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High-content analysis of single cells directly assembled on CMOS sensor based on color imaging

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ABSTRACT

A complementary metal oxide semiconductor (CMOS) image sensor was applied to high-content analysis of single cells which were assembled closely or directly onto the CMOS sensor surface. The direct assembling of cell groups on CMOS sensor surface allows large-field (6.66 mm × 5.32 mm in entire active area of CMOS sensor) imaging within a second. Trypan blue-stained and non-stained cells in the same field area on the CMOS sensor were successfully distinguished as white- and blue-colored images under white LED light irradiation. Furthermore, the chemiluminescent signals of each cell were successfully visualized as blue-colored images on CMOS sensor only when HeLa cells were placed directly on the micro-lens array of the CMOS sensor. Our proposed approach will be a promising technique for real-time and high-content analysis of single cells in a large-field area based on color imaging.

1. Introduction

Recently, imaging cytometry has been paid much attention to as a powerful tool for high-content cell analysis in place of flow cytometry (De Vos et al., 2010; Matula et al., 2009; Won et al., 2006). The measurement principle is based on scanning microscopy of cell groups on flat substrates, such as glass slide, culture dish or microtiter plate. Imaging cytometry has an advantage for use in real-time imaging of cell groups at a single-cell level. However, time-resolution is limited because a laser scanning is required to obtain two-dimensional (2D) images. In order to overcome these obstacles, 2D image sensor-based analysis of cell groups has been proposed as one of possible approaches. In this assay format, cell groups are two-dimensionally placed and extremely closed to 2D photosensor at a minimum of 100 μm from the surface (Ozcan and Demirci, 2008). This approach allows us to analyze cell groups simultaneously in a large field at a single-cell level as well as to miniaturize the system. For example, lens-less imaging has been reported using charge coupled device (CCD) or complementary metal oxide semiconductor (CMOS) sensor. Individual cells were recognized as diffraction patterns or shadow images projected onto CCD or CMOS sensor under light irradiation. Based on the above phenomenon, various types of cells in size and shape (e.g. mammalian cells or yeast cells) have been successfully distinguished by lens-less imaging (Cui et al., 2008; Seo et al., 2009; Su et al., 2009). However, until now, little information has been available on fluorescent or chemiluminescent detection of immune-stained or dye-stained cells.

On the other hand, our research group has proposed a novel DNA microarray analysis system based on fluorescent and chemiluminescent detection using a 2D photosensor, thin film transistor (TFT) photosensor. The fluorescent and chemiluminescent signals based on DNA hybridization were successfully detected (Hatakeyama et al., 2008, 2009; Tanaka et al., 2006). Furthermore, circular DNA spots were clearly visualized by the direct immobilization of probe DNA onto the photosensor surface because DNA hybridization occurred just above the photosensor surface. The direct imaging technique will enable us to efficiently detect light signals from cell surface or intracellular molecules based on fluorescence or chemiluminescence.

In this study, 2D imaging of cell groups was investigated using CMOS sensor by placing cell samples directly on the micro-lens array of the CMOS sensor. Furthermore, color imaging of stained cells were examined at the assay conditions. Our proposed cell analysis system will allow us to develop a novel miniaturized cytometer for high-throughput cell profiling.

2. Materials and methods

2.1. Materials

A commercially available CMOS sensor (IUCM130FO2; Trinity, Saitama, Japan) was used as a 2D photosensor. The CMOS sensor with RGB color filter array composes of 1.3 million pix-

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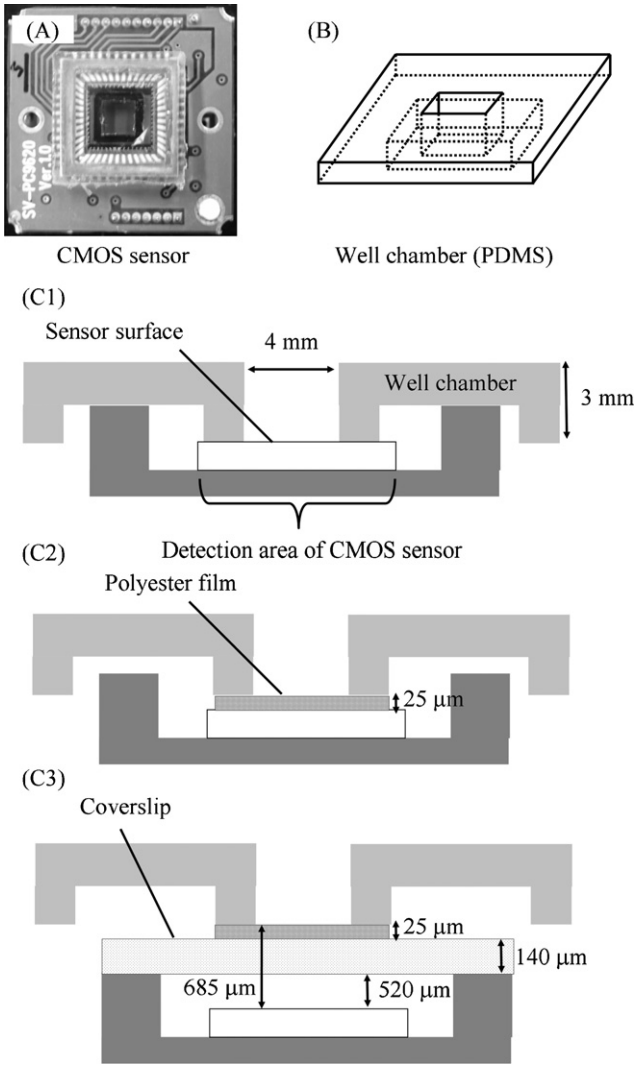

Fig. 1. (A) Photograph of CMOS sensor. (B) Schematic of PDMS well chamber. (C) Sectional view of CMOS sensor fabricated with well chamber. The distances between the well chamber and the sensor surface are 0 μm (C1), 25 μm (C2) and 685 μm (C3).

els with 5.2 μm × 5.2 μm in each size and 6.66 mm × 5.32 mm in entire active area. TViewer (Trinity, Saitama, Japan) was used for imaging software. A flat light-emitting diode (LED; CR-30S; 60 mm × 60 mm) was purchased from Nissin Electronic (Tokyo, Japan) as a white light source. Particle Size Standard Kit (average diameter: 10.2 or 15.3 μm), Blank Calibration Particle (average diameter: 20.9 μm) and biotin-labeled polystyrene particles (average diameter: 15.3 μm) were obtained from Spherotech (IL, USA).

2.2. Fabrication of well chamber on CMOS sensor

To evaluate the effect of separation distance from the sensor surface on the CMOS sensor imaging, polydimethylsiloxane (PDMS) well chamber was fabricated for placing HeLa cells (or micro-particles) at various distances from the sensor surface (0, 25 or 685 μm from the surface) (Fig. 1). At first, the protection glass attached onto CMOS sensor with adhesion bond was removed by using a cutter knife to make sensor surface exposed. The surface of micro-lens array on pixels was defined as sensor surface at zero distance (Z = 0 μm). A well chamber was directly attached on the surface to perform the cell imaging at zero distance (Fig. 1(C1)). To keep the distance between sample and sensor surface at 25 μm, a polyester film with a height of 25 μm (T-UV; TOCHISEN, Tochigi, Japan) was attached to CMOS sensor, and the well chamber was fabricated onto the film (Z = 25 μm) (Fig. 1(C2)). Furthermore, to construct the well chamber with the distance between sample and sensor surface at 685 μm, a coverslip with a height of 140 μm was put 520 μm above the sensor surface and a well chamber was attached to the coverslip across a polyester film of 25 μm (Z = 685 μm) (Fig. 1(C3)).

2.3. CMOS senor imaging of micro-particles and mammalian cells

To evaluate the effect of separation distance between sample and sensor surface on imaging, various micro-particles were visualized by CMOS sensor under light irradiation condition. Micro-particle (diameter: 10.2, 15.3 or 20.9 μm) suspensions (approximately 10^6 particles/ml) in phosphate buffered saline (11.8 mM phosphate buffer containing 137 mM NaCl; PBS (pH 7.4)) (5 μl) were introduced into the well chamber (Z = 0, 25 or 685 μm). After covering the well chamber with polymethyl methacrylate (PMMA) lid, 2D white LED light was irradiated on the sensor surface, and subsequently signal measurements were performed by CMOS sensor.

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37 °C in a humidified incubator containing 5% CO_2. The harvested cells were washed and re-suspended in 500 μl PBS. The CMOS sensor imaging was performed in the same manner as mentioned above. To prepare trypan blue-stained cells, the suspension of HeLa cells was heated at 80 °C for 5 min to induce the damage to cell membrane. After the cell suspension (100 μl) was mixed with 5 μl of trypan blue (0.4%, w/v), 4 μl of the solution was applied onto the well chamber (Z = 0 μm) of the CMOS sensor. Subsequently, the stained cells were visualized by the CMOS sensor.

2.4. Chemiluminescent detection by CMOS sensor

Chemiluminescent imaging of micro-particles labeled with horseradish peroxidase (HRP) on CMOS sensor was performed by the following protocol. At first, 50 μl of biotin-labeled micro-particle was mixed with 500 μl of 5 μg/ml HRP-conjugated streptavidin (SA) (HRP-SA; Thermo, IL, USA). After 30-min reaction at room temperature, the particles labeled with HRP-SA (HRP-particles) were washed twice with and re-suspended in 200 μl PBS. The HRP-particle suspension (3 μl) and a chemiluminescent substrate (40 μl) (SuperSignal ELISA Femto Maximum Sensitivity Substrate; PIERCE, IL, USA) were introduced into the well chamber. Subsequently, CMOS images were taken in dark with 7.5 fps frame rate. Background images (the well chamber was filled with only PBS) were subtracted from raw images to remove background signals at each pixel.

Furthermore, chemiluminescent imaging of HeLa cells was investigated. Prior to the application of HeLa cells onto CMOS sensor, the sensor surface was coated with 0.1% gelatin solution (ultrapure water with 0.1% gelatin; Millipore, MA, USA) for 60 min to improve the cell adhesion. Subsequently, HeLa cells in 60 μl of DMEM were introduced onto the well chamber (Z = 0 μm) at a concentration of 3×10^4 cells/ml and incubated in CO_2 incubator for 3 hours to attach cells onto the sensor surface. After washing with PBS using a micro-pipette, biotin-labeled anti-CD44 IgG antibody (BD pharmingen, CA, USA) was added according to the manufacture's protocol and incubated at room temperature for 30 min. After washing with PBS to remove excess the biotin-labeled anti-CD44 antibody, HRP-SA (BD pharmingen, CA, USA) solution was added and incubated for 30 min at room temperature. After washing with PBS, the chemiluminescent substrate was added to the well chamber and chemiluminescent imaging was carried out in a dark condition with 7.5 fps frame rate using CMOS sensor.