

## MODULE 5: KOHLER ILLUMINATION

**Objective:** This module aims to identify the importance of Kohler Illumination in modern scientific light microscopy, and how to achieve it. After this module, students should be able to consistently adjust and execute Kohler Illumination following the steps below.

### **Why do we do this?**

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 (for example a halogen lamp filament) is not visible in the resulting image. It requires additional optical elements which are more expensive and may not be present in more basic light microscopes. (from [https://en.wikipedia.org/wiki/K%C3%B6hler\\_illumination](https://en.wikipedia.org/wiki/K%C3%B6hler_illumination))

### **KOHLER ILLUMINATION**

1. Set up the microscope for brightfield imaging. Check your light path.



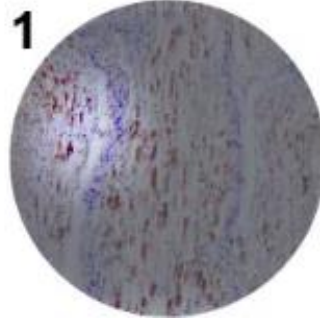
2. Adjust objective to low magnification (10X).



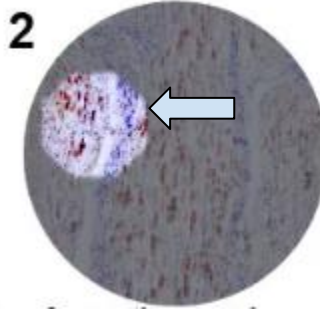
3. Focus on the specimen. **Do not** readjust your focus during the rest of the steps.



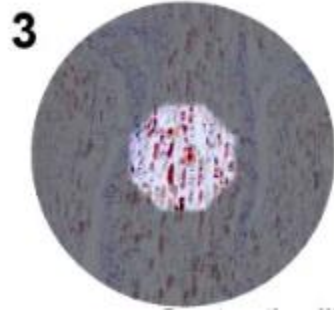
4. Close field stop all the way.



5. Focus the condenser to ensure pinhole edges are sharp.



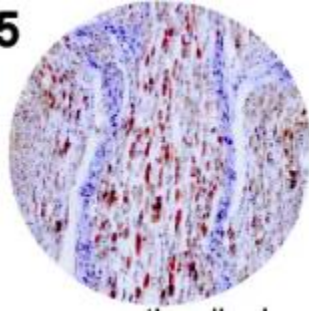
6. Place the pinhole in the center of the field of view using the adjuster knobs.



7. Open the field stop.



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Images from: <https://rsscience.com/kohler-illumination/>