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Effects of laser excitation saturation in measurements of biomolecule binding by FCS. YOU LI, GUOQING SHEN, LLOYD M. DAVIS, The University of Tennessee Space Institute — Fluorescence Correlation Spectroscopy (FCS) is a technique developed by physicists in the 1970's that is now widely used for determination of the nanomolar concentrations of fluorescentlylabeled biomolecules within microliter-sized droplets of solutions. The conventional theory, which dates from the early papers, states that the amplitude of the autocorrelation function of fluctuations in the fluorescence signal is inversely proportional to the mean number of fluorescent molecules in the focused laser beam. However, we have recently shown that excitation saturation causes dependence of the autocorrelation function on laser power even in the limit of infinitesimal laser power, and thereby a bias in the determined concentration. Here we use computer simulations to investigate the effects of such bias on two-species concentration determinations for application to measurements of molecular binding in pharmaceutical drug discovery.

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