Arcturus^{XT}™ Laser Capture Microdissection System
Tips Sheet – FLUORESCENCE
Nikon Eclipse Ti-*E* Microscope Base

Prior to starting fluorescence experiment, check the following items:

1. The fiber optic cable is fully inserted into EXFO cone attached to scope.
2. The fiber optic cable is fully inserted into the back of the EXFO box.
3. Check the EXFO cone is properly seated into the insert attachment to the scope.
4. Shutter located in the fluorescence turret is in the Open "O" position [Figure 1A].
5. Analyzer and Polarizer (used with DIC) are in the "OUT" position.
6. DIC prisms are removed (found beneath 10x, 20x, 40x and 60x objectives when DIC option is installed).
7. Fluorescence aperture should be open (pulled out) and centered. If needed, center the aperture by using the 2 set screws [Figure 1B].
8. Neutral density fluorescence filters (there are 2), located behind the EXFO cone are in their "OUT" position [Figure 1C].

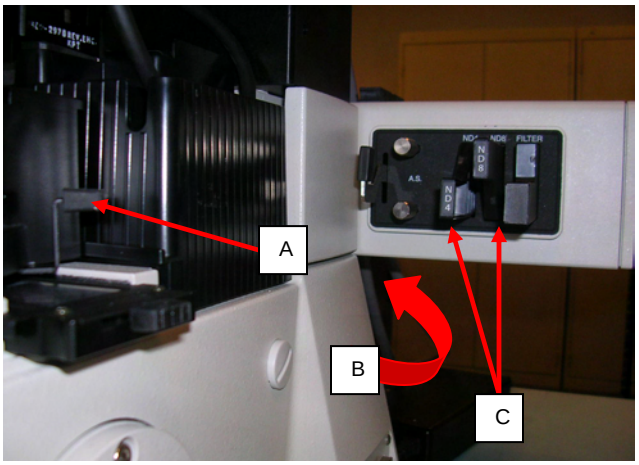

Figure 1. Fluorescence Components

Working with Fluorescently Labeled Samples:

1. Click on the fluorescence icon in the Inspect Tool Pane [Figure 2].
2. Open the Inspect Options dialog box; the fluorescence tab will automatically come up.
3. If Microscope Lamp Intensity is on, uncheck box to turn off [Figure 3A].
4. Rotate fluorescence turret to appropriate filter cube.
5. Locate signal:
   - 5.1 Set Fluorescence Lamp Intensity to 100% [Figure 3B].
   - 5.2 Set Camera Gain to maximum setting [Figure 3C].
   - 5.3 Adjust brightness setting (exposure time) in the Inspect panel until fluorescence signal is seen [Figure 4].
   - 5.4 Focus image.
   - 5.5 If needed, allow minimal brightfield light by clicking back on the microscope lamp [Figure 3A] and adjusting light accordingly.

Figure 4. Brightness (exposure time)

6. Once the sample has been located and focused, adjust the following to optimize image:
   - 6.1 **Camera Gain** [Figure 3C]: Adjust to the lowest possible value to still allow sufficient signal.
     Note: High values = increased image pixelation.

Figure 2. Fluorescence Icon

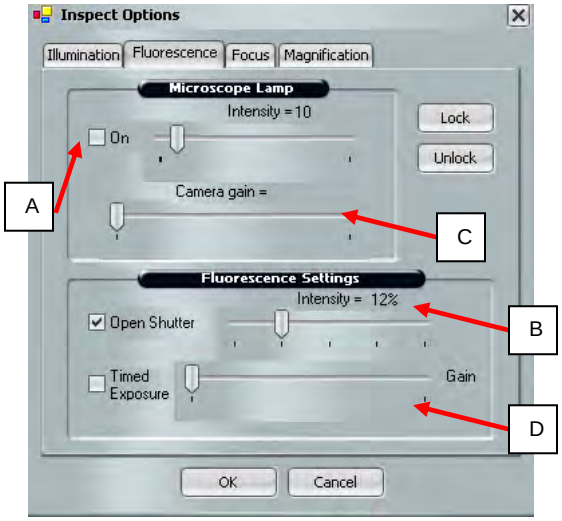

Figure 3. Fluorescence Components

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6.2 **Brightness** (exposure time) [Figure 4]: Set to 1s or less.
Note: Higher values will result in significant delay in live image updates.

6.3 **Fluorescence lamp intensity** [Figure 3B]: Lower intensity if a really bright fluorescence intensity is seen with the live image.

If need to toggle back and forth from fluorescence and brightfield illumination (i.e. to perform IR test fires):

1. Set Camera Gain [Figure 3C] for optimal fluorescence image and then adjust to appropriate microscope lamp intensity [Figure 3B].
   Note: Use the intensity setting found in the fluorescence tab and NOT the intensity setting found in the Illumination tab.
2. A good starting point for the Microscope Lamp Intensity setting is 30. Adjust from this point to get enough light as needed.

Important Notes:

1. Microscope Lamp Intensity and Camera Gain settings found in the Fluorescence tab are completely independent of those in the Illumination tab. That is, any changes to these settings while in the Fluorescence tab apply **only** while using fluorescence illumination. Once you switch off the fluorescence, the settings in the Illumination tab are applied.

2. Camera gain in the fluorescence tab controls the gain of both brightfield and fluorescence illumination.

3. Always keep the Microscope Lamp Intensity found in the fluorescent tab OFF when not in use. If it is On even at a minimal setting, it will contribute to the photobleaching of the sample.

4. The Gain setting found next to Timed Exposure [Figure 3D] is used only with this feature. Adjustment of this setting outside of Timed Exposure will NOT result in a change.

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