

## MOLECULAR IMAGING IN PDT USING FLIM AND SLIM

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Various problems arising during molecular imaging of different fluoroprobes and metabolites used in photodynamic therapy could be circumvented by focusing on time-resolved detection. For this, an interesting new method seems to be time-correlated single photon counting, where a time-to-amplitude converter determines the temporal position and a scanning interface connected to the scanning unit of a laser microscope determines the spatial location of a signal. In combination with spectral resolved detection (spectral lifetime imaging, SLIM) the set-up achieves the features of highly sophisticated lifetime imaging systems.

The photoactive substance on which 5-ALA PDT is based, is protoporphyrine IX which is synthesized in mitochondria. Alternatively, other metabolites from 5-ALA could be involved. Subcellular differentiation of those metabolites without extensive extraction procedures is not trivial, because of highly overlapping spectral properties. Measuring the fluorescence lifetime on a subcellular level could be a successful alternative.

To record lifetime images ( $\tau$ -mapping) a setup consisting on a laser scanning microscope equipped with detection units for time-correlated single photon counting and ps diode lasers for short-pulsed excitation was implemented [1]. The time-resolved fluorescence characteristics of 5-ALA metabolites were investigated in solution and in cell culture. The lifetimes were best fitted by a biexponential fitting routine. Different lifetimes could be found in different cell compartments. During illumination, the lifetimes decreased significantly. Different metabolites of 5-ALA could be correlated with different fluorescence lifetimes. In addition cells were coincubated with the nuclear staining dye DAPI, in order to investigate the cell cycle. In contrast to ALA, the lifetime of DAPI, which was best fitted monoexponentially did not change during photobleaching, making this dye a perfect internal standard.

[1] M. Kress, Th. Meier, R. Steiner, F. Dolp, R. Erdmann, U. Ortmann, A. Rück, „Time-resolved microspectrofluorometry and fluorescence lifetime imaging of photosensitizers using ps pulsed diode lasers in laser scanning microscopes,” *Journal of Biomedical Optics* **8** (1), 26-32 (2003).

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