Implantable CMOS Image Sensor Using Multilayer Filter Emission and Fiber Coupled Laser Excitation

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Abstract We proposed a new method in combining composite emission filters with fiber coupled laser excitation for lens-free fluorescence imaging applications. The composite filters which comprise an interference filter and an absorption filter produce a high-quality band-pass spectrum. On the other hand, the low numerical aperture (NA) optical fiber provides a blue narrow-spectrum light source as well as fully controllable illumination features. The in vitro experiment confirmed that image sensor capable to capture green fluorescence protein (GFP) emission from brain slice moderately.

Keywords: an implantable imager, lensless imaging, fluorescence, laser-coupling, interference filter.

1. Introduction

An implantable imager is powerful tools to elucidate animal brain function under freely moving due to its low invasiveness and lightweight [1]–[4]. Unfortunately, it was difficult to achieve high excitation rejection for lensless fluorescence imaging system. One of the challenges is the insufficient performance of the emission filter [5].

Recently, we have proposed a novel filter to overcome the problem by the complementary components of an interference filter and an absorption filter via a fiber optic plate (FOP). This composite filter structure exhibits high-performance rejection of excitation light even in lensless setup [6]. However, it is too thick and not suitable for implantable devices.

In this work, we propose a thin multilayer emission filter comprises an interference filter and an absorption filter. We directly stacked the interference filter on the absorption filter. This technique reduces the device thickness without loses complementary emission filter features. In addition, we introduce fiber coupled laser excitation light to overcome broad spectrum and illumination direction problems as in light emitting diode (LED). Also, the effect of temperature rises on the sample can be avoided since the light source is placed away from the detection area.

Thin composite emission filter shows high-quality band-pass transmission characteristics, which is relatively associated with the GFP emission. This combination of decent filter and high-quality light source leads to fluorescence detection enhancement.

As a result, an implantable imager capable to identify the fluorescence emission from the GFP in a brain slice noticeably.

2. Device Fabrication

Fig. 1 shows the schematic of the CMOS image sensor with multilayer filter emission and fiber coupled laser excitation. Multilayer filters comprise a short-pass interference and absorption filters which are directly stacked each other. The laser excitation was coupled on the fiber and illuminated blue light almost parallel with the sensor.

We used a 550-nm short-pass interference filter to reduce angled blue excitation light due to the spectrum shift with the incident angle as well as reflects the red fluorescence from the tissue. As the excitation light is almost parallel to the imaging area, the scattered component is taken into account. This scattered excitation light is absorbed by the yellow absorption filter. Meanwhile, the red angled fluorescence is reduced by green absorption filter. As a result, it produced band-pass transmission characteristic which is associated with the observation targets (i.e GFP).

The fabrication of imaging device is started with stacking multilayer filter by spin coating sequentially. Next, attaching the imaging device with the filters with epoxy resin. We used a laser lift-off technique to separate the filters from the substrate and then placed the sensor on printed circuit board (PCB). Finally, the fabricated device was coated with a parylene film for waterproofing after being electrically connected by a wire bonding technique. The photograph of an implantable device

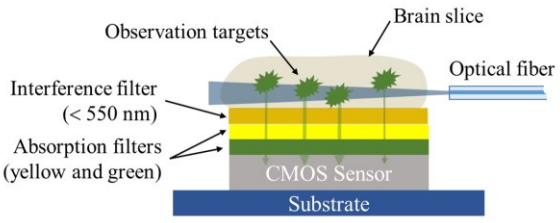

Fig. 1. Schematic of the image sensor with a multilayer filter and fiber coupled laser excitation.

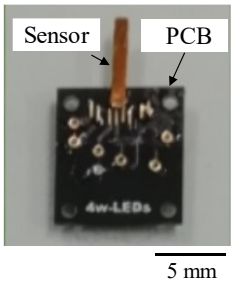

Fig. 2. Photograph of the fabricated device.

can be seen in Fig. 2.

The sensor chip was designed in our laboratory and fabricated by 0.35-µm 2-poly 4-metal standard CMOS technology (AMS). For imaging function, the chip uses a 3-transistor active pixel sensor (3-Tr APS) with a size of 7.5 µm × 7.5 µm. A number of pixels are 40×400.

3. Experiment Results

Fig. 3 shows the sensitivity spectrum of the image sensor. Its transmission is approximately 510 – 560 nm. This band-pass spectrum is relatively associated with the GFP emission. All the lights out of transmission band are almost negligible.

This transmission profile is a result of a composite filter mechanism. The wavelength longer than 560 nm is rejected by

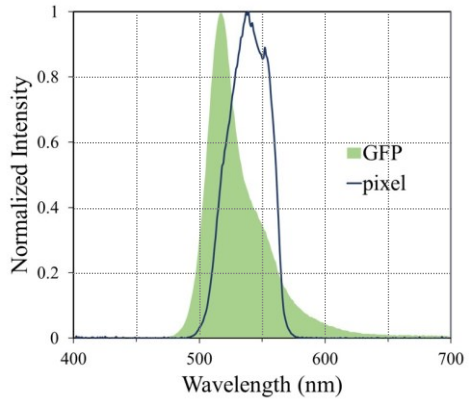

Fig. 3. Normalized sensitivity spectrum of the image sensor with the emission filters compared to the GFP spectrum

the short-pass interference filter and for the shorter than 510 nm is undertaken by yellow absorption filter. From this profile, auto-fluorescence from the tissue is expected can be largely suppressed.

In addition, as the examination performed with a stationary light source, the interference filter angle dependent was not observed. It transmits normal incident excitation light.

To confirm fluorescence detection of the fabricated device, we performed an in vitro experiment using 100-µm-thick brain slices obtained from an adult mouse (GAD67) which had genetically modified by GFP. The brain slice was directly placed onto the surface of the images sensor.

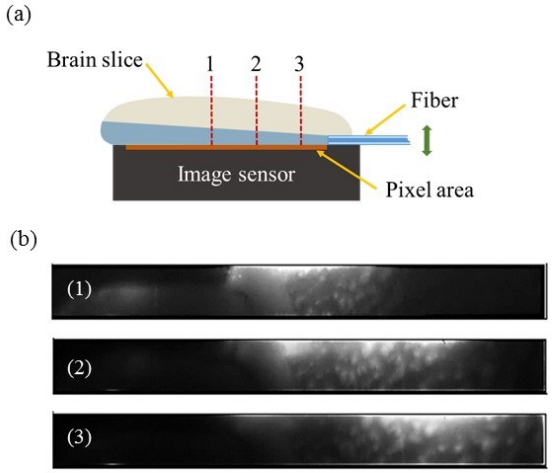

Fig. 4. Fluorescence detection experiment: (a) Illustration of the setup. b) Fluorescent image from the various position of excitation light (1 – 3) obtained by the image sensor.

In this experiment, we were able to control both positions of the fiber and the image sensor mechanically. This features facilitated in delivering the excitation light to the specific area of samples as well as image sensor position adjustment for best imaging result at the same time. Moreover, the light source position distinction may confirm the fluorescence shift due to the incident angle of excitation light.

Fig. 4 (a) shows the illustration of fluorescence imaging in the brain slice. We used a blue laser (λ_{laser} = 473 nm) which is coupled on multimode optical fiber (ϕ_{core} = 25 µm, NA = 0.1) and placed the fiber at the edge of the imaging area. The excitation light was delivered at the various positions of the brain tissue (position of excitation light emission: 1, 2, and 3).

The fluorescent images that were obtained by images sensor at each position are shown in Fig. 4 (b). All of these images showed that fluorescence emission from GFP could be identified noticeably. On the other hand, it seems that fluorescence is not shifted as the apparent difference in the images was the product of shadow.

4. Conclusion

The composite emission filters combined with fiber coupled laser excitation exhibited the capability of capturing fluorescence emission from GFP in the brain slice noticeably. We expect this method to open up an entirely high-quality fluorescence imaging applications with implantable imagers.

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