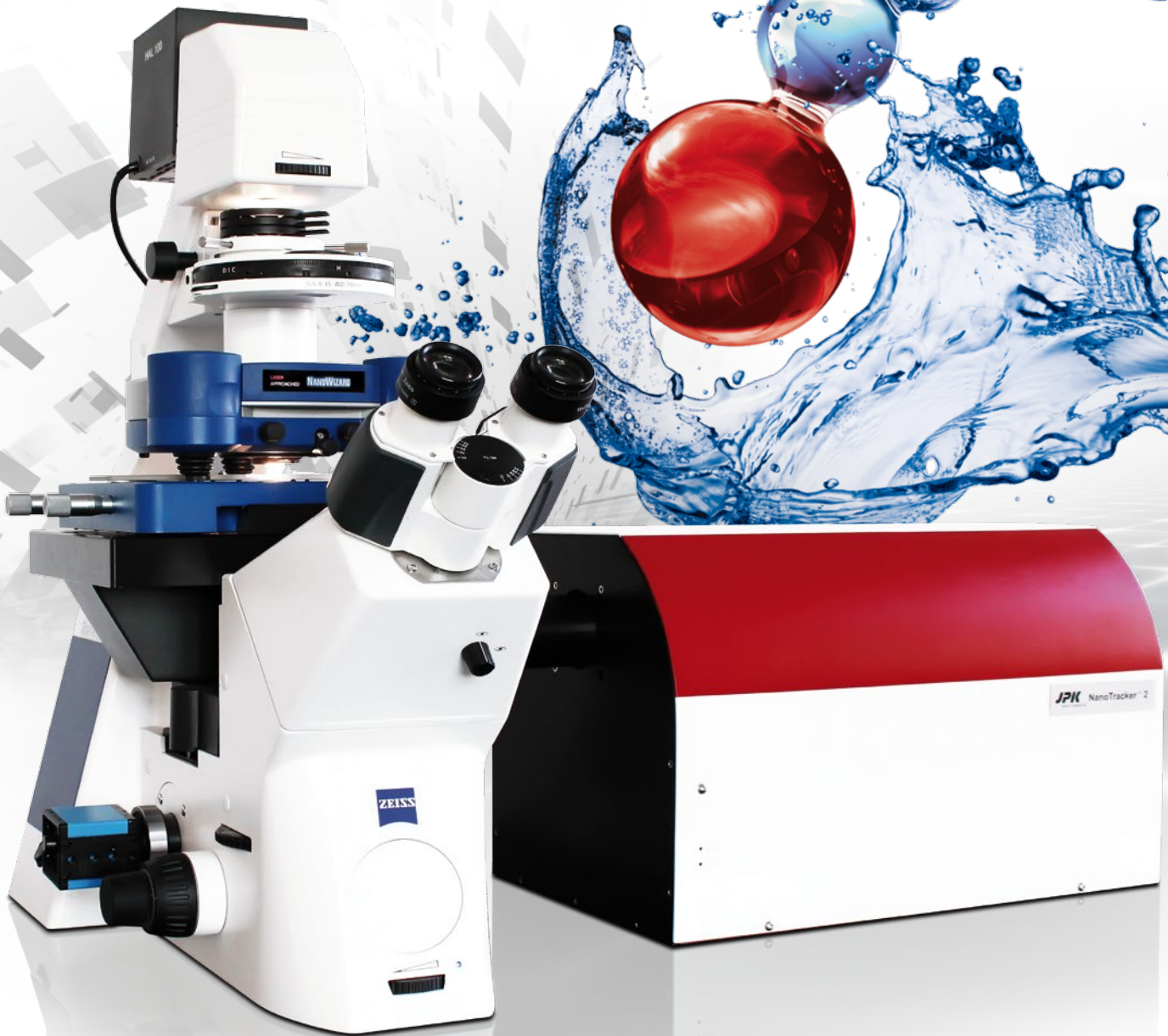


Powerful Optical Tweezers & AFM Combination: NANOTracker[®] & NANOWizard[®]

OT-AFM COMBI SYSTEM



Imaging, positioning, and manipulation experiments from single molecules to living cells

Measure forces in 2D and 3D, from 500 fN to 10 nN, on the same sample

Fully flexible and modular design, with the widest range of modes and accessories

Comprehensive integration with optical microscopy techniques such as TIRF and confocal

NANOWizard® AFM COMBINED WITH NANOTracker™ OPTICAL TWEEZERS

EXTENDING THE FORCE RANGE FROM NANONEWTONS TO FEMTONEWTONS

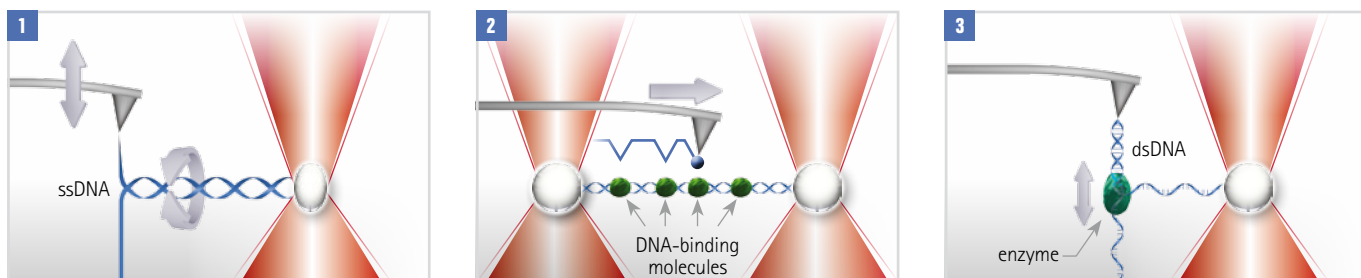
JPK pairs the exceptional surface force measurement and imaging capabilities of AFM with the ability of optical tweezers to apply and measure smallest forces in 3D. The unique combination of 3D positioning, detection, and manipulation provided by OT and the high-resolution imaging and surface property characterization of AFM opens up a whole new spectrum of applications.

SINGLE-MOLECULE APPLICATIONS WITH UP TO 14 DEGREES OF FREEDOM

With a multitude of available handles, interaction, and detection sites, OT-AFM significantly extends the range of single-molecule applications.

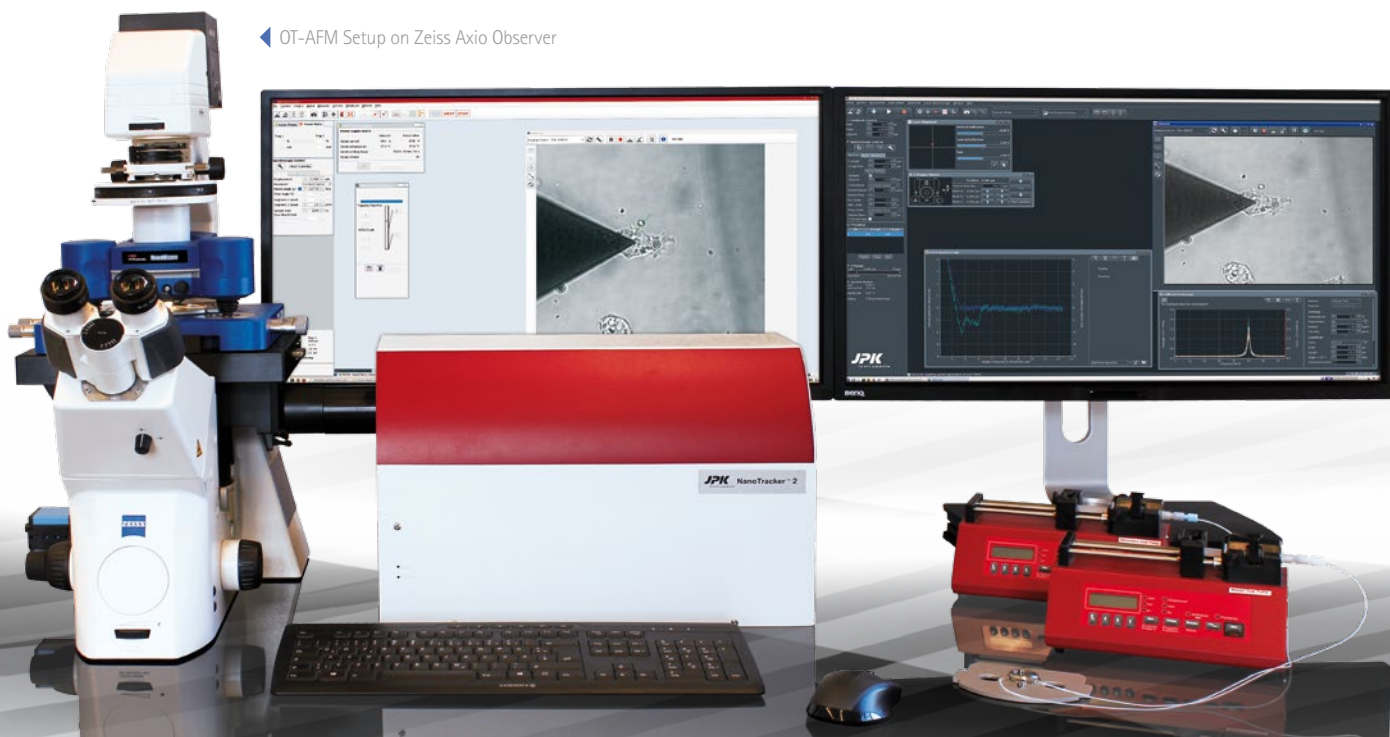
NANOWIZARD® AND NANOTRACKER™ TEAM UP – THE PERFECT TOOLBOX FOR IMAGING AND FORCE APPLICATIONS

The combined setup fulfills the highest demands on mechanical stability, flexibility, and modularity. A specially designed OT-AFM ConnectorStage™ is the key to combining any AFM of the NanoWizard® or CellHesion® family with the NanoTracker™ optical tweezers on a research-grade inverted optical microscope. JPK's established hardware and software integration of high-end optical methods like TIRF or confocal fluorescence microscopy with both AFM and OT provides correlated data easily. Dynamic processes can be controlled with the non-invasive power of light, while data is being simultaneously collected with the AFM. Single molecules and living cells can be manipulated in 3D with additional degrees of freedom in force measurement. Dual force measurement applications are supported by JPK's camera-based OT force detection.



1 DNA hairpin unzipping (AFM) while the optical trap can be used to suppress (high laser power) or to quantify rotation (low laser power). **2** Scanning of a decorated DNA molecule. The molecule with DNA binding proteins (green) is spanned between two optically trapped beads. A functionalized AFM tip (blue) scans along the molecule and whenever interactions between the DNA-attached proteins and the tip occur, these can be detected in the AFM and OT signals. **3** Monitoring of DNA-enzyme (e.g. polymerase, helicase) dynamics. With one strand attached to an optically trapped particle, the step-wise motion can be tracked. Closed-loop force clamping allows maintaining a constant force on the single strand.

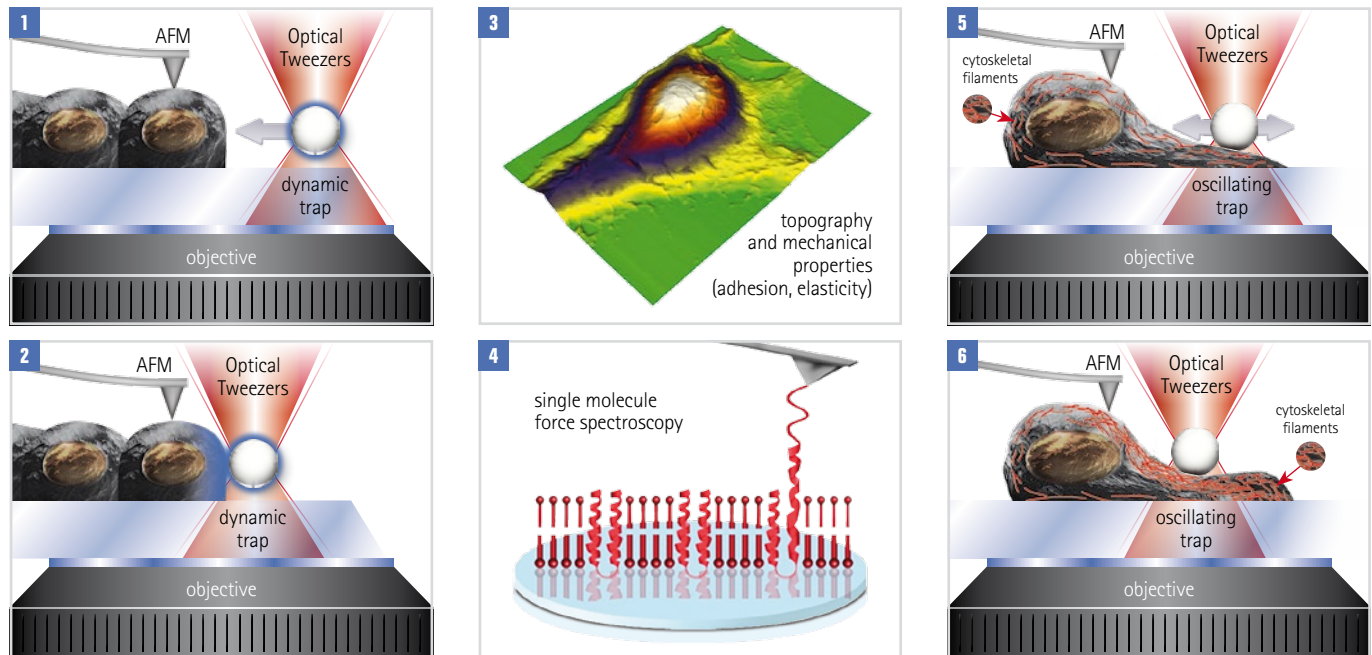
◀ OT-AFM Setup on Zeiss Axio Observer



THE BEST OF TWO WORLDS NOW IN ONE SETUP

SPECTACULAR LIVE CELL APPLICATIONS WITH THE OT-AFM SYSTEM

Cellular response, cell-cell or cell-matrix interactions, immune response, infection or bacterial/virus/nanoparticle uptake processes are just some of the examples that can be investigated with JPK's new state of the art OT-AFM platform. JPK's proven AFM and OT core technologies, combined with fluorescence microscopy, have set the ultimate benchmark for live cell applications.

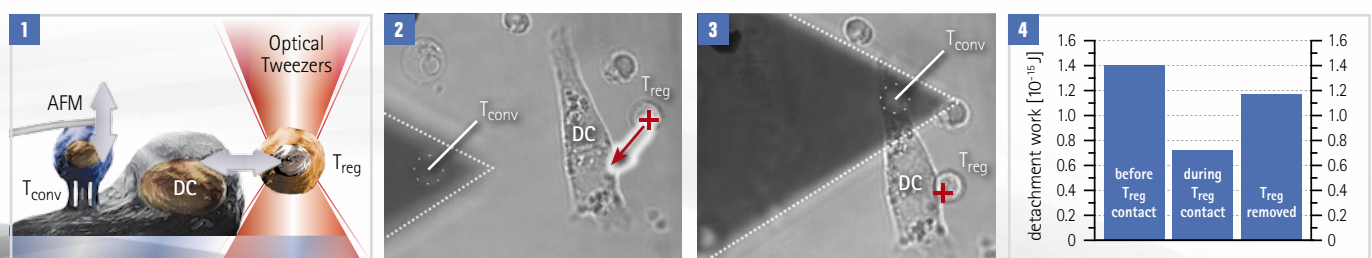


1 + 2 Activation of cells with functionalized beads, parallel AFM measurement. Signaling molecules on the surface of a microparticle are brought in contact with the cell at defined positions and time points. **5 + 6** A mechano-sensitive cell is stimulated by a periodic force, exerted by an optically trapped particle. Internal rearrangements of the cytoskeleton alter the mechanical properties of the cell. These properties are easily accessed with AFM methods like force mapping or JPK's Quantitative Imaging Advanced (QI™-Advanced). **3 + 4** AFM can be used in parallel to monitor changes in the cell structure, e.g. by monitoring mechanical properties throughout the process or by molecular recognition force spectroscopy that investigates the distribution and mechanical behavior of membrane proteins.

TRIGGERING IMMUNE SIGNALING THAT AFFECTS CELL ADHESION

Triggering cellular responses by using functionalized particles or modified microorganisms is a common method. The resulting changes in cellular structure, dynamics, and mechanical properties can be investigated using AFM-based methods. However delivering objects to specific regions of interest on the cell is very difficult to achieve. OT provides the perfect tool for manipulating the sample and triggering cellular response, at a precise time and location. This significantly improves the throughput, flexibility, and reproducibility of these studies. In this application, the influence of signaling between dendritic cells (DCs) and regulatory T-cells (T_{reg}) on the adhesion of conventional T-cells (T_{conv}) to the same DC is quantified by OT-AFM.

1 Adhesion experiment with dendritic cells (DC) and conventional T-cells (T_{conv}). The T_{conv} is attached to a tipless cantilever, then approached to the surface-bound DC. The cantilever is pulled up and the adhesion forces are measured. A regulatory T-cell (T_{reg}) is attached to and removed from the DC with optical tweezers to test its influence on the binding strength. **2 + 3** Measurement setup. The optical trap (red cross) moves the T_{reg} while adhesion measurements are performed with a cantilever-attached T_{conv} . **4** Detachment work measured for the three situations. T_{reg} attachment reduces DC- T_{conv} interactions. After the T_{reg} is removed, the adhesion level is almost restored. Sample courtesy of Yan Shi, University of Calgary/Tsinghua University, Beijing. The original experiment was designed by Yan Shi et al. (publication in print).



SPECIFICATIONS FOR THE NANOWIZARD® / NANOTRACKER™ COMBI-SYSTEM

Combined system specifications

- All NanoTracker™, NanoWizard®/CellHesion® 200 systems can be combined to become a complete OT-AFM-platform
- The ConnectorStage™ can combine the AFM with the tweezers hardware on all major inverted optical microscopes from Zeiss, Olympus, Nikon, Leica
- Two separate controllers drive the two systems independently
- NanoWizard® AFM controller with software
- NanoTracker™ OT controller with software
- Compatible with different sample holders and stages for every application
- Motorized precision stage for sample positioning
- Manual precision stage for sample positioning
- TAO™ module with 2 or 3 axis for sample positioning or scanning
- Compatible with different coverslip sample holders
- BioCell™ with perfusion and heating/cooling
- PetriDishHeater™ for standard Petri dishes with perfusion and heating capabilities
- CoverslipHolder with perfusion
- Transmission illumination with a standard condenser for brightfield or DIC
- Simultaneous fluorescence imaging (epifluorescence, TIRF, confocal)
- Real-time position/force detection in the optical trap via particle tracking in the live video image
- User-programmable software
- Powerful Data Processing (DP) functions with full functionality for data export, fitting, filtering, edge detection, 3D rendering, FFT, cross section, etc.

NANOWIZARD® AFM

AFM specifications for combined system

- Tip-scanning system for undisturbed optical integration
- High-resolution and low noise system for imaging and force measurements
- High performance sample property mapping with JPKs unique QI™ mode
- Liquid-safe scan head technology perfect for live cell measurements
- Unique design for transmission illumination with standard condensers

For details see www.jpk.com or NanoWizard® brochure.

NANOTRACKER™ 2

OT specifications for combined system

- Ultra-stable, custom designed laser with 3W, 5W or 10 W output, continuous power adjustment
- Single beam or dual beam configuration
- AOD option for fast scanning and up to 250 traps with multiplexing
- 3D trap positioning
- Modular design provides flexible hardware upgrade paths

For details see www.jpk.com or NanoTracker™ 2 brochure.

NanoWizard, CellHesion, TAO, BioMAT, NanoTracker, ForceRobot, Vortis, DirectOverlay, HyperDrive, ExperimentPlanner, ExperimentControl, RampDesigner, ForceWatch, TipSaver, HybridStage, BioCell, SmallCell, ECCCell, HTS, HCS, HCM, TopViewOptics, PetriDishHeater, QI, ForceCube, and ConnectorStage are trademarks or registered trademarks of JPK Instruments AG.

