

Simulation modelling for the analysis and the optimal design of SPAD detectors for time-resolved fluorescence measurements

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ABSTRACT

This paper describes a simulation model (implemented in MATLAB[®]) of a typical setup used for time-resolved fluorescence measurements, including: a laser source, basic fluorescence sample, optics, single-photon avalanche diode and read-out electronics. The correctness of the model has been verified by setting up a simple time-resolved fluorescence measurement using a CMOS SPAD-based detector. The solution of fluorophore (CdSe/ZnS quantum dots in toluene) in a glass capillary was placed above the detecting surface and excited by laser pulses. We have used a time-gating technique with 10-ns observation window shifted at 60-ps time steps across the appropriate time interval. The observed curve corresponds to the convolution of the fluorescence emission and the 10-ns observation window. Simulation accuracy has been verified by comparing the experimental fluorescence decay with the simulated one using chi-square test. The proposed model allows researchers to simulate the behaviour of SPAD detectors with a good accuracy and demonstrates how imperfections in the experimental system can affect the result. The model enables the design of SPAD-based detectors with the best performance for a specific application area.

Keywords: single-photon avalanche diode, fluorescence measurement, time-correlated single photon counting, time-gating, simulation modelling

1. INTRODUCTION

Due to their properties, single photon detectors find their applications in astronomy [1], laser ranging [2], quantum cryptography [3], single molecule detection [4], fluorescence decay [5,6], etc. Historically, photomultiplier tubes were the first detectors used in fluorescence measurements. They provide a low noise and fairly high quantum detection efficiency in the visible spectrum. At the same time they are bulky, fragile, and expensive, require high supply voltage (2-3 kV) and are sensitive to electromagnetic fields and mechanical vibrations. Single photon avalanche diodes (SPAD) have become an attractive alternative to photomultiplier tubes [7] due to the advantages of solid-state devices, such as: magnetic field immunity, robustness, long operative lifetime, small sizes, lower cost, lower bias voltage, suitability for inclusion in integrated systems. There has also been much progress in terms of improved detector time resolution, quantum efficiency and noise performance.

Recent progress in CMOS technology allows SPAD detectors to be fabricated alongside integrated read out electronics, which ultimately reduces the sensitive node capacitance and allows additional processing features [8,9]. This leads reduced system costs and the ability to produce monolithic arrays for large area detection. Co-integration of a SPAD and an electronic circuit on the same substrate provides advantages in terms of time and noise characteristics. The main disadvantages of the CMOS SPAD are the high dark count rate (DCR) and low photon detection efficiency at longer wavelengths (above 800nm) [10].

At the present time, the area of SPAD detectors is being developed constantly and quickly. Many new sensors with enhanced characteristics are produced every year. Fully integrated SPAD-based detectors with extensive on-chip processing features have been recently proposed [8,9,11,12,13,14]. However, current research works focus mainly on the improvement of particular characteristics using different performance metrics without consideration of their integral usefulness for experimenters. Moreover, the importance of each SPAD characteristic depends on the application. We

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propose to study all the characteristics of a SPAD detector taking into account the conditions of the experimental setup and measuring technique, in order to design a detector which has the optimal overall figure of merit for a specific application.

In this paper, we present a simulation model of a SPAD-based detector and all the essential parts of a fluorescence lifetime measurement experiment. Two measurement techniques, namely, time-correlated single photon counting and time-gating, were modelled as well. Lifetime measurements of quantum dots with a CMOS SPAD sensor were made to validate the correctness of the model.

The paper is organized as follows. Section 2 is a brief explanation of SPAD operation and fluorescence measurement techniques used in this work. An overview of the model and simulation methods is carried out in Section 3. A comparison of experimental and simulated data is reported in Section 4.

2. THEORY OF SPAD AND FLUORESCENCE MEASUREMENTS

2.1 Single-Photon Avalanche Diodes

The operation principle of an ideal SPAD is the following: in the quiescent state, when no current flows through the device and an excess bias voltage V_e is applied to the SPAD, the electric field in the depletion region is high enough to create an avalanche caused by the smallest fluctuation. A single photon absorption in the depletion region results in the creation of an electron-hole pair that is immediately accelerated. This action results in a self-sustaining impact ionization avalanche process. The current immediately grows up to a constant level that is dependant on the excess bias voltage and diode series resistance. The external quenching circuit reduces the applied bias voltage, V_b , to a value lower than the breakdown voltage V_{bd} , which leads to the quenching of the avalanche. The operation cycle is completed by the reset of the excess bias voltage to its initial value. Thus the output of the detector is the current pulse with a constant peak amplitude. The leading edge of this pulse indicates the time of photon arrival. The detector is insensitive to any photons arriving at a time between the start of an avalanche and the bias voltage being reset. This period is called the dead time of the SPAD.

Obviously, real SPADs differ from ideal ones. The first characteristic of imperfection is the time jitter. Time jitter or time response is a statistical distribution of the delay between actual arrival time of the photon and the leading edge of the output pulse. A typical SPAD time resolution curve has a fast peak followed by a slow exponential tail. In some SPADs reported in the literature, the diffusion tail was greatly reduced by changing SPAD structures [15,16]. The next characteristic of a real SPAD is its photon detection probability (PDP), which is defined as the ratio between the number of incoming photons and the number of corresponding output current pulses. The typical PDP values for CMOS SPAD are around 30% in blue-green range. Internal noise of the device is a characteristic which strongly affects the performance of the detector. It is called dark count rate – the avalanche triggering rate of a detector held in darkness. Another noise component is afterpulsing, which is a re-triggering of the avalanche caused by trap level generation in the absence of photon absorption. Afterpulsing can be reduced either by increasing the dead time of the detector, which leads to worse time-performance, or by decreasing of the excess bias voltage, which affects the photon detection efficiency.

All the characteristics described above, except photon detection probability, depend on the quenching circuit being used. The task of the quenching circuit is to limit the maximum current flow through the device and to reset the device, so it can count subsequent photons. The simplest way to quench an avalanche is to connect a quenching resistor R_q in series with the cathode of SPAD, so it will stop the self-sustaining avalanche current. The avalanche current discharges the total capacitance and induces a voltage drop over R_q . During the recovery time, when the diode voltage is higher than the breakdown voltage, a photon or trapped carrier can trigger an avalanche; however, the avalanche triggering probability depends on the time and is lower than the PDP value. Active quenching does not have those drawbacks associated with passive one. As soon as an avalanche is detected the circuit forces the quenching and reset to the initial state by applying an additional voltage. This takes a shorter time compared to the case of passive quenching. This type of quenching leaves an opportunity to deal with afterpulsing: the bias voltage can be reduced below the breakdown voltage and retained for a time sufficient to release trapped carriers.

2.2 Fluorescence lifetime measurements

Fluorescence lifetime measurements can be done in the time domain using two techniques: time correlated single photon counting (TCSPC) and time-gating detection. In the former, the arrival time of the first photon is recorded. A fluorescence decay curve is obtained by the detection of arrival time of multiple photons. Fluorescence lifetime in this

case can be obtained using the following scheme. Firstly, the convolution of assumed fluorescence decay with a known instrumental response is calculated. Then the result is compared with the measured experimental decay curve using statistical fitting criterion, such as the chi-square test. The goodness of the fit can be judged by the chi-square value and the autocorrelation of weighted residuals. The advantage of the described experimental technique is that the actual fluorescence decay is measured directly; the disadvantage is its relative slowness. In the time-gating detection, the number of photons detected during a fixed time interval is collected. For the measurement of a mono-exponential fluorescence decay, two time intervals are typically enough. The fluorescence lifetime τ in this case is calculated using:

$$\tau = \frac{T_1 - T_2}{\ln(V_1 - V_2)}, \quad (1)$$

where T_1 and T_2 are the time delays between the excitation pulse and the onset of the first and the second time intervals, respectively; V_1 and V_2 are integrated intensities (i.e. number of counts) of these time intervals. In the case of multi-exponential fluorescence decay (which is the case for vast majority of biological samples) more time intervals and a correction instrumental response are required.

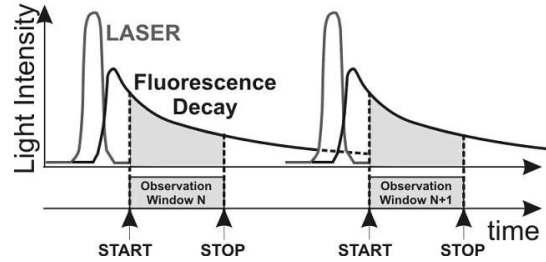


Fig. 1. Modified time-gated measurement technique [13].

A slightly modified time-gated technique has been utilized in the SPAD detector used here. Photons are collected during nanosecond observation window (OW) synchronized with the light pulse. Measurements are repeated a prescribed number of times to obtain a statistically significant result. The number of detected photons is allocated to a time slot. The observation window is then shifted by a picosecond time step and the measurement repeated from the start. Fluorescence decay convolved with the rectangular OW is prepared when a time range of interest has been fully scanned.

3. MODEL AND SIMULATION METHODS

In this work, we have used Monte Carlo simulation, a powerful tool for complex systems modelling [17]. The model of fluorescence measurement setup consists of a set of independent blocks. Each of them simulates an appropriate part of the experiment. A schematic diagram of these blocks, their inputs and outputs is shown in Figure 2. The main input of the simulation is the repetition rate of light source (synchronizing pulses) and duration of the experiment. The description of particular inputs, outputs and details of the simulation on each step are presented below.

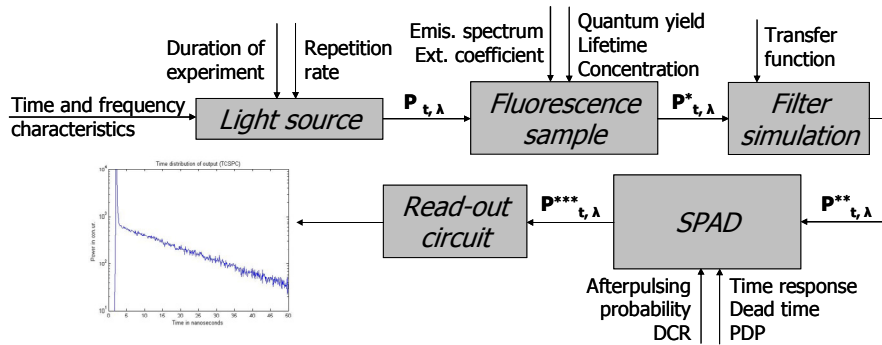


Fig. 2. Schematic diagram of the simulation model.

Light source simulation. The input consists of the full width at half of maximum (FWHM) of the light source in ps, its main wavelength in nm and the width of its spectral characteristic. As a first approximation the time and frequency distribution of a light source are simulated as 2-dimensional normal distribution with corresponding mean and standard deviation values:

$$f(t, \nu) = \frac{1}{2\pi\sigma_t\sigma_\nu} \exp\left(-\frac{1}{2}\left[\frac{(t-m_t)^2}{\sigma_t^2} + \frac{(\nu-m_\nu)^2}{\sigma_\nu^2}\right]\right), \quad (1)$$

where m_t , m_ν and σ_t , σ_ν – are the mean and the standard deviation of time and frequency, correspondingly. The number of photons is calculated from the repetition rate and experiment duration. The output of the light source simulation is a set of photons with time and wavelength characteristics generated by a rejection method [18], which allows us to use any empirical probability density function (PDF) in place of Eq. 1.

Fluorescence simulation. The input here is the output of the previous step, the concentration of the fluorophores and the characteristics of the fluorescent molecules, such as quantum yield, extinction coefficient and emission spectrum. At the current stage of this research we have made several assumptions concerning fluorescent sample:

- 1) the light absorption obeys the Beer-Lambert law;
- 2) fluorophores have uniform distribution;
- 3) the optical density of the fluorescent sample is negligible;
- 4) fluorescence decay is monoexponential;
- 5) there are no other processes except fluorescence.

These assumptions noticeably decrease the computational costs and at the same time they are still in good agreement with the real world.

The number of absorbed photons is calculated based on the quantum yield, extinction coefficient and fluorophore concentration. Time and wavelength are then simulated for each absorbed photon. Times are generated by the inverse function method:

$$t_i = -\tau \ln z_i, \quad (2)$$

where τ is a fluorophore lifetime, z is a random variable uniformly distributed from 0 to 1, i is a counter from 1 to the number of absorbed photons. Wavelengths are generated by a rejection method according to the emission spectrum of a fluorophore. A typical emission fluorescence spectrum is shown in Figure 3. The output on this step is an array of fluorescent photons with new time and wavelength values and scattered photons with time and wavelength simulated on the first step.

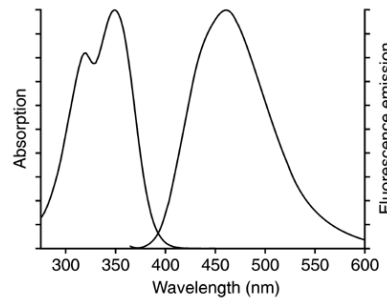


Fig. 3. Absorption and fluorescence emission spectra of quinine sulfate, dihydrate in 0.5 M sulfuric acid [19].

Optics simulation. At the current stage of the project, we have been interested in simulation of filters only, but not lenses. The output of the previous step and a filter transfer function are used as input for this part. During the filtering, the photons with unsuitable wavelengths are deleted from simulation. The output contains an array of photons with wavelengths that passed through the filter. Generally, it is made in order to attenuate light source and keep only fluorescence.

Single-photon avalanche diode simulation. The input here is the output of the previous step, detector photon detection

probability versus wavelength, time resolution characteristic, dark count rate value and afterpulsing probability density versus time. The SPAD simulation starts with the generation of dark counts. This has been implemented by modelling Poisson flow with the rate parameter equal to the DCR of the detector. Occurrence times in this case are modelled by the following recurrence equation:

$$t_i = t_{i-1} - \ln z / \lambda, \quad (3)$$

where λ is rate parameter of Poisson flow, z is random variable uniformly distributed from 0 to 1. Avalanche events caused by fluorescent photons are simulated depending on PDP for corresponding photon wavelengths. For each successful event the time jitter is modelled. The sum of the fluorescent time and the time jitter for each photon is put into the same time array where dark counts were written down heretofore. Further, the afterpulsing probability is calculated on the basis of DCR, dead time and afterpulsing characteristic of a detector. The afterpulsing events are drawn for each fluorescent photon and dark count and are put into the time array. Then this array is sorted in an ascending order. The last step is the removal of events occurring during the dead time of the previous event. The output of this step provides the times of avalanche events caused by fluorescent photons, dark counts and afterpulses. A block diagram of the SPAD simulation is shown in Figure 4.

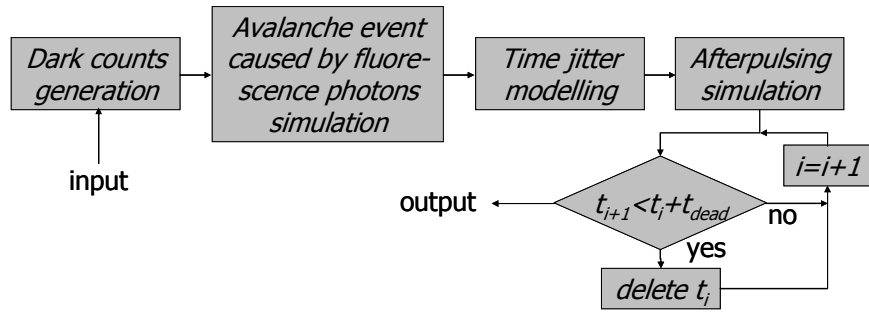


Fig. 4. The block diagram of single-photon avalanche diode simulation.

Read-out electronics simulation. The input here is the output of the previous step, observation window and time step (in case of time-gating). For TCSPC we consider only the first events between two consecutive synchronizing pulses. The output of the simulation in this case is a timing diagram of the delay between the synchronizing pulse and the first detected event. In the case of time-gating techniques, all events occurred during a nanosecond observation window are summarized and the obtained value is saved in a time slot corresponding to the beginning of the observation window. The observation window is then shifted at a picosecond time step and the simulation is repeated from the beginning until the time range of interest is covered. The output of the simulation in this case is represented by a convolution of the measurable fluorescence decay and the observation window.

4. RESULTS AND DISCUSSION

To validate the simulation model, a simple fluorescence decay measurement described in [14] was performed. CdSe/ZnS quantum dots in toluene [20] in a glass capillary were put in immediate proximity to the detector. A Picoquant LDH-P-C-470 pulsed diode laser with 80-ps FWHM and 1-MHz repetition frequency was used as the excitation source. The practical and simulated laser pulses are shown in Fig. 5. As one can see, the modelled laser shape doesn't have a long tail and thus it is a little bit narrower under the same FWHM.

Neither lenses nor optical filters have been used. A single CMOS SPAD pixel was used to detect fluorescence. The detector uses active quenching and has the following characteristics:

Table 1. The main characteristics of SPAD in the measurement setup.

Dark count rate	4kHz
Afterpulsing probability	~ 4.5%
PDP	max 32% (at 450 nm)
Dead time	520ns
FWHM	160ps

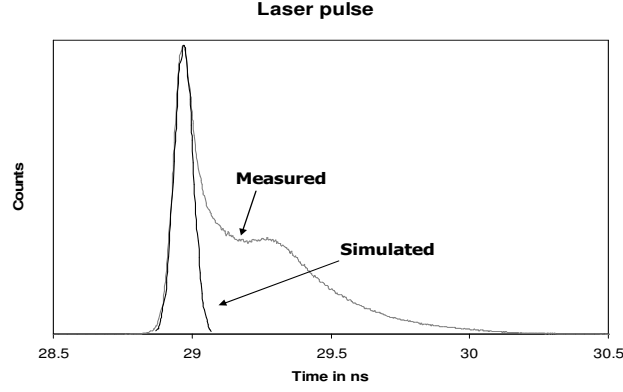


Fig. 5. Simulated (black) and practical (grey) laser pulse.

Theoretical lifetime equal to 16ns has been utilized to simulate fluorescence decay. The 10-ns observation window and 60-ps time step were used for time-gating. The experimental and simulated fluorescence decay curves are shown in Figure 6. The difference of intensity during the first 10ns between the experimental and simulated data can be explained by non-metering of light dispersion into fluorescent sample. All unabsorbed photons hit the detector, whereas in the real world a proportion of them are lost. The lifetime for each curve has been extracted using fitting of fluorescence decay by mono-exponential curve. The experimental and simulated lifetimes are 16.21 ns and 16.39 ns correspondingly.

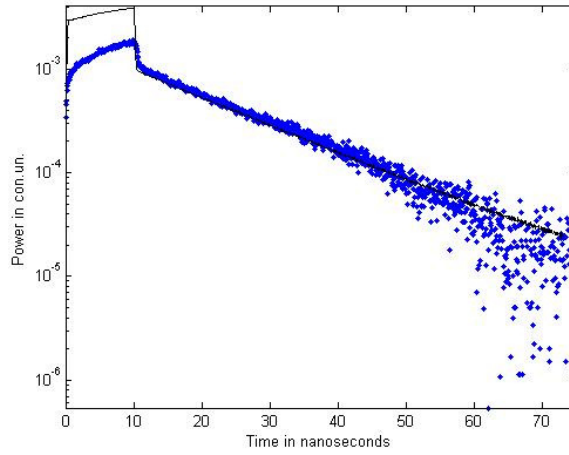


Fig. 6. Fluorescence decay measurement performed with single pixel SPAD CMOS sensor and time-gated technique (grey/blue line) and simulated by our system (black line).

The quality of simulation has been checked by chi-square test [21]. This test measures the deviation of experimental data from theoretical data (in our case, simulated data from measured data) and can be represented by the following equation:

$$\chi^2 = \frac{1}{N-1} \sum_{k=1}^m \frac{(v_k - I_k)^2}{(I_k + v_k)/2}, \quad (4)$$

where N – number of measurements; m – number of bins; v_k - theoretical mean value of events in k -bin; I_k - experimental mean value of events in k -bin. If $\chi^2 < 0.8$, it means that data level is not enough. If $\chi^2 > 1.2$, it stands for wide disagreement of theoretical and experimental data. In our case, $\chi^2 = 1.5$ that confirms a good agreement between simulated and measured data.

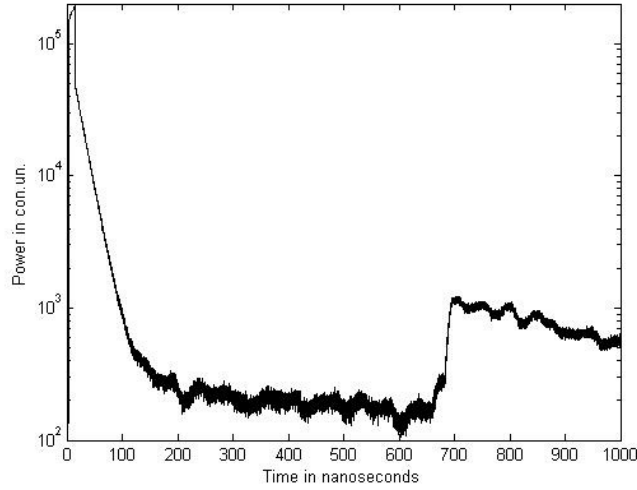


Fig. 7. Simulated fluorescence decay with evident second peak at long times.

By scanning the total time interval between two consecutive laser pulses, we have discovered the second intensity peak at the times comparable with dead time of the detector. Simulated fluorescence decay on long time scale is presented in Figure 7. We believe that this peak represents afterpulsing of the detector. Since the simulated SPAD has an active quenching circuit, any avalanche event can't be detected before the end of dead time. Moreover, this effect can be observed only with a time-gated technique, because in TCSPC technique, after the detection the first avalanche event read-out circuit waits for the next synchronizing pulse which comes with the next laser pulse. Thus, all avalanche events occurring during this time will be ignored. The experimental measurements of this effect are under investigation.

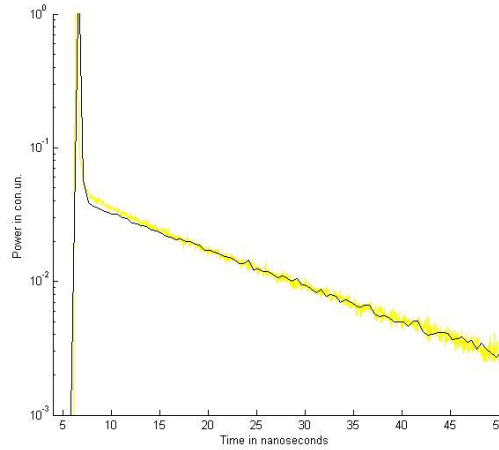


Fig. 8. Fluorescence decay measurement performed with single pixel SPAD CMOS sensor and TCSPC technique (grey/yellow line) and simulated by our system (black line)

We have also performed a simulation of fluorescence lifetime detection by time correlated single photon counting technique (see Fig. 8). The result has been compared with experimental data obtained with the same setup as in the previous case except the utilization of an external commercial TCSPC instrument [22]. As before, the simulated data are in a good agreement with the measured one.

5. CONCLUSIONS AND FUTURE WORK

A simulation model of SPAD-based detection of fluorescence decay has been presented. The model incorporates all the essential parts of a typical setup of time-resolved fluorescence measurements. It enables researchers to simulate the

behaviour of SPAD detectors with good accuracy and shows how imperfections in the system (noise, read-out strategy, etc.) affect the results. Preliminary tests of the model with a single pixel of a CMOS SPAD sensor and time-gated technique are in a good agreement with experimental data.

This work is research in progress in our research group. Future work will implement simulation of system geometry, laser intensity, optical lenses and effects related to passive quenching. Furthermore, an application of an optimization algorithm to the simulation model will provide an opportunity to fit SPAD and experimental setup parameters and to achieve the optimal system performance.

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