Supplementary Information

Signal Discrimination Between Fluorescent Proteins in Live Cells by Long-wavelength Optical Modulation

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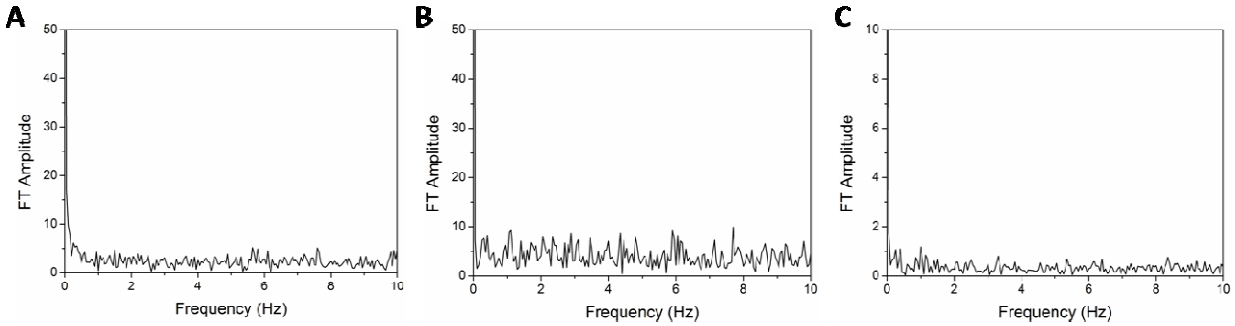

Figure S1. EGFP is not modulatable (1Hz secondary laser modulation frequency) using 476 nm primary and 561 nm secondary co-illumination. Fourier transform of dual laser-illuminated EGFP in solution using (A) primary intensity of 1.9 kW/cm^2 and secondary of 23.4 kW/cm^2; and (B) primary intensity of 4 kW/cm^2 and secondary of 43.7 kW/cm^2. C. Fourier transform of bulk EGFP fluorescence trajectory when immobilized in polyacrylamide using 1.9 kW/cm^2 primary and 23.4 kW/cm^2 secondary.

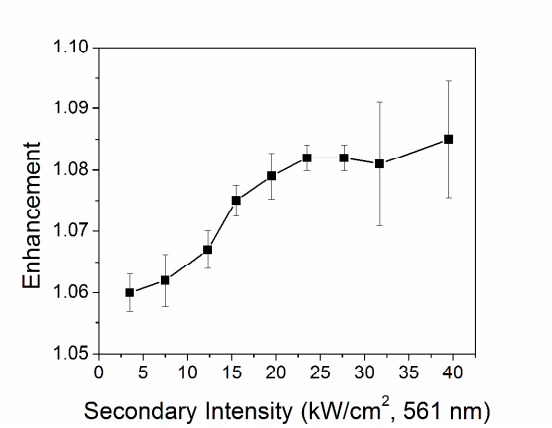

Figure S2. Fluorescence enhancement vs. secondary laser intensity. Primary intensity was held at 1.8 kW/cm^2. Enhancement is calculated by the fluorescence intensity of both lasers divided fluorescence with primary excitation alone.

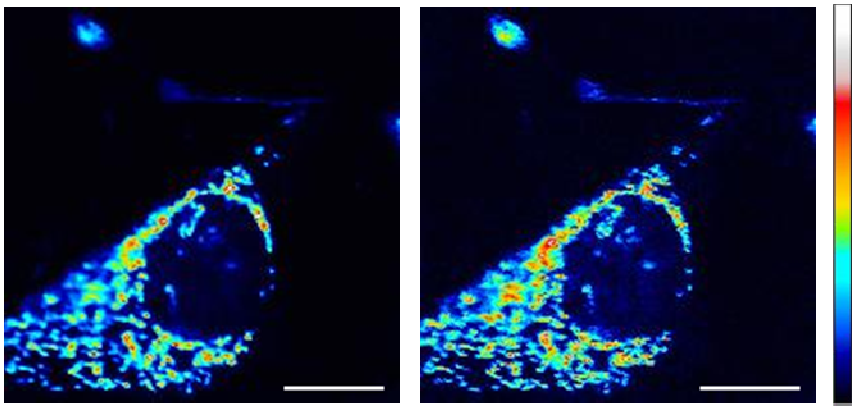

Figure S3. Fixed NIH-3T3 cells expressing mitochondria-targeted AcGFP. Primary intensity was held at 5.9 kW/cm^2 and the secondary intensity (64 kW/cm^2) was modulated at 300 Hz. (Left) Raw fluorescence image of AcGFP-labeled mitochondria. Min to max color bar is 0 to 10 × 10^5 photons. (Right) Demodulated image of AcGFP as lock-in amplitude. The color map on the right is linear from min to max in each image, with no threshold applied. The raw fluorescence image intensity is in photon counts, and the demodulated image intensity is in mV after lock-in amplification. Scale bar: 10 μm.

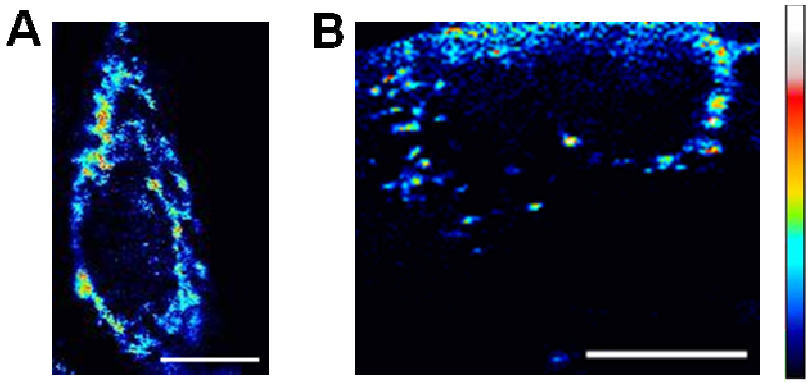

Figure S4. Demodulated images of fixed (A) and live (B) NIH 3T3 cells used to generate ratioed images in Figures 3A and B. The color map on the right is linear from min to max in each image, with no threshold applied. The demodulated image intensities are in mV after lock-in amplification. Scale bar: 10 μm.

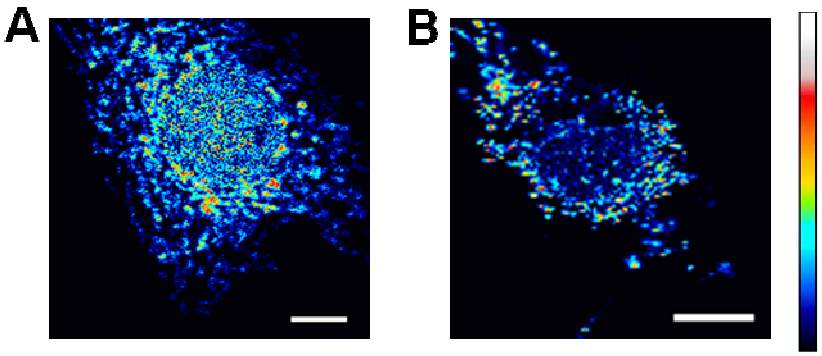

Figure S5. Demodulated images of fixed (A) and live (B) NIH 3T3 cells used to generate ratioed images in Figures 4A and B. The color map on the right is linear from min to max in each image, with no threshold applied. The demodulated image intensities are in mV after lock-in amplification. Scale bar: 10 μm.