MULTICOLOR TWO-PHOTON MICROSCOPY OF ENDOGENOUS FLUORESCENCE BY WAVELENGTH MIXING FOR MULTIPARAMETRIC METABOLIC IMAGING

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Multiphoton microscopy of endogenous fluorophores is emerging as an effective approach to study dynamic changes in cells and tissues metabolism in vitro and in vivo. Quantifying the metabolic coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) through optical redox ratio (FAD/(NADH+FAD)) and Fluorescence Lifetime Microscopy (FLIM) provides sensitive information on the relative balance between oxidative phosphorylation and glycolysis.
However, multiphoton microscopy of endogenous fluorophores in live cells and tissues is limited by the challenge of efficient simultaneous multicolor imaging. Being able to efficiently image NADH and FAD simultaneously would make it possible to track physiological and pathological processes in dynamic systems. Endogenous fluorophores have non-overlapping two-photon absorption maxima in the range between 700 nm and 1000 nm and are present in a wide variety of concentrations in living tissues. Metabolic imaging of NADH and FAD is usually performed by sequential excitation at different wavelengths using a tunable laser, leading to difficulties in ensuring pixel-level registration between the channels in the case of a dynamic sample.
A distinct advantage of wavelength mixing for ratiometric measurements in a point-scanning multiphoton microscope is that the fluorescence images are intrinsically co-registered at the scale of the diffraction-limited excitation volume. [1] Here we present the application of wavelength mixing to the excitation of spectrally distinct endogenous fluorophores and optical redox ratio imaging in tissues and live zebrafish embryos, overcoming the difficulties associated with sequential excitation at different wavelengths such as motion artifacts. [2] Through simultaneous excitation of NADH and FAD in living tissues, reliable 2-photon ratiometric redox imaging and simultaneous FLIM of NADH and FAD is achieved with similar signal levels in both channels. We used this property to measure NADH and FAD lifetime gradients associated to cellular differentiation in living tissues such as reconstructed human skin and *c. elegans* germline. [2]

References:

[1] Mahou, P., et al. (2012). "Multicolor two-photon tissue imaging by wavelength mixing." *Nature Methods* 9 (8): 815–818

[2] C. Stringari, L. Abdeladim, G. Malkinson, P. Mahou, X. Solinas, I. Lamarre, S. Brizion, J.-B. Galey, W. Supatto, R Legouis, A.M. Pena, E. Beaurepaire, "Multicolor two-photon imaging of endogenous fluorophores in living tissues by wavelength mixing," *Sci. Rep.* 7, 3792 (2017)