

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.\*\*\*

iLas <sup>2</sup>						
iLas <sup>2</sup>	HIIIII ■ ■ • Widefield TIRF Area > TIRF	405	<b>491</b>			
Calibration	Image: Wavelength Selection       Penetration Depth         Active       Angle (*)       Penetration Depth         405       0.06       963       nm         491       0.06       963       nm         561       0.06       314       nm         642       0.06       359       nm         Midefield       0.06       Equalize	0.0	642			
MMAutomation successfully loaded. v1.2.3						

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.

Macquire			
Acquire Save 'Acquire L' Save w/Sequence	🔁 far red		
Exposure Time:	Digitizer:	20 MHz (EM Gain)	[
20 🕂 ms 💌	Gain:	Gain 1 (1x)	[
AutoExpose	Gain 2:	Gain 1 (1x)	[
Binning: 1 🕂	EM Gain:	111 🗄 💶 🕨 🕨	1
Camera Area:	EM Gain 2:	111 🕂 4 🔹 🕨 🕨	]
-> Fuil Chip	Camera Shutter:	Always Open 💌	[
	Clear Mode:	CLEAR PRE SEQUENCE	[
	Clear Count:	2 🔹 Info	
	Trigger Mode:	Normal (TIMED)	[
	Live Dual State:	Optimize for Synchronization	[
	Live Trigger Mode:	Normal (TIMED)	[
	Show Focus Indicator		
	Frames To Avg:	20 🕂	

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'':

Stand Split View				
Split Align Configure Overlay				
Source image:       far red         Alignment Options       Alignment image         Subtraction       Image         Color Overlay       Image				
Region size Height: 512 ÷ Width: 512 ÷	Subtraction Constant:			
H Shift:         V Shift:         Multiplier:           W1:         0         ↓         100         ↓           W2:         512         ↓         0         ↓         100         ↓	Region type: • None • Cross-hair • Center quadrant			
	Reset			
	Close			

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).