



BASIC MICROSCOPY CONCEPTS



KOEHLER ILLUMINATION

THE PERFECT MICROSCOPE ILLUMINATION

A high quality illumination is regarded as a key issue for a perfect information transfer from specimen to target (human eye/camera chip). The illumination angle, definable by the condenser diaphragm, directly affects the resolution power of the microscope system. The final illumination setup should create a homogenous image background with a high dosage of sample details “on top”.

The human eye compensates illumination defects and pretends depth of focus. On the contrary, the microscope camera is brutally honest to a suboptimal setup and reveals any deficit in illumination. No coincidence that August Karl Johann Valentin Koehler (1866-1948) developed an optimized microscope illumination while working on photomicrography problems.

Following A. Koehler, a perfect microscope illumination has to fulfill the following requirements:

The illumination aperture (=angle) should be adaptable to the NA (=opening angle) of the objective in use.

In order to reduce stray light, the illuminated object area should be definable.

Illumination aperture and illuminated area should be adjustable independently.

Illumination for each image point has to be identical.



1 Condenser with Aperture Diaphragm



2 Field Diaphragm on microscope base

Aperture diaphragm **(1)** and Field Diaphragm **(2)** are the important variables of the microscope illumination and enable the user to follow Koehler’s requirements.

HOW TO DO A PROPER KOEHLER SETUP?

The first 4 steps have to be taken by using the field diaphragm:

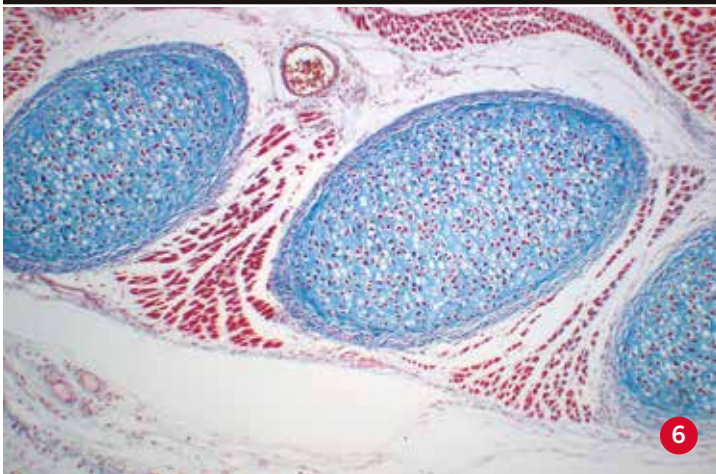
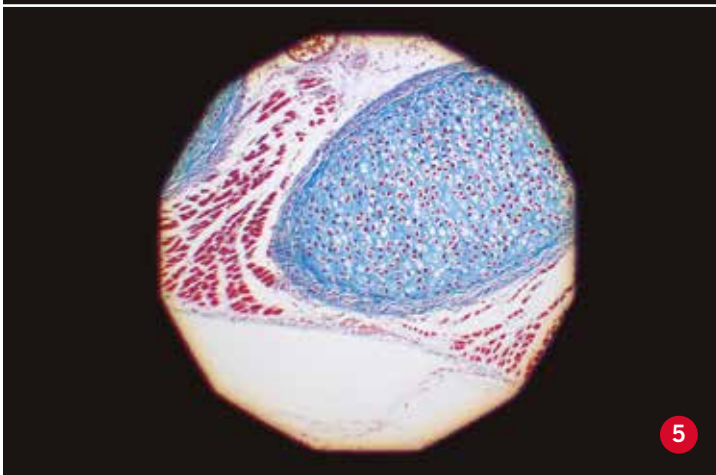
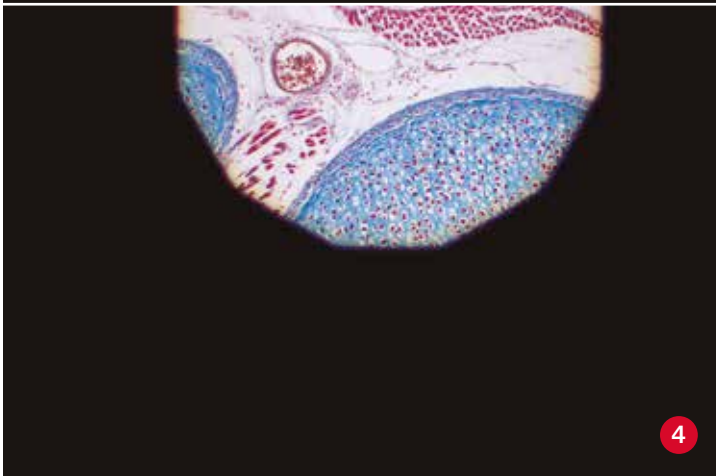
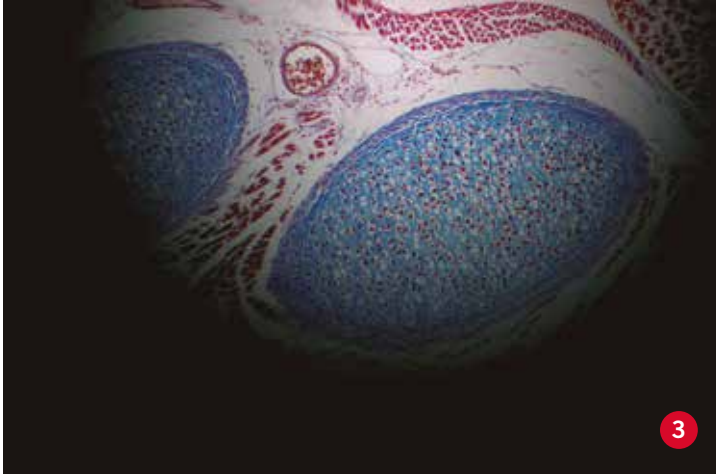
Focus on sample by focus drive.

Close Field Diaphragm (3)

Focus image of Field Diaphragm by adjusting condenser height (4)

Center image of Field Diaphragm by using condenser centering screws (5)

Open Field Diaphragm for complete field (6)



THE FINAL ADJUSTMENT

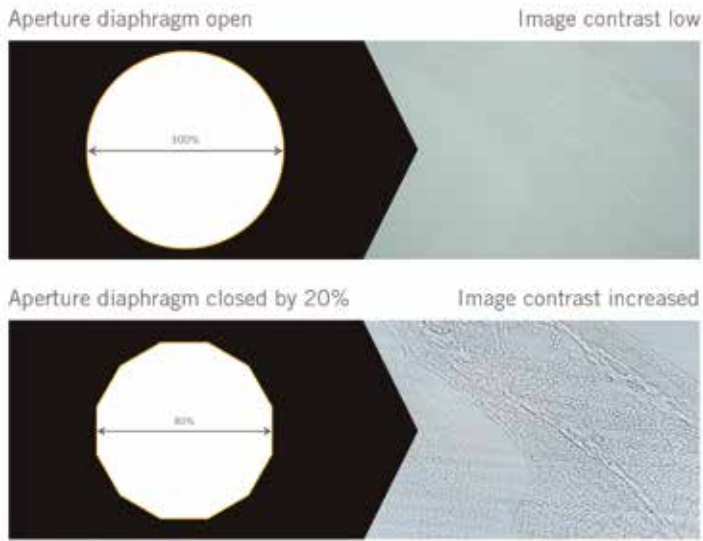
The final adjustment has to be done with the aperture diaphragm (7).

Especially unstained specimen (native smears, water samples) require a stronger closure of the aperture diaphragm to achieve contrast, while stained histological sections are less demanding.

The chart (8) may help to understand the consequences of the aperture diaphragm setup.

Using the aperture diaphragm will balance the image parameters (contrast, resolution, depth of field, brightness), always depending on the sample characteristics. Please do not use the condenser diaphragm to reduce the image brightness. The light setting in most cases is too high to observe delicate structures, be careful not to outshine them.

Follow August Koehler, and you will install best preconditions for your maximal understanding of the sample.



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APERTURE / IMAGE	Contrast	Resolution	Depth of Field	Brightness
● OPEN	Low	High	Low	High
● CLOSED	High	Low	High	Low

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You Tube Know more about how to adjust the Koehler illumination in this useful video



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