LiTone XL light-sheet microscope Standard Operation Protocol

CPOS IMAGING AND FLOW CYTOMETRY CORE

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Contents

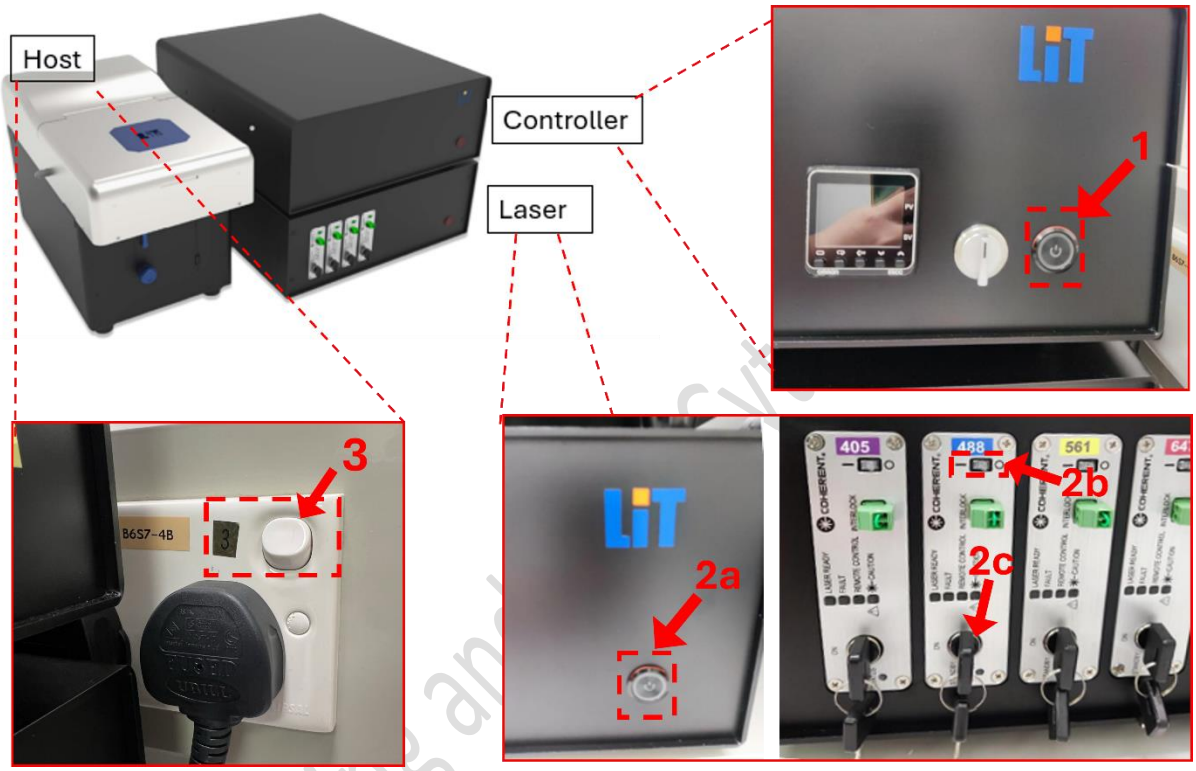
1. System Power On

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

1.1 Press the power button of controller (Caution: Don't switch the key beside the power button 1)

1.2 Press the laser power button (2a), toggle the switch from "o" to "—" to warm up the laser channel(s) (2b), wait for the light to stabilize and stop blinking, turn the corresponding key from "standby" to "on" (2c).

1.3 Toggle the switch of the camera unit at the back of the Host (3).



1.4 Turn on Computer. Log in user account with the password attached on the bottom of the monitor.

1.5 Log in PPMS tracker with your UID and 6 digit-code from Authenticator.

1.6 Double click to launch the software (LitScan XL 3.3.0) for initialization.

! CAUTION Do not insert the sample holder before starting the software.

2. Optical path adjusting

2.1. Installation of the sample chamber

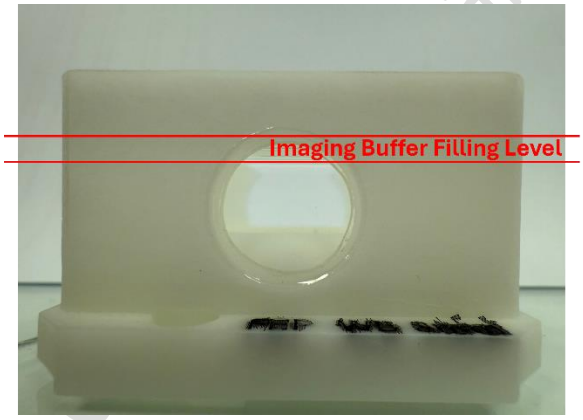
2.1.1 Objective choice for imaging should be indicated in the booking request. Any change of objective lens will only be performed by CPOS technical staff prior to the start of your booked sessions.

2.1.2 Select the sample chamber compatible to the objective lens. (The chamber selection sheet is based on objective lens and immersion buffer you are using.)

i. Measure the thickness of your sample and select the chamber with longer chamber working distance than your sample thickness.
ii. Select the chamber that is compatible with the objective intended.
iii. Pick the chamber dedicated to your immersion buffer property.

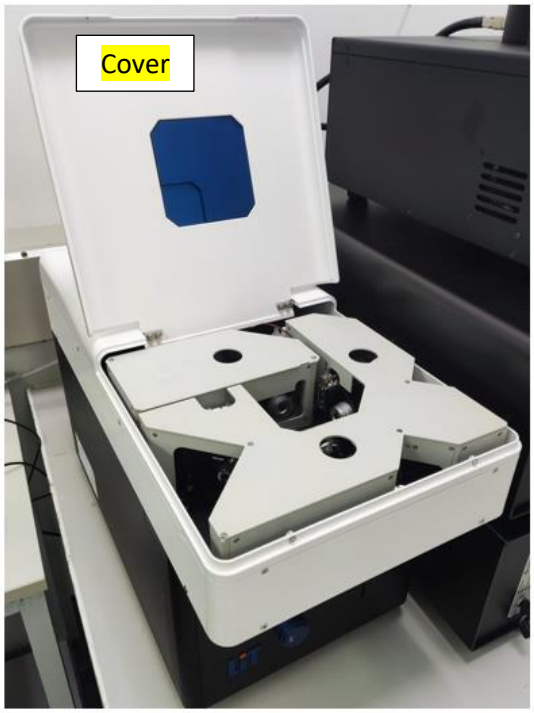
Chamber ID	Chamber structure	Compatible Objective	Chamber W.D.	Immer^{n} buffer
Olympus 4x Air	3D-printed polymer chamber with lens	Olympus 4x 0.28 N.A., 25 mm W.D.	6 mm	Aqueous
Olympus 4x Air	3D-printed polymer chamber with lens	Olympus 4x 0.28 N.A., 25 mm W.D.	8 mm	Aqueous
Olympus 4x Air	3D-printed polymer chamber with lens	Olympus 4x 0.28 N.A., 25 mm W.D.	10 mm	Aqueous
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	**3D-printed polymer chamber with FEP film**	**Olympus 10x 0.6 N.A., 8 mm W.D.** **Olympus 25x 0.95 N.A., 8 mm W.D.**	**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
Nikon 20x	**3D-printed polymer chamber with FEP film**	**Olympus 10x 0.6 N.A., 8 mm W.D.** **Olympus 25x 0.95 N.A., 8 mm W.D.**	**8 mm**	**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 4x 0.28 N.A., 25 mm W.D.** **Olympus 10x 0.6 N.A., 8 mm W.D.** **Olympus 25x 1.0 N.A., 8 mm W.D.**	**8 mm**	**Organic**

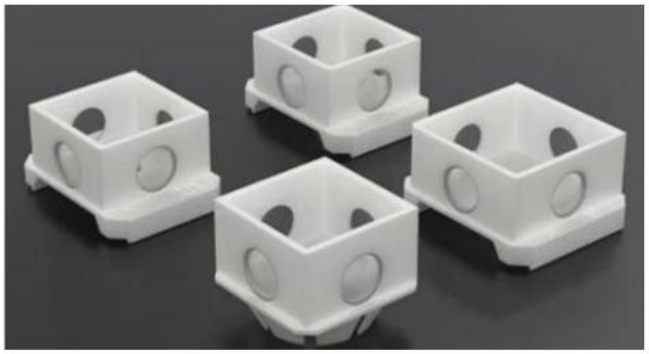
2.1.3 Add imaging buffer into the chamber to the **level** covering the top edge of all the lens on each side of the chamber.

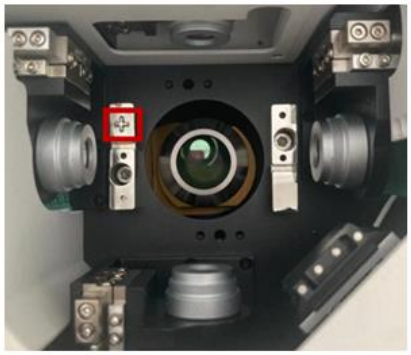


2.1.4 Open the cover of the host. Pinch the four corners of the chamber with your fingers, carefully put it into the host, align the cross "+" socket of the chamber with the metal cross protrusion in the instrument (the position highlighted in red), gently press to ensure a tight fit, and close the cover of the host.

! CAUTION Spilling liquid on optical or electronic components can damage the equipment. If any imaging liquid is spilled, please contact CPOS staff immediately.

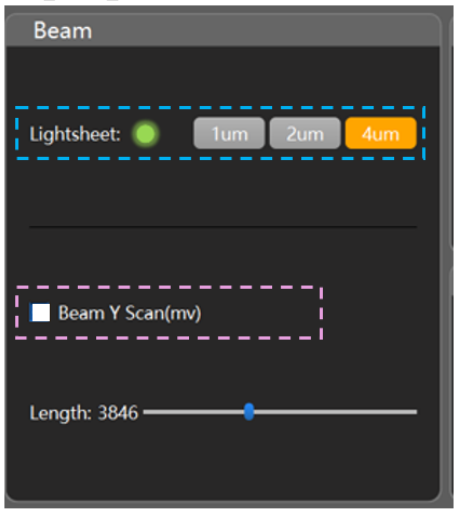




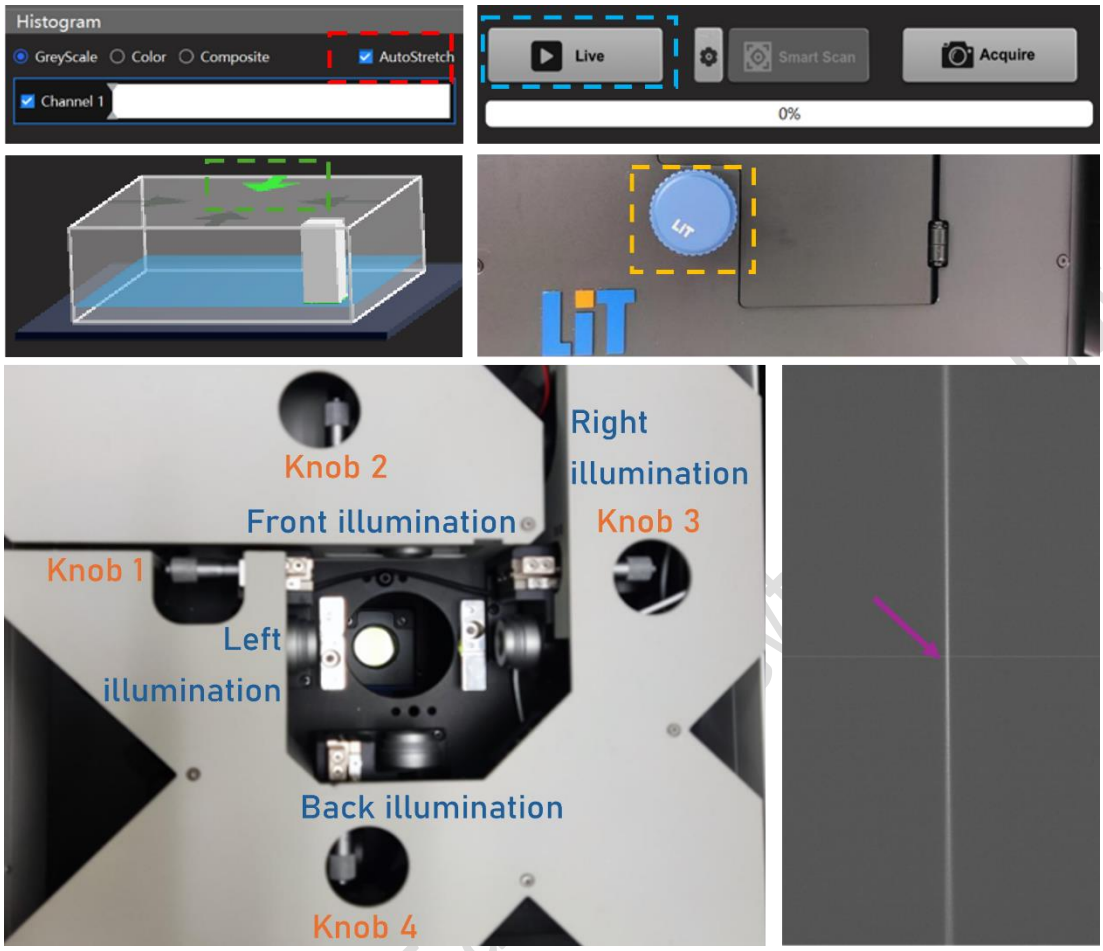


2.2. Adjust Optical path

2.2.1 On LitScan XL 3.3.0, **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**.



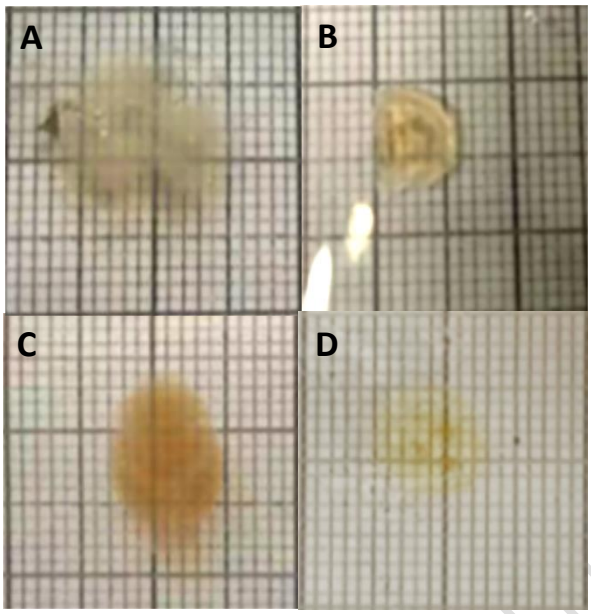
2.2.2 Check "**AutoStretch**" to adjust the contrast, turn on the **front illumination** in software and click "**Live**". Adjust the **focusing knob** so that the **beam** in the live window appears the finest. Adjust the **knob 2** so that the beam waist is in the center, then click **Stop Live**.



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).

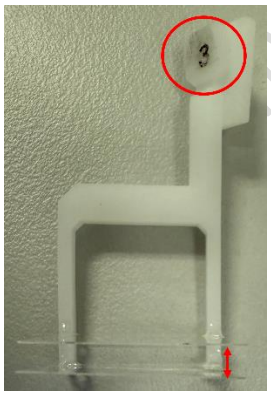


Sample after clearing:
Sample D is ideal

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).



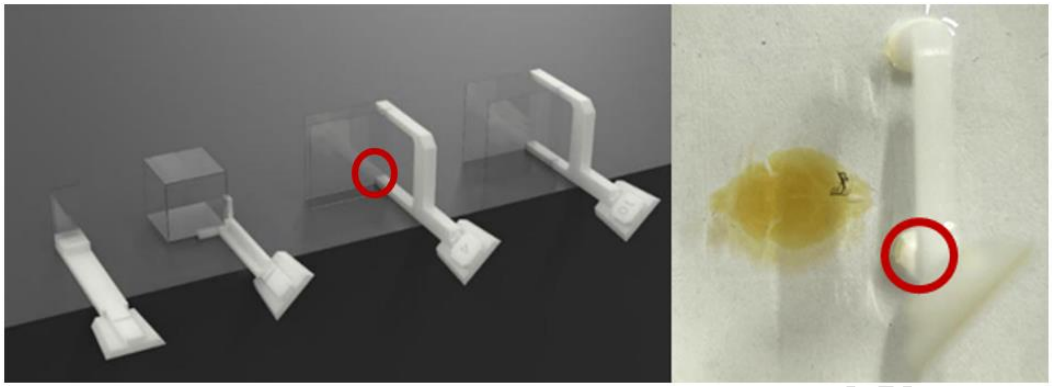
Holder size is indicated on the mounting arm of the holder.(**Sample holders are available for order through PPMS**)

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

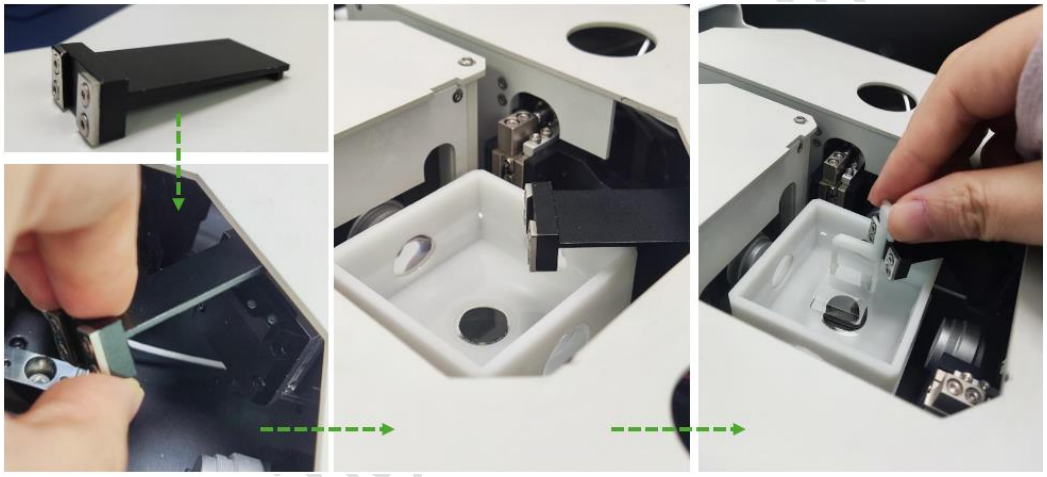
3.3 Sample loading

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.

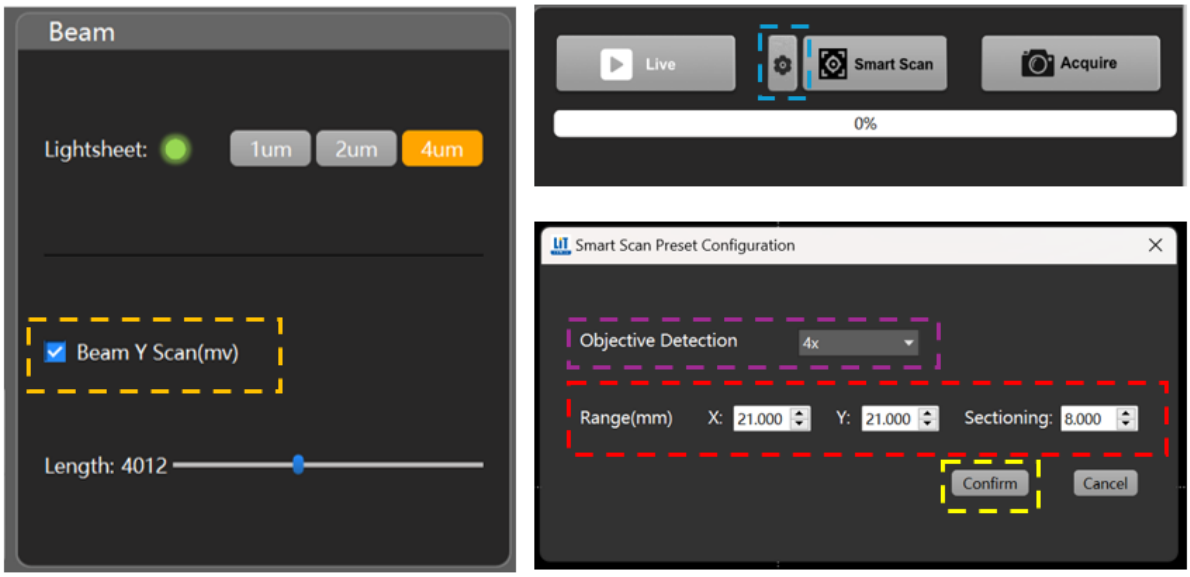
iii. Mount the **cantilever** to the sample stage (magnetically fit) and insert the sample holder to the cantilever slot from the top. (Spilling liquid on optical or electronic components can damage the equipment. If any imaging liquid is spilled, please contact CPOS staff immediately.).



4. Sample Smart Scan

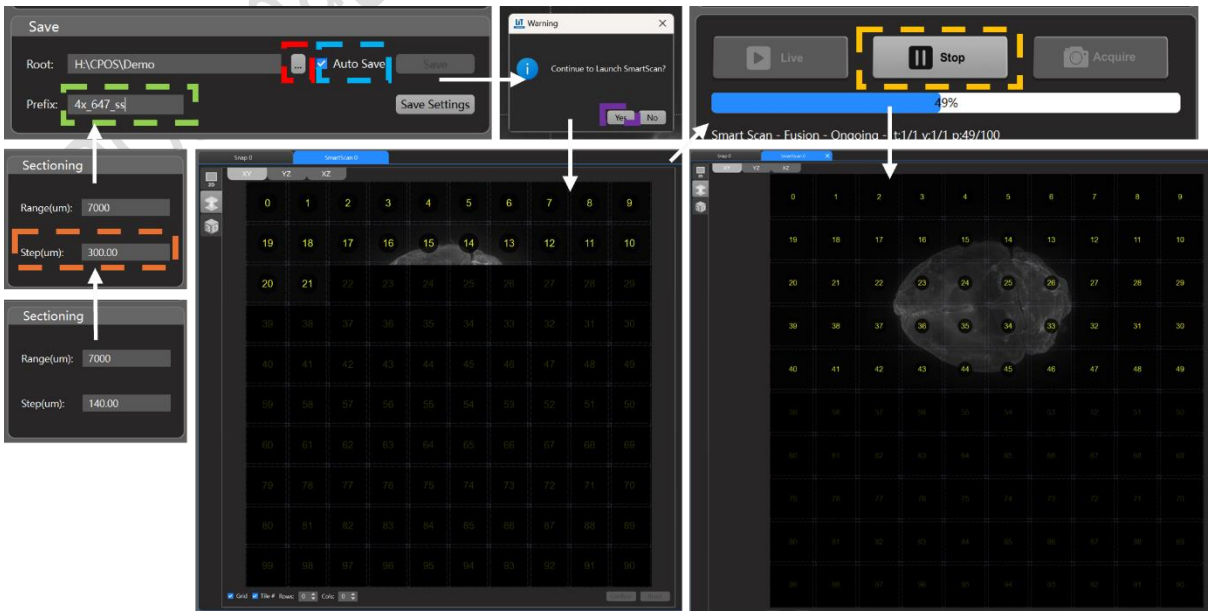
To initiate sample imaging, it is recommended to do a sample Smart Scan to quickly locate and determine the imaging dimensions.

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**" 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.



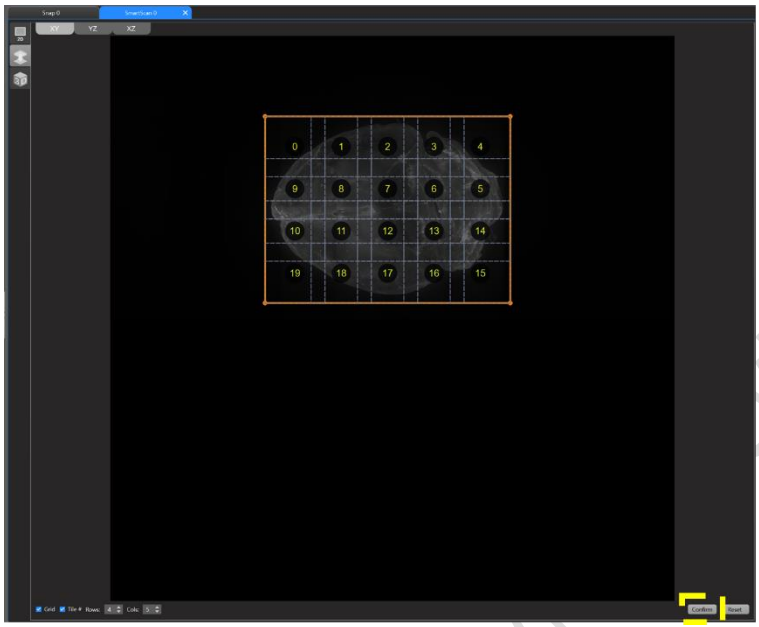
5. Set up imaging protocol

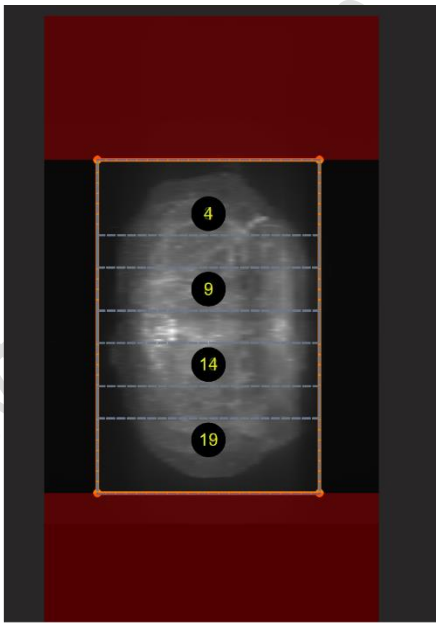
5.1 After Smart Scan, the 3D preview of the sample is displayed in XY; XZ and YZ projection views.

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.

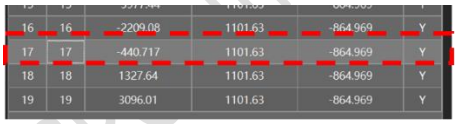
Confirm the scanning volume to update the multi-view grid selection.

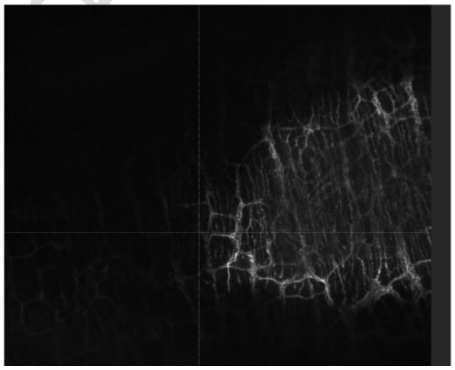


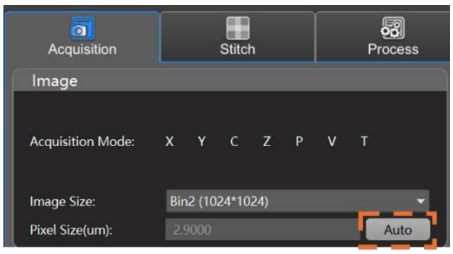


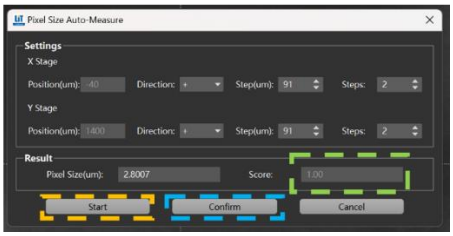
5.2 Auto pixel correction

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**.



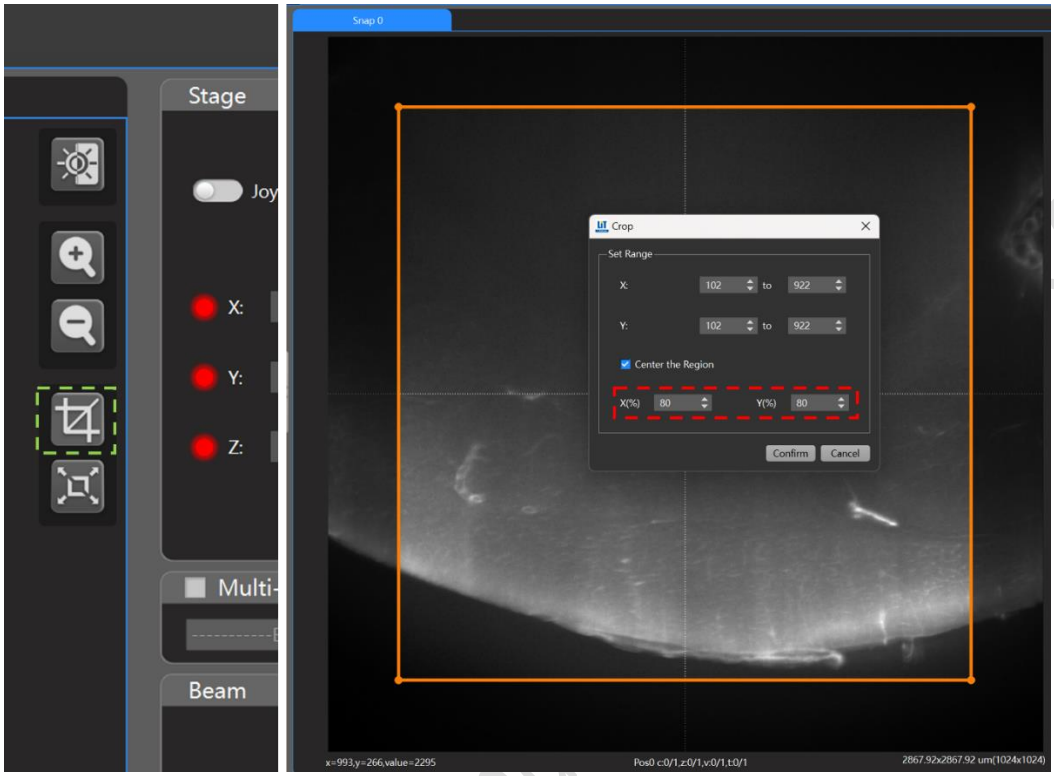






5.3 Crop factor adjustment

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%.

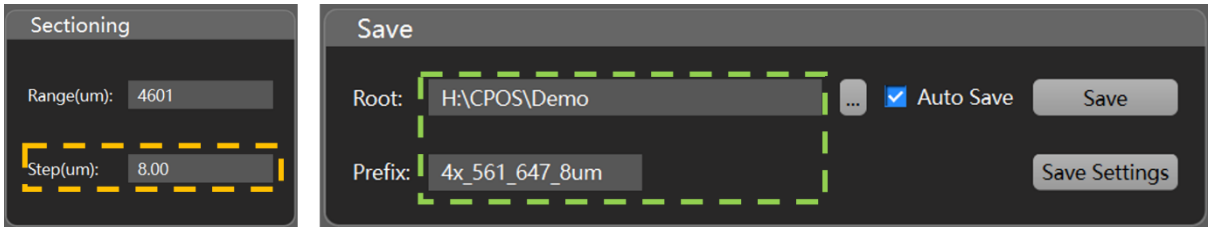


5.4 Update step size

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.

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%



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

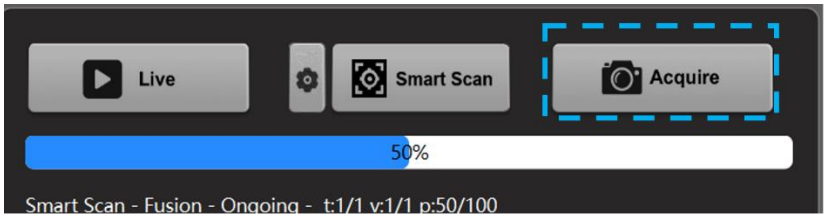
2. Select the **channel** for imaging and go LIVE to check the exposure of each channel.

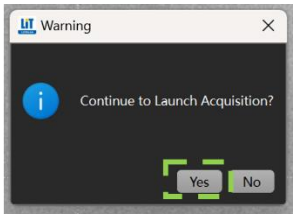
3. Set the **Exposure** time. Default is 20ms, increase to 30ms if the signal intensity is too low.

4. Adjust **Laser Intensity** to achieve a mean signal range between 5000-20000 or a maximum signal intensity ranging from 15000 to 30000.

5.6 Data acquisition

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





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.

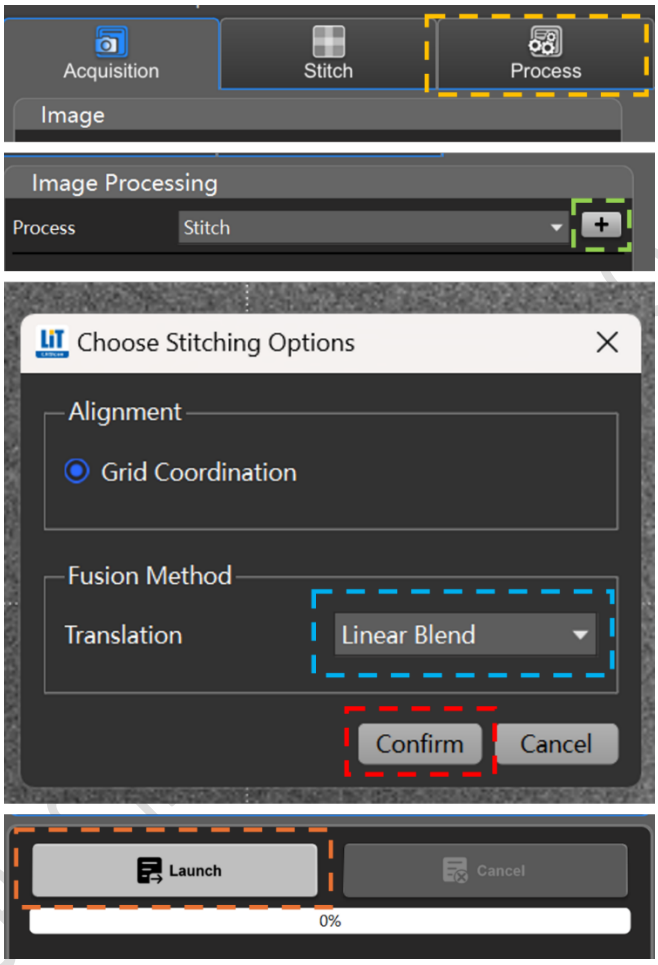
(a)To do the stitching directly after acquisition,

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

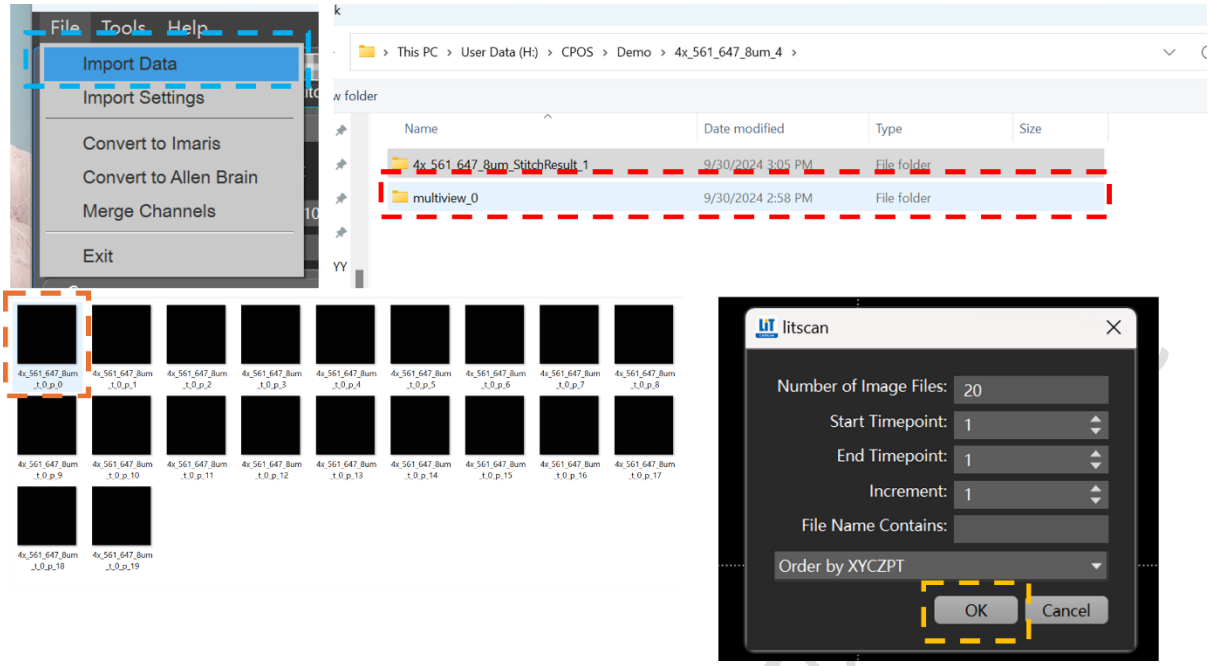
Alternatively, you can import the acquired dataset into the LitScan software on an offline workstation and perform stitching.

(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

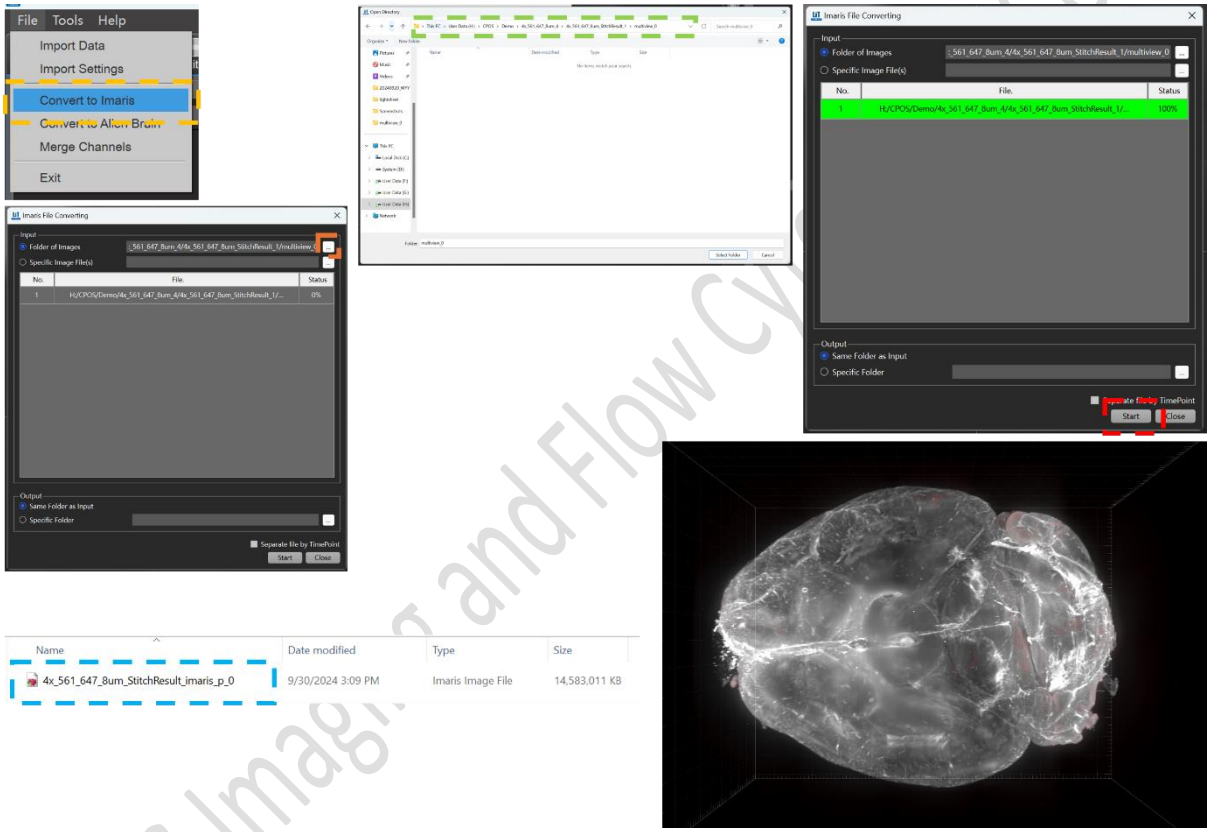
The stitched images can be exported as IMARIS compatible format for downstream analysis.

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.



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!