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Fluorescence lifetime imaging by multi-dimensional time correlated single photon counting

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Abstract Fluorescence lifetime imaging (FLIM) techniques for biological imaging have to unite several features, such as high photon efficiency, high lifetime accuracy, resolution of multi-exponential decay profiles, simultaneous recording in several wavelength intervals and optical sectioning capability. The combination of multi-dimensional time-correlated single photon counting (TCSPC) with confocal or two-photon laser scanning meets these requirements almost ideally. Multi-dimensional TCSPC is based on the excitation of the sample by a high repetition rate laser and the detection of single photons of the fluorescence signal. Each photon is characterised by its arrival time with respect to the laser pulse and the coordinates of the laser beam in the scanning area. The recording process builds up a photon distribution over these parameters. The result can be interpreted as an array of pixels, each containing a full fluorescence decay curve. More parameters can be added to the photon distribution, such as the wavelength of the photons, the time from a stimulation of the sample, or the time with respect to an additional modulation of the laser. In this review, the application of the technique will be described for the measurement of molecular environment parameters within a sample, protein interaction experiments by Förster resonance energy transfer (FRET), autofluorescence measurements of cells and tissue, and in-vivo imaging of human skin and the fundus of the human eye.

Contents

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Introduction

Techniques based on fluorescence detection have found broad application in life sciences because they are extremely sensitive and able to deliver information about biochemical interactions on the molecular scale (see the accompanying paper of Suhling et al., 2015 for a more detailed explanation). Carefully applied, fluorescence techniques do not induce changes in the biology of live cells and tissue. The techniques experienced a significant boost by the development of labelling techniques which permit the fluorophores to be placed into defined biological targets. The real revolution came with the introduction of fluorescent proteins (Chalfie et al., 1994, Tsien, 1998). Proteins expressed by cells can now be genetically modified to express fluorophores at exactly defined target positions. Another improvement was the introduction of confocal and multiphoton laser scanning microscopes (Wilson and Sheppard, 1984; White et al., 1987; Minsky, 1988; Denk et al., 1990; Masters, 2006; Pawley, 2006). By confining the detection to a well-defined focal plane, these instruments significantly improved the resolution of the images recorded.

Fluorescence signals are essentially characterised by three parameters. The intensity of the fluorescence depends on the fluorescence quantum efficiency and the concentration of the fluorophore. Images of the fluorescence intensity thus show where in a sample the fluorophore is located, i.e. show the spatial structure of the specimen. The fluorescence spectrum is characteristic of the fluorophore. Images containing spectral information thus allow the fluorophores in the individual pixels of an image to be identified. The shape of the fluorescence spectrum is, within reasonable limits, independent of the fluorophore concentration. The fluorescence lifetime or, more exactly, the fluorescence decay function, depends on the type of the fluorophore but not on its concentration. It depends, however, also on the molecular environment of the fluorophore. It is influenced by the presence of fluorescence quenchers and their local concentration, by binding of the fluorophore to different biological targets and their folding state, or by the presence of other optical absorbers to which it may interact by Förster energy transfer. Fluorescence decay functions and, consequently, fluorescence lifetime images, can therefore be used to obtain information on the molecular environment of the fluorophore molecules (Bastiaens and Squire, 1999; Lakowicz, 2006; Berezin and Achilefu, 2010; Becker, 2012a).

There are a number of different techniques to measure fluorescence lifetimes, and to combine fluorescence lifetime measurements with imaging. The techniques can be classified into time-domain and frequency-domain techniques, photon counting and analog techniques, and point-scanning and wide-field imaging techniques. It also matters whether a technique acquires the signal waveform in a few time gates (Buurman et al., 1992) or in a large number of time channels (Becker et al., 2004b), and whether this happens simultaneously (Becker, 2005; Buurman et al., 1992) or sequentially (Dowling et al., 1997; Straub and Hell, 1998). Virtually all combinations are in use. This leads to a wide variety of instrumental principles. Different principles differ in their photon efficiency, i.e. in the number of photons required for a given lifetime accuracy (Ballew and Demas, 1989; Gerritsen et al., 2002; Köllner and Wolfrum, 1992; Philip and Carlsson, 2003), the acquisition time required to record these photons, the photon flux they can be used at, their time resolution, their ability to resolve the parameters of multi-exponential decay functions, multi-wavelength capability, optical sectioning capability, and compatibility with different imaging and microscopy techniques (see Becker and Bergmann, 2008 and Becker, 2012a for an overview).

Time-correlated single photon techniques

One-dimensional techniques

Time-correlated single photon counting (TCSPC) makes use of the special properties of signals detected by a high-gain detector: If a sample is excited at a repetition rate in the MHz range, the detection rate of the fluorescence photons is lower than the pulse repetition rate of the excitation source.