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Development of novel multi-SiPM systems for biomedical applications

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"En ma Fin gît mon Commencement"

"In my end is my beginning"

- Mary, Queen of Scots -

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Introduction

The Silicon Photomultipliers (SiPMs) are the state-of-the-art semiconductor photodetectors with unprecedented photon number resolving capability and single photon sensitivity. They consist of bi-dimensional arrays of hundreds to thousands single-photon avalanche diodes (SPADs), called cells, with typical dimensions between $10\ \mu\text{m} \times 10\ \mu\text{m}$ and $100\ \mu\text{m} \times 100\ \mu\text{m}$. Typical SiPM areas range from 1 to 6 mm². Each cell is operated in Geiger mode avalanche (with an intrinsic gain of the order of 10^6) with a bias voltage of a few volts above the breakdown voltage. The output signal is the same regardless of the number of impinging photons on the cell, therefore each cell is a binary photon counter. Since in a SiPM the output of each SPAD is connected in parallel, the SiPM as a whole is an analogue detector, that can measure the incoming light intensity. They have several advantages over conventional photomultipliers. They are compact, robust and cheap. Moreover, their low operational bias voltage and insensitivity to the magnetic field represent crucial and high potential features in fundamental and applied science. These peculiar characteristics are the basis of the growing interest in this technology. In addition to this, the need to integrate various sensors (from tens to hundreds thousand) to cover large

sensitive areas is becoming urgent and requires compact readout. Application Specific Integrated Circuits (ASICs) can address this issue. In recent years, new ASICs, specifically designed for SiPMs, reach a sufficient mature technological stage to appear on the market. This demand is lead by fundamental physics, high energy physics and astrophysics, but also other fields, as biomedical applications, can benefit from these innovations [30].

The SiPMs was attractive for applications in medicine since the very beginning, in particular in the medical imaging field with the development of hybrid Positron Emission Tomography and the Magnetic Resonance (PET/MR) scanner. It is expected that in future SiPMs can play a crucial role also in dosimetry [31]. With the introduction of novel treatment techniques, the complexity of the actual treatment planning systems increases, requiring comprehensive quality assurance tools and in-vivo dosimetry. This latter significantly impacts on brachytherapy treatments, where sealed radioactive sources are placed inside or close to the tumour through interstitial or intracavitary applications. In brachytherapy, the relative risk of treatment errors is high due to the many steps in the procedures that involve human/manual tasks and because the source activities in use are very high (hundreds of GBq), but the frequency and severity of these errors are largely unknown as there are limited in-vivo dosimeters available to identify and report errors [32]. Nowadays, optical fibre based dosimeter readout with SiPMs represents the most promising technology for brachytherapy in-vivo dosimetry, thanks to their small dimensions (usually maximum 1 mm of core), flexibility and being lightweight.

Biophotonics, methods for the investigation of biological systems using the emission, scattering or absorption of light, represents another field where the potentiality of SiPMs can be exploited in the next years. The availability of on-purpose kits for the quantification of various metabolites has shifted the range of application from the academy to industry, increasing the panel of potential users and calling for the development of

simpler, stealthier and more flexible detection systems. Since the combination of light emission properties (mainly single photons) and the concentration range (some orders of magnitude) of metabolites sets stringent requirements on the detection system, nowadays the impact of SiPMs is still marginal, but their compactness, cost-effectiveness and immunity to damage from light overexposure, can address the needs with systems (possibly portable) that ensure rapid detection and examination of small volumes of biological samples at low cost [33].

In this Ph.D. work, the possibility to implement SiPM based systems in biomedical applications is presented. In particular, the development and characterisation of an in-vivo dosimeter, for brachytherapy treatments, and a platform for biological measurements, able to monitor the calcium gradients in living cells, are described. The former was carried out as part of the European project ORIGIN, within the HORIZON 2020 framework programme. It aims to address the urgent need to provide real-time in-vivo dose imaging methods, by the development of a new optical fibre based sensor systems (exactly 16 fibres) to support diagnostics-driven therapy through brachytherapy. The biological system was developed in collaboration with a group of biophysicists at the University of Piemonte Orientale. It aims to determine the requirements for the detection of bioluminescence, i.e. light emission due to chemical reactions in the biological sample. In particular, studies on the capability to investigate, through bioluminescence, the calcium gradients, related to the metabolism and the ability of cells to respond and adapt to the external stimuli, were carried out and published in [28, 34].

Each of the above mentioned topics will be examined in this thesis, that can be divided in three sections according to the following order. The first one presents an overview of SiPMs, from their operating principle to their main application fields (chapter 1) and a characterisation of the ASIC CITIROC1A, by Weeroc, for SiPM readout (chapter 2). This latter will be

exploited in the final prototypes as the front-end of choice for all the systems described in this thesis. The main features of this ASIC are qualified, in relation to its use for the different applications, through a new readout board, developed by CAEN S.p.A. and Nuclear Instruments s.r.l.. Then the integration of SiPMs in specific application systems is reported in the other sections. Firstly, the development of a brachytherapy dosimeter is described. Chapter 3 gives an overview of the brachytherapy techniques and dosimetry systems, specifically for prostate and cervical cancers (subject of the ORIGIN project). Then, since the proposed dosimeters embed scintillators, i.e. materials that emit light when excited by ionizing particles, the physical properties of these materials are qualified in chapter 4. Finally, chapter 5 reports the characterisation of the dosimeter in a clinical environment, exposing one fibre to an ^{192}Ir brachytherapy source at Ospedale di Circolo e Fondazione Macchi in Varese (Italy). The last section (chapter 6), after a brief introduction about the importance of calcium in the cell metabolism, presents the performances of a SiPM based system for the bioluminescence detection.

SiPM: Silicon PhotoMultipliers

Between the late '80s and early '90s, a new class of photodetectors, called *Silicon Photomultipliers* (SiPMs), also known as Multi-Pixel Photon Counters (MPPCs), was developed, tested and eventually patented (Russian patent number 1702831). Nowadays, this new technology represents the most competitive alternative to the usual photomultipliers (PMTs) for light detection in several applications.

Their rapid evolution and successful use in a waste range of applications are based on previous studies about Single-photon Avalanche Diodes (SPADs), carried out from from the '50s to the '80s (an exhaustive overview of SPADs applications can be found in [35]). Furthermore, they take advantage of the rapid evolution of the Silicon technology and the investment of different companies in terms of cost production, design flexibility and high-quality mass production. In general, the principal features of these detectors are: high internal gain (at the level of 10^6), single-photon sensitivity, high photon detection efficiency, proportional response to weak and fast light flashes, excellent timing resolution, low bias voltage, ruggedness and insensitivity to magnetic field [36]. They lead to an increasingly widespread use of SiPMs in varied fields of application: from the fundamental research (as high energy physics) to industrial application (as LIDAR-Light Detection and Ranging systems).

1.1 Basic operating principle

SiPM consists of a bi-dimensional matrix of independent SPADs with densities up to 10^4 per mm^2 , arranged on a common substrate and joint together in parallel to a single output [35, 37]. Each SPAD usually is referred to as cell or pixel and its dimension can vary from $10 \times 10 \mu\text{m}^2$ to $100 \times 100 \mu\text{m}^2$ (figure 1.1). Each cell is operated in Geiger-Müller regime and is biased at few volts above its breakdown voltage V_{bd} (30-100 V depending on the producer). V_{bd} is defined as the voltage where the multiplication factor, i.e. the number of secondary carriers produced per each primary one diverges [38]. In such conditions, due to the very high electric field (few 10^5 V/cm), a single charge carrier, generated into the depletion layer by photon absorption or by thermal excitation, can trigger a self-sustaining avalanche with a gain of about 10^6 . The current increases rapidly to a macroscopic level. It would continue to flow until the avalanche is quenched, through quenching resistor R_q , by lowering the bias voltage to or below the breakdown voltage. The bias voltage must then be restored to enable the cell to detect another incoming photon (reset phase).

In Geiger-Müller regime, the SPAD gives approximately the same signal, regardless of the number of photons that have triggered a discharge, operating as a binary device. Otherwise, SiPMs work as analogue detectors. In the analogue Silicon Photomultiplier, the information, related to the light intensity, is retrieved connecting the independently operating cells, through quenching resistors, to the same output and obtaining a signal proportional to the number of all the single SPAD discharges that occurred. Alternatively, in the digital Silicon Photomultipliers each SPAD is integrated with conventional CMOS circuits on the same substrate and is connected to its own readout electronic circuit. They provide the digital counting of the number of photons and the precise detection time, making the sensor less susceptible to temperature variations [39]. Both configura-

tions allow the SiPM to detect and count photons with good resolution and with single-photon sensitivity [40, 41].

SiPMs are produced with different cell size (pitch), typically ranging from $10\text{ }\mu\text{m}$ to $100\text{ }\mu\text{m}$, shown in figure 1.1, even if some new studies on SiPMs with a pitch of $5\text{ }\mu\text{m}$ are carried out by Fondazione Bruno Kessler (FBK) [42]. Arranging these single cells, SiPMs areas from $1\times 1\text{ mm}^2$ to $6\times 6\text{ mm}^2$ are available. Moreover, to cover larger areas, arrays up to $24\times 24\text{ mm}^2$ (16 SiPMs with an area of $6\times 6\text{ mm}^2$ connected with Through Silicon Via interconnection technology) [43]. In more detail, as sketched in

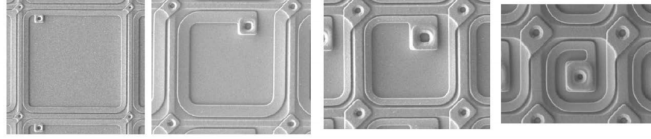


Figure 1.1: Microphotograph of different cell sizes. From left to right: 50, 25, 15 and $10\text{ }\mu\text{m}$ [1].

figure 1.2, a SiPM cell is made of an abrupt junction either n^+/p or p^+/n (in 1.3 the differences between these two types are shown). The n^+ layer has a thickness of about $0.1\text{-}1.5\text{ }\mu\text{m}$ and collects the impinging photons entering through a window (usually covered by an anti-reflective coating). The p -side consists of three layers with different doping levels to tailor the field distribution across the structure (as shown on the right of figure 1.2), when a reverse bias is applied. The top part of the field is the multiplication region (active area of the sensor), while the bottom part is the drift region. The conditions for Geiger mode discharge take place in the multiplication region. In particular, it is originated in the thin depletion region ($0.7\text{-}0.8\text{ }\mu\text{m}$) between the n^+ and π layers, where a high electric field ($3\text{-}5\cdot 10^5\text{ V/cm}$) is created by the dopant profile of the low doped side of the junction. The drift region is localized between the high-field region and the backside contact which has a highly doped silicon substrate. It drifts

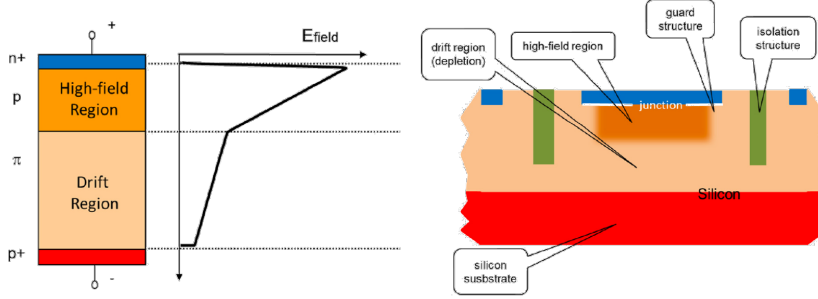


Figure 1.2: Doping structure of a SPAD in n+/p configuration with the electric field generated in operation mode (left). Schematic cross-section of a single cell of a SiPM (right) [2].

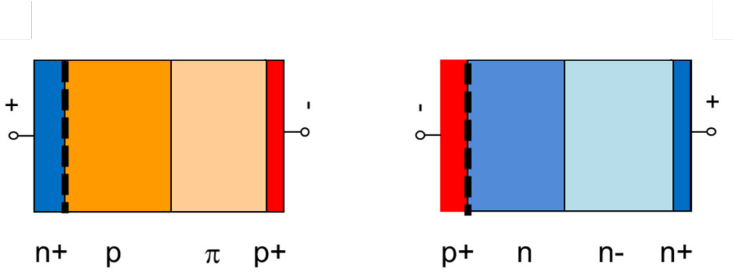


Figure 1.3: The two possible SiPM configurations: n+/p (left) and p+/n (right) [2].

the generated electrons to the multiplication region [2, 44].

1.1.1 Electrical model

The equivalent circuit, depicted in figure 1.4, models the operation principle of a SiPM. Figure 1.5 shows the currents flow through the switch I_{INT} and externally I_{EXT} during the discharge of a SPAD.

The avalanche photodiode is modelled as the parallel connection between the resistance of the avalanche region R_S (from a few hundred Ω to various k Ω [35]) and the junction capacitance of the inner depletion layer C_D . Each cell has in series a quenching resistor R_Q , which works as a fast

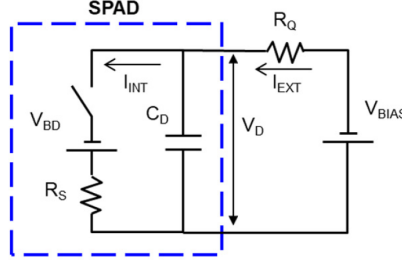


Figure 1.4: The equivalent circuit of a SPAD with the quenching resistor and the external bias. The dashed rectangle represents a SPAD operating in Geiger-Müller regime [2].

discharging path for the charge delivered during a trigger event [45, 46]. The pre-breakdown state corresponds to the switch in OFF condition (the switch is open), with C_D charged at the operating voltage $V_{bias} > V_{BD}$. No currents flow in the circuit in this condition. When a carrier passes through the high field region, it may initiate an avalanche with probability P_t . If the avalanche starts at $t = t_0$, the switch is closed and the system is in the ON condition. The capacitance C_D discharges to V_{BD} through the resistor R_S . The current flowing through the switch (I_{INT}) decreases exponentially with a time constant of $t_D = R_S \cdot C_D$ (typically ~ 100 ps), while the external current I_{EXT} increases with the same time constant, tending to the asymptotic value of $(V_{bias} - V_{BD}) / (R_Q + R_S)$ [45]. At this state, the avalanche may be quenched with a certain probability P_q at $t = t_1$. I_{INT} suddenly goes to zero, while I_{EXT} starts reducing exponentially with the time constant $t_R = R_Q \cdot C_D$. The electric field in the depletion region becomes too weak to maintain the Geiger mode and the self-sustained avalanche is quenched. The switch returns in OFF position and the capacitance, now charged at V_{BD} , starts recharging to the bias voltage V_{bias} with the time constant t_R . This characteristic time is defined as cell recovery time and, during this period, the cell is not sensitive to other impinging photons.

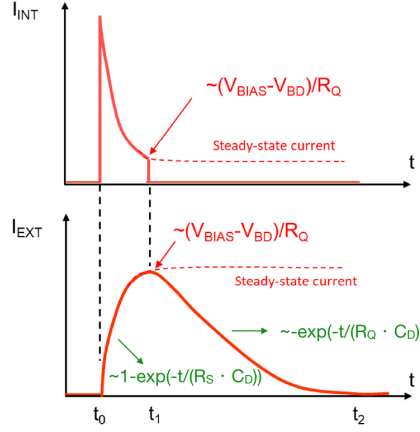


Figure 1.5: The description of the currents flowing in a SiPM cell during the detection process [2].

The total recovery time ranges between 20 and 250 ns, accordingly to the quenching resistor and to the cell capacitance.

The two parameters that dominate the detection process are the triggering (P_t) and quenching (P_q) probabilities. P_t corresponds to the percentage of the carriers produced by photons that will generate a Geiger avalanche. It is strictly related to the detection efficiency of the sensor, as described in section 1.2.2. On the contrary, P_q is defined as the mean duration of the steady-state current when the switch is closed [47]. If R_q is high enough (typically hundreds of k Ω), the current is so low (below 10-20 μ A) that statistical fluctuations reduce the number of carriers reaching the high-field region and the duration of the steady-state current, quenching the avalanche [2, 36, 48]. Finally, the SiPM current signal is the sum of the overall I_{EXT} rising from each fired SPAD. Its amplitude and charge are proportional to the impinging light intensity.

1.2 Basic operating parameters

A close relation exists between the SiPM parameters and its performances. In particular, three operating parameters are strictly related to the implemented technology and have to be carefully evaluated in every system development, depending on the different applications. They are the breakdown voltage, the gain and the Photon Detection Efficiency.

1.2.1 Breakdown voltage and SiPM gain

The breakdown voltage V_{BD} is a crucial parameter for SiPMs operation. It represents the minimum bias above which the SPAD works in Geiger-Müller regime. Moreover, it can be identified as the voltage where the reverse current-voltage curve starts increasing very sharply.

The internal structure of the cells and the doping of the junction affect the value of V_{BD} [49]. For instance, thicker depletion regions result in higher values of V_{BD} . In general, thinner depletion regions reach more reproducibility in terms of breakdown voltage [50]. Another influencing parameter is the temperature. The dependence on temperature is explained by the process of ionization: each carrier in the high field is accelerated and has a certain probability of hitting an atom in the reticle. In case of a collision, a minimum energy to generate a second e-h pair exists. At increasing temperature, the rate of collision rises and, thus, the mean free path and the energy of accelerated carriers decrease. Under these conditions, the effect is an increase of the critical electric field, needed to generate a self-sustained avalanche, resulting in a higher breakdown voltage [51, 3], as reported in figure 1.6(a).

The breakdown voltage deals with the gain of SiPMs. The gain G is the charged produced in a single avalanche, stored in the cell capacitance C_D :

$$G = \frac{C_D(V_{bias} - V_{BD})}{q} = \frac{C_D \cdot V_{ov}}{q} \quad (1.1)$$

where $q = 1.602 \cdot 10^{-19} C$ is the elementary charge, while V_{ov} is called over-voltage and it is equal to the difference between the operating bias voltage V_{bias} and the breakdown voltage. Since the overvoltage is of the order of few volts and C_D is ~ 50 fF, the gain usually ranges between 10^5 and 10^7 electrons [36, 52]. Figure 1.6 shows the SiPM gain as a function of the operating bias voltage at different temperatures. At a given temperature, G has a linear dependence on the V_{bias} and, consequently, the V_{BD} can be defined as the voltage at which the gain is zero. Furthermore, due to the linear proportionality with V_{BD} , the gain decreases with increasing temperature. At

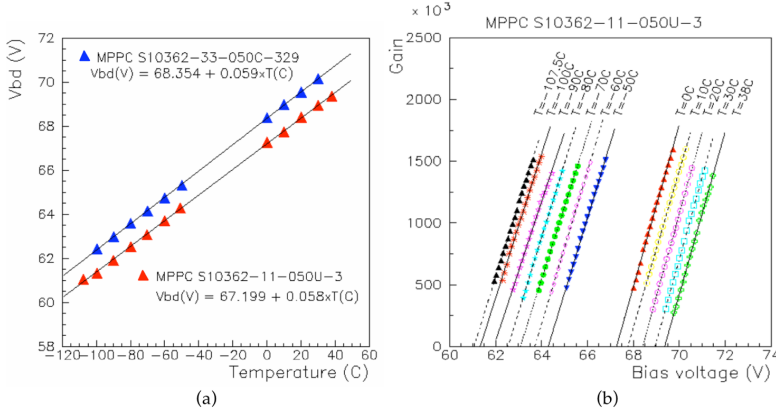


Figure 1.6: (a): The temperature dependence of V_{BD} measured in two Hamamatsu SiPMs 1×1 and 3×3 mm² with a temperature coefficient of $\sim 58.5 \pm 0.5$ mV/ $^{\circ}$ C for both sensors. (b): The G as a function of V_{bias} for the 1×1 mm² SiPM at 12 different temperature [3].

a given overvoltage, higher G can be reached with bigger SiPM cells, since they have larger capacitances.

This high gain assures a very good photon number resolving capability. This capability is well portrayed by the charge spectrum, called multiphoton peak spectrum, reported in figure 1.7. The entries of this spectrum correspond to the signal integral over a certain time window synchronous

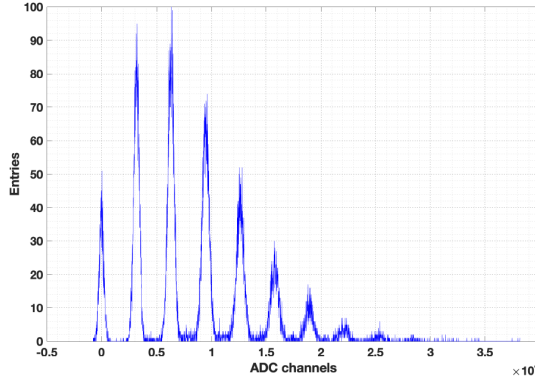


Figure 1.7: Multiphoton peak spectrum: response of a Hamamatsu MPPC 13360-1350CS, illuminated by a LED, biased at +3 V of overvoltage at 21°C.

with the light pulse, produced by a LED. The well-separated peaks are the different number of fired cells, occurring with a probability associated at the first order to the light intensity fluctuations. The peak-to-peak distance Δ_{pp} is related to the single cell gain and, thus, it grows linearly with V_{ov} [8]. Moreover, it represents the parameter that allows to convert the digitized charge ($ADC_{channels}$) in number of photoelectrons (phe) as:

$$phe = \frac{ADC_{channels} - pedestal}{\Delta_{pp}} \quad (1.2)$$

The lowest peak corresponds to no detected photons and its width σ_0 provides a measurement of the electronic noise. The widths (σ_i) of the other i -peaks are described by the equation [52]:

$$\sigma_i^2 = \sigma_0^2 + i \cdot \sigma_G^2 \quad (1.3)$$

where σ_G^2 takes account of the gain fluctuations. Indeed, although the high uniformity in structure production, the avalanches generated in different cells can reach differences of about 10% in the number of carriers generated [53]. The achievable resolution capability can be expressed through the ratio:

$$\frac{\sigma_G}{G} = \frac{\sqrt{\sigma_1^2 - \sigma_0^2}}{G} \quad (1.4)$$

Possible V_{BD} drifts due to temperature variations limit the device performances, shifting the distance between the peaks of the spectrum and reducing the resolution capability of the system [3]. To control these effects, a cooling system (Peltier) of the sensor can be implemented encapsulating the sensor in vacuum. This technique requires space because it introduces a distance between the sensor and the light source [1]. Another possibility is a correction of the operating bias (and thus of V_{ov}), in order to keep constant the gain. In this case, a continuous temperature measurement can be performed placing thermistor pins as close as possible to the sensor [54, 55].

1.2.2 Photon detection efficiency

The Photon Detection Efficiency (PDE) quantifies the probability that a SiPM produces an output signal in response to an incident photon, i.e. the percentage of detected photons out of the impinging ones [36]. It can be defined as the product of three different terms [44]:

$$PDE(\lambda, V_{ov}, T) = QE(\lambda) \cdot P_t(\lambda, V_{ov}, T) \cdot FF \quad (1.5)$$

where QE is the quantum efficiency, i.e. the probability for an impinging photon to create a primary electron-hole pair in the active volume; FF is a geometric factor, called fill factor, that corresponds to the ratio of the sensitive area to the total area; P_t is the avalanche triggering probability. Except for the geometric factor, all others depend on the photon wavelength and the V_{ov} , as described below. The influence of each of these on the PDE is sketched in figure 1.8: FF is wavelength independent, giving a first constant reduction; The second wavelength-dependent decrease is related to the QE. Finally, the last reduction is related to P_t , which is voltage-dependent at higher λ , where it is still growing with the V_{ov} .

¹An example of a SiPM with a Peltier system can be found at: <https://www.hamamatsu.com/eu/en/product/type/S13362-3050DG/index.html>

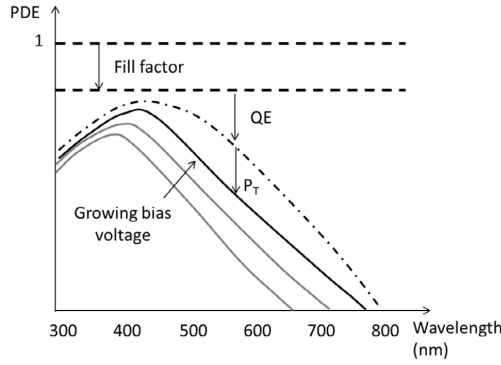


Figure 1.8: Schematic representation of the SiPM PDE, highlighting the influence of each terms of the PDE formula [1.5](#) [\[2\]](#).

Quantum Efficiency

The QE takes into account two different phenomena: the probability for a photon to cross the anti-reflective coating and the probability for a photon to generate a carrier that can initiate a discharge in the high field zone. The first factor should be optimized according to the application, while the second is related to the absorption depth of photons in Silicon. The behaviour of the PDE is dominated, in general, by the photon absorption depth in Silicon. The latter increases with the wavelength as shown in figure [1.9](#) and ranges from 0.01 to few μm for wavelengths $300\text{ nm} < \lambda < 700\text{ nm}$ [\[36, 56\]](#). Depending on the application, the SPAD structure should be designed accordingly to the photon wavelength. On one hand, in case of short wavelength, the drift region can be removed and a proper design of the anti-reflective coating is fundamental. Indeed, as reported in [\[57\]](#), adjustments of the layers made of SiO_2 and Si_3N_4 are necessary because these materials absorb significantly for $\lambda < 250\text{ nm}$. On the other hand, in case of long wavelength, the drift region thickness should be adequately increased to absorb the photons. At 850 nm , e.g., the absorption length in Silicon corresponds to $\sim 18\text{ }\mu\text{m}$ [\[4, 57\]](#). This solution, however, implies

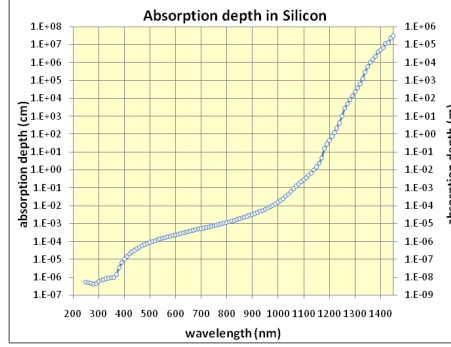


Figure 1.9: The absorption depth as a function of wavelength measured at 300 K [4].

distortions of the electric field that can be accounted for in the fill factor (increasing the dimension of the cell according to 1.5). Another possibility is to place a mirror on the opposite side of the entrance window, reflecting back the light [57].

Although SiPMs have high QE (close to 100% for optical photons, like other silicon-based photodetectors), the overall PDE is smaller because of other additional contributions, described below.

Avalanche Triggering Probability

The triggering probability P_t is strictly correlated with the probabilities for electrons ($P_e(x)$) and holes ($P_h(x)$) to trigger an avalanche at a certain position x in the detector. In a photo-generation, the two carries e-h are generated and travel in the opposite directions. Their different contribution to P_t are combined as following [48]:

$$P_t(x) = P_e(x) + P_h(x) - P_e(x)P_h(x) \quad (1.6)$$

The two probabilities $P_e(x)$ and $P_h(x)$ depend on the the ionization rate that is always higher for electrons than holes (roughly a factor two with a high field of $5 \cdot 10^5$ V/cm [58]). Moreover, $P_e(x)$ and $P_h(x)$, consequently P_t ,

increase with the electric field, thus with the overvoltage, and their difference decreases with increasing field [45, 48]. The figure 1.10 shows $P_e(x)$, $P_h(x)$ and P_t as a function of interaction depth x both for n+/p and p+/n junction configurations. The maximum of P_t corresponds to the drift region, in the n+/p configuration, and to the adjacent region to the junction within the p+ layer, in p+/n one. For instance, considering a n+/p type

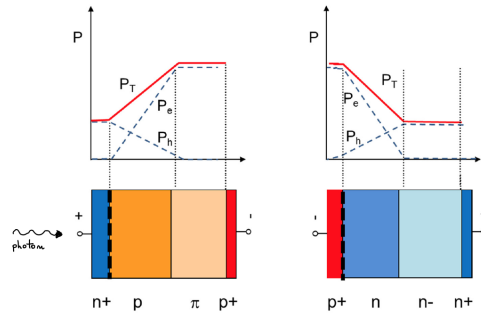


Figure 1.10: Triggering probability P_t as a function of the cell depth x for both configurations n+/p (left) p+/n (right) [2].

with constant high electric field in the p-side, if a pair is created in the n-side, the electron is immediately collected at the n+ terminal and only holes can trigger the avalanche. Symmetrically, if the pair is created in/near the p+ layer, there is only the contribution of P_e . Otherwise, if the generation occurs in the central high field region, both carriers contribute to P_t [45, 48].

On one hand, in case of blue/UV light, both junction types can be adopted because the photon absorption occurs within 500 nm. In a n+/p configuration the n+ layer thickness has to be as thin as possible, due to the absorption very close to the surface [56]. With a p+/n, otherwise, the electrons generated close to the p+ layer enter immediately in the electric field where P_t is maximum, as shown in figure 1.10, and trigger an avalanche. On the other hand, with long wavelengths, a n+/p junction is always preferable because, due to the high absorption depth, all the photo-

generations happen in the drift region.

Fill Factor

The fill factor FF represents the ratio between the active area and the total area of the sensor. This geometrical factor accounts for the fact that each cell in the SiPM has some dead areas on its periphery: the quenching resistor, the guard ring (figure 1.2), which is necessary for reducing quickly the electric field at the borders, and the isolation structure, required for optical and electrical isolation between cells (figure 1.11). Among the isolation

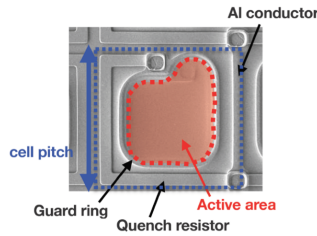


Figure 1.11: An example of a SPAD structure for a $25\ \mu\text{m}$ cell pitch SiPM. The active area is highlighted in red [5].

structure, the trenches are worth of mentioning. They consist in narrow and deep cut off region, surrounding the SPAD, and filled with dielectric material. The importance of these structures are well explained in section 1.3.2. Studies [5, 59] on the materials used for the quenching structures progress, allowing reaching higher FF (also up 90% for $100\ \mu\text{m}$ of pitch) as reported in figure 1.12. A higher FF results in higher PDE and gain, but also in higher capacitance, longer recovery time and less dynamic range (less cells in the same SiPM area), suitable when the application deals with a low number of photons.

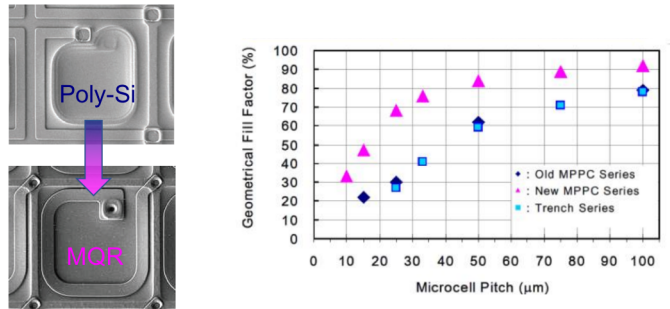


Figure 1.12: Left: Adopting new technologies as the Metal Quenching Resistor, the FF can be improved. Right: The FF is represented as a function of the cell pitch for Hamamatsu S10362 and S12571 series with and without the optical trenches [6].

1.3 Stochastic Effects

An ideal detector produces identical a signal proportional to the number of photons. First of all, as just discussed, the cell-to-cell gain variation introduces signal fluctuations. Furthermore, various sources of noise degrade the ideal signal. These sources are:

- the dark counts, i.e. the *primary noise*. This identifies the avalanches triggered by thermal generation or tunnelling in the high field region;
- the *correlated noise*: all the avalanches triggered subsequently to a primary event (a signal or a primary noise event). This secondary generation is due to the so-called optical crosstalk (inside neighbouring cells of the primary event) and afterpulse (inside the same cell of the primary event) effects.

1.3.1 Primary noise: dark count rate

The principal source of noise, which limits the single photon resolution, is the Dark Count Rate (DCR). It can be approximately determined by counting all the generated pulses by thermal generation or tunnelling in a time interval Δt . The dark events occur randomly and independently from each other. They follow a Poissonian distribution with a mean number of events in Δt equal to $\mu = \Delta t \cdot DCR$. Since SPADs in a SiPM are connected in parallel, the noise of the SiPM is the sum of the DCR of each SPAD. At room temperature, the thermal generated free carriers in the bandgap, i.e. the Shockley–Read–Hall (SRH) generation–recombination effect, dominates the DCR [7]. Furthermore, the impurities and the crystal defects mediate the SRH effect: higher the defect concentration, lower the lifetime of carriers and thus higher the DCR. When a high electric field is applied, the SRH effect is enhanced and the rate increases due to the tunnelling (FE-SRH condition). Finally, minority carriers can be generated also in the neutral regions, diffuse to the depleted one and trigger an avalanche [2]. As reported in figure 1.13, two operation parameters affects the DCR: the overvoltage and the temperature.

In a pure SRH condition (that dominates in the drift region of the junction), the rate of dark events increases with increasing V_{ov} because the triggering probability is higher. When P_T approaches 1, the DCR should remain constant, instead it continues growing due to the tunnelling effect. The DCR decreases exponentially with temperature from a few hundreds of kHz/mm² (at room temperature) to tens of Hz/mm² or KHz/mm² (at 200 K), depending on the producers. A factor of 2 every 8-10° was measured [3]. Each effect presents a different dependence on temperature, as described in figure 1.14. On one hand, the tunnelling shows the weak dependence with T , but it is the dominant factor to the DCR at low temperature. On the other hand, at higher temperature, the diffusion current in

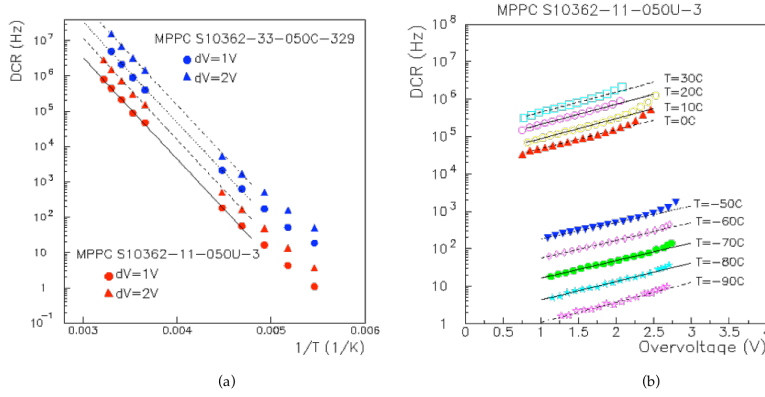


Figure 1.13: (a): The DCR variation with T at two different constant V_{ov} for the SiPMs area of $1 \times 1 \text{ mm}^2$ and $3 \times 3 \text{ mm}^2$. (b): The DCR trend versus V_{ov} for different T for the a SiPM with an area of $1 \times 1 \text{ mm}^2$ [3].

neutral regions becomes more significant. In order to work with low DCR

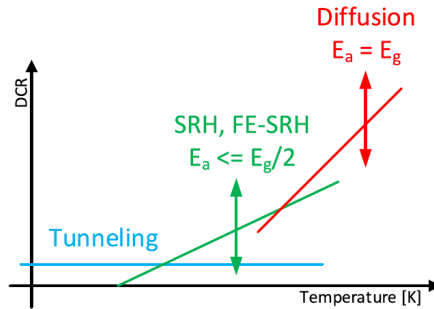


Figure 1.14: The dependence of dominant effects in the generation of dark events as a function of the temperature [2].

values, the SiPM should be operated at low V_{ov} and temperature, employing small sensitive area. Nowadays, the DCR values range usually at room temperature between $\sim \text{MHz}$ for $6 \times 6 \text{ mm}^2$ sensors to some tens of kHz for $1 \times 1 \text{ mm}^2$ ones.

1.3.2 Optical Crosstalk

Since 1955 it is known that during each primary avalanche, secondary photons are generated with energies between 1.15 and 1.4 eV or more [60, 61]. The emission of these photons is believed to take origin from transitions of hot electrons in the conduction band. Their intensity is of the order of $1\text{-}5 \cdot 10^{-5}$ photons per charge carrier, that corresponds to 10-50 visible photons, on average, considering a SiPM gain of 10^6 [60, 7]. The photons are emitted isotropically and can reach a neighbouring cell and initiate a new avalanche. This phenomenon is defined as optical crosstalk (OCT). Because it is a correlated noise, it is possible to classify two types of OCT on the basis of the inter-arrival time between the OCT and the primary event: “prompt” or “direct” optical crosstalk (DiCT) and “delayed” crosstalk (DeCT) [7]. The differences between these types are depicted in figure 1.15. In case of prompt crosstalk, the absorption and subsequent

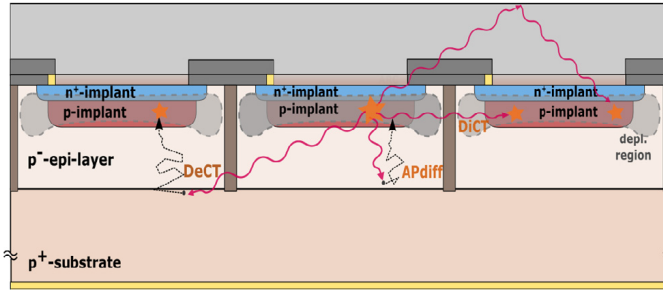


Figure 1.15: Sketch of the correlated noise types: delayed (DeCT) and direct crosstalk (DiCT), afterpulsing optically induced and/or diffused (APdiff) and external crosstalk [7].

avalanche occur in the active area of a nearby cell. The inter-arrival time is of the order of ps and, with typical electronics, the two avalanches are considered instantaneous. The SiPM output results in a signal with a double or higher amplitude, even though the primary event is only one. In

case of the delayed crosstalk, instead, the optical photons are absorbed inside the neutral region of another cell. The carriers generated can reach the depleted zone by diffusion and then enter the high electric field towards the drift region. Both these processes (diffusion and drift) require more time to happen, thus the primary avalanche can be distinguished from the correlated one. Finally, it is worth of mentioning the possibility that photons exiting the SPAD and then reflecting back in another cell, can trigger a secondary avalanche. The reflection could occur due to the presence of external reflectors as the top surface of the resin or the glass layer, placed to protect the SiPM surfaces from external stresses (“external” crosstalk).

The probability of having crosstalk in a given SPAD is proportional to:

- the gain of the SPAD, i.e. the number of carriers crossing the high field region;
- the emission coefficient of photons by the hot carriers ($1\text{-}5 \cdot 10^{-5}$);
- the optical collection efficiency in the second SPAD;
- the P_T of photons to trigger an avalanche.

Since the probability of crosstalk depends on the gain and P_T , it increases at higher overvoltage, as reported in figure [1.16](#). Moreover, a dependence on the cell size is observed: with higher pitch cell at the same gain the OCT probability is lower, since the generated photons have to travel a higher distance, on average, before hitting a neighbouring SPAD. For an ideal detector, the probability of two or more simultaneous thermal excitations should be negligible such that without correlated noise DCR has an average amplitude equal to one photoelectron. However, for a real SiPM, the OCT induces avalanches resulting in higher amplitude pulses, leading to a slower drop in the DCR. The procedure to determine the crosstalk is based on the analysis of DCR events. The amplitude of the SiPM signal in dark condition is discriminated at different voltages. For each set threshold of

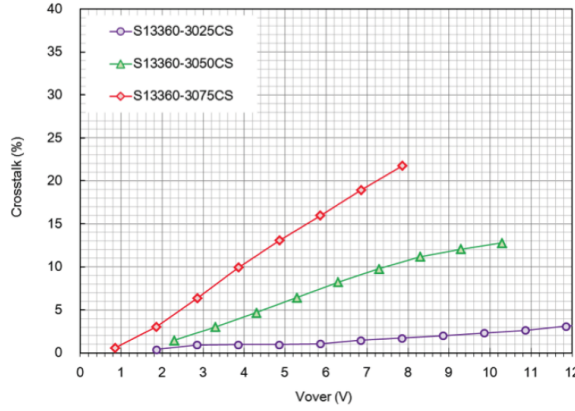


Figure 1.16: OCT probability as a function of V_{ov} for Hamamatsu SiPM series 13360 with three different pitch: 25, 50 and 75 μm [1].

the discriminator, the number of signals above the threshold is counted for a fixed time interval, forming the typical “staircase” (figure 1.17). The DCR exhibits a first plateau, corresponding to the fraction of pulses with an amplitude exceeding half the signal of a single Geiger discharge ($\nu_{0.5phe}$). Subsequent drops appear each time the applied threshold is higher than the value of the signal amplitude corresponding to a finite number of photoelectrons [8, 53, 39]. By comparing the event rate above one photoelectron ($\nu_{1.5phe}$) threshold with the total dark rate measured $\nu_{0.5phe}$, an estimation of OCT probability corresponds to:

$$OCT = \frac{\nu_{1.5phe}}{\nu_{0.5phe}} \quad (1.7)$$

A significant suppression of OCT phenomenon can be achieved, making the active area as thin as possible, compatibly with the wavelength². Another solution, more adopted by a lot of the producer, relies on the implementation, in the active area, of deep trenches surrounding each SPAD [56]. The trenches are filled with materials that reflect or absorb light.

²This can be a good solution for UV light detection

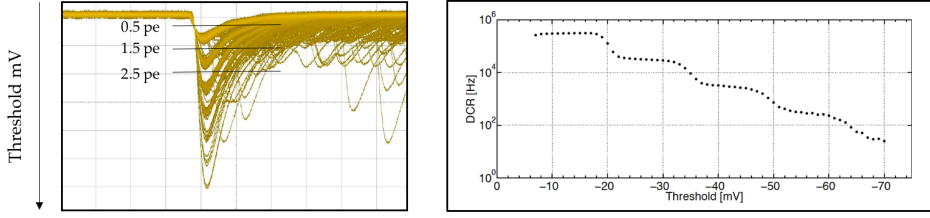


Figure 1.17: Response of a SiPM Hamamatsu MPPC S10362-11-100C illuminated by a light pulse (left). The DCR as a function of the discriminator threshold (right) [8].

Moreover, to reduce also the delayed crosstalk, the trenches can be extended up to the neutral region or a substrate with short diffusion length, i.e. carrier low lifetime, can be used [2, 41].

1.3.3 Afterpulsing

The second type of correlated noise is the afterpulsing (AP). During a Geiger discharge many carriers are generated and some of them can be trapped in the high-field region. The trapped carriers eventually are released and trigger an avalanche within the same cell as the original avalanche, but delayed in time. The delay spreads from ns up to a few of μ s. The correlated pulse has an amplitude (A_{AP}), that depends on the time delay and the recovery state of the involved cell. Since the diode voltage is still increasing towards V_{bias} with the time constant t_R (recovery time), the amplitude of afterpulse exhibits an exponential dependency (as presented in figure 1.18):

$$A_{AP}(t) = A \cdot (1 - e^{-t/t_R}) \quad (1.8)$$

where A is the amplitude of a primary single fired cell. Moreover, several studies demonstrate that multi-exponential law describes the AP event time distribution from the first one, since different trap levels exist in the energy bandgap of Silicon [52, 9]. Since the probability density of carriers

re-emission is exponential in time, most of them are released immediately after the primary avalanche. The AP probability is proportional to the car-

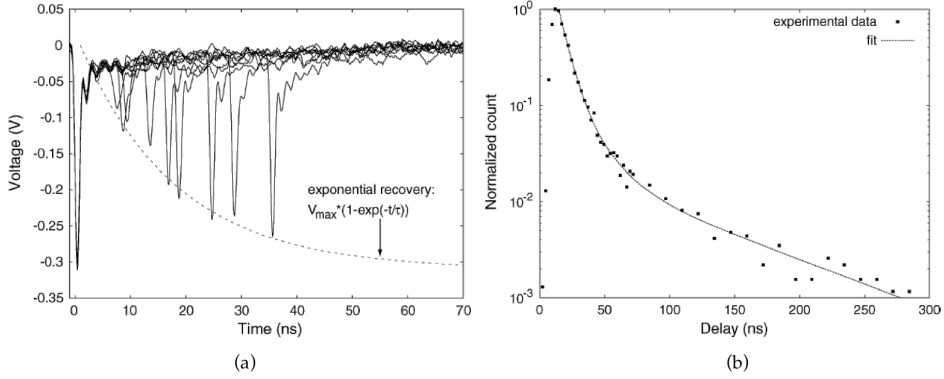


Figure 1.18: (a): Afterpulse examples, showing the exponential dependency of the amplitude at different delays in respect of the primary signal. (b): Spectrum of the delay time from the primary pulse to the afterpulse event, showing three different decays, corresponding to different trap levels in the energy bandgap [9].

riers (i.e. to the gain) and the triggering probability. Therefore it grows super-linearly (roughly as the second power) with V_{ov} . At room temperature and down to 110 K, no temperature dependence on AP probability is observed; instead for $T < 110$ K it starts rising as T decreases, as reported in figure 1.19 [10]. A possible explanation is that at low temperature new traps are activated.

Another source of AP generation is an optical induced phenomenon, similarly to the delayed optical crosstalk. Indeed photons, generated in the primary avalanche, can be absorbed in the neutral region of the same cell and then reach the active area, triggering a discharge (see figure 1.15). The time constant for this AP corresponds to the lifetime of the carriers in the un-depleted region. If this lifetime is shorter than the recovery time of the cell, this contribution is negligible. The AP probability depends on the sensor technology, in particular, on the number of traps. Therefore, solu-

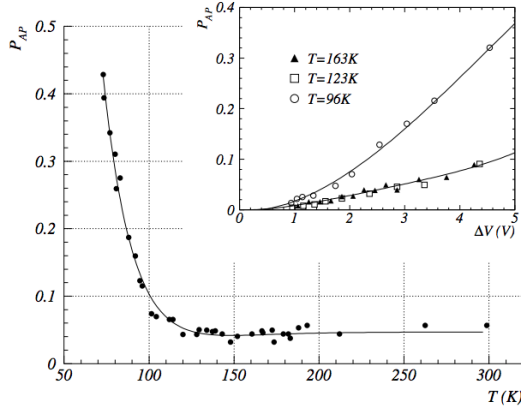


Figure 1.19: Afterpulsing probability as a function of T at a fixed overvoltage for a $1 \times 1 \text{ mm}^2$ FBK SiPM. The inset reports the same probability as a function of V_{ov} [10].

tions, to reduce the effect, are to minimise the impurities and the defects of the Silicon, implement a substrate with short diffusion length (as for the OCT) and operate the SiPM at low overvoltage.

1.3.4 Excess Noise Factor

All the described stochastic processes introduce an inter-cell correlated noise that changes the measured distribution with respect to the real distribution of the impinging photons (usually Poissonian one with mean value μ_R), as shown in figure 1.20. Two parameters are exploited to describe the worse performance of a non-ideal detector: the excess charge factor (ECF) and the excess noise factor (ENF).

The ECF corresponds to the average excess charge associated to a primary event, due to the OCT and the AP³. It is defined as:

$$ECF = \frac{\mu_{det}}{\mu_R} \quad (1.9)$$

³In an ideal situation with no correlated noise it is equal to one

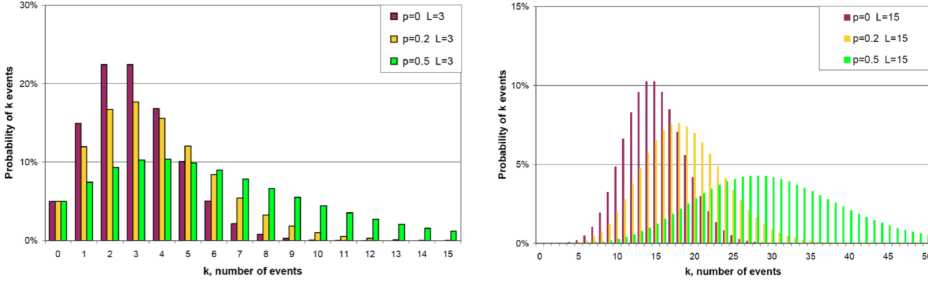


Figure 1.20: Compound Poisson distribution function with a mean value of photoelectrons equal to 3 (left) and 15 (right), for three different values of $p=[0, 0.2, 0.5]$ [11].

where μ_R corresponds to the mean charge of the Poissonian distribution of photons (ideal situation), while μ_{det} is the mean charge of the distribution effectively detected by the device. The ENF takes into accounts the rms deviation (σ_{μ_R} and $\sigma_{\mu_{det}}$) of the two distributions and it is equal to the ratio between their signal-to-noise ratio [62]:

$$ENF = \left(\frac{SNR_{in}}{SNR_{out}} \right)^2 = \frac{(\mu_R / \sigma_{\mu_R})^2}{(\mu_{det} / \sigma_{\mu_{det}})^2}. \quad (1.10)$$

The total output, considering the stochastic noise effect, belongs to a compound Poissonian distribution with a mean value equal to $\mu_{det} = \sqrt{\mu_R / (1 - p)}$, where p represents the probability to detect OCT and AP events [11]. Therefore, from equation 1.10 the ENF is $1+p$. Moreover, considering that the detection of a photon occurs with a probability equal to PDE, the total ENF is equal to:

$$ENF_{tot} = \frac{ENF}{PDE} = \frac{1 + p}{PDE}. \quad (1.11)$$

It can be measured from the single photoelectron charge distribution and calculated by means of [62]:

$$ENF = 1 + \frac{\sigma^2}{G} \quad (1.12)$$

where G is the SiPM gain, obtained as the total charge released by the mean number of fired cell, and σ^2 is its variance. Nowadays, ENF ranges from 1.01 to 1.5 [56, 62].

1.4 Dynamic Range

An implementation of a SiPM with high gain, high PDE, single photon-detection and high dynamic range is hardly achievable within the same device. The finite number of cells intrinsically limits the number of photons which can be detected simultaneously by a SiPM. This leads to a non-linear behaviour and to the saturation of the device response, limiting the dynamic range of the SiPM. Since the single charge is approximately the same for each pixel of a SiPM, the SiPM response is linear, until the number of incident photons in a pulse N_γ , multiplied by the PDE, is small with respect to the total number of available cells N_{tot} . When the intensity increases, the probability that one cell is fired twice or more is not yet negligible [36, 63]. The number of fired cells (N_{FC}) is a random variable, therefore, its mean value can be estimated considering a random distribution of $N_\gamma \cdot PDE$ photoelectrons on N_{tot} available cells. Moreover, supposing that the photons impinge on the sensor and trigger avalanches simultaneously and uniformly, the number of fired cell (N_{FC}) can be estimated as [64]:

$$N_{FC} = N_{tot} \left[1 - \left(1 - \frac{1}{N_{tot}} \right)^{N_\gamma \cdot PDE} \right]. \quad (1.13)$$

When $N_\gamma \cdot PDE$ and N_{tot} are large, the formula can be approximated by:

$$N_{FC} = N_{tot} \left(1 - e^{-\frac{N_\gamma \cdot PDE}{N_{tot}}} \right). \quad (1.14)$$

A constant term can be added in order to consider the contribution of fired cells due to the dark count noise [63, 12].

In a real SiPM, however, the response to incident light is influenced by three factors: the cell pitch, the cell recovery time and the inherent SiPM

noise (in particular the cell occupancy due to dark counts). Methods to address the influence of the noise are reported in [63, 65]. Moreover, due to the recovery time, the dynamic range depends on the arrival time distribution of the photons. When the time distance between two photons is of the order of t_R or more, the effective dynamic range can appear higher [36, 12]. If the photon time spread is higher than t_R , the cell can be partially recovered before the next photon arrives and, thus, the same cell can initiate another avalanche. A measurement of this effect, for a Hamamatsu S10362-050C SiPM, is reported in figure 1.21. In this study, the authors conclude that as long as the number of photons triggering simultaneously avalanches and the cell occupancy by dark counts is less than $\sim 20\%$, the response is almost linear and independent of the arrival time of the photons. Otherwise, the response becomes non-linear and strongly depends on the photons arrival time distribution [12].

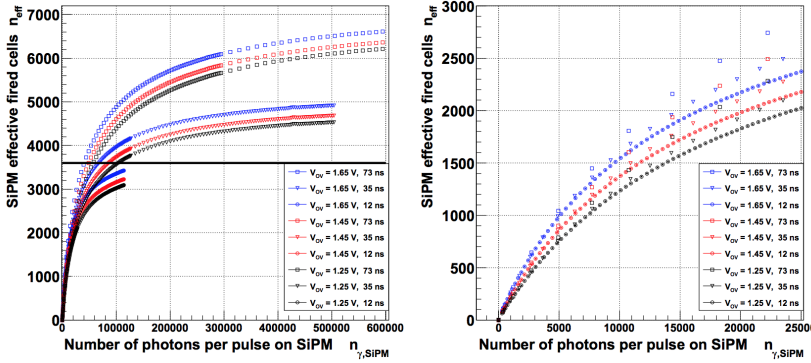


Figure 1.21: N_{FC} as a function of the number of photons per pulse on the SiPM sensitive area, for three different pulse durations. The black line on the left hand side represents the total number of available cells. The plot on the right hand side is a zoom of the previous one [12].

1.5 SiPM applications

The peculiar features of SiPMs (high internal gain, compactness, low operational voltage and insensitivity to magnetic fields) are the basis of the growing interest for this technology, rising from very different and varied fields of application [40]. Nowadays they are approaching a mature technological stage. Different producers invest in order to guarantee high quality mass production of SiPMs (allowing also a significant cost reduction for a single piece). This allows to consider them a valid alternative or the first choice platform for the development of novel solutions in a wide range of applications [66].

One of the most addressed field from SiPMs is the medical one [31]. In particular, their insensitivity to magnetic fields enables the opportunity to construct hybrid PET/MR (Positron Emission Tomography and Magnetic Resonance) systems [67]. Moreover the excellent timing properties of SiPMs make them suitable especially for TOF-PET (Time of Light Positron Emission Tomography) scanners. In [13] a 420 nm picosecond laser (produced by PiLas) with a pulse width of 42 ps (FWHM) was used to measure the Single Photon Timing Resolution (SPTR) of various SiPMs, with similar characteristics in term of pitch and sensitive area, as a function of the V_{ov} . A single photon time resolution close or below 100 ps FWHM has been measured, as shown in figure 1.22, roughly independently of V_{ov} . The TOF-PET principle is based on the measurement of the time difference in the detection of the two annihilation photons. The Coincidence Time Resolution (CTR) is correlated to the improvement of the image signal to noise ratio [68]. Thanks to this enhancement, the TOF-PET reconstruction algorithms converge faster with a better quality image result [69]. This is equivalent to a reduction of scan times and/or radioisotope administered activity (consequently dose), resulting in important advantages for typical medical staff workflow. The CTR, in addition to the SPRT, depends

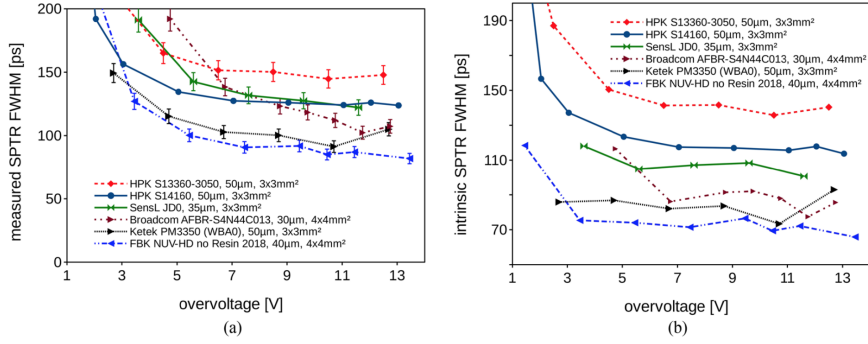


Figure 1.22: (a): The measured SPTR considering all the contributions: laser pulse width, electronic noise and acquisition jitter. (b): The real intrinsic SPTR of the tested SiPMs: the additional terms were quadratically subtracted [13].

also on the light characteristic of the emitted light when particles interacts with scintillating crystals. With LSO:Ce:Ca inorganic crystal results close to CTR=100 ps was reached, while with the BGO (Bismuth Germanate) higher values (ranging from 150 to 200 ps) were measured in laboratory [13]. Although the possibility to reach these resolutions were demonstrated in laboratory, the current systems don't exhibit so optimal features [31, 70]. For instance, the first clinical TOF-PET/CT system based on SiPMs was developed and installed in different hospitals by Philips. It is based on the coupling of LYSO crystals with digital SiPMs and presents a CTR of ~ 300 ps FWHM.

Apart from the medical imaging, SiPMs are playing a crucial role also in radiotherapy dosimetry. With the introduction of novel treatment techniques (as volumetric modulated arc therapy VMAT), the complexity of the actual treatment planning systems increases. Therefore, the possibility to implement a dosimetric system based on optical fibres readout by compact photodetectors as SiPMs allows to have comprehensive quality assurance tools and real-time dosimetry. Indeed this dosimetry is easy to handle and operate, radiation hard, with a excellent spatial resolution (the

fibre dimensions can reach 1 mm of core) and with a good scalability for a multi-channel system. Moreover the low operation voltage ensures that SiPMs and fibres are a reliable alternative for in-vivo dosimetry [71, 72]. In the last years, different prototype were proposed both for external beam radiation therapy and brachytherapy (the use of sealed radioactive source inside or close the areas to be treated). In general, it is reported that they reach a spatial resolution lower than 1 mm and absolute difference with the treatment planning system lower than 5% [71, 73].

Nowadays, SiPMs are also adopted in several high energy physics detectors. In particular, the capability to readout a huge number of optical fibres with a small, compact and low biased photodetector with low power consumption is exploited for the new generation of trackers and calorimeters. For instance, LHCb experiment at CERN replaces the silicon strips based tracker with the innovative concept of a full scintillating fibre tracker readout by SiPMs, reconstructing the particle position through center of gravity of the detected light on several adjacent SiPMs [74]. However, calorimetry is the field in high energy physics where the use of SiPMs nowadays is dominant. One of the most interesting examples is the upgrade of the barrel and endcap of the CMS experiment hadronic calorimeter. For the next phase of the LHC, CMS will replace the Hybrid Photo-Diodes (HPDs) with about 16000 SiPMs. This will provide a higher calorimeter longitudinal segmentation, increasing the particle ID capabilities and the shower separation abilities of the detector also in the external regions of the calorimeter [75].

Finally, a field, where SiPMs are not so widespread and currently are not the emerging technology, but the situation could evolve rapidly, is biophotonics. Biophotonics is a multidisciplinary research area, that aims to study biological systems through light-based technologies. Although the impact of SiPMs has been so far marginal, the potentiality of these detectors can become the key for developments of new systems. In particular, as

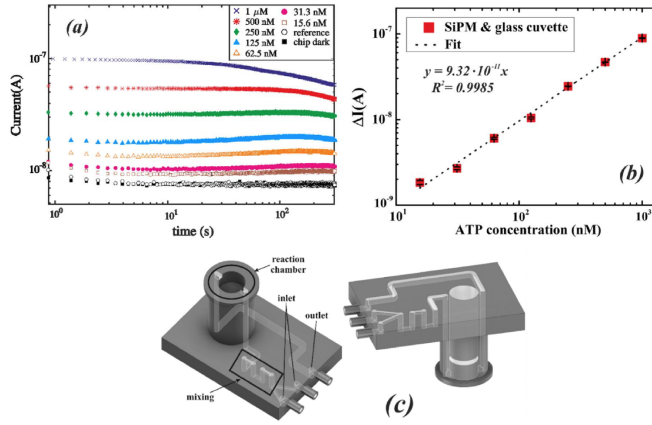


Figure 1.23: (a) ATP bioluminescence induced signal versus time for different ATP concentrations. (b) Calibration curve of SiPM response to ATP concentrations. (c) 3D printed microfluidic chip [14].

reported in [33, 76, 77], the small size, the cost-effectiveness and the immunity to damage from light over exposure, make possible to integrate SiPMs into systems to ensure rapid detection and examination of small volume of biological samples at low cost. For instance, a 3D printed microfluidic chip, shown in figure 1.23, was developed for the detection of Luciferase bioluminescence induced by ATP (adenosine triphosphate), retrieving the signals generated by low ATP concentrations down to a few of nanomolar [14]. Another interesting example is a time-resolved single photon detection module proposed by Martinenghi et al. in [78]. It is dedicated to the reconstruction of fluorescence decay patterns in biological samples and is able to reach an SPTR of ~ 100 ps, performing better than some commonly used PMTs [78].

SIPM Readout System: ASIC CITIROC1A

The growing need to integrate several (from tens to hundreds thousand) SiPMs to cover large sensitive areas requires the possibility to provide compact readout systems based on ASICs (Application Specific Integrated Circuit). This demand comes mainly from high energy physics, medical physics and astrophysics. The upgrades of the next-generation experiments at CERN and the construction of the Cherenkov Telescope Array requires to readout from hundred thousands to millions of SiPMs [79]. However, also other applications, as dosimetry or biosensing systems as explained in chapters 4 and 6, can benefit from the possibility to readout tens or more SiPMs. Therefore, since 2006 dedicated ASICs started to be designed and produced. A class of ASICs produced by Weeroc¹, a spin-off company from the OMEGA lab, reached the maturity for the market and some of them presents versatile features, suitable for different applications. The first readout of the class, designed in 2006, was the SPIROC chip, fabricated for the readout of SiPMs coupled to scintillating tiles for the CALICE collaboration [80]. It is a 36 channels ASIC, with two gain branches for charge measurements (on chip) up to 320 pC with 12 bit granularity. The original design quickly generated other architectures. In particular the EASIROC (the Extended Analogue Silicon photomultiplier In-

¹<https://www.weeroc.com/>

tegrated Read Out Chip [81]) and CITIROC (for the CTA experiments [82]) are inspired by it: the analogue part is very similar, while the digitalisation was removed and performed externally². Moreover, since the interest for time measurement at the level of hundreds of ps is increasing, Weeroc designed also a dedicated ASIC, called PETIROC [82], optimized to reach a bandwidth of 1 GHz to reduce the electronic jitter that spoils the time measurement at small signal amplitude [7, 30]. Time can be measured either with an internal TDC (resolution ~ 50 ps) or an external one, leading to a nominal resolution of ~ 15 .

The feature of the CITIROC1A can be exploited in some biomedical applications in which no time information is requested. In this chapter, after a brief description of the CITIROC1A and the front end board hosting it, a full characterisation of the most commonly used features in biomedical applications and the operation modalities is illustrated. It results a versatile readout for SiPMs, with the possibility to exploit it in charge and counting (also at the level of single photoelectron) measurements.

2.1 CITIROC1A: description

A block scheme of one CITIROC 1A channel is sketched in figure 2.1. Figure 2.3 reports the signal processing for the charge measurements in the CITIROC1A. A fine-tuning of the SiPM bias is implemented through an 8-bit Digital-to-Analog Converter (DAC) over a 4.5 V range. Each channel provides two parallel measurement branches with different gain, high gain (HG) and low gain (LG), with ratio 1 to 10, when operated in same conditions. At the first stage of the electronics chain, AC coupled voltage preamplifiers with variable gain allow increasing the reachable dynamic range up to 400 pC. This value corresponds to ~ 2500 photoelectrons for a SiPM with a gain of the order of 10^6 . Both preamplifiers can be tuned

²This reduces the dead time of the chip [30]

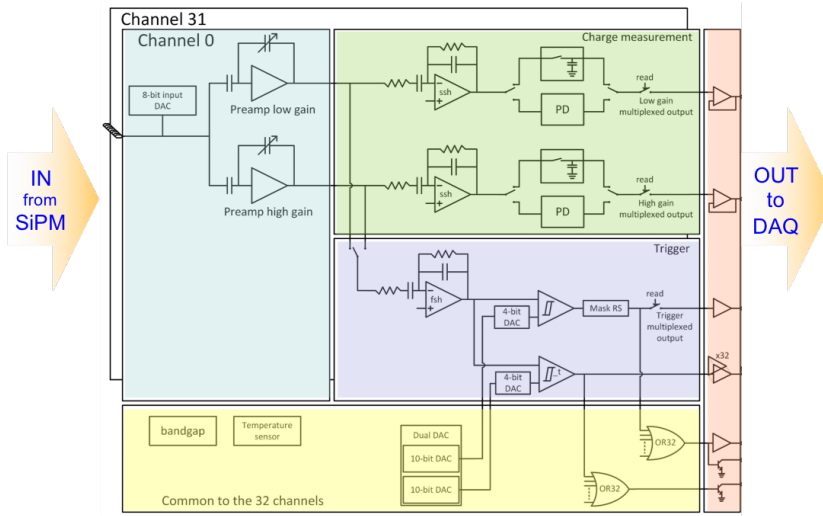


Figure 2.1: General block scheme of one channel of the CITIROC1A [15]. The light blue panel represents the fine bias adjustment and the pre-amplifier circuits. The green one is the charge measurement block with the bipolar slow shapers and the two peak sensing modes. The purple one is the trigger line, which embeds a fast bipolar shaper followed by two discriminators. The yellow panel shows the common parts for the trigger threshold set. Finally the orange one represents the output signals.

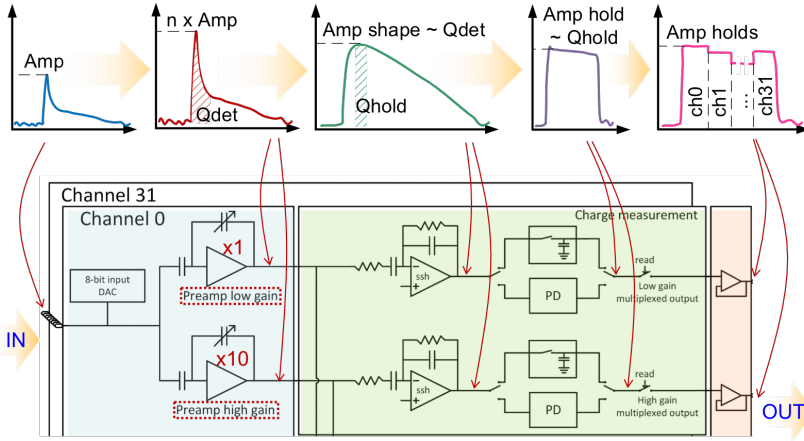


Figure 2.2: Signal processing for charge measurements in the CITIROC1A.

varying the feedback capacitance from 25 fF to 1575 fF with a step of 25fF (the input capacitance is equal to 1.5 pF for LG and 15 pF for HG). The charge measurement is ensured by two bipolar slow shapers connected to the outputs of the preamplifiers. Seven different peaking times of each slow shaper are available: from 12.5 ns to 87.5 ns with 12.5 ns of step. The peaking time is common for all the channels, but can be set differently for LG and HG branches. Two modalities are integrated to sample the peak of the output signal of each slow shapers: the so-called Track&Hold and Peak Detector. The selection of the peak sampling solution is independent between HG and LG³, but is common for the 32 channel of each branch. The working principle of both modalities is represented in figure 2.3. The Track&Hold modality returns the pulse height at the given sampling time, in other words at a fixed time. The Peak Detector mode, otherwise, provides the maximum of the signal in a predefined time window, allowing getting the maximum of the peak even if it is not precisely defined in time. This feature was added in this ASIC for the first time. The peak research

³This is true for the ASIC circuit, however on the readout board and its software, described below, only one modality can be chosen for both branches

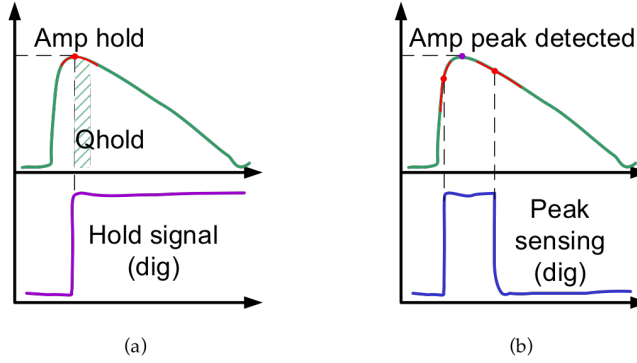


Figure 2.3: Different working principle of the two modalities of the peak sensing implemented in CITIROC1A: Track&Hold (a) and Peak Detector (b).

window can be either opened internally through the channel charge trigger or externally using an external and global trigger, common to all the 32 channels. In principle, the Track&Hold is suitable when the spread of the signal peak is negligible (i.e. the photons arrive on the sensor simultaneously with a few of ns of spread), while the Peak Detector is the solution when the spread of photons is higher. The charge value is readout in serial through two multiplexed analogue outputs. The information both from LG and HG of the same channel, are extracted at the same time.

The trigger line embeds a fast bipolar shaper, with fixed peaking time of 15 ns, and two discriminators (one called charge trigger and one time trigger). The fast shaper can be connected either to the LG or HG preamplifiers. For both discriminators, the threshold is supplied by a common 10-bits DAC and a 4-bits DAC for the fine adjustment of individual channels. The charge trigger features a programmable mask to exclude undesirable channels and provides, in a multiplexed output, the pattern of triggered channels (hit register). The time trigger returns 32 outputs with accurate time information (resolution of ~ 100 ps) [15].

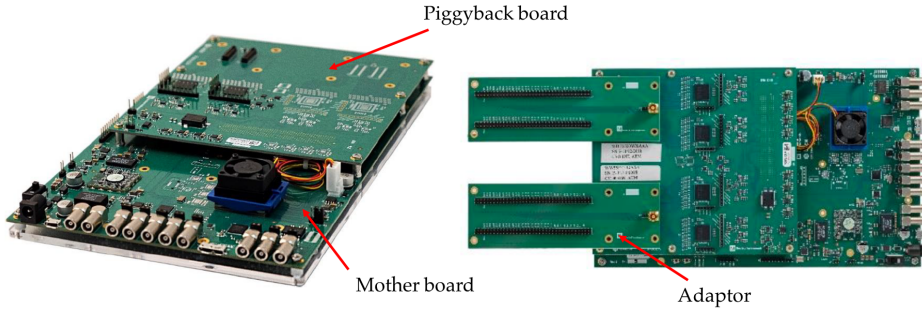


Figure 2.4: General view of the CAEN DT5550W board.

2.2 CITIROC1A: readout board

The readout board for the CITIROC1A, the DT5550W⁴, designed and developed in collaboration by CAEN S.p.A and Nuclear Instruments s.r.l., was tested in order to evaluate and characterise the ASIC performances. The system, reported in figure 2.4, is divided in two boards: the mother-board, hosting an user programmable FPGA, USB 3.0 for the communication with the host computer and a 14-bit Analogue-to-Digital Converter (ADC), and the Piggyback board, equipped with up to four ASICs, detector connectors and a power supply for common detector biasing (20 - 85V). The 2V peak-to-peak ADC perform the analogue readout of the ASIC multiplexed outputs and the monitor of the ASIC analogue probe: the preamplifier outputs, the slow shaper signals (charge line) and the output of the fast shaper (trigger line).

The Piggyback board can host up to four ASIC (for a total of 128 channels) and connect directly SiPM matrices, as hamamatsu S13361-3050AE-08, thanks to ultra fine pitch Samtec connectors. In order to use different SiPM sensors or matrices an adapter is provided or, in any case, a compatible remoting cable can be used. On the Piggyback board, close to each

⁴<https://www.caen.it/products/dt5550w/>

ASIC, some test pins are available, to allow the user to debug the internal operation of the chip itself as: the analog signal outputs for both LG and HG, the hit flag indicating which channels fired the trigger and the ASIC temperature sensor output. In general the firmware allows the following operations: configure ASIC parameters (gain, shaping time etc.) and the triggering logic (auto-trigger or external, the mask of the different channels etc.), multiple ASICs and multiple boards synchronization etc. The global trigger of the board is the OR of all the possible triggers (internals and external). Moreover, with this board, the CITIROC 1A readout can be performed in two ways:

- photon counting: the firmware just counts the transitions of the trigger output above a threshold for each channel. Only the ASIC trigger line, i.e. the fast shaper and comparator, is used. It is possible to extract also the information of the number of photons per channel in a specific integration window opened by a start signal. The start signal could be generated internally with a periodic signal (frequency between 1 Hz and 1 MHz) or by an external signal provided by one of the LEMO input available on the motherboard. The window width can vary between 100 ns and 4 s.
- analogue multiplexed readout: in this case the readout is performed using the ASIC analog multiplexed output and the external ADC, which is mounted on the motherboard, allowing the charge measurements.

2.3 CITIROC1A: characterisation

A full characterisation of the ASIC performances useful for some biomedical applications was carried out with the DT5550W. In particular, the behaviour of the preamplifier was measured. Then the investigation of the

two peak sensing method were performed. Finally the linearity of the ADC for the charge measurements and the counting capability of the system down to 0.5 photoelectrons was determined. The board under test is equipped with 2 ASICs.

2.3.1 Preamplifier gain

First of all, the gain variation of the two preamplifiers was studied. The circuits of the preamplifiers are depicted in figure 2.5. 64 different gain are available each corresponding to a different value of the capacitor in the preamplifier feedback. In these studies, only one Hamamatsu S13360-

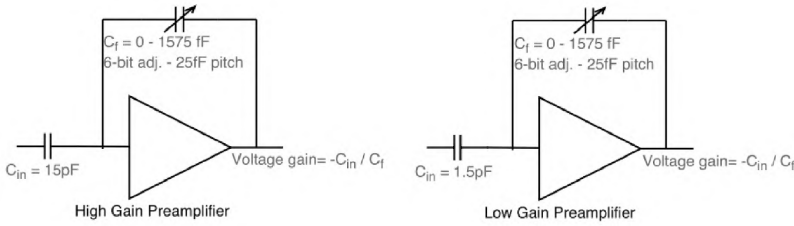


Figure 2.5: Preamplifier circuits for the high gain and low gain branches.

1350CS SiPM $1.3 \times 1.3 \text{ mm}^2$ with $50 \mu\text{m}$ of pitch was connected to the ASIC. It was illuminated with a PLS-8-2-443 sub-nanosecond-pulsed LED produced by PicoQuant. The SiPM was biased at its nominal $V_{bias} = 55\text{V}$ (corresponding to an overvoltage of $\sim +2.5 \text{ V}$). The gain of each preamplifier can be varied through the software, changing a code bit from 63 (the higher gain) to 0 (the lower one). In the measurements, 15 different code: 62, 61, 60 and then from 60 to 5 with step of 5. For each gain code, the SiPM was illuminated with a number of photoelectrons, in excess of 30. The intensity of the light was kept constant for the duration of the scan. For each gain, the charge of 50000 events was recorded, synchronously with the LED, and their mean value was computed. Figure 2.6 illustrates an example of the recorded spectrum for both the high gain branch and the low

gain one. The trend of the mean charge, pedestal subtracted, as a function

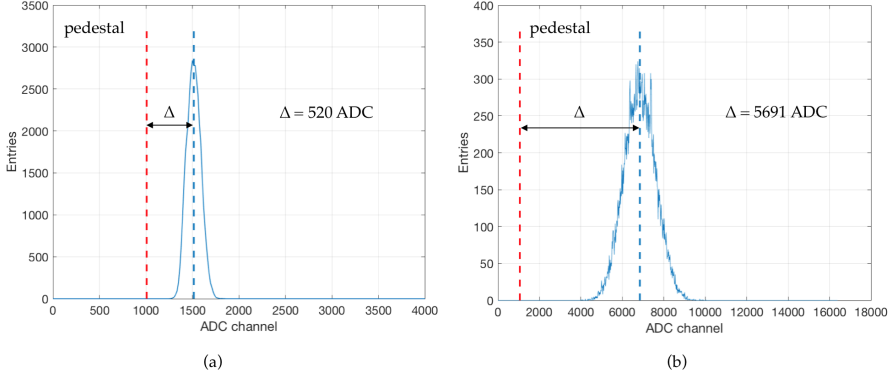


Figure 2.6: Charge spectrum of both branches (a) high gain and (b) low gain for a code gain equal to 50. The red dashed lines represent the pedestal of each brach (1005 ADC channels for LG and 1055 for HG one), while the blue ones the mean value of the charge distribution.

of the code preamplifier gain is shown in figure [2.7](#). With respect to the

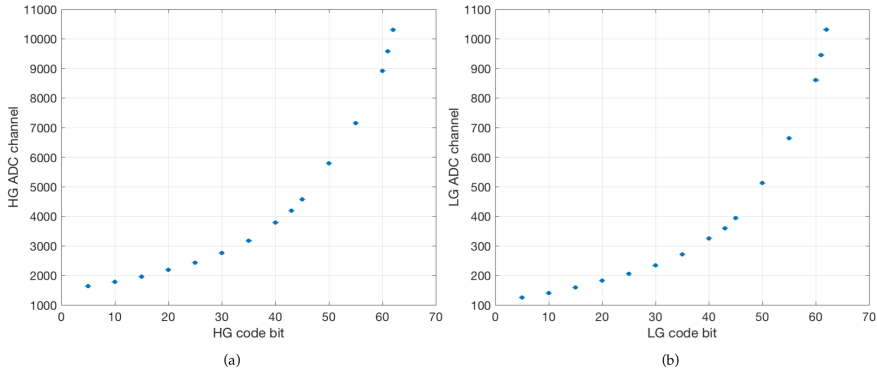


Figure 2.7: The mean charge, pedestal subtracted, as a function of the code preamplifier gain for the high gain branch (a) and the low one (b).

datasheet, a softer dependence was measured, especially at highest values of gain. The factor ~ 10 between the two branches, when operated with the

same code gain, was confirmed.

2.3.2 Track&Hold and Peak Detector modalities

As discussed above, two peak sensing modalities are embedded in the CITIROC1A. The Track&Hold is controlled by a hold signal (one hold signal for each branch). The hold signal rising edge should correspond to the peak of the bipolar shaped output signal in order to obtain the proper information. The hold signals are high (in hold mode) until the readout sequence has been completed (figure 2.8). This modality requires an excellent

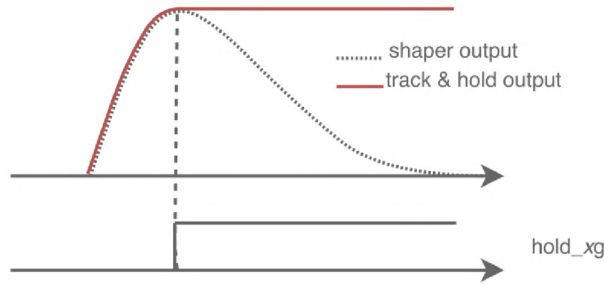


Figure 2.8: Track&Hold working modality. An example of shaper output (top) and the hold signal (bottom) are displayed, on the bottom the hold signal.

alignment between the shaped signal and the hold signal (generated after the trigger) and a negligible spread in time of photons. In the DT5550W there is the possibility to delay the hold signal with step of 8 ns, as required. To test the performances of the Track&Hold, the S13360-1350CS Hamamatsu SiPM is illuminated with few ns spread pulse light emitted by the LED in order to reconstruct the multiphoton peak spectrum. The distance between two peaks, the so-called distance peak-to-peak (Δ_{pp}) is evaluated for two different delays of the hold signal. The high branch shaping time constant was fixed to 37.5 ns, producing a nominal peaking time of ~ 50 ns. The board was triggered externally and synchronously with the

LED. The two multiphoton peak spectra, reported in figure 2.9, were acquired with two hold signals delayed of ~ 100 ns and ~ 130 ns. Since the delay between the trigger and the light pulse is 100 ns, the Δ_{pp} decreases from (550 ± 3) ADC to (491 ± 2) ADC at the higher delay time because it does not correspond to the peak amplitude of the signal, as shown on the bottom row of figure 2.9, where a small ripple on the analogue probe out of the shaped signal indicates where the hold signal sample the amplitude value. It is worth mentioning that a systematic shift in the ADC measured charge was observed. This behaviour caused an unexpected excess of events in the valley regions between the peaks. The problem was brought to the attention and discussed with the developers of the board. Its origin at the time of writing is still under investigation.

These results were compared to the Δ_{pp} retrieved with the Peak Detector modality. The Peak Detector acquisition presents a track phase and a peak detection one (the working principle is shown in figure 2.10). In the first one, before any trigger, the peak detector is in track mode (no recording any data). When a trigger occurs, the peak detector switches to peak detection phase. This phase keeps and memorises the maximum value once it has been reached. This phase continues until the rising edge of the hold signal is provided. With this modality, multiphoton peak spectra with 10000 events per run were recorded, increasing the search window of the signal maximum from 37 ns to 124 ns. Figure 2.11 shows the peak to peak distance as a function of research window length. As expected, the Δ_{pp} approaches a plateau value ($\Delta_{pp} = 553 \pm 4$ ADC) once the peak of the signal is kept. Therefore, it is possible to properly sample the amplitude of the shaped signal in both modalities, retrieving compatible Δ_{pp} values. However due to the higher versatility and the possibility to account the higher fluctuations of the photon time spread (for instance in case photons emitted by scintillators), in the final version firmware was implemented only the Peak Detector modality.

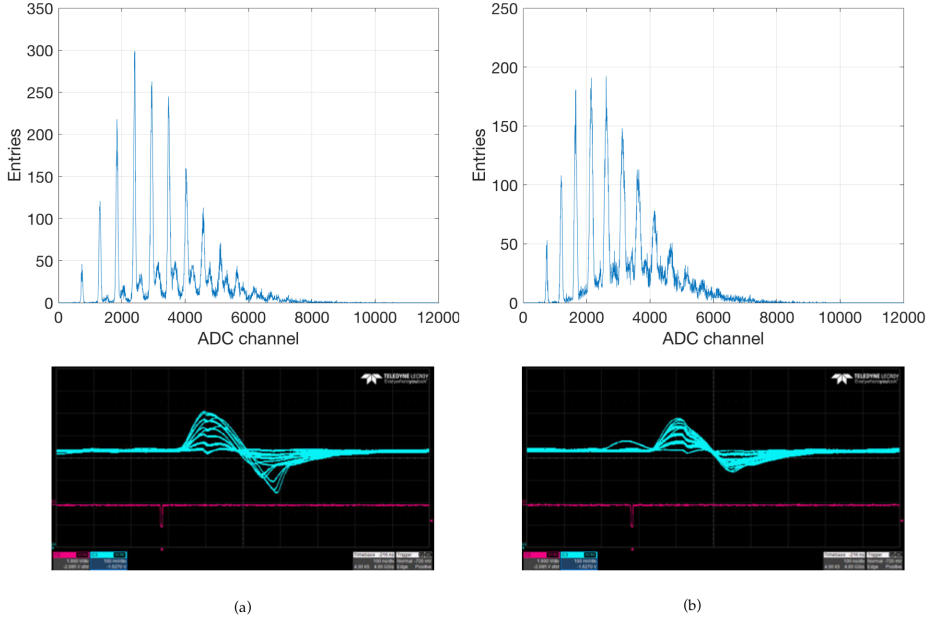


Figure 2.9: In the first row two multiphoton peak spectra are acquired illuminating a S13360-1350CS Hamamatsu SiPM with a LED with a few photoelectrons sampling the signal with two different times in the Track&Hold mode: 100 ns (a) and 130 ns (b). Exemplary signals are shown in the bottom row, highlighting where the signal amplitude are sampled through a ripple: (a) the peak of the signal is sampled properly, while in (b) it is sampled on the decreasing tail. In both figures on the bottom row the purple signal is the trigger output of the LED.

2.3.3 Linearity

The linearity of the HG and LG branches is a necessary requirement for many applications (e.g. calorimetry in high energy physics or γ spectroscopy). The CITIROC1A datasheet reports a dynamic range of 400pC. Using the S13360-1350CS SiPM with an internal gain of $\sim 1.7 \cdot 10^6$ at $V_{ov} = +3$ V [24], is not possible to test all the available ASIC dynamic range without saturating the sensor. Since 400 pC corresponds to ~ 1470 photoelec-

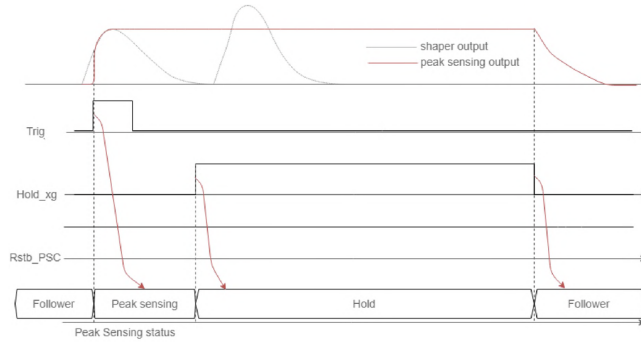


Figure 2.10: Peak Detector working modality. The trigger starts the peak sensing phase, while the rising edge of the hold signal stops this phase.

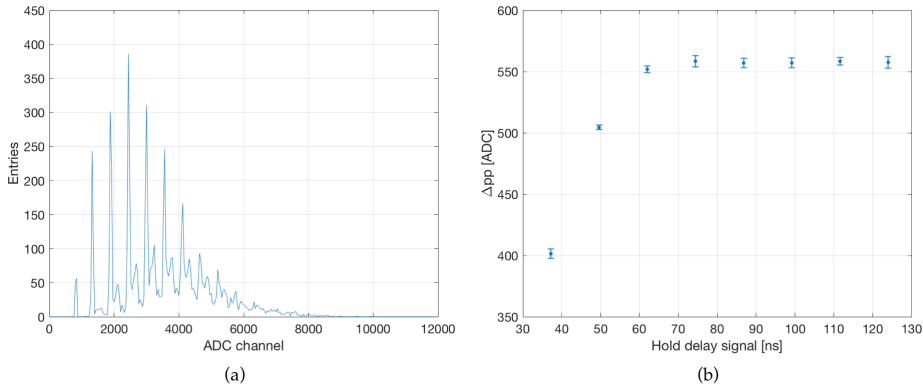


Figure 2.11: (a): Example of the multiphoton peak spectrum acquired with the Peak Detector search window 99 ns long, illuminating a S13360-1350CS Hamamatsu SiPM with a LED. (b): Δ_{pp} as a function of the increasing searching peak window in Peak Detector modality.

trons, while the sensor in use has only 667 available cells. Thus, in order to test the linearity, a Hamamatsu S14160-1315PS SiPM with an area of $1.3 \times 1.3 \text{ mm}^2$ and $15 \text{ }\mu\text{m}$ pitch (7296 total available cells) is used. The internal gain of this SiPM is $3.6 \cdot 10^5$ at $V_{ov} = +4 \text{ V}$ [83] that allows testing all the dynamic range of the ASIC (in this case 400pC corresponds to ~ 7000

photoelectrons). Two strategies were adopted. Firstly, a high preamplifiers gain was used and the dynamic range was tested with a not so high number of photoelectrons (~ 700) in order to avoid the intrinsic SiPM non linearity (described in section 1.4). Secondly, the preamplifier gain was reduced and the response of the SiPM was compared to the expected non linear behaviour.

The SiPM was illuminated by a 500 Hz externally triggered LED (Pico-Quant), with five increasing light intensities. In order to avoid the intrinsic non-linear response of the SiPM at high number of incident photons, an independent measurement of the light intensity was performed with a Photomultiplier Tube (PMT)⁵. The gain code bit of the two branches are set to 53, high enough to allow on the HG branch discriminating the peaks of the multiphoton peak spectrum and inter-calibrate the response of the PMT in terms of photoelectrons, as shown in figure 2.12. Indeed, the maximum value of measured photoelectrons on HG branch was ~ 50 (extrapolated by the formula 1.2, known the $\Delta pp = 136 \pm 2$ ADC/pe). Since the occupancy of the cells in this condition is less than 1%, the non-linear response of SiPM, evaluated by the equation 1.14 is negligible ($< 0.5\%$), proving the reliability of the conversion. Knowing the amplitude value of the PMT signal corresponding to a single photoelectrons (~ 0.175 mV), is possible to estimate the number of photoelectrons measured at the higher intensities. Linearities within few percent ($< 5\%$ for HG and $< 1.5\%$ for LG) was measured for both HG and LG branches, over all the ADC dynamic range with up to 700 photoelectrons on LG line, as reported in figure 2.12. Moreover, it is worth mentioning that the slope of the response on the LG branch is $\sim 13.1 \pm 0.2$ ADC/pe, roughly 10 times smaller than the value measured with the HG one, as expected.

In order to test the linearity also at higher number of impinging photoelectrons, the LG gain was reduced by a factor ~ 2.7 and the bias was de-

⁵Hamamatsu photosensor module H5783P

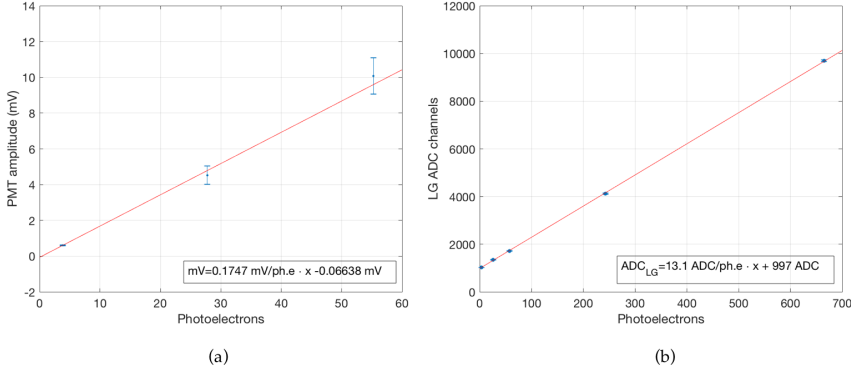


Figure 2.12: (a): Calibration curve used to convert the signal of the PMT in phe. The number of phe is extracted from the HG branch Δpp of the multiphoton peak spectrum. Roughly a PMT amplitude equal to 0.17 mV corresponds to 1 phe. (b) The ADC channels of the LG branch as a function of photoelectrons. The slope corresponds to the ADC channels for 1 phe, while the y-intercept corresponds to the pedestal i.e. $1005 \pm 10 \text{ ADC}$.

creased by $\sim 2V^6$, reducing the gain by a factor $\sim 1.8^7$. In these conditions, more than 4000 photoelectrons ($\Delta pp_{LG} = 2.79 \text{ ADC/pe}$) can be measured. The curve of photoelectrons detected by SiPM as a function of the expected number of impinging photoelectrons ($PDE \cdot N_\gamma$), detected by the PMT, is reported in figure 2.13(a). The behaviour is compatible with the non-linear SiPM response described by the equation 1.14. Indeed, a fit of the data with the function $y = a \cdot (1 - e^{\frac{-b \cdot x}{a}})$ returns a value of PDE (parameter b of the fit) equal to 0.37 ± 0.02 in good agreement with the value reported in the datasheet of 0.32 at an overvoltage of 4V. In order to exclude the possibility that the non-linearity is due also to the amplifier, a measurement with a

⁶biasing the the sensor at the recommended $V_{bias} = 42.5V$ ($V_{BD} = 38.5V$)

⁷Measured through the difference of the Δpp of the multiphoton peak spectra and confirmed by the trend of the gain as a function of overvoltage in the SiPM datasheet

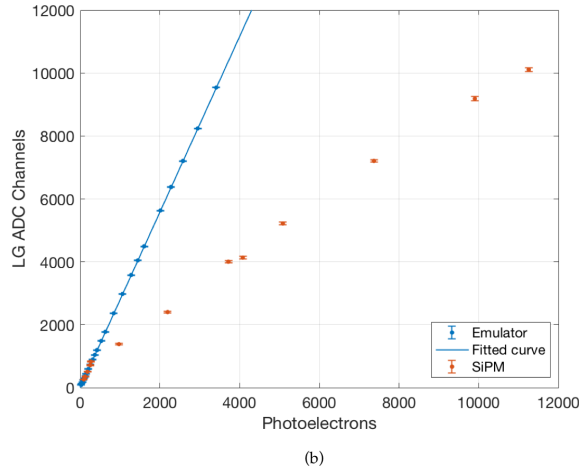
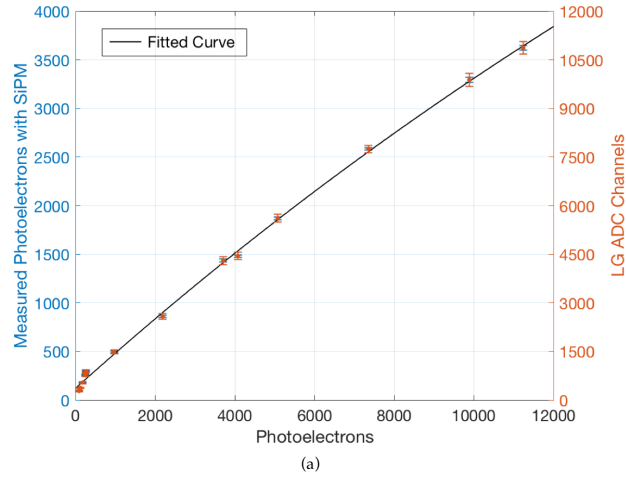


Figure 2.13: (a): the number of photoelectrons detected by SiPM as a function of the expected number of impinging photoelectrons detected by the PMT (blue points). The same curve is reported also in ADC channels, covering all the available dynamic range. The fit of the data represents the expected non-linear behaviour of the SiPM in use. (b): The linearity of the amplifier and the ADC is measured on the full available dynamic range (blue points). For comparison, in red are reported the ADC channels recorded in similar conditions with the SiPM.

signal emulator (DT5810B by CAEN S.p.A.^[8]) was performed. In this case, the input signal of the ASIC, shown in figure 2.14 is simulated as follows:

$$S_{in} = A \cdot (1 - e^{-t/\tau_1}) \cdot (e^{-t/\tau_2}) \quad (2.1)$$

where τ_1 is the rise time and is equal to 3 ns, τ_2 is the falling time equal to 50 ns and A is the peak amplitude in mV (at increasing A corresponds proportional increasing charge)^[9]. As shown in figure 2.13(b), linearity within

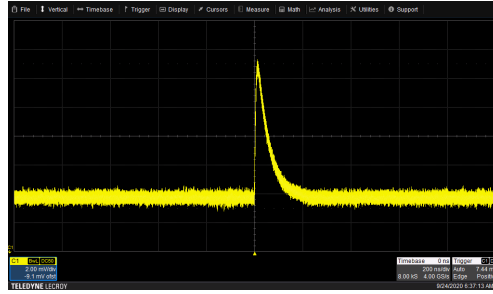


Figure 2.14: An example of simulated signal by the DT5810B.

1.5% is measured, confirming that the non linearity is due only to the SiPM intrinsic behaviour. It is worth of mentioning that this method was applied also on the HG branch and also in this case linearity within 1.5% is reached. These results are also confirmed by other works reported, for instance, in [84].

2.3.4 Counting Mode

The counting capability of the system is assessed through the characteristic SiPM staircase measuring the counting rate as a function of discriminator threshold. As described in section 1.3.2, this evaluation allows extrapolating the SiPM rates of primary and correlated noise. This method is based on measurements of the trigger rate, provided by the ASIC time trigger

^[8]<https://www.caen.it/products/dt5810/>

^[9]The signal parameters are close to the ones of the tested SiPM

line in photon counting mode, as described above, as a function of the discriminator threshold in dark noise regime [53, 39]. By the software, it is possible to define the range of the discriminator threshold, the variation step and the width of the counting window. The rate of each channels is recorded and is displayed also online. The SiPM under investigation belongs to the series S13360-1350CS; it is biased at the nominal operating bias $V_{bias} = 56V$ ($V_{ov} = +3V$). The staircase curve was recorded for the single SiPMs connected to different ASIC channels. Figure 2.15(a) shows an exemplary staircase. The first plateau corresponds to thresholds <1 photo-

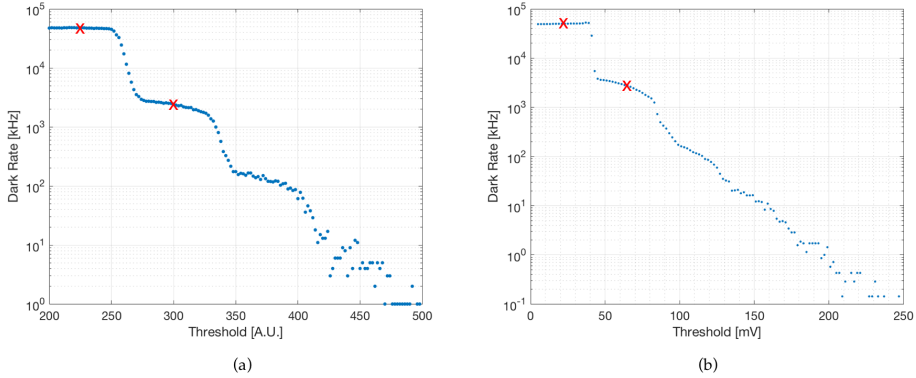


Figure 2.15: Noise rate of a SiPM Hamamatsu S13360-1350CS operated at an over-voltage of 3 V as a function of the discriminator threshold, measured with the ASIC and the DT5550W (a) and the the CAEN Silicon Photomultiplier Kit (b). The red crosses indicate the threshold and the rates used to estimate the DCR and the crosstalk probability.

electron (pe), provides a value of the SiPM DCR, that is equal to 49.00 ± 0.22 kHz. As described in section 1.3.2, the OCT probability is estimated as:

$$OCT = \frac{\nu_{1.5pe}}{\nu_{0.5pe}} = \frac{2.6kHz}{49kHz} = 5.3\% \quad (2.2)$$

The obtained values for DCR and OCT probability are in agreement with the SiPM datasheet and, moreover, the accuracy of the measurement was assessed with another independent measurements on the same sensor, performed with the CAEN Silicon Photomultiplier Kit [8, 39, 85]. In particular, the measurement was carried out, exploiting the leading edge discriminator, integrated in the two channel SP5600 CAEN Power Supply and three-stage Amplification Unit (PSAU). The DCR value of 51.00 ± 0.71 kHz and OCT probability equal to 5.5% confirm the accuracy of the values obtained with the ASIC.

A possible electric crosstalk between channels, that could induce an increase of the measured rates when more SiPMs are connected, is tested too. All 32 ASIC channels are coupled to 32 SiPMs (S13360-1350CS series), as shown in figure 2.16. The measurement with all the SiPMs connected to the

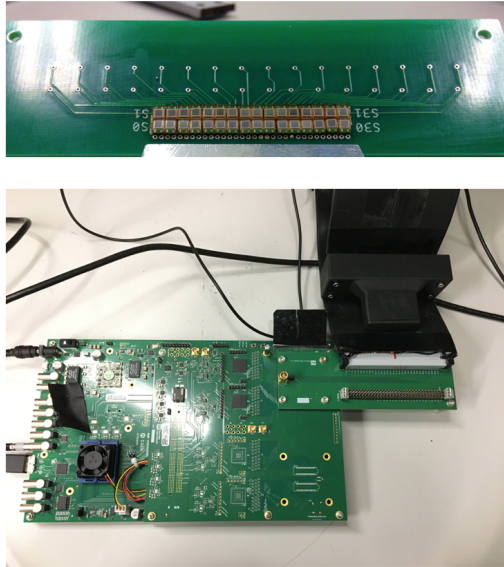


Figure 2.16: On the top the photo of the 32 SiPMs arranged in matrix 2×16 . On the bottom the photo of the set up shows the connection of the array, placed in a light tight box, to the DT5550W through a dedicated rectangular connector.

ASIC is performed providing the nominal bias voltage for each SiPM. The comparison with only one biased SiPM (but all 32 electrically connected to the board) was possible thanks to the High Voltage adjustment for each ASIC channel. Therefore, as shown in figure 2.17, the sensor bias was reduced below the breakdown voltage, switching it off, for all the SiPMs except one. In figure 2.18, an exemplary staircase, recorded by one ASIC

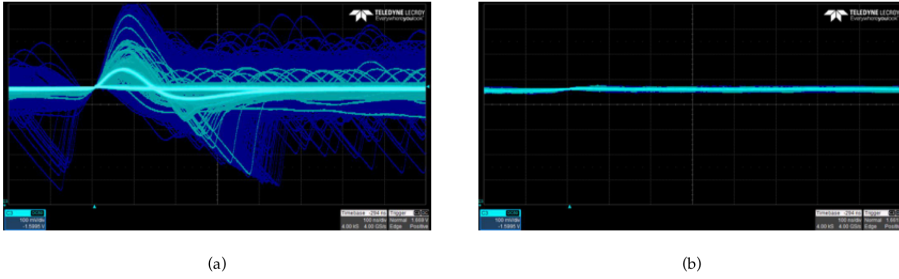


Figure 2.17: (a) Output signal of the SiPM, biased at the nominal bias, after slow shaper at the oscilloscope. (b) Output signal of the SiPM, after the reduction of the applied voltage below V_{BD} through the DAC.

channel, is reported. Moreover, the discrepancy between rates measured by one ASIC channel, when only one or all 32 SiPMs are connected, is evaluated and a good compatibility¹⁰ (on average less than 1σ and maximum at 3σ) is found, assuring an absent or at least a negligible electrical crosstalk inter-channels. Finally the counting capability of the system at different input frequencies was tested. In this case the same SiPM was illuminated with LED, externally triggered at different frequencies, ranging from 500 kHz to 5 MHz, with a mean number of photoelectrons of 13 phe. The discriminator threshold for the counting measurements was fixed at 5.5 phe (negligible DCR). The linearity (within 1%) of the measured count rate as a function of the trigger LED frequency is reported in figure 2.19.

¹⁰Measured through the normalized variable $Z = \frac{Rate_{oneSiPM} - Rate_{32SiPMs}}{\sqrt{\sigma_{Rate_{oneSiPM}}^2 + \sigma_{Rate_{32SiPMs}}^2}}$

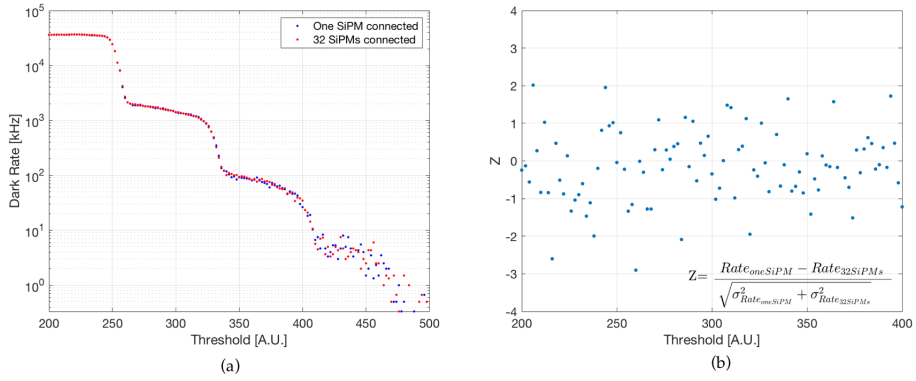


Figure 2.18: (a): Staircase acquired when only one SiPM out of 32 is biased as input of one ASIC channel (blue points) and when all 32 are biased to each 32 ASIC channels (red points). (b): the discrepancy between the two measurements are displayed and it is evaluated normalizing to square root of the sum of errors.

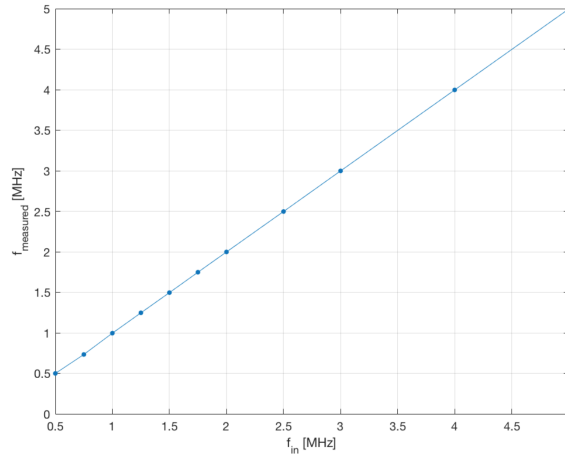


Figure 2.19: Measured count rate ($f_{measured}$) as a function of the trigger LED frequency (f_{in}). The threshold count is fixed at 5.5 phe.

2.4 Conclusion

A full characterisation of the main features of the CITIROC1A ASIC is performed, qualifying the ASIC and the readout front-end (DT5550W in par-

ticular) as a suitable and valid platform for future detecting systems. It allows to perform charge and counting measurement. Each modalities implemented for the signal peak sampling are tested. Both can be used considering a proper shaping time of the signal, although the Peak Detector looks to be the more versatile and easier to manage in order to obtain suitable measurements in different light conditions. The linearity of the two preamplifiers is verified on all the dynamic range available within 5% and 1.5% for HG and LG respectively, avoiding any measurement distortions. Furthermore, the accuracy of the counting capability triggering down to 0.5 photoelectron is demonstrated comparing the count rate measured by the ASIC and DT5550W with another independent system.

This ASIC is the readout of choice for the development of an innovative dosimeter system for brachytherapy in the European project ORIGIN¹¹: 16 optical fibres coupled to SiPMs readout by the CITIROC1A (described in chapters 4 and 5). Moreover the possibility to exploit SiPMs and this ASIC is investigated for biophotonic applications, in particular the detection of light emission following intracellular calcium concentration variations (reported in chapter 6).

¹¹<https://origin2020.eu/>

ORIGIN: Optical Fibre Dose Imaging for Adaptive Brachytherapy

The ORIGIN project (Optical Fibre Dose Imaging for Adaptive Brachytherapy) is an international project approved by the European Commission within the HORIZON 2020 framework programme. It aims to develop a dose imaging and localisation system for brachytherapy treatments.

In 2012, following the political declaration on Non-Communicable Diseases (NCD), World Health Organization (WHO) developed a global monitoring framework in order to track the progress in preventing and controlling diseases like cardiovascular and chronic lung diseases, diabetes and cancer. At the 2012 World Health Assembly, nine global targets were endorsed and, in particular, member states agreed to a goal of reducing rates of premature death from NCDs by 25% by 2025 [86]. Actually, individual country and cancer site-specific studies suggest that the age-adjusted incidence of many common cancers has increased in European populations over the past two decades and, both in low and high-income countries, in the next decades, cancer could become a major cause of morbidity and mortality [19]. It is expected that the burden of mortality for cancer will increase by ~45% in 2030¹, because of both the growth and the ageing of the

¹The incidence of all cancers is estimated to increase from 12.7 million new cases in 2008 to 22.2 million by 2030

population [87, 88]. Moreover, also the costs for public spending on health could become an important factor, considering that in 2013 the annual cost of the essential package of cancer services would be about US\$13.8 billion and US\$1.4 billion, for high-income countries and low-income ones respectively [88]. A key point in the fight against cancer is the identification of new therapies and the improvement of promising or qualified ones. In particular, new methods for radiotherapy and new instruments to verify the treatment plan are expected to play a role, since 50% - 60% of patients undergo a radiotherapy treatment at a certain point, both through external beam, with linear accelerators (LINACs), and brachytherapy, with the placing of radioactive seeds inside the body close or in the tumour [89]. The ORIGIN project aims at developing instruments and methods for real-time dosimetry and source localisation during brachytherapy treatments, in case of prostate and cervical cancers, through an optical fibre and SiPM based sensor system.

In this chapter a brief and not exhaustive introduction to the brachytherapy and its applicability for prostate and cervical cancer treatment is presented.

3.1 Brachytherapy

Radiation therapy can be applied in the treatment of cancer either as a single therapy modality or in combination with surgery and/or chemotherapy. Radiotherapy exploits the higher sensitivity to radiation of some tumour tissues, destroying the cancerous cells with a prescribed amount of radiation expressed in terms of dose, i.e. deposited energy per unit mass (gray, Gy). Radiation therapy can be administrated through two modalities, also in combination: external beam radiation therapy (EBRT) and brachytherapy (BT) [17]. Up to 1950, most of the external beam radiotherapy was carried out with x-rays generated at voltages up to 300 kVp.

The subsequent development of higher-energy machines (linear accelerators with energies up to a few of MeV) and the increasing popularity of the cobalt-60 units in the 1950s and the 1960s resulted in a gradual demise of the conventional kilovoltage machines. External beams (gamma and x-rays, but sometimes also electrons) deposit part of the radiation dose along the way through the body tissue up to cancer. Instead, brachytherapy, from the Greek term $\beta\rho\alpha\chi\upsilon\varsigma$ - brakhus, which translates to “short”, is a method in which sealed radioactive sources delivered radiation at a short distance to the tumour through interstitial, intracavitary, or surface application. This method allows a very high dose and local delivery to the tumour, sparing the healthy tissue and the surrounding Organs At Risk (OARs), thanks to the inverse-square law, i.e. the radiation dose is inversely proportional to the square of the distance from the source. In the past, Radium and Radon sources were mainly used, while, currently, radionuclides such as ^{137}Cs , ^{192}Ir , ^{198}Au and ^{125}I , are more widespread [17]. The physical characteristics of the various radionuclides are reported in table 3.1

Radionuclide	Half-life	Photon energy (MeV)	Half-value layer (mm lead) ²
^{226}Ra	1600 years	0.047 - 2.45	12
^{222}Rn	3.83 days	0.047 - 2.45	12
^{60}Co	5.26 years	1.17 - 1.33	11
^{137}Cs	30 years	0.662	5.5
^{192}Ir	73.8 days	0.380 (on average)	2.5
^{198}Au	2.7 days	0.412	2.5
^{125}I	59.4 days	0.028 (on average)	0.025
^{103}Pd	17 days	0.021 (on average)	0.008

Table 3.1: Physical characteristics of the radionuclides used in brachytherapy [17].

²It represents the thickness of absorbing material, lead in this case, needed to reduce the incident radiation intensity by a factor of two.

A few years after the discovery of Radium by Pierre and Marie Curie (1898), it was used as brachytherapy treatment for skin carcinoma in Russia. Soon some limitations in the use of Radium became evident. The accurate position of seeds was difficult, because radium needles were rigid structures, and required high levels of surgical skill to achieve good clinical outcomes. Therefore, the working conditions were unsafe, exposing the medical staff to high dose [90, 91]. For these reasons, soon the brachytherapy with radium declined, in favour of the EBRT. When in the 1960s the first remote afterloading system (shown in figure 3.1) was developed, the possibility to transfer (robotically) the sources from a shielded safe container to their treatment position in the implanted applicators in the patient, brought back to life brachytherapy, supported by radiographic methods for an optimal positioning of the sources [16, 18]. These systems are

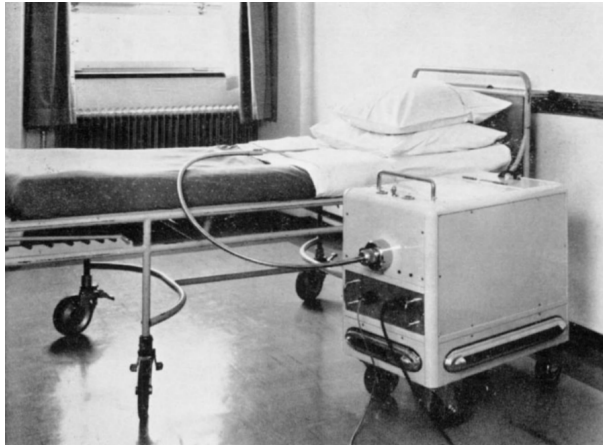


Figure 3.1: Picture of one of the first single-channel remote afterloading system [16].

expensive, but allow eliminating or reducing operating room staff exposure and undoubtedly provide superior techniques for optimisation and reproducibility of the treatment. Moreover, roughly in the same period, another relevant progress contributed to a renaissance of brachytherapy:

artificial radioactivity induced by particle accelerators. Initially, needles and wires of ^{60}Co and ^{137}Cs were the most widespread, nowadays ^{125}I and ^{192}Ir have a higher impact on brachytherapy treatments [91].

Brachytherapy sources are usually applied in three different ways: external applicators (actually not popular any longer), interstitial implantation and intracavitary therapy. The choice between these techniques is imposed by the type, the size and the localisation of cancer. For example, external applicators were used to treat small and superficial areas, like lips or ears. The applicators were prepared to conform to the surface to be treated. Typical distances between source and surface were 0.5 – 1 cm [92].

Nowadays, the other two methods are more widely used. In intracavitary therapy the applicators, containing the sources, are introduced in body cavities close to the tumour. They are mostly used for cancers of the uterine cervix and vagina. Different applicator geometries were designed in the past to hold the sources in a fixed configuration (figure 3.2) [17, 18]. In

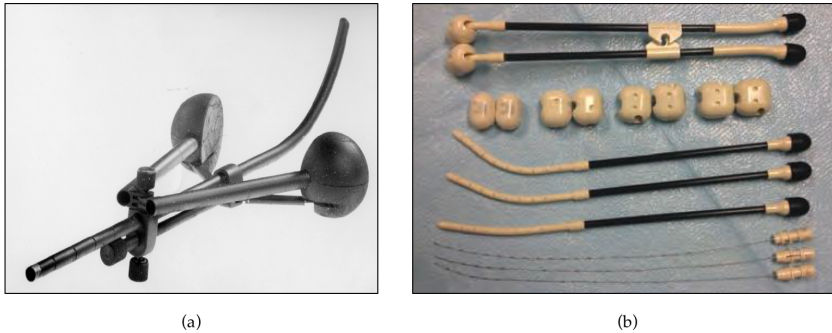


Figure 3.2: Examples of intracavitary applicators: (a) Fletcher-Suit Implants [17] and (b) Henschke's stainless steel afterloading applicator [18].

interstitial implantations, radioactive sources (produced as wires, needles and seeds) are inserted directly into the target volume (tissue) to be treated through catheters. This is the treatment of choice, for instance, in the case

of prostate cancers.

Moreover, it is usual to distinguish between temporary and permanent implantation. In the first case, the radioisotopes are removed after a certain time when the desired dose has been delivered. While, in the second one, the sources are left permanently in the implanted tissues [17]. In general, with temporary implantations is possible to reach a better control of radioactive distribution and, thus, dose delivery than with the permanent ones. However, the permanent implants are a one-time procedure and are preferred for some tumours such as those in the abdominal and the prostate [93].

Another important classification in brachytherapy takes into account the source rate, distinguishing between High Dose Rate (HDR) and Low Dose Rate (LDR). The International Commission on Radiation Units & Measurements refers to a dose rate of 0.4 to 2 gray per hour (Gy/h) as a low dose rate, 2 to 12 Gy/h as a moderate dose rate, and in excess of 12 Gy/h as a high dose rate [94]. This classification also implies different involved seed activities: usually, in case of LDR, few tens MBq, while for HDR some hundreds of GBq [17]. The LDR brachytherapy is the older procedure and it is, generally, performed with ^{125}I or ^{103}Pd as radioactive seeds. In this case, various radioactive seeds are inserted in the patient's body, according to a pre-set arrangement, and in a permanent way. A detail description of possible configuration that assures dose uniformity delivery within $\pm 10\%$ can be found in [17]. The most common diseases, treated with LDR brachytherapy, are some types of prostate cancers [93].

The diffusion of HDR brachytherapy was possible thanks to the introduction of the remote afterloading technology, that allow delivering precise treatment. Although LDR brachytherapy can be delivered through the same technology, prolonged treatments, that could require also hospitalisation of the patient, make HDR brachytherapy in the recent years a valuable and very widespread alternative to LDR one. Due to the high activities,

HDR treatments are often outpatient procedures, suitable for treating large numbers of patients. Another important advantage of HDR brachytherapy is the greater control over the dose distribution, that allows treating well-localized tumours with high dose. A typical HDR remote afterloading unit is shown in figure 3.3. It contains a single, high activity source, usually ^{137}Cs or ^{192}Ir . The source is a line (with diameter 0.3-0.6 mm and length 3.5-10 mm) welded to the end of a flexible drive cable. The unit presents several channels to which an indexer system can direct the source through catheters connected to the applicators in the patient's body. The common diseases treated with HDR brachytherapy are endobronchial obstruction by lung cancer [95], endometrial carcinoma [96] and localized prostate cancer [93, 97].



Figure 3.3: An example of an HDR brachytherapy unit by microSelectron.

3.2 Brachytherapy for prostate and cervical tumours

The aim of the ORIGIN project is the development of a dosimetry system for LDR and HDR brachytherapy during treatments of prostate cancers and cervical ones.

Prostate cancer is the most frequent neoplasia among males in Europe.

In 2020 the incidence is more than 23%³. It is the highest in northern and western Europe with more than 200 cases per 100.000 inhabitants, but also in the eastern and southern Europe, it is continuously increasing, as shown in figure 3.4⁴. This increase is due probably to better diagnostic techniques thanks to the introduction of prostate-specific antigen testing and consequent biopsy in asymptomatic men [19].

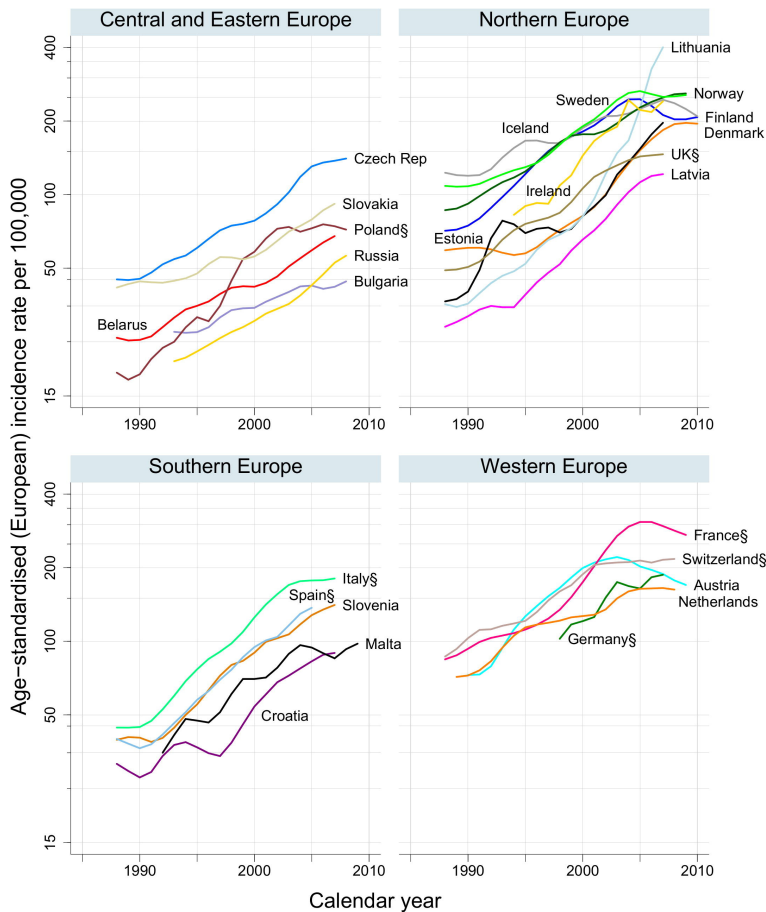


Figure 3.4: Incidence of prostate cancer in Europe [19].

³EUREG database <https://ecis.jrc.ec.europa.eu/>

⁴The 5-years survival rate is ~91% to be thorough.

Localised prostate tumours can be treated with different options, mainly based on radiotherapy (either EBRT or BT) and/or radical prostatectomy. The latter one can be carried out with traditional open surgery or with a more modern robot-assisted laparoscopic prostatectomy. The potential for a therapy (brachytherapy) that is equally efficient, but less damaging than others, is more attractive for early prostate tumours. Indeed brachytherapy is increasingly being used in the treatment of localised prostate cancer. At the moment, there is no evidence, in comparative studies, of the prevalence of one therapeutic approach. However, there has been a perception that brachytherapy is associated with a decreased morbidity and collateral effects [98].

The treatment plans, in case of brachytherapy, employ either permanent or temporary interstitial implantations with the dose delivered at either LDR or HDR. As already mentioned, the permanent implantations, that deliver low dose rates, usually use ^{125}I or ^{103}Pd (see table 3.1 for physics characteristics). Instead, the HDR therapy for prostate cancers uses ^{92}Ir seeds. The use of permanent implants firstly was pioneered by Withmore and Hilaris in the 1970s [17]. The modern approach was introduced in the 1980s and consists in inserting the radioactive seeds into the prostate gland, in a non-surgical way, with the help of transrectal ultrasonography and perineal templates, as shown in figure 3.5 [93].

The transverse and sagittal ultrasound images are necessary in order to obtain a gland volume study and, thus, draw the implantation target. Then a computer software allows the placement of seeds in the template grid to optimise the dose delivery to the target volume and try to minimise the dose at the organs at risk (OARs), like urethra, rectum and bladder. The typical single seed activity is 0.3 - 0.6 mCi (11.1 - 22.2 MBq) for ^{125}I and 1 - 1.8 mCi (37 - 66.6 MBq) for ^{103}Pd [93]. Usually 12 - 18 seeds are placed on the periphery of the gland at an inter-distance of ~ 1 cm. In the inner part (5 mm away from the urethra) other 4 - 7 seeds are inserted [93]. If

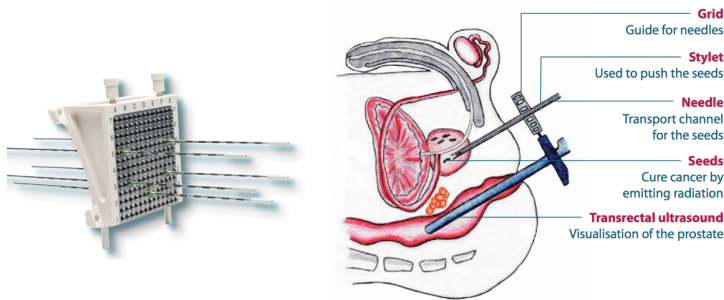


Figure 3.5: Current brachytherapy delivery templates for prostate (contour prostate template by Eckert & Ziegler - BEBIG).

compared to LDR, HDR brachytherapy offers different advantages. Firstly, from a practical point of view, there are no free sources used, no possibility to lose one of them, and, since it is a temporary procedure, less problem of radioprotection. Secondly, from a biological point of view, the ability to deliver intermittently high dose per fraction allows to reach high successful rates of cancer treatment. HDR brachytherapy is delivered either as a boost following an external beam radiotherapy treatment (the most widespread case) or as monotherapy. Treatments are usually based on the use of two to nine fractions of dose. Usually, two to four fractions are preferred, delivering a total dose of 26-36 Gy. The procedure is not very different from the LDR one. It exploits the transrectal ultrasound guided approach, fixing the single catheter position through a template. The modern HDR afterloading systems allow to define and manipulate the dose delivering using 2.5 or 5 mm dwell positions.

With respect to the prostate cancer, a different, more encouraging, situation is described by data for the cervical cancer. It is the fourth-most common cancer among women worldwide, with an annual incidence, in 2018, of 570000 cases and 311000 deaths, corresponding to an incidence

rate of 13.1 cases per 100000 women and to a death rate of 6.9 deaths per 100000 women [99]. In the last decades, the incidence decreases also by 50% thanks to improved screening methods and the human papilloma virus vaccine. However, a striking difference between low and high-income countries exists: approximately 84% of all diagnosed cervical cancer and ~88% of all deaths occurs in developing countries (it represents the second leading cause of cancer death among women). This implies an incidence rate of 26.7 cases per 100000 women in low-income countries, that drops to 6.9 cases per 100000 women in high-income ones [99]. In Europe the incidence is at the level of 2% (roughly a factor 10 lower than prostate cancer), although it increases to 4-5% in the 0-44 age group [5].

For this type of cancer, the standard care has evolved from EBRT alone to EBRT combined with brachytherapy and/or chemotherapy. The latter serves as boost of the disease control and survival, since it is the only method that provides the high dose required to control the cancer growth without undesired effects to OARs [6]. All women, that develop locally advanced cervical cancer, usually should be considered to be treated also with brachytherapy [100]. In the past, the treatment was based on LDR sources, with 1-2 fractions, using the isotope ^{137}Cs with a dose rate < 0.4 Gy/hour to the target volume. Since the beginning of 2000, HDR brachytherapy has overcome the LDR. The higher dose rate (~ 12 Gy/hour) is reached with ^{192}Ir isotope. At the moment, no clear evidence of the excellence of one of the two techniques exists, both in terms of outcome efficiency and toxicities [101]. Brachytherapy treatments are performed using intracavitary and interstitial approaches or a combination of them. The first one uses an applicator through the vaginal cavity and treats upper vagina, cervix

⁵Data from <https://ecis.jrc.ec.europa.eu/>

⁶Several investigations have demonstrated clinical correlation between rectal and bladder dose volume and toxicity. Less correlation has been observed for urethra and vaginal mucosa [100].

and uterus. In the second one, the transperineal/vaginal catheter template places the small tubes in and around residual disease. The choice, as usual, depends on the patient anatomy and the tumour extent [100].

3.3 In vivo dosimetry in brachytherapy

The ultimate goal of the ORIGIN project is the design and the qualification of an *in vivo* dosimeter for brachytherapy. Radiation dosimetry is a quantitative method for the determination of the energy deposited (dose) in a given medium directly or indirectly by ionizing radiations. The instruments that provide the reading of the dose are called dosimeters. Different dosimetry aspects, that influence the dose delivery accuracy, can be identified in brachytherapy processes. In figure 3.6 an example of these processes for HDR brachytherapy is reported. Four main different influ-

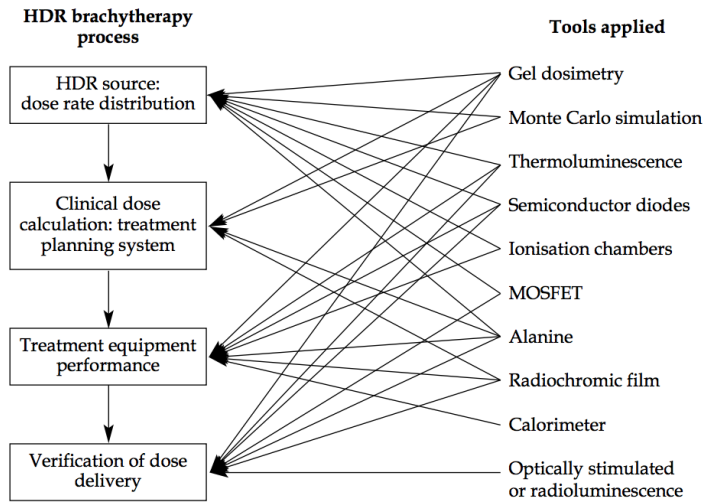


Figure 3.6: HDR brachytherapy dosimetric chain with some of the tools reported in the literature that have been applied to assess dosimetric accuracy [20].

encing factors can be highlighted [20]: (1) the source dosimetry, that allows to know the dose field around the source and any possible anisotropy; (2)

the treatment planning (i.e. the clinical dose), that has become more precise and accurate thanks to new model-based dose calculation algorithms; (3) the treatment equipment performance (e.g. the autoradiography of source dwell position), that has to be comprehensively commissioned and then regularly controlled; (4) the dose delivery verification to assess, through the in vivo dosimetry (IVD), the accuracy and effectiveness of the treatments. The ORIGIN project will contribute to the last point.

The increasing complexity and multiplicity of modern cancer care introduces the need of a comprehensive patient-specific quality assurance of the dose delivery during treatments. Indeed, the awareness of the potential risks associated with an incorrect dose delivery, especially to the OARs, is of growing interest. Although the recent introduction of 3D image-guided brachytherapy, nowadays various uncertainties persists. In vivo dosimetry may lead to improvements in situations with low energy emitted photons and in case of applicator and/or organ movements. Furthermore, the doses per fraction in brachytherapy are very large (>5 Gy). This entails that fractional error may be very significant, if compared to other modalities as EBRT. The risks are related both to underdosing (causing possible recurrence) and overdosing (inducing toxicity and, in extreme case, death). Unfortunately, IVD is not performed systematically. This lack of interest, in part, is due to the challenges of precise detector positioning and to the low number of available dosimetry systems.

Different errors and accidents occurs during a brachytherapy treatment as reported in ICRP reports number 86 [102] and 97 [103]. They are usually related to human errors and sometimes to malfunction of the equipment. In HDR brachytherapy there may be afterloading positioning errors, while in LDR possible source migration after the placement. Although ultrasound or computed tomography imaging is performed immediately before the dose delivery, organ or applicator movements in between imaging and treatments are a limitation of the planning treatment accuracy [104].

Moreover, in modern treatment planning systems persist some errors in dose calculation to tumour and OARs due to lack of corrections for heterogeneities and patient scatter [105].

An in vivo dosimeter should deal with some specific challenges. First of all, a dosimeter should exhibit a linear response and present a radiation energy independent response. Moreover, it should not show directional or angular dependence, a characteristic particularly important for IVD. The small size is another essential feature for IVD, allowing to develop minimally invasive dosimeters. Finally, an easy handling and functioning for the clinicians are fundamental features to reach a wide spread.

Although IVD is not performed usually in the brachytherapy workflow, various systems were developed and tested. They are based on thermoluminescent dosimeters (TLDs) [106], field-effect transition (MOSFET) [107], semiconductor diodes [108], alanine detectors [109], radioluminescence (RL) and optically stimulated luminescence (OSL) detectors [110]. A comprehensive review of these systems is reported in [32]. There is not yet an ideal detector for brachytherapy because every type presents some limitations as self-attenuation, angular anisotropy and energy dependence. In particular, TLDs have been used extensively for in vivo dosimetry. TLDs can provide integral dose readings at multiple points but do not provide any real-time information, because they require post irradiation processing to obtain a readout. Alanine dosimetry exhibits negligible energy dependence, high stability and small size, but, as TLDs, they do not provide real-time response. In addition to this, they are expensive. They also have some temperature and humidity dependence. Finally, semiconductor diodes can be used to provide data in real-time, however they exhibit a strong angular dependence and their sensitivity varies with the distance from the source. Optical fibre dosimeter represents a valid alternative that seems to present the most favourable combination of characteristics for dosimetry in HDR brachytherapy [110, 111].

3.4 In vivo dosimetry in brachytherapy: optical fibre sensors

Their small size, flexibility and lightweight make optical fibre based sensors suitable for minimally invasive in vivo dosimetry. Therefore, they are the sensor of choice for the ORIGIN project, due to the potential of being placed close to or in the tumour and/or alongside the radioactive seed. These sensors exploit the physical phenomenon of luminescence, i.e. the emission of light when radiation is absorbed. According to the type of exciting radiation, three kinds of luminescence can be distinguished: thermoluminescence (excitation through heat), photoluminescence (optical or UV light) or radioluminescence (directly or indirectly ionizing particle). The first two types will not be discussed because they are not inherent to the ORIGIN project, but some details can be found in [110, 32].

The phenomenon of radioluminescence is known also as scintillation. It exploits the capability of certain materials, called scintillators, to emit light, usually in the visible spectrum, when interact with a particle. A light detector, as Photomultiplier Tube (PMT) or SiPM, converts the light into an electrical signal [71, 32]. Scintillators are divided, according to the material in use, in two categories: organic and inorganic. The former are composed of aromatic hydrocarbons and the scintillation occurs at molecular level. They are classified as liquid or plastic. The inorganic one are crystals; thus the scintillation is due to the electron transition bands. In scintillator and optical fibre based-dosimeter the coupling between the fibre and the scintillator is a critical issue. Different configurations were proposed in the past. In some cases the scintillator is coupled to the tip of the fibre [112], in other cases the tip was coated with a mixture of scintillating material and glue [113]. A new manufacturing method was proposed by Woulfe et al. [114]: the tip was micromachined in order to create a hole of a few of mm long that hosts the scintillating powder mixed with an epoxy glue, increasing the coupling efficiency. On one hand, organic scintillators

present some advantages for dosimetry, since they are water equivalent and linear with dose [32]. A drawback, recently revealed, is the light yield temperature dependence [115, 116]. This can lead to significant differences between measurements performed in phantom and in vivo [116]. On the other hand, inorganic scintillators are often used in medical applications, especially in case of imaging thanks to their higher detection efficiency in case of X and γ rays because of a higher atomic number (Z) [117], even if they are not water equivalent. Since the atomic number is higher than water ($Z_{water} = 7.51$ [17]) for these materials, correction factors may have to be applied due to the different photon absorption cross-section. Two advantages of these scintillators are high sensitivity and high light yield (more than 5 times the plastic scintillator one) [117]. Finally a large variety of inorganic scintillators emitting in green/red wavelength region, are available. This feature allows to overcome the so-called stem effect [118].

3.4.1 Stem effect

The stem effect is the Cherenkov light produced in the optical fibre exposed to the radiation. Cherenkov light is produced in materials with refractive index (n) > 1 and when the speed of the particle, interacting with it, is $> c/n$, where c is the light speed. Since the PMMA refractive index is ~ 1.48 - 1.60 , Cherenkov light appears for an electron travelling in the medium at energies above 145-180 keV [119]. For any radiation with energy above this threshold level, the presence of fluorescent light generated in the optical fibre should be considered. A number of different techniques have been shown to minimise or remove this contribution. In case of plastic scintillator, a possible method to overcome the problem is the two fibres approach: one fibre hosts the scintillator in the tip, the second one, placed side-by-side, is a bare fibre (only PMMA). Assuming that for both fibres the irradiation conditions are the same, the signal of the bare fibre

can be subtracted to the scintillating one [120]. With the inorganic scintillator, emitting green/red light, the stem effect removal can be simplified. Indeed in this case the characteristics of the Cherenkov emission spectrum are exploited. The spectrum is continuous, it peaks in the ultraviolet, but decreases with the wavelength (λ) as λ^{-2} [119]. For these reasons, using a scintillator that peaks in the green or red spectrum region is more suitable than the plastic scintillator that emits in the blue one. The higher light yield of this type of scintillators in that part of the spectrum, it is possible to filter out the Cherenkov contribution interposing a bandpass filter between the fibre and the light detector [121].

Optical fibres represent an ideal solution for in vivo dosimetry due to their small size and electrical passiveness. Moreover their flexibility allows to guide them in or close to the tumour or alongside the radioactive seed within the existing equipment, without interfering with the usual medical staff workflow. Two examples of dosimeter based on this technology are briefly presented below. Both were developed and tested not only in phantom, but also during HDR brachytherapy treatments on patients. The first is the BrachyFODTM dosimeter (by Suchowerska et al. [122, 21]), shown in figure 3.7. It integrates a Bicron BC400 scintillator butt-coupled

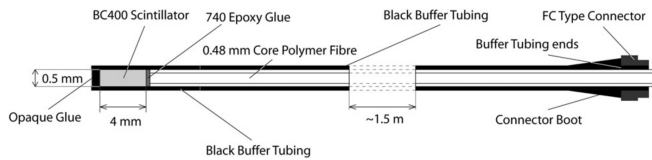


Figure 3.7: Sketch of the BrachyFODTM dosimeter. A Bicron BC400 scintillator (4 mm long and 0.5 mm diameter) is coupled to a 0.5 mm diameter PMMA optical fibre. To avoid environment light contamination it is covered with a black polyvinyl toluene tube [21].

to a PMMA optical fibre; different diameters, fitting the dimension of the standard catheters, were tested (1 and 0.5 mm). The scintillator is coated with a layer of aluminium to increase the amount of photons reaching the end of the fibre. The generated light impinges on a PMT, located outside the brachytherapy treatment bunker. The output of the PMT was measured through a Keithley 617 electrometer. During test on patients, a maximum variations of 9% from the calculated treatment planning system dose is measured. Although these excellent results, recent reports on the temperature dependence of plastic scintillators may impact their future use in radiotherapy applications. Another example of optical fibre based dosimeter consists of a small $\text{Al}_2\text{O}_3\text{:C}$ crystal coupled to a PMMA optical fiber (by Andersen et al. [123]). The peculiarity, but also the major complexity source, of this prototype is the capability to operate either in the radio-luminescence (measuring the absorbed dose rate in real-time) or photoluminescence mode (measuring cumulative absorbed dose post-irradiation). The light detector is a photomultiplier module (MP982 by Perkin Elmer) with 395 - 440 nm bandpass filters for luminescence detection. The system demonstrated excellent linearity with dose, with a reproducibility of 1.3%. The uncertainties in the comparison with the expected dose by the treatment planning system ranges from 5% to 8% in phantom. Fading problems, after ~ 80 days, were observed reporting energy dependence response that depends on the dose history of the system. At the moment all of these dosimeter consist of only one probe used for monitoring the dose at OARs, in particular at urethra.

Moreover, optical fibres represent the best alternative to the only dosimeter system nowadays available on the market and integrated in an afterloader system⁷ PTW Multichannel Dosimeter VIVODOS[®] (T10018) and PTW semiconductor probe type T9112/T9113 (PTW, Germany). Five probes are implemented, providing independent monitoring of the doses to the

⁷The HDR afterloader system is the SaviNova[®] by Eckert & Ziegler BEBIG

rectum and bladder/urethra (OARs) during HDR brachytherapy. The diameters of these probes are 7 mm and 3 mm for the rectum (schematic view in figure 3.8) and bladder probes respectively. The overall uncertainty of

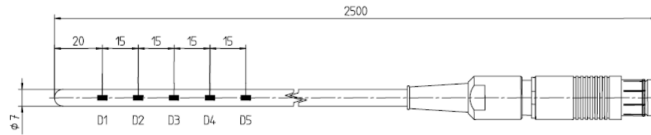


Figure 3.8: Scheme of the rectum dosimetric probe designed by PTW, hosting five diodes. The diameter of this probe is 7 mm. In figure all the dimensions are in mm. The bladder probe is similar. It has a smaller diameter (3 mm) and hosts only one diode [22].

the PTW T9112 probe relating to patient dosimetry measurements is reported to be 10%, with clinical use reporting differences between calculated and measured doses of up to 42% [124]. Such limitations have restricted its routine adoption in a clinical setting.

The ORIGIN project will exploit the optical fibre potentialities to develop an in vivo dosimeter, integrating an array of 16 fibres, optimising the existing technology in terms of optical coupling efficiency, scalability and mass fabrication. The principle of the proposed dose monitoring system is based on radiation-induced scintillation in inorganic material attached to a polymer optical fibre tip. The emitted light will be detected by SiPMs. Further processing of the dose information will provide precise localisation of the radiation for real-time source tracking during treatment. The higher number of single probes with respect to the PTW system will allow a better monitoring not only of the OARs but also close the tumour. Moreover, the small dimensions (1-2 mm of diameter), allow guiding them within existing brachytherapy delivery tubes or monitoring other targeted areas via conventional standard catheters and not only into anatomical orifices, like rectum. Finally, the ability to couple a large number of optical fibre sen-

sors paves the way for a significant advancement beyond the state of the art, by providing dose imaging capabilities. This will deliver dose-volume measurements in real-time during treatment, allowing for adaptive (patient specific) brachytherapy.

ORIGIN: scintillator characterisation

The dosimeter proposed by the ORIGIN collaboration exploits the phenomenon of scintillation. The light emitted by scintillators is transmitted to the SiPM through polymethyl methacrylate (PMMA) optical fibre. In order to perform a point dose measurement, only the tip of the optical fibre is sensitive to radiation^[1]. At the moment two different techniques to couple the scintillator and the fibre are adopted by two partners of ORIGIN. In the case of the HDR brachytherapy probe (assembled by DoseVue N.V.) the scintillator, glued at the end of the fibre, has a spherical shape with 1 mm diameter. Instead for the LDR one (prepared by University of Limerick), a cavity (700 μm in diameter and 7 mm in length) in the tip is micromachined and then filled with the scintillating material [114]. Currently, the optimal coupling technique for the final prototype is under investigation. Two different powder scintillators are tested^[2] $\text{Gd}_2\text{O}_2\text{S:Tb}$ (Gadolinium oxysulfide, GOS) for LDR probe and $1\text{Y}_2\text{O}_3\text{:Eu}+4\text{YVO}_4\text{:Eu}$ (Yttrium oxide + Yttrium orthovanadate, DV) mixture for HDR one. The choice of these scintillators are based on previous works [114, 118], which showed sensitivity to detect dose up to 3 cm (typical prostate dimension). Moreover, they enhanced the

¹In an ideal case, the stem effect is not present or at least should be negligible.

²Both are manufactured by Phosphor Technology <https://www.phosphor-technology.com/>

possibility to reduce the stem effect filtering the signal since their emission peaks in green/red visible light, where the Cherenkov light is less dominant. GOS is a typical material used in medical imaging, especially for X-ray detection [117]. The size grade of the powder particle is $4\text{ }\mu\text{m}$ with density 7.5 g/cm^3 . The emitted light shows three peaks at 490 nm, 545 nm (main peak) and 590 nm. The two scintillators of HDR mixture exhibits, according to the manufacturer, a size grade of the particles equal to $\sim 7\text{ }\mu\text{m}$. $\text{YVO}_4\text{:Eu}$ peaks at 619 nm and has a density of 5.3 g/cm^3 ; $\text{Y}_2\text{O}_3\text{:Eu}$ emits at 611 nm and presents a density of 5 g/cm^3 . Since both scintillating materials are powder, they are mixed with NOA61 glue, a liquid photopolymer that will cure when exposed to ultraviolet light, to guarantee an efficient coupling and manufacturing. The density of NOA61 is 1.231 g/cm^3 . The light yield (LY, i.e. emitted photons per deposited MeV) and the decay time of these scintillating powders are not provided by the producer. Moreover, various values are reported in literature [117, 125, 126]. The decay time, for instance, varies from a few hundreds of μs to ms. The variability may depend on the scintillator mixture ratio, the grain size of the powder particle and the presence of the glue. The physical characteristics of the scintillators are crucial for the development and qualification of the proposed dosimeter and their qualification is reported in this chapter. Initially, the scintillating materials and the energy deposited by radioactive sources are modelled using a GEANT4 simulation, a Monte-Carlo based toolkit for the studies of the particle interactions in the matter. Then, through laboratory measurements, the number of photons impinging on the SiPM (the effective light yield) and the time distribution of photon arrival are determined.

4.1 Scintillator characterisation

In order to correlate the energy, deposited by ionising particles emitted by γ or β source, to the light emitted, a simulation based on GEANT4.10.3.p01³ was implemented. The scheme at the base of this investigation is outlined in figure 4.1. The deposited energy (E_{dep}) along the ionisation path is con-

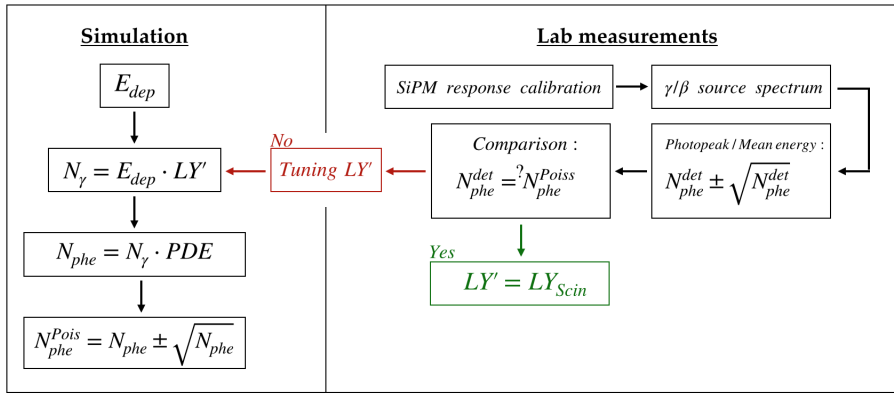


Figure 4.1: Scheme of the idea in order to exploit GEANT4 simulation and laboratory measurements to estimate the photons impinging on the sensor.

verted into number of photons (N_γ), assuming a certain light yield (LY'). The number of photons detected (N_{phe}) is estimated using the photon detection efficiency of the detector. Finally, the LY' is tuned in order to obtain the number of photoelectrons measured in laboratory (N_{phe}^{det}). This latter is measured in laboratory converting the deposited energy of a γ photopeak or the mean deposited energy by a β source in photoelectrons (the SiPM response is calibrated in photoelectrons through the multiphoton peak spectrum).

³<https://geant4.web.cern.ch/node/1>

4.1.1 GEANT4 simulation: interaction of γ s and β particles within the scintillators

Dedicated scintillator samples (mixed with the NOA61 glue) were prepared by the partner group at Vrije Universiteit Brussel. The samples of each scintillator, shown in figure 4.2(a), are cylinders with 5 mm diameter and 0.5, 1, 3 and 5 mm thick. The scintillating material is inserted in a PMMA ring-shaped holder (4 cm in diameter). In these samples there is not a reflective coating material.

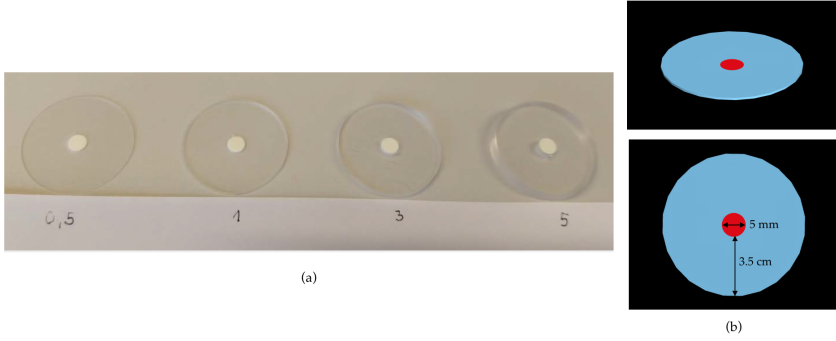


Figure 4.2: (a): A picture of the four different samples prepared for the tests for GOS scintillating powder. (b): Simulated geometry of the samples. The red disk corresponds to the scintillator, while the light blue disk is the PMMA surrounding the scintillators.

The sample geometry and materials are defined in the simulation toolkit. The simulated sample is shown in figure 4.2(b). The holder volume is filled with PMMA, according to the characteristic reported in the NIST database [127]. In GEANT4, a composite material made from other materials is simulated as a single homogeneous material, with the atomic number, evaluated according to the formula:

$$Z_{eff} = (Z_1^{2.94} \cdot a_1 + Z_2^{2.94} \cdot a_2 + \dots + Z_n^{2.94} \cdot a_n)^{\frac{1}{2.94}} \quad (4.1)$$

where $Z_1, Z_2, \dots, Z_n$ are the atomic number of each element of the mixture⁴ and $a_1, a_2, \dots, a_n$ are the fractional contributions of each element to the total number of electrons in the mixture [129, 128].

The $\text{Gd}_2\text{O}_3\text{:Tb}$ is defined with 0.05% Terbium (Tb) doping into Gd_2O_3 . The percentage was measured through a SEM (scanning electron microscope) analysis of the pure scintillator, performed by University of Limerick. The Z_{eff} of this scintillator is 60.10. Instead, in the case of DV scintillators, the percentage of Europium (Eu) is unknown. It was tuned in order to obtain the same Z_{eff} reported in [118], that is the reference for DoseVue N.V., ($Z_{eff} = 36.1$ for $\text{Y}_2\text{O}_3\text{:Eu}$ and $Z_{eff} = 31.1$ for $\text{YVO}_4\text{:Eu}$). This percentage corresponds to 0.25% of Eu for both. Then the composite mixture is defined by the weighted average of the two materials in ratio 1:4, obtaining a mean value of Z_{eff} of 32.10 and a mean density of 5.24 g/cm^3 . All the physical characteristics of the sample materials are reported in table 4.1.

Material	Atomic number Z_{eff}	density ρ [g/cm^3]
PMMA	6.64	1.19
$\text{Gd}_2\text{O}_3\text{:Tb}$	60.13	7.50
$1\text{Y}_2\text{O}_3\text{:Eu}+4\text{YVO}_4\text{:Eu}$	32.10	5.24

Table 4.1: Physical characteristics of the sample materials

To validate the simulation and the definition of the materials, measurements using a γ source have been performed. In particular, a comparison between the obtained results and the γ mass attenuation coefficient in the NIST database⁵ is performed. Unfortunately, only data concerning the Gd_2O_3 are present in the NIST database⁶. Therefore to reinforce the

⁴The exponent 2.94 results considering the dependence of the photoelectric and Compton scattering cross sections [128].

⁵<https://www.nist.gov/pml/x-ray-mass-attenuation-coefficients>.

⁶In the NIST is not considered the Tb doping, but the effect on the results is negligible since its influence on Z_{eff} is less than 0.004%.

procedure, a third scintillator, present in the database, is simulated: caesium iodide (CsI). 100000 monoenergetic (500 keV⁷) γ events are generated through the General Particle Source (GPS) in the same position: just above the centre of the scintillator. The possible lateral leakage contribution is investigated increasing the diameter of the scintillator and it was found to be always negligible ($\leq 0.1\%$). The attenuation is studied for the 4 different thicknesses: 0.5, 1, 3, 5 mm. Exemplary spectra of the simulated deposited energy are reported in blue in figure 4.3 for the GOS and DV scintillators 0.5 mm thick. The percentage of the attenuated γ is reported in table 4.2. The theoretical attenuation is calculated as:

$$\frac{I(x)}{I_0} = e^{-\mu \cdot x} \quad (4.2)$$

where x is the material thickness, I_0 is the initial intensity of the beam and μ is the attenuation coefficient extrapolated from NIST: 0.80 cm^{-1} for GOS and 0.44 cm^{-1} for CsI. The differences do not exceed a few per mille, confirming the reliability of the material construction and validation in the simulation. Then the mixture with NOA61 glue is considered. It is sim-

Thickness [cm]	Percentage %				
	CsI		GOS		DV
	Measured	Theoretical	Measured	Theoretical	Measured
0.5	2.2	2.2	3.9	3.9	2.2
1	4.2	4.3	7.6	7.7	4.4
3	12.1	12.4	20.9	21.3	12.4
5	19.5	19.8	32.2	32.9	19.7

Table 4.2: Percentage of attenuated γ in three different materials: CsI, GOS ($\text{Gd}_2\text{O}_2\text{S:Tb}$) and DV ($1\text{Y}_2\text{O}_3\text{:Eu}+4\text{YVO}_4\text{:Eu}$).

ulated as an epoxy glue ($\text{C}_{18}\text{H}_{19}\text{O}_3$) with a $Z_{eff}=6.31$ and density 1.231

⁷This value is the closest one to the ^{22}Na photopeak of 511 keV tabulated in the NIST database. The ^{22}Na is planned to be used in the experiments, as described below.

g/cm^3 . The densities of the resulting materials (60% scintillator + 40% glue) are defined as the weighted average and correspond to $4.99 \text{ g}/\text{cm}^3$ for GOS and $3.64 \text{ g}/\text{cm}^3$ for DV. The variations of γ attenuation are summarised in table 4.3. In figure 4.3 it is shown also how the spectra of the deposited energy changes when the glue is considered. As expected, due to the lower density and Z_{eff} the γ interaction cross-section decreases for both scintillators.

Thickness [cm]	Percentage %			
	GOS		DV	
	Without Glue	With Glue	Without Glue	With Glue
0.5	3.9	2.6	2.2	1.6
1	7.6	4.9	4.4	3.1
3	20.9	13.9	12.4	9.1
5	32.2	22.0	19.7	14.7

Table 4.3: Percentage of attenuated γ considering the mixture of GOS (60%) and NOA61 glue (40%) and DV (60%) and glue (40%) or pure scintillators.

Once the materials are validated, the second step of the simulation consists in the implementation of the γ and β radioactive sources. As explained in figure 4.1, this is a necessary step that allows a comparison between the results extracted by the simulation and the measurements performed in laboratory. The quantification of the scintillator light yields can be determined by the possibility to detect a γ photopeak or exploiting the average energy deposited by a β particle. The two sources used for the laboratory tests were ^{22}Na and ^{90}Sr . According to the sources available in laboratory and produced by Spectrum Techniques, the radioactive material is placed inside a plastic disk of 2.56 cm diameter and 3 mm thick, uniformly deposited only in a small area of 6 mm of diameter, at a height (from the bottom) of 1 mm for ^{22}Na and of 0.5 mm for ^{90}Sr . Figure 4.4 reports an example of the distribution of the simulated particle initial po-

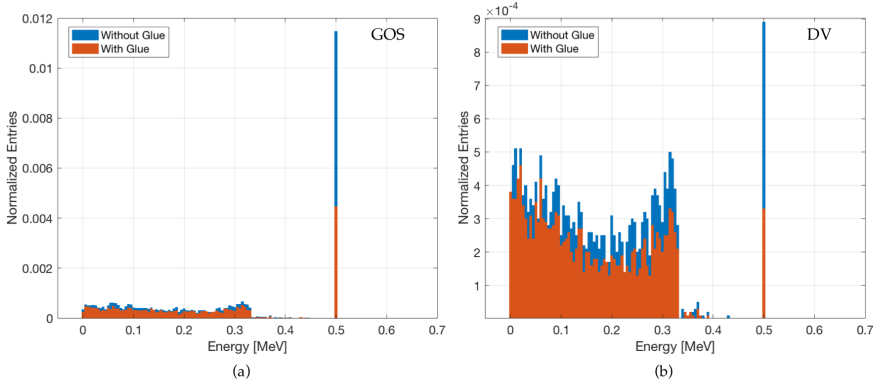


Figure 4.3: Energy deposited spectra of 500 keV γ rays in GOS (a) and DV (b) considering the pure scintillator or the mixture with NOA61. As expected, the photopeak at 500 keV is present and it decreases when the glue is added because the Z_{eff} and the density are lower. The Compton edge corresponds to the energy of 330 keV, as expected from the theoretical formula $E_{edge} = \frac{2E_\gamma^2}{2E_\gamma + m_e}$, where E_γ is the energy of the γ rays and m_e is the electron mass in energy [23].

sition in the source disk. Figure 4.5 shows the decay schemes of the two

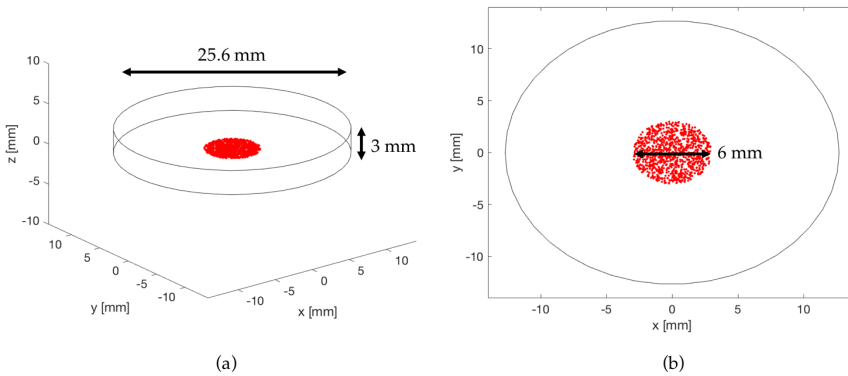


Figure 4.4: Simulated radioactive seed distribution inside the ^{22}Na plastic source disk.

sources; the branching ratios of the processes are already implemented in the adopted GEANT4 physics list.

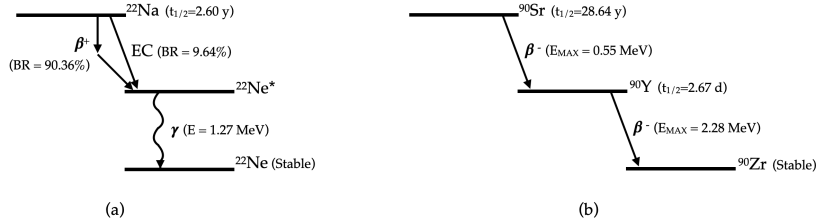


Figure 4.5: Decay schemes of the two sources implemented in the simulation: ^{22}Na (a) ^{90}Sr (b).

The ^{22}Na deposited energy spectra in the 0.5 mm thick scintillators⁸ are shown in figure 4.6. The ^{22}Na photopeak at 511 keV is more prominent in

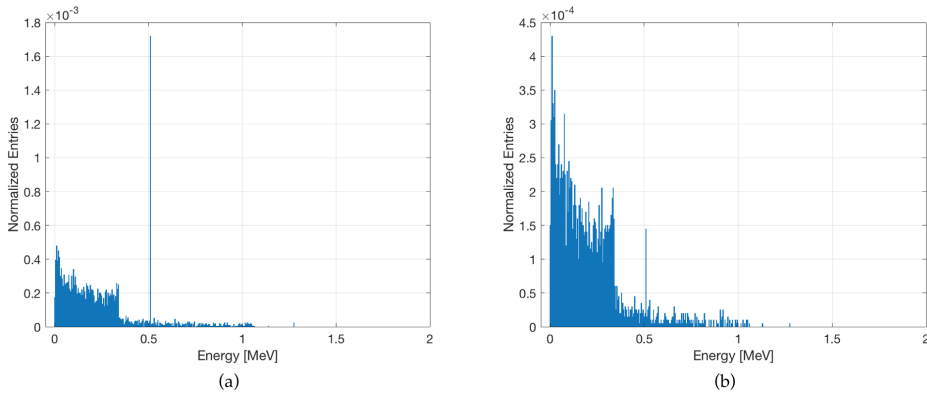


Figure 4.6: ^{22}Na Monte-Carlo truth absorbed energy spectra in the 0.5 mm thick for GOS (a) and DV (b) scintillators.

GOS, because of the higher γ cross-section with respect to DV. Instead, the photopeak at 1.27 MeV is not present in both scintillators. In particular, in DV the Compton effect is the dominant phenomenon.

⁸The results are shown only for this thickness because, as explained below, the measurements are performed only with it.

The ^{90}Sr deposited energy distributions are shown in figure 4.7(a) and 4.7(b) in 0.5 mm thick GOS and DV scintillators respectively. These spectra

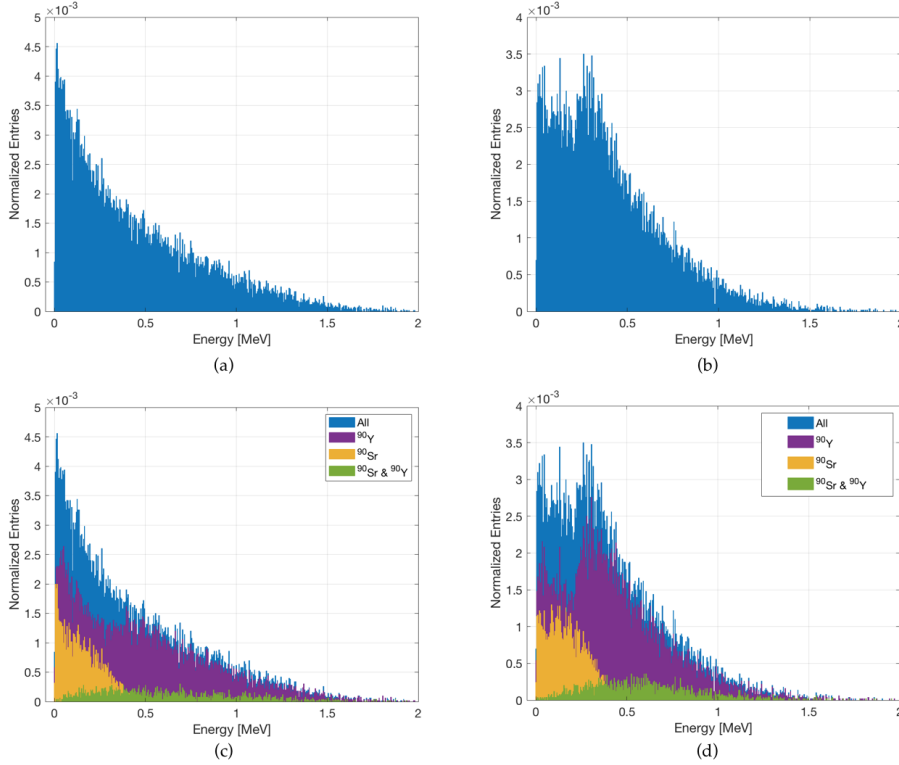


Figure 4.7: ^{90}Sr deposited energy spectra in the 0.5 mm thick for GOS (a) and DV (b) scintillators. The different contributions to the total spectra are highlighted for GOS (c) and DV (d). The purple distributions correspond to the deposited energies only when the ^{90}Y interacts in the scintillator. Similarly, the yellow distributions represent only the ^{90}Sr interacting events. Then the green ones describe the events with energies deposited by both elements.

are the superposition of contributions by ^{90}Sr and ^{90}Y in secular equilibrium. These contributions are highlighted in figure 4.7(c) and 4.7(d). The purple distributions represent the events where the energy is deposited only by ^{90}Y , where ^{90}Sr decays and deposits energy outside the scintilla-

tor or in the radioactive source disk or in the PMMA disk surrounding the scintillator. The yellow ones are due only to ^{90}Sr interactions, while the green distributions correspond to events in which both ^{90}Sr and ^{90}Y deposit energy. In the latter case the mean energy is 0.3 MeV, as expected by the theoretical distribution [130, 131]. In general, the average deposited energy, in this samples, corresponds to 0.40 keV for DV and 0.44 keV for GOS scintillators and these are the quantities that can be correlated to the detected photons.

4.1.2 Optical characterisation of the scintillating samples

As shown in figure 4.2, the scintillating materials are completely white, indicating a low transmittance, i.e. the ratio between the emerging and the incident flux of light intensity through a sample. At Vrije Universiteit Brussel the transmittance of the samples is measured. The evaluation occurs through a halogen source and a spectrum analyser with an integrating sphere. Figure 4.8 reports the transmittance as a function of the impinging light wavelength. A very low transmittance is measured for both scintillators: it corresponds to $\sim 11\%$ at 544 nm, the emission peak of GOS, in the 0.5 mm thick sample. At the same thickness the DV powder exhibits even a lower value: at 611 nm the transmittance is $\sim 4\%$. The transmittance drops with the thickness and it is almost negligible for the samples 3 mm and 5 mm thick.

4.1.3 Laboratory measurements

Due to the previous result, it was decided to perform lab tests with the radioactive sources only for the thinner samples (0.5 mm thick). Exposing the samples to ^{22}Na , a very low light emission (a few of photoelectrons) was observed, making difficult or impossible reconstructing the spectrum. To increase the probability of particle interaction ^{90}Sr was used, in order to re-

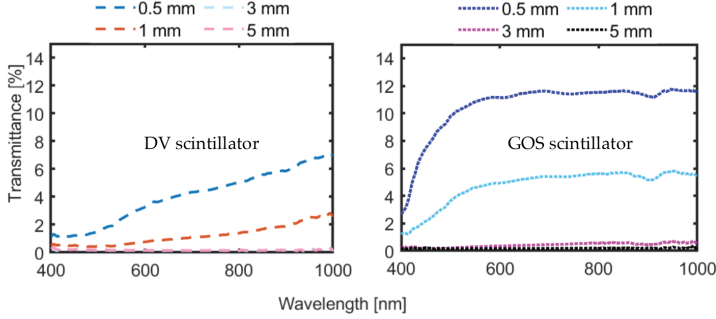


Figure 4.8: Measured transmittance as a function of impinging light wavelength for the samples of DV (left) and GOS (right) scintillators.

construct the energy deposited spectrum. The set up of the measurements is shown in figure 4.9. The scintillator disks were coupled to a $6 \times 6 \text{ mm}^2$ Hamamatsu S13360-6050CS SiPM operated at 55.5 V ($V_{ov} = 3 \text{ V}$). The signal was amplified by an AC coupled three stage inverter amplifier. Then a desktop digitizer DT5720 (CAEN S.p.A.) digitised 1.2 ms long waveforms with a resolution of 12 bit 2 V peak to peak and a sampling rate of 250 Ms/s⁹. To trigger the acquisition an undesired effect was exploited: the direct detection of β particle by the SiPM [132, 133]. Indeed looking at the signal, the presence of two classes of events was observed. As reported in figure 4.10, a fast and high amplitude signal (mean value $\sim 30 \text{ phe}$ with a decay time of 80 ns, compliant with the SiPM signal time development) is present and followed by a longer train of single photoelectrons. The former corresponds to a direct detection of the β particle in the SiPM, while the latter is the source-induced scintillation signal. The long and low intensity tail is highlighted averaging all the acquired waveform. Figure 4.11 shows the average signals for both scintillators. In case of GOS, the tail has a higher amplitude, therefore it is expected that more photoelectrons are

⁹The measurements were performed also with the desktop digitizer DT5730 with 14 bit and 500 Ms/s, but, due to the limited memory buffer, the DT5720 allows to record longer waveforms.

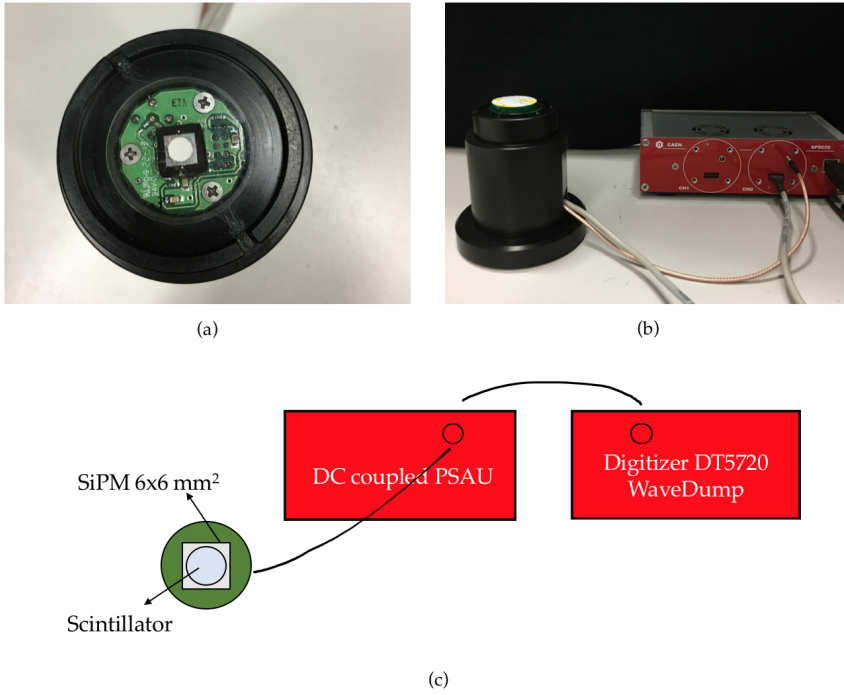


Figure 4.9: (a): top view of the scintillator disk, inserted in the PMMA holder, placed on the top of a $6 \times 6 \text{ mm}^2$ Hamamatsu S13360-6050CS SiPM. (b): front view of the set up for the measurements of the LY and decay time of the scintillators. (c): sketch of the setup: the SiPM output signal is amplified and digitised at 250 Ms/s (1.2 ms long waveforms are acquired).

detected by the sensors. Moreover, the hypothesis of correlating the trigger signal to the direct detection of the particle is confirmed by the spectrum of only the fast signal. It looks similar when the scintillator is interposed or not between the source and the SiPM, as shown in figure 4.12. When the scintillator is removed any train of photoelectrons is observed, as displayed by the mean signals in the top row of the figure 4.12(a). Although a 100% correlation between these two classes of events is not guaranteed, the direct detection phenomenon is exploited as trigger. Namely, if there

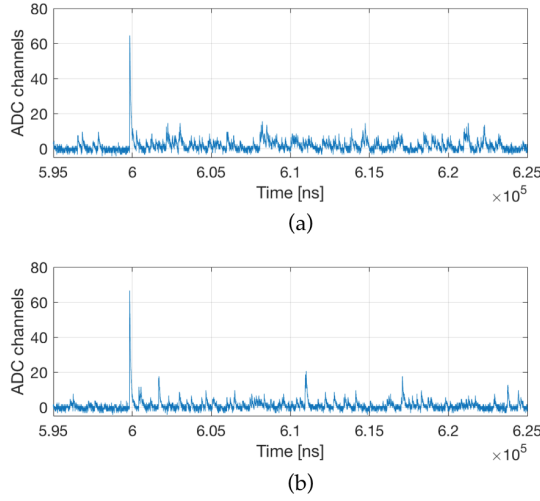


Figure 4.10: Zoom of exemplary recorded waveforms close to the trigger. The high signal, due to the direct detection of β particle, is followed by signals of few photoelectrons induced by the scintillation phenomenon. The same behaviour can be seen both in the GOS (a) and DV (b) samples.

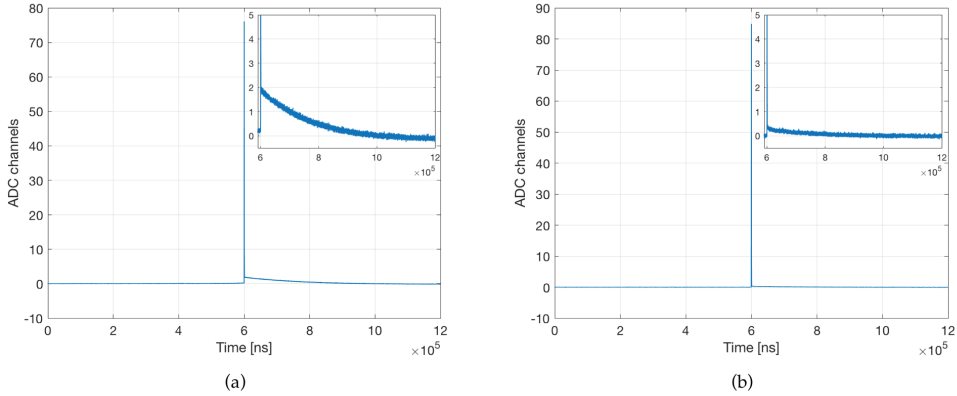


Figure 4.11: Average signals, sampled at 250 Ms/s and 12 bit, when β particles interact in the GOS (a) and DV (b) scintillator.

is a direct detection the particle has crossed the scintillator having a cer-

tain probability to interact within it, generating visible light. In the 1.2 ms digitised waveform the trigger signal lies in the middle at $600\mu\text{s}$.

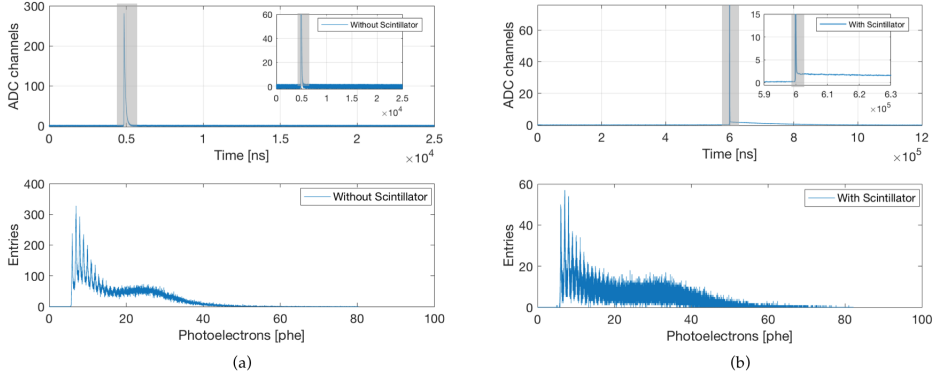


Figure 4.12: Comparison of the generated signals and the spectrum of the trigger signal in absence (a) and in presence (b) of the scintillator GOS between the β source and the SiPM. Different y-scales are due to different sampling of the signals. The signals without the scintillator is sampled with the DT5730 at 500 Ms/s and 14 bits, while with the scintillator the DT5720 at 250 Ms/s and 12 bits is used.

The information about the detected scintillation light intensity is retrieved by the statistics of the number of generated pulses in the $600\mu\text{s}$ after the trigger signal. Therefore event by event a procedure for the pulses discrimination were implemented and the difference between the number of photoelectrons after (DCR + scintillation) and before (only DCR) the trigger is computed. The waveforms were smoothed in order to reduce the noise fluctuations, reducing errors in the peak identification. The smoothing method consists of a moving average filter. It replaces each data point with the average of the neighbouring points in a defined data subset¹⁰.

The peak identification is performed through a post-processing procedure, based on the Differential Leading Edge Discriminator (DLED) algorithm [134]. As shown in figure 4.13, it reproduces a baseline-subtracted

¹⁰In this case the number of neighbouring points are fixed to 3, i.e. 12 ns.

signal, computing the difference between the SiPM signal (blue one in the top of the figure) and its replica delayed by ΔT_{del} (orange one in the top of the figure). It exploits the difference between the rising and falling edge

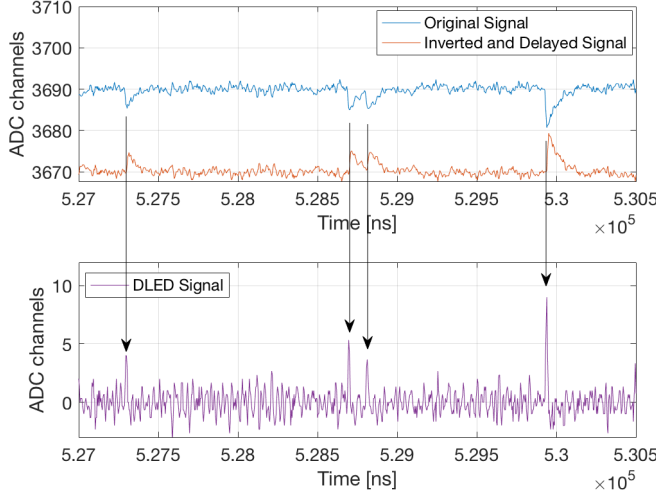


Figure 4.13: Exemplary digitised signal in which the DLED algorithm is applied. In the figure on the top the original signal (blue) and the ones delayed (orange) of 12 ns (3 digitised points) are displayed. On the bottom, the resulting signal when the DLED is applied is shown. The arrows highlight the peaks identified by the post-processing procedure.

of the signal, in order to obtain a better separation of pulses [134] and count all the pulses even if overlapped. Then the shortened signal (purple one in figure 4.13) can be discriminated computing the number of pulses above a fixed threshold. The tuning of threshold was performed previously on a noise run, where the signals are generated only by DCR. Finally the pulses before (B.T.) and after (A.T.) the trigger windows were counted and the difference between them was computed. DCR values of 1.1 MHz was measured. Any differences between the two windows should be detected in the noise run and it is confirmed by the distributions in figure

4.14. The difference distribution peaks around zero. The results on this run was summarised in table **4.4**

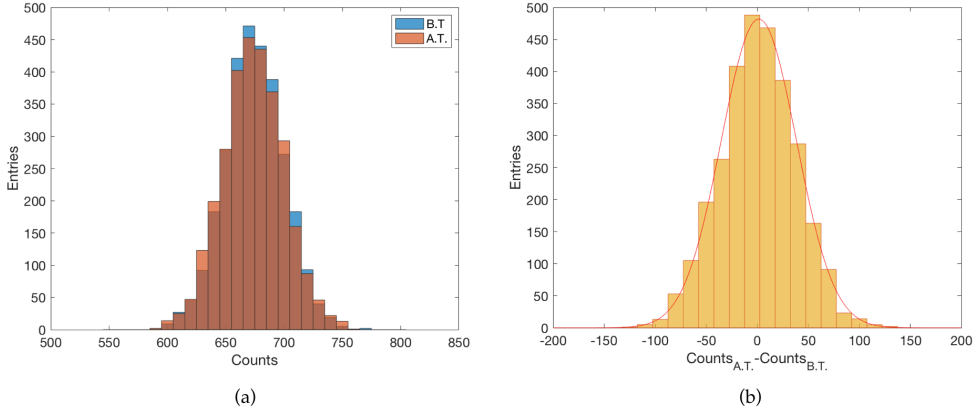


Figure 4.14: (a): noise count distribution before (blue) and after (orange) the trigger. (b): difference between the counts after and before the trigger. Its mean value (from the gaussian fit) is 0.1 ± 0.5 counts.

DCR at the oscilloscope	DCR in the B.T. window	DCR in the A.T. trigger
1 MHz	$1.1 \text{ MHz} \pm 777 \text{ Hz}$	$1.1 \text{ MHz} \pm 801 \text{ Hz}$

Table 4.4: Comparison of the SiPM DCR values obtained counting the pulses at the oscilloscope and then obtained on the recorded waveforms after the post-processing procedure was applied. The errors are evaluated as $\sigma_{DCR} / \sqrt{N_{events}}$ with $N_{events} = 2970$, i.e. the number of acquired waveforms.

The same procedure, applied on runs with the scintillators and β source, shows an excess of photoelectrons in the A.T. window compared to the DCR, as shown in the signals in figures **4.15(a)** and **4.15(b)** for GOS and DV respectively. In figure **4.16** the distributions of the number of signals in B.T. and A.T. windows are shown. The difference in counts between these two window is displayed in figure **4.17**, highlighting an excess of detected photoelectrons. In GOS the distribution has a mean value of 529.3

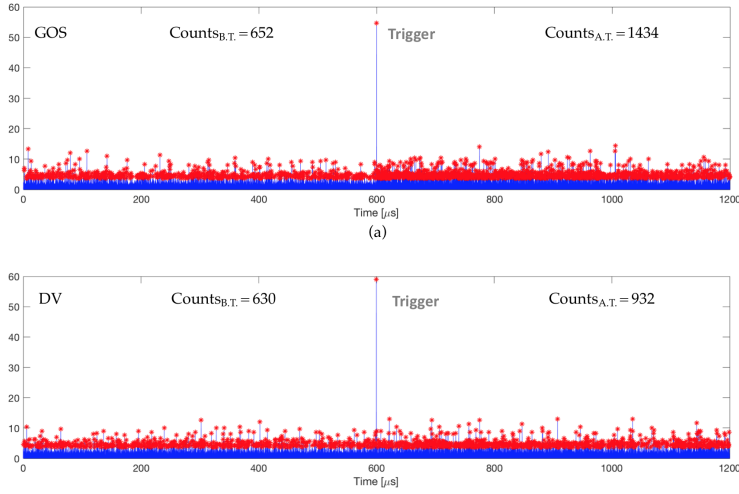


Figure 4.15: Exemplary waveforms of GOS (a) and DV (b) where the red points represent the identified pulses after the DLED algorithm and the peak identification.

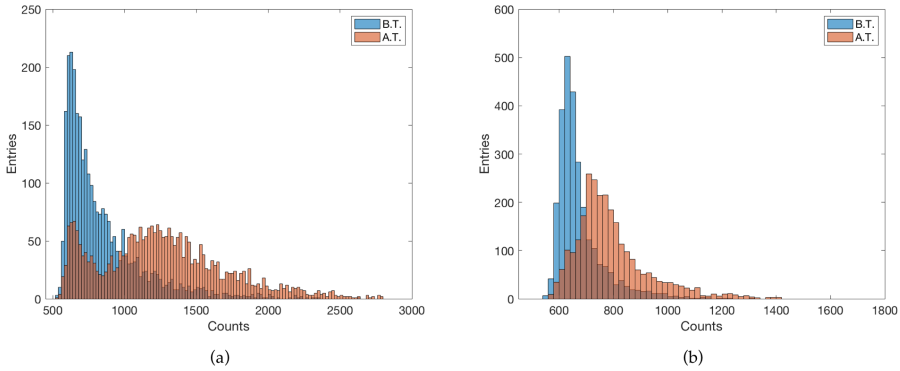


Figure 4.16: (a): Counted pulse distribution before (blue) and after (orange) the trigger with the GOS scintillator. (b): The same distribution for the DV scintillator.

phe, while in DV of 119.5 phe. They highlight also that different classes of events are present in the samples. In particular, in the events corresponding to the peak centred at 0 there is no scintillation (only direct detection).

It is clearly visible in the data for GOS scintillator, the separation is made more difficult in DV by the lower light emission.

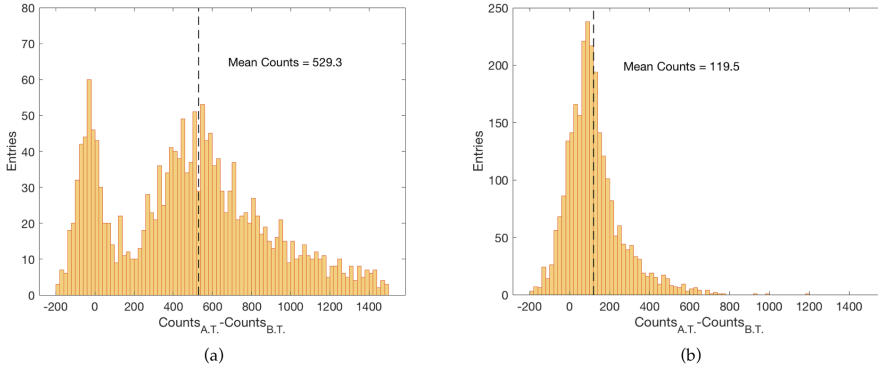


Figure 4.17: (a): The distribution of the pulse excess due to the scintillation phenomenon when the β particles interact for the GOS scintillator. (b): The same distribution for the DV scintillator.

Event selection

Different classes of events may be distinguished in the sample: proper scintillation events occurred after the trigger, events with only direct detection and pile-up events (with more than one scintillation signal in the recorded waveform). The classification of these events can be carried out counting pulses above 0.5 phe in a fixed time window ($\Delta T = 10\mu s$). The window scrolls through the entire waveform event by event before and after the trigger, as shown in figure 4.18. The counts in the first $10\mu s$ A.T. and B.T. sides are computed, then in the second $10\mu s$ and so on. In the following analysis this method is referred as “differential method”. Ideally, as shown in figure 4.19(a), in the B.T. side a flat distribution of counts is expected, since the DCR is constant. In the A.T. side, instead, a decreasing distribution of photoelectrons, due to the scintillation, is expected. In first approximation, a linear fit approaches the exponential decreasing of the

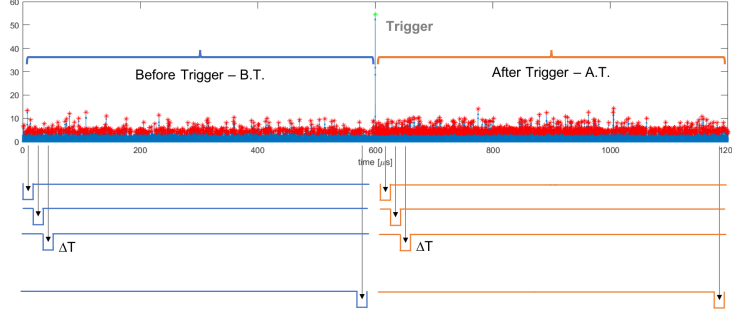


Figure 4.18: Sketch of the differential method, where for both B.T. and A.T. side, the time window, kept constant to $\Delta T = 10\mu s$, is moved through the whole waveform.

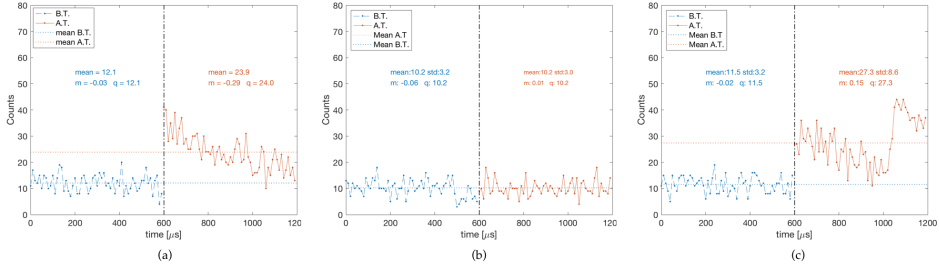


Figure 4.19: (a): An example of counts distribution in the B.T. and A.T. sides in the ideal waveform. (b): The counts distribution when only direct detection is registered. (c): an example of the counts distribution in case of an uncorrelated scintillation event in the A.T. side.

emitted photons and the correlation between the angular coefficient (m) and the y-intercept (q) can be exploited for the selection. A good event has m negative and q corresponds to the mean value of counts (greater than the noise counts). When there is no scintillation (figure 4.19(b)) both in the A.T. and B.T. sides $m = 0$ and $q = C_{noise}$, where C_{noise} corresponds to

the counts due to the DCR (1 MHz) in $10 \mu\text{s}$. Instead, when a scintillation event occurs uncorrelated with the trigger, in the A.T. side, the angular coefficient is positive (figure 4.19(c)). Finally, the scintillation event that occurs before the trigger are avoided imposing a cut on the noise distribution in the B.T. window. The cuts on for this selection are defined on the linear fit of the counts in a noise run (without radioactive source on the scintillators). The noise distribution for the two variables q and m in $10 \mu\text{s}$ is shown in figure 4.20. The gaussian fit of the distributions reports

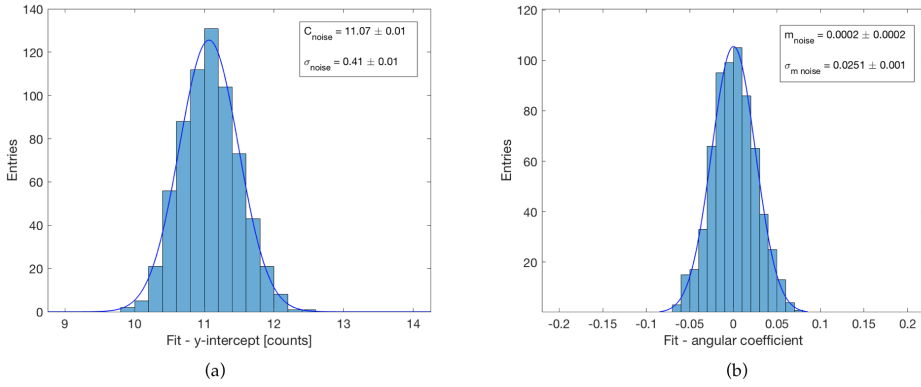


Figure 4.20: (a): y-intercept (q) distribution of the linear fit of the counts in $10 \mu\text{s}$ in a noise run. The mean value of the distribution is 11.07 ± 0.01 with a standard deviation, extracting from the gaussian fit, of 0.41 ± 0.01 . (b): angular coefficient (m) distribution of the linear fit of the counts in $10 \mu\text{s}$ in a noise run. The mean value is 0.0002 ± 0.0002 , compatible with zero, i.e. a flat count distribution in time, with a standard deviation from the gaussian fit, of 0.025 ± 0.001 .

a mean value of $q = C_{noise} = 11.07 \pm 0.01$, with a standard deviation of 0.41 ± 0.01 . The mean value of the angular fit m is 0.0002 ± 0.0002 , compatible with zero, i.e. a flat count distribution in time, with a standard deviation of 0.025 ± 0.001 . Therefore, only the events with a number of counts $C_{cut} \leq C_{noise} + 3 \cdot \sigma_{noise} = 12.30$ counts in the B.T. side are considered. Then in the A.T. side, the events with counts higher than $C_{cut} = 12.30$

counts and with a negative angular coefficient higher than $m_{cut} = -0.075$ counts/s ($3\sigma_{mnoise}$) are selected. In figure 4.21 the scatterplot of the two variables of the linear fit in the A.T. side for GOS scintillator reveals the capability to distinguish different types of events.

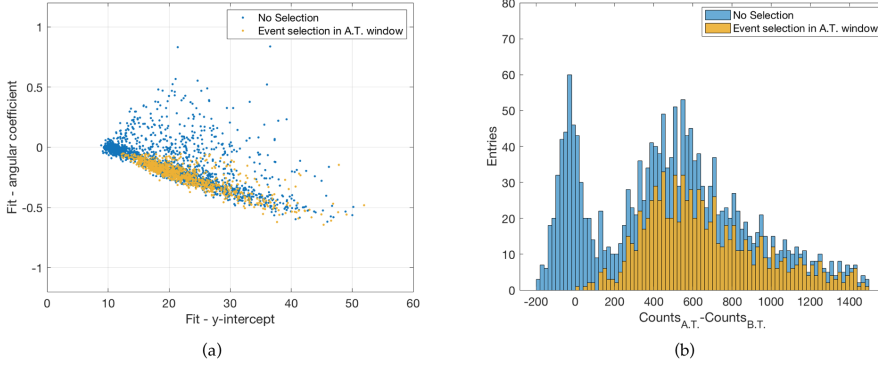


Figure 4.21: (a): The scatterplot of the angular coefficient and the y-intercept of the linear fit performed on the counts as a function of time per each waveform. The blue points correspond to all the events, the yellow ones are the selected events ($\sim 36\%$ of the total) (b): The distribution of the pulse excess in GOS with the event selection (yellow) and without (blue).

This selection allows to obtain the yellow distribution of detected photoelectrons, shown in figure 4.21(b), with a mean value of 719.5 phe. Unfortunately, the same method is not conclusive with the DV scintillator. At the moment other methods are under investigation, but in any case preliminary results considering all the events for this material are presented, knowing that the retrieved scintillator characteristic parameters may be slightly underestimated.

Light yield and decay time estimation

An estimation of the decay time and effective LY is performed with two methods: the differential method (DM) and the cumulative method (CM).

The first one is the same described in figure 4.18 for the selection of the events in the A.T. side: counting the pulses in the fixed gate ($\Delta T = 10 \mu s$) along the whole waveform event by event. The mean value of the distribution of these counts in each time window is retrieved. Figure 4.22 reports the trend of the mean counts as a function of the time for both scintillators. On the top row the counts before and after the trigger are shown. As expected, the counts before the trigger are constant (mean counts equal to 11 and 11.5 for GOS and DV respectively), while after the trigger the expected decreasing trend is highlighted. Assuming the exponential time distribution of the emitted photons by the scintillator, detected by the SiPM, $N_{ph}(t) = N_0 \cdot e^{-t/\tau}$, characterised by the decay time τ and the effective light yield N_0 , the number of photons detected (photoelectrons) in an interval ΔT ($N_{det}(t)$) as a function of time is described as follows:

$$N_{det}(t) = \int_{n\Delta T}^{(n+1)\Delta T} \frac{dN_{ph}}{dt} dt = \int_{(n+1)\Delta T}^{n\Delta T} \frac{N_0}{\tau} e^{-t/\tau} dt = N_0 e^{-n\Delta T/\tau} \cdot [1 - e^{-\Delta T/\tau}] \quad (4.3)$$

Therefore, the counts noise subtracted as a function of time are fitted with the formula 4.3, as shown in figure 4.22(c) and 4.22(d). The parameter of the fit, corresponding to τ and effective LY (i.e. N_0 , expressed in photoelectrons) are summarized in table 4.5.

In the cumulative method the approach is similar, but in this case the window of counting is increased of a fixed interval ($10 \mu s$), as shown in figure 4.23. In this case, the noise pulses in B.T. window increases linearly with time (DCR constant), as shown in the top row of figure 4.24. Instead, the trend of the number of photons detected after a certain time $\bar{t}$ (noise subtracted) $N_{tot}(t)$ as a function of time, reported in figure 4.24(c) and 4.24(d) for GOS and DV respectively, is described by the cumulative function as follows:

$$N_{tot}(t) = \int_0^{\bar{t}} \frac{dN_{ph}}{dt} dt = N_0 \cdot [1 - e^{-\bar{t}/\tau}] \quad (4.4)$$

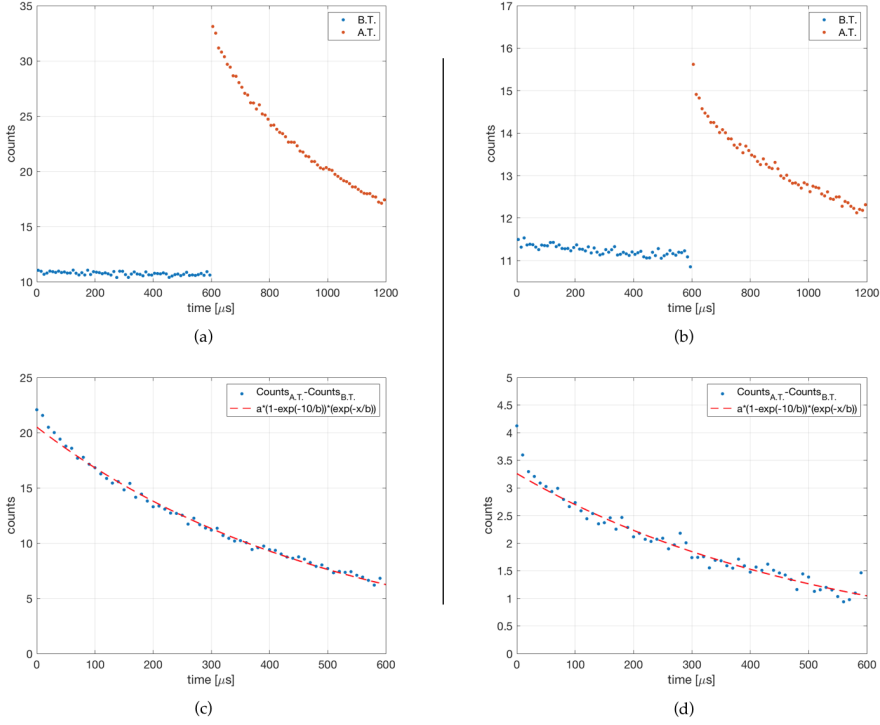


Figure 4.22: (a) and (b): The mean counts as a function of time calculated with the differential method in the B.T. window (blue points) and in the A.T. one (orange points) for GOS and DV scintillator respectively. (c) and (d) The mean difference counts between A.T and B.T. windows as a function of time for GOS and DV scintillator respectively. The trend is fitted with the formula [4.3](#)

The characteristic parameters for the two scintillators are reported in table [4.5](#). The decay times τ are compatible to the values reported in literature [[125](#), [135](#), [126](#)], that ranges from $500\mu\text{s}$ to 1 ms . Therefore the scintillators have comparable τ of $\sim 500\mu\text{s}$, while the effective LY (expressed in phe detected) is about 6 times higher for GOS. These latter are translated in photons impinging on the sensor, considering the SiPM PDE. The PDE trend as a function of the wavelength of the incident light, for the SiPM in use, is reported in figure [4.25](#). By datasheet, at 544 nm it is equal to 35%

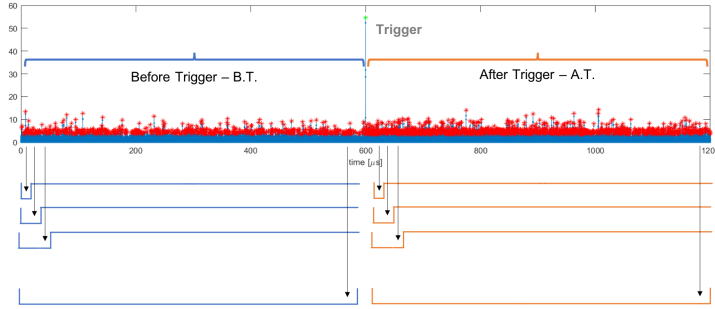


Figure 4.23: Sketch of the cumulative method, where for both B.T. and A.T. side, the time window per each step is increased of a fixed $\Delta T = 10\mu s$.

	Differential Method		Cumulative Method	
	GOS	DV	GOS	DV
τ (μs)	504.7 ± 5.3	527.7 ± 14.3	510.1 ± 2.3	533.9 ± 3.3
LY (phe)	1045.3 ± 7.0	173.6 ± 3.1	1047.7 ± 2.5	174.4 ± 0.6

Table 4.5: Summary of the characteristic parameters (τ and LY) of the two scintillators obtained fitting the trends with formulas [4.3](#) and [4.4](#) in the differential and cumulative methods respectively.

and to 26% at 611 nm. Moreover, in section [4.1.1](#), the average deposited energy retrieved for GOS and DV samples is 437 keV and 399 keV respectively. Therefore the LY of the photons impinging on the sensor for GOS is equal to 6848 ± 524 photons/MeV; for the DoseVue powder is 1677 ± 280 photons/MeV.

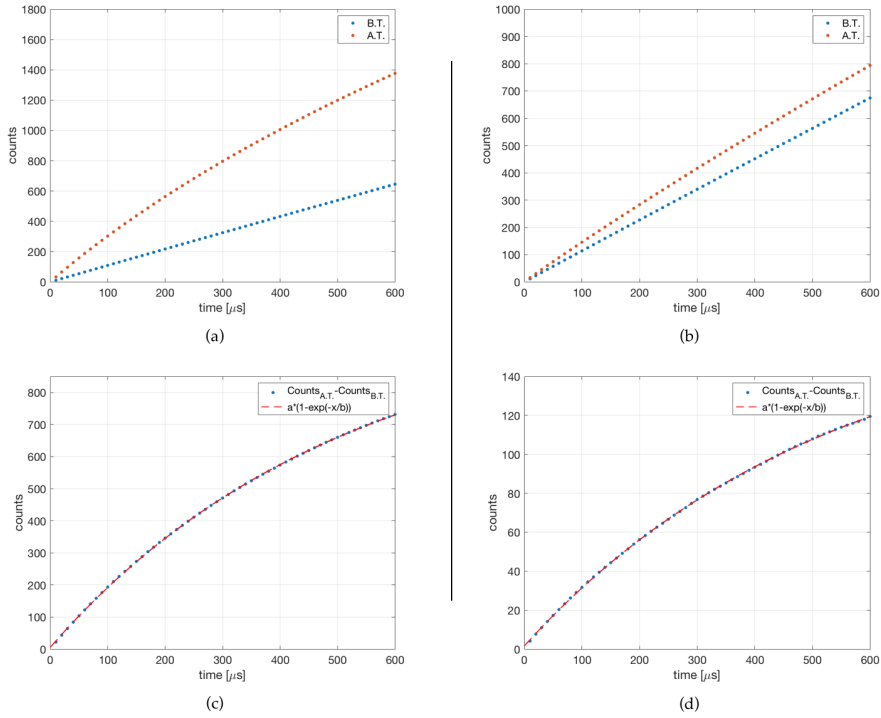


Figure 4.24: (a) and (b): The mean counts as a function of time calculated with the cumulative method in the B.T. window (blue points) and in the A.T. one (orange points) for GOS and DV scintillator respectively. (c) and (d) The mean difference counts between A.T and B.T. windows as a function of time for GOS and DV scintillator respectively. The trend is fitted with the formula [4.4](#).

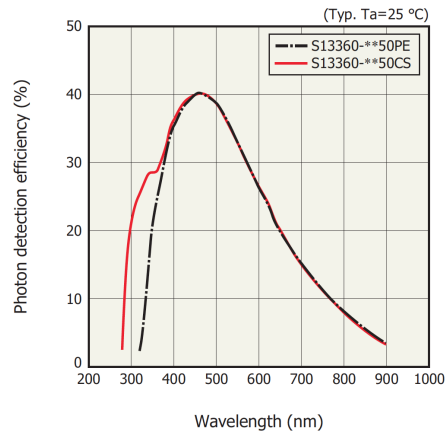


Figure 4.25: $6 \times 6 \text{ mm}^2$ Hamamatsu S13360-6050CS with $50 \mu\text{m}$ of pitch as a function of the impinging light wavelength, measured at $V_{ov} = 3 \text{ V}$ [24].

ORIGIN: dosimeter response characterisation

Once the features of the scintillating material were defined, the optical fibre based dosimeter was characterised and validated. First of all, the SiPM operational parameter were determined. Then the response of the fibre dosimeter scintillator was qualified and studied in laboratory using an X-ray beam (max energy 35 keV) and counting the pulses generated in the scintillator. Finally, the HDR dosimeter prototype, in which the DV mixture powder was used, was tested in clinical conditions exposing the fibre to an ^{192}Ir HDR brachytherapy source. Its response is qualified in terms of repeatability and dependence on the distance from the HDR brachytherapy source. The results are further compared with the theoretical dose calculations obtained by the Treatment Planning System (TPS) performed using TG-43 protocol. The performances are discussed and compared with two other brachytherapy dosimeters, briefly presented in chapter 3. The first one, based on the semiconductor technology, is produced by PTW and is the only commercially available system. The second one, proposed by Andersen et al., exploits the same working principle of ORIGIN (the radio-luminescence) and it represents a research prototype at an advanced stage of technological development.

5.1 System characterisation

5.1.1 Sensor characterisation

The light detectors in use for the characterisation of the single fibre dosimeter prototype are the Ketek $3\times 3\text{ mm}^2$ PM3325-WB SiPMs. The main characteristic of these sensors are reported in table 5.1.

Parameter	value
Size	$3\times 3\text{ mm}^2$
Pitch	$25\text{ }\mu\text{m}$
Number of cells	13920
V_{BD} temperature dependency	22 mV/K
Gain at +5 V_{OV}	$1.74\cdot 10^6$
PDE at 430 nm at +5 V_{OV}	45%
DCR at +5 V_{OV}	100 kHz/mm ²
OCT probability at +5 V_{OV}	$\sim 30\%$
AP probability at +5 V_{OV}	$\sim 5\%$

Table 5.1: PM3325-WB SiPM characteristics from datasheet [29].

They were mounted on a readout PCB equipped with a pin socket for an easy assembly of the SiPM (top line figure 5.1). A light tight enclosure, shown in figure 5.1, was designed in order to host the sensor and the PCB. The fibre was interfaced to the sensor by an SMA connector. Since the core of the fibre¹ is 1 mm while the detector sensitive area is $3\times 3\text{ mm}^2$, possible misalignments between fibre and sensor are presumed not to have an impact. For the preliminary characterisation, two readout PCBs were available: one AC coupled and one DC coupled². The connection schemes

¹The diameter of a standard brachytherapy catheter is $\sim 2\text{ mm}$, thus the fibre matches easily with the existing hospital equipment.

²The final product will present the SiPMs connected directly to the board hosting the ASIC CITIROC 1A.

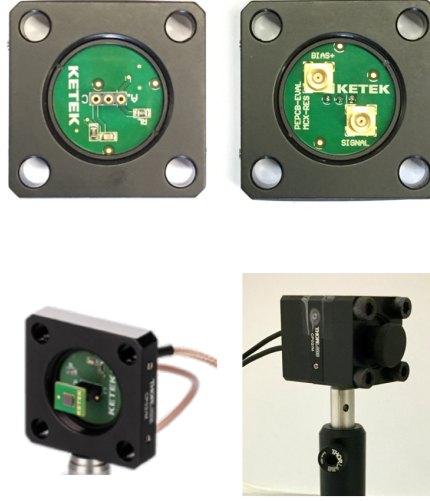


Figure 5.1: On the top row the pin socket for easy assembly of the SiPM on the left. On the right, the back view of the PCB with the MCX connectors for the SiPM bias and the output signal. On the bottom row, the Ketek $3 \times 3 \text{ mm}^2$ sensor mounted on the PCB board and the light enclosure hosting the sensor and the board.

for both boards are shown in figure 5.2. Both were tested because they present complementary features. The AC coupled one has two outputs: the

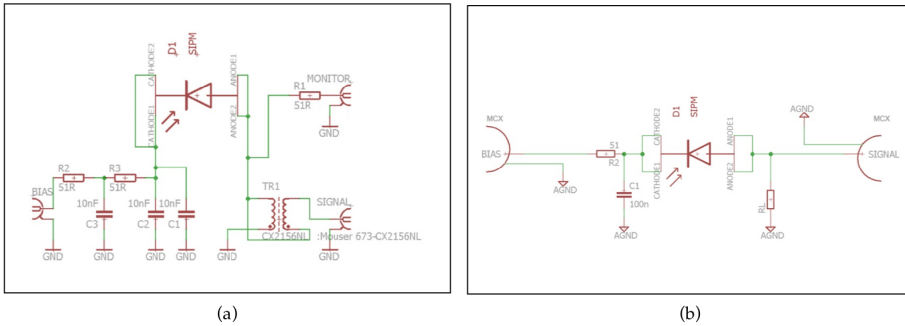


Figure 5.2: Readout scheme connection for the AC (a) and DC (b) coupled board.

so-called “signal” (90% of the initial SiPM signal) and the “monitor” one

(10%). Two exemplary signals of this board are reported in figure 5.3(a) and 5.3(b), when the SiPM is illuminated with a high light intensity (in excess of hundred photoelectrons). The “monitor” signal is faster (~ 100 ns full-time development), suitable for counting measurements. However, they present a very pronounced and long undershoot (20% of the amplitude of the signal and hundreds of ns long), possibly impacting on the baseline restoration and affecting the counting frequencies at high rates. The DC coupled board presents only one output signal (figure 5.3(c)), with a full time development of 300 ns, slightly slower than the fastest signal of the AC board. This latter is the board of choice for the single fibre-SiPM prototype, since it is reasonable fast and the absence of the undershoot is expected to guarantee a better counting capability.

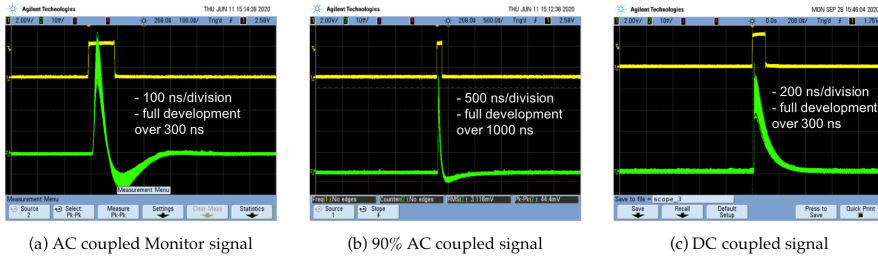


Figure 5.3: Direct output SiPM pulse shape at 50Ω load, when it is illuminated with a huge amount of light (more than hundred photoelectrons) for the AC coupled signal (a) and the monitor (b) and for the DC coupled board signal (c).

Firstly, the breakdown voltage V_{BD} of this SiPM, was evaluated at a temperature of 20°C ³. The linear gain-voltage dependence, reported in equation 1.1, is exploited to extrapolate the V_{BD} : since the Δpp is related

³During the measurements with the HDR brachytherapy source in hospital, described in 5.4 the temperature was kept under control and no significant variations were observed.

to the SiPM gain, at a given temperature, a linear increase between Δpp and V_{bias} is expected.

A typical multiphoton peak spectrum recorded at 29.3 V and the corresponding Δpp are shown in figure 5.4(a). Figure 5.4(b) reports the resulting Δpp for 12 different bias voltages, ranging from 26.9 V to 30.5 V. The different bias were provided by the Ketek power supply⁴. Each point

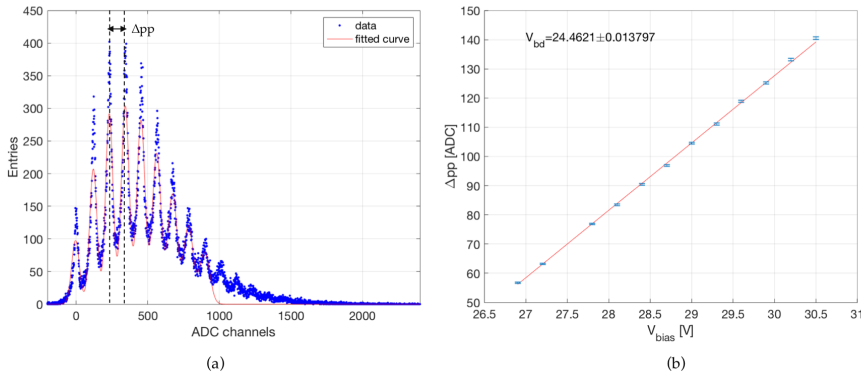


Figure 5.4: (a): Example of a multiphoton peak spectrum acquired illuminating the 3×3 Ketek PM3325-WB at $V_{bias} = 29.3$ V. (b): The linear relation existing between the extrapolated Δpp and the bias voltage applied to the sensor is exploited to determine its V_{BD} .

was taken at 20°C, illuminating the SiPM with few photons coming from a sub-ns pulsed led (PicoQuant PDL 800). The SiPM signal is amplified by a three stage inverter amplifier hosted in the Power Supply and Amplification Unit (PSAU SP5600 by CAEN S.p.A.⁵) and the signal is integrated over 350 ns. The V_{BD} is extracted considering the linear fit of the data $\Delta pp = m \cdot V_{bias} + q$ as the biasing voltage at which $\Delta pp = 0$. In this case $V_{BD} = -q/m = 24.46 \pm 0.01$ V. The error associated to V_{BD} was calculated

⁴<https://www.ketek.net/store/products/sipm-bias-source/>

⁵<https://www.caen.it/products/sp5600e/>

considering the correlation between the m and q parameters in the fit. The recommended operational voltage is $V_{bias} = V_{BD} + 5$ V.

Moreover, the Ketek SiPM was characterized in terms of DCR and OCT probability at this operational voltage, through the staircase curve, i.e. the count rate of the noise signal as a function of increasing discriminating thresholds. The signal was amplified through the PSAU at 42 dB. The count rate per each threshold was acquired for 100 ms. Figure 5.5 reports the resulting staircase curve. The sensor presents a DCR at 20°C at the level of 1 MHz and an OCT probability of 30%.

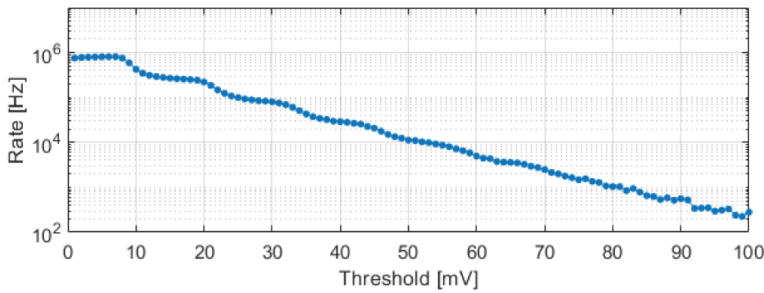


Figure 5.5: 3×3 Ketek PM3325-WB Dark Count Rate staircase at $V_{bias} = 29.3$ V.

5.1.2 Counting setup for dosimeter response

Since the decay times of the scintillators are very long, the emitted photons are so spread that the technique implemented for the readout of the dosimeter response is based on counting. The counting measurements are performed through the setup, summarised in figure 5.6. The bias to the SiPM is provided by Ketek power supply. The SiPM signal is amplified by PSAU SP5600 at 42 dB. The signal is shortened by the pole-zero cancellation circuit and digitized by the DT5730⁶. It is discriminated at different

⁶<https://www.caen.it/products/dt5730/>

thresholds through the leading edge discriminator, hosted in the desktop digitizer. Finally the discriminator output signal is counted over a time window of 100 ms exploiting the counter hosted in the second channel of the PSAU. The count rate is monitored and recorded by the CAEN S.p.A. software. The pole-zero cancellation circuit is necessary, since the counting capability is limited at high frequency of impinging light by the presence of the pile-up phenomenon, i.e. the superposition of one signal over the tail of the previous one. Therefore, reducing the full-time development from 300 ns to 30 ns, as shown in figure 5.6, by means of a custom made pole-zero cancellation circuit can enhanced the counting capability. Linear response up to some MHz of pulses frequency is expected, as discussed in more details in chapter 6 section 6.2.1.

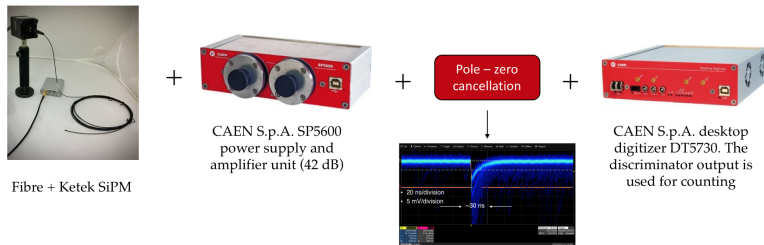


Figure 5.6: The scheme of the counting set-up used for the dose rate measurements of the standard HDR brachytherapy source.

In order to qualify this setup, a fibre hosting in the tip the DV scintillator (a sphere of 1 mm diameter) is exposed to an X-ray beam with maximum energy 35 keV at increasing current (from 0.1 mA to 1 mA). The use of the X-ray beam allows to reach higher rates of produced photoelectrons with respect to the ^{90}Sr source, testing the linearity of the system as a function of the current of the tube in counting mode. Actually, the activity of



Figure 5.7: X-ray apparatus with Molybdenum anode by LD Didactic (model 554 801). The tube voltage and tube current can range from 0 to 35 kV and from 0 to 1 mA respectively.

the HDR brachytherapy sources are of the order of GBq, therefore, they are not available in laboratory for radioprotection reasons. The X-ray offers a flexible and easily accessible system to test the system response to a continuous flux, simulating a very high activity source.

The count rate at different signal threshold is shown in figure 5.8(a) for all the tested currents. Figure 5.8(b) displays the count rate as a function of the X-ray tube current for three thresholds: 0.5, 1.5 and 2.5 phe. The linearity is guaranteed up to 4-5 MHz, as shown by the linear fit overlapped to the data at threshold 0.5 phe. This results were also confirmed by tests presented in chapter 6 section 6.2.1. Considering that the DCR of this SiPM is 1 MHz, performing measurements at thresholds higher than 0.5 phe, exploiting the high OCT of the sensor, allows detecting linear responses at higher intensities of X-rays and, consequently in hospital, at higher HDR brachytherapy source activities.

5.2 HDR brachytherapy source experimental setup

For the qualification of the HDR dosimeter prototype, the ORIGIN fibre dosimeter (with the DV scintillator) was exposed to a HDR brachytherapy

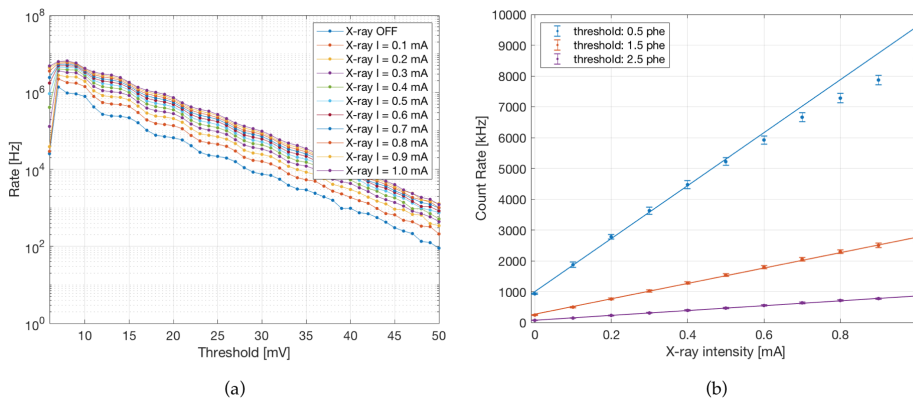


Figure 5.8: Assessment of the counting capability of the fibre dosimeter, with DV scintillator, exposed to X rays. (a): Measured count rate in a signal threshold scan, using the setup of figure 5.6. The dosimeter is exposed to different X-ray tube currents. The tube voltage is fixed to 35 kV. (b): Count rate for three different signal thresholds (0.5, 1.5 and 2.5 phe) as a function the tube currents. The number of generated pulses increases linearly with the current up to 4-5 MHz, as shown by the linear fits overlapped to the data.

^{192}Ir source in order to measure the dose rate (dose per unit time) at different distances. The measurements were carried out at Ospedale di Circolo e Fondazione Macchi in Varese (Italy). The HDR source is hosted in the afterloader system microSelectron HDR V3-Digital⁷ with 6 channels, shown in figure 5.9. The ^{192}Ir source, shown in figure 5.9, is enclosed in a stainless steel capsule, wound to a flexible cable, that allows to drive the source into the applicator or retract it in the afterloader. The measurements were performed two times (dates: 24/08/2020 and 9/10/2020) with two different source activities: 1.740 Ci (64.38 GBq) and 5.507 Ci (203.76 GBq)⁸.

During the experiments, both the fibre dosimeter and the source were

⁷<https://www.elekta.com/dam/jcr:cbc09f98-d04e-4262-a082-37d7d189577a/MicroSelectron%20brochure.pdf>

⁸The initial activities were 11.139 Ci (421.43 GBq) and 10.894 Ci (403.08 GBq) respectively and the decay time of ^{192}Ir is 73.8 days.

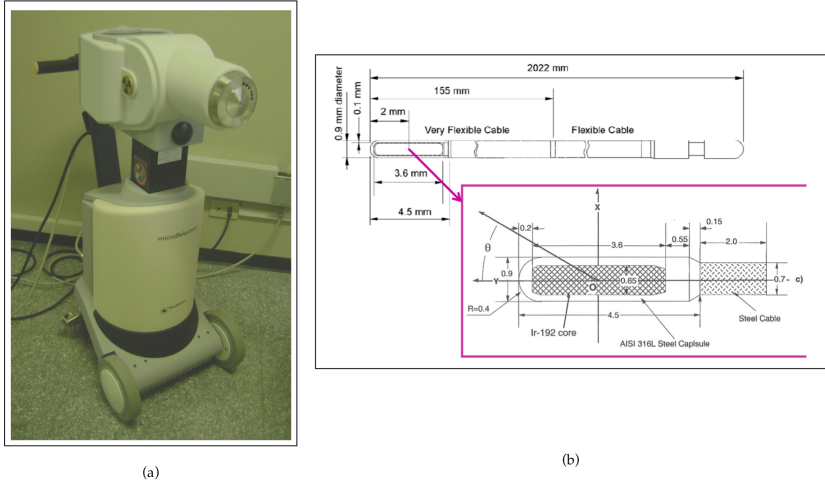


Figure 5.9: (a): picture of the microSelectron HDR V3-Digital afterloader. (b): mechanical design of microSelectron HDR ^{192}Ir source. The pictures are provided by the medical physics staff at Ospedale di Circolo e Fondazione Macchi in Varese.

placed into dedicated catheters (with 2 mm diameter) and allocated in a solid water phantom (by CIRS⁹). This final system was immersed in a distilled water tank, as shown in figure 5.10, with $\sim 4 - 5$ cm of water on the top of the phantom in order to guarantee that the scintillator was surrounded by water in all directions. The phantom has an area of $10 \times 10 \text{ cm}^2$, with 11 tunnels, arranged as reported in figure 5.11.

5.3 Repeatability

First of all, the repeatability of the sensor response was investigated. It is a critical parameter indicating the stability of the system. Although there are not strict recommendations, reproducibility at a few percent level is desirable [136, 137]. The source catheter laid in the first tunnel, while the

⁹<https://www.cirsinc.com/products/radiation-therapy/plastic-water/>

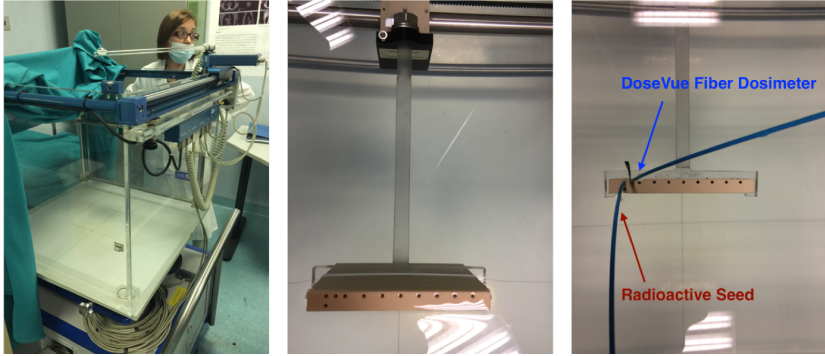


Figure 5.10: Photos of the set up (phantom and fibre dosimeter) for the dose rate measurements with the HDR brachytherapy source.

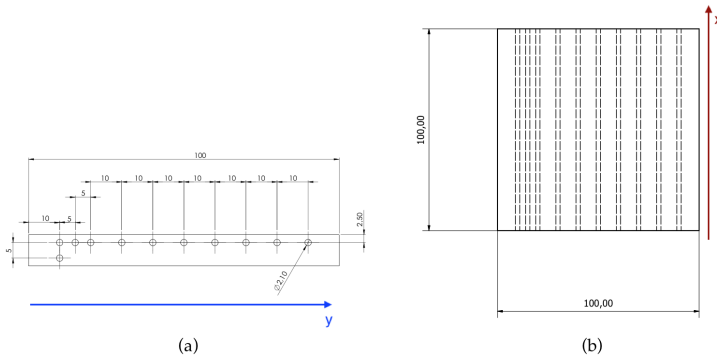


Figure 5.11: Solid water phantom drawings: side view (a) and top view (b). All the dimensions are expressed in mm.

dosimeter was positioned in the tunnel at a distance $y = 1$ cm in the phantom. From now on, the distance between the seed tunnel and the dosimeter one is denoted as y . The source (with activity 5.507 Ci) was inserted and removed three times, nominally in the same position in front of the dosimeter (5.7 cm from the bottom of the phantom¹⁰).

The dosimeter response was measured through the counting SiPM pulses

¹⁰It is referred as 18th seed dwell position in the next sections.

set-up, described in section 5.1.2 in figure 5.6. The signal, after the pole-zero cancellation, was discriminated at 1.5 phe threshold. This threshold allows to have a lower residual noise (it is due to the $\sim 30\%$ of OCT) to retrieve the information about the scintillation pulses, having a higher sensitivity to lower frequency. The pulses were counted in a pre-defined window (gate width) and the rate was averaged on an user-defined number of samples, as sketched in figure 5.12. In these measurements the gate width was fixed at 10 ms and the number of samples was equal to 10 (total counting window $\Delta T = 100\mu s$).

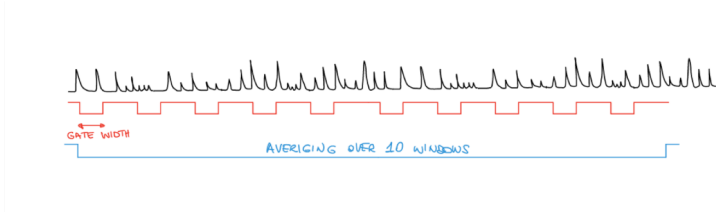


Figure 5.12: Sketch of the software operating principle for the counting measurements.

Figure 5.13 shows the traces recorded for the three repetitions. The first 25 s correspond to the noise rate (equal to 313.7 ± 1.7 kHz). When the source is inserted the rate sharply increases. The average count rate for each repetition (M_{rep}) and its relative error (Err_{single}) are reported in table 5.2. The mean value of the three measurements (M_{tot}) is equal to 2495.7 ± 5.4 kHz. The variations with respect to M_{tot} , always below 1%, correspond to 0.26%, -0.65% and 0.38% (compatible with the statistic fluctuations). The results are listed in table 5.2. The obtained results are comparable or even better than the repeatability levels reached by other prototypes based on different technologies. For instance, the fiber-coupled $Al_2O_3:C$ luminescence dosimetry (based on the radioluminescence working principle as ORIGIN dosimeter) proposed by Andersen et al. has a reproducibility of 1.3% [123]. Another in-vivo dosimeter based on the MOSFET technology,

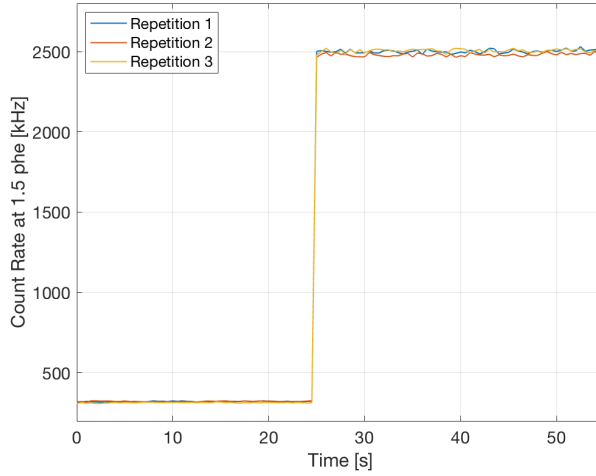


Figure 5.13: Repeatability figure of merit. Response of dosimeter (in terms of count rate) when the radioactive seed is inserted in the phantom three different times in the same position. The first 25 s correspond to the noise of the system. Then the increased rate is due to the presence of the source.

MOSkin (Centre of Medical Radiation Physics, University of Wollongong, Australia) exhibits a reproducibility of 1.1% during ^{60}Co HDR intracavitary brachytherapy [138]. Finally this prototype ensures repeatability levels at the level of the only existing in-vivo dosimetry for brachytherapy on the market. Indeed, the PTW Multichannel Dosimeter, VIVODOS, on average features a reproducibility at the 0.5% level, as confirmed by different studies [124, 139].

n°	M_{single} [kHz]	Err_{single}	Percentage variation
1	2502.4 ± 9.7	0.39%	0.26%
2	2479.4 ± 9.9	0.38%	-0.65%
3	2505.3 ± 9.7	0.39%	0.38%

Table 5.2: Summary of the repeatability measurements variations.

5.4 Dosimeter dose rate response as a function of distance

The response of the dosimeter as a function of the distance from the radioactive source was investigated and compared with the expected trend. To perform this measurement, the source catheter stayed in the first tunnel, marked in red in figure 5.14, while the fibre dosimeter was positioned in parallel to the source at different distances y , from 0.5 cm to 3 cm (blue dots). The source was moved into the catheter, along the tunnel, at dif-

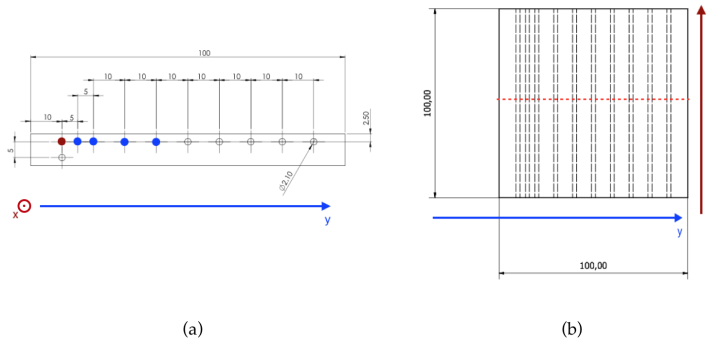


Figure 5.14: Solid water phantom drawings: side view (a) and top view (b). The red dot represents the source position, the blue one the position of the fibre dosimeter during different measurements. All the dimensions are expressed in mm.

ferent dwell positions with a minimum step of 2.5 mm and an accuracy of ± 1 mm¹¹ (the position of the source will be called x). Two different configurations were tested: one, with 33 steps, when the fibre was at the distance $y=0.5$ cm, and another of 23 steps for the other distances ($y=1, 2, 3$ cm), covering a full range of ~ 8 cm (± 4 cm from the maximum dose rate). The two configurations are shown in figure 5.15, where the 18th step corresponds to the maximum measured dose rate and is defined as $x=0$. In

¹¹It is provided by the producer

each step, the radioactive seed stayed in the same position for 60 seconds (dwell time).

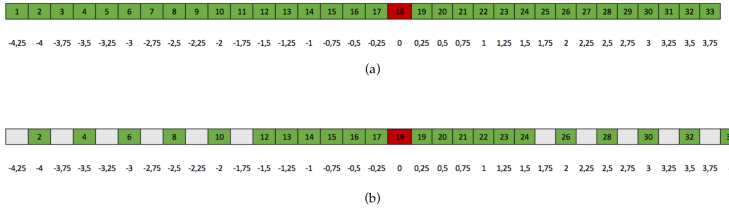


Figure 5.15: The planned position of the source in the catheter for the measurements at $y=0.5$ cm (a) and $y=1, 2, 3$ cm (b). Each step corresponds to a movement of 2.5 mm. The maximum dose rate is measured in the 18th dwell position, defined $x=0$. The numbers below the configuration drawings are the relative positions with respect $x=0$, expressed in cm

Due to the low amplitude expected signals, two discrimination thresholds were used: 1.5 phe and 2.5 phe. Figure 5.16 shows a typical acquired raw dataset: the count rate at each x position at fixed y was recorded. The ^{192}Ir stayed in the same position for 60 s. The time where the source moved to another dwell position is highlighted in figure by the red dashed lines. The first ~ 100 points correspond to the noise rate, then each step corresponds to the increase in the counting rate, due to source activity. In order to measure any change in the DCR, possibly due to temperature variations, the counting rate was monitored again at the end of the scan. For each step, the mean counting rate and its standard deviation were computed. The DCR at 1.5 phe was 309 ± 2 kHz and at 2.5 phe was 51 ± 0.9 kHz. For each step the DCR was subtracted, considering a linear variation between the noise recorded at the begin and the end of the

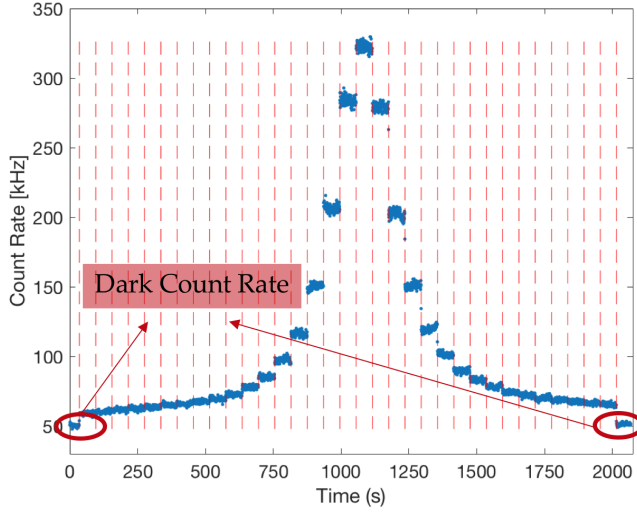


Figure 5.16: An example of the acquired raw data during an entire treatment plan according to the source dwell positions in figure 5.15(a) at threshold 1.5 phe.

file, assuming limited temperature fluctuations¹². DCR variations within 3 kHz between the begin and end of the run were measured. The count rates (noise subtracted) as a function of relative position x for each fibre dosimeter position y are displayed in figure 5.17 for both thresholds. It is expected that the dose rate decreases as $1/r^2$ as a function of distance between the dosimeter and the source ($r = \sqrt{x^2 + y^2}$) [17, 140]. This concept will be further discussed more in detail in section 5.5. To evaluate this trend, possible misalignments with respect to the reference position $x = 0$ (i.e. if the dosimeter is not well-centred around the 18th position) were accounted for in a parametric way, with the following fit:

$$\frac{a}{(x + b)^2 + (y + d)^2} + c \quad (5.1)$$

The x -misalignments, retrieved from the parameter b of the fit, were of the order of hundreds of μm ; the maximum value is $\sim 600 \mu\text{m} \pm 100 \mu\text{m}$

¹²Below 1°C .

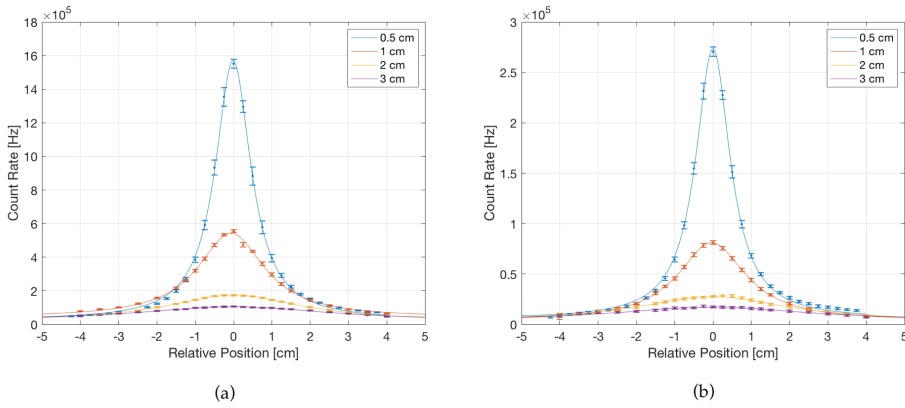


Figure 5.17: Measured count rate at threshold 1.5 phe (a) and 2.5 phe (b) with the CAEN kit for different dwell positions (x) and different dosimeter positions y (0.5 cm blue points, 1 cm orange ones, 2 cm yellow ones and 3 cm purple ones). The lines represent the fits using the equation [5.1](#).

(measured at $y = 1$ cm). The y-misalignments (parameter d) were less than $100\mu\text{m}$, compatible with the machining accuracy of the phantom tunnels. A 2D map of the dose (count) rate, in figure [5.18\(a\)](#) and [5.18\(b\)](#), summarises all the measurements performed. Figure [5.19](#) shows the same results considering the count rate as a function of the distance between the dosimeter and the source $\bar{r} = \sqrt{\bar{x}^2 + \bar{y}^2}$, where $\bar{x}$ and $\bar{y}$ are the positions corrected for the misalignments. The parameters of the curve ($y = a/x^b + c$) are in good agreement with the theoretical trend: the parameter b is compatible with 2 at a confidence level of 95%. The reliability of the measurements is also assured by the fact that the measured count rates (in all conditions) are well below the limit of 4 MHz, identified in [5.1.2](#), as the maximum rate before the deviation from linearity occurs.

In this first test, the ^{192}Ir source in use had a low activity (more than 2.5 decay times have passed), usually after 3 months, i.e. 1.2 decay times, the hospital staff change the source. This was not the usual clinical brachyther-

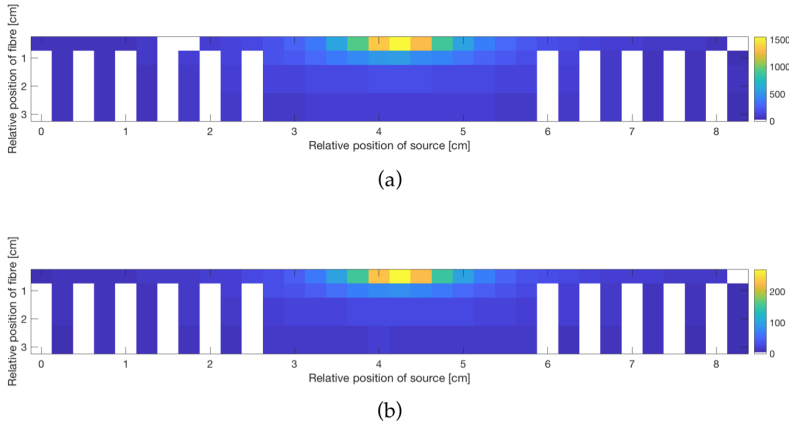


Figure 5.18: 2D map of the count rate at threshold 1.5 phe (a) and 2.5 phe (b), measured as a function of x (dwell seed position) and y (dosimeter positions).

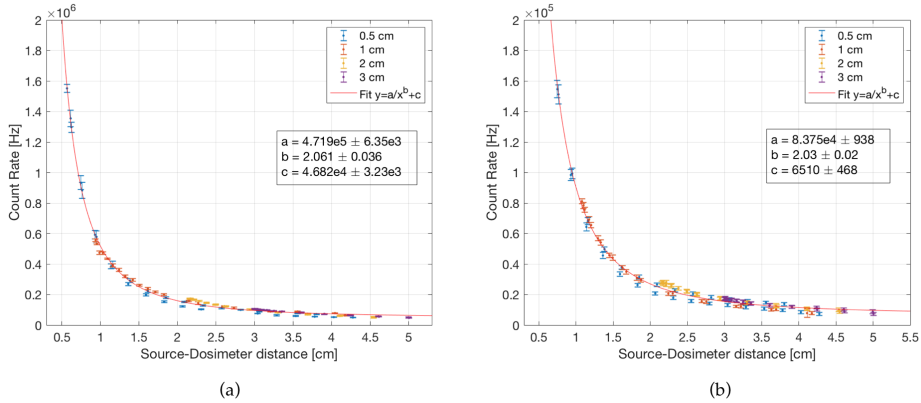


Figure 5.19: The count rate (i.e. the dose rate) measured with the CAEN kit as a function of the dosimeter-source distance $\bar{r}$ at threshold 1.5 phe (a) and 2.5 phe (b). The different colors correspond to the measurements at different dosimeter positions y : 1 cm blue points, 2 cm orange ones, 3 cm yellow ones and 4 cm purple ones. The red line is the best fit that reports a decreasing trend compatible at 1σ with $\bar{r}^2$.

apy activity, therefore the measurements were repeated with a higher activity source. In this case the activity was equal to 5.507 Ci (203.76 GBq). This test is important in order to define and identify any possible miscount due to too high frequency of the photons emitted by the scintillator, that can induce pile-up and deviation from a linear response. This time, together with the CAEN kit set-up (figure 5.6), it was also used the ASIC CITIROC 1A readout system based on the DT5550W board (figure 2.4). It will be the readout of choice for the final prototype with 16 fibres. The measurement configuration in phantom was the same of the previous measurements, but the fibre dosimeter was moved in the tunnels at $y = 1, 2, 3$ and 4 cm. The planned source positions were defined as reported in figure 5.15(b). First of all, since the dose rate is proportional to the source activity, the increase of the count rate induced by the higher activity of the new source is verified with the CAEN kit at threshold 1.5 phe. As demonstrated in figure 5.20, the count rate, corresponding to the peak of the source profile as function of x , increases (factor ~ 3.5) as the ratio of the source activities, confirming the reliability of the system.

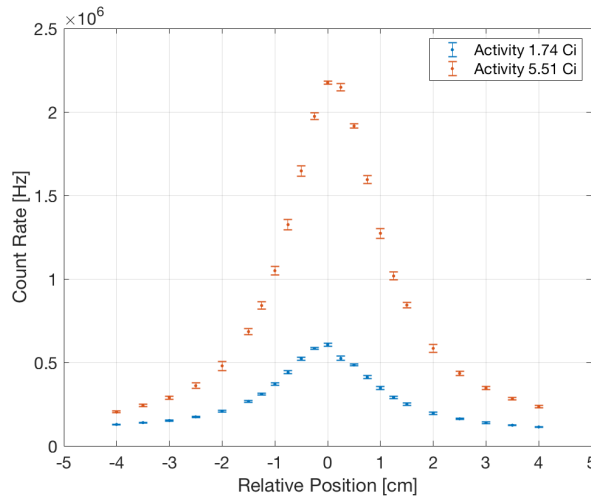


Figure 5.20: The comparison between the count rates measured with the CAEN kit at distance $y = 1$ cm for each source dwell position for both the lower activity used equal to 1.74 Ci (blue points) and the higher, corresponding to 5.507 Ci (orange points) at threshold 1.5 phe.

Unfortunately with the ASIC only the data at threshold of 2.5 phe were available, because at lower threshold the counting rate was affected by a pick-up noise of the same amplitude. At 2.5 phe the agreement between the two acquisition systems was good, a maximum difference of $\sim 10\%$ was observed. The noise rate in a counting window of 1 s at 2.5 phe threshold was equal to 100 ± 0.3 kHz. Figure 5.21 shows the count rates (noise subtracted) for every x and y position. In these measurements, the misalignment with respect to the center of the 18th position is evident, as confirmed by the shifts retrieved by the fit of the data corresponding to 1.5 mm, implying that the real center is roughly in the middle between the 18th and 19th positions. Figure 5.22 reports the 2D map of the count rate as a function of the position of dosimeter and radioactive seed and the trend of the count rates as $1/\bar{r}^2$ is reported in figure 5.23, confirming also in this case the good agreement (confidence level of 68%) between the performed measurements

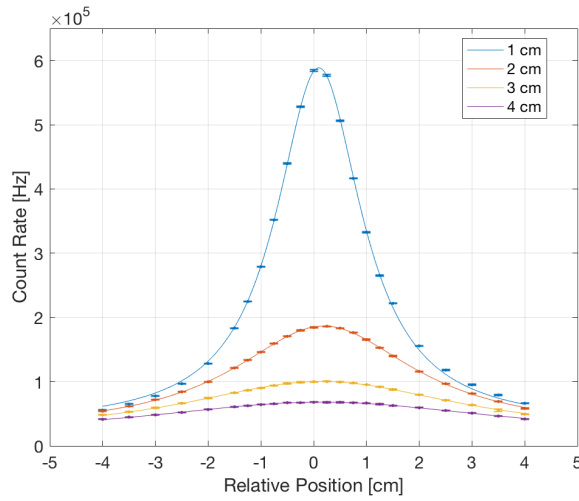


Figure 5.21: Measured count rate with the ASIC CITIROC1A for different dwell position (x) and different dosimeter position y (1 cm blue points, 2 cm orange ones, 3 cm yellow ones and 4 cm purple ones). The lines represents the fits with the equation [5.1](#).

and the theory: the retrieved exponent is equal to 1.990 ± 0.015 .

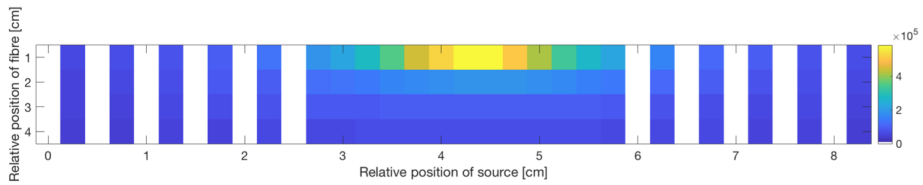


Figure 5.22: 2D map of the count rate measured with the ASIC CITIROC1A as a function of x (dwell seed position) and y (dosimeter positions).

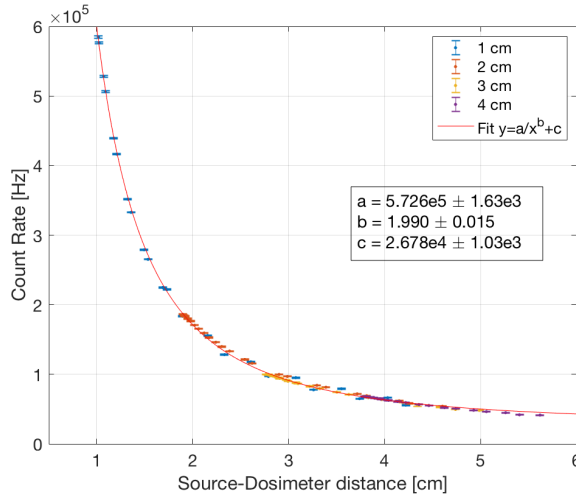


Figure 5.23: The count rate (i.e. the dose rate) as a function of the dosimeter-source distance $\bar{r}$. The different colors correspond to the measurements at different dosimeter position y : 1 cm blue points, 2 cm orange ones, 3 cm yellow ones and 4 cm purple ones. The red line is the best fit that reports a decreasing trend compatible at 1σ with $\bar{r}^2$.

5.5 Comparison with the treatment planning system

In order to assess the reliability of the system and to obtain a calibration curve, the last measurements (readout with the ASIC) are compared with the treatment planning system (TPS), based on the TG-43 protocol [141, 140]. It was published in 1995 and then revised in 2004 by the American Association of Physicists in Medicine (AAPM). According to this protocol the dose rate $\dot{D}(r, \theta)$ at the point $P(r, \theta)$ in a medium (see figure 5.24) is described by the equation:

$$\dot{D}(r, \theta) = S_K \Lambda \frac{G(r, \theta)}{G(r_0, \theta_0)} F(r, \theta) g(r) \quad (5.2)$$

where:

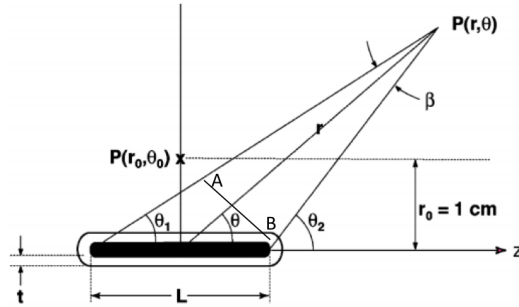


Figure 5.24: Coordinate system used for brachytherapy dosimetry calculations in the TG43 protocol for a line source.

- S_K is the air-kerma¹³ rate, a quantity related to the strength of the source in vacuum at a distance d (usually 10 cm) multiplied by d^2 , expressed in $\text{mGy m}^2 \text{h}^{-1}$.
- Λ is the dose rate constant, defined as the dose rate to water at a distance of 1 cm on the transverse axis of a unit air-kerma strength source.
- $G(r, \theta)$ represents the geometry factor and it accounts for the variation of the dose due to the spatial arrangement of the radioactive source. If the source extent is negligible, $G(r, \theta)$ corresponds to $1/r^2$ (point source approximation); otherwise it is equal to $\beta/(L \cdot r \cdot \sin\theta)$ (figure 5.24)¹⁴. For ^{192}Ir sources, the discrepancy between these two functions is below the 2% at distance r higher than 1 cm for every angles θ [141], assuring the consistency of the approximation of the

¹³It is defined as the amount of kerma, i.e. the kinetic energy actually deposited or absorbed in a unit mass (kg) of air

¹⁴With reference to figure 5.24, $L \sin(\theta) \sim \overline{AB}$ and $\frac{\overline{AB}}{\beta} \sim \frac{1}{r}$.

previous fits with $1/r^2$.

- $g(r)$ is called the radial dose function and accounts for the effects of absorption and scatter in the medium along the transverse axis of the source.
- $F(r, \theta)$ is the anisotropy function; it gives the anisotropy of the dose distribution around the source. These values are provided and computed by the producer of the HDR afterloader and TPS system through Monte Carlo simulation tools, as reported for instance in [142, 143].

A comprehensive explanation of all the parameters can be found in [141]. Therefore, by means of this protocol, the expected dose rate (TPS dose) in water, expressed in Gy/min, was provided by the hospital staff using the software Oncentra[®] Brachy by Elekta [144] at the point centred in the 18th position and $y=1$ cm. The expected behaviour is fitted with the equation

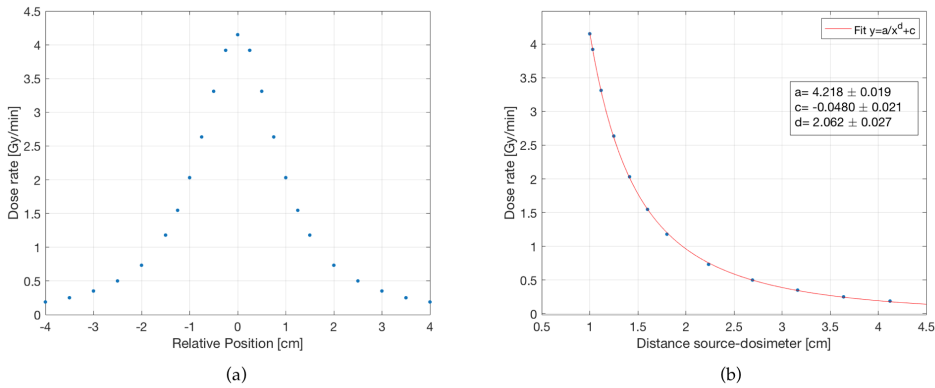


Figure 5.25: (a): Dose rate evaluated by the TPS through the TG-43 protocol as a function of relative position (with respect to the 18th position). (b): Planned dose rate as a function of distance r . The red line is the fit that best describes the data.

$y = a/x^d + c$. The parameter $d = 2.06 \pm 0.03$ results compatible with two, indicating that the point source approximation holds.

In order to compare the measurements with TPS, due to the different quantity of water above and below the phantom (there are only 4-5 cm of water above the top of the phantom, while more than 30 cm below) only the five central points (position [16th - 20th] in figure 5.15) are considered for all the y positions of the dosimeter. This choice should avoid differences in count rates due to the different scattered radiation.

The count rate is calibrated to the reference dose at the distance between source and dosimeter equal to $r = 1.929$ cm, as reported in figure 5.26(a). Figure 5.26(b) exhibits that there is a high discrepancy between the

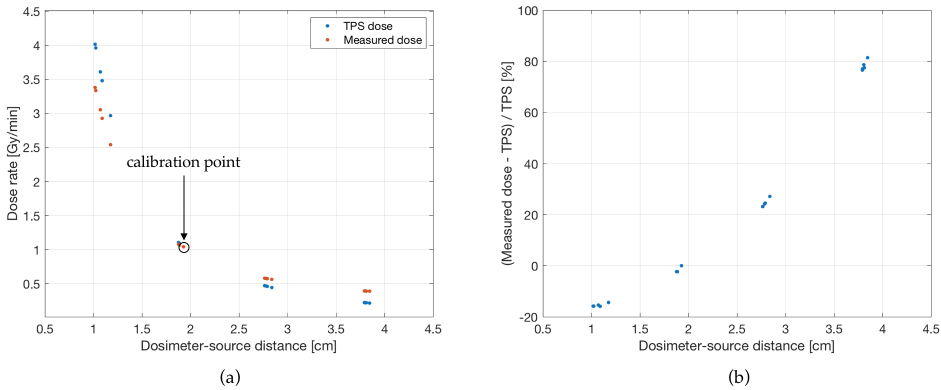


Figure 5.26: (a): Comparison between the TPS dose rate (blue points) and the measured one (orange points) as a function of the dosimeter-source distance. (b): Percentage variations between measured values and expected ones. They highlight a significant discrepancy due to the non water equivalence of the scintillator used in the tip of the fibre.

measurements and the model (reaching also values of 80%). This difference is due to the non water-equivalence of the dosimeter (the scintillating material). In fact, as reported in table 4.1, the scintillators have higher Z_{eff} (32.10) and density (5.24 g/cm^3) than water ($Z_{water} = 7.51$ and density = 1 g/cm^3), resulting in a different cross-section for gammas interactions¹⁵ and

¹⁵Considering also that the average photon energy varies as a function of the distance

thus in the absorbed dose. This requires the need for a calibration based on the ratio between the measured dose and the TPS dose as a function of r , as reported in figure 5.27(a). In order to verify the reliability of calibration curve, other points are corrected, corresponding at longer distances (dwell positions from 13th to 23th in figure 5.15). Figure 5.27(b) shows the comparison between the measured dose after the correction and the TPS (the points correspond to the measurements used in the calibration, while the crosses to the measurements added in order to confirm the procedure). The percentage difference is displayed in figure 5.27(c), demonstrating that, after a proper calibration, the dose measured by the fibre based dosimeter is in agreement with the TPS with an average accuracy of 1%, and a maximum deviation of 4%. The dispersion is below the limit of 5%, even if in brachytherapy treatment discrepancies between measurements and TPS up to 15% are allowed. For treatment delivery without patient movement, i.e. in ideal conditions, typical level of accuracy of 5% is requested, assuring that this prototype at least in phantom ensures the requirements for HDR brachytherapy dosimeter system¹⁶ [145, 146]. Finally it is worth of mentioning that also in this case the performances are comparable with other dosimeter prototypes after all the necessary corrections. The already mentioned fibre-coupled $\text{Al}_2\text{O}_3\text{:C}$ luminescence dosimetry (Andersen et al.) exhibits an overall uncertainty of 8% [123]. For the PTW system, instead, the average dispersion is $4.9 \pm 3.1\%$ [124], but uncertainty values up to 10% are reported [139].

5.6 Conclusion and future directions

A preliminary characterisation of the optical fibre dosimeter proposed by the ORIGIN collaboration was carried out. The investigation of the physi-

between source and dosimeter.

¹⁶This limit can increase to 7-10% in case of cervical intracavitary brachytherapy because of the higher complexity of the treatment plan [145].

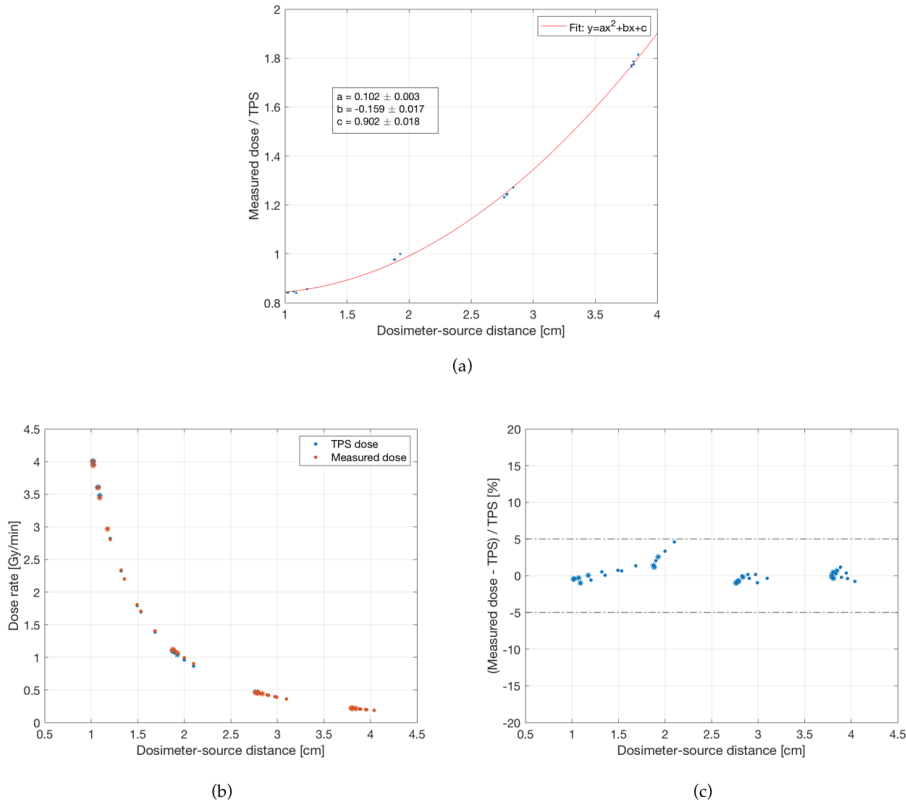


Figure 5.27: (a): Calibration curve for correcting the measured dose for the non water equivalence of the dosimeter. (b): Comparison between the TPS dose rate (blue points) and the measured one after the correction (orange points) as a function of the dosimeter-source distance. The points are the measurements used in the calibration, the crosses are other measurements used to confirm the calibration curve. (c): Percentage variations between measured values and expected ones. The black lines represent the limit of $\pm 5\%$. All the measurements are in agreement with the TPS with a maximum discrepancy of 4%.

cal characteristics of the scintillating material of choice indicates:

- similar decay times ($\sim 500 \mu\text{s}$) for both materials ($\text{Gd}_2\text{O}_2\text{S:Tb}$ and $1\text{Y}_2\text{O}_3\text{:Eu} + 4\text{YVO}_4\text{:Eu}$), although the number of photons impinging

on the sensor is higher for GOS;

- trains of single photoelectrons for both materials are expected, due to the time distribution over ms. Therefore, counting the pulses at the level of 1.5/2.5 phe is the optimal readout technique to retrieve the information related to the dose delivery. These thresholds guarantee sensitivity to the emitted light, also thanks to the high OCT ($\sim 30\%$) of the sensor, up to 3-4 cm (typical prostate dimension). Moreover, the choice of these thresholds allows working in the linear range of the measured frequency (below some MHz).

The prototype for HDR brachytherapy was tested in hospital in a solid water phantom in order to verify the system repeatability and its reliability to reconstruct the dose delivery to target areas and to monitor the dose absorbed in the OARs. The prototype exhibits stable working conditions: it has a reproducibility below 0.5% when consecutive measurements in the same conditions are performed. A comparative phantom study of Monte Carlo calculations based on the TG-43 formalism with the readings of the fibre dosimeter was carried out with ^{192}Ir source. This proof reveals an agreement between the fibre measured dose and the planned one at the level of 1%, after proper corrections. These performances also are demonstrated to be similar to other dosimeter system prototypes for brachytherapy, assuring the reliability of the system.

The stability of the SiPM-based readout will be addressed taking in consideration different and complementary solutions. Actually, effects of the SiPM gain drift due to temperature variations, for instance, could seriously limit the device performances. Detecting and counting low amplitude signals require keeping the SiPM V_{ov} constant in order to stabilise the counting threshold. Therefore, both a cooling system or a bias voltage feedback will be considered. The former keeps constant the temperature but it is more expensive, while the latter compensate the temperature variations

adjusting the bias voltage accordingly in order to keep the gain at a constant value. Moreover, the possibility to use another scintillating material, as Yttrium Aluminum Garnet doped with Cerium (YAG(Ce)), with different emitted photon decay time (70 ns), will be taken into account. This choice would guarantee to have higher amplitude signals, less vulnerable to SiPM gain variations, even if the manufacturing of YAG powder with proper grain dimensions and its integration in the existing fibre dosimeter production process have yet to be verified. Finally the characterisation of this dosimeter will be completed at the Northern Ireland Cancer Centre in terms of linearity, sensitivity and energy response. Moreover, in a similar way, the LDR brachytherapy dosimeter will be qualified. The impact of multiple fibres on the response of each fibre in the group will be studied to determine if the presence of multiple fibres affects the magnitude and type of scattered radiation. This is an important consideration for the use of the multi-fibre system. Any effects of multiple fibres on the radiation response will be corrected as required. A novel 3D printed anthropomorphic phantom will be designed and developed to allow testing of the fibre system in realistic geometric phantom constructed of tissue and bone like materials for both prostate and gynaecological cases. Future tests on this phantom will simulate prostate/cervix treatment confirming the possibility of using radioluminescence phenomenon to obtain a reliable dose estimation compared to commercial planning system outputs.

Bioluminescence

The peculiar characteristics of SiPMs can be exploited also in the field of biophotonics. Biophotonics is a set of methods and techniques for investigating processes of biological systems at molecular and cellular level, profiting from the emission, scattering or absorption of light. Biophotonic techniques can be divided in two types, depending on the emission light process. The light signal can be the result of chemical reactions in the biological sample (bioluminescence [147]) or of the interaction of the sample with an external source of light (fluorescence or phosphorescence [148]). Nowadays, bioluminescence techniques are widespread and with growing interest because of the increasing demand for biosensors in different fields of biophysics and biomedicine [147, 149]. The availability of on-purpose bioluminescence kits for the quantification of metabolites, related to the health state of cells or the level of contamination of food, air and water has increased the interest also in industry [150, 151]. However, this requires simpler, more compact, more flexible and, possibly, less expensive detection systems. Nevertheless, some challenges in bioluminescence detection are yet to be properly addressed, especially in terms of sensitivity both regarding the light level, often trains of single photons, and the metabolite concentration range.

Usually, the required specialisations are met by apparatuses integrating

PMTs. However, these luminometers are bulky and fragile. High voltage operation for PMTs is required (in excess of 1 kV) and the measurements can not be performed in magnetic fields. These features limit the full exploitation of the technique in industrial environments and diagnostics laboratories, as well as the further development of in loco assays, e.g. for the monitoring of water quality or in veterinary. Although until now SiPMs were sparingly adopted as sensors in biophotonics [33], the idea that they might eventually become the competitor or alternative to PMTs also in this field is emerging. Actually, different systems in recent years were proposed pointing out the need and the feasibility to implement a compact (in some case miniaturised) SiPM-based biosensing system [28, 76, 77, 14].

In this chapter, a characterisation of the performance of a multi-purpose and portable SiPM-based detection system in terms of low limit of detection (LoD) and linearity response is presented. It was tested with bioluminescence signals generated by aequorin, a chemiluminescent protein widely used as a genetically encoded intracellular probe for calcium (Ca^{2+}) gradient measurements [152].

6.1 Calcium signalling

Among all possible biological processes, calcium signalling is one of the most interesting and studied. Calcium (Ca^{2+}) plays a crucial role in cell metabolism and signalling, i.e. the ability of cells to perceive and respond to external stimuli in order to adapt to the environment changes [153]. The response occurs through a chain of chemical second messengers¹. Among these, Ca^{2+} is considered a universal second messenger because activates and regulates a plethora of proteins and, therefore, of processes [153, 25]. The Ca^{2+} signalling is constituted by four different functional units, as sketched in figure 6.1:

¹The first messengers are the external stimuli.

1. the stimulus, that generates Ca^{2+} -mobilizing messengers (signals);
2. the activation of the “ON mechanism” in order to allow Ca^{2+} enters into the cytoplasm (increasing the ion concentration);
3. Ca^{2+} -sensitive processes stimulated by the increase of Ca^{2+} concentration;
4. the “OFF mechanisms”, that remove, through pumps and exchangers, the excess of Ca^{2+} when the response finishes. They re-establish the initial conditions in the cytoplasm.

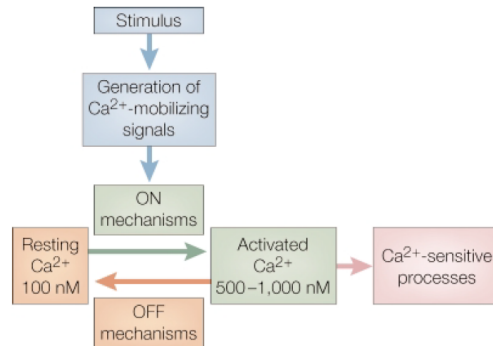
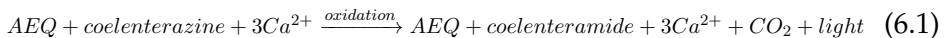


Figure 6.1: Sketch of the calcium signalling units: the stimulus generates Ca^{2+} -mobilizing signals, that induces an increase of Ca^{2+} concentration stimulating various processes. The response is then terminated extruding the excess of Ca^{2+} and restoring the initial conditions [25].

The Ca^{2+} concentration in cytosol ($[\text{Ca}^{2+}]_c$) when the cell is at rest is $\sim 50\text{--}100\text{ nM}$, while the typical values in extracellular environment ($[\text{Ca}^{2+}]_{ext}$) is $\sim 1.5\text{--}2\text{ mM}$. Actually, the cell continuously extrudes calcium from cytoplasm both by expelling it outside or storing it in subcellular organelles like mitochondria and lysosomes [153, 154]. Therefore, any variations of $[\text{Ca}^{2+}]_c$ occurs as the result of binding of specific membrane receptors in

response to a stimulus, following complex spatio-temporal dynamics. In the cell different Ca^{2+} -mobilizing messengers can co-exist because they are controlled by different receptors. This explains why Ca^{2+} regulates several processes. Some of these are: skeletal muscle and cardiac cell contraction, cell proliferation, gene transcription, neural transmission, ATP (adenosine-triphosphate, the energy unit for the cell) synthesis and many others [25]. Some of these examples are summarised in figure 6.2 for three different types of cell. For this reason, Ca^{2+} is one the most significant phenomenon to investigate the cell physiology and pathology. Several methods have been developed for measuring calcium intracellular concentrations mainly based on fluorescent or bioluminescent probes², i.e. molecules that undergo chemical reactions in presence of calcium. In general, they are based on the measurements of the emitted light due to the oxidation of probes. The emission is proportional to the calcium concentration [26].

Among bioluminescent probes, aequorin (AEQ) represents a common tool for measuring calcium concentrations. It is a protein that, when binds three Ca^{2+} ions, catalyzes the oxidation of its substrate (coelenterazine) with a subsequent emission of one photon peaked 465 nm, as shown in figure 6.3:



Therefore, a direct proportionality between the concentration of calcium and the intensity (frequency) of photons exists. When after a stimulus the concentration increases, an increase of the emitted light is expected. The breakthrough in the use of aequorin occurred when became possible to target specific cellular sub-compartments, allowing measuring the calcium concentrations in each organelle [155, 26]. AEQ-based assays are also of interest for the sensitivity to a wide range of $[\text{Ca}^{2+}]$, from sub-micromolar

²Unlike bioluminescence, fluorescence needs an external exciting light source to perform measurements.

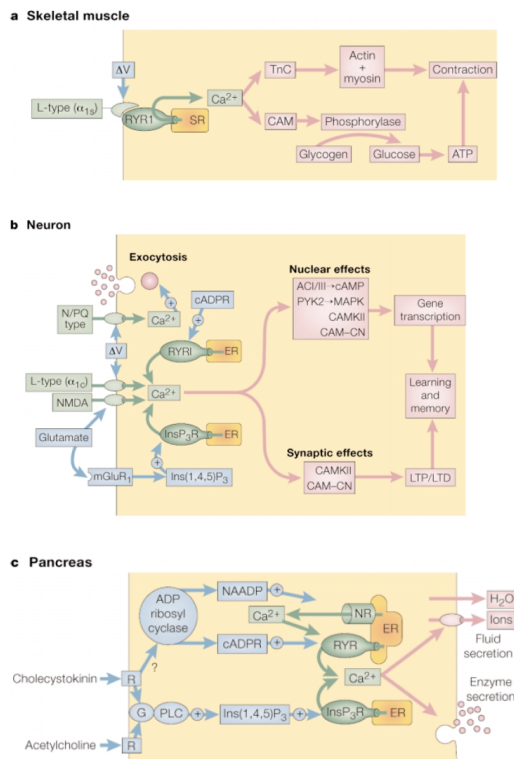


Figure 6.2: Description of some Ca^{2+} -sensitive processes in different cell types: skeletal muscle (a), Neuron (b) and Pancreas (c). The blue parts represents the different Ca^{2+} -mobilizing messengers involved to obtain the final response, i.e. the actuation of the Ca^{2+} -sensitive process (pink in figure). More details can be found in [25].

to millimolar. The major drawback of this technique is the low level of emitted light, consisting of a sequence of single photons [155, 26].

Usually the instruments dedicated to the detection of the luminescence phenomena are called luminometers. For every specific application they have peculiar features. However, in general, they consist of a sample chamber with reagent dispenser (sometimes with thermostat) for placing biological materials, a detector, signal processor methods and dedicated software

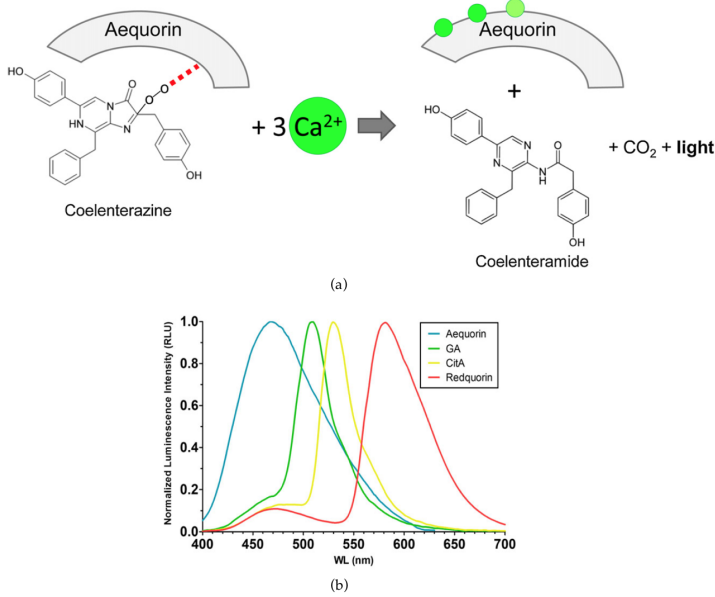


Figure 6.3: (a): Description of the chemical reaction that allows to use aequorin as Ca^{2+} probe [26]. (b): The Ca^{2+} -triggered bioluminescence emission spectrum of aequorin in blue. The spectrum is peaked at 465 nm with a FWHM of ~ 100 nm [27].

for the output results. The versatility and portability of SiPM based systems are crucial features that can be exploited for on-site measurements³, offering at least comparable performance with a lower cost [33, 76, 77].

6.2 Set up characterisation

The characterisation of a detection unit for aequorin bioluminescence based on SiPMs is carried out with a single SiPM Hamamatsu S13360-6050CS 6×6 mm² with 50 μm of pitch (14400 cells in total). The optimal bias voltage equal to 55.5 V ($V_{ov} = +3$ V) was provided by the CAEN Power Supply

³For instance, delicate cellular model requires specific competencies and expertise to be managed and cannot be easily delivered to distant laboratories for bioluminescence testing.

and Amplifier Unit (PSAU - SP5600).

Since the typical signals (figure 6.4) in bio applications and, in particular, in the proposed experiments for the Ca^{2+} detection (sections 6.3 and 6.4), are single photons emitted with a frequency that depends on the metabolite concentration, the measurements can be performed in two different ways: single photon counting (SPC) and charge integration (CI). In

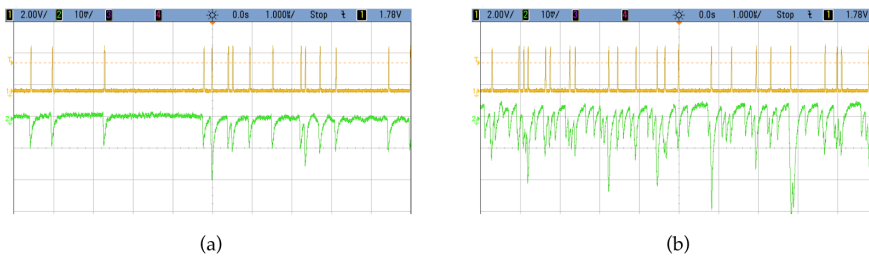


Figure 6.4: Examples of typical signals (in green) recorded in different experimental conditions, as explained in section 6.4.2.

SPC each pulse above the threshold of 0.5 photoelectron is counted in a predetermined time window ΔT . In CI the charge integrated in ΔT is retrieved. Both modalities were tested because they present complementary advantages and drawbacks. The phenomenon of pile-up, i.e. the overlap of single photon signals, spoils the measurements at high frequencies in SPC. The pile-up probability increases exponentially with the photon frequency, leading to non-linearity and saturation effects starting at a few of MHz. Charge integration overcomes the problem of the pile-up.

In order to qualify the two working modalities for a system with high dynamic range and low sensitivity, the SiPM was illuminated with a Poissonian light produced by a PLS-8-2-443 sub-nanosecond-pulsed LED by PicoQuant⁴, stochastically triggered. The trigger was generated discrim-

⁴<https://www.picoquant.com/products/category/picosecond-pulsed-sources/pls-series-sub-nanosecond-pulsed-leds>

inating the white noise, originated by an Agilent 33250A waveform generator, with a frequency ranges from few kHz to some MHz. The SiPM output signal was amplified by a two branches custom amplifier⁵. The two branches present different gain: 34 dB ($\times 50$) and 20 dB ($\times 10$). The full-time development of both output signals is 150 ns. The high gain branch is AC coupled (with an output capacitor of 1 μ F), while the low gain one is DC coupled. The latter assures the system sensitive to continuous photon fluxes, i.e. to photon frequencies so high that the baseline restoration is not possible. The 34 dB branch is exploited for the SPC measurements, while the 20 dB for the CI ones.

6.2.1 Single photon counting

For the characterisation of SPC, the intensity of the impinging light was set to an average value of $\mu_{phe} \sim 5$ phe/pulse, as shown in the multiphoton peak spectrum in figure 6.5. With this mean number of photoelectrons, the probability to have pulses without photoelectrons is below 1%, assuring that at least one photoelectron reaches the SiPM. It is estimated from the light Poissonian distribution as $P(0) = e^{-\mu_{phe}}$. The SiPM signal was digitized and discriminated by the Desktop Digitizer DT5730 (14 bit 2 V peak to peak). The discriminator output was then counted by a scaler (N1145 by CAEN S.p.A) over 10 s. For each frequency, a dark measurement (LED off) was also performed. On average the DCR measured was equal to 805.0 ± 0.9 kHz. Therefore, the frequency of photons reaching the SiPM was evaluated as the difference between the average count rate with LED on and the dark count rate. Figure 6.6 reports this difference as a function of the set LED trigger frequency. It is shown a non-ideal behaviour. The figure shows a clear non-linearity as the pulsing frequency exceeds 1 MHz. The system is no longer able to discriminate the pulses because

⁵The operational amplifier (AD8099) features ultra-low noise ($0.95 \text{ nV} / \sqrt{\text{Hz}}$) and high gain bandwidth (3.8 GHz).

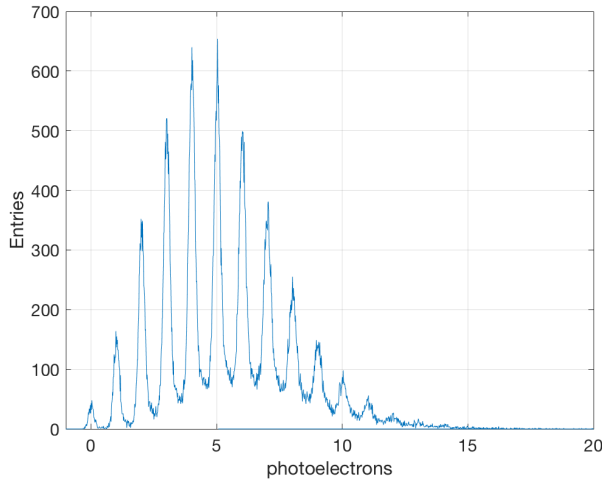


Figure 6.5: Multiphoton peak spectrum acquired with Hamamatsu S13360-6050CS (sensitive area of $6 \times 6 \text{ mm}^2$) for SPC measurements. The SiPM is illuminated with a pulsed LED at a frequency of 200 kHz. The mean number of photoelectrons is ~ 5 , assuring a null photon emission probability below 1%.

of the pile-up, as displayed by the oscilloscope screenshot in figure 6.6(c). In this example also a significant shift (some mV, with a Δpp of 5 mV) of the baseline is present, affecting the correct counting procedure at a fixed threshold. Fitting the data in figure 6.6 with the formula $y = a \cdot (1 - e^{-b \cdot x})$, a value $a = 5.0 \pm 0.2 \text{ MHz}$ is found, indicating that the system saturates at a few of MHz of incident light frequency, compliant with a full-time signal development of 150 ns. Moreover, also at low frequency, the system seems to underestimate the LED pulse frequency, even the above phenomenon should be negligible. Indeed a coefficient angular of the linear fit equal to $m = 0.78 \pm 0.01 < 1$ is retrieved.

The SPC capability can be enhanced by shortening the signal by means of a pole-zero cancellation circuit. Indeed, the pile-up probability depends on the count rate and the signal full-time development: shorter the signal higher the frequency reachable before pile-up arises. Therefore a filter,

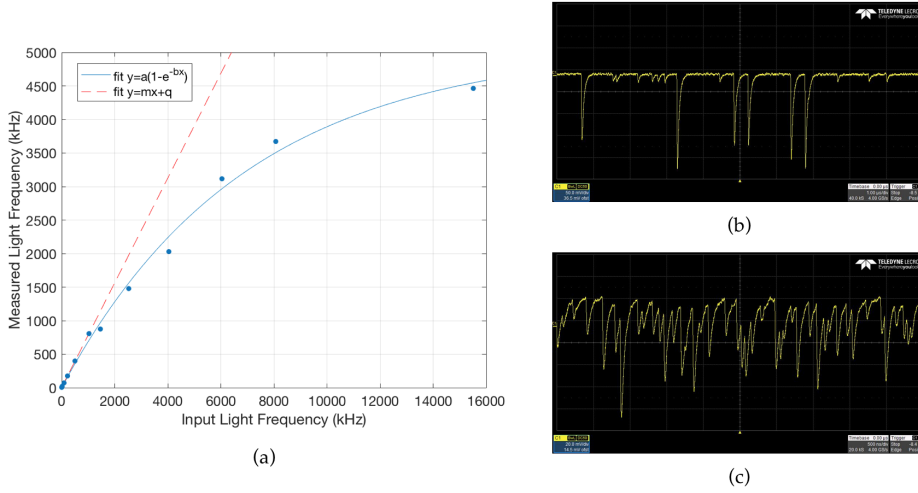


Figure 6.6: (a): The pulse detection frequency as a function of the nominal LED trigger rate. The blue curve highlights the saturation effect, while the dashed red curve points out the non-linear behaviour from MHz. Oscilloscope screenshots of the SiPM output signals are shown when it is illuminated with the LED triggered at 200 kHz (b) and 8 MHz (c).

able to produce an output signal with 30 ns full-time development, was designed⁶. An example of the reshaped SiPM signals is reported in figure 6.7. The measurements were repeated using the zero-pole filter to shorten the amplified signal. The measured frequencies as a function of the input ones are shown in figure 6.8. It points out that the linearity of the system is improved up to ~ 4 -5 MHz. Moreover the fit $y = a \cdot (1 - e^{-b \cdot x})$ leads to a higher saturation frequency $a = 27.9 \pm 0.6$ MHz, compliant with the signal full-time development of 30 ns (1/30 ns corresponds to a maximum distinguishable frequency of ~ 30 MHz). The increased discriminative capability of the system is shown also by figure 6.8(c), where the SiPM response to an

⁶The filter is passive, therefore, the amplitude is reduced of a factor ~ 2 . However, the signal remains high enough to be still discriminated.

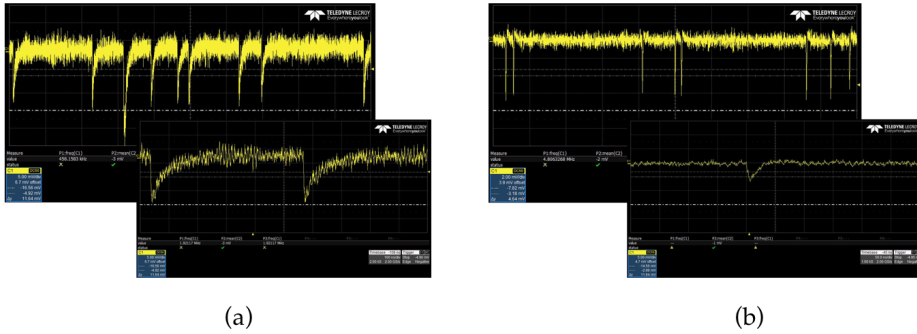


Figure 6.7: (a): the amplified SiPM signal without zero-pole cancellation. The zoom shows 150 ns full-time development of the signal (b): the SiPM signal after the zero-pole cancellation exhibits a full-time development of 30 ns. The zooms were acquired with a x-scale temporal division of 100 ns/division.

impinging frequency of 8 MHz is shown. An underestimation of the impinging light frequency is still present; the angular coefficient of the linear fit, in this case, is equal to $m = 0.88 \pm 0.01$.

Since the SiPM DCR is not negligible ($\text{DCR} \approx 805.0 \pm 0.9 \text{ kHz}$), the miscount can be due to the simultaneous arrival of one photon due to the DCR and one due to the LED illumination. This phenomenon becomes more evident at higher frequencies and implies a wrong subtraction of the noise pulses. In order to address this issue, coincidence measurements were performed. A signal long 100 ns and synchronous with the trigger LED was correlated with the discriminator output of the digitizer through the N405 coincidence logic unit (CAEN S.p.A.). The above pulse duration was chosen according to the time required for a ~ 5 phe analogue SiPM signal to return below threshold (0.5 phe) after a detection event. In this case, the rate of coincidences should approach the input light pulse frequency, when pile-up and baseline shift effects are negligible. As shown in figure 6.9, the angular coefficient without the pole-zero cancellation is

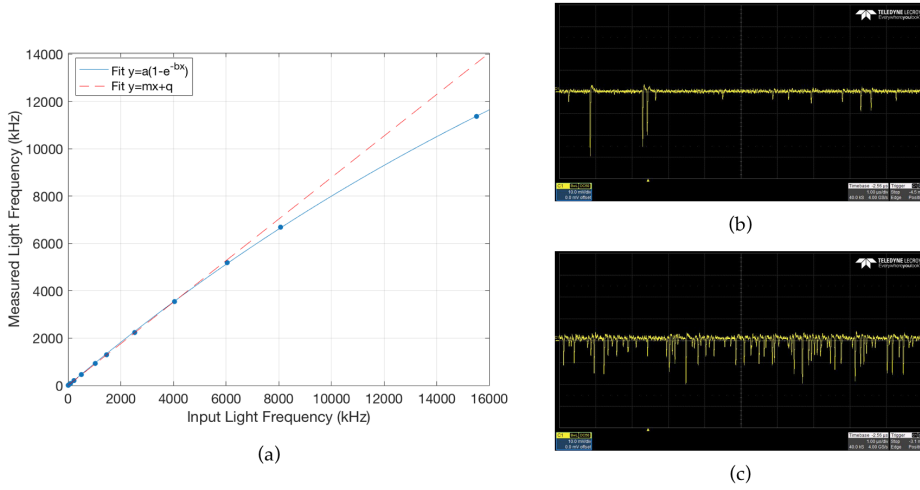


Figure 6.8: (a): The pulse detection frequency as a function of the nominal LED trigger rate when the SiPM signal is shortened through a pole-zero cancellation filter. The blue curve represent the saturation effect, that occurs at higher frequencies. The dashed red curve reports the increased linearity range to some MHz. Oscilloscope screenshots of the SiPM output signals are shown when it is illuminated with the LED triggered at 200 kHz (b) and 8 MHz (c).

0.91 ± 0.01 and with the filter is 0.96 ± 0.01 , which indicate an almost ideal detection efficiency. Indeed, the discrimination of pulses closer than 100 ns is not possible by the system. Therefore, the probability of losing events within $\Delta T = 100$ ns is $1 - e^{-\mu}$, where μ represents the expectation value of counts within this window. For a LED pulse frequency of 100 kHz (plus the DCR), this corresponds to $\sim 8\%$. In the measurements with the pole-zero cancellation filter, the closer value to 1 could be explained considering that subsequent pulses, being shorter, are on the average more distant in time [34]. Obviously the coincidence method can not be used in case of bioluminescent measurements, but, however, the SPC system has been shown to have a linear response up to some MHz with pole-zero cancellation.

Another important parameter is the limit of detection of the SPC modal-

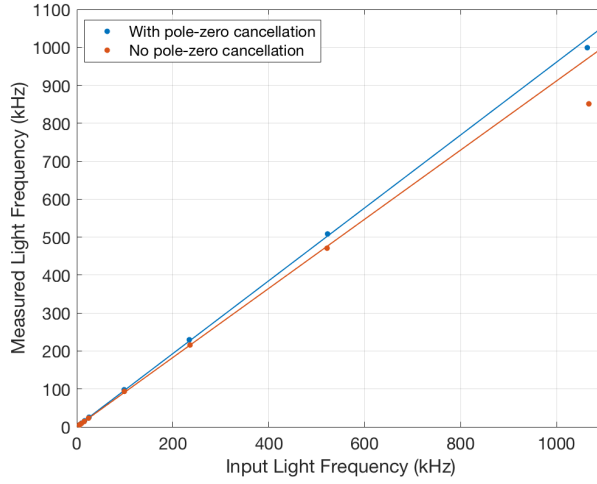


Figure 6.9: Coincidence rate with (blue points) and without (orange points) the pole-zero cancellation filter. The lines represents the linear fit describing the trends.

ity (LoD_{SPC}). It represents the minimum detectable variation frequency. Considering a Poissonian process, LoD_{poiss} can be defined as:

$$\text{LoD}_{poiss} \geq 3\sigma_{FreqPoiss} = 3 \frac{\sqrt{\Delta T_{count} \cdot DCR}}{\Delta T_{count}} \quad (6.2)$$

where ΔT_{count} is the time window in which the pulses over the threshold are counted. A signal is classified as such if it is higher than 3 standard deviations of noise. During the measurements performed on biological samples, described in section 6.3, the count window was kept fixed at $\Delta T_{count} = 100$ ms resulting in a LoD_{poiss} equal to ~ 8.5 kHz. This latter corresponds to the LoD_{SPC} measured in experimental conditions, as shown in figure 6.10, where a recorded DCR trace is displayed and $3\sigma_{DCR}$ corresponds to 8.45 kHz.

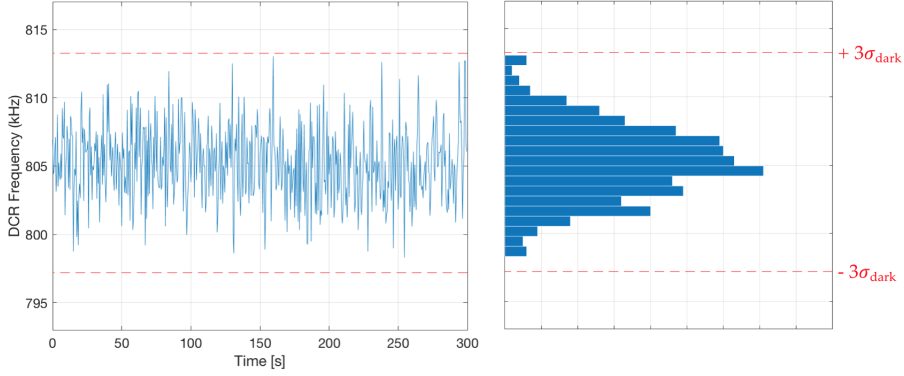


Figure 6.10: (a): Dark Count Rate trace, measured at 0.5 phe threshold, in a counting window equal to $\Delta T_{count} = 100$ ns. (b): The pertaining frequency histogram, showing a DCR mean value of 805 kHz. In both figures the red dashed lines represent the experimental LoD_{SPC} equal to $DCR \pm 3\sigma_{DCR}$ (8.45 kHz).

6.2.2 Charge integration

The charge integration measurements exploit the low gain branch of the amplifier. The charge integration was performed using an analogue integrator QDC (model V792N, CAEN S.p.A.). In this case, in order to simulate the light conditions of biological experiments, the mean value of photoelectrons impinging on the sensor was kept at ~ 1 phe, as shown by the multiphoton peak spectrum in figure 6.11. Indeed during the aequorin detection, trains of single photoelectrons are expected. The signals were integrated over a gate of $5 \mu s$ ⁷ both in light and dark conditions. The sampling rate of the QDC was fixed at 35 Hz. The mean values of the two distributions μ_{light} and μ_{dark} and their difference $\Delta = \mu_{light} - \mu_{dark}$ were computed. Figure 6.12 shows the trend of Δ as a function of the impinging frequency. A linear behaviour over roughly 4 orders of magnitude is retrieved, with an angular coefficient m equal to 0.087 ± 0.005 ADC/kHz

⁷By datasheet integration gates up to $40 \mu s$ are possible, but $5 \mu s$ is chosen according to the conditions used during biological measurements to avoid QDC saturation.

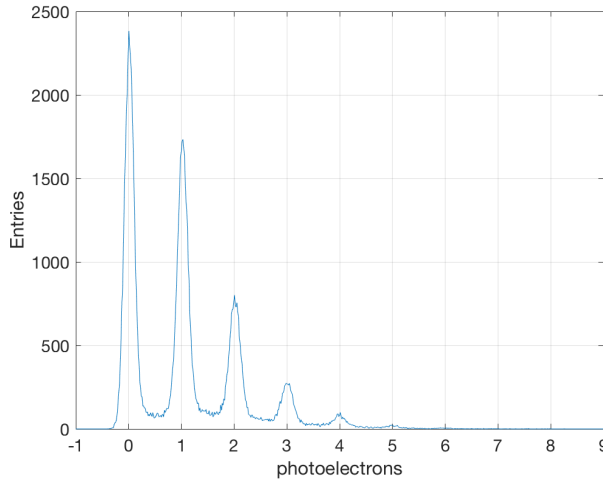


Figure 6.11: Multiphoton peak spectrum acquired with Hamamatsu S13360-6050CS (sensitive area of $6 \times 6 \text{ mm}^2$) for CI measurements. The SiPM is illuminated with a pulsed LED at a frequency of 90 kHz and the signal is integrated over ~ 200 ns. The mean number of photoelectrons is ~ 1 , assuring to recreate similar light conditions to the biological experimental ones.

and the y-intercept is compatible with zero within 2σ confidence interval.

In order to compare this acquisition modality with the SPC one, the LoD of the system, defined as the minimum detectable variation frequency, is evaluated as:

$$LoD_{CI} \geq 3 \frac{\Delta}{\sigma_{dark}} \quad (6.3)$$

i.e. the charge variation such that it is distinguishable from noise fluctuation (σ_{dark}) at a confidence level of 3σ . In these measurements, σ_{dark} is equal to 67 ADC channels. The LoD, considering the linear relation with a coefficient of 0.087 ADC/kHz, results in a minimum detectable variation frequency of ~ 2.3 MHz. Figure 6.13(a) reports the charge distribution of light OFF (the blue one) and light ON (the orange one) conditions when the LED is triggered at a frequency of 2.5 MHz. As displayed, the mean value of light ON distribution (red line) is above the $3\sigma_{dark}$ threshold (dashed

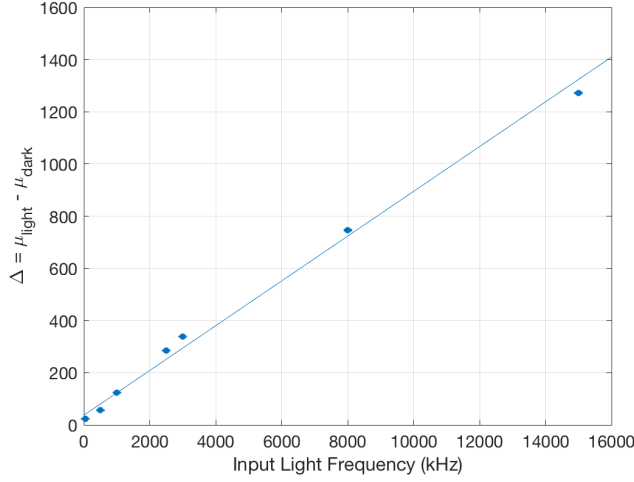


Figure 6.12: Variations Δ of the integrated charge with and without the light impinging on the sensor as a function of the impinging photons frequency measured by the analogue integrator QDC. The blue curve represents the linear fit of the data.

blue lines). It is worth of mentioning that this threshold is higher than the $\text{LoD}_{\text{poiss}}$ expected due only to Poissonian noise fluctuations. Indeed, from equation 6.2, taking into account an integration window of $\Delta T_{\text{int}} = 5 \mu\text{s}$ and a DCR of 805 kHz, the $\text{LoD}_{\text{poiss}}$ is equal to 1.2 MHz (a factor ~ 2 lower than the measured one). This is due to non-Poissonian fluctuations of the baseline, introduced by the amplifying stage. Indeed, temperature drifts and instability observed in the power supply of the amplifier induce shifts of the baseline that results in higher noise variations.

The LoD_{CI} can be reduced averaging the recorded charge over a number of samples $N=17$. This number was chosen to reduce the sampling rate of the QDC to 2 Hz (the SPC setup sampling rate) used in the bioluminescence measurements, described in section 6.3. Therefore, the standard deviations of the distribution scale as $\sqrt{N} = 4.12$. It results in a LoD_{CI} reduced of a factor ~ 4 , i.e. ~ 500 kHz. Indeed, as shown in figure 6.13(d), the

mean value of the charge distribution when the SiPM is illuminated with a photon frequency of 500 kHz, is distinguishable over $3\sigma_{dark}$.

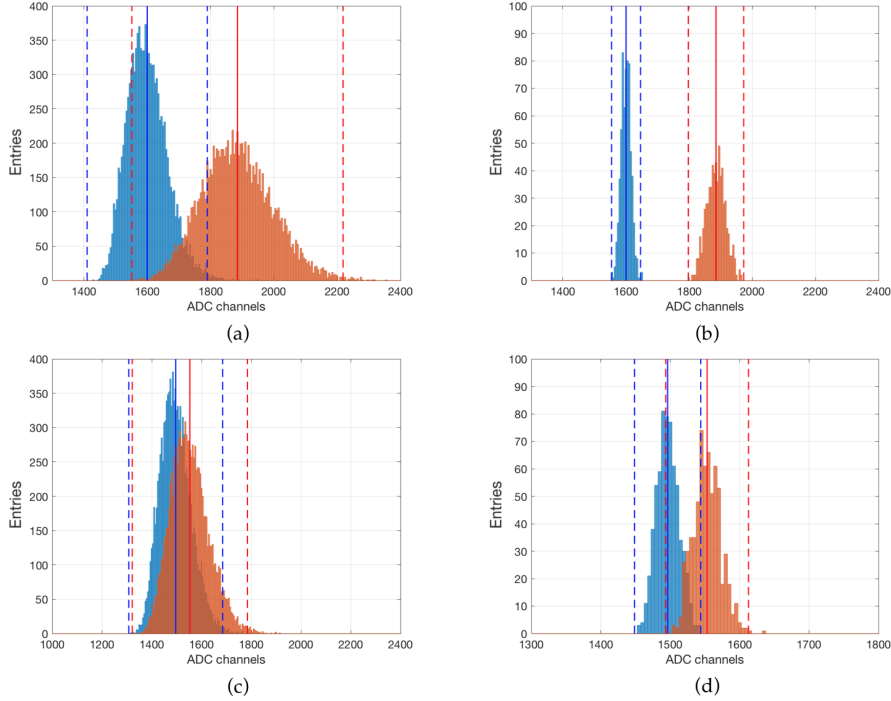


Figure 6.13: Figure of merit for the LoD_{CI} . (a) Charge distribution: in blue, the dark current of the biased SiPM (amplifier noise + SiPM DCR). The blue line corresponds to the mean value (μ_{dark}), while the two dashed lines set the limits $\mu_{dark} \pm 3\sigma_{DCR}$ in an integration gate of $5 \mu s$. In orange, the recorded charge when a Poissonian light generated at a frequency of 2500 kHz is displayed. The red line is the mean distribution value μ_{light} . (b): The charge distribution after average over $N=17$ samples in dark conditions (blue) and with 2500 kHz of light (orange). (c): Similarly to (a) with a light frequency of 500 kHz. (d): The dark and 500 kHz light distribution after average over 17 samples.

Therefore, due to the non-Poissonian fluctuations of the signal baseline, CI exhibits a lower sensitivity with a LoD_{CI} of 500 kHz, while SPC has a LoD_{SPC} equal to 9 kHz, keeping constant the sampling rate of events at

2 Hz. Although single-photon counting approach is plagued by a smaller linearity range due to pile-up phenomenon, it is more suitable than CI for the detection of faint signals.

6.3 Detection of bioluminescence: assessment of linearity with cell lysates

From the preliminary results, it is expected that the combination of the two possible working modalities of SiPM allows covering a dynamic range of at least 5-6 orders of magnitude in terms of single photon frequency. In order to verify this prospect and correlate it to Ca^{2+} concentrations, preliminary experiments on biological specimens were carried out. Initially (this section), a proof-of-concept based on the lysate, i.e. a fluid containing lysed cell, namely cells whose membranes are destroyed, was performed. Since it consists of “destroyed cells”, their response variability is reduced as compared to the live-cells and, therefore, it represents simplified experimental conditions from a biological point of view. Secondly (section 6.4), a first attempt to detect Ca^{2+} in live-cells was carried out. For all these measurements, human cervical adenocarcinoma cell lines (HeLa cells) were used. They stably expressed (i.e. they produce) cytosol-targeted-aequorin (cyt-AEQ) after infection processes, described elsewhere [152], and they were stored at -80° until needed.

The coverslip with the biological specimens (lysate or live-cells) was directly placed in contact with the sensitive area of the SiPM. Optical grease was interposed between the sample and the sensor in order to assure the optimal index matching of the refractive index. Figure 6.14 shows pictures of the setup.

For the lysate measurements, the HeLa cells with cytosol aequorin were grown in order to reach the confluence⁸ and then were centrifuged for 5

⁸The cells adherently cover all the surface of the culture dish where they are grown.

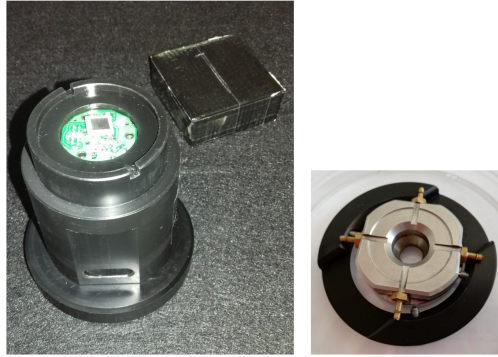


Figure 6.14: Pictures of the sample holder for the 6×6 mm² and chamber for the placing of the bio-specimens.

minutes obtaining the suspensions of cellular components⁹. Then, in order to detect different calcium concentrations, 11 lysate dilutions were prepared, adding a buffer solvent (composed of Tris, i.e. tris(hydroxymethyl) aminomethane, plus EDTA, i.e. ethylenediaminetetraacetic acid). More details on the biological protocol can be found in [28]. AEQ concentration ([AEQ]) was progressively halved, but keeping constant the volume of the sample (500 μ L) on the sensor.

These measurements allow assessing the operational dynamic range reducing at the minimum the biological variability. The bioluminescent light was detected in parallel with the two acquisition modalities. Figure 6.15 sketches the experimental set up. For the SPC mode, the pole-zero cancellation circuit was used. Then, the discriminated signal was counted by a 16-bit counter hosted in the PSAU SP5600 over a time window $\Delta T_{count} = 100$ ms. The sampling rate of the this system was 2 Hz. Instead the charge integration is performed with the QDC at a sampling rate of ~ 11 kHz¹⁰

⁹The cell membranes are destroyed and thus all the sub-cellular components are mixed

¹⁰The higher sampling rate allows averaging over a higher number of events, 5600 as described below, to reduce the LoD_{CI} .

in $\Delta T_{int} = 5\mu\text{s}$. For 30 seconds the noise of the system is recorded and

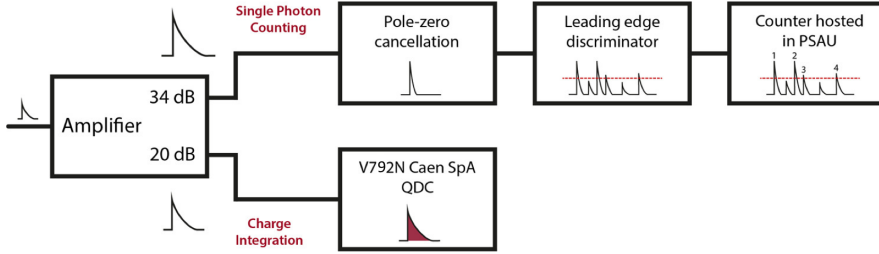


Figure 6.15: Block diagram of the SiPM-based experimental setup for bioluminescence measurements.

then the Ca^{2+} is injected. Exemplary traces recorded for both acquisition modes are reported in figure 6.16. The noise, estimated as the mean count rate/charge measured in the first 30 seconds, was subtracted. The response

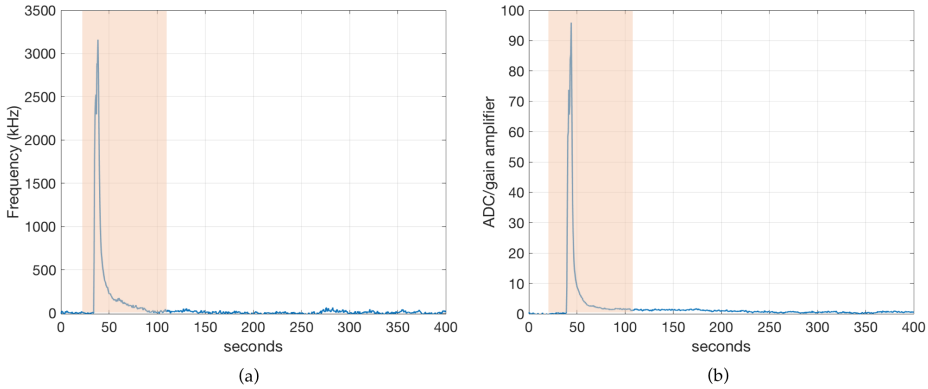


Figure 6.16: Response of the system in SPC (a) and CI mode (b) when Ca^{2+} is injected in lysate at a [AEQ] corresponding to a dilution factor of 2. The signal in the orange rectangular corresponds to the light emitted when the chemical reaction between Ca^{2+} and aequorin occurs.

to the injection of Ca^{2+} corresponds to the integral of the seconds-long spike, highlighted in orange in figure 6.16. It is proportional to the total

number of coelenterazine oxidation reactions, i.e. the AEQ concentration. The integral signal as a function of [AEQ] is reported in figure 6.17 for both acquisition modalities. To direct compare the results, the charge integration values are converted in count rates through the coefficient 272 fC/phe, deduced by the datasheets, considering the SiPM gain equal to 1.7×10^6 [24] and 1 ADC = 100 fC [156]. Figure 6.17(a) represents the ex-

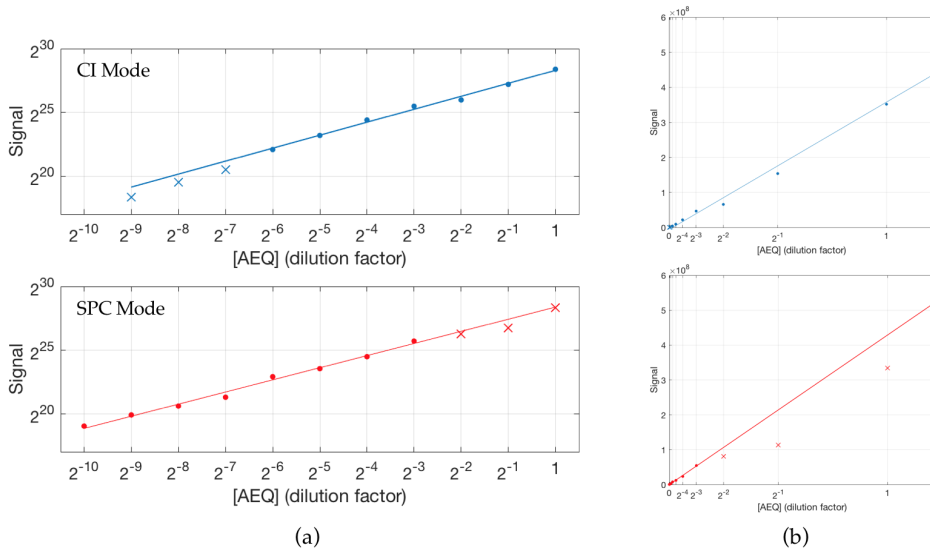


Figure 6.17: Linearity of the system response over 3 orders of magnitude. (a): The integrated intensity of the Ca^{2+} signal as a function of [AEQ] dilution factor for CI (blue points and crosses) and SPC (red one) mode in log-log scale. The signal corresponds to the integral, i.e. the sum, of all the Ca^{2+} spike points. The crosses indicate the excluded points from the linear fit. (b): Similarly in linear scale. In SPC mode (red) the deviation from the linearity, due to the pile-up phenomenon, is emphasized.

perimental behaviour in $\log_2$ - $\log_2$ scale¹¹. Since a linear relation between

¹¹It is a standard scale used in biological measurements when dilution is used to identify the trends.

the signal integral (called here counts) and the [AEQ] is presumed, a fit in log-log scale should give an angular coefficient equal to 1. Actually, a function of the type $y = mx^a$ is mapped in $\log_2$ - $\log_2$ scale in a line with $slope = a$ and y-intercept $q = \log_2(m)$. Figure 6.17 suggests a linearity of the system over ~ 3.3 order of magnitude ($\log_{10}(2^{11})$). Fitting all the data with a linear model, the obtained angular coefficients $a_{CI} = 1.107 \pm 0.030$ and $a_{SPC} = 0.921 \pm 0.027$, although close to the linearity, indicate slight deviations. In particular, SPC mode shows an underestimation at higher concentrations (more evident in linear scale in figure 6.17(b) bottom row), due to the pile-up phenomenon, that affects some of the points of the recorded bioluminescent signal, as explained below. Excluding the three higher concentrations, the slope of the fit increases to $a_{SPC} = 0.95 \pm 0.05$, compatible with 1 at 1σ . On the contrary, in CI the three lower signals are excluded because the integrals are underestimated as compared to SPC ones, due to a lower sensitivity of the system and a drift of the baseline that invalidates the measurements. The slope a_{CI} improves to 1.02 ± 0.04 , compatible with linearity. Instead, the intercepts are indicators of the sensitivity of the system. They are equal to $q_{CI} = 17.09 \pm 0.34$ and $q_{SPC} = 17.90 \pm 0.02$ respectively for SPC and CI. The higher value q_{SPC} indicates superiority of the SPC modality. Indeed, the difference $\Delta q = q_{SPC} - q_{CI}$ is related to the ratio between the sensitivities of the two working principles. In particular, the quantity $2^{\Delta q} = 1.75$ denotes that the counting is sensitive to a minimum concentration ~ 2 times lower than the CI. This conclusion is also confirmed by the experimental data, where the [AEQ] diluted 10 times is recorded only with the SPC modality (figure 6.17(a)).

In single-photon counting mode, the measurements at higher concentrations are affected by the pile-up. This can limit the accuracy in reproducing the temporal gradient evolution. As figure of merit to emphasise this issue, figure 6.18 shows the count rate of the Ca^{2+} signal peak detected in SPC (red points) and CI (blue ones) as a function of [AEQ] dilution factor.

A saturation trend over 3 MHz in SPC branch with respect to the CI one is evident. As demonstrated by the digitised SiPM signals during the Ca^{2+} in figure 6.18, the miscount is ascribable to the pile-up, loosing events at the level of single photon. The pile-up, as said previously, also affects the trend of the Ca^{2+} signal intensity, however, in the latter, the issue is mitigated, because only a few points around the peak are significantly distorted. CI

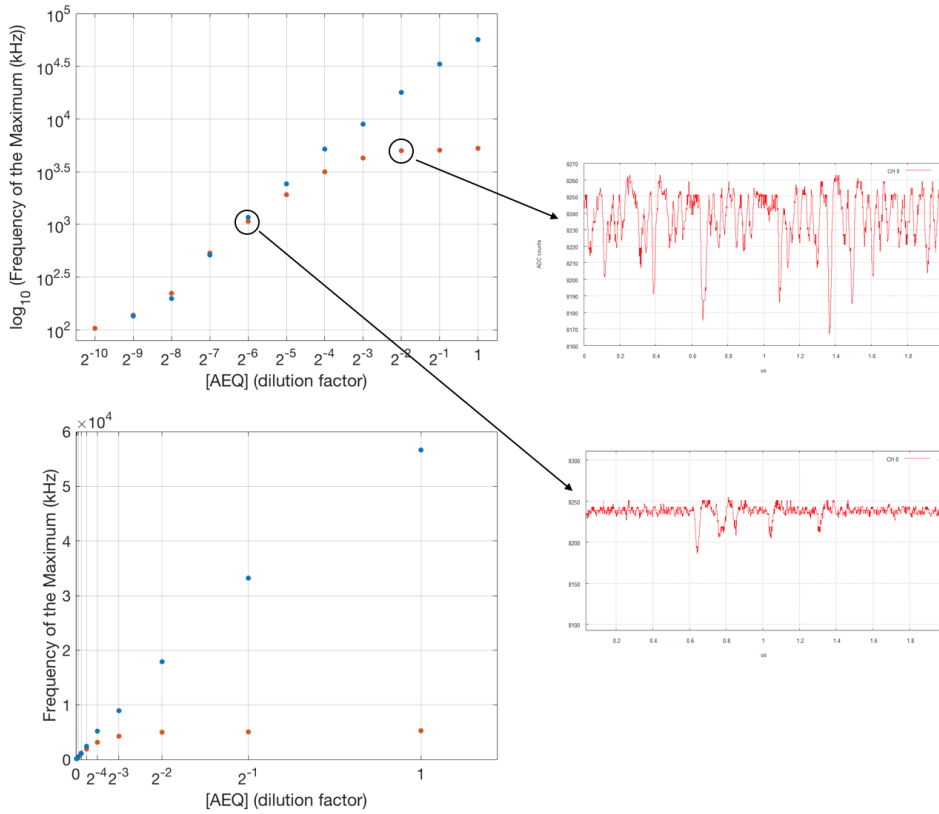


Figure 6.18: The Ca^{2+} signal peak frequency values detected in SPC (red) and CI (blue) as a function of AEQ concentration in the log-log plot (top) and linear scale (bottom). On the right, two examples of digitised SiPM signal at two different concentrations demonstrate how the highest concentrations are affected by pile-up.

branch is able, instead, to determine the peak also at lowest concentrations. Therefore, the limitation, in this case, is in accurately reconstructing the Ca^{2+} spike (in particular the tail) because of the lower sensitivity as compared to SPC one. It is worth of mentioning that, during these measurements, the QDC is externally triggered at ~ 11 kHz. Therefore, the calcium gradient reconstruction is performed with a higher time granularity. Averaging over a number of consecutive samples $N = 5600$, in order to reach the same time granularity of SPC system (~ 2 Hz), the LoD_{CI} can be reduced to ~ 31 kHz¹² (the LoD_{SPC} is ~ 9 kHz). This LoD_{CI} is 18 folds less than the value retrieved in the characterisation phase in section 6.2.2 i.e. it scales as the square root of ratio of the average number samples ($\sqrt{5600/17}$).

The ability of a SiPM-based system to quantify the chemical reactions of aequorin, when Ca^{2+} is directly injected from outside, is proved. Linear response over more than 3 orders of magnitude is retrieved exploiting the advantages of both working principles of SiPM, overcoming their intrinsic drawbacks.

6.4 Detection of bioluminescence: assessment of response to live-cells

For the procedure of Ca^{2+} measurements in living intact cells, the HeLa-cyt-AEQ cells were plated, 24 hours before the experiment, at decreasing densities on 24 mm round coverslip.

6.4.1 Live-cells response in charge integration

A first series of experiments was carried out recording Ca^{2+} gradients in CI mode. The cell densities were 100000, 50000 and 25000 cells/well. The external stimulus, that triggered intracellular Ca^{2+} release in the cells, con-

¹²The LoD_{CI} in $\Delta T_{int} = 5\mu\text{s}$ is found to be equal to 2.3 MHz. Since it scales as $\sqrt{N} = \sqrt{5600}$, it is reduced to 31 kHz.

sisted of the administration of adenosine-triphosphate (ATP) 100 μ M. After some minutes, injection of a surfactant (Triton X-100) destroyed the cellular membranes and all the residual AEQ was discharged with external administration of Ca^{2+} (in form of CaCl_2). This latter provides a sort of normalisation of the ATP-induced signal.

Figure 6.19 reports the traces of the two biological signals from living cells. ATP is a stimulus usually used to test the cell viability through bioluminescence detection¹³ [14], since it is able to activate various mechanisms that allow Ca^{2+} enters in the cytosol. Therefore, ATP injection (A) induces an increase of bioluminescence that lasts 1-2 minutes, as displayed in the inset of figure 6.19. Then the Triton-induced signal (T) is recorded. The CI measurements are compared to those acquired with a custom-designed PMT-based (H7360-01 Hamamatsu Photonics) aequorinometer produced by Cairn Research¹⁴, available at the Department of Pharmaceutical Sciences of the University of Piemonte Orientale. Figure 6.20 displays the recorded traces. In order to verify the agreement with the two different systems, the integral of the two induced signals (noise subtracted) and the average ratio (A/T) between these two quantities are computed. This quantity can be averaged over all the cell number because since the response to the two external stimuli is proportional to the [AEQ] expressed, their ratio is constant. The value results to be equal to: $A/T_{QDC} = 0.037 \pm 0.009$ and $A/T_{PMT} = 0.032 \pm 0.009$ for the SiPM-based and PMT-based aequorinometer respectively, confirming the reliability of the measurements performed with the SiPM in current integration mode.

¹³It can be considered a simple and effective first messenger; every cell responds to it, if not there are some problems in the cell homeostasis.

¹⁴<https://www.cairn-research.co.uk/>

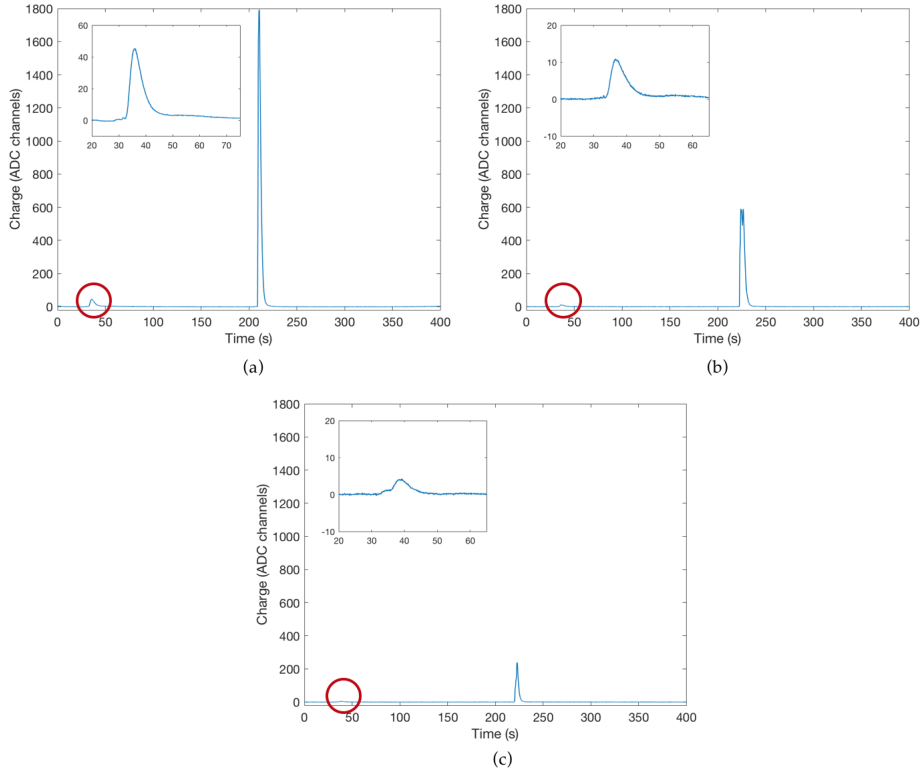


Figure 6.19: Bioluminescence signals (charge versus time) from AEQ-transfected HeLa cells, recorded in charge integration mode. Traces for three cell densities on the plate: 100000 (a), 50000 (b) and 25000 (c) cells/well. For each trace, the red circles highlight the response to the ATP injections, which is shown in the inset on the upper-left corner. The higher subsequent signal is induced by the Triton X-100 surfactant.

6.4.2 Live-cells response in single photon counting

Other samples with less cell/well densities (down to 3000 cells/well) were prepared to quantify the possibility to detect faint signals from a few numbers of cells¹⁵ in SPC acquisition mode. However, the need to discharge all

¹⁵Considering that 3000 cells are plated over a 24 mm diameter coverslip, only a few hundreds of cells lie on the SiPM sensitive area.

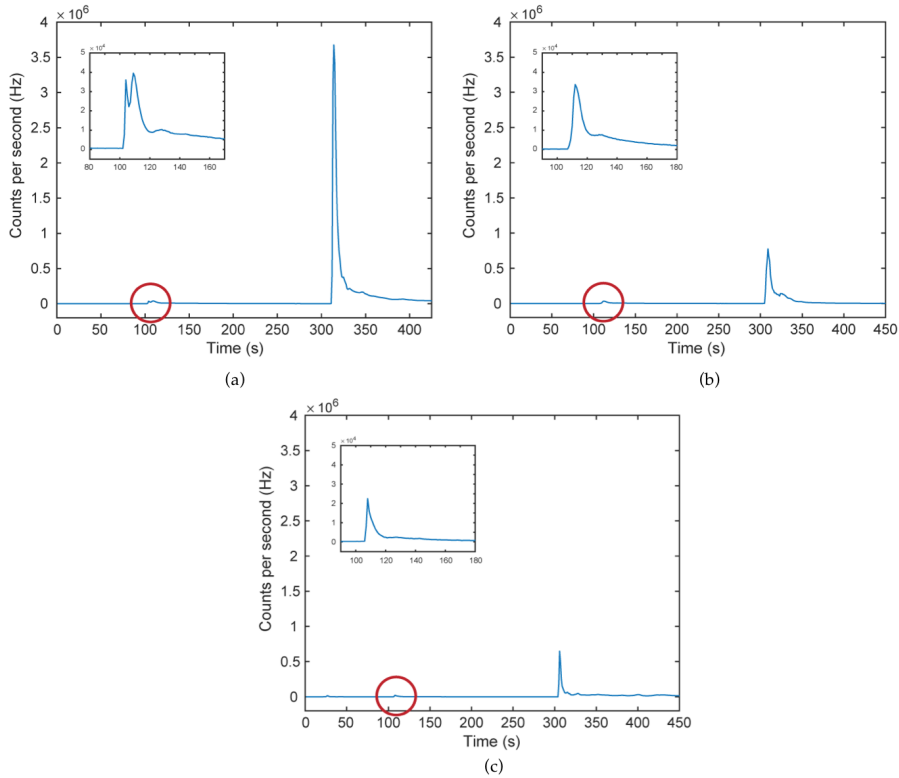


Figure 6.20: Bioluminescence traces from AEQ-transfected HeLa cells, recorded by the PMT-based luminometer for three cell densities on the plate: 100000 (a), 50000 (b) and 25000 (5) cells/well. The red circles highlight the response to the ATP injections, which is shown in the inset on the upper-left corner. The higher subsequent signal is induced by the Triton X-100 surfactant [28].

the residual aequorin through the delayed disruption of the cells induces a significant difficulty, since a factor ~ 25 -30 between the ATP and Triton signals is expected¹⁶. Figure 6.21 reports two exemplary traces recorded in SPC with cell densities of 30000 (a) and 50000 (b) cells/well. At higher densities ATP can induce a spike that could reach some MHz and, there-

¹⁶Considering different signal temporal evolutions, around the peak also a factor 45 can be reached.

fore, the expected Triton should exceed 30 MHz, while the SPC system is limited at some MHz. The Triton signal can not be accurately reconstructed because affected by intensive pile-up, as demonstrated by the oscilloscope screenshots in figure 6.22(b).

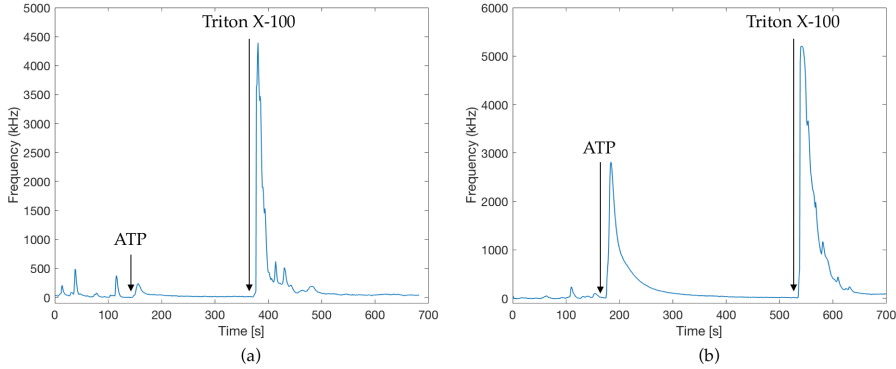


Figure 6.21: Bioluminescence signals (frequency versus time) from intact cytochrome AEQ-transfected HeLa cells, detected through PSAU discriminator in SPC mode for two different cell densities: 3000 (a) and 50000 (b) cells/well. The ATP and Triton X-100 induced signals are labeled by a black arrow. Spontaneous $[Ca^{2+}]_{cyt}$ signals are detectable before the ATP injections.

However, it should be emphasized that the higher sensitivity of the SPC system allows to measure very faint signals at lower densities. Moreover, the signals induced by the spontaneous $[Ca^{2+}]_{cyt}$ oscillations are detectable. In figure 6.21 there are some examples of these signals before the ATP injection. They are due to an interplay between calcium releases from intracellular stores and Ca^{2+} influx through the membrane, controlled by the Ca^{2+} itself or by intracellular messengers (for more details [157]). Spontaneous oscillations control fundamental cell processes as fertilization and differentiation. In HeLa cells, they are thought to play a role in stages of the cell division cycle [158]. This is also an example of the above-cited cell variability: not in all the samples they are present and in part they contribute to

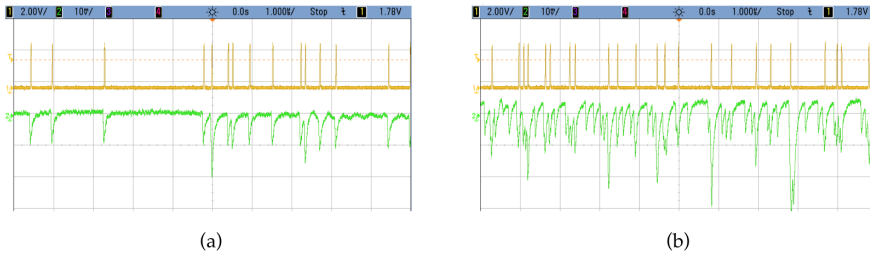


Figure 6.22: Bioluminescence single photons emitted by 3000 cells over the plate in response to the injection of ATP (a) and Triton X-100 (b). The SiPM signals are in green, while the yellow ones represent the discriminator output. From the oscilloscope screenshots, it is evident that the pile-up affects the measurements of the Triton induced signal also at a low number of cells. Instead the reconstruction of ATP signal is reliable.

discharge aequorin before the ATP stimulus, modifying the response from one cell sample to another.

6.5 Conclusion and future directions

In conclusion, the use of two different readout schemes for the SiPM signal processing allows addressing the challenging use of SiPMs for bioluminescence induced by Ca^{2+} gradients from living cells. Charge integration and single-photon counting are complementary alternatives to manage the bioluminescent signals (both strong and weak). Agreement between the CI-operated system and PMT-based luminometer, retrieved at 1σ of confidence level, proves the possibility to exploit SiPMs to reconstruct accurate Ca^{2+} gradients.

This proof-of-concept represents a starting point for instruments able to conduct more performing studies and tasks, not exclusively related to calcium signalling. SiPMs can be integrated (due to their compactness) in portable setups for on-site measurements, but, for instance, SiPM matri-

ces can be exploited for the development of microplate readers, used for drug screening. Currently, they feature a single PMT as detector. Therefore, the microplate has to mechanically slide over the sample in a sequential mode, increasing the screening time and avoiding parallel time-course recording. SiPM arrays, where each SiPM is placed under each well of the microplate, allow a parallel and high-throughput real-time acquisition for each biophysical parameter under investigation (in this case also more than one can be tested in parallel with different probes). Moreover, SiPM matrices can be implemented in multicolour luminescence detector. Different organelles (as mitochondria and cytosol) can be targeted with different probes (aequorin itself), emitting at different wavelengths for studies, for instance, about the cell energetic balance through detection of ATP exchanges. Other of these possible future evolutions are reported in [28].

6.6 Future directions: qualification of a multichannel system

A key configuration and natural evolution for future developments is a compact and multichannel system, naturally profiting from an ASIC read-out. A prototype was designed, constructed and commissioned, based on the CITIROC 1A and a Hamamatsu S131615-1050N 8×8 SiPM matrix (figure 6.23). It represents the first step towards an imaging SiPM-based system. Each SiPM of the array has a $1 \times 1 \text{ mm}^2$ sensitive area with $50 \text{ }\mu\text{m}$

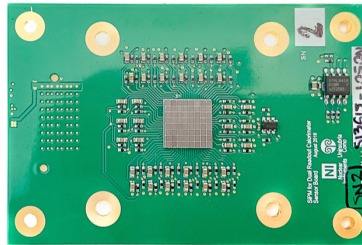


Figure 6.23: Picture of the Hamamatsu S131615-1050N 8×8 SiPM matrix.

pitch (396 total cells). Considering also the dead space between SiPMs, the matrix has a dimension of $9.56 \times 9.56 \text{ mm}^2$. The 64 SiPMs are readout by the two CITIROC 1A hosted in the DT5550W. Since charge integration measurements can not be performed with this readout system, only the single-photon counting mode is considered.

With a single $6 \times 6 \text{ mm}^2$ SiPM, at higher concentrations of aequorin in lysate, a peak frequency of $\sim 35 \text{ MHz}$ was measured (see figure 6.18). Moreover, frequencies of the same order of magnitude are expected in Triton induced signals. The 8×8 array covers a higher area with respect to the single SiPM. However, each $1 \times 1 \text{ mm}^2$ SiPM should sustain a rate that is roughly a factor 36 lower. Although the single SiPM array DCR is $\sim 50 \text{ kHz/mm}^2$ instead of 22 kHz/mm^2 of the $6 \times 6 \text{ mm}^2$ SiPM, a LoD_{SPC} of 2 kHz ¹⁷ should guarantee enough sensitivity. Therefore, in this configuration, the SPC mode could be used to obtain accurate bioluminescent measurements.

6.6.1 Sensor characterisation

Initially, every sensor in the array was characterised in terms of breakdown voltage, DCR and OCT. The V_{BD} was evaluated illuminating the sensors with a pulsed LED and calculating the Δpp at different V_{bias} ¹⁸. The relation $\Delta pp = mV_{bias} + q$ leads to $V_{BD} = -q/m$, as in section 5.1.1. Figure 6.24(a) shows, for one SiPM, the multiphoton peak spectrum used to extrapolate the Δpp at $V_{bias} = 53.5 \text{ V}$. Figure 6.24(b) reports the linear trend between Δpp and V_{bias} . The extrapolated breakdown voltage is equal to $V_{BD} = 50.48 \pm 0.03 \text{ V}$. The V_{BD} dispersion of the SiPMs is less than 100

¹⁷The LoD_{SPC} is evaluated considering a counting time window of $\Delta T_{count} = 100 \text{ ms}$. This ΔT_{count} is the same then used in the measurements.

¹⁸The gain of the high gain branch preamplifier was fixed at the bit code 60 (the maximum is 62) and the charge was retrieved with the Peak Detector mode searching in a time window of 180 ns.

mV. In order to assess the single-photon counting capability, the noise is

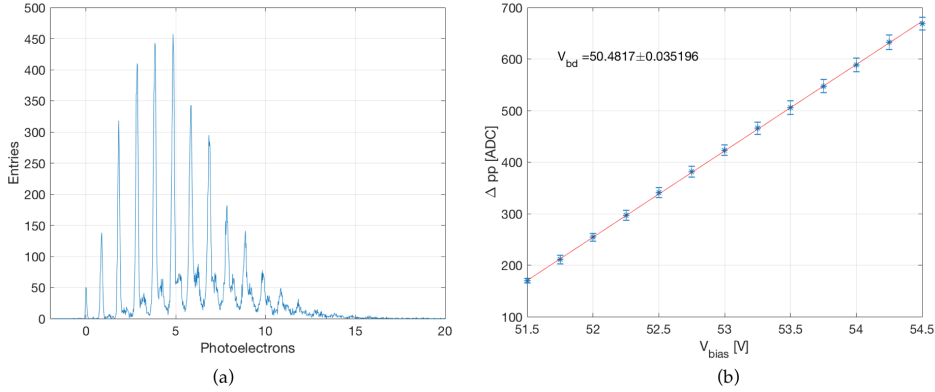


Figure 6.24: (a): Multiphoton peak spectrum measured for one SiPM, at the $V_{bias}=53.5$ V, illuminating it with a pulsed LED. (b): Extrapolated Δpp from multiphoton peak spectra as a function of SiPM V_{bias} . The V_{BD} is obtained by the linear fit (red curve) and is equal to 50.48 ± 0.03 V.

determined through the staircase curve, i.e. the noise count rate as a function of the discriminating signal threshold. The matrix is biased at 53.5 V ($V_{ov}=+2$ V), the recommended operation bias by the producer, through the common bias provided by the DT5550W. Figure 6.25(a) illustrates the staircase curve, while 6.25(b) the DCR at 0.5 photoelectron for all the SiPMs¹⁹. The count rates are evaluated in a time window of 1 s. On average, the SiPMs exhibit a DCR of ~ 46 kHz with a sensor to sensor dispersion of 1 kHz. This DCR results in a LoD_{SPC} equal to 2 kHz, expected to guarantee the sensitivity to the lowest level of lysate concentration investigated with the single 6×6 mm² SiPM. Finally, the sensor exhibits an OCT probability of 13%, in agreement with the values reported in the datasheet.

¹⁹It is found that three SiPMs don't work and, thus, they are excluded in the future analysis.

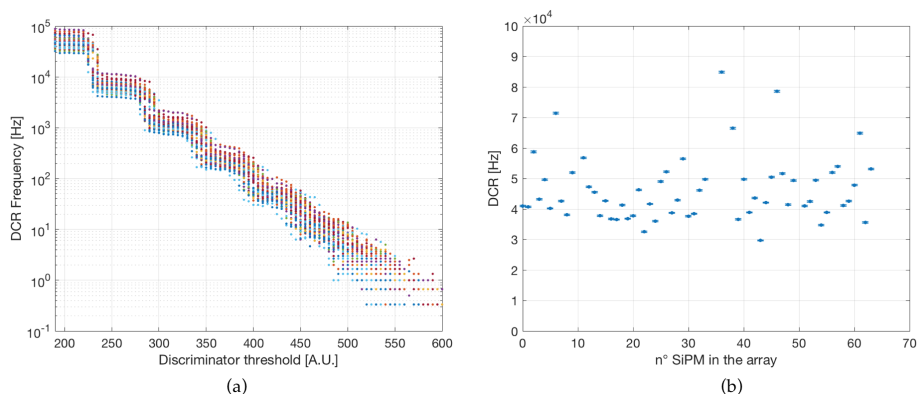


Figure 6.25: Figures of merit of the matrix noise. (a): Staircase curve measured for each SiPM in the array. (b): Extracted DCR values at 0.5 phe threshold (corresponding to threshold 205 A.U. in the staircase).

6.6.2 Portable luminometer system

A dedicated compact setup for biological experiments was designed (figure 6.26). A box hosts on the bottom part the perfusion chamber (figure

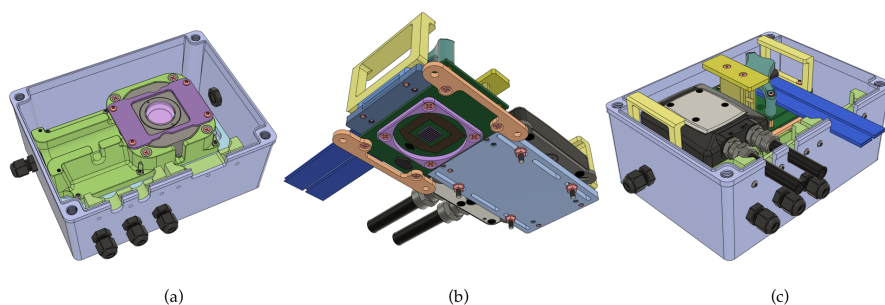


Figure 6.26: Drawing of the designed set up. (a): top view of the box with perfusion chamber holder placed on the bottom. (b): bottom view of the SiPM array holder, with the cooling system. (c): top view of the box once the sensor is placed in contact with the chamber holder.

6.27(a)), where the biological sample are placed, and the board with the

array is located above the sample (figure 6.27(c) and 6.27(d)). The alignment between these two components is assured by magnetic screws. The detector placement over the sample ensures that during the experiments possible leaks of liquids do not reach the sensor and the electronics. The refractive index matching between the sensor and the glass of the coverslip is obtained interposing 2 mm thick silicon rubber EJ-560 optical sheet²⁰. On the box side, some holes guarantee the possibility of the inlet and outlet of reactants. Moreover, the design takes into account the possibility to cool

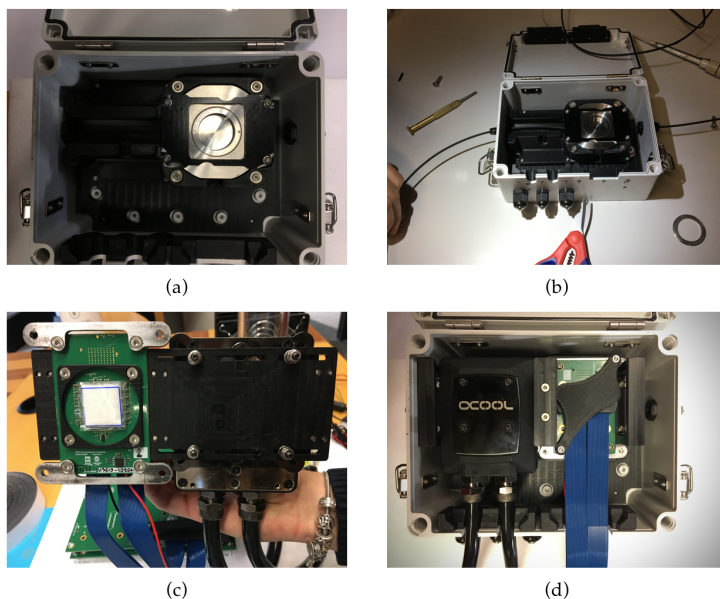


Figure 6.27: Pictures of the dedicated setup for bioluminescence measurements. (a) and (b): The perfusion chamber holder placed on the bottom of the box. Also the holes for the reactants inlet and outlet are visible. (c) and (d): The SiPM array board holder with the heat exchanger, placed on the top of the perfusion chamber.

down and keep constant the temperature through a Peltier system²¹. In particular, a 1 mm thermal conductive sheet and a 3.2 mm thick Peltier

²⁰<https://eljentechnology.com/products/accessories/ej-560>

²¹The idea is to cool down the sensor to $\sim 10^{\circ}\text{C}$ - 15°C .

element (TEC-30-32-127 by Wakefield-Vette²²) are put on the back of PCB board sensor. Four thermal flat pipes remove the heat laterally with respect to the sensor. Above the pipes, a heat exchanger (water-based) removes the heat from the box through tubes, in which cold water (18-20°C) circulates. A micro-controller regulates the current variation of the Peltier in order to reach the required temperature²³. Two temperature sensors are used. One monitors the environment temperature, while the second one is placed close to the SiPM array. The Peltier element acts on this latter. Operating SiPMs at a temperature of $\sim 13^\circ\text{C}$ ensures to decrease the noise of a factor ~ 2 , reaching a DCR and, consequently, a LoD_{SPC} comparable with the measurements performed with the $6 \times 6 \text{ mm}^2$ at 20-22°C. Figure 6.28 exhibits the staircase of the array SiPMs at 13°C with the cooling system prototype. A mean DCR of $\sim 23 \text{ kHz}$ is evaluated, resulting in a $\text{LoD}_{SPC} = 1.4 \text{ kHz}$ in $\Delta T_{count} = 100 \text{ ms}$, reaching the desirable sensitivity.

6.6.3 Assessment of linearity with cell lysates

The capability to detect bioluminescent light, produced by Ca^{2+} injection in the lysate of cells expressing cytosol-aequorin, is verified with the new system, in order to qualify the operational dynamic range, as previously done in section 6.3. Unfortunately, during these measurements instabilities on four channels, that increase the count rate, were observed. They seem to be related both to the Peltier system control²⁴ and the power supplied used for the DT5550W. This phenomenon is not yet completely understood and other tests are under investigation. Therefore, it was decided not to per-

²²https://www.mouser.it/datasheet/2/433/Wakefield_Vette_Thermoelectric_Cooler_Full_Data_Sh-1501217.pdf

²³Both a pulse width modulation and a linear drive are, at the moment, under investigation. However, the linear one seems to be the more performing in terms of stability and noise interference with the readout system.

²⁴Also some interferences when the soldered pin, that allows the readout of the SiPM temperature, is touched or connected to the Peltier control induces higher noise

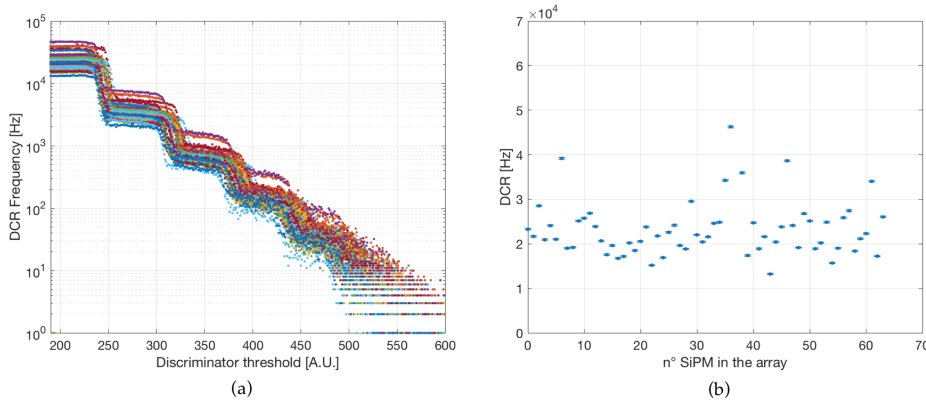


Figure 6.28: Figure of merit of the matrix noise. The system is cooled to 13°C. (a): Staircase curve measured for each SiPM in the array. (b): Extracted DCR values at 0.5 phe threshold (corresponding to threshold 209 A.U. in the staircase).

form the temperature control and the problematic channels were excluded in precautionary way. Only the heat exchanger was kept on allowing water at 20°C to circulate in the tubes and reach the thermal pipes to remove the heat from the box.

Applying the protocol described in section 6.3, 7 aequorin dilutions were performed. The calcium spikes recorded by each SiPM were summed, after noise subtraction, to obtain the total luminescent signal. Figure 6.29 reports the sum signal for the highest [AEQ] concentration (a) and the lowest one (b). For the same conditions, in figure 6.30 an exemplary single SiPM signal is shown. One single SiPM does not measure more than 800 kHz (850 kHz if noise is considered), while, when all the contributions are summed, the total signal does not exceed 40 MHz. Therefore, since the system doesn't seem to suffer the pile-up phenomenon²⁵, the linearity response should be recover over all the [AEQ] concentration tested. Figure

²⁵From datasheet, the sustainable count rate per each ASIC channel is ~ 20 MHz since the fast shaper signal output, used for trigger and count measurements, is 50 ns long.

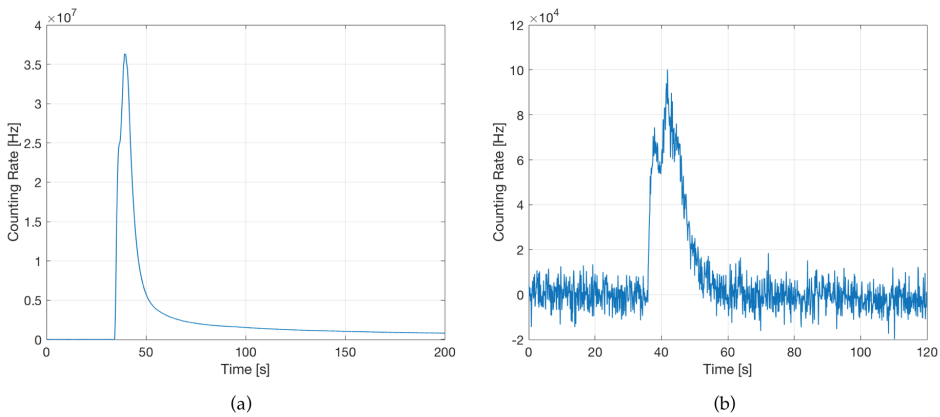


Figure 6.29: The traces of the sum signal of bioluminescence in lysate, noise subtracted, measured at the highest aequorin concentration (a) and at the lowest one (b). The double peak, especially evident in (b), is due to the injection operation; during this particular measurement the Ca^{2+} was injected in two steps that result in two peaks.

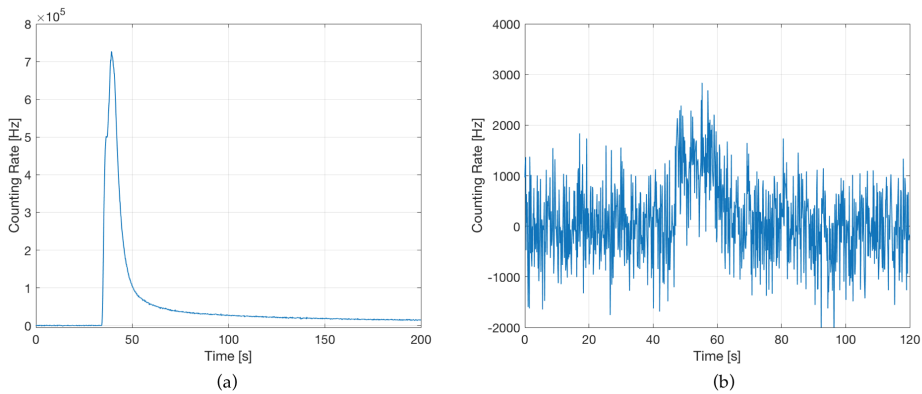


Figure 6.30: The response to Ca^{2+} in lysate for one SiPM in the array, measured at the highest aequorin concentration (a) and at the lowest one (b).

6.31 points out the linear response of the Ca^{2+} signal as a function of the aequorin concentration for both the sum of all the SiPMs (top row and blue points) and for one of them (bottom row and orange points). One point is

excluded from the fit because it results underestimated with respect the others. The linearity is confirmed by the angular coefficients of the log-log plot. They are equal to $a_{sum} = 1.059 \pm 0.077$ and $a_{single} = 1.065 \pm 0.080$, both compatible with 1. Moreover, the y-intercepts, equal to $q_{sum} = 18.53 \pm 0.45$ and $q_{single} = 12.70 \pm 0.52$, indicate that the sensitivity scales linearly with the effective sensitive area. Indeed the difference $\Delta q = 5.83$ leads to a ratio between the sensitivities of $2^{\Delta q} = 2^{5.83} \sim 57$, i.e. the number of SiPMs summed in the total signal.

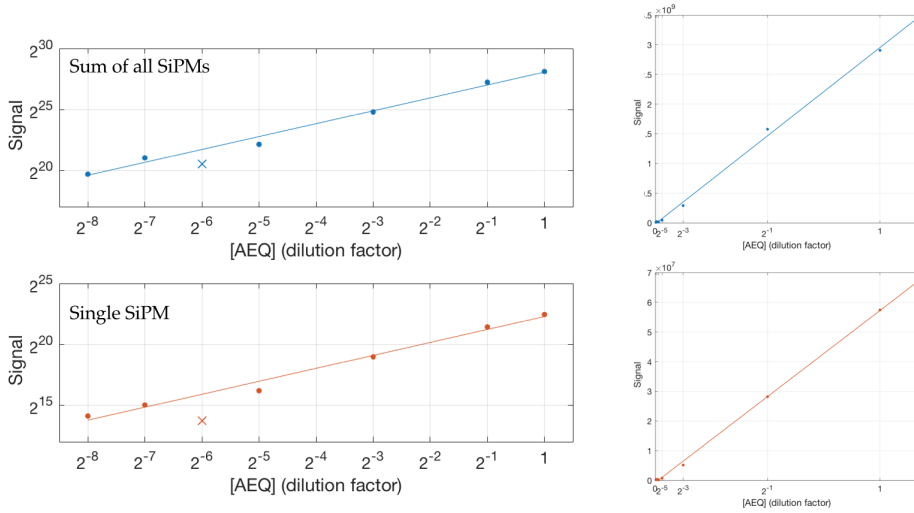


Figure 6.31: Linear operational dynamic range of the system. (a): The integrated intensity of the Ca^{2+} signal as a function of [AEQ] dilution factor for the sum signal (blue points and crosses) and the single SiPM signal (red one) in log-log scale. The crosses indicate the excluded points from the linear fit. (b): Similarly in linear scale.

The interesting possibility, offered by the 8×8 sensor, to observe the spatial evolution of the propagation, throughout the perfusion chamber, of the liquid with Ca^{2+} reacting with the lysate, can be considered the first step towards an imaging system. Figure [6.32](#) shows the images of the ar-

ray SiPMs count rates for six different instants. The increase of the count

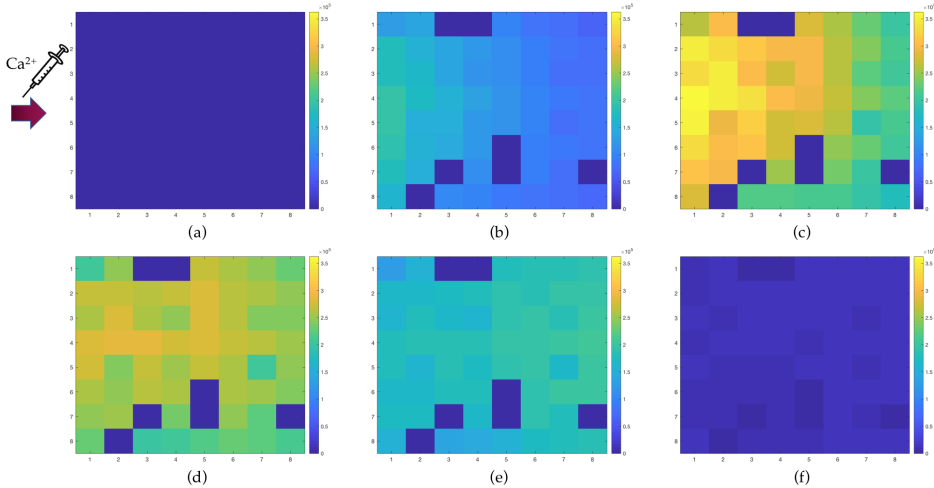


Figure 6.32: Images of the 8×8 SiPM array during the experiments. The intensity corresponds to the measured count rate, noise subtracted. The images are referred to different instants t . (a): $t=0$ s, (b): $t=1.9$ s, (c): $t=3$ s, (d): $t=3.2$ s, (e): $t=4.8$, (f): $t=41.8$ s.

rate, observed during the experiment, started from the injection side and then is uniformly distributed on the sensor, once the liquid propagated in the chamber. Obviously a resolution of 1 mm does not allow performing imaging on biological samples at the level of the single cell (the typical dimension of a cell is $\sim 50\text{--}100\ \mu\text{m}$). However, this proof-of-concept represents the starting point for future developments. For instance, the coupling of a 10× or 20× microscope objective lens could give the possibility to focus the light emission of few of cells on 1 or 2 SiPMs, studying, therefore, the pathways in or between the closest cells. Otherwise, with the advancement of the technology of digital SiPMs, as the single cells of the sensor are indexed and complex functionalities are implemented on chip, they can be implemented as the sensors of choice, without the need to use optical lens.

It is worth of mentioning that a quantitative comparison in terms of

[AEQ] with the previous measurements (based on $6\times 6\text{ mm}^2$ SiPM) can not be carried out, since the absolute concentration is unknown and is beyond the scope of these experiments. However, the two systems present both a linear response over ~ 3 orders of magnitude (2.7 with the array and 3.3 with the $6\times 6\text{ mm}^2$), guaranteeing operation working conditions for biological measurements with bioluminescent probes. Moreover, since the single SiPM of the array does not exceed 850 kHz, it is expected to be able to detect higher concentration without pile-up related issues.

Conclusions

The main purpose of the work described in this thesis is to identify and delineate the requirements for SiPM based systems in order to lead their application in innovative dosimeter systems and bioluminescence detection platforms. Moreover, the possibility to integrate several SiPMs and readout them through an ASIC (the CITIROC1A) is verified in order to improve the performances. SiPMs have proved to have enormous potential in dosimetry applications in medicine and in biophotonics, overcoming some of the main limitations of the current techniques and bringing to the development of breakthrough methods. The two examples, reported in the thesis, delineate this potentiality.

Firstly, the development of an in-vivo dosimeter for brachytherapy treatments verification was presented. The dosimeter, proposed by the ORIGIN collaboration, is based on the phenomenon of the scintillation. The scintillating materials in use were characterised. They exhibit a $\sim 500 \mu\text{m}$ long decay time and, therefore, the generated photons, when ionizing particles interact within the scintillator, are so spread in time that the scintillation signal consists of trains of single photons. Photon counting technique was

implemented to retrieve the information about the energy deposited by particles. However, because of the OCT of the sensor, a fraction of the detected scintillation photons results in signals of double or triple amplitude, easier to be counted due to the higher signal-to-noise ratio and the lower rate, guaranteeing linear response. The so-characterised dosimeter prototype was tested in a clinical environment (in phantom) in order to verify the system repeatability and its reliability to reconstruct the dose delivery to target areas. The agreement between the measured and the planned dose is at the level of 1%. These performances also are demonstrated to be similar to other dosimeters for brachytherapy, assuring the reliability of the system. Further tests, in terms of linearity, sensitivity and energy response, for both HDR and LDR brachytherapy sources will be carried out at the Northern Ireland Cancer Centre. When all the 16 fibres will be integrated, their output will be processed to develop a 3D image of the dose and a source localisation algorithm for HDR brachytherapy. This latter will be based on the trilateration, a mathematical technique in which the location of a point in space is calculated using the distances from such a point to other known points, as the tip of the fibres.

Additionally, a proof of principle for recording of intracellular Ca^{2+} dynamics using an aequorin-based probe and a SiPM sensor as bioluminescence detector were described and characterised. Bioluminescence detection remains challenging since the combination of bioluminescent emission properties and the concentration range of metabolites to be quantified sets stringent requirements on the detection system. Namely, single photon sensitivity, the ability of discriminating from noise very dim light signals (low detection limit) and wide dynamic range (some orders of magnitude of metabolites concentration) The work provides evidence of linearity of the overall system across three orders of magnitude of aequorin concentration. Charge integration and photon-counting proved to be two alternative and complementary modalities for handling the SiPM output signal,

coping with strong and weak bioluminescent signals. Moreover, the first steps towards a multi-sensor system readout with the CITIROC1A, that will allow the detection of metabolites, as calcium, in different organelles in cellular populations, was presented.

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