CMOS image sensor for fluorescent beads counting

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1. Introduction

Single molecule enzymatic assay is a highly efficient method by which ultra-low concentrations of proteins in complex samples are measured [1-3]. This method has been applied to bead-based enzyme-linked immunosorbent assay (ELISA) [4]. Target molecules are captured by beads with specific antibodies, and detected with fluorogenic substrates. By enclosing each bead into femtoliter chamber and counting the number of beads with fluorescence, measurement of low concentrations of proteins is achieved. This method is applicable to highly sensitive and earlier diagnosis for diseases and infections.

In the conventional system, a fluorescence microscope is used to detect the beads with target molecules. For practical use, it is important to simplify and realize a portable system. Complementary metal-oxide-semiconductor (CMOS) image sensors for bioimaging [5-6] are suitable for this system. By fabricating a femtoliter chamber array on an image sensor, very compact counting device for fluorescent beads as shown in Fig. 1 is realized. In this study, we fabricated a special CMOS image sensor and demonstrated imaging of fluorescent beads.

2. CMOS image sensor with light guide array for counting fluorescent beads

In order to detect fluorescence from beads efficiently, characteristics of color filters for standard color image sensor are not enough. In this work, an interference filter is employed because the cut-off or cut-on wavelength can be designed and high extinction ratio is achieved. Indeed, interference filters are widely used fluorescence microscopy. However, its transmission spectrum depends on incident angle. Thus, when a fluorescent sample in the vicinity is observed, scattered excitation light is transmitted even if the sample is illuminated by normal incident light. To solve this problem, we propose to use a Si-based femtoliter chamber array plate based on the light guide array we have previously reported [7]. The chamber array plate is a Si plate with a hole array and cut off the angled incident component. Because the normal incident component of the excitation light can be filtered out by the interference filter, high excitation light rejection is achieved by the combination of these devices.

Figure 2 and Table I show the micrograph and specification of the fabricated sensor module with the chamber array.
The sensor was fabricated with a 0.35-µm 2-poly 4-metal standard CMOS technology of austriamicrosystems. The interference filter is designed to be transparent for light at wavelengths longer than 500 nm. A chamber array plate is placed on the filter. The diameter of the holes is approximately 4 µm, the period is 15 µm. For waterproofing, bonding wires of the sensor were covered with epoxy resin.

3. Imaging experiment

Figures 3 show experimental setup. In this experiment, commercially available yellow fluorescent beads (FP-4052-2, Spherotech) are used for the demonstration of fluorescence imaging capacity. The absorption and emission peaks are 470 nm and 485 nm, respectively. The average diameter is 4.1 µm. The concentration was 0.1 %w/v.

The suspension was dispersed on the sensor and rinsed with pure water (Fig. 3(a)). Blue light at wavelengths of 455-490 nm is irradiated to the sensor with an incident angle of 45 deg (Fig. 3(b)). The frame rate of the CMOS sensor was set to 2.2 frames per second. The fluorescence microscopy and the CMOS sensor images are shown in Fig. 4 (a) and (b), respectively. For comparison, the overlaid image of (a) and (b) is shown in Fig. 4 (c). The holes of the chamber array are fabricated every 4 pixels of the sensor. They are on the intersection points of the vertical and horizontal grid that are guides for eyes. The yellow points are the points where fluorescent beads are observed in both of the images. All red points are on the intersection points of the grid and overlaid with green points, while some green points deviated from the intersection points are not overlaid. These results show that the beads in the holes are successfully and selectively detected with the proposed system.

4. Conclusions

We demonstrated a CMOS image sensor for counting fluorescent beads. By integrating the interference filter and the light guide array plate, the excitation light is successfully filtered out and fluorescent beads were imaged. This simple detection system would be applied to realize a portable single molecule enzymatic assay system.

Acknowledgements

This work was supported by Japan Science and Technology Agency, Core Research for Evolutional Science and Technology (JST-CREST). This work is supported by VLSI Design and Education Center (VDEC), The University of Tokyo with the collaboration with Cadence Corporation and Mentor Graphics Corporation.

References