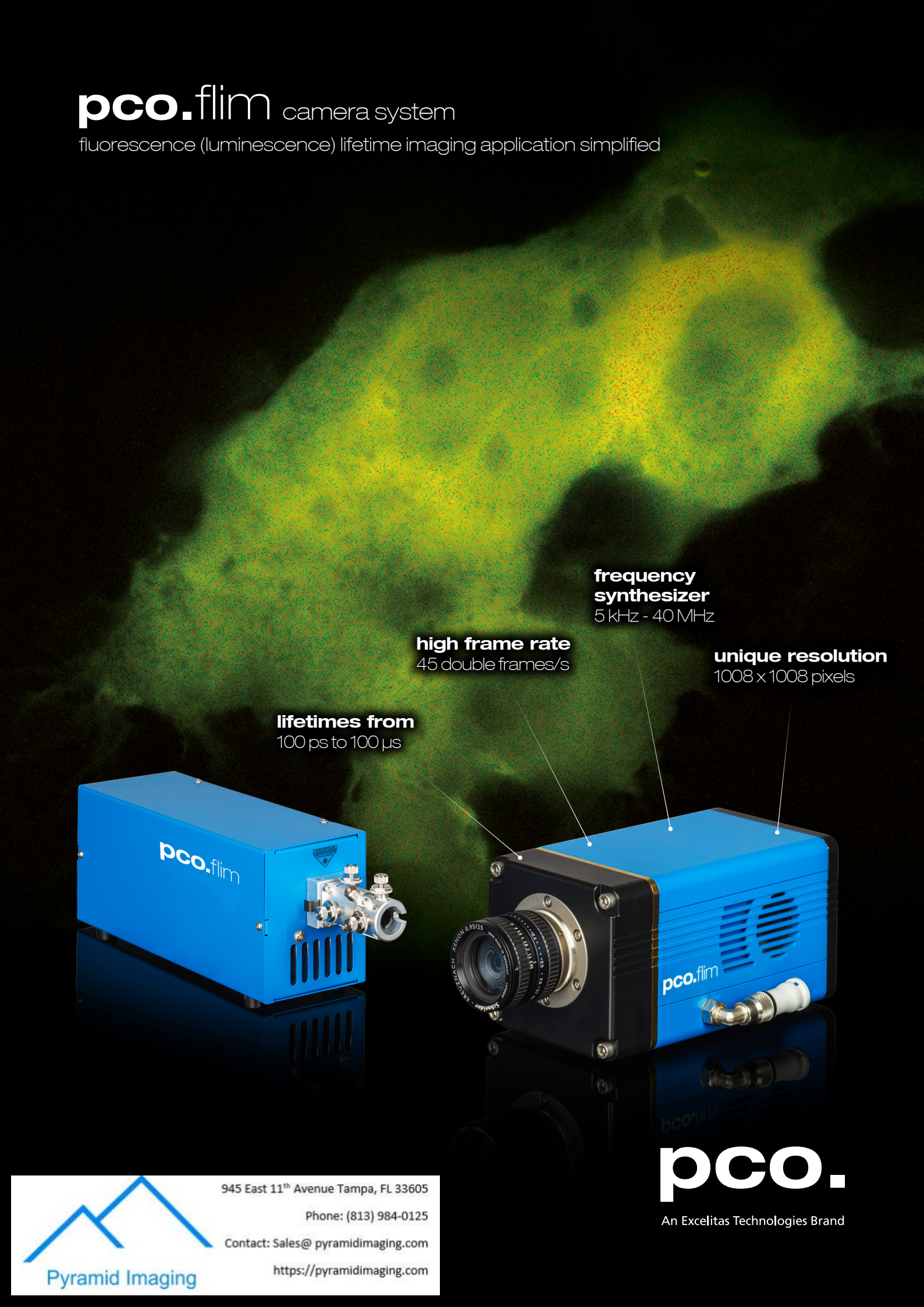


pco.flim camera system

fluorescence (luminescence) lifetime imaging application simplified



frequency synthesizer
5 kHz - 40 MHz

high frame rate
45 double frames/s

unique resolution
1008 x 1008 pixels

lifetimes from
100 ps to 100 μ s



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pco.

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Frequency vs. time domain luminescence lifetime measurements

In theory there is no difference regarding the information content of the two types of decay or lifetime measurements, since both methods give the same results, but with different experimental requirements.

The frequency domain measurement requires a reference measurement to cancel out the influence of the optical path, which might not be necessary for the time domain measurement, but time domain measurements with image sensors are not possible down to the nanosecond range, since the fastest available CMOS image sensors still have minimum exposure times of more than 100 nanoseconds, while the frequency domain camera system pco.flim, even at a modulation frequency of 30 MHz, can resolve 100 picoseconds. For example the differences between the pollen grains (yellow in figure 5 middle image) and the leaf cells (blue and green in figure 5 middle image) were in the range of 1.2 ns.

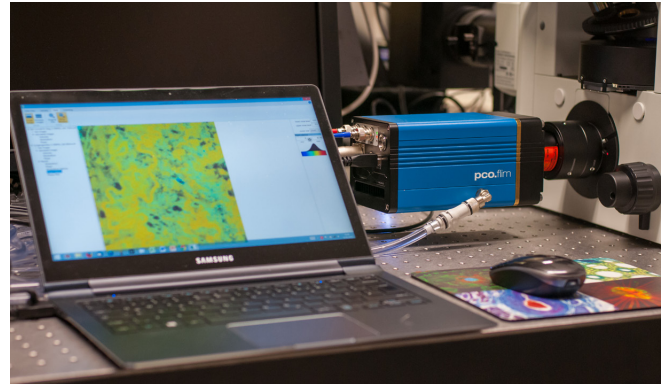


Figure 4: The pco.flim FLIM camera system attached to the camera port of an inverted microscope with the controlling PC.

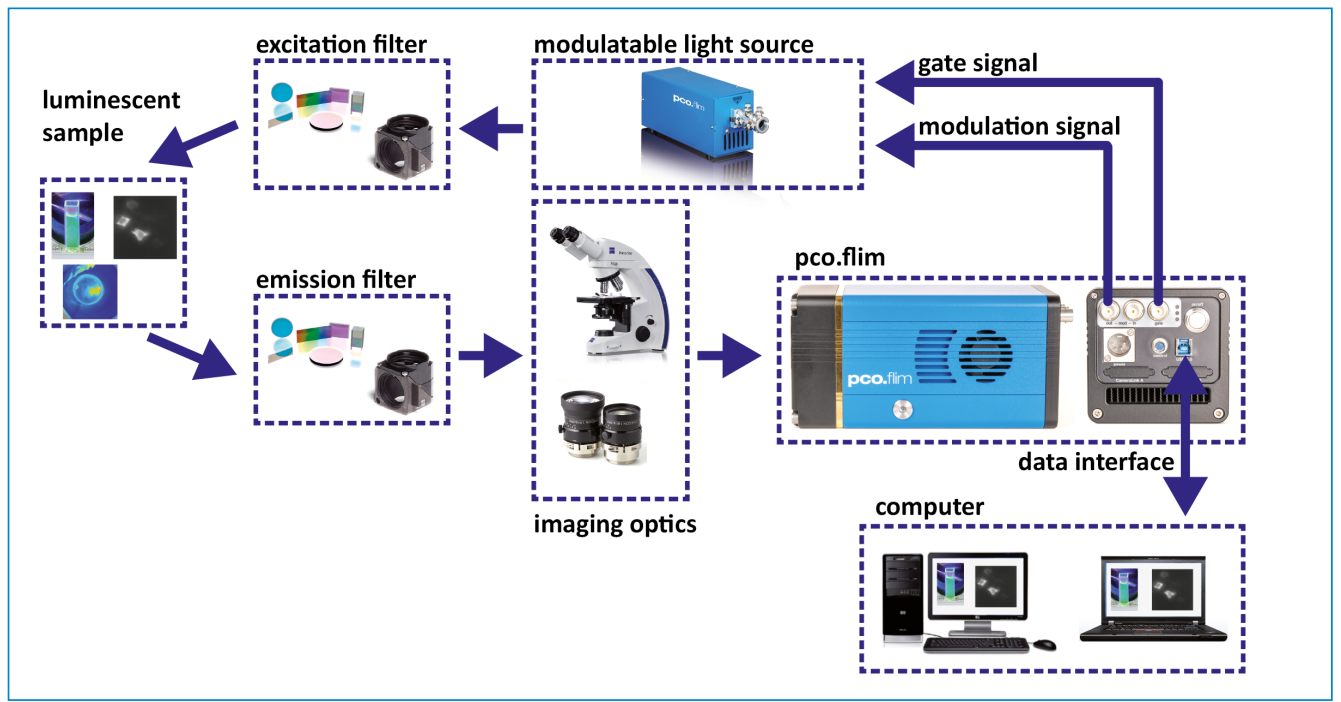


Figure 3: Structural overview of a set-up for luminescence lifetime imaging with a pco.flim camera system.



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pco.flim camera system

The pco.flim camera system, consisting of pco.flim camera and pco.flim laser, includes a complete frequency synthesizer, which is required for the generation of the modulation signals in the frequency domain.

The pco.flim has a resolution of 1008 x 1008 pixels and can read out a maximum of 45 double frames/s. The effective frame rate is about 20 frames/s, due to the fact that a minimum of 2 double frames have to be read out for a proper sine fit and this has to be done twice for a proper asymmetry correction. The camera system can be operated at a single frequency or multiple frequencies in the range of 5 kHz – 40 MHz and it can perform an asymmetry correction even before image readout.

With its widely used USB 3.0 interface it can connect to all sorts of computers. A thermo-electrical Peltier cooler keeps the image sensor at 5 °C by using either a fan or a water cooler to dissipate its own lost heat. With the C-mount it is easy to connect to any microscope or lens. Therefore the camera system significantly reduces the required efforts and costs for operation and research.

Application simplified

Integrated into an optimized software environment the measurement of 2D fluorescence lifetime distributions now has been simplified. Instead of an image intensifier camera, light source and timing or frequency generators, the application requires only a CMOS camera and a light source, which should enable a broad range of applications that were previously not feasible due to the complexity of the existing system requirements.

Therefore, numerous applications, including FRET applications for measuring the donor fluorescence to determine how much FRET has occurred, the measurement of auto-fluorescence lifetimes in natural tissue or the measurement of the luminescence lifetime for sensing purposes, ranging from optical chemical sensors on a cellular scale up to the use of pressure sensitive paint in wind tunnels, can all benefit from the new FLIM system.

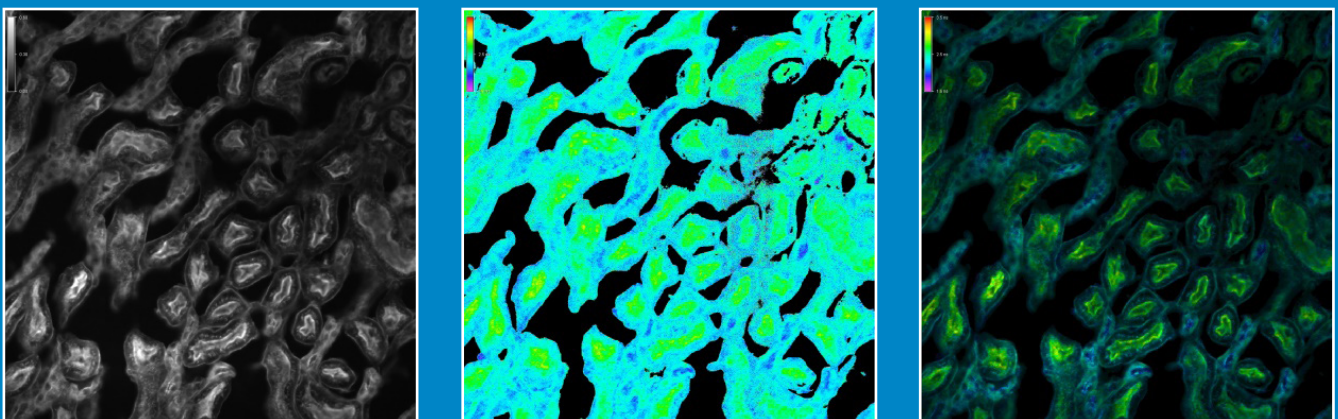


Figure 5: A ThermoFisher FluoCells® prepared slide, which contains a section of mouse kidney stained with a combination of fluorescent dyes. The visible marker is Alexa Fluor® 488 wheat germ agglutinin, a green-fluorescent lectin, which was used to label elements of the glomeruli and convoluted tubules, which were excited with 488 nm at a modulation frequency of 30 MHz. The left image shows the fluorescence intensity of the mouse kidney sample (20x air objective). The middle image shows the phase angle derived distribution of fluorescence lifetimes of the Alexa Fluor 488® in the range of 1.5 – 3.5 ns (NIS Elements, colorbar 1.5 – 3.5 ns). The right image shows the lifetime distribution image weighted by the fluorescence intensity image.

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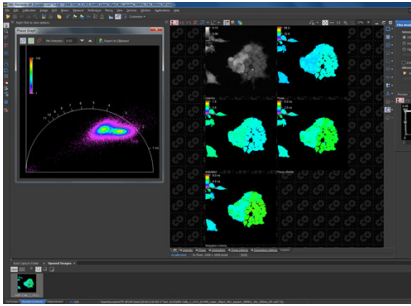
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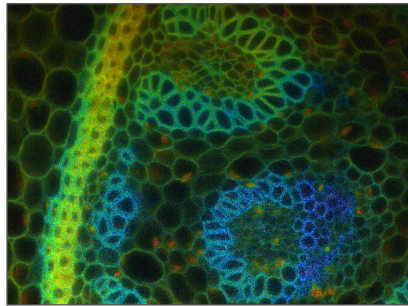


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Applications



Seamlessly integrated into Nikon NIS Elements AR software to use the pco.flim camera for homodyne frequency domain fluorescence lifetime imaging with all referencing and phasor plot feedback.

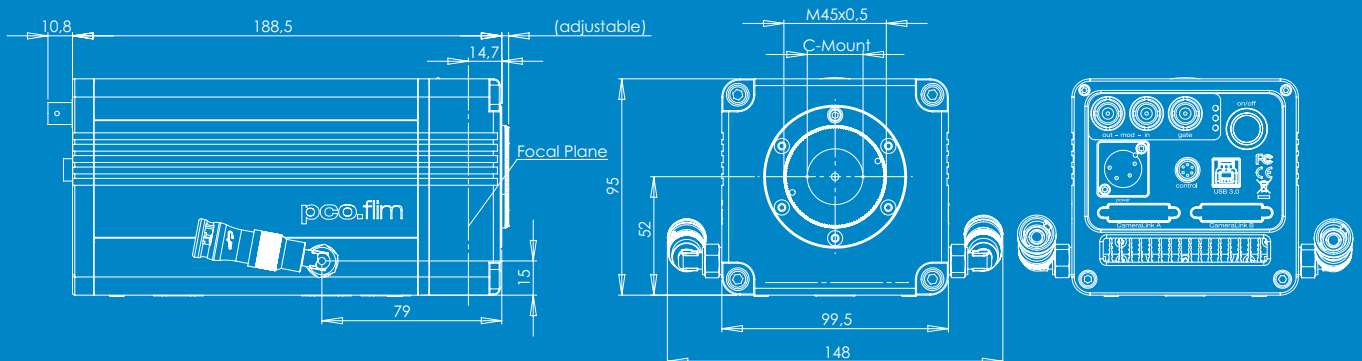


Endogenous fluorescence of a *Convallaria* (lily of the valley) slice sample. The image shows the endogenous fluorescence lifetime distribution derived from the measured phase angle in false color coding and weighted by the fluorescence intensity. The displayed lifetimes range from 0.5 - 4 ns.



HEK-293 cells co-expressing a fusion protein with Cyan Fluorescent Protein (CFP) and with Yellow Fluorescent Protein (YFP). Dimerization of this protein is detected by FRET as judged by the reduction in CFP lifetime. The image shows the fluorescence lifetime distribution derived from the measured phase angle in false color coding and weighted by the fluorescence intensity. The displayed range is from 0 – 4 ns (see color bar, courtesy of Prof. Dr. F.S. Wouters and Dr. G. Bunt, University Medicine Göttingen).

Dimensions



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Technical Data Camera System

The pco.flim camera system is the first luminescence lifetime imaging camera using a new modulatable CMOS image sensor. It offers all the required generation of frequency domain signals (5 kHz – 40 MHz) and also allows the use of external modulation signals in a limited frequency range (500 kHz – 40 MHz).

It has a USB 3.0 interface for image data transfer and control of all camera operation modes. Further, a variety of trigger input / output signals for integration of the camera into any application framework is available. The next tables gives an overview of the performance data of the camera system.

image sensor

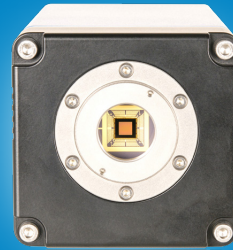
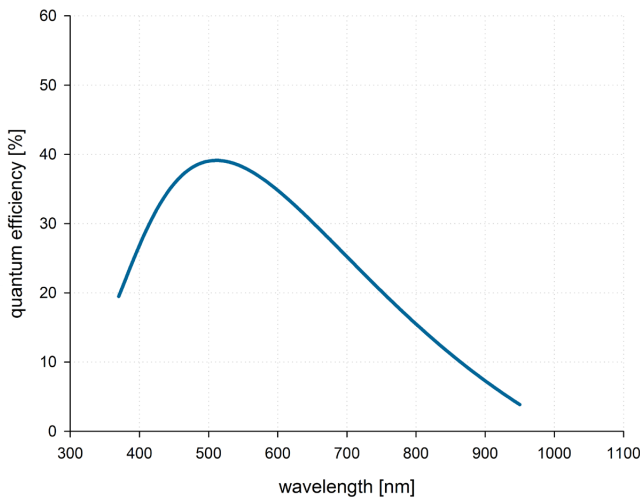
type of sensor	CMOS
image sensor	proprietary
resolution (h x v)	1008 x 1008 pixels
pixel size (h x v)	5.6 µm x 5.6 µm
sensor format / diagonal	5.7 mm x 5.7 mm / 8.1 mm
shutter mode	rolling reset / global exposure
fullwell capacity	52 000 e ⁻ (typ.)
readout noise	48 e ⁻ rms (typ.)
dynamic range	> 1 000 : 1 (60 dB)
quantum efficiency	appr. 39 % @ peak
spectral range	370 nm ... 780 nm (FWHM)
dark current	1220 e ⁻ / (s.pixel)
DSNU	56 e ⁻ rms
PRNU	0.7 %

camera

max. frame rate (full frame, full resolution)	45 double frames per s (2 tap readout)
modulation frequency	internal 5 kHz ... 40 MHz external 500 kHz ... 40 MHz
modulation signal shape	sinusoidal / rectangular
exposure / shutter time	1 ms ... 2 s
dynamic range A/D	14 bit
A/D conversion factor	3.4 e ⁻ /count
region of interest	steps of 16x1 pixel
thermoelectrically cooled	+5 °C
nonlinearity	< 1 %
trigger input signals	exposure start (phase sequence trigger)
trigger output signals	exposure, busy, gate (light enable)
modulation signal output	1 V _{peak-peak} in 50 Ω, AC coupled
modulation signal input	max. +/- 5 V in > 1 kΩ
data interface	USB 3.0

general

power supply	90 ... 260 VAC (12 VDC opt.)
power consumption	40 W max.
weight	2.4 kg
ambient temperature	+5 °C ... +40 °C
operating humidity range	10 % ... 90 % (non-condensing)
storage temperature range	-20 °C ... +70 °C
optical interface	C-mount
CE / FCC certified	yes



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pco.flim laser

The pco.flim laser is an excitation and illumination light source designed for use with the FD-FLIM pco.flim camera system. It features a digital (square wave) modulation frequency range of 0 – 250 MHz, which ideally covers the modulation frequency range of 5 kHz – 40 MHz offered by the pco.flim camera. There is a full range of different wavelengths available (375 nm – 395 nm – 405 nm – 445 nm – 473 nm – 488 nm – 515 nm – 638 nm). The system is optimum suited for the measurement of fluorescence lifetimes in the range of nanoseconds.

System connections

There are different optimal cabling connections between the pco.flim camera system and the pco.flim laser, depending on the main application the system should be used for. In case the fluorophores are reasonably stable and the samples are fixed (non-living) the camera and the laser should be connected in the following way:

The modulation output [mod - out] signal of the camera (see fig. 6) should be connected to the digital input [Digital] of the laser by means of a coaxial 50 Ohm cable. The dark gate [gate] output signal of the camera should be connected to the laser enable [Laser Enable] input signal of the laser with a similar cable.

If the laser control software settings are done properly, this connecting scheme achieves to switch OFF the laser for image readout only, which means, that even when no images are recorded the laser light is switched ON, which helps for looking at the sample with the eyes. In case photo bleaching of the fluorophores should be minimized, then for example the mechanical shutter of the microscope should be used to stop the illumination, or the control software can be used to switch OFF the laser or turn it to standby.

For living cell measurements, for fast bleaching fluorophores and for time lapse measurements, the excitation light impact on the samples has to be minimized, therefore preferably the excitation light should be only ON, when images are recorded. For that purpose the pco.flim camera also has a breakout cable which connects to the “control” connector on the back plane of the camera. There is an exposure output [exposure] signal of the camera which should be connected instead of the gate output signal to the laser enable [Laser Enable] input signal of the laser (see fig. 7).

If the laser control software settings are done properly, this connecting scheme achieves to switch ON the laser ONLY for image exposures, which means that the excitation light is always switched OFF if no images are recorded. In case the user wants to switch the light ON for orientation in the sample, the camera has to be used in preview mode (NIS Elements “Find”), then continuously images are recorded and the “flickering” light (for image readout the light is switched OFF) can be used to pick out a location of interest in the sample or to focus the sample plane properly.

pco.flim laser options

The pco.flim laser can either be ordered for homogeneous illumination of the sample in a widefield microscope application with a 3 mm liquid light guide cable, or it can be ordered for applications like Total-Internal-Reflection-Fluorescence (TIRF) microscopy, light sheet fluorescence microscopy (LSFM) and confocal spinning disk microscopy equipped with a kineflex adapter and a single mode fiber optic cable. Especially for Nikon microscope applications it can be ordered with the Nikon double-safety-shutter option.

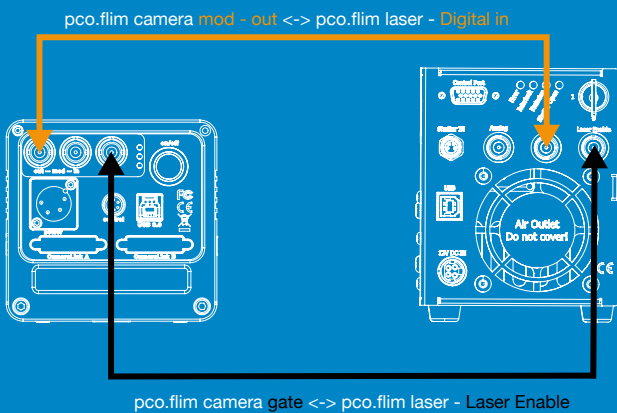


Figure 6: Cable connection scheme between pco.flim camera and laser if fixed samples should be measured with single measurements.

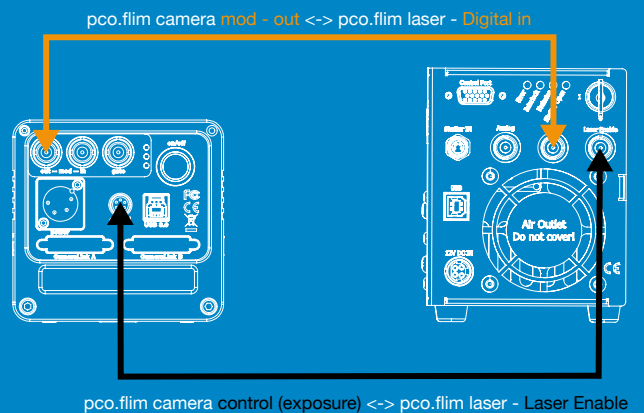


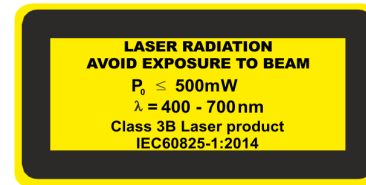
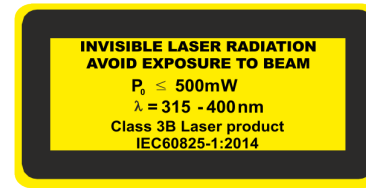
Figure 7: Cable connection scheme between pco.flim camera and laser if time lapse or living cell measurements should be made.

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Technical Data Laser

wavelengths & powers (selectable; other wavelengths and powers on request)	<ul style="list-style-type: none"> • 375 nm / 70 mW • 395 nm / 120 mW • 405 nm / 120 mW • 445 nm / 100 mW • 473 nm / 100 mW • 488 nm / 100 mW • 515 nm / 100 mW • 638 nm / 150 mW
opto-mechanical interface (selectable)	<ul style="list-style-type: none"> • 3 mm liquid light guide • FC/APC • FC/PC • FCP8 • 0.7 mm collimated beam
long term power stability	< 0.5 % / 8 h
RMS noise 20 Hz .. 20 MHz	< 0.2 % (CW)
analogue modulation input signal type	> 3 MHz (0.5 V @ 1.2 kOhm or 0.1 V @ 50 Ohm - user configurable)
digital modulation input signal type	> 250 MHz (+/- 0.5 V @ 50 Ohm)
laser enable (electronic shutter) input signal type	> 500 kHz (full ON/OFF) (LV-TTL @ 2 kOhm)
operation modes:	
mode 1	CW operation (ACC - automatic constant current)
mode 2	CW operation (ACC - automatic power control)
mode 3	analogue modulation
mode 4	digital modulation
mode 5	analogue + digital modulation
rise and fall time	analogue < 100 ns digital < 1.5 ns laser enable < 100 ns
extinction ratio	analogue > 1000 : 1 digital > 250 : 1 laser enable: infinite (full ON/OFF)
supply voltage	12.0 VDC +/- 0.5 VDC
control interface	USB 2.0
dimensions (l x w x h)	270 mm x 92 mm x 116 mm
additional shutter	optional: Nikon laser safety shutter

laser safety classification



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