

Basic Microscope Alignment: A Brief Tutorial

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Lamp Alignment:

To ensure even illumination, it is important to **periodically** check the alignment of the transmitted light bulb in your microscope. This is especially true after the bulb has been replaced. To align, turn the brightness knob down to a fairly low setting, then remove the frosted glass filter from the light path. On an upright microscope place a piece of lens paper over the field diaphragm to see the image of the filament. On an inverted microscope use the bertrand lens (*centering telescope*) to see the image of the filament (*this may require some focussing*). Use the adjustment knobs on the lamp to center the image of the filament. (*Note: some microscopes do not provide the knobs/screws that will allow the user to align the bulb. Consult your microscope service person at your next annual cleaning to ensure that the bulb is properly aligned. Some microscopes are designed to use pre-aligned bulbs.*)

Adjusting the Eyepieces:

Binocular microscopes can be adjusted to better “fit” the user. This quick check should be done **every time** a user sits down at a microscope.

- The distance between the eyepieces (*the interpupillary distance*) can be adjusted so that a user can see the image with both eyes without difficulty. To adjust, try gently pushing together or pulling apart the eyepieces to decrease/increase the distance between them (*occasionally there is a “thumbwheel” to make this adjustment*).
- One of the two eyepieces will have an adjustment to compensate for eyes of different “strengths” (*eyeglasses prescription*). The setting is typically ± 3 diopters. To adjust, start out by carefully focussing the image in the non-adjustable eyepiece. Now turn the adjustable eyepiece until the image is in focus for the other eye (*it may be necessary to start out at the 0 setting, there is often a dot or a ring to designate this “neutral” setting*). If you wear your glasses to use the microscope, the diopter setting should be at 0 since your vision is already corrected.

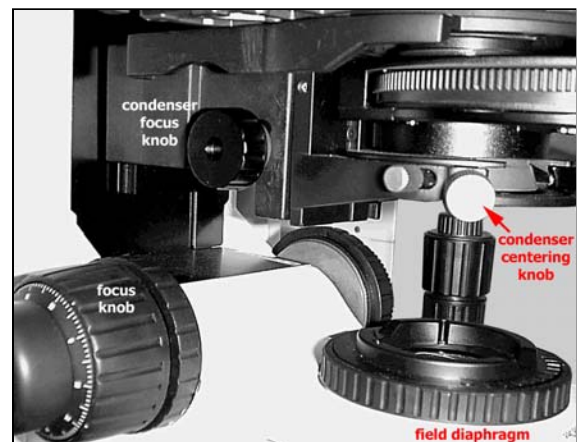


Note: Users that prefer to wear their glasses when working with a microscope may want to investigate purchasing eyepieces that have a slightly higher focal point (*if they don't already have such eyepieces on their microscope*). So-called “hi-eye” eyepieces are designed for eyeglass wearers (*make sure that you don't mix regular & hi-eye eyepieces on the same microscope*). Rubber shields for the microscope eyepieces also help to prevent scratches on eyeglass lenses (*contact your microscope vendor for more information*).

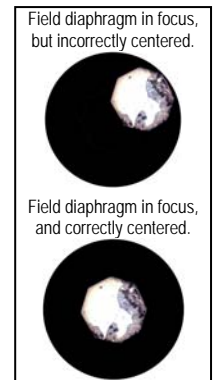
Alignment for Koeller Illumination:

Originally developed in 1893 by Professor August Koeller, this technique aligns the light path of a microscope to ensure the highest quality images. (*Note: some less expensive microscopes do not have an adjustable condenser lens and therefore cannot be aligned in this manner*). This alignment should be done **every time** a user sits down at a microscope.

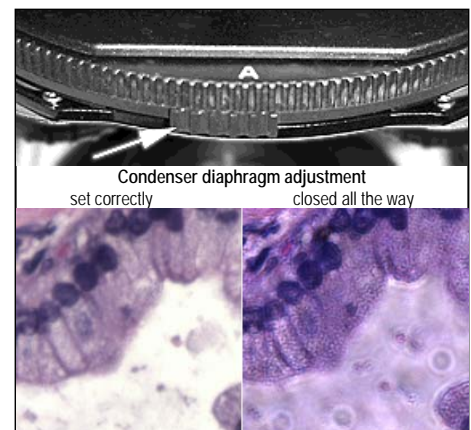
- 1) Adjust the intensity of your light source to a comfortable setting.
- 2) Select a low magnification objective lens (10X) and bring your sample into focus. The safest method for focussing a microscope is to carefully move the specimen close to the lens using the coarse focus knob, watching its approach “by eye,” and then adjust the focus by moving the specimen away from the lens.



- 3) If your microscope has a multi-function condenser (*brightfield, phase contrast, DIC/Nomarski*), make sure you are on the brightfield setting.
- 4) Close down the field diaphragm. This will either be in the base of an upright microscope, or up top near the lamp on an inverted microscope.
- 5) Using the condenser focus knob, adjust the image of the field diaphragm until the edges of the diaphragm can be seen.
- 6) Use the condenser centering screws to move the image of the field diaphragm into the center of the viewing area visible in the microscope.
- 7) Open up the field diaphragm so that the edges of the diaphragm are just outside the viewing area visible in the microscope. Opening the field diaphragm any farther results in loss of contrast.
- 8) Adjust the condenser diaphragm by closing it down until the specimen just starts to get dark and no further. While this “by eye” adjustment is usually satisfactory, a more accurate assessment can be done by removing an eyepiece and checking to see that the condenser diaphragm covers about 75% of the field.



Note, opening the condenser diaphragm too wide increases resolution, however, this also decreases contrast and the image tends to “wash out”. Closing the condenser diaphragm too much decreases resolution and increases contrast to the point that diffraction artifacts start to appear in the image (light halos next to edges, scratches and dirt in the light path, etc).



- 9) If you change objective lenses, you should repeat steps 3-7.

Note, if you are using lower magnification lenses (1X-5X) in combination with a condenser lens that requires you to “flip” a small lens out of the way, you cannot perform an alignment for Koehler illumination. In this case, open the condenser diaphragm all the way, and adjust the illumination with the field diaphragm.

Resources:

Molecular Expressions (*sponsored by the University of Florida, along with Leica, Nikon, Olympus & Zeiss*) is the premier light microscopy site on the WWW. They have an excellent description of transmitted light Koehler illumination that includes several interactive JAVA tutorials. See their tutorial at: <http://micro.magnet.fsu.edu/primer/anatomy/transkohler.html>

Optimizing Light Microscopy for Biological and Clinical Laboratories (May 1997), by Barbara Foster, American Society for Clinical Laboratory Science, Kendall/Hunt Publishing Company; ISBN: 0787235385. This book can be ordered at: <http://www.mme-microscopy.com/Book.htm>

About the author:

Mr. Cromey is the manager of the Cellular Imaging Core, a service that provides training & technical expertise to SWEHSC investigators interested in using microscopy and scientific imaging in their research. The SWEHSC is funded by the NIEHS, grant # ES06694. The Cellular Imaging Core is also host to *Microscopy & Imaging Resources on the WWW*, located at: <http://swehsc.pharmacy.arizona.edu/exppath/>

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