

Kohler Illumination SOP

Equipment

Leica Microscope

Contacts

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Why Should I use this Technique?

Illumination of the specimen is one of the most important variables when trying to achieve a high-quality image. Kohler Illumination has been devised to microscopes optics to align and focus a beam of light. Kohler Illumination allows for even illumination of the specimen, high contrast and resolution, reduced heating of the specimen, and reduced glare. Once the process is learned, it takes a minute to complete and provides a replicable method to ensure correct alignment and a usable image.

Supplies Needed

- Sample to be viewed

Safety and Instrument Care

- Wear the proper PPE: glasses and gloves
- Look from the side when adjusting the stage to not crush the sample
- Do not clean objectives unless qualified, as glass is easily scratched by any residue

Operation User Guide

1. In your lab notebook, record relevant data about the sample, objective, and camera settings used.
2. Take the cover off the microscope
3. Mount your sample to a slide and label it.



4. Attach the slide holder to the microscope.



5. Mount the slide to the slide holder.
6. Turn the master switch on. (marked with the red arrow)
7. Turn the transmission light on. (marked with the yellow arrow)
8. If using epifluorescence, turn the epifluorescence light on. (marked with the green arrow)
9. Adjust power on the transmission light to 20 percent intensity. (marked with the blue arrow)



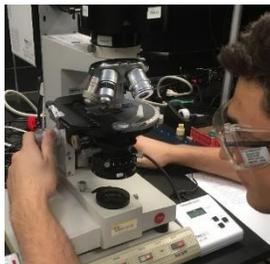
10. Start on a low objective by rotating the holder to objective 4.
11. Set polarizer to zero.



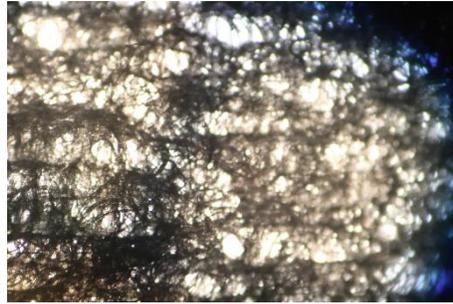
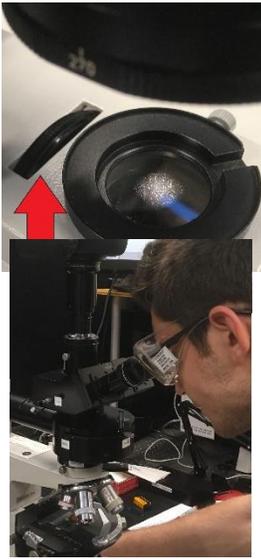
12. Set analyzer to parallel.



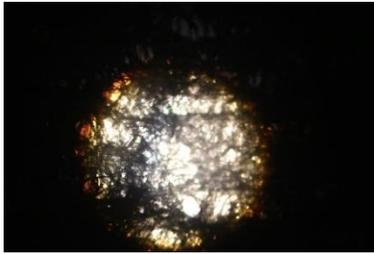
13. While looking at the sample, roll the stage up so that the sample is as close as possible to the objective.



14. While looking into the eyepiece, roll the stage down until the sample is in focus.
15. Minimize the aperture (marked with the red arrow).



16. While looking into the eyepiece, use the adjustment knobs to center the image.
17. While looking into the eyepiece, bring the substage condenser into focus by rotating it



- until the ring around the edge of the image is colorless.
18. Open the aperture until it touches the edge of the viewing field. If the previous steps have been done correctly, the circumference of the image should come into contact evenly with the edge of the viewing field at all points.



19. If you want to take a picture, pull the camera button out, turn on the camera, and take a picture with the glossy black button which is on the camera.
20. Take a scale photo.
21. Transfer your photos.
22. Turn the camera off.
23. Turn the epifluorescence off.
24. Turn the transmission light off.
25. Turn the master switch off.
26. Cover the scope.

Canon EOS Phone Image Transfer



1. Download the EOS app on your smartphone. It should be called Canon camera connect.
2. On the camera, press the menu button.
3. In the menu, scroll to the tab with a small image of a wrench with three dots.
4. Scroll down and press Wi-Fi function.
5. Press exit.
6. Press connect to smartphone.
7. Press review/change set.
8. Press change set.
9. Press ok.
10. Make sure easy connection is selected, press ok.
11. On your phone, go to the Wi-Fi settings.
12. Find EOS6D_1 in the available connections.
13. Connect using the encryption key which should be given by the camera.
14. Start the EOS app which you downloaded.
15. When prompted by the app, press on Canon EOS 6D.
16. On the camera, press ok.
17. Press ok again.
18. On the app, press "Images on Camera".
19. Press on an image, and then download it by pressing on the small down arrow which points into a box.