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Irving Langmuir Prize Talk: Single-Molecule Fluorescence Imaging: Nanoscale Emitters with Photoinduced Switching Enable Superresolution.

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In the two decades since the first optical detection and spectroscopy of a single molecule in a solid (Phys. Rev. Lett. **62**, 2535 (1989)), much has been learned about the ability of single molecules to probe local nanoenvironments and individual behavior in biological and nonbiological materials in the absence of ensemble averaging that can obscure heterogeneity. The early years concentrated on high-resolution spectroscopy in solids, which provided observations of lifetime-limited spectra, optical saturation, spectral diffusion, optical switching, vibrational spectra, and magnetic resonance of a single molecular spin. In the mid-1990's, much of the field moved to room temperature, where a wide variety of biophysical effects were subsequently explored, but it is worth noting that several features from the low-temperature studies have analogs at high temperature. For example, in our first studies of yellow-emitting variants of green fluorescent protein (EYFP) in the water-filled pores of a gel (Nature **388**, 355 (1997)), optically induced switching of the emission was observed, a room-temperature analog of the earlier low-temperature behavior. Because each single fluorophore acts a light source roughly 1 nm in size, microscopic imaging of individual fluorophores leads naturally to superlocalization, or determination of the position of the molecule with precision beyond the optical diffraction limit, simply by digitization of the point-spread function from the single emitter. Recent work has allowed measurement of the shape of single filaments in a living cell simply by allowing a single molecule to move through the filament (PNAS **103**, 10929 (2006)). The additional use of photoinduced control of single-molecule emission allows imaging beyond the diffraction limit (superresolution) by several novel approaches proposed by different researchers. For example, using photoswitchable EYFP, a novel protein superstructure can now be directly imaged in a living bacterial cell at sub-40nm resolution (Nat. Meth. **5**, 947 (2008)). These important advances provide the impetus for the further development of both new imaging schemes with 3-D capability as well as invention of new photoswitchable single-molecule emitters for use in polymers and in biological systems (JACS **130**, 9204 (2008); J. Phys. Chem. B **112**, 11878 (2008)).