

# *In-vivo Imaging of Neural Activity with Dynamic Vision Sensors*

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**Abstract**—Optical recording of neural activity using calcium or voltage indicators requires cameras capable of detecting small temporal contrast in light intensity with sample rates of 10 Hz to 1 kHz. Large pixel scientific CMOS image sensors (sCMOS) are typically used due to their high resolution, high frame rate, and low noise. However, using such sensors for long-term recording is challenging due to their high data rates of up to 1 Gb/s. Here we studied the use of dynamic vision sensor (DVS) event cameras for neural recording. DVS have high dynamic range and a sparse asynchronous output consisting of brightness change events. Using DVS for neural recording could avoid transferring and storing redundant information. We compared the use of a Hamamatsu Orca V2 sCMOS with two advanced DVS sensors (a higher temporal contrast sensitivity 188x180 pixel SDAVIS and a 346x260 pixel higher light sensitivity back-side-illuminated BSIDAVIS) for neural activity recordings with fluorescent calcium indicators both in brain slices and awake mice. The DVS activity responds to the fast dynamics of neural activity, indicating that a sensor combining SDAVIS and BSIDAVIS technologies would be beneficial for long-term in-vivo neural recording using calcium indicators as well as potentially faster voltage indicators.

**Keywords**—Neural imaging, high-speed imaging, fluorescence microscopy, calcium imaging, dynamic vision sensor (DVS), dynamic and active pixel vision sensor (DAVIS), event-based, spike-based, neuromorphic engineering, silicon retina

## I. INTRODUCTION

To study the brain and understand its complexity, it is important to measure neuronal activity in living animals, ranging from single synaptic events to firing patterns across millions of neurons. Consequently, neuroscientists are developing technologies able to measure neural activity with increasing spatio-temporal resolution and in larger tissue volumes, where the numbers of recorded neurons has doubled approximately every 7 years [1].

Optical imaging is especially promising as it allows chronic recordings of activity patterns in large populations of cells with fluorescent indicators. Several types of fluorescent markers have been engineered to measure the dynamics of neurons at the level of single spike resolution [2], [3]. They exploit physiological mechanisms that take place during an action potential, such as vesicle release, changes in neurotransmitter concentration, transmembrane voltage and intracellular calcium dynamics. Among those, calcium imaging is the most common modality for recording neural activity, allowing studies of

neural dynamics, coding, dendritic processing, and synaptic function. During rest, the calcium concentration is maintained at a low level inside cells. When an action potential occurs, calcium ions rapidly enter the cell and can bind intracellularly to genetically engineered fluorescent protein indicators, leading to a change in fluorescence signal.

The resulting temporal contrast in fluorescence relative to baseline ( $\Delta F/F$ ) can reach  $>20\%$  for a single action potential. The fluorescent signal lasts typically several 100ms. Thus the events reported by calcium indicators have a fast rise time and a slow decay time. The experiments reported here used GCaMP6f, a widely used fast and sensitive genetically encoded calcium indicator, that can measure action potentials in single cells with high reliability [2].

To image large populations of neurons, the simplest approach is to excite and record fluorescence across field-of-views (FOVs) of 0.5 to 10mm (“wide-field imaging”), covering tens to thousands of active cells. For this purpose, scientific CMOS (sCMOS) cameras are typically used, with the disadvantage of producing huge amounts of data (several Terabytes per day for typical experiments) that needs to be stored and processed with of powerful hardware.

Dynamic vision sensors (DVS) and their later improvements [5] could play an important role in wide-field imaging by enabling long term recording at a much lower data rate. The DVS can report changes in brightness with temporal precision down to the sub-millisecond range with a wide dynamic range ( $>100\text{dB}$ ). Each pixel responds asynchronously to log intensity changes and its information is transferred at the time it occurs. The result is a stream of digital pixel address, following the address-event representation (AER) protocol [6]. DVS respond only to changes, thus reducing temporal redundancy at the focal plane by selectively reporting active cells. The high temporal resolution, the wide dynamic range and the reduction in stored data make the DVS sensor an ideal candidate for neural imaging applications.

Feasibility studies of the application of event-based sensor in neural imaging was first reported in a mock experiment in [7] and in in-vitro slices in [8] (currently under review). This paper improves upon [8], by comparing the SDAVIS used in [8] with a previously unpublished back-side-illuminated BSIDAVIS sensor (see next section). We demonstrate recordings from neurons expressing GCaMP6f in brain slices and in awake mice, using a cranial window preparation allowing optical access to the brain.

## II. THE SDAVIS AND BSIDAVIS SENSORS

The Dynamic and Active Pixel Vision Sensor, **DAVIS** [9], combines the DVS pixel circuit with an active pixel sensor (**APS**) circuit sharing the same photodiode. This way, the DAVIS allows the simultaneous output of asynchronous events, from DVS, and synchronous frames, from APS. The DVS output can be used to detect fast changes in the fluorescent signal from the neurons. Each pixel responds to positive and negative changes in brightness by generating positive ON events or negative OFF events. The APS output allows recognition of the imaged cells, adjusting the focus and measuring the exposure.

Here, two improved versions of the DAVIS sensor have been investigated: the Sensitive Dynamic and Active Pixel Vision Sensor (**SDAVIS**) [8], and the Back Side Illuminated Dynamic and Active Pixel Vision Sensor (**BSIDAVIS**).

Table I. compares key specifications of these sensors with the sCMOS camera. Both DAVIS are fabricated in Towerjazz 180nm 6M CMOS image sensor technology that provides antireflection coatings, large microlenses (for front illuminated sensors), and buried photodiodes with optimized dark current.

SDAVIS increases the sensitivity for temporal contrast compared to the previous DAVIS [9], through the adoption of a photoreceptor preamplification stage [10]. Contrast sensitivity is improved from 10% down to 1% for OFF events and 3.5% for ON events at sufficient illumination level, giving a more detailed image. A smaller intra-scene dynamic range, is mitigated by a digital operating point control circuit capable of extending the overall dynamic range beyond 100dB, as in [9]. However, circuit limitations in the current version of SDAVIS limit operation at low light intensities, resulting in a higher minimum threshold of 13% at the experimental illumination and in higher number of noise events.

Conventional front illuminated sensors have quantum efficiency (**QE**) that is limited by the photodiode fill factor and metal stacking. This limitation is severe in DVS sensors with their complex pixel circuits. In back-side-illumination (**BSI**), the imaging wafer is flipped and bonded to a carrier wafer. The imaging wafer is thinned and passivated. Now light illuminates the back of the original wafer, thus potentially increasing the QE to nearly 100%. The BSIDAVIS was processed by imec for BSI after Towerjazz wafer fabrication. The starting silicon had 18 $\mu$ m-thick p-epi and it was thinned to 18 $\mu$ m.

Fig.1 compares the measured quantum efficiencies of SDAVIS and BSIDAVIS. The QE below 350nm is probably reduced by 0.55mm borosilicate cover glass absorption. The BSI processing increases the QE by a factor of about four. This feature makes the BSI sensor more suitable for applications in microscopy, where the amount of fluorescence light is at most  $10^{-6}$  of the incident light. The QE measured for the BSIDAVIS is comparable to the 80% QE @ 525nm for the sCMOS sensor used in the experiments.

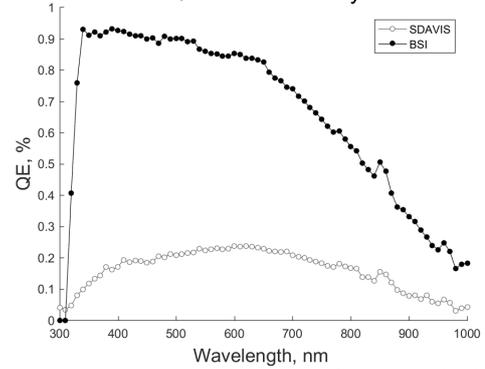


Fig.1 Quantum Efficiency (QE) spectrum of SDAVIS and BSIDAVIS

TABLE I. SENSORS SPECIFICATIONS

	sCMOS <sup>a</sup>	SDAVIS	BSIDAVIS
Array size (mm <sup>2</sup> )	13.3x13.3	3.6x3.5	6.4x4.8
Pixel size ( $\mu$ m <sup>2</sup> )	6.5x6.5	18.5x18.5	18.5x18.5
#pixels	2048x2048	188x180	346x260
Camera power (W)	70	3	3
Interface	CamLink	USB3.0	
DVS dynamic range(dB)	91.36	110	110
Readout time (ms)	10	0.02	0.02
QE @525nm	80	21.5	89.1
Conversion gain (e <sup>-</sup> /s)	NA	23	23
APS dark signal (e <sup>-</sup> /s) <sup>b</sup>	0.06	16k	18k
Min event threshold at 1mW/m <sup>2</sup> focal plane illumination (%) <sup>c</sup>	NA	13	19
Background DVS activity rate (Hz/pixel) <sup>c</sup>	NA	5.4	0.06
Readout noise, APS (e <sup>-</sup> RMS)	1.4	61	54.7

<sup>a</sup>. Hamamatsu Orca Flash 4.0 V2

<sup>b</sup>. sCMOS is actively cooled to  $-10^{\circ}$ C. DVS dark current is smaller by unknown amount due to readout path not having extra transistors

<sup>c</sup>. Inferred from experimental conditions

In the following experiments, we measured background sensor focal plane irradiance<sup>1</sup> of 800/400ph/ $\mu$ m<sup>2</sup>/s = 0.3/0.15mW/m<sup>2</sup> in the in-vitro/in-vivo experiments. Thus if we take 600ph/ $\mu$ m<sup>2</sup>/s as typical exposure rate, we see that the pixel exposure signal is about 180k e<sup>-</sup>/s, which is about 10 times the APS dark current signal from Table I.

The dark background of the neural image produces sufficient light to overwhelm the considerable DVS dark signal that dwarfs the negligible dark signal of the actively cooled sCMOS.

## III. WIDE-FIELD IMAGING IN BRAIN SLICES

To test the capabilities of the DVS for functional recordings in neuronal tissue, we started with an in vitro approach by virally expressing the calcium indicator GCaMP6f in hippocampal organotypic cultures<sup>2</sup>.

The DAVIS sensors were connected to an upright microscope (Axioskop FS, Zeiss) using a 40x NA0.8 water dipping objective. A 50/50 splitter allowed simultaneous

<sup>1</sup> Irradiances in-vitro/in-vivo experiments were measured using DAVIS APS output (exposure 44/40 ms), QE, conversion gain, and DN value (171/89 [V])

<sup>2</sup> Animal procedures follow the Guidelines for Laboratory Animals of the University of Zurich, approved by the Cantonal Veterinary Office.

imaging with the DAVIS and the sCMOS cameras. The fluorophores were excited using a 470nm LED and the emission light was bandpass-filtered (525/39nm). Bicuculline perfusion blocked inhibitory receptors to increase excitability.

The spontaneous action potentials from the imaged neurons were recorded. The BSIDAVIS and the SDAVIS both have capability of responding to the neural activity from individual neuron cell bodies present in the scene.

Fig.2 compares snapshots of 100ms bins of DVS activity in the BSIDAVIS (Fig.2A) and SDAVIS (Fig.2B). The SDAVIS reaches 11 events per pixel in the most active area while the BSIDAVIS reaches only 1 event per pixel. This difference in response is from the lower event threshold possible in SDAVIS, which makes it also more susceptible to noise. The background in the SDAVIS is noisier than the BSIDAVIS, where the image is visually cleaner and free of noise events coming from the non-active areas of the scene. This BSIDAVIS works better at low level of illumination, due to the higher QE. In the SDAVIS the dark background is out of the intrascene dynamic range which results in the pixels exposed to be noisy and self-oscillate [8], resulting in uninformative data that is difficult to filter.

Analysis of noise background events in the two sensors shows that the SDAVIS produced 5.4events/pixel/s while the BSIDAVIS produced only 0.03events/pixel/s. For this experiment, the event thresholds were set to 13.3% for the SDAVIS and 60% for the BSIDAVIS.

Fig.3 compares from three Regions of Interest (ROIs), the 30ms bins of BSIDAVIS events (blue traces), with  $\Delta F/F_0$  (black traces).  $\Delta F/F_0$  is the APS gray scale value [DN, Digital Number] normalized with respect to the baseline rest state DN of the cell. Fig.3A&B show that the BSIDAVIS is clearly able to follow the fast onset dynamics of the selected ROIs when  $\Delta F/F_0 > 0.05$ . The fast transients are correlated with the onsets of fluorescence, while the slow decay time courses of the calcium transients are not visible. For  $\Delta F/F_0 < 0.05$  (Fig.3C) the correlation is less clear and the noise is higher. However, the derivative of  $\Delta F/F_0$  of the same ROI, (Fig.3D) clearly shows the capability of the sensor in detecting the fast changes in fluorescent.

The BSIDAVIS allows an easier recognition of the spiking cells than with SDAVIS, and 30ms bins are enough to identify the ROIs. The SDAVIS needs 100ms bins for the ROIs to be visually recognizable from the background noise.

#### IV. IN-VIVO MOUSE BRAIN WIDE-FIELD IMAGING

The in-vitro results encouraged us to move to an in vivo setting by trying to record functional signals in a living mouse brain. Imaging in vivo is challenging due to the highly scattering tissue and the need for a cranial window to optically access the surface of the brain. In addition, the microanatomy of the brain with neurons, blood vessels and supporting cells densely packed in a small volume means that no single imaging plane can adequately sample the 3D cell population in a single brain area. Luckily, the sparse activity patterns of neurons in the superficial layers of the brain (Layer 2/3) mean that while single pixels correspond to cell bodies and dendrites of many

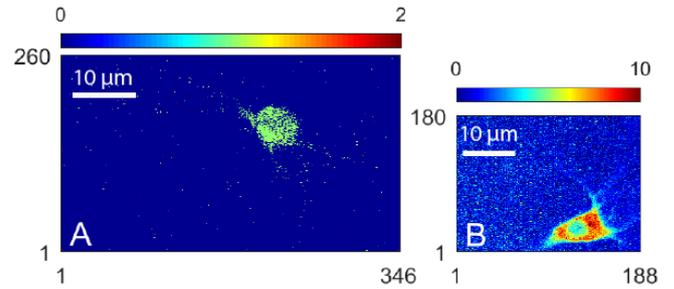


Fig.2 Comparison between the BSIDAVIS DVS output (346x260) (A) and the SDAVIS DVS output (188x180) (B) events count per pixels in 100ms, imaged cell size about 10 $\mu$ m.

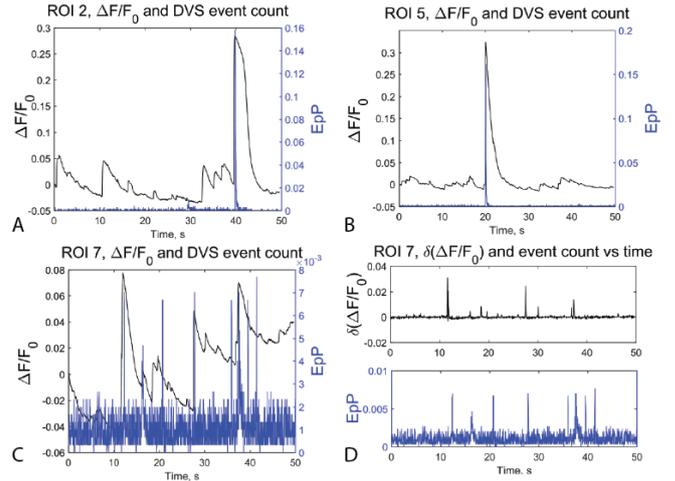


Fig.3 (A-B-C) Synchronized  $\Delta F/F_0$  of several ROIs from the Orca camera aligned in time with the BSIDAVIS 30ms binned event count per pixel (EpP). (D) Discrete difference derivative  $\delta(\Delta F/F_0)$  (ROI 7) compared with the BSIDAVIS 30ms bins

different cells, these neurons are unlikely to be coactive and the recorded calcium transients can be assigned to a single neuron. The very low baseline fluorescence of GCaMP6 is also beneficial as inactive cells are indistinguishable from the background signal.

The BSIDAVIS sensor was used to record neural activity through a 4.4mm window of a mouse genetically engineered to express the calcium indicator GCaMP6f in excitatory cells in layer 2/3 throughout the neocortex [11] (Fig.4A). The window was implanted above primary and supplementary motor areas (M1 and M2). The DAVIS was mounted on a custom wide-field fluorescence microscope with a 16x NA0.8 Nikon objective and a 135mm tube lens leading to 10.8x magnification. For overview images, a 4x NA0.28 objective was used. The filter set was similar to the in vitro experiments. The 470nm LED radiant power was about 10mW. The recordings were done using both the APS and the DVS signals from the BSIDAVIS. Fig.4B shows DAVIS APS and DVS binned image 50ms for different times. Only a single cell is spiking at each time. The DVS output shows only the active cells at different time, reducing the amount of data.

Fig.5 shows the results from the cell a) highlighted in Fig.4B of 50ms time bins of DVS activity (ON and OFF events) compared to the BSIDAVIS APS value, normalized respect to baseline. The event threshold was 27% (ON and

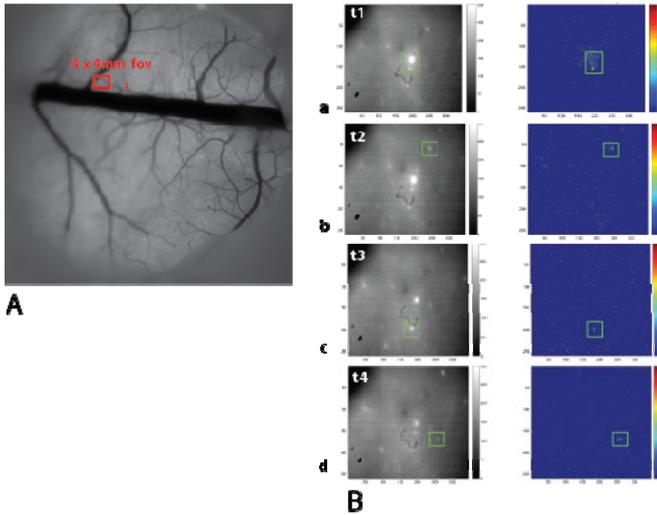


Fig.4. **A**: Wide-field overview image with Hamamatsu Orca V2 sCMOS camera, **B**: BSIDAVIS APS image and DVS events 50ms bins, FOV 4.4mm. Dark spots and worm shape are from contamination. The white spots are neurons that were active in this time period.

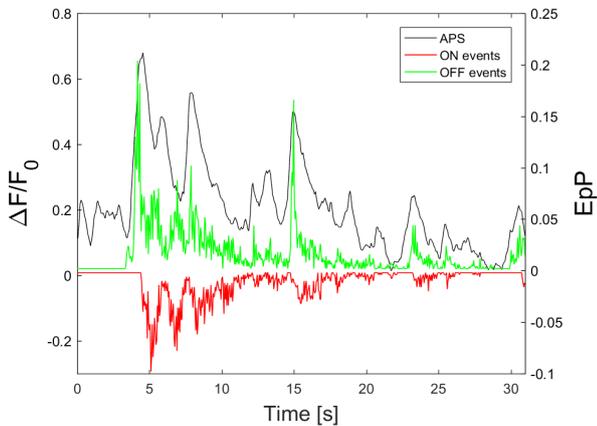


Fig.5 From Fig.4B cell a):  $\Delta F/F_0$  respect to baseline, (black line), aligned in time with the DVS 50ms bins Event count per Pixel, ON events (green line) and OFF events (red line). OFF events are plotted as negative

OFF events), comparable to [9]. The BSIDAVIS can clearly follow the fast ON and OFF dynamics of the neurons. The average event rate for this recording was 0.11/0.03Hz/pixel before/after filtering out uncorrelated events.

## V. CONCLUSION

DAVIS sensors have promising features for application in neural imaging due to its high temporal resolution that cannot be beaten by frame-based imager. They are capable of following the fast transient dynamics of spiking neurons with millisecond onsets, but fail to respond to the slow dynamics of the calcium indicator due to the GCaMP6f dynamic. The experiments performed with SDAVIS and BSIDAVIS suggest the development of a sensor that combines their main advantages. The SDAVIS has lower event threshold (13% in the experiments) and acceptable signal quality for fast spiking neurons [8] but a large amount of noise at low intensity. The

BSIDAVIS increases the QE to 89%, improving the performance in dark conditions and reducing the background noise, but has higher minimum event threshold. These experiments point out a clear path for large-format scientific DVS of combining SDAVIS and BSIDAVIS with additional modifications to reduce background leak event rate [12].

DVSs may be particularly suitable candidates for imaging with faster indicators, such as genetically encoded voltage indicators (GEVI). These have latencies below 0.2ms, allowing studies of voltage dynamics in cellular compartments, resolution of individual spikes in fast spike trains, and precise estimates of spike timing. Although current GEVIs have a much lower temporal contrast of typically  $\Delta F/F_0$  below 2% [8], upcoming technologies could result in sub-millisecond GEVI dynamics and contrasts exceeding 50%/100mV [3].

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## REFERENCES

- [1] I. H. Stevenson and K. P. Kording, "How advances in neural recording affect data analysis," *Nat. Neurosci.*, vol. 14, no. 2, pp. 139–142, Feb. 2011.
- [2] T.-W. Chen *et al.*, "Ultra-sensitive fluorescent proteins for imaging neuronal activity," *Nature*, vol. 499, no. 7458, pp. 295–300, Jul. 2013.
- [3] Y. Gong *et al.*, "High-speed recording of neural spikes in awake mice and flies with a fluorescent voltage sensor," *Science*, vol. 350, no. 6266, pp. 1361–1366, Dec. 2015.
- [4] B. F. Grewe, D. Langer, H. Kasper, B. M. Kampa, and F. Helmchen, "High-speed in vivo calcium imaging reveals neuronal network activity with near-millisecond precision," *Nat. Methods*, vol. 7, no. 5, pp. 399–405, May 2010.
- [5] P. Lichtsteiner, C. Posch, and T. Delbruck, "A 128 x 128 120 dB 15  $\mu$ s Latency Asynchronous Temporal Contrast Vision Sensor," *IEEE J. Solid-State Circuits*, vol. 43, no. 2, pp. 566–576, 2008.
- [6] S.-C. Liu, T. Delbruck, G. Indiveri, A. Whatley, and R. Douglas, Eds., *Event-Based Neuromorphic Systems*. John Wiley and Sons Ltd., UK, 2015.
- [7] M. Yang, S.-C. Liu, and T. Delbruck, "A Dynamic Vision Sensor With 1% Temporal Contrast Sensitivity and In-Pixel Asynchronous Delta Modulator for Event Encoding," *IEEE J. Solid-State Circuits*, vol. 50, no. 9, pp. 1–12, Sep. 2015.
- [8] D. P. Moesy and *et al.*, "A Sensitive Dynamic and Active Pixel Vision Sensor for Color or Neural Imaging Applications," *IEEE Trans. Biomed. Circuits Syst.*, submitted 2017.
- [9] C. Brandli, R. Berner, M. Yang, S.-C. Liu, and T. Delbruck, "A 240x180 130 dB 3  $\mu$ s Latency Global Shutter Spatiotemporal Vision Sensor," *IEEE J. Solid-State Circuits*, vol. 49, no. 10, pp. 2333–2341, Oct. 2014.
- [10] T. Serrano-Gotarredona and B. Linares-Barranco, "A 128 x 128 1.5% Contrast Sensitivity 0.9% FPN 3  $\mu$ s Latency 4 mW Asynchronous Frame-Free Dynamic Vision Sensor Using Transimpedance Preamplifiers," *IEEE J. Solid-State Circuits*, vol. 48, no. 3, pp. 827–838, Mar. 2013.
- [11] L. Madisen *et al.*, "Transgenic Mice for Intersectional Targeting of Neural Sensors and Effectors with High Specificity and Performance," *Neuron*, vol. 85, no. 5, pp. 942–958, Mar. 2015.
- [12] B. Son *et al.*, "A 640x480 Dynamic Vision Sensor with a 9 $\mu$ m Pixel and 300Meps Address-Event Representation," in *2017 International Solid State Circuits Conference (ISSCC) Tech. Dig.*, San Francisco, CA, USA, p. 4.1.