Review

Using Optics to Measure Biological Forces and Mechanics

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Spanning all size levels, regulating biological forces and transport are fundamental life processes. Used by various investigators over the last dozen years, optical techniques offer unique advantages for studying biological forces. The most mature of these techniques, optical tweezers, or the single-beam optical trap, is commercially available and is used by numerous investigators. Although technical innovations have improved the versatility of optical tweezers, simple optical tweezers continue to provide insights into cell biology. Two new, promising optical technologies, laser-tracking microrheology and the optical stretcher, allow mechanical measurements that are not possible with optical tweezers. Here, I review these various optical technologies and their roles in understanding mechanical forces in cell biology.

Key words: laser-tracking microrheology, laser tweezers, optical forces, optical stretcher, optical tweezers, single-beam optical trap

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Mechanical forces play key roles at all levels of biology. At the molecular level, chemical forces condense DNA into chromatin, and its structure mechanically impedes the progress of polymerases. At the subcellular level, the cytoskeleton forms dynamic networks that mechanically impede the progress of trafficking organelles. At the cellular level, cells control adhesive properties and increase their pulling forces just to crawl. Not only is there an implied mechanical consequence for each of these biological processes, optical techniques have provided new insights for all of these biological forces.

In this review, I focus on laser-based approaches that have been used to quantify biological forces. The most successful of these techniques is the single-beam optical gradient trap, often called laser or optical tweezers. Because photons have momentum, a laser beam can exert ‘radiation pressure’ and move microscopic objects. Optical tweezers are the simplest device that uses radiation pressure to trap and manipulate particles. The larger part of this review concerns optical tweezers, their technical enhancements and their applications. The maturity and commercial availability of optical tweezers have made them familiar to many cell biologists as micromanipulators. However, state-of-the-art force measurements require recent innovations that are not yet incorporated into commercial devices. Overall, the impact of optical tweezers on cell biology is still growing.

Optical tweezers are not the only method using laser-based optics to measure biological forces. Two new optical techniques, both spin-offs of efforts with optical tweezers, are worth the attention of cell biologists. In one, the ‘optical stretcher’ applies large optical forces that can deform whole cells. In the other, ‘laser-tracking microrheology’ measures mechanical properties without pulling or pushing particles, but by monitoring their Brownian motions. Depending on application, both techniques can measure mechanics that are beyond the capabilities of simple optical tweezers. The advantages and disadvantages of various optical approaches will be discussed.

This review is split into two parts. Technologically oriented, the first part reviews the origins of optical forces, optical configurations and spin-off technologies. Applications oriented, the second part is organized by size level, from molecular to cellular. At each size level, it considers alternative technologies for measuring biological forces.

Technology

History and basis of optical forces
In describing the genesis of optical trapping and micromanipulation, it is difficult not to pay homage to Arthur Ashkin. Although his scientific record speaks for itself, Ashkin’s recollections of key discoveries are insightful (1,2). Optical forces, or radiation pressure, on microscopic objects were first documented by Ashkin in 1970 (3). While demonstrating that a laser beam can exert axial forces and push particles across an aqueous chamber, Ashkin also showed that the beam exhibited unexpected radial trapping (see Figure 1A). With two opposed beams (see Figure 1B), the axial forces can be balanced and a particle is stably trapped between the two beams (3). This achievement foreshadowed the optical trapping of atoms (4,5) and of particles (6,7) with simpler optical geometries. Although he called it a single-beam optical gradient trap, Ashkin invented today’s popular optical
tweezers using infrared lasers, and demonstrated their safety for manipulating biological specimens (8).

The fundamental principles are straightforward. As photons of light propagate, they possess momentum. The stream of photons in a ray of light should exert ‘radiation pressure’ as the light interacts with matter. In principle, whenever light is absorbed, re-emitted, scattered, reflected and refracted by an object, there is a change in the momentum of the light. Conservation of momentum demands that radiation pressure generates a force on that interacting object. Until the advent of lasers, these optical forces were only significant for astronomy, but were negligible for more terrestrial considerations. Using lasers, Ashkin demonstrated the existence of significant optical forces on the microscopic scale (3). Only lasers have sufficient intensity and coherence to generate the requisite density of photons with uniformly directed momenta.

The physics of light interacting with matter is outside the scope of this review, but some generalizations may be useful. On the macroscopic level, light rays, reflection and refraction are the common notions underlying geometric optics. On the microscopic and atomic level, absorption, re-emission, scattering and the electromagnetic nature of light are the notions that guide thinking. Clearly, the macroscopic properties must be built from these microscopic and atomic phenomena. The dividing line between the two modes of thinking depends on the size of the object and the wavelength of light. When objects are significantly larger than the wavelength of light, notions of geometric optics are operant. When objects are significantly smaller than the wavelength of light, the so-called Rayleigh regime, concepts of light scattering, electromagnetic nature of light and electric dipoles in materials become operant. Indeed, for slowing and trapping atoms into an ‘optical molasses’, physicists rely upon Doppler-shifted absorption and re-emission of light. For cell biologists using biologically compatible infrared lasers, most particles are near wavelength (0.8–1.0 \( \mu \text{m} \)) in size and larger. Hence, geometric ray-optics notions provide satisfactory explanations for optical tweezers (9).

To describe the optically generated forces, Ashkin decomposed them into two components: scattering and gradient components. For a weakly focused beam (see Figure 1A,B), the gradient component is oriented orthogonally to the axis of the laser beam, and the scattering component is parallel. In this configuration, the inwardly directed radial forces arise from the intensity gradient of a TEM\(_{00}\) laser beam. Such a beam is brighter in the middle and decays radially. For a single-beam optical trap, the gradient forces are both axial and radial. For the purposes of this review covering different optical geometries, I will avoid these terms that suggest the origins of optical forces, and will only use geometric terms. Axial forces are parallel to the laser beam, and radial forces are perpendicular to the beam.

**Basic design of instrumentation**

The most popular configuration using optical forces is the single-beam configuration often called optical tweezers. A number of reviews have already been written describing the theory, construction, and practice of optical tweezers in the single-beam configuration (e.g. 10–15). User-friendly, commercial devices are available through Cell Robotics, Inc. (Albuquerque, NM, USA) and P.A.L.M. Microlaser Technologies, AG (Bernried, Germany). For those wishing to build their own optical tweezers but who are not familiar with optics, the primer by Mara Prentiss (16) is an excellent resource for building a simple instrument. Describing more complicated issues...
related to optical tweezers, many articles have been collected into a special issue of Methods in Cell Biology by Michael Sheetz (17).

Even though constructing optical tweezers is beyond the scope of this review, familiarity with their essential features are required. In constructing simple optical tweezers, the goal is to expand the laser beam to fill the back aperture of a high-quality microscope objective. Either water-immersion or oil-immersion objectives of high numerical aperture (NA > 1.2) are adequate. Typically replacing the fluorescence module of a microscope, a dichroic mirror reflects the laser beam into the objective. External optics expand the beam, direct it towards the dichroic, and shape the beam to match the tube length of the objective (either infinity-corrected or 160 mm). Filling the back aperture ensures that the full numerical aperture is used so that axial trapping is maximal. For large particles, the axial trapping requires the highly convergent rays (see Figure 1C). For very small particles, the large numerical aperture increases the intensity gradient both axially and radially, thus improving the trapping efficiency. Axial trapping is weakest in the single-beam configuration.

For general manipulations, there are two options for optical tweezers: either move the specimen or move the laser beam. In the simplest configuration, the microscope’s specimen translation stage is ergonomic and often sufficient. Depending on which optical port of the microscope is used to introduce the laser beam, controlling the laser beam may not be convenient for general use. Fancier systems use remote-controlled beam deflectors or mirrors to control the position of the optical trap.

**Optical tweezers and technical innovations**

To quantify and control optical forces, much effort with optical tweezers has been directed to measuring and controlling the position of particles relative to the optical tweezers. Because all traps, including optical tweezers, are like mechanical springs, optical forces depend on the particle’s displaced distance from the center of the trap. Most optical tweezers are ~150 nm in radius (18), so video-based tracking (5 nm at 30 Hz) (19) with stationary optical tweezers can be adequate. Faster tracking (~1 kHz) is possible by imaging the particle, including fluorescence imaging, onto quadrant photodiodes (14,20–23). Rather than imaging the particle in this way, it is more sensitive to use the plentiful forward-scattered laser light from particles in the optical trap (24–28). Historically, interferometry of forward-scattered laser light provided key scientific insights (29,30), but position measurements were only 1-D along the optical shear axis. Furthermore, placing quadrant photodiode detector conjugate with the back-focal plane of the objective eliminates the need to reposition the detector when moving the optical tweezers (31–33). At least 2-D position measurements are always relative to the center of the optical trap, and this feature offers distinct advantages when a single optical trap ‘juggles’ multiple particles (33–35). Tracking in 3-D using quadrant photodiodes has been described (27,28). In principle, two-photon fluorescence induced by the high-intensity optical tweezers can also provide high spatial resolution (27,36,37).

Controlling the position of the optical tweezers has also matured significantly. The simplest configuration is to have a stationary optical trap and to employ the microscope’s specimen stage or a custom motorized stage. More sophisticated versions use galvanometer-driven mirrors to displace the optical tweezers (e.g. 34,38). The most sophisticated approach is to use acousto-optical deflectors to move the trap at near MHz speeds (e.g. 20). With such deflectors, it is possible for a single optical trap to time-share amongst multiple particles (33–35). Alternatively, two optical traps can be synthesized using polarizing beamsplitters (e.g. 20). These multitrap configurations are critical for studying nonprocessive molecular motors such as myosin (20,21,39).

With accurate position detection and control, it is a natural, albeit challenging, development to build feedback control. Because the optical tweezers are like a mechanical spring, they are not suited for steady-state forces except when stalling motion. So-called ‘force-clamp’ feedback (33,40,41) will reposition the optical tweezers so that particle displacements, and hence optical forces, are constant. For RNA polymerase, force-clamp was necessary for force-velocity measurements (40), and for kinesin, force-clamp allows mechanical loading at high ATP concentrations (41).

Also a natural development, optical tweezers have been employed as atomic force microscopes (AFM) to measure surface topography, including cells (24,27,36). As the optical tweezers are scanned in x and y, the height of the trapped particle is monitored to generate surface topography.

**Newer approaches**

Although applications for optical tweezers are still growing, there are certain applications for measuring mechanical properties where optical tweezers are insufficient. Optical tweezers are excellent for very delicate forces generated by few molecules. However, cellular-level forces are beyond the abilities of most optical tweezers. Optical tweezers generating forces much larger than ~100 pN risk optical or thermal damage to most cells. Typically, such levels require more than 250–500 mW of laser power (1064 nm) irradiating cells, probably corresponding to ~10°C temperature increase due to the absorption by water (42,43). Although shorter wavelengths should provide less heating and higher forces, cellular forces are in the nN range and beyond the range of optical tweezers (44). In addition, the speed of dynamic cellular processes requires sophisticated automation of optical tweezers that has yet to be implemented. For example, phagocytic cells are often highly motile, but phagocytosis can be very fast, lasting only a few seconds (45). These two limitations have prompted the development of two different optical approaches, ‘laser-tracking microrheology’ (26,45,46) and the ‘optical stretcher’ (47,48). Both approaches have their roots in optical tweezers technologies, but work by different principles.
Laser-tracking micro rheology: Without applying any forces, merely tracking the Brownian motion of particles can quantify mechanical properties of complex viscoelastic materials (26,49,50), such as cytoplasm (46). We call this approach laser-tracking microrheology (LTM). It is a marriage of tracking techniques developed for optical tweezers with the mathematical physics developed for using light scattering to measure the rheology of complex materials (51). Rheology is the study of how materials flow and deform with different forces. For flexible polymers in solution, LTM is extremely accurate and matches the rheology measured by traditional macroscopic techniques (26).

Of interest for cell biologists, LTM can measure mechanical properties of both reconstituted networks and within living cells. Measurements of pure F-actin networks (49,52,53) and cross-linked F-actin networks (54) compare well with traditional techniques. Unlike tracking studies with small particles that could percolate through the actin network (55,56), LTM uses large particles trapped in the cytoskeletal network, thus revealing its bulk mechanical properties. In cellular applications, the highly localized measurements by LTM allows mechanical mapping of subcellular domains within living cells (46), and to monitor active phagocytosis by Dictyostelium discoideum (45). With implications for organelle trafficking, LTM can detect changes in the microenvironment when particles associate or dissociate from F-actin (54,57). The same technology showed that Listeria monocytogenes moved with actin-monomer sized steps (58).

Although LTM is very powerful, it has its limitations. As advantages, LTM is very well suited for fast phenomena where local mechanical properties change quickly. Unlike macroscopic rheology which requires large homogeneous samples, very small sample sizes are required for mechanical characterization by LTM, reducing prep-to-prep variations. As disadvantages, LTM is not suitable for measuring slow mechanical properties. For slow flow on the time scale $\sim 1$ min, $10$ min of particle tracking would be needed. Direct application of force with optical tweezers would complement the shortcoming of LTM in such situations. A more fundamental limitation is the need for a large particle in the zone of interest within cells. Luckily, some cells are rich in particles, but many cells are not.

Optical stretcher: Reminiscent of Ashkin’s dual beam optical trap (see Figure 1B) but using optical fibers (59), the optical stretcher uses two opposed laser beams to trap cells (47,48). Because cells are elastic, deformable structures, a subtle optical force manifests. When a photon enters a material with a higher index of refraction, its momentum increases as its wavelength decreases (momentum $p = \hbar n/c$, where $\hbar$ is Planck’s constant, $n$ the material’s index of refraction, and $c$ is the speed of light in a vacuum). By conservation of momentum, ‘pulling’ forces are applied to the optical interface by photons increasing their momenta, even when the light rays are not refracted. Ashkin first demonstrated this phenomenon with an air–water interface (60). Josef Kas and colleagues harnessed this effect, and showed that it is strong enough to stretch fibroblasts as well as red blood cells (47,48). The elasticity measured is comparable to previously measured values.

Although the optical stretcher is still very new, some of its advantages are clear. Unlike optical tweezers and Ashkin’s dual beam device, the laser beams are not focused, thus allowing higher laser powers without damaging cells. The optical stretcher generates forces that bridge those generated by optical tweezers and by atomic force microscopy. Because the elasticity of the whole cell cortex is measured, the optical stretcher summarizes whole cell behavior at the expense of subcellular information. Such global behavior is challenging to assess with optical tweezers alone. The optical stretcher provides information much like micropipet aspiration (e.g. 61), but has the potential advantage of high-throughput whole-cell mechanical measurements. Additional insights for cell biology should be forthcoming.

Applications in Cell Biology

General considerations

Optical approaches, such as optical tweezers, have unique advantages for cell biology. As a micromanipulator, it is unmatched in its delicacy of forces, local control, and ability to completely release trapped objects. This last feature allows weak associations to be detected (e.g. 62). An example of this exquisite control is the ability to tie a knot with a strand of F-actin or a strand of DNA (63). Typically polystyrene or silica particles, artificial ‘handles’ must be attached to these and other filamentous structures for micromanipulation by optical tweezers (e.g. 18).

Because of the high intensity required for optical tweezers, laser wavelength and power must be considered. The intensity of optical tweezers is clearly strong enough for stimulating multiphoton effects, such as fluorescence (27,36,37). Using a tunable laser on both animal cells and E. coli, the safest wavelengths are $\sim 800$ nm (64–67). Unfortunately, titanium-sapphire tunable laser systems are expensive. The popular alternative, Nd:YAG or Nd:YLF lasers, which emit $1.06 \mu m$ are very well tolerated by cells and reasonably priced. Laser diodes emit in the optimal range, but their beam quality requires significant optics for single-beam applications. However, for a dual beam trap, these laser diodes are very effective (25).

Of course, the final consideration is whether optical tweezers or related technologies are truly appropriate. The other noninvasive options for measuring biological forces are magnetic (e.g. 68), acoustic (69), and Brownian motion (e.g. 46). If mechanical contact is feasible, atomic force microscopy (70), micropipet (71) and microneedle (72) techniques should be considered.

In compiling the subsequent listing of various applications of optical tweezers to cell biology, it was clear that such a listing
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would not be comprehensive. The use of optical tweezers is so common, that many papers don’t include it as a key word. Rather than list all studies, I’ve chosen to list applications of optical tweezers that are well suited for the biological scale of study.

Molecular applications

Optical tweezers have made their greatest impact in understanding the molecular biophysics in biochemically reconstituted systems. It is no longer possible to give a comprehensive bibliography of applications of optical tweezers at the molecular level. The early efforts on kinesin (18,22,29,30,73,74), myosin (20,21,75–77), cytoplasmic dynein (78) and RNA polymerase (79) are well documented. More recent efforts with the optical tweezers in the force-clamp mode have data on kinesin that are different from the early studies without force-clamp (41,60). The latest optical tweezers efforts on myosin mechanics examine mutations associated with human diseases (81,82) as well as the elemental power stroke (83,84).

Other applications of optical tweezers in reconstituted systems are rotation of the bacterial flagellar motor (85,86), unwinding of chromatin DNA from nucleosomes (87), and importing DNA across reconstituted nuclear membranes (88). Mechanics of individual filamentous molecules such as titin (89,90) and individual filaments, such as F-actin (91) and microtubules (92), have been measured. Clearly, molecular applications of optical tweezers to reconstituted systems are numerous and very effective.

Subcellular applications

In living cells, studying subcellular properties with optical tweezers has not been as prolific as studies using reconstituted systems. Although the original studies demonstrating the noninvasive properties of optical tweezers were subcellular (93), it is often difficult to identify organelles so that specific hypotheses can be tested. During Drosophila embryogenesis, vesicle trafficking is developmentally regulated. Optical tweezers can measure discrete force levels when stalling the saltatory motion of lipid droplet trafficking, and klar appears to regulate the coordination of forces on these droplets (94).

Cell surface/adhesion

Optical tweezers have greatly aided studies of cell surface receptors. Leading edges of cells are enhanced to cytoskeleton (95), whereas the trailing edge is enhanced for detachment from cytoskeleton (96). Not only associating with cytoskeleton, certain cell surface receptors can stimulate cytoplasmic actin polymerization in Aplysia neurons (97,98). In fish keratocytes, traction forces are similar for both dorsal and ventral surfaces (99). Indeed, when extra load is applied to membrane receptors mediating traction forces, fibroblasts can reinforce or increase traction forces (100). For membrane proteins that do not mediate traction forces, optical tweezers can drag membrane proteins in the plane of the membrane, thus probing the cortical structure of the cytoskeleton (101–104). Clearly, different membrane proteins have different physiological roles and experience different forces. Optical tweezers will continue to help decipher these interactions.

Long-Term Prospects

In the last decade, studies incorporating optical approaches to measure biological forces have increased tremendously. For the most popular single-beam optical gradient trap, or optical tweezers, technological development seems to have stabilized. Because of its maturity, it is unlikely that there are any new technical capabilities that have not already been explored. Although subsequent development will largely focus on ease of use and robustness, such maturity bodes well for cell biologists. We have yet to saturate on applications for optical tweezers. However, addressing the limitations of optical tweezers, newer optical approaches to measuring biological forces are still being developed. Clearly, there is a ‘bright’ future.

References

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