Agilent 6000 ILM AFM

New Extended Capabilities System for Optical and Atomic Force Microscopy

Data Sheet

Figure 1. The Agilent 6000 ILM AFM.

Features and Benefits

- New incubator perfusion cell sample plate facilitates dynamic studies in liquids and gases
- New top-view video optics provide ability to see opaque samples while scanning
- New single-pass electrical characterization capabilities
- New force-volume spectroscopy capabilities
- New PicoView flexibility with plug-ins and scripting
- Exclusive PicoTREC toolkit for real-time molecular recognition imaging
- Simple point-and-shoot AFM imaging based on optical view

Applications

- Cell membranes
- Surface structure of cells
- Single DNA/RNA strands
- Individual proteins
- Single molecules
- Biopolymers

Overview

The Agilent 6000 ILM AFM seamlessly integrates the capabilities of an atomic force microscope (AFM) with those of an inverted light microscope (ILM) or an inverted confocal microscope, letting life science researchers go beyond the optical diffraction limit to achieve nanoscale resolution without special sample preparation.

The stable, robust mechanical design of the 6000 ILM minimizes non-linearity and helps ensure high-precision AFM measurements. Powerful yet easy-to-use Agilent software provides impressive built-in system functionality, such as point-and-shoot AFM imaging, automatic photodetector/laser alignment, and the rapid creation of precisely registered overlays of fluorescence/DIC and AFM images—valuable capabilities that are standard with every 6000 ILM.

A brand new top-view video optics package is also available for the system, giving researchers the ability to see opaque samples while scanning. Moreover, specially designed 6000 ILM sample plates now offer versatile new incubation and perfusion capabilities as well as the industry's most precise heating control.

Agilent Technologies

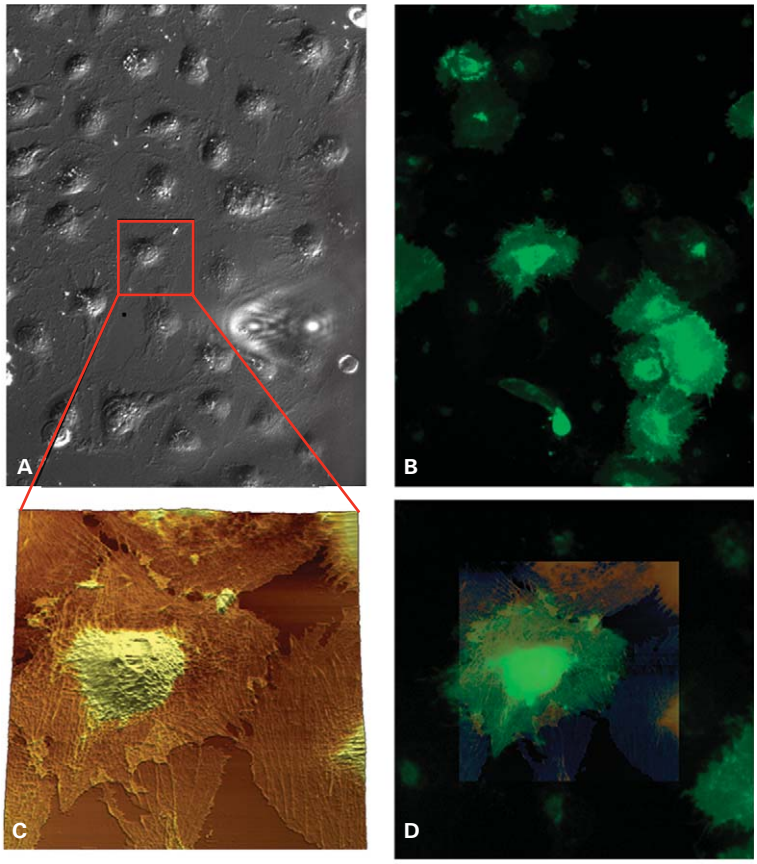

Figure 2. Optical and atomic force microscopy images of living T24 transfected to express GPI-(DAF)-GFP anchored to DAF cells. (A) The morphology of the T24 cell sample using DIC imaging. (B) Lipid raft distribution measured by GFP fluorescence of the same area as in panel A. From the fluorescence it can be seen that the expression level varies from cell to cell. (C) AFM topography (AAC mode, 90 x 90µm) of an area where a highly GPI-(DAF)-GFP protein expressing cell interacts with cells expressing hardly any GPI-(DAF)-GFP. The cytoskeleton was clearly resolved. In addition several connections between individual cells were found. (D) Overlay of topography and fluorescence image.

Additional advantages of the 6000ILM platform include unrivaled in-liquid AFM imaging via Agilent's patented MAC Mode; a broad range of new force-volume spectroscopy measurements and data utilizing Agilent's easy-to-use PicoView software; Agilent's new PicoScript interface for customized experiments; single-pass kelvin force microscopy (KFM) or electric force microscopy (EFM) capabilities; and real-time molecular recognition imaging via Agilent's exclusive PicoTREC toolkit.

Enhanced Optical and AFM Imaging Capabilities

The 6000ILM extends the capabilities of optical microscopes by allowing molecular imaging, live-cell imaging, force studies, and mechanical stimulus studies to be conducted with a single-system solution, all while preserving an efficient, natural workflow.

For ultimate ease of use, the 6000ILM uses a computer-controlled laser with automated photodetector alignment and offers simple point-and-shoot AFM imaging based on an optical image. A high-stability, low-noise motorized stage directs the movement of the sample beneath the AFM tip for measurement.

With the 6000ILM, researchers can perform fluorescence or DIC imaging simultaneously with AFM imaging and quickly create high-precision overlays of the resultant images. The 6000ILM facilitates high-resolution topography and mapping, the ability to collocate points of interest sequentially, and the acquisition of more detailed information about a sample's structure and material property domains.

The 6000ILM supports a wide variety of scanning probe microscopy (SPM) imaging modes, including contact mode, acoustic AC mode, phase imaging, lateral force microscopy (LFM), intermittent contact mode, magnetic AC mode (MAC Mode), kelvin force microscopy (KFM) and electric force microscopy (EFM).

Advanced optics, a standard 0.55 NA condenser, and an AFM scanner Z-range of 30 microns ensure excellent resolution. To ensure the best possible optical imaging performance, the 6000ILM fully supports several popular high-sensitivity scientific cameras from a number of leading manufacturers. The 6000ILM can also now be configured with top-view video optics, enabling researchers to see opaque samples during scanning.

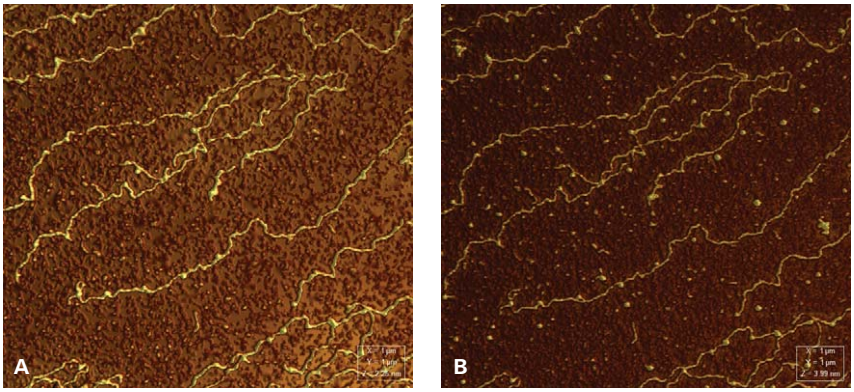

Figure 3. (A) Lambda phage DNA imaged in solution (aqueous buffer), and (B) in air, using the 6000ILM AFM in MAC Mode. Scan size: 1µm.

Superior In-Fluid Imaging with Patented MAC Mode

Agilent Technologies was the first to offer SPM for imaging in liquid and controlled environments, a technological legacy that continues to manifest itself in a wide range of superior solutions for in-fluid and soft-sample imaging. We hold more than 40 AFM-related patents and our leading-edge R&D is committed to the pursuit of advancements that will make atomic force microscopy easier to use.

Agilent's patented MAC Mode is a gentle, nondestructive AFM technique that has been designed for imaging extremely delicate samples. It is particularly useful for studies that require high resolution and force sensitivity, such as biology, polymer science, and surface science.

MAC Mode allows researchers to image submolecular structures that cannot be resolved with any other AFM technique. It offers the best control available for oscillating probe technology, thereby providing a tremendous benefit for imaging in fluids and imaging soft samples.

With MAC Mode, a paramagnetically coated cantilever called a MAC Lever is driven by an oscillating magnetic field. The magnetic field is applied directly to the MAC Lever. Digital lock-in amplifier technology is used to precisely determine the oscillation amplitude and phase response of the cantilever, resulting in excellent force regulation and high-quality phase images.

This amplifier technology eliminates spurious responses that may be generated by a cantilever-holding mechanism, the surrounding fluids, or the sample itself. Thus, there is less system noise and the cantilever can be operated at much smaller amplitudes. Subsequently, sample damage is decreased, probe sharpness is preserved, and resolution is greatly improved.

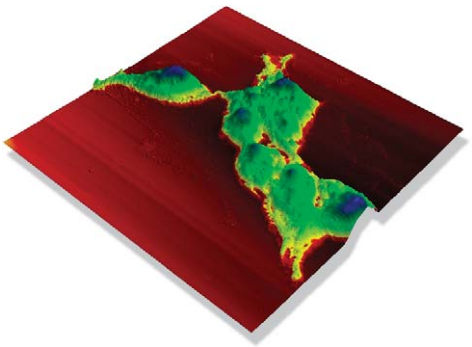

Figure 4. The human embryonic kidney 293 cell (HEK 293) imaged in contact mode and post processed in Pico Image. The image shows that the cells are relatively smooth and display a bulbous low profile (not more than several µm) morphology.

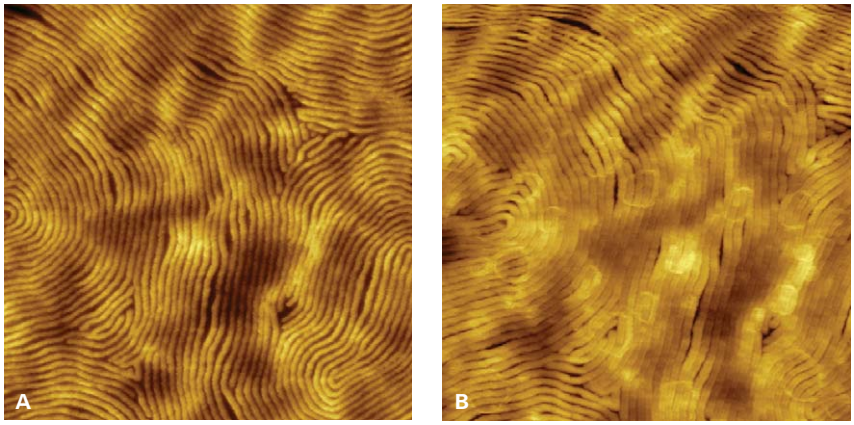

Figure 5. (A) Tri-block copolymer in air, and (B) in water. The high resolution image, (B), shows air bubbles on the surface in liquid.

Figure 6. Temperature sample plate.

Figure 7. Perfusion sample plate.

Figure 8. Petri dish sample plates.

New Incubator Perfusion Cell Sample Plate and More

Agilent offers a selection of sample plates specially designed for the 6000 ILM. These versatile plates help optimize imaging results, are simple and convenient to use, and can be cleaned quickly without undue effort. Their unique design affords superior stability as well as easy sample loading.

The 6000 ILM cover slip sample plate accommodates round cover slips (dia. 22 – 30 mm). The cover glass used by Agilent is thin (~170 µm) to facilitate light microscopy, yet very stable to allow high-precision AFM imaging.

The 6000 ILM microscope slide sample plate holds standard 1"x 3" (25 mm x 75 mm) slides, and the 6000 ILM Petri dish sample plate, designed for high-resolution live-cell imaging, facilitates simple mounting of 35 mm and 50 mm Petri dishes. A controlled-temperature sample plate that holds a 35 mm Petri dish and offers heating from ambient to 80°C with ±0.1°C stability is also available.

For dynamic studies in fluid, Agilent's new 6000 ILM perfusion cell sample plate provides continuous perfusion, offers variable flow-through, and maintains liquid level. The perfusion cell sample plate is compatible with round cover slips (dia. 22 – 30 mm) and can be sterilized by autoclaving.

Taking experimental control even further, Agilent's new 6000 ILM incubator perfusion cell sample plate has a sealed chamber, in and out ports for flow-through of liquids and gases, and the ability to heat from room temperature to 40°C with ±0.1°C stability. Cell viability can be maintained for several hours. The incubator perfusion cell sample plate provides continuous perfusion, offers variable flow-through, and maintains liquid level. It is compatible with round cover slips (dia. 22 mm) and can be sterilized by autoclaving.

Figure 9. Cover slip sample plate.

Figure 10. Glass slide sample plate.

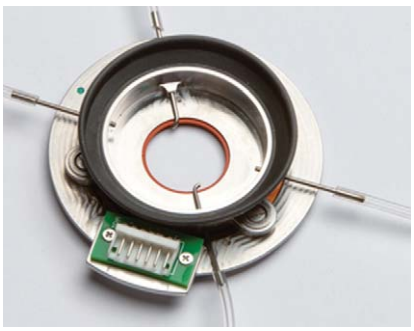

Figure 11. Incubator/perfusion cell sample plate.

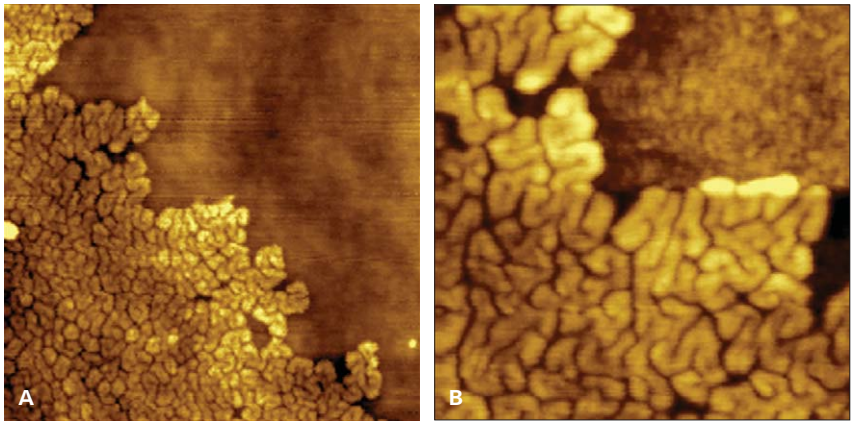

Figure 13. (A) EFM and (B) KFM images of $F_{14}H_{20}$. EFM scan size: 1 µm. KFM scan size: 5 µm.

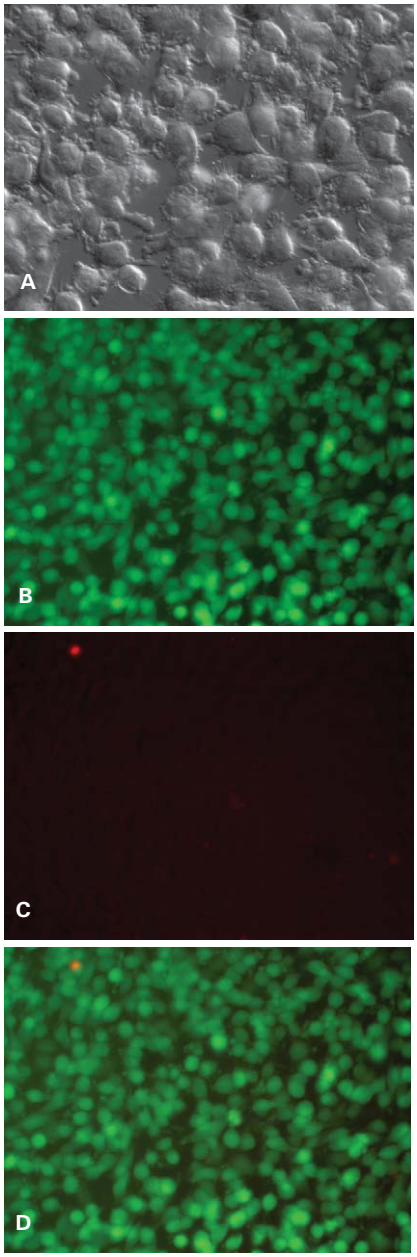

Figure 12. Images of live HeLa cells. (A) 40x DIC image. (B) 20x fluorescence image. (C) HeLa cells that died after 5 hours. (D) Overlay image of green fluorescence live cells and red fluorescence dead cells.

New Top-View Video Optics

The state-of-the-art 6000 ILM system can also be configured with a special top-view video optics package, which includes a color camera, enabling researchers to see opaque samples during scanning. Furthermore, the ability to view AFM tip placement from above helps make positioning and focusing easier than ever.

Figure 14. The 6000 ILM system with top-view video optics package.

New Single-Pass Electrical Characterization Capabilities

The 6000 ILM now lets researchers perform single-pass electrical characterization (KFM or EFM) concurrent with AFM topographic imaging via the system's easy-to-use PicoView software. When configured with an Agilent MAC Mode III controller, the 6000 ILM AFM offers advanced, customizable multifrequency capabilities. Simultaneous, high-accuracy topography and surface potential measurements are enabled by a servo-on-height cantilever approach that is not susceptible to scanner drift. MAC Mode III provides three user-configurable lock-in amplifiers and two expansion slots, affording researchers virtually limitless application possibilities and unprecedented speed.

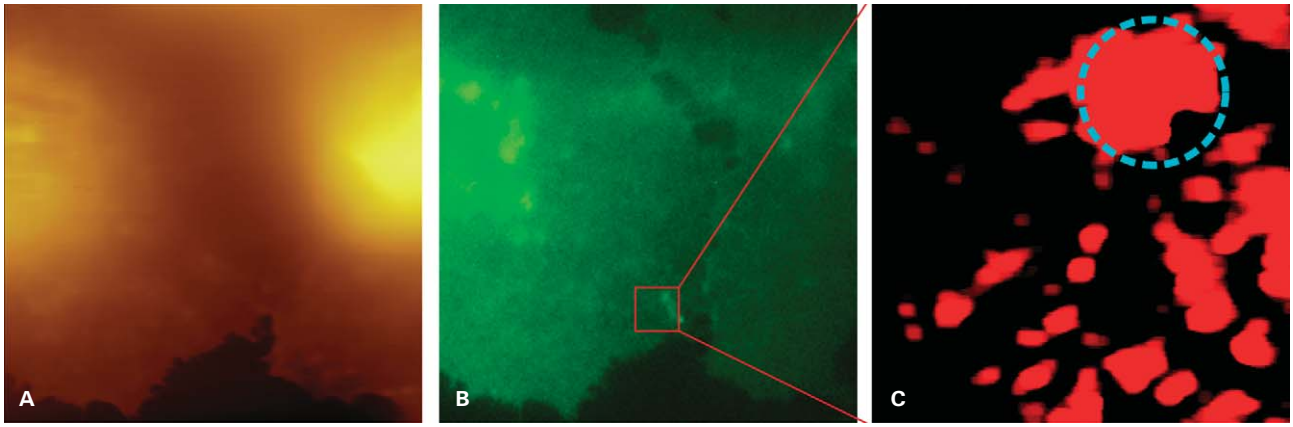

Figure 15. AFM topography of fixed T24 cells expressing GPI-GFP. Scan size: 50 µm. The image was measured with MAC Mode in PBS by using an Agilent type VI MAC Lever oscillating at 16kHz and at about 7nm with an imaging speed of 15 µm/s. The imaging force was so gentle that the filaments of the cytoskeleton underneath the plasma membrane can hardly be seen. Lamellipodia at the cell border can however be clearly detected. (B) Fluorescence image (FITC filter set, 100x magnification) of the same cells showing the expression level of GPI-GFP. Some bright regions close to the nucleus indicate the endoplasmic reticulum (ER) where the GPI anchored proteins are synthesized. Beyond the ER regions, the distribution of GPI-GFP in the plasma membrane looks homogeneous except several bright dots (e.g. those in the red square). (C) By using the cantilever functionalized with the anti-GFP antibody, the PicoTREC image of the region marked with red square in (B) revealed nano-domains with the size of about 200–300 nm. In the region marked with dashed blue circle, the nano-domains have been aggregated which correlates well to the micro-domain shown in the fluorescence image. During the recognition imaging the cantilever was oscillating at 16 kHz and at about 7 nm with an imaging speed of 6.5 µm/s. Scan size: 5 µm.

Molecular Recognition with Agilent-Exclusive PicoTREC

Agilent's unique PicoTREC option gives researchers the ability to detect and map molecular binding events while simultaneously generating topographic images in real time. Using PicoTREC and AFM tips that have been modified with specific biochemistries, researchers can rapidly identify areas on samples that are engaged in molecular binding events and generate a molecular recognition map along with a MAC Mode AFM topography image of the sample.

Alternatively, the information that is generated with PicoTREC and unmodified AFM tips can often be correlated to adhesion or other nonspecific interactions between the AFM tip and the sample.

As an award-winning breakthrough tool for AFM applications, PicoTREC allows researchers to pursue new avenues of discovery in nanotechnology. With PicoTREC, 6000 ILM system users can explore the dynamic properties of biological processes such as antibody-antigen, ligand-receptor, drug-receptor, DNA-protein, and DNA-DNA interactions.

The PicoTREC toolkit's specialized hardware and electronics also enable many advanced AFM spectroscopy applications. Force-distance spectroscopy measurements, molecular manipulations, and other AFM applications can now be performed easier, faster, and with much greater confidence and precision.

PicoTREC facilitates AFM force-distance spectroscopy studies, for instance, by quickly pinpointing areas of molecular interaction. In situations where quantitative information is not required, PicoTREC can even eliminate the need for force-distance spectroscopy experiments altogether.

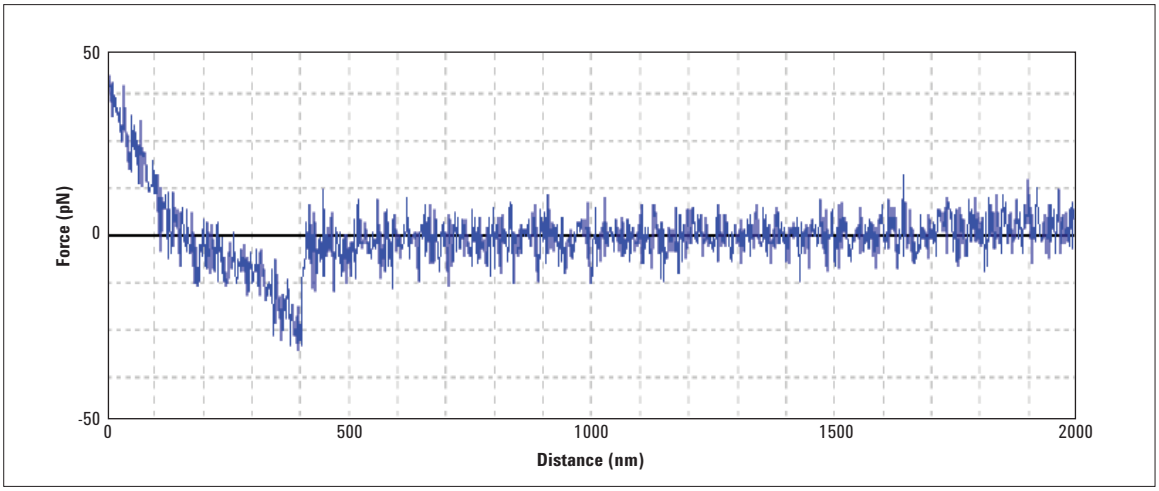

Figure 16. Force distance curve of a binding event on a T124 cell.

New Powerful PicoView and Pico Image Software

The ability of the 6000ILM to perform simple point-and-shoot AFM imaging based on an optical image is powered by Agilent PicoView. This extremely stable, 32-bit, Microsoft® Windows®-based software package also enables the easy creation of high-precision overlays of light microscopy and AFM images.

PicoView now gives 6000ILM system users an extensive range of AFM force-volume spectroscopy capabilities. Results are fast and reliable. Researchers can use their own algorithms and plug-ins, select their own data points on-the-fly, acquire force-curve measurements on any data point, and change experimental parameters in real time.

PicoView math plug-ins support the Python (PSF) programming language, providing a quick and simple way to customize the 6000ILM for special needs. Just write some code, drop it in, and run. For instance, the Math plug-ins can be used to process data associated with both flex-grid and volume spectroscopy applications. PicoView offers complete control of all scanning parameters and affords the flexibility required for more complex experiments.

Agilent PicoScript is an optional scripting package that is integrated into PicoView. It greatly enhances the capabilities of Agilent PicoView enabling the user to write scripts to automate tasks and create custom experiments. It provides the ability to build applications library for multiple users. PicoScript comes with an application programming interface (API). Scripts can be developed utilizing Python, C/C++, and MathWorks MATLAB. PicoScript also provides virtual instruments (VIs) for National Instruments LabVIEW.

Agilent's post-processing software, Pico Image Basic with co-localization, is also included with every 6000ILM AFM. This easy-to-use desktop publishing environment enables fast, accurate report generation and surface imaging and analysis workflow for full metrological traceability. Advanced and expert packages are available as well.

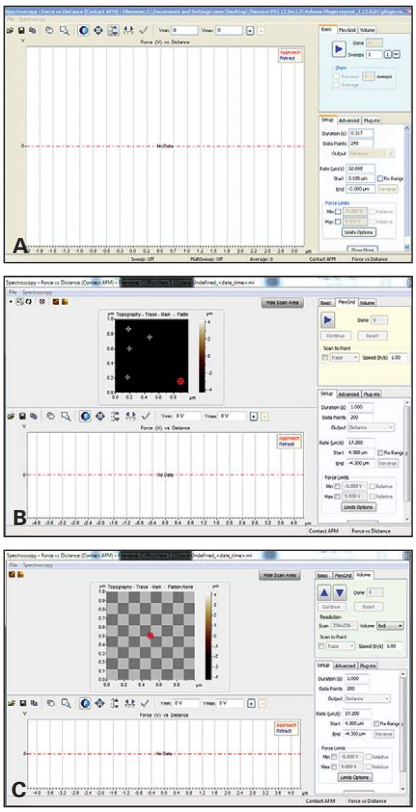

Figure 17. PicoView supports Python math plug-ins for customized applications. Spectroscopy example (A) Basic, (B) Flex Grid, (C) Volume.

Specifications

AFM Scanner		
X-Y (Closed Loop) Scan Range	100 µm x 100 µm	
Z (Closed Loop) Scan Range	30 µm	
Scanner Noise	0.3 nm (X–Y) 0.1 nm (Z)	
Scanner Laser	Infared (980 nm)	
Supported Microscopes		
Zeiss	Axio Observer (A1, D1, Z1)	
Olympus	IX71, IX81	
Stages	**Zeiss**	**Olympus**
Stage Travel	10 mm x 8 mm	4 mm x 6 mm
Motorized Stage Accuracy	±2.5 µm	±2.5 µm
System Noise		
	< 0.25 nm RMS (Air)	
	< 0.25 nm RMS (Fluid)	
Optical Imaging Modes		
Zeiss, Olympus	Phase contrast, DIC, TIRF, Confocal, epifluorescense, FRET	
Condenser Compatibility		
Zeiss	0.55 NA	
Olympus	0.5 NA	
Top-View Video Optics		
Working Distance	96 mm	
Magnification	97x – 628x	
Field of View	5.7 mm – 0.9 mm	
Resolution	≤ 5 µm	
Available Sample Plates		
Cover Slip		
Size	22 – 30 mm dia	
Thickness	>170 µm	
Microscope Slide		
Size	1" x 3" (25 mm x 75 mm)	
Petri Dish		
Size	35 mm x 10 mm or 50 mm x 9 mm Plastic or plastic with glass bottom	
Controlled Temperature Stage		
Size	35 mm x 10 mm Petri dish	
Temperature Range	Ambient to 80°C (± 0.1°C)	
Perfusion Cell		
Cell Volume	300µL (when nose is in contact with cover slip)	
Flow Rate	50–150 µL/min	
Autoclavable (cell only)		
Incubator Perfusion Cell		
Temperature Range	From room temperature to 40°C (±0.1°C stability)	
Cell Volume	300µL (when nose is in contact with cover slip)	
Flow Rate	50–150µL/min	
Autoclavable (cell only)		

AFM Instrumentation from Agilent Technologies

Agilent Technologies offers high-precision, modular AFM solutions for research, industry, and education. Exceptional worldwide support is provided by experienced application scientists and technical service personnel. Agilent's leading-edge R&D laboratories are dedicated to the timely introduction and optimization of innovative and easy-to-use AFM technologies.

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