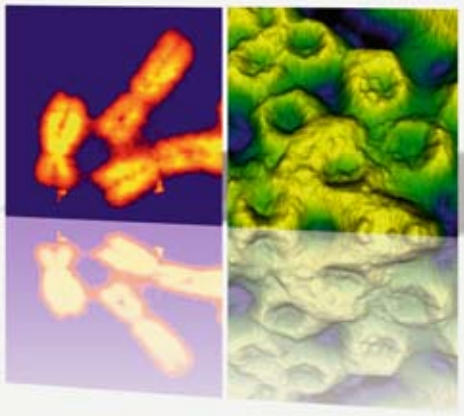
NanoWizard®II from the Pioneers of BioAFM. New Standards for Soft Matter and Life Science AFM. Performance Redefined.



JPK Instruments

Nanotechnology for Life Science

There is One Magic Rule for the Demands of Bio Applica

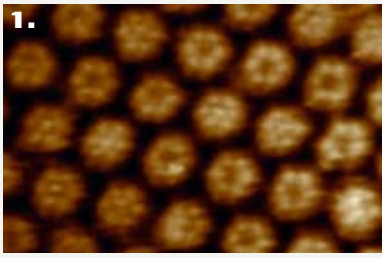
The JPK NanoWizard® AFM paved the way for a multitude of applications in Soft Matter and Life Science research. With German precision engineering and a pioneering spirit, JPK established the BioAFM as a new class of instrumentation with a tremendous global success. There are imitators, but only JPK seriously addresses the needs of life science researchers by concentrating on solutions for their applications. With this strong focus, JPK is the technology leader in this field, and the NanoWizard®II sets a new standard for the next generation BioAFM.

BioAFM – unlimited applications

AFM has crossed over from its roots as a technology for nanoscopic measurement tasks in material sciences to establish major AFM application fields in the life sciences:

1. High Resolution Imaging – Scanning Electron Microscopy resolution on untreated samples
2. Force Measurements down to the single molecule level – single bond forces are easily accessible
3. Stiffness and Elasticity Measurements – the mechanical response of a surface over a large range of forces provides stiffness or adhesion information
4. Nanomanipulation/Lithography – the AFM tip used as a nanoscopic manipulator

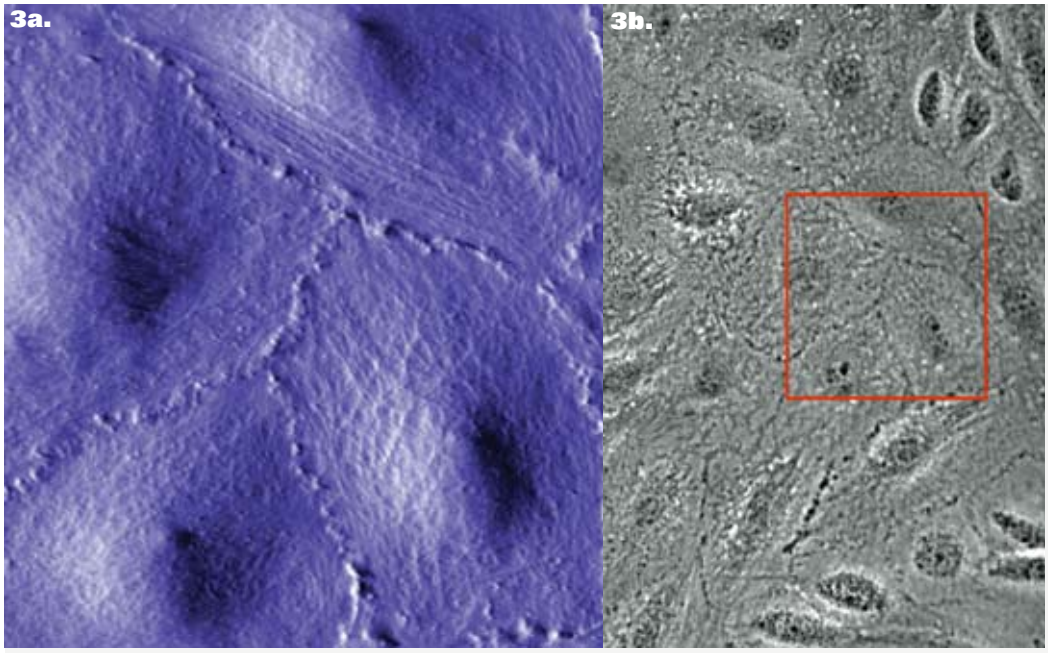
In life science applications all these techniques require perfectly controlled conditions, in solvents or buffered solutions, while handling the softest and most fragile samples that can be imagined.



1. HPI on mica, imaged in closed-loop in buffer, scan area 100 x 65 nm². Image courtesy of P. Frederix, group of A. Engel, BIOTEC Center, University of Basel, Switzerland.

On the cover:
(left): Human metaphase chromosomes. Sample courtesy of L. Costa and S. Thalhammer, LMU München, Germany.
(Right): Nuclear pore complexes prepared on a coverslip, scan size 550 nm. Sample courtesy of A. Kramer, group of H. Oberleithner, University of Münster, Germany.

BioAFM Innovation. Meeting tions. Without Compromise.



3. Monolayer of living ref52 fibroblasts imaged in the BioCell™, 95 µm AFM image (a.) and optical phase contrast overview (b.).

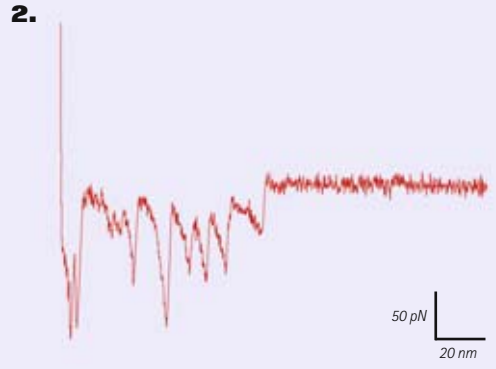
Overcoming the biggest challenge – an AFM built for Bio

Although rewarding, the application fields of the BioAFM are also the most challenging within the range of SPM applications and require patience and experience. So researchers should not have to worry about the instrument. JPK has taken on this challenge and developed the NanoWizard® product line. It provides flexibility and high performance along with stress-free operation, enabling optimal liquid handling, optics integration, high resolution and long term stability. This outstanding achievement was reached through close cooperation between AFM experts around the world and the interdisciplinary team at JPK, which comprises engineers, physicists, software developers, along with chemists and cell biologists. JPK's philosophy is to put the needs of the user first. Consequently, a team of application scientists with broad experience in BioAFM – from single molecules through polymers to cell biology and optics – is on hand to support our customers.

By introducing the NanoWizard®II, JPK sets the new standard in BioAFM, accomplishing even the most difficult AFM measurement tasks with ease. As a result, our users achieve results faster.

2. Unfolding of NhaA membrane protein in buffer in closed-loop. Data courtesy of A. Kedrov, group of D. J. Müller, BIOTEC Center, TU Dresden, Germany.

4. Nanomanipulation in closed-loop on a polycarbonate surface, "e" character size 168 nm. Outline path read into AFM software from a scalable vector graphics file.



Surprising Even AFM Inside

But a Whole New Class of I

Key BioAFM design criteria

BioAFM users are as varied as their applications. Chemists and biologists want easy use and clear answers to their questions. Biophysicists and optics experts need direct access to data and experimental flexibility. Integration with optics is a key factor, because optical methods are the standard for life science applications. Everything relevant to the life sciences happens in fluids, so AFM equipment developed for engineering and physics is totally unsuitable. JPK's AFM approach is completely different, meeting the following key design criteria for a BioAFM:

Best AFM performance

- AFM measurements work at the first shot
- Measuring single molecules demands highest resolution in imaging and force measurements, particularly in fluids
- Long-term experiments demand the utmost stability and zero drift – an ideal closed-loop scanner is essential

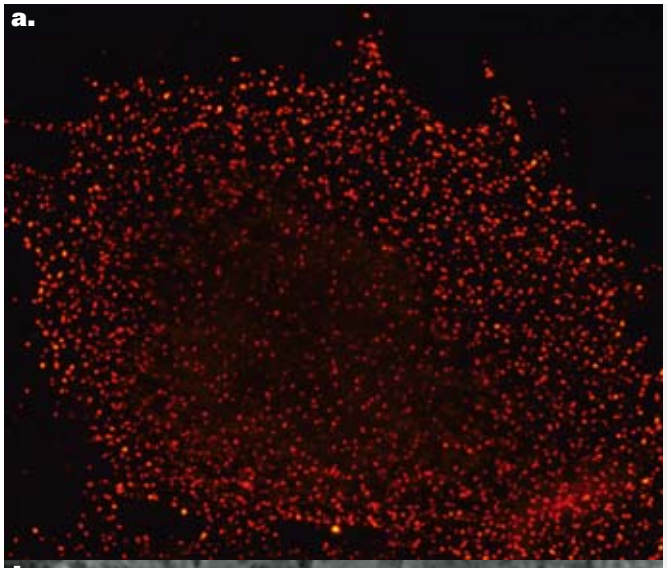
Uncompromising integration with optical microscopy

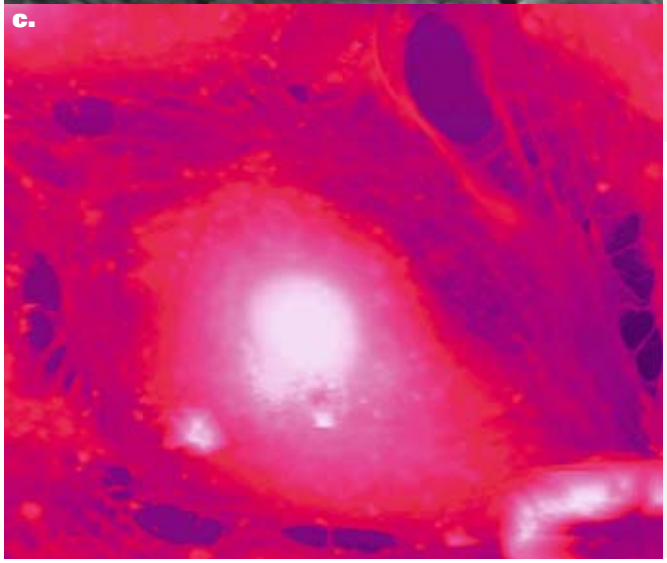
- Biological samples require optical contrast enhancement methods like phase contrast and DIC – a real optical condenser is needed
- Fluorescence techniques such as TIRF, FRET, FCS, FRAP, FLIM, Ca^{2+} response, and laser scanning microscopy require that the sample does not scan – only a cantilever tip-scanning AFM can provide this
- The AFM and optical microscope must be software integrated and without interference – optical filters and an infrared light source allow truly simultaneous operation

Easy and safe operation with liquids

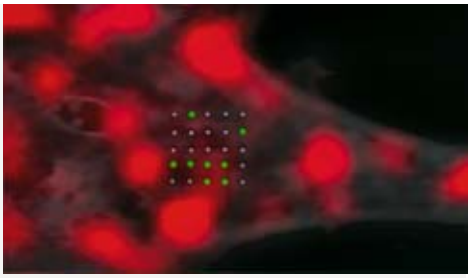
- Working in liquid demands that no sensitive parts be positioned below the sample level – a sample scanning approach is not suitable
- All parts in contact with the sample must be easy to exchange, replace, and rigorously clean
- The AFM must be completely sealed and offer different fluid cells for the full range of applications

rs: Not Just a New AFM, nstrumentation.





Ptk2 cells, transformed with PLAP, which was clustered and labelled with TRITC imaged in buffer in the BioCell™. Overlay of AFM topography, force map points and fluorescence of clustered rafts. Green points mark positive recognition events for the antibody-labelled tip against the antibody used to cluster proteins at surface. Sample courtesy of D. Meder, group of K. Simons, MPI-CBG Dresden, Germany.



Flexibility

- Optimum results for all different applications, i.e., a large scan range, but also the highest resolution
- Electronics, hardware and software must allow user-defined experiments and integration with external equipment, e.g., electrical or optical devices
- The entire spectrum of AFM modes and a comprehensive range of accessories are needed for complex experiments, e.g., for environmental control

JPK has followed these principles and provides the optimum BioAFM solution with the NanoWizard®II. The NanoWizard® has proven its performance in almost every research field, from nanomanipulation to cell adhesion measurements, from surface chemistry to DNA high resolution imaging, from bacteria to pharmaceuticals, from electrochemistry to polymer melting. Our continuously growing global user base and the scientific publication output of our customers are convincing proof of this.

Ptk2 cells, transformed with PLAP, which was clustered and labelled with TRITC. Images in buffer in the BioCell™, scan area 70 x 58 µm²:

a. Fluorescence of clustered rafts (63x immersion objective)
b. Phase contrast (40x)
c. AFM topography height channel.

Sample courtesy of D. Meder, group of K. Simons, MPI-CBG Dresden, Germany.

Maximum Resolution and T

The Next-Generation BioAFM

The BioAFM Head

A multitude of improvements and technological advances have been packed into a new finite-element modeled (FEM) housing. As a result, the AFM head meets all the requirements for stability, performance, handling, and modern industrial design. The NanoWizard®II is compact, robust, and stable for ultimate measurement results.

At the leading edge of closed-loop performance

The proven flexure scanner technology, custom-designed by Physik Instrumente (PI), has been further developed in recent years. The NanoWizard®II uses their latest technology to provide the ultimate performance in x, y, z closed-loop. The industry-leading lowest positioning noise and highest accuracy allows time-saving accurate zooming, long-term stability and the highest possible closed-loop AFM resolution.

A further improved z-sensor noise level enables the most demanding force measurement experiments, even in closed-loop operation. The fast time response of the decoupled, low-mass z-scanner allows rapid imaging even of soft samples and also benefits force experiments. The new scanner system provides better results faster. New materials and the optimized housing mean even better stability, minimal drift and the lowest susceptibility to acoustic noise.

NanoWizard®II setup on a Carl Zeiss Axiovert 200 microscope with joystick-controlled motorized stage and ForceWheel™ device.

rue Optical Integration:

Surpasses all Expectations.

Optical microscopy and AFM perfectly integrated

The NanoWizard®II uses a tip-scanning concept, so scanning in x, y and z is performed by the tip only and the sample remains fixed on the microscope stage. The patented head design means that optical images using standard condenser illumination or fluorescence are available at all times, fully simultaneous to the AFM operation. Sample-scanning AFMs have the problems of blurred optical images during scanning, sample shaking (not acceptable for living cells), and the complete loss of synchronization with laser scanning microscopes.

To optimize simultaneous AFM and optical microscopy, an infrared AFM laser operates far from the visible range of the optical microscope and filters eliminate any crosstalk. From the beginning, the NanoWizard® has had an enviable track record of successful operation on fluorescence microscopes, together with TIRF, and coupled to laser scanning microscopes. The NanoWizard®II allows the use of standard condensers for optical phase contrast and DIC and has an even smaller working distance for use with specialized condensers.

Perfect engineering down to the smallest detail

The NanoWizard®II head provides many improvements that make the life of the user easier. The selection between fluid and air operation is now just the simple turn of a button. New linear drives make the approach faster and much more accurate, while fluid seals protect the motors. A unique automated algorithm corrects sample tilt to minimize the required z-range. The new design makes tip and sample much more accessible from the side, for laser coupling or micro-manipulators. Of course, some original features cannot be made any better – such as the vertically incident AFM laser beam path, that keeps the laser spot visible at any time and allows cantilever adjustment without any external devices – switching from cantilever to cantilever on the same chip at the turn of a button.

The BioAFM Stages

The best instrument is nothing without a solid base

The stages for the NanoWizard®II have been developed for maximum stability. The solid alloy base plates provide the stability required for highest resolution imaging, even on the inverted optical microscope. The manual or motorized stages provide a travel range of 20 x 20 mm^2 – sufficient to conveniently navigate around the sample with an accuracy that allows sensitive movement within the field of view of a 100x objective. The joystick or software control of the motorized stage allows automated measurements on different sample spots. If transmission optics is not required, the stages are easily removed from the optical microscope and the AFM can be operated as a stand-alone.

Made for True Optical Integ

Powerful Electronics Combin

The SPMControl II Electronics

To reach the full potential of the NanoWizard®II head, a high-end controller is essential. State-of-the art control electronics has been developed using the experience gained through the continuous improvements of the NanoWizard® hardware. Sixteen channels for data acquisition and a virtually unlimited number of pixels/points for images, force curves, and force maps provide an ideal basis for cutting-edge experiments with sufficient bandwidth for any AFM measurement.

Performance needs power

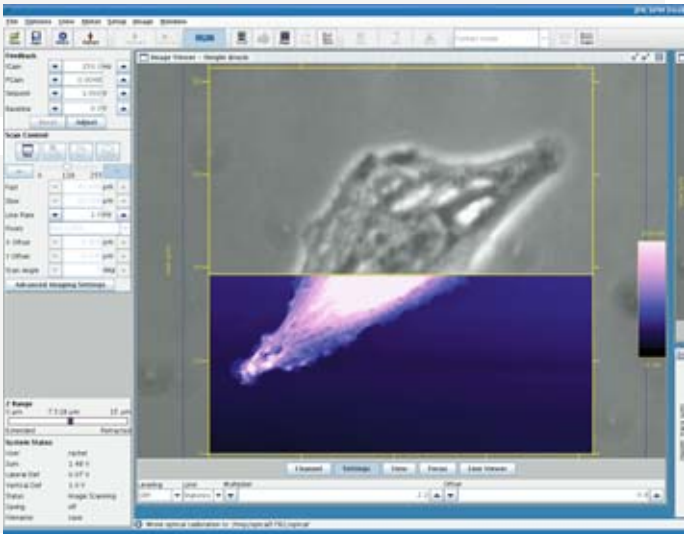
To free the user of residual noise sources that are common to conventional SPMs, a rigorous approach was chosen to eliminate electronic interference. The AFM electronics is galvanically decoupled from the PC by a high-speed optical fiber link, and an uncompromising power supply is used to provide the lowest possible noise. An intelligent grounding concept avoids ground loops, especially if external equipment is used together with the AFM. Users have access to clean DC power with multiple voltages through the Signal Access Module (SAM), which also allows direct access to analog or digital signals. All this makes building your own experiments much more straightforward.

The SPMControl II Software

One of the success factors of the NanoWizard® family is the user-friendly yet powerful software. Further premium features include the access to all AFM imaging modes, the fully comprehensive force measurement module with advanced cantilever calibration, force mapping, and the powerful nanomanipulation and lithography functionality. The NanoWizard®II introduces a number of new functions for Soft Matter and Life Science experiments. This next generation software provides batch processing of force curves, a hands-on interface for force curve functions (the ForceWheel™), an intelligent cantilever drift compensation function (the ForceWatch™ mode) and a built-in optical overlay function.

Seeing is believing – The DirectOverlay™

One highlight of the NanoWizard® software is the picture-in-picture display, automatically overlaying new and old scans. With this function, zooming into the nanoscale is straightforward and fast, because the closed-loop scanner takes you exactly where you want to go, without repeated scans that could contaminate the tip. Measurement or manipulation points can also be set directly within the scanned area.



DirectOverlay™ feature in action. Online import of optical image into AFM software allows calibrated overlay of AFM and optical images. AFM scan region and measurement points are selected directly in the background optical images.

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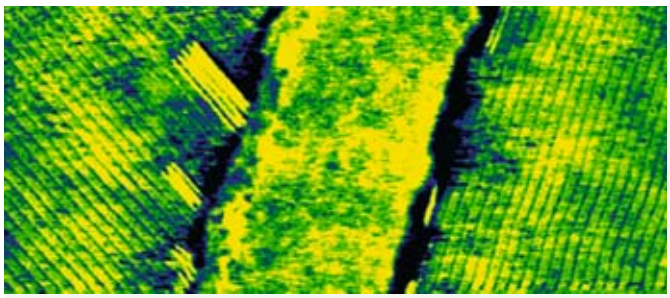
ed with Intelligent Software.



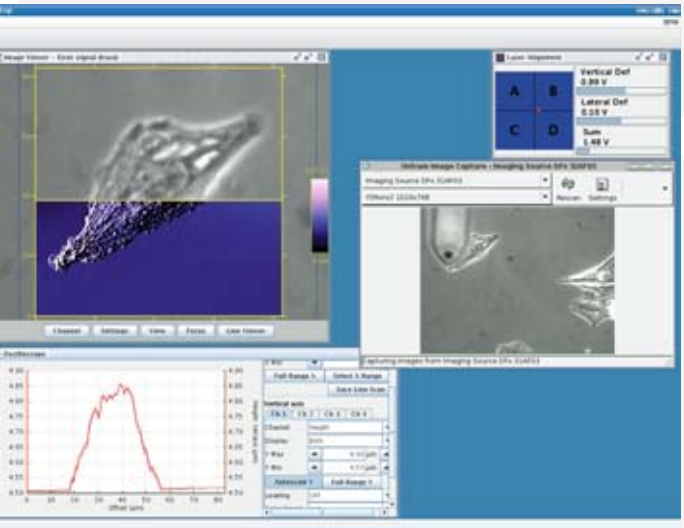
Alkane stripes – hexacontane adsorbed on HOPG, scan area 500 x 200 nm². Closed-loop imaging.

The NanoWizard®II offers an amazing extension to all these functions, with the new direct import of optical images into the AFM software. The calibrated optical image can be imported, for instance, to directly set force spectroscopy points on special features, even without previously imaging the surface so that chemically modified tips are not damaged.

There are many challenges in combining AFM and optical images – the orientation and scan area are offset, with different magnifications, and there are common distortions in the optical images. Previously this required time-consuming image post-processing, cutting and stretching, but now a patent-pending automatic algorithm determines the exact position of the optical image with respect to the AFM scan area and eliminates all distortions and deviations between the two images. This DirectOverlay™ benefits all experiments where data from transmission optics, fluorescence and AFM are combined. With one touch of a button, DirectOverlay™ is achieved within the AFM software and the calculation is independent of the sample features. This means that precise and independent colocalization measurements between AFM and optical images are possible for the first time.

Flexibility is key

The software is designed to get both newcomers and experienced users straight to producing results with the NanoWizard®II. The user interface is intuitively structured and provides all necessary parameters with a single click. A comprehensive range of advanced settings are just a second click away. For experienced users, the full internal command library of the SPMControl II software is accessible through a command line interface for direct control and override. User-developed programs to extend specific functionalities can be written using the script language. Our philosophy is for the operator to participate in the ongoing development of the AFM software; our users get free software updates for the lifetime of the instrument and an unlimited number of installations.



BioAFM – More Than Just th

Smart Accessories for Prec

BioAFM design and handling have to focus on different issues than conventional AFM because of the special demands on it. The NanoWizard®II is robust enough to be used every day by people that are not technical experts. This is reflected in the ergonomic design of the head, the industry-standard PC components, and the multi-user operating system. In fact, experience shows that the NanoWizard®II is truly hard to destroy. Frequent imaging in liquid poses no problems because the liquid guard package completely seals the head against fluids and vapors from the sample. The tip-scanner is also inherently safe because fluids will flow downwards, always moving away from the sensitive electronics in the head. In contrast to a sample scanning AFM design, there are no sensitive electronic parts below the "fluid level".

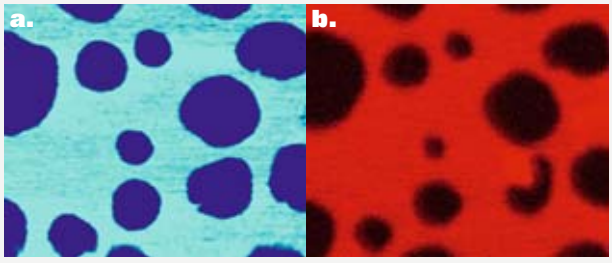
Accessories make the NanoWizard®II system solution complete

No BioAFM can be complete without fluid cells and temperature control options, because most samples in biology or biochemistry require immersion in a buffered solution and precise environmental control. JPK's patent-pending BioCell™ is the only AFM fluid cell on the market that allows temperature control between 15°C to 60°C, perfusion and gas flow, all on the basis of standard cover slips.

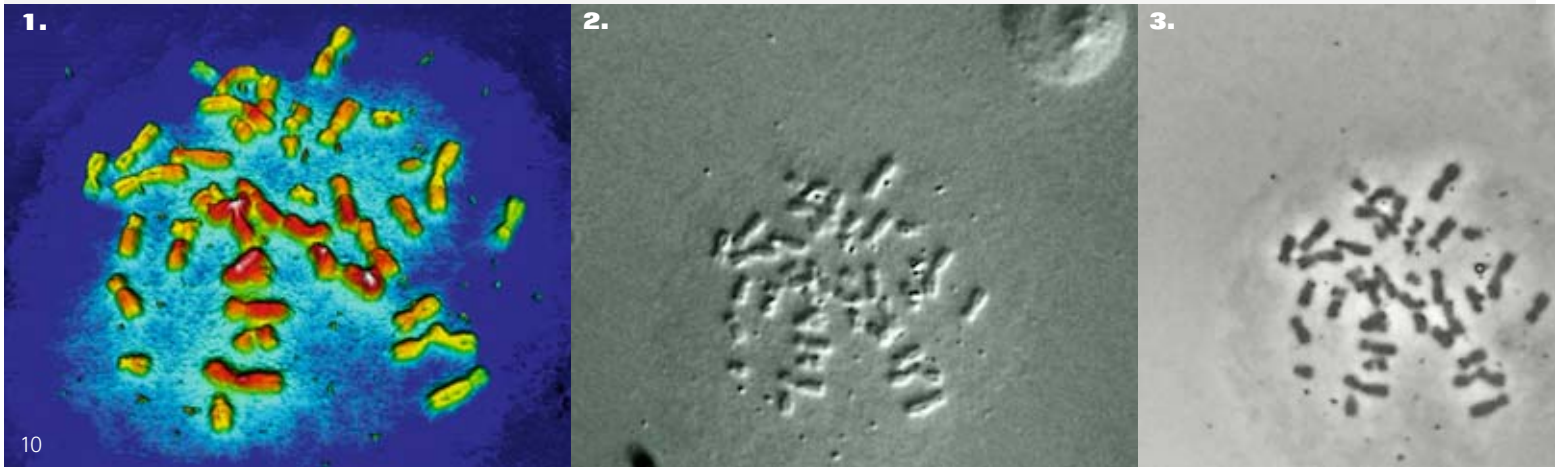
The BioCell™ allows both high resolution optical experiments using high-NA immersion lenses and high-resolution AFM measurements down to the single molecule level. It is therefore ideal for live cell studies and single molecule fluorescence applications. If temperature control is not required, the JPK CoverslipHolder offers the same capability for ambient temperature experiments.

Supported phase-separated lipid bilayer (a.) with simultaneous fluorescence image (b.) from Carl Zeiss 510 Meta confocal. Scan area 8.6 x 7.5 µm².

Image courtesy of S. Chiantia, group of P. Schwille, BIOTEC Center, TU Dresden, Germany.



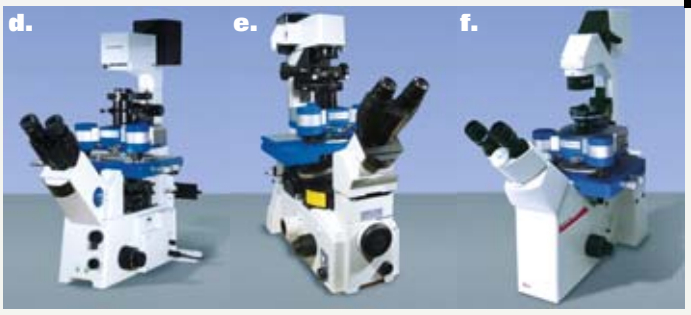
Human metaphase chromosomes, sample courtesy of L. Costa and S. Thalhammer, LMU München, Germany. 3D-topography (1.), DIC (2.) and phase contrast (3.).



e Sum of Its Parts.

ise Environmental Control.

A range of temperature control systems covers temperatures between 0°C and 300°C. Specific fluid cells are available for measurements with strong solvents or for ultra-small volumes, when costly chemicals are used. Unique for commercial AFM systems, the temperature-controlled electrochemistry cell ECCell™ provides a capability that is in high demand. Please see the accessories brochure and the JPK web site for more details.



The NanoWizard® AFM system is compatible with all major inverted research microscope platforms such as c. Carl Zeiss Axiovert 100/135/200 with LSM 510 confocal laser scanning microscope, d. Olympus IX 70/71/81 e. Nikon TE 300/2000 f. Leica DMI. All life science stages can also be unmounted for stand alone operation within one minute.

NanoWizard®II head with motorized stage as stand-alone system.

NanoWizard®II specifications

NanoWizard®II head

- *Tip-scanning stand alone system, the only choice for simultaneous AFM and laser scanning experiments*
- *Best closed-loop AFM on the market for reproducible tip positioning and long time position stability*
- *Position noise level 0.3 nm RMS in x, y (closed-loop) and < 0.15 nm RMS sensor noise level in z*
- *< 0.3 nm RMS free cantilever deflection noise at 20°C in fluid (spring constant 0.03 N/m)*
- *Atomic lattice resolution on inverted microscope (< 0.055 nm RMS z noise level)*
- *100 x 100 x 15 μm^3 scan range for the head in closed-loop mode*
- *Flexure stage scanner design with decoupled, low-mass z-scanner*
- *Built-in liquid guard package for maximum liquid safety*
- *Transmission illumination with standard condensers for precise brightfield, DIC and phase contrast, etc.*
- *IR laser optical system for zero interference and fluorescence free from cross-coupling*
- *Built-in optical filters for fluorescence without crosstalk*
- *Self-diagnosis functionality*

Stages

- *Liquid-safe, robust and drift-minimized design for highest stability*
- *Motorized precision stage with 20 x 20 mm^2 travel range with joystick or software control*
- *Manual precision stage with 20 x 20 mm^2 travel range*
- *Independent positioning of tip and sample with respect to the optical axis*

Sample holders

- *Sample holders for Petri dishes, coverslips or microscope slides*
- *Special holders and liquid cells*
- *Ø 140 x 20 mm^3 free sample volume*

SPMControl II electronics

- *State-of-the-art controller with lowest noise levels*
- *Signal Access Module (SAM) up to 16 channels*
- *TTL access and power supply for external equipment*
- *High-speed fiber link and intelligent grounding concept for maximum bandwidth and performance*
- *Z-range adjustable in real time*

SPMControl II software

- *All standard AFM modes – contact mode, Lateral Force Microscopy (LFM), AC modes, force modulation, force spectroscopy, force mapping, nanomanipulation, nanolithography, etc.*
- *Comprehensive force measurement with TipSaver™ and RelativeForce™ mode*
- *Automated cantilever calibration using thermal noise method*
- *Patent-pending DirectOverlay™ for picture-in-picture functionality on the fly*
- *ForceWheel™ handheld device for sensitive force control*
- *Automated sample tilt correction via the stepper motors*
- *Advanced oscilloscope functionality and online measurement of distances, cross sections etc.*
- *Improved ForceWatch™ mode for force spectroscopy and imaging for cantilever-drift free measurements*
- *True multi-user platform*
- *User-programmable software*
- *Pixel resolution: > 131 000 pixels for force curves; 5000 x 5000 $pixels^2$ for image scans*
- *Powerful image processing (IP) functions with full functionality for data export, fitting, filtering, edge detection, 3D rendering, FFT, cross section etc.*
- *Outline™ mode for precise selection of a new scan area even in the optical image*
- *IP ImageViewer for picture-in-picture display and export, including calibrated optical images*
- *Batch-processing for force curves*

Optical configurations

- *Inverted research microscopes from Carl Zeiss, Leica, Olympus and Nikon*
- *Fully simultaneous operation with optical phase contrast and DIC, using standard condensers*
- *Compatible with commercial confocal microscopes and fluorescence techniques such as TIRF, FRET, FCS, FRAP, FLIM*
- *TopViewOptics™ video optics for opaque samples*

Add-ons (see accessories data sheet)

- *Temperature control, gas flow and liquid cells (including patent-pending BioCell™ and SmallCell™)*
- *Cameras and light sources for video imaging or fluorescence*
- *Sample holders for all kinds of substrates*
- *Patented cantilever holders to eliminate cross-contamination in liquid*
- *Vibration and acoustic isolation from leading suppliers*

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