EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes

Protocols: Köhler Alignment

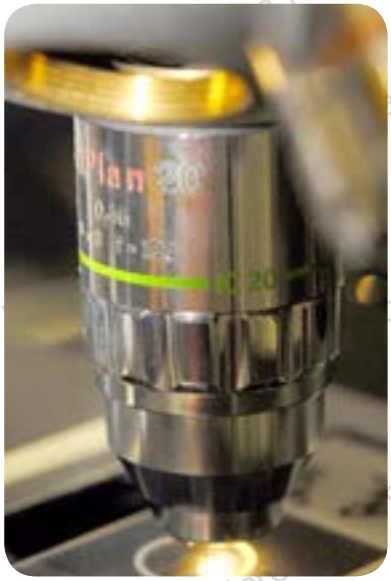
Köhler Alignment for the Optical Light Microscope (OLM)

The brain is an excellent image processor and can ignore image defects in real time but when viewing a captured image, that image stands alone and the brain then sees any defect. Performing a Köhler alignment will ensure that most common defects, such as uneven illumination, will not be present in the final image. However, the process is all too often an ignored concept in OLM and should be performed before every viewing session, especially when capturing images. **NOTE**: The most basic OLMs may not have the optical elements that are needed in order to perform the alignment.

A properly executed Köhler alignment produces an extremely even illumination of the sample and ensures that an image of the illumination source is not visible in the resulting images.

Directions

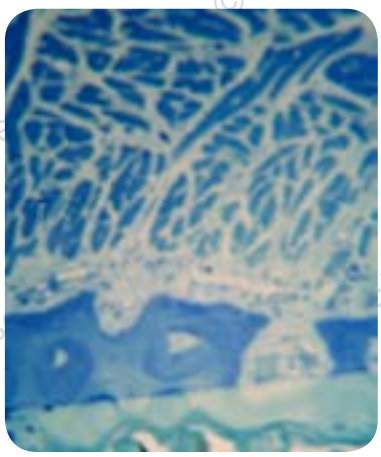
1. Select low power objective (10x) and place slide on stage.



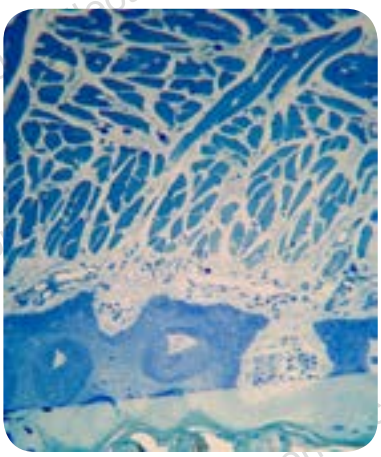
2. Set IPD (inter-pupillary distance)

3. Focus image by:
   - 3a. Bring stage up close to objective lens.
   - 3b. Looking through oculars move specimen stage DOWN until a focused image is obtained.

Out of Focus

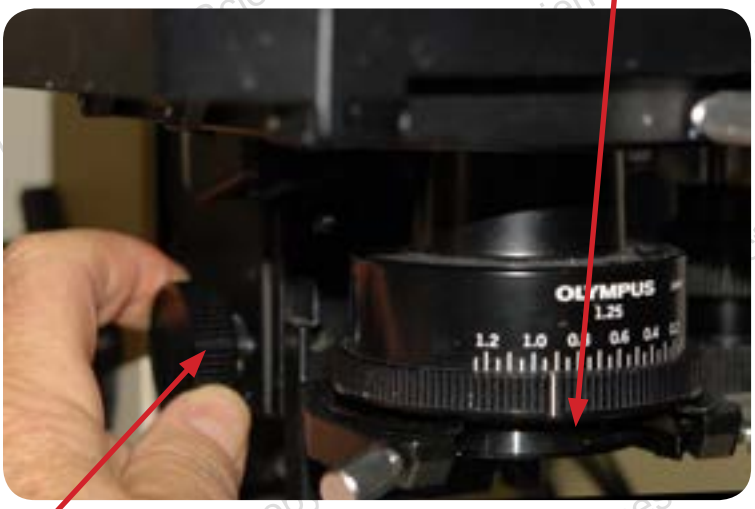


In Focus



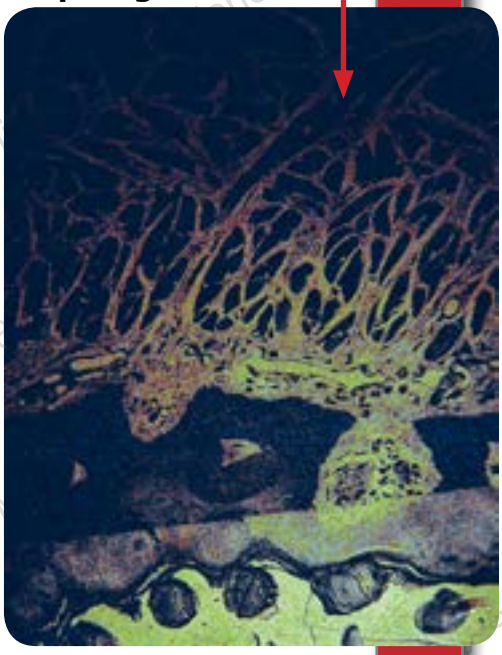
4. Adjust dioptric eyepiece for proper focus:
    4a. Close eye on the adjustable ocular.
    4b. Using focusing knob bring image into sharp focus.
    4c. Close other eye and turn adjustable ocular until image is sharply focused.

5. Open iris or condenser aperture wide open.



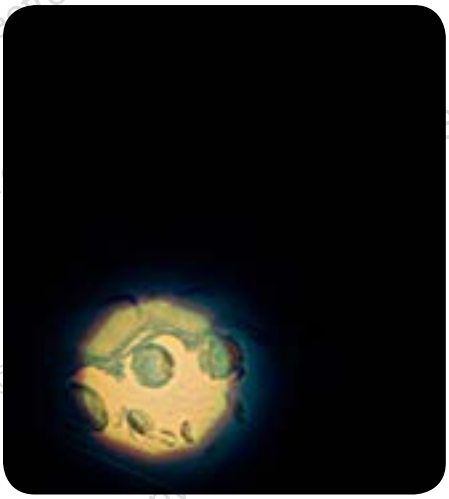
6. Close field diaphragm all the way.

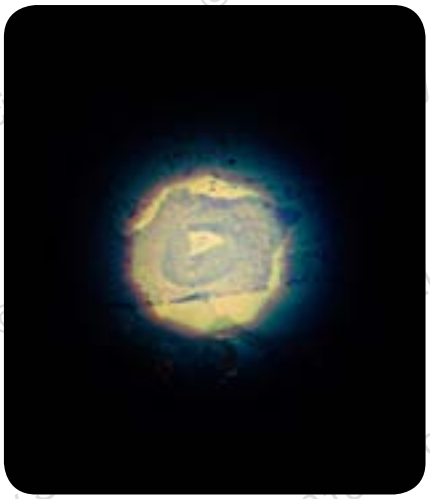
Image with Field Diaphragm Closed

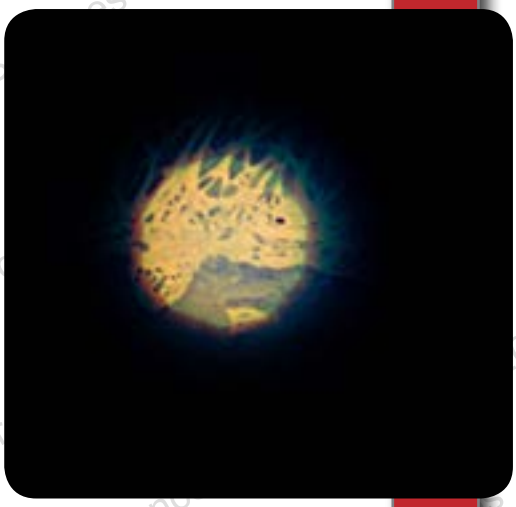


7. Adjust condenser height until the edges of the field diaphragm come into sharp focus.

8. Center illumination using the condenser centering screws. Open the field diaphragm some to be sure of position.

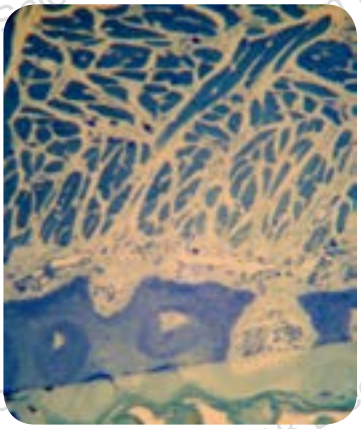




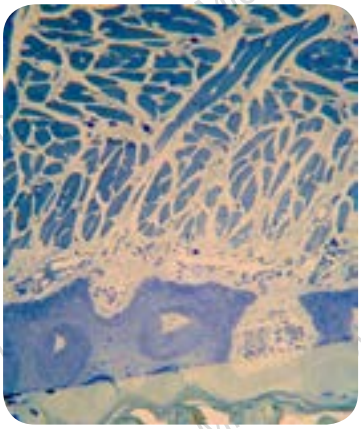


9. Open field diaphragm until it is just outside the field of view. (Image A).

A



B



10. Close the iris or condenser diaphragm down to the point where there is a marked increase in the image sharpness or contrast (Image B). **NOTE**: This should match the Numerical Aperture on the lens.

11. **NOTE:** Steps 6-9 should be done for each objective used, but always start at the lower magnifications first.

$$R \text{ (resolution)} = \frac{0.612 \times \lambda}{NA}$$

NA (numerical aperture) = n (sin α)
n= index of refraction