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.
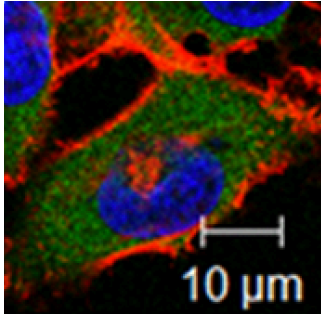
Fig. 1 Fixed cells imaged with a fluorescence microscope

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

   Answer: 60 x 40 um = 2,400 um = **2.4 mm**

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.

   Answer: Assuming green light of wavelength 550 nm (its ok to assume anything else in the visible or near IR spectrum) resolution will be d = 0.550/(2*1.4) = 0.196 um = **196 nm**

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 from the table on the next page.

Answer: We could use 405 to excite the blue fluorophore, 491 to excite the green fluorophore, and 561 to excite the red fluorophore. (To get it perfect matched, we would need to look at the excitation and emission spectrums.)

Specifications

Product	Wavelength
Cobolt Zouk™	355 nm
Cobolt Twist™	457 nm
Cobolt Calypso™	491 nm
Cobolt Fandango™	515 nm
Cobolt Samba™	532 nm
Cobolt Jive™	561 nm
Cobolt Bolero™	640 nm (coming soon)
Cobolt Flamenco™	660 nm
Cobolt Rumba™	1064 nm

If you are unsure of which Series, product or wavelength you need

Wavelength guide

355
405
445
457
473
488
491
515
532
553
561
594
633
638
647
660
785
1064
Mid-IR

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 semrock or chroma optical filters).

e) **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)**

Answer: Lets assume we are imaging green light. We calculated in Question 1b that we get 196 nm resolution. According to the Nyquist sampling criterion, we need to sample at twice this resolution. That means sampling at 98 nm in the sample and if we use a 60x objective this corresponds to 60 x 98 = 5,880 nm = 5.9 um in the sample. This means we should have pixels of that size. Our pixels are bigger, so we are undersampling. We could get a small problem with aliasing. Its not really perfect, so depending on the application we could choose to use a higher magnification objective to improve the sampling.

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.

Answer: Put arrow iris pupil Stop (Fourier Plane)

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 __close down_ the iris to __decrease____ the NA.

FIELD STOP
(Specimen Plane)

PUPIL STOP
(Fourier Plane)

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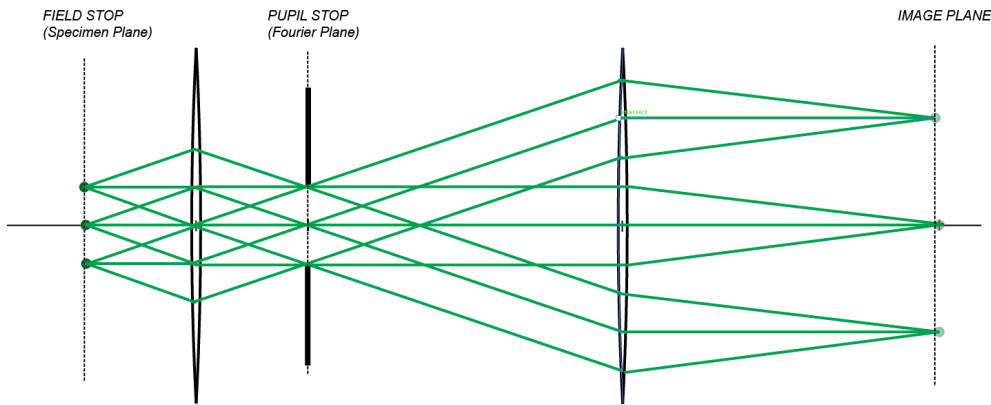
3. The eye forms an image on our retina. Label the critical anatomical parts of the eye in Fig. 2 and explain their function in the image formation process.

Answer: The cornea and the lens refract light to focus it onto the retina which records the image information. The pupil (the "aperture" in the iris) adjusts its size depending on how much (visible) light is hitting the retina.
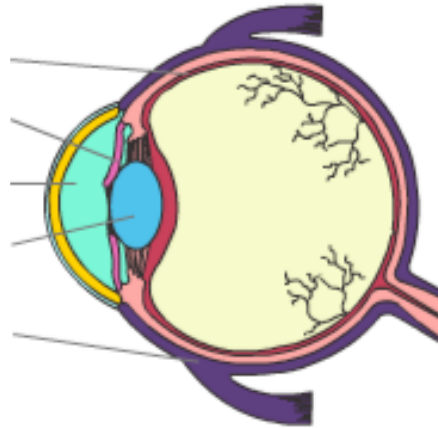

Fig. 2 Optical components of the eye. What is their function?

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.)
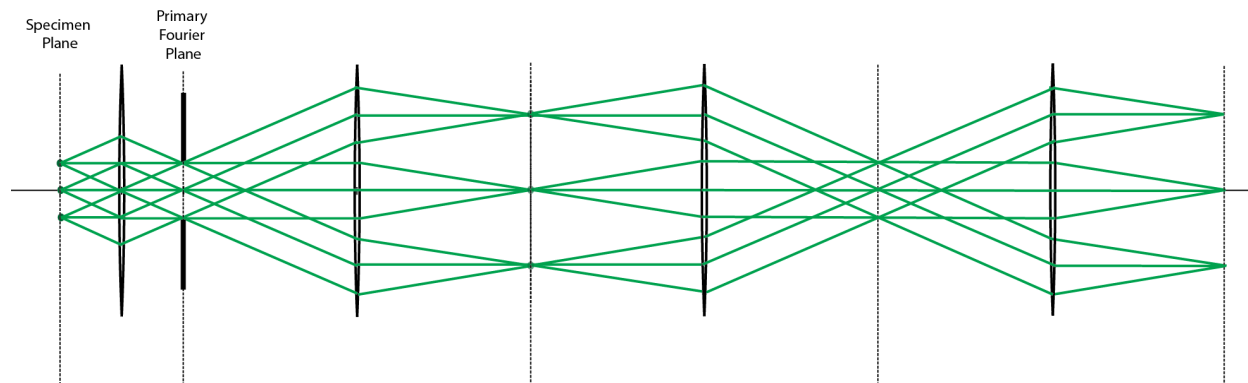

Fig. 3 Diagram of three principal rays from three points through a microscope with a relay lens system.

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.**
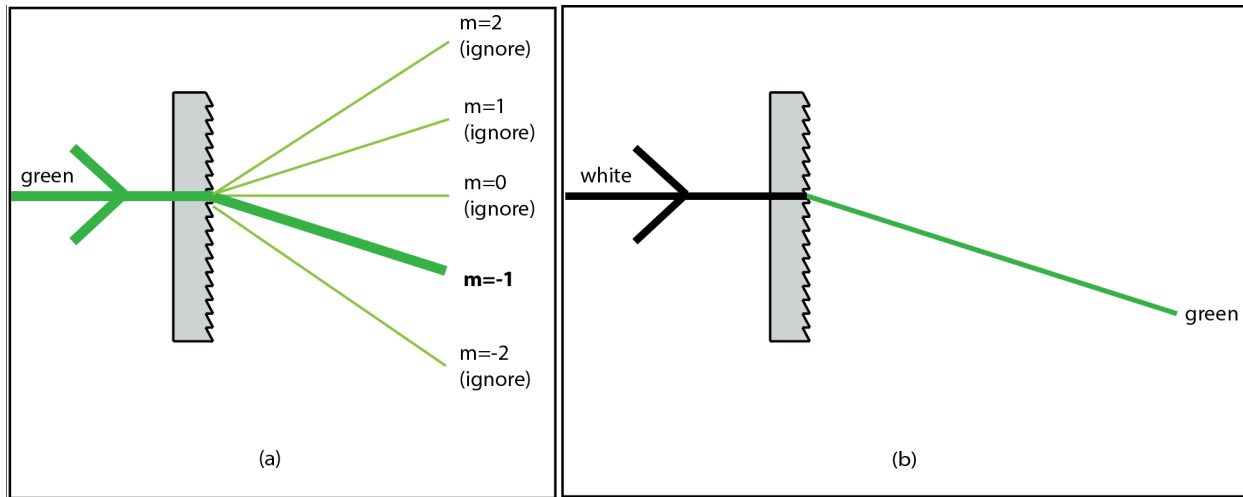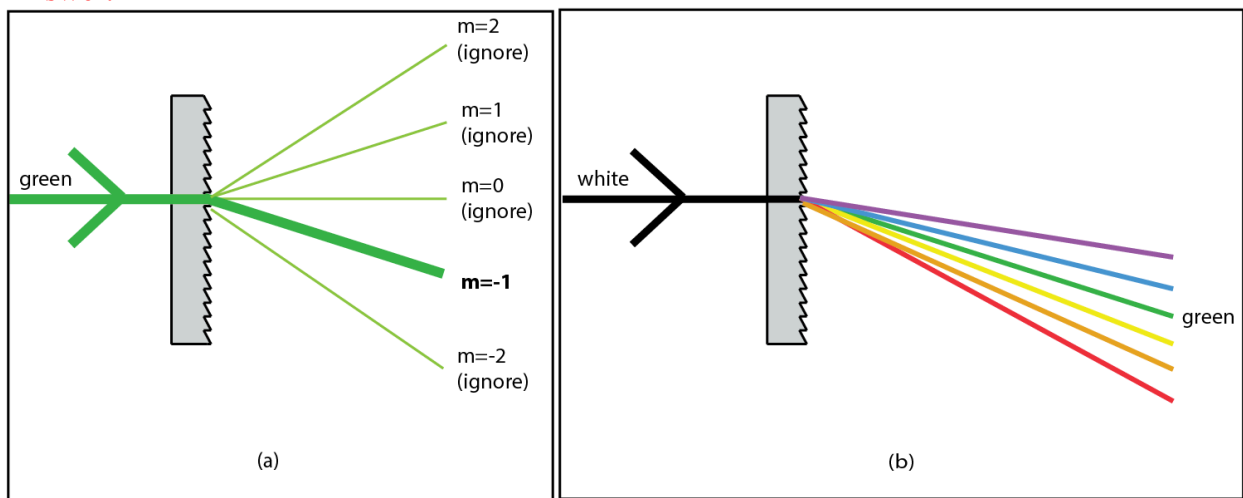

Answer:

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