32-Channel Single Photon Counting Module For Ultra-sensitive Detection of DNA Sequences

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ABSTRACT

We continue our work on the design and implementation of multi-channel single photon detection systems for highly sensitive detection of ultra-weak fluorescence signals, for high-performance, multi-lane DNA sequencing instruments. A fiberized, 32-channel single photon detection (SPD) module based on single photon avalanche diode (SPAD), model C30902S-DTC, from Perkin Elmer Optoelectronics (PKI) has been designed and implemented. Unavailability of high performance, large area SPAD arrays and our desire to design high performance photon counting systems drives us to use individual diodes. Slight modifications in our quenching circuit has doubled the linear range of our system from 1MHz to 2MHz, which is the upper limit for these devices and the maximum saturation count rate has increased to 14 MHz. The detector module comprises of a single board computer PC-104 that enables data visualization, recording, processing, and transfer. Very low dark count (300-1000 counts/s), robust, efficient, simple data collection and processing, ease of connectivity to any other application demanding similar requirements and similar performance results to the best commercially available single photon counting module (SPCM from PKI) are some of the features of this system.

Keywords: Avalanche photodiode (APD), single-photon avalanche diode (SPAD), photon counting, single-photon counting, single-photon detection, multi-channel photon counting, multi-channel single-photon detection, C30902S-DTC, DNA sequencing, multi-lane DNA sequencing.

1. INTRODUCTION

Single photon detection using SPAD is the most sensitive method for detecting low light levels and as a result it finds application in variegated fields such as in astronomy[1], single molecule detection (SMD) [2], optical fiber testing in communication[3-5], VLSI testing[6], quantum cryptography[7] and DNA sequencing for weak fluorescence detection[8, 9].

High speed photon counting is not possible without a high speed quenching circuit. The concept of active quenching, pioneered by Dr Sergio Cova, is well described in literatures[10-14]. A quenching circuit senses the rise in avalanche current and immediately lowers the voltage across SPAD below breakdown. After a precisely controlled quenching time, it switches back the SPAD bias voltage above breakdown. We have designed a quenching circuit based on logic gates and delay IC's with a minimum dead time of ~35 ns[15].

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Advanced Photon Counting Techniques, edited by Wolfgang Becker, Proc. of SPIE
Vol. 6372, 63720C, (2006) · 0277-786X/06/$15 · doi: 10.1117/12.685838

Arrays of large area SPADs would have been an ideal choice of detector for designing such a system, but still, such arrays are not available commercially. Biggest advantages offered by such arrays would be significantly less area and cost compared to a system based on individual diodes. It would also offer certain dis-advantages in the areas of optical system design for light delivery, quenching circuit design, data collection and processing and cross talk minimization techniques would be required. Complexities in quenching circuit and data processing can be eliminated if each pixel has its own quenching circuit. Again, individual quenching circuit would require more area, but using integrated circuits can solve this problem. Hence, unavailability of large area SPAD arrays, our desire to design high performance photon counting systems and excellent performance obtained from our 16-channel module[15], when integrated into our DNA sequencing machine gave us confidence to continue further research in this direction. High performance modules based on individual diodes are available to order[16], but they are very highly priced. Since our future goals were to periodically increase number of channels in parallel for high throughput DNA sequencing, keeping the cost at minimum, and design systems that suited our requirements, it was necessary to develop in house expertise.

This paper is an extension of our previous work. For detailed description of the quenching circuit, fiberization system and optical system refer to[15]. In this paper we describe a 32-channel single photon detection system based on individual SPAD model C30902S-DTC[17] from PKI and present some preliminary results for the same.

2. 32-CHANNEL DETECTION MODULE

Architecture (left) and implementation (right) of the system is shown in Fig.1. The 32 channels of the detector module comprise of 32 cooled SPADs, (Fig.1, right top) connected to individual PC boards (Fig.1, right bottom), each consisting of quenching and temperature controller circuit respectively, maintaining the temperature constant within (±0.1)°C. The system needs five power supplies viz, +250V for SPAD bias, +25V and +12V to generate quenching pulses and two +5V supplies for logic IC's and temperature controller circuit. .

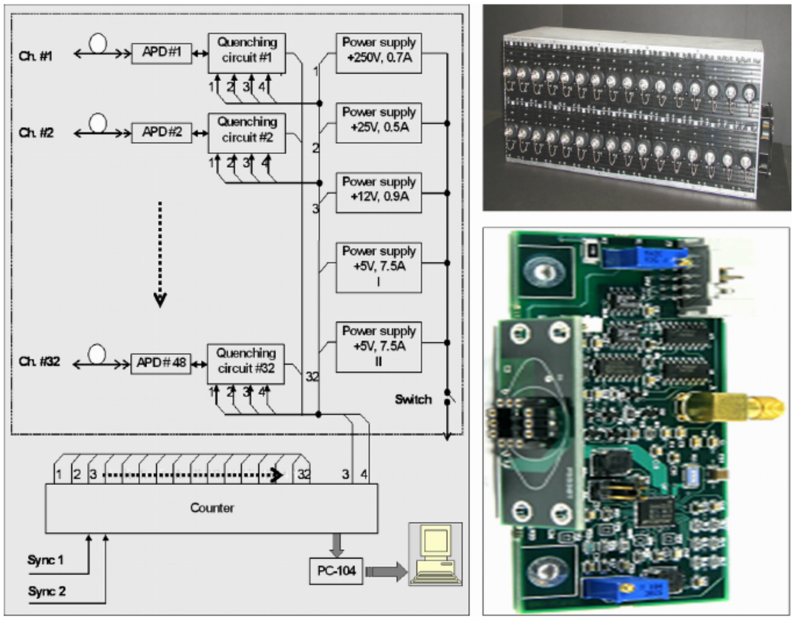

Figure 1: Architecture (left) and implementation (right) of the 32-channel single photon detection module. Front view showing 32 fiberized SPADs (top right) and PCB for one channel (right bottom).

The multi-color fluorescence detection is performed as follows: the fluorescence is passed through the rotating filter wheel comprising 4 band-pass filters and coupled to fiberized APD detectors. During each revolution of the wheel, the module performs measurement of the fluorescence light intensity that passes through each filter, producing four measurements per revolution.

The comparator at the input of quenching circuit generates an output pulse corresponding to the photons registered by the SPAD. A TTL line driver IC is used to retain the shape of the pulse and maintain sufficient amplitude for further processing. A 32-channel counter counts the pulses from the comparator. The counter has 32 identical TTL-compatible counting inputs and is controlled by signals from two sensors. The two sensors form encoding signals using a 2-bit Gray code to identify the band-pass filters in the filter wheel. The change of the code word at the sensor output indicates the change of the filter in alignment. The counter uses this filter code to assign the photon count to individual filters. The counting of the input photon pulses in each channel is performed by summation of pulses arriving to the channel input during the time intervals when a particular band-pass filter is in alignment with the photo detector.

The obtained data is transferred to PC-104 through LPT. The data management system takes the data from PC-104, performs data processing and transfers the data to the database and the data bank through Ethernet. One data sample is collected during one full revolution of the filter wheel. The frame consists of count values obtained in four fluorescence detection bands (4 color filters) for each detection channels. The frames are sent in order of their generation, determined by the filter code on the wheel and the direction of its rotation. Each frame starts with a 6-byte header, which includes the following fields: 1-byte counter type, 2-byte frame number, 1-byte color code (filter number) and a 2-byte counting period length. The frame number contains the number of the current frame. The number is incremented by 1 for each following frame thus forming a rising sequence with overflow. The frame numbers serve as synchronization marks and are used by the data processing software to find data frames in a continuous data stream. Frame numbers are also used for verification of data integrity and for finding errors introduced by interference in the transmission line. The duration of the counting period is measured in milliseconds and is represented by a two-byte value. The time duration when the filter is “on” is measured separately for each filter and is used by processing software to calculate the photon count rate. During the normal operation of the system the filter wheel performs 10 revolutions per second resulting in data transfer rate of 10 frames per second.

In previous system[15], all the diodes were mounted on a common metal plate that also formed the heat sink. Hence, it was difficult to tune or change one channel. This module is designed in such a way that each of the 32 channels can be easily removed from the system for testing or tuning purposes. Because of the number of components involved, enormous amount of power is dissipated from the system. Some important SPAD properties such as breakdown voltage, which increases with temperature and dark count depend on temperature. Hence, apart from the fan on the side of the module each SPAD is mounted on an individual heat-sink (Fig.1, top right) for excellent temperature stabilization.

3. EXPERIMENTAL RESULTS

During further research, we were able to extend the linear range of our quenching circuit from 1MHz to 2MHz without any linearity correction techniques, which is the upper-limit for the SPADs in use. Linearity can be further improved using linearity correction techniques described in[18]. Fig.2 shows the linear range as well as the maximum count-rate achieved by our circuit. We have set SPCM module from PKI as our comparison standard since it is one of the best, commercially available, single photon counting module (SPCM) [19] based on large area SLIK (Silicon with low k) device, and we have achieved comparable performance. The maximum count rate for this particular device tested (C30902S-DTC) is ~10MHz. We have found that this range varies from 10-14 MHz for different devices. This can be attributed to variable device capacitance.

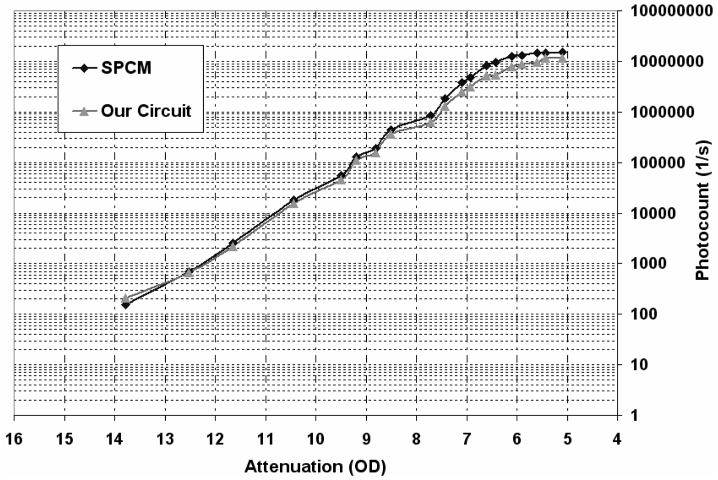

Figure 2: Linear range and maximum count-rate of our circuit and comparison with high performance SPCM module from PKI

We have also found that each device is different and some devices are significantly different, the reason for which should be different fabrication batches. Because of this, we have found that for same over-voltage, which means same sensitivity level, the variation in count rate is quite noticeable. Hence, in order to obtain consistent results (same sensitivity) from each channel, we need to adjust the over-bias for each channel individually. After adjusting, we measured the count rate on each channel for 5 different light levels and dark count (Fig.3). This graph gives an idea about the amount of spread one can expect for a particular constant light level, for example the dark count rate varies from a minimum of 200 to 1100 counts/s for a particular device (Fig.3, channel 3). We should stress here that we had to replace a couple of diodes in the process, as their dark count was almost 10 times higher than the other devices.

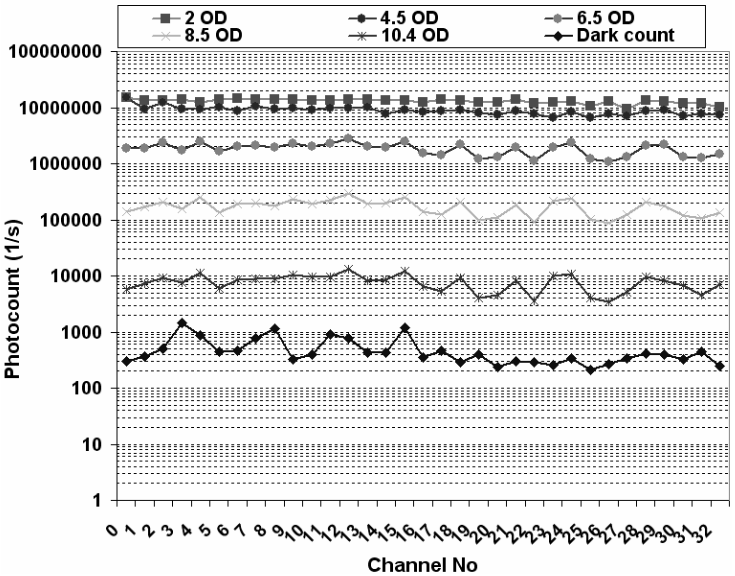

Figure 3: Count spread of 32 channels and SPCM for 6 different count values. Channel 0 is SPCM.

For DNA sequencing applications, the dynamic range of interest is from dark to 1MHz (maximum 2MHz). Hence, we measured the linearity of all the 32 channels in this range of interest and found that all the channels give consistent results (Fig.4).

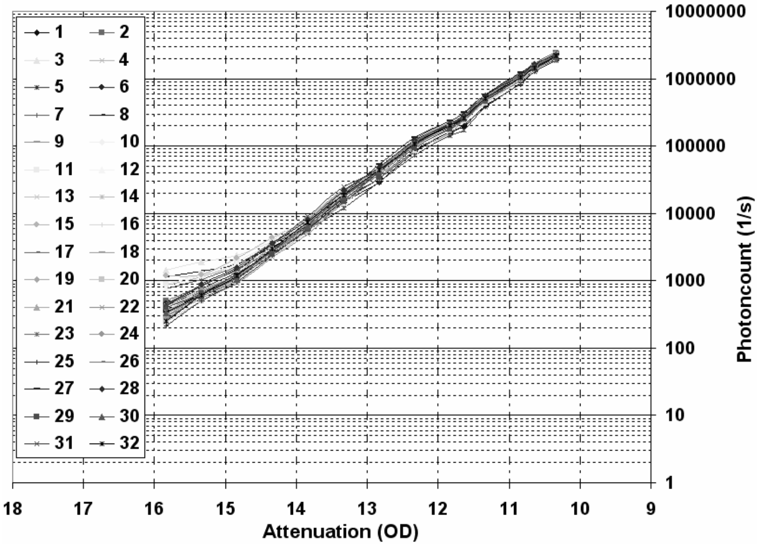

Figure 4: Linearity and maximum count-rate of our circuit

4. CONCLUSION

A novel, high performance, 32-channel single photon detection system has been designed, developed and tested. We have tuned each of the 32 channels to similar sensitivity levels and demonstrated high-speed photon-counting capability. In the near future, this module will be integrated into our 32-lane automated DNA sequencing system and extensively tested for DNA sequencing capabilities. Next task of interest would be to perform statistical study of important performance parameters. Since we have in all 48 SPADs (16 channel + 32 channel system), we will be able to get good statistical data. Such statistical data will give the buyer of a single SPAD an idea of the spread of certain performance parameters.

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