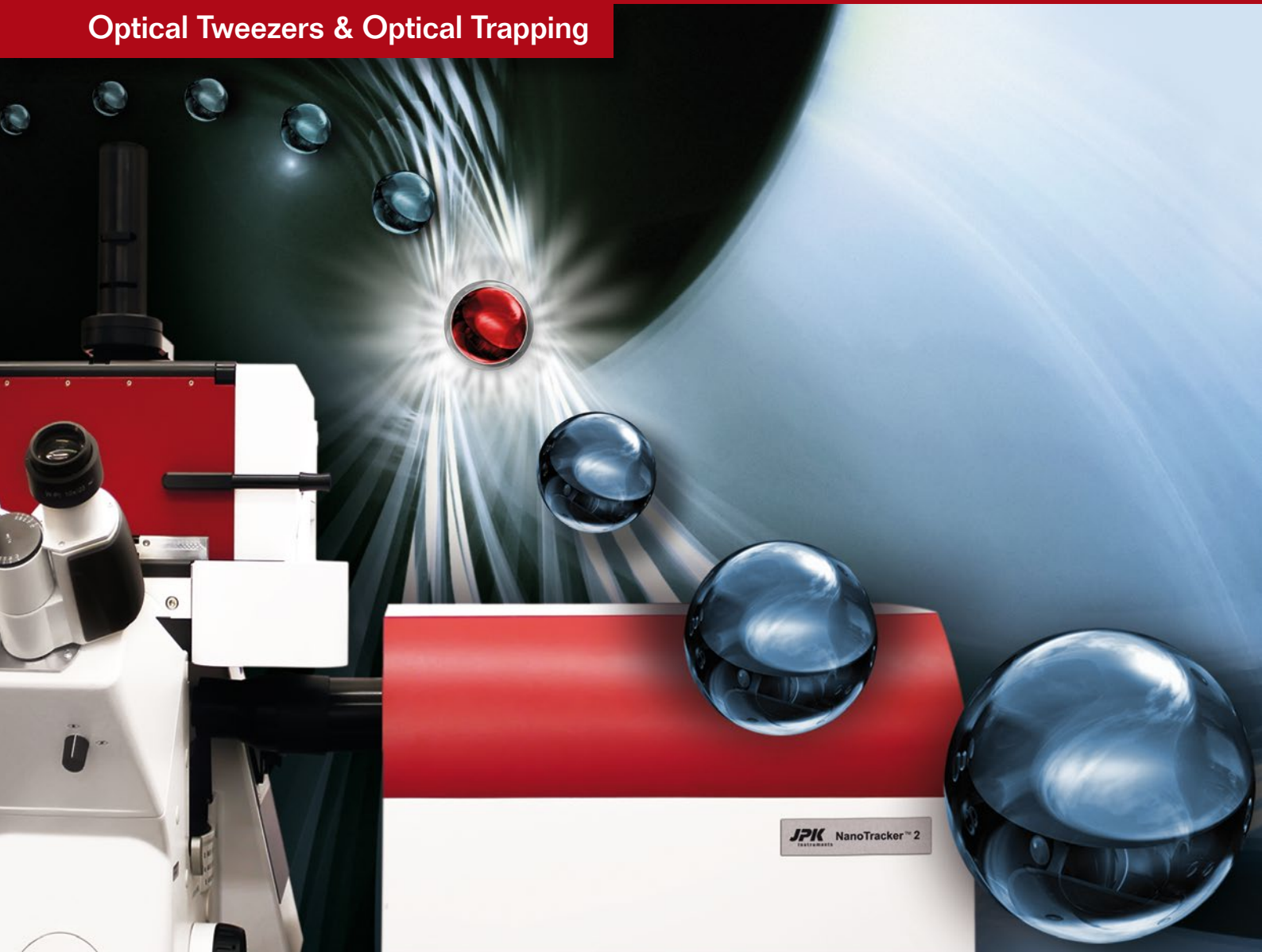


NanoTracker™ 2

Force-Sensing Optical Tweezers

Optical Tweezers & Optical Trapping



3D force measurements with femto-Newton sensitivity and sub-nm precision

Highest stability and lowest noise level for the most accurate measurements

Simultaneous fluorescence imaging

Powerful, flexible control and data analysis software

Class 1 laser certified

Flexible, modular design for applications ranging from single-molecules to living cells

Designed For Quantitative Force Measurements

Optical tweezers: tracking forces on the nanoscale

Optical tweezers are a tool that employs light to manipulate objects. This manipulation takes place on the microscopic scale, allowing interrogation of small objects such as individual cells, cell compartments, single nanoparticles and (bio)molecules.

The NanoTracker™ 2 is an optical tweezers platform based on research-grade inverted optical microscopes and designed for sensitive manipulation, force and tracking experiments. It provides the flexibility required by experienced users through open software and electronics architecture. As an off-the-shelf platform, the time-to-result for scientists can be greatly reduced: scientists can spend their time on experimentation rather than instrumentation.

Being a world-wide recognized leading SPM-manufacturer, JPK has gained vast experience in the development and manufacture of high-precision scientific equipments. JPK's knowledge of building nanometer-precise AFMs has enabled the company to develop the NanoTracker™ 2 optical tweezers with extraordinary stability, lowest drift and noise and capable of sub-picoNewton force measurements. The new head design allows the smooth integration of conventional and advanced microscopy techniques for simultaneous utilization with optical tweezers. An important source for improvements to the original instrument has come from the experience of users in a wide range of applications. JPK's development team works in close cooperation with all our users and, as a result, the continuous knowledge exchange leads

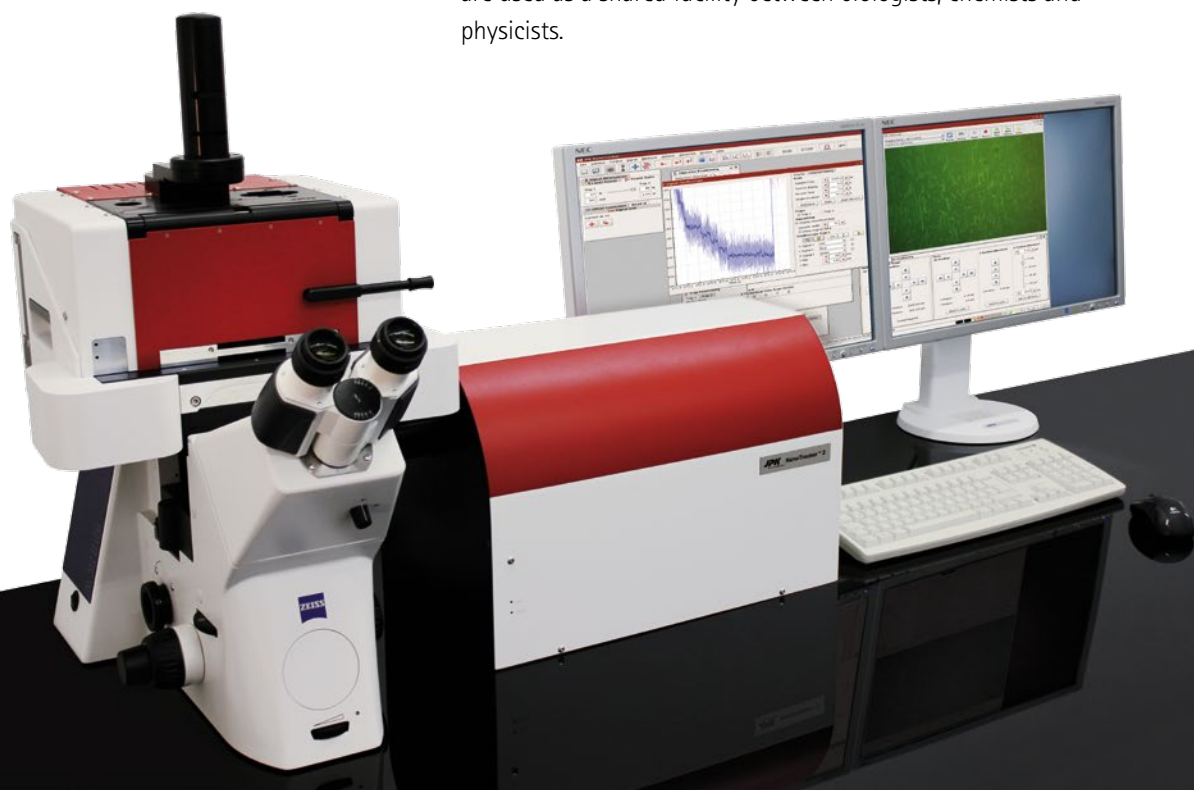
to the development of new instrumentations such as the NanoTracker™ 2 system. JPK guarantees the user the outstanding quality of the instrument and a truly 'Made in Germany' product.

Optical trapping is a technique for direct nm-scale manipulations, force measurements and 3D tracking. Special attention in the NanoTracker™ 2's solid design is paid to the quality and stability of the trapping beam as well as to the mechanical stability of all optical components including trapping and detection objectives mounts. The completely reworked head of the NanoTracker™ 2 and the improved steering unit with new hardware options provide higher spatial, time and force resolution. Sensitive parts like the piezo sample scanner are well protected from buffer or cell culture medium.

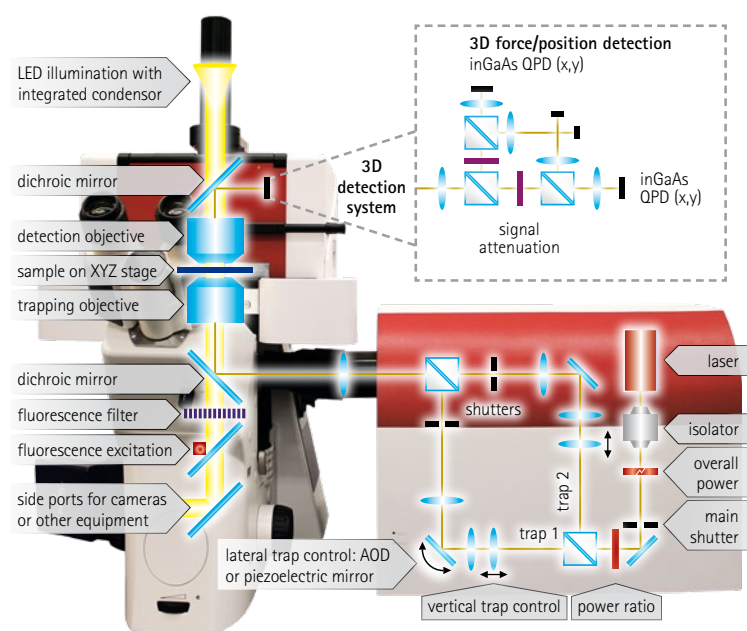
The key to versatile applications is the modular and flexible design. The NanoTracker™ 2 is based on the inverted research microscopes from Zeiss, Nikon and Olympus. NanoTracker™ 2's new head on top of the microscope is not blocking any of its optical ports making it possible to use the whole range of standard or high-end equipment including cameras, shutters, filters, detectors and illumination options.

The NanoTracker™ 2 is designed as a Class 1 laser product. This means that operation of the instrument does not require safety goggles and no special training. The NanoTracker™ 2 is not required to have a specially equipped laser laboratory. It is perfect choice for multidisciplinary environments where optical tweezers are used as a shared facility between biologists, chemists and physicists.

NanoTracker™ 2 setup
on Zeiss Axio Observer



Highest Performance & Modular Design



source by polarization splitting. This makes the system ultra-stable against drift. The new back focal plane interferometry detection unit of the NanoTracker™ 2 is equipped with individual detectors for each trap having separate diodes to lateral (XY) and axial (Z) displacements of the trapped bead. Such a detection approach, in combination with software-controlled dimming filters, allows the use of the full dynamic range of the detectors, achieving the highest possible sensitivity for any selected bead types, laser intensities and trap split ratios. Important for exact force measurements are precise calibration of the traps, lowest position noise and a flat trap stiffness profile over a large field of view. The new precise and flexible one-button trap calibration procedure is independent from bead size and medium viscosity. The cross-talk between trap signals in the detection is drastically reduced.

NanoTracker™ 2 system design

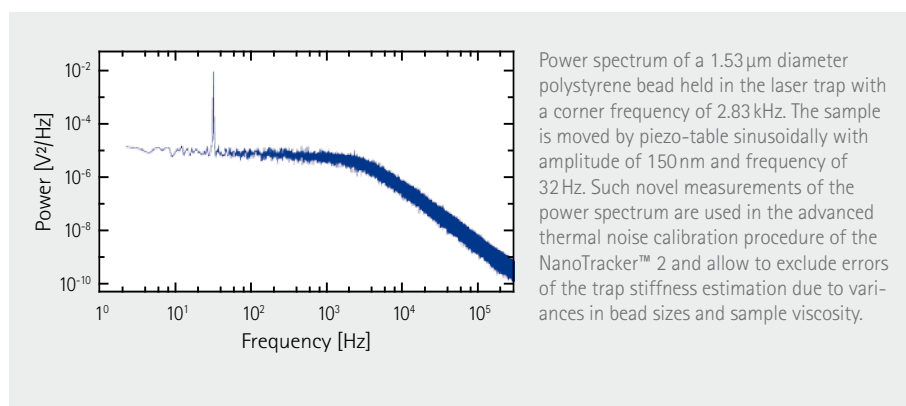
The new system is designed to detect the smallest forces and manipulate particles or molecules with the highest precision. Special laser stabilization and newly designed detection electronics in the head provide to very low noise levels. Additionally, the compact folded design of the laser beam path makes the system immune to drift.

Double-beam or multi-beam configurations, combined solutions for coarse and extra precise sample positioning give the user flexibility. Several beam steering options including the newly designed pivot-point piezo-driven mirrors and fast acousto-optic deflectors (AODs) perfectly match requirements of any application. In addition to extensive sample positioning control including a customized closed-loop piezo sample stage option, the traps can be steered individually in 3D through the sample. Moreover, the laser power can be controlled for both traps independently. This freedom is required to allow a wide range of experimental assays and geometries. The two traps are available full time and are generated from a single laser

Fast and low noise electronics

The electronics unit controlling the NanoTracker™ 2 is optimized to perform at lowest possible noise levels with the highest possible bandwidth. Data acquisition can be performed at up to 60 MHz. The signal access module (SAM) on the front of the controller provides an easy-to-use interface for feeding in auxiliary analog or digital signals. In addition, all internal signals can be monitored from the SAM. Similarly, external equipment such as separate cameras (e.g. EM-CCD's), spectrometers and detectors (e.g. PMT's or APD's) can be connected or triggered using TTL pulses. All electronic signals (trap signals, sample or trap positions and several auxiliary channels) can be recorded and streamed to disk with high bandwidth. The dynamic range of the trap signals can be controlled for optimum sensitivity.

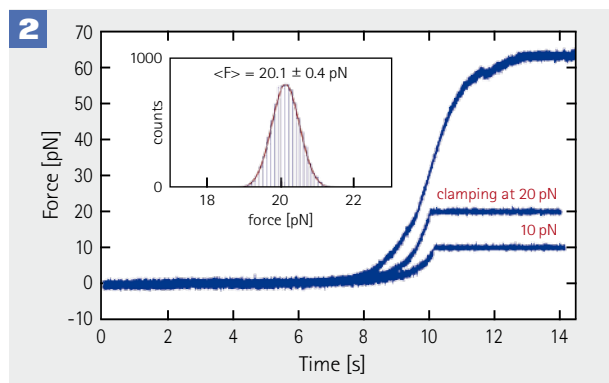
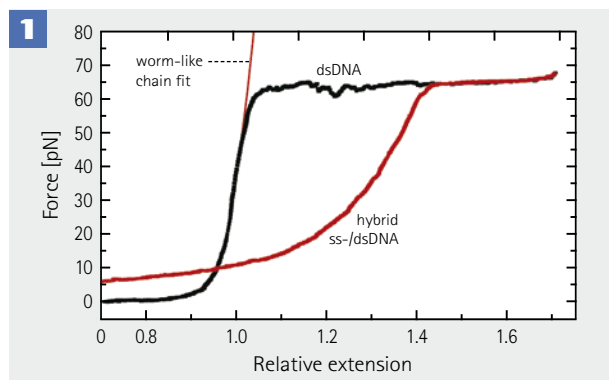
NanoTracker™ 2 controller with Signal Access Module



Single-Molecule Mechanics with Optical Tweezers

Highest spatial, temporal and force resolution

Single-molecule mechanics and manipulation experiments are very demanding. To acquire data with the highest precision, the instrument setup must be designed with lowest noise level and highest stability. The NanoTracker™ 2 is the result of continuous efforts in order to achieve this goal. All components are optimized for sub-nm, femtoNewton and μ sec resolution. The software has several built-in modes to reproducibly perform various standardized experiments. This includes a comprehensive ForceSpectroscopy package with the new JPK RampDesigner™ for either standard pulling experiments or more advanced ones involving force ramps, force clamps, etc. The built-in CalibrationManager is used for online calibration of the force and displacement detection system. This calibration is based on an entirely passive measurement of the thermal noise in the trap and thus does not affect the sample.

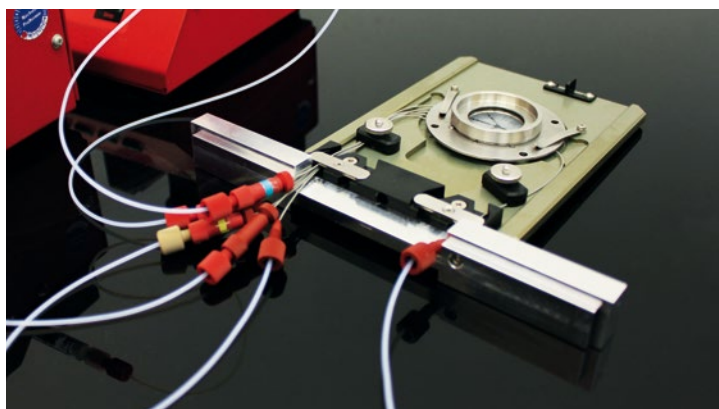


1 DNA elasticity measurement using the NanoTracker™. As the DNA is pulled to larger extensions, it undergoes several phases of distinct elastic behavior. The red line is a fit to the so-called worm-like chain polymer model. The overstretching transition, typical for double-stranded DNA, is readily seen. The black curve shows the stretching of a regular B-form DNA molecule; the red curve shows that of a hybrid molecule consisting of both double-stranded and single-stranded DNA regions.

2 Force-clamping mode of the NanoTracker™ applied to ds-DNA stretching between two trapped beads. Plots show clamping of a single ds-DNA molecule at forces of 10 and 20 pN and a full overstretching plot (obtained in separate experiments). Each curve is plotted with 0.5 s offset relative to others. Inset shows the diagram of detected force variations during clamping phase at 20 pN. (Samples courtesy of Yan Jie's lab, National University of Singapore.)

Multichannel LaminarFlowCell (LFC™)

For many biophysics and biochemistry applications, precise control over the well-timed addition of reagents is crucial. JPK offers a microfluidics flow cell designed for integrated use in the NanoTracker™ 2. The application of fluid flow can be extremely helpful in applying forces by means of viscous drag, e.g. for extending individual molecules such as proteins or DNA. The NanoTracker™ 2 LFC™ consists of up to five independent laminar flow channels. These channels can be flexibly laid out and merged: users can individually design the channel pattern of the flow cell, for example, by using polymeric spacers. Additionally, JPK provides multiple designs of channel patterns.



Key features

- Multichannel, flexible design with up to 5 inputs and one output
- Laminar (non-mixing) flow, keeping channels separated
- Variable channel height
- Software-controlled syringe pumps for automated fluid flow and exchange

Applications

- Single-molecule flow stretching
- Drag force measurements
- Biochemical triggering using sub-second buffer exchange
- Enhanced microfluidics and single-molecule optics experiments

NanoTracker™ 2 with two software controlled standard syringe pumps connected to the LFC™



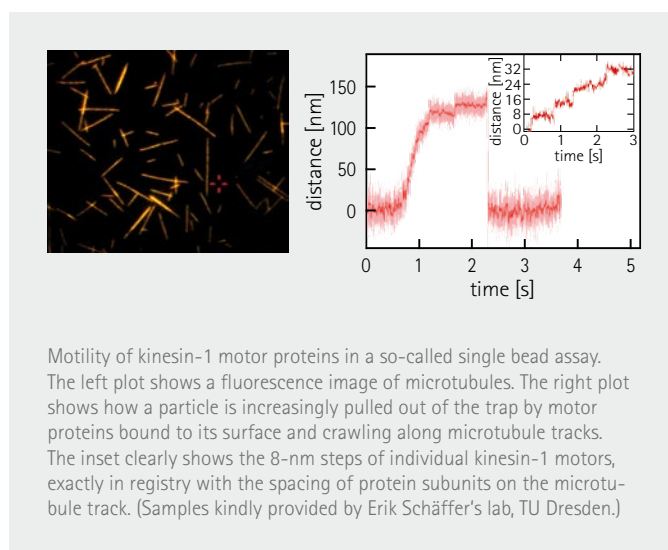
Ready for Simultaneous Single-Molecule Fluorescence

Superb optical integration

The NanoTracker™ 2 was designed to seamlessly integrate with research-grade inverted optical microscopes from Zeiss, Nikon or Olympus. The mounting of the high-NA objectives has been modified from the microscope standard in order to optimize the trapping stability. The optical coupling of the tweezers laser into the optical microscope is done by an additional port designed by JPK. The optical filters used for coupling-in the trapping laser were chosen to keep the entire visible spectrum (400-900 nm) open for other optical microscopy applications. Importantly, the standard microscope filter wheel is untouched by the NanoTracker™ 2 design so that fluorescence imaging can be performed simultaneously and independent of optical trapping. This opens a wide range of applications that require a combination of several optical microscopy techniques. Cameras from various manufacturers are fully embedded in JPK's data acquisition software, including Jenoptik and Andor Technology to cover standard fluorescence experiments up to high-end single-molecule fluorescence imaging applications.

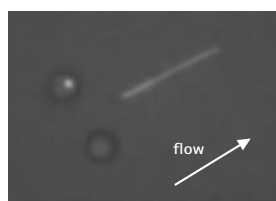
NanoTracker™ 2 software

The software is the main interface between the NanoTracker™ 2 and its users. Therefore, its user-friendliness is the key to getting



Motility of kinesin-1 motor proteins in a so-called single bead assay. The left plot shows a fluorescence image of microtubules. The right plot shows how a particle is increasingly pulled out of the trap by motor proteins bound to its surface and crawling along microtubule tracks. The inset clearly shows the 8-nm steps of individual kinesin-1 motors, exactly in registry with the spacing of protein subunits on the microtubule track. (Samples kindly provided by Erik Schäffer's lab, TU Dresden.)

Force spectroscopy experiments can be straightforwardly combined with sensitive fluorescence imaging as shown here for DNA coated with Alexa-555 labeled Rad51 proteins. The molecule is "caught" from the constant flow by second non-coated DNA, which is held between two optically trapped beads. (sample courtesy of M. Modesti's lab, CNRS, Marseille.)



results faster. The well-organized graphical user interface allows the operator to quickly and intuitively control all motorized components in the system from the laser and steering unit to the external components such as syringe pumps, temperature controllers or cameras. The 'Static Camera Image' function of the NT software allows long time experiments even for very sensible fluorescent samples. After taking a fluorescent image and shutting down the excitation source, it is possible to use the static camera image to position the traps accurately and perform experiments without further photo bleaching of the sample. A "live" optical image is seamlessly integrated. Using JPK's Point and Trap™ functionality, the position of the traps are intuitively controlled by clicking and dragging in the image. Trap multiplexing is available as a software mode to generate many traps by time-sharing. For designing complex experiments with many parameters, the operator can use the JPK ExperimentPlanner™ module. For even more advanced experiments, both a command line tool and a scripting center are available that allow access to all software functions. For data analysis, the dedicated JPK DataProcessing software package is provided. It includes overlay functionality of different data channels, filtering routines, various models for single-molecule force spectroscopy processing (including worm-like chain, freely jointed chain, etc.) and state-of-the-art step-fitting routines based on Kerssemakers et al. Nature 442 (2006) 709-712.

MagneticTwister™ option

The MagneticTwister™ add-on provides the NanoTracker™ 2 with an additional degree of freedom for manipulation experiments. Using this tool, the user is not only able to apply forces with light but also to apply torque to magnetically sensitive materials. Equipped with special magnets, the MagneticTwister™ generates a homogeneous magnetic field of 0.7 Tesla at a distance of 1 mm and has therefore the ability to create forces in the order of several pico-Newtons on the sample. Accessible by the software, the add-on is an easy to use and precise manipulation tool. It is also possible to change between the detection objective and MagneticTwister™ add-on without turning off the system.

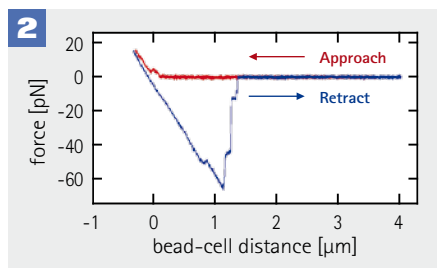
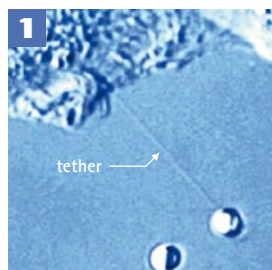
Image of the unmounted MagneticTwister™ option. Controlled by an electric stepper motor it is possible to rotate the magnets 360° with an accuracy of 4° using the provided software. ▶



Live Cell Applications

'Escape force' measurements, adhesion experiments and manipulation of objects inside of cells

The combination of hardware components, available accessories, advanced detection approaches and flexible user-friendly software allows the use of NanoTracker™ 2 in a number of advanced live cell experiments. The user is able to obtain reliable quantitative data such as bacteria- or virus-to-cell force probing, detection of protein-ligand binding events in the cell membrane and studying viscoelastic properties of a cell via membrane tether pulling or rheology. Complementary to force measurement applications, NanoTracker™ 2 can be used to directly manipulate cells with optical traps which allow cell sorting, deformation, relocations and stretching experiments. It is also possible to measure the force and the trajectory of moving or escaping cells such as sperms or bacteria, or to manipulate particles or organelles inside of living cells. All of the manipulation or force experiments can be done simultaneously to fluorescence imaging or optical spectroscopy.



1 DIC image of a CHO cell with a membrane tube pulled by an optically trapped protein coated bead. **2** Corresponding force vs. distance plot from picture 1. The retract curve shows a bond rupture in several steps. (Cell courtesy of A. Herrmann's lab, HU Berlin.)

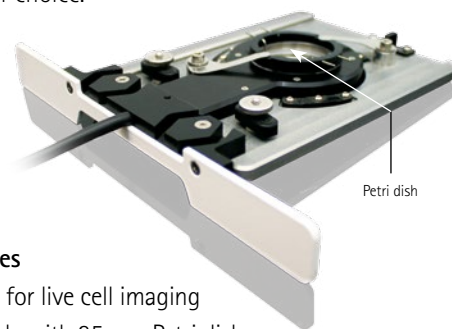
Minimized drift for long term stability

For long-time experiments, which are often required for live cells, or optical tweezing in combination with techniques like TIRF or confocal microscopy, the minimized sample surface drift and trap stability become crucial characteristics. The NanoTracker™ 2 is equipped with a state-of-the-art integrated

LED illumination unit in standard Köhler configuration, which allows brightfield or DIC in transmission to visualize the cells. Standard side- and back-ports of the inverted microscope provide the freedom to combine several measurement techniques or use different kind of analyzers or detectors such as VIS or Raman spectrometers, different types of cameras or single photon counters (PMTs, APDs) at the same time.

JPK PetriDishHeater™ for live-cell experiments

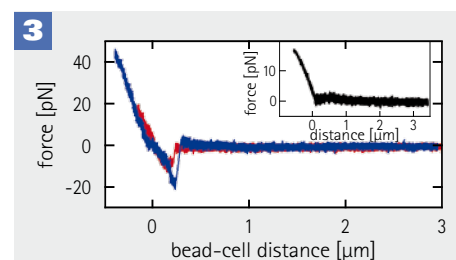
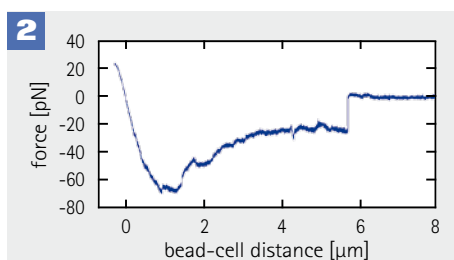
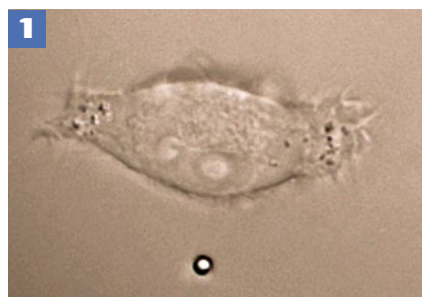
The uncompromising requirement of nearly all live cell experiments is a suitable environmental control. This is fully satisfied in the NanoTracker™ 2 with the availability of several accessories and the closed head design. For cell cultures, users may work with conventional glass-bottom Petri dishes. The temperature is software controlled by a specially designed Petri dish heater which includes possibilities for buffer exchange and to support a CO₂ atmosphere. In the open geometry of such a Petri dish, a water-dipping objective lens is the detection objective of choice.



Key features

- Designed for live cell imaging
- Compatible with 35 mm Petri dishes from standard suppliers such as BD, Corning and Wilco
- Heating from ambient to 60 °C with 0.1 °C precision
- Perfusion control employing standard syringe pumps and CO₂
- Drift minimized design

1 Bright field image of a MDCK cell approaching and retracting a 2 μm carboxyl-coated polystyrene bead held in the optical trap. Such configuration of a bead-cell experiment is typical for acquisitions of force vs. distance curves to characterize interactions of cells with target molecules on the bead surface, viscoelastic properties of membrane tethers, ligand-receptor bindings, virus-cell interactions etc. **2** Corresponding force vs. distance plot from picture 1. The retract curve shows phases of elastic membrane extension and breakage of multiple unspecific bonds with final unbinding of the bead from the cell surface at about 6 μm separation. **3** Single-virus force measurements using optical tweezers. Influenza virus-coated beads were moved toward a cell until touching, and subsequently retracted. Single rupture events of different height were recorded. When the bead surface was blocked with BSA, no interaction could be detected (see inset). (Adopted from C. Sieben et al., PNAS 2012, vol. 109 pp. 13626-31.)



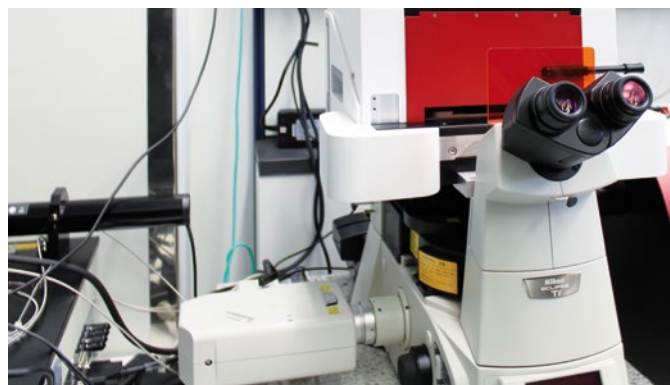
Advanced Measurements from Particles to Cells

Rheology applications

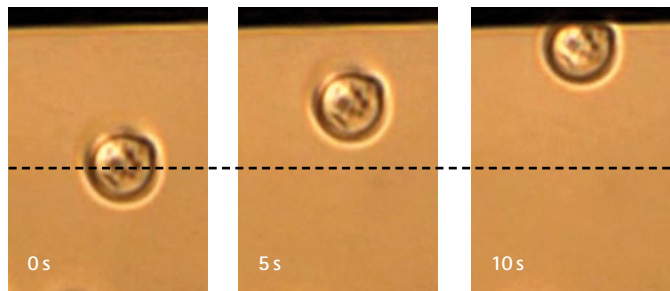
High performance electronics and flexible hardware options of the NanoTracker™ 2 enable rheological studies on cells or gels. The AOD steering option allows applying advanced trap calibrations methods in high-viscosity environments where standard thermal noise method is not applicable. The rheological characterization of a wide range of materials is possible due to the broad-band electronics of the NanoTracker™ 2. These enable the collection of beads movement spectra up to 3.5 MHz. The ability to calibrate traps in high-viscosity media makes it possible also to perform trap calibrations trapping beads inside cells.

3D and active tracking

Passive 3D and active tracking (also called force- or position-clamping) are two of the main tools used to investigate processivity and force generation of big family of motor proteins, cell membrane trafficking, binding events and DNA-polymerase interactions. The force-clamping feature of the NanoTracker™ 2 allows feedback assisted tracking and to record the movements of the single-molecules or live cells displacements with nanometer precision applying minimal necessary and well-controlled forces.



NanoTracker™ 2 mounted on a Nikon Eclipse Ti confocal microscope

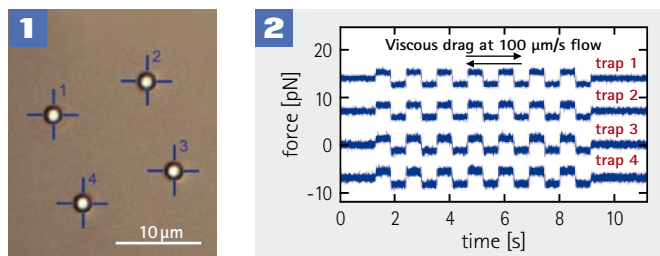


A time sequence of optical images of a trapped yeast cell approaching a glass wall. This is a model experiment of using NanoTracker™ 2 optical tweezers in implant materials research, biofouling etc.

Multi color image of Xylem cells acquired with Nikon C1/TE2000 confocal microscope combined with NanoTracker™ 2. ▶

AOD multiplexing and de-multiplexing

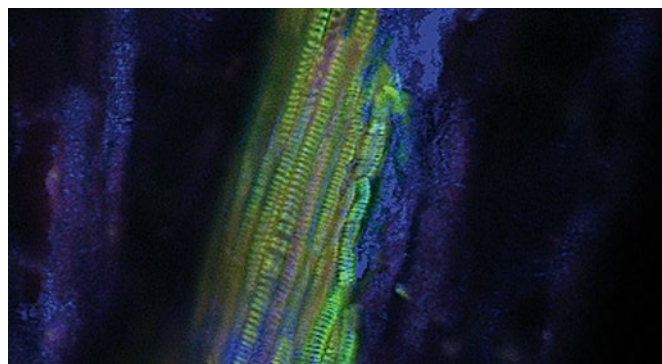
For some applications like the condensation of the bacterial chromosome or tracking dual-head motor proteins, one requires an optical tweezers with four and more independently controllable optical traps. Such advanced configurations can be achieved in the NanoTracker™ 2 due to the utilization of fast AODs and the laser time sharing principle. Such trap multiplexing allows sensitive control of multimolecular complexes. Furthermore, it is crucial to be able to calibrate these multiple traps and control their stiffness as well as measure forces quantitatively with highest resolution.



1 Bright field image of four polystyrene 2 μm beads held by multiplexed traps of AODs-equipped NanoTracker™ 2. Laser time-sharing technique (or multiplexing) allows creation of many traps with full control over their stiffnesses and positions. A multiplexing with AODs is often the only solution for experiments where more than two traps are required. **2** Force measurements obtained from four multiplexed traps (shown in image 1) during viscous drag experiment, where a piezo was oscillating with constant speed of 100 μm/s. The demultiplexing feature of the NanoTracker™ 2 makes it possible to record forces simultaneously from up to 8 multiplexed traps.

Combination with confocal microscopy or TIRF

The ability to combine optical tweezers with confocal microscopy becomes decisive in studies involving tissues, cells and their internal compartments such as organelles. Confocal microscopy often is the only microscopy method which allows finding regions of interest inside a cell. The proper alignment of optical tweezers relative to specific points of the specimen and performing highly selective force measurements or manipulations is guided by the fluorescence. The integrated and stable design of the NanoTracker™ 2 is important here. All ports from the inverted optical microscope can be used for confocal coupling as usual. The NanoTracker™ 2 components are not blocking the confocal beam path.



NanoTracker™ 2 Technical Specifications

System overview

- Flexible platform for trapping and tracking of nanoparticles for measurements from single-molecules to entire cells
- Compatible with inverted microscopes for combined experiments, e.g.,
 - Carl Zeiss Axiovert 200 & Axio Observer
 - Nikon TE 2000 & Eclipse Ti
 - Olympus IX70/71/73
- Compatible with all major light microscopy techniques such as DIC and fluorescence
- Spectral range of 400-900 nm for undisturbed fluorescence imaging
- Optical components for trapping are optimized for 1064 nm wavelength
- Automated calibration for enhanced productivity
- All major components are motorized and computer-controlled
- No safety goggles needed (Class 1 laser) and no alignment by hand
- Optional external standard modules such as confocal units, CCD cameras, detectors



NanoTracker™ 2 head

- Closed head design prevents stray light and airborne noise for noise-free measurements
- Liquid-safe, robust and drift-minimized design for highest stability
- Optional sample positioning piezo stage with up to 100 x 100 x 100 µm³ travel range and closed-loop control for accurate trap calibration, fast vertical scanning and experimental flexibility
- Software controlled motorized precision stage with 20 x 20 mm² travel range
- Oil or water immersion lenses for high power or low spherical aberration for advanced trapping applications
- Four sensitive InGaAs photo detectors with up to 3.5 MHz bandwidth (16 bit sampling)
- Decoupled detection of XY and Z for optimized Z sensitivity
- Precise focus adjustment via software

NanoTracker™ 2 sample holder options

- Standard slides, cover glasses or Petri dishes can be used
- Multichannel LaminaFlowCell (LFC™) is a coverslip-based fluid cell with laminar flow perfusion capabilities for single-molecule applications
- Living cell studies with temperature control (ambient to 60 °C), perfusion and gas flow (CO₂) for 35 mm Petri dishes with glass bottom from Wilco, BD, and Corning

NanoTracker™ 2 laser unit

- Ultra-stable custom-designed laser (1064 nm)
- < 0.5 % intensity stability
- 3W laser power (other options available)
- Class 1 laser certification

NanoTracker™ 2 steering unit

- True 3D fast and continuous beam steering through the full field of view
- One or two beams with adjustable power distribution
- One beam with XY steering by advanced piezo mirrors or AOD, and Z steering by fast linear drive
- 500 µsec response time for steering unit

NanoTracker™ 2 controller

- State-of-the-art controller with lowest noise levels
- 60 MHz bandwidth with 16 bit for XY and Z detection
- Signal Access Module (SAM) with more than 20 input/output channels
- TTL access and power supply for external equipment
- High-speed Ethernet link and intelligent grounding concept for maximum bandwidth and performance

NanoTracker™ 2 software

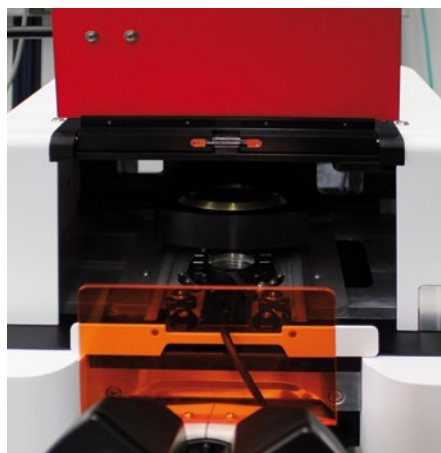
- Easy to use Java™-based user interface for intuitive instrument control
- Point and Trap™ beam steering
- Automated force versus displacement calibration
- JPK's proven DirectOverlay™ functionality for precise matching of trap and sample position
- Embedded camera control for high-end EM-CCD's
- Advanced oscilloscope functionality and online measurement of distances, cross sections, and many more
- Powerful force spectroscopy with the new RampDesigner™ for advanced force clamp or force ramp experiments
- JPK's ExperimentPlanner™ for easy design of advanced experimental routines
- Advanced and high-speed batch processing force curve analysis with huge number of fitting models including WLC, FJC or step fitting algorithms
- Complete environmental control via software
- User-programmable software for advanced experiments
- User-definable shortcut buttons

Optional modular items for enhanced flexibility

- Single-beam/dual-beam configuration
- Detection unit: equipped as single-beam or dual-beam
- AOD option for faster beam steering and optimized trap multiplexing
- Closed-loop piezo stage configurations: 3-axes, 1-axis (only Z) or none
- Axial trap control for independent focus of traps and microscope
- Lasers with different output power
- Choice of microscope objectives: magnification, immersion medium, TIRF
- MagneticTwister™ add-on

Instrument Modes

- 3D particle tracking
- Point and Trap™ for easy trap positioning
- Online calibration based on power spectrum
- Advanced Force Spectroscopy including Force Clamp and Force Ramp with the new JPK RampDesigner™
- Active and passive force mapping
- Micro-rheology mode
- Optical sorting
- Trap oscillation
- Multiplexing for 3D trap arrays
- Line traps & circular traps
- Microfluidics control
- Nanoassembly
- Optical z-stacking



Applications

Single-molecules & biopolymers

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

Cell biology applications

- Membrane organization (e.g., lipid rafts)
- Trans-membrane processes, trafficking
- Intracellular forces
- Receptor-ligand experiments
- Cell mechanics and cell motility
- Membrane tether dynamics
- Micro-rheology of cells and gels

Cell-particle interaction and infection studies

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

Advanced measurements

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

Advanced optical microscopy modes

- Brightfield transmission illumination (standard)
- Differential Interference Contrast (DIC) in transmission (standard)
- Epi-fluorescence microscopy (standard)
- Raman spectroscopy
- TIRF microscopy
- Confocal microscopy
- FRET microscopy and more

NanoTracker 2, JPK ExperimentPlanner, JPK RampDesigner, Point and Trap, PetriDishHeater, MagneticTwister, LFC are trademarks or registered trademarks of JPK Instruments AG.

Open NanoTracker™ 2 head with mounted PetriDishHeater™