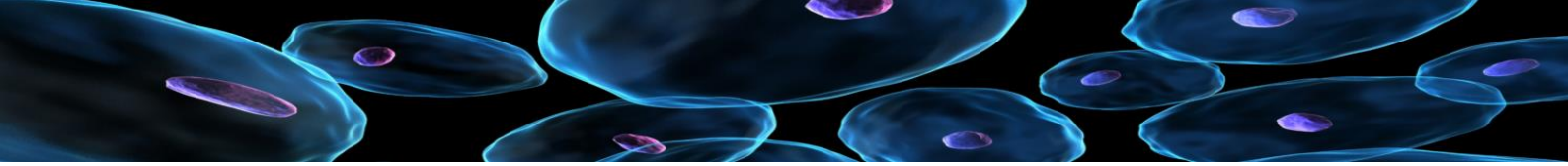

Optical trapping and force spectroscopy of non-spherical rod-shaped bacteria and diatoms

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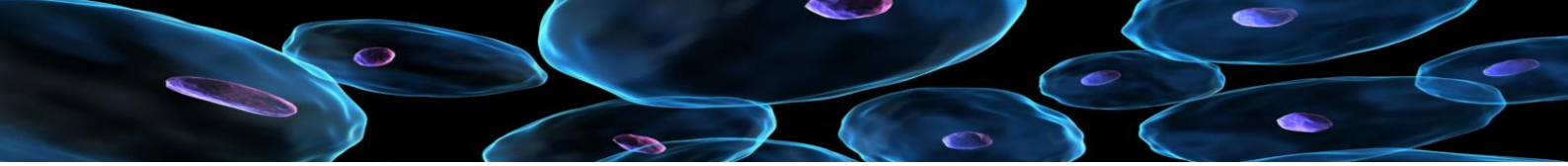
Keywords: MMI CellManipulator, optical tweezers, optical trapping, Cell manipulation, bacteria motility, diatom

Feburary 2022

Figure 1: MMI CellManipulator mounted on Nikon ECLIPSE Ti2 inverted microscope

Abstract

Optical tweezers provide non-invasive manipulation of cells and subcellular organelles to study their physical characteristics and biological properties. In this white paper, we have employed optical tweezers (MMI CellManipulator, Molecular Machines & Industries GmbH, Eching, Germany, illustrated in figure 1) to trap and immobilize bacteria and diatoms. Their motility and adhesion to surfaces is associated with a wide range of biological processes. *Marinobacter adhaerens* bacteria are small and rod-shaped, and they adhere to surfaces or are very dynamic in the medium. *Thalassiosira weissflogii* diatoms, in contrast, are larger in size. They have non-spherical elongated shape. Since optical tweezers are known to work best with round objects, the optimal and non-toxic manipulation of *M. adhaerens* and *T. weissflogii* is critical and challenging. In this study, we show that the MMI CellManipulator reliably detached the bacteria from the cover slip surface to immobilize and translocate them. As well, diatoms were trapped and translocated with this optical tweezers setup. The applied trapping force caused the bacteria and diatoms to rotate and orient their long axis parallel to the optical propagation axis of the optical tweezers. They returned to their original orientation when released form the optical trap.



Introduction

Single-molecule manipulation techniques have gained attention for many years, specifically optical tweezers which were discovered by Ashkin[1,2]. Optical tweezers allow for a contact-free non-invasive manipulation and biophysical studies of single cells[3]. Moreover, optical tweezers have been used intensively for single molecules manipulation such as DNA and proteins, with molecular motors for stretching, folding and adhesion forces. Optical tweezers are also used for cell sorting in microfluidics, separating cells, trapping bacteria and more[4,5].

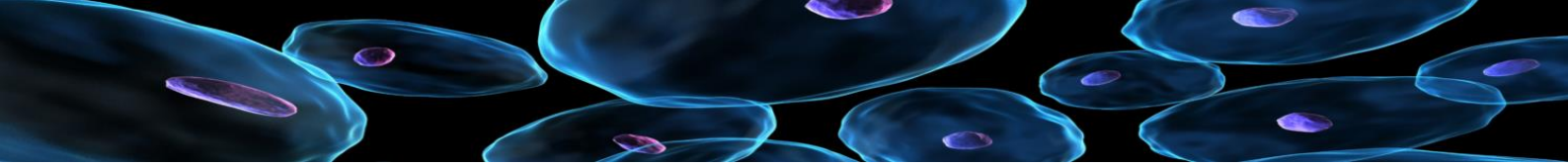
Optical tweezers are known to work best with round objects, such as microbeads. In this white paper, we have used optical tweezers from Molecular Machines & Industries (MMI CellManipulator) to manipulate and immobilize diatoms and bacteria as models for rod-shaped living microorganisms (Figure 1).

Diatoms are photosynthetic eukaryotes considered as a major group of algae. Diatoms are unicellular with various size ranging from 2 µm to 100 µm. The study of diatoms is important since they play a major role in the global cycling of carbon and silicon. It is considered that diatoms contribute at least 20 % of synthesis of organic compounds from atmosphere or aqueous carbon oxide known as the annual primary productivity. Their large size make them difficult to be handled by common optical tweezers[6,7].

Bacteria can adhere to surfaces but can also be motile in the medium. Their behavior and functionality are widely studied. Optical tweezers provide an opportunity to discover further aspects of the mechanisms behind their behavior and as well their functionality[8,9,10].

Optical tweezers principle

Optical tweezers focus infrared laser to a diffraction-limited spot on the sample plane to trap the target. The optical trap is able to hold, move, rotate, join, separate, stretch or otherwise manipulate the target. Once a laser beam is encountering an object, the light will be refracted and since the light carries momentum, the optical tweezers will exert force on the target. Near the laser beam focus, the gradient force pushes dielectric particles with high refractive index (in relative to the medium refractive index) toward the focal point, where the laser intensity is high (Figure 2)[5,11]. Light momentum which is transferred to the object (conservation of momentum) is always directed towards the laser focused spot. At the same time, beside of the gradient force, the optical radiation pressure force resulted from the absorption or reflection of the light by the object, will be acted on the object. This force will push the target away from the focus. These two forces are acting against each other. Thus, highly optimized optics including high numerical aperture (NA) objectives are required to create a diffraction limited spot at the object plane to allow the gradient force to overcome the radiation pressure force. The applied concepts and principles in the optical tweezers, strongly depend on the size of the object relative to the wavelength of the optical tweezers laser (1,070 nm), the optical and physical properties such as refractive index and shape of the object. When the object is much bigger than the optical tweezers laser wavelength, ray optics principles are applied. If the object is much smaller than the light wavelength, the dipole approximation is adopted. When the object size is comparable to the light wavelength, the wave optics models are applied[5,11] (Figure 2).



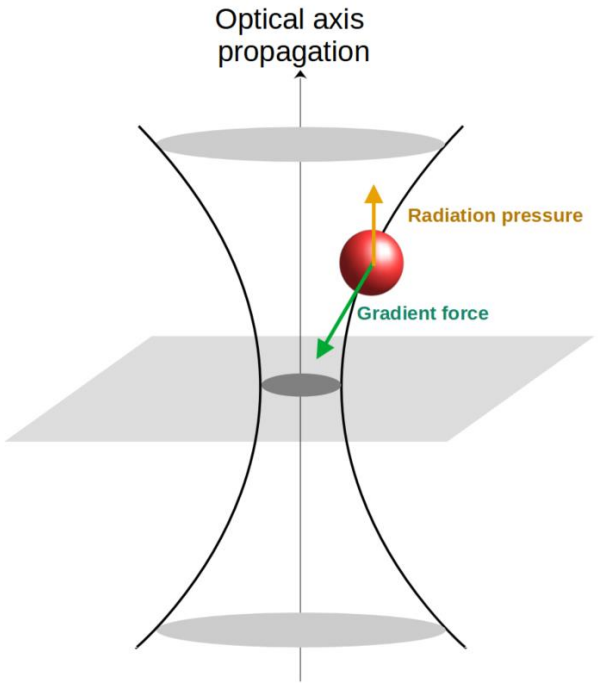


Figure 2: Optical tweezers principle – Optical tweezers laser is focused to create diffraction limited spot on the sample plane. Light momentum is transferred to the objects due to the energy conservation. Gradient force and radiation pressure force are acting on the target against each other.

MMI CellManipulator

Molecular Machines & Industries (MMI) is providing solutions for single cell Micromanipulation, Microdissection and Imaging. The MMI CellManipulator was introduced in 1992 as one of the very first commercial optical tweezers systems. The long history and success behind the MMI CellManipulator makes it the most reliable, effective and comfortable optical tweezers tool in the scientific community. The MMI CellManipulator can easily be implemented on top of the major microscope systems from Nikon and Olympus (Figure 1, more details can be found at https://www.molecular-machines.com/products/cellmanipulator).

Besides of its powerful trapping capabilities, MMI force spectroscopy modules enable precise force measurement of the samples. It has optical force in the range of 1-2,000 piconewtons (pN) on targets with size ranging from few nanometers (nm) up to several micrometers (µm) in sub-millisecond time resolution. The MMI CellManipulator system offers up to 20 traps with time sharing with two independent beams. MMI CellManipulator consist of an infrared laser (1,070 nm, 10 watt continuous-wave) which is focused by the microscope objective (100× oil immersion). This powerful laser brings necessary power required for trapping larger samples. A fast two-axis galvanometer is used to give the flexible trap positioning across the field of view. Figure 3 illustrates configuration of the MMI CellManipulator mounted on a Nikon ECLIPSE Ti2 inverted microscope. The infrared laser is coupled to the scope through a second

vertically separate light path without interfering with the microscope performance.

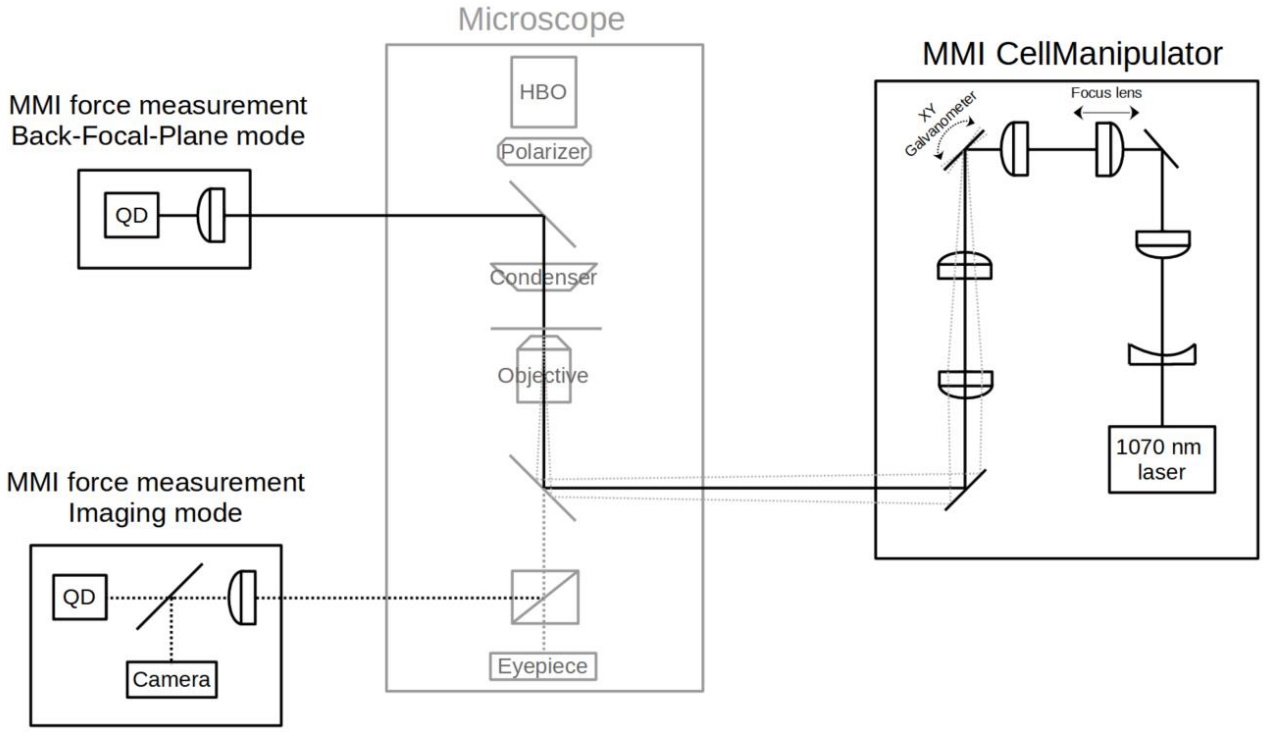


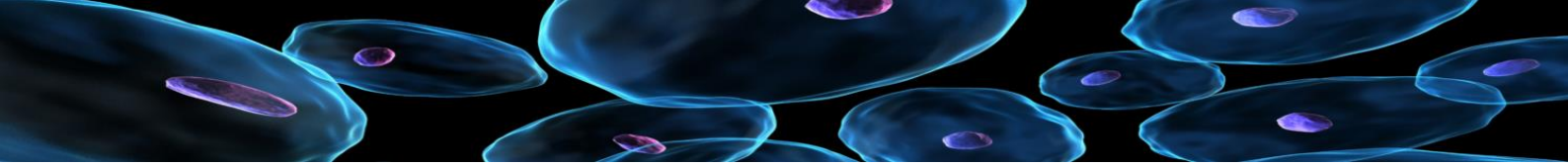
Figure 3: Schematic of MMI CellManipulator, mounted on a Nikon ECLIPSE Ti2 microscope. The MMI CellManipulator is shown on the right side, microscope in the middle and force measurement modules are illustrated on the left side.

MMI CellManipulator offers two types of force spectroscopy technologies refereed as imaging mode force spectroscopy, mounted on a camera port of the microscope, and back focal plane force spectroscopy, mounted in front of the condenser lens. In this study we have used the imaging mode force spectroscopy. It contains a four segment photodiode quadrant detector (QD) used for measuring the position of the trapped object[12]. A lens transfers image of the target object on a quadrant photodiode detector. Their fast response time with high precision and stability is required for the target position detection. The quadrant photodiode detector of the MMI CellManipulator features multiple noise reduction layers with adjustable signal amplifications. The force calibration process is performed by following the instruction within the MMI software CellTools. Depending on the research question, force calibration can be done on target cells or beads. After force calibration, force measurement can be utilized.

Sample preparation

Bacteria

Marinobacter adhaerens HP15 (wild type) was used in this work. A single colony was picked from 2216 agar plate, inoculated into 3 mL of 2216 medium, and cultured overnight in a shaking incubator (300 rpm at 30 °C).



Diatom

Thalassiosira weissflogii (CCMP1336) was obtained from the Bigelow culture collection. This culture is axenic and was maintained in L1 medium prepared from autoclaved and sterile-filtered artificial seawater (a salinity of 36 ‰). A photon irradiation at ~100 µmol photons m^{-2} s^{-1} was provided over a 14-hour/10-hour day/night cycle. Cells were inoculated in fresh L1 medium at a 1:100 (v/v) dilution three days before an experiment.

Polystyrene beads

A 2 µm aqueous polystyrene bead dispersions with relatively low polydispersity (Polysciences, Inc.) were used. The bead dispersion was diluted 1:100 in water and a drop of it was placed on a cover slip. Beads were allowed to settle for approximately 30 min prior to the experiment.

Results

Trapping motile bacteria

Bacteria are very motile organisms. Their motility which is mediated by different mechanisms, such as bacterial flagella, is crucial to their development and has been studied in various researches[8]. The MMI CellManipulator was used to trap motile rod-shaped bacteria. The force generated by optical tweezers was optimized to trap and immobilize the actively swimming bacteria. Figure 4A shows a screenshot of bacteria, some adhere to the surface and some are motile. 100× oil immersive objective was used to image and trap a bacterium. To demonstrate the trapping effect of the optical tweezers, a swimming bacterium was trapped and immobilized at position A shown in figure 4B. The bacterium was held in this trap briefly and when the bacterium was released from the trap, it recovered the motility. Then the bacterium was freely moving for a short time, before it was trapped again at position B in figure 4. We observed that when bacteria were released from the trap, they oriented themselves parallel to the surface but when they were trapped, they rotated and aligned themselves parallel to the optical tweezers laser propagation axis as illustrated in figure 4C.

Detaching adherent bacteria

As mentioned previously, some bacteria adhere to the coverslip surface. The optical trapping force of the MMI CellManipulator was strong enough to overcome the adhesive forces between the bacteria and the surface. As shown in figure 4D, the bacterium which was adhered to the surface at position A, was trapped and immediately it changed the orientation parallel to the optical tweezers light axis. The bacterium was translocated with the optical tweezers on a random path to position B and was released from the trap. Consequently, the bacterium adhered itself again to the surface and immediately aligned parallel to the surface (Figure 4D).

Trapping large diatoms

In this work, we used diatoms to illustrate the capability of the MMI CellManipulator to trap and move large non-spherical biological samples without damaging them. As shown in figure 5A, this diatom is elongated in one axis with the size of approximately 15 µm. By pointing the optical tweezers laser to the diatom, it rotated and aligned itself to the optical axis propagation (Figure 5B, C). Next, the trapped diatom was translocated by the optical tweezers. The diatom kept its orientation and followed the optical tweezers trap. After releasing the diatom from the trap, it was returned to its orientation of long axis parallel to the surface (Figure 5C).

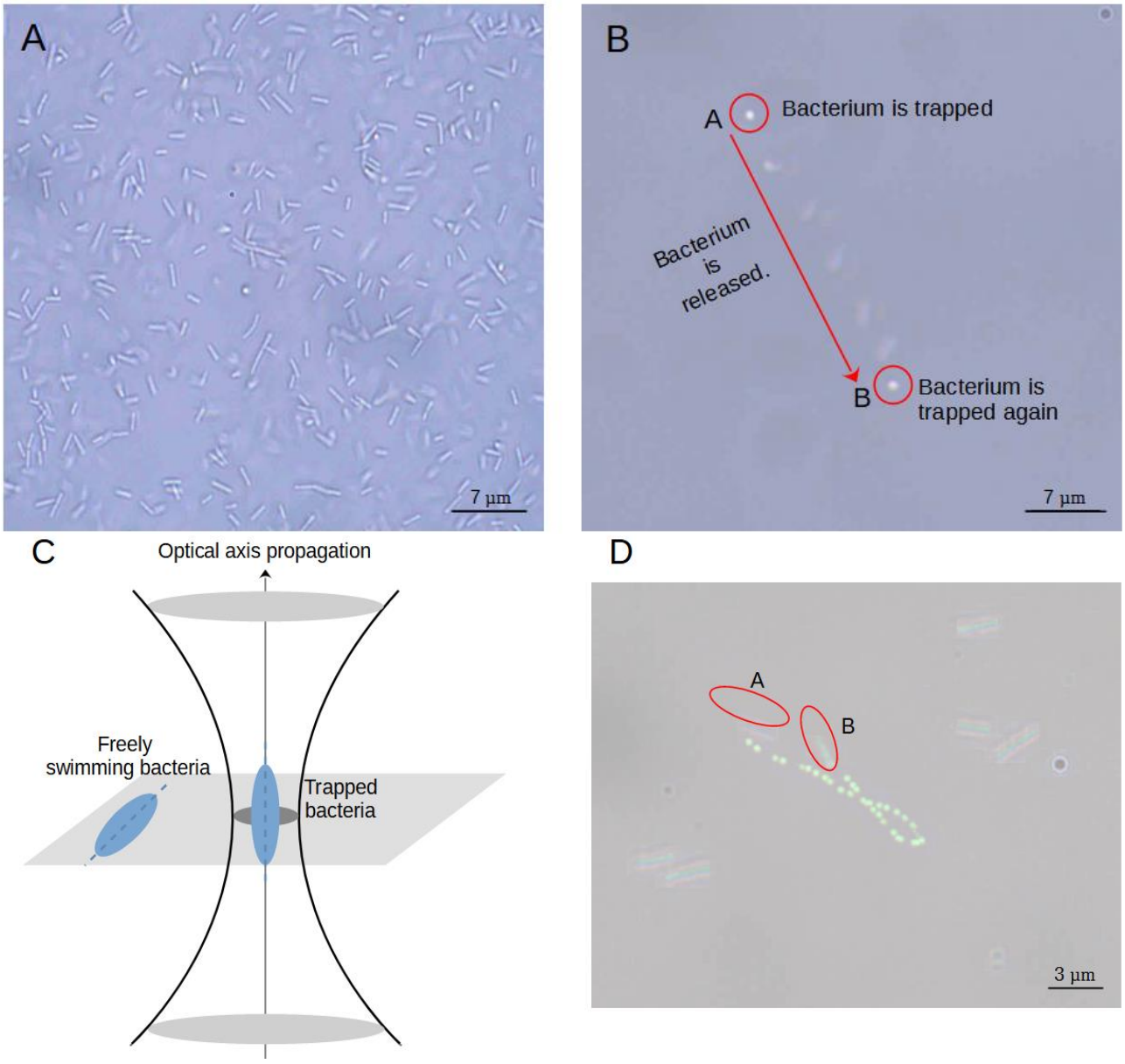
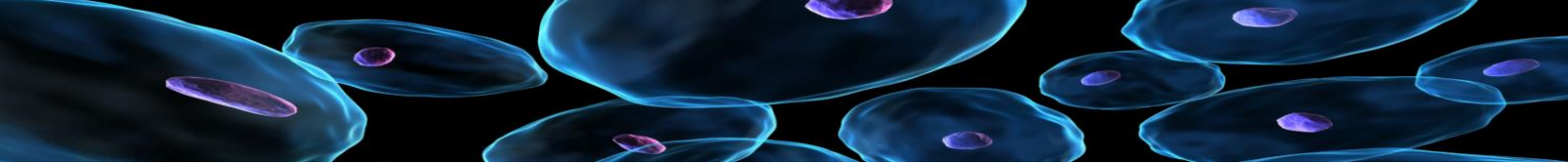


Figure 4: Trapping and manipulating bacteria with the MMI CellManipulator optical tweezer. A) Screenshot of bacteria – some adhere to the surface while others are motile. B) A motile bacterium is trapped and after releasing the bacterium from the trap, it starts swimming again. C) Optical tweezers create strong forces, causing the bacteria to jump into the trap and align themselves parallel to the laser optical axis. D) A bacterium, that adhered to the surface of the cover slip, was detached from the surface and moved to different positions by the MMI CellManipulator. Watch the videos at https://youtu.be/Wxcedu1TeFE and https://youtu.be/wdhOjrp4tvc



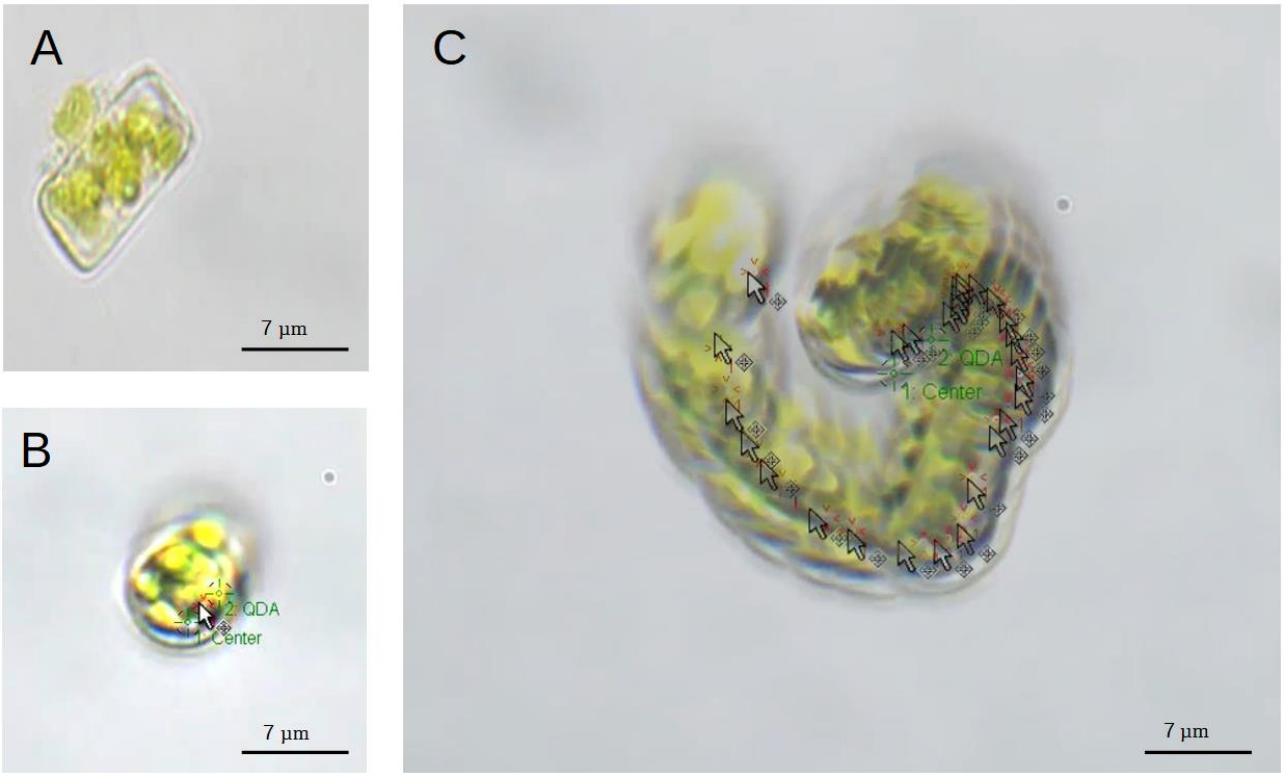
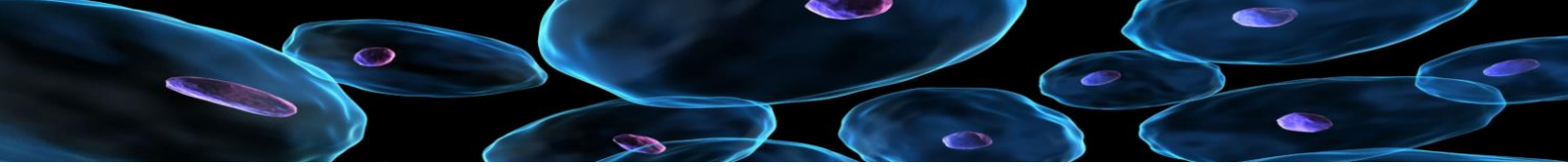


Figure 5: A) Bright field image of a diatom with wide field microscope and 100X oil immersion objective. B) A diatom is trapped by the MMI CellManipulator. C) The trapped diatom followed the optical tweezers trap movement and was translocated to another position. Watch the video at https://youtu.be/Z_pjB-B3VGE

Bacterium force spectroscopy

We would like to measure the exerted force on the bacterium. To do so, first we follow the calibration procedure within the MMI CellTools software with a 2 µm bead in the same medium as the bacteria. When the force calibration is completed, force measurement can be utilized. To perform the force measurement, a swimming bacterium was trapped and was oscillated in one axis with a 3 µm amplitude. The exerted forces on this bacterium were measured during the movement. The measured force is shown in figure 6. In this figure, x-axis is the position of the bacterium in µm and y-axis is the exerted force in pN. The trap force measurement can be done in x- or y-axis oscillation with different oscillation amplitude or as well with linear movement of the trap. At the same time, the force can be measured and visualized live on the MMI CellTools platform. All the data can be exported for publication purposes. Analyzing the mechanisms behind these forces are of interest for many researches.



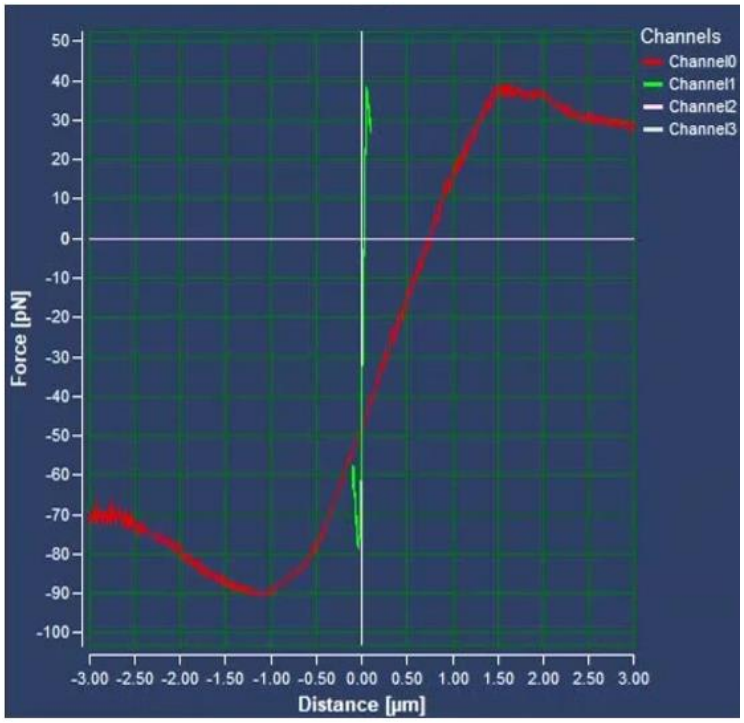


Figure 6: Force calibration is done on a 2 µm bead by following the calibration procedure within the MMI CellTools software. After calibration, a bacterium was trapped and was oscillated with 3 µm amplitude (x-axis). The exerted forces on the bacterium were measured during the oscillation (y-axis). The x-axis is position of the bacterium in µm and the y-axis is the exerted force in pN.

Conclusion

Optical tweezers are emerging tools for manipulating living cells and sub-cellular structures in a contact-free and contamination-free way. The MMI CellManipulator offers modular design with specialized optics to achieve maximum trapping forces and stability. In this study, we demonstrated that the MMI CellManipulator optical tweezers system is an easy to use system to create stable and adjustable trapping forces which is applied to manipulate rod-shaped living organisms such as bacteria and diatoms. Optical tweezers are known to sufficiently trap spherical particles but facing difficulties in manipulating the elongated objects. This study highlights that optical trapping can also be applied for elongated cells. Interestingly, the rod-shaped cells orient themselves along the laser beam axis. This study opens the door to further trapping experiments with rod-shaped organisms, especially including force spectroscopy measurements in physiological conditions.

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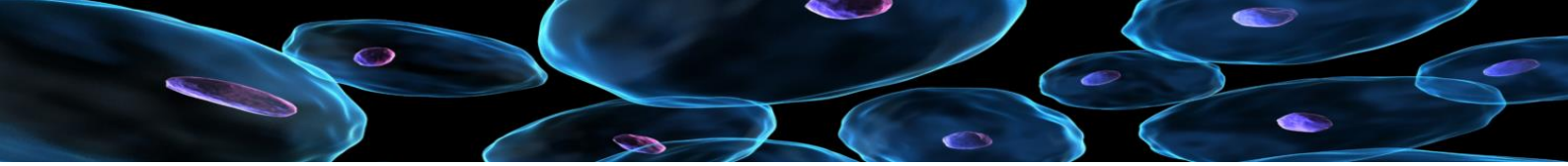

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