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Cholesterol testing on a smartphone

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Cite this: *Lab Chip*, 2014, **14**, 759

Home self-diagnostic tools for blood cholesterol monitoring have been around for over a decade but their widespread adoption has been limited by the relatively high cost of acquiring a quantitative test-strip reader, complicated procedure for operating the device, and inability to easily store and process results. To address this we have developed a smartphone accessory and software application that allows for the quantification of cholesterol levels in blood. Through a series of human trials we demonstrate that the system can accurately quantify total cholesterol levels in blood within 60 s by imaging standard test strips. In addition, we demonstrate how our accessory is optimized to improve measurement sensitivity and reproducibility across different individual smartphones. With the widespread adoption of smartphones and increasingly sophisticated image processing technology, accessories such as the one presented here will allow cholesterol monitoring to become more accurate and widespread, greatly improving preventive care for cardiovascular disease.

Received 21st October 2013,
Accepted 28th November 2013

DOI: 10.1039/c3lc51194d

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Introduction

The alarming increase in premature deaths due to heart disease in the developed world has resulted in numerous efforts to make blood cholesterol measurements accessible outside the clinical setting.[1,2] It is estimated that 60% of adults in the US have high cholesterol (over 200 mg dl^{-1}), with 37 million among them having very high cholesterol (over 250 mg dl^{-1}).[3] Long-term studies on the effect of serum cholesterol on coronary heart disease mortality indicate that there is a 17% increase in mortality rate for every 20 mg dl^{-1} increase in serum cholesterol levels above 210 mg dl^{-1}.[4] Monitoring cholesterol levels is important because it can empower people to make lifestyle choices for preventing heart disease later in life. For some people, improving diet and increasing exercise is enough to lower overall cholesterol, but in some cases medication needs to be prescribed. Products such as Cardiochek PA (Polymer Technology Systems Inc, Indianapolis, USA) and Cholestech LDX (Hayward, USA) have been on the market for over a decade; however home cholesterol testing is still not common.[5] A recent study suggested that current cholesterol kit users are interested in easier ways of tracking results and that they would test more frequently if supplies were more affordable.[6] The accuracy of those devices is also a major user concern and has been addressed in several publications.[7–9]

Smartphones have the potential of addressing all these issues by eliminating the need for separate test kits. The test strips could be imaged directly on a smartphone and the processed data can be stored for tracking or sent *via* e-mail directly to a physician. Increasingly sophisticated camera technology on smartphones can also improve the accuracy of cholesterol monitoring. Smartphone accessories for the detection of biomarkers in bodily fluids have been the subject of extensive investigation because they have the potential of greatly decreasing the cost and increasing the availability of heath care in the world.[10,11] In a recent paper we have demonstrated a system for colorimetric monitoring of biomarkers in sweat and saliva.[12] Several academic groups are also developing smartphone platforms for biomarker detection[13] and smartphone-based image processing for quantifying colorimetric changes on paper-based immunoassays.[14]

In this paper we present and characterize our smartCARD – *smart*phone Cholesterol Application for Rapid Diagnostics – system. The system can quantify cholesterol levels from colorimetric changes due to cholesterol reacting enzymatically on a dry reagent test strip. It consists of a smartphone accessory that allows uniform and repeatable image acquisition of the test strip and an app that analyzes parameters such as hue, saturation and luminosity of the test area, quantifies the cholesterol levels and displays the value on the screen. In the Methods section, we begin by discussing how the accessory was optimized to improve the accuracy and sensitivity of the colorimetric reaction imaging process. We then discuss the correlation between cholesterol and different image parameters such as saturation and luminosity before presenting the algorithm used to process those parameters and calculate the blood cholesterol levels. In the Results section, we present a series of human trials using the device and characterize the accuracy of the system and

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reproducibility of the measurements across different smartphones. We conclude by discussing how the system and the test strips can be further improved to increase test accuracy and reproducibility.

Methods

Smartphone accessory for colorimetric analysis

The smartCARD accessory attaches around the camera of the smartphone as shown in Fig. 1a. It has been designed to allow quantification of the cholesterol colorimetric reaction that occurs on a dry reagent test strip over the entire range of physiological cholesterol values. We have investigated different designs and types of lighting sources in order to increase the robustness of the system and ability to deal with misalignment of the test strip. In the end, we opted to use the smartphone's flash to illuminate the strip as it provided more uniform lighting for accurately imaging the colorimetric reaction on the test strip. Typical smartphone acquired images of the colorimetric reaction, at low ($<$100 mg dl^{-1}) and high ($>$400 mg dl^{-1}) cholesterol concentrations are shown at the bottom of Fig. 1. The accessory is designed in such a way as to illuminate the test strip from the bottom as can be seen in Fig. 1a. This ensures better uniformity of lighting on the circular detection area of the test strip.

In order to improve the sensitivity of the system to variations in the color of the test strip and to reduce the effect of test strip misalignment into the device, we have incorporated a light diffuser over the flash as can be seen in the inset of Fig. 1a. The effect of integrating different diffusers in the smartCARD on the measured saturation values at different points on the detection area is shown in Fig. 1b. It can be seen that at low cholesterol concentrations a light diffuser is needed so that the color change can be quantifiable. When no diffuser is used or only PDMS is used the strips appears as white with either 100% or 0% saturation levels. Diffusers made of black PDMS and FullCure, an acrylic-based photopolymer material, allowed for the saturation value on the low cholesterol test strip to be quantifiable with standard error of 0.16% and 0.42% respectively across a 200 px section at the center of the strip. This is important because it indicates that misalignment of the test strip will have little effect on the measured saturation value.

The sensitivity of the image acquisition system, defined as the ability to differentiate between colorimetric test strips at different cholesterol concentrations has also been investigated. As can be seen in Fig. 1c the smartCARD accessory with the black PDMS diffuser has on average a 36.6% point decrease in lightness when imaging the high cholesterol test strip compared to the low cholesterol one. The effect is much lower, only 5.2%, when a FullCure diffuser is used. Consequently, black PDMS was used as the diffuser material because it not only allows for uniform illumination of the strip but also maximizes the range of colorimetric variation on the strip.

The test strips used in this section are dry reagent strips manufactured by CardioChek (Polymer Technology Systems Inc, IN, USA). When the user applies a drop of blood on one side, it first goes through a series of filter papers that separate plasma from red blood cells and direct some of the plasma towards an analyze-specific reaction pad. At that point, HDL is separated from LDL and VLDL fractions and precipitated by the reaction with phosphotungstic acid. An enzymatic reaction then converts total cholesterol and HDL cholesterol to cholest-4-en-3-one and hydrogen peroxide. The peroxide then reacts with disubstituted aniline to form quinone imine dyes.[15] The color change from the last reaction is then imaged inside the smartCARD accessory by the smartphone camera.

Correlation between cholesterol levels and colorimetric reaction

In order to quantify the colorimetric reaction and to obtain the blood cholesterol concentration value, we have developed a calibration curve linking cholesterol to the HSL (Hue Saturation

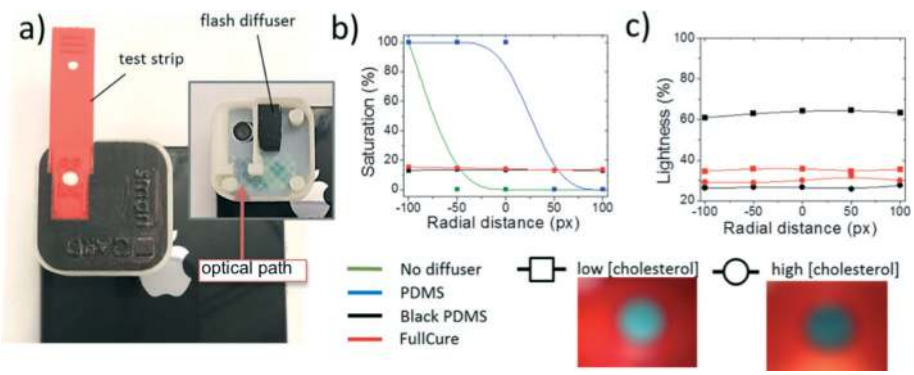

Fig. 1 a) Picture of the smartCARD accessory and the test strip used; the inset shows the inside of the accessory with the black PDMS diffuser and the optical path of the flash used to illuminate the strip. b) Variation in saturation across a 200 px area in the center of the low cholesterol ($<$100 mg dl^{-1}) test strip for different diffusers in the smartCARD. c) Variation in lightness across a 200 px area between the low cholesterol strip ($<$100 mg dl^{-1}) and high cholesterol ($>$400 mg dl^{-1}) strip for 2 different diffusers. The bottom of the figure shows the legend for different diffusers used as well as the 2 test strips used for the data.

Lightness) cylindrical-coordinate representation of the RGB (Red Green Blue) color values at the center of the cholesterol test strip. The advantage of using HSL coordinates over RGB for smartphone-based colorimetric imaging has been demonstrated in several publications.[12,16,17] Hue (H) has a piecewise definition and in the region of interest of the cholesterol colorimetric reaction can be written as a function of the red (R), green (G) and blue (B) color values:

$$H = (B - R)/C + 2 \text{ if } M = G \text{ or } H = (R - G)/C + 4 \text{ if } M = B \quad (1)$$

In the equation above $C = M - m$ where $M = \max(R, G, B)$ and $m = \min(R, G, B)$. In addition the lightness (L) and saturation (S) are described by the following equations:

$$L = \frac{1}{2}(M + m) \quad (2)$$

$$S = (M - m)/(1 - |2L - 1|) \quad (3)$$

For the calibration curve, human serum (Sigma Life Science, USA) was used and augmented using Cholesterol Lipid Concentrate (Rocky Mountain Biologicals Inc, MT USA) in order to cover the whole range of physiological cholesterol levels. At each cholesterol concentration in the relevant physiological range (140 mg dl^{-1} to 400 mg dl^{-1}) the test strip was first analyzed using the CardioChek portable Blood Test System and then imaged using the smartCARD system. In Fig. 2a we show the variations in lightness and saturation for images acquired using the smartCARD system. The cholesterol reading is first obtained using the CardioChek portable Blood Test System. The hue values show very little variation across the whole range of cholesterol values and are not shown in Fig. 2a. However, as we will show later in the Results section, hue values can be used to indicate if a test is successful or if it fails due to image acquisition or test strip issues. The relationship between concentration and saturation can be described by a second order polynomial.

$$[\text{Chol}] = 0.08S^2 - 4.56S + 196.84 \quad (4)$$

As can be seen in Fig. 2b this allows almost perfect matching with a maximum error of 1.8%.

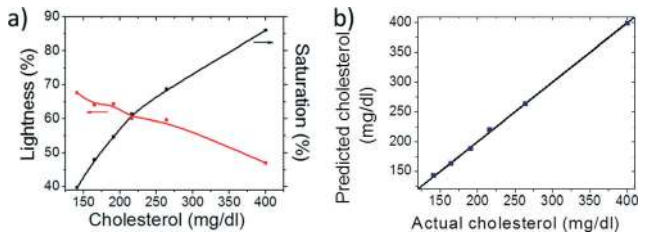

Fig. 2 a) Variation in lightness (red) and saturation (black) *vs.* cholesterol levels. b) Predicted cholesterol concentration *versus* actual cholesterol concentration defined by CardioCheck PA.

Smartphone image acquisition and processing

Several groups have developed algorithms for test-strip colorimetric analysis on a smartphone and have demonstrated their application measuring simple colorimetric reactions such as pH.[12,14] In those cases instantaneous reactions make it easy to image. Slow enzymatic reactions involving whole blood separation steps such as for cholesterol monitoring have the additional complication of being time dependent and more variable due to strip manufacturing variability. For such cases, Mohapatra *et al.*[18] has proposed using a personal glucose meter to quantify active enzyme analytes as a way of reducing the effects of lighting conditions, focus and camera differences. Other groups have focused on improving the algorithm for image processing and combining it with an accessory that block external lighting for the analysis of time varying reactions such as fluorescent assays.[19,20]

Here we have developed a smartphone application for the iPhone iOS platform that in combination with the smartCARD accessory allows for image acquisition and colorimetric analysis of the cholesterol enzymatic reaction. A screenshot of the app is shown in Fig. 3a. When the user presses "analyze" on the app, an image of the colorimetric color changes is acquired through the iPhone camera. As shown in the Fig. 3b schematic, the app then executes several processing steps before the cholesterol value is displayed on the screen. First, a 100 px by 100 px calibration area is selected at the bottom right corner of the image. The average RGB value is computed and converted to HSL. This average HSL value is then compared to a reference value and a background shift is computed. The whole image is then is subjected to this background shift. After the background shift, a 100 px by 100 px area in the middle of the detection circle is then selected and the same computation as before is done to obtain the average HSL value of the test area. The algorithm then verifies if the test is valid by comparing the average hue value to the typical value of the cholesterol test, which as we will show in the Results section is constant across physiological cholesterol values (H ~ 180) both for serum

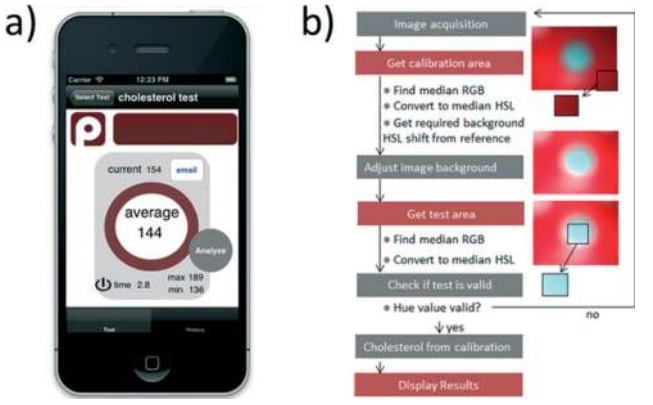

Fig. 3 a) Screenshot of the app showing current cholesterol reading as well as average reading for one user. b) Algorithm used for image processing and implemented in iOS app.

samples and blood samples during test trials. In order to decrease fluctuations due to lighting conditions, the strip is imaged 3 times and the average hue value over those 3 images is taken. If the hue value falls within the range of expected hue values, than the cholesterol level is calculated using the calibration curve obtained in the previous section. The ability of identifying bad samples is a major advantage over specialized hand-held devices, such as CardioChek PA, that use reflectance photometry to quantify colorimetric reaction.

Results and discussion

Accuracy and reproducibility

A critical issue to consider for point-of-care testing is the accuracy of the measurement. Once the user applies a drop of blood on the strip it takes some time for the colorimetric change to occur on the other side of the strip since the blood goes through several separation steps and chemical reactions and the colorimetric change occurs gradually as can be seen at the bottom of Fig. 4. If the strip is imaged before the reaction has terminated then we will get a misleadingly low value for the blood cholesterol level. In order to determine the approximate time required for the reaction to occur we have monitored the color change for a serum sample with an actual concentration of 178 mg dl^{-1}. As can be seen in Fig. 4a it takes about 60 s for the colorimetric change to stabilize. The variation in predicted cholesterol levels are contained within less than 3.9% of the actual value after that however the value shifts up as time elapses. It is therefore important to be consistent by building in the algorithm a time frame for imaging the test strip. In addition averaging several acquired images during that time frame can helped further improve the accuracy. Therefore in next section the image acquisition is all done between 60 s and 80 s and the predicted cholesterol is the average of the predicted cholesterol on 3 different images. As will be shown in the next section the 3.9% inter-assay variation that was observed here for the smartCARD system is lower than what was observed for other lipid point-of-care device.

Another important feature of the smartCARD system is that it can be used on different smartphone platforms with small variability. Fig. 4b shows the imaging of the same test strips with 3 different smartphone cameras across a wide range of physiological cholesterol values. The maximum difference between readings is 5.8% which is significant but on the same order as the CardioChek PA system reading error. This error between phones can be minimized by designing custom strips that allow for better calibration as we showed in our previous publication.

Validation of smartCARD for human trials

In order to demonstrate the ability the smartCARD system for determining blood cholesterol levels we ran user experiments and compared the predicted cholesterol levels to the actual cholesterol levels as indicated by the CardioCheck PA lipid point-of-care device. As can be seen in Fig. 5a from the 9 readings taken, the maximum difference between our predicted value and the CardioCheck PA reading was 5.5% in one instance with less than 3% difference for all the other readings.

For the CardioCheck PA the observed inter-assay imprecision has been recorded by Shephard *et al.*[15] as 4.4% and observed bias with respect to a CDC-certified laboratory method has been recorded as 12.1%. Other studies produced similar results for lipid point-of-care devices.[9,21,22] Inter sample and comparative difference for the smartCARD system indicates that the error is lower than for such point-of-care systems.

In addition, the smartCARD algorithm can identify erroneous readings caused by misalignment of the test strip in the accessory or insufficient amount of blood on the test strip. During the human trials, two readings were discarded because the recorded hue didn't fall within the normal range of hue values (170° to 190°) as shown in Fig. 5b. A low hue value can indicate that the amount of blood applied on the test strip is not sufficient or that the sample was contaminated. Further investigation into the reasons why some tests fall outside the normal hue range would be useful to improve the system but is beyond the scope of this paper.

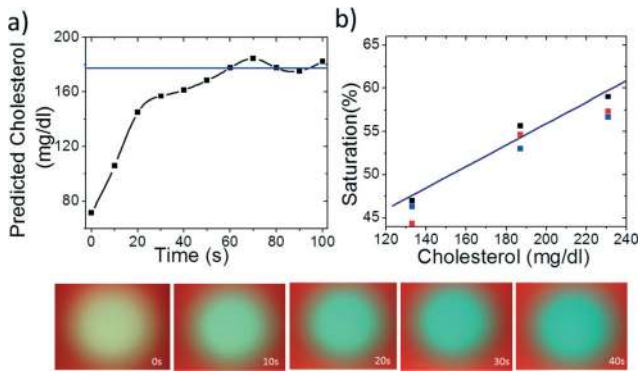

Fig. 4 a) Variations in predicted cholesterol levels *vs.* time for a test strip with the clue horizontal line representing the actual cholesterol level of 178 mg dl^{-1}. b) Variation in readings with different 3 different iPhones. The bottom of the figure shows the imaging of the test strip during the first 40 s.

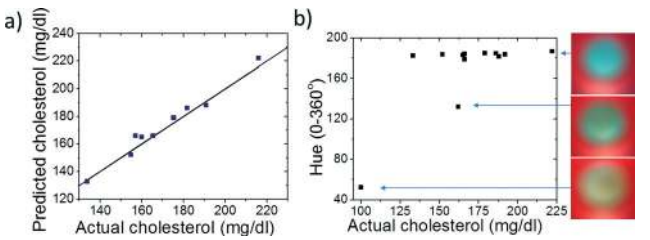

Fig. 5 a) Difference between CardioChek PA reading and smartCARD for human trails. b) Hue differences for all the human test trials showing that discarded tests give hue values outside of the normal range (170–190°).

Conclusion

In this paper we demonstrate a system that can be used to measure and track cholesterol levels directly on a smartphone. The smartCARD system is designed in a way that allows for optimal image acquisition. We showed the ability to measure cholesterol levels within 1.8% accuracy in the relevant physiological range (140 mg dl^{-1} to 400 mg dl^{-1}) by looking at the acquired image's saturation. We also demonstrated interphone repeatability before performing user experiments and measuring blood cholesterol levels with the system we have developed. The smartCARD predicted cholesterol values for the user testing were compared to the measured value using the CardioChek PA system and found that the maximum difference between our predicted value and the CardioCheck PA reading were less than 5.5% in all cases. In addition, we demonstrated the system's ability to identify erroneous readings, which is something current commercial devices cannot do.

In the future it is possible to design the cholesterol test strips in such a way as to further minimize analysis fluctuations. We have demonstrated in a previous publication that by incorporating and imaging a white reference area on the test strip, we can adjust the white balance and get more reproducible results across different smartphones.[12] Finally, using the smartCARD system presented here it is possible to measure other commercially available colorimetric test strips for LDL, HDL cholesterol, and triglycerides.

Acknowledgements

D.E. acknowledges funding support through a CAREER award #0846489 from the US National Science Foundation and the Academic Venture Fund of the Atkinson Center for a Sustainable Future at Cornell University. In addition, V.O. acknowledges the support of the National Science and Engineering Research Council of Canada (NSERC) through a Postgraduate scholarship. M.M. would like to acknowledge a National Science Foundation Graduate Research Fellowship under grant no. DGE-0707428.

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