Wide-field FLIM for minimal invasive long-term imaging of biological micro- and nanostructures

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Picosecond fluorescence lifetime imaging microscopy (FLIM) provides a most valuable tool in biology and biophysics because it allows time-resolved investigations of the interaction of fluorophores with their local environments in living cells and biological micro- and nanostructures. In the currently most widely used FLIM method the exciting laser beam is focused to a diffraction-limited spot and a fluorescence lifetime image is obtained by the scanning technique. This leads to very high peak light intensities at the sample causing in many cases detrimental photodynamic effects.

In order to avoid this disadvantage we use a non-scanning wide-field FLIM method (Europhoton GmbH) based on time- and space-correlated single photon counting. In this equipment the laser is not focused to a diffraction-limited spot, but illuminates the complete image area. The fluorescence image is projected on the photocathode of a novel position sensitive microchannel plate photomultiplier with quadrant anode (QA-MCP). This allows parallel acquisition of time resolved images under minimal invasive low-excitation conditions and long-term fluorescence lifetime imaging of biological micro- and nanostructures.

The potential of the technique is demonstrated by presenting results obtained from measurements of the fluorescence dynamics in individual chloroplasts of moss leafs and living cells of the chlorophyll *d* containing cyanobacterium *Acaryochloris marina.*

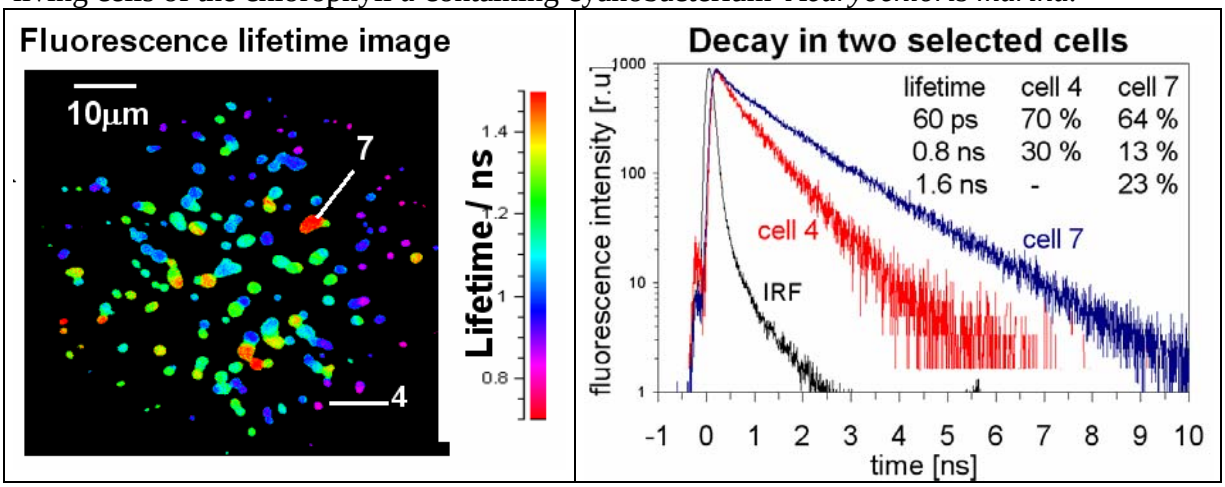

Fig. 1: Fluorescence dynamics in individual cells of the cyanobacterium *Acaryochloris marina*[1].

[1] H.-J. Eckert , Z. Petrášek, and K. Kemnitz (2006): Application of novel low-intensity non-scanning fluorescence lifetime imaging microscopy for monitoring excited states dynamics in individual chloroplasts and living cells of photosynthetic organisms. Proc. SPIE Vol. 6372, 637207/1-9