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Ashis Gopal Banerjee
Sagar Chowdhury
Wolfgang Losert
Satyandra K. Gupta

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Ashis Gopal Banerjee,^a Sagar Chowdhury,^{b,c} Wolfgang Losert,^{d,e,f} and Satyandra K. Gupta^{b,c}

^aMassachusetts Institute of Technology, Computer Science and Artificial Intelligence Laboratory, Cambridge, Massachusetts 02139

^bUniversity of Maryland, Department of Mechanical Engineering, College Park, Maryland 20742

^cUniversity of Maryland, The Institute for Systems Research, College Park, Maryland 20742

^dUniversity of Maryland, Department of Physics, College Park, Maryland 20742

^eUniversity of Maryland, Institute for Physical Science and Technology, College Park, Maryland 20742

^fUniversity of Maryland, Institute for Research in Electronics and Applied Physics, College Park, Maryland 20742

Abstract. Optical tweezers have emerged as a promising technique for manipulating biological objects. Instead of direct laser exposure, more often than not, optically-trapped beads are attached to the ends or boundaries of the objects for translation, rotation, and stretching. This is referred to as indirect optical manipulation. In this paper, we utilize the concept of robotic gripping to explain the different experimental setups which are commonly used for indirect manipulation of cells, nucleic acids, and motor proteins. We also give an overview of the kind of biological insights provided by this technique. We conclude by highlighting the trends across the experimental studies, and discuss challenges and promising directions in this domain of active current research. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3579200]

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1 Introduction

Tightly focused laser beams or optical traps exert small optical forces of the order of picoNewtons (pN) on freely diffusing components that are smaller than tens of micrometers and up to a few nanometers in fluid medium. These forces are sufficient to “trap” the components that are present in the vicinity of the beam focal region. Using this property, optical tweezers have been developed to successfully manipulate (e.g., trap, translate, rotate, and stretch) micro- and nanoscale components of many different sizes and shapes.¹ Optical tweezers provide several beneficial features that make them particularly attractive options for manipulating a whole host of biological objects such as cells, DNA, RNA, kinesin, myosin, cell organelles, actin filaments, lipid molecules, and biopolymers. For example, they do not exert forces through a physical contact point with the manipulated object and, hence, avoid potential damages due to, e.g., contact point friction or surface chemistry. Objects can also be simply released from the optical traps by switching off the laser beams. A large number of objects can be manipulated in parallel unlike magnetic and electrophoretic techniques by employing beam shaping techniques via diffraction or rapid scanning mirrors that multiplex a single laser beam into many. And, while optical tweezer systems require excellent objectives to focus the laser beam onto a diffraction-limited spot, they can use relatively low power lasers and video cameras.

There are two different ways to manipulate biological objects. The first method involves trapping them directly using laser beams. The second method is to trap them indirectly

without focusing the laser beams directly on them. Instead, tweezers are used to trap dielectric components (made of latex, polystyrene, silica, etc.) that are attached to the ends or boundaries of the objects. Such trapped beads act as “handles” or “grippers” to hold the objects in order to perform useful functions such as cell sorting or to provide insight on biological processes such as DNA folding. Indirect manipulation may be needed due to the following two reasons. First, the biological object is too small to be effectively trapped by the laser at a reasonable power. Second, there is a risk of damaging the object by directly exposing it to the laser light. In this paper, we exclusively focus on indirect manipulation.

Several review articles have been published on optical manipulation of biological objects. Wright et al.² were the first ones to provide a comprehensive survey of laser trapping in cell biology. Uchida et al.³ provided a survey of different optical trapping techniques for manipulating whole cells. Mehta et al.⁴ gave a detailed review of an investigation of single-molecule biomechanics using optical methods. Allaway et al.⁵ provided a short review of the application of optical trapping over a wide spectrum of biological research. Zhang and Liu⁶ gave an updated review of the extensive body of work in the field of single-cell studies using optical tweezers. Another recent survey article on optical manipulation for single-cell studies is available in Ref. 7. Perkins⁸ provided an overview of optical trapping for single-molecule biophysics. Ou-Yang and Wei⁹ reviewed the use of optical tweezers in investigating mechanical properties of biological systems. Stevenson et al.¹⁰ emphasized the impact of optical tweezers in both single-cell and single-molecule studies.

Many of the review articles focused on a specific research topic. Bockelmann¹¹ surveyed single-molecule optical

Address all correspondence to: Satyandra Gupta, University of Maryland, 2181 Martin Hall, College Park, Maryland 20742. Tel: 301-405-5306; E-mail: skgupta@umd.edu.

manipulation of nucleic acids, while Zhuang¹² reviewed the work on DNA condensation. Liao et al.¹³ surveyed the progress in trapping and stretching of red blood cells, Herbert et al.¹⁴ reviewed the single-molecule studies of RNA polymerase, and Li et al.¹⁵ and Woodson et al.¹⁶ surveyed the literature on unfolding and refolding of RNA. Chemla¹⁷ reviewed the work on stepping dynamics of nucleic acid motor proteins, Mauritz et al.¹⁸ focused on the study of malaria-infected red blood cells, and Sung et al.¹⁹ surveyed single-molecule studies using dual-beam optical traps by considering myosin as the object of interest.

Quite a few survey papers also reviewed the role of optical tweezers in investigating biological systems in conjunction with or comparison to other manipulation techniques. Bustamante et al.²⁰ gave an in-depth review of investigations of single molecules of DNA using both optical tweezers and AFM. Ozkan et al.²¹ provided a survey of optical manipulation of cells in microfluidic devices. Neuman and Nagy²² compared the capabilities and limitations of optical tweezers, magnetic tweezers, and AFM in the context single-molecule force spectroscopy. Gross et al.²³ described how optical tweezers, single-molecule fluorescence microscopy, and microfluidics have been effectively combined for DNA-protein interaction studies. Tinoco et al.²⁴ reviewed the application of both fluorescence resonance energy transfer and optical tweezers on single RNA molecule reactions.

We provide a significantly different perspective from the aforementioned articles by viewing indirect manipulation of biological objects as robotic gripping of small scale objects, where the dielectric components (beads) act as the gripper fingers. This point of view allows us to identify an effective gripping strategy and equipment setup based on the shape and size of the biological objects and the type of manipulation operation required. We review the current literature on indirect trapping of three different types of widely-studied biological objects and show that this framework can explain the successful setup designs. Thus, we believe that this paper will be helpful to new researchers (particularly experimentalists) in providing them with general guidelines on how to select and build an indirect optical trapping setup for biological objects. It will also lay down some key challenges and research milestones that need to be achieved for more widespread use of indirect optical manipulation.

2 Robot Gripping-Based Indirect Optical Manipulation Framework

We first present a framework for the choice of the experimental setup based on the shape and size of the biological objects and the desired type of manipulation. Extending our analogy with robotic gripping, we can say that instead of pneumatic, hydraulic, or electrical actuation, in our case the grippers work as a result of piezoelectric actuation or trap reconfiguration using dynamic holograms. Unlike macroscale systems where the grippers are attached to robotic arms that rest on some solid supports, the dielectric beads are kept in place by optical trapping, suction, or other tethering forces. We refer to the force that is responsible for supporting the gripper as the localization force. Also, an additional coating on the gripper fingers (beads) is often required to provide better adhesion between the fingers and the gripped biological object as in the case of nucleic acids

and motor proteins. We now discuss some of the specific factors controlling setup designs in greater detail.

2.1 Admissible Size Range of Gripped Object

Optical tweezers have been shown to be very useful in trapping objects that are between a few hundred nanometers to about ten micrometers in diameter. Thermal or Langevin forces dominate below this size range such that high laser intensities are required to provide sufficient counteracting trapping forces. Gravity and viscous drag (drag only for moving particles) forces are dominant for larger sized objects such that high laser intensities are again required to ensure stable trapping. Now, even though laser beams are not focused directly on the biological objects in the case of indirect optical manipulation, some amount of light is always incident on the objects. Hence, high laser intensities are potentially damaging for the biological objects that we are trying to manipulate. So, the size of the gripper beads is usually restricted to lie within this range where tweezers can work satisfactorily at reasonable intensities.

2.2 Role of Gripped Object Size

There is a strong correlation between the size of the gripped biological object and the number and size of the gripper finger beads. Just as larger, stronger robotic grippers are required to grasp bigger objects, relatively larger and a greater number of beads are necessary to indirectly manipulate the cells as compared to the much smaller nucleic acids and motor proteins (in terms of axial diameter or neck thickness). This can be easily explained from the fact that larger and a greater number of beads experience stronger optical trapping forces and, hence, can exert stronger contact forces to manipulate the gripped object. We should note here that although thermal and drag forces are smaller for larger objects, the enhanced effect of gravity more than counterbalances this decrease.

2.3 Impact of Gripped Object Shape and Manipulation Type

The shape of the object and the desired form of manipulation play a significant role in governing the number of beads and the type of localization force. Since nucleic acid molecules are axially elongated, either one or two beads need to be attached at the ends of the molecules to manipulate them. Both the beads can be optically trapped or one of the beads can be conveniently held in a micropipette by means of suction force or even tethered to the coverslip surface. Two alternative arrangements are shown for DNA molecules in Fig. 1. Motor proteins are typically so small that just a single bead is sufficient for manipulation. However, if the objective of the experiment is to study the interaction between the motor protein and the walking medium, then three beads are often used. One is attached to the molecule itself, while the other two are attached to the two ends of the axial microtubule or actin filament, thus holding a piece of the scaffolding on which the motor proteins move. For manipulation of a suspended cell, different multibead arrangements are possible as depicted in Fig. 2. A two-bead arrangement works well if the purpose is to stretch the cell from two sides. On the other hand, a four or six bead arrangement is more suitable if the aim

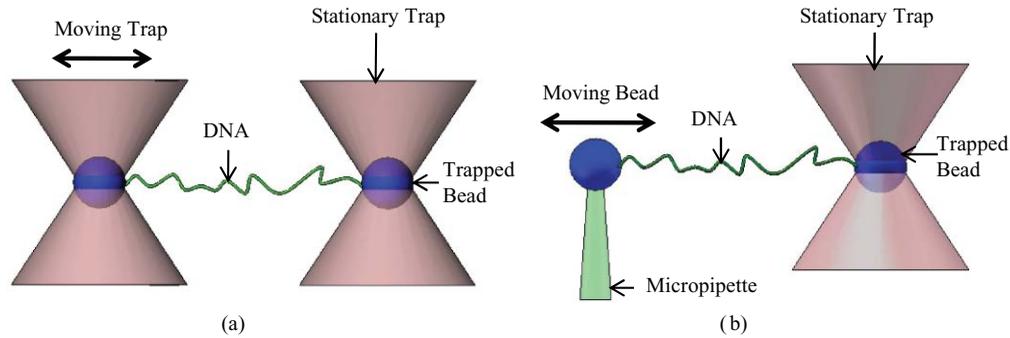


Fig. 1 Schematic illustration of two bead arrangement to manipulate DNA molecule; (a) (adapted from Ref. 25) shows a setup where both the beads are optically trapped, whereas (b) (adapted from Ref. 26) depicts a setup where one of the beads is held in a micropipette. Note that the figure is not drawn to scale.

is to grasp a cell strongly and transport it at a reasonable speed. As cells are commonly translated and rotated instead of being only stretched, the beads are not usually held in micropipettes or tethered to coverslip surfaces, and are always optically trapped.

Based on these observations, we can summarize some general principles for designing indirect optical manipulation setups as follows:

- The diameter of the gripper beads usually lies between a few hundred nanometers and tens of micrometers to provide stable optical trapping at reasonable laser intensities.
- Relatively more and larger sized beads are used to grasp bigger biological objects as compared to smaller ones since they provide greater trapping forces.
- Suction or tethering forces can replace optical trapping if only stretching of axially elongated objects is required.
- One or two beads are sufficient to indirectly manipulate axially elongated objects; however, three or more beads are necessary to localize and transport spherical objects.
- Small biomolecules must be attached to the gripper through chemical bonds, while larger objects such as cells can be gripped using just contact forces.

These principles provide justification for the successful experimental setups that have been designed so far as shown in the next three review sections. We also believe that they will prove to be very useful to future researchers who are planning to develop their own systems. As an example, if one wants to build a system for indirectly manipulating cell organelles such as vesicular networks of endoplasmic reticulum, the person can follow

the basic principles that we stated above and select a small size of the beads (roughly the same as that used for motor proteins), and attach two properly-coated beads to the ends of the network strands. It turns out that this is exactly the arrangement which is used in Ref. 29. Thus, our simple analysis provides the foundation for a systematic and informed design of manipulation setup for any kind of biological object that resembles the geometry of the three commonly-studied systems presented in the paper.

3 Indirect Manipulation of Cells

Optical tweezers were initially used to manipulate cells directly. However, soon it was observed that direct trapping can lead to considerable photodamage of trapped cells, including the death of cells as noted by Ashkin et al.³⁰ Many in-depth studies show the adverse effects of optical micromanipulation on cell health to some extent.^{31–34} The low light threshold for cell damage is also of great concern for the use of optical micromanipulation. Using 1064-nm wavelength laser, Ayano et al.³⁵ showed that cell damage to *E. coli* was linearly dependent on the total dose received and found that cell division ability was affected at a dose of 0.35 J. Rasmussen et al.,³⁶ using internal pH as a measure of viability, found that the internal pH of both *E. coli* and *Listeria* bacteria declined at laser intensities as low as 6 mW. Aabo et al.³⁷ also found that exposure of yeast cells to 1070-nm light over several hours had no apparent threshold in the amount of laser light that would negatively affect cells and that both laser power and total dose affected cell health adversely. All of these studies caution that direct cell trapping may not be desirable.

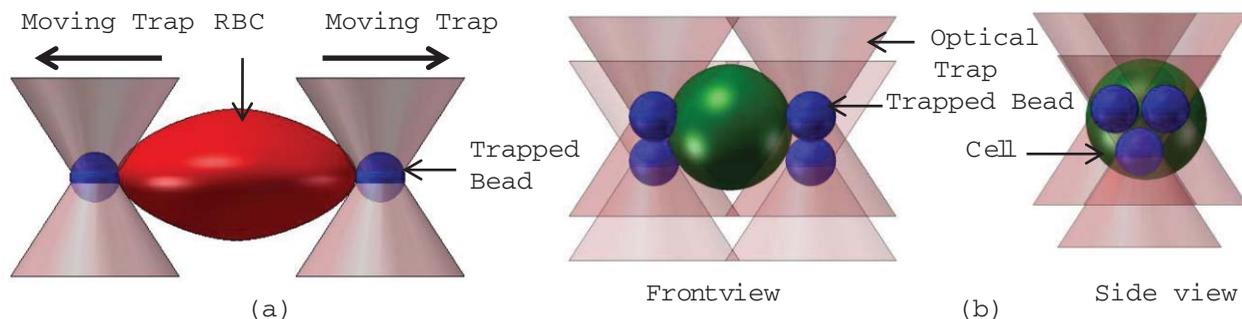


Fig. 2 Schematic illustration of two and six bead arrangements to manipulate cells; (a) (adapted from Ref. 27) is useful for stretching red blood cells, while (b) (adapted from Ref. 28) is useful for transporting cells. Note that the figure is not drawn to scale.

Cells exhibit greater diversity in terms of shape, size, and physical properties as compared to nucleic acids and motor proteins. As discussed in Sec. 2, since the gripper configuration depends on the object being gripped and on how the objects will be manipulated during the experiments, many different types of experimental designs can be found in the literature. This section reviews most of the representative experimental setups and also compares this approach with other manipulation techniques.

3.1 Representative Work in Indirect Manipulation

Many researchers have investigated the properties of cells using indirect optical manipulation. Laurent et al.³⁸ measured the viscoelastic properties of alveolar epithelial cells using magnetic twisting cytometry and optical tweezers. Li et al.²⁷ studied the deformation of the erythrocyte cells by stretching them through optically trapped beads. Fontes et al.³⁹ developed a new method to measure mechanical and electrical properties of red blood cell (RBC) rouleaux using double optical tweezers. Wei et al.⁴⁰ used a micro, rheometer based on oscillatory optical tweezer to measure the extracellular and intracellular complex shear modulus for alveolar epithelial cell. Python et al.⁴¹ studied the viscoelastic properties of microvilli using optical tweezers.

In order to measure the mechanical force constants (i.e., perform tensile tests), cells need to be held from one side while applying force on the other side. Indirect manipulation systems based on optical tweezers have proven to be appropriate for these kinds of experiments because of their ability to localize a bead on the surface of the cell precisely to hold it from one side or apply force. Henon et al.⁴² used optical tweezers to measure the shear modulus of RBC. Using a two bead arrangement, Li and Liu⁴³ measured the transverse and longitudinal strains of RBCs both experimentally using the optical tweezer system and theoretically using the finite element analysis (FEA) model analysis. The best matched results were then used to calculate the elastic constants of RBCs. In a similar experiment, Wu et al.⁴⁴ and Sleep et al.⁴⁵ measured the elasticity of RBCs with increasing osmotic pressure. Another research group led by Li et al.⁴⁶ attached one side of RBC with the coverslip and applied force on the other side using an optically trapped bead to measure the mechanical properties. Tan et al.⁴⁷ used a similar procedure for mechanical characterization of RBCs.

Some researchers have investigated the response of cells to external stimuli using optical tweezers. Miyata et al.⁴⁸ studied the effect of temperature and opposing force on the gliding speed of the bacteria *Mycoplasma mobile*. Kress et al.⁴⁹ investigated the binding mechanism of cells during phagocytosis using an optically-trapped bead as a local probe. Taka et al.⁵⁰ studied the dynamic behavior of a fibroblast cell membranes. Pozzo et al.⁵¹ used optical tweezers to study the chemotaxis behavior of a flagellated micro-organism when exposed to a gradient of attractive chemical substance. In order to understand the role of the pili of *E. coli* during adhesion to the host tissues, Andersson et al.⁵² studied the biomolecular properties of pili.

Some researchers have also experimented with new optical tweezer setups. For example, Ferrari et al.⁵³ used two different setups to create multiple traps for indirect manipulation of biological objects. One of the setups used acousto-optic deflectors

to achieve deflection of laser fast enough to maintain multiple traps by sequential sharing of the laser beam. However, this setup could only provide planar trapping configuration. The second setup used diffractive optical elements. The optical tweezer setup developed by Mejean et al.⁵⁴ was capable of measuring the mechanical coupling force between the cytoskeleton of *Aplysia* bag cell and neuron cell adhesion molecule.

Some researchers have also used objects other than microspheres as handles. For example, Sun et al.⁵⁵ used an irregularly shaped diamond as handles for the controlled rotation and translation of cells. Ichikawa et al.⁵⁶ proposed a new method for manipulation of micro-organisms by instantly creating and destroying the microtool. The microtool was formed by local thermal gelation using the laser power. After manipulation the microtool was dissolved by stopping the laser. Zhang et al.⁵⁷ successfully manipulated RBCs under various physiological flow conditions by attaching microbeads using optical tweezers.

Many researchers have been interested in cell sorting using optical tweezer systems. Dholakia et al.⁵⁸ performed passive cell sorting operation inside the microfluidic chamber by applying optical forces. Cells were tagged with microspheres to provide variations in refractive indices which enhanced the speed of the sorting process. Paterson et al.⁵⁹ used the same idea of tagging cells with microspheres in order to sort them using Bessel light beams. Mthunzi et al.⁶⁰ tagged mammalian cells with microspheres in order to improve the manipulation forces.

3.2 Comparison with Other Approaches

As cell manipulation is an important area both for medical applications and making fundamental advances in biological sciences, several different techniques have been developed for manipulating cells. Dielectrophoresis has been successfully used to manipulate cells.⁶¹ Magnetic manipulation involves tagging cells by magnetic particles and then using the time varying magnetic field to move the particles, and, hence, the cells.⁶² Both of these methods impose restrictions on the type of cells that can be manipulated by these methods and the environments in which the cells should be manipulated. Moreover, it is very difficult to achieve an independent placement control over multiple cells concurrently.

Recent advances in silicon and polymer-based microelectromechanical systems have been exploited to develop microscale grippers that can hold individual cells and arrays of cells.⁶³ These methods utilize customized grippers to grasp the cells. These grippers are used in conjunction with mechanical micromanipulators to move the cells. These grippers are not reconfigurable to allow for changes in the cell shapes. Moreover, only a limited field of view is available for imaging while the gripper is holding the cell. Integrating multiple mechanical manipulators together to perform multiple independent operations is challenging due to workspace limitations.

Microfluidics, when combined with, e.g., electro-osmotic actuation, can be a powerful tool to steer a small number of objects. It has been shown to be a useful technology for cell manipulation.⁶⁴ However, fluids are incompressible, making it harder to aggregate cells than optical traps. Microfluidics also generally requires a closed system for controlled flows and, thus, makes further manipulation of the sample by inserting a

micropipette or a chemoattractant difficult unless they are integrated with the microfluidics device.

4 Indirect Manipulation of Nucleic Acids

Biological molecules have been the workhorses in driving the development of optical micromanipulation even though the absolute precision with which the position of an object can be estimated is diffraction-limited and, thus, is much larger than the size of typical molecules. The key to the success of an optical tweezer is its amazing ability to measure the displacements of objects very precisely down to sub-nanometer accuracy. Hence, it has been very successful in investigating the changes in the shapes of biomolecules.

Most of the studies on nucleic acid molecules focus on stretching them to investigate their force-extension properties as well as to develop a fundamental understanding of the mechanism behind folding, unfolding, and transcription. As discussed in Sec. 2, motion is imparted to the bead that is held in the micropipette or tethered to the coverslip to stretch the molecule. The extension is measured by observing the displacement of the other optically trapped bead using video microscopy or reflections off the bead using quadrant photodiodes. The assumption of constant trap stiffness for small displacements of the beads from the trap centers is typically used to measure the forces acting on the molecule indirectly.

Although a lot of research has been performed in manipulating DNA and RNA with other types of techniques, optical tweezers provide greater resolution for manipulating individual molecules as compared to electrokinetic and magnetic tweezers as well as fluid flow-based approaches. Even though atomic force microscopy (AFM) provides better spatial resolution than optical tweezers, AFM tips are very stiff compared to optical grippers and often causes damage since the smallest forces that can be exerted by AFM are still quite large at the molecular level.²²

4.1 Manipulation of DNA

DNA strands are too thin for simple direct manipulation, and, hence, virtually all work on DNA involves indirect manipulation. As is done throughout this paper, the focus is on the experimental setups being used and readers are advised to go through the review articles mentioned in Sec. 1 for details on the scientific breakthroughs in this active field of research.

Several methods have been used to apply forces to DNA strands at multiple points. Perkins et al.⁶⁵ first studied the relaxation of single DNA molecules by attaching optically trapped 1 μm latex microspheres to one end of the molecule and pulling the other end via fluid flow. A feedback stabilized motor was used to move the microscopic stage at a constant speed to generate fluid flow around the stationary, trapped beads that stretched the molecules. Smith et al.⁶⁶ attached microscopic latex beads to both ends of DNA molecules, one of which was trapped by a laser tweezer and the other one was held by suction on a glass micropipette. The DNA was extended by moving the micropipette relative to the optical trap.

Shivashankar and Libchaber⁶⁷ grafted DNA-tethered beads onto silicon substrates such as AFM cantilevers using optical tweezers. The other end of the DNA molecules were attached

to coverslip surfaces. Shivashankar et al.⁶⁸ also studied the flexibility of DNA molecules by using an optical tweezer instead of an AFM. A collinear red laser light beam was used to probe the fluctuations in bead position with nanometer accuracy based on the direction of backscattered light. Wang et al.⁶⁹ used a similar setup but moved the coverglass with respect to the optical trap using a piezo-driven stage, while the bead position was recorded with nanometer-scale precision. A feedback circuit was activated to prevent bead movement beyond a preset clamping point by modulating the light intensity, thereby altering the trap stiffness dynamically.

The interaction of DNA with proteins has also been investigated. Bennink et al.⁷⁰ studied the interaction of DNA with Rec A and YOYO-1 molecules by capturing the DNA molecule between two polystyrene beads using biotin-streptavidin linkers. Wuite et al.⁷¹ used a similar setup to study the relation between the DNA strand tension and the activity of polymerase proteins bound to DNA. In one of the more unconventional studies, Arai et al.⁷² studied the mechanical properties of DNA molecules by continuously controlling the radius of curvature of the molecular strand by tying a knot in it.

At larger scales, Cui and Bustamante⁷³ studied the forces responsible for maintaining the higher-order structure of chromatin fibers using optical tweezers. They connected the two ends of a single biotinylated chromatin fiber between two avidin-coated polystyrene beads inside a flow chamber. Identical to the setup used in Ref. 74, one bead was trapped in a dual-beam optical tweezer, and the other was held atop a glass micropipette.

Bocklemann et al.⁷⁵ performed force measurements on single DNA molecules using optical tweezers to study the high sequence sensitivity of strand breakage. They created a molecular construction, wherein both strands of the DNA molecule to be unzipped were prolonged by linker arms of 2.5- μm length each, consisting of double-stranded DNA with multiple, modified base pairs at the ends. Hirano et al.⁷⁶ showed that the ends of single DNA molecules could be gripped by clustering microparticles using optical tweezers. As many as 40 latex beads of 0.2- μm diameter were aggregated at 400-mW laser power and moved at a speed of 40 $\mu\text{m/s}$.

Davenport et al.⁷⁷ studied transcriptional pausing and arrest by *E. coli* RNA polymerase in real time over large template distances using a combination of optical tweezer and flow-control video microscopy. The RNA polymerase-DNA complex was tethered between two 2.2- μm streptavidin-coated beads and kept in a continuous buffer flow. Soni et al.⁷⁸ combined optical tweezers with micropipettes to develop a setup that was capable of operating autonomously at constant force, constant velocity, or constant position. The authors conducted three different experiments that had sub-pN force sensitivity and a nanometer scale positioning accuracy to highlight the usefulness of the system.

Larson et al.⁷⁹ investigated the mechanism of transcription termination of bacterial RNA polymerase by creating a two-bead assay with unequal size of the optically-trapped polystyrene beads; the polymerase was attached to the smaller bead via a biotin-avidin linkage and the DNA template was attached to the larger bead via a digoxigenin-antidigoxigenin linkage. Terao et al.⁸⁰ manipulated single chromosomal DNA molecules by using a microhook and a microbobbin structure, both of which were driven using optical tweezers. The microhook was used to

capture a molecule at any desired point and two microbobbins (one revolving around the other) were used to wind the molecule.

More recently, Galburt et al.⁸¹ studied the dynamics of transcriptional elongation of RNA polymerase II by using a dual-trap optical tweezer setup similar to the one described in Ref. 79 except for the fact that they used beads of identical size. Kleimann et al.⁸² investigated the binding kinetics of Triostin-A to λ -DNA by measuring the force-extension curves of the DNA-ligand complex. Landry et al.⁸³ characterized the damage caused by optical traps on DNA tethered between optically-trapped polystyrene beads. Lin et al.⁸⁴ developed an optically-induced dielectrophoresis platform to elongate and rotate single DNA molecules by tethering one end of the molecule to the substrate and binding the other end to a polystyrene bead.

Mameren et al. used a combination of fluorescence microscopy, optical tweezers, and microfluidics to resolve the structural basis of DNA overstretching. They held and extended double-stranded DNA molecule by attaching both ends to an optically-trapped microsphere. The same setup⁸⁵ was also used to study the DNA strand tensions needed to disassemble (or shed) aggregates of proteins that can form around DNA strands, e.g., filamentous aggregates of RAD51. Mossa et al.⁸⁶ evaluated the equilibrium free energy differences in pulling DNA hairpins by adopting the common procedure of attaching beads at the two ends; one of them was held in a pipette, whereas the other one was placed inside a moving optical trap. Murade et al.⁸⁷ studied the force extension of DNA molecules in the presence of oxazole yellow dyes by integrating fluorescence microscopy imaging in an optical tweezer setup.

Carter et al.⁸⁸ developed an optical trapping assay with one DNA basepair resolution by employing active stabilization techniques to reduce the 3D surface motion and the effect of different sources of laser trap noise. Fuji et al.⁸⁹ presented a technique for fabricating a single DNA nanowire by using laser local heating at Au/water interface, wherein an optical tweezer was used to compress a bead attached to the DNA molecule to the solid surface, thereby resulting in pinning of DNA. Ommering et al.⁹⁰ characterized the bonding between streptavidin-coated polystyrene and superparamagnetic particles and a biosensor surface by tethering the particles with double-stranded DNA molecules and manipulating them using magnetic fields in the presence of a laser beam. Zohar et al.⁹¹ demonstrated the usefulness of modified peptide nucleic acids (PNAs) in manipulating DNA molecules by attaching one end of the PNA-DNA-digoxigenin complex to an optically-trapped, antidigoxigenin-coated polystyrene bead and the other end to a streptavidin-coated bead that was held in a micropipette.

4.2 Manipulation of RNA

Single stranded RNA comes in a wide variety of arrangements in terms of secondary and tertiary structures, which have been investigated in depth. Liphardt et al.⁹² first applied mechanical force to induce unfolding and refolding of single RNA molecules by attaching them to polystyrene beads using DNA-RNA hybrid handles. Similar to the setup used in much of the reported work, one of the beads was held in a force-measuring optical trap and the other one was linked to a piezo-electric actuator through a micropipette. Three different RNA secondary structures were

investigated, namely an RNA hairpin, a three-helix junction, and the P5abc domain.

Harlepp et al.⁹³ probed RNA secondary structures by combining single molecule stretching experiments with stochastic simulations. The RNA structures were hybridized to two double stranded DNA extensions, which were attached to beads and surfaces using biotin-streptavidin and digoxigenin-antidigoxigenin linkages. Mangeol et al.²⁵ probed the unfolding/refolding hysteresis behavior of RNA molecules after first validating their two-bead experimental setup by conducting experiments on stretching of DNA molecules. The mechanical unfolding, force-quench refolding, and the hopping rates of RNA hairpins were studied and a comparison of experimental and simulation results with theoretical analysis were conducted in Ref. 94.

Li et al.⁹⁵ investigated the mechanical folding kinetics of single RNA molecules by using dual-beam optical tweezers within the standard two bead setup, where the antidigoxigenin-coated polystyrene bead was held by suction in a micropipette and the other streptavidin-coated bead was held in an optical trap. Green et al.⁹⁶ characterized the mechanical unfolding of RNA pseudoknots formed from an infectious bronchitis virus using the same two bead, dual-beam optical tweezer setup. Wen et al.⁹⁷ also studied the effects of different experimental variables on the RNA folding/unfolding kinetics on a model RNA hairpin (P5ab) by using the two bead, dual-beam setup. In a companion article, Manosas et al.⁹⁸ applied a mesoscopic model to simulate the kinetics under comparable conditions and obtained good agreement with the experimental results.

5 Indirect Manipulation of Motor Proteins

As mentioned in Sec. 2, almost all the manipulation methods for motor proteins are similar in the sense that they attach optically-trapped, dielectric beads (often coated with proteins) to one end of the proteins or to the substrate for investigating the motion of the proteins on the walking medium, namely, microtubule and actin filaments. The walking medium can be localized either by attaching two beads at the ends or by immobilizing it on the substrate. Most of the operations involve bringing the motor proteins in close contact to the substrates and then analyzing their motions as the beads are first pulled away and then retracted by the traps. Although a lot of researchers have looked into manipulating them with other types of techniques, optical tweezers offer certain advantages as discussed earlier in the context of nucleic acids.

5.1 Manipulation of Kinesin

Block et al.⁹⁹ used optically-manipulated silica beads to measure movement of kinesin molecules along microtubules. The beads were coated with carrier proteins, exposed to varying kinesin concentrations, and individually manipulated by single-beam optical traps. Svoboda et al.¹⁰⁰ directly observed that kinesin moves with 8-nm steps. The motion was analyzed under varying laser power (and, consequently, trapping force) conditions using optical interferometry, which combined an optical tweezer with a dual-beam interferometer. The motion analysis was done by tracking the bead position, keeping the trap stationary after depositing the bead. Once the molecule would get released after traveling for a certain distance along the

microtubule, the bead would return to the trap center, reattach, and fresh movement would start. Thus, an individual molecule motion could be studied for several minutes and up to hundreds of mechanochemical events, until the molecule failed to bind to the microtubule or got stuck irreversibly. Svoboda and Block¹⁰¹ also used the same principle to measure the force-velocity curve of single, silica bead-attached kinesin molecules moving on microtubules.

Kojima et al.¹⁰² studied the motion of kinesin molecules by adsorbing them onto optically-trapped latex beads, and then bringing them in contact with axonemes that were bound to a glass surface. Visscher et al.¹⁰³ studied how the chemical energy is coupled to mechanical displacements in single kinesin molecules by adsorbing them on to optically-trapped silica beads and moving them on immobilized microtubules. As the precision of the measurements increased, the position in the third dimension needed to be taken into account to compute forces and displacements. Jeney et al.¹⁰⁴ studied the mechanical properties of single kinesin molecules by recording the 3D positions of kinesin-coated, optically-trapped glass beads, tethered to cover-slip adsorbed microtubules, with a spatial precision in the nanometer range and a temporal resolution in the order of few tens of microseconds. Unlike in Ref. 103, where high loads were used to reduce Brownian motion, optical forces were kept to a minimum (of the order of fN) here to allow thermal fluctuations to dominate the probe measurements.

Carter and Cross¹⁰⁵ investigated the mechanics of kinesin stepping by attaching a single molecule to an optically-trapped spherical bead that was steered toward an immobilized microtubule. Bormuth et al.¹⁰⁶ used optical tweezers to characterize the frictional drag force of individual yeast kinesin-8 (Kip3p) molecules interacting with microtubule tracks in the presence of ADP. Kip3p-coated microspheres were dragged near immobilized microtubules at a low enough myosin concentration to ensure that only single molecules would interact with the microtubule. The microtubule was moved back and forth relative to the laser trap and the position of the microsphere was recorded. Guydosh and Block¹⁰⁷ provided useful insight on the interactions of the individual motor heads with the microtubule.

Gutierrez-Medina et al.¹⁰⁸ measured the torsional properties of kinesin molecules by attaching them with fluorescence-marked polystyrene beads and then trapping them in the solution medium using optical tweezers. The captured kinesin-bead complex was then placed near a microtubule that was immobilized on the coverglass surface. The optical trap was switched off after kinesin-microtubule binding took place, allowing free rotation of the tethered bead due to thermal forces. Bruunbauer et al.¹⁰⁹ investigated the regulation of heterodimeric kinesin-2 motor molecules by moving them on a microtubule tract that was attached to the coverslip surface. The molecules were coated on an optically-trapped polystyrene bead that was moved out and then pulled back into the trap focus during binding-unbinding with the tract, where the restoring force was provided by a piezoelectric stage clamped to the coverslip surface. Butterfield et al.¹¹⁰ conducted measurements of power strokes of kinesin-14 molecules using a three-bead geometry, where a biotin-coated microtubule was suspended between two streptavidin-labeled, optically-trapped silica beads. The microtubule was attached to the third, larger diameter bead that was sparsely coated with the motor molecules.

5.2 Manipulation of Myosin

Finer et al.¹¹¹ measured the force and displacement resulting from the interaction of myosin with an actin filament where the substrate of myosin was micromanipulated. An actin filament was attached to polystyrene beads at each end and held in place by two optical traps. Just as in the kinesin studies, measurements were performed in the constant trap stiffness region by pulling the actin filament using one of the beads. Then it was brought close to the coverslip surface so that it could interact with one or a few myosin. Once contact was established, a quadrant photodiode was used for high resolution position detection of the other trapped bead that started moving along the direction of the filament.

Veigel et al.¹¹² used the three-bead setup described in Ref. 111 to measure the stiffness and working stroke of a single actomyosin structure. Wakayama et al.¹¹³ studied the motion of myosin actively sliding along actin filaments suspended between two immobilized microbeads which were trapped by double-beam optical tweezers. Clemen et al.¹¹⁴ used single beam optical tweezers with a force feedback that allowed for a large range of motion to study the stepping kinetics of myosin-V molecule under controlled forward and backward loads. Polystyrene beads exposed to myosin-V were optically trapped and positioned over fluorescently labeled, surface-anchored actin filaments. Cappello et al.¹¹⁵ used optical tweezers to bring a myosin-coated bead in contact with the actin filaments and the motion of the bead was recorded parallel and perpendicular to the filament axis with nanometer accuracy microseconds time resolution.

Instead of following the common three-bead setup, Arsenault et al.¹¹⁶ used dielectrophoresis to suspend actin filaments across a trench that was created between gold electrodes to study the helical motion of myosin molecules, which were attached to a bead held by an optical tweezer. One of the main advantages of this hybrid setup was to provide clearance beneath the filament to allow unhindered motion of the bead. Kaya and Higuchi¹¹⁷ measured the step size and stiffness of skeletal myosin molecules interacting with actin filaments that were suspended between two streptavidin-coated, optically-trapped beads. Streptavidin-coated quantum dots were also attached to the actin filaments to reduce the uncertainty in the linkage stiffness and single myosin molecules, embedded in myosin-rod co-filaments, were tightly bound to the filaments. Sellers and Veigel¹¹⁸ investigated the reversibility of the power stroke of myosin-Va motor heads; they made direct observations on the interactions of the myosin molecules present on surface-attached beads with F-actin filaments that were held between two optically-trapped polystyrene beads.

6 Conclusions

6.1 Trends

6.1.1 Lasers and objectives

Certain common trends can be observed across this research domain. For example, Nd:YAG and Nd : YVO₄ are the two most popularly used types of lasers. The lasers are always operated in the infrared regime, although the specific wavelengths may vary from 790 to 1064 nm. Usually, the laser power is kept quite low (mostly below 300 mW), even though in a few cases higher values are used. Typically, very high magnification (100×) and

numerical aperture (1.2 to 1.4) objective lens are used. Lens having 40, 50, or 63 \times magnification, and numerical aperture of 1.0 or 0.6 are utilized only in a few cases.

6.1.2 Gripper finger size, linkage material, and manipulation type

As expected from our discussion in Sec. 2, relatively more variation is observed in the case of the gripper finger size. Although in quite a few cases, bead size within the range of 1 to 2.5 μm are selected, in certain cases, beads as small as 75 nm in diameter are used, whereas in other cases, beads as large as 10 μm diameter are utilized. Biotin-streptavidin and digoxigenin-antidigoxigenin are commonly used to link the beads with the biomolecules. Stretching or pulling is the most prevalent form of manipulation due to its simplicity. Nevertheless, rotation is also now becoming possible as evident from Refs. 119 and 120.

6.2 Challenges and Research Directions

Various approaches for indirect micromanipulation of cells and biomolecules are now well-established. In particular, some of the techniques are quite optimized for manipulating biomolecules. However, the slow speed of optical manipulation of cells, confinement to single-cell studies, and lack of widespread usage in cell biology laboratories and clinics indicate that a more systematic approach to design and control this complex system may be valuable for broader implementation. Hence, we believe that there are many promising areas of future research. We list them and briefly discuss how they may help in addressing the current challenges.

- **Parallelization/multibeam tweezer systems:** While single-cell studies using optical tweezers provide us with a lot of insight on biomechanical and other physiological properties, they are inherently inefficient and restrict us to only certain kinds of applications. Studies on intercellular signaling, response of cells to pathogens, etc., require creating cellular assays (often in regular, geometric patterns), which cannot be formed or manipulated using just one or two optical traps. Instead, holographic, rasterized scanning mirror-based or other types of multibeam tweezers need to be used in order to manipulate several cells in parallel.
- **Hybridization:** An alternative to multibeam tweezer systems for achieving multicell manipulation lies in combining optical traps with other forms of manipulation techniques, most notably electrophoretic and microfluidic. Although researchers^{121,122} have already developed hybrid systems to pattern cells or separate them, to the best of our knowledge this has not been done in the context of indirect manipulation. We believe that the combination of microfluidic and indirect optical manipulation systems holds the greatest promise in providing high speed of operation and positional accuracy simultaneously. In such systems, the gross motion will be imparted by the fluid flow, whereas the fine and precise positioning of cells at their final locations will be performed by the optical grippers.

- **Optimized setup selection:** Further work is needed in designing an optimized setup for indirect optical manipulation of cells in terms of the number of beads required, size of the beads, and contact point locations. Three key steps are involved in doing that. First, an accurate modeling of the contact forces between the beads and the cells, along with the other forces present in the system (optical trapping, thermal, viscous drag, and gravity) is required. Such modeling needs to account for the geometry of the cells, laser beam cone, and the experimental apparatus parameters. Second, a suitable numerical scheme such as finite difference or finite element method needs to be employed to compute the forces as it is expected that exact analytical solutions will be quite hard to obtain. Third, an appropriate parametric optimization technique has to be applied to determine the desired quantities based on the computed forces.
- **Automation:** Operation automation is very important since manual intervention and low throughput are major hurdles against wide adaptation of optical tweezers. Although some work has been done on automating transport of colloidal microspheres,^{123–125} significant advances in image processing and planning and control are necessary for developing reliable autonomous systems to indirectly manipulate cells. Specifically, automation will tremendously help in re-adjusting trap and gripper positions by compensating for the constant Brownian motion of the cells, planning optimal trajectories to transport the cells to desired locations in the assays, and selecting appropriate trap intensities and speeds to maximize the operation efficiency.

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