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Optical Trapping: A Versatile Technique for Biomanipulation

INTRODUCTION

The ability to manipulate biologically important molecules individually has made great impact in the ongoing biotechnological revolution. One of the most versatile and effective manipulation techniques is the optical trap, also known as laser tweezers. Tight focusing of high-intensity beams of single wavelength radiation (lasers) with high numerical aperture objectives allows for the generation of intense and localized electro-magnetic fields or photon fluxes. Although photons have no mass, they can impart momentum. For example, at 1 W and assuming 10% of the laser light is reflected at the particle surface, a 1 μm bead can be accelerated at $\sim 10^6$ g.¹ Through an appropriately shaped spatial distribution of light intensity, highly focused intense laser light can be exploited to exert force for manipulating small particles in room temperature solutions under biologically relevant conditions. This optical force, or radiation

pressure, is the phenomenon upon which optical traps are based. Pioneering work by Ashkin and co-workers employed a highly focused laser beam to trap nano- and micro-sized particles effectively in a variety of media.¹⁻³ Since these initial demonstrations, the applications of optical traps have continued to grow, especially in the areas of biophysics and biotechnology.

Laser tweezers, for example, have been used in biomedical fields as a tool for assistance in cellular sorting and patterning,⁴⁻⁷ in microsurgical procedures,^{4,8,9} and in the manipulations of various types of cells.¹⁰⁻¹⁵ In addition to controlling cells, biological and synthetic vesicles can be manipulated using optical traps and utilized as effective microanalytical devices for both the introduction to and collection from an array of biological samples. Such individually positioned and controlled vesicles, for example, can be used for the analysis of cellular microenvironments.¹⁶⁻²¹ Other bioanalytical applications using optical trapping entail the assistance of dielectric beads for providing platforms for DNA sequencing as well as investigating particular enzymes.^{22,23}

One of the most celebrated applications of optical tweezers is perhaps its implementation in studies designed to unravel the workings of molecular motors and biopolymers.²⁴⁻²⁷ Specifically suited for applications involving small, piconewton (pN) quantities of force, optical traps offer convenient means to measure the mechanical properties of individual biomolecules. Using beads as effective handles attached to the individual molecules, high-resolution displacement measurements of the bead, and thus, of the molecule, can be readily achieved. Further demonstrating the wide applicability of laser tweezers are recent advances in trapping individual globular DNA molecules without the assistance of a bead handle.^{28,29} These abilities open new opportunities for the investigation of biopolymers that are not currently attainable by using bead handles. The ability to trap and confine individual molecules in a particular conformation, for example, shows promise as a technique for precisely controlling the initiation of conformational transitions in biopolymers.

The diversity of applications realized with optical trapping stimu-

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lates great current interest. Here, we discuss both previous achievements afforded by the versatility of optical trapping as well as its potential for further advancement in fields concerned with studying biological systems. In particular, we describe the possibility of applying optical traps as a reaction initiation device for studying the dynamics of individual biopolymers.

THEORY AND IMPLEMENTATION OF OPTICAL TRAPPING

Theoretical descriptions for optical trapping forces can be divided into two regimes: (1) Mie regime, for particles much larger than the trapping wavelength, and (2) Rayleigh regime, for particles much smaller than the trapping wavelength. Because the wavelengths used for most applications fall between ~ 800 nm of a near-infrared diode laser and the 1064 nm of a continuous wave (CW) Nd:YAG laser, the optically trapped particles discussed in this paper (e.g., cells) lie primarily in the Mie regime ($r_{\text{particle}} \gg \lambda$). However, it has been difficult to model quantitatively the trapping forces involved in the Mie regime due to considerations of boundary conditions and to optical inhomogeneity of biological particles. Despite such difficulties, ray-optics models that quantitatively describe the trapping forces on spherical particles in the Mie regime have been developed.^{30–32} Many excellent manuscripts, which further describe the theory behind optical trapping, are also available.^{27,33,34} Here, we briefly outline the mechanism for trapping in both the Rayleigh ($r_{\text{particle}} \ll \lambda$) and Mie ($r_{\text{particle}} \gg \lambda$) regimes and then describe a common configuration used for optical trapping. Many intracellular particles (e.g., organelles and large DNA fragments) have sizes comparable to the wavelengths in the near IR and are intermediate between the Mie and Rayleigh regime, which makes it difficult to describe quantitatively their interaction with the optical trap.

Rayleigh Regime. In a single-

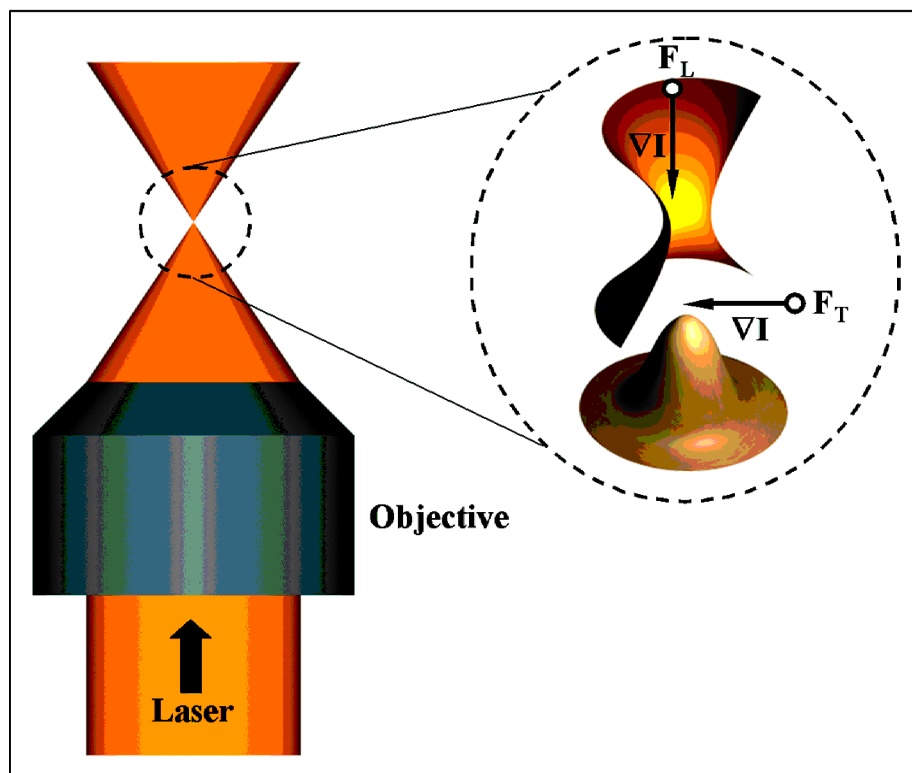


Fig. 1. Three-dimensional drawing of an expanded view of the laser focus of a single-beam gradient trap. The Gaussian laser beam is sent through a high numerical aperture objective to produce two intensity gradients (∇I) at the focus. F_T represents the transverse force exerted on the particle from the intensity gradient produced by the TEM_{00} mode of the laser. F_L is the longitudinal force on the particle due to the intensity gradient generated by the tight focusing of the objective.

beam gradient trap, a Gaussian (TEM_{00} mode) laser beam is focused tightly onto the sample through a high numerical aperture objective (e.g., N.A. 1.3). Figure 1 illustrates the tight focus that provides a three-dimensional (3D) gradient of laser intensity, which is created by the Gaussian profile of the laser output in the transverse dimension and by the tight focusing of the high N.A. objective in the longitudinal dimension. A particle in the vicinity of this 3D intensity gradient will experience an interaction with the laser beam, as described by the relation:

$$U = -\mu_{\text{induced}} \cdot E \quad (1)$$

where U is interaction energy, E is electric field of the laser, and μ_{induced} is induced dipole moment. Because

$$\mu_{\text{induced}} = \alpha E \quad (2)$$

the magnitude of the induced dipole

is directly proportional to the strength of the electric field and is related by the proportionality constant, α , the polarizability of the molecule. The resultant gradient force (F_{grad}) is caused by the presence of the three-dimensional intensity gradient created at the laser focus, and can be described by:³

$$\begin{aligned} F_{\text{grad}} &= -\frac{n_b}{2} \alpha \nabla E^2 \\ &= \frac{n_b r^3}{2} \left(\frac{m^2 - 1}{m^2 - 2} \right) \nabla E^2 \end{aligned} \quad (3)$$

where n_b is the refractive index of the medium, α is polarizability of the particle, ∇E^2 (or ∇I) is the intensity gradient, r is the radius of the particle, and m is the effective index of refraction (which is the refractive index of the particle minus the refractive index of the medium). The gra-

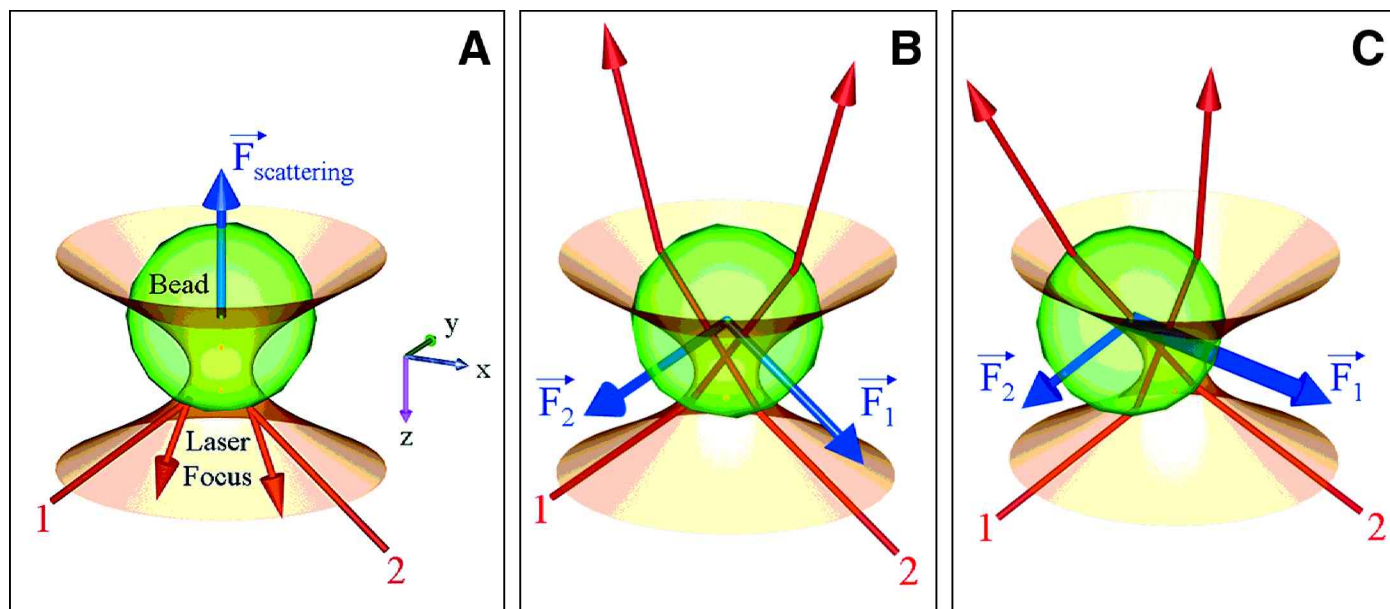


Fig. 2. A three-dimensional ray optics model illustrating the scattering (A) and the gradient forces (B, C) in the Mie regime. Rays 1 and 2 represent straight-line paths of the laser beam that are focused by a high numerical aperture objective. The dielectric bead (green) is optically trapped in the z-direction slightly above the laser focus (orange). Forces on the particle are shown in blue. (A) Scattering force arises from reflection at the solution–particle interface, due to the refractive index mismatch between the particle and media, and points mainly in the direction of beam propagation. (B) Gradient force along z results from refraction of light passing through the bead and points in the direction counter to beam propagation. (C) Gradient force in x–y plane centers the displaced bead at the focus because of an angular difference between rays 1 and 2, thus leading to a corresponding difference in the resultant forces ($F_1 > F_2$).

dient force is proportional to the third power of the radius of the particle because the number of polarizable molecules in the particle scales linearly with the volume (and thus r^3) of the particle. This nonlinear decrease in the gradient force with particle radius makes the trapping of small particles (<25 nm for beads) difficult at reasonable laser powers. Because polarizability of the molecule is related to the square of a particle’s index of refraction, the efficiency for trapping increases proportionally with the polarizability of the molecule at the molecular level and with the index of refraction of the particle at the macroscopic level. The gradient force also scales linearly with the intensity of the laser. As the laser power increases, however, multiphoton absorption by the particle may lead to significant photo-damage or even photo-destruction of the trapped particle. Typical output powers, therefore, are limited to tens to hundreds of milliwatts for most applications.

In opposition to this attractive gradient force is the scattering force, which is described by the following equation:³

$$F_{\text{scat}} = \frac{I_0}{c} \frac{128\pi^5 r^6}{\lambda^4} \left(\frac{m^2 - 1}{m^2 + 2} \right)^2 n_b \quad (4)$$

where I_0 is light intensity and c is the speed of light. The instability caused by the scattering force is mainly in the direction of beam propagation and experimentally makes trapping in the z-direction difficult compared to that in the x–y plane.

Mie Regime. Descriptions of optical trapping in the Mie regime use a simple ray-optics model in which incident laser beams are depicted as straight-line paths of light.^{30,35} Figure 2 shows a 3D representation of the optical forces involved in trapping in the Mie regime. Similar to the Rayleigh regime, two primary forces exist: gradient force and scattering force. Scattering force (Fig. 2A) points in the direction of laser beam propagation and arises from reflec-

tion of light at the surface of the bead, due to a mismatch in refractive index between the particle and the medium. The magnitude of the scattering component is angle dependent and reaches a maximum value when the incident ray is near normal to the surface of the bead. Early experiments performed by Ashkin et al. exploited this scattering force to levitate² and accelerate particles in solution.¹ In opposition to this scattering force, gradient forces act to restore the position of a displaced bead towards the center of a laser focus. Figure 2B shows trapping in the z-direction, which is based on the difference between the angle of light entering and exiting the bead. The high N.A. objective directs incident rays to impinge on the particle with a large cone angle ($\theta = \sim 60^\circ$ for N.A. 1.3), which has a small z-component. Refraction as the light passes through the bead, however, changes the directionality of the ray and increases the z-component of the exited beam. By conservation of mo-

mentum, this angular change in the light path causes a corresponding transfer of momentum to the bead, thereby pushing it back in the z-direction towards the laser focus. The gradient force in the x–y plane arises from an angular difference in the rays impinging on the surface of the bead (Fig. 2C). Ray 1 experiences more bending through the dielectric particle as compared to ray 2, thus causing a difference in the exit angle of the two rays. In other words, ray 1 transfers more momentum onto the particle than ray 2, which results in a larger force ($F_1 > F_2$) that pulls the displaced particle back towards the center of the focus thus holding it tightly in the x–y plane. Practically, trapping in the x–y plane is much easier than in the z-direction due to the presence of scattering force.

General Considerations. The most widely used trapping configuration in biological applications is the single-beam gradient force trap,³ owing both to its ease of implementation and to its compatibility with other microscopy techniques. Figure 3 schematically depicts the optical setup we used for carrying out simultaneous trapping and fluorescence imaging. For imaging, we use the 488 nm wavelength from an Ar⁺ laser to excite fluorescence. To form a homogeneous illumination area without specular patterns, we pass the Ar⁺ laser through a spinning diffuser to remove the spatial and temporal coherence of the laser prior to sending the beam into the microscope. The ability to image under fluorescence is particularly useful for trapping small, sub-micrometer particles for which detection sensitivity becomes an issue for adequate visualization.

There are a number of practical considerations in implementing a single-beam gradient trap for biological applications: (1) the wavelength of the laser should fall in the near infrared (~800–1200 nm) because most biological samples do not absorb in this region, which minimizes heating and other forms of optical damage to the sample;⁸ (2) the back aperture of the objective should be

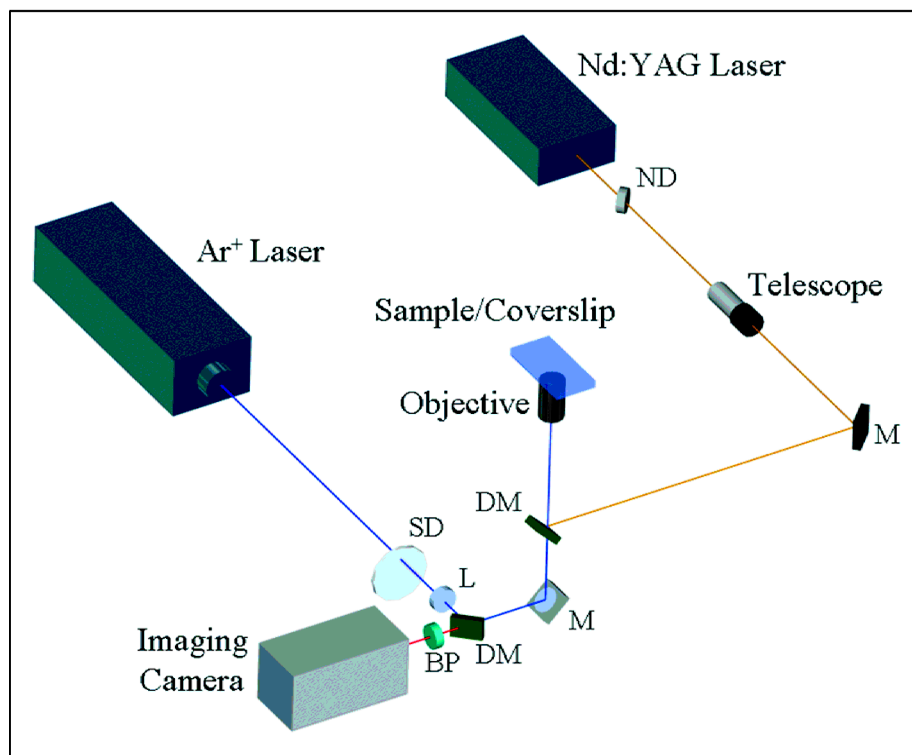


Fig. 3. Schematic depicting the optical trapping/fluorescence imaging setup we used. An Ar⁺ laser and an Nd:YAG laser were used for exciting fluorescence and optical trapping, respectively. ND: neutral density filter; M: mirror; DM: dichroic mirror; BP: bandpass filter, L: singlet lens; SD: spinning disk (for dispersing the beam).

slightly overfilled to ensure optimal focusing and the creation of a steep z-direction intensity gradient; (3) the z-direction position of the trap should be controllable and made to coincide with the image plane, which is best achieved by sending the laser beam through a telescope to control the divergence of the beam as it enters the microscope objective; and (4) the laser beam should be spatially coherent with a good Gaussian profile. Incidentally, due to their typically poor beam quality, many commercially available high-powered diode lasers are incapable of producing a diffraction-limited focus and thus a stable trapping center. In addition to using optimally shaped laser beams, high-quality oil-immersion objectives also are important in implementing a single-beam gradient trap because of their high numerical aperture (N.A. > 1.3). One drawback with high N.A. oil-immersion objectives, however, is their limited working distance, which usually restricts

trapping to objects that are within ~50 μm (for a 100×, N.A. 1.3 objective) from the surface of the coverslip. Beyond this distance, the z-direction trap is no longer stable: the particle will still be attracted to the focus because of stable trapping in the x–y plane, but will be ejected along the z-direction away from the trap as a result of the scattering force and the instability of the z-direction trapping. To overcome this limitation, water-immersion objectives (N.A. ~ 1.2) are sometimes used for applications that require trapping particles deep in solution.³⁶ The disadvantages with water-immersion objectives are their relative high cost and weak z-direction trapping in comparison with high N.A. oil-immersion objectives. Other designs to achieve trapping deep in solution exist and include the use of two counter-propagating beams that are aligned and slightly focused by two opposing low numerical aperture (~0.85 N.A.) objectives.³⁷ These de-

signs, however, are often difficult to align and are prone to drift.

MECHANICAL STUDIES OF BIOLOGICAL MOTORS

Of the many applications provided by optical traps, one of the most revered has been their use in force and displacement measurements on a variety of individual biological molecules, particularly molecular motors. Compared to other force measuring devices (e.g., atomic force microscopy,³⁸ surface force apparatus,³⁹ magnetic force configurations⁴⁰) the pliant nature of optical traps offers the ability to measure exquisitely small forces (pN), a force range that encompasses most biological motors. A typical experiment to measure forces generated by single biomolecules involves the chemical or biochemical attachment of the biomolecule to a micrometer-sized polymer bead (usually polystyrene), which acts as an optical handle permitting facile manipulation by laser tweezers. Methods for carrying out sensitive force measurements with laser tweezers generally require a design that monitors nanometer-scale bead displacements (which arise from an external force) and is coupled to a feedback mechanism that increases the laser power needed to restore the position of the slightly displaced bead. Early methods of choice for monitoring bead displacements employed interferometry.^{35,41} More recently, however, quadrant detectors used to measure deflections of a helium–neon laser focused on the trapped bead have gained popularity, owing to better resolution, less noise, and ease of implementation in comparison with interferometric devices.⁴² Common methods used to calibrate the force exerted involve applying a defined amount of drag force—produced by a constant-velocity flow—against a trapped bead present in a flow cell.²⁷

By employing these force measurement techniques with optical tweezers, a wealth of biophysical information has been reported on interesting biological systems. Early experiments exploited optical traps

as force transducers for investigating the torsional compliance of single live bacteria, *Escherichia coli* and *Streptococcus*.⁴³ Quantitative and qualitative biomechanical studies were carried out for flagellar motors of *E. coli*,^{44,45} transport of mitochondria along microtubules,⁴⁶ motile human sperm cells,⁴⁷ and the movement of chromosomes during mitosis.⁴⁷ In addition, many exciting advances have been made in understanding forces and movements of single molecular motors. Optical-trapping-based force measurement devices have elucidated mechanical information (e.g., force and displacement) for kinesin,^{35,48–52} myosin,^{53–58} RNA polymerase,^{59–61} and the packaging of DNA by bacteriophage p29's portal motor.⁶² The availability of optical tweezers has generated much of our new understanding on the workings of molecular motors. Because there exist many excellent reviews on these biomechanical usages of optical tweezers,^{25–27,63} in the following we focus on the bioanalytical and other non-biomechanical applications of optical trapping.

BIOANALYTICAL APPLICATIONS OF OPTICAL TRAPPING

Overview. Optical trapping has proven to be a versatile technique for the manipulation of biological particles, specifically cells, intracellular organelles, and lipid-bilayer vesicles. The powerful manipulation capability of optical trapping, for example, has been applied to cellular sorting and assaying,^{4–7} positioning of cells for single-cell electroporation and fusion,^{18,64} studies on a variety of cellular interactions,^{10–14} investigations of cellular membrane characteristics,¹⁵ manipulation for cellular microsurgery and laser induced fusion,^{4,8,9} and cell patterning.⁵

Unlike their cellular counterparts, synthetic vesicles, also called liposomes, have been used to a lesser extent in experiments that use optical trapping. Vesicles offer many promising potential applications as protective nanocontainers^{16,17} and as microanalytical devices.^{18–21} In contrast to

vesicles, dielectric beads have been extensively used in microanalytical devices and in optical trapping experiments, such as in single-molecule DNA sequencing and in studies of enzymes.^{22,23}

A number of advantages of optical trapping have contributed to its popularity in biologically related applications: (1) optical tweezers are easily integrated into microscope imaging systems and offer a sterile and noninvasive means to manipulate biological particles ranging from tens of nanometers to many micrometers; (2) in comparison with other techniques, such as glass micropipettes, optical tweezers offer a more versatile and facile method for micromanipulation. To multiplex the laser beam for trapping many particles simultaneously is straightforward, a feat that is difficult to achieve with micropipettes; (3) optical tweezers offer excellent spatial resolution and dexterity in micromanipulation. Using a properly shaped laser beam, for example, a particle can be trapped then rotated;⁶⁵ and (4) the near-infrared wavelength (~800–1064 nm) used in most optical traps produces rather minor effects, if any, on the function of biological particles and the viability of cells.^{8,66,67} The main drawback to using optical tweezers in some applications is the limited amount of force (typically in the pN range) that it can produce in comparison with other manipulation techniques. Micropipettes, for example, can readily exert nanonewtons (nN) of force on a particle. Nevertheless, the forces produced by optical tweezers are sufficient for most biologically relevant applications. With these advantages, there is little surprise that optical trapping has been applied widely in biological and analytical studies.^{68,69} A comprehensive discussion of these applications is beyond the scope of this article to provide, rather we have chosen to report on some of the recent achievements in the bioanalytical usages of optical trapping.

Manipulations of Cells Using Optical Traps. *Cellular Sorting and Manipulation.* Since early ex-

periments on single-cell manipulation by Ashkin and co-workers,⁷⁰ many other examples of cellular manipulation using laser tweezers have emerged. An automatic cell-sorting system based on optical trapping, for instance, has been developed recently by Grover and co-workers.⁷ By combining image recognition and processing for identifying cells of particular morphologies with optical trapping for single-cell selection and transport, they demonstrated the isolation of an individual erythrocyte from a human peripheral blood sample containing a mixture of leukocytes, erythrocytes, and platelets. This demonstration is particularly interesting because it provides an automated method for selecting and isolating individual cells in solution and offers the potential for selective single-cell bioanalysis and characterization from complex samples that contain many different cell types. With ongoing efforts to probe biological processes at ever smaller length scales, the biomedical community would benefit from a flexible cell-sorting device that permits rapid and straightforward analyses of many different types of cells at the single-cell level.

Optical trapping can also be easily integrated with other single-cell platforms, such as single-cell electroporation and electrofusion.^{18,21,64} In single-cell electroporation, for example, the selected cell can be positioned by laser tweezers in between the micrometer-sized gap of two opposing microelectrodes and then permeabilized by the application of a short (ms) and intense (kV/cm) voltage pulse. Electroporation of single cells provides a means to introduce cell-impermeant compounds into the cellular interior, which could be dye tags for fluorescence imaging, plasmids for selective gene expression, or other cell-impermeant molecules for affecting the intracellular chemistry. Similar to electroporation, single-cell electrofusion assisted by laser tweezers can be used to alter the genetic makeup of individual cells.⁶⁴ Unlike bulk experiments, the facile and precise mechanical positioning of cells

using optical trapping combined with the capability of single-cell electro-transformation opens new opportunities for designing and building complex and well-defined cellular networks *in vitro*.

Single-Cell Microsurgery. The near-infrared wavelength employed in optical tweezers can be combined with the ablative power of a pulsed UV laser as a platform for carrying out microsurgery on single-cells: optical trapping has been used for “grabbing” the cell or intracellular organelle of interest while the pulsed UV laser acts as a “scalpel” to cut the biological structure. The resolution of this microsurgery technique is diffraction limited, which is ~ 500 nm for the near-IR light and ~ 200 nm for the UV wavelength. This microsurgical procedure has been applied to a number of biological areas, including pharmacology, immunology, and *in vitro* fertilization (IVF). In IVF, for example, the UV laser can be used to “burn” a hole in the wall of the oocyte, while optical tweezers trap and translate a single sperm cell to and across this hole into the oocyte.^{4,8} Naturally, there are concerns over the possible deleterious effects produced by optical trapping on human sperms and by UV ablation on the egg. Much effort in this area, consequently, has been focused towards characterizing and understanding these potential effects on cell motility and viability.^{8,66,71} The ability to perform dissection at the single-cell level represents not only an impressive technological advance, but also opens new possibilities for studying the cellular landscape.

Laser microsurgery is especially suited for the manipulation and dissection of plant cells, which have a tough cell wall and are larger in comparison with mammalian cells. Using this microsurgical technique, Weathers and co-workers reported an experiment in which they created a micrometer-sized hole in the plant cell's wall,⁹ followed by selective transport of micro-particles, such as polystyrene beads and bacteria, through the hole and into the cellular interior. Such studies arouse interest-

ing possibilities for monitoring plant and mammalian cell responses to the selective insertion of foreign particles such as biomaterials and micro-organisms. In addition to ablating holes on cell surfaces, pulsed UV lasers can also be used to induce fusion between two cells after they have been properly positioned and aligned using optical trapping.⁷² Although the long-term effects of such laser-induced fusion are poorly characterized, this technique may offer a convenient route to the creation of hybridoma at the single-cell level and to the study of cellular processes stimulated by cell fusion.

Controlled Patterning of Cells.

The ability to create well-defined patterns of cellular networks is an important capability in both fundamental studies (e.g., cellular communication and ecology) and engineering sciences (e.g., the generation of artificial tissues and cell-based screening devices). One approach towards achieving this goal has been realized through the manipulation powers characteristic of optical trapping. For instance, using laser trapping and fluorescently labeled cells for visualization, Seeger and co-workers demonstrated the selective deposition of cells onto substrates of which the surface had been modified and lithographically patterned with adhesive proteins.⁵ Optical trapping was used to select and transport cells from adjacent chambers, followed by deposition and immobilization onto the adhesive patterns on the surface of the central cell-patterning chamber. The advantage in using optical trapping for cell-patterning lies in the precision and flexibility of the technique and its compatibility with microscopy-based imaging. The main drawback was the serial nature of the method, which limited both the throughput of the procedure and the extent of the area over which cells could be patterned.

To partially address this drawback, methods for generating multiple traps have been developed.⁷³⁻⁷⁵ One example has been the use of computer-controlled galvano or piezoelectric mirrors for rapid scanning

of the laser beam, similar to their use in a scanning confocal microscope.^{74,75} Rather than using the scanning to generate an image, rapid movement of the laser focus in the object plane can be used to sequentially create multiple locations for trapping. This method is based on laser beam time-sharing and the finite diffusion time that is required for a particle to leave a defined position: multiple particles can be manipulated if these particles trapped at the different sites do not diffuse away substantially from their location when the laser beam is scanned among the particles to restore their original positions. Unfortunately, these patterning methods are limited both by the sizes of particles used as well as by the area occupied by the designed pattern—smaller particles diffuse faster and larger areas mean longer scanning times. To overcome such constraints, improved scanning rates and larger deflection angles for high-quality actuating mirrors may offer the ability to trap smaller particles within larger areas. Despite these limitations, the use of multiple traps offers a convenient and flexible platform for controlling and defining spatial relationships among different cell types, which is a useful and necessary capability for studying cellular interactions and communications.

Microanalytical Procedures Using Optically Trapped Vesicles and Beads. *Vesicles.* While somewhat overshadowed by applications involving cellular manipulations, biological and synthetic vesicles offer several unique opportunities for small-volume chemical analyses. The chemical profiles of individual secretory vesicles (~0.5–1 μm in diameter) from the mollusk *Aplysia californica*, for example, were obtained by Zare and co-workers, in which they combined optical trapping with microscale chemical reaction (nanoliter on-column derivatization) and separation (capillary electrophoresis).¹⁹

Figure 4 illustrates this experiment, in which an individual vesicle was optically trapped (Fig. 4A) and then transported near the inlet of a tapered

separation capillary (Fig. 4B). By applying a small electric potential, the vesicle was electrokinetically introduced into the capillary (Fig. 4C). After the vesicle was introduced and immobilized at the inlet of the tapered capillary, the capillary tip was transferred to a solution containing a fluorogenic reactive dye, naphthalene-2,3-dicarboxaldehyde (NDA). The introduction of this reactive dye solution causes the immobilized vesicle to lyse and its contents to be tagged with fluorescent molecules. Figure 4D shows an electropherogram of the separated contents from one vesicle. By analyzing many vesicles, Zare and co-workers showed distinct variations in chemical contents between vesicles and the existence of sub-populations of these secretory vesicles, which may offer interesting insight into the biology of these mollusks.

In addition to the chemical analyses of single biological vesicles, individual synthetic vesicles with well-defined constructs (i.e., membrane proteins and intra-vesicular molecules) may be synthesized and employed as microanalytical devices and localized sensors. In this context, optical trapping is a flexible and convenient enabling technique for positioning such micrometer-sized vesicles at defined locations at the cellular exterior to sample and to provide information about the extracellular microenvironment.¹⁸ Figure 5 is an overlay image that illustrates this concept, in which a fluorescent vesicle was optically trapped then moved sequentially from positions 1 through 6 along the process of an NG 108–15 cell. By combining precise manipulation with optical trapping and the incorporation of selective transporter proteins into the vesicle wall, individually manipulated vesicles may be used as microsampling devices for spatially resolved collection and concentration of species released from single cells. Multiple traps, which can be created by using various scanning mirror configurations, will permit the simultaneous positioning of many vesicles followed by the spatially and tem-

porally resolved sampling of cellular signals and metabolites released from various regions of a single cell.

Few methods exist for studying the chemical composition contained in and released from the different locations of the highly heterogeneous cell. The use of optical trapping for the selective isolation of cellular organelles and for the sampling of extracellular environments will complement existing microanalytical approaches and is critical to furthering our understanding of the myriad chemical interactions that occur with a cell.

Beads. In contrast to using vesicles for microsampling of species released from single cells, beads coated with cell-signaling molecules can be used for selectively inducing responses in different regions of a cell. Optical trapping was used by Cahalan and co-workers to place an antibody-coated bead in contact with a selected portion of a T-cell,¹¹ thereby stimulating a spatially resolved biochemical response from the T-cell that can be monitored with calcium-sensitive dyes and fluorescence imaging. By translating the antibody-coated beads along the periphery of the T-cell, sensitivity of different regions of the T-cell to the antibody could be mapped. Their results showed interestingly that the leading edge of the T-cell responded with a greater intensity than that of the trailing end by as much as 10-fold when using 6.2 μm coated beads to initiate a response. This experiment, which is made possible by optical manipulation, demonstrates the importance of understanding spatial organization of a single cell.

Another example of a biological study carried out using optically trapped beads is by McLaughlin and co-workers,²³ in which they explored the enzymatic properties of phosphoinositide-specific phospholipase C- δ_1 (PLC- δ). In their experiment, a bead was first coated with a bilayer membrane composed of phosphatidylcholine (net neutral charge) and two-percent negatively charged phosphatidylinositol 4,5-bisphosphate (PIP₂). Optical trap-

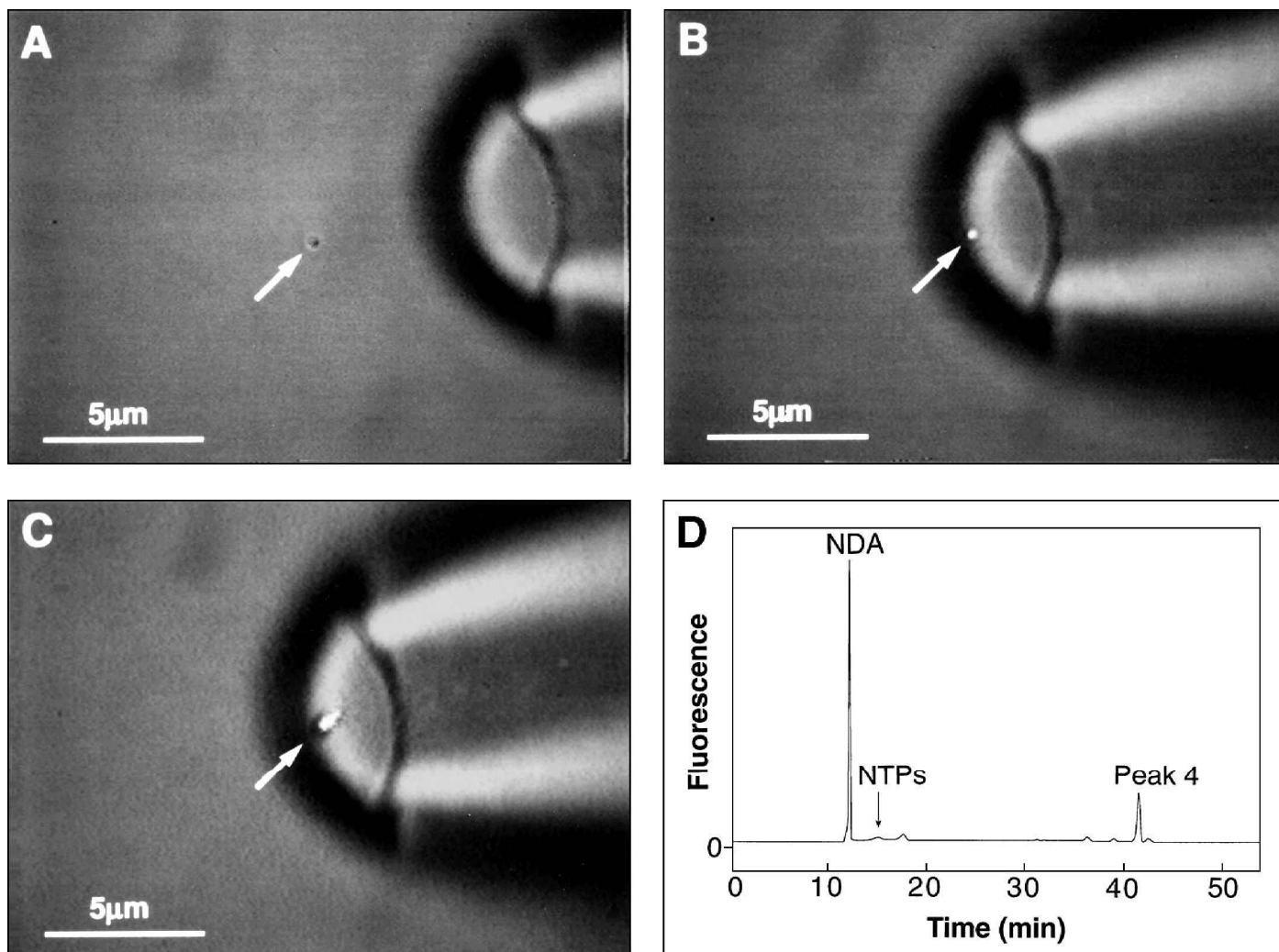


Fig. 4. A–C show the injection of a single vesicle ~ 500 nm in diameter into the tip of a tapered capillary. The vesicle was first optically trapped (A), then moved (B), and finally electrokinetically injected (C) into the capillary tip. After injection, the vesicle was lysed, its contents were derivatized with a fluorogenic dye (naphthalene-2,3-dicarboxaldehyde), then separated by capillary electrophoresis and detected by laser-induced fluorescence. (D) shows the resultant electropherogram. (Reprinted with permission from Chiu et al., *Science (Washington, D.C.)* 279, 1190, Copyright 1998 American Association for the Advancement of Science.)

ping was then used to position and retain the bead between two electrodes. Application of an ac field across the bead caused a displacement—measured using a quadrant diode detector—proportional to the number of negatively charged lipids that coated the bead. Because the enzyme PLC- δ selectively hydrolyzes the negatively charged PIP₂ into a neutral lipid, the activity of PLC- δ could be measured precisely based on changes in the magnitude of the bead displacement. The ease with which these types of cellular and enzymatic experiments can be

carried out using optical trapping again illustrates the versatility and the potential of the technique.

In addition to biological studies, optical manipulation of beads has found use in a number of analytical applications. One example is in single-molecule DNA sequencing proposed by Keller and co-workers in which a trapped bead attached with a single fluorescently labeled DNA was positioned in a sheath flow stream.²² The bead was positioned approximately twenty micrometers upstream from a detection region. Introduction of an exonuclease into

the flow stream around the bead-attached DNA led to the progressive cleavage of each of the fluorescently-labeled DNA bases, which would then be detected (and ultimately identified) downstream via laser-induced fluorescence; the sequence detected from each cleaved base would then correspond to the sequence of the original DNA. In principle, such single-molecule DNA sequencing techniques offer the potential to directly sequence long DNA molecules (tens of thousands of bases) more quickly and cheaply than is currently possible.

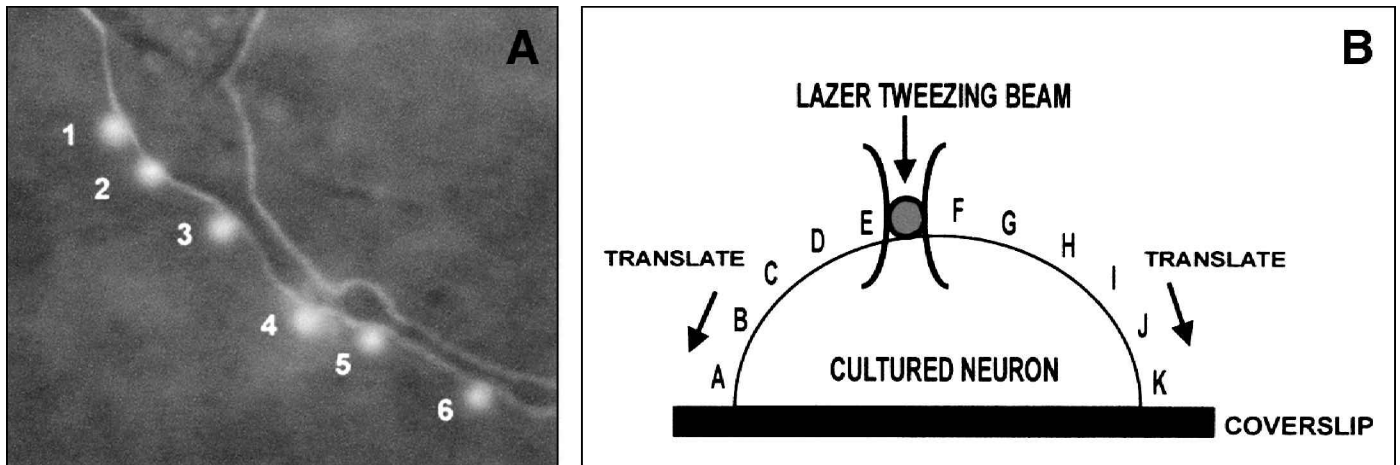


Fig. 5. (A) An overlay image showing the controlled positioning and translation (from positions 1–6) of a vesicle along the processes of an NG 108–15 cell using optical trapping. The vesicle was labeled with ~2% DiO, a membrane stain. (B) Schematic illustrating the concept of using single vesicles, which can be positioned at different locations outside a neuronal cell, as high-spatial-resolution microsampling devices. (Reproduced from Ref. 18.)

Another analytical application for optical trapping involves its combination with spectroscopy and offers an exciting new avenue for future uses in biology.^{76,77} Recently, the Harris group demonstrated a method in which they could optically trap single silica particles while simultaneously monitoring the Raman spectra of a solid-phase peptide synthesis reaction that was occurring on the bead.⁷⁷ This technique points to the possibility of obtaining spectroscopic information from single biological particles (e.g., cells, intracellular organelles, and large DNA fragments). Although the amount of obtainable information is limited by the sensitivity of the spectroscopic techniques, their integration with optical trapping would offer new chemical and structural information not currently available on single, trapped bioparticles.

MANIPULATIONS OF BIOPOLYMERS

Use of Bead Handles for Trapping. Most optical trapping experiments on the physical and biological properties of single biopolymers require the use of beads as handles. Initially, studies employed one bead to allow for optical manipulation of individual DNA molecules. Using this strategy, Chu and co-workers

were able to gain important insights into the polymer physics and behavior of DNA molecules.^{78,79} More recently, to mechanically stretch single biopolymers, beads are attached to both ends of the molecule rather than to just one end. In such experiments, one bead is usually held in an optical trap to allow for force and displacement measurements, whereas the other bead is immobilized on the surface of the coverslip by a micropipette or with another optical trap. In addition to DNA,^{78,80–83} optical manipulation experiments have also elucidated intriguing biophysical information on the filamentous protein titin,⁸⁴ actin filaments,⁸¹ chromatin fibers,^{85,86} RNA,⁸⁷ and collagen.⁸⁸

Figure 6 demonstrates the dexterity of optical trapping: individual fluorescently labeled DNA molecules were tied into knots.⁸¹ In this experiment, Harada and co-workers first attached streptavidin-coated beads to both ends of a biotinylated DNA molecule. The two beads on either end of the DNA were then optically manipulated using a dual beam configuration. Individually controlling each trap to manipulate the DNA, knots could be tied in less than one minute within a dense solution of sucrose, actin filaments, and salts: viscous solutions were helpful in allowing for more facile manipulation ow-

ing to reduced Brownian motion of the DNA. These experiments have provided information on the flexural rigidities of DNA and may offer insight into biological processes that require the bending of DNA molecules, which occurs during the binding of proteins, such as RNA polymerase. Furthermore, carrying out these types of experiments using techniques other than optical trapping, such as dielectrophoretic¹⁰ or magnetic,⁴⁰ would be quite difficult if at all possible.

Trapping Without Bead Handles. Single DNA molecules also can be manipulated optically without the use of bead handles. Chiu and Zare demonstrated this feat by first collapsing the DNA into a dense globular state, followed by optical trapping.²⁸ Several studies have shown that DNA undergoes an extended-to-globular transition when placed in solution that contains a condensing agent, such as poly(ethylene glycol) (PEG) with various cation salts,^{29,89} polyglutamic acid,⁹⁰ spermidine,⁸⁹ cetyltrimethylammonium bromide (CTAB),⁸⁹ and acidic pH.^{28,91} Studies of systems that induce DNA condensation are of particular interest to biology because DNA is tightly packed within the nucleus of cells and in viral capsules.

Figure 7 shows the direct optical

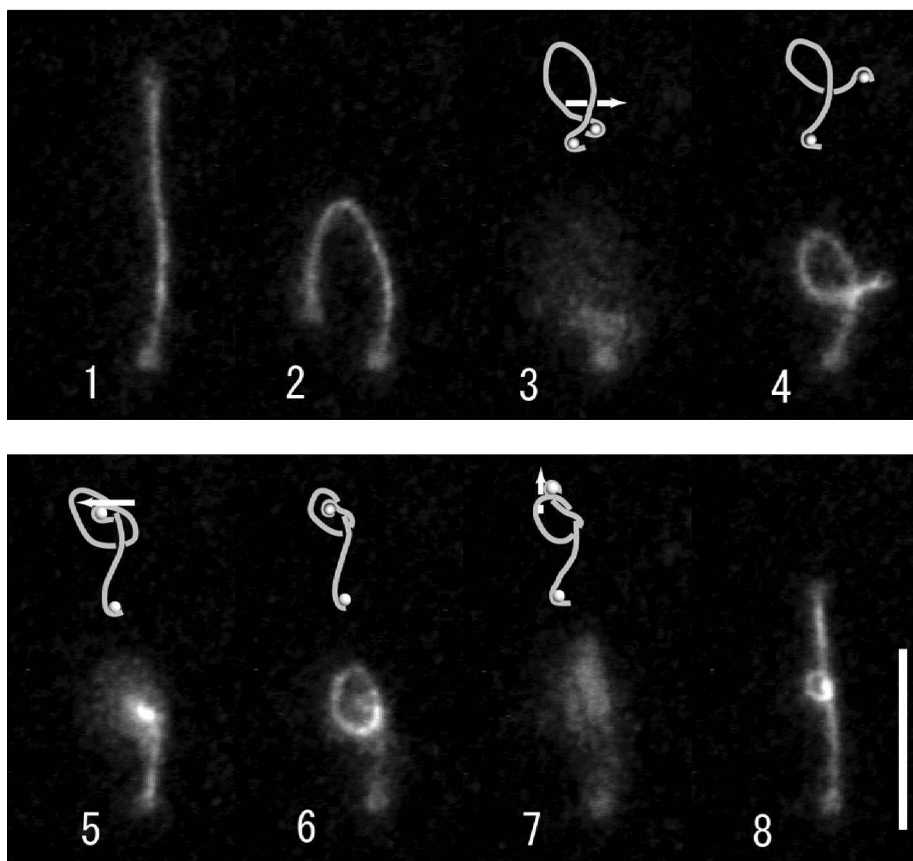


Fig. 6. Sequential images showing an individual DNA molecule being tied into a knot. The accompanying drawings show the progression needed to result in a knot. Scale bar, 10 μm . (Obtained from Y. Harada, *The Tokyo Metropolitan Institute of Medical Science (Rinshoken)*.)

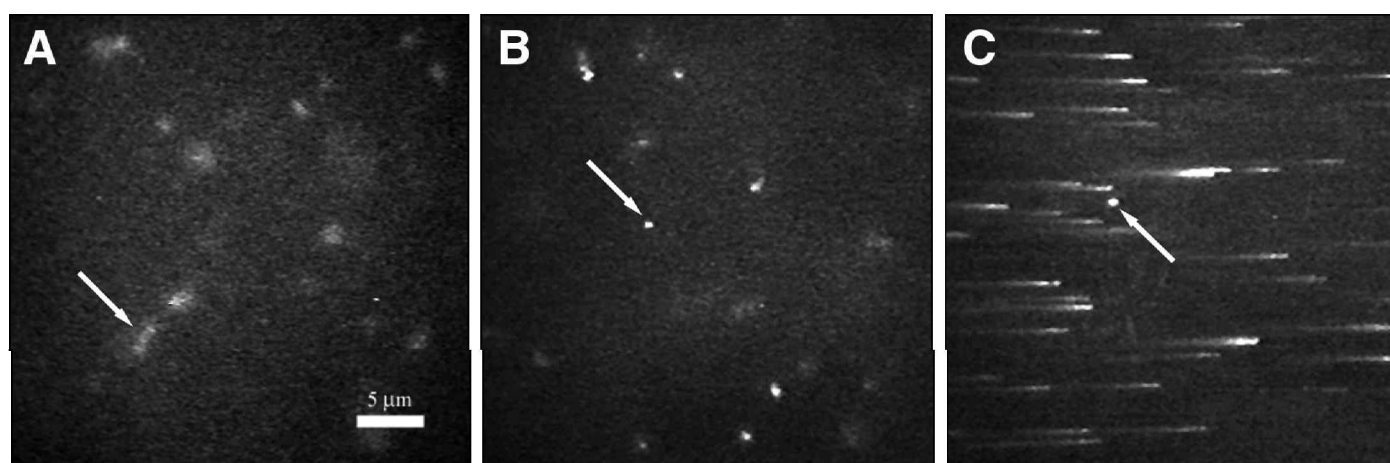


Fig. 7. Fluorescent images of individual λ -DNA ($\sim 48\,500$ base pairs) molecules intercalated with YOYO-1 dye. (A) λ -DNA adopts an extended conformation, which appears blurry in the image owing to Brownian motion, in a pH 8 buffer solution (containing 10 mM Tris, 1 mM EDTA, 2 mM NaCl, 1% β -mercaptoethanol, and 0.1% glucose). (B) λ -DNA compacts into a globular structure when the pH of the buffer is lowered to ~ 5.7 . (C) In this globular conformation, individual DNA molecules can be trapped and moved optically; the blurry streaks were caused by the immobilized DNA on the coverslip while a single trapped DNA (marked by an arrow) was translated in solution. Each solution also contained 125 $\mu\text{g}/\text{mL}$ glucose oxidase and 25 $\mu\text{g}/\text{mL}$ catalase to reduce photobleaching of YOYO-1.

trapping and translation of single λ -DNAs ($\sim 48\,500$ base pairs) that were intercalated with a fluorescent dye (YOYO-1) for visualization. In a solution of pH 8, λ -DNA took on an extended, floppy conformation (Fig. 7A), which extends over several micrometers, and could not be trapped optically. This extended, random-coil form compacted into a globular state having sub-micrometer dimensions when the pH of the solution was decreased to \sim pH 5.7 (Fig. 7B). In this dense and compact form, individual DNAs could be trapped easily and translated at will in solution (Fig. 7C).

Surprisingly, we have observed that an optically trapped globular DNA could be confined to its globular conformation even after the surrounding solution had been returned to the initial pH of 8, which favors the extended form. Once the trapping beam was blocked or turned off, however, the globular DNA transformed back into its extended form. Figure 8 illustrates this observation. Here, a single λ -DNA molecule was trapped in a solution of \sim pH 5.7. While trapping the DNA molecule, the pH of the surrounding solution was returned to \sim pH 8; the presence of higher pH caused the

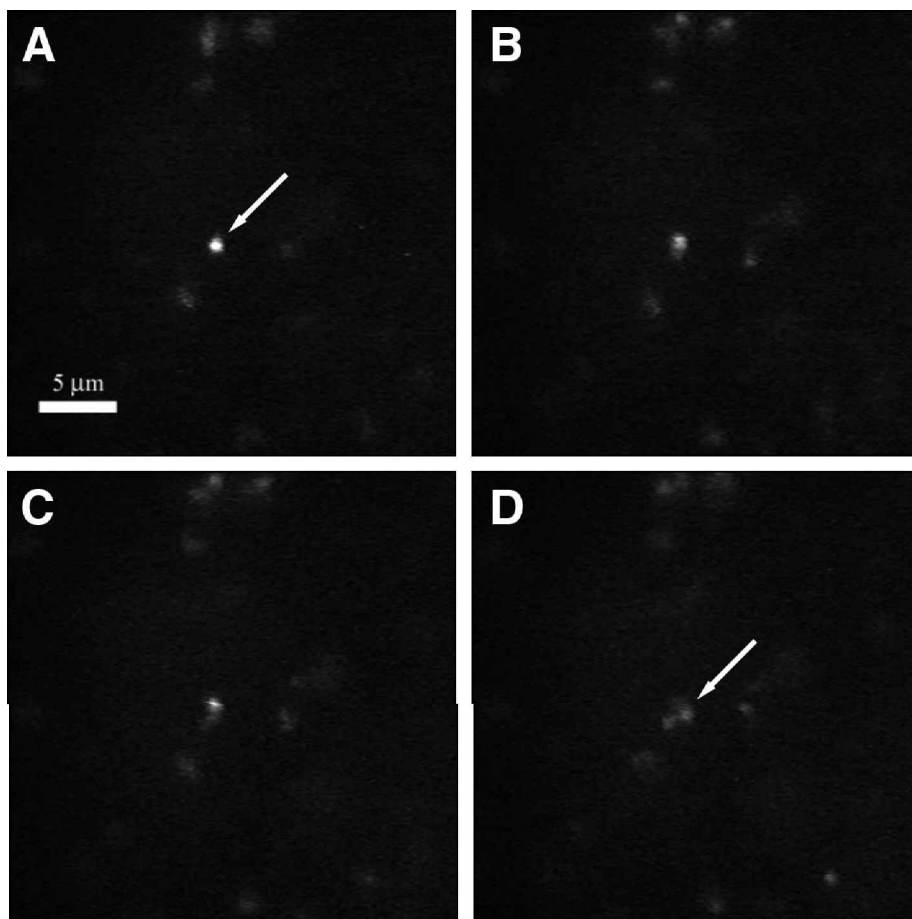


Fig. 8. Fluorescent images showing the sequential uncoiling of a single λ -DNA molecule ($\sim 48\,500$ base pairs) from a globular state to an extended conformation. (A) A single λ -DNA (marked by an arrow) was first optically trapped in a pH 5.7 solution. The pH of the solution was then raised gradually until the surrounding DNA molecules adopt an extended structure; the optically trapped DNA was confined to its globular state. (B–D) Upon blocking the trapping laser, the trapped globular DNA uncoiled to its extended form.

neighboring DNA molecules to return to their extended form (Fig. 8A). Upon blocking the trapping beam, which was done right before the image shown in Fig. 8B was taken, the globular DNA unfolds progressively into its extended conformation (Figs. 8B–8D). The time-scale for B–D was ~ 150 ms or three successive frames collected at 20 frames per second. This observation opens an exciting avenue of research: optical trapping can be used as a reaction initiation device. Because the trapping laser beam is turned on or off with high time resolution and because only one molecule can be studied at a time, a very sharp time zero can be defined for

the start of the reaction. By precisely controlling the initiation of DNA conformational transitions, the dynamics of these processes can be studied in detail and with high temporal resolution. Such experiments may provide a better understanding of the dynamics of DNA conformational changes within the nucleus of the cell, an environment in which pH is believed to play an important role in enzyme activation. Certain endonucleases, for example, are active only in an acidic solution.⁹²

CONCLUSION

With exciting advances in the spectroscopic imaging of biological

molecules and the onset of single-molecule detection, optical trapping offers a complementary, versatile, and powerful technique for manipulating a wide array of biologically relevant particles in solution. From cells, vesicles, molecular motors, and beads attached to biopolymers to the trapping of freely diffusing globular DNAs, the future outlook of using optical forces for exerting nanoscale control is bright. Optical trapping offers useful means for measuring forces in the piconewton range, for observing displacements with nanometer resolution, and—as demonstrated in this paper—for the controlled initiation of dynamic processes with precise start times. The ease with which optical trapping can be integrated with microscopy techniques will make it an invaluable addition to a biologist's toolbox. In a sense, optical traps afford researchers the ability to grab onto single molecules using their “own hands” to hold, pull, stretch, and control objects at the nanometer-size scale.

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