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Metabolic monitoring using UV-excited cellular autofluorescence in the presence of a quencher¹ CHONG KAI WONG, DYLAN PALO, PAUL URAYAMA, Miami University — Reduced nicotinamide adenine dinucleotide (NADH) is an important endogenous fluorophore for metabolic monitoring due to its increasing use as a biomarker. Thenoyltrifluoroacetone (TTFA) is an inhibitor of cellular respiration, known to quench NADH fluorescence. TTFA added to NADH in solution also changes the emission spectrum shape, possibly due to the differential quenching of the various NADH conformations. In vivo, the sequential additions of TTFA and cyanide to a cellular suspension of baker's yeast yields insight into the interpretation of pharmacologically-induced changes in the autofluorescence (i.e., endogenous fluorescence) spectrum shape. At 1 mM TTFA concentration, subsequent addition of cyanide produces no change in autofluorescence intensity, as seen at lower TTFA concentrations. Despite the lack of intensity change, cyanide addition does produce the expected change in autofluorescence spectrum shape. Because spectrum shape is increasingly shown to correlate with NADH conformation both in solution and *in vivo*, spectrum shape may be useful as a metabolic monitoring parameter even for situations where intensity is affected by non-metabolic artifacts.

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