

# Shining Light in Mechanobiology: Optical Tweezers, Scissors, and Beyond

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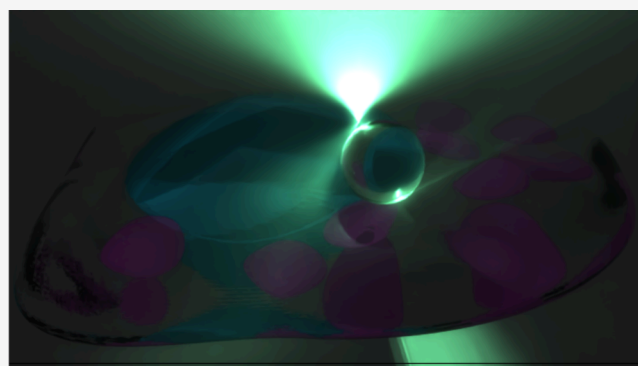
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**ABSTRACT:** Mechanobiology helps us to decipher cell and tissue functions by looking at changes in their mechanical properties that contribute to development, cell differentiation, physiology, and disease. Mechanobiology sits at the interface of biology, physics and engineering. One of the key technologies that enables characterization of properties of cells and tissue is microscopy. Combining microscopy with other quantitative measurement techniques such as optical tweezers and scissors, gives a very powerful tool for unraveling the intricacies of mechanobiology enabling measurement of forces, torques and displacements at play. We review the field of some light based studies of mechanobiology and optical detection of signal transduction ranging from optical micromanipulation—optical tweezers and scissors, advanced fluorescence techniques and optogenetics. In the current perspective paper, we concentrate our efforts on elucidating interesting measurements of forces, torques, positions, viscoelastic properties, and optogenetics inside and outside a cell attained when using structured light in combination with optical tweezers and scissors. We give perspective on the field concentrating on the use of structured light in imaging in combination with tweezers and scissors pointing out how novel developments in quantum imaging in combination with tweezers and scissors can bring to this fast growing field.

**KEYWORDS:** *Mechanobiology, optical tweezers, laser scissors, quantum light, cell biology*



The recent developments of optical or light technologies on the nano- and microscale have enabled unprecedented advances in our understanding of the life sciences. More specifically, the key advantage of optical tweezers, scissors, and structured light illumination over mechanical prodding, poking, and sorting is that they normally do not damage the fragile and soft structures that make up the cells of living organisms. There exist a large number of techniques that enable measurements of the biomechanics of cells and molecules. In this Perspective, we concentrate our attention on predominantly optical tweezers, and scissors, digital microscopy, as well as light-based neuroscience studies as applied in cells and molecules. The methods we discuss are predominantly light based as many innovative studies have benefited from the application of versatile optical fields and optical potentials. These can be created using structured light. We review the methods that enable creation of these types of optical fields and discuss some of their applications in in-depth studies of complex biological systems. In conclusion section of this Perspective, we give a brief account of perspectives of the field in future that can be used for further studies in mechanobiology.

## ■ INTRODUCTION TO OPTICAL MECHANOBIOLOGY

Cells sense and respond to external mechanical stimuli and internal forces generated by surrounding cells and the environment. It has been widely recognized that these processes are key drivers for physiological, developmental, and functional processes. Research into the mechanisms that govern these processes led to the birth of mechanobiology. Today, mechanobiology is a highly interdisciplinary field at the interface of biology, physics, material science, and bioengineering. The emphasis in this field is on understanding how cells sense and transduce mechanical forces. Mechanobiology also led to research that aimed at building an understanding of how changes in the mechanical properties of cells change or alter their behavior. Another question that the field tried to tackle

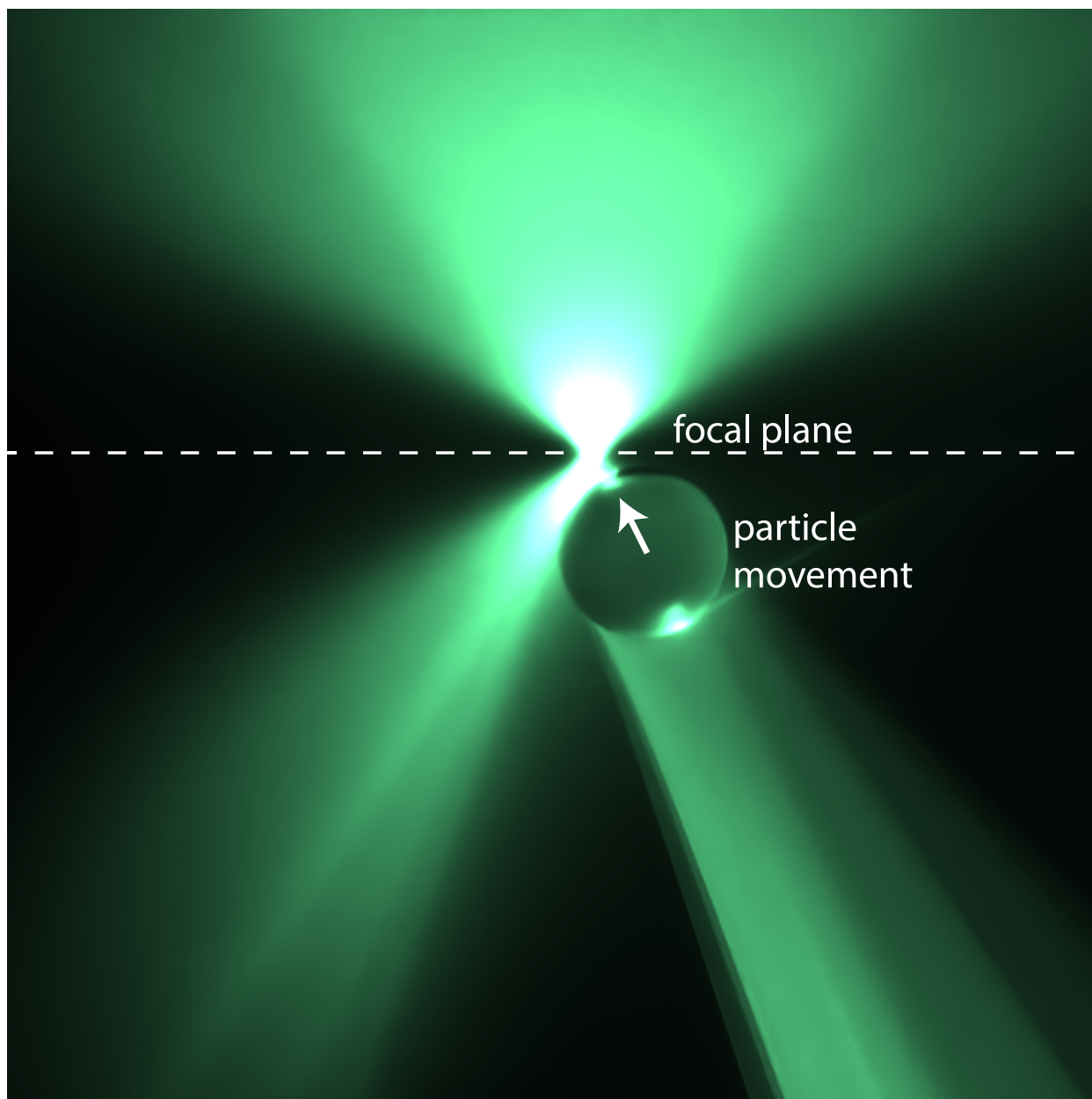
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**Figure 1.** Optical tweezers create an optical trap using the deflection and scattering of light by transparent glasses and plastics. In this visualization, light is focused to a point from the top of the image, a small glass particle deflects the light to the right. It correspondingly receives a force moving it to the left by conservation of the momentum of light. The light is more focused after refraction through the particle and so it also receives a force moving it up toward the focus.

was concerned with the influence that the forces acting on a cell or around it can have in altering the development of normal and unhealthy tissue and subsequent functioning of it.<sup>1–3</sup> In this Perspectives, we concentrate on optically based methods for studies in mechanobiology where we define optical microbiology as a study of the forces and their effects on biological systems when using optically based methods. Other-words we interrogate mechanobiology through probing light–matter interaction.

1976 saw the first publication that demonstrated that all cells have an internal molecular skeleton that is made of contractile actomyosin filaments (that was given the name “cytoskeleton”) which was an unprecedented development in cell biology.<sup>4</sup> A few years later, scientists were asking whether mechanical

forces play a significant role in cell function as biological regulators, rather than just relying on chemical cues.

In one of the earliest papers on mechanobiology by Wang et al. (1993)<sup>5</sup> the authors were studying mechanotransduction while the cell was exposed to mechanical stresses. The stresses were applied directly to the cell surface using a magnetic twisting device. They showed that cytoskeletal stiffness increased proportionally to the applied stress and that the transmembrane extracellular matrix receptor, integrin  $\beta 1$ , transferred mechanical signals to the cytoskeleton by a specific molecular pathway. So, it acted as a mechanoreceptor and transferred mechanical signals to the cytoskeleton. The final result of this paper was that mechanotransduction was mediated simultaneously at many locations inside the cell and happened through force-induced rearrangements within

the cytoskeleton. The field of mechanotransduction studies has extensively grown since then.<sup>6</sup> A vast range of quantitative tools have been developed where well-defined forces in well-defined geometries have been applied to study both to cells and processes within them. Those methods secured quantitative tools that enabled further developments in mechanobiology. One example of such studies is the use of a model system to study the behavior of cells exposed to deformation using confocal and histological imaging of fibroblasts.<sup>7</sup> Another very active area of study in mechanobiology is tissue fracture. It can be studied using a variety of experimental methods that can detect the motion and behavior of single-molecules. This has been carried out using atomic force microscopy (AFM), optical single-molecule force spectroscopy combined with optical tweezers, micropipette cell separation, shear test on cell pairs, or Microtensile test on cell pairs.<sup>8</sup> A further study of how mechanical stimuli are transmitted into biomechanical signals was demonstrated by Wang et al. (2005).<sup>70</sup> This study was done using mechanical stimulation provided by applying optical tweezers traction on fibronectin coated beads adhering to the cells and monitoring fluorescence resonance energy transfer signal. It made it possible to monitor mechanotransduction in live cells with spatiotemporal characterization and to establish that induced activation is a dynamic process that directs signals via the cytoskeleton to spatial destinations. This study was an important step in establishing new experimental techniques as powerful tools for studies of mechanobiology (we cover this work in more detail in section *Cell Signaling*). Further work looked at remodeling of cells' local extracellular matrix in response to mechanical and biomechanical properties of the fibrous environment.<sup>9</sup> The method employed in this study was based on using multi-axis optical tweezers active microrheology. It demonstrated that peri-cellular measurements are needed in order to understand better cellular mechanotransduction. A recent mini-review by Wang et al. (2022)<sup>10</sup> point out that noninvasive methods of measuring forces and torques acting on particular objects, such as optical tweezers, are very attractive as they do not otherwise mechanically disturb the system studied.

Optogenetics has an expanding role in mechanobiology. Optogenetics is commonly associated with the studies of neuronal signaling.<sup>11</sup> However, for example, it can elucidate the process of embryonic and adult stem cells exposure to diverse mechanical signals from the extracellular matrix (ECM) and enable a study of how these signals influence their fate.<sup>12</sup> Optogenetics in combination with Fluorescence Resonance Energy Transfer (FRET) helps to decipher the sensing of mechanical forces acting on cells and neurons. These mechanical stresses can be sensed by specialized force sensors located in the cell membrane or the intracellular compartments.<sup>13</sup> We show how structured light can be utilized in neurosciences, in particular in optogenetics for studies of neuronal networks.

Overall, new research developments in the field of light–matter interaction enable more detailed studies of important behaviors of live cells. In the current perspective paper, we concentrated our efforts on elucidating interesting measurements of forces, torques, positions, viscoelastic properties, and optogenetics inside and outside a cell attained when using structured light in combination with optical tweezers and scissors. We describe the principles of optical tweezers as a microscale ruler for studies of whole cell motility, as well as

microviscoelasticity inside living cells. We also describe the use of optical scissors for studies of chromosomal processes and forces acting during cell division. We present a discussion on the use of structured light for these types of studies as well as methods for creating a variety of structured light depending on their subsequent use. These are exemplified by studies of chromosomes when forces are applied to them during mitosis. We describe how these techniques can be used in mechanobiology to elucidate biomechanical processes such as chromosome movement on the mitotic spindle.

## ■ OPTICAL TWEEZERS AS A MICROSCALE RULER

Optical tweezers successfully probe biological systems ranging from single molecules<sup>14–16</sup> to whole cells and embryos.<sup>17–19</sup> They confine objects such as cells and microscopic dielectrics using minute (on the scale of pico- to nano-Newton scale) forces induced by highly focused light.<sup>20</sup> The momentum transfer to a solid or liquid is determined by the bulk refractive index. Transparent objects such as glass, plastic, biological membranes, and many proteins are readily controllable with light with wavelengths over several bands in the infrared which avoids damaging heating that comes with absorption.<sup>21–23</sup> When strongly diverging light passes through a transparent spherical particle it acts as a small lens and reduces the divergence of the scattered light to overcome radiation pressure from the transmission of light.<sup>20</sup> On the other side of the focus the opposite occurs—the particle increases the divergence of the light. On the near side of the focus, the particle is pushed forward and on the far side, it is pulled backward—in both cases moving toward the focus, producing the “optical tweezers effect” and an optical trap. When the particle moves in and out of the beam to the side the same kinds of refraction apply as shown in [Figure 1](#) producing an optical force that confines the particle.

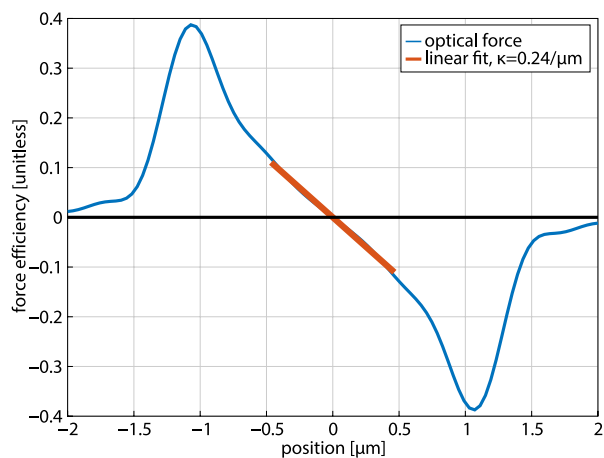
The transfer of light momentum to the particle changes linearly with small displacements. Thus, the system can be calibrated, enabling study and accurate measurements on a wide range of biological activity at the nano- and microscale.<sup>10</sup>

**Linear Model of Optical Trapping.** Characterizing the optical forces generated by optical traps and tweezers—turns the control supplied by the optical tweezers into a microscale force and position sensor. The amount of momentum transferred per second to an object by light (force) for some small displacement can be effectively modeled as a Hookean spring:

$$F(x) = F_0 - \kappa x \quad (1)$$

where  $F_0$  is some constant force (which is zero at equilibrium),  $\kappa$  is the stiffness of the optical trap (trapping strength), and  $x$  is the displacement such that  $F(0) = F_0$ . Over large displacements, this assumption breaks down because the optical trap is finite in extent.<sup>20,24,25</sup> A force–extension curve for optical tweezers trapping a polystyrene particle of radius 1  $\mu\text{m}$  in water is shown in [Figure 2](#) (generated using the Optical Tweezers Toolbox software package<sup>26</sup>). Near the equilibrium, a linear force response is a good model of the optical trap behavior. The maximum optical force occurs as the displacement from equilibrium approaches that of the width of the particle. At larger displacements, the particle emerges from the beam and the optical force becomes negligible.

**Calibration.** Quantitative measurement with optical tweezers has motivated the development of calibration techniques. Depending on the details of the problem, there



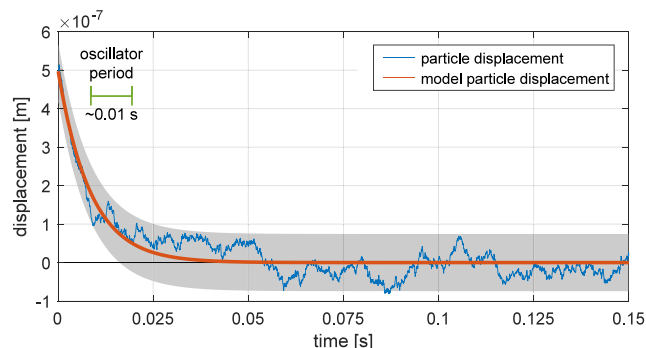
**Figure 2.** Optical force (dimensionless units) calculated for a  $1\ \mu\text{m}$  radius polystyrene particle trapped with  $\text{NA} = 1.2$  microscope objective in water using the Optical Tweezers Toolbox software package.<sup>26</sup> The equilibrium for the particle is at the center of the beam.

are several requirements: precision and bandwidth<sup>27–33</sup> and coverage of large displacements and particle shape.<sup>34–38</sup>

High measurement sensitivity and speed are needed to measure the force of single events on a microscopic scale. There are two commonly utilized schemes to measure forces with optical tweezers: microscopy and light deflection measurements. Microscopy techniques are good because they can be performed on multiple particles and regions of interest simultaneously. Light deflection measurements are high sensitivity and bandwidth, but they are limited to single particles.

The force and position of a particle in an optical trap are related. A quantitative optical tweezers measurement requires that a portion of the force–extension curve (Figure 2) is accurately characterized. The force efficiency as a function of position can be used to determine the force acting on a particle when the medium refractive index and laser power are known.<sup>24</sup> One way to go about converting the force to a position is to observe the behavior of the probe particle in the optical trap and compare it to a known ruler. The data obtained from scattered light contains statistical information from the Brownian motion of the particle and can be fit using a variation of maximum likelihood estimation in either the time or frequency series.<sup>39</sup> The sheer number of calibration methods is astounding and there is an overwhelming choice of analysis techniques for optical tweezers measurements.

No matter if a calibration scheme is in the time or frequency domain it is inevitably based on either passive or active observation of the probe particle. Passive observation will typically mean that the Brownian motion is used to characterize trap response which will be sufficient for small displacements. Active observation involves agitating or otherwise exciting the particle so that large excursions from the equilibrium can be measured. If all we're after is 10% accuracy in the trap stiffness a small number of measurements are needed, particularly for active measurements where the signal-to-noise ratio is large. Figure 3 shows position traces demonstrating the difference between the random motion of the particle on its way to the center of the optical trap and its fit to a model of exponential approach to the equilibrium position. As a particle enters the optical trap, the behavior of an



**Figure 3.** A particle moving into an optical trap from a large distance follows a characteristic exponential approach to the stable trap position in the absence of Brownian motion. The characteristic motion is that of an overdamped harmonic oscillator, with a characteristic time scale represented by the green bar. Over this time, one could expect the particle to move from one side of the optical trap to the other as the trap's force response is weak due to frequency band limits. The exponential approach behavior is hidden by Brownian motion near the equilibrium. The shaded region represents a  $\sim 95\%$  confidence interval of where the particle can be found in an exponential approach model centered at the exact starting position.

exponential approach to the stable trap position is apparent and measurable on a short time-scale<sup>33,40,41</sup> and thus the trap may be characterized quickly. This measurement may not be possible for various reasons ranging from sampling windows in detection, blurring, and limited ability to predict oncoming trapping events. Mechanical oscillation of the sample can be used to create a systematic shift from the trap equilibrium (and obtain a high signal-to-noise relative to passive observation) and thus quickly and accurately determine the trap strength.<sup>15,42</sup> A passive measurement relies on the Brownian motion to drive particle dynamics. These forces are often quite small and thus require long sampling times to overcome various sources of noise from the detectors used to perform the measurement. On the other hand, passive trap characterization requires mere observation of the particle, and in some schemes—with position sampled at random intervals. Under Brownian motion, only the thermal states of the trap are occupied. Over sample times much larger than the trapping period, a random sample of the positions of the particle will exhibit a normal distribution. The trap strength can thus be estimated using the distribution width,  $\langle \Delta x^2 \rangle \sim k_b T / \kappa$ , where  $\kappa$  is the trapping strength. These sorts of displacements are small and thus have poor signal-to-noise that can only be overcome by increasing sampling length, improving proportionally to the root number of samples, uncertainty  $\propto 1/\sqrt{n}$ . Where a reasonable sample rate in excess of the trapping oscillator frequency is possible, one of the more popular calibration techniques is power spectrum calibration which analyses the linear optical trap response in frequency space.<sup>15,29,32,42</sup>

For a comprehensive overview of many other calibrations that will accommodate various limitations in measurement capability, see Gieseler et al.<sup>39</sup>

**Modes of Particle Detection.** Optically trapped probe particles are visible in high-numerical-aperture microscopes and so measurements can be made using either camera,<sup>39,43,44</sup> or position sensitive detector,<sup>32,34,35,38</sup> and a variety of other measurements that are not covered here. These two modes of detection offer different capabilities. A camera is an excellent



means of tracking a field of particles,<sup>31</sup> but it will not deliver a good measurement when attempting to track a single particle at high precision over short time scales. The deflection of the trapping- or an ancillary probe laser beam can be detected at high accuracy and precision, but only for a single particle. The difference in capabilities arises from the means by which information is extracted. An illuminated sample imaged by a camera contains local information about each particle over a small number of pixels with light that is diffused over the entire sample. A single probe beam targets a single particle, and so all information about motion is contained in the deflected beam and this means that sample times can be short, even to a level far in excess of the trap frequency.<sup>32</sup> A camera, on the other hand, will need to capture for a significant amount of time before the signal-to-noise approaches an acceptable level to extract information about the particle position at a “single instant”. A single instant is not resolvable under such a scheme without also increasing the illumination level which could damage a biological sample. When particles accelerate over periods of time on the order of the exposure time the position of the particle is blurred. Blurring has to be accounted for to obtain valid calibration.<sup>33,44</sup> Thus, a measurement of position in a dynamic situation is not the true position and parameters such as trap stiffness must be corrected to account for this. Care must be taken in determining if the parallel tracking capability of camera detection is worth trading off the precision and accuracy afforded by detection with single probe beams.

## ■ APPLICATIONS OF OPTICAL TWEEZERS IN MICROBIOLOGY AND MECHANOBIOLOGY

**Molecular Scale Studies.** Optical tweezers have an excellent track record determining forces at a molecular scale.<sup>15,16</sup> The level of precision and accuracy that optical tweezers afford enables the determination and characterization of the forces of single molecule events such as the “hand-overhand” of kinesin stepping,<sup>45</sup> and the function of cadherin proteins,<sup>46</sup> and more recently to investigate the role of mechanical structures on gene expression,<sup>47</sup> and determine the free energy landscapes of Titin as it generated force,<sup>48</sup> naming just a few of the many studies performed on single molecules. Each one of the studies involves the characterization of the locomotion of proteins as they perform activities that are vital to the proper functioning of organisms. These studies demonstrate levels of sensitivity ranging from the pico-to-femto Newton scale on length scales down to a subnanometer.<sup>49</sup> In the case of a number of important molecules such as certain DNA fragments, and proteins, single-molecule studies that measure stepping and binding forces can be performed with off-the-shelf reagents. Optical tweezers can be moved within the sample potentially offering measurements over distances ranging from a few nanometers to over hundreds of micrometers with the appropriately actuated system.

Optically trapped probes, such as silica, latex, or even metallic nanoparticles are introduced in many optical tweezers studies. These particles are excellent probes in optical tweezers as they are simply characterized as shown in section [Calibration](#), and are readily functionalized using standard techniques, for example, amines or bioconjugate systems such as avidins, biotins, digoxigenin, and others.<sup>50</sup> The crucial step in any biomolecular measurement is the purification of the desired protein or other molecule—a step which will take the most time and effort in any novel experiment. Once a

functionalization scheme is perfected, a suite of apparatus can be utilized to extract exquisite quantitative and time-resolved information about single molecules using optical tweezers in both translational and rotational modes of measurement. For a comprehensive review of apparatus and techniques, see Bustamante et al.<sup>51</sup>

As optical tweezers are a physical technique at the core, the capability to measure subpiconewton forces on subnanometer scales depends not only on the quality of microscope components and detector sensitivity, it is also strongly affected by environmental noise such as air and floor vibration and electromagnetic interference. For experiments where piconewton and nanometer precision is desired then standard biological safety level 1/2 (often labeled as BSL-1/BSL-2) laboratories will be more than sufficient for routine and successful molecule-scale assays. High-accuracy measurements reaching levels of femtonewton and attometer precision are more difficult to accommodate as they require environments with low acoustic vibrations and electronic noise. In these cases, specially designed laboratories may be needed.

**Microtubules and Molecular Motors.** One of the areas we want to focus on are optical tweezers measurements of important biomolecules moving inside cells. Microtubules play key roles in the motility of whole cells (see next section on sperm motility) and of molecules and structures inside of cells. In this regard, they are fundamental to the movement of chromosomes on the mitotic and meiotic spindles. However, the precise molecular mechanism of how individual microtubules move an organelle is still unclear. The initial predominant theory was that as microtubules grow (polymerize) at one end they shorten at the other. In this model, the organelle (a chromosome for example) is attached to microtubules at its kinetochore (centromere) region. At the cell pole, the microtubules depolymerize (shorten) and thus pull the kinetochore-attached chromosome toward the pole.<sup>14</sup> However, this simplistic model of chromosome movement has been replaced with a “molecular motor” theory that relies upon forces from optical tweezers. In these studies, streptavidin-coated latex beads were conjugated to the molecule motor protein kinesin. It was possible to reversibly stop the translocation of single microtubules by single kinesin molecules by applying optical trapping forces to the kinesin-conjugated beads. The microtubules escaped from the optical trap when the trapping force was less than of 1.9 pN. This study established the use of optical traps to measure the force parameters of single intracellular molecules. Surprisingly, 1.9 pN is in the same range as the force pulling chromosomes on the mitotic/meiotic spindles. Current models of chromosome movement on the mitotic spindle also include a second motility generating molecule, dynein. In addition to interacting with individual microtubules as a motor protein, dynein also localizes to the kinetochore of the chromosome where the microtubules attach and where “checkpoint” proteins prevent the chromosomes from moving along the spindle. Apparently, a function of dynein is to remove the checkpoint proteins so the chromosomes can move toward the pole.<sup>52</sup> Thus, it appears that the mechanism to move chromosomes along the spindle utilizes two motor proteins that function in concert to generate the amount of force needed to translocate the chromosomes from the metaphase plate to its proper pole.

**Beyond Trapping to Measurements in the Ballistic Regime.** The work of Madsen et al. (2021)<sup>32</sup> is likely the highest bandwidth and sensitivity measurement (near the

theoretical quantum limit of measurement) of an optically trapped particle in a fluid on the  $2\ \mu\text{m}$  size scale. In this work, a single estimate of the viscosity of several fluids was obtained on the order of 2 ms using the thermalization of the velocity autocorrelation function. High-accuracy estimates were produced from an average around 10 such 2 ms measurements. The experiment used structured detection to improve the specificity with which the antisymmetric component of the scattered light wave function was detected. The trap stiffness was sufficiently low that it in effect provided only a low-frequency localization of the particle—the high-frequency free-ballistic-motion can be determined as if the optical trap were not there. This type of measurement is of particular importance when studying complex biological systems over ultra short time scales. This would be for example of particular interest during binding events.

**Cell Scale Studies.** The demonstration of whole cell control appears very shortly after the first demonstration of optical tweezers in Ashkin et al.<sup>17</sup> In this study, whole yeast and *E. coli* were moved in speeds of excess of  $100\ \mu\text{m s}^{-1}$ —a concrete demonstration of the use of optical tweezers to manipulate cells on the  $1\text{--}10\ \mu\text{m}$  scale. Computer-actuated tweezers control has also been applied to the manipulation of swimming cells in excess of the  $10\ \mu\text{m}$  scale using a novel optical configuration.<sup>53</sup> Computer-controlled optical tweezers/laser scissors (see section [Laser Scissors](#) as well) systems have been demonstrated to manipulate and optically inject bilipid membrane impermeable molecules into embryonic and other cells<sup>18,54</sup> which represent an outstanding development and technical achievement and are of great use for mechanobiology. Indeed, there have been many other advances that offer great flexibility and control on the cellular and subcellular levels. For example, optical tweezers have been used to measure mechanical properties within a mechanically strained cell.<sup>55,56</sup>

Cell sorting and identification is one of several successful applications of optical tweezers. In this mode of operation, light is weakly focused or otherwise shaped into a surface where cells can be either actively or passively transported within the sample.<sup>57–59</sup> An optical stretcher uses light emitted from opposing fibers to create a large area trap where video microscopy can be used to measure whole cell stiffness<sup>60</sup> with force differentials between 10s and 100s of piconewtons between one side of the cell and the other. Coupled with real-time analysis, such devices can be used to simultaneously measure the physical properties and sort cells.<sup>61</sup>

Characterization of motility of swimming cells have also been determined from optical tweezers measurements. The swimming efficiency of bacteria such as *Escherichia coli* has been determined to be about 2% using optical tweezers measurement by modeling the hydrodynamic interaction within the optical trap.<sup>62</sup> In this study, analysis of the trapping beam allowed for direct measurement of rotation rates of both the head and body of *E. coli* strains HCB30 and YK4516. An excellent demonstration of detailed measurement on single cells with parts moving at a rate of  $\sim 100\ \text{Hz}$ . Force measurements on swimming cells have been extended to three dimensions through the application of a specially designed optical filter on the transmitted trapping light.<sup>63</sup> Using this technique the tumbling and swimming motion phases of *E. coli* strain MC3400 showed a typical flagellar swimming force of about 0.53 piconewtons along the beam

propagation axis that would normally be inaccessible in optical tweezers experiments.

In the sections below we focus on two areas: Sperm and cell signaling.

**Sperm.** One of the most important factors in reproduction is the transmission of genetic material in zygotes—such as sperm cells. Optical tweezers have an excellent track record in measuring their biomechanics. Individual vertebrate sperm cells are highly motile. They have to navigate a significantly long distance in the female reproductive tract, and exert a burst of speed (and force) at the end of their journey to achieve successful fertilization.<sup>64</sup> An aspect of sperm motility that was not widely studied prior to the introduction of optical tweezers in the late 1980s was the swimming force. Optical tweezers have provided a significant way to measure sperm motility. In an early study, the swimming force of sperm was measured when they were exposed to an extract from crushed cells from the cumulus oophorus surrounding the unfertilized egg. Three motility patterns were studied: linear, hyperactivated, and cumulus-related. Mean escape power from a 760 nm optical trap for spermatozoa displaying linear motility was  $59 \pm 43\ \text{mW}$ , for hyperactivated motility  $122 \pm 67\ \text{mW}$  ( $P < 0.0001$ ) and for cumulus-related motility  $200 \pm 44\ \text{mW}$  ( $P < 0.0001$ ). This study showed that human spermatozoa generated more relative force upon exposure to the cumulus mass. The combination of small-amplitude lateral head displacement and higher relative force may produce a “drilling” effect which is synergistic with the enzymatic digestion of the cumulus matrix during the fertilization process.<sup>65</sup> Another study combined optical tweezers with laser scissors to achieve fertilization from a dilute preparation of cattle sperm. In this study, optical tweezers were used to trap the sperm and move them to a laser scissors drilled hole in the surface of the ovum. Successful fertilization occurred when at least 3–5 sperm were trapped and inserted directly through the laser-perforated egg.<sup>66</sup> The ability to measure swimming force in sperm led to a series of studies related to their evolution and physiologic energetics. The evolutionary hypothesis being tested was that sperm from primates where females mate with only one male (such as gorillas) as opposed to females from species where the females mated with many different male partners, such as chimpanzees and Rhesus macaque, should swim faster and with more force than sperm from the single partner species. The rationale being that in the multipartner species sperm competition is greater, thus resulting in the evolution of faster and stronger swimming sperm. Structurally, the midpieces (the structure that has a large concentration of the ATP-generating mitochondria in sperm) were larger in sperm from multipartner species than in single partner species.<sup>67</sup> This is consistent with higher motility in the multipartner species. Despite the complexity of obtaining sperm from the different species, the hypothesis was confirmed: regression equations for VCL (curvilinear swimming velocity) and  $F_{\text{esc}}$  (escape force from the trap) were found to be statistically different for all four primates ( $p < 0.05$ ) (Figure 2 from Nascimento et al. 2008<sup>68</sup>). Interestingly, human sperm fell in between the fast and slow swimming sperm, suggesting a sociological component in human sperm evolution. The figure in that publication overlaps the plots of  $F_{\text{esc}}$  (pN) versus VCL (mm sK1) for each primate species analyzed. This figure reflects the range in sperm competition, showing that as the level of competition increases, the swimming speed distribution

increases to faster velocities and the escape force distribution increase to stronger swimming forces.

The ability to track and trap sperm in real time led to further studies on sperm physiology, specifically on sperm energetics. The combination of optical tweezers, fluorescent imaging, and real-time automated tracking and trapping (RATTS) can measure sperm swimming speed and swimming force simultaneously with mitochondrial membrane potential (MMP). This approach was used to study the roles of the two sources of ATP in sperm motility: oxidative phosphorylation, which occurs in the mitochondria located in the sperm midpiece and glycolysis, which occurs along the length of the sperm tail (flagellum).<sup>69</sup> The prominent role of the glycolytic pathway in supplying the ATP for sperm motility has caused a major reassessment of sperm energetics.

**Cell Signaling.** The combination of Forster resonance energy transfer (FRET) biosensors with optical tweezers has allowed us to visualize mechanotransduction in cells.<sup>70</sup> A optical trap was used to apply a small amount of force (300 pN) to 8  $\mu\text{m}$  diameter beads coated with an extracellular matrix protein (ECM) fibronectin. This allows the engagement of the beads to the integrin receptors on the surface of cells expressing a FRET biosensor whose conformational change is triggered by Src kinase (Src) activity. As such, the force imposed by the optical trap on the beads can be physically transmitted to the cells, triggering molecular reactions inside the cells which can be reported by the biosensors change in emission. Src kinase activity and ATP will cause a drop in FRET to occur and thus an increase in the emission from the acceptor fluorochrome. Pulling on the beads resulted in an alternating wave of strong and weak Src kinase activity across the cell membrane away from the force-pulling site, providing a real-time view of a cell sensing an external force and communicating this to other areas of the cell. When the experiment was modified such that the cells were treated with drugs to disrupt the actin and microtubule cytoskeleton, the FRET propagation wave was abolished. The ability to view internal cellular molecular dynamics in real time, has opened a whole new vista of experimental biology.

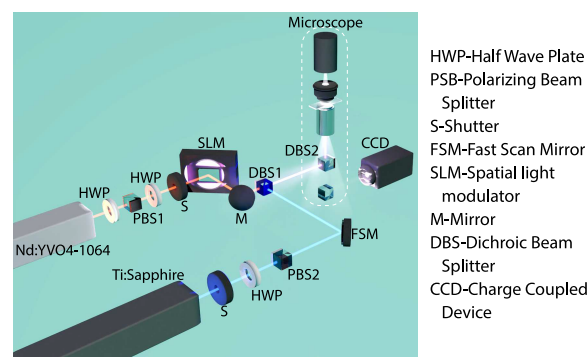
In a recent paper by Sergides et al.,<sup>71</sup> the authors discuss the need for a combination of several techniques for the study of mechanotransduction in living cells that as mentioned earlier involves the measurement of force in multiple cell locations with molecular specificity, as well as the simultaneous imaging of biochemical and genetic signals transduced by the cell. In order to achieve that they device a system that is a combination of optical tweezers, FRET-based molecular tension microscopy and fluorescence imaging with single-molecule sensitivity. They demonstrate the usefulness of this system when the cell is mechanically stimulated and the imaging of the propagation of mechanical and biochemical signals inside the cell is carried out simultaneously. This system should be very useful for future studies on the transmission of mechanical forces from the outer cell membrane to the cell's cytoskeleton and nucleus. It will also help to understand the transduction of the forces into other types of signals and cell responses, resulting in a deeper understanding of mechanisms involved in mechanotransduction in living systems. Mohammed et al.<sup>72</sup> points out that in order to understand molecular mechanisms that are involved in outside-in and inside-out mechanotransduction signaling pathways we need to develop integrated strategies that combine super-resolution fluorescence with biophysical probes and multipatterning of

proteins as well as adding FRET biosensors that could be used to examine force transmission across the cytoskeleton to nuclear envelope proteins, chromatin remodeling or mechanically induced changes within the nucleus.

## LASER SCISSORS

Laser scissors provide a vast contribution to the studies of cells functions in cell differentiation under different condition. They can provide both physical and genetic knockout of biological function that impacts mechanobiology. The application of laser scissors are summarized in several excellent foundational articles, reviews, and books.<sup>73–77</sup>

In the 1990s two-photon microscopes were commercially available, and a large number were sold for multiphoton imaging, the same systems could be used for scissors-ablation studies, thus expanding the use of laser scissors. A Ti:sapphire pulsed laser was used for multiphoton ablation of cells and organelles.<sup>78</sup> Figure 4 shows a combined trapping and scissors system capable of trapping at multiple points with the infrared laser and performing steerable ablation using the femtosecond pulsed laser.<sup>79</sup>



**Figure 4.** Visualization of setup for multipoint trapping and rapid scanning ablation of biological samples, modeled on the system used in Ono et al.<sup>79</sup> The system uses a continuous wave infrared laser for trapping that can trap objects at multiple points with light shaped by an SLM (see section [Spatial Light Modulators \(SLMs\)](#)). A femtosecond pulsed Ti:sapphire laser is used for the ablation/microsurgery of cells. A fast scan mirror allows the high-intensity pulsed beam to be precisely positioned within the sample.

### Applications. Gene Inactivation and Manipulation.

Laser scissors can be used for genetic manipulation and control of genetic expression. The first example of this application can be seen in the work of Berns et al. which showed that they could target the nucleolar organizer rDNA (rDNA) region of a chromosome in early prophase.<sup>80,81</sup> This resulted in gene inactivation of that region and in the daughter cells (of the irradiated cells) which displayed intact (not truncated) chromosomes.<sup>80,81</sup> These studies demonstrated the utility of laser scissors toward genetic manipulation. When the short pulse lasers in the nanosecond and picosecond regime became available, gene and chromosomal ablation were adopted by other research groups.<sup>75,82,83</sup> The mechanisms of ablation were shown to be via multiphoton inactivation and/or plasma-generated ablation.<sup>84,85</sup> The replication of the irradiated chromosome with its normal morphology was perplexing at the time. However, recent laser studies showed that mitotic chromosomes are not only capable of initiating DNA repair but that full repair may occur of mitotic DNA damage since



cells expected to carry damage were able to undergo replication, a process that does not occur in the presence of DNA damage.<sup>86</sup>

Current studies on genetic manipulation have focused primarily on the use of lasers for microdissection and genetic sequencing of chromosome fragments. Such investigations are critical toward the understanding of functions related to whole chromosome regions which may not be captured using other methods.<sup>87,88</sup> Furthermore, the use of scissors and tweezers for genetic manipulation was recently demonstrated to be useful in performing chromosome rearrangements in a technique called chromosome welding.<sup>89</sup>

**DNA Damage and Repair.** Mutations in DNA repair mechanisms have been shown to lead to developmental disorders, accelerated aging and cancer.<sup>90</sup> Therefore, it is of great interest to study and understand how the cell handles damaged DNA. Microscope-based laser scissors have been an excellent tool in elucidating DNA repair responses in both interphase and mitotic cells as it allows for spatiotemporal control of damage production at the submicron level. This gives researchers the ability to monitor recruitment kinetics and localization of repair proteins when combined with fluorescently tagged proteins. When coupled with optical tweezers, the laser nanosurgery microscope provides even greater possibilities for trapping and cutting subcellular organelles in order to elucidate structure and function.<sup>91</sup>

In this section, we review research findings on DNA repair following subcellular photonic damage and put them in the context of other methods used. We demonstrate that laser nanosurgery is a unique tool which permits greater elucidation of DNA damage responses which may affect the way in which cells undergo cytokinesis. This process involves the coordinated action of various proteins and mechanical forces.

Ionization radiation has been a common method of DNA damage induction for DNA double strand break repair studies. Following damage, several DNA damage repair factors form foci which are termed ionization radiation induced foci (IRIF). However, the visualization of IRIF is dependent on a protein clustering step which differs from the initial recruitment.<sup>92–94</sup> Kong et al. showed that the green and NIR lasers are suitable for the detection of double strand break factors that do not form IRIF.<sup>94</sup> Furthermore, studies using the focused laser have allowed for the detection of repair proteins on damaged mitotic chromosomes which have been previously shown to be absent when damage was elicited with ionization radiation.<sup>86</sup> This is likely due to the ability of the laser to produce a submicron region of DNA damage which permits the visualization of factors which were likely below the detection limit in the previous studies. Serial sections of a chromosome damaged by the Ti:sapphire laser suggested that a volume of  $0.65 \mu\text{m}^3$  was damaged.<sup>95</sup> Human chromosomes have been estimated to have a density of  $16 \text{ Mbp} / \mu\text{m}^3$ .<sup>96</sup> Thus,  $\sim 10 \text{ Mbp}$  were affected by the laser. This is significantly larger than that found in an IRIF where a single foci may consist of a single DNA double strand break.<sup>97</sup>

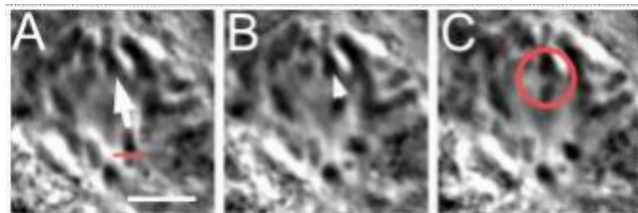
Several studies using ionization radiation or radiomimetic drugs led to results which suggested that DNA repair in mitosis is repressed or truncated. Nevertheless, it was recently shown that mitotic cells are capable of DNA repair synthesis when damage is elicited in metaphase with a NIR femtosecond laser or UV light.<sup>86</sup> This is significant given that the prevailing view is that damage is marked in mitosis for repair in later phases of the cell cycle. These results demonstrate that the mitotic DNA

repair response is more complex than previously thought and not all damage is repaired in later phases. Furthermore, these results demonstrate the need for further analysis of mitotic DNA damage responses using laser nanosurgery and other methods.

Laser scissors can be used to target a specific region of the chromosome such as the telomere, or the nucleolar organizer region to study the DNA damage responses.<sup>98–101</sup> The telomere has been demonstrated to have an important role in aging as its shortening can lead to cell senescence or cell death. Baker et al. investigated the response to DNA damage of chromosome tips (telomere containing region) after anaphase onset and compared it to damage created in other parts of the chromosome.<sup>102</sup> They found that damage to telomeric regions leads to a delay in cell division/cytokinesis in 71% of cells. Later experiments investigated the responses to damaged telomeric regions during metaphase. A delay was once again identified and activation of the DNA repair response was confirmed suggesting that a DNA damage-mediated checkpoint may have occurred.<sup>101,103</sup> Furthermore, differences in protein recruitment were observed at the telomeric region when compared to chromosome arms. These results provide tantalizing evidence suggesting a role of telomeres and associated DNA repair in the fundamental biomechanical process of cell division.

## ■ LASER MICROSURGERY COMBINED WITH OPTICAL TWEEZERS

The combination of laser microsurgery with optical tweezers has proved invaluable in the probing of recently discovered intertelomeric tethers which have been shown to impart forces on chromosomes during anaphase in a variety of animal cells including those from humans.<sup>104</sup> Tether forces are in addition to the normal poleward forces exerted by kinetochore-attached microtubules. Tethers occur between the tips of sister chromosomes and functional assays of the tethers consist of using laser microsurgery to cut a chromosome tip and monitor it as it moves away from its pole toward its sister partner.<sup>104–106</sup> Figure 5 demonstrates a chromosome arm



**Figure 5.** Anaphase intertelomeric tethers result in the pulling of chromosome fragments toward sister chromatids. (A) A red line indicates the region targeted by the laser and an arrow indicates the direction that the cut chromosome will move and the tip of the sister chromatid. Scale bar = 5 microns. (B) 20 s after irradiation the chromosome fragment has moved toward its sister. (C) After 45 s the fragment has reached its partner.

that is cut and moves toward its partner. These studies have determined that the tethers are elastic and that the elasticity is dependent on phosphorylation.<sup>107,108</sup> Additionally, Forer et al. have found that as the tethers elongate and the cell progresses through anaphase, the tethers lose elasticity.<sup>104,105</sup> When optical tweezers were used to trap a fragment tip that was created with laser scissors, the force that the tethers imparted



on its partner was found to be  $\sim 1.5$  pN.<sup>79</sup> The function of the tethers is not completely understood. However, it has been shown that when kinetochore microtubules and tethers were cut during anaphase, the chromosome movements to the respective poles sped up, suggesting that the tethers may act to regulate the speed of chromosome movement.<sup>106</sup> This study is reminiscent of studies by Aist and Berns<sup>109</sup> on the fungus *Fusarium*. This organism has prominent bands of microtubules extending between the separating nuclei that were assumed to exert pushing force to separate the two nuclei. However, when the band of microtubules was severed by laser ablation, the separation speed of the two nuclei sped up to  $22 \mu\text{m min}^{-1}$  as opposed to  $7 \mu\text{m min}^{-1}$  of cells with unsevered microtubule bundles. These experiments required a major rethinking of the nature of the forces in chromosome movement in fungi, higher plants, and animal cells. The composition of tethers is not known. However, DNA and microtubules have been ruled out.<sup>110</sup>

In addition to tethers, other forces have been identified to act on chromosomes during anaphase which do not require the classic kinetochore to pole microtubule attachments. Studies using lasers to cut kinetochore fibers connecting to the poles demonstrated that sister kinetochores can move away from each other through the sliding action of bridging fibers. These are microtubule fibers that connect sister kinetochores to each other.<sup>111</sup> Therefore, a more complex model for chromosomal segregation during anaphase is beginning to form where chromosome segregation is coordinated by various mechanisms involving more than the classically accepted model which involves the attachment of kinetochores to the pole via microtubules.

#### Forces During Cell Division (Mitosis and Meiosis).

One of the most perplexing and unresolved questions in cell biology is “how much force does it take to move a chromosome on the mitotic/meiotic spindle.” This is an important question because all eukaryotic cells have a microtubule based spindle that plays a key role in the cell division process, and aberrations in the process are a major cause of birth defects, cancer, and a host of other diseases. The difficulty of measuring what must be small forces inside a cell is significant. The only experimental measurement (preoptical tweezers) was conducted by R. B. Nicklas in 1983<sup>112</sup> in which he used a very fine needle to skewer individual chromosomes on the meiotic spindle of the grasshopper spermatocyte. By quantifying the bend in the needle exerted by the microtubule attachment to move the chromosome, a value of 700 pN was obtained. Optical tweezers based studies demonstrated that the force was orders of magnitude smaller. In studies on large mitotic chromosomes of the salamander *Taricha* cells, 30 pN was needed to move a single chromosome fragment cut with laser scissors,<sup>113</sup> and in cells from the marsupial *Potorous* only 4 pN of trapping force was needed to stop the anaphase movement of chromosomes.<sup>114</sup> In a novel series of studies using an optical trap in meiotic spindles of crane-fly and flat-worm spermatocytes, the trapping force to stop chromosome movement were 2–3 and 6–10 pN, respectively.<sup>115</sup> When the force was removed, the chromosomes continued to move at the same rate as pretweezers.<sup>115</sup> Not only did this array of experiments on diverse organisms determine the forces during cell division, but the amount of force was only a few pN, 2 orders of magnitude less than the 1983 experiments posited. The lower force values determined with optical tweezers are consistent with the theoretical calculation of forces based on

elasticity and viscosity in a diverse group of organisms ranging from 0.06 pN for chromosome movement in water lily *Haemanthus* to 20 pN in the salamander cells comparable to those used in Liang et al. (1991).<sup>114</sup> A picture of intertelomeric tethers pulling chromosome fragments are shown in Figure 5.

## STRUCTURED LIGHT IN BIOSYSTEMS

Structured light beams with their distinct spatial or spatiotemporal properties have become a particularly useful addition to the arsenal of biophotonic techniques which can be brought to bear on problems in mechanobiology. The use of light as a stimulation tool allows the targeting of single cells or communities of cells in a spatially and temporally precise way. This offers an opportunity to disrupt both cellular networks and subcellular organelles. As the ability to create structured light fields in 2D, 3D,<sup>116,117</sup> and in time,<sup>118</sup> has become increasingly available, so too has the demand increased for this technology. Though many examples of this rely on the generation of images or patterns in a particular plane, beam shapes with more complex beam profiles have also found use in biophysics experiments. Examples of this are Bessel<sup>119</sup> and Airy<sup>120</sup> beams, among others. In biophysics experiments, Bessel and Airy beams have proven indispensable for tasks like noninvasive imaging, cell trapping, and the study of intricate cellular dynamics.

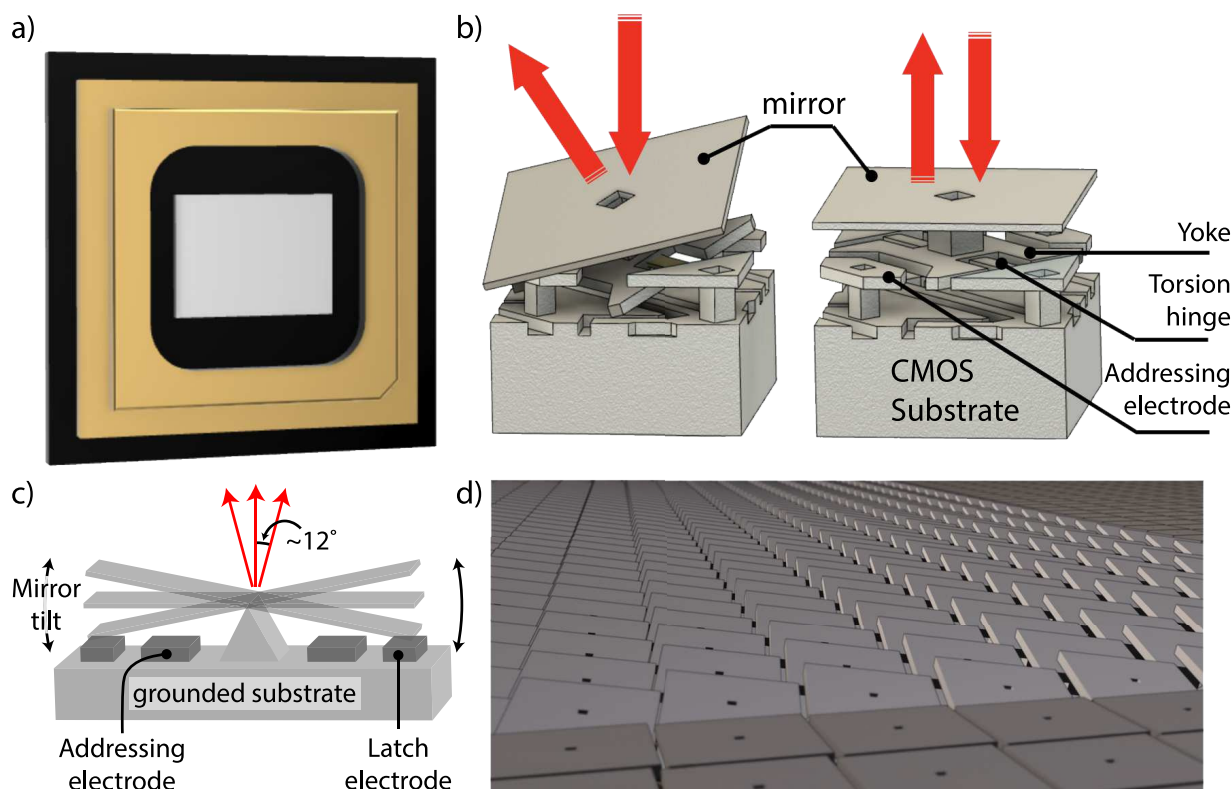
To generate suitable beams for mechanobiology applications, a variety of new hardware is increasingly being utilized. These devices which are capable of generating structured beams range from newly developed meta-materials to commercial devices such as DMD projectors. This has created an interesting reciprocal relationship between the commercial and research spaces which serves to drive the field forward. Here, we will discuss some of the techniques and technology currently being employed.

**Spatial Light Modulators (SLMs).** Spatial light modulators (SLMs) are computer-controlled devices that enable the user to interact with the optical characteristics of a light beam via computer. At the most basic level, SLMs allow the user to adjust either the phase, intensity, or polarization of a light field. SLMs have proven to be useful in a variety of applications, including, fluorescence imaging,<sup>121</sup> interference microscopy,<sup>122,123</sup> neurobiology,<sup>124,125</sup> stem cell research,<sup>126</sup> optical tweezers,<sup>127–129</sup> among others.

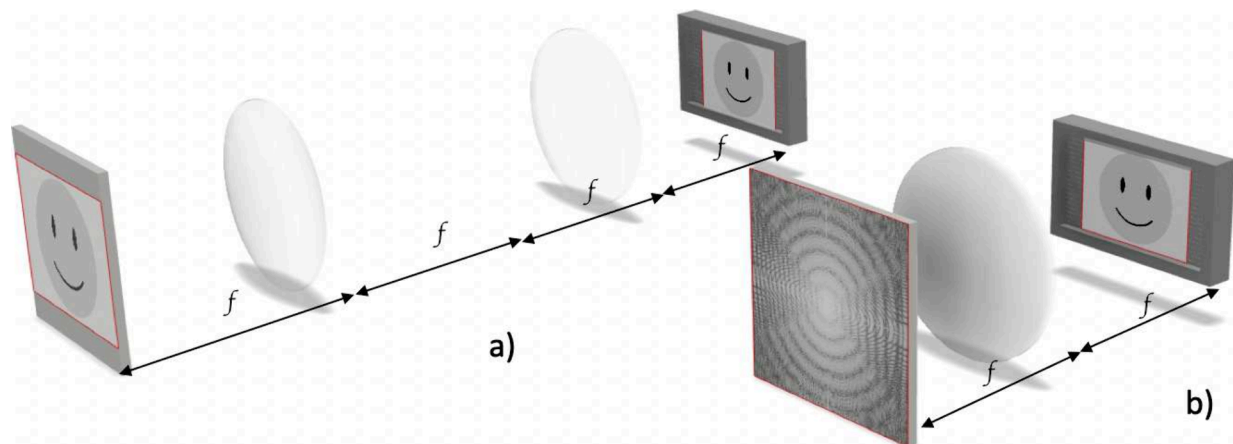
SLMs with a variety of addressing and modulation technologies have been created over the years. Though early systems which were based on projector technology focused on amplitude modulation,<sup>130</sup> phase-only modulation has become the most dominant variety.<sup>131</sup> This is mainly due to considerations of optical efficiency.<sup>132</sup> It should also be noted that amplitude modulation can be achieved even with a phase-only SLM.<sup>133</sup>

Phase modulation of light can be created by several electro-optic processes but the majority of SLMs rely on applying electric fields to liquid crystal cells contained between optical flats. These devices thus work as window or mirror depending on whether they are transmissible or not.

In recent years substantial improvements have been made in electrically addressed devices, which have shown significant technical improvements in aliasing, update speed, pixel count and diffraction efficiency. This improvement has led to an increased usage of SLMs in many imaging and light-shaping applications. Most recently developmental metamaterial



**Figure 6.** DMDs consist of arrays of tilting mirrors: (a) DMD micromirror device; (b) individual micromirrors showing actuation mechanism; (c) deflection electrodes are addressed by a CMOS chip enabling electrostatic attraction of the mirror and tilting of incident light; and (d) tilting multiple cells at once contribute to especially varying optical flux.



**Figure 7.** Two common modes of projection used by SLMs and DMDs. (a) Projection of modulator (the object plane) to the imaging plane. (b) Projection from the object to conjugate image plane (a.k.a. Fourier projection).

devices have also become more popular leading to the promise of GHz refresh rates.<sup>134,135</sup>

**Digital Micromirror Devices (DMDs).** Micro-electro-mechanical systems (MEMS) have become a staple of many modern light projection systems and are thus excellent platforms for flexible light delivery into a wide variety of optical systems. Their advantage over SLMs is their high-speed—capable of display updates from subsecond to microsecond rates. Their ability to manipulate the amplitude of light at extremely high speeds is crucial for various applications in biology. In the study of dynamic cellular processes, for instance. However, this comes at a cost, they can

only manipulate the amplitude of light without using the principles of Fourier optics. A schematic of the general structure of the device can be seen in Figure 6. High speeds are possible through the use of electronically microactuated mirrors using the electromechanical field effects.

**Physical Principles—Working with Modulators.** The exact physical setup of structured light systems is of course highly dependent on the application. The type of projection that the devices perform are either in standard imaging where the object plane (of the modulator) is imaged at the focus of an optical system or the Fourier plane is imaged. These imaging schemes can be seen in Figure 7. Figure 7a represents

imaging of the beam shaping device, and Figure 7ab is a Fourier projection mode of imaging. In this mode of operation, the modulator itself is not imaged directly but rather the Fourier Transform of the modulator. This may seem unintuitive but when well applied it has several advantages. The creation of a small spot near the center of the focal plane can be made with almost all the energy incident on the modulator at resolutions far in excess of the pixels (elements) of the device used. This is particularly useful in applications that require comparatively high beam powers such as optical tweezers (some early examples of the technique can be seen in refs 136 and 137) or laser scissors—which as we have seen throughout this review have many applications to the study of mechanobiology (sections: [Molecular Scale Studies](#), [Cell Scale Studies](#), and [Laser Scissors](#)).

The use of computer controlled diffractive optical elements is powerful and can manifest as the ability to move optical traps in beam foci at nanometer resolutions for precise placement of probes.<sup>138</sup> This level of precision is invaluable in biological applications, such as single-molecule studies and cellular biomechanics research, where researchers need to precisely interact with biological structures at the nanoscale. One drawback though is that the intensity of such patterns produced in the Fourier plane is typically modulated by a Sinc function produced as a consequence of the boxcar function associated with the SLM.<sup>139</sup> However, if light is sacrificed this can be mitigated by multiplicative masking.<sup>140</sup>

**Structured Light in Biology.** With the advent of fluorescence microscopy, structured light has been useful in improving the resolution of imaging systems. One obvious example of this is the development of stimulated emission depletion microscopy (STED),<sup>141</sup> which uses a vortex beam to create a reduced excitation volume at the focus of a scanning beam. The emitted fluorescence signal then can be used to produce a high-resolution image. The understanding that optical vortices can be used for super-resolution imaging is not limited to STED microscopy. Label-free technologies such as plain vortex imaging have also been studied. Specifically, a variety of imaging systems have been developed by several authors.<sup>142,143</sup> Light shaping of beams has also been used to improve other types of imaging. Airy beams have been used as an alternative to light-sheet imaging giving enhanced axial resolution.<sup>120</sup> Spatial control of wavefronts can modify the temporal or spatiotemporal characteristics of light beams. This can be particularly useful for shaping beams such as in multiphoton microscopy.<sup>144</sup>

Structured light is ideal for profiling the geometry and topography of object in the optical domain. Measurements of reflective particles can be performed using a geometric pattern (often composed of lines or spots) that is projected onto them. The deformation of the pattern is measured via a camera and the three-dimensional geometry is inferred from the pattern deformation. Several variations of this technique exist, including color modulation, phase shifting, and coded patterns.<sup>145</sup> In microscopic systems, these techniques can still be employed at scales close to the diffraction limit.<sup>146</sup> However, when objects are transparent to light, other methodologies must be employed.<sup>147</sup> The diffraction limit of the optical system limits the resolution of the images produced by these techniques. One answer to this is structured illumination microscopy (SIM),<sup>148–150</sup> which applies periodic illumination patterns to a sample to utilize an increased frequency space and thereby improve image resolution.

Further, DMDs and SLMs can be used to improve image clarity by enhancing contrast or engineering the point spread function of a microscopic system.<sup>151</sup>

Beam shaping and structured light based techniques are particularly important for biological samples such as cells which often absorb light weakly and cannot be seen well in conventional bright field imaging. By imaging phase changes or polarization changes in light passing through a cell rather than intensity changes, image enhancement can often be achieved. Quantitative phase microscopy utilizes interference to make measurements of phase changes in the light transmitted through microscopic objects such as cells.<sup>147,152</sup> Digital holographic microscopy uses a similar approach, interfering a coherent reference beam with the laser light transmitted through the sample. Spatial light modulators can be employed in this context to produce known phase shifts between the beams, enabling phase recovery from a series of 3 or more interference images.

Careful design of optical system projecting structured light with retro-reflection can enable manipulations, levitation, and movements of large cells.<sup>53</sup> Other related techniques can be used to greatly increase optical trapping strength by choosing ideal phase patterns to transform the optical potential restraining dielectric particles<sup>153</sup>—which could potentially be used on cells—without retro-reflection. Recently, impressive demonstrations of precise positioning and rotation of groups of cells using feedback to control a structured optical potential has been demonstrated.<sup>154</sup>

## ■ LIGHT-BASED NEUROSCIENCE

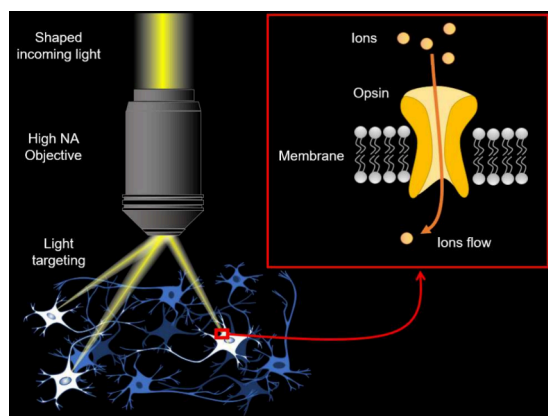
Innovation in light-based technologies has recently made it possible to observe the neural mechanical properties, behavior, and growth of neurons as well as neuronal networks across small intact brains (e.g.: mice, *Drosophila*, zebrafish, *C. elegans*) while perception and behavior occur. These new capabilities have opened doors to an unprecedented description of neural communication pathways, growth, and dynamics, as well as brain-wide sensory systems. Optogenetics has an expanding role in mechanobiology. It can be used to investigate mechanical signals from the extracellular matrix (ECM) and it influences adult stem cells.<sup>12</sup> Optogenetics techniques can help to decipher the sensing of mechanical forces acting on cells and neurons. These mechanical stresses can be sensed by specialized force sensors located in the cell membrane or the intracellular compartments.<sup>13</sup>

**Foundation of Optogenetics in Mechanobiology.** A wide range of new optical technologies, exploiting the very nature of light, have been integrated to conventional microscopes reaching unprecedented flexibility, precision, and depth for the visualization of neuronal processes and networks as described in a previous section.

One quintessential example in neuroscience is optogenetics.<sup>11</sup> Optogenetics is a combination of optics and genetics, where the presence of a specific wavelength of light on a genetically modified neuron expressing light-sensitive proteins, can force its stimulation or silencing. Genetically, the neurons' DNA is modified to express on its membrane light-sensitive ion gated channels, also called opsins. Its exposure to a specific wavelength of light would open or close the ion gated channels, forcing the build-up or decrease of an action potential and therefore, forcing the triggering or silencing of a signal. While original optogenetics systems based their light delivery method on holographic methods originally used in



optical tweezers, considerable advances have now been made in developing new technologies and adapting them to studies of the brain, in particular since the combination of two-photon excitation (2P) lasers and SLMs for holographic optogenetics.<sup>155</sup> Using spatial light modulators one can generate holograms and target very specific individual isolated neurons as well as a sequence of individual neurons as part of a network or a cluster (ensembles of neurons firing together) (Figure 8).



**Figure 8.** Illustration of light targeting and optical manipulation of neurons through opsins and laser light.

Constant effort is placed in the development of new opsins, in particular in the engineering of their sensitivity, signal-to-noise performance, and spectral characteristic.<sup>156,157</sup> In optics, state-of-the-art optical devices able to alter the laser beam wavefront and correct for scattering in biological environments with high flexibility have allowed great advances in the specific targeting of neurons, specific parts of axonal branches and axon terminals.<sup>158,159</sup> These technologies include DMD, SLMs, optical fiber, and adaptive optics (see discussion of these methods in section **Structured Light in Biosystems**).

The technologies described above offer the ability to target arbitrary combinations of neurons *in vivo*. This field of view for these illuminations can range from brain-wide in small models such as zebrafish, *Drosophila*, and *Caenorhabditis elegans*, to a single brain region or subset of neurons in larger models such as mice or rats. In all cases, however, it allows a significant number of possible stimulation patterns during experimentation: neuronal recordings or behavioral assays. Understandably, the power of such a tool is unprecedented and a careful consideration of the arbitrary combinations of neurons to be perturbed would be of great value for these studies. The stimulation of one neuron at a time is the simplest approach and is useful to determine single neuron necessity and functional interactions within a network. In practice, the effects of most single neurons are small or unlikely to generate rich downstream neuronal dynamics; therefore, careful consideration of the experimental method (e.g., multiple stimulation), neuronal structures targeted, and their projections (embedded network and its role) is necessary. Some examples are discussed in the following.

Interestingly, recent studies have shown that between 2 and 20 neurons chosen on the basis of their physiological responses could influence behavior and, therefore, significantly disturb the network. In some of these studies, the authors targeted neurons on the basis of their shared response to a sensory or cognitive feature (for example, the orientation of a grating or

the direction of a cued action).<sup>160–162</sup> In one study, computational analysis of the physiological data identified specific neurons that appeared to have a very high degree of functional coupling with many other neurons (putative “hub-like” or “pattern completion” neurons).<sup>163</sup> In another study, the authors used a process-of-elimination strategy to identify the minimal ensemble that could still elicit behavior (in this case, tail bending in zebrafish).<sup>164</sup>

Further and more complex analysis of the network involves the introduction of neuronal activity feedback-loop and by extension closed-loop holographic optogenetics,<sup>165–167</sup> where the neuronal activity is acquired and determine (based on the conditions set by the user) the following optogenetics stimulation. These technological advancements could now allow testing hypotheses such as how connections between neurons elicit behavior, and more broadly, how neurons in a network lead to population dynamics.

**Effects of Laser Scissors in Neuroscience.** Beyond the generation of targeted illumination, laser beams can be used as laser scissors<sup>91</sup> or precise ablation tools for various medical applications<sup>168–170</sup> and in particular the regeneration of various components of the nervous system such as axons<sup>171,172</sup> or neuromasts.<sup>173</sup>

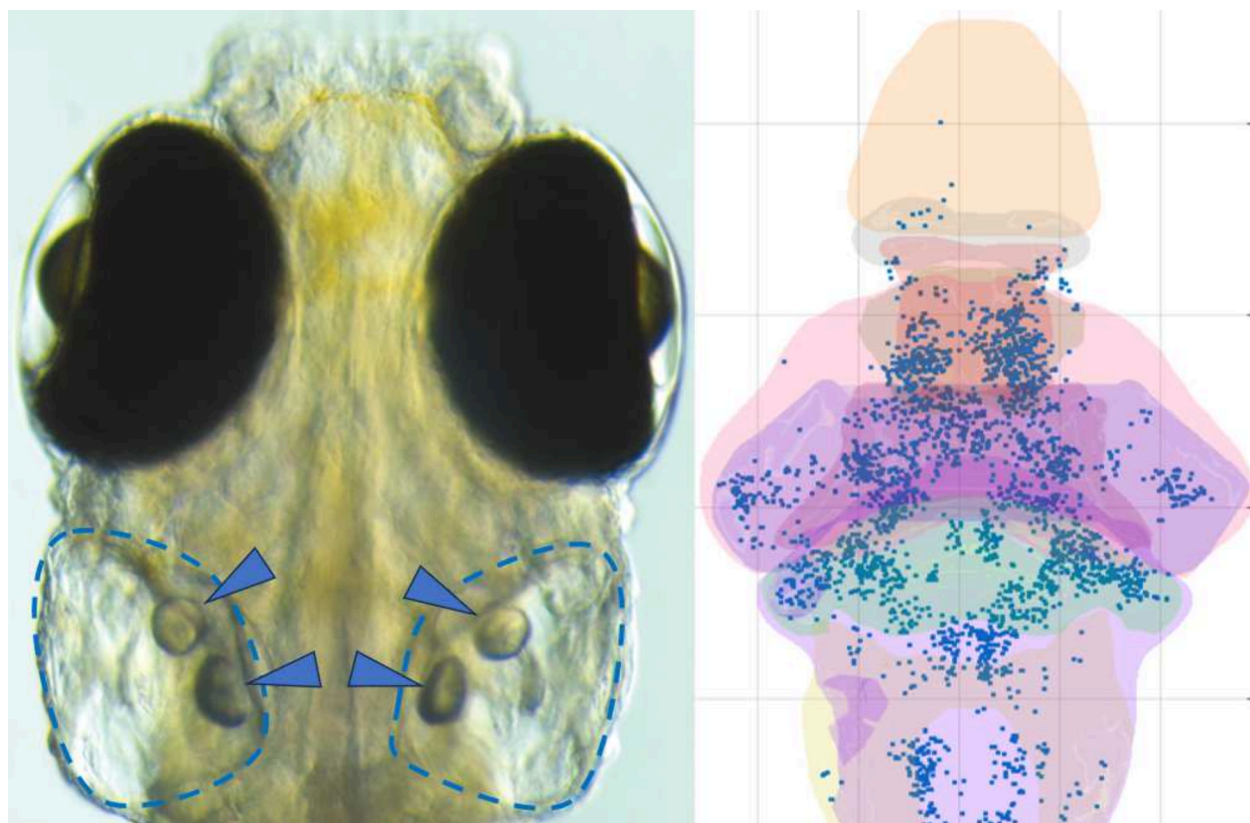
Stepping up in power, lasers are also used to generate shock waves. A laser shock wave is generated through the building up of energy at a defined point in space. This concentration of energy generates a cavitation bubble which expands and by this means creates a mechanical wave equivalent to a shockwave.<sup>174</sup> This method has been used to simultaneously monitor the neuronal response to shear stress and nearby cell death or injury.<sup>175</sup>

Intensely focused laser beams can also be used for removing one or multiple cells from a network. In neuroscience, this method has been proven useful when investigating the necessity of targeted neurons in a neuronal network or behavior, or for the study of neuronal development and network plasticity.<sup>176,177</sup>

An interesting example is the study of the necessity of the optic tectum brain region in zebrafish optokinetic and optomotor responses. The optic tectum is known to be the largest visual brain region where retinal ganglion cells project information from the eye to the brain. However, Roeser et al.<sup>178</sup> show that the laser ablation of retinal input to the optic tectum provided further insights into the understanding of the tectum functions: the tectum is required for the correct pacing of saccades during the optokinetic responses, however, layers of the tectum receiving optical information appeared to be dispensable for the execution of optokinetic and optomotor responses.

**Mechanobiology of Neurons.** Another important advance in optical methods for neuroscience is the use of optical tweezers for the measurement of mechanical properties of neurons. The complexity of neuronal processes, complex networks, and their influence on behavior and cognition has generated a strong incentive for a wide range of combinations of optical methods. Optical tweezers, as described in previous sections, is a relatively flexible technology, and in many cases can be integrated into optical systems and combined with advanced imaging techniques. Interesting combinations include electrophysiology, epifluorescence, confocal fluorescence, TIRF and FRET, Raman spectroscopy, differential interference contrast (DIC), interference reflection microscopy (IRM), super-resolution microscopy (STED), or phase





**Figure 9.** Optical manipulation of ear stones in zebrafish inner ear. Left: Top view of 6 days post fertilized zebrafish. Blue outline delimits the inner ear. Blue arrows show ear stones. Right: Example of distribution of regions of interests (ROIs, blue dots) involved in auditory processing. Brain is outlined in color.

contrast. To create forces beyond optical tweezers capabilities, they have also been combined with other trapping technologies such as acoustics tweezers, magnetic tweezers, and mechanical systems such as microfluidics. Some key examples are discussed in the following.

**Lamellipodia and Filopodia Forces.** Lamellipodia and filopodia are cytoplasmic projections and highly motile structures that explore the extracellular environment, determine the direction of neuronal growth as well as guide the extension of the axon. Using optical tweezers, forces generated by such crucial structures have been measured. Using the Brownian motion of optically trapped particles attached to lamellipodial membrane,<sup>179</sup> measured the distribution of the particles' velocities, and calculated forward and backward "jumping" times (elementary events underlying force generation) to be between 0.1 and 0.2 ms with amplitudes varying around 20 nm. They also measured the frequencies and amplitudes of those jumps and measured their changes in the presence of different molecules concluding on their effects. Another interesting example is the measure of forces exerted by filopodia and lamellipodia. Using optical tweezers, Cojoc et al. (2007) placed a trapped bead against isolated filopodia and lamellipodia and measured single filopodial forces not exceeding 3 pN, and lamellipodial forces of at least 20 pN.<sup>180</sup> These values suggest that an isolated filopodium does not have the capacity to alter the environment while lamellipodia can. These results explain why filopodia change its direction of growth when encountering large objects, while lamellipodia can move or lift large structures, modify the environment and facilitate the growth of axons.

**Altering Neuronal Growth.** The study of neuronal growth or guidance using optical tweezers includes the direct manipulation of lamellipodium (optical pull).<sup>181–184</sup> Despite its small size (about 200 nm thick and 2 to 4  $\mu\text{m}$  long) past studies have shown the influence of optical tweezers on lamellipodium growth. However, due to the possible thermal changes at the optical tweezers location, it is disputed whether the neuron's guidance is due to thermal effects rather than the expected optical force gradient.<sup>185,186</sup> The heat generated within the trap focus may in addition trigger a biochemical signaling cascade which could result in the chemical guidance of cell growth.<sup>187</sup>

Another study of neuronal guidance is the creation of localized fluid flows through the spinning of particles trapped in optical tweezers and rotated with angular momentum (section: **Rotational Microrheometry**). By changing the rotation direction and location of a trapped bead, Wu et al. (2011) have been able to create a local flow (on the micro scale) and consequently, a shear force that influenced the growth axon's development, showing that the environmental physical dynamics directly influence neuronal growth.<sup>188</sup>

**Neuronal Disorders.** Significant efforts have been placed into the understanding of how the brain and neurons recover or adapt from neurodegenerative conditions. Studies have shown that the mechanical properties of the brain deeply influence neurodevelopment,<sup>189,190</sup> and are correlated with a number of developmental disorders.<sup>191,192</sup> Therefore, research studies have been directed toward the understanding of the biomechanics and rheological differences between healthy versus unhealthy brains.

One example of a method used is indentation. Indentation allows to physical deformation of brain tissue on a large scale (brain slices), in order to measure its stiffness and elasticity.<sup>193,194</sup> This method however presents some drawbacks: it cannot be performed in vivo and lacks precision. Techniques such as optical tweezers, magnetic tweezers, atomic force microscopy, micropipettes have allowed precise measurements and significant progress into mechanotransduction studies.<sup>195,196</sup> Optical tweezers, in particular, offers many advantages over large-scale indentation. They allow non-invasive measurements, applies comparatively large forces, can probe cells individually and provides precise force measurements in 3D.<sup>197–201</sup> A good example is the use of optically trapped silica particles by Dagro et al.<sup>202</sup> to deform neuroglia and measure the local mechanical properties of cells grown in a 3D environment. They successfully measured their stiffness and elasticity for high and low strain rates which makes optical tweezers as a valuable method for indentation on a small scale and a method to investigate how mechanical properties influence neurodevelopment.

**Neuronal Networks.** Another significant area of neuroscience is the determination of the neuronal code. While the information processed by the brain is known to be operated by a succession of neuronal events, the overall code is an ambitious question. A large number of studies have therefore focused on breaking down this question into the study of more elemental processes such as sensory processing, cognition, or memory.

An excellent example is the investigation of hearing and acceleration sensing with light and our use of optical tweezers on zebrafish otoliths (ear-stones located in the inner ear). In a series of publications,<sup>203–206</sup> it was shown how optical tweezers technology combined with fluorescence imaging were applied to the inner ear of zebrafish to simulate mechanical vibrations equivalent to sound or acceleration and record whole brain activity, simultaneously. With optical tweezers manipulation ranging from 1 Hz to 1 kHz this Bio-Opto-Acoustic (BOA) technique allows to displace all of the four ear-stones (Figure 9) at a chosen frequency and stimulate the neurons responding to natural tones. It is worth emphasizing that optical tweezers can optically manipulate individual ear stones; a manipulation that is not possible with natural mechanical waves. The results show that the neuronal network of individual ears projects to the contralateral ear which was shown in different models. In addition, the responsive neurons showed responses profile dependent on optical tweezers configuration across the whole brain which suggests that individual ear-stone neuronal networks do not fully overlap and information is treated differently from ear-stone to ear-stone. In particular, it was revealed that the utricular and saccular otoliths—previously described as having separate biological functions—are in fact part of an integrated system and work cooperatively. Tail bends and eyes rolls in response to individual or multiple otoliths optical manipulation was also observed. Results suggest that the fish is compensating behaviorally for the perceived, but nonexistent, acceleration. Combining optical tweezers and fluorescence imaging with behavioral imaging of the eyes and tail in zebrafish exemplifies the role that optogenetics plays in novel studies of mechanobiology.

Since optical tweezers offer high spatial precision, we could manipulate single elements of the inner ear and precisely map the neural networks that responded, providing important

information about the separate and shared circuits involved in hearing and vestibular perception.

## ■ MICRORHEOLOGY USING OPTICAL TWEEZERS

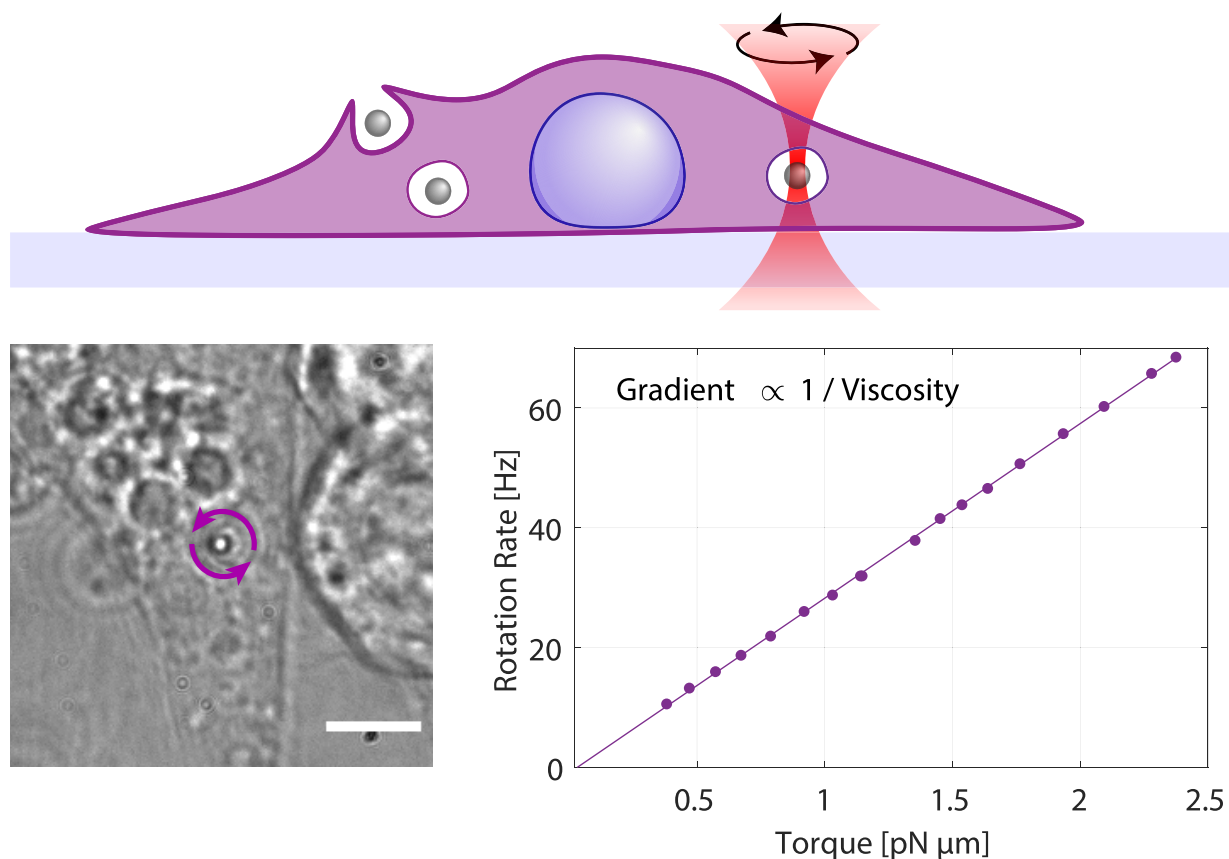
The mechanical properties of fluids within the cellular environment, both intracellular and extracellular, can affect the behavior of cells and the motion of biomolecules. This has led to the field of biomicro-rheology, which considers the average response of fluids and elastic macromolecules to mechanical stress.<sup>207</sup> On the microscale, stress can result from a variety of sources including the motion of individual cells, their protrusions, vesicles, or the diffusion of individual biomolecules. Characterizing these mechanical properties on different scales can provide insight into cell behavior and functions.

Intracellular dynamics depend on the diffusion and trafficking of molecules, proteins, and vesicles within a cell and are affected by local fluctuations of viscoelasticity.<sup>207</sup> The average viscoelasticity of the cytoplasm can describe the physiological properties of the cell. Similarly, local extracellular viscoelasticity can impact the internalization of nearby particles including nutrients and signaling molecules. On a slightly larger scale, the mechanical properties of whole cells provide physiological insight.<sup>208</sup> For example, human epithelial cells and red blood cells have increased rigidity with age,<sup>209</sup> while breast cancer epithelial cells have a lower viscosity than normal cells.<sup>210</sup>

The microrheological properties of the extracellular matrix (ECM) have far-reaching influences on cell<sup>211,212</sup> and tissue behavior including stem-cell differentiation,<sup>213,214</sup> cell migration,<sup>212</sup> tissue organization,<sup>215</sup> and facilitates the development and proliferation of malignant tumors.<sup>210,216–218</sup> Micro-rheological studies provide insight into these processes, open avenues for potential treatments, and improve physiological models.<sup>212</sup>

Understanding the rheology of biological systems across these different scales provides crucial functional and developmental insight and has led to a wide range of microrheometry techniques. A distinction can be made between viscoelastic fluids which are predominantly more viscous, and viscoelastic solids which are predominantly more elastic. Investigations of membrane, whole cell, and tissue rheology fall more closely in the latter category. Experimental methods often involve a variety of stretching and shearing techniques (including optically based), atomic force microscopy,<sup>219</sup> ultrasound elastography<sup>220</sup> or magnetic resonance elastography.<sup>221</sup> This section will restrict its discussion to considering the average response of viscoelastic fluids to mechanical stress.

Microrheometers function by observing the interaction between a probe and the surrounding fluid, where the probe's motion causes stress on the fluid that in turn generates drag. Analysis of the probe's trajectory enables the determination of rheological properties which can be quantified by its frequency-dependent complex shear modulus,  $G^*(\omega) = G'(\omega) + G''(\omega)i$ , where  $G'(\omega)$  is the elastic storage modulus and  $G''(\omega)$  is the viscous loss modulus.<sup>222</sup> This frequency-dependent response is measured using either passive or active techniques, synonymous to section Calibration. Passive measurements, where the motion is thermally driven, are more useful to determine high-frequency responses and those from mostly viscous mediums.<sup>223</sup> However, long measurement times required to average out the Brownian motion for low-frequency rheometry limits measurements of dynamic



**Figure 10.** Intracellular microrheometry using rotational optical tweezers. A macrophage macropinocytoses birefringent microspheres which are trapped and rotated with circularly polarized light. The rotation rate increases with torque inversely proportional to the viscosity. Scale bar is  $5\ \mu\text{m}$ . Microscopy and data is from Watson et al.<sup>242</sup>

systems.<sup>224,225</sup> Instead, active methods can be implemented generating longer relaxation times and enabling measurements of low frequency responses.<sup>223,224</sup> Additionally, the shear viscosity alone often provides sufficient information, especially in systems that are predominantly viscous and can be measured by determining the Stokes drag on the particle.

There are a variety of measurement techniques capable of performing microrheometry featuring different methods of particle tracking and driving methods.<sup>226</sup> Optical methods have had broad success determining viscosity and viscoelasticity due to their high spatial and temporal resolutions and are especially useful for cellular systems as noninvasive methods. Yamada et al. introduced the use of Laser-tracking microrheology as a method for passive intracellular microrheometry, monitoring laser deflection by perinuclear granules to determine their trajectories and hence the cytoplasmic viscoelastic modulus.<sup>227</sup> In addition to precise measurements of position, optical tweezers have the capacity to generate and measure forces and torques, making optical tweezers-based methods a widely utilized and successful microrheometer. Typically, these methods measure the trajectory of an optically trapped probe as the position relaxation is temporally correlated, enabling statistical methods including mean-squared-displacement (MSD), autocorrelation, and power spectrum methods to model the trajectory and determine the frequency-dependent complex shear modulus. Frequency-independent viscometry can also be performed by determining the drag on the particle and relating it to the Stokes drag at low Reynolds numbers.

Passive optical tweezers methods track the trajectory of an optically trapped probe experiencing thermal motion as done with passive calibration techniques. The position relaxation occurs on very short time scales which restricts measurements to the high-frequency regime. The harmonically trapped particle is modeled with a generalized Langevin equation which can be analytically solved in frequency space, expressing the complex shear modulus in terms of measured quantities of MSD<sup>224</sup> or position autocorrelation.<sup>129</sup> Similarly, the power spectrum can be used to determine the frequency-dependent viscosity of a purely viscous substance.<sup>29</sup> These methods have enabled intracellular measurements, where internal probes are optically trapped and their position measured. The choice of probe has ranged from injected particles, phagocytosed particles,<sup>228,229</sup> and even naturally derived particles such as intracellular granules and vesicles.<sup>42,227,230</sup>

Active optical tweezers methods drive the motion of the probe with an external force to generate longer relaxation trajectories that can be modeled using the aforementioned statistical methods. These trajectories are often sourced from the probe falling into an optical trap after a disturbance from equilibrium that is greater than thermal motion. This has been achieved by translating the sample stage relative to the optical trap,<sup>224</sup> having a probe switching between two slightly displaced traps that alternate on and off,<sup>129</sup> and through blinking optical tweezers where the trap is periodically switched on and off.<sup>231</sup> It is important to consider whether the probe remains in the Hookean regime to ensure accurate trajectory models.<sup>225</sup> These methods are similar to the



calibration of optical tweezers and enabled intracellular measurements.<sup>29,42</sup> These measurements have predominantly focused on the cytoplasm linking changes in viscoelasticity to cell cycle,<sup>230,232,233</sup> the nonthermally driven motion of intracellular components,<sup>229,234</sup> intracellular crowding,<sup>233,235</sup> and correlating extracellular and intracellular stiffness.<sup>236</sup>

**Rotational Microrheometry.** Microrheometry in the rotational regime can be achieved by exploiting the transfer of angular momentum in rotational optical tweezers (ROTs) to generate optical torques. In the case of spin angular momentum transfer, birefringent particles can alter the polarization state of the incident beam which changes its angular momentum. Under conservation laws, the change in spin angular momentum is transferred to the particle as an optical torque causing the particle to rotate. Furthermore, the change in polarization of the beam can be directly measured enabling the calculation of optical torque and angular position of the particle. With careful control of the beam's polarization state and choice of birefringent particle, either an alignment torque or constant torque can be generated.<sup>237,238</sup> The rotational regime requires less volume for microrheology as the center of mass of the probe can be tightly constrained making it ideal to study systems with limited free volume.<sup>239–242</sup> It also benefits from reduced effects of nearby surfaces with Faxén's corrections compared to the translational case.<sup>243</sup>

For shear viscosity measurements, a birefringent particle rotating under a constant torque will reach terminal angular velocity due to a drag torque from the fluid. In the case of a rotating sphere, the Stokes drag torque at low Reynolds numbers is given by  $8\pi\eta a^3\dot{\theta}$ , where  $\eta$  is the shear viscosity of the fluid,  $a$  is the radius of the microsphere, and  $\dot{\theta}$  is the angular velocity. With knowledge of the optical torque, it is straightforward to determine the viscosity of the fluid.<sup>244</sup> This has recently been achieved within the vesicle of a living macrophage cell as shown in Figure 10 demonstrating the use of rotational optical tweezers for intracellular rheological measurements.<sup>242</sup> A birefringent microsphere was internalized into a macrophage vesicle via macropinocytosis, optically trapped, and rotated using 1064 nm light. As the trapping power increases the rotation rate increased inversely proportional to the viscosity. Tracking the changes in the viscoelasticity as the vesicle matures could provide insight into a number of cellular pathways that utilize this internalization method.

For viscoelastic measurements, ROTs can generate a harmonic alignment torque on a birefringent microsphere. Analogous to the translational case, the angular trajectory of the probe can be used to determine the frequency-dependent complex shear modulus with respect to the Fourier transform of the angular displacement autocorrelation. Passive measurements have been performed where thermal torques cause small deviations from the probe's equilibrium orientation<sup>240</sup> and by monitoring the out-of-plane orientation.<sup>245</sup> Active measurements have been achieved where the probe is rotated between two alignments enabling access to the low-frequency regimes.<sup>246</sup> This has been extended into the nonlinear regime reducing the error in measurements of viscoelasticity<sup>225</sup> applied to investigate time-dependent changes of viscoelasticity in the tear film of contact lenses<sup>246</sup> and within lipid structures<sup>247</sup> demonstrating its applicability to biological systems. Recently, passive rotational viscometry was performed

within the cytoplasm of MCF-7 cancer cells and shown to match measurements in the translational regime.<sup>248</sup> In colloidal complex fluids like the cytoplasm, the rotational motion may generate different stresses than translational motion based on the mechanical properties of the elastic biomolecules. For a full mechanical description of intracellular fluids, it is essential to investigate the rotational regime.

## CONCLUSIONS AND PROSPECTS

A number of developments and building of further understanding of mechanobiology has been enabled when using optical nano and micromanipulation. In this perspectives paper we have concentrated on elucidating developments in a few specific areas of complex biological systems.

At the cell scale, laser scissors have been useful in genetic manipulation, DNA repair studies and investigations on the contribution of bridging fibers and intertelomeric tethers to the mechanical separation of mitotic chromosomes. Optical tweezers have allowed researchers to measure forces required to move chromosomes, as well as the contribution of molecular motors toward chromosome separation. The contributions of laser scissors and tweezers have been significant in that they have led to the re-examining of the complexity of mechanics involved in mitosis. Several questions remain on the contribution of all these mechanisms toward cancer, aging, and developmental disorders. Such investigations will continue to benefit from the optical methods described in this perspectives paper.

Structured light beams have become valuable tools in mechanobiology enabling both the imaging and manipulation of biological samples. Advancements in hardware and technology have significantly improved our ability to do this. Modern modulation devices are now capable of approaching communications speeds. Furthermore, the use of sophisticated algorithms have contributed to an advancement in the speed and major advances in the way in which light can be projected.

The use of structured light has made significant contributions to microscopy and imaging. It is also being employed very successfully for three-dimensional profiling, enhancing contrast and improving image resolution. Furthermore, the contribution of structured light to fluorescence microscopy in particular has been sizable, with developments such as STED microscopy which allows super-resolution imaging of microscopic nano/microscopic phenomena. Overall, the field of structured light has seen significant technological advancement in the last 20 years. New algorithms for designing structured light have led to novel imaging techniques that vastly improve resolution and enable 3D imaging. Further developments in this area will see numerous new applications arise and improve the precision in optical nano and micromanipulation and the imaging of complex biological systems.

Optical methods have resolved a large number of challenges in neuroscience. From molecular processes to large-scale neuronal networks, light-based technologies have allowed unprecedented precision and insight into previously out of the reach processes. Significant discoveries in the dynamics and growth of neurons, neuronal disorders, and formation and alteration of networks have produced significant steps forward in the field of neuroscience.

Additionally, optical methods have helped develop microrheological techniques, enabling measurements of local viscosity and viscoelasticity across biological scales ranging from intracellular measurements to those of whole tissues.



Optical tweezers-based methods offer a powerful biophysical tool to noninvasively measure intracellular properties to help fully characterize the mechanical properties of cells and describe cellular processes.

The future of the field of optical mechanobiology is bright. A major opportunity for discoveries in mechanobiology comes from development and use of several new classical and quantum optics-based techniques. These techniques show promise in ramping up interactions between probe and system and therefore enable better measurements of subtle effects. Additional *in situ* capabilities such as voltage sensors based on a nanodiamond platform<sup>249</sup> are exciting as they add optical electrical field sensing opportunities in biomechanics. Up-conversion particles<sup>250–252</sup> allow for stronger interactions with light and can thus improve signal-to-noise ratio. Improvements to signal-to-noise can enable deep measurements within tissue at rates on the order of kHz-MHz. Another important development is the maturation of the design and fabrication of diffractive surfaces—also called metasurfaces—that allow for bespoke interactions with light.<sup>253,254</sup> This will enable novel forms of passive measurements and control. Other new quantum technologies such as nonlinear quantum microscopes<sup>255</sup> could also have application to measurements in mechanobiology by allowing real time measurement of active molecules without tagging.

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## DEDICATION

This Perspective is dedicated to the memory of our dear friend and mentor, Professor Michael Berns, who was one of the original authors of this Perspective when we started writing it, but who unexpectedly passed away during our work. Michael was a giant of our field. He was the first person to perform subcellular surgery of chromosomes (Berns, M. W. et al. *Laser Microsurgery in Cell and Developmental Biology*. *Science* **1981**, 213, 505–513. [10.1126/science.7017933](https://doi.org/10.1126/science.7017933)). Michael helped to pioneer laser nanosurgery and was the father of laser microbeams (Berns, M. W.; Olson, R. S.; Rounds, D. E. *In vitro* Production of Chromosomal Lesions with an Argon Laser Microbeam. *Nature* **1969**, 221, 74–75. [10.1038/221074a0](https://doi.org/10.1038/221074a0)). His lab was the first to combine the optical tweezers and laser scissors techniques, and the first to combine the optical tweezers technique with fluorescence resonance energy transfer (FRET) microscopy and fluorescence microscopy (Wang, Y.; Botvinick, E. L.; Zhao, Y.; Berns, M. W.; Usami, S.; Tsien, R. Y.; Chien, S. Visualizing the mechanical activation of Src. *Nature* **2005**, 434, 1040–1045. [10.1038/nature03469](https://doi.org/10.1038/nature03469)), all of which contributed significantly to our understanding of mechanobiology.

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