Fast frequency-domain FLIM for live cell bioimaging

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The fluorescence lifetime, or the average decay time of the excited state of a fluorescent material, is a tell-tale signature of a fluorescent material. FLIM (Fluorescence Lifetime Imaging Microscopy) is a technique to map the spatial distribution of lifetimes within living cells, tissues and whole organisms. The fluorescent lifetime is affected by a range of biophysical phenomena, and hence the applications of FLIM are many: from ion imaging and oxygen imaging to drug discovery and medical diagnosis. The most used phenomenon in biological applications of FLIM today is Förster Resonance Energy Transfer (FRET), which is used extensively in studying cell function and cell disease in quantitative cell-biology. Changes in fluorescence lifetime as small as several hundreds of picoseconds can, for example, indicate interactions between proteins in living cells.

The LIFA system for widefield FLIM developed by Lambert Instruments allows acquisition of entire lifetime images within seconds. Its key component is a fast and sensitive FLIM camera based on a modulated image intensifier that is fiber-optically coupled to a CCD camera. The system achieves picosecond lifetime accuracy, is compatible with every major brand microscope, and is very easy to use and maintain. Here attention is given to the latest technological developments relevant for live cell applications, including a new and even faster FLIM camera and a new confocal FLIM combination, in which the LIFA is attached to the VT-Infinity3 multi-beam confocal scanner.