Imaging Protocol for FISH

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1) Open Image-Pro Plus 7.0. Under the Acquire Heading choose Video Digital Capture

2) Under the Macro heading choose the macro management subtitle. A window will open up. Click on change and choose the Digital_Probe.ipm file. This file should have macros suitable for imaging.

3) The macro Save saves images in order DAPI first then CY3. Thus the DAPI image has to be the last image taken. It saves the images in the C:\Imaging folder as DAPI#1, CY3#1 to DAPI#12, CY3#12. In order to save images, go to the macro header and choose Probe_Save.

4) Make sure the imaging folder is open so you can see the images saved. Also open the folder where you want the images moved to. After each set of 6-12, you need to move the images as the macro will overwrite the images and will NOT prompt.

5) The Exp Pvw and Exp Acq should be set to either 00.100.000 ss.mmm.μμμ OR 00.050.000 ss.mmm.μμμ. If not, select the more button and under select setting switch to Probe Imaging. This defaults to one of the above.

6) Turn the microscope on and get the slides that need imaging. I usually do one probe at a time, leaving the rest in the fridge or freezer.

7) The following instructions are for the Olympus AX70 and Qimaging digital camera so can be adapted for your system.

8) The slide under UV light (Filter Cube B) is usually very bright and so four methods are used to darken the image.

   Neutral density filter (one at the back of the scope) is always in
   The AX_AN filter (analyser for Nemarsky) is usually in but can be left out
   In Image-Pro Plus the video capture window must be on the more setting with no integration clicked
   The filter cube must be set to B – UV light.

9) The slide under CY3 light (Filter Cube A) is usually very dark and so four methods are used to lighten the image.

   Neutral density filter is always out
   The AX_AN filter (analyser for Nemarsky) is always out
   In Image-Pro Plus the video capture window must be accumulate
   Set by frames
   - Use 2-4 frames for Alt, Vibrio, Roseo and Neg
   - Use 5-7 frames for SAR11 and Eubac
   - REM that this is usual and might need adjusting for your specific slide.

   The filter cube must be set to A – NG light.

10) Snap 12 images (six sets). I usually take CY3, Probe first and then DAPI so they are in pairs. If you mess up, try to take a second picture so you delete in pairs. OR you can delete the bad files and save the pairs taken and start again in another folder (more, extra).

11) Save files using Save in the marco. These will save to the imaging folder on the c drive.

12) Move these images to the correct folder. They will be called Image#1DAPI, Image#1CY3 etc. Then start on the next filter.

13) Imaging usually takes 1 hour to do 8 filters. It can be speeded up a little (1hour to do 12 filters).