Experimental biophysics Spring 2020: Optical tweezer
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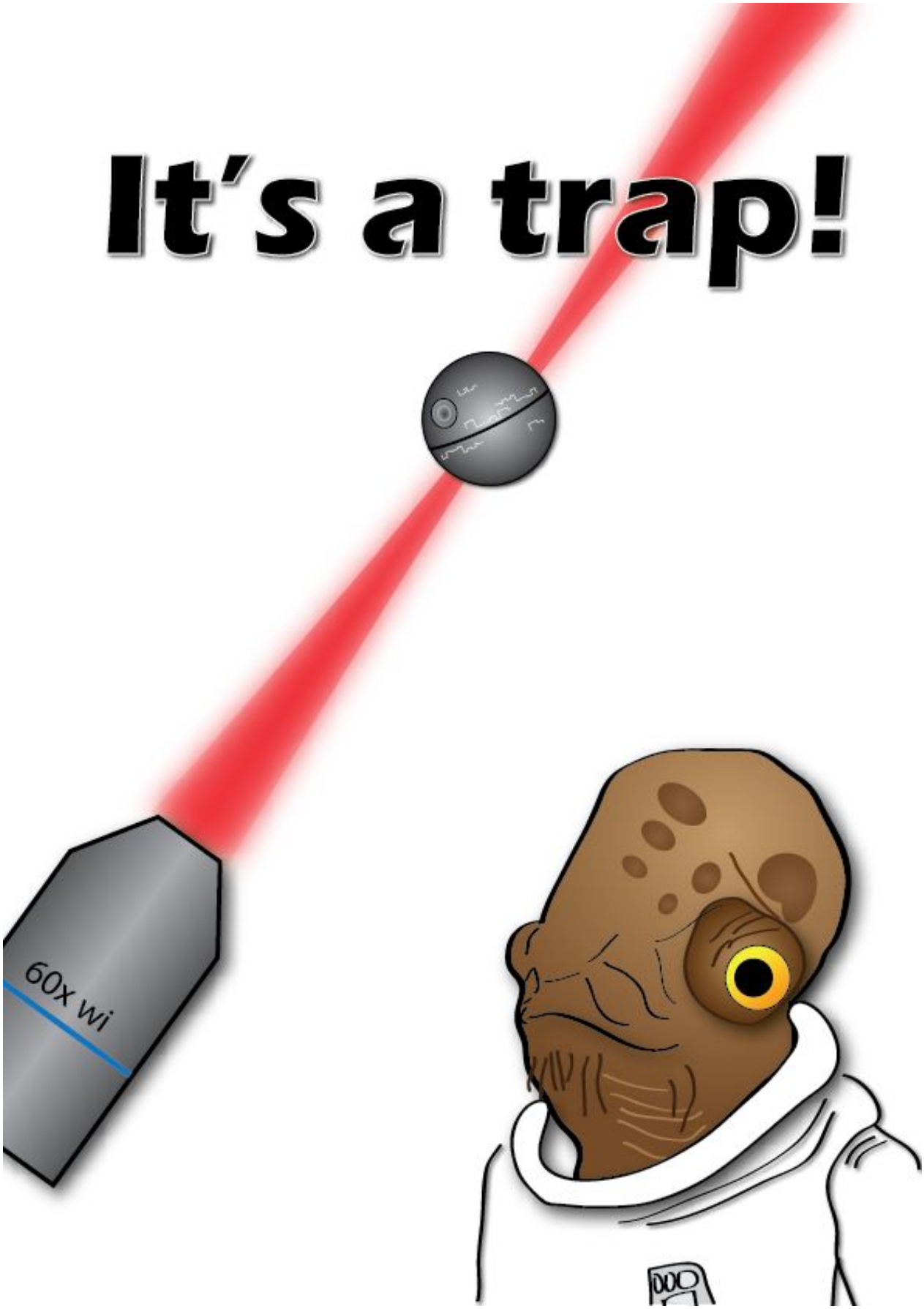

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Optical Tweezers

Introduction

In this lab exercise you will familiarize yourselves with optical tweezers, an important tool for the manipulation of single molecules and cells where light is used to capture particles. This handout will describe the physics behind the tweezers as well as our experimental setup which is very similar (not to say identical) to the FRAP-setup. You should also read the review article

> *Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy*, Keir C Neuman & Attila Nagy, Nature Methods, 2008

to learn more about applications and limitations of the tool (you don't have to read the parts about magnetic tweezers and AFM unless you want to). It is recommended that you read this handout before you read the article. On the lab, there will be a short demonstration of the tweezers and then you will be given a problem to solve.

In Figure 1 the general principle behind the tweezers can be seen.

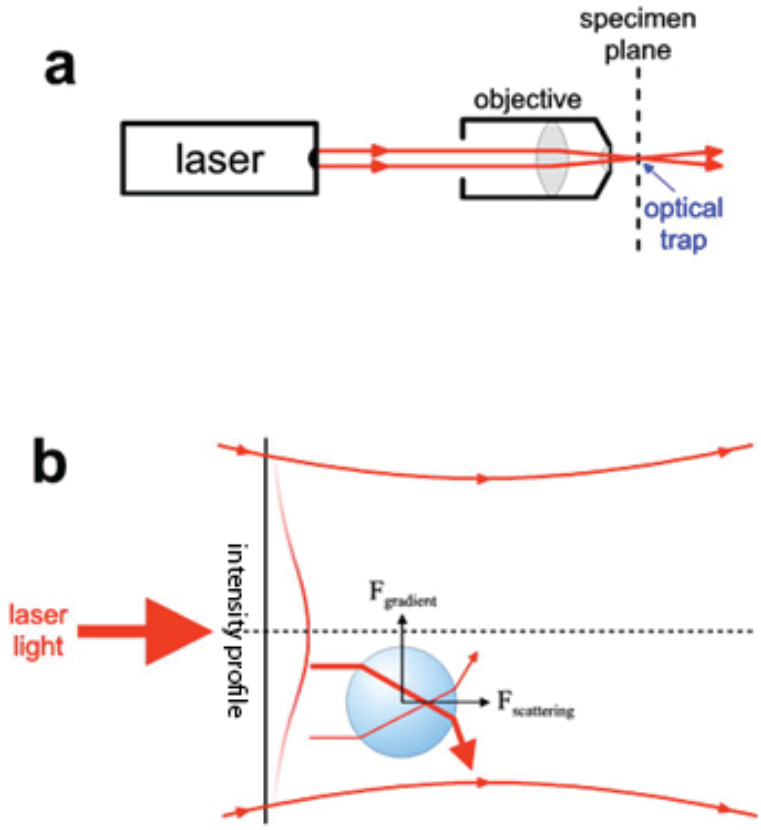

Figure 1 The general principle behind the optical tweezers.

Underlying physics

The physics allowing us to manipulate small particles, from the size of cells down the atoms, can be explained in two different ways (though only one explanation is given here). The manipulation of larger particles can be explained by basic optics.

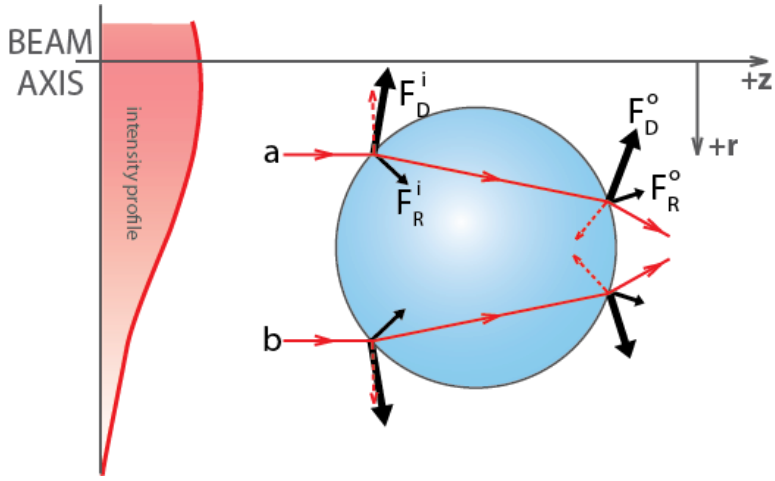

Figure 2 The force exerted by light on a particle can be explained using ray optics.

A pair of rays a, and b, hit a sphere of higher index of refraction than the surrounding medium as seen in Figure 2. Consider ray a. The ray will be both reflected and refracted at both surfaces of the sphere. Because the ray changes direction during each of these processes, due to conservation of momentum, some momentum will be imparted to the sphere. Due to reflection at each surface the sphere will experience forces F_R^i and F_R^o, and due to refraction F_D^i and F_D^o. F_R^i and F_R^o are both small compared to F_D^i and F_D^o and they also cancel each other to first approximation. F_D^i and F_D^o add to give a net force with a component in the +z direction and a component in the –r direction.

Considering ray *b* we see that the situation will be mirrored leading to a force in the +r direction and another in the +z direction. Integrating over all pairs of rays gives us the total force on the sphere. The sum of the forces in the +z direction is called the scattering force. If the laser has a linear profile then all the radial components cancel each other leading to no radial force but if the laser is focused, as in the diagram with a Gaussian profile, those rays closer to the center, ray *a* for example, will have a higher intensity and will impart a greater force than those further from the focus, for example ray *b*. See Figure 3.When all the forces are summed the result is a net force in the –r direction or in general toward the focus. This radial force is called the gradient force.

In a sufficiently well focused and aligned setup the scattering force can have a component in the –z direction when beyond the focus. This leads to true three dimensional trapping at the focus.

It is important to understand that the difference in refractive index plays an important roll with regards to the force the particles experience. If the particles have a lower index of refraction than the surrounding medium then they will experience a force away from the focus!

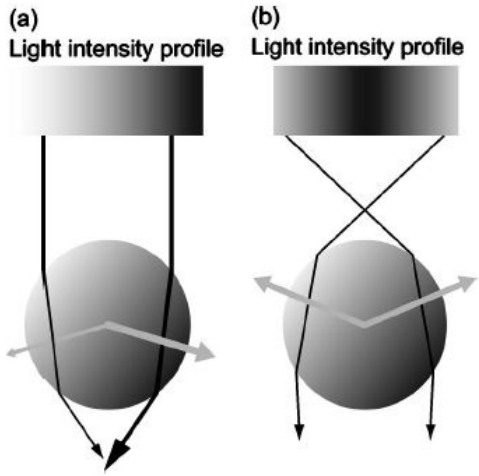

Figure 3 The forces (grey arrows) experienced by a particle in light beams with different intensity profiles.

A trapped particle experiences a spring force (of the form $F= k_{spring}x$) acting in the direction of the maximum intensity of the laser beam. By considering the equipartition theorem of thermal energy ($E = \frac{1}{2} k_B T$ in 1D), an expression for calculating the spring constant *kspring*, can be obtained since the mean potential energy of a trapped particle ($E_{pot} = \frac{1}{2} k_{spring} \langle x \rangle^2$) and the thermal energy is in equilibrium (provided that the viscous drag is negligible) and thus

$$k_{spring} = k_B T / \langle x \rangle^2$$

where $\langle x \rangle$ is the mean distance from the center of the trap.

Some applications

Below are some examples of applications involving optical tweezers.

- A stable position clamp, where the position of a particle is held constant, to allow for the measurement of small forces in the pN range, *e.g.* the study of motion of motor proteins [Brouhard].
- A force clamp, where a feedback system keeps the particle at a constant distance from the trap center, applying a constant force to the system [Block].
- Tracking of particle movement, *e.g.* in the study of molecular motors [Vrljic], and the tracking of membrane protein movement [Oddershede].
- Confinement of particles and cell sorting [Grover].
- The manipulation of cell organelles within living cells [Felgner] and insertion into cells [Buer].
- Elasticity measurements, *e.g.* of red blood cells [Smith], or strands of DNA [Cluzel].
- Local viscosity measurements by studying Brownian motion of the object trapped.
- The assembly and polymerization of structures for microfluidic devices [Teray], [Katsura], [Cronin-Golomb].
- Cooling of atoms (i.e. *laser cooling*) [Chu]
- Optically guided neuronal growth [Gunn-Moore]

Experimental setup

The experimental setup is shown in Figure 4. Notice the similarities with the FRAP setup.

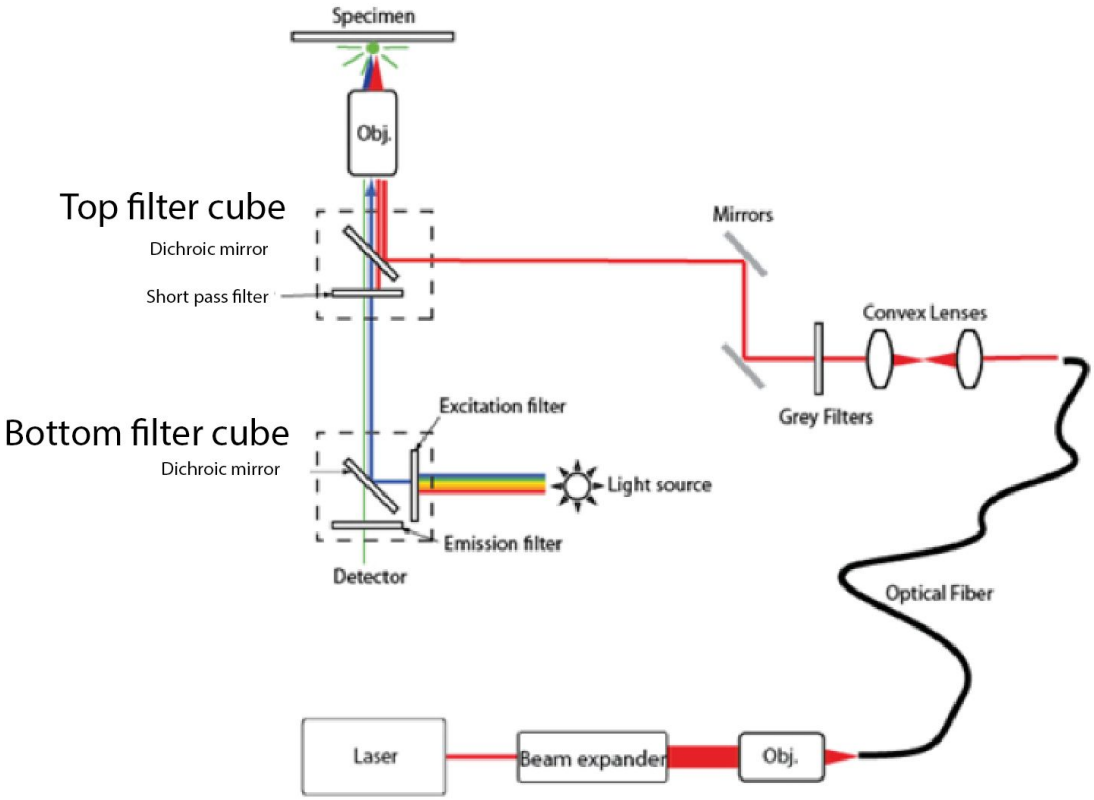

Figure 4 The experimental setup for the optical tweezers.

The different components have the following purposes

- **Laser:** generates the laser beam.
- **Beam expander:** expands the laser beam to fill the objective so the entire lens is utilized.
- **Objective:** focuses the expanded beam onto the entrance of the optical fiber. The numerical aperture of the objective is less than that of the optical fiber, meaning that the entire light cone from the objective can be collected by the optical fiber.
- **Optical fiber:** leads the light from the laser to the microscope
- **Convex lenses:** these are used to be able to adjust the focus of the laser beam in the specimen (by adjusting the distance between them).
- **Grey filters:** control the intensity of the laser.
- **Mirrors:** lead the light into the microscope.

- **Top filter cube:**
    - **Dichroic mirror:** reflects the laser to the specimen but allows excitation and emission light to pass.
    - **Short-pass filter:** the dichroic mirror does not reflect 100% of the laser so this filter is needed to ensure that no laser reaches the camera (its high intensity would damage the camera greatly)
- **Objective:** Focuses the laser. In order to create the steep intensity gradient required to move the particles an objective with a high numerical aperture is required. (We will use a 60x water immersion objective.) The objective also focuses excitation light and collects the emission light.
- **Bottom filter cube:** as in normal fluorescence microscopy.

Key questions for the lab:

Be prepared to discuss these questions at the start of the lab. More importantly, make sure you understand the answers to these questions at the end of the lab.

1. What are optical tweezers?
2. How do optical tweezers work? Is a low or high numerical aperture objective preferable?
3. Why is it important to have a well-focused laser?
4. What happens if the refractive index of either the particle or the medium is changed?
5. How can you measure the trapping force? Give a few examples!
6. In the lab, what trapping forces can we achieve?
7. Can you think of any applications for optical tweezers? Can you see any advantages over mechanical micromanipulators?
8. What alternatives to optical tweezers are there for force measurement applications?
9. Why is the optical fiber supposed to have a higher numerical aperture than the objective focusing the light beam?
10. We will be using fluorescent beads during this exercise, is there anything one has to consider when choosing beads or is the choice arbitrary?