ANALYSIS OF TIME FILTERING OF EXCITATION LIGHT IN FLUORESCENCE MEASUREMENT WITH SIMULATION MODELLING

Marina Repich [a,b], David Stoppa [b], Gian-Franco Dalla Betta [a]

[a] Department of Information Engineering and Computer Science, University of Trento, Italy;
[b] Fondazione Bruno Kessler - FBK-IRST, Trento, Italy

This paper presents a simulation model of a typical fluorescence measurement setup. The model includes the simulation of light source, basic fluorescent sample, single-photon avalanche diode detector, read-out electronics and setup geometry. In this paper we employ our model to investigate the possibility of using time filtering instead of conventional optical filtering in fluorescence detection. In order to achieve this, we have performed a number of simulations of time-resolved fluorescence measurements with time filtering of the excitation light. The light source was simulated as a micro-LED array, the fluorescent sample as a solution of CdSe/ZnS quantum dots in toluene, and the detector as a CMOS SPAD array. The detected fluorescence was processed with time-correlated single photon counting technique.

INTRODUCTION

Due to their properties, single-photon avalanche diodes (SPAD) have become an alternative to the conventionally used detectors, such as photomultiplier tubes and micro-channel plates in time-resolved fluorescence detection [1]. Today, SPAD manufacturers produce new SPAD detectors with increasingly better performance. However, different research groups use different performance metrics as the importance of each detector characteristic depends on the device application area. We propose to consider SPAD within the system context, taking into account all features of the experiment setup, from light source to measurement technique. In order to achieve this, we developed a simulation model of a typical time-resolved fluorescence measurement experiment including the following parts:

- geometry of the setup;
- light source;
- optical filter;
- fluorescent sample;
- SPAD-based detector;
- measurement technique.

The model has been already verified against experimental data [2, 3]. In this paper we employ our model to investigate the possibility of using time filtering instead of conventional optical filtering in fluorescence detection.

EXPERIMENTS

A range of experiments with optical filter have been carried out to bootstrap the simulator. A vertically integrated micro-system described in [4] was used. The system consists of a blue micro-LED array and a CMOS SPAD detector array. The fluorescent sample (CdSe/ZnS quantum dots in toluene [5]) in a micro-cavity slide and an optical filter (Semrock, LP02-514RU-25 [6]) were placed between the excitation and detection planes. An external time-correlated single photon counting (TCSPC) module (Becker and Hickl, SPC-130 [7]) was used to build fluorescence decay curve. For the simulation we selected a corner single pixel in the SPAD array and the nearest LED from the LED array above (see Fig. 1).

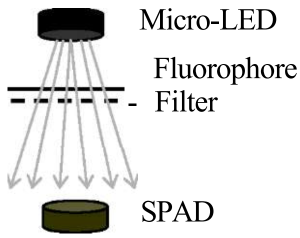

Fig. 1. Simulated system

The experimental and simulated results are presented in Fig. 2. As one can see, the optical filter does not cut off the laser light completely; a part of it is still present in the detected curve. It happens due to the very high intensity of the excitation light and finite ability of optical filter to cut out this light. Such a situation usually does not happen in conventional fluorescence detection, where emitted light is detected at right angle with respect to the incident beam. However, in micro-size systems the situation of straight-line detection is quite common.

One of the possible ways to filter out the excitation light in this case is to use time filtering. Time filtering can be implemented with two approaches. The first one is to keep the detector turned on during all the time, but start photon counting only after the end of laser pulse. However, this approach does not perform a "true" time filtering. As soon as SPAD detector is switched on, it can be fired by laser photons. After such an event, the SPAD will not be able to detect any photons for a certain period of time, called "dead time" (from units to hundreds of nanoseconds). This means that part of fluorescent signal will be lost due to the early detection of excitation light. The higher the intensity of the laser is, the higher is the probability for SPAD to detect an excitation photon and not to be ready to detect other photons during fluorescence decay.

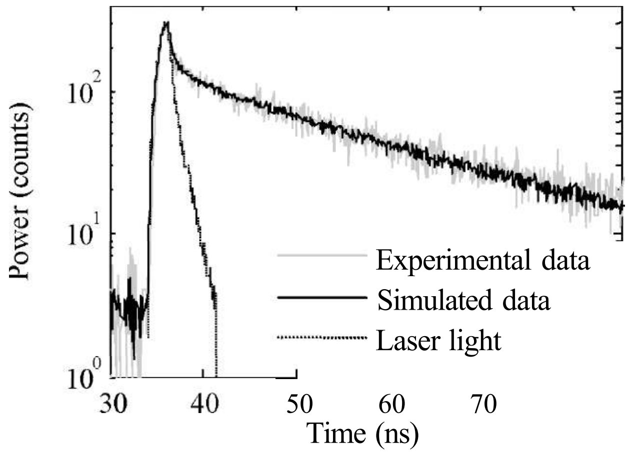

Fig. 2. Experimental and simulated data of considered system with optical filtration of laser light.
The laser light intensity has been normalized to the peak of detected curve and does not display the real value

The drawbacks described above can be avoided by another approach to time filtering implementation. In this approach, the detector is switched off during the laser pulse, and then turns on after the end of this period and can immediately detect photons. It should be noted, however, that SPAD turn-on time can be up to tens of nanoseconds, depending on quenching circuits and stray capacitances. In the system under consideration the SPAD had passive quenching with turn-on time of 35ns (which in case of passive quenching equals to the dead time). The simulated results of time filtering by this approach are presented in Fig. 3.

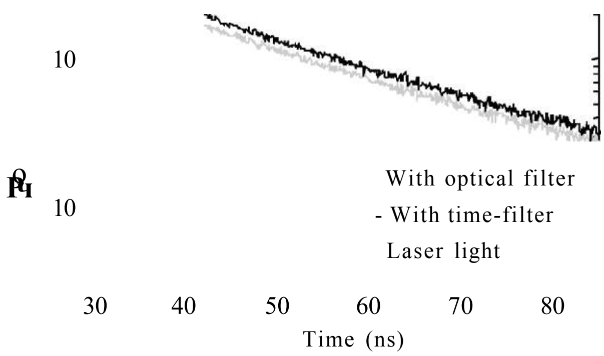

Fig. 3. Comparison of optical and time filtration of laser light.
The laser light intensity has been normalized to the peak of detected curve and does not display the real value

As one can see, the time filter has completely cut out the excitation light. Also, a small part of fluorescence corresponding to the duration of the laser pulse is missing, but in the rest of time period the detected fluorescence intensity is higher with the time filter than with the optical one.

CONCLUSION

The simulation has demonstrated the good performance of the time filtering in SPAD detector. We found that by switching off the SPAD for 7.6 nanoseconds it is possible to completely remove the laser light from detection. At the same time, this does not affect the fluorescence decay because the fluorescence emission takes at least one order of magnitude longer time than the SPAD switching-off time.

The time filtering approach has several advantages in comparison to the conventional optical filter. First of all, if the time filtering is used, the excitation wavelength can be selected at the peak of absorption spectrum even for the fluorophores with small Stokes shift. In the case of a very weak fluorescence signal, the optical filter (which has high but not 1jj% cut-off and transmission efficiency) will not be able to satisfactorily reduce the excitation signal without significant attenuation of the fluorescence signal.

A possible direction for the future work is to find an optimal trade-off between time- and optical-filtering.

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