Technology Offer

eeFILM – Extended Excitation for Faster Measurement of Fluorescent Lifetimes

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The estimation of excitation lifetimes based on fluorescence or phosphorescence emission is an essential technique in basic and applied science. It provides information on molecular interactions as well as on physical properties. Measuring the emission lifetime with spatial resolution provides an imaging modality, which in case of fluorescence is called Fluorescence Lifetime Imaging (FLIM). FLIM is applied extensively in biological imaging in order to determine the conformation, activation, interactions and redistributions of key molecules involved in signal transduction. In biotechnology, fluorescence decay measurements are used for high-throughput screening (e. g. via binding assays) of prospective diagnostic or therapeutic molecules with designated targets.
The experimental difficulty in conventional time-domain measurements, in particular FLIM, is the need for short excitation light pulses (duration less to or comparable to the decay time), which deliver low energy, and the superposition of exponential emissive decay functions. Thus, data analysis of the detector response function for deriving the decay time requires deconvolution. Here, complex mathematical procedures based on iterative minimization are involved, requiring considerable computation time – even with current computers extending to minutes. Most FLIM applications are based on the mean decay lifetime computed from the individual decay times and their corresponding amplitudes. Deriving a mean value still requires a complete decay analysis in order to obtain the number of components and their parameters. This limitation applies to all current FLIM or single channel lifetime techniques.

Technology

Here we provide an improved emission lifetime measuring method and apparatus, avoiding dis- advantages of conventional techniques. The basic concept is to replace the very narrow conventional excitation pulses by a pulse encompassing a constant plateau section longer than the longest excited state lifetime in the sample (s. fig. 1). Thus the sample achieves an equilibrium excited steady-state showing a constant fluorescence intensity.

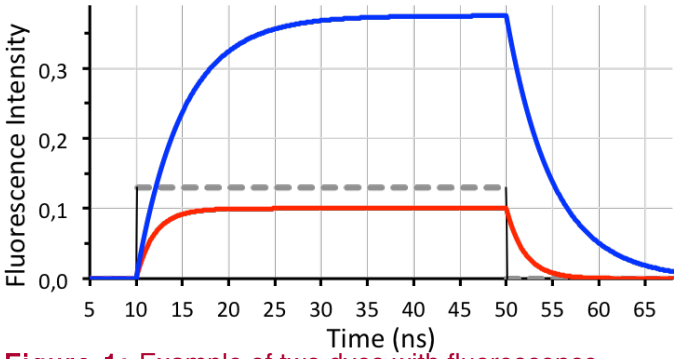

Figure 1: Example of two dyes with fluorescence lifetimes of 2 ns (red) and 5 ns (blue); the dashed line represents the excitation period.

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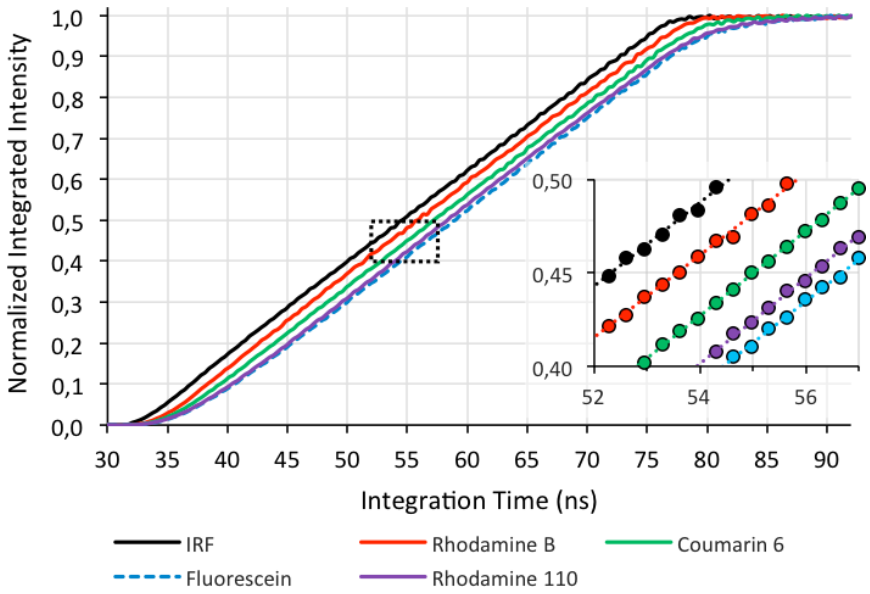

Figure 2: Fluorescence lifetimes of different dyes determined with eeFLIM; IRF: scattered light reference (lifetime: 0 ns); inset: magnification of outlined area.

Integration of the fluorescence signal leads to a detector response function including a linear response section (s. fig. 2). This section is characterized by an intercept on the temporal axis with a displace- ment corresponding to the mean fluorescence lifetime. Since this value can be directly derived by means of only two points and a linear equation, the calculations for FLIM imaging are drastically simplified, thereby not only accelerating image capture but also allowing real-time imaging of complex biological or physical processes.

Advantages

- Longer excitation pulses have much higher intensity and energy and are easier and cheaper to generate
- Broad range of samples can be investigated: solid, liquid and gaseous samples with single or multiple electronic transitions
- Measurements in a broad time range covering fluorescence or phosphorescence
- Detection of very short lifetimes with high sensitivity and photon efficiency
- Any detector (single, array, camera) incorporating a suitable gating feature is feasible
- Enhanced signal-to-noise ratio
- Fast signal processing and reduced complexity of algorithms in signal processing
- Compatible with TCSPC technology

Patent Information

- EP patent application filed in January 2016.

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