Leica TCS SMD Series

Single Molecule Detection Platform

Technical Documentation

Living up to Life

Leica
MICROSYSTEMS

General Specifications of SMD Prepared Leica TCS SP5

Microscopes	Upright	Leica DM6000 CS
		Leica DM6000 CFS
	Inverted	Leica DMI6000 CS
		Leica DMI6000 CS bottom port
Microscope anti-vibration table	**Specification**	**For imaging**
	Vibration insulation	Passive
Z-drive	SuperZ galvanometer stage	1500 µm travel range/3 nm stepsize
	Motorfocus (stand)	Travel range depending on mechanics of microscope/15 nm step size
Continuous wave lasers	**Laser type**	**For imaging**
	VIS	Diode, 40 mW: 442 nm
		Ar, 65 mW: 458, 476, 488, 496, 514 nm
		HeNe, 1 mW: 543 nm
		HeNe, 2 mW: 594 nm
		HeNe, 10 mW: 633 nm
		DPSS, 20 mW: 561 nm
	UV	Diode, 50 mW: 405 nm
Pulsed lasers	**Laser type**	**For imaging**
	IR	TiSa 1.2 ps 1 W 690...1040 nm (various ranges)
	VIS	–
		–
	UV	–
Excitation modulation	**Modulation type**	**For imaging**
	AOTF VIS	Up to 8 channels
	AOTF UV	Up to 3 channels
	EOM IR	Yes
	Pulsed laser driver	Optional

Additional SMD Specific Specifications

TCS SMD FCS	TCS SMD FLIM	TCS SMD FLCS
Yes	Yes	Yes
Yes	Yes	Yes
Yes, recommended	Yes	Yes, recommended
Yes	Yes	Yes
For FCS	**For FLIM**	**For FCS and FLIM**
Passive	Passive	Passive
1500 µm travel range/3 nm stepsize	1500 µm travel range/3 nm stepsize	1500 µm travel range/3 nm stepsize
Travel range depending on mechanics of microscope/15 nm step size	Travel range depending on mechanics of microscope/15 nm step size	Travel range depending on mechanics of microscope/15 nm step size
For FCS	**No cw laser excitation for FLIM**	**For FCS**
Diode, 40 mW: 442 nm	–	Diode, 40 mW: 442 nm
Ar, 65 mW: 458, 476, 488, 496, 514 nm	–	Ar, 65 mW: 458, 476, 488, 496, 514 nm
HeNe, 1 mW: 543 nm	–	HeNe, 1 mW: 543 nm
HeNe, 2 mW: 594 nm	–	HeNe, 2 mW: 594 nm
HeNe, 10 mW: 633 nm	–	HeNe, 10 mW: 633 nm
DPSS, 20 mW: 561 nm	–	DPSS, 20 mW: 561 nm
–	–	–
Pulsed excitation for FCS not required	**For FLIM**	**For FLIM and FLCS**
–	TiSa 1.2 ps 1 W 680 ... 1040 nm (various ranges)	TiSa 1.2ps 1 W 680 ... 1040 nm (various ranges)
–	Diode, up to 3 mW average @ 40 MHz, 470 nm (software controlled selection of pulse frequency: 5, 10, 20, 40 MHz), < 90 ps ... < 500 ps (depending on selected power level)	Diode, up to 3 mW average @ 40 MHz, 470 nm (software controlled selection of pulse frequency: 5, 10, 20, 40 MHz), < 90 ps ... < 500 ps (depending on selected power level)
–	Diode, up to 4.5 mW average @ 40 MHz, 640 nm (software controlled selection of pulse frequency: 5, 10, 20, 40 MHz), < 90 ps ... < 400 ps (depending on selected power level)	Diode, up to 4.5 mW average @ 40 MHz, 640 nm (software controlled selection of pulse frequency: 5, 10, 20, 40 MHz), < 90 ps ... < 400 ps (depending on selected power level)
–	Diode, up to 3 mW average @ 40 MHz, 405 nm (software controlled selection of pulse frequency: 5, 10, 20, 40 MHz), < 70 ps ... < 300 ps (depending on selected power level)	Diode, up to 3 mW average @ 40 MHz, 405 nm (software controlled selection of pulse frequency: 5, 10, 20, 40 MHz), < 70 ps ... < 300 ps (depending on selected power level)
For FCS	**For FLIM**	**For FCS and FLIM**
Up to 8 channels	–	For FCS: 8 channels
–	–	–
–	Optional for MP FLIM	Optional for MP FLIM
–	For VIS and UV FLIM: up to 3 channels	For VIS and UV FLIM: up to 3 channels

General Specifications of SMD Prepared Leica TCS SP5

Optics	**Features**	**For imaging**
	Number of laser ports	Up to 3 (UV - VIS - IR)
	Number of lasers	Up to 8
	Excitation – emission splitting	Acousto Optical Beam Splitter (AOBS®) or dichroic beam splitters
	Detection range	400...800 nm
	UV and IR imaging	Sequential or simultaneous
	Field upgradable	To IR: yes
	UV correction	Individual precise correction optics (up to 6 positions)
	Pinhole	Alignment stable single pinhole
	Pinhole diameter control	Motorized by software, automatic mode available
Scanner	**Scanner design**	**For imaging**
	Scanning concept	Optically correct scanning at low inertia
	Switch conventional – resonant scanner	Conventional and resonant scanner in one system (optional)
	Conventional scanner	**For imaging (PMT and APD)**
	Maximal line frequency	2800 Hz
	Minimal line frequency	1 Hz
	Scan speed granulation	1400
	Maximal frame rate 512 x 512	5 Hz
	Maximal frame rate 512 x 16	50 Hz
	Beam park	Yes
	Maximal frame resolution	8192 x 8192 pixel
	Scan zoom	1.0 ... 64 x
	Panning	Yes
	Field rotation	200° optical
	Field diameter	22 mm

Additional SMD Specific Specifications

TCS SMD FCS	TCS SMD FLIM	TCS SMD FLCS
For FCS	**For FLIM**	**For FCS and FLIM**
1 (VIS)	2 (UV & IR, or UV & VIS)	For FCS: 1 (VIS) For FLIM: 2 (UV & IR, or UV & VIS)
Up to 8	Up to 3	For FCS: up 8, For FLIM: up to 3
AOBS	For pulsed lasers: dichroic mirrors	For FCS: AOBS, For pulsed lasers: dichroic mirrors
Depending on filter cube used	Internal SP FLIM: 400 … 800 nm External FLIM: Depending on filter cube used	Depending on filter cube used
–	–	Internal SP FLIM: 400 … 800 nm External FLIM, FCS, and FLCS: Depending on filter cube used
To FCS: yes	To FLIM: yes	–
UV not required for FCS	Required for UV FLIM	To FLCS: yes
Alignment stable single pinhole	Alignment stable single pinhole	Required for UV FLIM
Motorized by software, automatic mode available	Motorized by software, automatic mode available	Alignment stable single pinhole
For FCS	**For FLIM**	**For FCS and FLIM**
Beam park	Optically correct scanning at low inertia	FCS: Beam park FLIM: optically correct scanning at low inertia
Conventional scanner required	Conventional and resonant scanner in one system (optional)	Conventional scanner required, resonant scanner optional
No scanning during FCS measurement	**For FLIM data acquisition**	**For FLIM data acquisition**
–	1400 Hz	1400 Hz
–	1 Hz	1 Hz
–	1400	1400
–	5 Hz	5 Hz
–	50 Hz	50 Hz
–	Yes	Yes
–	512 x 512 pixel	512 x 512 pixel
–	1.0 ... 64 x	1.0 ... 64 x
–	no	no
–	200° optical	200° optical
–	22 mm	22 mm

General Specifications of SMD Prepared Leica TCS SP5

Scanner	**Resonant scanner**	**For imaging**
	Maximal line frequency	16000 Hz
	Minimal line frequency	8000 Hz
	Scan speed granulation	1
	Maximal frame rate 512 x 512	28 Hz
	Maximal frame rate 512 x 16	290 Hz
	Beam park	No
	Maximal frame resolution	1024 x 1024 pixel
	Scan zoom	1.7 ... 64 x
	Panning	Yes
	Field rotation	200° optical
	Field diameter	15 mm
Scan modes	**Scan options**	**For imaging**
	xt	Yes
	xy	Yes
	xyt	Yes
	xyλ	Yes
	xz	Yes
	xzλ	Yes
	xyz	Yes
	xyzλ	Yes
	xyt	Yes
	xzt	Yes
	xyzt	Yes
	xytz	Yes
	Automated brightness control of FLIM images	–
	Maximum number of FLIM images	–
Beam park positioning	**Beam park options**	**For spot bleach (in general software)**
	xy	Yes
	xyz	No
	xz	No
	xzy	No
	Maximum number of FCS/FLCS measurements	–
	Maximum number of distinct measurement points	–
	Maximum number of measurement repetitions at a point	–
	Maximum number of measurement cycles	–

Additional SMD Specific Specifications

TCS SMD FCS	TCS SMD FLIM	TCS SMD FLCS
No scanning during FCS measurement	**For FLIM data acquisition**	**For FLIM data acquisition**
–	8000 Hz	8000 Hz
–	8000 Hz	8000 Hz
–	1	1
–	28 Hz	28 Hz
–	290 Hz	290 Hz
–	No	No
–	512 x 512 pixel	512 x 512 pixel
–	1.7 ... 64 x	1.7 ... 64 x
–	No	No
–	200° optical	200° optical
–	15 mm	15 mm
No scanning during FCS measurement	**For FLIM data acquisition**	**For FLIM data acquisition**
–	No	No
–	Yes	Yes
–	Yes	Yes
–	Yes (SP FLIM)	Yes (SP FLIM)
–	Yes	Yes
–	Yes (SP FLIM)	Yes (SP FLIM)
–	Yes	Yes
–	Yes (SP FLIM)	Yes (SP FLIM)
–	Yes	Yes
–	Yes	Yes
–	Yes	Yes
–	No	No
–	Yes	Yes
–	1000	1000
For automated FCS, FCCS, FLCS data acquisition series (in FCS wizard)	**For single point lifetime measurement (in general software)**	**For automated FCS, FCCS, FLCS data acquisition series (in FCS wizard)**
Yes	Yes	Yes
Yes	No	Yes
Yes	No	Yes
Yes	No	Yes
1000	–	1000
100	–	100
100	–	100
100	–	100

General Specifications of SMD Prepared Leica TCS SP5

Internal confocal detection	**Detection features**	**Up to 5 PMT for confocal imaging**
	Emission separation	Highly sensitive prism spectral detector
	Maximum number of confocal channels	5
	Tunability of emission bands	Yes
	Spectral detection range	400 – 800 nm
	Tuning steps of emission bands	1 nm
	Minimal detection range	5 nm
	Sensors	High sensitivity low noise PMT: R 9624
	Dark current	–
	TTS FWHM	–
External confocal detection	**Detection features**	**2 APDs for confocal imaging**
	Emission separation	User-exchangeable beam splitting filter cubes
	Confocal channels	2
	Sensors	APDs from PE (SPCM-AQRH series) or MPD (PDM series)
	Quantum efficiency	PE APD: wavelength dependent, typ. 65% @ 670 nm MPD APD: wavelength dependent, typ. 45% @ 550 nm
	Dark counts	PE APD: < 250 cps MPD APD: < 250 cps
	Jitter FWHM	Not relevant
	Dead time	Not relevant
Non-confocal detection	**Detection types**	**For Imaging**
	Transmitted light detector	Optional, allowing BF, DIC, Ph etc.
	Non descanned transmitted light channels	Up to 4 channels (MP)
	Non descanned reflected light channels	Up to 4 channels (MP)
Electronics	**Devices**	**For imaging**
	Scanner control	Digitally at high performance (FPGA, field programmable gate arrays)
	Trigger in/out functions	Yes
	Auxiliary data input channels	Up to 2
	Max channels in parallel	12
	Computer	High performance PC workstation
	Integration of third party software	–
	–	Programmable control panel with LCD function & value display

Additional SMD Specific Specifications

TCS SMD FCS	TCS SMD FLIM	TCS SMD FLCS
Not for FCS data acquisition	**Up to 2 FLIM-PMTs for spectral FLIM**	**Up to 2 FLIM-PMTs for spectral FLIM**
–	Highly sensitive prism spectral detector	Highly sensitive prism spectral detector
–	2 for FLIM + 3 for imaging	2 for FLIM + 3 for imaging
–	Yes	Yes
–	400 – 800 nm	400 – 800 nm
–	1 nm	1 nm
–	5 nm	5 nm
–	SP FLIM PMT (Hamamatsu R7400U series, active cooled)	SP FLIM PMT (Hamamatsu R7400U series, active cooled)
–	< 300 cps @ 15 °C	< 300 cps @ 15 °C
–	MP FLIM: < 300 ps UV & VIS FLIM: < 400 ps	MP FLIM: < 300 ps UV & VIS FLIM: < 400 ps
2 external detectors for FCS & FCCS	**2 external detectors for FLIM**	**2 external detectors for FCS, FCCS, FLCS, and FLIM**
User-exchangeable beam splitting filter cubes	User-exchangeable beam splitting filter cubes	User-exchangeable beam splitting filter cubes
2	2	2
APDs from PE (SPCM-AQRH series) or MPD (PDM series)	APDs from MPD (PDM series)	APDs from MPD (PDM series)
PE APD: wavelength dependent, typ. 65% @ 670 nm MPD APD: wavelength dependent, typ. 45% @ 550 nm	Wavelength dependent, typ. 45% @ 550 nm	Wavelength dependent, typ. 45% @ 550 nm
PE APD: < 250 cps MPD APD: < 250 cps	MPD APD: < 250 cps	MPD APD: < 250 cps
Not relevant	MPD APD: 400 – 500 nm: typ. 200 ps > 500 nm: down to 50 ps	MPD APD: 400 – 500 nm: typ. 200 ps > 500 nm: down to 50 ps
Below typical count rate (40 ... 70 ns)	Below typical count rate (40 ... 70 ns)	Below typical count rate (40 ... 70 ns)
None for FCS	**None for FLIM**	**None for FLIM and FCS**
–	–	–
–	–	–
–	–	–
For FCS	**For FLIM**	**For FCS and FLIM**
Digitally at high performance (FPGA, field programmable gate arrays)	Digitally at high performance (FPGA, field programmable gate arrays)	Digitally at high performance (FPGA, field programmable gate arrays)
Not required	Required	Required
Used for APD imaging	Optional: Used for APD imaging	Used for APD imaging
12	12	15
Second workstation for FCS data acquisition and analysis	Second workstation for FLIM data acquisition and analysis	Second workstation for FCS and FLIM data acquisition and analysis
Client server network connection between workstations for full system control and data transfer	Client server network connection between workstations for full system control and data transfer	Client server network connection between workstations for full system control and data transfer
Required	Required	Required

General Specifications of SMD Prepared Leica TCS SP5

Extensions	**Devices**	**For imaging**
	Fast ROI-spectrometer	Optional
	Auxiliary emission port	Optional
	Environment accessories	Various options
Software (LAS AF)	**General**	**Intuitive and guiding user interface**
	Context sensitive online help system	Included
	Multi-dimensional data acquisition	Included
	Region of interest (ROI) scan	Included
	Excitation line/frame sequential scan	Included
	Emission spectrum recording	Included
	Quantification tools	Included
	Multi-color restoration, spectral unmixing	Included
	General time lapse experiment control tile scanning (mosaic scan)	Included
Software options (LAS AF)	**Dedicated application wizards**	**For imaging**
	Live Data Mode	Interactive data recording also allowing job sequencing and online evaluation
	Advanced Mark & Find	Combines Mark & Find with sophisticated 3D recordings, Live Data Mode etc.
	3D visualization	Maximum and other projections, simulated fluorescence process, rotation animations, stereo pairs, red-green anaglyphs, height color coded extended depth of focus images etc.
	Colocalization	Histogram based colocalization and area measurements
	Deconvolution	Deconvolution option for widefield and confocal images
	MicroLab	FRAP wizard, FRAPxt wizard, FLIP wizard, FRET SE wizard, FRET AB wizard etc.
	SMD FCS wizard	–
	SMD FLIM wizard	–

	Additional SMD Specific Specifications		
	TCS SMD FCS	TCS SMD FLIM	TCS SMD FLCS
	For FCS	**For FLIM**	**For FCS and FLIM**
	Not possible	Optional with internal SP FLIM Not possible with external FLIM	Not possible
	Not possible	Optional with internal SP FLIM Not possible with external FLIM	Not possible
	Various options	Various options various options	Various options
	Included	**Included**	**Included**
	Included	Included	Included
	Included	Included	Included
	included	included	included
	Included	Included	Included
	Included	Included	Included
	Included	Included	Included
	Included	Included	Included
	Included	Included	Included
	For FCS/FCCS	**For FLIM**	**For FCS/FCCS/FLCS and for FLIM**
	–	–	–
	–	–	–
	–	–	–
	–	–	–
	–	–	–
	–	–	–
	FCS wizard for optimization and automation of FCS and FCCS measurement series	–	FCS wizard for optimization and automation of FCS, FCCS and FLCS measurement series
	–	FLIM wizard for optimization and automation of FLIM measurement stacks and time series	FLIM wizard for optimization and automation of FLIM measurement stacks and time series

General Specifications of SMD Prepared Leica TCS SP5

Software features SymphoTime	**General**	**For imaging**
	Supported time-resolved analysis methods	–
	One-line visualization for time-resolved data acquisition	–
	FLIM/FCS options	**For imaging**
	Data acquisition method	–
	TCSPC channel width	–
	Resolvable lifetime range	–
	FLIM analysis	**For imaging**
	Data processing	–
	Fitting models	–
	Optimization methods	–
	Error test/assessment	–
	Error analysis	–
	FCS analysis	**For imaging**
	Correlation method	–
	Fitting model	–
	Optimization methods	–
	Error test/assessment	–
	Error analysis	–

Additional SMD Specific Specifications

TCS SMD FCS	TCS SMD FLIM	TCS SMD FLCS
For FCS/FCCS	**For FLIM**	**For FCS/FCCS/FLCS and for FLIM**
FCS, FCCS, FRET, Scripting language for user-defined analysis routines	FLIM, FLIM-FRET, Lifetime Histogram, Scripting language for user-defined analysis routines	FLIM, FLIM-FRET,FCS, FCCS, FLCS, FRET, Lifetime Histogram, Fluorescence Time/Lifetime Traces, Scripting language for user-defined analysis routines
Auto- or Crosscorrelation, Intensity time-trace	FLIM image, Intensity-time trace, TCSPC histogram	Auto- or Crosscorrelation, FLIM image, Intensity-time trace, TCSPC histogram
For FCS/FCCS	**For FLIM**	**For FCS/FCCS/FLCS and for FLIM**
Time-Tagging of photon arrival times using Time-Correlated Single Photon Counting (TCSPC) electronics	Time-Correlated Single Photon Counting (TCSPC)	Time-Correlated Single Photon Counting (TCSPC)
–	min. 4 ps	min. 4 ps
–	< 100 ps to some µs (depending on system configuration and experimental conditions)	< 100 ps to some µs (depending on system configuration and experimental conditions)
For FCS/FCCS	**For FLIM**	**For FCS/FCCS/FLCS and for FLIM**
–	Whole image or ROIs (arbitrary shape)	Whole image or ROIs (arbitrary shape)
–	1 to 4 exponentials, iterative reconvolution or tail fitting	1 to 4 exponentials, iterative reconvolution or tail fitting
–	Least squares, MLE, Marquardt-Levenberg, Monto Carlo	Least squares, MLE, Marquardt-Levenberg, Monto Carlo
–	Chi-Square, distribution weighted residuals	Chi-Square, distribution weighted residuals
–	Asymptotic standard errors	Asymptotic standard errors
For FCS/FCCS	**For FLIM**	**For FCS/FCCS/FLCS and for FLIM**
Software correlation (auto- and crosscorrelation)	–	Software correlation (auto- and crosscorrelation)
Pure diffusion, triplet-state, conformational, protonation, 2D/3D Gaussian PSF	–	Pure diffusion, triplet-state, conformational, protonation, 2D/3D Gaussian PSF
Least squares, Marquardt-Levenberg, Monto Carlo	–	Least squares, Marquardt-Levenberg, Monto Carlo
Chi-Square, distribution weighted residuals	–	Chi-Square, distribution weighted residuals
Asymptotic standard errors, bootstrap and support plane analysis	–	Asymptotic standard errors, bootstrap and support plane analysis

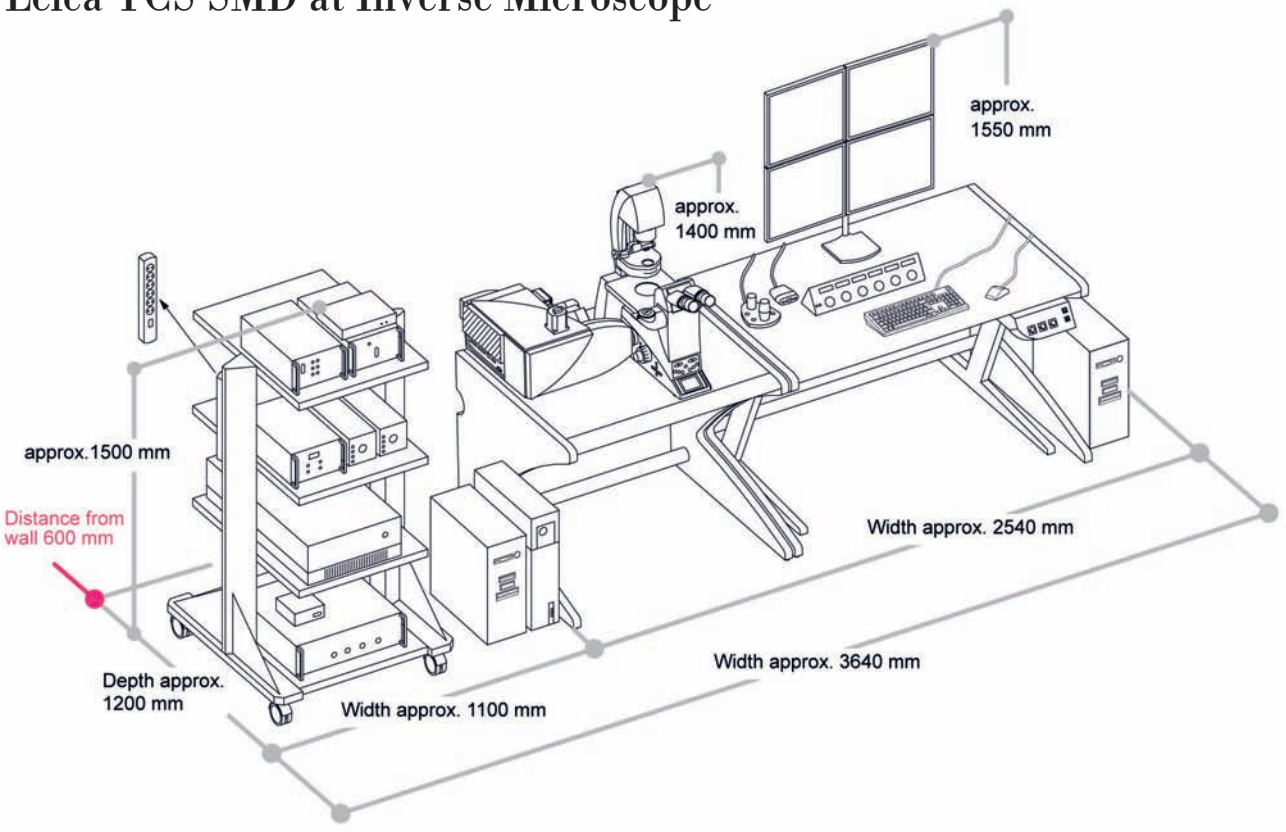
Installation Requirements

Weight base system	VIS	Max. 320 kg
	IR	Optical bench 900 x 1500 mm: + ca. 280 kg IR laser system: + ca. 100 kg
	SMD specific components	+ up to 160 kg
Heat load max.	VIS	3.2 kW
	IR	6.2 kW
Separate cooling	IR laser	IR laser, air-cooled heat exchanger (chiller)
Electric supply	VIS lasers (with or without 405 nm cw laser)	3 x 100 … 120 or 200 … 240 V AC. 1600 VA, 50/60 Hz
	IR laser	+1x 100 … 120 or 200 … 240 V AC, 15 … 10 A, 50/60 Hz
	Chiller for IR laser	+1x 100 … 120 or 200 … 240 V AC, 10 A/6 A, 50/60 Hz
	SMD	+1x 100 … 120 or 200 … 240 V AC, 20 A/16 A, 50/60 Hz
Environment	Room temperature	+18 to + 25°C, avoid proximity to air conditioning equipment!
	Temperature for optimal optical behavior	+21 to + 23 °C
	Vibration velocity @ Frequency range 5 Hz–30 Hz @ Frequency range 5 Hz–30 Hz	 < 30 µm/s (effective value) < 60 µm/s (effective value)
	Pollution degree	Class 2, protect from dust!
	Relative humidity	20 – 80% (not condensing)
	Illumination	Room darkening recommended!
	Load carrying capacity	200 kg/m^2
	Door width	> 1.00 m

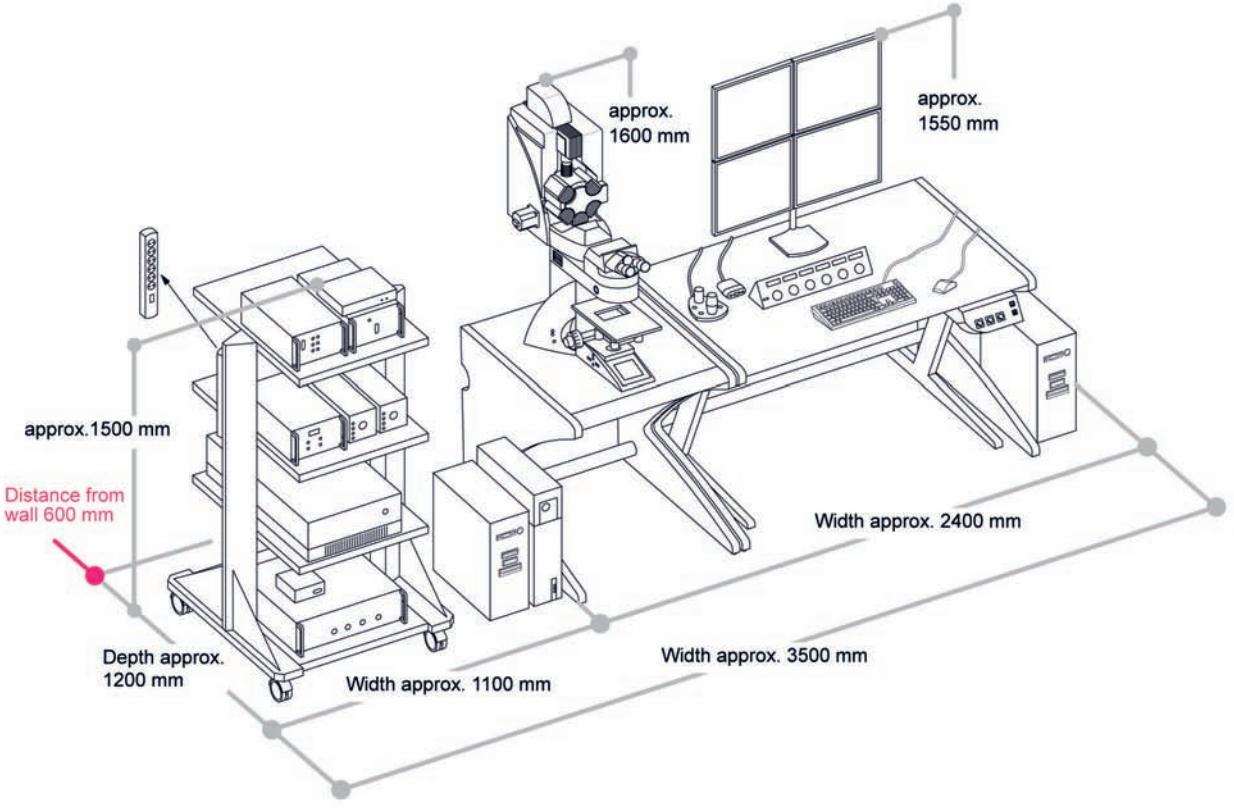
visible and ultraviolet radiation:

infrared radiation:

Leica TCS SMD at Inverse Microscope



Leica TCS SMD at Upright Microscope



“With the user, for the user”

Leica Microsystems

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The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems’ customers at the leading edge of science.

• Industry Division

The Leica Microsystems Industry Division’s focus is to support customers’ pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

• Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

• Surgical Division

The Leica Microsystems Surgical Division’s focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life**.

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