Chalmers Tekniska Högskola och Göteborgs Universitet
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Maj 1996

4 sidor

OA1

OPTICAL TWEEZERS

AIM.
The aim of this laboratory is to build a functioning set-up for optical tweezers and apply it for micro manipulation of particles.

PREPARATIONS.
As an introduction to the laboratory read the enclosed paper which describes the principles and applications of optical tweezers.
It is also required that you familiarise yourself with the laser safety manual.

Name:..... Course:.....

Date: ..... Supervisor: .....

Completion date: ..... Graded by: .....

OPTICAL TWEEZERS

The single-beam gradient optical trap enables the manipulation of microscopic particles using an optical force of the order of piconewtons. Aptly dubbed "optical tweezers", the trap consists of a single tightly focused laser beam which attracts neutral dielectric particles towards its focal region. The trapping action results from the domination of the optical gradient force over scattering and absorption forces.

The aim of this laboratory is to build a functioning set-up for the laser micro manipulation, characterise the laser beam which is going to be used for the manipulation and use the constructed set-up for

-sorting of particles in a water bath

-studies of the gradient force as a function of the laser power output

-measurement of the scattering phenomena of the tapped particles

-trapping and manipulation of particles using oil immersion microscope objective.

Through out this laboratory you will be using an Ar-ion laser with the output wavelength of 514 nm and varying output power (from few mW up to 5 W)!
REMEMBER: You have to be very careful when using the laser as the direct beam from the laser will cause a damage to your eyes. Even unwanted beam reflections of the laser light can be harmful. Read carefully the instruction manual about the laser safety.

Use the safety goggels!

Hints:

Start your experiment work by trying to characterise the laser beam. You are asked to investigate the beam profile. You might have found out in the literature that it is best to use a Gaussian beam for optical laser micro manipulation. How would you characterise the output beam from the Ar-ion laser?

Try to establish the characteristics of the laser beam which are needed for the most effective trapping when the laser beam is directed through the microscope objective towards the object which is going to be manipulated.

How can you observe that he particle is trapped in the laser beam?

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SAFETY INSTRUCTIONS:

The laser is a unique light source and exhibits characteristics which are different from conventional light sources. Its safe use depends on the user becoming aware of these characteristics and treating the instrument acordingly. For example, the high-energy output of the laser passing directly into the eye can cause serious damage with possible loss of vison. In fact, many light sensitive elements can be damaged by direct exposure to the beam, e.g., CCD cameras. Because it remains coherent, the beam might also cause damage to the eyes if contacted indirectly from reflective surfaces. For these reasons and others, the user is advised to follow the precaution below.

Use the "laser in operation" warning sign outside the door when using the laser.

Never look directly into the laser light, or at scattered light from any reflective surface. Never try to follow the laser beam with your eyes through optical components.

Use laser safety glasses.

Take off rings and clocks to avoid reflections.

Maintain experimental setups at low heights to prevent inadvertent beam-eye encounter at eye level. Avoid this potentially dangerous situation by closing off the restricted area with beam stops.

Do not work sitting on a chair with your eyes at the height of the laser beam or the scattered light.

During the setting up, use low power.

Do not place reflecting surfaces into the beam before having verified where the reflected beam will go.

As in the example (see next page), most accidents happen during an alignment procedure.

Comment

Accident victim's view

Because laser injuries to eyes are rare, workers tend to discount the importance of safety precautions. The following dramatic account by Dr. C. David Decker, a victim of such an accident earlier this year (LF Feb p4), was prepared in the hope that his experience may increase vigilance among his colleagues.

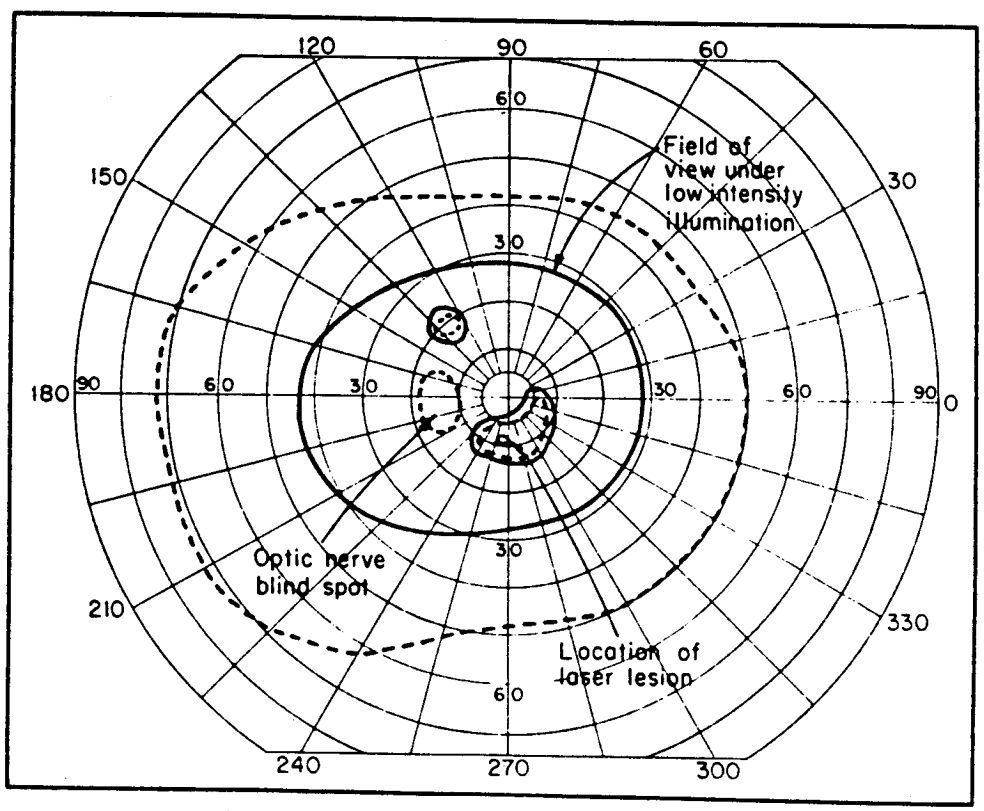
The necessity for safety precautions with highpower lasers was forcibly brought home to me last January when I was partially blinded by a reflection from a relatively weak neodymium-yag laserbeam. Retinal damage resulted from a 6-millijoule, 10-nanosecond pulse of invisible 1,064-nanometer radiation. I was not wearing protective goggles at the time, although they were available in the laboratory. As any experienced laser researcher knows, goggles not only cause tunnel vision and become fogged, they become very uncomfortable after several hours in the laboratory.

When the beam struck my eye I heard a distinct popping sound, caused by a laser-induced explosion at the back of my eyeball. My vision was obscured almost immediately by streams of blood floating in the vitreous humor, and by what appeared to be particulate matter suspended in the vitreous humor. It was like viewing the world through a round fishbowl full of glycerol into which a quart of blood and a handful of black pepper have been partially mixed. There was local pain within a few minutes of the accident, but it did not become excruciating. The most immediate response after such an accident is horror. As a Vietnam War Veteran, I have seen several terrible scenes of human carnage, but none affected me more than viewing the world through my bloodfilled eyeball. In the aftermath of the accident I went into shock, as is typical in personal injury accidents.

As it turns out, my injury was severe but not nearly as bad as it might have been. I was not looking directly at the prism from which the beam had reflected, so the retinal damage is not in the fovea. The beam struck my retina between the fovea and the optic nerve, missing the optic nerve by about three millimeters. Had the focused beam struck the fovea, I would have sustained a blind spot in the center of my field of vision. Had it struck the optic nerve, I probably would have lost the sight of that eye.

The beam did strike so close to the optic nerve, however, that it severed nerve-fiber bundles radiating from the optic nerve. This has resulted in a cresent-shaped blind spot many times the size of the lesion. The diagram is a Goldman-Fields scan of the damaged eye, indicating the sightless portions of my field of view four months after the accident. The small blind spot at the top exists for no discernible reason; the lateral blind spot is the optic nerve blind spot. The effect of the large blind area is much like having a finger placed over one's field of vision. Also I still have numerous floating objects in the field of view of my damaged eye, although the blood streamers have disappeared. These "floaters" are more a daily hinderance than the blind areas, because the brain tries to integrate out the blind area when the undamaged eye is open. There is also recurrent pain in the eye, especially when I have been reading too long or when I get tired.

The moral of all this is to be careful and to wear protective goggles when using highpower lasers. The temporary discomfort is far less than the permanent discomfort of eye damage. The type of reflected beam which injuried me also is produced by the polarizers used in q switches, by intracavity diffraction gratings, and by all beamsplitters or polarizers used in optical chains. —C. DAVID DECKER



EYE DAMAGE caused by laser-pulse is shown in this plot of field of view under high-intensity illumination **dotted lines** *and under low-intensity illumination* **solid lines**. *Outer circles show field of view; the two small regions inside the field of view are blind spots produced by laser damage. The blind spots are larger than the lesion and occupy a larger area under low illumination*

23 oktober 1997

EQUIPMENT FOR OPTICAL TWEEZERS

NR	EQUIPMENT	SPECIFICATION
1	Ar-ion laser	
7	Mirrors with mounts	High reflective for 514 nm
1	Mirror	Reflective for visible light
2	CCD-cameras	
2	Lens	Convex f=50 mm
2	Lens	Convex f=100 mm
1	Lens	Convex f=150 mm
1	Lens	Convex f=250 mm
1	Lens	Convex f=500 mm
1	Lens	Concave f=-25 mm
1	Lens	Concave f=-75 mm
5	Lens holders	
1	Beam splitters	
1	Dichroic mirror	
3	Optical rods	
13	Optical rod carriers	
1	Microscope objective	x 10
1	Microscope objective	x 20
1	Microscope objective	x 40
1	Microscope objective	x 100, NA = 1.25, Oil immersion
8	Magnetic bases	
1	Beam attenuator with holder	
1	Iris with holder	

4	Laser safety glasses	
3	Colour filters	
	Particles	
	Yeast	
	Water bath	with partition
	Microscope slides	
	Cover glass	
	Immersion oil	
	Pipettes	
2	Power meters	
	Beam stops	
	Post clamps	
1	xy-translation stage	for microscope
2	z-translation stage	for microscope
2	Illumination housings and lamps	
1	Condenser lens	
	Alcohol	for cleaning the optics
	Lens tissue	
	Paper towels	
2	Polaroidfilters with holders	
	Mounting posts	
4	Post holders	
1	PC	

Laser Tweezers

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Introduction

The single-beam gradient trap, or laser tweezers, is a unique tool for micro manipulation, enabling its user to access tiny objects without any mechanical contact. It consists of a laser beam focused through a powerful lens to a diffraction-limited spot. The beam then has very high energy density, and exerts relatively strong forces on particles near its focus.An example of such manipulation is shown on the cover of this issue and Figure 1.

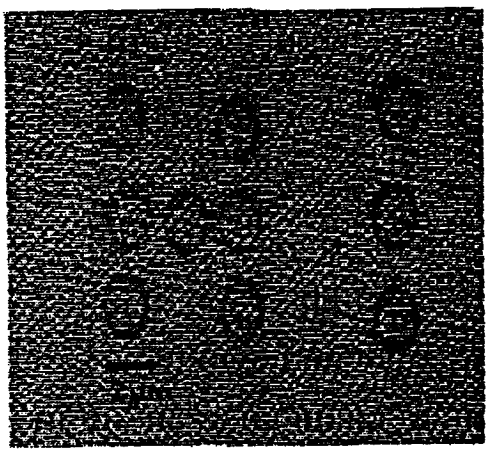

***Figure 1:** the word 'HI' was formed by manipulating polystyrene microspheres using laser tweezers. The microspheres are 2μm in diameter and are dispersed in distilled water*

There is as yet no general theory describing the forces exerted by a strongly focused laser beam for particles of arbitrary size. The difficulty in the treatment of forces arises when the particle's size is of the order of an optical wavelength. Particles that are much smaller than a wavelength can be treated as point dipoles, and forces on them described in terms of the electromagnetic field acting on the particle. For particles that are much larger than the wavelength, the forces can be calculated using a geometrical optics approach.

Forces on a point dipole are found from the non-relativistic Lorentz force on a polarisable particle. The Lorentz force is easily separated into an absorption term, a force due to the absorption of momentum by the particle, which will cause the particle to move in the direction of the wave vector, and a gradient term, the effect of the field on the particle due to its polarisability, which will cause the particle to move to the region of higher intensity if the polarisability is positive, in order to lower the energy of the system.

A particle which is mostly transparent (or has low absorption coefficient) and has refractive index greater than the medium experiences a gradient force which is greater than the absorption force when irradiated by a tightly focussed beam, and thus is trapped in the region of highest intensity. Under favourable circumstances this even means that these particles will move back along the beam axis to a beam waist, giving three-dimensional trapping. A highly absorptive particle will experience a much greater absorption force than gradient force, and will be 'pushed' away from the region of high intensity. These particles cannot be trapped three-dimensionally using a Gaussian beam. They can, however, be trapped two-dimensionally using a ring of light, or a 'doughnut' beam. Absorptive particles are pushed away from the intense region, either away from the beam or into the central dark region where they will be trapped.

We can find the forces acting on large particles (i.e. much larger than an optical wavelength) using ray optics[1]. When a light ray entering a particle with refractive index greater than that of the surrounding medium is bent toward the normal, the change in momentum of the light results in a force on the particle. This force can be resolved into two components, in a similar-manner to the force on a dipole: a gradient force $\mathbf{F_g}$ acts to move the particle into the region of high intensity and a scattering force $\mathbf{F_s}$ acts in the direction of the ray, as shown in figure 2(a). From figure 2(b), we can see that a displacement of the particle from the waist in any direction results in a restoring force. For a

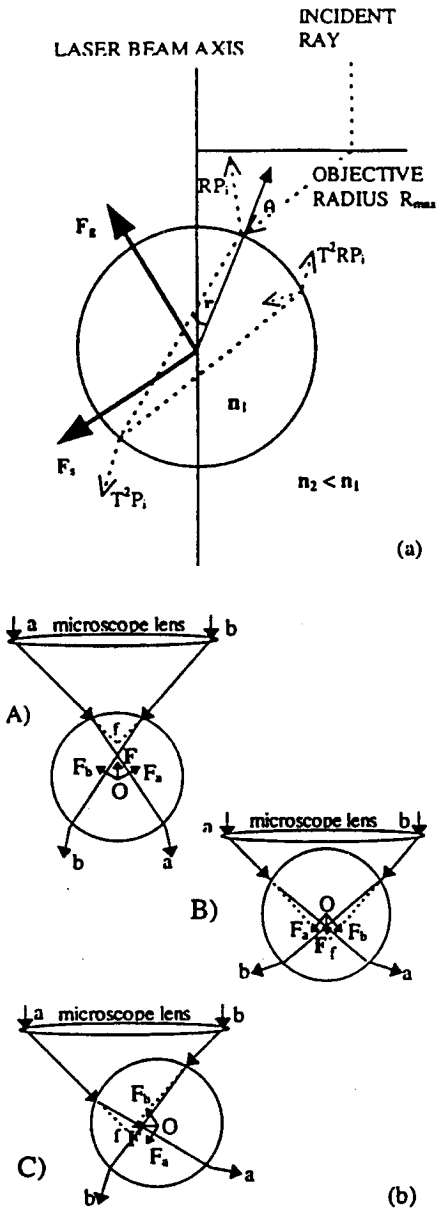

Figure 2: Ray Optics model for laser tweezers (after Ashkin [1]) (a) ray path for an incident ray, where T is the transmittance and R is the reflectance of the sphere(b) illustration of the restoring force on a sphere when it is displaced from the focus

transparent particle these forces are easily understood, however the geometrical optics theory is not easily transferable to the case of a highly absorptive particle, and more work is needed in this case.

To set up a single beam gradient trap in practice requires a laser, some optics, a high power lens and some method of observing the operations. Particles that are trapable range from tens of nanometres to tens of microns in size, so strong magnification is needed for viewing. Often the same lens is used for both focussing of the laser beam and viewing of trapped objects. This is usually done by introducing the laser beam into the optical path of a conventional microscope via some aperture in the body of the microscope and an inclined beam-splitter, as shown in figure 3.

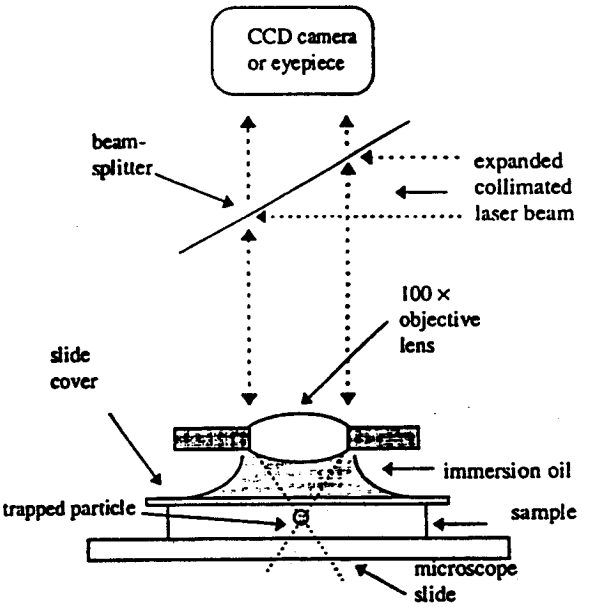

Figure 3: Experimental arrangements for optical tweezers

The width of the focussed beam at the waist can in principle be as small as a wavelength. To achieve the smallest possible spot size (to give the highest possible intensity gradient, and thus the most effective trap) requires that the beam be expanded to fill the back of the microscope objective lens. When the beam has been introduced into the microscope, manipulation can be performed by movement of the microscope stage with respect to the trap, and viewing through the eyepiece.

The wavelength and power of the laser used depends on the size and type of the particle being trapped. For medium sized particles (1 - 10 μm) between 10 and 100 mW should be sufficient laser power to trap and manipulate, providing the sample is not very absorptive at the laser wavelength. When trapping biological samples, the wavelength should be chosen so as not to cause damage to the specimens. Using infrared light from a Nd:YAG laser, up to 80 mW laser power at the focus has been used to trap bacteria without apparent damage, whereas some experiments report severe damage to biological specimens using visible argon-laser light at about 20 mW.

As an example of the manipulation of very small objects that is possible using optical tweezers, we show at the head of the article a simple greeting with the letters "HI" spelled out by assembling a number of 2μm diameter polystyrene spheres in water. The optical tweezers arrangement used was a simple setup as shown in figure 3, based on a 100x oil immersion objective and a 15 mW He-Ne laser.

Optical tweezers in the biological sciences

Optical tweezers have been extensively studied by a number of researchers around the world. The physical properties of the trap have been evaluated and possible applications in a diverse number of fields have been demonstrated. The functions of the optical tweezers give the opportunity to control and manipulate non-living as well as various types of living biological objects non-intrusively. In addition the forces in the optical trap are small but sufficient for micro-manipulation of biological and molecular objects and they can be carefully controlled so that no excessive force has to be applied to a fragile biological system. Moreover, the light is inherently sterile so that contamination risks can be minimised.

While we have conducted several proof of principle experiments at the University of Queensland using very simple and inexpensive equipment[2], computer controlled commercial systems using high power lasers are now available and progress in applications has been spectacular.

It has been demonstrated that the optical tweezers can be successfully utilised for capture, movement and positioning of a wide variety of single cells and sub-cellular particles without direct contact. It has been also established that these operations can be performed without causing any significant optical damage to the studied biological material. Several wavelengths have been used for trapping and it was verified that damage-free trapping was best achieved when using IR radiation due to lower absorption of the material in this wavelength region. A further advantage of IR as compared with visible traps is that, because of the larger focal spot size, a fourfold reduction in intensity will occur without a similar reduction in force.

To observe the reproduction of *Escherichia coli* within the optical trap, Ashkin *et al*[3] captured an individual bacterium and lifted it up from among a collection of bacteria to a clear region, where it was monitored continuously. In a long time run several life cycles were observed with all of the offspring remaining in the trap. This experiment gave clear evidence of achieving manipulation and carrying out subsequent studies of the living organism without causing optical damage. Damage-free trapping and manipulation of suspensions of human red blood cells and of organelles located within individual living cells of spirogyra were also achieved. Optical tweezers have also been used to study the compliance of bacterial flagella. The forces produced by optical tweezers are sufficient not only for trapping of a mobile bacterium but they are also able to overcome the torque generated by the flagella motor of a bacterium tethered to a glass surface by a flagella filament. The compliance of flagella can be determined by calibrating the trapping force against Stokes' drag and measuring the twist that is sustained by this force. Thus optical tweezers give novel means of studying the elastic properties of individual bacterial flagella, which are hard to obtain with more conventional techniques. Optical tweezers have been also used to probe the elasticity of the cytoplasm and membrane skeleton.

When two trapping beams are introduced (double beam optical tweezers) to the microscope studies can be performed on objects which have to be stretched, aligned or turned. Using this technique Chu *et al* studied recoil behaviour and visco-elastic properties of DNA molecules. The molecules were stretched out, fixed and viewed with scanning tunnelling or atomic force microscopes[4]. Further studies of de Gennes' reptation model were subsequently carried out using the single-beam optical trap on fluorescently labelled DNA molecules by fluorescence microscopy.

When optical tweezers are combined with a laser micro-beam (also called optical scalpel) controlled cell fusion can be carried out[5]. The basic idea of the use of a laser scalpel for intracellular microsurgery on biological objects is that the laser cutting beam is strongly focussed onto the object from a large aperture microscope objective (as is the beam of optical tweezers). This ensured that the laser scalpel

has very short effective depth of field implying that there will be enough power density for the desired effect only at very limited depth in the object (1-2 μm). Above and below this depth, the light intensity will cause no harm to the tissue. This ensures that the micro-beam surgery will be carried out in the interior of an unperforated cell with no damage to cell walls or membranes.

For induced cell fusion the optical trap is combined with a pulsed UV laser micro-beam. The two selected cells are brought into close contact by the optical tweezers. Once inside the trap, the two cells can be fused by applying several pulses of the UV laser micro-beam. With this technique a selective fusion of two cells is done without critical chemical or electrical treatment. Laser induced cell fusion should provide an increased selectivity and efficiency in generating viable hybrid cells in the future[6].

An important application of combined usage of optical tweezers and UV-laser micro-beam is manipulation of gametes and early embryos. In this way the fertilisation processes can be studied in more detail leading to increased efficiency of in-vitro fertilisation. The combination of UV-laser micro-beam and optical tweezers for assisted fertilisation was first suggested by Ng *et al*[7]. Subsequently Schütze *et al* [8] successfully drilled a hole into the zone pellucid and inserted a single sperm through the laser drilled hole into the pervitelline space using these combined techniques.

In summary, it can be concluded that a combined system of optical tweezers, laser micro-beam and laser induced fluorescence detection converts the ordinary light microscope from being a passive analytical instrument into a preparative and manipulative instrument that allows micro-manipulation of biological objects without any mechanical contact.

Optical trapping of non-transparent particles using doughnut beams

Higher order laser modes can also be used to trap particles. A focused TEM^*_{01} doughnut beam (called a doughnut because it is a bright ring of light) can be used to trap high refractive index transparent particles, in fact with higher efficiency than a Gaussian as the light is on the average inclined at a larger angle to the axis. This is not of great importance as the TEM^*_{01} cannot usually be generated with as high efficiency as the fundamental mode.

However, the doughnut can also trap particles which are repelled by a Gaussian beam, such as reflective or highly absorbing particles, or low refractive index particles or bubbles. For this reason, we have been studying trapping using doughnuts. In general, the wings of the beam will repel such particles, and there is no axial gradient trapping force, so the particles are always pushed against the microscope slide, but small particles can be very effectively transversely trapped in the dark central region of the beam and manipulated as desired.

Since it is not often easy to make a laser run on the TEM^*_{01} doughnut mode we have developed a series of computer generated holograms to convert a normal Gaussian TEM_{00} mode into a doughnut[9]. This has the further advantage that we can generate higher order doughnuts with relatively larger central dark spots which are rarely observed in lasers. Our first holograms were simple binary amplitude holograms made from 35 mm negatives of laser printed patterns. These had efficiencies of only a few percent. More recently we have made off-axis blazed phase holograms by contact printing continuous tone patterns onto holographic plates which are then bleached. The best of these produce a "charge 3" doughnut corresponding to a Gauss-Laguerre LG_{03} mode with an efficiency of over 50%, sufficient to allow us to trap a range of small particles using a 20 mW He-Ne laser.

The doughnut beams produced by the hologram have a very interesting structure. The reason for the persistence of the dark central spot in the beam as it propagates is that opposite sides of the beam are out of phase. Hence there is a phase singularity at the centre and this gives the beam a helical structure. Like a screw thread, this can be right or left handed, or single or multi-start. The helicity is preserved as the beam propagates or is focussed, but is reversed when the beam is reflected.

As a result of the helical structure, the beam carries angular momentum, even when linearly polarised: in fact each photon carries $l\hbar$ of

angular momentum (where l is the "charge", equal to the order of the Gauss-Laguerre beam or number of thread starts) as well as $\pm\hbar$ if it is circularly polarised.

We have been able to directly observe the transfer of this angular momentum to small particles trapped in the dark centre of a phase singularity, as it sets them into rotation. In our experiment, the 'doughnut' beam containing a charge 3 singularity was focussed using a high numerical aperture oil immersion microscope objective to a waist a few μm across in a cell containing small particles.

In one series of experiments we have used strongly absorbing CuO particles dispersed in kerosene. These particles are repelled by the wings of the laser beam, but small ones 1-2 μm across can be trapped in the dark central minimum and held there while their surroundings are moved by translating the microscope stage. Such particles are observed to rotate with speeds in the 1-10 Hz range. Because they are asymmetric, they tend to tumble and the speed is uneven, but the direction is consistent. We have made video recordings of many such particles, all rotating in the same direction. We have then reversed the hologram to reverse the helicity of the beam and observed that all the trapped particles now rotate the other way. To prove that the helicity of the beam is the factor which determines the direction of rotation we have introduced a Dove prism into the beam. Because the beam suffers one reflection within the prism, its helicity is reversed. We can then trap a particle and observe its sense of rotation. If we quickly remove the prism the same particle remains trapped but immediately begins to rotate in the opposite direction.

In a second series of experiments we used polystyrene microspheres dispersed in water. These are transparent enough to be trapable in either a doughnut or a Gaussian beam. The individual particles are perfectly spherical so we cannot detect if they rotate but we can easily observe the slow rotation of a small clump of particles trapped in the doughnut beam. Moving our hologram sideways effectively moves the singularity out of the beam. The clump of particles remains trapped but ceases to rotate.

These two series of experiments show conclusively that the observed particle rotation is caused by the helicity of the laser beam. A simple calculation supports the hypothesis of angular momentum transfer. We can estimate the rotation rate where the viscous drag torque on the particle balances the angular momentum flux absorbed from the beam - say 25% of the 4×10^{-18} mNs^{-1} - to be of order 10 Hz, consistent with our observations on the CuO particles.

Recalling the controversy over whether it was the absorption of linear momentum from a light beam which caused the vanes in a Crookes' radiometer to rotate (it wasn't!) the reader might be tempted to ask whether some thermal effect might explain our observations. We have been unable to find any way to estimate such effects in a liquid but have been able to exclude the possibility in the following way.

Imagine that the particles were not rotating in response to an externally applied torque due to absorption of angular momentum from the beam. In that case angular momentum must be conserved in the particle/medium system and rotation would result from thermal(?) forces between the particle and liquid. As a result, one would predict that the liquid surrounding the particle would move in the opposite direction to the particle. We have been able to test this prediction by observing the motion of small particles in the vicinity of a trapped rotating one. They don't move in the opposite direction: in fact they slowly circulate around the trapped particle in the same direction, presumably swept around by the fluid motion driven by the rotating particle, which in turn is driven by the beam.

All the experiments described above were carried out with linearly polarised light. In a fourth series of experiments, we are examining the effects of circular polarisation. We have not been able to observe rotation of clumps of weakly absorbing polystyrene spheres trapped in a circularly polarised non-helical TEM_{00} beam. We attribute this failure to the weak absorption and smaller angular momentum per photon. However, by inserting a quarter-wave plate in the $l = 3$ doughnut beam path, we can increase or decrease the angular momentum per photon by $\hbar$ by simply rotating the plate, maintaining the trapping continuously. This leads to a perceptible speeding up or slowing down of

the rotation of CuO particles trapped in the helical beam.

This proves that the helical and polarisation angular momentum effects are of the same order of magnitude as expected. Although the transfer of polarisation angular momentum from light to a macroscopic object was demonstrated by Beth in 1936 and Ashkin and Dzeidzic more recently rotated particles in a circularly polarised laser beam, we believe our experiments are the first to demonstrate transfer of the angular momentum associated with the helical beam structure, sometimes called "orbital" angular momentum to distinguish it from the "spin" angular momentum associated with polarisation.

Current work at The University of Queensland

One of the most exciting current developments using optical tweezers is in the field of scanning force microscopy (SFM). Scanning force microscopy involves the raster-scanning of a sharp stylus mounted on a soft spring across a sample. Movement of the stylus can be monitored with great precision, enabling much greater resolution than is available using optical microscopes. The choice of cantilever for an SFM is critical for achieving vibration isolation and resolution, and preliminary results by Ghislain *et al*[10] and Friese (unpublished) indicate that a stylus trapped by optical tweezers may be a suitable cantilever for SFM.

It is desirable that the spring constant of a cantilever for SFM be very low (below 1 Nm^{-1}) to achieve the required sensitivity, yet the resonant frequency must be high (greater than 10 kHz) to achieve isolation from low frequency building and equipment vibrations. The lower limits of the spring constants and upper limits of resonant frequencies of current micro-fabricated stylus-cantilever systems are determined by the lowest possible mass of the system. The use of optical tweezers to hold the stylus significantly decreases the mass of the system, so it is theoretically possible to achieve softer springs and higher resonant frequencies. This increase in sensitivity would enable the imaging of soft samples in aqueous environments, a development of considerable importance in many biological areas.

At the University of Queensland, we are currently working on a project aimed at producing a working prototype of a scanning force microscope with an optically trapped stylus. One of opticsí great contributions to science has been the development of microscopy, which has allowed observation of the fundamentals of life. With the addition of a laser, we are now in the position to actively manipulate, study and modify, rather than just passively observe.

References

1. A.Ashkin, Biophysics Journal **61**, 569 (1992)

2. C.D'Helon, E.Dearden, H.Rubinsztein-Dunlop and N.Heckenberg, Journal of Modern Optics **41**, 595 (1994)

3. A.Ashkin and J.Dziedzic, Science **235**, 1517 (1987)

4. T.T.Perkins, D.E.Smith and S.Chu, Science **253**, 861 (1991)

5. K.Schütze and A.Clement-Sengewald, Nature **368**, 667 (1994)

6. W.H. Wright, G.H.Sonek, Y.Tadir and M.W.Berns, IEEE Journal of Quantum Electronics **26**, 2149 (1990).

7. Ng *et al.*, J. Assist. Reprod. Genet. **9**, 191 (1992).

8. K.Schütze, A.Clement-Sengewald and A.Ashkin, Fert. Steril. **61**, 783 (1994)

9. H.He, N.R.Heckenberg and H.Rubinsztein-Dunlop, Journal of Modern Optics **42**, 217 (1995)

10. L.Ghislain and W.Webb, Optics Letters **18**, 1678 (1993)