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CBIS Widefield Fluorescence/Darkfield/TIRF system

WIDEFIELD FLUORESCENCE USER GUIDE

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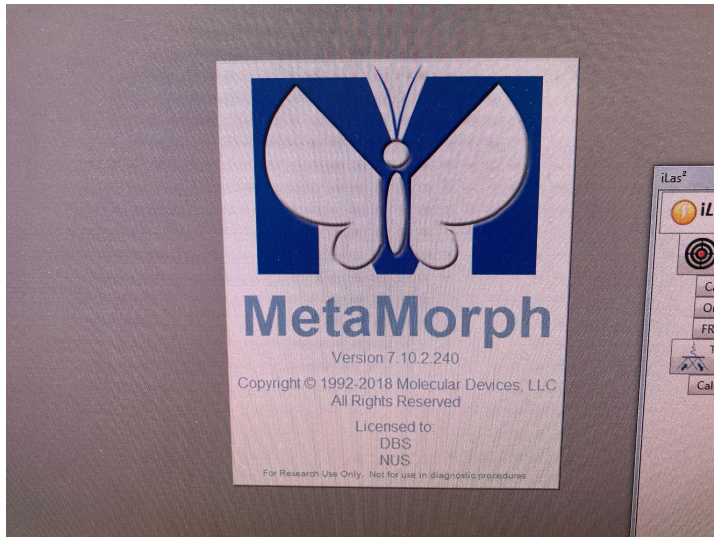


Turn on the WF switch on the wall. Leave the LASER switch off. Wait a few minutes for the camera to initialize, then turn on the computer.

Meanwhile, mount your sample. Remember coverslip face down.



This knob on the front of the microscope should be in this position (1x).



Launch MetaMorph software. Watch for the MetaMorph logo to appear in the software window (above). If it doesn't appear, it hasn't detected the camera and you have to restart the computer.



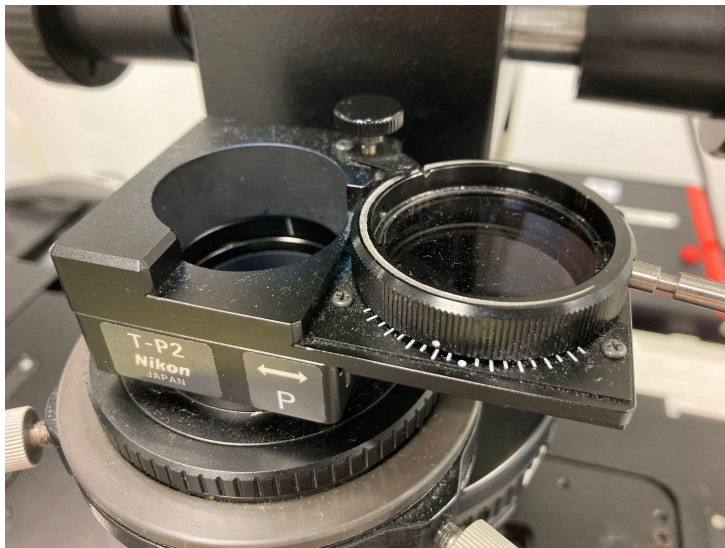
Select objective in the software (instead of the microscope touch screen) so the software will embed the correct scale information in the image.

TRANSMITTED LIGHT

If you don't need transmitted light, skip to the FLUORESCENCE section.



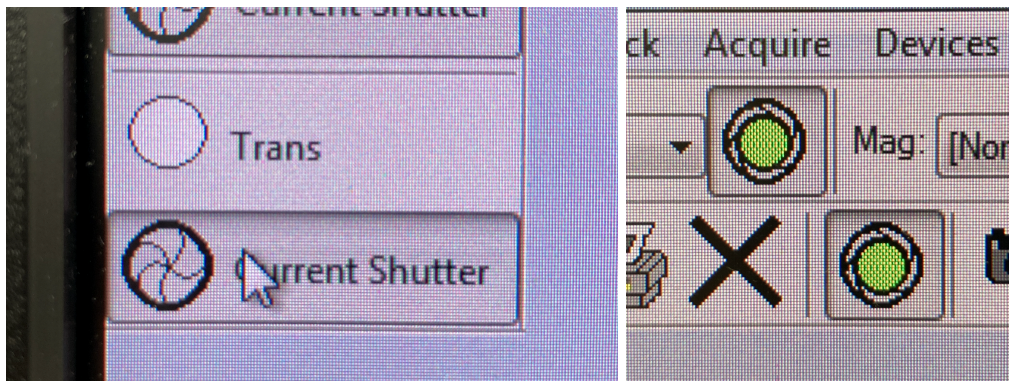
Swap in the bright-field condenser if necessary. Remember to align the notch at the back so it doesn't rotate, and tighten the set screw securely. Rotate condenser turret to position A.



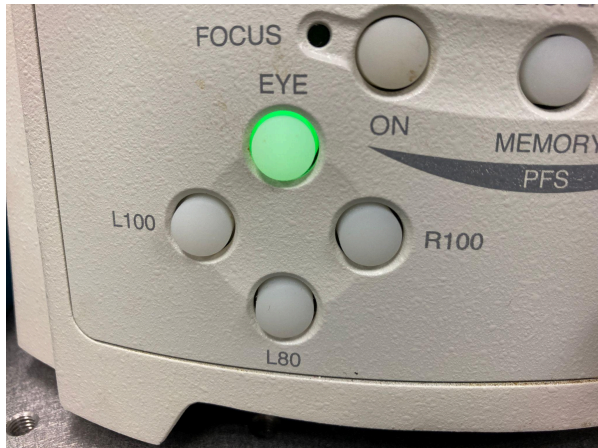
Polarizer should be out of the light path.



On the LED control panel, flip the right-hand switch to EXT for control using the software (below), or ON if you want to control it directly from the panel. The right-hand black slider controls brightness.



Click Trans followed by Current Shutter at lower left. The shutter icons at the top of the window should turn green.



Press the EYE button on the microscope if you want to use the eyepieces, or L100 if you want to view image on camera.

See later for camera operation.

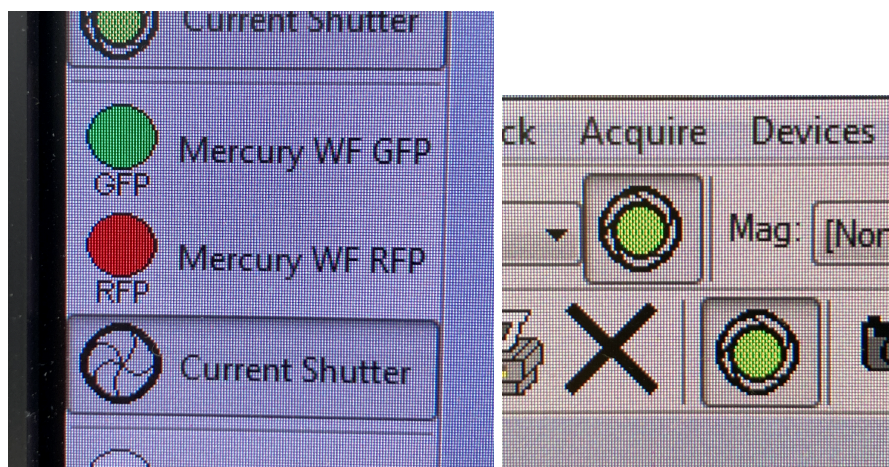
Focus the sample and perform Köhler illumination.

FLUORESCENCE

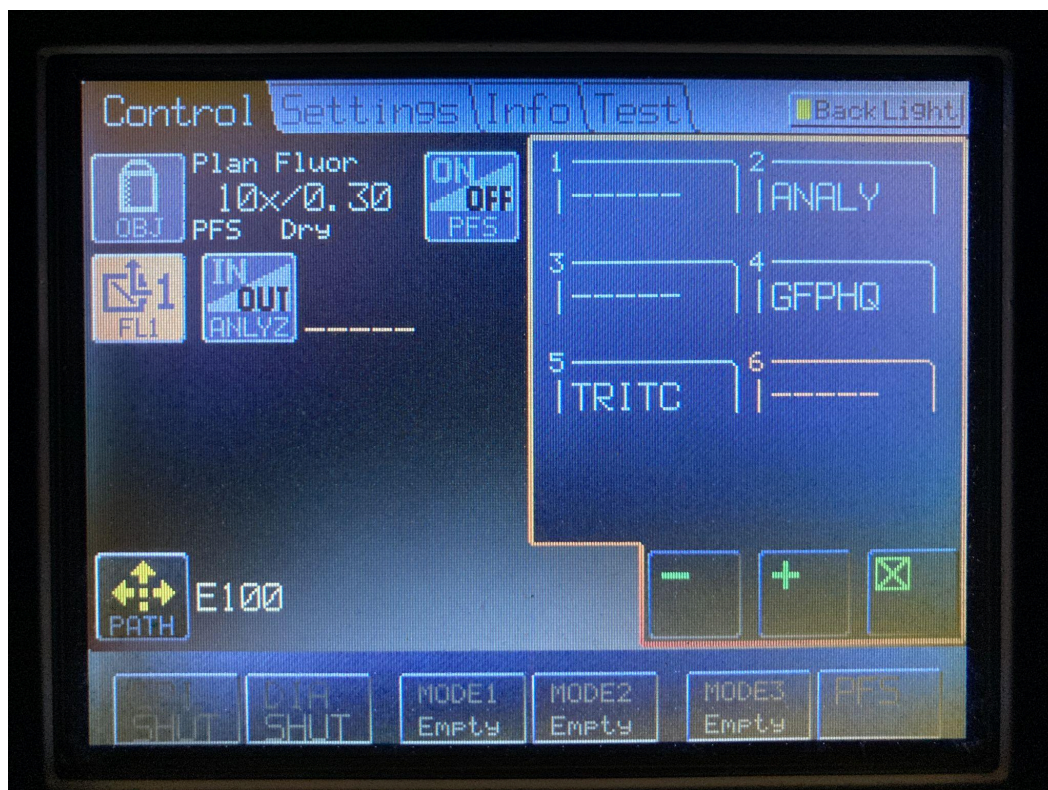
If you were using transmitted light, remember to turn off the light if you were controlling it directly from the panel.



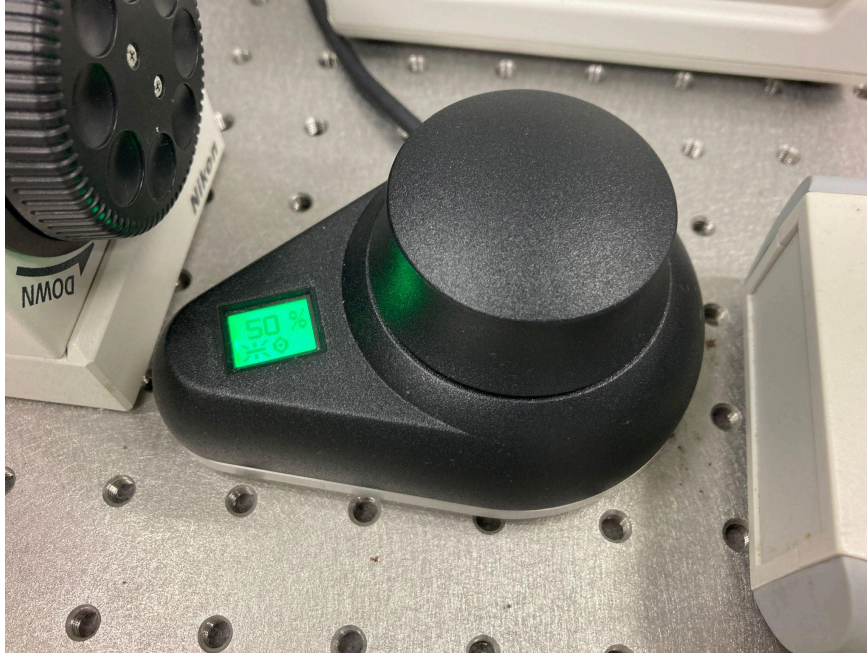
Push in the pin at the back of the microscope.



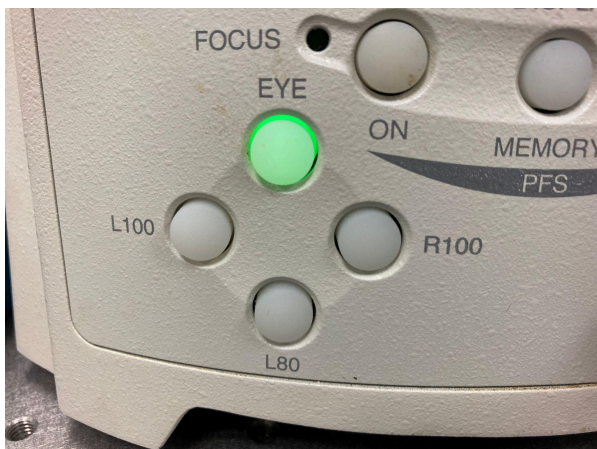
Click the required excitation wavelength at lower left followed by Current Shutter. The shutter icons at the top of the window should turn green. For DAPI see below.



For DAPI, press the FL1 button on the microscope touch screen and press position 6.



Tap this knob to turn the excitation light on and off (it is automatically controlled if you select excitation in software). Rotate the knob to change brightness. The small green panel displays the status.



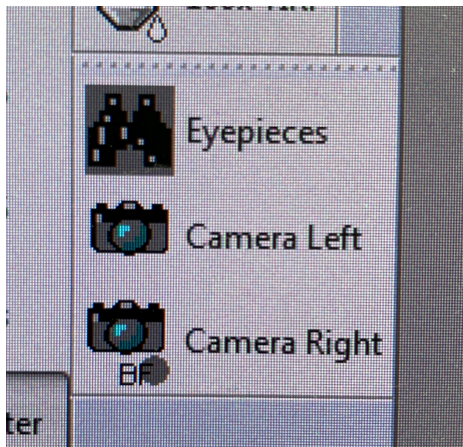
Press the EYE button on the microscope if you want to use the eyepieces, or L100 if you want to view image on camera.

See later for camera operation.

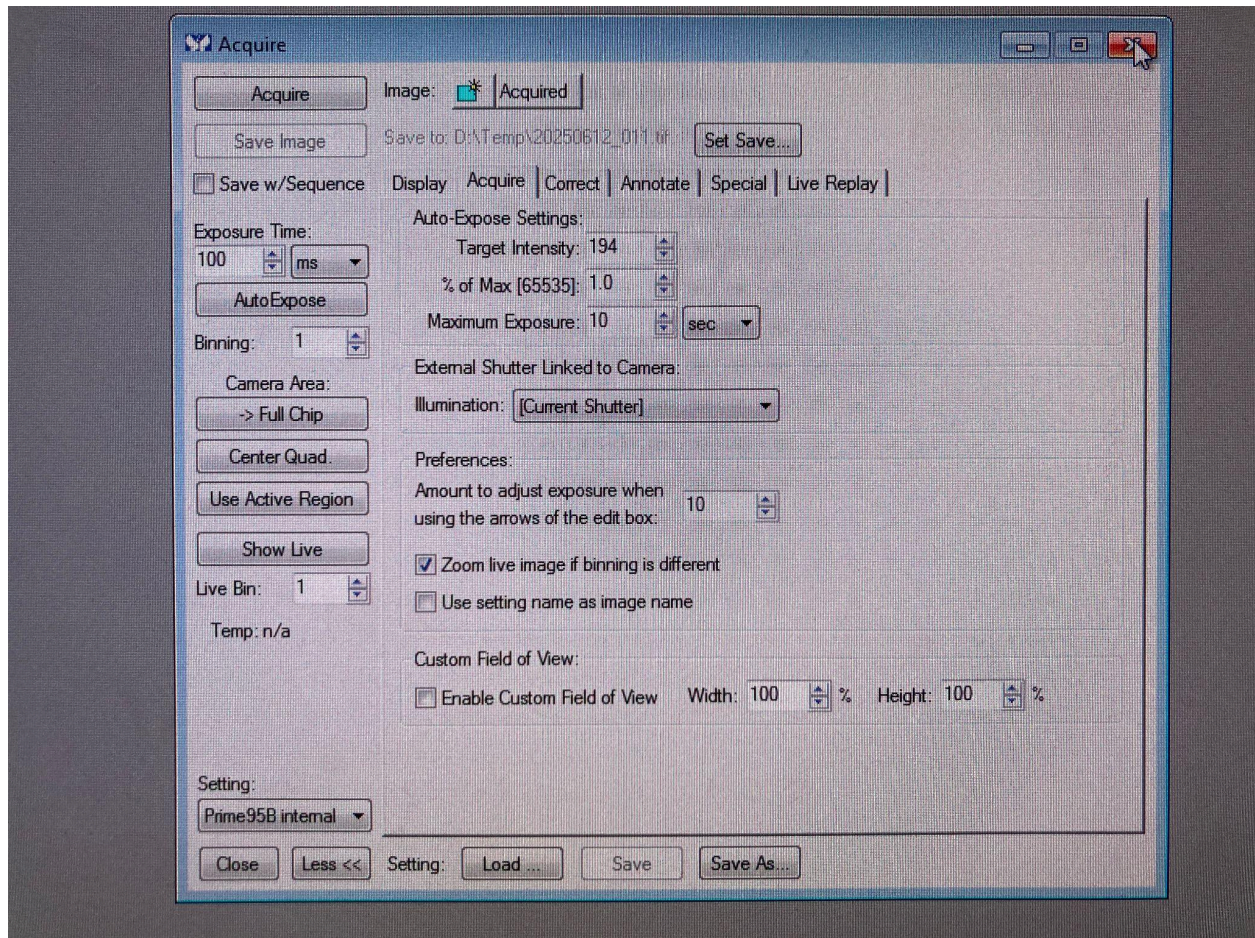


Focus the sample and perform Köhler illumination. The aperture levers are on the left and right sides of the microscope near the back.

CAMERA OPERATION



Click Camera Left in the software.



Go to Acquire --> Acquire in the menu. You can control Exposure Time here. Check that Binning and Live Bin are set to 1. Click Show Live or Acquire as desired.

Fine-tune sample focus.

Check histogram in image window and adjust its display range to assess exposure.

After acquiring an image, right click on it to save.