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High-sensitive and high-efficient biochemical analysis method using a bionic electronic eye in combination with a smartphone-based colorimetric reader system

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ABSTRACT

Bionic electronic eye (Bionic e-Eye), a developed smartphone-based colorimetric reader system, consists of smartphone or pad (iPhone 4s or iPad 3) as detection instrument and portable accessory as illumination provider integrating with a wide-angle lens, a piece of lowest-power electro luminescent and a custom-made dark hood. A 96-well microtiter plate (MTP) was positioned on the electro luminescent and Bionic e-Eye captures the detection images by the back camera of smartphone. Being similar to human visual system, the hue, saturation and value (HSV, also called hex cone model) color model was employed in image processing algorithm of Bionic e-Eye. Optimized system dimension was determined by the system steadiness experiment of different photograph distances. Moreover, the commercially available bicinchoninic acid (BCA) protein assay and cell counting kit (CCK8) assay were carried out to evaluate this Bionic e-Eye. Analytical performance of Bionic e-Eye had the better precision, higher sensitivity than microtiter plate reader (MTPR) and previous smartphone-based colorimetric reader for both two assays. Also, Bionic e-Eye using optical image detection had simultaneous and synchronous working mode, while MPTR using machine moving detection had asynchronous working mode in high throughput detection. Therefore, Bionic e-Eye will be an ideal point-of-care (POC) colorimetric detection device in the field of clinical application, industrial quality control, environment monitoring, and food assessment.

1. Introduction

In the recent decades, the microtiter plate (MTP)-based enzyme linked immunosorbent assay (ELISA) is the gold standard in various bioanalytical settings [1–3]. However, the microtiter plate reader (MTPR) is low-efficient and low-precision due to moving platform, which cannot solve the result differences induced by solution changes in the detection of several minutes. Therefore, a high-efficient, high-precision and user-friendly biochemical analysis device is demanded in "point-of-care" (POC) applications, especially biomarker diagnosis in clinical facilities. Devices for POC analysis should be portable, quick, high-throughput, and easy to use, which should also be especially critical for particular scenes such as emergency room, operating theaters, and intensive care units. For an integrated and self-standing device, it should perform the entire analytical process including high-speed data acquisition and processing.

Currently, smartphones are fully automated and equipped with a high resolution camera, a powerful processor with high storage capacity, wireless connectivity, real-time geo-tagging, secure data management, and cloud computing, so they can provide a hopeful digital detection and analysis platform for many fields such as point-of-care (POC) diagnosis, mobile healthcare, and biochemical analysis. As smartphones have extremely large users, they are widely used in commercial purposes for life day management. Smartphone-based bioanalytical platforms are becoming a new hotspot of biochemical analysis research. These applications include smartphone-based microscopy [4,5], fluorescent imaging [6–8], imaging cytometry [9], electrocardiography [10,11], lateral flow assays [12,13], surface plasmon resonance-based sensing [14], electrochemical sensing [15], immunoassays (IAs) [16–21], and other applications [22–27]. Also, the accessories of

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smartphone-based system appear one after another to improve detection performance [28,29]. A developed smartphone-based colorimetric reader (SBCR) [30] is validated as an extensive outreach and tremendous potential in healthcare and bioanalytical sciences by conventional procedures, which is a prerequisite for routine bioassays by employing any novel approach. Compared with the commercially-available bulky and expensive MTPR, SBCR can perform the *in vitro* diagnosis and bioanalysis assays at decentralized and remote facilities. However, the SBCR system structure has some shortcomings in system dimension and detection stability. In addition, the SBCR with RGB color model has not high enough sensitivity and uncertain color channel selection for different biochemical analysis assays.

In this study, bionic electronic eye (Bionic e-Eye) was proposed as a developed SBCR system, which improved the existing colorimetric reader in system structure, sensitivity and efficiency. Bionic e-Eye was high-efficient system using a smartphone (iPhone 4S and iPad 3) integrated with simple accessories including a piece of electro luminescent, a wide-angle lens, and a dark hood. The best system dimension was determined by the system steadiness experiment of different photograph distances. Bionic e-Eye innovatively employed HSV color model, which was similar to human visual system, as the core of the algorithm to promote system analytical performance. Two model assays (the commercially available BCA protein assay and CCK8 cell number assay) were employed to determine the performance of Bionic e-Eye. The analytical performance of Bionic e-Eye (dynamic range, sensitivity, precision, and detection limit) was evaluated and compared with that of MTPR and previous SBCR. All the details will be discussed in the following sections.

2. Materials and methods

2.1. Reagent and setup

Phosphate buffered saline (0.1 M PBS, pH 7.4) are procured from Invitrogen, Germany, while Tween 20 is from Carl Roth GmbH, Germany. MTPR is SpectraMax Paradigm (Molecular Devices, USA). The smartphone is iPhone 4s and the pad is iPad 3 (Apple, USA). Electro luminescent is made in the size of 118 mm × 160 mm (SuZhou JieMing GuangDian Science and Technology Co., Ltd., China). The wide-angle lens has 0.4 amplification factor (HangZhou Jiatu Transportation Facility Co., LTD., China). All buffers and solutions are prepared in autoclaved ultrapure water (MilliQ, Millipore, Germany). The BCA protein assay kit and the 96-well polystyrene plate are bought from Thermo Fisher Scientific, Germany. Bovine-serum albumin (BSA) is from Sigma-Aldrich, Germany. HepG2 cell line is bought from American Type Culture Collection (ATCC) and cultured in DMEM medium which includes 10% heat inactivated FBS and 0.5% antibiotic solution (10 mg mL^{-1} streptomycin and 1000 U/mL penicillin). Then it is incubated at 37 °C in humidified air with 5% CO_2 in an incubator (Thermo, USA). When the confluent cells reached 80%, 0.25%, Trypsin-EDTA is used to dislodge cells from the flask to 96-well plates. Each experimental scheme is repeated three times. All of the reagents of cell culture are purchased from Gibico, USA. CCK8 kit is obtained from Dojindo Lab., Kumamo-to, Japan.

2.2. BCA protein assay

The BCA protein assay is performed as the product instructions brochure. Initially, the working reagent is freshly prepared by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B. The BCA protein assay procedures involve the dispensing of 20 μL of BSA (varying concentrations from 0 to 500 μg mL^{-1}) into the MTP well followed by the addition of 200 μL of the working reagent to each well. The MTP is mixed thoroughly on a plate shaker for 30 s. Subsequently, the BCA protein assay is monitored for 1 h to record the readouts of MTPR every 3 min. The absorbance of the colorimetric solution is determined by MTPR at 560 nm. Meanwhile, the BCA protein assay is monitored by Bionic e-Eye every 2 min in 1 h. The assay is kept at 37 °C during the whole process.

2.3. CCK8 assay

As modified method of MTT, CCK8 assay is commonly applied to assess cell number or viability. Briefly, HepG2 are seeded in the 96-well MTP at different seeding densities. After cell cultured for 24 h, 30 μL CCK8 solution is added to each well of the MTP, and then cells are incubated for 1.25 h at 37 °C. Finally, Bionic e-Eye and MPTR detect the CCK8 assay every 15 min during the time. The absorbance of the colorimetric solution is determined by MTPR at 450 nm.

2.4. Bionic e-Eye and image analysis

Bionic e-Eye consists of smartphone or pad (iPhone 4s or iPad 3) as detection instrument and portable accessory as illumination provider (Fig. 1A). The portable accessory contains a wide-angle lens, a dark hood, and a piece of lowest-power electro luminescent, which could provide stable and low-power illumination for Bionic e-Eye. The wide-angle lens is fixed in the dark hood at the back camera of smartphone. The related dimensions of dark hood are shown in Fig. 1A. 96-well MTP, containing the immunoassay's colorimetric end-products, is placed at the designated illuminated positions on the electro luminescent. Subsequently, the dark-hood is placed on the base holder and the colorimetric images are taken by putting the smartphone inside the designated smartphone containment on the top of the hood. As shown in Fig. 1B, the image processing algorithm initially determine the center of each MTP well. Then square image (10 × 10 pixels) around the center is cropped from colorimetric image to calculate the mean pixel intensity. Bionic e-Eye employs HSV color model instead of RGB color model in the mean pixel intensity calculation (Eqs. (1)–(4)).

$$H' = \begin{cases} 0, & \text{if } \max(R,G,B) = \min(R,G,B) \\ 60 \times \dfrac{(G-B)}{\max(R,G,B) - \min(R,G,B)}, & \text{if } R = \max(R,G,B) \text{ and } G \geq B \\ 60 \times \dfrac{(G-B)}{\max(R,G,B) - \min(R,G,B)} + 360, & \text{if } R = \max(R,G,B) \text{ and } G \leq B \\ 60 \times \dfrac{(B-R)}{\max(R,G,B) - \min(R,G,B)} + 120, & \text{if } G = \max(R,G,B) \\ 60 \times \dfrac{(R-G)}{\max(R,G,B) - \min(R,G,B)} + 240, & \text{if } B = \max(R,G,B) \end{cases} \quad (1)$$

$$H = \frac{H'}{360} \quad (2)$$

$$S = \begin{cases} 0, & \text{if } \max(R,G,B) = 0 \\ \dfrac{\max(R,G,B) - \min(R,G,B)}{\max(R,G,B)}, & \text{otherwise} \end{cases} \quad (3)$$

$$V = \frac{\max(R,G,B)}{255} \quad (4)$$

where H, S, V, R, G, B stand for hue channel, saturation channel, value channel, red color channel, green color channel, and blue color channel, respectively. The HSV color model is built by A. R. Smith in 1978 and is similar to human visual system. The images captured by acquisition unit such as smartphone usually use the color space of RGB color model. Therefore, the equations of Eqs. (1)–(4) are employed to convert the values of RGB model to the values of HSV model. The ranges of hue, saturation and value are 0–360, 0–1 and 0–1, respectively. Eq. (2) is used to obtain the map value of hue channel in the range of 0–1.