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OPTICAL TRAPPING

ABSTRACT

Optical trap is a powerful tool used currently in many physical and biological applications. It allows for instance to manipulate and measure with high precision different cellular structure in biophysics and also to perform some experiments in biology such as cell sorting, single cell analysis…In this lab, the objectives are twice: i) To become familiar with the fundamentals of optical trapping and ii) To learn how to calibrate the optical traps for position detection and force measurement. Thus it will start with a brief theory on optical trapping system and the controls for it. Then the subsequent practical work will be as following: Load a sample slide into the trap for calibrating the position detector, and find a bead attached to the coverglass surface. Run the Matlab position calibration to relate the voltage output of the position detector to bead displacement in nanometers. Make a new slide with free beads, find bead that is not attached to the surface and trap it. Use two different methods to calculate the trap stiffness and compare their results. Finally, the data generated will be analysed using Matlab.

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I. THEORY

Optical trap or "tweezers" is a device used to apply piconewton sized forces and make precise measurements on a scale of roughly one micron. It can be created by applying a precisely focused laser onto a dielectric material. It allows scientists to make very detailed manipulations and measurements on several objects in the field of 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 and also in physical applications such as atom cooling.

1.1 History

The effect of light on matter has been known for over four hundred years, dating from Kepler's observation that comet tails always point away from the sun. Indeed, light from the sun can exert a pressure up to 5 mN/m^2 on a totally reflecting surface, ten orders of magnitude less than the force on a cube of the same dimensions due to gravity on the earth's surface. Although resulting in an extremely small force, radiation pressure from sunlight can be significant, for example as the driving force behind solar sails where gravity is negligible. At the beginning of the twentieth century, using thin plates suspended in a evacuated radiometer, Lebedev [1] was the first to experimentally measure the radiation pressure proposed by Maxwell-Bartoli, showing that the pressure for a reflective surface is twice that of an absorbing surface. Arthur Ashkin [2-4]discovered the method of optical trapping in 1970. He calculated that the momentum from a high power laser, focused entirely onto a micron bead would propel the bead forward with 100,000 g's of acceleration. Taken by curiosity he performed this experiment and found that not only was the intended bead pushed downstream by the laser but also that other beads in his solution were highly attracted to the beam-path and flew in laterally from other parts of his slide. He then created the first working trap by using two opposing laser beams.

At one point a bacterium that had contaminated a sample flew into the trap and was trapped [5], 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. The focus for this laboratory section is on the basic science behind how force is generated in an optical trap and how it can be calibrated and used to characterize the force spectroscopy of biomolecules.

1.2 The Physics behind Trapping

The interaction between light and matter is a complicated one which is not understood fully for all cases, but informative approximations are available under a number of limits. The origin of a force on matter because of an electromagnetic wave can be understood qualitatively by an electric field exerting a force on charges within a particle, and a magnetic field exerting a force on currents.

1.2.1 Gradient force

Light incident on a particle creates a dielectric response, due to the polarizability of the constituent atoms or ions. For one of these atoms or ions in a monochromatic, linearly polarized, continuous light field, ***E***, the time-averaged induced dipole moment is:

$$\vec{p} = \alpha \cdot \vec{E} \tag{0.1}$$

For a small particle in an aqueous medium where $\alpha = \alpha' + i \cdot \alpha'' = n_m^2 \tau^3 \left(\frac{n_c^2 - 1}{n_c^2 + 2} \right)$ is the relative complex polarizability of the particle to the surrounding medium (n_m is medium refractive index, and n_c is relative index of the particle n_p to the index of the surrounding medium n_m). The interaction of the induced dipole with the electric field of the light creates an electrostatic potential:

$$U = -\vec{p} \cdot \vec{E} \tag{0.2}$$

Thus in a light field with a spatially varying intensity, there is a gradient

$$\vec{F}_{grad} = -\nabla U = -p \nabla \vec{E} = -\alpha (E \cdot \nabla) \vec{E} \tag{0.3}$$

For a small particle of radius r_p, this leads to the force relation

$$\vec{F}_{grad} = -\frac{n_m^3 \cdot r_p^3}{2} \left(\frac{n_c^2 - 1}{n_c^2 + 2} \right) \nabla \vec{E}^2 \tag{0.4}$$

Thus the gradient force is linearly dependent on the spatial variation of the intensity of the light field and on the dielectric contrast of the particle to be trapped relative to the surrounding media, which can be described by the ***Clausius-Mossotti relation.*** For particles with a refractive index higher than the surrounding medium, the gradient force acts toward the point of highest intensity, that is to say the focal point of a diffraction-limited beam in optical tweezers. Conversely, particles with a lower refractive index can be trapped at a minimum in the light field intensity. The strength of the restoring gradient force in an optical trap of radius *r* can be characterized as a Hookean spring with stiffness, k, where the force is linearly proportional to small displacements ($d < r/2$):

$$\vec{F} = -\kappa \cdot \vec{r} \tag{0.5}$$

The laser trap produced by a focused Gaussian beam can be classified by an almost harmonic potential. For a μm particle in viscous fluid the friction force is ordered of magnitude larger than the inertial force. The particle shows an exponential dumped motion

$$x(t) = x_0 \exp\left(-\frac{\kappa}{6\pi\eta\tau} t \right) \tag{0.6}$$

With the trap period, a measure of correlation time is given:

$$\tau = \frac{6 \cdot \pi \cdot \eta \cdot r_p}{\kappa} \tag{0.7}$$

Where η is the viscosity of the surrounding medium, κ is the trap stiffness and r_p is friction constant according to the Stoke's law. A schematic of the axial and radial potentials and their resulting stiffness's is shown in Figure 1. Techniques for measuring the stiffness of an optical trap are described later, but for a 1 μm diameter polystyrene bead in a typical optical tweezers setup, the stiffness can be varied easily in the range 10^{-6}–0.1 pN/nm by adjusting the laser power from 10–1000 mW. These characteristics complement the stiffness of physical cantilevers such as AFM tips (10–10^4 pN/nm), which cannot be as easily tuned after fabrication.

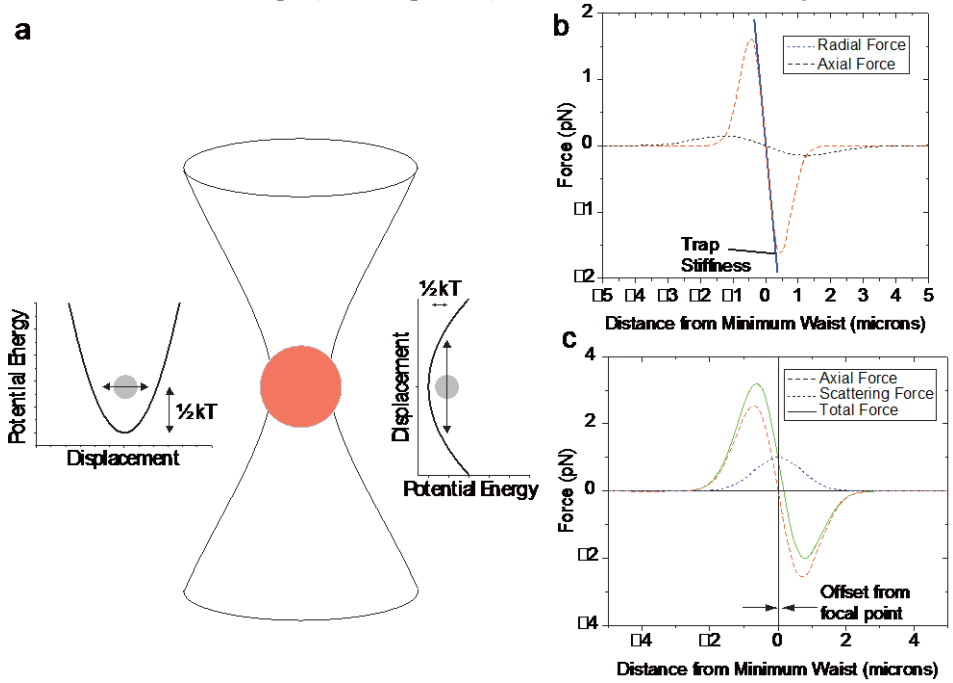


Figure 1 **(a)** The axial and radial trapping potentials of a bead in an optical trap lead to **(b)** differing stiffnesses and extents. **(c)** With the addition of the scattering force, the trap center is offset from the focal point.

The trap stiffness is important in determining the minimum force which can be measured through displacement detection and sets an upper limit for the maximum useful sampling rate through the trap frequency. Although not immediately obvious from these simple expressions, the trap stiffness is greatest when the particle to be trapped is the same size as the beam waist; as particle size decreases, the restoring force decreases rapidly, but decreases only modestly when the particle size increases.

1.2.2 Scattering force

The second force component in an optical trap arises from the scattering of light and is a consequence of photons having momentum. This force acts in the direction of propagation of the light and is dependent on the light intensity rather than the gradient. The momentum of a single photon of energy E is:

$$\vec{p} = \hbar \cdot \vec{k} = \frac{\vec{E} \cdot n_m}{c} \qquad (0.8)$$

A beam of incident photons can be scattered from the particle, resulting in two impulses: one along the direction of light propagation, and the other opposite the direction of the scattered photon. For isotropic

scattering, dependent on the size of the particle, the latter impulse has no preferred direction and results in a net force in the direction of light propagation. The change in momentum, or force, of a particle can be calculated by considering the photon flux impinging on and leaving an object under the conservation of momentum:

$$\vec{F}_{scat} = \frac{n}{c}\int\int \left(S_{in} - S_{out}\right)\cdot dA = \frac{n_m \sigma \langle S \rangle}{c} \qquad (0.9)$$

Where n_m is the refractive index of the surrounding medium, is the time-averaged Poynting vector, c is the speed of light, and s is the particle's optical cross section. In the case of a small, spherical, (much smaller than the wavelength of impinging light) dielectric particle, the Rayleigh scattering cross-section is:

$$\sigma = \frac{8}{3}\pi\left(\frac{2\pi n_m}{\lambda}\right)^4 r_p^6 \left(\frac{n_c^2 - 1}{n_c^2 + 2}\right)^2 \qquad (0.10)$$

Where r_p is the particle radius, $n_c = n_p / n_m$ is the refractive index contrast between the particle (n_p) and the medium (n_m), and $k = 2\pi/\lambda$ is the wave vector of the trapping light. The scattering force on a Rayleigh particle can then be written in terms of the light intensity I_0 [4]:

$$F_{scat} = \frac{128\pi^5 r_p^6}{3\lambda^4}\left(\frac{n_c^2 - 1}{n_c^2 + 2}\right)\frac{n_m}{c} I_0 \qquad (0.11)$$

Thus the scattering force is dependent on the photon flux or light intensity, the wavelength of the trapping light, the particle size, and its refractive index contrast against the liquid in which it is immersed. For larger particles ($r_p >> \lambda$), the scattering cross-section can be expressed as $\sigma = \mathrm{Q}_{\mathrm{scat}} \cdot \pi \cdot \mathrm{r}_p^2$ where Q_{scat} approaches the limit of 2.

However, for intermediate sizes, an accurate force $F_{\max} = \frac{0.49 n_m \cdot P}{c}$ estimate needs to be numerically evaluated using Mie theory [6], in part because the scattering of incident photons is no longer isotropic. To maximize the gradient force, the particle's radius should be comparable to the wavelength of the trapping laser and its associated minimum focal spot size and consequently is most appropriately described by the intermediate, Mie regime. The need to numerically solve Mie scattering theory is one of the complications in developing a simple model for optical tweezers and makes direct comparison of the gradient force and scattering force difficult. However, one variable which can be controlled and optimized is the ***refractive index contrast*** between the trapped particle and the surrounding medium. The optimal refractive index contrast is 1.2–1.3, which maximizes the gradient force with respect to the scattering force for the $F_{\max} = \frac{0.49 n_m \cdot P}{c}$ incident optical power P.

Conveniently, polystyrene beads in water have a refractive index contrast of n_c = 1.59/1.33 = 1.2, close to optimal, with a potential maximum force of $Fmax$ = 2.2 pN/mW, though optical tweezers generally operate at around 2/3 of this value [7].

Adding the two force components results in the equilibrium position for an optical trap being displaced a distance proportional to the light intensity from the minimum beam waist in the direction of the light propagation, typically 100–500 nm, as illustrated in Figure 1 c. This distance can be found experimentally by

translating a trapped bead into a surface and measuring the displacement of the bead in the trap when it is in the focal plane of the surface, or comparing it to a bead previously fixed to the surface.

1.2.3 Rayleigh Regime ($r << \lambda$)

In the two particle size limits, the Rayleigh regime ($r << \lambda$) and the ray optics regime ($r >> \lambda$), a theoretical treatment for calculating the radiation pressure is relatively straightforward and provides a number of useful insights. In the Rayleigh regime, particles can be treated as a collection of dipoles polarized by the envelope of the light field forming the trap, with the phase of the field being approximately constant throughout the particle. In the previous sections, equations were presented for the gradient and scattering forces on small dielectric particles; however, in practice, it is difficult to exert sufficient force to trap a dielectric particle below 100 nm in size with current optics and laser limitations.

The force exerted by the laser is proportional to the particle's polarizability which is in turn proportional to the volume of the trapped object: to trap a 10 nm object requires a million times as much input power as to trap a 1-micron object.

As particle size increases, the difference between Rayleigh and Mie scattering becomes measurable for particles larger than 200 nm for visible trapping fields [8], and Rayleigh approximations break down for most trappable objects. The dielectric constant can be enhanced to trap particles down to 5 nm in size by exploiting nonlinearities such as a plasma resonance, ionic resonance, or intensity dependent refractive index [9].

Alternatively, the medium surrounding the particle can be modified to minimize Brownian motion to the extreme of trapping and cooling small numbers of atoms in an ultrahigh vacuum chamber [10]. In general, however, the complications and limitations associated with these approaches mean that optical traps rarely operate in a pure Rayleigh regime, and predictions can be inaccurate without experimental validation.

1.2.4 Ray Optics Regime ($r >> \lambda$)

In the other limiting case, where the size of the particle to be trapped is much larger than the wavelength of light, and has a small refractive index contrast with the surrounding medium, the component forces can be modeled using ray optics.

An incident monochromatic light beam can be decomposed into individual rays with appropriate intensity, momentum, and direction. In a uniform, nondispersive media these rays propagate in a straight line and can be described by geometric optics. For a uniform dielectric sphere the optical forces, including the scattering component, can be calculated directly from ray optics [4]:

$$F_{scat} = \frac{n_m \cdot P}{c}\left(1 + R\cos(2\theta_R) - \frac{T_F^2(\cos(2\theta_R - 2\theta_T) + R\cos(2\theta_R))}{1 + R^2 + 2R\cos(2\theta_T)}\right) \qquad (0.12)$$

$$F_{grad} = \frac{n_m \cdot P}{c}\left(1 + R\sin(2\theta_R) - \frac{T_F^2(\sin(2\theta_R - 2\theta_T) + R\cos(2\theta_R))}{1 + R^2 + 2R\cos(2\theta_T)}\right) \qquad (0.13)$$

where R & T_F are the Fresnel coefficients, and θ_R and θ_T are the angles for reflection and transmission of the incident rays. Figure 2 schematically illustrates the origins of the axial and radial forces due to diffraction and how the component forces add together.

For nonspherical and complex particles approximations can be computed [11]. The ray optics regime is increasingly accurate for dielectric particles of radius $r_p > \frac{5n_c\lambda}{\pi n_c}$ though for these larger particles the radial trapping force diminishes. However, increasing the focal spot size to compensate would decrease the axial trapping force. As mentioned earlier, trapping efficiency is highest for objects which are approximately a wavelength in size and therefore fall in the intermediate regime between the Rayleigh and ray optics regimes.

These theoretical models generally assume a continuous, diffraction limited monochromatic beam focused by a high numerical aperture lens to trap a rigid dielectric sphere with a refractive index higher than the surrounding medium. All of these assumptions can be broken through choice of trap geometry, light source, and particle to be trapped. For the remainder of this section we will consider some of the trap designs, light sources, and particles which have been or can be used.

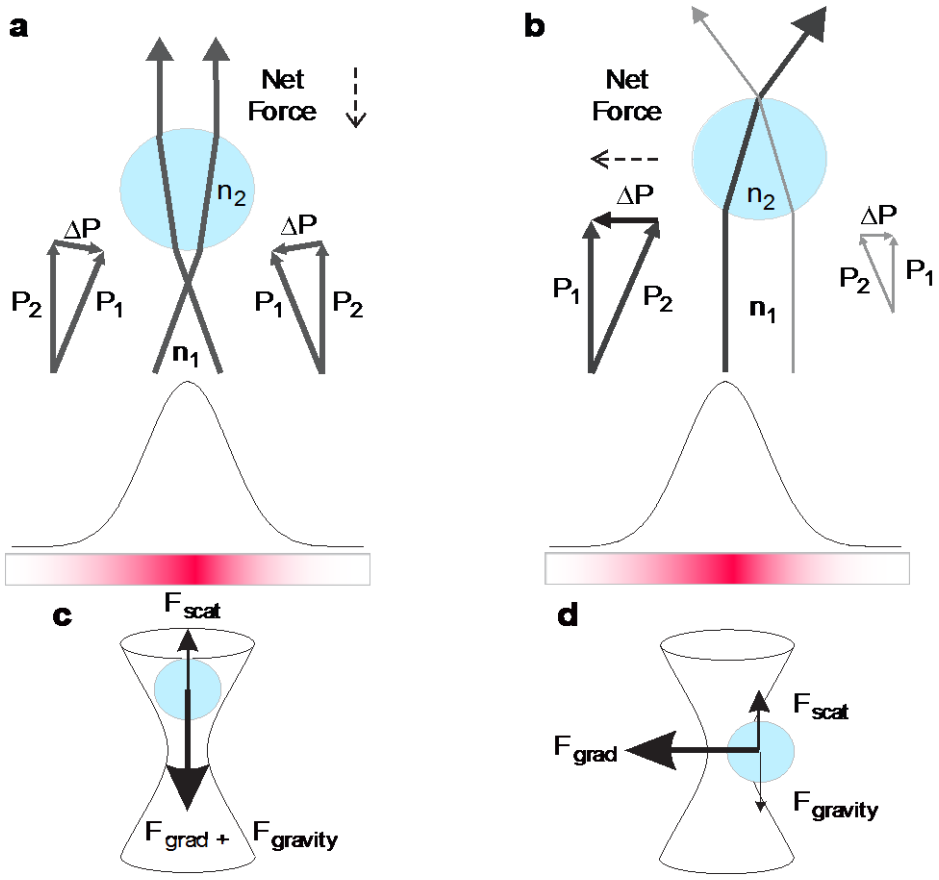


Figure 2 Schematic of the optical forces in the ray-optics regime. Summing the rays gives an **(a)** axial force due to vertical displacement from trap center; **(b)** radial force due to lateral displacement from trap center. Taking into account gravity and scattering, **(c)** the axial and **(d)** radial gradient force must be the dominant component to form an optical trap.

In order to trap a particle we need to create a **stable equilibrium**. The force that will be counteracting the movement in this case is the change in momentum (remember that force is defined to be dp/dt) of the laser light path (light carries momentum) as the trapped particle refracts and bends the light in various ways. In order to understand how it is stable, we only need to consider a couple of test cases. In the Figure 2 to the right we see an illustration of two possible laser-particle set-ups. The red region represents the laser at its focus point, the blue ball is the particle, and the black arrows are representative light rays whose thicknesses correspond to their intensities (note that the beam is brightest at its center). In case (a) the particle is dead canter and will not be pushed left or right. It is still reflecting some of the laser light hitting it dead on however and may be pushed "downstream."

To counter act this force we note that the two beams, though now symmetric, are still being bent inwards. This slight bending creates a force that pulls the bead back towards the laser source. This only happens very near the focus of the laser and only when the light comes in at an extremely crossed angle or at a high numerical aperture.

In case (b) we see that the particle has been moved slightly to the left, the two black arrows refract through the particle and bend inwards. The reactionary force vectors on the particle are also included in the image. We see that, because the particle is slightly to the left of center, the right ray is more intense (and thus carries more momentum) than the left ray. As a result of the ray's bending to the left, the particle will be pushed to the right in order to conserve momentum. Thus, a perturbation to the left causes a right directed force back towards equilibrium.

If a picture is worth a thousand words then a java applet must be worth a million. Inspect this applet to get a feel of how the light-bending forces work. Be sure to adjust the numerical aperture at the bottom in order to obtain a working trap. Also play with following java applet.

1.3 Quantitative measurements

1.3.1 Position detection

The laser light scattered from the trapped object is directed onto a Quadrant Photodiode (QPD) to provide a position signal for the bead location. The QPD outputs a voltage signal for the x- and y-axes of bead displacement. These signals must be related to the physical position of a bead, and our goal is to record voltage vs. position data for each axis. More information about this detection method can be found in Gittes and Schmidt. [12]

1.3.2 Force calibration

For calculating the forces exerted by the trap, the key parameter we need to know is its stiffness, analogous to the spring constant k of a spring-mass system. We will look at three different ways to measure it. In optical tweezers systems, the usual symbol for stiffness is α. In general, for small displacements x from the equilibrium position, the optical trap is considered to be a harmonic potential, which means that trapped particles experience a Hookean restoring force F = -αx, and the potential energy stored due to displacement is given by

$$U = \frac{1}{2}\alpha x^2 \qquad (0.14)$$

1.4 Optical Tweezers - Setup Description

Like many optical trapping systems, this one is based on an inverted microscope design. The structure of the inverted light microscope is constructed using

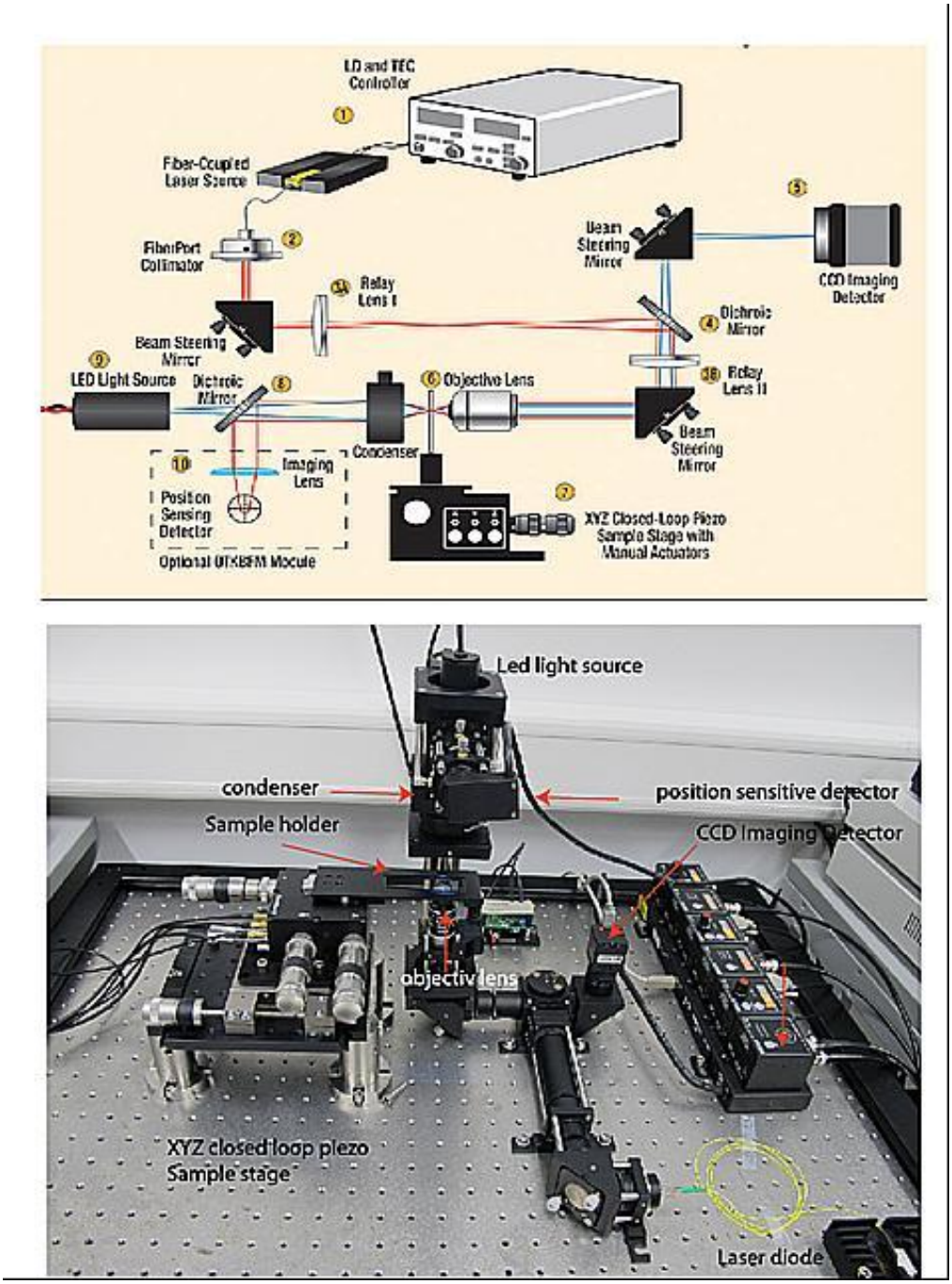


Figure 3. Schematic and photograph of an optical trap

① **LD and TEC controller**: The 980nm trapping laser source which a pigtailed Fiber Bragg Grating (FBG) stabilized single mode laser diode in a hermetically sealed 14-pin butterfly package. The integrated TEC element and thermistor in the butterfly package allow the **temperature** of the laser to be precisely controlled. The current controller regulates the **current** through the laser diode. This laser and controller combination was chosen to ensure that the output power (330 mW max) of the laser will be extremely stable, which is important to maintaining a constant trapping force.

② **FiberPort**: collimates the output of the trapping laser. It is a versatile collimator since it allows the aspheric collimation lens to be precisely positioned along 5 axes (X, Y, Z, Pitch, and Yaw). For polarization sensitive applications, the keyway on the FiberPort can be rotated about the optical axis so that the orientation of a linearly polarized collimated beam can be set.

③ **The two achromatic doublet**: relay lenses expand the collimated trapping laser beam by a factor of **two** so that it is approximately 5 mm in diameter. In addition, the relay lenses image the rotation point of the first right angle mirror onto the back aperture of the objective lens so that the KCB1 can be used to position the optical trap. The back aperture of the focusing objective is 5 mm in diameter, which means that objective is not over filled. However, the stiffness of the trap is still excellent. The second relay lens also serves to image the sample on the CCD Imaging Detector.

④ **The dichroic mirror**: reflects 980 nm light (trapping source) while passing visible light.

⑤ **Visible light from the LED light source**: illuminates the sample and is then imaged on the 1280 x 1024 pixel color CCD camera. The dichroic mirror in the light path in combination with a short pass filter prevents backscattered light from the 980 nm laser from saturating the CCD detector.

⑥ **100X oil immersion Nikon objective lens**: is used to focus the 980 nm laser beam down to form the optical trap. The calculated diffraction limited trap diameter is 1.1μm. A Nikon Condenser collimates the beam after the optical trap.

⑦ **The sample stage:** consists of a microscope slide holder mounted to a 3-axis (X, Y, and Z) translation stage that is mounted on a 1-axis long travel translation stage, which results in the following capabilities:

A) 2" (50 mm) of travel perpendicular to the beam path. This makes it easy to load the sample and coarsely position it near the trap.

B) 4 mm of travel in the X, Y, and Z directions using the coarse knobs (0.5 mm/rev) on the 3-axis stage actuators.

C) 300 μm of travel in the X, Y, and Z directions using the differential knobs (50 μm/rev) on the 3-axis stage actuators.

D) 20 μm of travel in the X, Y, and Z directions using the piezo actuators (20 nm resolution without using feedback from the internal strain gauge sensors, 5 nm resolution using the internal strain gauges for positional feedback) on the 3-axis stage. Three T-Cube Piezo Drivers are included in the kit. (Two T-Cube Strain Gauge Readers are included with the OTKBFM force module.) The stage assembly is mounted on a translating breadboard TBB0606 which facilitates loading/unloading of samples.

⑧ **Dicthroic mirror:** The visible light emitted from the LED passes through the dichroic mirror and illuminates the sample while the 980 nm laser beam is reflected down the optional Force Calibration Module.

⑨ **Single emitter white light LED**: The light from the LED illuminates the sample.

⑩ **The OTKBFM force measurement module**: contains the hardware needed to calibrate the trap. The back focal plane of the condenser is imaged on the Quadrant Position Detector (QPD) using a 40 mm focal length biconvex. The detector is silicon based segmented quadrant position-sensing detector with a rise time of 40nsec and ***a bandwidth of 150 kHz.*** The signal generated by the QPD is sensitive to the relative displacement of the trapped particle from the laser beam axis. As a result the output of the detector can be used to calibrate the position, stiffness, and force of the optical trap. A T-Cube Quadrant Detector Reader is included with this module.

1.4.1 Interfacing a optical setup with DAQ card and computer

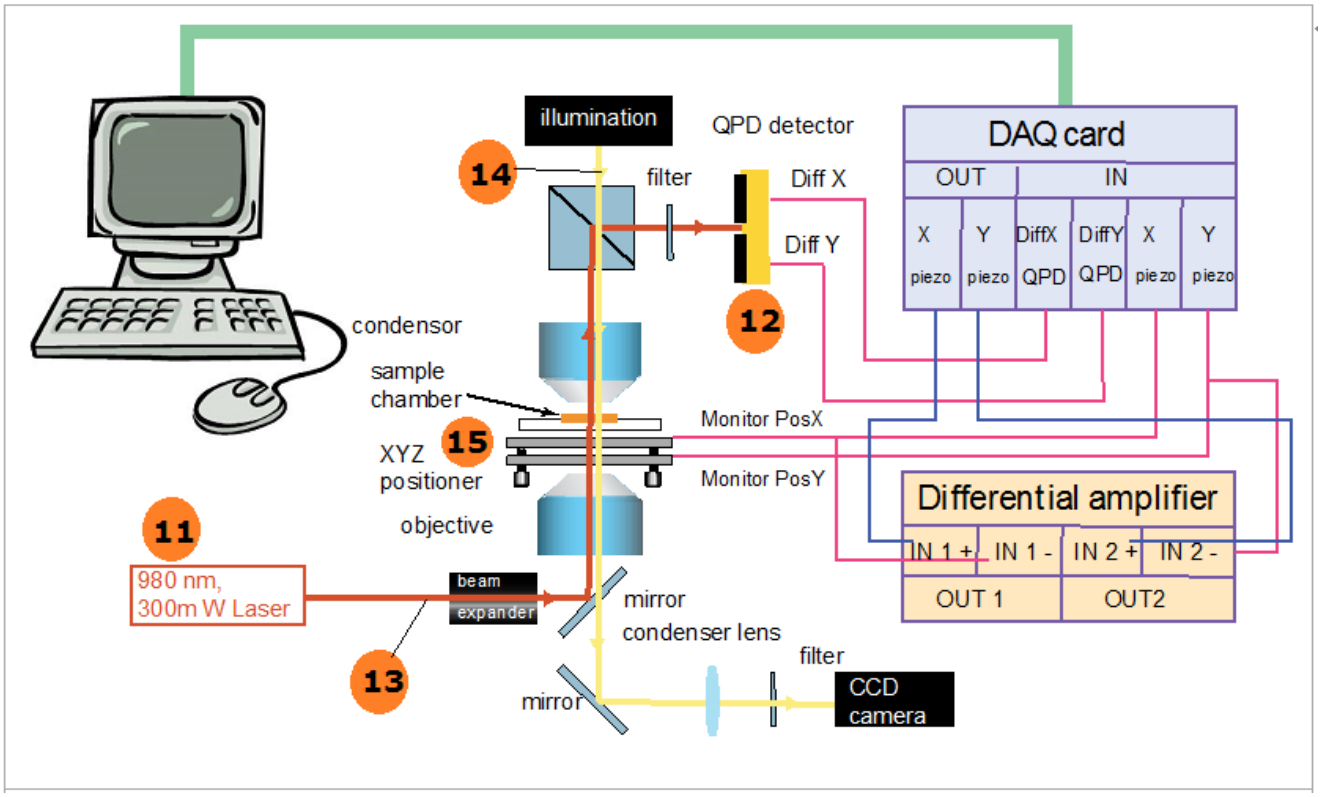


Figure 4 Schematic of the optical trap and how it is interfaced with the computer.

⑪ **Laser**: The Light Amplification by Stimulated Emission of Radiation diode has a maximum power output of 350 mW, though after collimation only a fraction of this power is retained. The diode produces near Infrared light (980nm) and is chosen so as not to heat up trapped living organisms. This Diode Laser is coupled to an optical fiber.

⚠ CAUTION (LASER MANIPULATION)

If this fiber is *broken or kinked it could cause backwards reflections of the beam, destroying the laser and, thus, the experiment*.

1. DO NOT touch the laser or any metal components it is in contact with because any tiny amount of static discharge, much below what you can feel as a shock, can destroy the laser.
2. Be careful when handling anything in the lasers optical path as adjustment will cause *misalignment.* If one optical device is misaligned it is much easier to correct than *if multiple are misaligned*. So if the laser becomes misaligned and/or is not trapping beads well, do not try to fix it, but notify the staff and realignment procedure will be arranged, which will likely delay experimentation. It is important that you AVOID TAMPERING WITH THE OPTICAL PATH in any way. To ensure proper functioning of the laser we need controllers (temperature and current).

⚠ SAFETY (LASER MANIPULATION)

The laser used for trapping in this experiment has a power output of up to 350 mW and a wavelength of 980 nm, which is near Infrared. Even a brief exposure to the focused beam at this power can cause *permanent damage to the retina of your eye*. Because the beam is invisible, you could be exposed without even realizing it. For this reason, the beam path is shielded wherever it is tightly focused. The measures below are essential for your protection.

1. NEVER place your hands or any reflective material, such as rings or watches, in the path of the laser. Due to the invisibility of the infrared laser, its position cannot be seen and hence, it may be scattered in undesired, potentially harmful directions. Accidental exposure to the laser may *cause blindness*.
2. NEVER bypass any safety device.

⑫ **Quadrant Photo-Diode (QPD)**: The signal from the diodes is amplified by low-noise preamplifies and then networked to calculate the X and Y position of the incident light beam. The infrared laser scatters off of a particle and is reflected by a dichroic mirror onto the Quadrant Photo Diode (QPD). The QPD is 4 photodiodes in a quadrant formation to allow X and Y position calculation. Within a certain range of light intensities, the output voltage of a photodiode scales linearly with the intensity of light incident upon the diode. The light incident upon each quadrant in the QPD generates a voltage. The analog circuitry then outputs a voltage Vx and Vy which are proportional to the actual X and Y position of the incident beam only around the center of the trap. As the light scatters in a predictable way off of the spherical beads, this information can be used to recover actual bead position within a narrow range around the center of the trap. Further information can be found in reference: Gittes and Schmidt.

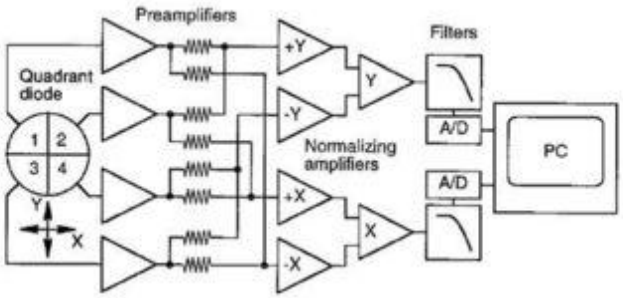


Figure 5 Schematic of the Quadrant Photo Diode position detection system

⑬ **Laser optic pathway:** The laser exits the diode and travels through a fiber optic cable to the back of a Beam Expander. This increases the width of the beam so that when it eventually enters the objective it fills the lens thus creating a narrower waist when it exits the objective, thus creating a stronger, more localized trap. The beam, though pictured as red, is near infrared, and thus invisible. Using a special type of photosensitive dye the beam can be detected. TA can show you how this looks like! The light is reflected from the Dichroic Mirror (this mirror reflect IR and passes visible light). Then the beam passes through the high Numerical Aperture Objective oil immersion 100X creating a narrow beam waist where particles are trapped. After this waist, however, the LASER acts like a point source and is highly divergent. The laser then passes through a condenser, which turns the highly divergent beams into parallel rays. This signal is then bounced off of another Dichroic Mirror and onto the surface of the QPD, producing a voltage signal. When a particle interferes with the laser by getting trapped at the waist, it causes the light to scatter, affecting the position of the laser on the Quadrant Photo Diode surface.

⑭ **Visible light optics pathway:** As the light is infrared and fairly low powered, it cannot illuminate the microscope to allow us to see what is happening. White light is used to illuminate the contents of the slide. This light then passes through a Dichroic Mirror and then the condenser, further focusing the LED light onto the objects in the microscope slide. The light then passes through the objective, magnifying the contents of the slide by 100 xs. The light then bounces off of a mirror below the stage and into the box, after passing through through another Dichroic Mirror, splitting off from the laser light path. The white light then passes through a 200mm 'eyepiece' which further magnifies the picture, this light hits the CCD camera lens and creates the picture.

⑮ **XYZ positioner:** The User will be able to adjust the X, Y and Z position of the stage. Familiarize yourself with the knobs for the X, Y and Z pos view and laser beam. By changing the Z direction of the stage, the waist of the laser beam can be moved up and down through the fluid on the slide allowing beads to be trapped away from either of the glass barriers. The X and Y position will allow stage movement which will be used for searching the microscope slide for particles and QPD calibration. The X and Y and Z position can be adjusted finely with nanopistioning stage.

After familiarizing with all optical elements of the trapping setup, you are ready to start experiments.

1.5 Questions (Theory)

Q1. Give a brief description of the two forces involved in optical tweezers and how they act to trap small glass or polystyrene beads suspended in water.

Q2. How does a diode laser work as compared to a typical optically pumped laser?

Q3. Briefly, what are the functions of the controller?

Q4. Explain briefly how a QPD works in the lab logbook, without going into details about the circuitry.

Q5. How does the Dichroic Mirror work?

Q6. Why is a piezo electric crystal used to generate small movements of the stage? Is its resolution comparable with the motion of some molecular motors?

Q7. How should the solution density change as a function of bead diameter to preserve the same bead to water concentration?

2. PRACTICAL WORK

2.1 Material requirements

- *Handling.* Safety goggles, gloves, tweezers, pipettes.
- *Machines* .Oprical trap, Pipettor: Finpette 10-100µL
- *Products*. Synthetic beads from Bangs Laboratories, http://www.bangslabs.com (10,4,1µm)

2.2 Polystyrene suspensions preparation

To make a solution with floating beads you will use the distilled water to create a 1:3000 dilution of 1% by weight 2 micron beads. Beads are located (and should be kept) in the refrigerator, and each of the vials is clearly marked with the size of bead that it contains. (Note: The sizes reported on the vials are mean particle diameter, not radius). These vials are often extremely concentrated and you may wish to create your own diluted solution to work with. One way to create a 1:3000 dilution of 2 micron beads in distilled water is to use a two vial process. The two vial process also will make a 1:500 concentrated solution which will be used in the experiments as well.

Remarks:

- Higher dilutions will be necessary for smaller beads and may be necessary for 2 micron beads. This will become evident when trapping of the beads is attempted. If the solution is too concentrated, it will be hard to find one bead alone to trap or, once one is trapped, there may be a constant barrage of other beads, knocking the original out of the trap.
- When making a solution, **DO NOT** dip the pipette tip into one bead solution and then into another. Discard the tip **EACH** time you sample from a different bead solution as the bead solution is 3 to 4 orders of magnitude greater in value than the tips and cross contamination can make the experiment difficult to run! It may be a good idea to label the tips for water, salt solution, etc. so that cross contamination is avoided.

1) Remove a 2μm bead vial from refrigerator and shake it vigorously to ensure it is mixed uniformly.
2) Using a NEW filter tip, extract 10μL from the previous vial and deposit into a new plastic vial.
3) Use the large volume micropipette to add 5mL of distilled water to the same plastic vial to make 1:500 dilution. Cap vial using a plastic vial top and shake it vigorously to ensure it is mixed uniformly.
4) Now using another small plastic vial, dilute about 5mL of the first solution in more water (Calculation!), creating a 1:3000 dilution. If the pipette with the 1-10mL volume intake is available, this type of solution can be made directly in 1 vial.
5) Once the solution is created in one of the small vials, shake and label it.

2.3 Floating bead preparation

Now that we have a proper vial of viscous bead solution made up we need to transfer a sample of it onto a slide so that we can observe the beads' behavior.

1) Take out a slide from its box and carefully rest in a position to minimize dust contamination.
2) Place a self-adhesive reinforcement ring onto the center of a new slide. This will create a well for the solution and keep it from drying out. See Figure 6.
3) Make sure that this label is well pressed down onto the slide to ensure that liquid isn't sucked out towards the open air. Rubbing the edge of another slide over the coverslip provides a good method of pushing down the well without contaminating the slide with oils from your hands.
4) Remove outer adhesive liner

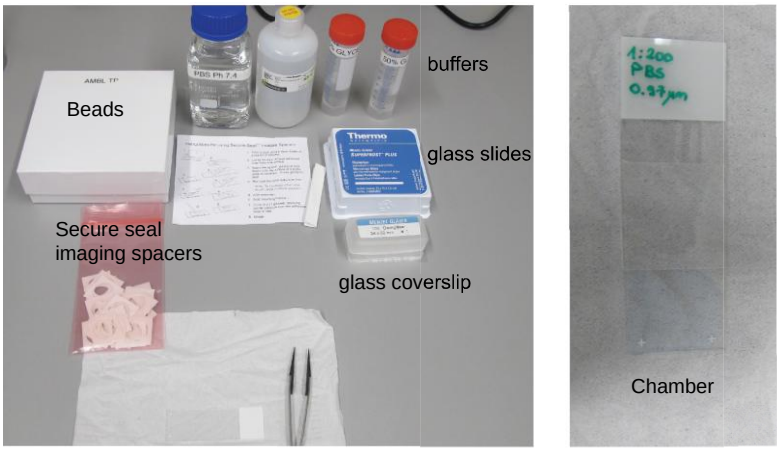


Figure 6. Make a beads slides

5) Use the pipette to transfer roughly 30-40µL of your bead solution into the center of the well
6) Cover the slide with one of the small 24 x 60mm coverslips. It is important to ensure that air bubbles do not form beneath the coverslip. To prevent this, rest one edge of the coverslip on the slide and then let the other side drop onto the slide. (Capillary action will adhere the coverslip to the slide).

2.4 Slide loading

1) Lower the objective lens, so that it will not be scratched (or even touched) by the microscope slide as it is being loaded.
2) Place one drop of the oil on the lens using the dispenser in the bottle of oil. This should be 1 small drop. Avoid touching the dispenser to the objective. Oil only needs to be added to the objective every other slide.
3) Place the slide into the slot on the stage with the cover slip down. This is an inverted microscope, after all!
4) Raise the objective until you see the oil make contact with the cover slip. This Z direction will have to be adjusted later to focus the microscope and laser.

2.5 Trapping bead

1) To illuminate the beads, you must turn on the white light by flicking the switch **LED ON/OFF** on the table.Turn on the ThorLabs's T-Cube controllers for piezo and force module switched them all in CLOSE LOOP. After loading the slide, open Matlab program and set the current directory to be the directory containing ***OtkbCalibration.m***. file. From either the Matlab figure or script file ***OtkbCalibration.m***, press the green arrow to launch the GUI. Maximize the figure that appears for position consistency (you will see why this is important in the following section see Figure 7).

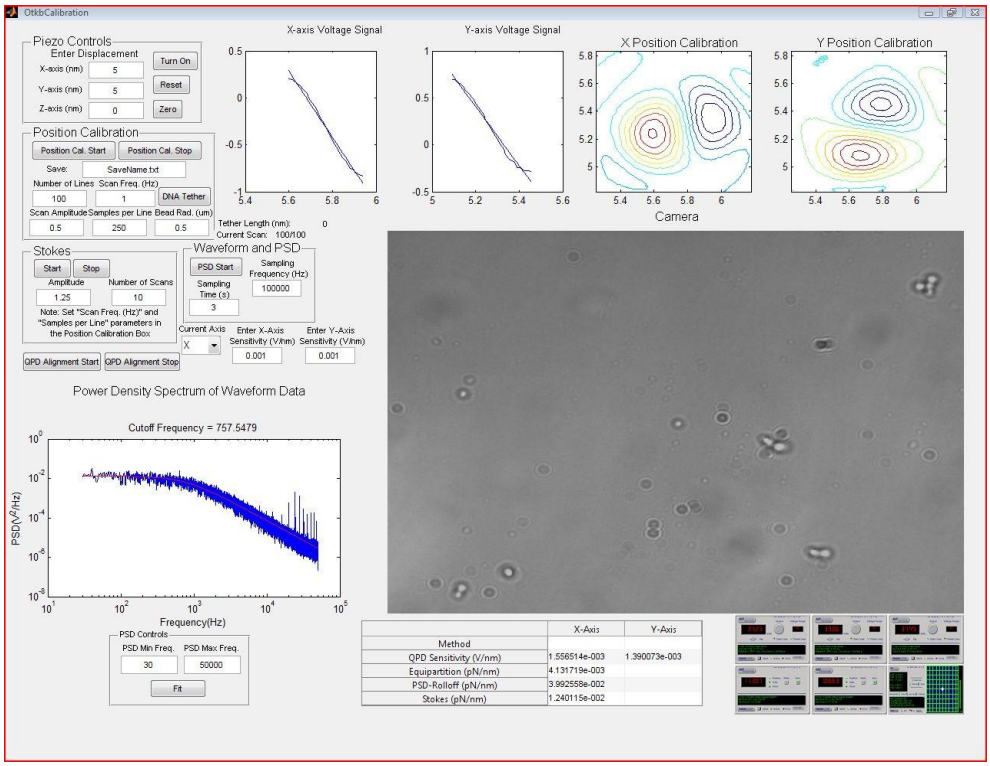


Figure 7 .GUI layout

2) When you run the GUI, you will notice several different panels, which will be used to control the stage and perform the aforementioned calibrations, and several axes whose purposes will be explained later. The GUI is initially launched with none of the piezo, strain gauge, or camera active-x controls displayed. In order to turn them on, press the "Turn On" push button in the upper left corner of the GUI in the "Piezo Controls" panel. You will notice that 6 small piezo, strain gauge, and QPD active-x controls will be initialized in the lower right of the GUI and the camera active-x control will be initialized in the middle right of the GUI. For the sake of this GUI, you can ignore the piezo and strain gauge controls as they will not be manually adjusted.

3) The camera active-x control is initialized with the 'AutoGain' and 'AutoExposure' properties turned on. You will need to adjust these properties. Most importantly Pixel clock should be set below 5M.

4) Now adjust the objective slowly with the Z positioning knob until you can see floating beads in the Camera window. If you advance too far you will lift the slide off of the stage, too little and you will not see the beads.

5) Refer to the laser operation section and, following all safety procedures, finally turn on the laser. To turn it you need to start the controllers first. Make sure that you turn ON the TEMPERATURE controller first (first red arrow left then red arrow right).

6) Now it is safe to turn ON also LASER DIODE controller (left green arrow). Don't change pre-set parameters (20 000 kΩ for T_{SET}). See Figure 8. They ensure proper functioning of laser diode. Now turn on the right green arrow and the laser diode should be on Yellow arrow Figure 8.

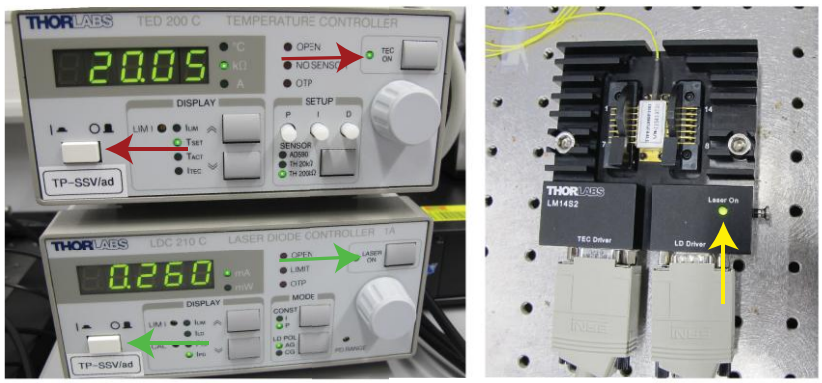


Figure 8. Laser diode controllers

7) Once you have turned up the power to the lasing power, you will be able to see the laser reflected off of the beads you trap or any glass you are focused on. To find the edges of the cell, first retract the objective in the Z direction until you see the laser reflected in a bright circular pattern on the near coverslip. To find the far side, advance the laser past the floating beads until you see the circular diffraction pattern on the microscope slide. The laser is in best focus just past the coverslip away from the microscope slide.

8) This section discusses how to use the GUI to zero the strain gauges, center the piezos, and use the nanometer precision positioning panel :

- Zeroing the strain gauge cubes:

Now you need to null the position reading on the strain gauge cubes. In order to do this, press the "Zero" push button in the upper left corner of the GUI in the "Piezo Controls" panel. This button will set the x and y piezo control outputs to 0V, set the strain gauge position to 0V, and then set both the x and y piezos in closed loop (feedback) mode. In addition, it will move the piezos to the center of their output

- Centering the piezo cubes:

This property of the GUI allows you to set all 3 piezo cubes (x, y, and z axes) to the middle of their output range, the approximate value of which is mentioned at the end of the previous section. In addition, it will zero the output from the two DAQ analog output channels. In order to execute this property, press the "Reset" push button in the upper left corner of the GUI in the "Piezo Controls" panel.

- Nanometer accuracy 3-axis positioning:

The three edit text boxes in the upper left corner of the GUI in the "Piezo Controls" panel allow you to move the stage by a specific displacement in nanometers. In order to move the stage, type the desired displacement in nanometers in the edit text box corresponding to the cardinal axis upon which you would like to move. Hit enter in order to move the stage. You can press enter multiple times to move the stage in increments of the entered value. This works for both positive and negative displacements. If you exceed the range of the piezos, which is

set to be between 0 and 75V (this value should not be changed), you will no longer be able to move the stage via this method. In order to counteract this, try to do the majority of the movement using the rough and fine adjust knobs on the actual stage, and use this property for final small displacements.

9) The easiest way to trap a bead is to find one near the coverslip that is not stuck, focus just past it and then move the center of the trap over the bead. It is highly recommended that at this time you mark the location of the center of your trap on the computer screen. Make an arrow-shaped piece of tape and use it to mark the center of the bead on the screen. This will make trapping much easier because you will have a rough physical reference to the location of the laser.

10) When the bead is trapped it will reflect the laser into the camera. If the bead is not stuck to the glass, you should be able to move it in the X and Y directions and then, very carefully, advance the bead away from the coverslip by advancing the objective by moving in the Z direction. If the bead falls out of the trap, try again with another bead near the coverslip. Trapping beads successfully away from the glass may take some practice.

11) Save an image of the trapped bead as shown on Figure 9.

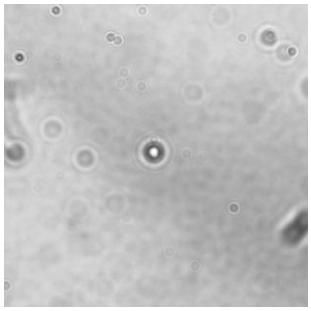


Figure 9. Trapped bead

2.6 Calibration

As is typical for instruments in AMBL the goal of this section is to relate the detector outputs to physical quantities we would like to measure. However, before you proceed, it's important to consider laser power dependence. Both the position calibration, as well as the stiffness of the trap depends greatly on the power output of the 975*nm* trapping laser. Therefore, you'll want to take data for 3-4 different trap power outputs in the range of 30, 60 and 90 mW (maximum power from laser diode is 90 mW at 260mA), and determine the (hopefully linear) relationship between **power and stiffness,** and power and QPD position readout. Keep in mind which of the measurements you make is dependent on accurate position calibration, since you'll need to recalibrate when you change the laser power. Observing specimens and trapping small objects is straightforward with the procedures described above. But to tap the full potential of the laser tweezers to precisely quantify size, position, and forces at nm and pN scales requires careful calibration.

To save time, we are providing you with calibrations of the microscope image scale and the step-size of stage movements by the picomotors. We leave to you the more interesting calibrations, including translating the voltage measurements from the QPD into the position of the trapped particle relative to the center of the trap (QPD position calibration) and the force exerted by the trap on the particle (trap stiffness calibration). There are multiple techniques used to calibrate traps, and we'll let you try a few of them and compare their results and appropriateness. Nevertheless, please read the paragraph below about how to calibrate.

2.6.1 *Voltage vs. position calibration*

What is Position Calibration? To calibrate the position detection, a relationship between the QPD output voltages and position data must be determine. Within a narrow range around the trap (about 100-200 nm), the voltages Vx and Vy from the QPD are linearly related to the distance of the bead from the center of the trap along each axis. The position calibration, also called sensitivity, allows us to translate our raw Vx and Vy data into distance data. Sensitivity, ρ is usually given in units of Volts/μm. Of course, the power setting of the laser will affect the light intensity incident on the QPD, and thus the voltage responses, so rather than a single conversion we really want the relationship between sensitivity and laser power. This normally is fairly linear, so measuring sensitivity at 3 power levels is plenty to characterize the relationship.

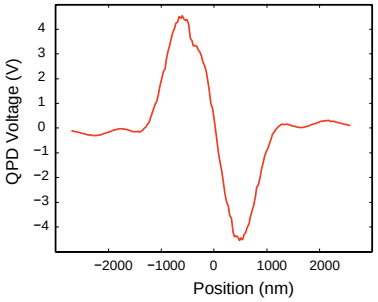
2.6.2 *Stuck bead calibration (done by TA beforehand)*

The calibration is performed by finding a 1μm bead attached to the glass surface (this sample is deliberately mixed in a high-salt buffer to make the beads stick to the glass by hydrophobic interaction).

To make a slide with stuck beads: use a ~1 M NaCl solution (provided) of 1:300 dilution of 1% by weight 3μm beads.. The best procedure to keep the beads from clumping is to first dilute the beads in a small amount of distilled water (maybe 500μL) and then add the 50μL 1M NaCl solution. For the stuck bead slide, it is best to let the slide rest for about 1/2 hour before using, to let the beads settle onto the surface of the glass.

Once the stuck bead slide is made, load it and scan a stuck bead along the x- and y-axis while recording the QPD signal. A more precise method of calibration involves moving the bead in a grid pattern using either the stage, or a separate optical trap, but our stage positioning does not have enough repeatability to enable this, so we will limit ourselves to measurements near the trap axes. *Estimated sensitivity is affected by focus.* This part of the exercises has been performed by TA's before. Typical sample position detection calibration curve for one axis is shown in Figure 10.

Remarks: 90 mW of laser power at sample plane corresponds to ≈260 mA on current controller, 60mW ≈175 mA and 30 ≈85 mA.



Laser power [mW]	Sensitivity α Vx ρ [V/nm]	Sensitivityα Vy ρ [V/nm]
30	0.0061	0.0064
60	0.0042	0.0044
90	0.0035	0.0037

Figure 10. Sample position detection calibration curve for motion along a single axis, Position detector output [V] vs displacement [x] curve, for a 0.97μm diam polystyrene bead.

Q8. How does sensitivity vary with power level? Why this relation?

2.7 Measurement of the trap stiffness

2.7.1 Power Spectrum Method

The thermal motion of a spherical bead of known size suspended in water is well characterized. As the laser power is turned up on a trapped bead, the Brownian motion of the bead is constrained more and more by the increasing trap force restoring the bead to the center of the trap. A statistical analysis of this motion allows us to estimate both the **sensitivity of the trap** and **its stiffness.**

The PSD Method stiffness measurement requires knowledge of the hydrodynamic drag on the particle. For a sphere, the Stokes drag relation is well known, and requires knowing the diameter of the bead and viscosity of the fluid. The Stokes relationship is not valid close to a wall, so a particle must not be near the surface of a slide or coverslip.

The OTkb program puts in the table the calculated trap stiffness by fitting your PSD data to Lorenzian. Now we explain what how program derives the trap stiffness from the PSD data: The Power Spectral Density (PSD) of a trapped bead has a Lorentzian profile described by:

$$S_{vv} = \frac{\rho^2 k_B T}{\pi^2 \beta (f_0^2 + f^2)} \qquad (0.15)$$

in V^2/Hz. Where $\beta = 3\pi\eta d$ is the drag, η is the fluid viscosity of water, d is the diameter of the bead, ρ is the sensitivity of the trap, and f is the frequency of bead vibrations. If the fluid is water then we can take: $\eta = 8.90 * 10^{-4} Pa * s$. Using this, a curve can be fit to the log of the data sets giving the rolloff frequency f_o. In addition the rolloff frequency relates to the relaxation time:

$$\tau_0 = \frac{1}{2\pi f_0} \qquad (0.16)$$

One useful analytic method for this part of the lab minimizes the least-squares distance of every point from the predicted curve, giving two parameters of the equation, alpha and f_o. The Lorentzian can be recast as the equation:

$$\log S_w(f) = \log \alpha - \log(f_0^2 + f^2) \qquad (0.17)$$

The rolloff frequency parameters found through fitting gives the trap stiffnesses $\boldsymbol{\kappa}$ from the following relation: $\boldsymbol{\kappa = 2\pi f_o \beta}$ again where $\beta = 3\pi\eta d$ is the drag, $\eta = 8.90 * 10^{-4} Pa * s$ is the viscosity of water, and d is the diameter of the bead (in meters). This is enough information to calculate and output trap stiffnesses.

2.7.2 Stokes drag

A second method of calculating the trap's stiffness is by calculating the drag force F=αx exerted on a bead as the stage is moved. The most basic formulation of the force exerted on a sphere by fluid flowing past it is

$$\alpha x = \beta v = 3\pi\eta d v \qquad (0.18)$$

Where v is the flow velocity $\eta = 8.90 * 10^{-4} Pa * s$ is -the viscosity of water, and d is the diameter of the bead (in meters). Note that this equation only applies for constant velocity. The Stokes subprogram will run the bead back and forth at various stage velocities it's therefore important that the stage micrometers have ample movement left and that there are few other beads in the area far from trapped bead. 7

Most theory about microspheres in traps involves the Stokes drag formula. However when a bead is close to a surface the formula doesn't hold anymore, and we need to use something called **Faxen's law**. Stokes equation then needs to be corrected due to the proximity effects by factor *g* that can be approximated by Faxen's law.

$$g = \left(1 - \frac{9}{16}\left(\frac{r}{h}\right) + \frac{1}{8}\left(\frac{r}{h}\right)^3 - \frac{45}{256}\left(\frac{r}{h}\right)^4 - \frac{1}{16}\left(\frac{r}{h}\right)^5\right)^{-1} \qquad (0.19)$$

Where h is a distance from the coverslip and r is bead radius. This equation is valid for $h\text{-}r \geq 0.02r$. The factor *g* takes values between 1 and 3.

2.7.3 Data acquisition

Please record in your notebook the values generated by both methods, for the three different laser powers and enter corresponding sensitiveness given in table 1. To do so:

1) Starting with a slide loaded with a sparse suspension of beads in water, trap a bead and move it well away from the coverslip, slide, and from other beads. (If you have centered the QPD at the highest power setting, you won't have to release the bead and recenter between power settings.)
2) Before taking data align the signal on QPD by pressing QPD alignment start once the signal is centered on QPD.
3) Enter the correct value for bead diameter.
4) Specify the filename and directory to save your data. (You need to change a file name EACH TIME YOU CHANGE CONDITIONS laser power, or axis for example PSD30mWx or STOKES60mWy etc.)
5) Now press PSD. Start Default sampling frequency is 100 000 Hz (but it can be changed; if changed note it in your logbook). Default sampling time is 3 seconds. You can change axis by pressing Current axis button. Once done, software will display the PSD as a function of the frequency image as shown below on Fig 11.

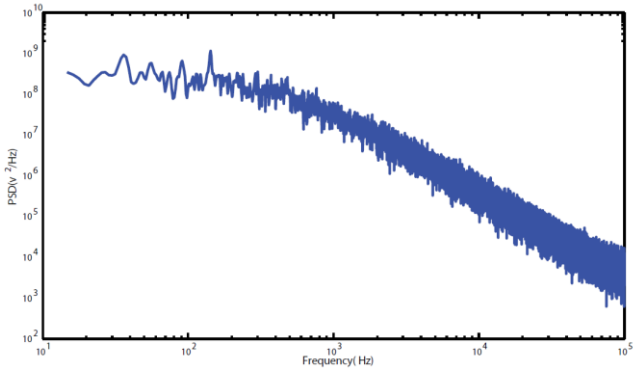


Figure 11. Power spectrum of V[t] for a 0.97μm beads in water solution at room temperature. Laser power is 90 m.

6) Then, Run the Stokes: watch the movement on the video capture. If additional beads fall into the trap, discard the data. If this is a consistent problem, ask for help from a TA - a more dilute bead solution may need to be made. Make sure to run the Stokes program for both the x- and y-axes and all there laser power, as for PSD the program calculates the trap stiffness.

7) Repeat data collection for both methods, at each of the laser power settings you have chosen for your calibration.

3. DATA ANALYSIS

1) There is some experimental data available for a 1μm trapped microsphere on the course server (filename: trapdata.txt, size ca 5Mb): This is a time-series of position data along one dimension for a microsphere. The data is collected at 200 kS/s so the time-interval between each point is 5μs. The units are in some esoteric unit preferred by the experimenter (raw output of a 16-bit AD-conversion)
   - Download the data, import it into MATLAB or some other data-analysis program of your choice.
   - You can remove the average of the data from all data points to center it around zero.
   - Calculate and plot the power spectral density in a log-log figure (in matlab this can be done with the pwelch function).
   - Fit your result derived in excel to the data and determine the roll-off frequency. You will need two fitting parameters: an overall constant that scales the curve, and the roll-off frequency. In matlab you can use the function lsqcurvefit to do the fitting.

Q9. What is the roll-off frequency? What is the stiffness of the trap?

2) Plot the trap stiffness for both axis as a function of laser power.

3) Compare the results obtained using PSD and Stokes drag method. Do you remark some differences? Why ?

4) Think or speculate about why Stokes formula doesn't work near a wall. (Hint: find a hydrodynamics book where Stokes law is derived and think about the assumptions. Search the library or the web and find out one formula (there are probably many) for Faxens law (it should look like Stokes law, but involve the distance to the wall somehow).

Q10. Your friend is doing an experiment with a 2μm microsphere which is trapped so that the center of the sphere is 5μm above a glass coverslip. How large is the error (in percent) he/she is making when using Stokes law?

5) Plot and fit PSDs for the data you took during the 1st session using the procedure you developed in 1).

Q11. Compute a histogram for the trapdata data set of the time-series and use the stiffness you calculated. What is the position sensitivity of the detector that has been used?

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Additional literature

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J. Bechhoefer, S. Wilson, Faster, cheaper, safer optical tweezers for the undergraduate laboratory. Am. J. Phys., 70 (4), Apr. 2002. Read Optical tweezer theory and Applications, part B and Appendix for Trapped-Particle Statistical Analysis explanation.

2. Optical Tweezers from Wikipedia.
3. J. W. Shaevitz A Practical Guide to Optical Trapping, a less technical description of the optical set up and detection.

(Not everything here is relevant to us but it serves as an excellent starter guide).