Luminosa

Single Photon Counting Confocal Microscope

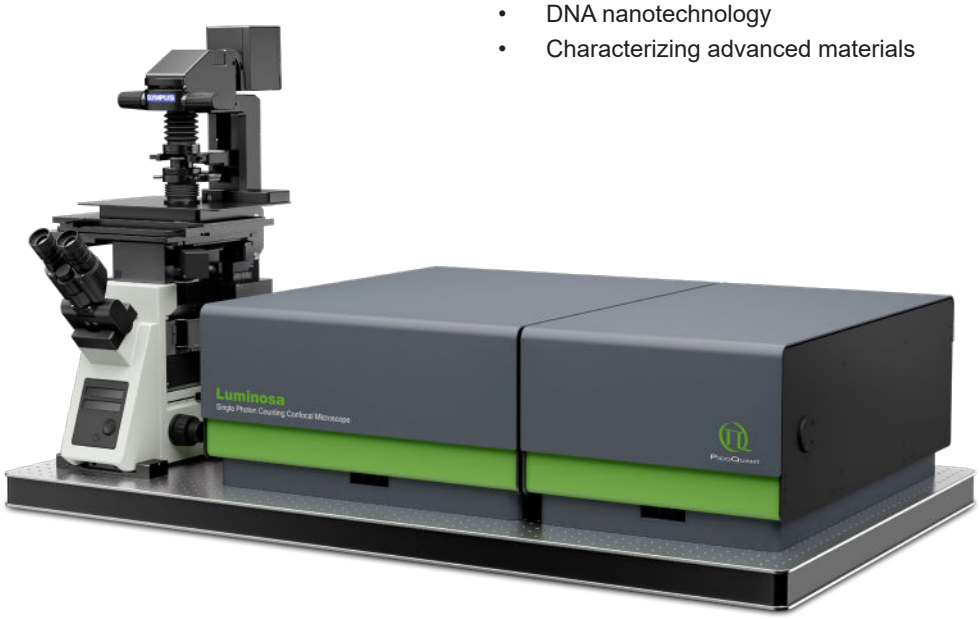
- Software-controlled confocal system based on an inverted microscope
- Versatile excitation system with laser wavelengths from 375 to 1064 nm
- VarPSF: fine-tuned observation volume for FCS and single molecule FRET experiments
- Motorized positioning stage for “tiling and stitching” in transmission, epi, DIC and confocal modes
- Scanning options: FLIMBee galvo scanner and piezo objective scanning
- Stage top incubator for extended live cell experiments
- Up to 6 truly parallel detection channels with SPAD and/or Hybrid PMTs
- PDA-23 add-on: an integrated SPAD array detector
- < 650 ps dead time per channel and 5 ps time bins
- One click auto-alignment for consistent optimal performance
- Fast results with minimal user interaction thanks to GPU accelerated algorithms and context-based workflows for FCS, FLIM, and single molecule detection
- Imaging scanning microscopy (ISM-FLIM): FLIM with more spatial resolution and higher contrast
- An open Python interface enabling adaptive workflows, custom acquisition and reporting

Core Methodologies

- Single molecule FRET (burst and time trace analysis)
- Fluorescence lifetime imaging (FLIM)
- Fluorescence correlation spectroscopy (FCS)
- FLIM-FRET
- Anisotropy Iimaging
- Fluorescence recovery after photobleaching (FRAP)
- Time-resolved photoluminescence
- Antibunching

Application Areas

- Dynamic structural biology
- Studying cellular mechanisms driven by phase separation
- Environmental sensing and marker multiplexing
- Mapping dynamics and structure of cellular membranes
- Characterizing functional nanovesicles
- Metabolic Imaging
- Studying chemical reactions at the single molecule level
- DNA nanotechnology
- Characterizing advanced materials



Specifications

Feature Highlights	
LumiFinder	• Automated detection and measurement of immobilised emitters • Automatically use different excitation power for emitter identification and time trace measurements to avoid unnecessary photobleaching • Advanced stopping criteria for the time trace measurement offer more flexibility in single molecule FRET measurements of immobilised emitters
FRETcompass	Automated determination of correction factors used in single molecule FRET experiments based on the benchmark study published in 2018 (Hellenkamp et al. Precision and accuracy of single-molecule FRET measurements – a multi-laboratory benchmark study. Nat Methods 15, 669-676 (2018))
InstaFCS	Online FCS fitting with automatic suggestions for model and parameters
InstaFLIM	• Provides first suggestions for FLIM species separation without user interaction • Suggested parameters can be fine-tuned • Simultaneous TCSPC and phasor analysis options for ROI determination
Sample-free Auto-alignment (SFA)	• One-click auto-alignment without any sample required • Ensures optimal performance for every measurement, even the most challenging ones
CalEx*	• Excitation laser power calibration allows setting and displaying excitation intensity in µW • No external power meter required
VarPSF*	Switch between diffraction limited and larger observation volume to: • Increase observation time window to check for dynamic transitions between states • Study diffusion properties of larger particles, 3-6 times larger volumes are achieved (depending on wavelength)
LumiPy	Enabling adaptive workflows and custom acquisition and reporting via a python interface allowing for Luminosa functionality to be exported to Python classes

Excitation	
	1 - 8 lasers with choice of wavelengths from 375 - 1064 nm • Optional: 2 additional exit ports available for coupling additional lasers • Optional: fs fiber laser from TOPTICA at 780 nm for metabolic imaging
Commonly used wavelengths	405, 440, 485, 532, 560, 595 and 640 nm
Individual laser operation modes	Pulsed and Continuous Wave (continuous mode availability depends on the laser model)
Repetition rates	1 - 40 MHz, for specific wavelengths up to 80 MHz
Laser driver	8 channel Sepia PDL 828 L
Supported multi-line operation	Pulsed Interleaved Excitation (PIE) or simultaneous emission modes (for specific wavelengths alternating excitation move after (ALEX) in µs and ms time scales also available)
Motorized main dichroic wheel	6 positions available for 18 × 25 × 3 mm single, dual, triple, and quad-band dichroics

Microscope	
	Inverted Evident IX73 (partially motorized)
Objective types	• UPLXAPO60XW X-Line PlanApochromat, NA 1.2, water immersion, 400 - 900 nm • UPLXAPO100x1.45 Oil X-Line PlanApochromat, NA 1.4, oil immersion, 400 - 850 nm • Other oil-immersion, apochromatically corrected, air-spaced, NIR-enhanced, or long-working-distance objectives are available
Observation modes	• Confocal, transmission • Optional: epi-widefield illumination, differential Interferometric Contrast (DIC)
Oculars	Yes
Camera	Optional CCD camera at the left side port
Sample holder	• Multi-functional sample holder compatible with several standard sizes of microscope coveslips and petri dishes • 96-well plate sample holder
Stage-top lincubator	Yes
Axial drift (typical values **)	• For 15 minutes measurement: 50 ± 10 nm • For 2 hour measurement: 100 ± 20 nm

Detection Options		
Point detection options	• Parallel detection with up to 6 point detectors in the spectral range of 300 - 1000 nm • 23 pixel SPAD array • Additional fiber coupled or free-space exit ports for customised detectors	
Point detection types	• Single Photon Avalanche Diodes (SPAD) for low dark-counts and maximum detection efficiency • Hybrid photomultiplier tubes (PMA-Hybrid-40) for optimum timing performance and when working at high count rates	
Type	SPAD (SPCM-AQRH)	PMA Hybrid - 40
Spectral range	400 - 1000 nm	300 - 720 nm
Dark counts (at 20 °C, typ. value)	< 250 cps	< 300 cps
Photon detection efficiency (PDE)	Typ. 50 % at 550 nm Typ. 70 % at 650 nm	Typ. 40 % at 500 nm
Timing response	200 - 400 ps (depending on wavelength)	< 120 ps
PDA-23 Array Detector with 23 independent Single Photon Avalanche Diode pixels		
Active area	100 µm x 110 µm	
Effective fill factor with microlenses	> 75 %, typical 80 %	
Photon detection efficiency (PDE)	• Typ. peak of PDE 55 % at 520 nm • PDE typically above 35 % for wavelengths from 440 to 660 nm • Usable spectral window 400 - 950 nm	
Dark counts at working temperature of 10 °C via integrated cooling	• Typ. 100 cps for most pixels • Median value of dark counts per second is < 100 • Typ. 1 hot pixel with > 1 kcps and below < 10 kcps • Hot pixel not in the center of the array	
Jitter	< 120 ps FWHM for wavelengths between 510 and 785 nm	

Deadtime of each pixel	50 ns
Crostalk probability between pixels	0.1 %
Afterpulsing probability	0.1 %
Maximum count rate per pixel	Typically > 7 Mcounts per second

Data Acquisition	
	• Based on Time-Correlated Single Photon Counting (TCSPC) in Time-Tagged Time Resolved (TTTR) measurement mode in T3 and T2 modes • Simultaneous data acquisition with up to 6 channels + PDA-23 • New dynamic binning format for FLIM imaging
TCSPC units	• MultiHarp 150 P (standard option) • MultiHarp 160 (for PDA-23)
Time resolution (bin width)	5 ps
Dead time	< 650 ps
Timing precision	< 28 ps RMS
Number of available detector inputs	8 for MultiHarp 150 P (standard option) 32 for MultiHarp 160 (for PDA-23)

Scanning Options	
FLIMbee XY galvo scanner	• Min. pixel dwell time: 0.5 µs, • Min. pixel size: 17 nm (60x objective) • Up to 5.2 frames per second for a 512 x 512 pixel image max. • Scan field: 200 x 200 µm (60x objective) • Possibility to bypass the FLIMbee galvo scanner for point measurements
XY objective scanning	• Min. pixel dwell time: 0.2 ms min. • Pixel size: 1 nm (independent of objective) • Max. scan field: 80 x 80 µm (independent of objective)
XY-scanning combined option	Combination of FLIMbee and objective scanning on the same system with software-controlled change between the two scanning modalities
Piezo-based Z-scanning	• Min. step size: 50 nm • Overall range: 100 µm (optional 400 µm)
Positioning stage with spiral scan mode, creating overview map by tiling and stitching	• Overall range: 121 x 81 mm • Max speed: 300 mm/second • Positioning repeatability: < 0.15 µm

Luminosa Software	
Operating System	Windows 11
GPU-based programming	Software-Open GL
Context based workflows for easy acquisition and analysis	For single molecule FRET burst analysis, single molecule imaging, FCS in vitro, FCS in cells, FLIM, FLIM-FRET, steady-state FRET, anisotropy imaging, and FRAP

General Info	
Operating environment	• Room temperature range 15 - 25 °C • Room temperature stability ± 1.5 °C (recommended) • Room humidity < 60 %
Optical table (minimum dimensions)	• For 1 - 4 detectors: 1500 × 900 mm • For 5 - 6 detectors: 2000 × 900 mm
Power consumption	• 6 A at 230 V AC (typ. EU) • 20 A at 110 V AC (typ. USA)
Operating voltage	115 or 230 V AC
Altitude	Guaranteed performance up to 2000 m above sea level

* Feature available only for PicoQuant lasers
** Under constant room temperature and after the sample and z drive have settled for at least an hour

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