
Encyclopedia of Nanotechnology



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Optical Trap

► Optical Tweezers

Optical Tweezers

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Synonyms

[Laser tweezers](#); [Optical trap](#)

Definition

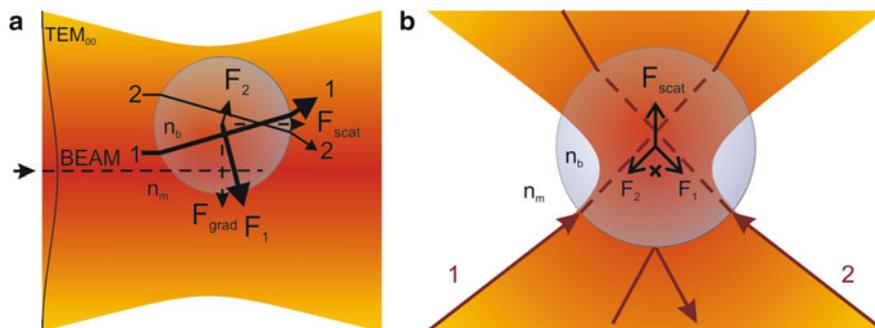
The technique of optical tweezers and manipulation of small neutral particles by lasers is based on the forces of radiation pressure. These forces arise from the momentum of the light itself.

Overview

It has been first documented by Arthur Ashkin in 1970 that optical forces, or radiation pressure, could be used to trap and accelerate dielectric polarizable micron-sized particles [1]. For these experiments, a stable optical potential well was formed using two, slightly divergent, counter-propagating laser beams. This pioneering study established the groundwork for the well-known optical tweezers (OT) technique, where a single TEM₀₀ laser beam is focused by a high numerical aperture (NA) objective lens to a diffraction limited spot.

In the ray optics regime, in which the dimensions of the trapped particle are larger than the wavelength of the laser light, known as the Mie regime, the origin of optical forces can be understood easily. [Figure 1](#) shows the principle of optical trapping in the Mie regime. In [Fig. 1a](#), a dielectric semitransparent sphere with a high index of refraction (n_b ; e.g., polystyrene $n_b = 1.59$) is shown. This sphere is immersed in a fluid with index of refraction n_m (e.g., water $n_m \sim 1.33$). When exposed to a slightly focused laser beam with a Gaussian intensity profile, the sphere is drawn into the high-intensity region of the laser and then accelerated along the beam axis. Consider two rays “1” and “2” impinging on the surface of the sphere in a symmetrical manner about its center. At this moment, surface reflections are neglected. Most of the rays get refracted through the bead and give rise to forces F_1 and F_2 in the direction of momentum change. For theoretical consideration on the forces arising, the reader is referred to Grange et al. [2]. Due to the fact that the intensity of ray “1” is higher than that of ray “2,” the force F_1 is higher than F_2 . Adding all such symmetrical pairs of rays striking the sphere, it is imaginable that the force can be separated into two components, a gradient force, F_{grad} , and a scattering force, F_{scat} . The gradient component arises from the electric field gradient pointing toward the highest intensity region of the beam. The scattering force component, F_{scat} , which is pointing in the direction of the laser beam, is caused by the photons scattering on the surface of the sphere. In [Fig. 1b](#), stable optical trapping in three dimensions is illustrated. If the laser beam is highly focused, a backward light force is generated. It can be shown that stable trapping occurs along the optical axis when the gradient force, which is composed of the sum of the two forces F_1 and F_2 , overcomes the scattering force F_{scat} . This explains why (1) high NA objective lenses have to be used and (2) the back aperture of the objective lens has to be overfilled to create the steepest possible gradient. When a particle is stably trapped, the sum of all forces acting on it is zero. If the particle is slightly displaced from the equilibrium point by applying an external force, the gradient force acts in the direction of the field gradient toward the center of the beam. This force depends linearly on the displacement similarly to a spring that follows Hooke’s law. Optical forces are however very small, since 100 mW of laser power at





Optical Tweezers, Fig. 1 *Optical forces.* (a) Origin of scattering force, F_{scat} , and gradient force, F_{grad} , for a dielectric high index of refraction (n_b) sphere in a medium (n_m) in a mildly focused laser displaced from the TEM_{00} beam axis. The gradient component is oriented orthogonally to the axis of the laser beam, and the scattering is parallel. (b) The axial trapping requires

highly convergent rays. A two-dimensional ray optics model illustrates the scattering and the gradient forces in the Mie regime. Since the axial trapping is weakest in the single beam configuration, counter-propagating laser traps have been developed [2, 16]

the focus only produce forces of tens of piconewtons. At the focal point, not only dielectric spheres can be trapped, but also biological organisms such as cells, viruses, or bacteria as shown in 1987 by Ashkin and coworkers. The trapping of objects much smaller than the wavelength of the laser light, such as cooled atoms, has been demonstrated. This work resulted in the Nobel Prize in physics in 1997. Trapping cooled atoms cannot be understood by simple ray optics. For a more detailed insight into the principle of trapping atoms, the reader is referred to the entries of this encyclopedia where atomic clocks or Bose-Einstein condensates are discussed. For a good overview on the evolution of optical trapping, a review from Ashkin is recommended [3].

Choice of Light/Laser Source

The high-intensity radiation of the laser beam used in optical trapping experiments has the potential of effecting materials exposed to the radiation. In some experiments, laser power of more than a hundred milliwatts (mW) is used as a source. When focused to a micron-sized region, the light power in the trapping spot is equivalent to 10 MW/cm^2 , which is over 600 million times greater than the flux of solar radiation on the Earth's surface on a sunny day. For chemical or other physical experiments, where the material manipulated is being modified, lasers with a wavelength in the visible regime are used. For biological research, laser light is in the near-infrared regime

(NIR: $\lambda \sim 800\text{--}1,100 \text{ nm}$) and the laser power is chosen depending on the system under investigation. In the high-power NIR, single biomolecules are less susceptible to optical damage than whole cells. The local heating of the surrounding liquid is in the range of only a few degrees Celsius. However, Steven Block's lab demonstrated in 1999 that live microorganism cells were affected by the radiation and showed physiological photodamage of live *Escherichia coli*.

Another technique, laser-tracking micro-rheology (LTM) was developed in the mid-1990s to probe the mechanical properties of living cells by examining cellular viscoelastic properties [4]. Not only cells can be probed but also rheological properties of liquids in general. Micron-sized trapped objects such as beads or individual cells are moved with OT and can be used to generate fluid flow in two and three dimensions around the item and this can be combined with rheological measurements. Thereby, the bodies' displacements from the trap center are analyzed during the motion or when the optical trap is temporarily switched off. Such LTM measurements are highly reproducible, minimally invasive, and can be extended to in-depth studies of the rheological properties of liquids, cells, or internal cell compartments and microstructures in lab-on-a-chip devices [5].

Meanwhile, OTs are a focal point for interdisciplinary research ranging from condensed matter physics and physical chemistry to biology [6]. The fast emerging applications for OT have led to various technical innovations, thus improving the versatility of optical traps. The instrumental advances involve



rapid position detection, sample positioning, trap stiffness determination, applying of controlled and calibrated forces. Multiple laser beams and computerized automation of laser beams have been used to study quasicrystals as well as polymer, metallic particles [7] and also aerosol droplets in air [8].

Most commonly TEM₀₀ lasers with a Gaussian intensity beam profile are used to form a trap (see Fig. 1a). But physicists also started to use other beam profiles, such as Bessel beams, to enable the manipulation of micrometer-sized particles in multiple planes. Asymmetric beams for instance are used as tools to study the transfer of angular momentum from light to particles for their rotation. An overview on such developments is given in Ref. [9], where a series of experts in the field describe their optical experiments.

Setup of an Experiment

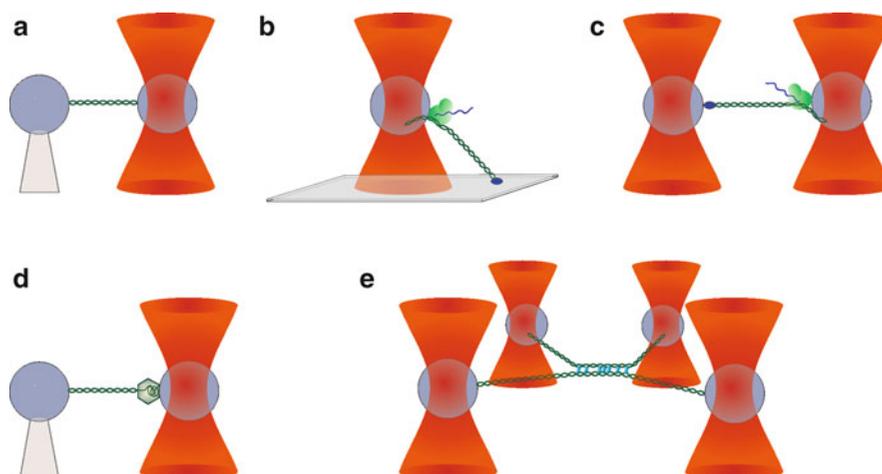
The interest for biologists in the OT technique comes from the fact that minute forces can also be measured with sub-pN accuracy and nanometer spatial resolution on the trapped object. Since such small forces are not accessible by conventional techniques, such as scanning-force-microscopy-based techniques (in aqueous/liquid environment, the natural “habitat” for many both *in vitro* and *in vivo* biological systems), OT has become a major investigating tool in biology (for recent reviews on instrumentation in optical trapping see [10–13]).

Biological molecules are often in the nanometer regime and are too small to be directly trapped by OT. The key to such experiments is the immobilization of the biological molecule of interest, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein or molecular motor, on the surface of a micron-sized sphere. The sphere serves as a handle for optical manipulation. In the commonly used OT setups, interacting partner molecules or the other end of the manipulated molecule is attached either to a cover slide or to a second bead (which can also be trapped in three dimensions) see Fig. 2. The external control parameters, which can be manipulated in such experiments, are the end-to-end distance of the investigated biological system linked to the spheres/surfaces or the externally acting force in the range of 0.1–150 pN. In experiments one can vary the distance between the spheres, and then the optical force

acting on the bead is recorded. The experimental plots of distance-versus-force give direct access to the mechanical properties of elongated molecules. Force-extension graphs are typically compared to worm-like chain or freely jointed chain models for polymers, and parameters that describe the polymer are extracted – most notably the persistence length, which describes the behavior of the polymer under thermal fluctuations. In the other experiments, where the position of one sphere is fixed and the force is kept constant, the molecular extension is the variable under observation. Such experiments allow direct examination of the kinetics of the involved molecules, may it be a biomolecular motor traveling along a defined path of another biomolecule (e.g., RNA polymerase along a DNA molecule [14]) or a change in molecular mechanics as observed in protein polymerization on DNA [15].

The single molecule approach to biology offers distinct advantages over the conventional approach of taking ensemble measurements. Parameters such as individual kinetics or motion can be investigated (e.g., observing a single molecular motor at work). One should keep in mind that the setup of these intricate experiments must be done in series, which is very time consuming. The required purity of the solutions and biological molecules is very important and different from “normal” molecular biological experiments where ensembles are investigated. The arrangement of an experiment in the OT starts with the production of modified molecules at a nano-molar concentration ($\sim 10^{11}$ /mL). Subsequently, the molecules which are often artificially tagged to enable specific anchoring on interfaces are linked with spheres at a concentration of 10^8 /mL. The spheres thereby serve as handles for manipulation in the OT device. The strongest linkage of biomolecules to the interface of the spheres requires covalent binding and chemical activation steps. Each additional step reduces the amount of successfully anchored molecules on the interface. Therefore, a bigger quantity of modified biomolecules has to be produced at the start to end up with one molecular complex available for the OT experiment. Ligand-receptor pairs, such as biotin-streptavidin, histidine tags nitrilotriacetic acid (NTA), or antibody-antigen pairs (e.g., digoxigenin and anti-digoxigenin), are commonly used to provide specific, oriented, and tight binding to microspheres. Biomolecular interactions do not have to be activated like chemical





Optical Tweezers, Fig. 2 *Geometry of optical tweezers systems used in single molecule experiments.* (a) One end of the DNA molecule is attached to a microsphere held in the optical trap and the other end to a bead held by a glass micropipette by suction. Pulling force is generated by moving the micropipette, which is mounted on a piezoelectric stage [2, 15, 16, 24]. (b) RNA polymerase is attached to the trapped microsphere and to one end of the transcribed DNA. The other end of the DNA is attached to the glass slide of the flow cell mounted on the piezoelectric stage. Force is generated by moving the

piezoelectric stage [12, 19]. (c) Dual optical traps are used to eliminate micromechanical noise coming from the glass slide or micropipette. Individual 0.34-nm base-pair steps of RNA polymerase translocation are observed in this setup (see Fig. 3c) [14]. (d) Geometry of the OT used for the investigation of bacteriophage ϕ 29 DNA packing motor [20]. (e) Sequences of nonspecific protein-DNA interactions are investigated using four optical traps. The DNA-H-NS-DNA complex is formed by trapping two DNA molecules simultaneously in the presence of nucleotide-associated protein H-NS. Schematics are not drawn to scale

reactions. This allows longer incubation periods to complete the interactions and often provides a successful coupling of the molecule to the surface of the spheres. Constant force experiments with forces exceeding 40 pN for long duration require either biotin-streptavidin or covalent linkage. The biomolecular “bonds” of single antibody-antigen pairs unbind after short periods while being pulled. The externally applied forces lower the activation barrier; therefore, the ligand unbinds faster from its receptor. To strengthen such interactions, some researchers apply more than one of these bonds in series. This principle has already been used in DNA pulling experiments where multiple digoxigenins were introduced as tags on the DNA to anchor the molecule to the antibody-activated spheres [16].

Biological Applications of Optical Tweezers

In the following sections, experiments with OT, which are used for holding and manipulating a variety of

nanometer- to micron-sized biological objects such as DNA, single molecules, membrane bilayers, intracellular structures, vesicles, and plant cells, are reported [12]. Trapping these distinct objects with laser tweezers enables measurements of their mechanical properties such as elasticity, stiffness, rigidity, torque, and dynamic behavior.

Single Molecular Systems in Optical Tweezers

To date several different homebuilt OT systems have been designed to manipulate single molecules of DNA and RNA, to investigate interactions between DNA and proteins and to measure the forces generated by single molecule motors. Thereby, the position of the trapped microspheres, which act as handles, can be tracked with sub-nanometer spatial resolution and sub-millisecond temporal resolution [17, 18].

Bustamante and coworkers performed one of the first single molecule experiments with OT in order to



study DNA elasticity. In their assay, one end of the manipulated DNA molecule was attached to a bead held in the optical trap and the other end to a bead, which was held by a glass micropipette by suction (see Figs. 2a and 3a) [2, 16]. This assay gave detailed insight into the mechanical properties of double-stranded DNA (dsDNA) molecules for forces between 1 and 100 pN [16] (Fig. 3b).

Protein-DNA interactions are the most fundamental biomolecular interactions in the living cell. DNA elasticity plays an important role in the interactions with proteins and protein complexes. It was demonstrated that by cooperative protein binding to DNA, proteins act as a powerful molecular machine [15]. The seminal experiments, which focused on protein-DNA interactions involved in transcription, copying, and packaging of DNA, were performed around the turn of the century by the Block or Bustamante labs [19]. Most of these processes are performed by molecular motors, i.e., proteins with dynamic structures capable of converting energy stored in nucleoside triphosphates (NTPs) into mechanical work. For example, RNA polymerase is an enzyme that constructs RNA chains from DNA as templates in a process called transcription. Single molecule studies performed with OT gave comprehensive insight into the stall force, transcriptional pausing, and backtracking of RNA polymerase. Please refer to the review papers [18, 20] for a good overview. One of the OT geometries used for studying DNA transcription is shown in Fig. 2b. The transcribing RNA polymerase was attached to a microsphere held by the optical trap and the DNA is attached to the surface of a flow cell. The position of the flow cell could be precisely controlled by a piezoelectric stage. During transcription, the polymerase moved along the DNA and therefore shortened the distance from the sphere to the end of the DNA. To maintain a constant force on the RNA polymerase, respectively on the trapped bead, the piezoelectric stage moved along the direction of the polymerase to keep the bead in the laser trap at a constant position (i.e., at constant force). In both experiments, the DNA elasticity experiment (Fig. 2a) and the RNA polymerase experiment (Fig. 2b), the biomolecules were linked to a surface, which was “connected” to the external mechanical environment of the OT (micropipette or flow-cell wall). This connection introduces a positional noise of a few nanometers to the experiment due to the inherent thermal

motion of the mechanical objects to which the biomolecules are attached.

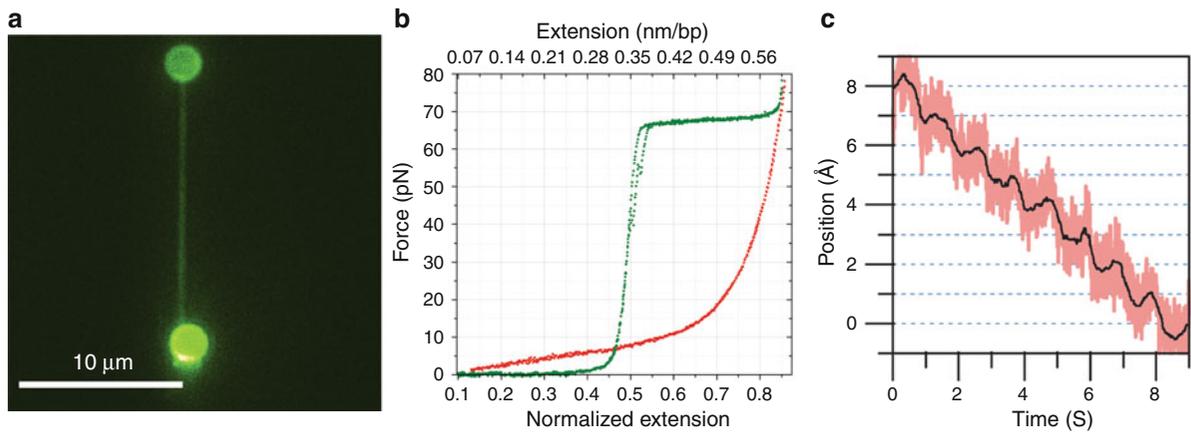
A much more stable OT design was obtained by decoupling the spheres from mechanical objects introducing a second optical trap into the system. Instead of using a micropipette or a glass slide, a high-power laser beam was split optically to receive another attachment point with a second sphere, as illustrated in Fig. 2c [17, 18]. One of the beams was then moved by steering the beam with one precise optical mirror. This bead-DNA-bead dumbbell configuration allowed Block and coworkers to observe amazingly individual 0.34-nm base-pair steps of RNA polymerase translocation [14] (see Fig. 3c).

When viruses are reproduced by infected cells, they are reassembled from proteins which form a shell (i.e., a capsid) and from their genetic material, which they need to infect subsequent cells. The packaging of viral DNA by a rotary protein motor into the viral capsids was investigated with OT in 2001 by the Bustamante lab [20]. One of these studies (Fig. 2d) revealed that a bacteriophage ϕ 29 motor can work against forces of up to 57 pN, making it one of the most powerful molecular motors known.

Optical traps have been used to probe the motion and mechanisms of various nucleic acid enzymes such as exonuclease, proteins that cut nucleic acids; helicases, proteins that unwind double-stranded DNA; and polymerases, proteins that synthesize RNA and DNA [18]. Today not only polymerase activity on DNA has been investigated on a single molecule level but also the translational activity of ribosomes acting on RNA or polypeptide chains [21, 22].

The genetic material of living cells is packed into condensed structures called chromosomes, the precise architecture of which is yet to be fully elucidated. The great complexity of chromosomal assembly is based on interactions between DNA and numerous proteins that bind DNA in sequence-independent manner. Optical tweezers proved to be an excellent tool for studying these interactions. In one study, researchers combined two optical traps to capture a pair of double-stranded DNA molecules and characterized protein-DNA complexes created in the presence of the DNA-bridging protein H-NS (Fig. 2e) [12].

Apart from protein-nucleic acid interactions, many biological interactions were investigated at a single molecule level. Kinesin and dynein, molecular motors



Optical Tweezers, Fig. 3 Single molecule experiments.

(a) Typical image of a combined fluorescence – OT experiment of DNA being stretched by externally applied forces. The DNA molecule in this experiment has a length of approximately 16 μm. The spheres are functionalized with streptavidin or are chemically activated to bind the ends of the modified DNA ends covalently. After successful tethering, the biomolecule is exposed to fluorescent dyes (i.e., SYBR[®] green) and extended. The individual laser power of each of the NIR ($\lambda = 830$ nm) counter-propagating trapping lasers is ~ 85 mW at the trap. The molecule can be stretched and relaxed numerous times without any change in its mechanics. To visualize the fluorophore-stained DNA, the molecule is illuminated for a couple of seconds by the built-in Argon laser ($\lambda = 488$ nm) and can be visualized with a confocal electron multiplying CCD. If the illumination

time by the Argon laser (~ 4 mW) at the trap is increased to few tens of seconds, the molecule is optically damaged and ruptures due to breakage of covalent bonds. (b) Typical force-versus-extension curves of unlabelled double-stranded DNA (contour length = 0.5), and single-stranded DNA (contour length = 1) in buffer (10 mM HEPES pH 7.2, 1 mM EDTA, 150 mM NaCl). Curves are normalized to the contour length of single-stranded DNA (assuming a base-to-base distance of 0.7 nm). The DNA base-to-base distance (obtained by multiplying the normalized extension by 0.7 nm) projected onto the direction of the applied force is shown at the top [2, 15, 16]. (c) RNA polymerase moves in discrete steps along DNA molecule: Steps resolved for a stiffly trapped bead moved in 1-Å increments at 1 Hz. Data were median filtered with a 5-ms (grey) and 500-ms (black) window [14]

that transport cellular cargo by “walking” along microtubules, and myosin, a molecular motor that moves on actin filaments and generates the forces in muscles, have been investigated in depth for many years. The interaction forces of these motor proteins were among the first ones to be elucidated by OT [23].

Since the size of biomolecules is in the order of nanometers, they are too small to be observed by classical diffraction limited optical microscopy. To overcome this limit, biomolecules can be fluorescently labeled and visualized using fluorescence microscopy. Fluorescently tagged molecules can be detected one by one. However, measuring single molecule forces and fluorescence at the same time is difficult due to the high level of background light from the optical traps, which is ten orders of magnitude greater than the light emitted by a single fluorophore. Over the last 5 years, instruments capable of simultaneous spatially coincident optical trapping and single molecule fluorescence

were introduced to gain a more detailed insight into the nature of biomolecules. This combination was enabled by using improved optical designs, high-performance spectral filters, and automated rapid data acquisition [24, 25].

When applying a mechanical load on single molecules with OT, their response could be monitored using fluorescent probes. In Fig. 3a, an experiment on a single dsDNA molecule is shown [2]. A DNA molecule of 16-μm length was labeled with SYBR[®] green fluorophores and subsequently stretched with OT. The polystyrene bead in the upper part of the image (diameter 2.9 μm, coated with streptavidin) was trapped by two counter-propagating laser beams and acted as an anchoring point for the biotinylated end of the DNA. For such typical stretching experiments, the bead on the micropipette (with aperture < 1 μm), to which the other end of the DNA was covalently coupled, was moved with nanometer precision by a piezoelectric



stage until the DNA was extended to $\sim 75\%$ of its contour length. Thereby, the force was raised above 1.5 pN and the DNA was then visualized in the confocal image plane of the microscope arrangement [2]. When extended further, the force on the molecule, which was measured by the change in light momentum flux, raised till the molecule reached a value of approximately 68 pN. At this force, the molecule undergoes a structural change and could be extended without much force to almost 1.7 times of its contour length [15, 16]. Further examples of combining optical traps with other analytical techniques will be presented later in this report.

Cells and Optical Tweezers

As mentioned before, one has to be careful with the choice of wavelength and the power of the laser used in optical trapping experiments. This is even more pronounced when whole cells are investigated. First experiments with whole cells, where cell membrane mechanics were investigated, were performed by the Sheetz group in the mid-1990s. A valuable application of OT could for instance be found in the study of bacterial motility and their adhesion to surfaces. Many types of bacteria express micrometer-long attachment organelles, the pili, which adhere to the host tissue and have an ability to uncoil and recoil. The pili facilitate bacterial conjugation, which is the exchange of genetic material from cell to cell. The folding mechanisms of piliated bacteria could be studied with force-measuring OT at single molecule level at a resolution in the low pN range [26]. For the first time, this method allowed the operator to assess forces mediated by individual pili on single living bacteria in real time. Recently, this technique was used to study how uropathogenic *E. coli* can withstand shear forces in a flow configuration [27]. Thus, force-measuring OT could help to understand these mechanisms enabling to fight infections caused by antibiotic-resistant bacteria. In Fig. 4, an image of a bacterium, which was manipulated with an OT, is shown.

Besides *E. coli*, also other mammalian cells such as red blood cells, nerve cells, and gametes were studied extensively by optical trapping [28]. Furthermore, OT could be used to spatially fix subcellular structures, which normally move inside a cell. Being optically

trapped, these structures could be investigated with confocal microscopy. A recent example of such laser-based nano-surgery was the displacement of the cell nucleus to study the relevance of its intracellular position for the cell division and the resulting cell shape and size [29]. How internal cell structures like a nucleus can be manipulated by OT is shown in Fig. 5 [30]. Here cell internal particles, which diffract light (such as granules or organelles), can be trapped and then be displaced within the cell body. This mechanical motion induces effects onto surrounding structures such as the cell nucleus, which can be monitored.

Trapping outer cell components can also be realized using OT. These studies gave insight into essential cell functions such as cell motility, vesicle-mediated release or uptake of soluble and membrane components, or nanotube-mediated cell-cell communications [31]. Analyzing cell membranes with OT could even help to promote aging research, for instance by exposing endothelial cells to vertical forces using optical trapping. This experiment simulated the effects of high blood pressure to the blood vessels, and cell stiffening was observed indicating that the cells undergo complex morphological changes under the effect of external pressure [32].

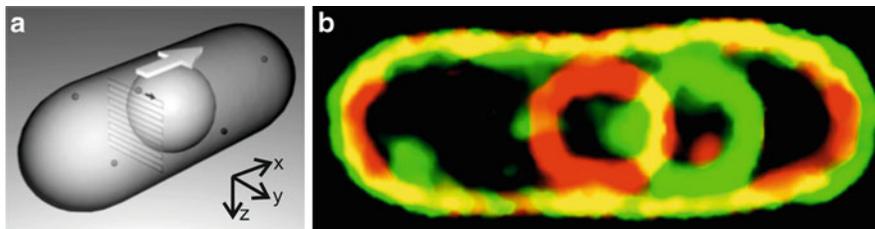
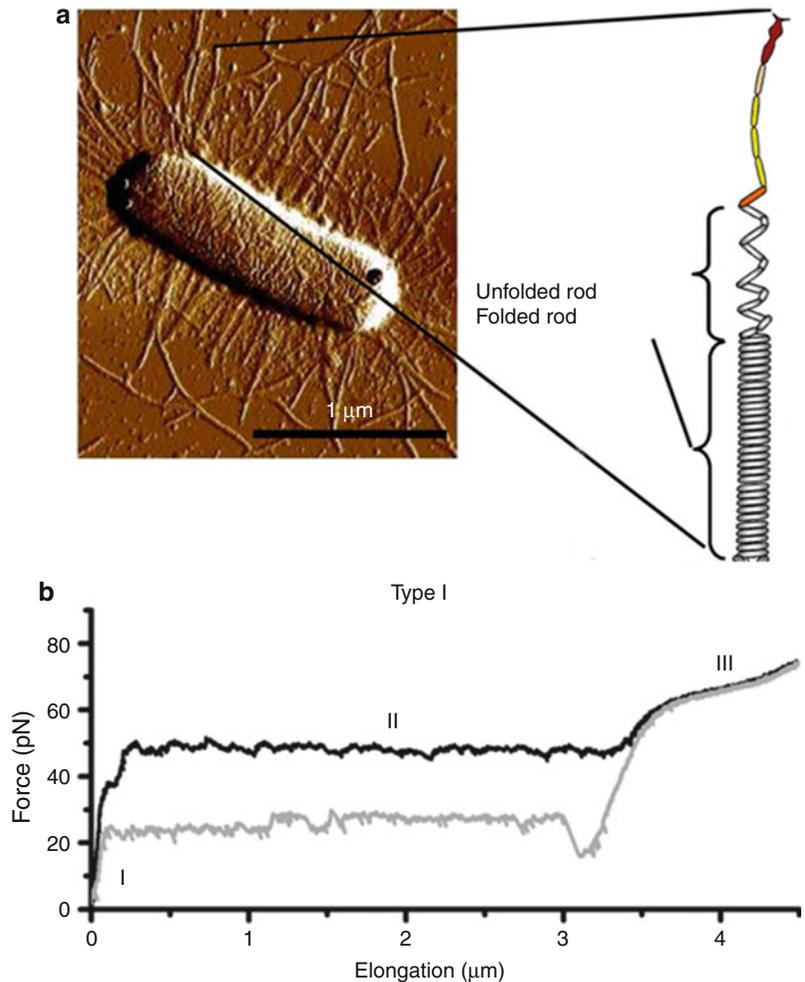
Optical Stretching of Cells

Normally, forces acting on a small scale like a single molecule or an organelle are investigated by optical tweezers, as reported above. Nevertheless the forces acting on whole cells are also important, for instance during cell differentiation. To enable whole cell investigations, the optical stretcher, where two opposed laser beams are used to trap a cell, has been developed. In this experiment, no point force acts on the object. The photons generate surface forces at the opposite membranes and act on the whole structure, thus leading to a stretching of the cell along the beam axis [33]. When stretched, the elasticity of a whole cell can be measured and yield its Young's modulus. Thereby, the cell damage is reduced significantly since the optical stretcher only uses unfocused laser beams. This allows the application of higher laser powers to biological materials. Hence, the forces exhibited by an optical stretcher bridge the gap between OT and atomic force microscopy.



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Fig. 4 (a) AFM image of a HB101/pPAP5 bacterium expressing pili. The schematic drawing shows the folded and unfolded parts of the pili. (b) Typical force-versus-elongation response of a single pili during unfolding (*upper curve*) and refolding (*lower curve*) at an elongation speed of $0.1 \mu\text{m/s}$ [27]. In region I, the pili are elongated up to a fraction of its relaxed length, and a linear force-elongation response is observed. In the following steady-state region II, a constant force is applied to the pili so that the unfolding process takes place. Region III originates from an overstretching of the pili



Optical Tweezers, Fig. 5 Scheme of the cell nucleus being displaced due to optical tweezers. (a) A lipid granule (small sphere) is trapped close to the nucleus (large sphere). The small granule is moved along the shown raster-scanning trajectory in the y-z plane (small arrow). At the same time, it is shifted

in x direction (big arrow), thus pushing the adjacent nucleus forward in x direction [30]. (b) Superposition of two images of a single cell: before (nucleus in the centre) and after (nucleus moved to the right) the manipulation of the nucleus. The nuclear envelope and cell membrane were marked with GFP [29]



The optical stretcher has already been employed to stretch human erythrocytes, mouse fibroblasts, and PC12 cells [33]. In recent studies, the viscoelastic properties of cells have been correlated with their metastatic potential so that optical stretching has become a valuable technique for gaining new insights into the processes of cell migration and metastasis.

Combining Optical Trapping of Cells with Other Optical Techniques

As already mentioned before, nowadays, OT is often combined with other analytical methods to form powerful tools in single molecule studies. Besides fluorescence microscopy, Raman spectroscopy could be used simultaneously with OT. In a recent study, red blood cells were placed under stress with OT to simulate the stretching and compression the cells experience when passing through small capillaries. Simultaneously, the oxygenation state of the blood cells was monitored by Raman spectroscopy revealing a transition between the oxygenation and deoxygenation states of the cells when being stretched [34]. Raman spectroscopy combined with optical trapping could also be used to identify micrometer-sized particles, e.g., bacterial

cells, in an aqueous environment. A single cell was immobilized in the laser trap and then the collection of its Raman signals could be maximized without any unwanted background signals from other particles or substrate surfaces. Thus, the OT analysis of the relative concentration of each type of organism in the aqueous solution was possible [35].

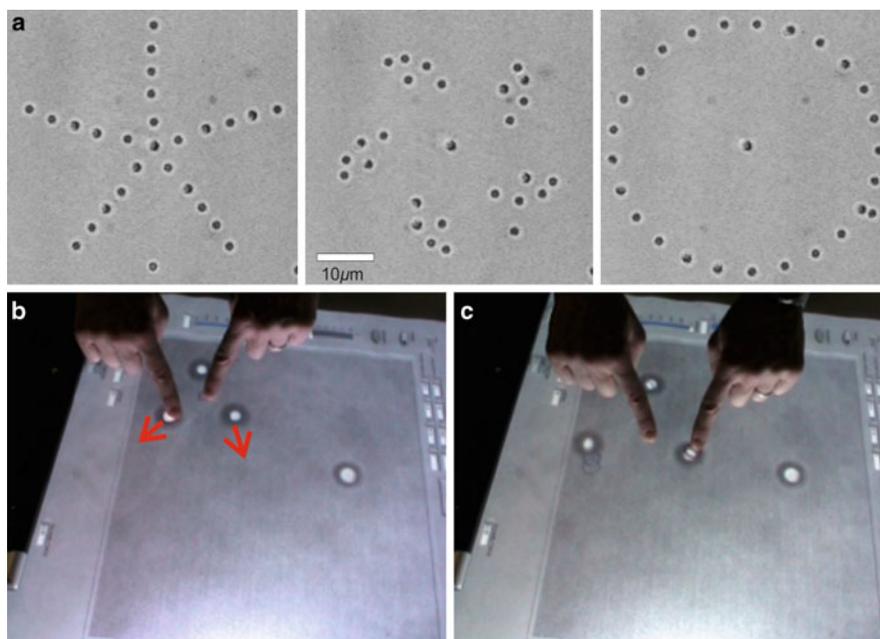
Holographic Applications of Optical Tweezers

With multiple laser beams, an array of several particles can be trapped at the same time. Such spatial arrangements of traps, also known as holographic tweezers (examples are shown in Fig. 6), can be achieved with two different methods:

- (a) Scanning between trap positions: A single beam scans rapidly between several discrete trap positions, which can be computer controlled. Thus, the single beam follows separate light paths, that are recombined before the beam enters the microscope. If the laser beam switches fast enough between the distinct positions, multiple objects can be trapped simultaneously because the viscous drag on them is high enough to keep them in the

Optical Tweezers,

Fig. 6 *Dynamic holographic tweezers in action.* (a) Polystyrene spheres with 990 nm in diameter are trapped simultaneously in dynamic tweezers and moved in the same plane from a star pattern into a circle [6, 37]: (left) the original configuration; (middle) after 16 steps; (right) the final configuration after 38 steps. (b) An arrangement of SiO₂ spheres is optically trapped, and the spheres can be seen in a multi-touch interface for holographic tweezers. (c) The arrangement was moved simultaneously in the direction of the arrows shown in Fig. 6b [39]



defined positions while the laser switches to the surrounding positions [36]. However, the number of objects which can be trapped with this technique is limited by the diffusion of the objects and the switching speed of the laser.

- (b) Separate laser beams: A computer-addressed spatial light modulator (SLM) is employed to impose a predefined phase shift on the incoming laser beam. Thus, the coherent beam is split into many separate beams, each of which is focused into an optical trap by a strongly converging lens [6, 37, 38]. Computer-generated holograms thereby configure the resulting pattern of traps, and each trap can have individually specified characteristics, arranged in arbitrary three-dimensional configurations. Recently, a novel multi-touch interface for the interactive real-time control of holographic OT was presented to control numerous optical traps independently but simultaneously [39].

With permanent three-dimensional arrangements of OT, several new applications arose. Tweezer-organized structures can for instance be fixed in place by sintering or gelling, depending on the material surrounding the trapped objects. In this nanofabrication process, the intense illumination of the laser tweezers can even be used to drive spatially resolved photochemistry for fabricating small complex three-dimensional structures [6]. Moreover, three-dimensional configurations of isolated cells such as *E. coli* have been obtained by trapping the cells within a gelatin matrix. When the trap was switched off and the gel was set, a permanent and vital cell architecture was maintained for several days, thus opening new perspectives in cell and tissue engineering using OT [40]. With such results, the development of holographic OT promises exciting new opportunities for research engineering and manufacturing at mesoscopic length scales.

Key Research Findings

The molecular actions of individual molecules or cells can be investigated in optical tweezer experiments in buffers that are very similar to their native environments. Experiments reach an accuracy of sub-nanometer resolution applying sub-piconewton forces. By scaling up to multiple traps using holographic techniques, new manufacturing of three-dimensional structures at mesoscopic length scales is possible.

Cross-References

- [Atomic Force Microscopy](#)

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Optimization of Nanoparticles

► [Integrated Approach for the Rational Design of Nanoparticles](#)

Optoelectrically Enabled Multi-scale Manipulation

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Synonyms

[Hybrid optoelectric manipulation](#); [Optoelectrofluidics](#)

Definition

Multi-scale manipulation refers to manipulation of objects with operational length scales ranging from several millimeters to nanometers. An optoelectric platform, which integrates multiple physical mechanisms, uses optically triggered electrokinetics to achieve multi-scale manipulation. These platforms are characterized by dynamic programmability and multi-tasking.

Overview

The growing demand for point-of-care analysis has motivated the rapid growth of the lab-on-a-chip (LoC) market. Considering the complexity, diversity, and multitasking nature of the technology,