

Super-Resolution Microscopy of the Synaptonemal Complex within the *Caenorhabditis elegans* Germline

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Materials

Name	Company	Catalog Number	Comments
100x/1.5 oil objective	Olympus	UPLAPO100XOHR	UPLAPO100XOHR
2-mercaptoethylamine (MEA)	Sigma-Aldrich	30070-10G	Dissolved in MilliQ water to 5 M solution, pH 8.7 adjusted with HCl. Aliquoted to a single-use volume, frozen, and kept at -80 °C.
Additional 640 nm booster laser	Toptica	IBEAM-SMART-640-S-HP	
AlexaFluor 594, NHS ester	ThermoFischer Scientific	A37572	Dissolved in DMSO to 1 mM solution, aliquoted to single use volume, frozen and kept at -80 °C
AlexaFluor 647, NHS ester	ThermoFischer Scientific	A37573	Dissolved in DMSO to 1 mM solution, aliquoted to single use volume, frozen and kept at -80 °C
anti-HA	Thermo Fisher Scientific	2-2.2.14	Mouse monoclonal, 1:250 (SMLM), 1:1,000 (STED Microscopy)
anti-HTP-3	a gift from Abby F. Dernburg	MacQueen et al., 2005	Chicken polyclonal, 1:250 (SMLM), 1:1,000 (STED Microscopy)
<i>Caenorhabditis elegans</i> strain YKM349	a gift from Yumi Kim	Hurlock et al., 2020	syp-5(kim9[syp-5::HA]) I; mels8[pie-1p::GFP::cosa-1, unc-119(+)] II
CF6680, NHS ester	Biotium	92139	Dissolved in DMSO to 1mM solution, aliquoted to single use volume, frozen and kept at -80 °C
Circular cover glass 12 mm No. 1	Menzel-Gläser; VWR	631-0713	
Circular cover glass 24 mm No. 1.5	Carl Roth	PK26.1	
Cylindrical lenses	Thorlabs	LJ1516RM-A, LK1002RM-A	
Egg Buffer (10x)		Edgar 1995	250 mM HEPES, 1.18 M NaCl, 480 mM KCl, 20 mM EDTA, 5 mM EGTA, pH 7.4
Ethanol (absolute for analysis)	Merck	64-17-5	
F(ab') ₂ fragment anti-chicken IgY	Jackson Immunoresearch	AB_2340347	Donkey polyclonal, 1:100 (SMLM), 1:1,000 (STED Microscopy)

F(ab') ₂ fragment anti-mouse IgG	Jackson Immunoresearch	AB_2340761	Donkey polyclonal, 1:100 (SMLM), 1:1,000 (STED Microscopy)
Fisherbrand Microscope slides T/F Ground 0.8-1.0 mm thick	Fisher scientific	7107	
Gauge Worm Pick 30 diameter 0.254 mm - Iridium 10%	Kisker	789265	
Glucose oxidase/Catalase enzyme mix (GIOX/Cat)	a gift from Jonas Ries	Hoess, Mund, Reitberger, & Ries, 2018	20x, 1916 U/mL glucose oxidase (Sigma G7141), 42350 U/mL catalase (Sigma C3155), 50 mM Tris-HCl pH 8.0, 51% glycerol, MilliQ water. Stored at -20 °C.
Imaging buffer base	a gift from Jonas Ries	Hoess, Mund, Reitberger, & Ries, 2018	50 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10% D-Glucose. Aliquoted to a single-use volume (950 µL), frozen, and kept at -80 °C.
Invitrogen ProLong Glass Antifade Mountant	ThermoFischer Scientific	P36982	
Leica Stellaris 8 STED FALCON	Leica	N/A	The microscope is equipped with the latest generation white light laser, a 775nm pulsed STED laser, the FALCON Fluorescence Lifetime IM aging module, HC PL APO CS2 100x/1.40 oil objective, and Leica HyD X detector. The system is capable of FLIM module enhanced Tau-STED which measures the specific fluorescence lifetime of a dye and is therefore capable of removing background signal based on differences in fluorescence lifetimes of the dyes, and dye conditions in the sample. Additionally, the resolution is increased by accounting for the variation of fluorescence lifetimes in different areas of the depletion donut.
Longwave channel emission filter	AHF Analysentechnik	F47-702	700/100 nm bandpass
Methanol (absolute for analysis)	Merck	67-56-1	
NaHCO ₃	Sigma-Aldrich/Merck	S5761-500G	100 mM NaHCO ₃ , pH 8.3
Near-infrared fiber-coupled laser	Toptica	IBEAM-SMART-PT-CD	Custom Design, 808 nm - 75mW
Objective lens piezo mount (PIFOC)	Physik Instrumente	P-726.1.CD	100 µm travel range
Orca Fusion BT sCMOS camera	Hamamatsu	C15440-20UP	
PCR tubes	Greiner Bio-One	673283	0.2 mL
Phosphate Saline Buffer (PBS 10x)		N/A	137 mM NaCl, 2.7 mM KCl, 10 mM Na ₂ HPO ₄ , 1.8 mM KH ₂ PO ₄ , pH 7.4
Pierce 16% formaldehyde (w/v), methanol free	ThermoFischer Scientific	28906	16% formaldehyde is transferred from the original glass ampule into 1.5 mL tube and kept at room temperature.
PlasmaPrep2 plasma cleaner	GaLa Instrumente GmbH	N/A	
Poly-L-lysine hydrobromide	Sigma-Aldrich/Merck	P2636-25MG	0.1% w/v solution was prepared in Milli-Q water, and stored in aliquots at -20 °C.
Primary dichroic (illumination reflecting)	AHF Analysentechnik	F73-866S	quad bandpass @ 405, 488, 561, 640
Quadnotch filter	AHF Analysentechnik	F40-072	405/488/561/640 nm
Quadrant photodiode (QPD)	Laser Components	SD197-23-21-041, LC301DQD-PV	
Razor blades	Apollo Herkenrath Solinger	N/A	
Refractive beam shaper	AdlOptica	PiShaper 6_6_VIS	

Roche Blocking Reagent	Roche	11096176001	10x solution was prepared according to recommendation. Frozen aliquots were stored at -20 °C.
Scalpel blade (Feather brand #11, No. 3)	Heinz Herenz Medizinalbedarf GmbH	1110911	
Scalpel removal box	Fisher scientific	10002-50	
Secondary dichroic (emission reflecting)	AHF Analysentechnik	F38-785S	750 nm longpass
Shortpass filter	Semrock	BSP01-785R-25	750 nm
Shortwave channel emission filter	AHF Analysentechnik	F37-677	676/37 nm bandpass
Single molecule localization microscope	EMBL Imaging Centre	Diekmann et al., 2020 with modifications	<p>The microscope provides widefield epi-illumination via a single-mode fiber-coupled laser engine, additional booster laser, and refractive beam shaper to provide a uniform illumination field (Stehr et al, 2019). Widefield images are captured on a sCMOS camera and appropriate relay optics for a system magnification of 61x and a pixel size of 106 nm.</p> <p>For ratiometric imaging of spectrally overlapping far-red dyes, an image splitter produces two spectrally distinct images on the camera (splitting dichroic: 665 nm long pass, shortwave channel emission filter: 676/37 nm bandpass, longwave emission filter: 700/100 nm bandpass. An additional 405/488/561/640 nm quadnotch filter and 750 nm shortpass filter are common to the two paths and provide additional laser blocking). A compound cylindrical lens provides the astigmatism required for 3D imaging.</p> <p>To maintain a fixed focus across acquisitions exceeding 2 hours in time (comprising 200 000 - 250 000 images), focus locking is achieved by total internal reflection of a near-infrared fiber-coupled laser from the coverslip and subsequent height sensitive detection on a quadrant photodiode (QPD). The QPD signal provided closed-loop control of the objective lens piezo mount.</p> <p>For access to this microscope, refer to https://www.embl.org/about/info/imaging-centre or contact ic-contact@embl.de</p>
Single-mode fiber-coupled multi-laser engine	Toptica	iCHROME MLE-LFA-HP	Provides widefield epi-illumination of 100 mW at 405, 488, 561, 640 nm
Splitting dichroic	AHF Analysentechnik	F48-665SG	665 nm long pass
Square cover glass 22 x 22 mm No.1	Menzel-Gläser; VWR	630-2882	
STAR 635P, NHS ester	Abberior	ST635P-0002-1MG	Dissolved in DMSO to 1 mM solution, aliquoted to single use volume, frozen and kept at -80 °C
Stereo microscope Stemi 305 Stand K LAB	Zeiss	N/A	
Tetramisole hydrochloride	Sigma-Aldrich/Merck	T1512-2G	1% (w/v) solution was prepared in Milli-Q water. Frozen aliquots were stored at -20 °C. Thawed aliquot was kept at 4 °C and used for several months.

TetraSpeck Microspheres	ThermoFischer Scientific	T7279	0.1 μ m, fluorescent blue/green/orange/dark red
Tris Saline Buffer (TBS 10x)		N/A	200 mM Tris-HCl, 1.5 M NaCl, pH 7.5
TWEEN 20	Sigma-Aldrich/Merck	P9416-50ML	Kept at room temperature in original packaging.
WormStuff worm pick	Kisker	789277	
XY microscope stage	Smaract	N/A	Custom Design
Zeba Micro Spin Desalting Column	ThermoFischer Scientific	89877	7K MWCO, 75 μ L