

Köhler Illumination

Köhler Illumination for Brightfield

Köhler illumination is a method for evenly illuminating the sample with white light. This method is very important for brightfield imaging in widefield microscopy, and for using the T-PMT for confocal imaging. Brightfield illumination from light source focuses onto the sample via condenser, as a result extra steps are necessary to ensure the light-path is focused on the sample.

1. Bring the sample into focus
2. Close the field diaphragm (2) until you can see at least one edge
3. Adjust condenser height (3) until the edges of the diaphragm are crisp. You'll see an octagon.
4. Center the diaphragm (4) using the two centering screws
5. Open the field diaphragm (2) just until the image fills the field of view (FOV)

Link for setting up Köhler illumination

ZEISS Microscopy How-to: Set up Köhler Illumination on your ZEISS Axio Imager (Upright):

<https://www.youtube.com/watch?v=Qk8dbT-N1xw>

MicroscopyU has a great interactive on guide for Köhler illumination:

<https://www.microscopyu.com/tutorials/kohler>

