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Super-resolution imaging of multiple fluorescent proteins with highly overlapping emission spectra in living cells MUDALIGE GUNEWARDENE, University of Maine, FEDOR SUBACH, Albert Einstein College of Medicine, TRAVIS GOULD, Yale University , GREGORY PENONCELLO, MANASA GUDHETI, University of Maine, VLADISLAV VERKHUSHA, Albert Einstein College of Medicine, SAMUEL HESS, University of Maine — Diffraction limits resolution in far field microscopy. Single molecule localization based superresolution imaging has surpassed such limitations and is rapidly gaining popularity, yet limited availability of cell-compatible photoactivatable fluorescent probes with distinct emission spectra have impeded simultaneous visualization of multiple molecular species in living cells. We introduce PAmKate, a monomeric far-red photoactivatable fluorescent protein (PAFP), which has facilitated simultaneous imaging of three PAFPs in biological samples with fluorescence photoactivation localization microscopy (FPALM). Successful probe identification was achieved by measuring the fluorescence emission intensity in two distinct spectral channels spanning approximately 100 nm of the visible spectrum. Raft-, non-raft- and cytoskeleton- associated proteins were simultaneously imaged in both live and fixed fibroblasts co-expressing Dendra2-hemagglutinin, PAmKate-transferrin receptor and PAmCherry1-β-actin chimeras, revealing evidence for specific interactions between membrane proteins and membrane-associated actin structures.

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