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Lab on a Chip

Cite this: *Lab Chip*, 2012, **12**, 4240–4243

www.rsc.org/loc

TECHNICAL INNOVATION

Point-of-care colorimetric detection with a smartphone†

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Received 1st July 2012, Accepted 7th September 2012
DOI: 10.1039/c2lc40741h

Paper-based immunoassays are becoming powerful and low-cost diagnostic tools, especially in resource-limited settings. Inexpensive methods for quantifying these assays have been shown using desktop scanners, which lack portability, and cameras, which suffer from the ever changing ambient light conditions. In this work, we introduce a novel approach of quantifying colors of colorimetric diagnostic assays with a smartphone that allows high accuracy measurements in a wide range of ambient conditions, making it a truly portable system. Instead of directly using the red, green, and blue (RGB) intensities of the color images taken by a smartphone camera, we use chromaticity values to construct calibration curves of analyte concentrations. We demonstrate the high accuracy of this approach in pH measurements with linear response ranges of 1–12. These results are comparable to those reported using a desktop scanner or silicon photodetectors. To make the approach adoptable under different lighting conditions, we developed a calibration technique to compensate for measurement errors due to variability in ambient light. This technique is applicable to a number of common light sources, such as sun light, fluorescent light, or smartphone LED light. Ultimately, the entire approach can be integrated in an ''app'' to enable one-click reading, making our smartphone based approach operable without any professional training or complex instrumentation.

1. Introduction

Paper microfluidic analytical devices have emerged in recent years,[1,2] leading to development of a number of point-of-care (POC) analyses, including HIV chips,[3,4] paper ELISA,[5,6] and low-cost colorimetric diagnostic assays.[7–9] Such paper microfluidic assays are gaining popularity as a simple and fast way of disease screening in resource limited environments.[10–12] Although the colorimetric results of these assays can be viewed by naked eye, it is difficult to precisely quantify the analyte amount.[13] Promising colorimetric detection results have been demonstrated using video cameras,[14] digital color analyzers,[15] scanners[16] or custom portable readers.[8] A key drawback of all these methods is the need for specialized instrumentation and for image analysis with a computer.

A smartphone (or tablet) offers an attractive alternative for imaging, analysis, and communication of results in the field. With 6 billion subscriptions worldwide, cell phones are becoming ubiquitous.[17] Indeed, several investigators have already demonstrated the use of camera phones for on-site diagnosis in dermatology,[18] ophthalmology,[19] and colorimetric diagnostics.[20–22] However, cell phones have yet to gain popularity for colorimetric detection due to three key challenges. First, integrated color balancing functions of a conventional cell phone are optimized for photography in high ambient light, and are not suited for images when accurate quantitative measurements must be performed. Second, lighting conditions during imaging can be difficult to control, especially outside of controlled environments such as a laboratory. Third, analysis of images can be challenging especially when small color changes are present, and the red, green, and blue (RGB) intensity values alone are not necessarily sufficient. For these reasons, the use of camera phones has not yet been fully exploited for POC.

In this work, we introduce a simple approach to quantify colorimetric paper test strips with a cell phone (Fig. 1). We use the International Commission on Illumination (CIE) 1931 color space for quantification of multiple elements in colorimetric diagnostics. Differences in lighting conditions when taking images with a phone can be compensated, yielding accurate measurements as we demonstrate using commercially-available urine test strips and a simple pH paper test. Thus, a smartphone can provide a simple solution to quantitative POC colorimetric analysis, especially when naked eye alone is not sufficient, and is a good alternative to a scanner (see ESI1 for details).

2. Color quantification

Smartphone cameras use CMOS arrays, which are low-cost and integrate a range of automated functions, such as *Auto White Balance* (*AWB*), designed to provide good color reproduction by adjusting the detected RGB signals at different ratios. The

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2lc40741h

Fig. 1 Quantification of colorimetric measurements (*e.g.*, urine test strips) with a smartphone

resulting images are brighter and more pleasing to our eyes, making the automatic function popular with non-professional photographers. However, changes in RGB values skew measurements when attempting to use a smartphone camera for quantitative measurments.[23] The best solution to this challenge is to fully control all camera functions to preserve consistency through the tests. Indeed, we used this approach in our previous work with optical oxygen sensors and fluorescence detection.[23] However, this approach is not applicable to cell phones since these functions are not accessible in a fully integrated smartphone camera. An alternative approach is needed if a conventional smartphone is to be used as photodetector.

We designed a reference chart containing 12 color regions with known color intensities to stabilize the smartphone camera functions and compensate for ambient light difference in the subsequent data processing. Our reference chart is analogous to the color-rendition chart in photography, in which 24 regions are included.[24] Our chart contains seven grayscale regions, and five color regions that range from short wavelength (blue) to long wavelength (red). This reference chart nearly eliminates effects of automatic camera functions, making images reproducible and quantifiable. Skewing the reference chart in the direction of warm colors by including yellow, orange, and red regions allows to overcome the tendency of AWB to decrease gain in the *red* channel of the CMOS imaging array, since Si exhibits better responsively at these longer wavelengths. The reduced complexity of the chart permitted simpler and faster analysis, with little sacrifice in quality. This is further supported by our ambient light compensation tests, which we discuss later on, illustrating that our 12-region reference chart is sufficient to build a conversion curve to compensate for differences in ambient light.

While our reference chart allows accurate colorimetric imaging, processing of these images must be considered. An obvious approach of directly converting RGB values into the corresponding analyte concentrations does not yield useful data. A clear illustration of this can be observed from imaging pH paper, which turns colors from red to dark blue according to pH value of the test solution. The RGB values of images for pH increasing from 1 to 12 are plotted in Fig. 2, illustrating that RGB intensities do not exhibit any discernible trend and are difficult to correlate with test solution pH values. Some investigators have attempted to address this inadequacy of the direct RGB measurement by using hue values,[25] taking a ratio

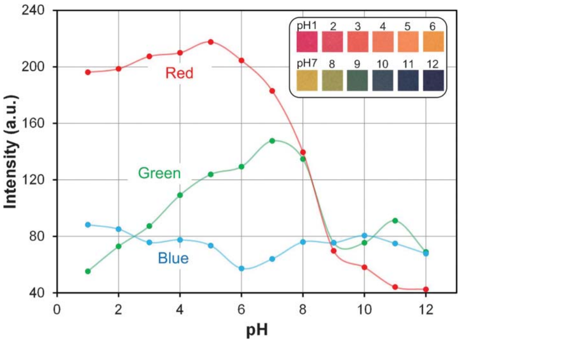

Fig. 2 RGB intensities of pH paper color at each pH value. None of the individual channel intensities correlate with the pH values.

between red and green channels,[22] or using subtractive CMYK and hue-based HSL values.[26] However, these methods are not sensitive to dark colors and are application specific.[27] Thus, an effective method of quantifying color is still needed for quantitative measurements and building calibration curves.

We chose CIE 1931 color space to code the colorimetric image to overcome inadequacies of the simple RGB analysis. The CIE system[28] is the most recognized method in which color is represented by parameters x and y determining chromaticity of a color and Y parameter representing luminance (brightness) of a color. The color space is then represented in a 2-D chromaticity diagram and can be used to predict outcome of color mixtures,[29] which potentially can be useful in more complicated colorimetric assays. Notably, the hue and saturation of a color, on which the widely used HSV and HSL color space models are defined, can be derived from its location on the xy diagram.[30] Conversion of the smartphone camera image data into the CIE 1931 color space involves three steps, in which the color space terms are derived from the conventional RGB values (details are in ESI2). Considering these assets of the CIE 1931 color space, our approach to quantifying colors is versatile and works well as we demonstrate with pH measurements below.

3. Measurement of pH

The described color quantification method can be applied to commercially available colorimetric test strips. Here, we demonstrate the approach using colorimetric pH indicator strips (Micro Essential Laboratory) which were dipped into a range of pH buffer solutions and then imaged with a smartphone camera. The mean RGB intensities of the region of interest (ROI) were calculated and converted to the chromaticity values x and y. Fig. 3a illustrates that the 2-D diagram not only intuitively reflects the color change of the pH strip, but also the corresponding pH change. The pH value appears to be a function of the chromaticity values x and y in the 3-D space (Fig. 3b). A calibration plane can be constructed for pH quantification using chromaticity values of various standard solutions of known pH. By substituting the x and y values, the corresponding pH value can be obtained. Fig. 3c illustrates the standard curve for measuring pH in the range from 2 to 10. The observed sensitivity of $\sim$0.5 is limited by the pH strip, and can be improved by using narrow range pH strips for more accurate measurements.

We should note that the calibration curves developed above show dependence on the smartphone CMOS chip. Our tests with HTC and BlackBerry phones show slight variations (<5%), indicating necessity of re-calibration for each new smartphone model used if higher precision is desired. Nevertheless, only initial calibration is necessary

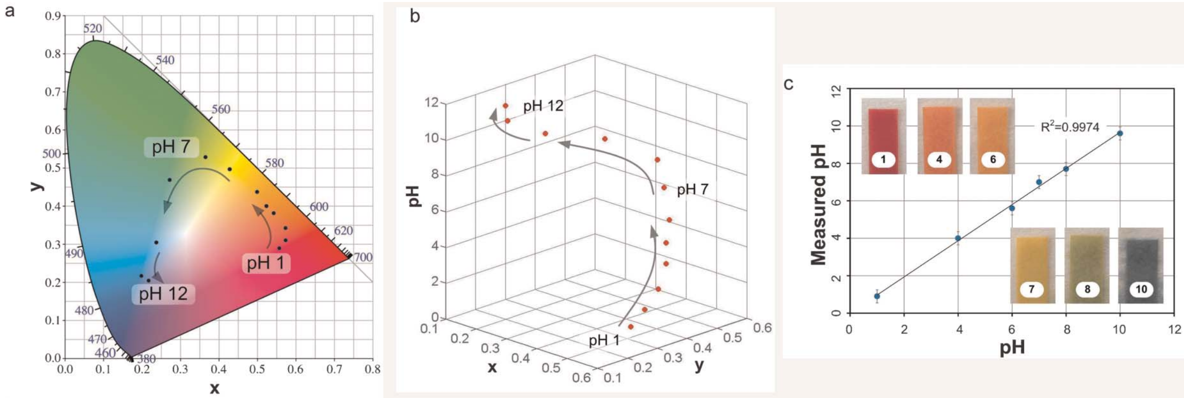

Fig. 3 (a) pH paper colors in CIE 1931 color space. (b) The 3-D view of the relation between *xy* coordinates and the pH values. (c) Standard curve of the smartphone reader response to different pH buffer solutions.

and the process does not need to be repeated for each measurement. We should also note that additional measurement errors can be caused by differences in ambient light conditions when test strips are imaged. Thus, we next examine the effect of ambient light and propose an approach for its compensation.

4. Ambient light compensation

While the benchtop results above demonstrate the capability of using a smartphone for quantitative colorimetric analysis, a critical challenge still prevents us from taking it out of the lab and into everyday use. This is due to the variation in ambient light conditions which are determined by the position of the light source, light temperature, or outdoor lighting environment. An object absorbs light in a specific wavelength range while reflecting the rest. The CMOS pixel measured intensity of the reflected light is determined by many factors, such as the ambient light wavelength, the reflection, the color of the object and the RGB responsivity of the CMOS pixels. A practical way is to treat all these factors as a black box and build a mapping algorithm based on the measured RGB intensities of a color reference chart. After measuring the 12 regions on the reference chart, we found that the measured intensities between different ambient light conditions had a linear relationship. As is illustrated in Fig. 4a, the measured intensities at one light condition I_1 can be mapped to another I_2. We calibrated the test strips responses and the corresponding 3-D fitting model using a 5000 K fluorescent light source, and ran detection tests under other light conditions (see ESI3). By building the compensating equations in the red, green, and blue pixel channels, we can map RGB intensities of any imaged sample to the 5000 K exposure and calculate the corresponding chromaticity values. This color mapping approach provides an excellent way to compensate for errors caused by the ambient light changes, making the cellphone detection approach applicable to any lighting environment from indoors to outdoors, from sunshine to overcast.

We calibrated our detection algorithm for pH measurement at the 5000 K ambient light condition. Then the measurement was repeated at the 3500 K ambient light condition. Fig. 4b illustrates the measured chromaticity coordinates of the pH strips' color response to samples with pH. The chromaticity values measured under 3500 K ambient light exhibit an approximately 0.03 shift in x or y which causes large error in pH detection. After the processing, the different intensities caused by the ambient light were compensated, resulting in a new series of chromaticity coordinates which matches the calibration at 5000 K very well. The slight deviation is caused by material of the reference color chart and the test strip paper with different reflection. If the reference chart is printed directly on the test strip, we expect the accuracy of this method can be further improved.

Our light compensating method creates mapping of signals detected at any light conditions to the calibration light condition, enabling improvisatory calibration of the test. To get an accurate measurement, light sources with high color rendering index are still recommended and the image of the color reference and test strip should be taken with care. The method assumes that the ambient is uniformly shining on the sample so that the intensities of the reference colors can be used to build a precise conversion curve for the unknown sample. If the smartphone is too close to the sample, it may block the light and generates shadow which breaks the uniformity and causes false measurement. Thus, the smartphone should be placed at a proper position and height, depending on the location of the camera, to get accurate readings.

5. Discussion

With the experimental results reviewed, we will now highlight potential advantages and applications. First, any cell phone or smartphone can be used for imaging colorimetric tests. According to the International Telecommunication Union (ITU), mobile-cellular subscriptions reached 6 billion in 2011, with 75% in developing countries; a 3–8 megapixel camera is a standard feature for the most of the phones.[17] Using cell phone for imaging colorimetric tests may offer a simple and convenient way to read results (and potentially transmit data to a physician *via* cellular network). With a smartphone, it may even be possible to process images with the phone for immediate display. Furthermore, using a smartphone for data analysis does not require trained personnel, and can be accomplished in seconds by a novice. Coupled with low-cost paper test strips, smartphones may offer a simple approach to disease screening in developing countries and resource limited settings. We do not believe that smartphone based colorimetric detection will replace traditional microscopic or spectroscopic based diagnostic, but it may offer a low-cost solution for *a priori* screening of a large number of potential patients.

Second, and most important, the approach can be easily extended to images taken by other means. Indeed, we have

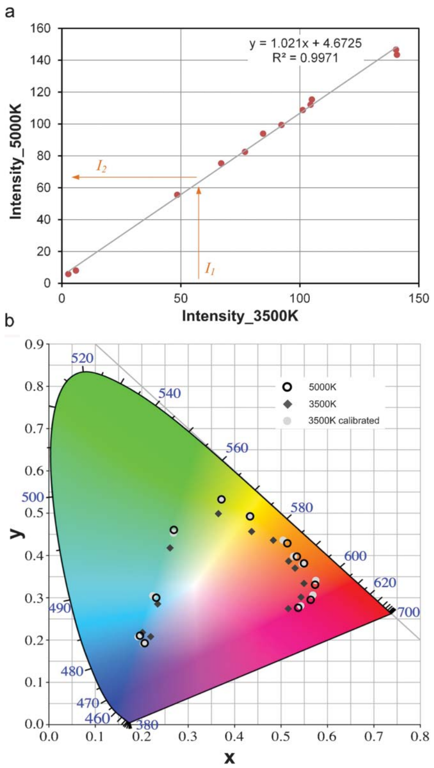

Fig. 4 (a) The linear relationship of the reference chart intensities between the 5000 K and 3500 K fluorescent light. The color intensities of the sample can be substituted to the fitted curve to derive the corresponding intensities at which the device was calibrated (5000 K). (b) Shift in color space coordinates of the pH test strip images due to different ambient light conditions. The ambient light correction technique effectively compensates the error caused by ambient light.

successfully applied it to images obtained by a tablet, a microscope and a scanner. Using Matlab, the color conversion process can be automated for processing on a laptop computer or a tablet running Win7 (*e.g.*, Fujitsu Q550). The CIE 1931 color system projects all the human visible colors onto a 2-D plane regardless of brightness, and is inherently resistant to changes in intensity in ambient light. Our approach is simpler and more accurate than the direct RGB measurement using hue values[23] or ratios of red and green channels.[22] Using color space conversion leads to accurate quantification and ambient light compensation. Further, as already mentioned, with a tablet or smartphone one could take advantage of cellular network transmission and cloud-based data storage, or perform analysis outside laboratory.

Thirdly, the color conversion analysis technique could be extended beyond imaging colorimetric test strips, and could be applied to analysis of any color images including fluorescence data. The recent development in paper microfluidic immunoassays[31,32] and ELISA chips[33] make these accurate methods directly accessible to end users, without performing complicated sample handling steps. We envision our smartphone based color conversion analysis technique will provide a user-friendly approach that matches these assays. Not reported herein, but under development is a second generation color conversion platform that automates the process in an "app" with cross-platform functionality. This new platform further simplifies analysis in multiplexed applications, and will be reported in a future publication. Ultimately, we believe the technique can be broadly applied to POC diagnosis with any type of colorimetric test strip, or to any sensor systems that provide colorimetric response.

Acknowledgements

This work was supported by funds provided by the Air Force Office of Scientific Research and by the Human Effectiveness Directorate, 711 Human Performance Wing, Air Force Research Laboratory.

References

1 A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 2007, **46**, 1318–1320.
2 D. A. Bruzewicz, M. Reches and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3387–3392.
3 S. Wang, F. Xu and U. Demirci, *Biotechnol. Adv.*, 2010, **28**, 770–781.
4 M. A. Alyassin, S. Moon, H. O. Keles, F. Manzur, R. L. Lin, E. Hæggstrom, D. R. Kuritzkes and U. Demirci, *Lab Chip*, 2009, **9**, 3364–3369.
5 S. Wang, L. Ge, X. Song, J. Yu, S. Ge, J. Huang and F. Zeng, *Biosens. Bioelectron.*, 2012, **31**, 212–218.
6 A. W. Martinez, *Bioanalysis*, 2011, **3**, 2589–2592.
7 W. Wang, W. Y. Wu, W. Wang and J. J. Zhu, *J. Chromatogr., A*, 2010, **1217**, 3896–3899.
8 D. Lee, B. G. Jeon, C. Ihm, J. -. Park and M. Y. Jung, *Lab Chip*, 2011, **11**, 120–126.
9 M. A. Nash, J. M. Hoffman, D. Y. Stevens, A. S. Hoffman, P. S. Stayton and P. Yager, *Lab Chip*, 2010, **10**, 2279–2282.
10 W. G. Lee, Y. Kim, B. G. Chung, U. Demirci and A. Khademhosseini, *Adv. Drug Delivery Rev.*, 2010, **62**, 449–457.
11 P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam and B. H. Weigl, *Nature*, 2006, **442**, 412–418.
12 A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3–10.
13 L. Yu, C. M. Li, Y. Liu, J. Gao, W. Wang and Y. Gan, *Lab Chip*, 2009, **9**, 1243–1247.
14 K. Tohda and M. Gratzl, *Anal. Sci.*, 2006, **22**, 383–388.
15 K. Suzuki, E. Hirayama, T. Sugiyama, K. Yasuda, H. Okabe and D. Citterio, *Anal. Chem.*, 2002, **74**, 5766–5773.
16 D. J. Soldat, P. Barak and B. J. Lepore, *J. Chem. Educ.*, 2009, **86**, 617–620.
17 International Telecommunication Union, http://www.itu.int/ITU-D/ict/statistics/index.html..
18 E. M. T. Wurm, R. Hofmann-Wellenhof, R. Wurm and H. P. Soyer, *J. Dtsch. Dermatol. Ges.*, 2008, **6**, 106–112.
19 V. F. Pamplona, A. Mohan, M. M. Oliveira and R. Raskar, *Proc. of Frontiers in Optics, Adaptive Optics for the Eye (FTuB)*, FTuB4, Rochester, NY, 2010.
20 S. Wang, X. Zhao, I. Khimji, R. Akbas, W. Qiu, D. Edwards, D. W. Cramer, B. Ye and U. Demirci, *Lab Chip*, 2011, **11**, 3411–3418.
21 A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas III, H. Sindi and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3699–3707.
22 A. García, M. M. Erenas, E. D. Marinetto, C. A. Abad, I. De Orbe-Paya, A. J. Palma and L. F. Capitán-Vallvey, *Sens. Actuators, B*, 2011, **156**, 350–359.
23 L. Shen, M. Ratterman, D. Klotzkin and I. Papautsky, *Sens. Actuators, B*, 2011, **155**, 430–435.
24 C. S. McCamy, H. Marcus and J. G. Davidson, *J. Appl. Photogr. Eng.*, 1976, **2**, 95–99.
25 K. Cantrell, M. M. Erenas, I. De Orbe-Payá and L. F. Capitán-Vallvey, *Anal. Chem.*, 2010, **82**, 531–542.
26 S. Paciornik, A. V. Yallouz, R. C. Campos and D. Gannerman, *J. Braz. Chem. Soc.*, 2006, **17**, 156–161.
27 M. H. Saad, H. I. Saleh, H. Konbor and M. Ashour, *Int. J. Comput. Theory Eng.*, 2011, **3**, 701–706.
28 K. Nassau, *Color for Science, Art and Technology*, Elsevier, New York, 1998.
29 C. Arbizzani, M. G. Cerroni and M. Mastragostino, *Sol. Energy Mater. Sol. Cells*, 1999, **56**, 205–211.
30 G. Wyszecki and W. S. Stiles, *Color Science: Concepts and Methods, Quantitative Data and Formulae*, Wiley, New York, 1982.
31 H. Jiang, X. Weng and D. Li, *Microfluid. Nanofluid.*, 2011, **10**, 941–964.
32 E. Fu, T. Liang, P. Spicar-Mihalic, J. Houghtaling, S. Ramachandran and P. Yager, *Anal. Chem.*, 2012, **84**, 4574–4579.
33 J. H. Cho, S. M. Han, E. H. Paek, I. H. Cho and S. H. Paek, *Anal. Chem.*, 2006, **78**, 793–800.

