

# IMPROVED UNDERSTANDING OF FLUORESCENCE MICROSCOPY EXPERIMENTS BY USE OF SIMULATIONS

Lloyd M. Davis, GuoQing Shen, and David A. Ball,  
Center for Laser Applications (CLA)  
University of Tennessee Space Institute  
Tullahoma, Tennessee 37388, USA  
E-mail: ldavis@utsi.edu

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## **ABSTRACT:**

Excitation saturation, triplet photophysics, photobleaching, detector dead time, multiple species with different binding configurations, and optical misalignment are examples of practical effects that often occur in microscopy experiments involving single-molecule detection (SMD), fluorescence correlation spectroscopy (FCS), and fluorescence cross-correlation spectroscopy (FCCS). In fluorescence resonance energy transfer (FRET) experiments, the problem of cross-talk, which is caused by direct acceptor excitation, and/or detection of the donor emission in the acceptor channel, may be compounded by other effects, such as triplet and saturation phenomena. Theoretical approaches to account for such effects involve approximations that limit their applicability. Recently, a computationally fast approach has been developed for *ab initio* Monte Carlo simulations of fluorescence microscopy experiments [1]. Instead of using a small fixed time step, time increases continuously for all processes except molecule transport. The simulation, which can provide updated graphics during execution, gives an autocorrelation function (ACF) that agrees well with theoretical predictions when triplet crossing, saturation, photobleaching, optical misalignment, background, and detector dead-times are turned off. It can model 2 independently aligned laser beams, 2 single-photon detectors, several specified chromophore types, with possibility of FRET and excitation or emission cross-talk, and several molecule types with different diffusional or flow mobilities, labeled by specified numbers of the defined chromophore types. The improved understanding of practical effects exhibited in SMD, FCS, and FCCS experiments by use of the simulation, the study of systematic and statistical errors, and the comparison of new and previously published data analysis strategies are discussed.

[1] L.M. Davis, P.E. Williams, D.A. Ball, K.M. Swift, and E.D. Matayoshi, "Data reduction methods for application of fluorescence correlation spectroscopy to pharmaceutical drug discovery," *Curr. Pharm. Biotech.* **4**: 451-462 (2003); an example of the simulation may be downloaded from [www.utsi.edu/ldavis](http://www.utsi.edu/ldavis).