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High-Speed Hyperspectral Nanoscopy for Studying Dynamic Protein Interactions

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Many cellular signaling processes are initiated by dimerization or oligomerization of membrane proteins. However, since the spatial scale of these interactions is below the diffraction limit of the light microscope, the dynamics of these interactions have been difficult to study on living cells. We have developed a novel high-speed hyperspectral microscope (HSM) to perform single particle tracking of up to 8 spectrally distinct species of quantum dots (QDs) at 30 frames per second. The distinct emission spectra of the QDs allows localization with $\sim$ 10 nm precision even when the probes are clustered at spatial scales below the diffraction limit. The capabilities of the HSM are demonstrated by application of multi-color single particle tracking to observe membrane protein behavior, including: 1) resolving antigen induced aggregation of the high affinity IgE receptor, FcϵR1; 2) dynamic formation and dissociation of Epidermal Growth Factor Receptor dimers; 3) four color QD tracking while simultaneously visualizing GFP-actin; and 4) high-density tracking for fast viscosity mapping of the cell membrane.

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