# Force Clamping in Arbitrary Directions

## Introduction

Ever since the discovery and description of optical trapping by Arthur Ashkin in the 1970s [1], this technique has been used in various applications from the well-defined manipulation of micrometer-sized particles and cells to the highly precise detection of forces and molecular dynamics in the course of biochemical processes. The underlying physics is the same for all applications: In an electromagnetic gradient, dielectric particles with a higher refractive index than the surrounding medium will experience a force pointing towards regions with higher light intensity. This phenomenon is the basic principle of optical tweezing where a tightly focused laser beam generates a three-dimensional potential well resulting in a restoring force on optically dense objects. These are thus 'trapped' in the center of the beam.

While simple manipulation and force measurements have been sufficient for many applications in the past, the growing interest, for example, in single molecule studies calls for new modes of trapping and tracking. These experiments include protein unfolding [2] or the precise tracking of molecular motors [3], [4].

In order to fulfill the high accuracy and stability requirements of these studies, the NanoTracker™ 2 system has been equipped with the ability to hold particles under defined forces in arbitrary directions within the focal spot over a specific period of time.

## Principle of Optical Trapping

The basic principle of optical trapping and force detection is very well explained in one of our earlier technical reports titled: 'Quantitative force measurements with optical tweezers: The JPK NanoTracker™' (follow link to download).

In particular, there is one key fact about optical trapping one has to keep in mind. Stable trapping of optically transparent objects is only possible at the focal spot of the trapping laser. Only there do the forces acting on the particle cancel each other out. Any displacement from this equilibrium position leads to the rise of restoring forces $\vec{F}$, directing the particle back towards the focus.

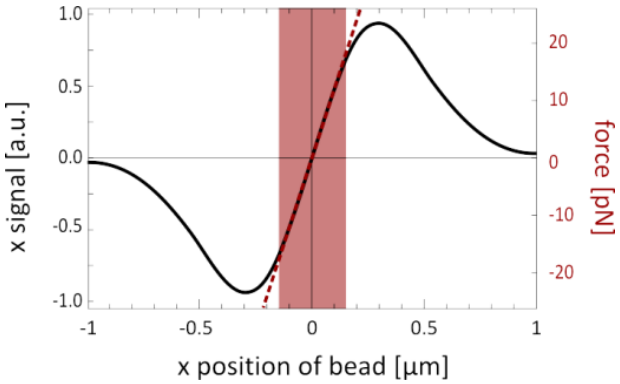


***Fig. 1**: S-shaped detector response for a sphere $d = 0.5\mu m$ moving through the optical trap. The central linear range (shaded) can be calibrated to displacements or forces as indicated by the dotted line.*

This type of system can be modeled as a Hookean spring, at which the restoring forces are linear for small displacements from the center (see figure 1). The typical application of this basic optical trapping mode is to expose the trapped particle to external forces, e.g. by attaching it to dynamic systems or by exposing it to viscous drag. These forces can then be calculated from the measured displacement of the bead from its equilibrium position.

## Force Clamping

In a variety of applications it is not only necessary to measure the forces acting on a particle but to keep these at a constant level. In the context of optical trapping, this *clamping* of the force level is achieved through monitoring of the bead displacement (equivalent to a force) and the precise repositioning of the trap in the respective direction so that the set force is reached again. Typically, two spherical beads are attached at opposite ends of the sample of interest (e.g. a macromolecule) and both are optically trapped. One is used as sensor for feedback control while the second particle attached to the other end of the probe is repositioned by the respective trap in order to maintain a constant stress. If the setup features a piezoelectric sample scanner, the whole sample chamber can be

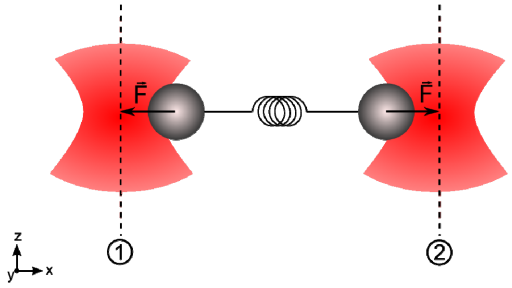


***Fig. 2**: Force clamping using two trapped particles with an elastic linker in between. Shown is the x-z-plane with the displacement of the particles relative to the trap center and the generated restoring force.*

repositioned so that surface bound molecules, particles or cells attached to a glass slide can be exposed to well-defined (local) stresses.

The most widely used type of systems consists of two beads connected with an elastic linker e.g. DNA (figure 2). Each of the beads is held in one of the traps. Force clamping is now realized by moving the control trap (e.g. trap 1) away from the other trap. Because of the elastic linker the second bead will be moved away from its equilibrium position until the predefined set point for the clamping force is detected in the feedback trap (e.g. trap 2). The control trap (1) is then moved such that the particle in the feedback trap (2) is kept at a constant position relative to the trap center (corresponding to a constant force, see figure 2). This principle is not only applicable along the $x$- or $y$-axis as in other optical tweezers systems but in arbitrary directions within the focal plane as illustrated in figure 3.

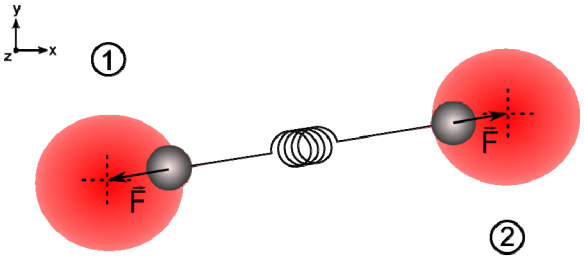


***Fig. 3**: Force clamping using two trapped particles with an elastic linker in between. Shown is the x-y-plane with the displacement of the particles relative to the trap center and the generated restoring force.*

The restoring force for displacements in arbitrary directions is calculated using a linear combination of the $x$- and $y$-displacement ($\Delta x, \Delta y$):

$$\vec{F} = k_x \cdot \Delta x \cdot \hat{x} + k_y \cdot \Delta y \cdot \hat{y}$$

The measured force $\vec{F}$ is then projected onto the pulling direction $\hat{n}_\theta$.

$$F_n = \vec{F} \cdot \hat{n}_\theta = k_x \cdot \Delta x \cdot \cos(\theta) + k_y \cdot \Delta y \cdot \sin(\theta)$$

In both equations $k_x$ and $k_y$ describe the spring constants of the trap in $x$- and $y$-direction, $\hat{x}$ and $\hat{y}$ are the unit vectors and $\theta$ is the angle of pulling, measured counter-clockwise with respect to the positive $x$-axis. Both sketches (figures 2 and 3) show examples of possible spatial configurations.

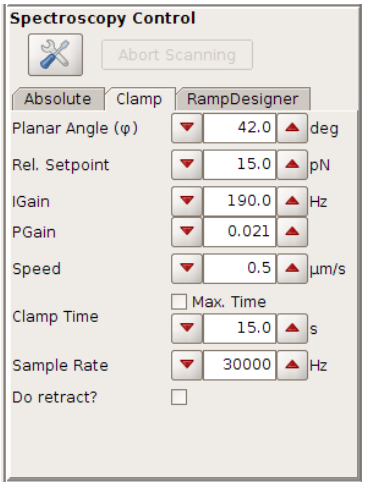


***Fig. 4**: NanoTracker™ 2 force clamping control options. The direction and magnitude of applied force, as well as feedback para-meters (integral and proportional gain), clamp duration and the sampling rate can be adjusted. In combination with a piezo-electric sample re-positioning system, this provides maximum flexibility.*

During development, our focus was on the highest possible flexibility for custom applications. Thus, in the NanoTracker™ 2 control software, several clamping criteria like amount and direction of applied force as well as clamping duration and also force feedback parameters are readily accessible (see figure 4). As mentioned above, either of the available traps can be used for force detection and feedback control while the actual force is applied through the other trap or the sample chamber. With this convenient and intuitive control at hand, a multitude of configurations is possible.

Equipped with acousto-optical deflector (AOD) based trap positioning, the force feedback loop employed in JPK's NanoTracker™ 2 performs real-time trap repositioning at a rate of 50 kHz in within a field of more than 100 µm in the $x$-$y$ plane. Among other cases, this is of great use when the adaption of large-scale molecular systems to external stresses (e.g. through relaxation) is investigated or when the motion of a particle is to be tracked over longer periods of time.

As will be discussed later, the accuracy of the constant force is in the sub-piconewton range. This high accuracy of the clamping force is achieved through high quality, noise-minimized hardware as well as innovative software solutions developed by JPK Instruments. Equipped with our reliable and versatile control unit and software routines, the outstanding performance in feedback control known from the NanoWizard™ 3 atomic force microscope system is now also available for the NanoTracker™ 2.

## Position Clamping

The basic quantity being measured in optical tweezing is the displacement of a particle from the trap center which can only be converted to a force after the trap has been calibrated. Of course, this parameter can also be passed directly to the feedback mechanism, resulting in a *position clamp* rather than a *force clamp*. Here, the trapped particle is held at a constant position while the force required to maintain this position can be recorded. Equivalent to the sub-pN force accuracy that will be demonstrated in the following paragraphs, the position of the trapped particle can be detected and held constant with deviations as small as a few nanometers.

Classic position and force clamp setups based on atomic force microscopy (AFM) have produced excellent data in protein unfolding [2] and other studies. However, these setups are restricted to surface bound samples, a limitation that can be overcome with the multiple-trap configuration of the NanoTracker™ 2. Suspended samples from single molecules [5] to whole cells [6] can be held under constant stress or spatial constraints while their response is measured without any surface contact effects negatively influencing the sample behavior. In combination with JPK's multichannel LaminarFlowCell, easy and rapid variations of the surrounding medium allow the investigation of different factors on single-molecule dynamics and mechanical properties.

## Application

As described above, the NanoTracker™ 2 provides a variety of possible trap and sample configurations and in combination with the force clamping feature, the system now covers an even wider field of applications including single molecule force spectroscopy. The following paragraphs will explain and discuss some basic experiments in order to demonstrate the capabilities of the NanoTracker™ 2 force clamping mode.

**DNA** By activating both traps and the force clamping feature, it is easily possible to elongate a single molecule such as double-stranded DNA (ds-DNA) and to apply a defined tension. In this type of experiment, other properties like the enthalpic regime of DNA at different extension levels can be studied. The course of the experiment is schematically shown in the micrographs in figure 5.

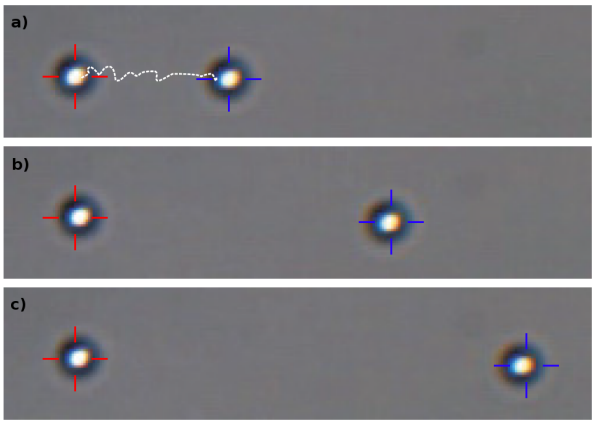


***Fig. 5**: Screenshots of ds-DNA force clamping. In the first image, the DNA strand is represented by the white line. The images depict the following situations: a) starting configuration with relaxed DNA strand between the two traps b) clamping at 10 pN and c) clamping at 50pN.*

Figures 6 and 7 highlight different strengths of the feature. The plots in figure 6 correspond to a single ds-DNA strand being pulled until pre-defined force levels are reached. For clarity the different clamping situations were

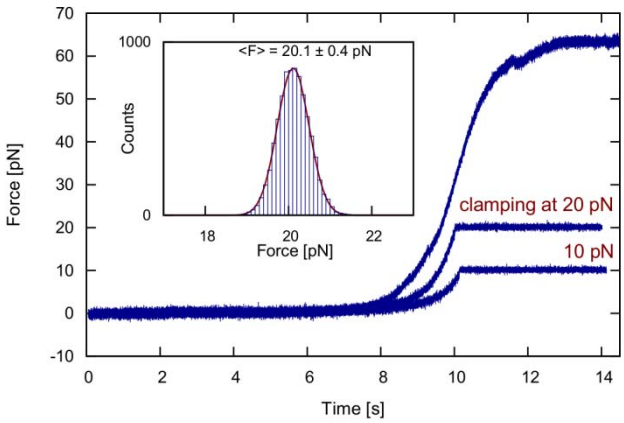


***Fig. 6**: Clamping of a ds-DNA molecules at forces of 10 and 20 pN and a full overstretching plot (obtained in separate experiments). Each curve is plotted with 0.5 s offset relative to the others. Inset shows the histogram of measured forces during clamping phase at 20 pN. The standard deviation of 0.4 pN reflects the high accuracy of the clamping feedback. (Sample courtesy of Yan Jie's lab, University of Singapore)*

separated by slightly shifting the respective curves in time. The histogram in the inset of figure 6 displays the data recorded during 20 pN force clamping and clearly shows the sub-piconewton accuracy with which the force is kept constant. Furthermore, the top curve shows the overstretching of ds-DNA for comparison.

Figure 7 demonstrates the method's independency of the force angle. Once more, ds-DNA was elongated until the desired force value was reached, in this case 20 pN, but

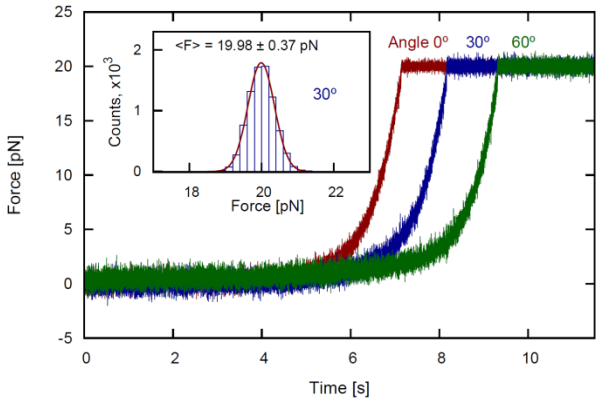


***Fig. 7**: Force clamping of ds-DNA for different clamping directions using a two trap system. Curves representing different clamping angles were shifted in time. The histogram plot of the clamping data shows the accuracy of the clamping force for the 30° direction. (Sample courtesy of Yan Jie's lab, University of Singapore)*

now at different angles. Example data is shown for 0°, 30° and 60° (measured counterclockwise starting with the positive $x$-axis). This feature, however, is not restricted to specific directions but works at arbitrary angles. The histogram demonstrating that the standard deviation of measured values is well below 1 pN is shown in the inset of figure 7. It was plotted from the data recorded with an angle of 30°.

**Motor Protein Tracking** Measurements of motor protein kinetics will also benefit from this useful measurement technique. Using this feature allows the application of constant forces smaller than the stall force of the motor protein while following its movement. By observing the displacement of the trap over time, it is possible to record individual steps of the motor with high accuracy.

Furthermore, unlike with a static trap, it is possible to follow a single motor protein for a long time, in many cases for the complete movement over the substrate (e.g. a microtubule) depending on the environmental conditions.

Additionally, the new angle independent clamping mechanism offers the possibility to use arbitrarily oriented tracks like microtubules in the sample plane. This freedom enables the user to omit time-consuming alignment steps in sample preparation without sacrificing accuracy and to spend more time on data acquisition and analysis.

## Conclusions & Outlook

Having the possibility to keep biological samples under a constant, well-defined force opens access to a wide field of new experiments and applications. Most biological reactions come along with conformational changes which in turn cause tension within molecules or multi-molecular complexes. Being able to keep the forces on the system constant allows eliminating one variable and studying others in more detail.

Further potential applications include the study of whole cell relaxation processes and mechanics or of drug delivery through biological membranes. It is known that the permeability of membranes for different types of molecules strongly depends on the local mechanical conditions [7]. Employing the new force clamping mode

will allow studying the dependency of the transportation process on the stress and strain of the cell membrane and vice versa.

Generally speaking, the ability to define the forces under which biological systems have to operate with high stability and accuracy offers new control and research applications. Additionally, due to the angle independency of this feature, it is highly flexible and very easy to use.

## Literature


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