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Lights, action: optical tweezers

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Optical tweezers were first realized 15 years ago by Arthur Ashkin and co-workers at the Bell Telephone Laboratories. Since that time there has been a steady stream of developments and applications, particularly in the biological field. In the last 5 years the flow of work using optical tweezers has increased significantly, and it seems as if they are set to become a mainstream tool within biological and nanotechnological fields. In this article we seek to explain the underpinning mechanism behind optical tweezers, to review the main applications of optical tweezers to date, to present some recent technological advances and to speculate on future applications within both biological and non-biological fields.

1. Introduction

It is 15 years since Ashkin *et al.* [1] published their seminal paper 'Observation of a single-beam gradient force optical trap for dielectric particles'. The technique is now referred to as 'optical tweezers' or 'optical trapping' and their original paper has received 400 citations, half of which appeared during the last 5 years. In essence, optical tweezers rely upon the extremely high gradient in the electric field produced near the beam waist of a tightly focused laser beam, which creates a force sufficient to trap micron-sized dielectric particles in three dimensions. Commercial tweezers systems are now available (Cell Robotics International Inc., Albuquerque, New Mexico, USA; PLAM GmbH, Bernried, Germany), and although originally devised by physicists, it is mainly biologists who have put optical tweezers to use. However, technology does not stand still and tweezing techniques are at present undergoing a further spate of development. The future of this cross-disciplinary field is bright.

2. The mechanism behind optical tweezers

Thirty years ago when Ashkin [2] started to experiment with optical traps he realized that an unfocused laser beam would draw objects of high refractive index towards the centre of the beam and propel them in the direction of propagation. An arrangement of two counter-propagating beams allowed objects to be trapped in three dimensions. In these experiments he was able to observe the effects of radiation pressure and to overcome the usually much larger radiometric (heating) effects of light by using relatively transparent objects in a relatively transparent medium. He later discovered that a single, tightly focused laser beam could be used to capture small dielectric particles in three dimensions. This technique enables small particles to be picked up and moved at will using a beam of visible light and hence was christened optical tweezers. When trying to understand the origin of the forces acting within optical tweezers, two distinct approaches may be adopted, one based on ray optics, and the other on the electric field associated with the light.

2.1. *Describing the forces generated by optical tweezers*

In all cases it is useful to agree on how the forces generated by light can be compared and described. As every photon carries energy $h\nu$ and momentum h/λ, it is straightforward to state that, if absorbed by an object, the momentum transferred from a light beam of power P, leads to a reaction force F on the object, given by

$$F = \frac{nP}{c}, \tag{1}$$

where c is the velocity of light and n is the refractive index of the surrounding medium.

The efficiency of any particular optical configuration can then be described in terms of a dimensionless quantity Q,

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whereby the force generated by the light beam in the optical tweezers is given by

$$F = Q\frac{nP}{c}. \quad (2)$$

This paper mainly concerns the optical forces imparted to small dielectric or metal particles for which Q typically lies in the range between 0.03 and 0.1.

2.2. *Tweezers force: ray optics*

Analysis of the forces required to achieve optical tweezing is quite complicated as the vectorial nature of these forces needs to be taken into account. Ashkin [3] himself published a detailed analysis of the forces acting on a dielectric sphere, calculated in the ray optics regime. At the centre of this approach is the understanding that, since a light beam carries a linear momentum of h/λ per photon, the refraction of light by a transparent object results in a change in photon momentum and a corresponding reaction force acting on the object. This approach can also allow for forces generated by reflection or scattering from the interface; however, as the trapped object is usually suspended in a fluid of similar refractive index, the resulting Fresnel reflections and corresponding recoil forces are small and in the main are ignored. Figure 1 shows the refraction of light rays at the surface of a dielectric sphere and the resulting forces acting upon it. By adopting an approach similar to that of a ray-tracing package used for lens design, the effect of a complete optical beam can be modelled using a bundle of rays, with each individual ray weighted according to its intensity.

With reference to figure 1, the counter-intuitive aspects of optical tweezers are immediately apparent. Firstly, the intensity profile of the beam cross-section results in a force acting to move the object into the centre of the beam, that is the force arising from refraction of the light can be in the opposite sense to that from light scattering alone. Secondly, if a beam incident from above is tightly focused, then it is possible to generate a force that acts to lift the object up towards the focus, thereby creating a three-dimensional trap with a single laser beam. Finally, we note that reversing the direction of a ray does not change the direction of the force; this illustrates nicely that the forces associated with refraction are linked to the beam intensity rather than to the direction of propagation.

For objects larger than the wavelength of the laser, the ray optics approach gives remarkably accurate estimates of

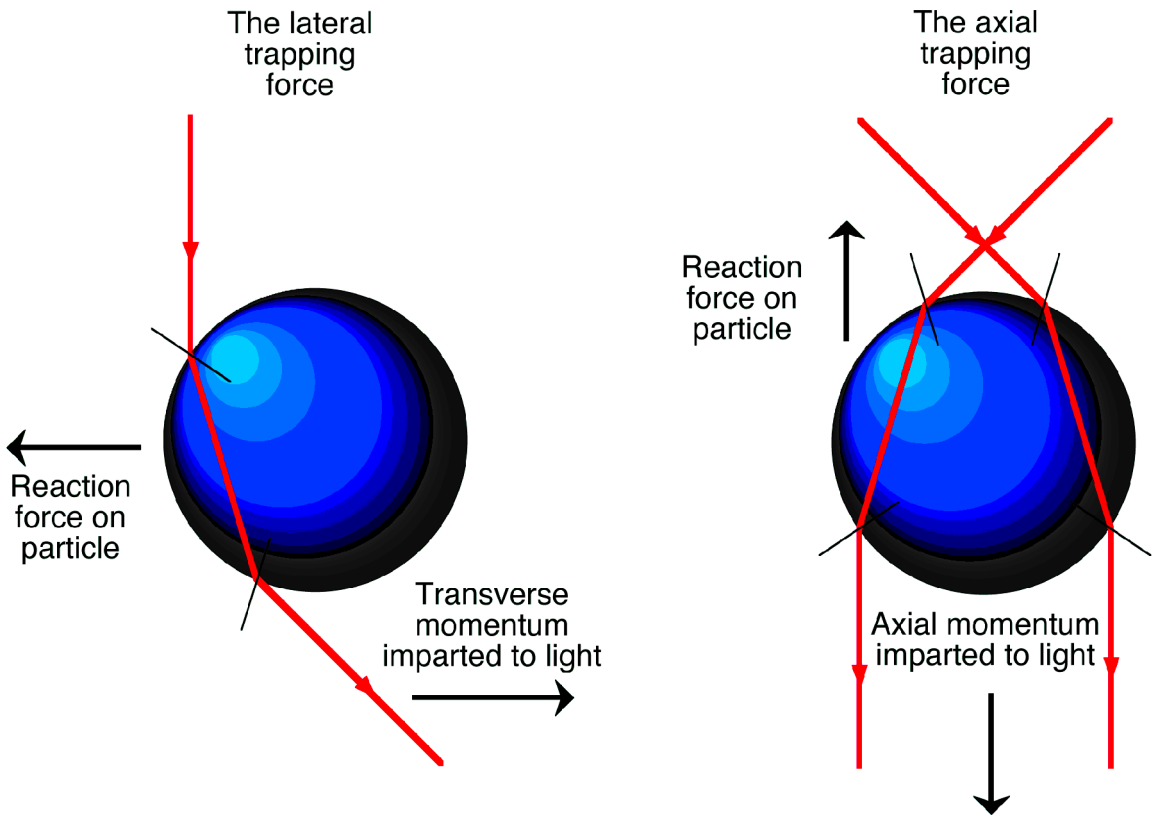

Figure 1. The ray optical origin of the lateral and axial trapping force within optical tweezers.

the observed values of Q [4]. It can readily be applied to calculation of Q obtainable when using different beam profiles such as for Laguerre–Gaussian modes [5]. The Laguerre–Gaussian modes, which we shall encounter later, have annular intensity profiles and carry an orbital angular momentum.

2.3. *Tweezers force: electromagnetic field*

For particles smaller than the wavelength of the laser beam, the ray optical approach is less satisfactory and it is better to consider the forces in terms of the electric field near the trapped particle. As before, the forces can be divided into those arising from scattering of the light and those arising from an intensity gradient.

For an object of radius r and light beam of intensity I_0, the force resulting from the light scattering is,

$$F_{\text{scat}} = \frac{I_0}{c}\frac{128\pi^5 r^6}{3\lambda^4}\left(\frac{N^2-1}{N^2+2}\right)^2 n, \tag{3}$$

where N is the ratio of the refractive index of the object to the index n of the surrounding medium. Although not apparent from this form of the equation, the scattering force is directed perpendicular to the wavefronts of the incident light, that is objects are pushed in the direction of light propagation.

The intensity gradient near the beam focus gives rise to a gradient force, which is equivalent to the refraction of the light rays, given by

$$F_{\text{grad}} = \frac{-n^3 r^3}{2}\left(\frac{N^2-1}{N^2-2}\right)\nabla(|E|^2). \tag{4}$$

Explicit in this equation is that the force is directed towards the region of highest light intensity.

As the scattering force acts in the beam direction whereas the gradient force acts towards the beam focus it follows that, for a beam directed downwards, the stable trapping point lies just below the focus. For a full three-dimensional trap to be established, we require the gradient force to exceed the scattering force. Both these forces scale linearly with light intensity; adjusting the laser power alone is therefore not sufficient to form a trap, instead we have to concentrate upon maximizing the intensity gradient in the beam. This can be most readily achieved by extremely tight focusing of the trapping laser; use of oil-immersion microscope objective lenses with high numerical aperture and high magnification is the norm.

2.4. *Trapping of high-index and low-index particles*

So far we have assumed that the refractive index of the trapped particle is higher than that of the surrounding media. If, however, a particle such as an air bubble or hollow sphere is in an intensity gradient, then the direction of the gradient force is reversed and the particle experiences a force away from the maximum intensity region. We shall see later that this realisation allows us to trap both low-index and high-index particles.

2.5. *Additional forces in optical tweezers*

The gradient force is central to the operation of all optical tweezers and provides a restoring force which, over distances up to several hundred nanometres, is a linear function of displacement x. The equation of motion governing the behaviour of a trapped object of mass m, in a medium that gives a viscous damping β (see below) is a balance between inertial, viscous and elastic forces:

$$m\frac{\partial^2 x}{\partial t^2} + \beta\frac{\partial x}{\partial t} + \partial x = 0, \tag{5}$$

where κ is the elastic constant or stiffness of the optical trap. In the absence of any damping (i.e. in air [6] or in vacuum) the result would be an oscillator with resonant frequency f_{res}, given by

$$f_{\text{res}} = \frac{1}{2\pi}\left(\frac{\kappa}{m}\right)^{1/2}. \tag{6}$$

In typical biological applications, the stiffness of the optical tweezers is around 0.05 pN nm^{-1} (5×10^{-5}N m^{-1}) and the trapped objects are around 1 μm diameter (corresponding to a mass of 5×10^{-16} kg). Hence, the resonant frequency is approximately 50 kHz. However, because biological experiments must be performed in an aqueous medium, significant damping force arises. For micron-sized particles of radius r, moving in a fluid of viscosity η, the Stokes drag constant β is

$$\beta = 6\pi r\eta. \tag{7}$$

For a sphere 1 μm diameter in water, $\beta = 1\times10^{-8}$ N s m^{-1}. The combination of viscous damping and the spring-like stiffness of the optical tweezers gives rise to a single-pole low pass filter with -3 dB frequency f_0 given by

$$f_0 = \frac{\kappa}{2\pi\beta}. \tag{8}$$

For typical biological application we find that the roll-off frequency is well below 1 kHz. Since this is much lower than the resonant frequency, the motion is very over-damped. In fact, this means that inertial and gravitational forces can be ignored altogether. In addition to providing the damping force, the surrounding fluid has the advantage of providing cooling to minimize the heating effect of the laser light.

Biological experiments must be performed at around room temperature (i.e. 300 K) and, because the mechanical system that we have described is over-damped, we find that the damping source is also a source of thermal energy input

given by the product of the Boltzmann constant and absolute temperature, $k_B T$. The random bombardment of the trapped object by surrounding water molecules gives rise to a fluctuating thermal force which, from the theory of equipartition of energy, produces a resulting mean-squared deviation in position along one axis, $\langle x^2 \rangle$, calculated from,

$$\frac{1}{2}\kappa\langle x^2\rangle = \frac{1}{2}k_B T. \tag{9}$$

Again, substituting typical values for temperature and tweezers stiffness we find that the rms deviation in position is about 10 nm. Note that this also means that it is very unlikely that the trapped particle will spontaneously diffuse from the grasp of the optical tweezers, which have a capture range of about 300 nm. However, when we wish to use optical tweezers to measure molecular scale events this 10 nm is a significant distance (see section 5).

Finally, to obtain a full description of the observed thermal motion we find that it is distributed over a Lorenzian power density spectrum where the amplitude A_f of motion over each frequency interval f is given by

$$A_f = \frac{4k_B T\beta}{\kappa^2(1 + f/f_0)^2}. \tag{10}$$

For the non-specialist, this spectrum resembles white noise that has been subjected to a single-pole low-pass filter. Acoustically, this would sound like a muffled hiss or a rumble.

3. A standard tweezers configuration

Although optical tweezers can be commercially obtained, they are also surprisingly simple to assemble by anyone with an expertise in building optical systems. Given that optical tweezers have many of the components in common with a high-magnification microscope, a research grade microscope represents a good starting point for a custom-built instrument.

3.1. *Obtaining a tightly focused beam*

The requirement for tight focusing of the trapping beam means that a laser with high spatial coherence is used as the optical source. Most optical tweezers use diode-pumped yttrium aluminium garnet lasers, chosen for their good beam pointing stability and low absorption of the near-infrared laser line (1064 nm) by biological materials. To produce a diffraction-limited focal spot, a high-magnification microscope objective lens with a high numerical aperture is the obvious choice of focusing lens. Traditionally, microscope objectives were designed to operate at finite conjugates, producing an image of the sample plane 160 mm above the rear shoulder of the objective housing. Such designs are extremely convenient in that the only additional optic then required is an eyepiece or camera, the physical constraint means that inserting additional filters, camera or illumination sources can be problematic. It is now common to design objective lenses that produce an image at infinity. Although requiring an additional 'tube lens' to produce an image such 'infinity corrected' objectives place no restriction on the physical size on the optical system. For optical tweezers they are particularly appropriate as they take a collimated laser beam and focus it exactly to the sample plane (figure 2).

For biological applications the sample is usually immersed in a liquid and contained beneath a thin glass coverslip which prevents contamination. To minimize the resulting image aberrations the objective lens is specifically designed to operate with a thin layer of oil between itself and the coverslip, thereby eliminating any air gap. Lenses are typically available with a numerical aperture of 1.3, which allowing for an index-matching fluid with refractive index 1.56, corresponds to a focused light beam with a full cone angle of 110°! Such extreme focusing not only gives the maximum possible optical resolution in the microscope image but also ensures the tightest of focused spots for the trapping laser beam. To obtain the smallest possible focused spot one requires that the incident laser beam fills (or slightly overfills) the back aperture of the objective lens.

One further subtlety is that, although we are maybe familiar with a microscope which looks down on to the sample plane, many biologists favour an inverted geometry where the sample is held above the objective lens as this allows easy access to the sample plane. In this case, the sample cell can be thought of as an open-topped bucket with a thin transparent bottom.

3.2. *Beam steering within optical tweezers*

Obtaining a tightly focused laser beam in the centre of the field of view of the microscope is not a problem. However, for many applications it is useful to be able to steer the beam around, thereby manipulating the trapped object. For an optically efficient instrument we require the beam steering mechanism to produce a collimated laser beam which is always centred on the back aperture of the objective lens. An angular displacement about this point will then produce a lateral displacement of the focused spot in the sample plane without any additional loss of light. It is possible to devise a number of optical relay systems that achieve this end, but perhaps the simplest and most straightforward is shown in figure 3. The collimated laser beam is incident upon the main beam steering mirror. This plane is then re-imaged using an afocal telescope to the back aperture of the objective lens. Angular adjustment of the beam steering mirror then produces a lateral displacement of the focal spot. Clearly, beam steering of this kind can be automated, and used in conjunction with an automated sample stage,

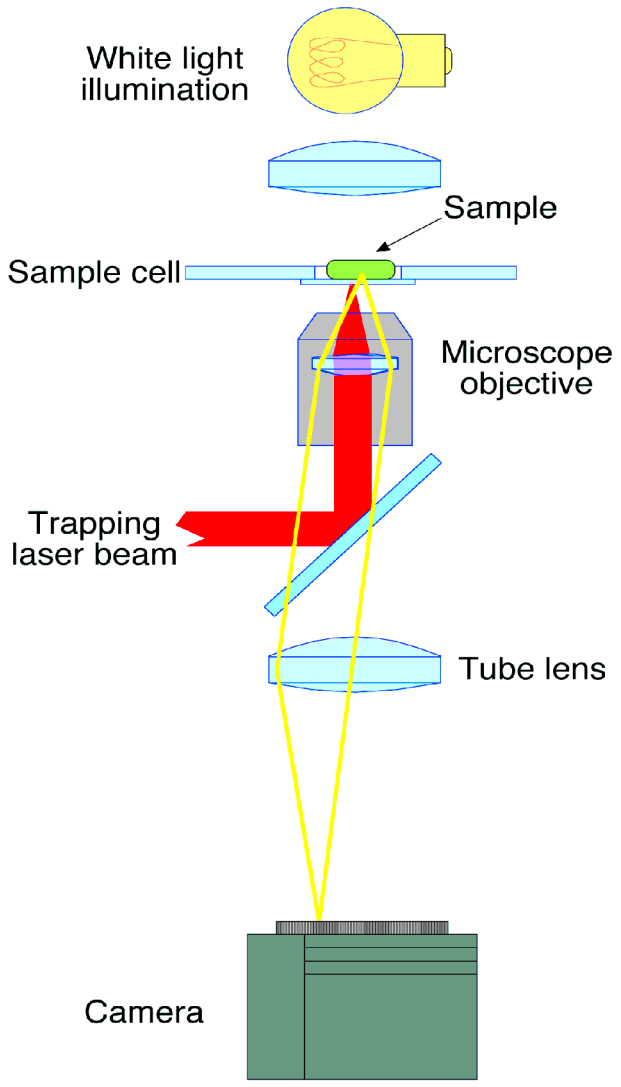

Figure 2. A simple optical tweezers arrangement based on an infinity corrected microscope objective.

enabling both absolute and relative movement of the trapped object with respect to its surroundings.

4. Multiple tweezers

Optical tweezers have also been configured using multiple beams to trap more than one particle simultaneously. These have been implemented by the rapid scanning of a single beam between two or more trap positions [7, 8], splitting the beam early in the optical circuit to produce two separate light paths which are later recombined before entering the microscope [9] and using computer-generated holograms to give multiple beams simultaneously [10]. Such multiple beam traps allow independent positioning of different objects, or parts thereof.

4.1. *Rapid scanning between trap positions*

If the laser beam is moved with sufficient speed, then multiple objects can be held and manipulated by time-sharing a single beam [7]. Multiple optical tweezers can be created in this way because viscous drag on the trapped objects is sufficiently high to provide positional 'persistence' while the laser beam is elsewhere, servicing another object. In practice, the laser is made to dwell at a number of discrete positions that can be controlled by computer. If two objects are held, then the duty ratio (time spent by the laser at each position relative to cycle time) is 50%, for three objects this falls to 33% and so on. Also, the trapping beam must revisit each object position often enough that the object has not diffused a significant distance. For a typical biological application, an object 1 μm in diameter has a diffusion coefficient D given by Einstein's relation:

$$D = \frac{k_B T}{\beta}, \tag{11}$$

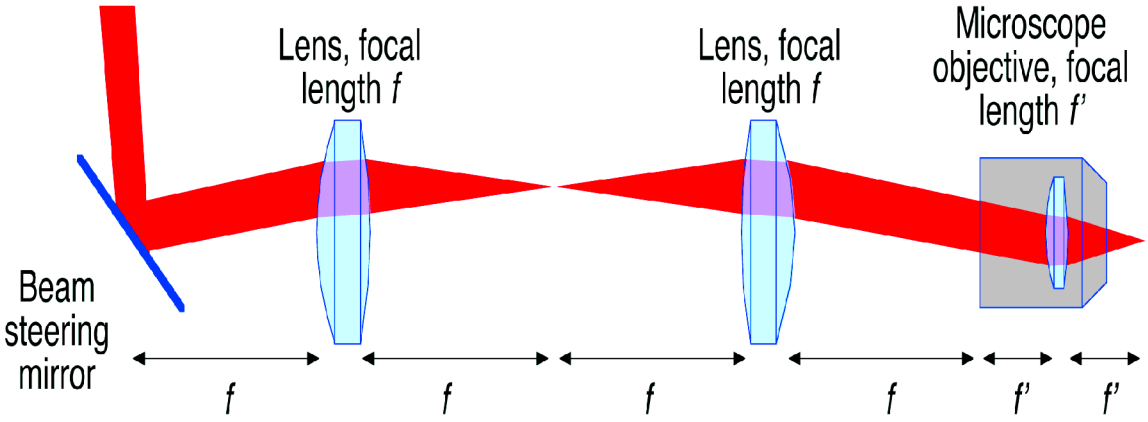

Figure 3. A simple optical relay system to give lateral control of the beam position in the sample plane of optical tweezers.

and a rms diffusion distance d over time t given by

$$d = (2Dt)^{1/2}. \quad (12)$$

For example, for an object 1 μm in diameter suspended in water, the diffusion coefficient is $D = 4 \times 10^{-13}$ m^2 s^{-1}. If the optical tweezers are absent for 25 μs (e.g. two optical tweezers synthesized at 20 kHz) the rms diffusion distance is about 5 nm. This represents a maximum limit to the accuracy to which the spheres can be positioned or their position measured.

We can see that, like a circus performer juggling balls, there is a limit to the number of objects that can be handled in this way. However, if rapid acousto-optic beam deflectors are used, up to eight optical tweezers have been demonstrated. Figure 4 shows four latex beads 1 μm in diameter being held and manipulated using a rapid scan multiplexed system.

Mechanical beam deflectors (e.g. galvanometer mirrors) are less suitable for this approach because they are slow and likely to overshoot or resonate at a high switching speed. However, the mechanical approach offers a wider range of movement together with higher efficiency of light transmission and is therefore better suited to some applications. Note that, because computer control of this system is straightforward to implement, complex optical tweezers geometries can be devised and, if in addition position sensors are used, the separate objects being held can be independently servo-controlled [8] (see section 5).

4.2. *Separate light paths*

A conceptually simpler method to produce dual optical tweezers is to divide the laser into two separate beams [9]. This method requires that the two light paths have separate $x-y$ deflector systems; so control is more complicated (and expensive) and positional stability is harder to achieve. However, unlike the time-sharing method described above, the fact that both optical tweezers are present at all times is appealing when one considers experiments such as stretching single polymer molecules.

4.3. *Holographic methods*

Computer-generated holograms are used widely to convert the fundamental Gaussian mode emitted from most commercial lasers into a beam with a different intensity and phase structure [11, 12]. Although the term 'hologram' is correct, it is more informative to describe such an element as a computer-generated diffraction pattern which gives a diffracted beam of the desired form. In its simplest form, a computer-generated hologram is produced from the calculated interference pattern that results when the desired beam intersects the illuminating laser beam at a small angle. This pattern is then transferred to high-resolution holographic film. When the developed hologram is placed in the original laser beam, a diffraction pattern results, the first order of which has the desired amplitude and phase distribution. When photographically processed as a phase hologram, conversion efficiencies of over 50% are achievable. Although such holograms can be fabricated to produce any beam or combinations thereof, recent advances in display technology and computing power means that holographic patterns can be calculated in real time and transferred to spatial light modulators, giving a dynamically adjustable holographic element.

In 1999, Reicherter *et al.* [10] reported optical trapping with computer-generated holograms written on a liquid crystal display. They adapted a miniature display, removing the polarizing layer to produce a 640×480 pixelated phase shifter with an update rate of 30 Hz. By calculating successive holographic patterns they were able to manipulate three particles independently. In their original system they were limited by the time that it took to calculate each frame and indeed the low diffraction efficiency (less than 10%) meant that a high power laser was required. Subsequently, the same group refined their apparatus to use a more complex display element with a higher diffraction efficiency, a faster computer, and a modified optical system to give independent particle control in three dimensions, using both standard and 'doughnut' modes [13]. Excitingly such a technique seems to combine the

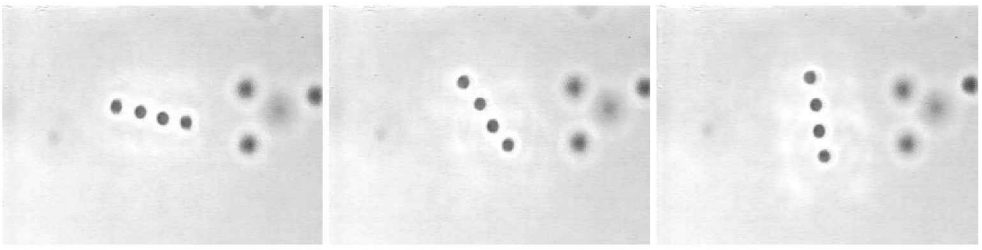

Figure 4. Video images of spheres held by four optical tweezers, which have been synthesized by rapidly scanning a single laser beam using an acousto-optic beam deflector. The single beam was time-shared between the different locations at 10 kHz. Successive frames show how the four trapped plastic microspheres 1 μm in diameter can be independently manipulated.

simplicity of rapid scanning using modulators with the simultaneous trapping of the multibeam approach. Although slight concerns exist over the discreet nature of each frame and the precision with which the resulting beams can be manipulated, this technique can only improve as the associated technologies undergo development. One alternative approach reported recently reduces the required computational power by writing the desired intensity pattern directly to the phase modulator and a phase contrast technique to project this pattern to the trapping plane [14].

5. Biological applications of optical tweezers

Biologists were quick to take advantage of optical tweezers as a tool for purposes such as measuring the compliance of bacterial tails [15], the forces exerted by single motor proteins [16] and the stretching of single deoxyribonucleic acid (DNA) molecules [17]. Optical tweezers have also been combined with an additional laser to form optical scissors [18] or used as part of fluorescence [19], confocal [20] or scanning force [21] probes. In this section we discuss some of the early biological studies and some of the recent high resolution single molecule-experiments.

The first biological studies were made on material that was large enough to manipulate directly using optical tweezers. Ashkin and co-workers used optical tweezers first to capture bacteria and small numbers of tobacco mosaic virus [22], then to manipulate single cells [23] and cell organelles [24] and finally to measure the force of cell organelle movement inside living cells [25]. In 1989, Block *et al.* [15] made the first calibrated measurements of the compliance of bacterial flagellae, using the tweezers to grab and forcibly to rotate bacteria that had become tethered to a microscope coverglass by their flagellum. They calibrated the forces applied by the optical tweezers from the time constant of elastic recoil of the bacterium in the viscous medium. This study paved the way to making calibrated measurements using optical tweezers as a force transducer.

In most of these studies, rather than manipulating the biological material directly it is usual to attach it to a latex (polystyrene) microsphere. Biological molecules (e.g. DNA and proteins) are less than 25 nm in diameter and are therefore too small to be manipulated on their own. Also, the reproducible size and even shape of the synthetic microspheres used allows easy calibration of the system. When a microsphere is used indirectly to manipulate material, it is often referred to as a 'handle'.

5.1. *Optical tweezers based single molecule force transducers*

Until the mid-1980s, much of what was known about the mechanical properties of biological materials was derived from testing of bulk material. From such experiments the properties of individual molecules were inferred by modelling the entire system. Such models gained molecular scale information from structural studies made before or after testing. However, the modelling exercise often depends critically upon a number of uncertain parameters, for instance the number of molecules being strained during the test, how they are connected in series and parallel and, perhaps most importantly, whether the individual molecules undergo structural rearrangements gradually or whether such structural changes are rapid and catastrophic. Before going further, it is helpful to consider the sensitivity and range of a transducer required to study different biological processes, in terms of the energy, force, position and time.

Energy: 1 photon = 400 pN nm; hydrolysis of 1 adenosine triphosphate (ATP) molecule = 100 pN nm; 1 ion moving across a biological membrane = 30 pN nm; thermal energy = 4 pN nm.

Force: required to rupture a covalent bond = 1 nN; required to convert DNA from a double helix to a ladder = 50 pN; required to break most protein-protein interactions = 20 pN; produced by most motor proteins = 5 pN.

Length: diameter of a bacterium and optimal size for beads held in optical tweezers = 1 μm; resolution of light microscope = 300 nm; diameter of eukaryotic cell organelles = 100 nm; large protein assemblies and virus particles = 25 nm; work stroke produced by motor protein = 5 nm; diameter of hydrogen atom = 0.1 nm.

Time: cell division = minutes; cycle time of many biochemical processes = seconds to milliseconds; individual biochemical steps = milliseconds to microseconds; protein conformational changes = nanoseconds; molecular dynamics = picoseconds.

In order to convert optical tweezers into an instrument for measuring small forces (a force transducer), we require the addition of a sensor capable of measuring the position of the trapped objects. The layout of a typical transducer, suitable for making measurements on biological molecules is shown in figure 5.

As mentioned earlier, because proteins and DNA are so small, modern studies use plastic microspheres as handles to manipulate the material under test indirectly. From the preceding sections we know that, for single beam tweezers to work, we must use transparent objects with diameter of about 1 μm. The position of such uniform microspheres, held in optical tweezers, can be

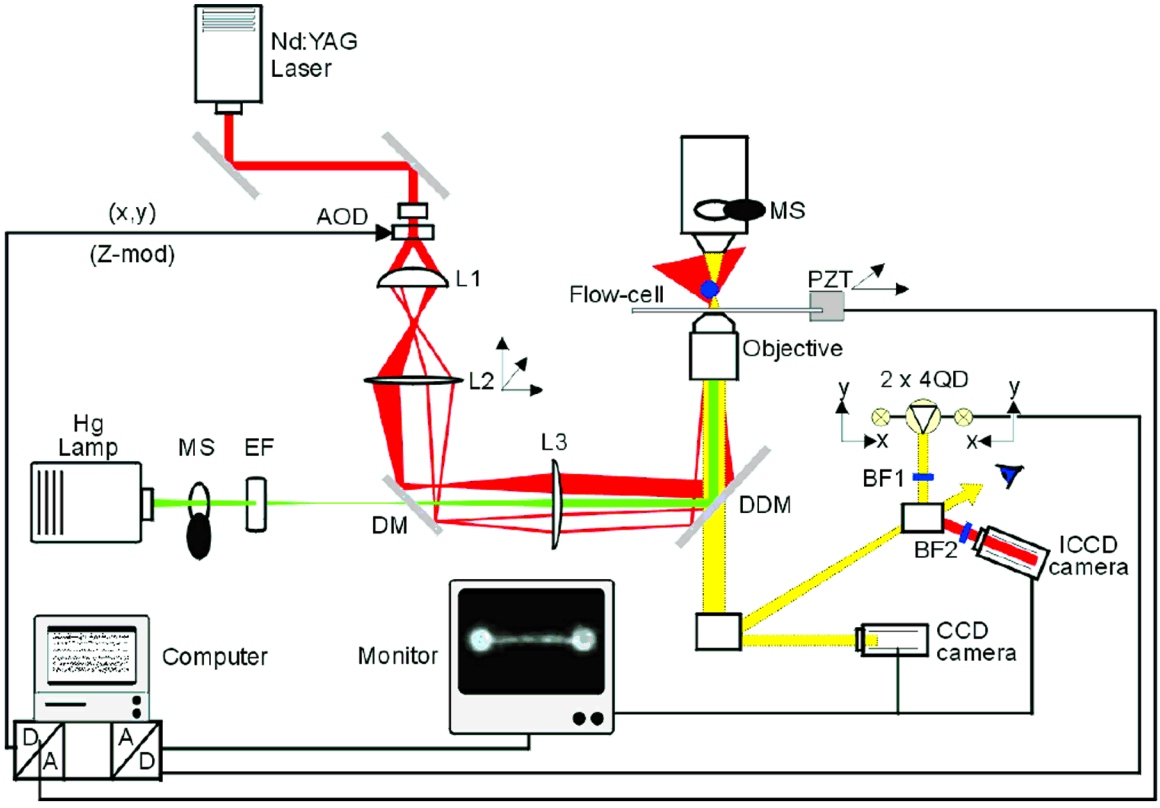

Figure 5. Layout of a multiplexed dual optical tweezers transducer for making measurements of force and movement produced by single molecules. The apparatus is based around a conventional research grade inverted microscope (e.g. Zeiss Axiovert). The optical tweezers are produced by a diode-pumped neodymium-doped yttrium aluminium garnet (Nd:YAG) laser introduced using a light path similar to figures 2 and 3. A dual dichroic mirror (DDM) is used to reflect infrared and green light but to transmit red light from fluorochromes. Mechanical shutters (MS), dichroic mirrors (DM), excitation filters (EF) and barrier filters (BF) together with an intensified charge-coupled device (ICCD) camera allow observation of fluorescent proteins. Central to this particular design is a computer that controls the piezoelectric transducer (PZT) on the microscope, the beam steering optics (acousto-optic deflectors AOD) and also collects data from the two four-quadrant imaging detectors (4QD) that monitor the position of both objects. Computer software allows a closed feedback loop to control the bead positions to compensate for any detected motions.

determined using a four-quadrant photosensor to measure the 'centre of gravity' of the object, either using conventional imaging (bright field, dark field or phase contrast) or by interferometry. If a very intense illumination source is used, then it is possible to measure nanometre displacements over a bandwidth of 0.1 Hz to 5 kHz. The sensor and detection circuitry is calibrated by moving either the sensor itself or the trapped objects through a known distance. Once the position calibration factor is known, then the optical tweezers stiffness can be calculated either from analysis of the thermal motion of the trapped object (knowing equation (9) [figure 6 (*c*)]) or by application of known viscous drag forces (either from spectral analysis, equations (8) and (10) [figure 6(*b*)] or by applying a viscous drag force $F = \beta v$). Usually, a combination of approaches is used to check that they are consistent. Most apparatus are based around research grade fluorescence microscopes, which enable fluorescently tagged proteins to be observed simultaneously. Computer control is an essential feature as it allows many different types of experiment to be performed, automatic calibration routines and rapid data collection, storage and analysis.

At the beginning of this section, we introduced the typical distances, forces and energies involved in making single-molecule measurements. From this, we know that, to measure the movement produced by a single protein conformational change, we expect displacements in the nanometre range. Knowing the energies involved, this requires optical tweezers with a stiffness of about 0.02–0.1 pN nm^{-1}, needing 5–30 mW of laser power. At such low stiffnesses, we find that, in the absence of any biological

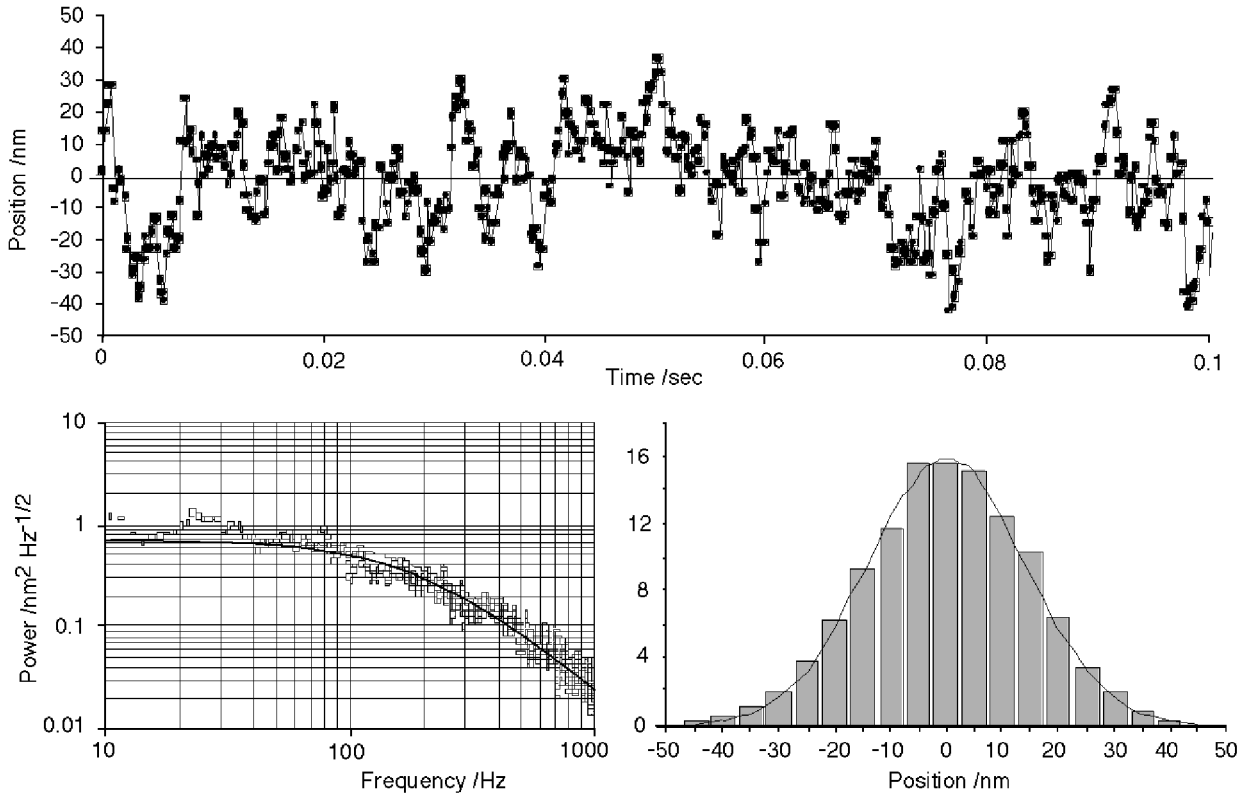

Figure 6. Operation of an optical tweezers transducer. (*a*) The motion of a plastic microsphere 1.5 μm in diameter held in optical tweezers of $\kappa = 0.018$ pN nm^{-1}. Note that the bead position fluctuates on a relatively slow time scale (tens of milliseconds) because of the high viscous damping and low tweezers stiffness. (*b*) Spectral analysis of the movement shows the expected Lorenzian behaviour with characteristic single-pole roll-off. (*c*) The histogram shows how the number of observations of the bead at position x is determined by a Boltzmann distribution given by $N = \exp(-\kappa x^2/2k_BT)$.

forces, the motion of a trapped microsphere is determined by the balance between thermal Brownian motion and the tweezers restoring force (figure 6).

As we can see from figure 6, the behaviour of an optical tweezers transducer is very different from that of a macroscopic force transducer; this is because it works in the very low force regime, where thermal forces are significant. It is also rather different from the atomic force microscopes because the motion of an atomic force microscopy probe is dominated by its relatively high mass and high stiffness; so the probe tip shows resonant behaviour. It is essential that the behaviour of the transducer is well understood and appreciated before we move on to discuss some of the biological measurements that have been made. The idea of the remainder of this section is simply to whet the reader's appetite; there are to date nearly 500 publications that use optical tweezers to make measurements from biological materials. To do each study justice would require an explanation of the biological system as well as the measurement made—an impossible task.

5.2. *Observing single biological motors at work*

One of the best-studied and most interesting problems in biophysics is the muscle contraction mechanism. In recent years, it has become clear that all living cells contain a wide variety of *molecular motors* that take chemical energy and convert this to mechanical work. They perform a multitude of functions that are essential to life, from DNA replication, ribonucleic acid (RNA) transcription and protein synthesis to cell division, vesicle trafficking, cell locomotion, endocytosis and of course, the best known example, muscle contraction. There are two types of motor. 'Rotary motors' are usually embedded in membranes and are driven by the flow of ions across transmembrane electrochemical gradients; the bacterial flagellar motor is a good example. 'Linear motors' work in an isotropic chemical environment and derive energy from chemical reactions, usually the hydrolysis of the chemical ATP to adenosine diphosphate (ADP) and phosphate. Linear motors move along anisotropic (or polarized) protein tracks that therefore confer direction-

ality to the movements produced; a good example is the muscle protein system.

The linear motors can be further subdivided into two classes: 'porters' and 'rowers' [26]. Linear motors that take many successive steps and effectively walk along their filament track without diffusing away, are known as 'porters' (processive enzymes), while those that interact in an intermittent fashion producing just a single tug and then releasing from the track, are called 'rowers' (non-processive enzymes). For example, the *kinesin* motor that 'walks' along *microtubules* in your nerve cells, carrying membrane bound bags of neurotransmitter from your spine to your finger tips is a *porter*, while the motor protein in muscle called *myosin* is a *rower*, as it acts as part of a large team of molecules, that each give a quick tug on the *actin* filament track as it slides past causing muscle to shorten. Like a porter, kinesin rarely drops its bags and like a rower, muscle myosin works in a team to produce large external forces and rapid shortening.

The rotary motors and both classes of linear motor (porters and rowers) have been studied using optical tweezers. We have already heard how Block *et al*. [15] teamed up to make the first calibrated measurements of the stiffness of the bacterial flagellum. Since then, Berry and Berg [27] have made a more detailed study of the bacterial rotary motor mechanism by measuring the force produced by both its forward and its reverse rotation. They used a plastic bead held in an optical tweezers as a handle to push against the bacterium and to rotate it around its fixed flagellum, thus applying torque to the motor system. They found that the torque required to push it backwards is only a little more than that which stops it. This indicates either that the motor can slip, or that the mechanism is reversible and the transition from motor to pump (dynamo) is seamless. Such studies give great insights into the molecular mechanism of this biological electric motor.

A landmark in the study of single motor proteins came in 1993 when Svoboda *et al*. [28] measured the individual steps taken by the molecular porter, kinesin, as it walked along a fixed microtubule track. In their experiments, a single kinesin molecule was bound to a plastic microsphere and this was then held close to its microtubule track, which had been fixed to a microscope coverslip. The bathing medium was a buffered salt solution containing the chemical fuel ATP. When the kinesin motor and microtubule track interacted, the bead was pulled along by the kinesin and the nanometre scale displacements and piconewton forces produced were measured. The crucial observation made, and indeed a testament to the incredible sensitivity of their method, was that they could identify discrete 8 nm steps taken by the kinesin molecule. Rather than moving smoothly like an ensemble would do, the single molecule moved in a stochastic jerky fashion. They found that the motor paused for a random interval after taking each step as it waited for a fresh ATP molecule to arrive. The excitement of these measurements should not be forgotten! Molecular-scale motions powered by just 1 ATP molecule (equivalent to less than one tenth of the energy of a single photon) were being observed in real time, with no signal averaging required. Figure 7 shows data from a more recent study (N.J. Carter and R.A. Cross, unpublished data) using the same protein system.

The next challenge was to make similar measurements from myosin, the molecular rower obtained from muscle. One might think that this would be a straightforward progression from the Svoboda *et al*. study, however, it turned out that in order to measure the activity of intermittent biomolecular interactions a different approach was required. In 1994, Finer *et al*. [16] devised a way to hold the two proteins, actin and myosin, close to one another for long periods of time so that many individual interactions could be recorded from a single molecule. Their method required a dual optical tweezers system (figure 8 (*a*)). Using the twin tweezers, to hold a single actin filament suspended between two plastic microspheres the filament could then be placed in the vicinity of a third fixed microsphere that had been sparsely coated with myosin. Because the bathing solution contained the chemical fuel ATP, when the proteins made contact, one molecule of fuel was broken down and a single kick or displacement of actin occurred. The resulting movement was measured by monitoring the position of one of the trapped beads. A year later, Molloy *et al*. [28] performed a similar study but they realized that because the intermittent movements produced were so small (only about 5 nm) the starting position for each interaction observed was being randomized by the thermal vibrations of the beads in the tweezers. By monitoring the variance of the position signal to detect myosin binding (the variance $\langle x^2 \rangle$ falls when myosin binds because the system stiffness is increased) they were able to measure many hundreds of myosin binding interactions and then later to deconvolve the myosin induced motion, x_0, from the thermal motion by fitting the distribution of step sizes to the equation $N = \exp\left[-\kappa(x - x_0)^2/2k_BT\right]$ (see captions for figures 6 and 8 (*b*)).

The studies on motor proteins are likely to be paradigms for future single molecule mechanical studies and the basic methods devised will be useful for studying systems in which the biomolecular interactions are either processive (porters) or non-processive (rowers). Readers who would like further information on optical-tweezers-based studies of rotary and linear motor proteins are directed to the excellent recent reviews [29, 30] and references therein.

Clearly, the mechanical properties of motor proteins are of interest since they have a direct mechanical function, but what about other biological molecules? We know that the mechanical properties of DNA and proteins are crucial.

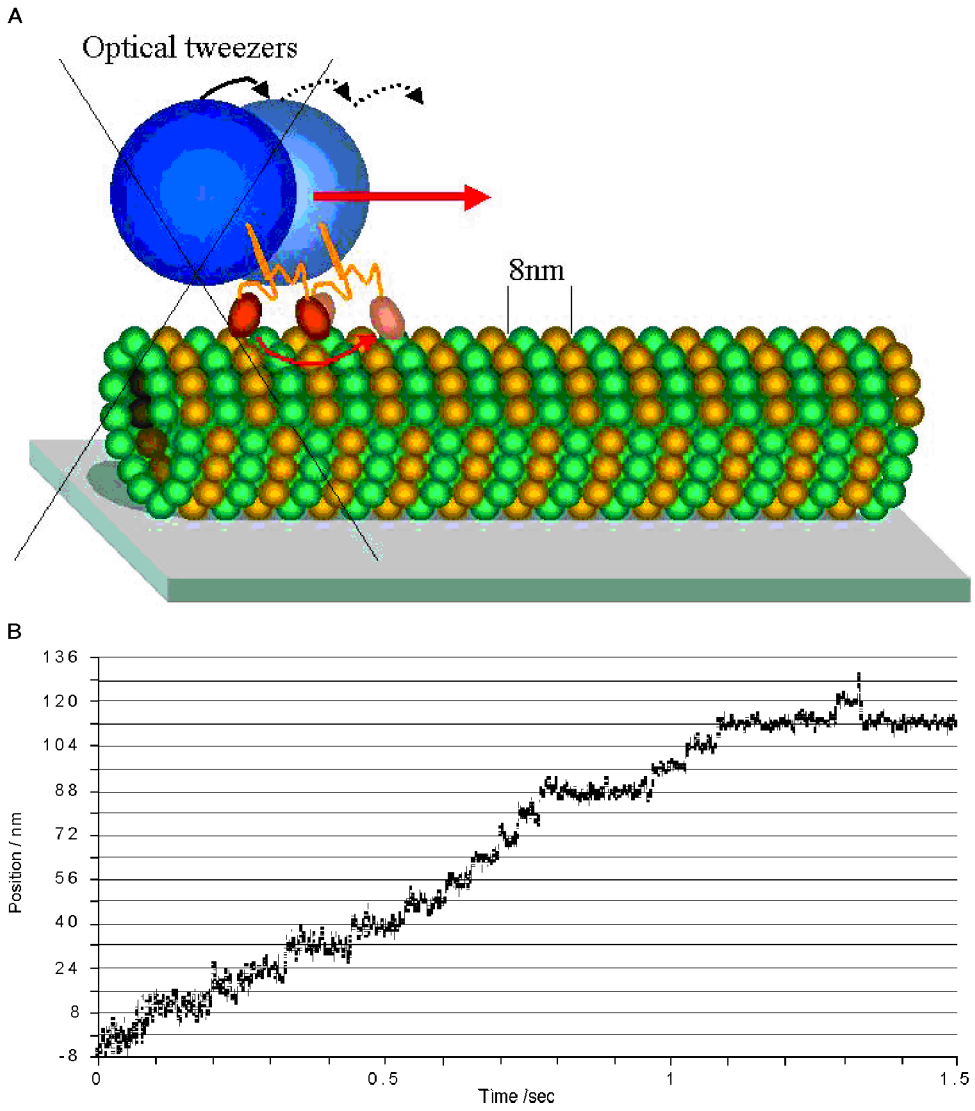

Figure 7. Mechanical recording made from a single processive kinesin, a molecular porter that walks along the microtubule track taking steps that are commensurate with the microtubule 8 nm lattice repeat. In the experiment a single (double-headed) kinesin molecule was attached to a latex microsphere held in optical tweezers. (*a*) The position of the microsphere was monitored using a four-quadrant detector, as the kinesin walked along a fixed microtubule track. (*b*) Note that the staircase structure to the position data is a direct result of the single kinesin pausing in between individual ATP cycles. This record was kindly provided by Dr N.J. Carter and Dr R.A. Cross, Molecular Motors Group, Marie Curie Research Institute, Oxted, Surrey. For further information, see http://mc11.mcri.ac.uk/motorhome.html.

For instance the formation of DNA loops, bubbles, hybridized strands, supercoiling, unwinding and the correct folding of proteins and dynamic conformational rearrangements are key to their function. In fact, many of the problems in biology can be framed and addressed in mechanical terms. People have already started to pull and bend DNA and to stretch proteins to make them unfold and then to refold. The technical problem with such studies is how to link the molecules specifically between the optical tweezers transducer (a plastic bead) and mechanical ground (usually the microscope coverglass). The obvious starting point for these studies has been to use large molecules so that attachment artefacts are minimized. We shall finish this section by briefly discussing mechanical studies of DNA and unfolding studies made on the giant protein titin.

The double-helix structure of DNA is probably the most famous discovery in structural biology. In one of the earliest mechanical studies using optical tweezers a length of DNA was attached to a plastic bead and then extended by dragging through a viscous medium in order to measure

its mechanical properties [31]. This gave useful information about its polymer mechanics. Knowledge of its mechanical persistence length (about 50 nm) informs the biologist about the probability that adjacent regions of DNA come in contact with one another. For instance, we know that, if two pieces of sequence are 20 nm apart, then the intervening length of DNA is relatively stiff, if they are 500 nm apart, then the linking region will be bendy enough to allow the two regions to come together. Later, the Bustamante group [32] applied very large forces (greater than 60 pN) to DNA and found that it suddenly over-stretches and breaks the double-helical B-form to give a parallel ladder.

With the ability to manipulate DNA and a proven track record of working with motor proteins like kinesin, Block and co-workers [33] set claim to another first when they measured the force produced by RNA polymerase as it transcribed a DNA gene. They attached a plastic bead to one end of a DNA strand and then allowed the other end to bind to an immobilized molecule of RNA polymerase. When 'transcription buffer' was added (containing all the necessary nucleotides for transcription), RNA polymerase moved along the DNA, proceeding with its job of transcribing the gene and producing a new RNA chain. At that moment, they grabbed the bead that was attached to the free end of the DNA and then measured the pulling force produced by the RNA polymerase. The force was about 25 pN; perhaps surprisingly this is five times greater than that produced by either of the studied motor proteins myosin or kinesin.

The ultimate polymers in nature are made from amino acids, the proteins. As we know, the amino acid sequence of a protein is coded by DNA and the amino acid chain is linked together by peptide bonds. Using just 20 different types of amino acid as basic building blocks, nature creates a huge diversity of different proteins; from structural proteins that are stronger than steel, to signalling molecules that make the human brain work to motors that are powered by single molecules of fuel. The secret to how all proteins work is held in the formation of their tertiary structure, how the amino acids fold up to build the protein. An exciting application of optical tweezers has been deliberately to unfold and refold protein modules or domains.

Tskhovrebova *et al*. [34] used optical tweezers to pull on a giant protein called titin, which consists of about 9000 amino acid residues. Titin is a structural protein obtained from muscle that helps to maintain muscle structure and also makes relaxed muscles slightly springy. They attached one end of titin to a plastic bead (the optical tweezers handle) and the other to a microscope coverslip. They pulled hard on the protein so that it experienced a force of about 100 pN; then, over a period of a few seconds, individual protein domains unfolded catastrophically to relax the tension in a stepwise fashion. The individual unfolding events could be quantified in terms of their lifetimes and by the extension produced as the tertiary folded structure unwound to give a linear chain of amino acids. The excitement of these measurements is that in the future it may be possible to follow the folding and unfolding of different amino acid sequences and so to deduce the energy profile of the folding process. Such studies will give great insight into the protein folding mechanism and it is crucial that we understand this fully, if we are to make sense of the new genomic data.

6. Alternative tweezers configurations

Clearly the biological applications of optical tweezers have been wide ranging; however, optical tweezers themselves have been undergoing interesting developments in a number of areas.

6.1. *Rotation within optical tweezers (optical spanners)*

The various methods that have been used for achieving rotation of particles held in optical tweezers are summarized in figure 9.

Physicists have used optical tweezers as a tool to study the transfer of angular momentum from light to particles. For particles trapped on the beam axis, both the spin and the orbital angular momentum of a light beam have been shown to cause rotation of birefringent [35] and absorbing [36, 37] particles respectively (figures 9 (*a*) and (*b*)). For absorbing particles, spin and orbital angular momentum can be transferred simultaneously and with the same efficiency so that the applied torque is proportional to the total angular momentum [38, 39]. Most recently, optical tweezers have been used to explore the intrinsic and extrinsic nature of a light beam's angular momentum by examining the motion of particles trapped away from the beam axis [40]. However, it should be emphasized that the prime motivation behind most of this work lay in the study of the optical properties of the beams rather than specifically as a technical tool.

For practical applications, rotation within optical tweezers has a history dating back to 1991 when Sato used the gradient forces associated with a rotating high-order Hermite–Gaussian mode to induce the rotation of red blood cells [41] (figure 9 (*c*)). As discussed earlier, the gradient force acts to draw a dielectric object towards the maximum intensity. In the case of a rectangularly symmetric beam, this forces an asymmetric particle, such as a cell, to take up a particular orientation (figure 10).

Rotation of the laser mode then leads to a direct rotation of the cell. Indeed, in their early work, Ashkin *et al*. observed that this inherent alignment of objects with the

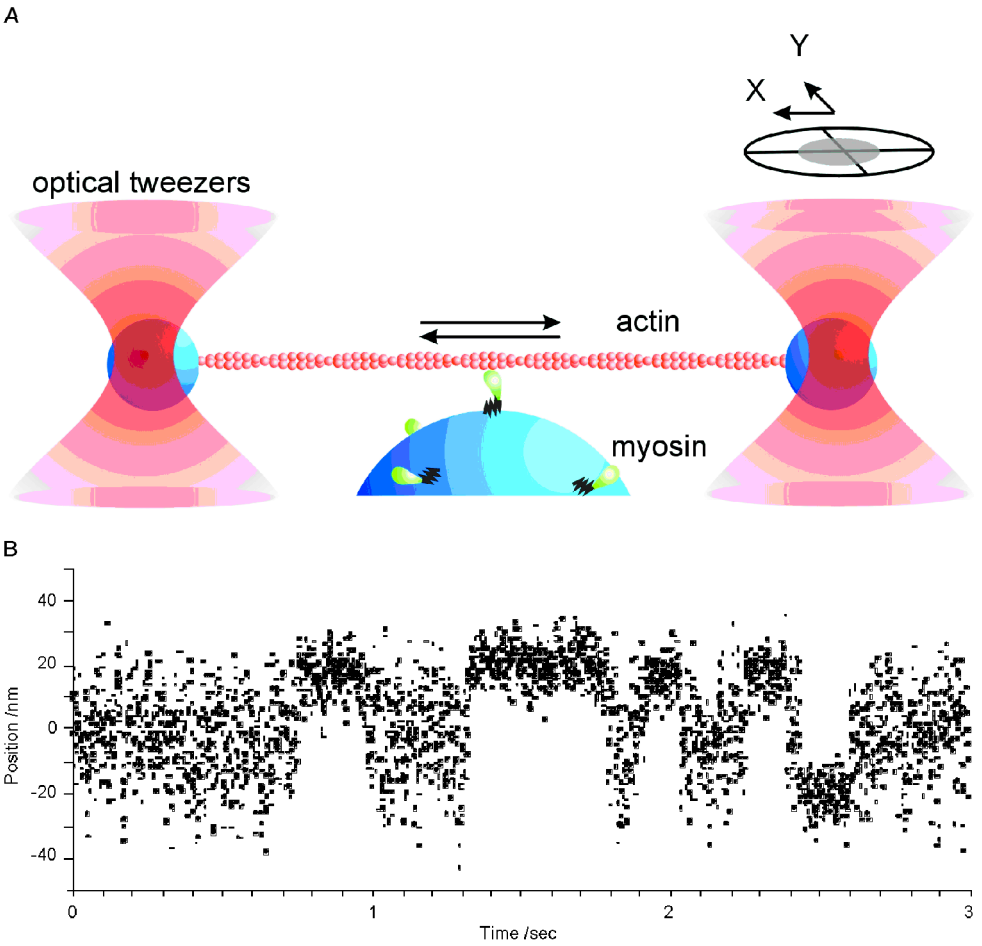

Figure 8. **Mechanical recording made from a single non-processive myosin, a molecular rower that interacts intermittently with actin. (*a*) The actin filament and myosin must be positioned close to one another so that multiple individual events can be observed. Myosin is coated on a large glass microsphere fixed to a microscope coverslip, while actin is suspended between two beads held using a dual optical. By monitoring the position of one or both beads held in the optical tweezers, individual binding events can be observed. (*b*) Note that the thermal noise is reduced during binding events because the actomyosin bond forms a stiff link to mechanical ground.**

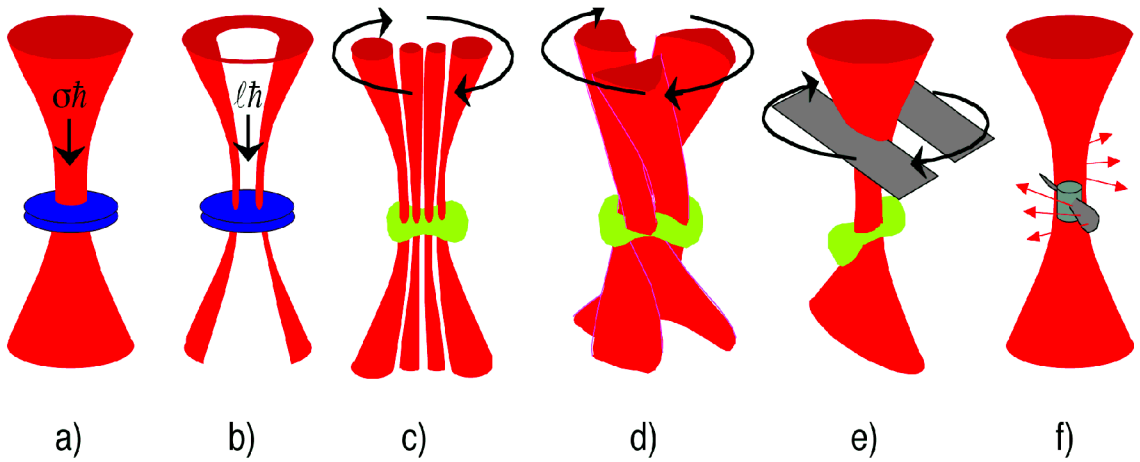

Figure 9. **The various schemes employed to achieve rotation of a particle within optical tweezers; transfer of (*a*) spin and (*b*) orbital angular momentum of the light beam to the trapped particle, torque generated by the gradient force created by (*c*) rotation of a high-order mode, (*d*) rotation of an asymmetric interference pattern or (*e*) rotation of an aperture and finally (*f*) the radiation pressure acting on propeller-shaped objects (see text for further details).**

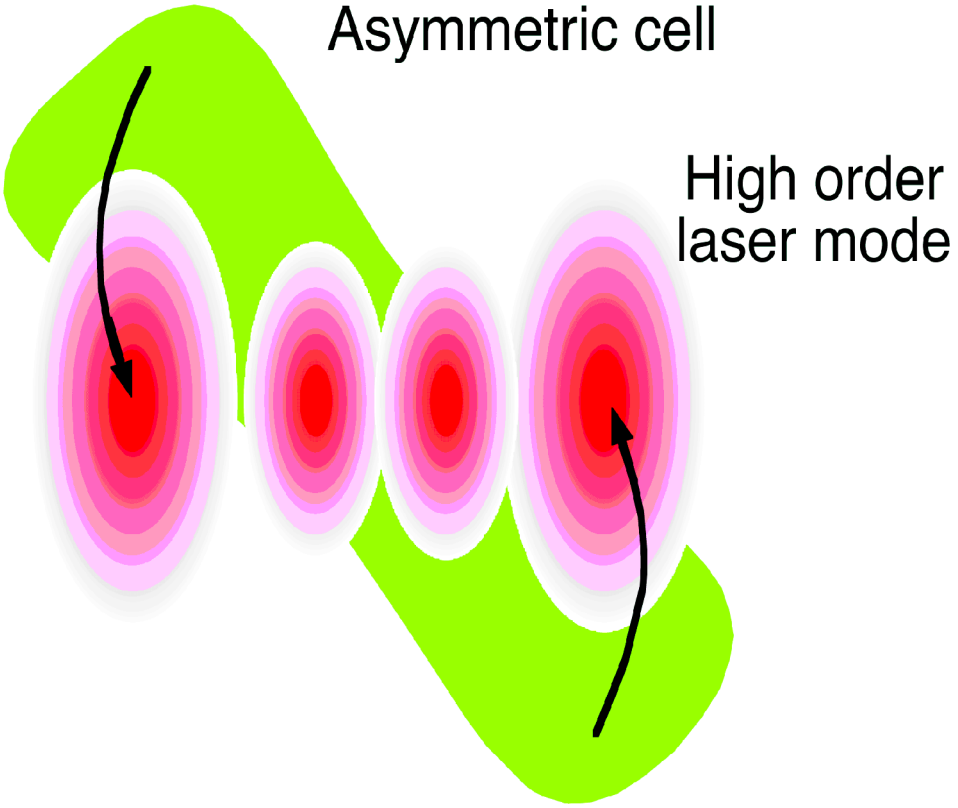

Figure 10. The gradient force associated with a rectangularly symmetric mode will create a torque on an asymmetric cell, causing alignment of the cell with the beam.

beam symmetry could cause rod-shaped bacteria to stand upright, aligned vertically along the trapping axis of the beam. It has also been proposed to use an elliptical beam for the same purpose [42]. Although simple in concept, the rotation of a laser beam exactly about its own axis is more difficult than might appear. For example, a rotating Dove prism is well known to rotate a transmitted image or beam, but such a prism requires precise angular and lateral alignment to a level that is difficult to achieve at optical wavelengths [43].

One novel way to obtain a rotating asymmetric intensity pattern is to cause a Laguerre–Gaussian light beam, which has helical wavefronts, to interfere with a plane wave (figure 9 (*d*)). The resulting spiral interference pattern can then be rotated by changing the path difference between the two beams. Such an approach has been applied to rotate a Chinese hamster chromosome [44]. The technique is adaptable in that changing the indices of the Laguerre–Gaussian mode changes the rotational symmetry of the interference pattern thereby potentially optimizing the shape of the intensity pattern to the shape of the object to be rotated.

Perhaps the most obvious way to generate an asymmetric beam is to introduce an aperture into the optical path (figure 9 (*e*)). Mounting the aperture within a rotation stage that is itself mounted on an $x-y$ translation stage means that its rotation axis is easily aligned to the beam axis. Indeed a rotating rectangular aperture mounted in the tweezing beam has been used to introduce a rotation in this way [45]. In that work, a rectangular aperture 4 mm wide was placed into a beam 10 mm in diameter, producing a focused spot in the sample plane with an ellipticity of approximately 2:1. Assemblies of silica spheres 2 μm in diameter were trapped in three dimensions and would rotate synchronously with the aperture (figure 11). Rotational rates of a few hertz are possible.

This simple method for rotational control does not require high-order modes, interferometric precision or computer-controlled optical modulators and can be easily applied to, or removed from, existing tweezers apparatus.

Another approach to inducing motion within optical tweezers is to rely upon the shape of the trapped object and the reflection or scattering of the light beam's linear momentum, that is the radiation pressure (figure 9 (*f*)).

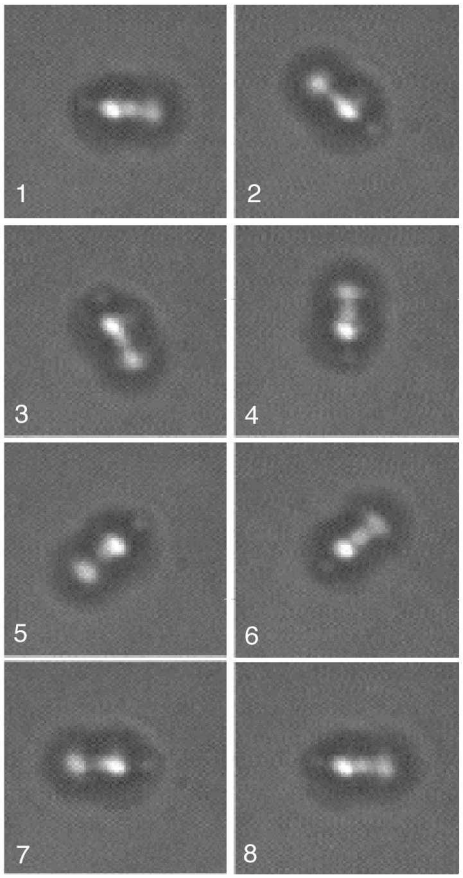

Figure 11. Video frames showing the rotation of an assembly of silica spheres, set into rotation using a rotating aperture. In this case, the asymmetric object consists of two silica spheres of 2 μm diameter fused together.

This method is analogous to that of a windmill where it is the shape of the blades that determines the sense and efficiency of rotation. In 1994, Higurashi *et al*. [46] fabricated four-armed rotors, 10–25 μm in diameter, from silicon oxide. They observed that, when placed in optical tweezers, these rotors were trapped in three dimensions and set into rotation. The sense of the rotation was determined by the handedness of the rotor construction. A further point of interest is that, unlike most micro machines that suffer from frictional wear, the three-dimensional trapping of optical tweezers requires no mechanical contact. Suitable rotors have also been assembled from partly silvered spheres and in that case, rotation speeds of several tens of hertz have been achieved [47].

Most recently [48], two-photon polymerization of light-curing resins has been used to fabricate micro machined gearwheels a few microns in diameter. One of the gearwheels was then held in the optical tweezers and set into rotation by the radiation pressure. Moving the trap position so that the teeth of this gearwheel engaged with a second gearwheel, set the latter into motion. The result was the demonstration of a multicomponent micromachine driven by light.

6.2. *Interferometric optical tweezers*

One new approach to optical tweezers was reported in 1997 when Choui *et al*. [49] used two optical beams to create an interference pattern, the intensity profile within which gave rise to a gradient force confining both spherical and 'rod-like' particles. In that work they used a 20× microscope objective of 0.4 numerical aperture and two interfering beams to give a fringe spacing in the sample plane of approximately 3 μm. Changing the path difference between the beams caused a translation of the interference pattern and the particles trapped within. One particularly interesting aspect of that work was that, by ensuring the two beams were slightly displaced, the interference fringes were confined to a central portion of the field of view with 'non-fringed', high intensity regions on either side. These spots of high intensity acted as 'end stops' for the motion, providing complete confinement of the manipulated particles. The same group also pointed out, and indeed demonstrated, that a similar intensity pattern could be obtained using a single beam to project an image of a Ronchi grating into the sample plane. In the latter case, movement of the trapped particles was achieved by translation of the grating itself.

Most recently this interference technique has been applied to the simultaneous trapping of both low-index and high-index particles [50], where the high-index and low-index particles are confined to the regions of high intensity and low intensity respectively.

6.3. *Optical tweezers with high-order Gaussian modes and other beams*

The first deliberate use of a laser beam within optical tweezers that was not a fundamental Gaussian mode was that discussed in section 6.1, namely a high-order Hermite-Gaussian mode to force the specific alignment of a asymmetric object within the optical trap. Following that work, significant interest by many groups has been shown in using computer-generated holograms to produce Laguerre–Gaussian light beams for use within optical tweezers. These beams can possess helical wavefronts which carry an orbital angular momentum [51], the transfer of which to particles has again been referred to in section 6.1. Associated with the helical wavefronts is an annular intensity distribution with a zero on-axis intensity, sometimes referred to as an optical vortex. The annular nature of these beams leads to two further applications within optical tweezers. When trying to trap particles

which have a lower refractive index than their surrounding media, the direction of the gradient force is reversed. Consequently, rather than being attracted to the highest intensity region, the low-index particles are repelled from the beam axis. Use of a Laguerre–Gaussian mode overcomes this problem because, once within the annular ring, a low-index particle experiences a gradient force again directed to the beam axis. Called 'vortex traps', optical tweezers based on Laguerre–Gaussian modes have enabled the three-dimensional confinement of hollow glass spheres between 2 and 50 μm in diameter [52, 53]. It is interesting to note that, for a beam directed downwards, the stable trapping position for low index spheres lies just above the beam focus. The same group demonstrated that these vortex traps could simultaneously confine high-index particles to the normal position of just below the beam focus.

In his early considerations of the trapping mechanism, Ashkin pointed out that, since the trapping force results from a change in ray direction, it is only the off-axis rays that contribute to the axial trapping force. Indeed, his calculations predicted that modest improvements in Q_{axial} were possible using a $\text{TEM}_{01}{}^{*}$ 'doughnut' mode, which has no on-axis rays. With their zero on-axis intensity, Laguerre–Gaussian modes also satisfy this condition. Indeed recent experimental results confirm that high-order Laguerre-Gaussian modes do, at least for spheres above 2 μm in diameter, improve the axial trapping efficiency of optical tweezers [54].

A further recent development in optical tweezers is the use of Bessel light beams. Bessel beams [55], which are frequently referred to as 'diffraction free' beams, consists of a bright central spot surrounded by concentric rings of decreasing intensity. Over a limited range, the central region of the beam propagates without changing shape, creating an intensity distribution which has no gradient in the propagation direction. In 2001, Arlt *et al*. [56] used Bessel beams within an optical tweezers to trap objects laterally in two dimensions and most excitingly to obtain stacking and guiding of the trapped objects along the bright

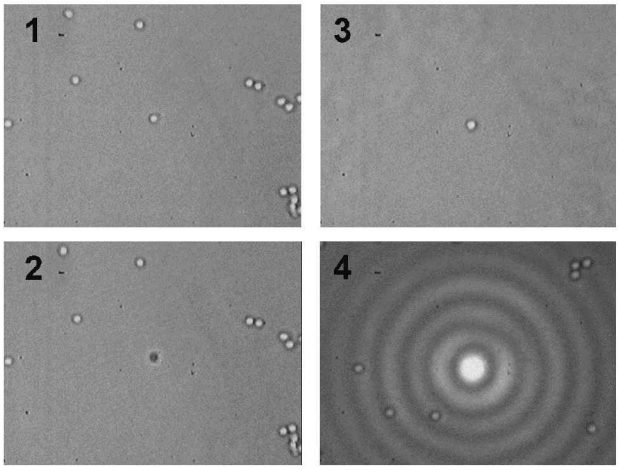

Figure 12. Successive video frames showing guiding of a 5 μm sphere (centre of frame 1) along the central maximum of the Bessel beam (see frame 4). Note that the sphere rises and goes out of focus in frame 2. Frame 3 is refocused at the top of the sample. This record was kindly provided by Dr J Arlt, Dr V. Garces-Chavez and Dr K Dholakia, School of Physics and Astronomy, University of St Andrews, Scotland. For further information, see http://www.st-and.ac.uk/~www_pa/group/atomtrap/.

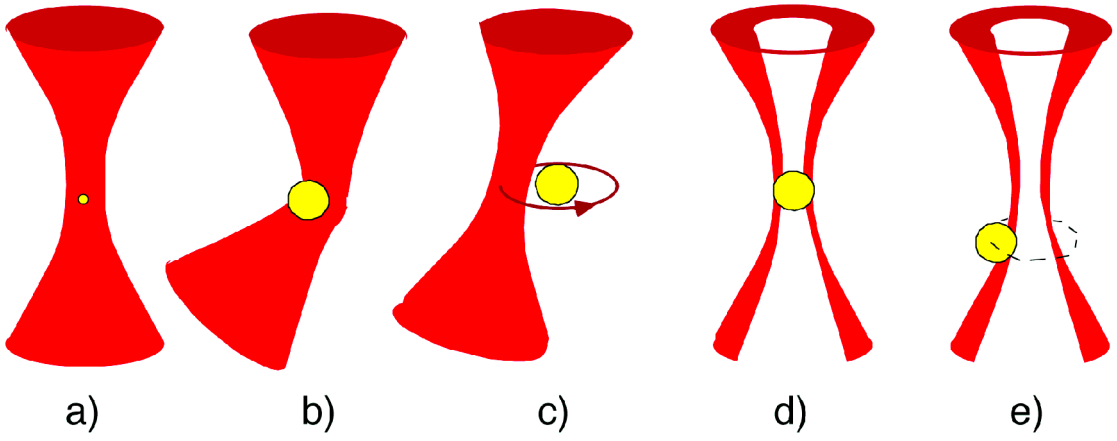

Figure 13. Schemes employed for trapping metal particles: (*a*) submicron particles trapped at the beam focus by the force arising from light scattering; (*b*) Mie particles trapped by the surface plasmon wave; (*c*) larger particles trapped by rotating or (*d*) annular beams to confine that particle or (*e*) by light scattering around the outside of a Laguerre–Gaussian mode.

central core of the beam (figure 12). In this case the Bessel beam was created from a standard Gaussian beam using a specially fabricated glass axicon, although computer-generated holograms could also be used for the same purpose.

6.4. *Optical tweezers using diode lasers*

When considering the practical applications of many laser based systems there is the obvious desire to make the laser as simple as possible. This invariably means asking the question: can we use a diode laser? It is perhaps a reflection of the fact that most optical tweezers work to date has been completed within specialist laboratories, that in the main, optical tweezers are configured using more complex, albeit more adaptable, laser systems. However, if optical tweezers are to become a routine tool, the transition to diode lasers is clearly attractive. The first use of a laser diode within optical tweezers was reported in 1991 [57]. In that work, a 1300 nm $In_{x}Ga_{1-x}As_{y}P_{1-y}$ diode was shown to trap both silica spheres and yeast cells with an efficiency comparable with a conventional laser. One advantage offered by diodes is that most biological samples are highly transparent in the infrared, and the absence of light absorption and the corresponding heating reduces the risk of inducing cell death, so called 'optication'.

6.5. *Optical tweezers for metal particles*

The various methods that have been used for trapping metal particles are summarized in figure 13. Metallic particles scatter, reflect and absorb much more strongly than the transparent particles normally held within optical tweezers. Consequently, the balance between the gradient and scattering force is different, making the trapping of metal particles much more difficult. In terms of refractive index, absorption corresponds to an imaginary component of the refractive index. Small (compared with the optical wavelength) Rayleigh particles (10–50 nm in diameter) have been trapped in three dimensions as in this size regime scattering from metal and scattering from dielectric particles are similar [58]. Larger Mie particles have been trapped in two dimensions, the forces arising from a creeping wave induced by a surface plasmon [59]. Metal particles have also been confined by annular rings [60] or rotating beams [61], which rely on light scattering to produce a repulsive force and to trap the particle in two dimensions. Three-dimensional confinement has also been achieved to an annular region around the outside of a Laguerre–Gaussian mode [62]. When the trapping beam is directed upwards, the scattering force provides both vertical and radial restoring forces.

7. Possible future uses of optical tweezers

Regarding the technical development of optical tweezers it seems as if the ongoing development of spatial light modulators will have a significant impact. Not only will these allow the building of simple multibeam tweezers without problems associated with limited dwell times but also they will replace the static holograms currently used for alternative beam generation. In the latter case, a real time addressable light modulator will allow the user to switch between different trapping beams, allowing independent control of the axial and lateral trapping force.

In general, few of the non-biological applications of optical tweezers have been explored. Optical forces associated with scattering, the gradient force or angular momentum transfer have been postulated as a means to drive micromachines and so far the scattering force in particular has looked promising. Another area in which optical tweezers have not been exploited is in the assembly of the micromachines themselves, or microstructures such as photonic crystals.

Biological applications of optical tweezers are moving to a new level. Biologists thirst for more detailed mechanical and biochemical information on how single molecules work. Recent advances by the Yanagida group [63] in which optical tweezers have been combined with single-molecule fluorescence imaging are a very exciting development. However, biologists also need advances in the tweezers design itself. They now want to grapple with single biological molecules and to sense the vibrations of individual domains and side chains with high time and high spatial resolution. The desire for higher-resolution data throws down the gauntlet to physicists to devise new breeds of optical tweezers that are sharper and more dextrous than those in use today.

Acknowledgements

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[1] Ashkin, A., Dziedzic, J. M., Bjorkholm, J. E., and Chu, S., 1986, *Optics Lett.*, **11,** 288.
[2] Ashkin, A., 1970, *Phys. Rev. Lett.*, **24,** 156.
[3] Ashkin, A., 1992, *Biophys. J.*, **61,** 569.
[4] Felgner, H., Müller, O., and Schliwa, M., 1995, *Appl. Optics*, **34,** 977.
[5] Simpson, N. B., Allen, L., and Padgett, M. J., 1996, *J. mod. Optics*, **43,** 2485.
[6] Omori, R., Kobayashi, T., and Suzuki, A., 1997, *Optics Lett.*, **22,** 816.
[7] Visscher, K., Brakenhoff, G. J., and Krol, J. J., 1993, *Cytometry*, **14,** 105.
[8] Molloy, J. E., Burns, J. E., Sparrow, J. C., Tregear, R. T., Kendrick-Jones, J., and White, D. C. S., 1995, *Biophys. J.*, **68,** S298.
[9] Warrick, H. M., Simmons, R. M., Finer, J. T., Uyeda, T. Q. P., Chu, S., and Spudich, J. A., 1993, *Method Cell. Biol.*, **39,** 1.

[10] Reicherter, M., Haist, T., Wagemann, E. U., and Tiziani, H. J., 1999, *Optics Lett.*, **24,** 608.
[11] Heckenberg, N. R., McDuff, R., Smith, C. P., and White, A. G., 1992, *Optics Lett.*, **17,** 221.
[12] Heckenberg, N. R., McDuff, R., Smith, C. P., Rubinsztein-Dunlop, H., and Wegener, M. J., 1992, *Opt. quant. Electron.*, **24,** S951.
[13] Liesener, J., Reicherter, M., Haist, T., and Tiziani, H. J., 2000, *Optics Commun.*, **185,** 77.
[14] Mogensen, P. C., and Glückstad, J., 2000, *Optics Commun.*, **175,** 75.
[15] Block, S. M., Blair, H. C., and Berg, H. C., 1989, *Nature*, **338,** 514.
[16] Finer, J. T., Simmons, R. M., Spudich, J. A., 1996, *Nature*, **368,** 113.
[17] Wang, W. D., Yin, H., Landick, R., Gelles, J., and Block, S. M., 1997, *Biophysics J.*, **72,** 1335.
[18] Liang, H., Wright, W. H., Rieder, C. L., Salmon, E. D., Profeta, G., Andrews, J., Liu, Y. G., Sonek, G. J., and Berns, M., 1994, *Exptl. Cell Res.*, **213,** 308.
[19] Liu, Y., Sonek, G. J., Liang, H., Berns, M. W., Konig, K., and Tromberg, B. J., 1995, *Optics Lett.*, **20,** 2246.
[20] Visscher, K., and Brakenhoff, G. J., 1991, *Cytometry*, **12,** 486.
[21] Ghislain, L. P., and Webb, W. W., 1993, *Optics Lett.* **18,** 1678.
[22] Ashkin, A., and Dziedzic, J. M., 1987, *Science*, **235,** 1517.
[23] Ashkin, A., Dziedzic, J. M., and Yamane, T., 1987, *Nature*, **330,** 769.
[24] Ashkin, A., Dziedzic, J. M., 1989, *Proc. natn. Acad. Sci. USA*, **86,** 7914.
[25] Ashkin, A., Schutze, K., Dziedzic, J. M., Euteneuer, U., and Schliwa, M., 1990, *Nature*, **348,** 346.
[26] Leibler, S., and Huse, D. A., 1993, *J. Cell Biol.*, **121,** 1357.
[27] Berry, R. M., and Berg, H. C., 1997, *Proc. Natn. Acad. Sci.*, **94,** 14433.
[28] Svoboda, K., Schmidt, C. F., Schnapp, B. J., and Block, S. M., 1993, *Nature*, **365,** 721.
[29] Vale, R. D., and Milligan, R. A., 2000, *Science*, **288,** 88.
[30] Mehta, A. D., Rief, M., Spudich, J. A., Smith, D. A., and Simmons, R. M., 1999, *Science*, **283,** 1689.
[31] Perkins, T. T., Quake, S. R., Smith, D. E., and Chu, S., 1994, *Science*, **264,** 822.
[32] Smith, S. B., Cui, Y., and Bustamante, C., 1996, *Science*, **271,** 795.
[33] Wang, M. D., Schnitzer, M. J., Yin, H., Landick, R., Gelles, J., and Block, S. M., 1998, *Science*, **282,** 902.
[34] Tskhovrebova, L., Trinick, J., Sleep, J. A., and Simmons, R. M., 1997, *Nature*, **387,** 308.
[35] Friese, M. E. J., Nieminen, T. A., Heckenberg, N. R., and Rubinsztein-Dunlop, H., 1998, *Nature*, **394,** 348.
[36] He, H., Heckenberg, N. R., and Rubinsztein-Dunlop, H., 1995, *J. mod. Optics*, **42,** 217.
[37] He, H., Friese, M. E. J., Heckenberg, N. R., and Rubinsztein-Dunlop, H., 1995 *Phys. Rev. Lett.*, **75,** 826.
[38] Simpson, N. B., Dholakia, K., Allen, L., and Padgett, M. J., 1997, *Optics Lett.*, **22,** 52.
[39] Padgett, M. J., and Allen, L., 2000, *Contemp. Phys.*, **41,** 275.
[40] O'Neil, A. T., MacVicar, I., Allen, L., and Padgett, M. J., 2002. *Phys. Rev. Lett.*, **88,** 53601.
[41] Sato, S., Ishigure, M., and Inaba, H., 1991, *Electron. Lett.*, **27,** 1831.
[42] Sato, S., and Inaba, H., 1996, *Opt. quant. Electron.*, **28,** 1.
[43] Courtial, J., Robertson, D. A., Dholakia, K., Allen, L., and Padgett, M. J., 1998, *Phys. Rev. Lett.*, **81,** 4828.
[44] Paterson, L., MacDonald, M. P., Arlt, J., Sibbett, W., Bryant, P. E., and Dholakia, K., 2001, *Science*, **292,** 912.
[45] O'Neil, A. T., and Padgett, M. J., 2002, *Optics Lett.*, **2,** in press.
[46] Higurashi, E., Ukita, H., Tanka, H., and Ohguchi, O., 1994, *Appl. Phys. Lett.*, **64,** 2209.
[47] Luo, Z. P., Sun, Y. L., and An, K. N., 2000, *Appl. Phys. Lett.*, **76,** 1779.
[48] Galajda, P., and Ormos, P., 2001, *Appl. Phys. Lett.*, **78,** 249.
[49] Chiou, A. E., Wang, W., Sonek, G. J., Hong, J., and Berns, M. W., 1997, *Optics Commun.*, **133,** 7.
[50] MacDonald, M. P., Patterson, L., Sibbett, W., and Dholakia, K., 2001, *Optics Lett.* **26,** 863.
[51] Allen, L., Beijersbergen, M. W., Spreeuw, R. J. C., and Woerdman, J. P., 1992, *Phys. Rev. A*, **45,** 8185.
[52] Gahagan, K. T., and Swartzlander, G. A., 1996, *Optics Lett.*, **21,** 827.
[53] Gahagan, K. T., and Swartzlander, G. A., 1998, *J. opt. Soc. Am. B*, **15,** 524.
[54] O'Neil, A. T., and Padgett, M. J., 2001, *Optics Commun.*, **193,** 45.
[55] Durnin, J., 1987, *J. opt. Soc. Am A*, **8,** 651.
[56] Arlt, J., Garces-Chavez, V., Sibbett, W., and Dholakia, K., 2001, *Optics Commun.*, **197,** 239.
[57] Sato, S., Ohyumi, M., Shibata, H., Inaba, H., and Ogawa, Y., 1991, *Optics Lett.*, **16,** 282.
[58] Svoboda, K., and Block, S. M., 1994, *Optics Lett.*, **19,** 930.
[59] Furukawa, H., and Yamaguchi, I., 1998, *Optics Lett.*, **23,** 216.
[60] Sato, S., Harada, Y., and Waseda, Y., 1994, *Optics Lett.*, **19,** 1807.
[61] Sasaki, K., Koshioka, M., Misawa, H., Kitamura, N., and Masuhara, H., 1992, *Appl. Phys. Lett.*, **60,** 807.
[62] O'Neil, A. T., and Padgett, M. J., 2000, *Optics Commun.*, **185,** 139.
[63] Ishijima, A., Kojima, H., Funatsu, T., Tokunaga, M., Higuchi, H., Tanaka, H., and Yanagida, T., 1998, *Cell*, **92,** 161.

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