High-speed Fluorescence Imaging System for Freely Moving Animals

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***Abstract*— An image sensor to be used in a miniature microscope system for recording brain activity over a wide cortical area (4-9 mm^2) is presented. The image sensor is a 32x32 pixel array fabricated in bulk CMOS process. Each pixel has a 74 μm x 34 μm photodiode. The SNR performance of the image sensor is comparable to current state-of-the-art voltage-sensitive dye imaging sensors. The power consumption and weight of the proposed microscope is much lower than other voltage-sensitive dye imaging systems and can be used on awake and freely moving animals.**

I. Introduction

Neuronal recordings of awake and freely moving animals are desirable because they provide one of the most representative views of brain function. Currently, there are no widely-used systems that can optically record rapid electrical events over a wide cortical area in freely moving animals. 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].

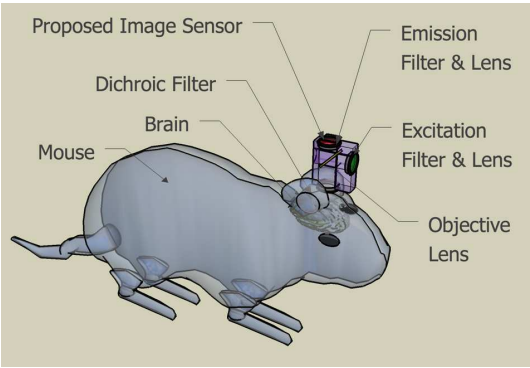


Fig. 1. Proposed VSD imaging system for freely moving animals.

The preferred imaging technique for measuring activity in the cerebral cortex is voltage-sensitive dye (VSD) imaging. The VSD is applied to the brain surface [2], and the dye molecules alter their fluorescent output in response to changes in transmembrane voltage. A method for measuring brain activity in awake mice exist , but this method is for calcium-sensitive dye (CSD) imaging.

VSDs have a couple advantages over CSDs. CSDs give larger signals than VSDs, but are slower ($<$ 100 Hz) and generally do not resolve single action potentials [3]. VSD imaging systems have temporal resolutions of more than 500 Hz, which is important in discriminating the timing of individual action potentials. Also, VSDs are better at detecting small subthreshold events or inhibitory potentials [4]. Also, CSD imaging has a smaller spatial observation field [5].

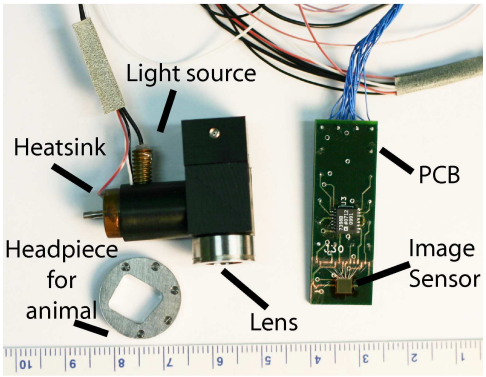


Fig. 2. The microscope housing holds all the optics, filters, light source and the image sensor.

VSD imaging techniques have advantages over other traditional methods of measuring neuronal activity [6],

as VSD offers better temporal and spatial resolution than most other techniques. Also, VSDs are not as invasive as electrodes which can damage the very neurons being observed. VSD imaging provides different information about cortical processes that cannot be readily obtained from other methods, such as propagating sub- and supra-threshold waves [7].

There are many challenges for adapting current VSD imaging systems for use on freely moving animals due to restrictions on weight and power. The nature of the VSD signal requires the image sensor to have an SNR of more than 13 bits, as the signal is 0.1-1% of the background illumination. A frame rate of greater than 500 Hz is required to sample fast neurophysiologic events [7], [8]. All components, including the light source, excitation and emission filters, and lens must be incorporated along with the image sensor in a light-weight ($<$ 10 g) and consume less than 15 mW. A custom engineered and manufactured miniaturized head-mounted imaging system that addresses all of these issues is shown in figure 2.

In Section II, the image sensor circuit and its characteristics are presented. Section III presents an in vitro experiment involving TRPV1 transfected HEK cells. An in vivo experiment imaging rat olfactory receptor neurons is described in Sectiono IV.

II. Image Sensor Design and Characterization

The pixel is shown in figure 3. The pixel uses a standard 3-T design composed of a PMOS reset transistor and a NMOS follower. The photodiode in every pixel was integrated for 25 ms at different light intensities to test the signal-to-noise (SNR) of the image sensor. At saturation, the image sensor has a SNR of 76 dB, which is enough for VSD applications.

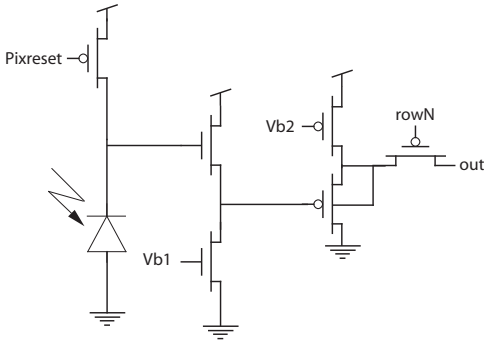


Fig. 3. Schematic of the pixel used in our image sensor.

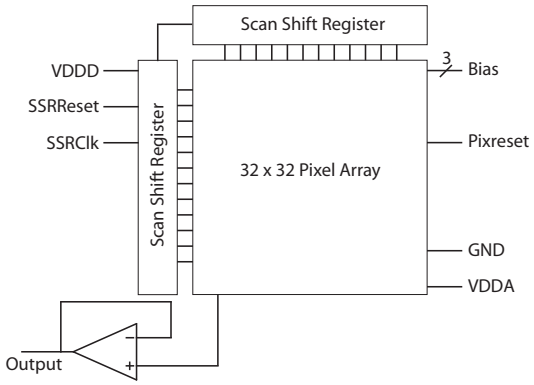


Fig. 4. Overview of our image sensor. Shift scan registers are used to address each pixel during readout. The *output* is read by the system through a global buffer.

TABLE I
Pixel Parameters

Technology	AMI 0.5-μm Bulk CMOS
Size	75 μm x 75 μm
Photodiode Size	74 μm x 34 μm
Fill Factor	45%
Photodetector Type	n-well
Voltage Swing	1.47 V
Well Size	700,000 e-
Conversion Gain	0.8 μV/e-

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 (from 32 x 32 pixels) 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 at 150 lux and 25 ms integration time, the standard deviation was 308 μV over a 1.298 V swing.

The quantum efficiency (QE) of the photodiode is shown in figure 5. A HORIBA Jobin Yvon FluoroMax-3 acted as the light source. The light power at different wavelengths was calculated by measuring the current generated by a Newport 818-UV photodiode which acted as a reference photodiode. Then, the integration time for a 1 V swing was measured using the same light source and the QE was derived from the collected data.

Next, two phantom images were taken using a typical VSDI setup. Figure 6(a) is taken with our image sensor and figure 6(b) is taken with Red Shirt Imaging NeuroCCD-SM256 [9]. NeuroCCD is commonly used for VSD and calcium imaging. The performance of the two image sensors are comparable at 500 frames per second as shown in figure 6.

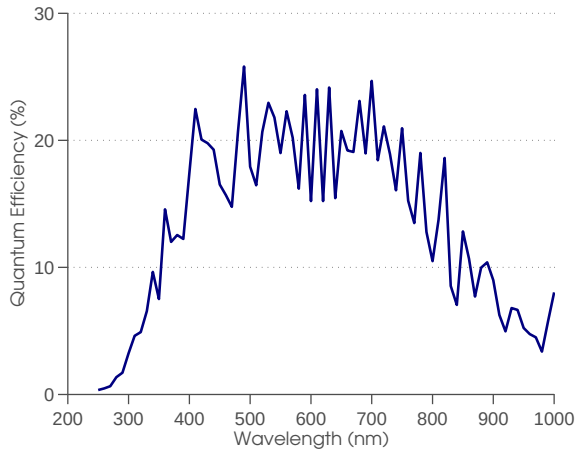
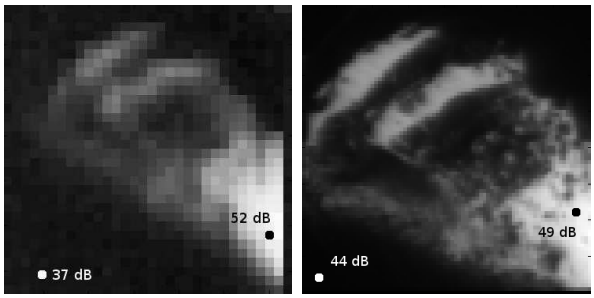


Fig. 5. Quantum efficiency curve of our image sensor.



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

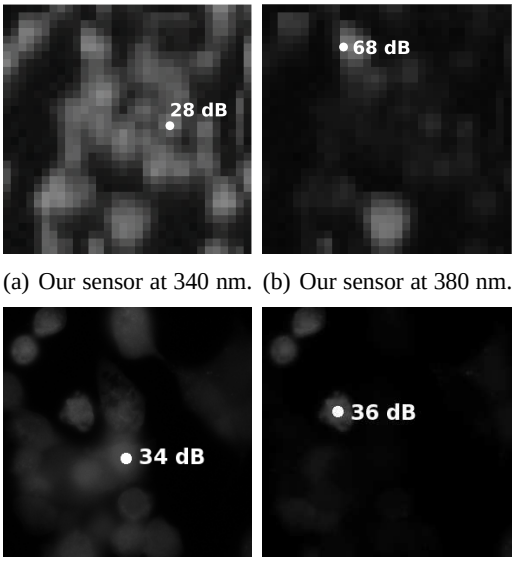
Fig. 6. Phantom images taken with our image sensor and Red Shirt Imaging NeuroCCD-SM256. The SNR of our image sensor is 37 dB in the dark region and 52 dB in the bright region. The SNR of NeuroCCD is 44 dB in the dark region and 49 dB in the bright region.

III. In Vitro Testing

The image sensor was then tested for fluorescent imaging of cultured HEK-293T cells. The HEK-293 cells were transfected with rat TRPV1. TRPV1 is a Ca^{2+}-permeable transient receptor potential ion channel activated by vanilloids such as capsaicin, the pungent ingredient in chili peppers. TRPV1 is expressed specifically in nociceptive chemosensory neurons and is sensitive to acidity and noxious heat.

Imaging and electrophysiological recordings were performed 1626 h after transfection. For imaging, cells were loaded with Fura-2 for 1 h and subsequently washed and imaged in standard bath solution. Ca^{2+} imaging was performed on an Olympus IX51 microscope with a Polychrome V monochromator (Till Photonics).

In the experiment, the activation of TRPV1 by capsaicin was observed. Fura-2 emission images were obtained at 340-nm and 380-nm excitation wavelengths. Our image sensor operated at 8 fps and the Sensicam at 2 fps. The desired result for this specific experiment was a visible confirmation of the activation of the receptor, which is shown by the fluorescing of the cells discernible from the background.



(a) Our sensor at 340 nm. (b) Our sensor at 380 nm.

(c) Sensicam at 340 nm. (d) Sensicam at 380 nm.

Fig. 7. Calcium imaging studies on HEK cells transiently transfected with TRPV1. The Vanilloid receptor TRPV1 is a non selective cation channel that is activated by Capsaicin, the pungent ingredient in chilli peppers. 5μM capsaicin induced a robust calcium signal in cells loaded with Fura-2 AM. The 340 nm and 380 nm images are used for ratiometric imaging. The exact same field of view for the image sensors was not possible, as the sample is free-floating and moved when trying to find the same field of view.

IV. In Vivo Testing

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 [11]. Odorants were diluted from saturated vapor of pure liquid odorant using mass flow controllers. A photoionization detector verified the linearity and stability of the olfactometer. An odorant delivery tube was positioned 6 mm from the animal's nose and connected to a 3-way valve controlled vacuum flow.

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 prior to imaging. After creating the optical window, a double tracheotomy was performed to control

sniffing. Imaging started 0.5 sec after the trigger and an odorant was presented from 4 to 9.5 s.

The ORNs were observed using both the NeuroCCD and our image sensor. Since our image sensor has a 32 x 32 resolution and the field of view is 1.5 mm x 1.5mm, the imaging area was smaller than that of NeuroCCD. However, the same area of the cortex was able to be observed using the two image sensors to measure the change in light levels.

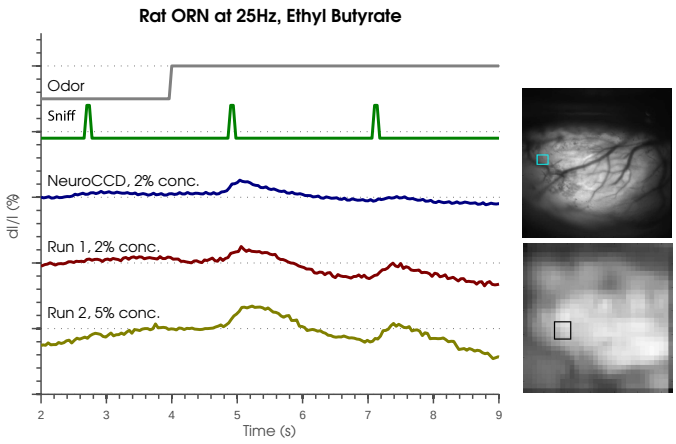


Fig. 8. Observed change in fluorescence. The curves are raw, single event data and not an average. Each major tick of the y-axis repsents a 10% change in light intensity. Both our sensor and NeuroCCD detected a similar change in light intensity once the odor was sniffed by the animal. However, our image sensor can be integrated into the miniature microscope (figure 2). The top right image is a frame from NeuroCCD and the bottom right image is a frame from our image sensor. The boxed outline in the frames is the olfactory bulb where the change was detected.

The change in light intensity over time is shown in figure 8. The change detected by NeuroCCD is shown by the third line. The change detected by our 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 the result after the increase in odor concentration. For the bottom line, the change in intensity is 2.94%. This is within the range of expected values.

V. Conclusion

This paper presented an image sensor for use in a miniature system for freely moving animals. The system is capable of recording electrical activity of the neural 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 commercial CCD sensors during in vivo and in vitro experiments for various calcium dye applications and phantom VSD samples. However, more VSD testing is needed to ensure that signals of interest can be observed with the image sensor at faster frame rates.

VI. Acknowledgments

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