34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

A CMOS-based on-chip neural interface device equipped with integrated LED array for optogenetics

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***Abstract*— A novel CMOS-based neural interface device equipped with an integrated micro light source array was proposed and demonstrated. Target application of the device is optogenetics. GaInN LED array formed on sapphire substrate was successfully assembled with a multifunctional CMOS image sensor which is capable of injecting current via any of the pixel. We demonstrated addressable LED operation with the present device. The device has advantages such as simultaneous multi-site stimulation and on-chip optical imaging, that are not available with previously reported LED array device for optogenetics.**

I. Introduction

Optogenetics is a technology in 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 valuable 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.

Fiber-based light introduction [4, 5] is one of commonly-used methods to perform light stimulation. It is compatible with *in vivo* experiments with freely-moving animals. However, the spatial resolution of light stimulation is limited and localized stimulation including two- dimensional patterned stimulation is difficult with the fiber- based technology. On the other hand, from a viewpoint of high-resolution localized light stimulation, microscope- based technologies [6, 7] are promising especially for *in vitro* experimental applications.

For *in vivo* optogenetics including freely moving situations, light emitting diode (LED) array will be a promising device platform [8, 9]. 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 on-chip light stimulator applicable for optogenetics. Since LED is two-terminal semiconductor device that can be fabricated in an array, one can operate any LED in an array taking X-Y matrix access scheme [8, 9].

In this work, we propose to use a multifunctional CMOS image sensor with on-chip current injection capability to drive an GaInN LED array. Figure 1 shows the concept of the CMOS-based neural interface device with an integrated LED array.

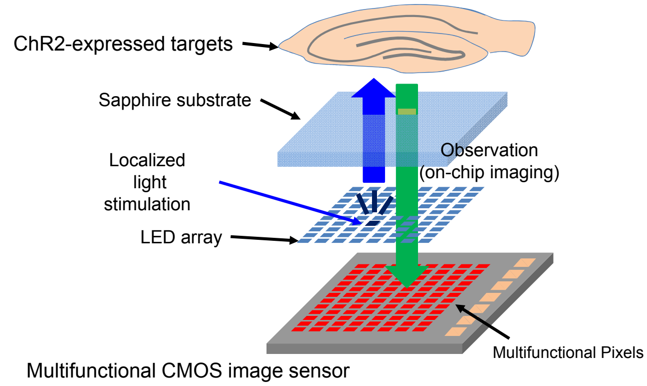

Figure 1. Concept of the CMOS-based neural interface device with an integrated LED array

Taking an advantage of CMOS circuitry, multi-site patterned stimulation is available with this device architecture. Furthermore, on-chip imaging capability is also available. This device architecture is expected to be a platform for a multi-medium neural interface device with electricity and light.

In this report, we present the concept, structure and fabrication of the neural interface device, including a design of the multifunctional CMOS image sensor. We also demonstrate the functionality of the localized light stimulation with the fabricated device.

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

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

A. Design of multifunctional CMOS image sensor chip

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 [10, 11]. Figure 2 shows (a) layout, (b) block diagram of the multifunctional CMOS image sensor, and (c) schematic of the multifunctional pixel circuit. Table I shows specifications of the multi-functional CMOS image sensor chip.

*Resrach supported by JST-PRESTO.

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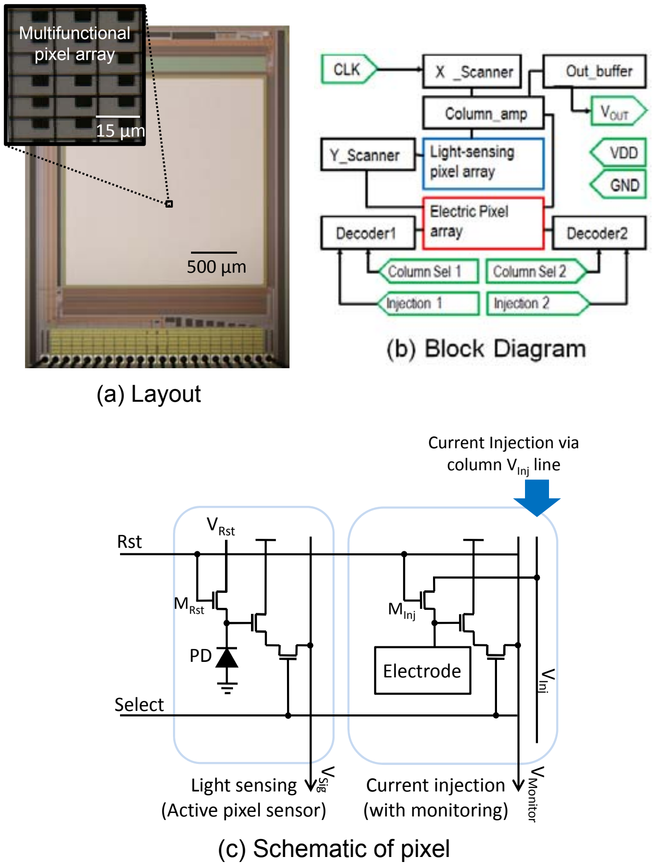


Figure 2. (a) Layout, (b) block diagram of the multifunctional CMOS image sensor, and (c) schematic of the multifunctional pixel circuit

TABLE I. SPECIFICATIONS OF THE MULTIFUNCTIONAL CMOS IMAGE SENSOR CHIP

Technology	0.35 µm 2-poly 4-metal Standard CMOS
Chip size	2236 µm ×3171 µm
Array size	128×268
Pixel size	15 µm×7.5 µm
Pixel type	3-Transistor APS
Operation voltage	3.3 V

The CMOS sensor was fabricated using 0.35 µm 2-poly, 4-metal standard CMOS technology. The size of the multifunctional pixel was 15 µm × 7.5 µm. A current injection electrode (pixel electrode) was formed with a top metal layer in each pixel. The pixel electrodes were exposed to establish connection to electrodes on the LED array wafer.

As shown in Fig. 2 (b) and (c), two selectable columnar current injection circuits were implemented on the CMOS chip. We can use these columnar current injection lines to operate LEDs. In this chip, we used row selecting function (vertical scanner) of the light sensing function to select electrodes for current injection. Thus, to operate on-chip LED array, we set two columnar lines for current injection and activate external current sources synchronously to the row selection function of the chip.

B. Integration of LED array on the CMOS sensor chip

Figure 3 shows (a) outlook of the LED array wafer and (b) structure of the neural interface device. The GaInN array formed on sapphire substrate was used as the micro light source array for the 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 x 10 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.

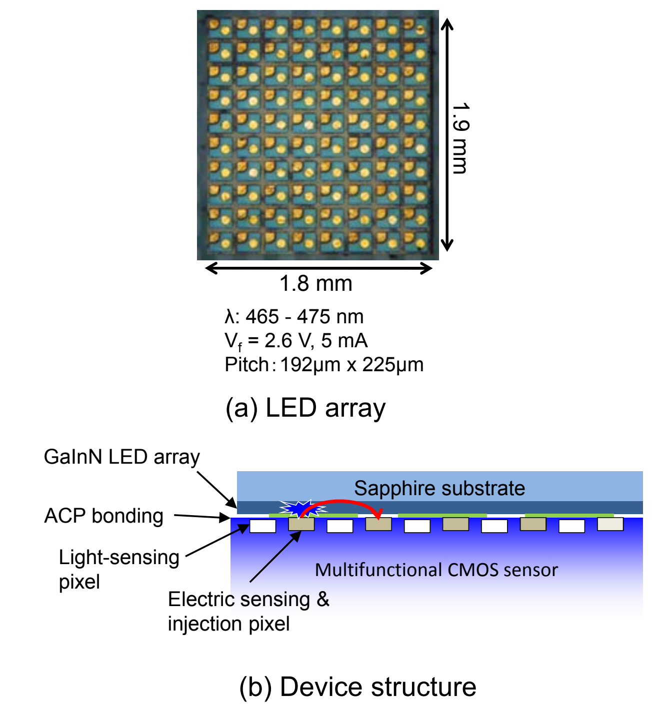


Figure 3. (a) Outlook of the LED array wafer and (b) structure of the neural interface device

We bonded the CMOS sensor chip and theGaInN LED chip using flip-chip bonding technique which is commonly used in semiconductor fabrication process. We used anisotropic conducting paste (ACP) for the bonding and we have succeeded to directly bond the two wafers without gold bumps. Figure 4 shows outlook of the bonded chips with a magnified image of the pixel array.

In Fig. 4, we can see pixel array through the LED array chip. This means that on-chip imaging is possible through the LED array chip. However, in order to utilize this imaging function for on-chip bioimaging, there are two important issues to overcome. Since anode and cathode electrodes on the LEDs are not transparent, the captured image includes the shadows of the electrodes (see Fig. 5 (b)). This shadow must be largely reduced or eliminated adopting a transparent material for the electrodes of LED. And, in order to realize on-chip fluorescent imaging, an optical filter layer should be implemented to suppress the signal caused by scattered excitation light.

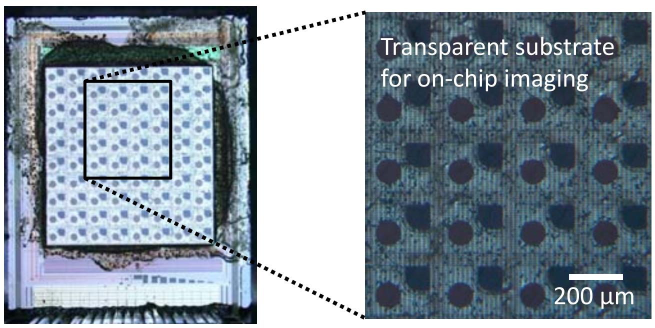
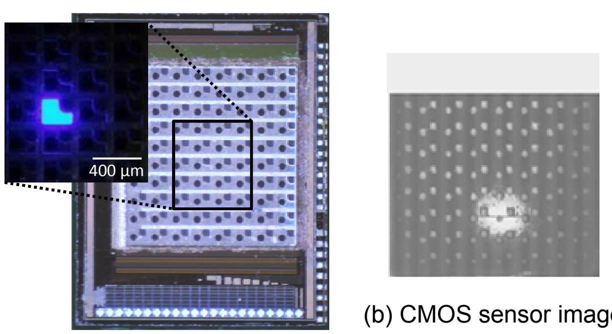


Figure 4. Outlook of the bonded CMOS and LED array chips

III. FUNCTIONAL EVALUATION AND DEMONSTRATION OF ON-CHIP LIGHT STIMULATION CAPABILITY

Figure 5 shows images of LED operation taken with (a) an external microscope and (b) the multifunctional CMOS image sensor. We confirmed the addressable LED operation as shown in Fig. 5 (a). Furthermore, it was confirmed that the on-chip optical imaging is available during the LED operation.

It means that we can use the on-chip optical imaging function to monitor the operation of the LED array. This unique feature is quite advantageous for *in vivo* light stimulation especially in freely-moving situations.



(b) CMOS sensor image

(a) LED operation

Figure 5. Images of LED operation taken with (a) an external microscope and (b) the multifunctional CMOS image sensor

In the present device, typical emission light intensity of 7.5 µW was obtained. This value approximately corresponds to 200µW/cm^2. Referring values reported in the literatures, the present device is applicable not all, but a part of neural stimulations in optogenetics. However, we have a clear idea to improve the emission power of the device. We have modified the current injection circuits in the multifunctional CMOS image sensor to be compatible with an operation voltage of 5 V. The result obtained with the new CMOS chip will be presented at the conference, too.

Not only single-site, but also multi-site stimulation is available with the present device. Figure 6 shows image of multi-site LED operation. Since we implemented two columnar current injection lines that can be operated independently, we can perform simultaneous dual-site stimulation. Combining columnar addressing and current source for LED operation that are operated synchronously to the row (vertical) scanner, we can perform two-dimensional patterned light stimulation. Two LEDs in the same row can be operated simultaneously and LEDs in different rows will be operated sequentially, as shown in Fig. 6.

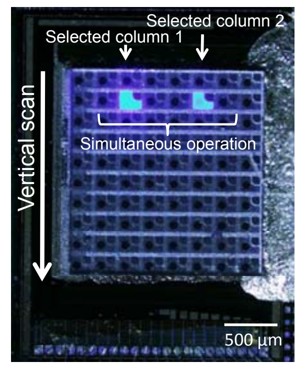


Figure 6. Multi-site LED operation performed by the present neural interface device

It should be noted that two LEDs for simultaneous dual-site stimulation should be in the same row in the current CMOS chip. However, this limitation is only due to the simple functional implementation that we take for this first CMOS chip. Refining the functional implementation, completely independent addressing can be realized. We have already designed another multifunctional CMOS image sensor with an improved addressing function, which is currently under evaluation.

Simultaneous multiple LED operation is not available with previously reported LED array device with simple X-Y matrix addressing scheme [8, 9]. Therefore, we expect this feature will be one of the largest advantages of the CMOS-based neural stimulator.

IV. CONCLUSION

A novel CMOS-based neural interface device with an integrated LED array was proposed and presented. Target application of the device is neural stimulation and observation in optogenetics. We combined a multifunctional CMOS image sensor which is capable of on-chip addressable current injection and GaInN LED array formed on sapphire substrate. LEDs in the array were successfully operated using the addressable current injection function of the multifunctional CMOS image sensor. We have also demonstrated simultaneous dual-site stimulation, which is the key feature of the present device.

ACKNOWLEDGMENT

This work was partially supported by the Japan Science and Technology Agency, Precursory Research for Embryonic Science and Technology (JST-PRESTO), and the VLSI

Design and Education Center (VDEC), the University of Tokyo in collaboration with Cadence Design Systems, Inc.

REFERENCES

[1] G. Nagel *et al.*, "Channelrhodopsin-2, a directly light-gated cation-selective membrane channel.," Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 24, pp. 13940-5, Nov. 2003.

[2] T. Ishizuka, M. Kakuda, R. Araki, and H. Yawo, "Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels.," Neuroscience research, vol. 54, no. 2, pp. 85-94, Feb. 2006.

[3] O. Yizhar, L. E. Fenno, T. J. Davidson, M. Mogri, and K. Deisseroth, "Optogenetics in neural systems.," Neuron, vol. 71, no. 1, pp. 9-34, Jul. 2011

[4] A. M. Aravanis *et al.*, "An optical neural interface: *in vivo* control of rodent motor cortex with integrated fiberoptic and optogenetic technology.," Journal of neural engineering, vol. 4, no. 3, pp. S143-56, Sep. 2007.

[5] D. Huber *et al.*, "Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice.," Nature, vol. 451, no. 7174, pp. 61-4, Jan. 2008.

[6] O. G. S. Ayling, T. C. Harrison, J. D. Boyd, A. Goroshkov, and T. H. Murphy, "Automated light-based mapping of motor cortex by photoactivation of channelrhodopsin-2 transgenic mice.," Nature methods, vol. 6, no. 3, pp. 219-24, Mar. 2009.

[7] K. Ueno, S. Sakai, T. Honjoh, and H. Yawo, "Patterned optogenetic activation of neurons and neural circuits: Multi-independent light stimulation system (MiLSS)," Neuroscience Research, vol. 71, p. e310, Sep. 2011.

[8] V. Poher *et al.*, "Micro-LED arrays: a tool for two-dimensional neuron stimulation," Journal of Physics D: Applied Physics, vol. 41, no. 9. p. 094014, May-2008.

[9] N. Grossman *et al.*, "Multi-site optical excitation using ChR2 and micro-LED array.," Journal of neural engineering, vol. 7, no. 1, p. 16004, Feb. 2010.

[10] T. Tokuda, A. Yamamoto, K. Kagawa, M. Nunoshita, and J. Ohta, "A CMOS image sensor with optical and potential dual imaging function for on-chip bioscientific applications," Sensors and Actuators A: Physical, vol. 125, no. 2, pp. 273-280, Jan. 2006.

[11] T. Tokuda, K. Tanaka, M. Matsuo, K. Kagawa, M. Nunoshita, and J. Ohta, "Optical and electrochemical dual-image CMOS sensor for on-chip biomolecular sensing applications," Sensors and Actuators A: Physical, vol. 135, no. 2, pp. 315-322, Apr. 2007.