


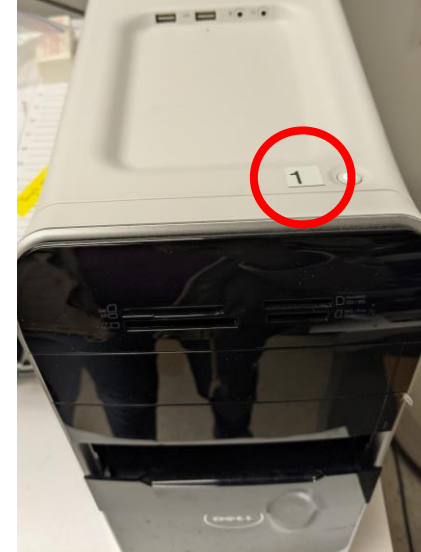
Leica M165 FC Stereoscope

User Manual

Microscopy and Imaging Facility
Shared Research Support Service
Dietrich School of Arts & Sciences
University of Pittsburgh
Pittsburgh, PA, USA

Turn on the Stereoscope

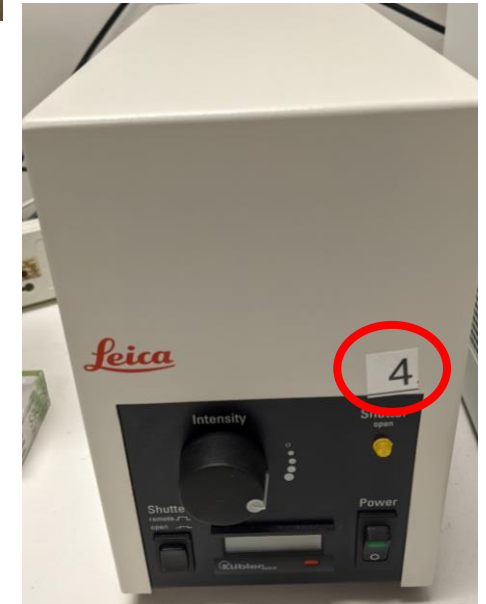
1. Sign on the log sheet before starting the system.
2. Check if the stage is clean. If not, please clean it before you start.
3. Turn on the microscope in the order of 1→4 as labeled on the equipment. The mercury lamp (4) needs to be on for at least 30 min before you can turn it off. If you just turned it off, wait for at least 30 min before you turn it on.
4. Log in to the computer with user account: Gurley lab, Password: planaria10
5. Click on the LAS V3.8 software icon on the desktop.  Make sure that all hardware needed is on before turning on the software.



Back of the microscope

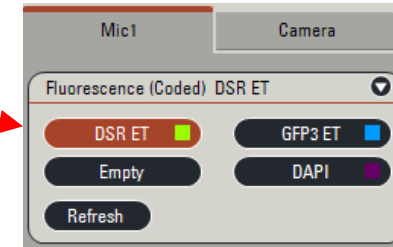
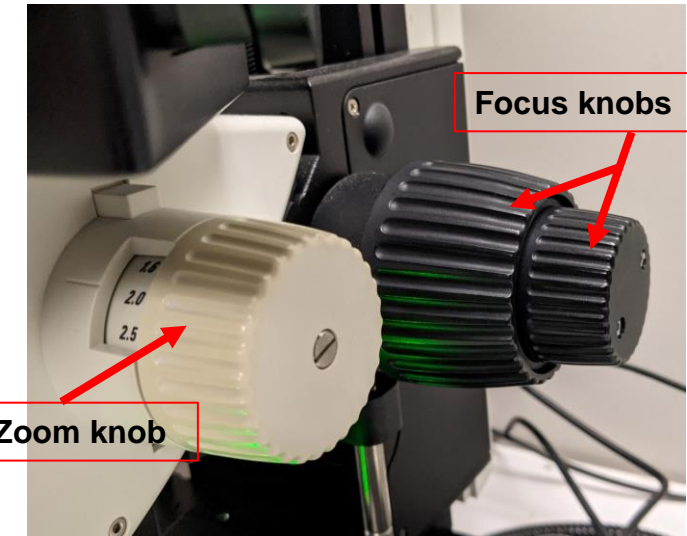
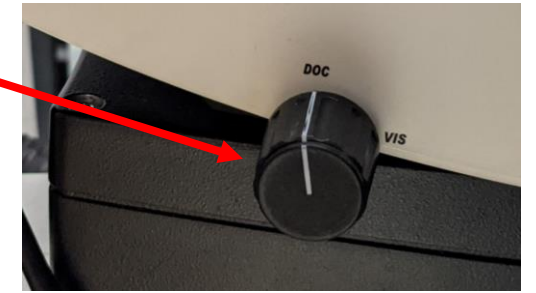
**Equipment No. 4, is fluorescent light source.
Equipment No.3 is brightfield light source.**

You only need to turn on the light source for your experiment. You can turn on 3 and 4 if you need both light sources.



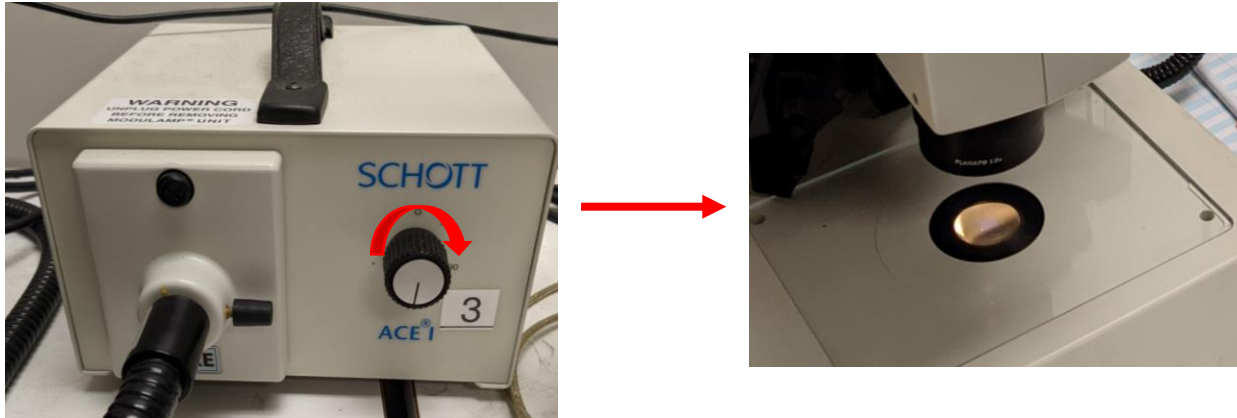
View samples through eyepieces (Fluorescent)

1. On the left side of the microscope head, there is a DOC/VIS knob. Turning to VIS directs light towards eyepieces. Turning to DOC directs light towards the camera. Turn the knob to VIS to start viewing your sample through the eyepieces/ binocular.
2. The focus knob and the zoom knob are located on the right side of the microscope. Zoom factors range from 0.73 to 12.
3. The auxiliary lens that's installed is 1x. There is also a 0.5x and a 1.6x objective available. If you need other objectives, please inform the facility staff to change it for you. Software configuration change is needed after changing the auxiliary lens.
4. For fluorescent imaging, on the front right side of the scope, there is a filter wheel to select different fluorescent channels. You can see the name of the cube as labeled on the filter turrets. (The empty position is for brightfield).
5. When a filter cube is changed manually, the filter name on the software under Mic1 section changes with it. But clicking on the software icons does not change the filter cubes.
6. Above the filter turret, there is a shutter that turns on and off the light. Check if the shutter is closed if you do not see any light come through. **Always close the shutter when you finish observation to avoid photobleaching** (Make this a good habit on any microscope.).



View samples through eyepieces (Brightfield)

- There are two transmitted light sources for brightfield imaging. One is the #3 box. It controls light that comes from the bottom of the stage. You can increase the brightness of the light by turning the knob on #3 box clockwise.
- The two knobs at the left side of the stage changes the angle of this transmitted light.



- The second transmitted light source is the LED light source from above the stage. The power switch and light intensity can be controlled manually or through the software

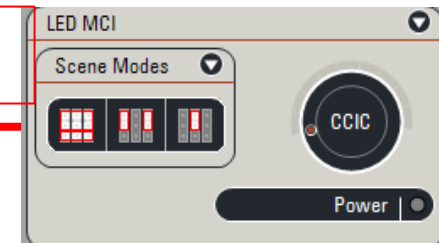
LED light
power
switch



Manual control



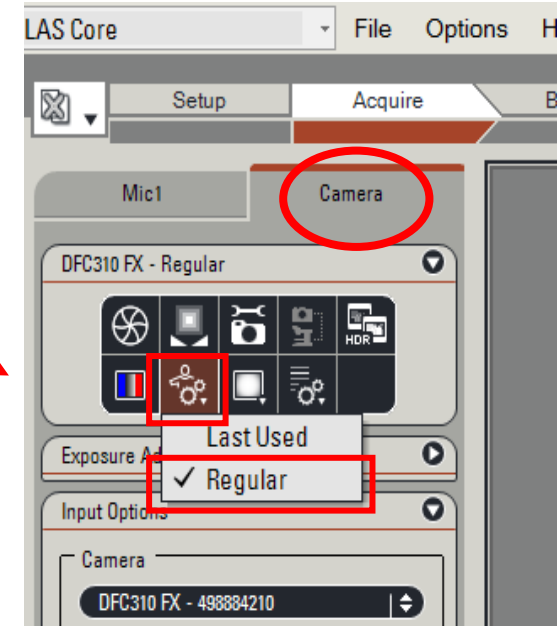
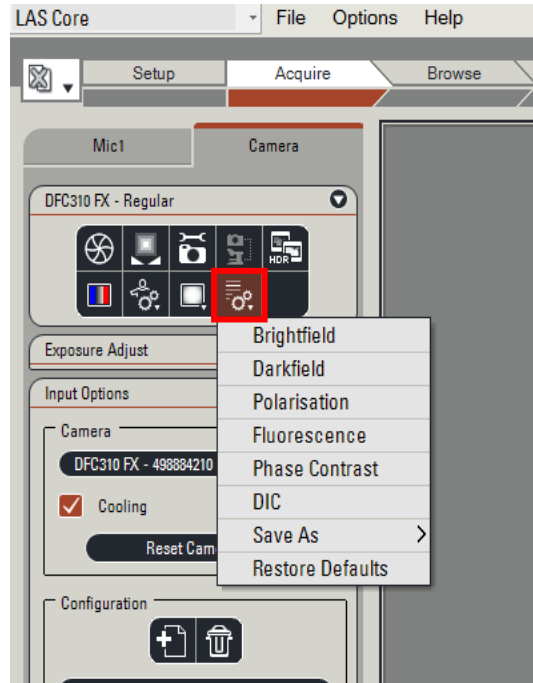
Adjust light angles
and intensity.



Software control

Image Acquisition

1. After eyepiece observation, **close the shutter of the fluorescent light**. Then switch the DOC/VIS knob to DOC. This will direct light towards the camera.
2. If you are doing regular fluorescent imaging, in the software, select Camera, then click on the configuration icon and select Regular. **(This is a very important step, do not skip.)**
3. If you are performing brightfield imaging, first click on Regular, then select one of the brightfield setups as indicated in the images.



4. For fluorescent imaging, open the shutter. Now you will see the image in the live view window. **No not forget to refocus since focus through the eyepieces is different from focus in the software.**
5. To adjust the brightness of the fluorescent light, you can increase exposure time under Camera → Exposure Adjust → Exposure.

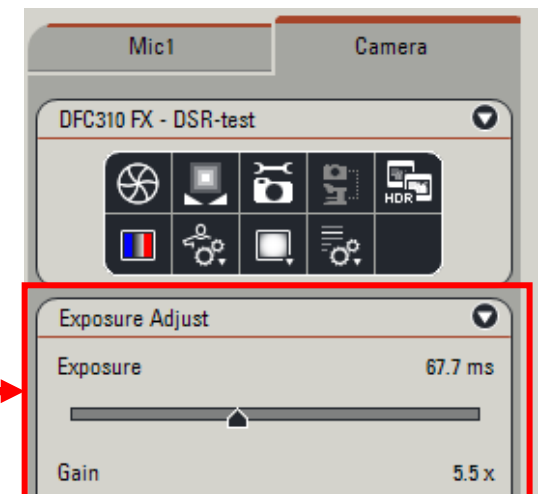
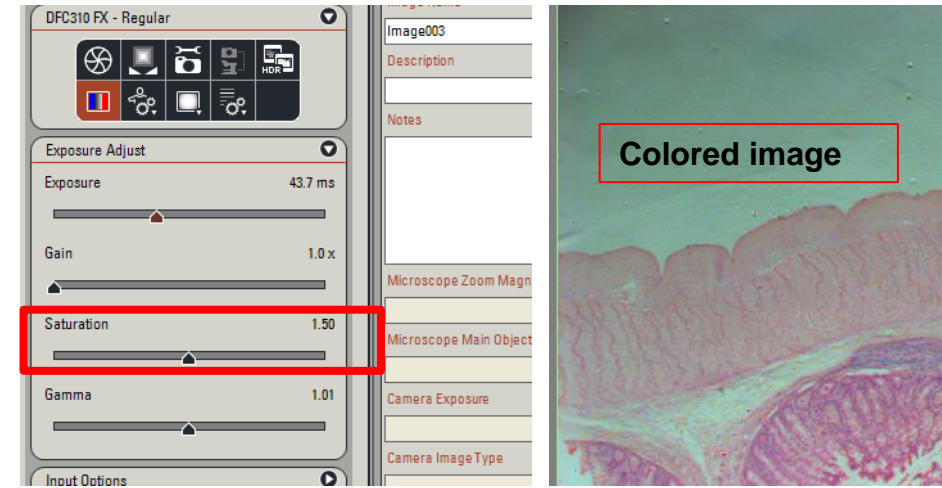
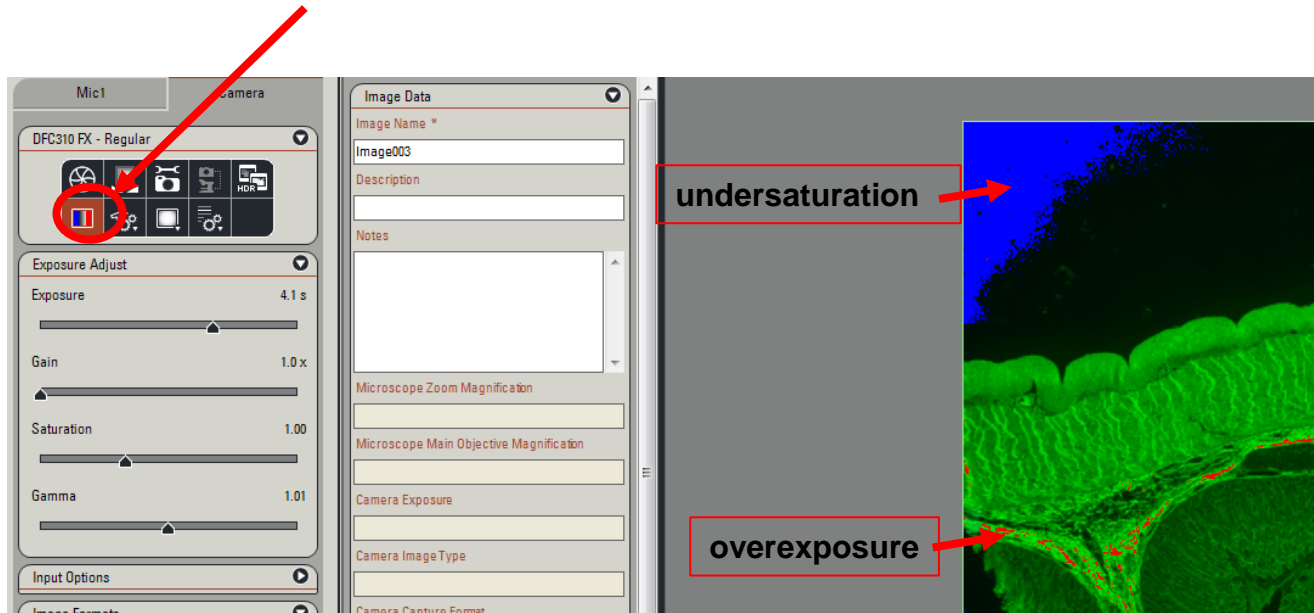


Image Acquisition

- Turn on the saturation indicator to check if any part of your image is overexposed. Red indicates overexposure and blue indicates undersaturation. Make sure that your region of interest is not overexposed and there are some blue in the background where there is no sample, but the background is not full of blue.



- Saturation at around 1 allows you to take a colored image (both fluorescent and brightfield). To capture a black and white image, turn saturation down closer to 0.
- For transmitted light/brightfield imaging (colored), you need to adjust the white balance of the image. Click here to adjust white balance.

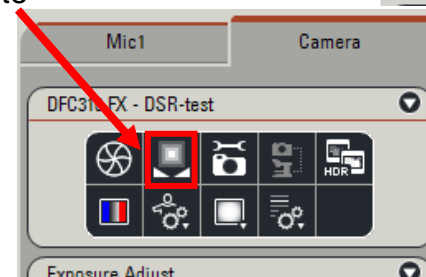
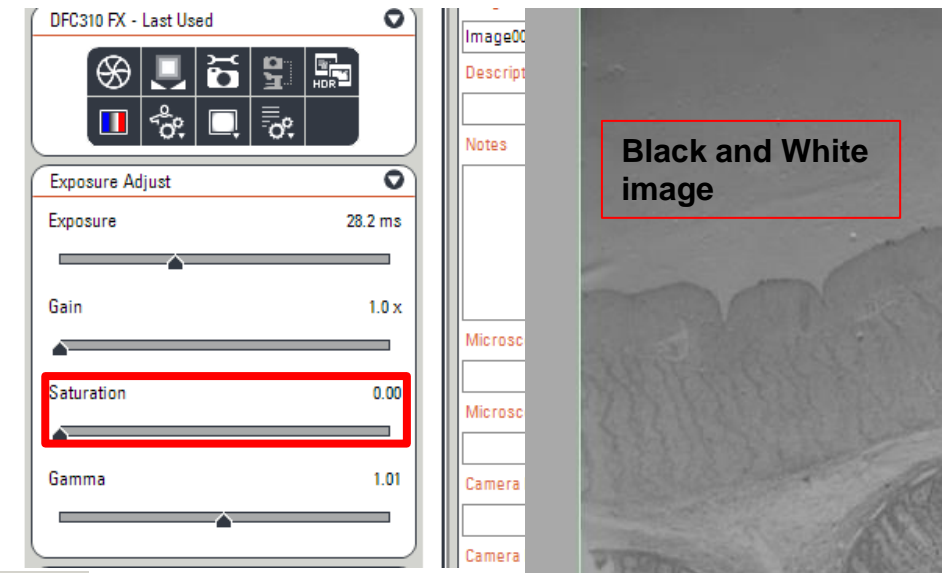
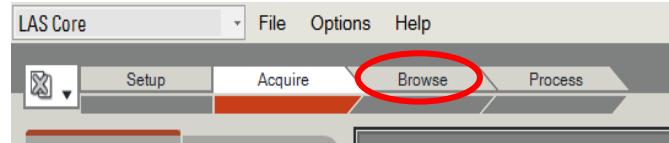
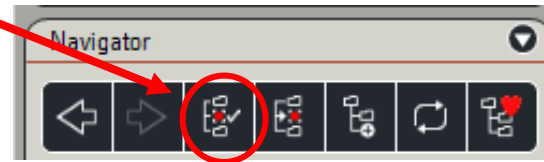


Image Acquisition & Saving

9. Once the image is in focus and well adjusted for brightness. Click Browse.

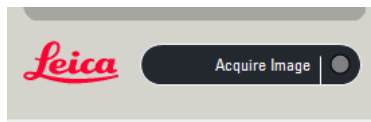


10. In the Browse section, under Folders, click on the folder you want to save the data into, so that it is highlighted in red. Then click on the Set Capture Location icon. Now you will find a red dot in front of your selected folder. All your captured images will be saved in this folder.

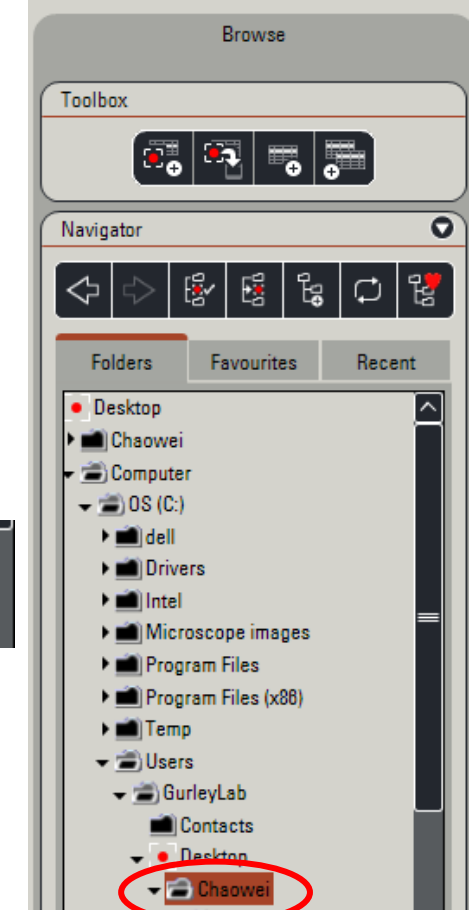
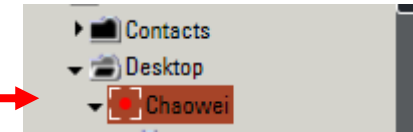
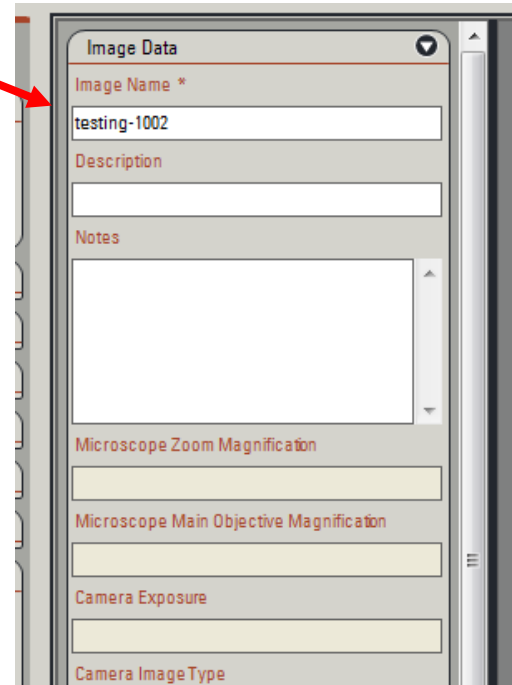


11. Go back to Acquire. On the left side of the Live view window is the Image Data section. Name the image under Image Name before acquiring an image.

12. At the bottom left corner, click on Acquire Image to capture the image.



13. This image is now saved.



Clean and turn off the system

1. If you spilled any medium, please clean the stage with alcohol. Do not leave any slides or samples behind.
2. Copy your data after each session due to limited space in the PC.
3. Please turn off the system in the order of 4→1.
4. Log off on the log sheet.