

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 6.5µm

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

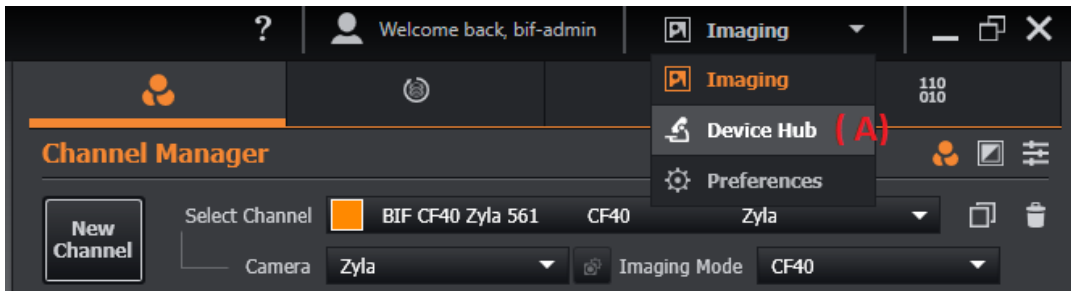


During logging in – deleting script will run in the background. It will restore all default settings (channels, protocols) in the Fusion.

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.



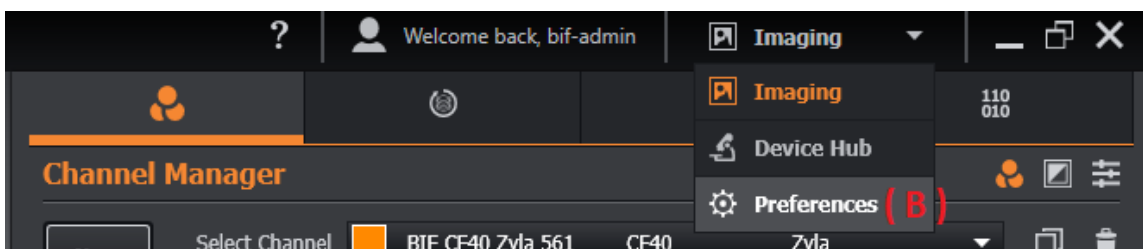
8. In the event of any startup errors you may check connections by going to the upper menu and selecting the DeviceHub option (A):



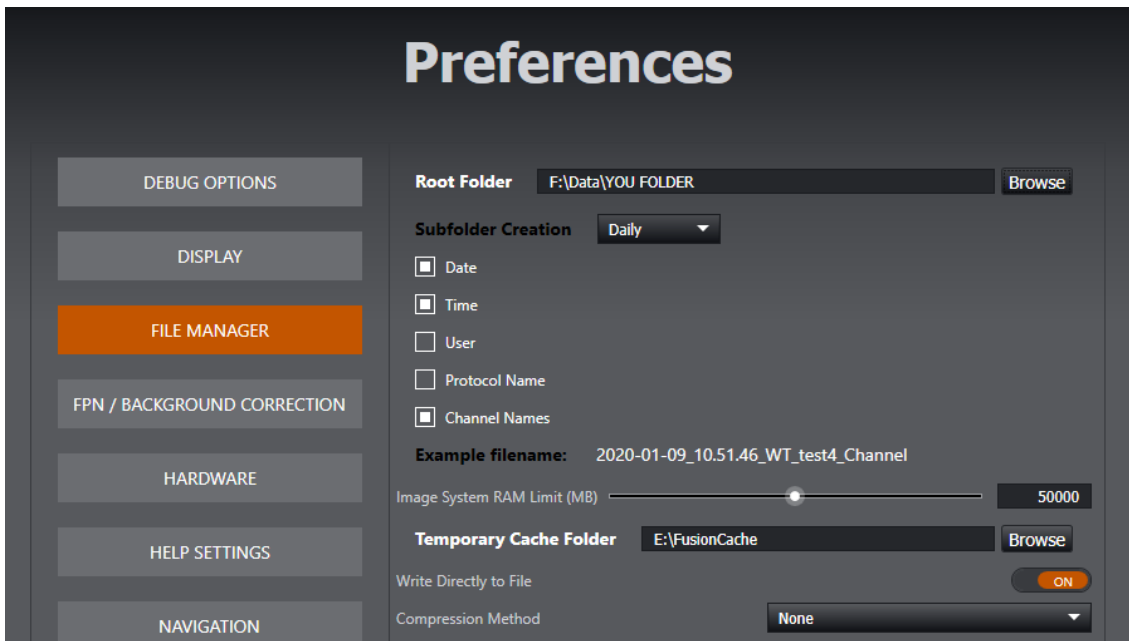
You can see a series of hardware devices and connection statuses. If any item appears with a RED marker or does not show a GREEN tick sign, you need to choose to reset the hardware connection by clicking the RECONNECT button.

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

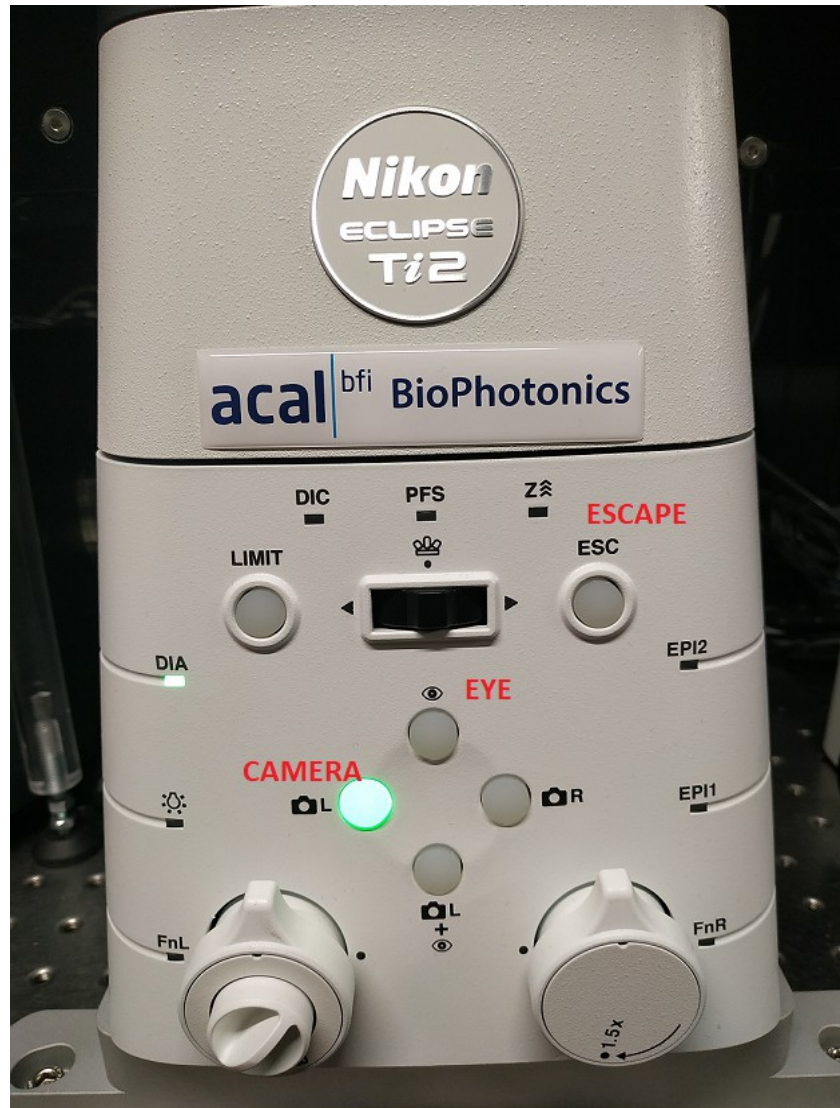
If you are planning to do live imaging experiment we recommend to turn on the heating chamber at least 1 hour before you start imaging. CO2 mixer can be turned on just before, but please consider that to work properly you need to have a correct sample holder. CO2 and humidity concentration have to be 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 your sample in the correct holder.
4. Press "EYE" in the front panel of microscope stand.

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.

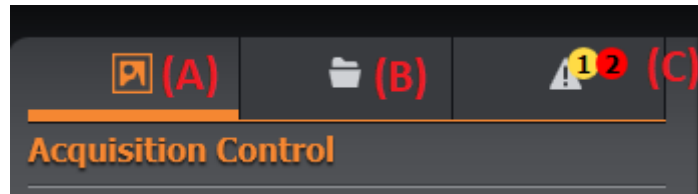
Changing Objectives

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

IMAGING WITH THE FUSION SOFTWARE

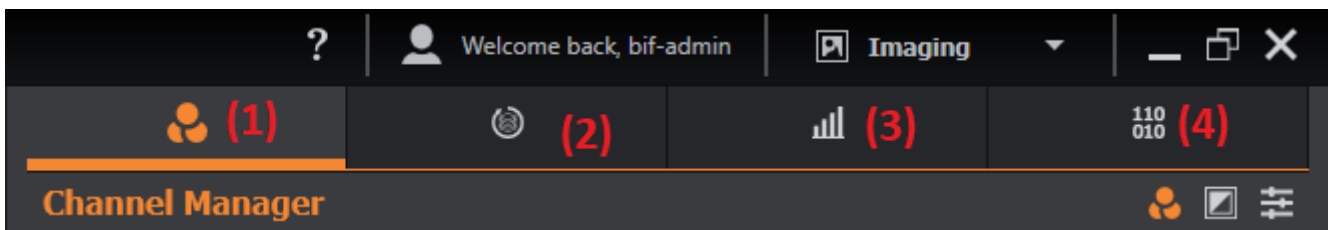
!!! Be careful – when editing any settings–Fusion will automatically update the settings without prompting to save, it is very easy to destroy protocol and channel settings.

Acquisition software tabs



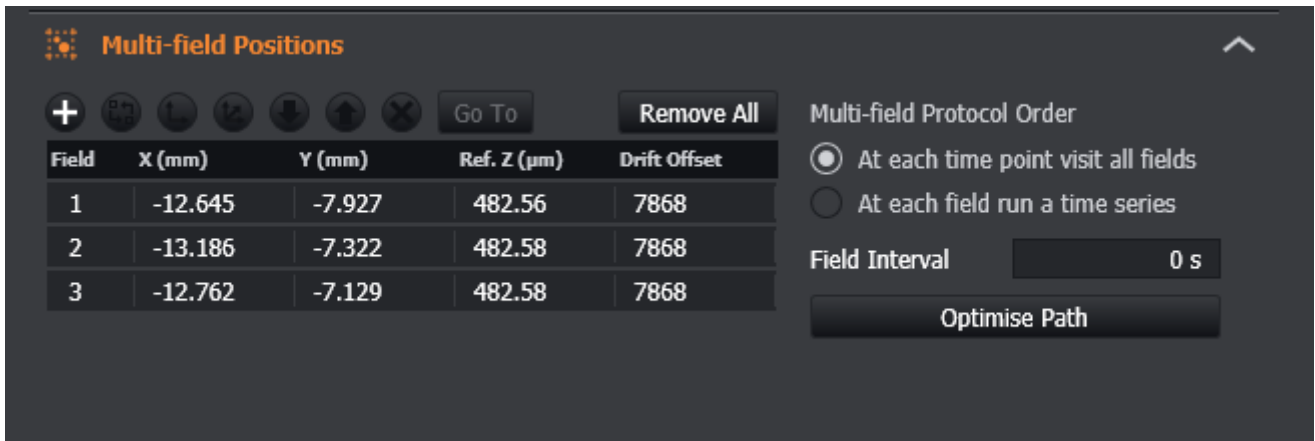
- to start image capture, ensure that you have the Acquisition Control (A) window selected
- tab (B) changes to the File Manager window
- tab (C) changes to the Error/Warning window

Main menu (Expander bar)



- (1). Channel Manager
- (2). Protocol Manager
- (3). Analysis
- (4). Image Processing

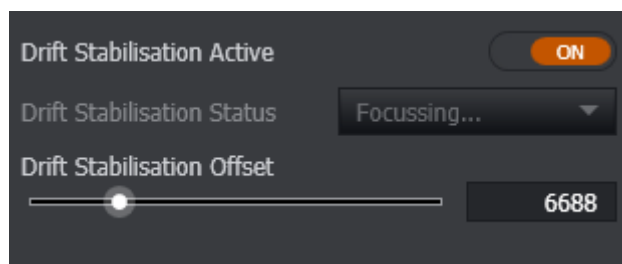
4. MULTIFIELD (MULTIPOSITIONS) SET UP



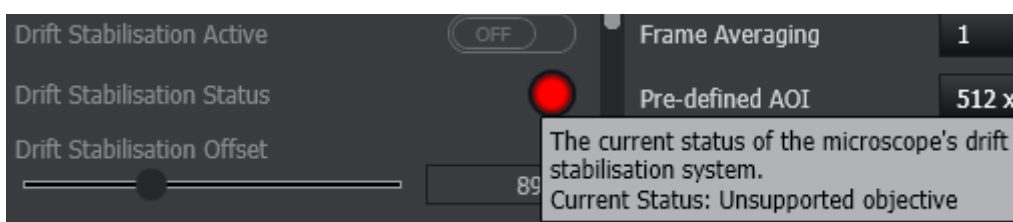
5. DRIFT STABILIZATION

This device works 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.



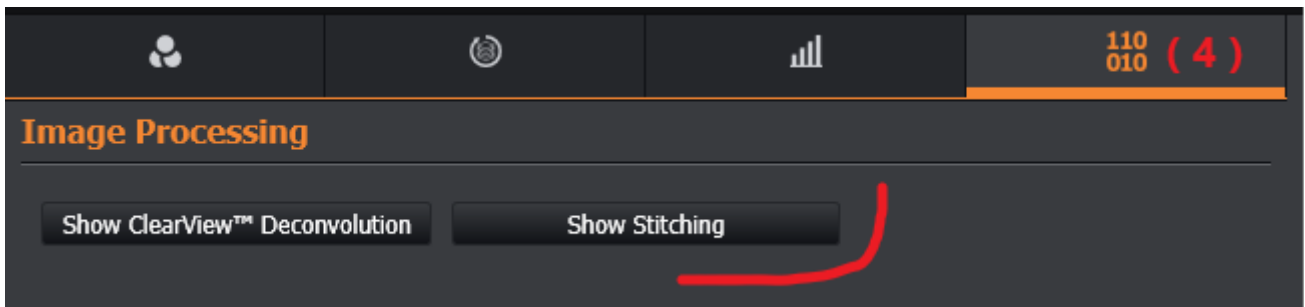
(4) IMAGE PROCESSING

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

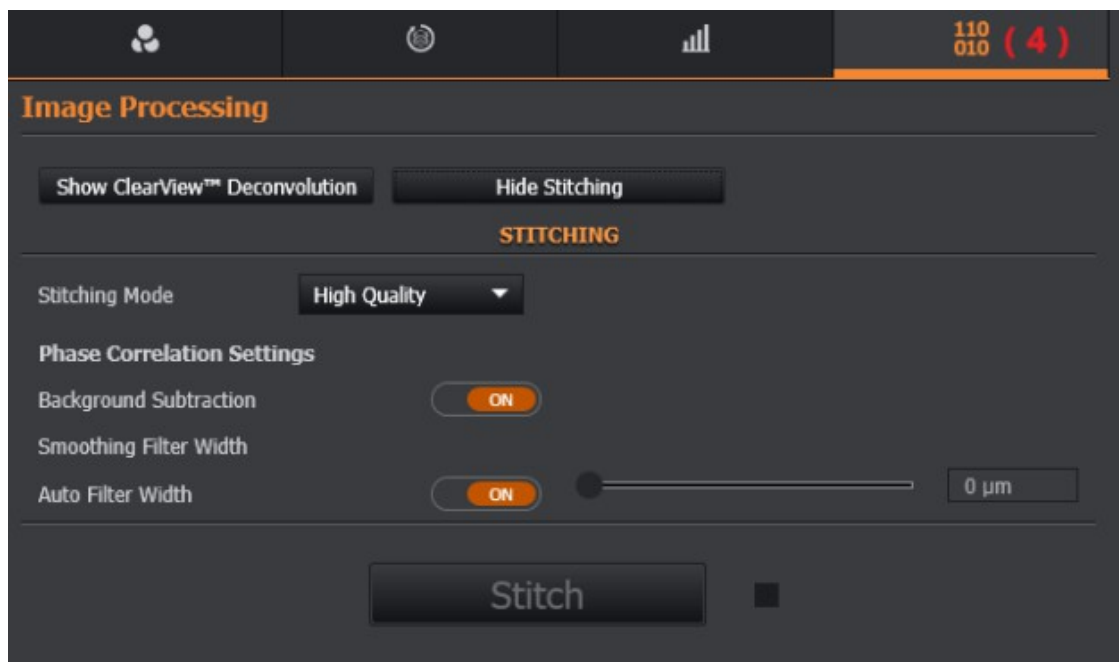
1. **STITICHING** - (also available on Workstations/Imaris: 1 floating licence)

<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>

Image Processing

Hide ClearView™ Deconvolution Show Stitching

CLEARVIEW DECONVOLUTION

Run Time: --:--:-- Remaining: --:--:--

Deconvolve GPU Compatibility: ●

PROCESSING OPTIONS

Preview

Deconvolution Preview Mode: OFF

Synchronise Zoom and Pan: OFF

Centre Preview Output: OFF

Size of Preview

X: 128 Y: 128 Z: 1

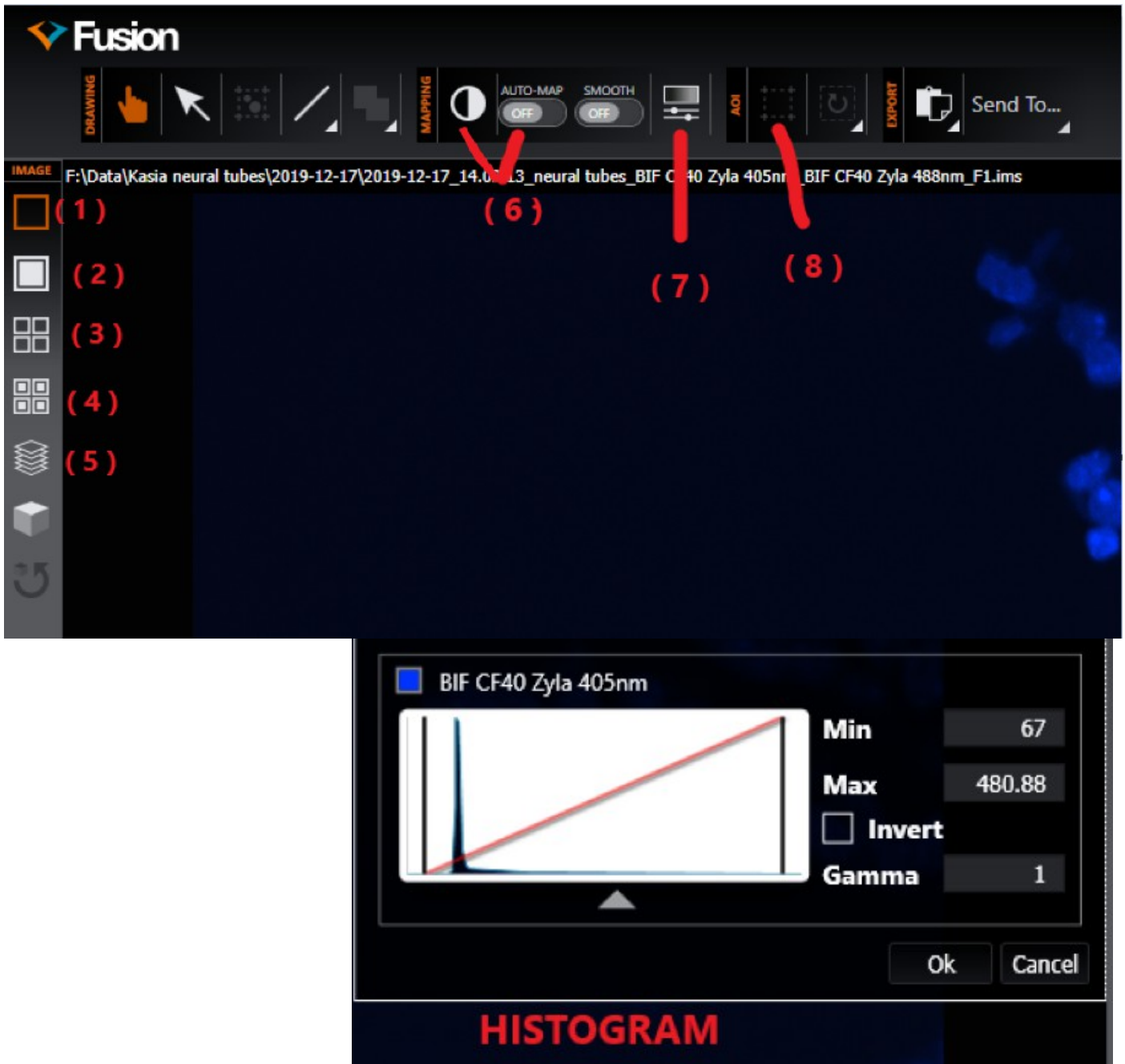
Central Positions of Preview

X: 1 Y: 1 Z: 1

Deconvolution Settings

Algorithm: Robust (Iterative) Number of Iterations: 10

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”**.

