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Real-Time Analysis of Endogenous Nuclear NADH in Differentiating Cells using the Spectral Phasor Approach

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NADH is an endogenous autofluorescent regulatory metabolite detected in the nuclear regions of live cells which when analysed for the bound and free form can aid in determining a cell's metabolic status and molecular activity. Detecting the spectral differences of free and bound NADH in live cells is currently limited due to the very small differences in emission. The Spectral Phasor technique enables not only examination of small shifts in spectral emissions but also provides the spatial location of spectrally different components in live cells without any prior knowledge of the species. The phasor representation enables direct comparison of either optical sections (i.e. different focal planes) of one cell or multiple cells for global analysis.

Here we describe the use of Spectral Phasors to spatially map NADH's spectral emission in the nucleus of live cells under normal culture conditions and those stimulated into the early stages of differentiation. A comparison of undifferentiated cells and those stimulated to differentiate demonstrate differing spatial distributions of emission spectra associated with NADH. Undifferentiated cells displayed shorter emissions cantered in the nucleus, while longer wavelengths were localised around the perinuclear boarder. Cells stimulated into the early stages of differentiation displayed a redirection of the shorter emissions to regional clustering predominately in one area close to the nuclear/cytoplasmic boarder, while the longer wavelengths were localised throughout the remainder of the nucleus. Here we show the application of the Spectral Phasor technique to identify discrete wavelength shifts associated with endogenous NADH autofluorescence in the nucleus, and observed changes in their spatial distribution in live cells during the early stages of differentiation. Work in part supported by NIH P41-GM103540 and NIH P50-GM076516.