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Some of the images in Fig. 9(b) are the same as Fig. 9(a) by our mistake. Figure 9(b) should be replaced by the figure below (color online). The discussions and conclusions are not altered by this correction.
Implantable self-reset CMOS image sensor and its application to hemodynamic response detection in living mouse brain

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A self-reset pixel of 15 x 15 µm² with high signal-to-noise ratio (effective peak SNR &gt;60 dB) for an implantable image sensor has been developed for intrinsic signal detection arising from hemodynamic responses in a living mouse brain. For detecting local conversion between oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) in brain tissues, an implantable imaging device was fabricated with our newly designed self-reset image sensor and orange light-emitting diodes (LEDs; λ = 605 nm). We demonstrated imaging of hemodynamic responses in the sensory cortical area accompanied by forelimb stimulation of a living mouse. The implantable imaging device for intrinsic signal detection is expected to be a powerful tool to measure brain activities in living animals used in behavioral analysis.

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1. Introduction

Neuronal activity measurement of the brain with high spatial and temporal resolutions in freely moving mice is expected to lead to a large step forward for revealing brain functions.1–3 Implantable devices for electrical4,5) or optical6,7) measurements enable the monitoring of brain neuronal activity of awake mice or rats. Optical approaches are advantageous over electrophysiological approaches in terms of high spatial and temporal resolutions and simultaneous spatial and temporal measurements.8) Recently, we have been developing implantable optical-imaging devices based on CMOS technology.9–11) Small-size and light-weight features of miniaturized CMOS imaging devices, as shown in Fig. 1, were expected to enable the observation of behavior-induced neuronal activity without disrupting freely moving mice.

In optical neuronal activity measurement, injection of fluorescent indicators12) or genetic engineering approaches13–15) are necessary in most cases. Although optical approaches of measuring neuronal activity are advantageous, the complex experimental procedures of fluorescent labelling sometimes limit their applicability. Intrinsic signals of hemodynamic responses, such as blood flow16) and optical absorption changes of brain tissues arising from local conversion between oxyhemoglobin (HbO) and deoxyhemoglobin (HbR),17,18) are generally observed around excited neurons. A local conversion between HbO and HbR is induced by neuronal activity, and optical absorption of brain tissues changes because of the difference in the absorption spectrum between HbO and HbR. Intrinsic signal detection is a promising approach to observing brain activity without the need for complicate experimental procedures of labelling. However, intrinsic signal detection is not straightforward since optical absorption changes in the presence of orange light (λ = 605 nm) were only 1% or less.18) Although intrinsic signals that show relatively large changes, such as blood flow changes,19) have been detected using implantable imaging devices, small signal changes such as the optical absorption changes arising from local conversion between HbO and HbR have not yet detected. To measure such small signal changes of less than 1%, an implantable image sensor should have a signal-to-noise ratio (SNR) of ~60 dB.

To realize intrinsic signal detection using an implantable image sensor, we developed a high-SNR image sensor with the self-reset function.20) The self-reset function enables the collection of images with a wide dynamic range of light intensity and high SNR. In addition, a self-reset image sensor is considered to be advantageous over other wide-dynamic-range sensors21–23) for detecting small changes in signals, such as intrinsic signals, because it can maintain its sensitivity in a wide range. The self-reset function increases the effective full-well capacity, which ordinarily determines SNR by recycling the well.24) Thus, the self-reset image sensor enables imaging in a wide dynamic range of light intensity with its sensitivity maintained at any range. A previously reported self-reset sensor achieved a peak SNR of nearly 60 dB at a very high light intensity, but the sensor consumed a relatively high power compared with another implantable sensor.20) Large power consumption generates heats, which damages biological tissues. Therefore, this serious problem should be solved for the self-reset sensor to be applicable to intrinsic signal detection in the brain of living animals.

In this study, we developed an implantable self-reset CMOS image sensor with reduced power consumption and heat generation. The architecture of a self-reset pixel of the low-power-consumption type was reported in a conference proceedings.25) We have developed the implantable self-reset...
CMOS image sensor and applied it to measuring brain functions. Intrinsic signal detection of hemodynamic responses was demonstrated in the primary sensory cortical area representing the forelimbs related to sense stimulation using our newly developed image sensor.

2. Experimental methods

2.1 Self-reset CMOS image sensor

2.1.1 Architecture of self-reset pixels.

A pixel with the self-reset function realizes a high dynamic range of light intensity and a high effective peak SNR by preventing the saturation of its in-pixel capacitance. The basic concept of the architecture of the self-reset pixels for an implantable image sensor was previously reported. Figure 2(a) shows a schematic diagram of the developed self-resetting pixel. The potential of the photodiode \( V_{PD} \) drops linearly with the intensity of illuminating light from the resetting voltage \( V_{rst} \) owing to the photoelectric conversion at the photodiode. The self-reset pixel resets \( V_{PD} \) when the \( V_{PD} \) becomes lower than the threshold voltage \( (V_{TH_{RST}}) \) through the Schmitt trigger inverter. The external resetting \( (\theta_{EXRST}) \) resets \( V_{PD} \) at the start of every frame. Figures 2(b), 2(c), and Table I show the pixel layout, a micrograph, and specifications of the self-reset image sensor, respectively. The pixel size of \( 15 \times 15 \mu m^2 \) was small enough to observe narrow blood vessels or cell bodies by contact imaging. A fine pitch of pixels is required for an implantable image sensor, because pixel pitch greatly influences the spatial resolution in contact imaging. The self-reset image sensor chip was fabricated using 0.35-\( \mu m \) 2-poly 4-metal standard CMOS technology.

2.1.2 Performance analysis of self-reset pixels.

To show the dynamic range of light intensity of the self-reset pixels, we measured the output signal and noise levels of the self-reset image sensor as functions of illuminating light intensity. The self-reset image sensor was illuminated with a uniform beam light using a green light-emitting diode (LED; Stanley Electric UG5305S, \( \lambda = 525 \text{ nm} \)). Images were taken at a frame rate of 11.3 frames/s and processed using a 14-bit analog-to-digital converter. The images of 1,024 frames at each illuminating light intensity were obtained to calculate the average output signal and noise levels.

2.2 Detecting intrinsic signals of hemodynamic responses in living mouse brain

2.2.1 Fabrication of an implantable imaging device with a self-reset image sensor.

To detect intrinsic signals in a living mouse brain using the self-reset image sensor, we developed an implantable imaging device. Figure 3 shows a photograph of the implantable imaging device with the self-reset image sensor and LEDs as light sources. The self-reset image sensor (length, 3000 \( \mu m \); width, 1050 \( \mu m \); and thickness, 650 \( \mu m \)) and four orange LEDs (Rohm SML-P12DT, \( \lambda = 605 \text{ nm} \)) were fixed using epoxy (Epoxy Technology EPOTEK 730) onto a flexible polyimide circuit substrate and electrically connected via Al wires by a bonder (West Bond 7700CP). Parylene C was vapor-deposited at 2 \( \mu m \) thickness to cover the entire device for water proofing using a parylene-coating chamber (Specialty Coating Systems PDS 2010).

2.2.2 Normal mode and self-resetting mode of a self-reset CMOS image sensor.

The normal mode is used to obtain images using the self-reset CMOS image sensor at a light intensity lower than that triggering self-resetting in any pixels. Using this normal mode, the self-reset CMOS image sensor was illuminated with a uniform beam light using a green light-emitting diode (LED; Stanley Electric UG5305S, \( \lambda = 525 \text{ nm} \)). Images were taken at a frame rate of 11.3 frames/s and processed using a 14-bit analog-to-digital converter. The images of 1,024 frames at each illuminating light intensity were obtained to calculate the average output signal and noise levels.
sensor can also be used as a normal image sensor without self-resetting. The self-resetting mode is used to obtain images at a light intensity higher than that triggering pixel self-resetting. Thus, the self-resetting mode enables imaging in a wide dynamic range of light intensity.

2.2.3 Image processing for reconstruction of original images from resetting-acquired images. To obtain original light intensity, reset counts were obtained under the same condition as those of the experiments of intrinsic signal detection, before performing the experiments. Using the obtained reset counts, original light intensity and images were reconstructed by calculating the sum of raw signal value and the multiplication of reset counts at each self-reset pixel. Reset counts were determined by gradually increasing the irradiation light intensity of LEDs from zero to light intensities appropriate for performing animal experiments, such as intrinsic signal detection. Animal experiments were performed after this counting process.

2.2.4 Animal preparation for intrinsic signal detection. All animal procedures conformed to the animal care and experimentation guidelines of the Nara Institute of Science and Technology. Device implantation procedures were performed as previously reported as follows. Urethane (1.0 g/kg) was intraperitoneally injected into an adult mouse (C57BL6, wild type) for anesthetization. The mouse head was fixed using a stereotaxic instrument (Narishige SR-5M). The abdomen of the mouse was kept warm with a heater. The skull was drilled and removed centering on the primary sensory cortical area representing the forelimbs. In this experiment, the dura was not removed to prevent injury and hemorrhage. The implantable imaging device was placed onto the brain surface. Figure 4(a) shows a schematic image of the experiment for intrinsic signal detection using the implantable imaging device. The imaging area shown in Figs. 4(b) and 4(c) corresponds to the primary sensory cortical area representing the forelimbs. Finally, to detect intrinsic signals in the sensory cortical area, electrical pulses (pulse width, 1 ms; pulse interval, 250 ms; amplitude, 1 mA; 12 shots in one stimulation) were applied to the forelimb or hindlimb of the mouse for stimulation using an electronic stimulator (Nihon Kohden SEN-3301) and an isolator (Nihon Kohden SS-202J). The implantable imaging device was connected to a controller and a personal computer (PC) to obtain images from the self-reset image sensor. The sensor was driven at a frame rate of 40.6 frames/s in experiments of intrinsic signal detection.

3. Results and discussion

3.1 Improvement of power consumption of the present self-reset pixel
The architecture of the present self-reset pixel described in this paper was designed to reduce power consumption compared with the previously reported architecture of self-reset pixel. Lowering the power consumption of image sensors is one of the most important issues of implantable devices for biomedical applications from the viewpoint of saving power supply from batteries and minimizing heat-induced damage to biological tissues. One difference between the present architecture of self-reset pixel and the previously reported architecture is the power supply voltage of the Schmitt trigger inverter ($V_{STI}$). Figure 5 shows the result of SPICE simulation of the power consumption of the Schmitt trigger circuit as a function of $V_{STI}$. The previously reported pixel, which uses 3.3 V for $V_{STI}$, consumed approximately 40 µW/pixel on average in the Schmitt trigger circuit. It is
mainly due to the shoot-through current that occurred under the transitional condition of the self-resetting. Figure 5 indicates that the power consumption decreases exponentially with decreasing $V_{\text{STI}}$. $V_{\text{STI}}$ was set as 1.7 V in the newly designed architecture of the self-reset pixel. The newly designed self-reset pixel was estimated to consume approximately 0.15 $\mu$W/pixel on average. Moreover, the current limiter $M_{\text{CL}}$ was added to the resetting circuit to reduce the peak resetting current $I_{\text{MB}}$. As a result, the average power consumption of the newly designed sensor was 20 mW, whereas that of the previously reported self-reset image sensor was 185 mW.20) A power consumption reduction by nearly 90% was achieved, which was a major improvement for the newly designed architecture of self-reset pixel.

### 3.2 Improvement of SNR of the present self-reset pixel

Figure 6(a) shows the level of output signals of the self-reset image sensor as a function of illuminating light intensity. Figure 6(a) shows that the level of output signals were proportional to the light intensity until the signal level reaches the reset level, and after self-resetting occurred in the pixels, the signal level increases again. The signal level can be reconstructed by adding the multiplication of reset counts by the magnitude of signal swing. The reconstructed signal level in Fig. 6(a) was proportional to the light intensity even after self-resetting. Therefore, the original light intensity profiles can be estimated from the reset counts. In the present self-reset image sensor, a reset counter was not integrated to reduce the circuit area in pixel. Thus, post-data processing was used to estimate original light intensity profiles, as described in Sect. 2.

As shown in Fig. 6(a), the noise level was almost constant for light intensities under $10^{-6}$ W/cm$^2$. After the first self-resetting at a light intensity of approximately $2 \times 10^{-6}$ W/cm$^2$, the noise level increased nearly twice that before the self-resetting. This increase originated from the additional reset noise generated by self-resetting, and this reset noise was added at each self-resetting. After the first self-resetting, noise level increased almost proportionally to the square root of light intensity. In this region, the photon shot noise was considered to be dominant.

Figure 6(b) shows the comparison of effective SNR between the present and previous self-reset image sensors.20) The effective SNR was calculated by dividing the reconstructed signal level by the noise level. Light intensity was converted to light energy using frame rate to compare the SNR at the same photon number per frame in Fig. 6(b). The SNR of the present self-reset image sensor is lower than that of the previous self-reset image sensor for light energy at a less than the $10^{-8}$ J/cm$^2$, because the sensitivity of the present pixel was decreased by the lower fill factor of the pixel. In the region of approximately $10^{-7}$ J/cm$^2$, the SNRs of the two sensors are almost the same. After self-resetting, the SNR of the present self-reset image sensor is approximately 5 dB higher than that of the previous self-reset image sensor. The effective peak SNR was estimated to be 64 dB. The improvement of SNR was considered to be achieved owing to less heat generation resulting from the decreased power consumption. The present self-reset image sensor achieved an SNR of over 60 dB at an illuminating light energy of approximately $2 \times 10^{-6}$ J/cm$^2$.

### 3.3 Reconstruction of original intensity profiles from the resetting-acquired images

Figure 7(a) shows an image of the brain surface of the mouse obtained using the self-reset image sensor. In Fig. 7(a), stripe patterns indicate the self-reset boundaries.
and originate from the difference in the reset count between the each imaging area. From the image data shown in Fig. 7(a), the image of original image profiles was reconstructed using a reset count map [Fig. 7(b)]. Figure 7(c) shows the reconstructed image with original intensity profiles. A fine image with original intensity profiles was acquired as shown in Fig. 7(c), which corresponds to the microscopy image shown in Fig. 4(c).

3.4 Comparison between normal mode and self-resetting mode for intrinsic signal detection

We compared the SNR in the intrinsic signal detection between the images taken in the normal mode and self-resetting mode. In the normal mode, the intensity of illuminating light was limited to not exceed the original dynamic range of the self-reset sensor. In this mode, the self-reset function can be ignored, and the sensor worked as a normal 3-Tr APS image sensor. Figures 8(a) and 8(b) show obtained intrinsic signals ($\Delta I/I$) related to the conversion between HbO and HbR after the stimulation of the forelimb measured in the normal mode and self-resetting mode, respectively. Approximately 1% changes of $\Delta I/I$ appeared 3–4 s after the stimulation in both modes. Although the noise level exceeded half of the signal change in the normal mode [Fig. 8(a)], the noise level was suppressed in the self-resetting mode [Fig. 8(b)] and this signal change level can easily be detected by a simple method such as setting a threshold value. Note that this data was obtained from a single image without data processing to improve SNR, such as integrating multiple images.

3.5 Intrinsic signal imaging of hemodynamic responses in self-resetting mode

Finally, to confirm whether the detected signals were the responses of brain activity, we compared the images of signals detected at the primary sensory cortical area representing the forelimbs following forelimb and hindlimb stimulation. Figures 9(a) and 9(b) show time-series images of the brain surface obtained using the self-reset image sensor following the stimulation of the forelimb and hindlimb, respectively. A signal change ($\Delta I/I$) of over 0.4% was detected in entire imaging area 4 s after the forelimb stimulation, as shown in Fig. 9(a). In particular, the top area of the images shows relatively larger responses than other areas. In contrast, almost no signal change was detected at any time in Fig. 9(b) after the hindlimb stimulation, except for the small response in a bold blood vessel in the image 3 s
after the stimulation. Consequently, the signal change was detected at the primary sensory cortical area representing the forelimbs only during the stimulation of the forelimbs of the mouse. The detected signal changes in the brain were considered to be intrinsic signals arising from the hemodynamic responses of the conversion between HbO and HbR in a living mouse brain. Thus, it is considered that the intrinsic signals related to brain functions were successfully observed using the implantable self-reset imaging device.

4. Conclusions

A self-reset CMOS image sensor with high SNR was developed for intrinsic signal imaging in a living mouse brain. The pixel of the sensor resets itself when each in-pixel circuit detects the voltage of the pixel capacitance exceeding some threshold value. The newly designed self-reset pixel showed improved power consumption and SNR compared with the previously reported sensor. Improvements in terms of a power consumption reduction by nearly 90% and a SNR of approximately 5 dB were attained. The effective peak SNR was 64 dB. The implantable imaging device was fabricated with the self-reset image sensor and illumination source orange LEDs for brain functional imaging. Intrinsic signal detection in a living mouse brain was demonstrated. Intrinsic signals of hemodynamic responses were clearly observed in the primary sensory cortical area as the responses to forelimb stimulation. The implantable self-reset CMOS image sensor is expected to be used for detection of relatively weak signals in the brain, such as intrinsic signals, with high SNR.

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Fig. 9. (Color online) Time-series images (1 s interval) of the brain surface after the stimulation. The change in rate was overlaid in red and blue. Images were averaged over 10 trials to reduce artifacts arising from physiological responses. (a) The right frontal limb was stimulated at 0 s. Signal change were detected in the entire imaging area at 4 s. Almost no signal changes were detected at the pixels on self-reset boundaries owing to artifacts of the image processing. (b) The right hindlimb was stimulated at 0 s. Almost no signal changes were detected in the entire imaging area at any time.