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SiPM as miniaturised optical biosensor for DNA-microarray applications

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ABSTRACT

A miniaturized optical biosensor for low-level fluorescence emitted by DNA strands labelled with CY5 is showed. Aim of this work is to demonstrate that a Si-based photodetector, having a low noise and a high sensitivity, can replace traditional detection systems in DNA-microarray applications. The photodetector used is a photomultiplier (SiPM), with 25 pixels. It exhibits a higher sensitivity than commercial optical readers and we experimentally found a detection limit for spotted dried samples of ~1 nM. We measured the fluorescence signal in different operating conditions (angle of analysis, fluorophores concentrations, solution volumes and support). Once fixed the angle of analysis, for samples spotted on Al-TEOS slide dried, the system is proportional to the concentration of the analyte in the sample and is linear in the range 1 nM–1 μM. For solutions, the range of linearity ranges from 100 fM to 10 nM. The system potentialities and the device low costs suggest it as basic component for the design and fabrication of a cheap, easy and portable optical system.

1. Introduction

Among the different application fields of biosensor, the biomedical sector attracts a special attention due to the countless needs to monitor disease-related parameters and parameters associated with the maintenance of health. Nowadays, biomedical systems should be miniaturisable, at low cost and easy to use in order to allow patient self-monitoring. Si photodiodes have the great advantage with respect to traditional detection systems of being highly miniaturisable, integratable and inexpensive. Hence, they are the best candidates for portable applications. For these reasons, an optical system based on silicon photomultipliers (SiPMs) for biomedical applications was developed.

SiPM is a pixelated photodetector where each pixel, formed by the series between a single photon avalanche diode (SPAD) and a quenching resistor, operates in Geiger mode [1–3]. The presence of the quenching resistor in each pixel ensures the avalanche shutdown, triggered by the generation of electron–hole (e–h) pairs in it. This device structure allows to the pixel to work as independent photon counter. Moreover, a common resistor collects the output signal of all pixels, connected in parallel. As a result, the output voltage is an analogue signal, proportional to the product between the number of pixels fired and the amount of charge produced in each avalanche event. However, avalanche breakdown can be triggered either an e–h pair thermal- or photo-generated or by free carriers generated during the avalanche of close pixels (optical cross-talk). To reduce optical interferences between close pixels, optical trenches were implemented in the device structure. They are fabricated all around each pixel, filled by metal or oxide, to limit interference phenomena between pixels, thus reducing the device noise [4–6].

Several works describe SiPM as fluorescence light detector [7–9], highlighting its higher sensitivity than commercial traditional detectors, its high response speed, its low cost and its small physical size that are key parameters to achieve inexpensive and portable devices. One of the biosensor applications in which SiPM was used, is the ssDNA microarray [10,11]. It consists of an array

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of ordered probes able to recognise a labelled nucleic acid (target) by direct hybridization with the complementary sequences contained in the probes anchored onto a surface [12]. In many applications, to improve the detectability, the target amount is amplified by means of a reaction as, for example, the polymerase chain reaction (PCR) [13]. In this paper we describe an optical system, based on SiPM technology, able to detect the fluorescence emitted by ss-DNA amino-terminated labelled with CY5, immobilized on Al-TEOS surfaces using GOPS protocol [14]. Aim of this work is to demonstrate that SiPMs are able to detected low-levels of fluorescence signals emitted by CY5 and that they are a promising substitute for traditional optical scanners. The results shown highlight the possibility to reduce the size of the detection systems, allowing the portability, and to make this kind of analysis less expensive; thus accessible to the entire population.

2. Materials and methods

Sensor details, sample preparation and experimental set up used to measure fluorescence signal emitted by DNA samples labelled with CY5 are described in the following subsections.

2.1. Sensor details

The sensor used in this work is 25 pixels SiPM with trenches, produced by the R&D of STMicroelectronics in Catania (Italy). It ensures the best signal-to-noise ratio if compared to same devices with a higher number of pixels with a physical size of $0.3 \times 0.3\ mm^2$. It has been inserted in a 32 pin open package, welded on a board, and placed inside a metallic box (miniDom [15]) to electrically and optically shield the device. In the miniDom, two BNC connectors allow one to bias the device and collect the output signal. Moreover, it has an optical window on the surface to allow the sensor to detect the fluorescence emitted by biological molecules.

2.2. Biological sample preparation

We studied SiPM limit of detection measuring fluorescence emission of 25 bp oligonucleotides CY5 labelled. We resuspended 30.7 nmol of GAPDH-Cy5 (/5Cy5/TGCCAACGTGTCAGTGGTGGACCTG/3AmMO/) lyophilized powder (from MWG Biotech) in 300 μl of water (DNase–RNase–Protease free); then, we centrifuged sample at 5500 rpm for 30′, after agitation in vortex, obtaining a 100 μM solution. A Sodium Phosphate Dibasic buffer (150 mM at pH 9.2) was used to dilute solution from 100 μM to 1 μM; 0.5 μM; 0.1 μM; 0.01 μM; and 0.001 μM, for optical measurements. After dilution, we dropped samples on 865 nm thickness aluminium-TEOS slides [12], following a specific layout (5×10 array); we spotted ten drops of 0.1 μl for each concentration in order to study SiPM sensitivity, stability and reproducibility of the measurement. We dried sample at room temperature for 10 min.

To experimentally test the sensor detection limit, we used the fluorophore Sulfo-Cyanine5 NHS ester (CY5, from Lumiprobe) diluted in DNase–RNase–Protease free water (Sigma Aldrich). We prepared seven fluorophore solutions (100 nM, 10 nM, 1 nM, 100 pM, 10 pM, 1 pM, 100 fM) for spotting. We dropped both 10 μl of samples and 2 μl of a mineral oil (used to prevent samples evaporation) on 12 wells chips following the layout shown in Fig. 1.

The spotting layout consisted of two 4×3 matrixes containing replicates of fluorophore dilutions from 100 nM to 100 pM (Fig. 1A) and 100 pM to 100 fM (Fig. 1B). A water–oil mix spot was used as reference. All preparation steps were performed in dark because of CY5 photosensitivity.

2.3. Experimental set up

The experimental set up used to detect fluorescence emitted by biological samples is based on: a HeNe fibre-coupled laser (633 nm) that hits at 60° with respect to the normal axis to the sample; a biological sample placed on a "*xyz* stage" that can move along three dimensions (*x*, *y* and *z*); a 25 pixels sensor placed on a goniometric system to monitor the signal behaviour at different emission angles. The miniDom optical window was equipped with a band pass filter centred at 670 nm (emission peak of CY5), in order to eliminate the background noise due to the excitation laser radiation reflected by the sample surface.

To measure the fluorescence emitted by CY5 solutions, the set up was slightly modified. In this case, the HeNe fibre-coupled laser hits normally to the sample placed on a "*x*, *y* Mount" parallel to the optical table. The sample can move along two dimensions (*x* and *y*) thereby intercepting the laser beam within the small well to be measured. The sensor, in this case, is placed at 45° with respect to the normal axis to the sample.

In both cases, the sensor is biased through a 236 Keithley Source Meter, that measures also the sensor output signal. The measurement system is based on a software opportunely developed in Labview©, that allows to control the bias voltage, the measurement conditions (angle of analysis and other key parameters), and to acquire the output signal automatically. The signal acquired is elaborated offline by a routine we developed in Matlab©.

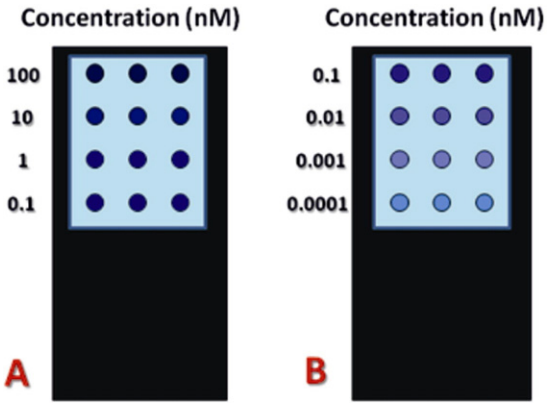

Fig. 1. Spotting scheme.

3. Results and discussion

3.1. Measurement method

The choice of the sensor bias voltage is very important since increasing the bias voltage both the sensor gain and its noise increase. In order to measure low fluorescence levels a trade-off between gain and noise must be found. For this reason we electro-optically characterised the sensor, measuring its current *vs* bias voltage (reverse *I–V* characteristic), as reported in Fig. 2.

We measured the sensor reverse current when during fluorophore emission (I_A, red line) and when no fluorophore is on the sample (dark condition, I_B, blue line). We chose the bias voltage of −30 V since it ensures the best signal-to-noise ratio. Once fixed the bias voltage at −30 V (green vertical line), we measured the net fluorescent current value is:

$$\Delta I = I_A - I_B \tag{1}$$

The current value reported in all the following figures (ΔI) is the net current measured as the difference between the current due to the fluorophore and the reference (laser radiation diffused by the support surface) using a device bias voltage of −30 V.

3.2. Evaluation of SiPM performances on microarray spots

In order to define the SiPM experimental detection limit, we measured the fluorescence emitted in different operating conditions in terms of analyte concentration and angle of analysis (Fig. 3). In particular, we analysed five different concentrations of ss-DNA labelled with CY5: 1 μM (black dashed line), 0.5 μM (red dashed line), 0.1 μM (blue dashed line), 0.01 μM (green dashed line), 0.001 μM (orange dashed line). We chose four angles of analysis to study the fluorescence behaviour: 35°, 40°, 45° and 50° with respect to the normal axis to the sample.

Fig. 3 shows that as the sample concentration increases, the emitted fluorescence increases as well. The current signal value at each concentration is roughly constant in the measurement angular range chosen, confirming the isotropic emission of fluorescence [9, 11,14]. In existing measurement systems, an image is acquired and elaborated offline, after acquisition, to achieve the quantitative signal. Our system, based on SiPM technology, provides directly a quantitative signal as output in real time. This simplifies the software of