

ATOMIC FORCE MICROSCOPY

NANOWIZARD® ULTRA SPEED 3

Fast automated imaging at up to 1,400 lines/sec Seamless integration into advanced optical microscopy

NanoWizard ULTRA Speed 3 Next-Level Performance

Bruker's NanoWizard ULTRA Speed 3 BioAFM sets new standards in speed and performance. Advanced automation features and an unprecedented scanning speed of 1,400 lines per second have been combined in one instrument, that can be seamlessly integrated into advanced optical microscopy and super-resolution techniques. The system delivers correlated sample measurements and the comprehensive nanomechanical characterization of an extensive range of soft matter and living biological samples. Its automated fast-scanning and analysis capabilities maximize throughput and deliver outstanding performance. Novel, cutting-edge automation features open new possibilities for long-term, self-regulating experiment series and provide unrivaled sensitivity, reliability, and precision.

Pioneering Innovation

NanoWizard ULTRA Speed 3 features a host of new advanced functionalities:

- Fully automated set-up, alignment, and re-adjustment of system parameters, for easy operation and increased productivity
- Bruker's 3D acceleration sensor and feed-forward technology for unrivalled stability in the tip-scanning motion and highest feedback bandwidth for excellent surface tracking
- **DynAsyst** for automatic adjustment and optimization of scan parameters in TappingMode™
- Internal LED lighting for stand-alone operation
- QR code reader for improved cantilever handling and management. Scans and automatically sets the parameters of Bruker's pre-calibrated cantilevers
- Advanced data management and processing capabilities



Morphology: The new software feature **DynAsyst** and improved **Dynamic PID** enable easy investigation of fragile, unfixed samples at higher speeds and lowest forces.

Dynamics: A scanning speed of 1,400 lines/sec allows the real-time visualization of dynamic processes.

Nanomechanics: Enables fast, label-free, multiparametric characterization of nanoscale biomechanical properties.

Microrheology: An additional, optional fast Z-scanner with an innovative sensor technology generates reproducible force curves at highest speeds, significantly extending the frequency range for microrheological measurements.

NanoWizard ULTRA Speed 3 sets benchmark standards by delivering fast AFM scanning capabilities that can be combined with advanced optical techniques. Its tip-scanner technology achieves scanning speeds previously unattainable with conventional AFMs.

Image top

NanoWizard ULTRA Speed 3 integrated into a Zeiss Axio Observer running the new Bruker SPM software version 8.1

Image bottom

TappingMode topography images of DNA origami (GATTA-AFM, Gattaquant, Germany) in TAE buffer acquired at 1,400 lines/sec. (inset)

Overview

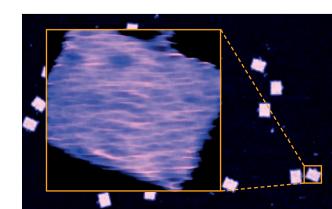
Scan size: $2.00 \,\mu\text{m} \times 1.25 \,\mu\text{m}$ Height range: $4.44 \,\text{nm}$

Inset

Scan size: 100 nm × 100 nm Height range: 2.25 nm

The new, automated NanoWizard ULTRA Speed 3 combines high-resolution and high-speed capabilities with excellent ease-of-use. It is ideal for the reliable investigation of single molecule phenomena.

Stefania A. Mari, Daniel J. Müller Lab. ETHZ, Basel, Switzerland



Empowered Research

Nanoscale structural analysis and nanomechanical characterization deliver crucial insights into functionality at the molecular, single-cell, and cellular levels.

NanoWizard ULTRA Speed 3 combines speed, automation, and precision with ease of use. Achieving high-caliber results is easier than ever before and paves the way for a host of new applications and groundbreaking scientific discoveries.

Providing unique advantages

- Stable scanning at speeds of up to 1,400 lines per second
- Seamless integration with advanced optical and super-resolution techniques
- Patented scanner technology
- Latest dynamic PID implementation
- Unparalleled multi-channel data acquisition rates and rapid analysis of complex, multiparametric data sets

Enhancing capabilities

The visualization of dynamic molecular and cellular mechanisms and quantification of the associated kinetics and reaction rates advance our understanding of fundamental biological processes. In conjunction with advanced optics, ULTRA Speed 3 enables multiparametric observation of in-situ dynamics.

- Single-molecule protein dynamics
- Protein folding
- Receptor-ligand interactions
- Mechanosensitive signaling pathways
- Enzymatic reactions
- Kinetics of surface parameters on nanometer scale

Top row images Image left

Atomic resolution of a calcite crystal plane recorded with TappingMode AFM in water at 60 Hz line rate. 2D FFT analysis (inset) gives rectangular lattice subunit dimensions of a = 0.526 nm and b = 0.847 nm.

Scan size: 19.5 nm × 19.5 nm Height range: 120 pm

Image center

TappingMode in liquid of puC19 DNA using Bruker probe FASTSCAN-D showing majorminor groove resolution (inset)

Scan size: 87.5 nm × 87.5 nm Height range: 3.5 nm

Image right

Gasdermin pore-forming proteins imaged in lipid membrane

<u>Overview</u>

Scan size: 240 nm × 240 nm Height range: 2.9 nm

Inset

Scan size: $36 \text{ nm} \times 36 \text{ nm}$ Height range: 3.5 nm

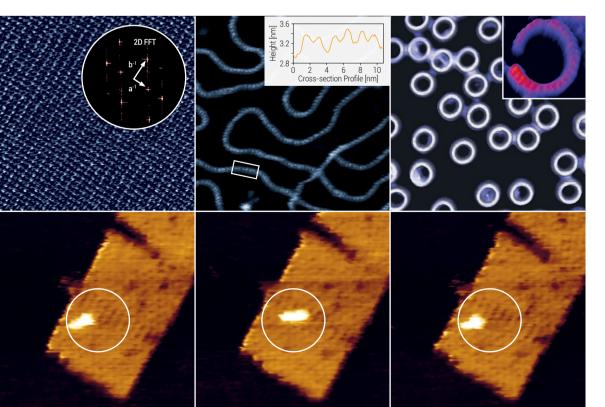
Sample courtesy Han Yu, Daniel J. Müller Lab., ETHZ, Basel, Switzerland

Bottom row images

Guided thermally driven protein kinetics on DNA nanostructures imaged in TappingMode at 1 frame/sec.

Scan size: 150 nm × 150 nm Height range: 6 nm

In collaboration with C.M. Domínguez, C.M. Niemeyer, Institute for Biological Interfaces (IBG-1), KIT, Germany



Next-Generation Automation

Enabling fast results and enhanced productivity

The culmination of Bruker's pioneering work in the field of BioAFM, NanoWizard ULTRA Speed 3 incorporates cutting-edge innovations and a host of new advanced features. Its high degree of automation, speed, and functionality deliver best-in-class capabilities, significantly increasing the number of samples and positions that can be probed, maximizing throughput and enhancing productivity.

Delivering unprecedented ease of use

ULTRA Speed 3, with an intuitive user interface, customized and favorite workflows, and on-screen context help, is the ideal choice for multi-user environments and imaging facilities.

Automated procedures and the choice between standard and advanced features enable experts and less experienced users alike to repeatably produce highest-quality data.

The advanced Data Processing software allows users to browse easily through thousands of images at specific locations, channels, and individual frames in their time-series data, all of which are efficiently saved in HDF5 container format.

Various output formats can be exported automatically and simultaneously, such as individual processed data files, image videos or numerical data from cross-sections or histograms.

Cover image

DNA origami 2D lattice formation in buffer with 50 mM NaCl imaged in TappingMode at 0.5 frame/sec.
Scan size: 1 µm × 1 µm
Height range: 3 nm

Page background image

2D DNA origami lattice imaged in buffer with 50 mM NaCl with 2048 × 2048 px and 4 µm × 4 µm scan size at 20 Hz line rate.

Images below

2D DNA nanostructure lattice formation in buffer with 75 mM NaCl and 10 mM CaCl₂ imaged in TappingMode at 1 frame/sec. Scan size: 1 µm × 1 µm Height range: 2.8 nm

Sample courtesy of Dr. Adrian Keller, Paderborn University, Germany

Taking AFM automation to the next level

Automated set-up and alignment

Single-click automated cantilever calibration

Automated measurement procedures and workflow

Single-click automated optical image calibration

Automated scan parameter adjustment with DynAsyst

Automated, high pixel density mapping and imaging

Intelligent adaptive scanning routines

Automated Force Spectroscopy

Fast, automated scanning of challenging, highly corrugated samples on an inverted microscope

Featuring advanced capabilities

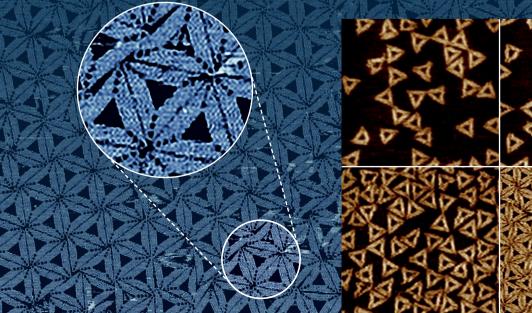
DynAsyst: Automated parameter adjustment in TappingMode for minimal force, utmost consistency, and unattended operation

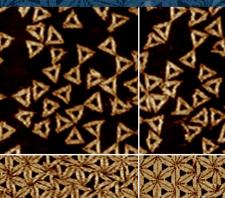
PeakForce Tapping® with ScanAsyst®: The gold standard for fast and easy high-resolution imaging

PeakForce-QI™: The symbiosis of PeakForce Tapping and QI modes delivers speed and advanced force control for highly delicate samples

ExperimentPlanner: Predefinition of settings and parameters allows complex experiments to run automatically

ExperimentControl: Remote monitoring of long-term lab experiments via a browser on any device





Quantitative Nanomechanics

Enhancing mechanobiology analysis

NanoWizard ULTRA Speed 3 comprises a comprehensive range of tools for the investigation of the nanomechanical properties of samples ranging from single-molecules to single-cells and beyond. An extensive range of modes enable the study of structure, mechanobiology, and dynamics on soft and challenging samples. Perform high-resolution imaging and quantitative nanomechanical characterization of properties, such as Young's modulus, adhesion, dissipation, and deformation, with optional modes, such as Peak-Force-QI, PeakForce QNM®, or QI Advanced.

- Multiparametric characterization in combination with optical microscopy
- Trigger and observe interaction and adhesion processes
- Determine viscoelastic properties
- Contact point imaging (CPI)
- True, real-time force curve monitoring
- Time-dependent, force-induced nanomanipulation
- Easy and precise batch processing of images and force curves

Redefining flexibility

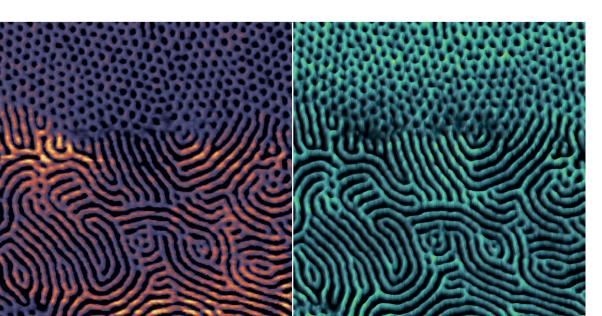
The novel SmartMapping feature allows the flexible selection of multiple, user-defined 2D force maps. Numerous regions of interest (ROI) can be selected in advance and examined automatically, enabling the systematic study of large sample areas. The optimal range of force acquisition is continuously evaluated and automatically adjusted by XY and Z-motors in combination with the piezo scanners, delivering a degree of precision and flexibility second to none.

Extending technology

The new NestedScanner microrheology feature extends the operation of multiple Z-piezos, allowing fast analysis of viscoelastic properties. By optimizing use of the Z-piezos, high-speed, high-resolution imaging of steep surface structures with heights of up to 8 μ m, e.g., living cells, bacteria, and tight junctions, is now simple.

The ease of integrating the ULTRA Speed 3 AFM with fluorescence microscopy and co-localizing measurements, even on living cells, is unique. Correlating fast multiparametric, nanomechanical data sets with advanced optical images allows cross-validation and delivers novel, reliable results.

Assoc. Prof. Clemens Franz WPI NanoLSI, Kanazawa University, Japan



Images

PeakForce-QNM image of a thin film of styrene-ethylenebutylene triblock copolymer (Kraton G1652) prepared on a silicon wafer.

The topography is shown on the left and the corresponding Young's modulus is shown on the right.

Scan size: $1 \mu m \times 1 \mu m$ Height range: 15 nmModulus range: 400 MPa

Integration into Advanced Optical Techniques

Setting new standards in correlative microscopy

NanoWizard ULTRA Speed 3 can be seamlessly integrated into advanced optical techniques.

Optical guidance allows the fast and easy combination of measurements, while optimizing output and efficiency.

Enabling groundbreaking discoveries

The ability to trigger, control, and observe molecular reactions and cell-cell or cell-surface interactions in real time can significantly improve our understanding of fundamental biological mechanisms and chemical and physical processes.

- One-click optical image import
- Advanced calibration algorithms and visualization routines for precise correlation of AFM and optical data
- Easy navigation around the sample and selection of ROIs in optical image for combined AFM experiments
- SmartMapping for selection of multiple, free-hand drawn ROIs for AFM operation
- Automated large-area, multi-region imaging (by combining DirectTiling, DirectOverlay 2, and MultiScan software features) extends the optical viewing field for atomic force microscopy

Transmission illumination (brightfield, phase, DIC) using standard condensers and reflection microscopy

Confocal microscopy and spinning disc

Ca²⁺ imaging

Macroscope combination

980 nm optical beam deflection (OBD) option

Optical super-resolution (STED, PALM/STORM, FLIM)

FRET, FCS, FRAP, TIRF, IRM, SIM

Upright Optics for tissues, implants etc.

Optical tweezers with OT-AFM

Images

Optics

Confocal laser scanning fluorescence image of live E.Coli bacteria labelled with Hoechst 33342 (blue - live) and Propidium lodide (red – dead) acquired on coverslip in buffer

AFM

NestedScanner TappingMode AFM topography images acquired at 5 Hz line rate (zoom) and 1 Hz line rate (overview)

<u>Overview</u>

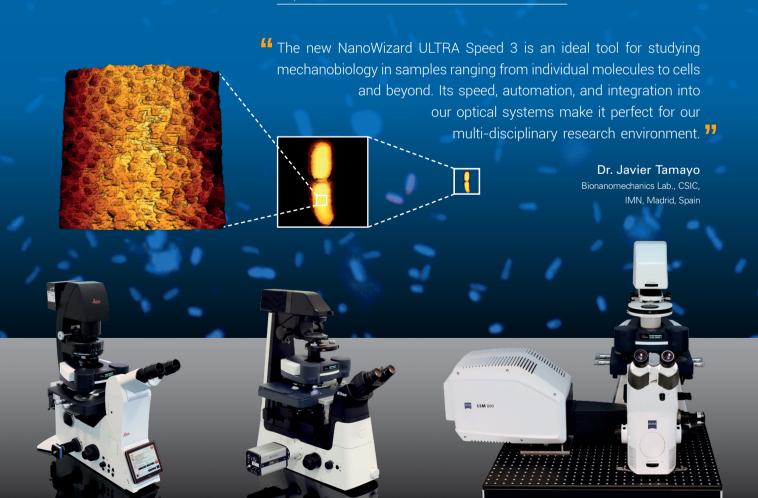
Scan size: 4 µm × 4 µm Height range: 1.4 µm

Zoom

Scan size: 200 nm × 200 nm Height range: 15 nm

Setup images

NanoWizard ULTRA Speed 3 integrated into Leica DMi8 (left), Nikon Ti2 (center), Zeiss LSM 800 (right)



Ultimate Versatility with Widest Range of Accessories and Modes

Providing flexibility and modularity

NanoWizard ULTRA Speed 3 supports the most extensive range of accessories and modes on the market today, greatly extending the range of applications and experiments possible. Accessories, such as the temperature and environment control options, e.g., gas/fluid exchange modules for perfusion and electrochemistry applications, enable the controlled, systematic study of complex phenomena.

The properties of Bruker pre-calibrated cantilevers, manufactured at our fabrication facility, can be integrated into the SPM environment by a single click with the new QR-code reader option, significantly simplifying the initial steps of the experiment.

Delivering maximum control

The new Vortis 2.2 controller delivers highspeed, low-noise DACs, and precise position sensor readout technology. Extended channel capabilities support the new proprietary 3D acceleration sensor technology for advanced feed-forward operation, high-performance data processing, and computing algorithms.

The latest FPGA technology and dual core PowerPC ensure benchmark data processing rates. The large number of feedback modes and a powerful HV amplifier module facilitate high-speed applications. Passive cooling lowers acoustic noise during the measurement.

Superior Versatility

Conductive AFM, EFM, and KPM modules (also with enclosed volumes)

New Ringing Mode option

STM module

Piezoresponse Force Microscopy (PFM) module

ECCell Electrochemistry cell

Scanning electrochemistry (SECM) package

Glovebox solutions on request

Extensive user management

Comprehensive Environment Control

Temperature control options:

- · Ambient to +300 °C with High Temperature Heating Stage (HTHS)
- · -35 °C to +120 °C with Heating Cooling Module (HCM)
- · -120 °C to +220 °C with CryoStage

Fluid cell options:

- · BioCell
- · PetriDishHeater (PDH) and PetriDishHolder
- · Humidifier for PDH
- · CoverslipHolder
- · SmallCell for harsh environments

Enhanced Usability

HybridStage and motorized stages for automated, large scale sample analysis

New head elevation kit for samples of additional 8 mm in height

Head-up stage for samples of up to 121 mm in height

StretchingStage for following sample property changes under mechanical load

TopViewOptics for opaque samples

Accessories Images below PetriDishHeater (left)

for temperature controlled, living cell experiments with AFM and advanced optics.

Heating Cooling Stage (center) designed for AFM experiments in air or liquids, from 0 °C up to 100 °C, with minimized drift in all dimensions.

QR code reader (right) reads properties of Bruker pre-calibrated probes into the SPM software simplifying the AFM experiment.

See Bruker BioAFM
"Accessories Handbook"
for a complete list of options.



	NanoWizard Ultra Speed 3 AFI
System specifications	Tip-scanning, stand-alone system, with a rigid low-noise design and drift-minimized mechanics Latest Vortis 2.2 SPM Controller generation True atomic resolution on inverted microscope in closed-loop
Software V8.1	Fully automated cantilever and detector alignment routines User-specific experiment design for unattended/remote long-term measurements True multi-user platform, perfect for imaging facilities Fully automated sensitivity and spring constant calibration using thermal noise or Sader method
Stages and sample holders	Stages are available for all major inverted optical microscope manufacturers, such as Zeiss, Nikon, Evident/Olympus and Leica Motorized Precision Stage with 20×20 mm² travel range, with joystick and/or software control Manual Precision Stage with 20×20 mm² travel range
Widest range of accessories and probes (see Accessories Handbook)	Large choice of temperature controls for ambient, liquid and gas, and liquid cells even for aggressive solvents
Optical configurations	Fully simultaneous AFM operation with commercially available transmission optical modes, such as, brightfield, optical phase contrast, and DIC using standard condensers Combine AFM with advanced commercial confocal microscopes and fluorescence optical techniques, such as FCS, FRET, TIRF, FLIM, FRAP, STED, STORM/PALM, SIM, and more
Standard operating modes	TappingMode, PeakForce Tapping and QI FastImaging with up to 1,400 lines/sec and Hi-Res mode with feed-forward technology NestedScanner capabilities for several modes Fast PeakForce Tapping Contact mode with lateral force microscopy (LFM)
Optional modes	PeakForce-QI and PeakForce QNM Advanced spectroscopy modes incl. various force clamp modes or ramp designs QI Advanced mode for quantitative data, perfect for soft samples SmartMapping ScanAsyst automated gain and setpoint adjustment in PeakForce Tapping, PeakForce QNM and PeakForce-QI Advanced AC modes such as FM and PM with Q-control & Amplitude Gain Control Microrheology in CellMech Package Kelvin Probe Microscopy MFM and EFM Conductive AFM Electrochemistry & Scanning Electrochemistry with temperature control and optical microscopy NanoLithography and NanoManipulation Scanning Thermal AFM



FM Specifications • Liquid-safe AFM with integrated vapor barrier, specialized encapsulated piezo drive and tip-moving design

• $30 \times 30 \times 6.5 \,\mu\text{m}^3$ scan range and $1.5 \,\mu\text{m}$ extra z range

- DirectOverlay 2 option for combined optical and AFM information
- Powerful Data Processing (DP) with full functionality for data export, fitting, filtering, edge detection, 3D rendering, FFT,
- ross section, video creation etc.

 Powerful batch processing of force curves and images including WLC,
 FJC, step-fitting, JKR, DMT model, and other analysis methods
- Holders for petri dishes, coverslips, microscope slides or metal
- Large Ø 140 x 18 mm³ free sample volume, up to 121 mm in z with Head-Up stage
- Complete range of probes for all operating modes Vibration and acoustic isolation from leading suppliers
- Upright Fluorescence kit enables the combined use of AFM and upright optical fluorescence microscopes
 TopViewOptics for opaque samples with 12× zoom
 BioMAT option for high-NA upright fluorescence optics combined with AFM on opaque samples
- Large range of cameras supported
- TappingMode with DynAsyst and PhaseImaging™
- ExperimentPlanner for designing a specific measurement workflow

 • Static and dynamic force spectroscopy
- Force Mapping
- ExperimentControl feature for remote experiment monitoring
- FluidFM® solution from Cytosurge®
- Additional XY and/or 7 sample movement stages available with CellHesion, TAO and HybridStage modules
 • STM
- Electrical spectroscopy modes
 Piezoresponse Microscopy (PFM)

Selected Publications using NanoWizard ULTRA Speed technology:

- 1. Pothineni, B. K.; Grundmeier, G.; Keller, A. Cation-Dependent Assembly of Hexagonal DNA Origami Lattices on SiO2 Surfaces. Nanoscale 2023, 15 (31), 12894-12906.
- 2. Webby, M. N.; Oluwole, A. O.; Pedebos, C.; Inns, P. G.; Olerinyova, A.; Prakaash, D.; Housden, N. G.; Benn, G.; Sun, D.; Hoogenboom, B. W.; Kukura, P.; Mohammed, S.; Robinson, C. V.; Khalid, S.; Kleanthous, C. Lipids Mediate Supramolecular Outer Membrane Protein Assembly in Bacteria. Sci. Adv. 2022, 8 (44), eadc9566.
- Berger, R. M. L.; Weck, J. M.; Kempe, S. M.; Hill, O.; Liedl, T.; Rädler, J. O.; Monzel, C.; Heuer-Jungemann, A. Nanoscale FasL Organization on DNA Origami to Decipher Apoptosis Signal Activation in Cells. Small 2021, 17 (26), 2101678.
- Zhao, L.-S.; Huokko, T.; Wilson, S.; Simpson, D. M.; Wang, Q.; Ruban, A. V.; Mullineaux, C. W.; Zhang, Y.-Z.; Liu, L.-N. Structural Variability, Coordination and Adaptation of a Native Photosynthetic Machinery. Nat. Plants 2020, 6 (7), 869-882.

Visit www.bruker.com/BioAFM/nanowizard-ultra-speed for a comprehensive list of key publications.

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