**Nikon Eclipse 80i (Upright) Operation**

**Viewing Only**

1. If using the mercury lamp for fluorescence microscopy, turn on the X-cite 120 illuminator (box to the left of the microscope) as well as the shutter (square green button on right side of microscope).

2. Turn on halogen light switch on back left side of microscope (above the power cord).
   - To adjust brightness use knob on left side of microscope.

**Reflector Cube Positions**

1. Blank
2. UV-2E/C (DAPI, Hoechst 33258/33342)
3. FITC HQ (FITC, Alexa 488)
4. G-2E/C (TRITC, Rhodamine, Propidium Iodide, Ethisedium Bromide, Alexa 546/555)
5. Y-2E/C TR (Texas Red, Alexa 594)
6. Blank

3. To view image through the microscope BOTH of the sliders need to be pulled OUT.

**Taking Pictures**

1. Turn on components
   1. Hg bulb (only if using fluorescence)
      a. Shutter (green square button on the right side of the microscope)
   2. Halogen bulb on back left side of microscope (above the power cord)

2. Open the SPOT software
3. Make sure BOTH of the sliders are PUSHED IN.

**For BRIGHTFIELD Images:**

1. Select Brightfield from the configuration drop box
2. Choose the objective you want to use and set the condenser adjustment ring to either “2-4x” for 2X, 4X, 10X, and 20X objectives and to “N2” for the 40X, 60X, and 100X objectives. These latter objectives are DIC objectives and are labeled “N2”.
3. Make sure reflector cube position 1 or 6 is chosen (blank)
4. Pull out both sliders on the right side of the microscope to view specimen through the oculars.
5. Focus on specimen
6. Move to a blank area on the slide
7. Push in both sliders on the right to send the image to the camera
8. Select compute white balance from the toolbar on the right
9. Pull out both sliders
10. Move back to the specimen
11. Push in both sliders
13. Choose Live to focus image
14. Take picture

For FLUORESCENT Images:

NOTE: if you are not actively looking at the image through the oculars, focusing, or taking a picture you need to CLOSE THE SHUTTER.

Single Dyes
1. Select the desired color fluorescence from the configuration drop box.
2. Choose the objective you want to use and set the condenser as in 2 above if you are collecting brightfield images as well.
3. Choose the reflector cube appropriate for the fluorochrome you are imaging (reflector cube positions list in the table below).
4. Pull out both sliders on the right to view specimen through the oculars.
5. Open the shutter and focus on image.
6. Shut the shutter.
7. Push in both sliders on the right to send the image to the camera.
8. Open the shutter and choose live to focus
9. Take the picture.

Multiple Dyes
1. Select Factory Defaults from the configuration drop box.
2. Choose the objective you want to use and set the condenser as in 2 above if you are collecting brightfield images as well.
3. Choose the reflector cube appropriate for the first fluorochrome you are imaging (reflector cube positions list in the table below).
4. Pull out both sliders on the right to view specimen through the oculars.
5. Open the shutter and focus on image.
6. Shut the shutter.
7. Push in both sliders on the right to send the image to the camera.
8. Open the shutter and choose live to focus
9. Take the picture.
10. You can change the color of the picture from grayscale to color by clicking on Edit→Set palatte→choose the desired color from the drop box.
11. Select the appropriate reflector cube for the next fluorochrome you are imaging and take the picture again.

Reflector Cube Positions
1: Blank
2: UV-2E/C ..................DAPI, Hoest 33258/33342
3: FITC HQ ..................FITC, Alexa 488
4: G-2E/C ..................TRITC, Rhodamine, Propidium Iodide, Ethedium bromide, Alexa 546/555
5: Y-2E/C TR .................Texas Red, Alexa 594
6: Blank