Fluorescence Lifetime Imaging (FLIM) made easy

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We report on the development of a compact, stand-alone prototype for real-time, wide-field fluorescence lifetime imaging (FLIM) in the frequency domain. The prototype yields a 2D map of fluorescent lifetimes in the nano- to microsecond range without the need for scanning. Its portable size, low cost and ease of use show potential for a variety of applications based on monitoring fluorescence lifetime.

Fluorescence signals are characterized by three main parameters: intensity, wavelength and lifetime[1]. The fluorescence intensity depends on the concentration of the fluorophore and its quantum efficiency. Fluorescence intensity images therefore yield the spatial distribution of a fluorophore. The emission spectrum is a characteristic of the fluorophore. Images containing spectral information thus allow different fluorophores to be identified. The third property, the fluorescence lifetime, depends on the type of the fluorophore and on the molecular environment surrounding it. It is influenced by the presence of fluorescence quenchers, by binding of the fluorophore to different biological targets, or by the presence of other optical absorbers to which it may interact. Fluorescence lifetime imaging (FLIM) can therefore be used to obtain information on the molecular environment of the fluorophore molecules, or to identify fluorophores based on their fluorescent lifetime[2]. Unfortunately, fluorescence lifetime imaging has traditionally been associated with complex and expensive equipment, and has therefore found limited applications outside of research laboratories.

To demonstrate the potential of FLIM and open the door to novel applications, we developed a compact, stand-alone prototype incorporating all the necessary hardware components for wide-field frequency-domain FLIM (Figure 1).

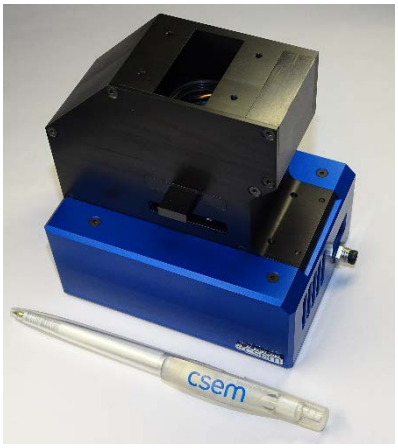

Figure 1: Compact stand-alone prototype for real-time, wide-field fluorescence lifetime imaging (FLIM) in the nano- to microsecond range.

The prototype includes a modulated light source (LED), a CMOS Time-of-Flight imager (256 × 256 pixels), dedicated FPGA-based electronics, and optical components for illuminating the probe and collecting the fluorescence emission.

The camera electronics is based on a stacked PCB approach, including a base board, a FPGA processing module and a sensor head PCB. A MATLAB GUI running on a separate PC is used to set the measurement parameters (such as modulation frequency, illumination intensity and integration time) and display the results. The optical modulation frequency can be varied between 3 kHz and 20 MHz, allowing the measurement of fluorescence lifetimes from hundreds of microseconds down to a few nanoseconds.

The FLIM prototype yields a 2D map of fluorescent lifetimes in a single shot (Figure 2), without the need for scanning. With the current optics an area of 6 × 6 mm is imaged. The emission wavelength can be selected by exchangeable spectral filters.

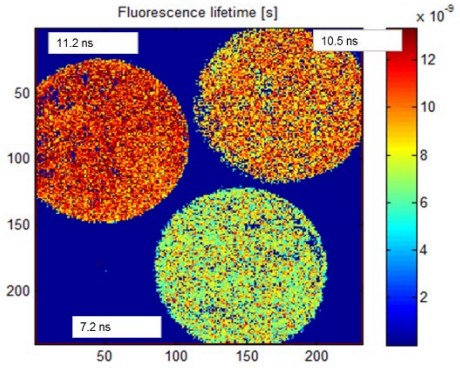

Figure 2: 2D map of fluorescence lifetime obtained with the FLIM prototype, showing lifetime differences on the order of a few nanoseconds.

The FLIM prototype was benchmarked against several high-end commercial FLIM systems on a wide range of fluorescent probes. In general a good agreement was found, although in some cases deviations between instruments (even commercial ones) were observed. The performance of the CSEM prototype was essentially on par with high-end systems, despite a considerably reduced size and cost.

Current applications of the FLIM system are in the field of wound monitoring and chemical sensing (oxygen, pH), but other applications in the nondestructive analysis of probes, either based on fluorescence tags (anti-counterfeiting, forensics, etc.) or on auto-fluorescence (food analysis, medical diagnostics, etc.) can be envisaged.

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[1] J. R. Lakowicz (Ed.), "Principles of fluorescence spectroscopy", Springer Science & Business Media (2013).

[2] M. Y. Berezin, S. Achilefu, "Fluorescence lifetime measurements and biological imaging", Chemical reviews, 110 (2010) 2641.