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The Spatial Mapping of the Metabolic Cofactor NADH within Live Progenitor Stem Cells

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NADH is a naturally occurring bi-product and regulatory metabolite associated with cellular respiration. The quantification using the difference lifetime of autofluorescence of free and bound NADH has the potential to enhance the understanding of a range of cellular processes including apoptosis, cancer pathology and enzyme kinetics. Fluorescence lifetime imaging microscopy (FLIM) enables not only examination of the spatial location of the cofactor within live cells but also of its state.

Here we describe the use of phasor FLIM to spatially map the fluorescence lifetimes of NADH in both free and bound form within live undifferentiated myoblast cells. The phasor approach graphically depicts the change in lifetime at a pixel level without the requirement for fitting the decay. The phasor representation enables the possibility for a direct comparison of either optical sections (i.e. different focal planes) of one cell or multiple cells to enable a global analysis. A comparison of myoblast cells induced to differentiate through serum starvation and undifferentiated cells show differing spatial distribution of the different forms of NADH. Cells due to undergo differentiation displayed a short lifetime representing free NADH situated around the cytoplasmic periphery and a longer lifetime attributed to the presence of bound NADH just outside of the nucleus. Differentiated cells displayed redirection of the distribution of free NADH located mainly within the nucleus while the bound form remained directly comparable to that of the other cells. Furthermore, there appears to be a spatial shift in the distribution of lifetimes at a pixel level within the phasor plot. We show that the states of differentiation of myocytes may be determined through the phasor FLIM analysis of the autofluorescent properties of NADH.