Andor Dragonfly 200 User Manual

Version 1.2

生物與軟物質物理公用實驗設施

Biological and Soft Matter Physics Shared Facilities

中央研究院物理研究所

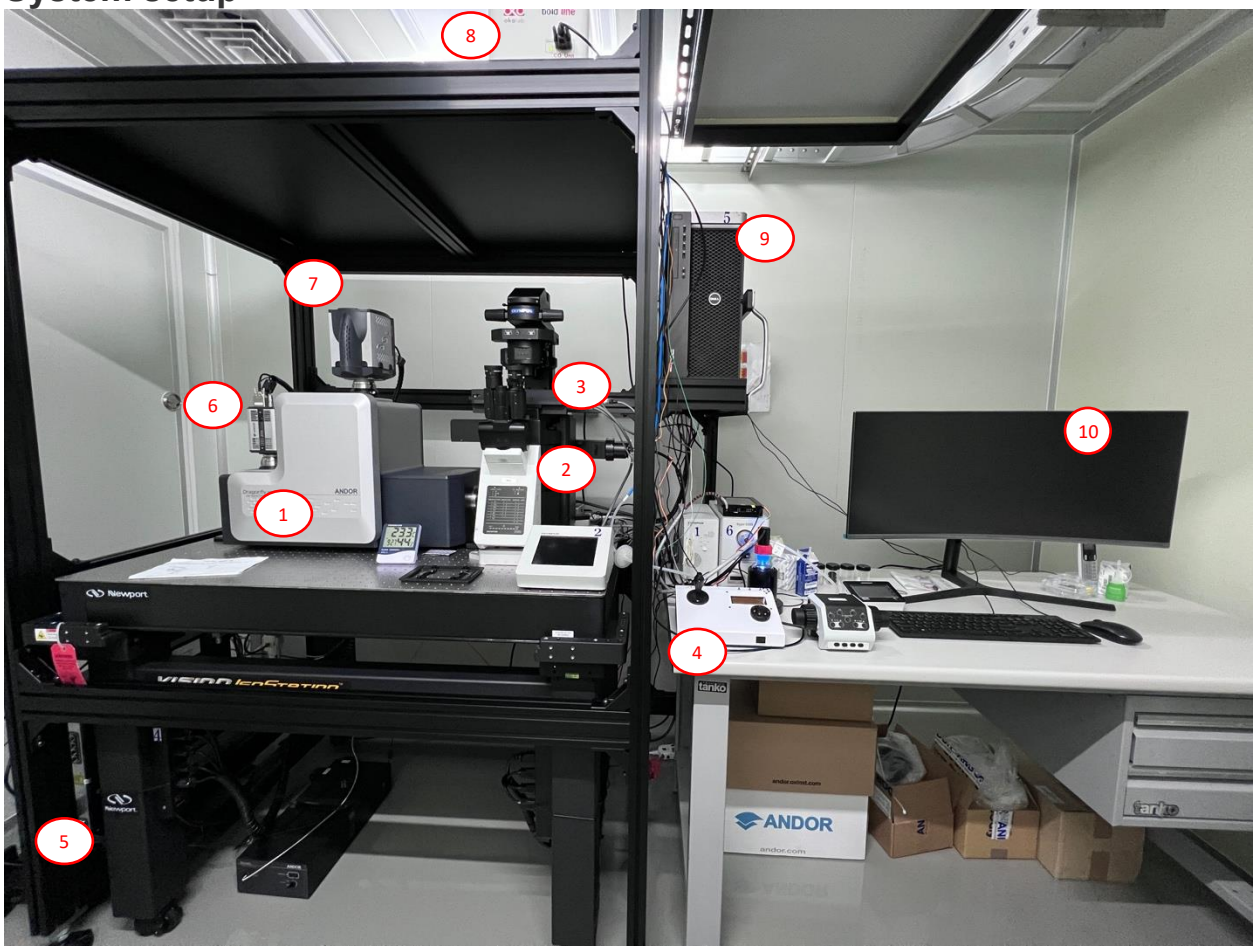
Institute of Physics, Academia Sinica

Edited by Arsene Chang (Andor Technology), Jie-Pan Shen (IPAS), Jung-Ren Huang (IPAS)

Hardware Introduction

Andor Dragonfly 200 is a high-speed confocal imaging platform that offers confocal, laser based widefield, transmitted light and deconvolution functionality in one system.

System setup



1. Dragonfly 200 confocal scanning unit
2. Olympus IX83
3. ASI Piezo Z stage
4. ASI Piezo Z stage controller
5. Lasers
6. Camera 1 (Zyla sCMOS)
7. Camera 2 (Life 888 EMCCD)
8. Incubator controller
9. PC workstation
10. Monitor

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Objective Specification

Magnification	NA	Immersion medium	Working Distance (mm)
10x	0.4	Air	3.1
20x	0.75	Air	0.6
40x	0.95	Air	0.18
100x	1.4	Oil	0.13
60x	1.3	Silicone oil	0.3

Laser Wavelength

Laser Wavelength (nm)	Power (mW)	Fluorescence Dye Example
405	100	DAPI
488	150	FITC/Alexa488
561	100	TRITC/Alexa568/Cy3
637	140	Alexa647/Cy5

Emission Filter Specification

450-050
525-050
600-050
620-060
700-075

Please visit Andor's official website (https://andor.oxinst.com/) or contact us (https://www.phys.sinica.edu.tw/~bssf/) for more information.

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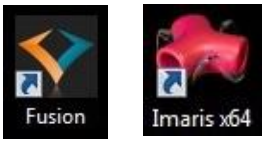
Software Introduction

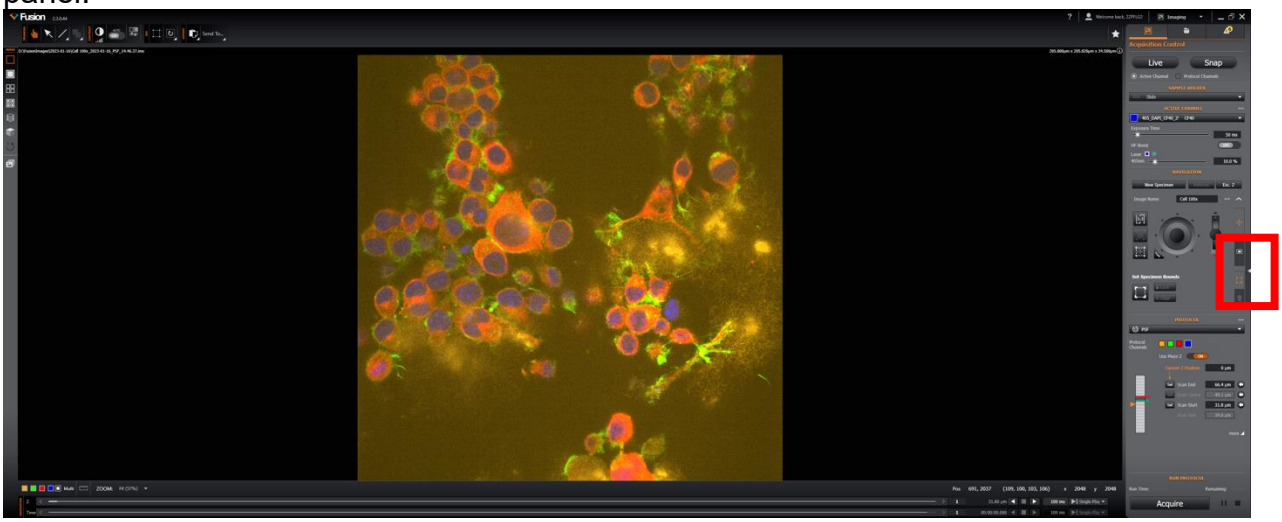
The system is controlled by **Fusion. Imaris** is also installed on the workstation for data visualization

Icons on the desktop:

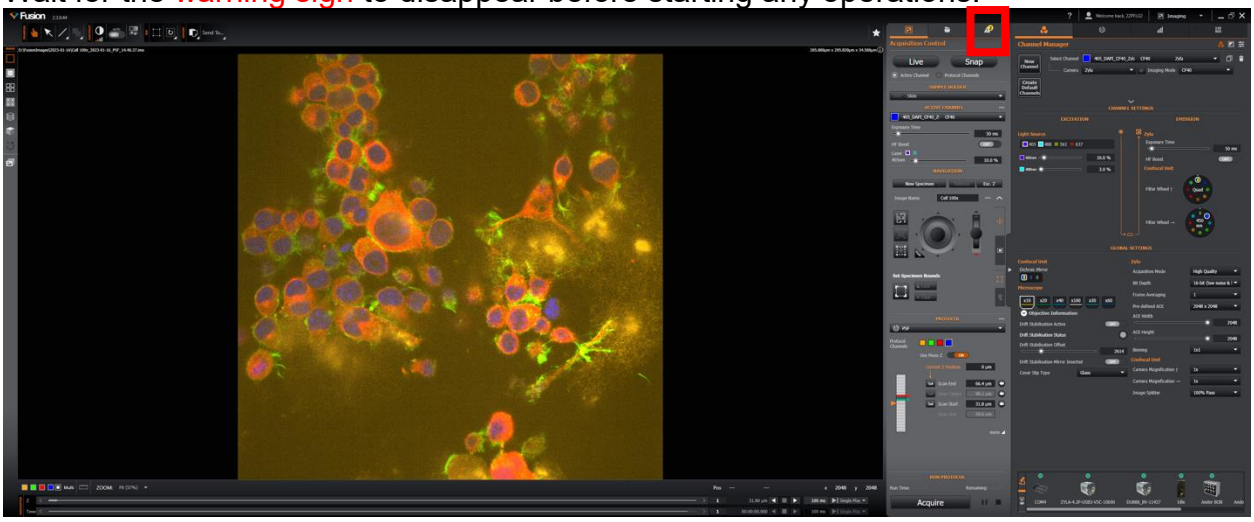


Fusion Interface

As Fusion is starting, click on the small triangle in the red box to expand the control panel.



Wait for the warning sign to disappear before starting any operations.



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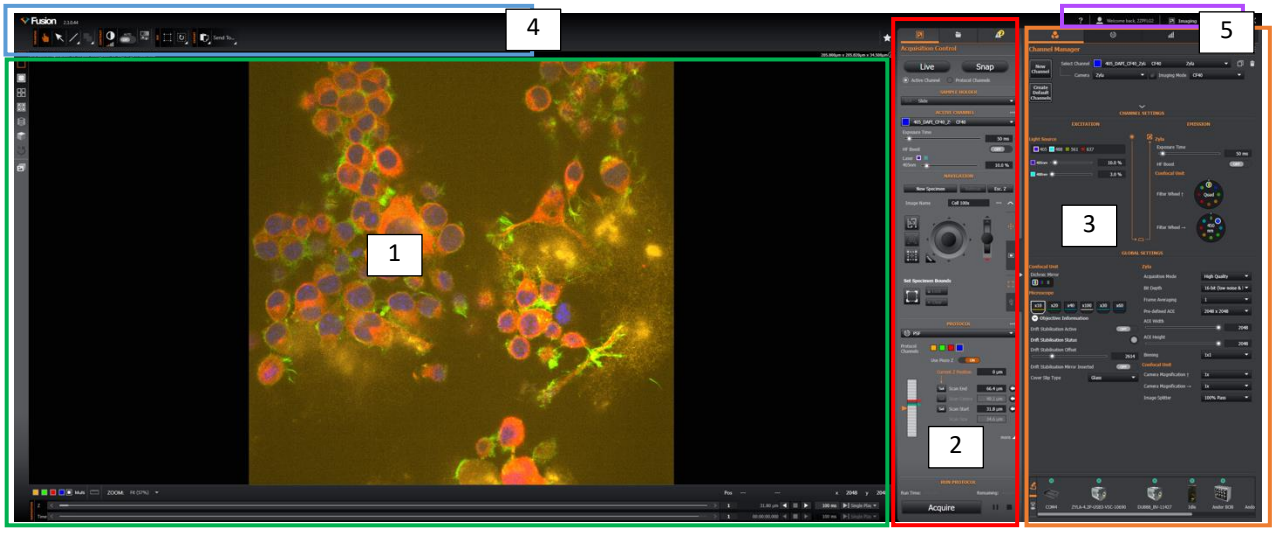
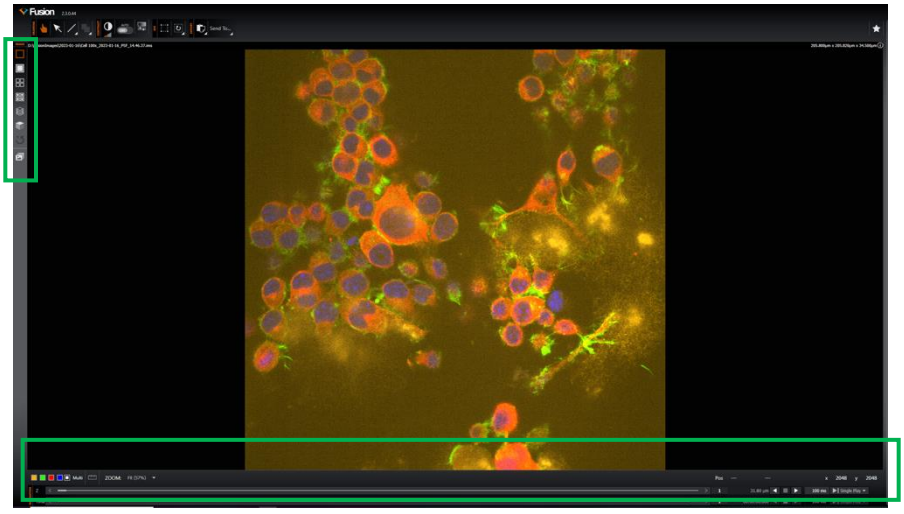
The Fusion window is divided into 5 sections of different functions:



1. Image display window
2. Basic control panel
3. Advanced control panel
4. Image tools
5. Menu

1. Image display window



Click on the icons on the upper left side to switch between different modes of displaying live or previously acquired images.

Switch on/off the display of the filter channels by clicking on the corresponding color buttons at the bottom. Right-click on the color button to change the software setting for the color of the channel.

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Images taken at different Z positions (only in 2D display mode) or time points or XY positions can be displayed by moving the slider at the bottom.

2. Basic control panel:

- Acquisition Control
- Managing Files
- Warning

2-1 Acquisition Control

1. Click to view/stop live imaging.

2. Snap an image using current channel and settings

3. Live/Snap using current channel or channel(s) in the protocol.

4. Select the sample holder: slide or multi-well plate (for multiple position protocol setup).

5. Choose active channel for live viewing

6. Camera exposure time, EM gain (only for EMCCD) and laser output power setup.

7. XYZ digital control and sample navigation functions. Click on the “3 dots” button to enter Preference menu to change the file name and location before acquisition. User should save data only in the folder designated by the administrator.

8. Quick protocol setup.

9. Start, pause, or stop acquisition under a protocol.

2-2 Manage Files

1. Open saved images; Export selected images to a different format; Open selected images in Imaris

2. Gallery of saved images

2-3 Warning

Red Warning: System is initializing; Device disconnected; Settings missing in protocol…etc.

You will not be able to operate Fusion with red warnings

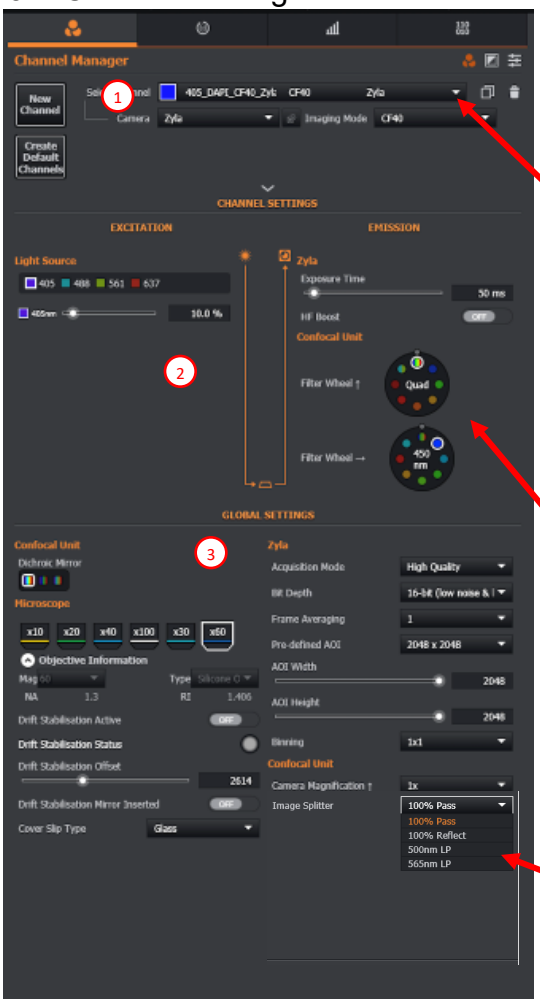
Yellow Warning: Laser is not stabilized yet; Camera cooling is not ready yet…etc.

You will still be able to operate Fusion with yellow warnings. However, the proper action should be waiting until the yellow warning disappears.

3. Advanced Control Panel

- Channel Manager
- Protocol Manager
- Analysis
- Image Processing

3-1 Channel Manager



1. Channel list: Select existing channel from the dropdown list or create a new channel.

Make sure you are using the channel with the correct camera (Zyla or iXon) you want to use.

2. Channel settings: These settings will change with channel, usually you only need to setup camera exposure and laser power here.

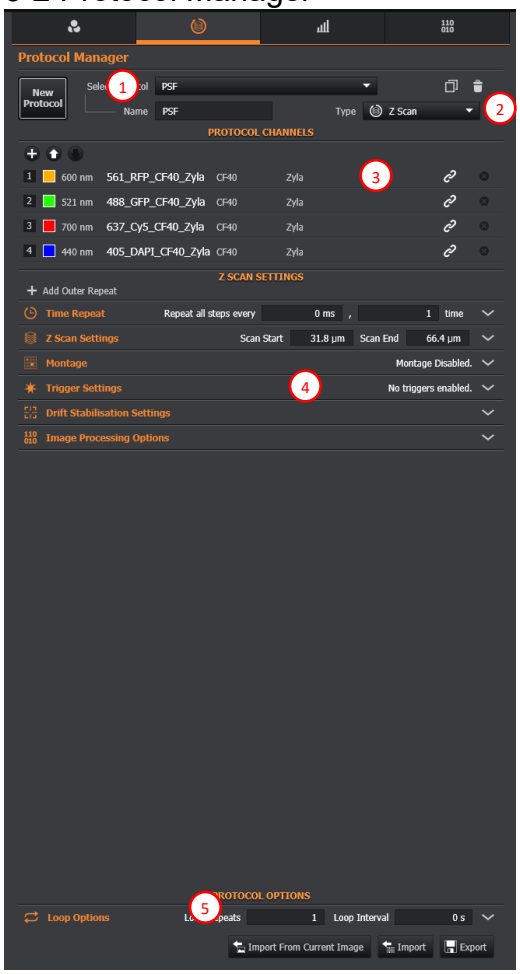
Don't change Filter Wheel settings by yourself without discussing with the system administrator.

3. Global settings: These settings will not change with channel, you can select objective and change camera ROI, binning…etc. here.

When using Zyla (camera 1), make sure Image Splitter position is at 100% Pass.

When using iXon EMCCD (camera 2), make sure Image Splitter position is at 100% Reflect.

3-2 Protocol Manager



1. Protocol list: Select existing protocol from the dropdown list or create a new protocol.

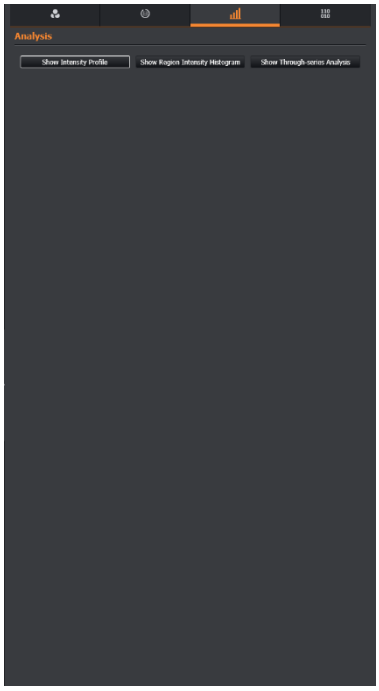
2. Protocol type: Choose the protocol type you want to setup (time series/Z scan/Multi position).

3. Protocol channels: Add/delete the channels you want/don't want to use in this protocol.

4. Setup for each protocol type.

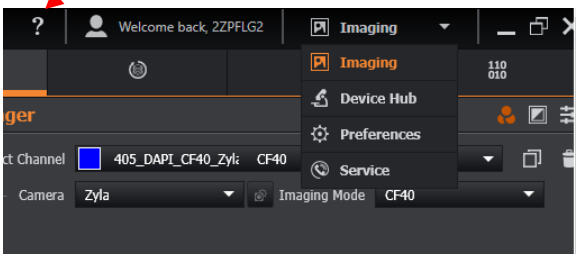
5. Import protocol from current image or a saved protocol file; Export current protocol to a protocol file.

3-3 Analysis

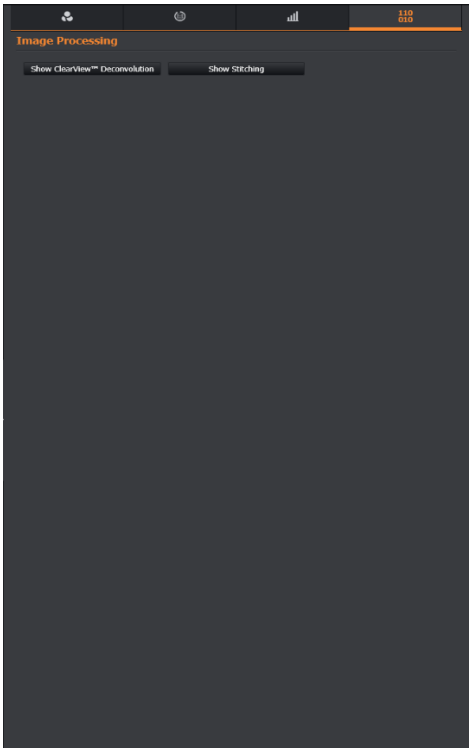


Basic image intensity analysis functions.

Please check Image Analysis section in “Fusion software guide” for more details

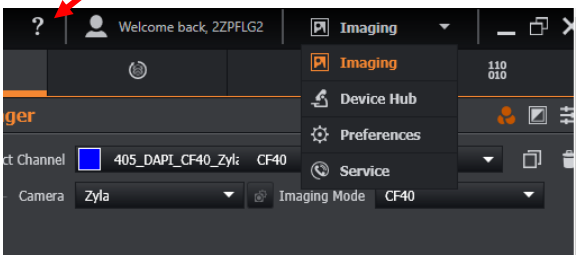


3-4 Image Processing



Perform ClearView Deconvolution or run Stitching for montage image.

Please check Image Analysis section in “Fusion software guide” for more details



4. Image Tools

1. Drawing tool for intensity analysis on the image.

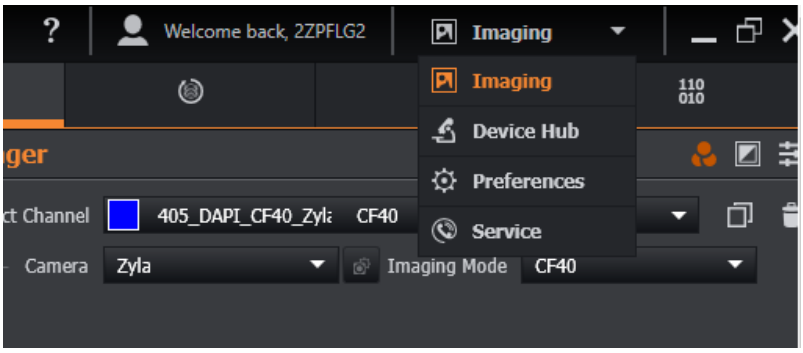
2. Contrast adjustment, you can switch “auto-contrast” on/off or adjust histogram by clicking on the histogram icon to open the histogram window for adjustment. Note: The adjustments here only change how images are displayed. No camera hardware parameters are changed.

3. Adjust the camera’s region of interest.

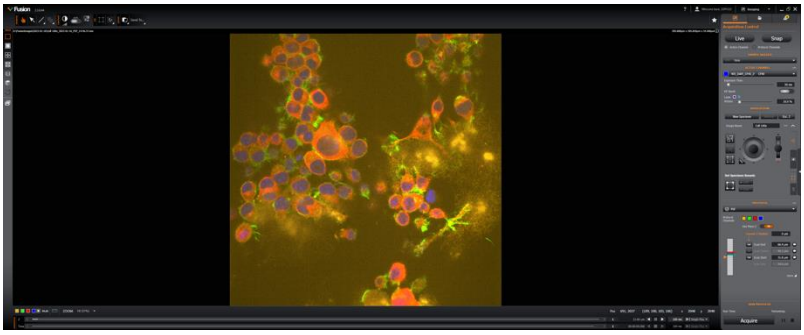
4. Export current display as a snapshot picture or open current image in Imaris.

5. Menu

You can visit “Imaging”, “Device Hub” and “Preference” interface by selecting from dropdown list



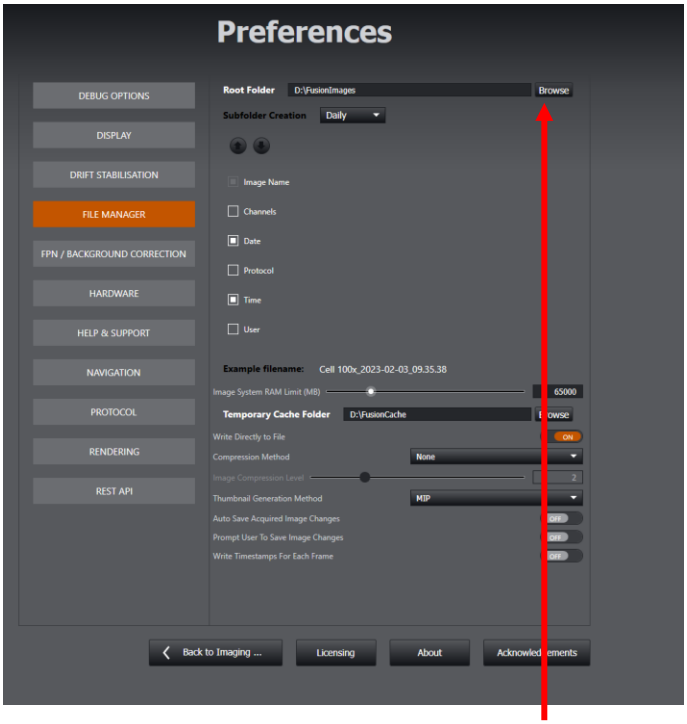
Imaging interface (Please see Sections 1-4 for details.)



Device Hub

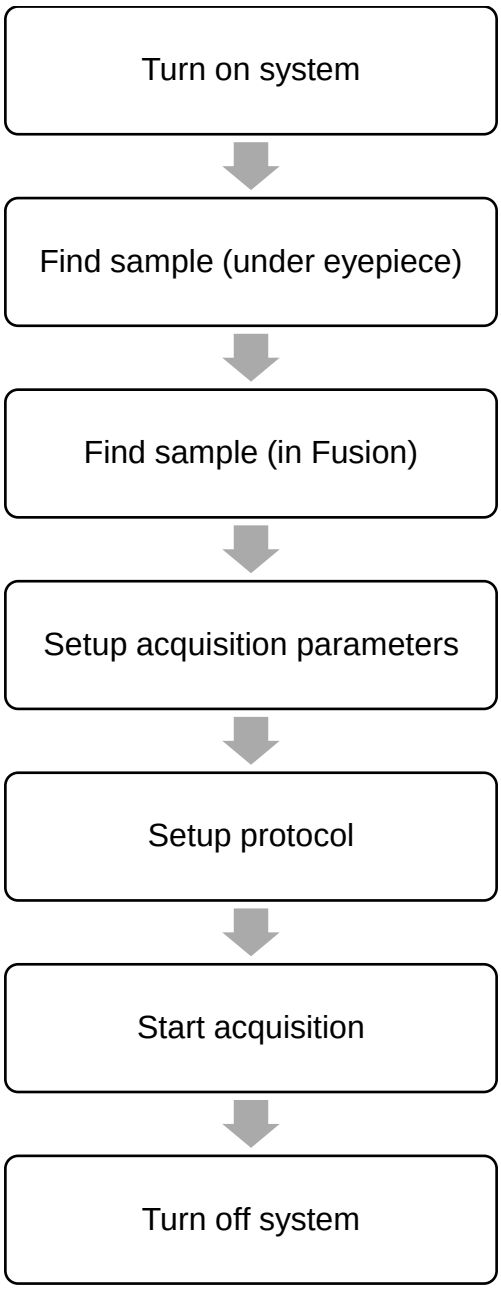
Here it shows all the hardware controlled by Fusion. Try to reconnect the corresponding hardware if there is connection issue showed in the warning. **Please do not click on other buttons without first asking the system administrator**

Preferences



You can change the file saving location in Preferences > File Manager. However, user should save data only in the folder designated by the administrator. **Please do not change other settings without first asking the system administrator**

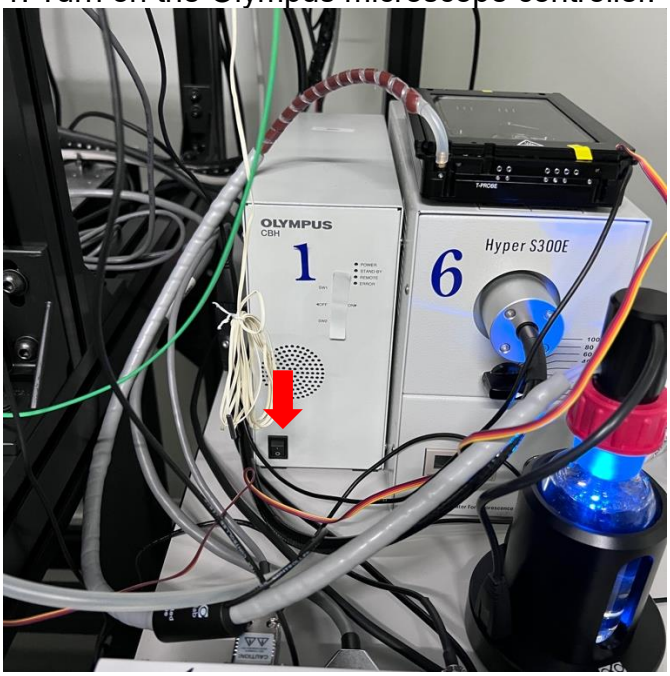
General Workflow



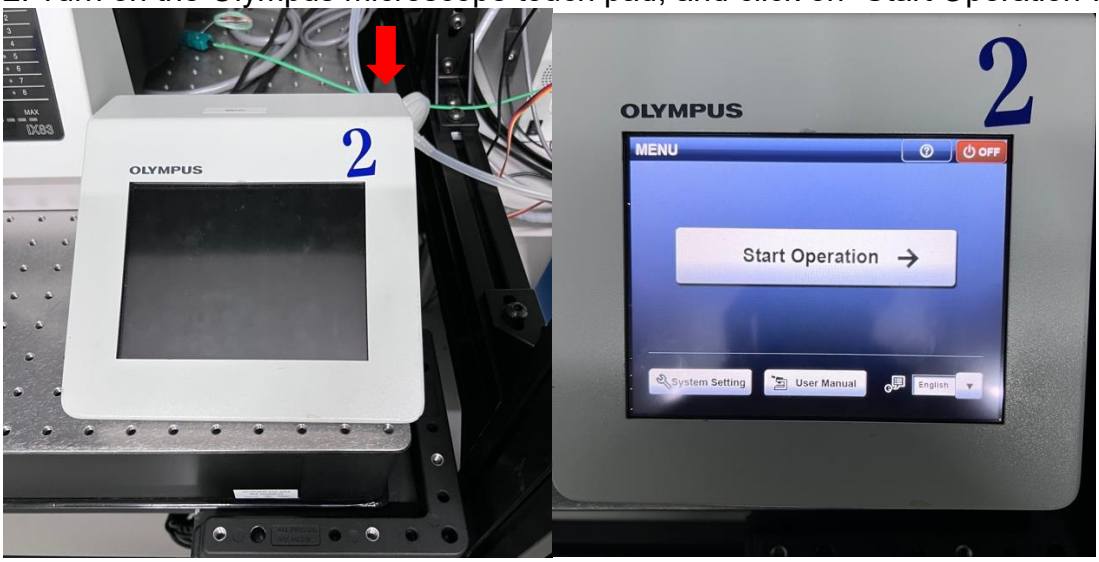
System Turn-on Sequence

Strictly follow the steps below for turning on the system to avoid hardware connection issue.

1. Turn on the Olympus microscope controller.



2. Turn on the Olympus microscope touch pad, and click on “Start Operation”.



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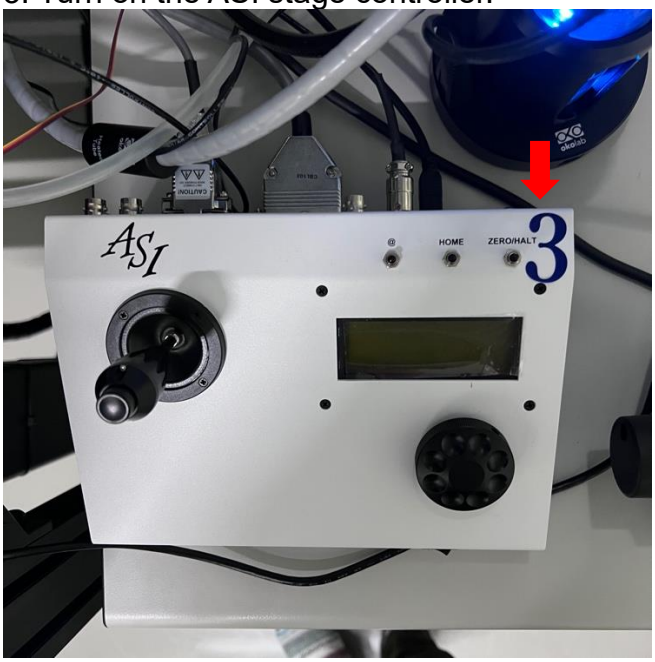
3. Turn on the ASI stage controller.



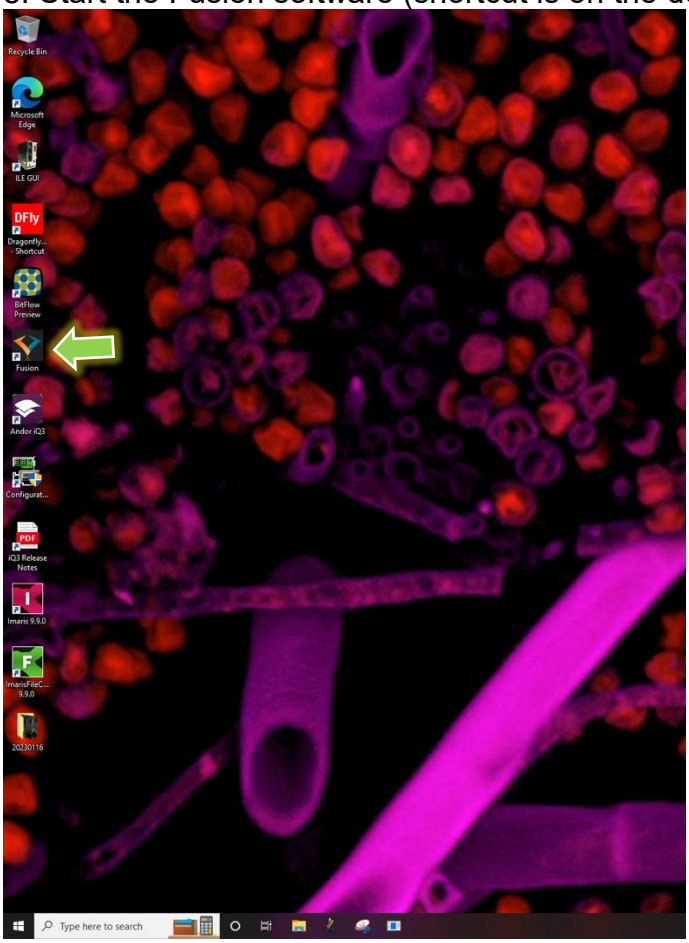
4. Switch on the power strip.

5. Turn on the workstation.

6. Turn on EPI fluorescence light source if you need to check fluorescence sample using the microscope eye pieces.

7. Turn on the monitor (if it is off).

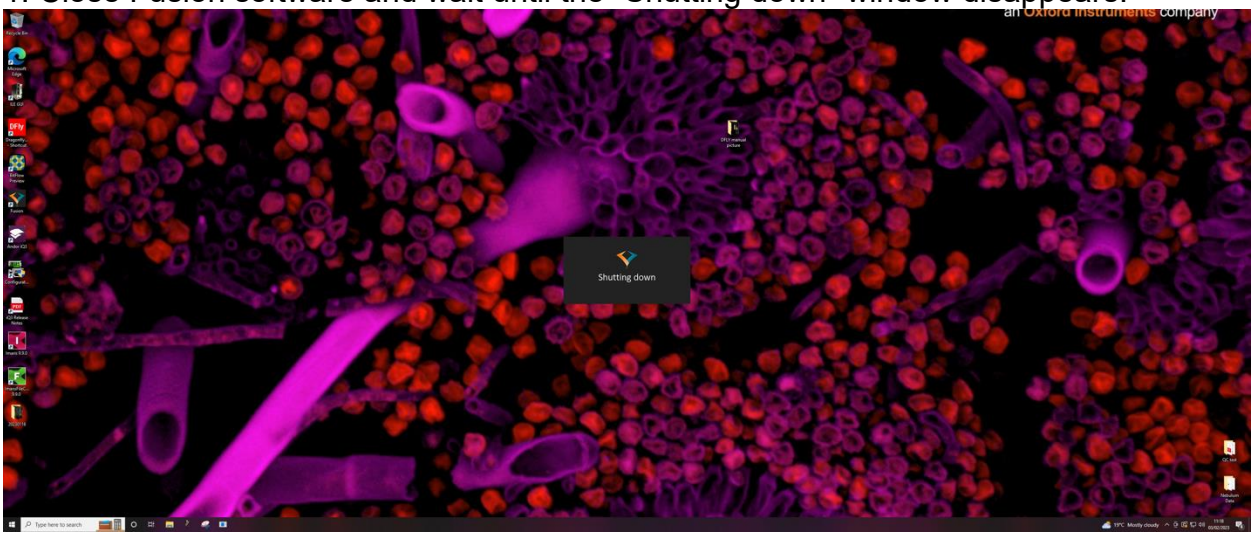
8. Start the Fusion software (shortcut is on the desktop of the workstation).



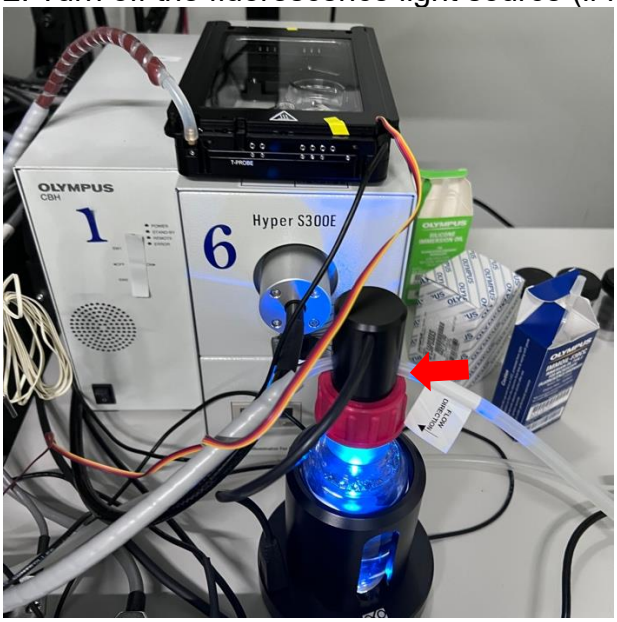
System Turn-off Sequence

Strictly follow the steps below for turning off the system to avoid hardware connection issue.

1. Close Fusion software and wait until the “Shutting down” window disappears.

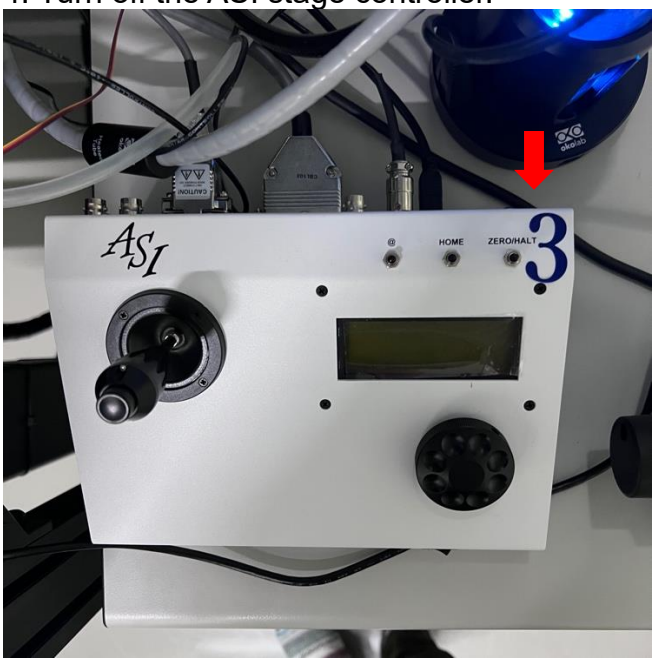


2. Turn off the fluorescence light source (if it is on).



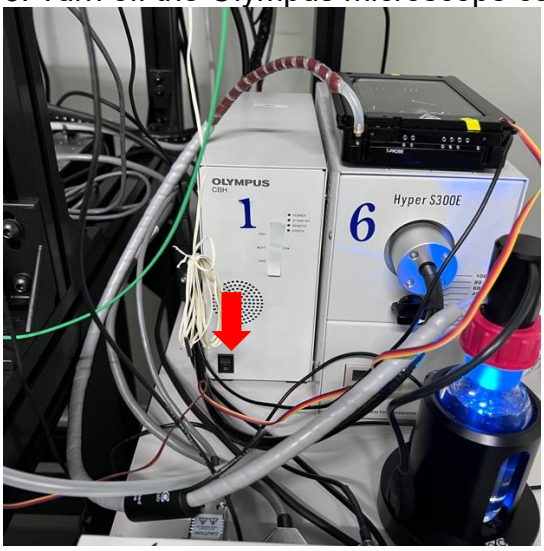
3. Switch off the power strip.

4. Turn off the ASI stage controller.

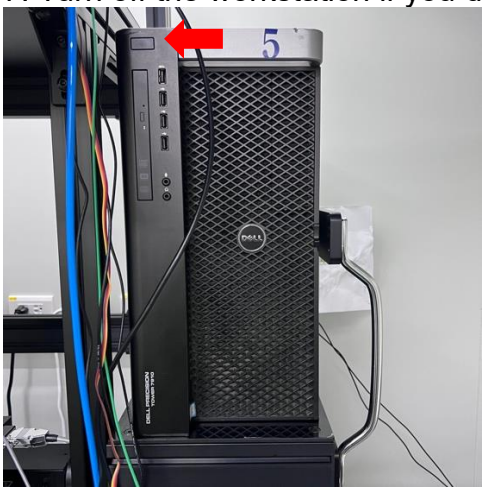


5. Turn off the Olympus microscope touch pad.

6. Turn off the Olympus microscope controller.



7. Turn off the workstation if you don't need to use it.



Find sample under eyepieces

1. Select the objective using the touch pad by clicking on the objective icon.

Make sure the correct immersion medium is used for the objective you selected (Air/Oil/Silicone oil).

2. Switch the microscope port to eyepiece by clicking on the port icon and the icon changes to “eye”.

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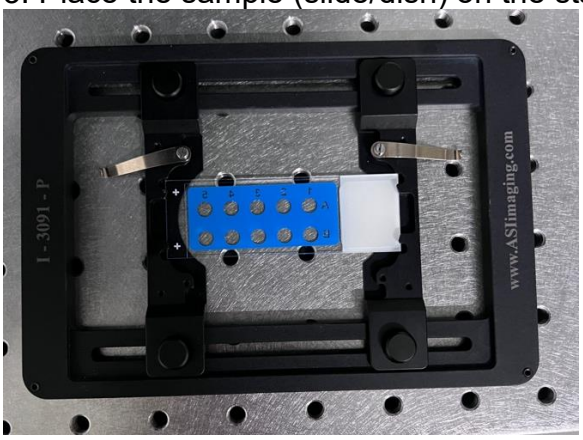
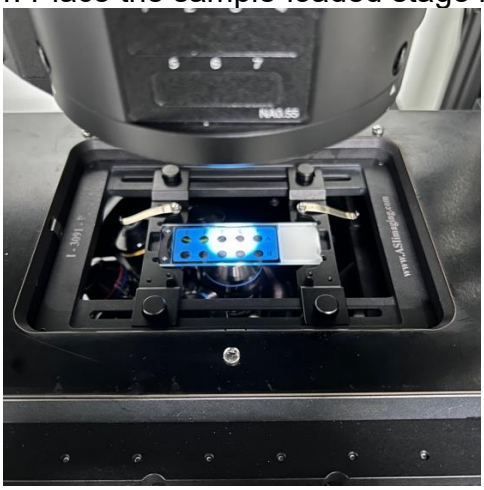
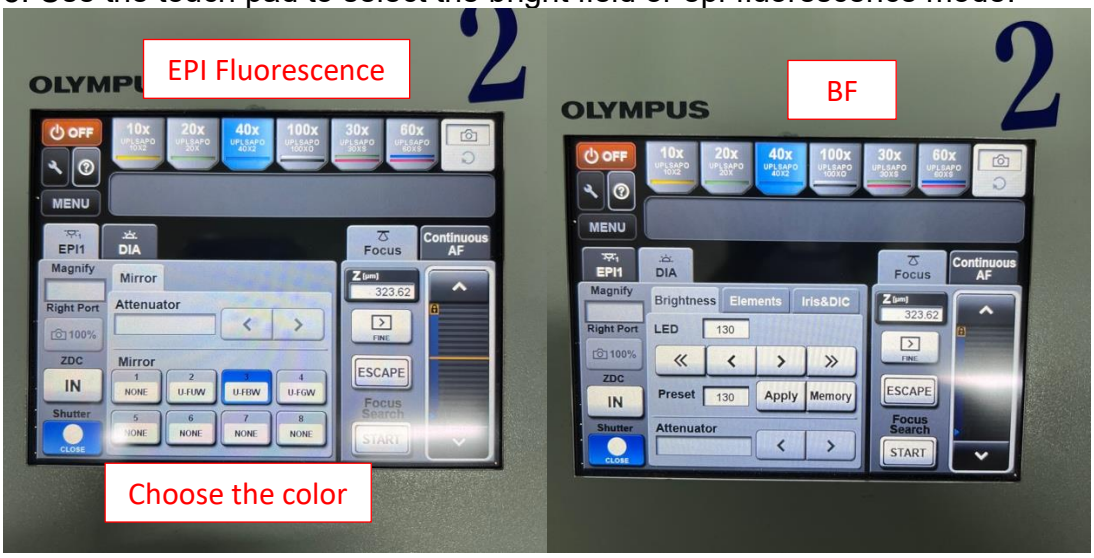
3. Place the sample (slide/dish) on the stage holder.



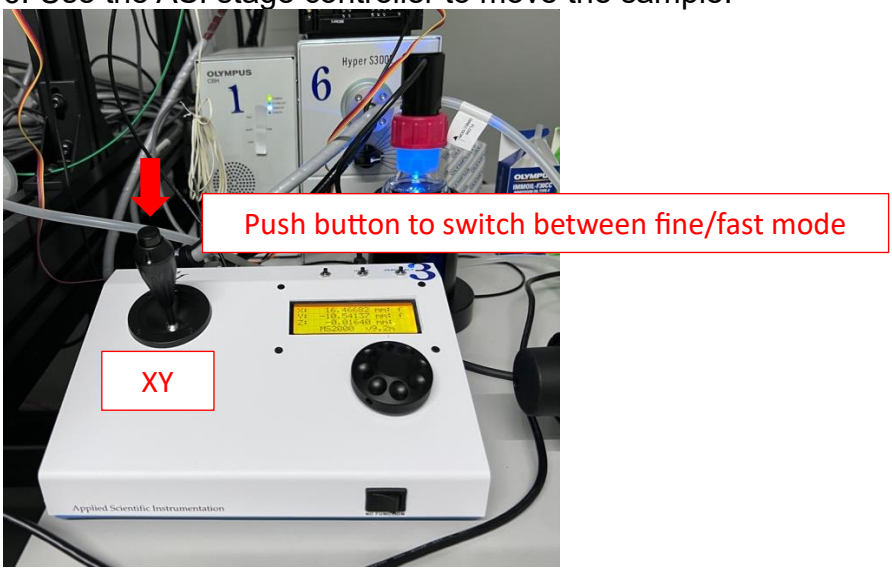
4. Place the sample-loaded stage holder on the ASI microscope stage.



5. Use the touch pad to select the bright field or epi-fluorescence mode.



6. Use the ASI stage controller to move the sample.



7. Use microscope Z controller to focus sample.

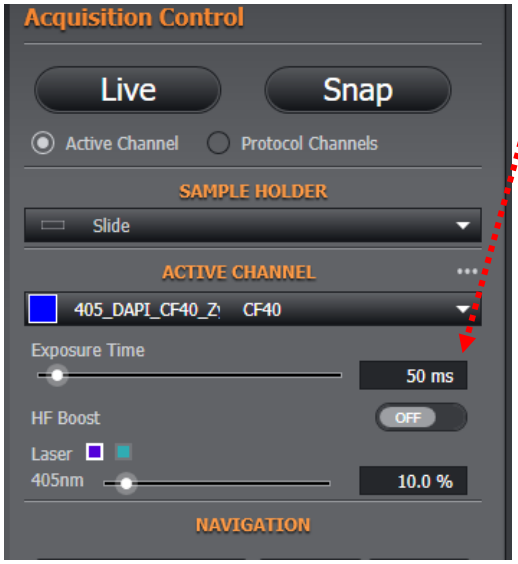
8. Now it is ready to view the sample and set up acquisition parameters in Fusion.

View sample & Set up acquisition parameters for each channel in Fusion

1. After seeing the sample under eyepieces, go to Fusion and select the channel for live images in Fusion from the active channel dropdown list.

Adjust exposure time and laser power properly (we recommend starting from values about 100ms and 10% for confocal, 50ms and 2% for WF), then click on “Live” to view live images.

Sometimes it is easier to first focus the sample under WF and then switch to CF after you can see the sample in live view under WF. If needed, refocus the sample after switching from WF to CF.



2. After the sample is in focus in live view, adjust camera exposure time and laser power for optimal imaging quality with this channel.

Turn on auto-contrast function and check the histogram for this channel (Section 4). By adjusting camera exposure and laser power, the value of “Max” changes accordingly. We recommend having “Max” at least 3x larger than “Min”, meaning the SNR (Signal to Noise Ratio) > 3

3. Repeat step 1-2 for all other channels you would like to use in a protocol for this sample

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*** Note: If EMCCD is in use, set identical EM gains for all channels**

Change from air objectives to oil-immersion objectives

1. Before changing the objective, find the current stage XY coordinate from the navigation panel. Record the XY coordinate values.

2. Press the Escape button on touch pad to lower the objective.

3. Switch to oil objective either via Fusion or touch pad.

4. Move the stage to an open position where you can drop the oil on the objective (please use the correct oil for the objective).

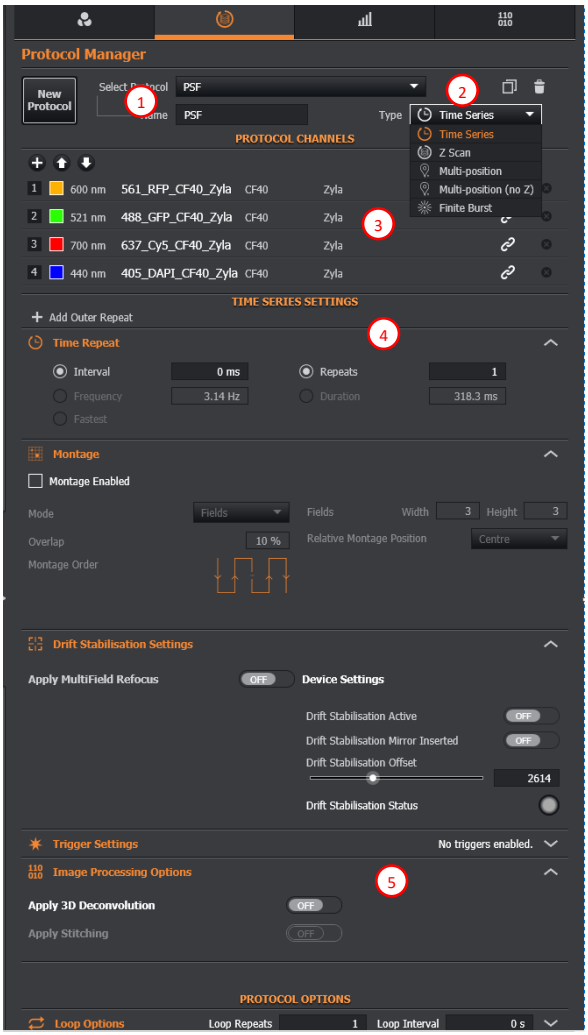
5. Enter the XY stage coordinate (recorded in Step 1) in Fusion navigation panel.

6. Press Escape button again to rise the objective.

7. Refocus the sample via eyepieces or live imaging.

Set up Protocol

1. After finishing setting up the acquisition parameters for each channel, go to “Protocol Manager” interface to set up the protocol for sample imaging.



1. Select from existing protocols, or create a new protocol with your preferred name.

2. Select which protocol type you would like to use.

3. Add channels you would like to use in this protocol.

4. Set up time repeat (time series) style.

5. Turn auto deconvolution or auto stitching on/off (recommend setting is off)

6. Make sure the file name and save location is correct

7. Start acquisition

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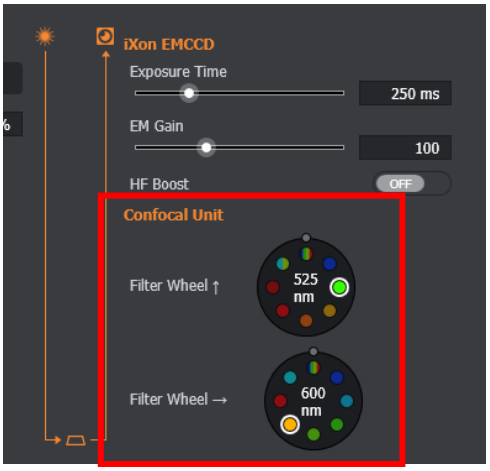
Set up protocol channel for dual camera acquisition, an example of green and red colors

Green (cam 2)/Red (cam 1) dual color acquisition can be achieved with 565LP dichroic mirror in position (Section 3-1: Channel Manager, Global Settings, Image Splitter -> select 565LP).

1. Set camera 1 (Zyla) to 2x2 binning to match the chip size with camera 2 (iXon EMCCD)

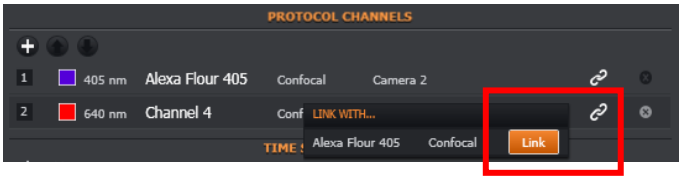
2. Make sure both emission filter wheel position settings are the same as the ones shown in the image below (561nm for camera 1 channel and 488nm for camera 2 channel in this example).

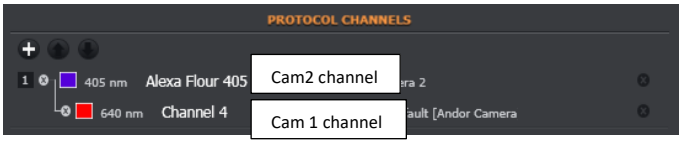


For 488/561 simultaneous acquisition, the positions of the two filter wheels should be 525nm for cam2 (Filter Wheel ↑) and 600nm for cam1 (Filter Wheel →).

3. Add cam 1-561 channel and then cam 2-488 channel to protocol channels.

4. On the cam 2-488 channel, click on the “chain” icon on the right to link the two channels.
Note: The images below are just for showing how to link two channels, which use 405nm and 640nm channels. For the example presented in the above (488nm and 561nm), the wavelengths in the images below “405nm” should be “488nm”, and “640nm” should be “561nm”.





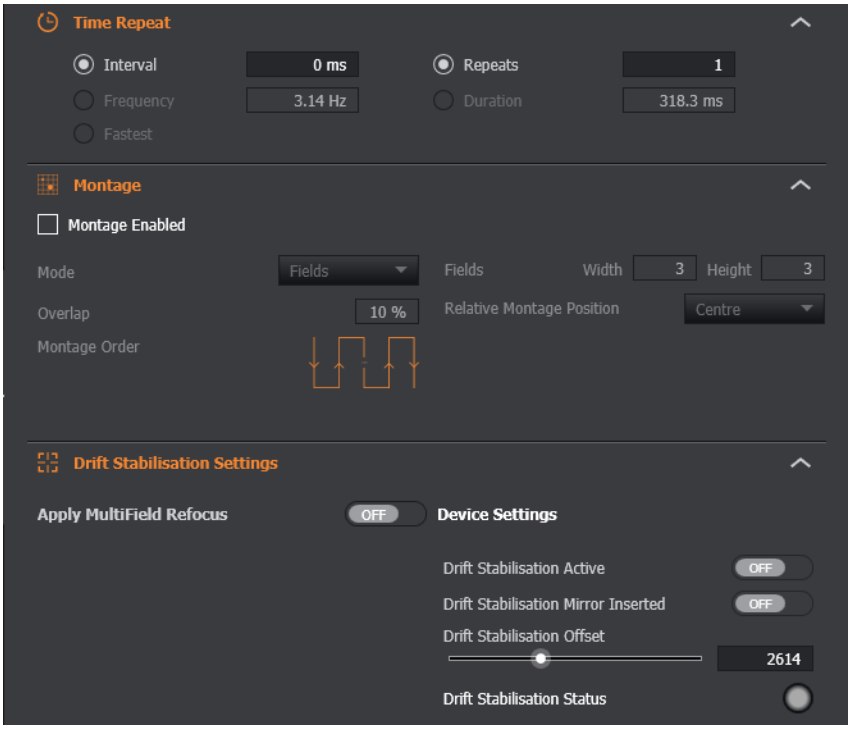
5. Now you can set up other protocols and start acquisition.

Time repeat (time series) protocol setup

Options: use either “Interval” or “Frequency”, and “Repeats” or “Duration”.

Ex: Acquiring all channels every 30 minutes and for 5 hours: Set “30m” in “Interval” and enter “11” in Repeats or “5h” in “Duration”.

Interval unit in ms/s/m/h/d.



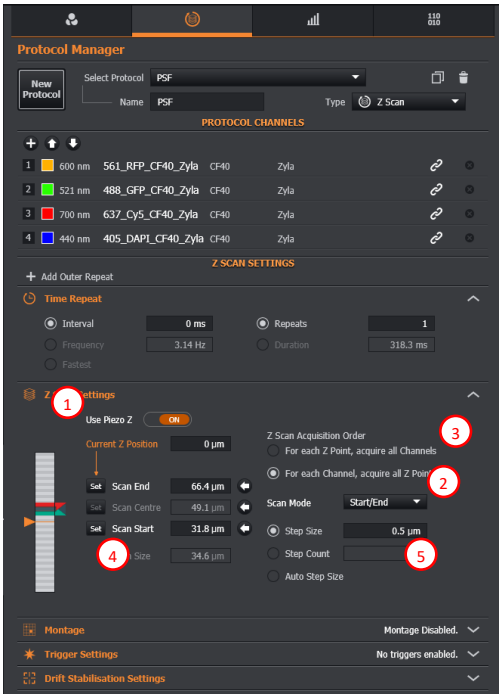
Montage function can be enabled with time series.

When montage is in use, please make sure the time needed to complete the montage at each time point should be less than the interval.

You can enable Olympus ZDC for time series by turning on “Drift Stabilisation Settings”.

Z scan protocol setup

You can run Z scan with time series and montage.



1. Choose either Piezo Z or microscope Z for z scan. We recommend using Piezo Z (as it is faster) unless the z scan range is > 500um.

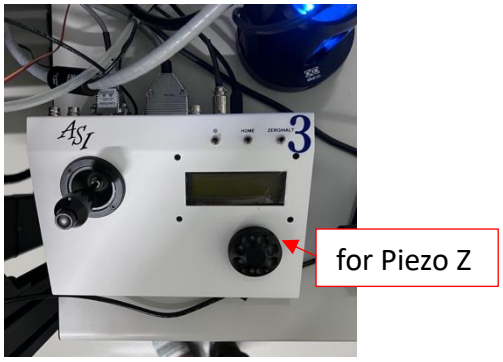
2. Choose scan mode “Start/End” or “Center/Size”.

   Start/End: Set Z position for start and end position, scan size is automatically calculated.

   Center/Size: Set current Z position as center position and determine the scan size. Usually this mode is used for multi positions with Z scan protocol and when “Drift Stabilisation Settings” is enabled for long time series.

3. Choose acquisition order, Z first (top one, slower but Z position is more accurate across channels, especially when the sample is not fixed) or Channel first (bottom one, faster but not suitable for moving sample).

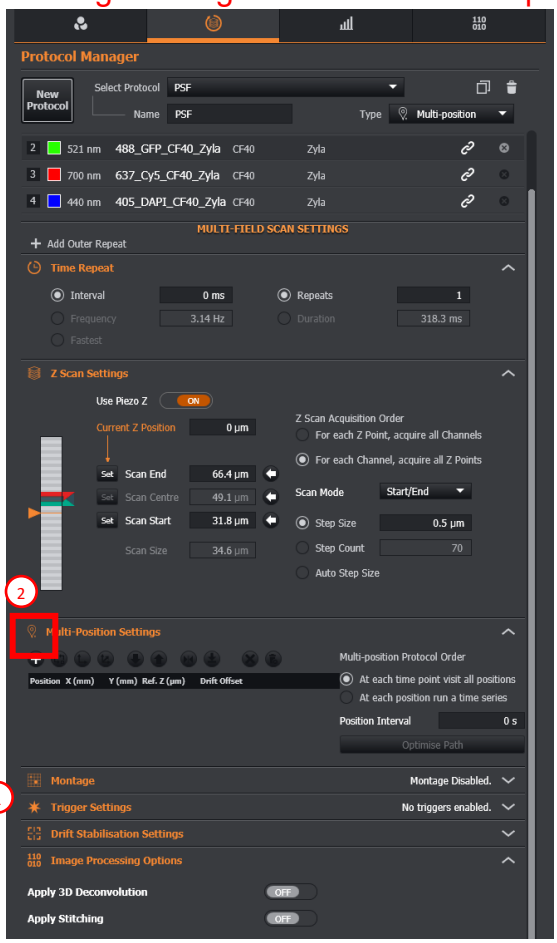
4. Set the z position for “Start/End” or “Center/Size” mode by live viewing.

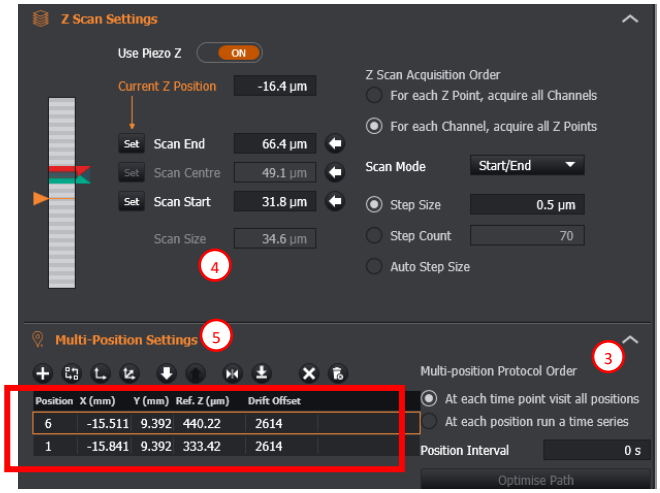


5. Select “Step Size” or “Step Count” or “Auto Step Size” for z step size between each z slices.

Multi-position protocol setup

You can run multi position with time series, Z scan, and montage (the z scan setting and montage setting are the same for all positions).





1. Enable “Drift Stabilisation Settings” if needed

2. Find the XYZ position of interest and then click on “+” button to add to the position list.

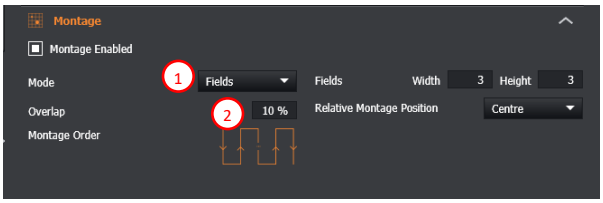
3. If combined with time series, determine time series setup parameters (p.29) and time interval of each position.

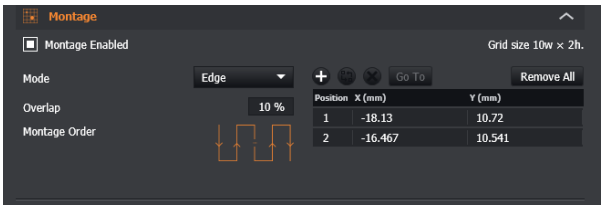
4. Setup Z scan settings (the same for all positions). Select protocol type “Multi position (no Z) if Z scan is not needed.

5. Modify positions in the list using the functions (icons under the “Multi-Position Settings” title), e.g., update position information, change the order of positions, go to the selected position, delete the position.

Montage setup

You can run time series, Z scan, multi-position protocol with montage by enabling the montage in protocol setup.





1. Choose “Fields” or “Edge” mode.

   Fields: Use current field of view as center (or specific corner) of the montage and acquire desired number of fields.

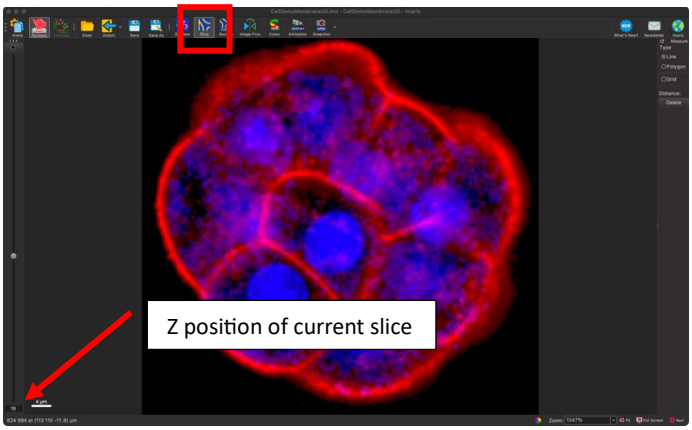
   Edge: User can define the edge of the montage (minimum 2 position) and Fusion will calculate the grid size automatically.

2. Determine the Overlap percentage, default is set to 10%

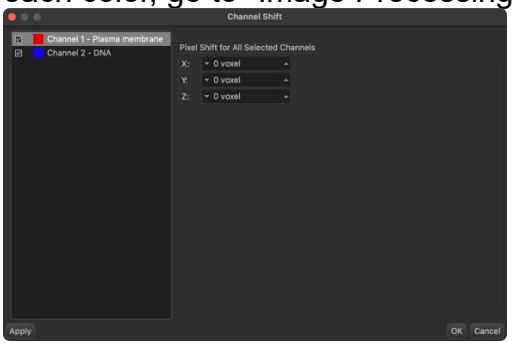
How to correct Z shift between colors in Imaris

1. Open the data file with “Open in imaris” button in Fusion or by double click the file in the folder.

2. In Imaris, go to “Slice View”, in the slice view you can check the shift between each color by going through the Z series and check the Z position number, you can use one color as reference and find shift of other colors based this reference color



3. Once you know the amount of Z shift (how many planes to be moved up or down) for each color, go to “Image Processing” menu > “Channel Shift”.



4. Adjust Z shift for each color. For example, if the red color data should be moved 2 Z-slices up to match the blue color data, then you can enter 2 in the Z section.

5. After the correction is done, remember to save the data.

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