

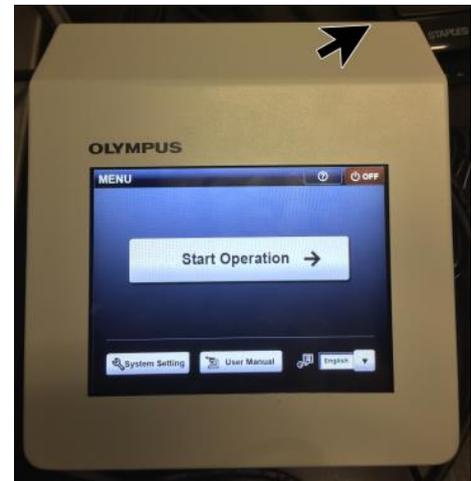
Andor Dragonfly Spinning Disk Confocal Microscope

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.
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.

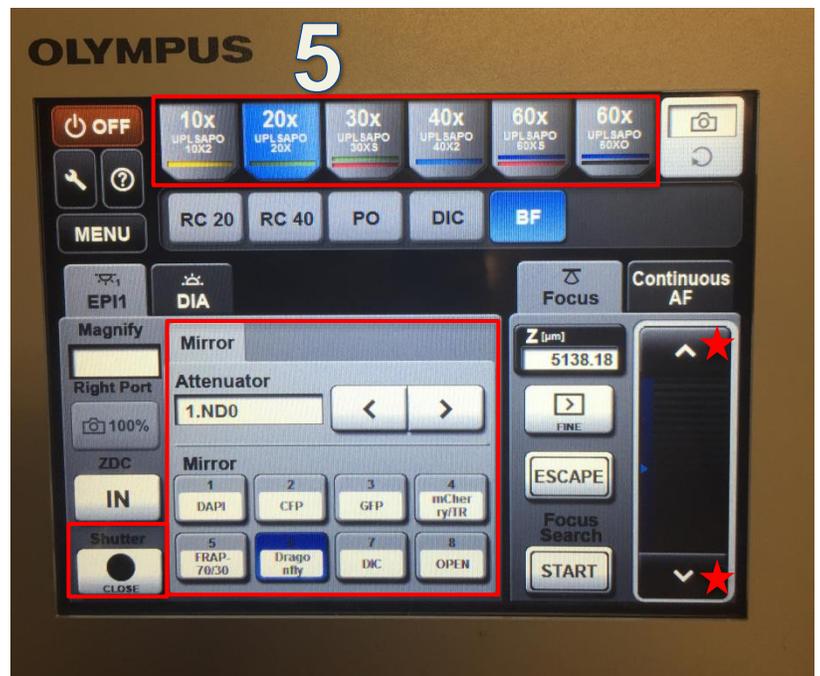
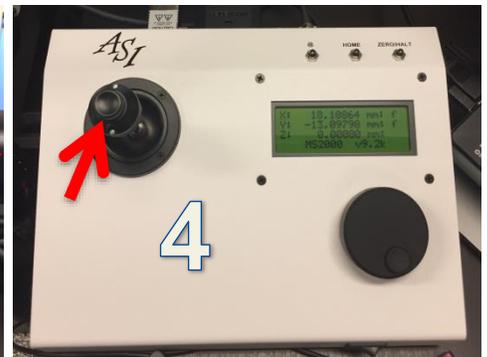
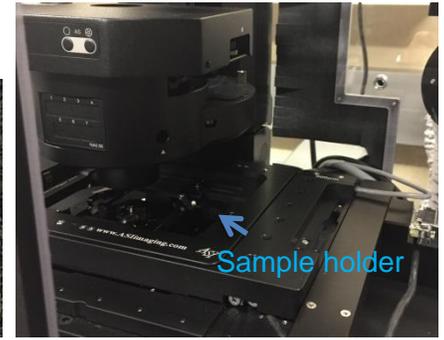
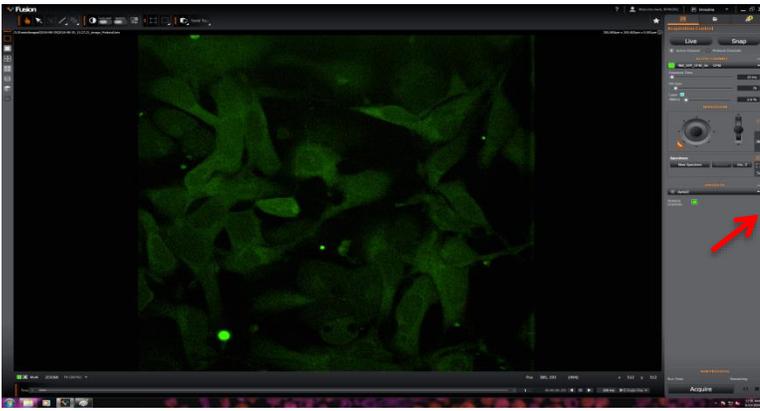
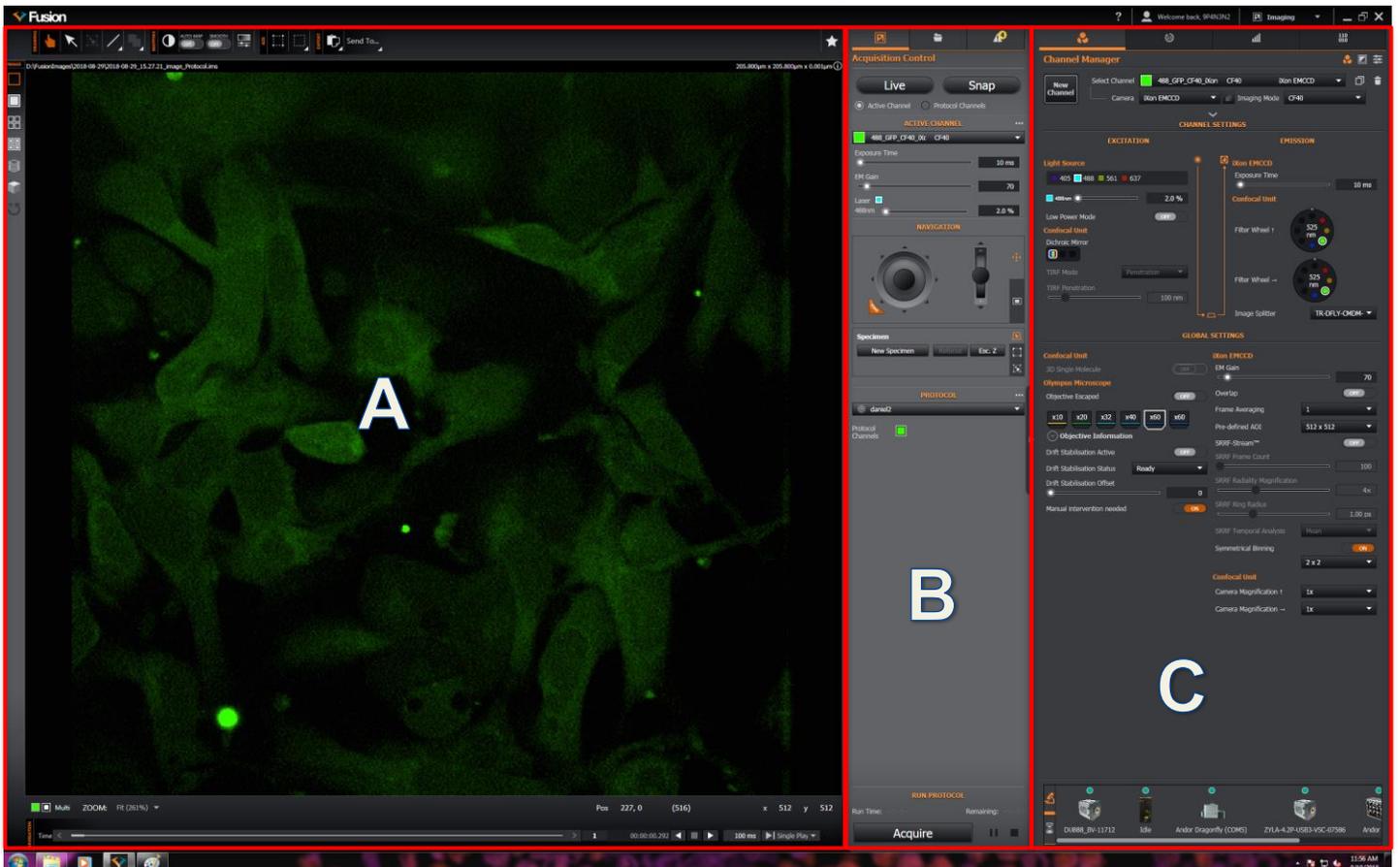


Image Acquisition with Fusion



Upon start-up, the Fusion interface will display imaging area and Acquisition control tab. A Click 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.

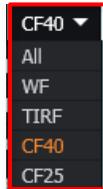
Select **Imaging Type** from the drop-down list; Z scan, Time Series, Multi-field, Multi-field (no Z), or Finite Burst.



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.

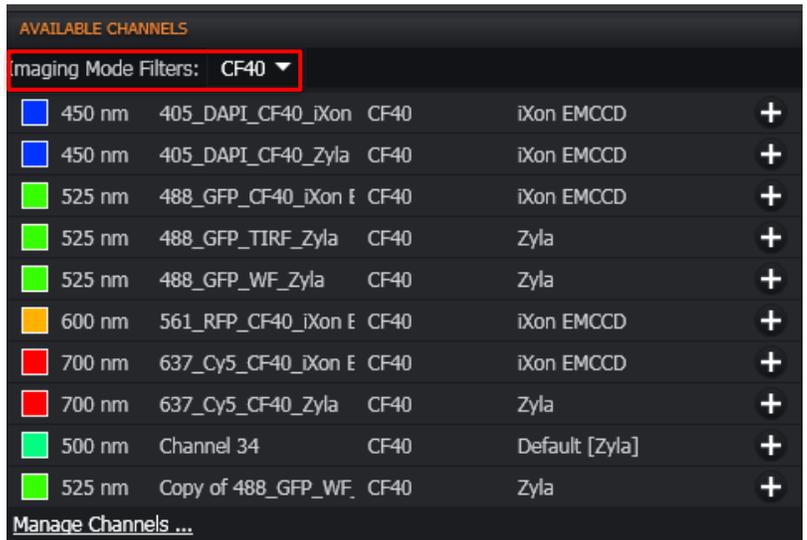
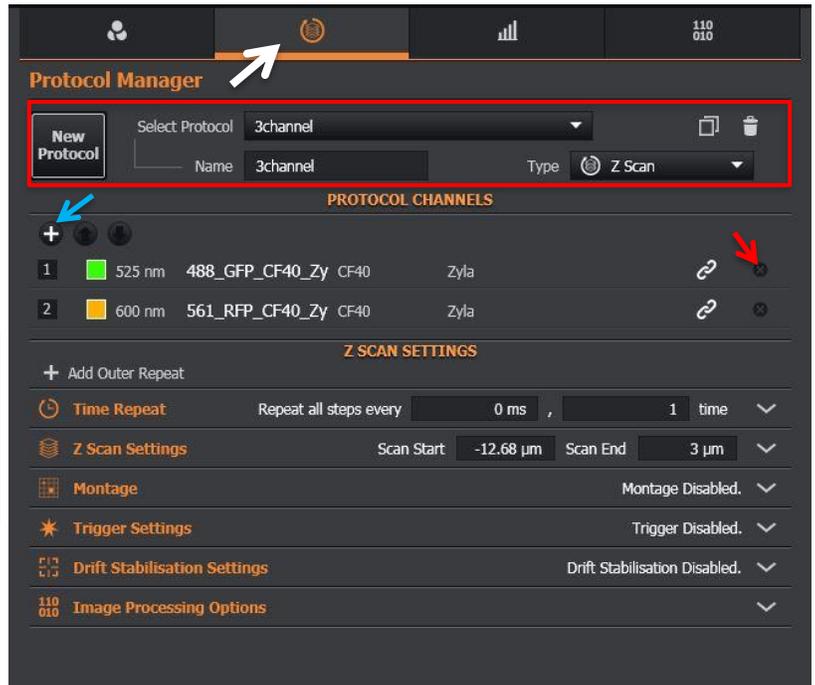
To add filter, click ⊕ (blue arrow) and it will show a list of available channels.



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

Select **CF40** for routine 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)

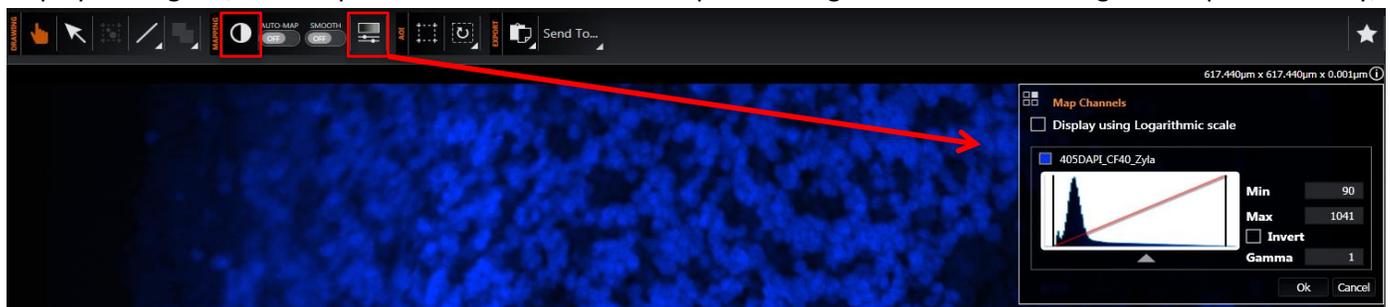
Click **Active Channel** button (2).

Click **Live** button to start scanning the sample. It will change to **Live**.

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)**.

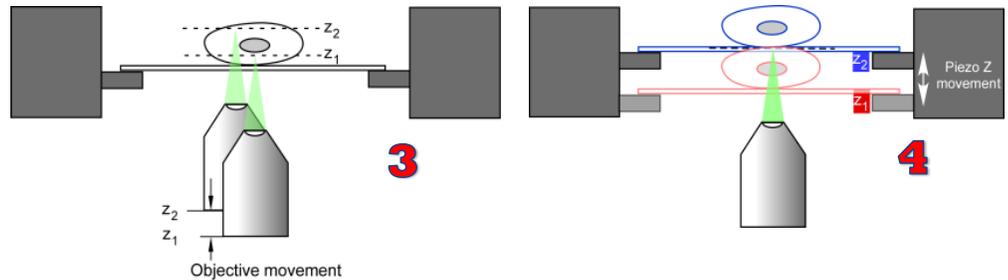
If you check **Active Channel** button, **Snap** will acquire the image of only the current active channel.

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 (3)** or fast **Piezo movement (4)** for focusing mechanism.



To use the **Piezo movement** (recommended), click **ON** for **“Use Piezo Z for scans”** (red box) 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.

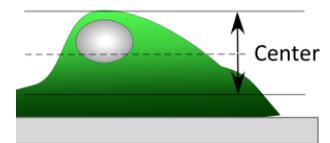


Click onto the yellow arrowhead and roll the mouse wheel up to focus one side and click **Set** button. Roll down to change focus to the other side and click **Set** button (red arrows).



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.

