Optical Trapping and Force Measurement

IONOVATION PicoTweezers

PicoTweezers Technical Note 01

The Optical Trap

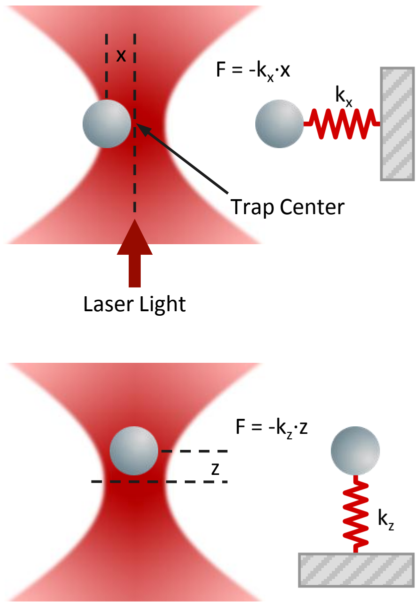
Microscopic objects - like individual nano- or microparticles, cells, bacteria, cell compartments, or clustered molecules - can be trapped securely inside the center of a strongly focused laser beam.

When an external force is acting on the trapped object, it deflects from the center of the trap. The deflection depends linearly on trap stiffness *k* and force *F*.

Forces

Any trapped object 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 proximity. Macroscopic fluid movements cause drag forces. Electric fields, bulk or surface charges may generate electrophoretic or electroosmotic forces, too.

Particularly, single molecules bound to a trapped object can induce forces of broad variety and magnitude. The application of a force generated by an optical trap to a single molecule will gain vast insights into its molecular structure and elasticity, binding properties and kinetics.

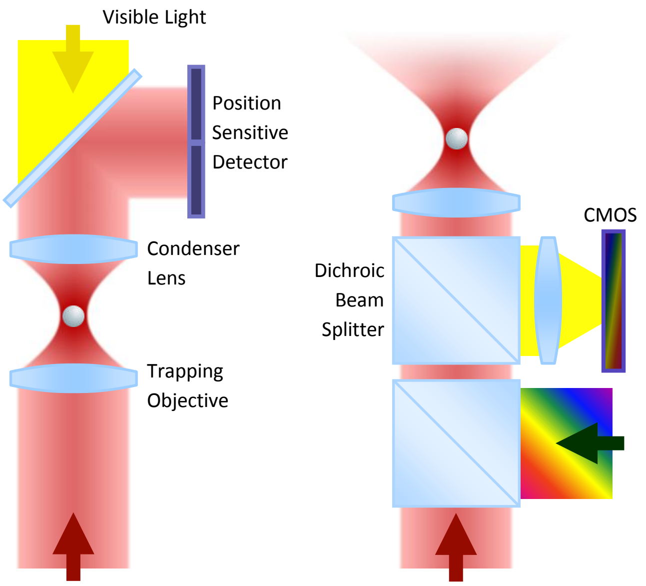


Lateral and axial forces acting on a trapped object.

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 accuracy and versatility. Thus, force detection must be accomplished by precisely measuring the deflection of the trapped particle in each dimension.

PicoTweezers utilizes a sophisticated and easy-to-use video analysis for particle detection, tracking and force measurements. It provides the largest field of application since it avoids common optical tweezers' calibration difficulties, system instabilities, as well as experimental and spatial restrictions.



Left: The common principle of force measurement. Infrared laser light (red arrow) 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 (ca. 1mm) to the trapping objective needs to be precisely adjusted. It significantly limits the experimental space and is susceptible against drift and misalignment.

Right: In video-based particle detection a great diversity of trapped particles can be video-analyzed and measured. The image of the trapped object - illuminated either by reflected or transmitted light - is directly monitored by a camera. This extremely robust setup allows high experimental freedom, where no detector alignment is required anymore, and an easy integration of fluorescence illumination can be established, too. Inside the system, the fluorescence excitation and emission light is reliably separated from the illumination light.

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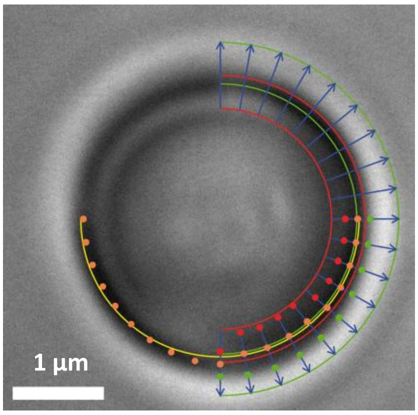
Benefits of Video-Based Force Detection

General Aspects of PicoTweezers

- Single or dual beam optical trap system integrated into an inverted microscope
- 3D real-time video-based force measurements with sub-pN resolution (400 Hz sample rate)
- Very fast, easy and reliable force calibration
- No detector alignment or adjustment is required in the beginning or during experimentation
- Achievable trapping force of 300 pN with 1 W-IR-Laser and up to 3 nN with 10 W-IR-Laser
- Manipulation of trapped objects with nanometer precision
- Compact and ultrastable modular system, extendable to fluorescence, STED, Raman, TIRF, or CLSM
- Programmable LabView™ software interface
- Customizable to other microscopes

The entire video detection is integrated into the optical pathway below the optical trap. Since the diameter of the trapped object is permanently monitored, any particles of interest can be trapped, analyzed or compared with previous ones on demand. Video detection is unsusceptible to disturbing particles that occasionally may be trapped together with the measured object. The video analysis delivers an interference-free force signal, even when particles of interest are trapped close to interfaces (bottom or ceiling of a sample chamber, artificial or biological membranes, etc.).



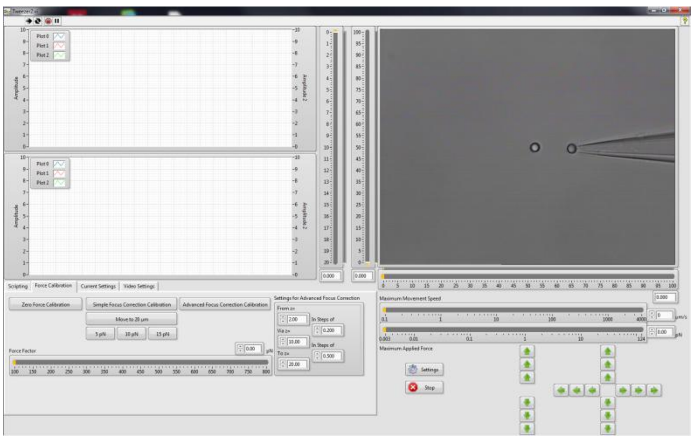
Video frame of a trapped microbead with different overlaid detection lines.

Video Detection and Analysis

The PicoTweezers system includes two cameras. The first camera is imaging the surrounding area of the optical trap, and the second high-speed CMOS camera simultaneously surveys the magnified image of the trapped particle. The software analyzes both the position and size of the particle in real-time by edge detection and translate them into a force.

Easy and Reliable Force Calibration

Force calibration in three dimensions is either conducted by an automated routine moving the surrounding medium via the 3D piezo stage using Stokes' drag force law, or by Brownian noise analysis using Allan variance.



LabView™ based trapping, calibration and measurement software.

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