Airyscan quick quide LSM 880

Calibration

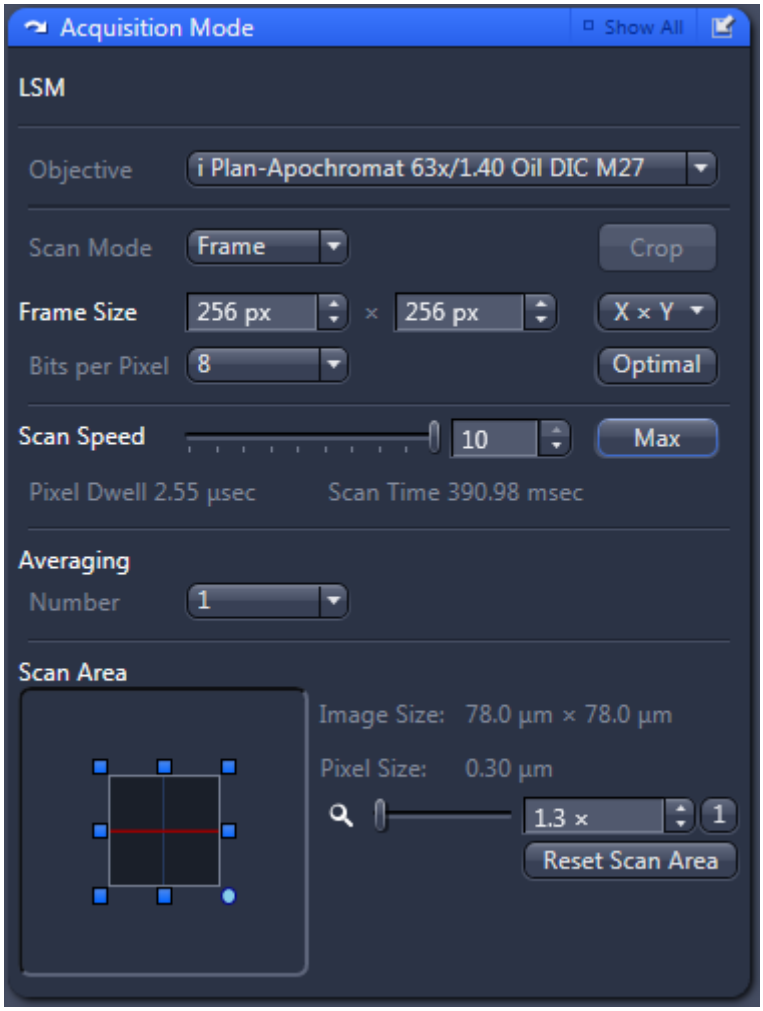
When using the **LSM 880** a calibration can be useful after the first start of the system and after each change of the objective

1. Put a bright sample with good contrast and intensity on (e.g. Fluocells)

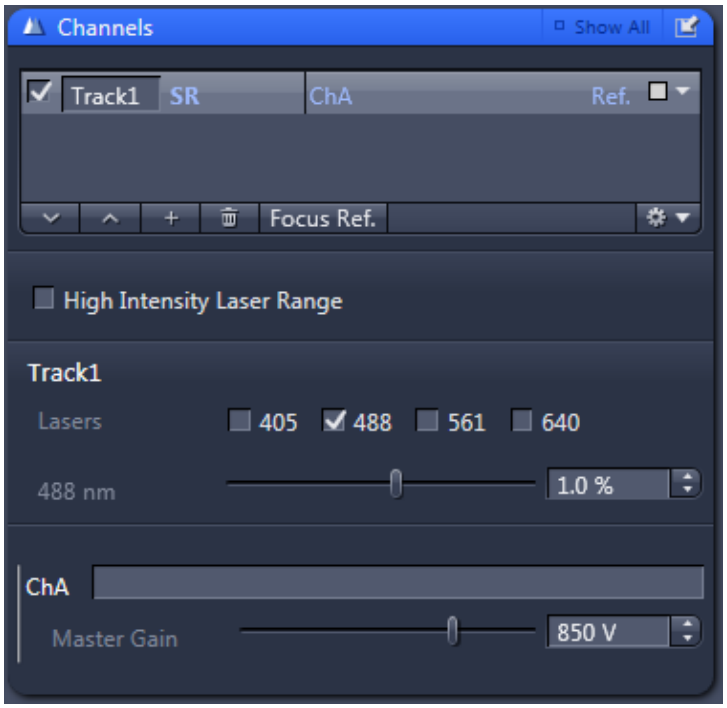
2. Setup an Airyscan experiment and focus the sample

3. Use 8 bit data depth (default), this is sufficient for Airyscan imaging

4. Choose appropriate zoom (at least 1.3x), a small frame size (e.g. 256x256 pixel), and a fast scan speed (here Speed 10)

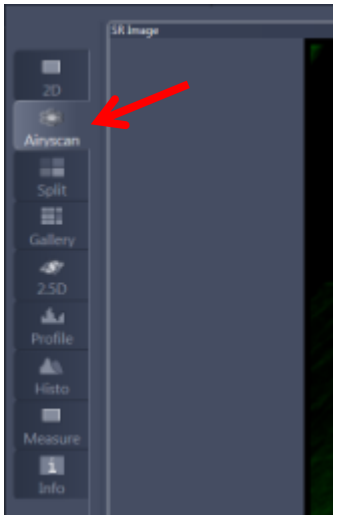


5. Choose a laser line that matches your sample and apply the same laser power you normally use for confocal imaging (here 488nm, 1%) and set the PMT gain to 850V (default for Airyscan)

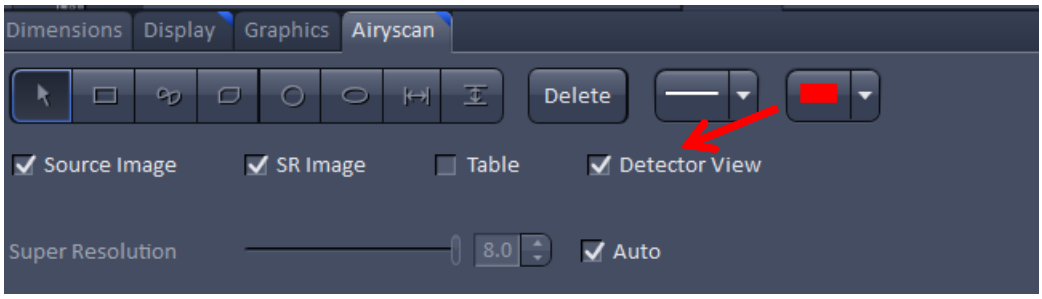


6. Start a continuous scan

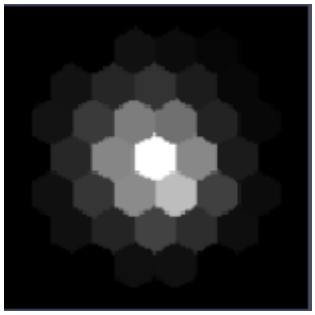
7. Switch to Airyscan view (left side of the image container)



8. Open the Airyscan tab below the image and activate detector view



9. The brightest spot should be in the center, or at least in the first ring.



a) If the brightest spot is not centered, but shifted to the second or third ring, increase gain to help the adjustment of the beam, if necessary, also increase LP → the brightest spot will move to the center in continuous scan
b) If all elements are completely white, decrease laser power and gain.
c) If the detector view shows a random intensity distribution, the signal might be insufficient. Check that the sample is properly illuminated and in focus.

9. Once the beam is centered, you are ready to start your experiments with Airyscan data acquisition.

Imaging

1. Now the real sample, if not already used for calibration, can be applied.

2. The settings have to be adjusted for optimal imaging. That means min. zoom of 1.8x, optimal number of pixels and, if z-stack is used, optimal z-slice interval

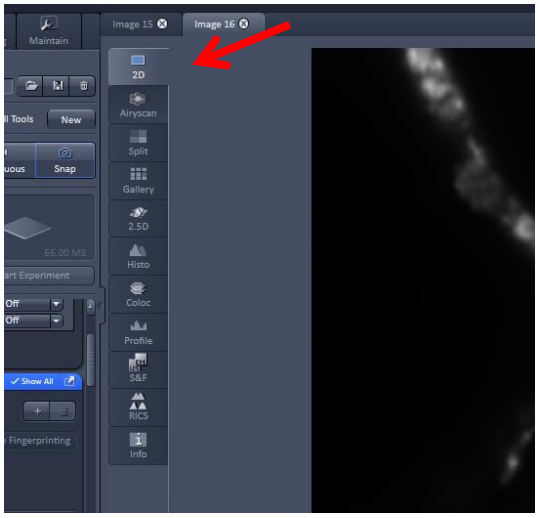
3. Maximum available scan speed can be used and usually no averaging is needed, 8bit data depth is sufficient.

4. Start with the same laser power you normally use for confocal imaging

   and a gain of 850V

5. Focus the sample with live or continuous

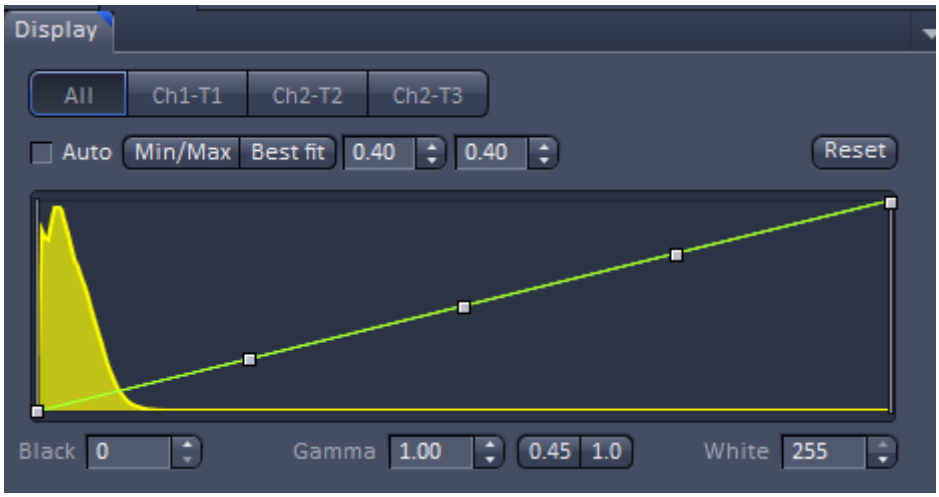
6. Go to 2D view (left side of the image)



7. To make sure, that none of the detector elements is saturated, have a look at the Range indicator. Red pixels show a saturation of one of the detector elements -

→ Make sure that for the range indicator, the histogram is linear as with the LSM 880 the range indicator is influenced by the chosen histogram setting

→ The 2D view shows the average of all 32 GaAsP elements. As the outer elements detect comparatively low intensities, a filling of the histogram up to 25% is sufficient.

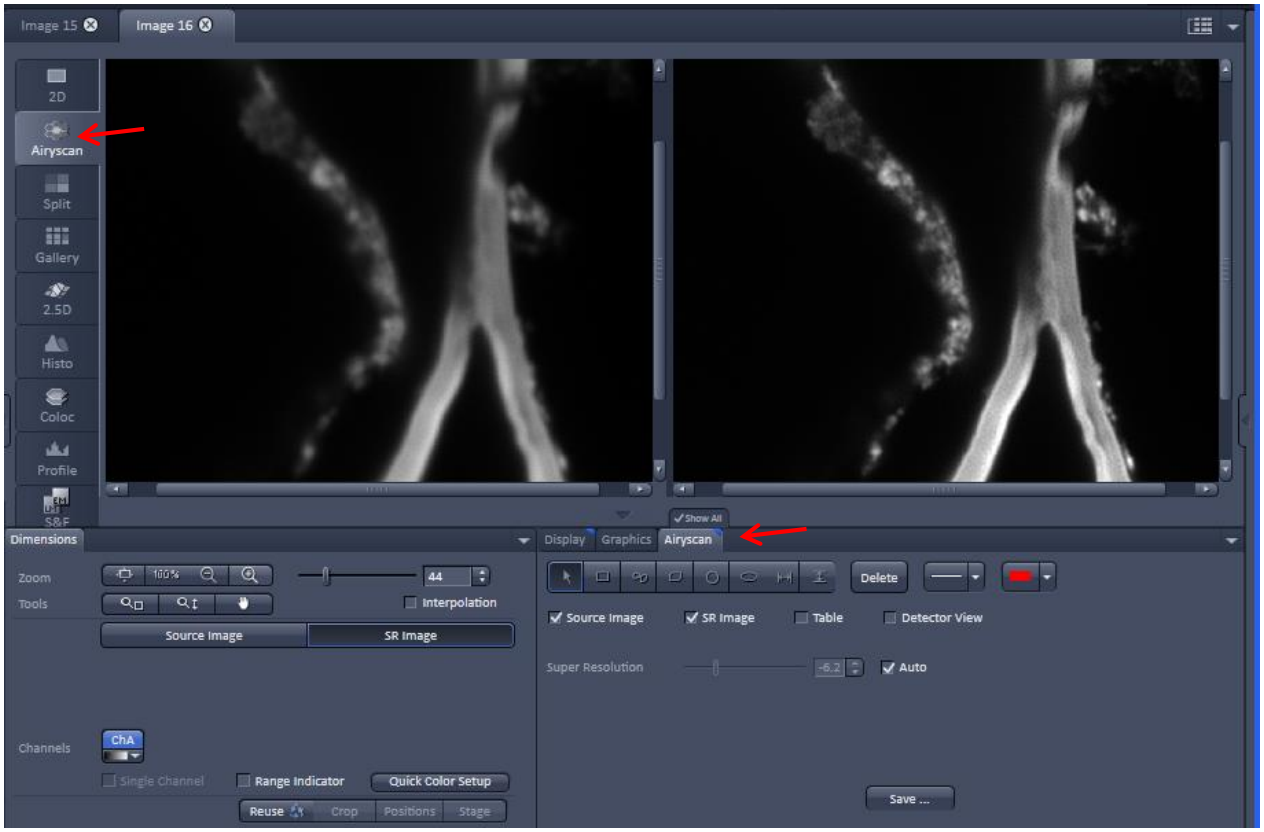


The PMT gain can be increased up to 900V and only if that is not sufficient, the laser power should be increased

8. Start Snap or Experiment

Processing

1. Once the imaging setup is finished and Snap/Start Experiment is pressed, go to Airyscan view tab, (left side of the image) and open Airyscan tab below the image



2. Activate Source image and SR image. The source image shows the same as 2D, an average of all detector elements and the SR image shows the SR image of the current z-plane.

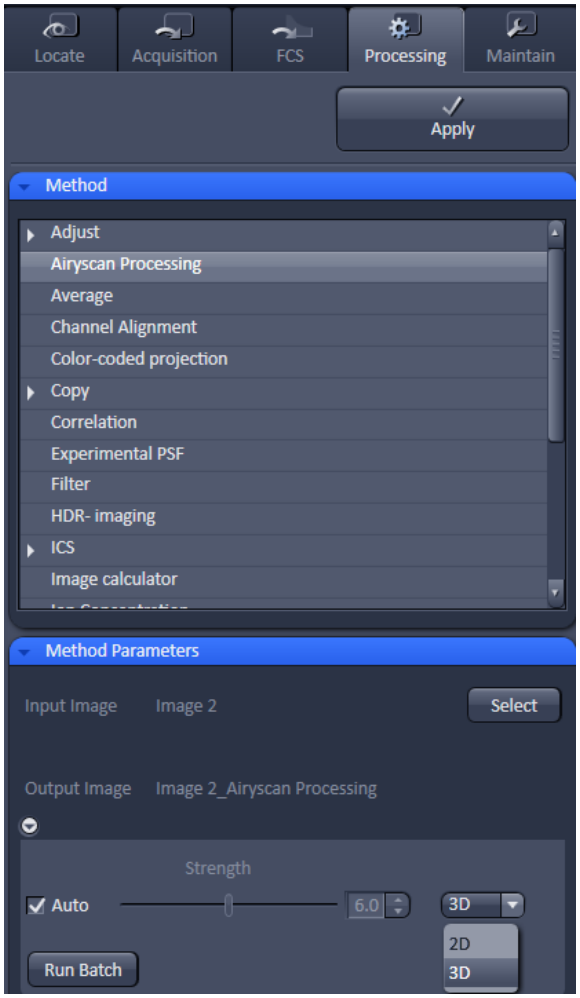
→ During the acquisition, the SR image does not show the final SR, but the Sheppard sum (rearranged pixel, no deconvolution). As soon as the acquisition is finished, the SR image will be refreshed and the fully processed Airyscan image is shown.

In the Airyscan tab of the LSM 880, the following processing options are available:

- Save: saves the currently displayed SR image (2D processed)

Further procession options are available in the processing tab, see 3.

3. Processing Tab



Choose Airyscan Processing method and select the image.

Airyscan Processing can also be used in a batch processing workflow.

If the image is a z-Stack with 5 or more sections, the 3D processing option can be chosen. This is necessary to get the resolution increase in Z.

For the beginning, leave the filter settings on Auto Filter. In the vast majority of biological samples, this works fine. The Airyscan processing uses a linear deconvolution algorithm.

If you prefer a crisper image appearance, check in the image Info of the processed image and check, which value for the filtering was used.

Then go back to the processing tab, load the original Airyscan image again, deactivate Auto Filter and manually choose a value for the filtering that is approx. 0.2 higher than the value used for Auto.

Optionally repeat this step with a different filter setting.