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A Multimodal Sensing Device for Fluorescence Imaging and Electrical Potential Measurement of Neural Activities in a Mouse Deep Brain

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***Abstract*—We have developed a multimodal CMOS sensing device to detect fluorescence image and electrical potential for neural activities in a mouse deep brain. The device consists of CMOS image sensor with on-chip electrodes and excitation light sources, all of which are integrated on a polyimide substrate. The novel feature of this device is its embedded on-chip electrodes which are partially transmit incident light so that the whole image can be acquired by the sensor. We have demonstrated the CMOS sensor device successfully operates in hippocampus area of an anesthetized mouse.**

I. Introduction

Neural activities in the deep brain are very important for learning and memory. To study the activities *in vivo* by using small animals such as a mouse, we face on difficulties of observing such activities with the present measurement tools such as fMRI (functional magnetic resonance imaging), PET (positron emission tomography) and NIRS (near infrared spectrometer). To address this issue, several devices have been reported [1], [2], but it remains still difficult to observe neural activities in deep brain region of mouse brain by using these devices.

We have developed a new CMOS-based imaging device with spatio-temporal resolution of sub-mm and sub-second and successfully demonstrated to monitor the time course of serine protease activities inside the mouse hippocampus [3]-[5]. Recently, we have succeeded in monitoring neural activities in the deep brain of a freely-moving mouse [6].

In this paper, we have demonstrated a novel type of a multi-modal CMOS-based device to simultaneously sense fluorescence and electrical potential in neural activities. Although our previously developed devices in [3]-[5] have functions with image sensing and recording electrical potentials, the two functions are separately integrated on one chip. Also, the captured image is not whole image, because the areas where the electrodes are placed are lack of pixels of the image sensor. We address this issues that the whole image can be obtained even if the electrodes are placed on the imaging area [8]. In the new device, the two functions are merged in one device, so that the image is not disturbed by the embedded electrodes on a chip.

In section II, we describe the structure and fabrication process of the device in detail. In the next section, we demonstrate the preliminary experimental results of the fabricated device including *in vitro* and *in vivo* experiments. Finally, future issues are addressed.

II. Device Specifications

A. Image sensor chip

The device consists of a CMOS sensor chip with embedded electrodes and an LED for excitation of fluorescence, all of which are placed on a polyimide substrate. The CMOS sensor chip is fabricated in a standard 0.35 μm CMOS process technology. The chip specification is described in Table 1.

TABLE 1: SPECIFICATION OF SENSOR CHIP.

CMOS chip		Technology	0.35μm 2P 4M CMOS
		Operating voltage (V)	3.3
		Chip size (μm^2)	1300 x 3846 (max)
	Pixel	Array size (μm^2)	450 x 2310 (max)
		Type	3-T Active Pixel Sensor
		Photodiode	Nwell - Psub
		Pixel size (μm^2)	7.5 x 7.5
		Fill factor (%)	35
		Number	13852
	Electrode	Size (μm^2)	90 x 90
		Type	Al Pad*
		Number	10
Mounted LED		Size (μm^2)	300 x 300
		Color	Blue
		Number	1
On-chip filter		Thickness	~ 2μm
		Color	Green

* Metal is removed over photodiode area.

The microphotograph of the chip is shown in Fig. 1. The length of the chip is about 3.8 mm to acquire fluorescence image in a mouse hippocampus area located at 2.5 - 3.5 mm in depth from the brain surface. The outline of the sensor chip is delineated as a shank-shape for smooth insertion to the brain tissue like the Michigan Silicon probe [7].

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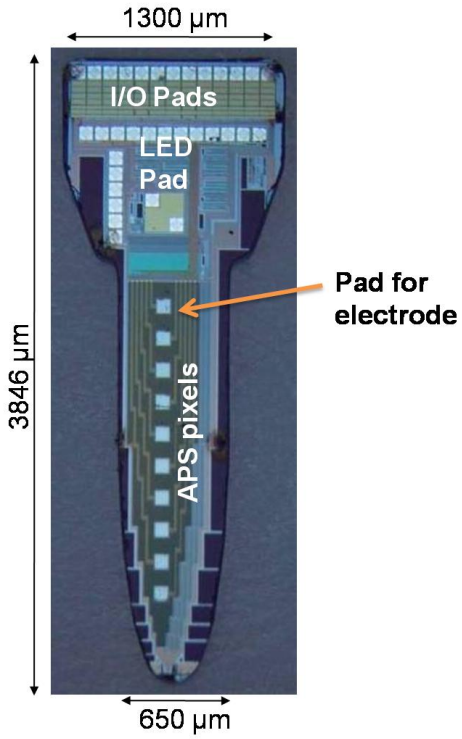

Fig. 1: Microphotograph of the sensor chip.

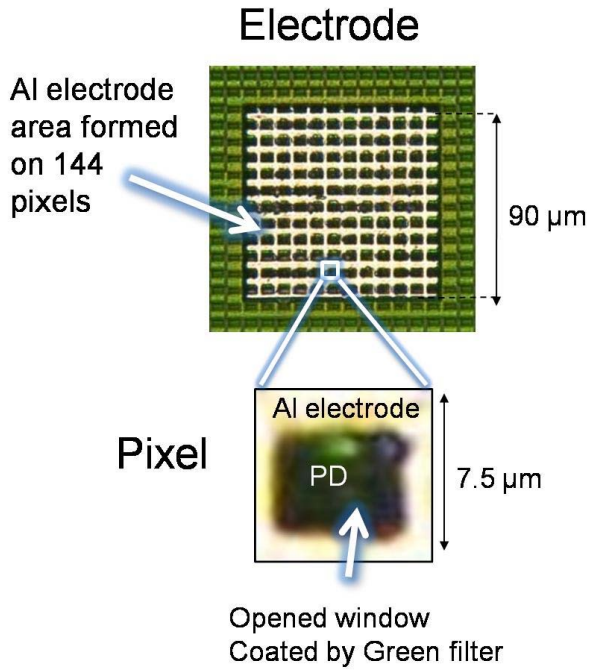

Fig. 2: Unit electrode area. Al was formed on the top of the pixels in the electrode area. The Al was removed only on the top of the PD. The PD surface was coated with Green filter to selectively transmitted fluorescence light.

The pixel design is the same as our previous device [3]-[5], except for the pixels the top of which Al is formed. This area is used as an electrode as shown in Fig. 2. The top Al layer is used as the base of the electrode. Layers of biocompatible metals such as Au, Pt, or W are deposited on the Al electrodes.

Figure 2 shows the photograph of the electrode, where Au was deposited on the Al pads. In this study, we used an Al electrode in the basic experiment to investigate the simultaneous multifunction of the CMOS sensor device. The windows are opened over the photodiode area so that the pixel under the electrode can detect incident light. There are 10 electrodes on the pixel array and unit electrode has 12 × 12 windows over the photodiode area.

Figure 3 shows an image taken by this sensor. Because the perimeter of the unit electrode is light-shielded as a marker under the experimental trials, the 10 square frames corresponding to the rim of electrodes appear in the captured image.

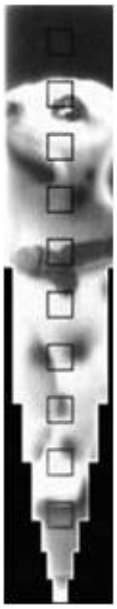

Fig. 3: Image taken by the sensor chip.

B. Device structure and fabrication process

The CMOS sensor device consists of an image sensor to detect fluorescence, an LED to excite fluorescence, a color filter resist transmitting fluorescence selectively to the pixel array, and electrodes to sense change in electrical potential in mouse brain. As shown in Fig. 1, the sensor chip is shaped like a shank, and it is packaged with water-resistant and biocompatible materials.

The fabrication process of the device is as follows. The Al layer was deposited on the back of the sensor chip as a metal mask and etched in the shape of a shank by deep reactive ion etching. A 300 µm × 300 µm LED was flip-chip bonded on the CMOS sensor chip using an Au bump. The mounted LED is shown in Fig. 4.

Then, the chip was bonded on a flexible polyimide substrate using epoxy resin. A 2-µm-thick green filter resist was spin coated onto only the pixel array. I/O pads were connected to the polyimide substrate using Al wires. Then, the polyimide substrate was also cut in the shape of a shank using an excimer laser. The entire device was coated with

parylene for water-resistance and biocompatibility. The green filter resist and parylene resin on the electrode were selectively removed by using a green laser. A photograph of the fabricated device is shown in Fig. 5.

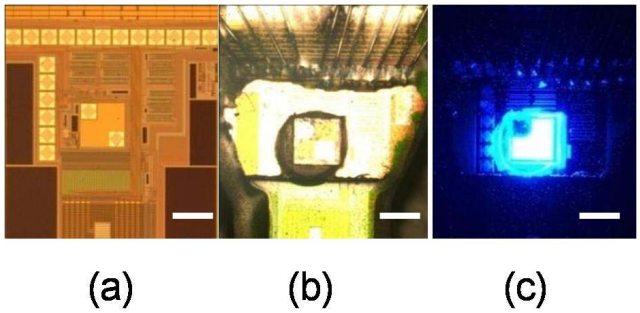

Fig. 4: Photographs of mounted LEDs on the CMOS chip. (a) the mounted area in the CMOS chip, (b) the mounted LED on the chip, and (c) LED with emitted blue light.

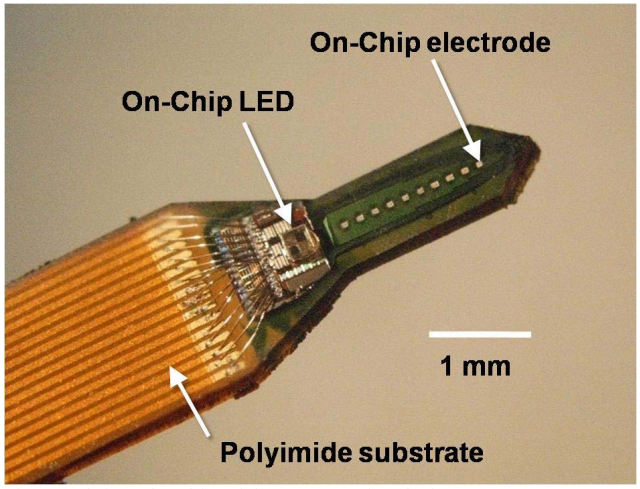

Fig. 5: Fabricated multimodal CMOS sensing device.

III. Experimental Results

A. *In vitro experiment*

To demonstrate the simultaneous imaging of fluorescence and electric potential in the same region, we performed an experiment using fluorescent beads and a brain phantom. In this experiment, the green filter resist was coated only on the photodiodes in the electrodes. The brain phantom was prepared using 6.6% skim milk mixed uniformly in 1% agarose gel. The properties of the phantom are similar to those of brain tissue. First, the pixel array was covered with fluorescent beads in 10-µm diameter. The peak excitation wavelength and peak emitting wavelength of the beads were 441 nm and 486 nm, respectively. The fluorescence bead solution (Fluoresbrite® YG Microspheres 0.10 µm, Polysciences, Inc.) was covered with the pixel array. The bead solution on the CMOS image sensor was then heated by a hot plate. After the solvent evaporated completely, the beads can be imaged using the CMOS image sensor. Next, the brain phantom was placed over the sensor array. A sine wave signal with an amplitude of 20 mV was applied to the brain phantom. Then, real-time simultaneous sensing of fluorescence and electric potential was performed.

The experimental result is shown in the Fig. 6. Figure 6 (a) shows the captured image of the excited fluorescence beads on the pixel array. Figure 6 (b) shows a magnified view of the fluorescent beads in the unit electrode. The square frame indicates the shielded area of the photodiodes. Each 10-µm bead was successfully imaged. The resolution of the imaged beads on the pixels was approximately 7.5 µm × 7.5 µm so that the imaging quality is in agreement with the imaging result. Figure 6 (c) shows the result of the sensing of the electric potential of the brain phantom. We used three different settings for the frame rate of the image sensor—no imaging operation, operations with 2 fps, and 4 fps. The output signals of the electric potential became noisy with an increase in the frame rate.

This noise may originate from the switching noise from scanner circuits and may be suppressed by changing timing of the operation for imaging and electrical sensing. Although the input signal level is larger than field potential signal level in neural activities, this result encourages us to realize the simultaneous detection of fluorescence imaging and electrical potential.

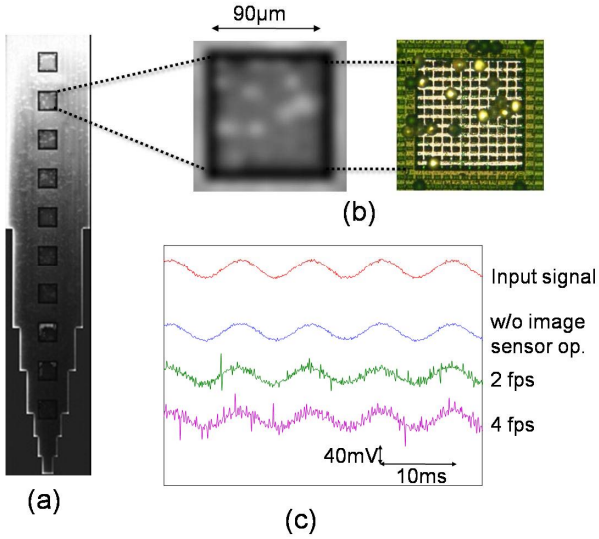

Fig. 6: *In vitro* experimental results of simultaneous detection of image and electrical potential. Fluorescent beads are placed on the device. (a) shows the captured image and (b) shows its magnified image of one pixel, and (c) shows the electrical detection results.

B. *In vivo experiment*

We implanted the fabricated CMOS sensor device into the hippocampus of an anesthetized mouse to demonstrate *in vivo* imaging in the deep brain. The CMOS sensor device was fully coated with a parylene layer. All the experiments were carried out in accordance with the Institutional Guidelines of the Nara Institute of Science and Technology. The results of the experiment are shown in Fig. 7. Figure 7 (a) shows images captured during the insertion of the device into the brain cortex. As the surface of the brain is covered

with a fluid that scatters light onto the pixel array, a bright area appears at the center of the captured image. Figure 7 (b) shows images captured by the device, the end of which was implanted in the depth of 4.5-4.6 mm from the brain surface. In the region, the some portion of the pixel array was implanted in the hippocampus. Areas in the electrodes were bright, implying that the photodiodes in the electrode sensed the light intensity through the windows of the electrode.

This result has demonstrated that the parylene-coated device can operate effectively, and that the photodiodes under the electrode can sense the light intensity in the hippocampus of an anesthetized mouse. After the *in vivo* experiment, the CMOS sensor device was found to operate normally, and the mouse remained alive.

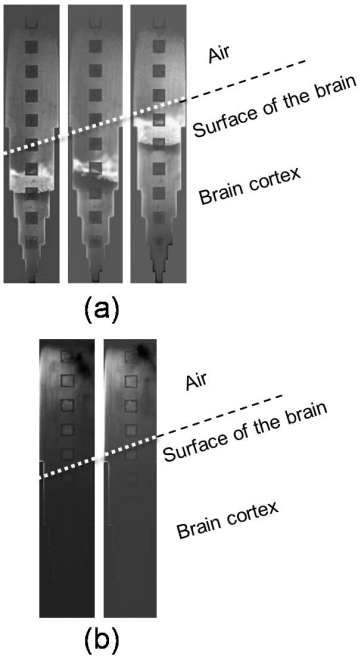

Fig. 7: Images taken by the CMOS sensor device which implanted in the mouse brain. In (a), the device is inserted just into the surface of the brain cortex, and in (b), it is inserted into the hippocampus region.

IV. Conclusion

We have developed a multimodal CMOS sensing device to detect fluorescence image and electrical potential for neural activities in a mouse deep brain. The device has successfully been demonstrated the simultaneous operation of imaging and sensing electronic signals under *in vitro* environment. Also the device is implanted in the deep brain of an anesthetized mouse and captures images.

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