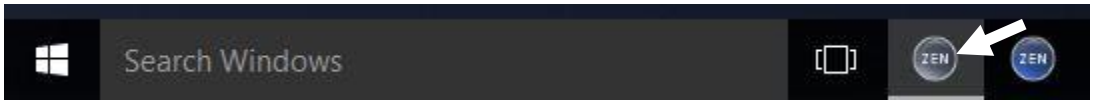
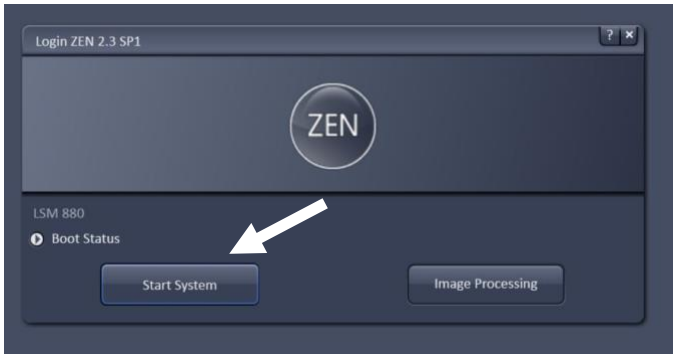
SOP for Turning on Zeiss LSM 880 system and imaging samples through the eye pieces

1. Make sure that the Water immersion 20X objective (in objective position 1) is in place and fully lowered (this is required for the software to know where all the objectives are).
2. Turn on the “Analysis” Computer system.
3. Turn on the system power using the Remote Switch Box located to the left of the scope body on the surface of the vibration isolation table (see image below):
   a. 

   b. Press MAIN SWITCH to the On position and wait 10 s (wait for two “click sounds).
   c. Press SYSTEM/PC Switch to the On position and wait 10 s.
   d. Press the COMPONENTS Switch to On and wait 10 s.
4. Turn on the System Computer (immediately to the right of the Scope).
   a. Press the on button as indicated in the image below:
   b. 

   c. Wait for system to boot up Windows 10 before proceeding to the next step (2-3 minutes – please be patient!).
5. Launch Zen Black software by clicking on black Zen icon in the task bar.
   a. 

   b. Once the Zen software opens, Click on the Start System button and

c. 

d. wait for all of the systems to come on line (shown by the blue progress bar).

e. f. 

6. Locating, Focusing and Viewing a sample through the eyepieces

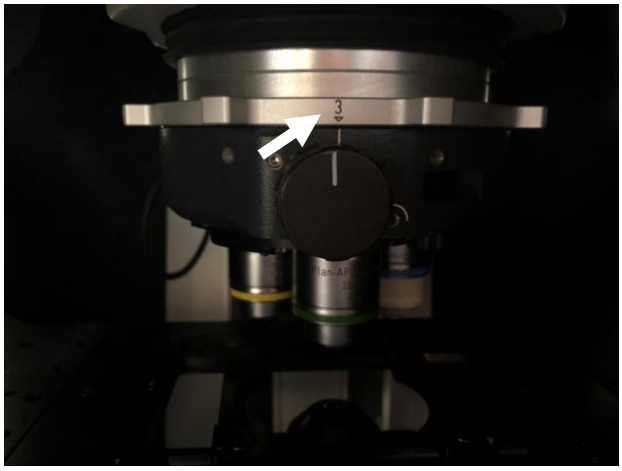
    a. Controls for the microscope transilluminator for Bright-Field (BF) and the LED light source for epiillumination and Fluorescence (Red, Green or Blue) are located under the **Locate Tab** Window.

b. 

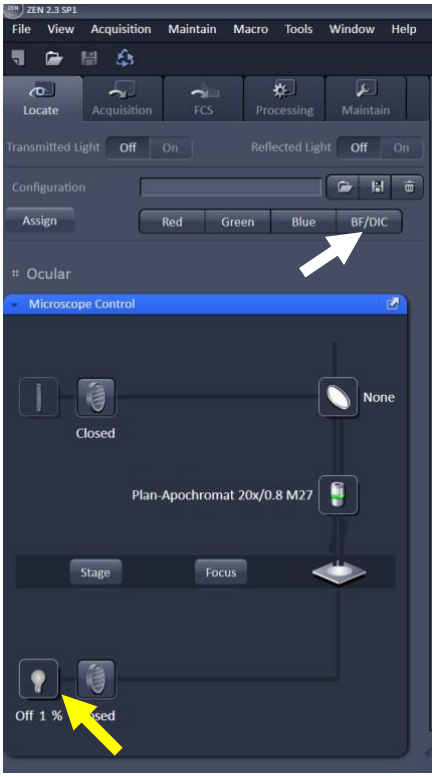
c. Place an appropriate sample on the stage of the microscope

d. Select the appropriate objective and line up its number with the line on the nose piece (see figure below).

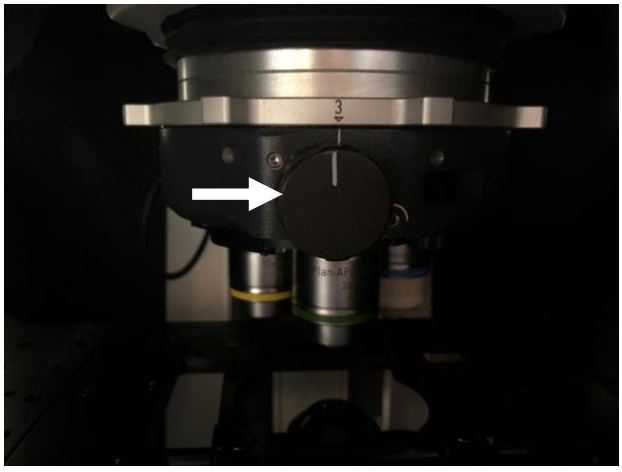
e.

f. 

g. Turn on the transmitted light by clicking on the BF/DIC button (see white arrow below). Adjust the illumination intensity by clicking on the light bulb icon (see yellow arrow below)

h. 

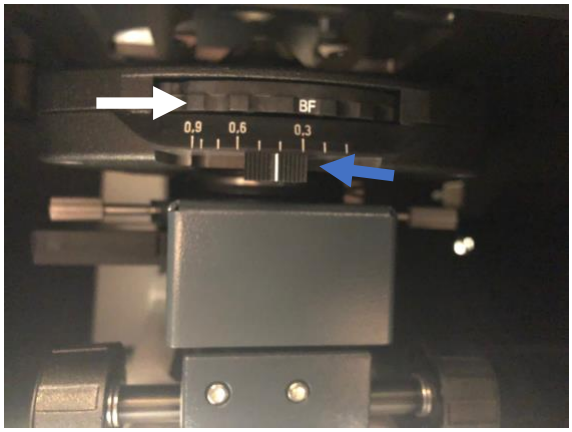
i. Lower the objective towards the sample using the knob on the microscope nose piece clockwise (see arrow in Figure below) while looking through the eyepieces (to make sure that you don't run the objective into the sample). Ideally, you should lower all the way. However, very thick samples may make this impossible, so lower with caution to avoid crushing your sample, breaking a slide and damaging the objective (you break it, you buy it!!!!)

j. 

k. To view your sample with transmitted light:

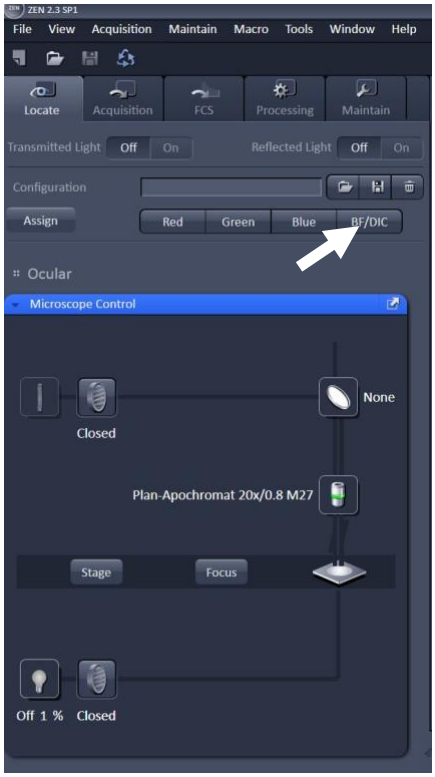
    i. Set the large dial on the condenser to BF (or the appropriate DIC setting if DIC will be used – covered later in this document) (see white arrow in

image below). The long working distance condenser does not have this option.

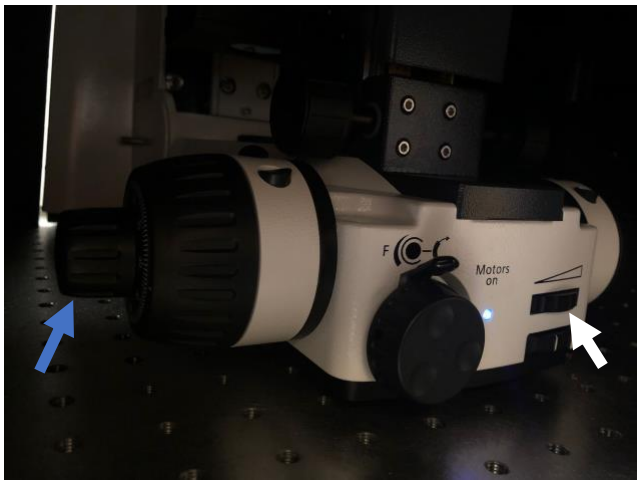
ii. 

iii. Position the aperture diaphragm to about its mid point or as needed to obtain an image in the eyepieces (see blue arrow in image above).

iv. Turn on the transilluminator by clicking on the BF/DIC button in the Locate Tab Window in the Zen Software (see image below)

v. 

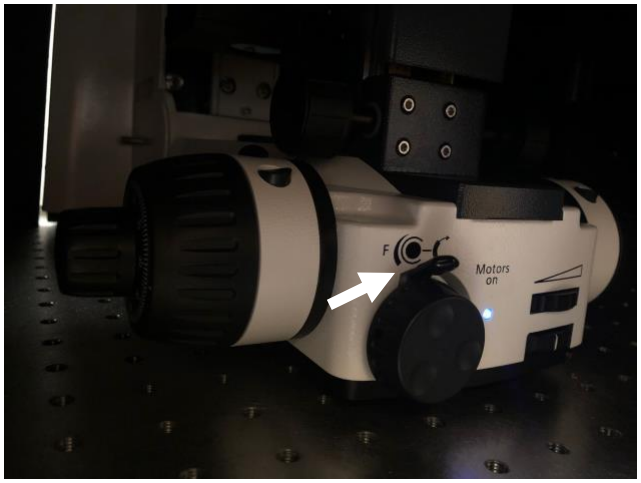
vi. The intensity of the transilluminator is controlled by the dial on the base of the microscope (see white arrow in image below).

vii. 

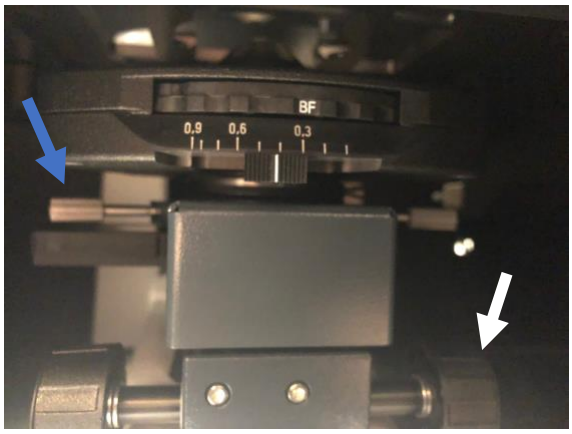
viii. Focus on your preparation using the focus knobs (left and right) on the base of the microscope (see blue arrow image above)

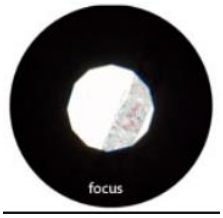
ix. You can change/move your sample in the x-y directions using the stage control joy stick to the right of the scope on the table.

x. Setup Köhler illumination (see http://zeiss-campus.magnet.fsu.edu/articles/basics/kohler.html)

1. Close the Field diaphragm below the condenser using the lever control (see white arrow in image below)
2. 

3. Use the small knobs on the left and right of the condenser to center the image of the edges of the Field Diaphragm (see blue arrow in the image below)
4. Lower or raise the condenser using the knobs on the lower right and left of the condenser (see white arrow in the image below) to bring the image of the Field Diaphragm into focus.

5. 

6. When finished, you should see something like the image below through the eyepieces:
7. 

8. Open the Field Diaphragm to fill the field of view.

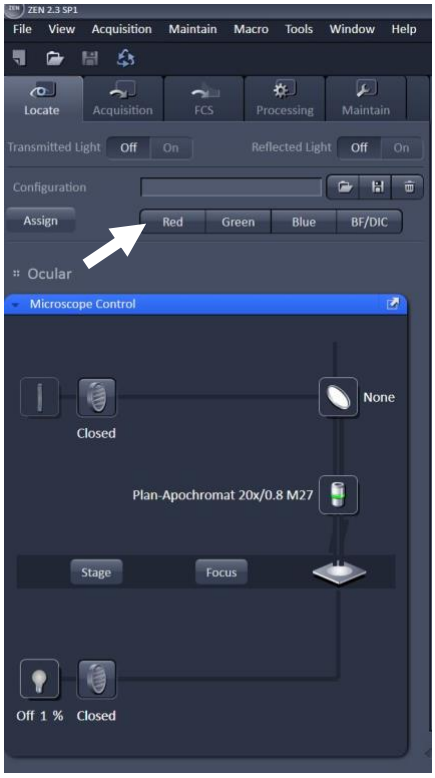
xi. Following this procedure will allow you to capture confocal transmitted light images along with images of Fluorescence.

xii. Turn off the transillumination by clicking the Transmitted Light Off button in the Locate Tab Window (see image below).

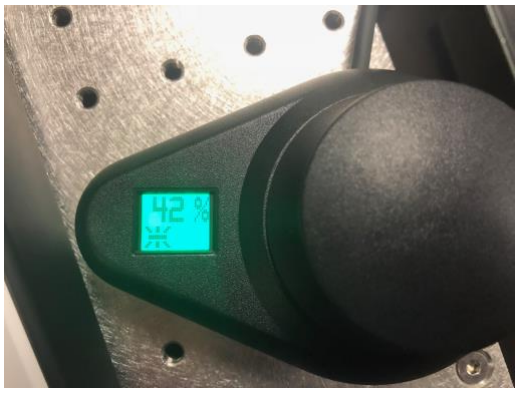
xiii. 

l. To view a fluorescent sample:

   i. Select the emission color of the fluorophore you are using (Red, Green or Blue) by clicking on the appropriate button in the Locate Tab Window (see arrow in image below). This will select the appropriate filters in the microscope and the appropriate LED from the LED light source.

ii. 

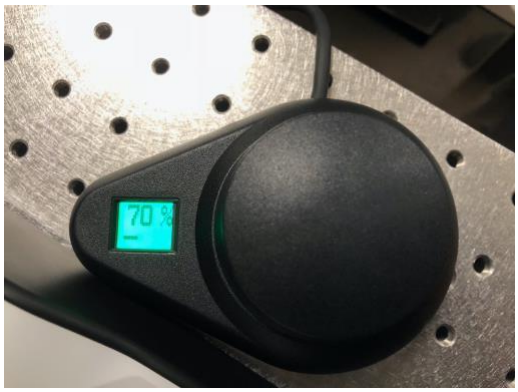
iii. To turn on the LED epi-illuminator, Tap/press down on the LED remote control button – you will see the “on” indicator come on in the display of the remote control as shown in the image below.

m. 

n. The intensity of the LED illuminator is controlled by turning the knob on the LED remote control.

o. Focus on your sample using the focus knobs on the base of the microscope (See image g.vii. above).

p. Turn off the LED illuminator by tapping/pressing on the knob on the LED remote control – you will see the “off” indicator come on in the display on the remote control (see image below).

q. 

r. Turn off the LED illuminator by clicking on the Reflected Light Off button in the Locate Tab Window (see image below).

s.