From the pioneers of BioAFM

NanoWizard® 4 BioScience AFM

- Fast scanning up to 100 Hz line rate for tracking dynamic processes
- Outstanding high-resolution quantitative imaging made easy
- Industry-leading technology integrating optical microscopy in real time
- Unique nanomechanics solutions for brilliant results
- Fully flexible design with the widest range of modes & accessories

JPK Instruments we have joined Bruker BRUKER

NanoWizard® 4 THE NEXT BENCHMARK FOR BIOAFM

Life sciences are constantly developing and JPK's NanoWizard® platform is a unique BioAFM system that grows with the demands of the users. NanoWizard® 4 is the latest generation to answer advanced and complex questions in tomorrow's research.

HIGH RESOLUTION AND FAST SCANNING – A NEW LEVEL OF PERFORMANCE

The NanoWizard® 4 BioScience AFM combines atomic resolution and fast scanning with line rates up to 100 Hz in a system with a large scan range of 100 µm. It is designed for highest mechanical and thermal stability on an inverted microscope for long term experiments even on living cells. It has the lowest noise level for all AFM components such as the closed-loop scanner and deflection detection system. The electronics ensure the highest quality data.

EXPANDED USABILITY AND EXPERIMENT CONTROL

The new ExperimentControl™ simplifies setting up the instrument and remote control of complex and long-term experiments is possible via the internet by a PC, tablet or smartphone, delivering a continuous status update.

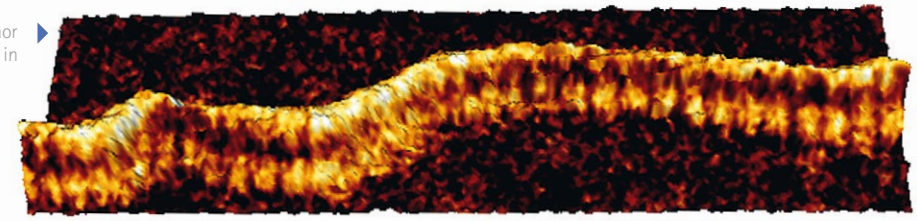
QI™ MODE – QUANTITATIVE IMAGING OPTIMIZED

The improved QI™ mode delivers quantitative imaging with the highest resolution for single molecules, live cells or tissues. The improved force sensitivity and perfect force control make the system ideal for any brittle, delicate, soft or sticky sample. Due to our new wizard-technology, the system is more intuitive and easy-to-use than any other BioAFM while providing total flexibility. The linear z-movement and a full set of force data in every pixel allow the extraction of quantitative data such as elasticity, adhesion, dissipation, chemical forces or molecular binding sites.

UNIQUE SOLUTIONS FOR PERFECT ENVIRONMENTAL CONTROL OF BIOLOGICAL SAMPLES

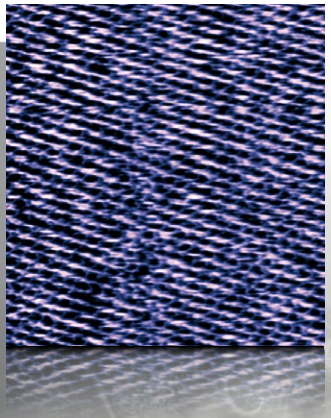
Imaging samples in liquids under physiological conditions is JPK´s core domain. NanoWizard® 4 comes with the largest number of accessories and modes of any AFM system on the market, providing full flexibility for any application.

3D image of lambda phage DNA showing major-minor grooves of DNA double-helix. Imaging in closed-loop xy in liquid (scan size: 100 × 20 nm², z-range: 2 nm).



NanoWizard® 4 BioScience setup on Zeiss Axio Observer with new user interface and tablet control

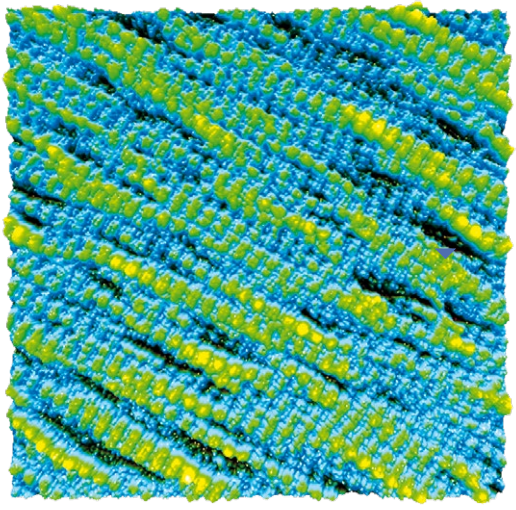
Atomic resolution of mica in liquid measured on an inverted microscope. Scan size 13 nm × 13 nm, closed-loop xy



FAST SCANNING CAPABILITIES TO TRACK SAMPLE DYNAMICS

BENCHMARK FOR FAST SCANNING WITH A LARGE SCANNER

The proven NanoWizard® ULTRA Speed scanner technology option, now available for the NanoWizard® 4, enables 100 Hz line rate – more than 30 times faster than other BioAFMs. The core elements of the new system include highest bandwidth for all components, accurate force control and fast feedback – even with a large scanner of 100 µm. Using the movie creator function, data collection is easier than ever before, and all this is possible simultaneously with optical microscopy.



Collagen type I nanolayer in liquid, scan size 2 µm, 70 Hz line rate, time per frame 3.6 s, closed-loop xy.

Dynamic morphology changes on a living KPG7 fibroblast cell, imaged in the JPK PetriDishHeater™ at 37 °C. Series of 40 µm AFM phase images (time per frame 15 s) show cell dynamic events such as vesiculation (circle) or cell protrusion. Sample courtesy of Prof. A. Herrmann, Humboldt University, Berlin.

FAST SCANNING BENEFITS

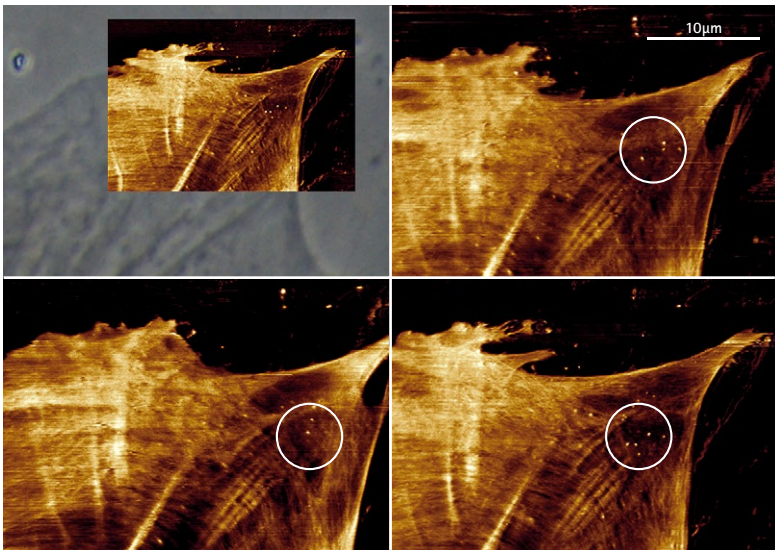
- Observe sample dynamics in real time
- Enhance productivity, probe more sample positions
- Time lapse studies on molecules or cells
- Capture living cells in a well-defined state
- Correlate all data with optical microscopy

CAPTURING SAMPLE DYNAMICS MADE EASY

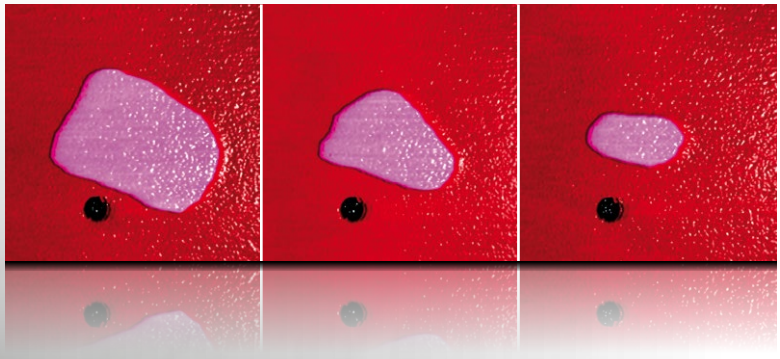
Dynamic experiments on living cells or single molecules with highest spatial resolution are driving modern life sciences. Experiments must be quick to capture living cells in their native state. Scanning times of several minutes per image are therefore not an option. The fast scanning capabilities of the new generation NanoWizard® 4 deliver the speed and accuracy to get stunning data in real time.

TRIGGER AND OBSERVE IN REAL-TIME

Experiments following sample dynamics often rely on triggering a reaction of the sample by changing the environmental conditions. The stimulus could be a change of pH, ionic strength or gas mixture (CO_2/O_2); or adding drugs or physical stimuli such as heating, cooling, mechanical stress from a pipette or liquid flow; or even stimulation by light. These are all possible simultaneously with measurements using JPK AFMs. The unique tip scanner makes the system incredibly flexible.



Melting of domains in a binary mixture of DPPC/DOPC supported bilayer under increasing temperature (left: 40 °C to right: 45 °C) in buffer solution. Scan range: 2 µm × 2 µm, 70 Hz line rate, time per frame 3.6 s.



CORRELATED AND SIMULTANEOUS DATA THROUGH SEAMLESS INTEGRATION OF AFM AND OPTICAL MICROSCOPY

TRUE CORRELATIVE MULTI-PARAMETER MICROSCOPY

Simultaneous observation of live cells or single molecules in real-time is possible using the NanoWizard® 4 with inverted or upright microscopes. To get synchronized data, both systems have to be intelligently integrated. JPK's DirectOverlay™ provides easy optically guided navigation to find an area of interest directly in the optical image. Synchronization of data acquisition by trigger signals delivers real correlated data. A broad range of cameras and detectors from Oxford Instruments/Andor, Jenoptik, Hamamatsu, Photometrics are supported. Additionally, JPK can run Andor's CMOS cameras with a speed of 100 frames/sec with high pixel density.

NANOWIZARD® AFMs – DESIGNED FOR PERFECT OPTICAL INTEGRATION

Data correlation in transmission illumination using standard condensers and reflection microscopy simultaneously with AFM is only possible by using a tip-scanning AFM with open physical access to the sample. JPK's core expertise lies in reaching highest stability on inverted microscopes, using high-NA immersion objectives and coverslip-based sample holders simultaneously. Environmental control solutions such as the BioCell™ or PetriDishHeater™ for long term cell studies are a great benefit. Thanks to the unique design, the NanoWizard® 4 fits all major inverted or upright microscopes and can be combined with advanced optical methods.

ERGONOMICS & EASE OF OPERATION FOR A RELAXED WORKFLOW

The new system comes with enhanced DirectOverlay™ software for direct correlation of AFM and optical data. Calibration algorithms, visualization routines and usability are expanded. The new graphical user interface with ergonomic design and "optical-style" contrast prevents eyestrain and makes operation more intuitive for long term optical experiments. A large single screen ensures a compact and streamlined workflow.

Unique side-view cantilever holder for cell interactions or microaggregation. Cell image courtesy of Dr. C. Gonnermann, Dr. D. Stamov and Dr. C. Franz, KIT Germany

ADVANCED OPTICAL TECHNIQUES SIMULTANEOUSLY WITH AFM

- Brightfield
- DIC, phase contrast or modulation contrast
- FRET, FLIM, FCS, FRAP
- Ca^{++} imaging
- TIRF and IRM
- Spinning disc
- Confocal microscopy
- Structured illumination techniques (SIM)
- Superresolution techniques such as STED, PALM/STORM

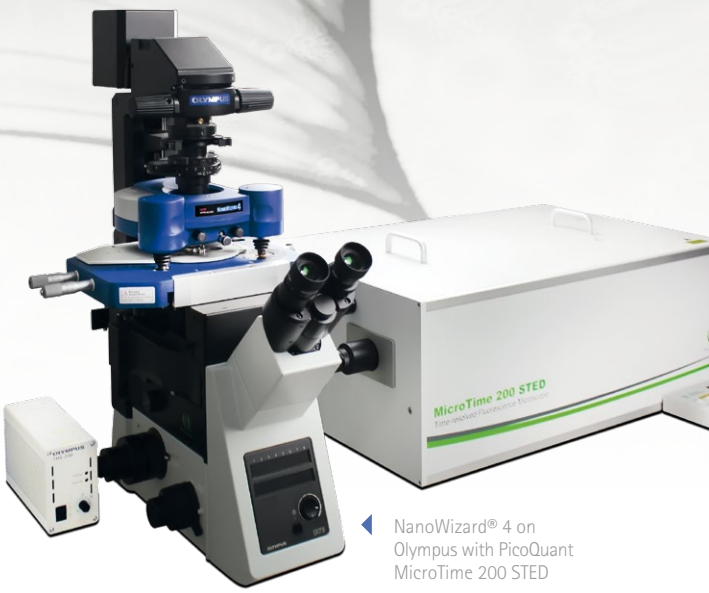
Upright Fluorescence Microscope (UFM) Kit for tissues or other large samples

BioMAT™ Workstation for high NA upright optics with Zeiss Axio Imager

NanoWizard® 4 setup on Zeiss LSM 880 confocal microscope with Airyscan

NanoWizard® 4 AFM and Superresolution Optical Microscopy – A Perfect Match

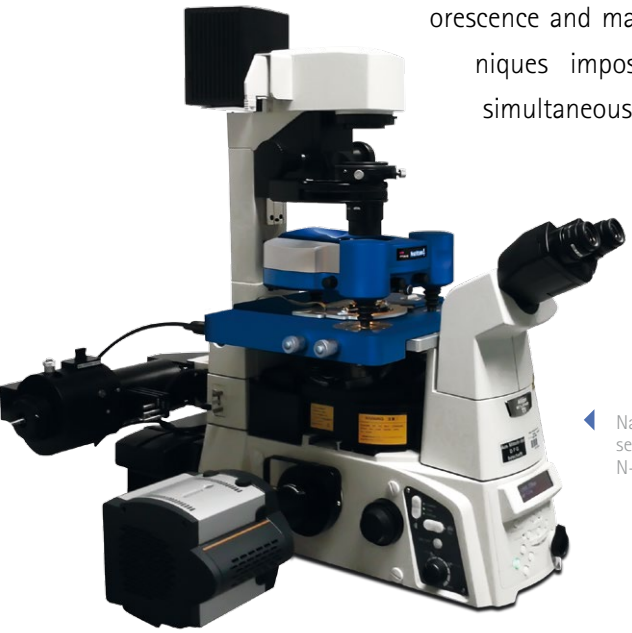
The discovery of Superresolution microscopy, recently recognized by the Nobel Prize, shifts the perspective of optics to the nanoscale. Techniques such as PALM/STORM, STED or SIM are becoming accessible for a broad range of life scientists. As Superresolution nears the spatial resolution of AFMs and fast scanning AFM reaches the temporal resolution of optics, correlation of data brings a new perspective to dynamic experiments on live cells or molecules.



NanoWizard® 4 on Olympus with PicoQuant MicroTime 200 STED

PERFECT INTEGRATION WITH SUPERRESOLUTION MICROSCOPY PLATFORMS

The new NanoWizard® 4 head comes with a 980 nm laser option for simultaneous use of "focus stabilization" systems used in optical microscopes. This is particularly important when performing long term experiments. Lower wavelength lasers used by other AFMs interfere with the fluorescence and make both techniques impossible to use simultaneously.

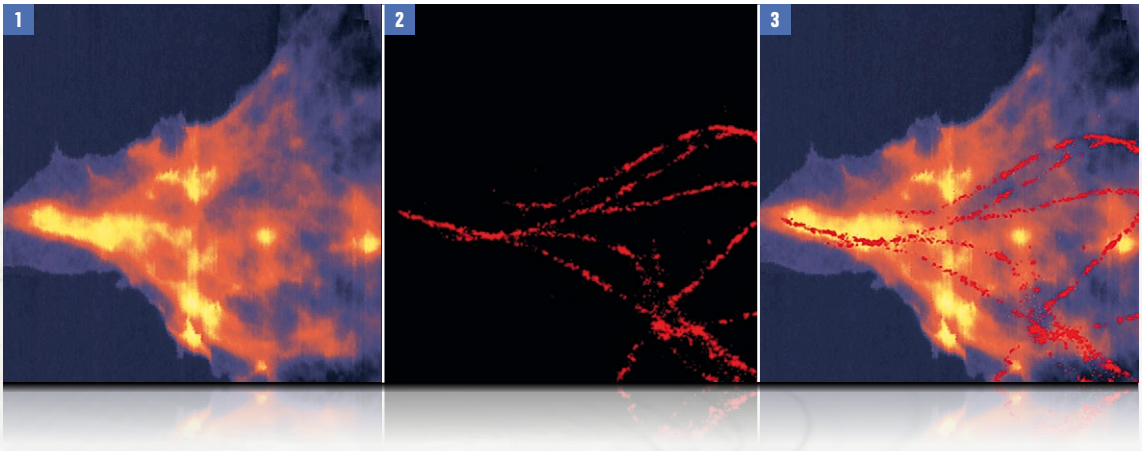


NanoWizard® 4 setup with Nikon N-STORM system

NanoWizard® 4 with its unique tip-scanning technology is ideal for integration into Superresolution microscopes from Zeiss (PALM/STORM, SIM), Leica (STED), PicoQuant (STED), Nikon (SIM, STORM) and Abberior (STED).

The HybridStage™ is a newly developed modular piezo-based sample scanner stage combined with motorized coarse XY sample movement. This enables accurate and highly reproducible z-stacks, important for image reconstruction, while the AFM stays in place.

Laser safety and temperature control are special issues when using Superresolution optics, therefore the whole system is equipped with a microscope-type incubator. A hassle-free NanoWizard® 4 integration into these standard incubators is easily possible.



1 HeLa cell measured in buffer solution and imaged in QI™ mode.
· scan size: 8 µm
· z-range: 300 nm

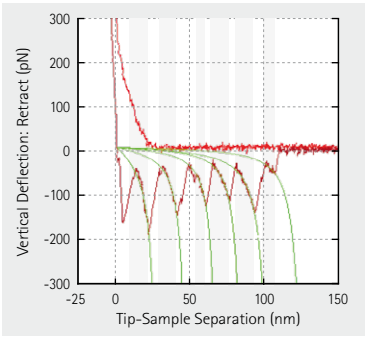
2 Microtubules were labelled with Alexa647-antibodies and measured with dSTORM.

3 Overlay of AFM and STORM image.

Sample courtesy: Dr. Josef Madl, Prof. Winfried Römer, BIOSS, Freiburg, Germany

OUTSTANDING QUANTITATIVE DATA FROM SINGLE MOLECULES TO LIVING CELLS

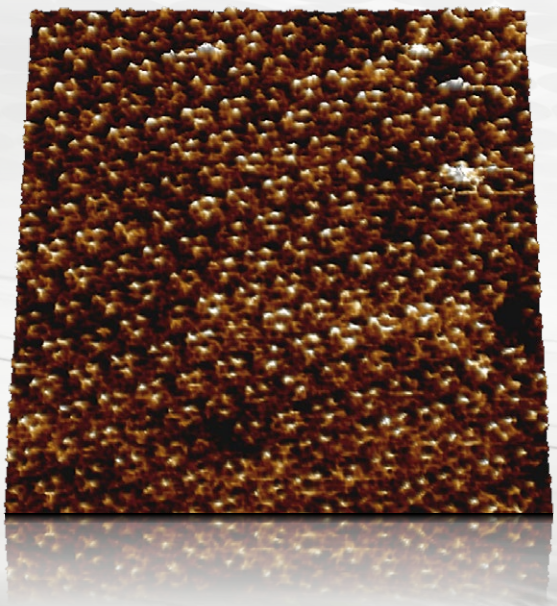
With over 15 years' experience, JPK is the leader in force spectroscopy for soft and biological samples. From single-molecule to single-cell force spectroscopy or the characterization of mechanical properties on substrates, cells and tissues, JPK offers THE perfect solution. The improved QI™ mode with lowest noise levels delivers outstandingly clear, quantitative data at the highest resolution from single molecules to live cells. QI™ is based on real force curves, so mechanical, chemical and biofunctional interactions are now more precise and easier to measure compared to other methods. Enhanced data analysis capabilities – especially for modulus calculation with its selection of models – is a great benefit. RampDesigner™ and ExperimentPlanner™, allow the user to design complex experiments. The new ExperimentControl™ feature gives the user remote access to their experiments. JPK offers a comprehensive toolset to control and vary sample conditions such as pH, ionic strength, adding drugs or other substances, temperature or CO_2 level.

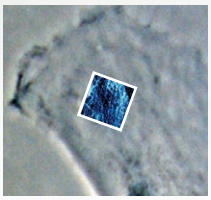


Unfolding curve of artificial polyprotein made of non-mechanical protein GB1. Characteristic saw-tooth pattern and equally spaced force peaks are visible (contour length: 18.0±0.5 nm).

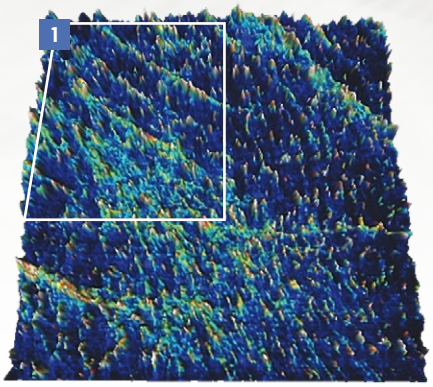
Sample courtesy:
Prof. Yi Cao,
Nanjing University, China

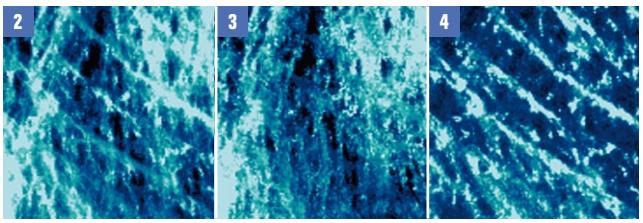
3D height image of bacteriorhodopsin using QI™ mode in buffer (scan size 100 nm, z-range 500 pm). Complete BR trimers as well as individual molecular defects are visible.





Optical 40× phase image of living KPG7 fibroblast cell measured in a Petri-DishHeater™ at 37 °C.





1 Overlay of height and Young's modulus image
2 QI™ mode height image on top of the cell (scan size: 7.5µm)
3 Corresponding Contact Point Image (CPI) with zero force
4 Young´s modulus image: While the surface in CPI mode looks smooth the Young´s modulus image reveals more details and it is possible to measure elasticity differences in high resolution at the same position.

JPK OFFERS COMPLETE HARDWARE AND SOFTWARE SOLUTIONS FOR FORCE SPECTROSCOPY ON BIOLOGICAL SAMPLES

- Highest resolution, quantitative data provided by QI™ Advanced mode
- Unique design of force curve pattern with the RampDesigner™
- Advanced algorithms for experimental workflow with the ExperimentPlanner™
- Powerful and fast batch-processing with comprehensive fitting routines for curves & images
- Full range of accessories for environmental control, vital for live cells
- Optional 100 µm z-range with CellHesion® options for live cell adhesion studies
- Automated mapping of sample properties over a large range with the new HybridStage™ for structured substrates, cells and tissue samples

COMPREHENSIVE NANOMECHANICS SOLUTIONS FROM LIVING CELLS TO TISSUES

JPK provides a complete set of methods to characterize mechanical properties such as hardness, viscoelasticity and adhesion of cells, gels, tissues or other large or corrugated heterogeneous samples. JPK's improved QI™ mode based on real force curves is an excellent improvement for obtaining quantitative data quickly and easily. Modes such as fast force mapping and single cell force spectroscopy (SCFS) are also available with NanoWizard® 4.

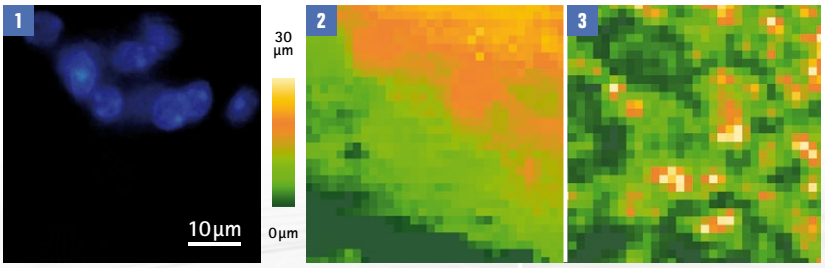
ADVANCED MODES FOR RECOGNITION IMAGING AND VISCOELASTIC SAMPLE PROPERTIES

- **Molecular recognition imaging** for localization of binding sites on single molecules or receptors on living cells with highest spatial and temporal resolution. Direct correlation of adhesion binding sites with topography and advanced optics
- **Micro-rheology mode** for frequency-dependent viscoelastic properties of polymers, gels and live cells
- **Contact Point Imaging (CPI)** comes with JPK's optional QI™ Advanced mode. It displays the topography at zero force unaffected by stiffness variations of the sample
- **Contact resonance imaging** for elastic moduli from a few to hundreds of GPa – perfect for polymers and cartilage

HYBRIDSTAGE™ BENEFITS

- Automated and streamlined workflow with the motorized HybridStage™ option
- Combined piezo and motorized stages for automated mapping or scanning of large areas
- Cell/cell or cell/substrate adhesion in 3D with a large pulling range up to 200×200×200 µm³
- Optional choice of XY or XYZ piezo flexure scanners with different travel ranges
- Large area automated mapping of mechanical properties from micron to millimeter range with simultaneous optical microscopy
- Application examples: tissue samples, cell culture scaffolds, plant cells, parasites, printed micro/nano 3D structures, spheres or capsules, cartilage, bones and many more

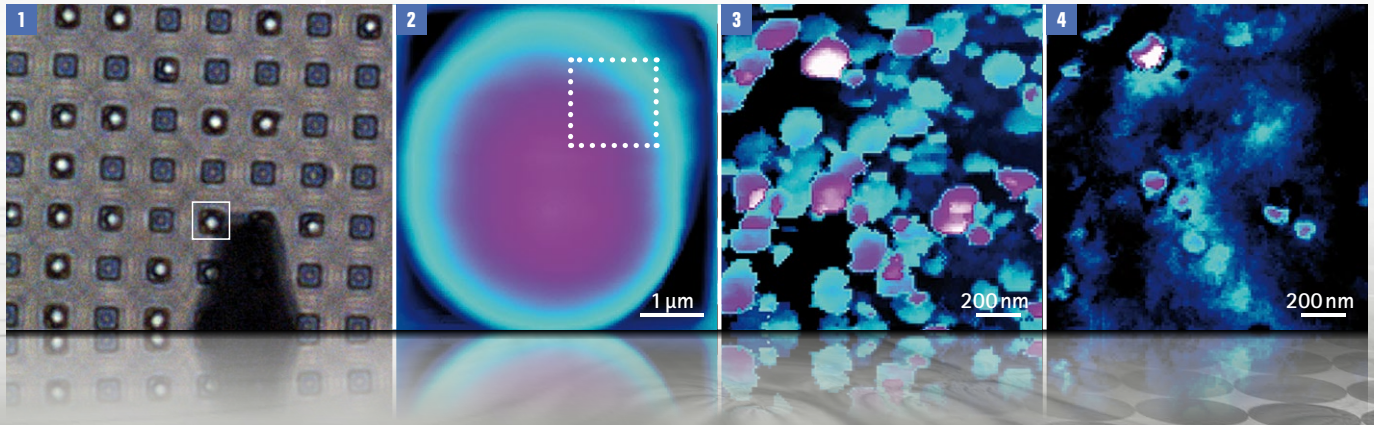
NanoWizard® 4 on HybridStage™



Fixed mouse cerebellum tissue embedded in 4% Agarose. BioMAT™ Workstation was used to overlay 1 63× upright fluorescent microscopy image of DAPI stained nucleus and 2 AFM force mapping height (z-range: 30 µm) and 3 Young´s modulus image (z-range: 5 kPa, scan size: 50 µm). Newly developed automatic height compensation was used to overcome the typical large height differences of tissue sample.

Sample courtesy:
AG Prof. Jochen Guck, Dr. Elke Ulbricht,
TU Dresden, Germany

1 Optical phase image 40× of living Candida albicans in a PDMS matrix. 2 Height image of single cell using QI™ mode (z-range: 2.5 µm). 3 Adhesion and 4 slope image of marked region. Adhesive nanodomains clearly correspond to stiffer cell regions. (adhesion range: 3.25 nN, slope range: 9.5-12.5 nN/µm). Data courtesy: Dr. Cécile Formosa, UCL, Belgium and Dr. Etienne Dague, LAAS, France



SPECIFICATIONS FOR THE NANOWIZARD® 4 BIOSCIENCE AFM

System specifications
- Atomic lattice resolution on inverted microscope in closed-loop (< 0.030 nm RMS z noise level)
- Ultra-low noise level of cantilever deflection detection system < 2 pm RMS free (0.1 Hz - 1 kHz) and high detector bandwidth of 8 MHz for high speed signal capture
- Tip-scanning, stand-alone system, with a rigid low-noise design and drift-minimized mechanics – the best choice for simultaneous AFM and laser scanning (LSM) experiments
- IR deflection detection light source with low coherence for interference-free measurements
- The only liquid-safe AFM with integrated vapor barrier, special encapsulated piezo drives and tip-moving design
- Transmission illumination with standard condensers for precise brightfield, DIC and phase contrast
- Scanner unit
  - Best closed-loop AFM on the market for reproducible tip positioning and long time position stability
  - 100 × 100 × 15 µm³ scan range
  - Position noise level < 0.15 nm RMS in xy (in closed-loop) and 0.06 nm RMS sensor noise level in z (3 kHz bw)

Vortis™ SPMControl electronics
- State-of-the-art digital controller with lowest noise levels and highest flexibility

SPMControl software
- True multi-user platform (perfect for imaging facilities)
- User-programmable software
- Fully automated sensitivity and spring constant calibration using thermal noise or Sader method
- Patented DirectOverlay™ for combined optical and AFM information
- Outline™ mode for precise selection of a new scan area in the optical image
- Improved ForceWatch™ mode for force spectroscopy and imaging for cantilever-drift free measurements
- Comprehensive force measurement with TipSaver™
- Advanced spectroscopy modes such as various force clamp modes or ramp designs, e.g. for temperature ramps, pulling speed or force feedback
- Powerful Data Processing (DP) functions with full functionality for data export, fitting, filtering, edge detection, 3D rendering, FFT, cross section, etc.
- Powerful batch processing of force curves and images including WLC, FJC, step-fitting, JKR, DMT model and other analyses

Stages and sample holders
- Stages are available for all major inverted optical microscope manufacturers such as Zeiss, Nikon, Olympus and Leica
- Motorized precision stage with 20 × 20 mm² travel range with joystick or software control
- Manual precision stage with 20 × 20 mm² travel range
- Holders for Petri dishes, coverslips, microscope slides or metal SPM discs are available
- Special holders and liquid cells possible
- Ø 140 × 18 mm³ free sample volume

Optical configurations
- Fits on inverted microscopes from
  - Zeiss (Axio Observer, Axio Vert 200, Axio Vert A1)
  - Olympus (IX line)
  - Nikon (TE 2000, Ti line) and
  - Leica (DMI/DMi lines)

  for AFM simultaneously with optical Microscopy
  - Fully simultaneous operation with optical phase contrast and DIC using standard condensers
  - Combine AFM with advanced commercial confocal microscopes and fluorescence techniques optical techniques such as FCS, FRET, TIRF, FLIM, FRAP, STED, STORM/PALM, SIM and more
  - Exact positioning and overlay of optical and AFM data with the unique JPK DirectOverlay™ software module
  - Upgradeable for scatter-type SNOM, Raman, TERS measurements
  - Superior stability when using immersion lens optics with highest NA in combination with a coverslip based sample holder
- TopViewOptics™ video optics for opaque samples
  - Maximum flexibility even if no fluorescence is needed (the sample stage can be mounted on an optical microscope within a minute)
  - Free access to the sample area for micropipettes or electrical connections
  - TopViewOptics™ with 12 × zoom
- BioMAT™ option (see BioMat™ brochure)
  - For high-NA upright fluorescence optics combined with AFM on opaque samples
  - Supports upright research microscopes such as Zeiss Axio Imager and Axioscope, Olympus BX51/53 and BX FM as well as LEXT, Leica DM 4000/5000
- Upright Fluorescence Microscope (UFM) Kit
  - Enables the combined use of AFM and optical fluorescence microscopes such as Zeiss Axio Zoom V16, Leica Macroscope Z16 ApoA, Olympus MVX 10 MacroView
- Large variety of cameras suported
  - High-end EM-CCD cameras such as models from Andor (iXon), Hamamatsu and Photometrics (Evolve)
  - sCMOS cameras from Andor (Zyla) or Hamamatsu (Orca)
  - CCD and CMOS cameras from Jenoptik, IDS or µEye

Temperature control options
- Ambient to 300 °C temperature range with 0.1 °C precision with the JPK High Temperature Heating Stage (HTHS™)
- -35 °C to 120 °C temperature range with 0.1 °C precision with the JPK Heating Cooling Module (HCM™)

Fluid cell options
- Inert glass standard cantilever holders for experiments in droplets or custom fluid cells
- JPK´s patented BioCell™ for high-NA immersion lenses and high resolution AFM down to the single molecule level, allows temperature control between 15-60 °C, perfusion and gas flow, made for standard cover slips
- CoverslipHolder offers the same performance as the BioCell™ for ambient temperature experiments
- Temperature controlled electrochemistry cell ECCell™ with transmission illuminations
- PetriDishHeater™ and PetriDishHolder perfect for living cells
- SmallCell™ small volume version for minimized volumes (< 60 µl) and with 3 perfusion ports

Integrated flexibility from a wide range of accessories
(see accessories handbook)
- Different sample holders, cantilever holders and stages for every application
- Large choice of temperature controls (for ambient, liquid and gas), liquid cells even for aggressive solvents
- JPK´s ForceWheel™ handheld accessory for most sensitive experiment control
- Full experimental control by scripting functionality and access to all signals
- CellHesion® module with extra 100 µm closed-loop z-range
- TAO™ module with 100 × 100 µm² or 100 × 100 × 10 µm³ closed-loop sample scanning stage
- Vortis™ Advanced SPMControl station for maximum flexibility
- Comprehensive selection of electrical measurement modes
- Vibration and acoustic isolation from leading suppliers

Modularity and future upgrade pathways
- Simple and cost-effective upgrade paths are available for NanoWizard® 3 to NanoWizard® 4
- System can be upgraded with a NanoWizard® ULTRA Speed head, a CellHesion® 200 head or a ForceRobot® 300 head

NanoWizard® 4 AFM with TopViewOptics™

STANDARD OPERATING MODES

Imaging modes
- Easy-to use QI™ mode for quantitative imaging
- Contact mode with lateral force microscopy (LFM)
- AC modes with phase detection

Force measurements
- Static and dynamic spectroscopy
- Fast Force mapping

OPTIONAL MODES

- Fast scanning option up to 100 Hz line rate
- QI™ Advanced mode for quantitative data
  - Mechanical properties such as adhesion, elasticity, stiffness, deformation
  - Conductivity and charge distribution mapping
  - Contact Point Imaging (CPI) with zero force
  - Molecular recognition imaging for binding site mapping
- HyperDrive™ mode for highest resolution imaging in fluid
- Advanced AC modes such as FM and PM with Q-control & Active Gain Control
- Higher harmonics imaging
- MicroRheology
- Kelvin Probe Microscopy and SCM
- MFM and EFM (see also QI™ mode)
- Conductive AFM (see also QI™ mode)
- STM
- Electrical spectroscopy modes
- Piezo-Response Microscopy
- Electrochemistry with temperature control and optical microscopy
- NanoLithography and NanoManipulation
- Nanoindentation
- Scanning Thermal AFM
- JPK ExperimentPlanner™ for designing a dedicated measurement workflow
- JPK RampDesigner™ for custom designed force curve segments for clamp and ramp experiments
- ExperimentControl™ feature for remote experiment control
- Environmental control options
- DirectOverlay™ for combined AFM and optical microscopy
- Additional xy or z sample movement stages available with CellHesion®, TAO™ and HybridStage™ module

JPK Instruments — we have joined Bruker — BRUKER

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