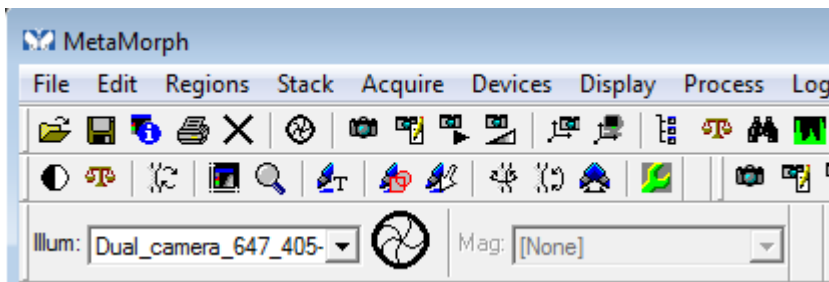


*Simultaneous dual-channel acquisition on the Roper iLas2 SMLM*

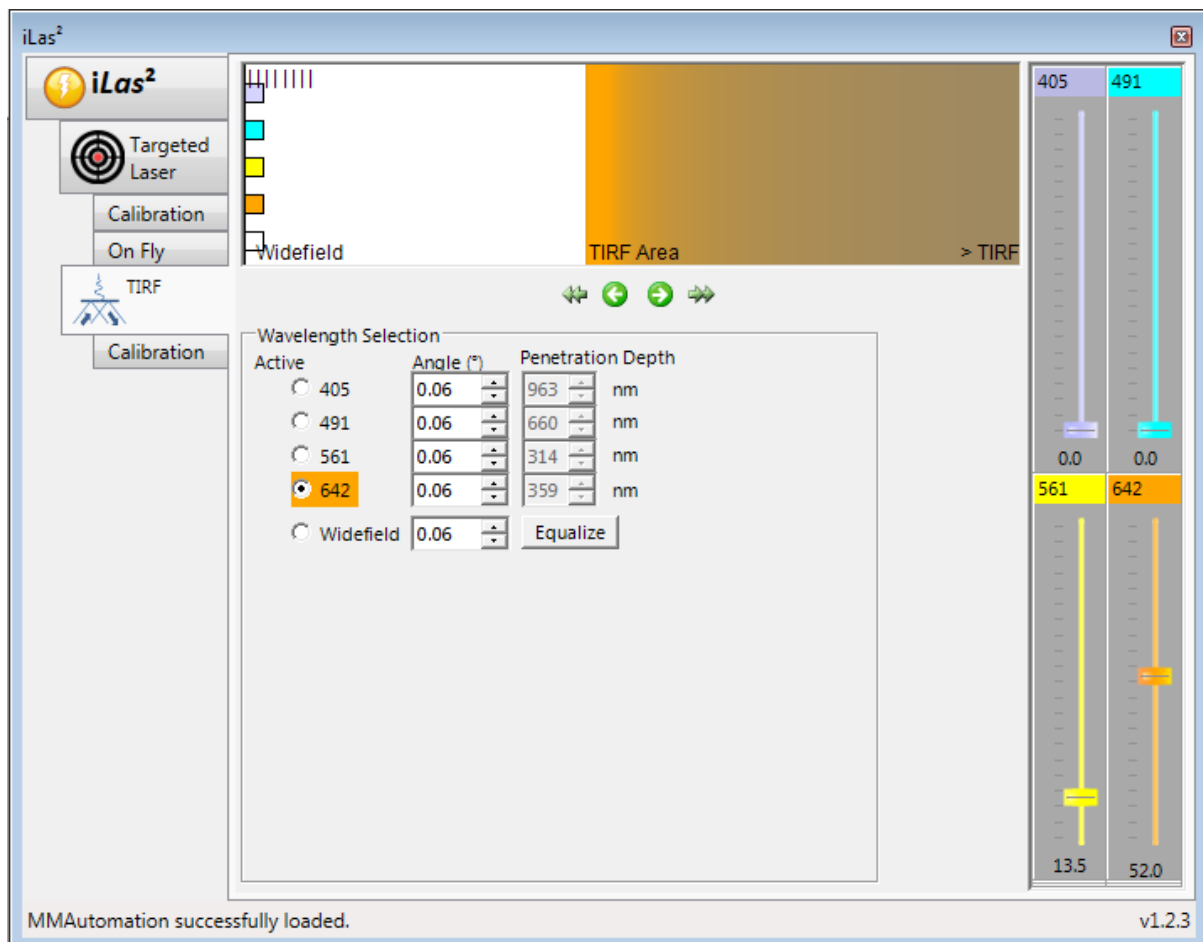


Cameras: 2 x Photometrics Evolve 512 Delta EMCCD cameras

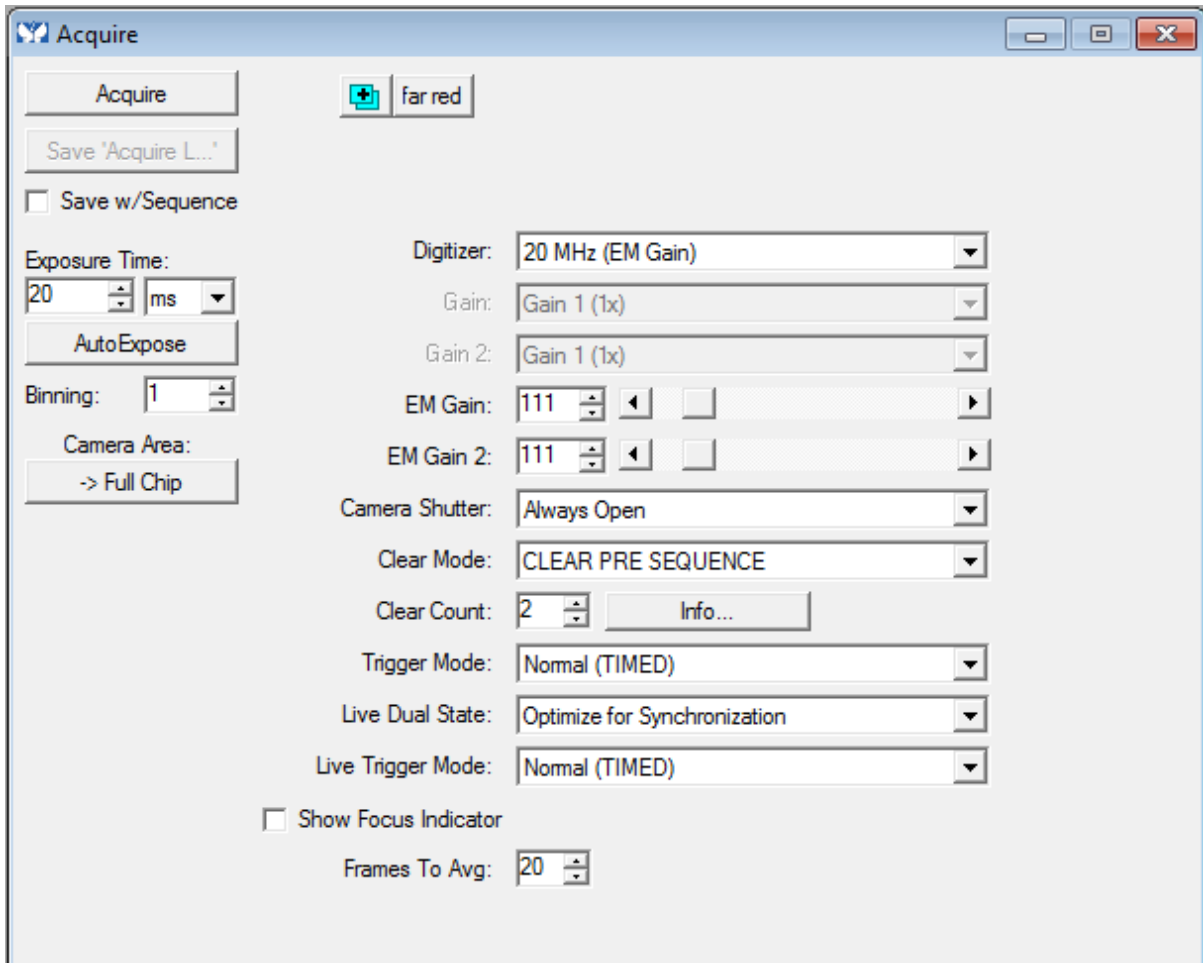
When doing dual-channel acquisition, please choose "Dual\_camera\_647\_405-491-561" for the Illumination setting:



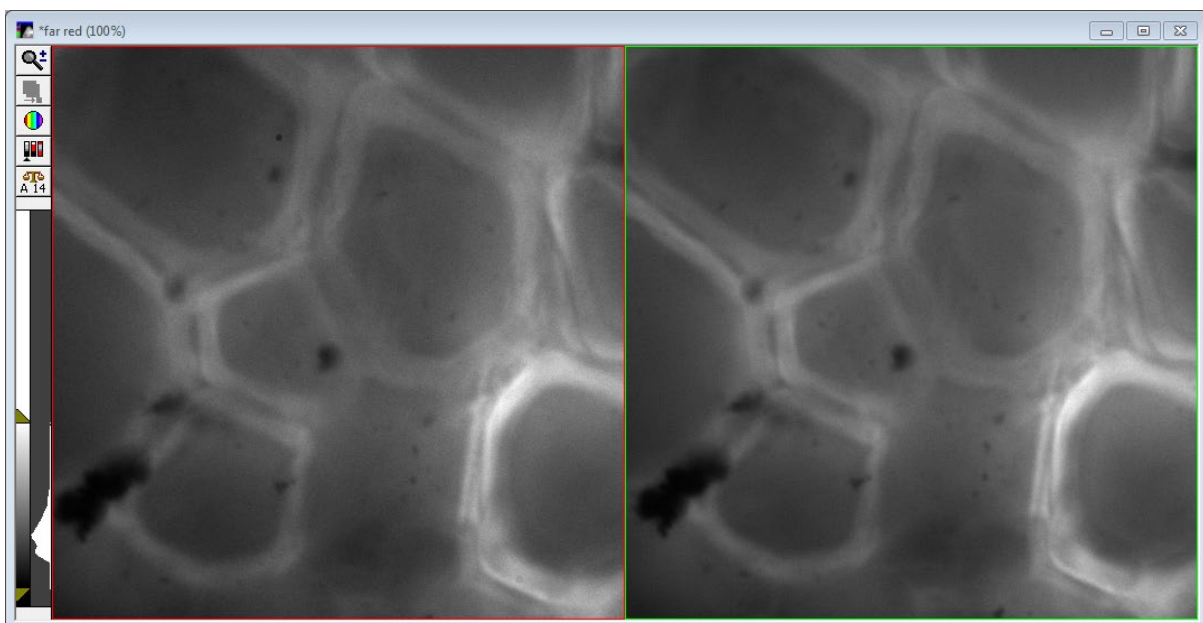
This lets through all four laser lines. In the iLas2 GUI, use the wide-field slider (white) to control the TIRF angle. The TIRF angle will be the same for all four lasers. \*\*\*For best results, please create a new calibration file.\*\*\*



To enable dual-camera acquisition, go to Acquire > Set Acquisition Channel > EVOLVE1 & EVOLVE2 - Monochrome 16-bit. When you now press Acquire (Acquire > Acquire), the two cameras are available simultaneously in the resulting window.

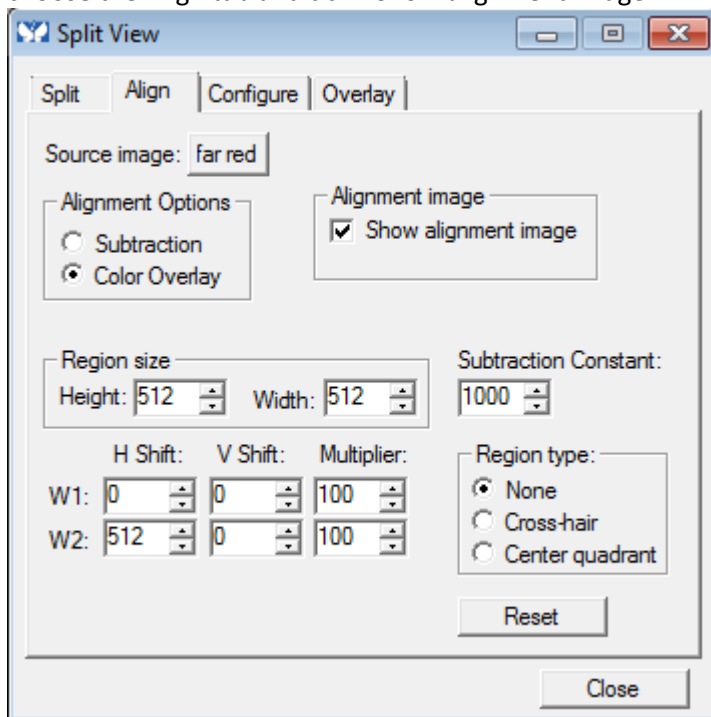


When you press Show Live, MetaMorph will now show the two camera views:

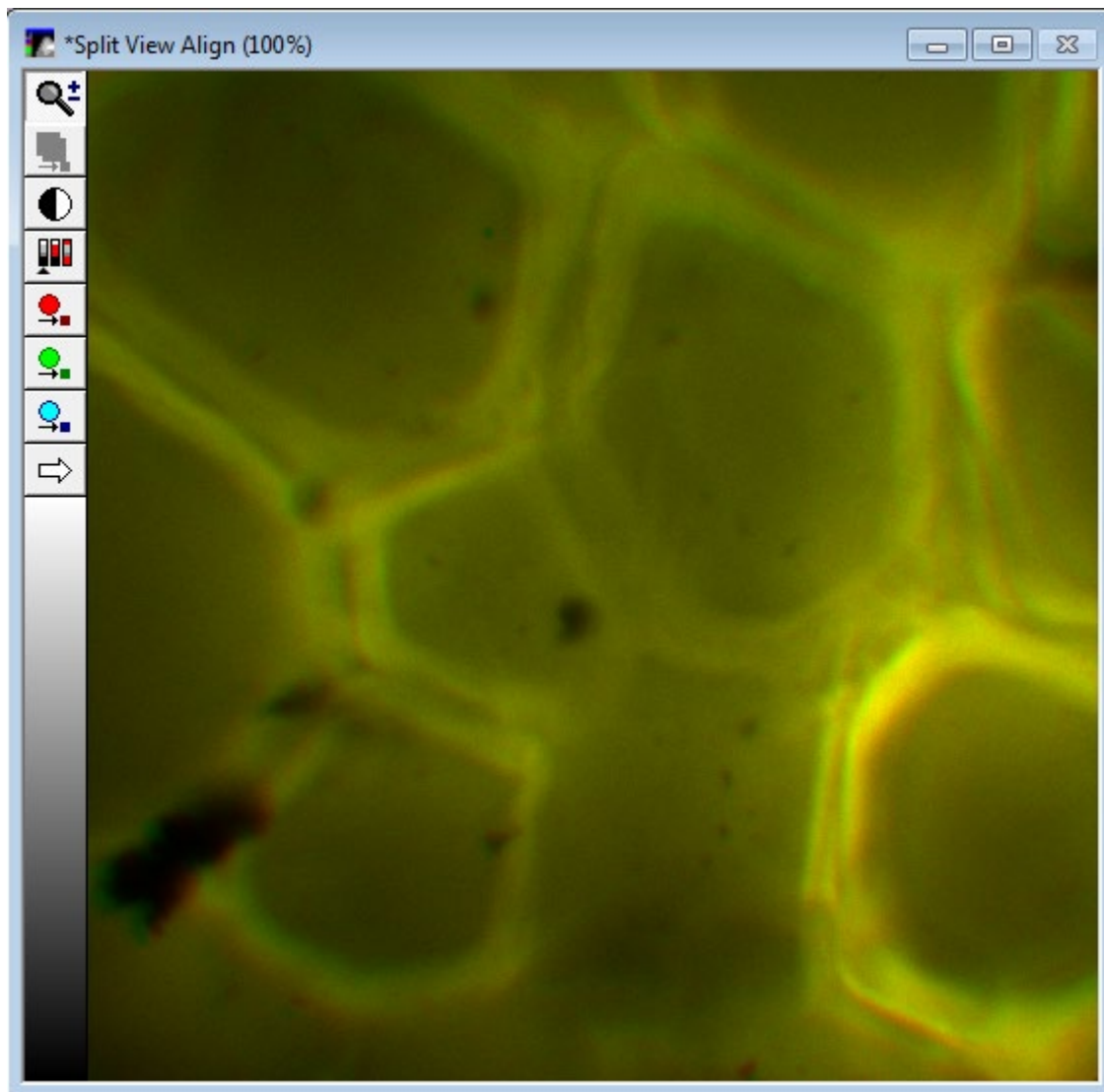


\*\*\*If you wish to stop live view, press Stop Live twice.\*\*\*

It could be helpful to overlay the images. You can do this by going to Display > Split View then choose the Align tab and tick "Show alignment image":



This then shows a two-channel overlay:



There are therefore two sets of optical filters on the Roper iLas2:

(1) This set is in the microscope body itself:

illumination dichroic = QUAD dichroic, Chroma ZT405/488/561/647rpc

emission filter = QUAD emission filter, Chroma ZET405/488/561/640m

(2a) This set is in the dual-camera splitter (which one can change, depending on the spectral split required):

"Normal" far red vs blue/green/red split:

emission path dichroic = Chroma ZT647rdc-UF1 (reflects blue/green/red, transmits far red)

emission filter, Transmission path (beside laser launch) = Chroma ET690/50m (far red)

emission filter, Reflection path (beside iLas2 controller) = Chroma ZET405/488/561m

(blue/green/red).

(2b) Green vs red split:

emission path dichroic = Chroma T565lpxr (reflects blue/green, transmits red/far red)

emission filter, Transmission path = Chroma ET600/50m (red)

emission filter, Reflection path = Chroma ET540/40m (green).