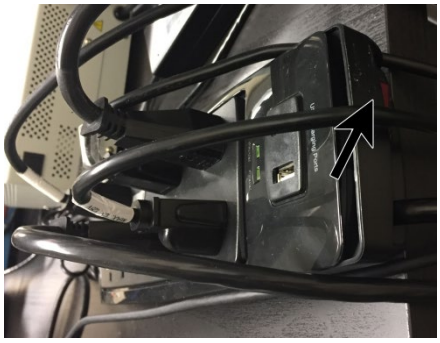
Andor Dragonfly Spinning Disk Confocal Microscope

System Start-up

1. Turn on the outlet switches. These will power up the entire system.



2. Turn on the **Olympus touch panel** by pushing switch on the **back** of the panel. When it is on, touch "**Start Operation**".

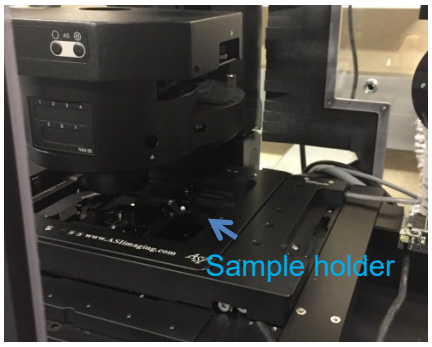
3. Turn on the computer.

4. Click on Fusion 

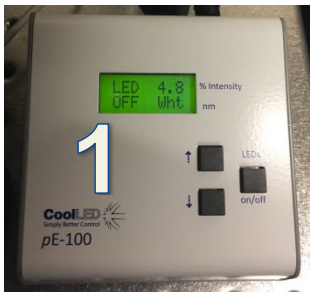
software.

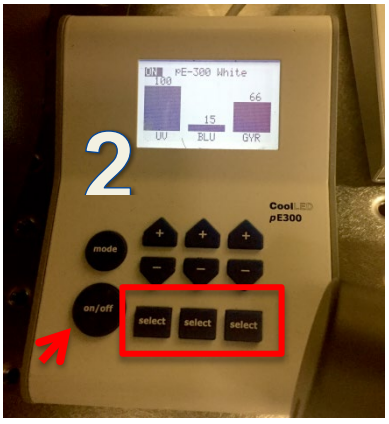
Microscope operation

Current version of Fusion software cannot control the microscope. Thus, basic operations, such as changing objective, filters, controlling light sources and lightpathway, are controlled by hardware themselves.

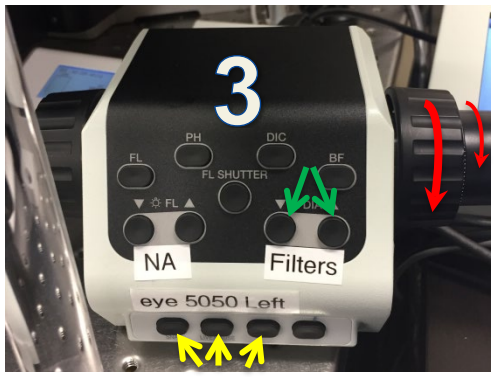


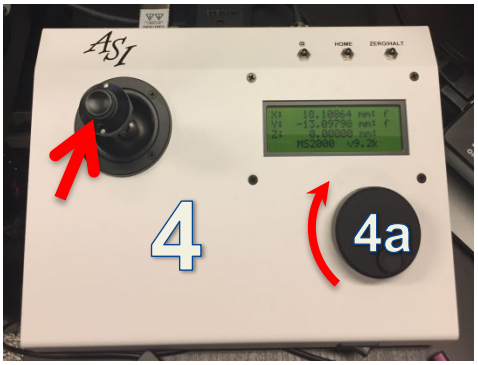
1. Put your sample with the coverslip facing downward to the objective. You can use the **foot switch** on the floor to turn on or off the light inside the environmental chamber.
2. The light sources LED bright field light **(1)** and Fluorescence light (2) can be turned on /off with ON/OFF button. With fluorescence light **(2),** you can turn on/off specific wavelengths (red box).





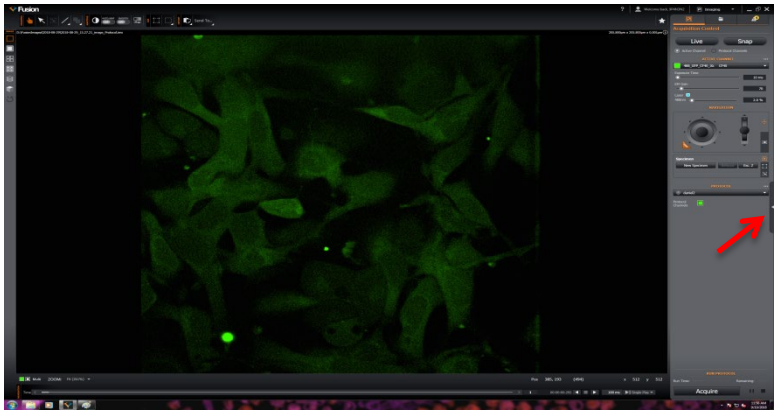
3. Click **eye button** (**yellow arrow**) on the **focus handle (3)** to view your sample with eye pieces.
4. Focus onto the sample by moving objective with focus handle (turning downward (red large arrow) will move objective up toward the sample; big knob for macro movement, small knob for micro movement). You can also move the objective by touching arrowheads (red stars) on the **touch panel (5).**
5. Move the sample by moving the stage with ASI joy stick **(4).** Push the button (red arrow) on the top of the joystick to change between fast and slow movement.
   Turn the **knob** (4a) clockwise will move the Piezo stage up and counterclockwise will move it down, which will change the focus.



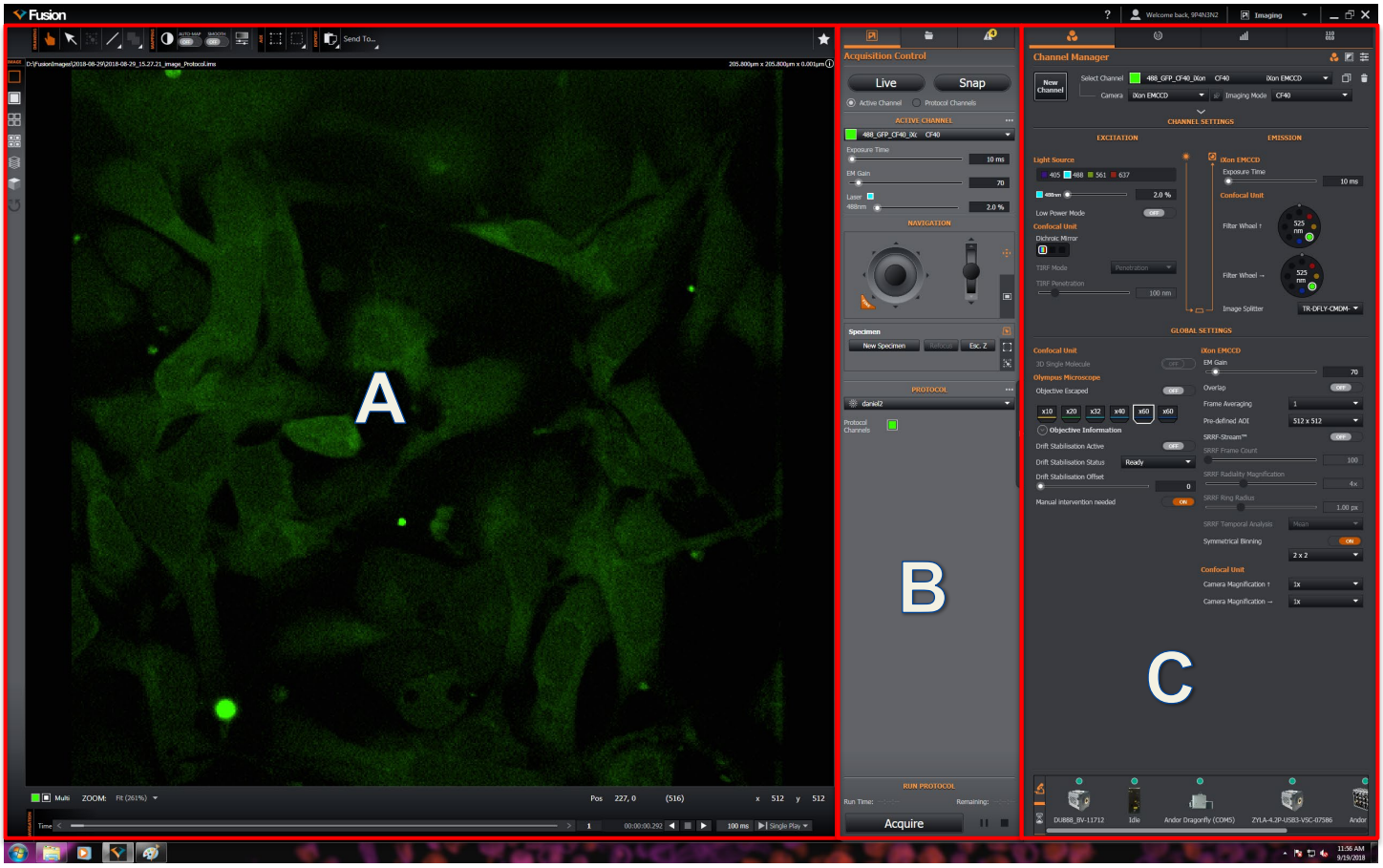


6. Change the objective by touching **objective icons** on **the touch panel (5).**
7. Light illumination to the sample area can be turned on/off with **Shutter** button on the touch panel.
8. Touch **EPI1** tab on the touch panel and select filter by touching **Mirror buttons** (1 for DAPI, 2 for CFP color, 3 for GFP green color, 4 for mCherry red color). You can change filters by pushing the **Filters buttons** (green arrows).
9. When you find area of interest (AOI) and ready for confocal scanning, push the **Left** button (yellow arrow) on the focus handle and touch **Dragonfly** (mirror 6) button on the touch panel to direct the lightpath to the laser scanner.

Image Acquisition with Fusion



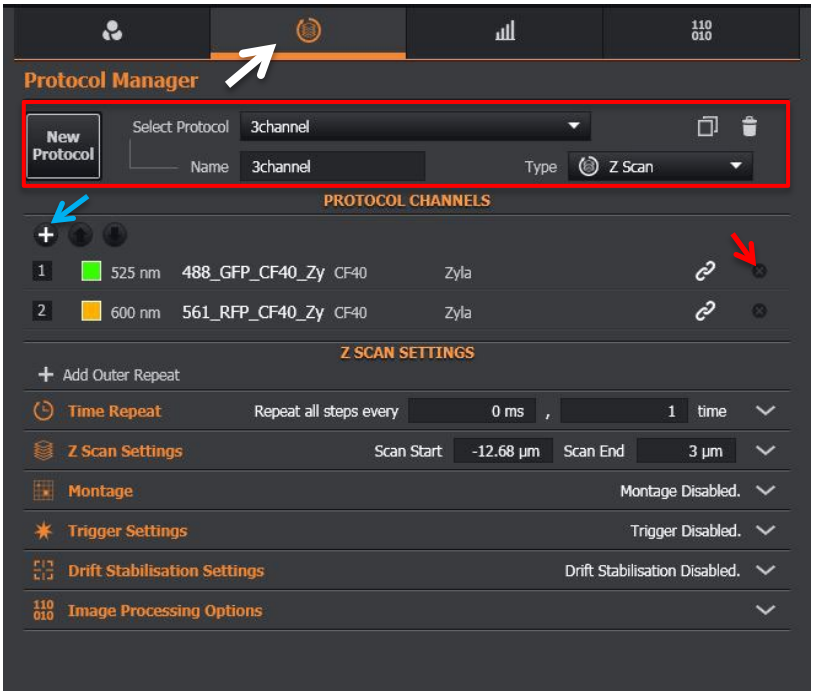
Upon start-up, the Fusion interface will display imaging area and Acquisition control tab.
AClick the arrowhead (red arrow) at the right middle border to expand the interface to display additional control tabs.



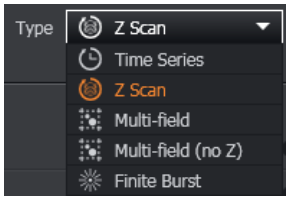
(A) Image Display: you can manipulate how image will be displayed, such as projection, simple 3D rendering, image histogram, and annotation.
(B) Acquisition Control: you can control laser power, exposure time, and selection of filters, and acquire images.
(C) Channel Manager: you set up acquisition conditions, perform image processing and analysis.

***Setting up imaging mode*.**

To image your sample, filters and camera need to be selected. Fusion has a list of fluorophore and camera combination you can select.



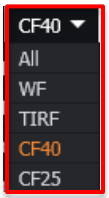
Click **Protocol Manger** tab (white arrow). You can select a protocol from the drop-down menu or create a New Protocol for your imaging needs. Put a name for your protocol.



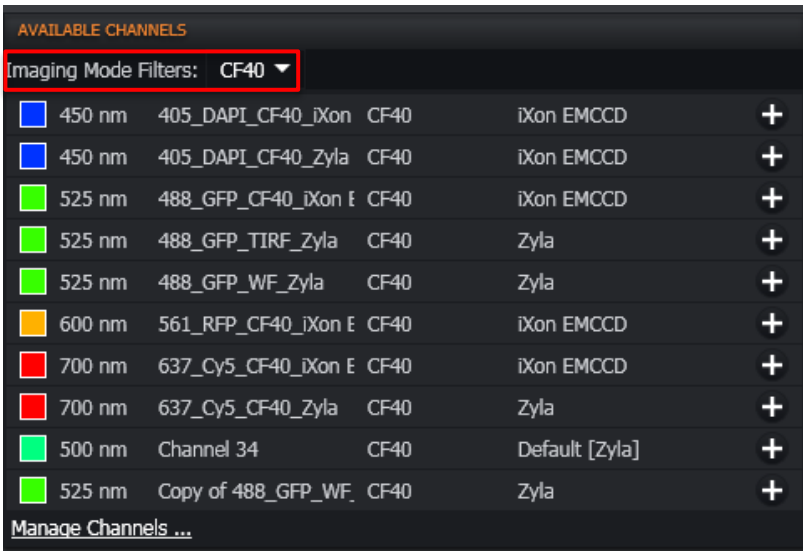
Select **Imaging Type** from the drop-down list; Z scan, Time Series, Multi-field, Multi-field (no Z), or Finite Burst.

For routine imaging, select Z scan.

In the **Protocol Channels**, you will select filter sets to match your sample fluorophore colors. The current filter is good, keep it. Otherwise click ⊗ button (red arrow) to remove from the selection.



To add filter, click ⊕ (blue arrow) and it will show a list of available channels.



Click Imaging **Mode Filters** to select a subset of filters for specific imaging mode (**WF**-wide field; **TIRF**; **CF40**-confocal spinning disk large pinhole (40x-100x objectives); **CF25**-confocal spinning disk small pinhole (10x-40x objectives).

For example, select **CF40** for confocal imaging and it will show available filters. Click ⊕ to add the desired filter. It is important to select all the filter sets with the same camera (either iXon EMCCD or Zyla). For example, if you want to image GFP and RFP with iXon EMCCD, you may select 525 nm 488_GFP_CF40_iXon EMCCD and 600 nm 561_RFP_CF40_Zyla.

Click Channel Manager tab. You will see your protocol with filter set you just selected.

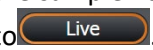
Since Fusion can't control Olympus hardware, it is important to match the objective that you will use **(7).**

Select one of the protocol channels in either **Active Channel (3)** or **Protocol Channels (6).**

Set low values for the exposure time (10 ms), EM gain (50) (only with iXon EMCCD camera), and Laser (5%). (4)

Click **Active Channel** button **(2).**

Click **Live** 

button to start scanning the sample. It will change to 

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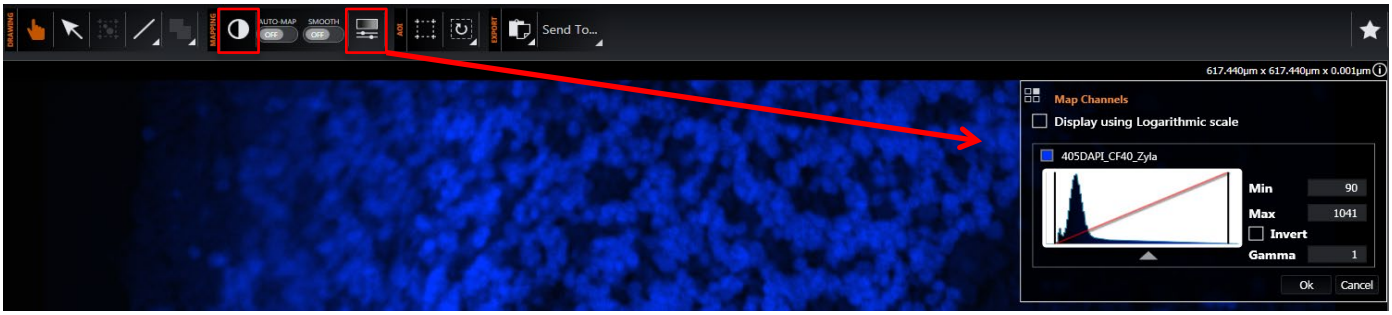
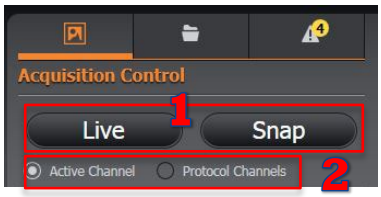
While scanning, change the exposure time, EM Gain, and Laser power to get good image on the display.
If image is not visible in the image window, click auto histogram  button. If the image display is not good, click Map Channels  button. It will open a histogram window. To change lower pixel intensity, click



left mouse button and drag left or right. To change higher pixel intensity, click right mouse button and drag left or right.

Once appropriate imaging condition is set, click **Protocol Channels (2)** and then click **Snap** button **(1).**
If you check **Active Channel** button, **Snap** will acquire the image of only the current active channel. **All the images will be saved to the Data drive automatically.**



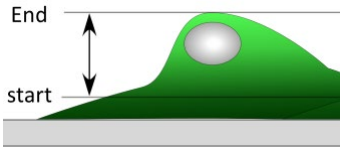
Once image acquisition is done, click back to **Active Channel**.

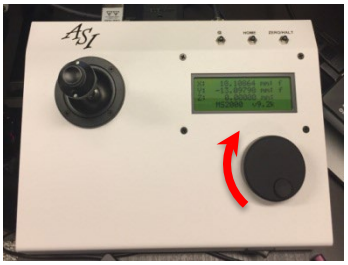
Z-series acquisition

For z series image acquisition, you can choose either slow objective movement or fast Piezo movement for focusing mechanism. Since the current Fusion can't control Olympus base, only Piezo Z works for z series.

To use the **Piezo movement** , make sure that **"Use Piezo Z for scans"** (red box) is **ON** either on **Protocol Manger tab** or **Acquisition Control tab.**

There are two ways to set up the range of z-series. The first one is to use a **Start/End** positions.

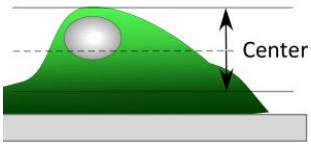


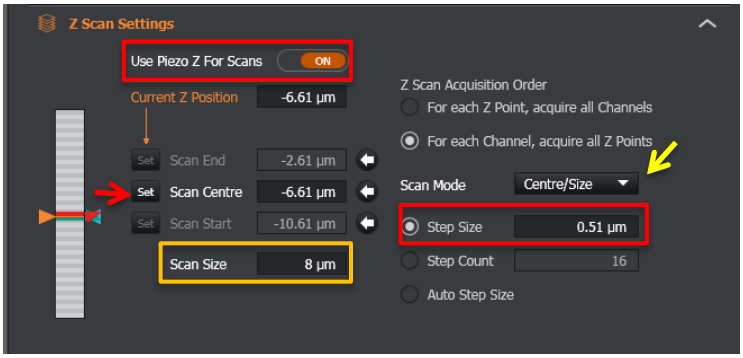


Click onto the yellow arrowhead and roll the mouse wheel up (***or turn the ASI knob counterclockwise***) to focus onse side and click **Set** button for **Scan End**. Roll down (**or *turn the ASI knob clockwise)*** to change focus to the other side and click **Set** button for **Scan Start** (red arrows). Type the **Step Size**, which represents focus movement in µm.

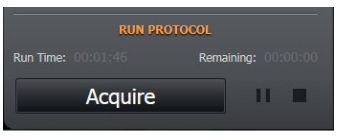
When you image multicolor (multichannel), you can select acquire either all Channels for each Z focus point or all Z slices for each channel (Blue box)

The other way is to set the current position as a center and then set the range up and down from the center. Select **Center/Size** from the **Scan Mode** list (yellow arrow). Focus onto the middle part of the range and click **Set** button of **Scan Centre**. Type **Scan size** for the range (yellow box); for example, type 8, it will range 4 µm up and 4 µm down from the **Scan Centre** position.





For starting image acquisition, click **Acquire** button on the **Acquisition Control** tab.

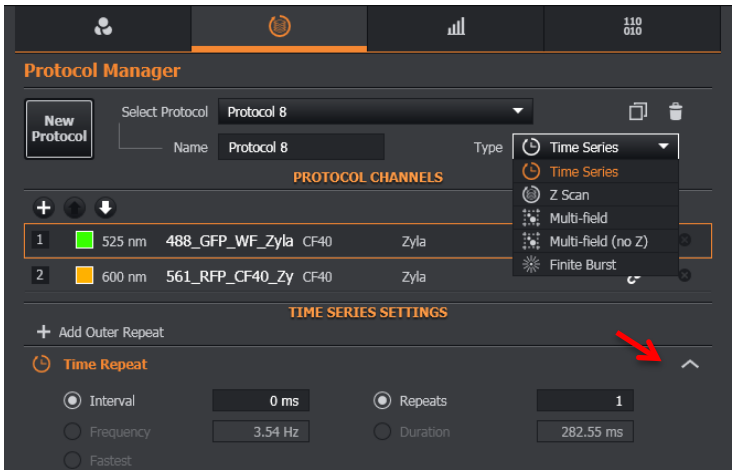


Time series acquisition.

To obtain time lapse image series, select **Time Series** in the **Type** menu on the **Protocol Manager**.

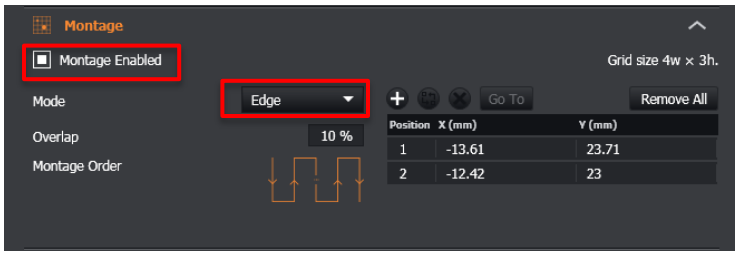
Click arrowhead to extend options form **Time Repeat**. Set the interval for image capturing and the number of repeats, it will give the total time of Duration.
Or, you can set interval and the Duration; it will calculate the number of repeats.

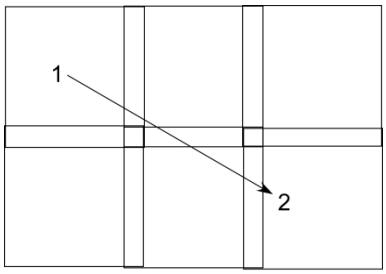
Click **Acquire** button on the **Acquisition control** tab to start acquisition.



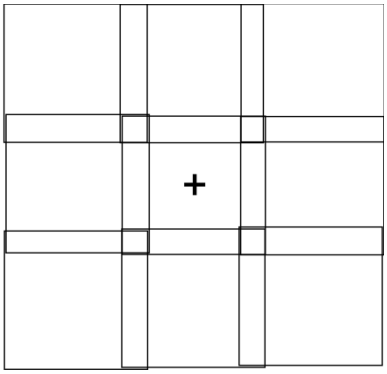
Montage acquisition

For capturing montage, click **Montage** option and click **Montage Enabled** box.
Select either **Edge** or **Field** Mode.





(1) Edge mode. You set upper left and lower right corners as position 1 and 2. Then Fusion automatically arrange image tiles with overlap (in % you set).



(2) Field mode. You place the position of interest in any position in the Relative Montage Position and type the number of Width and Height of tiles.

Multi-field image acquisition.

This mode allows you to acquire random multiple AOIs (area of interest) with or without Z series, such as image multiple cells on a slide or cells in a multiwell plate.

Find areas of interest and click ⊕ to add.

For time lapse imaging of the multi-field, select the **Multi-field Protocol order**.

Type time interval for **Field interval** (if necessary).

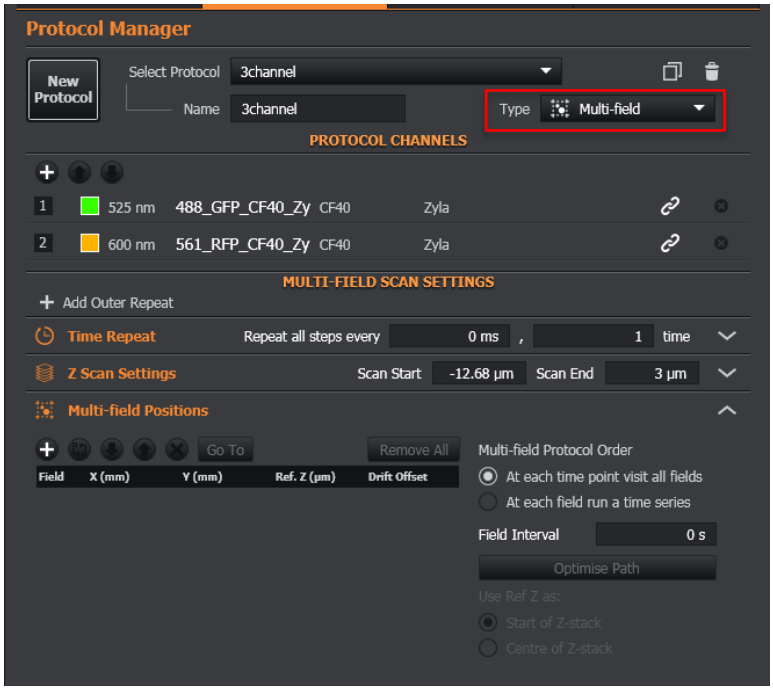
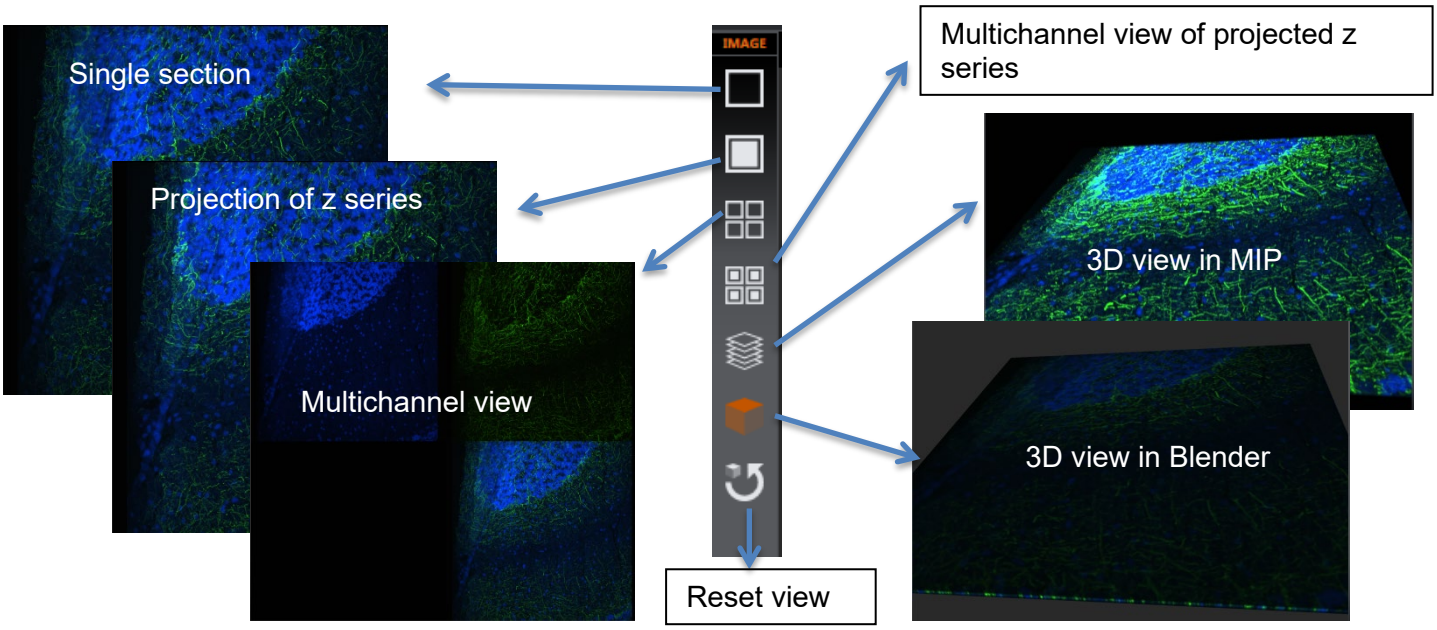
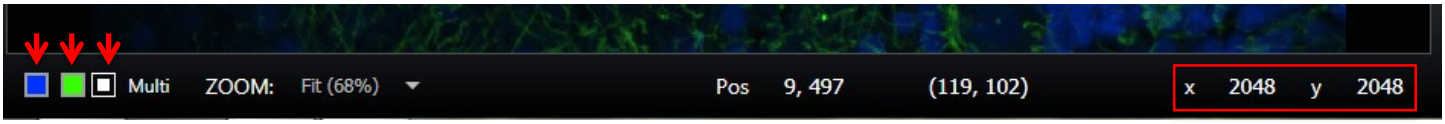


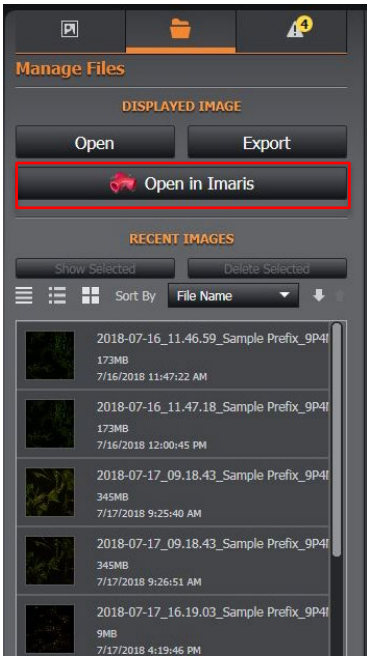
Image Display and Manipulation.



At the lower bar on the image display area, you can see the resolution of your image (red box) and can control the image display as merged or individual color channel by clicking the boxes (arrows).



These two buttons allows a simple 3D rendering for quick visualization. For more sophisticated rendering and manipulating, click **Open in Imaris** button on the **Manage Files** tab.



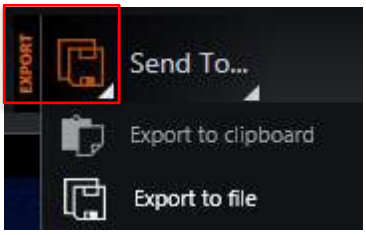
You can export the images into .tif file.

Roll the mouse wheel on the image to zoom in or out the display.

Exporting View Screenshots

Adjust and manipulate the image as you want such as projection, 3D, with annotations.

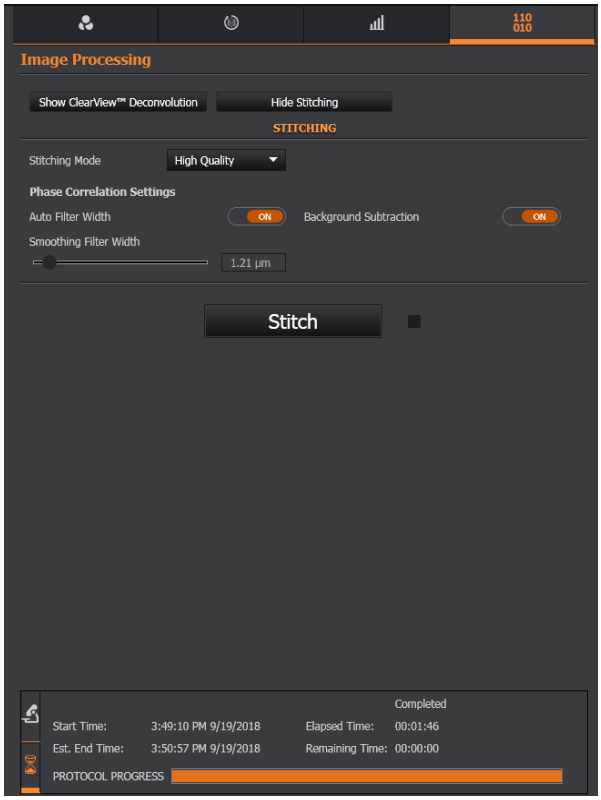
Select the **Disk icon** on the **EXPORT** button on the horizontal bar above the image and you have two options; Export to Clipboard and Export to file.

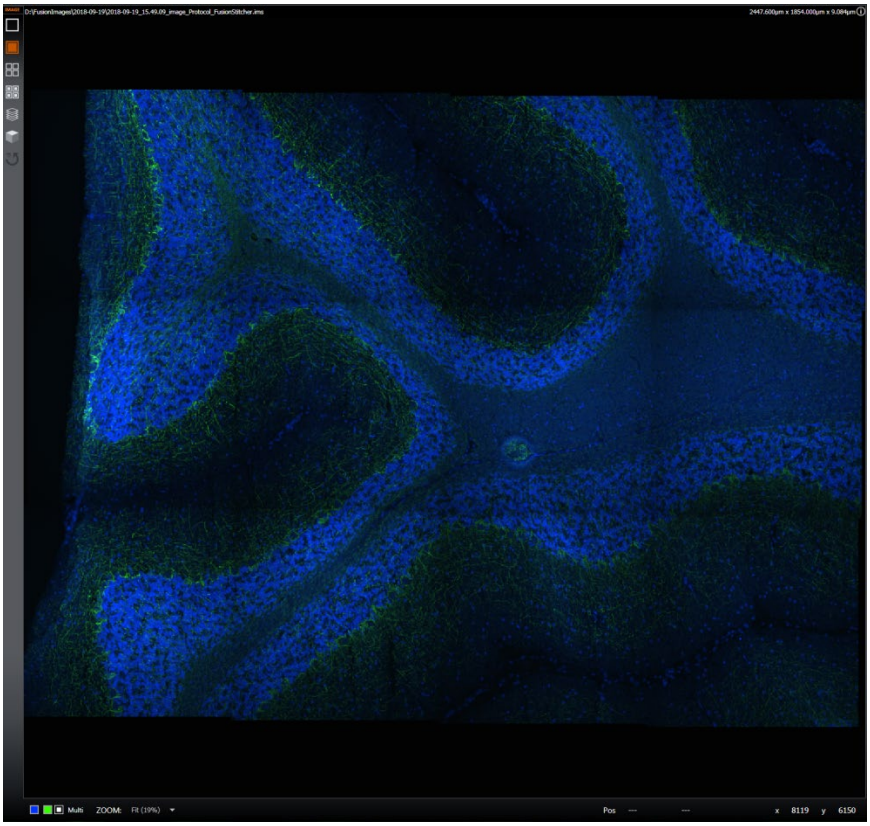


Montage image stitching.

When you acquire the montage images, the image will be display the individual tile images. By using Navigation bars, you can check individual tile images (and z stack if they are).

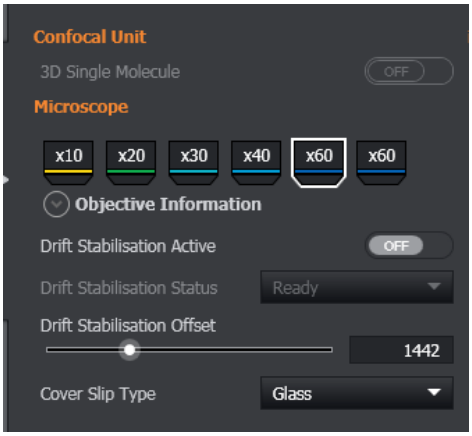
For stitch all tile images into a montage image, click **Image Processing** Tab. Under **STITCHING** section, choose **High Quality** Stitching Mode and keep other parameters as default. Click **Stitch** button. It will create a Montag image file.



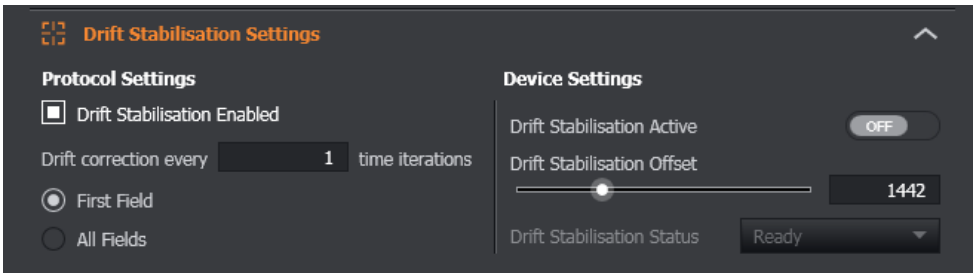


ZDC Drift compensation for stable focusing during timelapse imaging

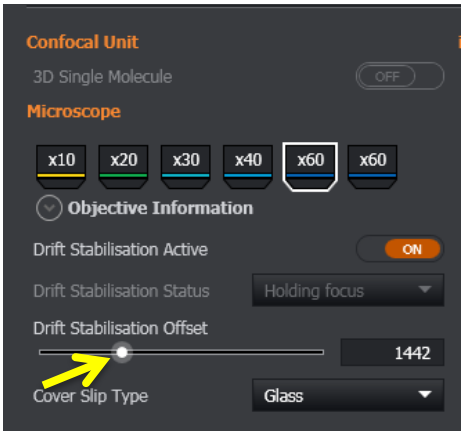
Focus onto the sample with the objective focus knob.

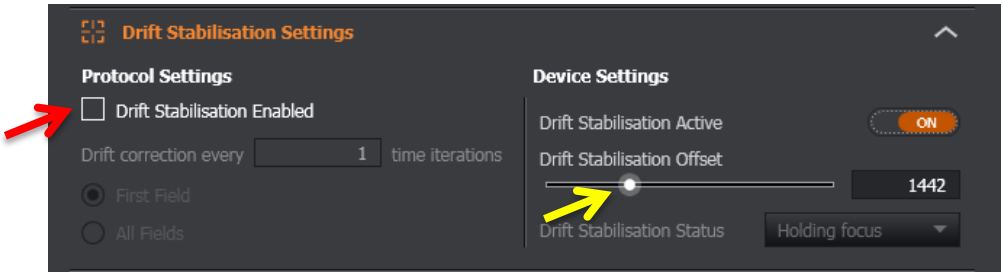


Turn on **Drift Stabilization Active** button to **ON** on either Channel Manager tab (Confocal Unit/Microscope) or Protocol Manager tab (Drift Stabilization Settings).
→ It will find an offset distance from the coverslip to objective and "beep" once if it finds it successfully. (The offset may not be necessarily good for maintaining the focus of your interest. So the focus may not be correct).



To get back to the focus of your interest, while **Drift Stablization Active** is **ON**, drag the **controller button** (yellow arrows) on either tab (or click mouse arrow to the controller button and use **Left** and **Right arrow keys** to change precisely)
You can see the focus changing.
Once the focus is restored, you are all set for drifting stabilization.





Check Drift Stabilization Enabled box (red arrow).
You can choose how often the Drift Stabilization will check by entering the number.
You can choose to check only the first field or all fields in multifield imaging.