# Simulation modelling of a micro-system for time-resolved fluorescence measurements

Marina Repich[*a, b], David Stoppa[b], Bruce R. Rae[c], Robert K. Henderson[c], 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;
[c] Institute for Integrated Micro and Nano Systems, School of Engineering, The University of Edinburgh, United Kingdom

## ABSTRACT

This paper presents the simulation modelling of a typical experimental setup for time-resolved fluorescence measurement. The developed model takes into account the setup geometry, characteristics of light source, detector and fluorescent sample as well as the adopted measurement technique. A qualitative verification of the model has been reported before. In this paper, we present a quantitative analysis and verification of the system versatility. For this we conducted time-resolved fluorescence measurements using a two-chip based micro-system, including a blue micro-LED array as a light source and a CMOS SPAD array as a detector. The sample of interest (CdSe/ZnS quantum dots in toluene) in a micro-cavity slide and an excitation filter were placed in the gap between the excitation and detection planes. A time-correlated single photon counting module was used to build fluorescence decay curves. A range of experiments with different excitation light pulse widths and using several setups have been performed. The simulated data are in good agreement with measured results and the model proves to be flexible enough to simulate different light sources and detector quenching/recharging circuits. This model can be used to predict qualitative and quantitative results for specific experimental setups, supporting the explanations of observed effects and allowing the realisation of virtual experiments.

**Keywords:** Single-photon avalanche diode, time-resolved fluorescence measurement, time-correlated single photon counting, simulation modelling.

## 1. INTRODUCTION

The features of single-photon avalanche diodes (SPADs) make these optical detectors an alternative to photomultiplier tubes and micro-channel plates [1] in different areas. SPADs are successfully used in astronomy [2], laser ranging [3], quantum key distribution [4], single molecule detection [5], time-resolved fluorescence measurement [6,7,8] and many others applications. Different techniques of SPAD fabrication produce devices with different characteristics and with different degree of applicability to specific applications. Our area of interest is CMOS SPAD-based detectors applied to time-resolved fluorescence measurement. CMOS technology allows miniaturisation of SPAD-based detectors without degradation of other characteristics. Many research groups are working in the area of CMOS SPAD development but they mainly focus on the improvement of SPAD characteristics using different performance metrics without consideration of the system context and specific application requirements. We propose to study the characteristics of SPAD-based detectors from a system perspective, taking into account the whole experimental setup and measurement technique. This will enable the development of electro-optical systems with the optimal performance for their target application.

To this purpose we developed a simulation model of a typical fluorescence measurement setup, including all blocks from light source to read-out electronics. The model takes into account:

- the geometry of the setup;
- power, time and frequency characteristics of the light source;

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[*] repich@fbk.eu

- filter transfer function;
- characteristics of fluorescence sample, such as thickness, concentration, lifetime, quantum yield and molar extinction coefficient.

The SPAD model implements the basic features of such detectors: photon detection probability, dark count rate, after-pulsing, timing characteristics, passive and active quenching. The system can model two different measurement principles: time-correlated single photon counting and the time-gating technique. The model can not only produce qualitative and quantitative simulation of existing experimental setups but also predict results of virtual experiments with a fictitious detector, light source, etc. This feature can be used to investigate the influence of individual system elements on final results. In addition, experimenters can use the system to investigate unexpected effects appeared in the measurements, such as, pile up effect, background light, etc.

The paper is organised as follows. Section 2 provides a brief description of the improvements of the model with respect to its previous version, presented in [9]. A comparison of experimental and simulated results for a range of experiments with different light pulse widths and different setups are reported in Section 3. Finally, Section 4 concludes the paper.

## 2. MODEL DESCRIPTION

To produce a quantitative simulation, the light source intensity should be considered and properly modelled. The previously developed model, proposed in [9], was operating as follows: each simulation block received a vector of photons with associated time and wavelength values, processed it in accordance with the block task and generated a new vector of photons. The length of the photon vector at the output of the block is always less than at the input. Depending on many factors the percentage of photons impinging on the detector ranges from units to tens of percents of initially generated vector.

To reduce the simulation time we switched from forward to backward simulation. We combined all the factors that decrease the number of photons into one coefficient. This coefficient includes:

- filtering,
- absorption by fluorescent sample without following radiation emission,
- geometrical losses,
- losses due to finite SPAD detection area.

Now only “survived” photons are generated at the beginning of the modelling and each simulation block changes the time and wavelength characteristics of photons but not their quantity. By doing so it is possible to reduce the calculation time allowing longer and more complex experiments.

In our previous implementation, time pulse and spectrum were represented by normal distributions with appropriate mean and variance values. Such approximation reduced simulation time but introduced an additional error. For curves similar to a Gaussian the error is less then 15%, whereas for other curves the error can reach 50%. For example, in case of simulation of Picoquant LDH-P-C-470 [10] pulsed diode laser with 80-ps FWHM the error was 53%. In current implementation, thanks to the backforward simulation, proper modelling of light source photons characteristics is possible. Now the simulator uses tabulated characteristics and the simulation error caused by photon generation is less than 1% (see Fig. 1).

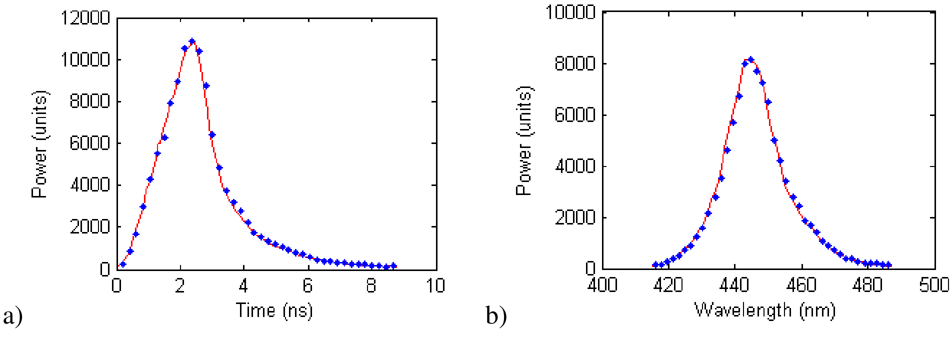

Figure 1. Simulated (dots) and tabular (solid line) light source time characteristic (a) and spectrum (b) for the blue micro-LED produced by the University of Strathclyde [11].

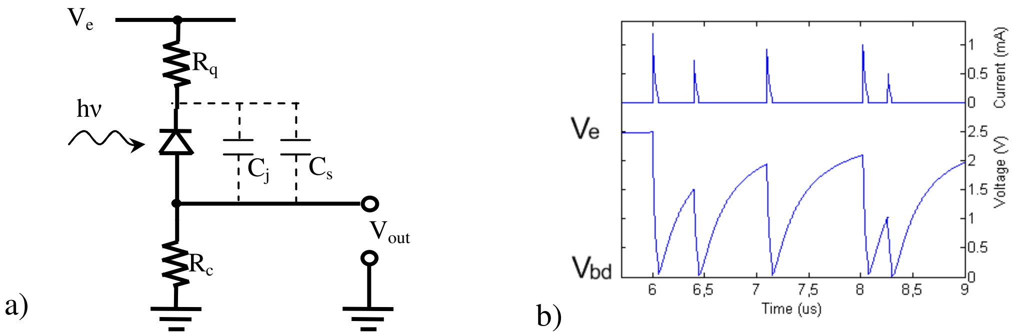

Figure 2. a) The simplest passive quenching circuit with quenching resistor $R_q$ in the order of a few hundred kΩ;
b) waveform of the avalanche current (upper) and of the voltage applied to SPAD (lower).

In order to extend the versatility of our model the passive quenching/recharging has been implemented. Although passive quenching is easier to be implemented in a SPAD, it is quite complex from the simulation point of view. The simplest way to quench an avalanche is to connect the quenching resistor $R_q$ in series with the cathode of SPAD, so limiting the self-sustaining avalanche current (see Fig. 2a). The avalanche current discharges the total capacitance C (given by the sum of the junction capacitance $C_j$ and the stray capacitance $C_s$) and induces a voltage drop over $R_q$. As can be seen in Fig. 2b the voltage on the diode decreases from the excess bias voltage $V_e$ to the breakdown voltage $V_{bd}$. Then the voltage starts to restore slowly with the time constant $R_qC$. During the recovery time, when the diode voltage is higher than the breakdown voltage but has not yet reached the intended final bias value, a photon can trigger an avalanche; however, the avalanche triggering probability depends on the time and is lower than that available at the final voltage. At the same time an avalanche can be re-triggered by a trapped carrier [12].

The simulation of passive quenching is implemented in the following way. After modelling all SPAD effects (photon detections, dark counts and afterpulses), the time intervals between these and previous events are calculated. Then the amplitude of current (voltage) peak is calculated by an exponential equation based on the RC time constant. All events for which the current (voltage) amplitude is lower than threshold are deleted. Remaining events are the output of the SPAD simulation. The block diagram of the passive quenching simulation is depicted in Fig. 3.

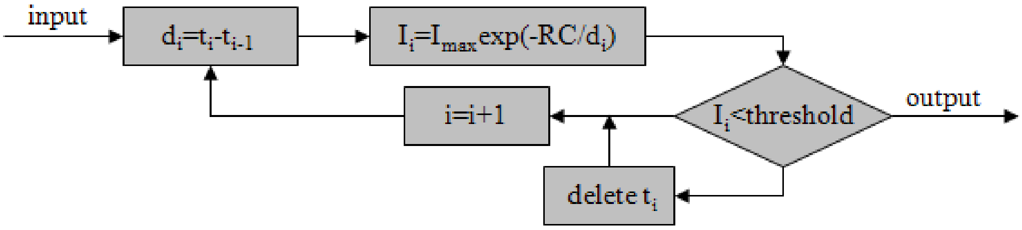

Figure 3. Block diagram of the simulation of passive quenching.

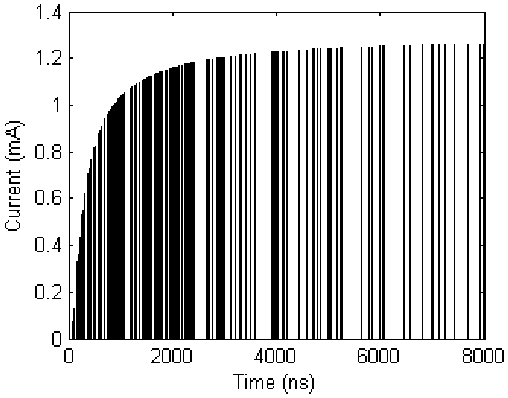

Figure 4. The simulated current pulses of a SPAD with passive quenching.

The simulated current pulses of a SPAD with passive quenching are shown in Fig. 4 where the cumulative picture of pulses occurred at a different time after the previous avalanche event is reported. The amplitude envelope corresponds to the voltage recovery graph (see also Fig. 2b).

## 3. EXPERIMENTAL VALIDATION

To validate the described improvements and make a quantitative analysis the following setup with the micro-system described in [8] was employed. This two-chip "sandwich" structure (see Fig. 5) includes a blue micro-LED array with a peak emission wavelength of 450 nm and a CMOS SPAD detector array where each pixel can extract time-gated measurements or send data to external photon counting hardware. For this experiment we have selected a single pixel in the SPAD array and the nearest LED from the LED array. The sample (CdSe/ZnS quantum dots in toluene [13]) in a micro-cavity slide and an excitation filter (Semrock, LP02-514RU-25 [14]) were placed in the 14mm gap between the excitation and detection planes. A time-correlated single photon counting module (Becker and Hickl, SPC-130 [15]) was used to build fluorescence decay curves.

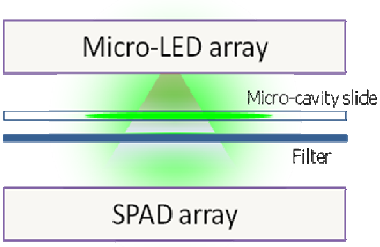

Figure 5. The two-chip "sandwich" structure includes a micro-LED array and a CMOS SPAD detector array. The fluorescent sample was located in a micro-cavity slide. An excitation filter was used to separate the LED light from the fluorescent light.

A range of experiments with different light pulse width was carried out to examine the sensitivity of the simulator. The simulated and experimental results for 3.1ns, 3.15ns and 3.3ns pulse width with respectively 3.77µW, 6.47µW and 9.08µW average optical power per pulse are presented in Fig. 6. The simulated curves are in good agreement with experimental ones.

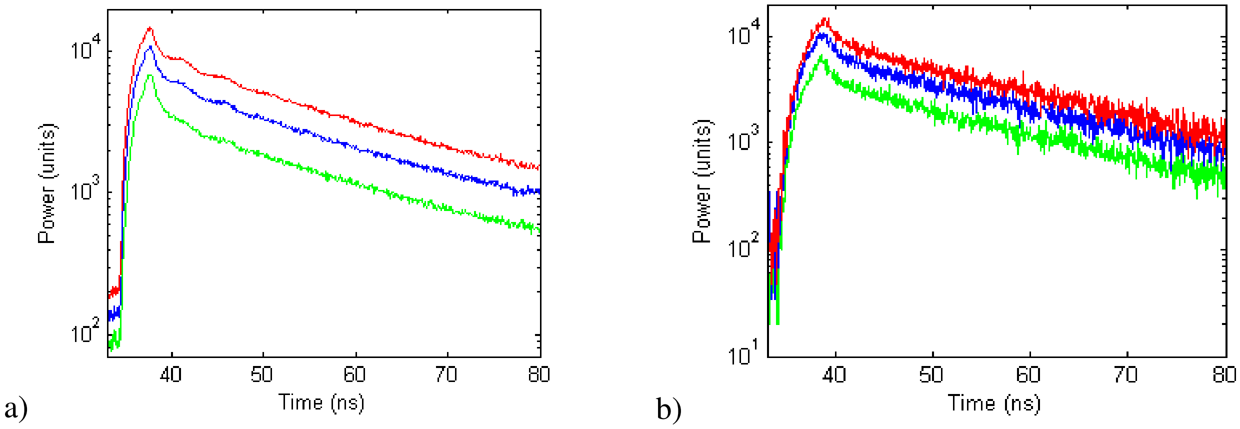

Figure 6. Experimental (a) and simulated (b) fluorescence decay with different light pulse width: 3.1ns (green), 3.15ns (blue) and 3.3ns (red).

The second range of experiments was used to estimate the quantitative simulation ability of the model. We used different setups with and without excitation filter and fluorescent sample. Selected results are presented in Fig. 7. In those experiments the dark count rate was artificially increased to simulate ambient light. Experimental and simulated data are in good agreement except in the peak area. This mismatch can be explained by the following reason. We did not have the experimental LED time curve at FWHM=2ns. The time curve shape for with FWHM=2.2ns has been squeezed to proper width. The distortion of curve shape has probably resulted in the disagreement described above.

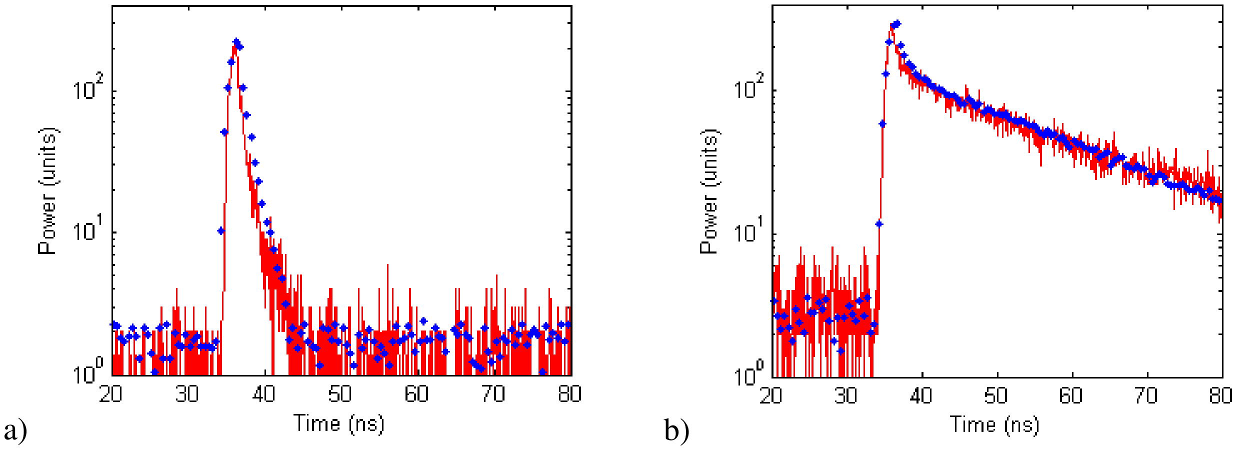

Figure 7. a) Experimental (solid line) and simulated (dots) curves measured without fluorescence sample; b) experimental (solid line) and simulated (dots) fluorescence decay.

## 4. CONCLUSIONS AND FUTURE WORK

An improved simulation model of a SPAD-based detection system for time-resolved fluorescence has been presented. The model has demonstrated flexibility in simulation of both micro-LED and laser light, passive and active quenching. The system is able to perform both qualitative and quantitative modelling of the fluorescence decay process. The simulation results are in good agreement with actual measurements.

This work will proceed in several directions. First, we will employ an optimisation algorithm to determine the optimal set of detector characteristics and experimental parameters. After that, further improvements to the simulation of SPAD, biological sample and optics will expand application areas, increase accuracy and enable researchers to analyse more complex and interesting problems.

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## REFERENCES

[1] Becker, W. [Advanced Time-Correlated Single Photon Counting Techniques], Springer Series in Chemical Physics, Springer, Berlin, pp. 213-262 (2005).

[2] Barbieri, C., Naletto, G., Occhipinti, T., Facchinetti, C., Verroi, E., Giro, E., Di Paola, A., Billotta, S., Zoccarato, P., Bolli, P., Tamburini, F., Bonanno, G., D'Onofrio, M., S., M., Anzolin, G., Capraro, I., Messina, F., Belluso, M., Pernechele, C., Zaccariotto, M., Zampieri, L., Da Deppo, V., Fornasier, S. and Pedichini, F., “AquEYE, a single photon counting photometer for astronomy,” Journal of Modern Optics, vol. 56, n. 2&3, pp. 261-272 (2009).

[3] Kodet, J., Prochazka, I., Koidl, F., Kirchner, G. and Wilkinson, M., “SPAD active quenching circuit optimized for satellite laser ranging applications,” Proc. SPIE, vol. 7355, pp. 73550W (2009).

[4] Zhang, J., Eraerds, P., Walenta, N., Barreiro, C., Thew, R .,and Zbinden H., "2.23 GHz gating InGaAs/InP single-photon avalanche diode for quantum key distribution," arXiv:1002.3240v1 [quant-ph], http://arxiv.org/abs/1002.3240v1.

[5] Ray, K., Ma, J., Oram, M., Lakowicz, J. R. and Black, L. W., "Single-Molecule and FRET Fluorescence Correlation Spectroscopy Analyses of Phage DNA Packaging: Colocalization of Packaged Phage T4 DNA Ends within the Capsid," Journal of Molecular Biology, vol. 395, n. 5, pp. 1102-1113 (2010).

[6] Gersbach, M., Boiko, D. L., Niclass, C., Petersen, C. C. H. and Charbon, E., "Fast-fluorescence dynamics in nonratiometric calcium indicators," Optics Letters, vol. 34, n. 3, pp. 362-364 (2009).

[7] Li, Day-Uei, Rae, B., Andrews, R., Arlt, J., Henderson, R., "Hardware implementation algorithm and error analysis of high-speed fluorescence lifetime sensing systems using center-of-mass method," J. Biomed. Opt., vol. 15, pp. 017006 (2010).

[8] Rae, B. R., Muir, K. R., Renshaw, D., Henderson, R. K., Girkin, J., Gong, Z., McKendry, J., Gu, E. and Dawson, M. D., "A vertically integrated CMOS micro-system for time-resolved fluorescence analysis," Proc. IEEE BioCAS, pp. 85-88 (2009).

[9] Repich, M., Stoppa, D., Pancheri, L. and Dalla Betta, G.-F., "Simulation modelling for the analysis and the optimal design of SPAD detectors for time-resolved fluorescence measurements," Proc. SPIE vol. 7355, pp. 73550O (2009).

[10] PicoQuant GmbH. Ultraviolet to infrared picosecond diode laser heads. On-line: http://www.picoquant.com/getfs.htm?products/ldh/spec_ldhseries.htm. Accessed on 8 March 2010.

[11] Jeon, C. W., Choi, H. W. and Dawson, M. D., "Fabrication of matrix-addressable InGaN-based microdisplays of high array density," IEEE Photonics Technology Letters, vol. 15, n. 11, pp. 1516 – 1518 (2003).

[12] Cova, S., Ghioni, M., Lacaita, A., Samori, C. and Zappa, F., "Avalanche photodiodes and quenching circuits for single-photon detection" Applied Optics, vol. 35, n. 12, pp. 1956-1976 (1996).

[13] Evident Technologies. Core and core-shell quantum dots. On-line: http://www.evidenttech.com/products/evidots.html. Accessed on 10 March 2010.

[14] Semrock. Lp02-514ru-25. On-line: http://www.semrock.com/Catalog/Detail.aspx?CategoryID=69&FilterPartID=164. Accessed on 10 March 2010.

[15] Becker & Hickl GmbH. Time-correlated single photon counting devices. On-line: http://www.becker-hickl.com/tcspc.htm\#spc130. Accessed on 10 March 2010.