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An Implantable CMOS Image Sensor with Light Guide Array Structure and Fluorescent Filter

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Abstract— We fabricated an implantable CMOS image sensor with a light guide array and an interference filter for high spatial resolution fluorescent imaging in a brain. By using a light guide array, incident angle of light into the pixel array of the image sensor is limited and spatial resolution degradation with the spacing between the sensor and a sample is reduced. We demonstrate spatial resolution improvement and wavelength selectivity by the fabricated image sensor.

I. Introduction

A Biomedical photonic LSI (BpLSI) is an implantable micro CMOS image sensor which can be implanted in a deep brain to obtain fluorescence images as shown Fig. 1 [1-8]. By integrating the image sensor, fluorescent filter and excitation light source, *in-vivo* brain imaging of a freely moving animal is allowed. The sensor can be placed near the observation target in a living tissue so that highly sensitive imaging is realized. Fluorescence imaging is a powerful tool for observing activities of living tissues. By choosing fluorescent materials such as dyes and fluorescent proteins, various activities can be visualized.

As methods to observe brain activities, Positron Emission Tomography (PET), functional Magnetic Resonance Imaging (fMRI), Near-Infrared Spectroscopy (NIRS) are widely used [9]. These are non-invasive but the observed animals must be held. On the other hand, the BpLSI realizes high spatiotemporal fluorescence imaging in a deep brain although there is some invasiveness because it is implanted in the brain.

We have demonstrated applications of BpLSI to *in-vivo* imaging. For example, serine protease activation in a hippocampus [5] and *in-vivo* imaging of mouse brain under freely moving condition [6] are successfully observed. One of the remained problems is that the micro sensor cannot be integrated with a lens. The spatial resolution is degraded drastically with the spacing between the sensor surface and the observation target. Thus, only the area in the vicinity of the surface is observed. In order to solve this problem, we proposed light guide array structure, which is a hole array with high aspect ratio [10]. Each hole corresponds to a pixel of the image sensor. By limiting the incident angle and allowing only vertical incidence, the spatial resolution is improved.

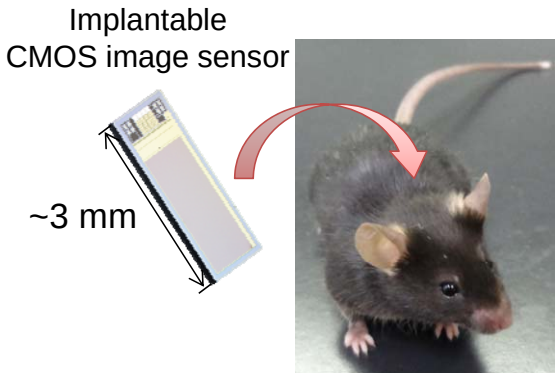


Fig.1. Concept of implantable image sensor for mouse brain imaging.

In this paper, an implantable CMOS image sensor for BpLSI integrated with a light guide array is fabricated. And, the optical characteristics of the sensor are measured. By using the fabricated sensor, fluorescent imaging of a living tissue is demonstrated.

II. Fabrication of Implantable Image Sensor with Light Guide Array

The observation target of the implantable image sensor is a living tissue stained with fluorescent materials. The fluorescent light from such samples is emitted isotropically. Thus, by using the sensor without an imaging lens, the spatial resolution is decreased as the spacing between the sensor and the observation target is increased. The implantable image sensor is designed for observation of the tissues contacted to the sensor surface. The spatial resolution is as high as neural

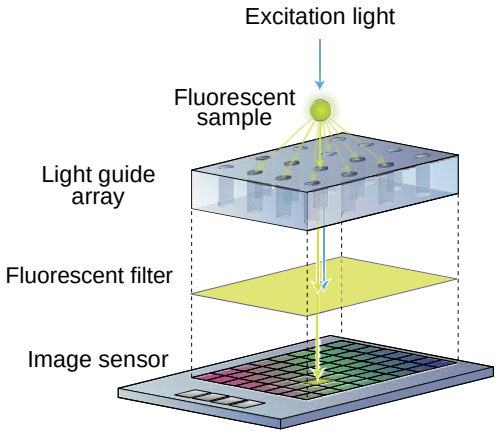


Fig. 2. Schematic of CMOS image sensor with fluorescent filter and light guide array.

cells (10-50 μm). However, the imaging depth of a few hundreds of μm are required because the neural cells are distributed three-dimensionally. The imaging depth was not sufficient.

Conventional lens optics is not suitable for the implantable sensor. It is difficult to reduce the thickness of the sensor module because some spacing is required to focus an image. In order to overcome this issue, we use a light guide array, which reduces the spatial resolution degradation with the spacing between the sensor and the sample by transmitting only vertical incident light as shown in Fig. 2. By using the light guide array, the spacing to focus an image is not required. Thus, a very thin module can be realized.

For fluorescent imaging, a fluorescent filter to eliminate excitation light is necessary. In fluorescence microscope systems, interference filters are usually used because high extinction ratio of emission and excitation wavelengths can be achieved. The transmission spectrum of a fluorescent filter depends on incident angle of light. Thus, the incident angle should be limited. The incident angle limitation feature of a light guide array is effective to solve this issue. By limiting incident angle by the light guide array, an interference filter can be applied to implantable sensors. In addition, this feature is also useful to improve extinction ratio of fluorescence and excitation lights by illuminate the fluorescent sample with angled excitation light because the excitation light is filtered out by the light guide array.

In this study, we fabricated an implantable image sensor with a fluorescent filter and a light guide array plate. The fabrication process is as follows: (1) a dielectric multilayer as an interference long-pass filter with a cut-on wavelength of 500 nm is deposited on a 60μm-thick silicon substrate. (2) The Si substrate is bonded on the image sensor we designed. Here, the filter side is faced to the pixel array of the sensor.

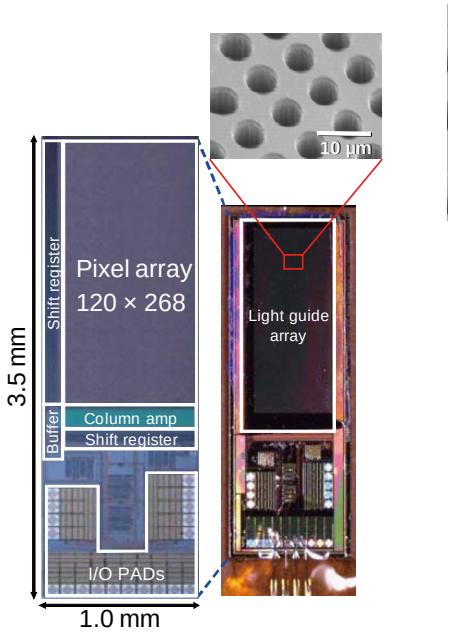


Fig. 3. Micrograph of a CMOS image sensor with light guide array. (inset) SEM image of the light guide array.

Amorphous fluoro polymer (CYTOP, Asahi glass) is used for bonding. (3) An Al layer with the thickness of ~200 nm is deposited and the light guide array is patterned by photolithography process. The Al layer is used as mask for the next etching process. (4) The Si substrate is dry-etched with high aspect ratio by deep reactive ion etching (DRIE) process, so that the light guide array structure is fabricated. (5) The sensor with the light guide array is fixed on a flexible substrate with epoxy resin. The pads are bonded with the lines on the flexible substrate by Al wires. (6) To make the sensor module waterproofed, the Al wires are embedded with epoxy resin. And, to supress the incidence of light into the photodiode array from the side of the sensor, the side of the sensor chip is coated with black resist.

The micrograph of the sensor is shown in Fig. 3. In this module, blue LEDs are also integrated. The hole diameter of the light guide array was approximately 5 μm.

III. Characteristics of the Image Sensor with Light Guide Array

By using the light guide array structure, incident angle received by the sensor pixel is limited, so that the spatial resolution is improved. In order to verify the effect of the light guide array, dependence of pixel output signal from the sensor on the incident angle was measured. A halogen lamp was used as a light source. The wavelength was chosen by a monochromator. The output beam was collimated by a plano-convex lens and launched into the sensor. The sensor was tilted to vary the incident angle.

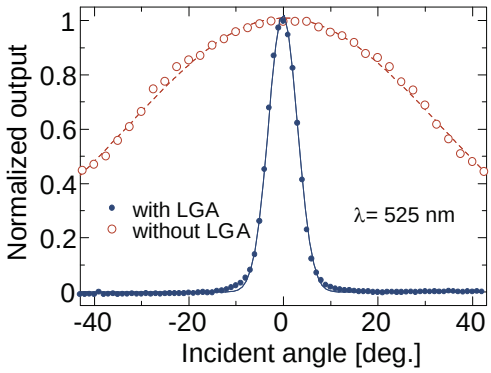


Fig. 4. Normalized output of the sensor with/without light guide array as a function of incident angle.

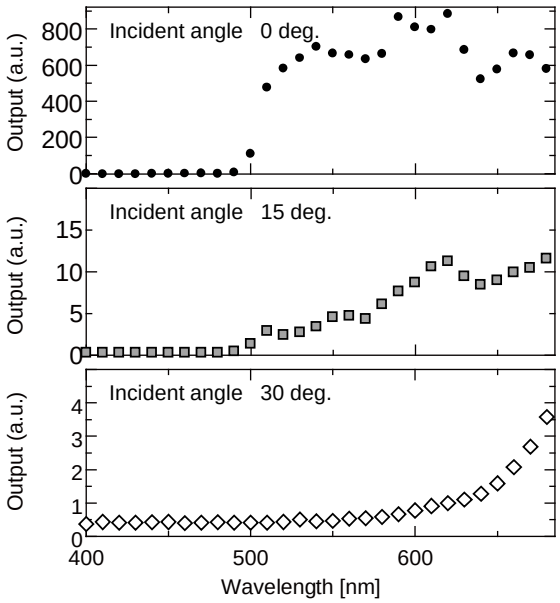
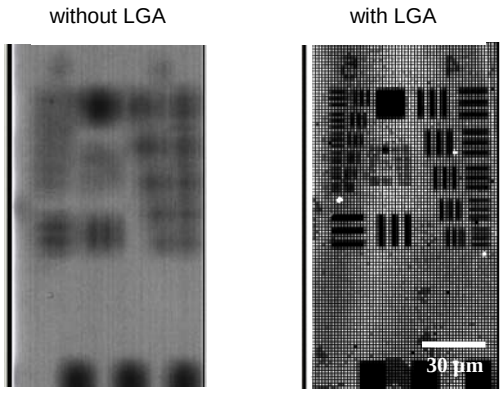


Fig. 5. Output of the sensor as functions of wavelength at incident angles of 0, 15 and 30 deg.

Figure 4 shows the normalized output as a function of the incident angle. The wavelength of the illuminated light was set to 525 nm, which is transmitted by the fluorescence filter on the sensor. The full width at half maximum of the incident angle is drastically decreased from 78 deg to 7.1 deg by applying the light guide array structure. This indicates that the incident angle is limited by the light guide array, so that the degradation of spatial resolution with the spacing between the sensor and the sample would be improved.

In order to evaluate the characteristics of the fluorescent filter between the light guide array and the sensor pixel array for green fluorescence imaging, dependence of the sensor output on the wavelength. Figure 5 shows the measurement results at three incident angles, 0 deg, 15 deg, and 30 deg. At the incident angle of 0 deg, the wavelength shorter than 500 nm is not transmitted. This indicates that the effect of fluorescent filter is appeared. At 15deg, the wavelength shorter than 500 nm is not filtered out as well as at 0 deg, and the output signal is increased as the wavelength become longer. At 30 deg, the filter effect at 500 nm is not appeared, only the signal increase with the wavelength is observed. From the result shown in Fig. 4, at the incident angle higher than 15 deg, it is estimated that the light directly entering the pixel is very weak. And, the output increase with the wavelength at 30 deg is exponential. These indicate that the light attenuation by the light guide array is dominant at 30 deg. It is considered that the output signal spectrum reflects absorption characteristics of silicon. At 15 deg, it is presumed that the transmission characteristics of the interference filter and the light guide array are comparable.



Spacing between sensor and chart: 200 μm

Fig. 6. Captured images by the sensors with/without light guide array.

The spatial resolution of the fabricated sensor was evaluated by using a 1951 USAF resolution test chart. The white light from a halogen lamp was filtered and light at 530±25 nm was obtained. The beam was diffused by an opal glass plate. The images of the test chart were captured with the spacing of 200 μm from the sensor surface. Figures 6 show the captured images by the sensors with/without the light guide array. There is one hole on the light guide for every 2 × 2 pixels. In the captured image by the sensor without the light guide array, all the lines are merged with their adjacent lines. On the other hand, with the light guide array, the line and space patterns are resolved. This shows that the spatial resolution is successfully improved by the light guide array.

IV. In-vivo Imaging of Mouse Brain Slice by the Fabricated Image Sensor

The implantable image sensor with the fluorescent filter and the light guide array was applied to imaging of a mouse brain slice in which green fluorescent protein (GFP) is introduced. Figure 7(a) shows the fluorescence image of the

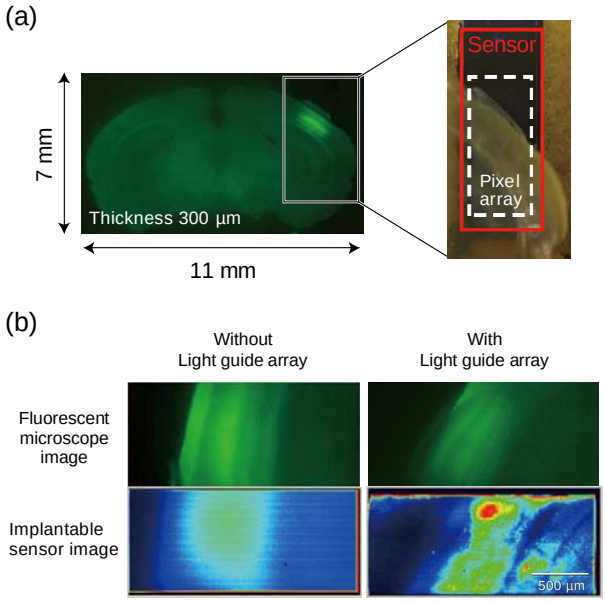


Fig. 7. (a) Observed area of mouse brain slice expressed GFP. (b) Photographs and images captured by the implantable sensors with/without light guide array.

slice. In this experiment, the wavelength of excitation light was set to 470±20 nm. The incident angle of the excitation beam was 45 deg.

Figures 7(b) show the images captured by a fluorescence microscope and our image sensors with/without light guide array. GFP fluorescence is observed in both of the sensor images. In comparison of with and without light guide array, the resolution is drastically improved by light guide array and the brain structures are observed. This result shows that the light guide array would be effective to high resolution in-vivo imaging.

V. Conclusions

We fabricated an implantable CMOS image sensor with a fluorescent filter and a light guide array for mouse brain imaging. The limitation of incident angle and the improvement of spatial resolution were achieved. The wavelength of detected light by the sensor was controlled by the interference filter between the light guide array and the sensor. In the fluorescence imaging experiment of the mouse brain expressed GFP, it is demonstrated that the proposed structure is effective. In the near future, it would be applied to *in-vivo* imaging for high resolution deep brain imaging.

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