Time-Correlated Fluorescence Microscopy Using a Room Temperature Solid-State Single Photon Sensor

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In this paper we present a two-photon fluorescence lifetime imaging microscopy (FLIM) system based on a two-dimensional single photon avalanche diode (SPAD) array fabricated in CMOS [1] [2]. To the best of our knowledge, this is the first demonstration of two-photon FLIM based on a CMOS detector. The sensor reported here exhibits picosecond accuracy, operates at room temperature, and requires no post-processing. Our two-photon FLIM setup consists of a high-power femtosecond mode-locked laser, time discrimination hardware, and a standard laser-scanning microscope. A SPAD consists of a p-n junction biased above breakdown, thus operating in Geiger mode. When a photon impinges on the surface of the SPAD triggers an avalanche in the multiplication region. A properly biased inverter senses the avalanche current transforming it into a digital pulse. After photon detection, the device remains inactive for a period known as dead time. The sensitivity of a SPAD is determined by the fillfactor of the SPAD array and the photon detection probability (PDP). The main performance figures of the SPAD detector, such as timing uncertainty, PDP, dead time and dark count rate (DCR) are summarized in Tab. 1.

In our experiments, a femtosecond Ti:Sapphire laser is used to illuminate the sample. The sample consists of a Oregon Green Bapta-1 (OGB-1) fluorophore, placed into contact with different calcium concentrations. The detection method used to determine fluorescence lifetime is the time-correlated single photon counting technique. A time-to-digital-converter is used to compute the arrival time of fluorescence photons for each excitation pulse. The resulting data are used to build a histogram. The measured response of the fluorophore in the presence of various calcium concentrations is shown in Fig.1. The measured lifetimes of OGB-1 match published data [1]. Future work will be on high-speed sensing of calcium concentrations in cells (e.g. neurons), using multi-beam excitation patterns and parallel data acquisition with 2D arrays of SPADs.

Parameter	**Typ.**	**Max.**	**Unit**
Array size	32x32		
Pixel size	58x58		μm^2
Fillfactor	1		%
PDP @ 500 nm	25		%
Active area diameter	7		µm
Timing uncertainty	67	80	ps
DCR	350		Hz
Dead time	25	40	ns
Afterpulsing probability	(<0.1)		%
Laser pulse width	100		fs
Excitation wavelength	800		nm

Tab. 1. Performance summary of the FLIM setup at room temperature.

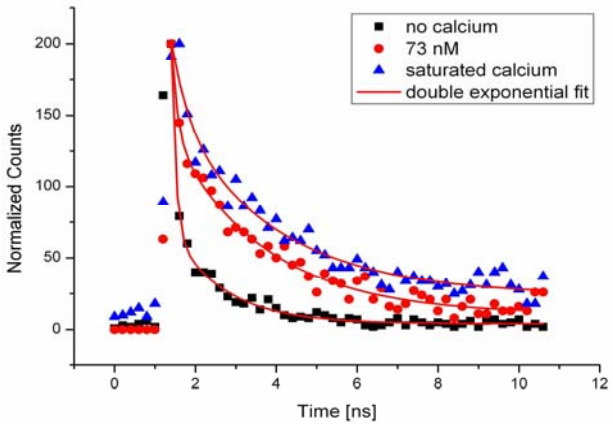

Fig. 1. Measured and numerically fitted fluorescence response of OGB-1 in the presence of various Calcium concentrations.

[1] A. V. Agronskaia *et al.*, *Journal of Biomedical Optics*, **9**, 1230-1237 (2004).
[2] C. Niclass *et al.*, *IEEE Journal of Solid-State Circuits*, **40**, 1847-1854 (2005)