Ver 2.1 Mar 2026

LiTone XL light-sheet microscope Standard Operation Protocol

CPOS IMAGING AND FLOW CYTOMETRY CORE

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Contents

1 System Power On

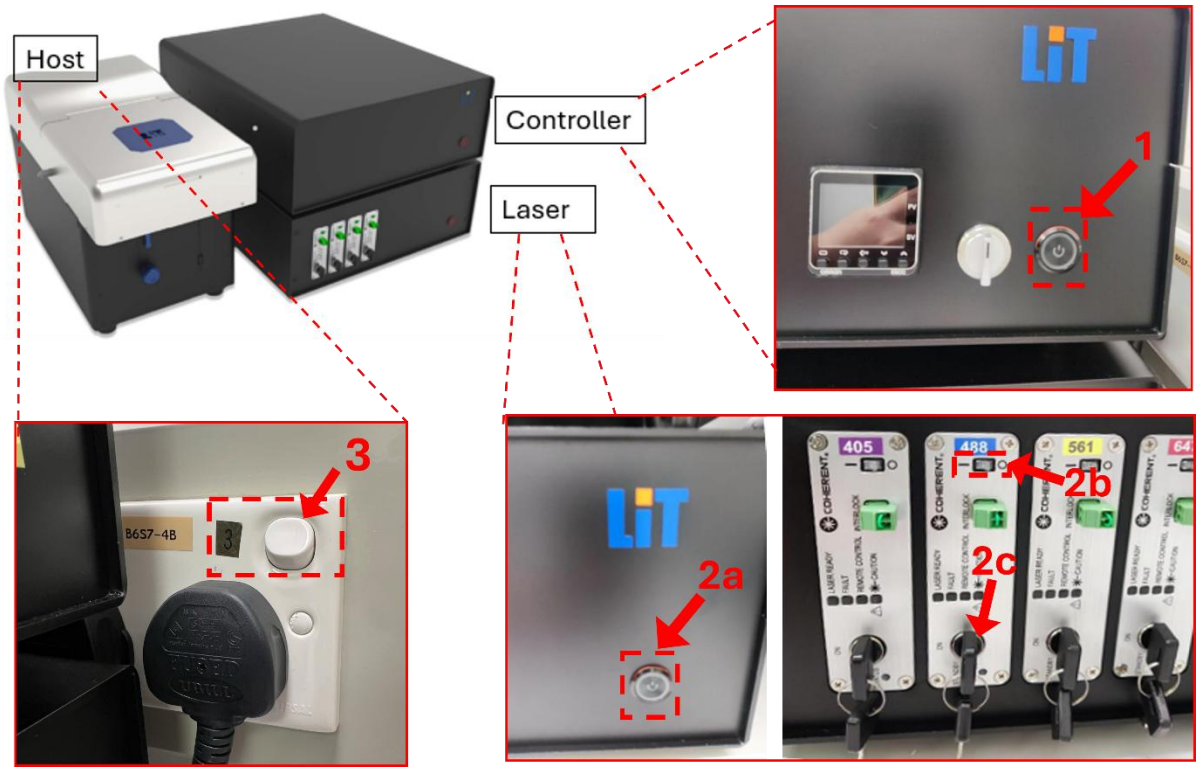
Follow the sequence to start LIT light-sheet scanning microscopy system:

1.1 Press the **power button (1) on the controller**.

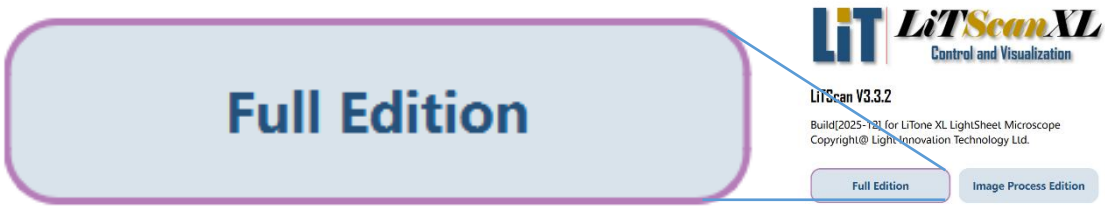
Note: Do **not** turn the key located beside the power button.

1.2 Press the **laser power button (2a)**.
Toggle the switch from **"o"** to **"—"** to initiate laser warm-up **(2b)**.
Wait until the indicator light stabilizes and stops blinking, then turn the corresponding key from **"Standby"** (vertical position) to **"On"** (**horizontal** position) **(2c)**.

1.3 Turn on the **camera unit (Host)** using the switch located at the side of the Controller and Laser units **(3)**.



1.4 Power on the **computer** and log in using the "User" account.
(The password is attached to the bottom of the monitor.)
1.5 Log in to the **PPMS tracker** using your UID and the **6-digit verification code** from the Authenticator app.
1.6 Double-click to launch **LitScan (V3.3.2)** and select **"Full Edition"** to complete software initialization.



⚠ CAUTION
Do not insert the imaging chamber until the software has been fully started and initialised.

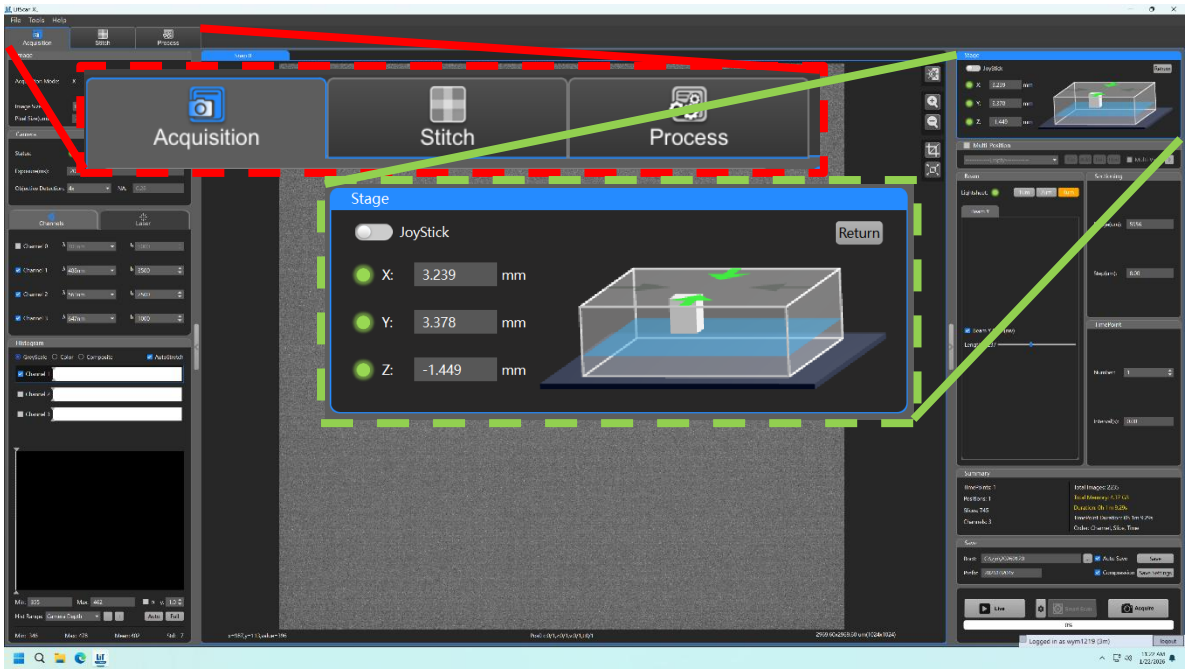
2 LitScan interface overview

2.1 Main Function Tabs

- **Acquisition** – Primary interface for setting imaging parameters and performing data acquisition.
- **Stitch** – Used for manual image stitching after acquisition.
- **Process** – Provides basic post-acquisition processing functions, including stitching, image flipping, and projection.

2.2 Stage Toolbox

- **Piezo-motorized Sample Stage Control Panel** – Controls precise movement of the sample stage.
    - Ensure that all **X, Y, and Z coordinates are reset to 0** before inserting the imaging chamber.



3 Optical path adjusting

3.1 Installation of the sample chamber

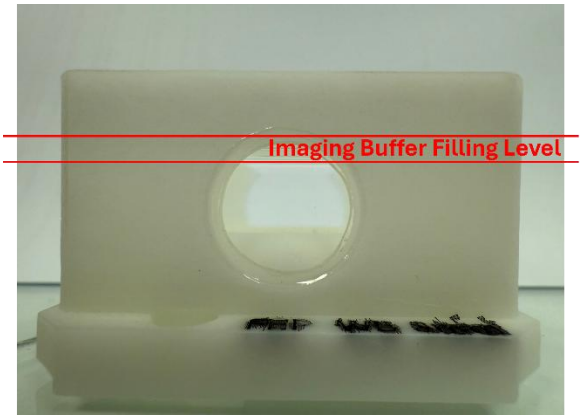
3.1.1 Users are strongly advised to specify the objective lens required for imaging at the time of booking. Changing of objectives will only be carried out by CPOS technical staff.

3.1.2 Select an imaging chamber that is compatible with the chosen objective lens. (Refer to the chamber selection table below, which is based on the objective lens and the immersion buffer in use.)

i. Measure the thickness of your sample and select a chamber with a working distance longer than the sample thickness.
ii. Ensure the chamber is compatible with the intended objective lens.
iii. Choose a chamber that is dedicated to the immersion buffer being used.

Chamber type	Chamber structure	Compatible Objective	Chamber W.D.	Immer^{n} buffer
Olympus 4x Air	**3D-printed polymer chamber with lens**	**Olympus 4x 0.28 N.A., 25 mm W.D.**	**12 mm**	**Aqueous**
Olympus 10x/25x	**Metal chamber with FEP film, magnetic alignment**	**Olympus 10x 0.6 N.A., 8 mm W.D.** **Olympus 25x 0.95 N.A., 8 mm W.D.** RI 1.33-1.40 **Olympus 25x 1.0 N.A., 8 mm W.D.** RI 1.41-1.52	**8 mm**	**Aqueous**
Nikon 16x	3D-printed polymer chamber with FEP film	Nikon 16x 0.8 N.A., 3 mm W.D.	3 mm	Aqueous
Nikon 16x O-Ring	3D-printed polymer chamber with O-ring	Nikon 16x 0.8 N.A., 3 mm W.D.	N/A	Aqueous
Olympus 4x Air	**Metal chamber with lens, magnetic alignment**	**Olympus 4x 0.28 N.A., 25 mm W.D.**	**12 mm**	**Organic**
Olympus 10x/25x	**Metal chamber with FEP film, magnetic alignment**	**Olympus 10x 0.6 N.A., 8 mm W.D.** **Olympus 25x 0.95 N.A., 8 mm W.D.** RI 1.33-1.40 **Olympus 25x 1.0 N.A., 8 mm W.D.** RI 1.41-1.52	**8 mm**	**Organic**

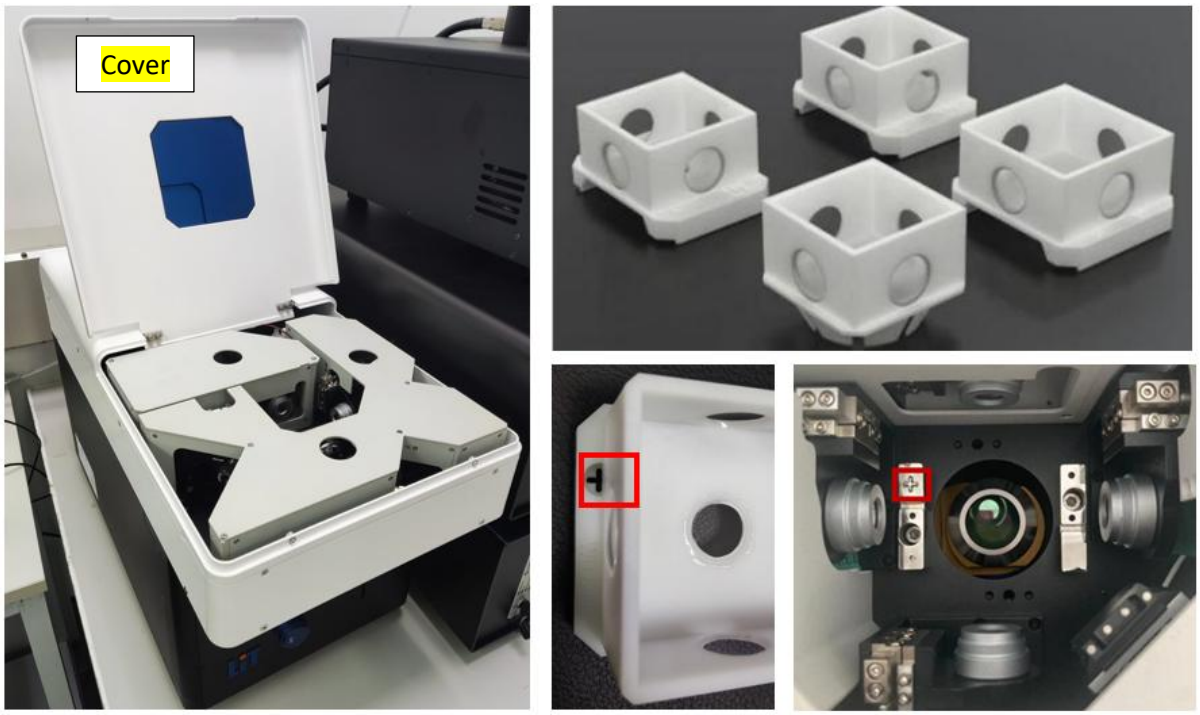
3.1.3 Add imaging buffer into the chamber to the level covering the top edge of all the lens on each side of the chamber.



3.1.4 Open the host cover. Holding the chamber by pinching its four corners, carefully place it into the host, align the cross "+" socket of the chamber with the metal cross protrusion in the instrument (the position highlighted in red), then gently press to ensure a secure fit. Close the cover of the host.

⚠ CAUTION

Spillage of liquid onto optical or electronic components may damage the equipment. If any imaging liquid is spilled, please contact CPOS staff immediately.

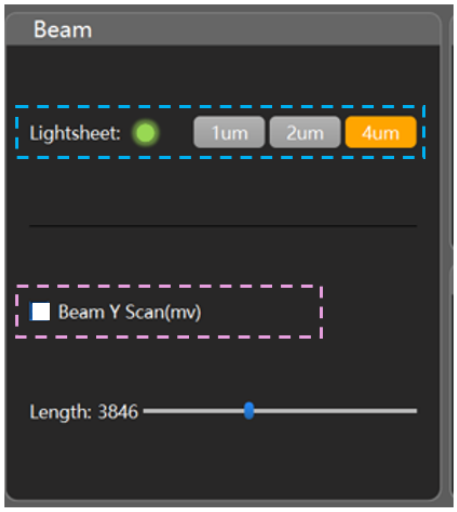


3.2 Adjust Optical path

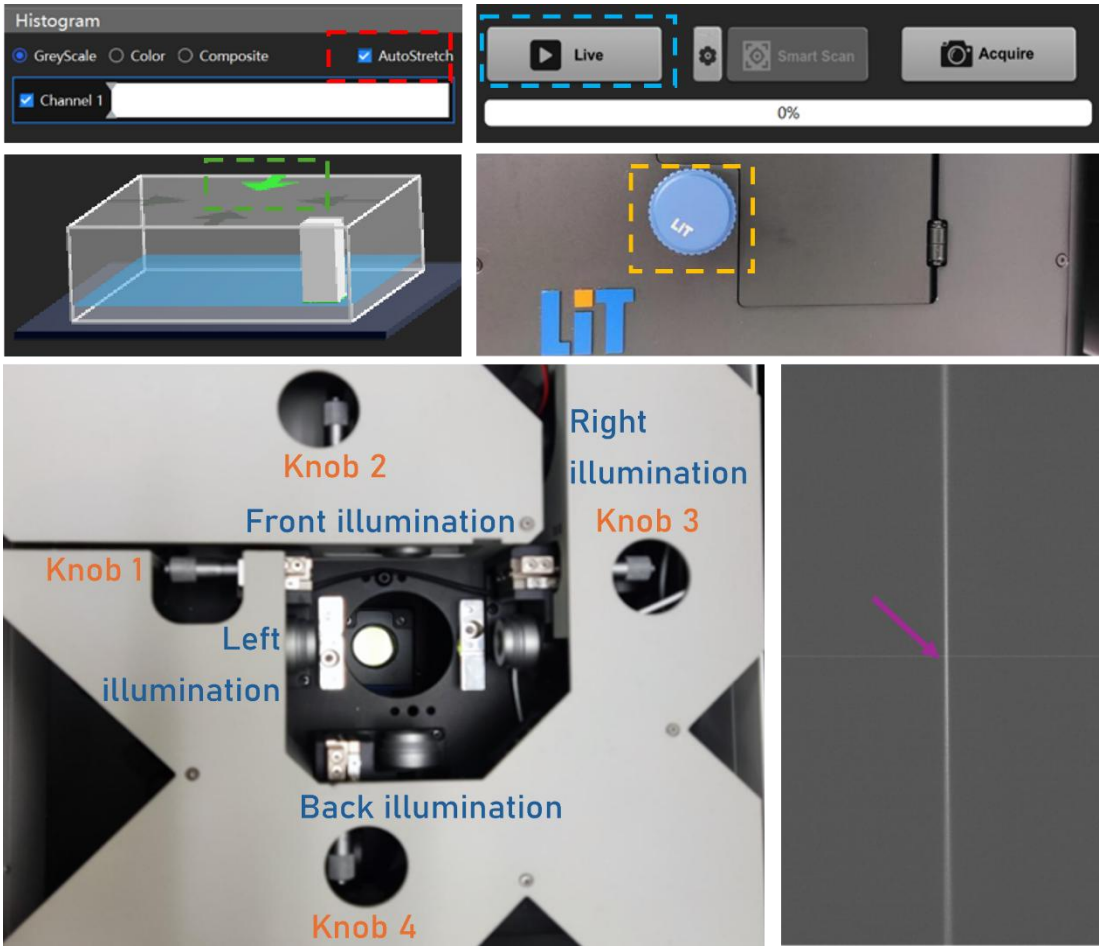
3.2.1 On LitScan V3.3.2, **select your objective lens magnification** and adjust the **exposure time to 50ms** in the Camera bar. Select **the thickness of the light sheet** (4X: 4µm; 10x / 16X: 2µm; 25x: 1µm) according to the objective installed. **Uncheck Beam Y Scan**. **Check the 488nm laser, adjust the laser intensity to 5000**.

Imaging parameter recommendation

Objective Magnification	Excitation Module	Lightsheet thickness	Step size	Crop Area
4x	4x	4 um	5-10 um	80% x 80%
10x	4x	2 um	2-3 um	80% x 60%
10x	10x	4 um	2-3 um	80% x 60%
16x	4x	2 um	2-3 um	80% x 50%
16x	10x	4 um	2-3 um	80% x 80%
16x	10x	2 um	1-2 um	80% x 50%
25x	10x	4 um	3-4 um	80% x 80%
25x	10x	2 um	2-3 um	80% x 50%
25x	10x	1 um	1-2 um	80% x 40%



2.2.2 Check "**AutoStretch**" to adjust the contrast, turn on the **front illumination** in software and click "**Live**". Adjust the **focusing knob** so that the **beam** in the live window appears the finest. Adjust the **knob 2** so that the beam waist is in the center, then click **Stop Live**.

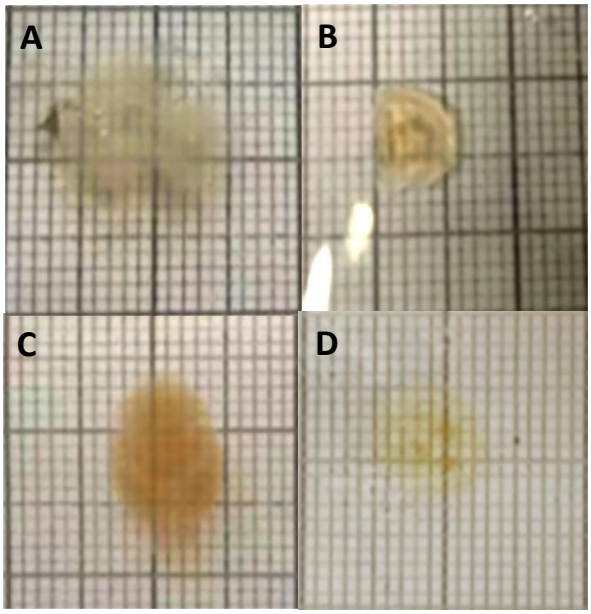


3. Sample loading

3.1 Examine the transparency and refractive index matching the sample

i. Transfer the cleared sample onto a petri dish and totally immerse the sample in imaging buffer.

ii. Place the petri dish containing the sample on the grid paper to examine the transparency and refractive index matching the sample. For optimal imaging result, the sample must be **completely transparent** (grid lines clearly visible) with **matched refractive index** (grid lines undistorted).

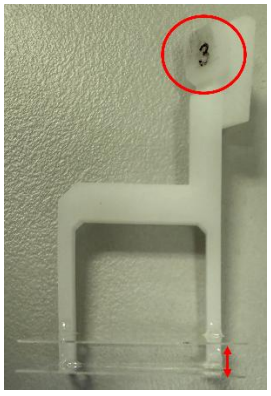


Sample after clearing:
Sample D is ideal

A. Opaque interior.
B. Unmatched refractive index.
C. Transparent sample pigment deposition.
D. High clarity sample with little to no pigment aggregation.

3.2 Choose sample holder

Estimate the size of your specimen according using the grid paper and select a proper size holder for your sample (it is recommended to choose a holder **0.5 mm smaller** than the thickness of the sample to avoid sample sliding during imaging).



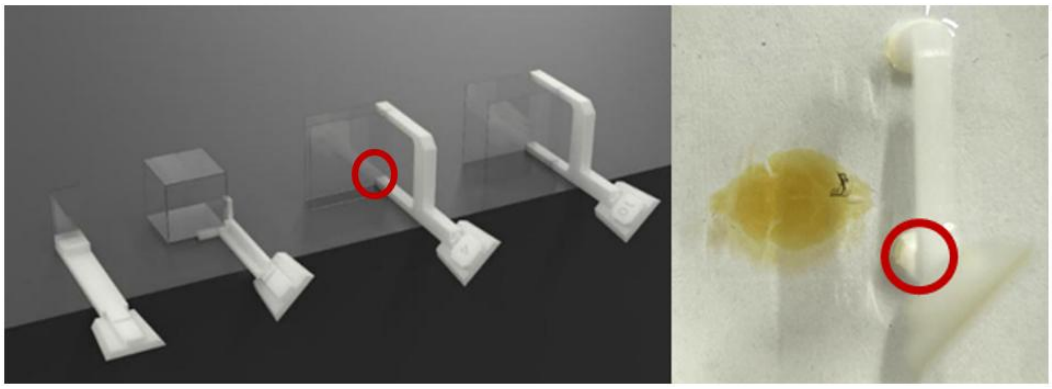
Holder size is indicated on the mounting arm of the holder.(**Sample holders are available for order through PPMS**)

Sample holder size:

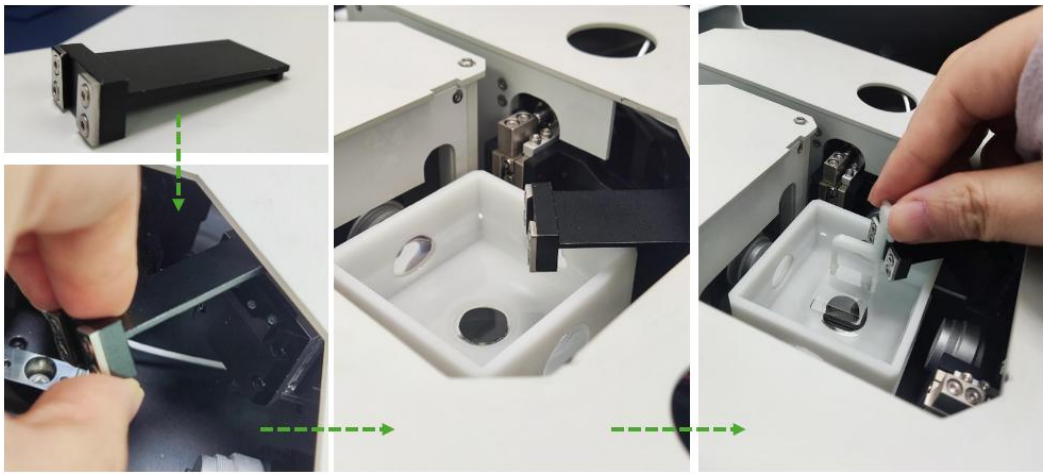
0.5 mm	1 mm	1.5 mm	2 mm	2.5 mm	3 mm
3.5 mm	4 mm	4.5 mm	5mm	5.5 mm	6 mm

3.3 Sample loading

i. Place the sample close to the lower right corner of the holder (red circle position) but beware of the pillar of the holder blocking the laser. For maximal optical penetration, align the long axis of the sample perpendicular to the light path.

ii. 

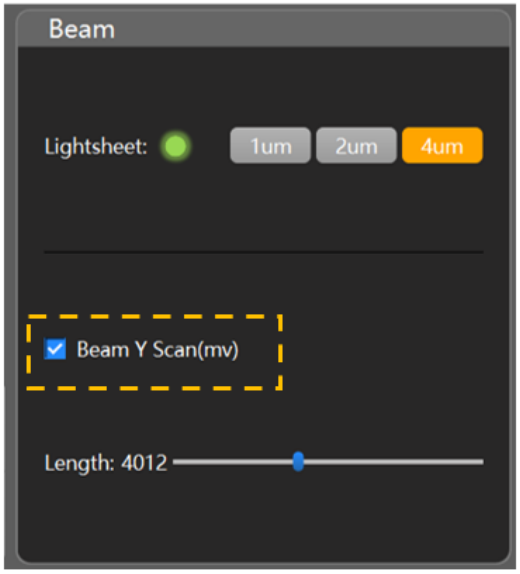
iii. Mount the **cantilever** to the sample stage (magnetically fit) and insert the sample holder to the cantilever slot from the top. (Spilling liquid on optical or electronic components can damage the equipment. If any imaging liquid is spilled, please contact CPOS staff immediately.).

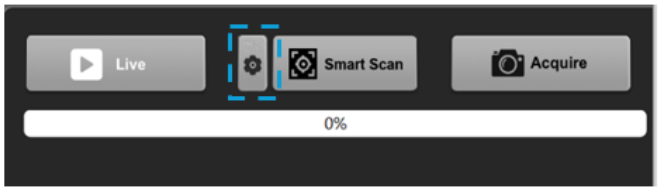


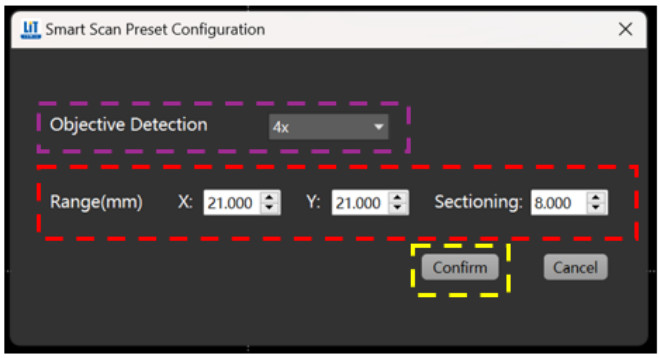
4. Sample Smart Scan

To initiate sample imaging, it is recommended to do a sample Smart Scan to quickly locate and determine the imaging dimensions.

4.1 Check "**Beam Y Scan(mv)**". Click the  **icon** to initiate the Smart Scan mode for the sample. In the pop-up options box, after **confirming the objective lens** magnification, keep the **XY range as suggested and 8mm in Sectioning** (which should cover the whole range of your sample carrier). Otherwise, you can also estimate the sample dimensions (mm), including the length (X) and width (Y) in the horizontal plane and the axial thickness. E.g. for a whole mosue brain (~4 mm x 6 mm x 4 mm) Click "**Confirm**".

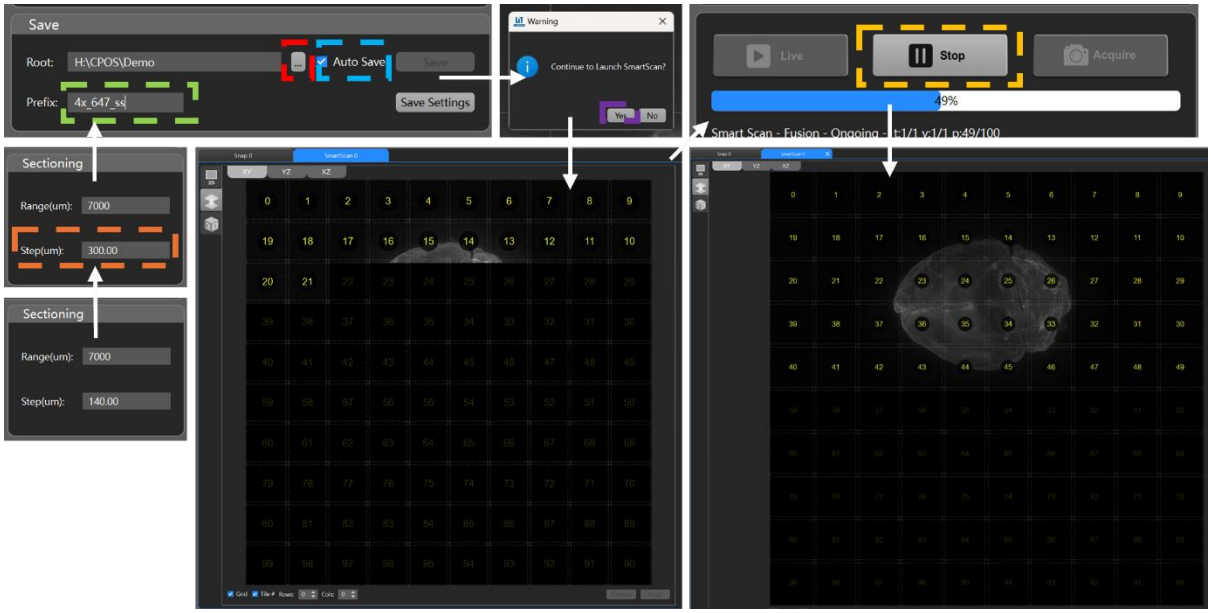






4.2 Select the **storage drive** and check "**Auto Save**" if you want to save the preview scan (optional) and **name the folder**.

Click on the "Smart Scan" button and "**Yes**" to begin the sample Smart Scan. The starting position of the Smart Scan is the corner closest to the sample holder arm. The smart scan default XY dimensions cover the whole holder area. You can stop the smart scan at any point during the scanning process. **Stop** the scanning process when you can see the whole sample that you when to image.



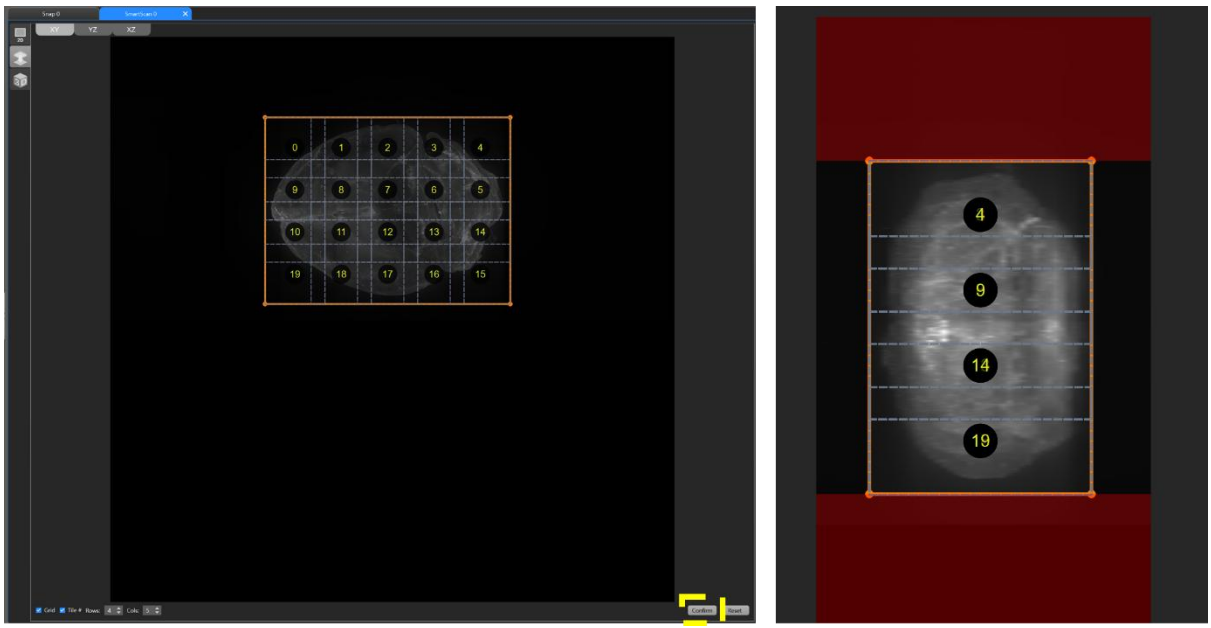
5. Set up imaging protocol

5.1 After Smart Scan, the 3D preview of the sample is displayed in XY; XZ and YZ projection views.

Select XY projection plane to preview the sample to be scanned. Drag and select the area covering the desired scanning area.

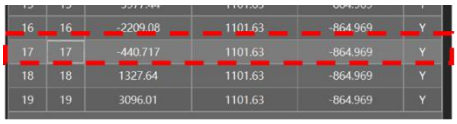
Click on XZ or YZ projection plane to preview the thickness of the sample. Drag and select the depth of the sample.

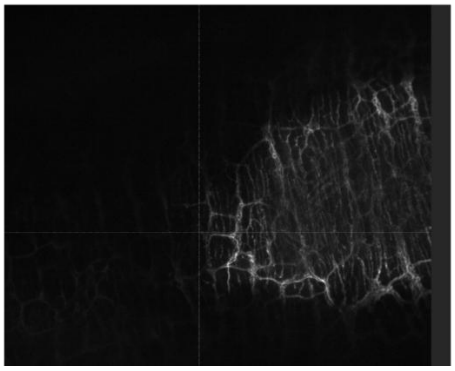
Confirm the scanning volume to update the multi-view grid selection.

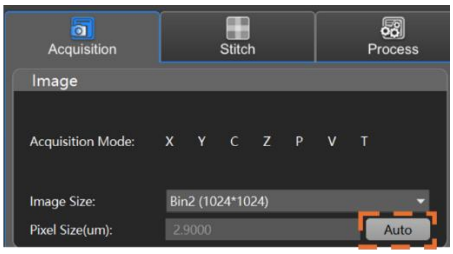


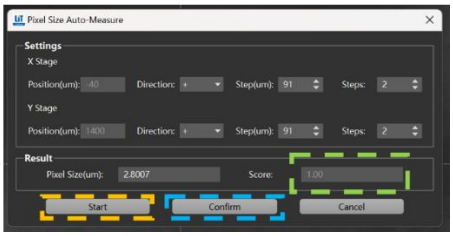
5.2 Auto pixel correction

Go to multi-view and **select one of the views** then click “Go”, “Live” for focusing. When the view is properly focused, select **Auto** from the Pixel Size setting. Click **Start** on the pop-out window, the system will calibrate for the optimal pixel size. When the calibration finish, check the adjusted pixel size, which should not be altered drastically. Check the **Score** on the right, it is ideal to have a score close to 1. Apply the adjusted pixel size by clicking **Confirm**.



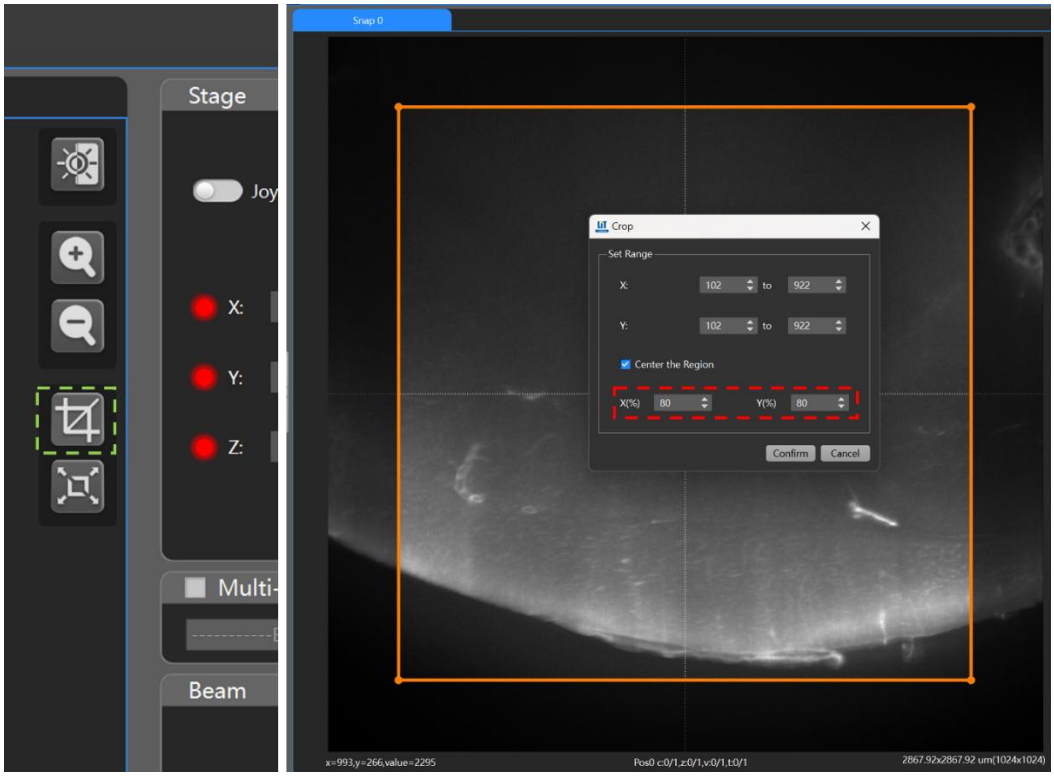






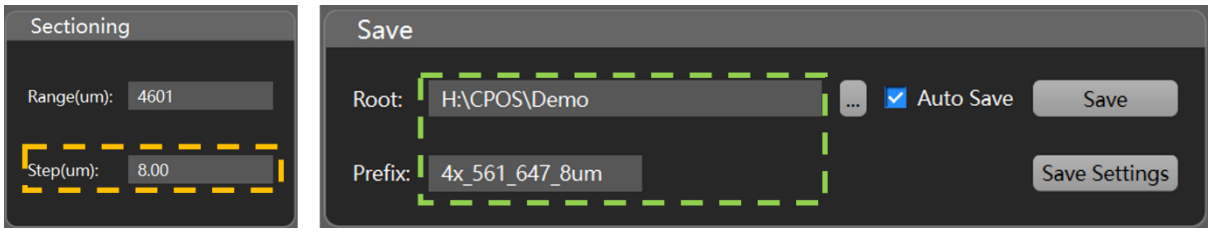
5.3 Crop factor adjustment

To crop the field of view for optimal resolution, click on the crop icon on the Snap 0 tab. Crop in the FOV to the area with best focus within the lightsheet. As a reference, for front and back illumination, 4x objective, it is recommended to set it to X: 80%; Y: 80%. For 10x objective, it should be set to X: 70%; Y: 50%.



5.4 Update step size

While step size during Smart Scan is set to be 100-300um for fast scan speed, step size during data acquisition should be adjusted to provide optimal z resolution for 3D reconstruction. It is recommended to set the **step size** to 8um for 4x objective and 3um for 10x objective.



5.6 Update data storage folder and file name

Update the **data storage folder and file name** for the data acquisition.

5.5 Channel adjustment

1. Check the image size and **binning** (resolution) configuration.

   For Smart Scan, use 512*512

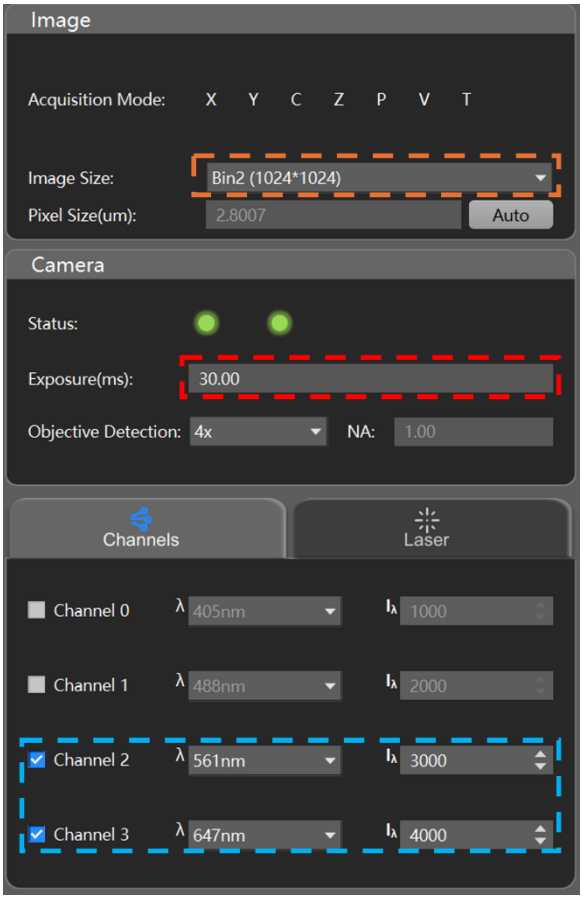
   For 4x and 10x objectives and moderate file size, use 1024*1024 (recommended)

   For maximum quality, 2048*2048

2. Select the **channel** for imaging and go LIVE to check the exposure of each channel.

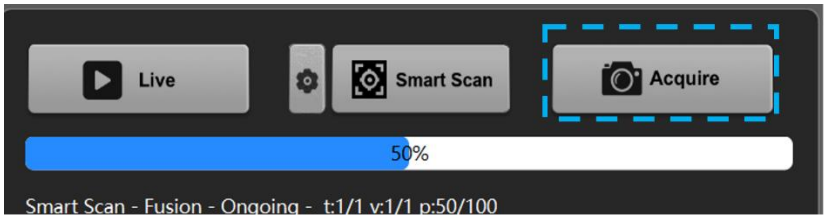
3. Set the **Exposure** time. Default is 20ms, increase to 30ms if the signal intensity is too low.

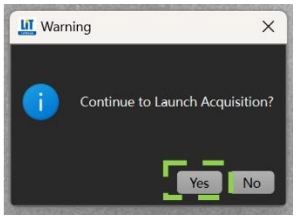
4. Adjust **Laser Intensity I_λ** to achieve a mean signal range between 5000-20000 or a maximum signal intensity ranging from 15000 to 30000.
MAX limit of I_λ is 2000



5.6 Data acquisition

When all imaging parameters are set, click **Acquire** and then **Yes** to start the image acquisition.


Live
Smart Scan
Acquire
50%
Smart Scan - Fusion - Ongoing - t:1/1 y:1/1 p:50/100


Warning
Continue to Launch Acquisition?
Yes
No

6. Image processing

6.1 Stitching of images

After image acquisition, the dataset should first be stitched together. To stitch the images, you may do it (a)directly on the LiTone XL workstation or you may do it (b) later on the LitScan workstation.

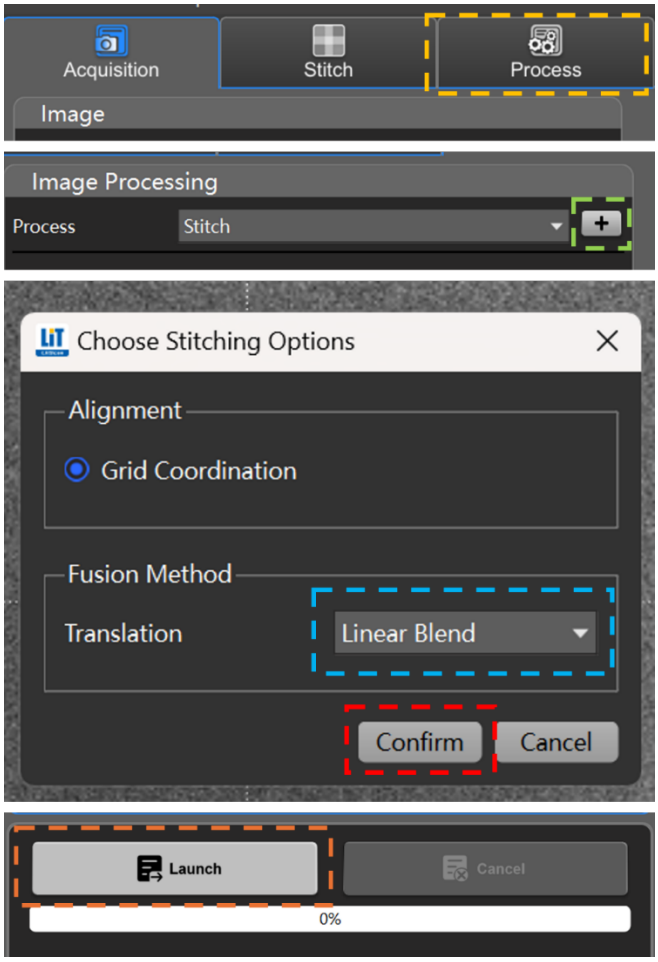
(a)To do the stitching directly after acquisition,

1. go to the top left-hand corner and select **Process**.

2.Select Stitch in the drop-down menu and click "+" to confirm.

3. In the popup window, select **Linear Blend** in the drop-down menu, then click **Confirm**.

4. Click **Launch** to start stitching.



6.2 Importing of data to LitScan on offline workstation

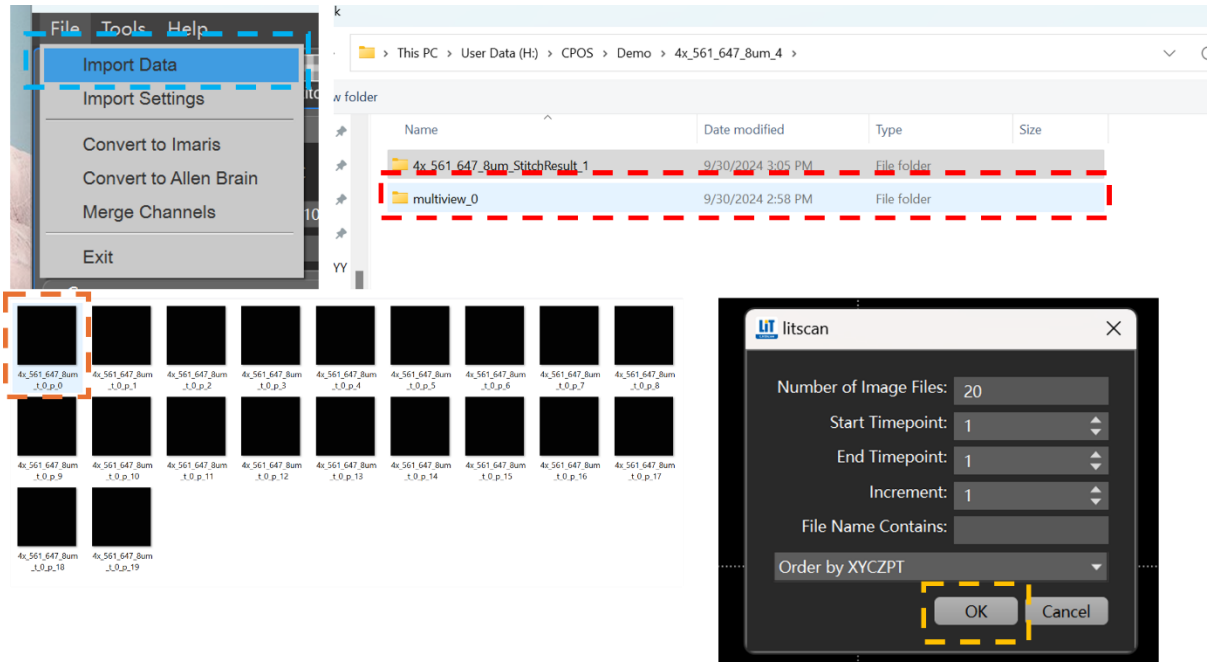
Alternatively, you can import the acquired dataset into the LitScan software on an offline workstation and perform stitching.

(b) Stitching on the offline workstation,

1. open the LitScan program. Select “File”, **Import Data**, the images of each FOV are saved in the folder name **multiview_0** under the dataset root folder.

2. Select the **first image** of the dataset and then click **OK** to start the import.

3. Continue with steps in 6.1 for the stitching procedures.



6.3 IMARIS export

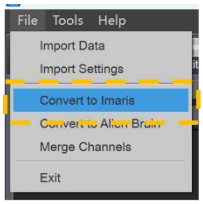
The stitched images can be exported as IMARIS compatible format for downstream analysis.

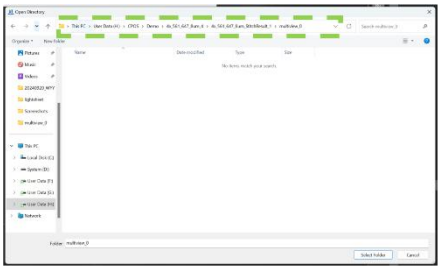
1. On the LitScan software, go to “File”, **Convert to Imaris**.

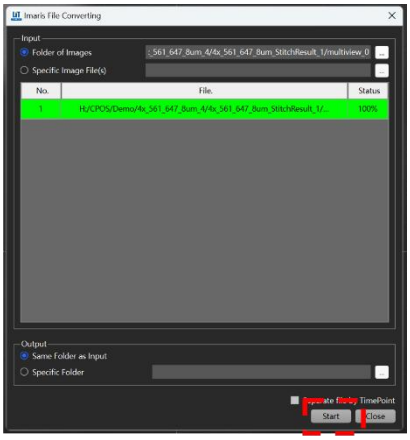
2. In the pop-up window, in “Input”, “Folder of images” click on the “**...**” button to pick the “**xxxx_StitchResult_xx/multiview_0**” to ”Select Folder”.

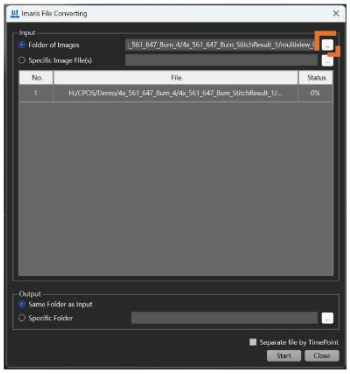
3. Click to **Start**. After the conversion, **the IMARIS file*** will be put in the newly created “Imaris” folder.

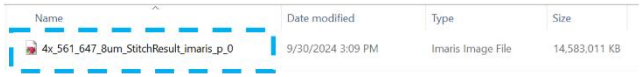
*You may open it with IMARIS viewer installed in both LiTone and Amira workstations or analyse it will IMARIS installed workstations in L6-11.

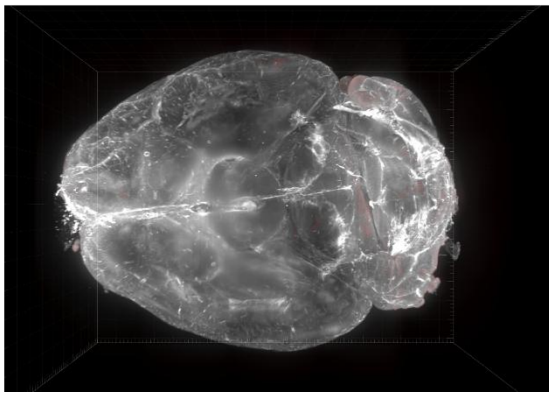










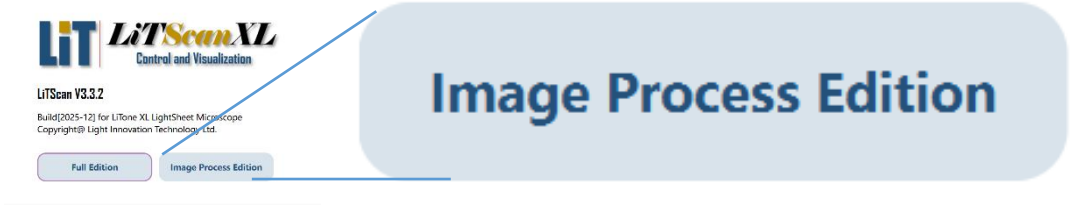


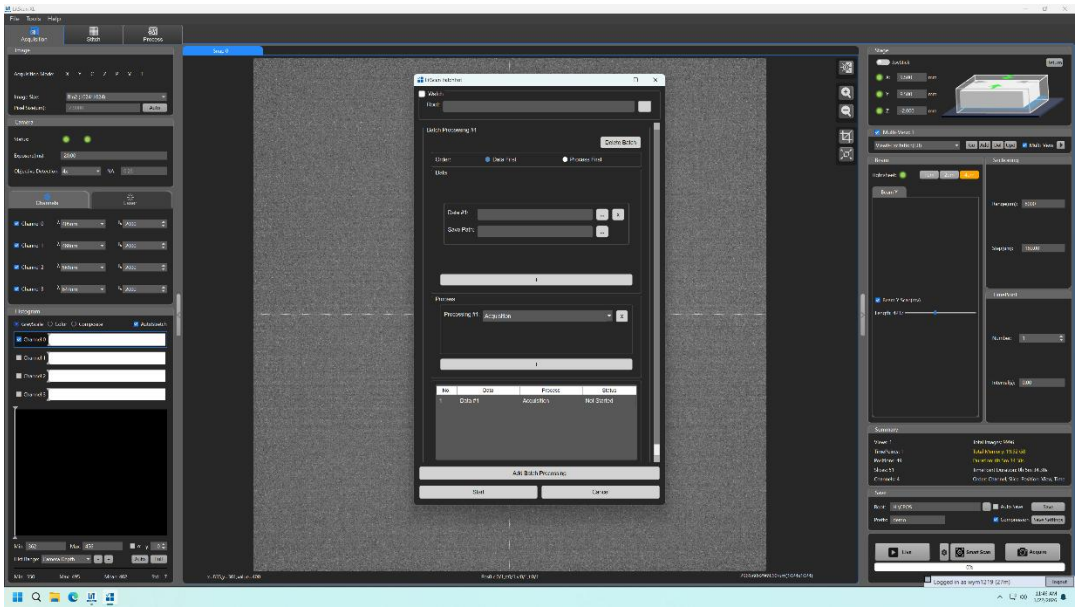
6.4 Batch processing

The stitching and subsequent IMARIS file conversion can be streamlined with the batch processing plugin tools of LitScan.

1. On the Desktop, open **LitScan**.

2. Select Image Process Edition. Wait till the software loaded, go back to Desktop and launch **LitScan_BatchBot**.





3. In the Data panel, go to ... designated Data #1 to indicate the path of your first set of data to be processed. Select the **mda_summary.json** of the dataset.

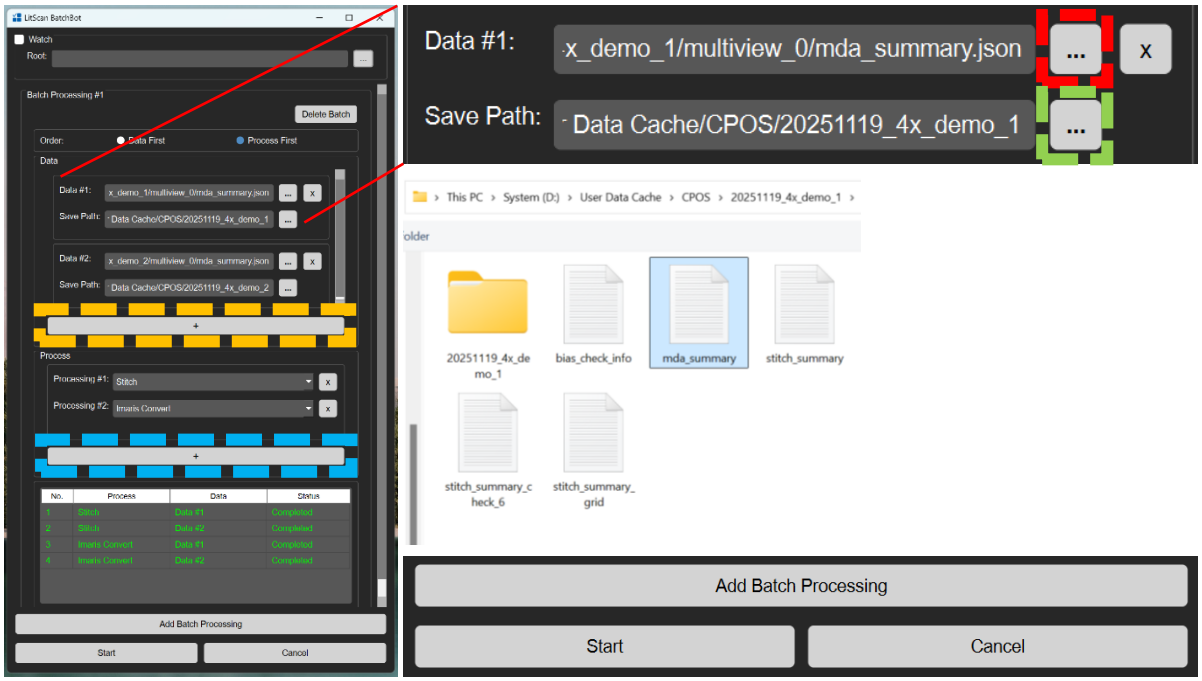
Select the save path of the output data by selecting ... in Save Path.

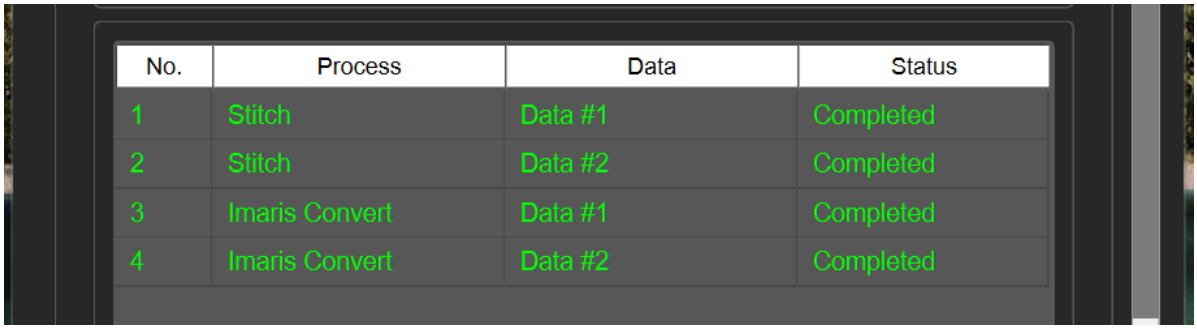
If you have more than one set of data to be processed, select + to add more data panel.

In the Process panel, select "**Stitch**" in the drop-down menu. Select + to add another process. Select "**Imaris convert**" in the select process.

Click "Start" to start the processing. During the process, don't click anything.

Wait until the whole processing progress to finish.





Batch processing finished.

7. Data transfer

While user data can be transferred through Data Transfer Server, the data size generated by LiTone XL is usually in the scale of tens of GB. As an alternative solution, we offered temporary SSD lending service to users for more efficient data extraction. Please contact our staff if you need one.

Borrowed SSD should be formatted and returned to L6-11 on the same date!