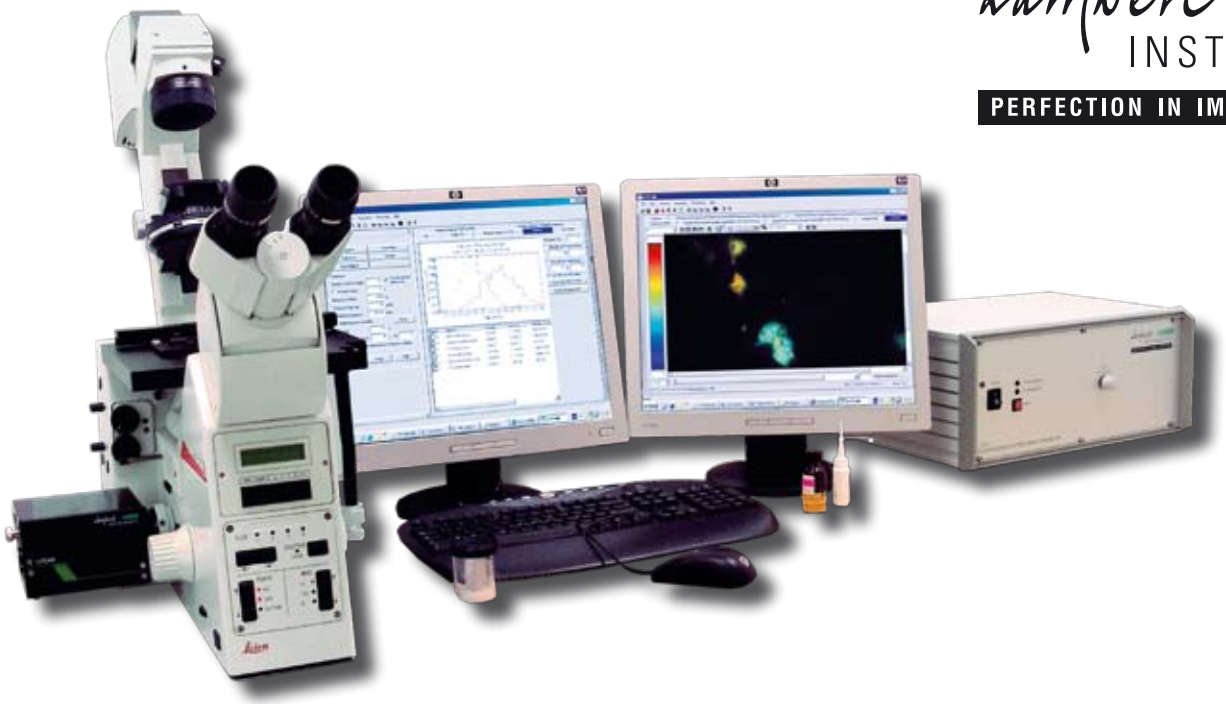
Lambert INSTRUMENTS

PERFECTION IN IMAGE DETECTION

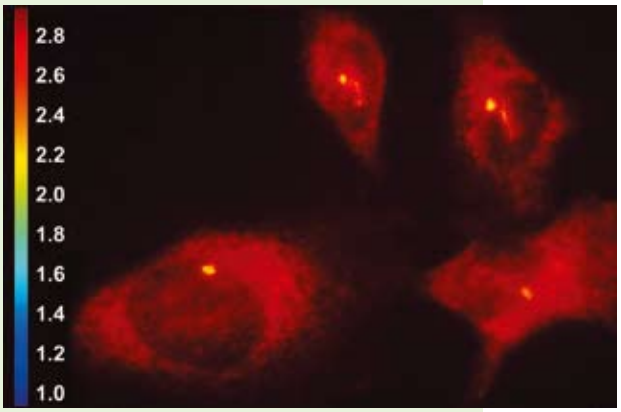


Fluorescence Lifetime Imaging Microscopy (FLIM)

Why lifetime imaging?

The fluorescence lifetime is the signature of a fluorescent material; it is the exponential decay in emission after the excitation of a fluorescent material has been stopped. FLIM (Fluorescence Lifetime Imaging Microscopy) is a technique to map the spatial distribution of lifetimes within microscopic images and it allows measurements in living cells as well as in fixed materials.

Because of the fact that some phenomena do affect fluorescence lifetimes, the lifetime is used to detect these phenomena leading to various applications such as: ion imaging (pH measurements), oxygen imaging, probing microenvironment, and medical diagnosis. Moreover, the most powerful FLIM-application in biology is Fluorescence Resonance Energy Transfer (FRET). When two fluorescent molecules (or two fluorescent labeled epitopes within a protein) are in very close proximity, i.e. less than 9 nm, the energy of the one fluorescent (donor) molecule (e.g. GFP) is transferred in a nonradiative process to the other fluorescent (acceptor) molecule (e.g. mCherry). In this way, the lifetime of the donor molecule decreases and this change can be measured quantitatively by FLIM.



FRET at the centrosomes (microtubule-organising centres), measured by FLIM. The mammalian cells are paraformaldehyde fixed and labelled with donor Alexa488 and acceptor Alexa568 fluorophores that are fused to two different proteins. At the centrosomes these two proteins interact with each other, as shown by FRET. In case of FRET, the lifetime of the donor is shorter (here: yellow) than in case of no FRET (here: red). (Courtesy of Diaspro, LAMBS, Italy).

Frequency domain method

The homodyne frequency domain FLIM method requires a modulated light source and a modulated detector. In the LIFA system these are the LED and the intensified CCD camera. Both are modulated at exactly the same frequency, but with an adjustable difference in phase. The emission intensity shows a phase-shift (or delay) with respect to the excitation as well as a decrease of modulation-depth. These two parameters depend on the fluorescence lifetime of the sample and the modulation frequency and are measured to calculate the fluorescence lifetime in each pixel of the image.

The Lambert Instruments FLIM Attachment (LIFA)… what is it?

The Lambert Instruments Fluorescence lifetime imaging microscopy Attachment (LIFA) is a dedicated system that allows image acquisition and generation of lifetime images in a matter of seconds. The system can be simply attached to any wide field fluorescence microscope and is easy to operate. The LI FLIM software allows acquisition at single-frequency or multi-frequency, time lapses and includes analysis features such as statistics per ROI, polar plot and multi-lifetime-component-fit. All data can be exported.

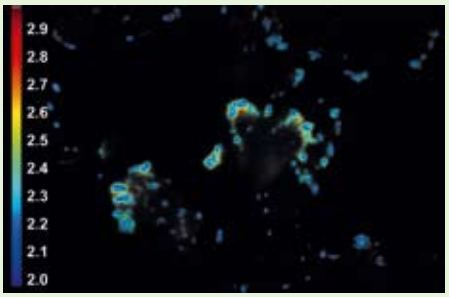
LIFA with other techniques

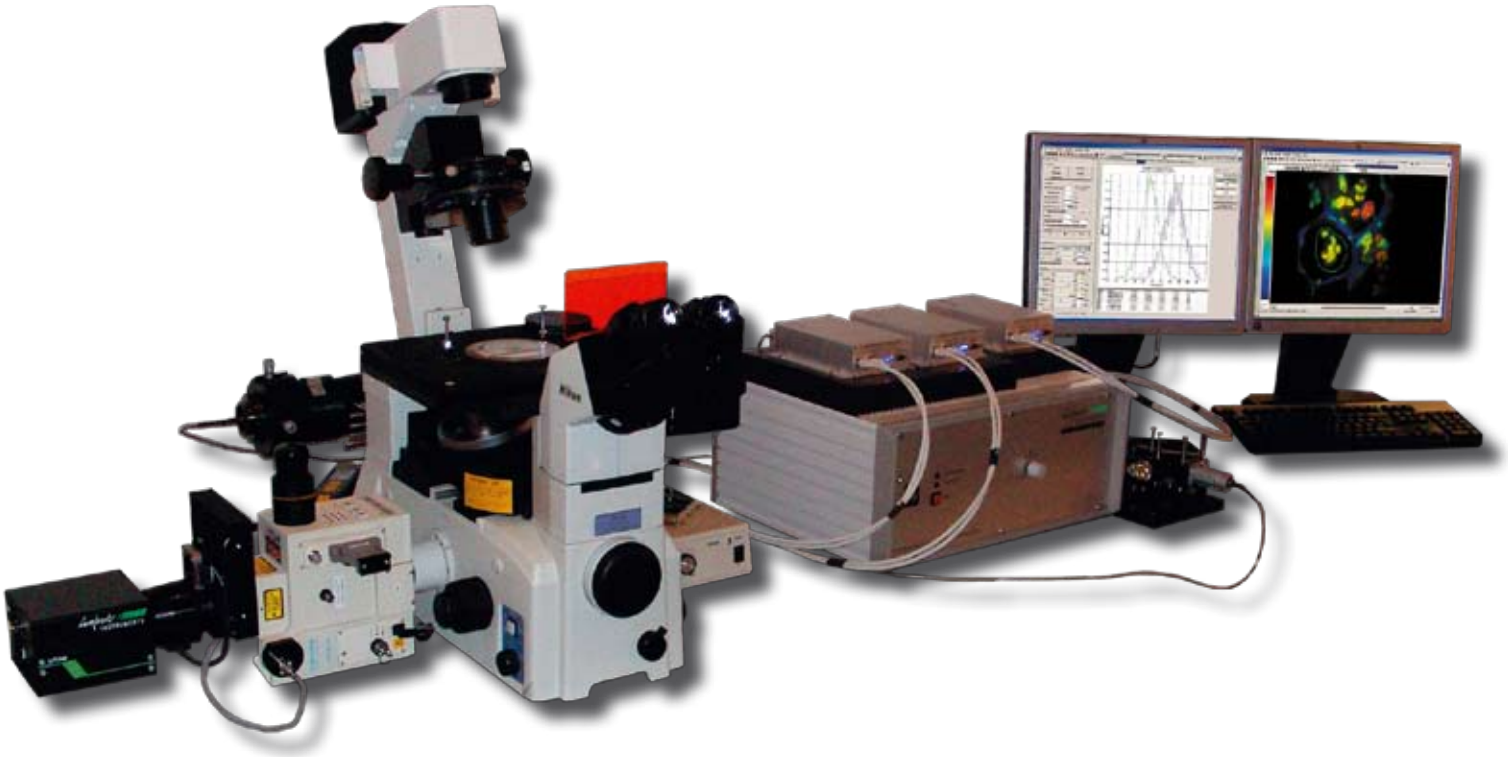
Widefield FLIM has the advantage of having a high speed of acquisition. However, one disadvantage is the out-of-focus light that lowers the contrast not only in the intensity image but also in the fluorescence lifetime data. This drawback is eliminated in multibeam confocal FLIM when using the LIFA with Yokogawa spinning disk and wide field microscope. A much better spatial resolution can be achieved at high acquisition rate. Moreover, 3D FLIM stacks can be generated with this set-up.

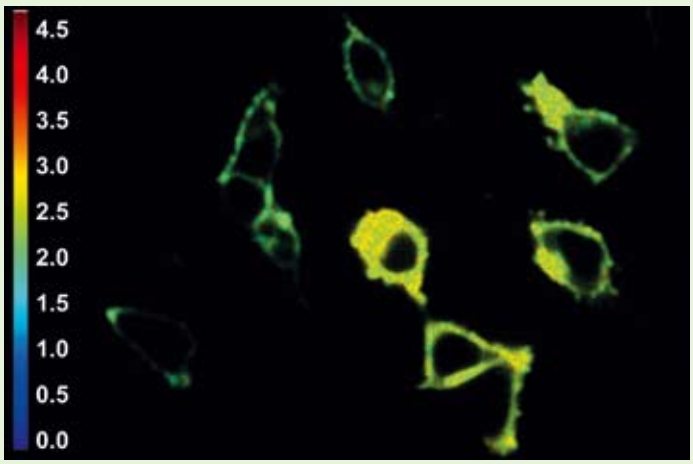
The LIFA can also be combined with laser-TIRF(Total Internal Reflection Fluorescence). The laser beam comes in at a large incident angle causing internal reflection in the cover glass which results in the excitation of fluorophores immediately adjacent to the cover glass-specimen interface. Fluorophores at more than a few hundred nanometers distance from the cover glass (e.g. nuclei in the cell) are not excited and do not contribute to the intensity. This results in a much better contrast in intensity and in the lifetime image of the phenomena of interest (e.g. in the cell membrane) close to the cover glass.

The LIFA with Yokogawa spinning disk or with laser-TIRF uses modulated diode lasers as excitation light source instead of LED's. The lasers are integrated with the LIFA to control their power and modulation via the LI FLIM software package that comes with the LIFA.

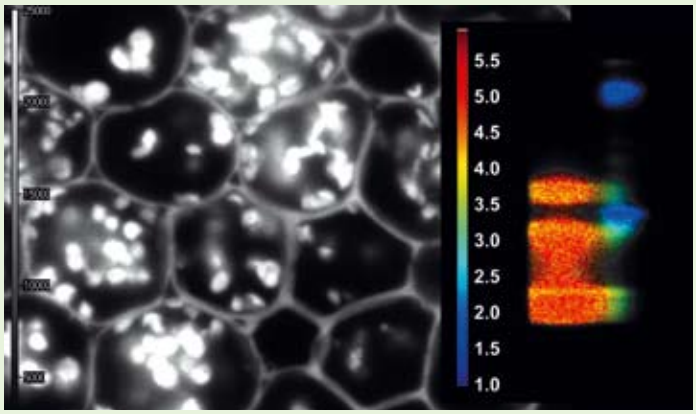
Spectrally resolved FLIM is done by the combination of LIFA with a spectrograph. One line out of the 2D intensity image is spectrally resolved by this spectrograph. Simultaneously the LIFA calculated the lifetimes per pixel resulting in a spectrally resolved fluorescence lifetime image.







Erythrocytic cells with membrane proteins fused to Cerulean or Citrine. FLIM image taken with LIFA combined to CSU22. Fluorescence lifetime in pseudo colors. (Courtesy of W. El Nemer, INSERM, and M. Christophe, platforme d'Imagerie Dynamic, Paris, France).



2D intensity image of a typical dicot root and lifetime image of one line from the intensity image, spectrally resolved with the fluorescence lifetime in pseudo colors. (Courtesy of Q. Hanley (Nottingham, UK).

Features

1. Frequency domain FLIM, used on wide field fluorescence microscopes.
2. Fast acquisition down to several lifetime images per second: convenient for moving objects in living cell samples.
3. The use of modulated LEDs or diode lasers allows excitation wavelengths from 365nm until 640nm.
4. Moderate excitation intensity reduces the chance to photo toxicity.
5. Multi frequency measurement allows the analysis of multi exponential lifetime components.
6. Combination with other microscopy techniques, like multibeam confocal, TIRF, spectroscopy, etc.

Build your own frequency domain FLIM system

For users who would like to build their own frequency domain FLIM system, the modulated intensifier II18MD is offered as a separate product. To complete the system, a modulated light source, CCD camera, and preparation of the acquisition and analysis software are required. The II18MD comes with a power supply / control unit and can be equipped with a signal generator (II18MDS). Modulated LED's and laser diodes are available as separate products as well.

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Reference sites

Europe:
Amsterdam, The Netherlands; Heidelberg, Germany; Cambridge, United Kingdom; Paris, France; Albacete, Spain; Goteborg, Sweden
Other continents:
Arkansas, USA; Melbourne, Australia; Singapore, Singapore; Okinawa, Japan.
Full details of test sites (address, system components) are available at our website.

Publications

Several publications have been prepared with our equipment. See our website for the full list of publications of LIFA and II18MD.

LIFA system components

- Modulated image intensifier (GenII) fiber optically coupled to digital CCD camera
  Optional: GenIII instead of GenII, for higher Quantum Efficiency (QE): better signal to noise ratio or shorter exposure time
  Optional: lens-coupled modulated image intensifier and CCD camera
- Modulated LED light source mounted in lamp housing
  Optional: modulated laser diode light source
- FLIM signal generator / control unit / power supply
- 2 USB 2.0 interfaces
- LI-FLIM software package
- Personal computer
- 2 LCD screens

Quick reference LIFA system specification	
Lifetime range	: 0-300 ns (in selectable ranges)
Lifetime resolution	: 80 ps
Modulation frequency	: 100 MHz max.
Measurement time	: 1 second typical
Speed	: 12 lifetime images/sec
Spatial resolution	: 39 lp/mm in DC mode 21 lp/mm in FLIM mode
Digital output	: 12 bit
ICCD Camera sensitivity	: down to 10^{-5} lx at video speed
Camera CCD pixel lay-out	: 1392(H) x 1040(V)
Binning	: 2 x 2, 3 x 3, 4 x 4
Camera field of view	: 14,4 x 10,8 mm
Camera mount	: C-mount 1x
Available LED wavelengths	: 365nm, 405nm, 448nm, 468nm, 489nm, 538nm, 630nm, etc.
Available laser diode wavelengths	: 375nm, 405nm, 445nm, 473nm, 488nm. More on request
Detection range (GenII, S20)	: 200-600nm
Detection range (GenII, Super S25)	: 450-850nm
Detection range (GenIII; GaAs)	: 450-900nm
Dimensions camera head	: 133(L) x 116(W) x 80(H) mm
Dimensions P.S./control unit	: 47(W) x 42(D) x 20(H) cm

Links are provided in the digital version of this brochure. Go to the "FLIM brochure" download at our website under Products – FLIM.

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