

A Second-Generation Imaging System for Freely Moving Animals

Joon Hyuk Park¹, Jelena Platisa², Vincent Pieribone^{2,3}, Eugenio Culurciello⁴

¹Department of Electrical Engineering, Yale University

²John B. Pierce Laboratory, ³Department of Cellular and Molecular Physiology, Yale University

⁴Department of Biomedical Engineering, Purdue University

joonhyuk.park@yale.edu, jplatisa, vpieribo@jbpierce.org, euge@purdue.edu

Abstract—An updated image sensor to be used in a miniature microscope system for recording brain activity over a wide cortical area (4-9 mm²) is presented. The image sensor is a 100 x 100 pixel array fabricated in bulk CMOS process. The sensor also contains a 10-bit ADC and two input channels to measure other physiological signals concurrently with the optical data. Another feature of the image sensor is the ability to observe dF over sequential frames in hardware, in order to reduce the amount of data that must be read out of the image sensor at high speeds. The speed and noise performance of the image sensor is suitable for voltage-sensitive optical probes in small systems for awake and freely moving animals.

I. INTRODUCTION

Neural recordings from awake and freely moving animals are desirable because the activity in cerebral cortex is severely suppressed and altered by anesthetics and physical restraints [1]. In order to record such data, genetically encoded optical probes (GEOP) can provide high spatial and temporal resolution, while being less invasive than traditional micro-electrode methods.

While it is possible to obtain high resolution images of neurons in animals with fiber optic tethered laser-scanning imaging methods [2], [3], these approaches lack the sensitivity and temporal resolution to record rapid and small fluorescence intensity changes produced by GEOP.

A small imaging system designed specifically for GEOP can be paired with a custom-designed miniature, head-mountable wide field epi-fluorescence microscope (Figure 1) to allow neural recordings from behaving animals.

The design challenges for such an imaging system is speed, sensitivity, weight and power. The imaging system must be able to resolve changes in light intensity below 0.3%. The capture speed must be greater than 100s of frames per second (fps) to sample fast neurophysiologic

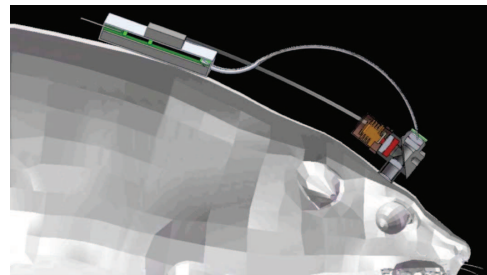


Fig. 1: Proposed imaging system for freely moving animals.

events [4], [5]. Such performance must fit into a compact system in order to be head-mountable on animals.

This work build upon our previous works in designing a small, head-mountable imaging system for awake and behaving animals [6], [7]. Previously, we designed a 32 x 32 pixel image sensor with an analog output. The updated image sensor uses a process optimized for image sensors, and also adds many more features to enable a complete lab-on-a-chip for optical imaging. In Section II and III, the image sensor circuit and its characteristics are presented. Section IV presents an in vitro experiment to test the sensitivity of the imaging system.

II. IMAGE SENSOR DESIGN

A functional diagram of the sensor is shown in figure 2. The sensor has a 100 x 100 pixel array. Each pixel is 30 μm x 30 μm . A schematic of the pixel is shown in figure 3. The n-well photodiode is sized to be 20 μm x 20 μm .

The pixel is a modified 3-T APS design to enable temporal differencing (dF) and correlated double sampling (CDS) to reduce noise. In normal image capture, all pixels in a row output to their respective shared column output line when $RSEL_n$ goes low. The output of the pixel at this point is the last stored value in the capacitor

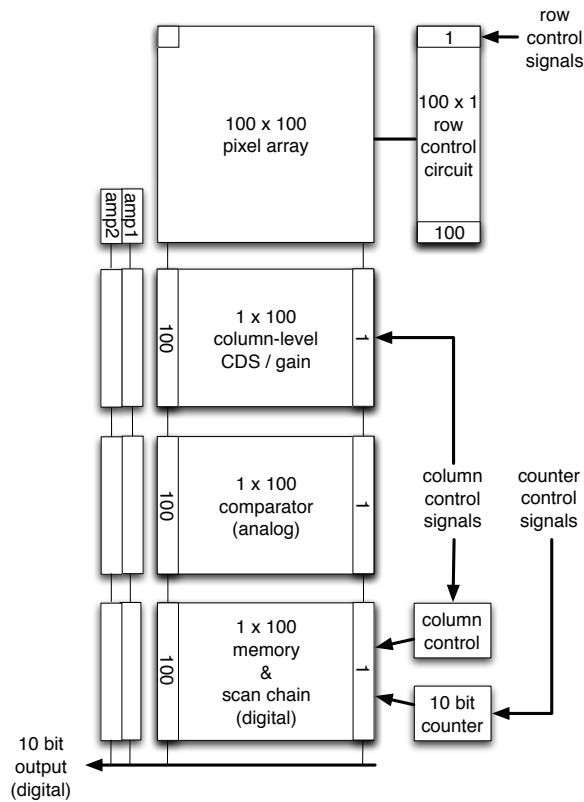


Fig. 2: Overview of our image sensor.

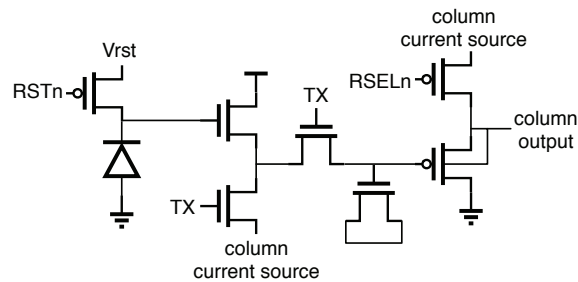


Fig. 3: Schematic of the pixel used in our image sensor.

(reset value). The output voltage is then sampled by the CDS circuit. Then, the TX signal goes high to transfer the current pixel voltage to the storage capacitor (MOSCAP) and the output line. This value is then sampled to get the CDS output. Then, the pixel is reset by pulsing $RSTn$. Then TX is brought to low, and the reset voltage is now stored in the storage capacitor, to be used in the next frame's CDS calculation.

Temporal difference mode is similar to the normal operation. However, the TX signal is brought low before $RSTn$ is pulsed, so that the current voltage is stored in the storage capacitor instead of the reset voltage. Thus, the CDS operation is done between the voltages of the

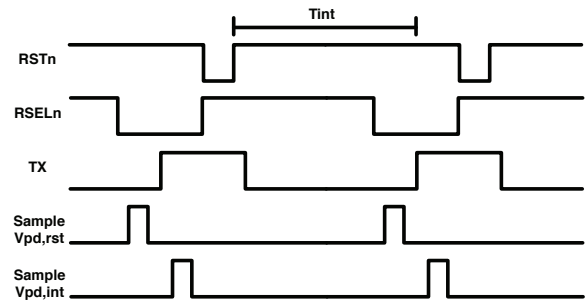


Fig. 4: Pixel timing for normal operation.

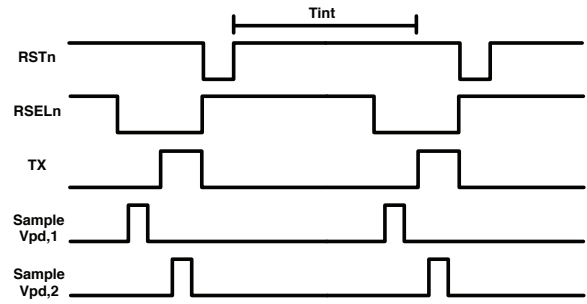


Fig. 5: Pixel timing for temporal difference operation.

two sequential frames.

The sensor also contains a column-level 10-bit single slope ADC. The single slope ADC is implemented by generating a ramp synchronized with a counter. When the ramp value is greater than the output voltage from the column-level CDS circuit, the counter value at that point in time is stored into on-chip, column-level memory. This digitized value is then read out in parallel.

III. IMAGE SENSOR CHARACTERIZATION

The photodiode in every pixel was integrated at 500 fps at different light intensities to test the signal-to-noise (SNR) of the image sensor. At saturation, the image sensor has a SNR of 54 dB, meaning that it can detect changes in intensity of 0.2%, which is acceptable this application.

The fixed pattern noise (FPN) was calculated by measuring the standard deviation of each pixel over 1000 frames and then taking the mean of the resulting 10000 (from 100 x 100 pixels) standard deviation values. For a short integration time of 2 ms in the dark, the standard deviation was 3.1 mV over a 5.4 mV swing. At near saturation and 22 ms integration time, the standard deviation was 3.9 mV over a 915 mV swing.

In order to test the performance of the imaging system at 500 fps, a sine wave was fed into an LED. The imaging system captured data at 500 fps and was able

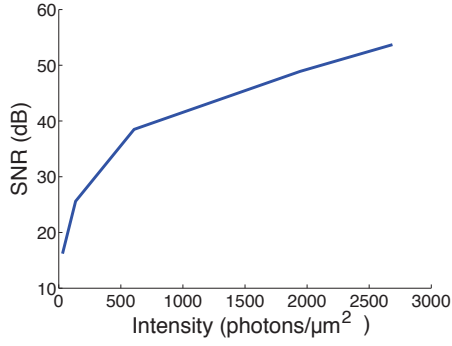


Fig. 6: Signal to noise ratio at different light intensities at 500 fps.

TABLE I: Pixel Parameters

Technology	ams 0.35 μm OPTO CMOS
Size	30 μm x 30 μm
Photodiode Size	20 μm x 20 μm
Fill Factor	44%
Photodetector Type	n-well
Voltage Swing	1.2 V
Well Size	253,000 e-
Conversion Gain	4.9 $\mu\text{V}/\text{e-}$

to capture the sine wave generated by the LED (Figure 7).

One improvement over the old design [6], [7] is a better implementation of the on-chip dF calculation circuit. Having such a circuit can cut down the data resolution by half, thus reducing the data throughput rate. This in turn results in faster frames rates. In order to test this circuit, an LED was pulsed at 50 Hz, and the temporal difference data was obtained at 500 fps (Figure 8).

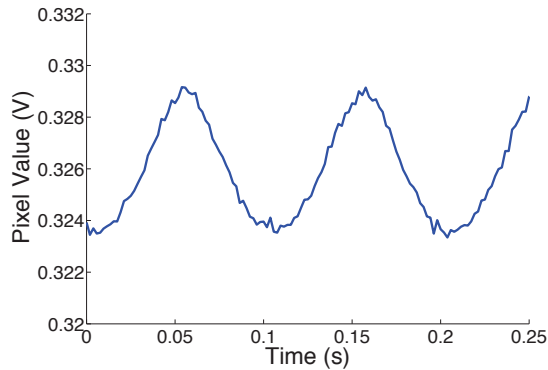


Fig. 7: A 10 Hz sine wave captured at 500 fps.

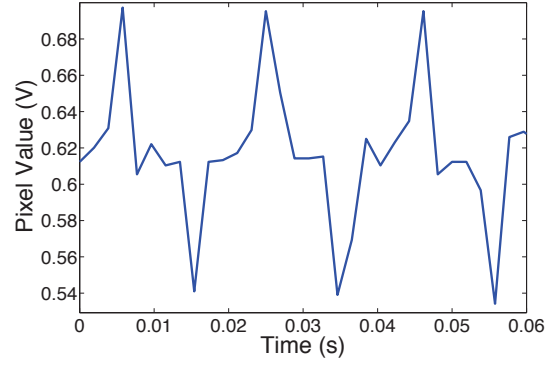


Fig. 8: A 50 Hz pulse with 50% duty cycle captured at 500 fps.

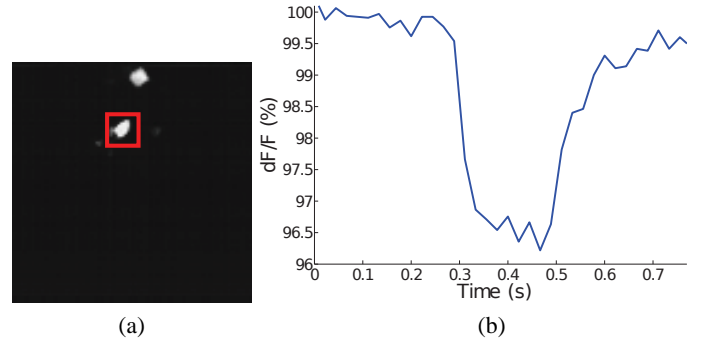


Fig. 9: A HEK293 cell expressing EGFP-based genetically encoded voltage sensor. (a) Image of the transiently transfected HEK293 cells. (b) Trace of a voltage-dependent fluorescence change recorded from the HEK293 cell outlined by the red box, derived from a 2 x 2 pixel average of 3 trials.

IV. IN VITRO TESTING

Human embryonic kidney (HEK293) cells were transiently transfected with an EGFP-based genetically encoded voltage sensor (to be published) using Lipofectamine 2000 (Invitrogen, NY). Whole-cell patch clamp experiments were performed using Patch Clamp PC-505B amplifier (Warner Instruments, CT) with cells kept at the holding potential of -70 mV. Voltage dependent changes in fluorescence intensity were provoked by 200 ms/+100 mV depolarizing steps recorded at 200Hz. Change of fluorescence was imaged with a 60x 1.40 N.A. oil immersion objective lens using a 150 W Xenon arc lamp (Opti Quip, NY). The result can be seen in Figure 9.

V. CONCLUSION

This paper presented a sensor for use in a miniature imaging system for behaving animals. The system has a resolution of 100 x 100 and is capable of recording electrical neural activity with optical probes at high speeds. The image sensor has a peak SNR of 54 dB. Currently, more experiments are being performed to further test the capability of the image sensor.

VI. ACKNOWLEDGMENTS

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