Paul Herzmark
University of California Berkeley
pherzmark@gmail.com
http://tinyurl.com/PaulHerzmark-microscopy

Microscope questions

How can you determine the focal length of a lens?

What are the two advantages of a high NA lens?

What planes are conjugate with the sample plane in a properly adjusted compound microscope?

What planes are conjugate with the condenser aperture.

Why does spherical aberration decrease the sensitivity of a microscope? How can you fix it / avoid it?

How do you separate the excitation light from the fluorescent light in a fluorescent microscope?

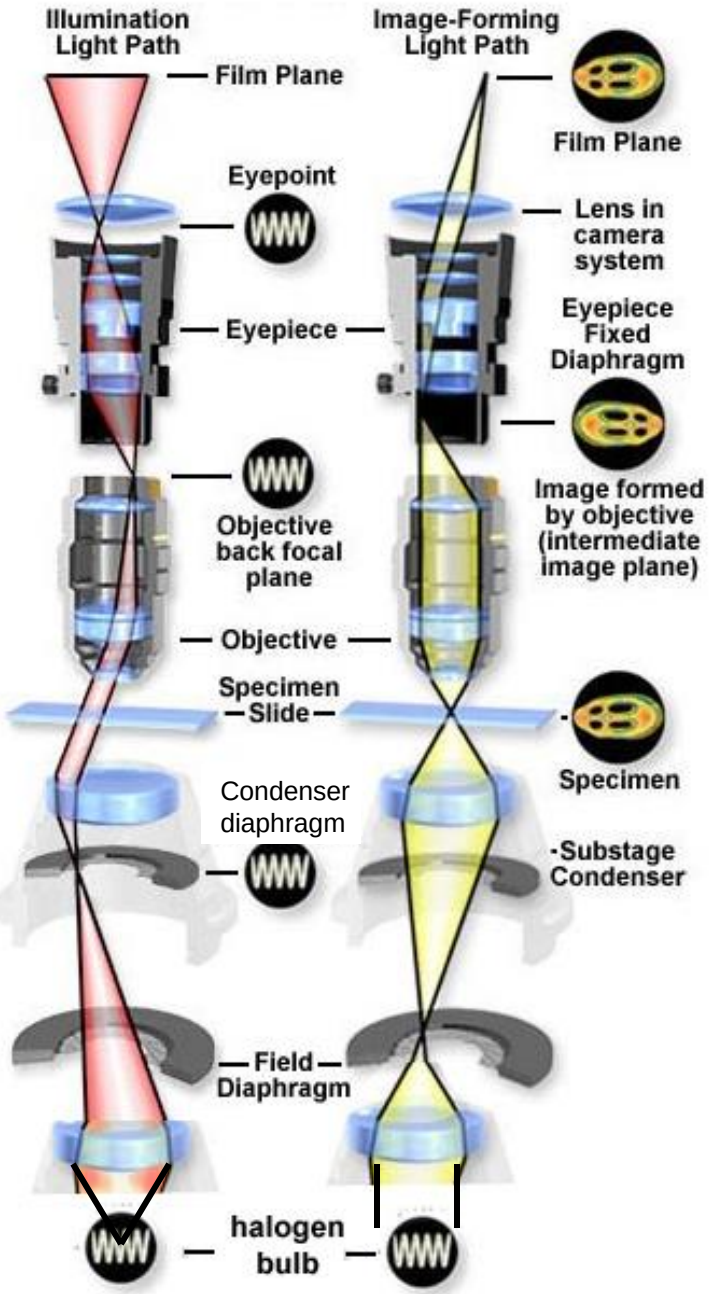
Why does a confocal microscope have to scan the specimen?

What compromises do you have to consider when you open or close the pinhole of a confocal microscope.

Your fluorescent sample is too dim. What can you do?

Name five things you can do when your cells are bleaching. Now list ten more!

Köhler Illumination



Koehler illumination / Bright field

1. Open the field diaphragm all the way. Close the condenser diaphragm almost completely.
2. Chose your objective lens and oil it if necessary.
3. Focus on your slide. (Nose oil makes a good target!) Focus away from the slide to avoid crashing into it.
4. Now don’t touch the objective focus anymore.
5. Close the field diaphragm all the way and use the condenser focus to make the edges of the diaphragm sharp. Center the illumination with the two screws
6. Open the field diaphragm until it just fills the area that you want to see.
7. Adjust the condenser diaphragm until you get the best compromise of resolution (more open) and contrast (more closed).

Phase contrast

1. Make sure you are using a phase contrast objective lens.
2. First set up Koehler Illumination.
3. Open the condenser diaphragm all the way.
4. Set the condenser position to match the objective phase number (1,2,3)

DIC Nomarski

1. Make sure you are using a DIC objective lens.
2. Insert the appropriate Wollaston Prism into the objective lens.
3. Set up Koehler Illumination
4. Put in the Polarizer and Analyzer
5. Turn the condenser Wollaston Prism to the correct position for the objective lens.
6. Looking through the eyepiece, rotate the polarizer until you get the view that you like.

Synonyms

Aperture diaphragm= condenser diaphragm= condenser iris diaphragm

Field diaphragm = luminous field diaphragm = field iris diaphragm