

FLIM combined with TIRF or confocal imaging improves image contrast

J. Siebring, H.M.J. Oosterveld-Hut and L.K. van Geest.

Lambert Instruments, Oosteinde 16, 9301 ZP, Roden, The Netherlands.

siebring@lambert-instruments.com ; www.lambert-instruments.com ; +31-50-5018461

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.

The fluorescence lifetime is independent of bleaching and intensity variations in the sample. Because some phenomena do affect fluorescence lifetimes, the applications of FLIM are various: ion imaging, oxygen imaging, probing microenvironment, and medical diagnosis. Moreover, the most powerful FLIM-application in biology is Fluorescence Resonance Energy Transfer (FRET)-microscopy. When two fluorescent molecules (or e.g. two fluorescent labelled epitopes within a protein) are in very close proximity, i.e. less than 9 nm, the energy of the one fluorescent (donor) molecule is transferred in a nonradiative process to the other fluorescent (acceptor) molecule. In this way, the lifetime of the donor molecule decreases and this change can be measured quantitatively by FLIM.

Lambert Instruments has developed a dedicated system (LIFA) that allows image acquisition and generation of lifetime images within one second: the Fluorescence Lifetime Imaging Attachment. The nanosecond lifetime information can be extracted pixel-by-pixel while visualising the intensity in grey scale and the lifetime in pseudo colours (Figure 1A).

The LIFA can be attached to any fluorescence wide field microscope and is compatible to several techniques, like Total Internal Reflection Fluorescence (white-TIRF as well as laser-TIRF) and multi-beam confocal microscopy (by spinning disk). The first, TIRF microscopy, is an ideal method for studying fluorescently labelled proteins that are located at the periphery of a cell, e.g. in the membrane, up to 100 nm from the coverslip (Figure 1B). This is because the fluorescent proteins of deeper regions in the cell are not excited by the evanescent wave that is generated in a TIRF set-up. The second compatible technique, multi-beam confocal microscopy, is a technique used to increase micrograph contrast, but then at any focus plane in the cell (Figure 1C). It is also used to reconstruct three-dimensional images by eliminating out-of-focus light in specimens that are thicker than the focal plane. The confocal image can be obtained by the Nipkow disk that has a spiral pattern of pinholes arranged to scan the specimen, with an array of light beams.

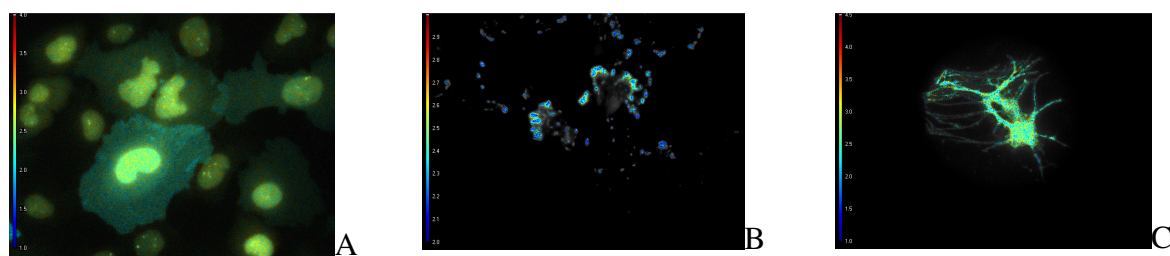


Figure 1. A, FLIM image with lifetime in pseudo colours and intensity in grey scale. The cells show a decreased lifetime (FRET) at the cell membrane. B, FLIM-laser-TIRF image. These cells show dSH2 in focal adhesions (courtesy of Leiden University, NL). C, Confocal FLIM image of GFP-expressing cell prepared with LIFA with the CSU-22 spinning disk.