Detecting stem cell differentiation using fluorescence lifetime microscopy (FLIM) by the phasor approach

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In fluorescence lifetime microscopy (FLIM) of live tissues a major issue is the assignment of auto-fluorescence to specific molecular components and their interactions within the physiological context. Analyzing the intensity decays with a multi-exponential fit is often not sufficient to properly describe this complexity. Here we use the phasor approach to FLIM to analyze complex decays in a live tissue. Each chemical species was identified and categorized by its specific location in the phasor plot. This phasor fingerprint reduces the importance of knowing the exact lifetime distribution of fluorophores and allows interpreting the FLIM images directly in molecular terms. The phasor signatures of different species have been used to separate many tissue components inside the testes of an Oct4-GFP transgenic mouse and to map the relative concentration of auto-fluorescence, GFP, collagen, retinol, retinoic acid, FAD and NADH. Furthermore the analysis of the fluorescence decay with higher harmonics of the phasor plot can separate different tissue components that have the same location in the phasor plot at one harmonic, but arise from different lifetime distributions. The phasor approach to lifetime imaging in live tissue provides a unique fit-free and straightforward method for interpreting complex decays in terms of molecular features the relative concentration of fluorophores. This method has the potential to become a non-invasive tool to characterize the local microenvironment and monitor differentiation and diseases in label-free live tissues.