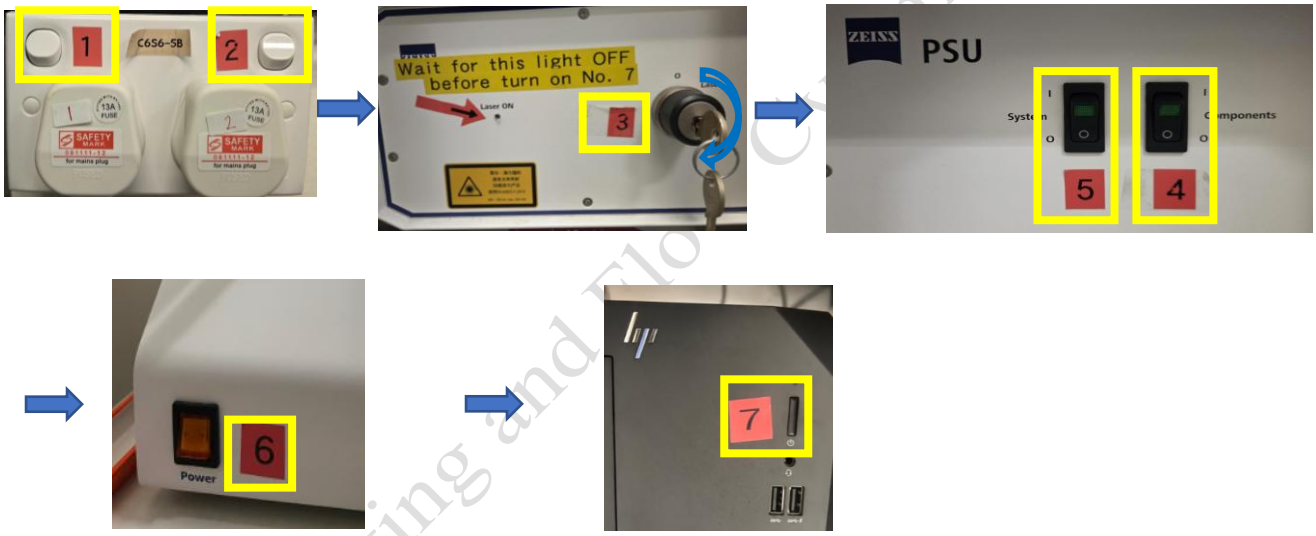
Inverted LSM900 Confocal Microscope

Standard Operation Protocol

System Start Up

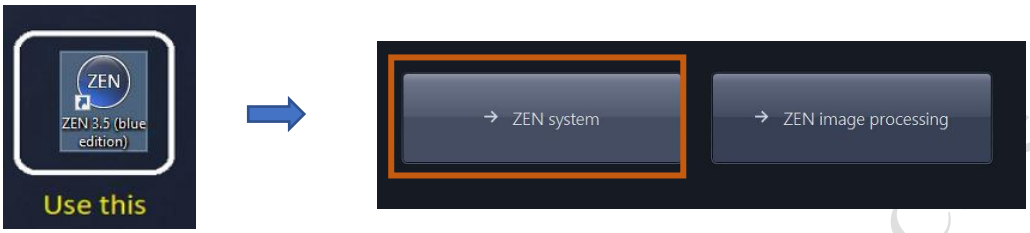
Sign on log sheet according to Actual start time (Please leave at least 5 seconds gap in between each step of machine Start Up process)

1. Switch on Main power switch ① →wait for 5 seconds
2. Switch on power switch ② on power socket → wait for 5 seconds
3. Turn the key switch ③ 90° clockwise for laser switch on → wait for 5 seconds
4. Switch on power switch ④ on power unit→ wait for 5 seconds
5. Switch on power switch ⑤ on power unit → wait for 5 seconds
6. Turn on the fluorescent light source power switch ⑥
   → wait for the light (near step 3) to be off
7. Turn on the computer power switch ⑦



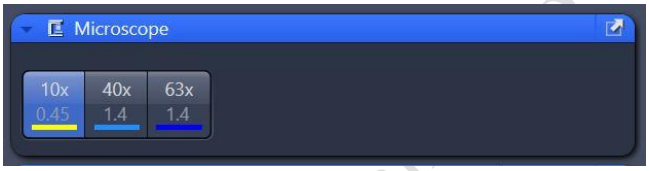
ZEN Software Initialization

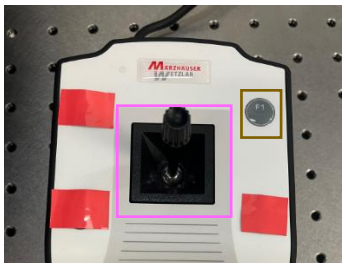
1. Log on computer with "**USER**" account
   (Please find USER account password posted under the bottom of computer screen)
2. Double click the **ZEN 3.5 blue** icon on desktop
3. Click **ZEN system** to run the software



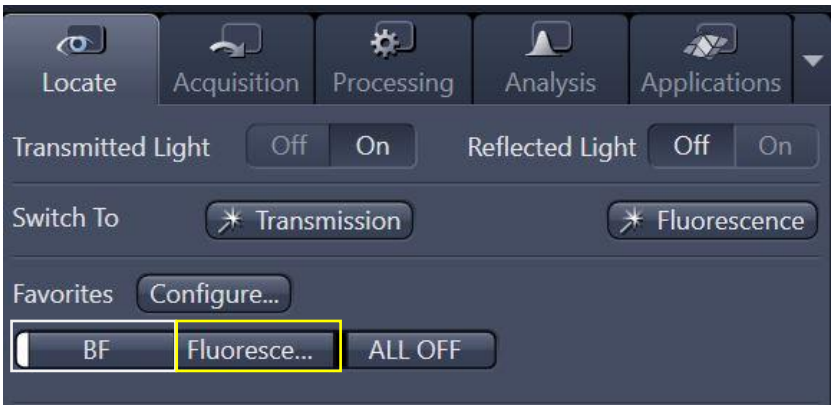
Locating Sample and Finding a Region of Interest

1. Select a **desired objective** from the touch screen. Apply a drop of oil onto the tip of the lens if 40x oil objective is used.
   Never put oil on Air objective!!! (10x)
   Note: Please make sure there is no oil or water at all on your slide when you are using air objective. Any Oil or water will cause damage to the air objectives





2. Place the sample (slide/dish) on the stage with coverslip facing down.
3. Move the region of interest to right above the objective lens with the **joystick**. Press the **top right button** to change the speed of stage movement (from fast to slow mode or in reverse).
4. Click "**Locate**" tab in the software and select a fluorescent channel of interest for direct observation via the eyepiece.
5. Click **BF** tab to set the microscope for brightfield observation.

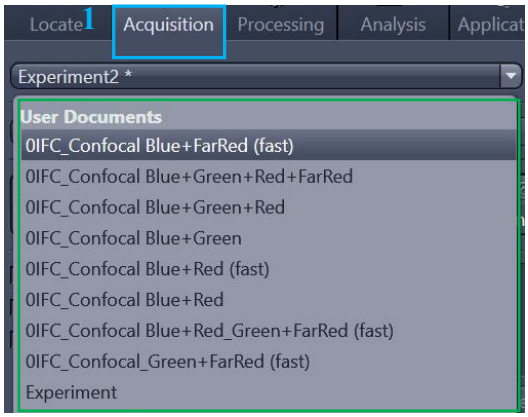


6. Click Fluorescence tab to enable fluorescence mode. Press on the intensity control knob on the external fluorescence light source controller.
Blue/**Green**/**Red**/**FarRed** button for blue emission/green emission/red emission /farRed emission observation respectively.
Fluorescence intensity can be adjusted by intensity control knob.
Clockwise: increase; anticlockwise: decrease

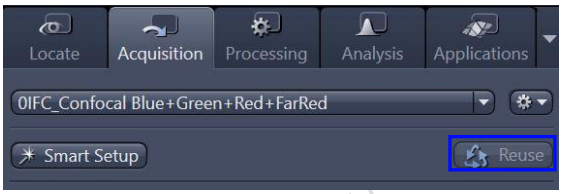
7. Focus the sample with Coarse/Fine focusing knob.
8. Once the region of interest is found and focused, switch the tab to "**Acquisition**".

Setting up the Scanning Track/Channel

Method 1
Load a **predefined configuration** of combination dyes from the **Load configuration list**.

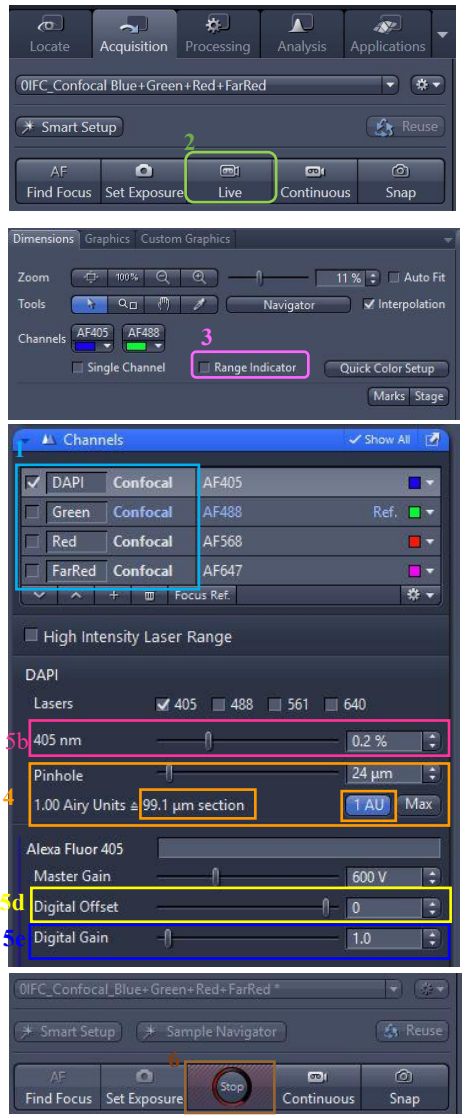


Method 2
Open any previous image with desire click "**Reuse**" button in Dimension panel.



Confocal Imaging Set Up

1. Select only **one channel** tool for parameter adjustment at a time.
2. Click **Live** for continuous fast scanning.
3. Activate **Range Indicator**. The scanned image appears in greyscale with red pixel indicating saturation (max. intensity) and blue pixel indicating zero (min. intensity). Advise to adjust the laser power and/or gain master until a little bit of red pixel is seen.
4. Set the **Pinhole to 1 AU (Airy Unit).** For co-localization studies, adjust the pinhole of each channel to the same **Optical Slice Thickness.**
5. To get optimal intensity and background signal,
   a. Increase **Detector Gain (Maximum of 800)** until a few red pixel (indicating saturation) appear in the image**.**
   b. Increase the **Laser Power** (too high cause bleaching), if increasing detector gain cannot achieve the desired intensity.
   c. Fine-tune the focus with the fine adjustment knob to the brightest or preferred z- position. And then adjust the detector gain and laser power to optimize the signal intensity.
   d. Decrease the **Digital Offset** to reduce background signal until the desired background region is filled with blue pixel.
   e. Increase **Digital Gain** to increase signal amplification if needed.
6. **Stop** the Live scan process and uncheck the Range Indicator.
7. Repeat step 1- 6 for other tracks.



Scanning Parameters Set Up

1. Go to Acquisition Mode.

2. Select a suitable **Scan Area** to capture image of interest (or use **Crop Area** function after a Live image).

3. Choose a **Frame Size** (change of **Pixel size**, thus change of resolution). 1024x1024 usually produces good results for general purpose. Click on the **Confocal** button for best resolution which depending on objective N.A. and λ.

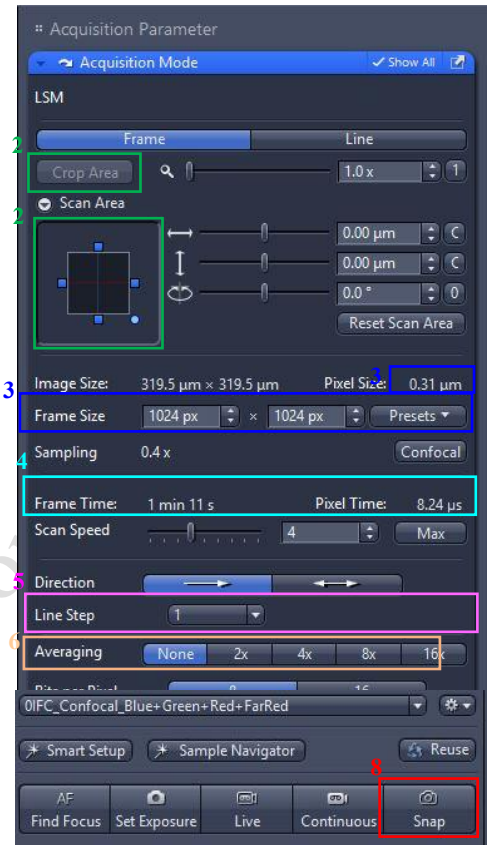
4. Adjust the scan speed with the **Speed** slider. Speed 4 or 5 usually produce good results. For samples with high background noise, use Speed 4.

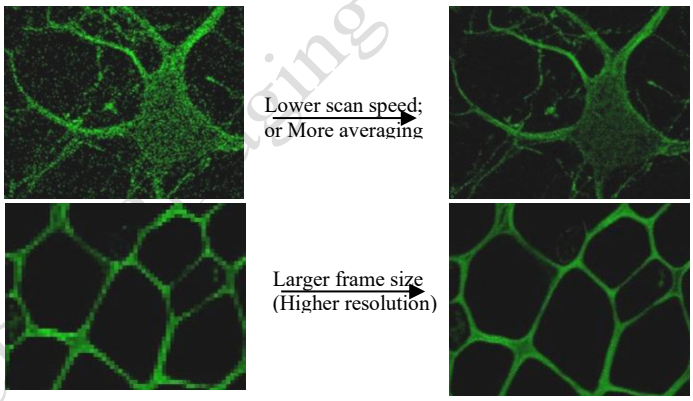
5. Select the number of **Average**. Averaging (usually in 2 or 4) improves the image quality by increasing the signal-to-noise ratio but it also increases the scan time.

6. Select the Bits per Pixel of 8 or 16 Bit in the **Bits per Pixel**. 16 Bit is recommended when doing quantitative measurements or other post-analysis.

7. Other setting can be kept as defaults (as in the right) which will be good enough for general purpose.

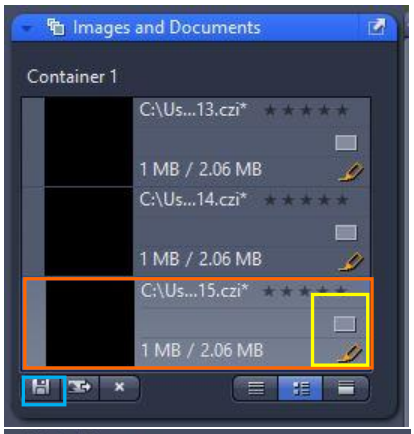
8. Click **Snap** to acquire a single frame (multi-channel) image.

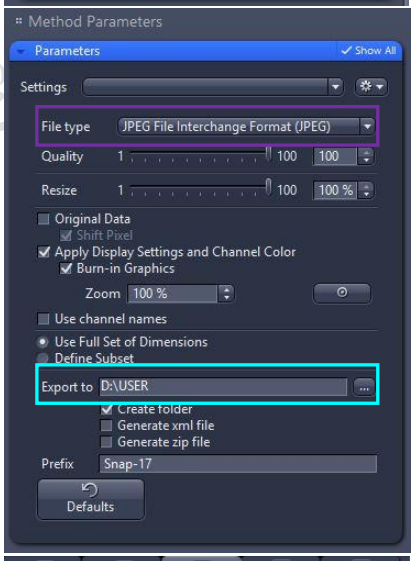




Storing and exporting image data

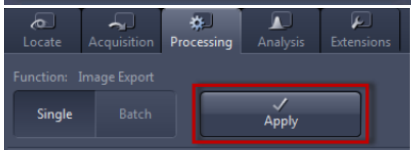
1. To save the acquired image→ highlight the desired image → click on **Save** icon button or Save As button in the File menu→ Create or choose your own folder in the D Drive (D:/user). Warning Icon indicated unsaved.
2. Create or choose your own folder in D:\user, enter a file name, select format as **CZI** and click on the **Save** button.
3. After saving the raw data as CZI format, Image can be exported as various Image format and series images can be export as video. Go to processing (or Go to File > Export)→Image Export→choose the **File type** as shown below→Choose export to→ select the image you want to export→click on Apply.



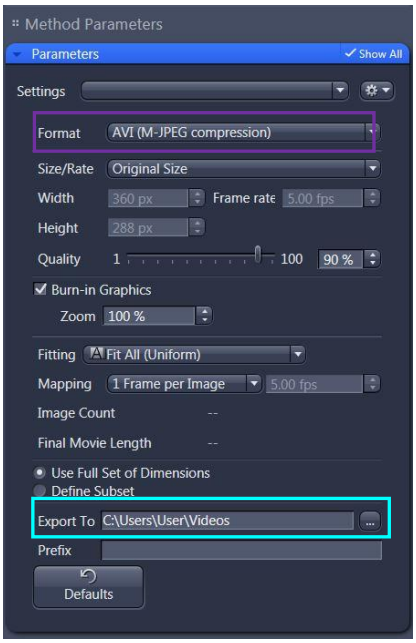


File type	JPEG File Interchange Format (JPEG) Windows Bitmap (BMP) Tagged Image File Format (TIFF) Tiff Format(64Bit) (Big Tiff) Portable Network Graphics (PNG) JPEG XR (WDP) DigitalSurf SUR (SUR)

4. For export as video→Go to processing (or Go to File > Export) →Movie Export→choose the **Format** as shown below→Choose export to→click on Apply.



Series (Gallery of images; Time series movie or 3D animation)	AVI (M-JPEG compression) AVI (uncompressed) AVI (DV) WMF (Windows Media) MOV (H.264) MOV (MPEG4) AVI (MS-Video1)



Z-Stack Experiment Set Up

1. Check the Box for Z-Stack → Open the Z-Stack module.

2. Choose a channel that has signal throughout the interested volume → start **Live**.

3. Use the focus knob to locate one end of the specimen → **Set First**. Focus to locate another end of the specimen → **Set Last** → stop **Live**.

4. Set the Z-stack **Interval** between each frame equal to/or less than the thickness of the thinnest **Optical Section** of the selected channel wavelength (so that you can scan all the space within the specimen). **Optical Section** (pinhole) of the channel is calculated according to the emission wavelength, objective lens, and the pinhole diameter.
   Optimal: double sampling with interval equal to half of the thinnest optical section and thus provide better 3D image reconstruction.

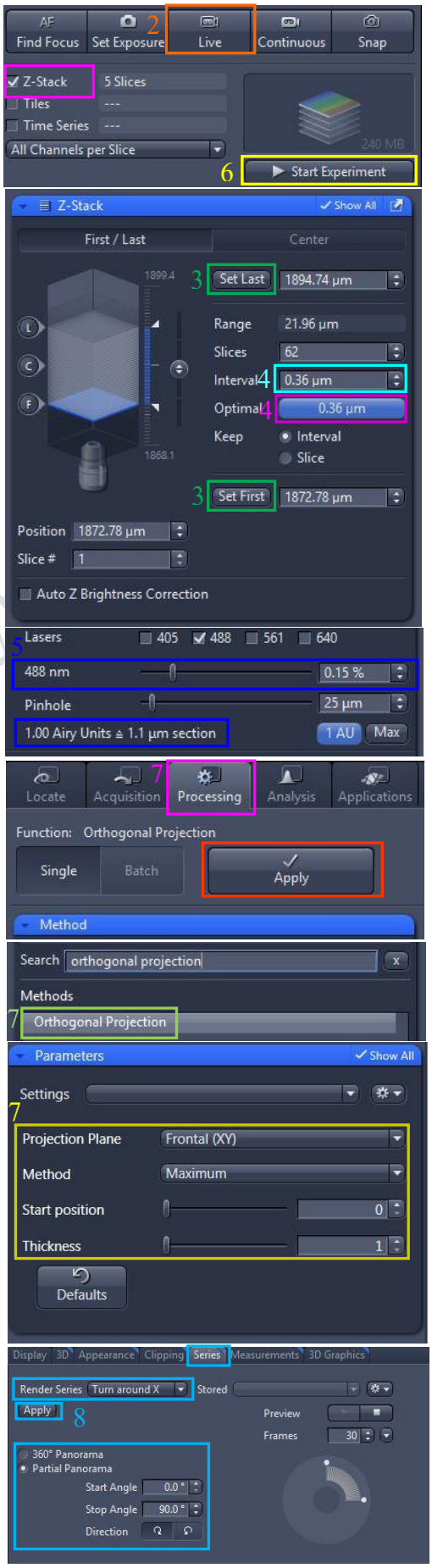
5. For colocalization studies, adjust the **Pinhole** of each channel in the Channels panel to the same **Optical Slice thickness** (so that each voxel contains data from the same volume of the specimen).

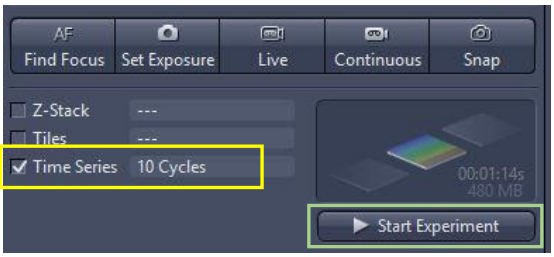
6. Click **Start Experiment** to start the recording of Z-Stack images.

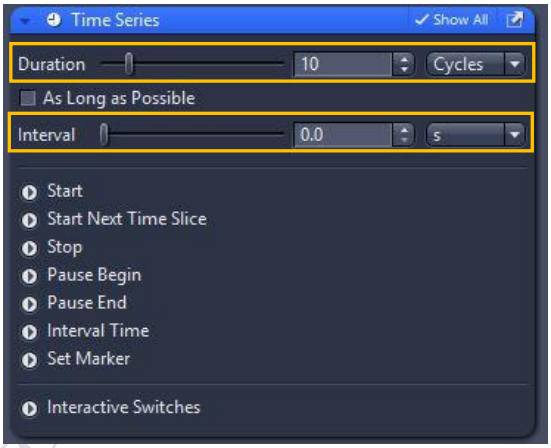
7. After saving the CZI format, a 2D stack up image can be generated, in which image in all frames will projected onto a single plane. Go to "**Processing**" tab → choose Orthogonal Projection → select projection plane, Method, set Start position and Thickness → **Apply**.

8. A video of rotating 3D image can also be made. Activate the **Series** tap, select the **Render Series**, number of frame and the rotation range (= **Total Frame** × **Difference angle**). Click **Apply** and the series images will be generated in a new tab.

   Go to File > Export, choose **movie export** and then choose a suitable **series** Adjust the playing speed by **Frame per Second** and **save** in your own folder.







Time Series Experiment Set Up

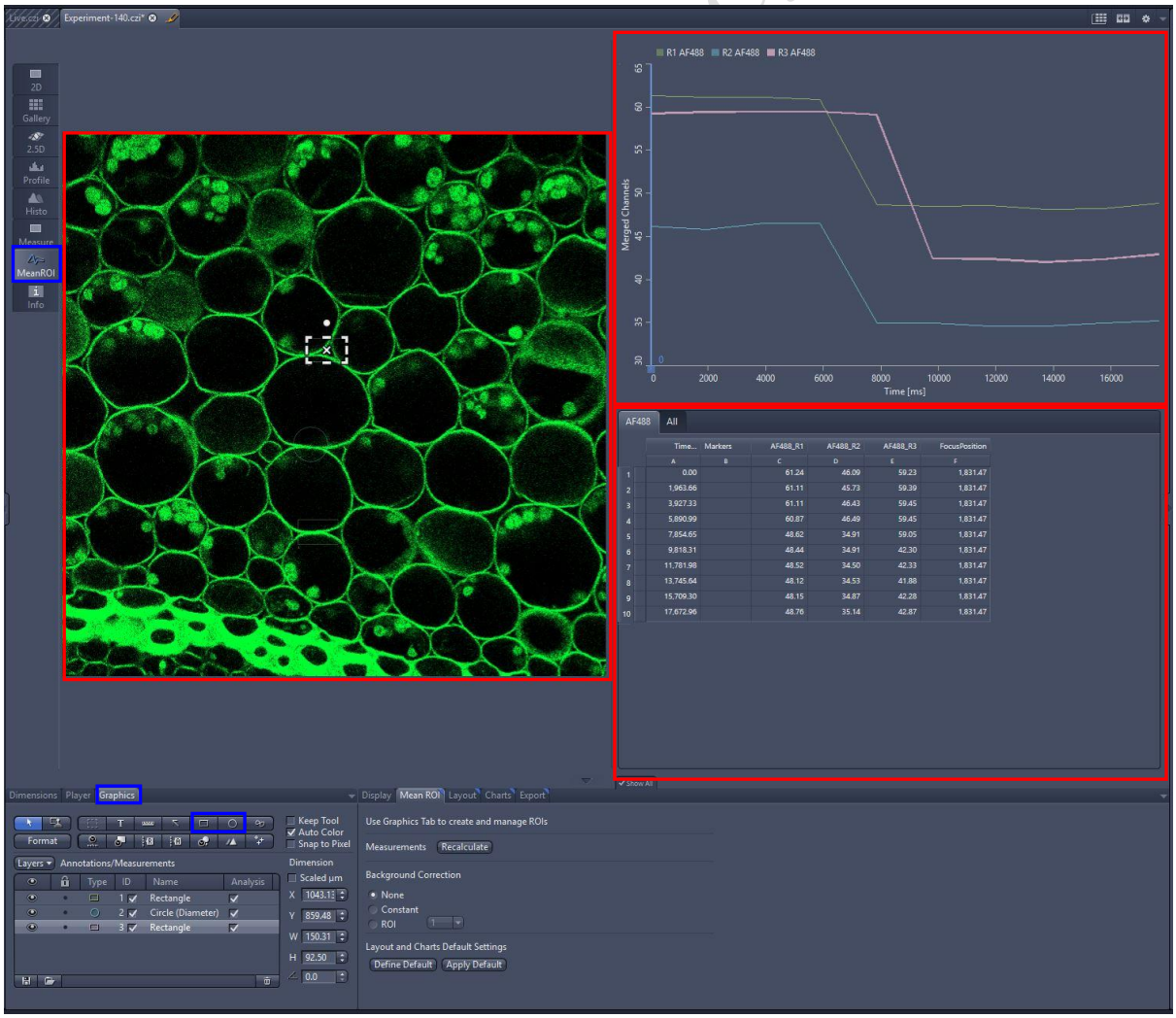
1. Check the **Box for Time Series** → open the **Time Series** module.

2. Set the number of **Cycles** and time **Interval** between each frame. (The scanning of each frame is included in the countdown of the Interval, therefore Interval time should be ≥ scanning time of one multi-color frame.)

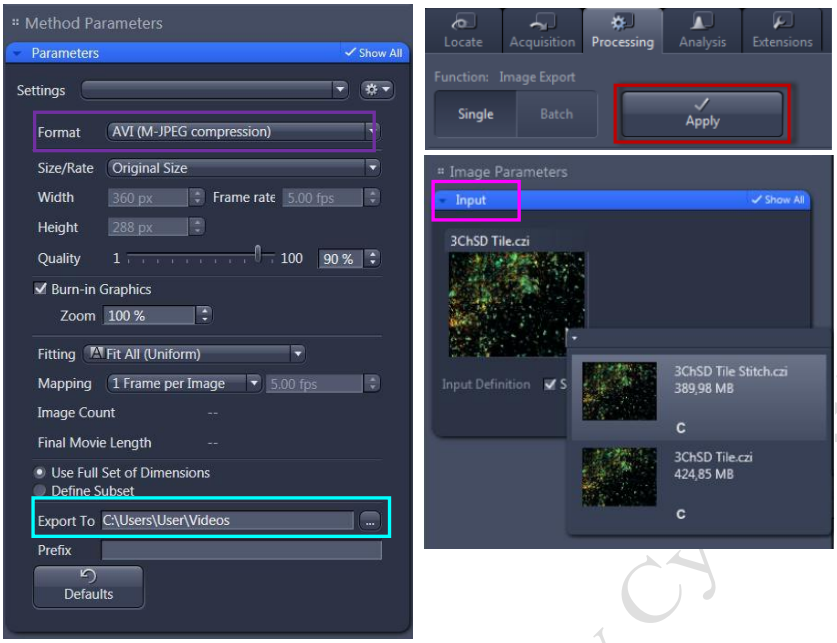
3. Set the channel and acquisition parameter if necessary and then click **Start Experiment**.

4. To quantifying changes in signal intensity after acquisition, click **Mean ROI** tab and create a **ROI** region with the drawing tool for the region of interest.

5. The **intensity profile** along the experiment duration will be shown in the graph while corresponding **data** will be shown in the table right.



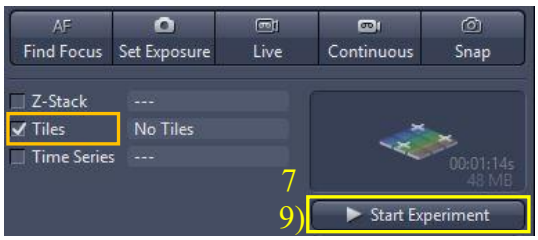
6. After saving the CZI format, video for the time series images can be exported. For export as video→Go to processing (or Go to File > Export) →Movie Export→choose the **Format** as shown below→Choose export to→click on Apply. Adjust the playing speed by **Frame per Second** and **Select file name** and **save** in your own folder.



Multiple Position Experiment Set Up

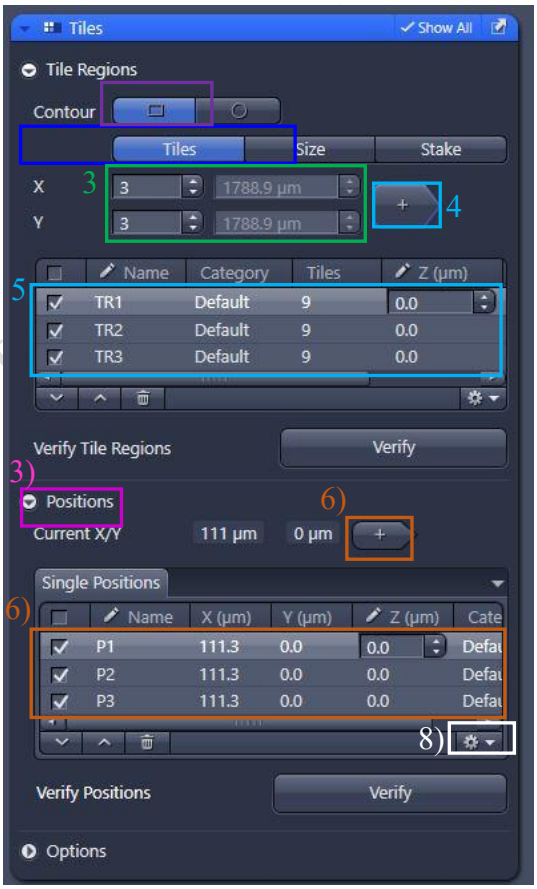
1) Open the Tiles tool in the Multidimensional Acquisition module and activate the Show all mode.
2) In the Tiles tool open the Sample Carrier section→Click on the **Select...** button→Choose a predefined **Sample Carrier template** and click **OK** (if applicable).
3) Open the Positions section.
4) Start the Live mode to use the stage to locate a position that you want to acquire.
5) Bring the specimen into focus using the focus drive.
6) Click on the  Add button→The current position is added to your experiment.
7) To add further positions, move the stage to another position on the sample and repeat the previous steps. (The added positions are shown in the list in the Single Positions section with their X, Y and Z-coordinates.)
8) Save the experiment (if applicable). To do this, in the Experiment Manager click on the  Options button and select the Save As entry. Enter a name for the experiment in the input field (e.g. Simple Tile Experiment).
9) Click on the Start Experiment button.

Tile Scan Experiment Set Up



1. Open the Tiles tool in the Multidimensional Acquisition module and activate the Show all mode. → Check the Box for Tiles Scan → open **Tile scan** module by clicking the Tile Regions
2. Choose the Contour
3. Set the scan mode with Tiles, Size or Stake.

   a. Tiles: Enter the number of X and Y to define the tile region (Example: X=3 and Y=3 equals to tile region containing 9 tiles)
   b. Size: Define the size of tile region
   c. Stake: Adding the area for tile scanning by input the edges of whole tile area. The software will define the total tile number base on the input region.
4. Click on the  Add button
5. To add further tile regions, move the stage to another position on the sample and repeat the previous steps. The added tile regions (TR1, TR2, etc.) are displayed in the tile regions list.
6. Save the experiment. To do this, in the Experiment Manager click on the Optionsbutton and select Save As. Enter a name for the experiment in the input field (e.g. SimpleTile Experiment).
7. Click on the **Start Experiment button**.



Turning off the system

1. Remove your sample and clean the stage
2. Clean the oil objective (with lens cleaning tissue only NOT Kimwipe).
    a. Remove immersion oil from the objective lens (40x/63x) with lens cleaning tissue.
    b. Repeat this step with a new area/piece of the lens cleaning tissue until no oil streaks are seen on the tissue.
3. Change objective to lowest magnification (10x) objective.
4. Click Load Position (on the touch screen)→Click Set Work Position(on the touch screen).
5. Transfer data through the Imaging and Flow Cytometry Core network storage server.
6. Please check the schedule and switch off the system if it will not be occupied within 1 hr.
   (Please leave at least 5 seconds gap in between each step of machine Turn Off process)
7. Shut down PC⑦ → 5 second.
8. Turn off the fluorescent light source power switch ⑥
9. Turn the Laser Key (labeled ③) 90° counter clockwise for switch off the laser→ wait for 5 second.
10. Turn off the components button④→ wait for 5 second
11. Turn off the System button ⑤ → wait for 5 second
12. Switch off the Main switch② mounted on the wall→ wait for 5 second
13. Switch off the Main switch① mounted on the wall→ wait for 5 second
14. Sign on log sheet according to **Actual** finishing time.