

STED (Stimulated Emission Depletion) Superresolution Fluorescence Microscope

Technical Specifications of Leica TCS STED (I) (preliminary, subject to change)

Confocal and Multiphoton Base System:

Inverted Research Microscope Leica DMI6000 CS

Fluorescence optical outfit with external illumination EL6000 and fiber coupling

Spectral Confocal Laser Scanning System Leica TCS SP5 with Tandem Scanner (resonant and conventional)

RGB visible lasers with AOTF control, fiber coupled

AOBS Acousto-optical beam splitter

Up to 5 spectral detector channels:

- 4 confocal/two Photon channels
- 1 STED channel

2 APD (Avalanche Photo Diode) channels (1 usable for STED)

Laser scanning transmission detector

Scanning stage including SuperZ Galvo

Optional: Non descanned transmitted & reflected light detectors

STED extension

- Excitation: Single wavelength excitation by 635 nm Diode Laser
- Depletion: Spectra-Physics MaiTai Broadband; usable for STED via fiber coupling (range 735-800 nm) or full spectral range via direct incoupling for conventional two photon microscopy
- xy resolution (FWHM) depends on dye, sample, wavelength, typical 90 nm, typical point object separation w/o deconvolution ~ 70 nm
- z Resolution (FWHM): ~550 nm
- Auto beam alignment of excitation beam and depletion beam for long term stability and high ergonomics
- STED coupling is occupying UV port of the Leica TCS SP5
- UV stainings can still be excited using the two photon laser in non-STED operation

STED (Stimulated Emission Depletion) Superresolution Fluorescence Microscope

Technical Specifications of Leica TCS STED (II) (preliminary, subject to change)

STED Extension (continued)

- Applicable STED dyes:
 - Atto 647N (750 nm depletion)
 - Atto 655 (780 nm depletion)
 - (other dyes under investigation)
 - STED Imaging:
 - Specially selected STED Objective
 - HCX PL APO 100x1.4 Oil CS STED
 - Scan field 75x75 μm^2 or smaller
 - Pixel size < 30 nm for full resolution transfer
- Typical attainable penetration depth with high STED efficiency:
10-15 μm
depending on:
- density
 - staining
 - refractive index
 - embedding medium
- Typical frame rate for STED imaging
 - 512 x 512 pixels: 1 minute
 - Faster frame rates in smaller scanning formats

STED detection is selectable (one STED channel):

APD (Avalanche Photo
Detector) for highest sensitivity

PMT (Photomultiplier) for
largest dynamic range

Basic room requirements (preliminary):

Temperature: 20° C \pm 1° C; 21° C \pm 1° C; 22° C \pm 1° C

Humidity : ca: 40-50% 1013 hPa

Max floor vibration amplitudes:

- frequency range 5 Hz ... 30 Hz: 30 $\mu\text{m}/\text{sec}$ rms

- frequency range > 30 Hz: 60 $\mu\text{m}/\text{sec}$ rms

Darkened room recommended

Power requirements:

minimum: three separate phases, 200 V~ to 240 V~, 50/60 Hz,
fuses 12 A to 16 A, depending on country-specific safety
regulations, alternatively (not to be combined):

minimum: three separate phases, 100 V~ to 120 V~, 50/60 Hz,
fuses 20A, depending on country-specific safety regulations

Max heat load 6,2 kW

Room must comply with country-specific regulations for Laser Class IV