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Multiple Optical Stimulation to Neuron Using Si Opto-Neural Probe with Multiple Optical Waveguides and Metal-cover for Optogenetics

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Abstract— **We have developed a Si opt-neural probe with multiple waveguides and metal cover for highly accurate optical stimulation. This neural probe had 16 recording sites, three optical waveguides, and metal cover for suppressing light leakage. We evaluated electrochemical properties of the recording sites, and confirmed that the neural probe had suitable characteristics for neural recording. We also demonstrated the optical stimulation to the neurons expressing ChR2 using our probe. As a result, we succeeded multisite optical stimulation, and observed that no light leakage from the optical waveguides because of the metal cover. From in vivo experiments, we successfully recorded optically modulated local field potential using the fabricated Si neural probe with optical waveguides. Moreover, we applied current source density analysis to the recorded LFPs. As a result, we confirmed that light induced membrane current sink in locally stimulated area. Our Si opto-neural probe with multiple optical waveguides and metal-cover is one of the most versatile tools for optogenetics.**

I. Introduction

Recently, lots of researches have dedicated themselves to developing medical treatments for brain diseases, analyzing brain functions, and realizing brain-machine interface (BMI). In these researches, various kinds of neural probes were developed and used for recording neuronal action potentials and other brain activities. Especially, a Si neural probe was one of the most important tools. As the Si neural probe was fabricated by micro and nano technologies used for MEMS and LSI fabrication, it was possible to realize high density recording[1-3]. We also proposed an intelligent Si neural probe system which had multifunctional properties[4-6]. Figure 1 shows a conceptual drawing of the intelligent Si neural probe system. The key part of this system was the Si neural probe with high density recording sites and various sensors. By mounting electrical and chemical sensors on the Si probe, we can record neural activities electrically and chemically. At the other end of the intelligent Si neural probe, electronic circuits such as low-noise amplifiers, multiplexers, and analog-to-digital converters were integrated. The neuronal signals recorded from the brain were amplified while keeping lower noise level. After that, the recorded neuronal signals from the brain were transmitted to the external recording apparatus by wireless connection. Therefore, once the intelligent Si neural probe system was embedded in freely moving animals, neural recording from the animal brain can be simultaneously achieved. We reported *in vitro* and *in vivo* high density recording with the double-sided Si neural probes, and successfully recorded neuronal action potentials from brains of a guinea pig and a macaque[4,5]. The Si neural probe with a microfluidic channel was also fabricated, and successfully demonstrated fluid delivery into the brain with a microfluidic channel[6].

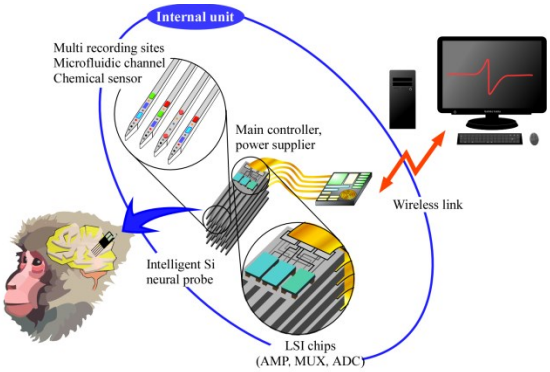

Fig. 1. Conceptual drawing of the intelligent Si neural probe system.

In this paper, we proposed a novel Si opto-neural probe with multiple optical waveguides and metal-cover for optical stimulation of neurons. Since several optical waveguides covered by metal layer were fabricated on the Si neural probe, multisite optical stimulation can be realized without light leakage. We fabricated the Si opto-neural probe with the multiple waveguides and evaluated the electrochemical and optical characteristics. Additionally, we demonstrated the optical stimulation for neurons expressing ChR2 using the fabricated Si opto-neural probe.

II. Proposal of Silicon Opto-Neural Probe with Multiple Optical Waveguides and Metal Cover

Direct optical stimulation is an effective method to realize a precise stimulation of neurons. In this method, the gene transfer technology was used, and neurons expressed a light sensitive channel protein such as Channelrhodopsin-2 (ChR2)[7-9]. Using the light for neural stimulation, a high spatial and time resolutions and less invasive of tissue can be realized. Moreover, we can electrically record neural reactions simultaneously during the optical stimulation[10].

To realize the optical stimulation to neuron, several kinds of probes capable of delivering light were reported[11-13].

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However, these neural probes used a chemically-thinned optical fiber which was manually attached with neural probes. Therefore, it was very difficult to control positions of optical stimulation site accurately. Moreover, it was also very hard to assemble lots of optical fibers precisely into the same probe.

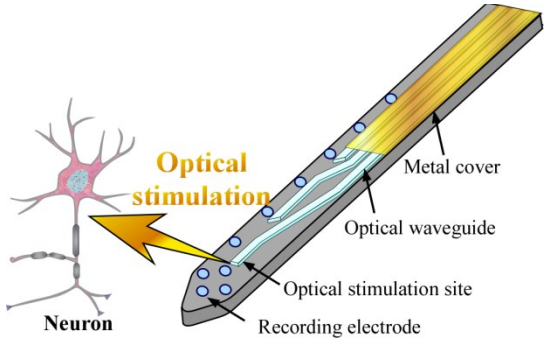

Fig. 2. Si opto-neural probe with optical waveguides and metal cover.

To overcome these problems, we proposed the novel Si neural probe with micro machined optical waveguides for the optical stimulation[14]. Figure 2 shows a conceptual drawing of our Si opto-neural probe. This probe had optical waveguides on the Si probe. These optical waveguides were fabricated by micromachining process. Therefore, optical stimulation sites were formed on the probe precisely. As recording electrodes were also formed on the probe, we can record the neural reactions elicited by the optical stimulation simultaneously. A size of optical waveguide was generally one hundredth times smaller than that of optical fiber. Thus, we can form several optical waveguides on the same probe and decrease an insertion injury caused by waveguides. Moreover, since material of the probe was Si, our probe can be easily integrated with Si-LSI. Using the Si opto-neural probe with multiple optical waveguides and metal cover, highly accurate and high density optical stimulations will be achieved.

III. Fabrication of Silicon Opto-Neural Probe

A fabrication sequence of our Si opto-neural probe is shown in Fig. 3. A 100-µm-thick Si wafer was used as the substrate of the probe. First, a 1-µm-thick SiO_2 layer was formed by thermal oxidation. Then, a 1-µm-thick SiN layer for waveguide core was formed by plasma-enhanced chemical vapor deposition (PECVD). Next, the SiN layer was patterned for optical waveguide shape using reactive-ion etching (RIE) with CHF_3 gases. Next, a 1-µm-thick SiO_2 layer for waveguide clad was also formed by PECVD. From these processes, optical waveguides were formed. Then, Au/Ti wirings were formed on the surface of the wafer by sputtering and wet etching with an iodine etchant and a dilute HF solution. Then, a 1-µm-thick SiO_2 layer was deposited by PECVD for isolation of the wirings. After that, contact holes were opened by RIE with CHF_3. Next, both Au recording electrodes and metal covers of optical waveguides were formed using a lift-off process. The metal cover prevented a light leakage from optical waveguides except for light outlets. Finally, the probe shape was formed by deep RIE with SF_6 and C_4F_8 gases. Using this method, we successfully fabricated the Si opto-neural probe having three optical waveguides and metal cover in this study.

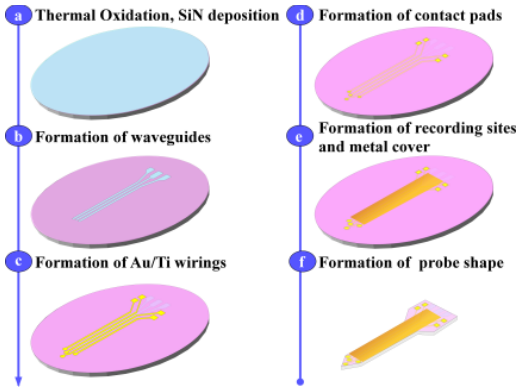

Fig. 3. Fabrication process of the Si opto-neural probe with optical waveguides and metal cover.

Figure 4(a) shows the fabricated Si opto-neural probe with multiple optical waveguides. The Si opto-neural probe was assembled on a printed circuit board (PCB). In addition, optical fibers were connected to the Si opto-neural probe. Using the optical fibers, it became possible to inject lights into optical waveguides of the Si opto-neural probe. Figures 4(b) and 4(c) show enlarged views of probe tip. From these Figures, we confirmed that Au recording electrodes, optical waveguides, and metal covers were well formed on the Si probe.

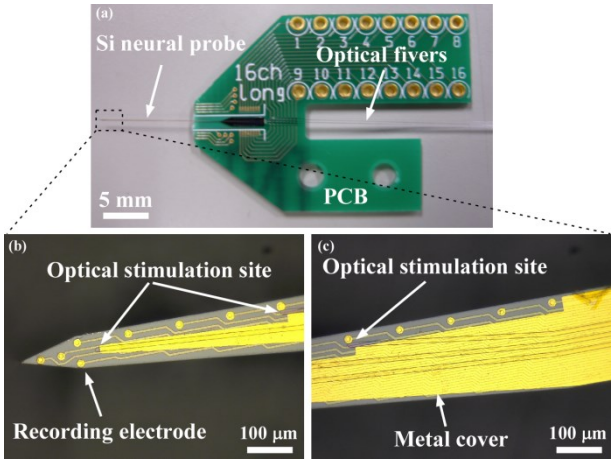

Fig. 4. Fabricated Si opto-neural probe with three optical waveguides and metal cover.

IV. Evaluation of Silicon Opto-Neural Probe

A. Electrochemical characteristics

At first, to confirm the capability of neural recording, we evaluated electrochemical characteristics of the fabricated probe. We measured electrochemical impedance of recording electrodes in an electrolyte. Measurements were performed with the 10 mV AC signal and the frequency ranging from 100 Hz to 10 kHz in phosphate buffered saline. In the measurement, we used Pt electrode as counter electrode and Ag/AgCl electrode to reference one. Figure 5 shows the electrochemical impedance spectroscopy of the Si opto-neural probe. The impedance value was about 1.5 MΩ at a frequency of 1 kHz. From this result, we confirmed that this probe had the suitable impedance value for neuronal

recording.

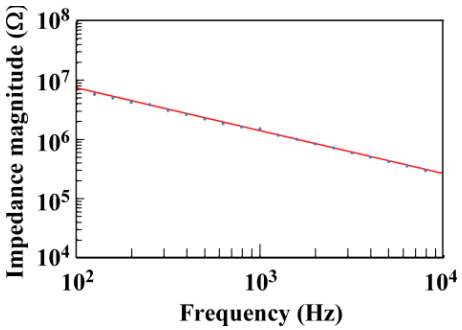

Fig. 5. Measured electrochemical impedance values of the recording electrode on the Si opto-neural probe.

B. Optical characteristics

We confirmed optical characteristics of the Si opto-neural probe. We performed the optical stimulation to neurons expressing the ChR2. Since sensitive wavelengths of a cell expressing ChR2 was 400–560 nm[8], a light source was a blue laser with wavelength of 452 nm. The Si opto-neural probe was connected with laser sources via optical fibers. Using this setup, we injected light into optical waveguides independently. Figure 6 shows light outputs from the multiple optical stimulation sites. Figure 6(a) shows probe tip, and three optical stimulation sites were observed. Figures 6(b) to 6(d) show the light output from the three optical stimulation sites respectively. From this result, we confirmed that it was perfectly possible to optically and independently stimulate multi points in the brain.

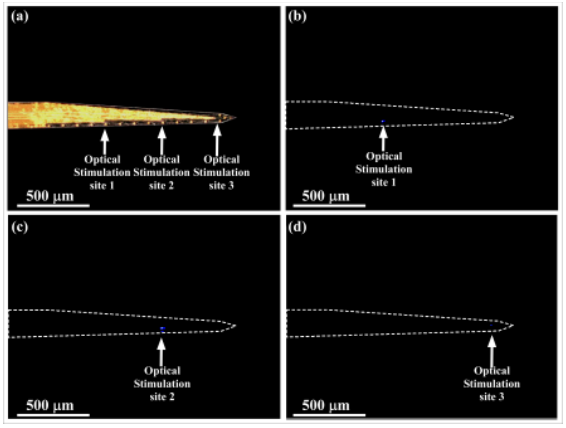

Fig. 6. Multisite optical outputs using three optical waveguides with metal cover.

We also evaluated light leakage from optical waveguides. Figure 7 compared the light leakage from optical waveguides between with and without metal cover. In Fig. 7(a), we observed the light leakage from the optical waveguide without the metal cover. As shown in Fig.7(b), however, there was no light leakage observed from optical waveguides with the metal-cover. From these results, using the fabricated opto-neural probe, we can optically stimulate neurons in multi points without stimulating an unwanted area.

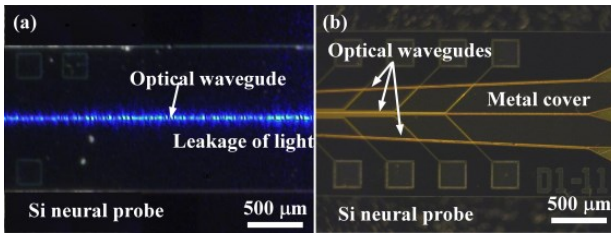

Fig. 7. Comparison of the light leakage from the optical waveguides with and without metal cover.

C. In vivo experiments

In this section, we demonstrated the optical stimulation for neurons using the fabricated Si opto-neural probe. All the animal experiments were approved by the Tohoku University Committee for Animal Experiments and were carried out in accordance with the Guidelines for Animal Experiment and Related Activities in Tohoku University as well as the guiding principles of the Physiological Society of Japan and the National Institutes of Health. In this experiment, we used the transgenic rat which expressed the ChR2 in neural tissue of the whole body. We also used a 452-nm wavelengths laser diode as the light source. The Si opto-neural probe was connected with this laser diode by an optical fiber. Figure 8 shows the insertion of the Si opto-neural probe into the rat brain. The rat was in anesthetized condition, and the opto-neural probe was inserted in an M1 area of the rat brain. The stimulation light exposed from the optical stimulation site 3 shown in Fig. 6(a). Then, we optically stimulated neurons and recorded local field potentials (LFPs) using the fabricated Si opto-neural probe.

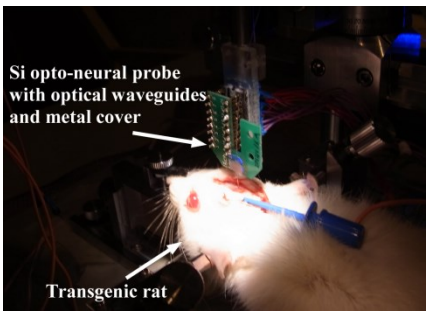

Fig. 8. Insertion of the Si opto-neural probe into the rat brain.

Figure 9(a) shows the averaged LFPs recorded by 16 recording electrodes, and Fig. 9(b) shows the electric potential distribution expressed by color. Blue color indicated lower potentials. We optically stimulated at a time of 0 sec in this graph. In the Fig. 9, light induced LFP changes were clearly observed in Ch. 1 to Ch. 4. These recording sites were formed around the optical stimulation site 3. Next, to identify current sources and current sinks of the neurons around the opto-neural probe, we adopted a current source density (CSD) analysis for the recorded LFPs[15]. The analyzed data are shown in Fig. 10. Fig. 10(a) shows the averaged membrane currents, and Fig. 10(b) shows the membrane current distribution expressed by color respectively. Blue color regions indicated the current sink. Neurons were stimulated at

the time of 0 sec. From this analysis, the current sink occurred only around light exposed area. From in vivo experiments, we succeeded that the local control of neural activity by the light stimulation using the Si opto-neural probe with optical waveguides.

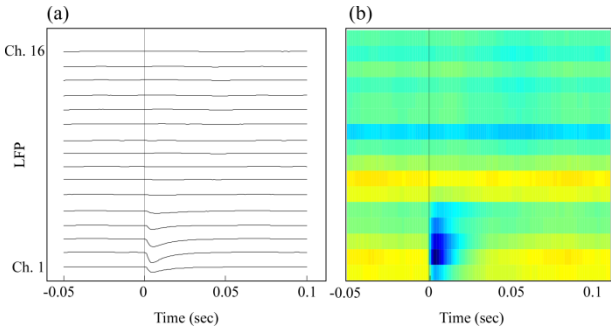

Fig. 9. Recorded LFP induced by an optical stimulation using the fabricated Si neural probe with optical waveguides.

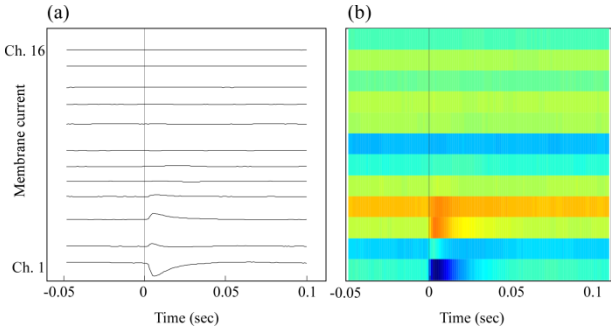

Fig. 10. Results of the CSD analysis extracted from recorded LFPs data.

V. Conclusion

We successfully fabricated the Si opto-neural probe with multiple waveguides and metal cover for multisite optical stimulation of neurons. Both SiN and SiO2 films were employed as a core and a clad of optical waveguide respectively. The Si opto-neural probe with optical waveguides was well assembled with optical fibers. From electrical experiments, it became clear that our probe had the suitable impedance for neuronal recording. Using the opto-neural probe, we successfully demonstrated the multi optical stimulations. Furthermore, the light leakage from waveguides was completely suppressed by metal cover over waveguides. We confirmed that we can stimulate neurons in multisite without stimulating unwanted neurons. Finally, we demonstrated the optical stimulation for neurons expressing the ChR2. We succeeded the recording of the light induced LFP changes, and confirmed the optically induced membrane current by the CSD analysis. From this experiment, we achieved the neural activity recording with higher spatial and time resolutions using the Si opto-neural probe. The Si opto-neural probe with multiple optical waveguides and metal-cover is one of the most versatile tools for optogenetics.

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