PHOTON-STATISTICS AND FÖRSTER RESONANCE ENERGY TRANSFER

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ABSTRACT Förster Resonance Energy Transfer (FRET) permits one to map biochemical events, e.g., protein-protein interactions and post-translational modifications, directly within the living cell. For this reason, FRET has become a widely used technique in the life sciences and a large number of methods have been implemented for the quantitative estimation of the FRET efficiency (i.e., the fraction of energy transferred from a donor to an acceptor fluorophore).

FRET causes a reduction in the donor fluorescence quantum yield and fluorescence lifetime with a concomitant sensitization of acceptor emission with reduced fluorescence anisotropy. These quantities can be determined with a variety of methods, including fluorescence lifetime imaging (FLIM), fluorescence anisotropy imaging (FAIM), spectral imaging, or a combination of these techniques.

Here, we provide a quantitative comparison of accuracy and precision of these FRET imaging methods, taking into account the effects of photon-statistics [1-3] through Monte Carlo simulations and theoretical studies. The results are analysed for a range of signal to noise ratios and discussed with relevance to current experimental practice. A goal is to provide the end user with practical guidance for the selection of an optimal FRET protocol in a given experimental situation.

References