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An application of optical tweezers

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AN APPLICATION OF OPTICAL TWEEZERS

BY

PATRICK W. SWANSON

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF
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IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
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Abstract

Oxycyte is a perfluorocarbon-based material that can mimic the oxygen carrying capabilities of hemoglobin and is in the testing stages of acting for use as a blood substitute. However, a problem associated with using Oxycyte as a blood substitute is that it clumps together too easily and does not coagulate. An approach for studying the problem uses optical tweezers to help model the forces between Oxycyte particles. Elements of optical tweezers, including laser beams, microscopes, and operation by trapping particles with light will be outlined. An experimental system created to study the forces between particles is presented. Experimental issues and successes will be discussed, along with further research that can be done using the proposed system.

Acknowledgments

I would like to acknowledge my mother, grandmother, and the rest of my family for the support they have given me throughout my pursuit of both undergraduate and graduate degrees. I would also like to thank Dr. Daniels for bringing me aboard for this research. I am also grateful to my employers, who wanted me to pursue a Masters degree in Science. Finally, I would like to thank the committee members who reviewed my work over the last three summers, Dr. Daniels, Dr. Methven, and Dr. Aryainejad. Thank you for the time you have spent teaching and supporting me through the last three years.

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Introduction

Blood is a substance that has been and will continue to be in high demand for emergencies and medical procedures like transfusions. A blood substitute would be beneficial to help ease the never ending need for a ready supply of blood. Developing a blood substitute has been a focus of the scientific community, and several promising possibilities have been invented over the last few decades. For example, Oxygen Biotherapeutics has developed a product called "Oxycyte" which shows possibilities of being a blood substitute. Oxycyte has the ability to absorb oxygen similar to red blood cells. Oxycyte consists of a perfluorocarbon group surrounded by a phospholipid layer. The perfluorocarbon group gives Oxycyte the oxygen carrying capability, while the phospholipid layer makes it water soluble so that it can simulate the consistency of blood. The problem with Oxycyte is that it readily clumps but does not coagulate, so it remains only in the testing phases. This project proposes a method to better understand the attractive forces between the Oxycyte particles and try to fix the clumping problem. Optical tweezers are to be used in order to measure the forces between the Oxycyte particles. Once the forces are modeled, it may be possible to discover a solution to the clumping problem of Oxycyte.

Literature Review/Theory

In this literature review, topics of emphasis will include lasers, microscope optics, and the elements of optical tweezers. The salient papers are discussed, and the first section will address laser operation and theory.

I. Overview of Lasers

A laser, or Light Amplification by Stimulated Emission of Radiation, was used for this experiment. This particular laser is an Argon Ion laser that operates at a wavelength of 488 nm, which is blue in the visible spectrum. Laser light often comes from atomic transitions in the medium of the laser, in this case, argon. If a collection of atoms have most of their electrons in an excited state, with the ground state relatively unpopulated, a photon of the proper energy is more likely to cause stimulated emission than it is to cause stimulated absorption. In stimulated emission, the electron is induced to make the transition from excited state to ground state, producing a photon with energy equal to the change in energy levels of the atom. Thus, one photon is incident on an excited atom and after the stimulated emission of the two identical photons, the atom will now be in its ground state. In a collection of excited atoms, one photon would become two, and those two may become four, and so on until a large number of photons are created. This multiplication of photons is the amplification of the laser. The photons also move in a coherent fashion, in the same direction.[1]

II. Microscope Optics

A microscope uses two convex lenses in succession in order to magnify small objects. The first lens, called the objective lens, is usually small and spherical, with a relatively short focal length. It collects light from a source near the object that will be magnified and projects a magnified, real image near the second lens, called the eyepiece. The eyepiece usually has a short

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[1] Pedrotti and Pedrotti. Optics and Vision. P. 242, 248, 253. Prentice Hall, 1998.

focal length as well, and the image formed by the objective usually lies within the focal length. Because the image formed is within the focal length, this will create a much more magnified, virtual image. Refer to Figure 1 for a ray diagram of a standard microscope. A real object, O, is placed near, but just outside, the focal point of the objective lens, L_1. As long as the object is outside the focal length, this creates a magnified, real image of the object, I_1. I_1 becomes the object for lens L_2. If I_1 is inside the focus of the eyepiece, L_2, the image created by the objective will be virtual and on the same side of the eyepiece as the object (I_2). In a typical microscope the final image is about 25 cm from the eyepiece so as to provide comfortable viewing for the human eye. In this experiment a camera is used for viewing.

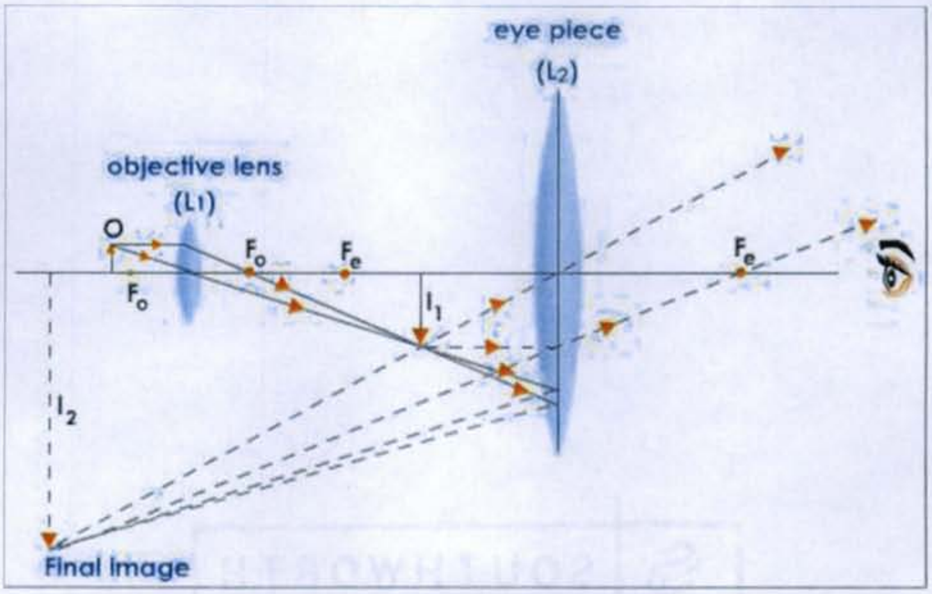

Figure 1[2]

Another factor that determines what is in the microscope's field of view is the tube length, or the distance between the eyepiece lens and the objective lens. The formula for the magnification of a microscope is

$$M= -(L)(25\text{ cm})/(f_0 f_e) \qquad (1)$$

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[2] http://www.tutorvista.com/content/physics/physics-ii/optical-instruments/compound-microscope.php

where L is the tube length of the microscope, f_0 is the focal length of the objective lens, and f_e is the focal length of the eyepiece. [3]

III. Optical Tweezers

In this experiment, a laser beam was used to trap particles in a manner similar to previous work done by A. Ashkin in 1969 at Bell Telephone Laboratories.[4] Ashkin was able to trap micron sized particles using only the force of radiation pressure from a monochromatic laser beam. Ashkin discovered that an unfocused laser beam shone off center through a 2.68 micrometer diameter sphere would simultaneously draw the bead in to the beam axis and accelerate the bead in the direction of the light. [5] He showed that the particles trapped by the laser beam no longer showed the effects of Brownian motion and wander off. Initially, this research was done with two laser beams, but as advances in technology grew, it was shown that particle trapping is possible using only one laser. Ashkin later discovered that a single beam would trap small dielectric particles in three dimensions.

Optical tweezers are used to hold very small, transparent objects in place using laser trapping. Every photon has an individual momentum, given by the equation

$$p=h/\lambda \qquad (2)$$

where h is Planck's constant and λ is the wavelength of the photon. Each individual photon has a very small amount of momentum, and when a large number of photons exist, their collective momenta can be used in optical tweezers.

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[3] Serway & Jewett. Physics for Scientists and Engineers, 8th edition. P. 1071 (2010)

[4] A. Ashkin. Acceleration and Trapping of particles by radiation pressure. *Phys. Rev. Lett.* 24, 156-159 (1970)

[5] A. Ashkin and J.M. Dziedzic. Optical levitation of liquid drops by radiation pressure. *App. Physics. Lett.* **19**, 283-285. (1971)

Laser light of a particular wavelength can be shone through a small, transparent sphere, and the results can be used to keep the sphere in place. When laser light hits the surface of the sphere, most of that light is refracted into the sphere. The angle the laser light is bent through the sphere can be calculated using Snell's Law,

$$n_i \sin\theta_i = n_r \sin\theta_r \qquad (3)$$

where n_i is the index of refraction of the material outside the sphere, n_r is the index of refraction of the material in the sphere, θ_i is the angle of incidence and θ_r is the angle of refraction. Because the light changed direction, its momentum changed direction as well. This means that if light changes direction upon entering a different medium, a small but finite and measurable force will be exerted on the object.

Figures 2 and 3 show how optical tweezers are able to trap particles. In Figure 2, laser beams 1 and 2 have equal intensity. If rays 1 and 2 are traveling parallel to each other when they enter the particle, ray 1 is bent to the right. Since the photons changed momentum to the right, there must exist an equal and opposite change in momentum in the particle to the left, this results in a force F_1 acting on the particle. In a similar manner, ray 2 is bent left, so there exists a force exerted to the right on the particle due to ray 2. The x components of the forces are equal and opposite, and the particle does not accelerate in the x direction.

The second part of the figure shows the net force on the particle if the beams are of unequal intensity. If beam 1 has less intensity than beam 2, the particle should be attracted to beam 2. This is because since beam 2 has more photons, when they refract through the sphere, the net momentum change for them is higher than beam 1, and the force from beam 2 should then be higher. This creates a trap in the lateral direction for the particle, and shows the particle should be attracted to the place of highest intensity of the beam.[6]

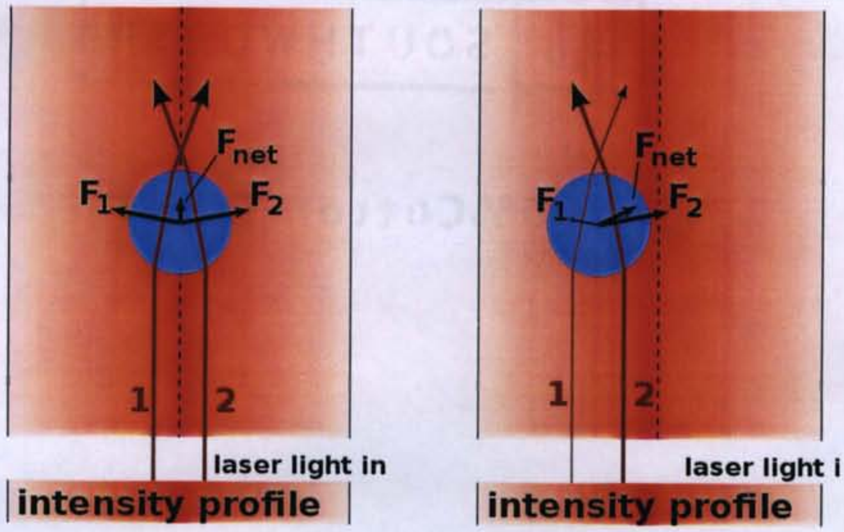

Figure 2[7]

Figure 3 demonstrates the particle is also attracted to the focus of the beam in the y direction. The beam is of Gaussian shape, and its highest intensity is in the center of the figure. The net force on the particles is directed towards the focus of the beam, creating an optical trap for the particle.[8]

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[6] A. Ashkin and J.M. Dziedzic. Optical trapping and manipulation of viruses and bacteria. *Science*. 1987 Mar 20;235(4795):1517-20.

[7] Roland Koebler, http://en.wikipedia.org/wiki/Optical_tweezers, Accessed 7/10/2012.

[8] A. Ashkin and J.M. Dziedzic. Optical levitation of liquid drops by radiation pressure. *App. Physics. Lett.* **19**, 283-285. (1971)

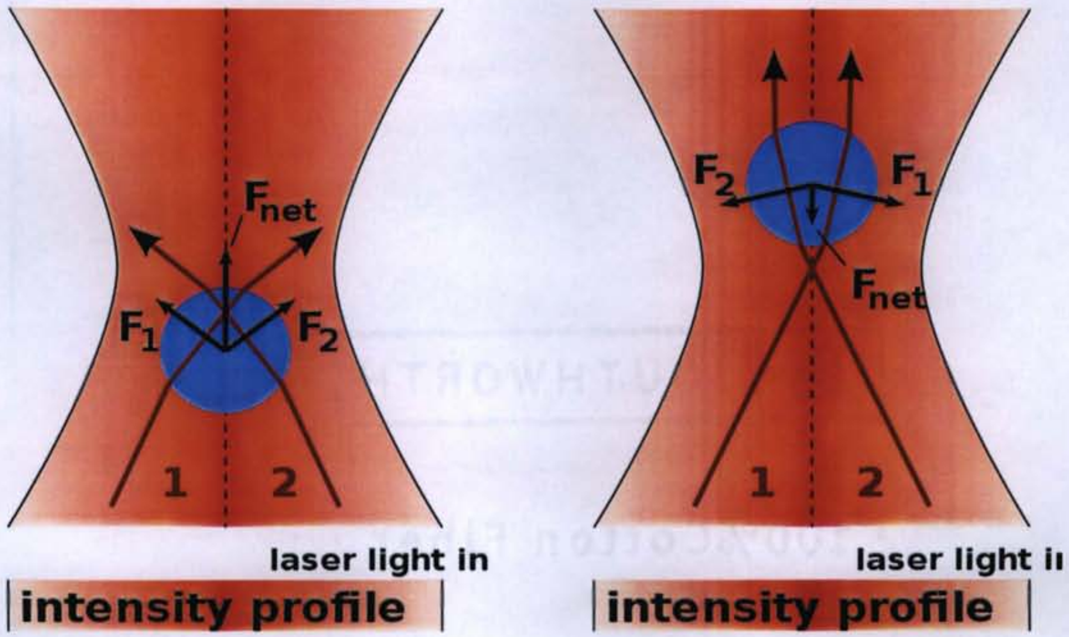

Figure 3[9]

Generally, for optical tweezers to work, the amount of light refracted must be much greater than the amount of light reflected at the surface. Hence, the particle to be trapped must be transparent in order for the particles to get trapped by the laser beam. The reason the laser beam needs to be focused is that it keeps the bead in the center of the laser in addition to keeping it at a fixed axial position. As the photons change direction due to their momentum change at both the entering and exiting interfaces of the particle, the force exerted from Newton's third law is towards the focus of the beam, regardless of whether the particle is displaced a small amount either in front of or behind the beam.[10]

As mentioned previously, a small but finite force is exerted on particles that are trapped. One of the ways this force can be calculated is by using the momentum flux passing through the beads. The momentum flux, or intensity of the light per unit area, where p is the total

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[9] Roland Koebler, http://en.wikipedia.org/wiki/Optical_tweezers, Accessed 7/10/2012.

[10] A. Ashkin and J.M. Dziedzic. Optical levitation of liquid drops by radiation pressure. *App. Physics. Lett.* **19**, 283-285. (1971)

momentum of the photons, n is the index of refraction of the material, S is the Poynting vector (or the direction of momentum of the photons and also the direction of the energy flux density), and c is the speed of light in vacuum, is given by

$$d\left(\frac{dp}{dt}\right) = \left(\frac{n}{c}\right) S dA \qquad (4)$$

From Newton's Second Law,

$$dp/dt = ma \qquad (5)$$

taking the antiderivative of the above equation will yield the force on the bead, so the net force *F* exerted on the particle is

$$F = \left(\frac{n}{c}\right) \iint (S_{in} - S_{out}) dA \qquad (6)$$

Where S_{in} is the incoming momentum flux, S_{out} is the outgoing momentum flux, and dA is a small element of area of the polystyrene bead. The total force is the incoming flux minus the outgoing flux, multiplied by the index of refraction of the material divided by the speed of light. This means that if light changes direction upon entering a substance, a small but finite and measurable force will be exerted on the object.

In addition, the greatest momentum change for the particles entering the bead occurs for the photons that enter at the edges, since they will refract or bend the most and result in the greatest gradient or trapping force. The forces on the particle push it towards the highest intensity of the beam. At the focal point the laser beam itself should be approximately the width of the object to be trapped. To do this, a specific lens is needed with a high numerical aperture. The numerical aperture, or NA, is defined as

$$NA = n \sin \alpha \qquad (7)$$

where n is the index of refraction of the material and α is half of the angle from the outside of the lens to the object.

A viscous drag force will be exerted on the beads as they move around due to Brownian motion. This force can be calculated using Stokes' Law,

$$F=6\pi\eta rv \qquad (8)$$

where η is the viscosity of the fluid, r is the radius of the bead, and v is the velocity of the bead relative to the fluid. If any measurements on the force on the bead are to be taken, this drag force must be accounted for and measured.

Once the forces are properly calculated, it has been noted that polystyrene spheres held by a laser beam experience an attractive force toward the focus of the beam. To first order, this restoring force is in most cases proportional to the distance between the center of the sphere and the focus of the lasers, or

$$F=-kx \qquad (9)$$

where k is the trap stiffness, analogous to the spring constant in Hooke's Law, and x is the displacement from the equilibrium position. For optical tweezers, a value of 50 pN/μm is reasonable.

In order to determine the trap stiffness, the system must be calibrated for background movement and Brownian motion of the particles. This is done through the use of eq. 8, Stokes' Law. Since the viscosity of the liquid is known and the polystyrene spheres have a diameter of 3 μm, only the velocity of the particles must be measured to calculate the drag force on the particles. One method involves trapping a bead, holding it perfectly still, and oscillating the cell at a fixed amplitude and frequency. If this is done using a video camera, the position as a function of time of the bead should be $x(t)= A\sin(\omega t)$. The velocity, or dx/dt, should then be $v(t)= A\omega\cos(\omega t)$. This velocity can then be substituted into Stokes' Law, and the drag force can be determined.[11]

Oscillating the system can be accomplished by a piezo driven translation stage. This stage can be computer controlled so that there is a clear connection between the motion of the stage and the bead position in the field of view. The development and implementation of this stage will be a future direction of this work.

The intensity of the laser beam can push the particles in the direction of the laser beam if the intensity is too high, but the particles will not be trapped if the beam is not intense enough. As a result, a balance of focus, intensity, and transparency that must be exactly right for trapping to occur. The laser focus needs to be approximately the size of the object held in place, so that almost all the light from the laser will hit the object. Most of these forces are in the picoNewton range (10^{-12} N). Forces on the order of picoNewtons are good for many applications in biology. For example, 60 pN is the force necessary to unravel the DNA double helix.[12]

Since lasers can be used to trap particles, they have been used to do research on many different things, such as studies in colloid science, cells, dipolar chains, and to trap sets of particles rather than single particles. Crude optical tweezers setups are now available commercially, but due to the nuances of particular experiments, and because of the incredibly

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[11] Mark C. Williams. Optical Tweezers: Measuring Piconewton Forces, in *Single Molecule Techniques*, Petra Schwille, ed., a volume of the *Biophysics Textbook Online*, L. DeFelice, editor-in-chief, Biophysical Society, Bethesda, MD

[12] Mark C. Williams. Optical Tweezers: Measuring Piconewton Forces, in *Single Molecule Techniques*, Petra Schwille, ed., a volume of the *Biophysics Textbook Online*, L. DeFelice, editor-in-chief, Biophysical Society, Bethesda, MD

precise measurements that need to be made, most setups are custom built by each lab so modifications can be made easily.[13]

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[13] C. Batters and J.E. Molloy. Optical Tweezers. Chemical Biology: Techniques and Applications. John Wiley and Sons Ltd. England, 2006.

Methodology

This study used optical tweezers to measure the forces between Oxycyte particles. A schematic of the setup built in the laboratory is shown. (Figures 4 and 5)

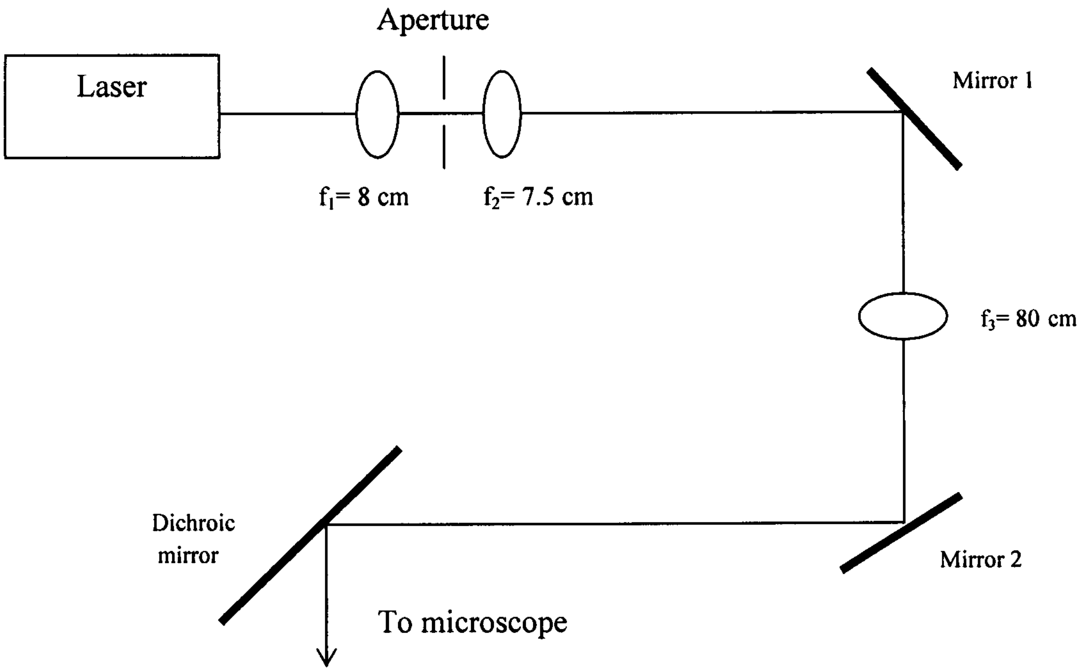

Figure 4

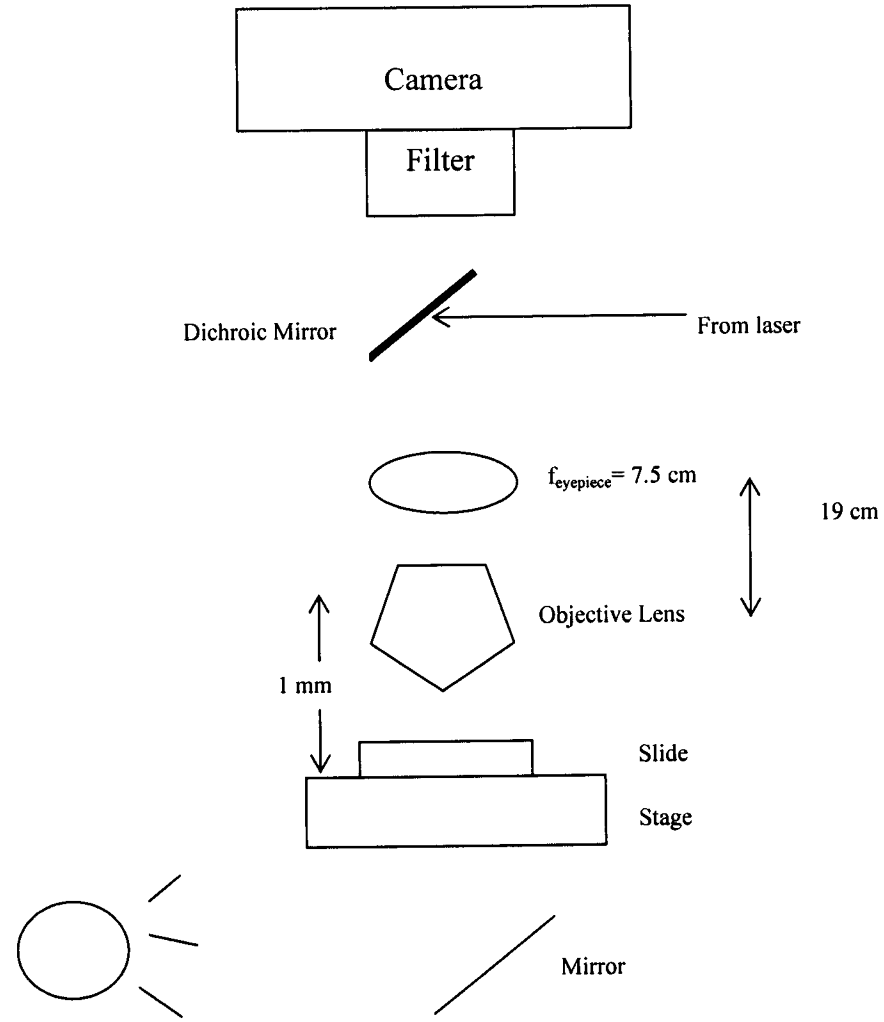

Figure 5

Figure 4 shows the path of the laser beam until it reflects off the dichroic mirror. A 4 W, 488 nm Argon-ion laser is shone through a spatial filter, which passes through two lenses, with focal lengths f_1 and f_2 of 8 cm and 7.5 cm, respectively, and an aperture located between the lenses. The spatial filter helps to make the beam more Gaussian in shape, which is necessary for the trapping particles. After passing through the spatial filter, the beam is reflected off two steering mirrors and focused through a lens of relatively large focal length f=80 cm. After hitting another steering mirror, the light is directed onto a dichroic mirror.

Figure 5 shows the vertical orientation of the objects in the plane the laser light travels. The laser light is reflected downwards through the eyepiece and the microscope through the objective lens onto the microscope slide, where it is focused to a point. The slide is backlit by a white light source for observing the particles as they would be found in a standard microscope. The camera and a filter are located above the Bausch & Lomb microscope. (Figures 6 and 7)

Figure 6

Figure 7

In order to see the objects to be studied, the microscope is used to help collimate and focus the laser beam as well as magnify the objects in order to see them. The dichroic mirror will reflect the blue laser light, which will then be focused through the microscope and be used to trap the polystyrene beads. White light from the backlit microscope is transmitted through the filter attached to the camera at red and orange wavelengths, allowing the viewer to see the bead. Any laser light reflected off the sphere or lens surfaces of the objective and eyepiece will come back through the microscope but will be reflected at the dichroic mirror and also filtered out of the light transmitted to the CCD camera so as not to overwhelm the detector. Parallel posts that contained the reflecting mirrors and the dichroic mirror were then connected together with a horizontal bar and two right angle clamps to increase the stability of the mirrors.

Polystyrene beads were chosen as the objects to "tweeze" because they are readily available in many sizes and also have the necessary properties to refract light, as they are transparent. In these preliminary studies, the polystyrene beads were 3.0 μm in diameter and that size scale serves to calibrate the position in the visual field. The beads can then be bonded with Oxycyte to study the attractive forces between molecules.

After setting up the system, the laser was turned on and the direction of the steering mirrors and lenses was fine tuned. The position of the microscope was adjusted so that the laser light went straight through it and would potentially capture the polystyrene beads on a test slide on the microscope stage. The microscope and steering optics needed to be aligned so that the laser optic axis and the microscope's optical axis were collinear to ensure the laser would be focused on the slide and the camera would see it. This part of the alignment turns out to be the most critical and difficult part.

Results

After much fine tuning, an optical tweezers device was built. Factors that helped achieve this success were inserting a spatial filter near the laser source and inserting a large focal length lens in between the two vertical steering mirrors.

Apparent trapping of the polystyrene beads has been accomplished, as video has been taken of the tweezers at work. The beads appear to be held in place by the laser compared to the background of the rest of the beads. An understanding of the optics of the system and the beads themselves was accomplished.

However, an unexpected problem occurred during this stage of the experiment. Video showed that polystyrene beads were drawn upwards toward the focal plane of the camera, sometimes drawing multiple beads at a time. This indicates that the laser is focusing in a place other than the focal plane of the camera. Though at this point Oxycyte has not been experimented with, it has been obtained in collaboration with Oxygen Biotherapeutics, and a possible method for investigating the clumping problem has been identified.

Discussion

Before research can be continued, the laser needs to be focused in the plane of the microscope slide to solve the "vacuum effect" problem. This will be most likely be solved by finding the optimal location of the 95 cm focal length lens between the two vertical steering mirrors. The option of inserting a lens of a different focal length also remains a possibility.

Optimizing the power of the laser should also be investigated. Different powers of the laser could lead to differences in trap stiffness of the optical trap. This could be important if the forces between Oxycyte particles lie in a certain range of values.

Once the optical tweezers are fully operational, the system should be calibrated using the piezo to find the force the tweezers apply to the beads. Once the piezo stage has been installed and operates properly, the system should be oscillated at a fixed amplitude and frequency, allowing for the use of simple harmonic motion to derive trap stiffness and drag force.

After trap stiffness is calculated, research on the chemistry between the polystyrene beads and Oxycyte must be conducted. The Oxycyte particles should bond with the polystyrene beads. It is also not known whether the Oxycyte particles are large enough to be seen with the equipment currently in place.

Significant progress has been made towards building a set of optical tweezers as a way of measuring forces between Oxycyte particles. A successful determination of the modeling parameters of Oxycyte may hold the key as to the viability of Oxycyte as a blood substitute. Future research on it promises to be exciting and potentially groundbreaking.

Sources

A. Ashkin. Acceleration and Trapping of particles by radiation pressure. *Phys. Rev. Lett.* 24, 156-159 (1970)

A. Ashkin and J.M. Dziedzic. Optical levitation of liquid drops by radiation pressure. *App. Physics. Lett.* **19**, 283-285. (1971)

A. Ashkin and J.M. Dziedzic. Optical trapping and manipulation of viruses and bacteria. *Science*. 1987 Mar 20;235(4795):1517-20.

A. Ashkin, J.M. Dziedzic, J.E. Borkholm, and S. Chu. Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* **11**, 288-290 (1986)

C. Batters and J.E. Molloy. Optical Tweezers. Chemical Biology: Techniques and Applications. John Wiley and Sons Ltd. England, 2006.

E.R. Dufresne and D.G. Grier. Optical tweezer arrays and optical substrates created with diffractive optics. *Rev. Sci. Instruments.* **69**, 1974-1977 (1998)

J.C. Crocker. Measurement of of hydrodynamic cross correlations between two particles in an external potential. *Phys. Rev. Lett.* **82**, 2211-2214 (1999).

K.C. Neuman and S.M. Block. Optical trapping. *Rev. Sci. Instruments.* **75**, 2878-2809 (2004)
Mark C. Williams. Optical Tweezers: Measuring Piconewton Forces, in *Single Molecule Techniques*, Petra Schwille, ed., a volume of the *Biophysics Textbook Online*, L. DeFelice, editor-in-chief, Biophysical Society, Bethesda, MD

Lin Ling, Fei Zhou, Lu Huang, and Zhi-Yuan Li. "Optical forces on arbitrary shaped particles in optical tweezers." *Journal of Applied Physics*, 108

McLaren M, Sidderas- Haddad E, Forbes A. Accurate measurement of microscopic forces and torques using optical tvíieezers. S Afr J Sei.2011;107(9/10), Art. «579,8 pages, doi: 10.4102/sajs.V107Í9/10.579

Micah J. McCauley and Mark C. Williams, "Mechanisms of DNA Binding Determined in Optical Tweezers Experiments." *Biopolymers* **85**: 154-168 (2007).

Pedrotti and Pedrotti. Optics and Vision. P. 242, 248, 253. Prentice Hall, 1998.

P.T. Korda, M.B. Taylor, and D.G. Grier. Kinetically lockied-in colloidal transport in an array of optical tweezers. *Phys.Rev. Lett.* **89**, 128301 (2002)

Roland Koebler, http://en.wikipedia.org/wiki/Optical_tweezers, Accessed 7/10/2012.

Serway & Jewett. Physics for Scientists and Engineers, 8th edition. P. 1071 (2010)