

# FLUORESCENCE RESONANCE ENERGY TRANSFER IMAGING BY MAXIMUM LIKELIHOOD ESTIMATION

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## ABSTRACT

Fluorescence resonance energy transfer (FRET) is a fluorescence microscope imaging process involving nonradiative energy transfer between two fluorophores (the donor and the acceptor). FRET is used to detect the chemical interactions and, in some cases, measure the distance between molecules. Existing approaches do not always well compensate for bleed-through in excitation, cross-talk in emission detection and electronic noise in image acquisition. We have developed a system to automatically search for maximum-likelihood estimates of the FRET image, donor concentration and acceptor concentration. It also produces other system parameters, such as excitation/emission filter efficiency and FRET conversion factor. The mathematical model is based upon a Poisson process since the CCD camera is a photon-counting device. The main advantage of the approach is that it automatically compensates for bleed-through and cross-talk degradations. Tests are presented with synthetic images and with real data referred to as positive and negative controls, where FRET is known to occur and to not occur, respectively. The test results verify the claimed advantages by showing consistent accuracy in detecting FRET signal and calculating FRET efficiency.