

BP210: Biological Light Microscopy

Lab 2: Kohler Alignment of a Research Microscope

Goal: Learn how the brightfield light path of a research microscope is laid out and how to properly focus and align the condenser for Kohler illumination. Look at the effect of illumination and detection NA on resolution.

Samples:

H&E stained samples

Diatoms

Protocol:

1. Identify the microscope condenser and brightfield lamp. Examine the objectives on the microscope. What are their magnifications and numerical apertures? What immersion media do they use?
2. Examine the condenser. What is its NA? Identify the field and aperture diaphragms and the condenser focus.
3. To set up Kohler illumination, first focus on an easy-to-find sample (an H&E stained pathology specimen works great).
4. Kohler illumination requires that the condenser be focused to the same focal plane as your sample. You do this by focusing the condenser so that the field diaphragm is in focus at the same time as your sample. Close down the field diaphragm and focus the condenser so that you can see it in focus, superimposed on your sample. Make sure it is as well focused as you can get it and that it is centered in the field of view.
5. Have someone mis-align the scope and then realign it yourself to figure out how to get everything back in alignment and proper Kohler illumination when starting from a badly aligned scope (as is often the case!).
6. Now look at the effect of the aperture diaphragm. How does the depth of field and contrast change with the position of the aperture diaphragm? This may be easier to see with a lower contrast specimen.
7. Diatoms make great resolution test samples, as different species have characteristic spacings between the holes in their frustule and these spacings are on the order of the resolution limit of the microscope. Put a diatom sample on the microscope. Figure out what the smallest frustule spacing you can see with a given objective. Close down the aperture diaphragm and see if you can see the decrease in resolution. Ideally, you should be able to find a diatom / objective combination where you can clearly resolve the hole spacing with the condenser diaphragm open, but not with it closed.

8. Fill a 35 mm petri dish with water and a few drops of milk or milk powder to see the light as it passes through. Put the dish on the microscope stage. Don't spill it! Focus the field diaphragm about halfway into the dish of milky water. Then look at it from the side and open and close the **field diaphragm** and the **condenser diaphragm**. You can also look at excitation light coming out of objective lenses with different numerical apertures.

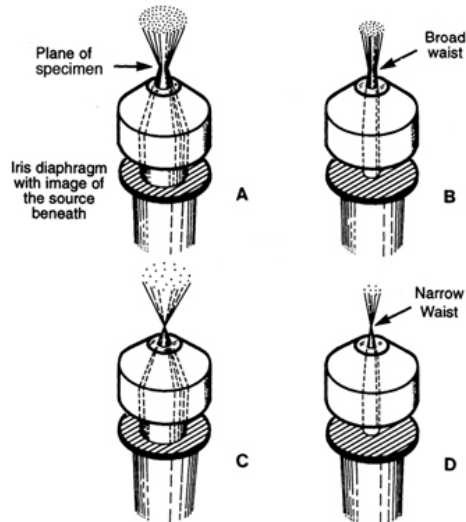
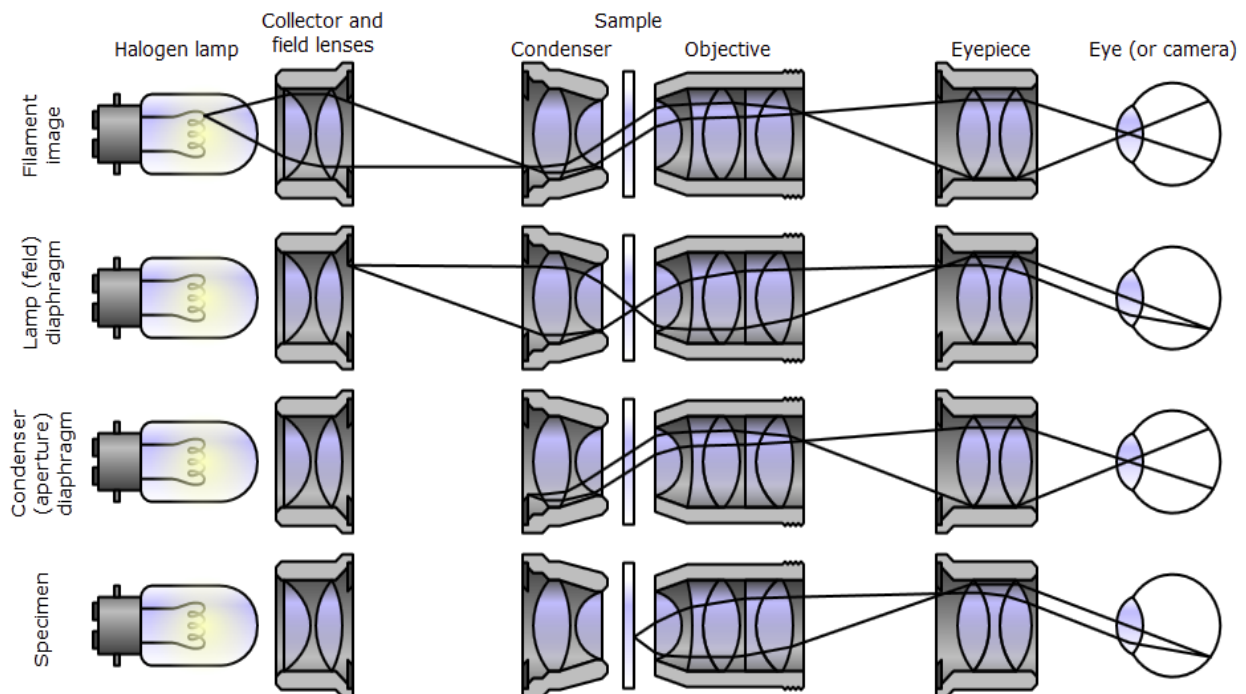


FIGURE 3-1. Effects of changing the field diaphragm and condenser iris diaphragm settings. A and C have identical condenser iris settings, as do B and D. Closing the condenser iris results in an illuminating cone of small angle (lower working NA; B and D); changing the field diaphragm results in a broad (A and B) or narrow (C and D) waist, without affecting cone angle. Effects such as these can be visualized by placing a fluorescent uranium-glass block on the condenser (or its conjugate above the ocular).

**Video Microscopy; The Fundamentals second edition
1997 Shinya Inoué and Kenneth R. Spring p.124**



References:

1. Molecular Expressions: Köhler Microscope Illumination
<http://micro.magnet.fsu.edu/primer/anatomy/kohler.html>
2. MicroscopyU: Microscope Alignment for Köhler Illumination
<http://www.microscopyu.com/tutorials/java/kohler/>
3. Wikipedia: Kohler Illumination http://en.wikipedia.org/wiki/K%C3%B6hler_illumination