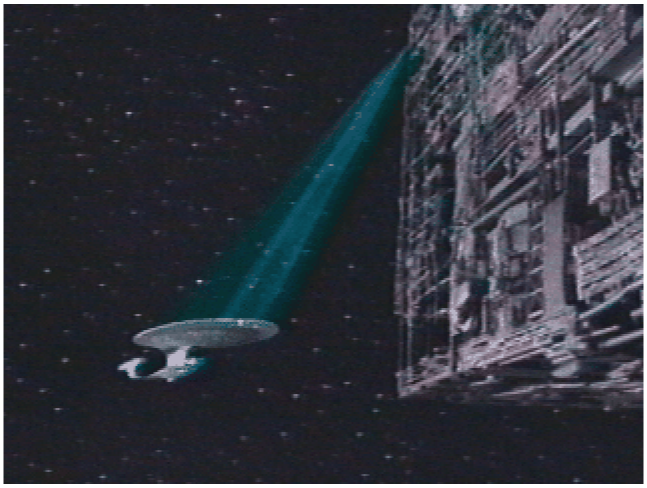
Bio-Photonics Lab: Optical Tweezers

You may not know what an optical trap is, but you've probably all seen something like it. It's essentially a tractor beam like one from an episode of Star Trek, only on a microscopic scale. Here, in an episode of Star Trek: The Next Generation, the Borg ship is holding the Enterprise in a Tractor Beam.



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Objective: During the course of this lab you will be able to grasp the basic knowledge of optical tweezers technique to study the micromechanics of soft condensed materials.

Introduction:

The physics behind the relatively young technique of optical tweezing has been known for centuries. In the seventeenth century, Johannes Kepler theorized that solar irradiance caused the tail of a comet to point away from the sun, and in 1873, James Clerk Maxwell proved theoretically that light can exert a force on matter (commonly know as radiation pressure, the "light force"). Sixty years later, Otto R. Frisch was able to deflect a beam of sodium atoms by bombarding the beam with light from a sodium lamp. In 1975, Hansch and Schalow proposed the idea of using lasers to trap atoms; a decade later, Steven Chu of Bell Laboratories was able to achieve three dimensional cooling in a technique nicknamed "optical molasses" (he would go on to win the Nobel Prize in Physics for the laser cooling and trapping of atoms with William Phillips and Claude Cohen-Tannoudji). These earlier techniques were all either optical two-beam traps or required an external force to be supplied by gravity or an electric field (these external forces provided trap stability). This paved the way for a single-beam gradient trap now known as optical tweezers. A year after Vladilen S. Letokov's proposal of atomic trapping by light beams, Arthur Ashkin at Bell Laboratories accelerated transparent latex spheres suspended in water using a laser beam (1986). By 1987, Arthur Ashkin of Bell Laboratories was able to trap living biological objects with a single laser, bringing the technique of optical tweezers, or optical trapping, to the scientific world.

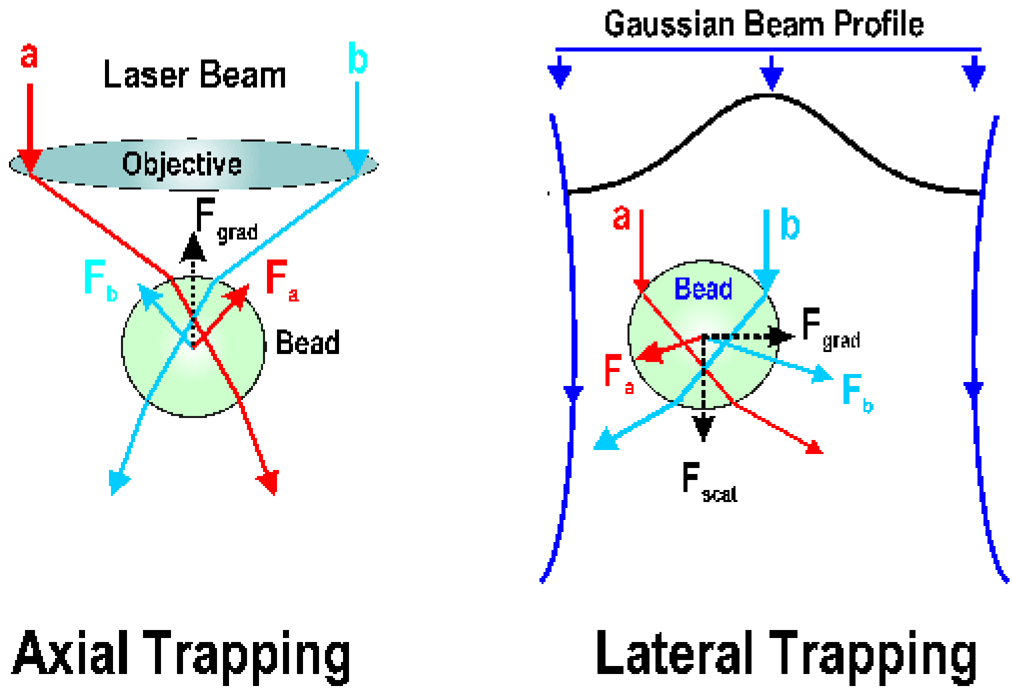
A single beam of tightly-focused laser light creates an extremely high electric field gradient in the vicinity of the focus. Similar to the force which draws a dielectric into the high field region of a capacitor, a dielectric particle falling within the laser beam focus will experience a force which is directed towards the focus of the beam. Provided the numerical aperture of the focusing optics is high, the optical system called optical tweezers gives rise to a 3-dimensional trap. The force due to the field gradient is sufficiently high to overcome the forces of both gravity and radiation pressure. The particles can be moved around in three dimensions. Optical tweezers consist of a single narrowly focused laser beam that can capture and move tiny particles a few millionths of a meter across. The laser beam produces a force which attracts the tiny particles, allowing them to be moved by moving the beam.

Using laser light to manipulate microscopic objects has broad applications in material research, biology, and biochemistry. The principle of optical tweezers is based on gradient force of a single tightly-focused laser beam. This gradient light force can trap particles as large as 100 μm and as small as an individual atom. Manipulation and control of dielectric spheres, living cells have all been achieved in laboratories around the world.

Principle of operation

The fundamental principle behind the operation of optical tweezers is the fact, that light carries momentum, which is proportional to the propagation vector of the electromagnetic field. When a light ray passes though a dielectric material suspended in a fluid media of lower refractive index (for example a polystyrene sphere suspended in water), the optical path is bent by refraction in the material which corresponds to a transfer of momentum from the light to the refracting particle[1]. The force exerted by the light or "radiation pressure" as is sometimes called is actually capable of pushing small particles around.

In a practical optical tweezer, we wish to trap and hold particles in all three spatial dimensions, rather than using the light to push them about. This requires that we simultaneously trap the particles both laterally (x-y plane) and axially (z direction). This can be accomplished if the laser beam striking the sphere has a sufficiently steep intensity gradient in all three directions as was first realized by Ashkin[2,3]. A Gaussian beam profile focused through a high numerical aperture microscope objective provides the required intensity profile for such a trap. The way in which the force components act to trap and hold the particle is shown in the figure. In this figure, the effects resulting in axial and lateral trapping have been separated for clarity.



Schematic diagram for the Axial and Lateral trapping of a bead in a Gaussian laser beam brought to a focus by a high numerical aperture objective. Axial trapping arises through the compensation of the scattering force (which pushes the bead in the direction of beam propagation) by the gradient force (which acts towards the focal spot). Lateral trapping arises form a gradient force acting towards the higher intensity region of the Gaussian beam profile

When placed in an intense laser beam with a Gaussian beam profile, a dielectric particle will experience a net lateral force acting towards the high intensity region of the beam as shown in the figure. The intensity gradient in the x-y plane causes the sphere to be pushed towards the centre of the beam; the sphere is thus effectively trapped in the x-y direction. There is also a scattering force component, acting to push the bead in the direction of laser beam propagation. For complete 3-D trapping, this effect of this scattering force must be counteracted. If the objective lens has a sufficiently high numerical aperture (NA), then there will also be a steep intensity gradient in the z-direction. This gradient has the effect of introducing a force directed towards the focal point of the lens opposing the scattering force arising from the radiation pressure in the z-direction.

This somewhat simplified description of the nature of radiation pressure, based on ray optics can be directly applied in an optical tweezer set-up. An expanded Gaussian laser beam directed though a microscope objective can therefore trap a particle in all three dimensions, the position of the particle being controlled by adjusting the focal point and the angle of the input beam.

Equation of Motion for an Oscillating Particle in a Viscous Fluid

The equation of motion for a particle trapped by an oscillating trap in a viscous medium is determined by two forces; the viscous drag force experienced by the particle, and the force imparted by the optical trap. Hookes' law, with an effective spring constant k_{ot}, can approximate the force produced by the laser on the trapped particle. As a reference, the force on a one-micron diameter polystyrene latex particle in water due to a laser is estimated to be 1 pN/mW of power at the trap center. Figure 3 shows the forces on a colloidal particle of radius a, in an oscillating optical trap residing in a simple viscous liquid.

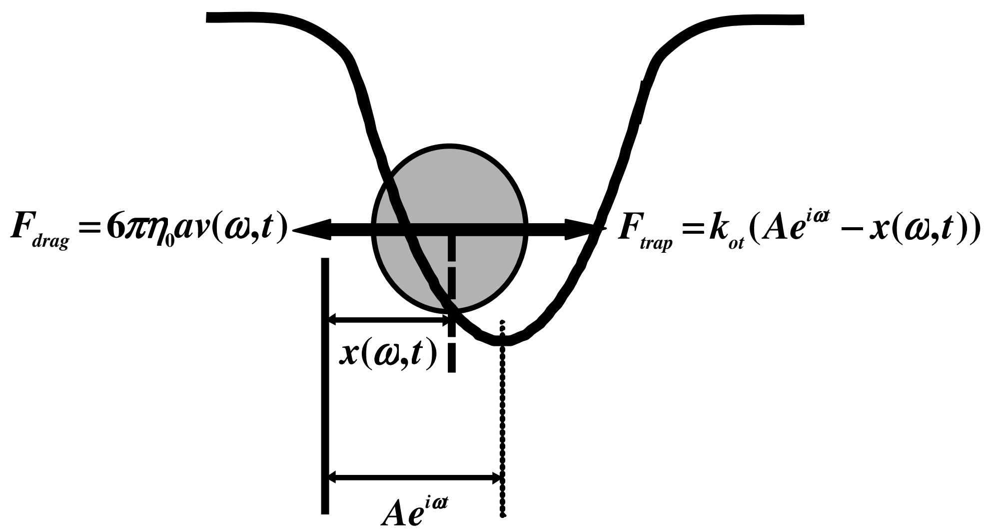


Figure 3: The solid vertical line represents origin of the system. The dotted line locates the center of the oscillating optical trap. Because the optical trap is oscillating in one dimension at constant amplitude, the distance between the origin and the center of the optical trap is given by $Ae^{i\omega t}$*. The distance between the origin and the center of the particle (shown by the dashed line) is the one-dimensional spatial coordinate of the particle* $x(\omega,t)$.

The optical trap as employed here can be approximated as a quadratic potential well that oscillates with amplitude *A*. For a simple viscous liquid, the drag force is taken to be the Stokes drag, $F_{drag} = -6\pi\eta_0 av$, where η_0 is the zero shear viscosity of the liquid, and v is the velocity of the particle. For a micron-sized particle, this expression for the viscous drag is an approximation because the inertia of the fluid has been neglected (low Reynold's number flow), making the expression valid for relatively small oscillation frequencies $(\omega = 1000\ rad/s)$. The force due to the trap is given simply as the effective spring constant multiplied by the distance between the center of the particle and the center of the trap. Newton's second law of motion then leads to the equation of motion for the particle as

$$m\ddot{x}(\omega,t) = -6\pi\eta_0 a\dot{x}(\omega,t) + k_{ot}Ae^{i\omega t} - k_{ot}x(\omega,t) \quad (1)$$

The above equation describes the motion of a particle of mass m in a damped, driven harmonic oscillator. A straightforward solution of the above equation provides the time-dependent position of the particle, expressed as a function of oscillation frequency ω, as $x(t,\omega) = x(\omega)e^{i\omega t} = D(\omega)e^{i(\omega t-\delta(\omega))}$, where the amplitude of the displacement of the particle is given by

$$D(\omega) = \frac{A}{\sqrt{\tau^2\omega^2 + (1-\omega^2/\omega_0{}^2)^2}} \quad (2)$$

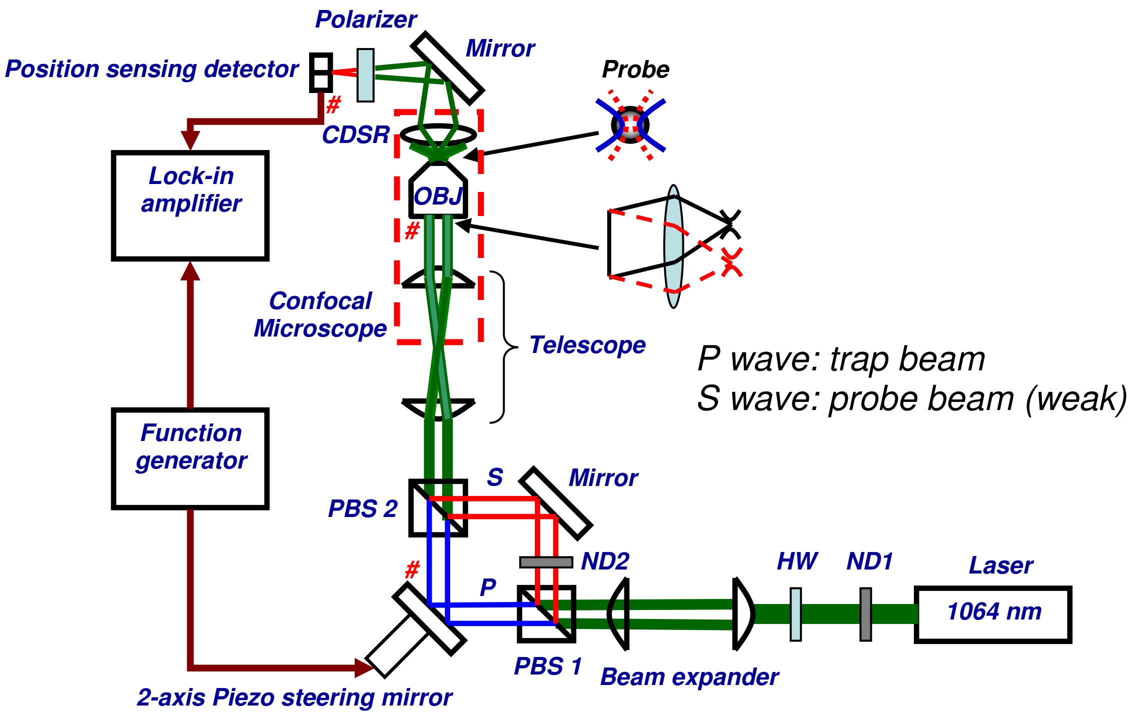
and the phase shift is given by

$$\delta(\omega) = \tan^{-1}\left(\frac{\tau\omega}{1-\omega^2/\omega_0{}^2}\right) \quad (3)$$

Here, $\tau = 6\pi\eta_0 a/k_{ot}$ and $\omega_0 = (k_{ot}/m)^{1/2}$.

To gain a physical understanding of the amplitude and phase of the particle's displacement, consider the limit when the particle mass is negligible (or $\omega^2/\omega_0{}^2 \approx 0$). In this limit, the amplitude of the displacement has two distinctive regimes. At low frequencies, namely, for $\tau\omega < 1$, the amplitude of the particle's displacement takes on the form $D(\omega) = A$. In this regime the elastic restoring force of the trap dominates the motion of the particle. At high frequencies, i.e., for $\tau\omega > 1$, the amplitude of the displacement takes on the form $D(\omega) = A/\tau\omega$. In this regime the viscous damping force dominates the motion of the particle. Over the entire frequency spectrum, the tangent of the phase is linear, given by $\tan\delta(\omega) = \tau\omega$. The crossover from elastically dominated behavior to viscously dominated behavior occurs when the in-phase component of the motion is identical to the out-of-phase component of the motion $(\delta = 45^0)$ at the frequency $\omega = 1/\tau$.

Experimental Setup:



Fig(3): A schematic of the dual-tweezers setup. HW is a half-wave plate; ND is a neutral density filter; PBS is a polarizing beam splitter; CDSR is a condenser lens. In the diagram, the sample is located immediately above the objective lens OBJ.

The experimental apparatus used to measure the motion of trapped particles is shown in Figure 3. A beam expander composed of two lenses is used to expand the laser beam (λ = 1064 nm, Spectra Physics, Nd:YVO_4) to the size of the back aperture of the microscope objective (Olympus, UPlanApo 100x NA 1.35). A half wave plate, HW, rotates the direction of polarization of the linearly polarized laser beam to allow the control of the relative intensity of the two beams exiting the polarizing beam splitter, PBS1. The first beam, S polarized, is reflected off a stationary mirror, and is used to create a stationary weak trap. The second beam, P polarized, is steered by a high frequency oscillating mirror, and forms the oscillating trap. A sinusoidal electric signal generated by a built-in frequency synthesizer in the lock-in amplifier (Stanford Research, SR830) is fed into a piezoelectric driver to drive the steering mirror (Physik Instrumente, PI-S224). Because a negative voltage would result in damage to the piezoelectric crystal, the piezoelectric driver is set to a DC offset of two volts. The level of the sinusoidal signal can be varied from 0.004 to 1 V_{RMS}. The beams recombine at the second polarizing beam splitter, PBS2, before entering the microscope (Olympus IX-81). The optical traps are formed at the microscope sample chamber by focusing laser beams through the high numerical aperture microscope objective.

Objective: Detection of the Motion of a trapped, oscillating particle, and calibration of effective spring constant of an optical trap.

To measure the in-phase and out-of-phase motions of a trapped particle relative to the motion of the oscillating trap, the light diffracted from a trapped particle is detected by a *position sensing detector* (PSM2-4 and OT-301, *On-Trak*). The electrical signals from the PSD are fed into a low-noise preamplifier (*Stanford Research, SR830)*. The lock-in amplifier measures the magnitude of the displacement and the phase shift between the particle's displacement and that of the oscillating trap. The signal detected by the PSD is purely due to the lateral translation of the particle relative to the laser traps.

Calibration of the Effective Spring Constant of an Optical Trap:

To calibrate the spring constant, a $1.5\mu m$ silica particle (Duke Scientific, Palo Alto, California) is trapped by an oscillating optical trap in water at room temperature ($\approx 23^{\circ} C$). The lock-in amplifier compares the oscillatory motion of the particle to the motion of the oscillating trap, and determines the amplitude of the particles' displacement, and the phase shift between the particle's displacement and the oscillating trap.

The data are corrected for the frequency dependent amplitude and phase shift induced by the electrical components. The displacement amplitude of the particle's motion is given by, $D(\omega) = D_{raw}(\omega) / D_{cor}(\omega)$, where D_{cor} is the frequency dependent amplitude induced by the electrical components. The phase shift of the particle's motion is given by, $\delta(\omega) = \delta_{cor}(\omega) - \delta_{raw}(\omega)$, where δ_{cor} is the frequency dependent phase shift induced by the electrical components. These corrections ensure that motion of the particle is determined relative to the driving signal, $Ae^{i\omega t}$. D_{cor} and δ_{cor} have been measured during system setup and will be provided to you. You will use them in your data analyses.

Procedure:

Sample Preparation:

1. Take 20 ml of ionized water. Using a 0.2µm filter, filter 5ml of water in two veils. Name these veils as A and B.
2. Add 10µl of 1.5µm silica particles in veil A.
3. Take 10µl of sample from veil A and put it in veil B.
4. Now prepare the sample chamber(provided) and fill it up from veil B.

Data Collection: Fix the sample slide on microscope. Make sure you use oil before fixing the sample on microscope. Since, we need index matching liquid between objective of microscope and the glass coverslip.

1. Move the focus of objective (using coarse and then fine focusing) to bring the objective focus to the chamber area.

2. Try to find a silica particle in the chamber. (Ask your instructor if you have any problem in locating the particles.)
3. Once you locate a particle, move the microscope state and bring it to the laser spot and make sure that the particle has been trapped by moving the focus up and down.
4. Make the particle such that it is 20μm above the clover glass surface (to avoid the effect of surface friction on the particle motion).
5. Go to computer. Click on the Lab View program.
6. Enter the filenames for phase and displacement of the particle.
7. Click on run and start recording data (Displacement and phase shift as a function of frequency).

To determine the force exerted by the optical tweezers, we first study a substance of known viscosity. By trapping and oscillating 1.5μm silica beads suspended in water and using the relations:

$$D(\omega) = \frac{A}{\sqrt{\tau^2\omega^2+1}} \; ; \quad \delta(\omega) = \tan^{-1}(\tau\omega) ; \quad \tau = 6\pi\eta_0 a / k_{ot}$$

where ω is the oscillation frequency and η is the viscosity of water (known). Plot *D/A* (normalized displacement) and δ as a function of ω, and fit, respectively, with the above two equations (on the left), using the only unknown parameter k_{ot}, as fitting parameter. The value k_{ot} (the average of the fit results from both *D* and δ) can then be used for any medium of unknown viscoelasticity.

Lab# 2
Objective: To calculate the viscoelastic moduli (G′, G″) of a bio polymer

Theory

By measuring the particle's displacement, D, and phase shift, δ, we can calculate the storage modulus, G', and the loss modulus, G'', of the local viscoelastic properties of biopolymer (k_{ot} is obtained in lab #1, *A* is the maxima of *D* from lab #1. You should take the average of 5 maximal *D* to reduce error.).

$$G' = \frac{k_{ot}}{6\pi a}\left(\frac{A}{D}\cos\delta - 1\right)$$

$$G'' = \frac{A}{D}\frac{1}{6\pi a}k_{ot}\sin\delta$$

Procedure: We will use 4% gelatin gel solution as a bio-polymer to calculate its viscoelastic properties.

Sample Preparation:

1. Weigh .8mg of gelatin using a weighing paper.

2. Mix it in 20ml of ionized water in a flask.
3. 4μl of 1.5μm silica particles from veil A (prepared in lab #1), andadd it to the gelatin solution.
4. Place a magnetic stirrer in the flask.
5. Put the flask on the hot plate (turn on the stirrer).
6. Stop the stirrer when the solution is clear (in about 15 minutes)
7. Prepare the sample chamber as described in previous lab and fill the sample chamber with gelatin solution.

Data Collection: Follow the same procedure as you did for water calibration.

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[1] *"Design and Applications of Oscillating Optical Tweezers for Direct Measurements of Colloidal Forces",* Chapter 15, Colloid-Polymer Interactions: From Fundamentals to Practice, Edited by Raymond S. Farinato and Paul L. Dubin, ISBN 0-471-24316-7, 1999 John Wiley & Sons, Inc

[2] A. Ashkin, Phys. Rev. Lett. **24**, 156 (1970).

[3] A. Ashkin, J. M. Dziedic, J. E. Bjorkholm and S. Chu, Opt. Lett. **11**, 288 (1986).