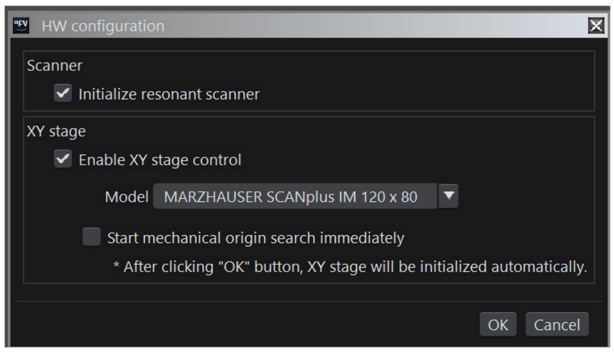
FV5000 Confocal Laser Scanning Microscope Instruction

1. **Preparation**
   Make sure the computer is turned on and you are logged in before turning on any other equipment.
2. **System Startup**
   Turn on the system components in the following sequence: 1 → 2 → 3 → 4 → 5.
   Double-click  to open the software.
   After startup, the following screen will appear. **Do not** change any settings; simply click "**OK**"



3. **Loading and Checking the Sample**
   After software open completely finished, move away condenser column and load sample, then move back the condenser column.
   Check sample under microscope:

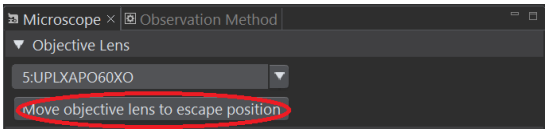
   - On the microscope control panel, push "**LIGHT PATH**" button to select 
   - Press "**EPI shutter**" button to select "close"
   - Press "**DIA ON/OFF**" to turn on lamp
   - Turn "**OBJECTIVE**" knob to select an objective (*Turn the knob one step at a time; turning it to the right increases the objective magnification*)
   - Turn "**EPI FILTER**" knob to select 8 (*for bright field mode*) or 7(*for DIC mode*) (*Turn the knob one step at a time; turning it to the right increases the number*).
   - Use joy stick to move sample, use focus knob on the microscope control panel to focus sample.

   For fluorescent imaging:

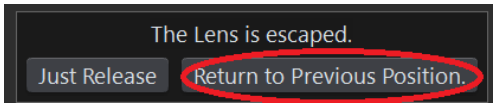
   - Press "**DIA ON/OFF**" to turn off lamp
   - Press "**EPI shutter**" button to select "open"

- Turn "**EPI FILTER**" knob to select an appropriate filter (2—DAPI, 3—FITC, 4—TRITC, 5—CFP, 6—YFP)
- Use joy stick to move sample, use focus knob on the microscope control panel to focus sample.
- Move the sample area to be scanned to the center of your eyepiece view .

- **Use immersion oil or silicon oil:**
  Immersion oil can only be used on 60x objective lens, Silicon oil can only be used on 30x objective lens!!!
  - ❖ Focus sample under 60x or 30x objective lens
  - ❖ In the "**Microscope**" tab, click "**Move objective lens to escape position**"



  - ❖ Move away the condenser column.
  - ❖ Use joy stick move away sample, then put one drop of immersion oil on 60X objective lens or one drop of silicon oil on 30X objective lens, then use joy stick move sample back.
  - ❖ Click "**Return to Previous Position**"



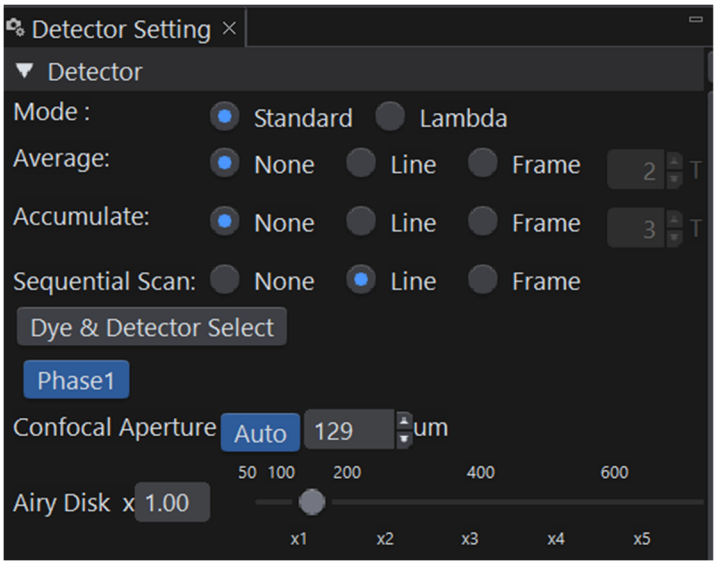
  - ❖ Move back condenser column

Clean off immersion oil or silicon oil from objective lens:

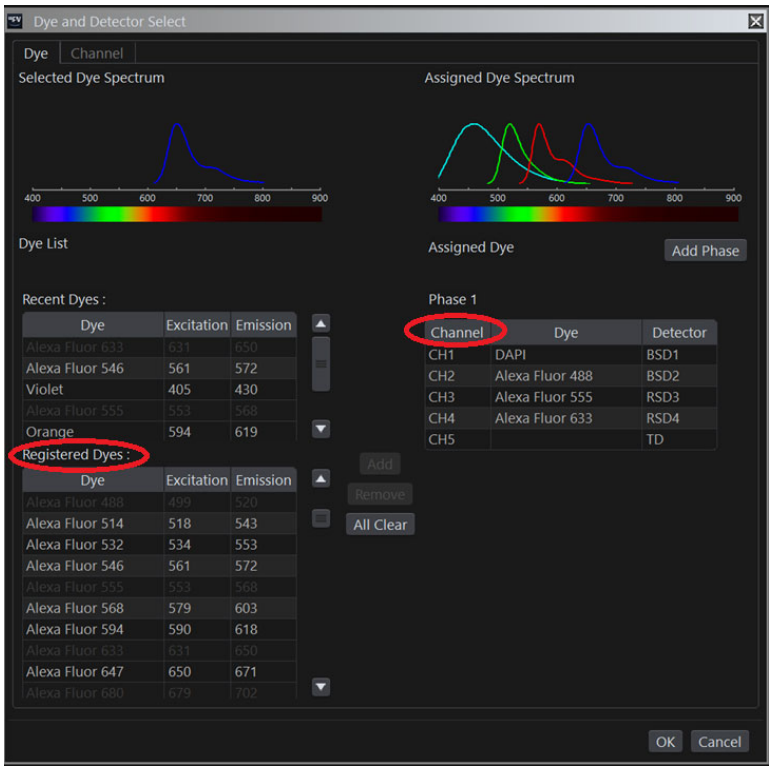
- ❖ Move away condenser column and unload sample
- ❖ Spray lens cleaner onto a lens tissue paper (**never spray lens cleaner directly on lens!!!**), then use the wet lens paper gently clean the lens. Let the lens air-dry completely before applying oil on it again. (**do not use dry lens paper to rub lens!**)
- ❖ Move back condenser column

4. **Scanning set up:**

- Press "**LIGHT PATH**" button to select 
- In the "**Detector Setting**" tab,
  Mode-------Standard
  Average----None
  Accumulate------None
  Sequential Scan-----Line
  Confocal Aperture----- Auto (blue)



- Click "**Dye & Detector Select**",
  Search for the dye in the "**registered Dyes**" list. Double-click the dye name to add it to the "**Channel Table**" on the right. Repeat this process to add all required dyes. To remove a dye from the "**Channel Table**," double-click its name. If bright-field imaging is not required, double-click "TD" to remove the bright-field channel.



- After selecting dyes, set the laser intensity for each channel to **1%** as the starting point. Adjust the laser intensity for each channel as needed based on the imaging results after scanning begins.

- In "**LSM Imaging**"tab,

  Scanner:

  Type------Galvano

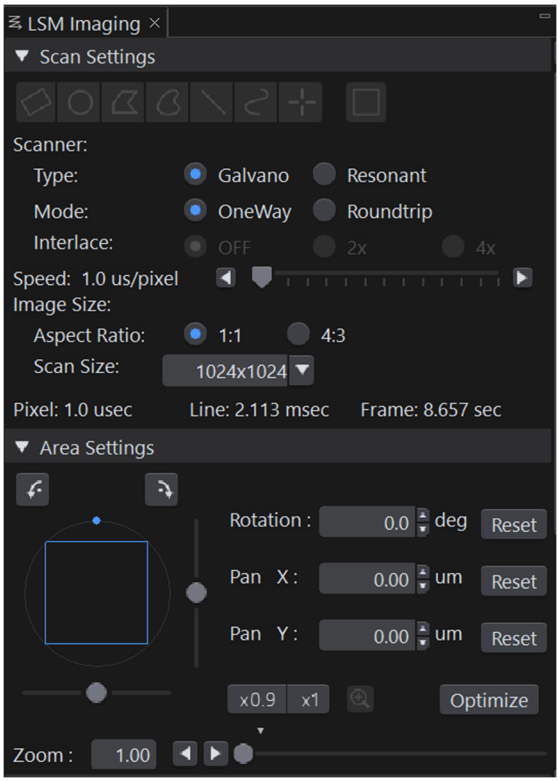
  Mode-----OneWay

  Speed------1 or 2 us/pixel *(normally)*

  Aspect Ratio-----1:1

  Scan Size------1024x1024

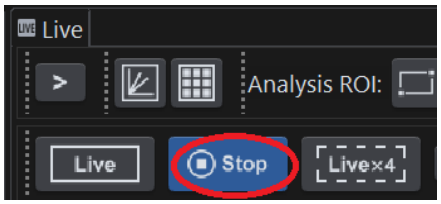
  Zoom-----1.0 (to begin with)



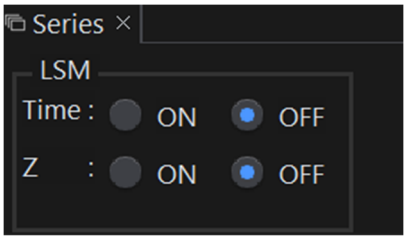
5. **For XY scan:**
   - In the "**Live**" tab on the right side, click "**LiveX2**" to preview scanning images.

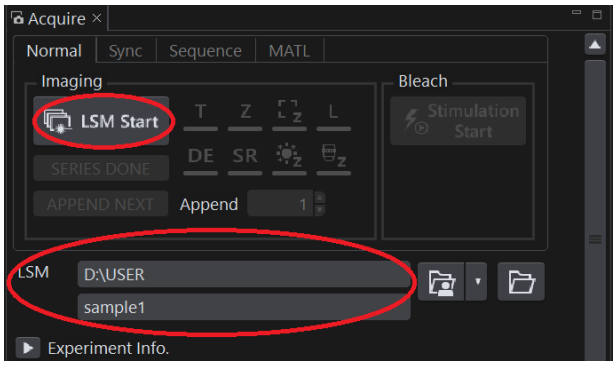
     ------ Use the **fine-focus knob** on the microscope control panel or **Ctrl + mouse wheel** to focus on the sample or select the desired scanning plane.
     ------ Move the image scanning area by **left-clicking and dragging**, or use the **"wherever double click on image will move to the center"** to move the selected area to the center.
     ------ Adjust laser intensity for each channel as needed in "**Detector Setting**" tab
   - Click "**Stop**" to stop the live scanning.

   - In "**Serials**" tab, set " **Time**" to **OFF**, and set "**Z**" to **OFF**.



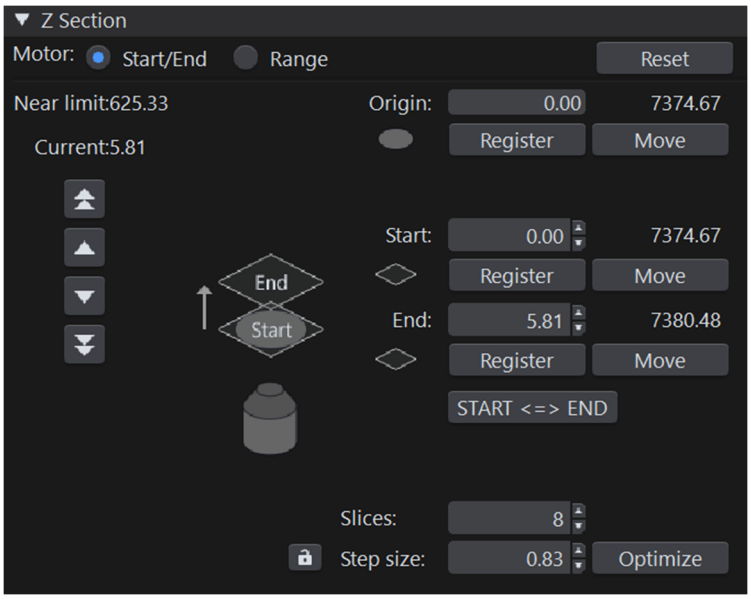
- In “**Acquire**” tab, select the folder in which you want to save the image data and enter a name for the image data. Then click “LSM Start” to begin scanning. When the scan finished, the image data will be automatically saved under the specified name in the selected folder.



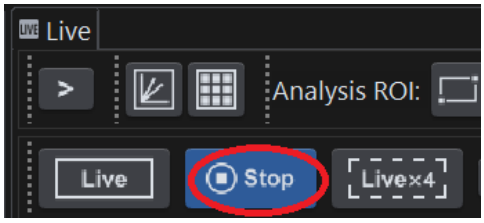
- To review the scanning result, click the tab containing the image name next to the “Live” tab.

6. **For 3 dimensional XYZ scan:**
   - In the “**Live**” tab on the right side, click “**LiveX2**” to preview scanning images.

   - ------ Use the **fine-focus knob** on the microscope control panel or **Ctrl + mouse wheel** to focus on the sample or select the desired scanning plane.
   - ------ Move the image scanning area by **left-clicking and dragging**, or use the **“wherever double click on image will move to the center”** to move the selected area to the center.
   - ------ Adjust laser intensity for each channel as needed in “**Detector Setting**” tab
   - In “**Z Section**” tab, Click “  ” until scanning image just disappears, click “ **Register**” next to “**Origin**” and click “ **Register**” next to “**Start**”; Then click “  ” until scanning image just disappears, click “ **Register**” next to “**End**”;
   - Enter the desired step size, or click “Optimize” to select the minimum step size based on the Z-axis resolution.

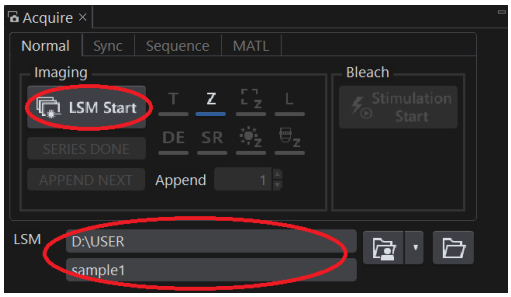


- Click “**Stop**” to stop the live scanning.

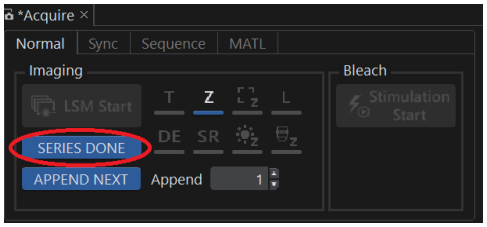


- In “**Serials**” tab, set “ **Time**” to **OFF**, and set “**Z**” to **OFF**.

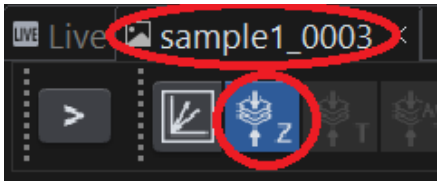
- In “**Acquire**” tab, select the folder in which you want to save the image data and enter a name for the image data. Then click “**LSM Start**” to begin scanning.



- When the scan is complete, click the flashing "**Serials Done**" button. The image data will then be automatically saved under the specified name in the selected folder.

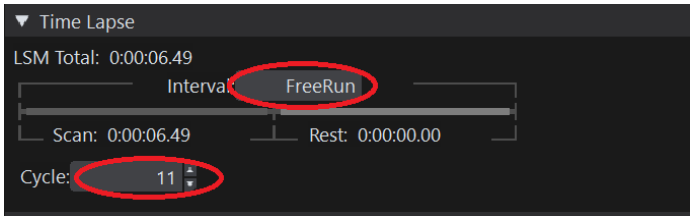


- To review the scanning result, click the tab containing the image name next to the "Live" tab, then click  to view Z stack scanning result.

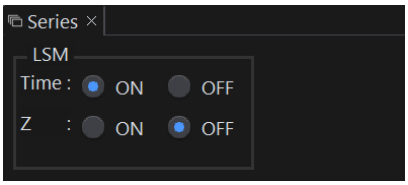


7. **For Time laps XYT scan**:
   - In the "**Live**" tab on the right side, click "**LiveX2**" to preview scanning images.

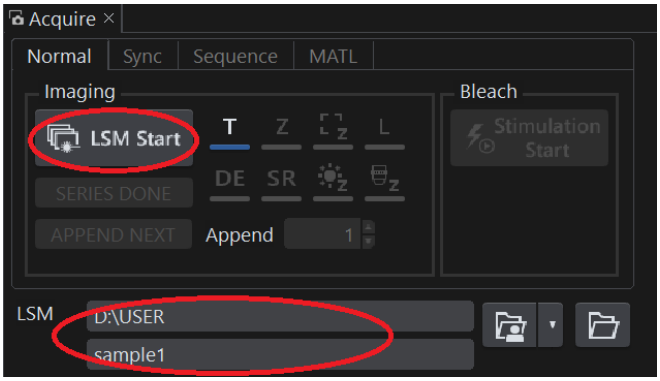
   - ------ Use the **fine-focus knob** on the microscope control panel or **Ctrl + mouse wheel** to focus on the sample or select the desired scanning plane.
   - ------ Move the image scanning area by **left-clicking and dragging**, or use the **"wherever double click on image will move to the center"** to move the selected area to the center.
   - ------ Adjust laser intensity for each channel as needed in "**Detector Setting**" tab
   - In "**Time Laps**" section under "**Serials**" tab, enter the desire **interval** time*(the time between each scan*), Select **Free Run** if you do not want any waiting time between scans. Then set the **Cycle** number, which specifies the total number of scans to be performed.



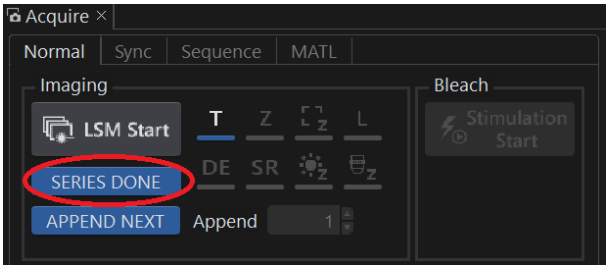
   - In "**Serials**" tab, set " **Time**" to **ON**, and set "**Z**" to **OFF**.



- In “**Acquire**” tab, select the folder in which you want to save the image data and enter a name for the image data. Then click “**LSM Start**” to begin scanning.



- When the scan is complete, click the flashing “**Serials Done**” button. The image data will then be automatically saved under the specified name in the selected folder.



- To review the scanning result, click the tab containing the image name next to the “Live” tab.

8. Adding a Scale bar

Click on the image tab, click  in the upper-right corner of the image tab, then drag the scale bar as desire length on the image.

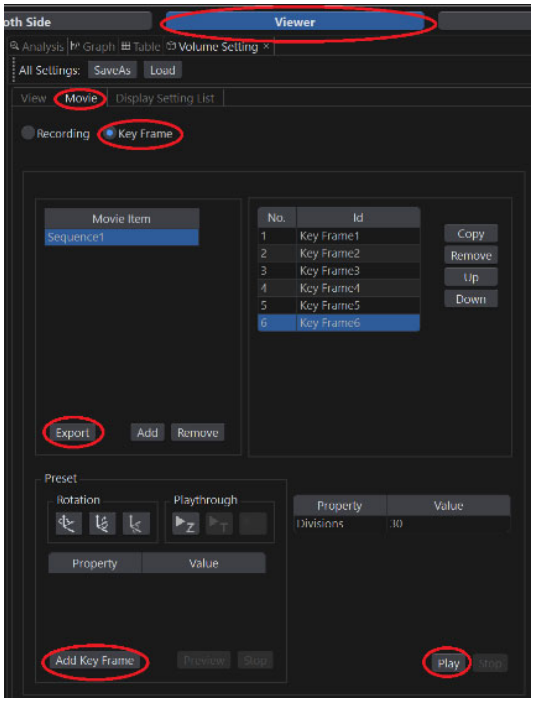
9. Changing channel color

Click on the image tab, click  in the upper-left corner of the image tab, then select the desired color for each channel.

10. Save images:

Click on your image tab,

- **Export**: Right click in your collected image window and select **Export**, Select a file location, enter a name for the image folder, and save. All channel images, including the merged image, will be saved as .tif files in the specified folder. (*Note: For Z stack images, exported folder only contains the individual images in the stack but does not include the final Z-projection image.)*
- **Save Display**: select "**Single**" on the top of image tab, select image channel(s) you want to save. Right-click the image and select "Save Display." Choose a file location and the .tif file type, enter a name for the image, and click "Save."
- **Save Z stack projection animation:** click off  , select "**Single**" on the top of image tab, select image channel(s) you want to save animation. Then right click on the image, select save animation, then select a file location and set the frame rate to 2.0, and type a name for your video, then click save.
- **Save Z stack 3D animation:** select "**Volume**" on the top of image tab, click "**Viewer**" tab, then select "**Movie**" tab and select "**Key Frame**". , left-click and drag to move the image to the desired position, then click "Add Key Frame." Repeat this process until all desired sample positions have been added. Click "Play" to preview the movie. You can edit the movie by removing or adding key frames. Finally, click "**Export**" select a file location, enter a name for the video, and click "Save".



- **Save Time laps animation:** select "**Single**" on the top of image tab, select image channel(s) you want to save animation. Then right click on the image, select save animation, then select a file location and set the frame rate to 2.0, and type a name for your video, then click save.

11. Turn off

- Move away condenser column and unload sample
- **Remember to clean the lens after using immersion oil or silicon oil!** (refer to instruction No. 3 above)
- Move back condenser column
- Close  software, ****Wait for the software completely close before proceed next!**
- Turn off the system components in the following sequence: 5 → 4 → 3 → 2 → 1
- Fill in the log book!!