Single-Photon, Deep Sub-Nanosecond Integrated Circuits for Fluorescence Lifetime Imaging Microscopy

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A time-resolved CMOS image sensor for fluorescence lifetime imaging microscopy (FLIM) is presented. The sensor pixel consists of a single-photon avalanche diode (SPAD), a deep sub-nanosecond time gating circuitry, and a 1-bit NMOS memory.

Fig. 1(a) shows the schematic of the time gating circuitry and the SPAD. Time gating is achieved via a sliding-time window scheme [1]. The timing diagram for time-correlated single photon counting (TCSPC) operation is shown in Fig. 1(b). 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. The sliding-time window is applied every 200ps time steps as described in [2]. Fig. 1(c) shows the chip micrograph, as well as a close-up of the pixel. The excellent median dark count rate (DCR), 53Hz at excess bias (V_e) of 3V, was measured at room temperature. The DCR per active area is as low as 1.88Hz/μm^2. The median photon detection probability (PDP) is 43.6% at V_e of 4V. Fluorescence images, mixed pollen grains (Carolina Biological Supply Company, NC, USA), were taken under the fluorescent microscope (BX51IW, Olympus, Japan). As shown in raw image (Fig. 2(b)), only 0.73% of the pixels have high noise (higher than 1kHz at V_e of 3V), which is more than a factor of 20 lower than that of [3]. Additionally, hot pixels are easily be removed by median filtering, as shown in Fig. 2(b).

New evidence of optical crosstalk in a large array format was observed for the first time. Fig. 3(a) shows a close-up of a typical noisy pixel which is saturated at a lower V_e (circled area in the lower portion). The crosstalk expands increasing V_e. Fig. 3(b) shows the DCR 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 it is caused by photon emission from noisy pixel. Therefore, a hot pixel elimination technique will become important for further image quality improvement.

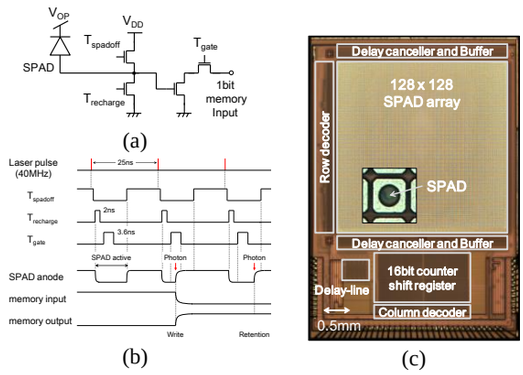

Fig. 1. (a) Schematic diagram. (b) Timing diagram. (c) Chip photomicrograph of the sensor. Time gating is achieved through transistors $T_{spadoff}$, $T_{recharge}$ and T_{gate}. The column decoder is used to serialize the data coming from the array onto two serial channels via the 16-bit shift resistor. The chip was fabricated in a 0.35μm HV CMOS technology. The pixel pitch and a total area are 25μm and 20.5mm^2, respectively.

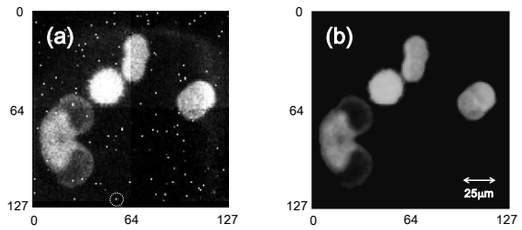

Fig. 2. Fluorescence image of mixed pollen grains obtained with 50k frames at a total exposure time of 2.46s. (a) Raw image. (b) After median filtering.

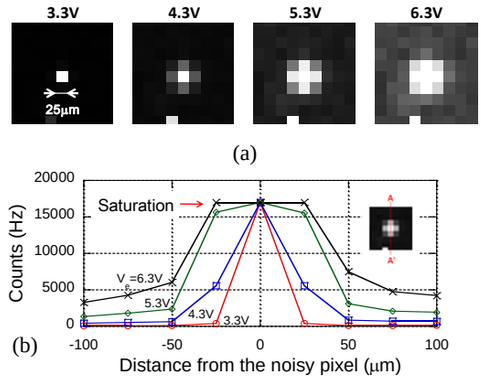

Fig. 3. (a) Close-up images of a hot pixel taken in the dark at various V_e from 3.3V to 6.3V. The images clearly show that the optical crosstalk is dominant over electrical crosstalk. (b) DCR distribution around a hot pixel. The photon emission from a hot pixel raises the DCR in neighboring pixels, which are located 25μm to 75μm away.

Table 1. Performance summary.

Parameter	Value
Array format	128 x 128
Pixel size	25 x 25 μm^2
Typical breakdown voltage	19.1 V
Fill factor	4.5 %
Median DCR @ V_e=3V	53 Hz
Peak median PDP @ 465nm	43.6 % @ V_e=4 V
Measurement range	9.6 ns
Jitter (FWHM)	230 ps
Frequency of operation	40 MHz
Frame rate	2,400 fps
Total IO bandwidth	40 Mbps
Power consumption @ V_{dd} = 2.5 V	360 mW

During the presentation, FLIM results taken from a multistained pine pollen grain will also be discussed. The paper also outlines advances in single-photon detection and data processing, monolithically integrated in deep-submicron CMOS technologies. Several examples are given of the single-photon detector arrays, their readout architectures, and functionality. The trends of single-photon detector arrays in other bioimaging fields, such as positoron emission tomography (PET), fluorescence correlation spectroscopy (FCS), super-resolution optical microscopy, are also discussed in detail.

Reference

[1] D. Stoppa, D. Mosconi, L. Pancheri, and L. Gonzo, *IEEE Sens. J.* 9, 9, pp. 1084-1090 (2009).

[2] Y. Maruyama and E. Charbon, in *Proc. of the Transducers'11*, pp. 1080-1083 (2011).

[3] C. Veerappan, J. Richardson, R. Walker, D. Li, M.W. Fishburn, Y. Maruyama, D. Stoppa, F. Borghetti, M. Gersbach, R.K. Henderson, E. Charbon, i*n Proc. of the ISSCC*, pp. 312-314 (2011).