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Tracking-FCS: Correlation Spectroscopy of Individual Particles
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Recently developed methods for trapping and tracking fluorescent particles have already been exploited for monitoring free diffusion with high spatial accuracy. These achievements suggest the possibility of performing fluorescence correlation spectroscopy (FCS) on an individual molecule as it moves freely in solution. Such an approach may enable, for example, the investigation of heterogenous folding dynamics in protein molecules in their native environment. In this talk, we will discuss our own experimental and theoretical approach to this problem. We use a spatially modulated Gaussian excitation laser and lock-in detection in order to track fluorescent particles in two dimensions. We show that Ornstein-Uhlenbeck statistics are appropriate for describing the fluctuations of a tracked particle, and we apply a generalization of the familiar (free particle) FCS equations to the situation in which a tracked particle is observed for a long period of time. Based on the same statistical model, we calculate absolute limits on the accuracy with which a particle of a given brightness and diffusion coefficient can be tracked using similar methods. Our results establish a framework for interpreting fluorescence time series and determining the feasibility of near-term goals in single-particle tracking experiments.

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