mHealth Dipstick Analyzer For Monitoring of Pregnancy Complications

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Abstract— **Dipstick-based urinalysis is routinely used for detection of early signs of such pregnancy complications, as preeclampsia and gestational diabetes. Usually it is done in doctor's office using an automatic dipstick analyzer. Here we present a novel smartphone-based colorimeter and demonstrate its application to the measurements of glucose and protein concentrations in biological samples. The key innovations of our approach was to combine powerful image processing encoded into a mobile phone application with a low cost 3D printed sample holder that allowed to control lighting conditions and significantly improved sensitivity. Different solutions with protein and glucose concentrations ranging from 0 to 2000 mg/dL were prepared and analyzed using our system. The detection limit for glucose was 40 mg/dL and for protein - 6mg/dL The smartphone-based colorimeter always correctly classified the corresponding reagent strip pads, what confirms that it can be used as a low cost alternative for commercial dipstick analyzers.**

Keywords— pregnancy complications, preeclampsia, glucose and protein detection, mHealth, urinalysis

I. INTRODUCTION

Recent advances in smartphone technology, such as greatly enhanced processing power, storage capability and wireless connectivity turned mobile phones into powerful computers integrated with high quality cameras and numerous sensors. Additionally, the price and size of the mobile phones have decreased so much, that they became available even in the countries with the lowest income. The worldwide mobile phone subscription recently reached nearly 7 billion users [1]. Some of the applications of the mobile phones in healthcare and biomedical fields include weight management [2], lens-free microscopy [3], hypertension monitoring system [4], label free immunoassays [5], monitoring system for Parkinson's disease patients [6], retinal disease diagnostic device[7], system for monitoring kidney metabolomics [8], flow cytometry [9] and many others [10].

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There are colorimetric smartphone software applications that determine a concentration of an analyte in a biological sample by conducting color analysis [11]. Similarly to traditional colorimeters, they are based on Beer-Lambert's law relating absorbance of collimated monochromatic beam in a homogeneous medium to the concentration of the absorbing species and the propagation distance through the absorptive medium [12]. Alternatively, mobile phone based colorimeters can use color analysis to determine a concentration a colored substance. For example, there was a smartphone based colorimetric reader for quantitative analysis of direct enzyme-linked immunosorbent assay (ELISA) for horse radish peroxidase (HRP), rapid sandwich ELISA for human C-reactive protein (CRP) and commercially available BCA protein estimation assay [13].

Smartphone based colorimeters are broadly classified into two categories: ones that are based on stand-alone applications, and others based on software combined with different hardware components. Mobile phone colorimeters of first kind are low cost alternative for expensive commercial colorimetric readers [11]. The main limitation of them is that the user needs to recalibrate them with even a slightest change in ambient light.

Mobile phones with an app combined with a dedicated light controlling hoods and integrated lighting setup works great in changing ambient lighting. The light controlling hood can also be used for holding a sample at constant position for continuous tracking of color change at particular location on the sample. Building a hood for a mobile colorimeter requires accurate fit to dimensions of the mobile phone and position of the camera. In addition to this, its operation requires additional battery powered light source, e.g. LED array, to keep the illumination constant. Usually such attachments to mobile phone are quite sophisticated, and assembly is challenging. Here we propose a simple and low cost hybrid point-of-care mobile phone based colorimeter. This device overcomes the limitation of both types of mobile phone based colorimeters and demonstrates great performance, high reproducibility, accuracy and stability under varying lighting conditions. Additionally, it can be used for very precise tracking of such critical indicators of preeclampsia and gestational diabetes, as concentration of protein and glucose in urine samples.

II. METHODS

In order to make a precise and reproducible mobile phone based colorimeters, we need to fix a number of hardware parameters, such as distance between a mobile camera and a sample, multiple settings of the camera and use a stable light source [14]. In order to keep a sample at constant distance from the camera, we introduce a 3D printed sample holder -Chroma-dock. The Chroma-dock, as shown in Fig. 1 (c) and (d), consist of a black box attached to a mobile phone and a removable cassette serving as a sample holder. This structure not only allows keeping the sample at constant distance from the camera, but also creates a controlled-light environment. This allows capturing very reproducible images and eliminates the background noise. The distance between the camera and the test strip is ~ 15 cm. The strip is placed into the groove at the center of cassette as shown in Figure 1 (b). Then the cassette is inserted into the black box which holds the strip in a fixed position during the color analysis.

Smartphone camera acts as a detector measuring color characteristics of the sample. Because of that, the camera parameters such as exposure rate, white balance, sharpness and ISO, play a vital role in error free colorimetric measurement. Exposure rate defines the amount of light per unit area that reaches the camera sensor. Our colorimetric measurements were performed inside a dark box where an auto settings can cause overexposure and loss of color details [15]. To avoid this, the exposure compensation value was set to the minimum.

A white balance setting adjusts the color of the captured image based on the light source used while shooting the picture. Color reproducibility for images captured under different light conditions can be achieved by using the automatic white balance setting [16]. Since our system is using a stable light source, the white balance was set to the constant mode. Specifically, the white balance was programmed in the mobile application to use the parameters of the 'daylight' mode that allows to normalize the color values based on standard daylight illuminant source D65.

Autofocus is a critical feature for stand-alone mobile phone colorimeters that use automatic focus at a specific region of interest (ROI). The movement of the camera might cause a blur in the image that can add error to the measurements [17]. Constant distance between the object and the camera eliminates the need for autofocus. Therefore, the mobile application was programmed to use 'fixed focus' mode. ISO number of a digital camera measures the sensitivity of the image sensor. The larger the ISO number, the worse is the signal to noise ratio (SNR) [18], so a lower ISO value (ISO 400) was programmed to yields a better SNR. In a case of a stand-alone application-based colorimeter that does not include any additional hardware, these settings have to be dynamically changed, according to varying environmental conditions, what introduces additional noise.

Finally, another important component of a stable mobile phone colorimeter is a high quality light source. While an external LED can be used for mobile colorimetric measurements, it would require an external power supply and wiring, what adds complexity to the system. Fortunately, contemporary cellphones have high quality built-in flashlight integrated with the camera. This light sources can be directly controlled using software installed on a mobile phone.

A. *Mobile software development*

Mobile application functionality for colorimetric measurements involves capturing, storing and analysis of an image of a sample. To determine a concentration of a substance, color information from an image has to be extracted and matched with the values from a calibration curve. Fig. 1 (a) shows the algorithm used for the colorimetric analysis and calibration.

B. *Color processing*

The image of the dipstick is taken using a built-in camera. After that a region of interest is chosen in the middle of a test pad responsible for the needed analyte. The ROI is a square 10x10 pixels. Color information in the ROI of the captured images as shown in Fig 1 (b) is extracted and red, green and blue (RGB) values of the corresponding pixels inside the ROI are obtained. RGB is a non-absolute color space as the color values depend on external factors like illumination, sensitivity of camera sensor, etc. [19-21]. CIE L*a*b* color model provide more accurate and uniform color representation [22]. L* value indicates lightness and it range from 0 to 100 (black to white). a* value indicates red/green color components (positive value represents red region and negative value represents green region). b* value indicates yellow/blue color components (positive value represents yellow region and negative value represents blue region). There is no direct standard formula to convert RGB to L*a*b* values. Mobile app is programmed to convert obtained RGB values to CIE L*a*b* values indirectly, by calculating XYZ tristimulus values. Standard illuminant D65 was considered for the RGB to L*a*b* color space conversion.

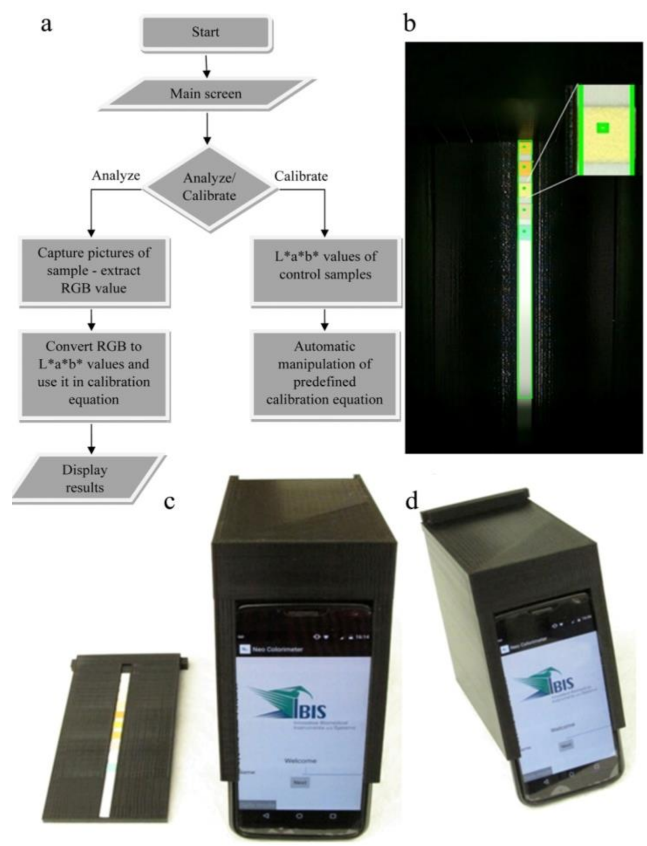

Fig. 1. (a) Diagram of the colorimetric analysis and calibration, (b) camera preview with region of interest (ROI) over the reagent pads of the test strip, (c) Mobile phone based colorimeter with a cassette and a holder, (d) the cassette is inserted.

C. Calibration

Color values obtained in the previous step were used to compute the concentration value of the substance. Equations fitting the calibration curves were built into the mobile app. Some substances change color non-linearly with linear change of concentrations, what adds complexity to the computation procedure. In this case, calibration equation of particular color component from L*,a* and b* is used to determine the concentration of the substance in the sample and the values obtained from the calibration equations of other color components are used in further decision making process.

D. Algorithm for Interphone repeatability

Different mobile phones working on android platform can be used for colorimetric measurements. Smartphone can be transformed into portable colorimeter, provided customized Chroma-dock module and a mobile software. Color response varies from camera to camera, what can introduce a significant error [23] in interpreting the concentration of the substance. Constant color response over different types of smartphones can be achieved by calibrating the device before the test. Stand-alone apps without any housing unit requires regular calibration whenever there is a change in ambient light conditions [11]. Our colorimetric module requires one-time calibration with a set of control samples before starting with the actual samples. Regular calibration is not required for our module as the change in environmental light does not have influence over the light conditions inside the Chroma-dock hood structure. Calibration button was added into the user interface screen of the mobile app that the user must select before performing the first sample test. In response to the input from the user the color values of the control samples are recorded and compared with the standard control values in the program. Difference in those values will be added to the predefined calibration equations extracted from the calibration curves. This algorithm provides a degree of freedom for transforming any smartphone into colorimetric reader by producing constant color response over the different cameras.

III. RESULTS AND DISCUSSIONS

Color stability of the pictures captured using Chroma-dock under different environmental lighting conditions were analyzed. This analysis was crucial to ensure the stability of the device performance and to evaluate the role played by the sample holder for controlling the variables during measurement.

A. Performance testing with different background colors

One of the challenges for stand-alone colorimetric apps is to control the white balance of camera during the measurements. Automatic white balance feature detects the white or neutral tone in the scene and automatically calibrates the rest of the image with respect to the neutral color temperature. This feature is frequently used in stand-alone colorimetric apps to counter-play the changes caused by the varying environmental conditions. Occasional errors in automatic white balance settings are inevitable [24,25] even when specialized techniques are used to minimize the influence of such errors.

Our experiments demonstrated that reagent test strip images taken on different background produced completely different outcomes as shown in Figure 2 (a) even when the auto-white balance, auto-focus and auto-exposure features were used.

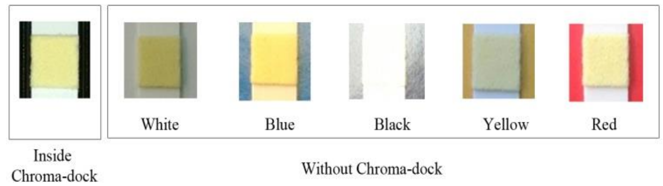

Fig. 2. Images of the same test strips taken on six different backgrounds

Table I. mean L*, a* and b* values for the same reagent pad of the test strip placed on six different backgrounds. From the table we can interpret that varying background affects the color values of the reagent pad. Using the Chroma-dock ensures the control over the background color and provides us with precise results.

TABLE I

Effect of varying background colors over L*a*b* values

Controlled illumination	**Intensities of color at 0 mg/dL of protein concentration**		
	L	**A**	**B**
In Chroma-dock (optimized)	67 ± 0.3	-21 ± 0.2	55 ± 1.6
White	41 ± 2.9	-21 ± 1.2	34 ± 0.8
Blue	67 ± 2.1	-4 ± 1.1	76 ± 0.5
Black	78 ± 0.1	-2 ± 0.3	12 ± 0.1
Yellow	57 ± 1.4	-4 ± 0.5	17 ± 0.5
Red	67 ± 0.6	-22 ± 0.5	37 ± 0.5

B. Performance testing under different lighting conditions

One of the important requirements for a colorimeter is to conduct reproducible measurement independently of changing lighting conditions. Mobile phone colorimeters with an attached box can completely eliminate the errors related to the changes in the lighting conditions. Here we demonstrate our experiments of stability testing under different lighting conditions with and without the Chroma-dock as shown in Fig 2 (b). The test was conducted under bright light illumination, dim light and in a very low light intensity.

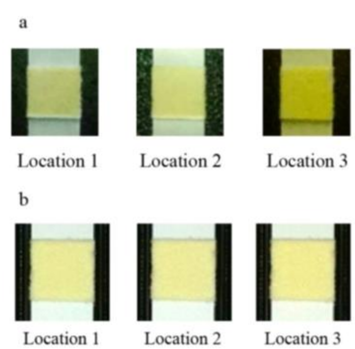

Fig. 3. Comparison between the same test strips imaged in different locations with different ambient lighting conditions. (a) Images taken without Chroma-dock and (b) bottom three images were taken with Chroma-dock. Excellent repeatability is observed when Chroma-dock is used.

Later on CIE L*a*b* color parameters were determined for the measurements conducted with and without the Chroma-dock. Table II provides the mean L*, a* and b* values for corresponding two categories. It can be noticed that the use of Chroma-dock resulted in stable measurements with a minimum standard errors. While measurements without the box resulted in large standard errors and highly non-reproducible measurements. This illustrates the importance of using the Chroma-dock.

TABLE II

Effect of varying ambient conditions over the L*a*b* values measured without and with Chroma-dock

Locations with different ambient light	External/Controlled illumination	Intensities of color at 0 mg/dL of protein concentration		
		L	A	B
Location 1	Without Chroma-dock	54 ± 15	-19 ± 14	56 ± 6
	With Chroma-dock	67 ± 0.3	-21 ± 0.2	55 ± 1.6
Location 2	Without Chroma-dock	67 ± 14	-15 ± 16	63 ± 7.1
	With Chroma-dock	67 ± 0.1	-21 ± 0.5	56 ± 3.1
Location 3	Without Chroma-dock	49 ± 11	-27 ± 3.1	77 ± 8.3
	With Chroma-dock	67 ± 0.2	-21 ± 0.3	55 ± 1.1

C. *Sample testing*

Clinical utility of the smartphone based colorimeter was demonstrated using Urinalysis Reagent Strips. Twelve samples with different concentrations of glucose and protein were prepared. D-(+)-Glucose solution (45%) and Bovine serum albumin from Sigma-Aldrich were used as a source of glucose and protein. They were added to the artificial urine solution from Flinn scientific inc. Eight samples with 0, 100, 250, 375, 500, 750, 1000 and 2000 mg/dL concentrations of glucose and another eight samples with 0, 15, 30, 100, 300, 750, 1500 and 2000 mg/dL concentrations of protein were prepared.

Test strips were briefly dipped into the artificial urine samples. After ensuring all the reagent pads on the test strip were moistened, excess of the sample were removed by wiping the edge of the test strip using standard test strip operation procedure. After time needed for reaction the test strips were placed on the cassette and loaded into the holder module. Mobile software with pre-programmed calibration equations for glucose and proteins was started. It captured and stored the images of the test strip and extracted the color values from those images. The calibration equations were used to calculate the concentrations of substances present in the samples which were displayed to the user and also saved in the database.

Reference color chart provided by the test strip manufacturer for glucose and protein were shown in the Fig. 4. (a) and Fig. 5. (a) and the respective color variation observed while measurement were shown in the Fig 4. (b) and Fig 5. (b).

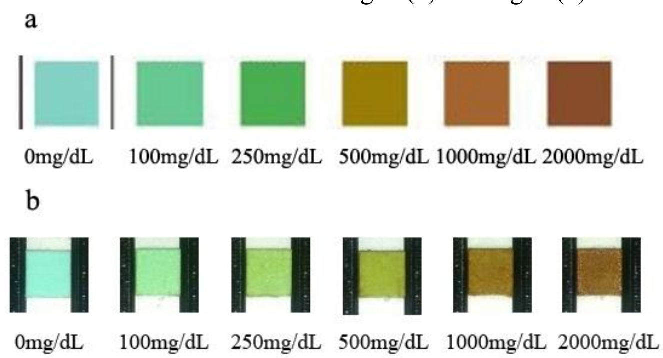

Fig. 4. Comparison between (a) reference chart from the manufacturer and (b) experimental color change for different concentrations of glucose.

Correlation graph between actual glucose concentrations and the values measured by smartphone colorimeter is in the Fig. 2 (a). It indicates good agreement between measured and actual values. In addition to detection of early sign of gestational diabetes, the software can be used to monitor other conditions related to excretion of glucose in the urine, due to untreated diabetes mellitus or renal glucosuria [26].

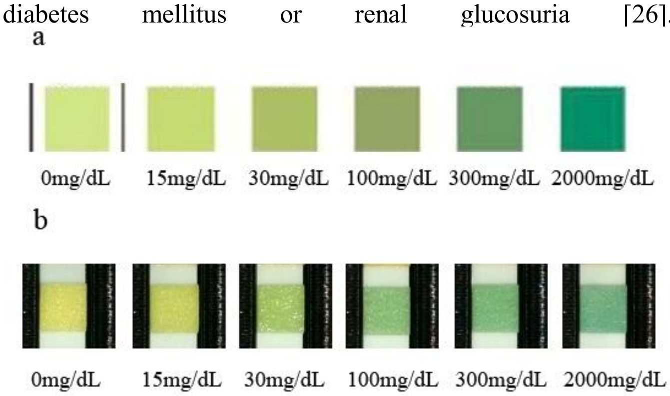

Fig. 5. Comparison between (a) reference chart from the manufacturer and (b) experimental color response for different concentrations of protein.

Correlation graph between the standard protein concentrations in the sample and the measured concentration values by smartphone based colorimeter is shown in the Fig. 2 (b) and (c). We can notice that the measured values are correlated well with the actual values. This can be used to proteinuria - excretion of protein in the urine, that may be due to preeclampsia, nephrotic syndrome, sickle cell disease, glomerular disease, diabetes mellitus, dehydrations, toxic lesions of kidneys, HELLP syndrome, etc [27].

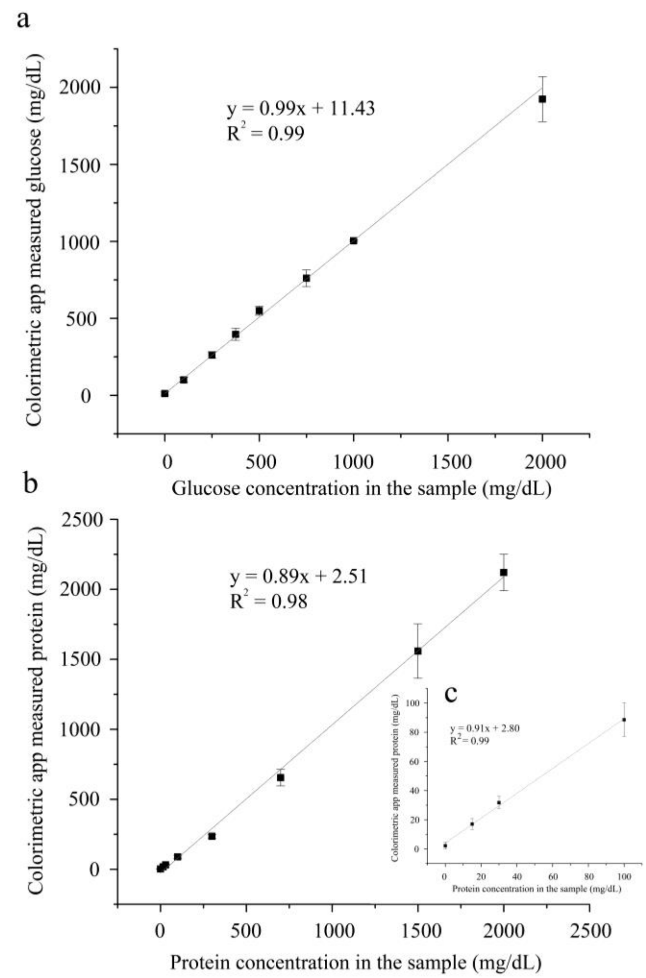

Fig. 6. Correlation graphs for (a) the glucose concentrations in the samples and values measured by the software, (b) and (c) the protein concentrations in the samples and values measured by the app. Panel (c) shows detailed view for the low concentration of protein.

IV. CONCLUSIONS

Advancements in smartphone technology paved the way for development of innovative point-of-care diagnostic devices. Here we demonstrated a mobile phone-based low cost portable colorimeter that can determine the concentrations of protein and glucose in a biological sample. Our device does not require regular calibration, as it is independent of external lighting conditions. The same app can be programmed to measure concentration of other urine components, like ketone, bilirubin, hemoglobin, nitrite, leukocytes, urobilinogen, and other, by adding corresponding calibration equations.

The total cost for manufacturing our smartphone based colorimeter was just several dollars in addition to the existing mobile phone. This makes our device, a low cost alternative for expensive commercial test strip analyzers that cost several hundred dollars. Our mobile app provides the optional feature of sharing these results with the registered members. In addition, this software can store the concentration values in the phone memory along with the user identity information and optionally the app can use cloud storage which makes it a potential product for telemedicine applications.

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REFERENCES

[1] ICT Facts and Figures – The World in 2015. 2015, ITU.

[2] Tsai, C.C., et al., Usability and feasibility of PmEB: a mobile phone application for monitoring real time caloric balance. Mobile networks and applications, 2007. 12(2-3): p. 173-184.

[3] Tseng, D., et al., Lensfree microscopy on a cellphone. Lab on a Chip, 2010. 10(14): p. 1787-1792.

[4] Logan, A.G., et al., Mobile Phone–Based Remote Patient Monitoring System for Management of Hypertension in Diabetic Patients*. American journal of hypertension, 2007. 20(9): p. 942-948.

[5] Giavazzi, F., et al., A fast and simple label-free immunoassay based on a smartphone. Biosensors and Bioelectronics, 2014. 58: p. 395-402.

[6] Arora, S., et al., Detecting and monitoring the symptoms of Parkinson's disease using smartphones: A pilot study. Parkinsonism & related disorders, 2015. 21(6): p. 650-653.

[7] Bourouis, A., et al., An intelligent mobile based decision support system for retinal disease diagnosis. Decision Support Systems, 2014. 59: p. 341-350.

[8] Kwon, H., et al., A smartphone metabolomics platform and its application to the assessment of cisplatin-induced kidney toxicity. Analytica chimica acta, 2014. 845: p. 15-22.

[9] Zhu, H., et al., Optofluidic fluorescent imaging cytometry on a cell phone. Analytical Chemistry, 2011. 83(17): p. 6641-6647.

[10] Preechaburana, P., et al., Biosensing with cell phones. Trends in biotechnology, 2014. 32(7): p. 351-355.

[11] Yetisen, A.K., et al., A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests. Sensors and Actuators B: Chemical, 2014. 196: p. 156-160.

[12] McNaught, A.D. and A.D. McNaught, Compendium of chemical terminology. Vol. 1669. 1997: Blackwell Science Oxford.

[13] Vashist, S.K., et al., A smartphone-based colorimetric reader for bioanalytical applications using the screen-based bottom illumination provided by gadgets. Biosensors and Bioelectronics, 2015. 67: p. 248-255.

[14] Caglar, A., et al., Could digital imaging be an alternative for digital colorimeters? Clinical oral investigations, 2010. 14(6): p. 713-718.

[15] Hirsch, R., Exploring Color Photography Fifth Edition: From Film to Pixels. 2013: Taylor & Francis.

[16] Hsu, E., et al. Light mixture estimation for spatially varying white balance. in ACM Transactions on Graphics (TOG). 2008. ACM.

[17] Brown, G., How autofocus cameras work. Internet Article, http://travel. howstuffworks. com/autofocus. htm, 2008.

[18] Foi, A., et al., Practical Poissonian-Gaussian Noise Modeling and Fitting for Single-Image Raw-Data. Image Processing, IEEE Transactions on, 2008. 17(10): p. 1737-1754.

[19] Mendoza, F. and J. Aguilera, Application of image analysis for classification of ripening bananas. Journal of food science, 2004. 69(9): p. E471-E477.

[20] Segnini, S., P. Dejmek, and R. Öste, A low cost video technique for colour measurement of potato chips. LWT-Food Science and Technology, 1999. 32(4): p. 216-222.

[21] Paschos, G., Perceptually uniform color spaces for color texture analysis: an empirical evaluation. Image Processing, IEEE Transactions on, 2001. 10(6): p. 932-937.

[22] Korifi, R., et al., CIEL*a*b* color space predictive models for colorimetry devices–Analysisof perfume quality. Talanta, 2013. 104: p. 58-66.

[23] Ilie, A. and G. Welch. Ensuring color consistency across multiple cameras. in Computer Vision, 2005. ICCV 2005. Tenth IEEE International Conference on. 2005.

[24] Hsu, E., et al. Light mixture estimation for spatially varying white balance. in ACM Transactions on Graphics (TOG). 2008. ACM.

[25] Fitzpatrick, J. *HTG Explains: What Is White Balance and How Does It Affect Your Photos?* How-To Geek 2013 06/19/2013; Available from: http://www.howtogeek.com/165794/htg-explains-what-is-white-balance-and-how-does-it-affect-your-photos/.

[26] Rose, B. and H. Rennke, Acid-base physiology and metabolic alkalosis. Renal pathophysiology—the essentials. 1st edition. Philadelphia: Lippincott Williams & Wilkins, 1994: p. 123-51.

[27] Simerville, J.A., W.C. Maxted, and J.J. Pahira, Urinalysis: a comprehensive review. Am Fam Physician, 2005. 71(6): p. 1153-62.

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