<u>Nikon Eclipse 80i Light Microscope instructions</u>

- Bring multiple CDs to save images. The image files are very large!!
- Microscope and camera set-up is located on the 4th floor of the Kips Bay building
- The sign up calender is located on the wall next to the monitor. Sign up for no more than 3 hours at a time. Be sure to record your name, time to use the microscope, your lab and the lab phone number.
- The microscope and camera are connected to a G5 Mac computer with a Dell monitor
- The computer is hooked up to a color printer for printing images

<u>Getting started…</u>
- Turn microscope on. The power switch is located on the back of the microscope on the lower left side.
- There is a green button on the bottom front of the microscope. When it is pushed in and green, it is on. This means that the camera will see 9 volts. This should be depressed if photographs will be taken.
- The computer should always be on, however, you may have to "wake it up" by pushing the space bar on the computer keyboard.
- The camera that is attached to the top of the microscope is on when the computer is on.
- You may have to log in to the computer. If so, use 'neuro' as the username and password, if needed.
- Be sure to create a new folder on the desktop to save your work. This will be deleted at the end of your session

<u>Microscope notes:</u>
- On this microscope, the objectives that are available include: 2x, 4x, 10x, 20x, and 40x. (There is also a 1x objective, but you will need to get Dr. Teri Milner to remove the stage before using this objective). To change objectives, grab the nosepiece, not the objectives themselves!
- There is also the option to modify the magnification if slight changes are needed. There is a dial on the upper right of the microscope that is always set at '1'. If less magnification is needed, you can turn the dial to a point less than 1. If more magnification is needed, you can turn the dial to a point greater than 1. Be sure that the dial is reset to '1' after your session is over.
- <u>When you change objectives, you must also change the condenser wheel, which is the black disk under the stage</u>. Each objective or set of objectives has a specific condenser setting and these are color-coded in gray scale. 2x and 4x share one condenser (2-4x); 10x has its own condenser (N1) and 20x and 40x share one condenser (N2). (Note: The color gray and codes are subtle!)
- When you change objectives on the microscope, the condenser should already be aligned and centered correctly. However, if they are not, you can re-center the condensers for the 10x, 20x and 40x objectives only. You cannot center the

condenser for the 2x and 4x objectives. If you need to center the 2x and 4x objectives, go to the 10x objective and center the condenser then turn to the 2x or 4x objective.

- o To center the condenser:
  - Using the vertical wheel on the lower right of the microscope, close the diaphragm so that the 16-sided shape is within the field of view
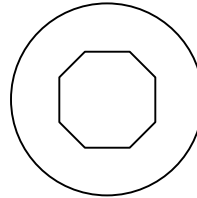
  - The object and the field of view may appear reddish. These should actually be slightly blue. Find the small dial located under the condenser disk on the left side of the microscope and adjust until the object and field of view are slightly blue.
  - When you spread the diaphragm out, the sides of the 16-sided shape should spread out evenly. If it does not, use the metal knobs that are located under the condenser plate to adjust and center the shape. Once it is centered, open the diaphragm fully. The condenser is now centered.
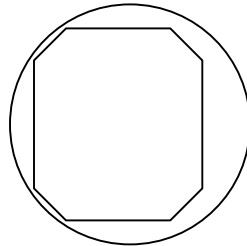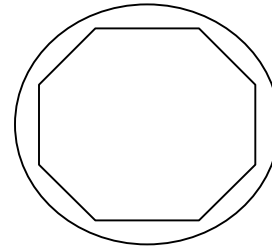
Off-center   Centered

- The stage of the microscope has been set to be somewhat loose for small movements and modifications (Example: Straightening out the image under the microscope or on screen for taking pictures.) Do not force the stage to move if it is tight. There are screwdrivers located on the top right side of the microscope to loosen the stage if needed. However, there should be no need to make any adjustments to the stage. **If in doubt, ask Teri for help!!
- There are two black-tipped metal shafts located on the top right of the microscope (close to the front). The bottom one sends light to the camera. When it is pulled out, the light is sent to the microscope. When the shaft is pushed in, the light is sent to the camera.
- There are 3 switches on the bottom right-hand side of the microscope. These constitute the 'Neutral Density Filters'. These are used to adjust the brightness of the image. The three switches are labeled 'ND8', 'ND32' and 'NCB11'. They can either be 'In' or 'Out' depending on the brightness needed. Please note the position of these switches for each picture.

- There is also a filter wheel that should be set to the light microscope filter. It is currently set to '3'. The microscope is equipped with a dark field option that can be used in cases of radioactive in situ hybridization. (There are also spaces on the filter wheel for placement of fluorescent filters).
- There also are "Differential Interference Optics" (DIC) for the 10, 20 and 40x objectives. The degree of DIC can be adjusted using the wheel on the top of the condenser. These add dimentionality to the photographs.

To take pictures:
- Place the slide on the microscope with the section side up. There is a guide wire that will help to place it correctly.
- Move the stage if necessary to straighten the image. If the stage does not move easily, don't force it! Be sure to check that the condenser is not jammed against the stage. To move the condenser up and down, there is a small dial under the condenser disk on the left.
- Push the bottom shaft in to send light to the camera
- Open up the IP Lab program. You will get a dialog box with the following options:
    o F1 – For a 1 x 1 fine resolution <u>color</u> picture; one plane (XY)
    o F2 – For a 1 x 1 fine resolution <u>black and white</u> picture; one plane (XY)
    o F3 – 'Z stack' – Takes pictures in and out of different planes of focus; Computer picks the best images and puts them together; multi-plane (XYZ)
    o F4 – '4 x 4 Grab' – For low resolution pictures
    o F5 – Crop
    o F15 – Set path for Save

- <u>For color pictures</u>, click 'F1' in the dialog box.
- You will get another dialog box saying to 'Send light to the camera'. Click 'OK'
- The image on the screen is what the picture will be. You can now make adjustments to the image. When focusing the image, be aware that there is a bit of a delay so move the focus dial slowly. Add or subtract magnification by turning the magnification dial. Use the Neutral Density Filter switches at any magnification to adjust the brightness of the image. (Be sure to do this before clicking the autoexposure option explained below).
- There will also be another 'Preview' dialog box that will appear for modifying and "grabbing" the picture.
    o In this box, the exposure time has been preset to 200, but you can also click "Auto" for auto exposure by the computer. (Be sure to note the exposure time if you choose this option!!).
    o There is also a magnification bar in the dialog box that is set so that the entire image can be seen on the computer screen. (This has no bearing on the actual magnification of the image). You can use this magnification bar to help focus the image by increasing the image on the screen, focusing the image and then returning the magnification bar to the default setting.

- o Click 'Color Balance' to get rid of the bluish tint on the image.
- Click 'Grab'. You will get a dialog box titled 'Alert' regarding white balance for the picture. Click 'OK'. A "Script Status" box will appear. Use the cursor to make a small square in a white or blank area in the background. Click 'Continue' in the Script Status box.
- The result is an untitled picture. To save this picture, click the 'F15 – Set Path for Save' option. You should have already created a folder on the Desktop for your work. Label each picture and save to your folder.
- With the color picture option (F1), you will get 2 pictures: The top picture is the adjusted, sharpened picture that can be used for printing, posters, etc. The bottom picture is the "raw" picture that has not been adjusted. This image can be adjusted in Photoshop later. You have the option of saving both depending on your needs.
- For black and white images: Click the F2 option. Adjust the image and grab the picture as described above. In this case, you only get one picture instead of two. You should always save the raw image for black and white pictures. When you open black and white pictures in Photoshop, click the 'grayscale' option, as this will make the pictures smaller. If you wish to move the picture directly to PowerPoint, choose the 'Normalize Image' option when prompted.
- All pictures should be saved as TIFF files.
- You will need to take pictures of the calibration bar for each objective. The calibration slides are located in the drawer to the lower left of the microscope. Be sure that the magnification dial is set to '1'. You will only have to do this once and can save these images for future experiments. However, if you choose to take a picture after modifying the image with the magnification dial, you will need to take a picture of the calibration bar under these particular settings. Example: If you are on the 2x objective and you change the magnification dial to 1.4x, you will have to take a picture of the calibration grids for the new 2.8x magnification setting. (2x objective magnification * 1.4x on the magnification dial = 2.8x magnification setting).
- You can use CDs, memory sticks or LaCie drives to save pictures (No Zips). You can use the 'Toast 6 Titanium' program to burn pictures onto Recordable CDs in different sessions (instead of all at once).
- To save:
  - o Open the Roxio Toast 6 Titanium program and the Desktop folder with your files
  - o Make sure the data option is selected in the Toast 6 Titanium program
  - o The data will be saved with a Pioneer DVD-RW-DVR
  - o Select the files to be saved on disk in the Desktop folder and then drag and drop the files into the Roxio Toast 6 file
  - o Push the red button on the bottom right of the Toast 6 dialog box.
  - o When prompted, eject the CD tray and insert disk
  - o Click 'Record'. Depending on the number of images you have, the time to save all of the data can take up to 3 minutes.
  - o After recording the data, click 'Verify'.
  - o When everything is finished, verify that your images have been burned to the CD prior to deleting the file. After verification, eject the disk. Close

the CD tray by clicking the button on the top right-hand corner of the keyboard or go to the toolbar at the top of the page, choose 'Recorder' and then 'Close Tray'.
  o Close Toast 6 Titanium program
- When finished, close the IP Lab program. Delete the folder you created from the Desktop by dragging the folder to the Trashcan icon. Turn off the microscope and place the protective covering over it.

Things to remember:
- **When you change an objective, make sure the corresponding condenser is set**
- **Always record the exact magnification, exposure time, neutral density filters and any other modifications you make for each picture**
- **Pictures of the calibration grids will have to be taken for each objective you use. This can be done once and saved for future experiments. However, if there are any adjustments made to the magnification dial, you will need to take a new picture of the calibration grids under these particular settings.**