Miniature Voltage Sensitive Dye Imaging System for In Vivo Experiments

Joon Hyuk Park[1], Vincent Pieribone[2], Eugenio Culurciello[1]
[1]Department of Electrical Engineering, [2]Department of Cellular and Molecular Physiology
Yale University
New Haven, CT 06520
joonhyuk.park, vincent.pieribone, eugenio.culurciello@yale.edu

***Abstract*— Currently, there are no widely used methods of optically recording rapid electrical events over a wide cortical area in freely moving animals. This paper presents an image sensor for recording electrical activity of large regions (4-9 mm^2) of the nervous tissue at high speeds ($>$ 500 Hz) using voltage sensitive dye imaging (VSDI) in freely moving animals. Each 75 μm x 75 μm pixel consists of a photodiode of 74 μm x 34 μm and a storage capacitor of 788 fF. The image sensor has a signal-to-noise ratio of 76 dB.**

I. Introduction

Neuronal recordings of awake and freely moving animals provide one of the most representative views of brain function. Animal experiments using anesthetics have dramatic effects on all levels of neuronal function [3]. The activity in cerebral cortex is severely suppressed with anesthetics as it alters and depresses neuronal activity. A conscious behavioral response is required to accurately study cognitive processing of stimuli.

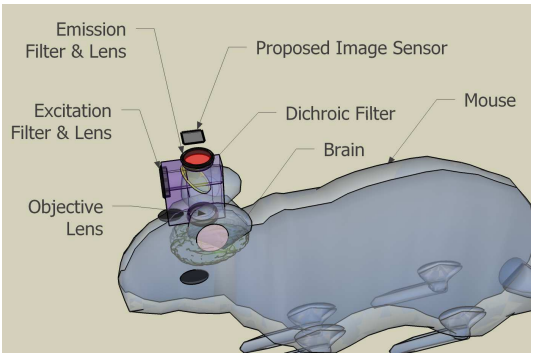


Fig. 1. Proposed VSDI system for freely moving animals.

In VSDI experiments, a small fluorescent dye is applied to the brain surface [6]. The dye molecules alter their fluorescent output in response to changes in transmembrane voltage. VSDI does not have a bias for any particular neuronal subtypes and allows the localization of voltage waves as they propagate across large areas (millimeters) of cortex.

There are many advantages of VSDI over other methods of measuring neuronal activity [**?**]. In addition, high speed (500-1000 Hz) wide field optical recordings of electrical activity such as VSDI provide different information about cortical processes that cannot be readily obtained from traditional electrophysiology methods, including propagating sub- and supra-threshold waves and state changes, subthreshold events, electrical events in neuron classes inaccessible to electrodes and glial responses [9].

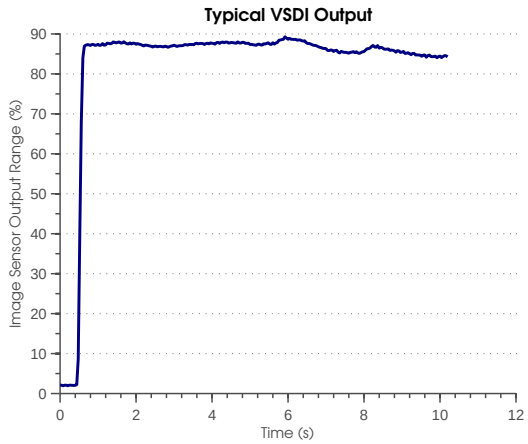


Fig. 2. Light intensity of a typical VSDI signal. The bright background light is turned on at 0.5 s, nearly saturating the image. Small fluctuations from 0.5 to 10 s is the signal of interest.

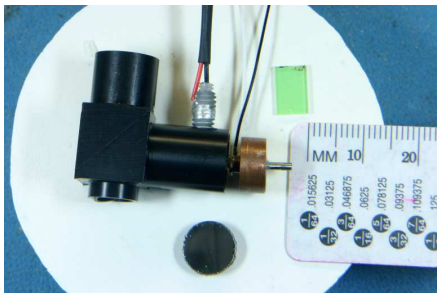


Fig. 3. Second generation microscope system. The housing holds all the optics, filters, light source and the image sensor.

Typical VSDI systems cannot be adapted for use on freely moving animals due to restrictions on weight ($<$ 10 g) and power ($<$ 15 mW). A miniaturized implantable VSDI sensor has many design challenges. The signal-to-noise ratio (SNR) must be larger than 13 bits, as the signal of interest is around 0.1-1% of the background illumination (figure 2). Thus a large photon-collection area per pixel must be paired with a 14-16 bit analog-to-digital converter (ADC). A high bandwidth

(> 500 frames/s) is needed to sample fast neurophysiologic events [9], [**?**]. Also, there are a number of dynamic noise sources that together degrade the signal. A custom engineered and manufactured miniaturized head-mounted imaging system that addresses all of these issues is shown in figure 3.

It is important to note that of the 16 bits collected per sample, 10-12 bits are from the unvarying background light (figure 2). Thus, only 4-6 bits are used to digitize the modulating signal from the tissue. For a head-mounted microscope for small animals, weight is a major concern, and having multiple 16 bit ADCs on the microscope is not feasible. Putting the ADCs off the system closer to a computer is also undesirable, due to the increase in wires and noise. To address this issue, and to exploit the fact that only 4-6 bits are used to digitize the signal of interest, frame-differencing on chip is implemented before the A/D conversion.

Previous imaging systems are not tailored for brain studies in freely moving animals. One CMOS sensor is compatible with a variety of clinical assays [**?**], but it suffers from a large inter-detector mismatch. Single large-area photodiodes for low-level luminescence sensing [**?**] lack necessary spatial resolution. State-of-the-art bio-luminescent sensors [**?**] do not possess the temporal resolution necessary for voltage sensitive dye imaging in the central nervous system (CNS). An attempt at resolving fluorescence lifetime signal was published [**?**] but the pixel pitch of this device is too large to detect small neuron populations. Recently, a miniaturized system for fluoresence microscopy in freely moving mice was presented [**?**], but it lacks the temporal and spatial resolution needed for VSDI.

In Section II, the image sensor circuit is presented. The characterstics of the image sensor is presented in Section III. Section IV describes the test set up for imaging olfactory receptor neurons. Results of preliminary experiments on an animal is shown in Section V.

II. Image Sensor Design

The pixel is shown in figure 4. The pixel uses a standard 3-T design composed of a PMOS reset transistor and a NMOS follower. The storage capacitor is a MOSCAP sized for 788 fF. A dummy switch (*s1N*) is used to reduce clock feedthrough and charge injection. The pixel value is stored into a capacitor and readout through *out1* or directly read out through *out2*. The two output lines allow the pixel to operate in a normal readout mode as well as temporal difference mode. For a normal readout, the integrated voltage of the photodiode can be stored into the storage capacitor by turning on *s1*. After integration, *s1* is turned off to isolate the storage capacitor from the photodiode. The stored voltage value is then read out by the circuit.

In temporal difference mode, the first frame is stored into the capacitor, just as in normal-image mode. However, *s1* is turned off once the first frame is integrated and stored in the capacitor. Once the storage capacitor is disconnected from the photodiode, the photodiode is reset. The second frame is then integrated by the photodiode and *out1* (the previous frame) and *out2* (the integrated voltage of the current frame) are read

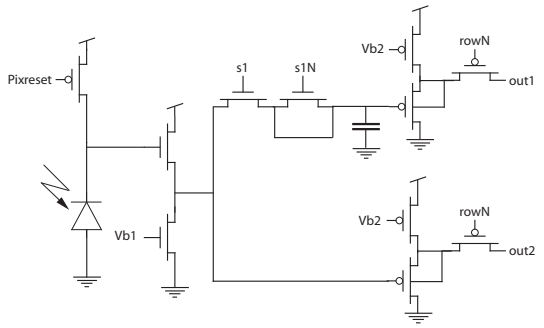


Fig. 4. Pixel used in the proposed image sensor. This pixel is capable of outputting 2 frames sequentially for temporal differencing on chip.

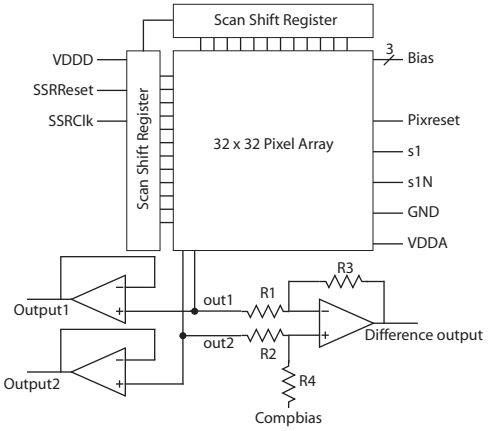


Fig. 5. Overview of the proposed image sensor. Shift scan registers are used to address each pixel during readout. *Out1* and *out2* are read by the rest of the system through two buffers. The temporal difference output it generated from the two outputs and read by the rest of the system through *Differential output* at a gain determined by the ratio between *R1, R2, R3* and *R4*

out simultaneously to an on-chip differential amplifier. The differential amplifier has a gain of 8, and amplifies a difference of a few hundred microvolts to a few millivolts.

Before the integration of a third frame, the current value of the photodiode is stored into the capacitor by quickly pulsing *s1*. This erases the first frame from the storage capacitor and stores the second frame into the capacitor. This cycle is repeated to produce a continuous temporal-differencing output. This feature has not been tested extensively yet and will not be discussed further in this paper.

III. Characterization of the Image Sensor

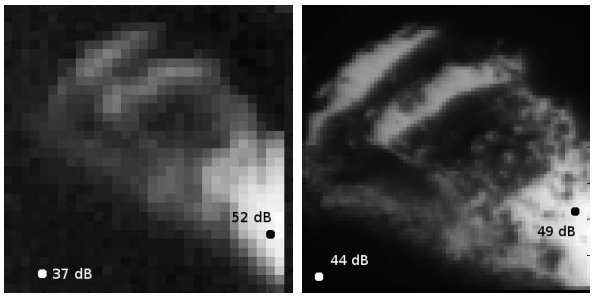
A test platform to measure the performance of the image sensor was built around an Opal Kelly XEM3001v2 platform. The FPGA on the Opal Kelly was used to control two 16 bit ADCs (for normal outputs), one 12 bit ADC (for the temporal difference output) and two 12 bit digital-to-analog converters that provided the appropriate biases to the image sensor. A control program was written in C++ and communicated with the Opal Kelly through an USB port.

To test the SNR of the image sensor, the photodiode in every pixel was integrated for 25 ms at different light intensities. The relationship between SNR and light intensity are given by

the equation: $SNR[dB] = 0.42 \cdot intensity[lux] + 31$ before saturation at around 150 lux. At saturation, the image sensor has a SNR of 76 dB. The SNR needs to be between 55 to 72 for VSDI applications.

In order to test the leakage of the capacitor, the photodiode was reset and *s1* was turned off immediately to isolate the capacitor from the photodiode. The voltage drop in the capacitor due to leakage was measured at different times. The voltage drop in 1 ms was 56 μV. For temporal-differencing, the value needs to be stored in the capacitor for 2 ms at 500 fps. In 2 ms, there will be a 112 μV drop, which is slightly above 1 LSB (for a 14 bit resolution of a 1.5 V swing). Improving the readout circuitry and increasing the framerate would minimize the effect of leakage in the capacitor. Since the storage capacitor is sized to be 788 fF, the leakage current in the capacitor can be calculated to be 44.8 fA.

The fixed pattern noise (FPN) was calculated by measuring the standard deviation of each pixel over 64 frames and then taking the mean of the resulting 1024 standard deviation values. For a short integration time of 20 μs in the dark, the standard deviation was 129 μV over a 3.1 mV swing. At near saturation (150 lux and 25 ms integration time), the standard deviation was 308 μV over a 1.298 V swing.



(a) SNR of proposed image sensor at 500 fps (b) SNR of NeuroCCD at 500 fps

Fig. 6. Phantom images taken with the proposed image sensor and Red Shirt Imaging NeuroCCD-SM256. The SNR of the proposed image sensor is 37 dB in the dark region and 52 dB in the bright region. The SNR of NeuroCCD is 44 dB in the dark region and 49 dB in the bright region.

Two phantom images were taken using a typical VSDI setup. Figure 6(a) is taken with the proposed image sensor and figure 6(b) is taken with Red Shirt Imaging NeuroCCD-SM256 [**?**]. NeuroCCD is commonly used for VSD and calcium imaging of anesthetized animals. The performance of the two image sensors are comparable as shown in figure 6.

IV. Animal Test Setup

Once the image sensor was characterized, experiments on anesthetized animals were run to compare the proposed image sensor against a commercial VSDI sensor.

Rat olfactory receptor neurons (ORN) were loaded with calcium-sensitive dye (Oregon Green BAPTA 488 Dextran 10kD) 7 days prior to imaging using a procedure similar to a procedure used for mice. Just prior to imaging, an optical window was installed over the dorsal surface of each olfactory bulb (OB) by thinning the overlying bone to 100 μm thickness and coating the thinned bone with ethyl 2-cyanoacrylate glue to preserve optical clarity. Odorants were diluted from saturated vapor of pure liquid odorant using mass flow controllers. Linearity and stability of the olfactometer were verified with a photoionization detector. Anodorant delivery tube (20 mm ID, 40 mm long), positioned 6 mm from the animals nose, was connected to a 3-way valve controlled vacuum flow. Switching this vacuum flow away from the tube assured rapid onset and offset of the odorant.

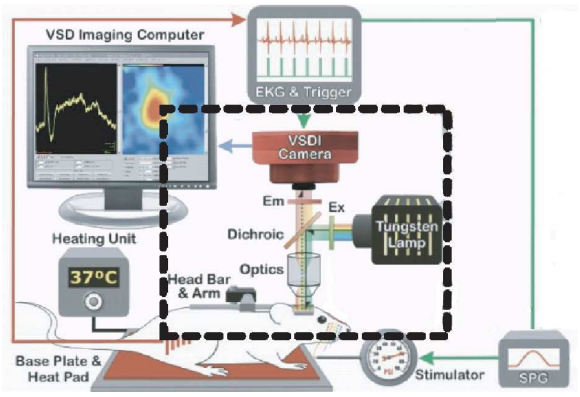


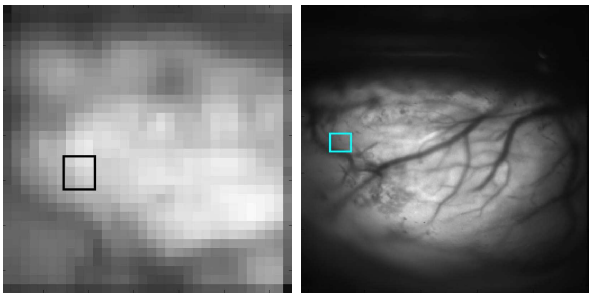
Fig. 7. Current VSDI setup for a fixed, anesthetized rat. The tungsten lamp and the dichroic filter provide the excitation (Ex) signal. The resulting fluorescent signal is passed through the emission (Em) filter and into a image sensor. The stimulator introduces an odor and controls the breathing of the animal. The VSDI camera component was replaced with the proposed image sensor and data was taken to compare the results with NeuroCCD. The proposed system (figure 3) will incorporate everything inside the dotted line into a compact system for use on freely moving animals.

After creating the optical window, a double tracheotomy was performed to control sniffing. To draw odorants into the nasal cavity, a teflon tube was inserted tightly into the nasopharynx. This tube communicated intermittently (250 ms inhalation at a 2 s interval) with a vacuum flow via a PC-controlled 3-way valve. A cyan LED was the light source. Neuroplex software initiated a trial by triggering the onset of the light source, resetting the sniff-cycle and triggering the olfactometer. Imaging started 0.5 sec after the trigger and an odorant was presented from 4 to 9.5 s. Figure 7 shows the test setup.

V. Animal Test Results

Figure 8 shows the area of the cortex observed by the proposed image sensor and NeuroCCD. Since the proposed image sensor is only 32x32, the field of view is much smaller than that of NeuroCCD. Still, the same area of the cortex was targeted to measure the change in light levels.

The change in light intensity over time is shown in figure 9. The change detected by NeuroCCD is shown by the third line. The change detected by the proposed image sensor is shown in the lines below. The fourth line is with 2% concentration of ethyl butyrate. During the experiment, the olfactory epithelium had adapted to the odor concentrations so the ethyl butyrate was increased to 5% concentration and the bottom line shows



(a) Proposed image sensor at 50 fps (b) NeuroCCD at 50 fps

Fig. 8. Area of rat cortex where activity was observed.

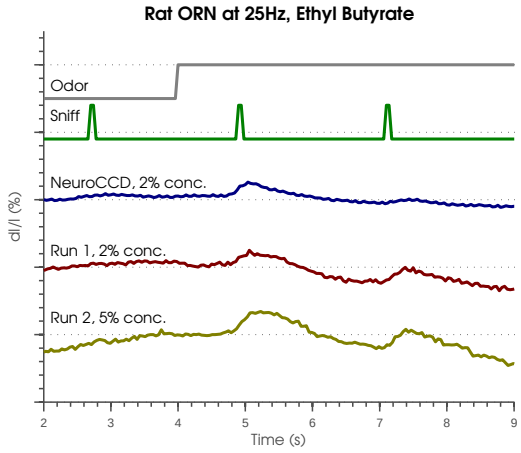


Fig. 9. Observed change in fluorescence. The curves are raw, single event data and not an average. Each major tick of the y-axis repsents a 10% change in light intensity. Both the proposed sensor and NeuroCCD detected a similar change in light intensity once the odor was sniffed by the animal. However, the proposed image sensor can be integrated into the miniature microscope (figure 3).

the result after the increase in odor concentration. For the bottom line, in terms of the absolute 16 bit ADC value at the normal output line, the reset level is 61800 and the background is 48200, for a voltage swing of 13600. At around 5.5 s, the value drops from 48150 to 47750, for a swing of 400. Thus the change in intensity is 2.94%. This is within the range of expected values.

VI. Conclusion

This paper presented an image sensor for use in a miniature VSDI system for freely moving animals. The system is capable of recording electrical activity of large regions (4-9 mm^2) of the nervous tissue at high speeds ($>$ 500 Hz). Each pixel is sized to be 75 μm x 75 μm and the photodiode is 74 μm x 34 μm. The image sensor has a signal-to-noise ratio of 76 dB.

The image sensor yielded similar performance to a commercial VSDI sensor when tested on a fixed animal. Future works includes completing the miniature microscope and running more experiments on freely moving animals. Also, the temporal differencing capability needs to be tested extensively and characterized to determine if the system can take advantage of this feature to reduce the stress on the readout ADC.

TABLE I

Image sensor properties

Technology	AMI 0.5-μm Bulk CMOS
Array Size	32 (H) x 32 (V)
Total Size	3 mm x 3 mm
Pixel Size	75 μm x 75 μm
Fill Factor	45%
Fixed Pattern Noise	129 μV (dark), 308 μV (150 lux)
Read Noise	67 e-
Output Voltage Swing	1.47 V
Well Size	700,000 e-
Storage Capacitor Leakage Current	44.8 fA
SNR	76 dB
Max Frame Rate	890 fps

VII. Acknowledgments

This project was partly funded by ONR grant number 439471 and 396490, and ARO contract W911NF-07-1-0597.

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