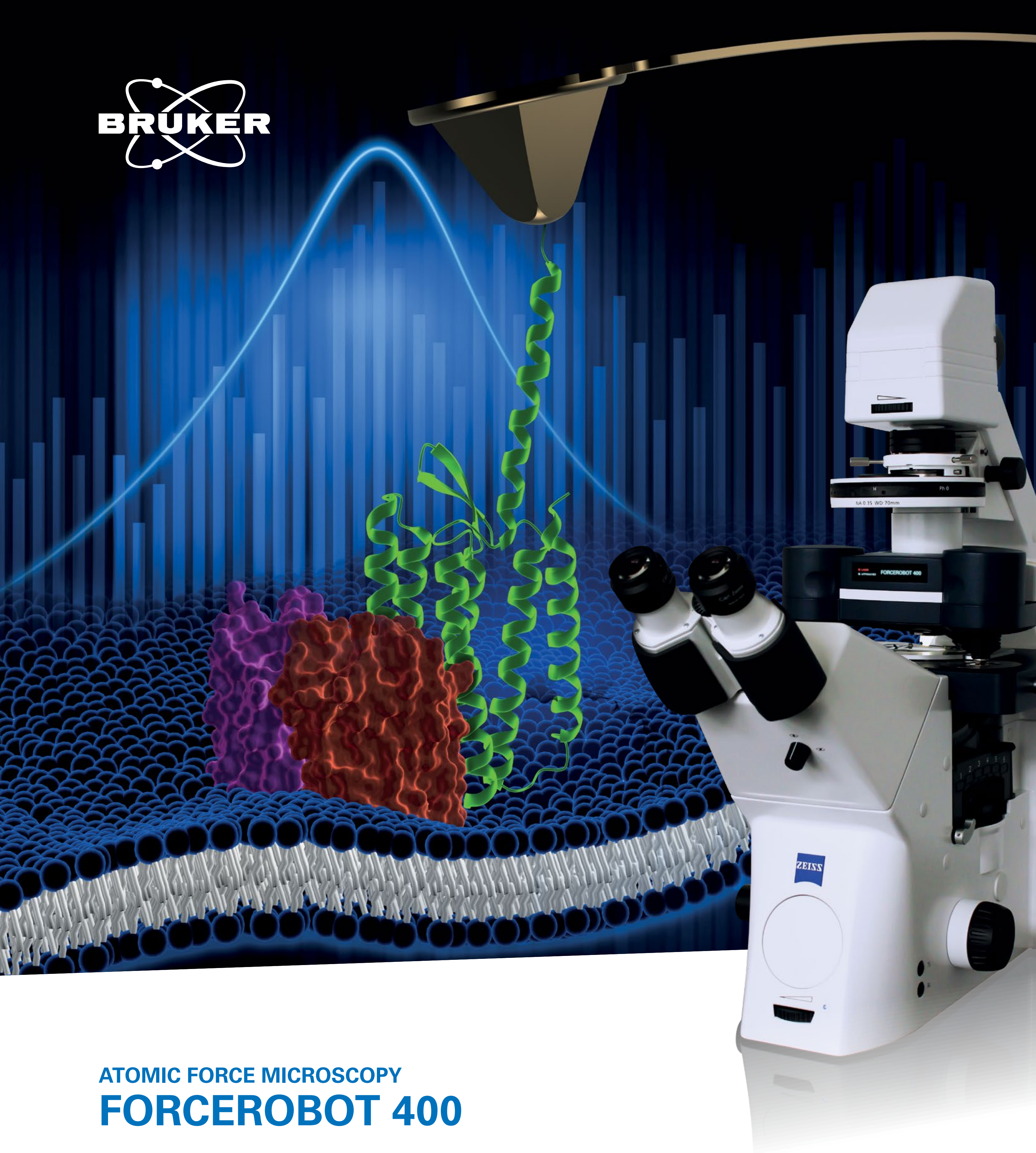


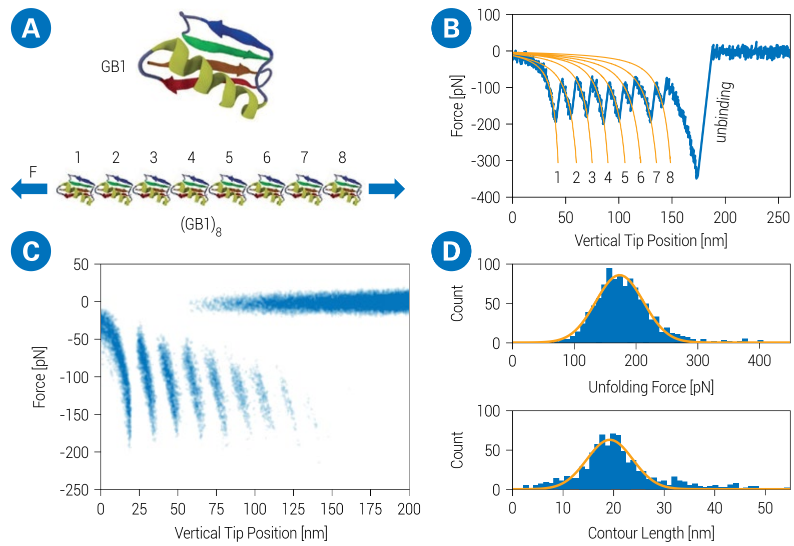
ATOMIC FORCE MICROSCOPY

FORCEROBOT 400

High-Throughput, Quantitative Single-Molecule Biomechanics

ForceRobot 400 BioAFM – Understanding the Mechanics of Single Molecules

Forces play a crucial role in molecular mechanisms, such as recognition, response, and signaling. Bruker's new ForceRobot® 400 BioAFM incorporates a number of unique force spectroscopy innovations to measure forces at the single-molecule level. It enables the quantification of the mechanical strength of individual molecular bonds and characterization of the force-dependent properties of molecular interactions and biomolecular complexes.



SMFS measurements on $(GB1)_8$ polyprotein with statistical analysis of force curves.

A: 3D representation of a single GB1 molecule (guanine nucleotide-binding protein) and corresponding polyprotein consisting of 8 tandem repeats of GB1. (Structure 2J52 from www.rcsb.org was used to display GB1.)

B: Exemplary force distance (FD) curve showing the complete unfolding of all 8 subunits of $(GB1)_8$. The contour length of each unfolded GB1 subunit was determined by fitting the FD curves with a worm-like chain model (orange).

C: Density plot of superimposed FD curves indicating the probability of unfolding up to 8 subunits.

D: Distribution of contour length and unfolding force values for GB1 subunits. Mean contour length determined is 19.2 nm, with an average unfolding force applied of 173 pN.

Sample courtesy of Prof. Yi Cao, Dept. of Physics, Nanjing University, China.

ForceRobot 400 is uniquely capable of providing crucial insights into functional biological mechanisms, opening new possibilities in such research fields as biophysics, biochemistry, developmental biology, and the development of novel therapeutic molecules.

Only ForceRobot 400 Delivers:

- 250,000 force curves per day
- Fully automated single-molecule force spectroscopy (SMFS) capabilities
- Statistically relevant, standardized datasets required in biomedical and preclinical research
- Advanced force curve designs and extensive fitting routines for flexible experiments

Use ForceRobot 400 to study:

- Protein function at the molecular level
- Molecular biomechanics and receptor-ligand type bonds
- Detection and kinetics of novel antibody-antigen complex formations for immunodiagnostics
- Viscoelastic properties of various materials
- Maximum adhesion force, mechanisms of individual binding and unbinding events
- Intramolecular properties of individual molecules and molecular arrangements
- Structural changes in macromolecules

"The increased level of automation, in conjunction with advanced optical techniques, makes the new ForceRobot 400 an outstanding tool for the study of biophysical phenomena at the single-molecule scale. Such force measurement capabilities enable the study of the mechanical properties of natural and synthetic polymers at the single molecular level, advancing the design and synthesis of novel macro- and nanomaterials."

Professor Yi Cao, Department of Physics, Nanjing University, China

Innovative Multiparametric Nanomechanical Mapping

ForceRobot 400 enables the label-free, nanomechanical measurement of individual molecules under near-physiological conditions. Correlative, multiparametric datasets of unprecedented quality can be collected and a comprehensive range of biophysical parameters derived automatically from each dataset.

Next-Generation Force Measurement Capabilities

Advanced Automated Force Spectroscopy

ForceRobot 400 is the ideal tool for SMFS, providing crucial insights into biophysical mechanisms at the molecular level.

Large-scaled z-motors and an enhanced motorized stage functionality enable the investigation of samples ranging in size from single molecules to larger biological complexes and regions of interest (ROI) that are far apart or in different sample compartments.

The latest optional **NestedScanner** feature has a fast z-scanner with a capacitive sensor. It generates reproducible force curves even at high speeds, significantly extending the frequency range for microrheological measurements.

The new **SmartMapping** feature allows the flexible selection of multiple, user-defined 2D force maps. Using optical tiling, multiple ROIs can be selected in advance and examined automatically, facilitating the systematic study of large sample areas.

Setting New Standards in SMFS Automation

ForceRobot 400 delivers advanced automation and analysis capabilities at unprecedented data acquisition rates. Automated alignment and calibration features, in combination with proprietary software tools, ensure autonomous operation and rapid results.

Ultimate Flexibility

The new ForceRobot 400 user interface, with intuitive user guidance, enables the fast and simple definition of operating parameters. Pre-defined experiment setups, such as force-clamp, force-ramp, or microrheology, along with easy-to-use scripting tools allow user-defined experiment designs. Long-term, self-regulating experiment series can be run thanks to the automated adjustment of system parameters.

Groundbreaking Capabilities:

- Over 10,000 force curves per hour
- Automated alignment of detection system and adaptation to environmental conditions
- **New** automated self-adjusting mapping mode for probing rough surfaces
- Characterization of viscoelastic properties with a modulation frequency of at least 5 kHz (requires optional z-scanner)
- Extensive range of environmental control accessories (humidity, temperature, ionic strength, buffer exchange, etc.)

Seamless Integration with Optical Microscopes

ForceRobot 400 can be seamlessly integrated with advanced optics and super-resolution techniques to deliver correlated nanomechanical data sets and the comprehensive characterization of an extensive range of biological samples.

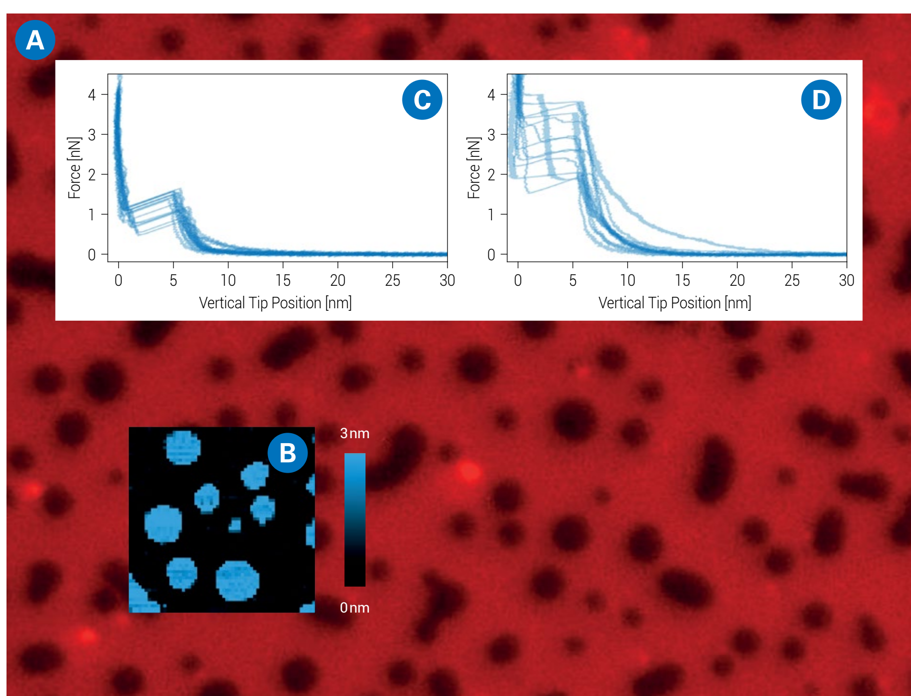
Correlative fluorescence microscopy and force mapping on phase separated DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) supported lipid bilayers containing cholesterol, sphingomyelin, and rhodamine-DOPE (1,2-dioleoyl-sn-glycerol-phospho-ethanolamine).

A: Fluorescence image of rhodamine-DOPE containing liquid disordered phase (red) and sphingomyelin-rich liquid ordered phase (black regions).

B: Height map showing elevated lipid rafts (liquid ordered phase) in liquid disordered phase. (Map size 20 × 20 µm² with 60 × 60 pixels.)

C and D: Force-indentation curves acquired on liquid disordered phase (C) and liquid ordered phase (D) showing breakthrough events at different forces.

Sample courtesy of Prof. Dr. Salvatore Chiantia, BB, Cell Membrane Biophysics Group, University of Potsdam, Germany.



BRUKER

ForceRobot 400 Specifications

System Specifications	• Automatic optical beam deflection system adjustment • Vortis 2.1 SPM Controller platform • Vertical travel range >19 mm; heights of z-piezo and head motors are compiled in a combined height channel • 15 µm capacitive z-scanner (SLD 880 nm) with closed-loop control	• Optional 15 µm capacitive z-scanner (SLD 980 nm) with closed-loop control • Optional additional 1.5 µm z-Piezo • Automated range and piezo adaption option in z-direction for highly corrugated surfaces
Software	• Version V8 • Automated cantilever and detector alignment • User-specific experiment design for complex measurement tasks • Data Processing (DP): data export, fitting, filtering, edge detection, 3D rendering, FFT, cross-section, video creation, etc.	• Batch processing of force curves and images, WLC, FJC, step-fitting, JKR, DMT model, etc. • Optional SmartMapping feature: flexible ROI selection, automatic z-position and z-motor adjustment
Stages	• Inverted optical microscopes: Zeiss, Nikon, Olympus, Leica • Motorized precision stage: 20 mm × 20 mm travel range	• Precision mapping stage: 100 µm × 100 µm piezo range
Sample Holders	• Petri dishes, coverslips, microscope slides, metal SPM discs	
Accessories (see accessories handbook)	• BioCell and CoverslipHolder for advanced optics with molecules (temperature control, perfusion, and gas flow (CO_2)) • PetriDishHeater and PetriDishHolder for handling of living cells in near-physiological conditions • Active humidity control for PetriDishHeater • Compatible with standard commercial incubators • SmallCell for small fluid quantities	• Stretching stage • SideView Cantilever Holder option • FluidFM ADD-ON from Cytosurge • Biocompatible probes • Easy cleaning and sterilization of all components that come in contact with samples
Optical Configurations	• Upright macroscopes and stereo microscopes: Zeiss Axio Zoom V.16, Leica Z16 APO A, Leica M205 FA, Olympus MVX10, etc. • TopViewOptics, video optics for opaque samples with 12× zoom	• Simultaneous operation with brightfield, optical phase contrast and DIC using standard condensers • Compatible with light microscopy techniques: DIC, phase contrast, fluorescence, and super-resolution techniques TIRF, FRAP, CLSM, STED, STORM, etc.
Standard Operating Modes	• Contact Mode Force spectroscopy • Force Mapping	• Advanced Force Mapping • Advanced spectroscopy: force clamp modes, ramp designs
Optional Modes	• Microrheology in CellMech Package • DirectOverlay	• DirectTiling • ExperimentPlanner for designing specific measurement workflow

Selected Overview of Peer-Reviewed Scientific Publications Using ForceRobot Technology:

1. Dupuy et al., Molecular Device for the Redox Quality Control of GroEL/ES Substrates. Cell 186 (5), 1039-1049.e17. (2023).
2. Li et al., Active Microrheology of Protein Condensates Using Colloidal Probe-AFM. Journal of Colloid and Interface Science, 632, 357-366 (2023).
3. Serdiuk et al., A Cholesterol Analog Stabilizes the Human β_2-Adrenergic Receptor Nonlinearly with Temperature. Sci. Signal. 15 (737), eabi7031 (2022).
4. Petitjean et al., Multivalent 9-O-Acetylated-Sialic Acid Glycoclusters as Potent Inhibitors for SARS-CoV-2 Infection. Nat Commun 13 (1), 2564 (2022).
5. Liu et al, High Force Catch Bond Mechanism of Bacterial Adhesion in the Human Gut. Nat Commun 11 (1), 4321 (2020).
6. Huang et al., Maleimide-Thiol Adducts Stabilized through Stretching. Nat. Chem. 11 (4), 310-319 (2019).

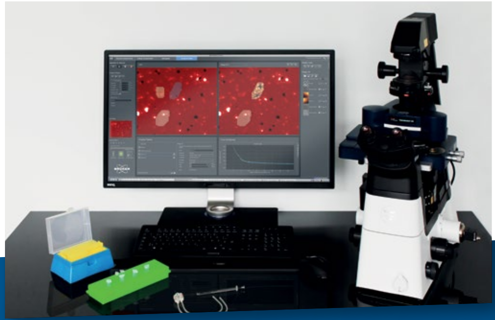
Visit www.bruker.com/bioafm/forcerobot for a comprehensive list of key publications.

JOURNAL CLUB
Join up here!

VIRTUAL SHOWROOM
Visit here!

LATEST WEBINAR
Watch on-demand

ForceRobot 400 on Nikon Eclipse Ti2 inverted microscope and intuitive software interface



JPK BioAFM Business
Nano Surfaces and Metrology Division
Bruker Nano GmbH

Am Studio 2D · 12489 Berlin, Germany
tel.: +49 30 670990 7500 · fax: +49 30 670990 30

www.bruker.com/bioafm

BioAFM Products

Contact us!