

Faster, cheaper, safer optical tweezers for the undergraduate laboratory

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We describe an optical tweezers experiment suitable for a third-year undergraduate laboratory course. Compared to previous designs, it may be set up in about half the time and at one-third the cost. The experiment incorporates several features that increase safety. We also discuss how to use stochastic methods to characterize the trap's strength and shape. © 2002 American Association of Physics Teachers.

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I. INTRODUCTION

A tightly focused beam of light can attract and trap micron-sized dielectric particles whose refractive index exceeds that of the surrounding medium. Although single-beam optical traps were developed only in 1986, they have already proven their worth in making possible an increasing number of experiments.¹ Optical traps have found particular use in biophysics, where they allow one to manipulate single molecules of DNA,² allowing one access to their physical properties and to the properties of attached molecules of biological interest. They have been used passively, to record the forces induced on a bead, for example, by kinesin molecules³ and myosin-V.⁴ In other applications, tweezers have played an active role, for example, to induce a “pearling” instability in lipid vesicles.⁵ The tweezer-induced motion of a bead also can be used to measure local elasticities and viscosities, for example, inside cells.⁶

The first designs of optical tweezers used large (> 1 W) lasers and expensive optical hardware, which placed them beyond the reach of undergraduate laboratories. Recently, however, Smith *et al.*⁷ developed an apparatus that is simple and cheap enough to be included in an undergraduate laboratory. This article explores improvements to their original design, the cumulative effect of which is to make the apparatus more practical and much cheaper. In addition, the design eliminates several possibilities for injuries, increasing the safety of the experiment. After the first version of this work was submitted, Moothoo *et al.* published a design with similarities to ours.⁸ There are, nonetheless, a number of differences worth discussing. In addition, a *two-beam* trap using a hollow-core fiber has also been described.⁹ It shares some of the advantages of the design described here, although lasers of much higher power are required.

In the following, we first briefly review the theory of optical tweezers, mostly to alert the reader to a recent theoretical advance that greatly simplifies calculations. We then discuss our design and its rationale, along with a careful discussion of one application for the tweezers.

II. BRIEF REVIEW OF OPTICAL TWEEZER THEORY

The theory for optical tweezers has been extensively discussed, for example, in Ref. 7; however, that discussion considers just two limits, one where the particle radius R is much smaller

mate the local energy density near the focus of a Gaussian laser beam as

$$U(r, z) = U_0 \exp\left(-\frac{r^2}{2w^2} - \frac{z^2}{2w^2\theta}\right), \quad (4)$$

where r is the radial distance from the beam axis, z is the distance along the axis, centered on the focus, and $U_0 = \frac{1}{2}\epsilon_0 E^2$ is the maximum energy density of the beam at the focus, $r=z=0$. Here, θ is the anisotropy of the energy density near the focus. For weakly focused light ($\text{NA} \ll 1$) [10]

by filters. But we need to remember the old adage that anything that can possibly go wrong eventually will.! By eliminating the eyepiece, we eliminate a whole series of unfortunate scenarios. Second, cheap microscopes are often unacceptably floppy when used with the 100X, oil-immersion objectives that produce the best results for trapping. Finally, they are often inflexible when we want to add nonstandard elements to the beam path.

For all of these reasons, we developed an “open microscope” based on commercially available optics and mounts, all placed on a standard optical breadboard.¹⁶ Previous designs using such optics have all been “inverted microscopes,” with the beam coming up through an objective and onto a horizontal sample stage. In our design, we opted for a *sideways* microscope where the beam path stays parallel to the optical table. This sideways configuration has several advantages:

Keeping the entire beam -laser and microscope! in one plane simplifies greatly the alignment and setup. Once a standard height is chosen -about 10 cm in our setup!, one can mark an index card at the proper height and quickly line up all elements approximately to the reference height. Having the microscope beam path at 90° vertical to the laser path is much more difficult to align correctly.

Having a low beam in one plane is safer. Students would have to stoop to put their eyes at the same level as the beam. In the traditional configuration, the beam will almost certainly pass eye level somewhere.

Our microscope design is as follows: The light source is a modified halogen desk lamp, whose 20 W bulb puts out ample light.¹⁷ We found that using two plano-convex lenses produced an acceptable condenser. The sample was held on an XYZ translation stage that served to focus and laterally displace the sample. The stage is the most expensive element of the microscope -\$650!, and a poor choice—one that lacks rigidity or whose movement is not smooth—will lead to much student frustration. After some trial and error, we settled on a 1/2" stage recently introduced by Thorlabs.¹⁸ As in Ref. 7, we use a student grade 100X oil-immersion microscope objective.¹⁹ Because the lens of the microscope objective and the sample glass slide are vertical, it is important to buy high-viscosity immersion oil.²⁰

Finally, the image from the microscope is directly projected onto a camera sensor. We used both a traditional CCD camera²¹ producing analog video output and a USB-based Web camera²² based on a CMOS sensor and producing digital output. -Firewire cameras have recently become available but remain more expensive.! The video camera was fed into a frame grabber²³ and into a computer. Although expensive, the camera and framegrabber provide a robust solution that is easily implemented. Web cameras are much cheaper but less flexible and less durable. They are made of plastic and tend to break and may be in the long run be more expensive to maintain. So many Web cameras are available that it is difficult to examine them all. The one we selected has features that are useful for the present design.

The lens can be removed -and replaced!, allowing us to project an image directly onto the CMOS sensor.

The legs detach, allowing us to fasten the camera easily to a standard 1/2" mounting post.

Other small improvements in our design include the following.

Because the beam is all in one plane, the laser beam encounters only one total-reflecting -“normal”! mirror and one

dichroic mirror. -The normal mirror is added only because we need two mirrors to independently fix the position and orientation of the laser beam with respect to the microscope’s optical axis.!

Previous designs used two lenses for a beam expander -to make the beam size equal to the back aperture of the microscope objective! and then a third lens to form an intermediate image at the standard 160 mm behind the objective. Here, both functions are accomplished by a single lens. -At the level of paraxial, Gaussian optics, a system of three lenses can always be reduced to a single-lens equivalent.! It is a nice exercise to ask the students to calculate the required focal length of this lens, given the approximate beam diameter from the laser module, the size of the back aperture of the objective, and the standard tube length -160 mm!. We find that we should use a lens of focal length $f = 160(D_1/D_2)$ mm, where D_1 is the diameter of the collimated laser beam and D_2 is the diameter of the back aperture of the microscope objective. As Svoboda and Block have noted,¹⁰ it is important to err by overfilling the back aperture, as underfilling will lead to a rapid decrease in effective NA and loss of trapping efficiency.

C. Aligning and operating the trap

Once students have set out all the pieces on the optical breadboard, they are faced with the sometimes frustrating task of aligning the elements to obtain trapping. One basic strategy is to separate the task of building the microscope from that of building the trap. The first step, then, is to align the microscope. This is not too difficult, but we need to make sure that we can make reasonably sharp, isotropic images of spheres in solution. One-micron polystyrene spheres are a good test of the performance of the microscope, and they make good objects to trap, as well.²⁴

move the intermediate lens along the optical axis in order to make the focus of the trap coincide with the focus of the microscope. Usually, we first reach a situation where the trap is located somewhere inside the glass of the coverslip, so that particles are trapped, in the radial direction by the optical forces and then pinned against the that

where the partition function Z is defined so that
 $\int_{-\infty}^{\infty} r(x) dx = 1$. For a parabolic potential, the expected dis-

It is safe to assume -and we can verify by looking at positional fluctuations in the high-power limit! that r_{shot}

$$M = 2k_B T g. \quad \text{-A10!}$$

The final form of the autocorrelation function is then

$$\hat{x}(t) \hat{x}(t + \tau) = \frac{k_B T}{k} e^{-|\tau|/t_0}, \quad \text{-A11!}$$

