CMOS On-Chip Optoelectronic Neural Interface Device with Integrated Light Source for Optogenetics

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Abstract. A novel optoelectronic neural interface device is proposed for target applications in optogenetics for neural science. The device consists of a light emitting diode (LED) array implemented on a CMOS image sensor for on-chip local light stimulation. In this study, we designed a suitable CMOS image sensor equipped with on-chip electrodes to drive the LEDs, and developed a device structure and packaging process for LED integration. The prototype device produced an illumination intensity of approximately 1 mW with a driving current of 2.0 mA, which is expected to be sufficient to activate channelrhodopsin (ChR2). We also demonstrated the functions of light stimulation and on-chip imaging using a brain slice from a mouse as a target sample.

1. Introduction
There have been drastic improvements in optogenetics in recent years [1-4]. It is a methodology that realizes optical stimulation of neural cells with the help of genetic modification. It provides a way to stimulate neural cells with good spatial selectivity and smaller invasion. Natural photosensitive protein plays an essential role in optogenetics. The protein works as a photosensitive ion channel on a cell membrane. It can be activated or deactivated by light with a specific wavelength and affects intracellular signaling.

Channelrhodopsin-2 (ChR2) is a photosensitive channel protein commonly used in optogenetics. ChR2 is activated by light with a wavelength of about 470 nm and shows a fast response to the light stimulation. It has been reported that light stimulation using appropriate illumination intensity and a frequency of around 20 – 50 Hz can evoke neural activity [4-6]. It has also been reported that the ChR2 protein does not disturb or change the physiological properties of modified neuron cells [7].

In our previous works, we demonstrated that our CMOS image sensor is capable of observing neural activity using an on-chip fluorescence imaging method [8,9]. In this work, we propose a new CMOS on-chip optoelectronic neural interface device, which integrates an array of light emitting diodes (LEDs) on our CMOS image sensor that are used as an addressable light source for optogenetically modified neural cells.
2. Design of CMOS image sensor chip for optoelectronic neural interface device

The CMOS image sensor is one of the primary parts of the proposed neural interface device. As reported in our previous work, this CMOS image sensor is capable of imaging the brain structure and activity in an on-chip configuration. The architecture of the CMOS image sensor consists of active pixel sensors as the pixel circuitry, analog signal processing circuitry, row and column selectors, and timing control circuitry [9]. The pixel substructure of our sensor uses a three-transistor type active pixel sensor (APS), which consists of a photodiode, select transistor switch, and reset transistor switch [10]. The photodiode is an important part of the APS and is used to change light into an electrical signal. The control and readout circuits for the sensor consist of a row selector at the left side of the pixel array and a column selector at the bottom of the pixel array. The row selector chooses the light sensing rows one-by-one, and the pixels in the selected row are connected to the column circuit via each column signal line. When the column selector is operated, the pixel signal levels in each column are read out consecutively [10-12].

| Table I. Specification of CMOS image sensor |
|--------------------------------------------|
| Technology       | 0.35-µm, 2-poly, 4-metal standard CMOS process |
| Operation voltage| 3.3 V |
| Chip size        | 1000 µm × 2965 µm |
| Pixel array size | 900 µm × 2010 µm |
| Pixel Type       | 3-transistor active pixel sensor |
| Pixel size       | 7.5 µm × 7.5 µm |
| Electrode size   | 90 µm × 90 µm |
| Electrode material| Al |
| Number of LED    | 12 |
| LED connection   | Parallel connection |
As the base chip of the proposed optoelectronic neural interface device, we designed a CMOS image sensor with an on-chip current injection electrode array. We used a 0.35-µm, 2-poly, 4-metal standard CMOS process for the fabrication. Figure 2 shows a layout of the CMOS image sensor chip for light source integration. Table I shows the specifications of the CMOS image sensor. As shown in Figure 2, we implemented an array of on-chip electrode pads to operate the LEDs on the sensor chip, with the capability of integrating 3 × 4 LEDs on the sensor. The pitches between the LEDs are 345 µm in the horizontal direction and 435 µm in the vertical direction. All of the pads for the cathodes of the LEDs are connected to the ground line. The LEDs’ anode pads are separately connected to connection pads aligned at the edge of the sensor chip for addressable operation. Thus, all of the integrated LEDs in the array are connected in parallel. In addition, the sensor has some area that do not contain pixel. This “empty” area has a size of 22.5 µm × 21.6 µm and was placed in each area of 8 × 8 pixel array (60 µm × 60 µm). This empty area was designed for making a hole on pixel array to deliver light through the sensor chip. However, the structure was not used in this work.

3. Integration of LED array on CMOS image sensor
We chose a commercially available InGaN Blue LED chip with a peak wavelength of 470 nm for integration. The chip size was 280 µm × 305 µm. The specifications of the LED are compatible with the excitation of the ChR2 used in optogenetics. We used a flip-chip bonding technique with gold bumps for the integration of the LED array on the CMOS image sensor.

![Figure 3](image)

Figure 3. (a) Integration process for multiple discrete LEDs on CMOS base chip, (b) LED array with gold bumps arranged on metal alignment plate

Figure 3(a) shows the process flow for integrating the LED array on the CMOS image sensor. Before we started the process, we formed gold stud bumps on both the anode and cathode pads of the LEDs. As shown in Figure 3(b), we used a metal alignment plate to align the LEDs before the bonding process. The LEDs were tentatively mounted on an adhesive film using the alignment plate as a jig. Then, we removed the metal alignment plate. Before we performed the flip-chip bonding, we covered the exposed adhesive film with polydimethylsiloxane (PDMS) to avoid the fixation of the film on the CMOS image sensor. This PDMS covering layer also helped to fix the position of each LED. We used anisotropic conduction paste (ACP) as an underfill for bonding. We used the following bonding conditions; pressure: 15 N, duration: 60 seconds, and temperature: 140 °C. After we bonded the LEDs, we glued the chip with the LEDs to a printed circuit board using epoxy resin and connected the connection pads of the sensor and the terminals of the printed circuit board with Al wire using a conventional wedge bonder. Then, we covered the surface of the device and the bonding wires with epoxy resin. To expose the backside surface of the LED array, we pressed the epoxy resin with a plate.
covered with silicone during the cure process. The refractive index of the epoxy resin was approximately 1.40. Since the current injection lines to drive the LEDs are separated each other, we expect crosstalk due to leakage current is negligible. On the other hand, since the gaps between LEDs are filled with transparent epoxy resin, light from one LED partly propagates through the sapphire wafer and epoxy resin. We have not characterized the optical crosstalk and it should be discussed in further works.

4. Characterization

4.1. Measurement setup

Figure 4 schematically shows the experimental setup for device operation. We designed an interface board for computer-controlled device operation and image capturing. The control signals from a PC (5 V digital) were transformed into 3.3 V digital signals by buffers on the interface board and supplied to the CMOS image sensor chip. The signals for controlling the sensor were compatible with those used in our previous works [12]. The CMOS image sensor output 3.3 V analog signals. An analog-to-digital converter (ADC) implemented on the interface board (Analog Devices, AD9225, 0 to 4 V/12 bit configuration) transformed the output signals from the CMOS image sensor into 12 bit-parallel digital signals. On the other hand, we used a simple approach to operate the integrated LEDs for light stimulation. The cathode lines of all the integrated LEDs were connected to ground, and the anode lines were separately connected to the terminals on the interface board. DC power supplies with current monitoring capability were used to drive the LEDs. We mounted the optoelectronic neural interface device under a conventional microscope (Olympus BX51WI), which we used to monitor the LED operation and on-chip imaging.

![Image](image_url)

**Figure4.** Measurement setup for CMOS on-chip optoelectronic neural interface device

4.2. Functional confirmation of LED operation

Figure 5 shows external views of the fabricated device in the following situations: (a) regular observation, (b) with LED illumination, and the images captured by the CMOS image sensor under (c) uniform illumination and (d) a dark situation with one LED operating. As shown in Figure 5(a), the LEDs were aligned on the CMOS image sensor using the flip-chip bonding technique. The LED array was molded with epoxy resin and a flat surface was obtained. Because the LEDs could be operated in parallel, we could perform not only single site stimulation, but also simultaneous multi-site stimulation, as shown in Figure 5(b). This is an advantage of our device compared to the previously reported LED array devices designed for optogenetics [13,14]. Because the LEDs were operated by currents injected
from external DC power supplies, we could control the illumination intensity by changing these currents. The current range for LED operation was 0.01-15 mA.

We measured the total illumination power emitted from the LEDs mounted on the chip. We obtained approximately 1 mW with an injection current of 2 mA, which was consistent with the specifications of the LED chip. Based on the literature, which suggests an intensity of 0.1-1 mW/mm² as a typical threshold [14-17], we consider the illumination performance of the on-chip LEDs to be sufficient to activate ChR2.

4.3. Characterization of imaging function

The images captured with portion of the CMOS image sensor are shown in Figures 5(c), and (d). Its imaging capability is one of the largest advantages of the present neural interface device, compared to the previously reported LED array device for optogenetics [13,14]. The frame rate was approximately 13 fps. The effective signal range in the image data used to show the intensity was approximately 10 bits. The control software could be used to perform inter-image subtraction, which is a very important function in imaging applications. We could also plot the values measured by selected pixels in real time, which will help with observations of the neural activities visualized by dyes such as Ca²⁺ indicating dyes or voltage sensitive dyes.

![Figure 5](image_url)

Figure 5. (a) LED array integrated on CMOS sensor with epoxy resin coating, (b) LED array illuminating CMOS sensor, (c) captured image of LED array on CMOS sensor, and (d) captured image of LED illumination

Figure 5(c) is an image taken under uniform illumination. The LED is not operating. Because we have pixel-less areas in each 8 × 8 pixel array, the image has data-less squares. We previously reported this in-array pixel-less design [18]. We can also see the pattern of the anode and cathode pads on the LED array in Figure 5(c). Because we used gold bumps for the flip-chip bonding, we could not avoid these shadows from the electrodes. We are currently developing a bonding technique without large gold bumps to avoid this image deterioration. Figure 5(d) shows an image captured under a dark situation. For Figure 5(d), we operated only one LED in the pixel array (LED number 8 shown in Figure 5(a)). We can observe the light emitted from the operating LED. This result suggests that we can observe and record the operation of the LEDs, as well as the situation of observation targets such as biological cells, brain slices, or the brain itself. It should be mentioned that we can take fluorescence images using the imaging functionality, if we integrate a high-performance optical colour filter with the image sensor [19, 20].
4.4. Functional demonstration: on-chip imaging of brain slice

As a preliminary functional demonstration, we performed an on-chip brain slice imaging experiment.

![Image of brain slice and LED illumination](image)

Figure 6. (a) Hippocampus brain slice on CMOS sensor, (b) LED illumination under Hippocampus, (c) captured image of brain slice on CMOS sensor

We used a mouse brain slice from around the Hippocampus area as the measurement target. All of the experiments followed our university’s procedure guidelines for animal experiments. The brain slice was fixed and kept in phosphate buffered saline (PBS) at a temperature of 2 °C [21]. The brain slice was placed on a culture dish (μ-dish, 33 mm, low). Prior to the observation, the PBS was drained from the culture dish, and 50% glycerine was dropped onto the slice to provide some moisture. Then, the culture dish was placed on the present optoelectronic neural interface device. Figure 6 shows: (a) the setup of the experiment, (b) demonstration of light emission from one LED, and (c) on-chip image captured under regular illumination (no LED operation). As shown in Figure 6 (b), the light from the LED diffuses within a diameter of approximately 500 μm. This result shows that the current resolution of the light stimulation is not suitable for cell-level stimulation, but region level stimulation. We could only perform region-level stimulation such as CA1, CA2, and CA3. In terms of on-chip imaging function, only a silhouette of the brain slice can be obscurely observed in the captured image, as shown in Figure 6(c). Currently, this imaging function can be used only as an assisting function to monitor the placement of the measurement target.

To improve the performance of the imaging function and realize a neural imaging in on-chip configuration, we need to implement an on-chip optical filter to eliminate excitation light. A filter resist layer will be formed between the CMOS chip and LEDs [22]. The shadow of the contacting electrodes on the LEDs is also an issue to overcome. We are also currently working on introducing a new LED electrodes and bonding materials.
5. Conclusions
We proposed a novel optoelectronic neural interface device for optogenetics. The proposed device is based on the technology of a CMOS image sensor. We integrated an array of GaInN LEDs on the CMOS image sensor with the capability of on-chip current injection. We successfully integrated multiple LEDs on the CMOS image sensor. We demonstrated the light-stimulation functionality of the LED array and performed an on-chip observation of the structure of a mouse’s Hippocampus.

The proposed architecture is advantageous because of its flexibility in arranging the number, type, and location of an LED array on a CMOS sensor. We can flexibly design optoelectronic neural interface devices that can fit a variety of targets such as culture cells, brain slices, and the brain itself under in-vivo situations.

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