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Polarized FCS: detection of rotational motions of macromolecules.

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Abstract

The use of fluctuation spectroscopy for the measurement of relatively slow rotations of large macromolecules in solution or attached to other macromolecular structures is discussed. Both simulations and experiment are presented to show the range of rotational correlation times and diffusion times under which this technique is applicable. In particular, we explore several analytical methods to analyze the polarization fluctuation data. We found that by first constructing the polarization function and then calculating the autocorrelation of this function, we can obtain the rotational motion of the molecule with little interference from the lateral diffusion of the macromolecule. However, this is only true if the rotational diffusion is faster than the lateral diffusion time. Surprisingly, for most commonly used fluorophores the autocorrelation of the polarization function is relatively unaffected by the photon statistics. In our instrument, two-photon excitation is used to define a small volume of illumination where a few molecules are present at any instant of time. The measurements of long DNA polymers labeled with the fluorescent probe DAPI show local rotational motions of the polymers in addition to translation motions of the entire polymer. For smaller molecules such as GFP, the viscosity of the solution must be increased to bring the relaxation due to rotational motion into the measurable range of our instrument. For some of the larger aggregates it is possible to follow the polarization trajectory. Under this condition, single particle analysis provides direct information on the rotational mobility of the macromolecule. Support: NIH, PHS P41 5 RR03155.