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An implantable needle-like shaped device and its performance in lensless fluorescence imaging of biological tissues

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Abstract

The availability of intravital fluorescence imaging method has provided great progress in brain research. We present an implantable needle-like shaped device designed to be inserted into deep brain tissue for lensless fluorescence imaging. Illumination condition of brain tissues using implanted LEDs and imaging performance using the implanted CMOS image sensor were investigated numerically and experimentally.

1. Introduction

Intravital fluorescence imaging method is a promising approach to study brain functions in living animals. Fluorescent probes enable to observe dynamic activities of neurons and biochemical reactions in an intact brain. Combined use of tabletop-type microscopes and optical fiber bundles [1] or gradient refractive index (GRIN) lens [2,3] enables obtaining microscopic images of brain, even in freely moving condition. However, these approaches using a series of optical lenses have limitations to miniaturize the size of the device because of volumes of optical lenses, and thus observing deep brain tissue is still challenging. Complementary metal-oxide semiconductor (CMOS) technology enables to miniaturize imaging devices without optical lenses [4,5]. In this paper, we present lensless fluorescence imaging method using an implantable needle-like shaped imaging device designed to be inserted into deep brain tissue. The needle-like shaped imaging device with 700-μm-width and 300-μm-thickness, which has small LEDs for illuminating biological tissues and a CMOS image sensor for obtaining fluorescent images, has been developed.

2. Methods

All experimental protocols were approved by the Animal Experiment Committee of Nara Institute of Science and Technology.

2.1 Lensless fluorescence imaging using needle-like shaped imaging devices

Figure 1 shows the schematics of lensless fluorescence imaging of mouse brain using implanted CMOS imaging device. A CMOS image sensor chip and small LEDs were implanted into a certain part of the brain of living mice. The brain tissue was illuminated with scattered light from the LEDs and fluorescence signal from fluorescent substances was detected by the CMOS image sensor.

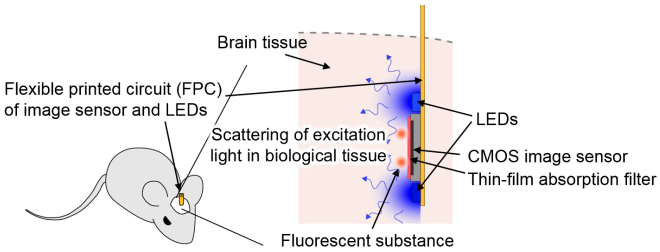

Fig. 1. Schematics of lensless fluorescence imaging of mouse brain using a needle-like shaped imaging device. (a) The imaging device is inserted into a brain tissue. A flexible printed circuit (FPC) can be connected to a personal computer (PC). (b) Brain tissue is illuminated by implanted LEDs and fluorescent images can be obtained by the CMOS image sensor with thin-film absorption filter.

2.2 Device design and fabrication

As shown in Figs. 2a and b, the CMOS image sensor chip and small LEDs were mounted on a flexible printed circuit (FPC). The absorption filter for filtering excitation light was directly coated onto the image sensor. The device was coated with a parylene film for waterproofing. The CMOS image-sensor chip and the LEDs were connected to a personal computer (PC) and a dc power supply via the FPC. Table 1 shows the specifications of the CMOS image sensor chip.

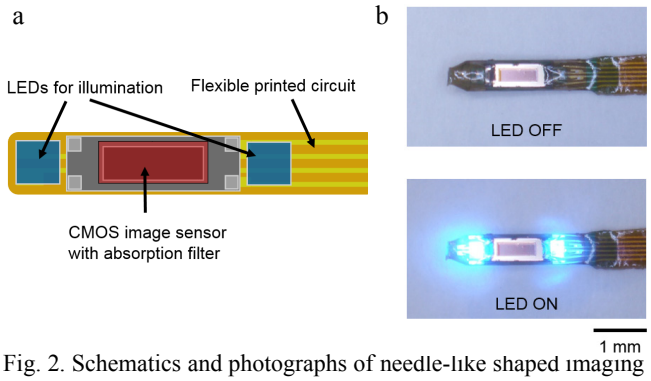

Fig. 2. Schematics and photographs of needle-like shaped imaging devices. (a) The imaging device is constructed with the CMOS image sensor chip with 450-μm-width, 1500-μm-length, and 150 -μm-thickness and the LEDs with 280-μm-width, 305-μm-length, and 90-μm-thickness, and the flexible printed circuit (FPC). (b) The LEDs for illumination was mounted beside the CMOS image sensor chip.

Table 1. Specifications of the CMOS image sensor chip

Process technology	0.35-μm 2-poly 4-metal standard CMOS process
Supply voltage (V)	3.3
Chip size (μm^2)	450 × 1500
Pixel array size	40 × 120
Pixel size (μm^2)	7.5 × 7.5
Pixel type	3-transistor active pixel sensor
Photodiode type	N-well–P-sub.
Fill factor (%)	44

3. Results and discussion

3.1 Illumination of brain tissues using implanted LEDs

Illumination in biological tissues results in a light intensity gradient in the tissues because of absorption and scattering. Therefore, to determine the spatial distribution of light intensity, the propagation of light in the brain tissue was investigated using optical simulation with the modified model described in the literature [5]. Figure 3a shows the light intensity distribution in the brain tissue illuminated by implanted LEDs. Figures 3b shows the light-intensity gradient at the surface of the image sensor. The calculated results of optical simulation can be utilized to computationally correct the obtained images under the condition of non-uniform excitation light distribution.

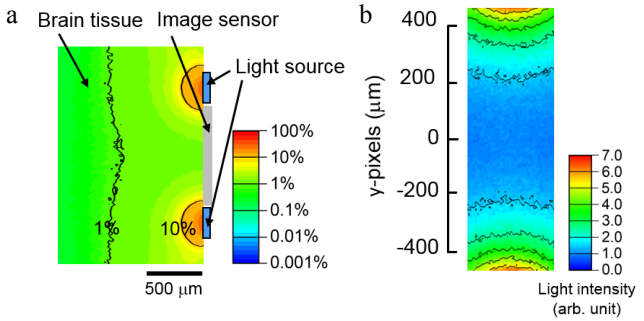

Fig. 3. Optical simulation of illuminating brain tissues using implanted LEDs. (a) Cross-sectional image of light-intensity distribution in brain tissues. Contours represent the surfaces where the light intensity drops to 10% and 1%. (b) Distribution of excitation light on the surface of the image sensor. Light-intensity gradient was appeared as the distance from the LEDs.

3.2 Lensless fluorescence imaging in biological tissues

The interaction of light and biological tissues, such as absorption and scattering, causes attenuation of fluorescence signals and loss of resolution. To demonstrate lensless fluorescence imaging in biological tissues, fluorescent microbeads of 15-μm diameter were imaged with the CMOS image sensor. To vary the distance between the fluorescent microbeads and the image sensor, D, a brain-tissue slice was placed onto the image sensor and the fluorescent microbeads were placed onto the brain-tissue slice, as shown in Fig. 4a. Fluorescent images of the microbeads embedded in biological tissues were obtained, as shown in Figs. 4b and c. Although signal attenuation and loss of resolution occur because of absorption and scattering as increasing the distance D, fluorescent signals from fluorescent substances can be detected by the image sensor.

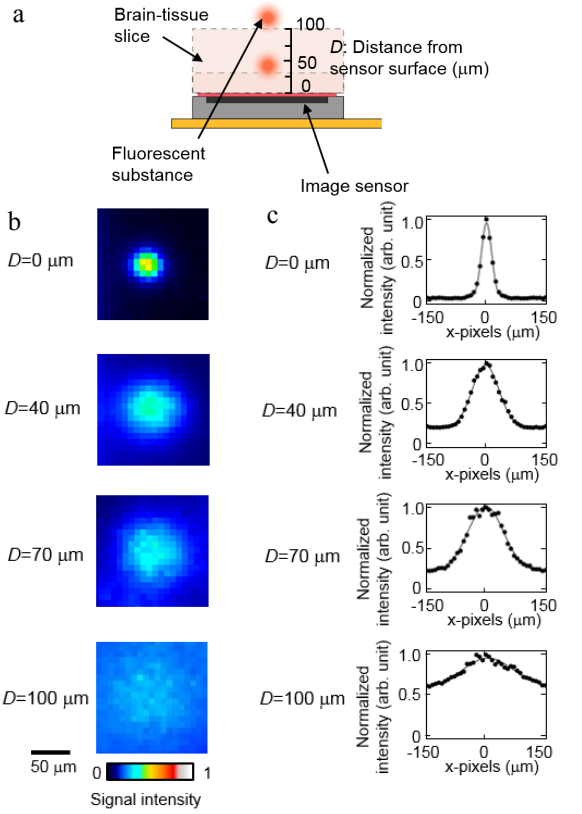

Fig. 4. Fluorescence imaging of fluorescent substances embedded in brain tissues. (a) Fluorescent microbeads were placed on the brains-tissue slices of 40, 70, and 100 μm in thickness, respectively. (b) Obtained images of fluorescent microbeads (ϕ = 15 μm) without a brain-tissue slice (D = 0 μm) and with brain-tissue slices of various thickness (D = 40, 70, 100 μm). (c) Representative intensity profiles of the microbeads. The experimental data (dots) were fitted by a Gaussian function (line).

4. Conclusions

In summary, the implantable needle-like shaped device was developed, which enables both illumination of tissues and lensless fluorescence imaging using the LEDs and the CMOS image sensor mounted on the device. Optical simulations showed the distribution of excitation light in the brain tissues illuminated by the implanted LEDs. Finally, lensless imaging of fluorescent substances embedded in the biological tissues was demonstrated. As a tool in brain research, lensless fluorescence imaging method enables fluorescence signal detection from the substances even in the deep brain tissue.

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