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A 65k pixel, 150k frames-per-second camera with global gating and micro-lenses suitable for fluorescence lifetime imaging

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Abstract

This paper presents our work on a 65k pixel single-photon avalanche diode (SPAD) based imaging sensor realized in a $0.35\mu\text{m}$ standard CMOS process. At a resolution of 512 by 128 pixels the sensor is read out in $6.4\mu\text{s}$ to deliver over 150k monochrome frames per second.

The individual pixel has a size of $24\mu\text{m}^2$ and contains the SPAD with a 12T quenching and gating circuitry along with a memory element. The gating signals are distributed across the chip through a balanced tree to minimize the signal skew between the pixels. The array of pixels is row-addressable and data is sent out of the chip on 128 lines in parallel at a frequency of 80MHz. The system is controlled by an FPGA which generates the gating and readout signals and can be used for arbitrary real-time computation on the frames from the sensor. The communication protocol between the camera and a conventional PC is USB2.

The active area of the chip is 5% and can be significantly improved with the application of a micro-lens array. A micro-lens array, for use with collimated light, has been designed and its performance is reviewed in the paper.

Among other high-speed phenomena the gating circuitry capable of generating illumination periods shorter than 5ns can be used for Fluorescence Lifetime Imaging (FLIM). In order to measure the lifetime of fluorophores excited by a picosecond laser, the sensor's illumination period is synchronized with the excitation laser pulses. A histogram of the photon arrival times relative to the excitation is then constructed by counting the photons arriving during the sensitive time for several positions of the illumination window. The histogram for each pixel is transferred afterwards to a computer where software routines extract the lifetime at each location with an accuracy better than 100ps. We show results for fluorescence lifetime measurements using different fluorophores with lifetimes ranging from 150ps to 5ns.

Keywords

single-photon avalanche diodes; image sensor; micro-lens array; global gating; fluorescence lifetime imaging microscopy

1. INTRODUCTION

Single-photon avalanche diodes (SPADs) make it possible to detect light with the highest level of precision: that of a single photon. With their precursors, avalanche photodiodes (APDs), being available since the 1960s, SPADs are the topic of numerous publications.^{1,2}

With the advent of mass-produced micro-electronics and more advanced production processes it became possible to integrate a large number of SPADs on a single silicon substrate.³ The mass-production of SPADs in standard CMOS technology has gained the interest of a large community and is an established research topic since.⁴

To permit the reliable fabrication of SPAD circuits, a CMOS process needs to attain some maturity, expressed predominantly in its cleanliness. SPADs utilize voltages above the breakdown voltage of one of their available junctions and hence they work outside of the operating conditions of most electronic circuits. The behaviour of a fabrication process under these conditions is often not well known and prompts further research in preparation of the production of SPAD circuits.

SPAD sensors amplify the arrival of a single photon to macroscopic levels. It becomes therefore possible to count and timestamp photon events at the level of single pixels. With a large number of single-photon sensitive pixels one can measure the distribution in time and space of photons in the area of the sensor. A good number of applications, especially in the microscopy domain are counted in the category of time-correlated single photon counting (TCSPC,⁵). Fluorescence lifetime microscopy (FLIM) is one that can profit particularly from the capabilities of SPAD based camera systems.⁶

In many applications it is beneficial to have a larger field of view and increased spatial resolution. In response to this demand we present SwissSPAD, a sensor with 512×128 pixels, and show its suitability for FLIM.

After describing our camera architecture in section 2 and the performance in section 3, we briefly evaluate the benefits of micro-lenses in section 4 before presenting FLIM measurements in section 5.

2. CAMERA ARCHITECTURE

For the fabrication of SwissSPAD we use a commercial $0.35\mu\text{m}$ CMOS process. A deep n-well implantation in the p-type substrate is used for the cathode below an anode of p+. This circular pn-junction is the active region of our SPAD and has a diameter of roughly $6\mu\text{m}$. A p-well guard ring guarantees the uniformity of the electrical field across the multiplication area. The construction of our SPAD allows for independent anode and cathode voltages. The sensitivity and noise characteristics are listed in Table 1.

One pixel of $24 \times 24\mu\text{m}^2$ contains the SPAD and twelve transistors. We use NMOS logic exclusively so that we can omit the n-well in the pixel area and achieve a tighter spacing and therefore higher fill factor. The pixel circuit in Fig. 1 can be viewed as three parts. The

photon sensitive front-end is composed of the SPAD and the shutter transistors controlling its anode voltage. The readout back-end is formed by the memory reset, value transfer and row access transistor. An NMOS latch used to memorize photon events sits in-between.

Transistors T2, T3 and T5 form the in-pixel shutter circuit. They are used to define the illumination time window where the SPAD is maximally photon sensitive and when it is effectively shut off. Through T2 the SPAD anode is lifted from ground, thus the excess bias voltage is removed and the SPAD is turned off. A pulse on the gate of T3 restores the excess bias voltage and makes the SPAD photon sensitive again. T5 finally prevents the false events generated by using T2 from toggling the latch. Global high-speed signals control the three transistors, the shutter of the camera. Their relative timing is critical and it is important that it is precisely controlled and simultaneous for all pixels.

A photon hitting a biased SPAD generates a macroscopic pulse on the anode and switches T4 to toggle the 1-bit memory cell of T6–T9. The memory value is accessed through T12 and reset through T10.

Fig. 2 shows the camera chip micrograph and Fig. 3 illustrates its architecture in a block diagram describing the full system.

As the pixels have one bit intensity resolution a higher number of measurements is needed to increase the dynamic range. A Xilinx®Virtex™ 4 FPGA is used to generate control signals and receive the column outputs. The readout part of SwissSPAD is operated at 80MHz generating a data rate of 10.2 Gbps over the 128 output lines. The USB2 interface, with a top speed of 480 Mbps, cannot sustain this data rate. The data is therefore accumulated in the FPGA in form of grayscale images and then written in large SO-DIMM buffer DRAMs. A double-buffering scheme is employed such that the full speed can be sustained at all times. The intensity resolution of images can be configured between 1 and 16 bits in order to obtain the optimal trade-off between memory space and time resolution.

3. PERFORMANCE EVALUATION

Much of the performance of the SwissSPAD camera is determined by the precision and speed of the shutter. The shutter window is defined through the three signals Spadoff, Recharge and Gate. The FPGA is generating these signals independently from the readout and they are distributed to all pixels in a well balanced manner such as to minimize the skew.

In the characterisation of this part of the circuit we used a picosecond laser with 40MHz repetition rate and 35ps FWHM pulse (Advanced Laser Diode Systems GmbH). The reference clock for the shutter generation is derived from the sync signal of the laser. This means that in practice, we evaluated the shutter precision of the whole chain from the laser through the FPGA and the SwissSPAD chip. The pulsed laser illuminates the chip and we choose a pattern for the shutter signals such that the photon detection window is shorter than the laser repetition period. Data is accumulated for a certain window position after which the window is moved with respect to the laser reference. We cover the whole repetition period of the laser with shutter windows in increments of approximately 20 ps.

For each of the 1280 positions in one 25 ns period we accumulate a 8 bit intensity value per pixel. The resulting intensity versus shutter position curve is in effect the convolution of the sensitivity window and the picosecond laser pulse. This response is then analysed as bilevel waveform according to *The IEEE Standard on Transitions, Pulses, and Related Waveforms, Std-181-2003*.

In Fig. 4 is the analysis of the gate window length over the array of 512×128 pixels. We perceive some systematic skew in the window length stemming from our shutter signal distribution on the chip. Additional measurements are in Table 1.

4. IMPROVING FILL FACTOR USING MICRO-LENSES

SPADs implemented in standard CMOS technologies may suffer from limited fill factor due to the need for guard rings and in-pixel electronics. The fill factor of SwissSPAD is approximately 5%, a low value especially for photon starved applications. To recover some of the area lost to guard rings and pixel electronics we decided to use a micro-lens array. A micro-optical concentrator is placed in front of each SPAD to collect the photons that would otherwise hit non-sensitive areas of the pixel. This means an additional fabrication step with increasing complexity and cost as sensors grow larger. A quartz mold of the lens array is fabricated with which the lenses are imprinted in a sol-gel polymer on top of the camera chip. The polymer is then UV-cured and stabilized by thermal cycling. Ref.⁷ provides more details on the fabrication process and performance analysis in a similar case.

To assess the performance impact, we compare measured light intensity of chips with and without micro-lenses. Due to the fact that our lenses are designed for use with collimated light they have very little impact when hit by diffuse light. Therefore we start our comparison by illuminating the chips with diffuse light of a fixed intensity. A TV objective with 16mm focal length and f-number* range from 1.8 to 16 is then used to collimate the light. As the f-number increases the light is collimated while at the same time the intensity is reduced. In this manner we can show that the measured micro-lens concentration factor increases with the f-number to reach a maximum of approximately 6 at f/16. At this stage the concentration factor is simply defined as intensity ratio between a chip with and one without micro-lenses and is calculated over an evenly lit area of the chips. Final figures with a calibrated illumination system should attain a factor of 8 or more.

5. FLUORESCENCE LIFETIME IMAGING

Fluorescence lifetime imaging microscopy (FLIM) is a powerful technique used for various chemical and biological measurements.⁸ In contrast to other fluorescence-based microscopy approaches, FLIM is not only interested in the intensity, but also in the decay time of fluorescent molecules. This extra information can reveal the type of molecules at different locations, by measuring the lifetimes of fluorophores associated with them. It can also probe the environment in which a fluorophore is located, when its lifetime is affected by its surrounding. The main advantage of lifetime augmented measurements over simple intensity

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*Focal ratio is equal to focal length divided by lens opening diameter.

measurements is the relative independence of the lifetime from the fluorophore concentration.

To extract the fluorescence lifetime of molecules, statistical information on the photon arrival times with respect to the exciting laser pulse is needed. One way to directly measure it is by using time-to-digital converters (TDC) connected to the pixels. In the extreme case each SPAD pixel has a TDC. For large arrays this negatively impacts the fill factor and chip complexity and it is in fact unnecessary as long as the light intensity is very low as is typically the case.

In SwissSPAD we do not have any TDCs, but a fast global shutter mechanism. The advantage of this approach is that it scales well with a large number of pixels with a much lower effect on the fill factor. The downside however is that we lose the photons arriving outside of our shutter window. This is less of a problem when the expected statistics are known in advance.

For the acquisition of fluorescent signals we use the characterisation procedure described in Section 3. Instead of shining the picosecond laser directly at the chip, we focus the excitation laser (PicoTrain, High Q Laser: 532 nm wavelength, 68 MHz repetition rate, 8 ps pulse) via a dichroic mirror into the back focal plane of a microscope objective lens to excite the fluorescence of different solutions of fluorophores characterised by their lifetime. The fluorescence signal is directed back through the dichroic mirror and a band pass filter to the SwissSPAD chip. By counting the photons arriving during the shutter window moving through the laser period we measure a signal corresponding to the convolution of the detection window and the fluorescent signal. A subsequent fitting of this signal with a decay model convolved with the shutter window, acquired separately, is then used to extract the lifetime parameter.

Typical waveforms for two different fluorophores are shown in red in Fig. 5 overlaid with the best fit of a mono-exponential decay of lifetime τ in blue. The computed lifetimes of the solutions are shown in Tab. 2 and they are in line with literature.

6. CONCLUSION

We presented SwissSPAD, a 512×128 pixel SPAD based image sensor with a fast global shutter. We explain our choices and trade-offs in the architecture and the characterisation of our system. We show one way to improve the low fill factor typical for large SPAD array sensors by using micro-lenses; the micro-lens array was characterised in terms of concentration factor and uniformity. With the presentation of fluorescence lifetime imaging measurements we show the suitability of the sensor for demanding applications, such as FLIM. We think that SPAD based sensors fabricated in commercial CMOS can offer advantages over traditional cameras in applications where conventional CMOS image sensors are currently at their limits. With SwissSPAD we show some of these advantages and the potential for further improvements.

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Figure 1.

Circuit schematic of the SPAD pixel. T2, T3 and T5 switch the SPAD on and off and define the photon sensitive time window. T1 can be used for passive quenching of the SPAD when shutter functionality is not needed. An NMOS-latch is created by T8 and T9, biased with T6 and T7. It is set with T4 from the SPAD and reset with T10. T11 finally transfers the memory value through T12 to the output line.

Figure 2.

Die micrograph of SwissSPAD with the pixel array in the center surrounded by logic on three sides. The inset is a detail of the SPAD cells. The construction allows for a double resolution system by abutting two sensors with minimal loss.

Figure 3.

A block-diagram of our camera system. The FPGA on the left is used to generate the control signals and receive the pixel data. The SwissSPAD chip has an array of 128×512 pixels. The power supplies and shutter signals are common to all pixels, while select and reset are connected by row and outputs are connected by columns.

Figure 4. Shutter length distribution over the pixel array. A systematic increase from top to bottom and from the center to the left and right edges is apparent. The total length variation is about 315 ps (FWHM) for the shutter signals used in these measurements.

Figure 5.

FLIM recording of different fluorescent samples using a 68 MHz (period: 14.6 ns), 532 nm wavelength laser with a 8 ps pulse duration.

Table 1

SwissSPAD performance parameters.

Parameter	min.	typ.	max.	Unit
Process	0.35 μm			HV CMOS
Chip size	13.5 $\times$ 3.5			mm ²
Format		512 $\times$ 128	512 $\times$ 256	Pixel
Pixel pitch	24			μm
Dead time		100		ns
Nominal fill factor		5		%
Micro-lens median concentration factor		6		
Micro-lens resulting fill factor		30		%
Photon detection probability ($V_e = 3.5\text{ V}$, $\lambda = 490\text{ nm}$)		22		%
Photon detection probability ($V_e = 4\text{ V}$, $\lambda = 490\text{ nm}$)		46		%
Spectral range ($V_e = 3.5\text{ V}$, $PDP > 5\%$)	350		850	nm
Median dark count rate ($V_e = 2.5\text{ V}$)		95		Hz
Median dark count rate ($V_e = 4.5\text{ V}$)		366		Hz
Electrical crosstalk	negligible			
Optical crosstalk ($V_e = 2.5\text{ V}$ @ 24 μm)		<3.5		%
Gating duration (FWHM)	4	20	6400	ns
Gating width accuracy (FWHM)			320	ps
Gating position accuracy (FWHM)			140	ps
Clock frequency		80		MHz
I/O bandwidth (80 MHz)		10.24		Gbps
Power consumption (idle)		660		mW
Power consumption (active)		1650		mW

Table 2

FLIM lifetime data.

Fluorophore	Reference life-time [ns]	Extracted life-time [ns]
CY3B	2.8	2.4–2.5
Alexa Fluor 546	4.1	4.2–4.4
ICG 40 μ M in Milk (3.5%)	0.580	0.550