Optical Tweezers

The Useful Micro-Manipulation Tool in Research

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Introduction

 One of the major goals of modern day science is to describe and characterize the world as it exist on the scales that is far beyond the scope of our own sense. The information can be used in various ways to benefit our lives. Manipulating small object is one of the quests in finding the information beneficial to our own. The challenge was to find a precise, easy to manipulate tool, and it must provide minimal damage to the studied object. Thus the development of optical trap were proposed over a century go. The ability of light to generate a force due to it change in momentum at and interface of media with differing refractive indices gave light the key feature to become an effective micro-manipulation tool in Cell biology and Biophysics research. This property of light has been understood since the time of Kepler and Newton. Radiation pressure was observed and confirmed by Maxwell in astronomical study. However, up until mid-twentieth century, the invention of laser has sparked the idea of invention of the optical trap. The earlier trap was utilized two sources of laser beams or the combination of one laser beam and gravitational force to achieve the stability of the trap by Ashkins 1980. In 1986, Ashkin et al. has published the first single beam optical tweezers. This optical tweezers satisfied the condition of stabilization of the trap that is the ratio of gradient force to scattering force is greater than unity. There are two regime depend on the size of the particles relative to the wavelength of the laser beam being used. In Rayleigh regime, the size of trapped particle is much less than the wavelength of light. On the other hand, the size of trapped particle is much more than the wavelength of the radiation beam being used in Mie regime. And the technique has been improved and customized to suit the researcher's need.

The practical applications of optical tweezers are endless. Currently the optical tweezers group at Neils Borh Institute has several researches which optical trap is used extensively. Physical properties of DNA and mRNA pseudoknots were studied to investigate the correlation between the strength of the pseudoknots with the frame shift efficiency of translation process in cell. Optical tweezers also used to mimic the action of molecular machinery and probe the physical properties of DNA and mRNA structure. Another research of the same institute is to use optical trap to manipulate certain individual membrane proteins. In this research, a protein is labeled and observed. Researcher was able to uncover the motility of individual receptor which correlated with metabolic rate of cell. Cellular adhesion, spreading, dynamic of mono-layers also studied with the use of laser trap. Weak interaction potentials within biological system were also studied using optical tweezers in this laboratory. Using the optical trap the researcher extracted the membrane tethers and investigated its force extension properties.

The goal for the experiment was to completely set up the optical tweezers. Furthermore, the force of the trap is investigated by varying the size of trapped particle. It is confirmed that as the size of the trapped particle increase the trap force decrease. Polystyrene sphere submerge in water was used to be the trapped particle. The index of refraction of polystyrene was approximately 1.58 which is higher than the submerged medium. This ensures the refraction beam which will balance out with approaching beam to form the laser tweezers.

The main drawback of optical trap is the limitation on the trap force which is in the range of 0.1pN to 500pN but the simplicity in design and other advantage feature of the trap still outweigh this limitation.

Physics of optical trap

Light caries a momentum that can be transferred when interact with a physical matter. When light is scattered by a particle, its momentum transferred and resulting force exerted on the particle. Optical tweezers make use of radiation pressure forces on its form of scattering and gradient force is trap dielectric particles. The polystyrene bead is used as trapped particle and for the simplicity the shape is assumed to be spherical. There are gradient forces come from the refraction of light through the sphere. This force points in direction of intensity gradient of the light. Scattering force is the resulted from the reflecting the light away from the object. This force points in direction of incident light.
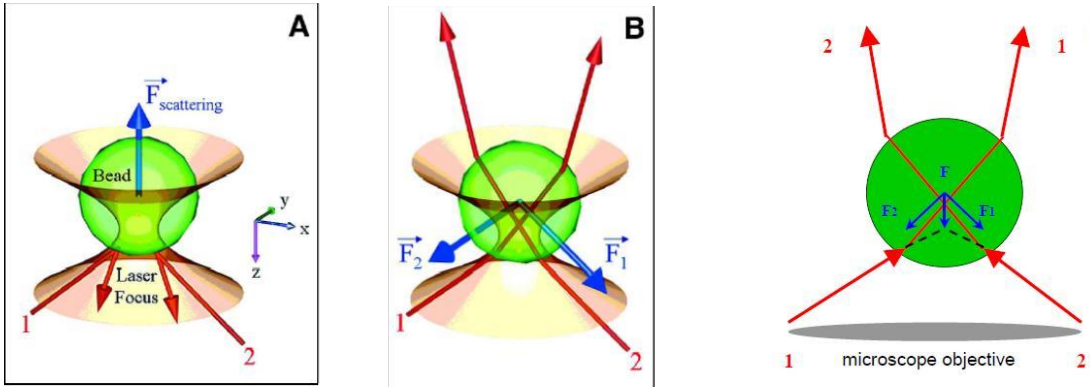Figure 1 shows the effect of the forces on the trapped particle in theory

Figure 1: Picture A shown the effect of scattering force on the bead in the opposite z direction of incident light Picture B convey the effect of gradient force act on the bead when it at the focused point of the incident beam The last picture depicted the total force where scattering force and gradient force satisfy the condition of stability of the trap.

Gradient force is determined by

$$F_g = \frac{n_1 P}{c}\left(R\sin 2\theta - \frac{T^2[\sin(2\theta - 2r) + R\sin 2\theta]}{1 + R^2 + 2R\cos 2r}\right)$$

And the Scattering force is determined by

$$F_s = \frac{n_1 P}{c}\left(1 + R\cos 2\theta - \frac{T^2[\cos(2\theta - 2r) + R\cos 2\theta]}{1 + R^2 + 2R\cos 2r}\right)$$

where n_1 is the index of refraction of the medium

θ and r are the angles of incidence and refraction

R, T are the Fresnel reflection and transmission coefficient of the surface at angle θ

c is the speed of light

P is the incident power

The stabilization of the trap is the balancing between these two forces. Thus the maximum restoring force of the trap can be found using

$$F = Q\left(\frac{n_1 P}{c}\right)$$

where Q is the scaling constant depend on the size of the trapped particle and the index differences between the sphere and the surrounding media.

Experiment set up

The apparatus used for the experiment was adapted from similar experimental designs used in previous attempt at building the optical tweezers (Smith et all, 1999). The trap is stabilized when the gradient force in the region beyond the focus overcome the scattering force. In practice, this condition is achieved by using a high numerical aperture objective lens guide the beam to the sample plane. The construction utilized all the tools and optical devices available in the laboratory. The trap set up is shown in Figure 2.
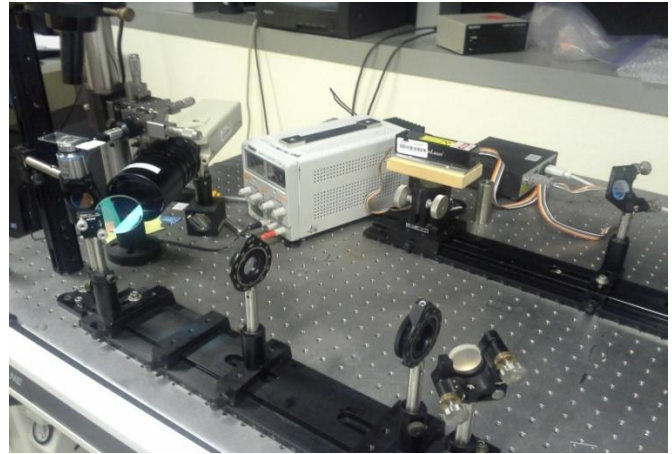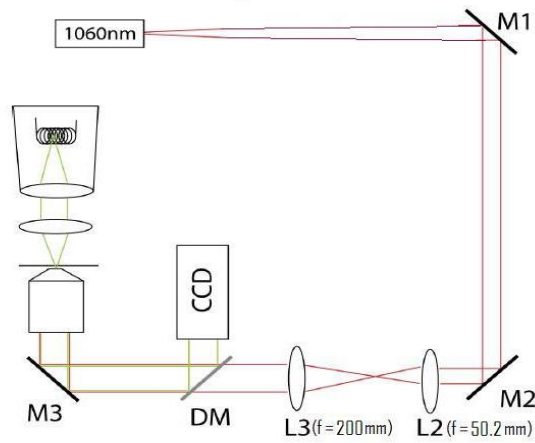

Figure 2: Schematic of the experiment and the picture of the actual apparatus

Air table was used to keep the whole apparatus as stable as possible. Infrared laser source (λ = 1060nm, power = 1065 mW) was guided by two reflected mirror M1 and M2. These two mirrors also act as the course and fine manipulation of the beam. The telescopic pathway composed of two lenses L2 (f = 50.2 mm) and L3 (f = 200mm). This help to focus and increase the spot size of the beam so that it will fill the back of the objective lens. The beam then sent through a dichroic mirror which allow low high frequency light through and reflect the lower frequency light. Mirror M3 reflect the laser beam upward toward the objective lens and to the sample plane. The objective lens is the infinity corrected oil immersion with NA = 1.4. The lighting system for the sample has built-in lens that help focus the light better. The sample was mounted on the 3-D micromanipulator. CCD camera was used to capture the activity of the trap.

Alignment procedure: Cleansing of the lenses and mirror is much needed for this experiment. Proper technique of cleaning optical devices is highly recommended to

ensure the clarity of the final images. The height of mirror and center of all the lenses should be the same. Pinholes were placed beyond the lens which assists the process of alignment. Each component of the trap should be aligned separately for the rough alignment. The beam was unexpectedly diverged that result in weak trap force. Thus, a small displacement of lens L3 on the telescope path is made. This results in a more focus, stronger beam on the sample plane and trapping success. By placing a converging lens between the laser source and the first mirror (M1) could potentially solve this problem. However, this has not been tested and proven to be sufficient.

Experiment result and further discussion:

The laser tweezers have been successfully built and tested. The trap force by vary the size of the polystyrene beads. The initially size was chosen to be 4 micron. The trap force was enough to maneuver the bead around the water medium slowly. The size change from 4 micron to 0.2 micron results in the significant increase in trapping force of the trap. As the trap move around the aqueous of bead, it sucked number of bead with much more rapid motion. The picture below depicts the event where the great force of the laser trap could move the bead from a distance. As the sample plane moved the focused laser trap sucks in the beads that is nearby in less than 1/10 of a second.
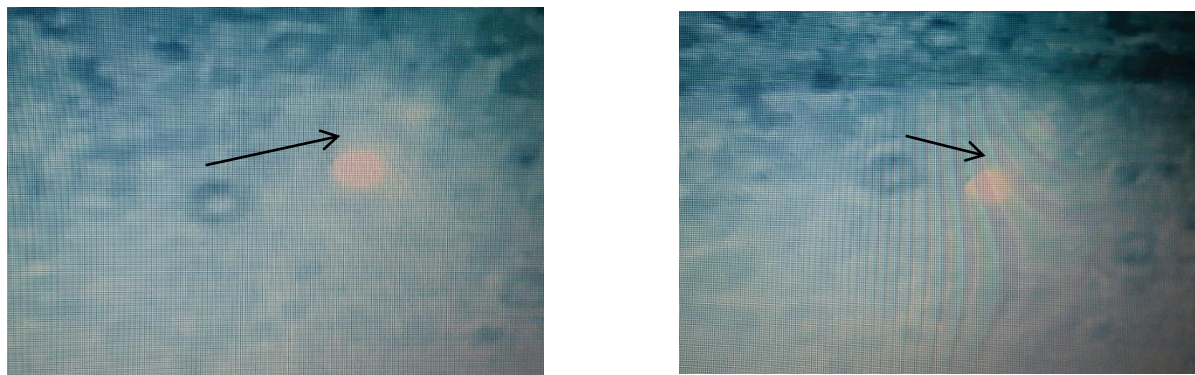

Figure 3: The nearby bead got sucked inside the laser trap

The apparatus of this experiment is the very basic but demontrate most of the basic and physics principle of a optical trap. With a very basic understanding about optic, the experiment provide a great deal of knowledge and practice for student. With the simplicity, optical trap offers a powerful instrument for laboratory research and great teaching tool for undergraduate students. The apparatus of this experiment can also be improve upon by replacing the manual control mirror with computerized scanning mirror. This addition improvement can provide much more precise measurement on trap force or finer manipulation of particle in medium.

Due to the constraint in time, there was no further testing or application for this optical tweezers. However if time permitted, controlled viral infection on mamalian cell in culture using optical tweezers is proposed. The idea is to have some what physical

control of the gene therapy process in which viral vector is used to inject genetic material into host cell.

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