Theodore L Hazlett and Enrico Gratton.

Photon counting and analog data acquisition in fluorescence correlation spectroscopy: issues of sensitivity and dynamic range.

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Abstract

Fluorescence Correlation Spectroscopy (FCS) has enjoyed an immense amount of attention in the past five years. FCS theory, data analysis tools, and instrument design have come to the state where non-specialist scientists are making use of prescribed protocols to garner information on the diffusion coefficients and fluorescence brightness factors for their biochemical and cellular systems. FCS is still a technique undergoing development and its application to complicated research areas, such as cell physiology and multisubunit organization brings new technical challenges. One point of difficulty in these areas is the presence of a heterogeneous population of structures that can result in a wide range in brightnesses. Because of the need for high sensitivity, photon counting detection electronics are predominantly used to collect FCS data. Unfortunately, photon counting technology has a limited dynamic range when compared to analog detection. For this work we have examined the sensitivity limits and the dynamic range of an H7422 Series PMT from Hamamatsu in photon counting mode or analog mode, as appropriate. Data for the two acquisition methods were collected on the same samples under identical conditions. The samples contain very bright particles to emphasize the limitation of the photon counting method. Details of the instrumentation and application are shown and the results are discussed in light of dynamic brightness ranges routinely found in cell and solution FCS studies. Supported by the NIH, PHS 5 P41-RRO3155, and by UIUC.