# Optical Trapping

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An optical trap or "optical tweezers" is a device which can apply and measure piconewton sized forces on micron sized dielectric objects under a microscope using a highly focused light beam. It allows very detailed manipulations and measurements of several interesting systems in the fields of molecular and cell biology and thus acts as a major tool in biophysics. They are used in biological experiments ranging from cell sorting to the unzipping of DNA. Similar principles are also used in physical applications such as atom cooling. In this experiment, you will measure the Brownian motion of a trapped silica microsphere in aqueous solution, both testing the theory of statistical mechanics and calibrating the "spring constant" of the trap. Then, using the calibrated trap, you will measure forces in biological systems, such as the actin-myosin molecular motors of vesicle transport in onion cells, the *E. coli* flagellar motor, or the restoring force of a stretched DNA molecule.

In its present form, large portions of this lab guide are derived from the literature for MIT Bioenhineering subject 20.309 [1] and UC Berkeley Physics subject Physics 111 Lab [2].

## PREPARATORY QUESTIONS

1. In the limit of ray optics, the trapping force on a dielectric sphere can be understood as arising as a reaction force to the change in linear momentum experienced by refracted light rays. To better understand how the scattering and gradient forces — and the trap's stability — vary with displacement from the trap center both vertically and horizontally, spend some time exploring this Java applet simulator developed by the lab of Roberto DiLeonardo, CNR-IPCF Dipartimento di Fisica, Universita di Roma Sapienza in Italy [3] `http://glass.phys.uniroma1.it/dileonardo/Applet.php?applet=TrapForcesApplet`. Describe and qualitatively sketch how a dielectric sphere slightly displaced from the center of a stable trap experiences a restoring force. Is the center of the trap at the same location as the focus of the light? Explain why high numerical aperature optics are used in the experiment. Finally, given the wavelength of the laser and the sizes of objects to be trapped in this experiment, do you trust the ray optics simulation to be quantitatively accurate?

2. Estimate the time and distance required for a mobile bacteria of typical bacterial speed in an aqueous environment to come to a halt under viscous drag. See the seminal work of Purcell (1976) [4]. How do these time and length scales compare to biologically relevant scales? How does $ma$ compare to the force needed to keep the bacteria moving at its initial constant speed (before it stopped), where $a$ is the average deceleration of the bacteria, and $m$ is its mass?

3. What are the principle safety hazards you could encounter in this experiment? How do you avoid danger from these hazards?

## SUGGESTED SCHEDULE

**Day 1:** Familiarize yourself with the apparatus. Make detailed notes on the effects of each control knob. Prepare an appropriate sample and trap a microsphere.

**Day 2:** Calibrate the QPD voltage to stage position using a fixed bead sample. Measure Brownian noise on a floating bead to obtain data for equipartition and PSD analysis. Obtain a first estimate of Boltzmann's constant and trap stiffness.

**Day 3:** Make an onion cell sample and trap a vesicle.

**Day 4:** Finish onion cell experiment. Optionally, do Stokes drag measurement — to refine Boltzmann's constant — or further biological experiments. Note that biological samples may take days to prepare, so you must plan ahead and communicate with your instructors.

The experimental goals are:

1. Measure Boltzmann's constant using equipartition theorem and Brownian PSD
2. Calibrate optical trap stiffness versus laser supply current
3. Estimate force and speed of molecular motors transporting vesicles in onion cells

## 1. INTRODUCTION

Light can impart a force, due to the fact that photons carry momentum. These forces are very small compared with those typical in the macroscopic world, but they can be very large relative to typical forces on single atoms, molecules, and small biological organisms, at the micrometer and nanometer scale. Focused laser beams can selectively impart force to atoms, to cool them from room

temperature to a few micro-Kelvin and below. They can also be used to push or trap microscopic dielectric spheres — or even entire, living, cellular organisms, inside biological media.

The method of optical trapping was discovered by Arthur Ashkin in 1970 [5] [6]. He calculated that the radiation pressure from a high power laser, focused entirely onto a micron-sized bead (or "microsphere"), would accelerate the bead forward at nearly $10^6$ m/s$^2$. When he performed the experiment to test this prediction, he found that while the target bead was indeed accelerated downstream, other beads in the solution were attracted laterally into the beam-path from other parts of the sample. He then created the first working optical trap by using two opposing laser beams. At one point a bacterium that had contaminated a sample became trapped in the beam, thus instigating the trap's revolutionary use in cell biology. Today optical traps are used extensively in both atom-trapping experiments and in biophysics labs worldwide.

In this laboratory experiment, you will explore the use of optical forces to trap dielectric microspheres held within a thin layer of water and vesicles in onion cells. The typical mechanical forces involved are on the scale of piconewtons ($10^{-12}$ N). Relative to this scale, hydrodynamical forces (drag and diffusion) on the microspheres and vesicles are substantial. Thus, the optical trap provides an excellent opportunity to study the physics of Brownian motion, which you will use to obtain a quantitative measurement of Boltzmann's constant. In the process, you will calibrate the dependence of trap stiffness (force/distance) on laser supply current. Biological motors, which are vital to intracellular transport and bacterial locomotion, also act with forces on this scale. You may thus employ the optical trap to quantify the speed and force of a molecular motor moving a vesicle along an actin fiber in an onion cell.

### 1.1. The Physics of Optical Trapping

The following material in this subsection is taken nearly verbatim from UC Berkeley's Junior Lab guide on their optical trap experiment [2].

The most straightforward mechanism to understand the physics of trapping is to consider the change in momentum of light that is scattered and refracted by the dialectic material, in our case a silica glass bead. Any change in the direction of light imparts momentum to the bead. This mechanism holds for objects much larger in diameter than the wavelength of the laser. A ray-tracing argument implies that the scattered light creates a *scattering force* in the direction of light propagation, while the refracted light creates an opposing *gradient force*. When the bead is in the center of the trap, these forces cancel. When a bead moves slightly away from the center, a net force is applied towards the center, making this a stable equilibrium.

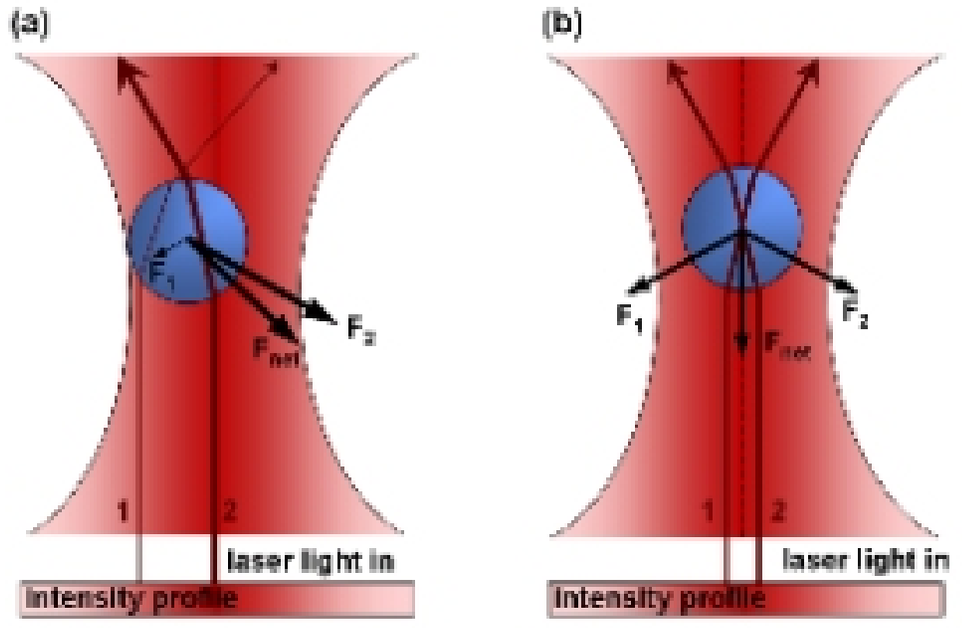


FIG. 1. A ray diagram showing how the gradient force stabilizes the trap laterally

In order to understand how the equilibrium is stable, it will help to consider how the gradient force responds to displacement of a bead from the center. As seen in Figure 1, the red region represents the "waist" of the laser at its focus point, with the laser passing upward through the sample chamber. The blue ball is the bead, and the dark red arrows (1) and (2) represent light rays whose thicknesses correspond to their intensities (note that the beam is brightest at its center). In case (a), with the particle slightly to the left of center, the two rays refract through the particle and bend inwards. The reactionary force vectors, $F_1$ and $F_2$, of each ray on the bead are shown as black arrows. Because ray (2) is more intense (and thus carries more momentum) than ray (1), the net force on the bead is to the right. Thus, a perturbation to the left causes a rightward-directed force back towards the trap's center.

In case (b) the particle is centered laterally in the beam and will not be pushed left or right. The net gradient force is downward, which is balanced by an upward scattering force (not shown) due to reflection of some of the light.

To better understand how the scattering and gradient forces and the trap's stability vary with bead displacement both vertically and horizontally, try this Java applet (`http://glass.phys.uniroma1.it/dileonardo/Applet.php?applet=TrapForcesApplet`) from the DiLeonardo lab [3] in Italy. The model used for this applet shows the importance of a high numerical aperture lens, as the extremal rays illustrated contribute disproportionately to the change in gradient force vertically. (Note that you must adjust the numerical aperture at the bottom of the applet in order to obtain a stable trap.) By moving the bead around and looking at the net force vector, you can get a pretty good feel for how the restoring force varies as a bead is displaced horizontally or vertically from the trap's center. Note particularly how the trap is less stiff as the bead is displaced above the trap's center. Remember this when you trap your first bead and try moving the bead with

the stage controls.

The ray optics approach described above holds for trapped objects whose diameter is much larger than the wavelength of the laser. For objects much smaller than this wavelength, ray optics are not valid. In this case, conditions for Raleigh scattering are satisfied and the object can be treated as a point dipole. The scattering force then is due to absorption and reradiation of light by the dipole, and the gradient force arises from the interaction of the induced dipole with an inhomogeneous electromagnetic field. This mechanism is detailed in the Neuman and Block review [7] and the Wikipedia article on optical trapping (`http://en.wikipedia.org/wiki/Optical_tweezers`). Since the 1 micrometer diameter beads we use in this lab essentially match the 975 nm wavelength of our laser, neither of these mechanisms is quite right. More complicated electromagnetic theories have been invoked to account for the observed forces [7] [8] [9]. However, these theories are not particularly useful in calculating forces from first principles; the ray optics approach is useful for guiding trap design and beam alignment, while calibration is based on direct measurements of bead motion.

### 1.2. Boltzmann's Constant and the Equipartition Theorem

The macroscopic world of masses and gasses connects to the microscopic world of atoms and particles through the laws of thermodynamics. It is in many ways remarkable that a collection of particles at some temperature $T$ gives rise to a macroscopic pressure $P$, when confined within a volume $V$, where a single constant relates the number of particles $n$ to the total kinetic energy of the gas. This constant is Boltzmann's constant (`http://en.wikipedia.org/wiki/Boltzmann%27s_constant`), $k_B$, and the relationship is the ideal gas law, $PV = nk_BT$.

How can one measure Boltzmann's constant? The crux of this challenge is the problem that it is unrealistic to be able to count the number of particles in a typical volume of gas. Thus, a direct approach based on the ideal gas law is difficult. However, the intrinsic connection between kinetic energy and temperature is also revealed through the *fluctuations* of the force imparted by the gas. The *equipartition theorem*, which is fundamental to thermodynamics, holds that each degree of freedom in a physical system at thermal equilibrium will have $\frac{1}{2}k_BT$ of energy. A single particle trapped in a harmonic potential — i.e., a mass on a spring — has energy $\frac{1}{2}\alpha x^2$, where $\alpha$ is the spring constant, and $x$ is the particle's displacement from the trap center. At thermal equilibrium with temperature $T$, such a trapped particle would have average energy

$$\frac{1}{2}\alpha\langle x^2\rangle = \frac{1}{2}k_BT \tag{1}$$

according to the equipartition theorem. Here, $\langle x^2\rangle$ is the statistical *variance* in the position of the particle, resulting from the fluctuation of the position of the particle due to random (Brownian) motion imparted by the medium at temperature $T$ with which the particle is in thermal equilibrium. If $\alpha$ and $T$ were known, and if $\langle x^2\rangle$ were measured, for example, by microscopic observation of the Brownian motion of a single particle, then Boltzmann's constant $k_B$ could be determined. This is exactly what we will accomplish in this experiment.

### 1.3. Brownian Motion and the Power Spectral Distribution (PSD) Function

The theory of Brownian motion predicts not only the variance of the trapped particle's position with time, but also the spectrum of these variations. Model the effect of the buffeting of the particle by a thermodynamically large number of individual molecules of the medium as a random time-dependent force $F(t)$. If each impact is truly random and uncorrelated, as one would expect from a gas of particles at thermal equilibrium, then the correlation time of the random forcing should be very short. Approximating it as zero, the resulting spectrum of the force is "white noise". Further approximating the motion of the bead as completely overdamped (that is, the viscous forces dominate over the inertia, known as the regime of low Reynolds number), the position $x$ of the bead in the harmonic optical trap of stiffness $\alpha$ is governed by the equation of motion

$$\beta\dot{x} + \alpha x = F(t), \tag{2}$$

where $\beta$ is the hydrodynamic drag coefficient $\beta = 3\pi\eta d$, $d$ is the bead diameter, and $\eta$ is the viscosity of the medium.

Using the Wiener-Khinchin theorem to define a "power spectral distribution" function (PSD) or "power spectrum" via the Fourier transform of the time-averaged autocorrelation function, the result is

$$S_{xx}(f) = \sqrt{\frac{k_BT}{\pi^2\beta(f^2 + f_0^2)}}, \tag{3}$$

where $f_0 = \alpha/2\pi\beta$. Note, this power spectrum, with units of $length/\sqrt{frequency}$, is different from, but closely related to the power spectrum defined as the complex norm of the Fourier transform, with which you may be more familiar. We have used the result that the power spectrum of the white noise is $\sqrt{4\beta k_BT}$ [10].

### 1.4. Molecular Motors and Forces in Microbiology

In this experiment, you will measure piconewton scale forces associated with the motion of individual (but large) molecules in microbiological systems.