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Quantifying colorimetric tests using a smartphone app based on machine learning classifiers

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ABSTRACT

A smartphone application based on machine learning classifier algorithms was developed for quantifying peroxide content on colorimetric test strips. The strip images were taken from five different Android based smartphones under seven different illumination conditions to train binary and multi-class classifiers and to extract the learning model. A custom app, "ChemTrainer", was designed to capture, crop, and process the active region of the strip, and then to communicate with a remote server that contains the learning model through a Cloud hosted service. The application was able to detect the color change in peroxide strips with over 90% success rate for primary colors with inter-phone repeatability under versatile illumination. The utilization of a grey-world color constancy image processing algorithm positively affected the classification accuracy for binary classifiers. The developed app with a Cloud based learning model paves the way for better colorimetric detection for paper-based chemical assays.

1. Introduction

The recent advances in smartphone technology propelled the fields of chemical and biological sensing for broad range of analytes [1,2]. The technical difficulties are overcome with the help of 3D printing technology and smart software [3–5]. The mainly used element of a smartphone is its camera that allows quantification of color images using colorimetry or photometry. Colorimetric analysis is a major chemical method using benchtop instruments such as spectrometers or specially designed test strip readers. Chemists frequently rely on color to follow through a reaction of interest, and yet have to use instruments to further extract quantitative data. Smartphone technology can help chemists for colorimetric analysis only if the captured image can be quantified. One way to achieve this goal is to use smartphone spectrometers that rely on captured spectral images and utilize Beer–Lambert Law to assess the absorbance of colored liquids [6,7]. Colors of liquids can be quantified using Hue-Saturation-Value (HSV) color space of taken images and applying non-linear fitting curve [8,9]. On the other hand, paper based sensors that change color when dipped into an analyte solution can be used for colorimetric analysis [10]. Nitrite and pH detection on a paper microfluidic sensor using a smartphone platform was demonstrated [11]. Test strips for alcohol content in saliva was evaluated for color change in various color spaces [12]. Photoluminescence intensity level of quantum dots was used to target glucose in biological fluids [13].

Quantitative data extraction from colored images is always followed by using a simple analytical model, such as fitting to singular parameters (R-G-B) from various color spaces. However, several problems exist when using analytical models to study concentration. Firstly, using JPEG images, which are greatly preprocessed before viewing, is not preferred for scientific data acquisition and processing [14,15]. Secondly, the methods to compensate for the disadvantages of JPEG images, such as black-white referencing [16,17], gamma-correction formula [18,19], and color referencing [20] cannot offer solutions to inter-phone repeatability and smartphone proprietary software for JPEG processing. Moreover, the success rate of simple analytical models decrease as the number of independent input parameters increase [21]. Multi-analyte sensors, such as paper based microfluidics or test strips, require extracting multiple analytical models to track each color change.

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Therefore, the aforementioned problems can only be solved using machine learning algorithms where the pre-obtained images are used to train a learning model, which can then automatically perform colorimetric tests as new data arrives [22–24]. Despite JPEG images can degrade the computation of simple analytical models, we recently showed that JPEG images show similar performance to other unprocessed formats (i.e. RAW images) when Least-Squares Support-Vector Machine (LS-SVM) was used as the learning model [25]. Here we develop an application-based solution to colorimetric testing of hydrogen peroxide (H_2O_2) strips using binary (LS-SVM) and multi-class (Random Forest) classifiers. The color images obtained from five different smartphones under seven different illumination conditions were used to train the classifiers, and the learning model was extracted and embedded into a remote server that is accessed by custom designed Android app for testing purposes using a Cloud-hosted service. The developed app and the learning model were tested under several illumination conditions and on various handsets to verify the success of the training set for inter-phone operability. No prior methodology was able to demonstrate an app based colorimetric detection using machine learning classifiers trained in very diverse experimental settings. The proposed methodology incorporating a Cloud based learning model to quantify colorimetric strips shows great promise in supplying ultimate solution to smartphone based colorimetric sensing.

2. Experimental section

2.1. Peroxide strip preparation

Stock solution of hydrogen peroxide (H_2O_2, Sigma–Aldrich) (500 ppm) was prepared in distilled water. The initial concentrations used in the experiments were prepared by serial dilution from the stock solution. Each strip was dipped into the test solutions for 1 s, and allowed to dry on tissue paper for 5 s. It is noteworthy that the accuracy of the color determination is pH-independent over the pH range of 2–9 at room temperature. The color chart on the purchased H_2O_2 test strips (Quantofix Peroxide 100) are shown in Fig. 1a. The test strips require tracking a single color as opposed to multi-color paper sensors. There are 6 main classes for 0, 1, 3, 10, 30 and 100 ppm with clearly distinct colors. We have prepared H_2O_2 solutions to obtain primary colors as well as secondary or in-between colors to increase the number of classes, which introduces a more challenging classification problem. The primary colors are pointed with red arrows in Fig. 1a and b. The secondary colors are obtained using H_2O_2 solutions with concentrations of 0.5, 2, 6.5, 20 and 65 ppm, respectively.

2.2. Experimental design and image capture

For machine learning training, we have designed an imaging experiment that involves controlled illumination conditions and multiple smartphones. Incandescent (I) and fluorescent (F) light bulbs, and sunlight (S) are used individually and together to provide 7 different illumination conditions: I, F, S, IF, IS, FS, IFS for each concentration of test strip (Fig. 1c). Incandescent (Osram 60 W) and fluorescent (Philips 12 W) light bulbs are suitably chosen to give warm (2700 K) and neutral colors (3500 K) while sunlight was used under shade with a clear sky (5000–6500 K). We should note that the number of illumination sources can be increased to give a much more variety of illumination conditions, however, exploring the diversity of illumination sources is beyond the scope of this work. The distance between the smartphones and the test strips were kept constant at 16 cm and the light sources provided homogeneous illuminated field at 35° angle of incidence.

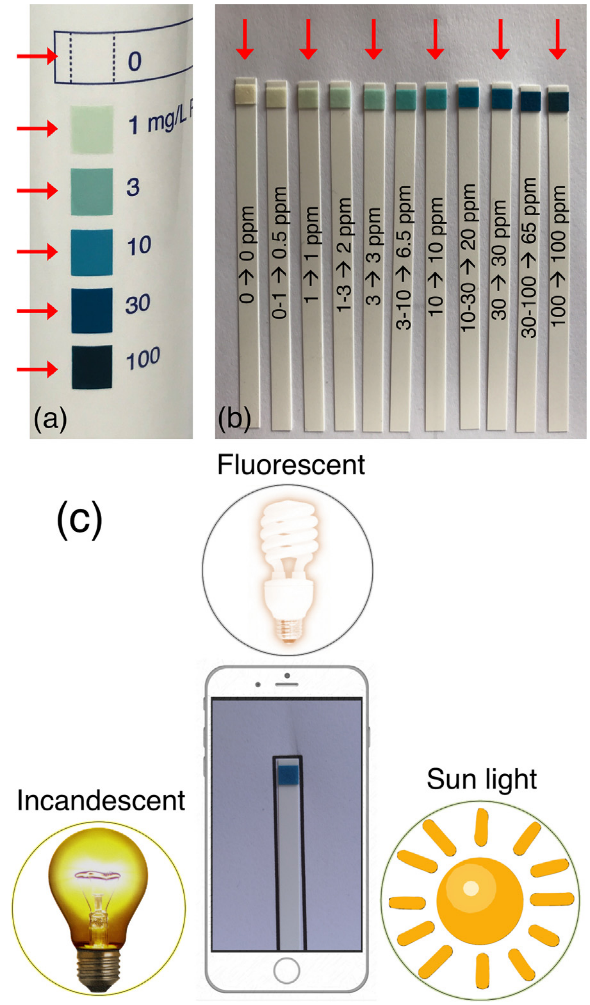


Fig. 1. (a) The peroxide test strips color chart showing the classification of colors based on concentration. (b) Peroxide strip images in JPEG format for peroxide content from 0.0 to 100 ppm showing secondary classes (11) with primary classes (6) pointed with red arrows. (c) The experimental setup for colorimetric detection of peroxide under incandescent, fluorescent and sunny illumination conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Even though the illumination conditions are kept under control, smartphones brands have different internal proprietary JPEG processing algorithms, which could result in a quite diverse training set. We have used 5 different Android based smartphones that are equipped with distinctive camera, optics and imaging software (Table 1). Each smartphone was positioned over the test strips at the same height and the automatic imaging mode was used to acquire 1 image under each illumination and concentration. The smartphone imaging settings such as color temperature, ISO, exposure time and shutter speed were automatically adjusted by smartphone's internal software. The total number of images obtained for each smartphone is 77 to result in 385 images for the whole data set.

2.3. Machine learning training

Both LS-SVM (binary class) and Random Forest (multi-class) were used as the machine learning classifiers for training and assessing the performance of app based colorimetric detection. To create a training set for the classifiers, all the images captured on five different smartphones with seven different illumination conditions were transferred to the workstation in order to perform pre-processing step in MATLAB environment. We created different

Table 1
The smartphones used to take images of peroxide strips of different colors for machine learning training.

Smartphone brand	REEDER P10	SAMSUNG Galaxy Note 3	LG G4	HTC One M7	SONY Xperia Z1
Image resolution	4160 × 3120	4128 × 2322	5312 × 2988	2688 × 1520	3840 × 2160
Optics	$f/2.0$	$f/2.2$	$f/1.8$	$f/2.0$	$f/2.0$

machine learning training sets for primary (6 classes) and secondary (11 classes) colors. The indicator pad on each strip was cropped manually to mimic the users who use the app during colorimetric tests. The cropped patches were in JPEG format that stores the pixel information in terms of RGB (Red-Green-Blue) values. The RGB values were also converted to HSV and LAB color spaces, which are more robust to illumination variation. The computed mean RGB, HSV and LAB values of the patches were used as the features fed to the classifier algorithms.

In order to account for the effect of various illumination conditions, one should further improve the training set such that the classifier performs robustly for versatile lighting sources. One way to increase the robustness is to achieve color constancy as it offers to perceive the colors independent from color of the light source [26]. The key idea behind the color constancy is to calculate actual color in captured image disregarding the illumination conditions. In other words, color constancy makes appearance of color stable against illumination variations. Many methods like white-patch, grey-edge and grey-world could be used for this purpose. In our application, however, the grey-world method was performed as it works under the assumption that deviation of the average color from grey is caused by the effects of the light source which is in line with the design of experiments in this study [27]. A traditional way of applying the grey-world method is to calculate average red (μ_R), green (μ_G) and blue (μ_B) values of the image $v = (\mu_R + \mu_G + \mu_B)^T$ [28]. Then, overall grey value for the image is calculated by taking average of these three values $g = (\mu_R + \mu_G + \mu_B)/3$. The scale factors $s = (g/\mu_R, g/\mu_G, g/\mu_B)^T$ are multiplied with each color component to obtain color constancy for the patches. As a result of achieving color constancy, each primary and secondary classes were extended to six forms by transformation in three color spaces (RGB, HSV and LAB) including with and without the grey-world algorithm, which leads to twelve training sets in total.

2.4. ChemTrainer mobile app

We developed a distributed system that enables multiple users with mobile devices to simultaneously classify test strips by taking their photographs. We use a message queue service to enable remote servers running a classification algorithm to serve multiple mobile devices simultaneously (Fig. 2a). Our servers run a classification algorithm that decides which class a photograph of a test strip belongs to. Our mobile device client application lets users take photographs of test strips and have the servers classify them.

The communication infrastructure of our system is based on Advanced Message Queuing Protocol (AMQP). We use a cloud-hosted RabbitMQ service running on CloudAMQP as the message-oriented middleware. This middleware enables us to easily set up and employ multiple computers as load-balanced servers that serve multiple mobile devices simultaneously over the Internet. This architecture permits both servers and mobile device clients to be behind firewalls as the connection is established through the cloud service.

We use RabbitMQ to implement a Remote Procedure Call (RPC) pattern of communication between servers and mobile devices. The server computers register to the RabbitMQ service for the same message queue so that they can share the workload. A mobile device client connects to the RabbitMQ service for this message queue and sends a request containing information about the cropped area of

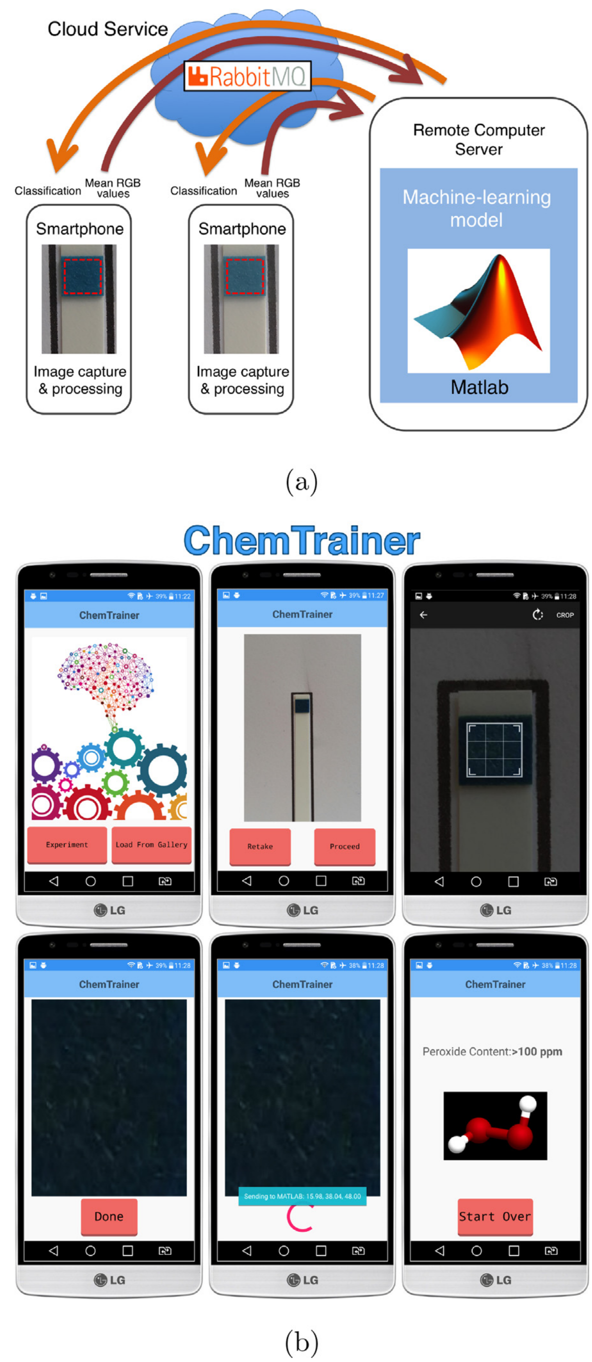


Fig. 2. (a) The communication infrastructure of the proposed smartphone based sensing platform. A Cloud service is used to access the remote server that attains the classification algorithm. (b) The activities and flow diagram of the developed ChemTrainer app.

the photograph, along with a temporary unique queue identifier that it starts listening to for the reply from a server. The RabbitMQ service chooses the server to respond to this request using a standard load-balancing algorithm that aims to keep the servers equally busy. The chosen server receives the request with the temporary queue identifier, decides on the class that the test strip belongs to, and replies back to the mobile device via the temporary queue. This way, our system utilizes multiple servers to simultaneously classify photographs sent by multiple mobile device clients.

In our system, any Windows, Mac OSX or Linux computer that runs MATLAB R2007b through R2016b and is connected to the internet, can act as a server. The classification algorithm implemented in MATLAB communicates with RabbitMQ through a standalone Java application based. The Java application registers to the RabbitMQ service to receive requests from mobile device clients. It then communicates with the running MATLAB script through the Java MATLAB Interface and executes our classification algorithm. It sends the result back to RabbitMQ, which is forwarded to the client mobile device.

Our mobile device client is implemented in Android and supports Android versions 4.0.3 (Ice Cream Sandwich) and above (Fig. 2b). The user either takes a new photograph ("Experiment" button) or loads a photograph from the gallery ("Load From Gallery" button). Then he/she crops the area of the photograph that contains the test strip using an adjustable cropbox. After clicking the "Done" button, the application computes the average red, green and blue values for the strip and sends this information to the RabbitMQ service. The application shows the user a progress animation until the result comes back from the server. Upon the arrival of the server's response, the application displays the class that the test strip belongs to.

2.5. Mobile app testing

The ChemTrainer app was uploaded to 6 smartphones, i.e. one extra Android phone was utilized, and the users were first asked to take pictures of primary and secondary peroxide strip colors in their offices under sunlight and a clear sky with automatic imaging mode. Then the users were instructed to take the same pictures with their office light on. Hence, a diverse testing environment was created for the machine learning model with both single and dual illumination settings. Each user took a total of 34 strip images for primary and secondary colors.

3. Results

A metric used for evaluating the performance of used machine learning algorithm is classification accuracy (*CA*), which is defined as, $CA = (TPos + TNeg)/(TPos + TNeg + FPos + FNeg) \times 100$, where *TPos* and *TNeg* are true positives and negatives, and *FPos* and *FNeg* are false positives and negatives, respectively. Based on the learning model developed with the training set, a strip image may or may not yield to a correct class. *CA* was calculated using 10-fold cross validation technique, where 9 subsets were used for training the classifier, and the remaining subset was used as test data in a randomized fashion. This procedure was repeated 10 times to obtain the final *CA* values.

We have computed the *CA* values for primary and secondary color sets with 6 and 11 classes, respectively. Each color set was also applied with the grey-world color constancy image processing algorithm before reevaluating the *CA* values to mitigate the effect of lighting conditions on the overall performance of the proposed approach. Fig. 3 shows the *CA* values obtained from the 10-fold cross validation for all experiments.

For the primary color set, the LS-SVM achieves over 99% *CA* for all peroxide contents using the RGB, HSV and LAB values as the feature set without the grey-world color constancy method. Using the grey-world algorithm, the *CA* values reach 100%, which is anticipated since the colors on each test strip in the training set were modified to have similar white balance setting. Thus, the LS-SVM is able to achieve perfect classification accuracy independent of the feature set. On the other hand, the *CA* values provided by the LS-SVM for the secondary color set are slightly decreased, yet over 98% using the RGB, HSV and LAB values as feature sets. This is due to the increased number of classes compared to the primary color set. In addition, the contribution of the grey-world algorithm to the performance of the LS-SVM is similar when used with the RGB values for the secondary color set.

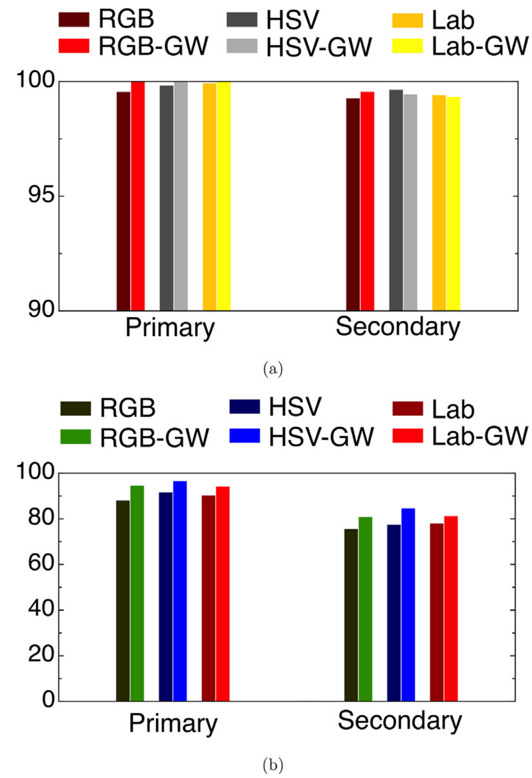
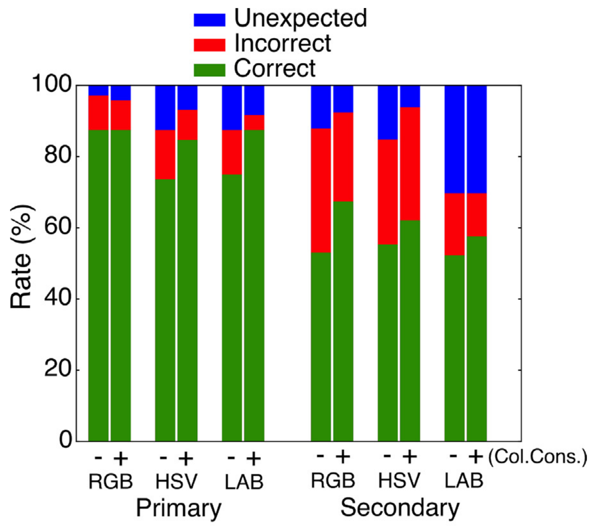


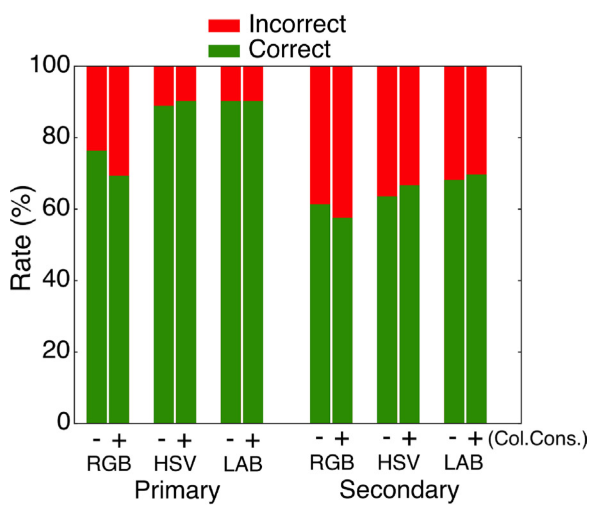
Fig. 3. Classification accuracy values obtained from 10-fold cross validation of (a) the LS-SVM classifier and (b) the Random Forest classifier using images with and without the grey-world (GW) algorithm.

For both the primary and secondary color sets, the *CA* values obtained from 10-fold cross validation of the Random Forest classifier are relatively lower than the ones provided by the LS-SVM classifier. However, the Random Forest classifier still performs well, i.e., over 87% and 76% *CA* for the primary and secondary color sets, respectively, where the RGB, HSV and LAB feature sets results in similar performance in distinguishing peroxide levels. Furthermore, the application of the grey-world algorithm enhances the *CA* of the Random Forest classifier for the RGB, HSV and LAB feature sets. Therefore, the white balancing consistently improves the overall capability of both the LS-SVM and the Random Forest classifiers, which proves the importance of accounting for versatile lighting conditions.

Apart from the cross validation of the proposed machine learning approach, we also evaluated the performance of the LS-SVM and Random Forest classifiers in testing the images provided by the ChemTrainer app in Android smartphones. For this, the responses from six users for each test strip were collected. The users compared the correct ppm value of the test strip with the response from the app and recorded the results to obtain classification accuracy values. For the LS-SVM classifier, aside from correct and incorrect results, the app accessible machine learning model may not always classify the calculated mean RGB, HSV and LAB features to a certain class. This is another type of misclassification and the app responds with "Unexpected" value in such a case. This is due to the one-vs-



(a)



(b)

Fig. 4. ChemTrainer app test results for primary and secondary classes with (+) and without (−) color constancy (grey-world) image processing algorithm for both classifiers. (a) The "Unexpected", "Incorrect" and "Correct" data gives the performance of LS-SVM learning model. (b) The Random Forest classification only give "Incorrect" and "Correct" results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

all approach in testing a captured image by the smartphone app since the LS-SVM is a binary classifier. In other words, the LS-SVM classifier attempts to assign a test image in one of the classes by checking whether the test image belongs to the first class or not. If the response is negative, the algorithm checks for subsequent classes one by one until the test image is assigned to a certain class. If the test image could be classified into none of the classes, then the result is labeled as "Unexpected".

In Fig. 4a, the "correct" classification rate of the LS-SVM for the primary color set using the mean RGB values is computed as 87.5% with and without the grey-world algorithm. In addition, the mean HSV values from the primary class data leads to 73.6% classification accuracy whereas application of the grey-world algorithm increases this rate to 84.7%. Similarly, the mean LAB values from the primary class data leads to 75% classification accuracy and application of the grey-world algorithm results in 87.5% classification accuracy.

On the other hand, the secondary class RGB, HSV and LAB datasets show relatively lower performance with over 50% success rate, while the application of the grey-world image processing algorithm consistently increases the success rate to 67.4%, 62.1% and 57.6% using the RGB, HSV and LAB values as features, respectively. The secondary class test sets demonstrate incorrect rates of over 25% for the RGB and HSV values. However more than 92% of those incorrect classifications belong to the neighboring class, either below or above the target classes. For instance, the 3ppm class was mostly confused with 1–3 ppm or 3–10 ppm class. Therefore, increasing the class number from six to eleven for the LS-SVM classifier decreases the chances of achieving a high success rate due to finer differences among peroxide levels while the application of the grey-world algorithm to the training and the test set increases the classification rate using the RGB, HSV and LAB features. The unexpected rates of the LS-SVM classifier for both the primary and secondary class test sets using the LAB features are higher compared to the ones provided by the RGB and HSV values.

In Fig. 4b, using the primary class RGB data with the Random Forest classifier results in lower performance, i.e., 76.4% and 69.4% before and after the application of the grey-world algorithm, respectively, compared to the LS-SVM. On the other hand, the primary class HSV and LAB values show much superior performance, where the HSV feature sets lead to 88.9% and 90.3% accuracy rate before and after the application of the grey-world algorithm, respectively, compared to the LS-SVM results. The secondary class HSV and LAB values also provide increased performance while secondary class RGB values show decreased performance compared to the LS-SVM secondary classes. An important observation from Fig. 4a and b is that the grey-world color constancy algorithm has a similar and a positive effect on the primary and secondary HSV and LAB results for both classifiers while the RGB features show declined classification rates for the Random Forest classifier. In other words, the color constancy algorithm persistently increases the performance of the LS-SVM classifier, while it decreases the *CA* of the mobile app test results and increases the *CA* obtained from cross-validation for the Random Forest classifier. Therefore, one should first carefully examine the effect of the grey-world algorithm when used with a particular classifier.

Although the classification accuracy rates obtained from the cross validation of both classifiers are well over 95% for the primary and secondary color sets (Fig. 3), classification of user data provided by the ChemTrainer app leads to a worse performance (Fig. 4). The change in classification success rate is partly due to semi-random conditions created by the ChemTrainer app users. For instance, the training set was obtained in a controlled laboratory environment that has uniform illumination conditions whereas the users tested the Android app in their own offices under distinctive lighting sources independent of the conditions in the laboratory environment. The proprietary camera software was set to automatic mode to freely adjust image settings, which are also independent of the settings in the laboratory environment. Furthermore, the crop sizes to calculate the mean RGB, HSV and LAB values for constructing the training set in the laboratory environment were adjusted uniformly, whereas the crop sizes chosen by the Android app were not uniform as the users were free to choose any crop size for the test strip as long as it was smaller than or equal to the indicator area. Hence, both the differences in the illumination conditions and the differences in the crop sizes bring new information that the training set formed in the laboratory environment does not involve, which causes decreased generalization ability, referred to as the performance of a classifier in classifying test patterns which were not used during the training stage [29], of both classifiers when used with the mobile app.

4. Conclusion

Herein, we proposed the ChemTrainer smartphone app that can automatically classify peroxide content based on the color of the test strip using binary and multi-class learning models accessed via Cloud hosted service. The LS-SVM and Random Forest classifiers were fed with the mean RGB, HSV and LAB values of the test strip image under various illumination conditions with and without the grey-world color constancy algorithm. The learning model was placed on a remote server where ChemTrainer can access to and receive a response from. The classification using the training data with 10-fold cross validation achieves over 95% accuracy while the mobile app user tests show as high as 90.3% correct classification for primary group with 6 classes. The misclassification rate increases with the number of classes as in the case of secondary group with 11 classes.

In this study, we illustrate that the proposed method to quantify colorimetric test strips using a smartphone app based on machine learning is a great prospect for any colorimetric assay with discrete levels of analyte. The classification accuracy rate could be increased by the use of additional handsets to form a more diverse and larger training set comprising of light sources with different color temperatures. Future work will focus on improving the machine learning by the ChemTrainer app further for multiple parametric sensing (e.g. water quality, urine, blood parameters) for healthcare and environmental monitoring in resource-limited settings.

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