

SYSTEM

LAMBER



#### Speed, sensitvity, and simplicity.

LIFA vTAU is a camera-based system for fast fluorescence lifetime imaging microscopy (FLIM), especially well-suited for live cell applications. Featuring vTAU, our versatile SPAD camera, the system allows for near instantaneous acquistion of lifetime images at unprecedented frame rates with high accuracy.

#### Complete solution

LIFA vTAU works with any brand of fluorescence microscope to form a fully integrated FLIM system for a complete solution from sample to data.

#### / Fast, adaptive dynamic range

Utilising the latest ultra-high sensitive SPAD detector, featuring micro exposures to optimise dynamic range, vTAU can capture up to 100 lifetime images per second in challenging light conditions.

#### I Broad lifetime range

Matching a wide range of capabilities, vTAU operates from the sub microsecond down to picoseconds range.

#### Multiple configurations

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Suitable for Widefield, Spinning-disk Confocal, Lightsheet, and TIRF systems, LIFA vTAU delivers an easy plug-n-play setup experience.

#### Next level sensitivity

With the unique properties of SPAD, vTAU features excellent light sensitivity to minimise measurement duration, and enables noise free readout of the detector.

#### Automatic data analysis

Dedicated LIFA software instantly calculates the fluorescence lifetime and presents it as a colour coded overlay on the original image.



# Why FLIM?

Fluorescence Lifetime Imaging Microscopy (FLIM) has become an important tool to assess the biochemical environment of fluorescent molecules and probes. Upon excitation, fluorescent molecules emit light and the fluorescence lifetime quantifies the decay rate of that emitted light. The fluorescence lifetime is a telltale signature of the molecules and their immediate environment.

FLIM is a technique which maps the spatial distribution of lifetimes in living cells and inorganic material. Fluorescence lifetime is independent of concentration, bleaching and intensity variations, making it an inherently quantitative technique, and a key advantage over light intensity.

## LIFA VTAU

The well-established frequency-domain detection technology offered by vTAU, our next generation modulated camera, allows near instantaneous acquisition of lifetime images with high accuracy.

## As it is a camera-based system LIFA vTAU is especially well-suited for live cell imaging.

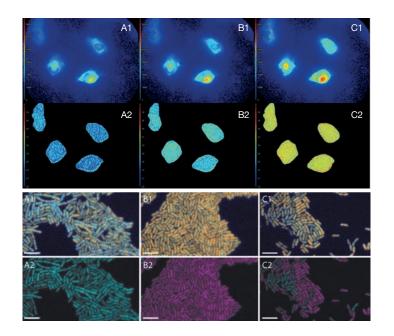
The standard, widefield system includes a Multi-LED modulated light source with high-power LEDs. Using a Multi-LASER engine it can be easily combined with Total Internal Reflection Fluorescence (TIRF) for TIRF-FLIM, and with multi-beam confocal spinning disk, for confocal FLIM.

## Compatibility The LIFA vTAU system is compatible with: Widefield Fluorescence Microscopes Total Internal Reflection Fluorescence (TIRF) Spinning-Disk Confocal Light Sheet Microscopy Hyperspectral imaging

LIFA vTAU system

#### LIFA vTAU system





#### Figure 1

Top Row - A1, B1, C1: Light intensity images (colourised).

**Bottom Row - A2, B2, C2:** Corresponding fluorescence lifetime images (colourised). The average fluorescence lifetime of the cells increases over time.

Image courtesy of the Netherlands Cancer Institute.

#### Figure 2

B.subtilis cells showing different lifetimes.

**Top Row - A1, B1, C1:** Original lifetime images of FRETing, non-FRETing and mixed cells.

Bottom Row - A2, B2, C2: Same cells as top row, but categorised and colourised based on average cell lifetime. Scale bar is  $5\mu$ m.

Image courtesy of University of Groningen.

#### How does it work?

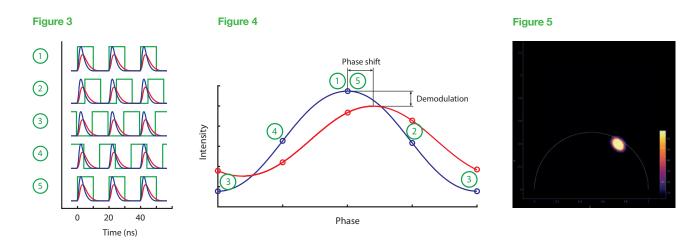
In frequency-domain FLIM, the fluorescence lifetime of a sample is acquired rapidly using a modulated light source (**blue curve**) and a modulated camera (**green curve**). Due to the fluorescence decay, the fluorescence emission from the sample (**red curve**) is phase-shifted and reduced in amplitude.

For both the excitation and the detection, the same frequency is used (homodyne detection), and at different camera phase settings (1-5 in Figure 3 below) a series of images of the fluorescence emission is taken. This results in a frequency-domain cross-correlation function (Figure 4, red curve) for each of the pixels in the image. The intensity of the emission image will depend on whether the detector sensitivity is partly (2 and 4 in Figure 4 example) or fully (1 and 5 in Figure 4 example) in phase with the fluorescence emission.

The key is that this function exactly mirrors the phase shift and the demodulation of the fluorescence emission in the time domain. These two parameters can be translated to a lifetime value per pixel. This frequencymixing approach is the basis of radio technology and is well known for its convenience, simplicity and strong noise suppression. This fluorescence lifetime is obtained simultaneously for all pixels by using a state-of-the-art modulated camera.

For further analysis, the frequency-domain lifetime data can be decomposed into exponential components. A popular alternative is to plot the measured phase shift and demodulation in a single diagram. The phasor plot offers a visual overview of the fluorescence decay in the image. (**Figure 5 below**)

In the phasor plot, the presence of different molecular species or the occurrence of FRET is visualized as data clustering in specific regions. The phasor plot analysis is fast and makes FLIM accessible to the non-expert in spectroscopy and data analysis.





## Experiments from start to finish

Our LIFA software guides you through your FLIM experiments from start to finish. All Lambert Instruments hardware is integrated seamlessly so you can focus on your experiment. Finding the right FLIM settings is easy with Live View from the camera. The software takes care of recording the FLIM data and instantly calculates the fluorescence lifetime.

#### Image acquisition

Recording a single fluorescence lifetime image takes less than a second. LIFA vTAU acquires the FLIM data and instantly calculates the fluorescence lifetime.

You can also record a time-lapse video of your sample to see how the lifetime changes over time. Simply set the duration and the interval between images and the software takes care of the rest.

### / Data analysis

Once you have recorded your FLIM data, the LIFA software automatically calculates the fluorescence lifetime. Results can be analysed as statistical data or in several visual representations, including histograms, scatter plots and the phasor plot.

All fluorescence images and graphs can be exported to common image formats or as raw data points.

Time-lapses can be exported to video files. Statistics can be copied from the LIFA software and pasted into third-party software, like Microsoft Excel.

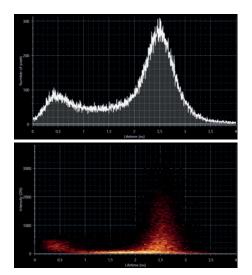
### Hardware control & automation

Lambert Instruments light sources can be controlled in the LIFA software, allowing you to switch between wavelengths with a single click.

The flexible system also supports a wide range of third party hardware, enabling the system to be updated and enhanced as experimental requirements change.

Take full control of the LIFA software by using the automation interface. With this, you can adjust all acquisition settings and trigger the camera to record FLIM images and time-lapses from any supported programming environment.







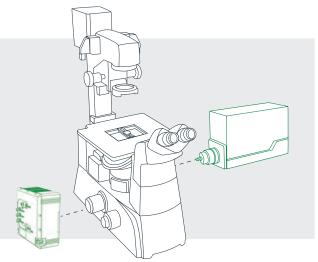


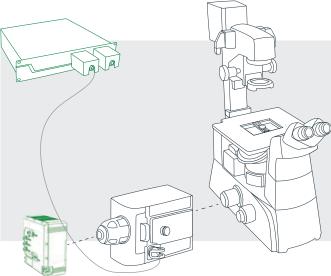
# LIFA vTAU configurations

The standard LIFA vTAU configurations are shown below. If your application requires a unique configuration, our applications specialist will be happy to discuss your requirements.

#### Widefield configuration

On widefield microscopes, the vTAU camera in combination with the Multi-LED offers a capable yet compact FLIM solution. vTAU is compatible with the camera port of widefield microscopes and the Multi-LED is compatible with the standard epifluorescence port of widefield microscopes.



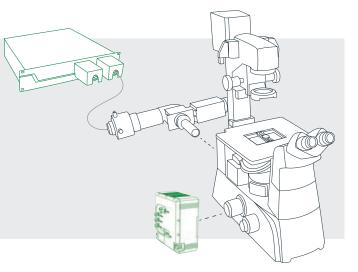


#### Spinning-disk confocal configuration

Being a camera-based system, the Lambert Instruments LIFA vTAU system for frequencydomain FLIM is compatible with multi-beam confocal microscopy techniques, most notably the Yokogawa CSU spinning disk series (based on the Nipkow disk scanner), and the VTInfinity series by Visitech International.

#### **I** TIRF configuration

Total Internal Reflection Fluorescence (TIRF) microscopy facilitates extremely high-contrast visualization and thereby high sensitivity of fluorescence near the cover glass. Typically, the optical section adjacent to the cover glass is about 100 nm. The unique combination of TIRF and frequency-domain FLIM makes it possible to measure lifetimes of, for instance membrane receptors in order to identify their signalisation pathway.





## vTAU... for versatile lifetime imaging



vTAU, our next generation FLIM camera, utilises the latest ultra-high sensitive SPAD detector, enabling FLIM up to 100 fps; simplifying experiments for researchers and imaging centres by combining excellent light sensitivity with easy image acquisition and data analysis.

This versatile camera, as part of the LIFA system, helps you minimise measurement duration, automate image acquisition and simplify data analysis, factors of great importance in cell biology, cancer research and high-throughput screening.

# FLIM images

#### Features

vTAU features a unique image sensor that combines excellent light sensitivity with advanced fluorescence lifetime imaging capabilities.

This image sensor was designed and optimized specifically for fluorescence liftime imaging applications and enables lifetime imaging at unprecedented frame rates.

Imaging modes of the camera include regular frequencydomain FLIM acquisition and time-lapse recordings.

Data analysis is done automatically by the LIFA software, which instantly calculates the fluorescence lifetime and presents it to the user as a colour coded overlay on the original image.

Compatible with any brand of fluorescent microscope, and suitable for multiple FLIM configurations, the versatility of LIFA vTAU provides a plug-n-play experience that allows for easy switching between setups.

# Large SPAD array 512 x 512

Parameter	Typical Value
Lifetime range	0.2 to 300ns
Pixel resolution	512 x 512
Pixel size	16 µm
Fill factor	50%
Frame rate	up to 300 fps
Sensor type	SPAD
Modulation frequency	10 - 60 MHz
Water cooling	Stabilised at 20°C
Dimensions (L x W x H)	156 x 80 x 160 mm
Camera interface	USB





#### Multi-LASER

The Multi-LASER is a light source for frequency-domain fluorescence lifetime imaging microscopy. The Multi-LASER contains up to 6 laser diodes with different wavelengths.

High-speed digital modulation up to 180 MHz, modulation depth of at least 250:1 (standard) or at least 2500000:1 (extreme). Power stability better than 2% (standard) or better than 0.5% (extreme).

We supply the Multi-Laser according to the wavelengths you require; most common wavelenghts requested are:

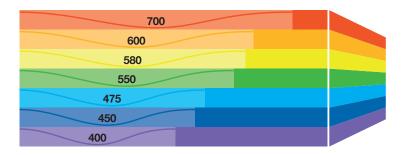
<400, 405, 445, 488, 515, 532, 561, 633, and 642 nm.

#### Multi-LED

The ultra-high sensitivity of the SPAD detector enables the use of LEDs, thus avoiding photobleaching and toxicity, providing a cost effective solution.

The Lambert Instruments Multi-LED is a versatile excitation light source for fluorescence lifetime imaging microscopy in the frequency-domain. The Multi-LED contains up to 4 LEDs that provide non-phototoxic illumination levels, have a low cost and a long lifespan.

All LEDs are high-quality modulating LEDs with a peak light intensity at wavelengths between 446 and 525 nm, 595 nm, 635 nm and 696 nm. Other wavelengths are available upon request.



#### Other LIFA systems

For Time-Domain FLIM for Widefield microscpes, LIFA can also be supplied with a gated **TRiCAM** camera. Typically more cost-effective than methods that require a confocal set-up, the LIFA with TRiCAM standard configuration offers an entry-level setup for FLIM measurements.



#### System Components



vTAU camera

Versatile, plug-n-play FLIM camera for quick and easy setup.

Ultra-high sensitive SPAD detector for up to 100 lifetime images per sec.

Regular frequency-domain FLIM and time-lapse image modes.

LIFA vTAU system also includes:

User Benefits

Versatile and simple setup

Long term life cell imaging

No alignment, plug-n-play setup

makes switching microscopes easy.

No maintenance, multi wavelengths system, with third party hardware

User training is quick.

Low phototoxicity

is now possible.

**Cost effective** 

support.

Easy to use



Light Source(s)

Multi-LED and/or Multi-LASER as required for your setup.

Excitation light sources for frequency-domain fluorescence lifetime imaging, supplied according to the wavelengths you require.

Computer with USB connectionInstallation by a Lambert engineer at your lab

### Applications

Molecular interactions Protein conformation Biosenors Oxygen imaging Metabolism Protein-protein interactions Ion imaging NADH/FAD fluorescence dynamics Viscosity imaging Membrane dynamics Membrane trafficking LED inspection Crude oil characterisation Solar cell MCL monitoring



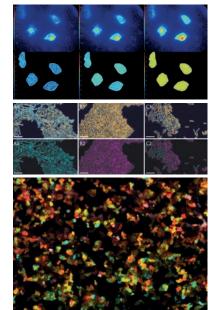
**LIFA Software** 

Seamless integration of all hardware for full system control

Guides users through FLIM experiments from start to finish.

Supports third party hardware for a flexible and expandable system.

/ One day of hands-on training/ Phone, email, and remote desktop support



Specifications subject to change

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Part of Tibidabo Scientific Industries

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Lambert Instruments is dedicated to development, production and worldwide sales of products for **time resolved imaging at low-light levels**.

Our mission is to enable our users to **reveal previously unseen phenomena**. Our products provide a possibility to record fast events at low-light conditions. Together with our software, we **reimagine detection** to offer complete solutions to challenging imaging problems.

#### www.lambertinstruments.com