A CMOS Optoelectronic Neural Interface Device Based on an Image Sensor with On-chip Light Stimulation and Extracellular Neural Signal Recording for Optogenetics

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Abstract A CMOS-based optoelectronic device is proposed for on-chip neural stimulation and observation with optogenetic methodology. The device is capable of local light delivery for stimulation and electrical neural signal recording. The device consists of an array of InGaN light emitting diodes (LEDs) and Au stacked bump electrodes integrated on a CMOS image sensor. Capabilities of on-chip light stimulation and signal recording were quantitatively characterized. We have also confirmed that neuron-like cells can be cultured on the surface of the device.

Keywords: Optogenetics, CMOS image sensor, Channelrhodopsin-2, field potential measurement

1. Introduction

Neurophysiology is one of the important fields of bioscience to explore behavior and mechanism of neural systems. Electric stimulation and measurement such as patch-clamp technique have been widely used in the neurophysiology. In terms of the electrical stimulation using an electrode, spatial resolution is limited due to dispersion of the current and it is difficult to realize non-invasive single-cell stimulation.

Optogenetics is a bioscientific technology to modify neural cells to be light-sensitive. With optogenetic approach, we can realize non-invasive neural stimulation with high spatial resolution. It is also possible to express the light-sensitivity in the specific cell types or positions. In the last decade, research strategies based on optogenetics have been widely introduced into many fields of medical and neuroscience.

Channelrhodopsin-2 (ChR2) is one of the mostly used proteins in optogenetics. A ChR2-expressed neural cell can be excited by light with wavelength of approximately 470 nm1–3). This protein acts as a gate that directly induce Na⁺ ion through the cell membrane. The induced Na⁺ ion changed the polarization of cell, so the target cell can be activated using a light stimulation4).

For wide-area optical stimulation, optical fiber is widely used in optogenetics. The widespread light stimulation is preferred to use for activated photosensitive neuron network than a localized light stimulation. An addressable light source device such as InGaN light emitting diode (LED) array is expected to be a platform for local light stimulation applicable for in vitro and in vivo neural experiments with optogenetics5,6).

Multifunctional devices have also be proposed to perform optical stimulation and electric neural measurement simultaneously. Optrode is a group of devices consisting of light-guide structure and electrode for electric measurement7–9). In most cases, one or several light-guides are implemented onto an electrode array. Light is delivered to the optrode using optical fibers. The optical fiber and wire harness prevent to use the optrodes in freely moving situations. A device with capabilities of local light stimulation and electric neural measurement with flexible interconnection is required for freely-moving situations.

In this work, we propose a CMOS-based optoelectronic device which is capable of not only stimulating the neural system with integrated micro light source array,
but also measuring neural activity with on-chip electrode array. Fig.1 shows (a) the concept of the device and (b) a photograph of assembled device for in vitro demonstrations. Integrating micro light source array and electrode array, we can simultaneously use optical and electrical schemes for neural stimulation and measurement. The proposed device structure is expected to be a promising platform of the multifunctional neural interface device for optogenetics. This device structure can be applied to bioimplantable devices for freely-moving animals using packaging techniques developed in our previous works.

2. Design of CMOS sensor chip

A CMOS image sensor with an on-chip electrode array was designed as the base chip of the proposed optoelectronic neural interface device. Fig.2 shows (a) block diagram of the sensor device, and (b) schematic of the pixel circuitry.

As shown in Fig.2 (a), the electrode array for LED operation and electric measurement of neural activity were implemented almost independently from CMOS image sensor block. Electrodes are parallel wired and no switching circuitry was implemented. For CMOS image sensor part, a conventional active pixel sensor was adopted, as shown in Fig.2 (b). A Y scanner was implemented for row selection. The pixel values (potential of photodiode node) of the pixels in the selected row are read out to readout circuit of each column. The values are sequentially selected by a X scanner and read out via output buffer.

As reported in our previous works, this CMOS image sensor is capable of imaging the brain structure and activities in on-chip configurations. For this work, the functionality of the sensor can be used to monitor the placement of observation target and operation of the LEDs for light stimulation.

We designed an array of on-chip electrode pads compatible to either current injection for LED operation and electric neural interfacing with microelectrodes. Top metal layer was used for the electrodes. Twenty four Al electrode pads were designed over the pixel array of the CMOS image sensor, and each electrode pad has a size of 90 µm x 90 µm.

Fig.3 shows the placement of the on-chip electrodes. A pair of anode and cathode electrodes was implemented for each of 3 x 4 LED sites. All of the cathode pads are connected to the ground line. Anode pads are separately connected to connection pads aligned at the edge of the
sensor chip for addressable operation. In the present work, we used half of the parallel-connected anode electrodes for electric stimulation and measurement of neural activities, as described in the next part.

3. Packaging of the optoelectronic neural interface device

We chose a commercially available InGaN LED for integration. The chip size was 280 $\mu$m $\times$ 305 $\mu$m. Since the LED was formed using sapphire substrate, it is almost transparent for visible light. Both anodic pad and cathodic bonding pads have a diameter of approximately 90 $\mu$m. The peak wavelength of the LED is 470 nm, which is compatible for excitation of ChR2 used in optogenetics.

We used a flip-chip bonding technique with Au bumps for the integration of the LEDs on the CMOS image sensor\(^{(3)}\). Au stacked bump electrodes were formed on the half of Al pads that were originally implemented as anode electrodes for LED operation. Each Au stacked bump electrode has a diameter of approximately 90 $\mu$m and same height as LED chips bonded on the CMOS sensor chip (90-120 $\mu$m). The placements of the LEDs and stacked Au bump electrodes are shown in Fig.3. After integrating the LEDs and stacked bump electrodes, the chip was mounted on a printed circuit board and was molded with epoxy resin. For in vitro applications, a cell culturing dish was attached surrounding the device (see Fig.1 (b)). Specifications of the assembled device are shown in Table 1. Fig.4 shows operation of integrated LED array with embedded Au stacked bump electrodes on the CMOS sensor. Table 2 shows the features of the present device in comparison with conventional optorodes reported previously\(^{(7),(9)}\).

4. Performance of on-chip LED array for light stimulation

The light power density reaching the target cells will determine the efficiency of light stimulation\(^{(15)}\). To estimate density of light, we emulated light propagation through brain tissue.

We used brain phantom to emulate brain tissue, because it has similar optical properties with a mouse’s brain. Brain phantom medium was prepared with 6.6% skimmed milk homogenized with 1% agarose gel\(^{(16)}\). Brain phantoms with thicknesses from 1 to 4 mm were prepared and placed on the top of the device. The light power and light distribution at the surface of the brain phantoms were measured.

<table>
<thead>
<tr>
<th>Table 1 Specification of the assembled device.</th>
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<tr>
<td><strong>CMOS image sensor</strong></td>
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<td>Chip size</td>
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<tr>
<td>Pixel number</td>
</tr>
<tr>
<td>Pixel size</td>
</tr>
<tr>
<td>Operation Voltage</td>
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<tr>
<td>Number of LED / electrode sizes</td>
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<tr>
<td><strong>Electrode connection</strong></td>
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<td><strong>LED array</strong></td>
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<tr>
<td>Typical size</td>
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<td>Light distribution diameter</td>
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<td>Typical Emission power</td>
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<tr>
<td><strong>Au stacked bump electrode array</strong></td>
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<tr>
<td>Impedance at 1kHz</td>
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<td>Baseline noise</td>
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Fig.4 LED array and Au stacked bump electrodes integrated on the CMOS sensor.

<table>
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<th>Table 2 A comparison between typical optorode and the present device.</th>
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<td><strong>Light source pitch</strong></td>
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<td>Electrode pitch</td>
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<td>Light dissipation at 2mm</td>
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*see section 4.

Fig.5 (a) shows total light power at the surface of the brain phantoms with different thickness. The light power density was 1.89 mW/mm\(^2\) at the center of the spot, when brain phantom was not placed on top of the device. When the 2 mm-thick brain phantom was placed on the device, the integrated light power was reduced to 75% as shown in Fig.5 (a). The light power density measured at the center was 0.85 mW/mm\(^2\). The light power density form the LED used in the present device was reduced to 45% at 2 mm. This light power density is significantly larger than optical fiber-based technologies. The light power density for optical fiber-based method was reported to be as small as 0.1% at the same distance\(^{(15)}\). This is a significant advantage of the LED as the light source for optogenetics.

We captured images of light distribution, and evaluated diameters of the distribution. Fig.5 (b) shows
light distribution at the surface of the brain phantoms as a function of the thickness of the brain phantom. The FWHM was 300 µm, when brain phantom was not placed on top of the device. When the 2 mm-thick brain phantom was placed on the device, the FWHM became approximately 4 times larger. Based on the literatures, a light with intensity range of 0.1~1 mW/mm² was suggested as typical threshold for neural activation. Our device has potential to stimulate ChR2 in brain tissue, when the target neuron is at a distance of 2 mm from the device surface.

5. Performance of stacked Au bump

In order to use the Au bump electrode as a neural signal recording electrode, we measured its impedance, and baseline noise in phosphate buffer solution (PBS). We also performed a measurement of simulated extracellular field potential signal.

Fig.6 (a) shows impedance of the Au stacked bump electrode in a frequency range of 50 Hz~100 kHz. In this experiment, we used two-electrode configuration with a Pt wire as a counter electrode. The measurement was done in PBS. Performance of recording electrodes in biological technology is typically characterized at 1 kHz. As shown in Fig.6 (a), the Au electrode has an impedance about 100 kΩ. This value is comparable with that of commonly-used planar multielectrode array (MEA). Therefore, we conclude the Au stacked bump electrodes are applicable as recording electrode.

We also characterized noise level and sensing performance to the emulated field potential in PBS solution using a measurement set up shown in Fig.6 (c). Amplitude of the baseline noise of Au stacked electrode was in an order of 100 µV which was measured with a stabled potential applied via an Ag wire electrode. The signal from neuron activity at extracellular region typically have amplitudes in a range of 100 µV - 1 mV, and the intensity of brain wave at surface of brain is as large as 10 mV. The sinusoidal potential signal with an amplitude of 1 mV and frequency of 200 Hz from a function generator was applied in PBS solution using an Ag wire electrode. The potential was measured using the Au stacked bump electrode. The measured signal was amplified by a low-noise preamplifier. The amplifier was configured with a gain of 10, and band-path setting from 0.1 Hz to 1 kHz. Fig.6 (b) shows a recorded waveform.
using the Au stacked bump electrode. The signal to noise ratio was approximately 20 dB. These experiments suggest that our device can be used for recording of extracellular field potential from neural cells.

6. Cell culturing on the device

We performed a preliminary experiment toward in vitro demonstration of device functions. We are planning to use ChR2-expressed Neuro2A cells as the first target neuron-like cell. Neuro2A is a cultured cell line that originates from mouse’s neuroblast. We coated poly-L-lysine on a surface of the device to enhance attachment of cells. We cultured Neuro2A cells using ATCC-formulated Eagle’s Minimum Essential medium was used as a medium culture. We incubated cultured cell at 37 °C, for 24–48h.° As shown in Fig.7, we succeed in culturing Neuro2A cells on the top of the device.

7. Conclusions

We proposed an optoelectronic device for neural interfacing with optogenetics. The device was designed based on a technology of CMOS image sensor. We integrated an array of InGaN LEDs and Au stacked bump electrodes on the CMOS sensor. We characterized light stimulation performances of the integrated LED array using brain phantoms, and demonstrated the functionality of field potential recording using the Au electrode. Our device is expected to be capable of activating ChR2-expressed neural cells in brain tissue at a distance of 2 mm, and recording an extracellular signal with an amplitude/frequency of 1 mV/200 Hz. Comparing with conventional optrode, the effective illumination can reach to a farther and wider area. We also succeeded to culture neuron-like cell on the top of our device, which is essential to perform in vitro experiments.

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