Lensless fluorescence microscope

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Abstract This paper presents a high performance lensless fluorescence microscope. We propose an emission filter composed of interference and absorption filters that shows high excitation rejection. Time lapse bright-field and fluorescence imaging during cell culturing is demonstrated.

Keywords: lens less fluorescence imaging, high performance emission filter, time-lapse imaging.

1. Introduction

Lensless imaging technique enables wide field-of-view with relatively small dimension due to its inherent simple optics [1-4]. This feature is very suitable for bio-imaging where very wide imaging area or high portability is required. However, most of recent researches of lensless imaging were based on bright-field imaging techniques. Although fluorescence imaging by using dyes or genetically introduced fluorescent proteins is also important, not so many researches have been reported. One of the reasons is that emission filter performance was not sufficiently high [5-7]. Thus, transmitted excitation was not negligible in fluorescence imaging. Recently, we have proposed and developed a novel filter that overcomes the problem [8]. In this paper, we report the feature of the filter and demonstrate highly sensitive lensless fluorescence imaging.

2. Hybrid emission filter

There are two types of emission filters, that are interference and absorption filters. In the fluorescence microscopy, the interference filter works well.

In a lensless imaging setup, an observation target is placed just above the imaging device. A part of excitation light is scattered by the target and launched into the interface filter with various incident angle. A transmission spectrum of inference filter has angle dependence. It shifts to lower with incident angle. Thus, a part of the scattered excitation light is transmitted.

Absorption filters do not have angle dependence. The transmittance of absorption filter is decreased exponentially with the thickness. However, this type of filters emits auto-fluorescence and overlapped with the fluorescence of the observation target. Thus, there is a lower limit of effective transmittance that cannot be improved with the thickness.

Recently, we developed a novel “hybrid” emission filter structure for lensless imaging that can resolve the problems described above. This filter is composed of interference and absorption filters. Here, these filters compensate their shortcomings each other. The most of excitation light is reflected by the interference on the top. And, the transmitted scattered light is reduced by the absorption filter that has no angle dependence. As a result, ultra-high excitation light rejection performance has been realized. By using this technique, we successfully reduced the transmission at 450 nm to approximately 10^{-8}.

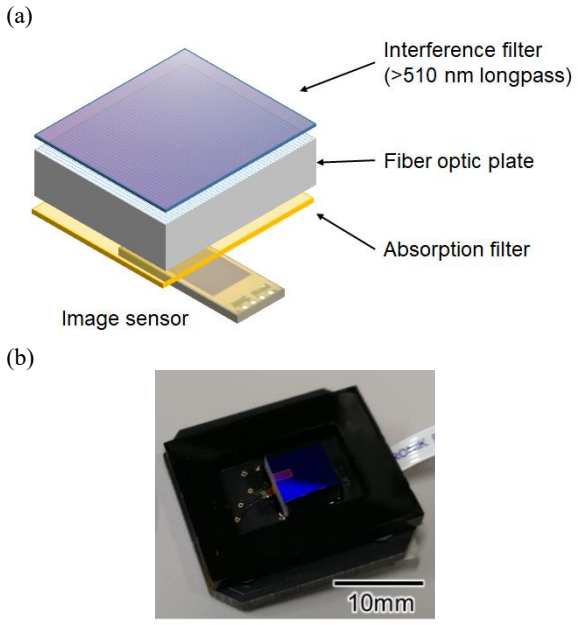

Fig. 1. (a) Schematic of an image sensor with a hybrid filter. (b) Photograph of the fabricated dvice.

3. Device Fabrication

Figure 1(a) shows a schematic diagram of an imaging device with the proposed hybrid filter. In lensless imaging device, the spatial resolution decreases with the effective thickness of the filter because fluorescence is emitted to various angles. Thus, the filter should be as thin as possible. In order to achieve sufficiently high absorption performance with thin structure, we prepared heavily dye doped filter. The weight ratio of the dye (Valifast yellow 3150, Orient chemical, Japan) and the base polymer (NOA63, Norland, NJ, USA) is 1:1. This filter is very soft and easily damaged by scratching. Also, it is difficult to fabricate an interference filter directly on it.

In order to avoid these problems, we used a fiber optic plate (J5734, Hamamatsu Photonics, Japan) as a substrate of the filters. The FOP is composed of a bundle of optical fibers and transmits an image from the one side to the other side. The spatial resolution does not depend on the thickness of the FOP. In this device, the thickness of the FOP is 2.54 mm. The height of the imaging surface is higher than the binding wires and flat target samples can be easily contacted. The core pitch of the FOP is 3 μm that is lower that of the image sensor pixel pitch

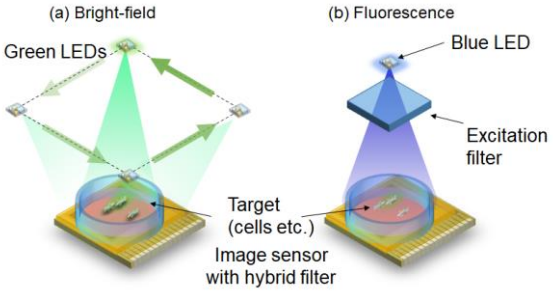

Fig. 2. Exmperimental setup of (a) brignt-field and (b) fluorescence imaging.

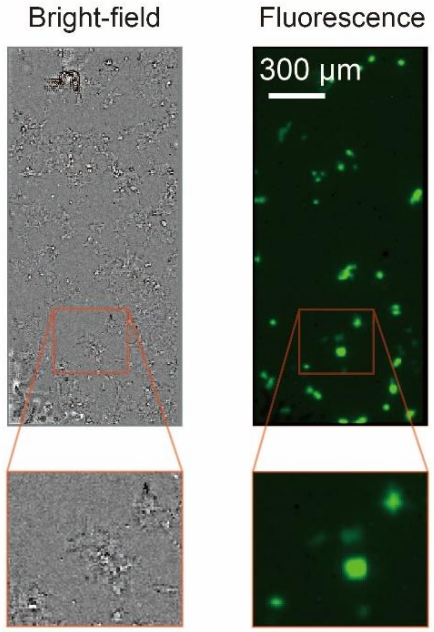

Fig. 3. Cell imaging exmaples of the fabricated lensless imaging device.

(7.5 μm). The FOP also works as a protection device of the soft absorption filter.

It is difficult to coat the absorption filter by spin coating technique because the area of the device is small. In order to avoid this problem, the filter is coated on a large substrate and transferred to the FOP.

Figure 1 (b) shows the photograph of the fabricated prototype device. The imaging area of the present device is not so large (= 1.0 mm × 2.7 mm). However, it is easy to the present fabrication technique to lager image sensors.

4. Imaging Experiment

An advantages of lensless imaging devices is their simple optics and small device seizes. In this work, we placed a chamber for cell culturing and carried out time-lapse imaging in a CO_2 incubator. Figure 2 shows the schematic of imaging setup. The light sources were blue and green LEDs. Here, the blue is for fluorescence excitation and the green is for bright-field imaging. For the blue LED, an exicitation filter is used to reduce the overlap of emission spectrum with the taget (green fluorescent protein). The bright field and fluorescece imagins were obtained sequentially. For bright field imaging, super resolution images were reconstructed from four images with difference illumination angles.

The examples of the obtained images are shown in Figs. 3. Here, green fluorescent protein is intoroduced to part of of the cells (HEK293) . By using the proposed hybrid filter, the green fluorescent emission was clearly detected with the lensless imaging device.

4. Conclusion

We porposed and demonstrated a high performance emission filter for lensless imaging device. The fluorescence sensitivity with this new filter is comparable to lens-based fluorescence microscope. This technique would expand the application area of lensless fluorescence imaging.

Acknowledgments

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