# A TIME-GATED 128X128 CMOS SPAD ARRAY FOR ON-CHIP FLUORESCENCE DETECTION

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## Abstract

An all-digital, time-gated 128x128 CMOS image sensor for on-chip fluorescence detection is presented. The sensor pixel consists of a single-photon avalanche diode (SPAD), time gating circuitry and a 1bit memory. The sensor allows on-chip fluorescence detection and fluorescence lifetime imaging microscopy (FLIM) for on-chip molecular detection such as DNA, protein and cancer markers.

## Introduction

A micro total analysis system (μ-TAS) is a miniaturized laboratory to perform rapid reactions, using extremely reduced sample volumes, and to efficiently process flow-based assays for applications in chemistry, biochemistry and the life science. To date, analysis is still performed by fluorescence microscopes, even though bulky and expensive, since they enable the highest flexibility.

On-chip fluorescence detection has been extensively studied and demonstrated based on CMOS image sensors targeting μ-TAS [1], point-of-care testing (POCT) devices [2], and *in vivo* fluorescence imaging [3].

In this paper we propose a low-cost, fully integrated on-chip fluorescence sensor based on a SPAD. In addition, the sensor has time-resolved imaging capability that is generally available only in the most advanced instruments.

## Chip architecture

The device is based on an array of SPADs capable of detecting single photons and their time-of-arrival at high accuracy. Fig. 1 shows the block diagram of the proposed system. To achieve time-resolved optical detection, a time gating circuitry, and a NMOS 1bit memory were implemented in-pixel. The necessary timing control signals are generated by a combination of an on-chip 600ps delay line and an off-chip 200ps delay line. Time-resolved imaging is achieved via a sliding-time window scheme [4]. Time-correlated, single-photon counting (TCSPC) is performed by activating and deactivating the SPAD as shown in Fig. 2a. Time gating is achieved by turning off $T_{spadoff}$ and subsequently turning on $T_{recharge}$ and $T_{gate}$, as shown in Fig. 2b. The sliding-time window is applied globally to the entire array. The in-pixel 1-bit memory holds the state of the SPAD between readout cycles. When the system is operating at 40MHz, 16,384 excitation laser pulses reach the fluorescent samples on-pixel each frame of 409μs while only the first successful detection is observed. Additionally, the stored data are read out in rolling shutter mode to minimize the inactive time of the pixel. This exposure method is sufficient to detect fluorescent objects in photon-starved regimes. The entire pixel architecture is shown in Fig. 2c.

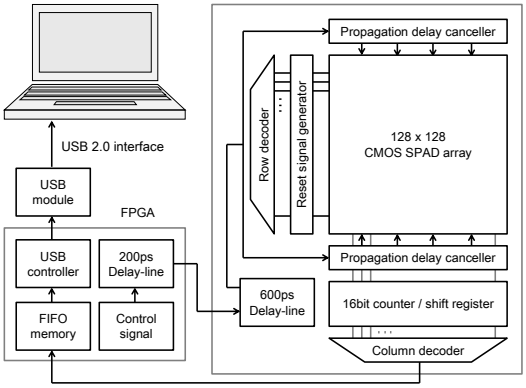
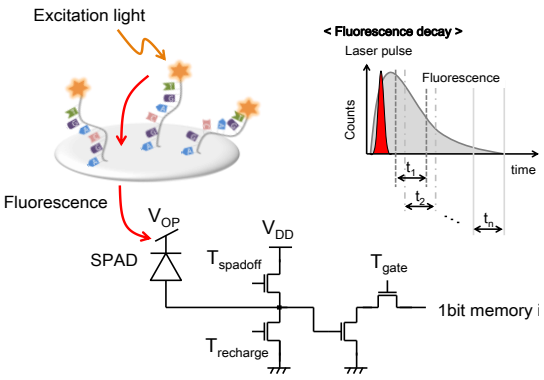
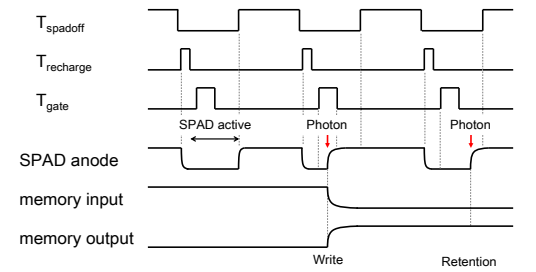



*Fig. 1. Block diagram of the proposed sensor. Each pixel's state is stored in a 1-bit memory that is read out in rolling shutter mode, accumulated and serialized on-chip, and formatted for USB communication off-chip.*




*(a)*

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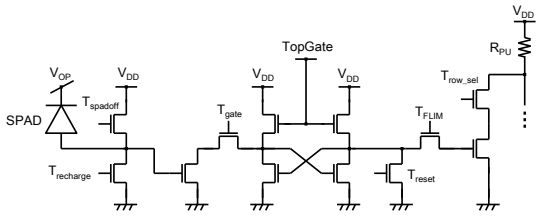



*(c)*

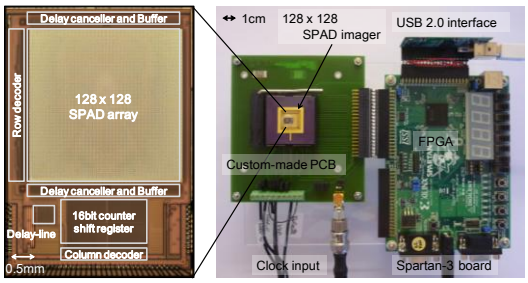
*Fig. 2. Schematic diagram of the pixel. (a) Time-gated fluorescence detection. Time gating is achieved through transistors $T_{spadoff}$, $T_{recharge}$ and $T_{gate}$. (b) Simplified timing diagram of the time-gated operation. The memory state is reset 1ns after readout cycle. (c) Overall pixel schematics, including the latch, reset controls, and readout interface.*

## Results

Fig. 3 shows a microphotograph of the sensor and a picture of the entire setup. The chip was fabricated in a 0.35µm standard CMOS technology. The pixel pitch and a total area are 25µm and 20.5mm$^2$, respectively.

Fig. 4 shows the dark count rate (DCR) cumulative probability plotted for different excess bias ($V_e$) conditions from 3.3V to 6.3V at room temperature. The median DCR of this chip, 186Hz at $V_e$ of 3.3V, is superior to that measured in [5], whereas the DCR per active area is 9.48Hz/µm$^2$.

Fig. 5 shows the raw and median filtered fluorescence image of a lily pollen grain sample. Thanks to its excellent DCR performance, a high quality image was captured in photon-starved regime (in this case, less than 17kcps per pixel). As shown in raw image, only 0.87% of the pixels have high noise (higher than 1kHz at $V_e$ of 3.3V).

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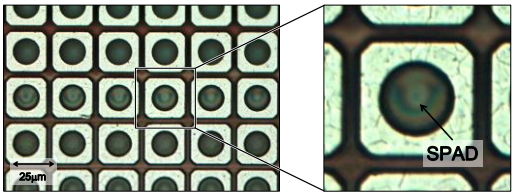



*(b)*

*Fig. 3. Time-gated, on-chip fluorescence detection system. (a) Photomicrograph of the sensor chip and a readout system. The column decoder is used to serialize the data coming from the array onto two serial channels via the 16bit shift resistor. (b) Close-up of the pixel.*

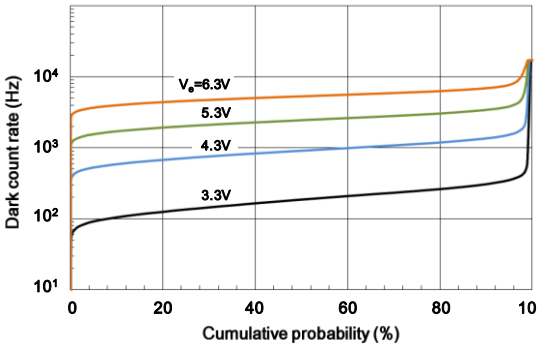
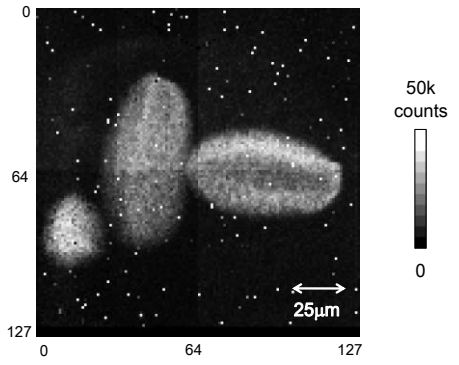



*Fig. 4. Dark count rate (DCR) cumulative probability under various excess bias conditions at room temperature.*

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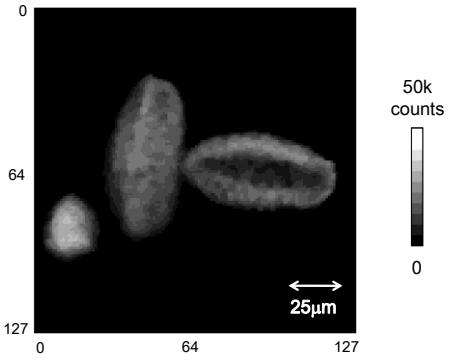



*(b)*

*Fig. 5. Fluorescence image of a lily pollen grain (Mixed pollen grains, Carolina Biological Supply Company, NC, USA) obtained with 50,000 frames at an integration time of 2.95s. (a) Raw image. (b) After median filtering.*

New evidence of optical crosstalk was observed for first time in this technology. Fig. 6 shows close-up pictures of a noisy pixel in saturation at a $V_e$ of 2.3V. The crosstalk expands increasing $V_e$. Fig. 7 shows the dark count rate change in neighbor pixels at different $V_e$ conditions. The activity of the high-noise or "hot" pixel radiates into neighbor pixels. This evidence implies that the crosstalk is caused by photon emission from noisy pixel rather than a voltage drop of power supply lines or electrical signal interference. Therefore, an on-chip hot pixel elimination technique will become important to improve image quality.

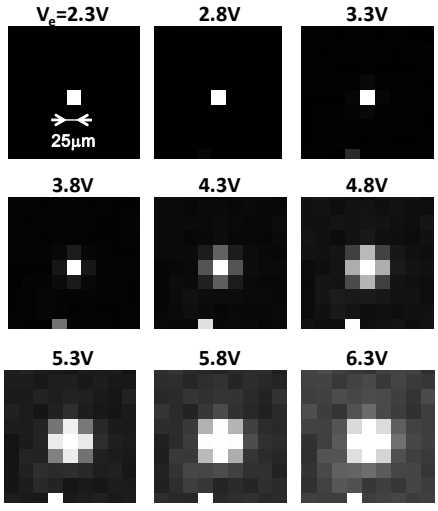



*Fig. 6. Close-up images of a "hot" pixel taken in the dark at various $V_e$ from 2.3V to 6.3V. The images clearly show that the optical crosstalk is dominant over electrical crosstalk.*

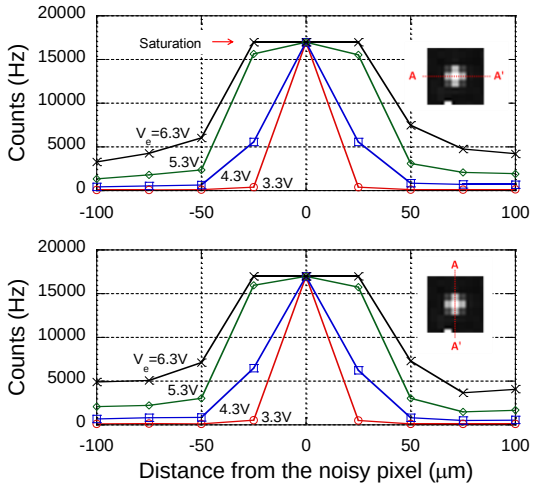



*Fig. 7. DCR distribution around a hot pixel. The photon emission from hot pixel raises the DCR in neighboring pixels, which are located 25μm to 75μm away.*

Finally, the chip was tested as an on-chip DNA detector based on time-resolved fluorescence imaging. Three different concentrations of Cy5 linked DNA (30mer), 36μM, 18μM, and 9μM, were placed on the sensor surface and dried. Time-resolved fluorescence detection was performed 3ns after the laser pulse for 8ns, as shown in

Fig. ***8***. Fig. 9a shows a 2D fluorescence image from each DNA spot. A limit of detection (3σ) of 14.6μM ($8.8x10^8$ molecules per pixel) was successfully observed as shown in Fig. 9b. Table 1 summarizes the measured data.

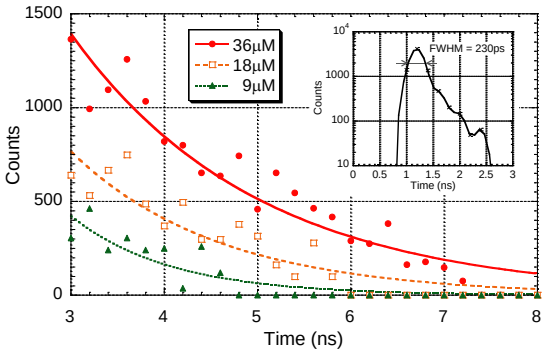
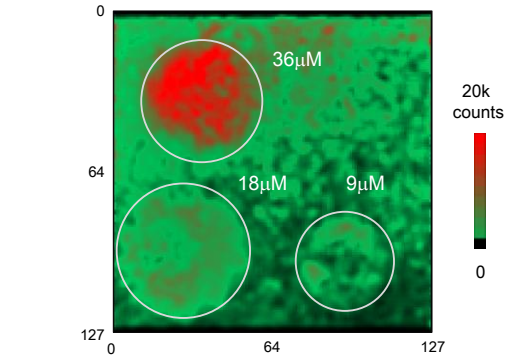



*Fig. 8. Histogram of photon counts from each DNA spot during time gating. The fluorescent label is Cy5 (λex=643nm, λem=667nm), a 637nm pulsed laser is used for excitation light source. The inset shows a median FWHM IRF of 230ps over the entire array.*

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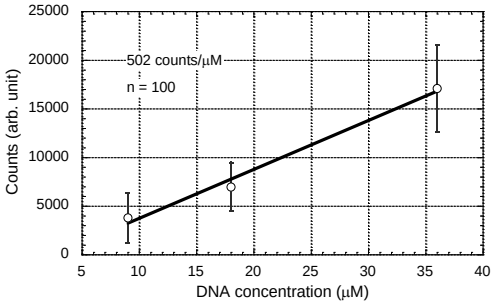


*(b)*

*Fig. 9. On-chip fluorescence detection. (a) 2D fluorescence image from each DNA spot with an integration time of 175ms. (b)The increment of 502 count/μM was obtained on average over 100pixels. An LOD of 14.6μM was measured without using any optical filters.*

*Table 1. Performance summary.*

| Parameter | Value | Unit |
|---|---|---|
| Array format | 128 x 128 | |
| Pixel size | 25 x 25 | $\mu m^2$ |
| Fill factor | 4.5 | % |
| Median DCR @ $V_e$=3.3V | 186 | Hz |
| Photon detection probability @ $V_e$=3.3V | 20 @ 465nm | % |
| Detection limit of DNA (LOD) | 14.6 | μM |
| Time resolution (LSB) | 200 | ps |
| Measurement range | 9.6 | ns |
| Jitter (FWHM) | 230 | ps |
| Frequency of operation | 40 | MHz |
| Frame rate | 2441 | Fps |
| Total IO bandwidth | 40 | Mbps |
| Power consumption @ $V_{dd}$ = 2.5V | 363 | mW |
| Photo response non uniformity (PRNU) | 3.5 | % |

## Conclusions

An all-digital, time-gated CMOS SPAD image sensor was proposed and demonstrated as an on-chip DNA sensor by employing the sliding-time-gating technique. The pulsed excitation light was successfully removed by proper setting of the SPAD activation start timing. Thanks to its single-photon sensitivity and time-resolved measurement capability, faint fluorescence signals were successfully detected from Cy5 labeled DNA molecules. The molecules were placed directly on the surface of the imager. The new approach for on-chip fluorescence detection reported here offers an exciting CMOS based analysis tool for μ-TAS and POCT systems.

## Acknowledgements

The authors acknowledge P. Brühlmeier of the École Polytechnique Fédérale de Lausanne (EPFL) for PCB layout. The authors would also like to thank M. W. Fishburn of Delft University of Technology for many invaluable discussions.

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