Imaging metabolic dynamics by label-free wide-field FLIM of NADH and FAD autofluorescence

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Abstract

Fluorescence lifetime imaging microscopy (FLIM) enables label-free determination of energy-related redox and protein-bound states by analyzing the autofluorescence of metabolically relevant molecules such as NAD(P)H and FAD.

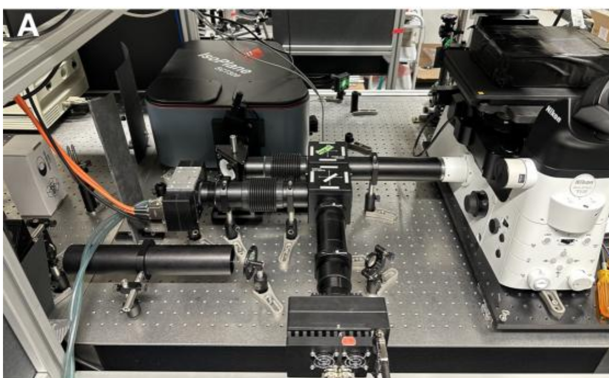
Therefore, the fluorescence lifetime of these intrinsic metabolites can be used to monitor activity- or pharmacologically-induced changes in cell cultures and tissue. However, observing small changes in active cells is challenging due to the low quantum yield of these metabolic molecules. In addition, credible FLIM measurements require robust statistics based on a sufficient number of collected photons. For this reason, a reliable FLIM system must meet the following criteria: (1) high sensitivity, (2) high signal-to-noise ratio, (3) temporal resolution in picoseconds, and (4) a detection range equivalent to the entire field of view.

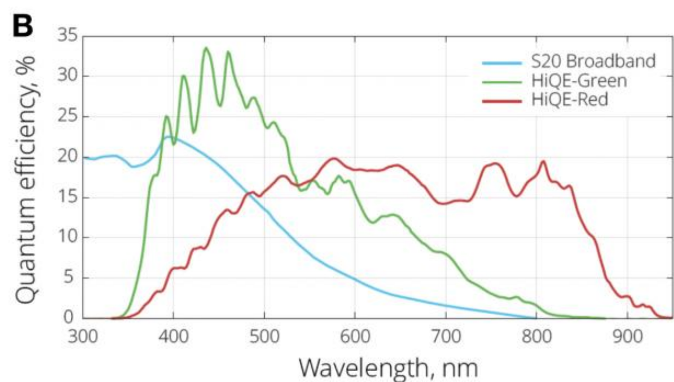
Here we present a highly sensitive wide-field FLIM method for NAD(P)H/FAD molecule detection using an innovative, commercially available camera system (LINCam, Photonscore GmbH, Germany) based on time-correlated single-photon counting (TCSPC). The imaging system enables tracking of small metabolic changes associated with brain cell activity over long periods under physiological conditions without invasive staining procedures and with minimal cell damage.

The detector features new photocathodes with high quantum yield, even in the near infra-red spectral range, a uniquely high signal-to-noise ratio, high temporal resolution (< 50 ps), and an ultra-high sensitivity, which allows it to work under extremely low light conditions (< 30 mW/cm2 on average). We show that the LINCam-based FLIM system can measure retinal cell metabolism under normal and pathophysiological conditions as well as changes in the metabolic activity of electrically stimulated cultured neurons.

Our experiments revealed a close correlation between neuronal activity and the dynamic changes of the observed metabolites. We show that the high sensitivity of the LINCam enables significant spatial scalability and high temporal resolution to resolve fluctuations in the molecular states of NAD(P)H/FAD during single-neuron imaging. After fixation, the recorded live-cell data can be merged with immunostainings to characterize the subcellular source of the measured metabolic activity.

In summary, metabolic FLIM can contribute to the development of minimally invasive diagnostic tools to identify pathological conditions in cells and tissue.





A: FLIM setup with LINCams for simultaneous acquisition of NADH and FAD autofluorescence; B: Quantum efficiency of different photocathodes