

Taking Pix

Startup

- 1.) Open Door to Chemostat Room – thermostat is in there and controls temperature in microscope room
- 2.) Turn on all parts of the scope → Lamp, shutter, scope, and camera: The lamp takes about 10 minutes to warm up (The LED on the lamp will stop flashing once it has warmed up).
- 3.) Log into computer and open Slidebook.

Setup

- 4.) Turn lamp up to 80% intensity by pressing the up button on the X-Cite lamp box until it reaches 80.
- 5.) On the computer, open focus window in Slidebook (the F with a circle around it from the buttons across the top of the window). Choose the 100x objective by clicking on the icon.
- 6.) Go to scope and put a drop of Olympus oil on the objective. Place slide face down in the holder – need to pull upper left corner locking mechanism back, place slide down, and gently release mechanism to lock slide in place.
- 7.) Use stage adjustment buttons on scope to bring objective with oil up into contact with slide – buttons are located on the front of the scope and are labeled as FOCUS with up and down arrows next to each button. You can tell when the oil comes into contact with the slide because the oil will start to spread out against the slide.
- 8.) Go back to focus window and click on the DAPI button to put the DAPI filter in place to find the cells, and click “Open Fluor” to turn the fluorescent lamp on. On the front of the scope next to the focus buttons is another button that switches the path of the light between the eyepieces and the camera. Press the button so that the light next to the eye is lit up.
- 9.) Use the knobs on the sides of the scope to focus the image. You can tell the image is in focus when a purple, rectangular box becomes illuminated.
- 10.) Once cells are focused press the light path button again so the light next to the camera is lit. Go back to the focus window and press start, and the focused image should come up in the live video window.

Setting Points

- 11.) Use the joystick to move around the slide. The more pressure used on the joystick the faster the stage will move. Check the position on the slide every now and then to ensure objective is still on the slide/coverlip. There are two options for controlling the z – you can use the knobs on the sides of the scope or the focus buttons at the bottom of the focus window. They are incremented from 10 microns to 0.1 microns, so use whichever option you prefer.
- 12.) Once an appropriate field has been found find the best plane of focus. Then click on the filter, which corresponds to the control dye, and find the best focus plane. Once the appropriate plane has been found, choose the XY tab in the focus window and click on set point. This saves the exact XYZ location of the point.
- 13.) Once all points of interest have been set, turn off the fluorescent light by clicking on “close fluor” in the focus window and stop the live video by clicking on “stop.”

Capture Window

- 14.) Click on the camera icon in the row of buttons at the top of the screen (next to the focus window button). This will first ask if you want to save your file to a specific spot. Save to the FISH folder using the appropriate naming system (Exp#_cy3gene_cy3.5gene_cy5gene). After saving the file, the capture window will come up.
- 15.) In the upper left hand corner of the capture window there is a pull down menu. Click on D.5_33_3.51_53 option from the pull down menu. This will bring up the saved settings for taking pictures.
- 16.) Make sure the following settings are chosen:
 - a. **Multipoint List**
 - b. **Range around Reference** with a total distance of 5 μm , 25 planes, and a 0.2 μm step size.
- 17.) At the bottom of the capture window there is a naming option. Give this the same name given above (all of the fields will be referred to by different position numbers).
- 18.) Recheck to make sure all of the appropriate options are chosen and press START. If possible, watch the first stack and make sure that everything works accordingly.
- 19.) Once acquisition is finished save Slidebook file and export – Image \rightarrow Export \rightarrow Export Channel Intensities as a 16-bit TIFF

Select destination and adjust name of file by doing the following:
Delete everything except %N_C%c – this will give the image name and the channel name.

Note: you may export before saving, and you can start another set of images. If the export is to a backed-up server, you may opt to not save the Slidebook file.

Closing Up

- 20.) After pictures are done being taken, be sure to click save icon button in the row of buttons at the top of the slidebook page.
- 21.) Use focus button on the front of the scope to lower objective from slide, and remove slide from stage. Wipe oil from objective using lens paper.
- 22.) Be sure to close slidebook before shutting down any of the equipment. Turn off lamp, shutter, scope, and camera, and log off computer.