FLUORESCENCE LIFETIME IMAGING MICROSCOPY WITH SPECTRAL RESOLUTION (SLIM)

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A multi-dimensional, time correlated single photon counting (TCSPC) technique was used to measure the fluorescent lifetimes of ECFP/EYFP-FRET systems. A bi-exponential decay was found for the ECFP fluorescent decay, as previously described [1]. No significant decrease in either the long or the short FRET lifetimes could be resolved unless filtering was used to separate the quenched donor emission from the sensitized emission. If donor and acceptor emission were selected by a bandpass 435-485 nm and 500-550 nm respectively, the emission specific decrease in ECFP fluorescence lifetime under FRET conditions was visible for the long lifetime component. In addition we observed a shortening in the lifetime of the short component. However, these data had to be examined more carefully, since the corresponding amplitude and lifetime parameter of this component were more difficult to determine numerically. These values were influenced by the shape and time-shift of the instrumental response function (IRF) of the measurement system. Moreover, both rapid intra- and intermolecular photophysical quenching reactions influence this lifetime. An improved determination of the fast transfer component is possible if the increase in the fluorescence at the acceptor site is taken into account. For this purpose an existing Zeiss LSM-510 coupled with an SPC-830/730 FLIM module can be extended with a second detector by deploying an external routing device described in [2].

Figure 1: Dual detector set-up for simultaneous acquisition of donor and acceptor fluorescence of FRET systems