ANALYSIS AND QUANTIFICATION OF MULTI-DIMENSIONAL CONFOCAL MICROSCOPY DATA

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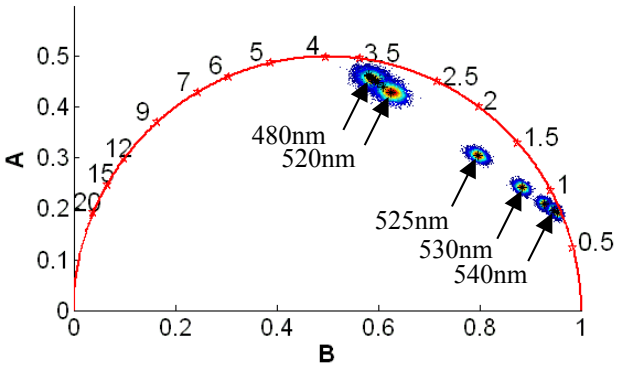
ABSTRACT: Multi-dimensional fluorescence microscopy seeks to measure many aspects of fluorescence emission, such as: excitation and emission spectra, lifetime and anisotropy, preferably in three spatial dimensions. In their combination these techniques enable optimal separation of several fluorescent species simultaneously. There are many examples where this is beneficial, such as label free tissue diagnostics or quantification of FRET. Hence multi-dimensional microscopy is becoming increasingly important in many biomedical applications [1-3].



In the literature there are few examples of excitation wavelength resolved confocal microscopy and in particular FLIM applications, because suitable light sources have not been available. Here, we report on the combined application of fluorescence excitation spectral scanning with lifetime imaging. Fluorescence decays were sampled for each excitation wavelength with Time Correlated Single Photon Counting (TCSPC), through use of a pulsed, fibre laser based supercontinuum source. Global analysis algorithms and AB-plot analyses were developed to recover the wealth of information stored in the resulting 4D (x, y, t, λ) datasets. An example AB plot displaying data from a dye mixture measured at several different excitation wavelengths is shown in Fig. 1. These strategies permit the quantitative unmixing of two spectrally similar fluorophores, providing information on molecular fractions.

References:

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