Development of a simulation environment for the analysis and the optimal design of fluorescence detectors based on single photon avalanche diodes

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• Introduction
  – Time-domain fluorescence detection
  – Single photon avalanche diode (SPAD) detectors

• Model of fluorescence measurement setup
  – General overview
  – Step by step simulation

• Validation of the model
  – Flexibility
  – Prediction ability

• Conclusions and future work
Road Map

Introduction

Proposed model

Validation of the model

Conclusions and future work
Typical experiment setup

- The photodetector determines the accuracy of the measurements.
- General performance of the setup is defined by all part, from light source to lifetime extraction algorithm.
Fluorescence detection

- **Features**
  - Nanosecond time scale
  - Typical lifetime 1ns – 20ns
  - Single photon sensitivity

- **Time-correlated single photon counting (TCSPC)**
  - Pulsed light source
  - Time-stamp of the first detected photon

- **Time-gating**
  - From 2 time intervals
  - More than one photon per pulse

$$I(t) = I_0 \cdot \exp(-t/\tau)$$
Single photon avalanche diode

SPAD is a class of solid-state photodetectors based on a reverse biased p-n junction.

Advantages
- Small size
- Low cost
- Magnetic field immunity
- Low voltage supply
- Suitable for matrices
- Real time data processing (only for CMOS SPAD)

Disadvantages
- Higher noise
- Narrow absorption spectrum
Photon detection

Simplified SPAD diagram

Avalanche process

- Excess bias voltage $V_e$ is applied to SPAD
- Electric field in depletion region is very high
- Single photon can create an avalanche
Avalanche current and voltage

- **t**<sub>1</sub>-t<sub>0</sub> – time jitter
- **t**<sub>2</sub>-t<sub>1</sub> – quenching time
- **t**<sub>3</sub>-t<sub>1</sub> – recharging time

**Typical time jitter curve**

A. Gulinatti et al., Proc. ESSDERC (2005)
Photon detection probability

\[ PDP = \frac{\text{output current pulses}}{\text{incoming photons}} \]

\[ PDP = QE(\lambda) \times P_{av}(V_e) \]

- QE – quantum efficiency
- \( P_{av} \) – avalanche triggering probability by the primary e-h pair

D. Stoppa et al., IEEE Sens. J. (2009)
Detector noise

Dark count rate (DCR) – avalanche triggering rate of the detector held in the darkness

- Thermal generation
- Band to band tunneling
- Afterpulsing

C. Niclass et al., SPIE Optics East (2006)
Passive quenching

- Simple to organize
- Long recovery time
- Triggering probability depends on time
- Not constant dead time
- Low maximum count rate
Active quenching

- Short recovery time
- Fixed and controlled dead time
- Adjustable hold-off time and afterpulsing
- High maximum count rate

SPAD in easy terms (video)
## SPAD performance

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>0.18μm CMOS</th>
<th>InGaAs/InP</th>
<th>Double-epitaxial</th>
<th>130nm CMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area</strong></td>
<td>2μm x2μm</td>
<td>Ø 25μm</td>
<td>Ø 50, Ø 200 μm</td>
<td>Ø 8μm</td>
</tr>
<tr>
<td><strong>Dead time</strong></td>
<td>3ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DCR</strong></td>
<td>70 kHz</td>
<td>1kHz, 50kHz</td>
<td>20Hz</td>
<td></td>
</tr>
<tr>
<td><strong>PDP</strong></td>
<td>45% at 1310nm</td>
<td>55% at 500nm, 68% at 550nm</td>
<td>20%-25% at 440-570nm</td>
<td></td>
</tr>
<tr>
<td><strong>Time response</strong></td>
<td>26.7ps (96.1ps)</td>
<td>30ps</td>
<td>35ps</td>
<td>200ps</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HV CMOS</th>
<th>0.35μm HV CMOS</th>
<th>130nm CMOS</th>
<th>0.35μm CMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area</strong></td>
<td>Ø 5μm</td>
<td>15.8 x 15.8μm</td>
<td>Ø 10μm</td>
<td>Ø 10μm</td>
</tr>
<tr>
<td><strong>Dead time</strong></td>
<td>200ns</td>
<td></td>
<td>100ns</td>
<td></td>
</tr>
<tr>
<td><strong>DCR</strong></td>
<td>50Hz</td>
<td>1kHz</td>
<td>100kHz</td>
<td>600Hz</td>
</tr>
<tr>
<td><strong>PDP</strong></td>
<td>34% at 470nm</td>
<td>32% at 450nm</td>
<td>34% at 450nm</td>
<td>35% at 460nm</td>
</tr>
<tr>
<td><strong>Time response</strong></td>
<td>80ps</td>
<td>160ps</td>
<td>144ps</td>
<td></td>
</tr>
</tbody>
</table>
Motivation

- Different manufacturing techniques result in SPADs with different characteristics
- Current works focus on particular parameters out of system context
- The importance of particular detector characteristic depends on application
- Trial-and-error is expensive and time consuming

How can we estimate the suitability of a SPAD-based detector for specific application? (without producing it)
Create a tool that will allow the performance analysis and optimization of a SPAD-based system

– Considering all essential parts of fluorescence measurement experiment

– Adjustable to different experiments
Expected benefits

The tool will enable the researchers to...

• Predict the results of planned experiments
• Explain the results of real experiments
• Determine the optimal trade-off between opposing characteristics
• Generate test data for evaluation of novel data processing algorithms
Road Map

Introduction

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Validation of the model

Conclusions and future work
Proposed model

- Pulsed light source
- Optical system
- Fluorescent sample
- Emitted light
- Photo detector
- Data processing

Simulation model

- Light source
- Fluorescence sample
- SPAD-based detector
- Filter
- Read-out circuit
- Measurement technique

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Simulation workflow (forward type)

- Duration of experiment
- Geometry
- Repetition rate
- Light intensity

Preprocessing

- Time and wavelength characteristics
- Emis. spectrum
- Ext. coefficient

Light source

- Quantum yield
- Lifetime
- Concentration

Fluorescence sample

- Transfer function

Filter

- Read-out circuit
- Dead time
- Time shift
- OW

SPAD-based detector

- Afterpulsing
- Quenching type
- RC constant
- DCR

- Time response
- Dead time
- Hold-off time
- PDP

Power (a.u., log scale)

Time (ns)
Simulation workflow (backward type)

Preprocessing → Light source → Fluorescence sample → Filter

Duration of experiment
Geometry
Filter transfer function

Repetition rate
Light intensity
Ext. coeff.
Fl. concentr.
Quantum yield

Time and wavelength characteristics

Lifetime
Emis. spectrum

Preprocessing

Read-out circuit

SPAD-based detector

Filter

Power (a.u., log scale)

Time (ns)

Dead time
Time shift
OW

Afterpulsing
Quenching type
RC constant
DCR

Time response
Dead time
Hold-off time
PDP
Light source simulation

• Gaussian approximation

- LED pulse
  Error – 12%

- LED spectrum

- Laser pulse
  Error – 53%

• Tabulated functions

- LED pulse
  Error – 0.4%

- LED spectrum

- Laser pulse
  Error – 0.8%

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Fluorescence simulation

Assumptions:
- Beer-Lambert law
- Uniform fluorophore distribution
- Negligible optical density
- Monoexponential decay
- No other processes
- Light source as a point
SPAD simulation

- **Dark counts generation**
  - Simulation of avalanche events caused by fluorescent photons
  - Time jitter modelling
  - Afterpulsing simulation

**Input**

**Output**

**SPAD detector**

- Quenching/recharging circuit model
Poisson flow

\[ P(\mu = n, t) = \frac{\lambda^n}{n!} \exp(-\lambda t) \]

where \( \lambda = \text{DCR} \)
Photon detection probability

Simulation of avalanche events caused by fluorescent photons

Dark counts generation

Time jitter modelling

Afterpulsing simulation

Quenching/recharging circuit model

Input

Output

Wavelength (nm)

Power (a.u.)

Wavelength (nm)

Wavelength (nm)

Power (a.u.)

Power (a.u.)

$PDP = \frac{1}{2} (\text{Fluorescent emission}) + (\text{Excitation light})$
Time jitter

Simulation of avalanche events caused by fluorescent photons

Afterpulsing simulation

Quenching/recharging circuit model

Input

Output

Dark counts generation

SPAD detector

Time jitter modelling

Initial laser pulse

Laser pulse with detector jitter

Power (a.u., log scale)

Time (ns)
Afterpulsing

- by rejection method
- for each fluorescence photon
- according to afterpulsing curve
Quenching/recharging circuit

- **Active quenching**
  - if $E_{i+1} < E_i +$ dead time $\rightarrow$ delete $(i+1)^{th}$ event
  - $E$ can be fluorescent photon detection, dark photon generation or afterpulse

- **Passive quenching**
  - $\Delta t$ between consecutive events
  - $V_i = V_{\text{max}} \exp(-RC / \Delta t_i)$
  - $V_i < V_{th} \rightarrow$ delete $i^{th}$ event

Dark counts generation $\rightarrow$ Simulation of avalanche events caused by fluorescent photons $\rightarrow$ Time jitter modelling $\rightarrow$ Afterpulsing simulation $\rightarrow$ SPAD detector

Input $\rightarrow$ Output

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Measurement technique simulation

- **TCSPC**
  - first photons
  - several photons

- **Time-gating**
  - all photon in dedicated time intervals

- **Modified time-gating**
  - all photons in one observation window (OW)
  - shift OW
Road Map

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Conclusions and future work
Experimental setup

- PicoQuant pulsed laser
- Quantum dots in a micro cavity
- MPD® SPAD
- PicoHarp 300 TCSPC module

Results

Test 1

Time-correlated single photon counting
Modified time-gating

- PicoQuant pulsed laser
- Quantum dots in a microcavity
- FBK SPAD
- Modified time-gating

Results

![Graph showing power vs. time with simulated and measured data.](image)

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Two-chip micro-system

- **Simulation**
  - Single pixel in SPAD array and nearest LED from LED array
  - Passive quenching
  - Backward simulation type

- **Experiment 1:** different LED pulse width
- **Experiment 2:** different setup

- Blue micro-LED
- Quantum dots
- Semrock filter
- CMOS SPAD
- Becker & Hickl TCSPC module
Different pulse width

Experiment

Simulation

• Conclusions:
  – The simulated and measured results are in good agreements
**Conclusions:**

- The simulated and measured results are in good agreements except the peak area.
- The mismatch can be explained by the lack of exact LED time curve used in simulation.
Pile-up effect investigation

- Picoquant pulsed laser 1MHz
- Quantum dots in a micro cavity
- MPD® single SPAD
- PicoHarp 300 TCSPC module
Pile-up with different ambient light

- Simulation of the same setup
- Noise level 1 < Noise level 2 < Noise level 3 < Noise level 4
- Conclusions:
  - the increasing number of counts near the end of TCSPC dead time is due to noise detection before laser pulse
  - higher noise level after TCSPC dead time is afterpulsing
Complete simulation of pile-up

Laser light

Fluorescence decay

- **Conclusions:**
  - These experiments demonstrated the ability of the proposed simulation model to help in explanation of some unexpected results of the real measurements.
• How perfect is the time-filter in comparison to the conventional optical filter?
• What is the influence of the “noisy” pixels on the lifetime estimation?
• How does the OW width affect the lifetime estimation?
Time filtering

0.3 ns SPAD switched-off time

0.25 ns SPAD switched-off time

Fluorescence emission

Excitation light

Power (a.u., log scale)

Time (ns)
Noise characteristic

- 80% pixels ~ 900Hz
- 20% pixels up to 150kHz
- Two time intervals
  - 10 ns and 10 ns
  - 6 ns and 6 ns
- Calculated:
  - Lifetime per pixel
  - Cumulative lifetime
10 ns time intervals

Per pixel and cumulative lifetime

Cumulative lifetime and noise characteristic

- Conclusions:
  - Cumulative lifetime has smaller statistical noise
  - In the case of very high noise per pixel cumulative lifetime starts increasing
  - It is better not to include information from noisy pixels to lifetime extraction
6 ns time intervals

Per pixel and cumulative lifetime

Cumulative lifetime and noise characteristic

• Conclusions:
  – In case of non optimal time interval width the extracted lifetime has worth accuracy
  – Using the average of several pixel improves the accuracy of lifetime extraction
Road Map

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Conclusions and future work
Summary

• A simulator of typical fluorescence measurement experiment has been designed
• It has been verified by real experiments
• The qualitative results are in a very good agreement to the real experiments
• The quantitative simulation is within the limits of accuracy of input parameters and made assumptions
Conclusions

The created model can...

• explain unexpected experimental results
• predict the results of real and planned experiments
• enable the preproduction-stage investigation of SPAD detectors
Future work

- Advanced models of experimental components
  - Fluorescence is not a point source
  - Lenses simulation
- More complex biological system
  - Energy transfer (for FRET)
- Optimization algorithm
- Conversion to a compiled programming language
Thank you