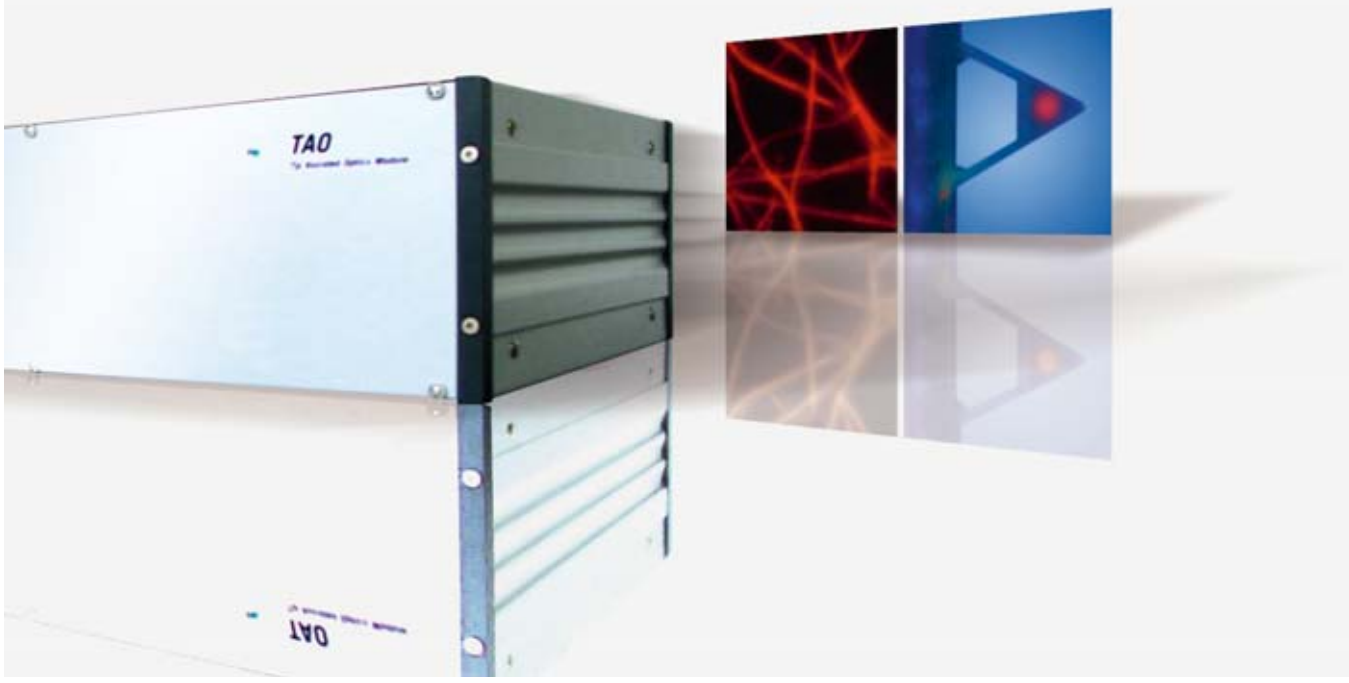
TAO™. Tip Assisted Optics Module for AFM. With Five Degrees of Freedom at the Peak of Advanced Single Molecule Research.



JPK Instruments

Nanotechnology for Life Science

Tip Assisted Optics. The M

Microscopy to a New Leve

Many of today's research activities are centered on studies at the level of individual molecules. Most of them employ tried and tested methods, but achieving meaningful results at the nanometer scale is challenging for both users and technologies. This is particularly true for demanding experiments using Atomic Force Microscopy (AFM) in combination with optical spectroscopy. Examples of this technique include TERS (Tip Enhanced Raman Spectroscopy) and scattering-type SNOM (Scanning Nearfield Optical Microscopy), but also methods for measuring FRET (Förster Resonance Energy Transfer), combining nanomanipulation of individual molecules with confocal fluorescence spectroscopy.

These methods – relatively new and usually the exclusive domain of specialists – are generally performed only with home built systems and are difficult to adapt to novel configurations. With the new TAO™ system, however, JPK offers an instrument that combines all important AFM components for such research in an integrated system and allows optics experts to enter this field without the need to develop deep AFM expertise or even their own AFM setup.

The TAO™ is integrated into the modular JPK concept, allowing you to choose an entire system, or to keep your options open by updating existing systems with a wide variety of additional components such as BioCell™, the patent pending coverslip based mini-incubator.

lost Advanced AFM Technol

el of Performance.

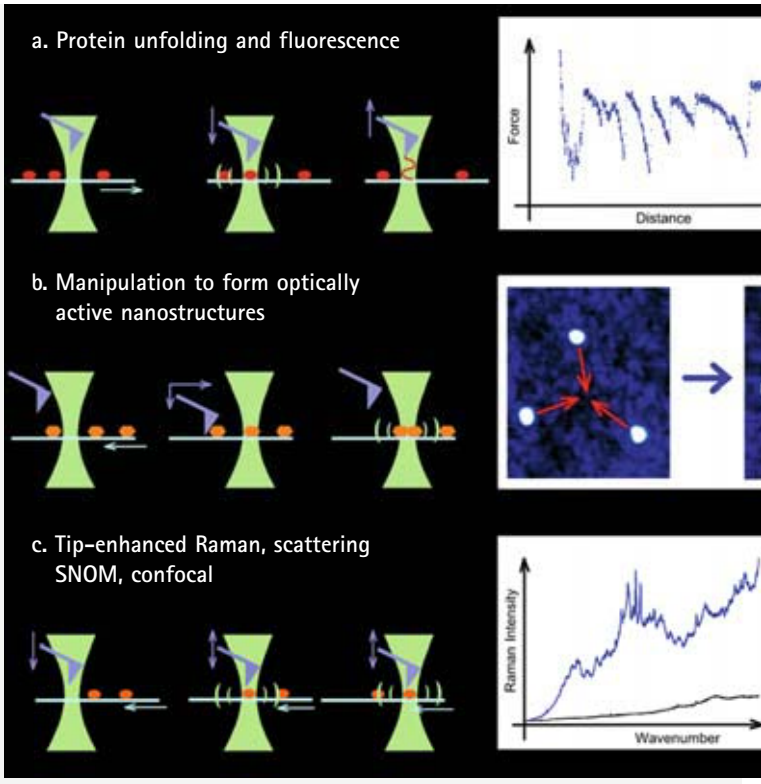
High performance AFM and optical spectroscopy: more than the sum of two parts – unlimited possibilities

The NanoWizard® opens up the entire spectrum of applications made possible by modern AFM technology. From high-resolution imaging to force spectroscopy, from nanomanipulation to nanolithography. From determining the morphology of cell surfaces, unfolding or stretching of DNA, and other single molecules to the measurement of material characteristics such as stiffness, elasticity and adhesion. In ambient or liquid environments, without the need for time-consuming freezing, staining or coating of the samples.

The TAO™ is a significant enhancement to the capability of the high-end AFMs from JPK, giving decisive advantages for single molecule optics with confocal detection. With the help of the AFM, classical optical spectroscopic methods can be expanded to the nanoscale, opening up applications in chemical analysis, advanced optical spectroscopies and for the combination of AFM force spectroscopy with optical methods such as FRET. Bringing the tip into the focus enables a whole new class of experimental methods that are demanding and rewarding at the same time.

Unequalled in precision and resolution: TAO™ dual scanner technology

Spatial resolution at the subnanometer level, excellent linearity and precise positioning – the power of the scanners in the integrated TAO™ system comes from piezo elements of PI * (Physik Instrumente) that have been custom-developed for JPK. The parallel kinematic scanners with friction-free flexure systems guarantee the best results in dynamics,

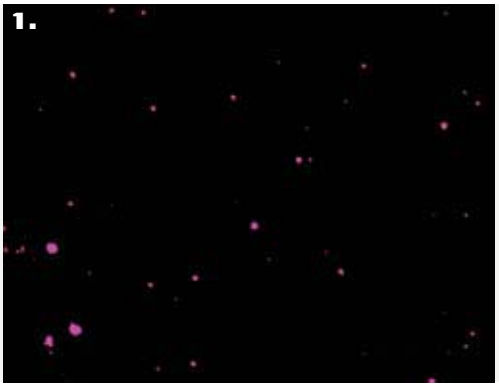


a. Molecules such as proteins can be positioned in the laser focus for simultaneous optical and mechanical unfolding measurements using the AFM tip.

b. Colloids or quantum dots can be manipulated with the tip to form optically active nanostructures in the laser focus. Scan area 500 nm x 500 nm.

c. Aligning the tip in the laser focus enables sample-scanning confocal measurements or tip-enhanced Raman spectroscopy (Raman data courtesy of V. Deckert, ISAS Dortmund and J. Popp, University of Jena, Germany).

** PI, headquartered in Waldbronn, Germany, is the world's largest supplier of piezoelectric nanopositioners and scanning systems, and a leading manufacturer of parallel kinematic components.*



ogy Brings Optical

resolution, and reproducibility. All axes are equipped with integrated capacitive sensors – a guarantee for absolute positioning accuracy and long-lasting stability during measurements. A positioning stability in the nanometer range and manipulation capabilities at the true nanoscale are the results of this uncompromising approach. The complete software integration with a multitude of operation modes enables the full capability of the hardware while leaving sufficient room for the user creativity and custom-designed experiments.

Key factor for cutting edge applications: system stability

Stability is a prerequisite for the highest possible resolution in AFM. All TAO™ components are designed that the entire platform – including the inverted optical microscope – will perform optimally, even during long-term experiments and with changing temperatures. The scanning is virtually drift free – especially important for TERS experiments where the laser has to remain precisely focused on the tip for a long time.

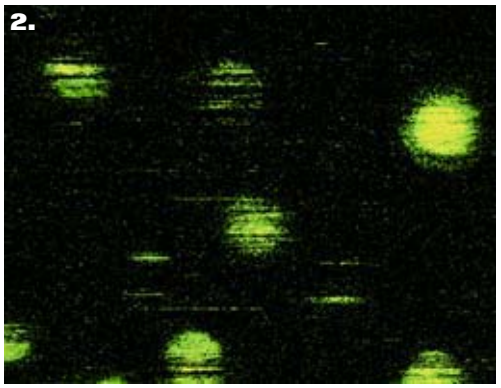
The results are convincing: highest-resolution images and absolute stability lead to peak performance for cutting-edge applications. A critical point in combining AFM and fluorescence is the cross coupling from the AFM deflection detection laser with other optical signals, e.g., fluorescence. The JPK infrared laser system for the cantilever deflection detection and special filters in the NanoWizard® AFM head, ensures not only fluorescence free of cross coupling and a complete suppression of the LED background emission but also avoid interference effects in force spectroscopy.

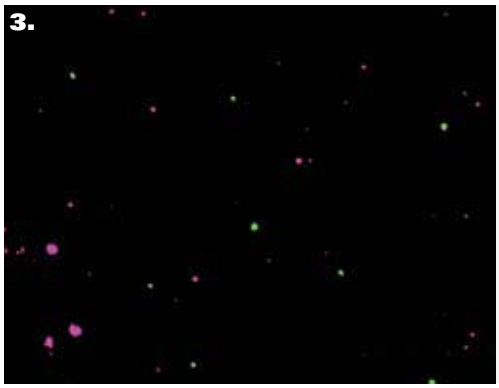
TERS: Tip Enhanced Raman Spectroscopy

Raman Spectroscopy and SERS (Surface Enhanced Raman Spectroscopy) are established methods in chemical analysis that until recently have been limited in spatial resolution. With the TAO™ system, however, Raman spectroscopy can now be extended to a true nanoscopic scale. In TERS measurements the excitation laser is coupled into an inverted microscope and focused through a high numerical aperture objective towards the AFM tip.

By full software control the NanoWizard® scan head positions the AFM cantilever tip into the focus with nanometer precision. Maintained in stable position, the metal coated AFM tip induces a local electromagnetic field enhancement which can increase the Raman scattering signal dramatically. The sample is then scanned, exposing the sample point by point to the excitation field close to the tip. The scattered signals are detected by a Raman spectrometer in parallel to this movement.

Software synchronization allows a semi-automatic data acquisition during these time consuming experiments. TERS enables the identification of the finger print of single molecules, in thin films or even biological materials in air or in liquid. It brings the sensitivity and spatial resolution of Raman measurements to a next generation level.





1. AFM height image of quantum dots. Scan area 7.5 µm x 10 µm.

2. Fluorescence image of the same area showing blinking of the quantum dots.

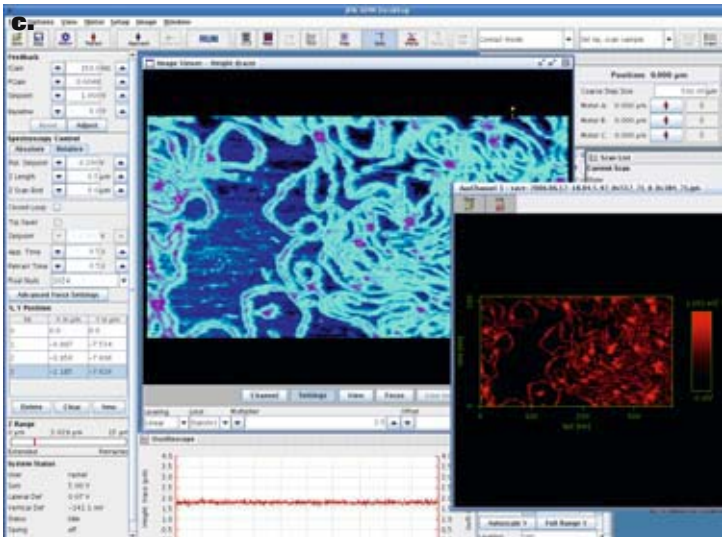
3. AFM topography with the quantum dots in regions of fluorescence highlighted.

Data courtesy of C. Heyes and G.U. Nienhaus, University of Ulm, Germany.

Tip scanner meets sample scanner: TAO™, the ultimate 5 axis system

TAO™ – Tip Assisted Optics – is the answer to cutting edge needs in the nanosciences. The TAO™ system consists of an electronics module, software and an additional scanner integrated in the NanoWizard® AFM stage. As a modular component of the NanoWizard® AFM system, it opens up almost unlimited possibilities with its unique combination of sample scanner (TAO™ Stage) and tip scanner (NanoWizard® AFM head). These independently controllable scanners provide a maximum of movement in a total of 5 axes: 100 µm x 100 µm x 15 µm in x, y, z (tip scanner in the head) plus 100 µm x 100 µm in x, y (sample scanner in the stage) – and this with full integration in inverted optical microscopes for techniques such as DIC, optical phase contrast, fluorescence, confocal laser scanning, TIRF, FRET and many other.

To reach the ultimate performance in optics simultaneous to the AFM, the use of coverslips is a prerequisite. Only with JPK's coverslip liquid cells (BioCell™ or CoverslipHolder) is it possible to obtain the best results in optical experiments and the highest resolution AFM in air or liquid. There is no room for uncertainty during single molecule AFM experiments and simultaneous single molecule fluorescence. The precise mechanical design and high performance scanner technology diminishes long term drift and noise that limit experiments in conventional designs.



a. TAO™ controller, front and backside view with BNC and digital connections

b. TAO™ stage with implemented x, y sample scanner and JPK CoverslipHolder liquid cell

c. Screenshot of the JPK SPM user interface

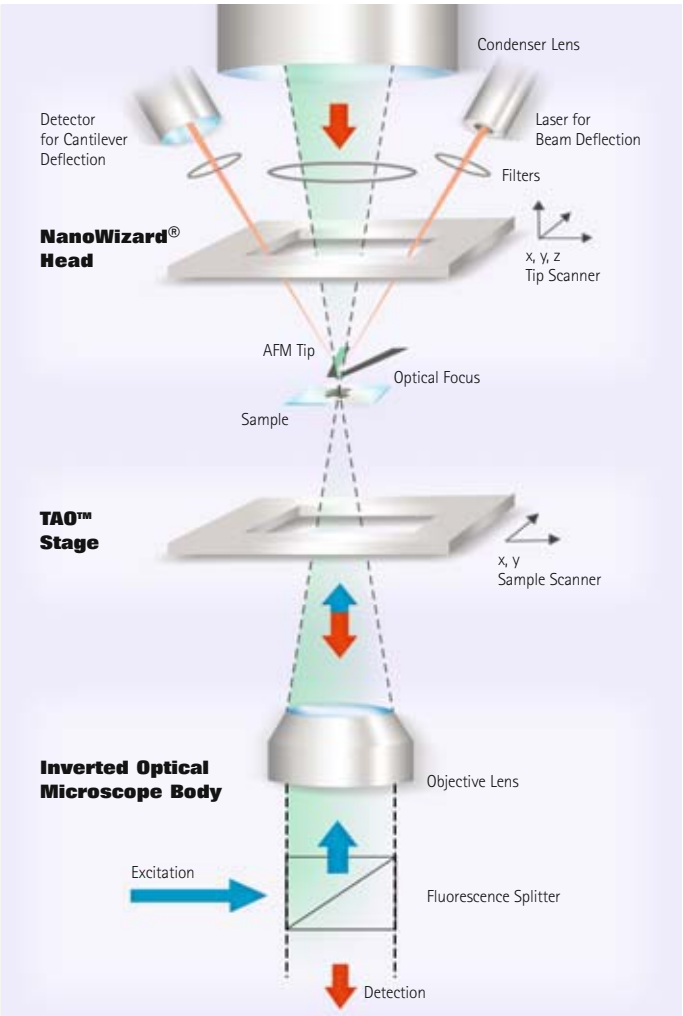
Single molecule FRET and Nanomanipulation within a confocal spot

FRET is a highly sensitive method for determining conformations and interactions between molecules such as proteins, lipids, sugars, DNA or other polymers. As it is used in liquid environments, the TAO™ system is a perfect match for extending this technique. For experiments at the level of individual molecules, fluorophore-labeled donor and acceptor molecules can be bound to a molecular chain, with a laser beam used as a photonic exciter.

Using both scanners of the TAO™, the molecule as well as the tip can be put to the confocal detection spot with an extremely high accuracy. By suitable chemical conditioning of substrate and tip, the molecule can be caught between them. By slowly moving the cantilever away from the surface the molecule is stretched in the vertical direction while the intra molecular forces are continuously measured with the AFM.

Events in the stretching force curve like unfolding events are therefore detected together with the changes in the fluorescence characteristics of the donor-acceptor system. This allows researchers to observe and measure distances, interactions, and even structural changes on single molecule level with the functional information of the optical spectroscopy at the same time. Of course those measurements are performed under native conditions, with temperature control, and an easily variable chemical environment.

Another type of advanced spectroscopic experiment is the manipulation of labeled nanoobjects or particles such as DNA molecules, proteins, quantum dots or other within an optical focus. The interaction between these objects leads to different optical properties depending on their geometric orientation such as shifting of excitation wavelengths, blinking, or quenching and leads therefore to a deeper understanding of optical phenomena on the sub-wavelength scale.



TAO™: peak performance at a glance

- Unique combination of tip and sample scanner
- Ultimate flexibility through simultaneous control of 5 axes with nm closed-loop precision (linearized with capacitive sensors):
- Tip scanner: 100 µm x 100 µm x 15 µm
- Sample scanner: 100 µm x 100 µm
- Imaging, force measurement, manipulation, mechanical characteristics plus spectroscopic applications at the level of individual molecules
- Simultaneous confocal optical and topographic information
- Precise compensation of the offset between confocal optical and AFM image
- Proven NanoWizard® technology
- Works in air and in native environments (real in-situ measurements in fluids)
- Synchronisation of external devices such as spectrometers via pixel clock and line clock TTL outputs

JPK Instruments

JPK Instruments AG

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

www.jpk.com