Optical Trapping Experiment - Optical Tweezers

PHY 431 Optics, Fall 2011

Sources:

Advanced Physics Laboratory, J. Shen et al., University of Toronto

Phys 2010, Optical Tweezers, Brown Universiyt

Optical Tweezers: Measuring Piconewton Forces, M. C. Williams, Northeastern University

Phys 5318, Optical Tweezers Experiment, Northeastern University

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SUMMARY

In this lab you will learn a fairly modern technique, trapping a small sphere using the field gradient of a focused laser beam. The spheres you will use are made of plastic and are 1.2 μm in diameter. After setting up the optics and aligning the trap, you will make some "movies" of the ball diffusing within the trap to measure the strength of the trap as a function of the laser power.

IMPORTANT SAFETY RULES

The lasers used in this lab are more than 30 times more powerful than our usual red lasers, so please use extra caution in avoiding eye contact.

1. INTRODUCTION

Optical tweezers, or optical trapping, is a powerful method of manipulating micron and sub-micron sized particles, and now has been applied to a number of research areas in biology, chemistry, physics and biophysics. For example, scientists use optical trapping to manipulate single bacterial cells, to measure the forces generated by singular motor proteins, or to trap individual molecules and atoms in low temperature and observe physical phenomena such as Bose-Einstein condensation. The goal of this lab is to understand basic theories of optical trapping, to trap beads with lasers served as optical tweezers, and to determine the stiffness constant k for several different laser intensities.

2. THEORY

The theory of optical trapping can be divided into two main regimes: when the particle's radius R is smaller than the wavelength of light λ incident upon the particle (R << λ), or conversely, when the particle's radius is larger than the wavelength of the incident light (R >> λ). For the later case, one can apply geometric optics in conjunction with conservation of momentum. Please refer to the appendix for discussion on this case.

For the case R << λ, particles are treated as a collection of dipoles polarized by a slowly changing electric field. The energy of particles is then:

$$W = UV = -\frac{1}{2}\alpha\varepsilon_0 E^2 V$$

where the parameter $\alpha = \frac{\varepsilon_p}{\varepsilon_0} - 1 \approx \frac{n_p^2}{n_0^2} - 1$, $V = \frac{4}{3}\pi R^3$ is the particle volume, and $U = -(1/2)\vec{P}\vec{E}$ is the electrostatic energy density with polarization P. Such an energy density is also proportional to the local light density I. From conservation of energy, the changing of light density can lead to gradient of W, and hence giving effective force on particle. The force can be calculated by differentiating the expression of W with respect to the particle coordinate. For α > 0, or the index of refraction of the particle is larger than that of the surrounding medium, and consequently the particle will be attracted to the region of higher intensity of light. This force allows one to trap dielectric particles near the focus of a microscope objective, where there is a local intensity maximum. If considering the destabilizing effect of radiation pressure, scientists found that using high-numerical-aperture objective could result in stable traps. In practice, oil-immersion objectives are often used with numerical aperture (NA) > 1.

Though there are two models to explain optical tweezers in the two limits, R >> λ and R << λ, the most effective trapping occurs when R ≈ λ, a regime where neither model is accurate. An approach was proposed to be valid for arbitrary particle size, assuming only a small index difference between trapping particle and surrounding fluid. Thus the energy of the particle can be written as:

$$W = -\alpha \int_V \frac{1}{2}\varepsilon_0 E^2 dV$$

Besides, the local energy density near the focus of Gaussian laser beam can be approximated as:

$$U(\rho, z) = U_0 \exp\left(-\frac{\rho^2}{2w^2} - \frac{z^2}{2w^2\delta}\right)$$

Where ρ is the radial distance from the beam axis, z is the distance along the axis, centered on the focus, $U_0 = \frac{1}{2}\varepsilon_0 E^2$ is the maximum energy density of the beam at the focus, and δ is the anisotropy of the energy density near the focus. For weakly focused light ($NA \ll 1$), $\delta \approx 1/NA$. If $\delta \approx 1$, applying small perturbation and we obtain the linear restoring force on a trapped particle:

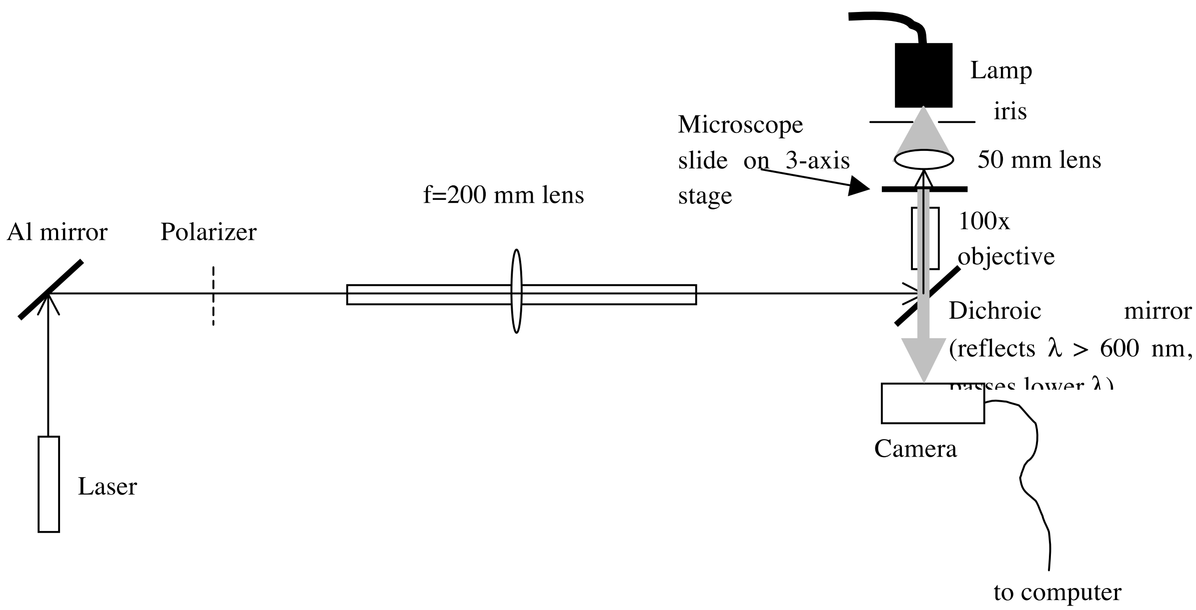
$$k = \alpha U_0 w \frac{4\pi}{3} a^3 e^{-a^2/2}$$

Where $a = R/w$ is the particle size relative to the width of laser beam.

Q1. Summarize the theory and extract the formula describing the linear restoring force (stiffness) on a trapped particle subject to small perturbations. Don't forget to define the variables used in the formula.

Q2. Comment on the differences for varying bead diameters and its effect on the respective trap stiffness.

3. Apparatus



Major components of the optical trapping apparatus are shown in Figure 1.

Using the schematic provided,

Q3. Describe the path of the laser leading up to the sample.

Q4. Provide a brief description of each major component comprising the optical trapping system. You should include component name and a short (<200 words) description each in a table format.

4. Optical tweezers - Measuring the Stiffness Constant k of the optical trap

A) Background

A bead trapped in an optical tweezer apparatus is not completely immobile. Ever present thermal fluctuations cause the bead to execute Brownian motion while trapped. What prevents the bead from escaping the optical trap is the fact that the trap has an inherent stiffness k, which is controlled by the power of the laser light. That is, an optical trap is taken to be a potential well with stiffness k. A spring with stiffness k prevents the mass attached to it from escaping and pulls it (or pushes it) back to the equilibrium position. Similarly, the optical trap tries to restore the bead to its equilibrium position, at the bottom of the trap's potential well.

B) Goal

The goal of this lab is to measure the trap stiffness k for several different laser intensities. You will also need to examine whether the trap stiffness in the x-direction is different from that in the y-direction.

C) How can one measure the stiffness k of the optical trap?

There are two ways to do this. Both ways involve the observation of the bead's Brownian motion while in the trap's potential well (i.e. while the bead is trapped). The methods are described below, but not in thorough detail. For more details on how one can derive the results mentioned below, one should refer to any statistical mechanics textbooks.

As mentioned above, both methods involve the observation of Brownian motion. In particular, the variable that is of interest to us is the position x of the bead with respect to an origin. Most commonly this origin is taken to be the bottom of the potential well (i.e. the center of the optical tweezer trap).

<u>Method:</u>

From the equipartition theorem we know that every degree of freedom has energy $k_B T/2$ where k_B is the Boltzmann constant and T is the temperature of the medium in Kelvin. So, for an optical trap in one dimension we get the equation:

$$\frac{1}{2}k_B T = \frac{1}{2}k\langle x\rangle^2$$

So, by measuring the average of the displacement squared when the temperature is known, one can find the trap stiffness.

Equipartition Theorem through a CCD camera

In order to decide trap stiffness, we apply the equipartition theorem for a trapped beam undergoing thermal fluctuations, which would be stated as following:

For each degree of freedom in the thermal motion of the particle, there will be $\frac{1}{2}k_B T$ of thermal energy, where k_B is the Boltzmann constant and T is the temperature in Kelvin. If the beads are not in trap, the thermal energy is totally converted into kinetic energy, causing bead to undergo random movement in the medium. If the bead is in a harmonic trap, the thermal energies become potential energies manifested in a small displacement. Over large sample sizes the fluctuations will give the result:

$$\frac{1}{2}k_x\left\langle x^2\right\rangle = \frac{1}{2}k_B T$$

Where $\langle x \rangle^2$ is the variance of the x-displacement. In this experiment, you have to record .avi videos or image frames from the CCD camera. It is important to note that precision of the positional data is critical for this method. Since the fluctuations in position are squared, it is highly sensitive to noise.

4.1. SAMPLE PREPARATION

a) b)

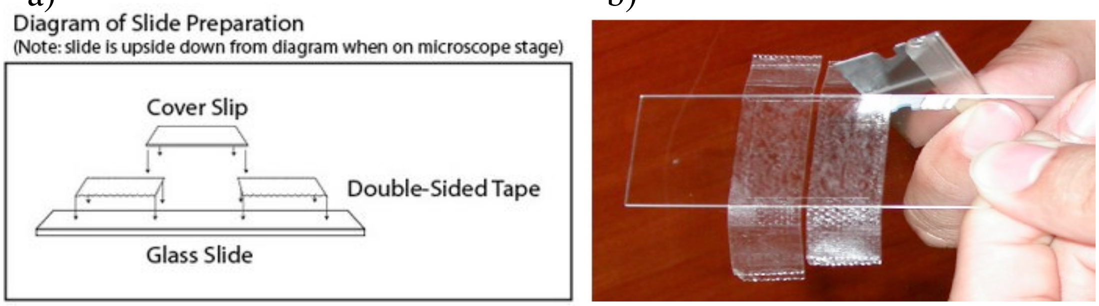


Figure 2: a) Components of the sample slide: microscope slide, two strips of double-sided tape, and a cover slip. [18]. B) Student trimming off the excess tape from the sample slide with a razor blade.

1. The following materials will be required: microscope slide, cover slip, double-sided tape, P200 micro-pipette, pipette tips, kim wipes, razor blade, diluted bead solution (beads – 1:100 dilution), and vacuum grease.

2. Clean both sides of the microscope slide with ethanol and kim wipes and ensure that it is kept clean. Dirt or oil can affect the measurements as it may cause scattering of the beam [18].

3. Place two pieces of double-sided tape separated by about 3-4mm across the centre of the slide.

   Place another layer of double-sided tape on the first layer. This creates a channel with sufficient height that the beads can move through. Press on the tape to eliminate air bubbles near the channel to ensure that the liquid will not escape the channel.

4. Clean the coverslip with ethanol and kim wipes and place it on top of the channel and the double-sided tape strips, which will hold it in place. Centre the cover slip such that it is not at the edge of the glass slide. Press on the tape contact to ensure that the liquid cannot escape from the channel, and carefully trim of the excess tape from the ends using a razor blade.

5. Adding the sample:

   a. Shake the sample solution thoroughly to evenly distribute the beads.

   b. Using a plastic pipette, pipette the sample up and down several times to mix the solution and obtain 15-20μL of the bead solution and insert it into the channel from one end just outside of the cover slip. The bead solution will fill the channel through capillary action and gravity.

6. Discard the plastic tip into the garbage bin.

7. Seal the open ends of the channel using vacuum grease and carefully recap the syringe.

8. Before loading the sample, place a drop of Immersion oil on the Nikon oil Immersion objective lens (Figure 7, B).

9. To load the sample, use the translating breadboard (Figure 7, C) to position the sample holder between the objective and condenser (Figure 7, A). Place the slide onto the sample slide holder with the cover slip facing down. Carefully press down the glass slide firmly into the holder.

Figure 3: A) Sample stage onto which the microscope slide containing the sample is placed. B) Nikon objective lens onto which a drop of oil must be added before loading the sample. C) Translating breadboard which can be moved horizontally to facilitate sample loading.

The beads in the channel should be somewhat scarce, so as to allow isolation and trapping. Once the sample is created and sealed appropriately, it may be used for up to 24 hours.

It is recommended that you use two bead concentrations during the course of the experiment – a higher concentration (eg. 10^{-5}%) to practice trapping and a lower concentration (eg. 10^{-8}%) to perform actual measurements.

The entire stage can be translated along the direction perpendicular to the beam path to facilitate loading and unloading of the sample.

WARNING: Please turn OFF the laser when changing samples.

"In-A-Hurry" 2-minutes Sample Preparation: To make a sample for the microscope, take a slide, put one drop of the bead solution on the right end of the slide (left end for the reversed setup) and cover with a coverslip. Place the slide in the holder with the coverslip facing the objective and advance the 3-axis stage towards the objective (this axis is the "focus"). When the slide is very close, add a little immersion oil between the coverslip and objective.

Q5. What is the N.A. (numerical aperture) of the objective? What is the purpose of the oil? What is the total effective N.A. of the microscope?

4.2. Camera Settings

The CCD camera is controlled through the Fire-i software. Before beginning, turn on the LED light to illuminate the sample by powering the LED (connect wire from the LED on top of the apparatus to the wire labeled “LED Power”). The standard operation can be referred as following:

- Start → All Programs →Fire-i
- Press the Play Camera button to display the live image.
- Press Settings→ Camera properties, to configure the camera.
- Format selection: Y_MONO with the highest possible resolution and frame rate should be selected.
- You can control the Exposure time through the Camera properties tab should the need arise. Exposure time should be set at approximately 15ms.

Note: no calibration is necessary.

4.3. Viewing the Beads

1. Turn on the white light and start the camera in “Preview” mode on the computer. You should see beads moving on the screen. If not, move the stage forward and backward until they come into focus.
2. The cover glass should be in contact with the immersion oil. Use small adjustments to move the Nikon objective upwards using the Z micrometer until you see the beads.
3. Use the X and Y micrometers (coarse and differential knows) to view the channel and adjust the brightness of the camera as necessary to improve visibility of the beads.

4.4. TRAPPING A BEAD

A. Now place the two mirrors in their holders and align the beam to the back of the objective. The two adjustment screws behind each mirror adjust horizontal (lower screw) and vertical (upper screw). The beam should run parallel to the table and along the grid of holes in the table. You can check this by measuring the beam position on a ruler at various points in the path. Finally add the lens to the rail. If the alignment by the mirrors is perfect, the beam should go straight through the lens. Slide the lens along the rail until the beam at the back of the objective is slightly smaller than the hole.

B. The final adjustment is to make sure the beam goes straight through the objective. This can be checked with a card behind the microscope slide (you might need to turn off the white light). The image should be bright fringes in an hourglass shape. Adjust the two mirrors until you see this image. Now look at the image on the camera. You should see a circular diffraction pattern that enlarges symmetrically as you change the focus. Adjust the mirrors until you see this. (If you see a red speckle pattern, it means the beam is not going straight through the objective).

C. Turn on the white light again and watch the beads. Adjust the focus until you see beads get trapped in the laser beam. With full power, you will probably see several beads get trapped. Put in the rotating polarizer and turn it to lower the power until you can trap just one bead (note the angle you set). Try carefully moving the slide vertically and horizontally to see if you can keep the bead trapped as you drag it around. (This is how researchers generally use this technique to apply small forces to molecules).

- To find the trapping position, scan the beam in the X and Y directions using the coarse and differential knobs. When a bead is near the trap axis, it will either be captured by the trap or be shot out of the field of view due to the scattering force.
- If you are having trouble trapping, gradually move the sample higher such that the focus is closer to the coverslip.

D. Once you have trapped a bead, record a video or sequence of frames.

- To record a video using the CCD camera:
  Go to *Video Capture* → Set the file path, change the file type to *JPEG* and *Max Frames* to 500 then press *Record*. The data will be saved in *.avi* format.
- To record a series of images:
  Stop the camera in preview mode and click on “frame capture.” Make the time between frames 50 ms and set the number of frames to 100. Also chose a directory to collect data (make a new folder for each data set) and click “OK.” Take data for a few seconds and stop. Look at the folder where the data was collected. You will probably need to delete the first frame because it won’t have a good image.

E. Repeat the measurement for at least four other power settings (total 5).

F. Perform this experiment for a bead with a different diameter.

5. Data Analysis

A. Use the Image/J program to convert the recorded video/images of the trapped bead from the camera into x and y position data.

B. Make a histogram of the x or y values of the displacement. If the histogram does not look like a Gaussian distribution, try adjusting the threshold in ImageJ and make a new displacement measurement. You can also try making the crop of the image smaller (the bead should take up most of the image). Find the variance in this histogram.

Repeat the measurement and analysis for at least four other power settings. The exact power in the trap can't be measured accurately but you can get a scale of the power by measuring the power after the polarizer. Plot the transmitted power versus the angle of the polarizer. Find the angle of minimum power, then turn 90° to get the maximum. Assume this power is 30 mW. The relative power of your other measurements are $30\cos^2(\theta)$, where θ is the angle between maximum power and your measured power (remember $P \sim \langle E^2 \rangle$) [Malus' Law].

Q6. Plot the variance vs. 1/P. What does the slope of this curve tell you?

Q7. Determine the spring constant. Plot the spring constant versus P.

APPENDIX

Predictions on the affect optical forces have on trapped beads are directly dependent on the diameter of the bead relative to the wavelength of the incident laser. The classic ray optics model is sufficient to explain how forces trap and displace bead particles only when the radius of the particle is a great deal larger than the laser wavelength. Once the diameter of a bead to be trapped is much smaller than the incident light wavelength, then the particle can be treated as an electric dipole and the electric dipole approximation can then be used to predict force interactions.

THE RAY OPTICS MODEL (SEE ALSO "OPTICAL TWEEZERS" ON WIKIPEDIA)

When the diameter of the trapped particle is far greater than the wavelength of the incident laser, the classic ray optics model of ray refraction can be used to describe the affect scattering forces have on trapped particles and the resulting gradient forces which withstand the scattering effects and keep the particle trapped just downstream of the beam center.

As emitted photons travel the path of the laser, they carry momentum. The momentum associated with each photon changes as it interacts with the trapped dielectric bead. This is because light particles colliding with the glass bead are refracted as they rebound of the particle. This refraction results in a change of photon direction and therefore alteration in the photon momentum. This change in momentum will cause a force to be exerted on the photon and, as a result of Newton's Third Law, a force of equal magnitude will also be exerted upon the trapped bead.

The laser beam used to operate the optical tweezers apparatus maintains a Gaussian profile. This means that the affect each photon has on the trapped bead is dependent upon its distance from the bead. As a result, the photons near the beam waist and trapped bead, which exert an attracting force and pull the bead towards the center of the beam, will have a more intense affect than those photons further from the trapped bead attempting to displace the particle from the beam waist [5]. When these distant scattering forces attempt to move the trapped particle to the left or right of the beam center, the high intensity attractive forces overcome this displacement and maintain bead position, as seen in Figure 1 [4]. These high intensity forces restoring the radial position of trapped particles are referred to as the gradient force [4].

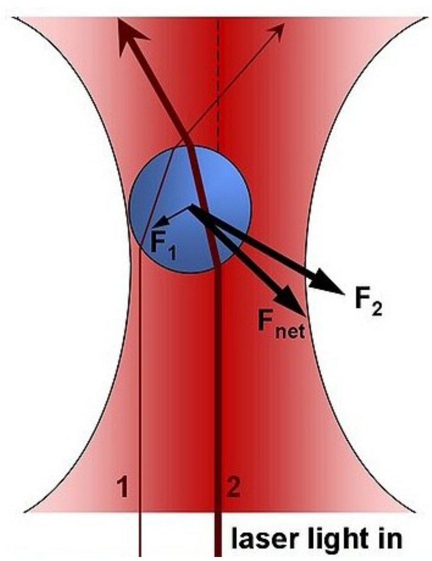


Figure 1: Any attempts to radially displace the trapped bead from the beam waist are overcome by the higher intensity attractive forces closer to the bead. [4]

Now, once the gradient forces have returned the bead position to the center of the trap, then all refracted photon rays will be symmetric about the bead, leading to a net momentum of zero. Without a change in momentum, there will be no forces pulling the bead from the center of the trap, and so it will remain in this lateral position. Thus, without a lateral net force, only the scattering forces in the axial direction of the beam path will be able to influence bead movement. It is these scattering forces which cause the radially centered bead to undergo lateral displacement and be trapped slightly downstream of the beam waist, as seen in figure 2 [5].

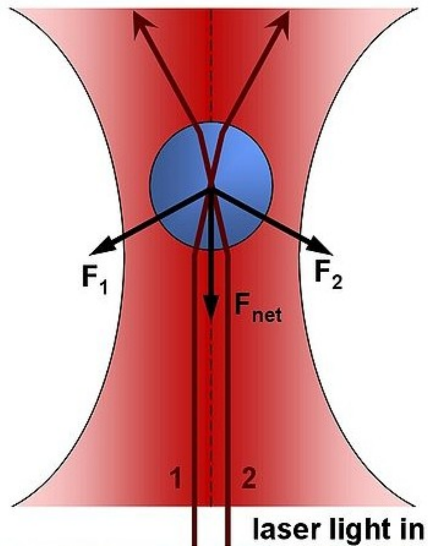


Figure 2: Once the trapped particle is centered radially, scattering forces in the lateral direction cause the bead to be displaced slightly downstream of the beam waist. [4]

THE ELECTRIC DIPOLE MODEL

Once the radius of the particle to be trapped is sufficiently less than the wavelength of the incident laser beams, then the electrical dipole model can be used to approximate the photon and particle interactions. Because the trapped bead is so much smaller than the laser wavelength, it can be thought of as a point dipole in the photon electromagnetic field.

The force acting on a single point charge placed in a magnetic field is called a Lorentz force [6] and can be mathematically described through the equation:

$$\vec{F} = (\vec{p} \cdot \nabla)\vec{E} + \frac{d\vec{p}}{dt} \times \vec{B} \tag{1}$$

Where $\vec{F}$ is the force [N], $\vec{E}$ is the electric field [V/m], $\vec{B}$ is the magnetic field [T], $\vec{p} = q\vec{\mathbf{d}}$ is the polarization of the dipole, q is the particle electric charge [C], and $\vec{\mathbf{d}}$ is the distance between charges [m]

Now, because we assume that the trapped point charge is linear, we can eliminate the dipole polarization from equation (1) through the use of the polarizability , α , where $\vec{p} = \alpha \vec{E}$. Equation (1) can be then be rearranged into the form of:

$$\vec{F} = \alpha \left[\left(\vec{E} \cdot \nabla \right) \vec{E} + \frac{d\vec{E}}{dt} \times \vec{B} \right] \ldots\ldots\ldots\ldots\ldots \qquad \ldots\ldots\ldots(2)$$

Which is simplified to:

$$\vec{F} = \alpha \left[\frac{1}{2} \nabla E^2 + \frac{d}{dt} \left(\vec{E} \times \vec{B} \right) \right] \ldots\ldots \qquad \ldots(3)$$

The last term on the right hand side of equation (3) is the time derivative of the Poynting vector, which represents the power flux through an electromagnetic field. During the optical tweezers experiment, the sampling frequencies are much shorter than the frequency of the laser beam, ~10^14 Hz, and so the power of the laser will be constant. Constant power will lead to a zero value of the time derivative of the Poynting vector and so this term can be removed from the equation. The force acting on the electric dipole can then be represented with the equation:

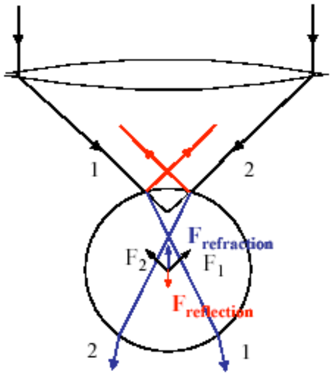
$$\vec{F} = \frac{1}{2} \alpha \nabla E^2 \ldots \qquad (4)$$

Because the term in equation (4) represents the electromagnetic intensity of the photons, the strongest light force acting on the particle will be those with the highest intensity. As the peak photon intensity occurs at the center of the beam waist, the forces acting on the bead to be studied will draw it to this position. These forces are then of gradient type, as they attract particles to the center of the beam.

As in the ray optics approximation, once the gradient forces have radially centered the beam, only axial displacement due to scattering forces can now occur. These scattering forces, represented by the Rayleigh approximation:

$$\vec{F}_{scat}\left(\vec{r}\right) = \frac{k^4 \alpha^2}{6\pi c n^3 \varepsilon_0^2} I\left(\vec{r}\right) \hat{z} \ldots \qquad \ldots\ldots\ldots\ldots.(5)$$

once again displace the trapped bead slightly downstream of the beam waist.



SUMMARY

For a particle with a radius much larger than the wavelength of light, the trapping force can be treated with geometric optics. In the figure below, the rays from the focused laser beam enter the beam at some large angle relative to the normal. If the index of refraction of the bead is higher than the surrounding medium (water), then some light will be refracted, producing a downward force, and some light will be reflected, producing an upward force. These forces are equal if the bead is centered in the focus of the beam; however, a restoring force is exerted if the beam or bead are displaced in any direction. Therefore the trap can be treated as a spring with a spring constant k proportional to the angles of the rays entering the bead (how tight the focus is) and the total power of the beam.

For a particle with a radius less than or equal to the wavelength, you can treat the system as a collection of dipoles where the energy of the particle is proportional to the energy density (or light intensity) of the trap. A large gradient in the energy density gives a gradient in the energy which also produces a spring-like restoring force. The particle can be visualized as trapped in a nearly harmonic potential well. The viscosity of the water acts to damp large excursions of the particle.

Since the bead is in water, even when it is trapped, it will receive random kicks from water molecules, making it jump about in the trap. How much it jumps, or diffuses, depends on the energy of the kicks and the strength of the trap. Since the system is in equilibrium we can equate two energies, the mean potential energy of the particle in the trap and the thermal energy:

$$\frac{1}{2}k_x\left\langle x^2\right\rangle=\frac{1}{2}k_BT$$

where k_B is Boltzmann's constant and T the temperature and $\left\langle x^2\right\rangle$ is the variance of the displacement in the trap in one direction. This model assumes that we can treat the particle as a 1-dimensional, damped harmonic oscillator driven by a random force.