OPTICAL TWEEZERS: THE POWER OF LIGHT

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ABSTRACT

An optical tweezer is a scientific instrument that uses a focused laser beam to provide an attractive or repulsive force. They have been a powerful tool with broad reaching applications in physics and in the study of biological systems. In biophysics, it is important to be able to manipulate particles in the micron-size regime without damaging them. Optical tweezers are very useful for this, because they can manipulate small particles very precisely without causing damage. In this study, we present an optical manipulation system that uses red light from a helium neon laser as tweezers. This experiment focused on the manipulation of optical tweezers by trapping a biological cell, yeast, and a living photosynthetic organism, algae. Stable optical traps of both yeast and algae, extending from one to three minutes, were observed.

INTRODUCTION

Optical tweezers is the name given to an instrument used for trapping and manipulating microscopic particles solely by the use of lasers. The manipulation of particles using diffracted light was invented by Arthur Ashkin [1] and has become a powerful tool in modern biological laboratories. It has provided a noninvasive method for manipulating biological cells. Optical tweezers can be used to trap individual cells as well as cell organelles and even DNA.[2]

Arthur Ashkin performed the first optical trap in 1971 and received the Nobel Prize for his research with optical tweezers. However, his main goal was not to use optical tweezers to move cells, but rather to trap them. The first application in biology came in the late 1980s when Ashkin and his colleagues used optical traps to capture a tobacco mosaic virus, an e.coli bacterium, and other bacteria cells. During their research, they observed that the green laser destroyed the cell by a process known as opticution – death by light. Consequently, they decided that an infrared laser with a much larger wavelength would be more efficient. They were able to trap algae, green plant cells, red blood cells and structures within the cell without damaging the cell wall. Besides the regular two-dimensional optical trap, Ashkin and his colleagues created levitation traps, which could make the particles move up and down as well as side-to-side.

Optical tweezers have had various applications since Ashkin's experiments, especially in biology. Scientists have used them to study processes inside the cell, sometimes performing "cell surgery," cutting and moving cell organelles. In addition, optical tweezers have been used to manipulate chromosomes during cell division to better understand mitosis. Moreover, optical scalpels, using femtosecond lasers,[4] have been very useful in DNA research, as a single fragment can be isolated from the rest of the DNA chain and captured by an optical trap. Alternatively, individual organelles can be separated from the cell and manipulated, a process known as cell sorting. The optical traps employ a spatial light modulator to split the beam into a series of individual lasers. As a result, a single beam can create several hundred traps. Each laser can then be individually controlled, and a whole system of particles, manipulated. This system is often used in cell sorting, that is, the movement and isolation of a specific set of cells into a container.[6] It is also a technique for studying cell-cell interactions. By forcing two cells into contact with one another, researchers can investigate the effects one cell has on a neighbor. Such experiments have increased the ability to observe cell structure and function.[7]

Further studies have shown that it is possible to use optical tweezers for *in vitro* fertilization. Scientists have manipulated sperm cells in three directions. However, because of possible genetic damage, such *in vitro* fertilization must be studied in more detail.

Optical tweezers have been usefully applied to cancer research. Because cancer cells grow and divide rapidly, they typically have a less developed cytoskeletal structure, meaning they are much less rigid than normal cells. Researchers have taken advantage of this fact by using optical tweezers to measure the elasticity of the cells with a technique known as optical stretching.[8] This technique can also discern malignancies, and detect and isolate stem cells, which also have a far less developed cytoskeleton.

Research with optical tweezers was first done at The College of New Jersey by Brandon Bentzley, who assembled the setup and wrote a manual for its assembly and use.[9] He tested the first optical trap by moving polystyrene spheres up to 10 micrometers in size. We have continued this work by using the optical trap to manipulate algae and yeast cells.

THEORY

The basic principle behind optical tweezers is the momentum transfer that occurs when light is bent. Optical tweezers use light to manipulate microscopic objects as small as a single atom. Light carries momentum that is proportional to its energy and in the direction of propagation. Any change in the direction of light, by reflection or refraction, results in a change in its momentum. If an object bends the light, causing a change in its momentum, conservation of momentum requires the object to experience an equal and opposite momentum change. The resulting force acts on the object.

The momentum of a single photon is defined as Planck's constant divided by the wavelength of the light, which is commonly referred to as radiation pressure.[10] The concept of radiation pressure was first conceived in 1871 by James Maxwell and later proven in 1900 by Lebedev. The knowledge that light could be used to manipulate objects was already firmly established in the 1970s by Ashkin, who explained that the idea that light could be used to trap particles occurred to him when he shined a laser into water and watched as the particles scattered.[11]

An optical tweezer works by focusing a collimated laser beam through a microscope objective. This creates an "optical trap" which can hold a small particle at its center. Since the index of refraction of the particle is different from its surroundings, it acts as a lens and any entering photon will be refracted. The optical forces experienced by the trapped particle result from the scattering of light and consequent gradient forces when the particle interacts with light.

The scattering force is present even in the absence of a stable trap, and pushes objects along the beam in the direction of propagation (see Figure 1a). The light that is deflected in the particle results in a gradient force that pushes the particle vertically towards the propagation of the laser beam, that is, the largest intensity of light in the middle of the laser beam. By focusing the light, the gradient force pushes the particle backwards. In general, the gradient force is caused by the photons coming in at the sides of the particle because they have the highest angle of incidence. The scattering force is caused by the photons in the center of the beam. A photon coming in at an angle is refracted after passing through the particle, and exits in a new direction (see Figure 1b). The conservation of momentum causes the particle to move in the direction opposite the momentum change. The resulting force on the particle is upwards and towards the center of the beam. If this force overcomes the propagation force of the laser beam the particle is stably trapped at a position slightly beyond the focal point of the beam (see Figure 1c).

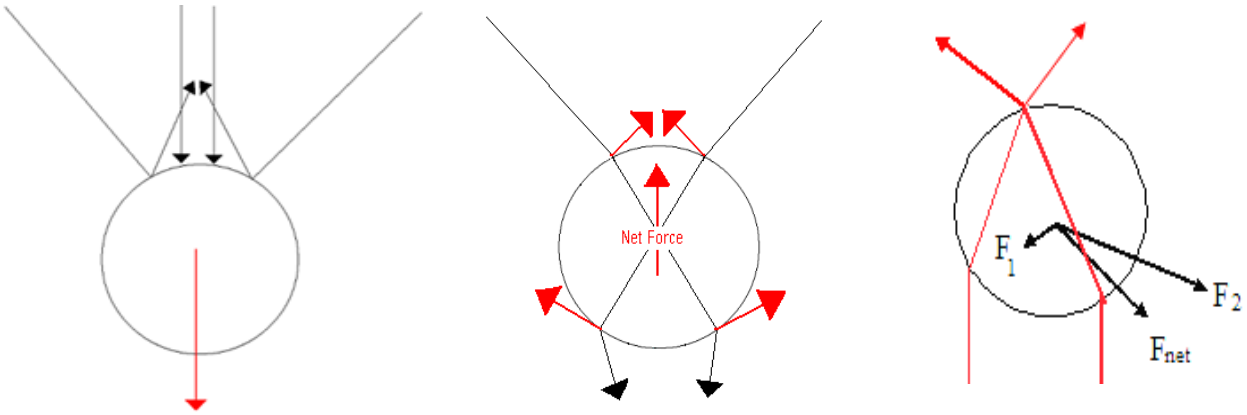

Figure 1:
a) Scattering force: A particle encountering the laser beam will be pushed towards the center of the beam, if the particle's index of refraction is higher than that of the surrounding medium. However, only a fraction of the photons striking the particle will be refracted. The reflected and absorbed photons, forces the particle downstream, in the direction of the laser propagation.
b) Gradient force: If the particle is located at the center of the beam, then individual rays of light are refracting through the particle symmetrically, resulting in no net lateral force. The net force in this case is along the axial direction of the trap, which cancels out the scattering force of the laser light. The cancellation of this axial gradient force with the scattering force is what causes the bead to be stably trapped slightly downstream of the beam waist.
c) Optical trap: if the particle is displaced from the center of the beam, the particle has a net force returning it to the center of the trap because more intense beams impart a larger momentum change towards the center of the trap than less intense beams, which impart a smaller momentum change away from the trap center. The net momentum change, or force, returns the particle to the trap center.

To achieve the tight focus required for trapping, a high numerical aperture objective lens is used. This allows the beam to be focused to a diffraction limited spot on the specimen plane, and for the rays to converge at the focus, which is important for stable axial trapping. The laser beam usually has a Gaussian profile, in which case it slightly overfills the back aperture of the objective.[1]

EXPERIMENTAL SETUP

The setup consisted of a series of lenses and mirrors used to focus the light from the laser onto the slide with the specimen through the microscope objective. In this experiment, we used a Helium Neon red laser with a wavelength of 632.8 nm. We need this laser and no other because earlier studies [1] show that red and infrared lasers are ideal in optical traps with live specimens. If a lower wavelength is used, the individual photons that form the beam have more energy and may damage or kill the cells.

After leaving the source, the beam is reflected off a plain mirror placed at a 45-degree angle with respect to the beam, and directed onto a convex lens with a focal length of 5 cm and then to another convex lens, with focal length of 30 cm. They were placed 35 cm apart, which is the sum of their focal lengths. These two lenses together constitute a 6x beam expander. It is necessary to expand the laser beam to fill the entire objective of the microscope, so that the light can enter the cell at the largest possible angle, therefore exerting the largest possible force. The light enters the first lens and is focused onto a point 5 cm away. Then the beam expands until it reaches the second lens. When it leaves the second lens, it is uniform and parallel, as well as magnified six times, which is equivalent to the ratio of the two focal lengths (see Figure 2).

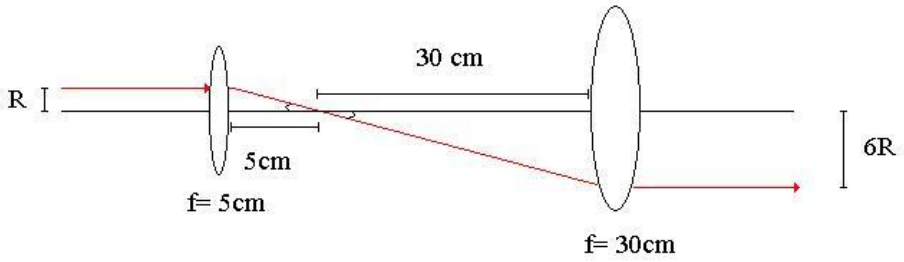

Figure 2:
Beam expander: light enters the lens with the smaller focal length first, and is magnified by 6 times its original radius by the time it leaves the second lens.

After the light passes through the beam expander, it is directed onto the gimbal mount mirror. This is a plane mirror the angle of which can be changed to control the direction of the beam. After the gimbal mount mirror, the light is directed through a third lens, which then focuses it on the microscope objective.

As the diagram shows (see Figure 3), the third lens is positioned so that it can focus the laser beam at the location of the trapped cell. After Lens 3, the light is reflected off the dielectric mirror down onto the microscope objective. The dielectric mirror reflects most of the light, but allows the light from the source to pass. Lens 4 and the CCD are placed above the dielectric mirror so that a clear, magnified, real image of the cells is created. The height of Lens 4 can be changed depending on the desired magnification, as well as to bring the trapped cell into focus.

To create a successful trap, the slide underneath the objective must be moved up and down very slowly to find the perfect distance to focus the laser light to exert enough force on the cells. From the CCD camera the image of the cells is sent to a monitor and a video camera, allowing us to observe the cells on a larger screen as well as record videos and transport them to a computer connected to the video camera.

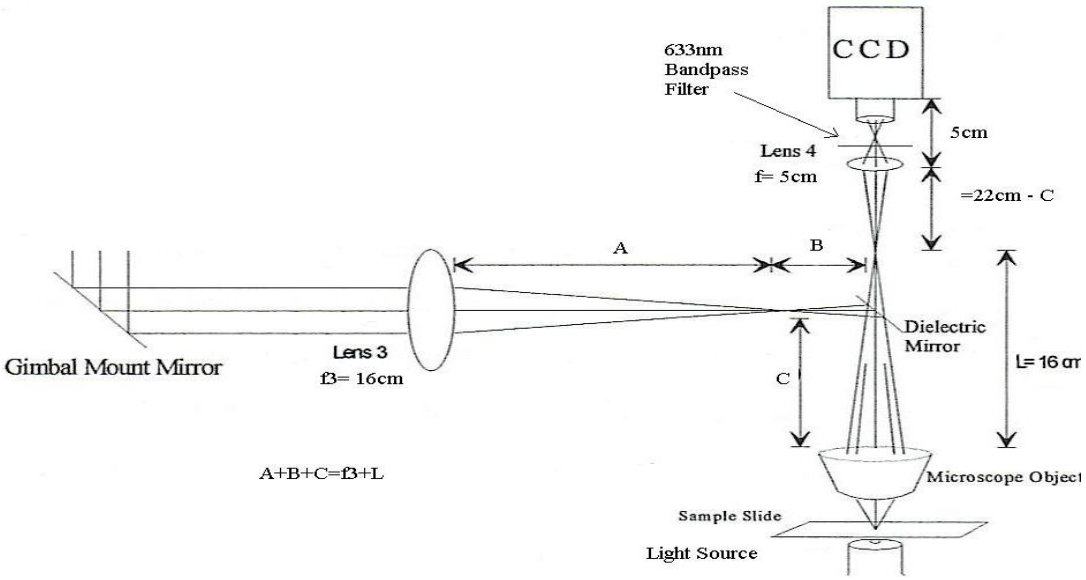

Figure 3: The light passes from the gimbal mount mirror, through Lens 3, and onto the microscope objective, with the aid of the dielectric mirror. It is then focused on the slide, creating the trap.

RESULTS

The first stage of experimentation was to set up the instruments that we would be working with and trap polystyrene spheres ranging in size from 2 μm to 10 μm. We were able to trap and record spheres of 6 μm to 10μm. For example, a 10μm sphere was trapped for about one minute before the trap weakened and the sphere escaped.

The second phase of experimentation consisted of trapping a living biological cell. Yeast, *Saccharomyces cerevisiae,* was chosen as the ideal eukaryotic microorganism because of its genome sequence. We were able to trap live yeast cells of different sizes. Figure 4A shows an image of a trapped yeast cell and 4B is a close up of the cell.

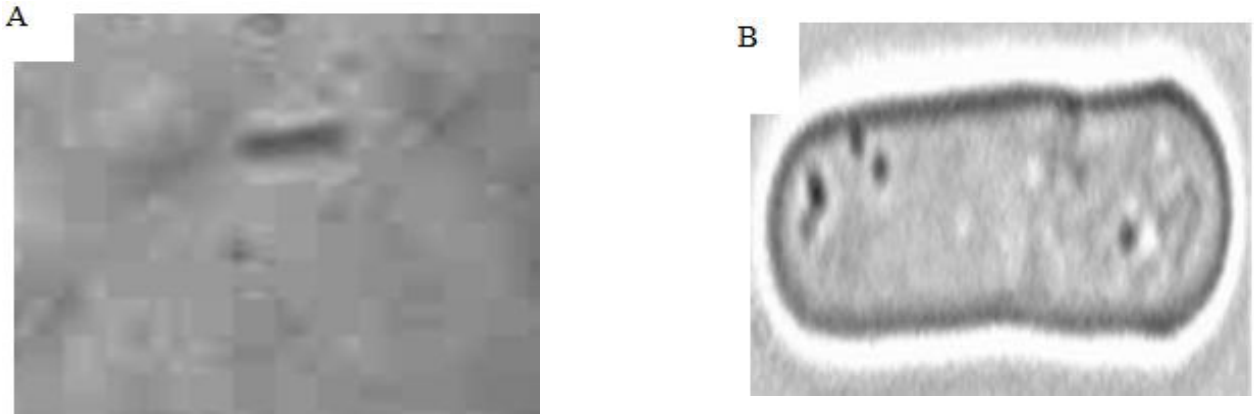

Figure 4:
A. Image of a trapped yeast cell, which was trapped for about 9 minutes. As other cells continued to move around it, the trapped cell was held in place by the laser beam.
B. Magnified image of a yeast cell.

We also were successful in using the optical trap to capture polystyrene spheres and to obtain a video of captured algae cells (see Figure 5). The cells stood still or spun as a result of angular momentum, while around them algae cells moved with Brownian motion. Cells that passed close to the trap sometimes slowed down visibly, affected by the force exerted by the fringes of the laser beam. The cells remained trapped for various periods of time, but rarely more than two minutes. Besides keeping them still, we were able to move algae cells in two dimensions, although in this case the trap didn't usually last as long and the cell would be lost. It was much easier to trap polystyrene spheres, because these did not move very much, whereas the algae cells moved very fast, which made it difficult to focus the laser on any one of them. It was nearly impossible to chase the fast moving cells; the best method was to set up a trap and wait until a cell would "stumble" into it while randomly moving around. Often cells slowed down as they came closer to the beam, but most had too much momentum and were able to continue moving. However, we were able to trap several of them and hold them in place.

Figure 5: An image of a captured algae cell, illuminated by the laser beam and held in place while other cells, which are also visible in the picture, continued to move. The other cells also slowed down whenever they came close to the focused laser beam.

DISCUSSION

We learned the theory of optical tweezers and set up the experimental apparatus. Our main objective was to trap a living cell in three dimensions. Initial studies were conducted on polystyrene spheres ranging in size from 2 μm to 10 μm. The particles were captured but tended to be displaced by the scattering force, and would remain trapped only for a few seconds before being forced downwards.

Biological samples were obtained using commercial grade baker's yeast and growing them in a solution of warm water and sugar for a few days. As a result, we obtained yeast cells of different sizes, which improved our chances of trapping them. Some of the yeast cells we found were very small, less than a micron in size. Others were too big and exceedingly difficult to trap. Long hours in the lab yielded yeast cells floating past the trap with some "sticking" briefly. This was far from a stable trap, unless the cells were caught exactly in its center.

The setup was quite sensitive. The strength of the trap varied daily. Components had to be continuously adjusted to achieve the most powerful trap possible. Redesigns and reassemblies of the equipment led us to optimize the setup. The improved setup allowed us to trap a yeast cell for about nine minutes.

Other biological samples were obtained from Lake Ceva. There were different species of organisms living in the sample water. The majority of the cells were either too large or too fast to be captured in the trap. We were able to trap some of the cells, and the experience of viewing them was very exciting in itself.

SUMMARY

The goal of this research, to use optical tweezers to trap living cells, was achieved, as we manipulated living algae and yeast cells. This means that the TCNJ optical trap is available for biological applications, which opens up endless possibilities for future research in that field. The trap can be used to study and control cell-cell interactions as well as some of the processes that occur within cells.

In the future, we hope to improve the setup, perhaps to measure distances moved by trapped objects. This could be done by adding and calibrating a Quad Detector, which allows for the observation of changes in the position of the laser, and therefore of the cell in the trap. This successful and rewarding study opens the way for new experiments with the optical tweezers.

REFERENCES

Ashkin, A. "Optical trapping and manipulation of neutral particles using lasers," Proc. Natl. Acad. Sci. USA, vol. **94**, 4853-4860, (1997).

Bentzley, Brandon. "How to build optical tweezers," The College of New Jersey, Ewing, NJ. 2005, private communication.

Bhattacharya, Shanoi. "Laser Beams sort stem cells by springiness", NewScientist.com news service. (April 12, 2005).

Hill, Murray. "He wrote the book on Atom Trapping," http://www.bell-labs.com/user/feature/archives/ashkin,(1997).

Liang, H., Wright W. H., Cheng, S., He, W., Berns, M. W., "Micromanipulation of chromosomes in PTK2 cells using laser microsurgery (optical scalpel) in combination with laser-induced optical force (optical tweezers)," Exp Cell Res. **204**, 110-20 (1993).

Louchev, O.A., Juodkazis, S., Murazawa, N., Wada, S., and Misawa, H. "Coupled laser molecular trapping, cluster assembly, and deposition fed by laser-induced Marangoni convection," Opt. Express **16**, 5673-5680 (2008).

Molloy, Justin E. and Padgett, Miles J., Department of Biology, The University of York, York. Department of Physics and Astronomy, University of Glasgow, Glasgow. http://www.optical-tweezers.org/UK/BT-tweezers/info/Light_Action_Optical_tweezers.htm (2001).

Plewa, Joseph, Del Sol, Timothy, Lancelot, Robert W., Lopes, Ward A., Mueth, Daniel M., Bradley, Kenneth F., Gruber, Lewis S. "Prospects for Holographic Optical Tweezers," Arryx Inc. Chicago (2004).

Schibli, T. R., Kuzucu, O., Kim, J. W., Ippen, E. P., Fujimoto, J. G., Kaertner, F. X., Scheuer, V., and Angelow, G., *IEEE J. Sel. Top. Quantum Electron.* **9,** 990 (2003).

Williams, Mark C. "Optical Tweezers: Measuring Piconewton Forces," Northeastern University, Department of Physics (2004).