FLIM and SLIM for sophisticated FRET imaging

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SLIM (spectral fluorescence lifetime imaging) is a highly sophisticated new technique, which combines spectral resolved and time resolved detection [1]. Real spectral information is achieved by using a grating in front of a PML-array, which allows time-correlated single photon counting (TCSPC). Whereas spectrally resolved fluorescence imaging alone has a reasonable sensitivity, the specificity of fluorescence detection can be improved by considering the fluorescence lifetime.

SLIM was realized on the basis of a laser scanning microscope (LSM410, Zeiss, Germany). The fluorescence light from the second descanned detection channel was coupled into a 600 µm multimode fibre. The end of the fibre was put into the input focal plane of an MS125 spectrograph (grating of 600 lines/mm, LOT-Oriel). A PML-16 multichannel PMT module (Becker&Hickl GmbH, Berlin, Germany), containing a 16 channel Hamamatsu R5900-01-L16 multi-anode PMT and the TCSPC routing electronics was attached to the output of the spectrograph. The grating yields a 200 nm spectral range spread over the 16 channels of the detector. The spectral bandwidth of the PMT channels was about 12 nm. For fluorescence excitation, a Ti:Sa laser or alternatively a ps diode laser was used.

The various possibilities which SLIM offers to improve cell diagnosis will be discussed as well as successfully realized applications. These include cancer diagnosis, and FRET measurements for multiple protein interactions. Pitfalls due to photobleaching and scattering under various excitation conditions will be discussed as well.