

Confocal microscope Leica with mupltiphoton excitation Leica SP8 WLL MP

Location

Room DaI / basement / room 010 (tel. 2582)

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Usage rules

See the document **General Usage Rules for Confocal Microscopes**

Microscope-specific Usage rules

Lasers in general must be turned off in LAS X software. Special attention must be pointed to Argon laser usage, which requires special system shut down procedure. DO NOT turn off the hardware laser power button, the system must stay turned on for about 15 minutes for Argon laser to cool down. Not following this rule will result in decreased service life of Argon laser or to its complete malfunction.

Detailed description of confocal microscope Leica SP8 WLL MP

1. Microscope. Inverted Leica DMI8 stand with binocular, motorized condenser, side port for camera for image acquisition in bright field (BF), DIC and fluorescence, motorized scanning table (with 127 x 83 mm scanning range, image acquisition for ROIs, TileScan, Mark&Find), with Super Z Galvo insert (z-range: 1500 μm)

2. Available of objectives.

- HC PL FLUOTAR (FLUOR) 5x/0.15 NA, WD=10 mm;
- HC PL FLUOTAR (FLUOR) 10x/0.30 NA, WD= 11 mm;
- Multiimmersion objective HC PL APO 20x/0.75 NA, IMM CORR CS2, WD=0.67 mm, DIC;
- HC PL APO 63x/1.20 NA W CORR CS2, WD=0.30 mm, DIC;
- HC PL APO 63x/1.40 NA OIL CS2, WD=0.14 mm, DIC.

(other objectives from the same producer can be used if agreed with the manager)

3. Scanning head UV-VIS-IR. Acousto-optical beam splitter (AOBS), conventional scanner (7 fps at 512 X 512 pixels unidirectional scan) and fast 8 kHz resonant scanner (14 fps at 512 X 512 pixels), spectral detector with tunable emission range 350 – 800 nm.

4. Illumination path.

- DMOD laser 405 nm (50 mW),
- Argon multiline laser 458 - 476 - 488 - 496 - 514 nm (65 mW)
- Pulsed supercontinuum “white light“ laser of 2nd generation WLL2 with wavelengths range 470 - 670 nm (laser power at each wavelength approx. 1.5 mW), with possibility to use up to 8 lines simultaneously by means of AOBS (tunable with a step of 1 nm), with independent laser power control, with fast modulation of the laser intensity by acousto-optical transmission filter (AOTF)
- Infrared tunable pulsed laser Chameleon Ultra I (Coherent Inc., CA) with tunable range of 690 - 1040 nm (step 1 nm), maximum output power 4 W, pulsing frequency 80 MHz, pulse width ~140 fs and with laser power and intensity controlled by attenuator and electro-optic modulator (EOM).

5. Detection path.

- Internal (confocal) - possibility to set gain and offset for each detector independently, as well as range of each detector.
 - 2 highly sensitive HyD spectral detectors with maximum quantum efficiency (QE) of 45 % at 500 nm, detection range of 400-720 nm with low noise and high dynamic range, photon-counting and gating capabilities.
 - 2 PMT sensitive detectors with QE = 30 % at 500 nm, range of detection 400-800 nm
- External (non-descanned) -1 transmitted light detector (TLD) - PMT detector for bright field mode (BF), DIC, or forward scattered SHG, suitable for image acquisition simultaneously with other channels (descanned or non-descanned detection)
 - 2 reflected light detectors (RLD) - highly sensitive HyD spectral detectors with maximum QE 45% at 500 nm, detection range of 400-720 nm, with low noise and high dynamic range, photon-counting and gating capabilities

6. Box incubator. chamber for live cell imaging (Oko-Lab) with controlled temperature by H301-T-UNIT-BL-PLUS (including objective heater), controlled concentration of CO₂ (0-20%) and O₂ (1-95%, hyperoxic conditions only if agreed with the manager concerning additional O₂ supply) by CO₂-O₂ Unit-BL [0-20;1-95], humidifier and a H301-K-FRAME with perfusion capability, GS35-M insert (for one 25x76 mm slide and up to two 35 mm Petri dishes with glass bottom) and with magnetic sample holders

7. FLIM module. One-channel system for time correlated single photon counting (TCSPC) Simple-Tau-150-D1 (Becker & Hickl GmbH, Berlin, Germany) – ultrafast system for single photon counting based on SPC-150 module with Magma PCI box, detector controller DCC-100 and cooled ultrafast PMC-100-1 detector based on PMT (for detection in 300 – 820 nm).

Also, standard non-descanned Leica HyD detectors (2x) can be used for FLIM and PLIM data acquisition by a dedicated SPC cards for simultaneous 2-channel FLIM and PLIM (kindly loaned by the Becker & Hickl GmbH).

8. Software. LAS X for microscope stand control as well as automatized image acquisition (including single images, time series, z-series as well as spectral series and their combinations) using different laser lines including sequential scans with laser re-tuning.

9. SW modules. Dye Finder, Live Data Mode, 3D Visualisation, Co-Localisation, MicroLab (for FRAP, FRET, FLIP experiments and ROI), FRAP Zoomer (for FRAP utilizing tandem scanner).

Useful websites

- [Reservation system](#) at the Institute of Physiology, Czech Academy of Sciences
- [Specification of optical microscopes](#) at the Institute of Physiology, Czech Academy of Sciences
- Information on the equipment that has been included into the project [Czech-BioImaging](#)
- [Training protocol – internal users](#) (including the rules for entering the facilities with laser scanning confocal microscopes at the Institute of Physiology, Czech Academy of Sciences, [template PDF](#))
- [Training protocol – external users](#)

- [LAS X Core Offline](#) software for opening Leica file formats
- [Nyquist online calculator](#) for theoretical calculation of pixel and voxel size

- Fiji (Fiji is Just ImageJ):
Bioformats (former LOCI Tools) [ImageJ](#)
[SLIM Curve](#)
[ImageJ world mailing list](#)

- Interactive dye spectra viewer:
[ThermoFisher](#) (Life Technologies) interactive Spectra Viewer
[Leica FluoScout](#)
[BdBioSciences Spectrum Viewer](#)
[BioLegend SpectraAnalyzer](#)

- [Tables of fluorescent dyes spectra](#)
- Other fluorescence relevant links:

<http://www.leica-microsystems.com/science-lab/fluorescent-dyes/>
<http://works.bepress.com/gmcnamara/9/>