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An *in vitro* demonstration of CMOS-based optoelectronic neural interface device for optogenetics

T. Tokuda, *Member, IEEE*, S. Nakajima, Y Maezawa, T. Noda,

K. Sasagawa, *Member, IEEE*, Y. Ishikawa, S. Shiosaka, and J. Ohta, *Member, IEEE*

***Abstract*— A CMOS-based neural interface device equipped with an integrated micro light source array for optogenetics was fabricated and demonstrated. A GaInN LED array formed on sapphire substrate was successfully assembled with a multifunctional CMOS image sensor that is capable of on-chip current injection. We demonstrated a functionality of light stimulation onto ChR2-expressed cells in an *in vitro* experiment. A ChR2-expressed cell were successfully stimulated with the light emitted from the fabricated device.**

I. Introduction

Optogenetics is a technology with which we can introduce light-sensitivity onto cells with a help of genetics [1-3]. In the last decade, various methodologies have been proposed and huge amount of research activities have been carried out. Channelrhodopsin (ChR2) is one of the major proteins used in optogenetics. ChR2 is a light-activated anion channel protein which is sensitive to light with wavelength of 470 nm.

For *in vivo* optogenetics including freely moving situations, light emitting diode (LED) array will be a promising device platform [4, 5]. To obtain light with wavelength of 470 nm, GaInN is the suitable material for LED. There are some reports that propose to use GaInN LED array as light stimulator applicable for optogenetics.

We have proposed and demonstrated a CMOS-based optoelectronic neural interface device with an integrated LED array for on-chip stimulation [6, 7]. Figure 1 shows the concept of the CMOS-based neural interface device. An LED array was integrated on a multifunctional CMOS image sensor with on-chip current injection capability. Taking an advantage of CMOS circuitry, multi-site patterned stimulation is available with this device architecture. This device architecture is expected to be a platform for a multi-medium neural interface device with electricity and light.

In this work, in order to obtain sufficient emission intensity for optogenetics, we improved light emission performance of the device. Then, we demonstrated the neural stimulation function in an *in vitro* experiment using ChR2- expressed cell cultured on the present device.

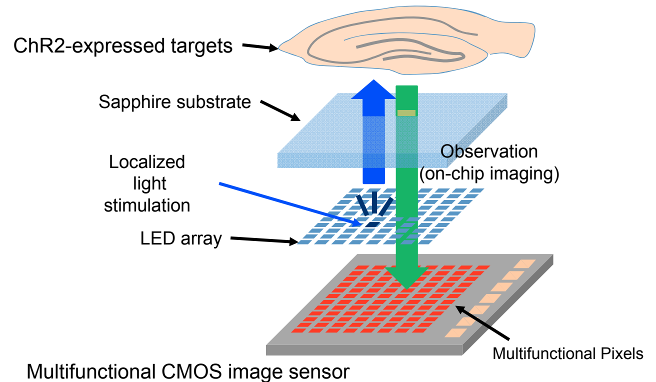

Figure 1. Concept of the CMOS-based optoelectronic neural interface device with an integrated LED array

II. Design and Fabrication of the CMOS-based Neural Interface Device with an Integrated LED Array

The CMOS-based optoelectronic neural interface device consists of two semiconductor chips that are bonded in face-to-face manner.

The base chip is the multifunctional CMOS image sensor chip made of Si. Based on a conventional CMOS image sensor equipped with 3-transistor active pixel sensor as light sensing circuitry, we implemented a capability of addressable current injection (and electric sensing) capability [8, 9]. Figure 2 shows (a) layout, (b) block diagram of the multifunctional CMOS image sensor. Table I shows specifications of the multi- functional CMOS image sensor chip.

The CMOS sensor was fabricated with 0.35 µm 2-poly, 4-metal standard CMOS technology. The size of the light-sensing pixel was 7.5 µm × 7.5 µm. An electrode array for current injection to LEDs was formed on the pixel array using top metal layer of the CMOS sensor chip. The on-chip electrodes were exposed to establish connection to electrodes on the LED array.

As shown in Fig. 2 (b), an addressable current injection line with a scanner ("Select-Scanner" in Fig. 2(b)) was implemented to operate the LED array. In our previous work, typical emission intensity for singe LED was as large as 7.5 µW, which corresponds to an illumination density of 200

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T. Tokuda, S. Nakajima, Y. Maezawa, T. Noda, K. Sasagawa, and J. Ohta are with Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, 630-0192, JAPAN (e-mail: tokuda@ms.naist.jp).

Y. Ishikawa, and S. Shiosaka are with Graduate School of Bioscience, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, 630-0192, JAPAN

T. Tokuda is also with JST-PRESTO, Japan Science and Technology Agency, 3-5, Sanbancho, Chiyodaku, Tokyo, JAPAN 102-0075

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μW/mm² [6]. In this work, to obtain a larger stimulation intensity that covers most of the optogenetics experiments (~10mW/mm²), we partly adopted a larger operation voltage (5.0 V) for current injection circuits than the previous design (3.3 V). We also reduced series resistance of the current injection lines using larger transistors and refined circuits.

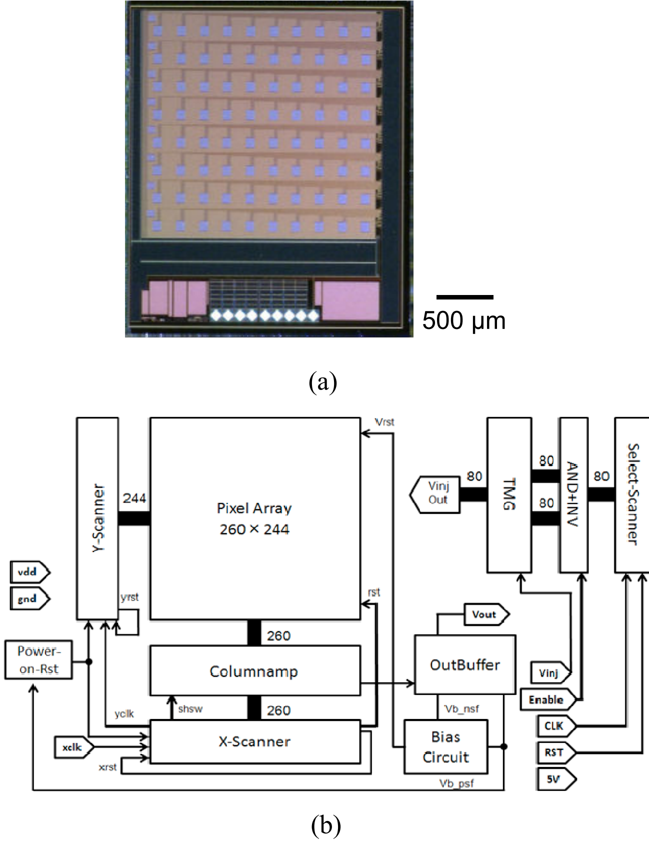


Figure 2. (a) Layout and (b) block diagram of the multifunctional CMOS image sensor

TABLE I. SPECIFICATIONS OF THE MULTIFUNCTIONAL CMOS IMAGE SENSOR CHIP

Technology	0.35 μm 2-poly 4-metal Standard CMOS
Chip size	2200 μm × 2500 μm
Array size	260 × 244
Pixel size	7.5 μm × 7.5 μm
Pixel type	3-Transistor APS
LED electrodes	8 × 10
Operation voltage	3.3 V (Imaging), 5.0 V (LED drive)

Figure 3(a) shows an outlook of the LED array wafer. The GaInN array formed on a sapphire substrate was used as the micro light source array for light stimulation. The emission peak wavelength is typically 470 nm, which matches the sensitivity peak of ChR2. Size of single LED is 192 μm × 225 μm, and the LED wafer was diced into 8 × 10 LED array. The LEDs were formed monolithically on the sapphire substrate and n-type layer of the pn junction was commonly connected for all the LEDs. Therefore, we can operate any one of the LEDs by injecting current from one of the p-type electrodes on the LED array. Since both the LED layer and sapphire substrate are transparent, we can perform on-chip optical imaging using the imaging function of the multifunctional CMOS image sensor.

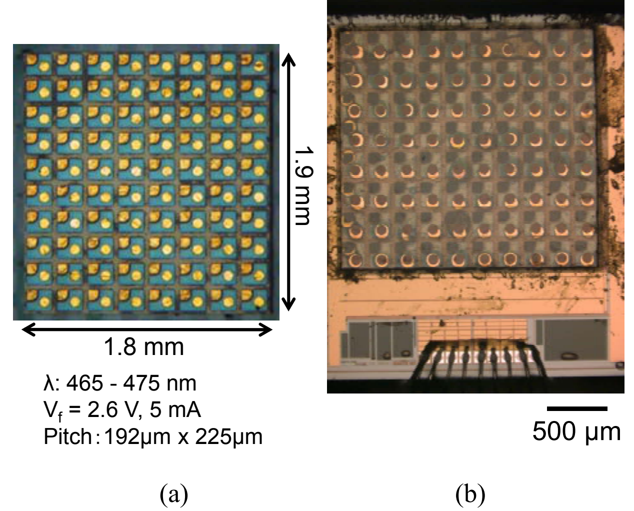


Figure 3. (a) Outlook of the LED array wafer and (b) assembled device.

We bonded the CMOS sensor chip and the GaInN LED array chip using flip-chip bonding technique which is commonly used in semiconductor fabrication process. We used anisotropic conducting paste (ACP) for the bonding with gold bumps. Figure 3(b) shows an outlook of the bonded chips.

III. FUNCTIONAL EVALUATION OF ON-CHIP LIGHT STIMULATION CAPABILITY

Figure 4 shows emission power of single LED as a function of injected current. Owing to the higher operation voltage and refined circuitry, an emission power of 2 mW (~48 mW/mm²) was obtained. This value is significantly larger than our previous device [6]. This illumination density covers most of reported conditions of optogenetic experiments. This result suggests that the present device architecture is a promising deice platform for *in vitro* and *in vivo* optogenetics.

The present device has only one current injection line for LED operation. However, we can use the select scanner to perform quasi-simultaneous two-dimensional LED operation. Figure 5 shows a two-dimensional patterned light emission (showing "NAIST") observed with (a) an external microscope, and (b) the device itself. We successfully performed the two-dimensional patterned emission. Furthermore, it was confirmed that LED operation can be clearly monitored using the capability of optical imaging. This function is an advantage to monitor the light stimulation especially *in vivo* applications.

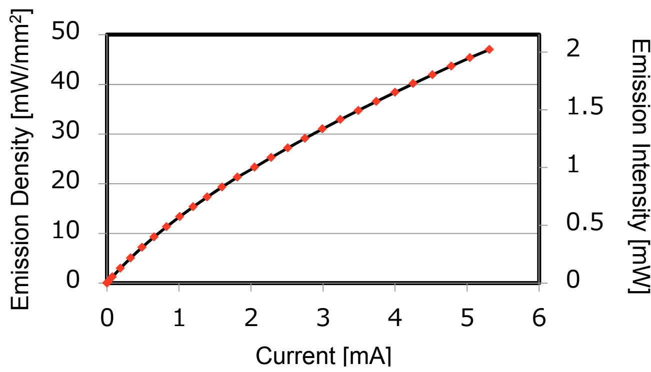


Figure 4. Emission power of single LED as a function of current.

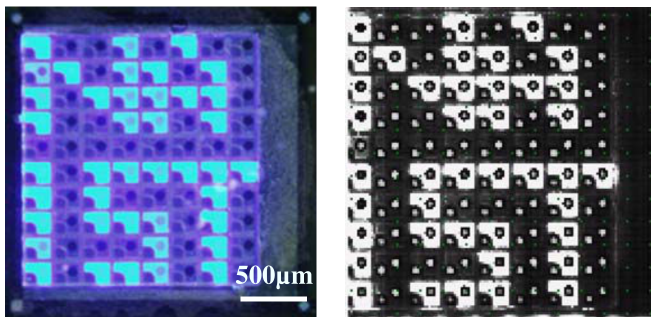


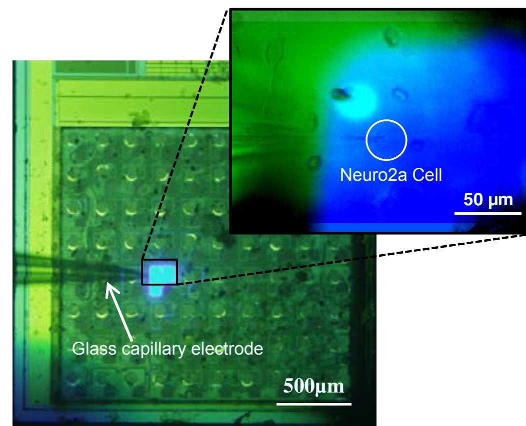
Figure 5. Two-dimensional patterned light emission (showing "NAIST") observed with (a) an external microscope, and (b) the present device

IV. *IN VITRO* DEMONSTRATION OF THE CMOS-BASED OPTOELECTRONIC NEURAL INTERFACE DEVICE

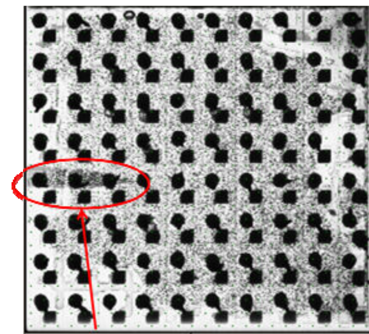
In order to demonstrate the applicability of the present optoelectronic neural interface device for optogenetics, we performed cell culturing and an *in vitro* light stimulation experiment. We used Neuro2a cell which originates in mouse neuroblastoma for the experiments. The surface of the present device is the polished back surface of the sapphire substrate. In order to enhance cell attachment onto the device surface, we treated the surface with poly-l-Lysine for more than 24h. Then we seeded Neuro2a cells. We performed a transfection using a plasmid with ChR2. After 24~48h, we performed the *in vitro* light stimulation experiment.

We observed the cell cultured on the device using patch clamp technique (voltage-clump mode). Figure 6 shows experimental setup observed with (a) an external microscope, and (b) the present device. Although the cells cultured on the device cannot be identified clearly in Fig. 6(b), the position and movement of the glass capillary can be monitored using the imaging function of the present device.

Figure 7 shows typical response of the channel current evoked by the light stimulation from the present neural interface device. A clear increase of the channel current was observed coincidently to the light stimulation. This result suggests the feasibility of the present device for optogenetics.



(a)



(b)

Figure 6. Situation of the *in vitro* experiment observed with (a) an external microscope, and (b) the presene device

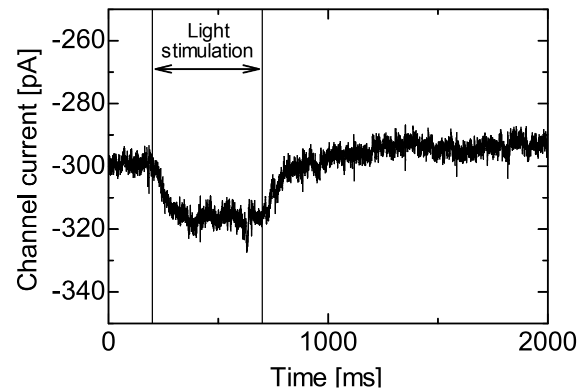


Figure 7. Channel current observed during the light stimulation

V. CONCLUSIONS

The CMOS-based neural interface device with an integrated LED array was improved and emission intensity compatible with most of optogenetic experiments was obtained. We also performed an *in vitro* experiment using cultured cell to show the capability of the neural stimulation. We successfully observed the response in channel current

evoked by the light stimulation from the present optoelectronic neural interface device.

We are working on an improvement of the imaging performance of the device, and a development of *in vivo* version of the device that can be used for living animals.

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