

ELYRA PS.1

Multi-functional fluorescence inverted widefield microscope enabling live-cell imaging, TIRF or HILO illumination and two super-resolution techniques: structured illumination microscopy (SIM) and single molecule localization microscopy (e.g. PALM).

Basic introduction to super-resolution microscopy can be found here:

<http://zeiss-campus.magnet.fsu.edu/articles/superresolution/introduction.html>

Application

- Fast and sensitive multi-color widefield imaging with TIRF, HILO or EPI excitation option
- Various measurement options: z-stack, time series, tile scan, multi positions and regions
- Long term live-cell imaging available
- Multi-color (up to 4 colors) super-resolution images obtained by SIM technique reaching lateral resolution of 120-160 nm and axial resolution around 300 nm
- Dual-color super-resolution images obtained by 2D and 3D single molecule localization methods (STORM, dSTORM, PALM; TIRF or HILO illumination available) with resolution improvement up to 10x compared to conventional optical microscopes

Technical specification

Motorized inverted fluorescence microscope Carl Zeiss Axio Observer equipped with Z-piezo stage insert, Definite focus (hardware autofocus system), Incubator XL dark and following units:

Software ZEN Black
 ZEN Blue

Illumination Transmitted light Illuminator VIS-LED, color temperature
5600 K

 Epifluorescence lamp module X-Cite® 120PC Q (Excelitas
Technologies)

Lasers

405 nm HR Diode Laser 50 mW
488 nm HR Diode Laser 300 mW
561 nm HR DPSS Laser 200 mW
640 nm HR Diode Laser 150 mW

Availability of fully motorized epifluorescence (EPI), high inclined and laminated optical sheet (HILO) and total internal reflection illumination (TIRF). Adjustment of TIRF angle is also motorized.

Filter Turrets

Filter set "2" (turret with 6 positions)		
MBS 405/561 + EF BP 420-480/BP 570-630/LP 740	laser excitation 405, 561, 640	fixed position
MBS 405/488/642 + EF BP 420-480/BP 495-560/LP 650	laser excitation 405, 488, 640	fixed position
MBS 405/488/561/642 + LBF -561/642	laser excitation 561, 640	fixed position
MBS 405/488/561 + LBF -488/561	laser excitation 488, 561	fixed position
Free position		position
Free position		position
Filter set "4" (turret with 6 positions)		
MBS 405 + EF BP 420-480 / LP 750	tailored to laser line 405	fixed position
MBS 488 + EF BP 495-550 / LP 750	tailored to laser line 488	fixed position
MBS 561 + EF BP 570-620 / LP 750	tailored to laser line 561	fixed position
MBS 642 + EF LP 655	tailored to laser line 642	fixed position
Tripleband Set DAPI/FITC/TRITC FSet77 HE Free position	epifluorescence lamp	exchangeable

Laser line 405 nm can pass on the excitation to serve as an activation laser for all filter cubes in PALM.

On demands, filter cubes from Carl Zeiss LSM 880 confocal microscope can be inserted to free positions of turrets. For visual inspection of image, filter cubes DAPI, FITC, mRFP and Cy5 are available in addition to Tripleband cube.

Objectives

Type	Immer- sion	Magni- f.	NA	WD [mm]	DIC/Correction Ring	Application
Plan- Achrom at	Air	10x	0.45	2	-	EPI
Plan- Achrom at	Air	20x	0.8	0.55	-	EPI
C- Achrom at	Water	63x	1.2	0.28	Corr (CG=0.14- 0.19mm)	EPI, SIM, PALM
alpha Plan- Achrom at	Oil	63x	1.46	0.10	DIC, Corr (CG=0.15- 0.19mm)	EPI, HILO, SIM, PALM

alpha Plan- Apochrom at	Oil	100x	1.46	0.11	DIC	EPI, HILO, TIRF, SIM, PALM
alpha Plan- Apochrom at	Oil	100x	1.57*	0.11	DIC, Corr (CG=0.165- 0.175mm)	EPI, HILO, TIRF, SIM, PALM

* Immersion oil with high refractive index (1.66) and HI cover glasses 0.17mm +/- 0.003 are recommended for imaging

On demand, objectives from Carl Zeiss LSM 880 confocal microscope could also be inserted.

Detection

EM-CCD Andor iXon DU 897 camera Active pixels 512 x 512; Pixel size 16 μm x 16 μm ; Frame rates up to 56 fps (full frame); QE 95%

sCMOS PCO Edge 5.5 camera Active pixels 1280 x 1280; Pixel size 6.5 μm x 6.5 μm ; Camera Link; Frame rates up to 100 fps (full frame); QE >60%

Optovar Lens is common for both cameras, swap of magnification lenses 1x and 1.6x is motorized.

Module for environmental control CO2 Module (CO2 concentration stable between 1 and 8% +/- 0.1%, adjustable) with humidity module, Heating Insert P and Heating Unit XL

SIM super-resolution platform

- Detection of up to four different fluorescent labels (sequentially)
- Five different grating frequencies for SR-SIM for optimal matching of illumination pattern to laser wavelength and objective lens (freely configurable)
- 3 or 5 rotations, 5 shifts
- Acquired on sCMOS camera, minimal time for single SR-SIM frame (1280px x 1280 px full frame, 3 rotations, 30 ms exposure time): 1.6 s
- Full software control of SR-SIM imaging
- Automatic selection and manual editing of processing parameters

PALM super-resolution platform

- Detection of up to two different fluorescent labels (sequentially or quasi simultaneously by fast sequential laser switching)
- Localisation precision: typically 20 nm - 30 nm lateral, 50 nm - 80 nm axial, given sufficient signal-to-noise
- TIRF angle adjustment
- Motorized TIRF field adjustment with three field size options (reduced FOV with the same laser power)
- Acquired on EM-CCD camera; up to 30 frames per second (full frame mode, 512 x 512 pixels)
- 3D imaging is achieved using an insert that produces a double-helix PSF in the image plane
- Z capture range typically 1.4 μm
- Full software control of PALM imaging
- Adjustable parameter settings for optimal results in PALM with different fluorophores

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