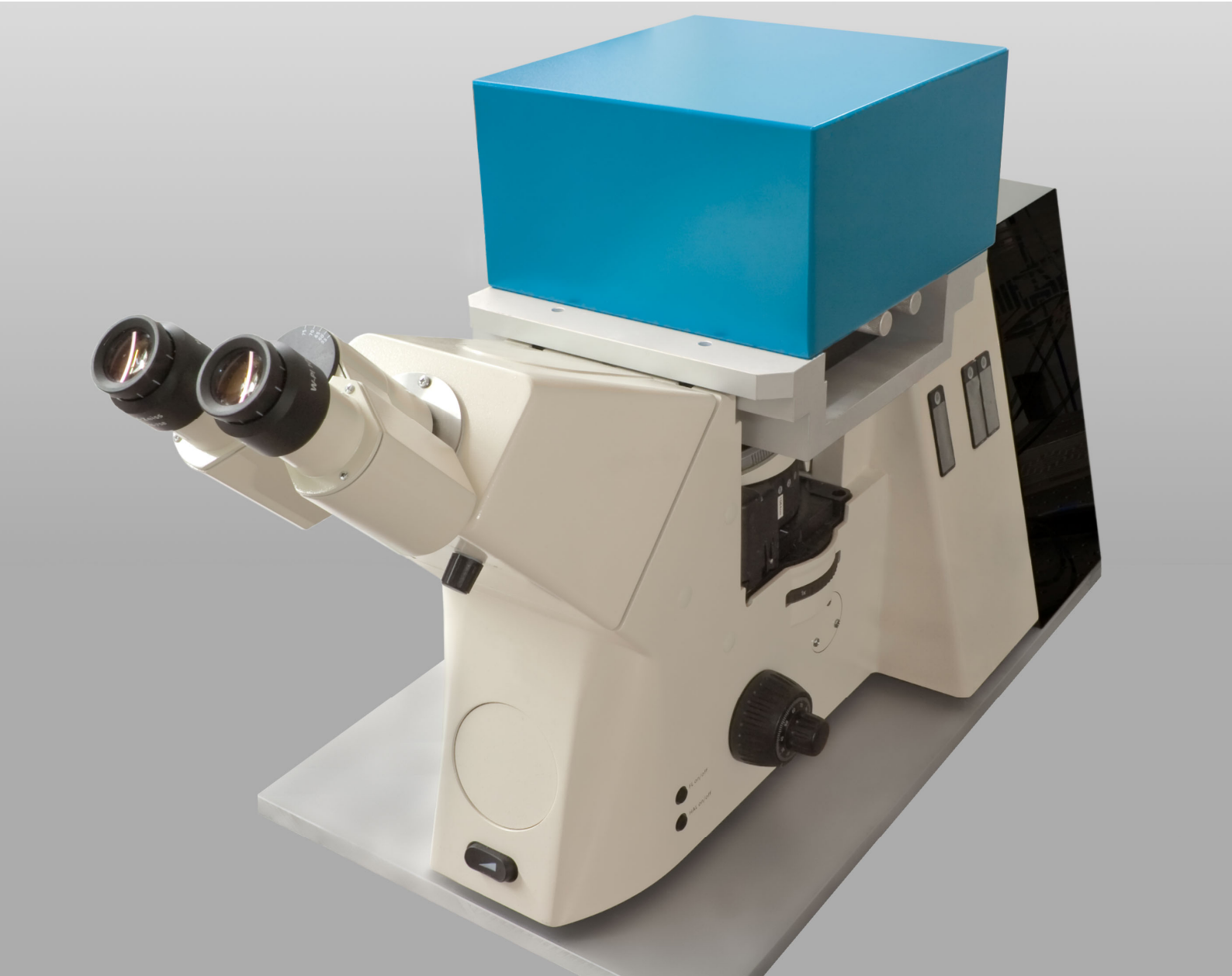
# PicoFocus

## FORCE SENSITIVE OPTICAL TWEEZERS

- Quantitative 3D Force Measurements with 0.1 pN Resolution
- Achievable Trapping Force of 400 pN
- Manipulation of Trapped Objects with Nanometer Precision
- Compact and Ultrastable Modular System
- Programmable LabView™ Software Interface
- Easy-to-use Force Calibration without Detector Alignment
- Scope of Applications: from Single Molecules to Living Cells
  from Polymer Elasticity to Microfluidics
  from Molecular Interactions to Nanopores

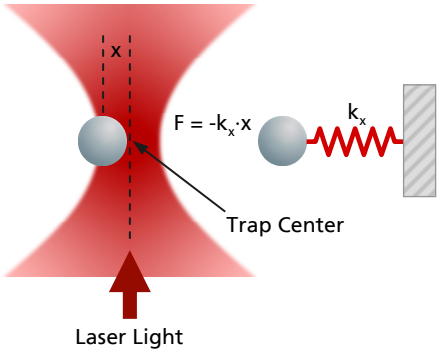


# Optical Trapping and Force Measurement

Optical tweezers are used to trap and actively manipulate microscopic objects. They also offer a vast area of applications by measuring forces applied to trapped objects.

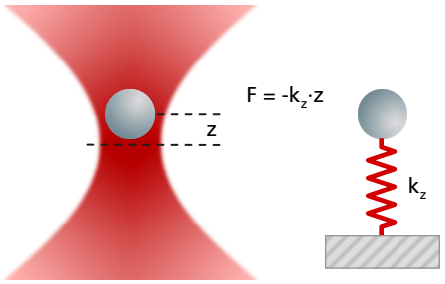
### The Optical Trap

Microscopic objects - like individual nano- or microparticles, cells, cell compartments, single or clustered molecules - can be trapped securely inside the center of a strongly focused laser beam.
When an external forces is acting on the trapped object, it deflects from the center of the trap as the deflection *x* depends linearly on trap stiffness *k* and force *F*.




### Forces

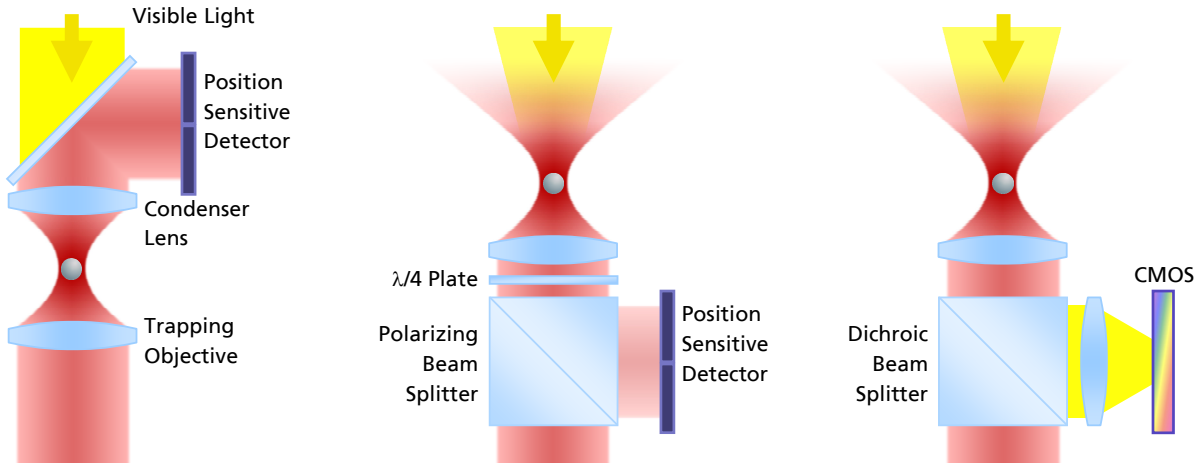
A trapped particle experiences various external forces. Atoms or molecules of the surrounding medium induce Brownian motion in all three dimensions, depending on temperature, viscosity and the presence of obstacles in the vicinity. Macroscopic fluid movements cause a drag force. Electric fields and bulk or surface charges may generate electrophoretic or electroosmotic forces.
Particularly, single molecules can induce forces of broad variety and magnitude while bound to the trapped object. On the other hand, the application of a force generated by an optical trap to a single molecule will gain vast insight into the molecular structure and elasticity, binding properties and kinetics.




*Lateral and axial forces acting on a trapped particle.*

### Deflection is the Essence

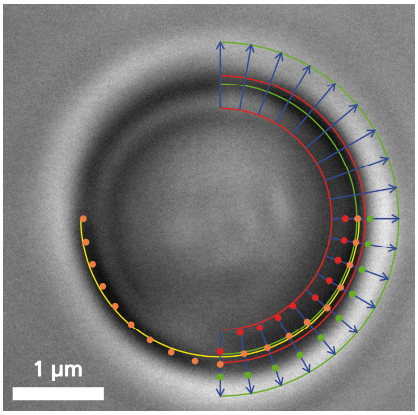
Generating and metering various forces requires a reliable force measurement capability in all three dimensions to allow for a maximum degree of experimental freedom and versatility. Therefore, force detection is accomplished by precisely measuring the deflection of the trapped particle in each direction.
**PicoFocus** utilizes a sophisticated and easy-to-use video analysis (right image below) for particle tracking, detection and force measurements. It offers the largest field of application since it clears common calibration difficulties, system instabilities, as well as experimental and spatial restrictions.




*The evolution of force measurement. Left: Laser light passing trough the trapped particle (forward scattered light) is collected and projected onto a detector sensing the particle's deflection. The condenser in close proximity to the trapping objective needs to be precisely adjusted. It is susceptible against drift and misalignment and limits the experimental space. Center: Backscattered light from the particle is collected by the trapping objective, separated from the incident laser light and projected onto the detector. This extremely robust setup allows high experimental freedom – the same applies for video-based detection method in the right image, where no detector alignment is required, too. In addition, a high diversity of trapped particles can be video-analyzed and measured.*

# Video Detection and Analysis

Video-based force detection is easy to calibrate and offers an alignment-free and unsusceptible method for all force measurements in three dimensions. It is embedded into the LabView™ platform.



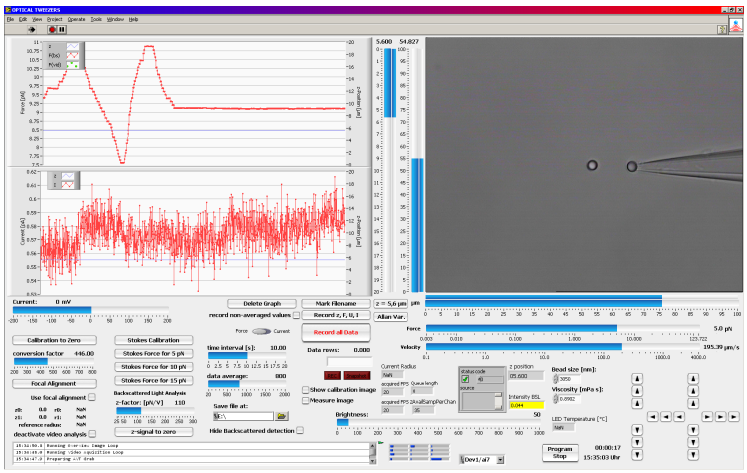

*Video frame of a trapped microbead with various overlaid detection lines.*

### The Principle

In addition to a video camera imaging the surrounding area of the optical trap, a second high-speed CMOS camera simultaneously surveys the magnified image of the trapped particle. The software searches for specific edges (as shown in the upper right quadrant of the image) in each frame and in real-time, determines gradients (lower right quadrant) and fits a circle (lower left quadrant) which correlates to the apparent particle diameter.
If an external axial force is acting on the particle, its apparent diameter changes, which the software translates into a z-force. Lateral forces only shift the center of the particle. These lateral deflections in the order of nanometers are then translated into x- and y-forces.

### Easy and Reliable Force Calibration

Force calibration in three dimensions is conducted by moving the surrounding medium via the piezo stage using Stokes' drag force law.
For specific applications, video-based force detection utilizes Allan Variance analysis and calibration. Here, smallest particle fluctuations are recorded and analyzed without the need of applying any frictional force. Both Stokes' method and Allan Variance do not require the determination of the trap stiffness *k*, though the video-analysis software can calculate it if desired.



*LabView™ based trapping, calibration and measurement software.*

### Benefits of Video-Based Force Detection

There is no need of detector alignment or adjustment in the beginning or during experimentation because the CMOS camera providing data for video detection and analysis is integrated into the optical pathway between laser and optical trap. Video detection is unsusceptible to disturbing particles that occasionally may be trapped together with the measured object. Since the diameter of the trapped object is permanently monitored, further particles of interest can be trapped and compared with previous ones. Specifically tailored Allan Variance for video analysis is a powerful calibration tool for experiments that take place in an environment that prevents other calibration or analysis methods. When trapping particles close to interfaces (bottom or ceiling of sample chamber, artificial or biological membranes, etc.), video analysis delivers an interference-free force signal.

### Detection Tandem

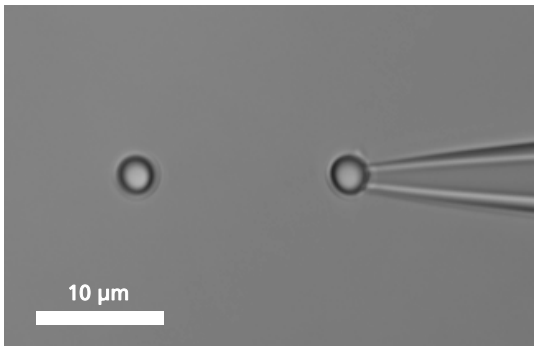
Optionally, **PicoFocus** can be equipped with additional backscattered light detection capability for simultaneous measurements or as stand-alone method, if experiments need to be conducted in absence of light or if particle fluctuations must be analyzed with highest sample rate in the kHz range.

# Applications – Single Molecules and Polymer Elasticity

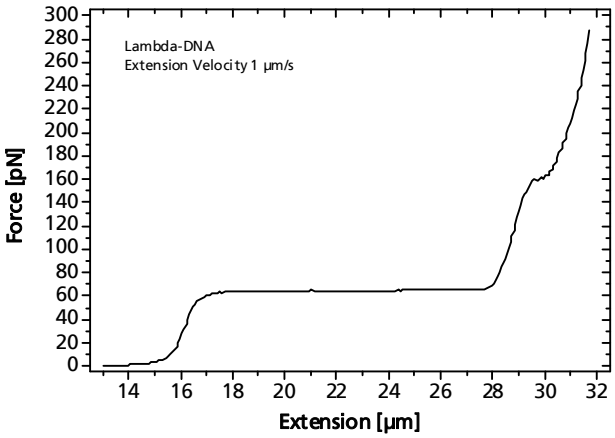
The elastic behavior of a single DNA-strand in absence or in presence of binding ligands can be reliably measured. Theoretical polymer models that are fitted to the results will deliver parameters, which characterize the polymer elasticity.

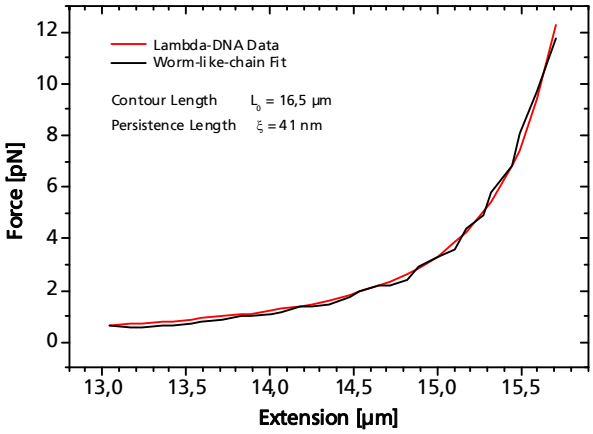
### Grabbing a Single Molecule

To bind a single DNA-strand between two coated microbeads, it has to be properly functionalized on both ends. Thus, it can be immobilized between two beads, of which one is optically trapped and the other is held on the tip of a micropipette. Increasing the distance between the beads by moving the piezo stage induces a controlled mechanical tension to the DNA.
As a response, the force-extension curve of the molecule exhibit characteristic mechanical properties, such as an entropic elasticity, an overstretching plateau and a melting transition region.

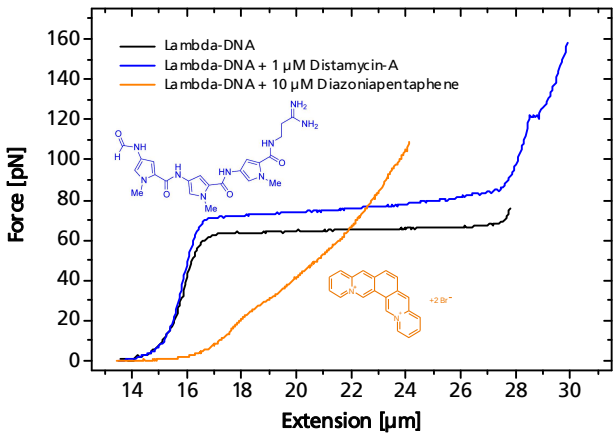



*A single DNA is immobilized between two microbeads.*




*Left: Force response of a single 48502 base pair long DNA molecule of bacteriophage lambda. In the force range up to 10 pN the entropic regime determines the elastic behavior of the molecule, whereas around 65 pN the characteristic overstretching transition occurs. The nature of this phenomena remains controversial, as well as for a less pronounced transition at 160 pN. Right: Fitting the Worm-like-chain model to the entropic regime yields two intrinsic elasticity parameters. For example, the persistence length strongly depends on salt concentration and on the presence of DNA-binding ligands.*



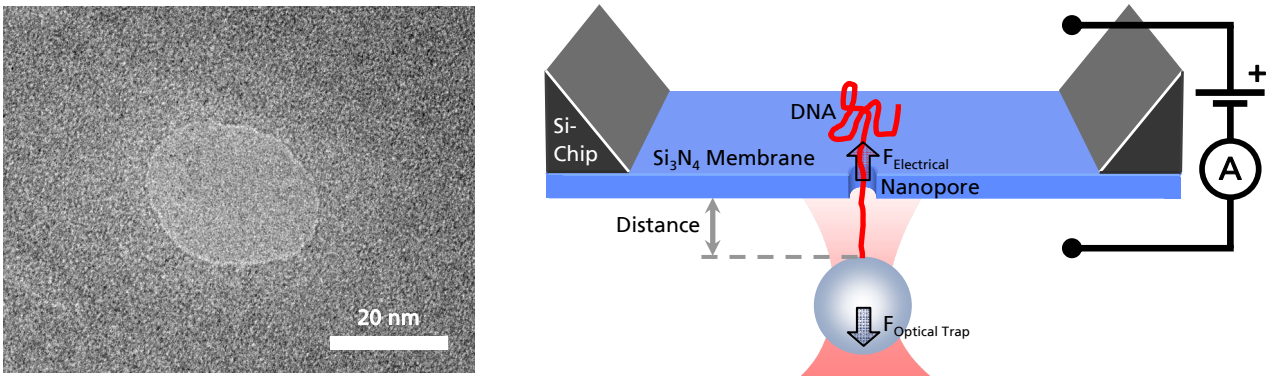

*Small or large variations in the elastic response of a DNA-molecule in the presence of binding ligands can be measured.*

### DNA as a Sensor for Foreign Molecules

The DNA strand can serve as a host for a variety of different molecules, such as small intercalators, groove-binders, proteins, enzymes or molecular motors.
The binding event of a single or a multitude of ligands can change the elastic response more or less significantly. As an example, the force curve of a DNA is shown in presence of the antibiotic distamycin-A that attaches to the minor groove of the DNA strand while stabilizing it and helping to resist the overstretching. On the other hand, diazoniapentaphene as an intercalator increases both contour and persistence length and renders the overstretching plateau to disappear.

# Applications – Translocation through Nanopores

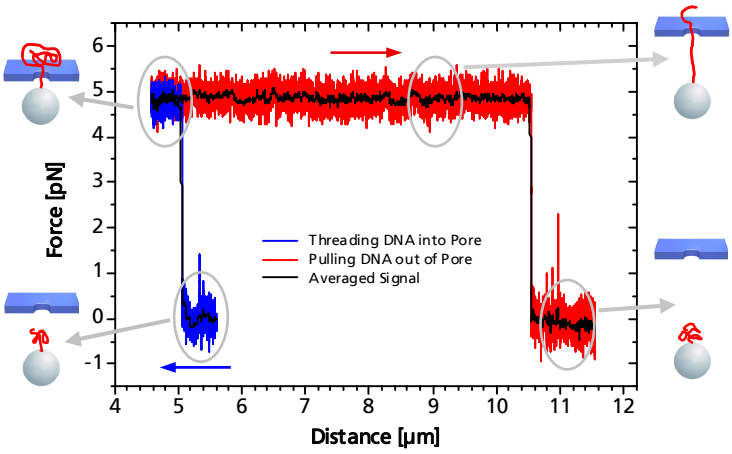
Nanopores play a major role in biology and they rapidly evolved into a new and promising technique in single-molecule detection. The controlled threading of a single DNA molecule or a DNA-protein complex into a nanopore allows investigation of the translocation dynamics and a localization of the bound protein.



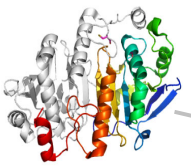

*Left: TEM image of a solid-state nanopore drilled with a focused ion beam machine into a $Si_3N_4$ membrane that serves as model system to study single molecule translocations. Right: Experimental setup of a DNA translocation measured with optical tweezers. When applying a voltage across the membrane, a single DNA molecule immobilized on a trapped microbead translocates through the pore. The electrostatic force acting on the molecule and the distance between bead and nanopore can be precisely measured.*

### Translocating a Single DNA Strand

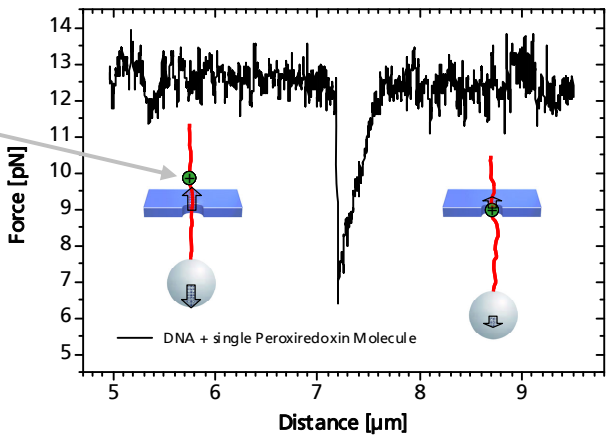
When the DNA on the trapped bead approaches the nanopore (to a distance of 5 µm) it is immediately threaded into the pore by electrostatic forces acting on the negatively charged DNA backbone. This effect can be monitored as an abrupt step of the force signal to a certain value, which remains constant even when retracting the bead. The measured force depends on the applied voltage, as well as on the diameter of the nanopore.
When the entire DNA strand with an end-end-distance of 10.5 µm is pulled out of the pore, the force drops back to zero.




*Controlled DNA threading into a 55 nm solid-state nanopore with an applied voltage of 50 mV.*



Peroxiredoxin plays a role in the defense against oxidative stress, decomposing reactive oxygen species and turning them into signaling events.




*Characteristic force signal of a single peroxiredoxin molecule bound to a DNA strand when both are translocated through a 35 nm nanopore.*

### Single DNA-Bound Protein

A distinct asymmetric force signal occurs when a single peroxiredoxin molecule bound to the DNA stand is actively pulled through the pore. This effect serves as a label-free localization of the protein binding site.
It can be understand as the result of an effective positive charge of the protein counteracting the negative DNA backbone charge and reducing the electrostatic force.

# We Design and Build Your Optical Tweezers.

**PicoFocus** is a stand-alone system, that can also be customized to your Zeiss Axiovert, Axio Observer A1 or D1. It will be equipped with a 1W or 3W IR fiber laser for highest spatial trap stability that deliver a trapping force of at least 400 pN or 1 nN, respectively. The 3D-piezo stage enables nanometer resolution in a range of 200 µm in x and y, as well as 20 µm in z-direction. Video-analysis can achieve a lateral and axial resolution of at least 2.5 nm, which results in a force resolution of 0.1 pN with a frame rate of 200 and 400 Hz in z- and x,y-direction, respectively.

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