CMOS Single-Photon Detector For Advanced Fluorescence Sensing Applications

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Abstract— **Fluorescence lifetime measurement is used in biological research to enhance the contrast of fluorescence images. The outstanding sensitivity that can be achieved with this method is obtained at the expense of a high data throughput. A substantial data reduction can be achieved using the time-gated technique, which consists in counting the number of photons occurring inside different time windows. Thanks to the recent developments in the realization of Single Photon Avalanche Diodes (SPAD) in standard CMOS technologies, this technique can be easily implemented at a pixel level. In this work, three different detectors fabricated within a 0.35 μm High Voltage CMOS technology will be described, focusing onto their use in lifetime imaging. The sensors have been designed for different optical setups and for different applications, ranging from Fluorescence Lifetime Imaging Microscopy to miniaturized Lab-on-Chip. The advantages and limitation of the proposed sensors will be pointed out and a case study of an application will be presented.**

Keywords-Single Photon Avalanche Diode; Fluorescence Lifetime; Time-correlated Fluorescence Spectroscopy

I. Introduction

Instruments capable of measuring the lifetime of fluorescent molecules, a characterization that is known as time resolved fluorescence spectroscopy, are becoming increasingly popular in biology research laboratories. This group of instruments can be classified in two categories: imaging instruments, that form the image of the specimen under analysis, and non-imaging instruments where sensors are typically close coupled to microarray of reaction sites.

The first group is mainly formed by microscopes able to perform Fluorescence Lifetime Imaging Microscopy (FLIM)[1]. One of the directions in FLIM detector development is towards arrays of optical sensors capable of increasing the frame rate of single-point scanning systems such as confocal scanning microscopes. One of the proposed solutions is the use of CCD cameras coupled to Gated Image Intensifiers (GIIs) [2], that suffers from the very low duty cycle of GIIs, but is capable of achieving the single photon sensitivity required by the application. The acquisition chain, even if the laser scanning of the specimen is avoided, is still bulky and complex, because the sensor needs to be connected to sophisticated time-measuring instruments.

If the specimen under analysis is a microarray assay of fluorescent probes spotted for analytical or diagnostic purpose, the analysis does not strictly requires to acquire a full image of the specimen. In this case, a non-imaging system can be built in a compact solution commonly addressed as Lab-on-Chip [3]. The characteristics of the optical detector in a Lab-On-Chip system are even more stringent because, if a large diffusion of the system is planned, the detector has to be also cheap, lightweight and easily operated.

One of the most promising solutions to achieve the detector required specifications in both imaging and not imaging instrumentation is the realization of matrices of Single Photon Avalanche Diode detectors (SPADs) and digital processing electronics in the same monolithic chip. This detector structure can be realized in a standard and cheap CMOS technology [4].

The construction of SPAD pixel arrays is challenging especially if the ratio between the photon sensitive area and the sensor area , namely the Fill Factor (FF), has to keep as high as possible. In principle every SPAD detector in the matrix needs to be surrounded by a guard ring structure, which limits the minimum distance between two neighboring light-sensitive areas. Moreover, decreasing the distance between two SPAD detectors, the probability of unwanted cross interference, such as Optical Cross Talk [5], increases.

Each SPAD must be coupled to a suitable quenching circuit and to a read-out channel suitable for fluorescence lifetime spectroscopy. The size of readout circuits in the SPAD arrays presented so far in the literature limits the sensor FF to a few percent, therefore strongly reducing the overall detection efficiency. The constraints of high FF is particular stringent in non-imaging devices where light propagates with a large angular distribution and therefore optical micro-lenses cannot be embedded on the top of the CMOS substrate to focus light on sensitive areas. Moreover, when the lifetime spectroscopy analysis is performed at the sensor level, the data-throughput between the sensor and the external system needs to be carefully considered.

In this paper we presents three different lifetime SPADs detectors, realized in a High Voltage CMOS 0.35 μm technology, and some experiments to validate their application to lifetime fluorescence spectroscopy in both imaging and non-imaging lab-on-chip setup.

In particular, in Section II we perform a comparison of the three detectors, that we separately detailed in [6],[7] and [8], analyzing the cited characteristics and explaining how the problems related to FF and data-throughput have been addressed. In Section III we describe the use of a SPAD array sensor in a lifetime spectroscopy experiment.

II. SPAD DETECTORS

The presented detectors operate using the Time Gate fluorescence detection principle [9]. The measurement is performed repeating a basic measurement cycle at a rate that is usually in tens of MHz range. The cycle starts with the excitation of the fluorescent molecules of the sample with a picoseconds laser pulse. Excited molecules return to ground state by emitting photons after a certain amount of time (typically some ns in biological applications), following an exponential-decay emission probability. The fraction of emitted fluorescence photons that reach the SPAD array is transduced in current pulses carrying the photon timing information with a picosecond resolution. The sensor readout channels measure the number of pulses inside two or more consecutive time-windows, thus allowing the estimation of fluorescence decay time. The number of photons detected in each time window after a large number of measurement cycles is stored in-pixel either in digital form or as an analog voltage value. Only the total number of photons detected for each time window are communicated to the external acquisition system and so the decay curve of the fluorescent light emission can be calculated with a low data throughput.

The three detectors have been developed in order to satisfy the requirements of three different target applications and therefore differ for the number of SPADs in the array, and for the characteristics of the front-end of the read-out channel. In Table 1 we resume the main characteristics of the sensors that in the following are labelled with the capital letters A, B and C.

The cross section of the SPADs employed in the three different detectors is depicted in Figure 1. The deep N-well implant can be shared between SPADs and this feature has been exploited to reach a higher FF. In the case of sensor B a remarkable FF of 48% has been obtained with a pixel pitch of 26 µm on a squared area of 260x260 µm. The noise of all SPADs has been measured and expressed as Dark Count Rate, i.e. the frequency of events that are detected in the absence of photons. This value is in the order of 1kHz for the 80% of the SPADs and a small percentage exceeds 100 KHz. The value depends on the excess bias voltage [5] and has been measured in all the three devices with an excess bias voltage of 4V. Among the three sensors, the one more severely affected by optical cross-talk issues is sensor B, which features the largest optical fill factor. Optical cross-talk between neighbouring SPADs measured on sensor B was found less than 4%.

Sensor A has been developed for Spectral Lifetime Fluorescence Microscopy. The different spectral components of a fluorescent emission collected by the objective of a FLIM system can be separated with the aid of a prism and focused on this linear array, with size 100x1660 µm, composed by 64 pixel. Each pixel contains 4 SPADs that are binned together and connected to the read-out electronics. The read-out channel of each pixels integrates the number of detected photons in four time-windows, storing the digital value of the four number in 8 bit shift register. With four time windows a double exponential decay can be detected.

Sensor B has been specifically designed as test structure for Lab-On-Chip application. The size of the structure, 260x260 µm, fits the dimension of sensing sites of spotted microarray assay of fluorescent probes. The sensor area is divided in four sectors, each composed by 25 SPADs. Light is integrated in two time windows and the number of events are recorded in 10 bit shift registers. The required bit rate of this sensor during lifetime measurements has been estimated not to exceed 160kbps. This low bit rate allows the use of a large number of these structures in the construction of Lab-on-Chip detectors.

Sensor C has been designed for imaging applications. For this kind of applications a digital read-out channel with the number of events stored in a shift register is not feasible due to the large amount of silicon area that would be required for each pixel. Therefore, an analog readout channel has been included in the pixel, and the number of the time windows has been reduced to a single one. The current pulses produced by each SPAD during the time window change the charge accumulated onto a capacitance integrated into the SPAD pixel and the pixel output voltage of the array is read-out as in common CMOS image sensors. An exponential emission decay can be measured combining the information stored in two or more images that are acquired with subsequent time windows.

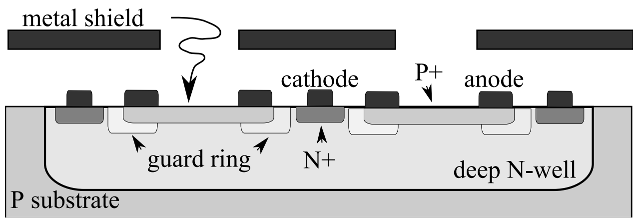

Figure 1 Device cross section.

TABLE I. DETECTORS CHARACTERISTICS

Label used in the text to identify sensors	A	B	C
number of SPADs	64x4	10x10	32x32
Read-out	Digital	Digital	Analog
Fill Factor	34%	48%	20.8%
Pixel pitch	26 µm	26 µm	25 µm
Dark count rate	1 kHz typ	2 kHz typ	500 Hz typ
Single SPAD disabling	no	yes	no
Gate Number	4	2	1
SPAD binning	4 block	5x5 block	no
Read out area for each pixel	50000 μm^2	75000 μm^2	200 μm^2

III. MEASUREMENT SETUP AND EXPERIMENT

An experimental setup based on sensor A has been built in order to validate gated detector operation in fluorescent lifetime measurement. Sensor A has the largest linear resolution and is suitable for a linear scanning system.

The measurement setup is illustrated in Figure 2. Light pulses produced by a laser diode (lp) (Picoquant, λ 470nm, pulse width 70 ps FWHM, repetition rate 40MHz) are directed onto the sample (s) that is mounted on a motorized linear stage holder (m). A lens system (l), that is coupled with an optical bandpass filter in order to cut the excitation wavelength (f), focuses the fluorescent emitted light onto the photosensor (p).

The sample is a 2D array of 16x16 silicon micro-reactors [10] filled with a solution 1µM of Fluorescein (Free Acid, Fluka) in carbonate buffer (0.1M, pH 9). The lifetime of free Fluorescein in high pH solutions is around 4 ns. The gate width of the sensor has been set to 4-4-4-4 ns. The micro-reactors array is a 100µm pitch 2D array of micro-wells, 50 µm in diameter, closed with 1µm thick membrane of $SiO_2/Si_3N_4/SiO_2$ multilayer.

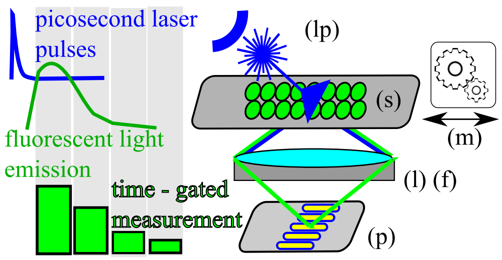

Figure 2 Setup scheme. See description in the text

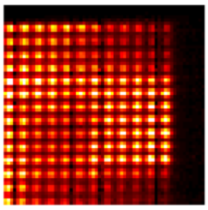

Figure 3 Intensity image of the sample (gate 1). The image shows a subset of 12x12 micro reactors of the whole array.

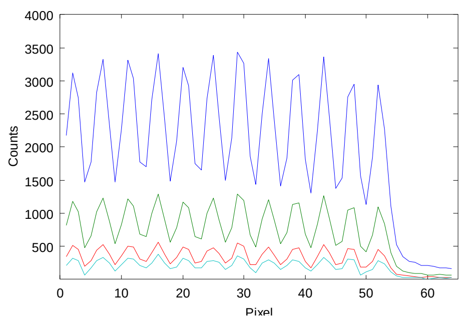

Figure 4 Light collected by the sensor in the four time windows, column mean.

The image of the sample, that has been obtained by scanning the specimen in the direction perpendicular to the sensor array, is shown in Figure 3. The acquisition time was 1s/line. The intensity profiles of the light collected in the four time windows is reported in Figure 4 for a single row of micro-wells. The mean of the calculated lifetime is 4.016 ns with a standard deviation of 68 ps.

IV. CONCLUSIONS

In this article we presented three different SPAD sensors specifically developed for time-gated fluorescence spectroscopy. The sensors active areas were designed to optimize the fill factor and the implemented readout circuits are capable of nanosecond gating with tens of MHz repetition rates. We have underlined the outstanding opportunities offered by the CMOS technology that has been used in the design of the detectors, pointing out how the structure of the sensors has been fitted to the needs of the applications. Finally we have reported preliminary experimental results in order to validate the use of the three sensors in fluorescence lifetime sensing applications. A more accurate characterization of the three sensors in different setups will be carried out in the near future.

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