Portable Microplate Analyzer with a Thermostatic Chamber Based on a Smartphone for On-site Rapid Detection

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A microplate method provides an efficient way to use modern detection technology. However, there are some difficulties concerning on-site detection, such as being non-portable and time-consuming. In this work, a novel portable microplate analyzer with a thermostatic chamber based on a smartphone was designed for rapid on-site detection. An analyzer with a wide-angle lens and an optical filter provides a proper environment for the microplate. A smartphone app-iPlate Monitor was used for RGB analyze of image. After a consistency experiment with a microtiter plate reader (MTPR), the normalized calibration curves were $y = 0.7276x + 0.0243$ ($R^2 = 0.9906$) and $y = 0.3207x + 0.0094$ ($R^2 = 0.9917$) with a BCA protein kit as well as $y = 0.182x + 0.0134$ ($R^2 = 0.994$) and $y = 0.0674x + 0.0003$ ($R^2 = 0.9988$) with a glucose kit. The times for obtaining the detection requirement were 15 and 10 min for the BCA protein kit and the glucose kit at 37°C, respectively; in contrast, it required more than 30 and 20 min at ambient temperature. Meanwhile, it also showed good repeatability for detections.

Keywords Thermostatic chamber, optical filter, smartphone, on-site detection, microplate method

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Introduction

The microplate method has been widely used in analytical research and clinical diagnosis. It has the advantages of high-throughput, reagent-preservation and wide-ranging application. The microplate method can be applied to the detection of absorbance, fluorescence and luminescence, including a large number of analytes, such as proteins, lipids, enzymes and environmental pollutants. Many of these detections are used for this purpose. As for the detection range, an enzyme-linked immunosorbent assay (ELISA), toxin detection, heavy metals and other substances can be detected and analyzed by a smartphone. The portability of a smartphone and its ability to communicate by the Internet also provide other advantages. Beyond these advantages, the smartphone satisfies the demands of on-site detection very well.

In this study, a portable microplate analyzer with a thermostatic chamber based on a smartphone was developed for on-site detection. A small dimension was applied to the analyzer for portability. An analyzer provided a light source, a dark room and a thermostatic chamber for taking images by the smartphone, which used the RGB component to complete the detection. For improving the detection performance, an optical filter was utilized to obtain narrow-band light. Then, the BCA protein kit and the glucose kit were applied to compare the performances of the analyzer and MTPR. Meanwhile, the dynamic changes of the kits were compared at 37°C and ambient temperature; 3 experiments were performed to verify the repeatability. All of the details are discussed in the following parts.

Method

Reagents and material

The smartphone used in the experiment was an iPhone 4s (Apple, USA). The microcontroller and analog-digital converter...
applied in the analyzer were a MSP430F149 and an ADS1148, respectively (Texas Instruments, USA). Pt100 temperature sensors (0.15°C) were obtained from Heraeus, Germany. The wide-angle lens had a 0.45 amplification factor (HangZhou Cherllo Science and Technology Co., Ltd., China). The electro luminescent was made in the size of 100 × 100 mm (ShenZhen Luminescence Technology Co., Ltd., China). The optical filter 80 × 80 mm had a pass band at 562 nm (ShenZhen Infrared and Laser Technology Co., Ltd., China). The BCA protein kit and the glucose kit were obtained from Solarbio (Beijing, China) and Applygen (Beijing, China); 96-well microplates were obtained from Corning (USA). The MTPR was a SpectraMax Paradigm (Molecular Devices, USA).

Design of the portable analyzer

An assembly drawing and related dimensions are shown as Fig. 1A. The analyzer consisted of a light source, a dark room and thermostatic chamber. The smartphone holder and sliding lid create a dark room, which are combined with the sliding lid and the base by a groove. A wide-angle lens, which is attached below the smartphone holder by hot-melt adhesive, is used to abridge the distance between the smartphone camera and the microplate. Electro luminescent and optical filters were fixed by a microplate holder. The chromogenic reactions in these kits were purple, so the optical filter had a pass band of around 562 nm.

A heating process is performed by a heating belt, which is controlled by a MSP430F149 microcontroller. Four Pt100 temperature sensors are placed above the microplate holder: the resistance values of Pt100 are converted by ADS1148. For enhancing the heating efficiency, a PMMA board is set in the sliding lid to separate the thermostatic chamber from the outside environment. The microcontroller uses these temperature data to control the heating belt, and shows the current temperature values and heating state of the belt on LCD. Both the DC adapter and a lithium-ion battery can supply the analyzer (Fig. 1B). Choosing the power supply flexibly can satisfy various situations in practical applications.

The workflow of the analyzer is shown in Fig. 1C. A homemade smartphone App—iPlate Monitor is used for image analysis, which is developed by Swift 1.0 and Objective-C in Xcode 7. The function of the iPlate Monitor includes calibration, sample measurement and data sharing. A blank microplate image needs to be taken by iPlate Monitor first. Next, a microplate with samples is prepared and incubated in the analyzer for a suitable time. When incubation is completed, the iPlate Monitor takes a microplate image for analysis. According to the color of the samples, a suitable RGB channel can be selected for a better performance. Blank calibration can be made by

\[ C = \text{Channel}_{\text{blank}} - \text{Channel}_{\text{used}}, \]

where \( C \), \( \text{Channel}_{\text{blank}} \), \( \text{Channel}_{\text{used}} \) are the RGB value of the sample, the RGB value of the blank microplate and the RGB value of the sample with a microplate, respectively. We then use the following equation for further processing:

\[ C_n = \text{C}_{\text{concentration}} - \text{C}_0 \]

where \( C_n \), \( \text{C}_{\text{concentration}} \), \( \text{C}_0 \) are the net RGB value of the sample, the original RGB value of the sample and the RGB value of the 0 concentration, respectively. There is a linear correlation between \( C_n \) and the corresponding concentrations. The iPlate Monitor can establish a calibration curve for detection by these data. The concentration of unknown samples can be calculated by the calibration curve. All of these data can also be sent to a laboratory by e-mail for further study, or to be archived.

Simulation for temperature distribution

Simulating the heating activity is necessary to set the proper temperature value for the temperature control system. A target temperature value can be set based on the temperature difference between the position of the sensors and the microplate. In this study, COMSOL Multiphysics was applied to simulate of the heating activity for an incubation temperature of 37°C.
Assay procedure

BCA protein assay. Reagents in the BCA protein kit include a standard solution of BSA (5 mg mL⁻¹), a BCA solution, a Cu²⁺ solution and phosphate buffered saline (PBS). At the beginning of the assay, a blank microplate should be read by the analyzer and the MTPR first. The detection solution is prepared by the BCA solution and the Cu²⁺ solution at a ratio of 50:1. Then, standard BSA solutions are added into each hole from 0 to 20 μL and each hole is made up to 20 μL by PBS. After adding 200 μL of the detection solution to each hole, the microplate is incubated at 37°C in the analyzer for 15 – 30 min. Finally, the absorbance of the colorimetric solution is measured by the MTPR, and an image of the microplate is analyzed by the iPlate Monitor in the G channel. For dynamic detection, images are taken every 2 min within 30 min from the beginning of the assay.

Glucose assay. Reagents in the glucose kit include a standard solution of glucose (10 mmol L⁻¹), a chromogenic agent and an enzyme solution. The detection solution is prepared by a chromogenic agent and an enzyme solution at a ratio of 4:1. The standard glucose solution is diluted into a lower concentration series, and adds 5 μL each of them to the microplate to establish calibration curves. After that, 195 μL of the detection solution is added into the microplate, which is...
incubated at 37°C in the analyzer for 15 – 30 min; the absorbance of the colorimetric solution is measured by the MTPR and an image of the microplate is analyzed by the iPlate Monitor in the G channel. Dynamic detection is also performed in the glucose kit.

**Results and Discussion**

**Results of simulation for the temperature distribution**

A simplified model of the heating chamber was constructed (Fig. 2A), which had an identical size to that of a practical model (150 × 122 × 42 mm). Each part of the model had been made of the same material used in the practical model. Figure 2B shows the result of simulation. When the temperature around the microplate reached approximately 37°C, the temperature difference between the outermost holes and innermost hole was 0.5°C (Fig. 2C). Three Pt100 were 10 mm higher than the microplate holder on the corner, where the temperature was about 3°C higher than that of the microplate (Fig. 2D). As a result, about 40°C could be set as the target temperature for a 37°C environment.

**Imaging quality with a wide-angle lens at different heights**

A proper height between the smartphone and the microplate could improve the imaging quality and reduce the dimensions of the analyzer. The images were taken directly and with a 0.45× wide-angle lens from 10 to 20 cm at an interval of 1 cm (Fig. 3A). The coefficient of variation (CV, the ratio of standard deviation and mean) of images at different heights was compared in the RGB channel. When the height was greater than 20 cm, or less than 10 cm, the microplate images were too small or incomplete for analyzing by a smartphone; 36 holes in the center of the microplate (B4 to G9) were chosen for the analysis. In order to make the edges of the microplate available in smartphone detection and for better image quality, the best height for each condition was 18 and 15 cm. The results of taking an image directly and with a 0.45× wide-angle lens are shown in Figs. 3B and 3C. Therefore, 15 cm was applied as the height between the smartphone camera and the microplate with a wide-angle lens.

**Detection results in the BCA protein assay**

A calibration curve was built by diluting the standard solution of BSA (5 mg mL⁻¹) into 0.5, 1, 1.5, 2, 3 and 4 mg mL⁻¹ BSA solutions. The standard solution was also applied for the calibration curve. Zero concentration was added by PBS. After incubation, the images without an optical filter and with an optical filter are shown in Figs. 4A and 4B; the microplate is shown in Fig. 4C. The G component of RGB in images and the absorbance were used to build the calibration curves. Because the RGB color intensity and the absorbance had a different range in detection results, normalization was necessary for further comparisons. The normalization results of a linear regression are shown in Fig. 4D. Normalization was conducted using

\[
C_{nor} = \frac{C}{C_{max} - C_0},
\]

where \(C_{max}, C_{nor}, C, C_0\) are the normalization value, the max value of the detection system, and the net RGB values of each sample and RGB value of zero concentration, respectively. The range of the RGB color intensity is 0 – 255 and the absorbance of MTPR is 0 – 3.5, so 255 and 3.5 were used as \(C_{max}\) in the analyzer and MTPR. The equation of the calibration curve without an optical filter, with a 562-nm filter and MTPR were \(y = 0.5675x + 0.013\ (R^2 = 0.9903)\), \(y = 0.7276x + 0.0243\ (R^2 = 0.9906)\) and \(y = 0.3207x + 0.0094\ (R^2 = 0.9917)\), respectively, where \(y\) is the normalization value and \(x\) is the concentration of BSA protein. We could conclude from the coefficient of determination \(R^2\) that the analyzer had a high consistency with the MTPR. At the same time, the sensibility improvement with the optical filter also reflected on the change of the slope: 0.7276 with an optical filter and 0.5675 without one. In a certain concentration range, a greater slope means a higher sensibility.

The incubation temperature can influence the performance of kits greatly under the on-site condition. Figures 5A and 5B show the change of the slope and \(R^2\) of the calibration curves with time. Under the 37°C condition, a better slope and \(R^2\) could be obtained at the same time. It took about 15 min for the BCA protein kit to reach the optimal performance under the
37°C condition, while more than 30 min was required at ambient temperature. This shows that a proper temperature can achieve a faster detection and better sensibility for a practical sample. Three experiments at 37°C and the slope and $R^2$ of the calibration curves are plotted in Figs. 5C and 5D.

Detection results in glucose assay

The calibration curves were established by the analyzer and the MTPR, respectively (Figs. 6A and 6B). The calibration curve was built by diluting the standard solution of glucose (10 mmol L$^{-1}$) into 0.125, 0.25, 0.5, 1 and 2 mmol L$^{-1}$ glucose solutions. Purified water was performed as zero concentration. The chromogenic reaction of the glucose kit is shown in Fig. 6C. The $G$ component and the absorbance were taken for calibration curves by the iPlate Monitor and MTPR. Then, the results of linear regression were converted into a normalized expression (Fig. 6D). The equation of the calibration curve without an optical filter, with a 562-nm filter and MTPR were $y = 0.1502x - 0.0003$ ($R^2 = 0.9956$), $y = 0.182x + 0.0134$ ($R^2 = 0.994$) and $y = 0.0674x + 0.0003$ ($R^2 = 0.9988$), respectively, where $y$ is the normalization value and $x$ is the concentration of glucose. Similarly, the analyzer had a high consistency with MTPR and an optical filter could enhance the detection sensibility (the slope of the calibration curve with a 562-nm filter was 0.182, rather than the one without an optical filter was 0.1502).

Figures 7A and 7B show the change of the slope and $R^2$ in the calibration curves with time. It took about 10 min for the glucose kit to reach the best performance at 37°C; at the same time, about 20 min or a longer time were needed to reach a stable condition in an ambient-temperature environment. The repeatability was verified by three experiments at 37°C, and the slope and $R^2$ values of the calibration curves were plotted in Figs. 7C and 7D.

Conclusions

In this study, a novel portable microplate analyzer with a thermostatic chamber based on a smartphone was developed for
respectively. It showed that there was a significant consistency
on rapid on-site detection; it can satisfy the requirements of on-site
kinetics experiments showed that heating in the analyzer can
on-site situ
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Fig. 7 Dynamic change of the slope (A) and $R^2$ (B) in calibration
curves at ambient temperature and 37°C, and the repeatability of the
slope (C) and $R^2$ (D) in calibration curves at 37°C in the glucose kit.

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References

1. I. Sitepu, L. Ignatia, A. Franz, D. Wong, S. Faulina, M.
2012, 91, 321.
2. X.-x. Li, G.-r. He, X. Mu, B. Xu, S. Tian, X. Yu, F.-r. Meng,
3. Y.-S. Cheng, Y. Zheng, and J. S. Vander Gheynst, Lipids,
2011, 46, 95.
5. G. Navarro, P. Carriha, J. Gandi, F. Ciruela, V. Casadó, A.
6. W. Wu, J. Li, D. Pan, J. Li, S. Song, M. Rong, Z. Li, J. Gao,
8. A. Roda, M. Guardigli, D. Calabria, M. M. Calabretta, L.
Cevenini, and E. Michelini, Analyst, 2014, 139, 6494.
George, P. Nath, and B. T. Cunningham, Lab Chip, 2013,
13, 2124.
11. V. Oncescu, D. O’Dell, and D. Erickson, Lab Chip, 2013,
13, 3232.
12. X. Wang, M. R. Gartia, J. Jiang, T.-W. Chang, J. Qian, Y.
13. Q. Fu, Z. Wu, X. Li, C. Yao, S. Yu, W. Xiao, and Y. Tang,
14. S. K. Vashist, T. van Oortd, E. M. Schneider, R. Zengerle,
F. von Stetten, and J. H. Luong, Biosens. Bioelectron.,
15. B. Berg, B. Cortazar, D. Tseng, H. Ozkan, S. Feng, Q. Wei,
R. Y.-L. Chan, J. Burbano, Q. Farooqui, and M. Lewinski,
ACS Nano, 2015, 9, 7857.
Express, 2014, 5, 3792.
18. L.-J. Wang, Y.-C. Chang, X. Ge, A. T. Omanson, D. Du, Y.
19. C. Yao, S. Yu, X. Li, Z. Wu, J. Liang, Q. Fu, W. Xiao, T.
20. S. Yu, W. Xiao, Q. Fu, Z. Wu, C. Yao, H. Shen, and Y. Tang,
Anal. Methods, 2016, 8, 6877.
21. Q. Wei, R. Nagi, K. Sadeghi, S. Feng, E. Yan, S. J. Ki, R.
22. S. Lee, V. Oncescu, M. Mancuso, S. Mehta, and D.
Erickson, Lab Chip, 2014, 14, 1437.
Actuators, B, 2017, 238, 1165.