

# Multi-Spectral Fluorescent Protein-based Live Cell Microscopy of Intracellular Dynamics

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The intracellular dynamics of organelles and the cytoskeleton typically involve the spatiotemporal co-ordination of several protein complexes. To date, the visualization of such processes has been limited by the number of proteins that can be imaged simultaneously in live cells. We have developed a modular fluorescent protein labeling system to tag various organelle and cytoskeleton reporter proteins with six spectrally separated fluorescent proteins. By combining cotransfection of multiple fluorescent protein fusions with multispectral confocal time-lapse microscopy, we are able to visualize the behavior of up to six fusion proteins simultaneously in live cells. Using Leica's SP5 tunable emission filter system, we have optimized rapid image acquisition of 6 fluorescent channels with less than 9% bleed-through between channels (fig. 1).

Six-color live-cell imaging is currently being used to investigate the spatiotemporal regulation of actin related proteins and how their co-ordination mediates cell motility. We are also investigating the spatial dynamics of organelles during cell cycle progression (fig. 2). Multi-channel live cell assays will allow us to better understand how intracellular co-ordination of organelles and the cytoskeleton mediate cellular response and fate.

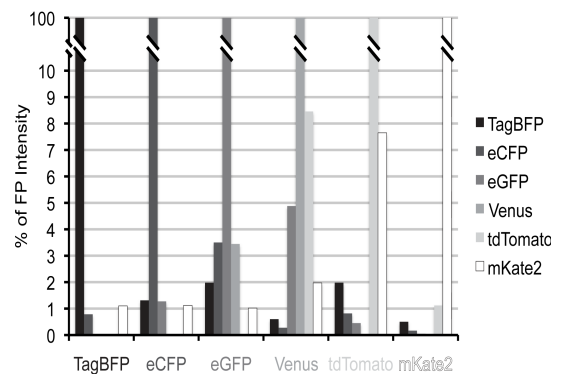


Figure 1. 293T cells were transfected with one of 6 fluorescent proteins and imaged in six spectral channel. Bleed-through was determined by measuring the fluorescent intensity of each channel for each fluorescent protein.

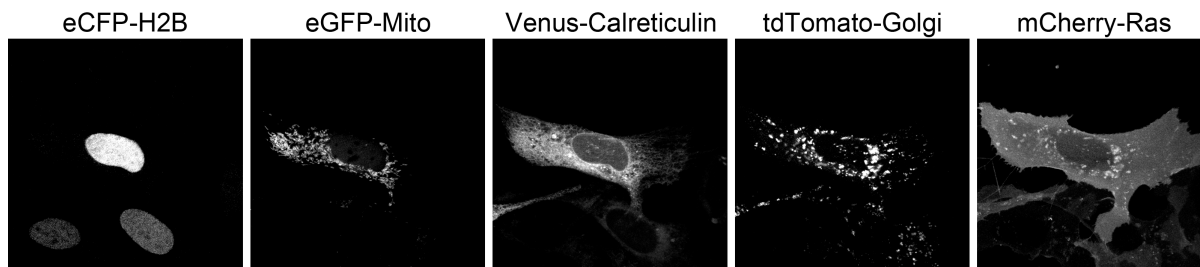


Figure 2. Co-expression of eCFP tagged H2B, eGFP-tagged mitochondrial targeting peptide, Venus-tagged Calreticulin (endoplasmic reticulum targeted), tdTomato-tagged golgi targeting peptide and mCherry-Tagged KRas (plasma membrane / golgi targeted) in U2OS cells.