FLuorescence Analysis Speedup to extremely High rates (FLASH)

Ivan Rech[1*], Giulia Acconcia[1], Serena Farina[1], Ivan Labanca[1], Andrea Giudice[2], Massimo Ghioni[1]

[1]Dipartimento di Elettronica Informazione e Bioingegneria, Politecnico di Milano Piazza Leonardo Da Vinci 32, 20133 Milan, Italy
[2]Micro Photon Devices, Via Stradivari 4, Bolzano, 39100, Italy
*Corresponding author: ivan.rech@polimi.it

ABSTRACT

Time Correlated Single Photon Counting is a widely diffused technique used in scientific experiments requiring the analysis of optical pulses with high timing precision. One of the major limitations affecting this tool are distortion phenomena at high count rates. As a result, experiments must be carried out at a slower operating rate than the laser excitation frequency. In this work we present a new detection system that combines single-photon sensitivity, picosecond temporal resolution, and unprecedented high throughput to acquire time-resolved fluorescence images. A similar system holds great promise to have a revolutionary impact on several fields of biology and medicine.

Keywords: TCSPC; SPAD; Single photon; Time resolved.

1. INTRODUCTION

One of the most time/cost-consuming stages of drug discovery is the identification of drug leads. This requires the measurement of hundreds of thousands of samples, which have been treated with varied dosages of a large number of drug leads. Fluorescence Lifetime IMaging (FLIM) widely demonstrated its potential in this field, but it is intrinsically too slow to be extended to a large scale. The main challenge in achieving fast FLIM has been in the lack of high-speed electronics combined with high-performance detectors. In this work we present a new detection system that combines single-photon sensitivity, picosecond temporal resolution, and unprecedented high throughput to acquire time-resolved fluorescence images. A similar system holds great promise to have a revolutionary impact on several fields of biology and medicine.

To overcome the FLIM speed limitation we combine in a single system three key elements:

- a high-performance Single-Photon Avalanche Diode (SPAD)
- fast, picosecond-precision front-end and processing electronics
- a new solution to guarantee zero-distortion at very high speed

In 2017, Cominelli at al. [1] demonstrated that a completely different approach with respect to what is currently used in this field can increase by an order of magnitude the acquisition rate in fluorescence imaging. The proposed solution guarantees zero-distortion at high-rates. Now, several aspects need to be addressed to make this solution available to end users. In particular, high-performance front-end electronics developed on purpose is of the utmost importance. To this aim, we developed a new complete Time Correlated Single Photon Counting (TCSPC) single-channel module providing both the theoretically estimated speedup by almost an order of magnitude with respect to classic pile-up limited acquisition chains, and very high performance. In principle, the same result could be achieved exploiting several channels operating in parallel. Nevertheless, numerous multichannel SPAD-based acquisition systems have been presented in literature so far. Unfortunately, the performance of most of them, especially those featuring a very high number of channels, are quite poor compared to the best results achieved with single-channel systems. Even worse, acquisition speed has not increased accordingly to the number of channels. Therefore, we present for the first time both unprecedented measurement speed and high performance with a TCSPC acquisition system.

Moreover, this approach can be even extended to multichannel systems to reduce the duration of fluorescence measurements by more than two orders of magnitude.

2. STATE OF THE ART

Currently available TCSPC systems suffer from a trade-off between acquisition speed and performance. Concerning single-photon detectors, SPADs have gained a prominent role in this field: they can be fabricated exploiting either the standard CMOS technology [2-4] or fabrication processes developed on purpose, usually referred to as custom technologies[5-7]. To date, best-in-class performance combining high photon detection efficiency, low dark counts and afterpulsing probability, and picoseconds timing jitter have been achieved with custom-technology SPADs. However, the inherent features of custom processes essentially prevent the integration of complex front-end and processing electronics on the same chip. As a result, TCSPC acquisition systems based on high-performance detectors have been limited to a low number of channels so far [8-9]. Unfortunately, these systems provide a low acquisition speed due to pile-up constraints that currently force the acquisition rate to be kept well below the excitation rate (typical range is between 1% and 5%) to avoid distortion. In principle, parallelism can reduce the overall time needed to perform the measurement. Above all, the exploitation of the standard CMOS technology, which allows the fabrication of both SPADs and electronics on the same silicon chip, has been leading to the development of systems featuring hundreds or even thousands of channels [2-3]. This approach, though, is limited by the fact that the detector is fabricated using structures (i.e., tubs and wells) that have been designed to optimize transistors performance; therefore, this approach intrinsically lacks the degrees of freedom necessary to pursue the best detector performance. Recently, other solutions including extra layers in the standard CMOS fabrication process, have been investigated, but the overall performance of these solutions is still mainly limited by the constraints that the use of a single technology for both the detector and the electronics imposes.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

We recently demonstrated the existence of an alternative pathway that no one has ever walked down yet: the acquisition speed of a single channel can be pushed beyond pile-up limits[1]. The FLASH project has translated a theoretical solution into a real compact module that can be used in real applications. To achieve this goal, a new module featuring one main characteristic has been designed: the overall dead time of the system has to be matched to an integer number of excitation periods. The actualimplementation of this solution has required new, high-performance front-end and processing electronics developed on purpose. In this paper we present such electronics for a single-channel module featuring an unprecedented speed and that can benefit of the exploitation of different technologies, each one specifically selected to optimize the performance of a specific part of the system. This paves the way to breaking the existing trade-off between acquisition speed and performance and it is compatible with a future expansion of this solution to a multichannel system pursuing an even higher speed increment.

. When it comes to experiments mainly limited by the measurement time, scaling this time by an order of magnitude means that in 10 years we can obtain results that prior to this couldn’t be obtained in the span of a scientist’s career; scaling this time of even two or three orders of magnitude will allow researchers to obtain the same results in the time span of a year or only of a month.

4. PROJECT RESULTS

Detection head

The core of the module is the detection head shown in Fig.1. A round-shaped Printed Circuit Board (PCB) has been designed to host the detector and the front end electronics. In particular, the SPAD has been placed on a Thermo-Electric Cooler (TEC) to allow a moderate cooling of the device (down to -20°C) with beneficial effects on dark count rate. The front end electronics consists of two separate circuits: first of all, a fast Active Quenching Circuit (AQC) with the structure reported in [10] has been connected to the cathode terminal to provide a finely tunable dead time as short as 12.5ns to match a 80MHz-laser period. Secondly, a fully-differential Pick-Up Circuit (PUC), also presented in [10], has been exploited to extract the timing information. The need of a fully-differential structure relies in the first main challenge of operating a TCSPC channel at high rates. While in a classic TCSPC measurement the impinging rate of photons is so low that is very unlikely to detect a photon nearly the AQC reset transition, the proposed solution aims at working at high speed so this scenario becomes very likely and the current pick-up circuit must be ready to acquire a photon event immediately after the bias conditions have been restored. To minimize the impact of the AQC reset transition, a fully-differential structure has been exploited: a SPAD and a dummy cell, consisting of another SPAD that is always kept off, are both driven by the same AQC. On the other side, the anode of the two sensors is connected to a fully-differential Pick-Up Circuit (PUC).

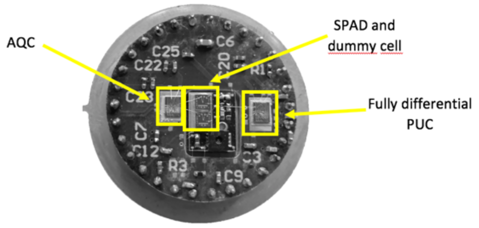

Fig. 1. The detection head of the module hosting a SPAD and a dummy cell, and two integrated circuits: a fast Active Quenching Circuit (AQC) and a fully-differential Pick-Up Circuit (PUC).

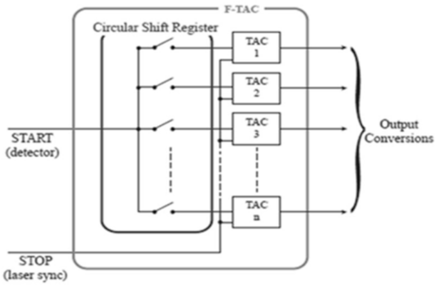

Fig. 2. F-TAC architecture to overcome the dead time limitation

In this way, the disturbance induced by the AQC during the reset results in a common mode signal at the input of the PUC avoiding any triggering of the avalanche current readout electronics. All the bias voltage required by the components hosted by the detection head are generated by means of a dedicated PCB that receives an external 12V power supply. This so-called power PCB also routes the timing signal from the detection head towards the external connector.

Fast time to amplitude converter (TAC)

The time measurement circuit plays a key role in a TCSPC acquisition chain: its precision and linearity set the overall performance of the system. Best in class results have been achieved with a Time to Amplitude Converter (TAC) architecture so far. However, the dead time of a TAC cannot be shrunk indefinitely since a finite amount of time is required at least to charge and discharge the conversion capacitor. To overcome this speed problem we developed a system based on the exploitation of multiple TACs preceded by a shift register : the overall structure, which is called Fast-TAC (F-TAC) and is sketched in Fig.2, requires just one Start signal and a single Stop signal, thus masking to the end user the presence of multiple TACs. The F-TAC architecture can be used in the design of a fast TCSPC acquisition chain to make the dead time of this block negligible. Indeed, it is possible to guarantee that the circuit is always ready to perform a conversion by choosing the number of TACs to achieve an average conversion rate equal to the detection rate of the single photon sensor. It is worth noting that the F-TAC architecture is intrinsically multi-hit: indeed, multiple Start signals are sequentially fed to the internal TACs and the minimum distance between two consecutive Start signals is only limited by the speed of the shift register, which can be in the order of 100ps.

We designed a single channel TAC able to provide excellent performance both in terms of precision and linearity with a Differential Non Linearity lower than 0.25% of the LSB rms (1.5% peak to peak) and a timing precision lower than 10ps Full Width at Half Maximum (FWHM).

Experimental results

Two main features have been analyzed in detail. First of all, we performed an indirect measurement of the reset transition applied by the AQC at the SPAD cathode. We estimated a reset time as short as 1.6ns. It is worth recalling that the theoretical study regarding the possibility to surpass pile-up limitations highlighted the need to discard any photon occurring during the reset[1].

Then, we tested the timing performance of the PUC at the end of the reset time. In Fig. 3 we report the Full Width at Half Maximum (FWHM) jitter of the system as a function of the time distance between the photon event and the end of the reset transition. As can be seen, a residual disturbance produces a light worsening of the timing performance right at the end of the reset transition; nevertheless, the timing jitter is always lower than 54ps.

1. FUTURE PROJECT VISION

In this paper, we proved that it is possible to increase the measurement speed of a single fluorescence acquisition chain by one order of magnitude while keeping zero distortion.

This is only the starting point. Indeed, all the electronics that we presented was conceived to allow the extension of the system to a multichannel structure. By doing so, we would gain another order of magnitude in the measurement speed using linear array, and other two orders of magnitude using two dimensional array.

When it comes to experiments mainly limited by the measurement time, being able to scale this time by two orders of magnitude , means that in few months we can obtain results that prior to this couldn't be obtained in the span of a scientist's career.

This the goal of the next phase of this project.

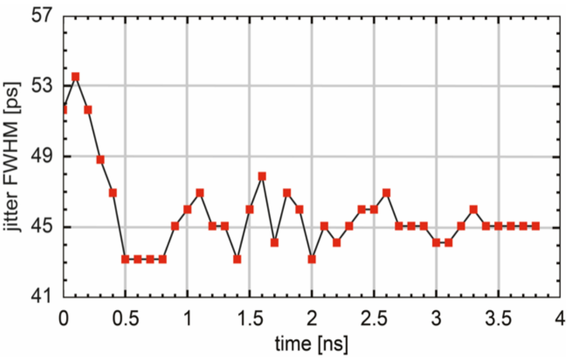

Fig. 3. Full Width at Half Maximum (FWHM) jitter of a thin custom technology SPAD with a fully differential Pick-Up Circuit (PUC) as a function of the distance between the photon event and the reset transition.

1.1. Technology Scaling

Thanks to the Attract project we moved from an initial TRL of 2, related to the theory presented by Cominelli et al.[1], to a TRL of 4 with a technology validated in lab on a complete working system. The next step has a twofold function: on one hand raising the TRL to 7-8 and potentially 9 thanks to the collaboration and interest of company like Micro Photon Devices; on the other hand further pushing the system's performance thus extending the ground breaking nature of this technology.

1.2. Project Synergies and Outreach

The great potential of this work has been recognized by the scientific and industrial community. Giulia Acconcia, the technical manager of this project, was awarded the Young Investigator Award in 2020 presenting the results of the FLASH project at the Biomedical Optics Symposium of SPIE Photonics West. The jury was composed by academic and industrial people and the award was sponsored by the German company Picoquant, that is a leader in the time-resolved data acquisition, single photon counting, and fluorescence instrumentation.

Starting from the success of the phase one, we aim at developing a multichannel system ready to be used on field in a real application.

To achieve such an ambitious goal, we will enlarge the actual consortium including

- Microscopy experts for the optical integration
- Biologists for the real application validation
- Leading company in the field of time-resolved data acquisition to transform it in a real product
- Computer science partner for the data management

We can already count on an extended network strongly engaged in our vision, people with whom we have been working in the frame of previous and current projects including NIH and HFSP grants.

We are just one step away from a revolution in fluorescence measurements.

1.3. Technology application and demonstration cases

The Time Correlated Single Photon Counting (TCSPC) technique is the gold standard in many time-resolved photon-starving applications as single-molecule analysis[11], underwater depth imaging[12], fluorescence lifetime measurements[13], [14] and many others. Along with its many advantages especially in terms of equivalent bandwidth and low noise, TCSPC is also widely recognized as a slow technique. Unfortunately, speed limitations have prevented the exploitation of this technique in many advanced applications so far. Studying cellular functions, for example, is by definition a multi-parameter problem, as in order to understand a molecular process, information from as many parameters as possible needs to be gathered simultaneously and dynamically. The ability to image multiple components in living cells can give key insights into how the right molecules come to the right place at the right time and the nature of the way they interact with each other, giving a base for gaining a better comprehension in medical issues, such as the origin and growth mechanism of tumors. A real technological breakthrough will be having real-time time-resolved and spectral-resolved single-photon cameras to combine for the first time the performance of the best in class single photon detector to the measurement speed required to obtain a real game change in this field.

1.4. Technology commercialization

Thanks to the continuous collaboration with the ex spin-off of the Politecnico di Milano Micro Photon Devices (MPD), the pioneering work in the field of Single Photon detectors by Prof Sergio Cova has been nowadays available to the scientific community for more than 15 years. MPD was a fundamental part of phase 1 of this project and has shown great interest in helping to make this technology a commercial tool available to all research laboratories.

1.5. Envisioned risks

The quality of the silicon foundry is a key element in the final device quality. During the Phase 2 two different foundries will be considered, the one currently used for our SPAD fabrication and a second one that was used to develop state of the art silicon photomultiplier (based on SPAD concept) demonstrating the suitability of this fab for the fabrication of this Single Photon Avalanche Diode. The second foundry, unlike the first one, is located in Europe and this would allow is to bring back to Europe the development of the device, a fundamental element for this project.

1.6. Liaison with Student Teams and Socio-Economic Study

As explained in the ATTRACT kick-off meeting and later reminded, ATTRACT Phase 1 provided the option to all the funded projects for collaborating with MSc. Level student teams. Those teams liaised with the volunteered projects for providing ideas and prototypes inspired by the projects technology for addressing Societal Challenges. This activity will be compulsory to all the projects funded in ATTRACT Phase 2.

This subsection should outline briefly and concretely your actions for enabling this activity (e.g. nominating an experienced person to facilitate MSc. level explanation materials of your technology, etc).

Also, ATTRACT Phase 2 will undertake an expert-driven socio-economic study of the ATTRACT initiative and ecosystem. You should explain as well how you would contribute with information to this study (e.g. interviews, technology impact references, etc).

2. ACKNOWLEDGEMENT

This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222

7. REFERENCES

[1] A. Cominelli, G. Acconcia, P. Peronio, M. Ghioni, and I. Rech, “High-speed and low-distortion solution for time-correlated single photon counting measurements: A theoretical analysis,” *Rev. Sci. Instrum.*, 2017.

[2] D. Bronzi, F. Villa, S. Tisa, A. Tosi, F. Zappa, D. Durini, S. Weyers, and W. Brockherde, “100 000 Frames/s 64 × 32 Single-Photon Detector Array for 2-D Imaging and 3-D Ranging,” IEEE J. Sel. Topics Quantum Electron., vol. 20, no. 6, pp. 354-363, Nov.-Dec. 2014.

[3] R. J. Walker, J. A. Richardson, and R. K. Henderson, “A 128×96 pixel event-driven phase-domain ΔΣ-based fully digital 3D camera in 0.13μm CMOS imaging technology,” Proc. IEEE ISSCC Dig. Tech. Papers, pp. 410-412, Feb. 2011.

[4] S. Burri, Y. Maruyama, X. Michalet, F. Regazzoni, C. Bruschini, and E. Charbon, “Architecture and applications of a high resolution gated SPAD image sensor,” Opt. Express, vol. 22, no. 14, pp. 17573-17589, 2014

[5] A. Gulinatti, I. Rech, F. Panzeri, C. Cammi, P. Maccagnani, M. Ghioni, and S. D. Cova, “New silicon SPAD technology for enhanced red-sensitivity, high-resolution timing and system integration,” J Mod Optic, vol. 59, no. 17, pp. 1489–1499, Oct. 2012.

[6] SPCM, Single Photon Counting Module datasheet, Excelitas, www.excelitas.com

[7] M. Ghioni, A. Gulinatti, P. Maccagnani, I. Rech, and S. D. Cova, “Planar Silicon SPADs with 200 μm diameter and 35 ps photon timing resolution,” presented at the SPIE Defense, Security and Sensing 2006 - Advanced Photon Counting Techniques, 2006, vol. 6372, p. 63720R.

[8] C. Cammi, A. Gulinatti, I. Rech, F. Panzeri, and M. Ghioni, “SPAD array module for multi-dimensional photon timing applications,” J Mod Optic, vol. 59, no. 2, pp. 131–139, Jan. 2012

[9] A. Cuccato, S. Antonioli, M. Crotti, I. Labanca, A. Gulinatti, I. Rech, and M. Ghioni, “Complete and Compact 32-Channel System for Time-Correlated Single-Photon Counting Measurements,” IEEE Photonics J., vol. 5, no. 5, pp. 1–14, Oct. 2013

[10] G. Acconcia, A. Cominelli, M. Ghioni, and I. Rech, “Fast fully-integrated front-end circuit to overcome pile-up limits in time-correlated single photon counting with single photon avalanche diodes,” Opt. Express, 2018.

[11] F. Gaspari, "An overview of cell phenotypes in HCS: limitations and advantages," Expert Opinion on Drug Discovery, vol. 4, no. 6, pp. 643-657, 2009.

[12] J. Lakowicz, "Time-Domain Lifetime Measurements," in Principles of Fluorescence Spectroscopy, New York, Springer, pp. 97-155, 2006.

[13] D. Bronzi, F. Villa, S. Tisa, A. Tosi, and F. Zappa, SPAD Figures of Merit for photon-counting, photon-timing, and imaging applications, Sensors Journal, IEEE, Volume: PP, Issue: 99, 2015.

[14] Y. Sun, R. Day and A. Periasamy, "Investigating protein-protein interactions in living cells using fluorescence lifetime imaging microscopy," Nature Protocols, vol. 6, no. 9, pp. 1324-1340, 2011.