Nikon Training for the Ti2 Ax System

Contents

Hardware – components

Ax Components - Scan head, Controller and detectors
Ti2 – controller and microscope main body
Laser Bed
PC

Ax confocal scan head

Controller, laser bed and Detector stack

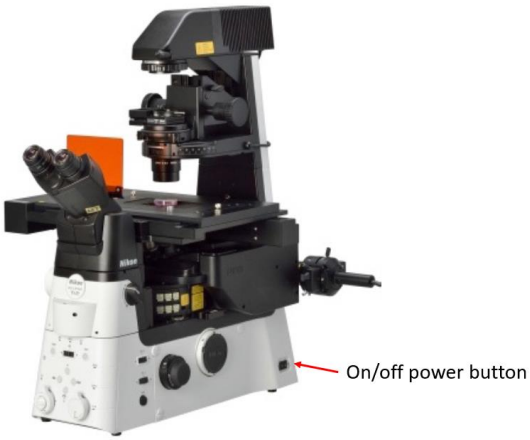
Ax detector unit

Laser bed

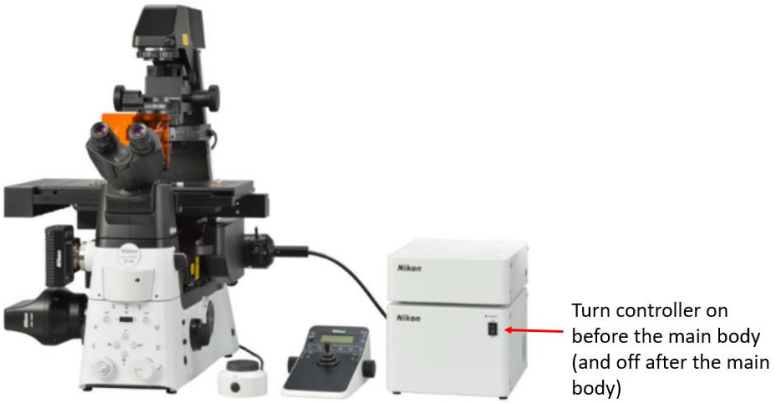
Ax controller

Press the power button on the front of the control unit

Ti2E Microscope main body



Ti2 Controller



Laser Bed

Press the power button on the rear of the laser unit and then turn the key

Z4 PC

Turning on the Equipment

1. Confocal controller (button on front of the unit at the bottom of the stack)
2. Laser Bed – Press power switch at the back of the unit (in the middle of the stack) then turn the key at the front.
3. Ti2 – controller and microscope main body – Turn on controller then the microscope. DO NOT leave a long gap between this.
4. PC
5. Start NIS Elements software

Turning off the Equipment

1. Turn off the NIS Elements software
2. Turn off the micrcoscope main body. Once the lights have stop flashing on the front of the microscope turn off the controller
3. Turn the key on the laser bed and then turn off the power button at the back
4. Turn of the button at the front of the controller
5. Turn off the PC

NIS Elements Software

Software interface

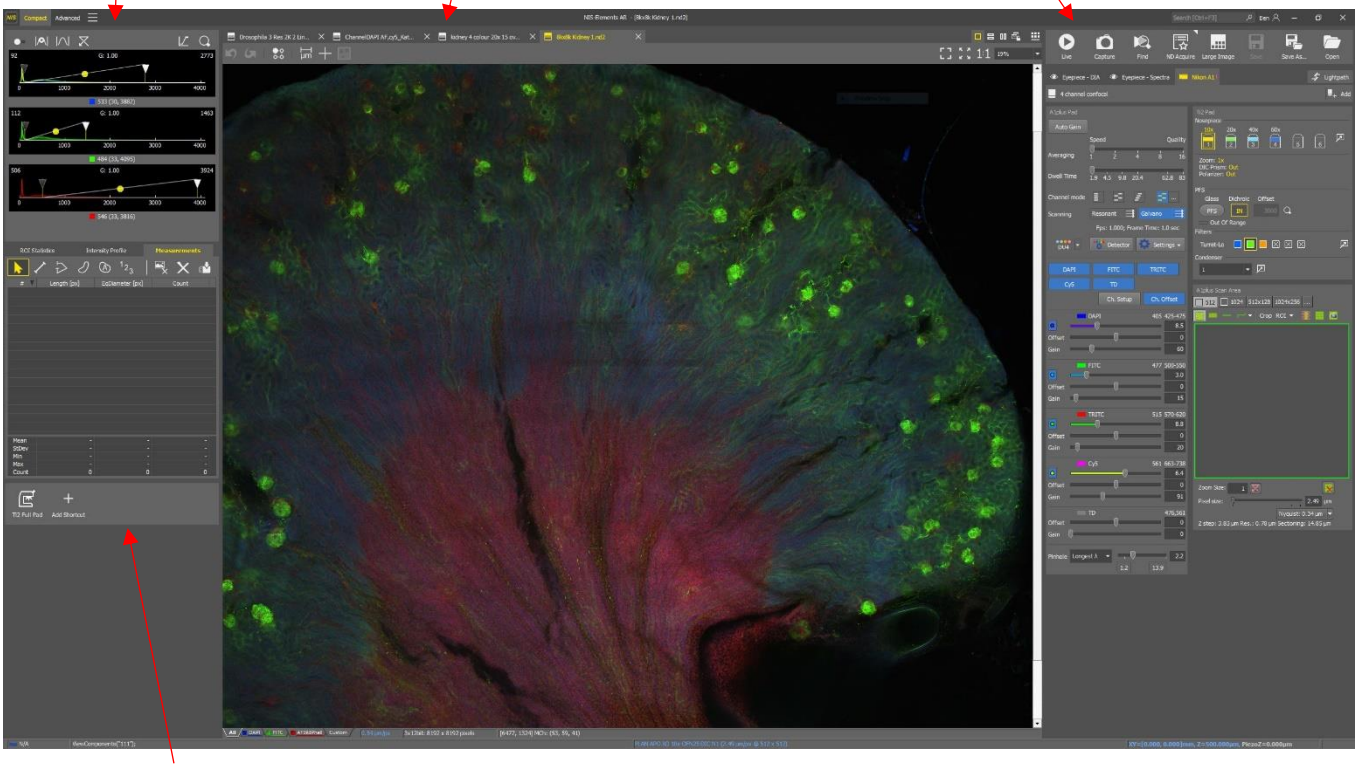
The NIS Elements interface is designed to be easy to use and flexible. There are two presets in the Ax confocal software; a fixed compact layout and an advanced layout that can be customized and added to as required.

Compact view

LUT – Look up

Multiple captured images tabbed for easy handling

Confocal Acquisition panel



Measurements and shortcut panel

Confocal Acquisition panel

Live – Start scanning

Capture single image

Find mode – uses line skipping to speed up acquisition for searching over a sample area

Lightpath selection

Open ND Acquisition

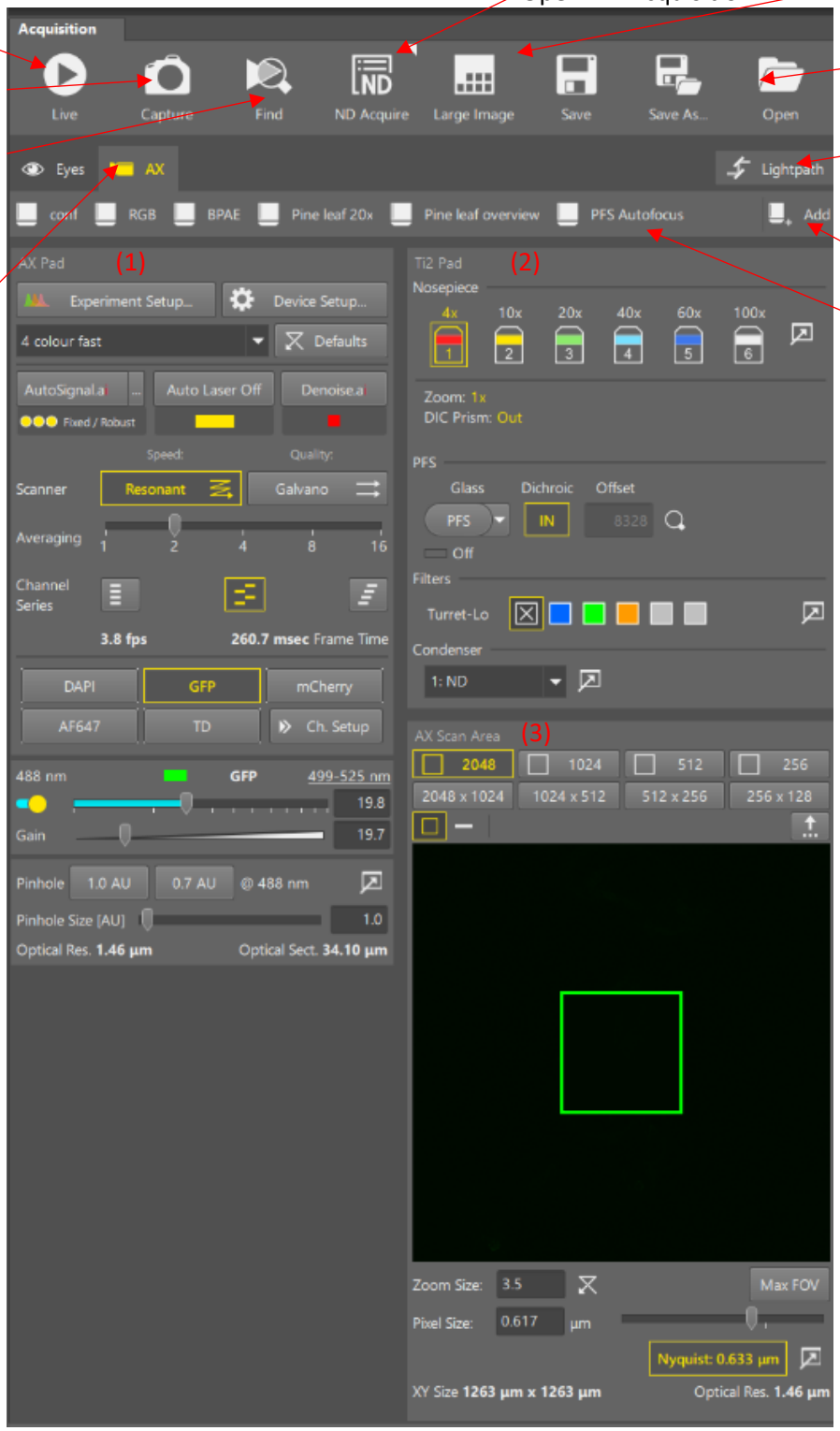
Open large image experimental setup

Save image/ open image folder

Opens up light path window

Create new OC

Optical configurations (OCS)



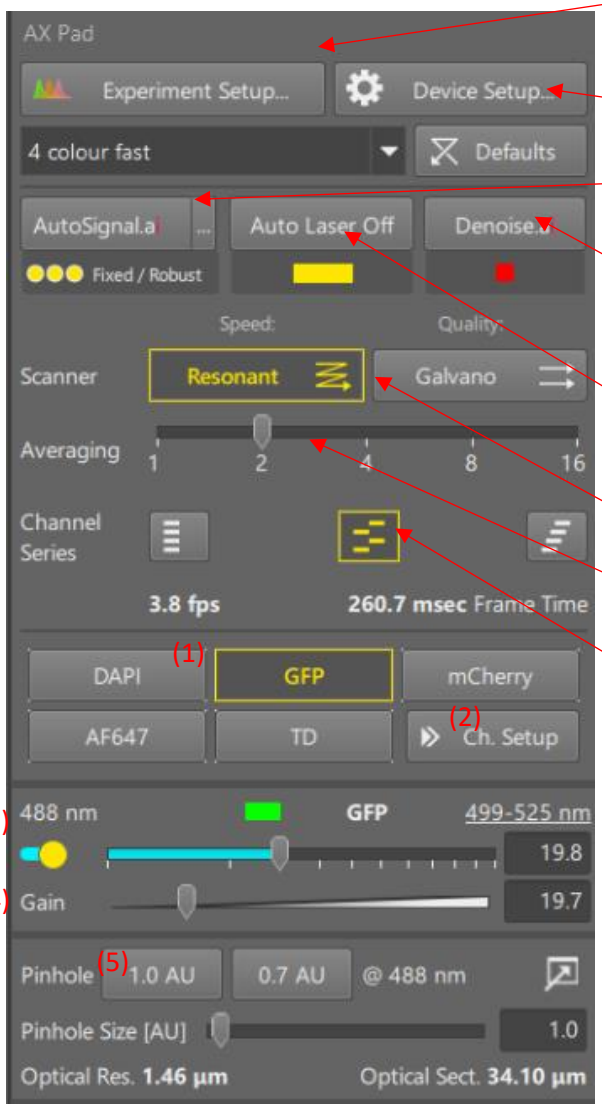
Control pads within the Acquisition window – Confocal Lightpath

- Ax confocal Pad (1)
- Ti2 Pad (2)
- Scan area pad (3)

Ax confocal GUI

The AxR has two scanning modes and the AX pad changes depending on which mode you are using.

Resonant Acquisition window



Experimental Setup – design the combination of lasers and detectors

Device Setup – Settings for the Ax including alignment and options for the Ax GUI window

Autosignal.ai – automatically sets gain and laser power for your sample type

Denoise.ai – Automatically removes shot noise from the live image. The quality indicator below should be amber or green for denoise to work well

Auto laser off – When selected will switch laser off in live mode when there is no change in the imaging window in terms of XY or Z. Prevents unnecessary light exposure

Resonant or Galvano - Select between scanning modes

Averaging - Select amount of averaging. Can be switched to integration in device setup

Channel series – simultaneous (fast). Balanced or minimal crosstalk.

Select which Channels (combination of laser and detector) are being used (1)

Channel setup – look at individual channel settings to allow easy adjustment of laser power and detector gain (2)

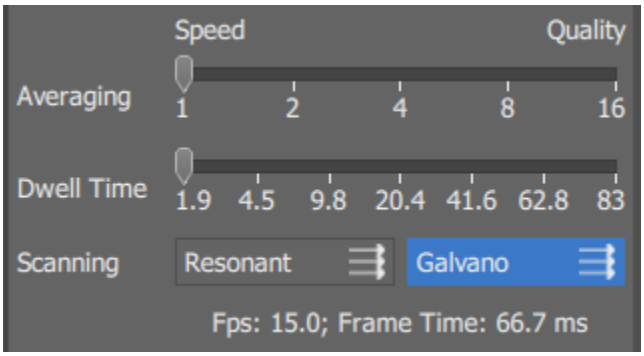
Select whether or not the laser is on (3)

Set gain (4)

Pinhole settings (5) 1.0 AU is optimum for confocal imaging, 0.7AU for enhanced resolution.

Galvano Ax pad

In addition to the AX pad settings shown above, the Galvano window, which is present when the Galvano scanner is selected, also has a sliding scale to set the pixel dwell time. A longer dwell time will reduce the frame per second but increase the image quality, a shorter dwell time increases the speed of acquisition giving a higher frame rate. The resonant scanner has a fixed pixel dwell time. At



Confocal – Lightsource and detector control

A laser bed is provided as the light source for the confocal. The Diode lasers can be controlled in terms of power and on/off state using the sliders at the bottom of the confocal control window (see below).

Turn laser on and off

Set power of laser

Set gain of detector

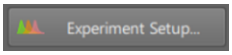
Select which channels you wish to use

Channel setup – In a multichannel expt you can select this to help you choose the right settings for each channel, one at a time.

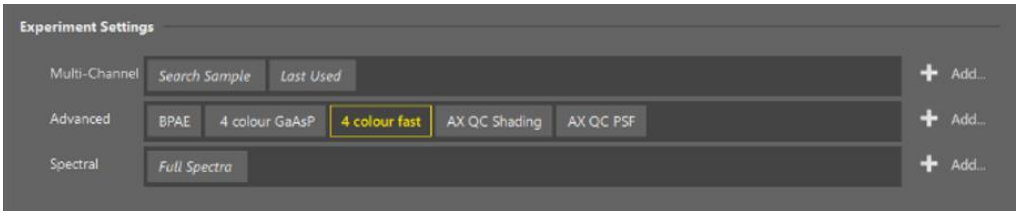
Details the bandwidth of the emission

The lasers and detectors shown in the Ax acquisition window is dependent on the experimental setup. You can have all lasers and detectors available but switch the combinations on and off by selecting the channel (see box in yellow). You may also have a detector on but the linked laser switched off by clicking on the laser button as shown above.

Experimental setup

Click on 

button to open up the Experiment Settings window. There you can create and adjust presets which can be then quickly selected from a pull-down menu in the Ax pad panel.



At the top of this window you have three options to choose from for named presets;

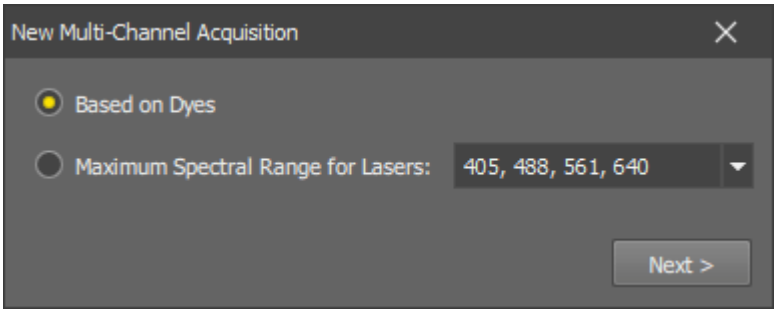
- Multichannel imaging – Has two presets setups and can be added to.
- Advanced imaging – Allows complete freedom in creating a bespoke experiment setup
- Spectral. Single preset and more can be created.

You can create more named presets by clicking Add… on the right or by duplicating an existing preset.

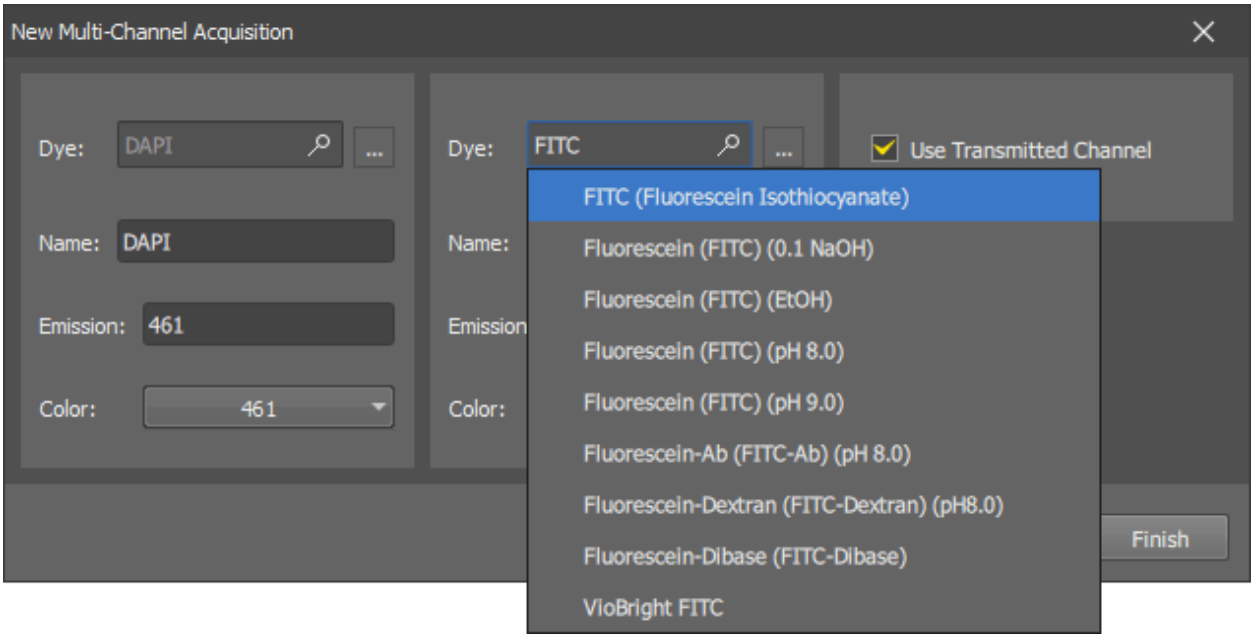
Creating an experimental setup

To create a simple experiment consisting of channels DAPI, FITC, TRITC and transmitted detect TD.

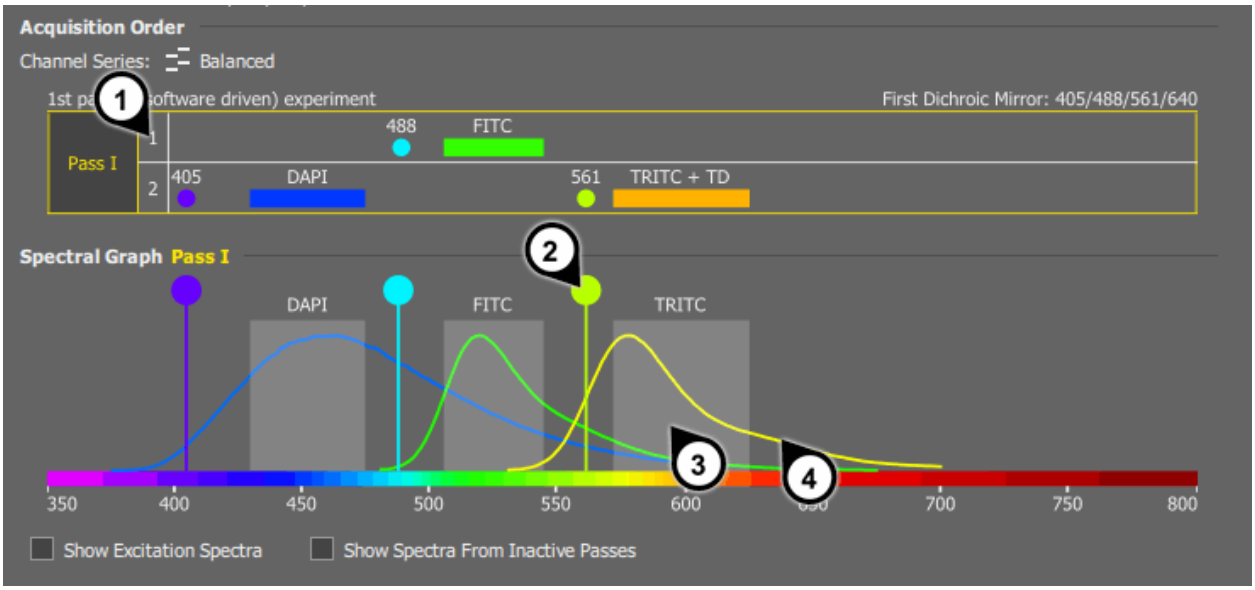
1. Make sure the *AX* light path is selected in the acquisition panel.
2. Click on the Add… button to the right of the multichannel presets
3. Select ‘Based on dyes’



4. The next window allows you to select your dye from a range of known dyes. Type “DAPI” in the first search field, select the actual dye you will be using and confirm it by Enter. Another search field appears. Add the FITC and TRITC dyes as well and select the *Use Transmitted Channel* option to engage the transmitted light detector in the experiment



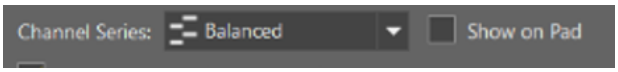
5. Click *Finish* to close the window. After you confirm the selection of channels, a window appears with the experiment setup visualized at the bottom



1. Channel Series = the acquisition order of channels, 2. Laser Wavelength, 3. Filter range

4. Emission curve of fluorescent probes

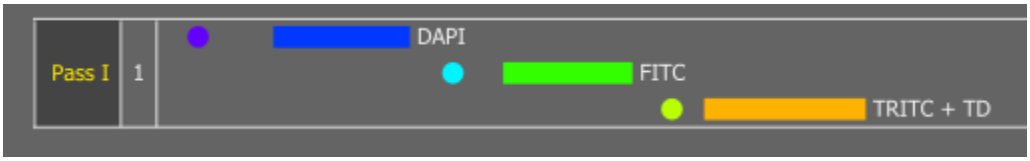
Channel Series



Select the preferred *Channel Series* mode in the pull-down menu above the spectral graph. The acquisition order of channels will be calculated automatically and indicated in the spectral graph as follows:

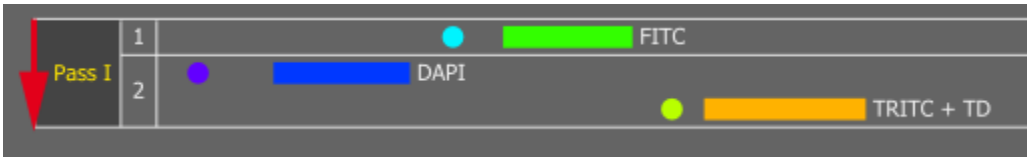
Fastest

All channels are captured at once. 1: DAPI + FITC + TRITC



Balanced

It is a trade-off between speed and minimized crosstalk. Wavelengths are captured in pairs so that there is a sufficient gap between the wavelengths. In our example, the sequence will be: 1: FITC, 2: DAPI + TRITC



Minimal crosstalk

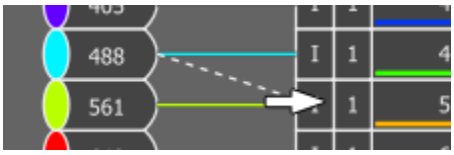
Each channel is acquired separately starting from the highest emission wavelength. 1: TRITC, 2: FITC, 3: DAPI

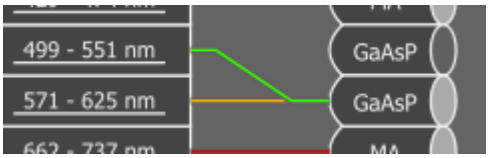
Ticking ‘show on pad’ will give the option to change the channel series within the AX pad window.

An experiment created as a multichannel set up automatically selects the detectors to be used for each. Once you are happy with the set up you can save it and it will appear as an option in the set up menu in the Ax pad. It is possible to further customize a setup by clicking ‘Move to advance’.

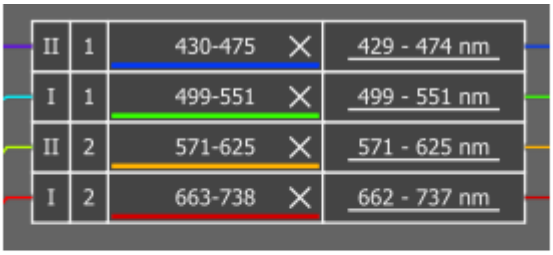
Advanced Experimental presets

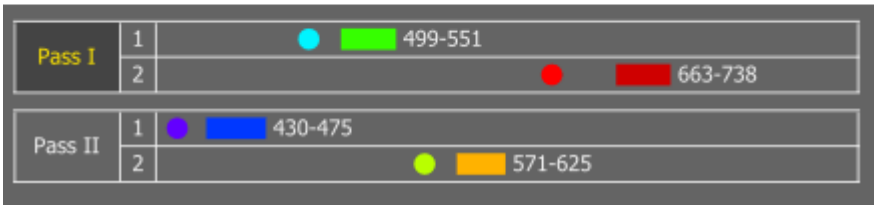
In the advanced setup the combination of lasers and detectors will once again be advised but now you can change the paths for each channel and freely select which laser line is used and which detector is linked to which line.





Channel series refers to the order in which channels are captured either simultaneously or sequentially line by line. Sometimes it is necessary to further separate channels frame by frame for example, if two channels are using the same detector. Then you have multiple passes (roman numerals) as well as channel series (1,2,3 etc).





Spectral

If you have a tuneable detector (DUX-VB) you have the ability to create a spectral experiment. To set up the Experiment preset;

Select the Ax lightpath and Exp. Setup window

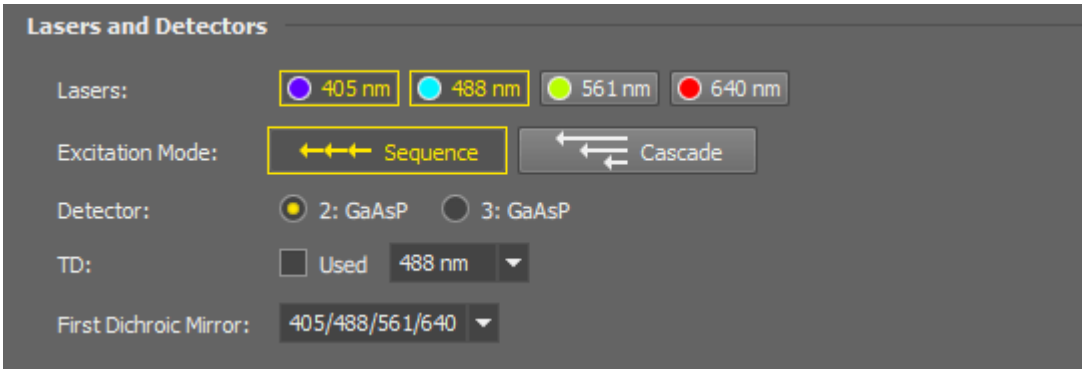
Similarly as in a multi-channel preset, add the dyes you will use to the preset:

Click the *Add...* button next to the list of *Spectral* presets.

Select the *Based on Dyes* option and click the *Next >* button.

Search for the dyes and add them to the preset.

Click *Finish* to close the window.



Lasers

Select lasers to be used for excitation during spectral acquisition.

Note

If one or more lasers are not compatible with the selection of the *First Dichroic Mirror* the corresponding laser is displayed as a dashed line in the spectral graph.

Excitation Mode

Specifies the way the lasers are turned ON and OFF. Channels are acquired from the longest emission wavelength to the shortest.

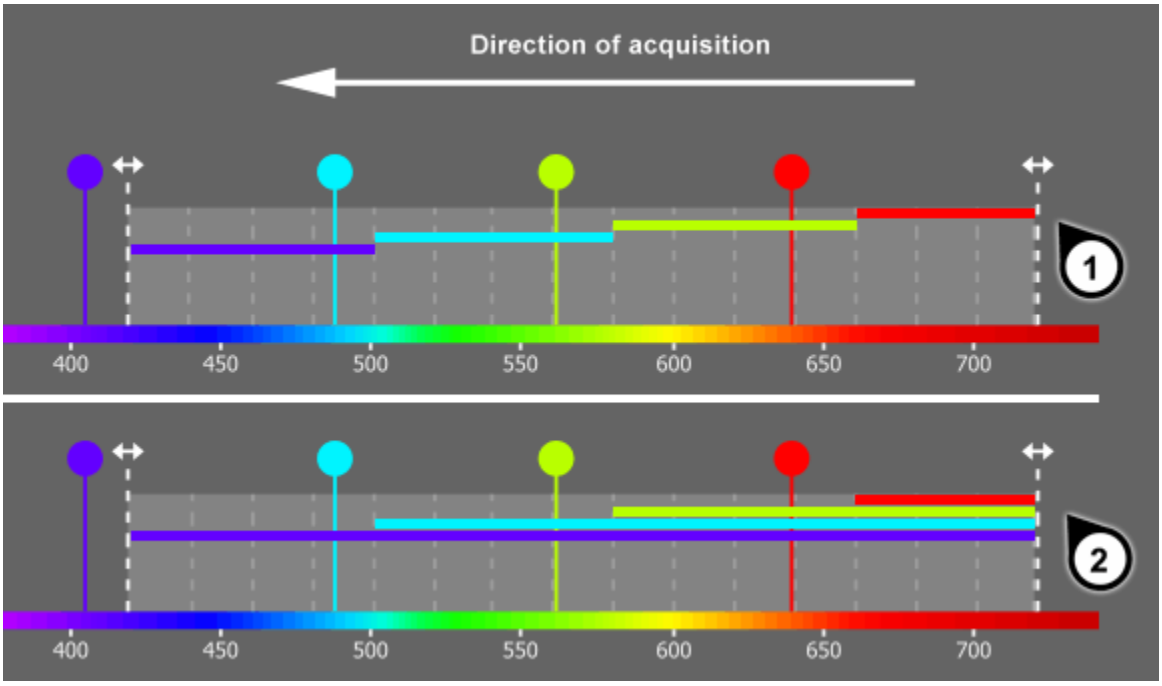
Sequence

Only the laser with the nearest lower excitation wavelength than the emission wavelength is turned ON.

Cascade

All lasers are turned ON at the beginning, once the filter range gets close to the excitation wavelength, the highest excitation wavelength is turned off.

Figure 202. Excitation modes: 1 - Sequential, 2 - Subtractive



Scan Area Window

Scan area size presets –Square or 2:1 rectangle

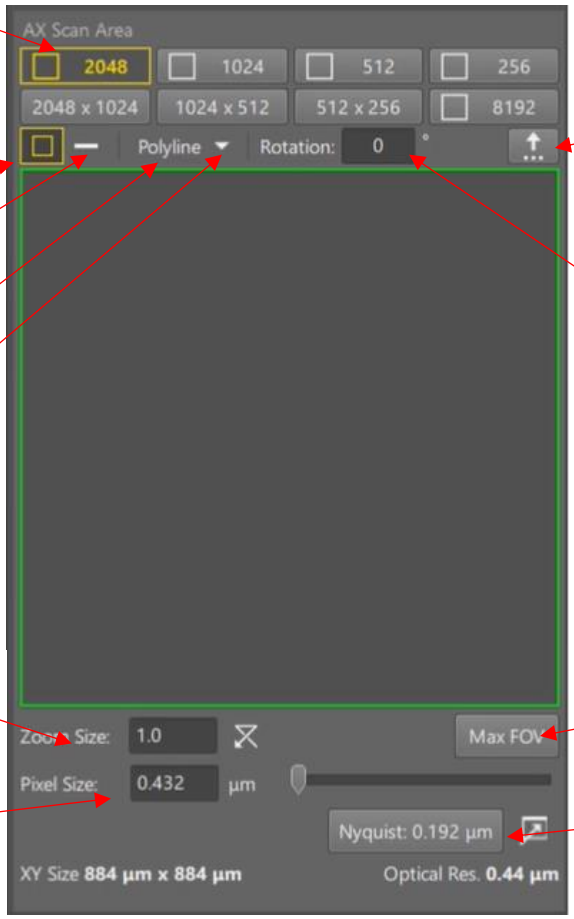
Any shape of the scan area can be specified. Select:

A rectangular (strict square or oblong) scan area.

A linear (1DT) scan.

Polyline A polyline-shaped 1 DT scan.

ROI The *Square Scan Area* can be restricted by defining a ROI.



… - Custom scan area can be set, either square or rectangle

Rotation/ The scan area can be rotated when galvo scan is selected.

Zoom Size - Zoom factor of the current scan area compared to full FOV.

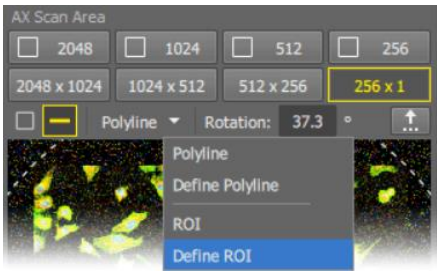
Pixel size – may be adjusted by typing or moving the zoom slider.

XY size – pixel size x number of pixels

Max FOV Sets the scan area to match the full FOV

Nyquist – Set the pixel size to match the objective for optimum resolution

Define ROI



1. Make sure the Live or Frozen image is displayed.
2. Select *Define ROI* command from the pull down menu in the Ax scan area window.
3. A simple ROI editor appears over the image. Select a tool and draw one or more ROIs of any shape to the image. Confirm the definition by the *Finish* button.
4. The ROI will appear in the preview area and the *ROI* button will be activated.
5. Once you run the Live image again, only the areas marked by the ROI(s) will be exposed to illumination. N.B. This does not affect the scan speed, it simply turns the lasers off in the excluded areas.

Specifying a Polyline (1DT) Scan Area

The procedure of defining a ROI and a Polyline are similar.

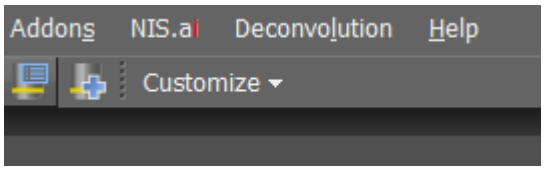
1. Reveal the pull-down menu and select *Define Polyline*
2. Start inserting node points of the polyline inside the preview area by the left mouse button.
3. Once the polyline is ready, click anywhere in the preview area by the right mouse button to finish it. The right-clicked point will not be part of the polyline.
4. Right-click once more to the preview area to activate the polyline for the live image.

Post-Acquisition Image processing

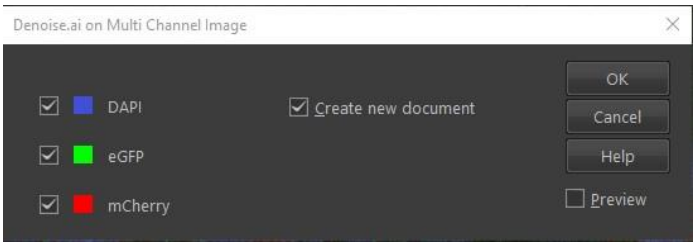
Denoise.ai

An optional processing method is called “denoise.ai”, an artificial intelligence filter that has learned to denoise resonant scanner images.

You can choose to denoise the raw data live by selecting denoise in the Ax window. Alternatively you can create a new denoised document post acquisition. The Denoise.ai can be found under the .NIS.ai heading.



Click on Denoise.ai and the software will open a new window asking which channel you want to denoise and whether you want to create a new document. .

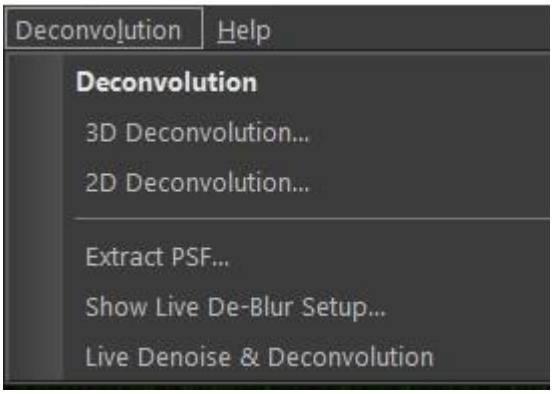


Deconvolution

An optional image processing method is an in-built deconvolution algorithm.

Top of the screen click on Deconvolution and select 2D or 3D deconvolution

There are a number of algorithms to choose from including Auto deconvolution, Richardson-Lucy and blind deconvolution. .



A new window will open and give you a series of choices for the deconvolution process. These are sample specific. You should keep the same parameters for images that will thereafter need to be compared. If Denoise.ai has already been carried out, set the noise level to clear

The software will open a new window with the deconvolved image.