Kohler (Koehler) Illumination

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VIBS Histology YouTube
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Kohler Illumination

Köhler illumination is a method of specimen illumination used for transmitted and reflected light optical microscopy. Köhler illumination acts to generate an even illumination of the sample and ensures that an image of the illumination source is not visible in the resulting image. Wikipedia

Köhler illumination reduces eye fatigue with long microscope use.
Kohler illumination
(order is important)

1. Focus specimen
2. Close field diaphragm
3. Focus condenser
4. Center condenser
5. Open field diaphragm
Kohler illumination

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Kohler illumination

1. Focus specimen
2. Close field diaphragm
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4. Center condenser
5. Open field diaphragm
Place slide on the stage with the labelled side up.
Kohler illumination

1. Focus specimen
2. Close field diaphragm
3. Focus condenser
4. Center condenser
5. Open field diaphragm
In view but out of focus

Focus image with fine focus knob

In view and in focus

Note: It is very important for the image to be in focus during the alignment procedure; however, do not use the image focusing knobs after the image is focused in the first step.
Kohler illumination

1. Focus specimen
2. Close field diaphragm
3. Focus condenser
4. Center condenser
5. Open field diaphragm
Close opened field diaphragm

Field diaphragm is closed
Kohler illumination

1. Focus specimen
2. Close field diaphragm
3. Focus condenser
4. Center condenser
5. Open field diaphragm
Focus condenser

Sharp edges of the diaphragm indicate focus of condenser on the field diaphragm.

Viewing edge

Light coming through of opening of diaphragm

Open field diaphragm to the viewing edge to use the viewing edge as a reference to center the condensed light.

Center condenser

When diaphragm opening is concentric with the viewing edge the condenser is centered.

Open field diaphragm just outside of view
Viewing edge

Light coming through of opening of diaphragm

Center condenser
Sharp edges of the diaphragm indicate focus of condenser on the field diaphragm.

Open field diaphragm to the viewing edge to use the viewing edge as a reference to center the condensed light.

When diaphragm opening is concentric with the viewing edge the condenser is centered.

Focus condenser

Center condenser

Open field diaphragm just outside of view
Kohler illumination

1. Focus specimen
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Equine hoof (H&E – BOX 28 & trichrome – BOX 29)

CCT of the corium will stain blue and the epidermal structures will stain red.

Primary & secondary epidermal laminae

horn tubules

non-tubular or intertubular horn