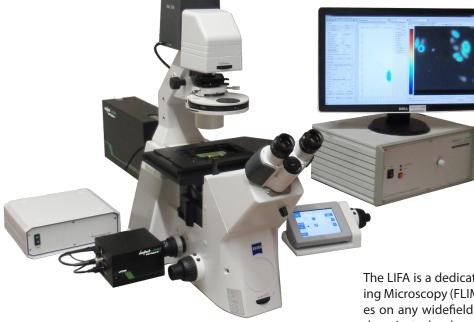


Fluorescence Lifetime Attachment

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#### **KEY FEATURES**

#### **Easy coupling**

Flexible and efficient coupling to all major-brand fluorescence microscopes.

**Fast acquisition** Record lifetime images in a matter of seconds.

#### High resolution image intensifiers

Gen II and Gen III image intensifiers offering the world's highest resolution and sensitivity in the UV, visible or near infrared.

Accurate lifetime determination Fluorescence lifetimes from 0–300 ns with an accuracy of 30 ps r.m.s. The LIFA is a dedicated system for Fluorescence Lifetime Imaging Microscopy (FLIM). It allows the generation of lifetime images on any widefield fluorescence microscope using frequency domain technology. The LIFA model consists of a TRiCAM M modulated intensified CCD camera, a modulated lightsource, a modulation control unit, a personal computer and the LI-FLIM software package. The intensified camera contains an image intensifier that is fiber-optically coupled to the CCD camera for maximum gain and speed.

### APPLICATIONS

Fluorescence Lifetime Imaging Microscopy (FLIM)

**FRET Efficiency Mapping** 

**Protein Interactions** 

**Biosensors** 

**Oxygen Concentration Imaging** 

NADH/FAD Fluorescence Dynamics

**Membrane Dynamics** 

**Molecular Interactions** 

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# **SPECIFICATIONS**

Specifications	Typical value
System Performance	
Lifetime range	0 - 300 ns
Lifetime resolution	less than 100 ps (single pixel)
Speed	2 lifetime images per second (full frame)
Spatial resolution	26 lp/mm (Gen II); 19 lp/mm (Gen III)
Sensitivity	single photon
Intrascene dynamic range	12 bit
Camera input format	1 inch
Effective pixel size	10.3 μm square (12,9 for lens coupled CCD)

## **CONTROL UNIT**

The control unit is used to generate and control the modulation signals for the lightsource and the intensified camera. It also contains the high voltage power supply generating the DC voltages for the image intensifier. All variables are controlled in the LI-FLIM software via a USB 2.0 interface.

Signal shape	Sinusoidal
Frequency range	1 MHz – 120 MHz
Frequency adjustment resolution	1 kHz
Frequency deviation	100 ppm max.
Harmonics suppression	Better than 25 dB
Output 1 Type a: MultiLED AC signal level DC current adjust	Capable of driving lightsource type a, optionally b and c Adjustable in 250 steps 0 – 1000 mA, 12 bit resolution
Optional lightsource outputs: Type b: Modulated laser diode AC signal level DC level adjust Type c: AOM for CW laser AC signal level Frequency Output 2 AC signal level AC signal after amplification	0.00 – 1.00 V peak-peak 0.00 – 1.00 V 0.00 – 1.00 V peak-peak Half the frequency of output 2 To image intensifier Adjustable from –50 to 0 dBm 15 Vpp nom.
External trigger input	LVTTL (phase shift on falling edge or rising edge)
Impedance	50 Ω
Phase adjustment range	0 - 360° in 1° steps
Phase deviation	< 0.01°
Cathode voltage DC	Adjustable from –150 V to +15 V
MCP voltage (gain) DC	Adjustable from 450 – 1000 V max.
Anode voltage DC	Fixed 6 kV

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## **MULTI-LED LIGHT SOURCE**

The Multi-LED is a modulated light source designed for fluorescence excitation. In the Multi-LED, up to four LED units are mounted having one common optical output to the microscope. Available LEDs cover the wavelength range from 360 to 660nm. The unit is cable-connected to the LIFA control unit that delivers the DC and AC power to the selected LED. LED selection, DC current, modulation frequency, amplitude and phase are controlled via the user interface of the LI-FLIM software. Optionally the Multi-LED can be temperature controlled for improved long-term stability. The Multi-LED is available for any brand of wide field microscope.

Peak wavelengths (nm), approximately	405 (CFP), 445 (cerulean, CFP), 470 (GFP), 535 (TRITC), 630 (CY5), others on request	
Number of mounted LEDs	3 (standard), or 4 (maximum)	
Modulation frequency	up to at least 80 MHz	
LEDs switching time	200ms	
Coupling	Compatible with existing Hg and Xe microscope adapters	
Lifetime drift	less than 30 ps*	
Optional Temperature stabilization for long-term (hours) lifetime stability	Active thermoelectric water cooling/heating system	
Control temperature range	5 – 45° C	
Temperature stability (constant load, constant ambient temp)	± 0.1° C	
Dimensions (l x w x h)	346 x 109 x 183 mm	
* Measured using stable fluorescent material with single lifetime component of 3.2 ns		

### **MULTI-LASER LIGHT SOURCE**

The modulated laser diodes of the Multi-LASER offer the high stability and high modulation depth required for high-accuracy lifetime imaging, and can also be used for regular fluorescence intensity imaging. The Multi-LASER uses a kineFLEX fiber delivery system to easily couple into your microscope stand. The Multi-LASER is a modulated light source for multi-beam confocal, TIRF, and widefield illumination.

Two versions are available: the standard version and the extreme version for even better power stability and less lifetime drift.

Wavelengths (nm)	375 (BFP), 405 (CFP), 445 (cerulean, CFP), 488 (GFP), 515 (YFP, Venus), 647 (CY5), others on request			
Number of laser lines	Standard: up to 3 or up to 6; Extreme: up to 4. All combined into one optical fiber output			
Output power (mW)	20 mW-300 mW, depending on the wavelength			
Modulation frequency	Up to 120 MHz (standard)			
Input signal	Digital: 01 V / 50 Ω			
Control interface	RS232			
Coupling	KineFLEX system, with a single-mode polarization maintaining fiber and either FC-APC, FC-PC or FC-P8 output connector			
Power stability	Standard: 2%*, Extreme: 0.5%*			
Lifetime drift	Standard: less than 50 ps*, Extreme: less than 20-30 ps*			
Dimensions (I x w x h)	19" rack housing. MultiLASER-3: 480 x 484 x 88 mm; MultiLASER-4 and 6: 610 x 484 x 132 mm			
Options	<ul> <li>Multi-channel excitation</li> <li>Two single-mode optical fiber outputs</li> <li>Custom fiber outputs on special request</li> </ul>			
* Measured using stable fluc	* Measured using stable fluorescent material with single lifetime component of 3.2 ns			

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## TRICAM M INTENSIFIED CCD CAMERA

The TRiCAM M is the modulated version of the TRiCAM modulated intensified camera manufactured by Lambert Instruments. The TRiCAM is a compact camera based on a 18 mm MCP image intensifier that can be gain-modulated at high frequencies to allow frequency-domain imaging. The intensifier can also be operated DC to allow focusing and selecting the object of interest. Optionally, the TRiCAM can be provided with gating to allow time-gated imaging. AC and DC voltages are provided from the control unit via a cable that is permanently connected to the camera for maximum performance. The output of the intensifier can be either fiber-optically or lens coupled to the high-resolution progressive scan CCD camera with 12 bit output. The image acquisition and camera parameters as exposure time, binning, ROI, triggering are controlled via the USB2.0 interface.

Resolution on input	Minimum	Typical	Units
Intensifier tube at nominal DC operation	Gen II: 55	Gen II: 65 Gen III: 57	lp/mm
Including relay lens or fiber optics, at DC operation and CCD camera		Gen II: 40 Gen III: 34	lp/mm
Including relay lens or fiber optics, at 40 MHz gain modulation and CCD camera		Gen II: 26 Gen III: 19	lp/mm
Image intensifier	Minimum	Maximum	Units
Input diameter	17.5		mm
Input window	Borosilicate glass		
Cathode sensitivity (Gen II: Super S25, Gen III: GaAs)*			
@ 2850 K @ 800 nm @ 850 nm	Gen ll: 400, Gen lll: 1000 35 25	1200	μΑ/lm mA/W mA/W
Phosphor	P43		
Luminance gain	Gen ll: 3180 Gen lll: 3600		cd/m²/lx lm/m²/lx
Equivalent Background Input		Gen II: 0.25 Gen III: 0.5	μlx
Modulation frequency range	1	120	MHz
Quality area	Gen II: 14.4 x 10.7; Gen III: 13	.5 x 10.0	mm

The number of spots exceeding a contrast with their surrounding area of 30% is less than or equal to the number indicated in the table below. The size of non-circular spots is determined on the basis of equal area to circular spots. When the distance between two spots is less than the maximum dimension of either spot, the two spots are considered to be one spot.

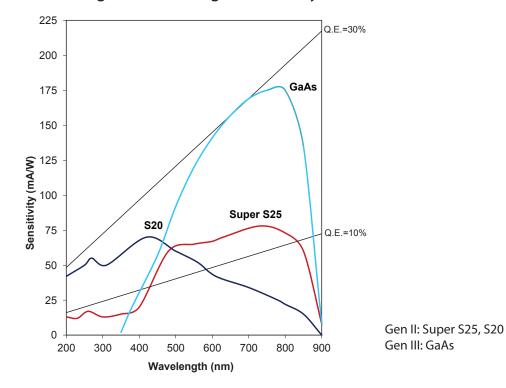
Gen II		Gen III		
Size of spots	Max. number of spots within effective area	Size of spots	Max. number of spots within effective area	
			Dark spots	White spots
> 300 μm 225 – 300 μm 150 – 225 μm 75 – 150 μm < 75 μm	0 1 3 6 Minimal	> 150 μm 100 – 150 μm 75 – 100 μm 50 – 75 μm < 7 μm	0 2 8 Minimal Minimal	0 0 4 6 Minimal

\* Photocathodes with different spectral response are available, see curve:



CCD Camera		
CCD	2/3" interline, progressive scan	
Resolution	1392(H) x 1040(V) pixels	
Pixel size	6.45 μm square	
Dynamic range	66 dB	
Synchronized with	Control Unit	
Integration time control	1ms – 2 min	
Selectable Region of Interest 1392 x 1040 1280 x 1024 1024 x 768 800 x 600 640 x 480	<ul> <li>@ 15 fps</li> <li>@ 16 fps</li> <li>@ 20 fps</li> <li>@ 24 fps</li> <li>@ 30 fps</li> </ul>	
Binning	2 x 2, 3 x 3 and 4 x 4	
Fastest mode (4x4 binning = 348x256)	@ 50 fps	
Digital output	Software selectable 8 or 12 bit, via USB	
External trigger input	LVTTL	
Trigger output	LVTTL	
Pixel clock	21 MHz	
Programmable camera gain	Up to 23 x	
High light level protection	Switches off all DC and AC power	
Environmental temperature	0° to 40° C	
Lens mount	C-mount	
Dimensions $(I \times w \times h)$	133 x 116 x 80 mm	

#### Image intensifier light sensitivity



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#### PERSONAL COMPUTER (MINIMAL SPECIFICATION)

Operating system	Windows 7 Professional 64-bit
Processor	Intel Core 2 Duo, 3GHz
Memory	4GB
Hard disk	300 GB
Monitor	24" LCD monitor, resolution: 1920x1080
System includes	DVD R, IEEE1394, LAN/Ethernet, USB2.0, keyboard, optical mouse
Software	
LI-FLIM	Acquisition and lifetime modules, full digital control of hardware, viewer and image analysis tools. External control via ActiveX.
Software Development Kit for CCD camera and Control Unit	Offers full programmatic (C/C++) control of camera and of Control Unit functionality.