STED imaging on the Leica SP8 STED 3X

Important consideration when choosing dyes for STED Imaging:

- We have 3 STED lasers: 592, 660, 775
- Excitation should be between 470 and 670nm
- Dye should not be excited by STED wavelength
- Dye should have some emission in STED wavelength

This guide assumes you are familiar with confocal imaging on this instrument.

1) Start the LASX software and select STED imaging on the initial configuration window.
2) Set up confocal imaging with the excitation wavelengths and emission bands suggested in the list of dyes for STED imaging using sequential frame acquisition. Optimize laser power, detector parameters, and number of averages.
3) If you want to capture both confocal and STED images copy each sequence onto a new sequence.
4) For the STED sequences, increase the laser power 2-3 fold and the detector gain 2 fold.
5) Activate the STED lasers,
   Click on Actvate STED, this opens a new window. Turn the 592 and any other needed STED laser on and set them to 70% power. Close this window
6) To optimize parameters:
   i)   Turn bidirectional scan off.
   ii)   Set the number of line averages to 32.
   iii)   Set the STED laser power to 0.01
   iv)   Turn gating off.
   v)   Click "Capture Image" – this will start capturing a slow image (if acquisition is too fast, increase the number of line averages). This is a confocal image, once the sample starts appearing increase the STED laser power, there should be a noticeable difference. Find the minimum amount of STED laser power that gives the best difference.
   vi)   If there is no difference, stop the capture, go to configuration at the top of the screen.

   Select STED on the left to open a new window:
   Click "align beams" and wait for beam alignment to be completed.
   Once completed return to the acquire tab at the top.

   vii)   After the beams are aligned, repeat step v and find the optimum STED laser power.
   viii)   Turn the detector gating on and increase it to improve the image, typically around 1 ns.
7) Once you have found the optimum parameters, turn bidirectional scan on, find a new region to image, lower the number of line averages to 2-3 times the optimum for confocal imaging.
8) Set a z-stack if desired using the confocal acquisition.
9) Click "start" to collect the images.