(Keynote Speaker)

Building An Optical Tweezers For Life Science Researches

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ABSTRACT

Optical Tweezers has evolved as a valuable tool to trap and manipulate particles. Since it was introduced in 1986 by Ashkin and colleagues, Optical Tweezers has been used in many research areas. Its applications are found on research in atomic physics, medical physics, chemistry, and more notably in biophysics and cell biology. The simplest optical tweezers can be built from a commercial optical microscope either upright or inverted. The tweezing force comes from the radiation pressure of laser light which is sent to the back of the objective lens then is focused by the lens to the particles in a cuvette or slides. The particles can be any things from atoms, molecules, bacteria, viruses, blood cells to DNA. The optical tweezers are very useful in studying biological samples because it can provide picoNewton and nanometer ranges of force hence one can study the motor properties of the particles without damaging them. There are many parameters can describe a reliable optical tweezers. These parameters depend on the laser wavelength used and the size of the particles. In this article, the process to build an optical tweezers and what optical components needed will be described. The optical tweezers described has been used to trap two size-polystyrene beads with 830 nm laser light from a diode laser. This optical tweezers will be prepared to trap and study chlorophylls of mango leaves in searching for the new method in identifying its diversity. In the future, a portable and affordable optical tweezers for life science researches will be built.

Key words: *Optical Trap, Optical Tweezers, Diode Laser, science research*

INTRODUCTION

Optical Tweezers is an optical trap which was developed by Ashkin in 1986 (**Ashkin, 1986**). Ashkin had demonstrated that Latex particles could be trapped by laser light by shining or focusing the laser light to a cell containing the particles. His experiments which were started in 1970s in trapping particles using laser light have evolved into two divisions of research. First, the trap principle has been used to trap and cool atoms and molecules. This technique is knownas laser cooling and trapping. Here, the laser light frequency is tuned below of above the resonant frequency of atoms or molecules. This research division has very much developed hence one can cool many kinds of atoms to nano Kelvin temperature and create Bose Einstein Condensation (BEC) (**Anderson, 1995**). The second direction of the research is to trap life particles or micron size particles without cooling it. This technique is known as an Optical Tweezers (OT).

Since it was developed in 1986, OT has been used for many kinds of experiments involving life or micron size particles. It was used to measure the total angular momentum transfer from laser light to the trapped particles. In this experiment, the rotation of the trapped particles was investigated with the variation of laser light mode and polarization (**Simon, 2006**). OT was also used to manipulate or transfer particles in nano devices (**Nam, 2009**).Study on DNA and protein interaction has been realized using an Optical Tweezers. DNA Isolation and Activity characterization in Biochemistry research are often estimated using average values hence most molecule activities can not be measured. Using an OT, the activity of an individual molecule can be measured. It can be done by attaching the small size DNA and Protein to micron- size polystyrene beads (**Allemand, 2007**). In medical physics research, it can be used to study disease or develop a medical diagnosis. For example, blood cell which was contaminated by malaria parasites is trapped in OT and compared to normal blood cell. The normal blood cell can rotate faster as the laser light power increased hence possible for Malaria diagnosis(**Mohanty, 2004**).

The OT has been useful for life particles experiments because it can trap particles without damaging them hence can be used in biology cell experiments.
Recently, a complete commercialOT can be purchased but it costs relatively very expensive especially for an undergraduate laboratory (**Thorlabs.com, 2012**). However, one with optics and electronics expertise can build his own Optical Tweezers. A simple OT can be constructed using alaser beam which is focused to a group of particles in a container or a cell. The particle refractive indices have to be larger than the refractive index of the medium in the container.

RESEARCH METHODS

The trapping force of Optical Tweezerscomes from laser light radiance pressure. The idea of radiance pressure came from Kepler who tried to explain a comet's tail which always points away from sun. The theoretical analysis of the trapping force depends on the wavelength of laser light and the size of particles trapped. There are two main forces working on the particles due to the laser light. They are a scattering force and a gradient force. The scattering force is a non conservative force while the gradient force is a conservative force. The scattering force is caused by photons scattered off the particles, its directionis the same direction as the laser light and its magnitude is increased as laser optical power increased. When electromagnetic field from laser light interacts with particles, it will polarize the particles and creates a gradient force. It happens if the particles refractive index is larger than the medium refractive index. The gradient force direction is the same as the gradient of light intensity and its magnitude is also increased when the laser power increased. In addition, at the focal point, the scattering force tends to push the particles out while the gradient force tends to pull the particles in. The stable trap is when the gradient force is larger than the scattering force.
Based on the particle size and the wavelength of laser beam, theoretical analysis of OT is divided into three regimes. They are Mie Regime, Rayleigh Regime, and Intermediate Regime. Mie regime analysis is used when the particle size is larger than the laser wavelength. In this regime, the conservation of momentum and geometrical optics or rays optics are used to describe OT properties. In the Rayleigh regime, the particle size is smaller than the wavelength therefore the electromagnetic wave theory is applied. The challenging case is when the size of the particles is comparable to the wavelength of laser beam. This regime is called Intermediate regime. The theoretical analysis for the two former regimes have been developed, the intermediate regime analysis still needs more development. One can apply both theories. The life particles or biological particles fall to the intermediate regime. Besides it isrich in application, the theoretical analysis for this regime need more research. In addition, the trap becomes more effective when the particle size is close to the wavelength of laser beam.

Optical Tweezersworks like a spring because there is a push and pull between the scattering force and the gradient force. Just like a spring, an OT can be characterized by its spring constant or stiffness. The magnitude of this stiffness depends on the force acting on the trap and the position of the particles. For example, for position variation of particles 100-300 nm (10^{-9} m), the trapping force needed is from 1 – 100 pN (10^{-12} N). This magnitude is very small but 10 pN forcesare enough to pull E. coli bacteria in water where the speed of water is ten times of the speed of the bacteria (**Ranaweera, 2004)**. The OT need to be calibratedbeforeit is used for an application. There are some calibration methods that have been developed such asStokes drag, equipartition, andpower spectrum. The first method is to use a cell such that there is a flowing particles in the cell, this method uses Stokes Theorem. Another method is by assuming the particles moves in Brownian motion in a harmonic potential. This method uses Equipartiton Theorem. The other method that is more accurate is to measure the trap power spectrum using a Quadrant Photodiode (**Appleyard, 2007**). In this experiment, the Equipartition method is used. There have been many calibration methods developed over last years.

A simple Optical Tweezers composes of a laser source, a controllable stage for particles container, an objective lens to focus laser light, and a camera or detector system to monitor the trapped particles. It can be constructed using affordable optical and electronics components(**Bechhoefer, 2002**).An upright or inverted microscope or a self-built microscopeis usually used as the stage for cuvette or microscope slides and for the objective lens. Figure 1 shows a scheme of an Optical Tweezers system that has been built to trappolystyrene particles. This system uses a Leybold microscope used for undergraduate laboratory. One of its objective lens was replaced by a good quality objective lens from Edmudoptics.com that has 100x magnification and 1.25 NA. This lens is an immersion oil lens. ACoherent diode laser with power 50mW and 830nm wavelength is used as light source because it is affordable. Some people use a very expensive laser as a light source because it can provide high power and good quality beam. However, commercial diode lasers are available in the market with variable wavelengths and power. The laser power about 10 mW before reaching the objective lens is enough to trap polystyrene particles. The disadvantages of using a diode laser are its beam quality and stabilization. Most diode laser has an elliptical shape and must be stabilized from temperature and current fluctuations but some laser companies now sell diode lasers that have circular beam using built in micro lens or a single mode fiber optics and are also stabilized.These lasers of course are more expensive than the elliptical one but still cheaper than solid state lasers such as Nd:YAG or Ti:Sapphire laser. The laser used here has an elliptical beam hence it needs an anamorphic prism pair (AP) to make it circular.The laser beam also needs to be collimated and expanded using a pair of lenses (L1 = 30 mm and L2 = 100 mm) before reaching the objective lens. M1 to M5 are mirrorsthat are used to turn the laser beam. Since the laser light is in Infra Red, all the mirrors are coated in Near Infra Red region in order to minimize light absorption by the mirror. Some setups use a second pair of lenses with equal focal length after M5 mirror to stir the beam going into the objective lens hence can move the focal point of laser beam after the objective lens. Here, M5 and a lens are used to stir the beam. Another important component in an OT is a hot mirror or a dicroic mirror which is capable to separate laser beam based on its laser wavelength. Here it can reflect the laser beam from Coherent diode laser into the objective lens and transmit visible light from the lamp and the slides into the CMOS camera.

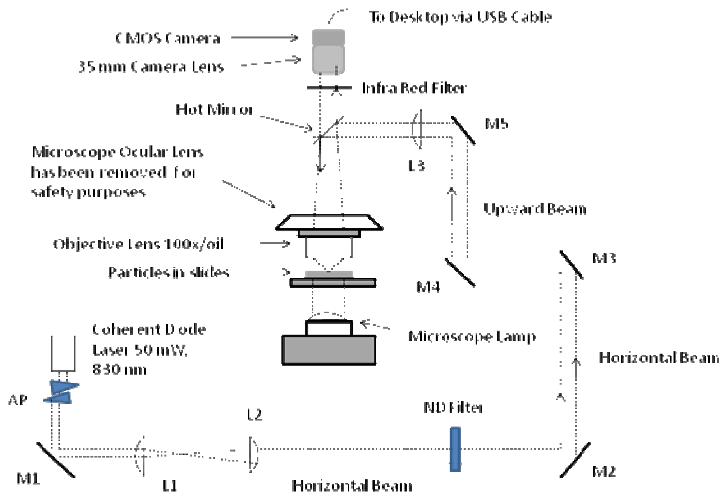


Figure 1.Setup of an Optical Tweezers system

In this experiment,a CMOS ThorlabsDCC1545M camera with 35 mm lens is used to monitor the trapped polystyrene particles. This camera has built-in software and can be connected to a computer by a USB cable. A CMOS camera is used rather than a CCD camera because it is cheaper but the performance is quite the same. A color filter and Neutral Density Filter are respectively used to filter unwanted light coming into the camera and to limit the laser power.
To trap particles, a container or a cell such as a cuvette or microscope slides is needed. In this experiment, polystyrene particles or beads with 3 μm and 10 μm in diameter are used. The particles are contained in a cell made from a microscope slide and a cover slip. The cell is made by using two strips of Scotch Double Stick Tape attached to the surface of the slide.Polystyrene particles are sold in a small 5 mL bottle that can have million of particles therefore it needs to be mixed with some liquid such as Glycerol or distilled water. Here, to get only some particles, 1 uL particles from 5 mL package ismixed with 250 mL distilled water. One drop of this solution is placed on the slide between the strips then closed by a cover slip to make a cell. The double tapes are used to bond the slide and the cover slip and to avoid the solution to dry out. The performance of the trap was investigated by measuring its stiffness. Here the stiffness was measured using Equipartition theorem that is by taking some videos of the trapped particles and analyzing the

images using a Scion Image program (Yogasari, 2012). One can also develop his own algorithm using MATLAB or LabViews.

RESULTS AND DISCUSSIONS

An optical tweezers system can be built if one has expertise in electronics and optics or one can collaborate with someone who is expertise in building an optical system. Nowadays, with advances in telecommunication such as internet and banking system, one can order electronic and optical components easily. One with a tight budget research can browse and compare over large number of companies which sell optoelectronic components such as Thorlabs, Edmundoptics, and Mellos Griot. Figure 2 shows the optical system that has been built to trap polystyrene particles. Most of the components are bought from Thorlabs.com because they are relatively cheaper but in high quality. Here, the some parts of a Leybold Microscope have been used as the part of the OT.

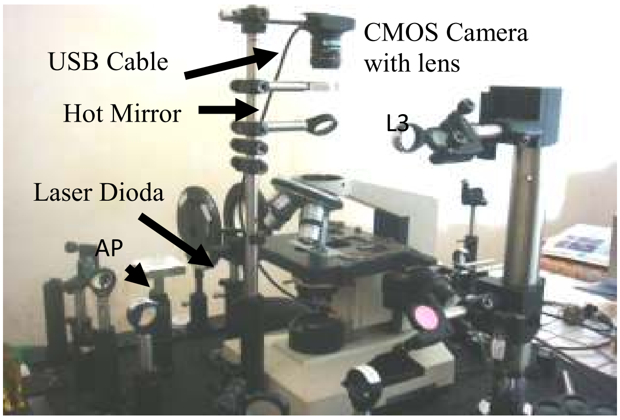
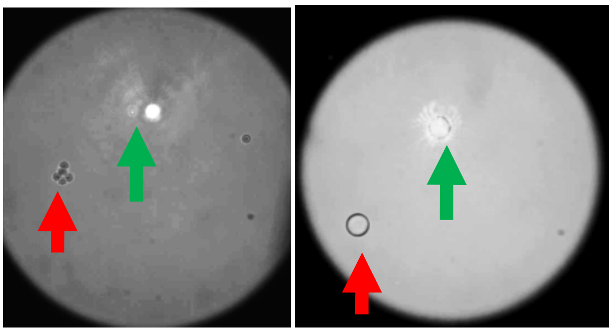


Figure 2. Optical Tweezers System



(a) (b)

Figure3.The trapped polystyrene particles, (a) 3 μm, (b) 5 μm

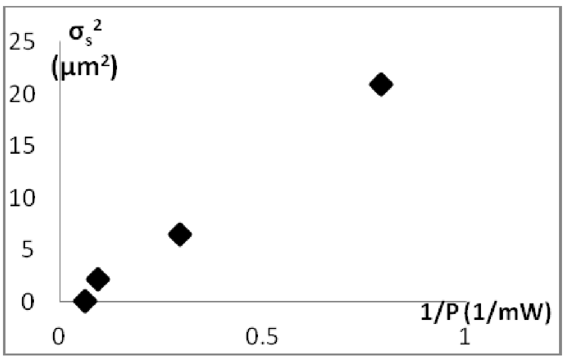


Figure 4.Variance versus 1/P laser Power for 3 μm particle.

Figure 3 shows the images of the trapped polystyrene beads taken by the CMOS camera. These images are taken in video mode by the camera software over 1 minute and converted to images to get 100 stacks images to get statistics of the images, then the variance of the trapped bead position for both particle sizes are calculated by Scion Image Program and ImageJ available online. The variances are calculated with the variation of laser power. The laser power is varied using Neutral Density filter with different Optical Density. One can also use a polarizer of a waveplate to vary the laser power. From the Graph of Variance (in x or y position of the particles) versus Laser power, the spring constant of the trapped is obtained as showed in Figure 4. From the figure, the spring constant for laser power 16.34 mW is 3.97×10^{-8} N/m. The spring constant or the stiffness increases linearly with laser power. The spring constant is lower for bigger particle at the same laser power value.

SUMMARY AND OUTLOOK

Building an Optical Tweezers is possible if one has experience in building an optical system. It is also affordable for an undergraduate laboratory. It can be simple or complex depending on the experiment one would like to pursue especially when trapping life particles of organisms. The Optical Tweezers that has been reviewed above will be modified to trap and analyze chlorophyll mango leaves for biodiversity study. For that purpose, an in-house inverted microscope are being constructed, the system will use 5 laser diodes with different wavelength. The calibration will be done using a Quadrature Photodiode (QPD) method.

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