

Objectives:

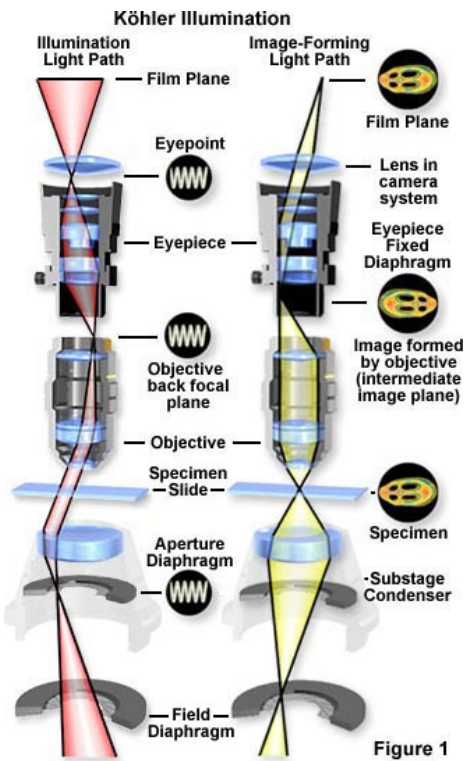
- Optical resolution
- Light path of the microscope
- Kohler illumination
- Lens aberrations

The microscope has been central to the practice of biophysics or physical biology. Beyond needing lenses of some sort to document phenomena, we use microscopes to look at stuff that is smaller than what we can resolve with our own eyes. Thus, an understanding of resolution is critical to choosing sort of microscope we would like to use. Additionally, in this lab we will get used to using the microscopes. Though the number of exercises is small, it will still take a bit of time as you learn how to set up microscopes (Micromanager), acquire data, etc.

In the required sections below, there are questions which you are expected to answer. If there are questions as to how to do certain things, or what the answer should be, please consult the TAs. We have been as specific as possible, but there may still be confusion.

Lab 1 – Getting to know your microscope (all required)

Brightfield microscopy. Undoubtedly, in the coming weeks, there will be times where the microscope seems to be not “working.” A good way to diagnose an overly dark image, or a non-uniformly illuminated sample, or trouble-shooting in general, is to think about what’s going on in the lightpath. The diagram on the left displays the brightfield illumination pathway of the microscope. What is constant between all the brightfield techniques we will be dealing with is the use of Koehler illumination. Koehler illumination means that the sample is illuminated uniformly, so any structure or hot spots in the lamp filament will be minimized; as well, in Koehler, the full numerical aperture of the condenser lens is utilized, which boosts the resolution of the microscope (see below).



1. Identify the brightfield pathway of one of the microscopes with your TA.
2. Align the microscope in Koehler Illumination.
3. Become familiar with Micromanager, which runs most of the microscopes in the lab.

4. *Edge-based resolution test:* Get one of the targets that has small bars on it. These edges with sub-wavelength sharpness. You will estimate how sharp the edges are using a 10-90% criterion in your homework. Since the edges are sub-diffraction, you won't be able to see a perfect corner. Measure the degree of imperfection of the edge. How wide is it? To get the width, you will need to figure out how to a) mount the slide onto a microscope, b) how to take an image of the slide, c) locate the image on the hard disk, d) import the image into Matlab, e) figure out how many pixels corresponds to the edge, and

f) how to convert pixels to a meaningful dimension, such as “micron” or “nanometer”. How does this value match what you expect from the response of the microscope? In your website, post an image of your resolution test. List all the parameters necessary for your measurement (objective NA, magnification, pixel size, etc.). Use $\lambda = 550 \text{ nm}$. Post also a line scan of the edge that you used. Your precision must have an error bar. All of this information and a helpful walk-through guide are in your homework assignment.

5. Become familiar with the *fluorescence light path*.

6. *Point-spread function*: We can characterize the optical response of a microscope by its “point-spread function”. Basically, the PSF tells us how the microscope images a point object – i.e., if we had a diffraction-limited object, then imaged that object through the microscope, the result would be the PSF. The PSF is a 3 dimensional object. Get some diffraction-limited fluorescent beads and take a z-stack of the beads. Reconstruct the PSF in 3D (a y-z or x-z plane will suffice). Why is there out-of-focus light? What are the limits of resolution in x, y, and z? Post your image of the PSF. Include measurements on the width of the PSF and the length (in z) of the PSF in real units. In this case, the wavelength of light is the wavelength of fluorescence. If you don’t know this, consult a TA.