FLI-Cam – a frequency-domain fluorescence lifetime imaging system based on a new directly modulatable CMOS image sensor

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For many years, the benefit of the luminescence lifetime as an analytical parameter has been described and many instruments including the 2D measuring setups with cameras have been developed and applied. However, since the instrumentation to perform either time- or frequency-domain lifetime measurements is rather complex, new developments in the technology of CMOS image sensors generated image sensors, which can be efficiently used for this purpose. While originally designed for distance measurements, the principle for these measurements shows a clear analogy to frequency-domain FLIM measurements, which also have been proven by researchers[1,2]. Based on this principle, a new CMOS image sensor has been developed and is investigated in line with a research project.

The image sensor has a resolution of 1024 x 1024 pixels with a 5.6 µm pitch and can be modulated up to 50 MHz. The first measurements show an effective dynamic range of 1:1000 (10 bit). The frame rate will be in the range of 40 frames/s. The camera system, which incorporates the sensor, also generates all required modulation signals from 1 kHz to 50 MHz (sinusoidal and rectangular). It includes enough memory to store reference images and do image processing, such that the user can read out either raw data, to test and improve custom algorithms, or images, which represent lifetime distributions of the sample (at a later stage). The modulation frequency can be freely adjusted and frequency sweep operation will be possible with up to 16 selectable frequencies.

First performance results are shown and discussed with the help of the phasor approach[3], that has been established to provide a more global view to pixel-wise fluorescence lifetime data and compare time- and frequency-domain results. Based on these results and the experiences of the on-going tests, it can be expected, that the FLI-Cam will ease the introduction of luminescence lifetime imaging systems to broader applications.

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References: [1] H. Heß, et al., *Proceedings Opto 2002*, **P7** (2002). [2] A. Esposito, et al., *Optics Express*, **13** (2005) 9812. [3] Michelle A. Digman, et al., *Biophysical Journal: Biophysical Letters* (2007) L14.

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