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Profiling Cancer Cell Intrinsic Fluorescence with the Spectral Camera-Phasor Analysis Method

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Advances in hyper- or multi-spectral based imaging cameras have expanded new areas of cellular research for the last 20 years. Most recently, intensity signals can now be detected with a line spectrograph in which light is transmitted over an imaging signal to an ultra sensitive CCD camera with fast readout speed. However, spectrally resolving these images is highly problematic given that most fluorescent emission spectra are broad and superimposed one with another. In order to spectrally resolve these images with high spectral resolution, we have developed the phasor analysis, used in the first and second harmonic, where each pixel in the image is used to construct a spectral profile that is Fourier transformed to produce the co-ordinates of the pixel in a polar plot. This graphical representation is free of fitting routines and can easily separate linear combinations of multiple spectral components. For this application we used the Andor’s iXon Ultra EMCCD camera to obtain spectral emission of cancer cell autofluorescence excited with a multiphoton laser source. Using the multispectral phasor analysis we have been able to identify autofluorescence of cell membrane, mitochondria, nucleus and other organelles. Given the complexity of these biochemical species, we treated the cells with various agents used to perturb metabolic states, membrane fluidity and cellular function to identify changes in spectroscopic signals. A map of these chemical species can provide important information to identify proliferation, stress and dysplasia at the single cell level and can be applied to in the study of cancer and other diseases. Work supported in part by NIH grants P50 GM076516 and P41 GM103540.