

Product Information Version 1.0

ZEISS ELYRA

Your Flexible Imaging System for 3D Superresolution Microscopy



Investigate the Cellular Universe Right Down to Molecular Level

> In Brief

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ELYRA is about choice: with ELYRA S.1 and superresolution structured illumination (SR-SIM) you reveal fine structural details while remaining free to label your samples with conventional dyes. Use ELYRA P.1 and photoactivated localization microscopy (PALM) for endogenously-expressed photo-switchable fluorescent proteins. Capture highly resolved structures of a whole cell in 3D in just one shot, while treating your sample so gently it stays fit for long-term observation. Your ELYRA system lets you reveal the ultra-structure of your object of interest, count molecules to quantify your results and see the arrangement of proteins within a structural context.

The choice is yours: The ELYRA product line puts the two most powerful and versatile superresolution technologies at your your disposal. You can even combine them in one system with the renowned LSM 710 or LSM 780. All that, plus ELYRA works seamlessly together with your ZEISS SEMs in a correlative workflow.





Somatic nucleus of rye (Secale cereale), Chromatin stained with DAPI (blue); cetromeric histone variant CENH3 stained with antibodies conjugated to Rhodamine (red); CAP-D3 protein labeled with antibodies conjugated to Alexa 488 (green). Courtesy of V. Schubert, IPK Gatersleben, Germany

Simpler. More Intelligent. More Integrated.

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Count on Highest Reproducibility

Motorized control of all hardware components makes it easy to switch between the fluorescence imaging modes widefield (WF), SR-SIM, PALM and laser scanning microscopy (LSM). You can acquire multicolor data with up to four channels using AOTF-controlled laser lines and a wide choice of filters. Add to that specialized and selected objectives, and advanced EM-CCD and sCMOs camera detectors adapted to each technology, to achieve precise results, over and over.

Never Compromise on Image Quality

ELYRA's specially-designed gratings let you select the best resolution for each wavelength. Choose the precise field-of-view (FOV) size to capture a whole cell or increase laser power densities for more efficient photo-switching. Render images in 3D using localization precision information. With a range of illumination schemes (EPI, HILO, TIRF) and powerful algorithms for drift and color correction, image quality is a given.

Put Flexibility First

Start by selecting the technology to implement: ELYRA S.1 for SR-SIM, ELYRA P.1 for PALM, or ELYRA PS.1 to combine PALM with SR-SIM. Then integrate the ELYRA system with your laser scanning microscope of choice: LSM 710 or LSM 780. Finally, use correlative microscopy to navigate between information from different imaging modes via ZEN Shuttle & Find. Maximize your freedom to explore.







Your Insight into the Technology Behind It

In Brief	Superresolution	Structured Illu	mination Micr	oscopy
The Advantages	Raw Imag	e		
The Applications	AMINARY		Widefield Image	
The System	ANNIN' AN		77 Y	
Technology and Details				1
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				2
			Star Lat	5
		Col	mpletion Percentage	
	Fourier Spec	trum		
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In SR-SIM, a grid pattern is projected onto the image plane. The grid structures interfere with sample structures, creating Moiré fringes. These contain high frequency information (synonymous with high resolution information) transformed down to low frequencies that can be captured by the optical system. Then all you need do is back-compute these lower frequencies to their true value in the final image. In this way you can double the resolution in all three directions.

Animation from www.zeiss.com/campus, © Mike Davidson, FSU, Tallahassee

Your Insight into the Technology Behind It

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Animation from www.zeiss.com/campus, © Mike Davidson, FSU, Tallahassee

In PALM, photo-switchable fluorescent molecules are sparsely activated so that only one out of many will be in its on-state within a single point spread function (PSF). This lets you determine its center of mass with a localization precision that far exceeds the extension of the PSF. Once recorded the molecule is turned to its off-state – for example, by photobleaching – and the cycle of activation/ deactivation is repeated again and again until all molecules are captured. The localizations are plotted in a new image to create the super-resolved image. If the PSF shape codes for the z-position, the method works in 3D as well. Expect to achieve resolutions in the range of 20-30 nm laterally and 50-80 nm axially.

Expand Your Possibilities

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ELYRA gives you more than just structural information. As your needs grow, ELYRA grows with you, forming the basis for a number of enhancements. Among them:



Dual Color PALM

Fast sequential laser switching to capture two colors quasisimultaneously in PALM.

CHO cells (Chinese Hamster Ovary cells). Dronpa fusion of Paxillin (Pax; red) tdEOS fusion of Vinculin (Vin; green). Courtesy of H Shroff, H. Hess, HHMI Janelia Farm, Ashburn, USA.



3D-PALM Image Fully rendered images, not just localized molecules.

8C interphase nucleus of Arabidopsis thaliana. Chromatin: DAPI (to visualize nuclei; not shown), Centromeric repeat stained by FISH using DNA conjugated to Alexa 488. Courtesy of V. Schubert, IPK Gatersleben, Germany.



Multi-emitter Fitting Algorithms Allow for denser labeling and enhance acquisition times up to 10-fold in PALM.

Escherichia coli. RNA Polymerase (labeled with Alexa 561 conjugated antibodies), top: multi emitter fit, bottom: single emitter fit. Courtesy of U. Endesfelder, M. Heileman, University of Frankfurt, Germany.



Microtubules with 1.4 μm Capture Range Excellent capture range in z with typically 1.4 μm for 3D-PALM.

Sample U2OS cell (human Osteosarcoma cell). α-Tubulin (labeled with Alexa 647 conjugated antibodies). Courtesy of S. Proppert, University of Würzburg, Germany.



Definite Focus

Definite Focus and Piezo Scanning stage minimize axial and lateral drift, respectively.

NIH 3T3 fibroblast cell. α-Tubulin (labeled with Alexa 647 conjugated antibodies). Courtesy of S. Niwa, N. Hirokawa, University of Tokyo, Japan.



Neuronal Cell Huge field-of-view, extendable by tiling and stitching in 3D-SIM.

Cultured primary hippocampal neurons. IPTK-N66, an F-actin binding protein (green; labeled with Alexa 488 conjugated antibodies); tdTomato used as a cytosolic filler (red); Tiling & Stitching to create 227 µm x 125 µm field of view. Courtesy of M. J. Schell, Uniformed Services University, Bethesda, USA.

Tailored Precisely to Your Applications

> In Brief	Typical applications, typical samples	Task	ZEISS ELYRA offers	
> The Advantages	Evolving organisms, such as Drosophila, C. elegans and	Resolve structural detail in 3D with high penetration depth.	SR-SIM: Water objectives for deep tissue imaging with up to 100 μm;	
> The Applications	Zebrafish embryos		tiling and stitching to cover a large area; level-adjustable stage to avoid a tilt in your sample	
> The System	Fixed Cells	Probe the structural organization of a whole cell.	SR-SIM: Large FOV to capture a whole cell in one go	
> Technology and Details		Investigate arrangement of cellular components and proteins.	SR-SIM:	
> Service		Explore interaction of molecules.	Up to four colors with optimized resolution for each wavelength, color correction with channel alignment tool	
		Reveal the ultrastructure of organelles.	PALM: Fast sequential laser switching for dual color acquisition:	
		Probe the ultrastructure of molecular assemblies.	fiducial-based drift and color alignment of the two channels	
		Map protein localization onto a structural context.	PALM: Adaptation of FOV to achieve higher laser power densities; fine tuning of activation laser power	
			3D-PALM: use photo-switchable proteins; excellent z capture range	
			SR-SIM & PALM Correlative methods with ZEN Shuttle & Find	
	Live Cells	Track many molecules and retrieve diffusion behavior.	PALM: Particle tracking software	
		Study structural changes of slower dynamics.		
			PALM:	
			SR-SIM-	
			Ontimized arid movement for 3D acquisition	

ZEISS ELYRA at Work

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- Investigate the structural arrangement of a protein.
- Study the arrangement of multiple proteins.



SR-SIM: Neuromuscular junction of Drosophila melanogaster larva, Bruchpilot (Brp) (labeled with Alexa 488 conjugated antibodies). Courtesy of C. Klämbt, H. Aberle, University of Münster, Germany.



3D-PALM: U2OS cell (human Osteosarcoma cell). CEP152, a centriolar protein (labeled with Alexa 647 conjugated antibodies); acquisition with 10 ms / frame. Courtesy of T. Klein, University of Würzburg, Germany.

- Reveal the ultrastructure of cell organelles in 2D.
- Reveal the ultrastructure of cell organelles in 3D.



SR-SIM: Cultured primary hippocampal neurons. ITPKA-N66, an F-actin binding protein (green; labeled with Alexa 488 conjugated antibodies), Bassoon, a pre-synaptic marker (red; labeled with Alexa 561 conjugated antibodies). Courtesy of M. J. Schell, Uniformed Services University, Bethesda, USA.



CLEM (SR-SIM/PALM): Saccharomyces cerevisiae. Ultrathin section, yeast wall (Calcofluor White; blue), hA1aR (Adenosin receptor (fused to Cerulean and labeled with Alexa 647 conjugated antibodies in red); Section imaged with FE-SEM, yeast wall imaged by SR-SIM, hA1aR imaged with PALM. Courtesy of K. Czymmek, University of Delaware, Newark, USA.

- Map molecules within a structure.
- Count molecules.



PALM: Xenopus laevis A6 cells (epithelial kidney cells). gp120, a nuclear pore complex protein arranged in an eightfold symmetry (labeled with Alexa 647 conjugated antibodies); acquisition with 8 ms/frame. Courtesy of A. Löschberger, M. Sauer, University of Würzburg, Germany.



SR-SIM/PALM: 8C interphase nucleus of Arabidopsis thaliana. Centromeric repeat stained by FISH using DNA conjugated to Alexa 488, imaged with SR-SIM (gray) and PALM (yellow). Courtesy of V. Schubert, IPK Gatersleben, Germany.

Your Flexible Choice of Components

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1 Microscope

- Axio Observer. Z1 (inverse stand)
- Incubator XL dark
- Motorized Piezo XY scanning stage
- Z-Piezo stage insert
- Port for LSM attachment
- 2 camera ports

2 Objectives

- C-APOCHROMAT 63x/1.2 Water (DIC)
- Plan-APOCHROMAT 63x/1.4 Oil (DIC)
- Plan-APOCHROMAT 100x/1.46 Oil (DIC)
- Plan-APOCHROMAT 100x/1.57 Oil HI Corr (DIC)

3 ELYRA Illumination and Detection

- Fiber coupled solid state or diode pumped solid state lasers
- Available lines:
 405 nm diode (50 mW),
 488 nm OPSL (100 or 200 mW),
 561 nm OPSL (100 or 200 mW),
- 642 nm diode (150 mW)
- Lasers shared between SR-SIM and PALM
- Andor iXon 897 EM-CCD camera (PALM)
- PCO edge sCMOS camera (SR-SIM)

4 LSM Illumination and Detection

- Fiber coupled lasers
- Available laser line: diode laser 405 nm (30 mW), diode laser ps/cw 405 nm (30 mW), diode laser ps/cw 440 nm (25 mW), Ar/ML 458/488/514 nm laser (25 or 35 mW), HeNe 543 nm (1 mW), DPSS 561 nm (20 mW), HeNe 594 nm (2 mW), HeNe 633 nm (5 mW), Tunable Laser In Tune (min. 1.5 mW), UV laser 355 nm (60 mW)

5 Software

- ZEN (black edition)
- SIM module
- PALM module
- 3D-PALM module (includes 3D VisArt)

System Overview



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Stand	Axio Observer.Z1, motorized inverted microscope for superresolution microscopy;	
Z drive	DC servomotor, opto-electronically coded; smallest Z step 25 nm	
XY Piezo Scanning Stage	motorized; range 130 mm x 100 mm; max sped 100 mm/s; resolution 0.2 μm; reproducibility: +/- 1 μm; absolute accuracy +/- 5 μm; suitable for mounting frames K 160 x 110 mm and Z-Piezo Stage insert	
2-Piezo Stage insert	for XY scanning stage, max travel range 100 µm; smallest Z step size 5 nm, sample holders available for standard 3''x1'' slides and 36 mm glass-bottom dishes; level-adjustable stage insert available for standard slides, glass-bottom dishes and LabTek™ chambers.	
Optical Filters for SR-SIM and PALM		
Filter sets	Five exchangeable filter sets available for multi-channel SR-SIM and PALM; each filter set with four precisely mounted ACR-coded ⁽¹⁾ filter modules for superresolution microscopy on a motorized six-position turret; two positions in each turret compatible with standard Push & Click filter modules (e.g. for visual sample observation).	
ilter slider	Manual filter slider with two positions (for emission filters or a Bertrand lens); fits into camera adapter of the microscope's side port; emission filters exchangeable for customizing detection conditions.	
Lasers		
aser module	Laser module for ELYRA P.1, ELYRA S.1 and ELYRA PS.1; laser coupling with polarization-maintaining single mode fiber (no adjustment of laser coupling by users required).	
aser Lines	405 nm (50 mW), 488 nm (100 mW or 200 mW), 561 nm (100 mW or 200 mW), 642 nm (150 mW); 405 laser can be attenuated by up to 1000 fold (used for activation and backpumping); high power lasers (150-200 mW) can be attenuated by two fold	
Cameras		
Camera for PALM	Andor iXon 897 back-thinned EMCCD camera; pixels: 512 x 512; pixel size: 16 μm x 16 μm; QE: 90% (camera specifications by ANDOR)	
Camera for SR-SIM	pco.edge sCMOS camera; effective pixels: 1280 x 1280; pixel size 6.5 μm x 6.5 μm; QE: 57%; dynamic range 15 bit; (camera specifications by PCO)	
	Liquid cooling system for EMCCD and sCMOS cameras	

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Illumination module	Fully motorized Epifluorescence (EPI), high inclined and laminated optical sheet (HILO) and total internal reflection illumination (TIRF);
	simultaneous TIRF illumination with VIS and 405 nm laser lines;
	individual triggering of lasers for synchronizing dye activation and illumination to camera read-out and transfer times;
	motorized TIRF angle adjustment; motorized TIRF field adjustment with three field size options.
3D-PALM module	Double phase ramp in pupil plane of back aperture of objective providing for phase ramp imaging localization microscopy (PRILM);
	z capture range typically 1.4 μ m; multi-plane acquisition possible to extend z range
Cameras	EMCCD camera (mounted to side port of microscope; left side port without LSM, right side port with LSM)
Objective lenses (PALM)	alpha "Plan-APOCHROMAT" 100x/1,46 Oil DIC, alpha "Plan-APOCHROMAT" 100x/1.57 Oil-HI DIC Corr (2D-PALM);
	alpha "Plan-APOCHROMAT" 63x/1.4 Oil DIC, C-APOCHROMAT 63x / 1.2 W Corr DIC (3D-PALM)
	ACR ⁽¹⁾ coding (optional)
Imaging modes	"Widefield (WF)" mode (sample illumination with arc lamp), "Laser WF" mode (sample illumination with laser),
	"LSM" mode (available if combined with LSM 710 or LSM 780)
Field of view (PALM)	Maximal field of view 51.1 x 51.1 μm (with alpha "Plan-APOCHROMAT" 100x/1,46 Oil DIC, full chip recording);
	81.1 x 81.1 μm (with "Plan-Apochromat" 63x/1.4 OIL DIC, full chip recording);
	HP field 2 x smaller, uHP field 2 x $\sqrt{2}$ smaller than TIRF field
Localization precision (PALM)	Typically 20 nm – 30 nm lateral, 50 nm – 80 nm axial, given sufficient signal-to-noise
Multi-color imaging (PALM)	Detection of up to two different fluorescent labels (sequentially or quasi simultaneously by fast sequential laser switching)
Acquisition speed (PALM)	TIRF (PALM) and widefield mode: up to 30 frames per second (full frame mode, 512 x 512 pixels);
	>100 frames per second in sub-array mode
Data recording and analysis (PALM)	Full software control of PALM imaging; software holding focus based on fiducial markers
	Online PALM processing for simultaneous data acquisition and analysis (2D-PALM only);
	manual editing of parameter settings for optimal results in PALM with different fluorophores;
	feature-rich rendering of PALM localization tables; export and import of localization tables for custom filtering;
	correction algorithms for lateral and axial drift;
	chromatic aberration correction (based on fiducial markers or prominent structures)
	Multi-emitter fitting algorithms allow to analyze overlapping signals with high precision.
	Up to 10 times higher labeling densities are possible speeding up acquisitions by the same factor.

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Illumination module	Fully motorized SR-SIM imaging;			
	five different grating frequencie	es for SR-SIM for optimal matchin	g of illumination pattern to laser wa	avelength and objective lens;
	motorized exchange of gratings in multi-color SR-SIM; fast piezo actuated phase stepping of gratings;			
	pattern rotation with adjustabl	e number of angle steps (3 or 5 ro	otations).	
Camera	sCMOS camera mounted on side port (left side port without LSM, right side port with LSM)			
Imaging Modes	"Widefield" modes for illumination with X-Cite 120 and lasers, "SIM" mode (three-dimensional SR-SIM), "I SM mode" (available if combined with I SM 710 or I SM 780)			
Objective lenses (SR-SIM)	Plan-APOCHROMAT 63x/1.40 Oil DIC, C-APOCHROMAT 63x/1.20 W Corr, alpha Plan-APOCHROMAT 100x/1,46 Oil DIC, alpha Plan-APOCHROMAT 100x/1.57 Oil-HI DIC Corr, ACR ^(II) coding (optional)			
Resolution (SR-SIM)	Lateral resolution (XY): 120 nm, axial resolution (Z): 300 nm (typical experimental FWHM values with objective lens Plan-APOCHROMAT			
Multi-color (SR-SIM Mode)	Detection of un to four different fluorescent labels (sequential detection)			
Max Field of view (SR-SIM)	21.25 v 21.25 µm (processed: 72.22 v 72.22 µm) full-frame recording (1200 v 1200 offective pv)			
	with Plan-APOCHROMAT 63x/1	.40 Oil DIC	stanig (1200 x 1200 checave px)	
Acquisition speed (SR-SIM)	Image Format	Single SR-SIM Frame ⁽²⁾	Time Series ⁽³⁾	Z-stack ⁽⁴⁾
	-	-	(10 SR-SIM frames)	(2 µm, 16 SR-SIM frames)
	1280 x 1280 px (full frame)	1.60 sec	14.2 sec	13.5 sec
	512 x 512 px (subarray)	1.55 sec	13.8 sec	12.8 sec
	256 x 256 px (subarray)	1.52 sec	13.7 sec	12.5 sec
	(2) 15 individual images recorded per SR-SIM frame (at three pattern rotations); 30 ms integration time.			
	(3) 150 individual images recorded without pausing representing 10 SR-SIM frames (same Z-level); 30 ms integration time.			
	(4) 240 individual images recorded corresponding to 16 SR-SIM frames at different Z-levels (spacing between Z-levels: 0.133);			
Data recording and analysis (CD SIM)		imaging		
	Multitracking (sequential multi-channel data acquisition with freely configurable change of gratings, filters and excitation lasers between			
	tracks); SR-SIM imaging in user-defined sub-array regions (ROI imaging);			
	Extension of imaged area possible with tile scanning and stitching.			
	Automatic selection and manual editing of processing parameters;			
	Channel-specific settings of processing parameters in multichannel data;			
	Selective processing of subsets of original data (subsets of Z-stacks, ROIs);			
	Batch processing; three types of output computed from original data (SR-SIM, widefield and deconvoluted);			
	I nree processing modes for Z-stack data ("2D", "3D", "3D" Large"); correction algorithm for chromatic aberration; Computation and viewing of Fourier transforms			

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ELYRA PS.1		
System information	ELYRA PS.1 integrates all imaging modes, hardware as well as software features of ELYRA P.1 and ELYRA S.1 (see above)	
Illumination module	Sample illumination in all widefield and superresolution modes by a single, highly integrated illumination module (with same set of lasers and a single ELYRA laser module)	
Cameras	Camera for PALM: Andor iXon 897 back-thinned EMCCD camera mounted to side port of microscope; left_sideport without LSM, right sideport with LSM Camera for SR-SIM: pco.egde sCMOS camera mounted to base port of microscope	
Combination with laser scanning microscope		
Combination with LSM	LSM 710 (systems with 2, 3 or 34 channel detection) and LSM 780 (32 channel GaAsP) with VIS lasers (including tuneable laser In <i>Tune</i>)	
Software		
Standard	ZEN imaging software (64-bit); operating system: Microsoft Windows 7 Ultimate	
	Full software control of image data recording in all imaging modes (incl. widefield, superresolution and LSM modes); Software-controlled switching between imaging modes; automated integration of different imaging modes into the same experiment; Full software control of data recording (multi-channel imaging, time series, Z-stack); Saving and restoring of user-specific configurations for data recording.	
Optional packages	ZEN 3D VisArt plus (sophisticated volume visualization); ZEN StitchArt plus (extension of field of view by tile scanning and subsequent stitching of tiles with 2D and 3D data)	
Accessories		
Definite Focus	Holding focus to compensate axial drift, typical z-position accuracy with an ELYRA system: 30 nm specified limits: 100 nm for 63x objectives; 90 nm for 100x objectives	
Incubation	Large chamber incubation with Incubator XL dark S1, which also prevents exposure to ambient light.	
	Stage-top incubation possible without z-piezo stage insert	
Optional packages	ZEN 3D VisArt plus (sophisticated volume visualization); ZEN StitchArt plus (extension of field of view by tile scanning and subsequent stitching of tiles with 2D and 3D data)	



Count on Service in the True Sense of the Word

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Because the ZEISS microscope system is one of your most important tools, we make sure it is always ready to perform. What's more, we'll see to it that you are employing all the options that get the best from your microscope. You can choose from a range of service products, each delivered by highly qualified ZEISS specialists who will support you long beyond the purchase of your system. Our aim is to enable you to experience those special moments that inspire your work.

Repair. Maintain. Optimize.

Attain maximum uptime with your microscope. A ZEISS Protect Service Agreement lets you budget for operating costs, all the while reducing costly downtime and achieving the best results through the improved performance of your system. Choose from service agreements designed to give you a range of options and control levels. We'll work with you to select the service program that addresses your system needs and usage requirements, in line with your organization's standard practices.

Our service on-demand also brings you distinct advantages. ZEISS service staff will analyze issues at hand and resolve it – whether using remote maintenance software or working on site.

Enhance Your Microscope System.

Your ZEISS microscope system is designed for a variety of updates: open interfaces allow you to maintain a high technological level at all times. As a result you'll work more efficiently now, while extending the productive lifetime of your microscope as new update possibilities come on stream.

Please note that our service products are always being adjusted to meet market needs and maybe be subject to change.







Profit from the optimized performance of your microscope system with services from ZEISS – now and for years to come.

>> www.zeiss.com/microservice

The moment your data change scientific minds. This is the moment we work for.

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We make it visible.