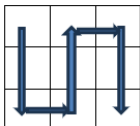# Stage Stitching on Spinning disc liveSR

This short manual is for stitching imaging on spinning disc liveSR.

- Scan Slide is the App used for multiple area scan. Scan direction is shown by an example 3x3 multiple area scan. You may need this information if you would like to use a 3rd part software for stitching.


- After sample scan, one needs go to Data Review tab on the App and click on "view image" to stitch image.

- For basic operation, please refer to CBIS manual CBIS_ liveSR_CSU.

- The APP has no focus map function. For uneven sample, two solutions are suggested:

  o Using PFS to image the sample in 2D, so the sample at the same distance from coverslip will be imaged. This is possible on the dry or water immersion lens for fixed slide in mounting medium.

  o Acquire 3D image, if using oil lens to image fixed slide. However, the data set is big and stitching will be more challenging. Normally data set less than 2G is still possible for the APP to carry out stitching.

Scan Slide Window: preview

To open the Scan Slide window: Acquisition -> Scan Slide.

1. Main: to define the folder, define objective, enter base name using objective which will be useful for the shading correction file saving in the acquistion tab. You may also save and load image acquistion settings in this tab.

   ==Make sure that a valid folder has been select for the "save directory" or the App will crash immediately after scan starts.==
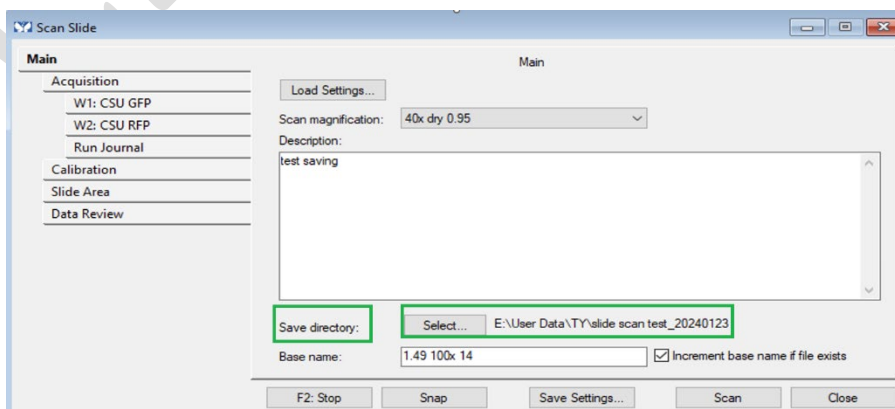
2. Acquisition: define channel numbers, channel, Z series. Choose "Ti Z" if you would like to use

PFS for 2D stitching. Choose "Piezo Z" if you would like to acquire 3D images.
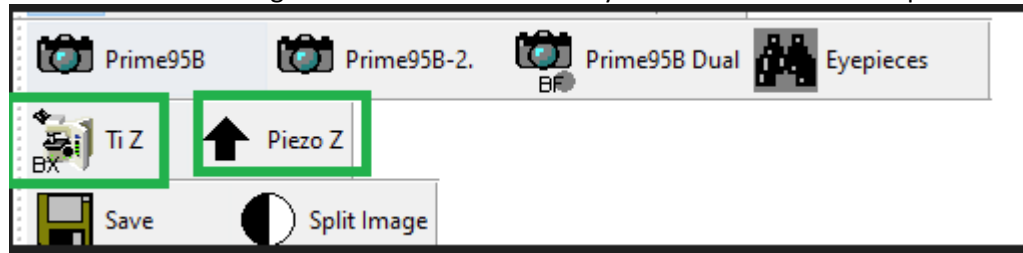
3. Calibration: for stage calibration to the camera so the stitching will be working properly.

4. Slide Area: to define the area to be scanned.

5. Data Review: to generate stitched image (and save it).

Procedure for multiple area imaging and image stitching:

Acquistion Tab:

(1) Enter number of channels/wavelengths to be imaged, choose light path accordingly.

(2) To improve the stitching effect, check box for "shading correction" and "Based on magnification and illumination settings". Move the sample slide to an empty area, click on "Acquire Shading Image" to generate a shading file. The file is named by the magnification and light path. Save the file so it would be applied during image stitching.
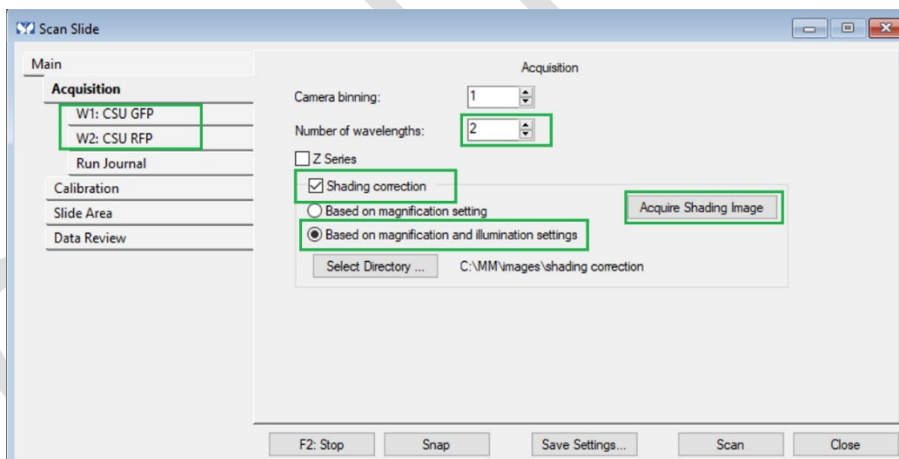

(3) You may crop the camera to centre FOV to improve the evenness of the illumination, which allows you get better stitched image even without applying shading correction.
   - Main tool bar-> Acquire-> open acquire window.
   - Select "acquire" tab on the right panel.
   - Check the box "Enable custom Field of View", e.g. enter "50" % for both Width and Height.
   - Click on "Use Active Region".
   - Click on "acquire" on the top of left panel to confirm that the cropping is on the place.
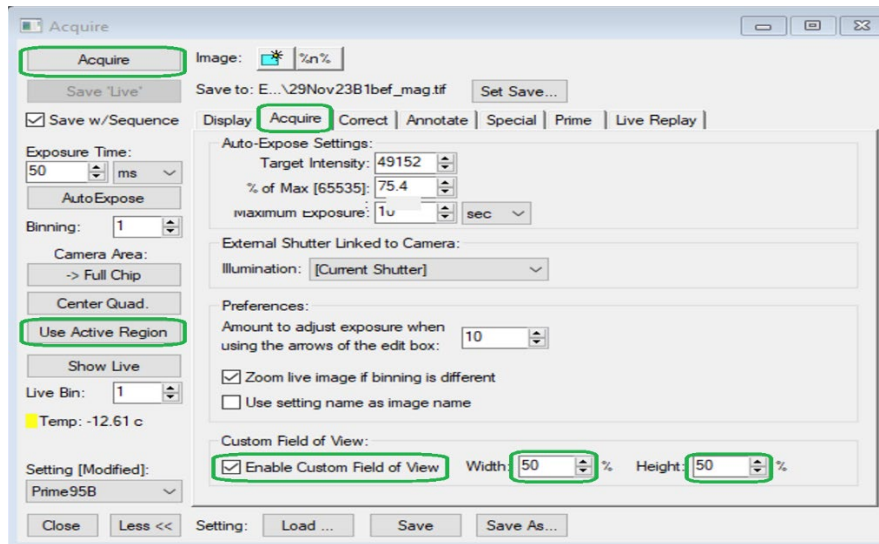
- Close the "Acquire" window.
- Switch back to scan slide App, click on "live" to make sure the crop function is on the place.

(4) Check "Z Series" option to enable Z-stack acquisition.
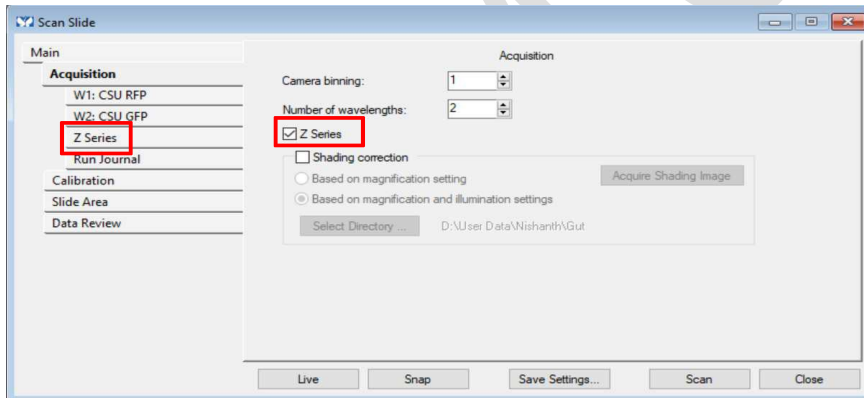
(5) Click on "Z Series" tab to open Z series window. Choose "range around current" to define range and step size or uncheck to define top and bottom.


3
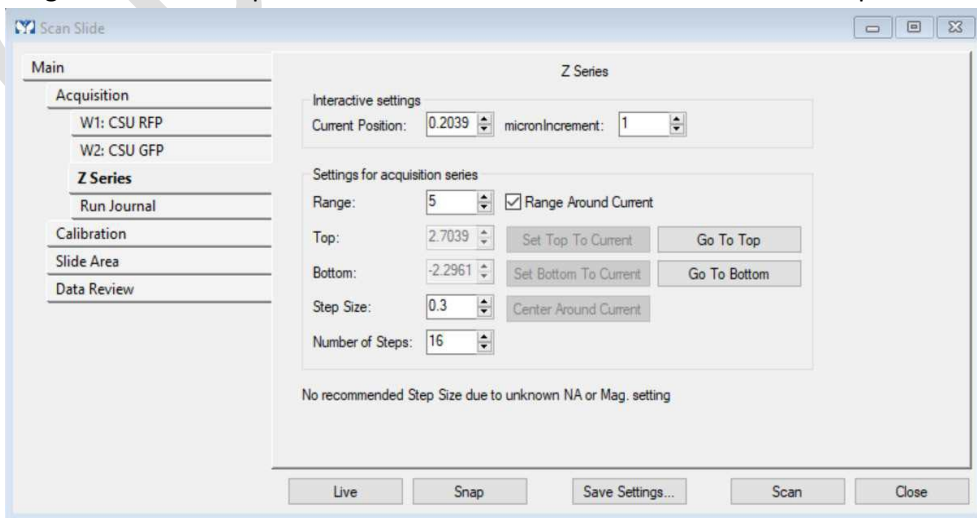
(6) By default, the system uses Piezo Z motor for Z series acquisition, which requires user to use the small up and down arrow to move the stage up and down, not the focus knob on the microscope.


Calibration tab:

(1) The following shows the calibration has been done for the objective as there are values um/pixel for x and y. However, you need to test the calibration by scanning a small area, e.g. 2x2 to make sure the current calibration works properly. Or you need to do the calibration with reference to the section on the bottom of this short manual.
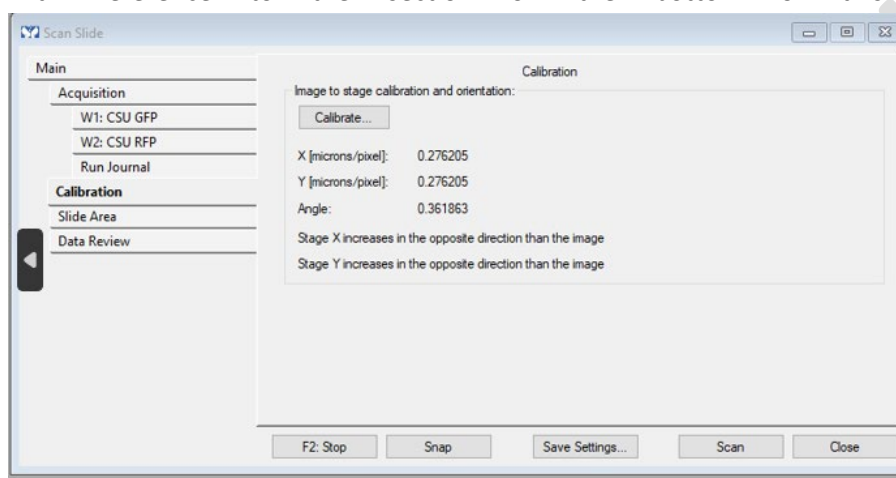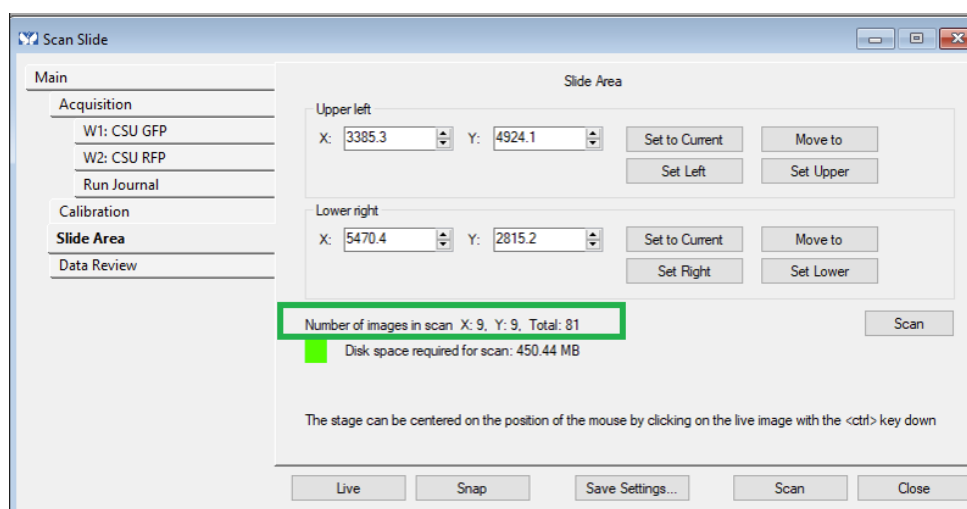

(2) If all the values are shown as 0 like below, it means calibration is not done yet. Please follow the steps under "Calibration" session.
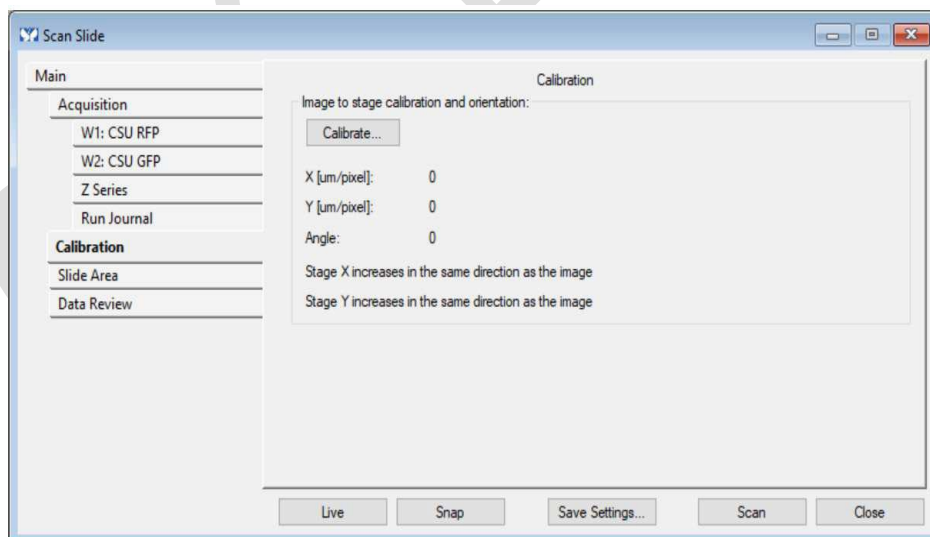

Scan Area tab:

(1) Define scanning area and take note of the number of images in scan. Click on "Scan" to start.

(2) After scanning has started, Z-stack at each site for each wavelength would be taken and a preview of stitched images would be shown.

(3) The stitched image shown on the screen now is a preview. That is not the final well stitched image.

Data Review tab:

(1) After scanning has finished, go to "Data Review".
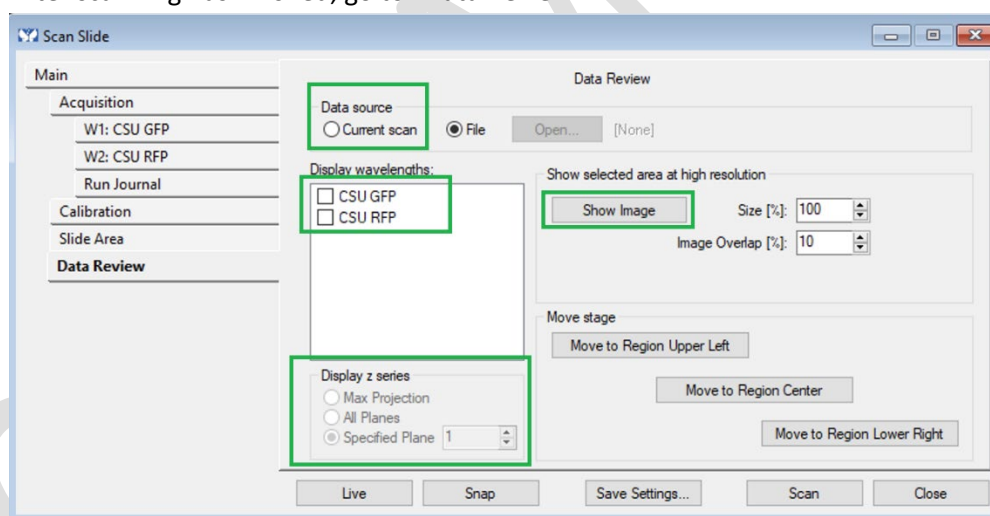
(2) Choose which mode you want to stitch and click "Show Image".

"**Max Projection**" -> Maximum intensity projection would be done for each Z-stack taken at each site for each wavelength. Then the maximum intensity projected image of each site would be stitched together.

"**All Planes**" -> Each plane of each site of each wavelength would be stitched together. In the end, you would get a stitched big image with all the Z planes you have taken.

"**Specified Plane**" -> You can choose to only stitch one of the planes in the Z-stack.
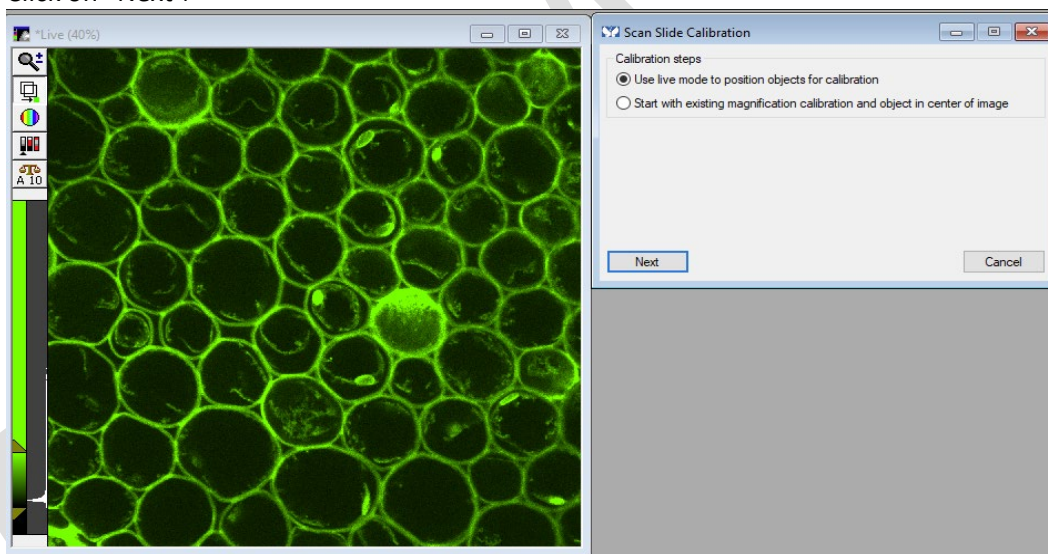
If the scanned area is very big and RAM size is not enough to open all, you may want to reduce "Size (%)" to compress the image to stitch and open.
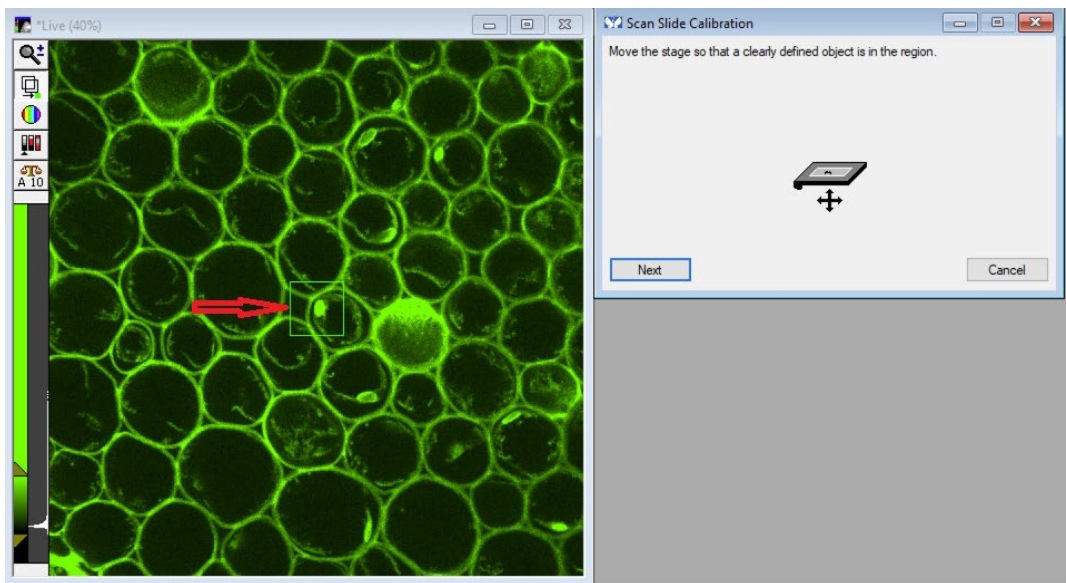
Procedure for Calibration:

1. Keep live and click on "Calibrate". Follow the wizard to finish the calibration. Please take note it is asking to move the stage or move the region in each step.
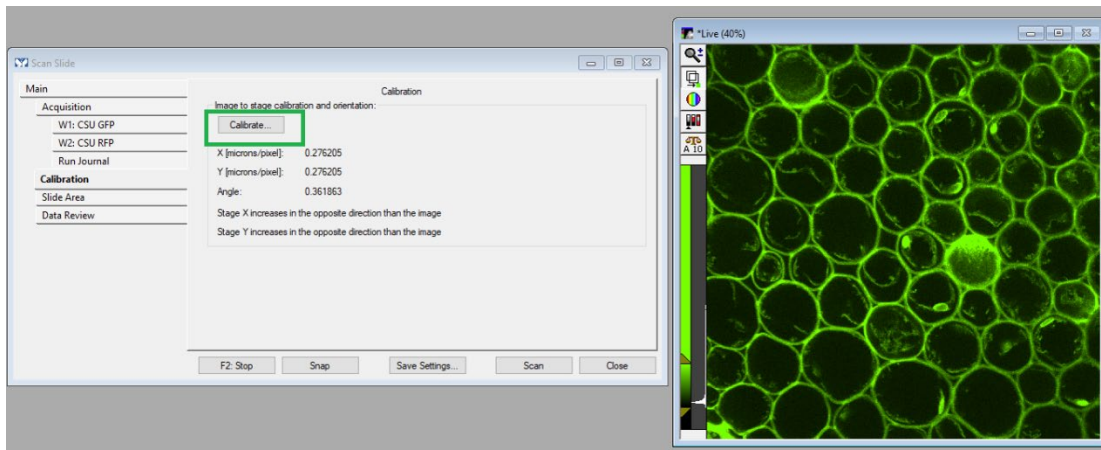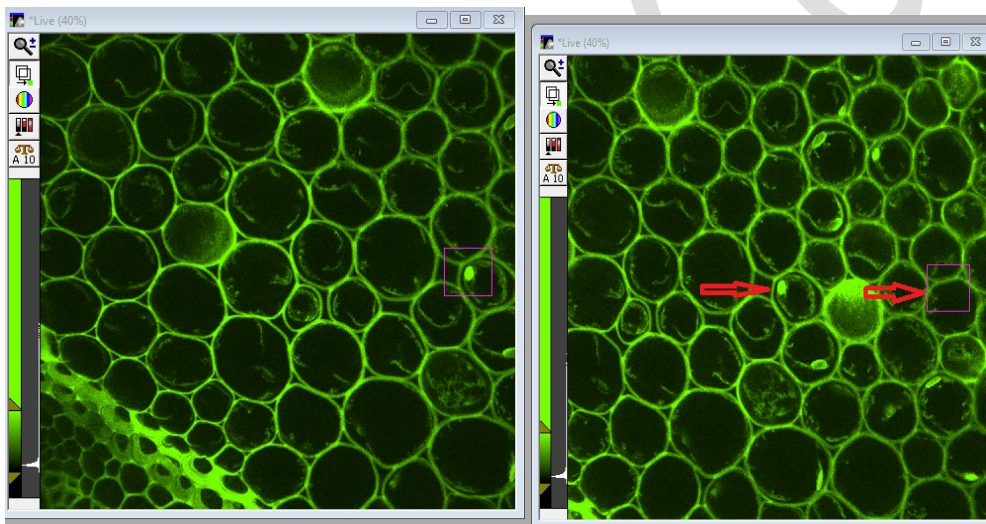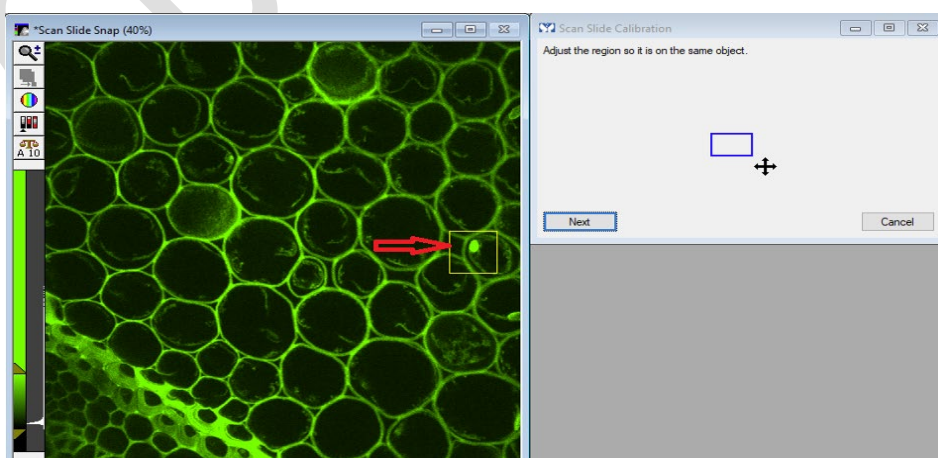

2. Click on "Next".

3. Identify a clear object and move the stage so the object is in the center of the green frame.
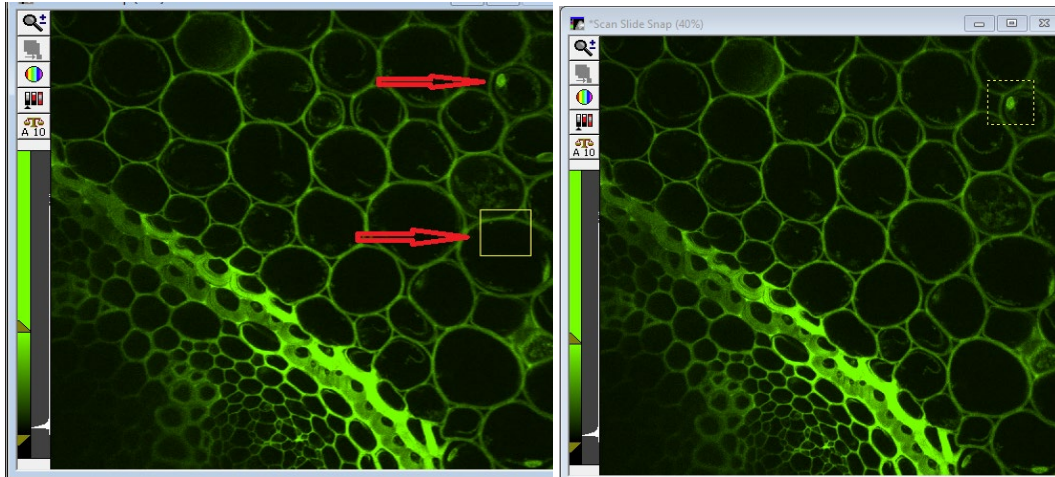
4. Move stage so the clear object is in the center of the purple frame.

5. Adjust the yellow region by mouse so the object is in the center of the yellow frame.

6. Move the yellow region up or down so the object is in the center of the yellow frame.

7. Click next to complete the calibration procedure.