

MULTISPECTRAL CONFOCAL FLUORESCENCE IMAGING OF FLAVIN AUTOFLUORESCENCE IN ISOLATED RAT CARDIOMYOCYTES

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1. INTRODUCTION. Visible-laser induced autofluorescence is naturally occurring endogenous fluorescence of living cells, having its origins mostly in flavins connected to the cell mitochondrial activity. However, complex spectral patterns and closely overlapping flavin spectra complicate its successful analysis. Here, we attempted to separate individual components of cardiomyocyte autofluorescence recorded directly in isolated cardiac cells by multi-spectral confocal fluorescence detection followed by linear unmixing.

2. SEPARATION OF CLOSELY OVERLAPPING EMISSION SPECTRA. In order to successfully separate flavin components with closely overlapping emission spectra, we first tested the linear unmixing approach using two-component computer-simulated data. We have observed and quantitatively estimated the decrease in the spatial resolution of the unmixed image, possibly linked to the decreasing Figure of Merit (relating the S/N ratio observed in the image to a case of noise-free photon-counting experiment) in the case of small signal intensities and/or large spectral overlap.

3. UNMIXING OF FLAVIN AUTOFLUORESCENCE. Components of flavin autofluorescence have been separated by linear unmixing using the reference spectra extracted by specific physiological mitochondrial modulation achieved by externally applied metabolic modulators, namely 2,4-dinitrophenol (uncoupler of mitochondrial oxidative chain) and cyanide (inhibitor of the respiratory chain IV). Difference spectra before and after application of the modulators constituted the reference component database (Fig.1A). The composite image of cardiac cells was successfully unmixed using this database (Fig. 1B). This technique greatly enhances quantitative analysis of autofluorescence, allowing its better understanding.

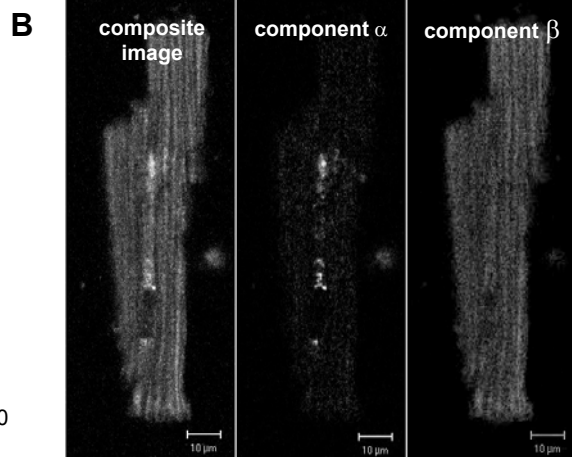
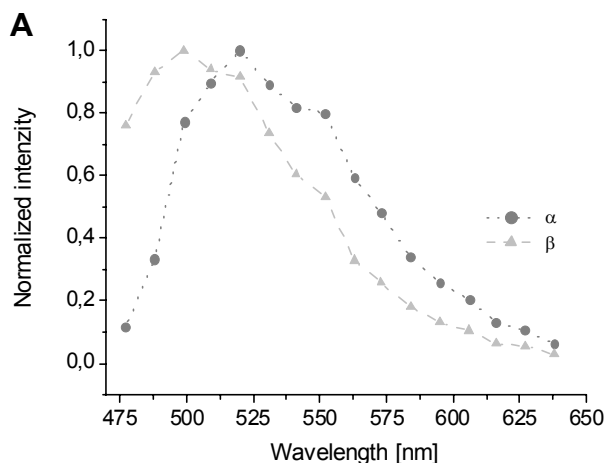


Figure 1: A) Difference autofluorescence spectra after modulation of isolated rat cardiomyocyte by 2,4-dinitrophenol (α) and cyanide (β). B) Linear unmixing of representative cardiac cell image according to the components of spectral database showed at A).