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A 48-pixel array of Single Photon Avalanche Diodes for multispot Single Molecule analysis

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Abstract

In this paper we present an array of 48 Single Photon Avalanche Diodes (SPADs) specifically designed for multispot Single Molecule Analysis. The detectors have been arranged in a 12×4 square geometry with a pitch-to-diameter ratio of ten in order to minimize the collection of the light from non-conjugated excitation spots. In order to explore the trade-offs between the detectors' performance and the optical coupling with the experimental setup, SPADs with an active diameter of 25 and of 50μm have been manufactured. The use of a custom technology, specifically designed for the fabrication of the detectors, allowed us to combine a high photon detection efficiency (peak close to 50% at a wavelength of 550nm) with a low dark count rate compatible with true single molecule detection.

In order to allow easy integration into the optical setup for parallel single-molecule analysis, the SPAD array has been incorporated in a compact module containing all the electronics needed for a proper operation of the detectors.

Keywords

Single-Photon Avalanche Diode (SPAD); Photon Counting; SPAD array; single molecule; FRET; high throughput

1. INTRODUCTION

Since first experimental demonstrations in the early 90's, single molecule analysis has gained increasing importance in scientific domains as diverse as super-resolution imaging, structural biochemistry, and single-protein tracking in live cells, yielding insights into outstanding fundamental biological questions. In contrast to ensemble measurements, which yield information only on average properties, single-molecule experiments provide information on individuals, such as distributions and time trajectories of properties that would otherwise be hidden. They make it possible to examine individual members of a

heterogeneous population and to identify, sort, and quantitatively compare their subpopulations^{[1][2][3][4][5]}.

Among all the techniques available for measuring the properties of a molecule, especially significant are those based on the detection of the fluorescence emitted by a molecule (or by a fluorophore attached to the molecule itself) previously excited by a suitable laser light. Many configurations do exist both for the illumination of the sample and for the collection of the emitted light. However, a very common approach is to shine the excitation light on the sample by means of a microscope objective with a large numerical aperture and to collect the emitted fluorescence by means of the same objective^[6]. Combined with a confocal scheme^[6], this approach reduces the volume which the light is collected from to a fraction of $1\mu\text{m}^3$. Provided that the sample is diluted enough, a molecule at most will be in the excitation volume and single molecule analysis will be possible.

If the case of immobilized molecules the excitation volume has to be scanned with respect to the sample in order to analyze multiple molecules; on the contrary, if the molecules are freely diffusing in a liquid, they randomly cross the excitation volume without the need of a raster scan.

The analysis of freely diffusing single molecules is especially demanding in terms of detector performance^[7]. In fact, in order to be suitable for this application, a detector must provide a very high sensitivity to cope with the limited number of photons that a molecule emits while crossing excitation volume. The detector must also provide a temporal resolution high enough to follow the variations of the optical signal determined by the random motion of the molecules that continuously enter in and exit from the excitation volume, this phenomena typically happening on a time scale of hundreds of microseconds. Because of these requirements, PhotoMultiplier Tubes (PTM) were rapidly superseded by the Single Photon Avalanche Diodes (SPAD) due to their better Photon Detection Efficiency (PDE), temporal resolution, compactness and ruggedness^[8].

One of the main limitations of the analysis of diffusing single-molecules is related to the dilution requirements. In fact, the sample has to be diluted enough to reduce to an acceptable level the probability of having more than one molecule at time in the excitation volume. As a consequence, most of the time, no molecules at all are in the excitation volume and the measurement has to be run for a long time before a sufficient number of molecules is analyzed. This fact not only limits the throughput of the measurement but also prevent the study of the samples that evolve on a time scale shorter than or comparable with the duration of the measurement.

In order to overcome this limitation, a parallel approach has been suggested. In particular, a suitable optics is used to focus the laser beam in N spots inside the sample therefore creating as many independent excitation volumes. The light is then collected and refocused on N detectors each conjugated to an excitation volume^[9]. Since the measurement is run in parallel on N independent sub-samples, the duration of the measurement is reduced by the same factor.

The development of such a system requires significant breakthroughs at multiple levels including excitation and collection optics, detectors technology and data analysis. To address these challenges, our research groups at Politecnico di Milano (Polimi, PI: M. Ghioni) teamed up a few years ago with the Weiss lab at University of California Los Angeles (UCLA, PI: X. Michalet). In the framework of a joint research project funded by the National Institute of Health (NIH, grant R01-GM095904), our collaborative effort aims at developing a complete setup for high-throughput analysis of freely diffusing single molecules. In particular, the system set up at UCLA will implement a 48-spot excitation and collection scheme and, among other single-molecule techniques, will exploit the Förster Resonant Energy Transfer (FRET). The latter is a phenomenon in which an excited fluorescent probe (the donor) can transfer its excitation to a lower excited state energy fluorophore (the acceptor) provided that they have overlapping absorption and emission spectra ^[10]. Since the probability of this phenomenon to happen is strongly dependent on the distance between the two probes, FRET is commonly used to measure the distance within a molecule (or between molecules) at a nanometer scale ^{[11][12][13]}. In order to measure the FRET efficiency, the system will include two parallel detection channels, one at the donor wavelength (in green region of the spectrum) and the other at the acceptor wavelength (in the red region).

In this paper we will present a photon detection module, based on an array of 48 SPADs, specifically designed for the donor channel of the aforementioned single-molecule setup. In particular, in Section 2 and Section 3 we will describe respectively the array of the detectors and the integrated electronics needed in order to operate them. In Section 4 we will introduce the 48-pixel photon counting module, while in Section 5 we will present a preliminary experimental characterization of the system.

2. 48-PIXEL SPAD ARRAY

In freely diffusing single molecule experiments the photons' stream from the sample is continuously acquired for a long time and then processed to identify single molecule bursts, each corresponding to the transit of a single molecule in the excitation volume ^[14]. Regardless from the method used to identify the bursts ^{[15][16]}, the key point is that a sufficient number of photons must be collected for every single burst in order to characterize the properties of the corresponding molecule with the required degree of accuracy. Increasing the duration of the overall measurement raises the number of collected bursts and therefore improves the confidence in the reconstruction of the statistical distribution of the population. However, the overall duration does not impact the signal to noise ratio achieved in the characterization of each molecule.

For this reason it is of the utmost importance that the detectors used in this kind of experiment provide the highest sensitivity and in particular the highest Photon Detection Efficiency. As a consequence, for the fabrication of the SPAD array, we resorted to a custom technology, i.e. to a technological process specifically designed to optimize the performance of the detector. Such an approach contrasts with the use of a standard CMOS technology. The latter has the advantage of allowing for the fabrication of high-density

SPAD arrays with complex in-chip electronics, but provides devices with a PDE and a DCR not as good as the ones achieved with custom technologies ^{[17][18]}.

In particular, in the last years we developed two different technologies for the fabrication of the SPAD detectors, one optimized for the green region of the spectrum (double epitaxial SPAD) ^{[19][20]} while the other for the red region (red-enhanced SPAD) ^[21]. Since the module discussed in this paper is intended for the use in the donor channel of the single molecule FRET setup, it will be operated at wavelengths around 550nm and therefore we based it on the double epitaxial technology. On the contrary, red-enhanced technology will be adopted in the future to develop a detection module to be used in the acceptor channel.

Figure 1 shows a sketch of the cross-section of a typical double epitaxial SPAD. The n+ region indicated as “shallow” constitutes the cathode of the detector while the active area of the device is defined by the p+ region called “enrichment”. The role of this region is twofold: on one hand it lowers the breakdown voltage in the device center thus preventing premature edge breakdown; on the other hand it precisely shapes the electric field profile in order to optimize the main device’s performance, such as DCR or PDE. The combination of the “sinker” and of the “buried layer” provides a low resistivity path for current flowing from the active region to the anode contact.

Finally the n+ region called “isolation”, in combination with the n+ substrate, forms a sort of p-well in which the detector is completely enclosed. This is especially important for the fabrication of the arrays because, by reverse biasing the junction between the anode and the isolation, it is possible to electrically isolate each pixel from its neighbors.

The Figure 2 shows the layout of two different arrays manufactured by using the aforementioned technology. In both cases the array includes 48 pixels arranged in 4 rows each containing 12 detectors. For each SPAD, the anode is connected to a bonding pad and made available for the connection to an external circuitry (see following section) by means of wire bonding. On the other hand, each cathode is connected to a common supply voltage by means of an integrated resistor of about 2k Ω . The latter is used to swiftly reduce the voltage across the SPAD therefore limiting the current flowing through detector in the timespan preceding the intervention of the external quenching circuit (see next paragraph). This limits the impact of all the phenomena related to the amount of charge flowing through the detector during the avalanche, such as for example afterpulsing probability ^[22] or optical crosstalk ^[23].

Both of the designs of Figure 2 are characterized by an elevated value of the pitch to active diameter ratio, this value being equal to 10 in both cases. Such approach allows for a reduction of the amount of light collected from non-conjugated spots ^[24]. The two configurations differ in terms of the active diameter of the detectors; in particular, for the array on the left, the SPAD diameter is equal to 50 μm (with a pitch of 500 μm) while for the array on the right it is 25 μm (with a pitch of 250 μm). While a smaller diameter is a definite advantage in terms of detector noise, a larger diameter, in principle, allows for a more efficient light collection from the conjugated excitation spot. However matching the pitch of the excitation spots with the larger pitch of the detectors requires a larger expansion of the

light beam into the optical path and therefore is more prone to optical the aberrations. This can in turn result in a reduction of the collection efficiency ^[24]. The two different structures have been designed to investigate further the trades-off between these phenomena.

3. ARRAY OF INTEGRATED ACTIVE QUENCHING CIRCUITS

In order to work properly, a Single Photon Avalanche Diode must be connected to a circuit that quenches the avalanche as soon as it gets triggered and that resets the initial biasing condition making the device ready for detecting a new photon ^[25]. The simplest approach to this problem is to use the so-called passive quenching circuit that relies on a single component (e.g. a resistor or a suitably biased MOSFET) to quench and reset the detector. However, despite its simplicity, this circuit is seldom used in real applications because of several intrinsic limitations mainly related to slow transients during bias restoring ^[25]. The common approach is therefore to use some active components in order to sense the avalanche and swiftly quench and reset the detector ^[26].

The implementation of such an Active Quenching Circuit (AQC) requires not only the availability of high-voltage transistors to drive the detector but also of low-voltage MOSFET to effectively sense the avalanche and build the required control logic. However, such components are not available in our custom SPAD technology since the fabrication process has been devised to optimize the performance of the detector. As a consequence we decided to adopt a hybrid approach in which the detectors are connected to the external AQCs by means of wire bonding. In particular in Figure 3.a it is possible to see the 48-pixel SPAD array, in the center, with two AQC chips on the sides.

Each chip contains an array of 32 AQCs; 24 of them are connected to the corresponding detector in the array, while the others have been arranged in view of a possible extension of the system. Compared to previous releases ^[26], the AQC has been completely redesigned to match the requirements of this application, with a special focus on the reduction of both area occupancy and power dissipation. These goals have been pursued both by using a highly scaled, 0.18 μ m High-Voltage CMOS technology and by adopting a new circuit structure that will be described elsewhere.

4. DETECTION MODULE

To easily integrate the detector into the multispot single molecule setup, we developed a compact module for parallel single photon counting (see Figure 3.b).

The 48-pixel SPAD array and the two AQC chips are housed into a hermetically sealable chamber separated from the rest of the module. During the assembling phase, in order to remove any residual humidity, some dry nitrogen can be circulated in the chamber by connecting two pipes to suitable inlet and outlet valves clearly visible in Figure 3.b. At the end of the process, the pipes can be removed and the chamber remains sealed.

The sealing in a dry atmosphere makes it possible to mount the SPAD array on a double-stage Peltier and to cool the detector down to temperatures well below 0°C without any moisture-related issues. This in turn allows for a significant reduction in the dark count

rate [27][28]. A C-mount at the top of the detector chamber allows also for an easy and reliable connection to the single molecule optical setup.

All the electronic circuitry needed for a proper operation of the detection system is housed outside the sealed chamber. In particular a power supply board generates all the voltage levels needed both by the detectors and by the AQC. The same board implements also a closed-loop temperature control by reading the detector's temperature (using a Negative Temperature Coefficient thermistor) and by suitably driving the Peltier cooler. A second board reads the output signals from the AQCs and assures the interfacing with an external system. In particular, for each channel, every single voltage pulse, corresponding to a photon's detection, is made available outside the module by means of a standard SCSI connector. This allows for an easy connection of the module to general-purpose boards for data acquisition and processing. Alternatively, thanks to an FPGA, a first basic processing of the data can be accomplished by the module itself and the results can be downloaded through a high-speed USB link.

5. EXPERIMENTAL CHARACTERIZATION

In this section we will report on a preliminary experimental characterization of the detection module described in the previous paragraphs. Measurements have been taken on a module incorporating a SPAD array such as that of Figure 2.b (i.e. with 25 μ m active diameter and 250 μ m pitch). The detectors are operated at room temperature, without turning on the close-loop temperature controller. A bias voltage common to all the devices guarantee an average overvoltage of 6V with a spread lower than 100mV owing to the good uniformity of the breakdown voltage across the array.

Figure 4.a represents the PDE for the 48 pixels of the array at three different wavelengths: 550nm (in green), 700nm (in red) and 850nm (in black). For 12 of these detectors (randomly chosen), Figure 4.b represents also the PDE for wavelengths ranging from 400nm to 1000nm. All measurements have been taken by using a standard setup for the evaluation of the PDE, based on a monochromator and an integrating sphere.

From Figure 4 it is evident that the PDE is highly uniform across the array, with a peak-to-peak spread of only a few percent. Moreover, the PDE peaks at 550nm, i.e. close to the wavelength of a typical donor channel in single-molecule FRET measurement. Its value exceeds 45% over all the pixels of the array, making it particularly suited for the purpose.

Due to the limited thickness of the absorption layer, the PDE decreases at the longer wavelengths, dropping to about 25% at 700nm; such value should be sufficient to use this module also for the acceptor channel, at least for the initial testing of the single molecule FRET setup [29]. However, in order to obtain high quality data and to study samples with lower FRET efficiency [30], in the future we will develop a new module for the acceptor channel based on a SPAD array fabricated by using a technology optimized for enhanced red sensitivity [31][32].

In addition to a high PDE, the successful detection of single-molecule bursts requires that the noise contribution from the detector is sufficiently low. In order to characterize this

aspect, we reported in Figure 5.a the DCR of every single detector belonging to the array, once again measured at room temperature with an overvoltage of 6V. The same data have been reported also in Figure 5.b sorted in increasing order to build an approximate cumulative distribution of the DCR.

The latter Figure shows that more than 90% of the devices have a DCR lower than 1000 cps, with only one pixel having a DCR slightly larger than 7000cps. With the exception of pixel number 39, such rates are adequate to perform high quality, single molecule measurements [33].

Nevertheless the cooling system will be exploited in the future to further reduce the DCR, especially for larger active area devices, or to allow for an operation of the detectors at higher overvoltage and therefore with an even better PDE.

6. CONCLUSIONS

In this paper we presented a photon detection module based on 48-pixel SPAD. The use of a custom technology for the fabrication of the detectors allowed us to obtain a combination of PDE and DCR suitable for single molecule analysis. While we will continue the experimental characterization of properties of the devices as a function of the operating condition, we will incorporate the module in the multispot single molecule FRET setup under development in the UCLA laboratory [9][24][29][33][34].

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Figure 1.
Cross section of a typical double epitaxial thin SPAD.

(a)

(b)

Figure 2.

Layout of two 48-pixels SPAD arrays. The chip on the left (a) is $6500\mu\text{m} \times 2400\mu\text{m}$ and contains $50\mu\text{m}$ -diameter SPADs with a pitch of $500\mu\text{m}$. The chip on the right (b) is $3500\mu\text{m} \times 1600\mu\text{m}$ and contains $25\mu\text{m}$ -diameter SPADs with a pitch of $250\mu\text{m}$.

(a)

(b)

Figure 3.

Picture of the 48-pixels SPAD array wired bonded with the two AQC chips (a). Picture of the photon timing module; in the center is visible the sealed chamber housing the detectors' array (b).

Figure 4.

Photon Detection Efficiency as a function of the detector number (a) and as a function of the wavelength (b). All the measurements have been taken at room temperature with the detector biased at an overvoltage of 6V. In the figure on the left the PDE has been reported only for three wavelengths while on the figure on the right it has been reported only for 12 detectors.

Figure 5.

Dark Count Rate (DCR) as a function of the detector number (a) and assessment of the DCR cumulative distribution (b). All the measurements have been taken at room temperature with the detector biased at an overvoltage of 6V. The figure on the right has been obtained by sorting in increasing order the DCR and rescaling the x-axis between 0 and 100%.