FORCE-SENSING OPTICAL TWEEZERS

NanoTracker 2

3D force measurements in combination with advanced optical microscopy techniques

NanoTracker 2

Manipulation and Force Sensing on the Nanoscale

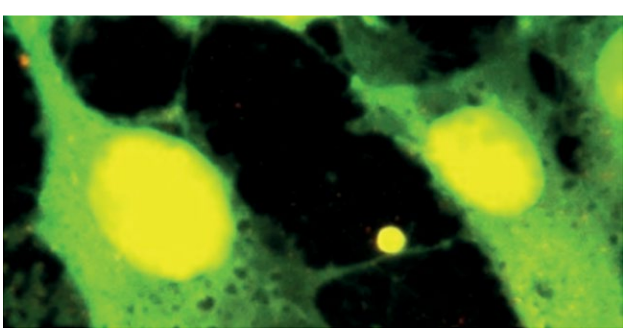
The NanoTracker 2 Optical Tweezers system enables the trapping and manipulation of microscopic objects on the nanoscale, and the exertion and measurement of piconewton-scale forces.

The unrivalled sensitivity and stability of NanoTracker 2 allows the investigation of nanoparticles and quantification of their interactions in materials science, as well as the study of samples ranging from molecules, proteins, and sub-cellular components to bacteria, emulsions, and polymers in the life sciences. Using the power of light, optical tweezers enable the non-invasive investigation of advanced materials, biological matter, and even living organisms, delivering profound insights into complex biophysical phenomena and the nanoscale properties of materials.

Unique Capabilities

This easy-to-use, turn-key platform delivers outstanding spatial, temporal, and force resolutions. NanoTracker 2 offers unrivalled flexibility. From straightforward manipulation experiments to the measurement and postition tracking of multiple traps, it generates fast results and highly accurate data. Only NanoTracker 2 delivers:

- Multiplexed trapping
- Investigation of microrheological properties
- Integration with advanced optical microscopy techniques, e.g., epifluorescence, STED, TIRF, confocal, DIC, Raman etc.
- Combination with AFM for innovative experiments
- Versatile, tunable laser power options up to 10 W

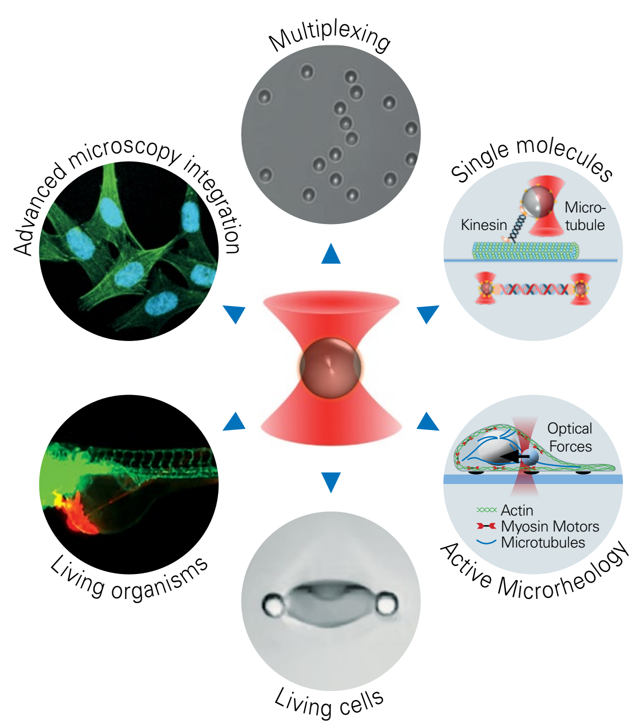


Combination of optical tweezers and confocal microscopy visualizes living fibroblast cells.

Unmatched Range of Applications

NanoTracker 2 enables an extensive range of unique applications:

- Single-molecule experiments
- Correlative optical experiments, e.g., complex single-molecule fluorescence setups
- Quantification of cell mechanics, adhesion, and dynamics
- Investigation of virus-host cell interactions
- Optical trapping and manipulation inside living cells and organisms
- Advanced materials research



Overview of applications using optical tweezers.

Unparalleled Versatility

Live Cell Experiments

Environmental control accessories, such as the PetriDishHeater, enable live cell experiments and the investigation of cell cultures in glass-bottom Petri dishes (35 mm). Study cell mechanics and dynamics under near native conditions with:

- Temperature control: ambient to 60 °C
- CO_2 atmosphere and fluid exchange
- Suitable for long-term experiments

PetriDishHeater enables environmental control and fluid exchange.

Multiplexing and Demultiplexing

The highly stable trapping beam in single and double-beam configurations allows flexible and precise sample manipulations. Fast acousto-optic deflectors (AODs) and a laser-time-sharing principle enable multiplexing and demultiplexing setups, for advanced experiments and precise control of multimolecular complexes. Only NanoTracker 2 delivers:

- Simultaneous 3D manipulation and control of up to 256 particles in individual optical traps
- Demultiplexing enables force measurements with up to 8 time-shared traps
- Individual calibration of up to 8 time-shared traps

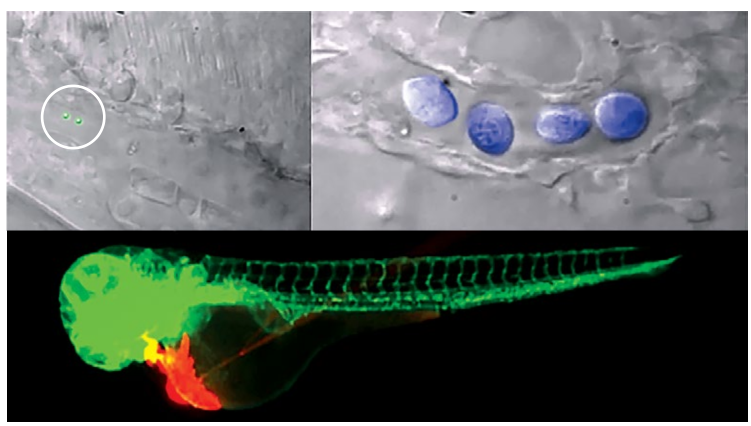
Multiplexing is ideal for the investigation of, e.g., bacterial chromosome condensation, tracking dual-head motor proteins, and protein-mediated DNA-DNA bridging.

Multiplexing in the study of DNA-DNA interactions.

Advanced Optical Integration

NanoTracker 2 operates on research-grade inverted optical microscopes and can be seamlessly integrated with advanced optical microscopy techniques for enhanced experiment designs that deliver crucial insights into fundamental biological mechanisms. The modular setup allows access to optical ports and the use of a wide range of commercial cameras, shutters, filters, detectors, and illumination options for unparalleled versatility:

- Localization of specific regions of interest that can then be investigated with optical tweezers
- Highly selective force measurements or manipulations guided by advanced optical techniques, such as fluorescence microscopy
- 1064 nm trapping laser does not inhibit the acquistion of the optical data



NanoTracker 2 combined with confocal microscopy: Manipulation of trapped particles (green) and cells (blue) inside a zebrafish embryo using optical tweezers (top) and simultaneous confocal imaging (Nikon C2) of living zebrafish (bottom). Images modified from Johansen, P., Fenaroli, F., Evensen, L. et al. Optical micromanipulation of nanoparticles and cells inside living zebrafish. Nat Commun 7, 10974 (2016). https://doi.org/10.1038/ncomms10974. To watch original videos, scan QR code.

Precision Design

High-Performance Hardware

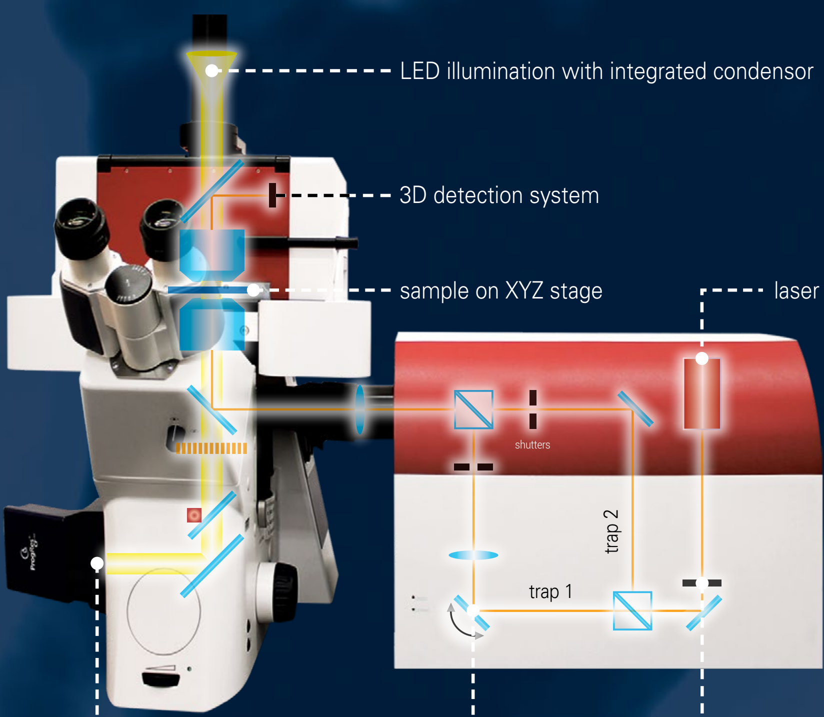
Mechanically robust design and select optical components guarantee stable laser intensities and trap split ratios, ensuring accurate, highly sensitive force and displacement measurements:

- Multiple beam-steering options, high-resolution piezo-driven mirrors, and fast AODs provide precise positioning and fast movement of traps and particles
- Minimized drift via a single, highly stable laser source and sensitive, drift optimized-detection electronics
- Optional piezo sample stage for precise sample positioning
- 3D recording of individual trap position and force via independent detectors for each trap, for minimal crosstalk

Precise trap calibration

Accurate force measurements require precise trap calibration. NanoTracker 2 delivers:

- One-click trap calibration
- Lowest position noise and consistent trap stiffness over a large field of view



Cutting-edge Electronics

At the center of the NanoTracker 2's stability, nanometer-precise position accuracy, and lowest noise levels is the high-performance electronics control unit.

External equipment, such as advanced cameras, spectrometers, and detectors (e.g., PMTs or APDs), can be integrated or triggered using TTL signals.

The high-bandwidth controller ensures real-time data acquisition, feedback, and response - essential for force clamp experiments and sensitive control of particle motion.

Intuitive Software

The straightforward, flexible, and user-friendly software of the NanoTracker 2 provides:

- Fast, intuitive control of all motorized components, e.g., objectives, sample stage, and syringe pumps
- Scriptable software interface for automated experiment workflows. Suitable for beginners and experienced users alike
- Powerful DataProcessing software with models for single-molecule force spectroscopy (worm-like chain, freely jointed chain, etc.) and step-fitting routines

Safe by Design

NanoTracker 2 is a certified Class 1 laser product and suitable for multidisciplinary environments. A designated laser laboratory is not required.

- No specific laser safety training necessary
- Suitable for biological experiments: All components are protected from liquids

Advanced Functionality

A wide range of add-ons, accessories, experimental modes, and features greatly extend the scope of applications possible. These include:

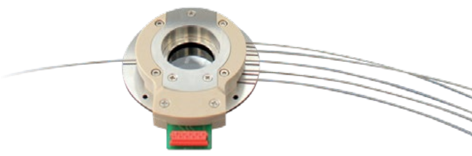
- Point and Trap functionality: a "live" optical image is seamlessly integrated for easy control of trap positions
- Absolute Force Spectroscopy: performs force-distance experiments by moving the trap or sample at user specified speed and direction
- Ramp Designer: for customized force spectroscopy experiments, such as force ramp and force clamp
- CalibrationManager: one-click calibration of trap stiffness and position detection system
- ExperimentPlanner module: easy design of customized experiment routines and automation scripts
- Precise calibration of optical image: enables highly precise positioning of trap

LaminarFlowCell (LFC) for Single-Molecule Applications

The multichannel LFC is a coverslip-based fluid cell with laminar flow perfusion capabilities. It is ideal for multi-component samples or multi-step experiments.

Features include:

- Up to five separate input channels, one output channel, and variable channel heights
- Software-controlled syringe pumps for automated fluid flow and exchange
- Sample heating up to 45 °C



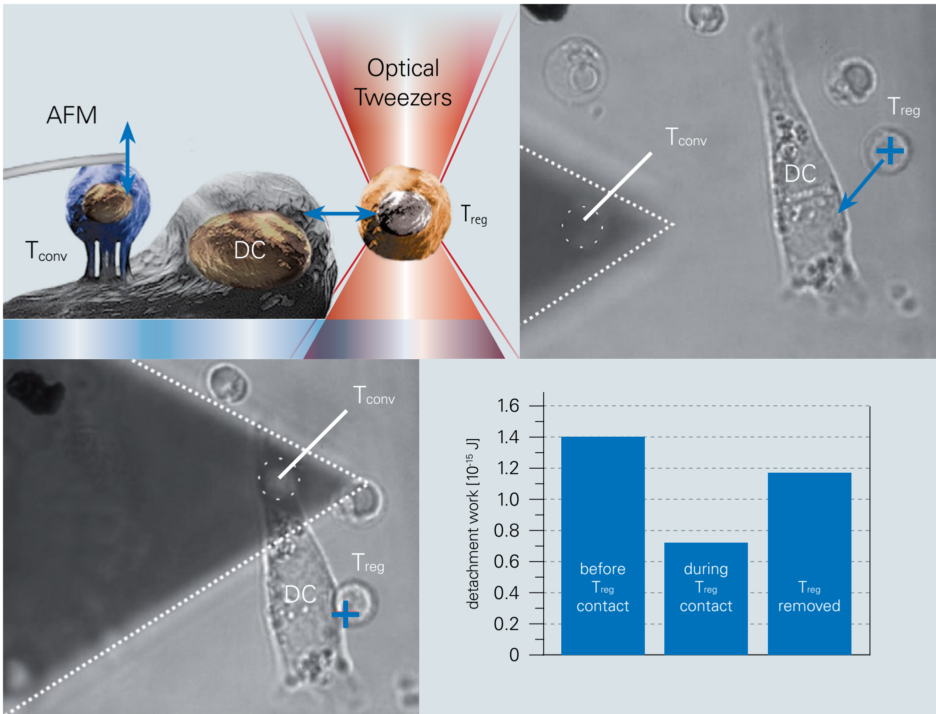
LaminarFlowCell with temperature control.

Combination with AFM (OT-AFM)

NanoTracker 2 can be seamlessly combined with Bruker's NanoWizard® AFM platform via the OT-AFM ConnectorStage.

This setup combines the 3D positioning and manipulation capabilities of optical tweezers with the high-resolution imaging, force detection, and surface property characterization capabilities of AFM, opening an entirely new spectrum of applications:

- Enables the measurement of forces in the range of 500 fN to 1 mN on the same sample
- Combine OT-AFM with optical techniques, such as TIRF and confocal microcopy, for comprehensive samples analyses



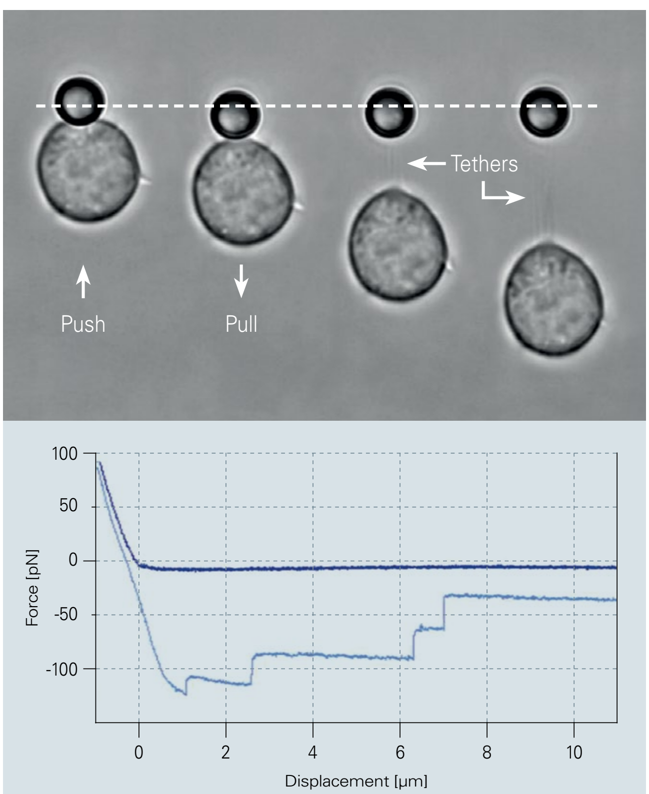
Immune signalling - cell adhesion experiment: cell adhesion experiment OT-AFM combination for spatio-temporal manipulation (OT) of cell-cell contact (regulatory T-cells and dentritic cells) and analysis of cell-cell interactions (conventional T-cells) with AFM.

Advanced Applications

Live Cell Applications

Advanced environmental control options allow the investigation of living cells under near-physiological conditions:

- Study the mechanisms of infection, immune response, and bacterial/virus/nanoparticle uptake processes. Quantify bacteria-cell or virus-cell adhesion and interaction forces
- Perform membrane tether pulling and cell indentation experiments
- Quantify the mechanical properties of living cells, their adhesion forces, and dynamic mechanisms
- Investigate protein-ligand binding events
- Trigger cellular response using functionalized particles or modified micro-organisms
- Study cell-cell, cell-surface, or cell-matrix interactions



Cell Manipulation

As well as applying and measuring forces, NanoTracker 2 can be used to manipulate cells directly with optical traps:

- Sort, deform, relocate, or stretch cells
- Manipulate particles or organelles inside living cells

Two beads bound to a red blood cell are used to dynamically stretch it.

Microrheology Applications

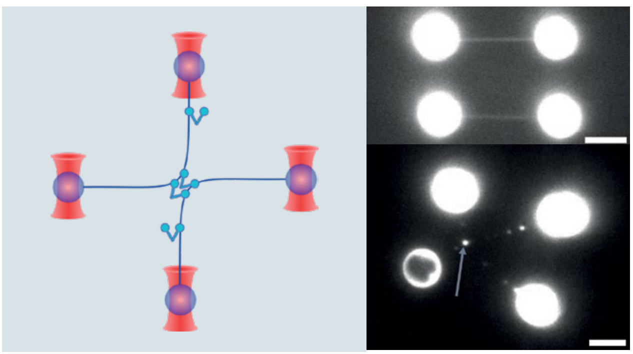
NanoTracker 2 enables the investigation of the microrheological properties of a broad range of samples, such as cells, soft matter, or gels.

- Quantify the viscoelastic and mechanical behavior of soft materials, biological matter, and emulsions
- Perform non-destructive, highly sensitive measurements in delicate systems
- Collection of bead movement spectra at up to 7.5 MHz
- High power laser option (up to 10 W) for advanced viscoelastic measurements in highly viscous environments

Multiple membrane tether pulling experiment via piezo-precision sample movement and force sensing > 100 pN with force-distance curve.

Single-Molecule Mechanics

The high spatial, temporal, and force resolutions of NanoTracker 2 provide the precision and stability necessary to investigate single-molecule mechanics and intramolecular forces. Perform manipulation experiments at sub-nm, sub-pN, and µs resolution, while executing reproducible, standardized experiments.

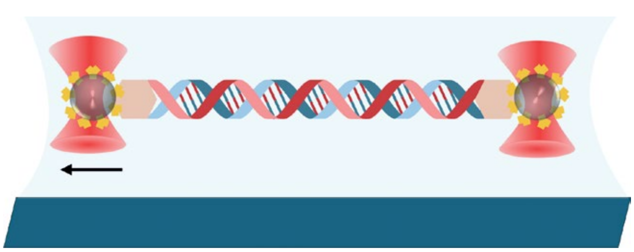


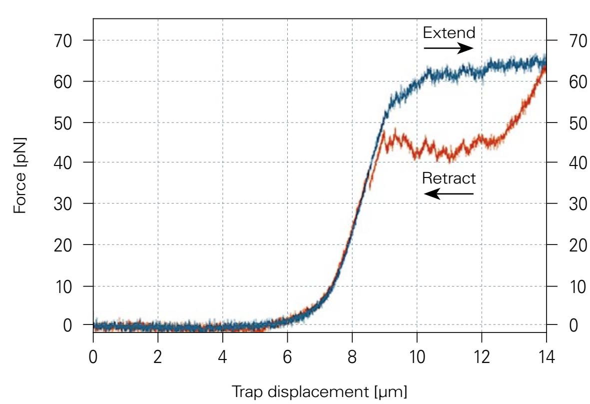
Complex arrangement of two DNA molecules. Study of DNA-protein interactions via multiplexing and fluorescence imaging.

3D Tracking

Passive and active 3D tracking (also called force- or position-clamping) are two of the main tools used to investigate processivity and force generation in motor proteins, cell membrane trafficking, binding events, and DNA-polymerase interactions.

The force-clamping feature of NanoTracker 2 applies a force to the sample that is kept constant via the feedback system. This allows researchers to study the response of a molecule to a specific force - useful in the investigation of unfolding or refolding events.





dsDNA is tethered between two optically trapped beads. One trap is steerable, allowing accurate DNA stretching (left). Force-distance curve of λ-phage dsDNA in PBS buffer (right).

Further Applications

Single-molecules and biopolymers

- Intra-molecular elasticity and protein folding dynamics
- DNA/RNA mechanics
- Protein-DNA binding
- Nanopores and 3D polymer network probing

Cell biology applications

- Membrane organization (e.g., lipid rafts)
- Trans-membrane processes and trafficking
- Intracellular forces
- Receptor-ligand experiments
- Cell mechanics and cell motility

Cell-particle interaction and infection studies

- Tracking of pathogen-host interaction forces
- Bacterial and virus adhesion forces
- Local gene or drug delivery
- Entrance mechanism studies
- Nanotoxicity and endocytosis studies

Advanced measurements

- Complex optical trap geometries
- Optical guiding and artificial crystal building
- Local field enhancement and Raman/SERS applications
- Brownian motion tracking, Photonic Force Microscopy (PFM)
- Colloidal and polymer meshworks force probing

NanoTracker 2 Specifications

System specifications	· Compatible with inverted microscopes: Zeiss, Nikon, and Olympus · Spectral range of 400-900 nm for undisturbed fluorescence imaging	· All major components are motorized and computer-controlled · Optional external commercial modules, such as confocal units, CCD cameras, detectors
NanoTracker 2 Head	· Closed head design: prevents stray light and ambient noise for precise measurements · Liquid-safe, robust, and drift-minimized design for highest stability · Detection unit: single-beam, dual-beam, or up to 8 trap demulitplexing option · Optional high-res. sample positioning piezo stage with up to 100 × 100 × 100 μm^3 travel range and closed-loop control	· Software controlled motorized precision stage with 20 × 20 mm^2 travel range · Oil or water immersion lenses for high NA and low spherical aberration for advanced trapping applications · Four sensitive InGaAs photodetectors with up to 7.5 MHz bandwidth (16-bit sampling) · Decoupled detection of XY and Z for optimized Z sensitivity · Precise focus adjustment via software
Sample holder options	· Suitable for use with standard slides, cover glasses, or Petri dishes · Temperature control (ambient to 60 °C), buffer exchange, and gas (CO_2) perfusion for 35 mm glass bottomed petri dishes	· Multichannel LaminarFlowCell (LFC): coverslip-based fluid cell with laminar flow perfusion
Laser unit	· Class 1 laser certification · Ultra-stable 1064 nm laser	· Various laser power options: 3 W, 5 W or 10 W
Steering unit	· 3D, fast, and continuous beam steering through the full field of view · Single-beam or dual-beam configuration	· XY steering via advanced piezo mirrors or AOD, and motorized Z steering
Controller	· 64 MHz ADC bandwidth · Optional Signal Access Module with over 20 input/output channels · Lowest noise levels	· TTL access and power supply for external equipment · High-speed Ethernet link and intelligent grounding concept for maximum bandwidth and performance
Software	· Java™-based user interface for easy, intuitive instrument control · 'Point and Trap' beam steering · Automated force/displacement calibration · DirectOverlay for precise matching of trap and sample position · Embedded camera control for EM-CCD's · Advanced oscilloscope functionality and online measurement of distances, etc.	· RampDesigner: Powerful force spectroscopy for advanced force clamp or force ramp experiments · ExperimentPlanner: easy design of advanced experimental routines · Advanced, high-speed batch processing force curve analysis with various fitting models, e.g., WLC, FJC, or step fitting algorithms · Complete environmental control via software · User-definable shortcut buttons
Correlative techniques	· AFM and Raman spectroscopy · Brightfield transmission illumination	· Epifluorescence and confocal microscopy · TIRF, FRET, DIC, and STED microscopy

Bruker Nano Surfaces and Metrology
Bruker Nano GmbH

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

Certified in accordance with ISO 9001:2015
Reg.Nr. DE-31624279 QM15

www.bruker.com/bioafm

www.bruker.com/bioafm/nanotracker