User Guide

004-1028-000

Document Revision History: BioScope Catalyst User Guide

Revision	Date	Section(s) Affected	Ref. DCR	Approval
D	2/12/2010	14.1.1, 14.2		Vinson Kelley
C	1/20/2010	NanoScope 8.10 revisions	N/A	Vinson Kelley
B	6/5/09	Update Chapter 5	N/A	D. Paszkeicz
A	5/15/09	New Manual	N/A	D. Paszkeicz

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Chapter 1 User Guide Overview

Volume 1, the *BioScope Catalyst User Guide* is meant to be read thoroughly by new users. Read in this fashion, it should take approximately 1-2 hours to complete. It provides information about a variety of topics that are essential to understand in order to operate the BioScope Catalyst safely and efficiently. Topics are covered at an introductory level in Volume 1, with frequent references to other areas in the manuals where users can find more in depth information. Volume 1 is meant to be used in conjunction with Volumes 2, 3, 4 & 5.

Volume 2 is the *BioScope Catalyst Experiment Guide*. Volume 2 is meant to help users learn how to run various microscope modes and applications with details on theory, hardware and probe selection, sample preparation, and detailed instructions.

Volume 3 is the *Nanoscope Software Version 8.00 User Guide*. This Volume includes specifics on how to run the BioScope Catalyst software.

Volume 4 is the *BioScope Catalyst Service and Support Guide*. This Volume provides details on facilities requirements for installation, calibration, maintenance and troubleshooting. This guide is invaluable for preparing your facility to receive a BioScope Catalyst.

Lastly, Volume 5 is the *MIRO 2.0 Microscope Image Registration and Overlay User Guide*. MIRO allows for the registration of optical and AFM images.

Taken together, these 5 volumes provide pertinent information to enable the BioScope Catalyst user to confidently run the microscope, while also obtaining great results.

Conventions and Definitions

- 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**.
- NSV is used to refer to a NanoScope V Controller.

Chapter 2 Safety

This chapter details BioScope Catalyst safety. This chapter is meant to detail the symbols and safety requirements of the BioScope Catalyst. Adherence to the information contained in this chapter is essential for the safe and successful operation of the BioScope Catalyst.

> **Note:** The safety information contained in this chapter does not includes information pertaining to the user-supplied inverted optical microscope. Please consult the user manual for the inverted optical microscope for all related safety issues.Veeco is not responsible for any safety issues that arise due to the inverted optical microscope.

Specifically, this chapter covers the following information:

- **Safety Symbols:** Section 2.1
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2.1 Safety Symbols

Figure 2.1a 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 personnels.
	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 Zustände oder Handlungen, die einen elekrischen Schock verursachen können.
	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.
	This symbol identifies a heavy object. Improper lifting can cause muscle strain or back injury.
	Ce symbole indique un objet lourd. Soulever cet objet de façon incorrecte peut entraîner des froissements musculaires ou des problèmes de dos.
	Dieses Symbol identifiziert ein schweres Objekt. Falsches Anheben kann Muskelzerrungen und Rückenverletzungen verursachen.
	This symbol identifies a hot surface hazard. Touching heated surfaces can result in burns or fires.
	Ce symbole identifie un risque de surface chaude. Les surfaces heated émouvantes peuvent avoir comme conséquence les brûlures ou les feux.
	Dieses Symbol identifizierent eine Gefahr der heißen Oberfläche. Rührende erhitzte Oberflächen können Brände oder Feuer ergeben.
	This symbol identifies a pinch point hazard. Pinch points can cause injury to hands, fingers or other body parts.
	Ce symbole identifie un risque de point d'invariance. Les points d'invariance peuvent causer des dommages aux mains, aux doigts ou à d'autres parties du corps.
	Dieses Symbol identifizierent eine Klemmpunktgefahr. Klemmpunkte können Verletzung verursachen den Händen, den Fingern oder anderen Körperteilen.

2.2 Safety Precautions

Because the BioScope Catalyst AFM system is connected to line voltages, deals with samples in fluids, utilizes a laser and generates secondary hazardous voltages, it is crucial that operators become familiar with precautions to avoid injury to themselves and/or damage to equipment and samples. This section of the manual should be read by ALL persons working with or around the system including those involved with routine cleaning and maintenance. Failure to observe safety and operating instructions contained throughout the manual may result in operator injury or death. Operation of the equipment in any manner or for any purpose not specifically documented may result in operator injury or death and/or incorrect results or equipment damage. All operators should be made aware that, if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

2.3 General Operator Safety

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 le règlement imposé par le gouvernement et l'entreprise. Le personnel non autorisé ne peut pas 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.

CAUTION: Contact Veeco for instructions before attempting to transport or ship the BioScope Catalyst AFM system.

ATTENTION: Contactez Veeco pour obtenir des instructions correctes avant d'essayer de transporter ou d'expédier le Microscope de Force Atomique (AFM) nomé BioScope Catalyst.

VORSICHT: Setzen Sie sich mit Veeco in Verbindung bevor Sie das BioScope Catalyst AFM transportieren bzw. versenden.

WARNING: Do not attempt repairs on electrical components. If it is necessary to enter the electrical chassis for any reason (e.g., to replace a computer card), power-down the entire system and disconnect it from its power source.

AVERTISSEMENT: N'essayez pas de réparer les parties électroniques. S'il est nécessaire d'accéder le boîtier électronique (pour remplacer une carte dans l’ordinateur par exemple), éteignez et débranchez tout le système.

WARNUNG: Versuchen Sie nicht, elektrische Komponenten selbst zu reparieren. Falls es aus irgend einem Grund notwendig sein sollte, ein Gehäuse mit elektrischen Bauteilen zu öffnen (z.B., um eine Computer-Karte auszutauschen), schalten Sie das gesamte System ab, und trennen Sie es von der Spannungsquelle.

WARNING: Voltages supplied to and within certain areas of the system are potentially dangerous and can cause injury to personnel. Power-down all components and unplug from power sources before doing **any** electrical servicing. (Veeco service personnel only).

AVERTISSEMENT: Les tensions utilisées dans le système sont potentiellement dangeureuses et peuvent blesser les utilisateurs. Avant toute intervention électrique, ne pas oublier de débrancher le système. (Réservé au personnel de Veeco, 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 Veeco Personal.)

CAUTION: Do not use acetone or other unauthorized cleaners/solvents to clean the BioScope Catalyst AFM.

ATTENTION: N'utilisez aucun produit solvable ou à nettoyage non autorisé pour nettoyer le BioScope Catalyst.

VORSICHT: Benutzen Sie keinesfalls Azeton oder andere unzulaessige Reiniger bzw. Loesungen, um das BioScope Catalyst AFM zu reinigen.

2.4 Laser Safety

The BioScope Catalyst AFM contains a superluminescent diode with the following characteristics:

- 850 nm wavelength (Infrared) laser, Class 3R
- Beam divergence: 3°-7°
- Maximum output: 1 mW
- Laser output power decreases over time as the laser diode ages

The label shown in Figure 2.4a will be attached to the BioScope Catalyst head.

Figure 2.4a Labels on BioScope Catalyst Head

Appropriate laser safety procedures must be followed to avoid risk of eye damage. All users should understand the cautionary notes regarding laser safety and risk.

WARNING: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

AVERTISSEMENT: Toute utilisation de controls, réglages ou performances de procédures autre de celles spécifiées ici, peut causer des expositions de radiations dangereuses.

WARNUNG: Einstellungen oder Handhabungen, die nicht in dieser Anleitung beschrieben sind, koennen gesundheitsgefaehrdende Strahlung zur Folge haben.

WARNING: Do not stare at the laser beam either directly or from a highly reflective surface

AVERTISSEMENT: Ne regardez pas vers le laser directement ou d'une surface très réflective.

WARNUNG: Vermeiden Sie in jeden Fall sowohl direkten als auch indirekten, durch Reflexionen verursachten Augenkontakt mit dem Laserstrahl.

WARNING: Viewing the laser output with certain optical instruments (for example, eye loupes, magnifiers and microscopes) within a distance of 100 mm may pose an eye hazard.

AVERTISSEMENT: Regardez le signal de sortie du laser en utilisant certains instruments optiques (par exemple une loup ou d'autres instruments a agrandir) d'une distance de 100mm peut causer des problèmes visuels.

WARNUNG: Das Betrachten des Laserstrahls mit optischen Geraeten (z. B. Lupe, Vergroesserungsglas, Mikroskop) in einem Abstand unter 10 cm koennen das Auge schaedigen.

2.4.1 Microscope

The BioScope Catalyst AFM is designed to prevent personal injury, equipment damage, and minimize procedural errors. The cautionary notes in this section and other chapters are integral to the safe use of the system. To avoid operator injury and equipment damage, observe the following cautions regarding the BioScope Catalyst microscope.

WARNING: If you use the equipment in a manner not specified by the manufacturer, you can impair the protection provided by the instrument.

AVERTISSEMENT: L'utilisation de l'équipement d'une façon non spécifiée par le fabricant, peut affaiblir la protection fournie par l'instrument.

WARNUNG: Falls Sie das Geraet in einer anderen als vom Hersteller vorgegebenen Art und Weise nutzen, riskieren Sie, die Schutzmassnahmen des Geraetes zu unterlaufen.

WARNING: Stage microscopes feature a motor driven X-Y stage and Z-axis capable of programmed movement. The movements of all axes are slow, but a hand caught in the stage of a BioScope Catalyst AFM could be injured.

AVERTISSEMENT: Le plateau du microscope fournie des axes motorisés des X, Y et Z et sont capables de mouvements programmés. Tout axe bouge lentement, mais une main coincée dans un axe du BioScope Catalyst peut causer des blessures.

AVERTISSEMENT: Stage Mikoroskope (Mikroskope mit beweglicher Plattform) verfuegen ueber ein in X-, Y- und Z- Richtung von Motoren angetriebene Stative. Die Bewegungen werden von einem Programm gesteuert und laufen daher selbstaendig ab. Obwohl die Geschwindigkeit der Bewegung in jede Achsrichtung sehr gering ist koennen Verletzungen an den Haenden verursacht werden

WARNING: The internal electronics of the microscope, controllers, and peripheral equipment feature high-voltage components. Because there are no user-serviceable parts, do not attempt system repairs. Disconnect faulty components and ship them to Veeco for repair or replacement.

AVERTISSEMENT: Les parties électroniques du microscope, du controleur et des équipements périphériques portent des elements fonctionnants à haut voltage. N'essayez pas d'éffectuer des réparations, car aucune des parties n'est concue pour être réparée par l'utilisateur. Déconnectez les parties défectueuses et envoyez les à Veeco pour réparation.

WARNUNG: Die Elektronik des Mikroskops selbst, der Steuergeräte und der externen Geräte ist mit Hochspannungselementen ausgestattet. Diese Elemente dürfen nur von geschultem Personal gewartet werden. Versuchen Sie nicht, das System selbst zu reparieren. Trennen Sie fehlerhafte Komponenten vom System, und schicken sie diese zur Reparatur oder zum Umtausch zu Veeco.

CAUTION: Avoid spilling fluids onto the AFM stage or into electrical assemblies, particularly the AFM head. Use only minimum necessary amounts of fluids -- no more than 3mm (0.125 in) depth in a petri dish. Refer to the procedure for spillage clean-up.

ATTENTION: Evitez de renverser du liquide sur la station de l'AFM ou sur les parties électroniques. Soyez particulièrement prudent avec la tête de l'AFM. Utilisez des montants de liquide minimaux -- pas plus de 3mm en profondeur dans une boîte de Pétri. Consultez la procedure de nettoyage si necessaire.

VORSICHT: Schuetten Sie keine Fluessigkeiten auf das AFM Stativ oder elektrische Bauteile, insbesondere auf den AFM Kopf. Benutzen Sie so wenig Fluessigkeit wie moeglich - maximaler Fuellstand in Petri-Schalen betraetgt 3mm. Beachten Sie auch die Anweisungen zum Reinigen des Geraetes.

WARNING: Do not remove or pull on ground cables.

AVERTISSEMENT: N'enlevez pas et ne tirez pas les cables de terre.

VORSICHT: Vermeiden Sie Zugbelastung auf Masse-Kabel und entfernen Sie diese nicht.

WARNING: Do not remove cover panels from any electronics devices when power is applied (PC, Controller, E-box, EasyAlign, BioScope, Catalyst Head).

AVERTISSEMENT: N'enlevez aucuns paneaux de couverage situés devant tout appareil électronique lorsque la prise est alimentée (ordinateur, controleur, E-box, EasyAlign, BioScope).

WARNUNG: Entfernen Sie keinesfalls Abdeckungen von elektrischen Komponenten (PC, Controller, Easy-Align, BioScope oder andere elektrische Bauteile) so lange das Geraet mit elektrischer Spannung verbunden ist. Zuvor Netzstecker ziehen!

2.4.2 Sample Safeguards

CAUTION: Do not change samples while the AFM is scanning. Verify that the stage is clear of tools, objects, and debris at all times. Use alcohol wipes periodically to keep the stage clean of dust. Dispose of wipes in an appropriately labelled solvent-contaminated waste container.

ATTENTION: Ne changez pas d'échantillon en cours d'utilisation. Vérifier que la station n'est pas encombrée, par des outils par exemple. Employer des tampons d'alcool régulièrement pour dépoussiérer le plateau de l'AFM.

VORSICHT: Tauschen Sie **keine** Proben aus, während sich das System im Betrieb befindet. Der Probentisch sollte von Werkzeug, anderen Objekten und Überresten ständig freigehalten werden. Benutzen Sie ein mit Alkohol getränktes Tuch, um den Probentisch regelmäßig von Staub zu reinigen.

2.5 Perfusion Cell & Heater Stage Safety Precautions

This section summarizes information which may be repeated in or referred to by other chapters where the information is directly pertinent. Safety symbols are described as well as general instructions for operator safety. The symbols will be used with appropriate messages at relevant locations throughout the manual.

2.6 Safety Precautions

This section describes cautions and warnings to observe when using the perfusion cell (See Chapter 13) and/or heater stage (see Chapter 12) with BioScope Catalyst.

Figure 2a. 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 qui peuvent endommager les équipments 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 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 Zustände oder Handlungen die einen elekrischen Schock verursachen können.
	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.
	This symbol identifies a thermal hazard. Touching could result in skin burns upon contact.
	Ce symbole indique un risque lié à hautes températures. Un contact peut entraîner des brûlures de la peau.
	Dieses Symbol bedeutet "Heiße Oberfläche" Berührung kann zu Hautverbrennungen führen.

CAUTION: Only qualified personnel aware of the hazards involved may perform service and adjustments.

ATTENTION: Toute réparation ou étalonnage doit être effectué par des personnes qualifiées et conscientes des dangers potentiels.

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

CAUTION: Follow company and government safety regulations. Keep unauthorized personnel out of the area when working on equipment.

ATTENTION: Il est impératif de suivre les prérogatives imposées tant au niveau gouvernemental qu'au niveau des entreprises. Les personnes non autorisées ne peuvent rester près du système lorsque celui-ci fonctionne.

VORSICHT: Befolgen Sie die gesetzlichen Sicherheitsbestimmungen Ihres Landes. Halten Sie nicht authorisierte Personen während des Betriebs fern vom Gerät.

CAUTION: When imaging fluid samples, use extraordinary precautions against spillage. Do not spill fluids on or around the sample holder, electronic boxes, or other components containing electronic parts. Avoid spilling all corrosive fluids on exposed surfaces; otherwise, damage may result! In the case of a spill, immediately clean and dry all affected surfaces carefully.

ATTENTION: Lors d'un travail en milieu liquide, prendre toute précaution pour éviter des fuites. Les liquides ne doivent pas se répandre sur la platine porte échantillons, le boîtier électronique ou tout autre partie du microscope contenant de l'électronique. Eviter tout fuite de liquide corrosif sur les surfaces exposées. Le non respect de cette recommandation peut entraîner des dommages. En cas de fuite, nettoyer et sécher immédiatement les surfaces touchées.

VORSICHT: Falls Sie Proben in Flüssigkeiten abbilden, lassen Sie äußerste Vorsicht walten, damit keine Flüssigkeit verspritzt wird. Flüssigkeiten dürfen nicht auf oder nahe der Probenhalterung, der Elektronikbox oder anderen Komponenten, die elektronische Bauteile enthalten, verspritzt werden. Vermeiden Sie bitte, korrosive Flüssigkeiten auf freiliegende Oberflächen zu verspritzen; andernfalls wären Beschädigungen die Folge! Falls Sie Flüssigkeit verspritzt haben, säubern und trocknen Sie alle betroffenen Flächen sorgfältig.

CAUTION: 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. (Veeco Metrology Group personnel, only).

ATTENTION: 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 Veeco Metrology Group seulement).

VORSICHT: 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 Veeco Metrology Group Personal).

CAUTION: 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.

ATTENTION: 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.

VORSICHT: 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 oder verstärken.

CAUTION: Always handle the atomic force microscope (AFM) head with care - it may be damaged if dropped or crashed with force into samples

ATTENTION: Prendre toute précaution en cas de manipulation de la sonde. Celle-ci peut être très abîmée si elle tombe ou "s'écrase" sur un échantillon.

VORSICHT: Bitte behandeln Sie das AFM mit Sorgfalt - ein kräftiges Aufsetzen des Kopfes auf die Probenoberfläche kann zu Beschädigungen des Piezos führen.

CAUTION: Due to occasional problems some users have experienced with moisture wicking up through fluid cell seals, it is recommended that you avoid prolonged immersion in fluids. After completion of your experiments, always remove the cell from the fluid, detach it from the scanner, and dry it thoroughly prior to storage. Any moisture present on the end of the scanner must be dried IMMEDIATELY to prevent shorting the piezo.

ATTENTION: A la suite de problemes occasionnels, certains utilisateurs ont experimentes des fuites du joint de la cellule liquide. Il est donc recommande d'eviter les immersions prolongees dans un liquide. Une fois l'experience termine, veuillez enlever la cellule de la solution, la retirer de la sonde de balayage piézo et la ranger apres sechage. Afin d'eviter un endommagement du piézo, l'extremite de la sonde de balayage piézo doit etre nettoyer et secher des que la moindre trace d'humidite et/ou de moisussure apparaissent.

VORSICHT: Da es zu Problemen mit Flüssigkeit kommen kann, die durch die Silikonabdichhtung an der Meßzelle bis hinauf zum Scanner wandert, beachten Sie bitte das Folgende: Es wird empfohlen, ein längeres Verweilen des Meßkopfes (fluid cell) in Flüssigkeit zu vermeiden. Nach Abschluß der Messungen entfernen Sie bitte immer die Meßzelle aus der Flüssigkeit, ziehen sie vom Scanner ab und trocknen sie vorsichtig und gründlich bevor sie gelagert wird. Jegliche Flüssigkeit am Ende des Scanners muß SOFORT entfernt werden, um einem Kurzschluß des Piezos vorzubeugen.

CAUTION: Do not squirt or splash fluid into spaces above the protective skirt or evaporation covers.

ATTENTION: Ne pas injecter (à l'aide d'une seringue par exemple) ou projeter de liquide dans les espaces situés entre les protections en coutchoucs de la sonde d'analyse.

VORSICHT: Vermeiden Sie Kontakt zwischen Scanner und Flüssigkeit oberhalb der Schutzabdeckung aus Silikongummi.

CAUTION: If the fluid level is too high, the end of the scanner tube may be submersed too far into the fluid - this should never be allowed to happen. Permanent damage may occur.

ATTENTION: Si le niveau du liquide est trop important, l'extrémité des céramiques piézo-électriques peut tremper dans le liquide. Ceci ne doit jamais arriver. Ce cas de figure peut entraîner des dommages définitifs sur les céramiques piézo-électriques.

VORSICHT: Falls der Flüssigkeitsspiegel zu hoch ist, könnte das Ende des Scannerröhrchens in die Flüssigkeit eintauchen-dies darf auf keinen Fall passieren. Es könnte sonst eine dauerhafte Schädigung des Scannerröhrchens eintreten.

CAUTION: When operating the BioScope Catalyst SPM at elevated temperatures in fluids, use extraordinary precautions against condensates coming in contact with the BioScope Catalyst AFM Head. ALWAYS use the evaporation cover to prevent accidental short circuits of electrical signal lines and permanent damage to the scanner piezoelectric elements. Never leave the BioScope Catalyst AFM head above hot fluids without the evaporation cover installed; evaporation can cause a short circuit inside the BioScope Catalyst AFM head. When removing the BioScope Catalyst AFM head, do not turn it upside down until the remaining fluid on the fluid cell and surrounding area has been removed.

ATTENTION: Lors de l'utilisation du BioScope Catalyst à température élevée en milieu liquide, utiliser toutes les précautions possibles pour éviter que les vapeurs n'entrent en contact avec la tête de l'AFM. TOUJOURS utiliser le couvercle pour empêcher des court-circuits ou des endommagements permanents de l'élément piézo-électrique. Ne jamais laisser la tête du microscope au dessus de liquides chauffés sans le couvercle anti-évaporation en place. Les vapeurs peuvent créer un court-circuit à l'intérieur de la tête du microscope. Lors du retrait de la tête, ne pas la retourner tant que du liquide se trouve encore sur et autour de la cellule liquide.

VORSICHT: Wenn das BioScope Catalyst SPM bei erhöhten Temperaturen in Flüssigkeiten benutzt wird, ist es notwendig, daß Sie besondere Vorsichtsmaßnahmen ergreifen, um den Dimension AFM-Mikroskopkopf gegen verdampfende Flüssigkeit zu schützen. Benutzten Sie STETS den Verdampfungsschutz, um versehentliche Kurzschlüsse zwischen den elektrischen Signalleitungen und dauerhafte Schäden an den piezoelektrischen Elementen des Scanners zu vermeiden. Montieren Sie den Dimension AFM-Mikroskopkopf NIEMALS oberhalb einer heißen Flüssigkeit, ohne daß der Verdampfungsschutz installiert ist; verdampfende Flüssigkeit kann Kurzschlüsse im Dimension AFM-Mikroskopkopf verursachen. Beim Entfernen darf der Mikroskopkopf ERST DANN auf den Kopf gedreht werden, wenn alle Restflüssigkeit aus der Flüssigkeitszelle und ihrer Umgebung sorgfältig entfernt wurde.

CAUTION: The temperature of the BioScope Catalyst Fluid Sample Heater is limited to 75°C. Temperatures greater than 75°C may cause damage to surrounding components and the SPM may not operate properly. The temperature setpoint of the temperature controller is limited to 75°C, by the software parameter “Setpoint Limit”, accessible from the CURVE ENTRY menu, to prevent raising the temperature above 75°C. The Setpoint Limit should not be adjusted and should never be set above 75°C. See Appendix for temperature controller default settings and reset procedures.

ATTENTION: La température du module de chauffe du BioScope Catalyst est limitée à 75 C. Des températures supérieures à 75 C pourraient endommager les équipements alentour et le SPM pourrait ne plus fonctionner correctement. La tempêraure cible du contrôleur est limitée à 75 C, par le paramêtre “setpoint limit” dans le logiciel, accessible depuis le menu “Curve Entry”, pour éviter d’élever la température au dessus de 75 C. La valeur cible ne devrait pas être ajustée et ne devrait jamais être supérieure à 75 C. Voir l’appendice pour les réglages des paramêtres par défaut du contrôleur de température et les procédures de réglage.

VORSICHT: Die Temperatur des Flüssigkeitsheizers (Fluid Sample Heater) des BioScope Catalyst ist auf 75ºC begrenzt. Temperaturen oberhalb 75ºC können Mikroskopbauteile beschädigen und Funktionsstörungen am SPM verursachen. Um Temperaturen oberhalb 75ºC zu verhindern, wird der Einstellpunkt des Temperaturreglers mit Hilfe des Softwareparameters "Setpoint Limit" auf 75ºC begrenzt. Den Parameter "Setpoint Limit" finden Sie im Menü "Curve Entry". Dieser Parameter sollte nicht verändert werden und darf nicht auf Temperaturen über 75ºC eingestellt werden. Im Anhang finden Sie eine Beschreibung der Standardeinstellungen des Temperaturreglers und wie dieser eingestellt wird.

CAUTION: When imaging under fluids at elevated temperatures, always monitor the fluid level or volume, which can decrease due to evaporation. If the fluid volume decreases below about 3ml, there is a possibility of damage to the sample or failure of fluid SPM operation.

ATTENTION: Lors de l'utilisation du BioScope II à température élevée, toujours controler le niveau de liquide ou son volume, qui peuvent diminuer par évaporation. Si le volume de liquide atteint moins de 3 ml, l'échantillon risque d'être abimé ou le microscope pourrait ne pas fonctionner correctement.

VORSICHT: Beobachten Sie STETS das Flüssigkeitsniveau beziehungsweise das Flüssigkeitsvolumen, wenn Sie bei erhöhten Temperaturen in Flüssigkeiten messen. Das Flüssigkeitsvolumen kann wegen Verdampfung abnehmen, wobei bei einem Volumen unter ca. 3ml die Möglichkeit besteht, daß die Probe beschädigt oder die Flüssigkeitsmessung des SPMs gestört wird.

CAUTION: Use caution when operating the BioScope Catalyst Fluid Sample Heater; the top surface of the heating plate is hot and can cause burning if touched. Never touch the surface of the BioScope Catalyst Fluid Sample Heater plate without adequate protection. If it is necessary to handle the BioScope Catalyst Fluid Sample Heater plate, make sure it has cooled adequately first.

ATTENTION: Faire attention lors de l'utilisation du module de chauffe du BioScope Catalyst. La surface de la plaque chauffante est chaude et peut entrainer des brulûres au toucher. Ne jamais toucher la surface de la plaque chauffante du BioScope Catalyst sans protection adéquate. Ne jamais toucher la plaque chauffante du BioScope Catalyst sans s'être assuré au préalable qu'elle est suffisamment refroidie.

VORSICHT: Betreiben Sie den Flüssigkeitsheizer des BioScope Catalyst mit Vorsicht. Die Oberfläche der Heizplatte ist heiß und kann bei Berühren Verbrennungen verursachen. Berühren Sie die Oberfläche des Flüssigkeitsheizers des BioScope Catalyst nur unter angemessenen Vorsichtsmaßnahmen. Lassen Sie die Heizplatte abkühlen, bevor Sie sie in die Hand nehmen.

Chapter 3 Theory of AFM Operation

This chapter details theory of AFM operation. Understanding of the information contained in this chapter is the basis for successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

- **History and Definitions in SPMs** Section 3.1
  - **History** Section 3.1.1
  - **Definitions** Section 3.1.2
  - **Other Forms of SPM:** Section 3.1.3
- **Contact Mode AFM** Section 3.2
- **TappingMode AFM** Section 3.3

3.1 History and Definitions in SPMs

3.1.1 History

Scanning Tunneling Microscope (STM)

- Developed in 1982 by Binning, Rohrer, Gerber, and Weibel at IBM in Zurich, Switzerland.
- Binning and Rohrer won the Nobel Prize in Physics for this invention in 1986.

Atomic Force Microscope (AFM)

- Developed in 1986 by Binning, Quate, and Gerber as a collaboration between IBM and Stanford University.

3.1.2 Definitions

Scanning Probe Microscopy (**SPM**): Consists of a family of microscopy forms where a sharp probe is scanned across a surface and probe/sample interaction is monitored.

The two primary forms of SPM:

1. Scanning Tunneling Microscopy (STM)

   Note: STM is not supported on the BioScope Catalyst.

2. Atomic Force Microscopy (AFM) (also called Scanning Force Microscopy (SFM)

   - There are 2 primary modes of AFM:
     - Contact Mode AFM
     - TappingMode AFM

3.1.3 Other Forms of SPM:

Lateral Force Microscopy (LFM)

Force Modulation Microscopy

Magnetic Force Microscopy (MFM)

Electric Force Microscopy (EFM)

Surface Potential Microscopy

Phase Imaging

Force Volume

Electrochemical STM & AFM (ECM)

Scanning Capacitance Microscopy (SCM)

Scanning Thermal Microscopy (SThM)

Near-field Scanning Optical Microscopy (NSOM or SNOM)

Scanning Spreading Resistance (SSRM)

Tunneling AFM (TUNA)

Conductive AFM (CAFM)

NanoIndentation

Torsional Resonance Mode (TR Mode)

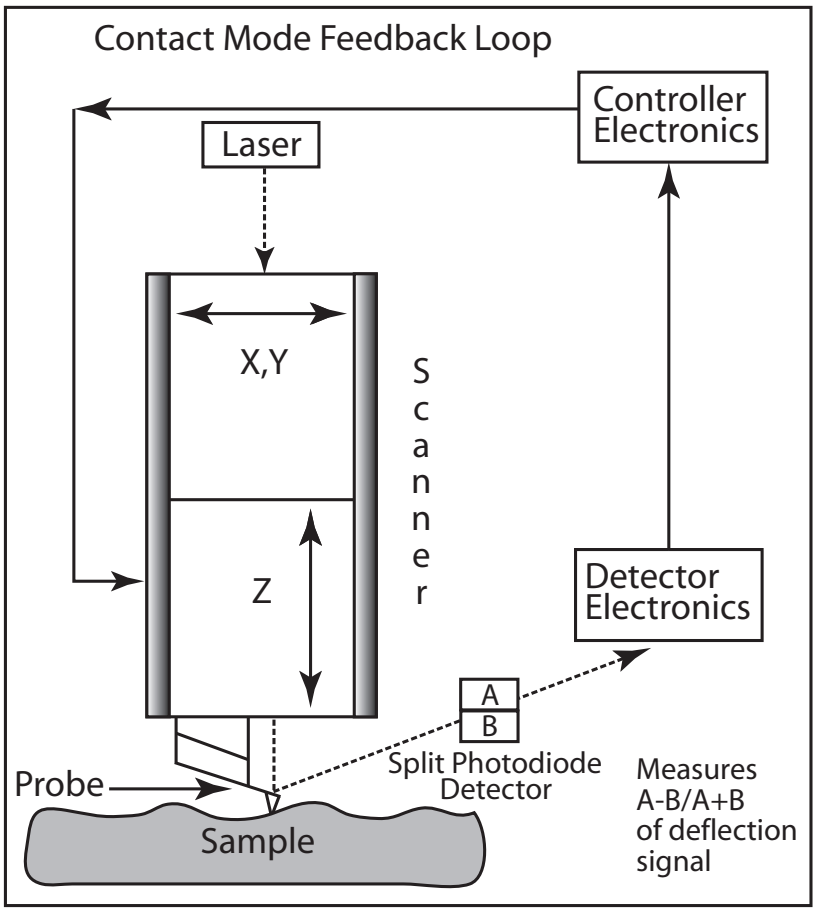
NanoLithography / NanoManipulation

SECPM

HarmoniX

3.2 Contact Mode AFM

Figure 3.2a Feedback Loop Maintains Constant Cantilever Deflection



Contact mode AFM operates by scanning a tip attached to the end of a cantilever across the sample surface while monitoring the change in cantilever deflection with a split photodiode detector. The tip contacts the surface through the adsorbed fluid layer on the sample surface.

A feedback loop maintains a constant deflection between the cantilever and the sample by vertically moving the scanner at each (x,y) data point to maintain a “setpoint” deflection. By maintaining a constant cantilever deflection, the force between the tip and the sample remains constant.

The force is calculated from Hooke's Law: $F = -kx$ where:

- F = Force
- k = spring constant
- x = cantilever deflection.

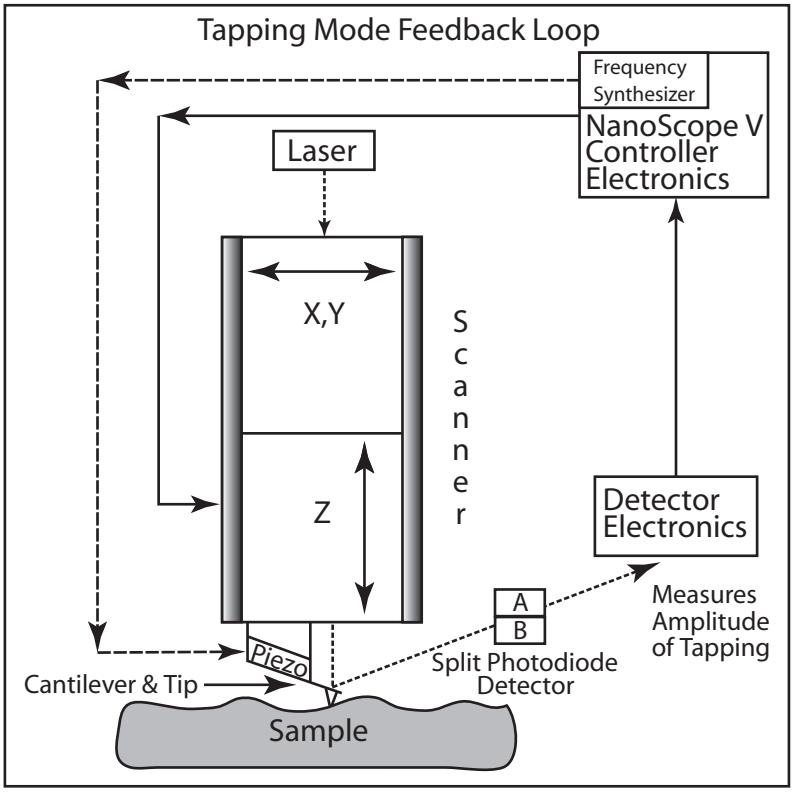
Force constants usually range from 0.01 to 1.0 N/m, resulting in forces ranging from pN to nN in an ambient atmosphere.

The distance the scanner moves vertically at each (x,y) data point is stored by the computer to form the topographic image of the sample surface.

Operation can take place in ambient and liquid environments.

3.3 TappingMode AFM

Figure 3.3a Feedback Loop Electronics



TappingMode AFM operates by scanning a tip attached to the end of an oscillating cantilever across the sample surface.

The cantilever is oscillated at or slightly below its resonance frequency with an amplitude ranging typically from 20nm to 100nm. The tip lightly “taps” on the sample surface during scanning, contacting the surface at the bottom of its swing.

The feedback loop maintains a constant oscillation amplitude by changing the Z position as the tip encounters features of different heights.

The vertical position of the scanner at each (x,y) data point in order to maintain a constant “setpoint” amplitude is stored by the computer to form the topographic image of the sample surface. By maintaining a constant oscillation amplitude, a constant tip-sample interaction is maintained during imaging.

Operation can take place in ambient and liquid environments. In liquid, the oscillation need not be at the cantilever resonance.

When imaging in air, the typical amplitude of the oscillation allows the tip to contact the surface through the adsorbed fluid layer without getting stuck.

Chapter 4 Microscope Components Overview

This chapter details the hardware and software components of the BioScope Catalyst. Understanding of the information contained in this chapter is essential for the safe and successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

- **SPM Hardware** Section 4.1
  - **Typical Arrangement** Section 4.1.1
  - **BioScope Catalyst Head** Section 4.1.2
  - **BioScope Catalyst Baseplate** Section 4.1.3
  - **Electronics Interface Box** Section 4.1.4
  - **Sample Substrate Clamps** Section 4.1.5
  - **Cantilever Holders** Section 4.1.6
  - **EasyAlign** Section 4.1.7
  - **Heater Stage (Optional)** Section 4.1.8
  - **Petri Dish Perfusion Cell (Optional)** Section 4.1.9
- **SPM Control Hardware** Section 4.2
- **SPM Control Software** Section 4.3
  - **Software Interface** Section 4.3.1
  - **The Select Experiment Window** Section 4.3.2
  - **The Main Screen Elements** Section 4.3.3
  - **Menu Bar Items** Section 4.3.4
  - **Toolbar Items** Section 4.3.5

- **The Workflow Toolbar** Section 4.3.6
- **NanoScope Version 8 Workspaces** Section 4.3.7
- **Scan and Ramp Parameter Lists** Section 4.3.8
- **The RealTime Status Window** Section 4.3.9
- **Image Windows** Section 4.3.10
- **Cursor Types** Section 4.3.11
- **Multiple Users Using NanoScope** Section 4.3.12

4.1 SPM Hardware

This section provides a brief overview of the major hardware components of the BioScope Catalyst. These components are described in more detail in following sections.

4.1.1 Typical Arrangement

The BioScope Catalyst is typically situated on a vibration isolation platform/hood in conjunction with an inverted optical microscope, as seen in Figure 4.1a. Each of the components are described in the Table 4.1a below.

Figure 4.1a BioScope Catalyst Components

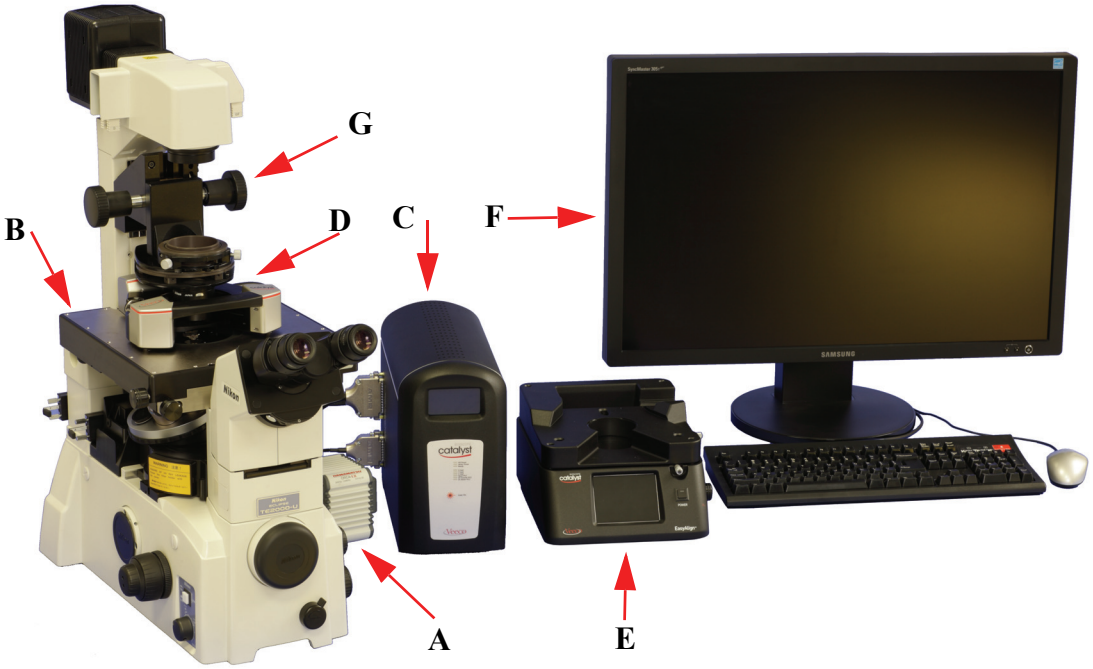


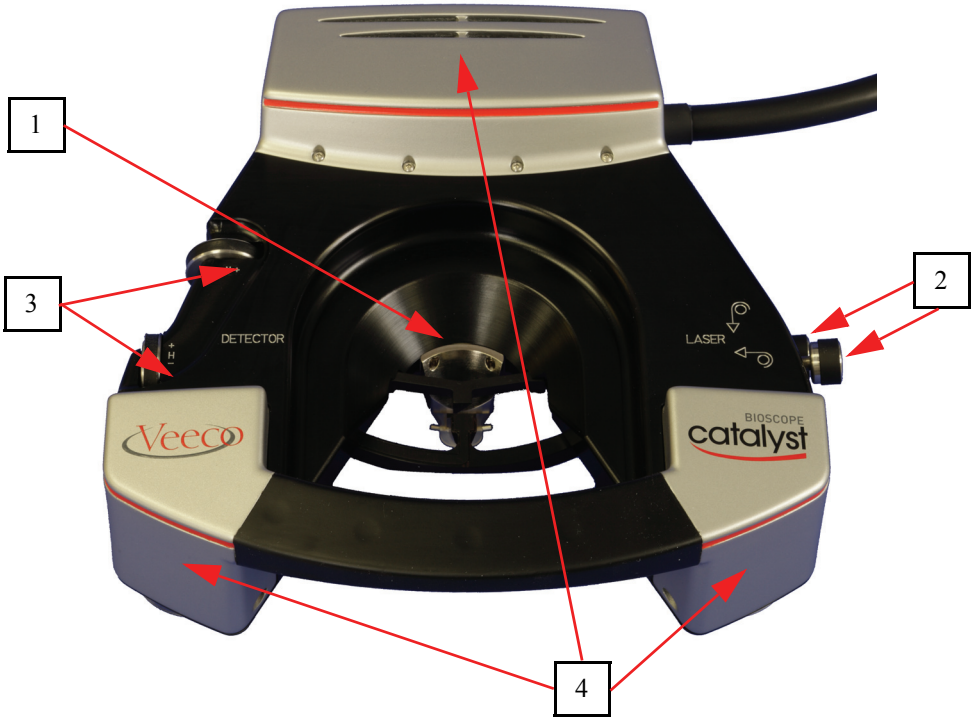
Table 4.1a BioScope Catalyst Hardware Components Descriptions

Code	Description	Function
A	Inverted Optical Microscope	Inverted microscope base (not included)
B	Baseplate	Provides XY piezo scanning and motorized XY stage for sample positioning.
C	Electronics Box	BioScope Catalyst Electronics Interface Box - Provides interface between SPM and NanoScope V controller and displays meter signals and system status information

Code	Description	Function
D	Head	Provides Z piezo scanning, cantilever deflection detection optics and electronics, and motors for coarse Z positioning and motorized SPM engage.
E	EasyAlign	Provides an optical view of the SPM probe and laser, for the easiest possible alignment of laser optics.
F	Monitor	Running NanoScope software and viewing images
G	Condenser	(Not included) Provides illumination for light microscope
H	Vibration table & acoustic hood	Optional, not included

4.1.2 BioScope Catalyst Head

Figure 4.1b BioScope Head



CAUTION: Never place the head directly on a work surface. The probe & head are easily damaged and care must be exercised to avoid contacting either.

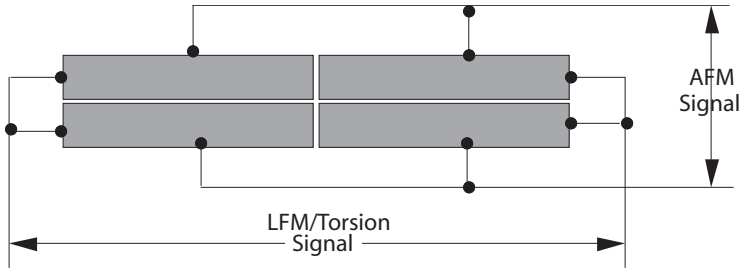
The BioScope Catalyst Head mounts on the baseplate in a three-point kinematic mount for stable and reproducible positioning. The head contains the following components and functions:

1. Z Scanner is a piezoelectric assembly which positions the cantilever holder (and therefore, the tip) along the Z-axis. The full (≥16µm) Scan range is produced by controller supplied -20 to +150 V_{dc} to the piezo element. An internal sensor provides Z-axis position data independent of the Z-scanner drive voltage.

2. Laser diode adjustment aligns a laser beam which reflects off the back of the cantilever to produce a spot on a quad photodetector. The beam is positioned onto the back of the cantilever using manual laser stage control knobs on the head to adjust X and Y positioning of the beam. (The EasyAlign and manual alignment methods are described in a later chapter.)

3. The photodetector provides different information depending on the operating mode. In all modes, outputs from the four elements combine to form the SUM signal. The amplified differential signal between the two top and two bottom elements provides a measure of the vertical deflection of the cantilever. This signal is used in Contact AFM mode. The differential signal feeds into a digital lockin amplifier for TappingMode operation. Similarly, the amplified differential signal between the sum of the two leftmost photodiodes and the sum of the two rightmost photodiodes provides a measure of the torsion in the cantilever (used in Lateral Force Microscopy). Figure 4.1c illustrates the arrangement of the photodiode elements in the BioScope Catalyst AFM head.

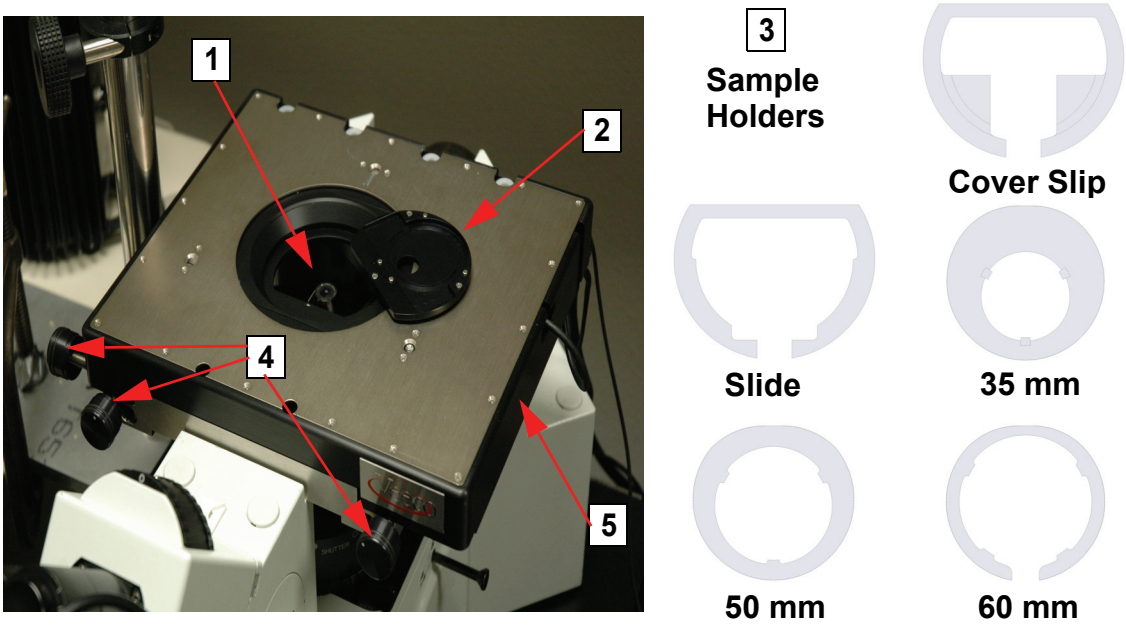
Figure 4.1c Quad Photodetector



4. Coarse Z-axis positioning over a total range of 8.4mm (0.33 in) is performed by three motors in the head. The positioning motors are computer controlled by the NanoScope V controller based on user input values or manipulation of a joystick attached to the PC.

4.1.3 BioScope Catalyst Baseplate

Figure 4.1d Baseplate Components, Controls and Accessories



1. XY Scan Stage: The XY scan stage is contained within the baseplate. Fine positioning during scanning (imaging) is performed by scanner piezo elements which are controlled by the NanoScope V controller based on user specified inputs. The range of scanning XY motion is nominally 150nm (full range in both X and Y). Integrated sensors allow closed-loop operation of the scan stage for accurate and stable imaging.

2. Sample Holder Plate (shown removed): The sample holder plate is mounted in the XY scan stage. The sample holder plate contains four magnets which mate with magnets in the sample substrate clamps. The sample holder plate may be removed for clean up of spills or replaced with an optional heater stage (a sample holder plate with an integrated heating element).

3. Sample Clamps: Clamps are available for a variety of different sample substrates. Magnets in the clamps mate with magnets in the sample plate holder to hold the sample securely for low-noise operation. The following (not included) sample substrates are supported (See Chapter 6 for more information):

    - Standard 25mm (1”) square microscope cover slip.
    - Standard 25mm x 75mm (1” x 3”) microscope slide.
    - Standard petri dish (35mm, 50mm and 60mm diameters).

4. Optical Axis Alignment Knobs: Three knobs are used to align the SPM axis (sample and probe) with the optical axis of the inverted microscope. Sample position relative to the probe is adjusted with the internal motorized XY stage (see next).

5. Motorized XY sample stage: Computer controlled motors in the baseplate assembly position the sample under the probe tip. The motors are controlled through the NanoScope V controller based on user entered values or manipulation of a joystick attached to the PC. The range of motor driven adjustment is nominally 6.25mm (0.25”).

4.1.4 Electronics Interface Box

Figure 4.1e BioScope Catalyst Electronics Box (“E-Box”)

Front Rear

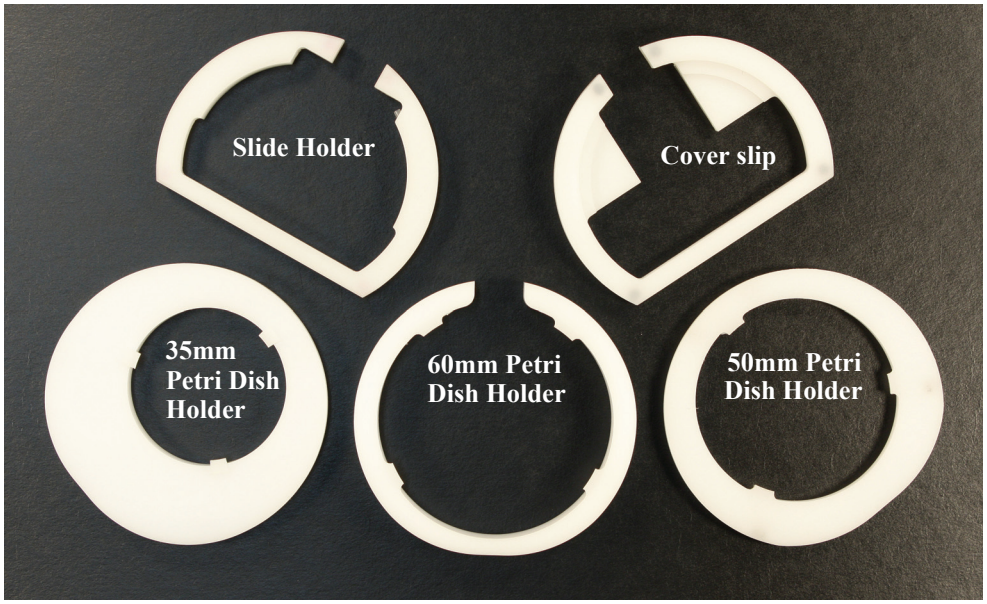
The BioScope Catalyst Electronics Interface box, seen in Figure 4.1e, serves several functions:

- Routes command, control and data signals between the head, baseplate and NanoScope V controller
- Drives the XY and Z stage motors via interface with the control PC
- Reads the capacitive XY and Z sensors and routes these signals to the NanoScope V controller
- Displays vertical and horizontal deflection, RMS tapping amplitude, and sum signal on the front LCD display
- LED indicators provide system status information (power, cable disconnections, XYZ motor limits, etc.)

4.1.5 Sample Substrate Clamps

Sample substrate clamps use magnets (on bottom of clamps) to hold substrates in place during imaging. Five clamps are available with the BioScope Catalyst. For more information on sample substrate clamps, please see Section 6.2.

Figure 4.1f Sample Substrate Clamps



4.1.6 Cantilever Holders

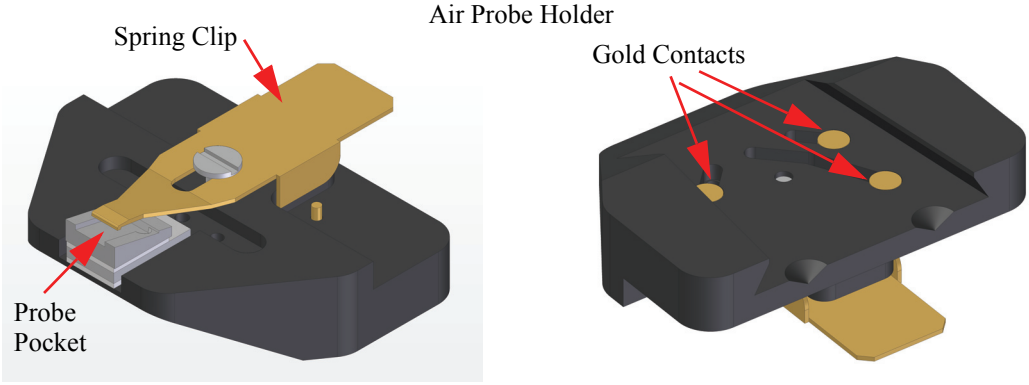
The cantilever holder, seen in Figure 4.1g, holds the cantilever (probe). It is an assembly which features:

- A dovetail feature for mounting on the Z scanner
- A spring clip to secure the probe and permit probe replacement
- A pocket to permit coarse positioning of the probe in the holder
- Three gold plated contacts provide electrical connection to tapping piezo and tip bias.
- A clear glass window to allow imaging in fluid.

Changing of probes and proper mounting of the cantilever holder to the z-scanner mount are discussed in detail in a later chapter.

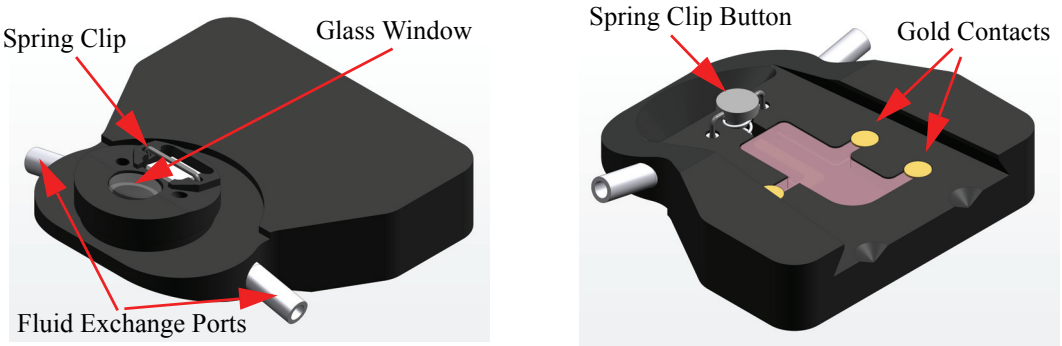
Figure 4.1g Air & Fluid Cantilever (Probe) Holders

Air Probe Holder



Top Bottom

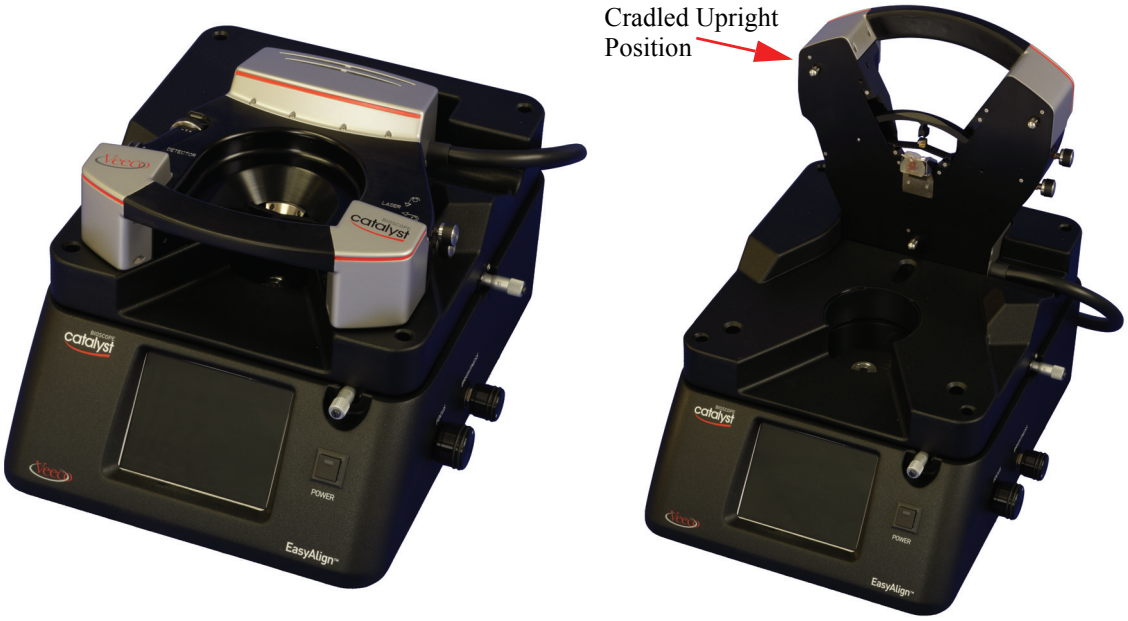
Fluid Probe Holder



Top Bottom

4.1.7 EasyAlign

Figure 4.1h EasyAlign with Head Mounted and in Cradled Upright Position

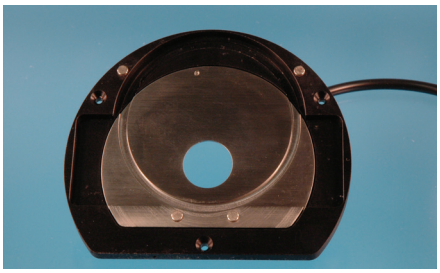


EasyAlign, seen in Figure 4.1h, is a standalone, separately powered unit which simplifies and facilitates aligning the laser beam onto the back of the cantilever. A magnified image of the cantilever and free air position of the laser beam is displayed on a LCD screen. Focus and illumination knobs on the side of the EasyAlign are used to optimize the image. Micrometer adjustments are used to position the probe in the field of view. Knobs on the BioScope Catalyst head adjust the beam onto the back of the cantilever. One should take care to turn EasyAlign off when not in use to conserve CCD life. When the head is not in use, it can be cradled in upright position on the EasyAlign. The EasyAlign includes a separate 12 V_{dc}/2 amp power supply.

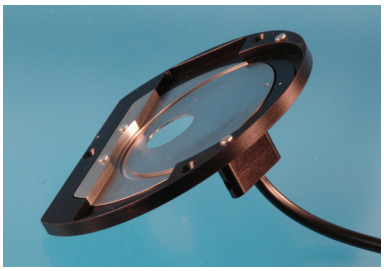
4.1.8 Heater Stage (Optional)

The optional heater stage replaces the sample holder plate (see figure Figure 4.1d). Similar in appearance to the sample holder plate, the heater stage includes an electric heating element for use when the sample is to be heated for/while imaging. Temperature is set using a dedicated controller.

Figure 4.1i Optional Heater Stage



Face

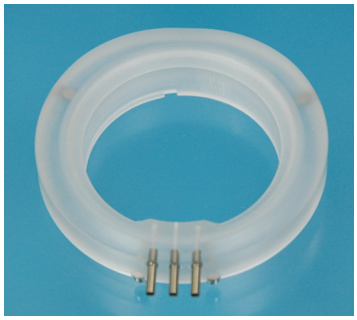


Side

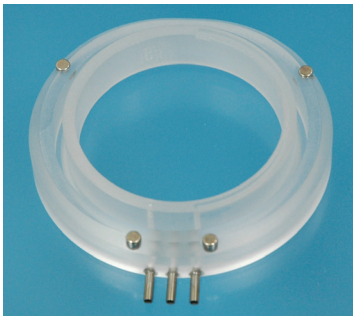
4.1.9 Petri Dish Perfusion Cell (Optional)

The petri dish perfusion cell is an optional accessory substrate clamp for 50mm petri dishes. The cell permits fluids or gasses to be introduced or exchanged from the petri dish while it remains in the BioScope Catalyst. The perfusion cell is described in detail in Chapter 13.

Figure 4.1j Optional Petri Dish Perfusion Cell



Top View



Bottom View

4.2 SPM Control Hardware

The BioScope Catalyst control hardware consists of the items described in Table 4.2a. A typical layout for the control hardware is show in Figure 4.2a.

Figure 4.2a BioScope Catalyst Control Station Components

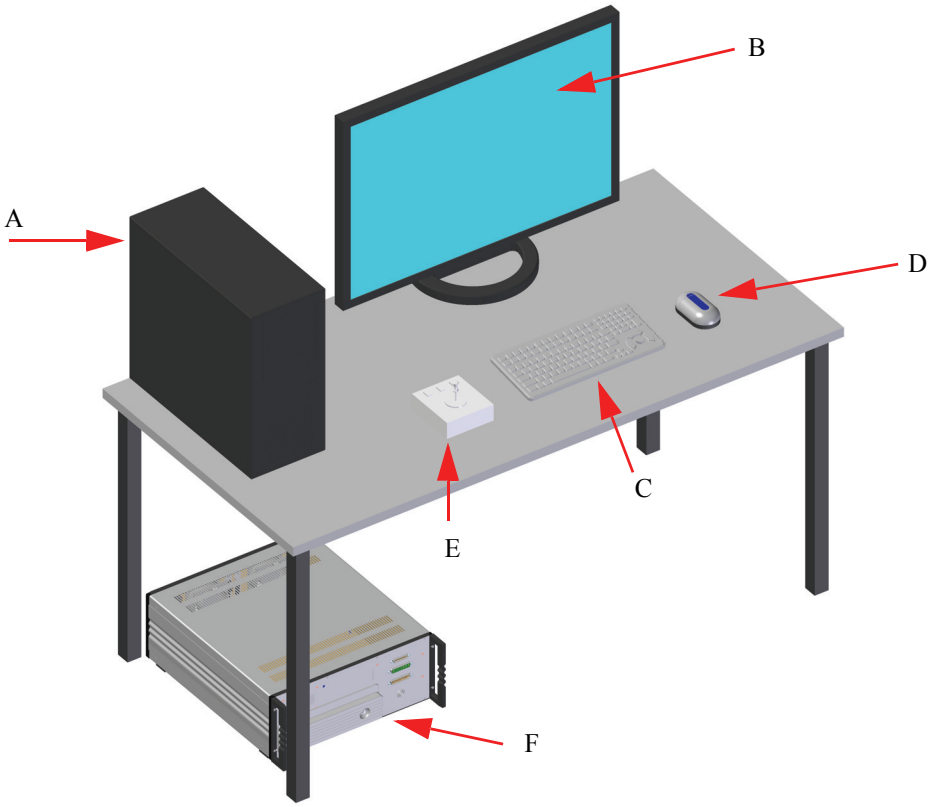


Table 4.2a BioScope Catalyst Control Station Components Descriptions

Code	Description	Function
A	Computer	Contains DSP board for interface to controller. Runs NanoScope control software.
B	Monitor	30 inch monitor display.
C	Keyboard	Standard
D	Mouse	Standard
E	Joystick	Controls X, Y and Z stage motors for tip and sample positioning.
F	NanoScope V Controller	Controls the BioScope Catalyst scan signals and data acquisition.

4.3 SPM Control Software

4.3.1 Software Interface

NanoScope version 8 software is organized by experiment type. Click **EXPERIMENT** > **SELECT EXPERIMENT** or the **SELECT EXPERIMENT** icon in the top left of the NanoScope software window. This opens the **Select Experiment** window, shown in Figure 4.3a.

Figure 4.3a The **Select Experiment**, Contact Mode, Window

The **Select Experiment** window forms the basis of the NanoScope version 8 user experience. Your experiment choice configures the **Scan Parameters** and, if necessary, the **Ramp Parameters** needed to run that experiment. This was done to simplify and make NanoScope software more intuitive and easier to use. That said, all functions from versions 6 and 7 remain accessible.

In addition to the **Scan Parameters List** and the **Ramp Parameters List**, **Select Experiment** also configures the **Workflow Toolbar**. The workflow toolbar, shown in Figure 4.3e, guides you through the experiment.

NanoScope software contains two modes of operation: **Realtime** (i.e., all operations related to controlling the microscope) and **Image Processing** or **Offline** (i.e., analysis and modification of captured images). In previous versions of NanoScope software, these modes were separate work environments. With versions 6, 7 and 8, both work environments have been combined.

4.3.2 The Select Experiment Window

Use of the **Select Experiment** window, shown in Figure 4.3b, is described below.

Figure 4.3b The **Select Experiment**, TappingMode, window

The Choose an Experiment Category Window

Highlight the **Experiment Category** that you wish to select in this window.

The Select Experiment Group Window

Highlight the **Experiment Group** that you wish to select in this window.

The Select Experiment Window

Highlight the **Experiment** that you wish to select in this window.

Experiment Description

A brief experiment **Description** is shown in this window.

Load Experiment

Click **LOAD EXPERIMENT** to begin real-time microscope operation.

Open Previous

Your previous experiment is saved in the **Previous Experiment** window. Click **OPEN** to start that experiment.

Change Microscope

Click **CHANGE MICROSCOPE SETUP** to open the **Microscope Select** window to select a new microscope or new microscope features.

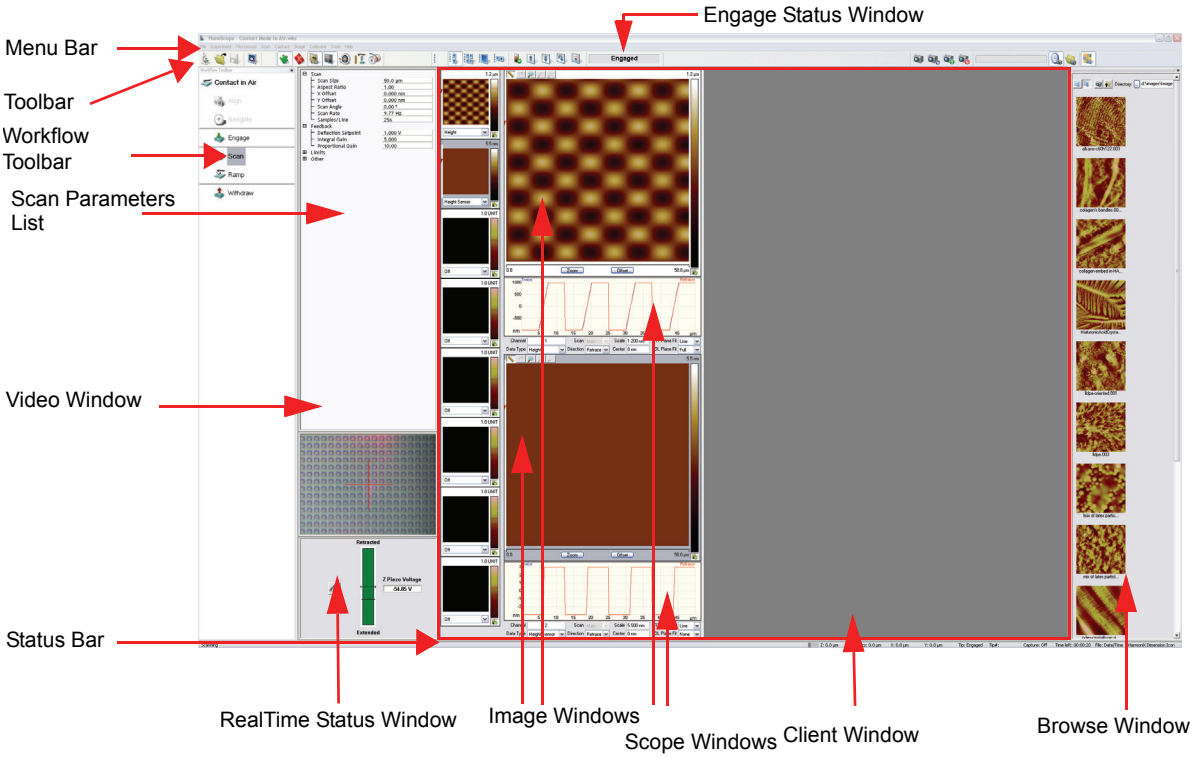
Cancel

Click **CANCEL** to close the **Select Experiment** window and continue to use the existing **Scan** and **Ramp Parameters**.

4.3.3 The Main Screen Elements

Workflow Toolbar	The left pane in the NanoScope window. The Workflow Toolbar sequentially organizes the steps (work) need to perform your experiment.
Client Window	A central window for viewing all Realtime and offline graphical displays, input parameters, results parameters and graphs.
Menu Bar	A group of items for executing commands or viewing files.
Toolbar	A group of icons for executing commands or viewing dialog boxes to configure input parameters.
Scan Parameter List	A list of the available scan parameters.
Status Bar	A read only list that displays the stage X, Y, Z coordinates and enabled functions (e.g., Capture: On).
RealTime Status Window	A dockable window in the client window displaying information about the Z piezo position.
Browse Window	A dockable window in the client window for browsing files. Available in list or thumbnail format.
Image Window	Windows that graphically display the 2D scan results.
Scope Window	Windows that display a real-time plot of the channel signal.
Engage Status Window	Window that prominently displays the engage status

Figure 4.3c NanoScope Version 8 Screen Elements



4.3.4 Menu Bar Items

The initial menu bar includes: **File**, **Experiment** and **Help** to begin the process of initializing and opening files in the client window. Once an experiment (workspace) is open, an expanded toolbar exists for running and configuring the microscope to scan or process images.

The menu items include:

- **File**—Accesses menu selections for opening, saving and printing files and documents.
- **Experiment**—Accesses menu selections allowing you to select and save experiments.
- **Microscope**—Accesses menu selections to administer commands during data collection.
- **Scan**—Re-starts the scan from the chosen location.
- **Capture**—Accesses menu selections that allow you to capture (save) images.
- **Stage**—Accesses menu selections that control stage movement.
- **Calibrate**—Accesses menu items for calibrating various settings.
- **Tools**—Accesses menu items for selecting various settings.
- **Help**—Accesses menu items for initializing and displaying the help screen, Technical Support contact information, probe purchase information and NanoScope information about your system.

4.3.5 Toolbar Items

The NanoScope version 8.00 toolbar, shown in Figure 4.3d, is described in Table 4.3a, Table 4.3b, Table 4.3c, Table 4.3d and Table 4.3e.

Figure 4.3d The NanoScope 8.00 Toolbar

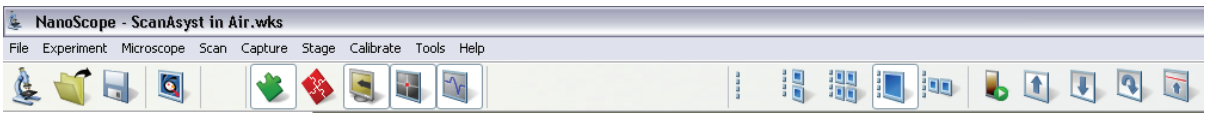


Table 4.3a NanoScope version 8.00 Toolbar menu

 **Select Experiment**	**SELECT EXPERIMENT** configures the **Scan** and **Ramp Parameters** needed to run an experiment. **SELECT EXPERIMENT** also configures the **Workflow Toolbar**.
 **Open**	Opens NanoScope files.
 **Save Experiment**	Saves the NanoScope Workspace.
 **MIRO**	Opens the (optional) MIRO (Microscope Image Registration and Overlay) package. See the *MIRO User Guide*, Veeco p/n 004-1027-000 for more information.
 **Simple Mode**	Makes only essential scan or ramp parameters visible, making simpler operation for users.
 **Expanded Mode**	Increases the number of visible scan, ramp and channel parameters for more advanced applications.
 **Video**	Turns the video window on and off.
 **Real Time Status**	Turns the real time status window on and off.
 **Point and Shoot**	Opens the **Point and Shoot** window. See NanoScope Software User Guide for more information.
 **High Speed Data Capture**	Opens the High Speed Data Capture window. See NanoScope Software User Guide for more information.
 **Thermal Tune**	Opens the **Thermal Tune** window. See the *NanoScope V Controller Manual*, Veeco p/n 004-992-000.
 **HarmoniX**	Opens the HarmoniX window. See the *HarmoniX User Guide*, Veeco p/n 004-1024-000 for more information.

Table 4.3b NanoScope version 8.00 Scan Toolbar menu

 **Thumbnails Only**	Shows 8 thumbnails only.
 **Two Channels Down**	Displays 8 thumbnails and two channels, arranged vertically.
 **Four Channels**	Displays 8 thumbnails and four channels.
 **One Channel**	Displays 8 thumbnails and one, large, channel.
 **Two Channels Across**	Displays 8 thumbnails and two channels, arranged horizontally.
 **AutoScale**	Automatically scales the vertical axis. Refer to the *NanoScope 8.10 User Guide* for details.
 **Frame Up**	The **Frame Up** command restarts the Realtime scan at the bottom of the frame. It is an easy way to begin to view an entire Realtime frame from the bottom. By clicking on this button, the Realtime scan restarts and moves up at the bottom of the frame. This allows you to go directly to the start of the frame and not have to wait for the previous frame to end.
 **Frame Down**	The **Frame Down** command restarts the Realtime scan at the top of the frame. This allows you to go directly to the start of the frame and not have to wait for the previous frame to end.
 **Frame Reverse**	Reverses the vertical scan direction from the existing location.
 **Skip to Line**	Restarts the scan at a user-specified line.

Table 4.3c NanoScope version 8.00 Ramp Toolbar menu

 **Plots Only**	Displays the ramp plots.
 **Plots and Controls**	Displays the ramp plots and ramp controls tabs.
 **Ramp Single**	Lowers and raises the probe tip *once* by a distance equal to the Z scan size, the halts.
 **Ramp Continuous**	The tip is continuously lowered and raised by a distance equal to the Z scan size. This is the normal, default motion during Force Calibrate.
 **Stop**	Halts all tip movement.
 **Single Approach**	The tip is lowered to the surface and raised in a single, controlled step. This process is halted if the surface is encountered by the tip, causing deflection exceeding the Step threshold amount. The resulting force curve is displayed.
 **Continuous Approach**	The tip lowers to the surface and raises in a controlled series of steps, then indexed by the Z step size (see Scan Mode panel) distance. This process continues downward until the tip encounters the surface. When tip deflection exceeds the Threshold Step amount, Continuous Approach halts and the resulting force curve displays.
 **Auto Ramp**	Begins auto ramping as defined by the parameters specified in the **Auto Panel**.
 **Update Sensitivity**	Updates the cantilever **Deflection Sensitivity**.

Note: For both **Continuous Approach** and **Single Approach**, if **Start mode** = **MOTOR STEP**, the motor is stepped towards the surface, not the Z piezo.

Table 4.3d NanoScope version 8.00 Capture Toolbar menu

 **Capture**	The **CAPTURE** command directs the software to save the next complete, uninterrupted frame to a file. A second click will force the software to save the next interrupted frame (i.e. - parameter changed).
 **Continuous Capture**	Continuously saves every frame until **WITHDRAW** or **ABORT CAPTURE** is selected.
 **Capture Now**	Immediately saves the uninterrupted image data before the frame is complete.
 **Abort Capture**	The **ABORT CAPTURE** command stops the capture process.
 **Capture Last**	Saves the last complete frame prior to the current frame.
 **Date and Time Stamp**	Sets the capture filename automatically using a date and time format.
 **Select Capture Directory**	Specifies the capture directory.

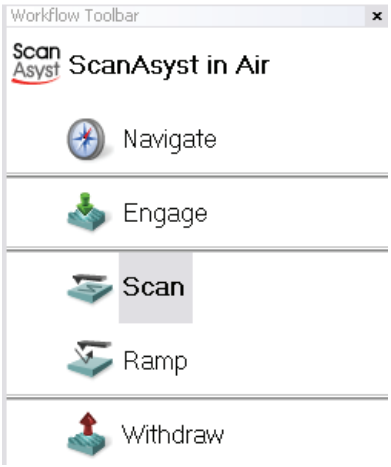
Table 4.3e NanoScope version 8.00 Browse Toolbar menu

 **Show/Hide Browse**	Displays the **Browse** window.

4.3.6 The Workflow Toolbar

The **Workflow Toolbar**, shown in Figure 4.3e, provides you with the steps, sequentially ordered, to run your experiment.

Figure 4.3e The **Workflow Toolbar**



Workflow Toolbar items are briefly described in Table 4.3f.

Table 4.3f NanoScope **Workflow Toolbar** menu

 **Experiment**	The name of the type of experiment that you have selected.
 **Align**	Opens the **Align** window, see NanoScope Software User Guide for more information.
 **Navigate**	Opens the **Navigate** window, see NanoScope Software User Guide for more information.
 **Tune**	Opens the **Tune** window, see NanoScope Software User Guide for more information.
 **Engage**	The **Engage** command brings the tip into contact with the sample surface and starts the Realtime imaging process.
 **Scan**	Scans the probe tip over the sample, producing an image. Additional information can be found in the NanoScope Software User Guide.
 **Ramp**	Opens the Ramp Parameter List, the ramp plots window and, if requested, the ramp controls window. Additional information can be found in the NanoScope Software User Guide.
 **Force Volume**	Opens the **Force Volume Parameter List** and the Force Volume GUI. Additional information can be found in the NanoScope Software User Guide.
 **Withdraw**	The **Withdraw** command stops the scanning process and withdraws the tip from the surface.
 **Generic Sweep**	Opens the **Generic Sweep** window, see NanoScope Software User Guide for more information.

4.3.7 NanoScope Version 8 Workspaces

A NanoScope workspace is a collection of views and parameters in NanoScope software. A NanoScope experiment is sometimes referred to as a workspace.

4.3.8 Scan and Ramp Parameter Lists

The Scan and Ramp Parameter Lists display the user-configurable (depending on options) scan and ramp parameters relevant to your experiment type.

Simple Mode

You can adjust the number of parameters shown in the **Scan Parameter List** using several methods.

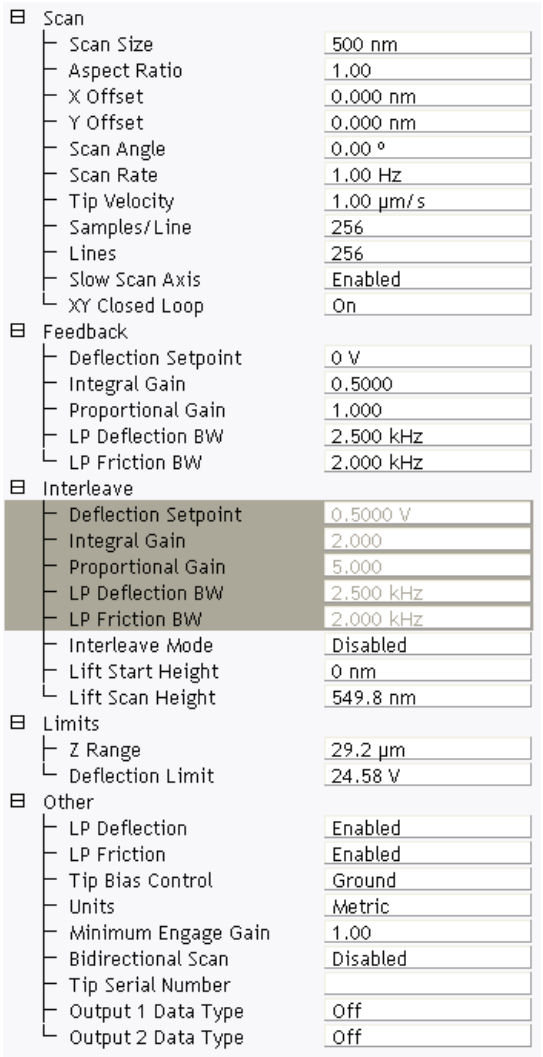
1. The default **SIMPLE MODE**, shown in Figure 4.3f, displays the essential parameters needed to make an image.

Figure 4.3f The **SIMPLE MODE** view of the **Scan Parameter List** in **Contact Mode**

Expanded Mode

1. The **EXPANDED MODE** view, shown in Figure 4.3g, increases the number of displayed parameters, providing access to more advanced options.

Figure 4.3g The **EXPANDED MODE** view of the **Scan Parameter List** in **Contact Mode**

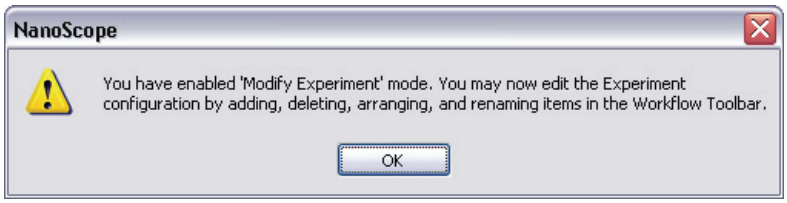


More advanced **Scan Parameter List** views are discussed below.

Show All

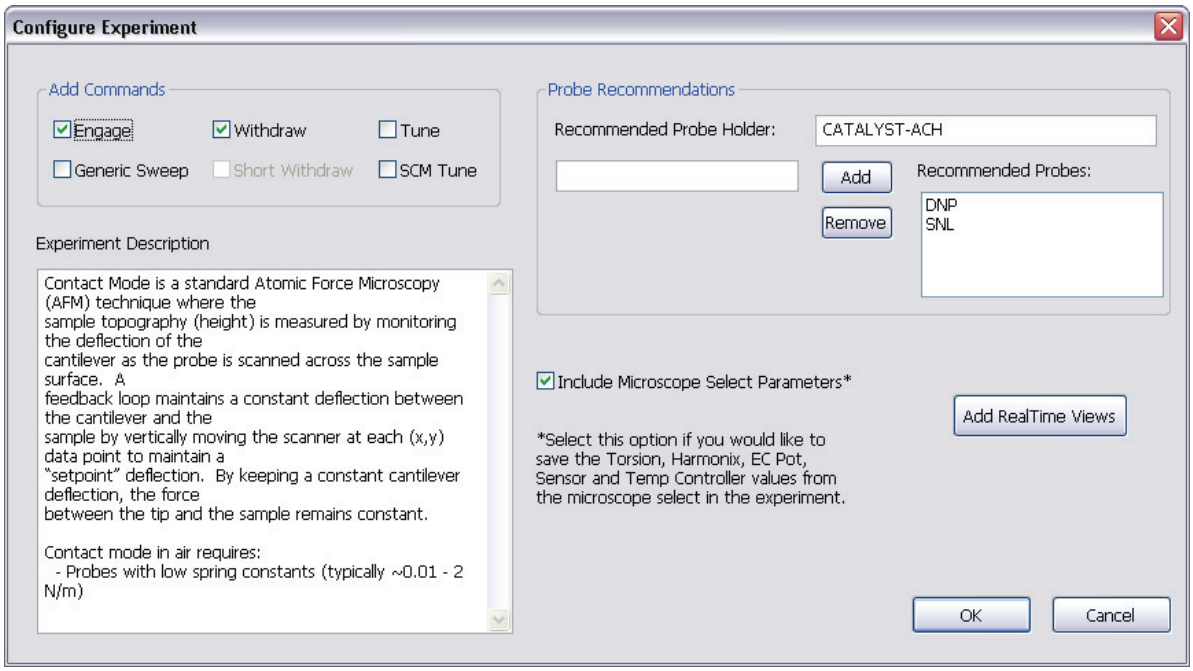
1. From the Menu bar, click **EXPERIMENT** > **CONFIGURE EXPERIMENT**. This opens an information window, shown in Figure 4.3h.

Figure 4.3h The Configure Experiment information window



2. Click **OK** to open the **Configure Experiment** window, shown in Figure 4.3i.

Figure 4.3i The Configure Experiment Window

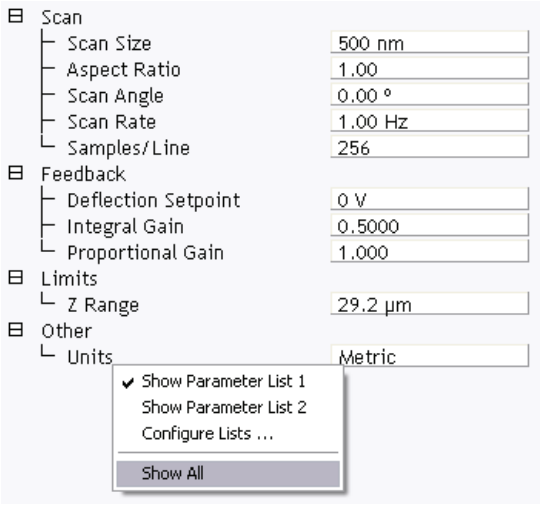


3. Check a box in the **Add Commands** panel to add that command to the **Workflow Toolbar**.

4. Click **OK** to accept your choices and close the **Configure Experiment** window.

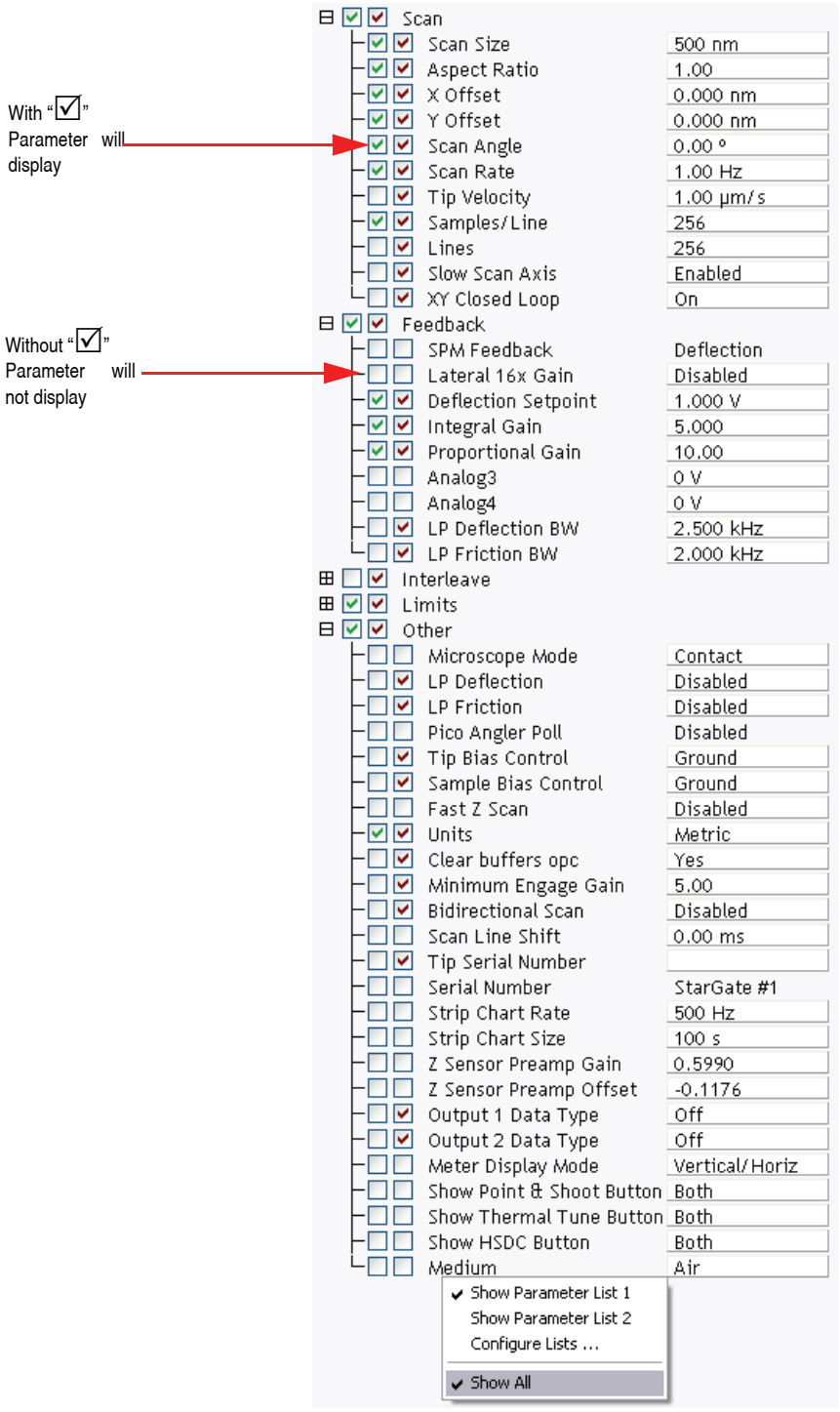
5. Right-click in the **Scan Parameter List** and select **SHOW ALL**, shown in Figure 4.3j.

Figure 4.3j Select **SHOW ALL** items



This makes all **Scan Parameters** visible along with two check boxes, the left, green, check box for the **SIMPLE MODE** and the right, red, check box for the **EXPANDED MODE**. See Figure 4.3k.

Figure 4.3k Enable Parameters



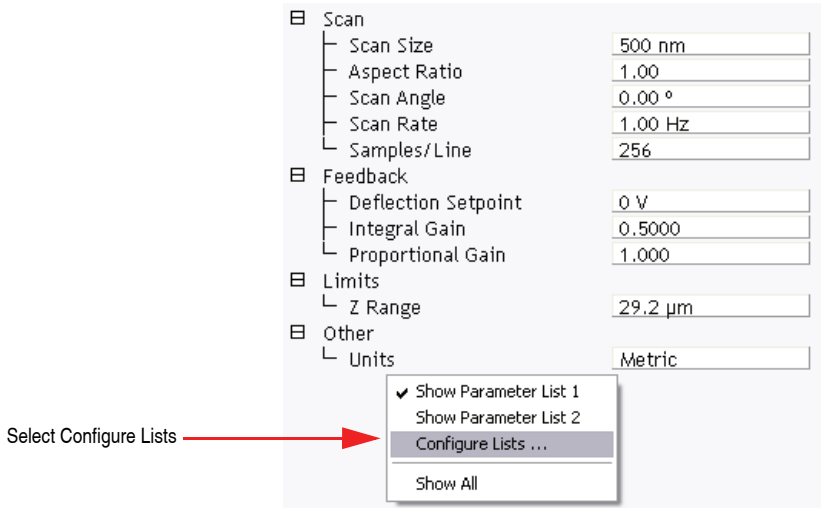
The checked ☑ parameters display in normal Real-time mode while those parameters without a ☑ will not display in normal Real-time mode.

Check the parameters that you want displayed and right-click in the **Scan Parameter List** and select **SHOW ALL** items to hide the unchecked parameters. The panel will once again appear in normal Real-time mode.

Configure Lists

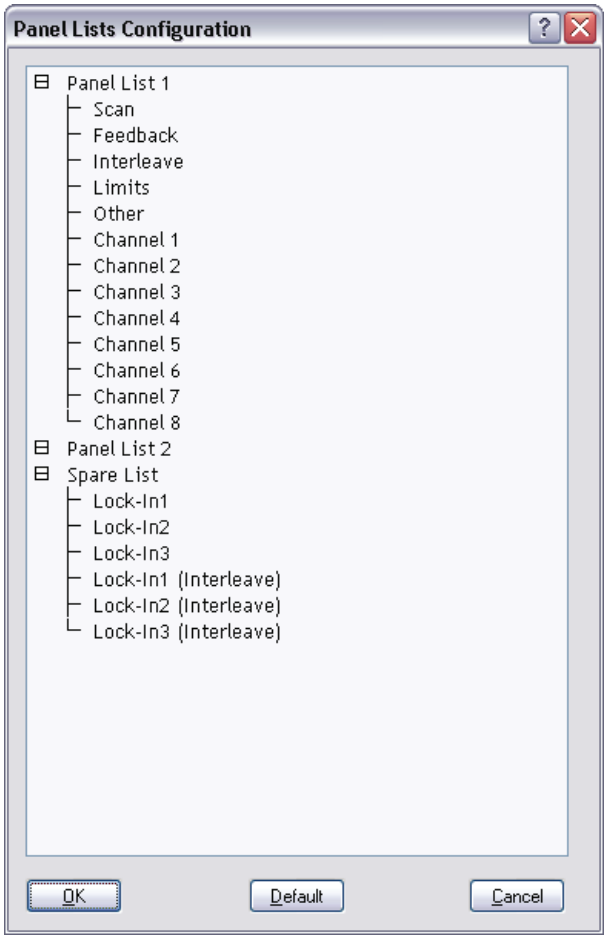
1. Right-click in the **Scan Parameter List** and select **CONFIGURE LISTS**, shown in Figure 4.31.

Figure 4.31 Select **SHOW ALL** items



This opens the **Panel Lists Configuration** window, shown in Figure 4.3m.

Figure 4.3m The **Panel Lists Configuration** window



2. Drag an item from the **Spare List** to either **Panel List 1** or **Panel List 2** and click **OK**.

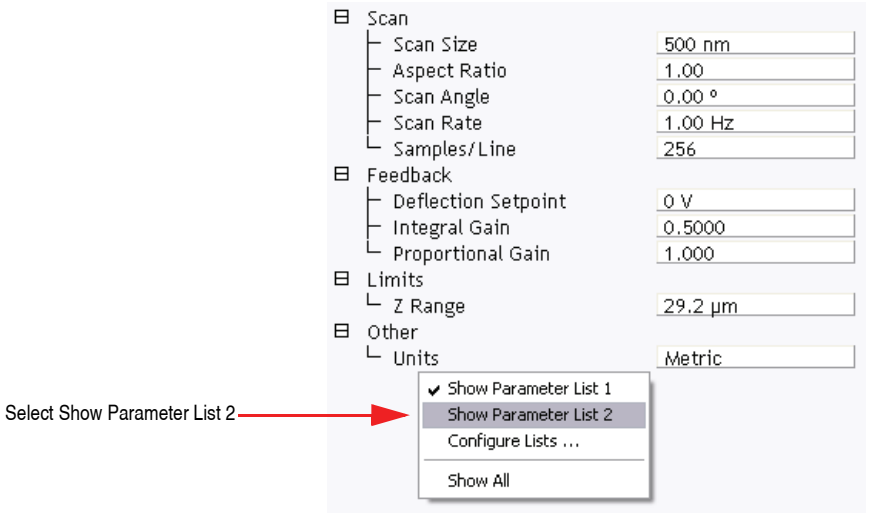
This adds selected items to the **Scan Parameter List**.

The **SHOW ALL** function works in the **Panel Lists Configuration** window the same way that it works in the **Scan Parameter List** window.

Show Parameter List 2

A second Scan **Parameter List** can be made visible by right-clicking in the Scan **Parameter List** window and selecting **SHOW PARAMETER LIST 2**.

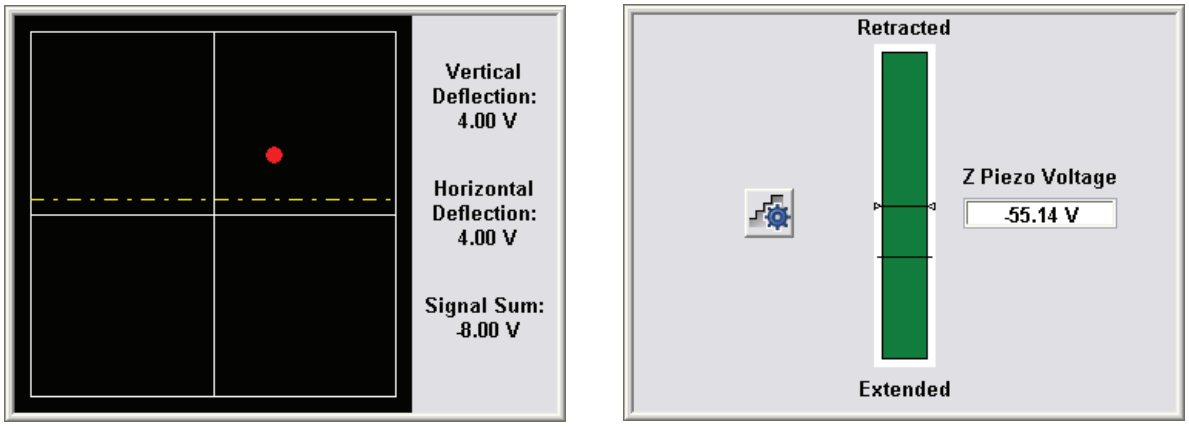
Figure 4.3n SHOW PARAMETER LIST 2



4.3.9 The RealTime Status Window

The Real Time Status window, show in Figure 4.3o, displays either the photo detector quadrant voltages or the Z piezo voltage.

Figure 4.3o Meter View (left) and Z position indicator (right) in the RealTime Status window.



4.3.10 Image Windows

Figure 4.3p A NanoScope Image Window

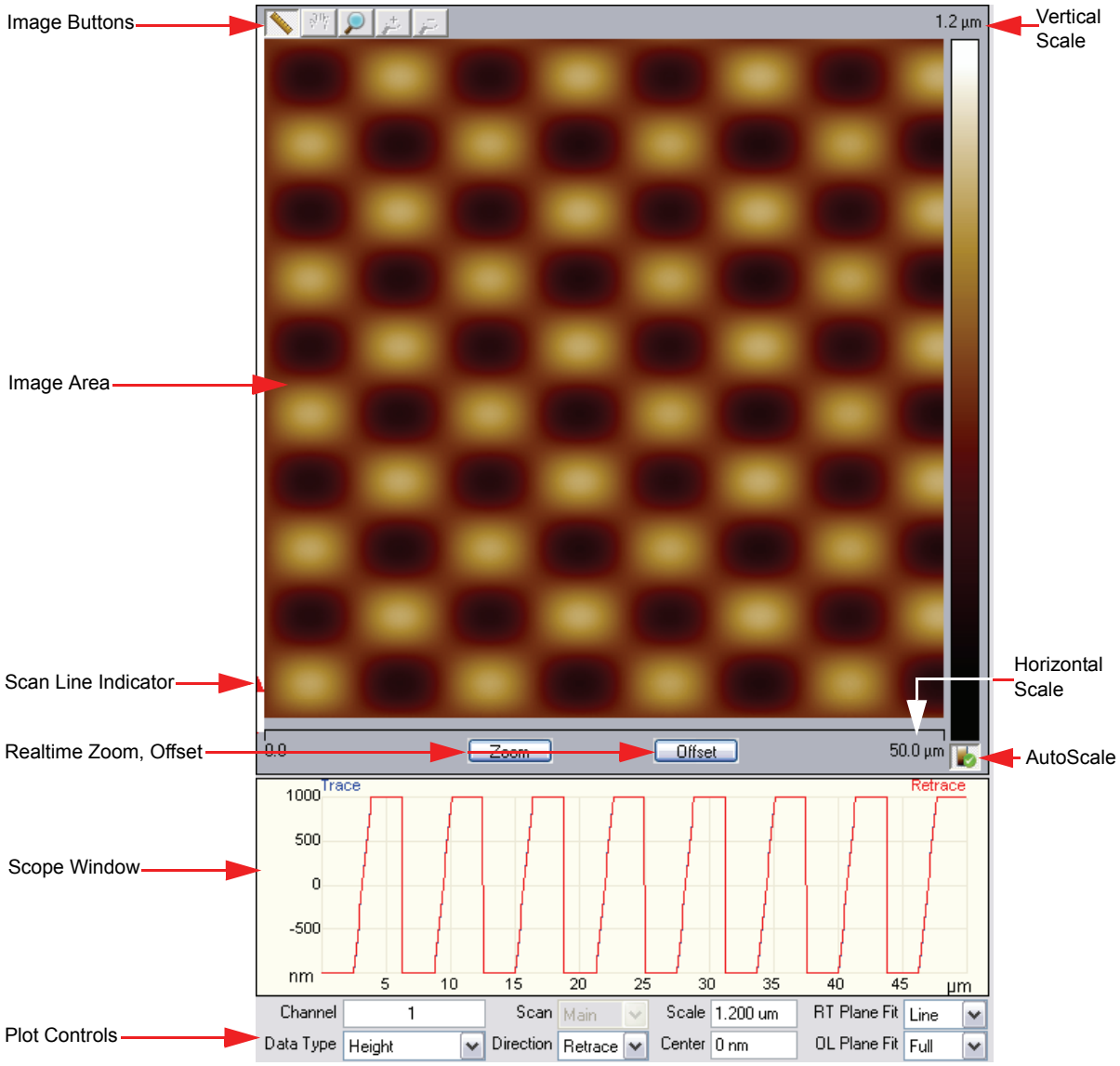


Image Area	The SPM image is displayed here.
Image Buttons	The image buttons, described below, allow pan, zoom and measurement functions.
Scan Line Indicator	An arrow showing the current scan line.
Vertical Scale	The vertical scale of the image.
Horizontal Scale	The horizontal scale of the image.
Realtime Zoom, Offset	Zooms and Offsets the scan. To Unzoom, you must change the **Scan Size**
Scope Window	Displays a real-time plot of the channel signal.
AutoScale	Automatically scales the data. Refer to the *NanoScope User Guide* for details
Plot Controls	Provides control of what is plotted in the image area.

The NanoScope image buttons, shown in Figure 4.3q, are described in Table 4.3g. These buttons operate on the scanned data and do not affect the real-time scanning. Realtime scaning can be controled with the real-time Zoom and offset buttons, shown in Figure 4.3r.

Figure 4.3q NanoScope image buttons

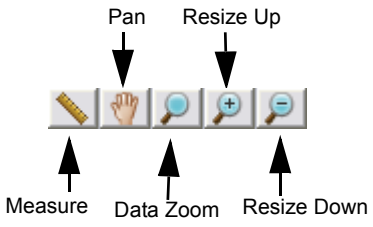


Table 4.3g NanoScope image buttons

Measure	Allows you to draw a box to make measurements, translate the image or offset and resize the image.
Pan	From a zoomed image, you can pan around to other areas of the original image.
Zoom	Allows you to draw a box to zoom in on an image.
Resize Up	Resizes the image up to the previous zoom level.
Resize Down	Resizes the image down to the previous zoom level.

Figure 4.3r Realtime Zoom and Offset buttons

4.3.11 Cursor Types

Within captured images, it may be necessary to do analysis or modification on a selected area or exclude this area from the analysis. Cursors allow for specifying this information.

The cursor types are as follows:

- **Lines**—Selecting specific data (e.g., lengths of features or sectioning features) along the line.
- **Boxes**—Selecting specific areas on the display for including or excluding data.
- **Grid Markers**—Horizontal or vertical line cursors within histograms and spectrum graphs for choosing data ranges or making measurements.

Using a Slider Cursor

In a graph or histogram, position the mouse within the blank area between the axis and the edge of the graph and drag the slider along the graph to position the cursor. The mouse cursor will change to ↔.

Positioning a Line or Box Cursor

- Click and drag the image to draw a line or box cursor.
- To resize an existing line or box cursor, click and drag on a corner edge or end of the object. Cursor will change to ↔ or ↕.
- To move an existing object, click and drag the center of the object to the desired location. The cursor will change to ✥.

4.3.12 Multiple Users Using NanoScope

The NanoScope computer can save multiple user preferences/settings in the computer registry. Once a user sets up an account on the computer, several settings are automatically saved for the user. These settings include:

- Previous image file type
- Previous directories
- Browse window settings
- Option to disable video while scanning
- Section View results to display

- Location of Abort dialog box
- Review curve settings
- Force Filter settings
- Help settings
- Script directory
- Default Parameter dialog box location
- Point and Shoot View settings
- Sweep dialog box settings
- Workspace settings
- Image control settings
- Grid control settings
- Meter View control settings
- Color control settings
- Z center control settings

Chapter 5 Getting Started

This chapter is meant to familiarize the user with the basic features of the BioScope Catalyst. Topics such as general system setup, probes and sample loading, and basic scanning are explained in detail. Understanding of the information contained in this chapter is the basis for safe and successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

- **Using the Planefit Function** Section 5.14.2
- **Using the Flatten Function** Section 5.14.3
- **Using the Section Analysis Function** Section 5.14.4
- **Saving Modified Files** Section 5.14.5

5.1 Powering Up the BioScope Catalyst

5.1.1 Power Up Sequence.

CAUTION: The power up/power down sequence is critical. Improper start up or shut down may damage the equipment. The NanoScope V controller should never be powered on (or left powered on) unless the PC is running.

CAUTION: Ensure that all power outlets or power strips provide proper grounding receptacles and are of the proper voltages as described *The BioScope Catalyst Service & Support Guide*.

1. Insure all cabling connections described in *The BioScope Catalyst Service & Support Guide* have been made and are secure. Special attention should be given to connections to and from the NanoScope V controller.

2. Turn on the PC control station components (Monitor has a push button on/off switch; PC has rocker switch on rear panel and push button on front panel).

3. Allow the PC to start up.

4. After the PC has fully "booted," turn on the NanoScope V controller using the rocker switch on the back panel. It is sufficient for the PC to have booted and not necessary to launch the NanoScope software prior to powering on the controller.

5.1.2 Recommendations for the Inverted Optical Microscope

A user supplied inverted optical microscope with associated illuminator and/or video camera may be attached to the BioScope Catalyst. Please follow the manufacturers guidelines for operation, maintenance, and safety of your Inverted Optical Microscope. The inverted optical microscopes currently supported for mounting to the BioScope base plate are included in Table 5.1a below.

Table 5.1a BioScope Catalyst Supported Configurations

Microscope Type	Supported Microscopes
Inverted optical microscopes	Leica DMI 6000, 4000, 3000 Zeiss Axio Observer A1, D1, Z1 (also Axiovert 100, 135, 200) Nikon Eclipse Ti-E/U/S (also TE2000-E/U/S) Olympus IX71, IX81 (also IX70) (also supported stand-alone operation)
Transmitted light condensers	Leica S28 (0.55 NA, 28mm WD) Zeiss LD (0.55 NA, 26mm WD) Nikon LWD (0.52 NA, 30mm WD) Olympus IX2-MLWCD (0.50 NA, 45mm WD) (also supports other models with longer working distances)
Confocal laser scanning microscopes	Leica TCS SP5 Zeiss LSM 5 and LSM 710 Nikon C1si and C1 plus Olympus FluoView 300 and 1000 (Inquire regarding other models)

For more details on Optical Inverted Microscopes, please refer to **Compatibility with Inverted Optical Microscopes** Chapter 10.

5.1.3 When to Power Down

System Power Down

Most users keep their BioScope Catalyst powered on at all times. If the equipment is scheduled to be idle for an extended amount of time, it is a good idea to consider shutting down the system. Upon restart, it will take approximately 1 hour for the system to warm up and provide stable data.

Easy Align Power down

When not in use, the EasyAlign should be powered down by pressing the on/off switch depicted in Figure 5.5b.

5.2 Starting NanoScope Software

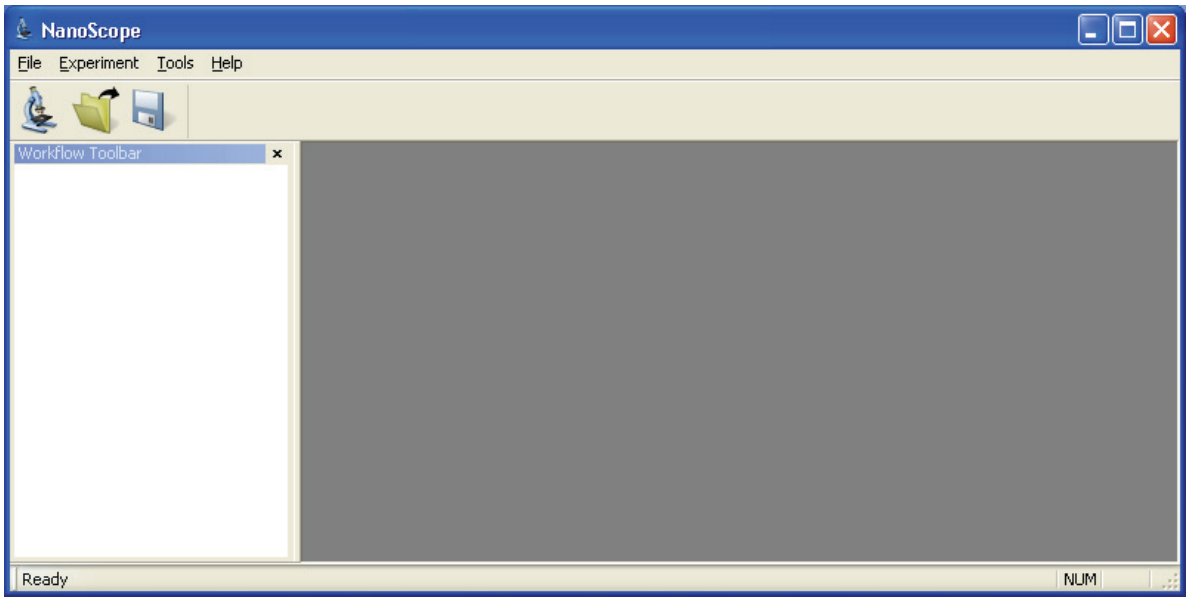
1. Start the PC and logon.

2. Power on the controller.

3. Launch NanoScope software by double clicking on the **NANOSCOPE 8.00** microscope desktop icon.

4. The screen in Figure 5.2a will appear

Figure 5.2a Opening Screen in NanoScope Software

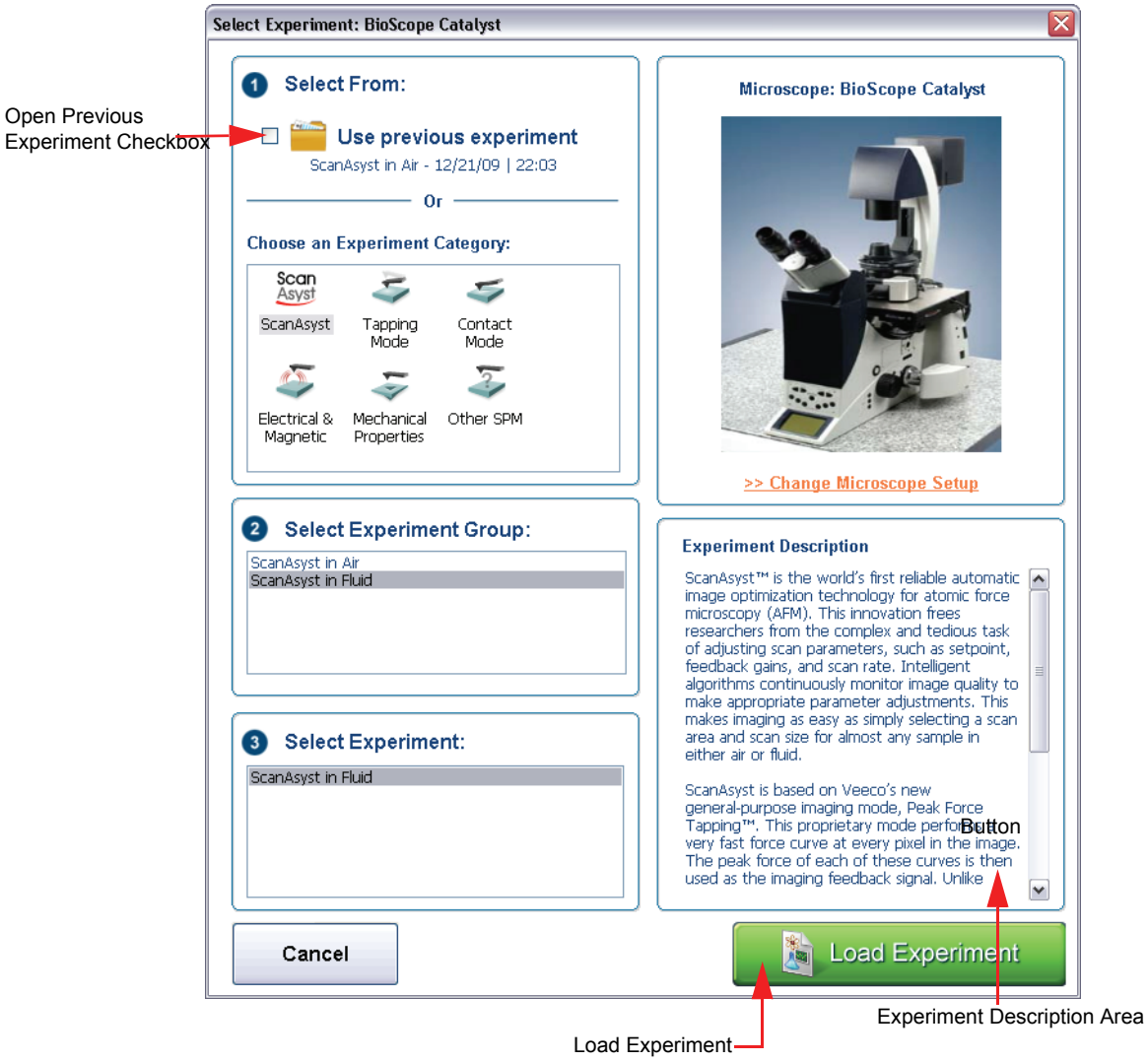


5.2.1 Loading an Experiment Configuration

This procedure is a continuation of the previous procedure found in Section 5.2.

1. NanoScope version 8 software is organized by experiment type. Click **Experiment > Select Experiment** or the **SELECT EXPERIMENT** icon in the top left of the NanoScope software window. This opens the **Select Experiment** window, shown in Figure 5.2b.

Figure 5.2b The Select Experiment Window



2. The **Select Experiment** screen allows for a few options. You can choose to Open a Previous experiment with the associated parameters or Choose an **Experiment Category**, **Experiment Group** and **Experiment Type** and the default parameters will be loaded. If you choose the latter, once you have chosen an **Experiment Category**, **Experiment Group** and **Experiment Type**, the **Experiment Description** area towards the bottom of the screen will describe the basics of the experiment chosen. See **Experiment Description** Area in Figure 5.2b.

5.2.2 More Information on Microscope Modes and Experiments

For more information on Microscope Modes and Experiments, please refer to the *BioScope Catalyst Experiment Guides* and *NanoScope Software 8.10 User Guide*.

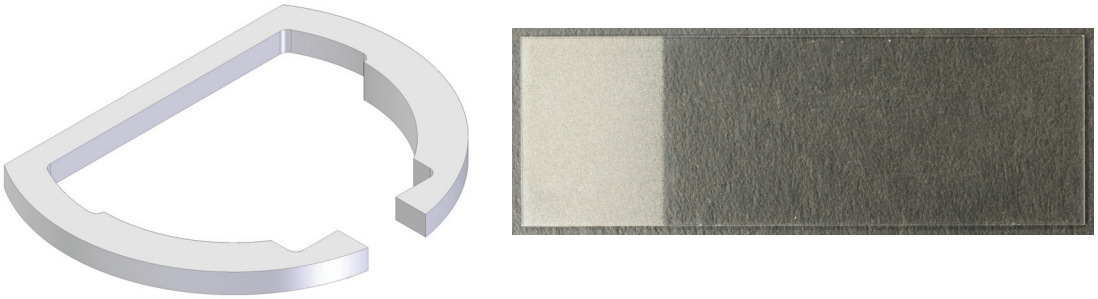
5.3 Preparing and Loading a Sample

5.3.1 Preparing a Cheek Cell Sample

The following components, shown in Figure 5.3a, are required in order to prepare a cheek sample:

- Standard microscope glass slide
- Isopropanol alcohol for cleaning the slide
- New cotton swabs
- BioScope Catalyst glass slide sample holder

Figure 5.3a A Sampling of the Components Required for Cheek Sample Preparation



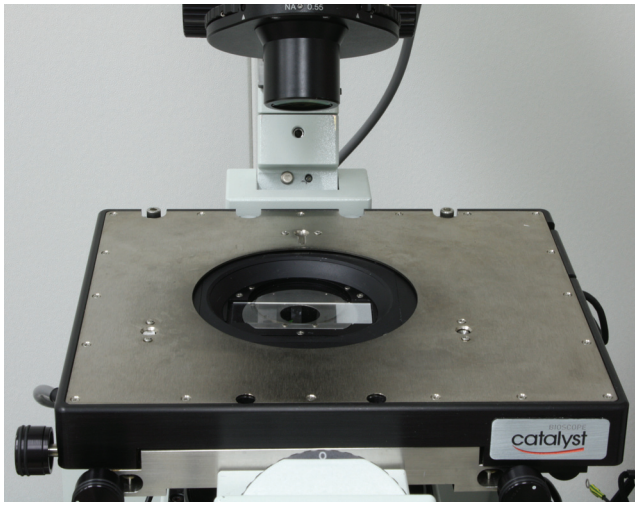
Sample Preparation

1. Obtain a standard microscope glass slide.
2. Clean both sides of the glass slide from particles with Isopropanol or other suitable method.
3. Let the slide dry appropriately.
4. Using a clean cotton swab, scrape the inside of your cheek.
5. Roll the cotton swab near the middle of the glass microscope slide using medium pressure.
6. Prepare to load the sample onto the BioScope Catalyst.

Sample Loading

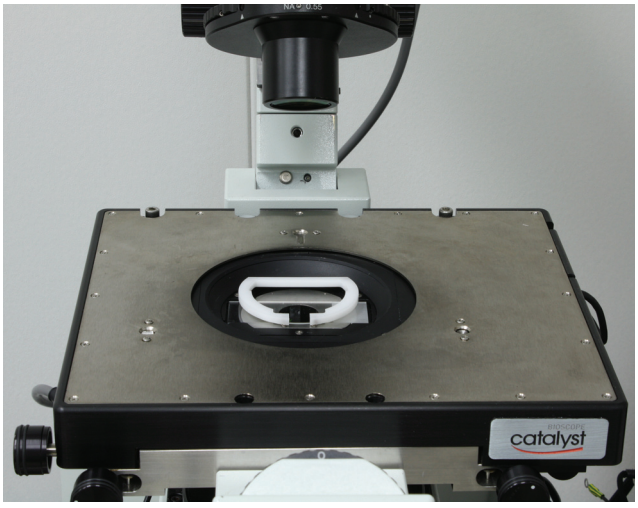
1. Place the glass slide into the base plate of the BioScope Catalyst, ensuring the sample side is face-up. See Figure 5.3b.

Figure 5.3b Glass Slide on the Baseplate of the BioScope Catalyst



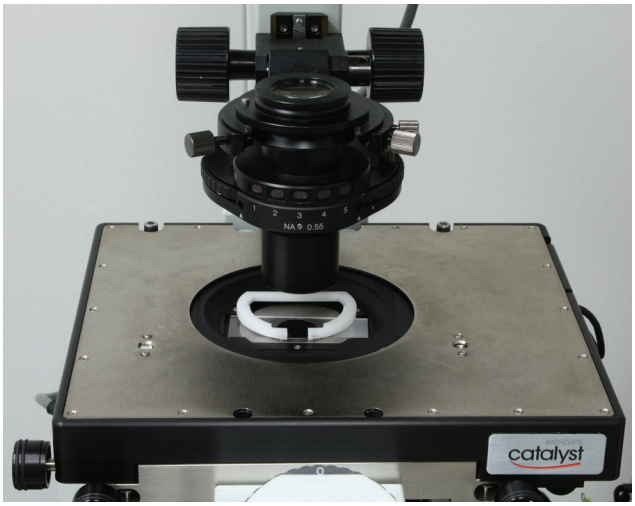
2. Place the sample holder on top of the glass microscope slide in order to lock the slide into place. See Figure 5.3c.

Figure 5.3c Sample Holder On Top of Glass Slide



3. View the sample surface by focusing with the optical microscope on the top of the sample slide. See Figure 5.3d

Figure 5.3d Optical Microscope In Place for Viewing of Sample Slide



4. View the desired area of interest.

5.3.2 More Information on Support Sample Types

For more information on supported sample types, please refer to **Supported Sample Types** Chapter 6.

5.4 Loading Probes

5.4.1 Loading a Probe In the Air Probe Holder

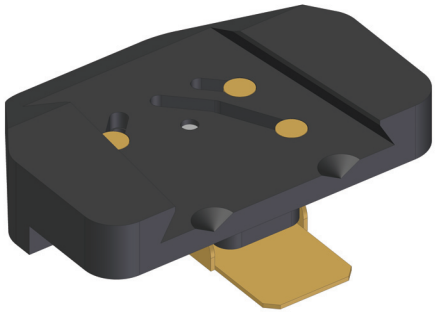
CAUTION: Probes are extremely fragile and should be handled touching only the substrate portion. Any physical contact with the cantilever or tip except during imaging is likely to damage the probe and render it useless.

The following components will be required in order to load a probe into the air probe holder:

- Air probe holder
- Probe stand

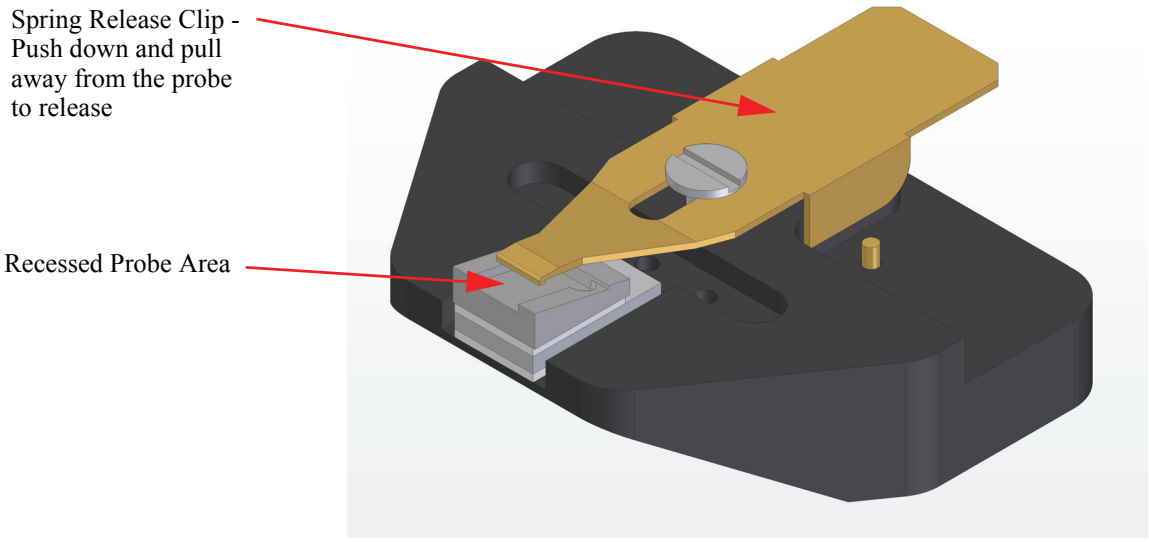
- Tweezers
- A few probes
- Optical microscope (recommended for new users)

Figure 5.4a Probe Holder - Dovetail Mounting Surface



1. Figure 5.4a and Figure 5.4b show the BioScope Catalyst probe holder. The surface shown in Figure 5.4a is the bottom dovetail that mounts onto the endcap of the z-scanner flexure of the head. The z-scanner mount has three gold plated contacts for connection to the corresponding posts on the z-scanner endcap.

Figure 5.4b Probe Holder - Probe Side



2. The probe is clamped in place by a spring loaded clip. The clip, identified in yellow in Figure 5.4b, is opened by pressing on the end of the spring release clip and pulling backwards away from the probe. Installing the probe under magnification is helpful, but experienced users will often mount the probe without magnification assistance. The probe is handled with sharp

tweezers touching only the substrate portion of the probe. If either the tip or cantilever are contacted by tweezers or other objects while handling, damage is extremely likely. The probe must be positioned carefully in the recess of the probe holder to ensure that it lies completely within the recess. It is recommended that the probe be positioned so that clearance is visible on both sides of the recess and as far back into the recess as possible but ensuring that the back edge of the probe remains within the recess.

3. It is typically easier to position the probe if the spring clamp is released to hold the probe, then gently nudge the substrate to position the probe as desired.

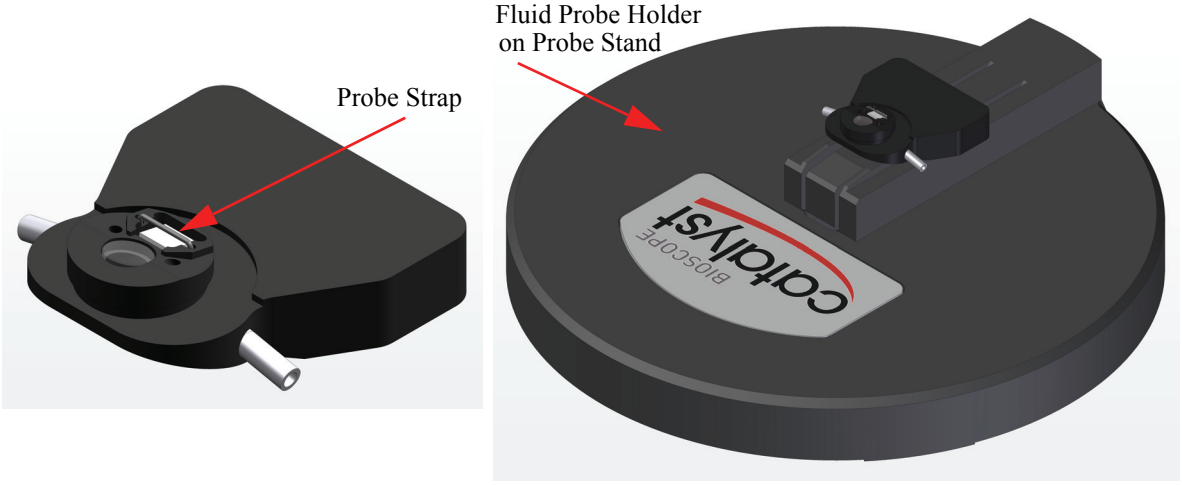
5.4.2 Loading a Probe Into the Fluid Probe Holder

CAUTION: Probes are extremely fragile and should be handled touching only the substrate portion. Any physical contact with the cantilever or tip except during imaging is likely to damage the probe and render it useless.

The following components, as seen in Figure 5.4c, will be required in order to load a probe into the fluid probe holder:

- Fluid probe holder
- Probe stand
- Tweezers
- A few probes
- A benchtop optical microscope (recommended for new users)

Figure 5.4c A Sampling of the Recommended Components for Loading a Probe into the Fluid Probe Holder



Probe Loading

1. Place the empty fluid probe holder onto the probe stand with the probe end facing towards the BioScope Catalyst logo. The fluid probe holder should be face-side up.

2. Slide the probe holder back (away from the Veeco logo) and press down gently. This will push the spring clip on the underside of the probe holder, allowing room for the probe to be inserted under the strap.

3. Using the tweezers, pick up a probe, being careful to only touch the sides of the cantilever.

4. Place the probe face up under the spring clip into the recessed area on the fluid probe holder. The probe must be positioned carefully in the recess of the probe holder to ensure that it lies completely within the recess. It is recommended that the probe be positioned so that clearance is visible on both sides of the recess and as far back into the recess as possible but ensuring that the back edge of the probe remains within the recess.

5. When you feel the probe is seated well, release pressure and set down the tweezers and gently pull the fluid probe holder off of the probe stand.

5.4.3 Mount Probe Holder onto Z-scanner Mount

1. Attach the probe holder with an etched silicon (tapping mode, TESP) probe onto the base plate of the z-scanner flexure of the head by sliding the cantilever holder firmly onto the dovetail. Ensure that the probe holder is pushed back securely.

Figure 5.4d Cantilever Holder Mounted on Scanner

.

WARNING: Invisible infrared laser may cause eye damage. Do not look directly at laser beam or at reflections from polished surfaces.

5.4.4 More Information on Probes

CAUTION: Silicon is extremely brittle.Be very careful to avoid any contact with the probe lever because it will immediately snap off.

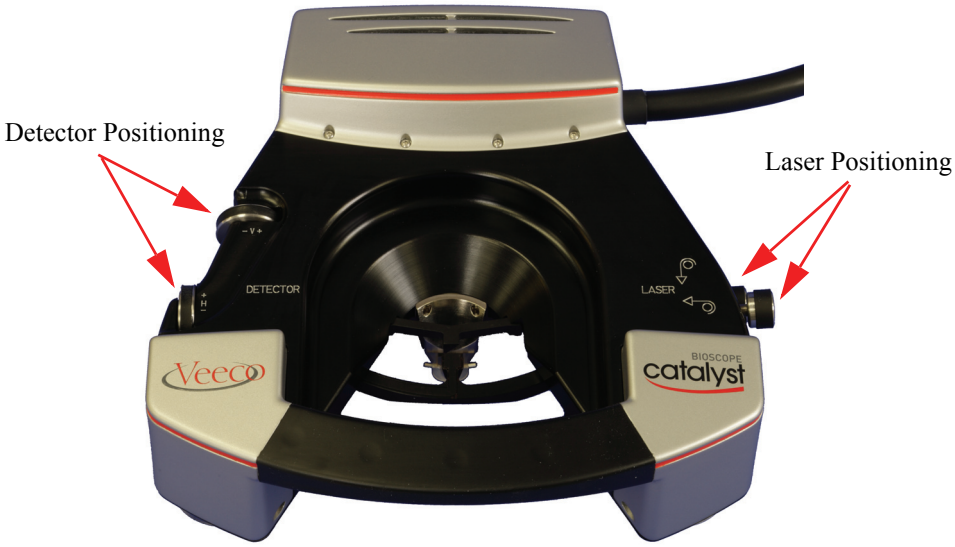
More information on probes and probe holders can be found in Chapter 7 and Chapter 8.

5.5 Aligning the Laser on the Probe

5.5.1 Coarse Align Laser

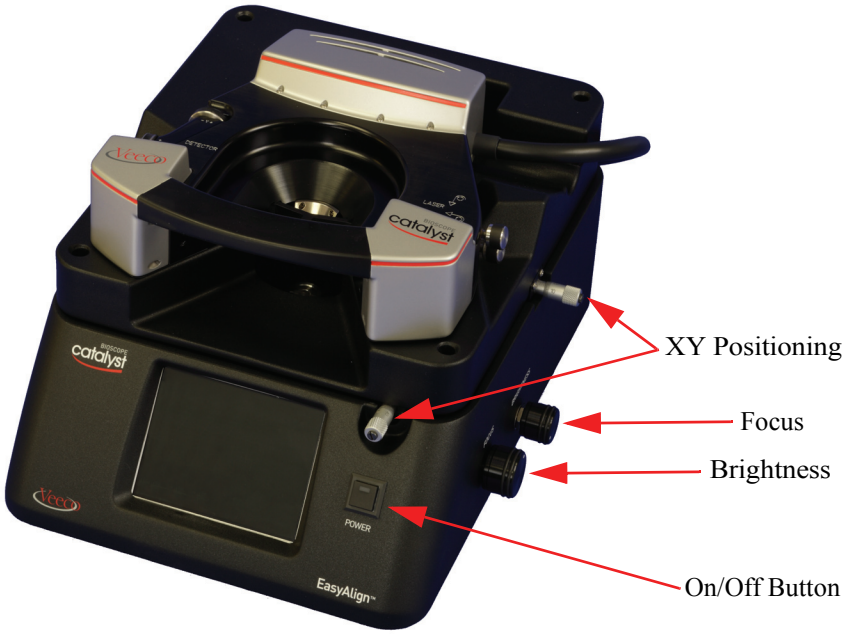
1. Figure 5.5a displays the laser control knobs located on the BioScope Catalyst head. Diagrams on the BioScope Catalyst head illustrate which direction the laser moves when turning the laser control knobs clockwise.

Figure 5.5a BioScope Catalyst Head Controls



EasyAlign controls and adjustments are shown in Figure 5.5b

Figure 5.5b EasyAlign w/BioScope Catalyst Head



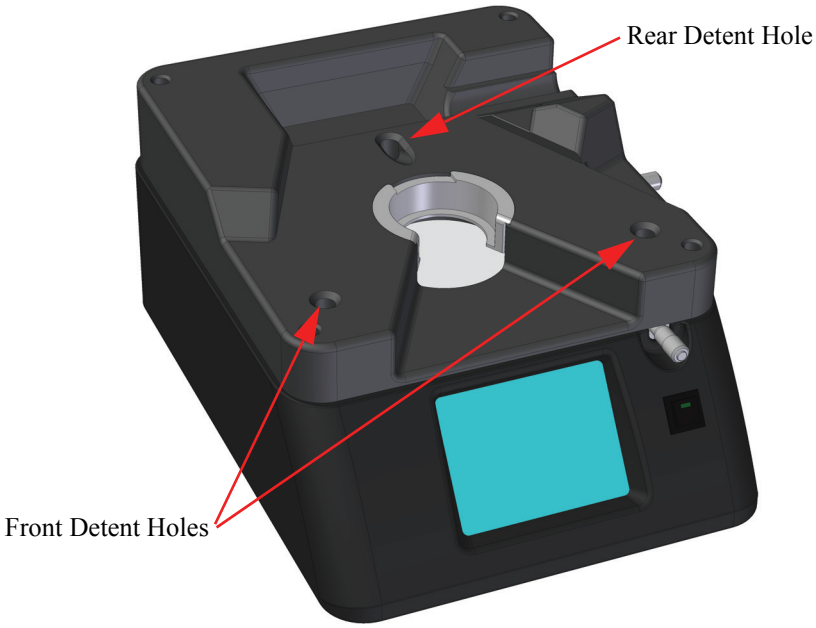
Note: Two styles of probe are commonly used with BioScope Catalyst:

- Silicon Nitride: Used in Contact Modeand Fluid Tapping. Four cantilevers (two per end of the probe) with the tip at the end of a triangle formed by two cantilever legs. When using this probe, it is important to ensure that it is properly installed so that the correct tip is one of the pair which are active and that laser alignment is made to the correct cantilever of the active pair.

- Etched Silicon: A single beam cantilever typically used in Tapping Mode imaging in air.

2. Place the head onto EasyAlign by positioning the rear “leg” of the head’s three point mount into the rear detent hole of the EasyAlign

Figure 5.5c EasyAlign Detent Holes



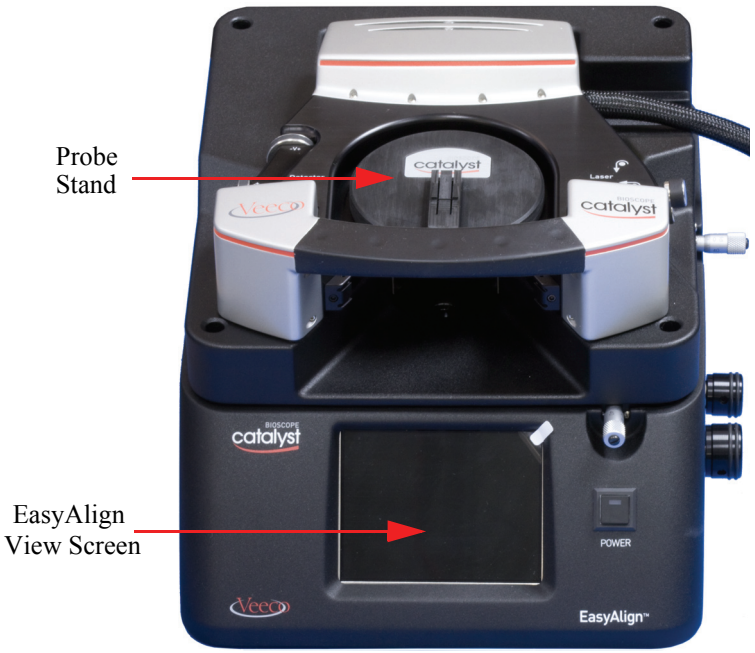
3. Lower the front end of the head so that the front two “legs” seat in the front two detent holes of EasyAlign.

4. “Wiggle” the head slightly to ensure that the head is properly seated in all three detents.

5. The on/off button is used to turn the EasyAlign on and off.

6. Place the probe stand on top of the head as show in Figure 5.5d. The probe stand will help to minimize stray light and enable better contrast on the EasyAlign view screen while the laser is being positioned on the cantilever.

Figure 5.5d Depiction of Probe Stand on Catalyst Head on Top of the EasyAlign



7. Adjust brightness and focus using the control knobs on the side of EasyAlign (ref. Figure 5.5b).

8. Adjust the XY position using the EasyAlign micrometer adjustments (ref. Figure 5.5b) to display the probe in the approximate center of the LCD screen.

9. Use the laser positioning controls on the head (ref. Figure 5.5a) to position the laser spot in the LCD display and onto the tip of the probe. On the E-Box LCD display, SUM should increase to indicate the laser is somewhere on the probe.

   Note: Sum may not increase if the photodetector alignment is badly adjusted. It may be necessary to adjust detector positioning (ref. Figure 5.5a) controls until HORIZ and VERT values on the E-box respond to the adjustments and to repeat Step 10.

10. Coarse alignment is completed. Normal optimum alignment is with the laser spot centered on the cantilever and as close to the tip as possible (some variation is used for special applications). Fine alignment will be performed using information on the E-Box LCD and is described in Section 5.5.3.

5.5.2 Sample/Probe Alignment

This section describes alignment of the probe and sample using an inverted optical microscope. This procedure aligns the optical view with the sample and probe. Although the basic alignment adjustments apply for a system without an optical microscope, the alignment will be very approximate and limited by the ability of the user to locate probe tip and sample using whatever

visual aids are available. If your system does not include an inverted microscope or the sample cannot be viewed in the optical microscope (viz. opaque sample), some suggestions are included [in brackets] on how achieve acceptable results without a microscope. Without a microscope, a magnifying glass or other magnification method will be helpful.

1. On the PC, open the **Navigate** window.

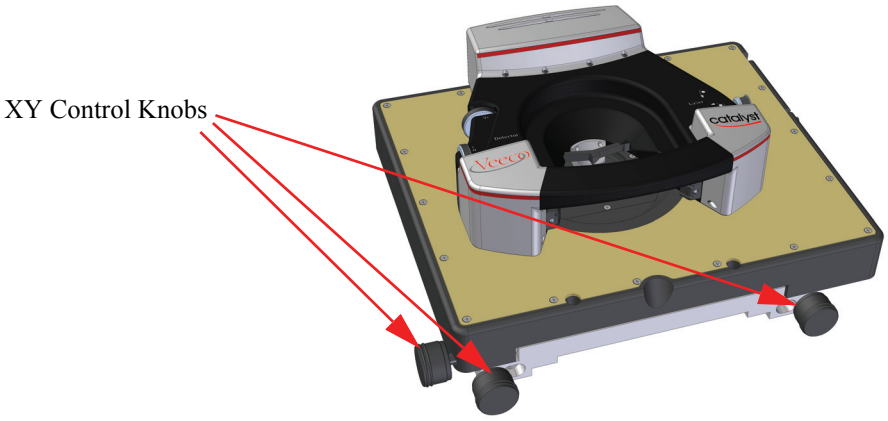
   Note: The arrow tools in the **Navigate** window may be used for positioning instead of the joystick description in the following sections.

2. Focus the microscope on the sample (refer to the microscope manufacturer's instructions). [Ignore this step if there is no microscope.]

3. Lower the probe using the joystick control until the probe is visible in the optical microscope but still out of focus. [Lower the probe but take care that it does not touch the sample.]

4. (Optional) To align the tip with the inverted microscope optics, use the XY control knobs on the baseplate as needed to position the probe in the approximate center of view of the optical microscope. [Ignore this step if there is no microscope.]

Figure 5.5e XY Knobs on Stage of Inverted Optical Microscope



5. Use the joystick without the button (x = left/right, y = up/down) to position the sample so that the area to be imaged is seen on the optical microscope to be beneath the probe tip. [Look through the opening in the head to position the sample.]

5.5.3 Laser Alignment - Fine

The purpose of fine laser alignment is to position the laser spot to be centered on the cantilever and as close to the tip as possible without loss of reflected (SUM) energy. This positioning provides optimum sensitivity (alignment may vary slightly for special applications). Fine alignment makes positioning adjustments based on readings on the E-Box LCD display. The head may be left on the EasyAlign.

1. While watching the E-box LCD display, adjust the x-axis laser positioning knob to maximize the value displayed for SUM. The maximum value indicates the laser spot is well centered on the cantilever

Figure 5.5f E-Box Front with Readings for Contact (Left) and Tapping Modes (Right)

2. Continue to watch the E-Box LCD readout and, using the x and y-axis laser position control knobs, adjust the laser spot position toward the tip until SUM cannot be kept at maximum. This indicates the laser spot is slightly off the cantilever.

3. Readjust the x-axis control to maximize Sum.

4. Readjust the y-axis control the minimum needed to maximize Sum.

5. The previous two alignment adjustment have optimized (for normal use) the laser alignment. Fine laser alignment is complete and the head is ready for photodetector adjustment.

5.6 Aligning the Photodetector

5.6.1 General Procedure

This procedure positions the reflected laser spot onto the center of the photodetector. Off center alignment may be useful for some special applications, however, the center position (HORIZ = 0, VERT = 0 in Tapping Mode) is most common and generally provides good results. (A typical setting for Contact Mode is HORIZ = 0, VERT = -2 which tends to place the spot in the center of the photodetector when the cantilever is deflected by contact.)

1. Make detector adjustments on head so E-Box HORIZ is zeroed then make adjustments to zero VERT. (A visual representation of photodetector alignment is displayed on the PC screen depicting a black box w/cross hairs).

5.7 Ensuring Adequate Tip-Sample Clearance

Before starting the procedure it is recommended that the BioScope Catalyst be in the following configuration:

- A probe is mounted onto an air or fluid probe holder which is mounted onto the head
- The head is in the upright position in the EasyAlign

5.7.1 General Procedure

1. Click on the **NAVIGATE** button to begin the navigation process. You can use either the joystick or the mouse and monitor to navigate in this procedure.

2. Before placing the head on the stage it is essential to move the head up to ensure there is sufficient sample clearance. You can move the head up by holding the Z button down on the joystick while simultaneously pushing the joystick up (towards the cord) or by doing the same with the mouse and corresponding up button on the monitor.

Figure 5.7a Joystick and Monitor Controls

3. Getting sufficient sample clearance is not an exact science. The goal is have enough clearance such that when you place the head on the stage you can visually ensure that the probe will not contact the sample when all 3 alignment pins are flush on the base plate. In this procedure it is better to have more than enough clearance, as failure to provide enough clearance can result in a broken probe or damage to the sample. One can always retract the head all the way back, however, this will make the engage process much slower. Once you are sure that the head is retracted sufficiently, proceed to step 4.

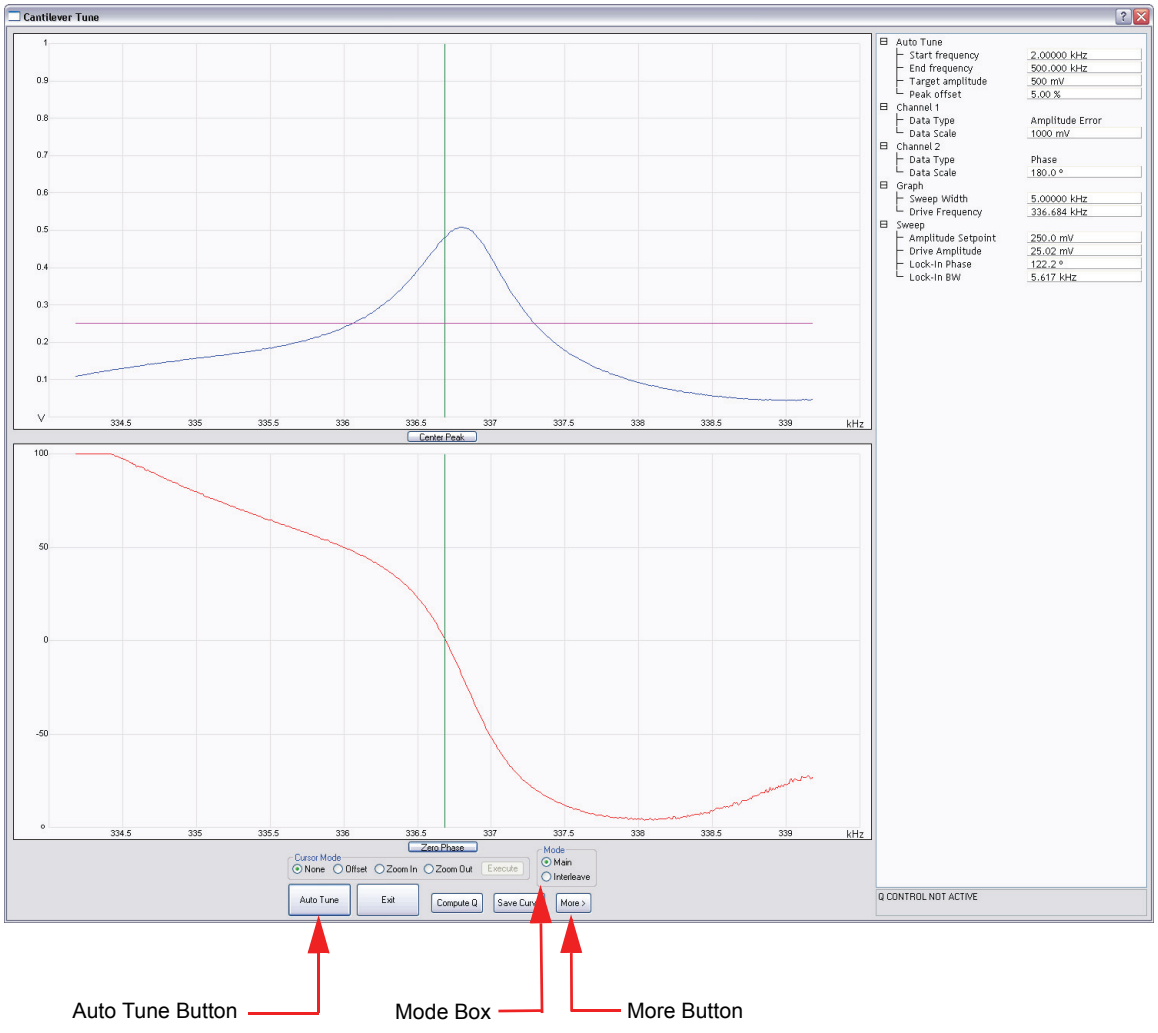
4. Place the head on the stage, inserting the back alignment pin first. With the first alignment pin in place, carefully lower the head to the base plate from back to front, noting whether there is enough sample clearance. If you are in doubt, it is best to put the head back into the Easy Align Cradle to retract the head some more, and then repeat this step. If you are sure there is adequate sample clearance, proceed with final placement of the head and the 2 front alignment pins. Gently wiggle the head to ensure it is seated properly.

5.8 Cantilever Tuning

This step is needed for tapping mode in air operation only. In TappingMode in air, the cantilever is mechanically oscillated at a frequency slightly below its mechanical resonant frequency (in the typical/weak flexing direction). Tuning is performed to determine the proper oscillation frequency.

1. In the **Workflow Toolbar**, click the **TUNE** icon.
2. In the new window that appears, click **AUTO TUNE** and wait for autotune to finish.
3. The **Cantilever Tune** window can be modified to: **change range**, **auto scale**, **change sweep limits**, **change dc offset**, etc. as desired.

Figure 5.8a Cantilever Tune Window



4. Close the **Cantilever Tune Window**

5.9 Understanding the Three XY Positioning Stages

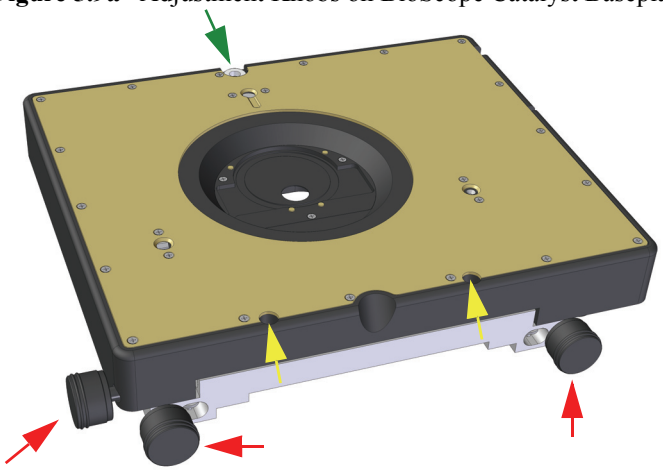
5.9.1 Overview

The BioScope Catalyst baseplate contains three XY positioning stages: the XY piezo scanner (>150µm range), a motorized XY sample positioning stage (+/- 10mm range), and a manual stage for alignment of the AFM and optical axes.

5.9.2 Aligning the SPM Axis with the Optical Axis

The AFM probe must be aligned with the optical axis of the microscope such that the probe is near the center of the optical field of view. This is accomplished using the three adjustment knobs on the Catalyst base plate, shown below in Figure 5.9a (red arrows):

Figure 5.9a Adjustment Knobs on BioScope Catalyst Baseplate



Before adjusting the alignment, be sure that the back base plate mounting screw(s) are loose (green arrow). Some baseplates have two screws along the back edge. Do not loosen the front recessed screws (yellow arrows). Then place the head, with probe installed, on the base plate and lower the head enough that you can focus on the probe. Initially it will be easiest to find the probe with a low magnification, 5X or 10X, objective. Using the three knobs, align the probe in the center of the field of view. The two front knobs should be turned together to move the stage forward and backward. Used individually, they rotate the stage. The left side knob moves the stage left and right. If you reach a stage motion limit before the probe is centered, it may be necessary to loosen the front mounting screws (yellow) and move the stage slightly in the required direction. Then retighten those screws and try adjusting again with the knobs. Once aligned with the low magnification objective, you may wish to fine tune the alignment with a high magnification objective. Once finished, tighten the back mounting screw(s).

Once initially aligned, only minor realignment should be necessary after exchanging probes, particularly when working with very high magnification objectives.

5.9.3 Aligning the SPM Axis with the Desired Imaging Location

Aligning the SPM axis with the desired imaging location can be done using the joystick or on screen control panel. Click on the **NAVIGATE ICON** and navigate to the preferred imaging location using the joystick or arrows on the control panel. Once at the desired location, proceed to the Engage section.

5.10 Engaging

5.10.1 Engage

This procedure lowers the probe to bring the probe tip into imaging position. The following procedure applies primarily to a BioScope Catalyst mounted on an inverted microscope. If your system does not include an inverted microscope, some suggestions are included [in brackets] on how achieve acceptable results without a microscope.

The Navigate and Real Time Video windows must be open. To open the **NAVIGATE WINDOW** click on the **NAVIGATE** icon in the **Workflow Toolbar**.

To open the **Real Time Video** window, click on the **VIDEO** icon. The joystick or monitor controls found in Figure 5.7a may be used for navigation.

1. Focus the optical microscope on the sample.

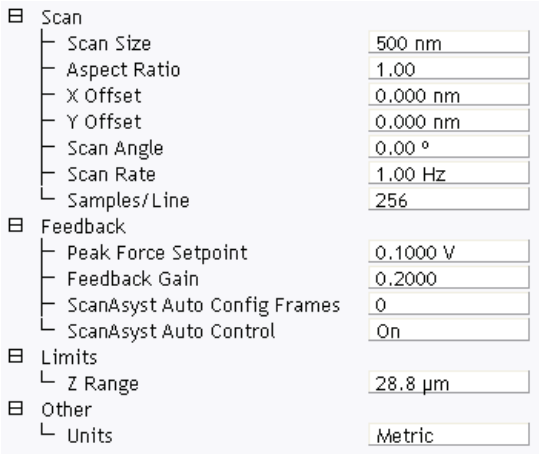
2. Hold the joystick "Z" button and move the probe closer to the sample by moving the joystick down (away from the cord connection). With the optical microscope, the image of the probe will begin to come into focus as the probe approaches the sample. The approach should be stopped well before the image of the probe becomes focused otherwise the probe will contact the sample and both may be damaged (probably destroyed) and require replacement. [It will be difficult to determine when the probe is close to the sample while looking through the head opening.] The liability of having the probe spaced far from the sample is that engaging will require more time than if the initial position is closer.

3. Click on the **SCAN WINDOW** icon.

4. Scan parameters are shown in the **SIMPLE MODE** (Green Puzzle Piece Icon). Alternatively, you can go into the **EXPANDED MODE** by clicking on the Red Puzzle Icon.

5. Check settings. Figure 5.10a shows settings that are good starting values.

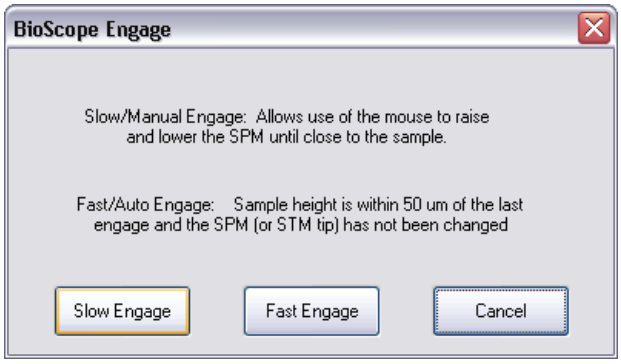
Figure 5.10a Initial Settings



6. When the probe has been positioned, click the **ENGAGE** icon located in the **Workflow Toolbar** (down arrow above a representation of the probe).

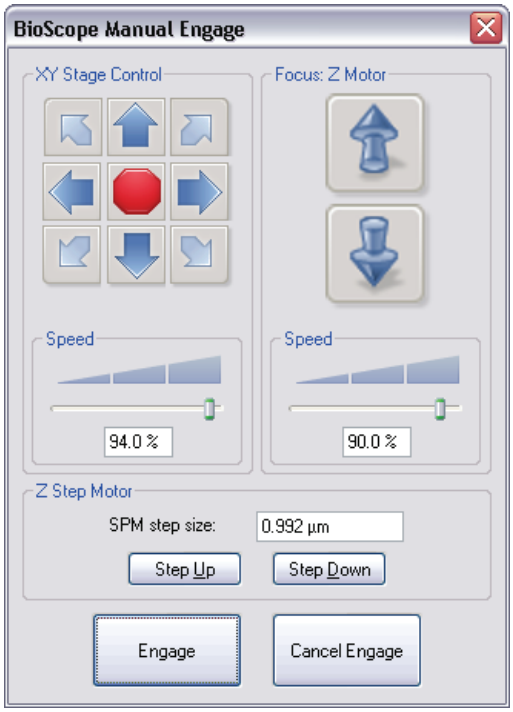
7. An **Engage** window, shown in Figure 5.10b, will open with the choices of **Slow Engage**, **Fast Engage** or **Cancel**. If this is the first time you are engaging on this sample, choose **Slow Engage**. The **Fast Engage** option should be used only if you have previously engaged the same tip on the same spot on the sample.

Figure 5.10b Initial Engage Window



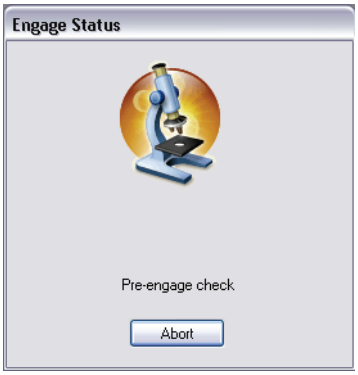
8. The **Final Engage** window, shown in Figure 5.10c, will open. This engage window includes navigation controls which allow the user to make final adjustments of tip to sample positioning prior to engaging. Once these final adjustments are complete, click **ENGAGE.**

Figure 5.10c Final Engage Window



9. The **Engage Status** window will open while the probe is lowered into imaging position.

Figure 5.10d Autoengage Screen



After Autoengage has completed, the window will close and scanning (imaging) will begin.

5.11 Optimizing Your Image

5.11.1 Adjusting the Amplitude Setpoint

The **Amplitude Setpoint** influences the tip/sample force that is maintained during scanning. It tells the feedback loop what amplitude to maintain during scanning. Set this parameter so that the smallest amount of force is applied during scanning while still maintaining a stable engagement on the surface. If the setpoint is too close to free air amplitude, the tip will not trace the topography properly.

The **Amplitude Setpoint** parameter can be adjusted by clicking on the **Amplitude Setpoint** field, as shown in Figure 5.10a. The choices are to manually enter a new setpoint by typing on the keyboard or make incremental changes by pressing the arrow right key to increase the setpoint and the arrow left key to decrease the setpoint.

5.11.2 Integral & Proportional Gains

The **Integral Gain** controls the amount of the integrated error signal used in the feedback calculation. The higher this parameter is set, the better the tip will track the sample topography. However, if the **Integral Gain** is set too high, noise due to feedback oscillation (Ringing) will be introduced into the scan, as seen in Figure 5.11b.

Proportional Gain controls the amount of proportional error signal used in the feedback calculation. Typically, it can be set to 1-2X the **Integral Gain**.

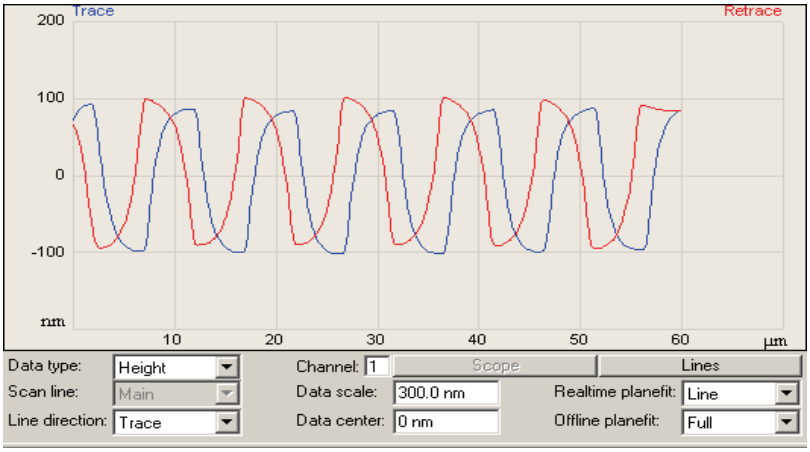
Integral Gain and **Proportional Gain** settings are adjusted to produce feedback to produce optimum images. Feedback is generally more sensitive to the **Integral Gain** setting than to the **Proportional Gain** setting. Gain settings are adjusted while observing the scope trace. Optimum gain settings (for most applications) produce trace and retrace images that mirror each other and are representative of the sample.

5.11.3 Set Gains

Note: If scope trace images are absent or obviously incorrect, it may indicate the probe is not properly engaged (possibly damaged). Reduce setpoint value to attempt to produce a usable trace.

1. In the example found in Figure 5.11a, the sample is a calibration grid consisting of vertically walled pits. The image shows poor gain adjustment.

Figure 5.11a Poor Gain Adjustment

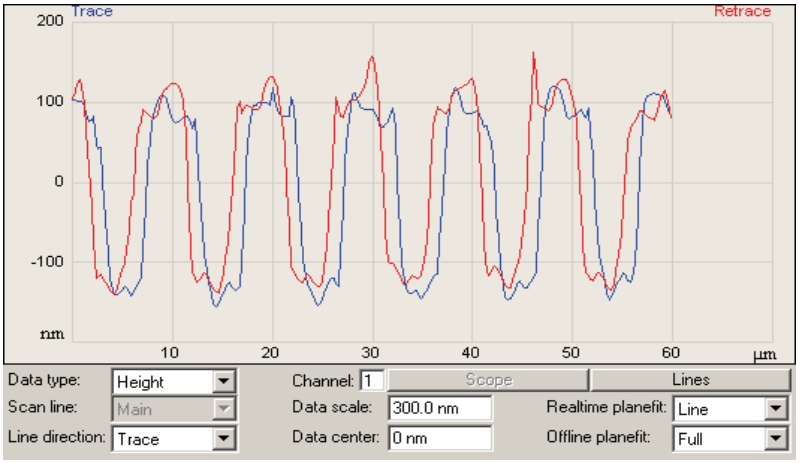


2. Change the **Scan Size** (**Scan Parameter List**>**Scan**>**Scan size**) as required to scan across features of interest.

3. To make gain setting easier, disable the **Slow Scan Axis** (**Scan Parameter List**>**Scan**>**Slow Scan Axis**) so that the same line is being scanned and re-scanned.

4. Increase the value of **Integral Gain** (**Scan Parameter List**>**Feedback**>**Integral Gain**) until the scope trace shows evidence of ringing (flat portions have “fuzzy” noise).

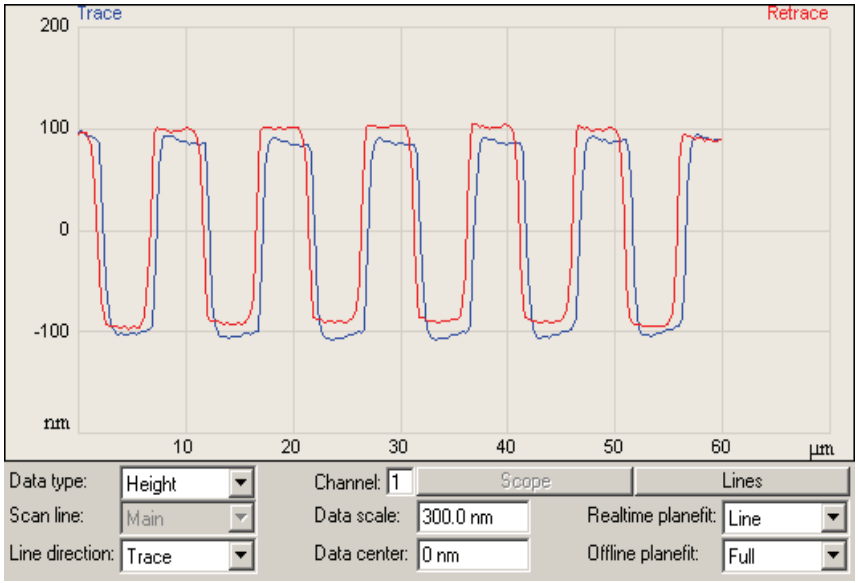
Figure 5.11b Gain Too High (Ringing Present)



5. Decrease the value of **Integral Gain** slightly until ringing stops.

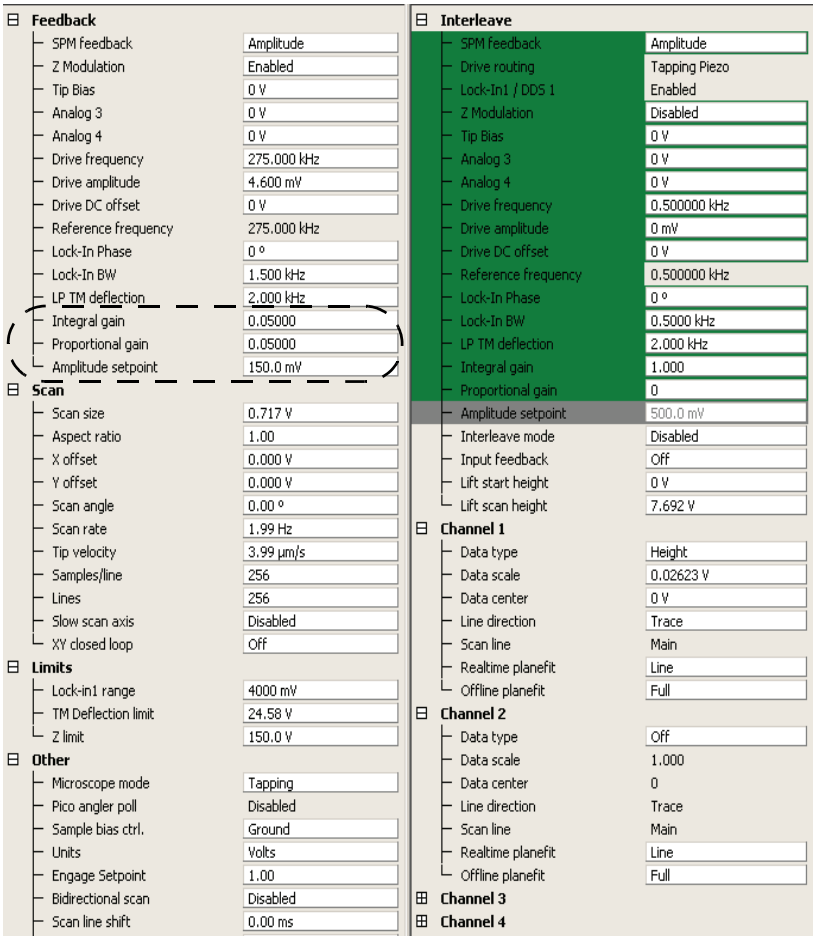
6. Adjust values of **Proportional Gain**, usually 2 to 10 times the **Integral Gain** value, (**Scan Parameter List**>**Feedback**>**Proportional Gain**) to optimize the image profile so that trace and retrace profiles appear similar to each other. Note that the image represents the known features of the sample.

Figure 5.11c Gains Set



7. Some adjustment of setpoint (**Scan Parameter List**>**Feedback**>**Amplitude Setpoint**) may also improve the trace profile.

Figure 5.11d Adjust Gains and Setpoint



5.11.4 Adjusting Scan Size and Offset

Scan Size adjusts the size of the X, Y scan area.

X Offset and **Y Offset** translate the scan area in X and Y directions without changing scan size (i.e X and Y Offset move you to a new scan area).

The **Scan Size** and X and **Y Offset** parameters can be adjusted by clicking on the **Scan Size** and X and **Y Offset** fields in the **EXPANDED MODE** window. The choices are to manually enter a scan sizes/offsets by typing on the keyboard or to make incremental changes by pressing the arrow right key to increase and the arrow left key to decrease each parameter.

5.11.5 Adjusting Scan Rate

The **Scan Rate** is the number of trace and retrace lines performed per second (Hz).

For example, with a **Scan Rate** set to 1 Hz, the tip will scan forward and back (trace and retrace) in 1 second.

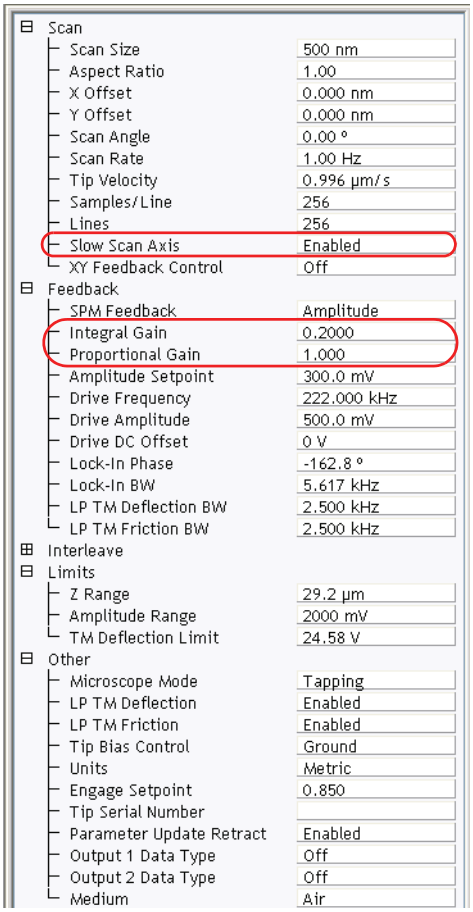
Scan rate should be set such that the feedback loop has time to respond to changes in the sample topography. Setting the **Scan Rate** too high will result in poor tracking of the surface.

The **Scan Rate** parameter can be adjusted by clicking on the **Scan Rate** field in the **SIMPLE MODE** or **EXPANDED MODE** window. The choices are to manually enter a **Scan Rate** by typing on the keyboard or to incrementally change the **Scan Rate** by pressing the arrow right key to increase and the arrow left key to decrease **Scan Rate**.

5.11.6 Produce Image

After a satisfactory profile image has been obtained, change the scan parameter settings to produce a scanned image in the scan windows.

Figure 5.11e Imaging Settings



Real-time Scanning Parameter Changes

1. **ENABLE Slow Scan Axis** to produce an image of the sample surface.

2. Adjust the image location either by entering **X offset** and **Y offset** values in the corresponding fields or by clicking on the **OFFSET BUTTON** below the scan and dragging on the scan image.

3. Adjust the size of the scan by changing the **SCAN SIZE** value in the **SCAN SIZE** field.

4. Clicking on the **ZOOM** tool button and drag on the image to zoom. (To unzoom, you must change the Scan size). Image location can be changed using the **OFFSET** or **PAN** tool buttons. Measurements can be made on the image using the **MEASURE** tool button.

5. Other parameter changes may be made to change scan rates and angles.

Figure 5.11f Additional Tool buttons

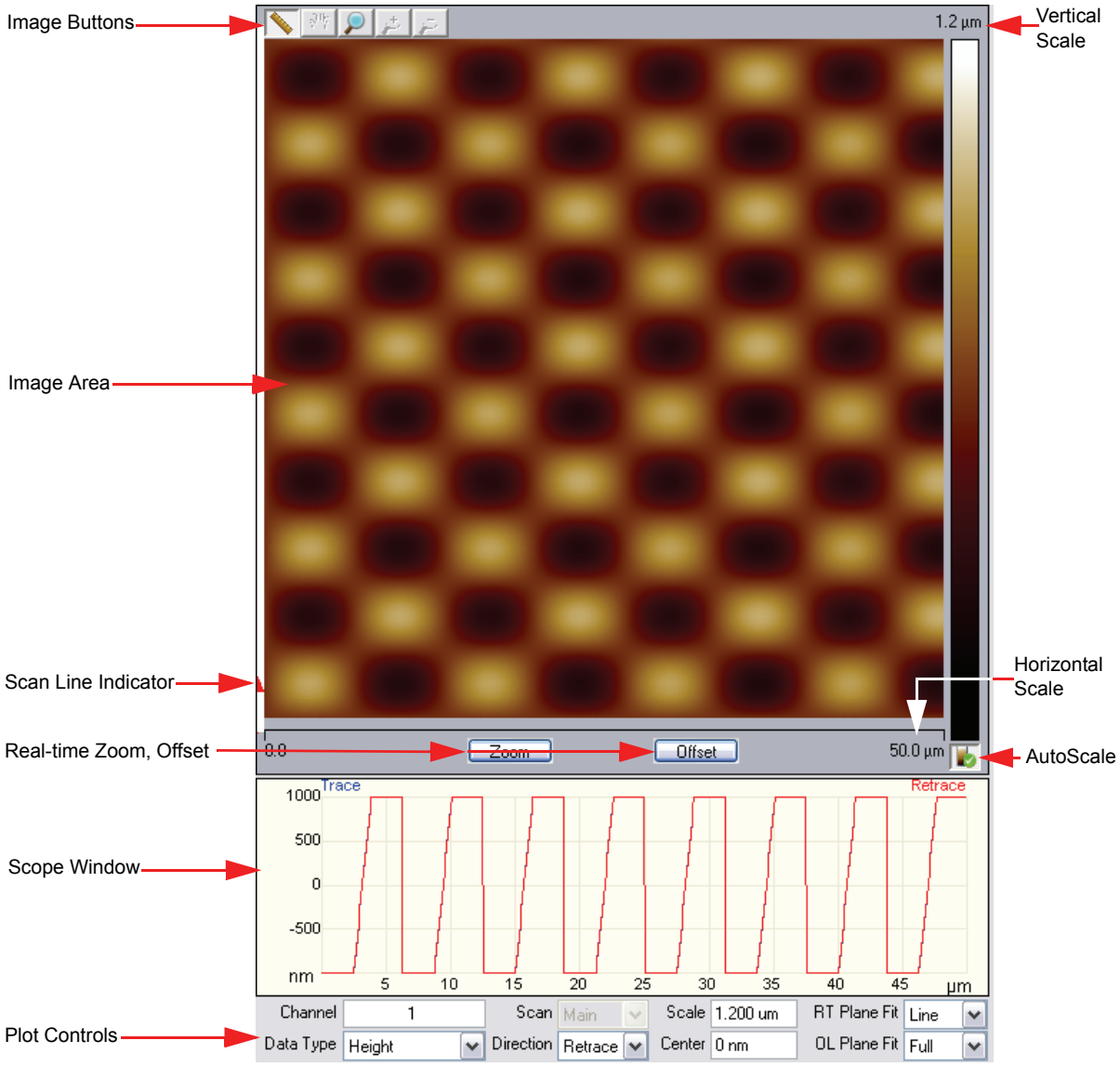


Image Area	The SPM image is displayed here.
Image Buttons	The image buttons, described below, allow pan, zoom and measurement functions.
Scan Line Indicator	An arrow showing the current scan line.
Vertical Scale	The vertical scale of the image.
Horizontal Scale	The horizontal scale of the image.
Real-time Zoom, Offset	Zooms and Offsets the scan. To Unzoom, you must change the **Scan Size**
Scope Window	Displays a real-time plot of the channel signal.
AutoScale	Automatically scales the data. Refer to the *NanoScope User Guide* for details
Plot Controls	Provides control of what is plotted in the image area.

The NanoScope image buttons, shown in Figure 5.11g, are described in Table 5.11a. These buttons operate on the scanned data and do not affect the real-time scanning. Real-time scanning can be controlled with the real-time Zoom and offset buttons, shown in Figure 5.11h.

Figure 5.11g NanoScope image buttons

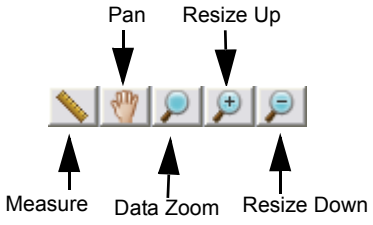


Table 5.11a NanoScope image buttons

Measure	Allows you to draw a box to make measurements, translate the image or offset and resize the image.
Pan	From a zoomed image, you can pan around to other areas of the original image.
Zoom	Allows you to draw a box to zoom in on an image.
Resize Up	Resizes the image up to the previous zoom level.
Resize Down	Resizes the image down to the previous zoom level.

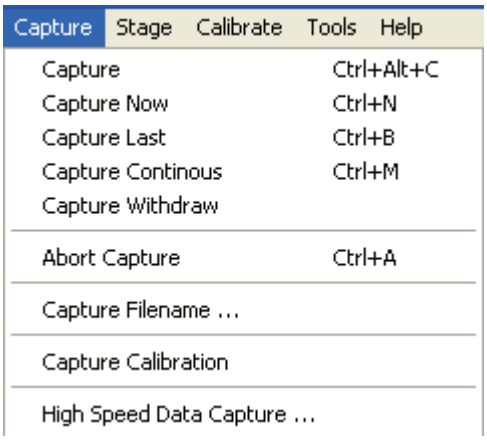
Figure 5.11h Real-time Zoom and Offset buttons

5.12 Capturing Images

A scanned image can be saved as a file which can be retrieved either as an image or as the underlying data which was used to produce the image.

1. Select (Menu items) **Capture - Capture Filename**.

Figure 5.12a Capture Menu



2. Specify file location and filename and click **CAPTURE**

3. Thumbnails of captured images can be seen by navigating to the location of the saved files using **View-Browse** in the Browser window.

4. Other Capture options include:

a. **CAPTURE NOW** - Captures the current image immediately and usually results in an incomplete image.

b. **CAPTURE LAST** - Captures the last completed images and is useful if a capture was desired but not taken before the next image was begun.

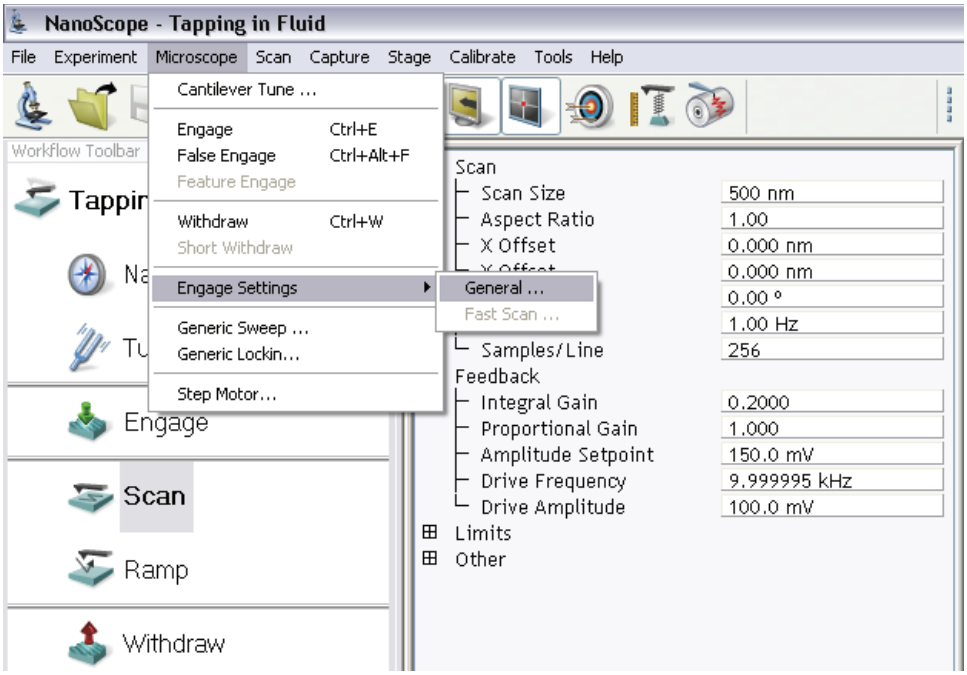
c. **CAPTURE CONTINUOUS** - Automatically captures every completed image until **ABORT CAPTURE** is selected in the menu.

5.13 After the Experiment

5.13.1 Withdrawing

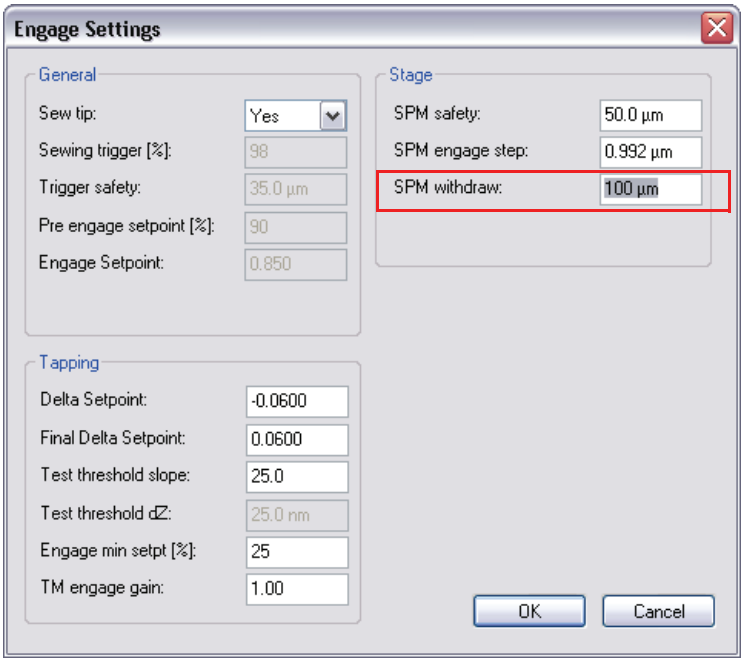
1. Go to the Workflow Toolbar and click on the **WITHDRAW** Icon.
2. The BioScope Catalyst will retract the probe to the default distance (100um).
3. This withdrawal default distance can be selecting **Microscope > Engage Settings > General** as described in Figure 5.13a.

Figure 5.13a Pull-down Window to Change Withdrawal Default Distance



4. The **Engage Stage Monitor Settings** window, shown in Figure 5.13b**,** appears.

Figure 5.13b Engage Monitor Settings Window with SPM Withdraw Box Highlighted



5. **SPM withdraw** can be changed in the highlighted box in Figure 5.13b.

5.13.2 Routine Cleaning

Major Components

CAUTION: If water or alcohol is used in cleaning the electronics portions of the BioScope Catalyst system, use only the minimum amount needed to lightly dampen a wipe. In no case should any liquid be applied directly to any part of the electronic equipment.

The BioScope Catalyst system is essentially a collection of electronic devices. Use the same care as with any laboratory electronics equipment. Cleaning is performed using soft, lint free wipes which may be used dry or lightly dampened with water or isopropyl alcohol.

Baseplate:

1. Remove the head from the baseplate and place it in the upward position on the EasyAlign.
2. As required, use soft laboratory wipes to clean away debris from the baseplate surfaces and around the control knobs.
3. Wipe the surfaces of the baseplate to remove any remaining debris or contaminants.

Head:

1. Remove the probe holder from the z-scanner mount.
2. As required, use soft laboratory wipes to clean away debris from the head surfaces, mounting points and around the control knobs.
3. Wipe the surfaces of the head to remove any remaining debris or contaminants.

Probe Holder:

CAUTION: Do not scratch the glass window while cleaning the probe holder.

1. Remove and store the probe appropriately.
2. Wash in a mild soapy solution.
3. Rinse in distilled water and (blot and blow) dry thoroughly.

Optical Microscope:

Refer to manufacturer's recommendations.

E-Box:

1. Leave cables connected.
2. As required, use soft laboratory wipes to clean away debris from the E-box surfaces with particular attention given to vent holes on the top cover.
3. Wipe the surfaces of the box to remove any remaining debris or contaminants.

EasyAlign:

1. Remove BioScope Catalyst head if required.
2. As required, use soft laboratory wipes to clean away debris from the EasyAlign surfaces and control knobs with attention given to the three head mounting detent holes.
3. Wipe the surfaces of the box to remove any remaining debris or contaminants.

Vibration Table:

Wipe down surfaces.

Controller:

1. Leave cables connected.
2. As required, use soft laboratory wipes to clean away debris from the controller surfaces with particular attention given to vents and fan covers.
3. Wipe the surfaces of the controller to remove any remaining debris or contaminants.

PC System (PC, Monitors, Mouse, Keyboard, Joystick)

Wipe down surfaces.

Other Components:

Sample Substrates (Slides, Cover Slips, Petri Dishes):

User defined procedures.

Sample Substrate clamps and Perfusion Cell:

1. Wash in isopropyl alcohol and a mild soapy solution.
2. Rinse in distilled water and (blot and blow) dry thoroughly.

Procedure for Clean-up of Spills:

The BioScope Catalyst is designed to permit analysis of samples in fluids but is subject to damage by fluid spill or spatter which has not been confined to the sample. Spills and splatter must be cleaned up before resuming analysis.

It is assumed that the user is aware of health and safety hazards associated with the fluids in use and will exercise appropriate safety and handling procedures during clean-up and disposal of spillage.

If a spill occurs, clean-up is performed in three stages depending upon the severity of the spill: Immediate action, Fluid removal and Final clean-up.

Immediate Action:

These are the initial actions to prevent spread of the spillage and put the AFM into a safe condition. Some of the actions may not be required for minor spills or spatter. Generally, the petri dish and clamp will provide a partial seal to slow migration of the spilled fluid and should not be removed until later in the clean-up process.

1. Stop the cause of spillage. Discontinue adding fluids, remove the fluid source.

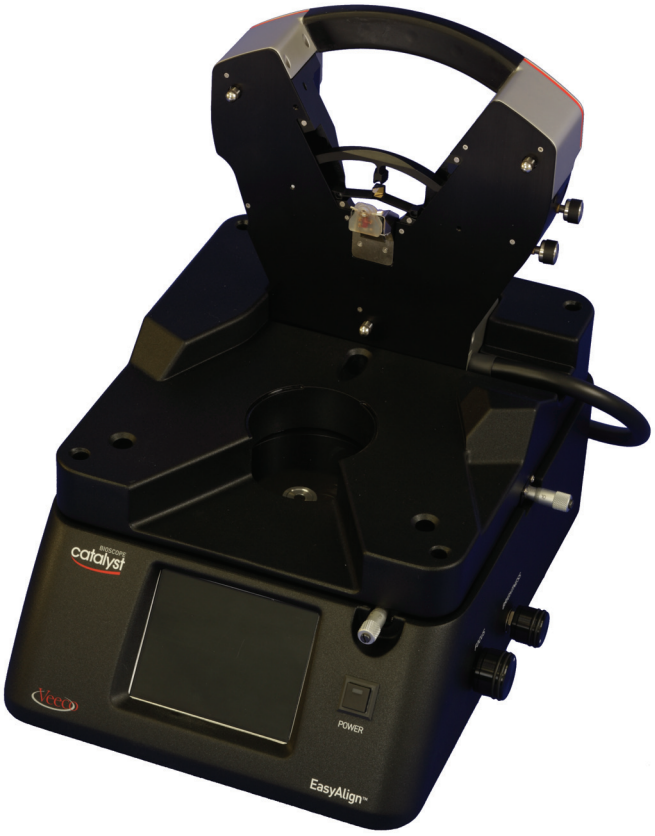
2. Using PC controls, stop any imaging and disengage the probe.

3. Shut down the NanoScope V controller and the external DC power supply connected to the E-box to remove power to the AFM.

4. Protect the inverted microscope (if installed). Retract the objective (turret) fully away from the sample and cover with an absorbent cloth/wipe. (A fluid impervious layer between the microscope and absorbent layer would be ideal for spills which could allow significant amounts of fluid to contact the microscope).

5. Remove the BioScope head (if in place), blot fluids to prevent running and place the head in the resting position on the EasyAlign.

Figure 5.13c Head in Resting Position on EasyAlign Cradle



Fluid removal:

The purpose of this stage is to remove obvious puddles or drops of fluid without spreading the fluid. Scrubbing, wiping or brushing should not be done during this stage. Air hoses or compressed air blowers should not be used.

1. If available, use a pipette or small lab vacuum to remove as much fluid as possible. Absorbent cloths/sponges/wipes/cotton swabs may be used for fluid removal but should be used to wick (gently blot up) and not wipe (spread the location of) the fluids.

2. As appropriate, remove fluid from the petri dish to avoid spillage when the dish is removed.

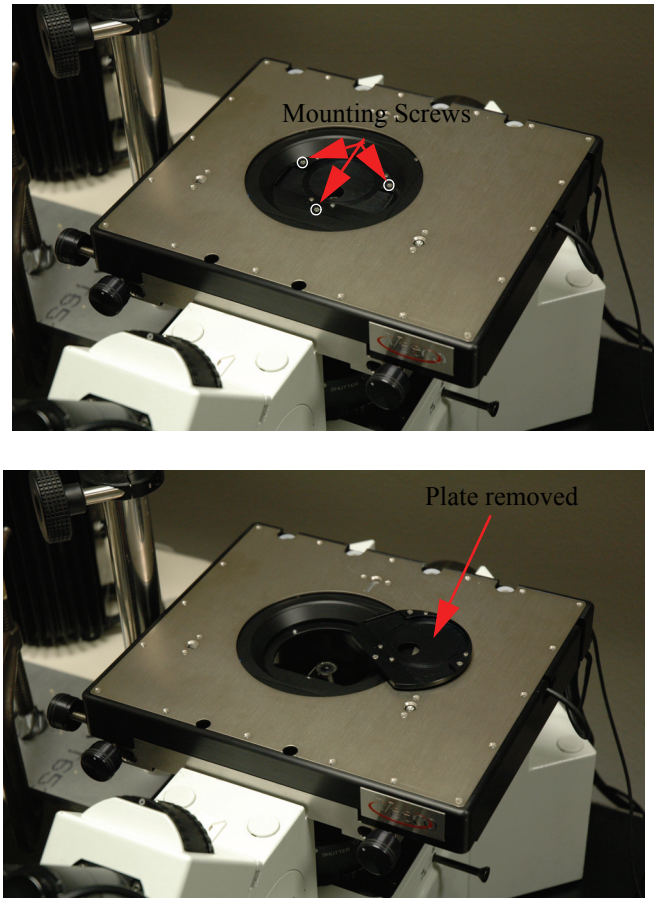
3. Remove the petri dish and substrate clamp and place them on an absorbent cloth/wipe for later attention.

4. Continue to remove fluid from the stage using the methods described earlier.

5. Remove the sample holder plate from the AFM stage by removing 3ea #1 phillips flathead (#2-56 UNF) screws and place the plate and screws on an absorbent cloth/wipe for later attention.

For reference, the figure shows the plate resting on the stage, however, a plate removed for spillage removal should be moved to an absorbent material away from the AFM.

Figure 5.13d Sample Holder Plate Removal



6. Continue to remove fluid from the AFM stage and sample holder plate cavity.

Final Clean-up:

After all obvious fluid has been removed, affected surfaces are cleaned and the AFM reassembled.

1. Use absorbent cloth/wipes/cotton tipped swabs/etc. to remove remaining fluid film from the AFM, Sample Holder clamp, petri dish, sample holder plate and mounting screws. Pay special attention to narrow crevices, small orifices and sharp corners.

2. Permit the AFM to air dry (no compressed air blow off) for an appropriate time which will depend upon the severity and extent of the spill. A minimum 1 hour is recommended for most spills but some spills may require 12 or more hours, depending upon environmental conditions, to insure adequate drying time.

3. If fluids have contacted the inverted optical microscope, refer to the manufacturer's instructions for cleaning.

After all parts have air dried, reassemble the AFM and resume analysis. Note: when reinstalling the sample holder, use minimum torque on the #1 Phillips screws for a "snug" fit -- do not overtighten.

5.14 Viewing an Image Offline & Basic Analysis

5.14.1 Using the Browse View

Open the Browse window by clicking the **SHOW/HIDE BROWSE** icon on the toolbar. Directory icons appear in the **Browse** window. Double-click a folder icon to browse that directory (see Figure 5.14a).

Figure 5.14a Browse Window

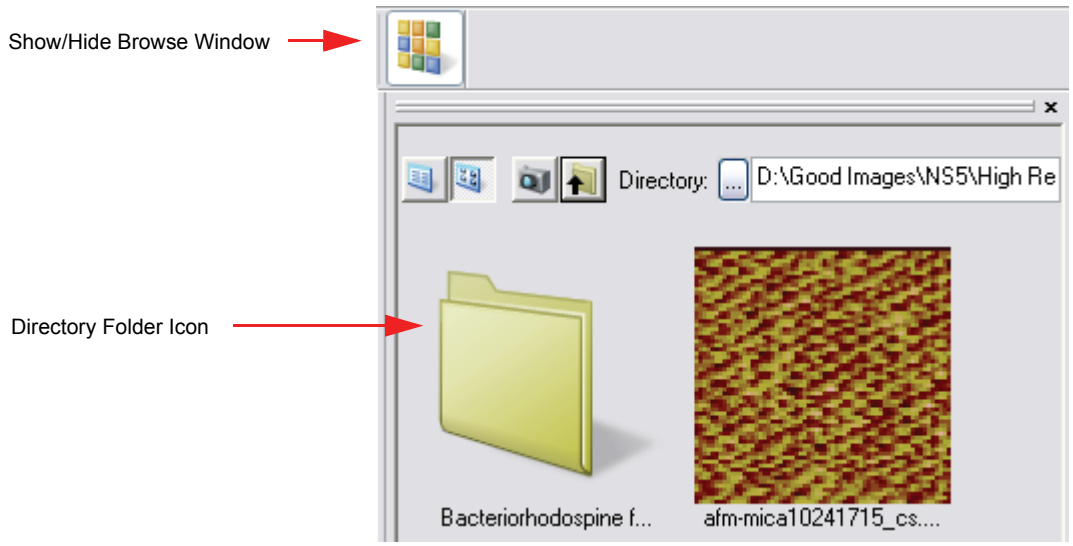
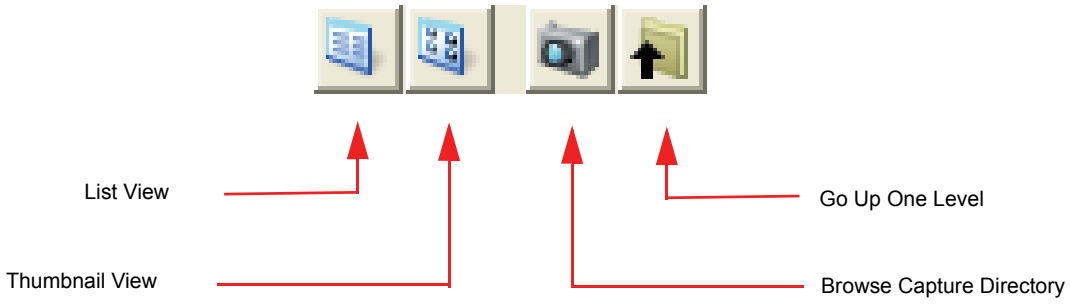


Figure 5.14b Browse Icons



For more detailed information on the Browse Window refer to the *NanoScope Software 8.00 User Guide*.

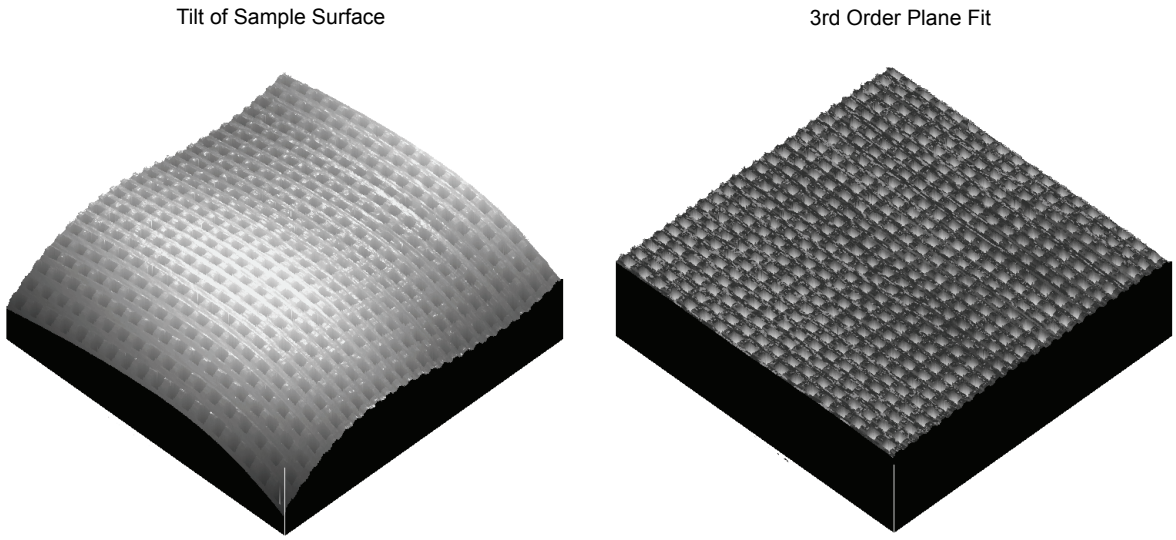
5.14.2 Using the Planefit Function

The **PLANE FIT** command computes a single polynomial of a selectable order for an image and subtracts it from the image. The **PLANE FIT** operation can be applied to either or both of the XY directions.

Box cursors or passbands allow specific points to be used in the calculation of the polynomial. Click on the image to start drawing a passband box. Right-click on a box to delete it or change its color.

Figure 5.14c illustrates an image with tilt and bow which could affect the analysis of the surface data.

Figure 5.14c Visual Representation of Plane Fit



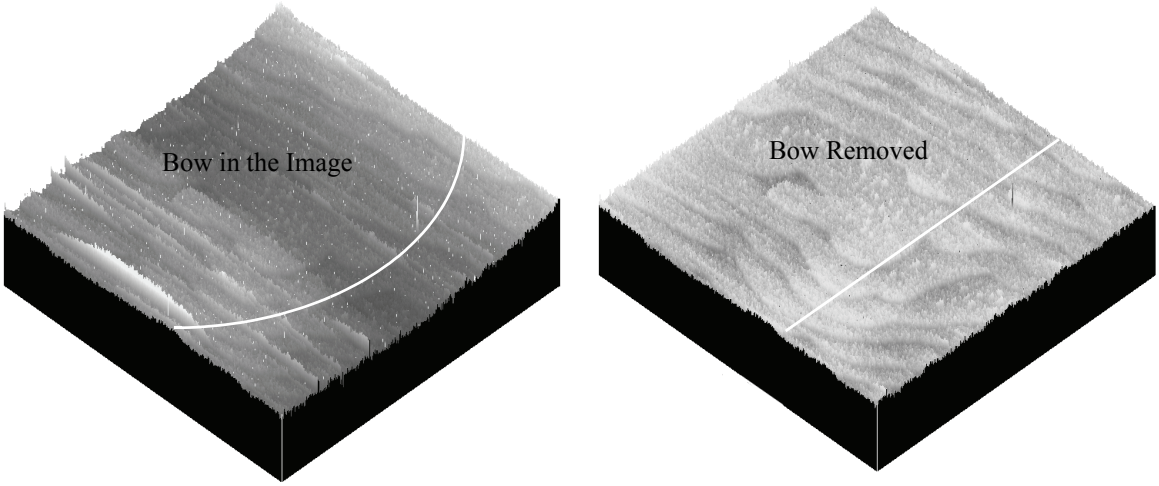
For more detailed information on Planefit Analysis please see the NanoScope Software User Guide.

5.14.3 Using the Flatten Function

The **FLATTEN** command eliminates unwanted features from scan lines (e.g., noise, bow and tilt). It uses all unmasked portions of scan lines to calculate individual least-square fit polynomials for each line.

FLATTEN is useful prior to image analysis commands (e.g., **DEPTH**, **ROUGHNESS**, **SECTION**, etc.) where the image displays a tilt, bow or low frequency noise, which appear as horizontal shifts or stripes in the image.

Figure 5.14d Image Flattened



For more detailed information on Flatten Analysis please see the NanoScope Software User Guide.

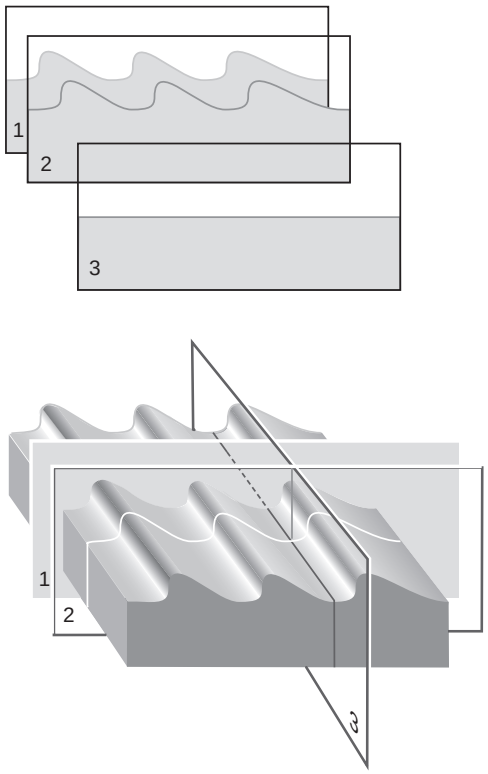
5.14.4 Using the Section Analysis Function

The **SECTION** command displays a top view image, upon which one, two or three reference lines may be drawn. The cross-sectional profiles and fast Fourier transform (FFT) of the data along the reference lines are shown in separate windows. Roughness measurements are made of the surface along the reference lines you define.

SECTION is probably the most commonly used Analysis command; it is also one of the easiest commands to use. To obtain consistently accurate results, ensure your image data is corrected for tilt, noise, etc. *before* analyzing with **SECTION**.

Samples are sectioned to learn about their surface profiles. The **SECTION** command does not reveal what is *below* the surface—only the profile of the surface itself. When sectioning samples, you should first ascertain surface topology. Depending upon the topology and orientation of the sample, the results of **SECTION** analysis may vary tremendously.

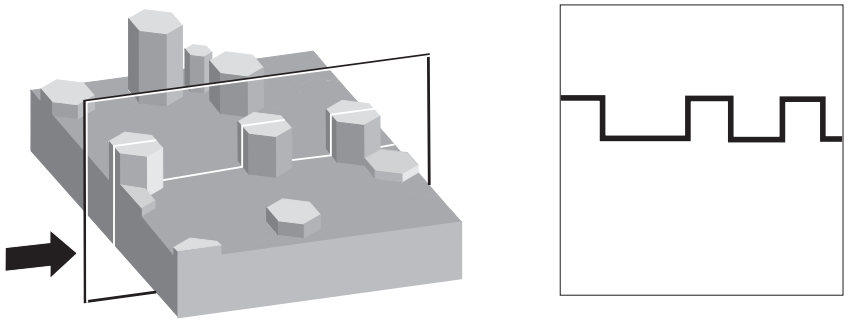
Figure 5.14e Section Analysis Orientation



In Figure 5.14e, the sample surface (a diffraction grating) is sectioned along three axes. Sections 1 and 2 are made perpendicular to the grating's rules, revealing their blaze and spacings. (Sections 1 and 2 may be compared simultaneously using two fixed cursor lines, or checked individually with a moving cursor.) Section 3 is made parallel to the rules, and reveals a much flatter profile because of its orientation.

The **SECTION** command produces a profile of the surface, then presents it in the **SECTION** grid (see Figure 5.14f).

Figure 5.14f Section Command Profile



Generally, **SECTION** analysis proves most useful for making direct depth measurements of surface features. By selecting the type of cursor (**Rotating Line**, **Rotating Box**, or **Horizontal Line**), and its orientation to features, you may obtain:

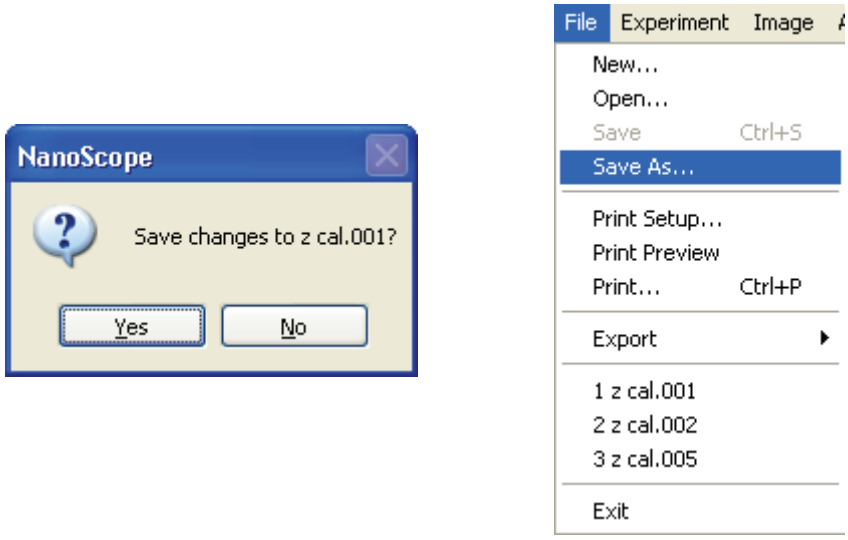
- Vertical distance (depth), horizontal distance and angle between two or more points.
- Roughness along section line: RMS, R_a, R_{max}, R_z.
- FFT spectrum along section line.

For more detailed information on Section Analysis, please refer to the NanoScope Software User Guide.

5.14.5 Saving Modified Files

Modified files can be saved in the same manner that modified Microsoft Windows files are saved. There are two choices. First, when closing a file after modification, the software will prompt the user if they would like to save changes to the file. See Figure 5.14g for an example of the dialog box to save a modified file. Alternatively, the user can go to the **File menu** and click on **Save As** to save the modified file. Similar to Windows, with either method the user has the choice to change the file name and/or location.

Figure 5.14g Options for Saving Modified Files



Chapter 6 Supported Sample Types

This chapter details the supported sample types for the BioScope Catalyst. Understanding of the information contained in this chapter is required for successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

6.1 Typical Sample Substrates

The BioScope Catalyst comes with the capability to use the following substrates:

- Microscope Slides
- Cover slips / cover glasses
- Mica discs and sheets
- Petri dishes / culture dishes

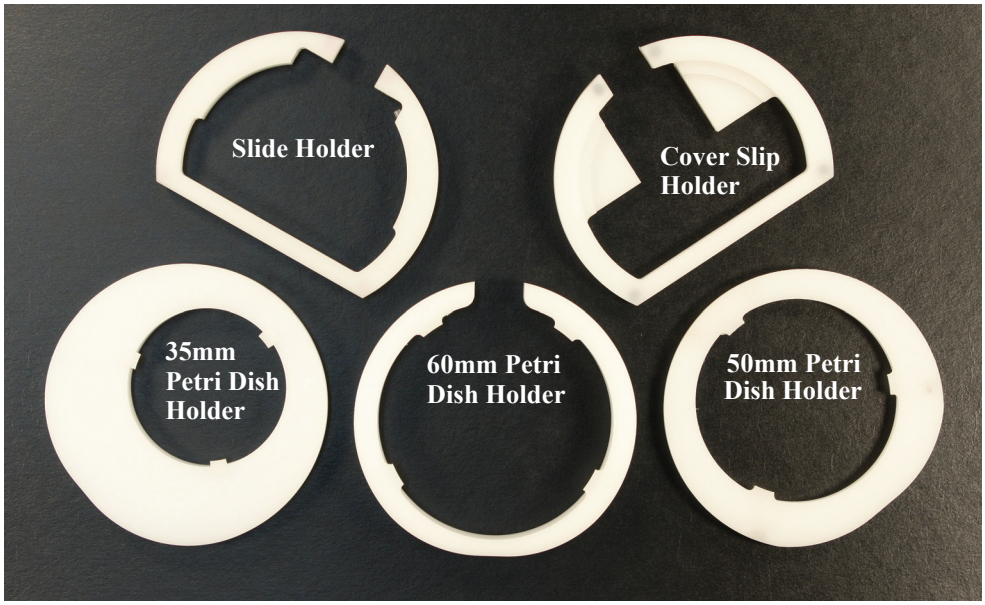
6.2 Sample Substrate Clamps

Sample substrate clamps use magnets (on bottom of clamps) to hold substrates in place during imaging. See Figure 6.2a. Five clamps are available with the BioScope Catalyst to hold the following substrates:

- 25mm (1.0 inch) square standard microscope cover slips
- 75 x 25mm (3” x 1”) standard glass microscope slides
- 35mm diameter petri dishes
  - Veeco recommends Corning model 430165 (tissue culture treated) or 430588 (not treated)
- 50mm diameter petri dishes
  - Veeco recommends WillCo Wells 50mm glass bottom petri dishes (type 5040)
- 60mm diameter petri dishes
  - Veeco recommends Corning model 430166 (tissue culture treated) or 430589 (not treated)

Note: Petri dish dimensions are nominal, so some brands may not be compatible. Please request samples from the dish vendor to confirm compatibility.

Figure 6.2a Top View of Sample Substrate Clamps.



6.3 General Sample Preparation Tips

6.3.1 Mica Substrate Preparation

Samples for atomic force microscopy imaging should be immobilized on a rigid support. Mica is a very common support because the surface is:

- Atomically flat
- Clean after cleavage
- Easy to cut to desired sizes
- Relatively inexpensive
- Negatively charged—but it can be modified to make the surface positive.

In order to prepare the mica for the sample, first, glue mica to a glass slide or cover slip using five minute epoxy or other suitable adhesive. After the glue has dried, cleave a fresh mica surface by first pressing some adhesive tape (i.e Scotch tape) against the top mica surface, then peeling off the tape. Repeat as needed until the mica cleaves cleanly without visible splintering, split layers, etc...

Mica Sources

Mica may be obtained from the following vendors:

- Spruce Pine Mica - Phone 828-765-4241 / Fax 828-765-7192
    - Bulk mica in random shapes
- Ted Pella, Inc. - Phone 530-243-2200 or 800-237-3526
    - Pre-cut mica shapes

6.3.2 The Importance of Good Sample Adhesion

Good sample adhesion is critical for obtaining useful AFM images. Using the following basic ideas as guides to imaging your samples will increase you chances of success.

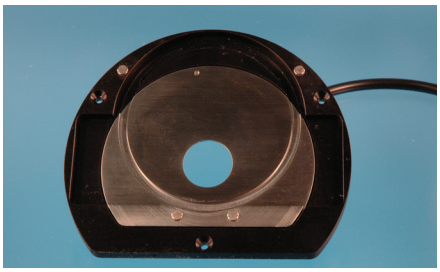
Specimen binding is usually accomplished using electrostatic attraction between charges on the specimen and charges on the mica surface. Proteins, for example, exhibit positive charges, especially when the buffer pH is lower than the isoelectric pH of the proteins. DNA, on the other hand, is negatively charged and can be bound either by creating positive charges at the surface (using a silanization process), or by adding a divalent metal counter ion (e.g. Mg^{2+}, Ni^{2+}) to the DNA buffer.

Sample preparation remains a challenging part of AFM studies in biology. It is still challenging to immobilize red blood cells or bacteria, for examples, to make them stick on a surface after immersion in a buffer solution. Tissues are also difficult to study for that reason, and also because of their softness. However, the steady progress in immobilization techniques explains the improved results being obtained daily. Keep in mind that it can take a while to find the right conditions to prepare and image a sample. Often, helpful ideas can be found in the scientific literature from previous work on similar samples.

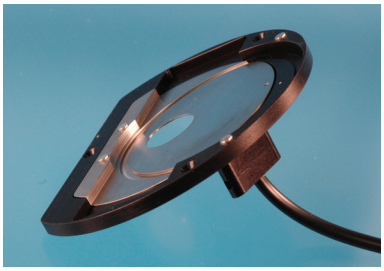
6.3.3 More Information on Sample Heating

The optional heater stage replaces the sample holder plate (see figure Figure 6.3a). Similar in appearance to the sample holder plate, the heater stage includes an electric heating element for use when the sample is to be heated for/while imaging. Temperature is set using a dedicated controller.

Figure 6.3a Optional Heater Stage



Face



Side

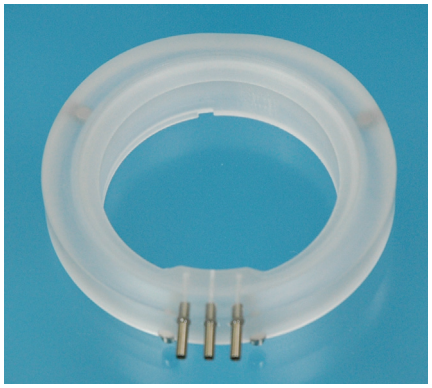
More information on sample heating is available in Chapter 12.

6.3.4 More Information on Fluid Perfusion and Culture Dish Incubation

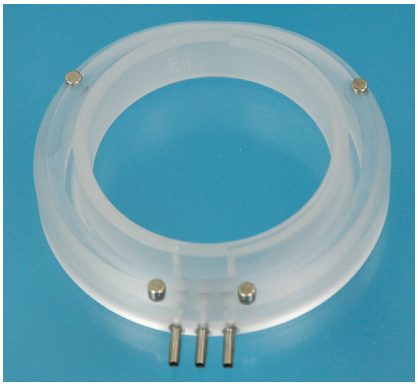
The perfusion cell is an optional accessory substrate clamp for 50mm glass bottom petri dishes. The cell permits fluids or gasses to be introduced or exchanged from the petri dish while it remains in the BioScope Catalyst.

More information on Fluid Perfusion and Culture Dish Incubation can be found in Chapter 11 and Chapter 13.

Figure 6.3b Optional Perfusion Cell



Top View



Bottom View

Chapter 7 Choosing A Probe

This chapter offers recommendations for choosing and working with AFM probes for life science applications. You can find a large selection of probes at Veeco Probes (veecoprobes.com).

Specifically, this chapter details the following information:

7.1 Choosing a Probe for Fluid TappingMode Imaging

Typical life science TappingMode imaging applications normally occur in fluid (water, buffers, etc.). TappingMode is frequently used for imaging single molecules (e.g. nucleic acids, adsorbed proteins), membranes (e.g. lipid bilayers, cell membrane patches), biomolecular assemblies (e.g. collagen fibrils, amyloid beta fibrils), and sometime living or fixed cells.

The most commonly used fluid TappingMode probe is the DNP-S, which is a sharpened conventional silicon nitride probe. On this probe, the short-thin "C" cantilever is most often used (k~0.32 N/m). This is a good starting point for almost any fluid TappingMode application.

A recently introduced alternative is the SNL probe, or Sharp Nitride Lever. The SNL probes have the same cantilever geometries as the DNP-S probes, but have etched silicon tips on the silicon nitride cantilevers. These tips are consistently much sharper than even the sharpened conventional silicon nitride tips. Again, the short-thin "C" lever is recommended for fluid TappingMode. Be aware that the sharper tips make force control even more important on delicate biological samples because the tapping force is spread over a small area. Therefore, it's recommended to start with a lower initial tapping amplitude than what you might normally use with a DNP-S probe. Once engaged, try to keep the tapping force as low as possible by keeping the amplitude setpoint as high as possible.

A less commonly used probe for fluid TappingMode is the MSCT probe, which is a sharpened conventional silicon nitride probe. The 0.1 and 0.5 N/m cantilevers on it have been used successfully for fluid imaging. Similarly, a new MSNL probes exists that offers very sharp etched silicon tips. As with the SNL probes, care must be taken to minimize imaging force to ensure best results.

7.2 Choosing a Probe for Fluid Contact Mode Imaging

Typical life science contact mode imaging applications normally occur in fluid (water, buffers, etc.). Contact mode is frequently used for high resolution imaging of protein membranes (e.g. bacteriorhodopsin) and imaging of living or fixed cells. In either case, it is usually critically important to minimize the imaging force to avoid sample damage. The same probes recommended above are still applicable, except the softer cantilevers should be used. For instance, on the DNP-S and SNL probe, the long-thin k~0.06 N/m cantilever, and on the MSCT and MSNL probes, the k~0.01 N/m or k~0.03 N/m cantilevers are recommended.

An important consideration for imaging cells is that a sharp probe can easily tear the cell membrane. For this reason, the unsharpened versions of these same probes are usually preferred, for instance DNP instead of DNP-S and MLCT instead of MSCT. The somewhat duller tips help prevent sample damage. The SNL and MSNL probes are generally too sharp to be use for cell imaging applications.

7.3 Choosing a Probe for Force Measurements

Many kinds of force measurements are being done in life science AFM research. These range from single molecule force spectroscopy applications (e.g. titin protein unfolding), to cell indentation measurements (e.g. cell elasticity). Another common application is to measure specific binding forces between a functionalized probe and the sample.

For any of these force measurements, it is important to choose a cantilever with an appropriate spring constant. If the spring constant is too large for the forces being measured then the cantilever deflection will be too small to measure (i.e. below the noise floor, typically several Angstroms). Likewise, if the cantilever spring constant is too small then the cantilever deflection will be very large, even exceeding the possible range of measurement. The photodetector response also becomes increasingly non-linear at large deflections, so it is best to keep the deflection within a smaller range. For best results, try to keep the measured deflection within about 2-3 V of the non-contact deflection value. Most life science force measurements applications fall into the fairly low force regime, so it's generally best to choose cantilevers with spring constants less that about k~0.1 N/m and often down to k~0.01 N/m.

The most common probes in this range include the aforementioned DNP, DNP-S, MLCT, MSCT, SNL, and MSNL probes. Of these, the MLCT, MSCT, and MSNL probes offer the softest (k~0.01 N/m) cantilever.

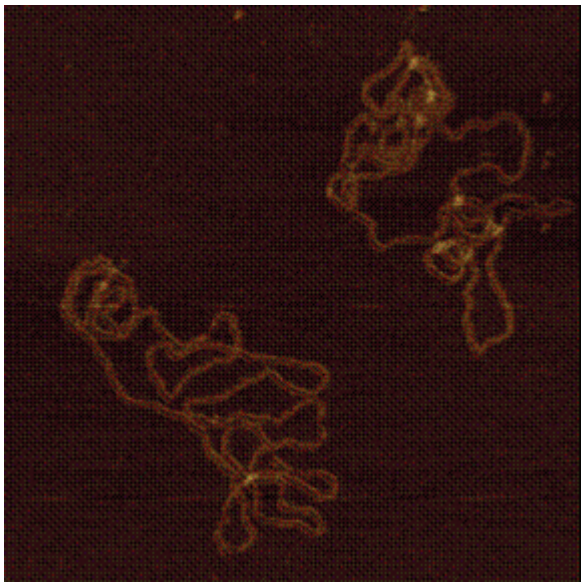
The softest probe available is the OBL probe, which has a cantilever with k~0.006 N/m. The cantilever is also quite small, which has the advantage of raising the resonance frequency compared to more conventionally sized probes. However, this also introduces some complications in usage as the probes tend to curl back on themselves, making setup challenging sometimes. They are only recommended for those applications that really take advantage of their unique features and users willing to exert extra effort in their use.

Tip shape is also sometimes an important consideration for force measurements. For single molecule force spectroscopy, tip shape is not normally thought to be very important so either unsharpened or sharpened probes can be used. However, for indentation measurements it is often advantageous to have a duller probe (e.g. MLCT or DNP) to avoid puncturing the cells or membrane. Sometimes round particles (e.g. glass or polystyrene microspheres) are glued onto probes to create large, well-defined indenter geometries. Tipless probes are available for these applications (e.g. NP-O and MLCT-O probes).

7.4 Recognizing Probe Artifacts

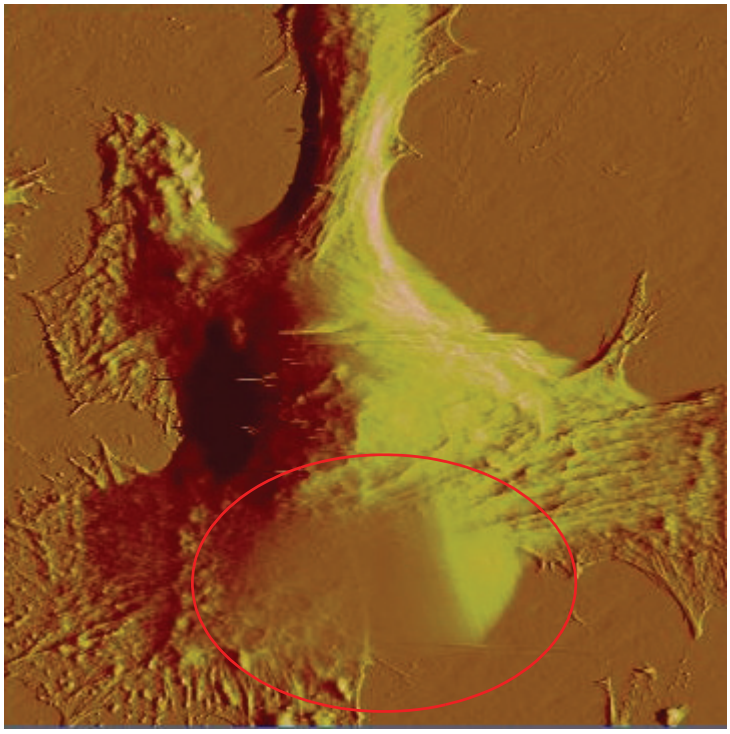
Two common tip artifacts are sometimes observed in life science AFM imaging. The first is the so-called "double tip," which occurs when the probe tip has more than one point in contact with the sample. This most often occurs when the tip breaks and leaves a jagged point. The image shown inFigure 7.4a is a typical example, where you can see that each DNA strand is doubled, like a shadow. The effect is usually quite obvious, but one way to diagnose it for sure is to change the scan angle, which changes the directionality of the "shadow." The only solution is normally to replace the probe. Sometimes double tips are caused by contamination rather than breakage, in which case sometimes the contamination disappears on its own or the probe can sometimes be cleaned.

Figure 7.4a DNA Image Showing "Double Tip" Artifact



A second tip artifact occurs on very tall samples or samples where the slope of the features is greater than the slope of the tip. This causes the tip sidewall to interact with the sample instead of the tip apex. The typical appearance is that of an almost linear ramp-like artifact around the feature, as shown in Figure 7.4b.

Figure 7.4b Cell Image Showing Tip Artifact (Circled)



7.5 Cleaning and Modifying Probes

Information on cleaning and functionalizing probes can be found in Veeco Application Note #44: "Choosing AFM Probes for Biological Applications." Note that some of the newer probes are not discussed in this publication. It is available from the Library section of the Veeco website.

Information on attaching microspheres to AFM probes can be found in Support Note #226: "Attaching Particles to AFM Cantilevers." Support Notes are available on request from Veeco's Customer Care Center.

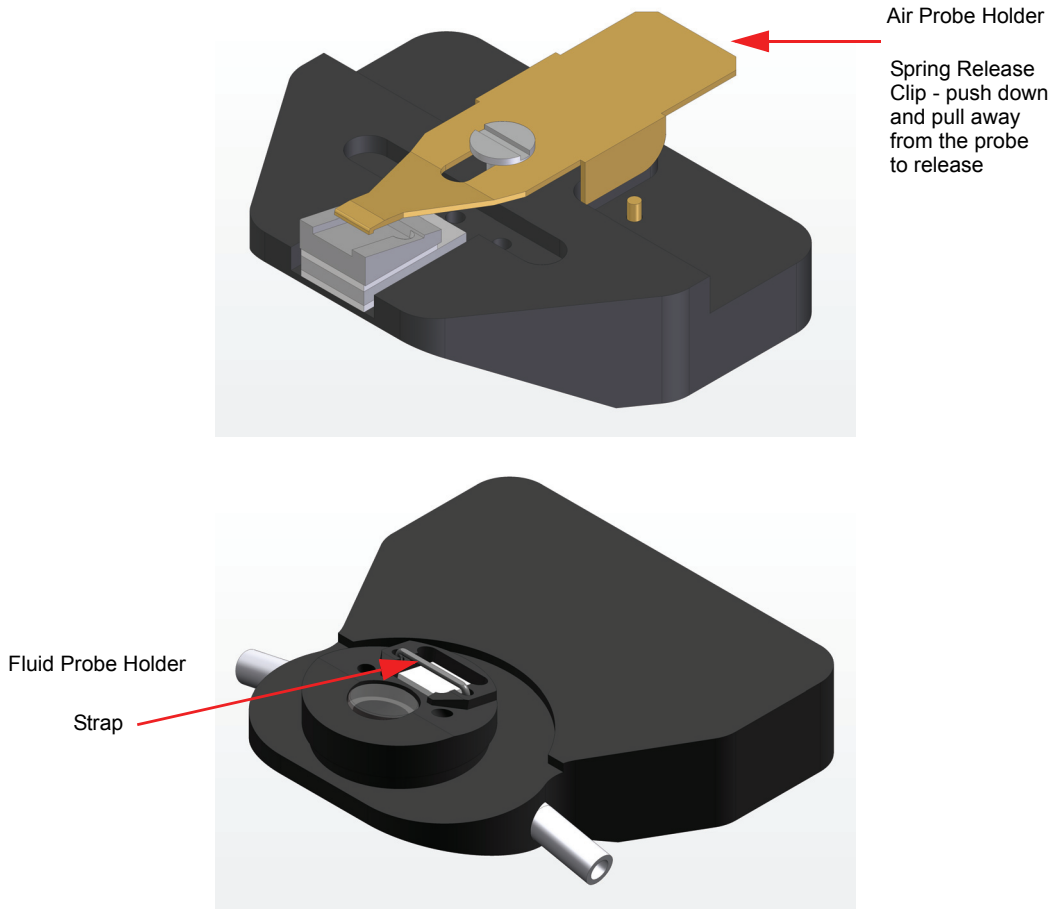
Chapter 8 Loading Probes

This chapter details how to load probes on the BioScope Catalyst. Understanding of the information contained in this chapter is required for successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

8.1 Air Versus Fluid Probe Holders

Figure 8.1a Air & Fluid Probe Holders



8.1.1 Identifying the Air & Fluid Probe Holders

The air and fluid probe holders are shown in Figure 8.1a. The best way to identify the probe holders is by the differences in shape. The Air holder has a large spring release clip while the fluid holder has tubing for the introduction and extraction of fluids during imaging.

8.1.2 Differences Loading Probes Into the Air & Fluid Probe Holders

There are some small differences when loading probes into the air and fluid probe holders. When loading a probe into the air probe holder, it is necessary to use your finger to push down and pull back the spring release clip in order to make room for the probe to be placed into the recess. On the fluid probe holder, no pushing and pulling of the spring clip is required. Rather, once you slide the

bottom dovetail portion of the fluid probe holder onto the probe load stand, it is necessary to put some gentle pressure on the probe load stand by pushing it down on a hard surface such as a desk. This, in turn, pushes the spring clip up releasing the strap that holds the probe down. With the strap raised, the probe can be placed carefully into the fluid probe holder recess. Gently removing pressure from the probe load stand will release the probe strap and lock the probe into position.

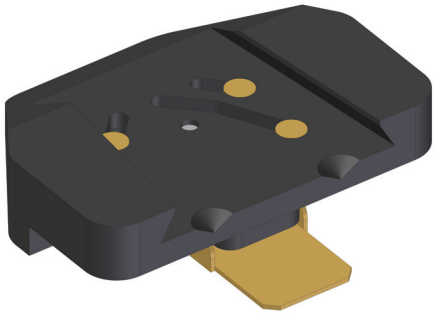
8.1.3 Loading a Probe In the Air Probe Holder

CAUTION: Probes are extremely fragile and should be handled touching only the substrate portion. Any physical contact with the cantilever or tip except during imaging is likely to damage the probe and render it useless.

The following components will be required in order to load a probe into the air probe holder:

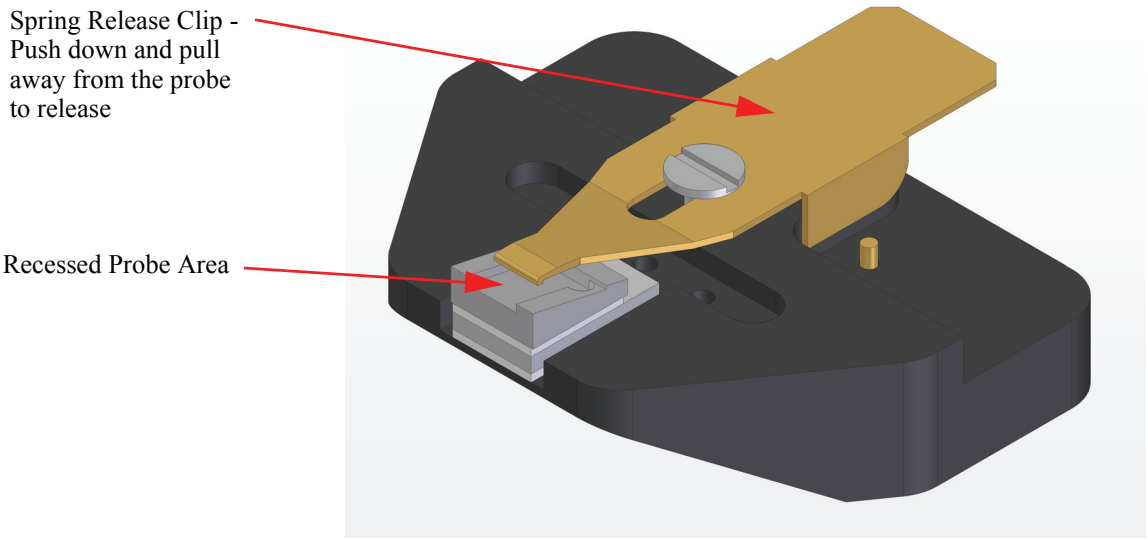
- Air probe holder
- Probe load stand
- Tweezers
- A few probes
- Optical microscope (recommended for new users - also known as a stereo microscope)

Figure 8.1b Probe holder - Dovetail Mounting Surface



1. Figure 8.1b and Figure 8.1c show the BioScope Catalyst probe holder. The surface shown in Figure 8.1b is the bottom dovetail that mounts onto the end cap of the z-scanner of the head. The z-scanner mount has three gold plated contacts for connection to the corresponding posts on the z-scanner end cap.

Figure 8.1c Probe Holder - Probe Side



2. The probe is clamped in place by a spring loaded clip. The clip, identified in gold in Figure 8.1c, is opened by pressing on the end of the spring release clip and pulling backwards away from the probe. Installing the probe under magnification is helpful, but experienced users will often mount the probe without magnification assistance. The probe is handled with sharp tweezers touching only the substrate portion of the probe. If either the tip or cantilever are contacted by tweezers or other objects while handling, damage is extremely likely. The probe must be positioned carefully to ensure that it lies completely within the recess of the probe holder. It is recommended that the probe be positioned so that clearance is visible on both sides of the recess and as far back into the recess as possible but ensuring that the back edge of the probe remains within the recess.

   Note: It is typically easier to position the probe if the spring clamp is released to hold the probe, then gently nudge the substrate to position the probe as desired.

8.1.4 Loading a Probe Into the Fluid Probe Holder

CAUTION: Probes are extremely fragile and should be handled touching only the substrate portion. Any physical contact with the cantilever or tip except during imaging is likely to damage the probe and render it useless.

The following components, as seen in Figure 8.1d, will be required in order to load a probe into the fluid probe holder:

- Fluid probe holder
- Probe load stand
- Plastic tip tweezers

- A few probes
- A benchtop optical microscope (recommended for new users)

Figure 8.1d A Sampling of the Recommended Components for Loading a Probe into the Fluid Probe Holder

Probe Loading

1. Place the empty fluid probe holder onto the probe load stand with the probe end facing towards the BioScope Catalyst logo. The fluid probe holder should be face-side up.
2. Slide the probe holder back (away from the BioScope Catalyst logo) and press down gently until it is securely in position on the probe load stand.
3. With the fingers of the non-dominant hand on the outside of the probe load stand, gently push down on the probe load stand. This action will push the spring clip on the underside of the probe holder upward, allowing room for the probe to be inserted under the strap.
4. Using the tweezers, pick up a probe, being careful to only touch the sides of the cantilever.
5. Place the probe face up under the spring clip into the recessed area on the fluid probe holder. The probe must be positioned carefully in the recess of the probe holder to ensure that it lies

completely within the recess. Veeco recommends that the probe be positioned so that clearance is visible on both sides of the recess and as far back into the recess as possible, but ensuring that the back edge of the probe remains within the recess. Be careful not to scratch the glass.

6. When you feel the probe is seated well, release the probe load stand and gently pull the fluid probe holder off of the probe stand. This will release the spring clip and secure the probe in place.

8.1.5 Cleaning and Maintaining the Probe Holders

Dirty probe holders can shed particles onto tips and samples and result in less than optimal imaging. As such, it is essential to keep your probe holders clean at all times.

Cleaning the Air Probe Holder

The air probe holder can be cleaned with tap water and liquid soap (non-abrasive). Once cleaned, it is best to rinse with de-ionized water. When finished rinsing, blow the the probe holder dry with compressed air or dry nitrogen, or let it air dry before loading another probe.

Cleaning the Fluid Probe Holder

The fluid probe holder can be cleaned with tap water and liquid soap (non-abrasive). Once cleaned, it is best to rinse with de-ionized water. When finished rinsing, blow the the probe holder dry with compressed air or dry nitrogen, or let it air dry before loading another probe.

Additionally, the tubing for the introduction and extraction of fluid can be rinsed with de-ionized water in order to clean it for future experiments.

Fluid probe holders should be cleaned immediately after use to prevent buffer salts from drying onto the holder.

Chapter 9 Calibrating Cantilever Spring Constants

This chapter details how to calibrate cantilever spring constants on the BioScope Catalyst. Understanding of the information contained in this chapter is required for successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

9.1 Overview

Atomic force microscopy (AFM) is being used in a great variety of force measurement applications, including investigating the unfolding pathways of native membrane proteins, probing the structure of single polysaccharide molecules, and monitoring the response of living cells to biochemical stimuli. All of these techniques rely on the accurate determination of the cantilever spring constant in order to yield quantitative results. For although the cantilever deflection can be measured with great accuracy and sub-Angstrom sensitivity, converting these measurements to units of force via Hooke's law, $F = -k \cdot x$, requires that the spring constant, k, be determined for each cantilever.

It has often been noted in the literature that spring constants can vary greatly from the values quoted by their manufacturers. In fact, these values are only provided as nominal indications of the cantilever properties and the manufacturers often specify the spring constant in a wide range that may span values up to four times smaller and four times larger than the nominal value. This is because the techniques used to fabricate the probes can result in substantially different cantilever dimensions, especially thickness, from wafer to wafer and smaller variations within a single wafer. While it is sometimes possible to achieve tighter tolerances, this generally is not practical for the economical production of probes for general imaging and force measurement applications.

Fortunately, this issue was recognized early while some of the first AFM force measurement applications were being developed and many techniques have since been proposed to characterize cantilever spring constants. These can generally be grouped into three categories: “Dimensional models” where fully theoretical analysis or semi-empirical formulas are used to calculate the cantilever spring constants based on their dimensions and material properties, “Static deflection measurements” where the spring constant is determined by loading the cantilever with a known static force, and “Dynamic deflection measurements” where the resonance behavior of the cantilever is related back to its spring constant. These techniques are reviewed in detail in Veeco Application Note 94: Practical Advice on the Determination of Cantilever Spring Constants.

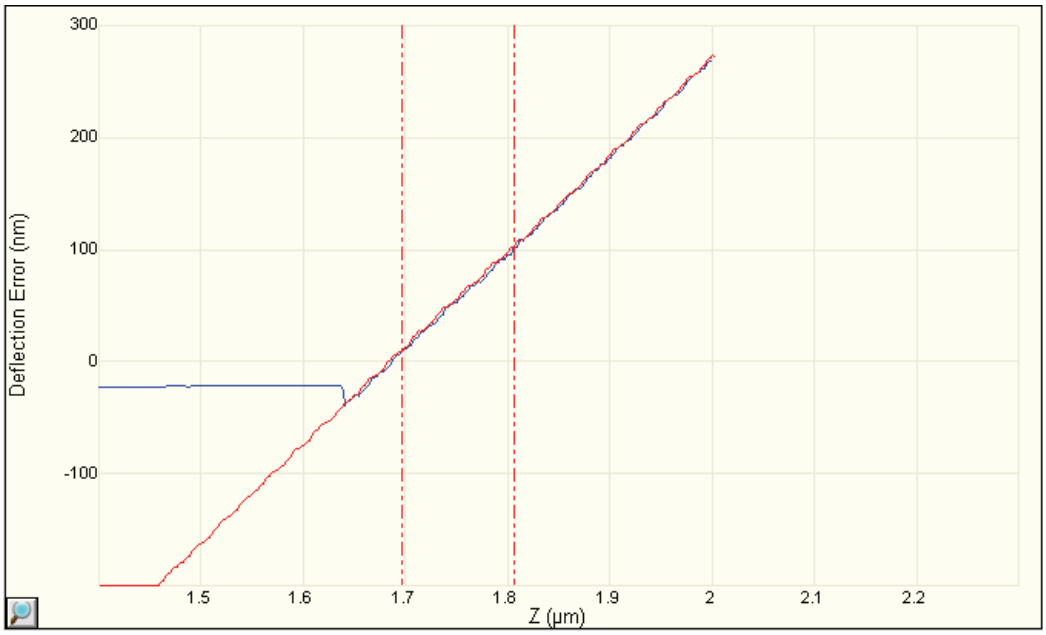
The most widely used and commonly applicable of these techniques is the “thermal tune method.” This technique is implemented in the NanoScope software, which allows the probe spring constant to be calibrated in either air or in fluid by simply calibrating the deflection sensitivity and then making a simple measurement in the thermal tune view. The thermal tune technique is best applied to probes with spring constants less that approximately 5 N/m (e.g. FESP) all the way down to the very softest silicon nitride probes (e.g. MLCT). For stiffer probes, please refer to the above application note for recommendations.

9.1.1 Thermal Tune in Air

1. Adjust the photodetector such that the non-contact deflection is near 0 V.
2. **ENGAGE** in contact mode.
3. Calibrate the deflection sensitivity over a small deflection (< 1 V), see below.

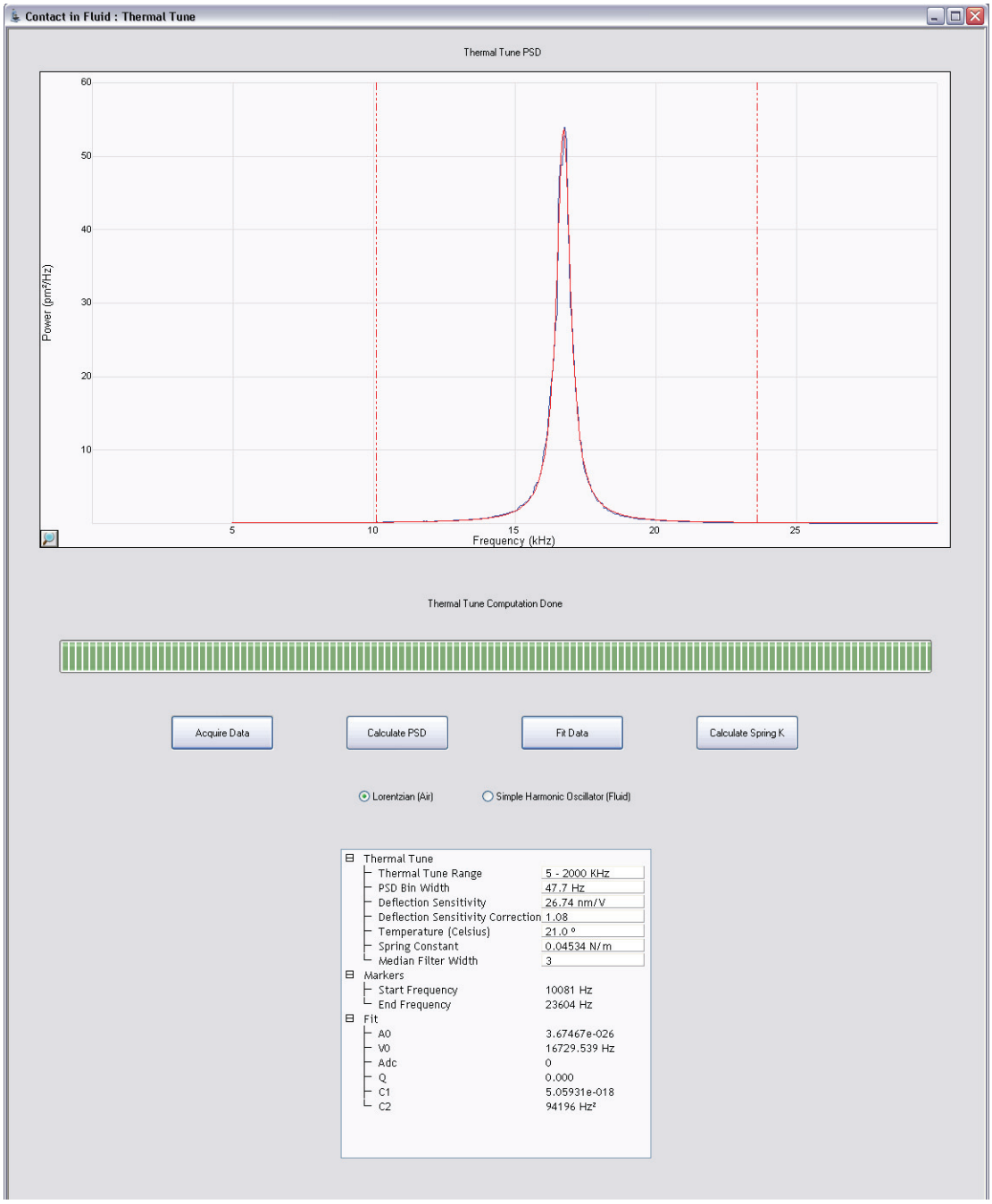
   Note: It is very important to calibrate the deflection sensitivity on a hard surface. Using a soft surface will overestimate the deflection sensitivity, which will result in spring constants that are too low.

Figure 9.1a Deflection Sensitivity Example



4. **WITHDRAW**. Confirm that vertical deflection is still near 0 V.
5. Enter the **Thermal Tune** view. Confirm that "Deflection Sensitivity Correction" is 1.144 for v-shaped cantilevers and 1.106 for rectangular cantilevers.
6. Click "**ACQUIRE DATA**", you should see the thermal tune curve appear in 10-20 seconds.
7. Position markers such that they are on the baseline to the left and right of the resonance peak, see below. Make sure that the Lorentzian (Air) fit is selected. Click "**FIT DATA**" and confirm that the fit is good.

Figure 9.1b Lorentzian Air Fit Example



8. Click "**CALCULATE SPRING K BUTTON**."

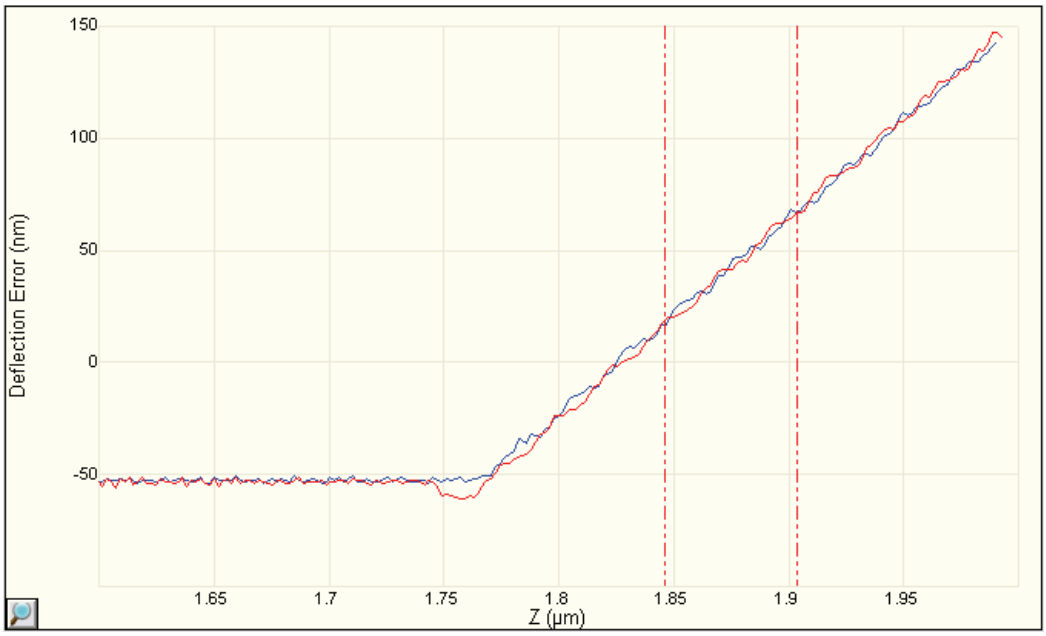
9. Repeat steps 6-8 a few times and take average of spring constant values.

9.1.2 Thermal Tune in Water

1. Adjust the photodetector such that the non-contact deflection is near 0 V.
2. **ENGAGE** in contact mode.
3. Calibrate the deflection sensitivity over a small deflection (< 1 V), see below.

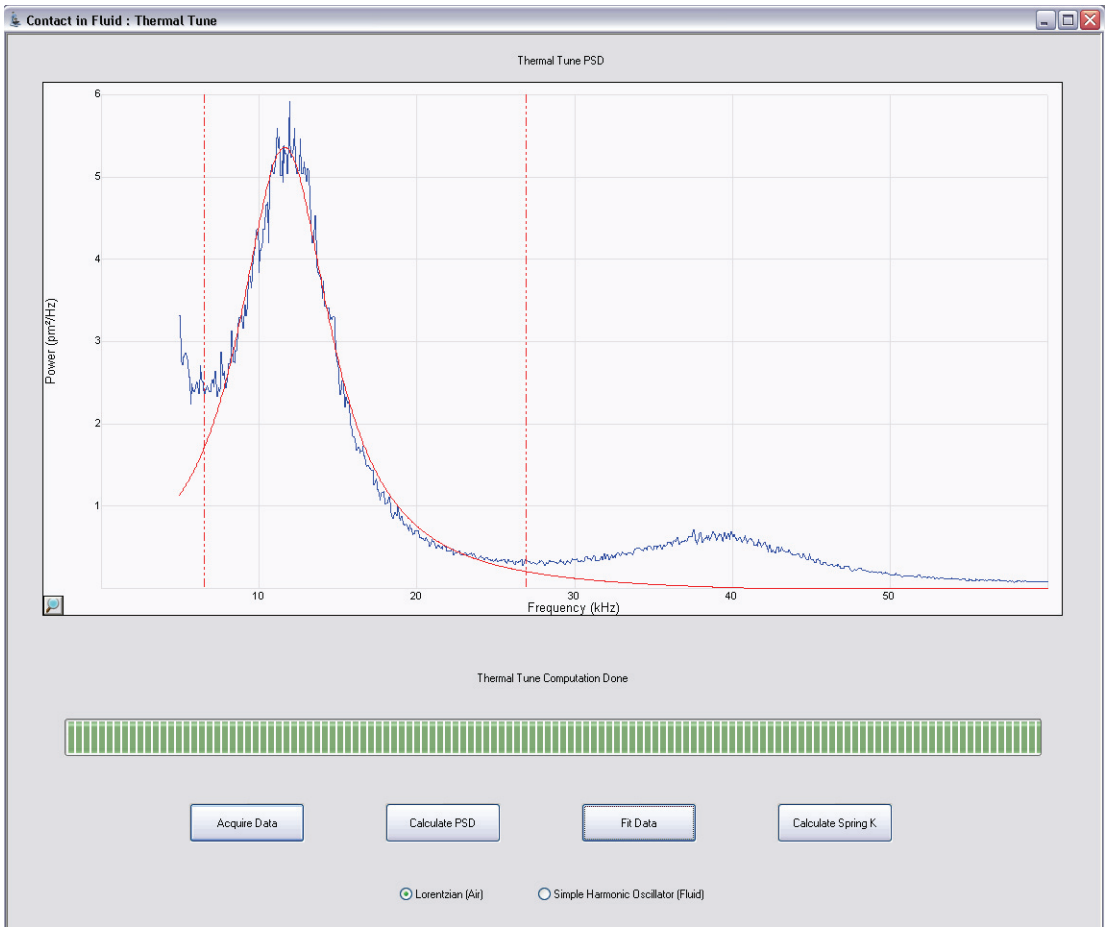
   Note: It is very important to calibrate the deflection sensitivity on a hard surface. Using a soft surface will overestimate the deflection sensitivity, which will result in spring constants that are too low.

Figure 9.1c Deflection Sensitivity Example



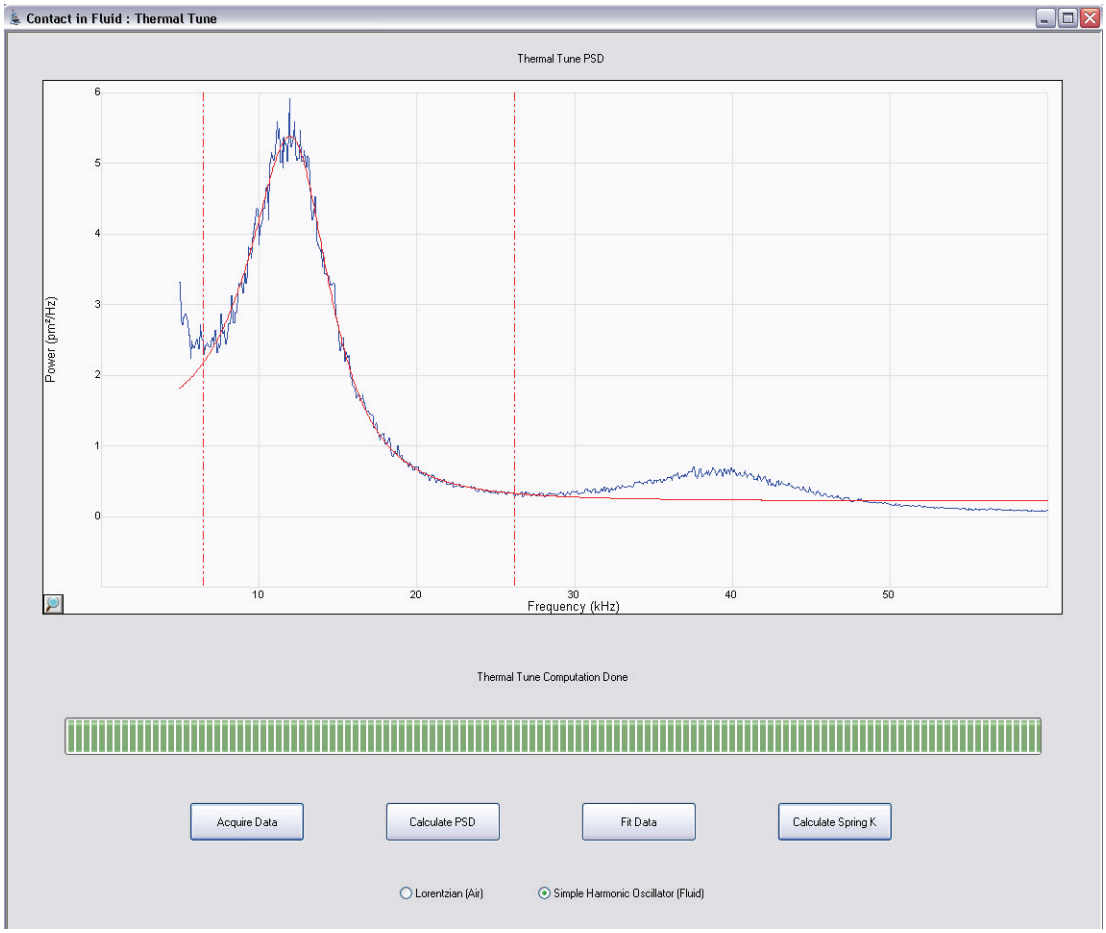
4. **WITHDRAW**. Confirm that vertical deflection is still near 0 V.
5. Enter the **Thermal Tune** view. Confirm that "Deflection Sensitivity Correction" is 1.144 for v-shaped cantilevers and 1.106 for rectangular cantilevers.
6. Click "**ACQUIRE DATA**", you should see the thermal tune curve appear in 10-20 seconds.
7. Position markers as shown below. The left marker should be as far to the left of the peak as possible while still being on the data (the data near DC is truncated to ensure that the plot range scales properly). If the resonance frequency is higher, such that the peak does not overlap DC, then place the left marker to the left of the peak at baseline level as you would in air. Place the right marker to the right of the peak at the lowest baseline level. The baseline may start rising again near a higher order resonance. If so, avoid placing the right marker in this region. Either the Lorentzian (Air) or Simple Harmonic Oscillator (Fluid) fit can be used, depending on which better fits the data. Both are shown below.

Figure 9.1d Lorentzian (Air) Fit



Note: Note that it fits peak quite well

Figure 9.1e Simple Harmonic Oscillator (Fluid) Fit



Note: Note that the fit overestimates the low frequency side of the peak and underestimates the right shoulder of the peak. This is typical.

8. If the resonance peak overlaps DC, you must reposition the left marker so that it is at DC (0 Hz). This extrapolates the curve fit all the way to DC. This is important because the entire area under the peak must be included in the calculation. See below for an example of the correct positioning.

9. Click "**CALCULATE SPRING K**".

10. Repeat steps Step 6 through Step 8 a few times and take average of spring constant values.

9.1.3 Additional Thermal Tuning Hints

Included below are a few more important thermal tuning hints:

- Keep the deflection at the same value, near 0 V is best. In any case, the deflection sensitivity and spring constant should both be calibrated at close to the same deflection value because the deflection sensitivity does vary slightly with the position of the spot on the photodetector.
- Calibrate the deflection sensitivity over a relatively small deflection (< 1V).
- It is VERY important that you calibrate the deflection sensitivity on a hard surface! This can be a problem on some samples, especially in fluid.
- In fluid it is VERY important to reposition the left marker after you fit the data.
- Make sure that you are far away enough from the surface during the thermal tune so that the tip is not interacting with the surface. This is especially important in fluid where the fluid can exert squeeze damping.
- Repeat the deflection sensitivity and spring constant calibrations a few times and take the average values.

Chapter 10 Compatibility with Inverted Optical Microscopes

This chapter details BioScope Catalyst compatibility with inverted optical microscopes (IOM). Understanding of the information contained in this chapter is required for successful operation of the BioScope Catalyst on an inverted optical microscope.

Specifically, this chapter covers the following information:

- **Supported Microscopes** Section 10.1
- **Safety** Section 10.2
- **Using High Numerical Aperture Objectives** Section 10.3
  - **Exchanging Sample Plates** Section 10.3.1
- **Using the Inverted Microscope Without the BioScope Catalyst** Section 10.4

10.1 Supported Microscopes

The BioScope Catalyst may be configured for compatibility with current and recent inverted light microscopes from Leica, Zeiss, Olympus, and Nikon. Since each light microscope model requires a different BioScope base plate / scan stage, this information must be included at the time of purchase.

The following table gives specific compatibility information on microscope models, confocal systems, and condensers:

Table 10.1a BioScope Catalyst Supported Configurations

Microscope Type	Supported Microscopes
Inverted optical microscopes	Leica DMI 6000, 4000, 3000 Zeiss Axio Observer A1, D1, Z1 (also Axiovert 100, 135, 200) Nikon Eclipse Ti-E/U/S (also TE2000-E/U/S) Olympus IX71, IX81 (also IX70) (also supported stand-alone operation)
Transmitted light condensers	Leica S28 (0.55 NA, 28mm WD) Zeiss LD (0.55 NA, 26mm WD) Nikon LWD (0.52 NA, 30mm WD) Olympus IX2-MLWCD (0.50 NA, 45mm WD) (also supports other models with longer working distances)
Confocal laser scanning microscopes	Leica TCS SP5 Zeiss LSM 5 and LSM 710 Nikon C1si and C1 plus Olympus FluoView 300 and 1000 (Inquire regarding other models)

10.2 Safety

The BioScope Catalyst AFM contains an 850 nm wavelength (Infrared) laser, Class 3R, with the following characteristics:

- Beam divergence: 3°-7°
- Maximum output: 1 mW
- Pulse duration: 1 nsec @ 500 MHz
- Laser output power decreases over time as the laser diode ages

The label shown in Figure 10.2a will be attached to the BioScope Catalyst head.

Figure 10.2a Label on BioScope Catalyst Head

Appropriate laser safety procedures must be followed to avoid risk of eye damage. All users should understand the cautionary notes regarding laser safety and risk.

WARNING: If the BioScope Catalyst is used in conjunction with an inverted optical microscope, use of an optical filter is required. Contact Veeco Technical Support for guidance on filter selection and installation.

AVERTISSEMENT: Si le BioScope Catalyst est employé en même temps qu'un microscope optique inversé, l'utilisation d'un filtre optique est exigée. Entrez en contact avec le support technique de Veeco pour des conseils sur le choix et l'installation de filtre.

WARNUNG: Wenn der BioScope Catalyst in Verbindung mit einem umgekehrten optischen Mikroskop benutzt wird, wird Gebrauch eines optischen Filters angefordert. Treten Sie Veeco mit technischer Unterstützung für Anleitung auf Filtervorwähler und -installation in Verbindung.

.

WARNING: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

AVERTISSEMENT: Toute utilisation de controls, réglages ou performances de procédures autre de celles spécifiées ici, peut causer des expositions de radiations dangereuses.

WARNUNG: Einstellungen oder Handhabungen, die nicht in dieser Anleitung beschrieben sind, koennen gesundheitsgefaehrdende Strahlung zur Folge haben.

WARNING: Do not stare at the laser beam either directly or from a highly reflective surface

AVERTISSEMENT: Ne regardez pas vers le laser directement ou d'une surface très réflective.

WARNUNG: Vermeiden Sie in jeden Fall sowohl direkten als auch indirekten, durch Reflexionen verursachten Augenkontakt mit dem Laserstrahl.

WARNING: Viewing the laser output with certain optical instruments (for example, eye loupes, magnifiers and microscopes) within a distance of 100 mm may pose an eye hazard.

AVERTISSEMENT: Regardez le signal de sortie du laser en utilisant certains instruments optiques (par exemple une loup ou d'autres instruments a agrandir) d'une distance de 100mm peut causer des problèmes visuels.

WARNUNG: Das Betrachten des Laserstrahls mit optischen Geraeten (z. B. Lupe, Vergroesserungsglas, Mikroskop) in einem Abstand unter 10 cm koennen das Auge schaedigen.

10.3 Using High Numerical Aperture Objectives

High magnification, high numerical aperture microscope objectives are typically much larger than other lower magnification objectives and typically have short working distances and require the use of immersion oil. Depending on the specific objectives, they may require a larger open aperture in the scan stage sample plate. Therefore two different sample inserts are provided, a standard sample plate with 14.5mm (0.571in) x 15° diameter aperture and a high NA sample plate with a nominal 21.75mm (0.856in) x 15° aperture, shown below:

Figure 10.3a Standard Insert

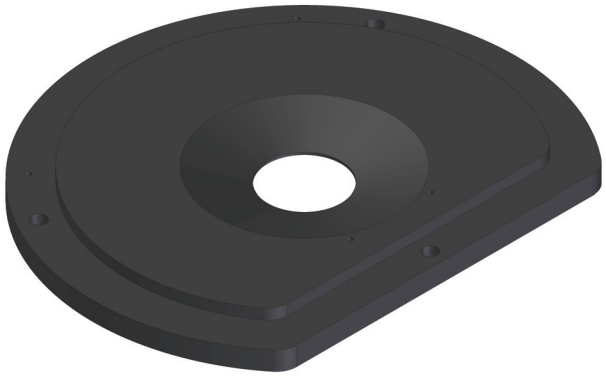
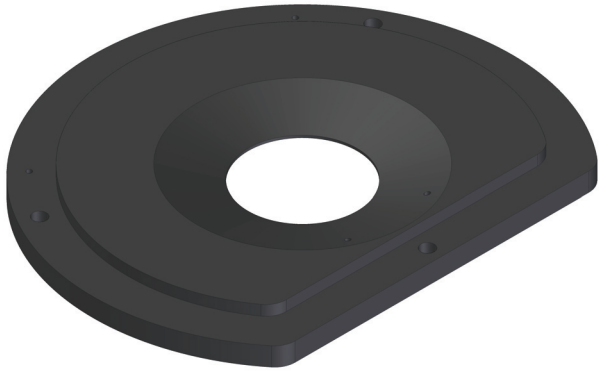


Figure 10.3b High NA Insert



Be aware that even with the high NA sample insert the mechanical clearance for objectives is limited and care should be exercised to avoid collision of the objectives with the sample place, especially when operating away from the center of the sample insert aperture. Also take care when rotating the objective turret, as some objective may need to be lowered before they can be rotated. Please familiarize yourself with the particular requirements of your light microscope configuration.

10.3.1 Exchanging Sample Plates

The sample plates can be exchanged while the scan head and sample are removed from the base plate. Three screws fasten the sample inserts in the XY scan stage. Simply remove the screws, lift out the sample insert, replace with the desired insert, and replace the screws. The screws should be fastened securely, but care should be taken to avoid over-tightening.

Usage Hints

The short working distance of high NA objectives requires the use of thin glass sample substrates like cover slips and glass bottom petri dishes. Be aware that these substrates are inherently less mechanically rigid than thicker substrates like glass slides and plastic petri dishes. Therefore you may note that imaging noise is somewhat higher, which is dependent on the exact configuration. Adding immersion oil is most easily accomplished before the sample is placed on the sample plate, though access underneath the base plate is also possible.

10.4 Using the Inverted Microscope Without the BioScope Catalyst

It may sometimes be desirable to use the light microscope while the Catalyst is not being used. Special requirements must be observed to allow the use of the motorized XY sample stage during these situations. The motorized XY sample stage can only be moved through the NanoScope software interface or the joystick when the entire system is powered and the NanoScope software is in the Navigate view. The scan head can remain on the EasyAlign and it is not necessary to have a probe installed or the laser aligned.

Chapter 11 Using the Small Volume Fluid Perfusion Cell

This chapter details how to use the small volume perfusion cell on the BioScope Catalyst. Understanding of the information contained in this chapter is required for safe and successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

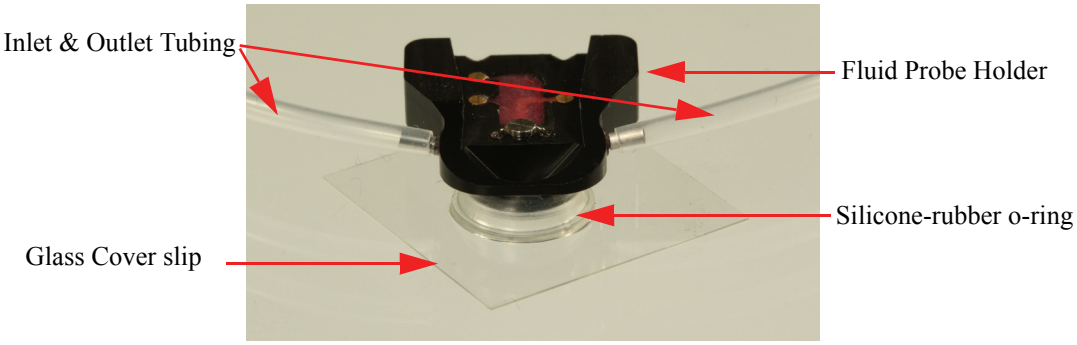
- **Hardware Configuration** Section 11.1
  - **The Perfusion Chamber** Section 11.1.1
  - **Recommended Substrates** Section 11.1.2
  - **Small Volume Perfusion Cell Setup** Section 11.1.3
  - **Fluid Flow Rate Recommendations** Section 11.1.4
  - **Recommended Fluid Delivery Methods** Section 11.1.5
  - **Heating Limitations** Section 11.1.6

11.1 Hardware Configuration

11.1.1 The Perfusion Chamber

A small volume fluid perfusion chamber is formed when a small silicone-rubber o-ring is seated onto the fluid probe holder. When pressed against a glass slide or petri dish, the small volume fluid perfusion chamber becomes a self-sealing chamber for fluid imaging. Tubing ports on both sides of the fluid probe holder provide access for the introduction and removal of fluid during imaging. The small volume fluid perfusion cell allows for imaging in < 50µL of fluid. Figure 11.1a shows the assembled small volume perfusion fluid cell seated on a glass slide. Additionally, Figure 11.1a shows the inlet and outlet tubing attached to the ports of the fluid probe holder.

Figure 11.1a The Small Volume Perfusion Cell Seated onto a Glass Coverslip



11.1.2 Recommended Substrates

The small volume fluid perfusion cell is best used with the following sample substrates:

- Glass slides or coverslips
- Petri dishes

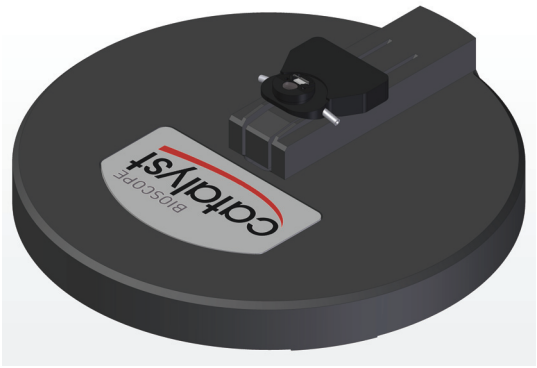
11.1.3 Small Volume Perfusion Cell Setup

The small volume fluid perfusion cell requires the following parts for correct setup:

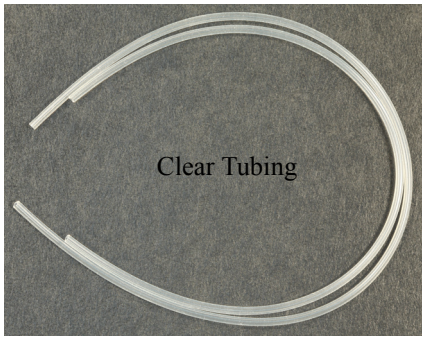
- Small volume fluid perfusion cell o-ring
- Stereo microscope (optional, but very helpful to ensure correct seating of the o-ring around the seat on the fluid probe holder)
- Tweezers

- Fluid probe holder
- Probe stand
- Clear tubing for the inlet & outlet ports

Figure 11.1b Parts Required for Small Volume Fluid Perfusion Chamber Setup



Fluid Probe Holder on Probe Stand



O-ring

The directions to setup the small volume fluid perfusion chamber are as follows:

1. Set up a stereo microscope and turn the light on.

   Note: The microscope allows easier visualization of the probe as it is inserted into the probe holder.

2. Under the microscope, load the fluid probe holder onto the probe stand.

3. Insert the preferred probe onto the fluid probe holder.

4. Remove the fluid probe holder from the probe stand. It is now time to attach the tubing to the fluid probe holder.

5. With the fluid probe holder in the non-dominant hand, and one piece of clear tubing in the dominant hand (all the while being very careful not to touch the probe), push the clear tubing onto one of the metal tubes of the fluid probe holder. The difference in diameter between the metal tubes and the clear tubing is very small, so it is helpful to squeeze the end of the clear tubing together in order to make it a little larger, and thus easier to attach to the metal tubing.

   Note: Moistening the metal tubes and tubing may facilitate easier tubing attachment.

6. When one side of the clear tubing is attached to the fluid probe holder, proceed to attach the second side. Now that both the inlet and outlet tubing are attached to the fluid probe holder, it is time to attach the small volume fluid perfusion o-ring to the probe holder.

7. In order to attach the small volume fluid perfusion o-ring to the fluid probe holder, position the small volume fluid perfusion o-ring such that the small diameter portion is on the bottom. See Figure 11.1c. Using the tweezers, pick up the small volume fluid perfusion cell and carefully seat it around the groove that surrounds the fluid probe holder. Figure 11.1d depicts the lip and seating area on the fluid probe holder where the small volume fluid perfusion o-ring should be properly mounted. Figure 11.1e shows the small volume fluid perfusion o-ring seated properly onto the fluid probe holder.

Figure 11.1c Small Volume Fluid Perfusion O-ring

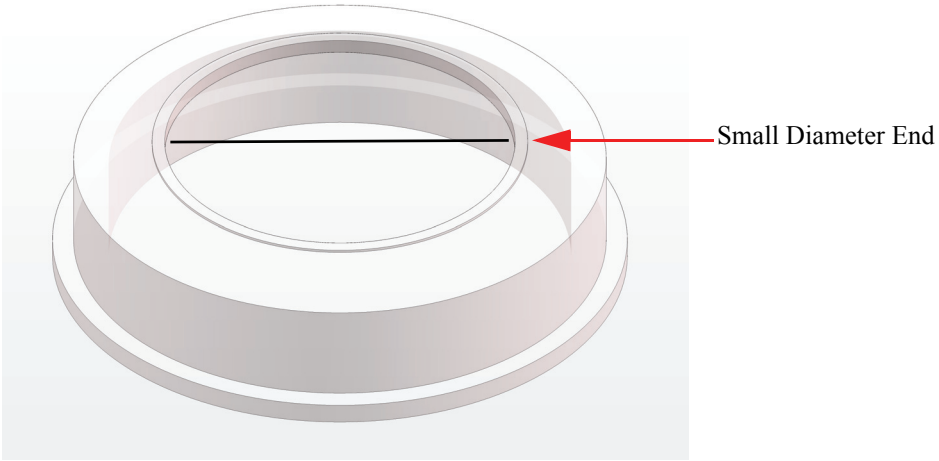


Figure 11.1d Lip and Seating Locations on Fluid Probe Holder

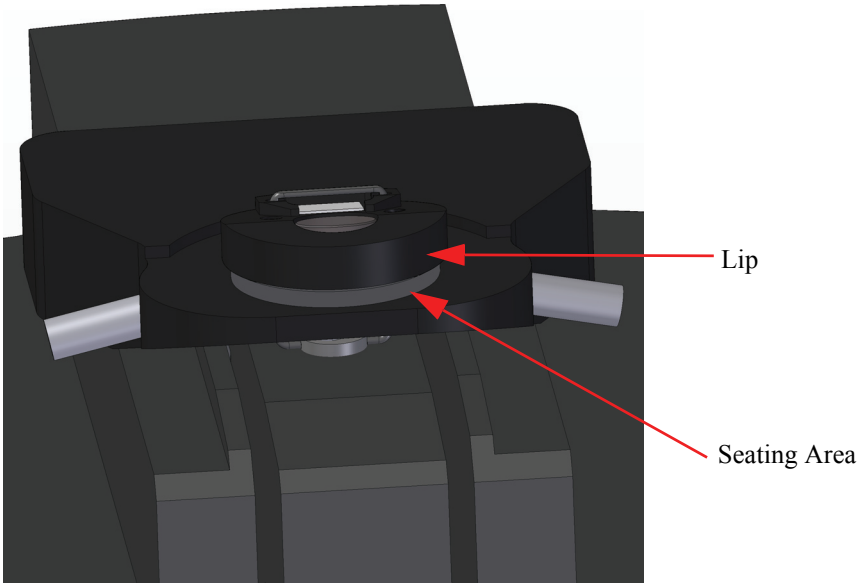
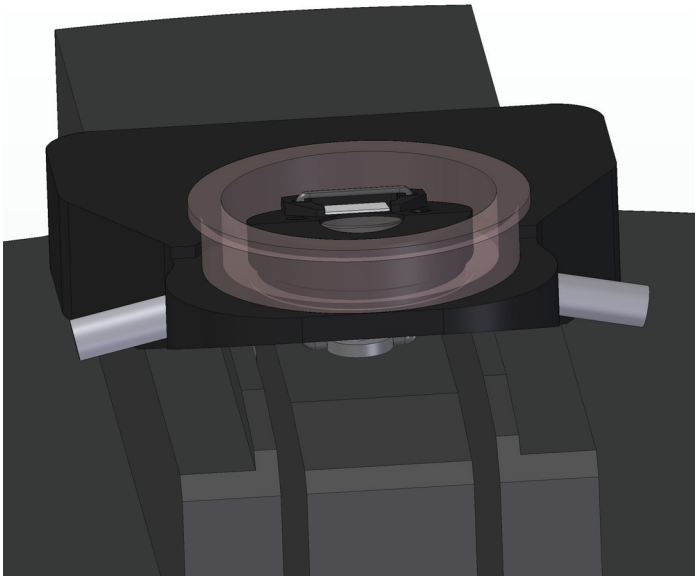


Figure 11.1e Small Volume Fluid Perfusion O-ring Mounted on Fluid Probe Holder



8. The fluid probe holder is now ready to be attached to the BioScope Catalyst head for scanning. Figure 11.1a shows the assembled small volume fluid perfusion chamber, with attached tubing sealed onto a glass cover slip.

11.1.4 Fluid Flow Rate Recommendations

- Adjustable flow rates: Excessive flow in and out of the perfusion cell will prevent proper imaging. Insufficient flow may prevent the desired environment during imaging. Metering of flow rates is optional and at user discretion.
- Maximum flow during imaging should be no greater than 1mL per hour (16µL/min)
- If it is required to flush the fluid in the cell at a higher rate than the suggested maximum flow during imaging, withdraw from the sample surface while exchanging fluid at a faster rate and slow down flow to re-engage for continued imaging.

11.1.5 Recommended Fluid Delivery Methods

Syringe Method

Hardware Requirements

- Syringe with an appropriate sized needle able to accommodate 0.05/0.03 inch outer/ inner diameter tubing connected to ports
- Inlet reservoir containing appropriate fluid for imaging
- Small tubing clamp(s) (optional)
- Small reservoir for collection of waste from outlet

Syringe Procedure

The syringe method is the simplest method for introducing fluid into the small volume fluid perfusion cell. After setting up the system for fluid imaging with the small volume fluid perfusion cell, simply introduce enough liquid into one of the tubing sides such that liquid comes out the other tube. This will indicate that the small volume perfusion cell is full. After verifying that the o-ring is not leaking and the small volume perfusion cell is full, clamp the side that the fluid was used to introduce fluid into the cell so that no additional air or bubbles can enter the cell. This will enable better fluid imaging.

Peristaltic Pump Method

A peristaltic pump with low flow can also be used as a viable fluid delivery method. Setup will be determined by the model of pump. The flow should be set such that it is in accordance with the maximum flow guidance provided earlier in this chapter.

11.1.6 Heating Limitations

Chapter 12 Sample Heating

This chapter details how to use the optional sample heating plate on the BioScope Catalyst. Understanding of the information contained in this chapter is required for safe and successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

- **Maintenance** Section 12.8
  - **Heater Stage** Section 12.8.1
  - **Cantilever Holder** Section 12.8.2
  - **Probes** Section 12.8.3
- **Troubleshooting** Section 12.9
- **General Specifications** Section 12.10
  - **Heater Stage Operating Specification** Section 12.10.1
  - **Temperature Controller Specification** Section 12.10.2
- **Appendix A - Front Panel Button Functions** Section 12.11
- **Appendix B - Controller Default Settings** Section 12.12

12.1 Safety Symbols

Before reading this chapter or attempting any procedures included in this chapter, please take the time to read and understand the safety precautions for using the BioScope Catalyst Heater Stage as defined in Chapter 2. If anything is not 100% clear, please contact Veeco before proceeding with using the heater stage.

Figure 12.1a outlines the safety symbols used throughout the BioScope Catalyst User Manual. It is important to become familiar with these symbols and to slow down and exercise caution when you encounter them in the documentation.

Figure 12.1a 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 personnels.
	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 Zustände oder Handlungen, die einen elekrischen Schock verursachen können.
	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.
	This symbol identifies a heavy object. Improper lifting can cause muscle strain or back injury.
	Ce symbole indique un objet lourd. Soulever cet objet de façon incorrecte peut entraîner des froissements musculaires ou des problèmes de dos.
	Dieses Symbol identifiziert ein schweres Objekt. Falsches Anheben kann Muskelzerrungen und Rückenverletzungen verursachen.

12.2 BioScope Catalyst Heater Stage Components

The heater stage accessory can be used with all approved configurations of BioScope Catalyst (i.e., optical microscope options, vibration table options, perfusion cell option).

The heater stage is installed on the BioScope Catalyst by replacing the existing sample stage with the heater stage, and connecting and powering the temperature controller. The heater stage is well suited for imaging living biological samples at a desired environmental temperature.

The following components are part of the heater stage accessory (see Figure 12.2a):

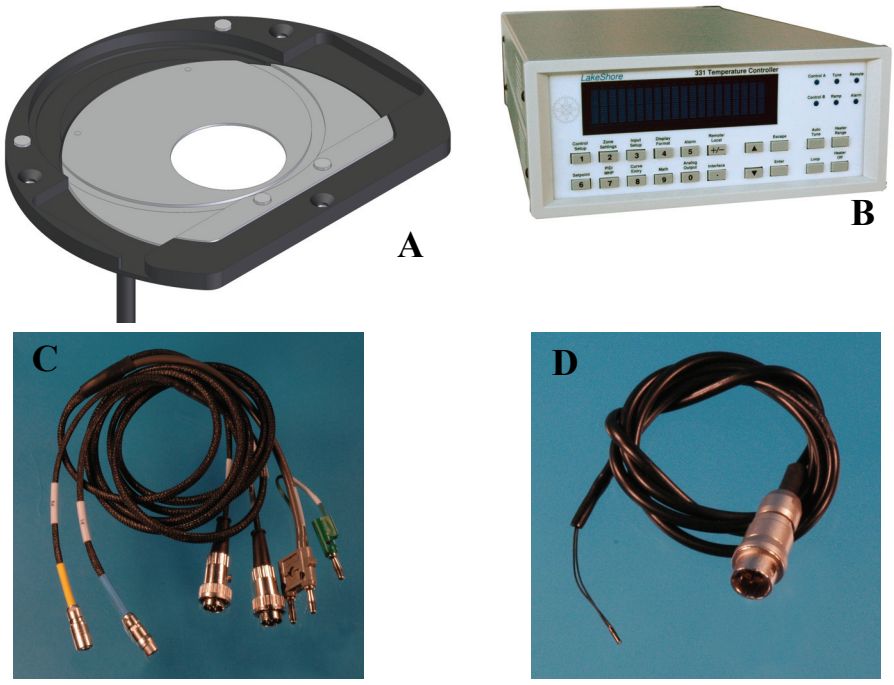
A. A heater stage with integrated temperature sensor, cabling and connectors

B. A Veeco/LakeShore 331S Temperature Controller and *User's Manual* to operate the heater and monitor temperature of the two sensors

C. Heater/sensor extension wiring and connectors

D. An auxiliary fluid thermistor temperature sensor (FLDSENS)

Figure 12.2a Heater Stage Option Components



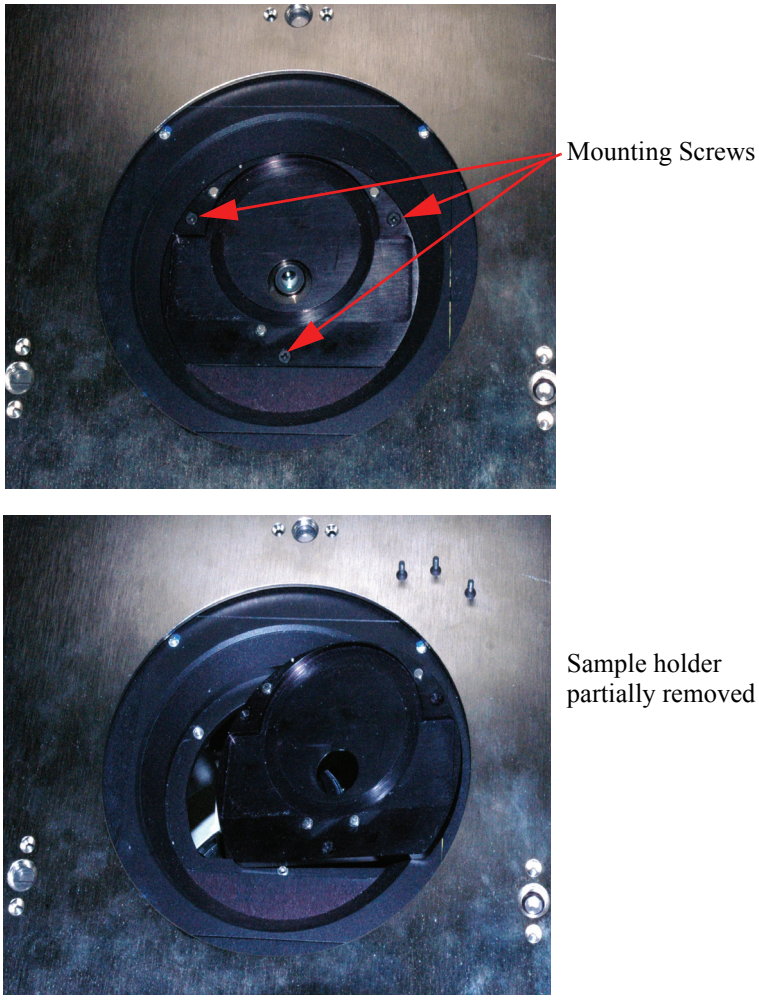
12.3 Installation

12.3.1 Mounting the Heater Stage on the BioScope Catalyst

1. Remove the BioScope Catalyst head from the stage and place it on the EasyAlign.

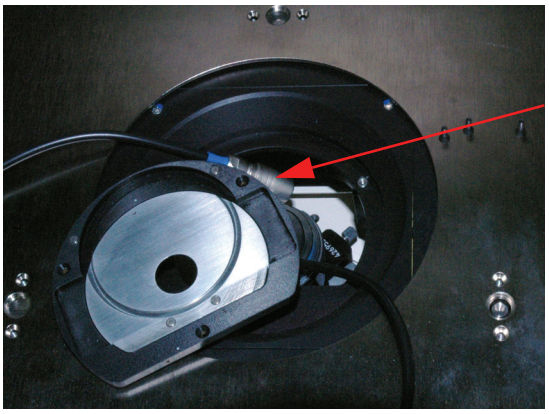
2. Remove the standard sample holder from the stage (see Figure 12.3a) by unscrewing the 3 (#0-80 flat head, #1 Philips) mounting screws. Save the screws for mounting the heater stage.

Figure 12.3a Remove Sample Holder



3. Route the approx. 26” (65 cm) long integrated cable of the heater stage through the opening in the BioScope Catalyst stage and out the side.

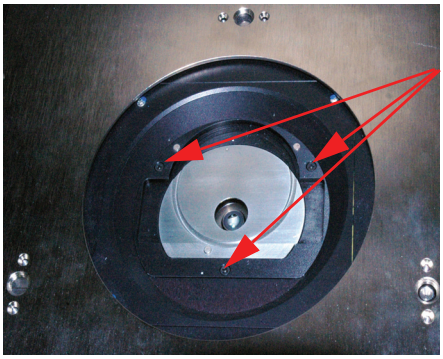
Figure 12.3b Route Heater Stage Cable



CAUTION: Be careful to avoid damaging the inverted microscope if present and ensure the cable is routed to avoid being kinked or pinched or interfering with the optical microscope.

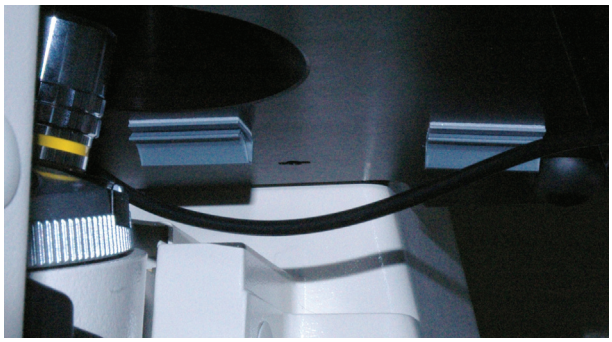
4. Secure the heater stage onto the BioScope Catalyst stage by re-installing the 3 original mounting screws. Do not tighten excessively.

Figure 12.3c Screw Heater Stage in Place



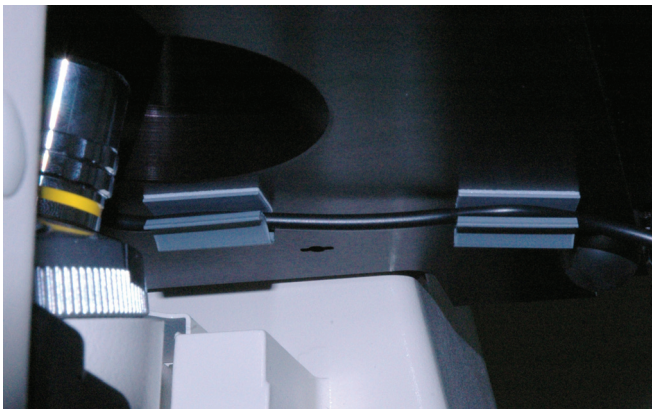
5. If the BioScope Catalyst is installed on an optical microscope, install adhesive mounted cable clips on the underside of the BioScope Catalyst stage. The clips are used to route the cable to avoid interference with the optical microscope turret rotation. If the BioScope Catalyst is table mounted (no optical microscope), cable clips are usually unnecessary. Place the clips to route the cable out of the way of the microscope turret and toward the desired cable exit direction.

Figure 12.3d Self-Adhesive Cable Clips on Bottom of BioScope Catalyst Stage



6. Secure the cable in the clips ensuring that it is not pinched or kinked and will not interfere with the optical microscope turret.

Figure 12.3e Cable in Clip



7. If your BioScope Catalyst is located in an ISBIO isolation hood, route the separate heater/ sensor extension wiring and connectors through the cable clamp with other BioScope Catalyst cables.

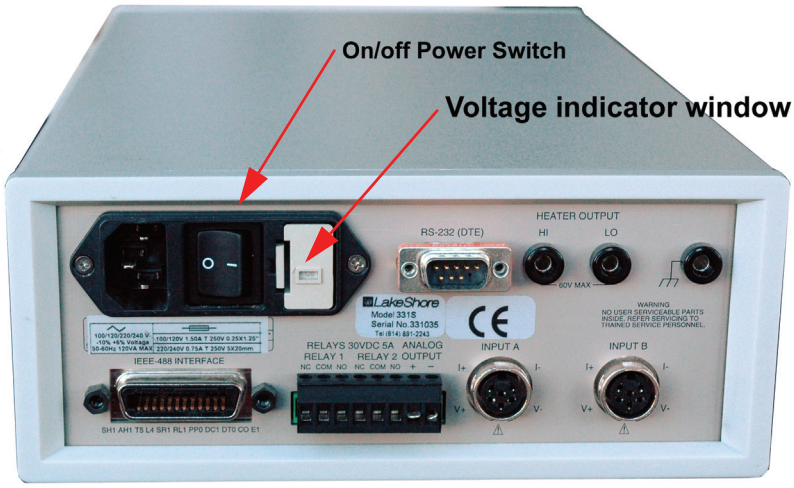
8. Connect the plate cables to the temperature controller cables, matching colors: blue to blue (the heater), green to green (the integrated sensor) and yellow to yellow (the auxiliary sensor).

Note: The heater plate may be used in place of the main sample holder plate, even if not used as a heater.

12.3.2 Cable Connections

1. On the temperature controller back panel, check that the AC voltage setting is appropriate to your location.

Figure 12.3f Voltage Setting



WARNING: If an incorrect voltage setting appears in the little window in the fuse box cover to the immediate right of the power switch on the back of the temperature controller, do not turn on power. See **Change of AC Operating Voltage Setting,** page 174.

2. Verify the power switch is in the **OFF** position.

3. Position the temperature controller for ease of viewing its front panel and within reach of the wiring from the sample plate. Plug all cables into the temperature controller (see Figure 12.3g):

   a. Attach the green color-coded cable to **INPUT A** and the yellow color-coded cable to **INPUT B.** The two sensor connectors are mated to the plate heater cable in Step 8 of Section 12.3.1.

   b. Attach the heater power connector (double banana plug) to **HEATER OUTPUT**, **HI** and **LO**.

   c. Attach the chassis ground (the single banana plug) to the adjacent connector marked with the ground symbol.

   d. Connect the AC power cord into the module in the upper left of the controller back panel.

Figure 12.3g Temperature Sensor Attachment to Temperature Controller

CAUTION: Do not block the ventilation holes in the sides of the temperature controller.

CAUTION: If the sensor connections are mistakenly exchanged and the external thermistor is used to directly control the fluid temperature in the sample holder, control will be poor and the temperature will oscillate over a range that may threaten living cells.

12.3.3 Power-Up

1. Plug the AC power cord into a wall outlet. Turn the power to the controller **ON**.

Figure 12.3h Lakeshore Controller Front Panel

12.4 Lakeshore Temperature Controller Operation

The operating procedures detailed in this section assume prior experience with BioScope Catalyst imaging. Refer to the BioScope Catalyst User Manual for details on scanning if any section below is unclear.

For more complete detail on the Veeco/LakeShore 331S Temperature Controller, refer to the *Model 331 Temperature Controller User's Manual*.

> **Note:** The temperature controller manual is also available from: www.lakeshore.com, the "Service" link.

Front Panel Displays

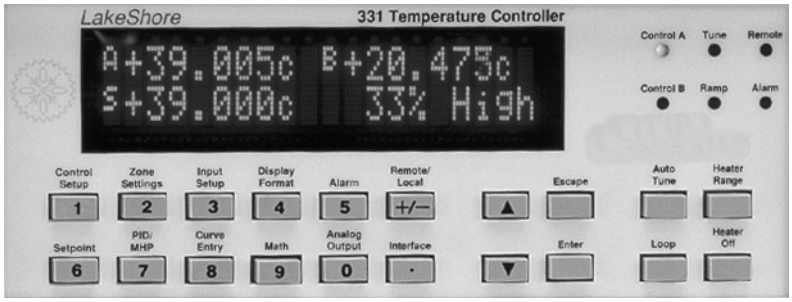
The temperature controller is pre-programmed with default settings (see **Appendix B - Controller Default Settings,** page 180) that are displayed on the front panel readout.

> **Note:** The default settings are appropriate for standard use of the temperature controller with the BioScope Catalyst heater stage; no modifications are needed.

The symbols displayed are:

- A - sensor input A B - sensor input B
- S - setpoint V - sensor units of volts
- W - sensor units: ohms mV - sensor units: millivolts
- K - temperature, Kelvin C - temperature, Celsius
- > - maximum value < - minimum value
- / - linear equation result

Figure 12.4a Front Panel Readout



On power-up, typically four parameter/setting subpanels are shown (see Figure 12.4a). To change what is displayed in one of the subpanels:

1. Press the **DISPLAY FORMAT** button (top row, button "4"). "Display Location 1" appears written in the lower half of the display.

2. Use the up/down arrow buttons to pick among Display Location 1 (upper left display subpanel), Display Location 2 (upper right display subpanel), Location 3 (lower left), etc. Press **ENTER**.

3. Use the up/down arrow buttons to select **INPUT A**, **INPUT B**, or **NONE**, with the additional option of **SETPOINT** for display location 3 and of **HEATER OUT** for display location 4. Press **ENTER**.

4. Use the up/down arrow buttons to select a source among options: **TEMP K, TEMP C**, **SENSOR**, **LINEAR**, **MIN** and **MAX**. Press **ENTER**.

   Note: If **SETPOINT** is entered for display location 3, only the first three source options are available. If **HEATER OUT** is entered for display location 4, there are no additional options to enter.

   Note: To display a calculated temperature in location 4, see **Advanced Operation 2: Math Functions,** page 165 and **Fluid vs. Plate Temperature Calibration,** page 166.

The six front panel lights (LED) "annunciators" indicate system status in normal operation. In normal operation, only the first light, Control A, is on.

- **Control A** (**B**)—is on when Input A (B) is the control input.

- **Tune**—is on continuously when Auto Tune is on, and blinks when Auto Tune is actively gathering data.

- **Ramp**—is on continuously when the Ramp feature is on, and blinks during a setpoint ramp.

- **Remote**—is on when in Remote Mode (i.e., controlled through the IEEE-488 interface or RS-232 port).

  Note: The IEEE-488 interface is not necessary for normal BioScope II heater stage function.

- **Alarm**—is on continuously when the alarm feature is on, and blinks when any alarm is activated.

  Note: Alarms have not been programmed as a part of BioScope Catalyst heater stage function, but can be used if desired.

Basic Front Panel Operation

There are twenty front panel function buttons (see Figure 12.4a). See **Appendix A - Front Panel Button Functions,** page 179 for the full set of functions. See **Appendix B - Controller Default Settings,** page 180 for the initial default values of these parameters. Only a few of the front panel buttons are regularly used:

- Enter a temperature setpoint: press **SETPOINT**, enter a number (use the numbered dual function buttons in the left half of the front panel buttons) and then press **ENTER**.
- Turn on the heater: press **HEATER RANGE**, press the arrow buttons to select **HIGH** and then press **ENTER**.
- Turn off the heater: press **HEATER OFF**.

Advanced Operation 1: Setting Alarms

In the default settings, alarms are disabled. Alarms may be set to indicate both over-maximum and under-minimum conditions.

> **Note:** Though alarms may be set to warn the experimenter of an (unlikely) equipment malfunction, a more common use is to monitor threats to living samples. For example, set alarms to sound if the temperature of the auxiliary sensor falls outside a prescribed small range (e.g., 35-39°C).

To setup alarms:

1. Press **ALARM** on the front panel (see Figure 12.4a).
2. Press the arrow buttons as needed to select **INPUT A** or **INPUT B**. Press **ENTER**.
3. Press the arrow buttons as needed to select **ALARM ON** or **ALARM OFF**. Press **ENTER**.
4. Press the arrow buttons as needed to select among: **TEMP C** (degrees Celsius), **TEMP K** (Kelvin), **LINEAR** ("MX+B" or "M(X+B)") or **SENSOR** (in volts, millivolts or ohms). Press **ENTER**.
5. Use the number keys to enter the low value alarm trigger point as a number consistent with the units selected above. Press **ENTER**.
6. Press the arrow buttons as needed to select **LATCHING ON** or **LATCHING OFF**. With latching on, the alarm remains on even after its trigger is removed (e.g., after the temperature is reduced from an over-maximum condition); **ALARM** must be pressed to silence it. Press **ENTER**.
7. Use the number keys to enter a numeric dead band value in the same units as the trigger point(s), then press **ENTER**. The dead band is a range of values just less than the maximum trigger point - and duplicated as the same range just greater than the minimum trigger point. A triggered alarm does not deactivate until the control parameter has exited the dead band adjacent the violated extreme.

8. Press the arrow buttons as needed to select **ALARM SETPOINT ON** or **DEAD BAND**. Press **ENTER**.

9. Press the arrow buttons as needed to select **AUDIBLE ALARM ON** or **AUDIBLE ALARM OFF**. Press **ENTER**.

Advanced Operation 2: Math Functions

Three **MATH** functions are provided to:

- Capture maximum and minimum sensor readings
- Apply a linear equation to the input data and display results
- Filter noise from sensor data.

To program math functions:

1. Press the **MATH** button. Press **ENTER** to continue. Alternatively, pressing the **MATH** button again clears (i.e., **RESET**s) the maximum and minimum readings recorded since the last reset.

   Note: The stored maximum and minimum readings are cleared when the controller is turned off.

2. Press the arrow buttons as needed to select **INPUT A** or **INPUT B**. Press **ENTER**.

3. Press **ENTER** for the Max/Min function. Press the arrow buttons as needed to select among: **TEMP C** (degrees Celsius), **TEMP K** (Kelvin), **LINEAR** "MX+B" or "M(X+B)" or **SENSOR** (in volts, millivolts or ohms). Press **ENTER**.

   Note: This concludes this procedure unless you are also enabling Linear Equation and/or Filter functions. Proceed to step 4 to specify a linear equation. Proceed to step 10 to specify filtering.

4. Press **ENTER** repeatedly until **LINEAR EQU MX+B** appears in the lower half of the display.

5. Press the arrow buttons as needed to select **MX+B**. Press **ENTER**.

6. Enter a numeric M value. Press **ENTER**.

7. Press the arrow buttons as needed to select among linear X variables: **TEMP C**, **TEMP K**, or **SENSOR**. Press **ENTER**.

8. Press the arrow buttons as needed to select among linear B variables: **+SP1**, **-SP1**, **+SP2**, **-SP2** or **VALUE**. **SP** stands for setpoint. Press **ENTER**.

9. Enter a numeric B value. Press **ENTER**.

   Note: This concludes this procedure unless you are also enabling the Filter function. Proceed to step 10 to specify filtering.

10. Press **ENTER** repeatedly until **FILTER ON** appears in the lower half of the display.

11. Press the arrow buttons as needed to select **FILTER ON** or **FILTER OFF**.

    Note: If you select **FILTER OFF** the controller returns to normal operation and you may exit this procedure.

12. Press the arrow buttons as needed to select between **02** and **64** filter points. The default value is **08**. Exponential filtering is applied to the moving average of the number of points selected. Press **ENTER**.

13. Press the arrow buttons as needed to select between a **01%** (the default) and **10%** filter window. If a single reading is greater than the filter window value, the reading is treated as intentional and the filter is restarted. Percentages are calculated over the full scale range.

12.5 Fluid vs. Plate Temperature Calibration

The controller may be programmed to estimate temperatures at locations other than where the sensors are positioned. For instance, the temperature within a fragile biological cell sample centered in the petri dish may be projected from the heater plate temperature based on a prior calibration of the setup. During the calibration, the auxiliary sensor, rather than a sample, is positioned in fluid at the dish center. Measure the temperature at the center of the dish on the plate, a relatively cool location, using the auxiliary sensor, while also monitoring the temperature of the plate. Repeat the measurement at various temperatures within the range of interest, allowing time for thermal stabilization between readings.

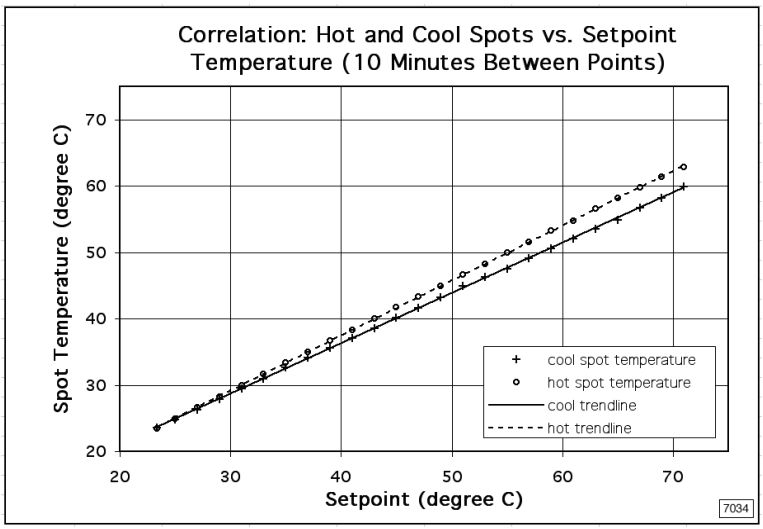
The temperature projection is a typical use of the **LINEAR EQU**ation **MATH** function. Express the correlation between the two temperature sensors by defining the dish center temperature as a function of plate temperature. The function may be fit offline to a linear equation and the coefficients, **M** and **B**, entered into the Veeco/LakeShore controller. Thereafter, the predicted center temperature, where the sample is located, may be displayed along with the plate and fluid temperatures.

The same procedure may be repeated at a “hot spot” - away from the center of the sample holder - directly above the heating element. Both correlation exercises have been performed at Veeco Metrology Group. Representative data (***for reference only***)is illustrated in Figure 12.5a. The linear fit equations summarizing these measurements are:

Hot spot: $T_{fluid} = 0.875 * T_{plate} + 4.25$
Cool spot: $T_{fluid} = 0.82 * T_{plate} + 4.25$, where T_{plate}: Input A (°C)

Actual result will vary with individual heater stages and ambient conditions.

Figure 12.5a Example of Data and Linear Fitting



The auxiliary temperature sensor used with the Veeco/LakeShore 331S must be calibrated to convert electrical potential readings in volts or resistance readings in ohms into temperature values. The BioScope Catalyst heater stage comes with calibration curve “21 User 2252” installed to make the appropriate conversion for the auxiliary thermistor supplied with the accessory.

Note: The temperature is lower in the petri dish placed on the heated sample stage than the reading given by the sensor integrated with the heating element. Therefore, in **Appendix B - Controller Default Settings,** page 180, the Veeco default **SETPOINT** is **39°C** to achieve 37ºC between “cool” and “hot” regions of the fluid.

12.6 Imaging with a BioScope Catalyst SPM

The BioScope Catalyst with a heater stage is designed to facilitate imaging live biological samples. The heater stage permits imaging of polymers, proteins and biomaterials at temperatures important to the characteristics of such specimens. The basic procedures for imaging nonliving or living samples are nearly identical to each other and are described in this section with any differences in procedure noted. This section is intended to be a guide for imaging with the heater stage. Details specific to imaging with the heater stage are described but other details are omitted and covered in other sections of the BioScope Catalyst User Manual.

The basic steps which are described are:

- **Setup the Lakeshore Controller and BioScope Catalyst SPM:** Section 12.6.1
- **Prepare the Specimen/Sample:** Section 12.6.2
- **Mount the Sample on the BioScope Catalyst:** Section 12.6.3
- **Engage and Realign the Laser:** Section 12.6.4

- **Perform Imaging:** Section 12.6.5

12.6.1 Setup the Lakeshore Controller and BioScope Catalyst SPM

This section assumes that the BioScope Catalyst with heater stage and Lakeshore controller have been properly and fully installed as described earlier in this document.

A. Setup the Lakeshore controller

Note: Setup the controller and start the heater stage to permit it to warm up while the steps in the remainder of this section are being performed.

CAUTION: Verify that a **SETPOINT LIMIT** is in effect:

1. Press **CURVE ENTRY**.
2. Press the arrow buttons as needed to select **EDIT CURVE**. Press **ENTER**.
3. Press the arrow buttons as needed to select curve "**21 USER 2252**".
4. Press **ENTER** as needed to advance to **SP LIMIT**.
5. Verify that the recorded value is **75C**; use the numerical keypad to enter the value otherwise.
6. Press **ESCAPE** to complete your verification.

See **Basic Front Panel Operation,** page 164, for description of the small subset of temperature controller functions used in imaging.

B. Install a probe in the cantilever holder.

It may be important to clean the cantilever holder prior to use to avoid contaminating the test specimen. This cleaning is especially critical when imaging living samples. The cantilever holder may be cleaned with most cleansers and disinfectants but will not withstand autoclave temperatures. The BioScope Catalyst User Manual chapter on fluid imaging describes cleaning of the probe holder.

C. Align the laser and adjust the photodetector.

The BioScope Catalyst head is on EasyAlign during this step. Follow the procedure for laser alignment and photodetector adjustment in the user manual.

12.6.2 Prepare the Specimen/Sample

Sample preparation is left to the expertise of the user but the following suggestions/ information should be noted.

- The BioScope Catalyst heater stage will accept samples in standard 50mm and 60mm petri dishes.
- Some additional liquid should be prepared in an appropriate dispenser (micropipette, syringe) and preheated to replenish any liquid lost to evaporation.
- It is assumed that the sample and additional liquid have been maintained at an appropriate temperature in an incubator or similar device to avoid thermal shock when the sample is transferred to the BioScope Catalyst.

Note: The perfusion clamp can be used in conjunction with the o-ring and the Fluid Condensation Window hardware in order to prevent evaporation during heated experiments. For more details on the Fluid Condensation Window hardware, please refer to Chapter 13.

12.6.3 Mount the Sample on the BioScope Catalyst

1. Check the Lakeshore display to ensure the heater stage is at the desired temperature.
2. Place the sample substrate (petri dish) onto the (preheated) heater stage taking care to avoid spillage.

   Note: It is advisable to reduce the amount of liquid to an acceptable minimum prior to mounting the substrate.

3. Clamp the sample substrate onto the heater stage.

   Note: If a standard substrate clamp is used, the surface of the petri dish will be essentially open to the ambient environment. The optional perfusion cell may be used with 50mm dishes and provides a somewhat sealed environment.

4. Install the external temperature sensor. For some analyses, the external sensor may be eliminated as an unacceptable source of contamination or physical damage to the specimen. If the external sensor is eliminated, the less direct measurement of temperature provided by the plate sensor must be relied upon for temperature control.
5. Add more (preheated) liquid as required

12.6.4 Engage and Realign the Laser

When imaging in fluid, refraction will alter the laser beam and the liquid temperature will affect cantilever deflection. This combination of effects requires adjustment of the laser alignment and photodetector positioning. When using the heater stage, ensure that the fluid and probe temperatures have stabilized before realignment. Stabilization time will vary with the liquid temperature and ambient environment. Realignment is described in detail in the *BioScope Catalyst User Manual*. Briefly, the procedure is:

1. Approach so that the probe is in liquid and near the sample surface.

2. Wait for temperatures to stabilize.
3. Realign laser and photodetector.
4. Retune cantilever oscillation (when appropriate).
5. Ready for Engage.

12.6.5 Perform Imaging

1. Engage. When imaging delicate specimens, such as living cells, position the probe tip to engage in a location which will not damage the specimen.
2. Perform imaging as described in the user manual.

12.6.6 Replenish Liquid

Imaging in liquid may require that the liquid be replenished. Some guidelines are:

- At 37°C, evaporation loss of water has been observed to exceed 4% per hour. Actual loss will vary with the temperature setting, liquid used and ambient conditions. Long imaging sessions may require replenishment of liquid.
- Do not add liquid while performing imaging or poor imaging will result. Withdraw/disengage, keeping the probe in the liquid when adding liquid.
- Add liquid to the petri dish only and do not allow liquid to spill or contact any portion of the BioScope Catalyst or cantilever holder. Liquid spillage can migrate and cause electrical problems as well as produce undesirable residues.
- Do not contact the probe or cantilever holder with the micropipette/syringe when adding liquid. The probe is extremely delicate so that any inadvertent contact is likely to produce severe damage. Bumping the cantilever holder may change its positioning/seating and affect imaging.
- Allow sufficient time for temperature to re-stabilize (readjust laser alignment and photodetector positioning if needed).

12.6.7 Change Temperature

The temperature setting can be changed if desired. Do not change temperature settings while imaging or a poor image or perhaps damage to the sample and/or probe may result.

1. Withdraw the probe, leaving it within the liquid.
2. Change the temperature by changing the **SETPOINT** on the Lakeshore controller.
3. Wait until the temperature has stabilized at the new temperature.

4. Realign the laser and photodetector as required.
5. Retune the cantilever if required.
6. Reengage and resume imaging.

12.7 Imaging Modes

Although Contact Mode imaging is a straightforward method of analysis, soft biological samples may be damaged using this mode. More commonly, Tapping Mode is used to minimize contact with the sample and reduce the likelihood of damage. Both modes are described in greater detail in the *BioScope Catalyst User Manual* and both modes can be used with the heater stage.

12.8 Maintenance

12.8.1 Heater Stage

The heater stage should be cleaned periodically to ensure good thermal conductivity between the stage and petri dish. Remove any debris or residue accumulation. Use lint free wipe either dry or dampened minimally with either distilled water or isopropyl alcohol. If a dampened wipe or swab is used it should leave no liquid on the surface or crevices since any free liquid will tend to wick and may result in damage to the equipment or a safety hazard.

Less frequently, it is recommended to remove the heater stage and clean the mounting surfaces of any residues or debris which may have accumulated from use.

CAUTION: Do not submerge the heater stage while cleaning. Use only minimally dampened wipes and dry immediately.

12.8.2 Cantilever Holder

The cantilever holder should be cleaned prior to storage following any imaging session. It is permissible to immerse the cantilever holder in distilled water or isopropyl alcohol for cleaning but it should be thoroughly blotted and blown dry before storage.

12.8.3 Probes

Probes are extremely delicate and can be cleaned only with the greatest of care. Refer to the *BioScope Catalyst Use Manual* for more information.

12.9 Troubleshooting

CAUTION: Verify that the Veeco default parameter values are in effect on the temperature controller. See **Appendix B - Controller Default Settings,** page 180.

Unresponsive Front Panel Buttons

Check the **Remote** LED in the upper right corner of the temperature controller front panel. If it is lit, the controller is no longer in Local mode. To return the controller to Local mode, press the **REMOTE/LOCAL** button. The **Remote** LED is turned off.

Display Reads "S. Over" or "S. Under"

The input is either at or over the full-scale value, or at or under the negative of the full-scale value in sensor units, respectively.

1. Check the Input A and Input B sensor cable connections at the back of the temperature controller (see Figure 12.3g) and the in-line connections of the yellow and green color-coded cables.

2. If the problem persists, verify that "Curve 21 User 2252" is available:

   a. Press the **CURVE ENTRY** button.

   b. Press the up/down arrows until **EDIT CURVE** appears.
   Press **ENTER**.

   c. Press the up/down arrows until **CURVE 21 USER 2252** appears. Press **ESCAPE**. If **CURVE 21 USER 2252** does not appear, it is no longer in the controller memory: contact Veeco Metrology Group for directions to restore the curve.

3. Check the input setup:

   a. Press **INPUT SETUP**. Select **INPUT A** or **INPUT B** with the up/down arrows. Press **ENTER**.

   b. Verify that correct type appears or press the up/down arrows until it does. Press **ENTER**.

   - Input A - 1000W Plat
   - Input B - NTC RTD

   c. Verify that correct curve is selected for Input A or press the up/down arrows until it does. Press **ENTER**.

- Input A - 07 PT-1000
- Input B - Curve 21 User 2252

d. Reversal = On (for both inputs).

Heater Output Display Reads “Htr Open”

1. Check the controller back panel heater connections: Heater Output Hi, Lo and Gnd (see Figure 12.3g).
2. Check the blue color-coded in-line cable connection.

Failure to Control Temperature

If the temperature controller fails to respond to changes in setpoint, make sure Loop 1 is selected.

If the Heater Output (display location 4, see **Front Panel Displays,** page 162) reads “Off L2”, or setpoint is in units of Kelvin (K), then the wrong loop is selected.

> **Note:** The BioScope Catalyst heater stage uses Loop 1. The temperature controller also offers control Loop 2, an analog output.

Press the **LOOP** button until **LOOP 1** is selected.

If the controller overshoots or undershoots or does not track the setpoint, the control parameters may have been replaced from their default settings.

1. To check the values of control parameters P, I and D, press the **PID/MHP** button. If the value reported for Proportional gain (P) is not **50.0**, enter **50.0** using the numbered buttons on the front panel and conclude by pressing **ENTER**.
2. If the value next reported for Integral gain (I) is not **20.0**, enter **20.0** using the numbered buttons on the front panel and conclude by pressing **ENTER**.
3. If the value reported for the Derivative parameter (D) is not **0.00**, enter **0.00** using the numbered buttons on the front panel and conclude by pressing **ENTER**, followed by **ESCAPE**.

> **Note:** To check the P, I and D parameters individually, press the **PID/MHP** button once, then press the **ENTER** button as often as needed to display the parameter of interest. Conclude by pressing the **ESCAPE** button.

Change of AC Operating Voltage Setting

If the AC line voltage indication showing in the little window in the fuse drawer cover to the immediate right of the power switch on the back of the temperature controller (see Figure 12.3f) does not match the local line voltage, then the AC line voltage is not set correctly and the wrong fuses may be installed. A “100” appears for 100V or “120” for 120V ground-referenced operation (typical of the United States). Similarly, “220” or “240” show for 220V or 240V operation, respectively. Use the following procedure to change the LakeShore temperature controller line voltage selection.

CAUTION: Verify the fuse value whenever line voltage is changed (see the following section).

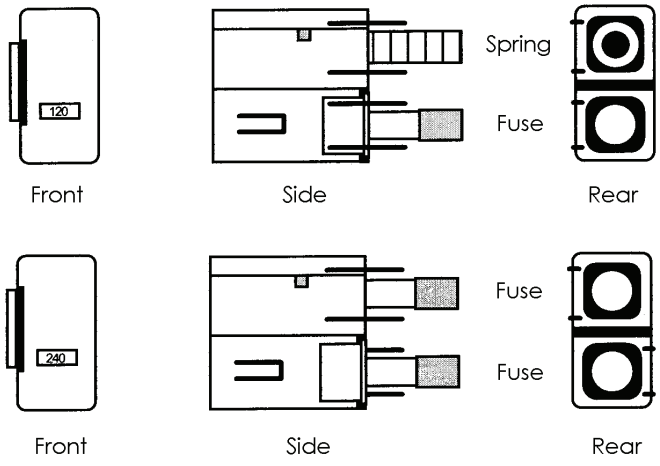
CAUTION: To avoid potentially lethal shocks, turn off the controller and disconnect it from AC power before performing this procedure.

1. Identify the line input assembly on the controller rear panel. The line input assembly consists of the power cord plug, power switch, and the fuse drawer.
2. Turn the line power switch to **OFF** (**0**).
3. Remove the controller power cord.
4. With a small flat head screwdriver, release and remove the fuse drawer holding the line voltage selector and fuse(s).
5. If necessary, locate the alternate fuse drawer assembly for the appropriate voltage. There are two types of fuse drawers, for 100/120V (beige) and 220/240V (black). The alternate fuse drawer is included in an accessory kit with the BioScope II heater stage (see Figure 12.9a).
6. Slide the removable plastic fuse holder out of the fuse drawer.
7. Rotate the fuse holder until the proper voltage is indicated in the small window, after it is re-installed into the fuse drawer.
8. Install and verify that the proper fuses are installed in the fuse holder(s) and fuse drawer.
9. Re-install the fuse drawer into the line input assembly.

10. Verify the proper voltage is indicated in the small window, after re-installing the fuse drawer.

11. Connect the controller power cord. and turn the line power switch **ON** (**1**).

Figure 12.9a Two Line Voltage-Specific Fuse Drawers



Above: Single fuse drawer for 100V/120V operation.
Below: Dual fuse drawer for 220V/240V operation.

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Changing Fuses

For proper operation with 100V or 120V supplied power, the Veeco/LakeShore 331S Temperature Controller uses a single 1.5A “slow blow” fuse (of size 0.25" by 1.25", 250V rating) in one holder of the fuse drawer and a spring in the other holder (see Figure 12.9a). For either 220V or 240V line voltage use the other fuse drawer, also supplied with the BioScope II heater stage, with a 0.75A “slow blow” fuse (of size 5mm by 20 mm, 250V rating) in each of the two holders (see Figure 12.9a). Contact Veeco Metrology Group for replacement fuses. Use the following procedure to change fuses..

WARNING: To avoid potentially lethal shocks, turn off the controller and disconnect it from AC power before performing this procedure

CAUTION: For continued protection against fire hazard, replace only with the same fuse type and rating specified for the line voltage selected.

CAUTION: Test fuse continuity with an ohmmeter. Do not rely on visual inspection alone.

1. Identify the line input assembly on the controller rear panel. The line input assembly consists of the power cord plug, power switch, and the fuse drawer.
2. Turn the line power switch to **OFF** (**0**).
3. Disconnect the temperature controller power cord from the controller.
4. With a small screwdriver, release the drawer holding the line voltage selector and fuse.
5. Remove and check the existing fuse(s) with an ohmmeter. Replace as needed with Slow-Blow (time-delay) 1.5A T 250V fuses (0.25"´1.25" size) for 100V or 120V operation; replace as needed with Slow-Blow 0.75A T 250V fuses (5´20mm size) for 220V or 240V operation.
6. Re-assemble the line input assembly in reverse order.
7. Verify the correct voltage indicator (i.e., 100, 120, 220 or 240) in the line input assembly window.
8. Connect the instrument power cord, first at the instrument, then to AC power.
9. Turn the power switch **ON** (**1**).

Note: If problems persist, or the symptoms are not addressed in this section, contact Veeco Metrology Group Technical Support for assistance.

12.10 General Specifications

12.10.1 Heater Stage Operating Specification

Veeco Metrology Group ensures accurate operation to specification of the BioScope Catalyst heater stage under these conditions:

Table 12.10a BioScope Catalyst Heater Stage Operating Specifications

Parameter	Nominal (typ.)	Comments
Temperature controller power requirement	120 VA	100, 120, 220 or 240VAC, +5%, -10%, at 50 or 60 Hz
Ambient temperature	20-30°C	-
Maximum relative humidity	80%	-
Maximum fluid temperature	60°C	Heating above 60°C is possible, but AFM operation is neither guaranteed nor tested.
Minimum fluid temperature:	ambient (25°C)	-
Recommended fluid height:	1.0-3.0 mm	Meniscus formed with fluid cell 1mm from specimen but do not immerse more than 3 mm.

Operating within the above fluid temperature and volume specifications, the following system characteristics have been measured by Veeco Metrology Group. The values reported here are guidelines and may vary with laboratory conditions (e.g. air temperature and velocity at the sample holder).

Table 12.10b Fluid Heating Guidelines

Parameter	Absolute Max/Min	Nominal / Typical	Comments
Temperature control stability (fluid):	± 0.5°C	± 0.25°C	Measured at fluid temperature of 37°C, ISBIO hood open. See Note 2.
Volume Loss (approximate)	N/A	15-20% in 4 hours @ 37°C	Varies with fluid temperature.
Temperature gradient (uniformity) within optical viewing area:	< 2°C @ 37°C < 4°C @ 60°C	1.0°C typical @ 37°C; 2.5°C typical @ 60°C	Varies with fluid temperature. See Notes 2 and 3.

Notes:

1. The BioScope Catalyst heater stage directly controls sample plate temperature, located underneath the petri dish of fluid. The fluid temperature is controlled indirectly using a calibration of the plate vs. fluid temperatures or by monitoring an external temperature sensor. Some specifications above are given for both the plate, under direct control, and the fluid, under indirect control.

2. Some specifications, including the temperature control stability and temperature gradient or uniformity, will improve with the BioScope Catalyst isolated in a ISBIO acoustic hood with its door closed and the inside temperature stabilized.

3. The fluid and petri dish surface are coldest at the center, over the optics aperture, where the bottom of the petri dish is uncovered and open to the environment. The hottest point is approximately halfway from the center to the edge, where the plate is in direct contact with the bottom of the petri dish. These are the points at which the temperature gradient is the largest.

4. There is essentially no (conventional) temperature overshoot for the fluid, since the plate temperature stabilizes very quickly, although error in the plate vs. fluid temperature calibration may cause overshoot of the desired final fluid temperature (see **Fluid vs. Plate Temperature Calibration,** page 166).

5. Stabilization times may vary for larger temperature changes and higher fluid temperatures.

12.10.2 Temperature Controller Specification

Thermometry:

Isolation: Sensor inputs optically isolated from other circuits but not from each other
A/D Resolution: 24 bit
Maximum Update Rate: 10 readings per second on each input
Filter: Averages 2 to 64 input readings

Control:

Proportional (P) Gain Settings: 0 - 1000 with 0.1 setting resolution
Integral (I) Reset: 1 - 1000 with 0.1 setting resolution

> **Note:** Setting I to zero turns the rest function off. I = 1000 / $I_{seconds}$. A system typically settles to a setpoint within several time constants. For example, I = 20 corresponds to a time constant of 50 seconds, so a 5 to 10 minute stabilization time.

Derivative (D) Rate: 1 - 200% with 1% resolution
Manual Heater: 0 - 100% with 0.001% setting resolution
Setpoint Ramping: 0.1 to 100 K/minute
Loop 1 Heater Output Type: Variable DC current source
Loop 1 Heater Output D/A Resolution: 18 bit
Loop 1 Maximum Heater Power: 50 watts

> **Note:** For the BioScope Catalyst heater stage, heater power is limited to 21.5 watts.

Loop 1 Maximum Heater Output Current: 1 ampere
Loop 1 Heater Output Compliance: 50 volts

Note: The BioScope Catalyst heater element has a resistance of 40W +/- 10%. At the maximum current rating, the heater output voltage is only 40 volts (40W).

Front Panel:

Display Update Rate: All readings twice per second
Temperature Display Resolution: 0.001° between 0° and 99.999°
Heater Output Resolution: 1%

General:

Size: 217mm (8.5") wide by 90mm (3.5") high by 317mm (14.5") deep
Weight: 4.8 kilograms (10.5 pounds)

12.11 Appendix A - Front Panel Button Functions

There are twenty front panel function buttons (see Figure 12.4a). Here they are listed from top-to-bottom and left-to-right and described briefly. See the Veeco/LakeShore 331 *User's Manual* for more detail.

- **CONTROL SETUP** - selects control input, setpoint units, closed vs. open loop control mode, power up enable, setpoint ramp enable, ramp rate, and display of heater output units.
- **SETPOINT** - is the target setting of the active control loop.
- **ZONE SETTING** - provides for entry of 10 temperature control zones, each with its own PID (see next) control parameter settings.
- **PID/MHP** - permits manual adjustment of control parameters Proportional (P), Integral (I) and Derivative (D), or Manual Heater Power (MHP) for the control loop.
- **INPUT SETUP** - selects among sensor types and associated temperature calibration curves.
- **CURVE ENTRY** - allows entry of up to twenty 200-point custom temperature calibration curves.
- **DISPLAY FORMAT** - enables programming the display.
- **MATH** - configures features Max/Min, linear equation and filter. Press twice to restore the default Max/Min settings.
- **ALARM** - is used to enable alarms and relays.

- **ANALOG OUTPUT** - configures the analog output feature.
- **REMOTE/LOCAL** - selects between the IEEE-488 interface and local operation of the controller.
- **INTERFACE** - sets the baud rate of the serial interface and sets IEEE-488 address an terminators.
- Ý - up arrow key for parameter selection and incrementing numbers.
- ß - down arrow key for parameter selection and decrementing numbers.
- **ESCAPE** - terminates a setting function without changing parameter values.

  Note: Press and hold **ESCAPE** to reset instrument and assign LakeShore default values (which are different from Veeco default values (see **Appendix B - Controller Default Settings,** page 180).
- **ENTER** - completes setting functions and returns to normal operation.
- **AUTO TUNE** - selects among tuning modes: **AUTOTUNE PID**, **PI**, **P**, **MANUAL PID** or **ZONE** for the control loop.
- **LOOP** - toggles between control loop 1 and 2.

  Note: The BioScope Catalyst heater stage employs control loop 1 exclusively.
- **HEATER RANGE** - selects among **HIGH**, **MED**(ium) and **LOW**.
- **HEATER OFF** - turns heater off.
- **0 - 9**, +/-, **.** - are used for numeric entries

12.12 Appendix B - Controller Default Settings

The Veeco/LakeShore 331S Temperature Controller has been programmed by Veeco Metrology Group with default settings applicable to BioScope Catalyst applications. Some of these are modifications of LakeShore's original defaults. Both settings are listed below.

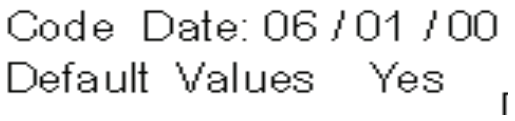
Note: The Veeco defaults are highlighted (**bold**) where they differ from the LakeShore default settings.

Reset Procedure: LakeShore Defaults

1. To reset the controller to the LakeShore default settings, press and hold the **ESCAPE** key until the contents of Figure 12.12a appear.

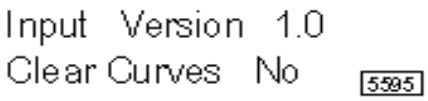
   Note: The "Code Date" in Figure 12.12a varies with firmware revision.

Figure 12.12a Controller Default Settings Reset Prompt Screen



2. Use the up/down arrow keys to select **YES** (to reset) or **NO** (to cancel), then press **ENTER**. A second screen appears (see Figure 12.12b).

Figure 12.12b Second Controller Default Settings Screen



3. Use the up/down arrow keys to select **NO**, then press **ENTER**.

CAUTION: Selecting **YES** to clear curves in the last step erases any stored curves other than the 21 standard curves. The BioScope II heater stage uses a nonstandard curve; it would have to be re-entered if curves were cleared in the last step. Contact Veeco Metrology Group Technical Support if the nonstandard curve used by the BioScope II heater stage must be re-input to the controller.

CAUTION: Verify that both Input A and Input B are set to the Veeco defaults.

Note: The acronym "PID" represents the proportional, integral and differential control loop parameters. "MHP" is short for manual heater power.

Reset: Veeco Defaults

Only the parameters appearing in **bold** font in the "Veeco Default" column have values different from the LakeShore default, so need to be changed. To reset individual parameters to the Veeco default:

1. Press the associated controller front panel function button.
2. Press **ENTER** (repeatedly, if necessary) until the parameter of interest appears.
3. Press the up/down arrow buttons to the value desired, or enter a numerical value.
4. Scroll (press **ENTER** repeatedly) until the end of options for the parameter, or press **ESCAPE** to quit the function button.

Table 12.12a Veeco Default Parameters for the Lakeshore Controller

Temperature Controller Button	Temperature Controller Parameter	Veeco Default	LakeShore Default
Control Setup:	Control Input	Input A	Input A
"	SP Units	**Temp C**	Temp K
"	Control Mode	Closed	Closed
"	Power Up	Disable	Disable
"	Heater Output Display	Current	Current
"	Setpoint Ramp	Off	Off
Zone Settings:	Setpoint Limit	0.000K	0.000K
"	Proportional (P)	50.000	50.000
"	Integral (I)	20.000	20.000
"	Derivative (D)	0.00	0.00
"	Manual Output	0.00%	0.00%
"	Heater Range	Off	Off
Input Setup - Inputs A:	Type	**1000W Plat**	Thermocouple/25mV
"	Curve	**07 PT-1000**	Type K
"	Reversal	**On**	N/A
Input Setup - Inputs B:	Type	**NTC RTD**	Thermocouple/25mV
"	Curve	**21 User 2252**	Type K
"	Reversal	**Off**	N/A
Display Format:	Location/Display/Source	**1/Input A/Temp C**	1/Input A/Temp K
"	Location/Display/Source	**2/Input B/Temp C**	2/Input B/Temp K
"	Location/Display/Source	3/Setpoint/-	3/Setpoint/-
"	Location/Display/Source	**4/Input A/Linear**	4/Heater Output/-
"	Alternate Location/ Display/Source	**4/Heater Output/-**	N/A
Alarm - Inputs A&B:	Alarm	Off	Off
"	Alarm Audible	Off	Off
"	Relay 1	Off	Off
"	Relay 2	Off	Off

Temperature Controller Button	Temperature Controller Parameter	Veeco Default	LakeShore Default
Remote/Local:	N/A	Local	Local
Setpoint	N/A	**39.000°C**	0.000°K
PID & MHP	Proportional (P)	**250.0**	50.00
"	Integral (I)	**100.0**	20.000
"	Derivative (D)	**100.00**	0.000
"	Manual Output	0.000%	0.000%
Curve Entry:	(Curve 21 already installed)	**21 User 2252**	N/A
Math - Input A:	Source	**Linear**	Temp K
"	Linear Equation	MX+B	MX+B
"	Linear Equation M Value	**0.82**	0.000
"	Linear Equation X Source	**Temp C**	Temp K
"	Linear Equation B Source	Value	Value
"	Linear Equation B Value	**4.25**	0.000
"	Filter	Off	Off
Math - Input B:	Source	Temp K	Temp K
"	Linear Equation	MX+B	MX+B
"	Linear Equation M Value	0.000	0.000
"	Linear Equation X Source	Temp K	Temp K
"	Linear Equation B Source	Value	Value
"	Linear Equation B Value	0.000	0.000
"	Filter	Off	Off
Analog Output	N/A	Off	Off
Interface:	Baud	9600	9600
"	IEEE Address	12	12
"	IEEE Terminators	CR/LF	CR/LF
"	Emulation Mode	331	331
Auto Tune:	Mode	Manual PID	Manual PID
Heater Range:	N/A	Off	Off

Temperature Controller Button	Temperature Controller Parameter	Veeco Default	LakeShore Default
Loop:	Selected	1	1
Keypad Locking:	Mode	Unlocked	Unlocked
"	Lock Code	123	123

Chapter 13 Petri Dish Perfusion Cell

This chapter details how to use the optional petri dish perfusion cell on the BioScope Catalyst. Understanding of the information contained in this chapter is required for safe and successful operation of the BioScope Catalyst.

Specifically, this chapter covers the following information:

- **Safety Symbols** Section 13.1
- **Introduction** Section 13.2
- **Perfusion Cell Construction** Section 13.3
- **Pump Selection** Section 13.4
- **Setup and Use** Section 13.5
- **Additional Considerations** Section 13.6

13.1 Safety Symbols

Before reading this chapter or attempting any procedures included in this chapter, please take the time to read and understand the safety precautions for using the BioScope Catalyst Petri Dish Perfusion Cell as defined in Chapter 2. If anything is not 100% clear, please contact Veeco before proceeding with using the perfusion cell.

Figure 13.1a outlines the safety symbols used throughout the BioScope Catalyst User Manual. It is important to become familiar with these symbols and to slow down and exercise caution when you encounter them in the documentation.

Figure 13.1a 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 personnels.
	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 Zustände oder Handlungen, die einen elekrischen Schock verursachen können.
	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.
	This symbol identifies a heavy object. Improper lifting can cause muscle strain or back injury.
	Ce symbole indique un objet lourd. Soulever cet objet de façon incorrecte peut entraîner des froissements musculaires ou des problèmes de dos.
	Dieses Symbol identifiziert ein schweres Objekt. Falsches Anheben kann Muskelzerrungen und Rückenverletzungen verursachen.

13.2 Introduction

The petri dish perfusion cell is an accessory for use with the BioScope Catalyst system. This section describes the perfusion cell and its operation.

The BioScope Catalyst is designed to perform analyses of a variety of samples, either dry or in fluid. Analysis of samples in liquid is typically performed with the sample, in a petri dish, which contains the fluid. In this type of analysis, the fluid is “static,” that is, there is no controlled method of cycling or replenishing the fluid. The perfusion cell is a special petri dish sample substrate clamp which allows the flow of fluid or gas into the petri dish during imaging. The perfusion cell has a removable silicone skirt which forms a partial seal for gases and provides some isolation from the ambient environment.

The perfusion cell module consists of:

- The perfusion cell
- O-ring
- Silicone rubber tubing

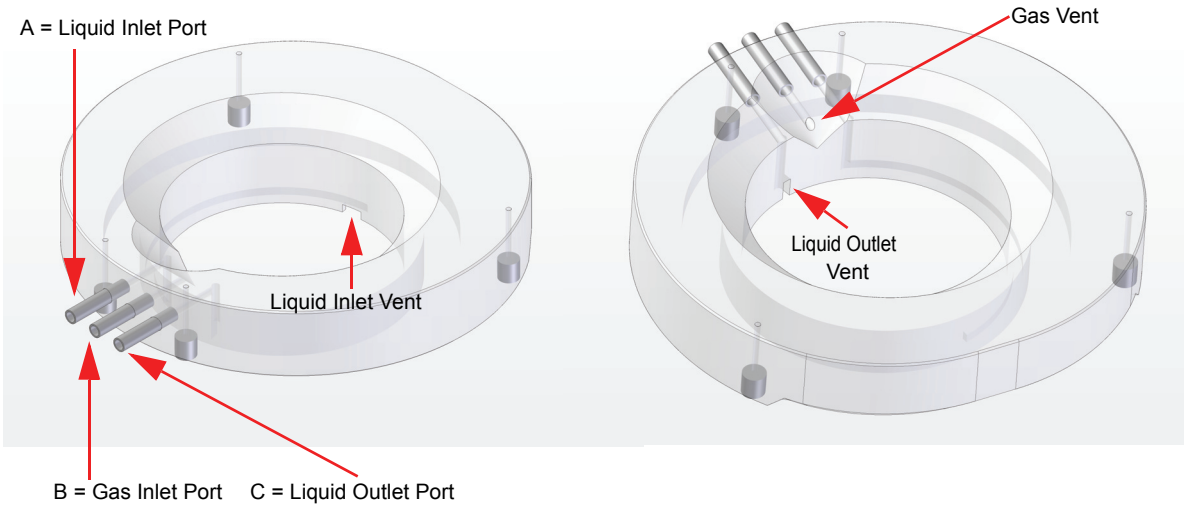
WARNING: Appropriate safety procedures must be observed when using hazardous gases or liquids with the perfusion cell.

CAUTION: Before using any liquids during BioScope Catalyst use, read the *BioScope Catalyst User Manual* section on clean up of spills. Clean up spills immediately according to the clean up instructions in the user manual.

13.3 Perfusion Cell Construction

The perfusion cell is made of Lexan and is equipped with a removable o-ring. The cell contains magnets which serve to clamp a 50mm glass bottom petri dish to the BioScope Catalyst stage. There are three stainless steel tubing ports for fluid or gas flow.

Figure 13.3a Perfusion Cell on 50 mm Petri Dish



A. Gas Inlet Port: Gas flows directly into the clamped petri dish through a gas vent slightly above the rim of the dish.

B. Liquid Inlet Port: Liquid enters the port and is ducted in an open slot near the bottom of the petri dish partway round the perfusion cell. The slot does not seal with the bottom of the dish and liquid may enter the dish from anywhere along the slot. The wall of the slot has three vents to allow fluid to enter the dish more freely.

C. Liquid Outlet Port: Liquid flows out of the petri dish through a vent which is near the port, approximately 1.5 mm farther from the bottom of the petri dish than the inlet duct wall.

13.4 Pump Selection

Several pumps are suitable for use with the perfusion cell. Both peristaltic pumps and syringe pumps have been used successfully. Alternatively, a gravity feed flow design can be a good low noise solution.

> **Note:** There must be appropriate flow in and out of the perfusion cell to prevent overflow.

The chosen fluid handling solution should have the following characteristics:

- Adjustable flow rates: Excessive flow in and out of the perfusion cell will prevent proper imaging. Insufficient flow may prevent the desired environment during imaging. Metering of flow rates is optional and at user discretion.
- Damped flow: Pulsation or other non-uniform flow may prevent proper imaging.

13.5 Setup and Use

It is assumed that the BioScope Catalyst has been prepared and configured for the particular application. Refer to the BioScope Catalyst User Manual for instructions on probe mounting, laser alignment and photodetector positioning.

1. Place all pumping equipment (pump, regulators, etc.) near the BioScope Catalyst but not in contact with the vibration isolation surface.
2. Attach tubing of sufficient length between the perfusion cell and pumping system.
3. Prepare the sample on a 50 mm glass bottom petri dish sample substrate.
4. Clamp the petri dish onto the BioScope Catalyst stage with the perfusion cell.
5. Ensure that the plastic tubing is not pinched or kinked.
6. Start pumping the fluid and wait until the tubing fills with the fluid and the fluid is entering the petri dish.
7. Make sure that there are no leaks and that the outlet flow is sufficient to prevent overflow.
8. Adjust flow rates as needed.
9. Place the BioScope Catalyst head into operating position and proceed with analysis as described in the appropriate section of the BioScope Catalyst User Manual.

13.6 Additional Considerations

- Improper fluid flow (too fast, not uniform) may affect imaging. It may be necessary to adjust flow rate.
- The petri dish perfusion cell is autoclaveable. The preferred autoclave temperature is 121°C at slow pressure.
- The petri dish perfusion cell can be used in conjunction with the heater stage. For detailed information on sample heating, please see Chapter 12. When these two are used in conjunction, it is recommended that the fluid condensation window is also used in order to minimize evaporation and prevent condensation on the mirror and photodetector. Figure 13.6a and Figure 13.6b show the fluid condensation window hardware standalone and on the Catalyst head. The fluid condensation window hardware attaches to the BioScope Catalyst head in the location shown in the top image of Figure 13.6b. Magnets on the BioScope Catalyst head hold the fluid condensation window hardware in place.

Figure 13.6a Fluid Condensation Window Hardware

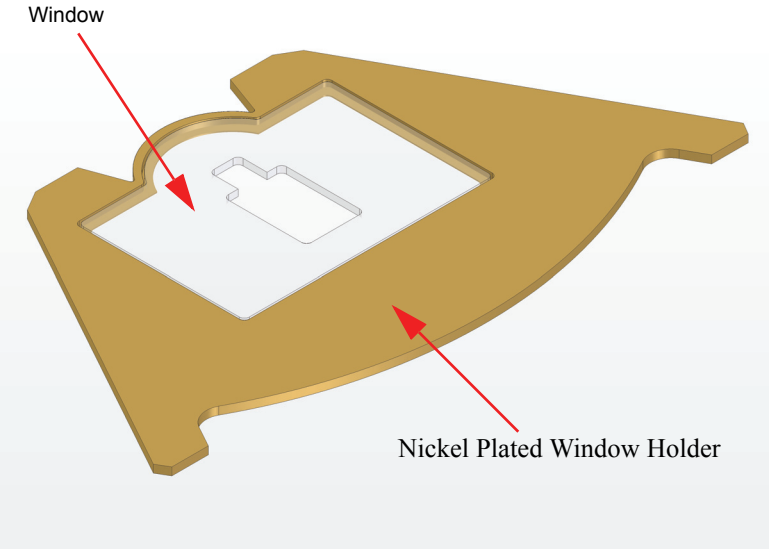
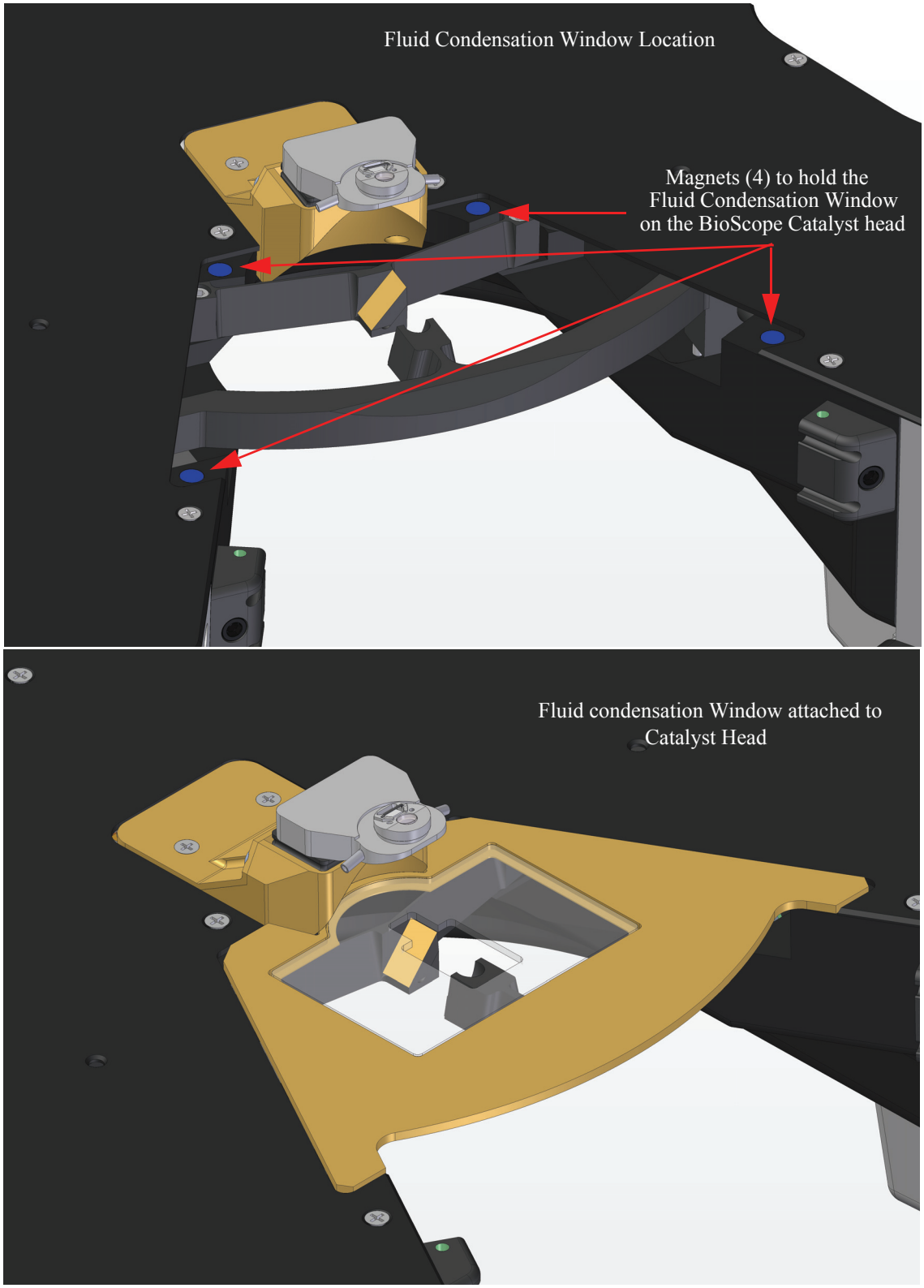


Figure 13.6b Fluid Condensation Window Location on the Catalyst Head



Chapter 14 Adaptive XY Scanning

This chapter discusses Adaptive XY Scanning, a Veeco-proprietary technology that enables faster scanning and less position noise than traditional closed-loop scanning.

Specifically, this chapter discusses the following areas:

14.1 Introduction

Adaptive XY control improves on conventional closed-loop XY control in two areas:

- Higher bandwidth tracking
- Improved position accuracy

Conventional closed-loop XY control, while greatly improving positioning accuracy and scan linearity compared to open loop operation, has two issues:

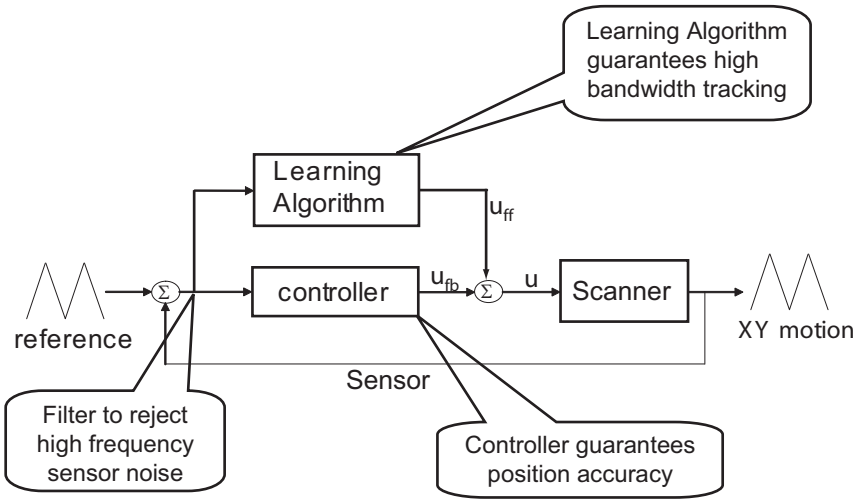
1. Increased XY position noise: The noise of the sensor is introduced into the feedback loop and propagates to the controller output (this is more prominent with scan sizes less than 1μm).
2. Feedback instability and limited tracking bandwidth: the nature of feedback requires that the tracking bandwidth is well below scanner's resonant frequency in XY direction for conventional PID closed-loop XY control.

Noise performance and tracking performance are two conflicting goals.

For AFM applications, the scanner is required to track a repetitive raster scan motion while compensating low frequency position errors introduced by creep and thermal drift. Adaptive XY control separates the two requirements with advanced digital signal processing which results in both increased tracking bandwidth and improved position accuracy.

Figure 14.1a shows a block diagram of the Adaptive XY control mechanism.

Figure 14.1a Block diagram of Adaptive XY control



14.1.1 Higher bandwidth tracking

Adaptive XY control takes advantage of a Veeco-proprietary control technology “Model Based Inverted Iterative Control” to achieve a tracking accuracy (e.g., peak error) of less than 1% of the total scan range in a minimum amount of time. This inversion-based iterative control (IIC) is based on the work of Zou et. al.[1]

After a user specifies a new **Scan Size**, **Scan Rate** or **Scan Angle**, the Adaptive XY algorithm automatically generates and optimizes a scan table. The scan table ensures high bandwidth tracking without introducing sensor noise.

For conventional PID closed-loop XY control, the tracking bandwidth is well below scanner's resonant frequency in the X and Y directions.

With Adaptive XY control, the tracking bandwidth can be even higher than the scanner's resonant frequency. Tens of Hertz can be achieved without compromising image quality.

1.Q. Zou et al., “Precision tracking of driving wave forms for inertial reaction devices,” Rev. Sci. Instrum. 76, 023701 (2005).

Figure 14.1b shows a scan without Adaptive XY control and Figure 14.1c shows the same scan with Adaptive XY control. The periodic artifact in Figure 14.1b is a product of XY scanner ringing. Adaptive XY control, shown in Figure 14.1c, avoids this ringing.

Figure 14.1b Adaptive XY control disabled

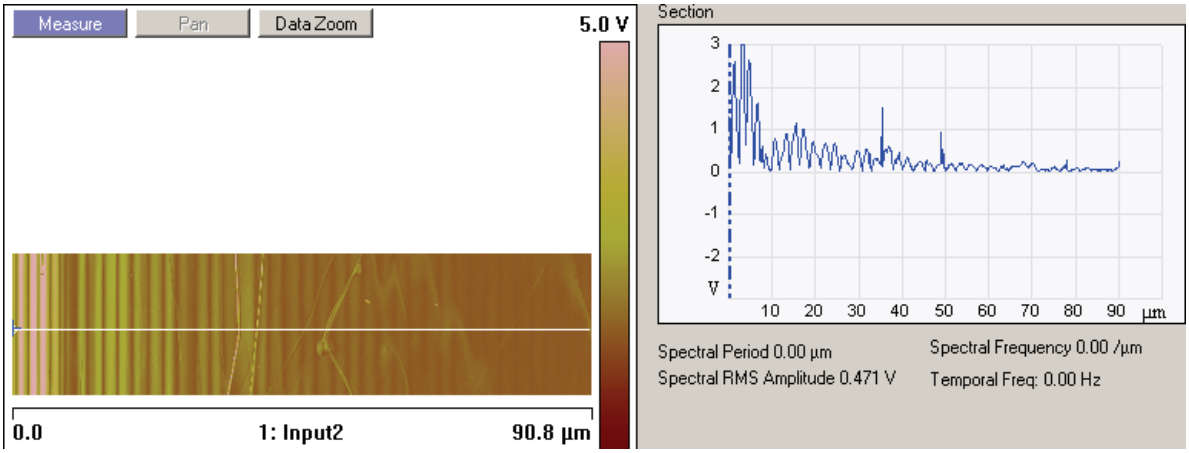


Figure 14.1c Adaptive XY control enabled

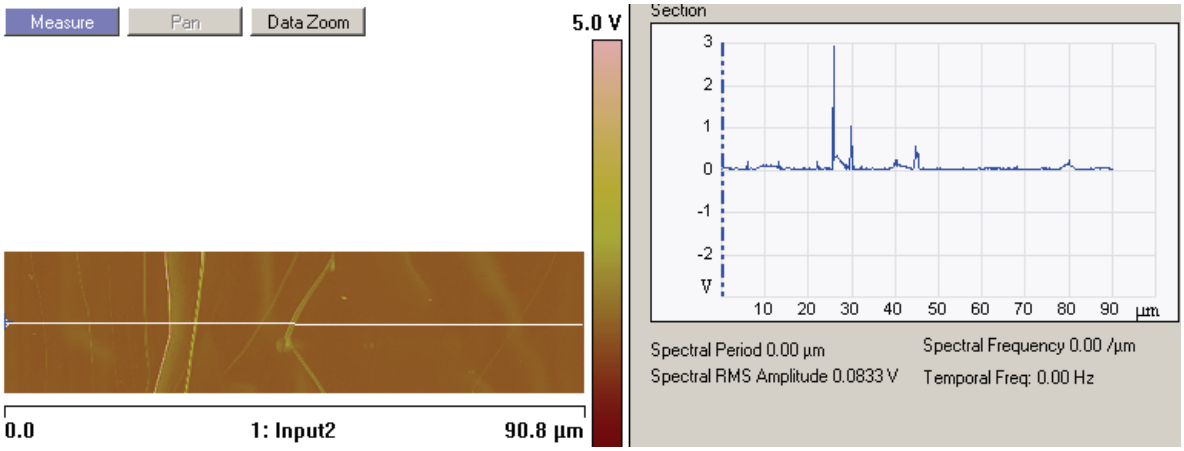
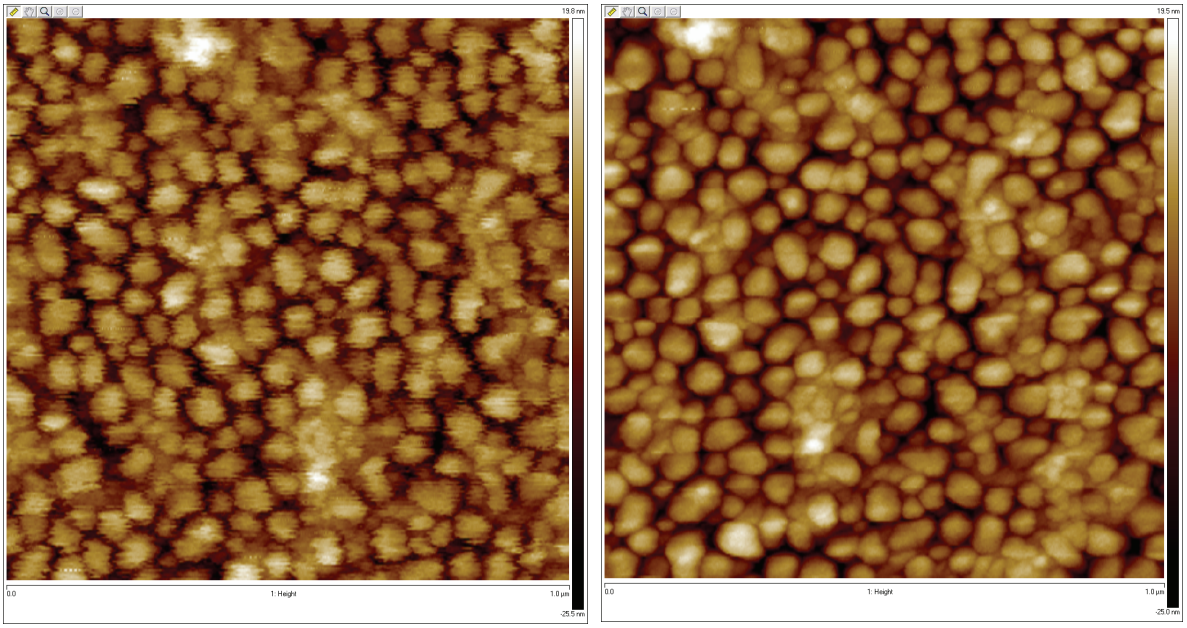


Figure 14.1d shows both analog (left) and adaptive XY (right) scans on poly Silicon showing improved XY resolution.

Figure 14.1d Analog (left) and adaptive XY (right) scans on poly Silicon showing improved XY resolution



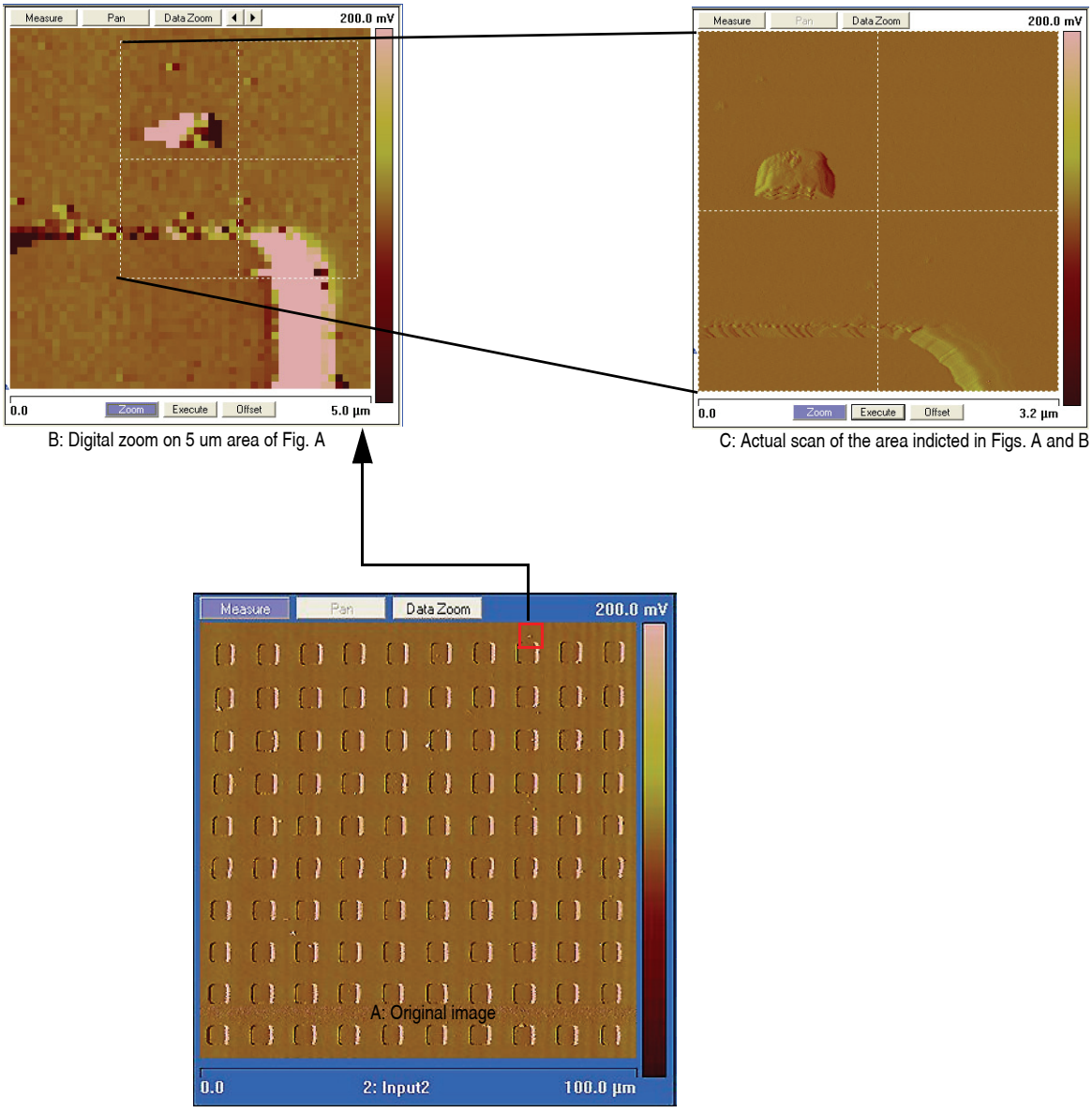
14.1.2 Improved position accuracy

Adaptive XY control uses a low frequency feedback loop to compensate for low frequency position errors introduced by phenomena such as thermal drift and creep.

The sensor signal is filtered to reject the high frequency noise and improve position accuracy.

Figure 14.1e shows a scanned image of calibration grating (10um pitch size, 100um scan size, 10Hz) along with a digital zoom of a 5 µm region of that scan (B). Figure 14.1e (C) shows a new scan (3.2um scan, 10Hz) at the area shown in (B). This shows low noise and precise positioning of the scanner. Creep is not evident.

Figure 14.1e Scanned image of calibration grating

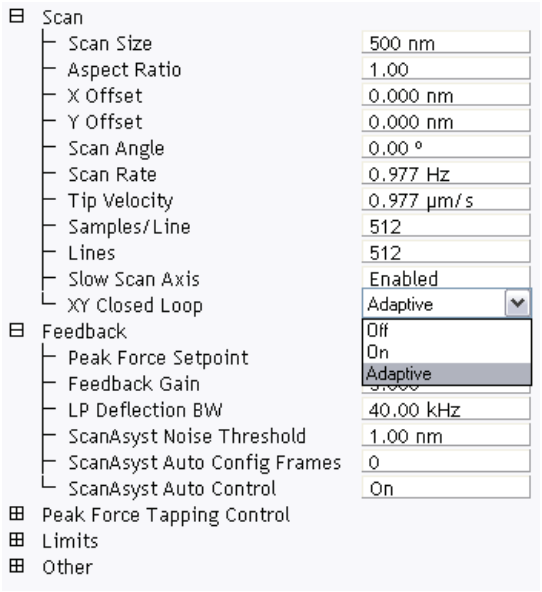


14.2 Operation

1. Click the **EXPANDED MODE** icon, to access the **XY Closed Loop** drop-down box, shown in Figure 14.2a.

2. Select **ADAPTIVE**, shown in Figure 14.2a.

Figure 14.2a Select **ADAPTIVE**



3. Click Calibrate > Scanner > **ADAPTIVE XY FEEDBACK** (see Figure 14.2b) to open the **Adaptive XY Feedback Calibration** window, shown in Figure 14.2c.

Figure 14.2b Select **ADAPTIVE XY FEEDBACK**

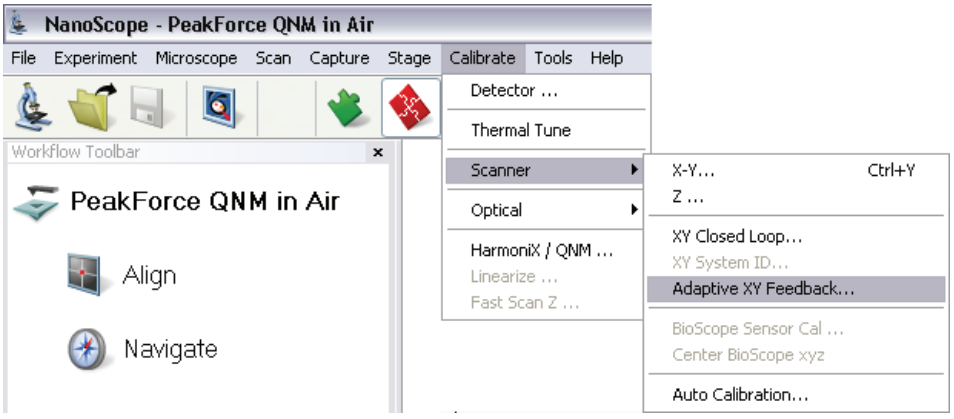
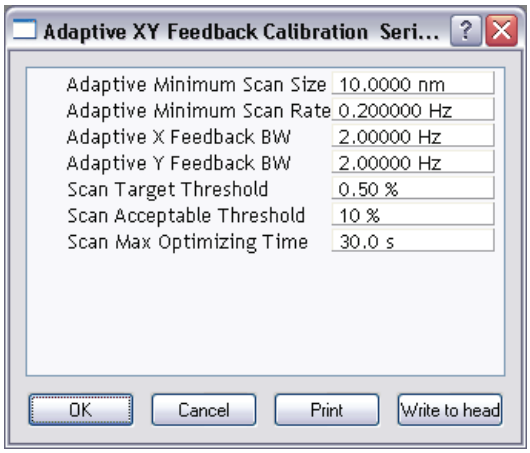


Figure 14.2c The **Adaptive XY Feedback Calibration** window



14.2.1 Adaptive XY Controls

The following controls are available for Adaptive XY scanning:

Adaptive XY Minimum Scan Size	To reduce sensor noise, the feed forward portion of the adaptive XY algorithm is turned off if the **Scan Size** is below this value.
Adaptive XY Minimum Scan Rate	The feed forward portion of the adaptive XY algorithm is turned off if the **Scan Rate** is below this value.
Adaptive X Feedback BW	This corresponds to the closed loop bandwidth. Lower values result in cleaner images.
Adaptive Y Feedback BW	This corresponds to the closed loop bandwidth. Lower values result in cleaner images.
Scan Target Threshold	The adaptive XY algorithm will stop converging when the linearity is below this value.
Scan Acceptable Threshold	The adaptive XY algorithm is deemed to have failed if the linearity is greater than this number. This usually indicates that something is wrong with the system.
Scan Max Optimizing Time	The maximum time allowed for the Adaptive XY algorithm to converge.

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