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Optimization of a nanosecond-gated spectrofluorimetry system used for the monitoring of cellular metabolism[1] MARTIN HEIDELMAN, PAUL URAYAMA, Miami University — Our lab has developed a system for the real-time monitoring of nanosecond-gated, UV-excited cellular autofluorescence (endogenous fluorescence). Time-gated detection using an intensified CCD coupled to a spectrograph enables spectral measurements at controllable time delays with respect to an excitation pulse, useful because autofluorescence is known to have many excited-state lifetime components. Under UV excitation (nitrogen laser, 337 nm wavelength, sub-ns pulse width), autofluorescence is primarily due to NAD(P)H with its "free" and "protein-bound" forms having short (˜300 ps) and long (1–5 ns) lifetimes respectively. Time-gated detection captures the long-lifetime emission separately from the emission as a whole. Further, quantifying spectrum shape using phasor analysis allows for the assessment of two-state behavior during chemically-induced metabolic transitions, providing information at the biochemical-pathway level. Here we present an optimization of the gate timing, maximizing rejection of short-lifetime emission while maintaining contrast in spectrum-shape change during the monitoring of metabolic transitions in a model cellular system.

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