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REVIEW

Fluorescence lifetime imaging (FLIM): Basic concepts and some recent developments

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Abbreviations: ADC, analogue-to-digital converter; AFM, atomic force microscopy; AMD, age-related macular degeneration; APD, avalanche photodiode; CARS, coherent anti-Stokes Raman scattering; CCD, charge-coupled device; CFD, constant fraction discriminator; CFP, cyan/cerulean fluorescent protein; CFP-Grb2, CFP-labelled growth factor receptor-bound protein 2; CMOS, complementary metal oxide semiconductor; EGF, epidermal growth factor; FCS, fluorescence correlation spectroscopy; FAD, flavin adenine dinucleotide; FGF9, fibroblast growth factor 9; FLIM, fluorescence lifetime imaging; FRAP, fluorescence recovery after photobleaching; FRET, Förster resonance energy transfer; GaAsP, gallium arsenide phosphide; GBP, glucose/galactose binding protein; GFP, green fluorescent protein; IRF, instrumental response function; MCA, multichannel analyser; MCP, microchannel plate; NADH, nicotinamide adenine dinucleotide; RFP, red fluorescent protein; RFP-^{WT}Shp2, RFP-tagged wild-type SH2 domain-containing protein tyrosine phosphatase 2; SNOM, scanning near-field optical microscopy; SPAD, single photon avalanche diode; STED, stimulated emission depletion; STJ, superconducting tunnel junction; TAC, time-to-amplitude converter; TCSPC, time-correlated single photon counting; TDC, time-to-digital converter; TIRF, total internal reflection fluorescence; TR-FAIM, time-resolved fluorescence anisotropy imaging; YFP, yellow fluorescent protein.

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Summary Fluorescence lifetime imaging (FLIM) is a key fluorescence microscopy technique to map the environment and interaction of fluorescent probes. It can report on photophysical events that are difficult or impossible to observe by fluorescence intensity imaging, because FLIM is independent of the local fluorophore concentration and excitation intensity. One prominent FLIM application relevant for biological concerns is the identification of FRET to study protein interactions and conformational changes, but FLIM is also used to image viscosity, temperature, pH, refractive index and ion and oxygen concentrations, all at the cellular level, as well as cell and tissue autofluorescence. The basic principles and recent advances in the application of FLIM, FLIM instrumentation, data analysis, molecular probe and FLIM detector development will be discussed.

Contents

Introduction

Much of our knowledge of biological processes at the cellular and sub-cellular level comes from the microscope's ability to directly visualize them: optical imaging is compatible with living specimens, as light is non-ionizing, non-destructive and minimally invasive. Fluorescence microscopy in particular combines advantages of single-molecule sensitivity, molecular specificity, sub-cellular sub-micron resolution and real-time data collection from live cells with negligible cytotoxicity. This allows not only the study of the structure of the sample, but also the observation of dynamics and function, in real time (Amos and White, 2003; Wouters, 2006).

Among the various fluorescence microscopy methods, fluorescence lifetime imaging (FLIM) has emerged as a key technique to image the environment and interaction of specific probes in living cells (Becker, 2012, 2015; Berezin and Achilefu, 2010; Borst and Visser, 2010; Wouters, 2006). There are several technological implementations of FLIM, but they all can report on photophysical events that are difficult or impossible to observe by fluorescence intensity