

**KOEHLER ILLUMINATION SYSTEM FOR THE MICROSCOPE**

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**Editions**

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# 1	24/02/2009	Creation

# KOEHLER ILLUMINATION SYSTEM FOR THE MICROSCOPE

## 1. Scope

This procedure explains the standard technique used to set the microscope with the Koehler illumination system.

## 2. References

- Anonymous, 2002. How to use a microscope and take a photomicrograph, Nikon Instech Co. LTD., 39 p.
- Kapitza, H.G., 1997. Microscopy from the very beginning. Carl Zeiss Iena GmbH, 2<sup>nd</sup> revised edition, 48 p. ([available online](#))
- Wastiaux, G. 1994. La microscopie optique moderne. Technique et documentation - Lavoisier, Paris-Londres-New York (ISBN: 2-85206-943-1), 269 p.
- Other available information on Internet: [Olympus website](#) ; [Nikon website](#)

## 3. General information

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. This technique is recommended to get uniformly bright and balanced contrast in the images. It allows the use of the microscope's full potential quality and is highly recommended to get the best possible pictures in photomicrography. This technique controls resolution, contrast and depth of field by the means of illuminating exactly the field of view and the uniform illumination of the object field, and by adjusting the illuminating aperture (see "setting the diaphragm aperture") to the power of the objectives.

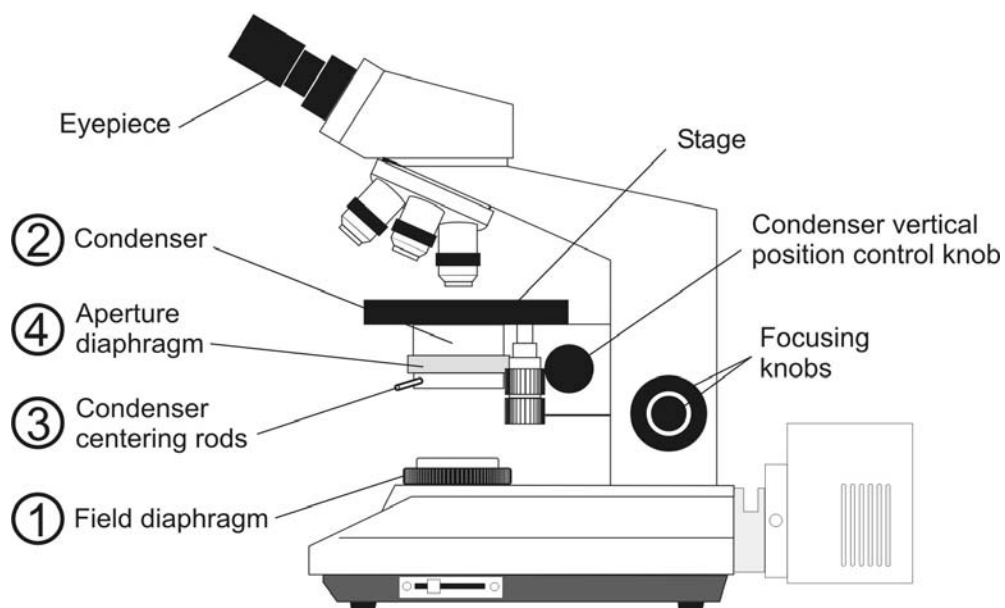
To allow a microscope to be set up for Koehler illumination, it must have two adjustable iris diaphragms: the aperture diaphragm at the substage condenser and the field diaphragm nearer to the lamp. The aperture iris diaphragm controls the angular aperture of the cone of light from the condenser, while the field iris diaphragm controls the area of the circle of light illuminating the specimen. The aperture diaphragm limits the projection of the light source (filament), which enables adjustment of brightness of the field of view. The field diaphragm is used to limit the area of illumination on the specimen and so to limit the light diffraction in the specimen tissue and avoid blurred images.

## 4. Equipment

Binocular microscope for histological and cytological examination, equipped with different normal dry objectives (10X, 20X, 40X and/or 60X) and a 100X (oil) objective with immersion oil. Be sure that the lamp filament is centred (see your microscope operating manual for more information).

## 5. Operating procedure

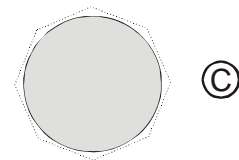
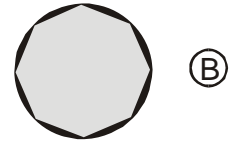
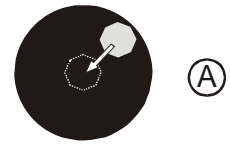
### 5.1. Description of the parts used in the process



## 5.2. Koehler illumination system

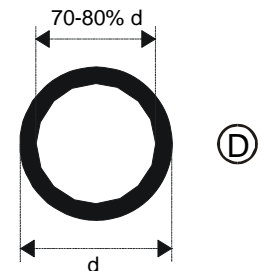
### 5.2.1. Setting the field diaphragm

- Place a stained specimen on the stage and focus using the 10X objective and the focusing knobs
- Open the aperture diaphragm ④
- Switch to the magnification objective that you want to use and focus the specimen
- Close the field diaphragm ①
- Focus the image of the field diaphragm by using the vertical position control knob of the condenser ② (see drawing A)
- Centre the image of the diaphragm using the 2 control rods ③ (see drawing A)
- Open the field diaphragm ① so that it is just inside the field of view (see drawing B)
- Centre precisely the image of the diaphragm using the control rods ③
- Open the field diaphragm ① so that it is just outside the field of view (see drawing C).



### 5.2.2. Setting the aperture diaphragm

- Remove one of the eyepieces and look into the tube : you can see the objective's back focal plane and the aperture diaphragm
- Close the aperture diaphragm so that it illuminates 70 to 80% of the observed area (see drawing D)
- Insert the eyepiece into the tube



The microscope is ready for observation

**Note: You should adjust the field diaphragm and the aperture diaphragm each time you switch objectives.**