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Development of a Smartphone-linked Optical Biosensing System for Point-of-care Testing Applications

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We report a miniaturized batteryless colorimetric biosensing system for measuring saliva components. Our system utilizes a CMOS image sensor and an LED light source embedded in smartphones as a part of the optical system. We measured the concentration of chemical substances by observing color changes of the coloring reagent DA-64, which is specific for hydrogen peroxide. The concentration calculated from the RGB scores of colorimetric images showed strong correlations ($r > 0.95$) with the concentrations of the samples. This result shows the possibility of biochemical measurements using miniaturized biosensing systems to determine physiological conditions.

1. Introduction

Advances in AI technology have strongly encouraged the spread of advanced and intelligent systems based on big data.[1,2] In particular, the use of vital information is increasing for individual patient-oriented health promotion, disease prevention, and optimal care.[3,4] Under these circumstances, the measurement of physiological status with smartphone-connected devices has become more promising than before the COVID-19 pandemic. Conventional smartphone-related healthcare systems often handle physical information such as heart rate, blood pressure, and weight. However, there are still many technical challenges in the use of chemical biomarkers such as lactic acid, glucose, and uric acid, which reflect physiological conditions such as oxygen metabolism, diabetes, and hyperuricemia, respectively.[5–7] Biochemical measurements using smartphone-linked sensors have been intensively studied.[6,8–11] For instance, wearable sensors for chemical components of interstitial fluid and sweat utilizing Bluetooth,[8] stretchable and screen-printed electrochemical sensors for glucose,[6,9] and mouthguard biosensors for salivary uric acid[10] have the potential to be used for personal health purposes. However, these measurement techniques require batteries for transmission, and there are various issues in terms of their size, weight, and cost.[11,12] Colorimetric analysis using smartphones is gaining popularity as one of the technical methods that do not require batteries to run the system.[13–17] With personal daily use in mind,

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smartphone-linked microsystems are desired to be miniaturized, easy to operate, and independent of the external environment. We focused on the use of cameras and LEDs embedded in smartphones as part of a miniaturized colorimetric system. This system enables biochemical measurement at any place and can evaluate components in body fluid such as saliva simply by photographing redox reactions. In this paper, we report on the visualization of enzymatic reactions using a coloring reagent, the fabrication of a miniaturized biosensing system, the results of hydrogen peroxide, lactic acid, and glucose measurements using our system, and the correlation between absorbance and RGB light.

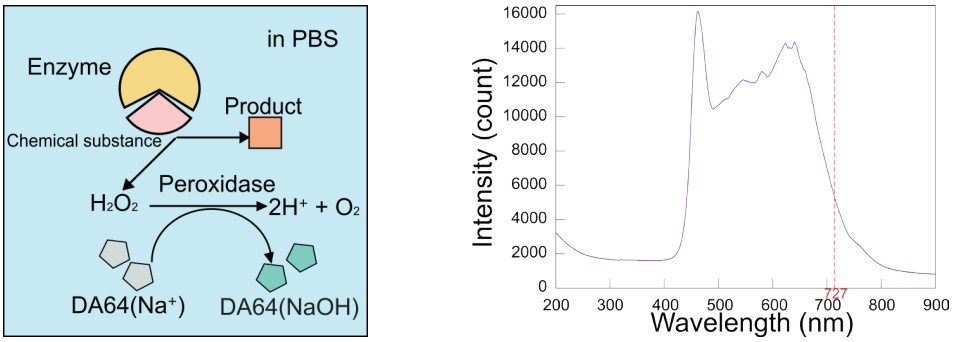
2. Experimental Method

2.1 Principle of smartphone-linked biosensing system

A miniaturized and batteryless system using optical elements in a smartphone determines the concentration of hydrogen peroxide, lactic acid, and glucose through colorimetric reactions. Figure 1(a) shows the reaction system. The hydrogen peroxide levels are converted into a color change of DA-64 (043-22351, for biochemistry, Fujifilm Wako Pharmaceuticals Corporation). Any reactions that result in the production of hydrogen peroxide can be converted to a change in the RGB value extracted from the images. In particular, in lactic acid and glucose measurements, hydrogen peroxide is produced by a redox reaction using lactate oxidase (LOD) and glucose oxidase (GOD), respectively. The reaction for lactic acid with LOD [Eq. (1)] and the reaction for glucose with GOD [Eq. (2)] are shown below. DA-64 takes on a cyan color in the presence of hydrogen peroxide. The absorbance of DA-64 is maximum at 727 nm, the emission edge of LEDs embedded in typical smartphones. [Fig. 1(b)].

$$\text{lactic acid} + O_2 \xrightarrow{\text{LOD}} H_2O_2 + \text{pyruic acid} \tag{1}$$

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} H_2O_2 + \text{gluconic acid} \tag{2}$$



(a) (b)

Fig. 1. (Color online) Scheme of smartphone-linked optical detection of chemical substances. (a) Color reaction using DA-64 for detecting redox conversion and (b) typical emission spectrum of LEDs of smartphones.

2.2 Reagents

The reagents used in the preparation are listed in Table 1. As buffer solutions, phosphate-buffered saline (PBS) solutions were prepared by dissolving potassium dihydrogen phosphate and disodium hydrogen phosphate anhydrate in 1000 mL of 0.9% sodium chloride solution, yielding a 50 mM phosphate buffer with a pH of 7.4. The coloring reagent, the DA-64 solution, was prepared by dissolving 1.86 mg of DA-64 and 0.5 mg of peroxidase from horseradish in 10 mL of PBS. Hydrogen peroxide solutions for measurement were prepared by dissolving respective amounts of hydrogen peroxide in PBS. Lactic acid solutions for measurement and selectivity evaluation were prepared by dissolving respective amounts of L-lactic acid in PBS. Glucose solutions for measurement and selectivity evaluation were prepared by dissolving respective amounts of D-(+)-glucose in PBS. Enzyme solutions (0.1 unit/μL), namely, LOD and GOD solutions, were prepared by dissolving LOD and GOD in PBS. Ammonia solutions for selectivity evaluation were prepared by diluting 10 wt% ammonia solution with PBS.

2.3 Spectroscopic study of colorimetric reaction of DA-64

Before the biochemical measurement with a smartphone, we verified the behavior of DA-64 in the presence of hydrogen peroxidase by conventional absorption spectroscopy. A DA-64 solution (80 μL) and hydrogen peroxide (10 μL) were dropped into a microplate. Then, the samples were incubated at 37 ℃ for 10 min and their absorbance spectra ranging from 600 to 850 nm were measured by using a microplate reader (51119250, Thermo Fisher Scientific, USA). We determined the concentrations of lactic acid and glucose by examining the coloring redox reaction. The DA-64 solution (80 μL), enzyme solution (5 μL), and either lactic acid or glucose solution (10 μL) were prepared in the microplate. The samples were then incubated at 37 ℃ for 10 min and the absorbance spectrum was measured.

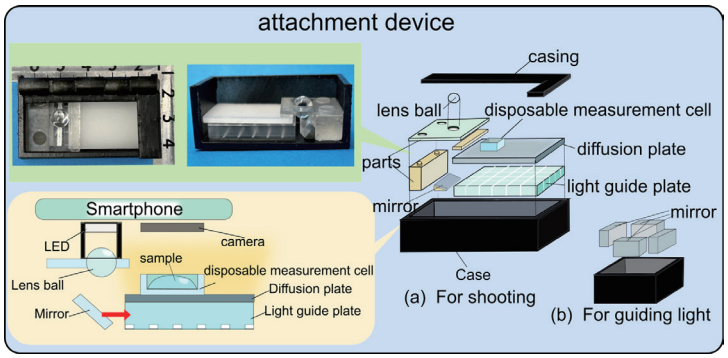
Table 1
List of reagents.

Reagent	Lot No.	Manufacturer
potassium dihydrogen phosphate	164-22635	Fujifilm Wako Co.
disodium hydrogen phosphate anhydrate	042-30055	Fujifilm Wako Co.
sodium chloride	195-15975	Fujifilm Wako Co.
DA-64	043-22351	Fujifilm Wako Co.
peroxidase	P8415-1KU	Fujifilm Wako Co.
hydrogen peroxide	086-07445	Fujifilm Wako Co.
lactic acid	129-02666	Fujifilm Wako Co.
D-(+)-glucose	049-31165	Fujifilm Wako Co.
LOD from Aerococcus viridans [LOX II]	T-47 (638-22641)	Asahi Kasei
GOD	G7141-10KU	Sigma
10% ammonia solution	013-17505	Fujifilm Wako Co.

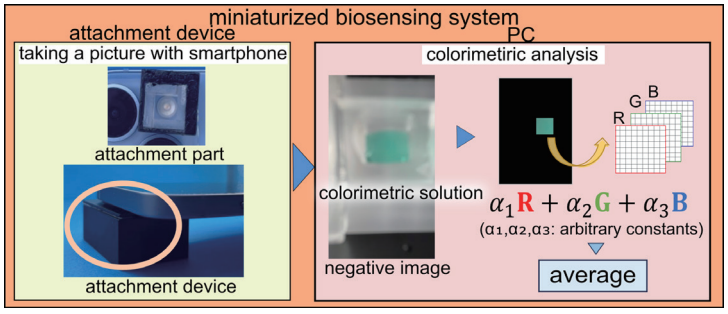
2.4 Fabrication of a batteryless colorimetric biosensing system

A batteryless colorimetric biosensing system consists of three components: a polydimethylsiloxane (PDMS) cell, an optical biosensing device, and an image processing software for colorimetric analysis. In the fabrication of the reaction cell, PDMS (SYLGARD 184 Silicone Elastomer Kit, Dow Silicones Co., USA) was mixed with a hardener catalyst (10 wt% of PDMS) and cast into a mold. It was cured in a constant-temperature dryer (OF-300B, AS ONE, Japan) at 85 °C for 20 min.

The optical biosensing device was composed of a lens ball, a light guide plate, and a diffusion plate. The LED light was uniformly diffused by the light guide plate and diffusion plate to illuminate the reaction cell from the back (Fig. 2). A lens ball with a focal length of 7 mm, which was fixed on a polymethyl methacrylate (PMMA) stay guided the emission of the LED to the side face of the diffusion plate via the lab-fabricated mirror. The light reflected by the mirror would be incident on their cross section and the surface of the illuminated light guide plate. In addition, attachment parts for LED light were designed to enclose mirrors in four directions. This enabled the collection of more light to the lens ball. To eliminate ambient light, we placed this structure in a black box that was fabricated by stereolithography 3D printing.



(a)



(b)

Fig. 2. (Color online) Schematic images of (a) attachment device and (b) measurement procedure of smartphone-linked optical biosensing system.

The colorimetric analysis software is a Python program using OpenCV, which is an image analysis library. When evaluating the colorimetric chemical reaction with a CMOS image sensor, the spectroscopic information (i.e., wavelength information) is replaced by the intensities of the light passing through red, green, and blue filters. Quantification is carried out by applying a filter that extracts features indicative of DA-64 concentration changes to a two-dimensional matrix in which the image is divided into red, green, and blue channels. This method enhances the flexibility and versatility so that the software can be used for other color reactions. This time, the priority was given to evaluating the feasibility of the DA-64 measurement rather than to optimizing the filter. For this reason, the CMOS images of hydrogen peroxide, lactic acid, and glucose were converted to their respective concentrations through the simple linear operation on each matrix.

2.5 Characterization of smartphone-linked biosensing system

Various redox reactions, including enzymatic reactions, can be measured as changes in hydrogen peroxide concentration. Therefore, the characterization of the smartphone-linked biosensing system started with the determination of standard hydrogen peroxide solution. 10 μL of hydrogen peroxide solutions of various concentrations were added to reaction cells containing 80 μL of DA-64 solution each. The samples were incubated at 37 ℃ for 10 min and a photograph of the mixture of DA-64 and hydrogen peroxide was taken for measurement. We also tested whether the system was suitable for measuring lactic acid and glucose. Lactic acid (10 μL) and LOD solutions (5 μL), instead of hydrogen peroxide solution, were added to the reaction cells containing DA-64. After incubation at 37 ℃ for 10 min, we measured the production of hydrogen peroxide resulted from the enzyme reaction, as explained above. Glucose was also measured similarly. We also confirmed the selectivity by using the reaction cells containing lactic acid, glucose, and ammonia.

In this test, the region of interest (ROI) was determined by cropping the region where the color intensity was relatively uniform. The software analyzed each pixel in turn, starting from the top left of the image, and divided the image into three RGB matrices. To estimate the concentration of DA-64, we employed an RGB score, which is the value obtained by arbitrarily weighting each matrix. The following equation represents the RGB score using the three RGB matrices extracted from the ROI. (***R***, ***G***, and ***B***: the array of the red, blue, and green components, $\alpha 1$, $\alpha 2$, and $\alpha 3$： arbitrary coefficients).

$$\text{RGB score} = \frac{1}{3}\left(\alpha 1 \cdot \boldsymbol{R} + \alpha 2 \cdot \boldsymbol{G} + \alpha 3 \cdot \boldsymbol{B}\right) \tag{3}$$

This time, as we focus on evaluating the feasibility of measurements using the DA-64, the coefficients in the algorithm for calculating RGB scores were fixed as shown in Table 2. Then ave_BR and ave_GR were used as indicators for measuring hydrogen peroxide concentrations. We compared the measurement result with that obtained by conventional spectroscopic measurement. The following equation represents the correlation coefficient for the comparison (x: concentration estimated from absorbance, y: concentration estimated from RGB score).

Table 2
RGB score coefficients.

indicator	$\alpha 1$	$\alpha 2$	$\alpha 3$
ave_BR	−1	0	1
ave_GR	−1	1	0

$$r = \frac{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - y)^2}} \tag{4}$$

3. Results and Discussion

3.1 Spectroscopic characteristics of DA-64

The absorption spectrum of the aqueous solution containing DA-64 showed maximum absorbance at a wavelength of 727 nm. The absorption at 727 nm was dependent on the concentration of hydrogen peroxide. The spectral behavior of DA-64 was similar in solutions containing redox enzymes and substrates (lactic acid and glucose), which resulted in hydrogen peroxide production. The typical absorption spectra of the DA-64 solution from 650 to 800 nm are shown in Figs. 3(a)–3(c). Compared with the direct measurement of hydrogen peroxide concentrations, the results for both lactic acid and glucose levels showed lower absorbances. This is due to the testing condition, in which substrates are not completely oxidized. Figures 3(d)–3(f) show calibration curves for hydrogen peroxide, lactic acid, and glucose obtained from absorption spectra, respectively. Regarding the result for hydrogen peroxide, absorption was linear up to 500 μM. From these results, we confirmed that DA-64 can be useful for the image-based system.

3.2 Image-based DA-64 measurement with the smartphone-linked biosensing system

Briefly, biochemical measurement with smartphone cameras, i.e., a combination of optical elements embedded in a smartphone (the LED, the lenses, and the CMOS image sensors), refers to obtaining the color profile of the solution from the database. The presence of hydrogen peroxide resulted in a color change of DA-64, as shown in Fig. 4(a). RGB values are the red, green, and blue intensities of the native image. The changes in the blue and green values were moderate compared with the significant decrease in the red value at elevated hydrogen peroxide levels. This can be associated with the cyan coloration of DA-64, which has an absorption peak of 727 nm. Since the color of each pixel is determined by a combination of red, green, and blue elements, there is insufficient information to make a measurement based solely on the value of red. Therefore, we defined the image-based RGB score as the average of a two-dimensional array of the red, blue, and green components of the ROI multiplied by an arbitrary coefficient

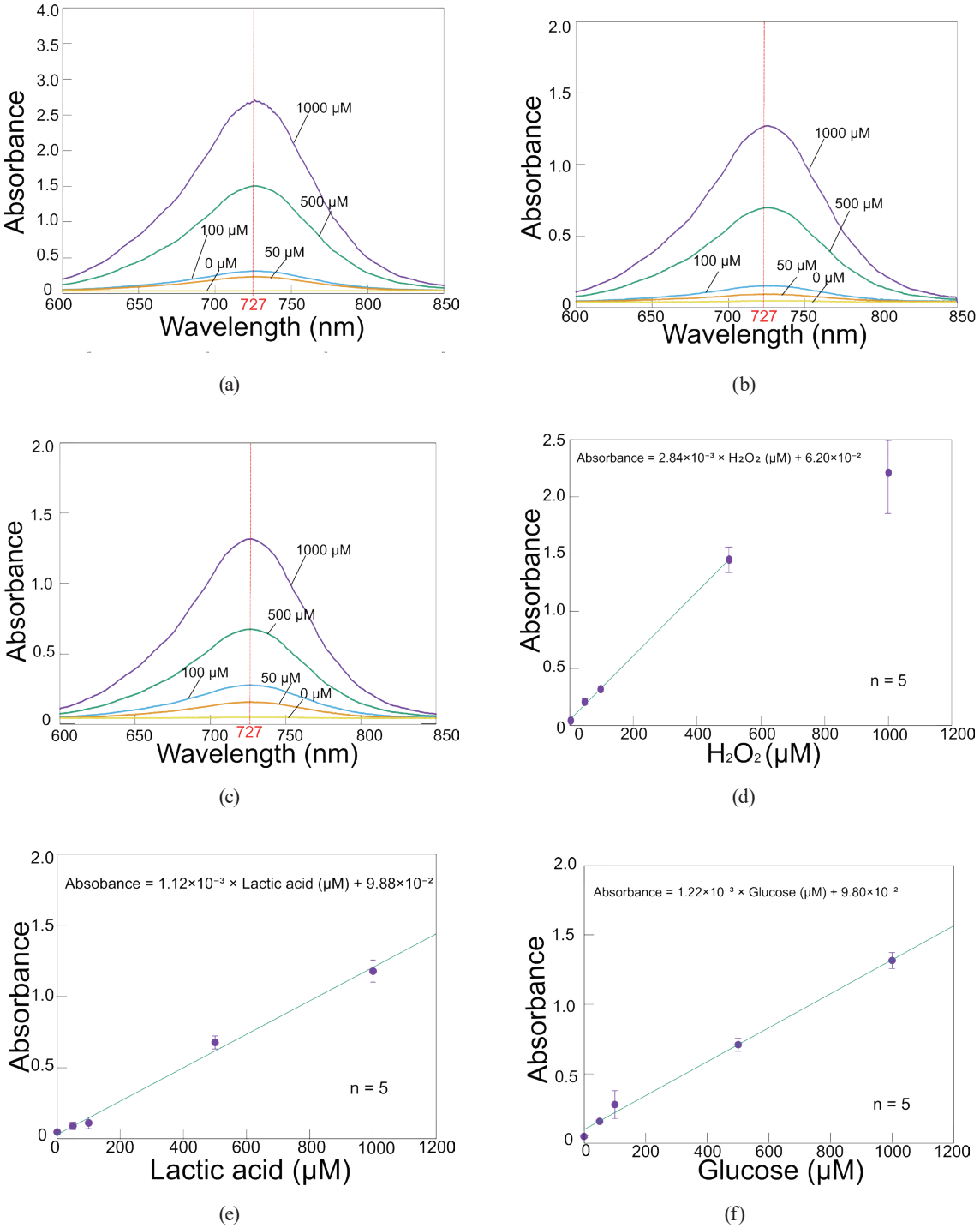


Fig. 3. (Color online) Absorption spectra of DA-64 in the presence of (a) hydrogen peroxide, (b) lactic acid, and (c) glucose. Calibration curves for (d) hydrogen peroxide, (e) lactic acid, and (f) glucose were also obtained from the spectra.

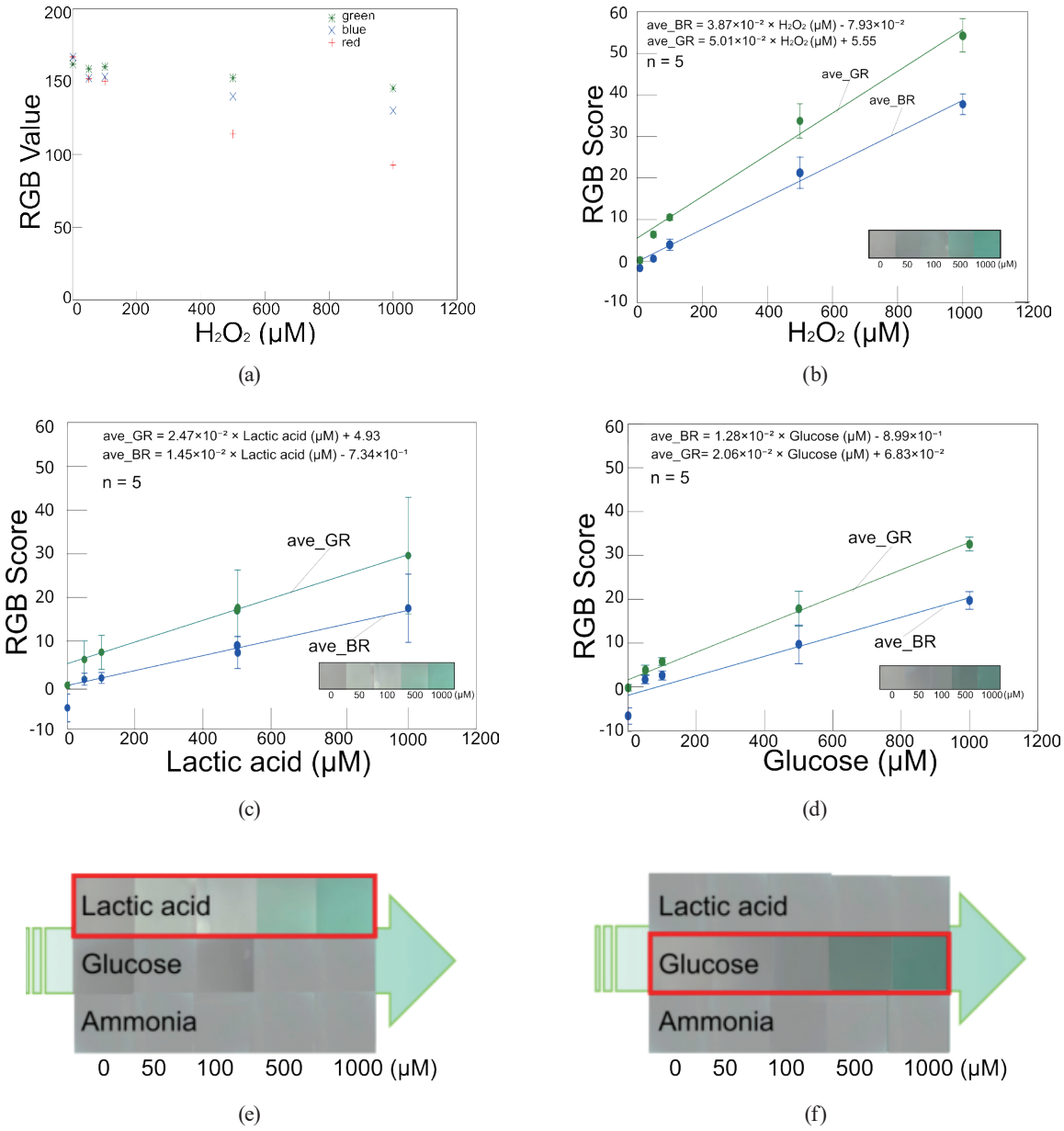


Fig. 4. (Color online) RGB scores for DA-64 solutions in the presence of hydrogen peroxide, lactic acid, and glucose. (a) Relationship between the concentration of hydrogen peroxide and RGB values obtained from the native image. (b)–(d) Relationships between RGB scores and hydrogen peroxide level, lactic acid level, and glucose level. Native images used in the image-based measurement of the DA-64 solution with LOD (e) and GOD (f).

(Fig. 2). In this measurement, ave_GB and ave_RB were employed as preliminary indicators of hydrogen peroxide concentration (Table 2). The value of this coefficient is based on the significant difference between red/blue and red/green as the hydrogen peroxide concentration increases (Fig. 1). Figures 4(b)–4(d) show the RGB scores obtained using ave_GR and ave_BR for various concentrations of hydrogen peroxide, lactic acid, and glucose. The native images for each test are also shown in the figures. The RGB scores, both calculated from ave_GR and ave_

BR, had a linear relationship with the concentrations of hydrogen peroxide, lactic acid, and glucose (50 to 1000 µM), respectively. This indicates that RGB scores have a potential for use in the determination of chemical substances even with little spectral information. Regarding the enzyme reactions, the coloration reflected the specific activities of enzymes [Figs. 4(e) and 4(f)]. This indicated that the RGB scores were less affected by possible interferences.

Finally, we also compared the results obtained from spectroscopic study and image-based sensing. Solutions with different concentrations of hydrogen peroxide were dispensed into the measuring reaction cell of the smartphone-linked biosensing system and into 96-well microplates, and the concentrations were measured by the methods described above. Figure 5 shows the correlation between the concentrations estimated from the absorbance using a conventional microplate reader and the RGB scores. As for the determination of hydrogen peroxide concentration with the smartphone-linked biosensing system, we used the calibration curve shown in Fig. 4, instead of calibrating the system with the sample used in the comparison.

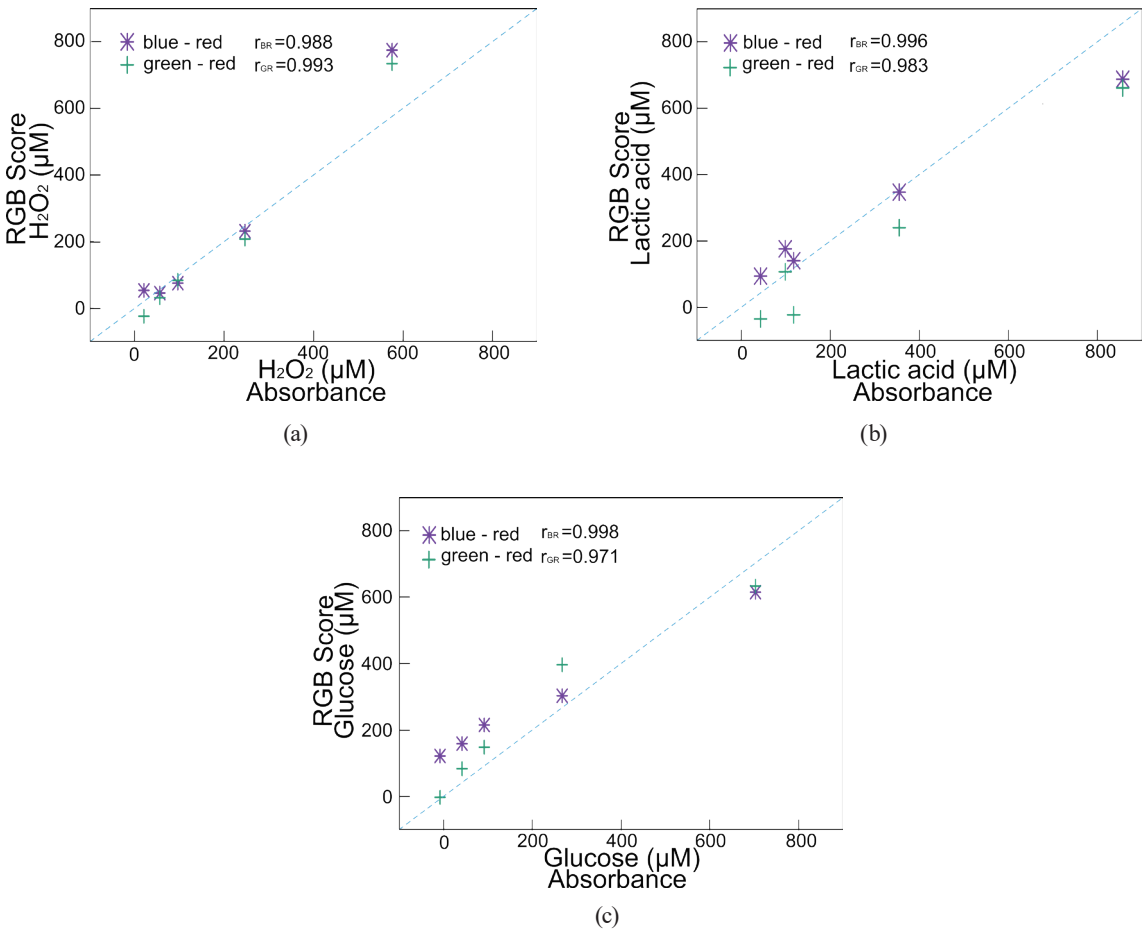


Fig. 5. (Color online) Correlation between the concentrations estimated from the absorbance using a conventional microplate reader and the RGB scores. (a) Correlation for hydrogen peroxide concentrations (0, 25, 75, 250, and 750 µM). (b) Correlation for lactate concentrations (0, 25, 75, 250, and 750 µM). (c) Correlation for glucose concentrations (0, 25, 75, 250, and 750 µM).

This is because a database-based calculation method is more appropriate for assessing reliability when measuring an unknown sample, although recalibration with the same sample as in the comparison experiment will clearly improve the correlation coefficient.

From these results, the blue/red score was found to have a better correlation for both lactic acid (r = 0.996) and glucose (r = 0.998). The results indicate that the smartphone-linked biosensing system has the potential for the rapid testing of body fluids.

4. Conclusions

A miniaturized smartphone-linked optical system for DA-64 determination was developed. With the emission of a smartphone LED as a backlight and a smartphone camera for measurement, the system enabled the rapid determination of hydrogen peroxide without the need for external batteries. Regarding image processing, a flexible and versatile algorithm based on weighted filters has also been proposed. An RGB score is expected to be useful for other colorimetric reactions with simplified procedures. The image-based RGB score used to estimate the concentration of hydrogen peroxide resulting from enzyme reactions showed strong correlations with the results obtained by the conventional spectroscopic method ($r > 0.971$) without the need for calibration for each measurement. The system was also little affected by possible interferences because of the specific activity of enzymes. Although there are several points to be improved, i.e., parameters for the determination of RGB scores, our system is expected to be used for personal healthcare purposes.

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