ANDOR DRAGONFLY 500 01.01.2020

System location: I04.UG.015

Acquisition Software:
Fusion

Visualisation Software:
Imaris

System description:

The Andor Dragonfly is an advanced Imaging platform with multiple imaging modalities and Borealis Perfect Illumination.

Imaging modalities/features:

1. **Confocal** (spinning disk)
2. Laser-based **Widefield**
3. **TIRF** (1 angle TIRF)
4. Astigmatic lens for 3D **SMLM** (single molecule localization microscopy) available soon!
5. **Super resolution** (SRRF)

File format:

Imaris files - .IMS. Possibility to export files as a TIFF.

Specification:

- Nikon Ti2 inverted microscope (single deck)
- 2 cameras: 1x EMCCD (13um pixel size) and 1x sCMOS (6.5um pixel size)
- Spinning disk with two pinhole patterns: 25 µm and 40 µm in confocal mode
- camera zoom optics of 1.0x, 1.5x and 2.0x for optimum resolution across different objective magnifications and camera pixel sizes
- illumination zoom optics of 4x, 16x & 36x power density
- Motorized stage:

- ASI XY stage: resolution of 100 nm for XY-axis
- ASIZ-piezzo & linear stage encoder: Linear XY encoders are available to provide 50 nm resolution
- Z positions with sub-nanometer accuracy (travel-range: 500 microns)

Light sources:

- CoolLED – pE-300 White Light source
- 405 nm laser: (100 mW) – Continuous wave
- 488 nm laser: (150 mW) – Continuous wave
- 561 nm laser: (150 mW) – Continuous wave
- 637 nm laser: (140 mW) – Continuous wave

Filter for visual examination:

- DAPI: Excitation: BP 377/50 Emission: BP 447/60
- GFP/FITC: Excitation: BP 482/18 Emission: BP 520/28
- DsRed/RHOD: Excitation: BP 531/40 Emission: BP 593/40

Objectives / position:

1. MRD00105: CFI P-Apo 10x Lambda / NA 0.45 / WD 4,0 mm
2. MRD00205: CFI P-Apo 20X Lambda / NA 0.75 / WD 1,00 mm
3. MRD01605: CFI P-Apo 60x Lambda / NA 1.40 / WD 0,13 mm – oil
4. MRD0169: CFI Apochromat TIRF 60x / NA 1.49 / WD 0,13 mm – oil

Extra high-end Objectives (book extra)

5. MRD77410: CFI Apo LWD 40x WI Lambda-S/ NA 1.15 / WD 0.60mm – Water (transmission : 405 – 950 nm)– **€€€**
6. MRD71120: CFI P-Apochromat 10x NA 0.50 / WD 5.50 mm – Glycerol – **€€€**
7. MRD77200 CFI P-Apochromat 20x NA 0.95 / WD 0.95 mm – Water– **€€€**

Cameras:

1. Andor iXon Ultra 888 Ultra EMCCD Camera. Active Pixels 1024 x 1024; Pixel Size 13 x 13 µm; Image Area (mm) 13.3 x 13.3;
2. Andor Zyla 4.2 Megapixel sCMOS. 2048 x 2048; Pixel size 6.5um

Camera Emission Splitter (for 2nd Port camera)

1. Dual camera switching mirror: use to switch emission light path between primary and secondary camera port.
2. Dual camera dichroic 565nm longpass: for dual colour GFP/RFP simultaneous imaging. (shorter wavelength is reflected to vertical camera port)
3. Dual camera dichroic 640nm longpass: for dual colour Cy3/Cy5 simultaneous imaging (shorter wavelength is reflected to vertical camera port)

Emission Filters wheel Camera 1 (iXon)

1. 445/46 bandpass filter: DAPI / BFP / Blueberry / Azurite (use with dichroics #1, 2 and 6)
2. 521/38 bandpass: GFP, Venus, mNeonGreen, Alexa488, FITC, mWasabi. (use with dichroics # 1, 2 and 6 only).
3. 594/43 bandpass: RFP/TRITC, mRuby, tDTomato. (use with dichroics #1, 5 and 6)
4. 620/60 bandpass: mCherry, Hc-Red1, mRuby
5. 685/47 bandpass: Cy5, Cy5.5, iRFP 670/682, mCardinal (use with dichroic #6 only)

6. Dual EM filter 488-561 – GFP/RFP
7. 478/37 band pass: CFP, mCerulean, mTurquoise, DAPI. (use with dichroics #3, 4 and 5)
8. Penta EM filter 405-488-561- 640: (DAPI/GFP/RFP/Cy5)

Emission Filters wheel Camera 2 (Zyla)

1. **445/46** bandpass filter: DAPI / BFP / Blueberry / Azurite (use with dichroics #1, 2 and 6)
2. **538/20** bandpass filter: YFP, Venus, mCitrine. (use with dichroic #5 only)
3. **521/38** bandpass: GFP, Venus, mNeonGreen, Alexa488, FITC, mWasabi. Use with dichroics # 1, 2 and 6 only).
4. **594/43** bandpass: RFP/TRITC, mRuby, tDTomato. (use with dichroics #1, 5 and 6)
5. **620/60** bandpass: mCherry, Hc-Red1, mRuby
6. **685/47** bandpass: Cy5, Cy 5.5, iRFP 670/682, mCardinal (use with dichroic #6 only)
7. **Dual EM** filter 488-561 – GFP/RFP
8. **Penta EM filter 405-488-561- 640:** (DAPI/GFP/RFP/Cy5)

Computer (Fusion-Imaris workstation PC):

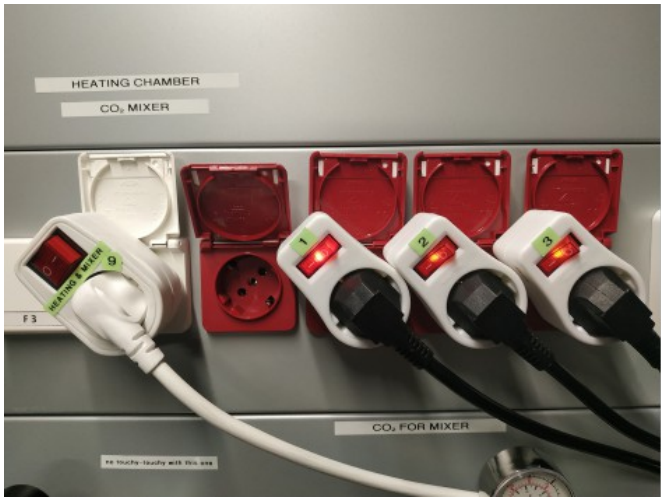
- Idual quad-core Xeon CPUs;
- RAM: 96GB ECC
- nVidia M4000 8GB
- AMD W7100 8GB
- 6x SSDs (4TB for images);
- 10 Terabyte disk drive upgrade.

FOR LIVE IMAGING: heating chamber and CO2 mixer

Contact persons:

- Anna Hapek
- Doreen Milius
- Yann Cesbron

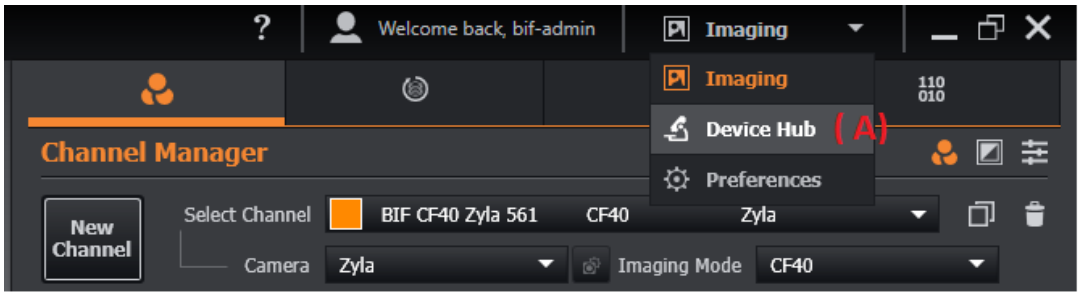
STARTING UP



During logging in – deleting script will run in the background. It will restore all default settings (channels, protocols) in the Fusion.

1. switch on three switches **#1** **#2** and **#3** on the wall
2. switch on the computer **#4**
3. FL lamp **#5** CoolLED is on by default, you just need to open the shutter (big blue button)
4. For heating chamber and the CO2 mixer, turn on the socket labelled **#9**
5. log in with a LOCAL ACCOUNT! Do not use your windows account here. **Software won't work in that case**
6. Start the Fusion software and **WAIT approx. 2 min** for the complete boot of the software+hardware.
7. Check if all components are connected, you can find it in right bottom corner. Green – fine, orange- in progress, red – not connected.

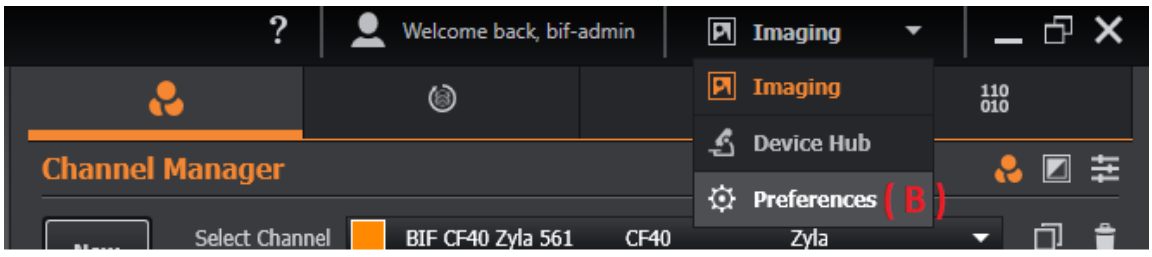
8. In the event of any startup errors you may check connections by going to the upper menu and selecting the DeviceHub option **(A)**:



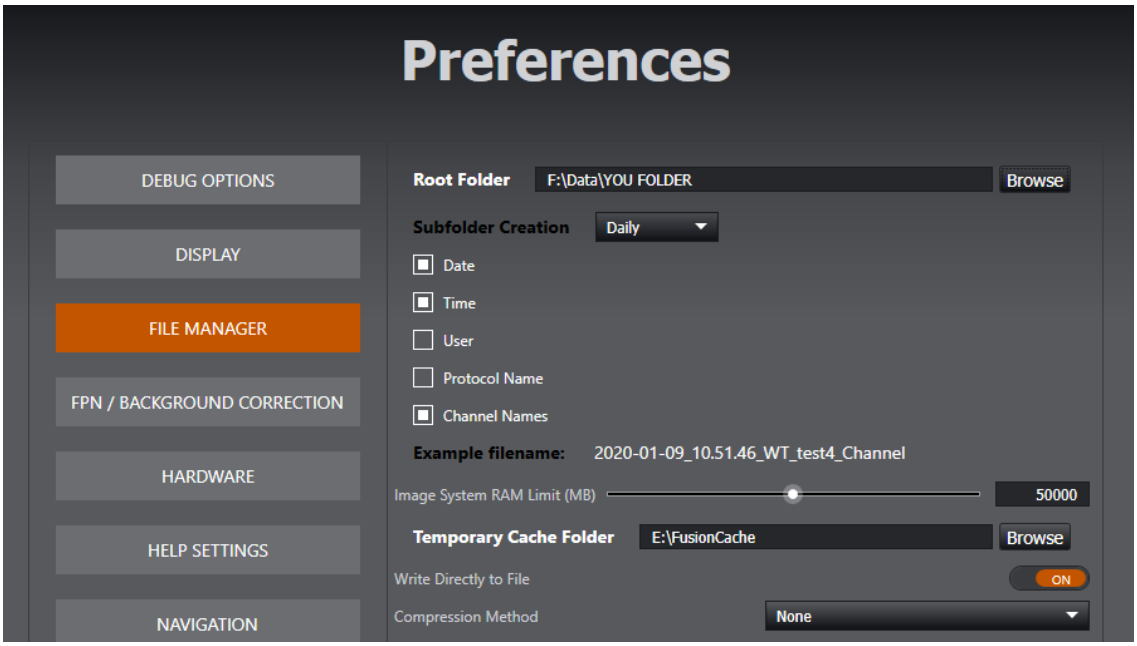
You can see a series of hardware devices and connection statuses. If any item appears with a RED marker or does not show a GREEN tick sign, you need to choose to reset the hardware connection by clicking the RECONNECT button.

9. **Initial Software settings:** Fusion automatically saves the captured images. Before you start imaging you have to choose the save location.

a. To edit the save location go to the upper menu and choose Preferences **(B)**



b. Choose a File Manager option.



Select drive F ---> Users → your group folder (example benkogrp, heisegrp, hippegrp)

Never save your data on drive C. From there images will be automatically deleted

- Subfolder creation: Daily is the best option.
- Adding both Date and Time options will ensure every file has a unique identifier. (You can also edit the file name in the main window during acquisition).
- Other options should be left as the default values.
- Go back to the imaging menu option.

LIVE IMAGING

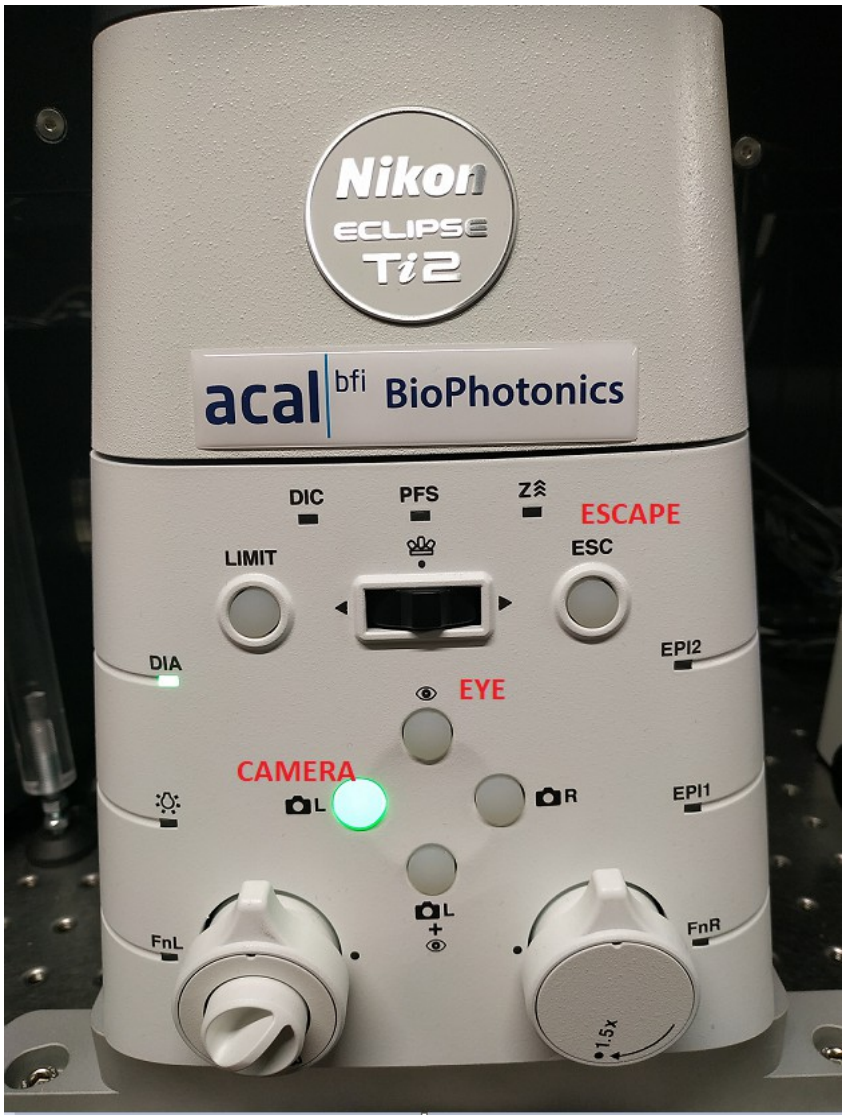
If you are planning to do live imaging experiment we recommend to turn on the heating chamber at least 1 hour before you start imaging. CO2 mixer can be turned on just before, but please consider that to work properly you need to have a correct sample holder. CO2 and humidity concentration have to be stable over the time therefore insert and the top lid must be sealed.

SHUTDOWN

Please make sure to log off from the computer after your session.

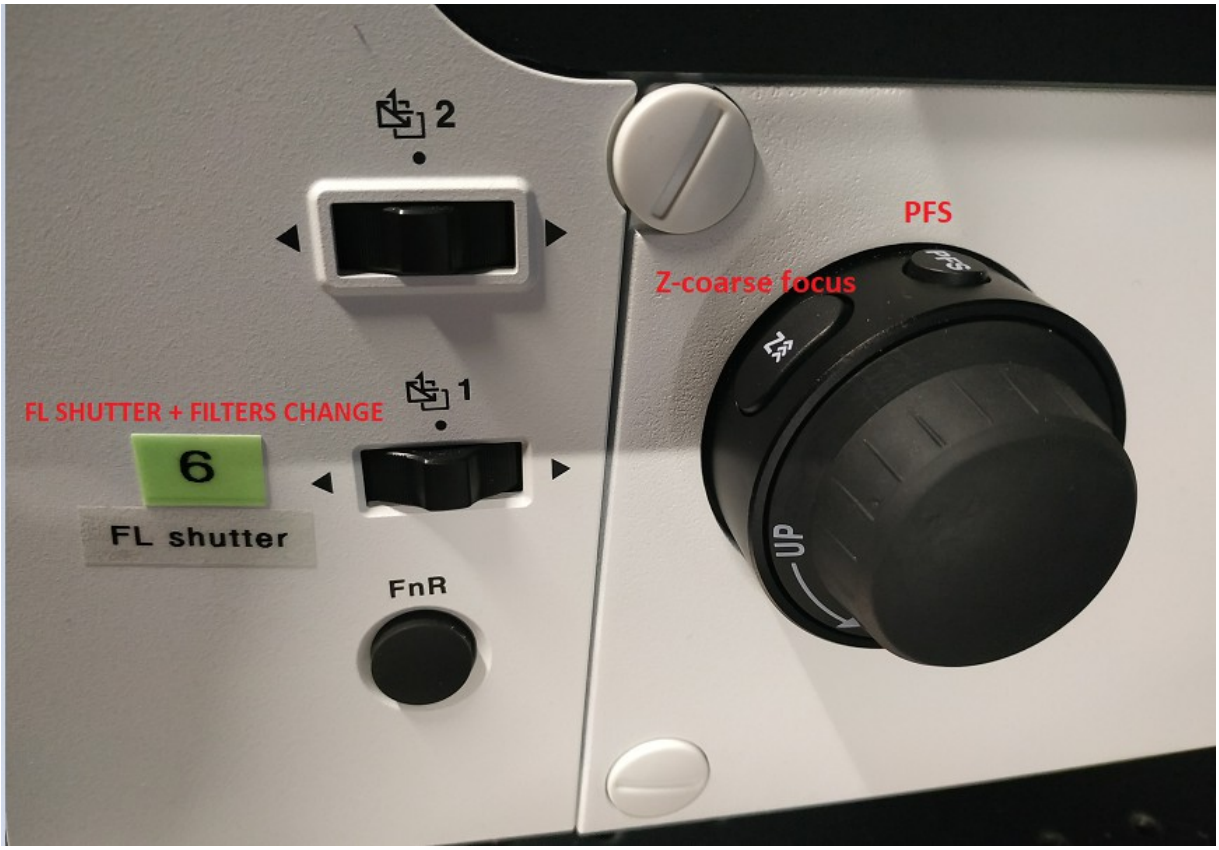
1. Copy your data to your file server.
2. Delete you images from the software Image List!
3. Clean the objectives!
4. Close the software.
5. Turn off heating chamber and CO2 mixer.
6. Shutdown the computer.
7. Turn off the switches: #3, #2 and 1# in this order
8. Clean the workspace.

MICROSCOPE STAND



Visual examination

1. Lower the objective using the ESCAPE button.
2. Choose the correct objective in the software. Make sure that you use correct immersion medium if necessary.
3. Place you sample in the correct holder.
4. Press “EYE” in the front panel of microscope stand.



5. Focus on your sample using a focus nob. Press the Z-coarse button if necessary.
6. If you want to check your sample with FL lamp: turn the lamp on (#5) and press the FL SHUTTER button (#6) on the right side of the stand. You can change filters by pressing the FL SHUTTER button to the side.
7. If you want to check your sample with TL: turn the lamp on and press the TL shutter (#7). You can regulate the light intensity with a nob "light intensity".

Perfect Focus (PFS)

a. mount your sample

b. Switch on the PFS button on the microscope stand (#8a) or (#8b) at Ti-E Joystick and the objective will automatically focus on your sample. You can also activate it in the software.

c. PFS control light at the front of the microscope stand will be ON upon activation

d. When PSF is activated ONLY drift stabilization offset in the software will work to change the focus position.

e. PFS keeps focus position for long term imaging.

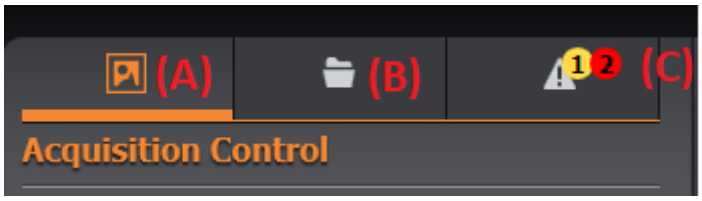
Changing Objectives

Please see document on the desktop: HOW TO CHANGE THE OBJECTIVE

IMAGING WITH THE FUSION SOFTWARE

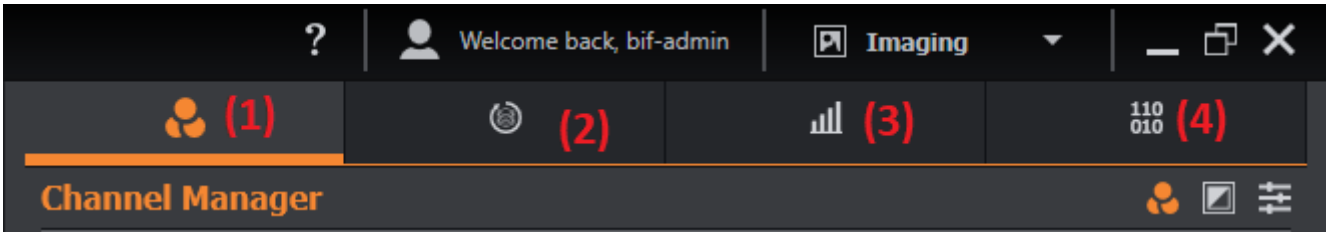
!!! Be careful – when editing any settings–Fusion will automatically update the settings without prompting to save, it is very easy to destroy protocol and channel settings.

Acquisition software tabs



- to start image capture, ensure that you have the Acquisition Control **(A)** window selected
- tab **(B)** changes to the File Manager window
- tab **(C)** changes to the Error/Warning window

Main menu (Expander bar)



(1). Channel Manager

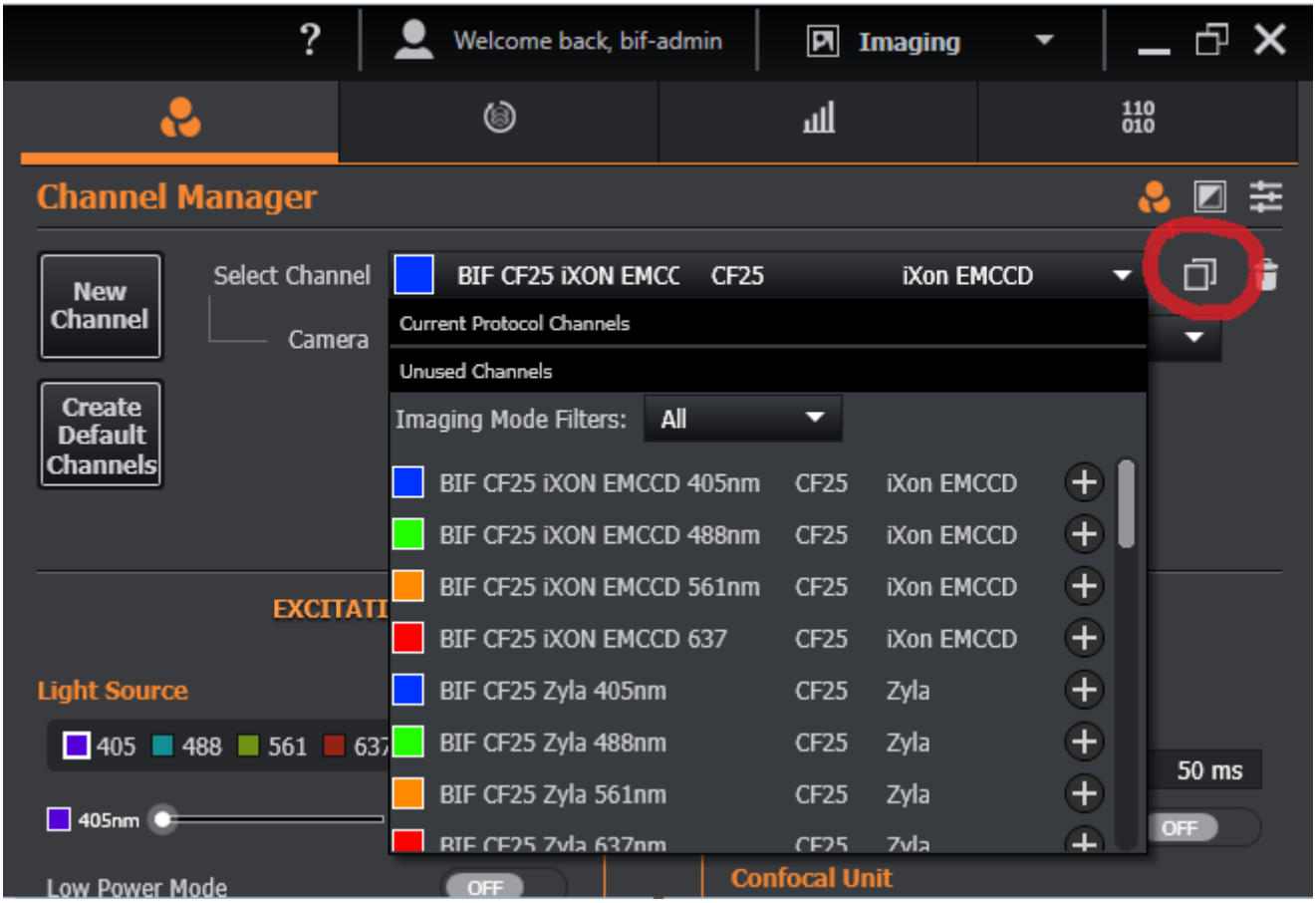
(2). Protocol Manager

(3). Analysis

(4). Image Processing

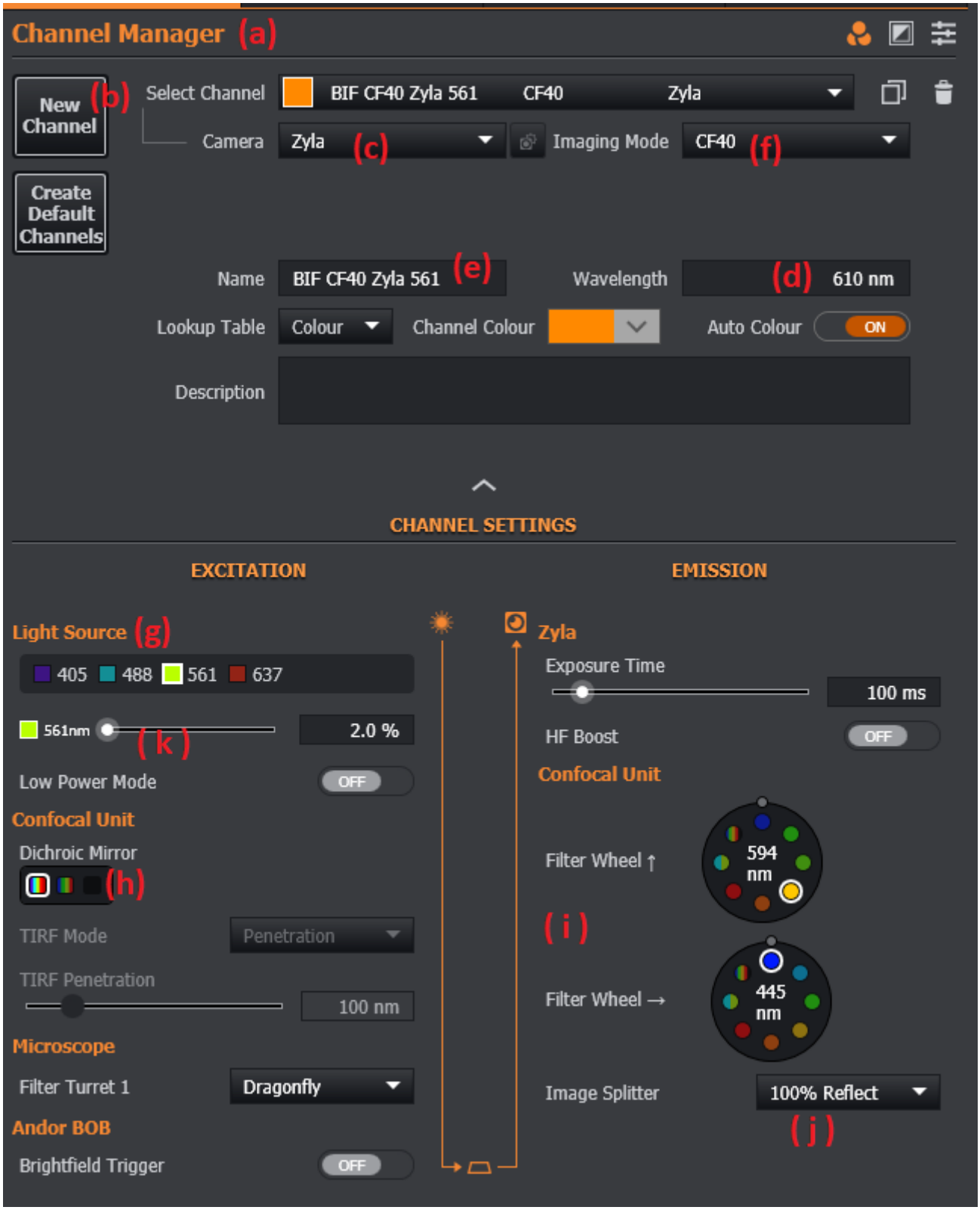
(1) CREATING A CHANNEL

Channels are settings files, created and managed in the Channel Manager. Channels specify all the settings for capturing an image of a specimen in a given Imaging mode, or with a specific fluorescence probe e.g. DAPI or GFP.



In the software you will find channels which were created by BIF e.g. BIF CF25 iXON EMCCD 405nm

To create a new channel:



a. Click on the Channel Manager tab.

b. Click on New Channel. You can also choose to duplicate the current channel (marked in red) (this is recommended and allows for editing the new channel while ensuring the original settings file is not altered)

c. Select your Camera from the Cameras available from the drop-down box (iXON EMCCD or Zyla)

d. Enter the Wavelength e.g. 405 nm.

e. Change the Name from the default name so that the new channel may be easily identified e.g. Alexa Flour 405.

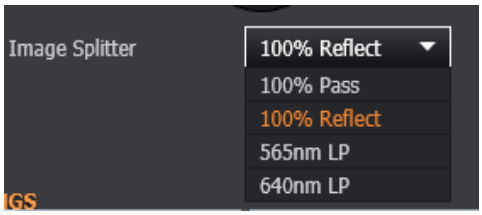
f. Select the Imaging Mode that the channel will run in e.g. TIRF.

g. Turn on the Light Source by clicking the Light Source options.

h. Select the correct dichroic filter set for the laser.

i. Select the emission filter for the chosen fluorophore on the Filter Wheel that matches your selected camera

j. Select the correct Image Splitter

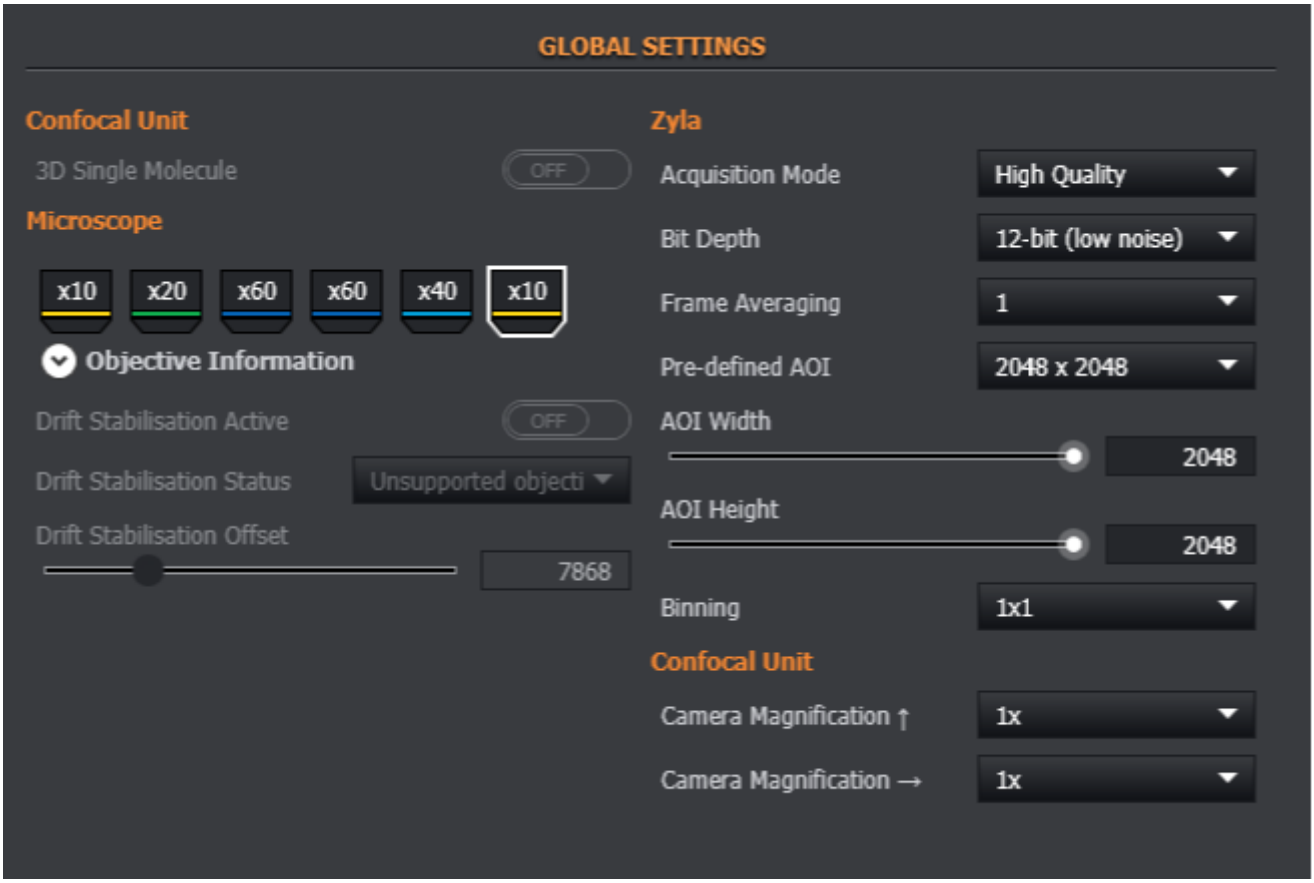


k. Set the laser intensity as required using the slider bar, or enter a value directly in the laser output field.

You can also duplicate an existing channel and modify it. Make sure that you rename it.

The new channel will now appear and may be selected in Protocol manager to add to your protocol.

GLOBAL SETTINGS



a. Click the objective lens to automatically change to this magnification.

b. The camera settings will be for the active camera in the chosen channel settings

c. Set the Acquisition Mode (High Quality or High Speed) and the Bit Depth (12-bit or 16-bit)

d. Set the number of frames to average the image over to improve signal to noise if required

e. AOI (Area of Interest) is a crop factor for the camera:

→ 2048x2048 is a full frame

→ Select other size settings from the dropdown list or custom size using the sliders or typing the number of pixels in for Width and Height

f. Apply binning if required to improve sensitivity at the cost of resolution

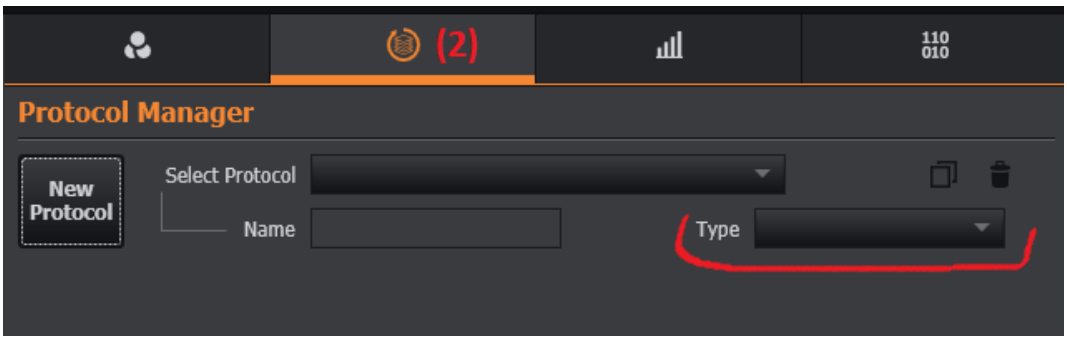
→A bin of 2x2 is equivalent of 1024x1024 pixels and gives 4x better sensitivity

g. The Camera Magnification options add either a 1x, 1.5x or 2x optovar magnification into the light path:

→This zooms the image but will not result in any increase in resolution

→ The extra optics will cause a decrease in sensitivity so the image will appear darker

CREATING A PROTOCOL (2)



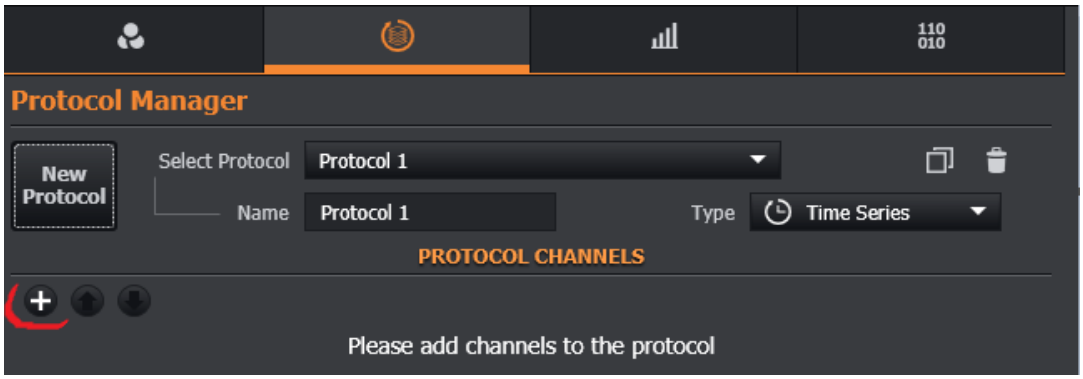
To create a new Protocol:

a. Navigate to the Protocol Manager (2) panel.

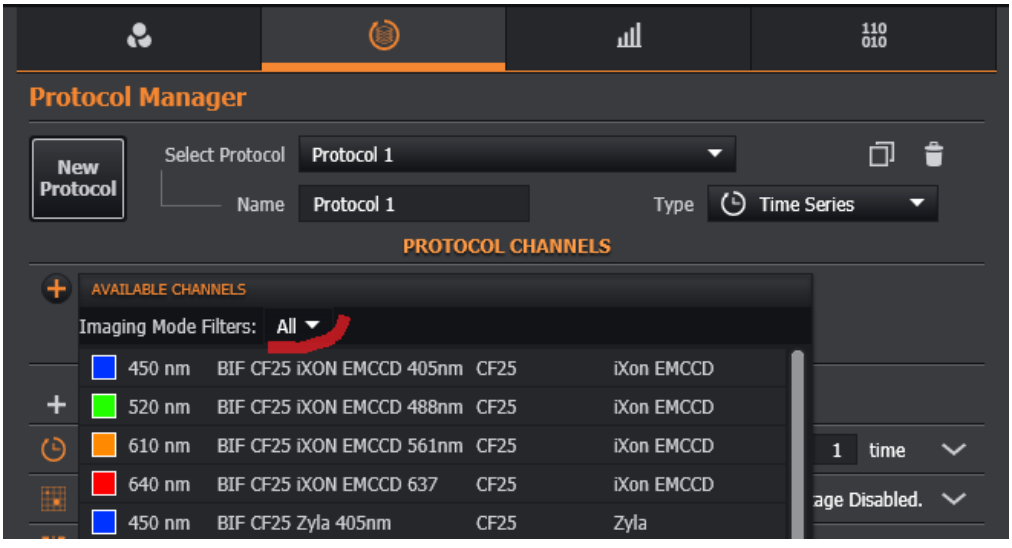
b. Click: New Protocol.

c. Select the Protocol Type **(marked in red).**

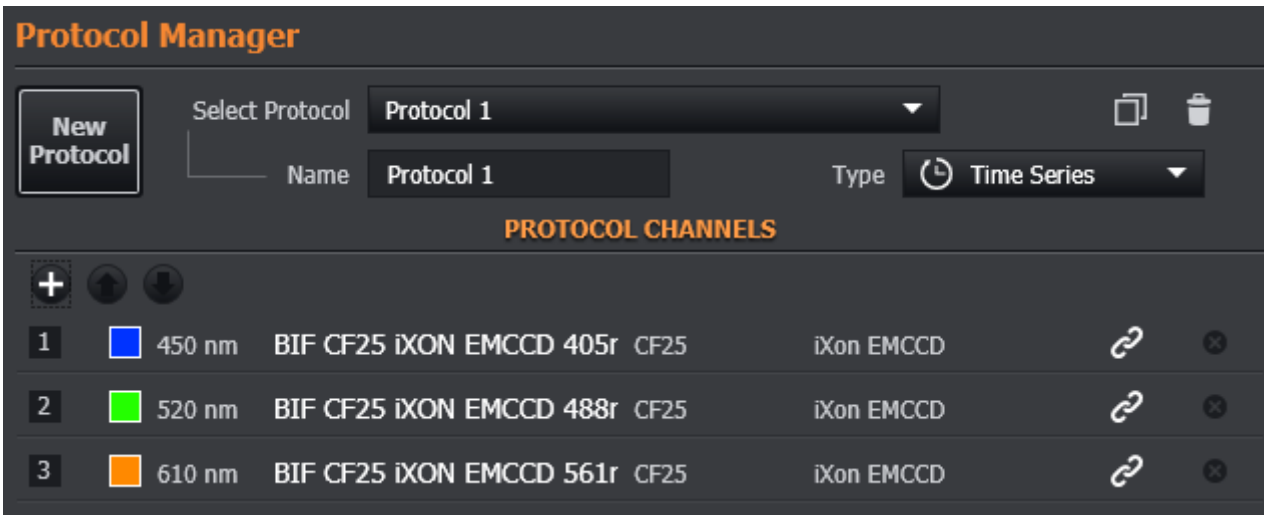
d. Add a channel to the protocol by clicking on + button.



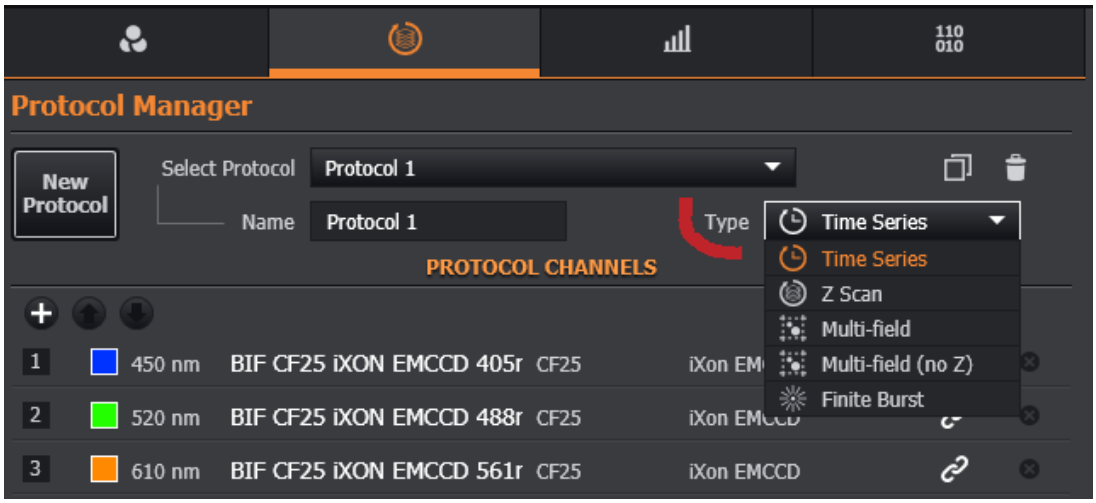
e. Select the required Channel from the Available Channels list. You can also filter channels by Imaging Modes **(marked in red).**



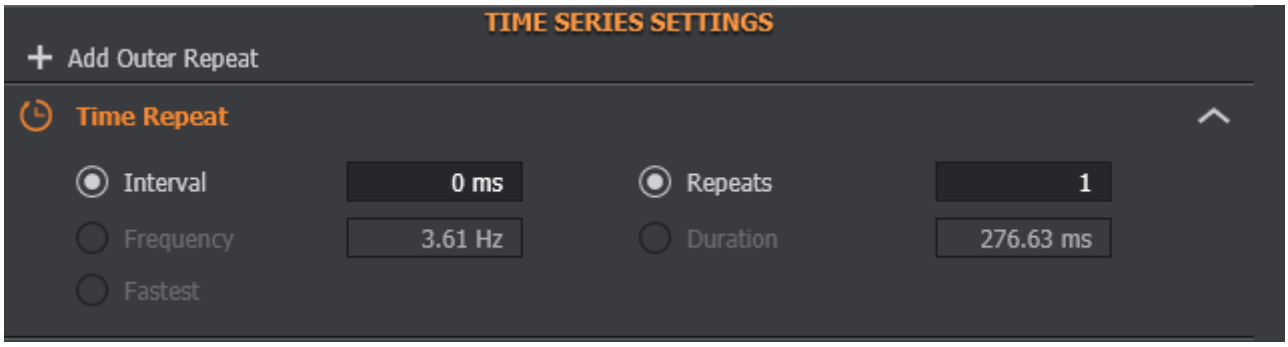
f. Channels are added to your protocol now. **Please make sure that you add channels after you set them up (checking exposure time, laser power etc).**



g. To set up an experiment: choose a Type **(marked red).**



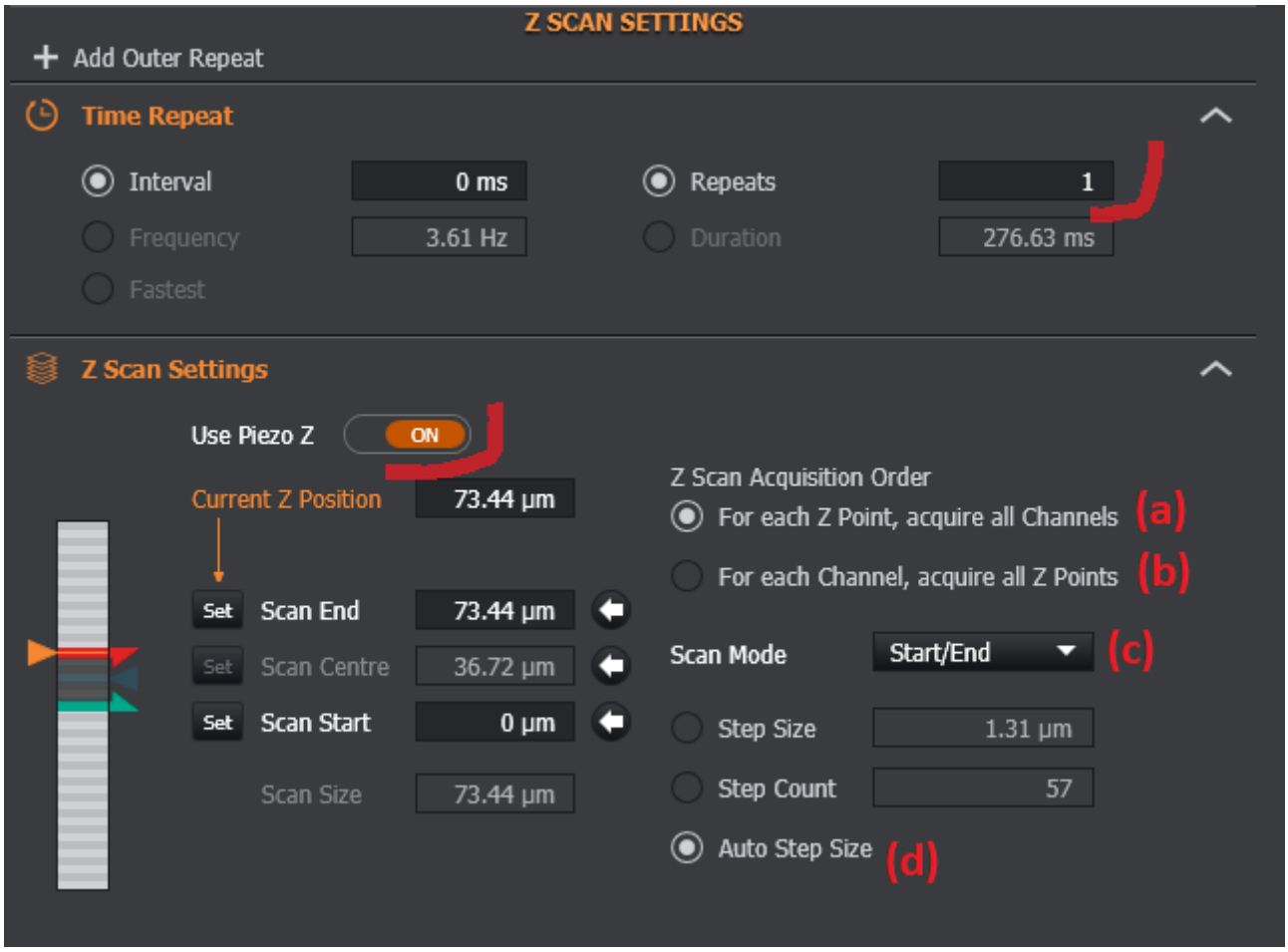
1. TIME SERIES SET UP



- Expand the **Time Repeat** section. Select a mode and define a value, one of:

Interval (set the minimum time between each repeat)
Frequency (set the max. rate at which repeats are performed)
Fastest (set the protocol to run at the fastest possible rate)
Select and define a value for either:
Repeats (run a specific number of repeats)
Duration (run protocol for a specific time)

2. Z SCAN SET UP



For a z scan you can use a piezo z drive **(marked in red)** or turn it off and use a motor drive.

Z Piezo drive range is 500um, for bigger z scan, please use the motor drive.

Together with z scan you need to set up a time series. It is a bug in the software. For a single stack please put "1" in the red marked window.

(a) option better for live imaging, imaging slower

(b) option better for fixed or not moving much samples, faster imaging

(c) Scan mode: START/END or CENTER/SIZE

(d) You can choose the step size. Auto Step – Nyquist. If you want to adjust it put step size or step count manually.

3. MONTAGE (TILES) SET UP

In the current version of Fusion, Montage can be added to a Time Series or a Z-stack Protocol.

a. Click the box that says "Montage Enabled" **(marked red)** to activate:

→ Set the Mode to **Edge** or **Fields**

- **Edge** allows you to add points of interest and the software will automatically create a tiled area to include all these position. Add points using the "+" button
- **Fields** allows you to specify a particular number of fields of view range (i.e. 3 x 3)

Select the starting point (i.e. **Centre, Bottom Right, Top Left** etc) where you want the tiles to start. This will be the current position of your field of view as the relative start position i.e. if you select **Centre** then the current stage position will be the centre of the overall tile scan.

b. Set the overlap percentage. Minimum 10%.

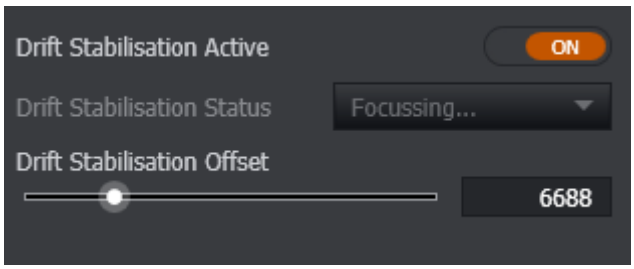
c. Each tile will be exported as a separate IMARIS file to the auto-save folder along with a stitch.

4. MULTIFIELD (MULTIPOSITIONS) SET UP

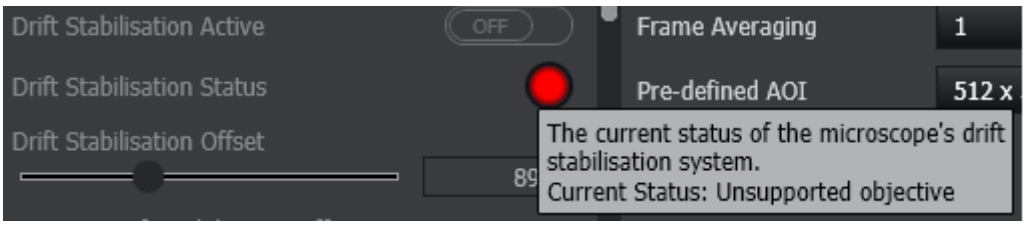
5. DRIFT STABILIZATION

This device work by detecting the reflection of an infrared (or near infrared) laser from the coverslip. This enables the microscope to maintain the correct distance between the objective and the coverslip over long (e.g. hours / days) periods of time. The user can set the focus (known as the offset in the Fusion software), so that the sample stays in focus. Bear in mind however, that if the sample moves away / towards the coverslip, the microscope cannot correct for this. It can only correct for changes between the objective and coverslip.

Drift Stabilisation Active - when ON, the microscope will be locked onto the sample, using the distance between the coverslip and objective as its reference. (This assumes that the microscope can see the coverslip reflection and that the objective currently in use is supported for this operation).



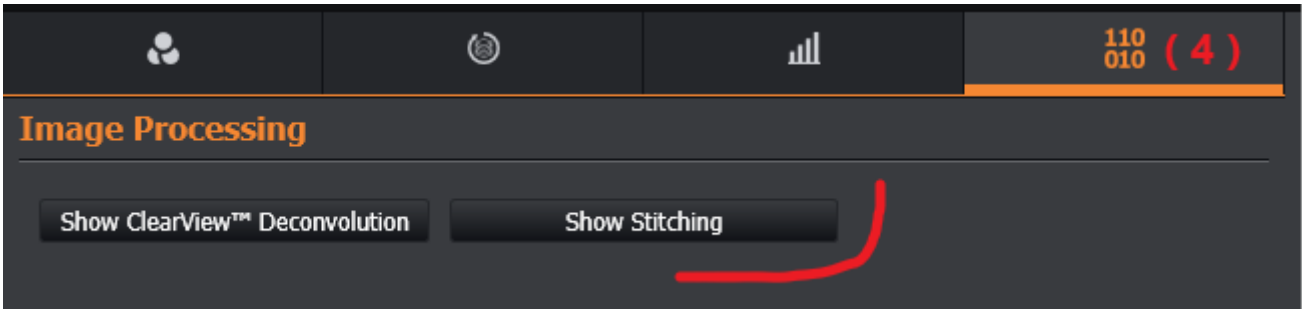
Drift Stabilisation Status -shows the microscope's status - Grey = Off, Yellow = Searching, Green = Holding Focus, Red = Failed State.



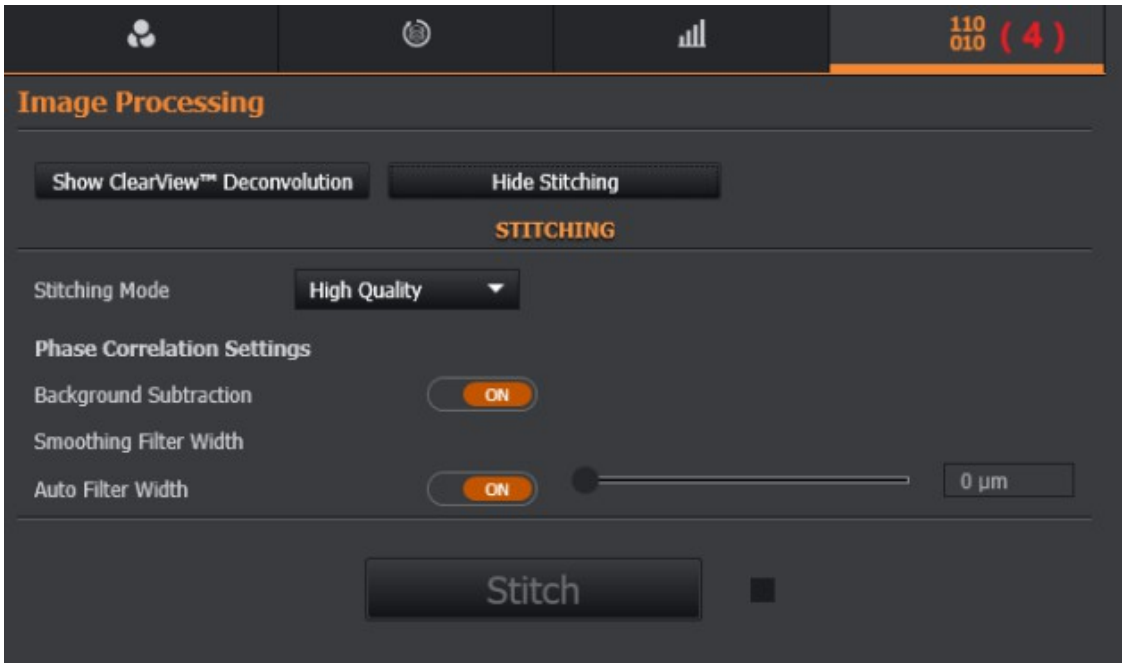
(4) IMAGE PROCESSING

NOTE: you can also use these options on our workstations. With big files, please do not use this computer for image processing

1. **STITICHING -** (also available on Workstations/Imaris: 1 floating licence)
   https://bif.pages.ist.ac.at/analysis-software
   Click on the tab at the top (4) and expand the Stitching option.



– Set the quality level, background subtraction and smooth filter and then click the Stitch button when ready to create a stitched version of the images

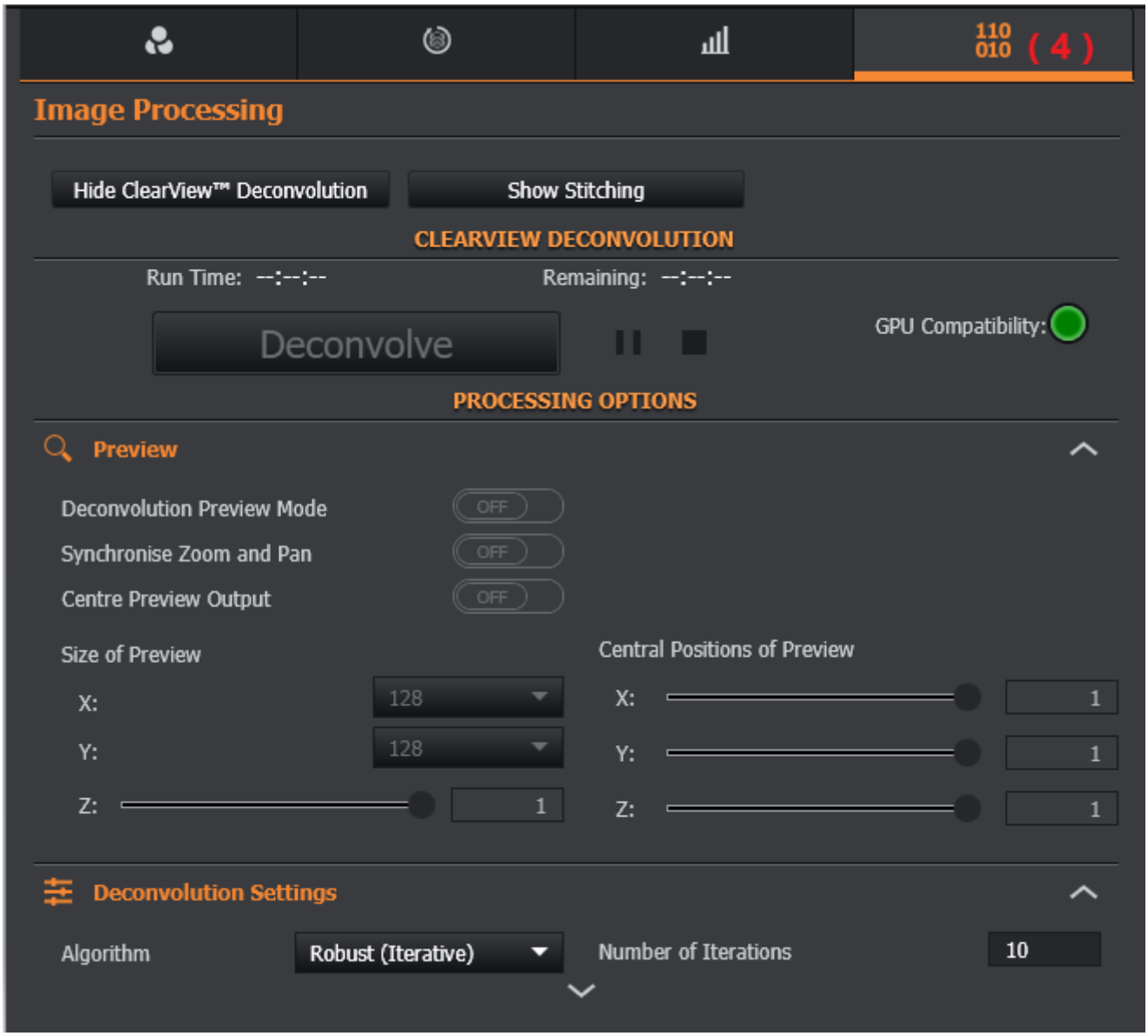


–

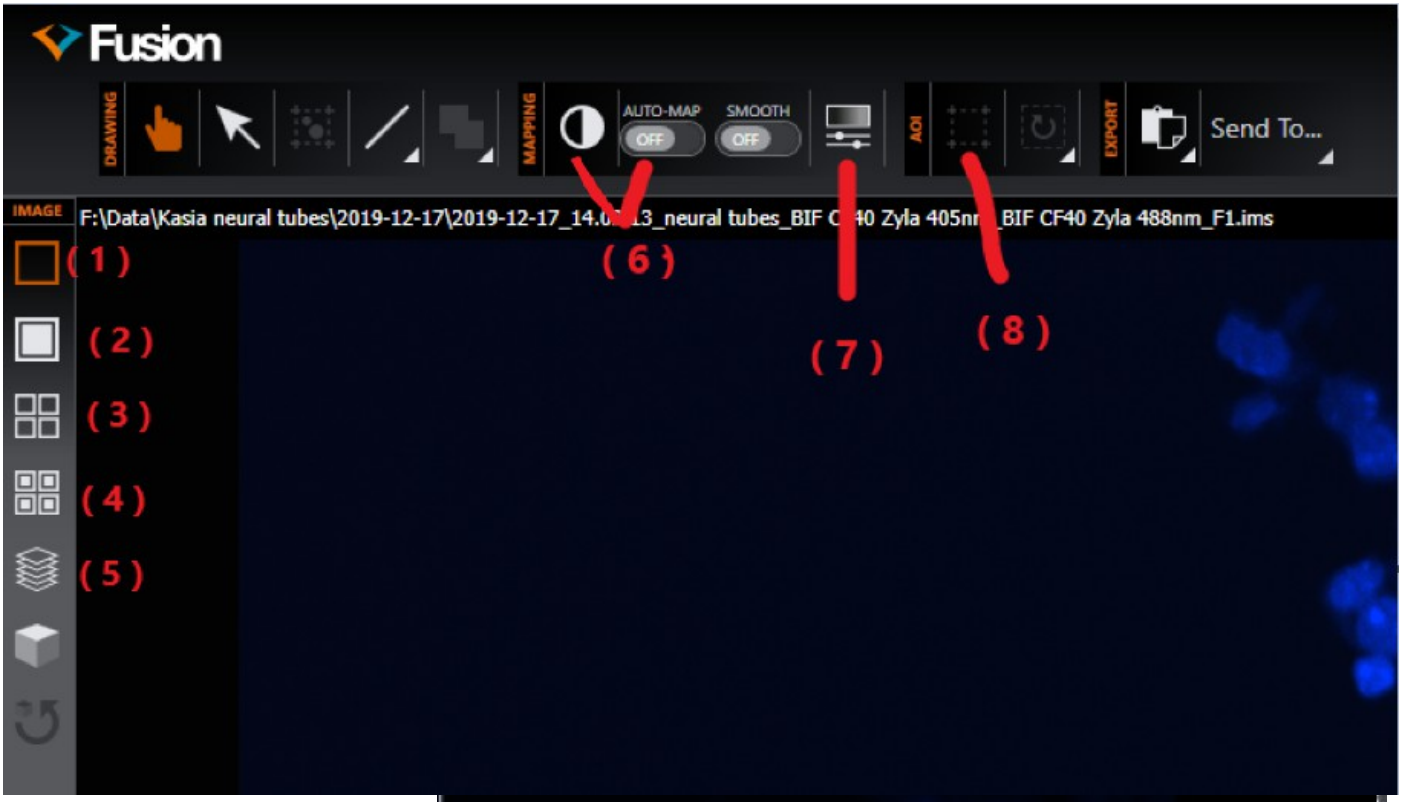
- The stitched version will create a separate file in the same folder as the original images

2. **Clearview DECONVOLUTION** (also available on Workstations/Imaris: 1 floating licence)

https://bif.pages.ist.ac.at/analysis-software



EXAMINING YOUR DATA



(1) single slice composite

(2) composite MIP

(3) single slice split-channel view

(4) split channel view MIP

(5) 3D volume render (problematic with a big data)

(6) quick display-range scaling and AUTO-MAP

(7) histogram

(8) AOI selection (reset AOI, Export image and Send to Imaris).

Please always delete your files from the list, after you are done with your experiment. Please, always check if there are no files in the list before you start imaging – if you see old files select all of them, press "Delete Selected" and then "Remove From List".