HIGH-SPEED FLIM FOR LIVE 3D-(LASER-SCANNING) MICROSCOPY

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KEY WORDS: TCSPC, FLIM, two-photon microscopy, confocal laser-scanning, in-vivo

In fluorescence laser-scanning microscopy the photon flux of common fluorophores can amount to 10^9/s at the detector. When pulsed or modulated radiation is used for excitation the temporal distribution of fluorescent photons with respect to the excitation gives unique information about the molecules local environment and binding condition. The analysis of the fluorescence decay is called Fluorescence Lifetime Microscopy (FLIM).
The present work demonstrates high-speed acquisition and analysis of FLIM data by means of a 16-fold multiplexed non-descanned time-correlated single photon counting (TCSPC) detector used in a two-photon microscope. The maximum recordable photon flux is only limited by the dead-time of the individual channels which is < 6ns. Typical lifetime frame rates reach those of standard PMT-based scanning microscopes. Fast new scanning techniques and analysis tools are presented. The detector allows for the first time to use fluorescence lifetime as a contrast parameter for live tissue and in-vivo imaging.