SINGLE-MOLECULE ENZYME ASSAY WITH A LENSLESS FLOUORESTCENT IMAGING DEVICE

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ABSTRACT

In this paper, we report on demonstration of digital counting of enzyme based on fluorescent femtoliter droplet array imaging by using a lensless CMOS image sensor. The result supports the device can be applied to digital enzyme linked immunosorbent assay (ELISA). Digital ELISA is an ultra-sensitive detection technique for a biomarker [1, 2]. In the previous work, we developed a miniature lensless CMOS fluorescence imaging system and demonstrated to image fluorescent dye in a microchamber array [3]. In this work, we improved the device to observe fluorescent signal from single enzymes in a microchamber array. As a result, we successfully performed single-molecule enzymatic assay with the lensless device.

KEYWORDS: digital ELISA, single molecule detection, CMOS image sensor, lensless imaging

INTRODUCTION

Recently, digital ELISA techniques have been developed to detect proteins/biomarkers with ultra-high sensitivity. In this technique, the reaction using in ELISA techniques are performed in a femtoliter chamber array. In the ultra-small chamber, fluorescent molecules (e.g. fluorescein) produced by even single enzyme molecule can be detected and counted using fluorescence microscopy. Thus, the target molecule can be indirectly counted and the detection sensitivity drastically improved. However, the conventional digital ELISA systems are relatively large because they are based on a fluorescent microscope. In previous work, we have proposed and fabricated a miniaturized system based on a lensless CMOS imaging device. In this work, we tried single-molecule enzyme assay with the device.

EXPERIMENTAL

The schematic and photograph of the device are shown Figs. 1, 2. A custom CMOS image sensor is used. The excitation light is reflected by total internal reflection on the bottom of the sample and absorbed by the yellow filter on the sensor. The thickness is approximately 20 μm. Droplet chamber

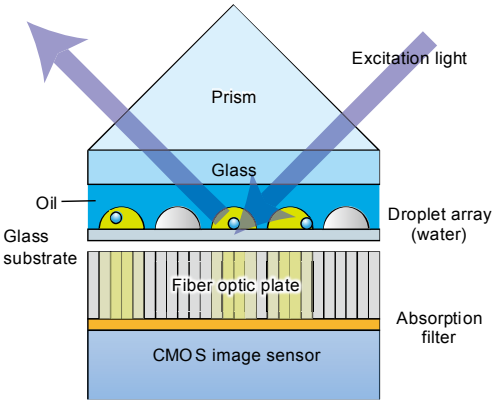

Figure 1: Schematic of lensless imaging device structure.

arrays are prepared as a specimen (Fig. 3). The substrate has hydrophilic-in-hydrophobic structures and water droplets were separated by oil (Fluorinert) [1]. The diameter and spacing of droplets are 5 μm and 20-100 μm, respectively. The substrate of the chamber is a 50 μm-thick cover glass plate (No.000, Matsunami).

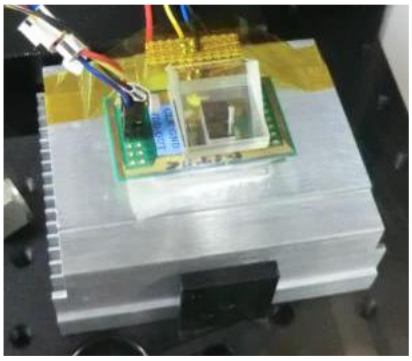

Figure 2. Photograph of the fabricated imaging device.

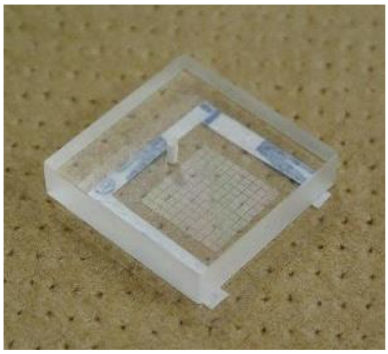

Figure 3. Photograph of a droplet chamber array device.

RESULTS AND DISCUSSION

We performed single-molecule enzymatic assay experiment by introducing fluorescence substrate, fluorescein di-β-d-galactopyranoside (FDG), and enzyme, β-galactosidase (β-gal). The device is illuminated by excitation light at 470 nm with an incident angle of 45 deg. The fluorescence from fluorescein generated as a result of the enzyme reaction was imaged by the sensor device and processed by a deconvolution technique (Fig. 4) [4, 5]. The β-gal concentration estimated form the number of the fluorescent chamber was in agreement with the sample concentration. And, the concentration as low as 0.3 pM was measured.

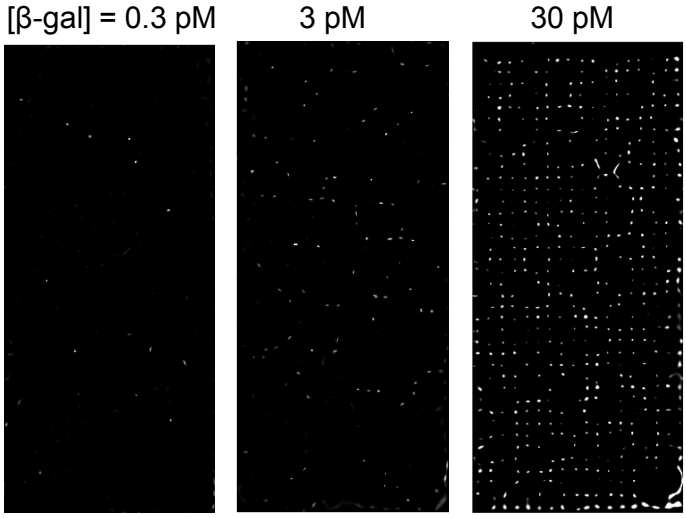

Figure 4. Fluorescence image obtained with the lensless imaging device. The images are deconvoluted for spatial resolution improvement.

CONCLUSION

We successfully demonstrated immunoassay on the lensless fluorescence imaging device by using a micro-droplet chamber array. The result shows that the device can be applied for digital ELISA measurement. The reaction time required to obtain enough fluorescent is several hours with the present device. However, it is expected that it can be reduced by optimizing the optics and the pixel size of the sensor.

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