Microscopy Shared Resource Facility
Icahn School of Medicine at Mount Sinai

Stereoscope Fluorescence Protocol

1) System Startup

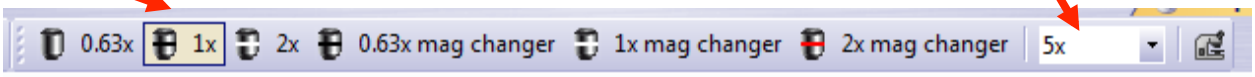
- Please note the sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
- **Follow each step** of the startup poster
- **Log into** the computer with you user account.
- **Open** cellSens software 

. You should see the startup screen when the software has successfully started.

2) Focusing and scaling

- Focus on your sample using the fast/slow wheel on the right of the microscope.
- Choose the appropriate fluorescent wheel from the filter turret.
  - 1: DAPI
  - 2: FITC
  - 3: TRITC
  - For Cy5 or CFP please contact CORE staff
- When switching from your eyes to the software, pull out the camera slider.
- Designate your lens selection and zoom factor on both the microscope and in the software.

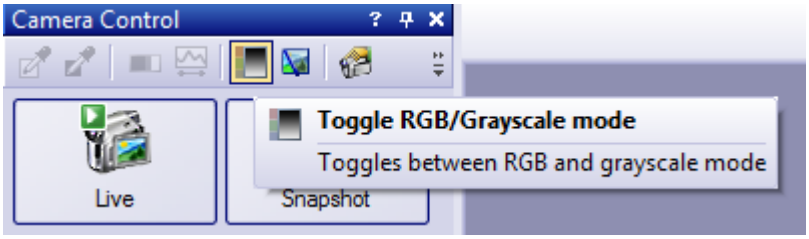
Lens: Zoom Factor:



* This step is critical for scaling. Please contact CORE staff if you need the 0.63x

3) Acquire your first image

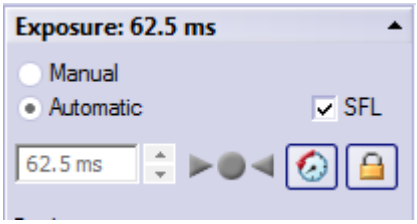
- Select the monochrome chip in the Camera Control window:



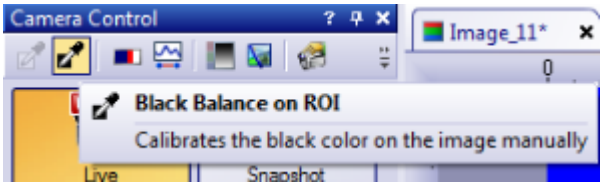
☞ Set up an exposure time.

- Click Live: 

- Adjust the focus.
- Set the exposure time.
  - Set it manually or automatically using the auto-exposure:



- If you used the auto exposure setting, turn it off.

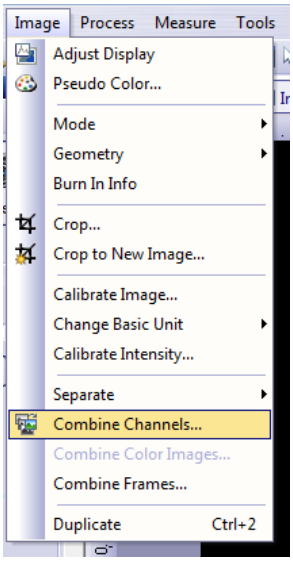
☞ Set the black balance. 

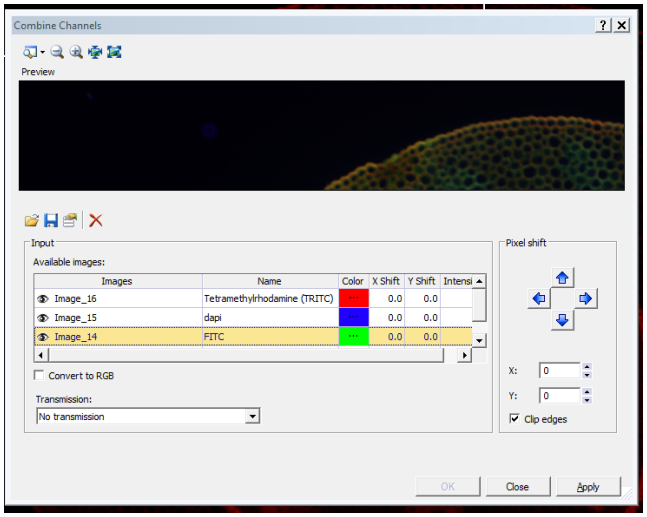
☞ Acquire the image. 

4) Adding a pseudo-color

☞ Go to the image menu

- Click "Combine Channels"
- Assign the image name to the appropriate channel color
- Click "Apply"
- Your overlay or individual image will appear in a new tab.
- Save the image.





☞ **Adding a pseudo-color** Go to the image menu

☞ Click "Burn in Info"