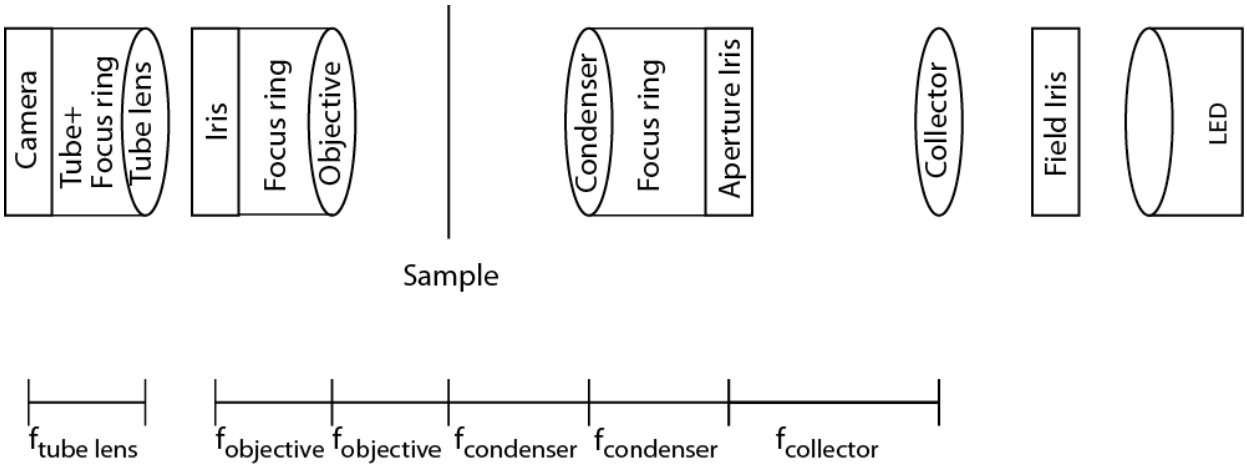
BP210: Biological Light Microscopy
Lab 1: Build your own microscope

Goal: To assemble a simple microscope using optical components, and get direct experience with where front and back focal planes are located and what information resides there.

Introduction: Modern scientific scopes are complex, but it's relatively straightforward to build a microscope with a light source, some lenses, and other basic components. Doing so is a good way to review the light path and the role of the different optics.

General setup of rail microscope (not drawn to scale!)



Protocol:

1. Your rail should come mounted with seven elements:
    a. LED with an aspherical lens so that the light source rays are focused at infinity.
    b. Field iris
    c. Collector lens (f=150mm)
    d. Condenser lens (f=50mm) attached to an aperture iris via a focus ring
    e. Sample Holder
    f. Objective lens (f=25mm) attached to a back focal plane iris via a focus ring
    g. Tube lens (f=150mm) attached to a camera via a focus ring and tube

2. Focus the elements with focus rings:
    a. Camera & tube lens- Take out all elements except the camera & tube lens. Install the camera software, turn on the camera and acquire images. Adjust the tube lens focus by rotating focus ring so something at infinity (i.e. very far away) comes into focus.
    b. Objective lens & back focal plane iris / Condenser lens & aperture iris. These should be pretty close to being focused, but it doesn't hurt to check. Detach

these elements from the rail, and place a piece of paper at the iris plane. Point the lens assembly at the overhead lights, looking through the paper, and adjust the focus ring so the lights come into focus.

3. Align the optical elements:
    a. It's easier to add and align optical elements sequentially rather than all at once. Each element has three degrees of freedom: vertical position, angle with respect to the optical axis, and lateral position along the rail.
    b. The vertical position and angle of each element should be set so that the optics are centered on the light path, and that the light path runs parallel to the rail. This means that the optics should be at the same height and should be perpendicular to the rail.
    c. The final lateral position of the optical elements should follow the diagram above. The important focal distances are shown.
        i. Objective and sample - With the camera still in place, put the objective assembly, the sample holder, and the LED on the rail with the LED close to sample. Put a sample in sample holder, and turn on the LED. Move the sample laterally so the image on camera comes into focus.
        ii. Condenser and sample - Move the LED to actual position. Put in the collector lens and condenser assembly. Move the condenser along rail so that it focuses onto the sample.
        iii. Collector and condenser - Move collector so it focuses an image of the LED onto the aperture iris plane.
        iv. Field iris- Place the iris between the collector lens and the LED. Its exact position isn't crucial.
    d. Use conjugate planes to check whether things are aligned/focused correctly. The field iris should be in focus on the sample and camera planes. The aperture iris should be in focus in the back focal plane of the objective. Use diffraction gratings to image at various conjugate planes.