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Optical Conformational Transition Pathways of DsRed, Elucidated by Polarization-Modulated Fourier Imaging Correlation Spectroscopy ANDREW MARCUS, ERIC SENNING, GEOFFREY LOTT, University of Oregon, MICHAEL FINK, Omega Optical, Inc — This work presents a novel 'phase-selective' approach to fluorescence fluctuation spectroscopy that simultaneously determines the joint probability distributions and two-dimensional spectral densities of protein conformational transitions, and nanometer center-of-mass displacements. Fourier imaging correlation spectroscopy (FICS) combines polarization- and intensity-modulated photo-excitation with phase-sensitive signal detection to monitor the collective coordinate fluctuations from a large population of fluorescent molecules (N $\sim$ 106). FICS is based on the principle that fluctuations of partially averaged molecular coordinates can be monitored through variations of an optical signal phase. Experiments are performed on DsRed, a tetrameric complex of fluorescent protein subunits, derived from a reef-building coral. Thermally induced conformational transitions of the DsRed complex lead to fluctuations in the optical dipolar coupling between adjacent chromophore sites. An analysis of polarization-resolved FICS fluctuation data, in terms of two-dimensional spectra and joint probability distributions, provides detailed information about cooperative 'transition pathways' between distinct dipole-coupled DsRed conformations.

Andrew Marcus
University of Oregon

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