Quantitative and high resolution imaging

NanoWizard® 3 BioScience AFM

From the pioneers of BioAFM

HyperDrive™ provides **SuperResolution** of soft samples in liquids, while **QI™ Mode** provides **Quantitative Imaging**

Designed for optimum imaging in liquid with excellent resolution, lowest noise & maximum usability

Unique integration with optical microscopy by tip-scanning design, **DirectOverlay™** mode & smart engineering

Comprehensive force measurements from single molecules to living cells with **ExperimentPlanner™** & **RampDesigner™** & **QI™ Advanced**

Expanded flexibility with the widest range of modes & accessories with the **Vortis™** controller

JPK Instruments

Nanotechnology for Life Science

JPK – the evolution of BioAFM

Launched in 2002, the **NanoWizard®** represented the beginning of a family of AFM systems that introduced multiple advances in imaging and the characterization of biological and soft materials. This success story of the pioneers of the world's first dedicated BioAFM is evidenced in the publication of more than 900 peer-reviewed papers citing the use of JPK systems.

JPK applications team

Building relationships with the SPM community and collaborating with users worldwide has enabled JPK to develop powerful and flexible systems. Upgradeability guarantees a safe investment for users and an international team of experienced scientists (see photo) takes care of service and support.

Engineered & made in Germany

JPK develops, engineers and manufactures instrumentation in Germany to the world-recognized standards of German precision engineering, quality and functionality. The company has a simple philosophy. As CTO Torsten Jähnke, says: "We have always designed our instrumentation after first listening to users and their challenges. Delivering successful answers for us means no compromises between usability and handling on one side and highest performance on the other side."

The milestones of development

1999 JPK was founded

Launch of the NanoWizard®: the first dedicated BioAFM in the world

Launch of CellHesion® module: for expansion of the NanoWizard® in the field of cell mechanics

Launch of TAO™ module: for tip-enhanced NanoOptics

Launch of NanoWizard® II: next generation BioAFM with our proprietary DirectOverlay™ feature which combines AFM and optical images

Launch of BioMAT™ Workstation: unique coupling of AFM with upright microscopy

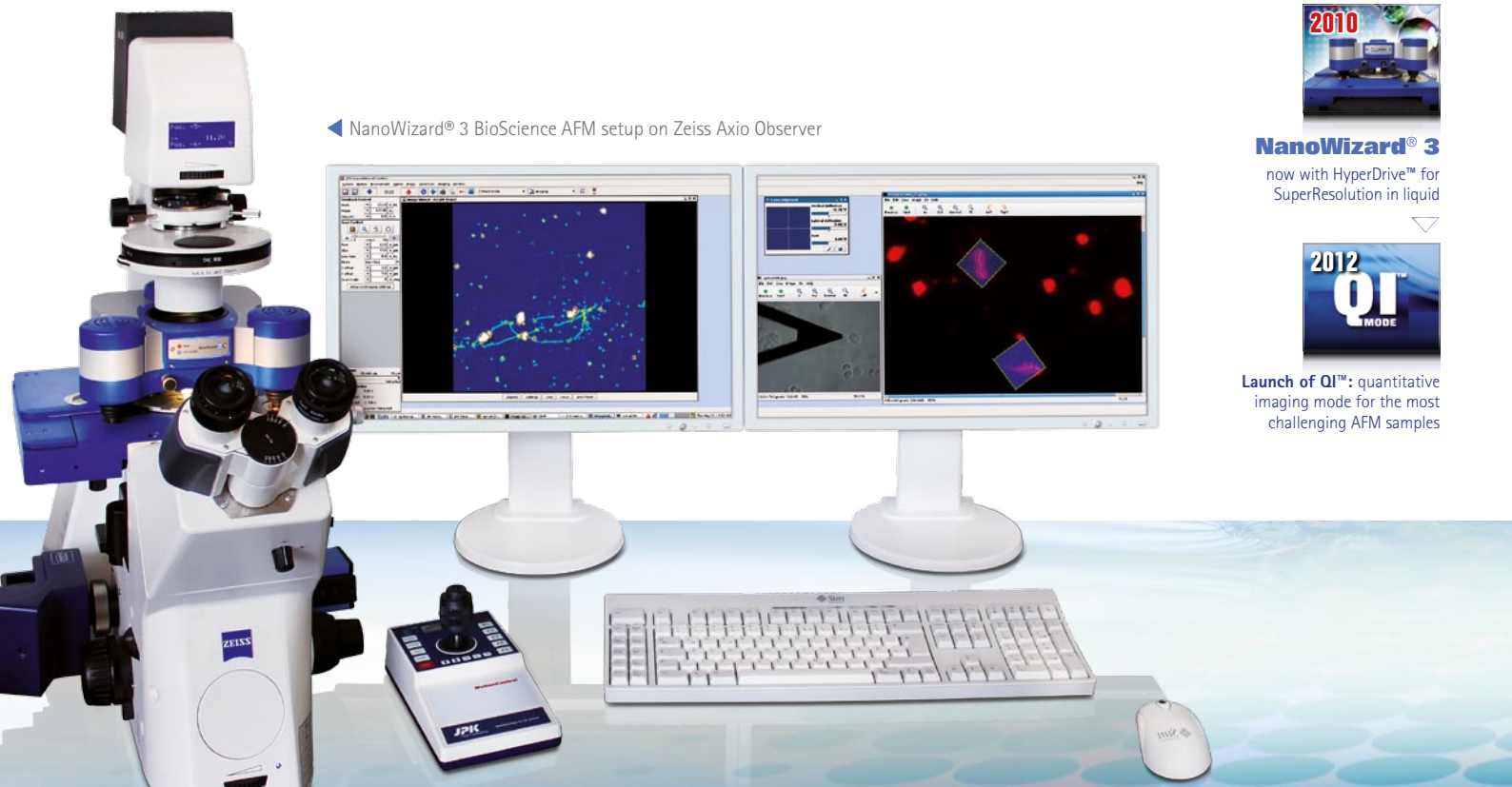
Launch of the NanoTracker™: first force sensing optical tweezers; **Launch of the CellHesion® 200:** first dedicated cell mechanics system

Launch of the ForceRobot® 300: worlds first dedicated Automated Force Spectroscope

NanoWizard® 3

now with HyperDrive™ for SuperResolution in liquid

Launch of QI™: quantitative imaging mode for the most challenging AFM samples



NanoWizard® 3 BioScience AFM setup on Zeiss Axio Observer

HyperDrive™ – SuperResolution AFM imaging in liquids

AFM imaging of soft samples has always provided much discussion. Which cantilever is correct to use? Should it be a DC or an AC mode? Does the sample get damaged? Are the measurements correct?

Remove these questions with **HyperDrive™**, a soft sample imaging technique in liquid which provides sub-nanometer lateral resolution with minimal tip-sample interactions.

HyperDrive™

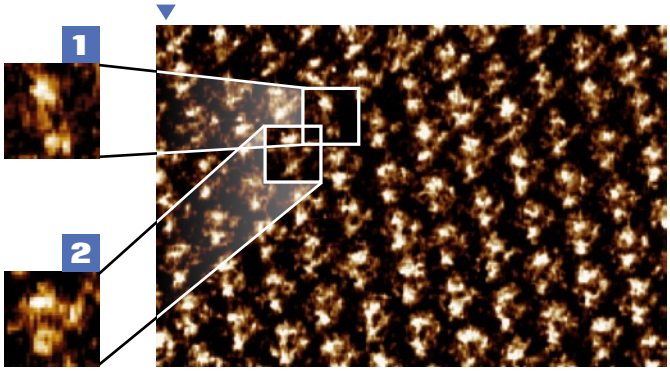
HyperDrive™ is available with the **NanoWizard® 3** AFM head and the **Vortis™** high bandwidth, low noise electronic control system. The electronics are extremely stable to drift and have the ability to detect the smallest cantilever deflections below a typical noise level of 2 pm RMS.

The **NanoWizard® 3** is the only AFM system on the market which is designed for optimal use in liquid and comes with a vapor barrier, encapsulated piezos and a variety of dedicated liquid cells for applications ranging from single molecules to living cells. Of course, one can use the system in air or controlled environment too.

HyperDrive™

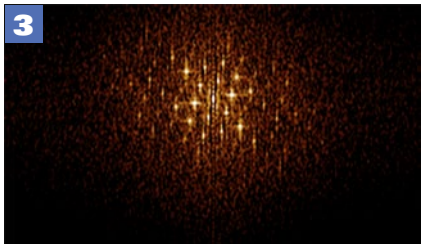
- is a sub-nm SuperResolution AFM imaging technique in liquids
- has extremely low tip-sample interaction
- is not damaging the sample and preserves the tip
- doesn't need a special cantilever
- is easy to use
- is unique to the **NanoWizard® 3** AFM

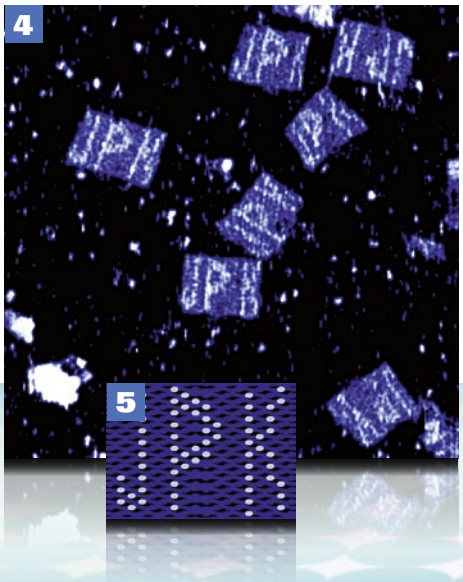
Height image of bacteriorhodopsin in buffer, Scan size 65 × 45 nm².
1 BR molecule with individual molecular defect.
2 Complete BR trimer.
3 Image shows the FFT.

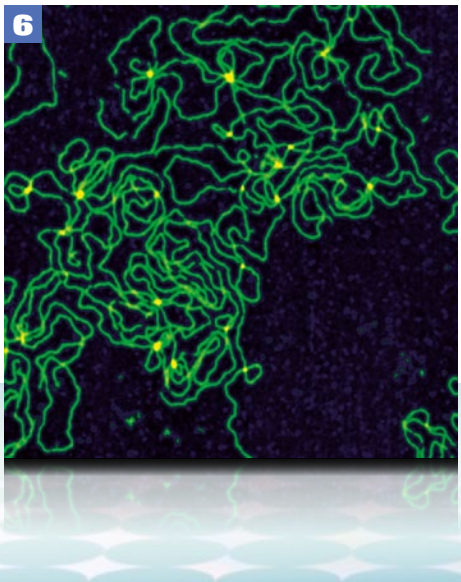


All images are in closed loop.

Sample courtesy of D.J. Müller group ETH Zürich, BSSE Basel







DNA origami imaged in buffer with the JPK letters marked in dumbell hairpins (4), scale bar 100 nm. One long DNA single strand runs through the whole structure, winding back and forth to fill the rectangular form. The second strand is made up of more than 200 short oligonucleotide "staple strands", which tie different parts of the long molecule together to form a three-dimensional structure by self-assembly. The higher points which form the letters are made up of individual hairpins in the staple strands. Inset (5) is the calculated JPK pattern. Sample courtesy of Simmel group, TU Munich.

Lambda phage DNA on mica (6) imaged in buffer solution (10 nM HEPES, 2 mM $NiCl_2$). Scan size 1 µm × 1 µm, Z-range 5 nm.

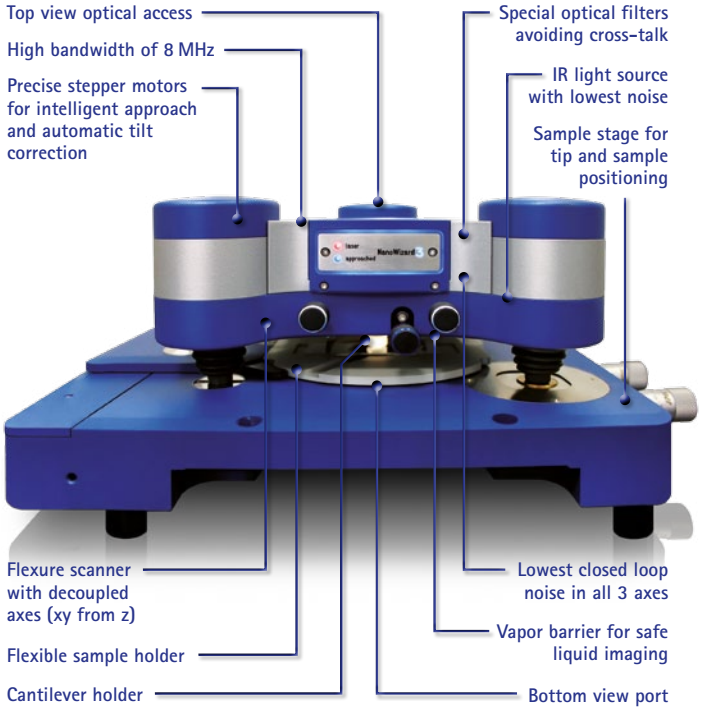
All images are in closed loop.

NanoWizard® 3 system design

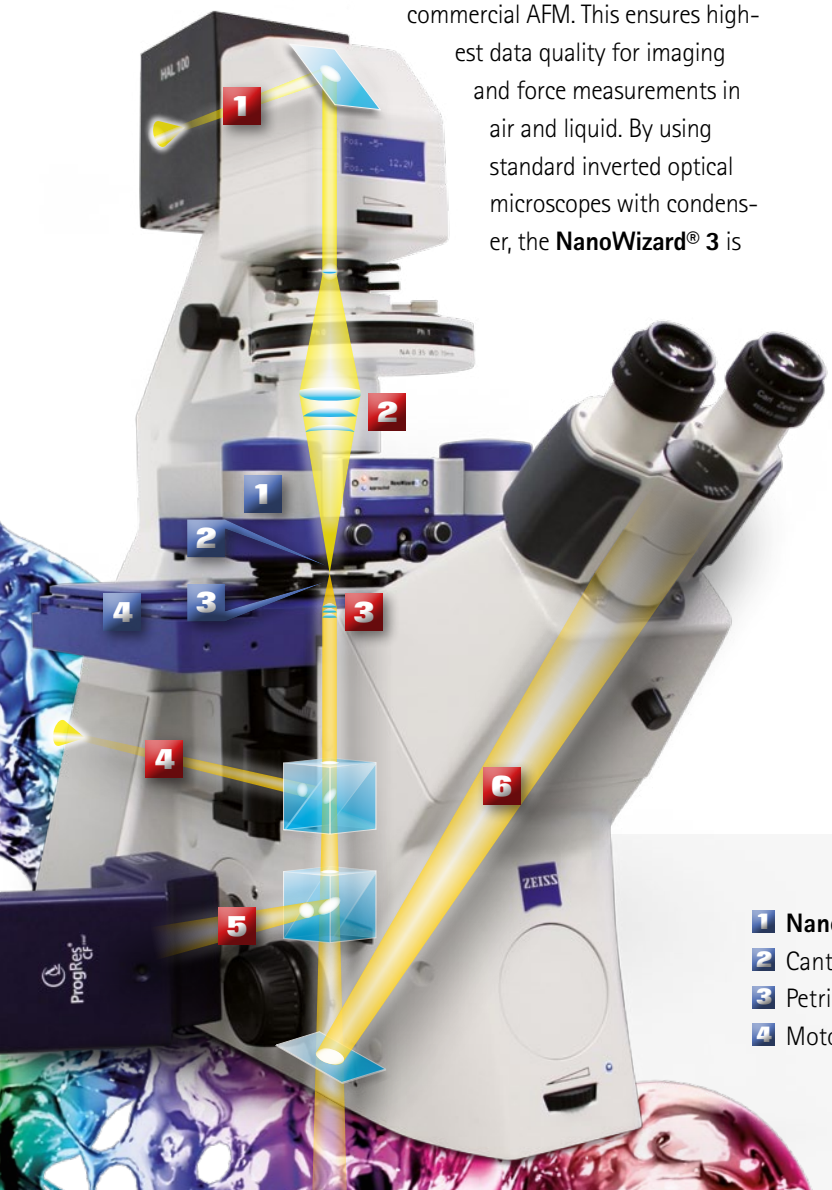
Where highest AFM performance and integrated AFM and optical microscopy come together

The **NanoWizard® 3** head comes with outstanding physical and optical access to the sample from front and side even when head and condenser are in place. The tip-scanning head equipped with a flexure scanner gives highest flexibility for a large variety of different samples; even larger ones without limitations on sample weight and geometry. Only a tip-scanning configuration allows the use of micropipettes or multiple electrical probes in contact with the sample simultaneous to AFM operation.

The **NanoWizard® 3** comes with the lowest possible noise level in the cantilever deflection detection system available on the market today. It is the only atomic-resolution AFM on an inverted microscope. The equally advanced **Vortis**™ SPM controller delivers cutting edge values for noise levels, speed, and highest versatility. Improved closed loop control on all three axes, highest resonance frequency in z and no cross-coupling between x, y and z axis delivers a scanner performance previously not available in a commercial AFM. This ensures highest data quality for imaging and force measurements in air and liquid. By using standard inverted optical microscopes with condenser, the **NanoWizard® 3** is easy to set up and run with full optical imaging capabilities in a matter of minutes, the user being able to choose high-NA immersion objective lenses and a wide range of detectors and cameras to meet their experimental requirements.



There is only one method of the simultaneous operation of an AFM with an optical microscope and its various contrast imaging methods. It is recognized by optical microscopists worldwide and requires a tip-scanning AFM scanning a stationary sample which is placed in the optical focal plane between condenser and objective. The reasons are quite logical and obvious. The optical access design offers stability for imaging and as the beam path is not disturbed, phase contrast and DIC imaging work perfectly. And so is single molecule fluorescence without cross-coupling of signals thanks to a special filter set. For Live Cell applications, the cells are not shaken as in sample scanning AFMs avoiding unwanted cellular response.



1 **NanoWizard® 3** head
2 Cantilever holder
3 Petri dish
4 Motorized stage

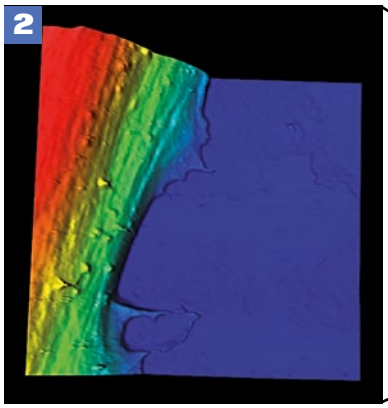
1 Transmission light beam path
2 Condenser lens
3 Objective
4 Fluorescence excitation path (backport)
5 Side port with fluorescence camera
6 Eye piece beam path

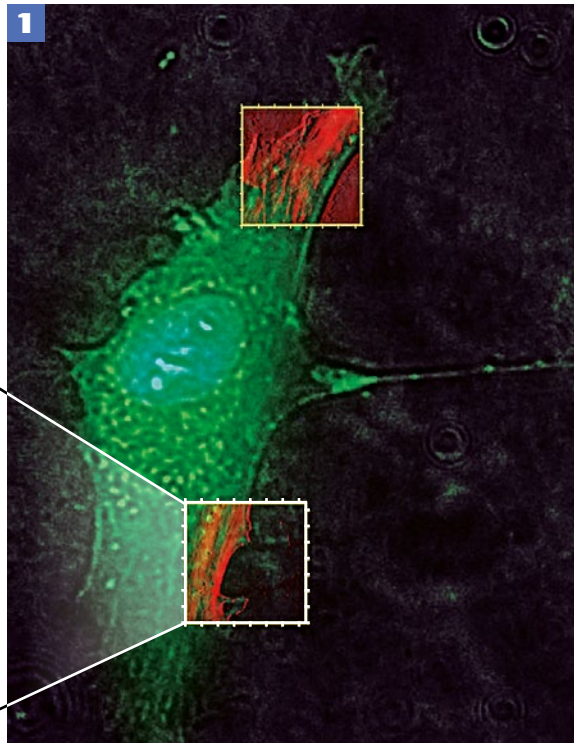
Fully integrated

AFM and optical microscopy by DirectOverlay™

The optical calibration and import feature of **DirectOverlay™** from JPK set a milestone in AFM when introduced in 2006. It has set the standard for the way AFM and optical microscopy should be combined to provide complementary information from the sample.

Additionally, complementary techniques such as epi-fluorescence, confocal laser scanning microscopy, TIRF, FRET, FCS, FLIM, FRAP, STORM, PALM, STED, spinning disc, etc., give insight about the behavior or location of particular features. It is now possible to combine AFM imaging **and** force measurements with these optical methods on the same spot at the same time on a routine basis. The display and understanding of combining different imaging methods becomes routine with the patented **DirectOverlay™** software module. With integrated software control, perfect overlay of optical and AFM data with sub-diffraction limit precision is available at a click of the mouse. Users may make direct "in optical image" selection of AFM measurements (imaging and force curves). This provides a dramatic reduction of overview image scanning in AFM, giving faster results and lower tip contamination. Finally, the optical image navigation to specific regions of interest is available even without AFM scanning. This also protects functionalized tips for stimulation or molecular recognition experiments, avoiding tip passivation from image scanning before the force measurements.





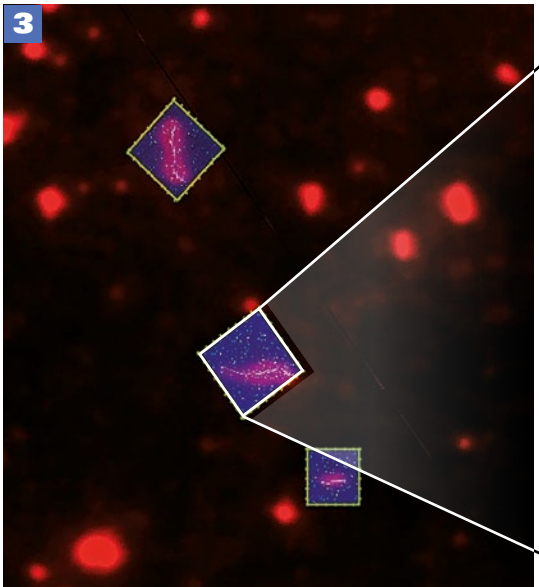
Multiple fluorescent labelling with brightfield together with AFM images (1). Cell nucleus – blue fluorescence, actin – green fluorescence, AFM images – red color scale. The inset image is another view of the lower AFM scan, shown as a 3D plot (2). Scan size 15 µm, height range 3 µm.

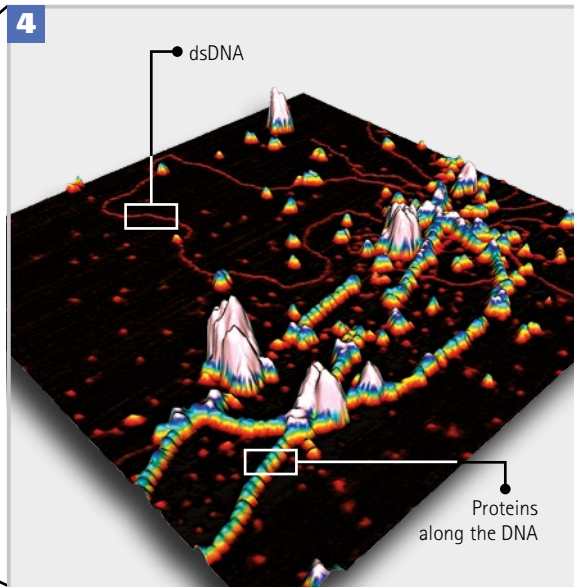
Courtesy of Spuler group,
Muscle Research Unit,
Charité University Medicine Berlin

Complementary optical methods

- Confocal LSM, TIRF, FCS, FRET, FLIM, FRAP, Ca^{2+} imaging, STORM, PALM, STED, spinning disc
- and many more together with highest resolution AFM imaging, all in air or liquids without compromising performance.

AFM and fluorescence images of Alexa555-labeled Rad51 proteins bound to DNA. Rad51 assembles into filaments along double-stranded DNA, which can be seen in the 3-D plot (4) of the 700 nm topography image. In the fluorescence overview image (3), the Rad51 filaments are red fluorescent, with the AFM scan regions superimposed in blue using the JPK DirectOverlay™ software. Each blue AFM scan encloses a single DNA molecule, partially coated with Rad51. The fluorescence image was captured with an Andor™ iXon+ 897 EMCCD camera, fully integrated with the AFM software.
Sample courtesy of Modesti group, CNRS Marseille.





Quantiative imaging & advanced force measurements

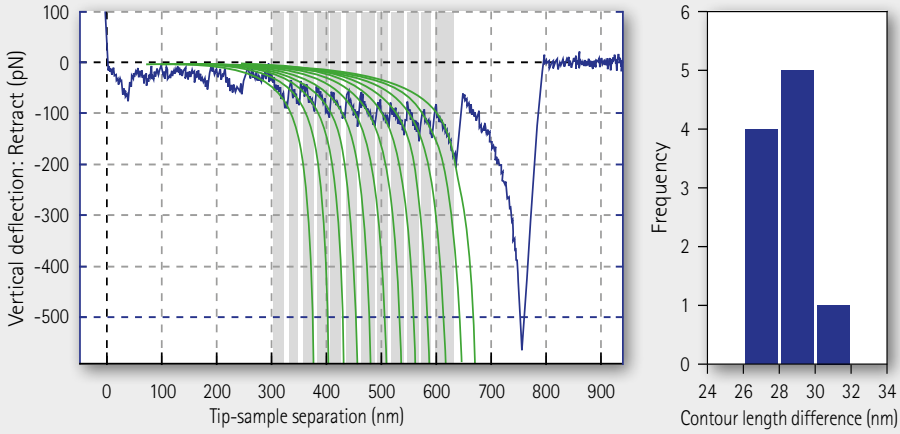
The advanced AFM head and stable, low noise control electronics raise the standard of routine force spectroscopy measurements with **NanoWizard® 3**. With **QI™**, the new force curve based imaging mode, the user has full control over the tip-sample interaction force at every pixel of the image. There is no longer a need for setpoint or gain adjustment while scanning.

The **RampDesigner™** can be used to create custom force curves, and the whole experiment and environment can be controlled through the **ExperimentPlanner™** interface. This allows convenient and customized force mapping and force ramp/clamp experiments. Advanced algorithms are in place for easy, rapid analysis of large data sets. Software design that reduces operator interactions may be important to some users while others prefer full access to instrument parameters. JPK's latest GUI design gives users control to the level they require.

Force measurement solutions from single molecules to living cells

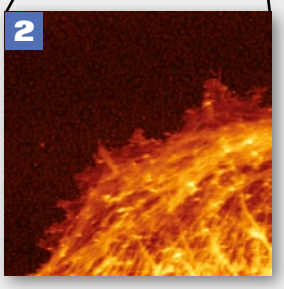
- JPK is the force spectroscopy expert with complete hard- and software solutions to meet individual experimental requirements
- Full data sets of force curves with QI™ Advanced imaging mode
- Advanced algorithms for experimental design & reporting
- Powerful & fast batch-processing with comprehensive fitting routines
- Full range of compatible accessories for environmental control
- Optional 100 µm z range with CellHesion® options

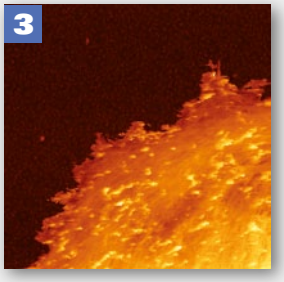
The **ForceWheel™** adds sensitive parameter control while running an experiment. Accessory versatility is important for users to control the experimental environment whether this is for working at different temperatures or being able to easily exchange buffer solutions. **NanoWizard® 3** comes with a broad range of liquid cells, gas flow solutions and sample heaters/coolers for imaging and force measurements.

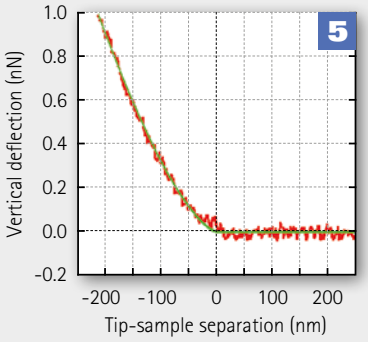


Unfolding curve of native fibronectin, a large modular extracellular matrix protein. The highlighted fit regions show the successive unfolding of 12 FNIII domains, with WLC fits to each event. There are 18 domains in a single fibronectin molecule, each has 90 amino acids, and therefore a contour length of around 30 nm. The histogram shows a clear peak at 30 nm. The JPK processing software allows fully automatic event finding and fitting to unfolding curves with WLC and FJC models, including fitting of multiple events.

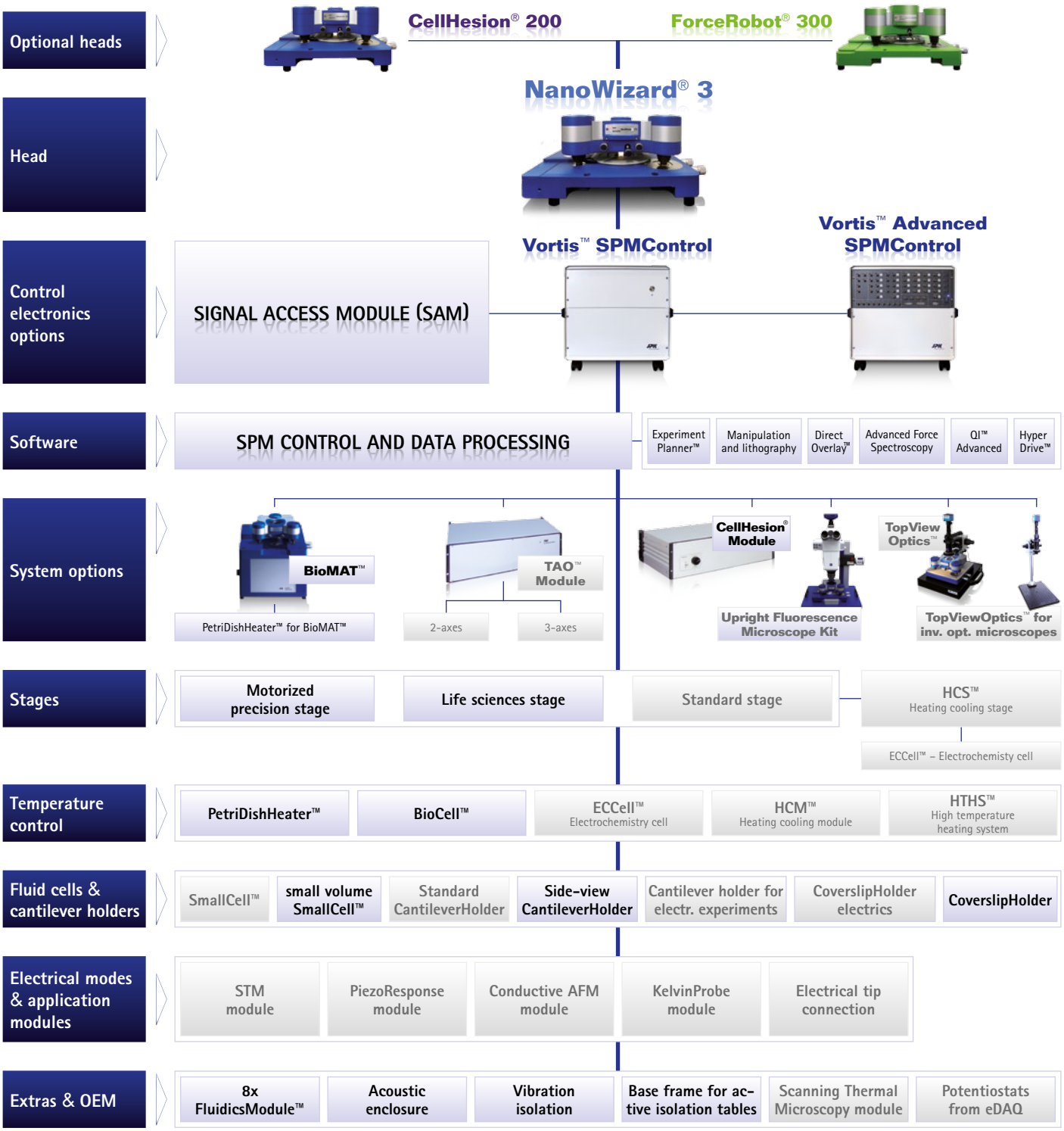
Living fibroblast cell, imaged in QI™ mode: Optical phase contrast image with inset region marked for AFM scan (1), height image at 1 nN (range 500 nm) (2), contact point height at zero force (range 1 µm) (3)) and calculated Young's modulus images (range 150 kPa) (4). Scan size 25 µm. (5) Force extend curve collected during the QI™ mode imaging at the point marked in (4). The Hertz fit curve is shown in green for Young's modulus calculation (22.4 kPa). Fibroblast cells courtesy of R. Schwarzer and Prof. A. Herrmann, Humboldt University at Berlin.







The NanoWizard® 3 AFM family



Setups and accessories for BioScience applications highlighted with LIGHT BLUE BOXES

Typical BioScience applications and research fields:

High resolution imaging in air and liquid of single molecules such as proteins, DNA/RNA, carbohydrates, lipids, Live Cell Imaging, bacteria and virus characterization, cellular adhesion and cytomechanics, molecular interactions, receptor-ligand, antibody-antigen, molecular unfolding, molecular recognition, biomaterials, compound and implant characterization, material properties such as adhesion, elasticity, hardness; drug formulation, encapsulation and aging studies and many more

Setups and accessories for NanoScience applications highlighted with GRAY BOXES

Typical NanoScience applications and research fields:

High resolution imaging in air and liquid of single molecules, thin films, nanoparticles or nanowires, surface properties characterization such as adhesion, stiffness, friction, conductivity, charges, hardness; polymer science, single molecule force measurements, smart organic materials, surface properties of conjugated polymers, biosensors, degradable materials, electrochemistry, colloidal probe, surface forces and interface phenomena; and many more

For more detailed information about all options see the brochures and product notes or visit www.jpk.com.

Specifications for the NanoWizard® 3 BioScience AFM

System specifications
- Atomic lattice resolution on inverted microscope (< 0.035 nm RMS z noise level)
- Ultra-low noise level of cantilever deflection detection system < 2 pm RMS (0.1 Hz - 1 kHz)
- Best closed-loop AFM on the market for reproducible tip positioning and long time position stability
- Tip-scanning stand alone system, the only choice for simultaneous AFM and laser scanning experiments
- IR deflection detection light source with low coherence for interference-free measurements
- NanoWizard® 3 can be operated:
  1) On top of an inverted research microscope for AFM simultaneously with optical microscopy
     - Find a measurement spot optically on your sample by fluorescent labeling
     - Combine AFM with advanced optical techniques such as confocal, FCS, FRET, TIRF, STED, PALM, STORM or others
     - Exact positioning and overlay of optical and AFM data with the JPK DirectOverlay™ software module
  2) Stand-alone based
     - 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™ optional
  3) BioMAT™ option
     - For high-NA optics combined with AFM on opaque samples
  4) Upright Fluorescence Microscopy Kit option
     - For co-localization experiments on opaque samples

NanoWizard® 3 head
- Rigid low-noise design and drift-minimized mechanics
- High detector bandwidth of 8 MHz for high speed signal capture
- Liquid-safe design with integrated vapor barrier, special encapsulated piezo drives and tip-scanning design
- Intelligent and automated approach with user defined parameters for soft landing even with functionalized tips
- Transmission illumination with standard condensers for precise brightfield, DIC and phase contrast
- Built-in optical filters for fluorescence without crosstalk
- Laser safety class 3R
- Scanner unit
  - Flexure stage scanner design with decoupled, low mass z scanner
  - 100 × 100 × 15 µm³ scan range for the head in closed-loop mode
  - Position noise level 0.2 nm RMS in xy (in closed-loop) and 0.06 nm RMS sensor noise level in z (3 kHz bandwidth)

Vortis™ SPMControl electronics
- State-of-the-art digital controller with lowest noise levels and highest number of signal channels
- High speed 16 bit AD conversion with 60 MHz for the photodetector signals
- 24 bit ultra precise ADC with 2.5 MSamples/s
- High speed lock-in amplifier technology for precise amplitude and phase detection
- High speed data capture with optional burst mode
- Modular hybrid analog/digital design with latest FPGA/PPC technology (PowerPC @ 660 MHz / FPGAs @ 240 MHz)
- Gigabit Ethernet interface for fast data link
- Number of data points that can be captured continuously: restricted only by HDD
- Thermal noise acquisition up to 3.25 MHz
- Optional Signal Access Module (SAM) with analog and digital connectors for maximum experimental freedom

SPMControl software
- Fully automated sensitivity and spring constant calibration using thermal noise method
- Patented DirectOverlay™ for combined optical and AFM information
- Outline™ mode for precise selection of a new scan area even in the optical image
- Improved ForceWatch™ mode for force spectroscopy and imaging for cantilever-drift free measurements
- Advanced oscilloscope functionality and online measurement of distances, cross sections etc.
- True multi-user platform
- User-programmable software
- Unlimited pixel resolution for imaging or force curves
- Comprehensive force measurement with TipSaver™
- JPK ExperimentPlanner™ for designing a dedicated measurement workflow
- JPK RampDesigner™ for custom designed force curve segments
- Advanced spectroscopy modes such as various force clamp modes or user-defined ramp design, e.g. for temperature ramps, pulling speed or force feedback
- Enhanced fast force mapping capabilities
- Automated filtering of curves without events
- Powerful Data Processing (DP) functions with full functionality for data export, fitting, filtering, edge detection, 3D rendering, FFT, cross section etc.
- DataProcessing Image-Viewer for picture-in-picture display and export, including calibrated optical images
- Powerful batch processing of force curves including WLC, FJC, step-fitting and other analysis

Stages
- Liquid-safe, robust and drift-minimized design for highest stability
- Motorized precision stage with 20 × 20 mm² travel range with joystick or software control
- Manual precision stage with 20 × 20 mm² travel range
- Independent positioning of tip & sample with respect to the optical axis
- Stages are available for all major inverted optical microscope manufacturers (see below)

Sample holders
- Holders for Petri dishes, coverslips, microscope slides or metal SPM stubs
- Special holders and liquid cells possible
- Ø140 × 18 mm³ free sample volume

Optical configurations
- Fits to inverted microscopes from
  - Zeiss (Axio Observer, AxioVert 200)
  - Olympus (IX line)
  - Nikon (TE 2000, Ti)
  - Leica (DMI line)
- 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
- Upgradeable for scatter-type SNOM, Raman, TERS measurements
- AFM and upright high-NA optics combination with the JPK BioMAT™ workstation (see BioMAT™ brochure)
- Large variety of high-end EM-CCD cameras supported
- TopViewOptics™ video optics for opaque samples

Temperature control options
- RT - 300 °C temperature range with 0.1 °C precision with the JPK High Temperature Heating Stage (HTHS™)
- -35 °C - 120 °C temperature range with 0.1 °C precision with the JPK Heating Cooling Module (HCM™)
- All heaters and heating/cooling solutions are software-controlled

Fluid cell options
- Inert glass standard cantilever holders for experiments in droplets or custom fluid cells
- JPK's patent-pending 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
  - for standard cover slips
- JPK CoverslipHolder offers the same capability as the BioCell™ for ambient temperature experiments
- JPK's temperature controlled electrochemistry cell ECCell™ with transmission illumination
- JPK's PetriDishHeater™ perfect for living cells
  - accommodates 35 mm Petri dishes even with coverslip bottom
  - ambient to 60 °C temperature range
  - perfusion and gas flow possible
- JPK SmallCell™ small volume version for aqueous solutions
  - Closed fluid cell for minimized volumes (< 60 µl)
  - 3 easily accessible sample ports, 2 for buffer exchange and 1 for adding drugs or proteins

Flexibility integrated
- Different sample holders, cantilever holders & stages for every application
- Large choice of add-ons such as temperature controls, 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

Options (see accessories data sheet)
- Sample holders for all kinds of substrates
- 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
- Electrical measurement modes
- Cameras and light sources for video imaging or fluorescence
- Vibration and acoustic isolation from leading suppliers

Standard operating modes

Imaging modes (air or liquid)
- HyperDrive™
- QI™ Quantitative Imaging
- Contact mode with Lateral Force (LFM)
- AC modes with Q-control
- Phase detection

Force spectroscopy mapping (air or liquid)
- Static and dynamic spectroscopy
- Force clamp & ramp
- Force Mapping
- QI™ Advanced imaging mode

Optional modes

- Advanced AC modes with PLL
- Higher harmonics imaging
- KPM and SCM
- MFM and EFM
- Conductive AFM and STM
- Electrical spectroscopy modes
- Piezo-Response Force Microscopy
- Electrochemistry with temperature control and optics
- NanoLithography and NanoManipulation
- NanoIndentation
- Scanning Thermal AFM
- Environmental control
- DirectOverlay™ for combined AFM and optics
- Additional xy or z stages available with CellHesion® or TAO™ modules

Visit the JPK web site for more information and sign up for our eNewsletter. Web address: www.jpk.com

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NanoWizard® 3 AFM on a Zeiss LSM 710 with FCS

JPK Instruments AG

Bouchéstraße 12
12435 Berlin, Germany
tel.: +49 30 5331 12070
fax.: +49 30 5331 22555
www.jpk.com