Resource Letter: LBOT-1: Laser-based optical tweezers

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This Resource Letter provides a guide to the literature on optical tweezers, also known as laser-based, gradient-force optical traps. Journal articles and books are cited for the following main topics: general papers on optical tweezers, trapping instrument design, optical detection methods, optical trapping theory, mechanical measurements, single molecule studies, and sections on biological motors, cellular measurements and additional applications of optical tweezers. © 2003 American Association of Physics Teachers. [DOI: 10.1119/1.1532323]

I. INTRODUCTION

The field of optical tweezers has enjoyed a wide range of applications since its inception in the early 1970s. By using light to trap microscopic objects noninvasively, optical tweezers provide a flexible tool for ultrafine positioning, measurement, and control. In practice, forces up to 200 pN or thereabouts may be applied with sub-pN resolution on objects whose characteristic dimensions are similar to the wavelength of light. Particle positioning and detection capabilities are therefore on a spatial scale of micrometers down to angstroms. The emerging applications of laser-based optical traps are quite diverse and extensive, ranging from atomic physics to the medical sciences. As a result, optical tweezers have been a focal point for interdisciplinary science.

Trapping apparatus ranges from simple, lens-based traps to complex instrumentation integrating multiple optical technologies. A variety of novel techniques have been developed for rapid position detection, trap stiffness determination, and applying controlled, calibrated forces. Instrument advances, such as the use of multiple laser beams, computerized automation of laser beams and sample positioning, and optical tweezers used in combination with other methodologies, such as fluorescence spectroscopy, micropipettes, and optical microbeams, have all helped to make optical tweezers an extremely versatile tool.

Owing to their exquisitely controllable force-exerting properties, optical tweezers are useful for a variety of nanomechanical measurements, particularly those with biological applications. Objects such as biopolymers (e.g., microtubules, DNA molecules), lipid membranes, intact or fractionated cells, and single biological macromolecules have all been studied successfully with optical tweezers. There are many broad areas of current research in biophysics, including the mechanical unfolding and refolding of proteins or nucleic acids, the strength of receptor-ligand bonding interactions, and the nanoscale mechanics of biological motors, which are especially well suited to work with optical tweezers.

Optical tweezers are also useful purely as manipulators and positioning devices. Tweezers can be used to confine or constrain microscopic objects, as well as to organize, assemble, locate, or modify them. In addition to studies of single proteins, biological applications such as intracellular particle tracking and positioning, selective cell harvesting, and probing the mechanics of cell membranes have all been pursued with vigor. Laser-based tweezers also have been used to study the interactions of many-particle systems, e.g., colloids and quasi-crystals.

A full theory of optical tweezers, covering the full range of spatial scales and levels of sophistication, has evolved comparatively slowly over the years, and lags somewhat behind experimental work at the present. Variations in the size, shape, and composition of trapped objects, the nonuniformity of the trapping light distribution, the fact that dimensions of trapped objects are often comparable to the wavelength of...
light, combined with the large numerical apertures employed (which preclude scalar paraxial approximations, necessitating a full vector treatment), have all conspired to make general theories difficult to develop. However, there has been much current progress, and many papers combine limited aspects of trapping theory with experiment.

Our goal for this Resource Letter is to provide a guide to the literature. Our strategy has been to organize selected papers into a few main categories, rather than to provide a comprehensive review of all literature. Thus, numerous articles were omitted, some of which can be found among the citations papers in the papers we list. We apologize to colleagues whose work may thereby have been underrepresented. Inevitably, some of the literature can be classified under multiple categories. Therefore, we strongly encourage reader to browse related titles and topics. For example, sections of research reports frequently include design details not necessarily covered in specific instrument papers.

We present a general section on optical tweezers first, including books and reviews on the subject. However, we caution readers that this is a fast-moving area, and much of the material found in books and early reviews is not particularly up-to-date. A focus on the earliest literature follows, including the seminal papers on optical tweezers. Papers relevant to optical instrument construction, calibration, and detection are listed next, followed by papers that deal mainly with optical trapping theory. The remaining sections are geared towards specific biological applications, including uses with cells, molecular motors, and additional applications of optical tweezers.

II. JOURNALS

The following are selected journals carrying articles on optical tweezers:

- Applied Optics
- Applied Physics Letters
- Biophysical Journal
- Cytometry
- Experimental Cell Research
- Fertility and Sterility
- Human Reproduction
- Journal of Applied Physics
- Journal of Modern Optics
- Methods in Cell Biology
- Nature
- Optics Letters
- Physical Review Letters
- Proceedings of the National Academy of Sciences
- Science

III. BOOKS, REVIEWS, AND GENERAL PAPERS


IV. OPTICAL TWEEZERS, CURRENT RESEARCH TOPICS

A. Earlier works on radiation pressure

B. Seminal studies on optical tweezers

27. “Observation of a Single-Beam Gradient Force Optical Trap for Dielectric Particles,” A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, Opt. Lett. 11 (5), 288–290 (1986). This is the original paper describing the invention of optical tweezers. Trapping of particles ranging from 10 μm to ~25 nm was observed in this single beam trap. (I)


C. Instrument design

The most common and straightforward method of building optical tweezers instrumentation is to custom-fit an optical microscope that already incorporates imaging capabilities and a good objective lens used for forming a trap. Attention to stable instrument construction and alignment details will improve the usability of the instrument. When deciding where to place an instrument, minimizing room temperature variations, acoustical noise, and mechanical vibrations should all be considered.

The references below describe a range of instruments from simple, single-beam traps to sophisticated multi-component systems. The incorporation of technologies in optical tweezers designs, frequently requiring ingenuity, has led to powerful new experimental methods. A broad range of components including trapping lasers, lenses, detection systems, calibration methods, and beam steering solutions has been incorporated into tweezers designs. Technologies for beam steering and multiple trap generation, including acousto-optic deflectors and galvanometer scanning mirrors, are outlined in some of the following papers. Computer control, automation, and data acquisition are critical components of optical tweezers experiments. The experimental requirements (speed of a motor, required position sensitivity, force regime desired) should provide a guide for optimizing the design of an instrument. Multiple feedback methods for force and position clamping have been implemented. Note that many research papers, found in other sections of this Resource Letter, contain instrument design details outlined in materials and methods sections.


49. “An integrated laser trap/flow control video microscope for the study
of single biomolecules,” G. J. L. Wuite, R. J. Davenport, A. Rappaport, and C. Bustamante, Biophys. J. 79 (2), 1155–1167 (2000). Detailed description of an instrument that combines optical tweezers and microelectromechanical systems. Video microscopy and deflection are used for detection. Forces are applied with optical tweezers and a computer-controlled flow system. Used to study the transcription of RNA polymers. (A)  


I. Detection method: video, quadrant photodiode, interferometry, and others  

Position detection may be achieved in many ways including video, quadrant photodiode, and interferometric methods. Time response and position sensitivity should be considered when deciding on a detection method. Video microscopy is straightforward and can be used to track a particle with sub-pixel resolution. Video detection has limited time response and is not as convenient for systems requiring fast positional feedback. Quadrant photodiodes, placed in either an image or back focal plane, can be used for two- or three-dimensional position sensing. Quadrant photodiode detection, which in some instances utilizes a separate detector beam for convenience, has both a faster time response and greater position sensitivity. Interferometry is another sensitive position-sensing method that is used to detect displacement along one axis.  


2. Calibration  

The force exerted on an object by an optical trap depends both on the trap (shape and intensity) and the object (size and composition). Detailed knowledge of the force exerted on a particle is a critical quantity in biochemical, kinetic, and mechanical trapping experiments. Force calibration is achieved by a number of methods, each with different advantages. The drag or escape force method is performed by moving an object or stage while monitoring an “escape” velocity, and is particularly useful to check the linearity of trapping potential in regions far from the trap center. The equipartition method, which is straightforward and fast, measures thermal fluctuations in position of a trapped particle. The power spectral method provides stiffness information in addition to a diagnostic for noise sources at various frequencies. In addition to the methods having different advantages, multiple methods provide a good consistency check of the overall trap stiffness.  


68. “Calibration of Light Forces in Optical Tweezers,” H. Felgner, O. Muller, and M. Schliwa, Appl. Opt. 34 (6), 977–982 (1995). It is shown that trapping in different axial positions is possible. (I)  


70. “Three-dimensional optical trapping and evanescent wave light scattering for direct measurement of long range forces between a colloidal particle and a surface,” A. R. Clapp, A. G. Ruta, and R. B. Dickinson,
Rev. Sci. Instrum. 70 (6), 2627–2636 (1999). Total internal reflection of a laser beam creates an evanescent wave that is used to determine particle position. (I)


3. Fiber-based traps

Light exiting from a fiber, because of its steep spatial gradient, can be used to trap objects, provided that the repulsive scattering force is more than balanced. The most common fiber-based trap involves two counter-propagating beams, to neutralize the scattering force in the central region. Because there are no local lenses, fiber-based traps have the advantage of being able to penetrate deep into solution. Fiber-based traps have also been used for cell stretching studies.


D. Theory of optical tweezers

A wide range of models and degrees of sophistication have been applied to the theory of optical tweezers. The size, shape, and composition of an object are important quantities when determining an appropriate theory. Laser focusing properties such as the mode, input beam diameter, and numerical aperture of the lens are also critical. Theories have been developed for describing the expected signal detection shapes. Many of the references below include both theory and experiments.


E. Experiments using optical tweezers

1. Mechanical and single molecule measurements

Mechanical properties such as elasticity, stiffness, rigidity, and torque can be measured using optical tweezers. Light is easily manipulated and relatively noninvasive, making laser-based mechanical measurements straightforward for studying biological systems. Cells, intracellular structures, filaments, and single molecules have all been probed. Multiple traps can be used to construct additional geometries for mechanical measurements. Combinations of optical tweezers and other methods, such as micropipettes, fluorescence microscopy, and microsurgery, provide very powerful tools for studying biological systems.

Single molecule mechanical measurements using optical tweezers, including biological motor motility, protein-protein unbinding, and protein unfolding, have experienced a tremendous growth in recent years. Throughout these papers, assay development remains a critical component, including details of slide/flow cell construction, methods for attaching samples to microspheres, and general assay conditions.

103. “Buckling of a Single Microtubule by Optical Trapping Forces: Direct Measurement of Microtubule Rigidity,” M. Kurachi, M. Hoshi, and H. Tashiro, Cell Motil. Cytoskeleton 30 (3), 221–228 (1995). The microtubule rigidity was found to be dependent on length. (I)


122. “Mechanical fatigue in repetitively stretched single molecules of titin,” M. S. Z. Kellermayer, S. B. Smith, C. Bustamante, and H. L. Granzier, Biophys. J. 80 (2), 852–863 (2001). Optical tweezers were used to repetitively stretch and release titin to study mechanical fatigue. (I)

123. “Detection and characterization of individual intermolecular bonds using optical tweezers,” A. L. Stout, Biophys. J. 80 (6), 2976–2986 (2001). Details of the instrument, technique and geometry for rupture force measurements are shown. (I)


2. Biological motors

Biological motors are excellent model systems for observing protein motions and conformational changes, and are a subject of intense research. Motor properties such as speed, force, processivity, working stroke distance, and substrate should be considered when designing an experiment. Many technological developments, including force clamping, the three-bead assay, and computer automation of trap and sample positioning have been used in biological motor re-


search. We encourage the reader to explore experimental innovations implemented in multiple motor systems.

**a. General motors**


**b. Kinesin**

Kinesin, which hydrolyzes ATP to move along microtubules, is a processive motor that takes about 100 steps before detaching. Kinesin’s processivity makes it ideal for optical tweezers studies. Optical tweezers measurements have identified that kinesin steps in discrete, 8 nm increments and hydrolyzes one ATP per step. Instrumental innovations specifically geared towards measuring kinesin motility have led to a number of advances in optical tweezers.


140. “Nucleotide-dependent single-to-double-headed binding of kinesin,” K. Kawaguchi and S. Ishiwata, Science 291 (5504), 667–669 (2001). Optical tweezers were used to measure the unbinding force of kinesin attached to microtubules under various nucleotide conditions. (I)


153. “A mutant of the motor protein kinesin that moves in both directions on microtubules,” S. A. Endow and H. Higuchi, Nature 406 (6798), 913–916 (2000). Optical tweezers were used to observe the directional bias of ncd and mutant motors. (I)


156. “Substeps within the 8-nm step of the ATPase cycle of single kinesin molecules,” M. Nishiyama, E. Muto, Y. Inoue, T. Yanagida, and H. Higuchi, Nat. Cell Biol. 3 (4), 425–428 (2001). This measurement was optimized to observe fast kinesin transients. (I)

**c. Myosin**

Myosin, which moves on an actin substrate, is the subject of intense research. A three-bead assay has been developed to measure the properties of skeletal muscle myosin, a nonprocessive motor. In this geometry, two trapped beads suspend an actin filament above a third motor-coated bead. Motor interaction and power stroke movement of the filament can be detected by monitoring fluctuations and movement of the double bead system. Many innovations have been implemented to both simultaneously generate multiple traps and detect position in this geometry. More recently, processive myosins have been discovered (myosin V being an example)
with properties somewhat similar to kinesin, and therefore amenable to many of the same techniques.


173. “Myosin-V is a processive actin-based motor,” A. D. Mehta, R. S. Rock, M. Rief, J. A. Spudich, M. S. Mooske, and R. E. Cheney, Nature 400 (6744), 590–593 (1999). A dual trap geometry, where the position was determined by oscillating one of the beads. (I)


**d. Nucleic acid-based enzymes**

RNA- and DNA-based enzymes with motor-like properties also have been studied with optical tweezers. Multiple geometries for motility assays have been implemented. The stretching properties of DNA have been used as a centering tool and as a ruler to monitor the progress of nucleotide motors. These motor studies have benefited enormously from powerful biochemical, as well as biophysical, methods available for manipulating nucleic acids.


labeled molecules of RNA polymerase were visualized using single molecule fluorescence excited in a total internal reflection geometry. (A1)


3. Measurements involving DNA

DNA stretching studies have been the subject of much experimental and theoretical development. Measurements ranging from base pair interactions to chromosome mobility have been studied.


206. "Entropy and heat capacity of DNA melting from temperature dependence of single molecule stretching," M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, Biophys. J. 80 (4), 1932–1939 (2001). Temperature ranging from 11 °C to 52 °C was used in this study. (I)

F. Cells and optical tweezers

Optical tweezers have numerous cell biology applications. Intracellular materials including organelles and chromosomes have been probed using optical tweezers. Cell function, in particular mitosis and motility, have been studied by methods such as laser inactivation and tweezers-assisted chromosome movement. Localized studies of membrane rigidity and fluidity have increased our understanding of cell morphology. Many cellular measurements involve combinations of optical tweezers with other methodologies, such as microscopy and fluorescence characterization, to form powerful tools for cell research.

I. General cells

Cell types including mammalian cells, Escherichia coli, red blood cells, nerve cells and gametes have been studied.


Micromanipulation of chromosomes in PTK-2 cells using laser microsurgery (optical scalpel) in combination with laser-induced optical force (optical tweezers),” H. Liang, W. H. Wright, S. Cheng, W. He, and M. W. Berns, Exp. Cell Res. 204 (1), 110–120 (1993). Microsurgery was used to laser-dissect chromosomes and optical tweezers were used to inhibit movement. The fate of the fragments is discussed and pictures of the process are presented. (I)


Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers,” J. Dai and M. P. Sheetz, J. Cell Biol. 168 (3), 988–996 (1995). IgG-coated beads were used to measure membrane mechanical properties through the exten- sion of filopodia-like tethers. Membrane viscosity in the presence of various reagents is presented. (I)


Micromanipulation of retinal neurons by optical tweezers,” E. Townes-Andersen, R. S. St Jules, D. M. Sherry, J. Lichtenberger, and M. Hassanain, Mol. Vis. 4, 12 (1998). Optical tweezers are used to position and group neuron cells. The outgrowth of manipulated cells is compared to unmanipulated cells. (I)

Keratoocytes pull with similar forces on their dorsal and ventral sur- faces,” C. G. Galbraith and M. P. Sheetz, J. Cell Biol. 147 (6), 1313–1323 (1999). A laser trap was used to place and hold a fibronectin- coated bead on the lamella of a keratoocyte to monitor cellular force and displacement. (I)

Elasticity of the red cell membrane and its relation to hemolytic disorders: an optical tweezers study,” J. Sleep, D. Wilson, R. Sim- mons, and W. Gratzer, Biophys. J. 77 (6), 3085–3095 (1999). Two beads were used to measure the force-extension relation of red cell membranes. (I)

A diffusion barrier maintains distribution of membrane proteins in polarized neurons,” B. Winckler, P. Foscher, and I. Mellmann, Nature 397 (6721), 698–701 (1999). In this study, optical tweezers are used to measure the lateral mobility of membrane proteins. (I)

Changes in Hechlian strands in cold-hardened cells measured by optical microsurgery,” C. S. Buer, P. J. Weathers, and G. A. Swartz- lander, Plant Physiol. 122 (4), 1365–1377 (2000). In this study concanavalin-coated spheres were inserted through an ablated hole in the cell wall and attached to a hechitian strand. (I)


Chiral self-propulsion of growing bacterial macrofilbers on a solid surface,” N. H. Mendelson, J. E. Sarlits, C. W. Wolgemuth, and R. E. Goldstein, Phys. Rev. Lett. 84 (7), 1627–1630 (2000). Optical tweezers were used to measure the Young’s modulus of the bacterial cell wall. (I)

Micromanipulation of chloroplasts using optical tweezers,” S. Bay- outh, M. Mehta, H. Rubinstein-Dunlop, N. R. Heckenberg, and C. Cristienly, J. Microsc. 203 (Pt 2), 214–222 (2001). Dual optical tweezers were used to probe chloroplast arrangement. (I)

Direct measurement of the area expansion and shear moduli of the human red blood cell membrane skeleton,” G. Lenormand, S. Henon,
2. Gamete cells

Optical tweezers can be used to manipulate and determine the force generation and swimming properties of sperm. Implantation and fertilization developments use combinations of zonal drilling with short-wavelength (blue-to-UV) lasers and manipulation with optical tweezers. Laser-assisted hatching has also been investigated to possibly improve implantation efficiency.


3. Cell damage

In general, optical tweezers are much more "cell friendly" than many alternative methods because of the noninvasive character of light. Cell photodamage remains an issue, however, one that has been investigated for various systems using a range of trapping wavelengths. The papers below discuss a number of relevant issues, and possible solutions to tweezers-induced cell damage.

4. Tools for cells


272. “Laser induced cell fusion in combination with optical tweezers: the laser cell fusion trap,” R. Wiegang-Stebuizing, S. Cheng, W. H. Wright, Y. Numajiri, and M. W. Berns, Cytometry 12 (6), 505–510 (1991). The optical trap is used to bring the cells together while a UV beam initiates the cell fusion. (I)


274. “Manipulation of cells, organelles, and genomes by laser microbeam and optical trap,” G. Weber and K. O. Greulich, Int. Rev. Cytol. 133, 1–41 (1992). The working principle of the optical trap is presented along with biological applications including cell fusion and cell wall perforation. Microdissection of chromosomes is also presented as a tool. Organelle movement is also presented. Includes many references. (I)


G. Trapping various objects

1. Particles, hard spheres, gels, and polymers


2. Vesicles and membranes

300. “Lateral movements of membrane glycoproteins restricted by dynamic cytoplasmic barriers,” M. Edidin, S. C. Kuo, and M. P. Shecht, Science 254 (5030), 1379–1382 (1991). Antibody-coated gold particles were moved across the cell surface with tweezers until a barrier was encountered. (I)


H. Nonstandard traps and trapped objects

1. Alternate trap shapes


2. Alternate trapped objects


I. Optical tweezers and other technologies


1. Two-photon generation


2. Optical probe microscopy


J. Additional applications of optical tweezers


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