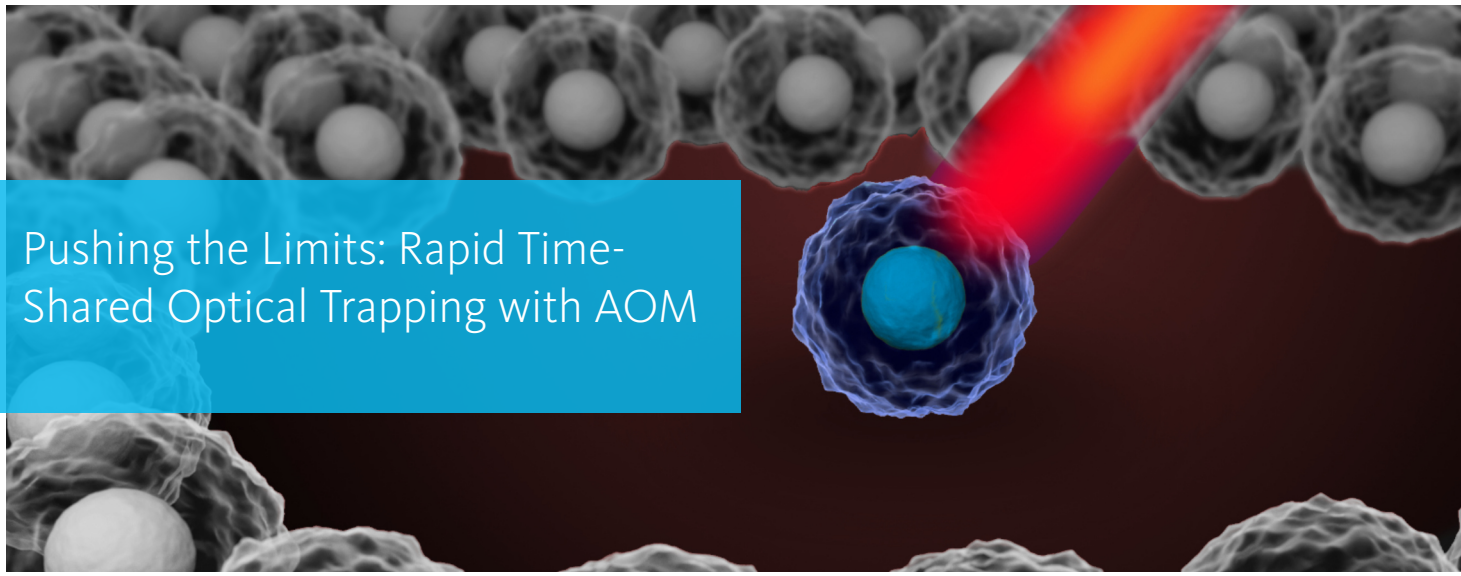
Technical Note

Acousto-Optic Modulator-Based Optical Trapping



Pushing the Limits: Rapid Time-Shared Optical Trapping with AOM

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Abstract

Trapping and manipulating many particles with an extremely rapid time-shared focused-laser beam is invaluable for researchers studying biological cells. This technique has immense potential to advance life sciences by accelerating discoveries at both intercellular and intracellular levels.

In this study, we demonstrated that the CellManipulator Optical Tweezers (OT) system can achieve stable time-shared trapping with a maximum visitation frequency of 10 kHz per trap using a dual-axis acousto-optic modulator (AOM). We successfully trapped single microparticles and cells and generated 256 trap points on a glass surface..

Finally, we performed trap stiffness calibration and force spectroscopy using the image-analysis mode.

Introduction

Basic of OT in Biology Applications

An OT provides 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 Zeiss Axio Observer 7 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, enabling a wide range of 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.

Beyond basic single- and multiple-trapping capabilities, the system supports quantitative force spectroscopy with resolution down to the sub-piconewtons range. Using galvanometric mirrors, the system can generate up to 20 time-shared traps through two polarization-split laser beams. With a high switching AOM, the system can generate more than 250 trap points using an unpolarized beam. These capabilities make the system ideal for a wide range of optical trapping applications, from studying biophysical interactions at the cellular and molecular levels to performing Raman spectroscopy on cells in a flowing microfluidic chamber [3, 8].

Time-shared based multiple trapping

An OT system typically operates with a single laser source, but this does not limit it to trapping only one particle. By rapidly switching the laser beam between multiple lateral positions, the system can generate multiple traps within a short timeframe—a technique known as time-sharing [9]. This method is implemented using one of two main approaches: galvanometric mirrors or AOM.

AOM uses the oscillating sound wave with the acoustic velocity of 650 m/s to modify the refractive index of a transparent crystal or glass [10]. The compression and the rarefaction of the material cause a change in density leading to periodic changes in the refractive index. It behaves like an optical grating, where the laser beam gets diffracted. Due to its high switching frequency, an AOM offers a response time more than 100 times faster than typical galvanometric mirrors. As shown in Tab. 1, the maximum response time for shifting the laser beam from one to another particle is 6700 µs for galvanometric mirrors and 50 µs for AOM. That means when users trap two particles, the visitation frequencies of the laser beam are:

$$f_v(\text{galvo}) = \frac{1}{6700 * 10^{-6} * 2} = \frac{75\ \text{Hz}}{\text{trap}}$$

$$f_v(\text{AOM}) = \frac{1}{50 * 10^{-6} * 2} = \frac{10{,}000\ \text{Hz}}{\text{trap}}$$

These represent the maximum visitation frequencies. The more the trap, the less frequent the trapped bead will be visited by the time-shared laser beam.

Tab. 1: The typical performance of commercial galvanometric mirrors vs. AOM

	Galvanometric mirrors	AOM
Device Switching Frequency (Max)[1]	7.14 KHz	20 KHz
Response time (Max)[2]	6700 µs	50 µs
Deviation angle	209 mrad	49 mrad

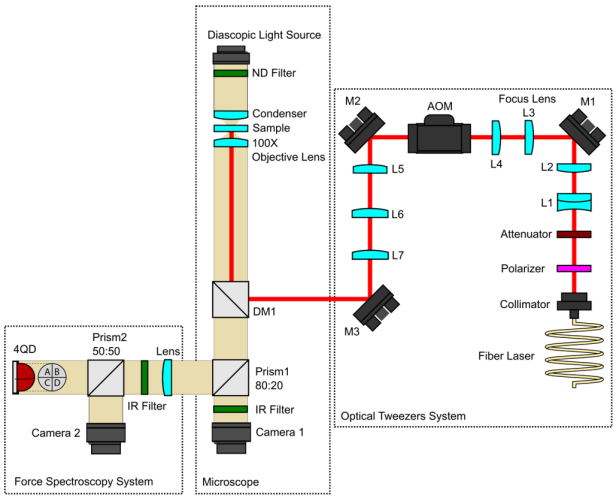

Fig. 2: Experimental Setup: The schematic of MMI CellManipulator OT system. The system consists of a beam control unit, an inverted light microscope, and a force spectroscopy unit.

Material and Methods

Polystyrene Beads

We prepared a working solution by diluting approximately 5 µl of polystyrene bead stock solution in 50 ml of distilled water and mixing it thoroughly. A droplet (ca. 20 µl) 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.

Pollen Cells

We prepared the sample by mixing approximately 5 mg of *Broussonetia papyrifera* (paper mulberry) pollen cells and mixed them thoroughly into 10 ml of distilled water. A droplet of the solution was then sandwiched between two glass coverslips, separated by the MMI membrane slide.

OT Setup

As shown in Figure 2, the diascopic light is generated by a light source and directed onto the sample through a neutral density (ND) filter and a condenser. The transmitted light is then collected by an objective lens and split in a 20:80 ratio between Camera A and the force spectroscopy unit. Within the force spectroscopy unit, the image undergoes further magnification before being evenly divided (50:50) between a four-quadrant detector (4QD) and a secondary camera.

Results

Single particle manipulation

In this study, we used polystyrene beads of 1, 2, and 3 µm, diluted in distilled water, to illustrate the most basic manipulation capability of the MMI CellManipulator OT system. As shown in Fig. 3, trapping the bead was achieved simply by clicking the bead of interest. By dragging the pointer, we moved the bead inside the field of view (FOV). Additionally, we tested the system's capability by trapping and manipulating a *Broussonetia papyrifera* pollen cell, which is approximately 10 µm in size.

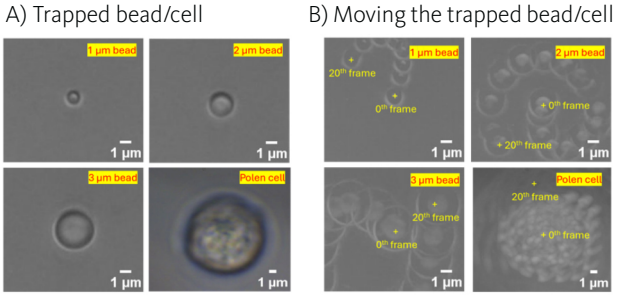

Fig. 3: (A) The single particle/cell trap. (B) The maximum intensity projection images generated from 20 frames of moving particle/cell images.

[1]Maximum jumps the device can generate

[2]Average time to jump from one trap to the other. For the relatively slower galvanometric mirror, during the "jumping" laser is turned off-on to minimize dragging movement on the trapped bead. The galvo movement during "jumping": up to 1500 µs, turning off: 50 µs, turning on 150 µs, trap time: 5000 µs. For AOM, the response time is considered 0 and no off-on timing. Therefore, only trap time contributes to the response time.

Group manipulation

In this study, we demonstrated the capabilities of the MMI CellManipulator OT system in generating a time-shared array of 256 trap points on a glass surface (Fig. 4). In this configuration, the maximum visitation frequency was around 80 Hz/trap.

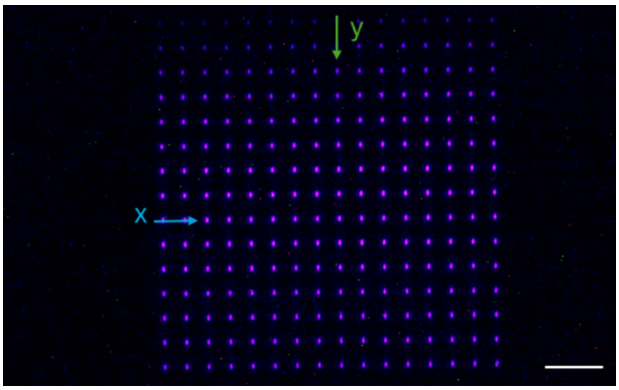

Fig. 4: Generating 256 trap points on the glass surface via 100x oil lens NA 1.3 using the MMI CellManipulator OT system. Scale bar = 10 µm. (T. Grawert master thesis)

Additionally, we showcased the system's ability to trap and manipulate 56 polystyrene beads, performing actions such as movement, stretching, rotation, and oscillation (Fig. 5).

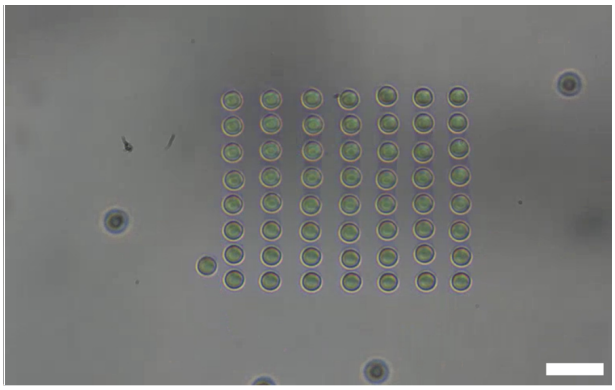

Fig. 5: Generating 256 trap points on the glass surface via 100x oil lens NA 1.3 using the MMI CellManipulator OT system. Scale bar = 10 µm. (T. Grawert master thesis)

Trap-stiffness calibration

In this work, we conducted voltage-displacement-stiffness calibration to demonstrate the capability of the image-analysis-based force spectroscopy system of the MMI CellManipulator As shown in Fig. 2, the image of the trapped bead was projected through the camera port onto a calibrated four-quadrant detector (4QD). Without this calibration step, the 4QD would detect only voltage differences resulting from bead movement within the sensing region.

To perform the calibration, we first translated the voltage into displacement. By oscillating the bead across the sensing region with an amplitude of 3 µm, we obtained the response curve (Fig. 6). We then selected the linear region around o V to determine the voltage-distance calibration factor.

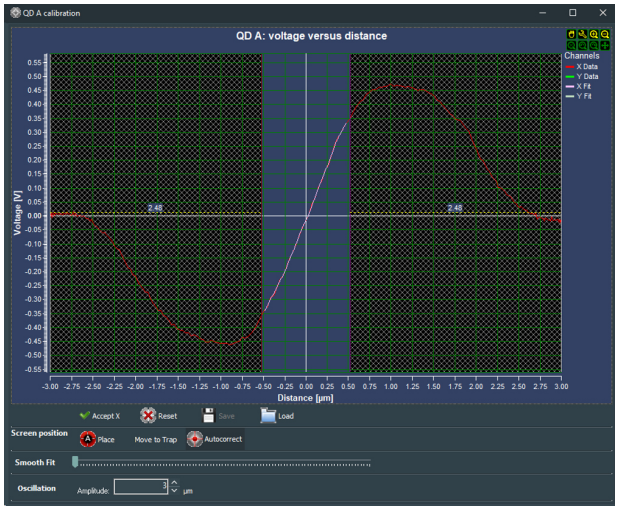

Fig. 6: Voltage-displacement calibration.

In this work, we conducted voltage-displacement-stiffness calibration to demonstrate the capability of the image-analysis-based force spectroscopy system of the MMI CellManipulator. Since the linear zone extended ±0.5 µm from the center, we set the maximum jump distance to 0.4 µm and the minimum to 0.1 µm, with a step width of 0.05 µm. As shown in Fig. 7, the 4QD accurately interpreted the jump distances.

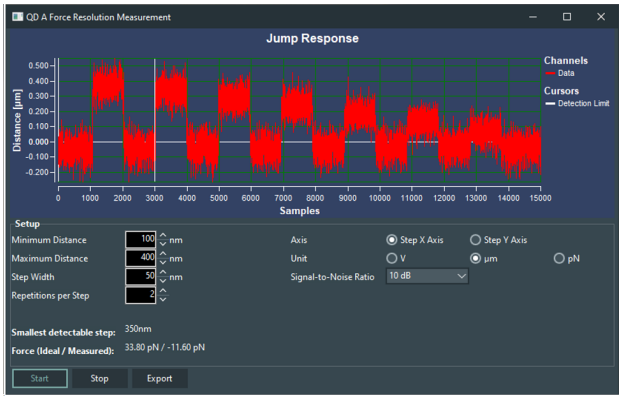

Fig. 7: Jumping experiments to verify the voltage-displacement calibration.

Next, we proceeded with the displacement-stiffness calibration. To do so, the MMI CellManipulator employs the harmonic oscillation method. The software automatically performed the bead oscillation by gradually increasing the frequency and measuring the phase shift at each frequency. Finally, the resulting curve was fitted to determine the trap stiffness (Fig. 8).

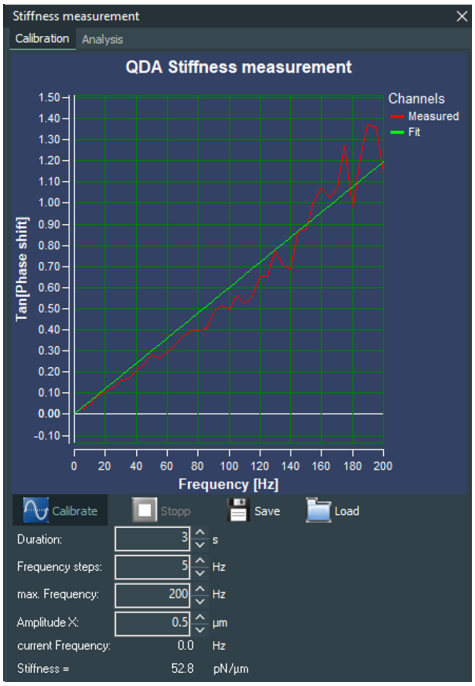

Fig. 8: Displacement-stiffness calibration.

The displacement-stiffness calibration was then performed using the harmonic oscillation method implemented in the MMI CellManipulator. The software automatically performed the bead oscillation by gradually increasing the frequency and measuring the phase shift at each frequency. Finally, the resulting curve was fitted to determine the trap stiffness (Fig. 8).

We then measured the trap stiffness for different bead sizes using a constant laser power of 88.57 ± 1.07 mW[3]. Tab. 2 shows the result.

Tab. 2: Trap stiffness of different bead sizes measured with a constant laser power (n = 50)

Bead size (µm)	Trap Stiffness (Average ± S.E) (pN/µm)
1	79.82 ± 1.21
2	78.02 ± 0.61
3	73.19 ± 1.09

Again, we verified the 4QD response of the displacement-stiffness calibration by performing jumping experiments in the x axis with exact parameters as before. As shown in Fig. 9, the forces were correctly measured by the 4QD.

Fig. 9: Jumping experiments to verify the displacement-stiffness calibration.

Force Spectroscopy

In this work, we measured the pushing force on the trapped bead. To do so, we trapped a 3 µm bead at the center of the 4QD sensing region and trapped another 2 µm bead, which we carefully manipulated to push the 3 µm bead.

We began by trapping the 3 µm bead and then performed the trap-stiffness calibration. Once the 3 µm bead was trapped, we used time-sharing multi-traps to trap a swimming 2 µm bead in the FOV and positioned it 1-2 µm away from the 3 µm bead.

[3]The laser power was measured at the sample plane through the 100x objective lens

After starting video recording and data acquisition, we carefully moved the smaller bead toward the trapped 3 µm bead along the x-axis direction.

In the case of using two time-shared traps with an AOM, the visitation frequency was around 10 kHz per trap, which corresponds to the data acquisition sample rate. As a result, the jump signal between two beads did not appear on the curve, which is a key benefit of having a high visitation frequency. When using galvanometric mirrors for two time-shared traps, the visitation frequency was around 75 Hz per trap. As depicted in Fig. 10, we were able to record the increasing pushing force until the 3 µm bead escaped the trap at approximately 25 pN.

A) Measuring the push force on the trapped bead

B) Force detected by 4QD

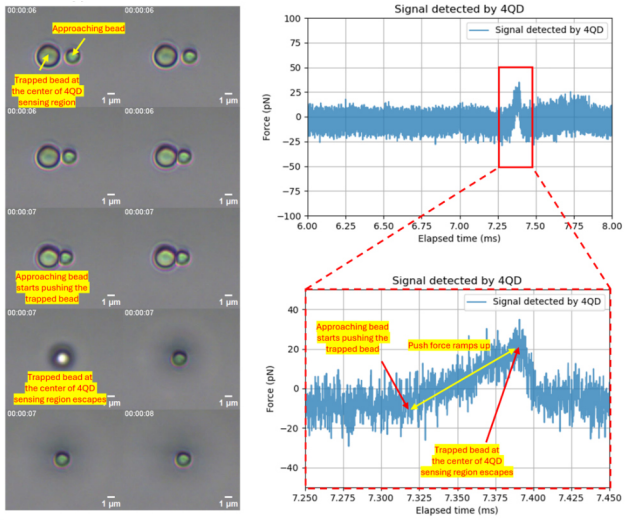

Fig. 10: (A) Measuring the pushing force on the trapped bead. A 3 µm bead was trapped at the center of the 4QD sensing region. Another 2 µm bead was trapped and was manipulated to push the 3 µm bead. (B) The increasing pushing forces on the 3 µm bead was recorded between 7.325 to 7.4 ms until the peak of around 25 pN was detected. After the peak, the 3 µm bead escaped the trap.

Conclusion

A versatile OT system, capable of manipulating multiple particles simultaneously and performing force spectroscopy, is highly valuable for studying biological cells. To demonstrate such capabilities, we developed an ultra-fast OT system with AOM as the beam steerer. With an extremely high switching frequency up to 20 KHz, we could manipulate single particles and group of particles simultaneously. We tested it not only on synthetic particles but also on cells.

Furthermore, we successfully demonstrated trap-stiffness calibration and force spectroscopy. This work expands the experimental possibilities of the MMI CellManipulator OT system, enabling a broader range of applications in optical trapping and force measurements.

Acknowledgements

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