Leica SP8X STED White Light Laser Confocal Microscope

- Complete spectral freedom
 - Up to 8 freely tunable laser lines simultaneously from 470 to 670nm White Light Laser,
 1.5 mw, in steps of 1nm, pulsed at 80 MHz
 - \circ 50 mw 405 diode laser
 - 592 nm STED depletion laser line output > 1.5 W
- AOBS (Acousto-Optical Beam Splitter)
- Two scanners mounted on Tandem scanner
 - Conventional Scanner scans 1-1800lines/s for high spatial resolution with largest FOV
 - Resonant Scanner scans at fixed 8000lines/s for high temporal resolution
- Objectives:
 - o <u>10X/0.4 HC PL APO CS, WD 2.2 mm</u>
 - o <u>20X/0.75 HC PL APO CS2, WD 0.62 mm</u>
 - <u>40X/0.85 HC PL APO CS, WD 0.21 mm, correction collar for 0.11-0.23 mm coverslip, not</u> for 405 nm excitation
 - <u>63/1.3 Glycerol HC PL APO CS2, WD 0.3 mm, correction collar for 0.14-0.19 mm</u> <u>coverslip, 20-40° C</u>
 - 100X/1.4 Oil HC PL APO CS2 STED, WD 0.13 mm
- Stage
 - SuperZ Galvo, Galvo Flow sweeping through live specimens in 4D
 - Adaptive Focus Control(AFC) actively and dynamically regulates the focus position
 - o Stage inserts for slide, petri dish up to 35 mm, labtek chamber, and multi-well plate
- Stage Top Incubator for heating, moisture and premixed gas flow control for live cell imaging
- 5-Channel Leica SP <u>Spectral Fluorescence Detection</u>, <u>three HyD</u> and two regular PMT detectors, 400-800nm, minimum 5nm
- HyD detector
 - Supersensitive photon detection with maximum quantum efficiency of ~45 % at 530 nm (twice as much as a standard PMT)
 - Very low dark noise to render the finest details
 - Photon counting capability for fluorescence detection
- <u>Super-resolution gated STED imaging down to sub-50nm sub-cellular structures</u>
- One Transmitted light detector for bright field, or DIC imaging
- FRET AB, FRET SE, FRAP, FRAP XT
 - o FRAP Wizard
 - Efficient bleaching by optional Zoom in and change of format during bleach
 - Fly Mode for fast recording of recovery by bleaching and dada readout in one frame
 - xyzt mode for pre-and postbleach acquisition for 3D FRAP analysis
 - Online and offline quantification of data
 - FRET Acceptor-Photobleaching Wizard
 - FRET measurements with fixed and immobile samples
 - FRET efficiency calculated for user-defined regions
 - Results displaced as FRET efficiency map
 - o FRET Sensitized Emission Wizard

- Correction of background fluorescence and cross-talk
- Online and offline quantification of FRET efficiency in user-defined regions
- Result displayed as FRET efficiency map
- Full spectral characterization of images, $\lambda \lambda$ scan of excitation-emission spectral image map
- <u>LightGate</u>
 - Zero background by removing reflection and autofluorescence by adjustable detection time gate
 - Removing non-wanted fluorescence to increase image contrast
- <u>Huygens Professional Deconvolution Software Package</u> restores STED/Confocal images back to original objects through mathematical de-blurring and de-noising. It enhances image resolution and signal/noise ratio, and removes noise background.



- Wide-filed Fluorescence Imaging
 - o ORCA-Flash4.0 V2 Digital CMOS Camera (High-end) for fast live imaging
 - 80% QE at 600nm
 - 2048 X 2048 resolution, 6.5 μm² pixel, 30 frames/s, 16 bit data output
 - full well 30, 000 e⁻, read noise 1.6 e⁻ rms (1.0 e⁻ median), dark current 0.06 e⁻ /pixel/s
 - Filter cubes
 - DAPI: EX: BP 350/50, EM: BP 460/50
 - GFP: EX: BP 470/40, DM 500, EM: BP 525/50
 - RFP: EX: BP 560/40, DM 595, EM: BP 630/75
 - Triple D/F/TX-S: EX BP 420/30, 495/15, 570/20; DM 415, 510, 590; EM: BP 465/20, 530/30, 640/40
 - o <u>MetaMorph Premier Imaging Acquisition Software</u>
 - Multi-channel, multi-site, and high-throughput time-lapse fluorescence and BF/DIC imaging