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Short communication

A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests

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ABSTRACT

A smartphone application algorithm with inter-phone repeatability was developed for both Android and iOS operating systems. The app transformed the smartphone into a reader to quantify commercial colorimetric urine tests with high accuracy and reproducibility in measuring pH, protein, and glucose. The results showed linear responses in the ranges of 5.0–9.0, 0–100 mg/dL and 0–300 mg/dL, respectively.

1. Introduction

The high mobile phone penetration and rapidly growing telecommunications infrastructure in the world represents an unprecedented opportunity for reading and transferring point-of-care diagnostic data [1]. Global mobile-cellular subscriptions have grown 70% over the last 5 years, reaching 6.8 billion as of 2013 [2]. Hence, exploiting the existing mobile phone infrastructure to monitor health conditions and the environment will accelerate the efforts toward diagnostics, as well as low-cost healthcare for existing and emerging diseases. The use of smartphone cameras has been suggested for diagnostic applications in dermatology [3], microscopy [4–6], ophthalmology [7], chemical analyses [8,9] and paper-based microfluidic devices [10–12].

Smartphone cameras have standardization challenges in optical analysis of colorimetric assays; integrated color balancing functions of camera phones are optimized for photography in bright ambient light. Recently, there have been several approaches to address issues related to ambient light variability during colorimetric test reading. For instance, assays have been read using a smartphone with a housing unit that eliminated the variation in lighting conditions and positioning of the camera. These solutions required a phone-specific external housing unit and possibly many other components including batteries, LED arrays (for reflection and transmission), and lenses [13]. In another study, a calibration chart and test assay images were captured using the phone camera, however image processing was performed externally with a computer [14]. Recently, there has been interest in smartphone applications from industry for point-of-care diagnostics [15]. It is our understanding that these applications have limited availability in the market to date. Fully integrated smartphone apps are needed to facilitate rapid low-cost testing, so that they can quantify different types of colorimetric tests on both iOS and Android platforms.

Here we report a smartphone app algorithm that runs both on Android and iOS to quantify colorimetric tests. This smartphone application can be used with dipsticks, lateral-flow tests as well as colorimetric tests in solution that are typically read by spectrophotometers or microplate readers (*e.g.* Bradford protein assay). The mobile app was designed to provide rapid on-site quantitative screening when fast diagnosis is needed. Hence, if further examination is needed, the subject can be referred to a clinical facility by means of the smartphone connectivity. We demonstrate the utility of our detection method through quantifying the concentrations of glucose, protein and pH in artificial urine. The tests were performed using a commercial urine dipstick (Fig. 1). The app can be adapted

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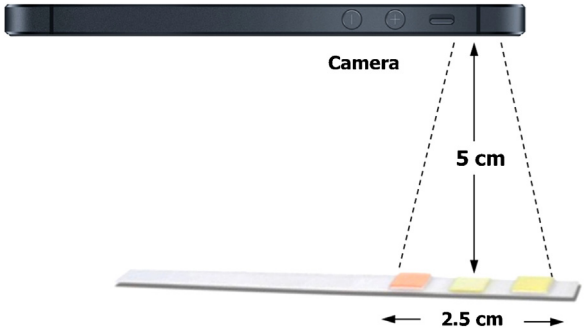

Fig. 1. Quantifying colorimetric tests through a smartphone reader. The smartphone captures and processes the image of the test zones, reducing time and errors related to visual inspection of the color reference chart.

for semi-quantitative analysis of commercially available colorimetric tests, making it an inexpensive and accessible alternative to more costly commercial readers.

The iOS (Fig. 2A–E) and Android (Fig. 2F–J) apps were designed to direct the user in all the steps necessary to perform an analysis of a point-of-care diagnostic test. First, the user may choose functions from sample testing, system calibration, sensor type selection and test history viewing (Fig. 2A and F).

Before sample testing, the user must first calibrate the system by choosing 'calibration'; this allows capturing and processing a set of reference images of the required concentration range. The calibration is then recorded under a given ambient light condition. When the ambient light changes (*e.g.* color, intensity and tone), the reader needs to be recalibrated. For sample testing, the user selects 'sensor type' function to specify the type of colorimetric test to be performed (Fig. 2B and G). Once the sensor type has been selected, the user captures the image of the corresponding test zones (Fig. 2C and H). The app processes the image information and transforms it into analyte concentrations by comparing the measured value with the calibration curve. Then, it displays the results in the subject's report (Fig. 2D and I). This report contains personal data (including, but not limited to name, age, weight, patient ID number and contact phone number) along with the medical information that may be uploaded, synced and transmitted to an offsite doctor's office. Following review, the doctor may resend the report and any additional comments back to the patient, or to a referring practitioner (Fig. 2E and J).

2. Materials and methods

The smartphone app measures electromagnetic radiation from the colored test zones with the complementary metal–oxide–semiconductor (CMOS) sensor present in the smartphone camera. The algorithm processes this information as concentrations of the analytes in each test zone and then the app displays the corresponding value on the smartphone screen. The sensitivity of the colorimetric measurement is based on the accuracy of the camera's CMOS sensor, on the color uniformity of the colorimetric reactions, and on the number of calibration points.

2.1. Calibration

This step collects the calibration curve for a given colorimetric sensor and ambient light conditions. Here, the user inputs (i) sensor type, (ii) target analytes, (iii) units of the concentration, and (iv) number of reference data points to be stored. Once this information is entered, the user captures the images of the calibration points. The smartphone is perpendicularly positioned over the assay at a fixed distance of 5 cm at a given ambient condition. The distance was kept constant in order to match the colorimetric zones with the evaluation area defined by the software. The measurements were performed at ambient temperature (24 °C). Capturing calibration points was achieved within ~1 min, and the calibration was stored in the smartphone memory for later use. The app was calibrated for measurements in pH, glucose and protein, based on 5, 4 and 5 data points, respectively. The app locates the reference colors (100 pixels) and transforms and averages the CMOS data into non-linear RGB (red, green, blue) values (R_c, G_c, B_c) for each pixel. Subsequently, the app linearizes the RGB values (R_l, G_l, B_l) through the following set of equations [16]:

$$R_l = \left(\frac{0.055 + R_c}{1.055}\right)^{2.4} \quad (1)$$

$$G_l = \left(\frac{0.055 + G_c}{1.055}\right)^{2.4} \quad (2)$$

$$B_l = \left(\frac{0.055 + B_c}{1.055}\right)^{2.4} \quad (3)$$

Next, linear RGB values are converted to tristimulus values, X, Y, Z by the following relationships [16]:

$$X = 0.1805B_l + 0.3576G_l + 0.4124R_l \quad (4)$$

$$Y = 0.0722B_l + 0.7152G_l + 0.2126R_l \quad (5)$$

$$Z = 0.9505B_l + 0.1192G_l + 0.0193R_l \quad (6)$$

Finally, X, Y, Z tristimulus values are converted into the 2D (x, y) CIE 1931 chromaticity space [17] using:

$$x_j = \frac{X}{X + Y + Z} \quad (7)$$

$$y_j = \frac{Y}{X + Y + Z} \quad (8)$$

After defining the values of x_j and y_j for the jth concentration data point C_j, the app saves the data points in an internal database to complete the calibration, before returning to the main screen.

2.2. Colorimetric measurements

The user captures the image of the target assay using exactly the same conditions as for the calibration points (*e.g.* distance, lighting and temperature), and the app follows the same steps performed for the calibration. Briefly, the app converts the CMOS data to RGB, which are linearized, converted to tristimulus values that are finally expressed as measured 2D chromaticity values (x_m, y_m) (see Supporting Information). Then, the app computes the final measurement by comparing the target data values with respect to the calibration curve. This is achieved by an interpolation algorithm similar to the nearest neighbor problem in computational geometry. For each point in the calibration curve (j), the shortest distance from the measurement value to the calibration point is determined by:

$$d_k = \sqrt{(x_k - x)^2 + (y_k - y)^2} \quad (9)$$

where k is an integer and goes from 1 to the number of stored x and y pairs (points) in the calibration curve, *i.e.* $k = j$. The algorithm stores two shortest distances to the sample point: d_{ks} and d_{kss}, respectively, obtained by Eq. (9). Their x and y values obtained by Eqs. (7) and (8), together with their concentrations C are stored in the app memory. The concentration range, d_c, of the nearest two data points is calculated as:

$$d_C = |C_{ks} - C_{kss}| \quad (10)$$

Fig. 2. Screenshots of the iOS and Android apps used to quantify a colorimetric test. (A) Main menu displaying sample testing, calibration and test history viewing. (B) The app displays sensor types. (C) User captures the image of the test zones. (D) The diagnostic test results are displayed to the user. (E) The information received from the physician displayed on the final screen may include user-specific instructions. (F–J) Android version of the same steps A–E, respectively.

where C_{ks} and C_{kss} are the concentrations of the points corresponding to d_{ks} and d_{kss}. The distance in x and y coordinates on the chromaticity space between the two nearest points on the calibration curve to the measurement point is calculated as (refer to Fig. S1 in Supporting Information for a graphical representation):

$$d_{xy} = \sqrt{(x_{ks} - x_{kss})^2 + (y_{ks} - y_{kss})^2} \tag{11}$$

The app then calculates the shortest distance from that measurement point to the line between the two calibration points, d_{sd}, using the following expression:

$$d_{sd} = \frac{|(x_{kss} - x_{ks})(y_{ks} - y_m) - (x_{kss} - x_m)(y_{ks} - y_{kss})|}{\sqrt{(x_{kss} - x_{ks})^2 + (y_{kss} - y_{ks})^2}} \tag{12}$$

The largest among d_{ks}, d_{kss} or d_{sd} is determined and stored. The $\pm$ variation, $v_{\pm}$, is calculated as a ratio or proportion, given the concentration range d_c for the largest distance as:

$$v \pm \frac{d_m d_C}{d_{xy}} \tag{13}$$

where d_m is the largest among d_{ks}, d_{kss} and d_{sd}. By using a similar proportionality approach, the distance from one of the corresponding calibration points to the point on the line to where d_{sd} is measured, is computed as the concentration (C_m).

$$C_m = \frac{d_p d_C}{d_{xy}} \tag{14}$$

$$d_p = \sqrt{d_{ks}^2 - d_{sd}^2} \tag{15}$$

The computed concentration, C_m, is located within the concentration range previously calculated, but it varies based on the distance between the measurement point and the calibration curve. After the algorithm ends, the app displays the diagnostic results for the calculated analyte concentrations.

3. Results and discussion

To demonstrate the clinical utility of the app, it was tested on both high and low-end smartphones (*i.e.* iPhone 5, 8 MP camera; and Samsung I5500 Galaxy 5, 2 MP camera) using a colorimetric urine test strip (cobas® Combur3 Test®, Roche). This three-patch

Table 1
The principles of colorimetric reactions in cobas® Combur3 Test®, Roche.

Assay	Test principle	Reactive ingredient (per 1 cm^2 patch area)	Range	Detection limit	Operating temp. (°C)
pH	Ingredients specifically react with H^+; the pH is the negative common logarithm of the H_3O^+ concentration. The tests pad change color from orange to greenish blue as the pH increase.	Bromothymol blue (13.9 μg), methyl red (1.2 μg) and phenolphthalein (8.6 μg)	5–9	N/A	Stable up to boiling point
Protein	Based on protein error principle of a pH indicator. The test pad changes color from yellow to greenish blue in the presence of albumin.	3′,3″,5′,5″-Tetrachlorphenol-3,4,5,6-tetrabromsulfophthalein (neutral form) (13.9 μg)	0–100 mg/dL	6 mg albumin/dL	Stable up to boiling point
Glucose	Based on glucose oxidase/peroxidase reaction. The reaction pad changes color from yellow to dark blue in the presence of glucose.	Tetramethylbenzidine (103.5 μg), glucose oxidase (6 U) and peroxidase (35 U)	0–300 mg/dL	40 mg/dL	<55

Table 2
Standard deviations of residuals (s_y), slope (s_m), intercept (s_b) and limit of detection for the pH, protein and glucose measurements.

	Assay	Std. of residuals (s_y)	Std. of slope (s_m)	Std. of intercept (s_b)	Limit of detection
pH	Android	0.459	0.068	0.0161	1.66
	iPhone	0.311	0.046	0.0048	1.21
Protein (mg/dL)	Android	11.44	0.077	12.21	41
	iPhone	8.95	0.060	7.51	33
Glucose (mg/dL)	Android	27.48	0.060	53.69	92
	iPhone	20.19	0.044	20.47	69

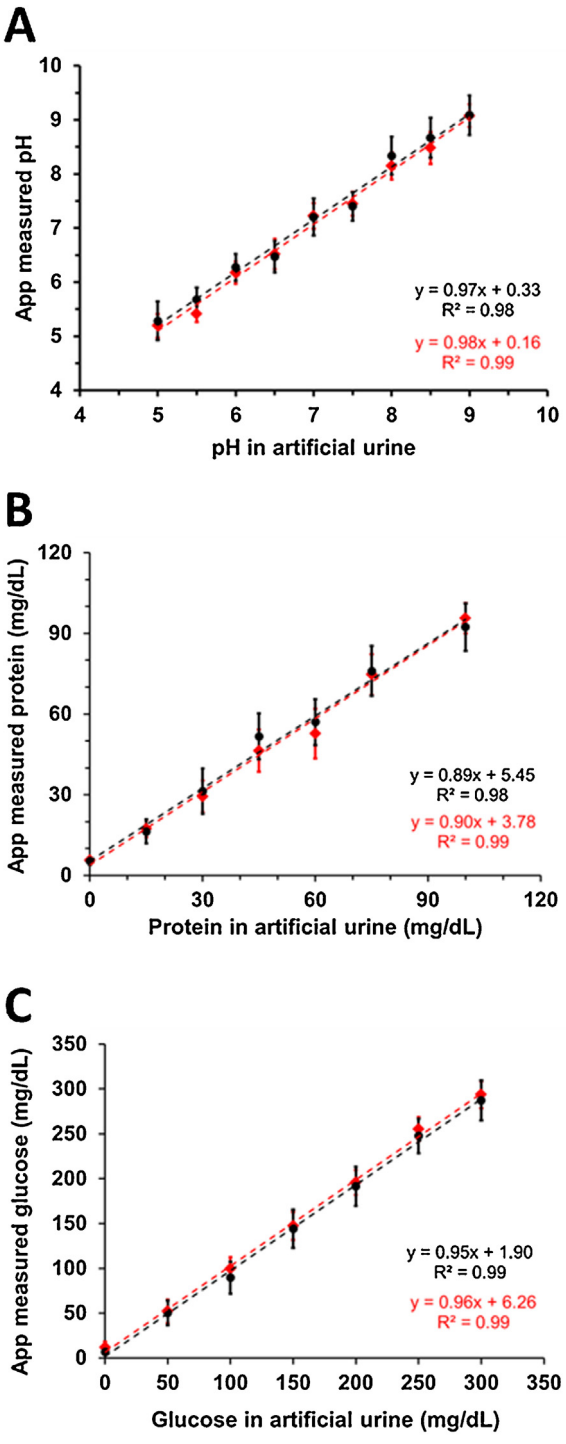

Fig. 3. Analyses of dipstick tests (cobas® Combur3 Test®, Roche) by iPhone 5 (shown in red) and Samsung I5500 Galaxy 5 (shown in black) for (A) pH, (B) protein and (C) glucose measurements in artificial urine. Standard error bars represent three replicates.

test strip is normally used for semi-quantitative determination of pH, protein and glucose in urine samples. Table 1 shows their sensing principles and limitations [18,19].

Artificial urine samples were prepared by following a protocol described previously [20] (see Table S2 in Supporting Information). The test strips were submerged into a range of artificial urine solutions, and the images of the test zones were analyzed with the smartphone app. The calibration was performed using the color reference chart on the product package provided by the supplier. The images were taken at a fixed distance under an ambient fluorescent light source, whose output was measured as $10 \pm 1\,\mu W$ (power meter, Coherent, Auburn, CA). Fig. 3 illustrates standard curves associated with pH (5.0–9.0), protein (0–100 mg/dL) and glucose (0–300 mg/dL) measurements. These concentration values are within the physiological range. The variation in the distance, if any, was negligible as corroborated by the error in the measurements. Standard deviations of residuals (s_y), slope (s_m), intercept (s_b), and limit of detection for the pH, protein and glucose measurements are shown in Table 2. The values given by the app showed accurate readings of the respective analyte concentrations in artificial urine, demonstrating the app's potential for reading colorimetric point-of-care diagnostic devices.

4. Conclusions

Smartphones have the potential to serve as low-cost point-of-care diagnostic device readers. We demonstrated an algorithm with inter-phone repeatability that allows a smartphone camera to read semi-quantitative tests rapidly with minimal operator intervention. The app utilized the smartphone hardware and software, and quantified the concentrations of protein and glucose, and pH. The method of quantitation was reproducible and highly sensitive for Android and iOS platforms (see Table 2). Our technology has utility both in resource-limited settings where trained healthcare professionals are scarce, and in resource abundant settings with limited healthcare budgets. Efforts are being directed at addressing several challenges with equipment-free smartphone-based readers for use in point-of-care testing, in particular, developing advanced apps that can automatically and robustly compensate for measurement variability (*e.g.* focus, angle, lighting conditions, shadow effects and sensor type). A step forward in this process is the design of algorithms that process data more efficiently into actionable information for the user. Notably, the color conversion technique we demonstrate in this app is not limited to urinalysis, but is also applicable to other established colorimetric assays such as colloidal gold, latex labels, solution-based assays, as well as emerging technologies such as microfluidic diagnostic devices [21–24]. Furthermore, cloud computing can potentially be adapted for transferring the medically relevant data to a centralized healthcare monitoring center, and it may be used for endemic or pandemic surveillance. We envision that our app will facilitate less expensive laboratory testing in developed nations and enable automated colorimetric point-of-care diagnostics in resource-limited settings.

Conflict of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2014.01.077.

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