Multiplexing autofluorescence and biosensors readouts for cellular metabolism studies

Christiane Weber, Matthias Richter, Anje Sporbert and Anca Margineanu

Advanced Light Microscopy, Max Delbrueck Centrum Berlin, Robert Roessle Str. 10, 13092, Berlin, Germany, E-mail: Anca.Margineanu@mdc-berlin.de

Keywords: autofluorescence, Peredox, multiplexed fluorescence, two photon microscopy.

Measurements of cellular and tissue autofluorescence have become an important tool to investigate cellular metabolism when applied in two photon microscopy. They have been used to discriminate between normal and cancerous cells/tissues or to follow the progress of stem cell differentiation. Two molecules are typically evaluated in these studies: NAD(P)H and flavins (FMN, FAD). The mitochondrial redox ratio NADH/FAD can be obtained by measuring the fluorescence intensity in two spectral channels, while the ratio between the free and bound NAD(P)H can be calculated based on the contributions of a short and long lifetime component respectively when using FLIM (fluorescence lifetime imaging).

Measuring these two molecules gives rather global information on cell metabolism, as they act as cofactors in multiple enzymatic reactions and discrimination of NADH and NADPH fluorescence is not straightforward. Moreover, they cannot give information on related metabolic reaction that do not generate autofluorescent molecules, nor on interrelations between metabolic and signalling pathways within cells.

To increase the complexity of the metabolic readouts in live cells, one could multiplex the autofluorescent signals with the fluorescence of endogenously expressed biosensors. However, detecting autofluorescence in the presence of overexpressed cytoplasmic fluorescent proteins is not easy, and the photophysical properties of these labels must be careful chosen. An alternative is to ensure a different spatial distribution of the biosensor (e.g. nuclear or membrane localisation). We have chosen the last approach, and we have multiplexed the autofluorescence readout with the readout of the biosensor Peredox. Peredox is sensitive to the NAD(P)H/NAD(P)+ cytosolic redox ratio and has been engineered with a nuclear localisation signal.

In this way, we are able to obtain simultaneous information on the changes of the mitochondrial redox ration NADH/FAD (an indicator of the oxidative phosphorylation) and of the cytosolic redox ratio NAD(P)H/NAD(P)+ (an indicator of glycolysis and of the pentose phosphate pathway). We have performed our measurements using two photon excitation. Optimisation of the excitation wavelengths for NAD(P)H, Sapphire and mCherry (the two fluorescent proteins of Peredox) and FAD have been realised in a first step. In a second step, we have tested the cellular responses after the addition of lactate/pyruvate. Other metabolic substrates and/or inhibitors will be further evaluated. Addition of fluorescence lifetime measurements will give us supplementary information on the free/bound NAD(P)H ratios, which have been recently related to the glycolysis/oxidative phosphorylation levels.

References