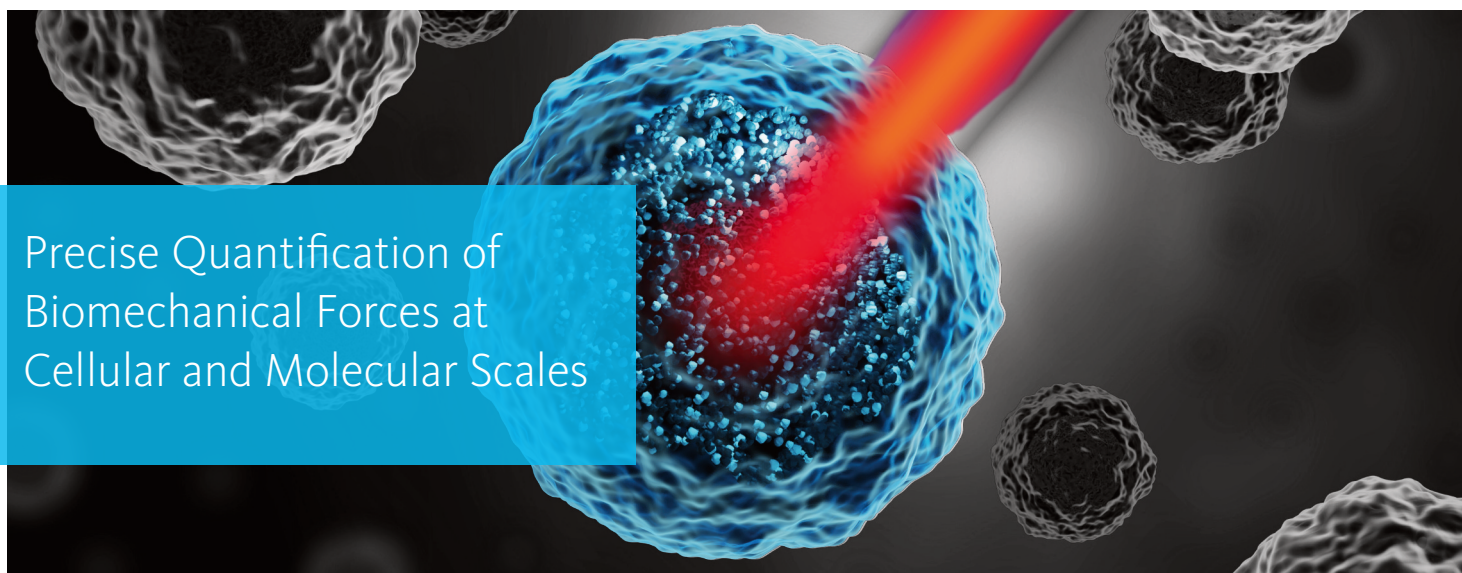


Technical Note

Forward Interferometric Position Based Force Spectroscopy using Optical Tweezers



Precise Quantification of Biomechanical Forces at Cellular and Molecular Scales

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Abstract

Performing quantitative force measurements with optical tweezers (OT) with a high spatial and temporal resolution is useful for researchers who study biological cells. It yields a huge potential to accelerate novel life-science understanding, not only at the inter- but also at the intra-cellular level.

In this work, we demonstrated that the MMI CellManipulator OT system equipped with a forward interferometric position detection system could be used to monitor the oscillation of the trapped bead at multiple locations within the field of view (FOV). Furthermore, we showed that the calibrated trap could function as a force probe to measure the stretching force of a polymer tethering a bead to the glass surface.

Introduction

Basic of OT in Biology Applications

An OT provide a non-invasive way to interact with single-cells and sub-cellular structures using a focused laser beam [1, 2]. Nowadays, this technique is widely used to measure molecular and cellular forces due to its high sensitivity [3, 4, 5].

OT use a focused laser beam, typically with a wavelength of 1070 nm, to manipulate microscopic objects [1, 6, 7]. Once the laser beam encounters a dielectric microparticle, a fraction of the beam will be reflected. The other fraction will be transmitted. As the direction of those reflected and transmitted beams differ from the incoming beam, the momentum and force associated with the beam change. It comes from the fact that the laser beam consists of photons carrying energy and momentum. When a photon is scattered by an object, its

momentum shifts, generating recoil forces on the particle, as described by the law of momentum conservation. In the case of OT applications in biology, where objects are typically much larger than the wavelength of light, recoil forces can be effectively described using geometrical or ray optics.

Recoil forces in optical trapping consist of the scattering force (the force pushing the particle parallel to the incoming light beam) and the gradient force (force directing the particle to the focal point, where the laser intensity is high) [1, 6]. When the particle has a higher refractive index than the surrounding medium, the gradient forces push the particle to the focal point near the laser beam focus. However, due to scattering forces, a collimated laser beam alone cannot effectively trap the particle. The most effective approach is to use a focused light beam through a high numerical aperture (NA) lens.

The CellManipulator

For cell researchers, who need to interact non-invasively with single cells and sub-cellular structures, The CellManipulator OT system is a scientific optical instrument that accelerates life science understanding at the inter- and intra-cellular level. Unlike competing products, the product is modular and can be integrated with almost all inverted and upright research microscopes on the market.

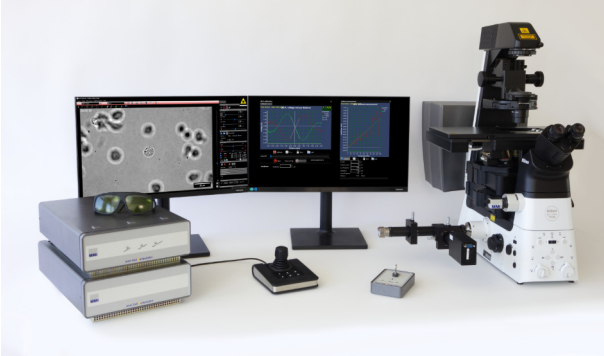


Fig. 1: The CellManipulator OT system on the Nikon Eclipse Ti2E inverted microscope. The system is modular and can be integrated with almost all inverted and upright research microscopes. Furthermore, the system can be combined with all MMI single-cell solutions for various research applications.

The CellManipulator focuses an infrared laser (1070 nm) beam to a diffraction-limited spot on a sample plane through a high NA objective [7]. The system can hold, move, rotate, join, separate, stretch or otherwise manipulate the target.

Besides the basic single/multiple trapping capabilities, the system also supports the quantitative force spectroscopy with resolution down to sub-piconewton range.

Forward Interferometric Position Detection

An optically trapped particle scatters the laser beam yielding the light field in the forward direction [6]. The light field is composed of the superposition of the incoming and the scattered light that produce an interference pattern. The forward interferometric position detection is a method to extract the position of trapped particle from its light interference.

A setup of a forward interferometric position detection typically consists of an objective lens to focus the trapping beam, a condenser lens to collect the interference pattern, and a photodetector conjugated to the back-focal plane (BFP) of the condenser. A 4 quadrant-detector (4QD) is one of the common photodetectors used as the position sensors. It measures the intensity between the opposite sides of the detection planes (i.e., left vs right / up vs bottom). The response of a 4QD is given by

$$x_{4QD} = \iint_{x_d > 0} I_{FS}(x_d, y_d) dx_d y_d - \iint_{x_d < 0} I_{FS}(x_d, y_d) dx_d y_d$$

$$y_{4QD} = \iint_{y_d > 0} I_{FS}(x_d, y_d) dx_d y_d - \iint_{y_d < 0} I_{FS}(x_d, y_d) dx_d y_d$$

$$z_{4QD} = \iint I_{FS}(x_d, y_d) dx_d y_d$$

As the 4QD is typically less noisy and much faster than digital cameras, this method can measure the position near the focal region with high spatial (down to sub nanometer) and temporal resolution (up to several megahertz).

Material and Methods

OT Setup

As shown in Figure 2, the MMI CellManipulator OT system was integrated into a dual-deck Nikon Eclipse Ti2 inverted microscope with a Nikon, Plan-Fluor, 100X oil immersion objective, NA= 1.3, 0.16 mm working distance (WD). The Ytterbium fiber laser 1070 nm (IPG Laser GmbH, Burbach) was used as the laser source. The sample was mounted onto a computer-controlled stage (Märzhäuser GmbH, Wetzlar, Germany). The trapping was performed with around 80 mW laser power¹.

Polystyrene Beads

We took approximately 5 μ l of polystyrene bead stock solution (1 μ m, Fluoro-MaxTM red fluorescent Ro100, Thermo Scientific), pipetted it into 50 ml distilled water, and mixed it well to prepare a working solution. A droplet (ca. 20 μ l) of working solution was then sandwiched between two glass coverslips, separated by the MMI membrane slide. The sample was mounted onto the stage holder of a widefield microscope equipped with the MMI CellManipulator OT system. After allowing the beads to settle on the upper surface of the bottom coverslip, we observed them using a 100x NA = 1.3 oil immersion objective with the MMI CellTools software and the CellManipulator Plugin.

Position Detection Setup

As shown in Figure 2, the setup of forward interferometric position detection (force spectroscopy system) consisted of a Nikon condenser lens NA = 0.85, WD = 5 mm, a condenser holder, a four-quadrant detector (4QD) (Electro Optical Components, Inc., California, USA), an auxiliary camera, mechanics, and optics (a lens, a filter, and a prism) to conjugate the back-focal plane (BFP) of the condenser lens to the 4QD. To ensure the image was fully covered by the 4QD active area, the optical arrangement was configured to magnify the interference pattern at the condenser BFP by approximately 0.5 $\times$ at the 4QD.

Positional-Force Calibration

To use OT as a quantitative tool, the system must be properly calibrated. For calibration, we followed by a slight modification the method described by Tolić-Nørrelykke et al [8]. The bead was oscillated along the x-axis at room temperature with an

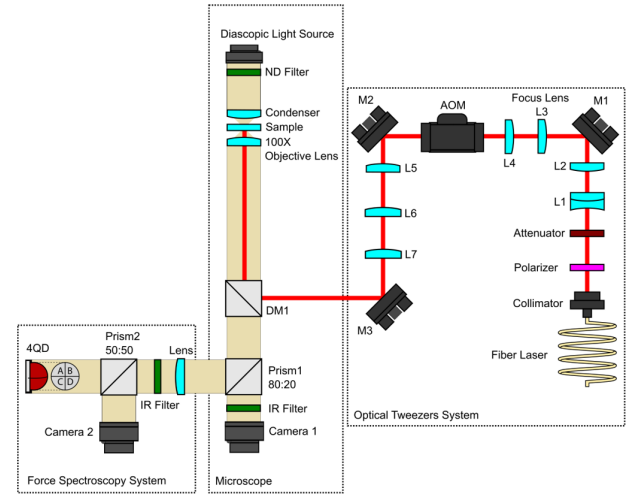


Fig. 2: Experimental Setup: The schematic of MMI CellManipulator OT system. The system consists of beam control unit, an inverted light microscope, and a force spectroscopy system.

amplitude of 500 nm and a frequency of 32 Hz to obtain the power spectral density (PSD). The sampling frequency was set to 65,536 Hz. The resulting curve was fitted with a Lorentzian's function to determine the sensor sensitivity $1/\beta$, the cutoff frequency f_c , and the diffusion constant D^{volt} . We acquired the trap stiffness by inserting the values into this formula

$$\kappa = 2\pi f_c \frac{k_B T}{\beta^2 D^{volt}}$$

Results

Position Detection

In this work, we demonstrated the capability of the 4QD to detect the presence/movement of the trapped particle. We began by aligning Köhler illumination to ensure that the sample receives a bright uniform light. Since the condenser also collected the interference pattern in the forward interferometric position detection, this alignment was particularly crucial. Additionally, we also verified that the condenser BFP was well resolved on the 4QD sensor by observing the image of a Nikon ELWD Ph1 phase-ring on the auxiliary camera 2 (see Figure 2).

¹The laser power was measured at the sample plane through the 100x objective lens

Then, we trapped a $1\ \mu\text{m}$ bead at the center of FOV. As shown in Figure 3, there was a qualitative difference between interference patterns generated by a negative control (the laser beam was turned on but did not trap a bead) (a) and an optically trapped bead (b).

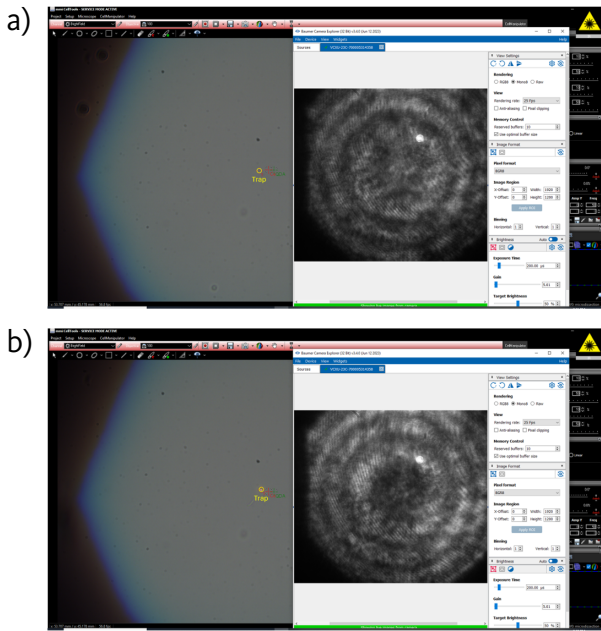


Fig. 3: Forward interference patterns of (a.) a negative control and (b.) an optically trapped bead. A prominent qualitative difference could be spotted.

We then verified the 4QD response by comparing the oscillation of the negative control vs the trapped bead along the x-axis. The oscillations were performed with the amplitude of $1\ \mu\text{m}$ and the frequency of 100 Hz.

As shown in Figure 4 and appendix 1, the negative control (a) did not generate a signal on the 4QD, whereas the trapped bead (b) produced clear oscillation signals, confirming the system's ability to detect bead movement accurately. The red curve represents the 4QD x-axis signal, which responds to the trapped bead's movement along the x-axis. The green curve represents the 4QD y-axis signal.

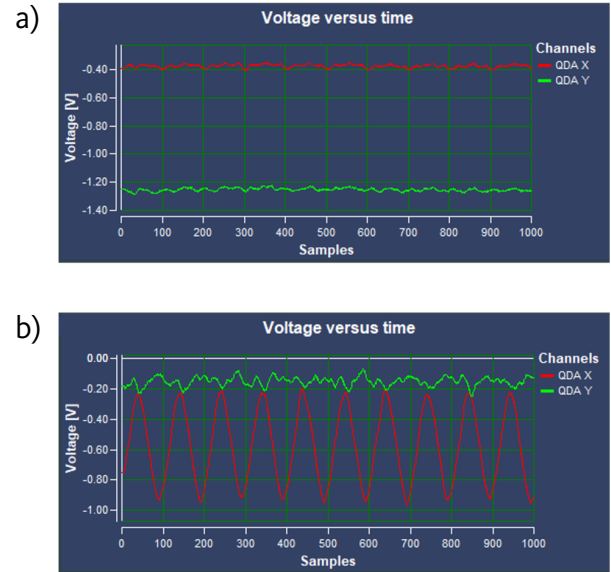


Fig. 4: The 4QD responses of (a) a negative control and (b) an optically trapped bead during oscillation along the x-axis.

Furthermore, we verified the 4QD response by oscillating the trapped bead at three different positions within the FOV to show that the quantitative measurements can be done at multiple places within the FOV. As shown in Figure 5 and appendix 2, the 4QD was able to detect the oscillation, represented by the red/green curves. The white curve represents the 4QD sum, which corresponds to the total current generated by the sensor. The sum signal is influenced by the amount of light striking the sensor and is calculated using analog electronics.

Trap Stiffness Calibration

In this work, we demonstrated the calibration of the trap stiffness. We trapped a $1\ \mu\text{m}$ close to the center of the FOV and performed the calibration. During the calibration step, we ensured the sum signal to remain under 10 V and as flat (no/ minimum oscillation) as possible. This step was necessary because, with galvanometric mirrors, the optical trap was not restricted to a fixed spot at the center. To move the focused laser in the FOV, the laser beam was deflected by two mirrors. The focused laser beam at the center of the FOV is typically calibrated to be deflected from the center of both mirrors. As a result, the focused laser beam strikes the glass surface at a perpendicular angle and scatters symmetrically at the calibrated

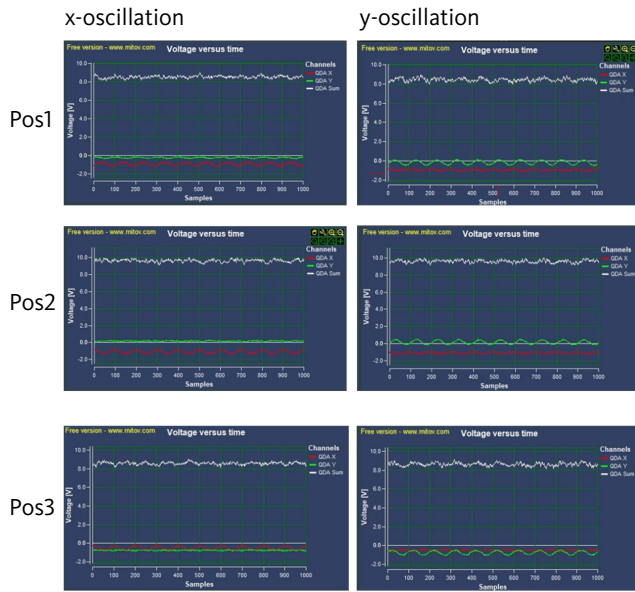


Fig. 5: The 4QD detected oscillations in both the x- and y-directions at three different positions.

spot. When we trapped outside the spot, the focused laser beam no longer strikes at a perpendicular angle. Depending on how far from the spot, the incoming angle differs, and the scattering pattern might lose the symmetry. As a result, this asymmetry affects the interference pattern collected by the condenser lens, which in turn influences the signal interpreted by the 4QD. In some cases, it can generate an overshoot or a significant oscillation in the sum signal.

As shown in Figure 6, the system was able to acquire the trap stiffness in x-axis (a.) and y-axis (b.) from the PSD curve. Additionally, we performed iterations of the trap calibration at three different locations within the FOV. Interestingly, as shown in Figure 7 (a), the stiffness values were interpreted differently by the 4QD depending on the trap position. In the position 1, the stiffness values of the x- and y-axis were 30.09 ± 0.61 pN/ μ m (mean $\pm$ S.E.) and 31.36 ± 0.61 pN/ μ m. In the position 2, the stiffness values of the x- and y-axis were 52.87 ± 0.92 pN/ μ m and 35.67 ± 0.93 pN/ μ m. Finally, in the position 3, the stiffness values of the x- and y-axis were 40.78 ± 0.67 pN/ μ m and 65.55 ± 0.97 pN/ μ m.

We suggest that the different values by the 4QD also comes from the variability of the scattering pattern (see Figure 7 (b)). In each location, laser beam hit the surface at different incoming angle generating a unique scattering pattern profile.

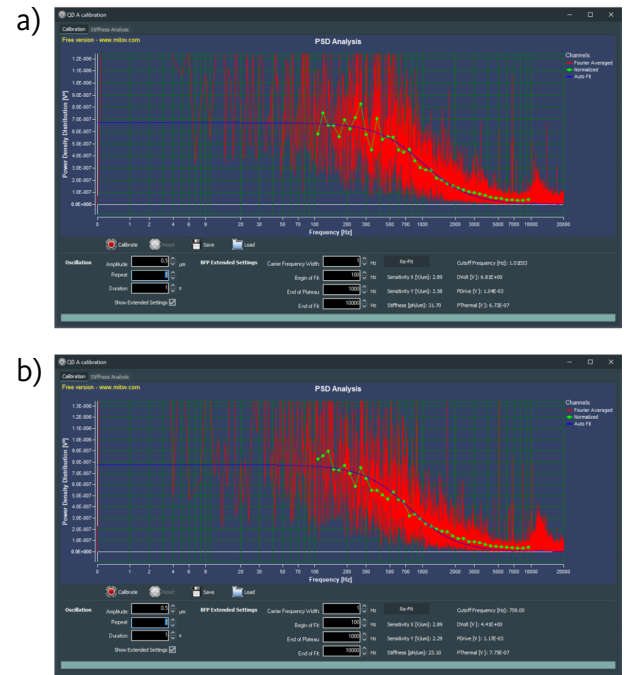


Fig. 6: PSD curves of (a) the calibration in x-axis and (b) the calibration in y-axis. The resulting curve was fitted with a Lorentzian's function to determine the trap stiffness.

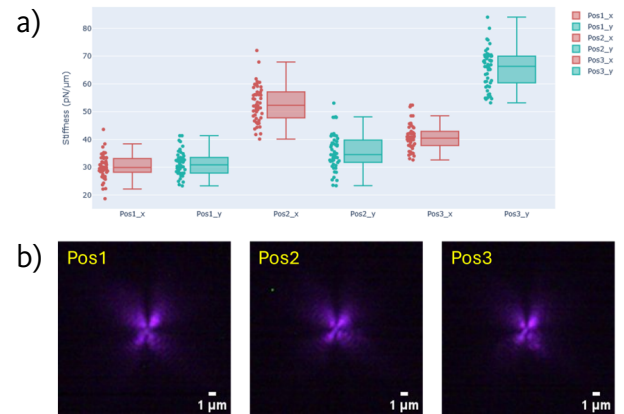


Fig. 7: (a) The trap stiffness values obtained at different location in the FOV. The red plots represent the calibration at x-axis. The green plots represent the calibration at y-axis. (b) The scattering patterns at different location in the FOV. They were imaged at approximately 2 μ m above the glass surface.

Force Spectroscopy

In this work, we demonstrated the capability of the MMI CellManipulator OT system to measure force. After completing the calibration, we trapped a 1 μ m bead that was tethered to the

surface, following an extended settling period of several hours on the glass surface. As shown in Figure 8, we stretched the polymer by moving the tethered bead in the positive x-direction. Force curves were obtained at different velocities (2,3,4,5 and 10 $\mu\text{m/s}$), allowing us to analyze the system's response. Finally, we determined that the length of the stretched tether was approximately 3.5 μm .

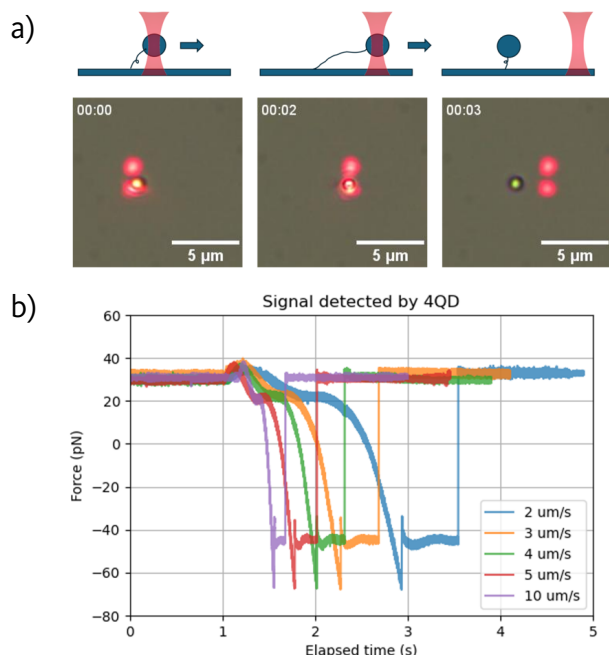


Fig. 8: The force spectroscopy experiment. The trap stiffness was 28.86 pN/ μm (a) Left: The bead tethered to the surface was optically trapped. Center: The trap moved the bead in x-direction with a linear speed. Right: The maximum tether extension was achieved. The bead escaped the trap. (b) The force curves generated at different moving speeds.

Conclusion

We developed a versatile OT capable of performing single and multiple trapping as well as force spectroscopy with a resolution down to sub-piconewton range, making it a powerful tool for studying cellular and molecular interactions. The system is equipped with a forward interferometric position detection mechanism, which extracts the position of the trapped particle by analyzing light interference collected by the condenser and relayed to the 4QD.

Our results demonstrated that the interference pattern of the trapped bead could be detected qualitatively using a camera and quantitatively using the 4QD at multiple positions within the FOV. Additionally, we showed that the trapped bead can function as a probe for stretching force measurements. This work significantly expands the experimental capabilities of the MMI CellManipulator OT system, enabling a broad range of applications, including precise force measurements at the cellular and molecular scale.

References

- [1] A. Ashkin, "Acceleration and Trapping of Particles by Radiation Pressure," *Physical Review Letters*, pp. 156-159, 1970.
- [2] A. Ashkin, J. Dziedzic, J. Bjorkholm and S. Chu, "Optical trapping and manipulation of viruses and," *Science*, pp. 1517-1520, 1987.
- [3] M. Andersson, A. Madgavkar, M. Stjern Dahl, Y. Wu, W. Tan, R. Duran, S. Niehren, K. Mustafa, K. Arvidson and A. Wennerberg, "Using optical tweezers for measuring the interaction forces between human bone cells and implant surfaces: System design and force calibration," *Review of Scientific Instruments*, vol. 78, no. 7, 2007.
- [4] U. Bockelman, P. Thomen, B. Essevaz-Roulet, V. Viasnoff and F. Heslot, "Unzipping DNA with Optical Tweezers: High Sequence Sensitivity and Force Flips," *Biophysical Journal*, vol. 82, no. 3, pp. 1537-1553, 2002.
- [5] S. C. Kuo and M. Sheetz, "Force of Single Kinesin Molecules Measured with Optical Tweezers," *Science*, vol. 260, no. 5105, pp. 232-234, 1993.
- [6] O. M. Marago, P. H. Jones and G. Volpe, *Optical Tweezers - Principles and Applications*, Cambridge University Press, 2015.
- [7] A. Sharghi, K. S. Lee, S. Niehren, H. M. Resch and R. Stocker, "Optical trapping and force spectroscopy of non-spherical rod shaped bacteria and diatoms," *MMI Application Note*, 2022.

- [8] S. F. Tolic-Nørrelykke, E. Schaeffer, J. Howard, F. S. Pavone, F. Juelicher and H. Flyvbjerg, "Calibration of optical tweezers with positional detection," Review of Scientific Instruments, vol. 77, no. 103101, 2006.
- [9] W. H. Guilford, J. A. Tournas, D. Dascalu and D. S. Watson, "Creating multiple time-shared laser traps with simultaneous displacement," Analytical biochemistry, vol. 326, no. 2, pp. 153-166, 2004.
- [10] P. Debye and F. W. Sears, "On the Scattering of Light by Supersonic Waves," PNAS, vol. 6, no. 409 - 414, p. 18, 1932.
- [11] K. S. Lee, M. Palatinszky, F. C. Pereira, J. Nguyen, V. I. Fernandez, A. J. Mueller, F. Menolascina, H. Daims, D. Berry, M. Wagner and R. Stocker, "An automated Raman-based platform for the sorting of live cells by functional properties," Nature microbiology, vol. 4, pp. 1035-1048, 2019.

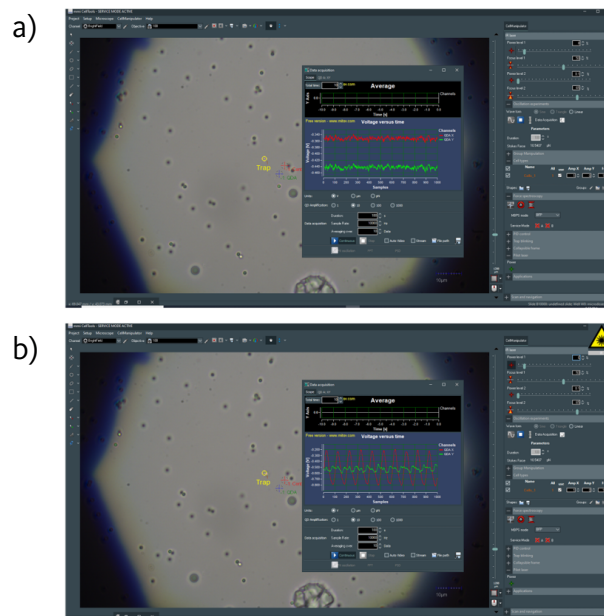
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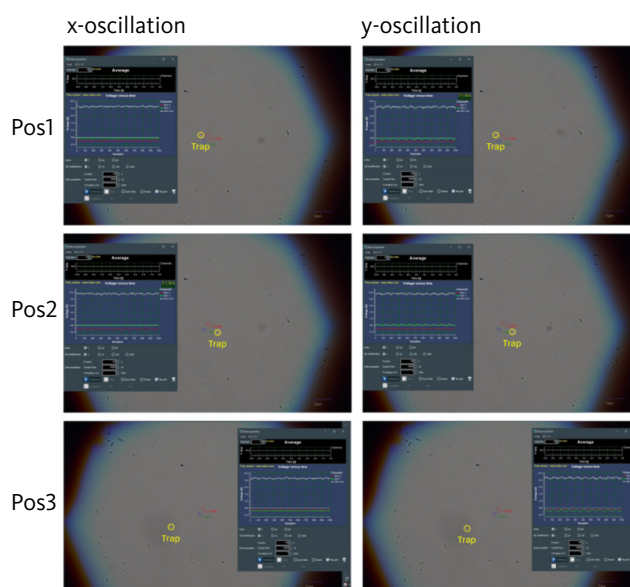
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Appendix

1. The comparison the 4QD response when a trapped bead was oscillated vs just the oscillation applied on nothing (negative control)



2. The 4QD detected oscillations at different locations inside the FOV



Technical Note_APN_011_A_Foward Interferometric Position Based Force Spectroscopy using Optical Tweezers

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