

Quantitative force measurements with optical tweezers: The JPK NanoTracker™

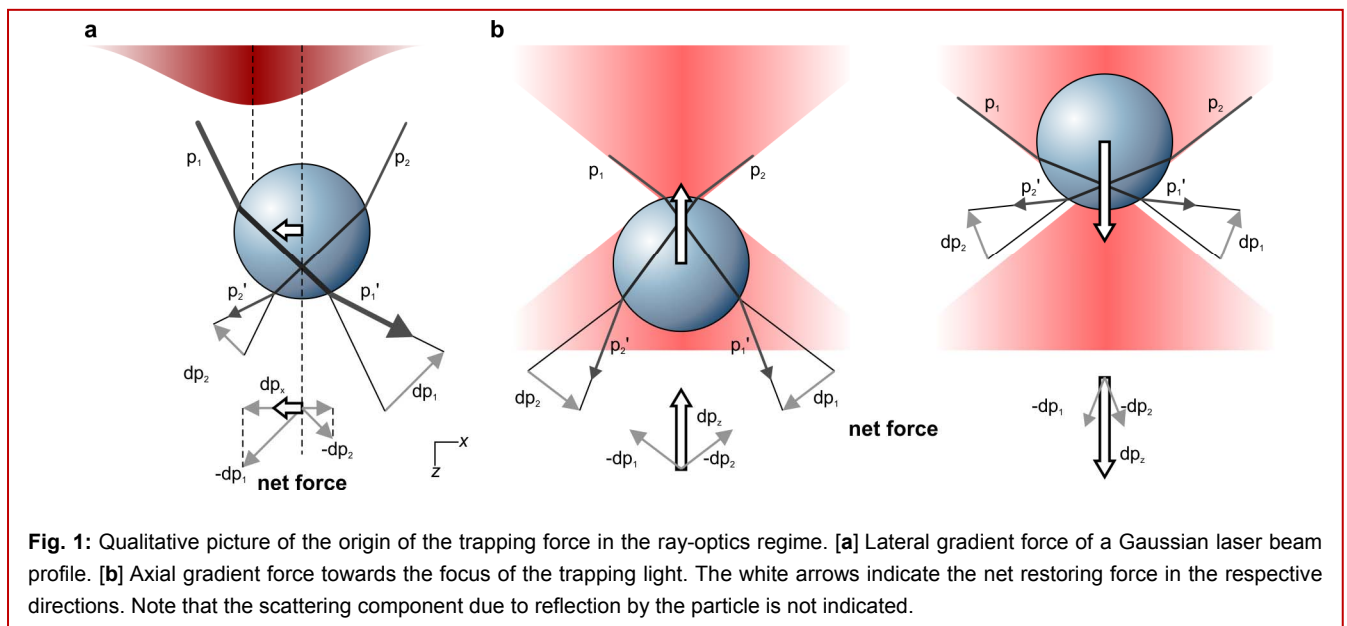
Introduction to optical tweezers

Optical tweezers methodology has developed from proof-of-principle experiments to an established quantitative technique in fields ranging from (bio)physics through cell biology. As the name suggests, optical tweezers are a means to manipulate objects with light. With this technique, microscopically small objects can be held and manipulated. At the same time, the forces exerted on the trapped objects can be accurately measured. The basic physical principle underlying optical tweezers is the radiation pressure exerted by light when colliding with matter. For macroscopic objects, the radiation pressure exerted by typical light sources is orders of magnitude too small to have any measurable effect: we do not feel the light power of the sun pushing us away. However, for objects of microscopic dimensions ($<100\ \mu\text{m}$) radiation pressure can have considerable effects. When tightly focusing a laser beam, using for example a microscope objective as a lens, the focus can be used to trap small objects in three dimensions: one speaks of an optical trap or optical tweezers. Optical tweezers have been used extensively not only to

manipulate biomolecules and cells, but also to directly and accurately measure the minute forces (on the order of fractions of picoNewtons) involved. Most often, the biomolecules of interest are not trapped themselves directly, but manipulated through functionalized microspheres.

The correct physical description of optical trapping depends on the size of the trapped object. One speaks of the 'ray-optics' regime when the object's dimension d is much larger than the wavelength of the trapping light: $d \gg \lambda$. In this case, diffraction effects can be neglected and the trapping forces of the light can be understood in terms of ray optics. The regime where $d \ll \lambda$ is called the Rayleigh regime. In this case, the trapped particles can be treated as point dipoles, as the electromagnetic field is constant on the scale of the particle. Obviously, if the laser is not focused, the particle will be propelled away due to the forward radiation pressure caused by light scattering.

In the ray-optics regime, the origin of the trapping force can be intuitively understood in terms of refraction of light rays between media with different indices of refraction. Figure 1 qualitatively depicts the origin of the trapping forces in this

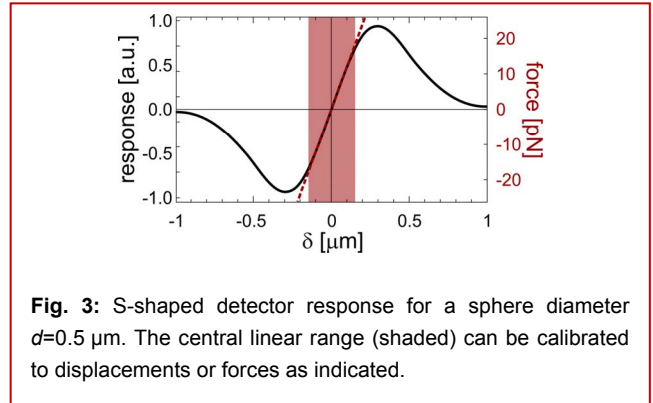
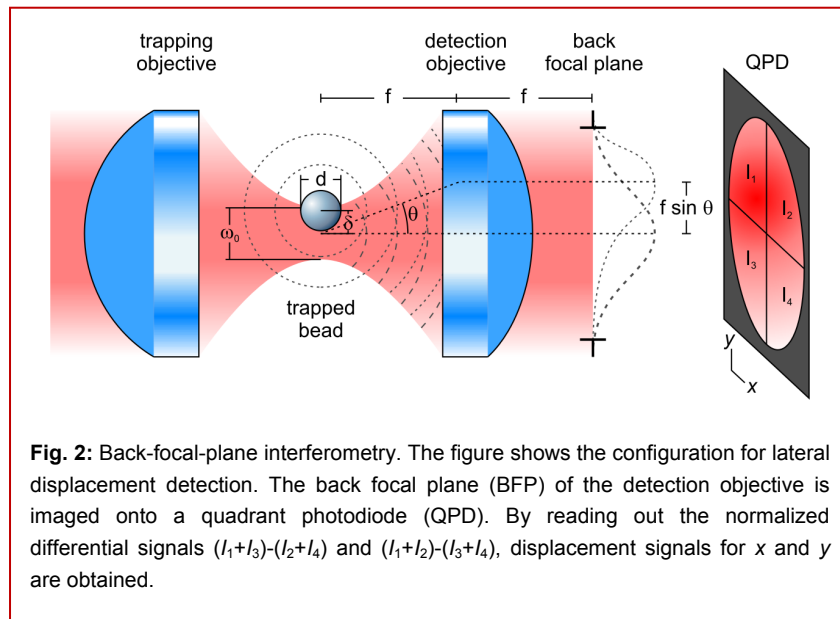


regime. The lateral gradient restoring force (Figure 1a) can be understood as follows: if rays p_1 and p_2 have different intensity, the momentum changes of these rays (Δp_1 and Δp_2 , respectively) differ in magnitude, causing a net reaction force on the refracting medium in the direction of highest intensity. The x -projection of this force Δp_x tends to counteract a displacement from the laser beam axis, pulling the particle to the center of beam.

The axial gradient force is similarly caused by momentum transfer upon refraction, resulting in a restoring force towards the focus, as in Figure 1b. The scattering force would cause the object to be propelled out of the focus (along the positive z -direction). The object is stably trapped only if the scattering force along the positive z -direction is compensated by the gradient force along the negative one. To achieve this, a tight focus is needed, with a significant fraction of the incident light coming in from large angles.

Measuring forces with optical tweezers

It is hard or even impossible to theoretically predict the force exerted by the trapping laser beam from first principles. However, it is possible to use the trapping light that is scattered by the object to get an accurate measure for external forces (i.e., other than the trapping force) acting



upon the trapped object. These external forces tend to push or pull the object from the center of the trap. The refractive object, in turn, acts like a little lens and refracts the rays passing through it. The far-field interference of the laser light with the scattered light from the trapped particle, collimated by a detection lens, can be used to get a sensitive measure for the displacements of the trapped particle from the focus. If the trap steering scheme is well-chosen, the intensity distribution in the back focal plane (BFP) of the condenser lens does not change when moving the optical trap around in the sample; the distribution is only affected by motion of the trapped object with respect to the laser focus. When imaging the light distribution in the BFP onto a position-sensitive detector, such as a *quadrant photodiode* (QPD: a light sensitive diode

which is divided into four equal segments), displacements of the particle in the trap can be measured. Figure 2 shows the configuration for the detection of the lateral particle displacement from the trap center. Similarly, the total light intensity arriving on the detector yields a measure for the axial particle displacement. This scheme for sensitive 3D displacement detection of a trapped object or the external forces acting on them is known as *back-focal-plane interferometry* [1, 2].

The S-shaped detector response for a microsphere displaced by a distance δ from the trap center is plotted in Figure 3 for a sphere diameter $d=0.5 \mu\text{m}$. As seen in the figure, for small displacements

($|\delta| < 150$ nm), the response is approximately linear (dashed line and shaded area).

If a force of known magnitude and direction is applied to the trapped microsphere and one records the corresponding response of the QPD signals, one can calibrate the signals to physically relevant units of either displacement or force. For instance, one can move the sample in which the trapped microsphere is suspended with constant velocity with respect to the position of the trap. The microsphere will be pulled out of the trap along with the sample motion due to the viscous drag of the fluid on the microsphere: for a fluid of viscosity η flowing with velocity v along a microsphere of diameter d , the force on the microsphere is $F = 3\pi\eta d v = \gamma v$, where γ is the drag coefficient.

Another, more accurate calibration procedure is to make use of the Brownian motion of the particle. The expected power spectrum of the diffusive Brownian motion exerted by a particle in a viscous liquid and held by a trap of stiffness κ has a Lorentzian shape:

$$S(f) = \frac{k_B T}{\gamma \pi^2 (f_c^2 + f^2)}$$

with $f_c = \kappa / 2\pi\gamma$ the characteristic roll-off or corner frequency of the trap. By fitting a measured, power spectrum of a trapped particle to this theoretical expression, both the detector sensitivity (in m/V) and the trap stiffness κ (in N/m) can be obtained. Hence, the detector signals can be calibrated either into nanometer displacements within the trap or picoNewton forces.

Experimental platform: JPK NanoTracker™

For the experiments in this report we have used the new JPK quantitative optical tweezers platform, the NanoTracker™, mounted on a Zeiss Axio Observer inverted optical microscope (see Figure 4). This compact system consists of a laser steering unit comprising two individually 3-dimensionally steerable beam paths, optimized for stability. The customized microscope body features both a long-distance motorized XY translation stage and a closed-loop 100x100x100 μm^3 piezo stage. On top, a dual-axis XYZ detection unit is positioned to independently detect 3-dimensional forces and displacements for the two traps. This detection is based on the interferometry scheme

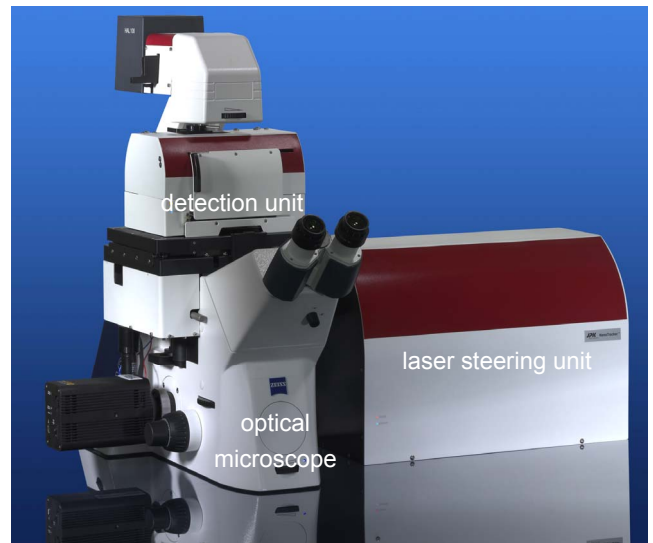


Fig. 4: The JPK NanoTracker™ optical tweezers platform.

described above. To increase sensitivity, separate detectors are implemented for lateral (XY) and axial (Z) detection. Moreover, axial detection is further enhanced by the application of an aperture stop to reject high-angle rays. The optical layout of the NanoTracker™ is schematically depicted in Figure 5.

Software-integrated calibration

The fully automated NanoTracker™ control and measurement software features a graphical user interface

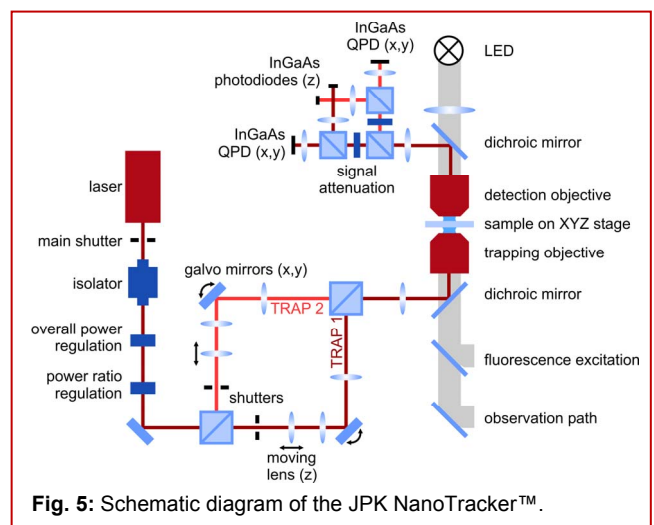


Fig. 5: Schematic diagram of the JPK NanoTracker™.

(GUI) that is easy to use for beginners yet still appropriate for advanced users. All hardware (motors, piezo stage, galvanometric mirrors, etc.) used for adjusting and steering of the optics and sample, including those for moving the two objectives, are conveniently controlled from this GUI.

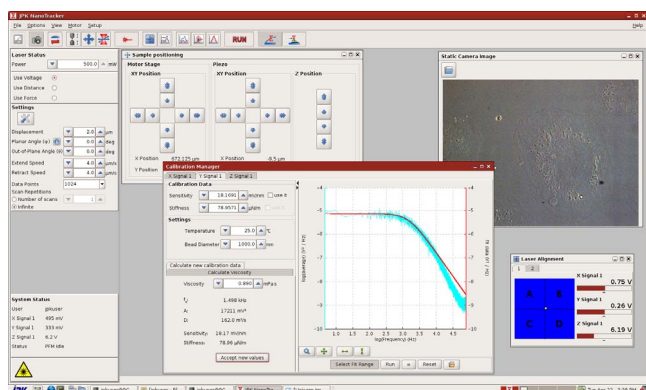


Fig. 6: Screenshot of the NanoTracker™ software, showing the online calibration manager.

The software includes the online high-bandwidth power spectrum calibration for all channels, which enables performing measurements and recording data in the units of choice. The calibration procedure relies on the latest Lorentzian curve fitting routines available in the literature [3].

Optical tweezers: down to sub-picoNewton

Measuring forces and displacements with optical tweezers can be done with very high accuracy over a wide range of forces. The typical forces exerted and explored by optical tweezers range between 0.1–300 pN. As such, optical tweezers are complementary to other force-sensing techniques such as atomic force microscopy, which is typically operated in a higher force regime.

It is important to realize that instrument resolution and sensitivity are intrinsically and fundamentally limited, independent of the instrumentation quality, by the physical principle of optical tweezers [4]. This is caused by the crucial interplay between probe stiffness (trap, cantilever, or other) and thermal fluctuations of a particle trapped in a viscous medium. The inherent variance of a force or displacement signal can be reduced by increasing the stiffness, yet at the expense of losing sensitivity.

Alternatively, signal filtering can increase the signal-to-noise ratio, yet at the expense of temporal resolution. For all single-molecule ‘nanomechanics’ experiments, this balance has to be well-chosen.

As an assessment of the power of force-sensing optical tweezers, a simple experiment was carried out. By moving the software-controlled piezo stage of the NanoTracker™ with constant velocity, the viscosity of the solvent induces a constant drag force on a trapped particle. As stated above, this force scales linearly with the flow speed. This renders it an excellent method to illustrate the sensitivity of optical tweezers. The piezo stage was moved back and forth with decreasing speeds, while the exerted forces were measured.

Figure 7 shows the result of this oscillatory stage movement for a particle dragged at three speeds. Whereas the steps in the signal are more and more obscured at lower drag forces, the filtered data retain a strong signature of the drag forces well below a picoNewton. This is even more clearly

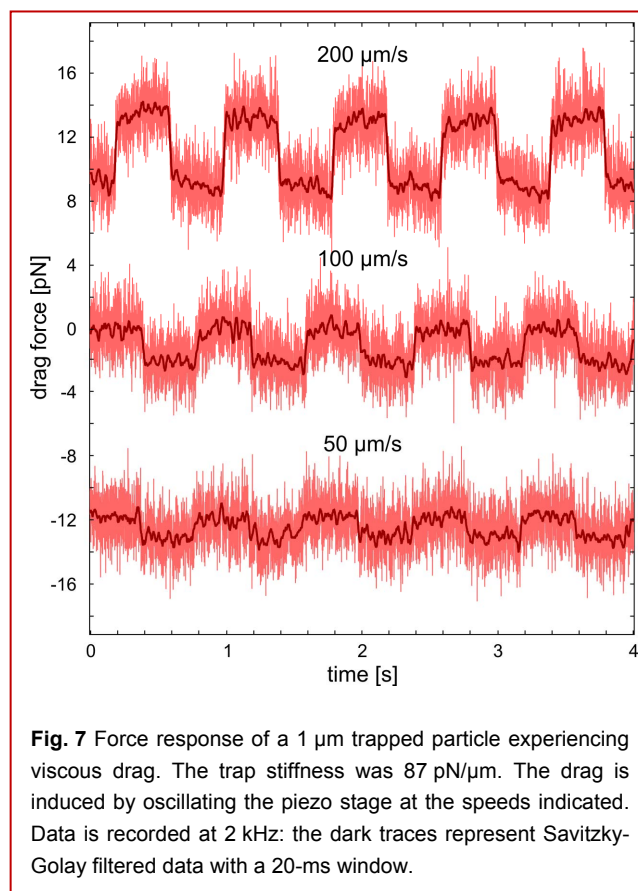


Fig. 7 Force response of a 1 μm trapped particle experiencing viscous drag. The trap stiffness was 87 pN/μm. The drag is induced by oscillating the piezo stage at the speeds indicated. Data is recorded at 2 kHz: the dark traces represent Savitzky-Golay filtered data with a 20-ms window.

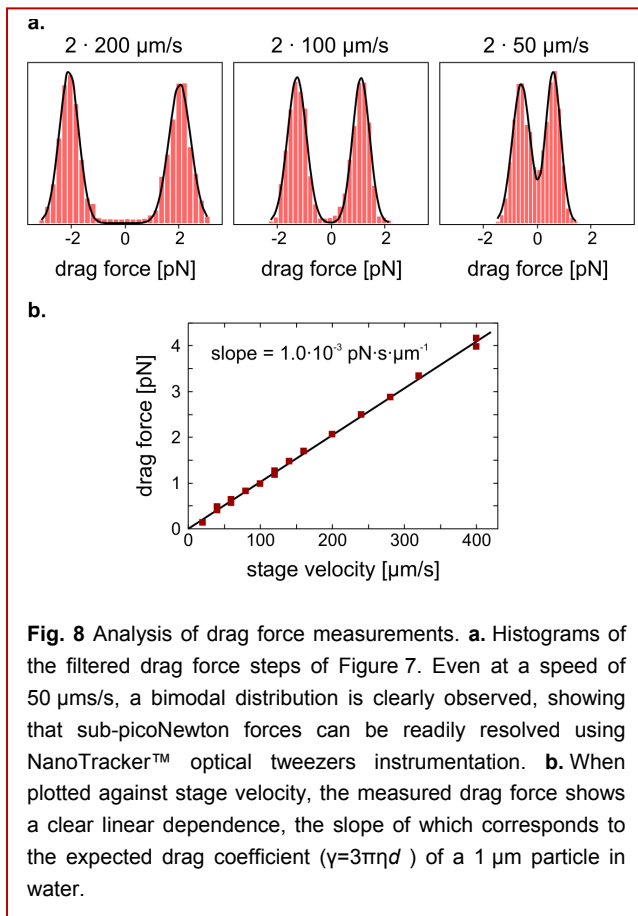


Fig. 8 Analysis of drag force measurements. **a.** Histograms of the filtered drag force steps of Figure 7. Even at a speed of 50 $\mu\text{m/s}$, a bimodal distribution is clearly observed, showing that sub-picoNewton forces can be readily resolved using NanoTracker™ optical tweezers instrumentation. **b.** When plotted against stage velocity, the measured drag force shows a clear linear dependence, the slope of which corresponds to the expected drag coefficient ($\gamma=3\pi\eta d$) of a 1 μm particle in water.

seen by binning the filtered data into histograms, as shown in Figure 8a.

Plotting the measured drag force for a range of velocities, the linear dependence is clearly revealed, reproducing the expected drag coefficient (Figure 8b) and thereby providing a direct control of the power spectrum force calibration.

Spatial resolution of optical tweezers

Depending on the application, optical tweezers can be alternatively applied to measure displacements from the trap center, rather than exerted forces. This can be employed for nanometer-resolution particle tracking, which, combined with the high bandwidth of quantitative optical tweezers, allows the real-time study of kinetic binding or motility.

As a demonstration of the high spatiotemporal resolving

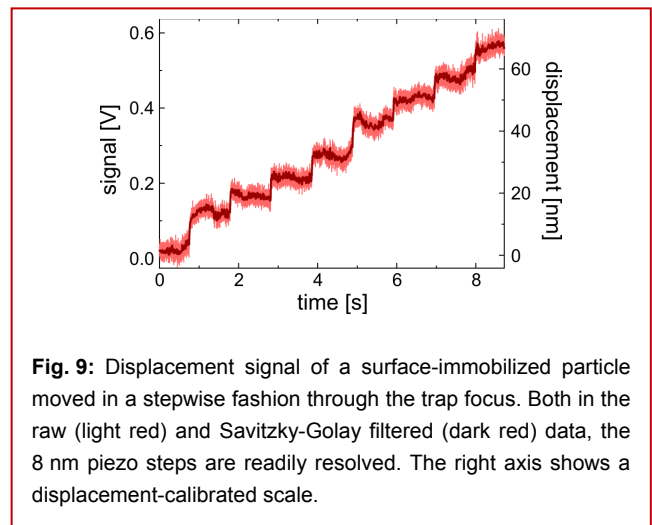


Fig. 9: Displacement signal of a surface-immobilized particle moved in a stepwise fashion through the trap focus. Both in the raw (light red) and Savitzky-Golay filtered (dark red) data, the 8 nm piezo steps are readily resolved. The right axis shows a displacement-calibrated scale.

power of optical tweezers, a 1 μm particle was immobilized onto the sample surface and scanned through the NanoTracker™ laser focus in 8 nm steps—a benchmark number in the single-molecule biophysics of kinesin motor proteins, as discussed below. Like shown in Figure 9, these steps are unambiguously seen, both in unfiltered and filtered traces.

Applications: from single-molecule to cell

Since their invention, optical tweezers have found their most prominent quantitative applications in the field of single-molecule biophysics. As such, they have on the one hand been used to unravel the complex elasticity of biopolymers—DNA, RNA, proteins—where measurements could complement those performed with other single-molecule force spectroscopy techniques such as AFM. On the other hand, they have contributed a great deal to the detailed understanding of how many ‘motor proteins’ convert chemical energy into work. Milestone papers in the kinesin and myosin field owed their first direct mechanical insights to optical tweezers measurements [5, 6], and have sparked many follow-up research efforts. A plethora of mechanically active enzymes have been studied since then, including many involved in DNA metabolism. The number of studies in this field increases steadily to date, as a lot of open biological and biophysical questions have not or hardly been touched. These efforts have helped in maturing

quantitative optical tweezers technology to its present-day level: the power of optical-tweezers instrumentation to directly and in real-time measure picoNewton forces and nanometer displacements, spot-on in the realm where many such biological motors are active, has secured a central place for this cornerstone technique.

More recently, optical tweezers have started to transcend single-molecule applications and to provide a tool for studying *in vivo* cell-biological questions. Starting out mainly as a tool to sort, manipulate, push and pull in a qualitative manner, the first reports have appeared where processes in or around live cells are analyzed using quantitative tweezers measurements, for example for the unraveling of phagocytosis mechanics [7]. Such applications of optical tweezers in the field of cell biology have great potential to yield new insights.

Conclusions

With the JPK NanoTracker™, JPK's new experimental life science platform, compact and off-the-shelf quantitative optical tweezers have become available. The measurements in this technical report show that the platform lives up to the benchmark standards of the strongly developed single-molecule biophysics field. At the same time, the instrument is versatile and user-friendly enough to allow easy operation by those researchers not used to working with lasers and optics. As such, the NanoTracker™ is an excellent platform to cover a broad range of quantitative nanomanipulation needs.

Literature

- [1] Gittes, F. & Schmidt, C. (1998), 'Interference model for back-focal-plane displacement detection in optical tweezers', *Optics Letters* **23**(1), 7-9.
- [2] Pralle, A., Prummer, M., Florin, E., Stelzer, E. & Hörber, J. (1999), 'Three-dimensional high-resolution particle tracking for optical tweezers by forward scattered light', *Microsc Res Techniq* **44**(5), 378-386.
- [3] Berg-Sorensen, K. & Flyvbjerg, H. (2004), 'Power spectrum analysis for optical tweezers', *Rev. Sci. Instr.* **75**, 594-612.
- [4] Neuman, K. C. & Nagy, A. (2008), 'Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy.', *Nat Methods* **5**(6), 491-505.
- [5] Svoboda, K., Schmidt, C.F., Schnapp, B.J. & Block, S.M. Direct Observation of Kinesin Stepping by Optical Trapping Interferometry. *Nature* **365**, 721-727 (1993).
- [6] Finer, J.T., Simmons, R.M. & Spudich, J.A. Single myosin molecule mechanics - picoNewton forces and nanometer steps. *Nature* **368**, 113-119 (1994).
- [7] Kress, H.; Stelzer, E. H. K.; Holzer, D.; Buss, F.; Griffiths, G. & Rohrbach, A. (2007), 'Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity.', *Proc Natl Acad Sci U S A* **104**(28), 11633-11638.