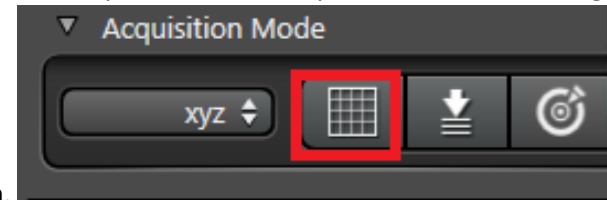


CBIS LM user manual: Leica Stellaris 8 Confocal (upright), for stage scan

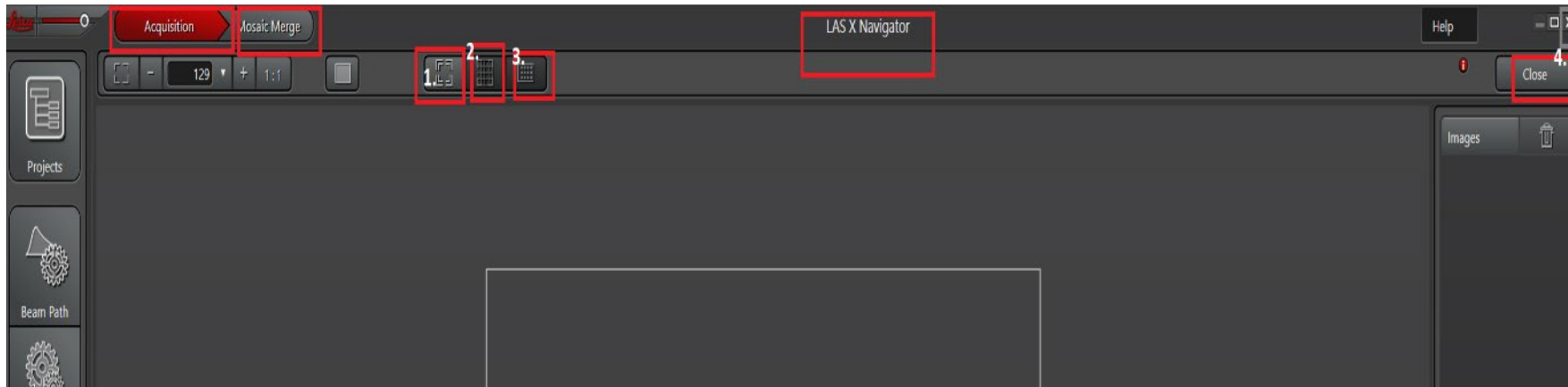
For normal 2D/3D confocal imaging, please refer to basic user manual for Stellaris 8.

1. After locate the sample and adjust brightness and contrast for each channel in main "Acquire" window , if you need to do stitching or multiple location imaging,



please open "LAS X Navigator" stage scan window by clicking on stage scan icon.

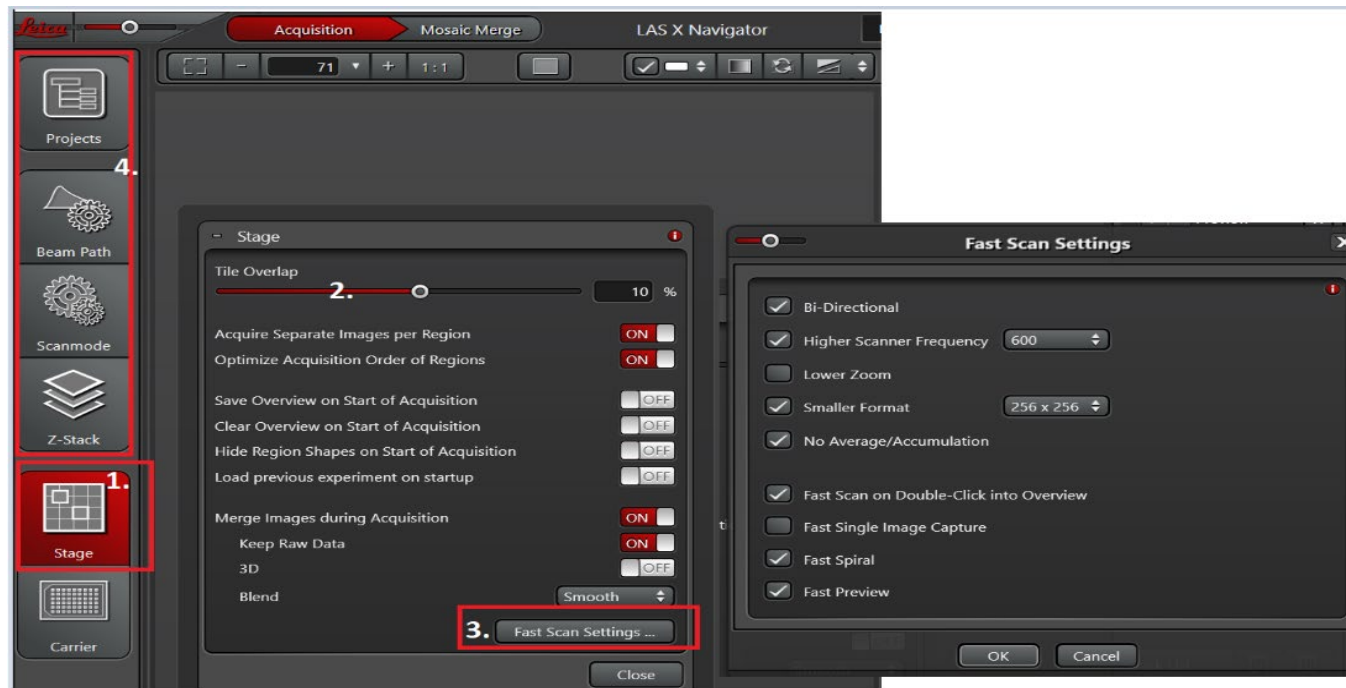
2. Preview of the Navigator window (viewer):



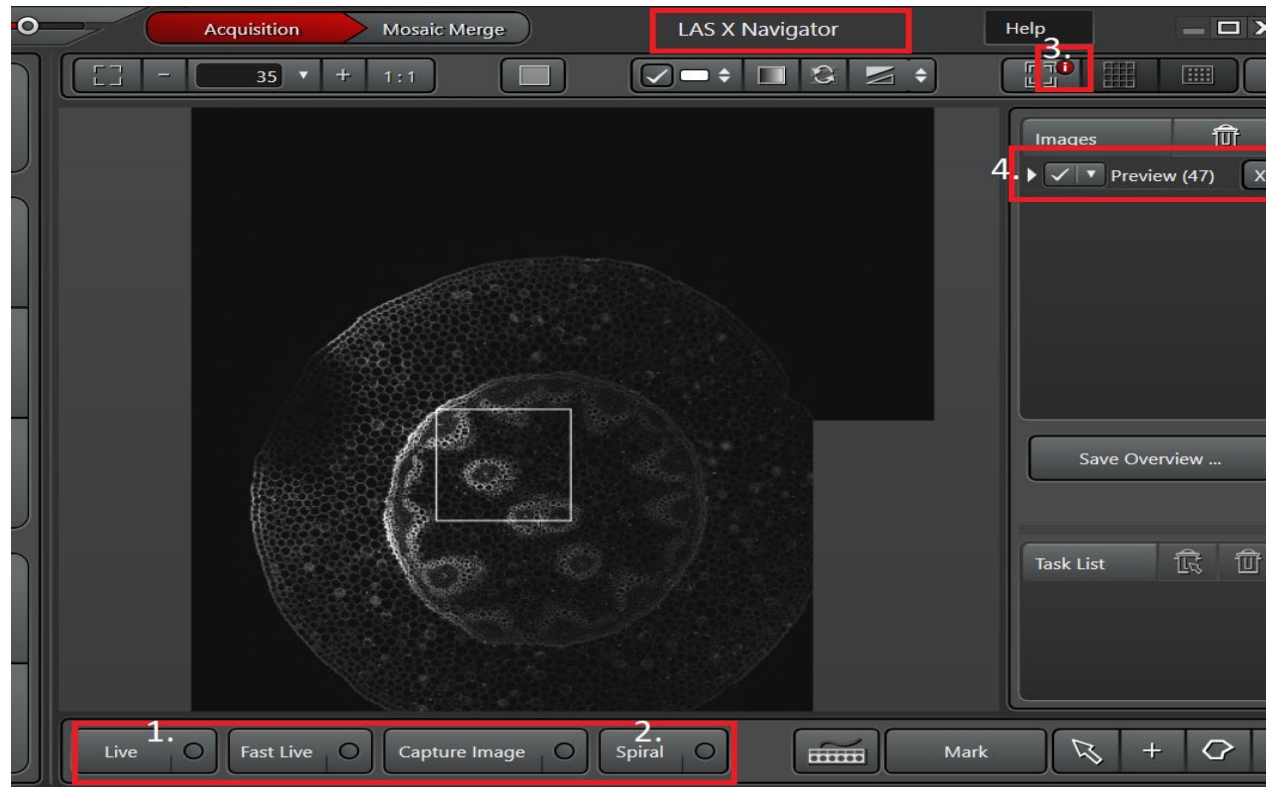
- A. "Acquisition" for previewing image and image acquisition.
- B. "Mosaic Merge": post acquisition image merging.
- C. "1" to move to current stage position
- D. "2" to Indicates all acquisition regions.
- E. "3" for full carrier region

- F. Closing the Navigator window (4) without exiting the Leica LAS X software, all settings/overview images are still saved in RAM and can be recalled properly when the Navigator window is opened again.
- G. Funtion of the mouse:
 - a. Acquire Image: Double-click to move stage to the desired position and an image is created.
 - b. Zoom: using the mouse wheel.
 - c. Moving: Move the displayed image by pressing and holding the left or right mouse button.

3. Go to stage (1), set "Tile Overlap" (2) at 10% and optimize "Fast Scan Settings"(3) to have a fast software response to any change you may make. "Projects", "beam path", "scan mode" (4) in the Navigator viewer will fetch information from the main "Acquire" window accordingly (they are synchroized).



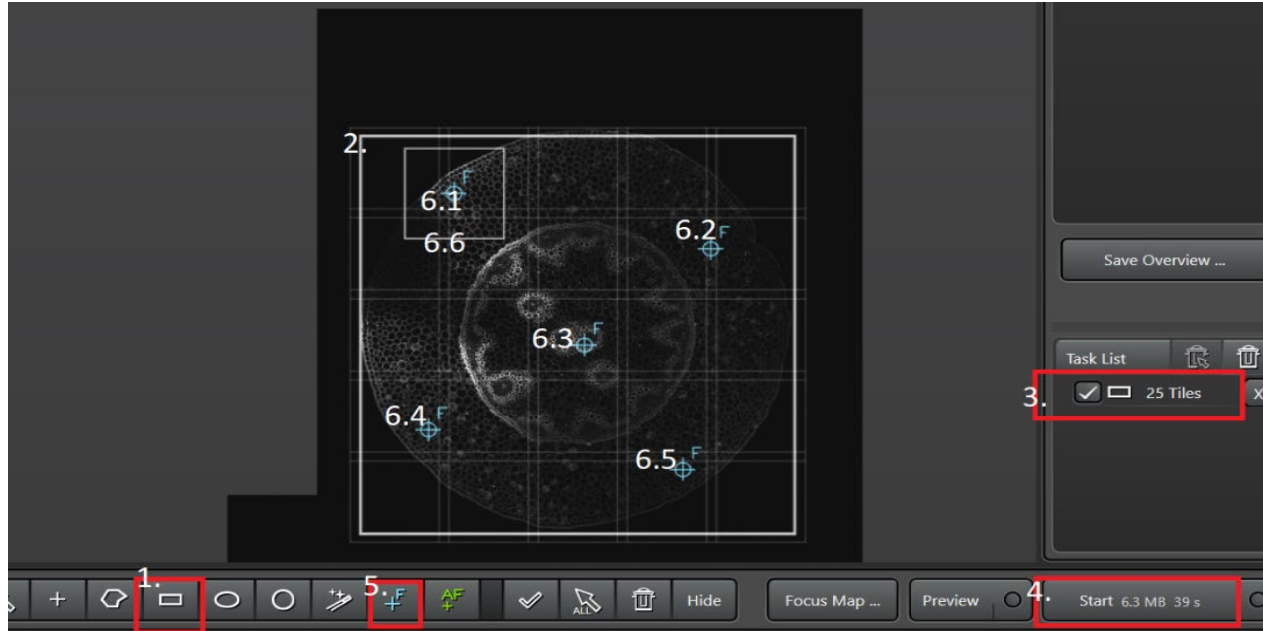
4. Keep "Fast Live" or "Live" (1) on, clicking mouse or moving stage manually to move stage while capturing images, and/or create a overview images. "Spiral" (2) starts at the current stage position and creates a quick overview of the sample. You need to stop "Spiral" each time once overview image is created. All overview images are saved in the preview (4) list. Click on "i" (3) for more details information.



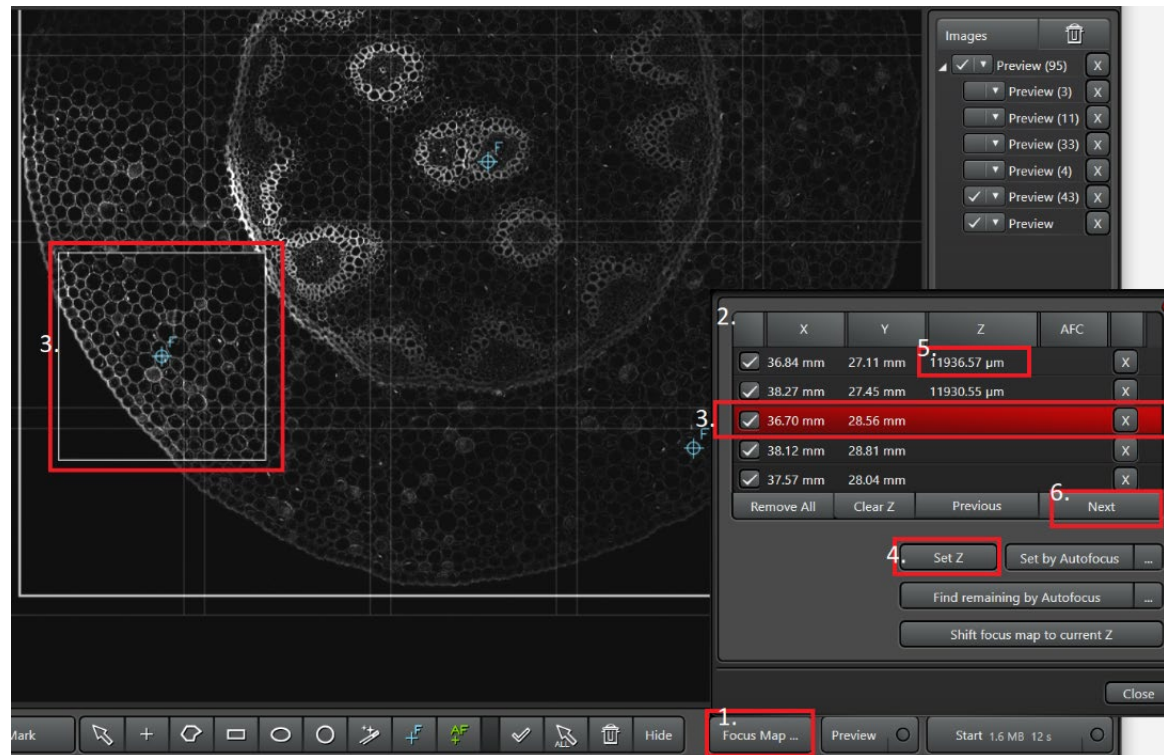
5. To setup tile scan:

- Use rectangular ROI (1) to define the desired scan region on the overviewed image (2).
- Software adds the ROI onto the Task list automatically (3).
- The acquisition duration based on current scan-mode and Z stack settings shown automatically (4).
- To maintain acquisition in-focus for a large view, click on "Focus Map Point" (5) and add focus points onto the overview image accordingly (6.1-6.5).

- e. Current stage position is shown by the small square (6.6) while the ROI tile region is shown by the big square (2).

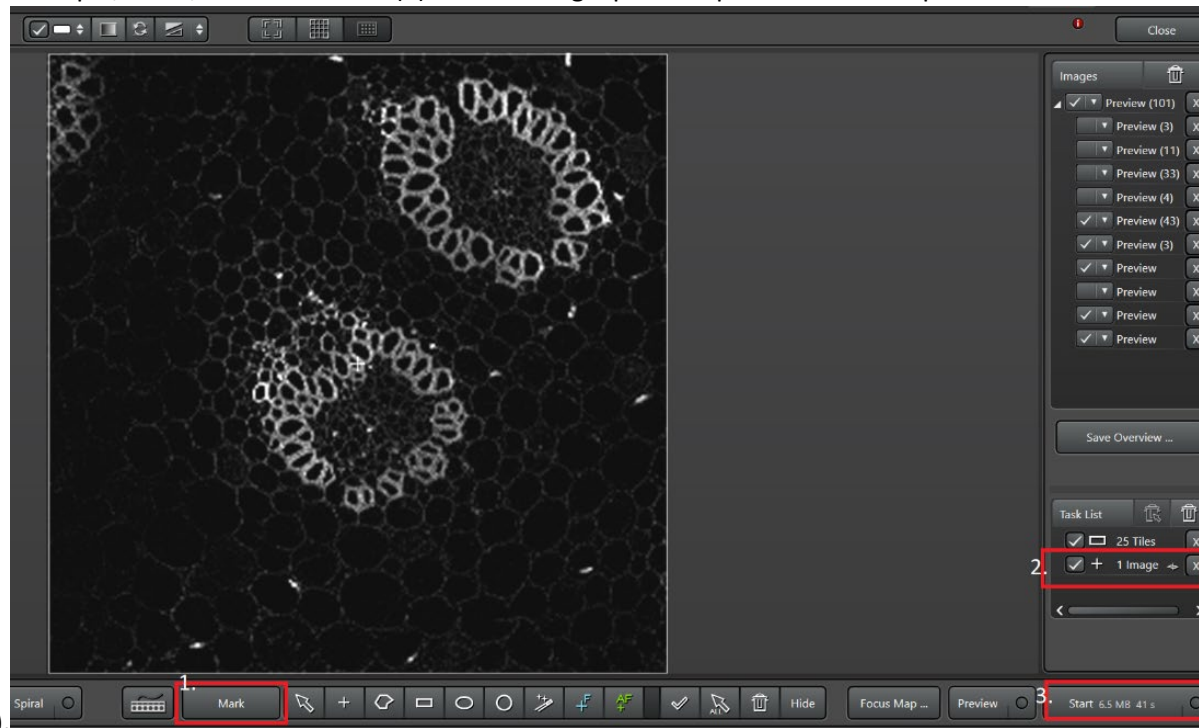


- f. Click on "Focus Map" (1) to call the focus map setup window (2) which lists all the focus map points created.
- g. Select by double clicking on the point to move stage onto that point, keep "fast live" scan and change objective focus until reach best focus. "Set Z" (4) to register the Z position for that point (5).
- h. The highlighted point (3) corresponds to the stage position in the small frame (3).
- i. Click on "Next" (6) until complete all the points.



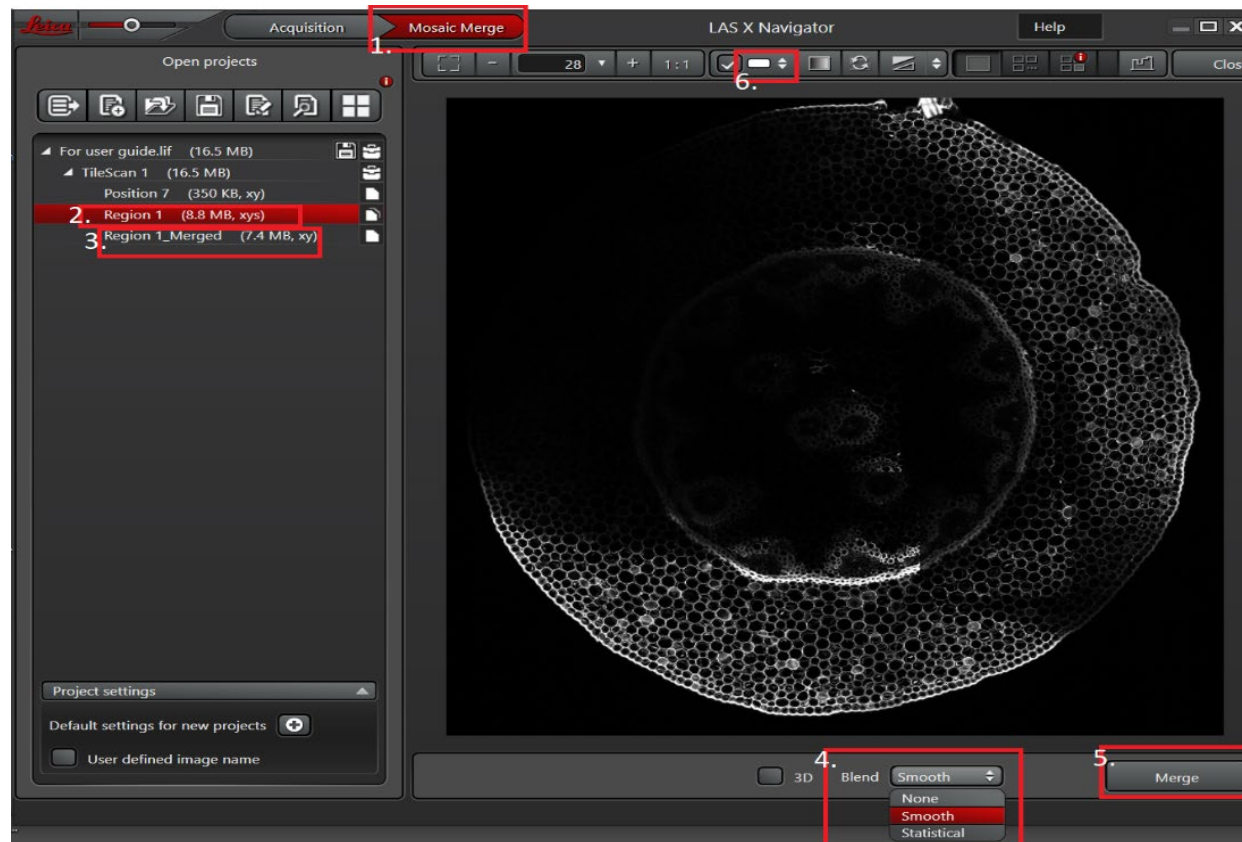
6. To setup multiple stage scan:

7. Move to next sample, focus, click on "Mark" (1) to add a single position point. Software updates the Task List automatically (2), as well as for the total acquisition



duration (3).

8. Click on "Start" to start stage scan acquisition (3).
9. After acquisition, go to "Mosaic Merge" (1). Tile image (2) has been merged automatically (3). You may try to use different algorithms (4) to "Merge" the image (5). Change image display contrast (6) when necessary.



- 10.** To setup multiple position stage 3D scan
- Find the sample, "Mark" the position.
 - Click "Z stack" under Navigator viewer (1).
 - Empty Z position (2).
 - "Turn off" Same stack size for all "regions" (3).
 - Define "Begin" (4) and "End" (5).
 - Define step size (6) based on the sample.
 - The Task list will update the position to 3D acquisition automatically (7).

- h. If you have changed the begin, end, step size, etc, click on "Redefine Stack" (8).

