An Enhanced Algorithm for the Quantification of Human Chorionic Gonadotropin (hCG) Level in Commercially Available Home Pregnancy Test Kits

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***Abstract*—Home pregnancy kits typically provide a qualitative (yes/no) result based on the concentration of human chorionic gonadotropin (hCG) present in urine samples. We present an algorithm that converts this purely qualitative test into a semi-quantitative one by processing digital images of the test kit's output. The algorithm identifies the test and control lines in the image and classifies an input into one of four different hCG concentration levels based on the color of the test line. The proposed algorithm provides significant improvement over a prior method and reduces the maximum false positive rate to less than 5%. This improvement is achieved by a careful choice of the color space so as to maximize the inter-concentration separability. Also, the proposed method increases the utility of the test kits by providing useful diagnostic information. Furthermore, the algorithm could be ported to a mobile platform to make it particularly helpful in remote rural health monitoring.**

I. INTRODUCTION

Home pregnancy test kits have become ubiquitous and are used as the default tool for early pregnancy detection. These kits are very affordable and provide fairly accurate results [1]. Typically, the test is performed on urine and is a qualitative test where the presence of hCG is determined by lateral flow immunoassays that contain antibodies specific to the β subunit of hCG. The appearance of a test line implies that the hCG is present beyond the detection threshold of the assay strip that is being used. The minimum detection threshold for most of the commercially available strips is around 25 mIU/ml. Though a qualitative test is sufficient to confirm pregnancy, quantification of hCG is necessary to predict different anomalies in pregnancy. For example, a normal pregnancy is indicated by a doubling of the hormone every 48 hours. Ectopic pregnancies or other problematic pregnancies that can occur in the case of infertility do not show such a progression. A quantitative test of hCG is carried out on urine samples and is based on Enzyme Linked Immunosorbent Assay (ELISA) technique. It is costly, time consuming and requires skilled technicians to perform the test. Semi-quantitative home kits can help patients and doctors diagnose such issues without undergoing laboratory tests.

Recently, there has been growing interest in using low cost imaging devices like mobile phones to quantify such tests at home [2] [3], [4]. The proposed algorithm is an attempt to reduce the cost by using cheap processing power available on mobile devices as opposed to relatively expensive ELISA plate readers or autoanalyzers in identifying the concentration of β-hCG and is an extension and improvement over the previous work done by Manasa K. et al [2]. Levels of the hormone between 5 mIU/mL and 1000 mIU/mL are relevant for clinical applications. The algorithm relies on the acquisition and processing of a digital image of the kit's output. Further, the algorithm was developed using data collected from commercially available off-the-shelf pregnancy detection kits.

The ultimate goal of this work is an application for smart phones and tablets that lets users photograph the result shown by the kit and be better informed of their physical condition. While β-hCG was considered in this work, the proposed algorithm is applicable to other tests performed on a lateral flow platform. The rest of the paper is organized as follows. Section II defines the problem, section III describes the proposed algorithm in detail. Section IV presents the results of the algorithm and section V discusses directions for future work and concludes the paper.

II. PROBLEM FORMULATION

The main problem considered in this paper is to explore the use of image processing techniques to determine the concentration of β-hCG in a commercial nitrocellulose-based kit. The input to this problem is a digital image of the output by the experimental protocol outlined in section III-A. As mentioned above, the commercial strip contains a test line and a control line. The test line's output color is a function of the concentration of β-hCG in the test sample. The test kit's output is photographed such that the test and control lines are clearly in view. The challenges to be addressed are discussed in the following:

**DD gratefully acknowledges support through grant no. 0002-02-02-01-01 from Grand Challenges Canada.

978-1-4799-2361-8/14/$31.00

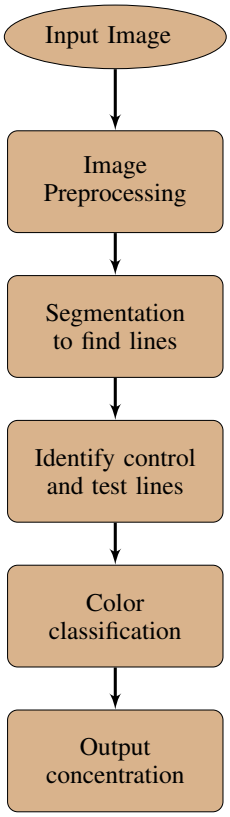


Fig. 1: Flowchart of proposed algorithm.

1) Identifying lines: For the technique to be useful and practical, identifying the control and test lines without manual input (i.e., automatically) is essential and is the first of the problems addressed. Further, it is desirable for the algorithm to make use of as few empirical thresholds as possible.
2) Color variability: In a real world setting, it is difficult, if not impossible to guarantee uniform lighting conditions. Further, color variability is also possible in the kits' response to the same concentration of β-hCG as shown in Fig. 2. For the algorithm to be practical, it is imperative for it to be illumination independent and minimize the effects of color variability.
3) Classification: After accounting for color variability, the final problem is to classify the test lines into concentration levels of β-hCG. The levels considered in the paper are 0, 25, 100, 250 mIU/ml. They have been chosen to be a representative subset of the possible concentrations in order to illustrate a proof-of-concept. The color variability and the classification problems are addressed jointly.

III. PROPOSED ALGORITHM

The proposed algorithm addresses the problem outlined in Section II and is summarized in Fig. 1. Each stage of the algorithm is discussed in detail in the following.

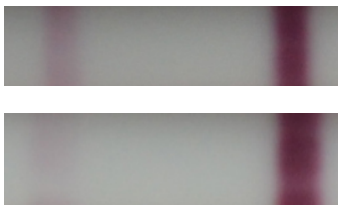


Fig. 2: Color variability at a concentration of 100 mIU/ml.

A. Image Acquisition

Sandwich immunoassay for β-hCG test was carried out on pilot batches of nitrocellulose chip with positive and negative spiked urine samples. Analyte (β-hCG) concentrations in the range from 25 mIU/ml to 1000 mIU/ml were spiked into human male urine samples. Test was performed by adding 3 drops (75ul) of reactive sample onto sample pad located upstream of the detection antibody zone of the commercial strip (Cipla, an Indian manufacturer). Lateral flow of sample along test strips through capillary action reconstitutes the conjugate and form gold-labeled detection antibody-analyte (β-hCG) complex. This labeled immune complex continues to migrate along the chip and is captured by the antibodies immobilized in the test and control line producing two red lines visible to the naked eye, indicating the successful binding of proteins. Images of these tested strips were captured under single bright white light lamp source from a distance of 15 cm by Nikon D5100 digital SLR camera fixed at tripod stand.

B. Image preprocessing

In a realistic setting, the image acquisition process would be far from ideal. We foresee inconsistent lighting to be the predominant issue during acquisition. We propose a preprocessing stage to address this problem. The image is traversed column-wise and processed such that the pixel values in a particular column are identical, and equal to the mean of the pixel values of that column. This accounts for lighting irregularities, if any, along the rows. A median filter of length 5 (chosen empirically) is applied along the rows to smoothen out any irregularities along the rows. This simple but effective preprocessing stage helps alleviate the effects of inconsistent lighting conditions during imaging.

C. Segmentation to find lines

The next step in the algorithm is to segment the image so as to find the lines of interest. The nature of the problem affords a simple segmentation technique - binarization [5]. A threshold is identified using Otsu's algorithm [6] and used to binarize the image. However, it was found that just using a threshold in the RGB (Red, Green, Blue) color space gives relatively poor results. In order to improve performance, we use the HSV (Hue, Saturation, Value) color space in addition to the segmented image from the RGB color space. The idea is to mark the areas of minima and maxima (> 0.9) in the first half of the strip (preferably upto 150 pixels), where the test line is likely to be located; and the areas of maxima (> 0.9) in the second half. The minima and maxima are of the sum

of the Hue and the Saturation values of these images, along a particular row.

D. Locating control and test lines

After segmenting the image to identify lines, the next step is to locate the control and test lines. It is assumed that the image is taken such that the control line is always to the right of the image. With reference to the algorithms defined for segmentation above, a region is taken as an area of interest if it is common to the regions located by both, the RGB as well as the HSV color space alorithms, as shown in Fig. 3. The regions marked black on the horizontal axis represent the regions of the test and the control lines respectively. While this step appears trivial, it is actually very important since the lines' locations are completely dependent on how the image was acquired. This step can be made completely deterministic if the following parameters are known: width of the lines, the camera pixel density, and the camera optics settings. In a practical setting, it is highly unlikely that the end user would have access to these operational parameters.

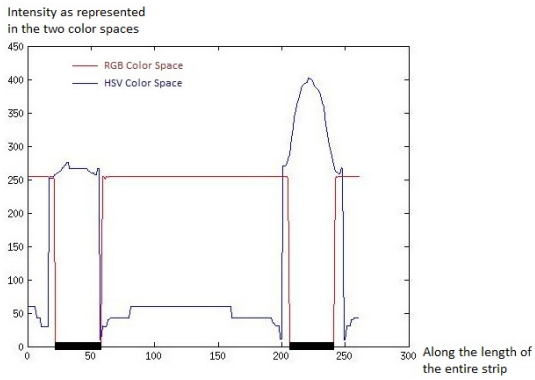


Fig. 3: Obtaining the region of interest - common to the RGB and HSV areas

E. Color classification

After line identification, the problem becomes a traditional data classification one. However, the intra-class intensity variability shown in Fig. 2 necessitates pre-processing of the data points. To handle this issue, training data for each class is sampled carefully to represent the variability. These samples are randomized before training the classifier. Moreover, it was found that using the image data in RGB color space gives poor results. A much better alternative for training the classifier was found to be the CIE 1976 L*u*v* color space. The CIE L*u*v* mimics the human visual system and takes into account the tristimulus values of the color, rather than just their intensity values as RGB does. The L*u*v* color space encompasses the entire gamut of human vision, making it all the more favourable for training the classifier [7]. The effectiveness of the L*u*v color space for inter-class separation relative to the RGB color space is shown in Fig. 4.

The popular SVM-based classification technique [8] is applied to solve the color classification problem. The number of output classes are chosen to be the following concentration levels of β-hCG - 0, 25, 100, 250 mIU/ml. This multi-class

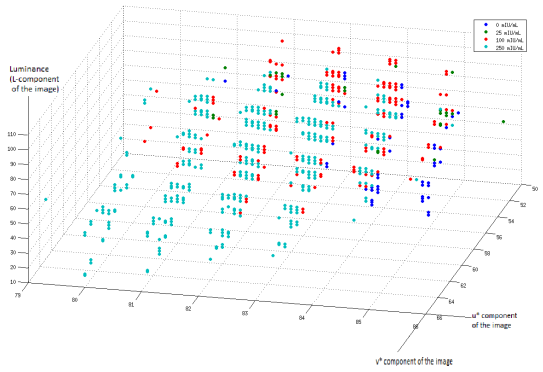


Fig. 6: Classifier test points.

classification problem is solved by applying the standard two-class SVM solution in a tree structure [9], [10]. It was empirically determined that a polynomial kernel SVM classifier gives the best results.

IV. RESULTS AND DISCUSSION

The output of each stage of the algorithm is presented in the following. The input image is shown in Fig. 5a. The results of the proposed algorithm are presented in the following.

A. Segmentation to identify lines

The input image has some luminance irregularities as shown in Fig. 5a. Therefore, after adjusting for the lighting irregularities, the output is as shown in Fig. 5b . After this step, all the rows become identical to each other.

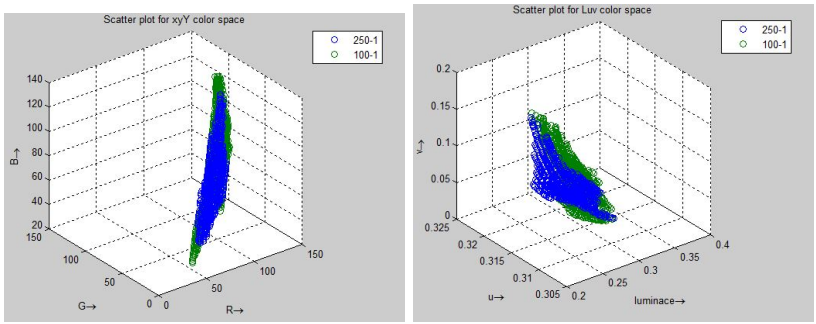
B. Locating control and test lines

The lines identified as control and test are shown in Fig. 5c. As shown, even though the lines of interest are of different widths but are clearly identified. However, as mentioned earlier, these are the regions of interest in the RGB color space.

C. Color classification

The SVM classifier was trained using 100 training samples per class. These samples were chosen randomly across test images represented in CIE 1976 L*u*v* color space corresponding to each class. Accordingly, the control and the test lines marked in the RGB color space, as in Fig. 5c were then represented in the L*u*v* color space, as shown in Fig. 5d. The classifier used a polynomial kernel. Performance was determined using test points from a randomly chosen set of images that contained a uniform sampling of all the four classes as shown in Fig. 6.

The results of the classification are shown as confusion matrices in Fig. 7. The first column of the table represents the input classes and the first row represents the output of the classifier. For example, the second row of Fig. 7a says that the classifier correctly classified 98.78% of input points belonging to the 25 mIU/ml class and misclassified 1.12% of the points as belonging to the 0 mIU/ml class. The classification performance is consistently higher than 95% across classes, which



(a) RGB color space. (b) L*u*v color space.

Fig. 4: Better separability of two concentration levels (250 and 100 mIU/mL) in the L*u*v color space.

(a) Sample commercial strip (Cipla) (b) Image after adjusting for lighting irregularities (c) Denoised lines in RGB color space (d) Denoised lines in LUV color space

Fig. 5: Segmentation results on commercial strip at 100 mIU/ml.

	Output (proposed method)			
Input	0	25 mIU/mL	100 mIU/mL	250 mIU/mL
0 mIU/mL	98.58	1.42	0	0
25 mIU/mL	1.22	98.78	0	0
100 mIU/mL	0	0	95.22	4.78
250 mIU/mL	0	0	1.45	98.55

	Output (method in [2])			
Input	0	25 mIU/mL	100 mIU/mL	250 mIU/mL
0 mIU/mL	100.00	0	0	0
25 mIU/mL	0	100.00	0	0
100 mIU/mL	0	0	80.92	19.08
250 mIU/mL	0	0	4.80	95.20

Fig. 7: Confusion matrices of classification results for proposed method and earlier method ([2]).

is a significant improvement over the previously obtained 80% in [2].

D. Discussion

Based on the results, a number of interesting observations can be made. First and foremost, there is a possibility to quantify lateral flow assays using sophisticated image processing techniques which can be ported onto simple imaging platforms. Secondly, repeatability of the flow path is a requirement for reproducible results and lateral flow assays may not have been optimized for such requirements.

The classification results are based on samples generated in a laboratory setting with inter sample variability carefully minimized and tested on four well-spaced concentration levels. In order to achieve high resolution classification, good training samples are absolutely essential. This in turn is directly dependent on sensitivity of the commercial strip.

While the proposed solution is similar in philosophy to [3], [4], we would like to point out two important differences. The images are acquired using a regular digital camera and does not require specialized acquisition devices. Further, the proposed algorithm uses a sophisticated SVM-based classifier to provide results that have a resolution higher than binary.

V. CONCLUSION AND FUTURE WORK

A robust algorithm for the classification of the β-hCG concentration in a novel fabric was presented. The proposed algorithm provides a proof-of-concept for converting the qualitative process of early pregnancy detection using self test kits into a semi-quantitative one. We believe that quantifying β-hCG levels would be beneficial both to the manufacturer and the end user. As part of our future work, we propose

to increase the number of classes in the classifier to make it more sensitive. Further, to achieve our bigger goal, the image processing would be done entirely on a mobile platform such as Android or iOS.

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