

4D Fluorescence Lifetime Imaging using Selective Plane Illumination Microscopy

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Fluorescence Lifetime Imaging (FLIM) delivers information about the local surrounding of the fluorochrome, for example the proximity of Fluorescence Resonance Energy Transfer (FRET) donors and acceptors, giving information of e.g. bound or unbound states of proteins in cells.

As the 3D distribution of the lifetime is of great interest (for 3D cell cultures, tissue engineering) we have combined a FLIM setup with a Selective Plane Illumination Microscope (SPIM) [1], providing optical sectioning capability and good spatio-temporal resolution. We use a FLIM setup in the frequency-domain and include reduction of photobleaching-induced artefacts by permuting imaging order [2]. This method does not increase calculation time and does not require additional recordings.

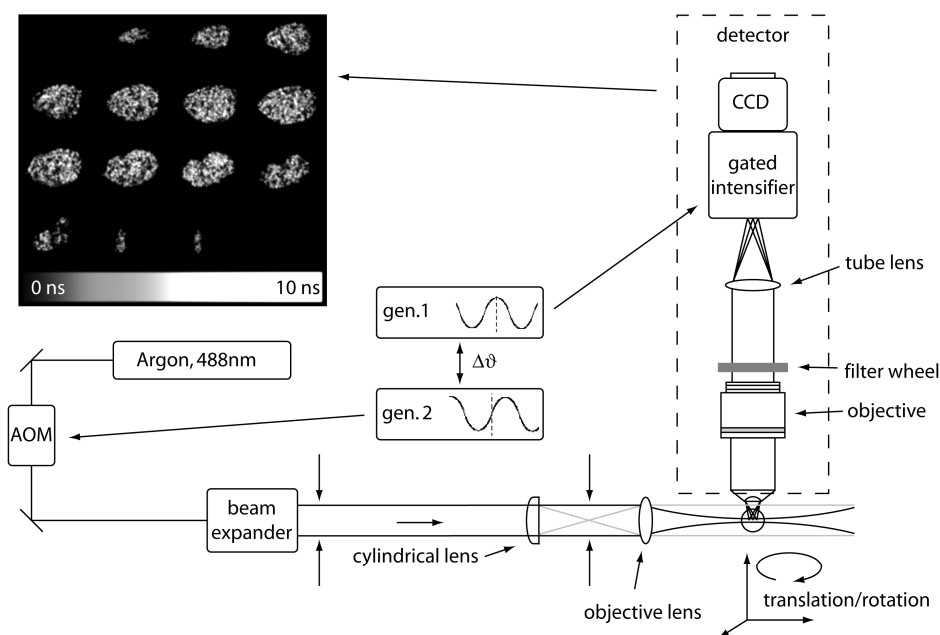


Figure 1: Overview of the FLIM-SPIM setup. The illumination (AOM) and the detection (gated intensifier) are modulated (gen. 1 and gen. 2) with the same frequency but changing phase difference ($\Delta\theta$), resulting in changing modulation depth and phase shift, dependent on the lifetime. The result is a 3D stack with the lifetime distribution in the cell, which is displayed here in grey-scale.

[1] J. Huisken, J. Swoger, F. Del Bene, J. Wittbrodt, E.H.K. Stelzer, "Optical sectioning deep inside live embryos by Selective Plane Illumination Microscopy", *Science*, **305**, 1007-1009 (2004)

[2] E.B. van Munster, T.W.J. Gadella, Jr., "Suppression of Photobleaching-Induced Artifacts in Frequency-Domain FLIM by Permutation of the Recording Order", *Cytometry Part A*, **58A**, 185-194 (2004)