ABSTRACT

Determination of Fluorescence Resonance Energy Transfer in Multi-fluorophore Systems: Theoretical and Experimental Analysis

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This thesis examines the determination of fluorescence resonance energy transfer (FRET) in multi-fluorophore systems from both a theoretical and an experimental perspective.

Theoretical models of energy transfer in multi-fluorophore systems are reported. It was found that the properties exhibited by models combining energy transfer processes with a parallel set of non-interacting fluorophores were dependent on two factors: 1) the specifics of the model, and 2) modulation frequency.

The photobleaching of two multi-fluorophore systems known to undergo FRET is reported. It was found that the model selected to best describe the mechanism of FRET for each multi-fluorophore system varied for the individual

wavelengths considered. The models selected incorporated either one or two energy transfer steps.

The phase and modulation behaviour of a multi-fluorophore system known to undergo FRET was investigated during photobleaching. It was found that the behaviour of the acquired phase and modulation data was consistent with a system in which excited-state processes are operating with a parallel set of non-interacting fluorophores.

Keywords: Toni Sharon Forde; Fluorescence lifetime imaging microscopy (FLIM); Fluorescence resonance energy transfer (FRET); Model building; Multi-fluorophore FRET; Phase-modulation fluorescence spectroscopy; Photobleaching FRET (pbFRET)