

H&E stained samples/Brightfield:

Make sure the **Koehler** illumination is setup. Make sure the DIC cube #424921 is out in one of the two filter boxes, if not take out the DAPI/FITC/Rhodamine filter turret under the objective section of the microscope and remove this filter from the position 5. To restore the histogram on the live window, uncheck the "shading correction" "enable" in the camera control tab "General", click on the "3200 K" and "Automatic" couple of times and also clicking "Autowhite balance" icon in the live window, clicking all of these three few times will restore/recover the histogram.

Automated DIC:

Make sure the DIC cube #424921 is installed in the DAPI/FITC/Rhodamine filter turret at position 5. Make sure the Polarizer is engaged above the condenser, both the prisms (one in the condenser and the other underneath the objective) are engaged and finally see if the person is using the "During Acquisition" set up containing the DICII (for 20x objective) or DICIII (for 40 and 63x objectives) methods. Note that the fluorescence image and the DIC image will not merge ideally because of the imperfection on the polarizer in the cube. But there is a "shift" routine available under 'Processing" menu, "Utilities" to correct the shift.

Manual DIC:

There is no need to install the DIC cube #424921. Note shift free images could be produced using the manual DIC slider (Analyzer) but note that cannot be used under automatic dual channel acquisition technique. This image should be taken manually by sliding in and out the Analyzer located on the left side of the scope, inside the chamber with adjustable polarizing angles. Make sure your setting in this produce optimum DIC effect and leave it at that position and this setting may different depending on your sample. Make sure it is disengaged, when you take fluorescent images as leaving the slide engaged will significantly reduce the fluorescence intensity of the sample going to the camera.

CO2 and Temperature Control Setup:

Some appointments put a request in the online calendar saying "Please turn on CO2 and Temp". In this case do the following.

Open the main valve of the CO2 cylinder, Turn on the CO2 controller power on the back, Turn on the Temp controller power on the front (located on the left side table of the microscope, turn on the green power button on the heating block (located on the left side of the microscope table itself), close all the doors and windows of the chamber covering the microscope. Make sure the temperature is reaching around 37 degrees and the CO2 indicator shows around 5 in 30 min.

VERY IMPORTANT!

Turn off only the heating block, CO2 controller and Shut the main valve of the cylinder when the imaging is done, the user should NOT turn off the TEMP CONTROLLER until it reads around 23 degrees C. We normally leave the TEMP CONTROLLER ON overnight and let it completely come down to ambient temp before turning it off. Failure to do so might blow the fuse in the heating block and should be returned to GERMANY for fixing.

NIR (685/780 nm) Diode laser set up:

First bring the Iris of the XCite 120 fluorescent lamp all the way down (do not turn off), take out the liquid light guide from the back of the microscope (after loosening the small screw holding it) and secure it on the scope table under a tape, take the other liquid light guide (yellow taped on the frame of the scope located on the back of the scope) and insert in the light path and secure using the same small screws, remove the DAPI/FITC/Rhodamine filter turret and remove the DAPI filter and insert the 685 filter set (at position number 1) located in one of the plastic boxes, same way, remove the FITC filter set and insert the 780 filter set (at position number 2) located in another plastic box, install the turret and turn on the DIODE power button on the laser holder, move the liquid light guide to appropriate wavelength and load the Ed ROY_NIR experiment and click the 685 (blue icon) "go" or 780 green icon "go" to get the signal. Use the PVCAM as the detector and adjust the exposures. Note these images cannot be taken automatically as one has to move the light source manually, take those two images separately and store. Also if you wish to have DIC images of the same positions install either the DIC cube filter #424921 in position 5 or use the "Analyzer" manually while taking the DIC images. In this case all three images need to be stored individually.

VERY IMPORTANT:

Bring the setup back to the original conditions after this appointment, i.e., remove the light guide from NIR source to XCite, remove the 685 and 780 filter sets and put back the DAPI and FITC filters in position, remove the DIC filter from position 5 (if used), install the filter turret, turn of the NIR main power.

As of June 16 2008: Questions? call Shiv 333-1214, 333-9896, 417-9593(cell)