**Leica SP8 FLIM**

Versatile inverted fluorescence confocal microscope equipped with 405 nm, 445 nm and white light laser, spectrally tunable detection and FLIM hardware. Presence of camera and epifluorescence lamp allows also widefield imaging. For more detailed microscope characteristics please see the section “Microscope”.

**Application**

* Fast and multi-color confocal imaging
* Fast and multi-color widefield imaging
* Various measurement options: z-stack, time series, tile scan, multi positions
* Spectral imaging
* Measurement of emission and excitation spectra
* Lifetime imaging, FLIM-FRET
* Measurement of e.g. membrane dynamics by FCS and FRAP
* Live-cell imaging available
* Brightfield, DIC, polarized light microscopy

**Microscope**

An inverted confocal microscope Dmi8 with a laser scanning confocal head Leica TCS SP8, a motorized microscope stage with a Super Z-galvo scanning insert for fast and precise Z movement, HW autofocus and Best focus control, transmitted light LED U-12V, square pinhole of tunable size and following units:

|  |  |
| --- | --- |
| Software | **LAS-X** |
| Epifluorescence  | CoolLED pE-300  |
| Lasers  | **405 nm** (max 40 MHz, 3 mW)**445 nm** (max 40 MHz)**WLL 470-670 nm** (max 80 MHz, 1.5 mW)- laser wavelength and intensity is controlled by a combination of AOTF and AOBS- the actual wavelength selectable with 1 nm step- up to 8 parallel laser lines |
| Additional beamsplitters | BP 440/40 |
| Filter turret – filter cubes for epifluorescence lamp | **DAPI**  325-375  400  435-485**FITC** 460-500  505  512-542**RHOD** 541-551  560  565-605**CY5** 590-650  660  662-738 Analyser |
| Objectives | HC PL APO CS **10x, NA 0.40**, WD 2.2 mm HC PL APO CS2 **20x, NA 0.75**, WD 0.62 mm, DICHC PL APO CS2 **63x WI, NA 1.2**, WD 0.30 mm, Correction Ring, DICHC PL APO CS2 **63x Oil, NA 1.4**, WD 0.14 mm, DIC  |
| Scanner | FOV SP8 scanner- maximal sampling 8192x8192px, - speed 1-1800 Hz (7fps@512x512 px)- hardware zoom 0.75x-48x |
| Fluorifier Disc Settings (notch filters) | NF 488 NF 445NF 514 NF 594 NF 458/514 NF 445/594NF 488/561/633 SMD1 NF 405/470 SMD2 NF 405/640 SMD3 NF 470/640 |
| Detection | **Transmitted light detector** (PMT)**Internal spectrally tunable detection unit*** **3x HyD detectors**  for FLIM (time-gating possible), GaAsP, 400 – 720 nm, modes: Standard, Counting, BrightR; QE=45%@500nm
* **2x PMT detectors**, 400-800nm, QE=30%@500nm

**Camera DFC365 FX** (Leica)* 1392 x 1040 pixels
* 6.45 μm x 6.45 μm
* CCD sensor Sony ICX285
* C-mount 0.7x
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| FLIM and FCS module | **HydraHarp400** (Picoquant)* Picosecond Event Timer & TCSPC Module
* Four channels available
* Enables detection of photon arrival times with respect to the beginning of the experiment, the excitation pulse and the channel in which the photon was registered (TTTR mode). In combination with a scanner controller, a histogram acquisition at different image positions is available.

**Symphotime64*** Software for image acquisition and analysis

Custom written software in LabView for image acquisition and data analysis. |
| Live cell imaging | OKOlab incubation system* regulation of temperature and CO2 and O2 level
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