

MULTI-EXPONENTIAL FLUORESCENCE LIFETIME IMAGING

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Multi-dimensional time-correlated single photon counting (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 in several detection channels. Each photon is characterised by its time in the laser period, its detection channel number, and the coordinates in the scanning area. Combined with a confocal or two-photon laser scanning microscope, multi-dimensional TCSPC makes a fluorescence lifetime imaging (FLIM) technique with multi-wavelength capability, near-ideal counting efficiency, picosecond time resolution, and the capability to resolve multi-exponential decay profiles [1]. We will show that double-exponential FLIM in fact adds three new dimensions to multi-dimensional microscopy. The technique is particularly useful for autofluorescence imaging of tissue and fluorescence resonance energy transfer (FRET) experiments in cells.

Autofluorescence is characterised by a large number of fluorophores in different binding states [2]. Resolving the resulting multi-exponential decay profiles yields images of the lifetime components and their relative intensities. These images show a wealth of detail, the biological meaning of which should be subject of further investigation.

Quantitative FRET measurements are faced with the problem that both the distance of donor and acceptor and the fraction of interacting donor are unknown [3]. Moreover, the labelling of the proteins by the donor and acceptor molecules is not necessarily complete. We show that double-exponential TCSPC FLIM is able to disentangle the effects of the distance and the interacting donor fraction. It thus yields more reliable FRET distances than single-exponential FLIM or sensitised-acceptor measurements.

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