

## 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	<b>LAS-X</b>
Epifluorescence	CoolLED pE-300
Lasers	<b>405 nm</b> (max 40 MHz, 3 mW) <b>445 nm</b> (max 40 MHz) <b>WLL 470-670 nm</b> (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	<b>DAPI</b> 325-375 400 435-485 <b>FITC</b> 460-500 505 512-542 <b>RHOD</b> 541-551 560 565-605 <b>CY5</b> 590-650 660 662-738 Analyser
Objectives	HC PL APO CS <b>10x</b> , <b>NA 0.40</b> , WD 2.2 mm HC PL APO CS2 <b>20x</b> , <b>NA 0.75</b> , WD 0.62 mm, DIC HC PL APO CS2 <b>63x WI</b> , <b>NA 1.2</b> , WD 0.30 mm, Correction Ring, DIC HC PL APO CS2 <b>63x Oil</b> , <b>NA 1.4</b> , 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)	<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">NF 488</td> <td style="width: 33%;">NF 445</td> </tr> <tr> <td>NF 514</td> <td>NF 594</td> </tr> <tr> <td>NF 458/514</td> <td>NF 445/594</td> </tr> <tr> <td>NF 488/561/633</td> <td>SMD1 NF 405/470</td> </tr> <tr> <td></td> <td>SMD2 NF 405/640</td> </tr> <tr> <td></td> <td>SMD3 NF 470/640</td> </tr> </table>	NF 488	NF 445	NF 514	NF 594	NF 458/514	NF 445/594	NF 488/561/633	SMD1 NF 405/470		SMD2 NF 405/640		SMD3 NF 470/640
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Detection	<p><b>Transmitted light detector (PMT)</b></p> <p><b>Internal spectrally tunable detection unit</b></p> <ul style="list-style-type: none"> <li>- <b>3x HyD detectors</b> for FLIM (time-gating possible), GaAsP, 400 – 720 nm, modes: Standard, Counting, BrightR; QE=45%@500nm</li> <li>- <b>2x PMT detectors</b>, 400-800nm, QE=30%@500nm</li> </ul> <p><b>Camera DFC365 FX (Leica)</b></p> <ul style="list-style-type: none"> <li>- 1392 x 1040 pixels</li> <li>- 6.45 µm x 6.45 µm</li> <li>- CCD sensor Sony ICX285</li> <li>- C-mount 0.7x</li> </ul>												
FLIM and FCS module	<p><b>HydraHarp400 (Picoquant)</b></p> <ul style="list-style-type: none"> <li>- Picosecond Event Timer &amp; TCSPC Module</li> <li>- Four channels available</li> <li>- 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.</li> </ul> <p><b>Symphotime64</b></p> <ul style="list-style-type: none"> <li>- Software for image acquisition and analysis</li> </ul> <p>Custom written software in LabView for image acquisition and data analysis.</p>												
Live cell imaging	<p>OKOlab incubation system</p> <ul style="list-style-type: none"> <li>- regulation of temperature and CO<sub>2</sub> and O<sub>2</sub> level</li> </ul>												