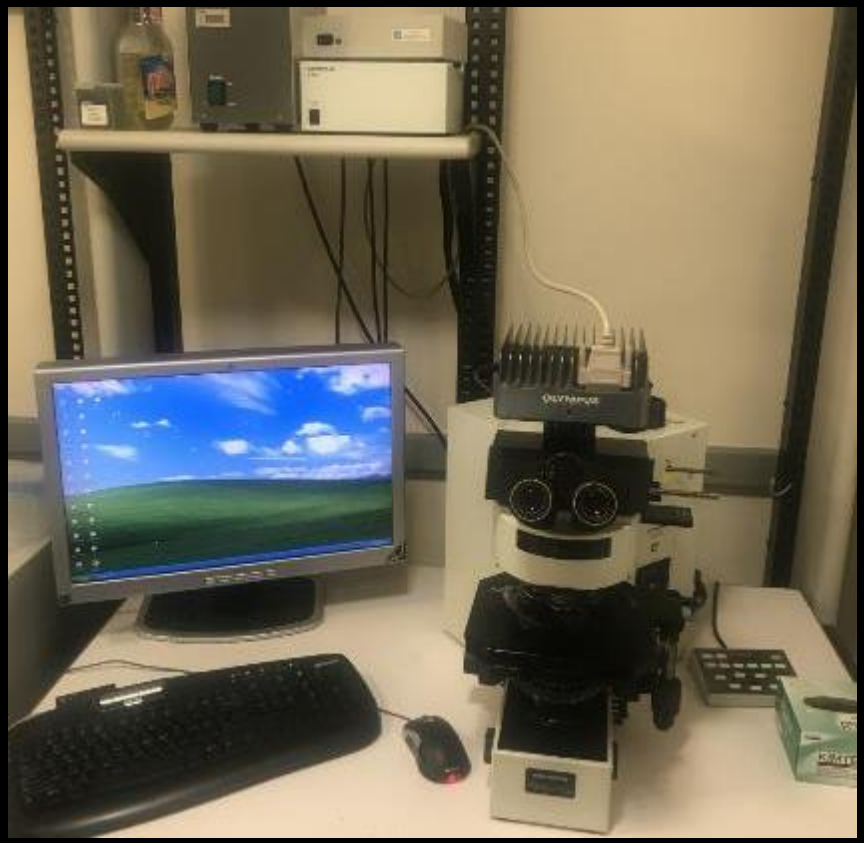
Olympus Provis: Brightfield Widefield Imaging



Brightfield

- Microscope setup
  - no Polarized light
  - with Polarized Light
- Software
- Microscope Shutdown
- Image processing

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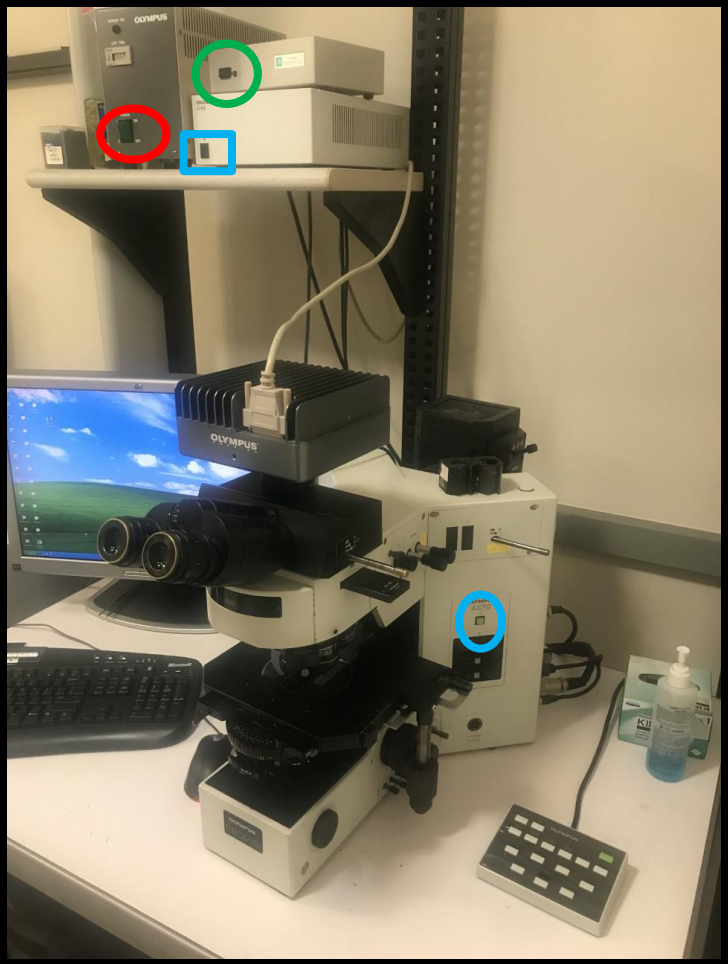
Order of Provis Microscope Startup/Shutdown

There are 4 total buttons to turn on/off the Provis microscope

1. Epifluorescent (EPI) lamp allows you to visualize a sample's fluorescence (red circle). This does not need to be on for imaging brightfield
2. Power supply for microscope camera allows you to see the signal (brightfield or fluorscence) on the screen and to take a picture (green circle)
3. Olympus microscope has two power buttons. One power button on the right-hand side on the base of the microscope (blue circle) and the white light source for Brightfield Imaging (blue square)



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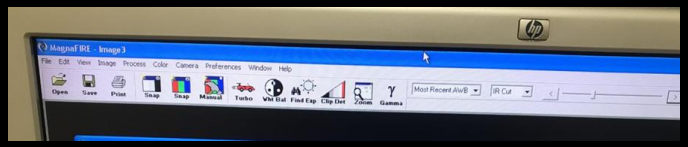
Provis Microscope Software (MagnaFIRE) Startup

After completing microscope start up (4 buttons), open MagnaFIRE Software on the computer desktop

- Light source (Brightfield or Fluorescent)
- Camera
- 2 microscope power buttons



Possible Error: MagnaFIRE software will not open

Fix: Confirm that the 4 microscope power buttons are powered on

or

Fix: Confirm there is not another window of MagnaFIRE already open

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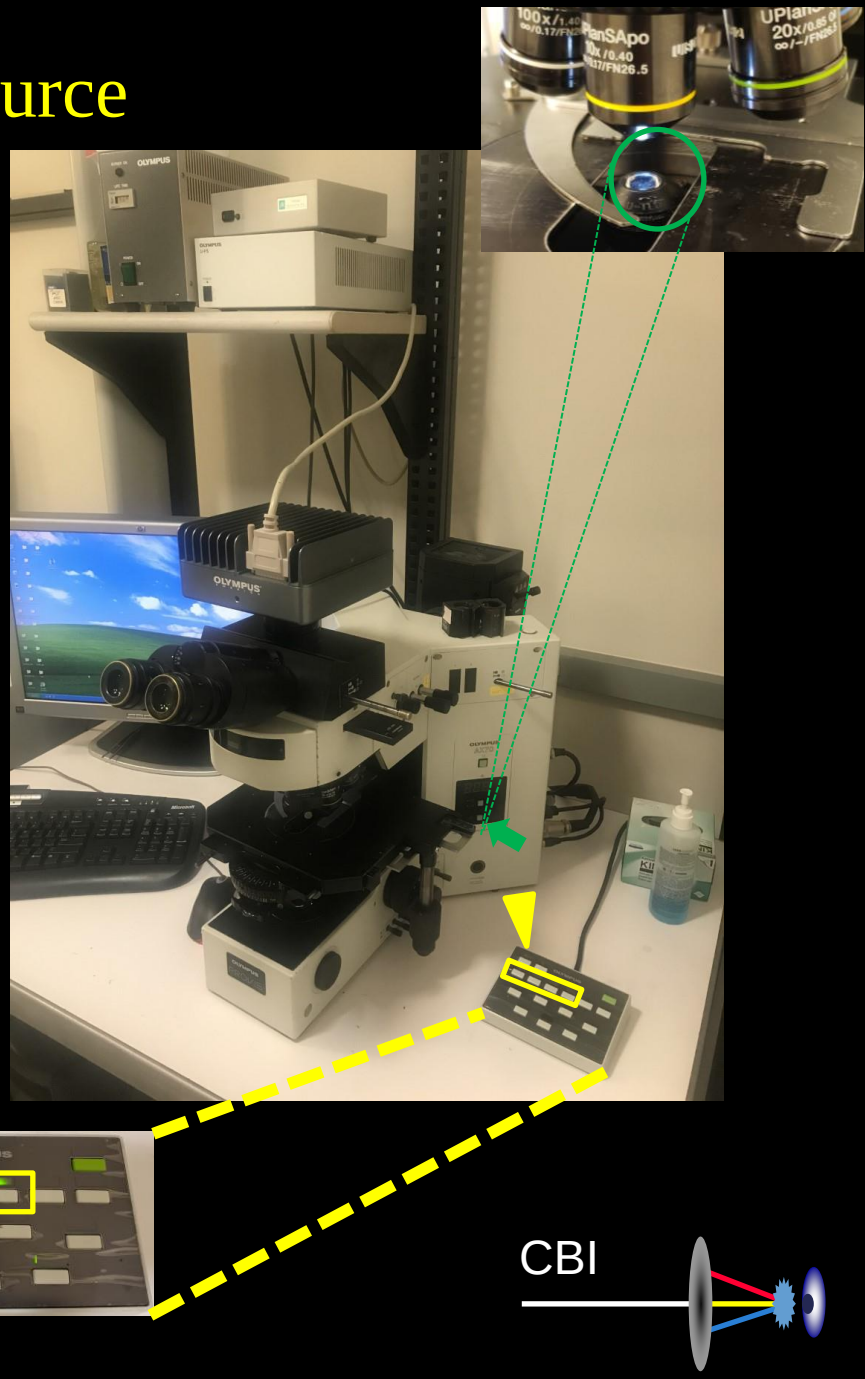
Provis Brightfield illumination source

- White light should illuminate from underneath the slide (green circle) by selecting the lower button (green arrow)
- Engage Transmitted light (yellow arrow, top left button) to illuminate field with white light, turn filter wheel to the position with no cube (yellow rectangle).
- Which button moves the filter wheel to the open position (no cube) may change but it is one of the 4 boxes within the yellow rectangle
- Carefully swing your objective of choice into place. **Only use oil if it built to be an oil objective.**



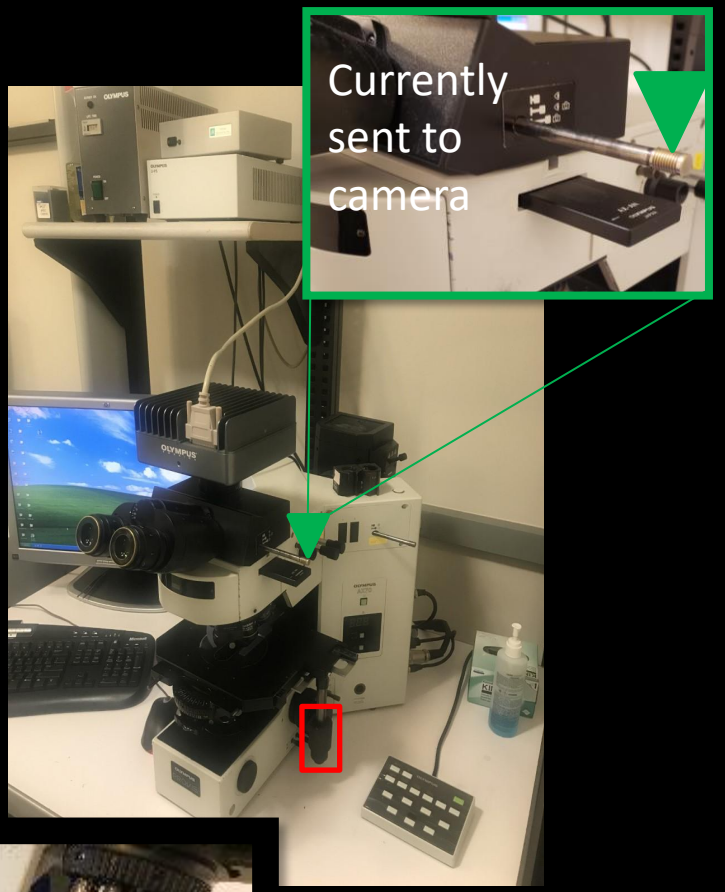
Focusing on the field of interest

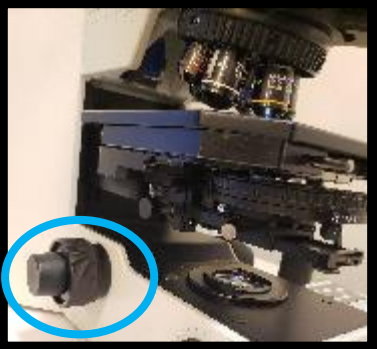
Place your slide on the stage with sample coverslip facing the objective.

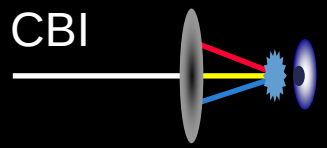
Adjust location of field of interest by moving the stage in the X and Y direction on the right side of the microscope (red box)

Select field of interest looking thru the eye port (metal bar slides toward the microscope base), once your field of interest is in focus send the image from the eyeport to the camera (green arrowhead) by sliding the metal bar to the right to view on the computer monitor

Readjust focus (blue circle) as the camera has a slightly different focal plane from when established thru the eyeport prior to when the image was sent to the camera (green arrowhead)







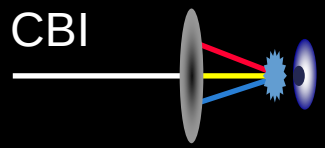
Köhler Illumination for brightfield image quality

Necessary for even field illumination, contrast and sharpness of the image

The components to be manipulated to set Köhler at each magnification are as follows:

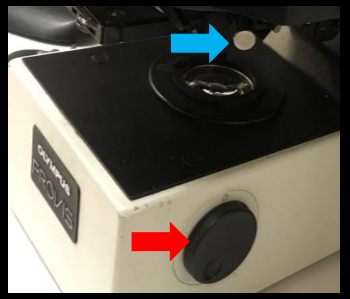
- distance of condenser from the sample
- size of field diagram affect spot of light
- location of spot of light within the sample
- inclusion of Bertrand Lens
- inclusion of Polarizing filter
- engagement of the condenser
- amount of light thru the condenser

Image by https://micro.magnet.fsu.edu/primer/anatomy/kohler.html



Location of microscope component for setting Köhler for optimal brightfield image quality

Alter amount of white light illumination by rotation of dial on right side of microscope base (red arrow)

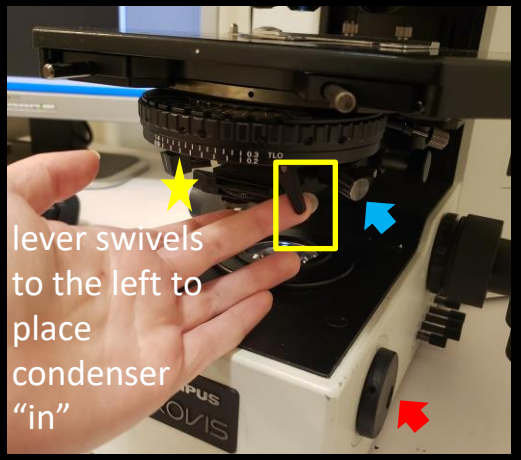


Adjust the field diaphragm size (green arrow) on the left-hand side of the microscope base

Adjust the height of the condenser (green arrowhead, black knob) on the left side under the stage adjusts condenser focus

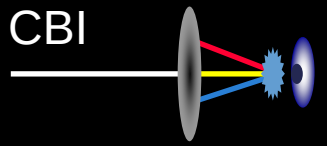
Center the octagonal spot of light within the center of the field using x- and y-translational adjustment silver screw(s) on each side of the condenser (blue arrow) under the stage

Engagement of the condenser (yellow arrowhead) by moving the lever to left (yellow box) moves the condenser to the "in" position. Only needed for (e.g., crystals)

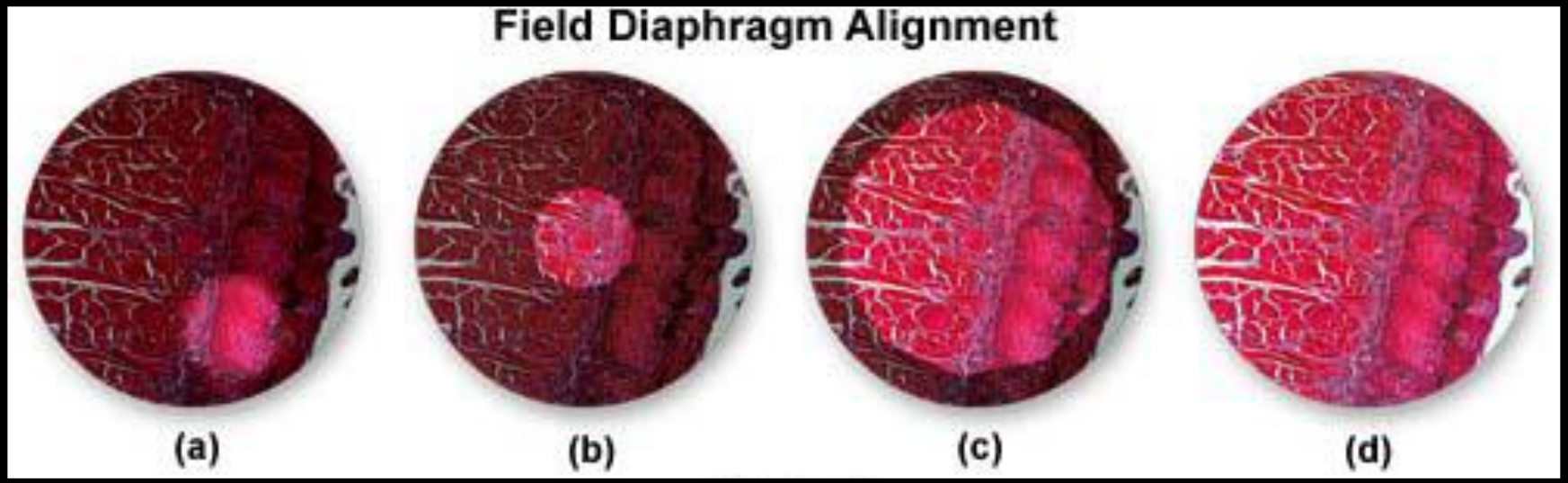


Polarizer filter (yellow star, black rectangular slider under the stage) should be pulled towards the front edge of the stage to be out of light path.

There is a black dial on the front edge of the polarizer that modulates amount of light that passes thru the polarizer and affects image contrast

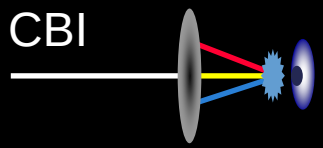


Optimizing Köhler to evenly illuminate the entire field



a. Close front aperture to reduce illumination spot size.
b. Center the illumination spot by altering condenser height, needs adjustment if the objective magnification changes.
c. Center the illumination spot of light by adjustment of the set screw(s) on the left and right side on the base of the microscope until you achieve a crisp octagonal beam of light in the center of your field of view.
d. Open the front aperture to spread the light completely over field of intrest.

Image by https://micro.magnet.fsu.edu/primer/anatomy/kohler.html

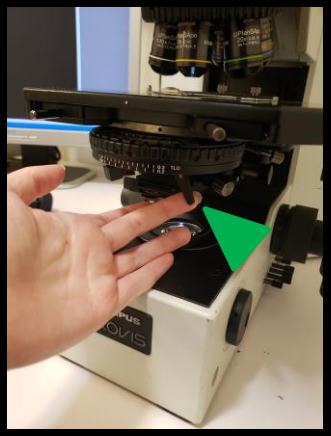


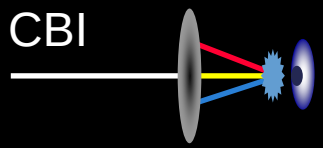
Brightfield Image Acquisition for magnification 10x or greater

Orange Box: Condenser aperture dial has settings from 0 to 8, with the dial showing a dot aligned with the 8 (orange box) allowing white light to pass thru the lens to the sample to adjust contrast

Green Arrowhead: Condenser lever swivel to the left to place “in position” (green arrow)

Red Box: Bertrand Lens to the out position, pulled to the towards the right, away from the base of the microscope



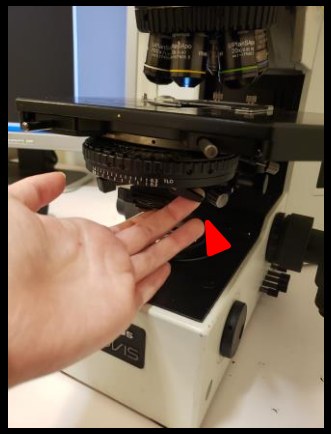


Brightfield above 10x

Condenser should be engaged by swiveling the front lever to the left to move the condenser to the "in" position

Brightfield below 10x

Condenser should be moved to the "out" of position by swiveling the front lever to the right to move the condenser to the "out" position



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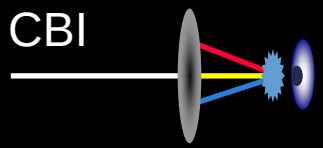
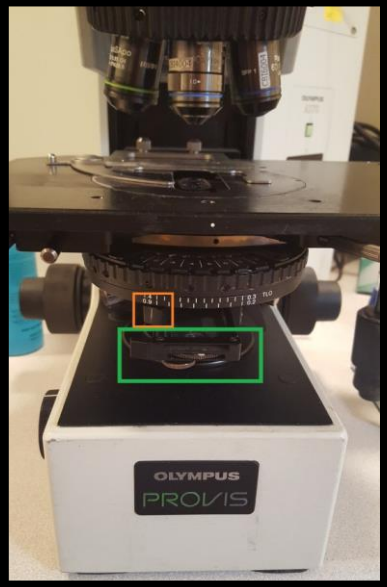


Filter Settings: Brightfield vs. Polarized Light

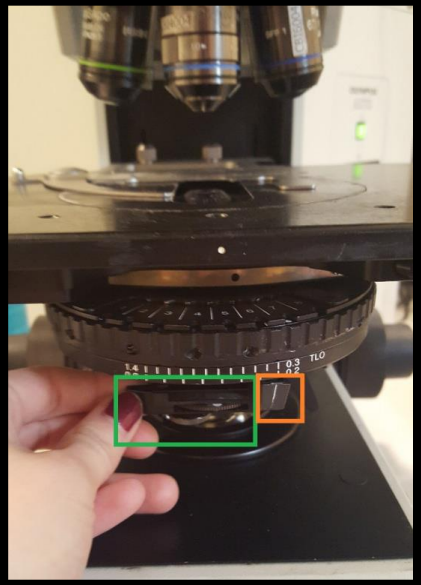


Brightfield Settings (above 10x)

Orange Box: Fully open polarizing filter front aperture by moving the slider to the left.

Green Box: Polarizer in the out position

Red Box: Bertrand Lens to the “out” position

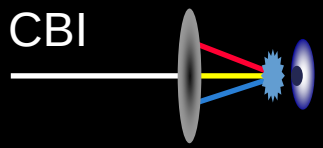


Polarized Light Settings

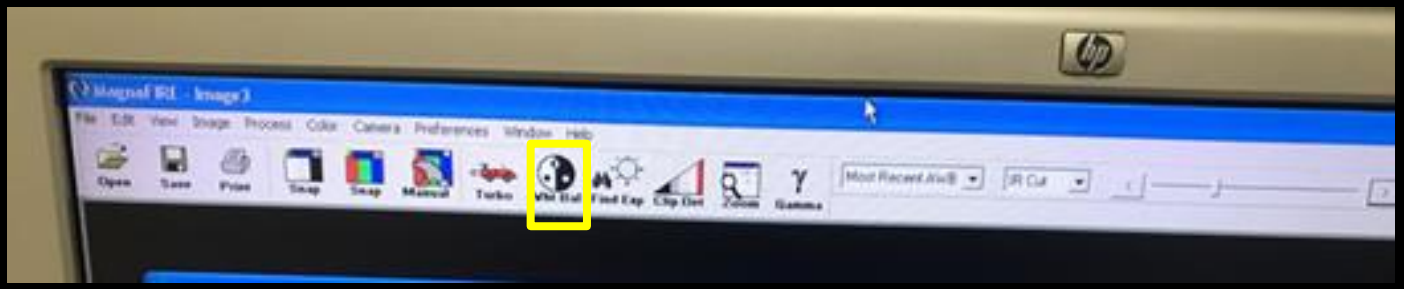
Orange Box: Close polarizing filter front aperture by rotating the front dial to the right

Green Box: Polarizer in the “in” position

Red Box: Bertrand Lens to the “in” position



Brightfield: White Balance



After establishing Köhler settings and microscope exposure time

- Move to an area of the slide without sample debri(s)
- Adjust fine focus so that image is a fraction out of focus
- Press white balance (yellow box) within MagnaFire software
- Under the File drop down tap, save these images as a Tiff for additional color correction in imaging software NIS Elements, Adobe Photoshop, or NIH Image J

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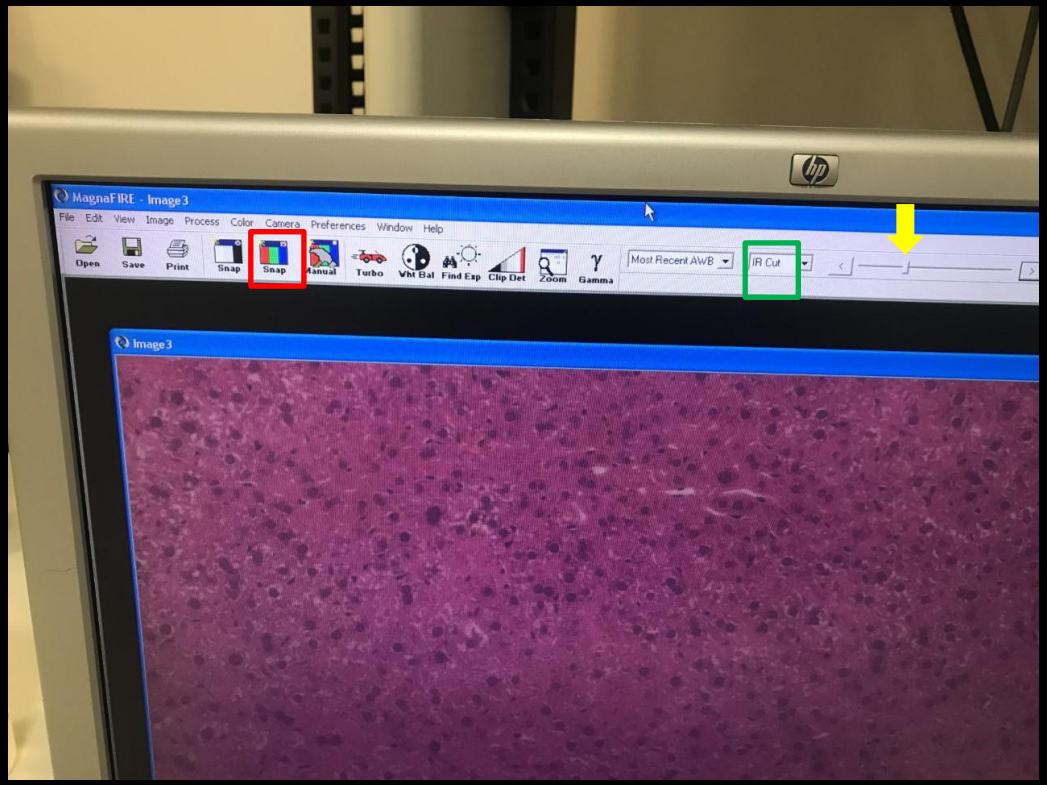
Set exposure of your sample for brightfield imaging within MagnaFIRE Software



Adjust exposure using slider (yellow arrow)

Immediately to the left of the exposure setting, drop down menu should select “IR Cut” (green box)

Image will be generated by pressing the Color SNAP icon (red box) that will generate the brightfield image

Under the File drop down menu, save the image as a 8-bit Tiff file

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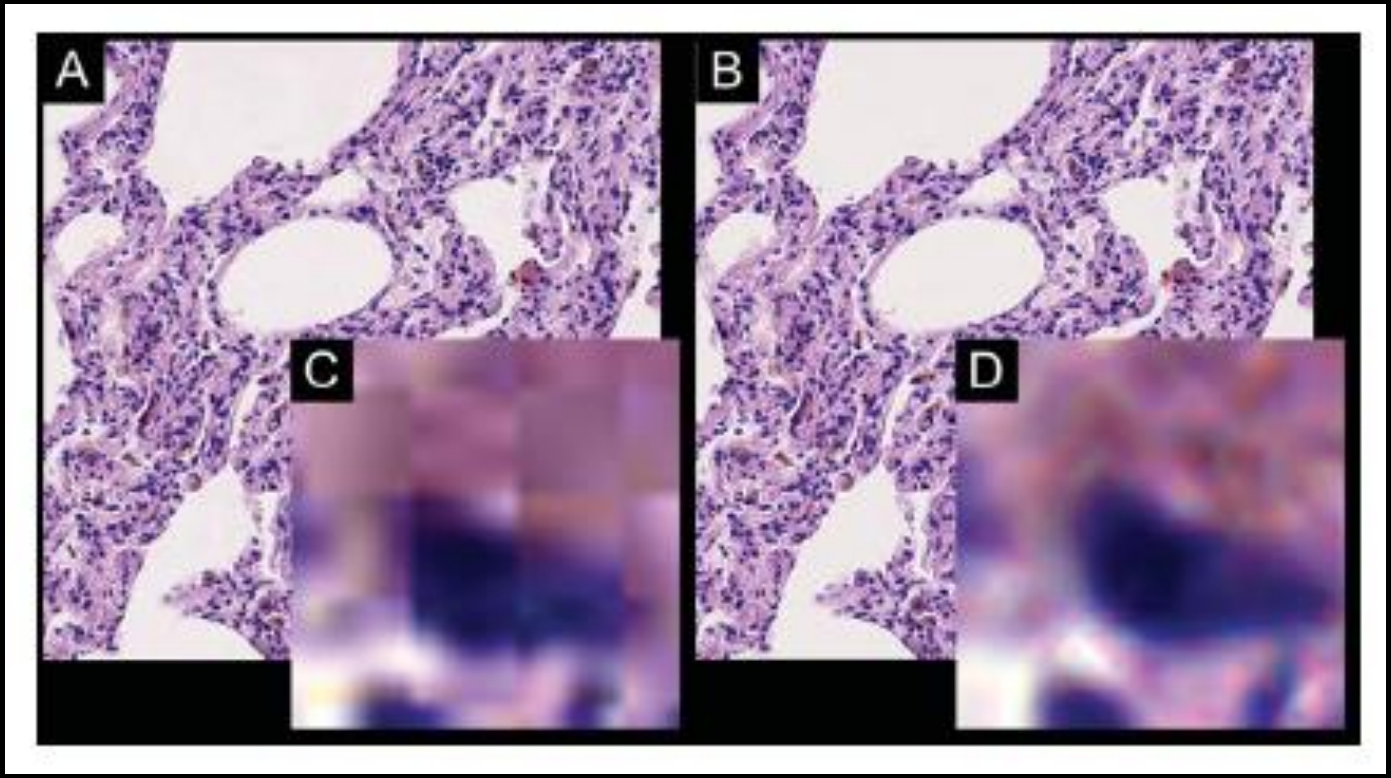
Tiff format 1024x1024 pixel is your friend



Comparison of file format and subsequent loss of detail when file is compressed. (A) RGB JPEG full field (B) RGB TIFF full field of view (C) region of interest with a digital zoom JPEG compression artifact (D) Region of interest with a digital zoom TIFF format of that same image. Best practice is to take a large area image at a lower magnification with a higher magnification of region of interest.

Image from Wallace *et al* CPCT 2018

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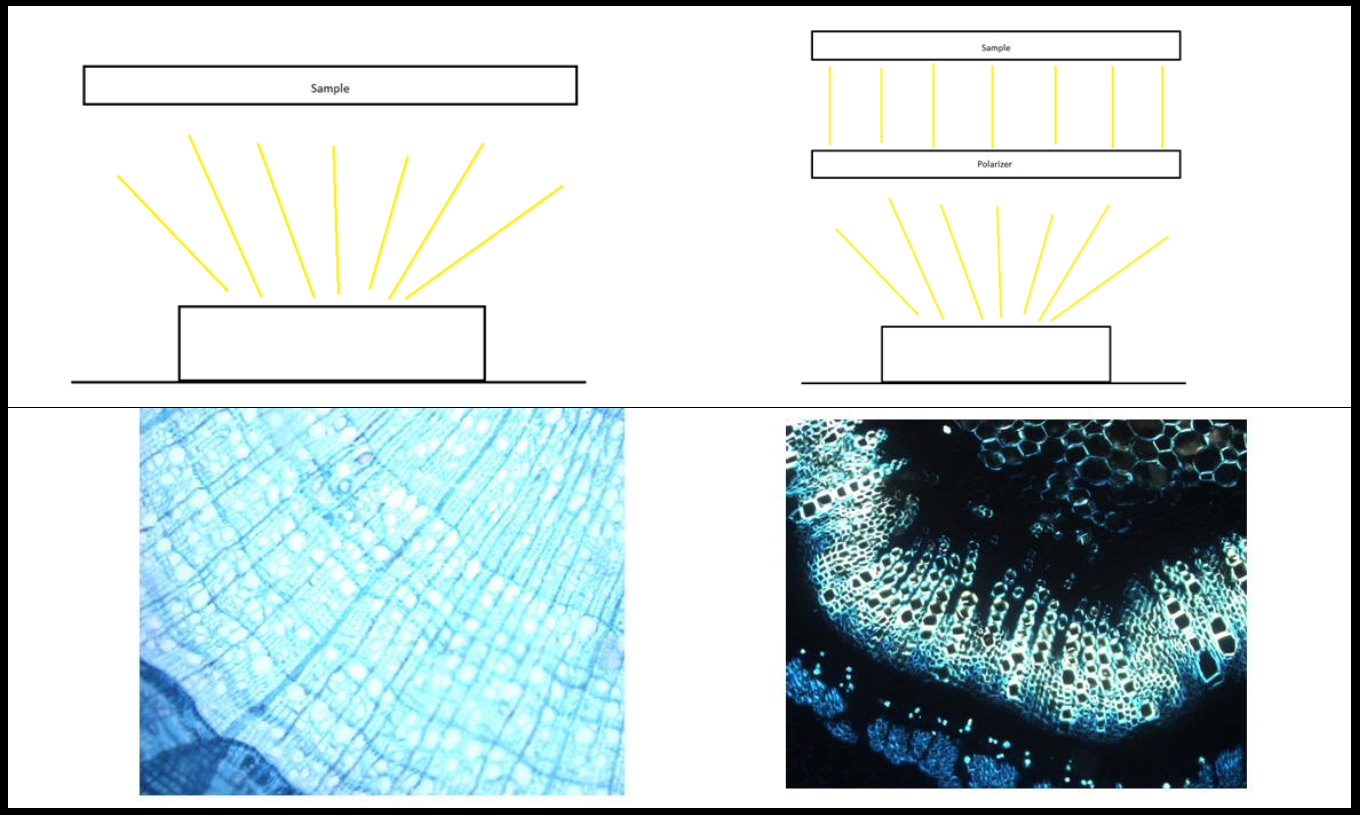
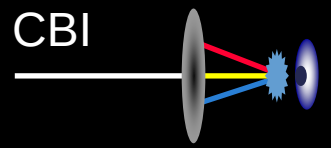
Brightfield with Polarized Light imaging specimen birefringence (e.g., for crystalline, lipid, fiber or mineral sample(s)

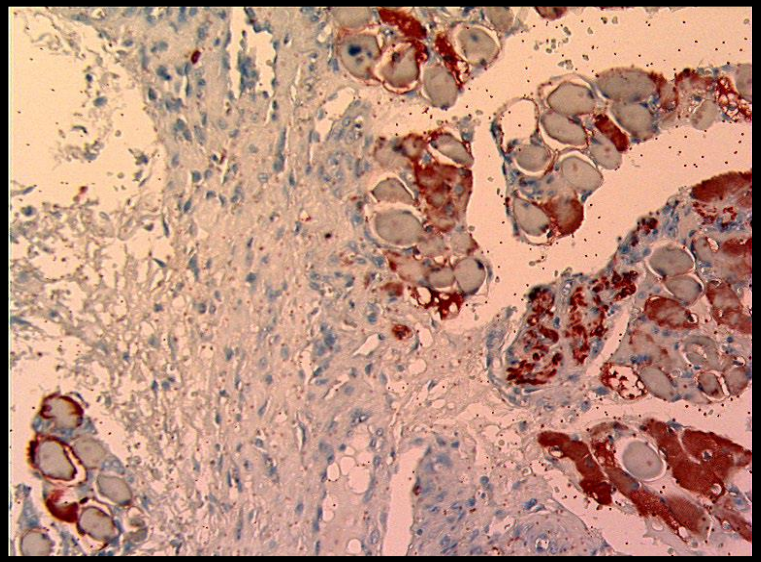
A **non-polarized** light image will show ALL the absorbed, refracted and reflected white light of the sample

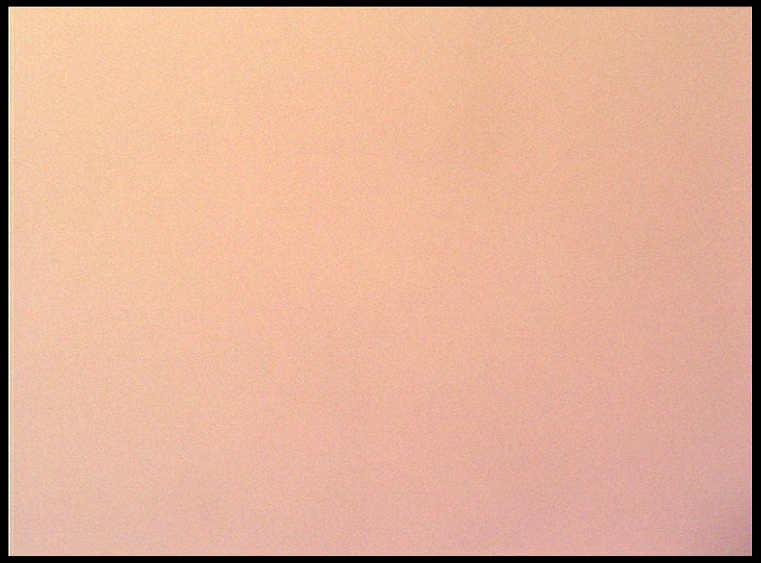
Polarized light image will show ONLY the light traveling in a single direction thru the sample



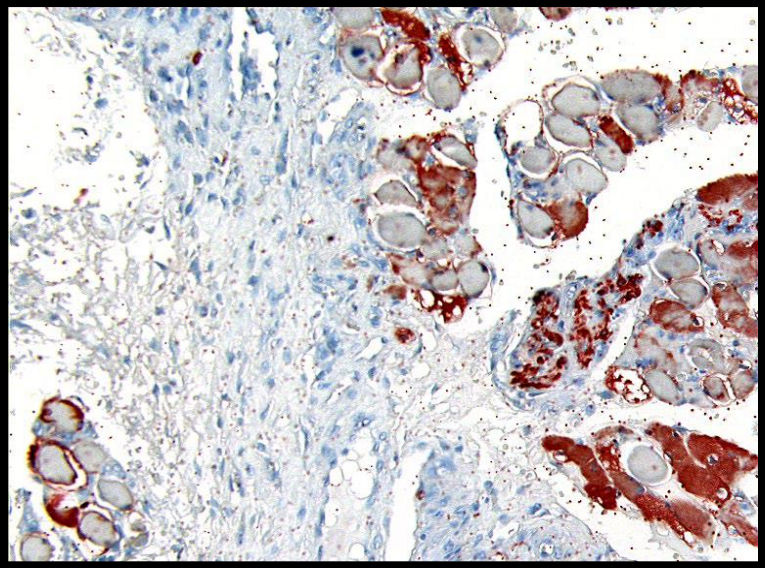


Additional Brightfield Color Correction AFTER imaging





Original/background corrects for non-white image by subtraction of the digital values of the image of an area of the slide without sample from an image of the slide with sample.



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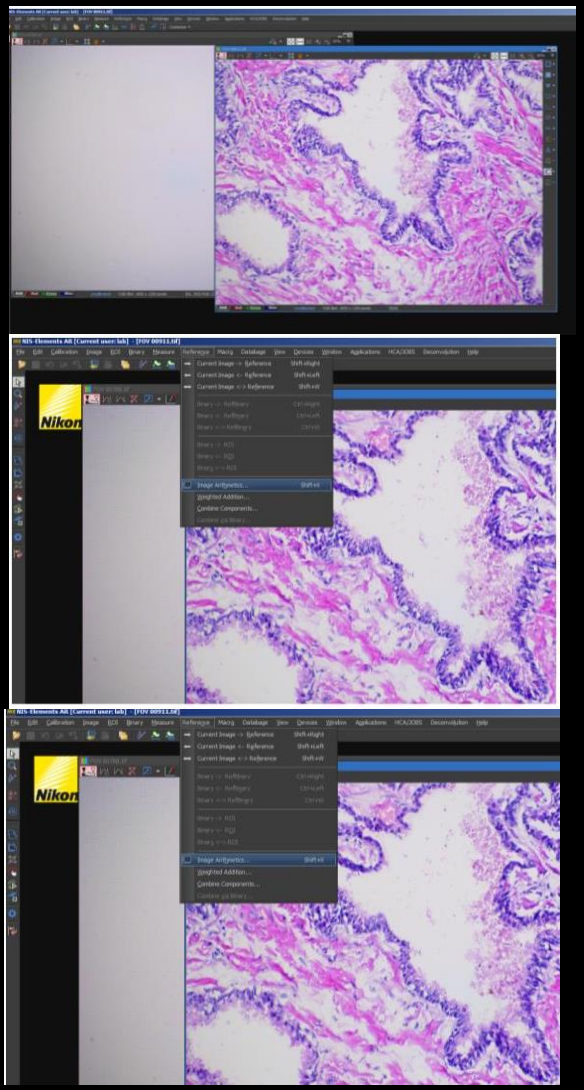
Alternative Brightfield Shading Correction in NIS Elements

- Open the background image that is not ideal as well as image of sample(s)
- Within NIS Elements
  - Go to reference and select the non-ideal background image as reference
  - Select your image of the sample and select “Image arithmetic” under the “Reference” drop-down menu
  - When software prompts, assign “Current<Shad>ref” and select OK

Debris or uneven shading will be removed

- Save your image

Within the same imaging set, you may apply shading correction to all images acquired during the same imaging session.



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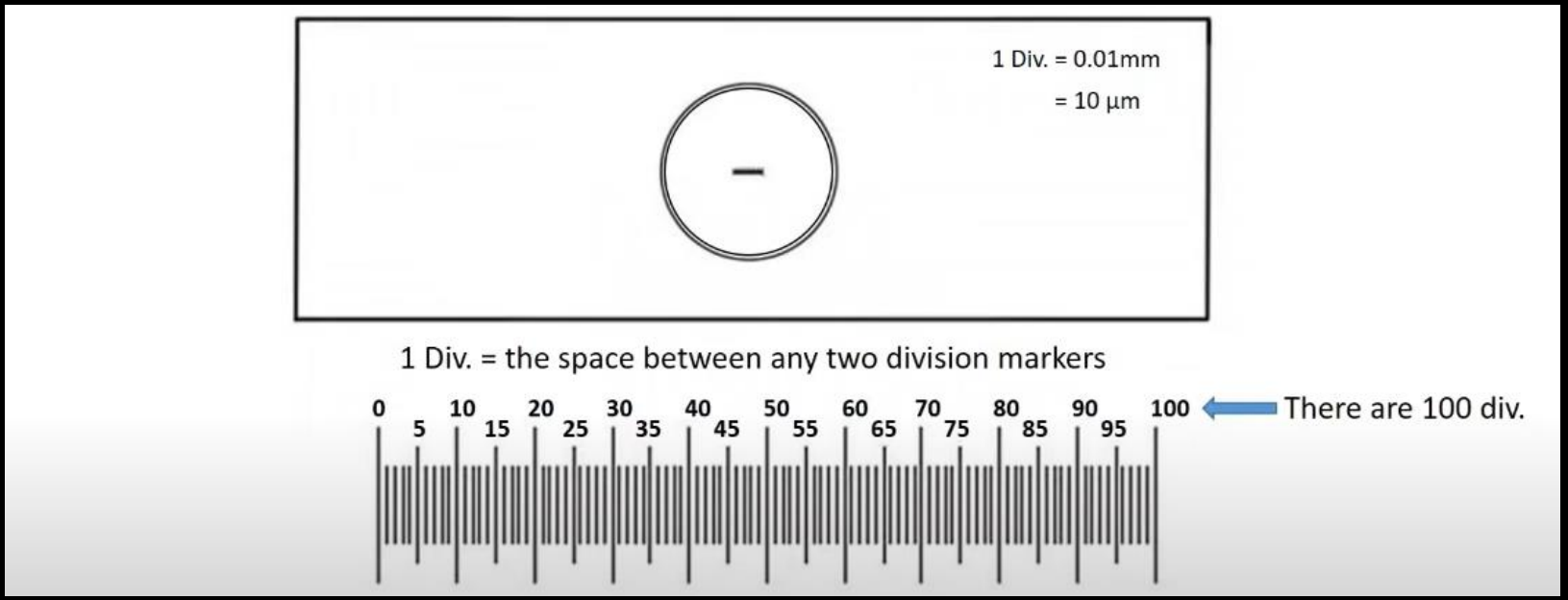
Addition of a calibrated ruler to image



Micrometer is a microscope slide with and engraved scale. Each unit on the 1mm micrometer subdivided into 100 units with each unit being equivalent to 0.01mm.

In general, an image with 10x objective should convert each unit to a 10-um distance; therefore, an image taken with a 100x objective would convert each visible micrometer unit to 1-um.

Images of a micrometer at different magnifications are in a folder on the desktop of each computer

Addition of a calibrated ruler to image(s) using imaging software NIS Elements, Adobe Photoshop, or NIH Image J

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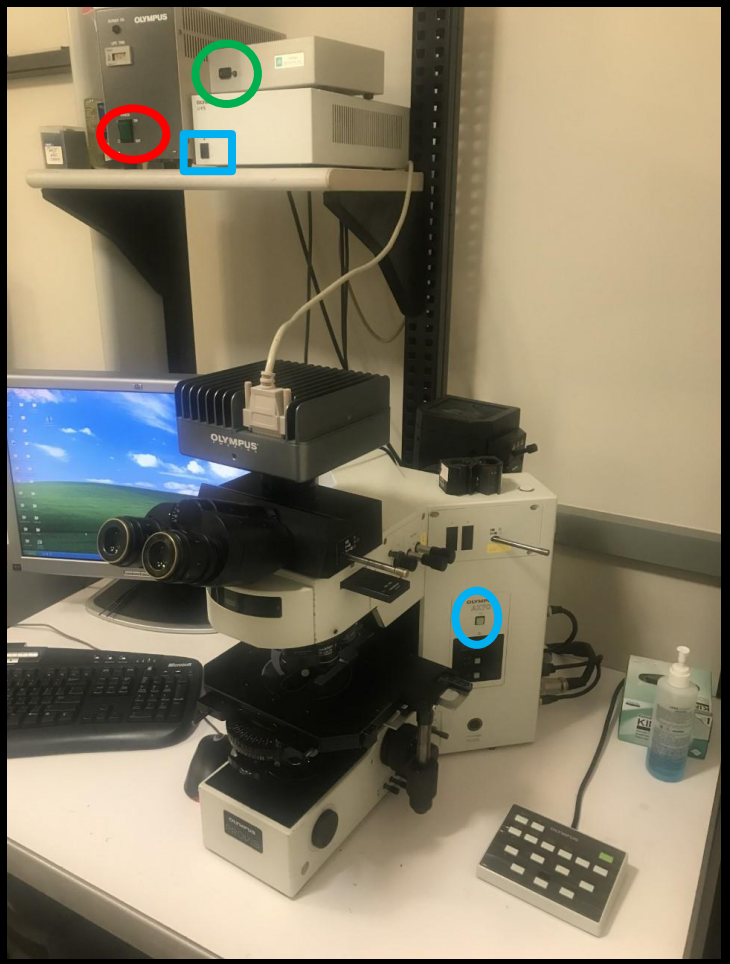
Order of Provis Microscope Shutdown

There are 3 instrument components to turn on/off the Provis microscope

1. Epifluorescent (EPI) lamp allows you to visualize a sample's fluorescence (red circle). This does not need to be on for imaging brightfield
2. Power supply for microscope camera allows you to see the signal (brightfield or fluorscence) on the screen and to take a picture (green circle)
3. Olympus microscope shutdown has one power buttons. The power button on the right-hand side on the base of the microscope (blue circle) automatically turns off when the white light source for Brightfield Imaging (blue square) is turned off

The Computer remains powered on, but you may exit the MagnaFIRE software



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