SPECTRAL IMAGING: FROM SINGLE MOLECULES TO CELLS

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We extended our home-built scanning confocal fluorescence microscope with a spectrographic facility, consisting of a prism and a CCD camera. The setup enables us not only to perform simultaneous multicolor excitation of different fluorophores but also to detect during scanning a full emission spectrum in every pixel of an image. The single molecule sensitivity allows us to measure emission spectra of individual fluorophores. From the measured emission spectra we can select any wavelength for representation in an image. In this way we can not only identify multiple fluorophores in one image, but we also can simultaneously localize them even within one diffraction-limited spot. In this contribution we will show spectral imaging results from quantum dots, single fluorescent molecules and from cells. The dynamic behavior of donor and acceptor labeled DNA molecules will be demonstrated from pixel to pixel variations in single pair FRET. Different mutants of the red fluorescent protein DsRed, exhibiting mixed green and red fluorescence, were studied with single molecule spectroscopy to investigate their complex photophysics. On the cellular level we analyzed spectral images for expression of GFP as well as for autofluorescence.

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