

Multispectral FLIM and FRET applications

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The fluorescence decay of a fluorophore in many cases does not show a simple monoexponential profile. A very complex situation arises, when more than one compound must be analyzed. A considerable improvement of the measurement could be achieved when time-resolved and spectral-resolved techniques are simultaneously incorporated. SLIM (spectral fluorescence lifetime imaging) is a new technique, which combines both. Time-correlated single photon counting (TCSPC) enables high counting efficiency for biomedical applications. For spectral resolved detection a polychromator in the detection path together with a 16-channel multianode photomultiplier tube and appropriate TCSPC routing electronics is a highly sophisticated system.

The various possibilities which SLIM offers to improve molecular imaging in living cells will be discussed as well as successfully realized applications [1]. Special attention will be focused on FRET (resonant energy transfer) measurements with respect to Alzheimer`s disease [2]. Improvement of FRET calculations were achieved by a global analysis using the phasor plot approach developed by Enrico Gratton.

[1] A. Rück, C.H. Hülshoff, I. Kinzler, W. Becker, R. Steiner, „SLIM: A new method for molecular imaging,” *Micr. Res. Tech.*,70, 485-492 (2007).

[2] A. Rück, F. Dolp, R. Steiner, C. Steinmetz, B. v. Einem, C.A.F. v. Arnim, „SLIM for multispectral FRET imaging,” *SPIE Proceedings, Photonics West*, 6860, 68601F-1 (2008).