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Correlated Fluorescence Parameters of Single Molecules CLAUDIU GRADINARU, University of Toronto, DAVID CHANDLER, CARL HAYDEN, Sandia National Labs — A novel detection system is used in a confocal optical microscope for measuring correlated fluorescence lifetimes and spectra. Fluorescence photons emitted from a sample are imaged through a dispersive optical system onto a time- and position-sensitive detector. For each photon the apparatus records the wavelength, the emission time relative to the laser excitation pulse and the absolute detection time, so that correlations among all the fluorescence properties are maintained. A histogram over many photons can generate a full fluorescence spectrum and a correlated decay plot at every pixel in a fluorescence image. The complex data structure allows mapping the time-dependent distribution of multiple fluorescent species in a sample and enables monitoring the dynamics of single molecules on a time scale that spans from picoseconds to minutes. Unique correlations between intensity, spectrum and lifetime prove useful for tracking changes in the nanoenvironment of fluorescent probes. The detection method also provides a more complete description of the fluorescence resonance energy transfer (FRET) than conventional microscopy techniques, as demonstrated by single-pair FRET experiments between dyes spaced apart by short peptides and by dsDNA chains.

Claudiu Gradinaru
University of Toronto

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