PHASOR-BASED MULTI-HARMONIC UNMIXING FOR IN-VIVO HYPERSPECTRAL IMAGING

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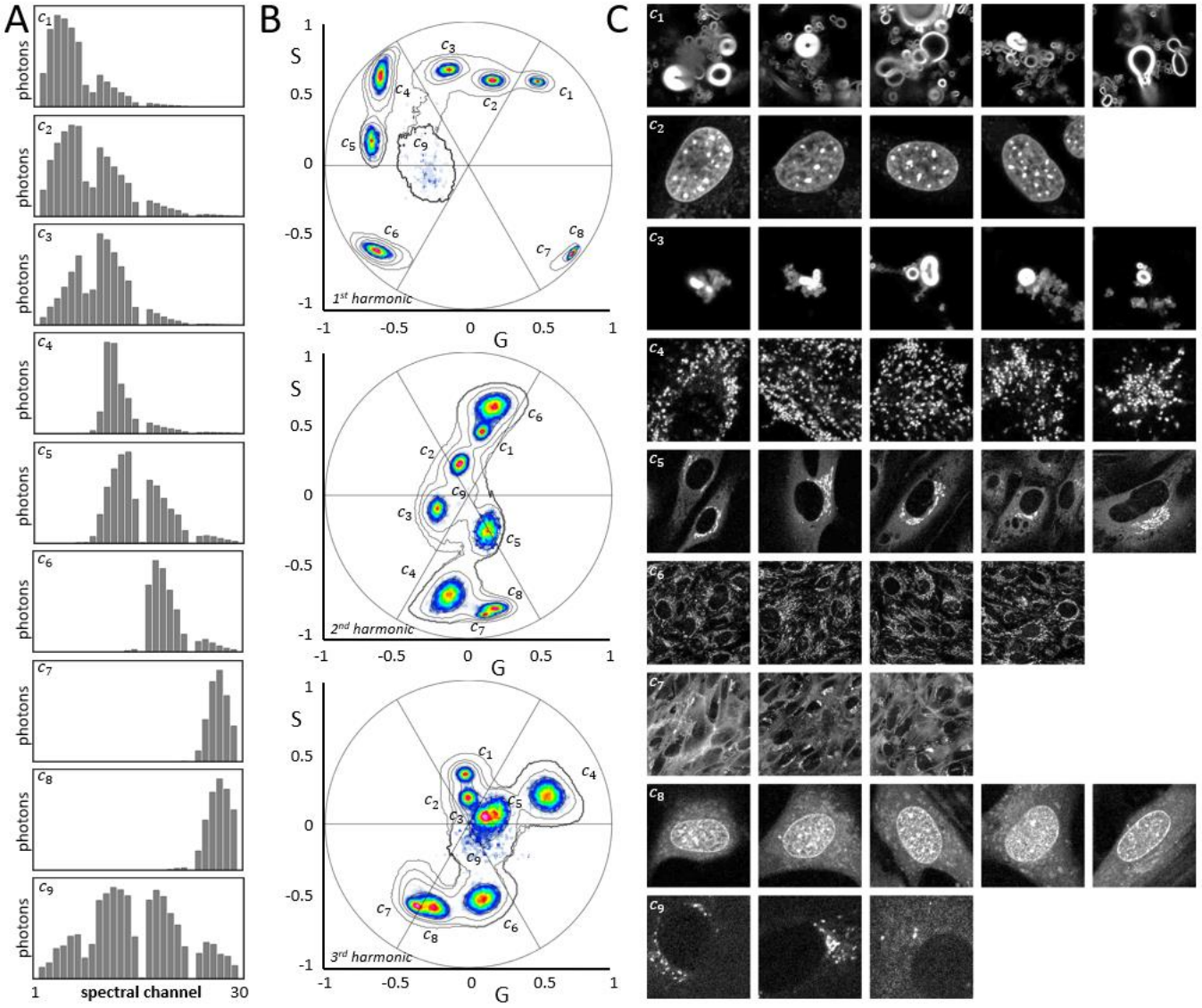
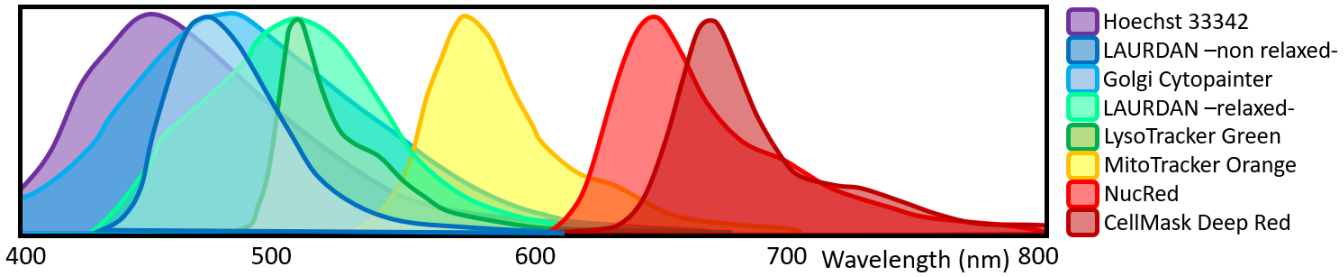


Supplementary Figure 1. Empirically measuring the phasor positions of the pure components. (C_1) Non-relaxed LAURDAN, (C_2) Hoechst, (C_3) Relaxed LAURDAN, (C_4) LysoTracker Green, (C_5) Cytopainter Golgi, (C_6) Mitotracker Orange, (C_7) CellMask, (C_8) NucRed, and (C_9) unlabeled cells (autofluorescence)). A) Average spectral emission for all pixels with more than 50 photon counts in each of the images (10^5 pixels per component, 10^7 photons per distribution). The gaps in the spectral distributions correspond to the emission dichroic band notches for the excitation laser lines. B) Spectral phasor distributions for the pixels of each set of images. C) Intensity image samples of each of the component sets.



Supplementary Figure 2. Theoretical emission spectra of the pure components. Spectra were obtained from the respective vendors, with the exception of the two LAURDAN emissions which were obtained from [1].

[1] Malacrida L, Gratton E and Jameson D M 2015 Model-free methods to study membrane environmental probes: A comparison of the spectral phasor and generalized polarization approaches *Methods Appl. Fluoresc.* **3**