

## Leica TCS-SP5 System Specifications

### Microscope

The system is built on an inverted Leica DMI6000 microscope, fully motorized (XY scanning stage and Super-z galvo stage), implemented with all necessary accessories (Temperature and CO<sub>2</sub> control) for live imaging and equipped with the following objectives and epifluorescence filter cubes:

Objectives	Oil	Numerical Aperture (NA)	DIC	Specifications
10x	No	0.40	No	HC PL APO 10x/0,40 CS ∞/0.17/A
20x	No	0.70	No	HC PL APO 20x/0.70 CS ∞/0.17/C
40x	Yes	1.25	Yes	HCX PL APO 40X/1.25-0.75 OIL CS ∞/0.17/D
63x	Yes	1.40	Yes	HCX PL APO 63x/1.40-0.60 OIL λ BL ∞/0.17/E

Filter cube	Excitation filter	Dichroic mirror	Emission filter	Example fluorophores
A	BP340-380	400	LP425	Dapi, Hoestch
GFP	BP470/40	500	BP525/50	GFP
I3	BP450-490	510	LP515	FITC, Cy2
N2.1	BP515-560	580	LP590	Cy3, dsRED

### Lasers

The Leica SP5 uses an Acousto Optical Tunable Filter (AOTF) for laser line selection and intensity control with microsecond precision. The system contains the following laser lines for the excitation of fluorophores:

- Diode laser: 405nm
- Multiline Argon Laser: 458nm, 476nm, 488nm, 496nm, 514nm
- Solid State DPSS Laser: 561nm
- HeNe Laser: 633nm

### Spectral detection system

An Acousto Optical Beam Splitter (AOBS®) replaces fluorescent filters and beam splitters in the light path. In combination with AOTF, the AOBS offers a more light efficient system. This optical element allows the excitation light to excite the sample and the emission passes straight through the crystal.

The Spectral Detector SP ® is free from barrier filters and secondary dichroic filters and therefore highly transparent and extremely sensitive. A high efficient prism disperses light emitted from the sample. The resultant spectrum is directed towards two tunable slits. 2 motorized sliders acts as barriers and transmit a step-lessly tunable band to the sensor. The full spectrum can be split into several channels without any loss of energy. The Spectral Sliders offer a flexible system for wavelength discrimination, which can be used to minimize cross talk.

The system is equipped with three highly efficient photomultiplier, SP, for simultaneous detection of different fluorescent dyes, one transmitted light detector for acquisition of DIC or bright field images.

### Scanning system

The galvanometric Scanner can be set at variable speeds, from 1 to 1400Hz and up to 2800 Hz in bidirectional mode. Scanning format (image resolution) covers from 16x16 to 8192x8192 px.

The system may acquire 5 Images/second at 512 x 512, ~54 Images/second at 512 x 16

It has a very big scan field with 21,2 mm (SFZ) Diagonal in the Intermediate image plan Hardware zoom, stepless 1x - 64x.

### Environmental control

Environmental control (temperature and CO<sub>2</sub>) for live specimens ("Brick" and "Cube" Leica devices).

### Software

The system is controlled under the Leica LAS software, which works in two modes:

- **Leica Application Suite Advanced Fluorescence (LAS AF)**, which controls all scanner and image acquisition parameters, provides tools for organizing and archiving image data sets ("experiments") as well as tools for measuring and processing them, and contains specific wizards to perform advanced applications such as FRAP and FRET.
- **Live Data Mode (LDM)**, which allows for recording manual and automatic workflows.