Single Photon Counting Module Based on Large Area APD and Novel Logic Circuit for Quench and Reset Pulse Generation

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Abstract—We present the design, implementation, and characterization of a single-photon counting module (SPCM) based on large-area avalanche photodiode (APD) and new logic circuit based on TTL integrated circuits (ICs) for generating precise quench and reset delays. Low dark count rate, high linearity of ∼2 MHz, maximum dynamic range of ∼12 MHz, and minimum dead time of 35 ns have been achieved with 0.2 mm² peltier-cooled single photon avalanche diode (SPAD) [model C30902S-DTC, Perkin Elmer Optoelectronics (PKI)]. The developed module was fiberized and demonstrated for the detection of fluorescently labeled DNA sequences. Detection sensitivity at the level of single fluorescent molecule has been demonstrated.

Index Terms—Active quenching, avalanche photodiode (APD), APD fiberization, photon counting, single photon counting module (SPCM).

I. INTRODUCTION

SINGLE-PHOTON counting using single photon avalanche photodiode (SPAD) is the most sensitive and widely used method for detection of weak signals [1]. This technology has opened up new ways for addressing problems in variegated fields, such as in DNA sequencing [2]–[4], single molecule detection [5], quantum cryptography [6], noninvasive VLSI testing [7], optical fiber testing in communications [8]–[10], astronomy [11], to name a few. Therefore, it has gained widespread importance in the recent past, replacing photo multiplier tubes (PMT) in the process.

SPADs alone cannot count photons. They have to be used with a special circuit that can quickly quench the avalanche generated by the SPAD and make it ready to detect another photon. Such circuit is aptly termed as quenching circuit. The concept of active quenching, pioneered by Cova, has been well documented since its inception [12]–[18].

The heart of the designed module is the high-speed quenching circuit. Innovativeness in our quenching circuit is introduced in the way nonoverlapping quench and reset delays are generated. The logic circuit is designed using only six NAND gates.

In the following sections, we describe the detailed design, implementation, and characterization of the developed module, and demonstrate its application in highly sensitive detection of DNA sequences.

II. DESIGN OF SINGLE PHOTON COUNTING MODULE (SPCM)

The aim was to design a compact, portable, fast, and robust photon-counting module for high-performance DNA sequencing. Main operating parameters such as bias voltage, dead time, and temperature are adjustable on the printed circuit board (PCB); hence, this module can be used with different SPADs and different applications having similar operating parameters. A compact external power supply module was built to supply all the necessary voltages for the module. Fig. 1 shows the block diagram, and Fig. 2 shows the implementation of the designed module. The block diagram essentially shows important blocks and all the parameters that are adjustable in the module. The light is delivered onto the device using a fiber optic cable that is connected to the fiberized detector using a standard fixed connection (FC) connector [Fig. 2(a)]. A coaxial connector is provided for the output signal. All the important designed sub-modules are discussed in this section.

A. Detector Selection

The geometry of the capillaries used in our DNA sequencer and the nature of fluorescence emitted from the capillary requires us to use large area detectors for maximum light collection. We selected SPAD model C30902S-DTC from Perkin Elmer Optoelectronics (PKI) [19], since it is the best commercially available large area SPAD having single photon sensitivity. The diode can operate up to a temperature of −20°C, and exhibits the smallest dark count in the series (100–700 Hz). Further, it has same sensitivity as the best commercially available SPCM (SPCM-AQ, PKI) [20]. In fact, the selected detector is also rather expensive ($1000 each, if not bought in bulk). Hence, we also tested the cheaper uncooled diode model C30902S ($200 per one diode) [19]. This diode exhibits the same sensitivity as the cooled version, but has a significantly higher dark count rate (∼30 000 Hz). Further, cooling these diodes is not feasible, due to problems discussed in [15]. Therefore, we chose the C30902S-DTC model for our SPCM development.
B. Fiberization System

Unlike the detectors in SPCM-AQ modules [20], the selected model is not fiberized, and tight focusing of light onto the receiving area of the detector is not an easy task. In applications similar to ours, it becomes a huge problem, since we need to couple the light onto the active area of the detector with maximum efficiency for best results. We solved this problem by designing a fiberization system that allowed us to couple the light onto the detector using an optical fiber with standard FC connector.

We faced two main design constraints: 1) large distance $L_2$ ($\sim 2.4$ mm) between the diode crystal and the glass window [Fig. 3(a)]; and 2) position of the APD crystal inside the diode case varies from diode to diode by $\sim \pm 0.1$ mm.

A universal fixture was designed to solve these problems as discussed here.

The fixture consists of two main parts, viz., the diode mount ring and the FC/PC connector with inserted gradient index (GRIN) lens [Fig. 3(b)]. The design of the fixture allows both lateral alignment of the GRIN lens and the diode crystal and vertical alignment of the GRIN lens enabling sharp focusing of the fluorescence delivered by the fiber onto the receiving surface of the diode. Since the size of the focused spot must be smaller than the receiving area of the diode, we have to choose different size GRIN lenses for fibers with different core diameters. We carried out calculations for SLW-1.8 GRIN lens (NSG, Japan) that fits the FC/PC format. Our calculations showed that for achieving maximum coupling efficiency, we have to use a GRIN lens of customized length. GRIN lens of size 4.26 mm was selected. With the selected GRIN lens and diode model, we can use up to fibers of $500 \mu m$. Fig. 3(c) shows the implementation.

C. Temperature Controller

Dark count, breakdown voltage, and after pulses vary with temperature; hence, precise control of temperature is a must. We have used Maxim IC Max1978 for precise temperature control. The controller gives excellent performance, and controls temperature with a stability of $0.001 \degree C$. When we start the photon counter, the actual temperature stabilizes to the set temperature in $\sim 10$ s. The controller IC is available in a 48-pin quad flat no leads (QFN) package (7 mm $\times$ 7 mm), which occupies very less space on the PCB and provides an excellent low cost solution.

D. Active Quenching Circuit

Fig. 4 shows the schematic block diagram of our active quenching circuit. All the switches are implemented using DMOSFET SST215, the comparator is implemented using IC LT 1719, all the NAND gates are implemented using IC 7400, line driver is implemented using IC 7407, and the delays U5 and U6 are implemented using IC series DS100X. To simplify the explanation, let us assume that quench switch (S1) is a P-channel device and turns on when a “0” is applied whereas a reset switch (S2) is an N-channel device that turns on when a “1” is applied.

Case 1: No photon is detected: When no photon is detected, the comparator output remains zero, the outputs of U1A, U1B, and U1D are 1, 1, and 0, respectively; hence, S1 and S2 both
are OFF. The output states of all other gates and devices are as shown in Fig. 4. The circuit continues to remain in this state until a photon is absorbed in the active region of the device and initiates an avalanche.

**Case 2: Photon is detected and an avalanche is initiated:**

When a photon is detected and an avalanche is initiated, large current flows through the SPAD. The large value resistor RL initially limits this current. When the voltage at the positive input of comparator crosses $V_{\text{ref}}$ or $V_{\text{threshold}}$, the comparator output goes high. Output of U1A, and hence, U1D remains same; hence, S2 continues to remain OFF. U1B output goes low; as such, S1 is turned on, and $V_{\text{quench}}$ is applied to the anode of the SPAD, quenching the avalanche. The output of U1B is also applied to U1C and the reset delay IC U5. The output from U1C propagates further through U2B, quench delay IC U6, U2A, U1A, and finally generates a “1” at the output of U1D, thus, turning on switch S2, and initiating the reset action. At the same time, output from U1A goes to U1B, which generates a “1” to turn off switch S1. The reset action is terminated after about the time determined by the reset delay IC U5 when the “0” at the input of reset delay IC U5 propagates through the same loop and turns off S2. Now, the SPAD is ready to detect another photon. This loop action takes place each time the SPAD fires and the current is large enough to trigger the comparator. In our circuit, the quench delay is the sum of delay generated by IC U6 plus 10 ns further explained in Section III-A, and reset delay is generated by U6. Both quench and reset delays are adjustable.

Fig. 5 shows the output pulse from the line driver IC that is sent to the counter for registering the photon. The rising edge of the output pulse is formed as soon as the comparator triggers, which initiates the quenching action. The output pulse ends when the voltage at the positive input of the comparator is pulled below threshold by the discharging SPAD anode during reset. Therefore, the width of the output pulse is $\sim 35$ ns, which is the sum of the quench and reset delay pulses. Fig. 6 shows the high voltage quench pulse that is applied to the anode of the SPAD during the reset delay time.

Some of the advantages of this method of generating quench and reset delay pulse are explained.

1) Small delay in quenching the avalanche (10 ns). This time is mostly dominated and limited by the component delays and it is desirable that it should be minimum. In our circuit, it is the sum of SPAD delay (to develop avalanche), time for the comparator input to rise above $V_{\text{ref}}$, comparator delay, one gate delay, and one transistor turn-on time. SPAD delay depends on the type of SPAD used, and can be quite low for thin-junction SPADs. Once the comparator output changes, there is just one gate delay and one transistor turn-on time before the quenching takes place.
III. DISCUSSION OF IMPORTANT CIRCUIT PARAMETERS

A. Quench Delay

During quench, the SPAD is biased below breakdown for a certain time, and it must be sufficient enough to allow all the carriers to be swept outside the junction. As mentioned earlier, the quench delay pulse [Fig. 7(a)] is the sum of delay time set by delay IC U5 plus an additional time of 10 ns, which comes from the loop delay. So, for setting a quench delay time of 25 ns, the delay IC U5 is set to 15 and 10 ns is added by the loop. In our discussion, we refer to the quench delay time as the total time. Quench delay is adjustable from 10 to 60 ns, which is sufficient for the device under test, and can be extended further by adding or changing delay line ICs.

We tried varying this time for a few devices of the same type, and found that most of the devices operate satisfactorily with a quench delay of 35 ns. But quite a few devices operate with a quench delay of 30 ns and very few even operate at 25 ns. Longer quench times help in minimizing after-pulsing but at the cost of larger dead times, and hence, nonlinearity. Hence, a tradeoff needs to be established and depending on acceptable dead time and after-pulsing, this time needs to be carefully selected.

B. Reset Delay

During the reset time, basically the capacitance between SPAD anode and ground ($C_{int} + C_{wire}$ in Fig. 4) is discharged through switch S2 to ground, essentially restoring the SPAD bias to make it ready to detect another photon. Hence, the reset time must be large enough to allow the complete discharge of this capacitance and ensure that the SPAD bias is completely restored in order to avoid retriggering of the comparator, and thus, the oscillations. On the contrary, reset time should not be very large, as it increases the total dead time of the circuit. In this case, large number of photons might not be registered during this time, introducing nonlinearity. Also, the SPAD is slowly recovering to its normal bias during this time; it is half-ready to detect photons. Therefore, if the reset time is too long, the probability that SPAD will detect another photon during this time will increase, not allowing the SPAD to completely quench itself for a long time, leading to diode heating and then after-pulsing. Most of these devices operate with a reset time of 10 ns and some require 15 ns. Connecting two discharge transistors in parallel instead of one does not show noticeable improvement. We also tried to employ discharge transistors at some critical high capacitance points in the circuit but did not see any further improvement.

IV. EXPERIMENTAL RESULTS

A. Experimental Setup

Experimental setup for characterizing the electrical properties of the module is shown in Fig. 8. The attenuator consists of two adjustable filter wheels for selecting filters with different optical densities (OD) from 0 to 5 OD, since the attenuator has a maximum 5 OD filter (total 10 OD for two filter wheels). For selecting lower light levels (>10 OD), we used extra neutral density filters, which can be fitted into the space in front of the lamp. A high-speed counter along with data recording software of our original design was used for data recording and processing.

B. Selection of Comparator Threshold Voltage

Comparator voltage should be properly tuned to ensure proper circuit operation. If the comparator voltage is selected too low, it will count unwanted noise signals, and if it is selected too high, it will reject useful pulses. We saw that for this device the output count remained quite stable over a large range of comparator threshold voltage (Fig. 9). Below certain threshold voltage (~3 mV for the device under test), the count increases abruptly, and the shape of the output pulse deforms and they do not look statistical in nature. To ensure stable functioning at all times and not lose useful signal, it is advisable to select this voltage little more than the point at which the circuit goes from
unstable to stable state. For example, for this circuit, this point would be between 3 and 4 mV (Fig. 9).

C. Dark Count

Current pulses are produced and the counts are registered even in the absence of light due to thermal generation, and are aptly called dark counts. Hence, dark count represents noise in the detector. Figs. 10 and 11 show the dependence of dark count on temperature and over-voltage, respectively. The dark count rate increases exponentially with temperature and almost linearly with over-voltage. Depending on the application and acceptable dark count rate, suitable temperature and over-voltage needs to be selected. For our DNA sequencing application, we are operating at low temperature (−20 °C) and measuring photocounts of the order of 100 000–500 000 Hz; thus, the dark count rate (300 Hz) can be easily neglected.

D. Noise

The noise in the photon counting system is determined by the temporal distribution of photon counts. In a correctly operating photon counter, we observe Poisson distribution where the variance of the photocount rate estimate (the number of photons counted over the given time interval) is equal to its mean value, setting the lower boundary for the signal-to-noise ratio of the photon detector. The photocount was collected during 0.1 s intervals, and recorded using our data recording software for ∼30 min for each illumination level. Fig. 12 shows the histogram of the photocount distribution at the output of our detector module compared to the Poisson distribution (solid line), demonstrating a good match between the experimental and theoretical distribution. This indicates that the measured noise is only caused by the stochastic nature of the photon fluxes detected by the photon detectors and that the detectors themselves and the circuits do not produce any additional noise.

E. Dynamic Range and Linearity

Dynamic range is the ratio between the smallest and largest possible values of a changeable quantity. Fig. 13 shows and compares the dynamic range of our module with SPCM. The maximum and minimum signal count rate for our circuit is ∼12 MHz and 1 KHz, respectively, giving a dynamic range of four decades. The maximum and minimum count rate for SPCM is 15 MHz and 0.5 KHz, respectively. Maximum count rate that can be achieved depends on the device as well as the speed of the quenching circuit. The upper limit on the maximum count rate that can be achieved is imposed by the device capacitance. We
were able to achieve the maximum saturation count rate for these devices as specified in the datasheet [20]. Fig. 13 also shows the linearity of our circuit. Linearity of a single photon counter depends on the dead time of the pulse shaping circuit.

It can be seen that our system behaves linearly for count rates up to ~2 MHz as compared to ~4 MHz for SPCM. Better SPCM performance can be attributed to the much better Si with low k (SLIK) devices [15] used in the SPCM module, whose active area (0.025 mm²) is about 2.82 times less than that of the device used in our module (0.2 mm²). Small active area means smaller device capacitance and higher speed, resulting in larger dynamic range and better linearity. Another source of the detection nonlinearity may be due to the decrease in bias voltage at the SPAD cathode. This effect can be seen at high illumination levels caused by a resistor between high voltage supply and SPAD cathode. Linearity can be further improved using linearity correction techniques discussed in [21].

**Fig. 12.** Histogram of photocount distribution at the outputs of the detection module compared with theoretical Poisson distribution.

**Fig. 13.** Linearity and dynamic range of our module compared with PKI SPCM-AQR module.

**F. Determination of Optimum Operating Point**

To find the optimum operating point for the module, we have extensively measured the response of the device by incrementing over-voltage in 1-V steps, up to an over-voltage of 17 V (Fig. 14). The topmost line is the plot for SPCM. It can be seen that for low over-voltages up to 7 V, the maximum linear count is low and the count rate saturates fast enough. Also, the maximum linear count rate does not increase much as we go to over-voltages above 10 V; hence, we select the optimum operating point for these diodes to be around 10 V. As expected, for each over-voltage, the count saturates at some point called the saturation point, and then starts to drop when more light is applied. High light levels should not be applied after the saturation point is reached, since at one point the diode goes blind and cannot see any more light as can be seen for $V = 195.2$ V. This result provides a good idea to tune the device based on the requirement of the application. It would also help in tuning this type of detectors in multichannel systems, since tuning each channel individually can become a tedious task [3], [4].

**G. Stability**

DNA sequencing experiments can take long hours, and as such, stability of the photon counter becomes very important. Also, if the photon counter is illuminated to high light levels for long period of time, it may get over-heated. If adequate heat sink is not provided, the device breakdown voltage may increase, and hence, the effective over-voltage will decrease, resulting in lower sensitivity levels. To measure this performance, we illuminated the photon counter with constant light, and recorded the count rate every hour for 6 h at $-20^\circ$C. We repeated the same for three different light levels, and observed that initially the count rate drops slowly between 0 to 30 min before settling to a stable value (Fig. 15). We believe that once the diode is illuminated, the temperature on the diode chip starts increasing depending on the illumination level. Also, the diode is mounted on a crystal that is glued or rested on a two-stage cascaded peltier element. The temperature-sensing resistor rests on the top of this two-stage peltier element. So basically, there is a ceramic piece...
between the top of the peltier element and the diode. It takes some time for the heat to pass through this ceramic and raise the temperature of the sensor. Once the temperature of the sensor changes, it adjusts the current on the peltier to accommodate for the diode heating and stabilize the temperature. Hence, we can conclude that this performance parameter is the property of the diode rather than the circuit design. The initial drop in count rate varies between 3%–4%; and once the count rate stabilizes, the maximum change in count rate is about 0.5%, suggesting that our module is quite robust for performing long experiments.

H. DNA Sequencing Tests

The developed single photon detector was tested with our DNA sequencer described in [2]. We carried out DNA sequencing runs of serially diluted BigDye DNA Sequencing Standard (Applied Biosystems, CA) using 0.5-mW laser power and fluorescence detection system based on designed photon-counter. We showed that for highly diluted DNA samples at very small fluorescence excitation power, our detection system could provide 450–600 base pair read length at 99% accuracy (Fig. 16).

In order to determine the number of labeled DNA molecules that can be detected by the developed module, we calibrated the detection system using serial dilutions of fluoresce in TSR buffer (Applied Biosystems, CA). We found that one fluorescent molecule gave \( \sim 200 \) photocounts/s at 1 mW of illumination power. Taking into account that the dark count in our photodetector does not exceed 1000 photocounts/s and that the count spread associated with the dark count is \( \pm 90 \) photocounts/s at the level of 3\( \sigma \), we can conclude that the developed single photon detector allows detection of single fluorescent molecules at signal-to-noise ratio higher than 2.

V. CONCLUSION

A compact general-purpose single photon counter based on large area SPAD has been developed that matches the performance of the best commercially available large area SPCM module. The device was successfully integrated into a single lane DNA sequencing instrument. Single fluorescent molecule sensitivity and excellent DNA sequencing results were obtained, thus, providing experimental evidence of the application of large area SPAD for high performance DNA sequencing. Overall, the developed fiberized SPCM is reliable, easy to operate, and can be easily integrated into any system requiring detection of weak fluorescence signals.

REFERENCES


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