

Laser tweezers and their applications

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Optical tweezers have recently become widespread tools for confining, controlling and manipulating micron to nanometre-sized objects with ease similar to that associated with mechanical tweezers (on much larger objects). While mechanical tweezers use frictional forces and mechanical pressure to manipulate millimetre-scale objects, optical tweezers make use of the radiation pressure exerted by a laser beam and the intensity characteristics of the beam to confine much smaller objects.

Keywords: Laser tweezers, optical tweezers, confining, controlling, manipulating, micron, nanometre, tweezers, radiation pressure, intensity, laser beam, radiation pressure, mechanical tweezers, frictional forces, mechanical pressure, millimetre-scale objects, much smaller objects.

1. Introduction

In the past decade, optical tweezers have become an essential tool for physicists, chemists and biologists alike. Indeed, in just the past year, over sixty papers[1] have been published relating to them, with over a quarter of those involving application in molecular biology[1]. This comes as no surprise, as they allow trapping and manipulation of objects ranging in size from complete living cells down to individual atoms, and non-destructive, precise observation or manipulation of sub-cellular structures within living cells[2].

Optical tweezers utilise the fact that laser beams typically have increasing intensity towards the beam centre, and that dielectric particles "bend" light. Light (and all other electromagnetic radiation) has associated electric and magnetic fields, the strength of these fields increases with the light intensity. The electric field across the dielectric particle produces a polarisation field within the particle, which partially opposes the external electric field[3]. This increases the refractive index of the particle, resulting in the individual rays being bent on entry to the particle and on exit*.

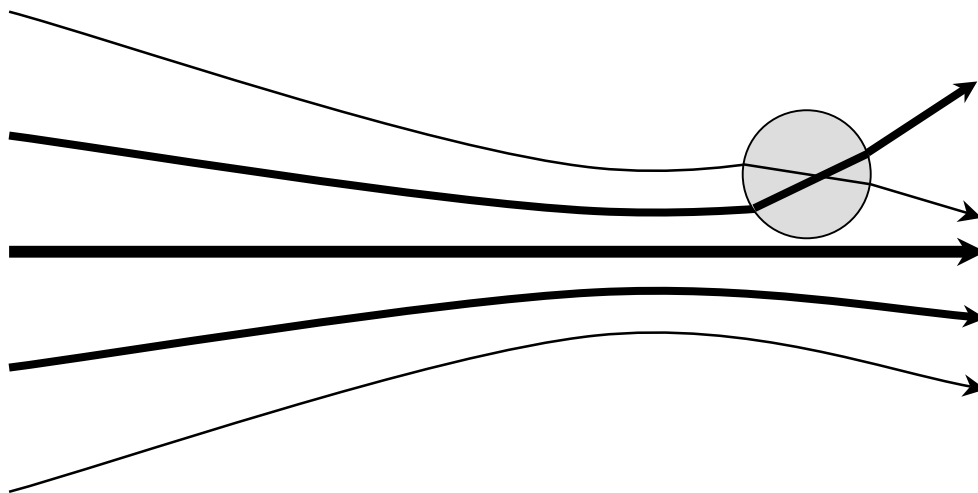


Fig.A: Part of a focused laser beam is refracted as it passes through a small particle. Thicker arrows indicate rays of higher intensity within the beam.

Light (and other electromagnetic radiation) is composed of streams of photons, which have an associated momentum[10], related to the photon energy by $p = E/c$, with c being the speed of light in a vacuum. As the more intense rays have a higher density of photons, the direction of the light passing through the particle is deflected slightly away from the centre of the beam, resulting in a change in the direction of the momentum of those rays. In order for the system to conserve

* Similar to light passing through a lens[11]

momentum, the particle therefore must gain momentum *towards* the centre of the beam[4], resulting in a net force on the particle directed towards the region of higher intensity (known as the *gradient force*).

For a highly focused laser beam, this gradient force pulls the particle towards the focal point of the beam. Some photons are reflected or absorbed by the particle (which therefore gains momentum), this creates a scattering force, which acts on the particle in the direction of the laser beam[4][10]. The net result of these forces is that the particle is attracted to a point on the beam axis, slightly further down the beam from the focal point[4].

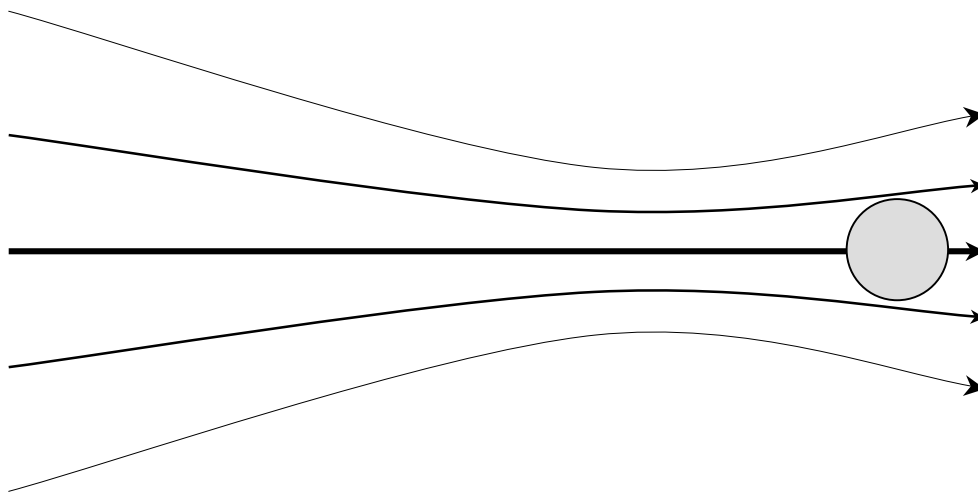


Fig.B: The particle is attracted towards a point on the beam axis by the gradient force. It is held a short distance beyond the focal point of the beam due to the scattering force.

The forces that may be applied with this technology range from tenths of femtonewtons to hundreds of piconewtons[5], allowing a wide range of uses. Several optical tweezers may be used simultaneously to control multiple particles and measure their interactions, or to assemble them to form larger structures. Similarly, several tweezers may be used with one particle, to bend and fold it or to measure mechanical responses (arising from properties such as elasticity) within it, or the viscosity of its surroundings[5].

The success of optical tweezers is largely due to all the doors that they open for scientists; for most purposes, there simply was no predecessor to them and the tasks that they accomplish with ease nowadays were impossible previously. For investigating protein folding (which would have previously required atomic force microscopy), optical tweezers allow precise three-dimensional measurements of positions and forces, whilst also allowing the experimenter to exert forces and influence the system[6].

2. Background and History of Development

While originally investigating scattering forces on lightweight particles in a fluid, Ashkin[4] noticed that the particles (in addition to being "pushed" by the beam) were also drawn towards the central axis of the beam. The *optical fountain*[8] operates by this principle. In this device, a diverging laser beam is directed upwards (i.e. opposite direction to gravity), and a particle may then be suspended in the beam[8]. The gradient force keeps the particle near the beam axis and the vertical position is determined by where the scattering force and gravitational force strengths become equal[8]. This confines a particle to a vertical axis, allowing one-dimensional movement by varying the output power of the laser. This design was preceded by a slightly more complicated trap, the *counter-propagating beam trap*[4][8]. In such a trap, two diverging beams are directed towards each other along a common axis[7]. The scattering force of a diverging beam weakens further along the beam, as the beam intensity decreases. This causes a particle in the beams to be pushed towards a point where the scattering forces from both beams cancel each other (i.e. are equal) [10]. The gradient force attracts the particle to the beam axis.

Probably the most common implementation of this concept is the *single-beam gradient trap*, commonly referred to as "optical tweezers". These use one laser beam, avoiding the alignment issues of the counter-propagating beam trap[8], but by focusing a wide beam through a lens of short focal length, a strongly diverging beam is created (beyond the focal point). This high divergence produces a much stronger gradient (both parallel and perpendicular to the beam axis) than is produced in the optical fountain, resulting in much stronger gradient forces. These can be comparable or considerably stronger than the gravitational forces, allowing three-dimensional control[9][5] of the position of the trapped particle in a vacuum or gaseous environment, with low-power lasers*. By simply moving the focal point of the beam (with lenses and mirrors), the object may be moved[11] along three-dimensional paths. Once the particle is very close to the beam axis, controllable rotation about the beam axis is also possible, resulting in such devices often being referred to as "optical spanners"[5]. While the theory involved is beyond the scope of this article[†], its application allows rotation and torque (twisting/levering forces) to be applied and measured via optical tweezers[5].

* According to [8], for a 1 μm sample, a trap of 0.2 pN nm^{-1} may be produced by a suitable laser of output 50 mW. For comparison, the average pocket laser-pointer has an output of up to 5 mW.

[†] For more information, see [5].

For most typical samples, confined by optical tweezers, the component of the gradient force perpendicular to the beam axis (the *restoring force*), which directs the particle towards the beam axis, has a magnitude proportional to the displacement from the axis; for a restoring force F and displacement x , that is: $\vec{F} = -k\vec{x}$. Analogous to the restoring force exerted by a spring on an attached mass (Hooke's law), k is referred to as the *stiffness* of the trap[10]. When the particle is in equilibrium (i.e. experiencing no net force), its displacement from the beam axis may be used to measure external forces acting on the particle, provided the trap stiffness is known. The resolution of such measurements may be on the order of, or less than a piconewton[10].

3. Design Considerations

In order to accurately exert and measure forces, the optical tweezers need to be calibrated to some known value[10]. One such method is to measure the movement due to Brownian motion of a small bead trapped in the beam. As ideal values for this can be calculated from thermodynamics[10], the dependence of the bead's range of motion on the trap stiffness may be used to calculate the force exerted on the bead by the optical tweezers.

If the particle under observation absorbs a sufficient amount of the laser radiation, it could be damaged/denatured. A beam with power on the order of tens of milliwatts in the visible part of the spectrum would be sufficient to damage human retinal tissue[12]. To prevent such damage from occurring, the wavelength of the laser must be carefully chosen. Ashkin *et al.* have demonstrated the use of relatively simple Nd:YAG* lasers (operating at 1064 nm wavelengths) for non-destructively† trapping and moving live yeast cells[13], using 80 mW lasers.

While being able to trap and move a particle is an interesting ability, it is considerably more useful if we have some means to measure the position of the particle. As the particle will typically be nanometre to micrometre sized, visual tracking technology of sufficiently high resolution has only been available for a few years‡. Before the availability of cheap high-speed digital imaging technology, the position of the particle would be calculated typically from how part of the laser beam is altered as it passes through the particle and reflections of a secondary light source off the particle[14].

* As an example of the availability of such technology, a small fraction of cheap green laser-pointers use Nd:YAG lasers to produce infrared, which is then converted to green light.

† Not only did these cells remain alive in the trap, but also they reproduced[13] while trapped in the 1064 nm beam.

‡ Assumed from the emphasis on low resolution and low frame-rate analogue video technology in *Direct Imaging Methods* section of [14]

Due to the high coherence of laser radiation, multiple trap beams generally cannot be used efficiently in an experiment. This is partly because the beams would interfere with each other[14]. One solution is to "flash" each trap in sequence at a high rate, similar to how the flickering of phosphors on a CRT television produces a steady image. This allows several traps of varying strengths to be produced by one laser and some automated optics[14].

Although the theoretical optical trap is very simple, practical use of it requires various complex optics and instruments, leading to a complicated lab setup:

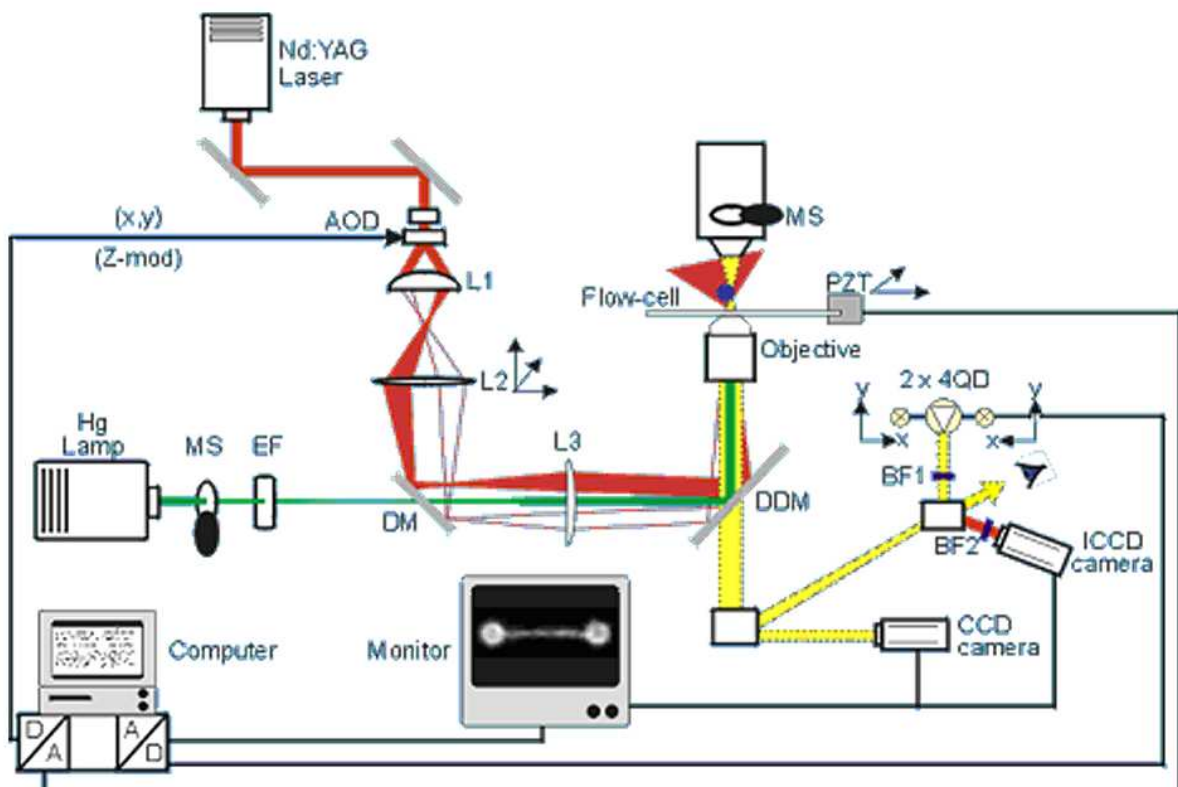


Fig.C: An example configuration of optical tweezers in a laboratory, with various auxiliary instruments to provide control and feedback from the tweezers and to record data.

Image adapted from: <<http://www2.bioch.ox.ac.uk/oubsu/ejknight/apparatus.html>> 10-Nov-2010

4. Applications

Optical tweezers have found uses ranging from breaking cryogenic records to re-arranging sub-cellular structures. Sparkes *et al.* used optical tweezers to stretch* and move organelles within living *Arabidopsis* (mouse-ear cress) cells[15], improving our knowledge of the protein transportation networks within cells. Liphardt *et al.* succeeded in demonstrating the folding and

* Technically not actually stretching, as the displacement of Golgi stacks induced growth of tubules, rather than an elastic extension.

unfolding abilities of an individual RNA molecule by attaching its ends to dielectric beads via other stiffer molecules, then using optical tweezers to trap one bead and a computer-controlled actuator to push/pull the other bead. By steadily increasing the space between the beads, and measuring the resulting force with the optical tweezers, a quantitative value of the minimum force needed to "un-zip" an RNA molecule was measured[16].

Optical tweezers have also been used to analyze the motion of Kinesin, a natural molecular motor used to transport material through cells via scaffolding known as the cytoskeleton. Kinesin molecules consist of two long chains that resemble legs. With optical tweezers, it has been found[17] that these molecules literally "walk" around the cytoskeleton, carrying material through the interior of the cell, and that they walk in steps of 8 nm [19] at a time!

Perhaps the most exotic use of optical tweezers however, is laser cooling. Previous cryogenic methods (for example, Doppler cooling) are first used to cool the sample atoms down to less than a thousandth of a kelvin[20]. Some of these atoms are then confined by an optical trap (optical tweezers). Due to energy being transferred between atoms during collisions, some atoms will have more kinetic energy than the rest (and thus contribute more to the temperature of the trapped vapour). Consequently, they will also have higher velocities. By occasionally disabling the trap for a short period, the atoms are "un-trapped" and allowed to move freely. The faster atoms will travel a longer distance from the trap point than the slower atoms. When the trap is then re-enabled, the slower atoms will be closer to it and more likely to be re-trapped in the tweezers. This progressive "boiling off" or "evaporating" of the hot atoms from the trap removes the atoms with greater energy, decreasing the average energy per atom in the trap, which results in a decrease in the temperature of the trapped vapour*.

5. Commercial devices

Nikon (commonly known for professional photography equipment) produce microscopes designed to accommodate optical trapping devices (laser tweezers). JPK Instruments have recently started producing a laser-trapping device targeted at biologists, for tracking and trapping particles ranging in size from 30 nm to 1 μ m [22]. This device is shown equipped onto a Nikon microscope, on the product's web page[†] [22].

* This technique works in an analogous way to sweating, where some sweat becomes significantly hotter than the skin due to thermal fluctuations and evaporates - slightly reducing the temperature of the skin.

[†] "Setup" tab

6. Future Development

It has been over four hundred years since Johannes Kepler first noticed the apparent momentum that light has and the pressure that this allows it to exert, as it pushes the tail of a comet away from the sun. Now with the assistance of gradient forces, this "pushing" quality of light (and other radiation) has been extended to pulling and twisting of invisible objects in just the past twenty-five years. Could this technology provide a "boom" for nanotechnology and micromanipulation in a similar style to the technological explosion caused by the arrival of the microprocessor?

Perhaps one of the most promising applications of laser tweezers currently being developed is their potential in the current "lab-on-a-chip" movement. In such a device, a fluid or tissue sample is (mechanically) taken and fed onto the "lab", which analyses the sample and produces a result without any other external machinery (besides a power supply). Once the "interesting" part of the sample has been identified, it must be manipulated and possibly extracted in order for analysis to be possible, a function that miniature laser-tweezers would be perfectly suited to provide[23]. In order to manipulate very small objects, the laser tweezers must overcome diffraction limits. This has motivated the development of so-called "Near-field" methods[24].

One notable variation on optical tweezers is *holographic tweezers*. A computer-generated hologram produces a three-dimensional light field, when illuminated by a suitable laser. This allows several traps to be created simultaneously[25], from one laser source and controlled without the need for moving mirrors or acousto-optical deflectors. Suitably high-resolution hologram projection technology is a very recent development, explaining why holographic tweezers are still mostly a physicists' toy rather than a widespread lab technique[24], however the ability to manipulate many particles simultaneously with one device will allow the development of this technology to accelerate as its technological dependencies become available.

While optical tweezers have developed incredibly quickly in the short time that they've been around, we have only begun to explore their many possibilities - whether in medicine, chemistry, biology or physics!

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8. Acknowledgements

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