Background on Optical Tweezers (Companion to the Guide to OT)

One of the major goals of modern day science is to describe and characterize the world as it exists on a scale far beyond the scope of our own senses. We can then use this information in a variety of ways that benefit society or simplify our lives. High on the list of challenges faced in pursuing this goal is manipulating objects that lie within this realm in a precise and controlled manner, be it to carry out fundamental experiments or to develop new technologies. One would also like to achieve this in a way that minimizes damage to the object being studied. It is also imperative that the investigator can observe the object, preferably in a direct manner. This cuts down on the noise and uncertainly in the experiment. This is why the development of the optical trap almost two decades ago was an important step in modern science, most notably to the tenets of Cell Biology and Biophysics.

Optical tweezers are proving to be a very useful tool in precisely manipulating micron and sub-micron sized particles. Early optical tweezers were all either optical two-beam traps or required an external force to be supplied by either gravity or an electric field for stability. In these early traps a competition between two forces provided the stability of the trap. These approaches were used to trap dielectric spheres which were large compared to the wavelength of the light, and thus ray optics can be used to describe the forces acting on the spheres (3). However, it is known that nonuniform electromagnetic radiation incident on a dipole causes a force which naturally divides itself into two components, the gradient force and the scattering force (4). The gradient force points in the direction of the intensity gradient of the light, while the scattering force points in the direction of the incident light (fig 1). The condition for stability of the dipole in the field is that the ratio of the gradient force to the scattering force be greater than unity (2). Essentially this means that the restoring force is greater than the force pushing the dipole out of the field.   As long as this condition is satisfied a single light beam can be used to trap a particle in the regime where the size of the particle is much less than the wavelength of light. This is called the Rayleigh regime. Early single beam optical traps were designed for rayleigh particles. It is found however that single beam optical traps can also be used to trap particles whose size is much larger than the wavelength of the incident light, this is called the Mie regime. It is also verified

experimentally that the criteria for stability is satisfied from the Rayleigh regime into the full Mie regime (2). Thus we are able to trap micron sized beads with an infrared laser whose wavelength (832 nm) is comparable to the size of the bead. Furthermore we can get a qualitative picture of the trapping using simple ray optics even though we are not strictly in the ray optics regime. Being able to trap micron-size particles also makes single beam optical tweezers a useful tool in biological research, where particles rarely lie within the Rayleigh regime.

In 1987, the same group showed that this technique could be valuable to biological research. They used a setup of single beam optical tweezers (another name for the optical trap) to trap bacterial cells and move them between cultures without incurring any discernable damage to the cells (5). The idea of laser trapping was combined to the use of a number of tools. An exemplary example is the laser scalpel, which is capable of cutting things as small as a fragment of DNA. This opened the door to a floodgate of biological applications, some of which are listed below (5).

- o   Gravity perception in plants
- o   Force estimation for Kinesin motors and other molecular motors
- o   Mechanical studies of bacterial flagella
- o   Chromosome manipulation during mitosis
- o   Chromosome dissection
- o   Microsurgery and manipulation of cells *in vivo*
- o   Controlled cell fusion
- o   DNA injection and/or incorporation
- o   Kinetic studies of DNA

The modern application of optical tweezers is seemingly almost exclusive to the fields within biology. A list of website that may prove useful are listed below.

(1) http://www.phys.umu.se/laser/twestat1.html
(2) http://www.nbi.dk/~tweezer/
(3) http://yakko.bme.virginia.edu/lab/presentation1/sld006.htm

Theory

I : The Physics:

The optical trap is based on the transfer of momentum between the beam of radiation and the object that it is passing through.   Specifically, it is predicated on the transfer of momentum from the photons of the beam to the particle being trapped, a result of the refraction of the photons themselves as they pass between the boundary separating object and medium. This refraction results in a force that effectively traps the particle in a 3D environment. However, the outcome of this interplay is dependent on the relationship between the index of refraction (n) of the object and its relation to the n of the environment it is immersed in. **In general, trapping requires that the particle have a higher index of refraction than that of its surrounding medium, with common ratios $n_{particle}$:$n_{surrounding}$ (n_p / n_s) being in the neighborhood of 1.1 to 1.2.** This is discussed below.

Students learn early on in lower division physics that when a beam of light passes through a boundary separating two media with disparate indexes of refraction that the beam is diffracted according to Snell's Law. Consider light moving from media A to B.

Snell's Law states: $n_a \cdot \sin \_ = n_b \cdot \sin \_$

where n_a and n_b represent the n of media A and B respectively. The angle _ represents the angle of the incident beam, as measured from the normal to the boundary surface where the beam crosses, and _ the angle of the resulting beam measured form the inward normal. Take home message, *higher index means less angle*. It is this simple law that plays a key role in understanding the trapping abilities of a setup of optical tweezers.

Now apply these ideas to the trap by considering fig (1) below. The bead is aligned along the incident beam axis, but is below the focal point of the objective lens. As the beam passes into the bead, it is refracted away from the incident beam axis, let this be the z-axis. This results in a transfer of momentum from the deflected photons to the bead itself. The magnitude and direction of this momentum is determined by conservation of momentum, and since $\partial \mathbf{p} / \partial z = \mathbf{F}$, this results in a force.
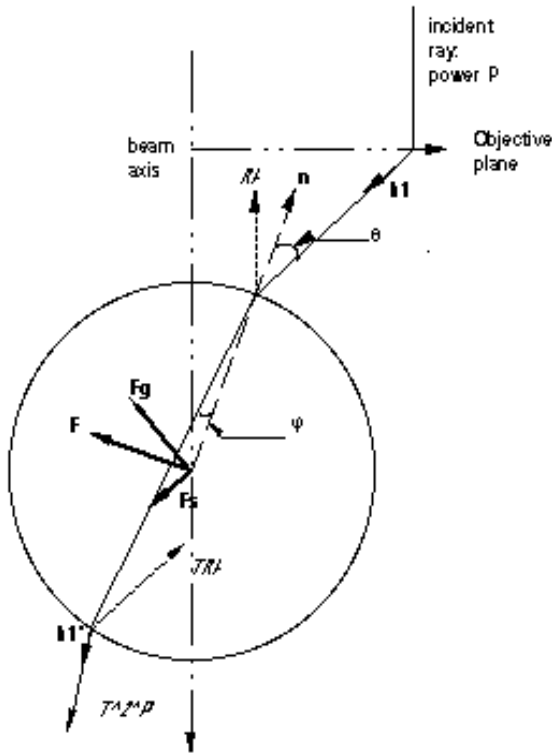
Fig (1)

This force points in the opposite direction of the change in momentum of the light, labeled in the diagram by the vector **F**. It can be broken into two components. The first is parallel to the original direction of the beam, as it left the objective. This is the scattering force F_s, and it can be thought of as the force the particle exerts as it hits the bead, effectively pushing it in its direction of propagation. The second component, F_g is perpendicular to the scattering force, its magnitude is determined by the vector relation:
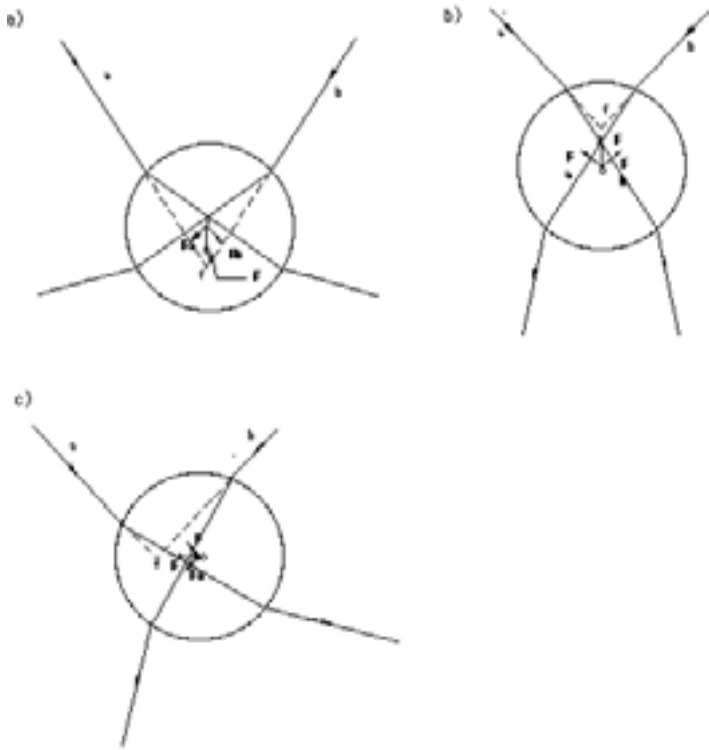
$$F = F_s + F_g$$

F_g is known as the gradient force. When the beam coming from the opposite side of the objective is taken into account and the resulting forces summed with the ones depicted here, all the lateral force components normal to the z-axis cancel each other out. This leaves the vertical force components along the z-axis to determine the net force on the bead. **Thus, the overall net force on the bead, F_t, can be reduced to a competition of forces between relevant components of the total scattering force F_S and the total gradient force F_G.**

$$F_T = F_G + F_S$$

It should now be apparent why the ratio n_p / n_s is critical to the effectiveness of the trap. If $n_p < n_s$, the angle _ will be greater than the angle _. This will skew the resulting force F_T, and its components F_S and F_G. In the example shown, this shifting of the force **F** will decrease the z-component of the gradient force and increase the magnitude of the z-component of the scattering force, which is pointing down away from the focal point of the beam. Thus, the total force F_T will tend to push the bead away from the focal point and out of the trap, rendering it ineffective. Some more examples showing how the bead

is sucked into the focal point of the beam are depicted below in fig (2). Figs 2a and 2b show how the bead will behave when the focal point of the beam is either below or above the center of the bead respectively. If the incident beam hits the bead from above the normal, the resulting gradient force will push the bead down. Conversely, if the beam hits from below the normal the bead is pushed up. In both cases the resulting force pushes the bead toward the focal point.

Another thing to note is the importance of the numerical aperture (N.A.). If the edge of the beam is not focused at a steep enough angle, the component of the scattering force will dominate the scattering/gradient relationship, pushing the particle out of the focal point. **Thus it is imperative to use the maximum N.A. possible to get the most out of your trapping force.** However,

Fig(2)

one must keep in mind that practically the purpose of the tweezers is to trap objects in a 3D environment, and N.A sacrifices trapping depth. This is discussed below.
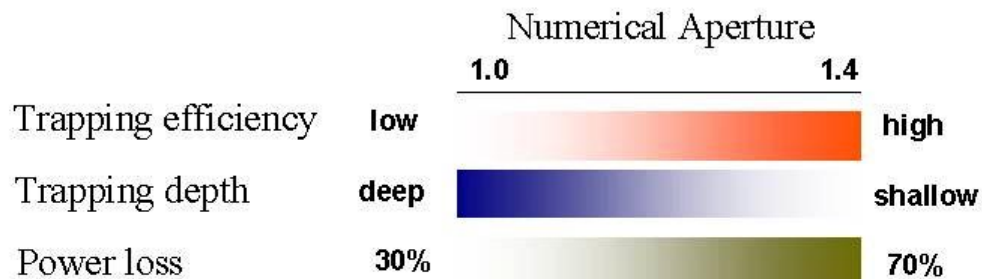
II : The Technical Challenges

In order to create a gradient force, which is capable of overcoming the scattering force, it is necessary to create a large gradient in the intensity of the incident light. This means that a high convergence angle of light is necessary. It is necessary then that the objective lens have a high numerical aperture (NA), defined as $NA = nsin\theta$, where n is

the refractive index of the lens, and θ is the maximum angle subtended by light entering the objective. The objective must also be completely filled by the incoming beam so that the beam achieves the maximum angle of convergence. This puts a strong constraint on the geometry of the experiment because the particle being trapped must be very close to the objective lens. The relationship between NA and trapping depth can be seen in fig (3) below. In general there is a tradeoff between trapping depth and NA. Thus, in order to trap at higher depths it is necessary to use a lens with a lower numerical aperture, and thus sacrifice some of your trapping efficiency.

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The N.A. quandary

- In the real world there tends to be a trade-off between numerical aperture, trapping efficiency, power loss and trapping depth.

Numerical Aperture

	1.0		1.4
Trapping efficiency	low		high
Trapping depth	deep		shallow
Power loss	30%		70%

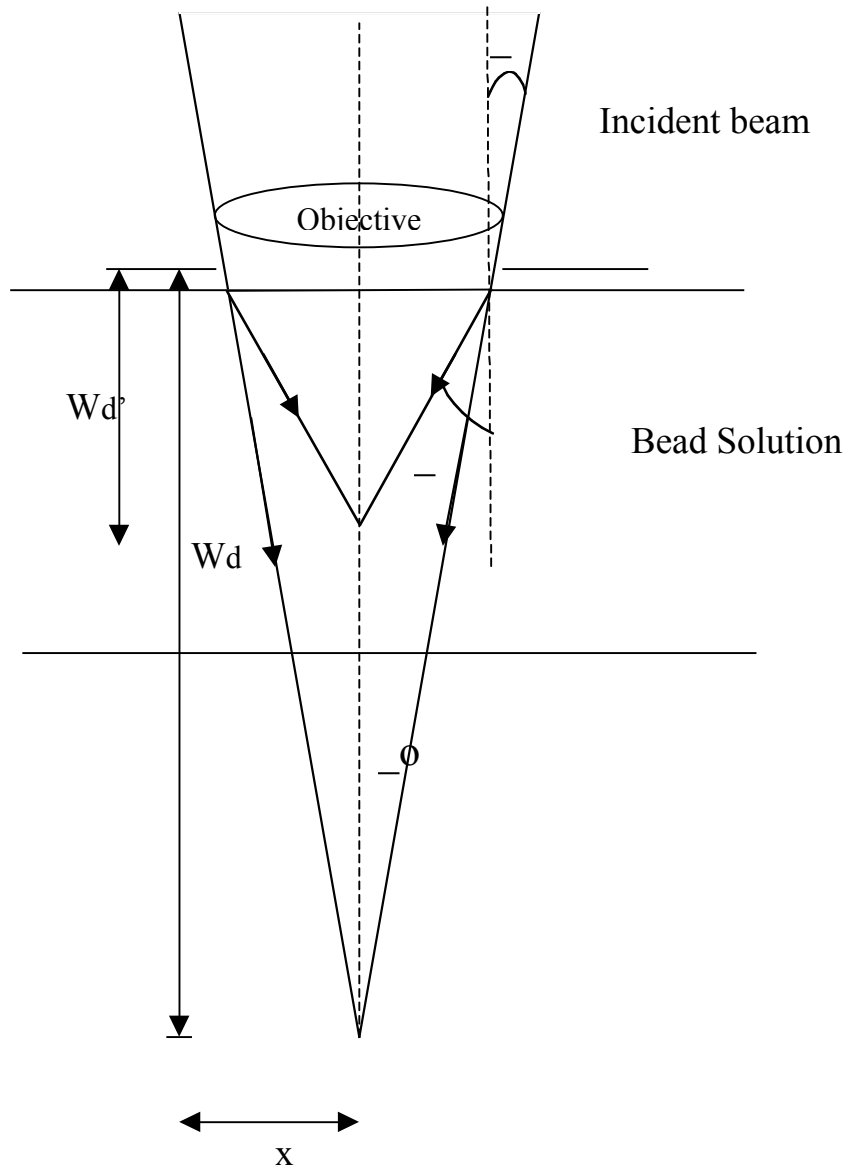Building Partnerships Between Engineering and Medicine

Fig (3)

The constraint on the trapping depth is further increased when the bead solution has a lower index of refraction than the immersion oil used with the lens. It is found that the working distance of the lens is further reduced by a factor of:

$$\sqrt{[(n_2)^2 - (NA)^2]} / \sqrt{[(n_1)^2 - (NA)^2]} : \text{ (see Appendix A)}$$

Which is typically around 1/3.

Another technical difficulty faced when setting up the laser beam is that the beam must be symmetric about the direction of propagation in order for the optical trap to be stable. This is achieved by adjusting the mirrors that lead up to the microscope so that the beam passes directly through the middle of each lens.

From the geometry above:

$\tan(\theta 1) = x/wd'$; $\tan(\theta o) = x/wd$

$\Rightarrow \tan(\theta 1)/\tan(\theta o) = wd/wd'$

using $\tan\theta = \sin\theta/\sqrt{(1-\sin^2(\theta))}$: $wd' = wd[\,(\sin\theta o/\sin\theta 1) * \sqrt{(1-\sin^2(\theta 1))} / \sqrt{(1-\sin^2(\theta o))}\,]$

now using the fact that the $NA = (n_1)\sin(\theta o)$, and $(n1)\sin(\theta o) = (n2)\sin(\theta 1)$,
where n1 = refractive index of oil and n2 = refractive index of water.
We get:

$Wd' = wd\{\,(n1)(n2)\sin(\theta o) * \sqrt{[1-(n1^2)\sin^2(\theta o)/(n2^2)]} / (n1^2)\sin(\theta o) * \sqrt{[1-\sin^2(\theta o)]}\,\}$

$= wd\{\sqrt{[(n_2)^2 - (NA)^2]} / \sqrt{[(n_1)^2 - (NA)^2]}\}$ Equation (1A)

8

References:

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