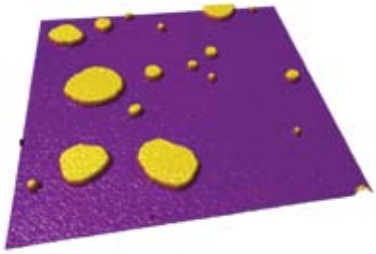
ASYLUM RESEARCH

The Only Full-Capability AFM on an Inverted Optical Microscope

MFP-3D-BIO™

Atomic Force Microscope

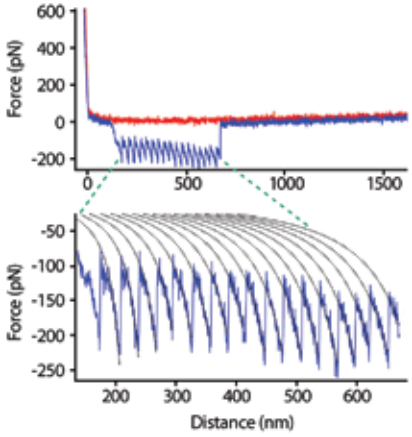
Uncompromised AFM on an Inverted Optical Platform



The MFP-3D-BIO provides the highest sensitivity and most accurate images and measurements possible on an inverted optical platform. The NPS™ closed loop nanopositioning sensors on all three axes ensure distortion-free images on samples as small as proteins and as large as cells – in both air and liquid. The MFP-3D measures the cantilever deflection to better than 20pm (8pm typical) without artifacts, making the MFP-3D ideal for force measurements such as unfolding single molecules or probing cell mechanics.

AFM Is More Than Just Pretty Images

From single molecule contour lengths to mammalian cell volumes, your published results are only as good as the data you acquire. The MFP-3D provides a truly quantitative three-dimensional map of your sample – a necessity for accurate measurements you can trust. Asylum's superior sensor and flexure stage technology coupled with our intuitive control software also gives you the ability to zoom and offset with a single mouse click - even from a previously captured image. Independent lateral (X-Y) and vertical (Z) positioning stages eliminate the crosstalk errors common to systems using tip-scanners. And the large Z range (up to 40µm) enables imaging of tall samples and cells, force curves on highly adhesive soft materials like gels, and other challenging applications. All standard AFM scanning modes are supported, including contact mode with lateral force, AC mode with phase, and advanced modes such as Dual AC™, nanomanipulation, nanoindentation and more.



The Best Force Measurements – from the Commercial Pioneer of Force Spectroscopy

Asylum Research pioneered commercialization of picoNewton-scale force spectroscopy with its first product, the Molecular Force Probe (MFP). Asylum continues that leadership to this day with the MFP-3D AFM (see sidebar at far right).

From left:
Dennis Discher,
Florian Rehfeldt,
Andre Brown

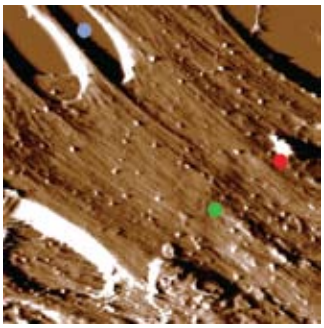
"We chose Asylum's MFP-3D-BIO AFM because it has the most powerful AFM capabilities of the inverted optical integrated systems. It excels in all aspects, from optical integration to high-resolution imaging and dimensional measurements to force spectroscopy and elasticity measurements of soft tissue matrices. Asylum's quality and reliability allow us to focus on the science."

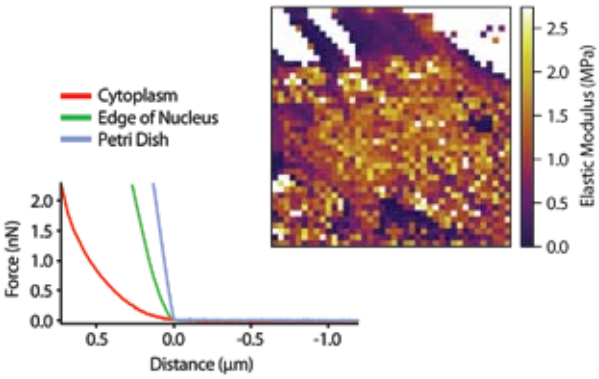
– Prof. Dennis Discher, University of Pennsylvania

No Hole in Our Head

Some competitors literally have a hole through their AFM head. While permitting the use of some commercially-available optical condensers, the hole compromises the integrity of the AFM optical lever design and the stability of the AFM stage. This adds noise to their scans, degrades imaging resolution, and hinders quantitative force and friction measurements.

In contrast, the MFP-3D measures picoNewton forces and resolves the smallest features of your samples. The flexure-mounted optical lever mechanism moves as a single robust unit, virtually eliminating non-linearities, off-axis motion and interference fringes. Our custom-designed condenser column provides unobstructed top-down sample viewing and enables standard optical microscopy modes, including phase contrast.





Force map on fixed MRC-5 fibroblasts, 30µm. Deflection image (left) shows location of selected force curves (center graph). Automated force curve analysis generates an elasticity map (right image) which displays the measured modulus by color at each of the 1600 force curve points taken on the cells and substrate. The lamellipodia at the top center of the force map appear much softer than expected due to the presence of a second cell underneath.

Leadership in Force Spectroscopy and Force Mechanics

- Simple setup, calibration, acquisition, display and analysis (any channel vs. any channel) with easy customization for advanced users.
- Superior 8pm deflection noise (typical), 20pm guaranteed.
- Accurate force measurements with no additional hardware required.
- Users can choose between open loop sensored force curves for the ultimate in low noise performance, or closed loop force curves for the most accurate velocity control.
- MacroBuilder™ graphical macro language allows custom force measurements to be easily programmed, even by beginners.
- Large vertical Z range (15µm standard, 40µm available) accommodates demanding applications such as adhesion measurements on cells and very soft gels.
- Force Mapping enables mechanical measurements at a grid of points with automated extraction of material properties such as elastic modulus.
- Force Clamping for single-molecule and bond-rupture force spectroscopy measures unfolding or rupture kinetics under constant force, allowing direct comparison to theoretical models.
- Perpendicular drive, flexured nanoindenter (optional) provides accurate force and displacement control with superior resolution, sensitivity and precision.

Top image, opposite page:
2.5µm scan of supported lipid bilayers (5nm tall) adsorbed onto mica and imaged in phosphate buffered saline.

Force Curve, opposite page:
The unfolding of fibril amyloid beta-sheets in algal adhesive reveals a distinct sawtooth pattern. The force curve was fitted to the worm-like chain (WLC) model (dotted lines) and a persistence length of 0.22nm was calculated. Data from Mostaert et al. (2006), J. Biol. Phys. 32(5):393.

Light Up Your AFM

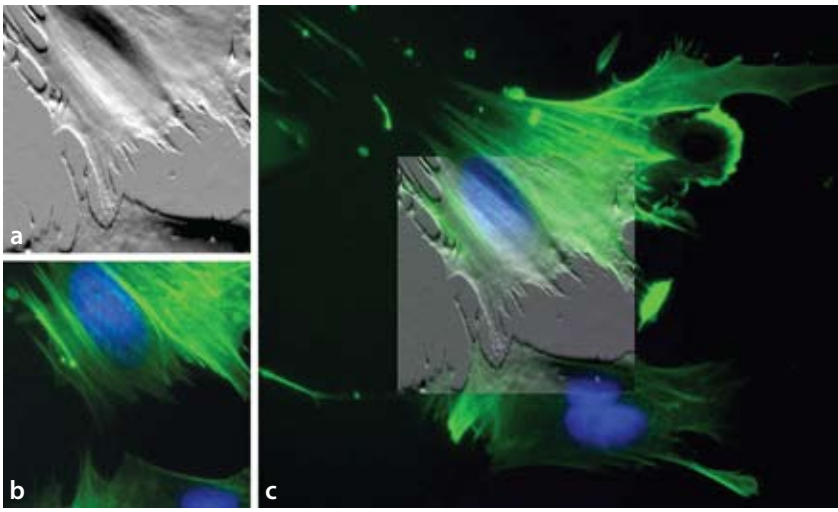
Seamless Optical and AFM Integration

The MFP-3D-BIO sets the industry standard for combining optical and atomic force microscopies in a single integrated tool specifically designed for biologists. Our team of biologists and optical engineers have optimized the MFP-3D for use with the leading inverted optical microscopes to ensure that you get the maximum benefit and productivity from the combination of these powerful techniques. High numerical aperture, water immersion, and TIRF objectives are all accommodated. No other AFM can match the MFP-3D's performance for optical integration, high resolution imaging, quantitative force measurements, and environmental controls. All of the following optical microscopy techniques are supported:

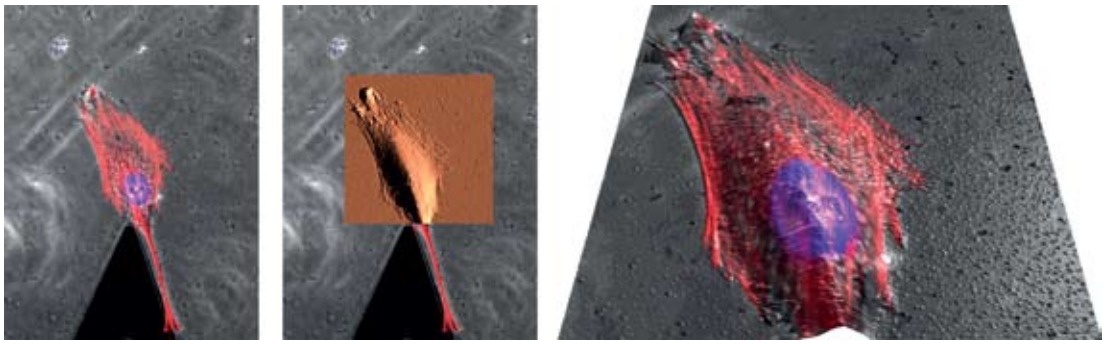
- Brightfield
- Phase Contrast
- Epifluorescence
- Confocal Laser Scanning
- TIRF
- FRET
- FCS
- FRAP
- Ion Indicators (e.g. Ca^{2+} response)

And the MFP-3D's exclusive X-Y scanned-sample design is essential and enabling for:

- Apertureless Near-field Microscopy (ANSOM)
- Raman and Tip-enhanced Raman Spectroscopy
- Tip-enhanced Fluorescence
- Fluorescence Lifetime Imaging (FLIM)



Multiply-labeled fibroblasts imaged in buffer using contact mode AFM (a), and fluorescence microscopy (b). The MFP-3D's standard overlay feature produced the composite image (c). Our proprietary IR filter blocks the AFM laser, enabling clean, full-spectrum fluorescence imaging – including far-red fluorophores.



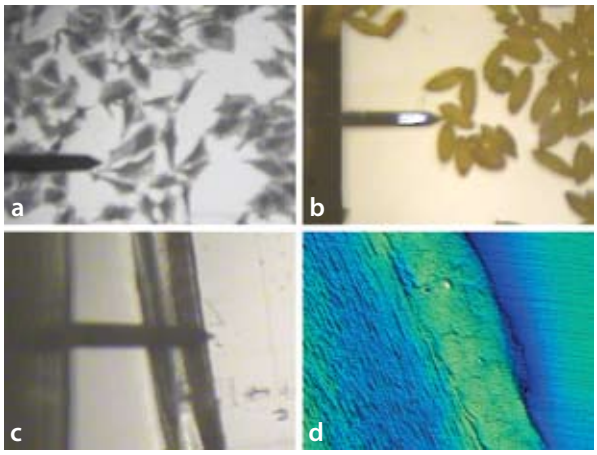
Locate a cell with Zernike phase contrast (gray) or fluorescence, examine features such as cytoskeletal structures (red) or the nucleus (purple), then zoom in for high-resolution topography or force measurements with AFM (copper). Overlay optical data on AFM topography for 3D analysis and presentation (right).

"The MFP-3D-BIO is a research instrument through and through and designed for the scientist. The optical integration is exceptional, and the flexibility of the platform offers almost endless possibilities."

– Prof. Jan Hoh, Johns Hopkins University

Versatility from Above: View, Illuminate, Navigate

The MFP-3D extends the capability of the inverted optical microscope by allowing you to view transparent and opaque samples from above while scanning with the AFM. The integrated top-view optics enable *in situ* laser alignment and tip positioning – without removing the AFM head and without ancillary equipment. Our unique design also allows the top-view objective lens to double as a high-quality condenser for phase contrast illumination. Top- or bottom-view optical images can be used to navigate the tip to any feature on the sample, scan that area at the nanoscale with the AFM, and then select specific locations for force curves – easily and seamlessly. Optical images can be overlayed on AFM data, and captured for documentation and publication.



Top view optical images: (a) HeLa cells on silicon, (b) pollen grains, and (c) mussel hold-fast fiber with corresponding AFM image (d). (a) J. Brison et al., *Surface and Interface Analysis*, submitted 2009. (c,d) Holten-Andersen *et al. (2007), Nature Materials* 6:669.

Control Your Sample Environment

Petri Dish Holder
Specially designed sample plate for imaging cultured cells and other biological samples in Petri dishes.

Petri Dish Heater
Petri Dish Holder with the additional capability for heating temperature-sensitive samples from ambient to 45˚C.*

Closed Fluid Cell
Sealed chamber with 10 inlet and/or outlet ports for exchange of liquid or gas media.†

BioHeater™
Closed Fluid Cell for imaging in liquid between ambient and 80˚C.*†

Fluid Cell Lite
Economical portless fluid cell. Ideal for individual users at multi-user facilities.†

CoolerHeater
Heats and cools samples with a Peltier element. Continuous temperature control from -35˚C to +120˚C.*

Humidity Sensing Cell
Measures humidity within a sealed sample cell.

iDrive™
Simplifies imaging in liquid with cantilever auto-tuning and elimination of mechanical resonance peaks.

Additional Options

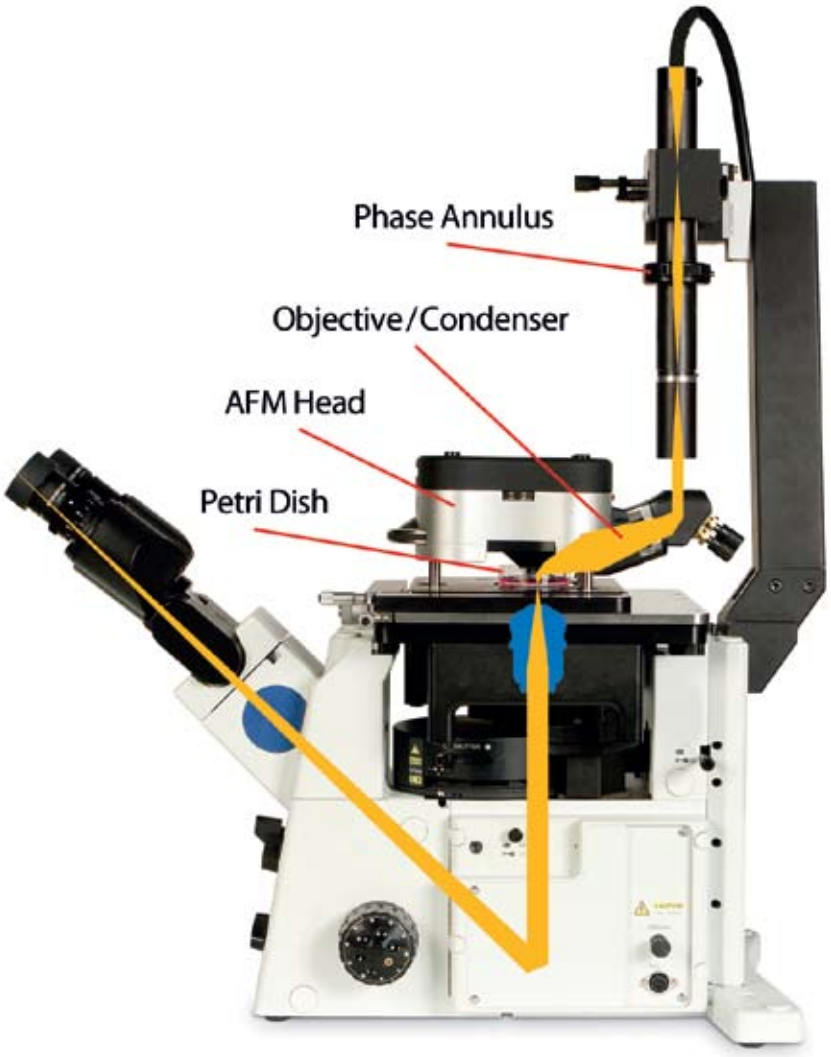
High-voltage PFM
Piezoresponse Force Microscopy for very high sensitivity, high bias and crosstalk-free measurements for piezoelectrics and biological systems.

ORCA™ Conductive AFM
Provides current-voltage measurement capability.

MFP NanoIndenter
True instrumented nanoindenting for quantitative measurements with unprecedented accuracy.

* Temperature stability ±0.1˚C.

† Compatible with glass cover slips used for high NA microscopy.

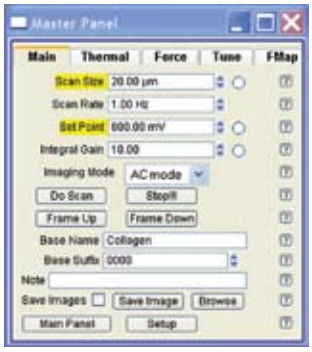


The optical path through the head of the MFP-3D-BIO utilizes a high-quality objective which can be used to view the top of opaque samples and to align the laser onto the tip. The objective can also be used as a condenser for brightfield or phase contrast illumination on transparent samples by using a series of lenses and mirrors to illuminate the sample. Using these optical elements, high quality illumination is achieved for observation with the inverted optical microscope objective while not undermining the fidelity of the optical lever or Z-flexure design of the AFM.

Choose the MFP-3D-BIO

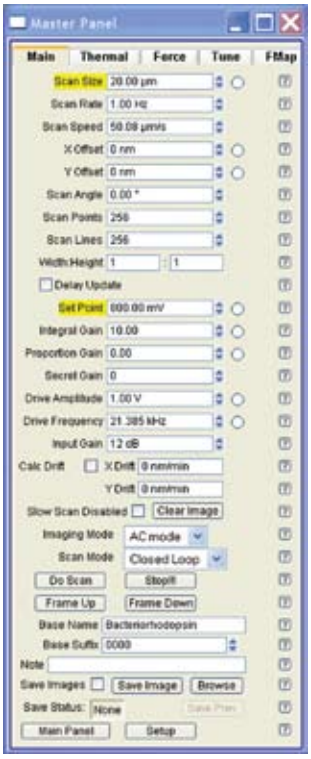
For Full Capability *and* Ease of Use

Asylum has made our full capability system easy to learn and use for novices while providing advanced functionality for the most experienced users. Don't compromise your research by choosing an "easy to use" system with limited capabilities. Choose the MFP-3D-BIO for both full capability *and* ease of use.



Walk

Start with the basics. Press one button in ModeMaster™ (top) and the AFM is configured for you, showing a simplified view with only the preset parameters you need for your experiment.



Run

Take control of your AFM. Control the instrument precisely for more advanced experiments, specifying exactly what you want to do in our full-featured interface. Don't be held back by inflexible software.

Fly

Automate your work. For your most demanding experiments, access the full programmability of the MFP-3D AFM easily from our visual MacroBuilder panel. Spread your wings – no computer coding required!

Legendary Product Support

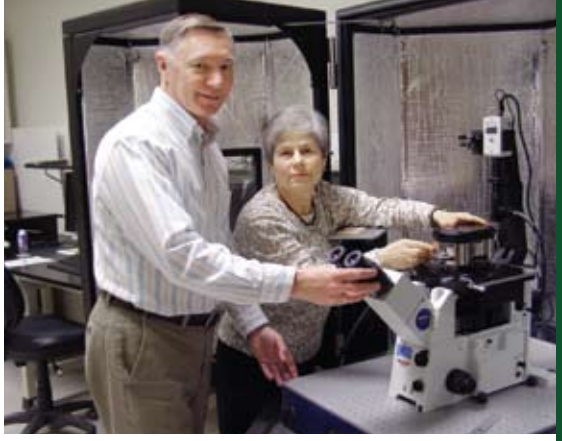
Biophysicist Deron Walters and biologists Irene Revenko, Sophia Hohlbauch and Nick Geisse have over 50 years of combined AFM experience. Our staff helps you succeed with AFM operation as well as sample preparation techniques and procedures.

"Working with the Asylum Research support team is like having an additional bio-AFM specialist on our staff."

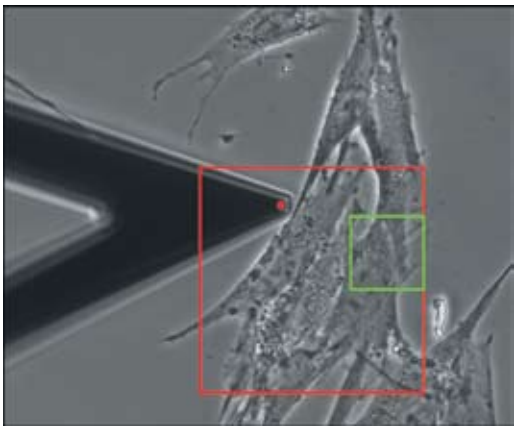
Prof. Miklos Kellermeyer, Semmelweis University

"Our MFP-3D is a highly reliable and key tool in our NanoImaging Core Facility. In addition to 'traditional' imaging in air, it has outstanding capabilities for imaging in liquid and performing reliable and accurate force measurements. The simplicity of the software allows us to easily train students, yet the open setup of the instrument makes it flexible and powerful for very high-end projects, including nanolithography and nanomanipulation."

– Prof. Yuri Lyubchenko, University of Nebraska Medical Center



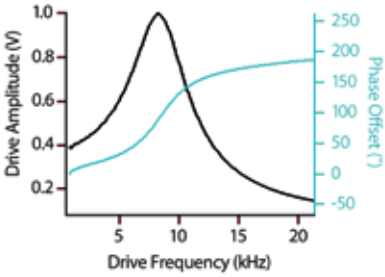
Yuri Lyubchenko with Luda Shlyakhtenko



Real-time Optical Navigation

Guide the tip to any location for AFM imaging, force mapping or individual force curves using any real-time optical input – brightfield, phase contrast, fluorescence, etc. This capability is of particular importance when working with functionalized tips or soft samples that preclude AFM scanning prior to force spectroscopy experiments.

Left: 40X phase contrast image of MRC-5 fibroblasts on a Petri dish. With a single click you can direct the tip (red dot) to any point, or select a new scan area (green box) within the scan range (red box).



Cantilever Auto-tune in Liquid

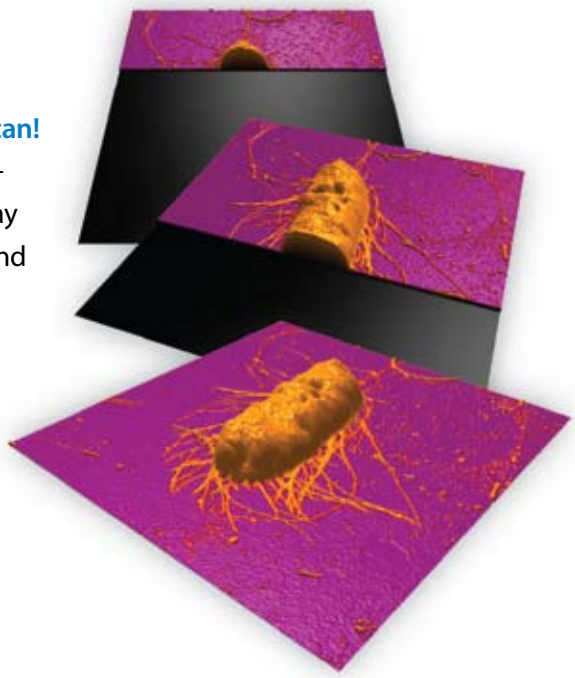
Tuning the cantilever for AC mode takes only a single mouse click with Asylum's exclusive iDrive electromagnetic actuation.

Real3D™ Imaging – View your data in three dimensions in real time as you scan!

The MFP-3D's exclusive real-time 3D display allows you to view a realistic representation of your sample while the image is still being acquired. You can overlay the AFM phase or other data channels, rotate the image to any point of view, and adjust the scaling and lighting – all during the scan. This powerful capability is only available on Asylum Research AFMs.

And once you've acquired your image our ARgyle™ image processing software helps you:

- Render your data for journal-quality presentation and publication.
- Control color height maps, lighting angle and intensity, dynamic data range scaling, interactive real-time rotation, zooming and panning.
- Create Quicktime® movies and 3D stereo anaglyphs.
- Overlay different AFM and optical channels. For example fluorescence or phase contrast can be mapped onto AFM topography to correlate the data/information (see center pages).



Sequence of real-time 3D renderings of *E. coli* on glass showing structure and fimbriae, 5µm scan.

Don't Just Take Our Word For It...

Christine Ortiz, Massachusetts Institute of Technology
"The MFP has worked great for us. I've used (most other AFM brands) and have found the MFP to be superior for force measurements to all of them. The machine is extremely easy to learn how to use. I have undergrads working on it with no problem. The instrument support is fantastic as well."

Tim Senden, The Australian National University
"...that seems to be AR's trademark. Great scientists behind great gear. You might think I'm being too effusive, but I haven't yet started..."

Thomas Gutsmann, Leibniz Center for Medicine and Biosciences
"We've used the MFP-3D since 2002 and it has been an extremely reliable and flexible instrument. On the one hand it is easy enough to use for training of students and on the other hand it is the most powerful AFM for high-end scientific research projects. The open access software offers a wide range of capabilities to enable your own ideas and visions."

Scott MacLaren, University of Illinois at Urbana-Champaign
"Many companies say they provide great service and support, but consistently fail to do so. Asylum Research, on the other hand, proves every day how much they care about their customers by providing the best service and support in the industry. Truly exceptional."

Yael Dror, Oxford University
(On Asylum's AFM in Biology Class)
"You all did a remarkable job in all areas! I am especially grateful for your sincere willingness to help each of us and the time and energy you spent with me to help, explain, guide and think together about my results. But above all you shared with us your love of the AFM, which couldn't possibly be ignored, and gave us an insight into a very special company."

Xiaohui (Frank) Zhang, Chinese Academy of Sciences, Shanghai
"It was such a wonderful experience at AR! Many thanks again for all the help and courtesy extended to me. I will for sure send my students to future Asylum Bio classes."

Go to www.MyAsylumAFM.com for more comments from our users.

Specifications

Note: Specifications in red are tested for 100% of MFP-3D-BIO AFMs shipped.

High Precision 3D Motion

Closed Loop Sensors on all three axes

X&Y axes: 90µm range in closed loop; resolution 0.5nm limited only by sensor noise. Sensor linearity better than 0.5%.

Z axis: >15µm range; resolution 0.25nm limited only by sensor noise. Sensor linearity better than 0.05%. Optional Extended Z Head with range >40µm and resolution 0.3nm.

Ultra-Quiet Z Drive: Voltage noise Adev <70µV in a bandwidth of 1Hz to 10kHz.

Height: Noise in the tip-sample distance is <0.06nm.

Measurement/analysis tools for noise and linearity are available to the researcher.

Molecular Scale Force Measurements

Cantilever Deflection Measurement: Noise <0.02nm; typical 0.008nm. Linearity better than 0.5%. Bandwidth 2MHz standard; up to 7MHz with PD-FAST option.

Low Coherence Source: Superluminescent diode (SLD) for ripple-free baseline.

Force Constant Calibration by the thermal noise and Sader methods.

Live Baseline Correction (linear and quadratic) for precise low-force triggers.

Flexible Interface allows recording or triggering from any channel during a force curve, including amplitude/phase from AC or Dual AC mode; user-supplied input voltages; and photon count rate (with optional Digital Access Module).

Slow Force Curves (up to 100 sec) and long dwell times (up to 500 sec) with variable data acquisition rate during motion and dwell.

Force Mapping: Up to 384x384 array of force curves; includes automated analysis to create maps of adhesion and elastic modulus.

Optical Microscope Integration

Includes stage unit for mounting on inverted optical microscope: Olympus IX81/71/51, IX70/50; Nikon Ti-E/U/S, TE2000-U/S; or Zeiss Axio Observer Z1/D1/A1. Inquire regarding other models.

All standard objective lenses including high NA oil- and water-immersion and TIRF objectives are supported.

Infrared Source: 860nm SLD for compatibility with far-red fluorophores.

Blocking Filters: Matched interference filters in AFM head and optical microscope completely block the SLD beam from fluorescence detectors and cameras.

Software Overlay of optical images on AFM data in both 2D (variable alpha) and 3D (topographic rendering).

Analog CCD Camera (640x480) included for positioning with transmitted light techniques.

Optically Guided region of interest (ROI) selection for imaging and force curves/maps.

Opaque Sample Capability: Incorporates Mitutoyo 10x/0.28 NA objective lens, optics module, and camera for SLD spot alignment and positioning tip on opaque samples.

Zernike Phase Contrast: Includes condenser and annuli for Ph2, Ph1, and Ph0/C/L operation concurrent with AFM imaging.

Compatible Techniques include brightfield, epi-fluorescence, darkfield, phase, confocal, Raman, TIRF, FRET, FRAP, FLIM, and more.

Superior Usability

Included Modes: Contact mode; AC mode with Q-control; AFM Phase; exclusive Dual AC mode; LFM; EFM; MFM; SKPM; piezoresponse force microscopy (PFM) including vector and switching spectroscopy PFM; Dual AC Resonance Tracking (DART); MicroAngelo™ nanolithography and nanomanipulation.

Optional Modes: Conductive AFM (CAFM) with ORCA module; enhanced LFM head; high voltage PFM; thermal analysis; nanoindentation.

Sample Format: 75x25mm typical. Maximum 80mm diam. x 5mm height, mass 2kg. Up to 22mm height with option.

Sample Adapters Included for coverslips (25mm diam. or 22x22mm) and Petri dishes (plastic and coverglass-bottom). See Options Data Sheet for heating, flow-through, and humidity sensing sample holders.

Computer: High-performance dual-monitor Windows™ computer (inquire for latest specifications and custom configurations).

Software: Open user interface based on IGOR Pro incorporates professional-quality analysis and graphing capabilities. AFM analysis includes section, histogram, roughness, particle analysis, and masking.

3D Rendering of real-time and offline data with color overlays using proprietary ARgyle module. Includes anaglyphs and STL or VRML stereolithography export.

ModeMaster allows one-push configuration; many preset modes included.

MacroBuilder graphical interface enables automation of data acquisition and analysis.

Extra Software Licenses: Free for MacOS or Windows. Each installation requires a valid IGOR Pro license.

Software updates: Free for lifetime of the microscope. May occasionally require IGOR Pro upgrade.

Toll-free phone support (US only), e-mail support, and user forum available.

Remote desktop streaming for online real-time support and training.

Vibration Isolation

Strongly recommended for all systems. See Options Data Sheet.

MFP-3D-BIO is a Class 1M Laser Product

Except as noted, noise measurements are specified as the average deviation (Adev) in a bandwidth of 0.1Hz to 1kHz. Sensor linearity is specified as the maximum absolute deviation divided by the full range of motion.

Cover Image: DNA origami triangles, ~120nm per edge, 1µm scan.
Sample courtesy of Paul W.K. Rothemund, California Institute of Technology. 5-2011

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