

OLYMPUS

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ILLUMINATION

Koehler Illumination



All too frequently, sophisticated and well-equipped microscopes fail to yield quality images because of incorrect use of the light source. Illumination of a specimen should be bright, glare-free and evenly dispersed in the field of view. Koehler illumination provides bright, even illumination and is the method of choice for the majority of modern microscopes.

What is Koehler?

Koehler Illumination is a process that provides optimum contrast and resolution by focusing and centering the light path and spreading the light evenly over the field of view. To allow a microscope to be set up for Koehler, it must have two adjustable iris diaphragms: the aperture diaphragm at the substage condenser and the field diaphragm nearer to the lamp. The aperture iris diaphragm controls the angular aperture of the cone of light from the condenser, while the field iris diaphragm controls the area of the circle of light illuminating the specimen.

Requirements for Koehler

- The substage condenser must be capable of being focused up and down.
- The substage condenser must be fitted with an aperture iris diaphragm that can be opened and closed by a lever or knob.
- The lamp must be fitted with a condensing lens, a collector and a field iris diaphragm that can be opened and closed.
- Also desirable is that the lamp filaments or bulb can be centred or pre-centred.

SETTING UP KOEHLER

(A) Focusing the condenser:

1. First place a stained specimen on the stage and focus using a 10x objective.
2. Now locate the field iris diaphragm control, located near the light outlet in the base of the microscope. Close this diaphragm right down while looking down the microscope - you will see a dark circle encroaching on the image.
3. Locate the focus control on the condenser, which is usually a knob on either or both sides of the condenser. By rotating the knob while observing the specimen you will be able to focus the condenser so that the edge of the dark circle (the blades of the iris) appear sharp. Do not move the main focus controls during this procedure.

(C) Adjusting the aperture iris:

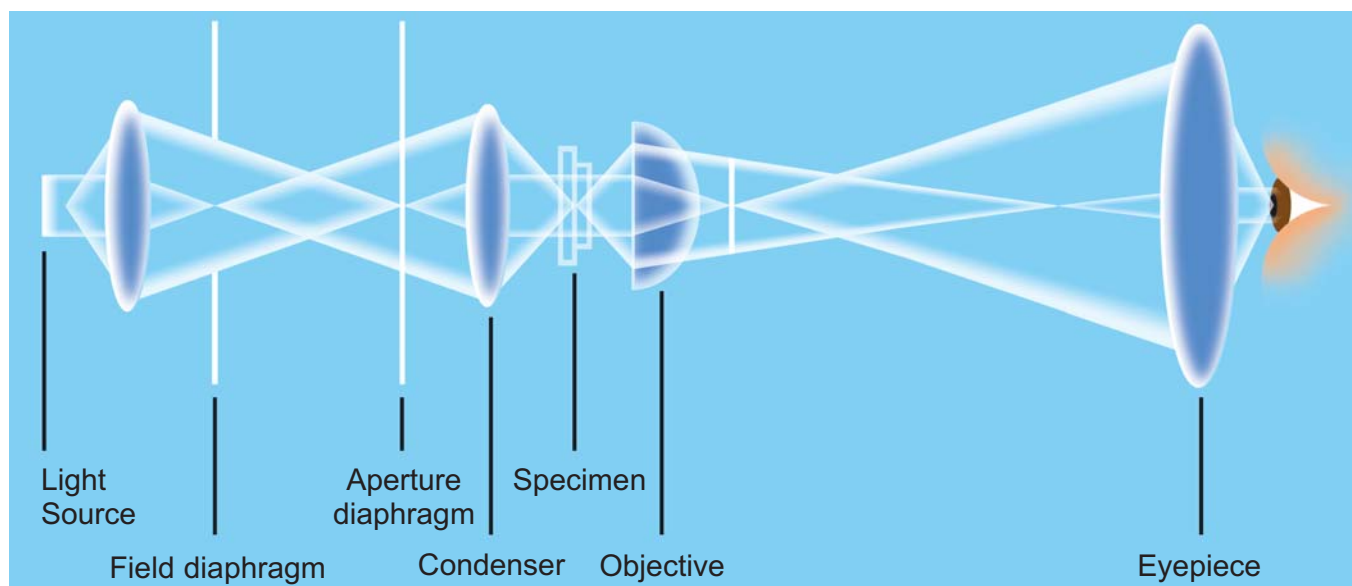
This important step is often neglected, leading to either sub-optimal resolution and/or poor contrast.

1. First locate the control for the aperture iris, which may be a ring around the condenser. If necessary, remove the condenser to locate the ring and check its action.
2. With the condenser in place, focused and centred, remove an eyepiece and look down the tube while turning the iris control. You will see a dark circle encroaching on the image at the bottom of the tube (the back focal plane).
3. This process can be made easier by slipping a centering telescope (used for setting up phase contrast) into the tube in place of the eyepiece. For most specimens, the iris should

be closed down so that it occupies the outer 20% or so of the field. This increases the contrast, making observation easier. Although some specimens may need variation on the 20%, beware of closing the iris too much as resolution will be drastically reduced.

A more accurate way of adjusting the aperture iris is to note the numerical aperture (or NA) on the objective, then set the NA on the condenser to 20% less. For example, with a 40x objective with an aperture of 0.65, set the graduation on the condenser to 20% less, approx. 0.5.

4. It can be seen that unlike the field iris setting, the aperture iris should be reset for each objective to match the NA of the condenser to 80% of the NA of the objective.



(B) Centring the condenser:

Find the controls for centring the condenser. These are generally two thin knobs projecting at angles from the front of the condenser carrier.

While observing the specimen, rotate either or both controls to bring the bright circle to the centre of the field of view. This process is made easier in the final stages by opening the diaphragm almost to the edge of the field of view so that the image of the edges of the blades can be aligned with the edge of the field of view.

Once the condenser has been focused and centred in this way, the diaphragm can be opened so that it is just outside the field of view. The condenser will remain centred when different objectives are selected, but the field iris diaphragm will have to be adjusted to just outside the field of view at different magnifications.

Many people regularly use a microscope that has not been properly 'Koehlered'. However, there is little point in having an expensive optical system if it is not producing the quality images of which it is capable - this is the equivalent of running a car with the engine out of tune. Although the human eye will accept sub-optimal images, once these images are captured by camera, the results will speak for themselves (take a look at some of the photomicrographs in scientific journals and judge for yourself!).

So there is Koehler illumination. It takes much longer to read about than actually do, so a little time taken to check your microscope before each use will be well worth the effort.

For further advice on Olympus microscopes
and imaging systems visit:

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