

## Experiment 10:

## Laser Tweezers

PHY 431

In this lab you will familiarize yourself with practices at a research laboratory that utilizes optical techniques. You will be setting up an experiment that employs laser tweezers, an optical technique allowing to hold and manipulate micron-sized particles. The technique is widely employed in research in physics and biology. The particles that you should be able to manipulate are spheres out of plastic  $1.2 \mu\text{m}$  in diameter, see Appendix.

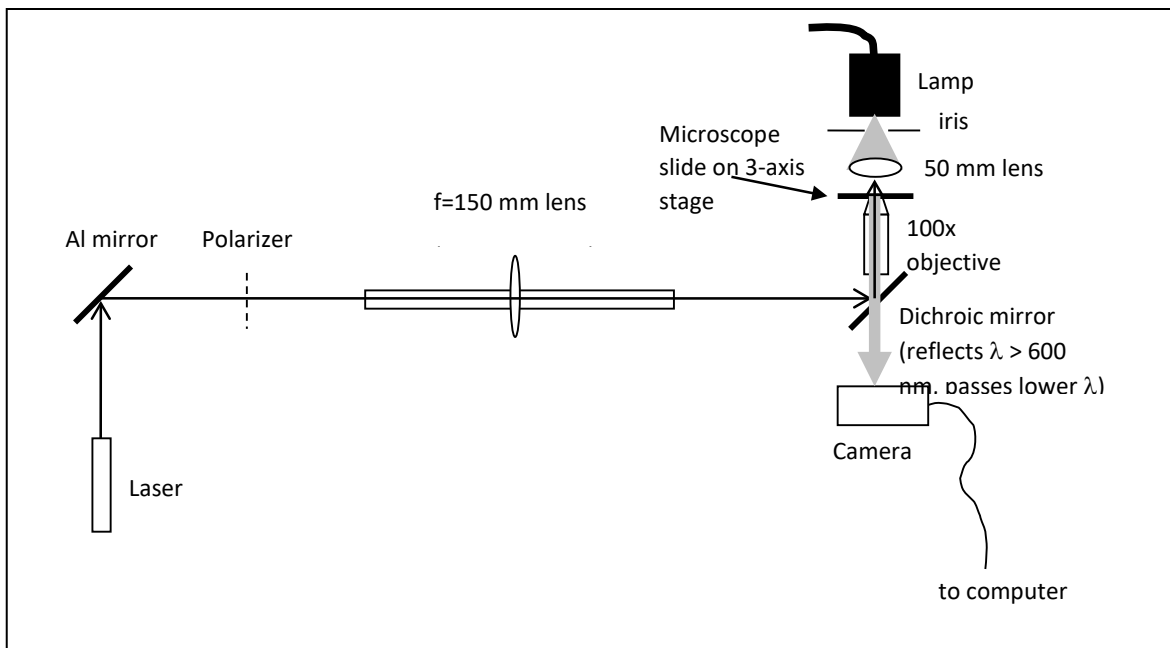
**SAFETY WARNING:** the lasers used in this lab are more than 30 times more powerful than our usual red lasers, so please use extra caution in avoiding eye contact.

You will be setting up this experiment on an optical table with a rectangular grid of holes 1" apart, tapped at  $\frac{1}{4}$ -20 (tripod) thread. The holes allow for various components to be bolted down along grid lines.

**Q1:** When two elements are bolted together, they are difficult to take apart. What forces hold them together? What features of the thread make these forces effective?

On occasions, when screwing a bolt into a hole, you will find that it will not fit. Do not try to force it in. The bolt may be too large or be threaded according to a different standard, such as metric instead of imperial. With different components manufactured in different countries following different standards, this is the reality of working at a laboratory. Look for a matching bolt or component. Error in manufacturing of the mirror for the Hubble Telescope stemmed from a confusion in conversion between imperial and metric units and from not fully adhering to the protocol of assessing device operation with independent methods.

The figure below shows a completed setup of your experiment



One complete or nearly complete setup will be there in the lab, serving as a reference for you.

## Procedure

- A. First try to put together the part of the setup between the camera and the illuminating LED lamp, excluding the dichroic mirror. To work together, optical elements need to share an optical axis. For setting up the elements and achieving this goal, use the laser later employed in the other part of the setup and a variable iris mounted on a post that is neither too low nor too high. **Q2:** Why should such an iris be convenient in synchronizing optical elements? Note: It may be convenient to use the same elevation of the iris above the table across the different teams. Put the laser in the place of the camera first, switch it on and adjust its beam to point horizontally along a grid line at the elevation set by the iris. The laser is likely placed in a spherical mount that allows for adjusting the direction of the laser beam and it is also likely to have a collimation adjustment. To test the collimation move a sheet of paper along the laser beam and look whether the beam changes in diameter. Adjust the collimation to get as little change as possible with position along the beam. Mount the microscope objective on the table, ahead of the slide mount, in such a way that it is close enough to touch the slide and that the axis of the objective is concentric with the laser beam. Test the latter in particular by placing the iris right where the beam enters the objective and reducing the iris opening. Bright light of the beam should shine through the objective even when the opening is of tiny size. Add the other elements for this part of the setup, eventually replacing the laser with the camera. Switch on the LED lamp and further test the lining up of the elements by putting a sheet of paper between the subsequent elements. The illuminated area of the paper should be round and uniform. You may dim the ambient lights for better visibility.
- B. The objective together with the camera should function as a microscope. Specifications of an objective are normally listed on its body. For details on objective operation see the page at Edmunds Optics in References. It is common for objectives to be optimized so that the image they yield is viewed by the end of the microscope tube, through an eyepiece that acts there as a simple magnifier. There are two standards in the optical industry for tube length, of 160 and 170mm. In our case, the image will be projected onto a camera sensor rather than viewed through an eyepiece. **Q3:** Where is the object placed in relation to the focal point of the objective, when using a microscope? Log into the computer and activate the program for looking through the camera.
- C. The slide for viewing under the microscope will be mounted in a holder that can be moved in 3 dimensions: up-down, left-right and towards and away from the objective. Prepare the slide by putting a drop of solution with microscopic beads onto the center of the slide and covering the drop with a coverslip. Place the slide in the holder in front of the objective with the coverslip towards the objective. Bring the slide close to the objective and put a drop of immersion oil between the objective and slide. **Q4:** Why is the immersion oil used in microscopes?
- D. Turn the lamp on to illuminate the slide from the back. Move the camera slightly around and the slide holder back and forth to see beads in the solution. With movement of the holder, the location that is observed to be in focus moves within and around the slide. The observed beads are likely to be drifting across the screen. **Q5:** What is the likely cause of

their motion? Gravity? Buoyant force? Other factors? Explain. Move the slide holder to observe beads crossing the volume of the water, beads moving along the water-glass interface and the water-air interface. The beads at a water-glass interface are going to be seen all simultaneously in focus and some might be stuck in position.

- E. Next turn to the remainder of the setup. Place the laser at its intended location in the setup and repeat the steps of aligning the beam with a grid line at the elevation of the iris and collimating the beam.
- F. Next concentrate on the first mirror that needs to change the direction of the laser beam by  $90^\circ$ . Place the mirror so that the reflection occurs at the intersection of the grid lines, at  $45^\circ$  to the mirror. The mirror has two adjustment knobs, a lower one that rotates the normal to the mirror about a vertical axis and an upper knob that moves the normal to the mirror about a horizontal axis. Make adjustments to ensure that the laser beam points in a horizontal direction along the grid line after the reflection. Use your reference iris for the purpose.
- G. Place next the dichroic mirror on the optical table and adjust it in a similar manner, making the laser beam enter the objective horizontally and along a grid line, while not obstructing the image formation at the camera sensor.
- H. The goal of the lens placed on the rail is to make the laser beam first focus at the image location for the objective, slightly less than one tube length of optical path from the objective, and then in the object region before the objective. The 150mm focal length indicated in the figure is a rough guess what might work best. After you place the lens in the way of the beam, the center for the beam entering the objective is likely to shift. **Q6:** Why is this the case? You can compensate for the shift by adjusting the angles for the dichroic mirror. As the beam enters the back of the objective it should slightly overfill the aperture there – this yields some forgiveness in the setup. If you cannot arrive at that you can put the collimating lens at the laser to work. Take the lens off the rail and use the collimating lens of the laser to make the laser light focus at the rear focal point of the objective, rather than infinity. Put the lens back onto the rail and adjust the dichroic mirror.
- I. By now you can dispose of the original slide into a dedicated container, as by now most of the water has evaporated from the slide and the beads got stuck in one place or another. Put a sheet of paper right in front of the objective and observe the pattern made by the laser light. Move the 150mm lens back and forth, adjusting the mirror angle if necessary. When getting the right focus you should observe a pronounced symmetric hourglass diffraction pattern in front of the objective. If you see a red speckle pattern, it means the beam is not going straight through the objective.
- J. Prepare a new slide and place it in the slide holder. Put a drop of immersion oil between the slide and objective. Sweep the field of view down the objective by moving the camera left and right and changing slightly the camera elevation. Your goal is to locate the point of focus for the laser beam in the field of view down the objective. The focus

should be in the form of a bright spot surrounded by circular rings. **Q7:** Why is the image of the beam not a single spot? As long as you keep the position of the camera and of the other optical elements fixed, the position of the focus for the laser beam relative to the camera should be fixed.

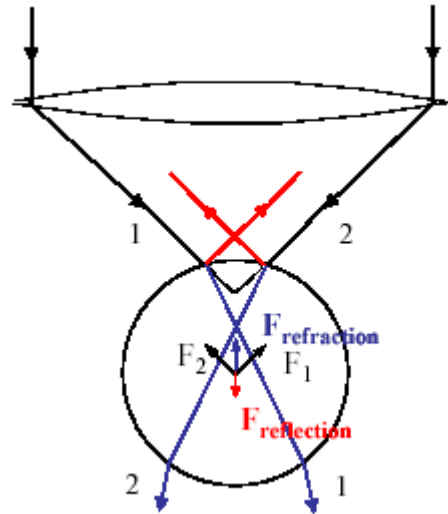
- K. Turn on the illuminating LED lamp on and move the slide holder trying to find some beads there. Try to make the laser spot location overlap with that of some of the beads. Note that some stray reflections can yield stray enhancements in intensity of laser light. The main laser spot should be very bright and clearly stand out. If you have a difficulty in seeing the beads, you can put a filter in front of the camera, suppressing the red light of the laser. See whether you can trap some beads with the laser beam - consult Appendix. In most cases you should be able to drag the beads along the water-glass interface or 2D. If your setup has good enough qualities, you should be able to drag the beads across the volume of the water or 3D. If you attempt to drag the beads too quickly, you are more at risk of losing them out of the optical trap. **Q8:** Why is that? **Q9:** Why is it harder to arrive at the 3D than 2D trap? If you run out of time or energy, you can manipulate the beads using the reference setup.
- L. The experimental setup can be augmented by putting a polarizer in the way of the laser beam, see the figure for the setup before. **Q10:** Given what you learned in the course so far, why the polarizer could be useful? Hint: Think of the Malus law. Feel free to consult the original article on this setup in References.

### References

- Edmund Optics website, [Understanding Microscopes and Objectives](https://www.edmundoptics.com/resources/application-notes/microscopy/understanding-microscopes-and-objectives/) (<https://www.edmundoptics.com/resources/application-notes/microscopy/understanding-microscopes-and-objectives/>)
- John Bechhoefer and Scott Wilson, [Faster, cheaper, safer optical tweezers for the undergraduate laboratory](https://doi.org/10.1119/1.1445403), American Journal of Physics 70, 393 (2002) (<https://doi.org/10.1119/1.1445403>)

## Appendix

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 bead 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:

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

where  $k_B$  is Boltzmann's constant and  $T$  the temperature and  $\langle x^2 \rangle$  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.