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An On-Chip Fluorescence Imaging System Using a Compact CMOS Image Sensor

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Abstract

Although fluorescence microscopy is the gold standard tool for biomedical research and clinical applications, their use beyond well-established laboratory infrastructures has been still limited. We present an on-chip fluorescence imaging system based on a CMOS image sensor. An ultra-thin glass bottom chamber was newly developed for fluorescence imaging of cultured cells using the on-chip platform and the resolutions for the contact imaging of fluorescence was evaluated. Fluorescence imaging of cultured cells and detection of fluorescent signal changes arisen from the cellular response to specific biological compounds demonstrated a promising use for the biomedical research and drug development. The on-chip fluorescence imaging system based on a CMOS image sensor has desirable features including compatibility with microfluidic chips and integratability with the electrical control system, and thus will fulfill the requirements of micro total analysis system and point-of-care testing devices.

1. Introduction

Fluorescence microscopy has been a powerful tool and is still considered as the gold standard for biomedical research and clinical applications. A wide variety of sophisticated fluorescent probes enables the high sensitivity and specificity of detection and microscopic observation of cellular activity. Conventional tabletop-type fluorescence microscopes, however, are relatively bulky and costly, because of their series of lenses and mechanical structures for focusing lenses. Thus, their use beyond well-established laboratory infrastructures with well-trained technicians has been still limited [1]. Complementary metal-oxide semiconductor (CMOS) image sensor for fluorescence imaging [2,3] enables simple and compact on-chip fluorescence imaging platform. Simple on-chip system based on a CMOS image sensor without optical elements, such as objective lens and relay lens, is considered to be cost effective and mass producible.

In this paper, we present an on-chip system for fluorescence detection of cultured cells based on CMOS image sensor technology.

2. Methods

Schematics of an on-chip fluorescence imaging system

Figure 1 shows a schematic diagram of the on-chip fluorescence imaging system with an ultra-thin glass bottom chamber and a compact CMOS fluorescence image sensor. The ultra-thin glass bottom chamber was designed for culturing cells, delivering chemicals to the cultured cells, and fluorescence imaging using the compact CMOS image sensor. Ultra-thin glass with 10 μm in thickness was used to minimize the distance between observing targets (e.g., fluorescent cells) and photodetectors in a pixel array of the CMOS image sensor.

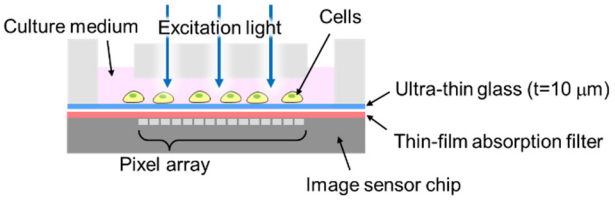

Fig. 1. Schematic diagram of the on-chip fluorescence imaging system using an ultra-thin glass bottom chamber and a compact CMOS image sensor.

An ultra-thin glass bottom chamber and a compact CMOS fluorescence image sensor

The ultra-thin glass bottom chamber was placed onto the compact CMOS fluorescence image sensor, as shown in Figs. 2a and b, and illuminated with excitation light using LEDs for fluorescence detection. An inlet and an outlet of the chamber was used for introducing culture medium, cells, and chemical compounds into the chamber. Cells were cultured in DMEM with 10% FBS at 37 °C in a humidified atmosphere with 5% CO_2. The compact CMOS fluorescence image sensor comprises two parts: a CMOS image sensor chip which had 120 × 268 pixels (pixel size: 7.5 μm × 7.5 μm) and a fluorescence filter (500-nm long-pass). The CMOS image sensor chip was fabricated using the 0.35-μm 2-poly, 4-metal standard CMOS technology.

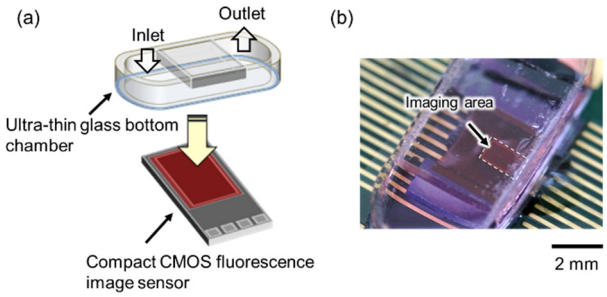

Fig. 2. (a) Schematic illustration of the ultra-thin glass bottom chamber and the compact CMOS fluorescence image sensor. (b) Photograph of the experimental setup for on-chip fluorescence detection.

3. Results and discussion

Evaluation of the on-chip fluorescence imaging system

To improve and the resolution of on-chip fluorescence imaging and the coupling efficiency of fluorescence signals to photodetectors, ultra-thin glass with 10 μm was used at the bottom of the chamber. To show the feasibility of the ultra-thin glass for on-chip fluorescence detection, we evaluated the resolutions for the contact imaging of fluorescence. Figures 3a and b show the representative images of the fluorescent microspheres of 10 μm diameter (Life technologies Inc., Grand Island, NY, USA) on the ultra-thin glass (10 μm in thickness) and a commercially available cover glass (120-170 μm in thickness). The representative intensity profiles of signal intensity were plotted in Fig. 3c. Estimated full widths at half maximums (FWHMs) were 37.6 ± 1.2 and 158.4 ± 5.6 μm with the ultra-thin glass and the cover glass, respectively.

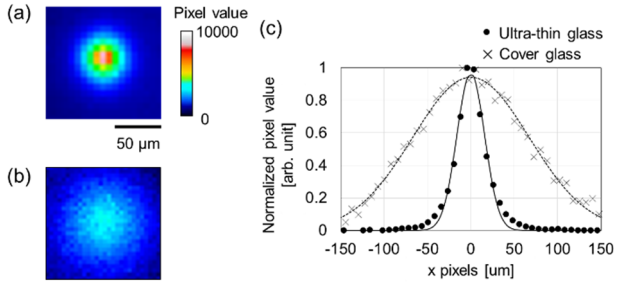

Fig. 3. (a) Fluorescence image with the ultra-thing glass (10 μm in thickness). (b) Image with the cover glass (120-170 μm in thickness). (c) Representative intensity profiles of the microspheres. The experimental data (dot and cross-mark) were fitted by a Gaussian function (solid line and dash line).

Imaging of cultured fluorescent cells

Förster resonance energy transfer (FRET) biosensor which comprises two fluorescent proteins of cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), for extracellular signal-regulated kinase (ERK), named EKAREV [4], was expressed inside nuclei of Hela cells. Thus, excitation light for CFP (~400 nm) was used and only fluorescent light from YFP (> 500 nm) was detected. FRET based fluorescent probes show large stokes shift because of intramolecular energy transfer, and thus, are preferred in the on-chip fluorescence imaging system. Figures 4a and b show representative images of fluorescent images obtained by using a tabletop-type microscope and the developed on-chip fluorescence imaging system, respectively. As shown in Fig. 4b, fluorescent images of nuclei of the cultured cells were clearly obtained.

Detection of cellular response to extracellular agents

The on-chip fluorescent detection system was applied to detection of cellular response to extracellular agents using the EKAREV. The EKAREV was designed for extracellular signal-regulated kinase (ERK). The EKAREV increases the FRET efficiency and fluorescent intensity of YFP upon perception of the extracellular signal. Cellular response to extracellular agents of Hela cells was induced by using endothelial growth factor (EGF) (10 ng/mL). Figure 4c shows the cellular responses to extracellular agents detected by the on-chip fluorescence imaging system. As soon as EGF was applied, a rapid increase in fluorescence intensity of YFP was shown. These results demonstrate a promising use of this on-chip fluorescence imaging system in biological research and drug development.

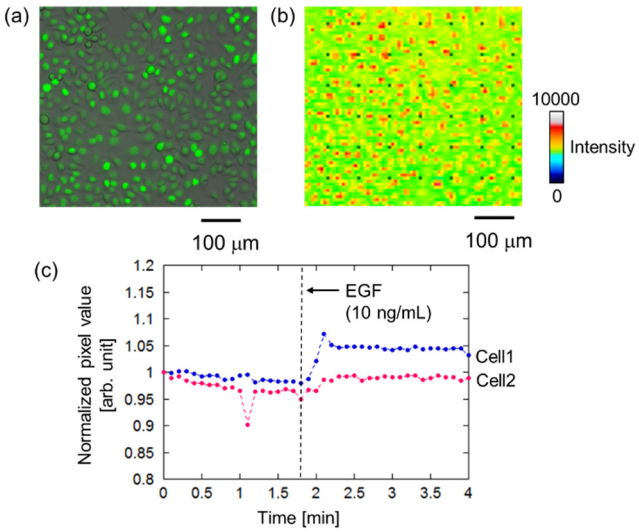

Fig. 4. (a) Microscopic image of EKAREV-expressed Hela cells obtained by a tabletop-type microscope. Merged image of bright-field image and fluorescent image. (b) Obtained image by the on-chip fluorescence imaging system. (c) Time course of detected fluorescence intensity in each individual cell.

4. Conclusion

We have developed the on-chip fluorescence imaging system based on CMOS technology. The ultra-thing glass bottom chamber was developed for on-chip fluorescence imaging of cultured cells. Fluorescence signals from cultured cells were successfully detected. The on-chip imaging system using the CMOS fluorescence image sensor has several desirable features including compatibility with plastic microfluidic chips and low-cost mass production, and integratability with the electrical control and cellular incubation system.

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