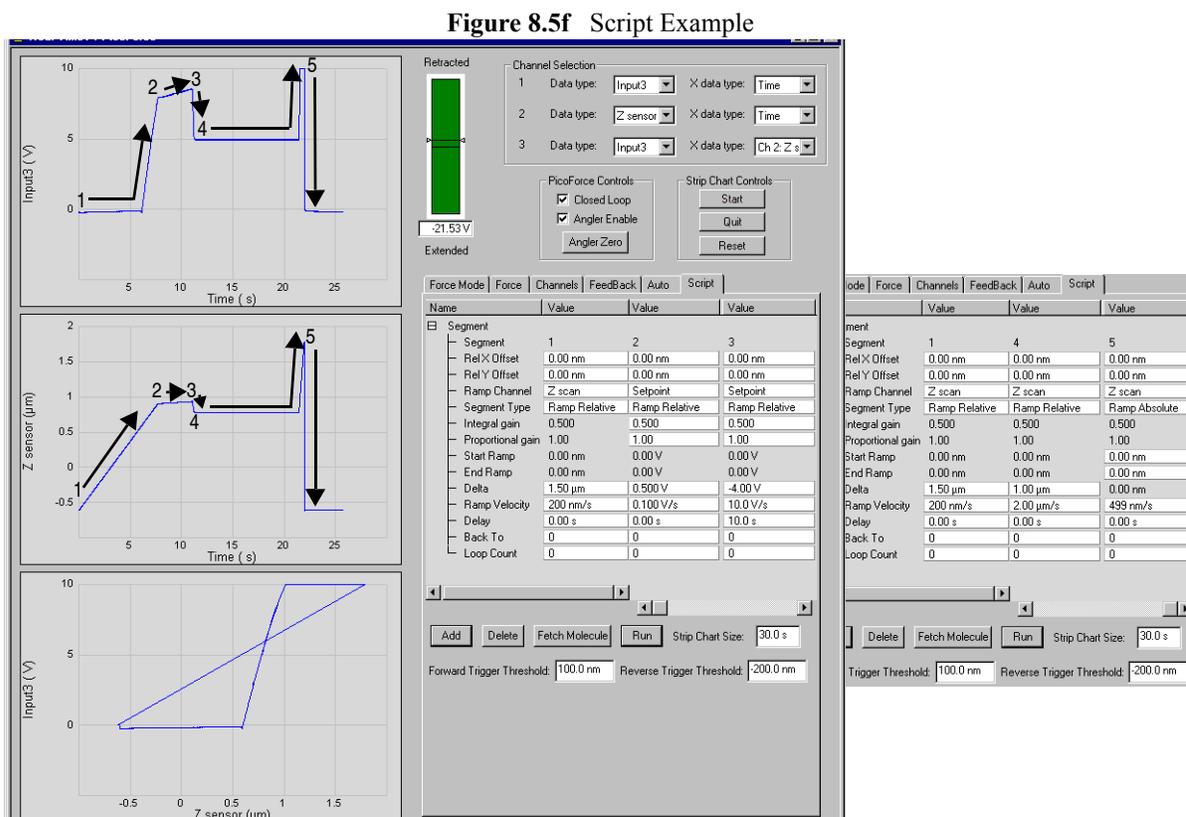
> **Note:** If ramping the **SETPOINT** more negative (i.e. increasing the tension on a molecule), the molecule may break or release from the probe tip. The piezo scanner will fully retract in an attempt to reach the desired adhesion. This is normal. Including an a **RAMP ABSOLUTE** of the **Z SCAN** channel to 0 as the last segment will return the piezo to the starting point.

8.5.2 PicoAngler Mode

The PicoAngler, shown in Figure 8.5g, provides a novel interface, modelled on the experience of fishing, for "catching" a molecule, pulling on it and feeling its resistance to unfolding from its resting position on the sample platform. The handheld PicoAngler displays numerically the Z-position offset added by the PicoAngler and, in digital light bars, cantilever deflection, force sensitivity and step sensitivity. The controls that the PicoAngler provides are: **Z-POSITION** (with simultaneous force feedback), **FORCE SENSITIVITY** and **STEP SENSITIVITY**. Each of these is described below.

- Z-position digital readout—displays in nanometers over a range of ±20µm, from fully extended (positive) to fully retracted (negative), the current Z-offset which the PicoAngler is adding to the Z-signal coming from the NanoScope Controller.

  Note: The PicoAngler adds a voltage to the low voltage Z signal coming from the NanoScope Controller before it is amplified in the closed loop Z-axis circuitry of the PicoForce Control Module. The low voltage Z signal from the NanoScope Controller can vary over ±10V, which corresponds to the full range of the PicoForce Scanner Z-range. The PicoAngler can add an offset to this voltage sufficient to move the Z-position anywhere in its range, regardless of the current value coming from the Nanoscope. For example, if the NanoScope voltage is at -10V (fully retracted), the PicoAngler can add 20,000nm (~20V) to that value in order to fully extend the stage. So while the PicoAngler is capable of adding ±20,000nm, you will only use the maximum PicoForce offset if the NanoScope Controller is fully extended or retracted. During normal operation when the NanoScope is directing the Z-stage to be near the middle of its range (~0 V), the useful range of the PicoAngler will be about ±10,000nm, corresponding to the ~20um range of the PicoForce Z-stage. Continuing to turn the knob when the stage has already reached its limit will not damage the unit in any way, but the position offset indicated by the LCD display on the PicoAngler will be inaccurate.

- Relative deflection display—is indicated by the horizontal light bar. A tip deflected downwards by attraction to the sample moves the light to the left of center (blue). A tip deflected away from the sample by contact with it moves the light to the right of center (red).

- **FORCE SENSITIVITY** knob—adjusts the strength of force feedback felt while turning the Z-position knob. The selection, 1×, 3×, 10×, or 30×, is indicated on the associated vertical 4-position light bar.

- **STEP SENSITIVITY** knob—adjusts the range of Z-motion in one full revolution of the Z-position knob. The selection, 1×, 3×, 10×, or 30×, is indicated on the associated vertical 4-position light bar.

- Z-position knob—adjusts the sample platform proximity to the probe in realtime while resisting adjustment in proportion to the induced deflection of the cantilever.

  Note: Manual control is ideal for exploratory force plots. Use the PicoAngler to bring the sample platform toward the tip arbitrarily slowly, then feel the cantilever deflect decidedly when the tip and sample begin to interact. Once the tip is pressed into the sample and deflecting monotonically as the platform is raised still further toward the tip, the decision to begin to retract is informed by tactile feedback, the Z-position Readout on the PicoAngler and displayed realtime force plot. For multiple force plots, triggered basic or scripted force plots may produce more repeatable results than the PicoAngler simply because the sample platform movement is pre-defined, so identical with each repetition.

Figure 8.5g The PicoForce PicoAngler
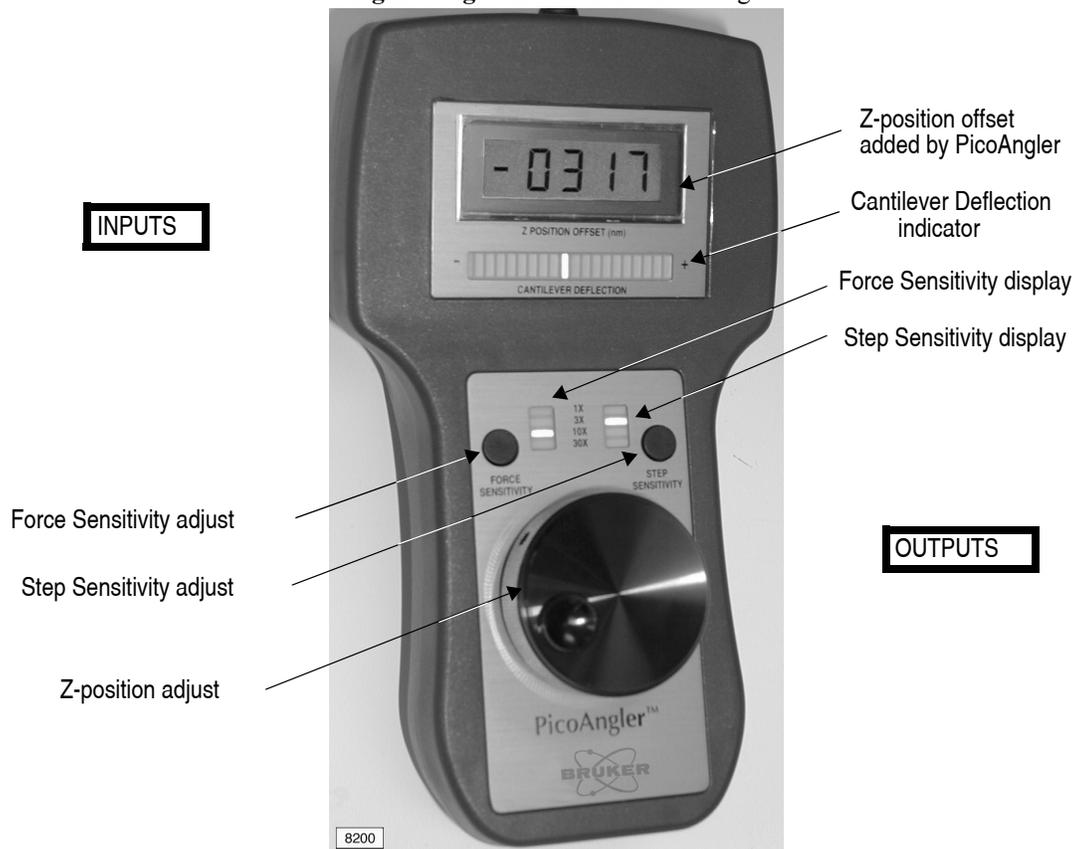
Directing a Force Plot Under Full Manual Control with the PicoAngler

Using the procedure below, you can make and record manual force curves by using the PicoAngler to control the tip-sample separation. When using the PicoAngler in this way, the Strip Chart Mode data collection is used, as described in the previous section.

1. Click PicoForce Controls > **ANGLER ENABLE** to activate PicoAngler (see Figure 8.4a).

   Note: Verify switch on PicoForce Control Module is set to **ANGLER INPUT**.

<table>
<tr>
<td>⚠️</td>
<td>**CAUTION:**</td>
<td>The PicoAngler adds a voltage to the Z-piezo drive signal otherwise set through the NanoScope software. The PicoAngler contribution, as displayed in nanometers by the Z-position digital readout (see Figure 8.5g) **should be near zero when first engaging the microscope**. This is most easily accomplished in **Force Mode View** by clicking PicoForce Controls > **ANGLER RESET** to zero the Z-position offset (see Figure 8.4a) or by manually turning the knob.</td>
</tr>
</table>

2. Click the **ZERO SETPOINT** button to set the noncontact force.

3. Click Strip Chart Controls > **START** (see Figure 8.4a).

    Note: Click Strip Chart Controls > **RESET** if the top or middle graph windows display anything from prior strip chart recording.

    Note: You may set the **SAMPLE RATE** and **DISPLAY SIZE** in the force menu.

4. Turn the Z-position adjust knob (see Figure 8.5g) clockwise to move the sample platform toward the tip.

    Note: Positive deflection is signalled by red lights to the right on the Cantilever Deflection indicator. Negative deflection is signalled by blue lights to the left on the same indicator.

5. Feel the force feedback through the Z-position adjust knob as the tip and sample interact. The top **Force Mode View** graph displays cantilever deflection as a function of time; the middle graph displays sample platform Z-position as a function of time.

    Note: The PicoAngler Z-position readout also displays sample platform Z-position in nanometers according to the same conventions:

    a. A negative Z-axis motion separates tip and sample. A positive Z-axis motion brings tip and sample closer together.

    b. The middle Force Mode graph plots absolute Z-position, where Z=0 roughly in the middle of the range of Z-axis travel. The PicoAngler Z-position readout displays relative Z-position, where Z=0 at the sample platform position when Picoforce Controls > **ANGLER RESET** was last clicked.

6. Continue to press tip and sample together to as high a cantilever deflection as desired (keeping in mind sample fragility, laser alignment and cantilever fragility).

7. Turn the Z-position adjust knob counterclockwise to retract the sample from the tip. Continue until you no longer feel or observe tip/sample interaction.

8. Click **STRIP CHART CONTROLS** > **PAUSE** to terminate (Z-position, deflection) data collection and freeze the three graphs.

9. The bottom graph displays a force plot by pairing data points determined at the same time on the top and middle graphs. The entire time interval is used in the force plot.

10. To zoom in on the force plot, position the cursor over either the left or right edge of either the top or middle graph, then drag a line marker into the graph. Repeat the action to provide a second marker line. Position the marker lines to bracket the time interval of interest in both the top and middle graphs simultaneously. The (bottom) force plot is redrawn, restricted to the subinterval of time defined by the markers.

Note: To zoom into the top or middle graphs to better view detail, press **CONTROL** on your keyboard and use the mouse to drag a box around the area you want to zoom. To zoom out: double click inside the graph.

Using the PicoAngler in Conjunction with Basic Force Plots

Besides being used as a tool for fully manual force curves, the PicoAngler is also very useful during basic force curves. In basic force curves, if no trigger is set and the continuous ramp button is clicked, the piezo begins to ramp up and down around the defined **Z START** position. This may not result in a force plot if the automated ramping is not bringing the tip close enough to the sample. Before PicoForce, the only way to guarantee force plot generation was to manually adjust the **Z START** position parameter in software or else define a trigger and turn on the "auto-offset" feature to find the surface. With the PicoAngler, however, you can conveniently and quickly find the surface by enabling the PicoAngler, beginning continuous force ramps in Basic Force Mode, and then using the PicoAngler to reduce the ramping tip-sample separation. This is functionally equivalent to moving down the **Z START** position, but it is more convenient since you only have to turn the knob instead of manually adjusting a numerical parameter. The force-feedback feature of the PicoAngler knob also makes it clear when you have found the surface. There, use the PicoAngler to fine-tune the offset of the force plot (how much of it occurs on the surface and how much is the retraction). You may find this a convenient alternative to using triggers in some applications.

⚠️	**CAUTION:**	While the PicoAngler is helpful in tip/sample engagement in Basic Force Mode, once on the surface, if not using the PicoAngler for a while, you may want to disengage it to avoid the possibility of accidentally bumping the Z-position adjustment knob when tip/sample separation is being controlled independently.

8.5.3   Switching Between Imaging and Force Modes

The range of cantilever deflection and the manipulation of the probe typically differ between Image Mode and Force Mode. For instance, see **Deflection Signal Clipped at Top of Plot,** for a comparison of the range of cantilever deflection in the two modes.

In switching between Force Mode and Image Mode, the role of the deflection setpoint changes. In Force Mode, one typically clicks **ZERO SETPOINT** with the tip away from the sample to associate zero applied force with the unengaged cantilever deflection. Before switching to Contact Mode Imaging, increase the Feedback Tab > **DEFLECTION SETPOINT** from its near zero value. A setpoint near zero in Contact Mode imaging can prevent engagement because the undeflected state achieves the setpoint value. A positive setpoint value requires the cantilever to be deflected by the sample to reach the goal of the applied force control system.

8.5.4   External Input Mode

Control of the sample platform Z-position may also be directed from a signal external to the MultiMode PicoForce system. The external input voltage is added to the Z-position voltage coming from the NanoScope Controller as is done with the PicoAngler offset voltage. Like the PicoAngler, the voltage applied with the external input can range within ±10V, but this voltage has twice the sensitivity of the voltage coming from the NanoScope Controller. Hence, ±5 V covers the full range of the Z-piezo provided the input voltage from the NanoScope Controller is 0V. The ±10V range is provided so that the full range of the piezo can be used regardless of the NanoScope voltage. For example, if the NanoScope is directing the Z-piezo to fully retract (-10V), then an applied external voltage between –10V and 0V has no effect because the piezo is already at –10V. External voltages between 0 and 10V cover the full range, 0V being fully retracted (-10V + 2*0V= -10V), and 10 V being fully extended (-10V + 2*10V= 10V). This final voltage controlling the Z-piezo can be monitored on the **MODIFIED LV OUTPUT** on the front panel of the PicoForce Control Module.

> **CAUTION:** When using the external input there are no safeguards against overextending the piezo and damaging the tip and or sample. The LCD display on the PicoAngler shows the offset being externally applied and the 20-bar LED display shows the realtime deflection. Carefully monitor tip deflection to avoid tip/sample damage.

> **Note:** It is possible to use the external input in conjunction with basic force curves as described for the PicoAngler in **Using the PicoAngler in Conjunction with Basic Force Plots,** page 72. However, take care to avoid tip/sample damage.

To direct sample Z-position externally:

1. Toggle the switch on the PicoForce Control Module front panel from **ANGLER INPUT** (the up position) to **USER INPUT** (the down position, see Figure 5.1a).

2. Attach to the adjacent connector labelled **MANUAL OFFSET INPUT** the BNC cable that will carry the external signal.

> **CAUTION:** The external signal should be at ground potential (0V) initially to prevent premature sample platform motion. When the signal is switched on, it should be constrained to stay within ±10V, which spans the full range of available Z-axis movement, from fully retracted to fully extended.

3. Click Strip Chart Controls > **START** (see Figure 8.4a).

4. Turn on the external Z-axis piezo drive input signal. Monitor cantilever deflection and sample platform Z-position on the top and middle Force Mode graphs, respectively.

5. When the desired tip/sample interaction data has been collected, turn off the external Z-axis piezo drive input signal.

6. Click Strip Chart Controls > **STOP**.

Note: The same steps 8 and 9 of the PicoAngler procedure (see page 71) to optimize the force plot apply to externally directed Z-positioning as well.

8.5.5   Auto Mode

The **Auto** Mode, shown in Figure 8.5h, allows the user to predefine grid points which are then used to acquire force curves. The user defines a grid of **N ROWS** by **M COLUMNS** with corresponding **ROW STEP**s and **COLUMN STEP**s.

Note: The maximum number of **ROWS** or **COLUMNS** is a function of tip position as well as the **ROW STEP** or **COLUMN STEP**:

$$\text{Row}_{max} = \left(\frac{DistanceToNearestEdge}{RowStep} - 1 \right) \times 2$$

The force curves are taken at (x,y) locations centered about the current tip position. Start the **Auto** force curves by clicking the **AUTO RAMP** icon, shown at left, in the tool bar. The tip is moved in x, along a row, incrementing the **TRIGGER THRESHOLD** by the **THRESHOLD STEP** for each new x. The **TRIGGER THRESHOLD** is reset at the beginning of every row. An abort button showing the current tip position is displayed while the **Auto** force curves are running. Each force curve is saved individually, using the capture file name (incremented for each curve), as a single force file. The force curves can be opened using the Multiple Curve Analysis (MCA) feature in PicoForce described in **Analyzing Multiple Force Plots, page 89**.

Figure 8.5h   Auto Panel
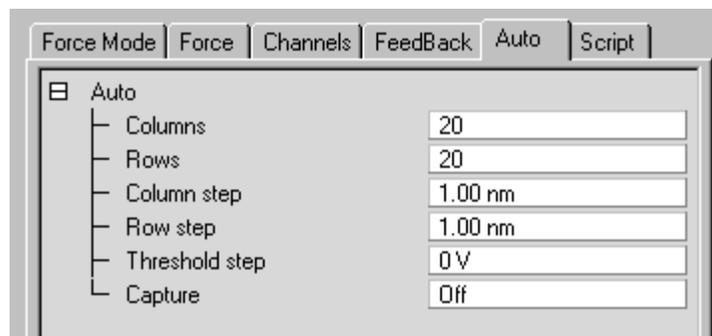
8.5.6 Point and Shoot View

The **Point and Shoot** View allows you to select specific points on an image (see Figure 8.5i). Use **Point and Shoot** to capture an image and/or collect a force curve for every point you designate.

When you click a point on an image, a crosshair (+) marks the location. You can designate individual points, or use the tools in the **Point and Shoot** dialog box to assign multiple points simultaneously.

Figure 8.5i Point and Shoot Dialog Box
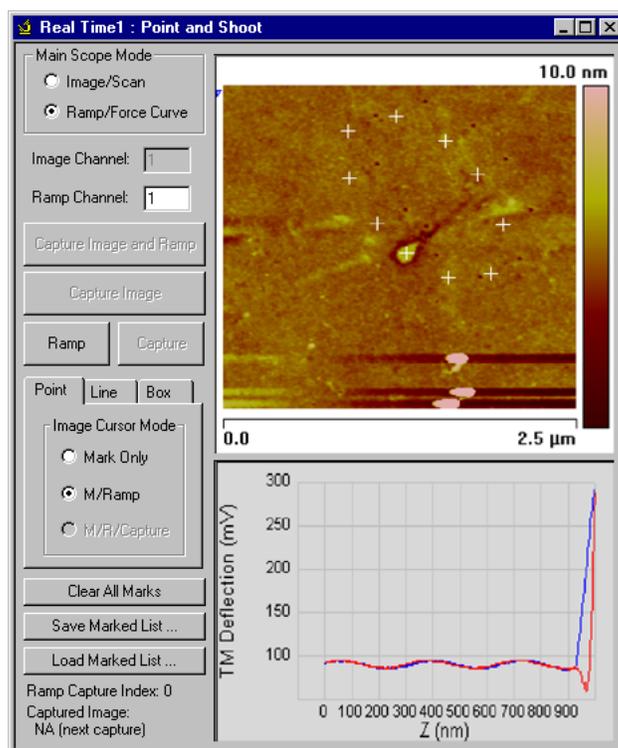
Image/Scan Parameters

Image/Scan	Allows you to select points and capture images of each point.
Ramp/Force Curve	Allows you to select points on an image, ramp each point, and create a force curve for each point.
Image Channel	Select the channel to use for the image.
Ramp Channel	Select the channel to use for the ramp.
Capture Image and Ramp	Click this button to ramp the each point, capture an image and save it in the Capture Directory.

Capture Image	Click this button to capture an image and save it in the Capture Directory.
Ramp	Click this button to ramp each point.
Capture	Click this button to ramp each point and capture the image and save it in the Capture Directory.
Point Parameters	Image Cursor Mode Settings:
	Mark Only—Select points of interest on the image.
	M/Ramp—Select points of interest. Software will automatically ramp each point.
	M/R/Capture—Select points of interest. Software will automatically ramp each point and capture an image.
Line Parameters	Draw a line to select specific points on an image.
	Point Number—Number of points in the line.
	Spacing—The distance in nm between each point. All points are equidistant.
	Clear Path—This button clears the current line and associated points.
	Convert to Points—Places a + in the location of each point in the line. The line disappears.
Box Parameters	Draw a box in the area you want to place a group of points. You can use the parameters below to create a grid of points.
	Row Number—Designates the number of rows of points in the grid.
	Column Number—Designates the number of columns of points in the grid.
	Row Space (nm)—Designates the distance in nm between each row of the box.
	Col Space (nm)—Designates the distance in nm between each column of the box.
	Clear Path—This button clears the current box and associated points.
	Convert to Points—Places a + in the location of each point in the grid. The box disappears.
Clear All Marks	Removes all user-defined marks from the Point and Shoot image.
Save Marked List...	Save the marks on the image as Path Files (*.psm).
Load Marked List...	Open a previously saved Path File (*.psm) which contains marks on a Point and Shoot image.

8.6 Realtime Analysis

By enabling CAPTURE while operating in **RealTime**, the three plots of a Force Mode session are named and saved. Most analysis of data taken in Force Mode is performed after acquisition rather than in realtime. A handy exception is determination of the conversion factor from cantilever deflection in volts to nanometers as described next.

8.6.1 Determine Deflection Sensitivity in Force Mode

Deflection sensitivity should be recalculated whenever the laser beam path changes due to laser alignment, probe change or re-seating or photodetector adjustment.

With a force plot displayed in Force Mode, drag two markers in from the left and/or right edges and position them as far apart as possible while both still within the contact region of the force plot. Click the **DEFL. SENS.** button in the Force Mode Tab (see Figure 8.4e). You are asked whether you accept the calculated value (see Figure 8.6a).

Figure 8.6a   Deflection Sensitivity Determination in Force Mode
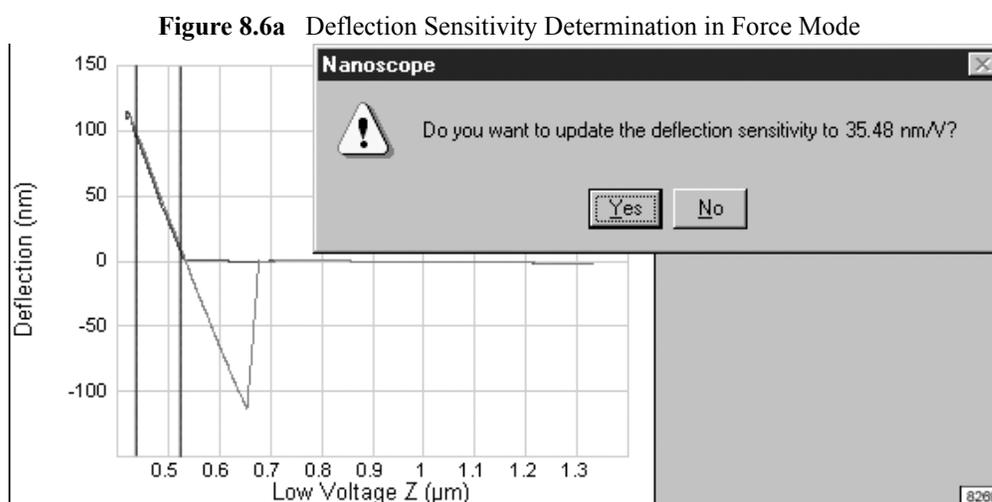
8.7 Determine Cantilever Spring Constant by Thermal Tune

The Thermal Tune method (see **Measuring Thermal Noise,** page 42) provides an automated and quick determination of cantilever spring constant.

		CAUTION:	Make sure the probe is withdrawn adequately from the sample before activating Thermal Tune. The probe should not interact with the sample during its self-excitation under ambient conditions. Four or five clicks of the **WITHDRAW** icon (shown) is sufficient to elevate the tip.

Determine the cantilever spring constant by the thermal tune method according to the following procedure:

1. Determine cantilever deflection sensitivity if you have not done so already (see **Determine Deflection Sensitivity in Force Mode,** page 77).

   > **Note:** A correction factor should be applied to this calibration. In air, the cantilever is constrained at only one end, but the cantilever is constrained at both ends when on a surface. The cantilever bends differently in the two situations and thus reflects the laser beam of the optical lever differently. When you calibrate the deflection sensitivity using a static force, you underestimate the amplitude of dynamic motion by approximately 8%. This correction factor is applied in the thermal tune panel (Figure 8.7c) (**DEFL. SENS. CORRECTION**).

2. **WITHDRAW** (click **WITHDRAW** 4 times).

3. Click **THERMAL TUNE** in the RealTime View (see Figure 8.7a).

Figure 8.7a Thermal Tune
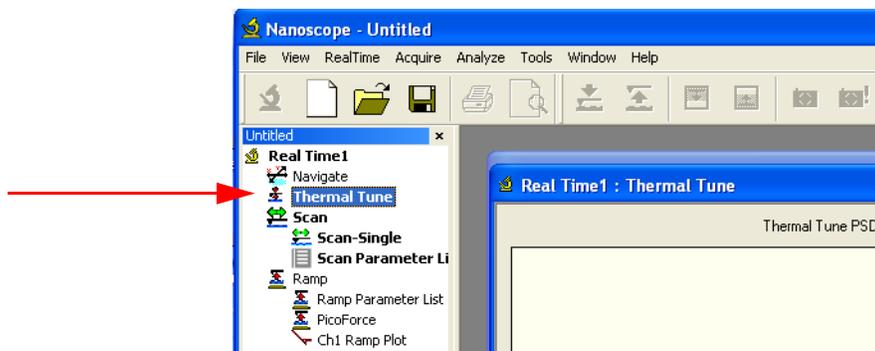
4. Select a frequency range over which you will tune (see Figure 8.7b) (Applicable only to the NS V controller. NS IIIa and NS IV(a) controllers are limited to a **THERMAL TUNE RANGE** of 1-30kHz.)

Figure 8.7b Select Thermal Tune Frequency Range
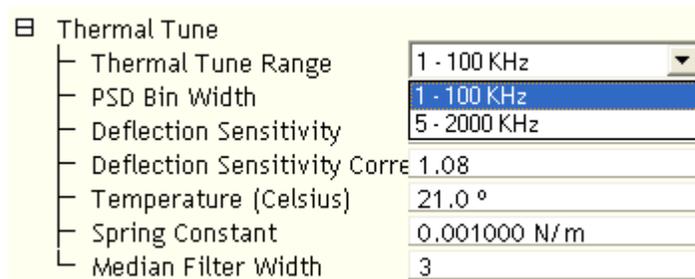
5. Click **GET DATA** in the **Thermal Tune** panel (see Figure 8.7c).

6. The microscope will acquire data for about 30 seconds.

Figure 8.7c   The Thermal Tune Panel
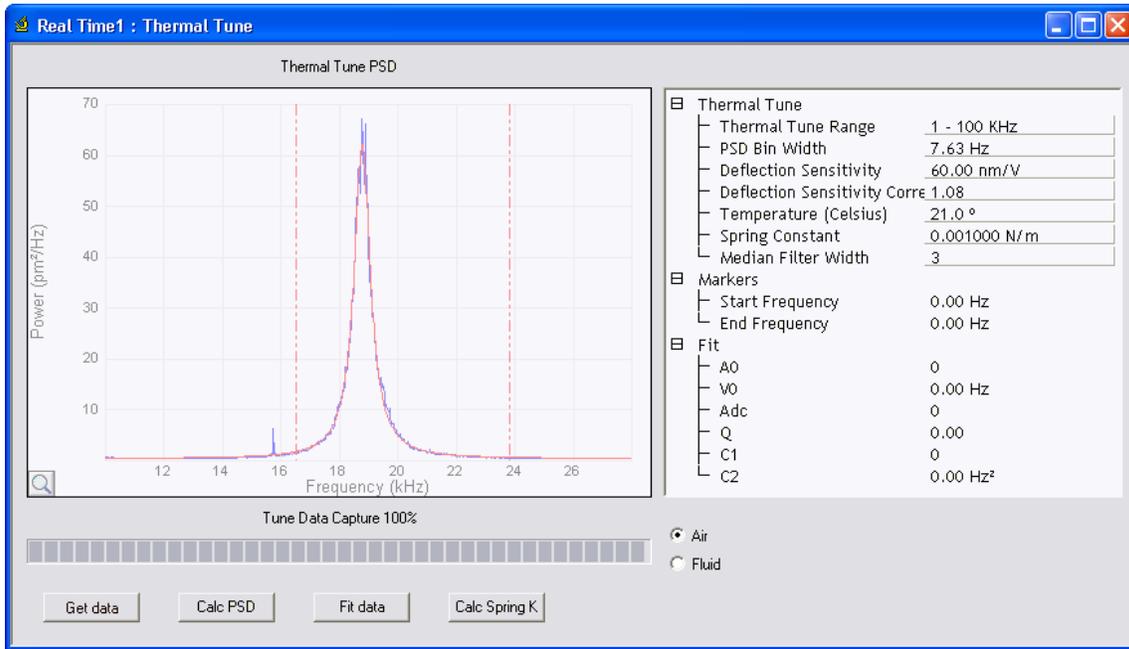
> **Note:** Thermal Tune computes the spring constant from the "Filtered Deflection" signal (see **Signals Available via the PicoForce Control Module,** page 116).

7. A power spectral density (PSD) plot of the cantilever response to ambient conditions is displayed. Click either **AIR** or **FLUID** to select a Lorentzian or a simple harmonic oscillator model, respectively, of the PSD to be least squares fit to the data.

> **Note:** The equations used to fit the filtered data are:
>
> Simple Harmonic Oscillator, for use in fluid
>
> $$A(\nu) = A_0 + A_{DC} \cdot \frac{\nu_0^4}{(\nu_0^2 - \nu^2)^2 + \frac{\nu_0^2 \nu^2}{Q^2}}$$

where: $A(\nu)$ is the amplitude as a function of frequency, ν
           A_0 is the baseline amplitude
           A_{DC} is the amplitude at DC (zero frequency)
           ν_0 is the center frequency of the resonant peak
           Q is the quality factor

Lorentzian, for use in air

$$A(\nu) = A_0 + \frac{C_1}{(\nu - \nu_0)^2 + C_2}$$

where: $A(\nu)$ is the amplitude as a function of frequency, ν
   A_0 is the baseline amplitude
   ν_0 is the center frequency of the resonant peak
   C_1 is a Lorentzian fit parameter
   C_2 is a Lorentzian fit parameter

8. Version 7 software: Adjust the **MEDIAN FILTER WIDTH**, shown in Figure 8.7d, to remove individual (narrow) spikes. This replaces a data point with the median of the surrounding n (n =3, 5, 7) data points.

Figure 8.7d   MEDIAN FILTER WIDTh
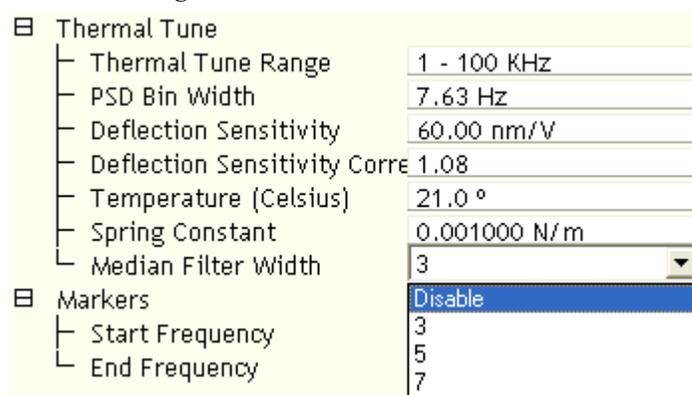
9. Version 7 software: Adjust the **PSD BIN WIDTH** to reduce noise by increasing the averaging.

10. Drag markers in from the left and/or right plot edges to bracket the bandwidth over which the fit is to be performed.

   Note: Typically the markers are located roughly where the spectrum rises from the noise floor. Precise placement is unnecessary; the fit is insensitive to the minimal power contributed from these frequencies far from the natural resonance (see right "shoulder" of waveform, Figure 8.7c). You may exclude a larger portion of a shoulder of the waveform from the fit bandwidth to ignore a noise spike (see left shoulder, Figure 8.7c). Experiment with repeated fits of the same acquired thermal tune data to become familiar with its sensitivity to bandwidth and choice of model.

11. Click **FIT DATA**. The curve fit is displayed along with the acquired data.

12. Adjust the markers for the bandwidth of the fit PSD to include in the spring constant calculation.

> **Note:** While the goal of setting the bandwidth for the curve fit was to achieve the highest signal-to-noise ratio by excluding noise, in setting the bandwidth for spring constant calculation from the (noise-free) fit curve the objective is to include all frequencies that contribute power to the spectrum. For instance, if you left out part of a shoulder in performing the curve fit, include it in determining the spring constant (see Figure 8.7e).

13. Enter the cantilever **TEMPERATURE**.

14. Click **CALC. SPRING K**. You will be asked whether you want to accept the calculated value of the spring constant, k (see Figure 8.7e).

Figure 8.7e Bandwidth for Spring Constant Calculation
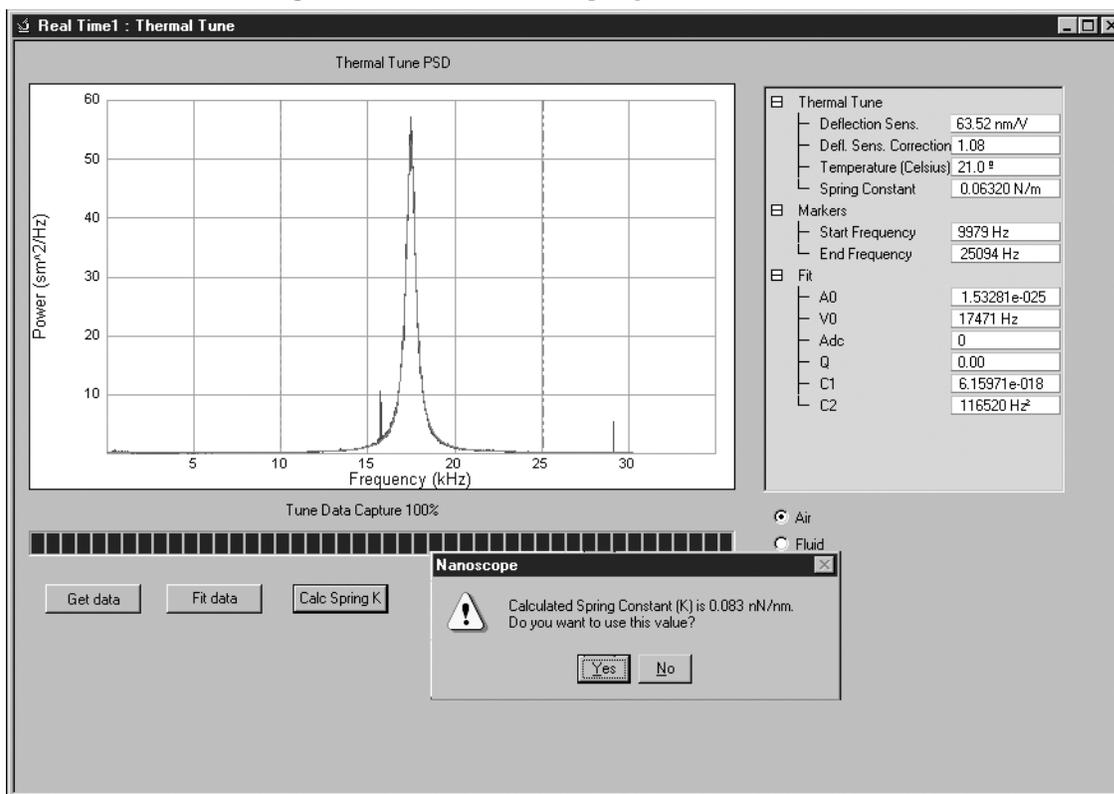
See **Problems with Thermal Tune,** page 120 for troubleshooting tips.

Chapter 9   Pulling: Offline Analysis

RealTime and Offline modes can operate concurrently in Versions 6 and 7 of NanoScope software, so are not distinguished as they were in previous versions.

> **Note:**   Offline functions operate more slowly when the SPM probe is engaged with a sample. Withdraw the probe to speed up nonrealtime activities.

Force plot files are named and saved in the same way as scanned image files. NanoScope Version 6 and 7 software features a dockable file browsing window initially at the right edge of the client window (see Figure 9.0a).

> **Note:**   Many subpanel edges are movable. Position the cursor over an edge and watch for the cursor appearance to change to a line segment with arrows at each end. Then drag (click and hold while moving the cursor) to re-position the dragged edge. For instance, the column widths (Name, Size, Type, etc.) can be re-sized as well as the window in which they appear itself.

Click the box to the right of the word **DIRECTORY** in the file browsing window to locate a folder in the directory listed to the right of the box. Selecting the first icon in the upper left of the file browsing window initiates a text presentation of file information (as in Figure 9.0a). The second icon in the upper left of the file browsing window causes thumbnail presentation of image files as illustrated in Figure 9.0b (the icon has been scrolled out of view at the top of the window of images). Click on a thumbnail to open the image for further analysis. The third icon in the upper left of the file browsing window displays file information, in either text or thumbnail presentations, of the capture directory (D:\capture).

Figure 9.0a   Browse for Folder Window Open Upon Clicking Directory
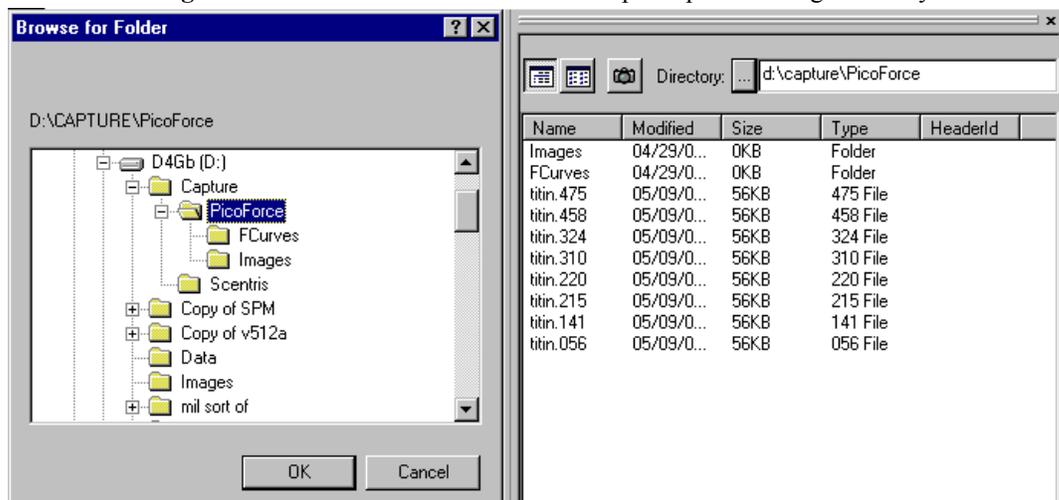
Once an image is open, traditional **Offline** image analysis icons appear (see Figure 9.0b) in the toolbar to the right of the Help icons ("?"). Refer to the *Command Reference Manual* and *NanoScope Software Version 6* and *7 User Guide* for description of their functions. From left to right, they are: **Image**, **Depth**, **Section**, **Roughness**, **3D Surface Plot**, **PSD**, **Zoom**, **XY Drift, Flatten, Planefit, Median, Low Pass and Erase**.

Figure 9.0b   Click an Image on the Right to Open It in the Central Client Window
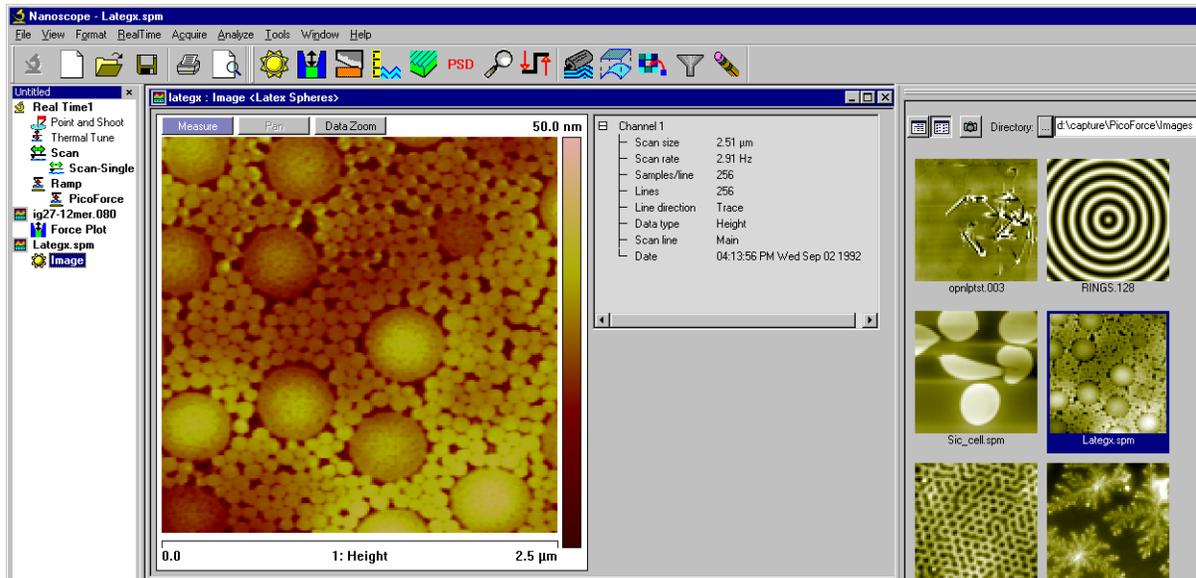
When a force file is opened (rather than an image file) from the file browsing window, opening it causes the three constituent plots to be displayed (see Figure 9.0c).

> **Note:** The same functions menu, opened by right click in the image, is available here in Image Processing Mode, just like in RealTime Mode (see Figure 8.4c and accompanying notes, such as for zooming in and out of a plot). For instance, select **EXPORT** to save a plot elsewhere in bitmap or JPEG format. Comma-delimited text format will also soon be available.

Two pairs of markers are available on each plot. Drag from a side into a graph to make a marker appear. For each marker pair, the marker/curve intersections are tabulated to the right along with the difference in coordinate values along each axis and the resulting slope and its inverse.

Cantilever deflection is listed in volts for each marker. If **DEFLECTION SENS** has been entered, cantilever deflection is also listed in nanometers. In addition, if a value is entered for **SPRING CONSTANT**, cantilever deflection appears as a force, in piconewtons, too.

> **Note:** See Bruker Application Note 97, "NanoScope Software Tools for Force Spectroscopy Data Analysis," available at http://bruker.com/library for a useful review of NanoScope Force Spectroscopy tools.

Figure 9.0c   Offline Presentation of a Captured Force Plot
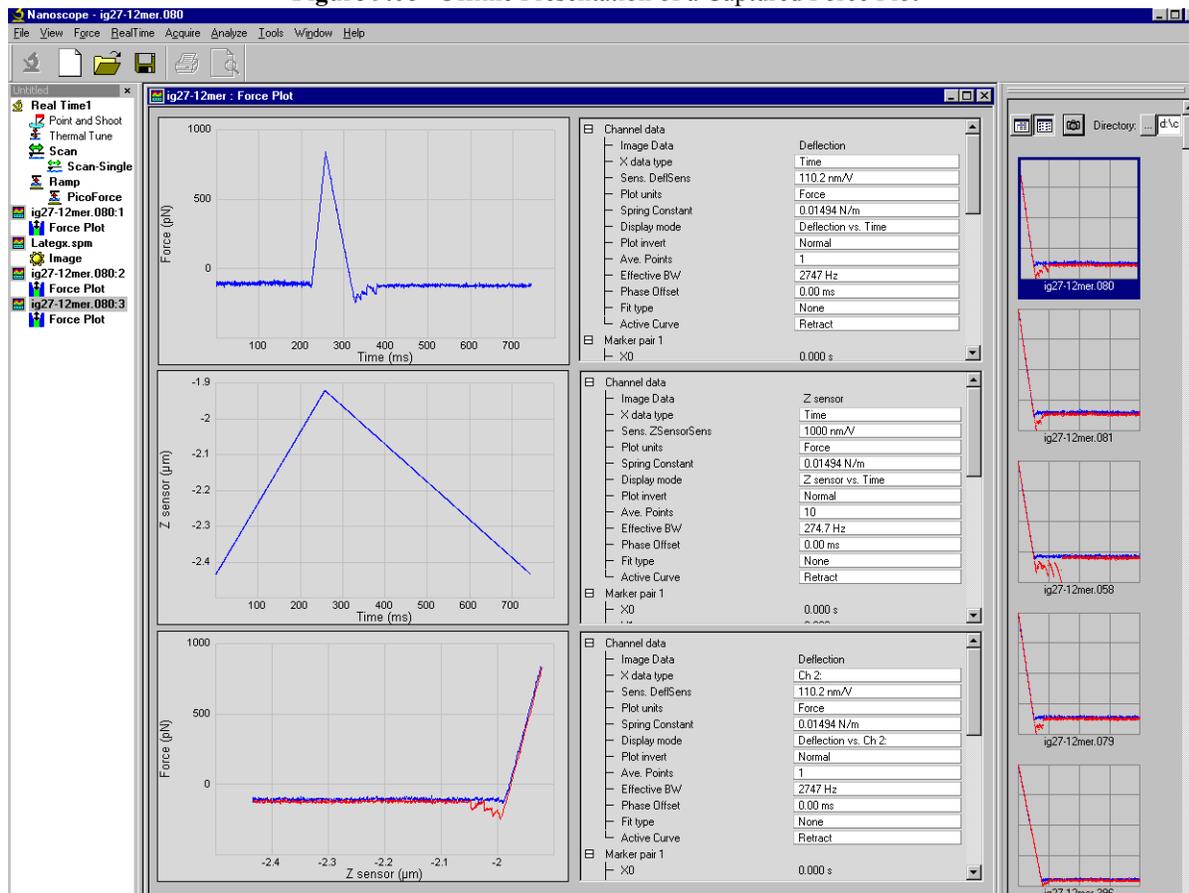
9.1   Fitting Force Plots to Models

Stored force curves may be fit to several built-in models: LINE, HERTZIAN or WORM-LIKE
CHAIN. To perform a fit:

1.   Select a force curve.

2.   Set the DISPLAY MODE to DEFLECTION VS. SEPARATION.

        Note: Calibrate the deflection sensitivity using a hard sample.

3.   Select the appropriate ACTIVE CURVE; APPROACH for the HERTZIAN model and RETRACT for
      the **WLC** model.

4.   If necessary zoom (see zoom, Page 55) in on the appropriate portion of the force curve.

5.   Drag a pair of markers (see markers Page 84) to bound the region of interest.

6.   Select the appropriate model, LINE, HERTZIAN or WORM-LIKE CHAIN.

a. The **LINE** model fits outputs m and b parameters for

$$y = mx + b$$

b. The **HERTZIAN** model, shown in Figure 9.1a, fits

$$f(x) = KR^{0.5} \cdot x^{1.5}$$

where f(x) is the force in Newtons, x is the distance in nm, R is the radius in nm and K is the fit parameter with units of Newtons/nm^2. This model is appropriate for pushing (Approach) a sphere into a hard surface.

Note: R is fixed at 1 nm. Scale for other values of R.

Figure 9.1a   Hertzian Fit
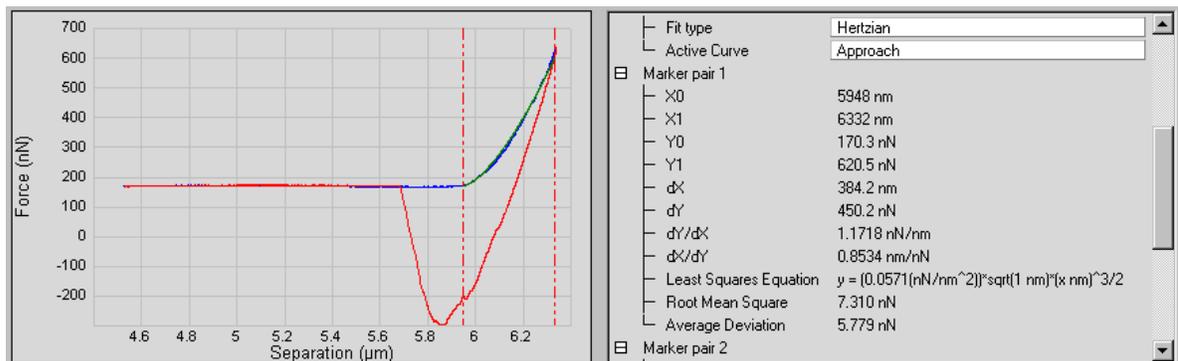
c. The **WORM-LIKE CHAIN** model, shown in Figure 9.1b, fits

$$f(x) = \left[\frac{k_B T}{4 L_p}\right] \cdot \left[\frac{1}{(1 - x/L)^2} - 1 + \frac{4x}{L}\right]$$

where f(x) is the force in Newtons, x is the distance in nm, k_B is Boltzman's constant, T is 298°K, L_p is the persistence length (in nm) and is an input variable and L is the parameter to be estimated.

Figure 9.1b   Worm-Like Chain Fit
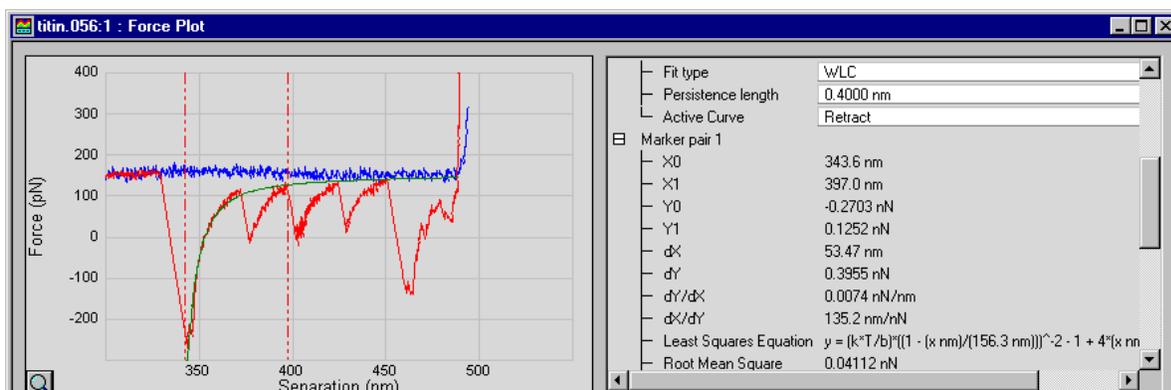
7. A green curve fit will be displayed in the plot window and the least squares fit equation, with RMS error and deviation will be displayed in the right window.

9.2   Reviewing Multiple Force Plots

A force spectroscopy session using the MultiMode PicoForce can yield dozens if not hundreds of individual force plots.

1. To facilitate comparison of plots and deletion of files deemed unworthy of inclusion, click **File** > **REVIEW FORCE CURVES...** or right-click on a force curve in the browse window and click **REVIEW CURVE**.

2. The **Review Force Curves** window opens (see Figure 9.2a).

    a. Enter the path to the **DIRECTORY** whose contents are the force plot files of interest.

    b. Select a **SORT** category: **DATE**, **NAME** or **TMR** (scanning trace minus retrace).

        Note: The TMR values are proportional to the area between the approach and retract curves.

    c. Select a **SORT** order: **ASCENDING** (box checked) or **DESCENDING** (box unchecked).

    d. Select the **PLOT UNITS** (vertical axis) units: **VOLTS**, **METRIC** or **FORCE**.

    e. Select the **PLOT** number.

    f. Select the **TRACE**(s) to plot: **EXTEND**, **RETRACT** or **BOTH**.

3. Advance through the ordered set of files by clicking the arrow buttons in the bottom left of the **File** text box or using the keyboard arrow keys.

    a. Click **MARK FOR DELETE**, **D**, or the keyboard **DELETE** key for any force plot you wish to discard from the set.

> **Note:** Files marked for deletion are not sent to the recycling bin until the **DELETE FILES** button is clicked. You may remove the check from the **MARK FOR DELETE** at any time prior.

Figure 9.2a The Delete Force Curves Window for Reviewing Multiple Force Plot Files
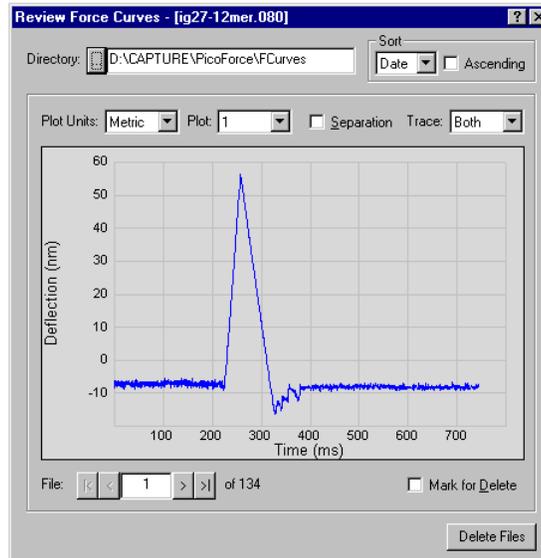
4. Zooming on a plot is accomplished by pressing **CTRL**, left-clicking and dragging in the plot area. Zooming out is done by double-clicking in the plot area.

9.3 Filtering Multiple Force Curves

Another way to quickly delete force curves uses the **FILTER CURVES...** function.

1. You may apply selection criteria to delete force curves by selecting, from the main menu, **FILE > FILTER CURVES...**

2. The **Filter Force Curves** window opens (see Figure 9.3a).

Figure 9.3a The Filter Force Curves Window
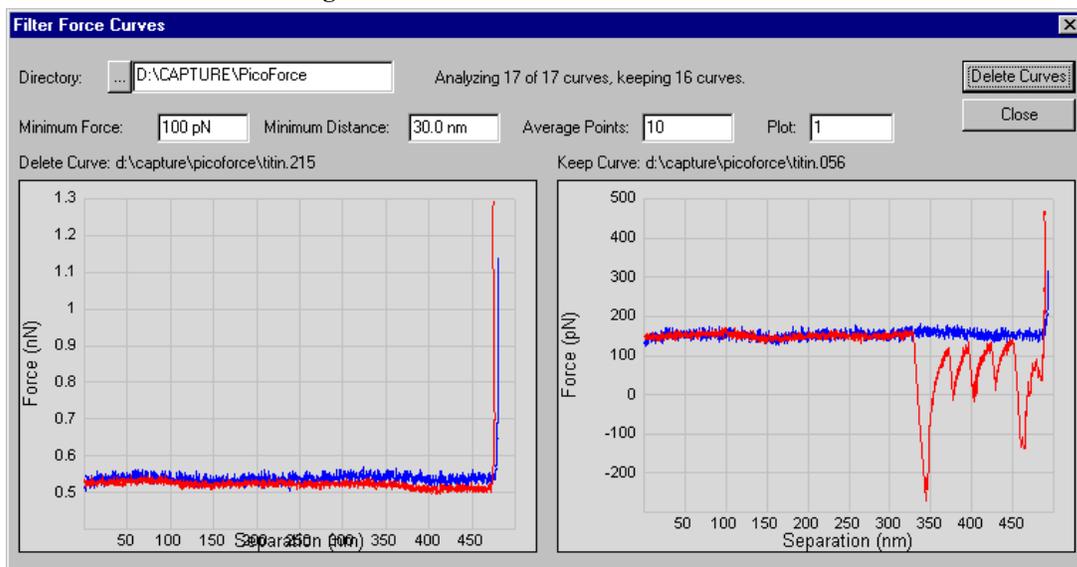
a. Enter the path to the **DIRECTORY** whose contents are the force plot files of interest.

b. Enter the **PLOT** number you wish to analyze.

c. Enter a **MINIMUM** (x) **DISTANCE** from the contact point below which data will be ignored.

d. Enter a **MINIMUM FORCE** from the contact point to qualify peaks.

e. Enter the number **AVERAGE POINTS** to use. To reduce noise, peak values are computed by averaging ±(**AVERAGE POINTS**)/2 around a point.

3. Click **DELETE CURVES** to analyze, using the criteria in the **Filter Force Curves** window, the force curves in the selected directory. Click **CANCEL** to abort the process.

4. Files meeting the deletion criteria will be shown in the left window, shown in Figure 9.3a, and a message, **Confirm File Delete**, will be displayed asking if you wish to send the specified file to the Recycle Bin.

9.4   Analyzing Multiple Force Plots

Large numbers of force plots can be evaluated using the PicoForce Multiple Curve Analysis (mca) function.

1. Click **File** > **New** > **MULTIPLE FORCE (*.MCA)** or select **MULTIPLE FORCE CURVES** in the browse window, right-click and then click **CURVE ANALYSIS** to open a **MultipleForce** window.

2. Click **ADD FILES...** in the **MultipleForce** window to open a file selection window and click the files to be analyzed (see Figure 9.4a). Click **ADD**. These files are then added to the file list in the **MultipleForce** window. Files in that list may be removed individually or multiply by clicking the file name and then clicking **REMOVE FILES**.

Figure 9.4a   Multiple Force Window
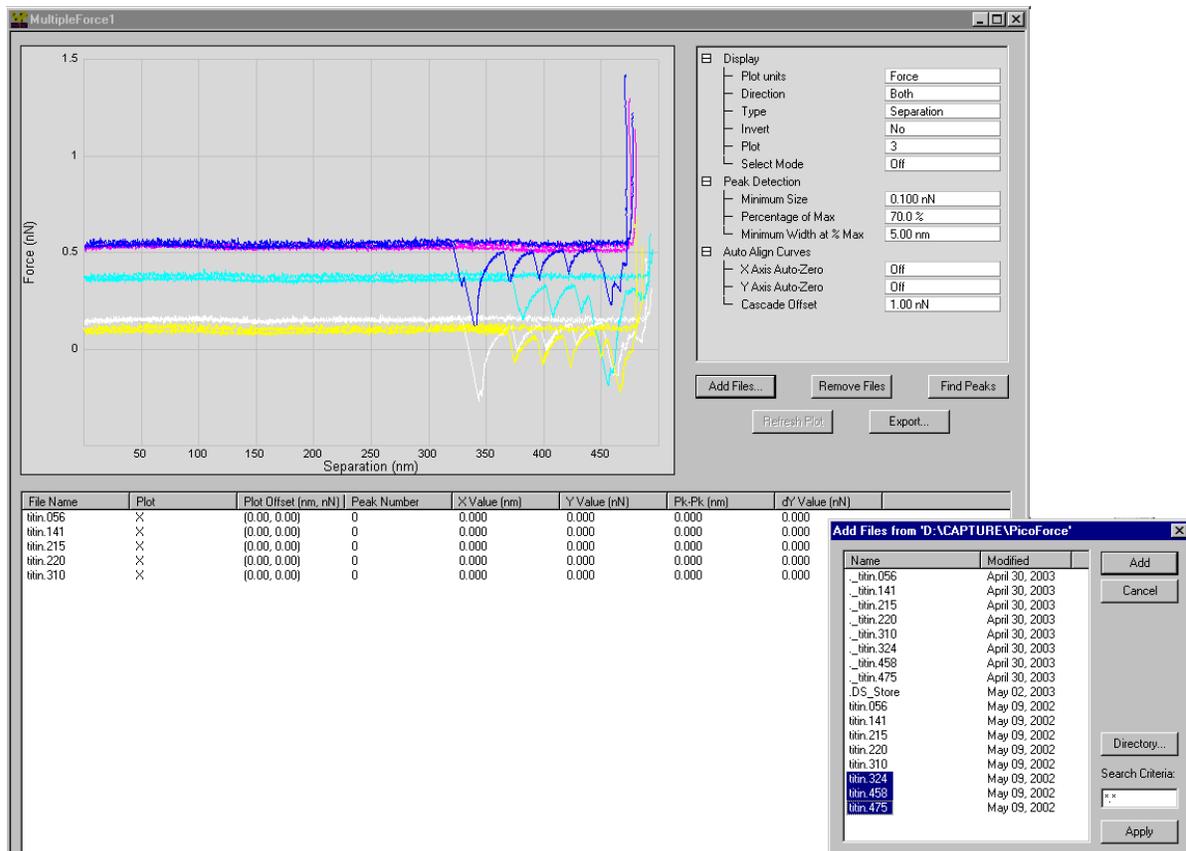
3. Individual or multiple files to be analyzed are selected from this list by checking the **PLOT** column.

4. Right-clicking a file in the **MultipleForce** window opens a menu (shown in Figure 9.4b) that allows checking or unchecking all files in the list as well as functions that affect plotting parameters, discussed below.

Figure 9.4b   Right-click Menu

Many display and analysis functions are available in the **MultipleForce** window:

- **DISPLAY**:

    - **PLOT UNITS**: The user can choose either **FORCE**, **VOLT** or distance (**METRIC**) on the Y axis.

    - **PLOT DIRECTION**: The **EXTEND** option plots and looks for peaks only in the **EXTEND** direction. The **RETRACT** option plots and looks for peaks only in the **RETRACT** direction.

    Note: The **EXTEND** curve will still be used to find the point of contact if that option is selected under **AUTO ALIGN CURVES** -> **X AXIS AUTO-ZERO**.

    - **TYPE**: Either **Z** or **SEPARATION**. **Z** displays the Z position of the piezo measured by a capacitive sensor. **SEPARATION** displays the corrected (for cantilever deflection) **Z** data.

    - **INVERT**: The Y axis is inverted when **YES** is selected.

    - **PLOT**: Any of three channels (see **Basic Force Mode:** Section 8.4) may be plotted.

    - **SELECT MODE**: **ON** turns all curves to color number one and the selected (by mouse or arrow key) curve to its plot color.

- **PEAK DETECTION**:

    - **MINIMUM SIZE**: Finds peaks greater than the value entered here.

    - **PERCENTAGE OF MAX**: The Y variable must fall by this percentage to be considered a peak.

    - **MINIMUM WIDTH AT % MAX**: A peak must have a width (at **PERCENTAGE OF MAX** of the peak's absolute value) greater than the **MINIMUM WIDTH AT % MAX** to be considered a peak.

- **AUTO ALIGN CURVES**:

    - **X AXIS AUTO-ZERO**: Either **ON** (at the contact point), **OFF** or **USER**.

- **Y AXIS AUTO-ZERO**: Either **ON**, **OFF**, **USER** or **CASCADE** which increments the Y offset of each plot by the **CASCADE OFFSET**.

- **CASCADE OFFSET**: Define the Y offset to be used here when the **Y AXIS AUTO-ZERO** is set to **CASCADE**.

Many data plotting commands are accessible by right-clicking in the plot area, shown in Figure 9.4c:

Figure 9.4c   Plot Command Menu: Right-Click
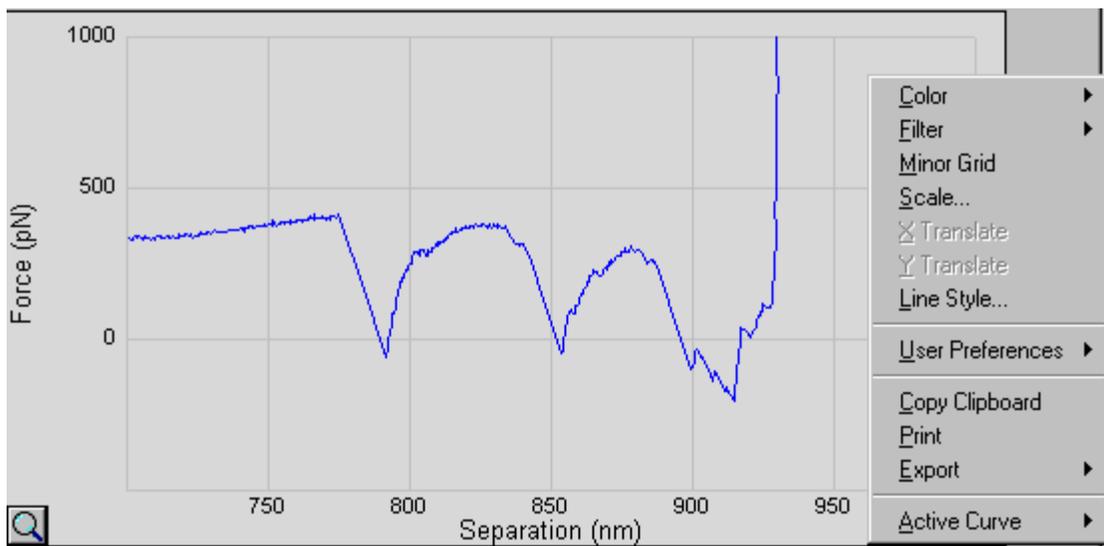
Additional data plotting commands are initiated by keystrokes:

- Zooming on a plot is accomplished by pressing **CTRL**, left-clicking and dragging in the plot area. Zooming out is done by double-clicking in the plot area or clicking the magnifying glass at the lower left corner of the plot.

- Arrow keys move a plot by an increment defined by the **Set Increments** menu.

Figure 9.4d   Set Increments and Set Offset Menus
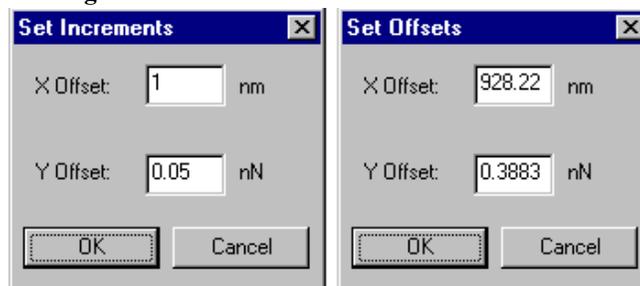
The following buttons, below the plot window are available:

- **ADD FILES...**:Opens a file selection window that allows files to be added in the **Multiple Force** window.

- **REMOVE FILES**:Deletes selected (by clicking) files from the **Multiple Force** window.

- **FIND PEAKS**: Finds peaks using the filters (rules) selected in the **PEAK DETECTION** functions to the right of the plot window. The **X OFFSET** and **Y OFFSET**, **PEAK NUMBER**, **X VALUE**, **Y VALUE**, **PEAK-TO-PEAK** and **DY VALUE**s are shown in the table below the plot window, as shown in Figure 9.4e.

- **REFRESH**: Used to refresh the plot window after selecting or deselecting files or changing inputs.

- **EXPORT...**: Saves the results from **FIND PEAKS** in a tab-delimited text file.

Figure 9.4e MCA: Find Peaks
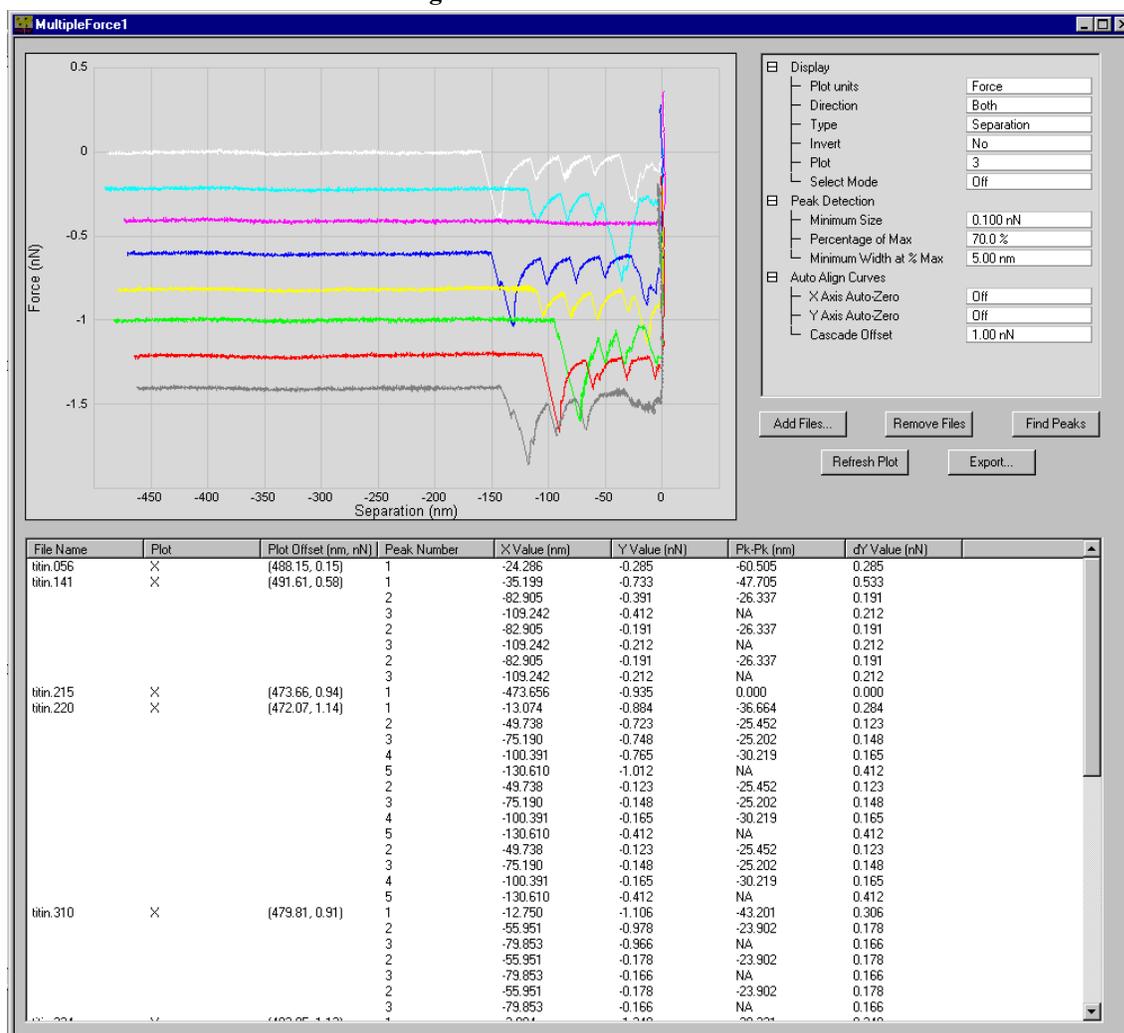
9.5   Exporting Force Plot Files

Stored images (see Figure 9.0b) and force plots (see Figure 9.0c) can be included in thumbnail format in the Browse window on the right of the PicoForce display screen. From there, files may be selected for export. If a force curve is open, its data may also be exported by clicking **FILE > EXPORT > ASCII**.

1. Click **View** > **BROWSE**.

2. Enter the path to the directory of interest at the top of the Browse window to select what is displayed. Drag the left edge of the window to the left to expand the window as needed.

3. Select the file(s) of interest.

    Note: The keyboard **CONTROL** and **SHIFT** keys have familiar capabilities here. Multiple files are included in a single selection by holding down **CONTROL** while clicking on each item. Consecutive files are selected by clicking on the first one, holding down the **SHIFT** key and clicking on the last one.

4. Within the selection, right click to display a menu with options: **OPEN**, **EXPORT**, **DELETE**, **AUTO PROGRAM** and **CURVE ANALYSIS**. Click **EXPORT > ASCII**.

5. In the **Export** window check the boxes that apply:

    a. Click **EXPORT TIME** to include the horizontal axis data from Channels 1 and 2, the sources of the vertical and horizontal axis data, respectively, of a force plot (Channel 3).

    b. Check the units preferred for **DEFLECTION** data (volts, nanometers or piconewtons).

    c. Check the units preferred for **Z SENSOR** data (volts or nanometers).

    d. Check which **TRACES** to include: (at least one of) **EXTEND** and **RETRACT**.

    e. Check **EXPORT HEADER** for inclusion of more detail on the conditions during data taking.

6. Click **SAVE AS...** to export the selected files.

7. In the **SAVE AS...** window provide a path to a directory and choose a file extension type to designate the file format.

    Note: If no file extension is provided, files are stored as text files (*.txt, see Figure 9.5a).

    Note: If more than one file are selected, a batch export is performed automatically.

Figure 9.5a The Data Export Window and (the Beginning of) a Sample Data File
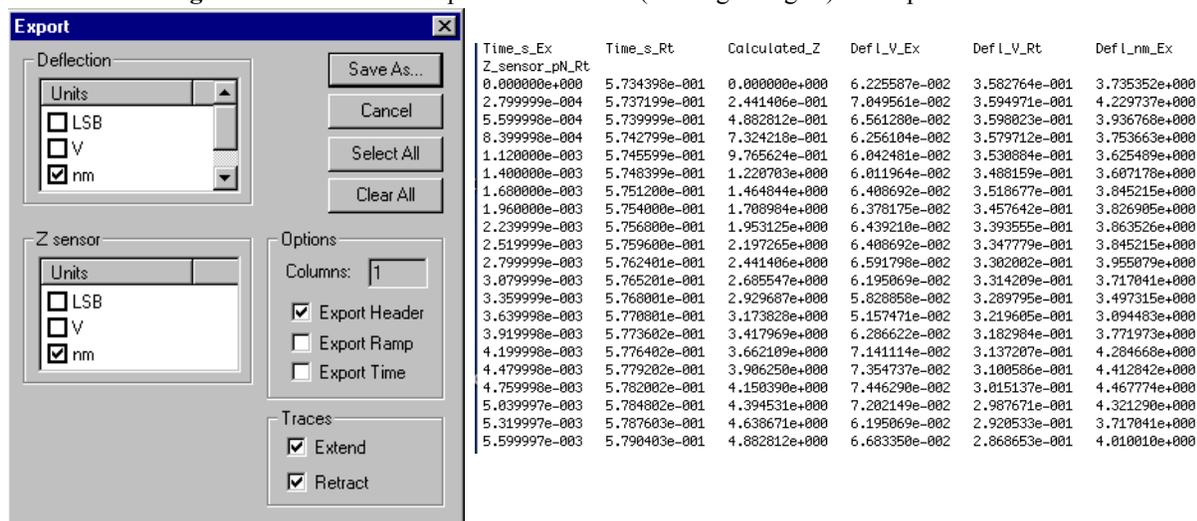
Force files may also be exported as a **BITMAP** or **JPEG** image file or as an **XZ DATA** file by right-clicking in the plot window.

Chapter 10   Pushing: Theory and Principles

10.1   Types of Pushing Applications

MultiMode PicoForce can be used to push as well as to pull a sample. A typical application is indentation: deliberate pressing into a surface to measure how it yields to pressure. Force plots, which entail both pushing and pulling, can be viewed as an application in the transition region between molecule pulling and nanoindentation. In particular, force plots are often performed on soft samples specifically to establish an upper bound on the pressure that may be applied before the sample is crushed or punctured. A force plot on a soft sample lacks a linearly sloped contact region (region 3 in Figure 7.1a) in which to define force sensitivity.

> **Note:**   For applications emphasizing in-sample-plane pushing, as opposed to out-of-the-sample-plane pushing, the NanoMan Option with a Dimension SPM may be preferable due to its closed loop XY-positioning of the tip. MultiMode PicoForce features closed loop Z-positioning of the sample.

An indentation force plot can be used to estimate the elastic modulus of a sample. A simple relationship has been shown to exist among contact stiffness, contact area and elastic modulus that is independent of the shape of the indenter used.

Other reasons to apply a force to a sample are deliberate scratching and wear testing, both of which add elements of scanning to the basic indentation process.

10.2   Analysis of Indentation Force Plots

The traditional force plot shown in Figure 7.1a illustrates an unyielding surface: the cantilever deflects linearly with applied force and follows the same trajectory retracting from the sample. Such a plot on a hard surface is ideal for establishing **DEFLECTION SENS**. In a typical indentation application, the surface yields to pressure and is crushed directly under a diamond tip. Thus, the tip follows a new trajectory when retracted. The initial slope of the retraction curve portion of a force-displacement plot is a measure of *unloading stiffness*, $S = dF/dz$. Assuming the indenter/sample contact area remains constant during initial tip withdrawal, the function $F(z)$ has been found to be:

$$F = \frac{4\mu a}{1 - \nu} z$$

where a = the radius of the contact area,
  μ = the *shear modulus* of the sample and
  ν = *Poisson's ratio* for the sample.

The *elastic modulus*, E, of a material is related to the shear modulus and Poisson's ratio by:

$$E = 2\mu(1 + \nu)$$

Differentiating force, F, with respect to displacement, z, completes the relationship among unloading stiffness, contact area and elastic modulus:

$$\frac{dF}{dz} = \frac{2}{\sqrt{\pi}}\sqrt{A}\frac{E}{(1 - \nu^2)}$$

where $A = \frac{1}{4}a^2$, the circular contact area with radius a.

Though originally derived for a conical indenter, the above equation has been shown to be valid for any indenter such that its contact surface is the body of revolution of a smooth function. Even for indenters with square or triangular cross sections, deviations from the relationship are by only approximately 1.2 percent and 3.4 percent, respectively.

Allowing for some deformation of the indenter itself as well, a reduced modulus, E_r, is defined by:

$$\frac{1}{E_r} = \frac{(1 - \nu^2)}{E} + \frac{(1 - \nu_i^2)}{E_i}$$

where i represents the indenter: E_i = the elastic modulus of the indenter and ν_i is Poisson's ratio of the indenter. The governing relationship then transforms to:

$$S = \frac{dF}{dz} = \frac{2}{\sqrt{\pi}}E_r\sqrt{A}$$

With the above equation, measuring the initial unloading slope and the maximum area of contact yields the reduced elastic modulus. Because MultiMode PicoForce is an imaging platform as well as an applicator and measurement device for nanoscale forces, the indentation contact area is readily measured.

Another material characteristic measurable from indentation is *hardness*, $H = F_{max}/A$, the maximum load divided by the contact area at peak load.

For further discussion and derivations of the above relationships, refer to "*An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments*", by W. C. Oliver and G. M. Pharr, in the Journal of Materials Research, Vol. 7, No. 6, June 1992, page 1564.

Chapter 11   Pushing: Operation

Into-the-sample-plane pushing applications are generally ranked by dimensionality. *Indentation* ideally involves pressing a probe into a single point. *Scratching* starts with pressing in at a point and proceeds with dragging the probe along a line segment. *Wear testing* typically entails a repeated raster pattern of scratches. Only certain probe types are designed to withstand the stresses of higher dimensional pushing applications.

11.1   Probe Selection

A particularly hard probe is preferred in most pushing applications so the results can be unambiguously interpreted as representative of the effect of the indenter on the sample and not vice versa, nor a mixture of the two. Bruker supplies the DNISP type probe for pushing applications: a diamond-coated tip of <25nm radius mounted on a stainless steel cantilever. Compared to other scanning probes, a typical indentation probe has a higher spring constant for imparting larger forces to a sample (see Table 11.1a). DNISP probes are supplied with their spring constant specified within ±10%. A silicon reflector is mounted on the top side of the cantilever to obtain a well focused laser spot on the photodetector (see Figure 11.1a). The stiff cantilever, durable tip and reflector add mass relative to standard scanning probes, lowering the cantilever resonant frequency. Indenting probes are still useful for TappingMode imaging as well, facilitating contact area determination following indentation.

Table 11.1a   Probe Characteristics Comparison

Application	Cantilever Spring Constant	Cantilever Resonant Frequency
Contact Mode scanning	0.01-1.0N/m	n/a
TappingMode scanning	20-100N/m	~300kHz
Pushing	100-300N/m	35-60kHz

The forces involved when indenting are typically in the range of 1—100µN for a standard probe cantilever. The diamond tip is in the form of a 3-sided pyramid (plus base) with an apex angle of 60 degrees. To promote symmetric dents, the diamond is mounted such that the vertical axis of the pyramid is approximately normal to the sample when mounted on the SPM.

Figure 11.1a A DNISP Type Indenting/Scratching/Wear Testing Probe
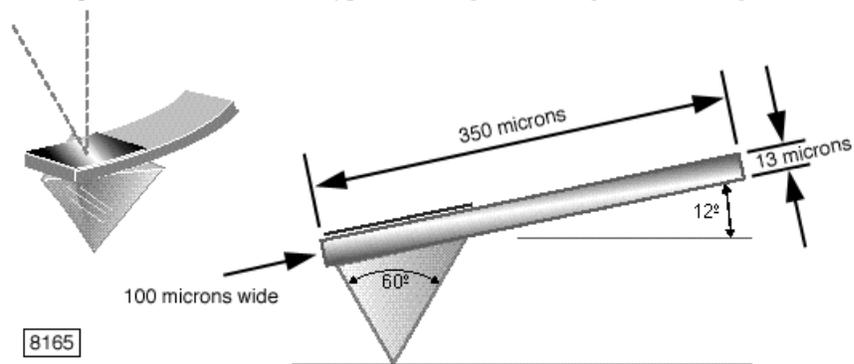
11.2 Indentation

A dent is made by forcing the tip into the sample surface until the required cantilever deflection is reached. The tip is then lifted to its initial Z position above the sample surface. For each dent, a force plot is recorded. You may execute an indentation array automatically using the **AUTO INDENT** command, specifying an initial force and a force increment in the X direction (and a constant force in the Y direction). Both the number of dents and the spacing between them—in both X and Y directions—can be preset.

Indentation is not presently supported in NanoScope Version 6. If you have a NanoScope IIIa or IV(a) Controller, exit Version 6 and start NanoScope Version 5 to perform indentation or scratching. If you have a NanoScope V Controller, refer to *Support Note 423, Nanoindentation for NanoScope Software Version 7***.**

Single-cycle Indentation

The basic nanoindentation procedure follows:

1. Load the sample and indentation probe into the SPM.

2. Align the laser on the reflector atop the cantilever.

3. Engage the surface in TappingMode with RMS amplitude of 0.25–0.3V.

    Note: Execute **CANTILEVER TUNE** to find the resonant frequency of the cantilever. Set the **DRIVE FREQUENCY** to the center of the resonance peak. Adjust the **DRIVE AMPLITUDE** until the RMS amplitude of the cantilever is from 0.25-0.3V (This is significantly lower than the RMS amplitude typically used with standard TappingMode probes).

    Note: When positioning the tip close to the sample surface before engaging, be aware that the diamond tip extends 100μm beneath the underside of the cantilever.

4. Image the sample to locate an area of interest for indenting.

> **Note:** Set RealTime **Scan Controls** and **Feedback Controls** imaging parameters to values appropriate for TappingMode imaging. Limit SCAN RATE to about **1Hz** for indentation probes. Engage with a small SCAN SIZE, about **1–3MM**, and increase SCAN SIZE after engaging if necessary. The standard INTEGRAL GAIN and PROPORTIONAL GAIN of **0.5** and **1.0**, respectively, are appropriate.

5. Click **RealTime** > **View** > **Force Mode** > **INDENT** and indent the surface.

> **Note:** The **Indent Controls**, **Feedback Controls**, **Display Controls**, and **Auto** panels, and three data **Channel** panels open. Open the **Indent Controls** panel using the **Panels** menu option, if it is not already open. The parameter settings shown in Figure 11.2a are a good starting point for indenting a new sample. The TRIGGER THRESHOLD is the cantilever deflection at which the controller stops pushing the tip into the surface. Click RUN SINGLE (icon shown) to make a single-cycle indentation.

Figure 11.2a Indent Controls Panel for Indenting

Indent Controls		Other Controls	
Z scan size:	10.0 V	Indent setpoint:	0.900
Z scan rate:	2.03 Hz	Start mode:	Indent
Trig threshold:	0.2000 V	Auto ramp size:	Enabled
X offset:	0.00 V	Lift height:	200 nm
Y offset:	0.00 V	Units:	Volts
X Rotate:	22.0 deg		
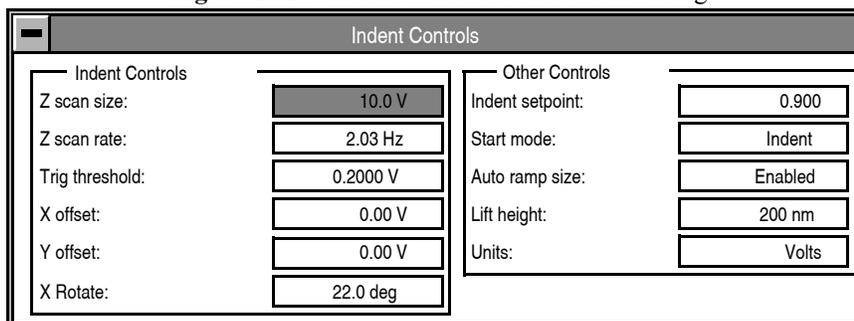
6. To save the displayed force plot, click the **CAPTURE** icon (shown).

7. Click **View** > **IMAGE MODE**, or click the Image icon (shown) to view the dents just created.

For more information about **Indent Controls** parameters also used in other SPM modes, refer to *Support Note 225F, Nanoindentation and Nanoscratching with SPMs for NanoScope Version 4.32 Software*, or the *Command Reference Manual*. The **X ROTATE** parameter in the Indent Controls subpanel and **INDENT SETPOINT** in the Other Controls subpanel are described here because they are unique to pushing operations.

X ROTATE controls lateral tip motion during indentation. This is useful because the cantilever is at an angle relative to the surface. **X ROTATE** can prevent the tip from plowing the surface laterally due to cantilever bending or coupling of the Z- and X-axes of the piezo scanner. At engagement, the tip is oriented normally. However, as the tip is pressed into the surface, it tends to pitch forward. By applying a slight X-axis offset, the tip is brought normal again (see Figure 11.2b). **X ROTATE** is typically set between **15** and **25 DEGREES**, often to **22.0 DEGREES**. **X ROTATE** is disabled when set to **0 DEGREES**.

Figure 11.2b   Maintaining the Tip Normal to the Surface During Indentation with **X ROTATE**
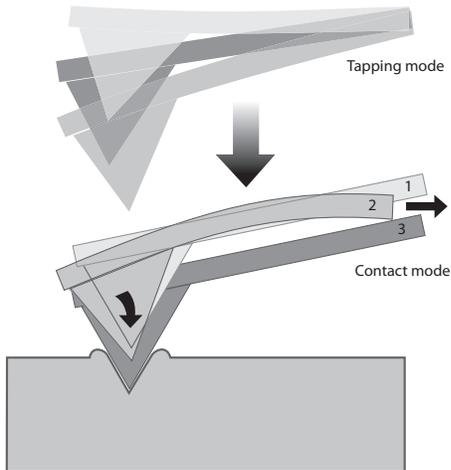
The three images in Figure 11.2c all use the same **TRIGGER THRESHOLD** value and demonstrate the effect of **X ROTATE** at various settings. The dent is largest for a value of **0.0 DEGREES**. Material is piled on the left, outboard side of each indentation depending upon the amount of correction. The pitching forward of the cantilever during nanoindentation tends to move the laser spot in a direction opposite to normal deflection. This produces reduced deflection at the photodetector for higher forces, the opposite of the desired mode of operation. Deeper, larger dents are made at lower **X ROTATE** values.

Figure 11.2c   X ROTATE Effect on Gold Under Constant **TRIGGER THRESHOLD**
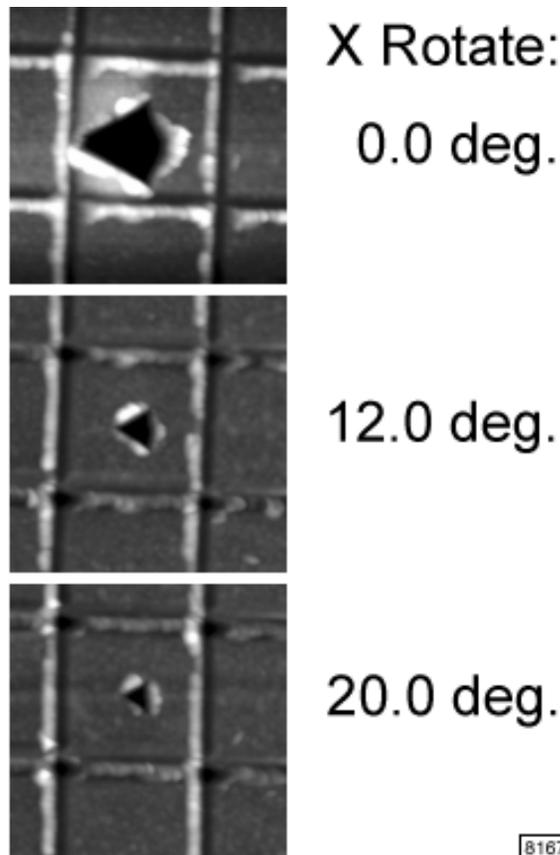
The **INDENT SETPOINT** parameter influences the transition from TappingMode during engage to Contact Mode for indentation. **INDENT SETPOINT** only has an effect during indentation and scratching. With a value typically between `0.5` and `1.0` (and usually `0.9`), **INDENT SETPOINT** multiplies **AMPLITUDE SETPOINT** to reduce its effective value during engage for indentation.

INDENT SETPOINT is useful in cases where the free-air (pre-contact) part of a force plot is not flat. In such cases, the maximum deflection and force during indenting vary depending on where the indentation is triggered. Applying the **INDENT SETPOINT** multiplier enables moving the surface contact point closer to where cantilever deflection begins (see Figure 11.2d). During the tip descent to the surface, the graph displays the surface contact point as a vertical yellow line.

CAUTION: If **INDENT SETPOINT** is set too low, the tip may be extended too far into the sample surface, threatening the tip. If the target oscillation amplitude is too close to the noise level, the tip may be pushed into the surface with the Z piezo fully extended. Also, if the **INDENT SETPOINT** parameter is set too high, the tip will be retracted from the surface as the control loop attempts to attain an RMS amplitude which is higher than the free air amplitude.

Figure 11.2d Shifting the Surface Contact Point Using **INDENT SETPOINT**
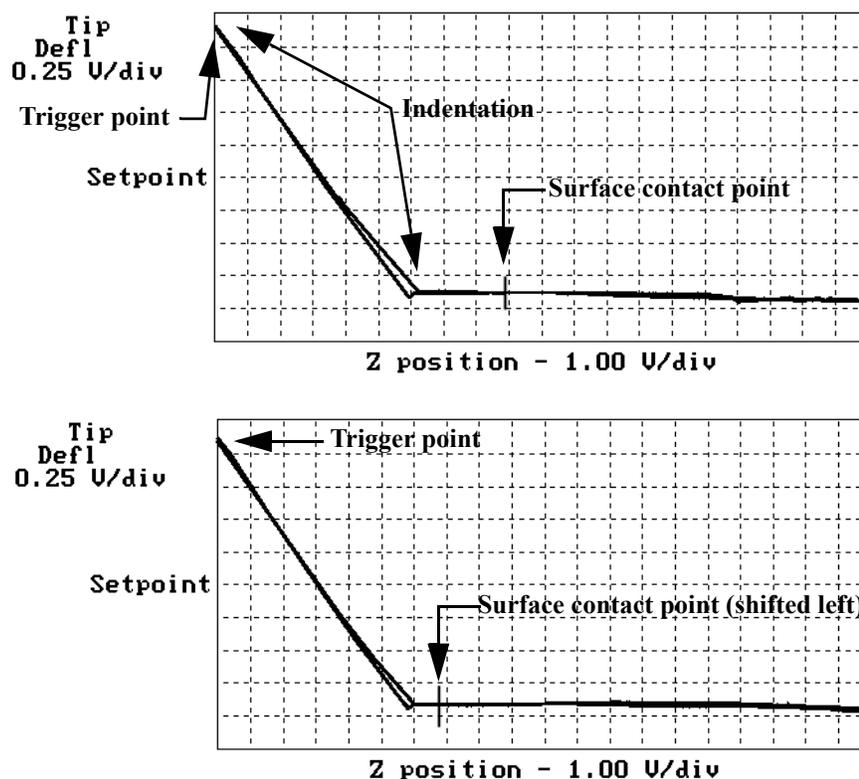
Automated Indentation Arrays

To generate an array of indentations initiated by a single command, begin with the first five steps of the standard single-cycle indentation procedure (last section). Then:

1. In the **Auto** panel (see Figure 11.2e), enter the size of the array of indents by providing the number of indents along the X-direction for **COLUMNS**, and along the Y-direction for **ROWS**.

2. Set the array spacing in the X- and Y-directions by entering values for **COLUMN STEP** and **ROW STEP**, respectively.

3. To increase the force applied with each successive indentation along the X-direction, enter a value for **THRESHOLD STEP**. The initial indentation in each row applies a force determined by **TRIGGER THRESHOLD**. Each subsequent indentation in the row concludes at a force higher than the previous indentation by an amount corresponding to **THRESHOLD STEP**.

4. Set **CAPTURE** to **ENABLED** before executing the array if you want to store the results.

Figure 11.2e   Example Auto Panel for Automated Indentation
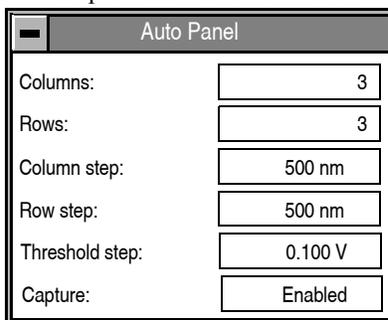
Auto Panel	
Columns:	3
Rows:	3
Column step:	500 nm
Row step:	500 nm
Threshold step:	0.100 V
Capture:	Enabled

5. Click **Probe** > **AUTO INDENT** in the Indent Mode menu to begin execution of the array.

   Note: During execution, the force plot for each indent is displayed along with the (X,Y) coordinates of the indent in the array.

6. Click **View** > **IMAGE MODE**, or click the Image icon (shown) to view the dents just created.

11.3   Scratching

Nanoscratching is essentially the same process as nanoindentation, except that the tip is moved laterally by a prescribed amount after the sample surface is penetrated. A scratch is made by forcing the tip into the sample surface until the required cantilever deflection is reached. Then, with the Z feedback turned off, the tip is moved laterally using the preset distance, direction and speed. The tip is then lifted to its initial Z position above the sample surface. As with nanoindentation, it is also possible to execute arrays of scratches automatically using the **AUTO SCRATCH** command.

   Note: **AUTO SCRATCH** is not available in NanoScope version 7 software.

Figure 11.3a Nanoscratching

8166
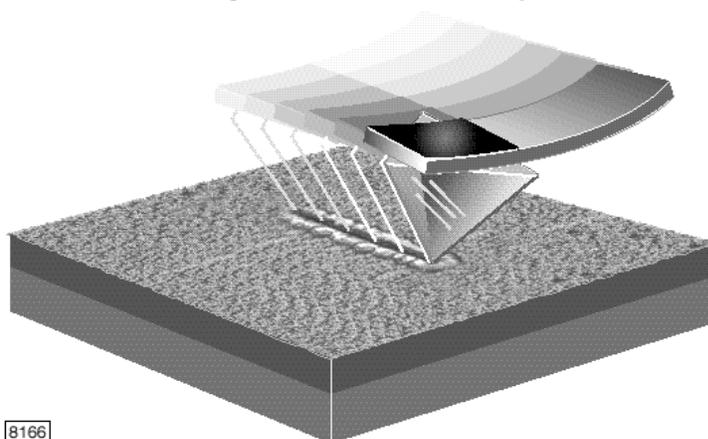
Single-cycle Scratching

The basic nanoscratching procedure follows:

1. Load the sample and indentation/scratching probe into the SPM.

2. Align the laser on the reflector atop the cantilever.

3. Engage the surface in TappingMode with RMS amplitude of 0.25–0.3V.

   Note: Execute **CANTILEVER TUNE** to find the resonant frequency of the cantilever. Set the **DRIVE FREQUENCY** to the center of the resonance peak. Adjust the **DRIVE AMPLITUDE** until the RMS amplitude of the cantilever is from 0.25-0.3V (This is significantly lower than the RMS amplitude typically used with standard TappingMode probes).

   Note: When positioning the tip close to the sample surface before engaging, be aware that the diamond tip extends 100μm beneath the underside of the cantilever.

4. Image the sample to locate an area of interest for indenting.

   Note: Set RealTime **Scan Controls** and **Feedback Controls** imaging parameters to values appropriate for TappingMode imaging. Limit **SCAN RATE** to about **1Hz** for indentation probes. Engage with a small **SCAN SIZE**, about **1-3MM**, and increase **SCAN SIZE** after engaging if necessary. The standard **INTEGRAL GAIN** and **PROPORTIONAL GAIN** of **0.5** and **1.0**, respectively, are appropriate.

5. Click **RealTime** > **View** > **Force Mode** > **SCRATCH** and indent the surface.

   Note: The **Indent Controls**, **Feedback Controls**, **Display Controls**, and **Auto** panels, and three data **Channel** panels open. Open the **Indent Controls** panel using the **Panels** menu option, if it is not already open. The parameter settings shown in Figure 11.3b are a good starting point for scratching a new sample.

Note the scratching-specific parameters in the Other Controls subpanel. The Indent Controls subpanel parameter **TRIGGER THRESHOLD** is lower than shown in Figure 11.2a for indenting. Click **RUN SINGLE** (icon shown) to make a single-cycle indentation.

Figure 11.3b   Indent Controls Panel for Scratching

Indent Controls		Other Controls	
Z scan size:	10.0 V	Indent setpoint:	0.900
Z scan rate:	2.03 Hz	Start mode:	Scratch
Trig threshold:	0.100 V	Auto ramp size:	Enabled
X offset:	0.00 V	Lift height:	200 nm
Y offset:	0.00 V	Scratch length:	1.00 μm
X Rotate:	22.0 deg	Scratch rate:	1.00 Hz
		Scratch angle:	90.0 deg
		Units:	Volts
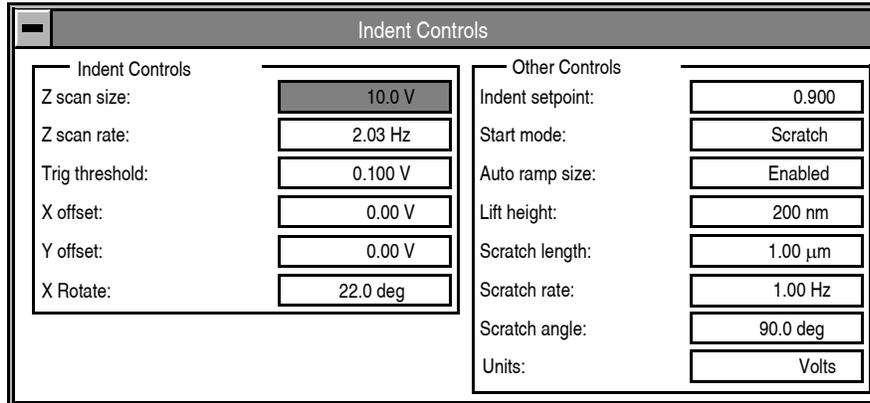
6. A force plot displays (deflection as a function of Z-position, the latter changing little during the scratch itself). To save the displayed force plot, click the **CAPTURE** icon (shown).

7. Click **View** > **IMAGE MODE**, or click the Image icon (shown) to view the dents just created.

Automated Scratching Arrays

To generate an array of scratches initiated by a single command, begin with the first five steps of the standard single-cycle scratching procedure (last section). Then:

1. In the **Auto** panel (see Figure 11.3c), enter the size of the array of scratches by providing the number of scratches along the X-direction for **COLUMNS**, and along the Y-direction for **ROWS**.

2. Set the array spacing in the X- and Y-directions by entering values for **COLUMN STEP** and **ROW STEP**, respectively.

   Note: Automated scratching uses the beginning of the scratches as the reference for the array. Thus, after an X and/or Y offset is performed, the scratch is made in the specified direction. Often, the **ROWS** parameter is set to **1** and **SCRATCH ANGLE** to **90 DEGREES**, resulting in a row of scratches parallel to the Y-axis.

3. To increase the force applied with each successive scratch along the X-direction, enter a value for **THRESHOLD STEP**. The initial scratch in each row applies a force determined by **TRIGGER THRESHOLD**. Each subsequent scratch in the row is made at a force higher than the previous scratch by an amount corresponding to **THRESHOLD STEP**.

4. Set **CAPTURE** to **ENABLED** before executing the array if you want to store the results.

Figure 11.3c   Example Auto Panel for Automated Scratching

Auto Panel	
Columns:	3
Rows:	1
Column step:	500 nm
Row step:	0.00 nm
Threshold step:	0.100 V
Capture:	Off

5.   Click **Probe** > **AUTO SCRATCH** in the Scratch Mode menu to begin execution of the array.

> **Note:**   During execution, the force plot for each indent is displayed along with the (X,Y) coordinates of the indent in the array.

6.   Click **View** > **IMAGE MODE**, or click the Image icon (shown) to view the scratches just created.

11.4   Wear Testing

Wear tests are performed by simply scanning the sample in Contact Mode using indentation cantilevers (see Figure 11.4a). With their spring constants more than 100 times greater than standard Contact Mode imaging cantilevers, indentation cantilevers cannot be used to image in Contact Mode. Worn areas can be imaged afterward in TappingMode with the same indentation probe.

Figure 11.4a   Wear Testing in a Rectangular Area
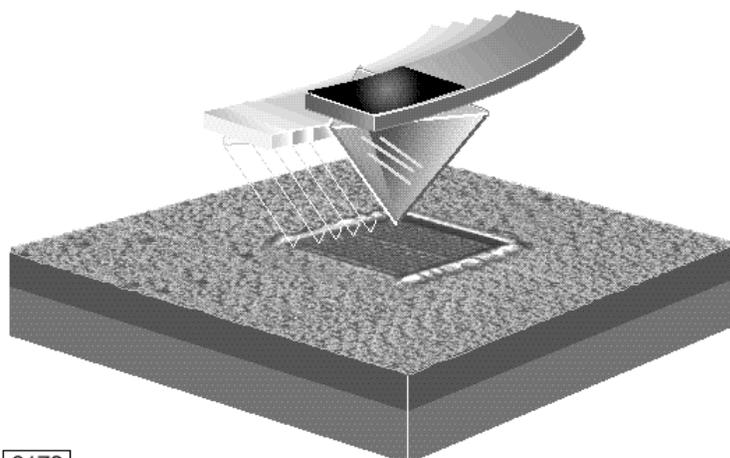
8173

The basic nanowear testing procedure follows:

1. Load the sample and indentation/scratching/wear probe into the SPM.

2. Align the laser on the reflector atop the cantilever so the reflected spot on the photodetector produces near-zero vertical and horizontal deflection signals.

3. Set RealTime **Scan Controls** and **Feedback Controls** imaging parameters to values appropriate for Contact Mode imaging. Set **SCAN SIZE** to `0.0` to prevent scanning/wearing initially, and set **DEFLECTION SETPOINT** within the range `0.3–0.5V` to minimize the engage force, while ensuring engagement. Set both the **INTEGRAL GAIN** and **PROPORTIONAL GAIN** to a value of `2.0`.

4. Manually lower the tip to near the sample prior to engaging.

CAUTION: The diamond tip extends 100μm beneath the underside of the cantilever. Focus on the top of the cantilever while positioning the probe.

5. Engage the surface by selecting the **RealTime** > **Motor** > **ENGAGE** command or the **ENGAGE** icon (shown).

6. Decrease the **DEFLECTION SETPOINT** by 1-2 volts to lift the tip off the surface. Check that the Z-piezo is retracted by looking at the image monitor. The z center position should move to the retracted side of the **Z Center Position** bar on the image monitor and the word "Limit" should appear in place of the Z center voltage value. It this does not occur, the Z-piezo is not fully retracted and the tip may still be on the surface. Decrease the **DEFLECTION SETPOINT** in 1V increments until the tip is retracted.

   Note: Engaging in Contact Mode using indentation cantilevers often produces a small dent at the initial point of contact. Offset to a fresh location on the sample to perform the test if you wish.

7. Set **SCAN SIZE** and **SCAN RATE** as desired for the wear test. Tip velocity equals **SCAN SIZE** times twice the **SCAN RATE**. Set **NUMBER OF SAMPLES** for the desired resolution; it determines the number of scan lines made during the test.

   Note: Keep **SCAN RATE**; **SCAN SIZE**; **NUMBER OF SAMPLES**; **INTEGRAL GAIN**; **PROPORTIONAL GAIN**, and **SETPOINT** fixed across all wear tests being compared.

8. Increase the **DEFLECTION SETPOINT** to achieve the desired applied force and immediately select **Frame** > **UP** or **Frame** > **DOWN** from the RealTime menu. As soon as the **DEFLECTION SETPOINT** is increased sufficiently, the tip returns to the surface and starts scratching.

   Note: The applied force also depends on the free-air vertical deflection, which is set to zero with the **ZERO SETPOINT** parameter (see **Basic Force Mode:** page 50). In general, cantilever deflection equals **DEFLECTION SETPOINT** minus the free-air **VERTICAL DEFLECTION**, in volts. **VERTICAL DEFLECTION** is displayed on the MultiMode base.

9. Click the **WITHDRAW** icon (shown) to stop wear scanning, for instance, at the end of a single scan,

     Note: If you choose to image the worn area after changing probes, take note of large nearby features for guidance in positioning the new tip over the worn area. The indentation probe may yield a low resolution image if the tip has picked up debris while scratching. First image with the indentation probe scanning in the same direction as wearing was performed to minimize the additional spread of debris. Increase the TappingMode **AMPLITUDE SETPOINT** while still tracking the surface and increase **INTEGRAL GAIN** to enhance image quality.

Chapter 12   Additional Signal Access

The PicoForce Force Spectroscopy Control Module provides access to some intermediate signals between the NanoScope Controller and the MultiMode which otherwise require a Signal Access Module (type I or III) to observe. In addition, a Signal Access Module may be used to input or output other waveforms to a MultiMode PicoForce system, so the more general method of signal access is described next, and the specific capabilities of the Control Module follow.

12.1   Introduction to Signal Access Modules

A Signal Access Module (SAM) enables monitoring of NanoScope SPM signals that are otherwise "internal." In addition, external signals can be introduced to the SPM for control or processing by connecting the signal sources through a SAM, also known as a *breakout box*.

The SAMIII (see Figure 12.1a) is the full-function SAM described next in this chapter. SAMIII interfaces twenty-five SPM signals and ten Application Module signals. Toggle switches enable switching each line separately between a normal, uninterrupted configuration and an external input signal. Output BNC connectors permit monitoring of both conditioned and uninterrupted signals.

SAM(I) provides the Top Panel functions of SAMIII (see Figure 12.1b) for access to the SPM, but not to Application Modules. For the Side Panel functions of SAMIII (see Figure 12.1c) in a separate module, access with its focus on Application Modules, there is the Signal Access Module Interface to Application Mode (SAM-I-AM). Each SAM is also detailed separately: *SAMIII*, Support Note 332; *NanoScope SAM—Description & Use*, Support Note 210; *NanoScope SAM-I-AM*, Support Note 285.

Figure 12.1a   Signal Access Module III; Labelling of Panels
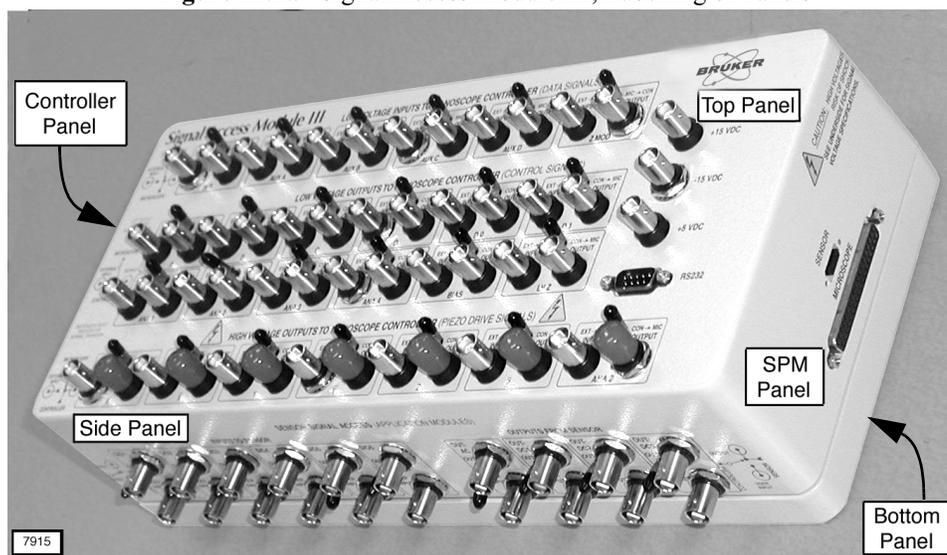
Figure 12.1b   SAMIII: Top Panel, Access to SPM Signals

Figure 12.1c   SAMIII: Side Panel, Access to Application Module Signals

12.2   Signals Available through SAMIII

Signal connectors are divided into five groups (see Figure 12.1a for top and side panel definitions):

- Low Voltage Inputs to NanoScope Controller (data signals) ⎫ Top Panel
- Low Voltage Outputs from NanoScope Controller (control signals) ⎬ (see
- High Voltage Outputs from NanoScope Controller (piezo drive signals) ⎭ Figure 12.1b)
- Low Voltage Inputs to Application Module ⎫ Side Panel
- Low Voltage Outputs from Application Module ⎭ (see Figure 12.1c)

The individual pairs of input and output signal connectors in the first three groups (above), those located on the top panel, are tabulated in Table 12.2a. In the bottom row, X and Y refer to scan position (i.e., MultiMode sample motion). Z refers to probe position with respect to the sample plane. A bar over a signal indicates the signal inverse (i.e., a sign change).

Table 12.2a   The Array of BNC Connectors as Labelled on the Top Panel of SAMIII

Low Voltage Inputs to NanoScope Controller (Data Signals)						
In0	AuxA	AuxB	AuxC	AuxD	Zmod	—
Low Voltage Outputs from NanoScope Controller (Control Signals)						
A	B	C	D	D0	D1	—
Ana1	Ana2 (LV)	Ana3	Ana4	Bias	LVZ	—
High Voltage Outputs from NanoScope Controller (Piezo Drive Signals)						
X	$\overline{X}$	Y	$\overline{Y}$	Z	$\overline{Z}$	Ana2 (HV)

Signal power limits are specified for SAM connections in Table 12.2b.

CAUTION: The X, Y and Z High Voltage Output (see Table 12.2b) signals are used to drive the piezoelectric elements in scanners and can be as large 220V. Observe the explicit precautions emphasized in detailed documentation of SAM (either *SAMIII*, Support Note 332; *NanoScope SAM—Description & Use*, Support Note 210 or *Access to Intermediate NanoScope IV Signals* in *The NanoScope IV Controller Manual*).

Table 12.2b   Signal Access Module Connector Ratings

ACCESS	SIGNAL	MAX. VOLTAGE	MAX. CURRENT	SIGNAL TYPE
SPM	In0	± 10 VDC	10 mA	Analog Input
SPM	AuxA	± 10 VDC	10 mA	Analog Input
SPM	AuxB	± 10 VDC	10 mA	Analog Input
SPM	AuxC	± 10 VDC	10 mA	Analog Input
SPM	AuxD	± 10 VDC	10 mA	Analog Input
SPM	Zmod	± 10 VDC	10 mA	Analog Input
SPM	A	± 5 VDC	500 mA	Open Collector Logic (OCL)
SPM	B	± 5 VDC	500 mA	OCL Output
SPM	C	± 5 VDC	500 mA	OCL Output
SPM	D	± 5 VDC	500 mA	OCL Output
SPM	D0	± 5 VDC	n/a	Transistor-Transistor Logic (TTL)
SPM	D1	± 5 VDC	n/a	TTL Output
SPM	Ana1	± 10 VDC	10 mA	Analog Output
SPM	Ana2	± 12 VDC	10 mA	Analog Output

ACCESS	SIGNAL	MAX. VOLTAGE	MAX. CURRENT	SIGNAL TYPE
SPM	Ana3	± 10 VDC	10 mA	Analog Output
SPM	Ana4	± 10 VDC	10 mA	Analog Output
SPM	Bias	± 10 VDC	10 mA	Analog Output
SPM	LV Z	± 12 VDC	10 mA	Analog Output
SPM	X	± 220 VDC	70 mA	High Voltage Output
SPM	X	± 220 VDC	70 mA	High Voltage Output
SPM	Y	± 220 VDC	70 mA	High Voltage Output
SPM	Y	± 220 VDC	70 mA	High Voltage Output
SPM	Z	± 220 VDC	70 mA	High Voltage Output
SPM	Z	± 220 VDC	70 mA	High Voltage Output
SPM	Ana2 (HV)	± 220 VDC	70 mA	High Voltage Output
App. Mod.	OUT-AC	± 10 VDC	10 mA	Analog Input
App. Mod.	OUT -DC1	± 10 VDC	10 mA	Analog Input
App. Mod.	OUT-DC2	± 10 VDC	10 mA	Analog Input
App. Mod.	OUT-DC3	± 10 VDC	10 mA	Analog Input
App. Mod.	SIG1	± 10 VDC	10 mA	Analog Output
App. Mod.	SIG2	± 10 VDC	10 mA	Analog Output
App. Mod.	SIG3	± 10 VDC	10 mA	Analog Output
App. Mod.	SIG4	± 10 VDC	10 mA	Analog Output
App. Mod.	SIG5	± 10 VDC	10 mA	Analog Output
App. Mod.	SIG6	± 10 VDC	10 mA	Analog Output
General	+15 VDC	—	500 mA	Power Supply
General	-15 VDC	—	500 mA	Power Supply
General	+ 5VDC	—	500 mA	Power Supply

SAMIII is always connected, either directly or indirectly, to an SPM via a D-37 type cable attached to SAMIII through the connector labelled **MICROSCOPE**. SAMIII is always connected, either directly or indirectly, to an SPM controller via another D-37 type cable attached to SAMIII through the connector labelled **NANOSCOPE CONTROLLER**.

Different signals are available depending on whether SAMIII is inserted in the system along the path of Cable A, B or C (see Figure 3.2f with its Options 1-3). Table 12.2c lists the available signals with SAMIII at each of the three cable positions with an extended MultiMode and Extender in Contact Mode operation. Table 12.2d lists SAMIII-accessible signals in an identical system configuration (i.e. Option 2), but operated in TappingMode. Table 12.2e and Table 12.2f provide the corresponding information for an extended MultiMode with Quadrex (i.e., Option 3) operating in Contact and TappingMode respectively.

> **Note:** In terms of signals available via SAMIII, Option 1, with the NanoScope IV Controller (and no Cable C), is a subset of Option 3 (i.e., with SAMIII in the Cable A and B paths) because Quadrex is included in the NSIV Controller.

> **Note:** "Filtered Deflection" is the signal used by the Thermal Tune algorithm. It is the **DEFLECTION** signal AC coupled and amplified (gain approximately 20).

Table 12.2c   Intermediate Signals: Contact Mode, MultiMode PicoForce System with Extender

Signal	Cable A	Cable B	Cable C
I**N**0	**Deflection (1MHz)**	**Deflection (1MHz)**	Deflection (1.6kHz)
A**UX** A	LFM	LFM	Handshake
A**UX** B	—	**Z-sensor Position**	**Z-sensor Position**
A**UX** C	—	Filtered Deflection	Filtered Deflection
A**UX** D	—	**Mod. LVZ**	LFM or **Mod. LVZ**

Table 12.2d   Intermediate Signals: TappingMode, MultiMode PicoForce System with Extender

Signal	Cable A	Cable B	Cable C
I**N**0	**Amplitude (1MHz)**	**Amplitude (1MHz)**	RMS Amplitude
A**UX** A	LFM	LFM	Handshake
A**UX** B	—	**Z-sensor Position**	**Z-sensor Position**
A**UX** C	—	Filtered Deflection	Filtered Deflection
A**UX** D	—	**Mod. LVZ**	Phase or TM Deflection

Table 12.2e   Intermediate Signals: Contact Mode, MultiMode PicoForce System with Quadrex

Signal	Cable A	Cable B	Cable C
I**N**0	**Deflection (1MHz)**	**Deflection (1MHz)**	Deflection (10kHz) Minus Setpoint
A**UX** A	LFM	LFM	LFM
A**UX** B	—	**Z-sensor Position**	**Z-sensor Position**
A**UX** C	—	Filtered Deflection	Filtered Deflection
A**UX** D	—	**Mod. LVZ**	**Mod. LVZ**

Table 12.2f   Intermediate Signals: TappingMode, MultiMode PicoForce System with Quadrex

Signal	Cable A	Cable B	Cable C
I**N**0	**Deflection (1MHz)**	**Deflection (1MHz)**	RMS Amplitude
A**UX** A	LFM	LFM	Handshake
A**UX** B	—	**Z-sensor Position**	**Z-sensor Position**
A**UX** C	—	Filtered Deflection	Filtered Deflection
A**UX** D	—	**Mod. LVZ**	Phase or TM Deflection

Note: The signal names shown highlighted in Table 12.2c through Table 12.2f are available from the PicoForce Control Module directly, without a SAMIII (see the next section).

Note: Refer to the first three columns of any of Table 12.2c through Table 12.2f for PicoForce operation with an NSIV Controller (Option 1 in **Hardware Configuration, page 13**).

12.3 Signals Available via the PicoForce Control Module

The front panel BNC connections of the PicoForce Force Spectroscopy Control Module (see Figure 5.1a) provide some "internal" signals otherwise only accessible via a signal access module such as SAM or SAMIII. Table 12.3a maps PicoForce Control Module front panel connectors to their signals.

Table 12.3a   MultiMode PicoForce Control Module Output Signal Access

Front Panel BNC	Signal	Description
Deflection Output	Deflection (1MHz)	Unfiltered cantilever deflection signal
Sensor Output	Z-sensor Position	Probe tip location, to/from sample
Modified LV Output	Mod. LVZ	The LVZ signal from the NanoScope controller plus the PicoAngler/User Input offset.
Manual Offset Output (with switch on **ANGLER OUTPUT**)	Knob offset	The offset to Z-axis actuation supplied by turning the PicoAngler Z-position knob
MANUAL OFFSET OUTPUT (with switch on **USER INPUT**)	Whatever is present on the **MANUAL OFFSET INPUT** BNC.	You may input a signal and observe it. Input signals should conform to the ratings specified in Table 5.1a.

Chapter 13   Maintenance and Support

13.1   System Maintenance

Refer to the Maintenance chapter of the *MultiMode Scanning Probe Microscope Instruction Manual* for MultiMode-specific issues.

MultiMode PicoForce does not require periodic maintenance if always operated at a room temperature comfortable to the operator.

⚠️	**CAUTION:**	Do not obstruct the cooling fans in the PicoForce Force Spectroscopy Control Module, nor in the NanoScope Controller (whether NSIIIa, NSIV(a) or NSV)

13.2   Troubleshooting: General

Additional troubleshooting tips particular to the MultiMode SPM subsystem can be found in the *MultiMode Scanning Probe Microscopy Instruction Manual*.

13.2.1   Fuses and Power Supply

If there is any indication of a lack of power to the instrument, especially failure of the top left light on the front panel of the PicoForce Control Module to illuminate when power is turned on, a fuse may be blown. Two numbered fuses are located toward the left of the back panel of the PicoForce Control Module (see Figure 5.2a). To replace a fuse, complete the following:

1. Toggle the **POWER** switch **OFF**, and unplug the PicoForce Control Module.

2. Replace any blown fuse(s) with the proper replacement as indicated in the table below and to the right of the fuses, and reproduced in Table 13.2a.

    Note: Type "T" indicates time delay, equivalent to "Slow Blow."

Table 13.2a PicoForce Control Module Fuse Ratings

Fuse	100V	120V	240V
1	800mA	800mA	400mA
2	800mA	800mA	400mA
ALL FUSES: 250V TYPE "T" SLOW BLOW			

Note: The appropriate fuse depends on the supply voltage in use: 100V, 120V or 240V. The maximum power drawn by the PicoForce Control Module as a function of supply voltage is listed in a table on the back panel of the module (see Figure 5.2a, page 28) and reproduced in Table 13.2b.

Table 13.2b AC Input Power Factors (Voltage and Current): Three Options

Voltage	Current	Frequency
100VAC	700mA	
120VAC	600mA	50 - 60 Hz
240VAC	300mA	

3. Reconnect Module power and toggle the **POWER** switch **ON**.

13.2.2 No Sensor Signal or Z-Feedback in Force Mode

Make sure the serial communication cable is installed (see **Hardware Configuration,** page 13, and Figure 3.2f, cable "D"). If you are using an NSIV Controller, make sure the PicoForce Sensor Module Jumper (dongle) is connected to the **SENSOR MOD.** connector on the back of the NSIV (see **Hardware Configuration,** page 13). If you are using a Quadrex Module with an NSIIIA Controller, make sure the PicoForce Sensor Module Jumper (dongle) is connected to the **APPLICATIONS MODULES.** connector on the back of the Quadrex Module (see **Hardware Configuration,** page 13).

13.3   Troubleshooting: Pulling Applications

13.3.1 MultiMode Sum Signal Goes to Zero During a Force Plot

The **Sum** display on the base of the MultiMode measures the intensity of the reflected laser light falling on all four quadrants of the photodetector. A high **Sum** signal is the criterion for laser alignment initially in preparing the optical lever for use with a given installed probe. Loss of the **Sum** signal indicates either an unsecured probe in the probeholder, a cantilever deflection so large

that the reflected laser beam falls outside the photodetector or a damaged probe, possibly due to one of the first two causes going undetected.

⚠️ 🔺	**CAUTION:**	If a relative trigger has been set and the photodetector is not accurately recording cantilever deflection, the probe may be driven into the sample without being triggered to stop.	
		Stop the force plot by clicking the **WITHDRAW** icon (shown) or by manually retracting the tip by turning The Angler knob counterclockwise.	

13.3.2 Probe Tip Stuck to Sample Surface

A low spring constant cantilever tapping in air can get stuck in the thin water layer on a sample surface as detected by its tapping amplitude dropping to zero suddenly upon reaching the surface. While a higher **DRIVE AMPLITUDE** may free the tip, at least a higher **AMPLITUDE SETPOINT**, if not a higher **DRIVE AMPLITUDE** is needed to keep the tip from getting stuck again. The higher impact force may not be acceptable either to the sample if it is fragile or to the tip if the sample is particularly hard. A stiffer cantilever may be necessary to resist the pull of the water.

Low spring constant contact mode probes may encounter the same difficulty: excessive cantilever deflection (more than the **DEFLECTION SETPOINT**) required to lift the probe off the sample surface. Switching probes is simplest for standard silicon nitride probes (Model NP) where the same substrate supports two V-shaped probes, one 200μm long and a stiffer one at half the length.

13.3.3 Deflection Signal Clipped at Top of Plot

If the deflection signal goes offscale (the top plot of three in Force Mode, see Figure 8.4a for RealTime Mode or Figure 9.0c for Image Processing Mode), it may be just as it appears: the cantilever is deflected beyond the range of the photodetector. The photodetector range, governed by the parameter **DEFLECTION LIMIT**, can, on an NanoScope IIIa controller, be set to either of two values: `20V` or `2.5V`. The **DEFLECTION LIMIT** range for a NanoScope IV(a) is `0V –20V`. The **DEFLECTION LIMIT** range for a NanoScope V is `4.096V – 24.58V`. Deflections are typically more restricted while imaging, so a smaller **DEFLECTION LIMIT** value is preferred in imaging because the same precision applied to a smaller range yields higher resolution. In Force Mode, larger deflections are expected and lower resolution is tolerated. Check that in switching from imaging to Force Mode, the Scan View > Other Tab > **DEFLECTION LIMIT** was not left at the lower value.

Another possible source of an offscale deflection signal plot is the choice of plot scaling employed.

1. Click in the plot to open the functions menu (see Figure 8.4c).

2. Click **SCALE** to open the **Scale** panel (see Figure 13.3a).

3. For manual scaling, make sure **AUTO SCALE** is not checked and enter a value for Vertical Axis **RANGE** and a selection for Center of Range to accommodate the full range of the deflection signal.

4. And, for the simplest resolution of the problem: check **AUTO SCALE**.

Figure 13.3a   The Scale Panel
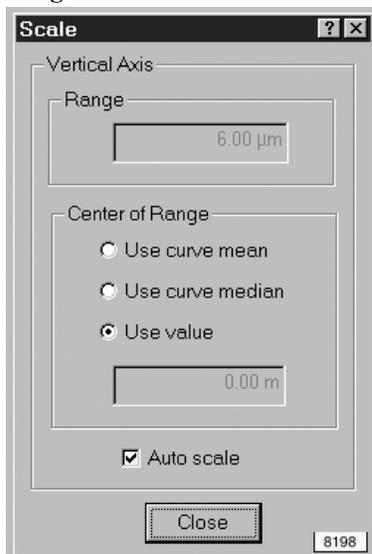
13.3.4 Problems with Thermal Tune

Baseline Not Flat

If the baseline is not flat, adjust the photodetector until the vertical deflection is approximately 0 V.

Sharp Noise Spike at 20 kHz

If there is a sharp noise spike near 20 kHZ, adjust the photodetector until the vertical deflection is approximately 0 V.

Sharp Noise Spike at 15 kHz

A sharp noise spike at approximately 15 kHz sometimes appears if the Sony video monitor that is commonly provided with he OMV option is turned on.Turn off the Sony monitor before clicking **GET DATA**.

13.4   Troubleshooting: Pushing Applications

13.4.1 No Sign of Indentation or Scratch

The force needed to deform a surface increases as the tip becomes duller (as a result of wear). To increase the force applied during pushing applications, increase the **TRIGGER THRESHOLD** in

0.05V (for scratching) or 0.1V to 0.2V increments (for indenting). Generally, a **TRIGGER THRESHOLD** < 0.2V is insufficient to leave a trace, while a **TRIGGER THRESHOLD** of 1.0V is sufficient. Indentations and scratches should be visible imaged with a **SCAN SIZE** between **1** and **3ᴜᴍ**.

13.4.2 Contaminated Indentation Probe

All pushing applications have the potential to produce debris, some of which may become attached to the tip. A debris-encrusted tip can create irregularly shaped dents, may require higher force to achieve the same pressure as a sharper tip and reduces imaging resolution. Comparing image resolution of a site before and after pushing provides a good indication of tip contamination during pushing.

To clean an indentation tip, perform several indentations at the same location, limiting the **TRIGGER THRESHOLD** to **0.5V** on hard samples (such as Diamond-Like Carbon, DLC) or to **2V** on soft samples (such as the 1um pitch grating gold sample provided). If these actions fail to improve image resolution, refer to the troubleshooting section of *Support Note 225, Nanoindentation and Nanoscratching with SPMs for NanoScope Version 4.32 Software* for more forceful measures.

13.5 Thermal Tune Filter Calibration

13.5.1 Purpose

The PicoForce Thermal Tune Filter Calibration procedure is done to set the Filter Gain in the NanoScope Controller into alignment with the Picoforce Controller. This procedure is needed when a new PicoForce system is setup, if PicoForce Controllers are changed, if Extender/Quadrex/NS IV are changed, or if the Scanner Calibration file is lost.

> **Note:** Thermal Tune Filter Calibration of NanoScope V Controllers is not discussed here and should be performed only by Bruker personnel.

13.5.2 Passband Gain Calibration

Purpose

This procedure connects TappingMode Drive line (Ana 1) to the deflection line (In0) going into the PicoForce controller and the In1B line going out of the PicoForce controller using the PicoForce Passband Gain Dongle (PPGD) (P/N 468-006-100). When the PPGD is connected between the PicoForce controller and the Extender or Quadrex (for NS3a) or the microscope connector of the NSIV, the PPGD allows the calibration of the passband gain of the filtered deflection line (In1C) relative to the In1B line. The expected **FILTER GAIN** values are ~20.

Figure 13.5a   PicoForce Passband Gain Dongle (PPGD

Procedure

1. With PicoForce Controller off and Nanoscope Controller off, connect PPGD to the PicoForce Controller Nanoscope Input Connector

2. Connect 37 Pin Monster Cable to other end of PPGD. Disconnect the cable to the MultiMode Microscope from the back of the Picoforce Controller.

Figure 13.5b   PicoForce Passband Gain Dongle In Situ
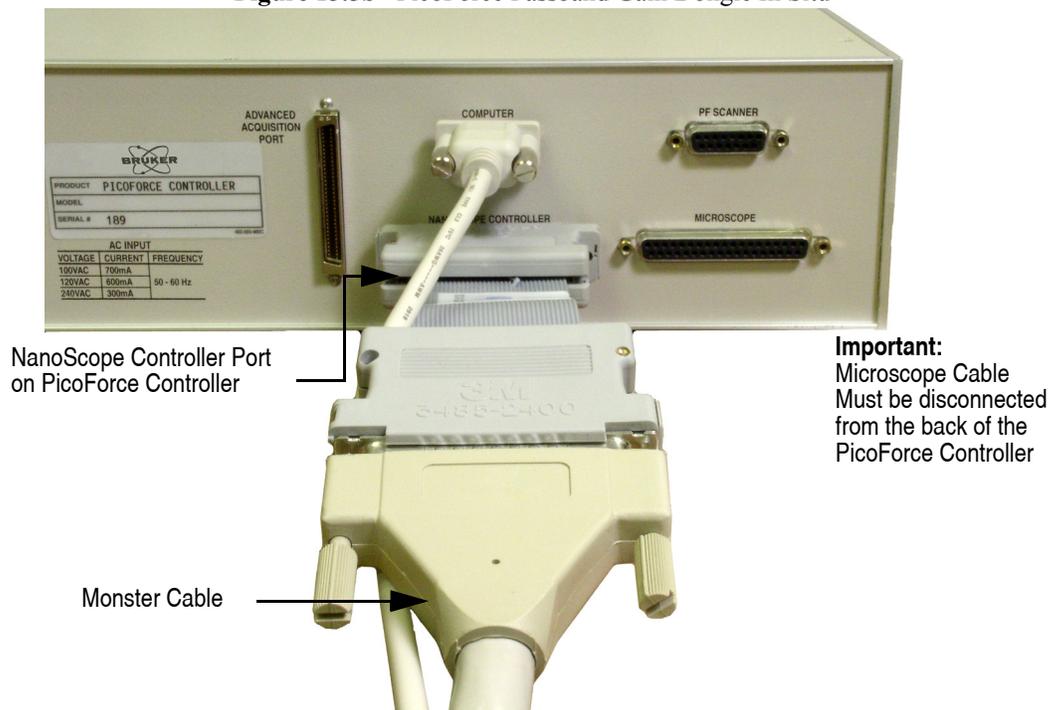
3.   Turn on the PicoForce Controller and the NanoScope Controller

4. Using Version 6 NanoScope software, enter the PicoForce workspace and open the **Thermal Tune** window, shown in Figure 13.5c.

Figure 13.5c   Thermal Tune Window
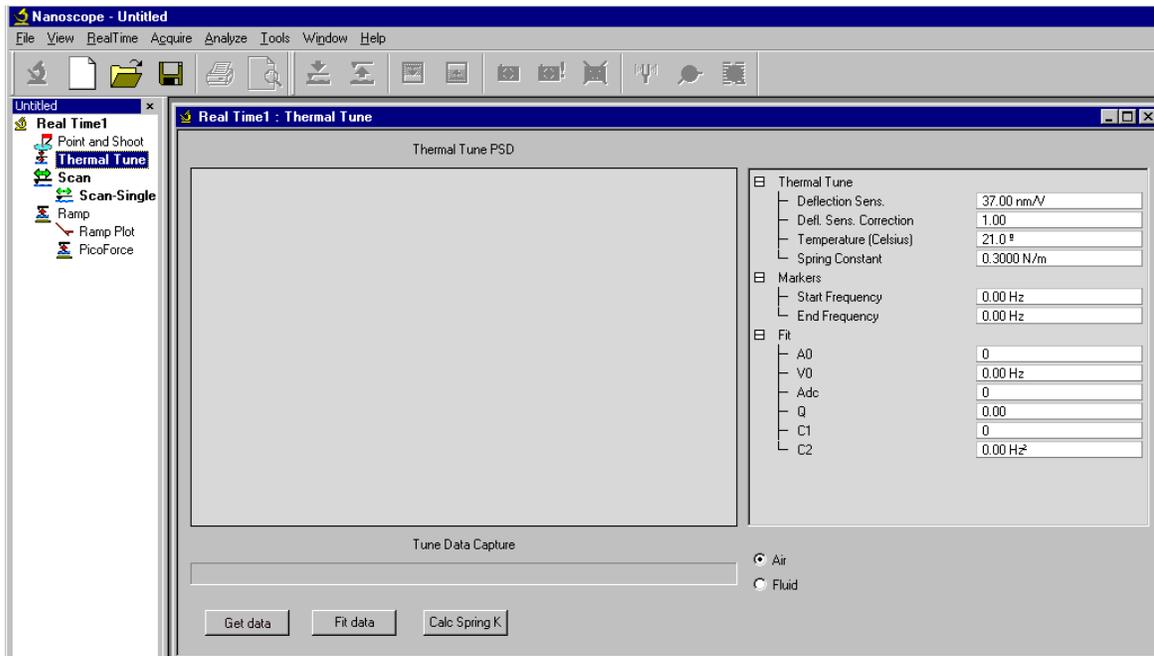
5. Click **TOOLS** > **RESET CONTROLLER**.

6. Enter the Classic mode by pressing **CTRL ALT L**.

7. Open the **System Debug** window, shown in Figure 13.5d, by pressing **ALT ~**.

**Figure 13.5d** System Debug Window
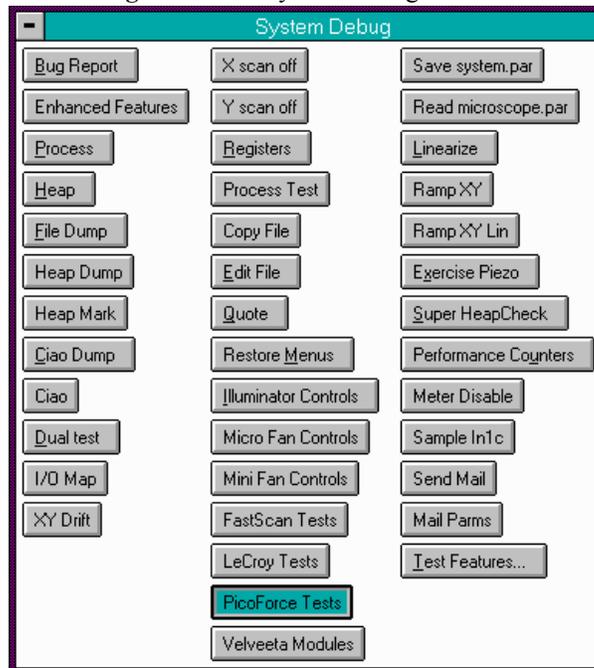
8. Select **PicoForce Tests**, shown in Figure 13.5e.

Figure 13.5e PicoForce Test Window
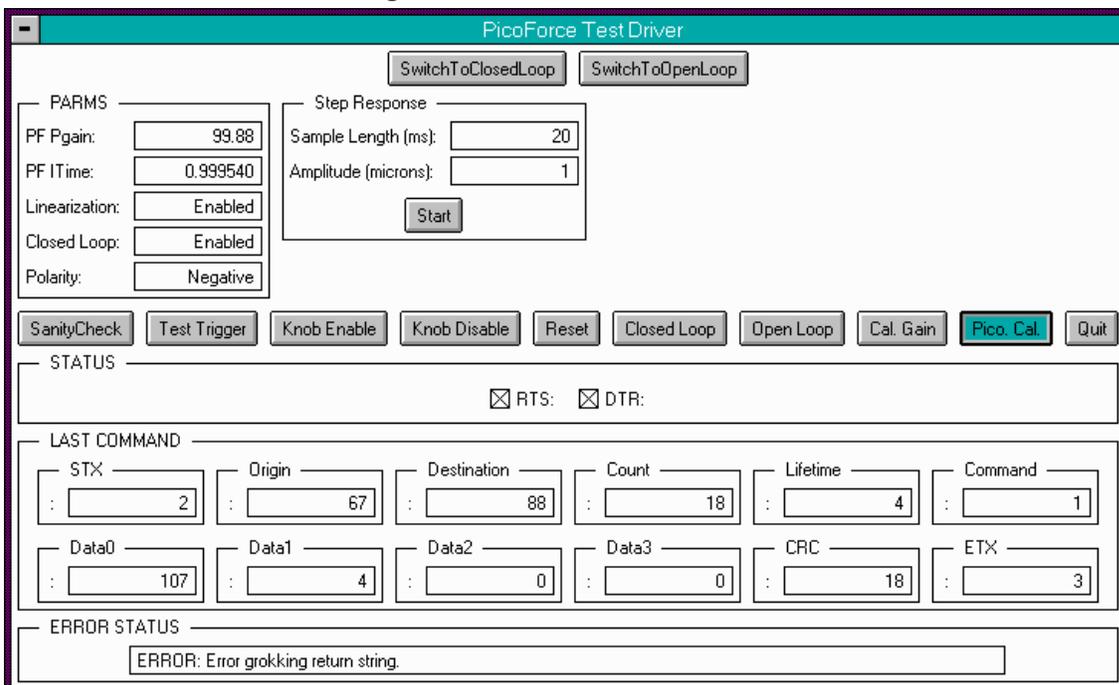
9. Select **Pico Cal**, shown in Figure 13.5f.

Figure 13.5f PicoForce Calibration Window
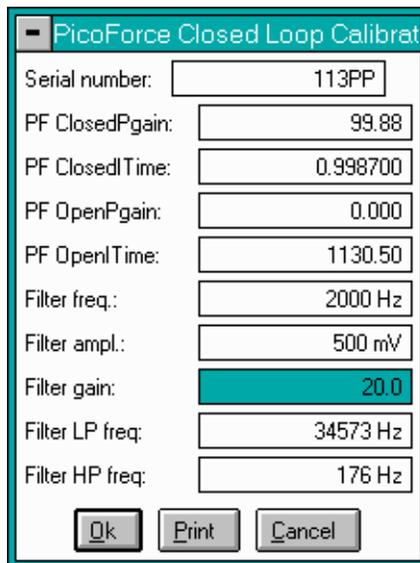
10. Check the filter parameters:

    • **FILTER FREQ**: `2000 Hz`

    • **FILTER AMPL**: `500 mV`

    • **FILTER GAIN**: `20`

    • **FILTER LP FREQ**: `34573 Hz`

    • **FILTER HP FREQ**: `176 Hz`

11. Click the **CAL. GAIN** button in the PicoForce Test Driver window, shown in Figure 13.5e. This calculates a new **FILTER GAIN**.

12. Accept the prompt to place the PPGD.

13. The NanoScope software will automatically false engage for 30 seconds, after which, it will prompt you to accept the newly measure Gain value: **RAW DEFLECTION GAIN IS ##.#, USE THIS VALUE?**

14. Click **OK** to accept the new **GAIN** value if it lies between 18 and 22.

15. If the **GAIN** lies outside this range, click **CANCEL** and then click **CAL. GAIN** again.

16. Perform steps 11 through 15 several times to verify that the **GAIN** does not vary by more than ±0.1. It is likely that first two measured **GAINS** differ from subsequent, stable values.

17. Return to the **PicoForce Calibration** window, Figure 13.5f, and verify that the **FILTER GAIN** is the value measured in step 14.

18. Leave the Classic mode by pressing **CTRL ALT L**.

13.5.3 Deflection Gain Calibration

Purpose

This procedure connects the Ana 3 line to the Deflection line (In0) and the In1B line with the PicoForce Deflection Gain Dongle (PDGD) (468-006-101).When connected to the microscope side of the Extender or Quadrex (for NS3a) or the Microscope Connector of the NSIV, the PDGD allows the calibration of the gain on the deflection line relative to the In1B line. The expected ratio is approximately 0.8.

Procedure

1. Unplug the PicoForce Passband Gain Dongle (PPGD) (P/N 468-006-100) from the PicoForce Controller and the NanoScope Controller.

Figure 13.5g   PicoForce Deflection Gain Dongle (PDGD) (468-006-101)

2. Plug the monster cable into the Extender or Quadrex.

3. Plug the PicoForce Deflection Gain Dongle (PDGD) (468-006-101) into the Extender, Quadrex or NS IV Controller To Microscope cable connector. See Figure 13.5h.

Figure 13.5h   PicoForce Deflection Gain Dongle (PDGD) In Situ

4. False Engage the microscope: **REAL TIME > FALSE ENGAGE**. See Figure 13.5i.

Figure 13.5i   False Engage
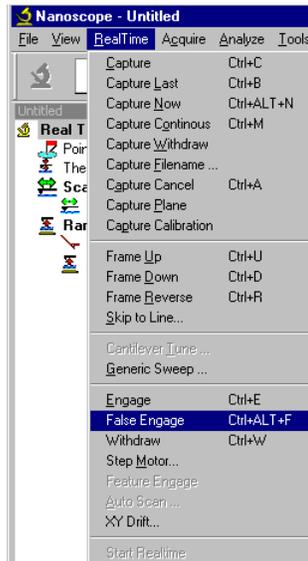
5. Set the **DEFLECTION SETPOINT** to `0V`, shown in Figure 13.5j.

Figure 13.5j DEFLECTION SETPOINT = `0V`
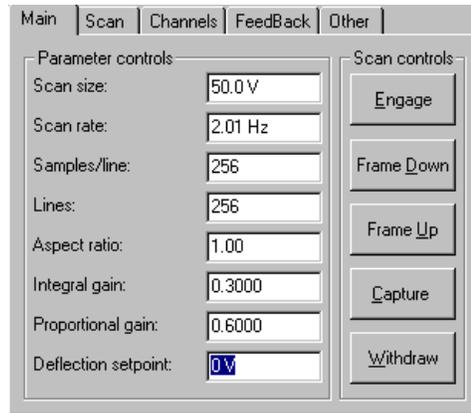
6. Switch to **Force Mode** and set CHANNEL 1 to DEFLECTION and CHANNEL 2 to Z SENSOR. See Figure 13.5k.

Figure 13.5k   Force Mode Window
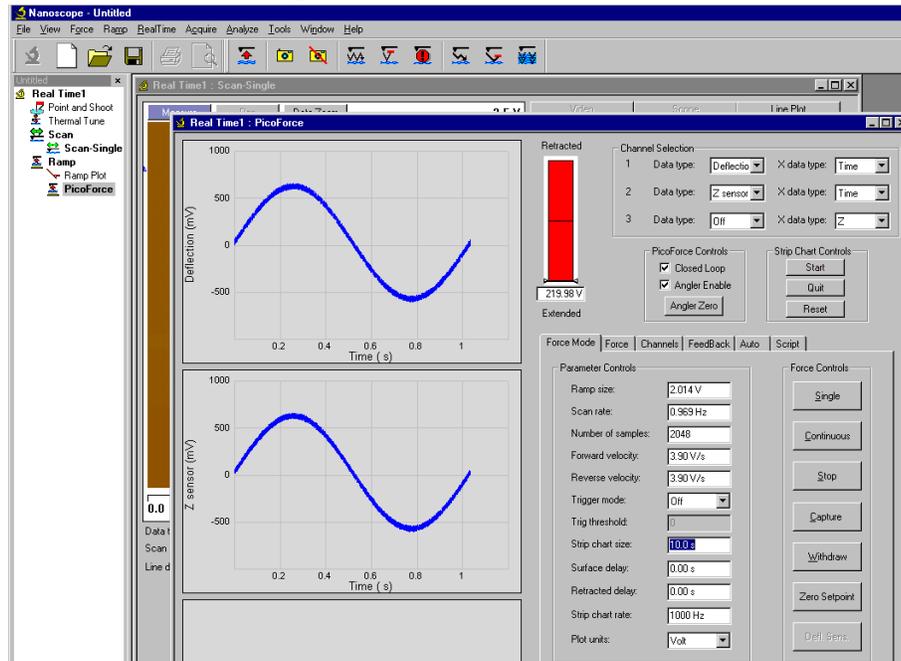
7. In the **Force Mode** window, set the PLOT UNITS to VOLTS, the STRIP CHART RATE to 1000Hz and the STRIP CHART SIZE to 10.0s. See Figure 13.5l.

Figure 13.5l Force Mode Tab
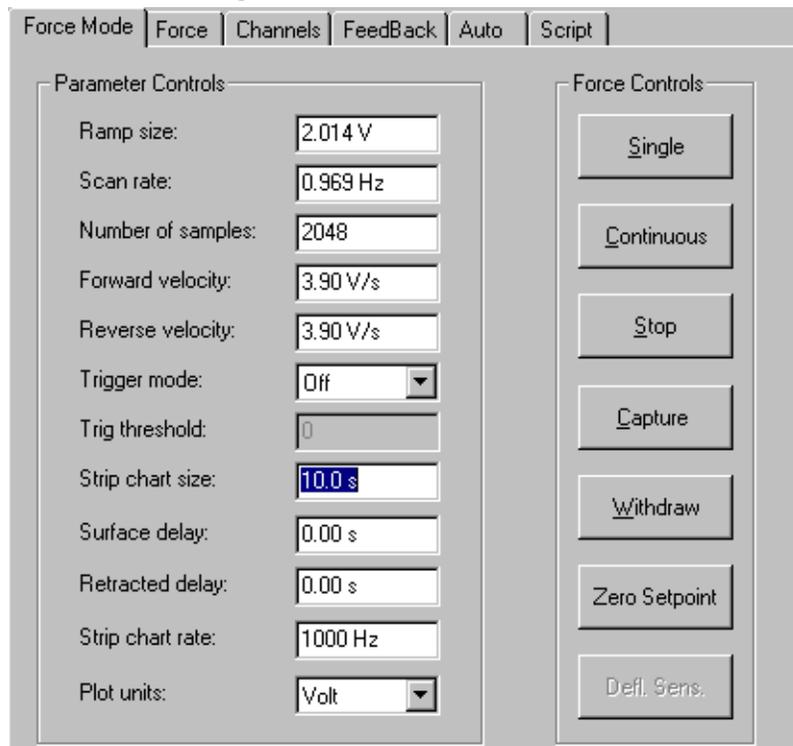
8. In the **Feedback** tab, set the **DEFLECTION LIMIT** to **20V**. See Figure 13.5m.

Figure 13.5m **Feedback**: **DEFLECTION LIMIT** = **20V**
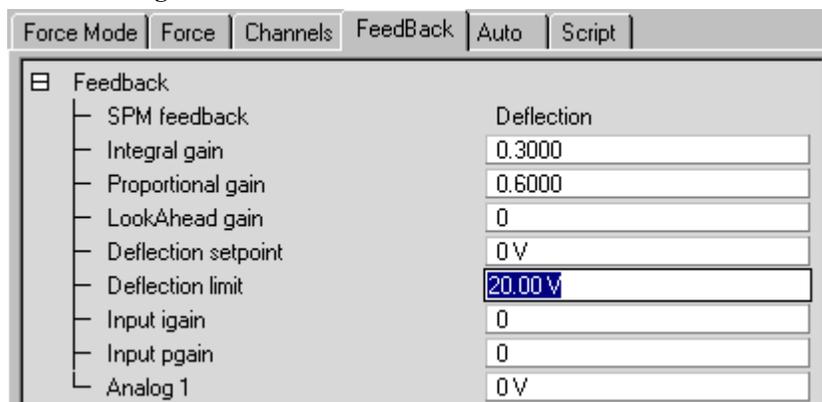
9. Enter the Classic mode by pressing **CTRL ALT L**.

10. Open the **System Debug** window, shown in Figure 13.5n, by pressing **ALT ~**.

Figure 13.5n System Debug Window
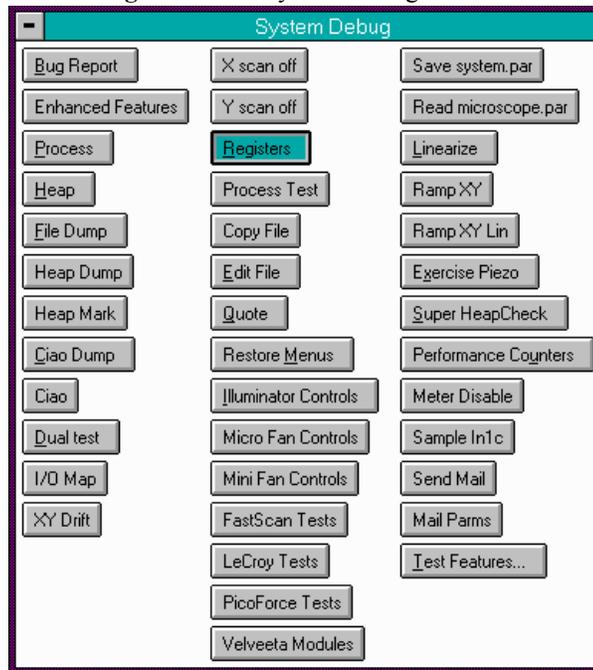
11. Select **Registers**, shown in Figure 13.5e and set **ANA3** to **7AAA**, shown in Figure 13.5o

Figure 13.5o PicoForce Registers Window
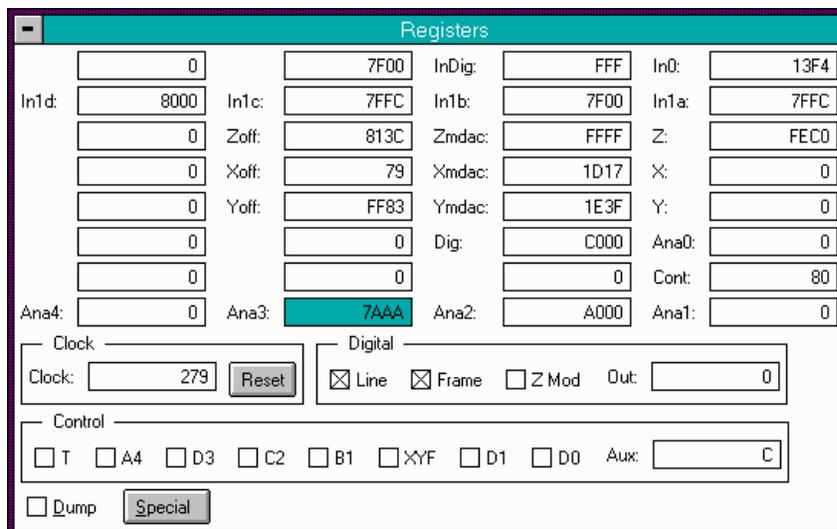
12. Return to the **Force Mode** and click **START** in the **Strip Chart Controls** area. See Figure 13.5p.

Figure 13.5p   Force Mode Window with Running Strip Charts on Channels 1 and 2.
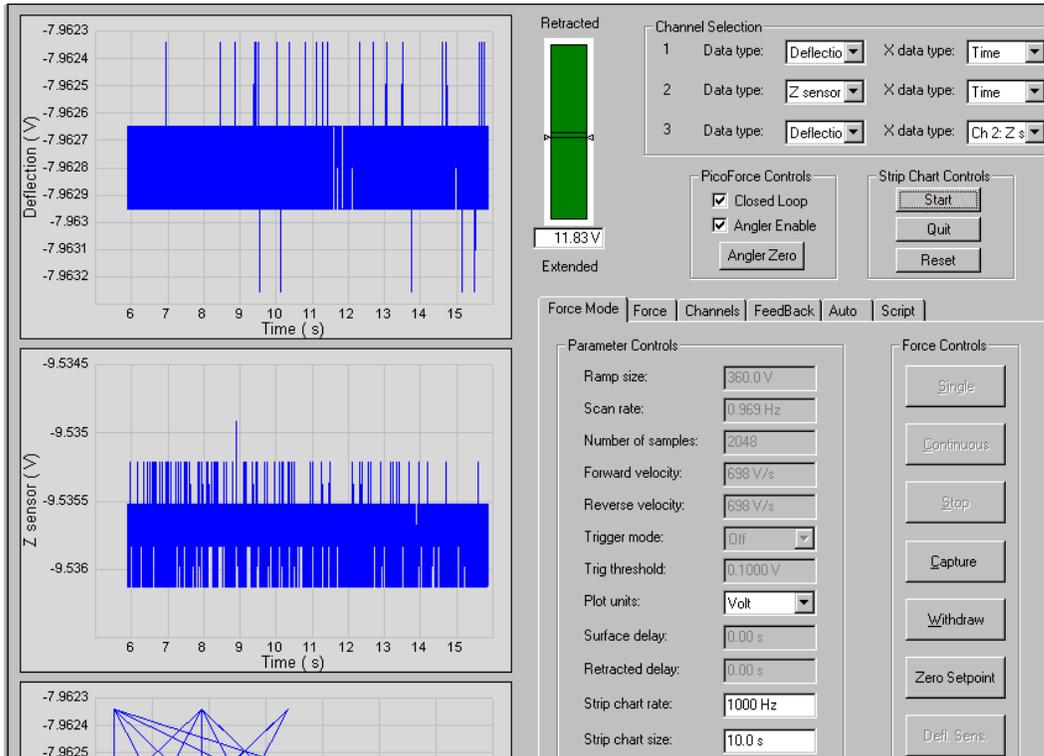
13. Divide the average value of the **Z SENSOR** by the average value of the **DEFLECTION** estimated from the Strip Charts and record the absolute value. The absolute value should be approximately 1.2. For the example shown in Figure 13.5q, <Z sensor> is -9.5357V and <Deflection> is -7.628V giving a ratio of 1.24.

Figure 13.5q   Running **Strip Charts** with **DEFLECTION** on Channel 1 and **Z SENSOR** on Channel 2
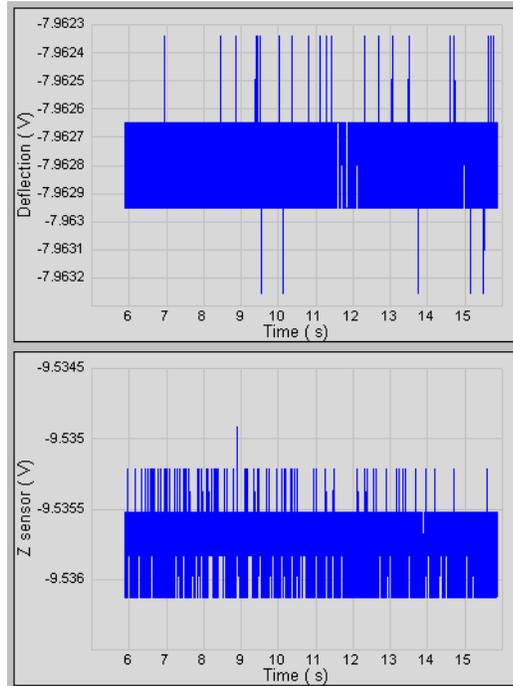
14. Enter the Classic mode by pressing **CTRL ALT L**.

15. Open the **System Debug** window, shown in , by pressing **ALT ~**.

Figure 13.5r   System Debug Window
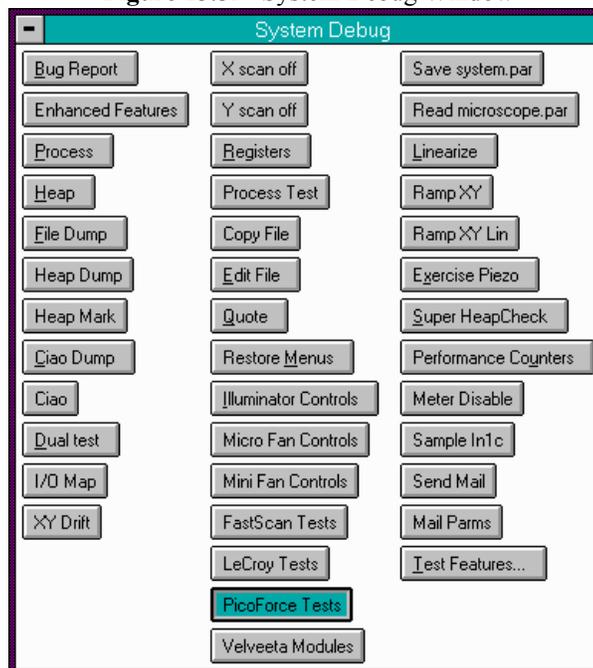
16. Select **PicoForce Tests**, shown in Figure 13.5s.

Figure 13.5s PicoForce Test Window
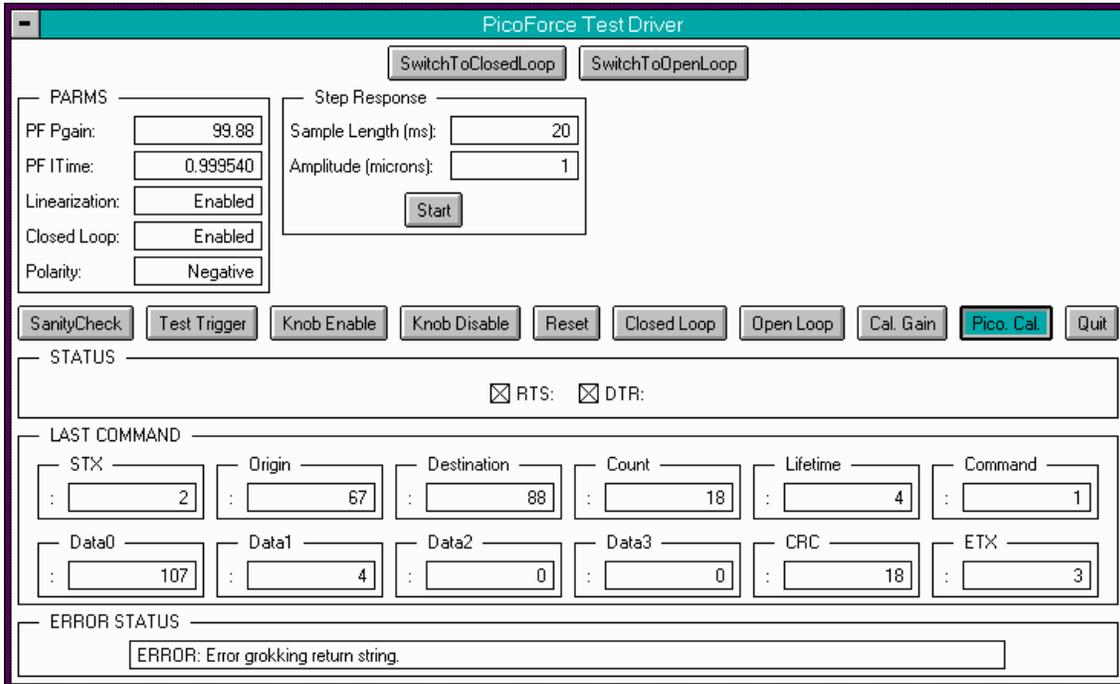
17. Select **Pico Cal**, shown in Figure 13.5t. Multiply the **FILTER GAIN** measured in Step 13 by the value in the **FILTER GAIN** field. Enter this number, typically 22-26, in the **FILTER GAIN** field and click **OK**.

Figure 13.5t PicoForce Calibration Window
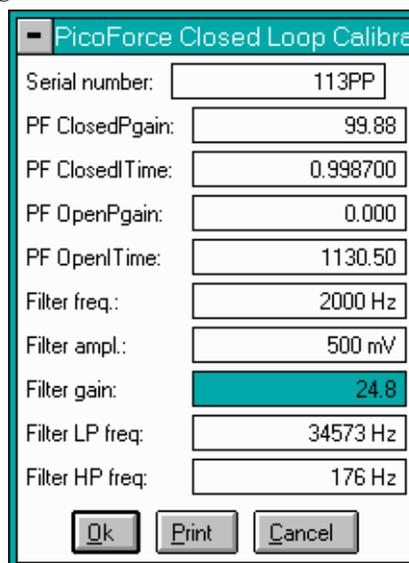
18. Leave the Classic mode by pressing **CTRL ALT L**.

13.6   MultiMode PicoForce Specifications

The key to precise picoscale force measurement is the closed loop Z-axis control of the scanner. The key to thermal noise based calibration of a cantilever spring constant is other noise sources contributing less Z-position uncertainty than do thermal perturbations.

Additional MultiMode SPM specifications apply and can be found in the *MultiMode Scanning Probe Microscopy Instruction Manual*.

Table 13.6a   MultiMode PicoForce System Characterization

Parameter	Specification
Scan Size in XY	greater than $40 \times 40\mu m$
Scan Size in Z	20µm
Z-axis nonlinearity	less than 0.2%
Deflection Signal Noise: free triangular cantilever with 30pN/nm spring constant at room temperature in fluid	less than 0.5nm RMS
Input impedance of **MANUAL OFFSET USER INPUT**	1M¾

For specification of the signals available through BNC connectors on the front panel of the PicoForce Control Module, and appropriate cautions when accessing them, see Table 5.1a, page25, and the immediately preceding text.

Index