NanoTracker™

Force-Sensing Optical Tweezers & 3D Particle Tracking Platform

Optical Tweezers

- 3D nanoscale force measurements with femto-Newton sensitivity
- Sub-nanometer precision measurements
- Simultaneous fluorescence imaging
- Intuitive and flexible software interface
- Laser class 1 certified
- More than 12 degrees of freedom plus environmental control

Prism Awards
FOR PHOTONICS INNOVATION

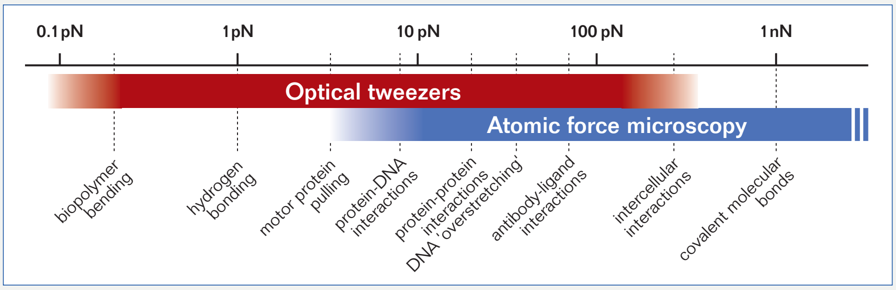
JPK Instruments

Nanotechnology for Life Science

NanoTracker™. Quantitative Force Measurement Platform.

Optical tweezers: tracking forces on the nanoscale

'Optical tweezers': not only the figurative name has secured a current prominent place in the nanobiosciences. Relying on lasers, optics and hydrodynamics, it is no miracle that the big push for the technique has come from the physics community. However, soon after its invention it was picked up and employed to study physical aspects of biological systems, and is now ready to find intriguing applications in polymer and colloidal sciences as well as in molecular and cell biology.



As the name hints at, optical tweezers are a tool that employs light to manipulate objects. This manipulation takes place on the microscopic scale, allowing interrogation of small objects like individual cells, cell compartments or even single nanoparticles or (bio)molecules. Two decades ago, it was Arthur Ashkin who for the first time experimentally demonstrated a working optical tweezers instrument. Several generations of technological improvements and refinements have brought optical tweezers to their present-day state: a cornerstone technique in the life sciences, employed for both micromanipulation and the accurate tracking of pico-Newton forces generated in biology at the (sub)cellular level. Most biologically relevant mechanical processes produce forces in this range. Importantly, the manipulation and force-sensing using optical tweezers is 'contact-free' and sterile, since no foreign objects are in touch with the often delicate sample.

The physical basis of optical trapping lies in the interaction of light with matter. For transparent materials, light passes through the material, but changes direction upon entering and leaving the material by refraction. A fraction of the light momentum is imparted onto the refracting material. When a laser beam is focused tightly by a strong lens, the effective radiation pressure pulls refractive objects towards the focus and holds it there: an optical trap. If the laser focus is moved through space, the trapped object is forced to follow. One can use an optical trap to manipulate microscopic objects in three dimensions.

When the refractive object is pulled away from the laser focus by an external force, the object will scatter the trapping light, yielding a slight deflection of the ongoing laser light. This allows for 3D tracking of the position or the force on the particle to sub-nanometer or sub-picoNewton accuracy and with up to MHz bandwidth.

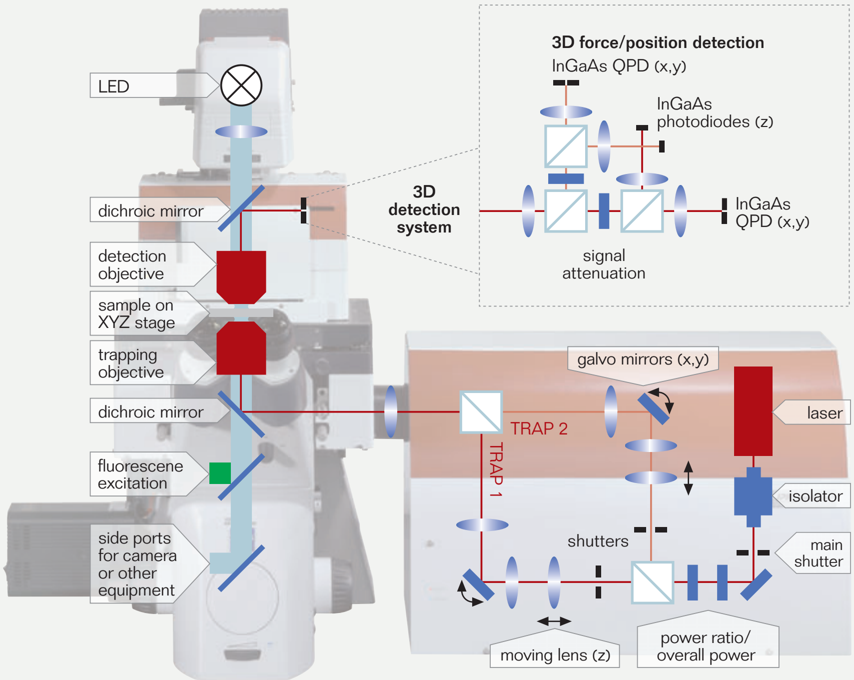
JPK NanoTracker™ – a new class of instrumentation

Up to now, state-of-the-art optical tweezers have been mostly used by physicists able to design and construct an instrument from scratch. The JPK NanoTracker™, a compact, table-top instrument brings the technology to a broader range of scientists. The NanoTracker™ is an optical tweezers platform based on research-grade inverted optical microscopes and designed for sensitive manipulation, force and tracking experiments. No compromises are made either in terms of stability or concerning flexibility. The system provides two full-time optical traps that can be moved in all three dimensions. It comes as a maintenance-free system that is particularly user friendly for starters. Yet, it provides the flexibility required for experienced users through an 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. For the first time, groundbreaking optical tweezers technology can be quickly exploited by scientists with no prior knowledge. Experienced users can push the technology to new frontiers harnessing our open software and electronics architecture.

Stability is the key

Long-term stability and operation without manual adjustments have been key design criteria. A highly stable laser source combined with a short, folded optical pathway in combination with a drift-compensated design guarantees the highest performance. With over ten years of expertise in high-end atomic force microscopy instrumentation, JPK Instruments has the necessary knowledge and experience in this area. The system lives up to the demanding expectations of even those researchers that would otherwise have to design and build such an instruments themselves: sub-nanometer precision and force resolution down to the femto-Newton regime are key.

For Single Molecules, Nanoparticles and Living Cells.



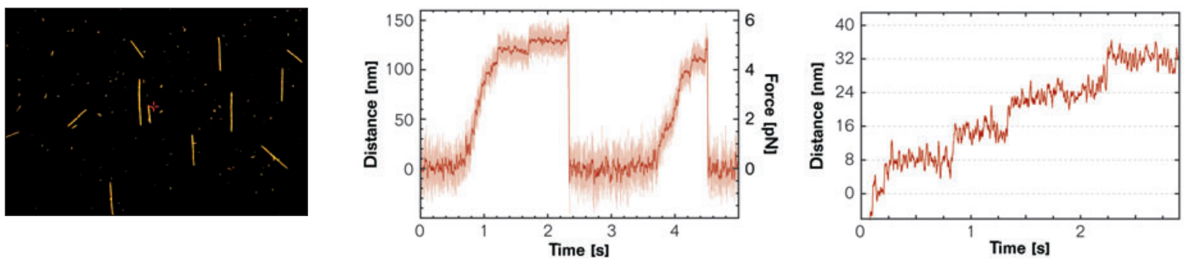
NanoTracker™ system layout

The NanoTracker™ is built around microscope bases from Zeiss, Nikon or Olympus, in order to retain the highest optical performance and the flexibility to mount cameras or other equipment onto side or back ports. Elements of the standard microscope design were reengineered by JPK to attain the required stability and drift compensation.

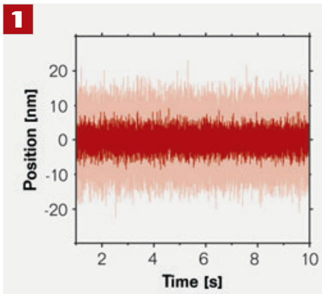
For maximum comfort, we designed the system as a Laser Class 1 product, avoiding the hassle of working with safety goggles. The closed design acts as an acoustic isolation and stray-light safe chamber. Laser safety is assured by interlocks. A large number of degrees of freedom are provided to the user. In addition to extensive sample positioning control including a closed-loop piezo translation stage, the two available traps can be steered individually in 3D though the sample. Moreover, the laser power can be controlled in a continuous manner 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 source by polarization splitting. Thus, the system is ultra-stable against drift.

To obtain the best possible detection sensitivity, separate detectors are installed for lateral (XY) and axial (Z) displacements, as indicated in the schematic.

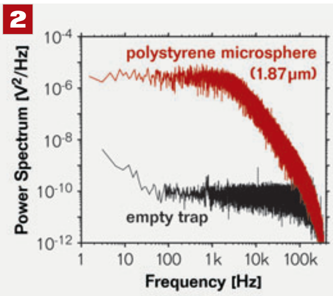
Measurements of the motility of kinesin-1 motor proteins in a so-called single bead assay. The left plot shows a fluorescene image of microtubules. The middle plot shows how a microparticle is increasingly pulled out of the trap by motor proteins bound to its surface and crawling along microtubule tracks. The motor proteins stall when the trapping force becomes too high. The right plot clearly shows the famous 8-nm steps of individual kinesin-1 motor proteins, exactly in registry with the spacing of protein subunits on the microtubule track. The resolution of this protein kinetics is one of the hallmarks of biophysics. (Samples kindly provided by Erik Schäffer's lab, TU Dresden.)



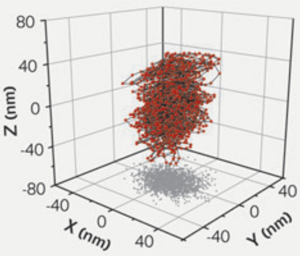
1



2



3



1 *Raw position data (sampled at 20 kHz and 1 kHz, respectively) of a 1.87-µm particle in a trap with 130 pN/µm stiffness shows both a positional accuracy that is limited only by thermal motion and an essentially drift-free signal.*

2 *Typical power spectrum recorded with 800 kHz, both of a 1.87-µm polystyrene particle and of an empty trap. Such measurements of the power spectrum are used to accurately calibrate the measurements.*

3 *Thermal motion plot of a 1-µm silica particle in the trap volume.*

Optical Tweezers with Advanced Optical Microscopy.

Superb optical integration

The NanoTracker™ was designed to seamlessly integrate with research-grade inverted optical microscopes from Zeiss, Nikon or Olympus and can typically be incorporated into an existing system. The system can be used with all kinds of high-NA objective lenses. The optical coupling of the NanoTracker™ 1064 nm laser beam into the optical microscope base is done by an extra 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™ design, such that fluorescence imaging can be performed simultaneous and independent of optical trapping. This opens a wide range of applications that require a combination of several optical microscopy techniques.

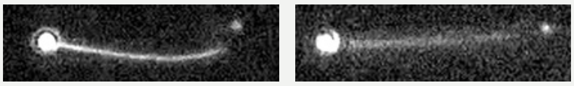
The mounting of the objectives was strongly modified from the microscope standard in order to optimize the overall system stability. It provides an objective changer that houses two objectives, to allow convenient switching between different magnifications or different immersion media objectives.

Brightfield microscopy is provided by a LED illumination unit in standard Köhler configuration. Possible fluorescence excitation sources include arc lamps, lasers and LED-based systems that can be used for multicolor imaging.

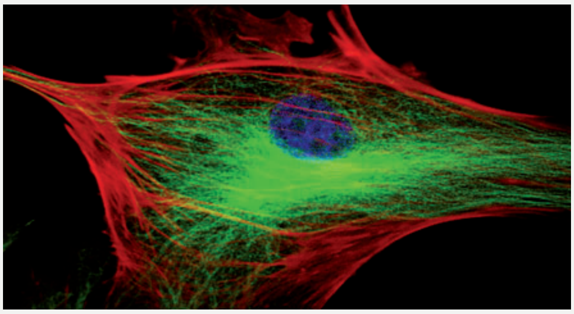
Cameras from various manufacturers are fully embedded in JPK's data acquisition software, including those from JenOptik and Andor Technology to cover standard fluorescence up to high-end single-molecule fluorescence imaging applications.

Advanced optical microscopy modes

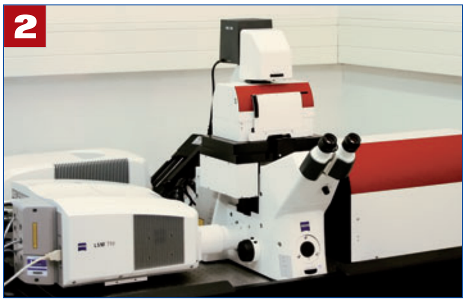
- Brightfield transmission illumination (standard)
- Differential Interference Contrast (DIC) in transmission (standard)
- Epi-fluorescence microscopy (standard)
- Raman spectroscopy
- TIRF microscopy
- Confocal Laser Scanning Microscopy (CLSM)
- FRET, FLIM and FCS imaging

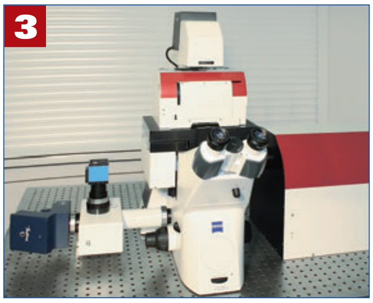


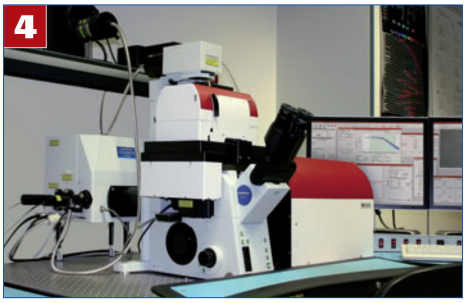
Force spectroscopy experiments on the NanoTracker™ can be straightforwardly combined with sensitive fluorescence imaging as shown here for DNA coated with Alexa-555 labeled Rad51 proteins. (Proteins courtesy of. M. Modesti's lab, CNRS, Marseille.)



Three-color fluorescence image (BPAE cell, FluoCells® slide #2).







The NanoTracker™ optical tweezers platform embedded in various optical microscopy configurations:

1. *NanoTracker™ on Nikon Eclipse TE2000 base equipped with an Andor iXon+ 897 EMCCD camera.*
2. *NanoTracker™ on a Zeiss AxioObserver base equipped with an LSM710 confocal laser scanning instrument.*
3. *NanoTracker™ on a Zeiss AxioObserver base equipped with a dual port adapter mounting, here with a JenOptik CFcool color fluorescence camera and a camera from The Imaging Source.*
4. *NanoTracker™ on an Olympus IX81 base with a FluoView 1000 confocal laser scanning instrument.*

Ready for Confocal Imaging & Single Molecule Fluorescence.

It's all in the software

The software is the main interface between the NanoTracker™ and its users. Therefore, its user-friendliness is the key to get results faster. The well-organized graphical user interface allows the operator to quickly and intuitively control all motorized components in the instrument, as well as the laser and steering unit. The software has several built-in modes to reproducibly perform various standardized experiments. This includes a comprehensive Force Spectroscopy 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.

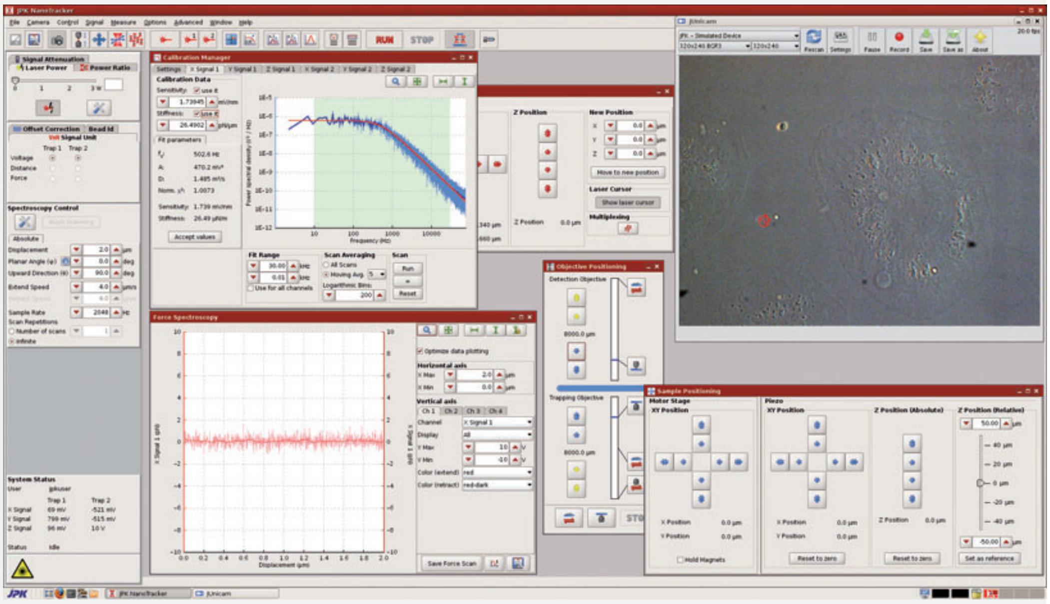
All electronic signals (trap signals, sample or trap positions and several auxiliary channels) can be recorded and streamed to disk with high bandwidth using any of the available real-time oscilloscope panels. The dynamic range of the trap signals can be controlled for optimum sensitivity.

A live optical image coming from any of the range of supported cameras 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 out of a single by time-sharing.

Naturally, the various sample handling options for the NanoTracker™ (for microfluidics and temperature control) are embedded in the software, too.

For advanced experiments, both a command line tool and a scripting center are available that allow access to all software functions from a script. Scripting can be exploited for experiment automation and user-defined experiments.

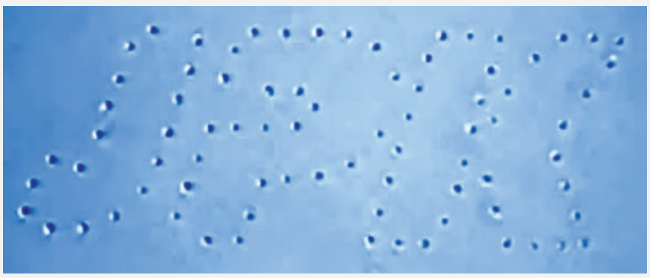
For data analysis, the dedicated JPK DataProcessing software package is provided. It includes overlay functionality of different data channels, filtering routines, various models for single molecule force spectroscopy processing (including worm-like chain, freely jointed chain, etc.) and state-of-the-art step-fitting routines based on Kerssemakers et al. Nature 442 (2006) 709-712.



Screenshot of the NanoTracker™ control software

State of the art electronics

The electronics unit controlling the NanoTracker™ is optimized to perform at lowest possible noise levels with the highest possible bandwidth. Data acquisition can be performed at up to 60 MHz (16 bit); at lower sampling frequencies 18-bit or even 24-bit A/D channels are available. 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, detectors (e.g. PMT's or APD's) etc. can be connected with analog signals or triggered using TTL.



JPK logo formed by an array of 1-μm silica particles held by multiplexed optical traps

Advanced Sample Handling and Environmental Control.

A variety of sample chambers for every application

The user can choose different standard substrates and sample chambers including

- Holder for standard cover glasses
- Petri dishes with cover glass bottom with a 35 mm diameter
- Slides from IBIDI (ask for model)

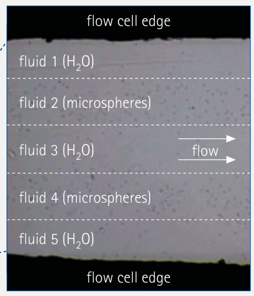
For more advanced experiments the operator has the choice between the Multichannel LaminarFlowCell and the PetriDishHeater™.

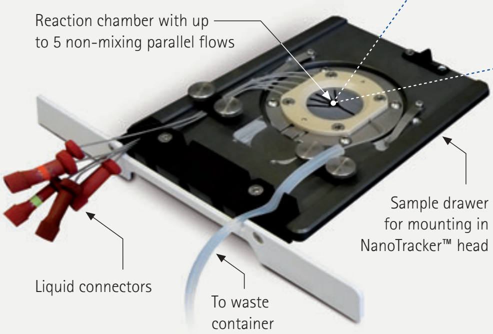
Multichannel LaminarFlowCell (LFC)

For many biophysics and biochemistry applications precise control over the well-timed addition of reagents is crucial. To this end, JPK offers a microfluidics flow cell designed for integrated usage in the NanoTracker™.

The application of fluid flow can be extremely helpful in applying forces by means of viscous drag, e.g. for extending individual polymeric molecules such as proteins or DNA. The NanoTracker™ flow cell 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 such as PDMS or Parafilm®.

Overview microscope image showing the five separated laminar flow channels. In the image, 2-µm microspheres flow from left to right in two channels, both separated on either side by a channel containing only water. The total width of the five channels is around 2 mm.





Key features

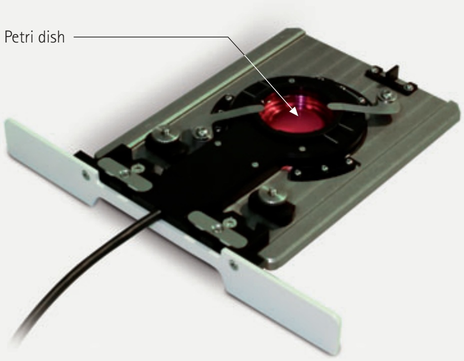
- Multichannel, flexible design with up to 6 inputs and/or outputs
- Laminar (non-mixing) flow, keeping channels separated
- Variable channel height
- Temperature-controlled from room temperature to 70 °C
- Software-controlled syringe pumps for automated fluid flow and exchange

Applications

- Single-molecule flow stretching
- Drag force measurements
- Biochemical triggering using sub-second buffer exchange
- Enhanced experimental control

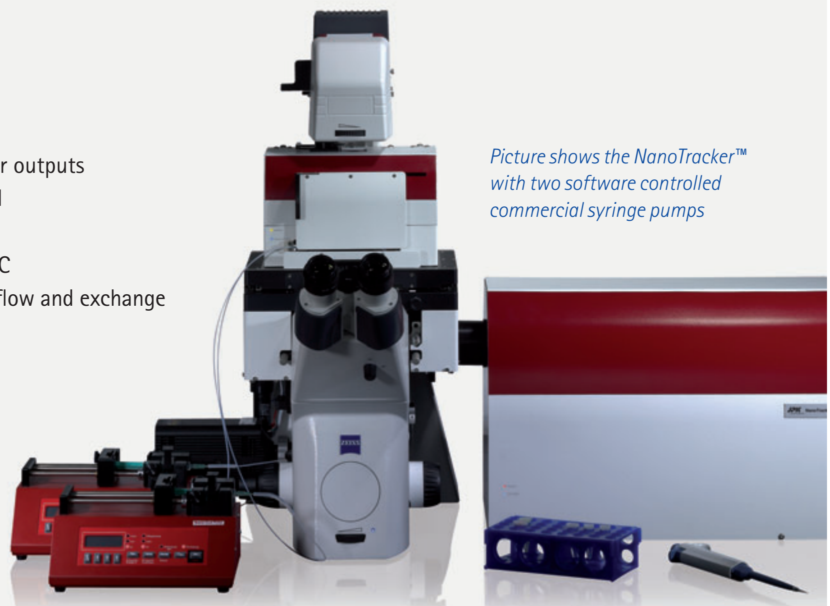
JPK PetriDishHeater™ for live-cell applications

Experiments involving live cells critically depend on the ability to optimize their environment. The PetriDishHeater™ allows control of the temperature of the medium as well as gas perfusion of samples mounted in Petri dishes. In the open geometry of such a Petri dish, a water-dipping objective is typically the detection objective of choice.



Key features

- Designed for Live Cell imaging
- Accommodates 35 mm Petri dishes
- Compatible with Petri dishes from standard suppliers such as BD, Corning and Wilco
- Heating from room temperature up to 60 °C with 0.1 °C precision
- Perfusion control employing standard syringe pumps and CO_2
- Drift minimized design



Picture shows the NanoTracker™ with two software controlled commercial syringe pumps

For an Unlimited Variety Of Applications.

Optional modular items for enhanced flexibility

- Single-beam/dual-beam configuration
- Detection unit: equipped as single-beam or dual-beam unit or video tracking only
- AODs for even 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
- Laser configuration: output power, non-Gaussian beam modes
- Choice of microscope objectives: magnification, immersion medium, TIRF

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
- Thermal fluctuations tracking in 3D of Brownian motion
- Microrheology mode
- Optical sorting
- Trap oscillation
- Multiplexing for 3D trap arrays
- Line traps & circular traps
- Microfluidics control
- Nanoassembly
- Optical z-stacking

Applications

Single biomolecules & biopolymers

- Intramolecular elasticity & protein folding dynamics
- Motor protein tracking
- DNA/RNA mechanics
- Protein-DNA interaction
- Nanopores & 3D polymer network probing

Cell biology applications

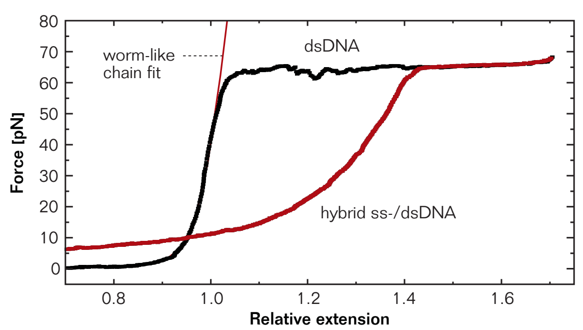
- Membrane organization (e.g., lipid rafts)
- Trans-membrane processes, trafficking
- Cell mechanics and cell motility
- Membrane tether dynamics
- Microrheology of cells and gels

Cell-particle interaction and infection studies

- Nano-toxicity & endocytosis studies
- Local gene or drug delivery
- Entrance mechanism studies
- Tracking of pathogen-host interaction

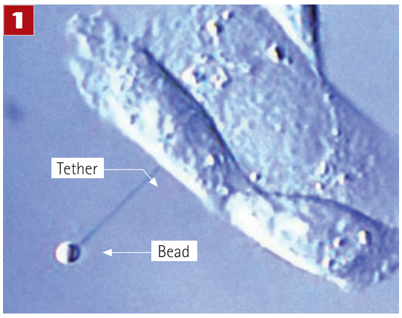
Advanced optical techniques

- Complex optical trap geometries
- Optical guiding & artificial crystal building
- Local field enhancement & Raman/SERS applications
- Video particle tracking

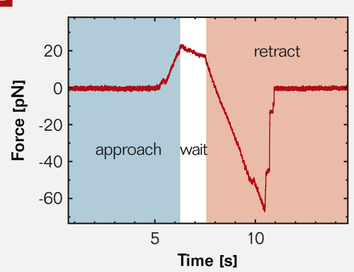


DNA elasticity measurement using the NanoTracker™. As the DNA is pulled to larger extensions, it undergoes several phases of distinct elastic behavior. The red line is a fit to the so-called worm-like chain polymer model. The overstretching transition, typical for double-stranded DNA, is readily seen. 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.

1



2



1 *DIC image of a CHO cell with a membrane tube pulled by an optically trapped protein coated microsphere.*

2 *Corresponding force vs. time plot from picture 1. Approach and retract curves are shown. The retract curve shows a bond rupture in several steps.*

(Cells courtesy of A. Herrmann's lab, Humboldt University, Berlin.)

NanoTracker™

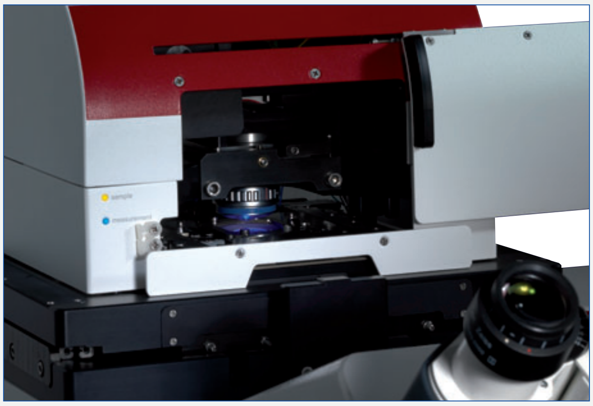
Technical Specifications

System overview

- Innovative 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 IX70/71/80
- Compatible with all major light microscopy techniques such as DIC, fluorescence and confocal techniques such as FRAP and LSM
- 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 (laser class 1) and no alignment by hand
- Optional external standard modules such as confocal units, CCD cameras, detectors

NanoTracker™ 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 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
- Patent pending objective exchange mechanism
- Precise focus adjustment via software



NanoTracker™ sample holder options

- Standard slides, cover glasses or Petri dishes can be used
- Multichannel LaminarFlowCell (LFC) is a coverslip-based fluid cell with temperature control and laminar flow perfusion capabilities for single-molecule applications
- Living cell studies with temperature control (ambient to 60 °C), perfusion and gas flow (CO_2) for 35 mm Petri dishes with glass bottom from Wilco, BD, and Corning

NanoTracker™ laser unit

- Ultra-stable custom-designed laser (1064 nm)
- < 0.05 % intensity stability
- 3 W laser power (different options available)
- Laser class 1

NanoTracker™ steering unit

- True 3D fast and continuous beam steering through the full field of view
- Fully independent steering of 2 beams with adjustable power distribution
- XY steering by galvano mirrors and Z steering by fast linear drive
- 500 µsec response time for steering unit
- AOD option available for one beam

NanoTracker™ 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 in/output channels
- TTL access and power supply for external equipment
- High-speed Ethernet link and intelligent grounding concept for maximum bandwidth and performance

NanoTracker™ 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
- 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

Opened NanoTracker™ head with mounted sample

JPK Instruments

JPK Instruments AG

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

www.jpk.com