

Abstract Submitted
for the SES09 Meeting of
The American Physical Society

Lifetime Resolved Fluorescence Fluctuation Spectroscopy PENG GUO, KEITH BERLAND, Emory University — Fluorescence correlation spectroscopy (FCS) has been widely used to investigate molecular dynamics and interactions in biological systems. FCS typically resolves the component species of a sample either through differences in diffusion coefficient or molecular brightness. Diffusion based assays currently have a major limitation which requires that the diffusion coefficients of component species in a sample must be substantially different in order to be resolved. This criterion is not met in many important cases, such as when molecules of similar molecular weight bind to each other. This limitation can be overcome, and resolution of FCS measurements enhanced, by combining FCS measurements with measurements of fluorescence lifetimes. By using global analysis on simultaneously acquired FCS and lifetime data we show that we can dramatically enhance resolution in FCS measurements, and accurately resolve the concentration and diffusion coefficients of multiple sample components even when their diffusion coefficients are identical provided there is a difference in the lifetime of the component species. We show examples of this technique using both simulations and experiments. It is expected that this method will be of significance for binding assays studying molecular interactions.

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Date submitted: 18 Aug 2009

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