Optical Tweezers Combined with Multi-Fluorescence Microscopy

Introduction

Fluorescence microscopy is a well-known and everyday tool for use in biology and biophysics. Furthermore, since the discovery of the green fluorescent protein (GFP) in 1962 by Osamu Shimomura [1], the technique experienced an enormous boost in the '90s with many new applications and more scientists working with fluorescence microscopy than ever before. The reason for this growth was the increasing interest in biological samples and the possibility to express proteins in the lab. Reflected is the large impact of GFP in more than 20.000 papers published in the last 20 years [2]. Additionally, the discovery of GFP led to the invention and development of several new microscopy techniques including fluorescence life-time imaging (FLIM) and super-resolution techniques such as STED, PALM/STORM [2].

At the same time as the biological field was being revolutionized with the possibility to express proteins in the lab, optical tweezers became a prominent and convenient tool for manipulation and force measurement in the fields of biological and biomechanical studies. One of the few instruments combining both techniques and being commercially available is the JPK NanoTracker™.

The optical tweezers system from JPK Instruments was designed around a standard inverted microscope. Furthermore, the approach chosen ensures that the beam path of the trapping laser is not interfering with any fluorescence experiment. The advantage of the design is the possibility to use multi-fluorescent samples and change the excitation and emission wavelength during the experiment. This provides an unchallenged optical tweezers system offering the highest flexibility to its users. Also, some major modifications to the optical microscope

Fig. 1: *The JPK NanoTracker™ 2 mounted on a Zeiss Axio Observer microscope. The trapping laser is coupled with a special side-port above the fluorescence filter wheel.*

are undertaken to improve the performance of the tweezers system even further.

Modifications and Improvements

JPK's optical tweezers system consists of several major components: The laser box is responsible for the generation and controlling of the laser beams while the detection unit enables high accurate force detection.
The latter is positioned on top of a modified research-grade inverted microscope. This microscope forms the basic framework of the set-up. Currently, NanoTracker™ is operational with the Nikon (TE2000, Eclipse Ti), Olympus (IX70/71/80) and Zeiss (Axiovert 200, Axio Observer) microscopes.

Two major changes are made for the NanoTracker™ integration. Firstly, the objective carousel was removed, because it is not mechanically stable enough to match the high accuracy standards in noise and resolution of JPK's beam detection. Instead, an in-house developed objective

holder is installed. The second improvement is the implementation of the trapping laser. Figure 2 is a schematic drawing showing the light paths within the inverted microscope. The original light path is not altered at all. The head of the NanoTracker™, housing the detection unit is put on top leaving a side port to couple in the infrared trapping laser.

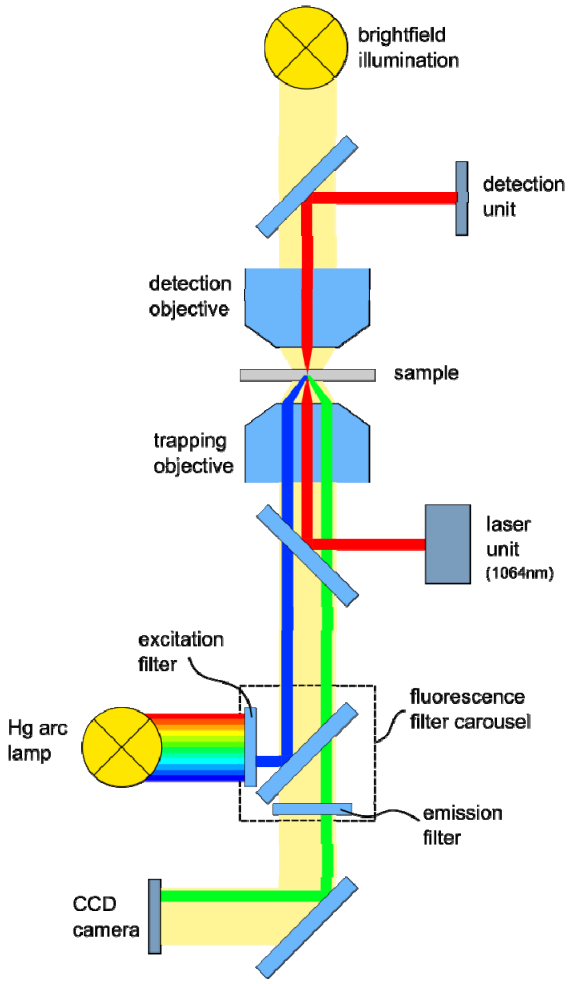

Fig. 2: *Schematic drawing of the light path within the inverted microscope used for the NanoTracker™ 2. Here an example of the beam path demonstrates the integration of the fluorescence microscopy.*

The advantage compared to an implementation using any of the side or the back ports of the microscope is the fact that the trapping laser is not directed through the fluorescence filter. Using this kind of set-up makes it possible to change the fluorescence filter during operation with the trapping laser running. If the chosen laser power is strong enough to overcome the small mechanical vibrations every filter change introduces into the system, the actually trapped sample is not lost during a change of fluorescence filters and the positions of the traps remain constant.

With the approach chosen for the integration of the trapping laser, it is easily possible to change between brightfield and fluorescence microscopy. Additionally, it is also possible to change to other microscopy techniques, such as differential interference contrast (DIC) microscopy, while the trapping laser is still running.
This versatility allows the user to perform different type of microscopy techniques at the same sample location without losing any trapped particle or any accuracy of the system.

Applications

All the data shown in this report was recorded with a NanoTracker™ 2 system mounted on a Zeiss Axio Observer.A1 inverted microscope. This configuration shows the same modifications as described earlier. A 63x Zeiss C-APOCHROMAT water-immersion objective with a numerical aperture 1.2 was used for trapping and fluorescence detection. The mounted CCD camera was an ImagingSource® DFK 31AF03. The fluorescence illumination was performed using an HXP 120 fluorescence light source.

To demonstrate the stability of the system, a benchmark experiment was performed (see figures 3 and 4). In this experiment, trap 1 (red crosshair) was at a constant

position, while trap 2 (blue crosshairs) was multiplied using an acousto-optic deflector (AOD) and therefore timeshared at 60 different positions forming a circle. The dwell time at each point was increased to one second leading to an overall time of 60 seconds per cycle.

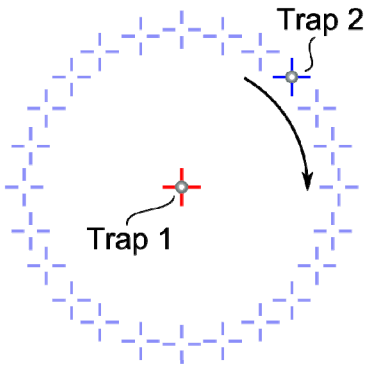

Fig. 3: *Schematic drawing to show the working principle of the benchmark experiments. While trap 1 stays exactly at the same position, trap 2 is automatically moved to 60 different positions. In both traps, a fluorescently labeled bead is captured.*

While trap 2 is orbiting around trap 1 the fluorescence filters were changed during operation. This possibility offers the customer the option to use multi-fluorescent samples and perform the same measurements at the exact same positions.

The initial state of the experiment is shown in figure 4.a), where two fluorescently-labeled, carboxyl-modified 2 µm beads (Sigma Aldrich (L4530); 2 µm carboxylate-modified polystyrene, fluorescent (yellow-green) beads; λ_{ex}~470nm, λ_{em}~505nm [3]) are trapped. The first one is captured in the stationary trap 1 and the second one in the moving trap 2 (see red arrows). While trap 2 is orbiting around trap 1, the fluorescence filters were changed without stopping the experiment. The change was performed in the following order: filter-set 47 (λ_{ex}: BP436/20; λ_{em}: BP480/40), filter-set 43 (λ_{ex}: BP545/25;

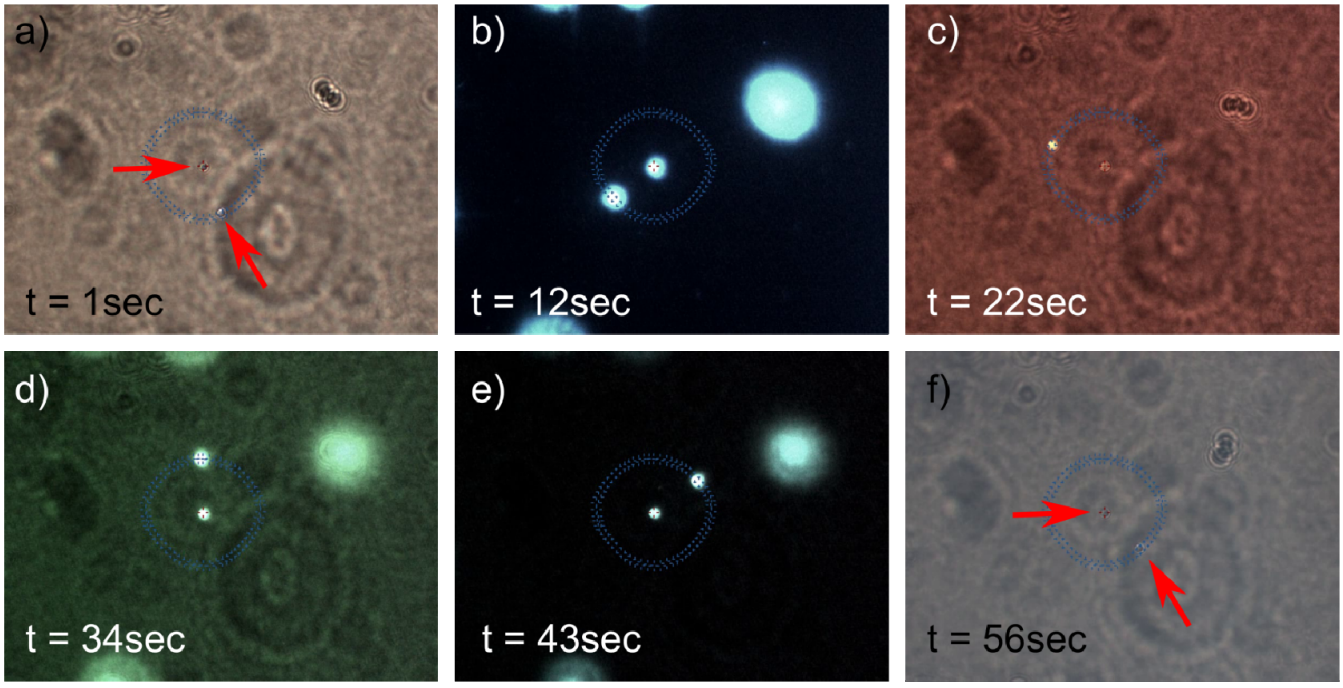

Fig. 4: *In this time-series, the possibility of changing the fluorescence filter during trap manipulation is demonstrated. Two fluorescent, carboxyl-modified 2 µm beads are trapped. While trap 1 (red crosshair) is not modified, trap 2 was multiplexed 60 times using an acousto-optic deflector (AOD). The so-created traps are arranged in a clock-like pattern. While the bead in trap 2 is orbiting around trap 1, four different fluorescence filters were introduced into the system. In all cases, the fluorescence of the trapped as well as the freely moving particles can be observed.*

λ_{em}: BP605/70), filter-set 17 (λ_{ex}: BP485/20; λ_{em}: BP515-565), filter-set 13 (λ_{ex}: BP470/20; λ_{em}: BP505-530) [4].

In total, four different fluorescence cubes were introduced into the beam path leading to different fluorescent responses (see figure 4.b)-4.e)). As expected, the emission for filter-set 47 is much brighter than the one for filter-set 43. The last image (figure 4.f)) again displays the situation without any fluorescence filter being introducedeven though the fluorescence lamp is still running. During the whole experiment the fluorescent beads were stably captured in the traps.

In conclusion, this time-series demonstrates the fact that changing the fluorescence filter cubes in the NanoTracker™ does not influence the trap positions or their capability of catching beads.

A video of this experiment, of which the single frames were extracted, can be found at JPK's YouTube channel: http://www.youtube.com/jpkinstruments

Conclusions & Outlook

This benchmark experiment demonstrates the fact that the modifications performed by JPK to the inverted microscope are not interfering with fluorescence or any other light microscopy technique. The two beam paths are not interfering with each other which makes it possible to use different kinds of microscopy techniques at the same sample position and with the same trapped beads. Also the possibility to use multi-fluorescently labeled samples and change the excitation and emission filters during operation is provided.

One of the next steps in the development of the NanoTracker™ 2 will be to improve the sensitivity of fluorophore detection. Another approach will be the development of camera based detection routines for both position and force tracking.

References

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