

FRET Imaging by Maximum Likelihood Estimation: A Quantitative Evaluation

Brian Northan, Tim Holmes, Yupeng Zhang, Yumin Yuan
AutoQuant Imaging, Inc.
Watervliet, New York 12189

E-mail: northanB@aqi.com, holmes@aqi.com

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Fluorescence resonance energy transfer (FRET) is a fluorescence microscope technique involving energy transfer from a molecule labeled with a donor fluorophore to a nearby molecule labeled with an acceptor fluorophore. The technique can be used to determine if protein-protein interactions occur and to measure the distance between proteins. We have developed a Maximum Likelihood Estimation algorithm that automatically searches for the FRET Image, donor concentration, acceptor concentration, filter efficiencies and FRET conversion factor. The algorithm is based upon a Poisson process since the CCD camera is a photon counting device. Other methods make assumptions about key system parameters. The advantage of our system is that it determines statistically the most likely set of parameters based on observed data. We show a quantitative comparison of our method versus other accepted approaches using a collection of FRET images with known amounts of donor and acceptor and using a set of negative and positive controls. In both cases we show that our algorithm produces FRET efficiency results closer to the theoretical values.

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