

Leica TCS STED

Beyond the Limits!

Technical Documentation

Leica TCS STED Features

- Fully integrated STED-software for immediate and easy access to superresolution
- Improved resolution purely optically achieved: special point spread function with optimal "zero"
- STED Platform: Leica TCS SP5 as well approved basis for high stability and minimized failure rate
- Software controlled auto-alignment of excitation and depletion beam: easy, time saving & long term stable
- STED penetration depth up to 20 μm (dependent on sample and embedding): inner regions of samples can be investigated with STED
- Special STED objective: perfect chromatic correction for maximized STED efficiency
- STED as Leica TCS SP5 upgrade available
- Combination of dynamic spectral photo detectors (PMT) and ultra sensitive Avalanche Photo Diodes (APD): sample adapted detection, less critical sample preparation
- Complete TCS SP5 AOBs with full range multiphoton for maximum flexibility: suited for imaging core facilities
- Fully integrated DFC360 FX: for fast visualization of invisible IR-emitting ATTO-dyes
- Combination of STED and up to 4 confocal detection channels: for highest resolution multicolor recordings

Leica TCS SP5 Highlights

- High speed live cell imaging and high resolution morphology - All In One
- Prism spectrometer for high transmittance and tunability
 - Fastest true confocal system with rates up to 200 frames per seconds
 - Acousto-optical beam splitter (AOBS): maximal transmissive and spectrally adaptive
- Region of interest spectrometer: fast spectra from living samples in situ
- Illumination regimes switchable in microseconds: fast dynamic measurements
- Software wizards for FRAP, FLIP and FRET



- 1 Inverted research microscope DMI6000 CS
 - 2 Scan head
 - 3 Laser and power supply
 - 4 Computer table
 - 5 Air damped optical table
 - 6 Control panel
 - 7 Supply control
 - 8 Beam routing for infrared laser
 - 9 STED module
 - 10 APD detection unit
 - 11 PDL-800 for 635 nm STED excitation laser
 - 12 Titan Sapphire laser
- (Not shown: DFC 360FX and NDD detection unit)

STED Modul		
Mechanics		Ultra stable and compact device, firmly fixed to scanner
Lasers	STED excitation	pulsed (80 MHz) diode 9 mW 640nm
	STED Depletion	pulsed (80 MHz) TitanSapphire, tuning range: 725- 850 nm, variable
output power		
Software		
Optomechanics		
	used imaging port	UV-port (no UV available)
	modulation of depletion PSF	automated beam adjustment for perfect alignment of excitation and
depletion laser, average duration: <2 min		

Specifications*

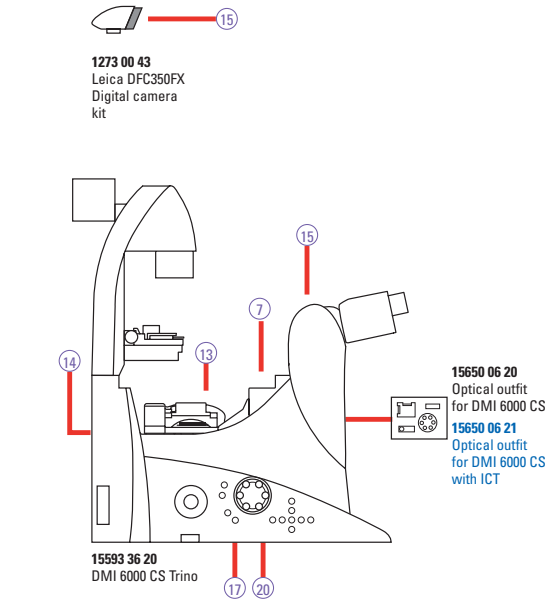
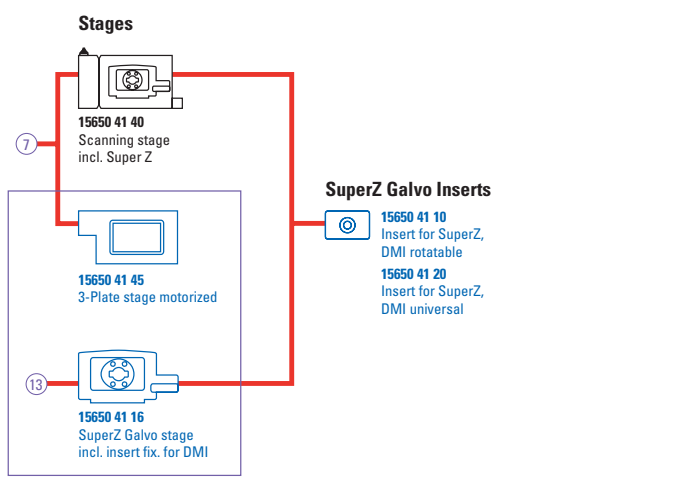
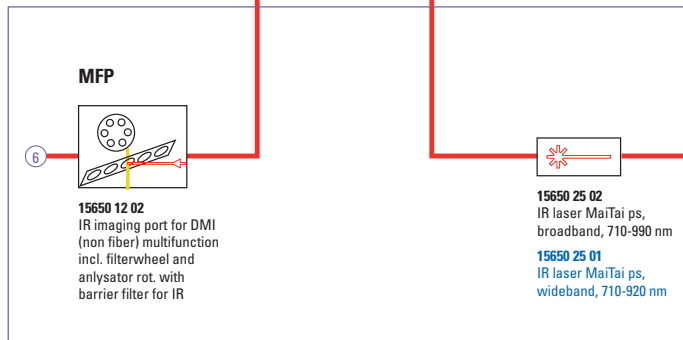
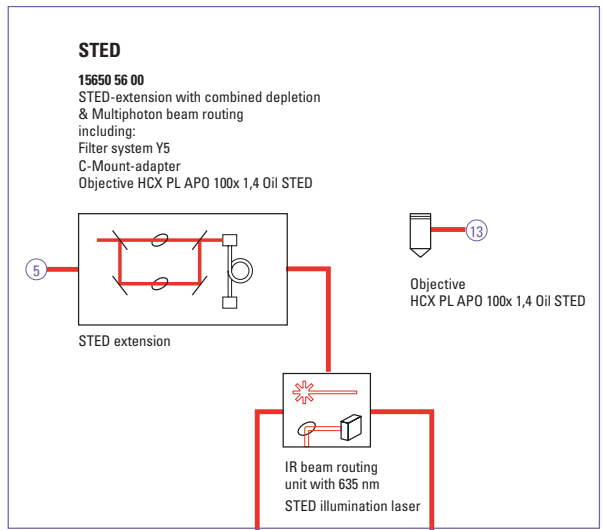
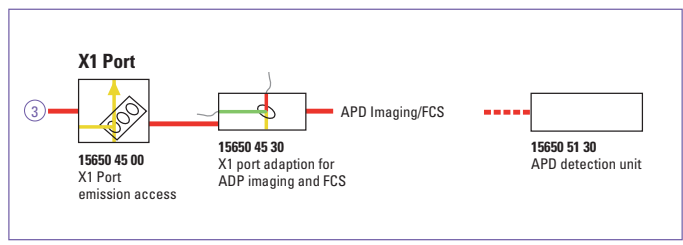
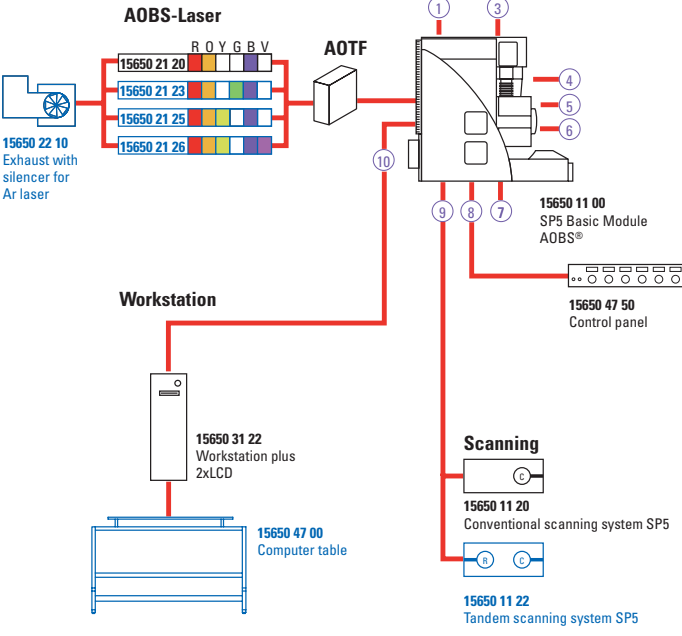
Microscope	inverted	Leica DMI6000 CS Trino
Z-drive	SuperZ galvanometer stage motorfocus (stand)	1500 µm travel range/3 nm stepsize travel range depending on mechanics of microscope/15 nm step size
Lasers	VIS	diode 18 mW 442 nm Ar 100 mW 458, 476, 488, 496, 514 nm HeNe 1 mW 543 nm HeNe 2 mW 594 nm HeNe 10 mW 633 nm DPSS 10 mW 561 nm
Pulsed lasers	IR	TiS 1.2ps 1 W 720...1000 nm (various ranges)
Excitation modulation	STED excitation	diode 9 mW 640 nm
	AOTF VIS	8 channels
Optics	EOM IR [AOM IR]	yes
	number of laser ports for imaging	3 (STED, VIS, IR)
	number of lasers for imaging	up to 8
	excitation - emission splitting	Acousto Optical Beam Splitter (AOBS®) or dichroic mirrors
	detection range	400...800 nm
	pinhole	alignment stable single pinhole
	pinhole diameter control	motorized by software, automatic mode available
Scanner	scanning concept	optically correct scanning at low inertia
	switch conventional - resonant scanner	conventional and resonant scanner in one system (optional)
	vibration insulation	active
Conventional (C)	max line frequency	2800 Hz
	min line frequency	1 Hz
	scan speed granulation	1400
	max frame rate 512 x 512	5 Hz
	max frame rate 512 x 16	50 Hz
	beam park	yes [no]
	max frame resolution	8192 x 8192 pixel
	scan zoom	1,0 ... 64x [2,0 ... 64x]
	panning	yes
	field rotation	200° optical [no]
	field diameter	22 mm [75 µm]
Resonant (R)	max line frequency	16000 Hz
	min line frequency	8000 Hz
	scan speed granulation	1
	max frame rate 512 x 512	25 Hz
	max frame rate 512 x 16	250 Hz
	beam park	no
	max frame resolution	1024 x 1024 pixel
	scan zoom	1,7 ... 64x [2 ... 64x]
	panning	yes
	field rotation	200° optical [no]
	field diameter	15 mm [75 µm]

* Text in brackets: deviant specifications when system is used in STED mode

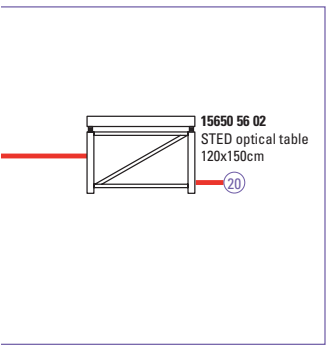
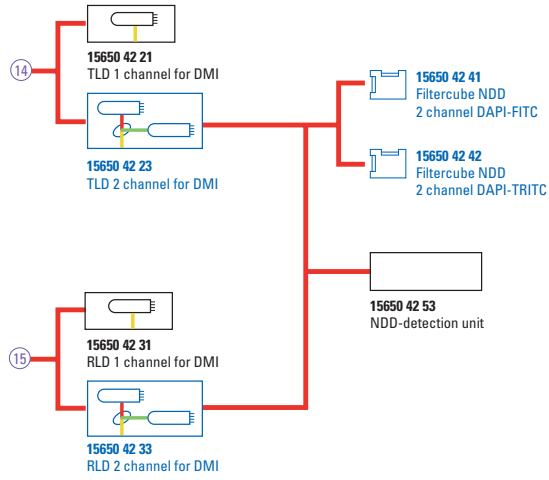
Scan modes	t	yes (conventional scanner)
	xt	yes
	xλ	yes
	xyt	yes
	xyλ	yes
	xz	yes
	xzλ	yes
	xyz	yes
	xyzλ	yes
	xyt	yes
	xzt	yes
	xyzt	yes
	xytz	yes
Detection	emission separation	highly sensitive prism spectral detector
	max spectral confocal channels	4 + 1 STED [PMT 4 with BP 680/60]
	tunability of emission bands	yes
	tuning steps of emission bands	1 nm
	sensors	high sensitivity low noise PMT: R 9624 ultra sensitive Avalanche Photo Diodes (APD): 2 [1]
	digitization	high speed CCD Camera: DFC360 FX, fully integrated into LAS AF
	max grey resolution	12 or 8 bit per channel
	read out frequency	16 bit imaging
	transmitted light detector	40 MHz
	non descanned transmitted light channels	optional, allowing BF, DIC, Ph etc
	non descanned reflected light channels	1 – 2
Electronics	scanner control	digitally at high performance (FPGA, field programmable gate arrays)
	trigger out functions	yes
	computer	high performance PC workstation programmable control panel with LCD function & value display
Software	general	intuitive and guiding user interface context sensitive online help system multi-dimensional data acquisition region of interest (ROI) scan excitation line/frame sequential scan emission spectrum recording quantification tools multi-color restoration, spectral unmixing general time lapse experiment control tile scanning (mosaic scan) fully integrated STED control
Software options	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 visualisation	maximum and other projections, simulated fluorescence process, rotation animations, stereo pairs, red-green anaglyphes, height color coded extended depth of focus images etc.
	Colocalisation	histogram based colocalisation and area measurements
	Deconvolution	deconvolution option for widefield images, confocal images and 4pi images
	MicroLab	FRAP wizard, FRAPxt wizard, FLIP wizard, FRET SE wizard, FRET AB wizard etc.

Laser:

- R: HeNe 10 mW 633 nm
- O: HeNe 2 mW 594 nm
- Y: DPSS 10 mW 561 nm
- G: HeNe 1 mW 543 nm
- B: Ar 100 mW 458, 476, 488, 496, 514 nm
- V: Diode 18 mW 442 nm



System Overview Leica TCS STED

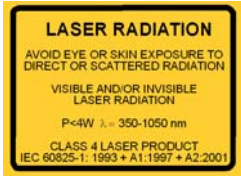


Legend:

Black elements: basic modules

Blue elements: optional modules

Installation Requirements



Including optical bench & IR laser system:	740 kg
Heat load max:	6,2 kW
Separate cooling:	IR laser, air cooled heat exchanger (chiller)
Electric supply:	Minimum three separate phases: VIS lasers: 100...240 V AC ± 10% 2 x 1600 VA, 50/60Hz (Power Input 1 + 2)
	IR laser: 100...240 V AC ± 10% 15...10 A, 60/60 Hz
	Chiller for IR laser: 110 V/230 V AC ± 10% 10 A/6, 50/60 Hz
Environment:	Temperature: 23°C ± 1°C
Humidity: ca: 40 – 50% 1013 hPa	
	Max floor vibration amplitudes: - frequency range 5 Hz ... 30 Hz: 30 µm/sec rms - frequency range > 30 Hz: 60 µm/sec rms
	Internet access for advanced remote diagnostics Room must comply with country-specific regulations for laser class IV Room darkening recommended Protect from dust Separate room for IR laser chiller recommended (fiber length: 3 m) to minimize vibrations, noise and heat

