1. Consider a regular wide-field microscope set up with a 60x, NA = 1.4 objective and a monochromatic digital camera with 8 um pixels, properly positioned in the primary image plane. This microscope is set up so that the field of view in your sample is 40 um x 40 um and your specimen is a fixed cell stained with blue, green and red fluorophores (displayed in true color) as shown in Figure 1.

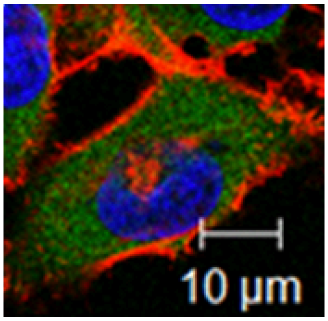


Fig. 1 Fixed cells imaged with a fluorescence microscope

a) How big will that physical image of the cells be your camera sensor?

b) What is the maximum resolution you can expect to get when imaging with this objective, if all optics are perfect and aberration-free? State what assumptions and simplifications you are making to be able to answer this.

c) Your microscope is already equipped with a dichroic --- or if you want to be perfectly correct, actually a "polychroic" --- mirror that can deliver the three laser excitation wavelengths you need to your sample. What wavelengths could these be? Select three in the table below.

d) You have only one monochromatic camera attached and you want record the image in Figure 1. Select an emission filter set that you can use to order to record the color image in Figure 1 from the filter catalogue sheet attached (not attached here, please use a website service such as Chroma).

e) Select the lasers that you need to excite the fluorophores from the laser catalogue sheet attached. What laser excites which fluorophore?

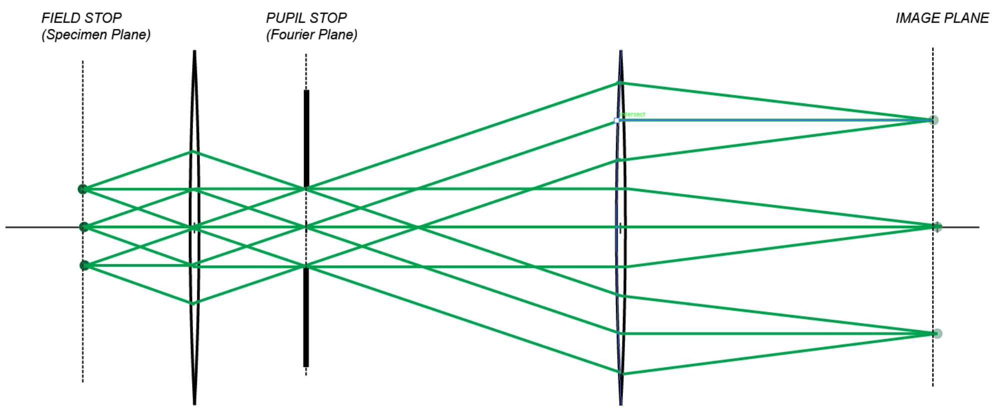
f) Are you sampling properly on the sensor??? (Remember: Nyquist criterion states that you need to sample at at least twice your resolution to avoid aliasing)

2. Figure 2 shows the light rays traveling from the specimen to the image through a fluorescence microscope. You have an iris that you can put somewhere to change the Numerical Aperture (NA) of your image to change its properties.

a) In which plane in Figure 2 would you change the size of if you wanted to change the effective NA of your image? Mark it out with an arrow.

b) If you wanted to *increase* the depth of focus in your image, would you open up or close down the iris? Would this increase or decrease the NA?

Answer: I would ______________ the iris to ________________ the NA.



3. The eye forms an image on our retina. Label the critical anatomical parts of the eye in Fig. 2 and explain their function in the image formation process.

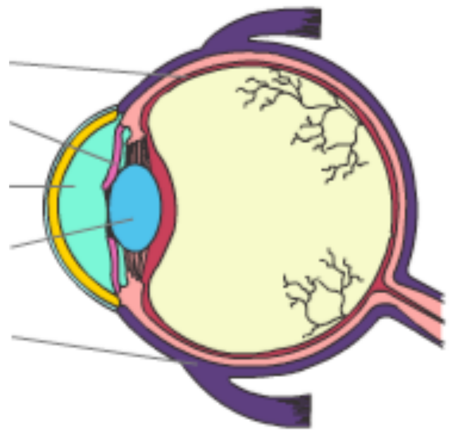


Fig. 2 Optical components of the eye. What is their function?

4. Figure 3 shows an imaging system consisting of a microscope objective and tube lens with a 4F relay lens system appended to it - just like in the laboratory session. Principal rays from three points in the specimen are traced through the system. The specimen plane is already marked out for you, and also the primary Fourier plane. Please, indicate in Figure 3 where the positions of the **primary and secondary image planes** and the **secondary Fourier** plane are located. (Hint: these three planes are marked with dotted lines.)

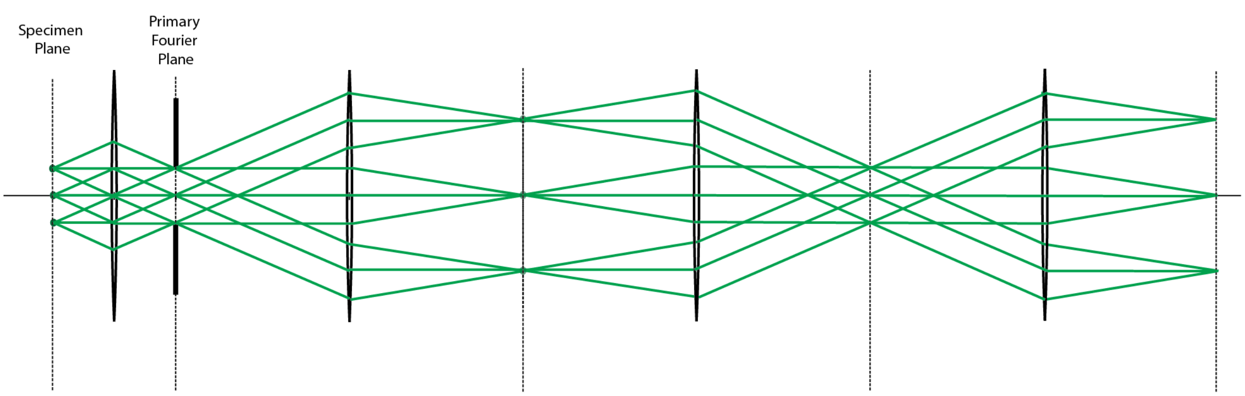


Fig. 3 Diagram of three principal rays from three points through a microscope with a relay lens system.

4. Chromatic aberration arises because light of different wavelengths λ experience different refractive index *n* when traveling through a medium, such as glass. One way to correct this aberration is by using an achromatic doublet consisting of a positive and a negative lens of different refractive indices. Look at Fig. 4, which shows how the rays travel through the achromatic doublet. Which of the two singlets has the higher refractive index?

Answer: For a given wavelength, the crown glass has _____________refractive index *n* than the flint glass.

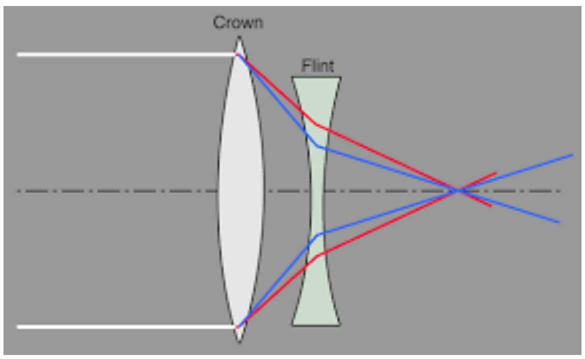


Fig. 4 This pair of singlet lenses is used to correct for chromatic (color) aberration. Look at the rays traced through the glass. Which one of the elements respectively has higher and lower refractive index?

5. Figure 5a shows how a blazed, transmission diffraction grating works for a monochromatic beam of light. It directs almost all of the diffracted light into the -1 diffractive order. Lets consider only this one order in this problem.

In Figure 1b you see an incident beam of white light --- containing all wavelengths in the visible spectrum --- hitting the blazed grating. Using the information about blazed gratings and your understanding of how diffractive optical components work in general --- i.e. how they diffract light of different wavelengths stronger or less strongly depending on wavelength --- please **draw out and color-label the other six "rainbow" color beams (red, violet, orange, indigo yellow, and blue) in Figure 1b.**

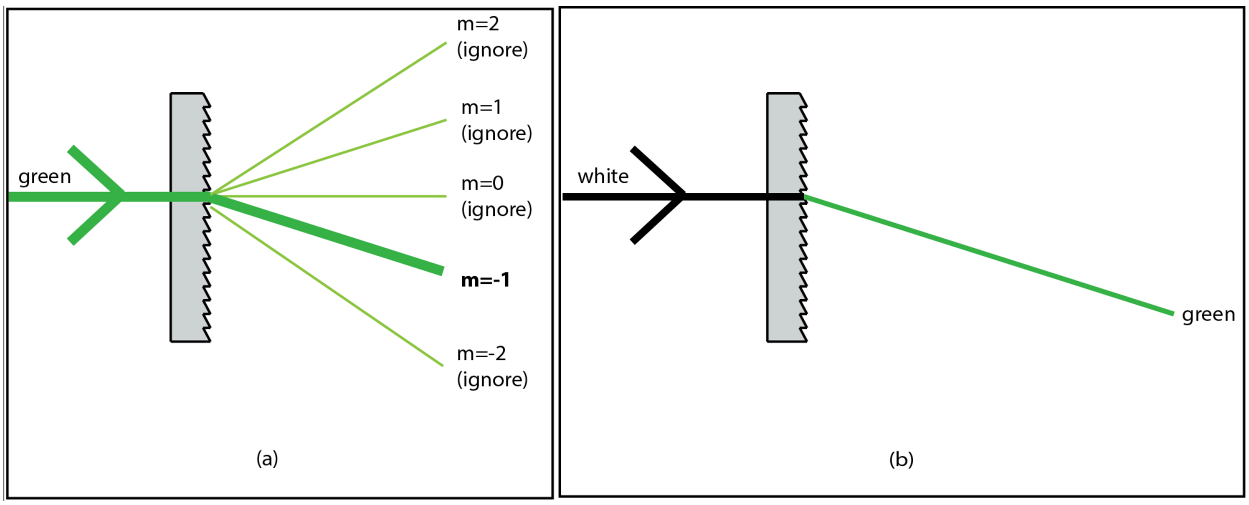


Fig. 1 Blazed grating and chromatic dispersion. **Please draw out the rainbow beams in Figure b.**