Imaging and Flow Cytometry Core

Centre for PanorOmic Sciences

ZEISS Lattice Lightsheet 7 Microscope

Standard Operation Protocol

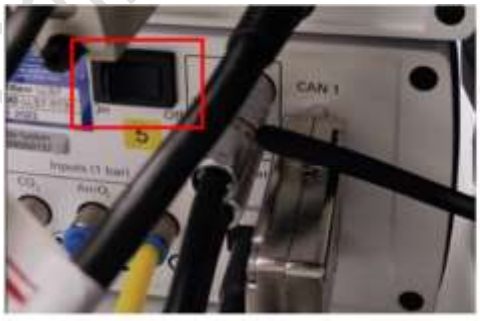
Contents

Startup of the System

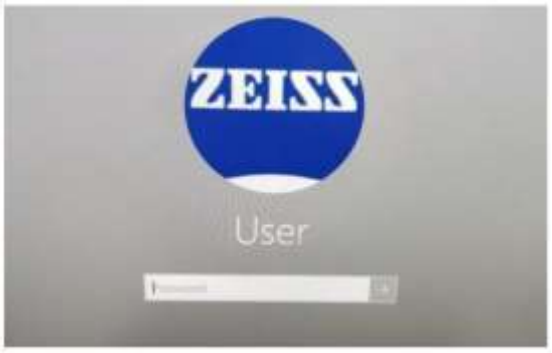
1. Sign on log sheet according to Actual start time. Follow the sequence 1—2—3—4 for the system start up.
   - Turn the key switch ① clockwise for 90 degrees
   - Turn on switch No. ②→wait for 5 seconds.
   - Turn on switch No. ③ → wait for 2 mins.
   - Press on No. ④ to turn on the workstation

Only applicable for live cell imaging, please skip this step if it is not needed

- (Optional) Switch on gas controller ⑤ and temperature controller ⑥.



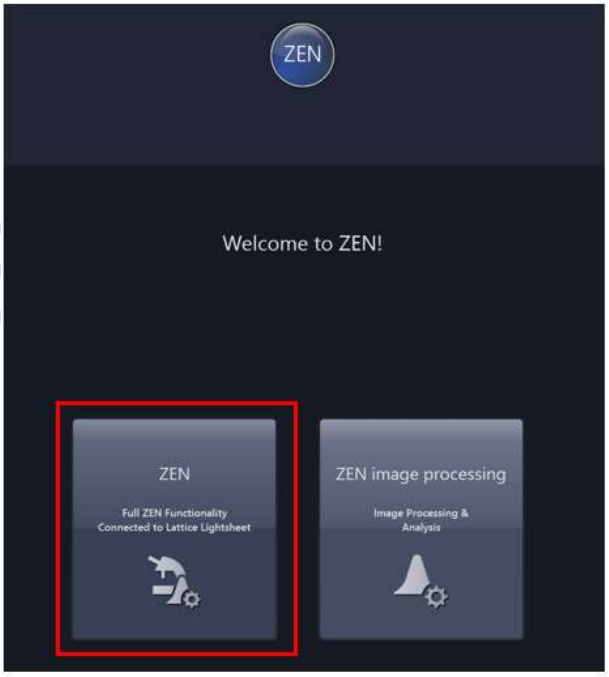
2. Log in to the **User** account in Windows, the password is attached on the stand of the monitor.



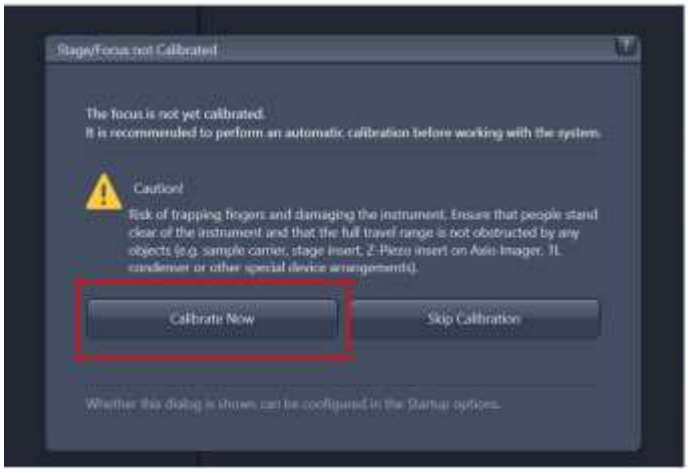
3. Login PPMS tracker with your HKU portal ID and the 6-digit code generated by the authenticator app of your mobile phone.

4. Double-click ZEN 

to initialize the acquisition software and select "Full ZEN Functionality Connected to Lattice Lightsheet". The software will need at least two minutes for the initialization.



5. Stage calibration window will pop out after software initialization, please make sure there is no sample carrier on the stage and the transmission arm is in the upper position. Click “Calibrate **Now**”. During the calibration, don’t hamper the movement of the stage.

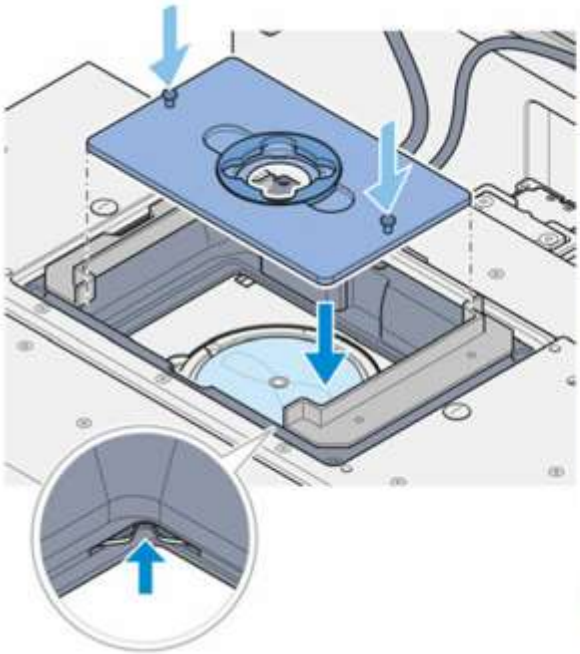


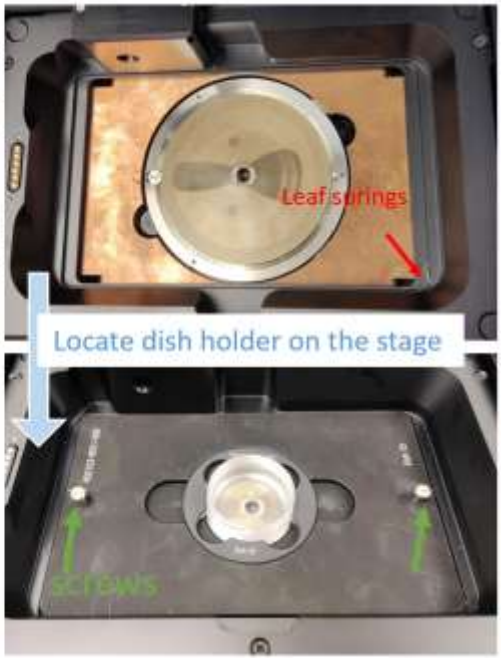
Locate Your Sample

1. Select the proper holder based on your sample carrier and secure your sample carrier on the holder.

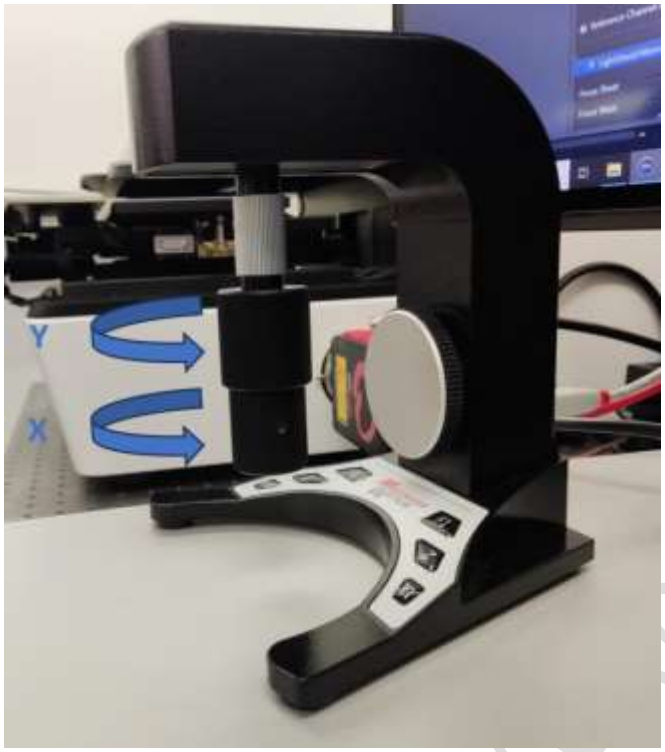
Carrier for multiwell plate	***Carrier for slide***	***Carrier for chamber slide***
		
Carrier for confocal dish		
35mm	**36mm**	**39mm**
		

2. Hold the two knobs to load the holder onto the stage. Press the holder on the two leave springs at the right bottom corner to lock the holder in place.

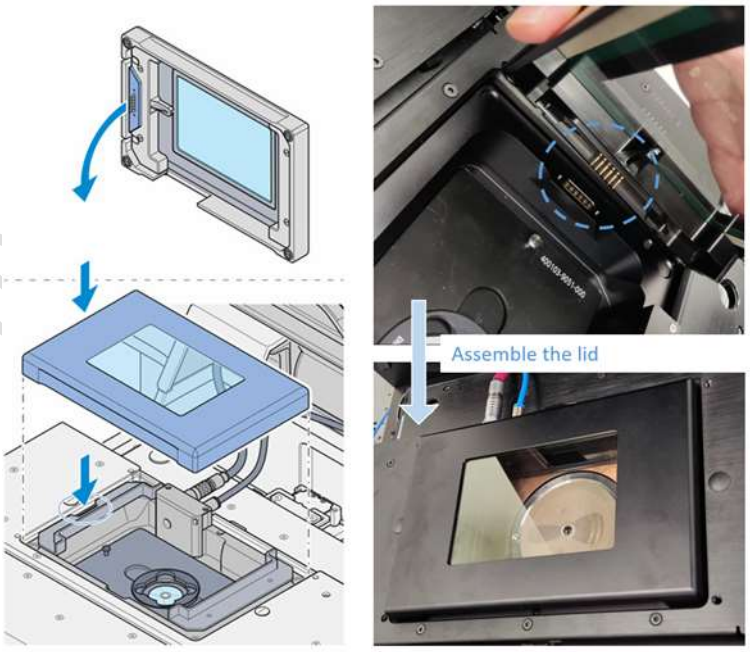




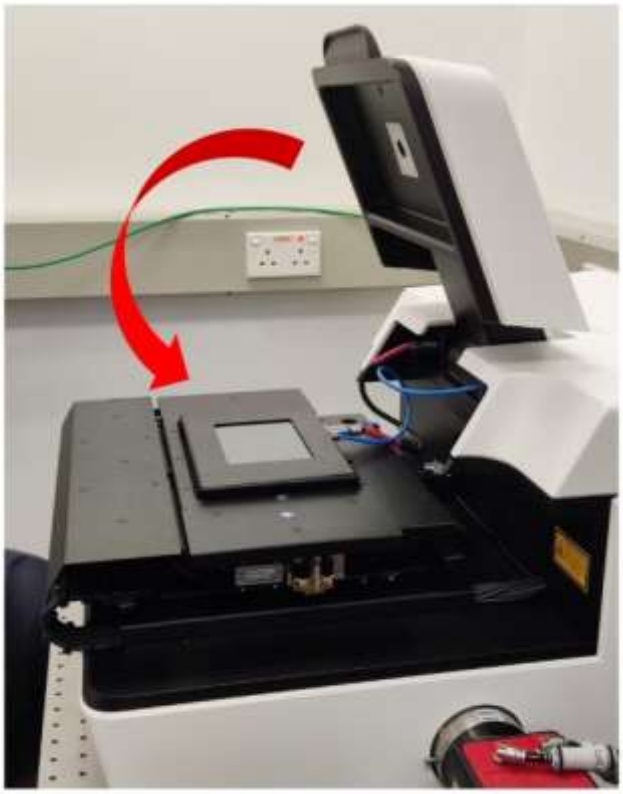
3. Turn the knobs of the stage controller to navigate onto your sample. The upper (Y) knob and the lower (X) knob correspond to the Y and X axis stage control respectively.



4. (Optional) Install the incubator cover. *
Only applicable for live cell imaging, please skip this step if it is not needed
*Make sure to align the electronic contacts of the incubator lid with the stage. The incubator lid is held in place by magnets.



5. Lower the transmission arm after installation.



6. (Optional) Setting the Environmental Conditions

Only applicable for live cell imaging, please skip this step if it is not needed

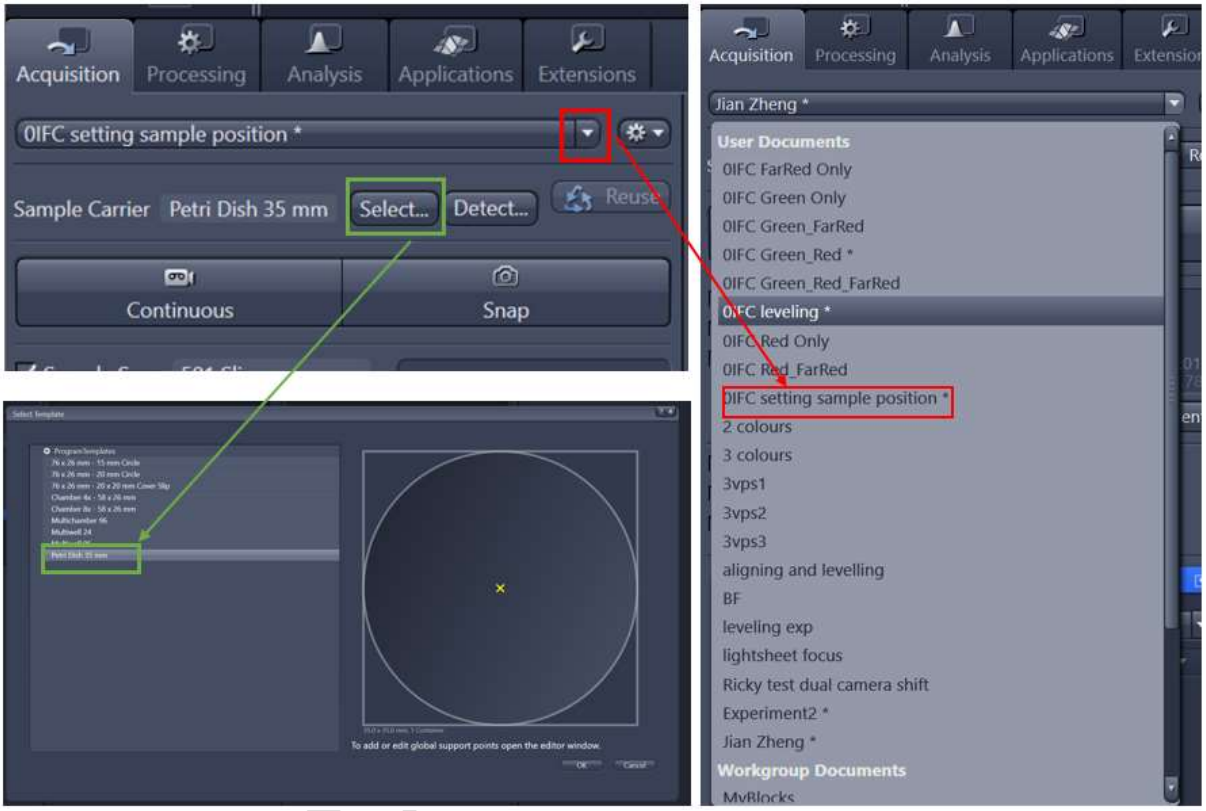
Turn on the gas valve which is labeled for **Lattice Lightsheet** to supply with CO_2.

Go to the "Incubation" panel in the software, to maintain sample temperature at 37 ℃， set the temperature for the "**Lid**", "**Tray**", and "**Base**" as 40.0, 38.0, and 38.0 respectively. Set the concentration for CO_2 and O_2 as 20.0% and 5.0% respectively. Set the humidity as 90.0%rH. If you need to maintain your sample at

other temperature instead of 37 ℃, contact the Technical Staff in Charge.

Setting the Sample Position

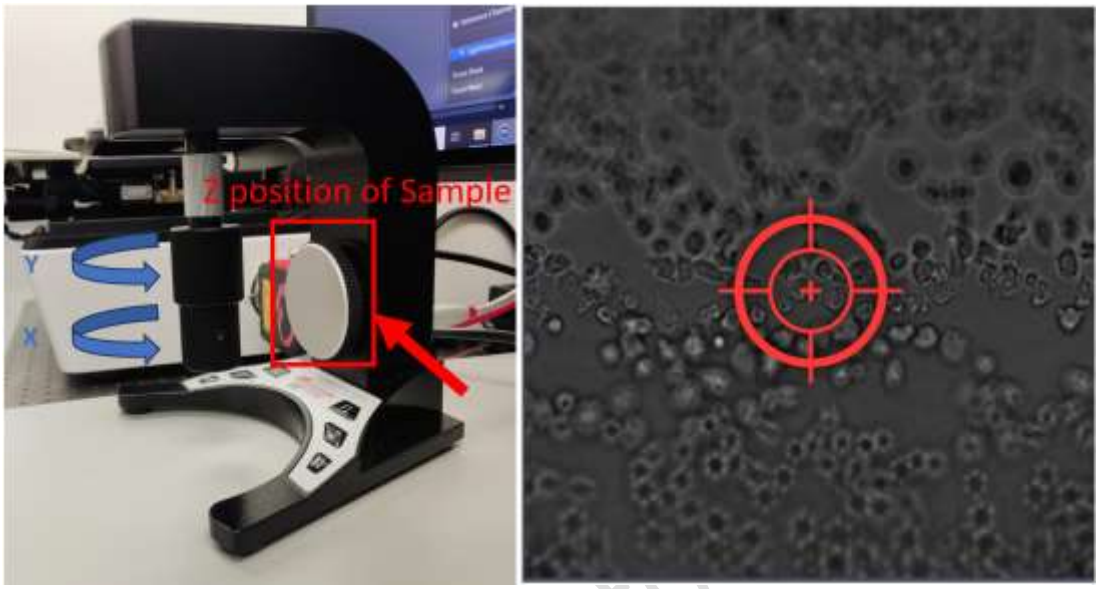
1. In the “Acquisition” tab, click the  icon to select the setting “0IFC setting sample position” in the dropdown list, the white LED lamp will be on. Select the sample carrier you used.



2. Go to the **Acquisition Mode** panel, and set the “Size” of the camera ROI to 2048x2048 px.

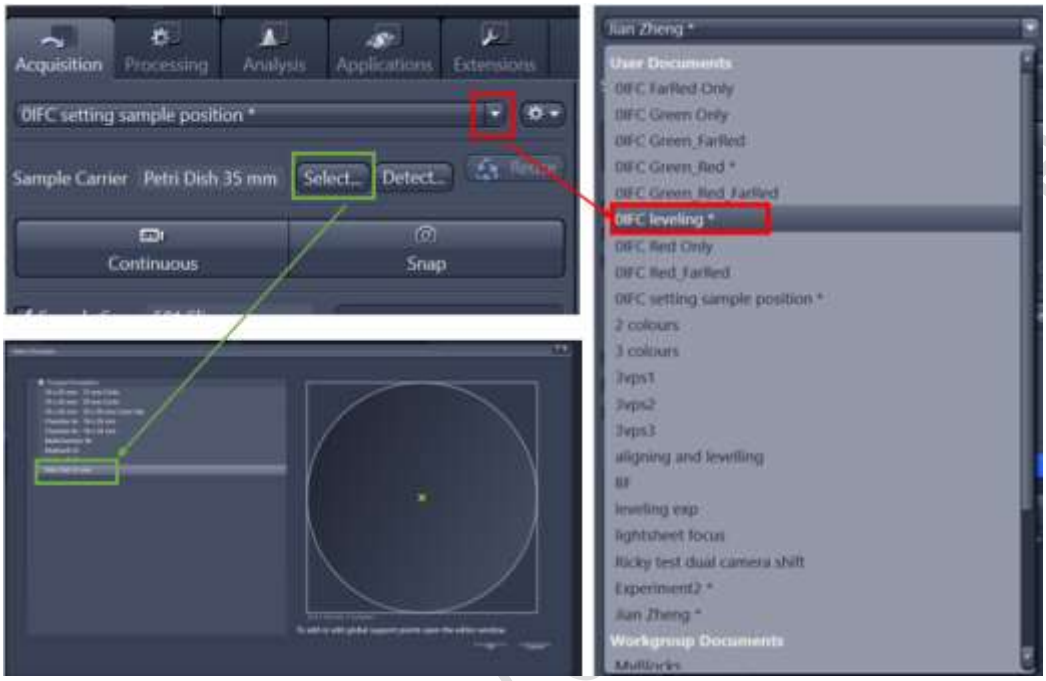
3. Click **Create Immersion**, this system will auto-immerse the water lens.

4. Click "**Continuous**", and an image with a sharp band is displayed. Move the stage in the X and Y axis until your interested region moves to the center of the live window. Use the scroll wheel to adjust the focus until the sharp band is centered around the crosshair.



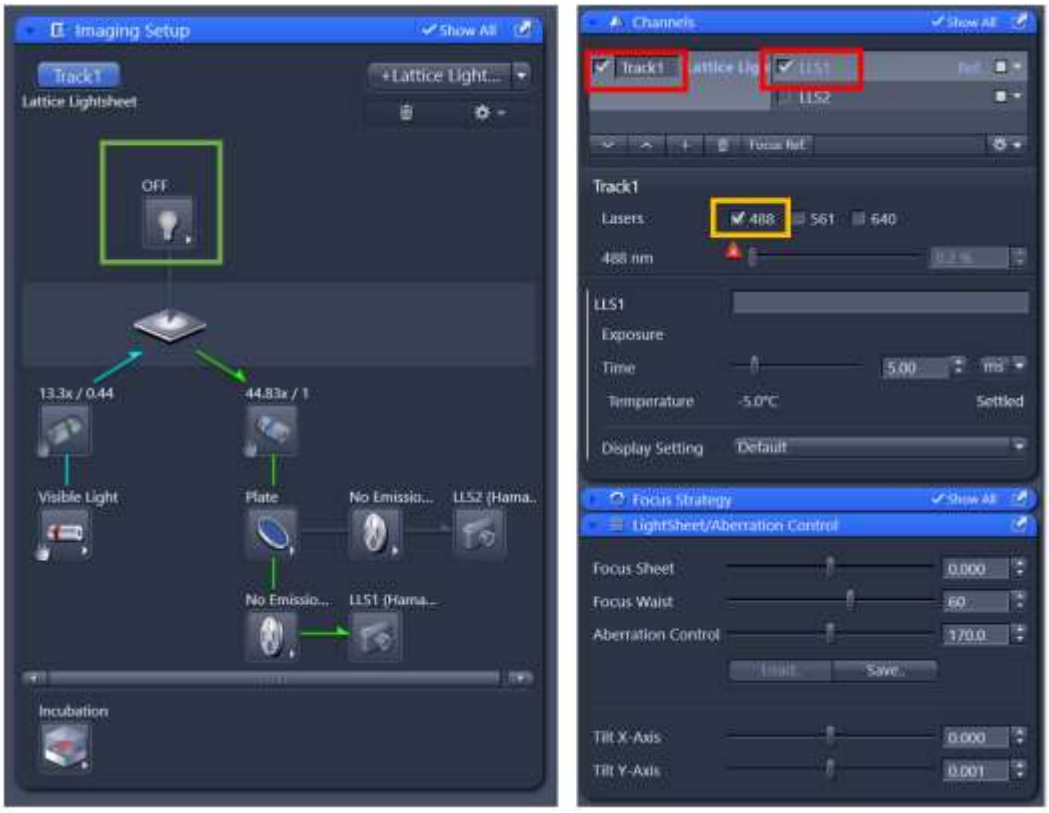
Aligning the Lightsheet and Levelling the Stage

1. In the “Acquisition” tab, click the icon  to select the proper setting “0IFC leveling” in the dropdown list.

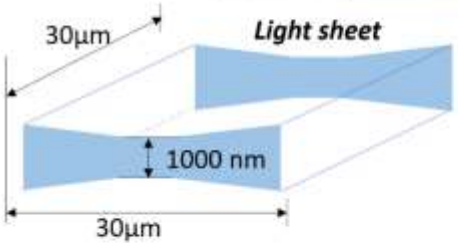


2. Turn off the white LED. Keep Track 1 and LLS1 checked. Select one laser to do lightsheet alignment and stage leveling. For example, you have Green (488), Red (561), and Fared (640) labeling in your sample, and you select the Green (488)

channel to align the lightsheet and leveling the stage.



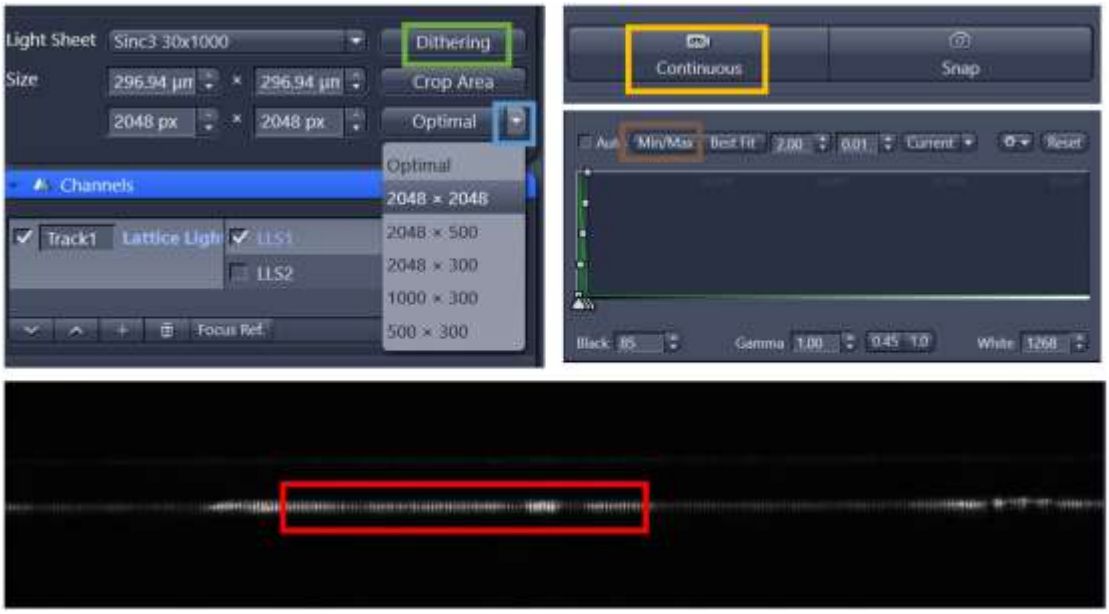
3. Go to the **Acquisition Mode** tool and select **Light Sheet** based on your sample type**.** The values in the **Sinc3** are the width and the thickness of the lightsheet. e.g. 30 x 1000 will work well with cells.



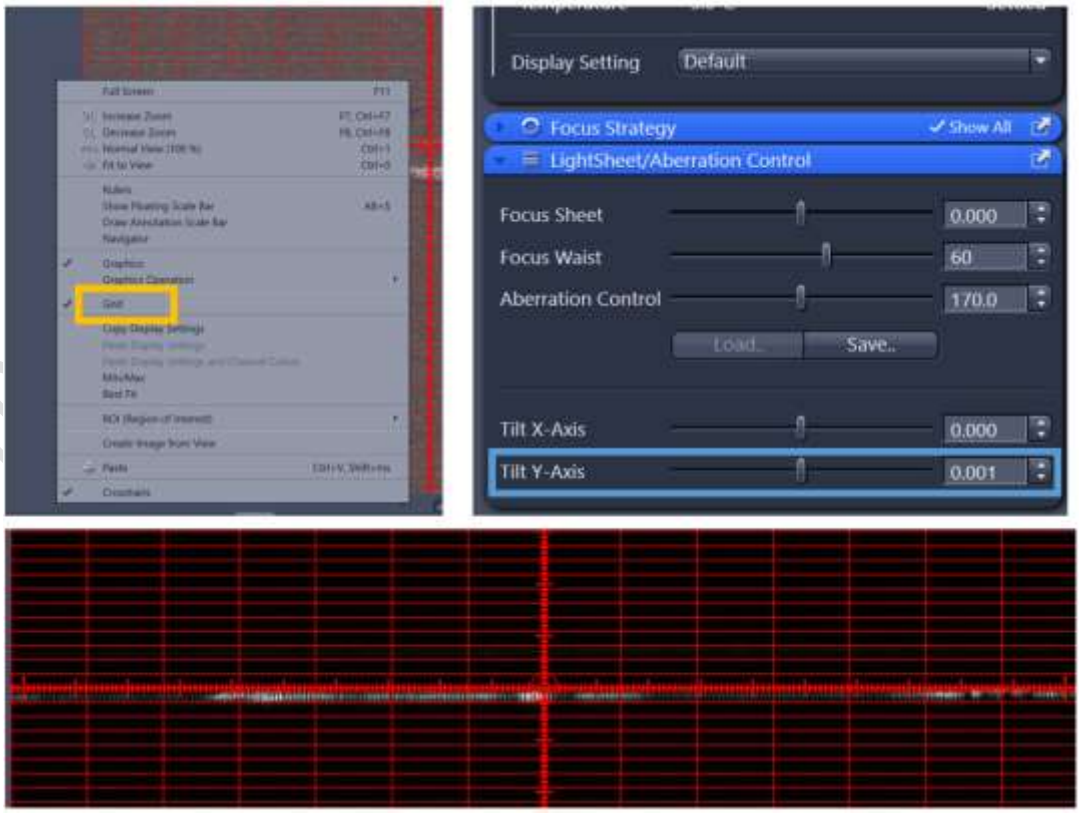
Light sheet: Sin3 30 x 1000
Width and length: 30 µm
Thickness (thinnest site): 1000 nm

Sinc3 15x550	Detailed subcellular structure
Sinc3 30x1000	Cell
Sinc3 100x1800	Spheroids or organoids

4. Switch off **Dithering**. Set the camera ROI to **2048x2048px**, click **Continuous**, highlight **Min/Max** to see the lattice structure in the live window. Focus the sample with the focus wheel until the lattice light sheet is as thin as possible and the lattice light sheet is in the central position of the live window.

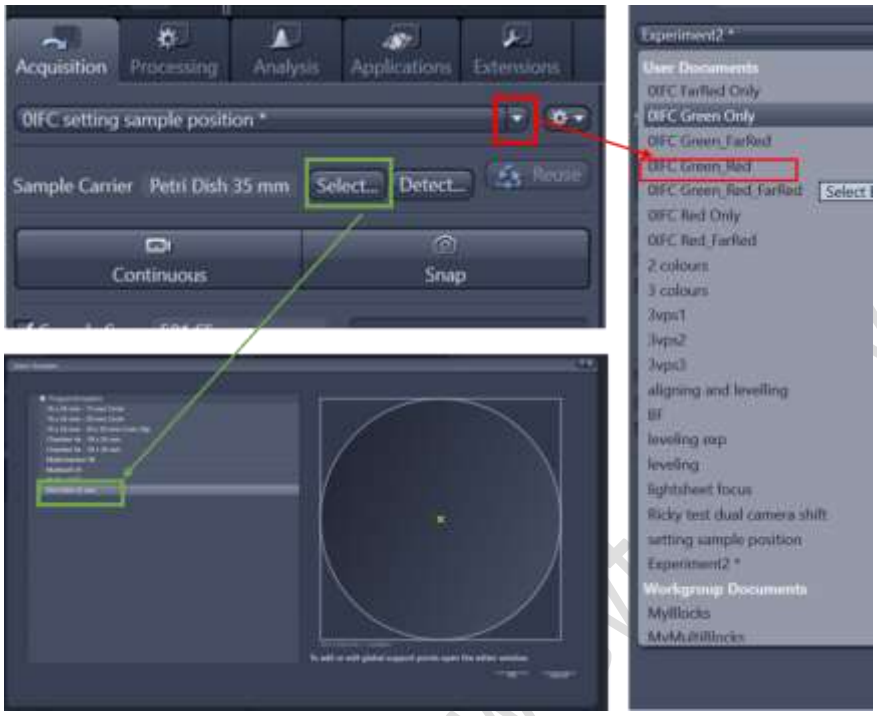


5. Right-click the live window to activate the "**Grid**" function. Move the stage in Y direction, to observe the light sheet for horizontal consistency. If the light sheet is shifting up or down when moving the Y stage, adjust the **Tilt Y-Axis** until the light sheet is parallel to the grid.

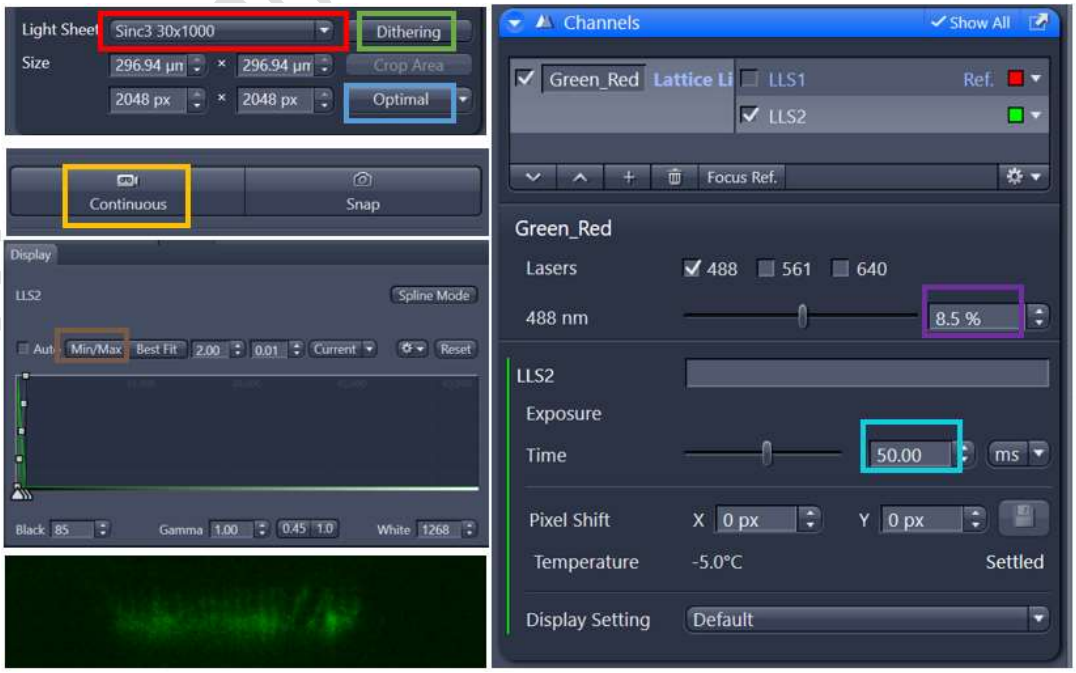


Setting the Lightsheet Focus

1. In the "Acquisition" tab, click the icon  to select the proper setting "**0IFC XXXX**" in the dropdown list based on the fluoresces labelling on your sample.

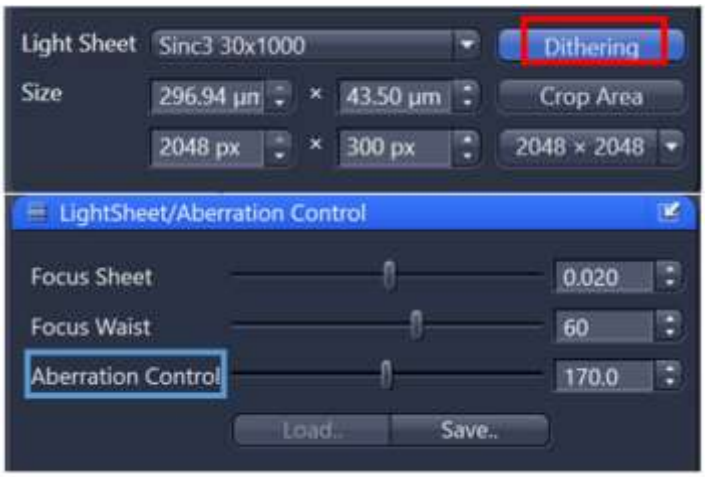


2. Select Light Sheet based on your sample type (illustrated in previous part 5.3). Switch off **Dithering**. Set the camera ROI to **Optimal**, click **Continuous**, highlight **Min/Max** to see the lattice structure in the live window. Adjust the laser power and exposure time if the signal is too weak to see the lattice structure.



3. Adjust **Focus Sheet** to bring the lightsheet into the focus plane of the detection objective. Use **Focus Waist** to minimize out-of-focus blur. Adjust the Focus Sheet and Focus Waist interactively until the lattice gets to achieve a clear contrast on the lattice.

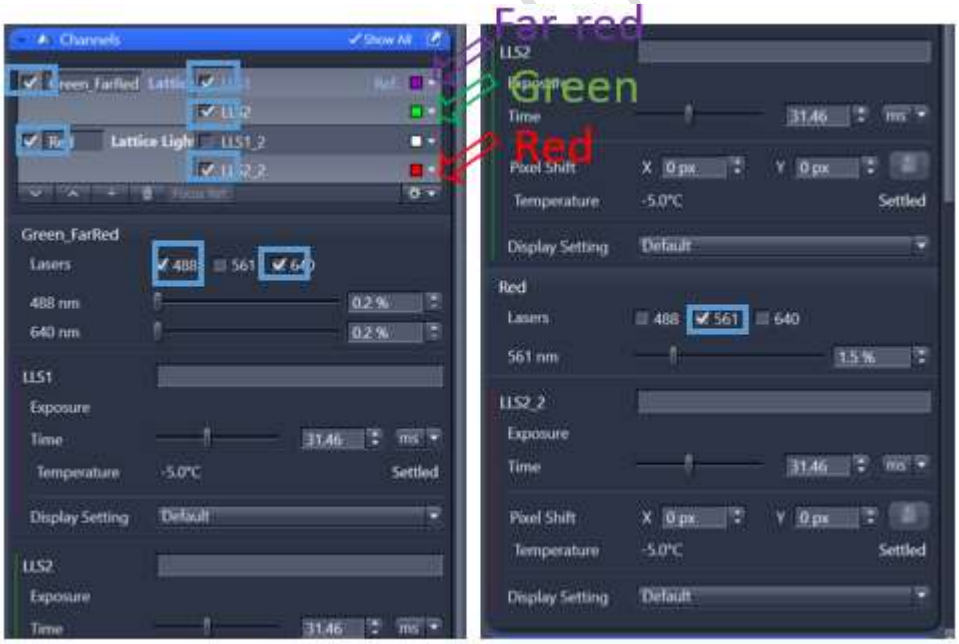
4. Switch on Dithering. Use **Aberration control** to maximize sharpness and contrast.



Setting the Channel and Scanning

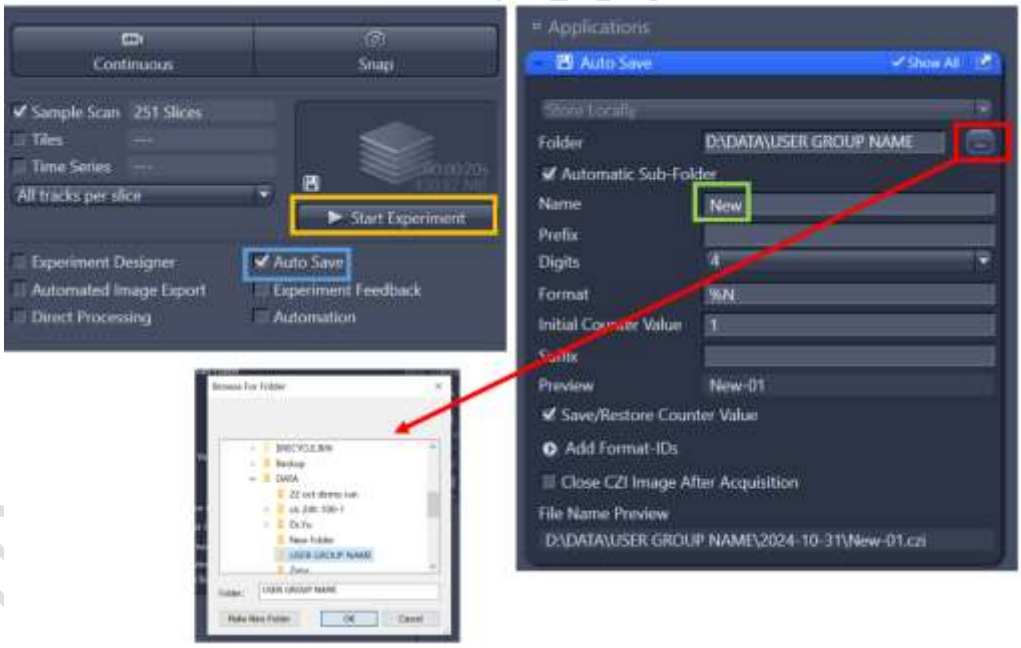
1. Set the imaging parameters of each fluorescence channel one by one. For example, if your sample has been labeled with three colors, and the green channel is set first. Tick and highlight the green channel, click **Continuous**, and adjust the laser power and exposure time, avoid oversaturation, which is presented in red in the live window when you check the **Range Indicator**. Repeat the channel adjustment until all the channels have been set.

2. Tick all the channels before you start your scanning.



3. Set the Range x that could cover your interested region, Set the **Interval** by clicking **Optimal**.

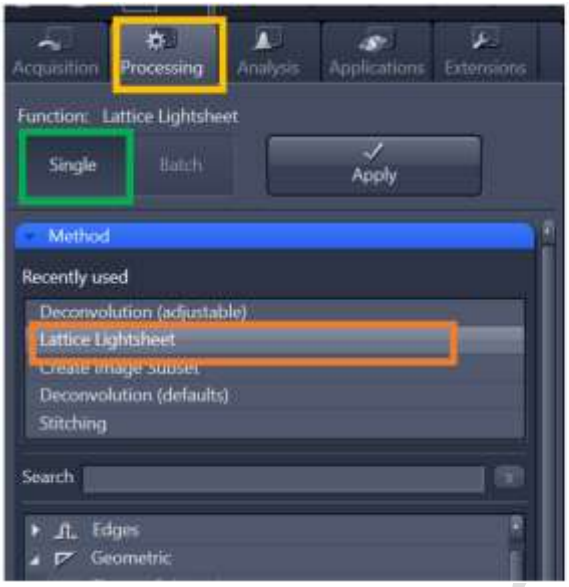
4. Check **Auto Save**. Navigate to the DATA folder and set up a new folder with your name. Type in the data Name. Click **Start Experiment** to start acquisition. The data will be autosaved in the folder you set.

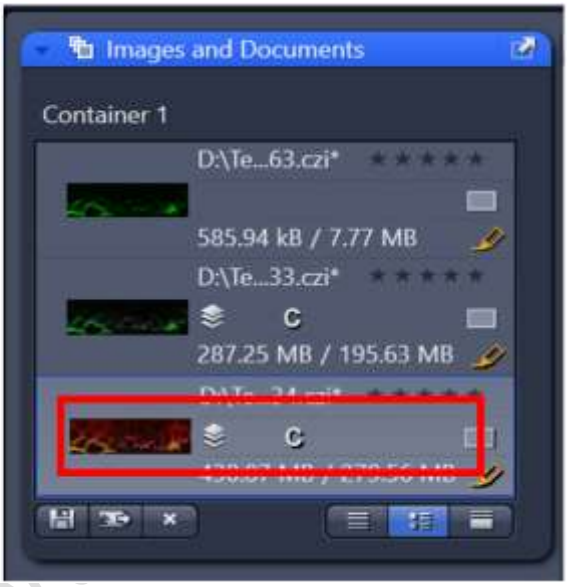


Data Processing

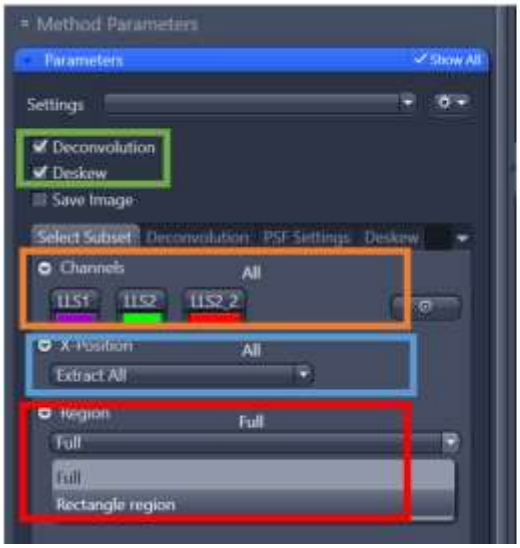
This part could be done on Lattice Offline PC

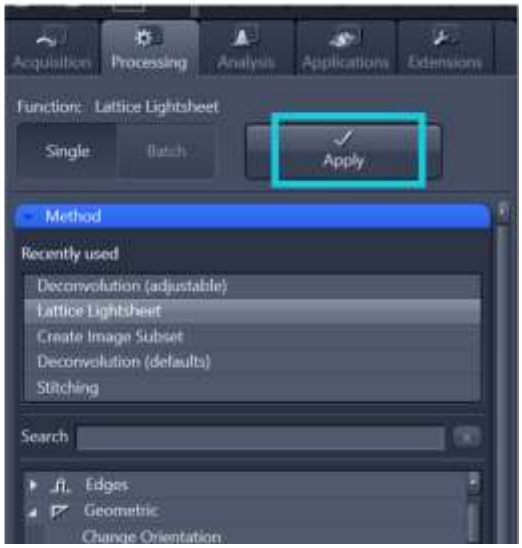
1. Go to the "**Processing**" tab. Select **Single**. Select **Lattice Lightsheet** in Method. Highlight the data you are going to process in the **Images and Documents**.





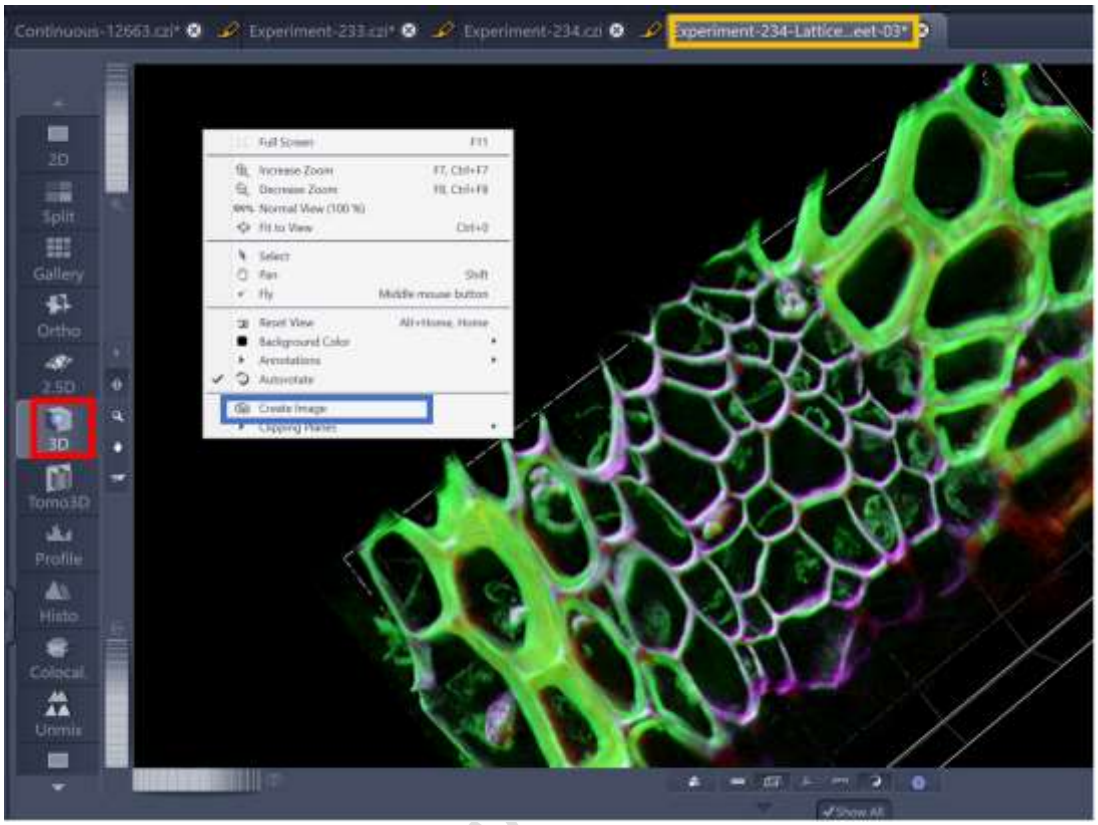
2. Check **Deconvolution** and **Deskew**. You could select the whole data or a part of the data to perform Lattice Lightsheet Processing. Click **Apply** to start. You can perform lattice Lightsheet Processing for the full dataset. Or you could select the channels, X-Positions or Region and click Apply for partial Lattice lightsheet processing.



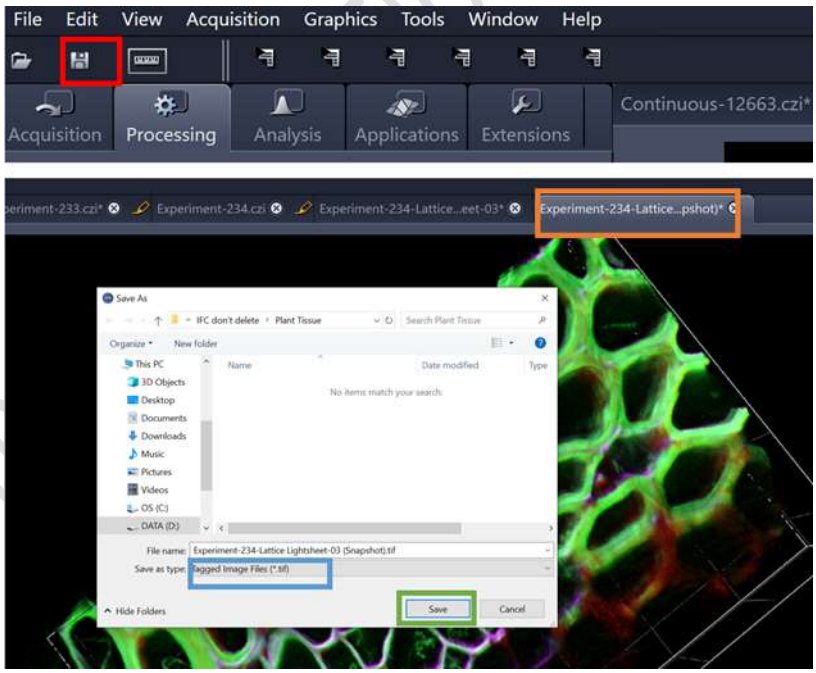


3. After lightsheet processing, processed data will be generated and displayed in the container. Select 3D in the processed data, a 3D render will be displayed in

the window. You may create snapshots of the render by right-clicking on the window and selecting Create Image.

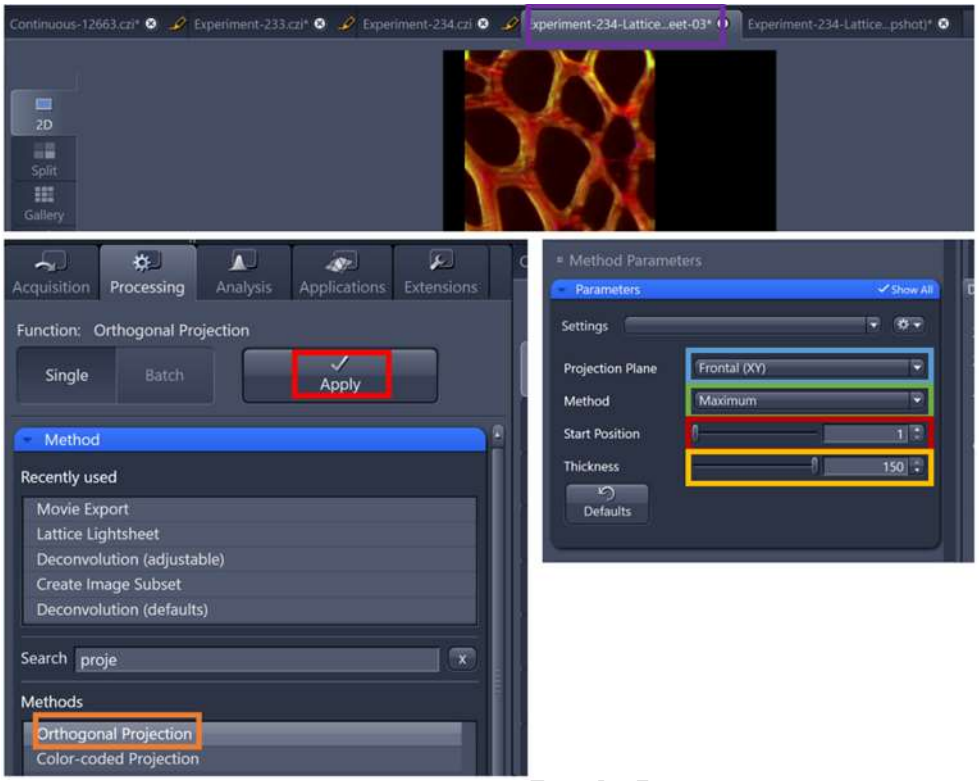


4. A snapshot of the 3D render will be generated, and it can be saved as a tiff.

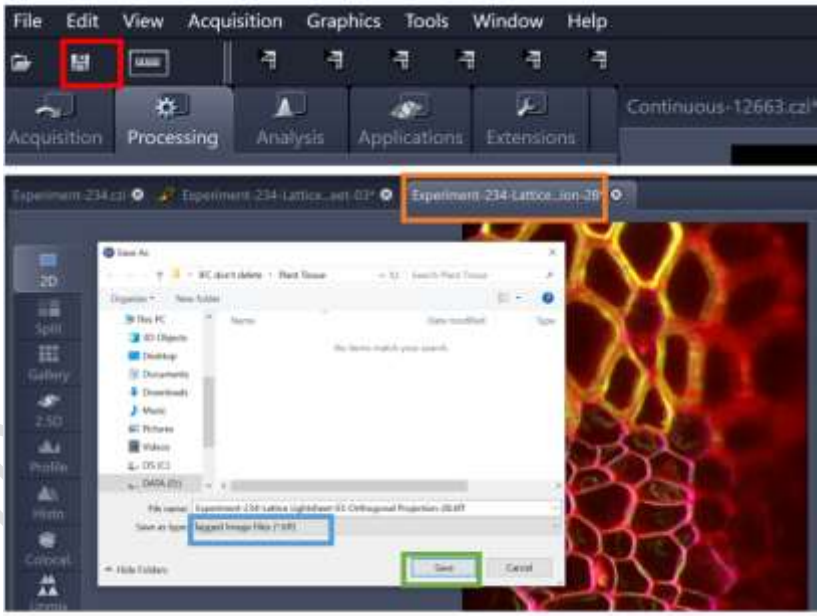


5. Go back to the Lattice Lightsheet processed data, intensity projection could also be done. Select Orthogonal Projection in the Methods and projection parameters

could be adjusted such as Projection Plane, Projection Method, Start Position, and Thickness. Click **Apply** to generate the projected data.

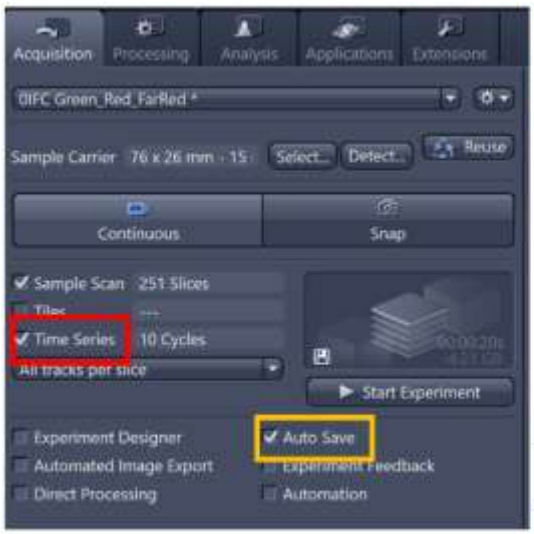


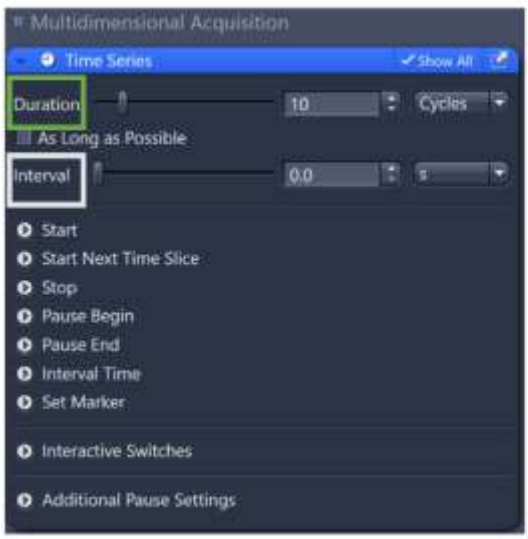
6. Save the projected data as tiff.



Time Lapse

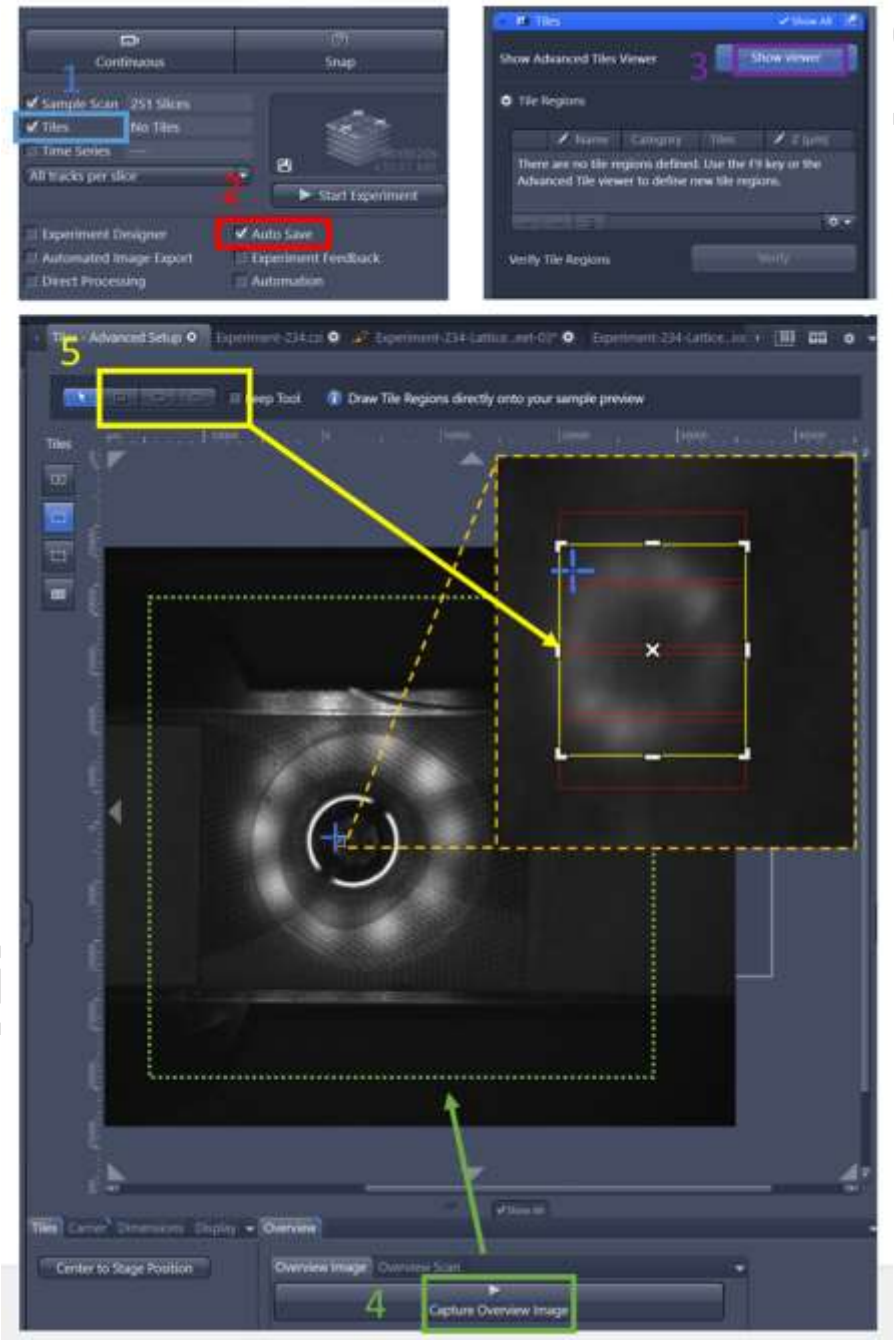
1. Tick **Time Series**, **Auto Save**, and **Auto Immersion***.
   *The auto immersion time interval is recommended to be set as 0.5h -1.5h, which should be based on the temperature and travel range for your imaging.
2. Set the Duration and Interval based on your experimental design.





Tile Scan

1. Tick **Tiles** and **Auto Save**. The Autosave path has been clarified in session 7.
2. Click **Show viewer** in the **Tiles** module, and the window **"Tiles – Advanced Setup"** window will be present.
3. Click **Capture Overview Image** and an overview of your sample carrier will be displayed in the **Tile-Advanced Setup** window.
4. Select an **ROI tool** to set regions of interest in the window.
5. Click **Start Experiment**. The system will start the tile scan.



6. Perform **Lattice Light Sheet Processing** firstly, subsequent processing including 3D reconstruction, stitching, and intensity projection could be further finished, which has been clarified in session **Data Processing**.

Shutdown System

Steps 1-3 are only applicable for live cell imaging, please skip the steps 1-3 if they are not needed

1. (Optional) For users that used the live cell incubation system, turn off the valve that is labeled "**Lattice lightsheet**".
2. (Optional) For users that used the live cell incubation system, uncheck all the options in the **Temperature** and **Atmosphere** in ZEN software.
3. (Optional) For users that used the live cell incubation system, turn off the gas controller (switch 5) and temperature controller (switch 6).

Steps 4-6 are applicable for all users in office hours

4. Transfer your data to your data transfer server.
5. Log off your PPMS tracker. Record the logbook.
6. !!! Don't exit ZEN software
   !!! Don't shut down the computer
   !!! Don't turn off the lattice lightsheet microscope.
   Technical staff will shut down the system at the end of the day.

Steps 7 is only applicable for experienced users during non-office hour.

7. For experienced user who need to shutdown the system during non office hour, please exit ZEN software, shut down the PC. After the PC is off, follow the sequence to shut down the system 1—2---3.