

Predictive Framework Development for User-Friendly On-Site Glucose Detection

This chapter is divided into two parts. The first part describes the design and development of a design capturing apparatus, for image dataset enhancement at various illumination conditions and incident lightning angles by utilizing different smartphones. While the second part deals with the development of a user-friendly disposable bio microsystem for on-site glucose detection by utilizing two different surface functionalization method with chitosan(C) and without chitosan (WC) for the color change analysis, which employs machine-learning algorithms to estimate various glucose concentrations.

4.1 DESIGN AND DEVELOPMENT OF IMAGE CAPTURING APPRATUS

To achieve high classification performance, machine learning-based approaches require training with a suitable dataset. The content and quantity of the dataset directly impact the classifier's performance, which can be improved by increasing the number of input data. To obtain image data at various ambient illumination conditions, incident angles, and camera optics. An apparatus was designed and used for capturing of images at different illumination condition, incident light angles, and camera optics.

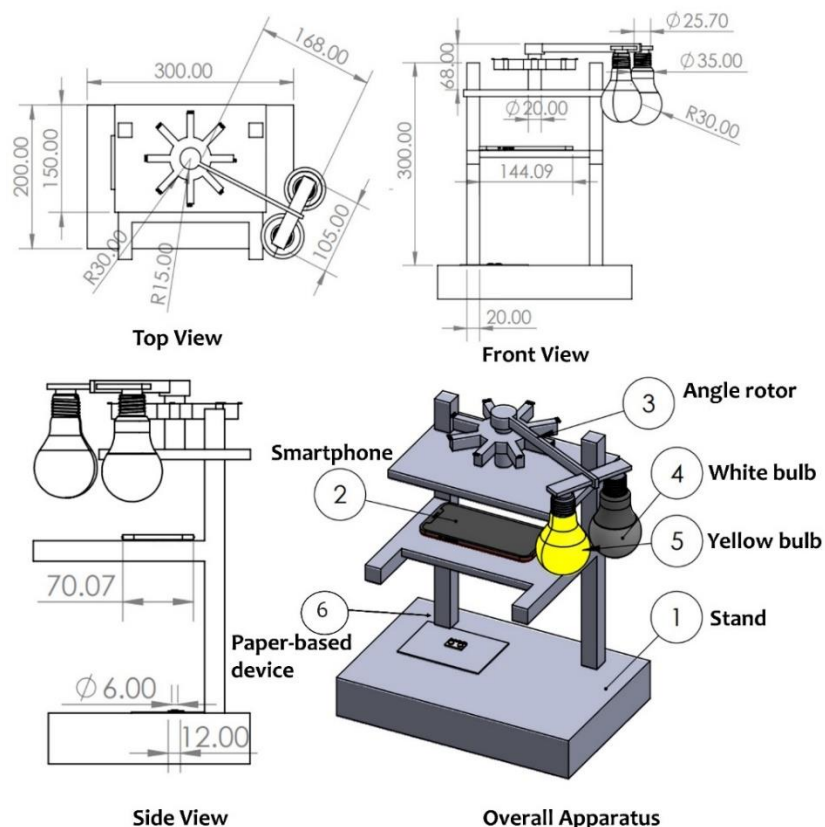


Figure 4.1 : Schematic representation of assembly and design of the utilized apparatus, all dimensions are in mm.

Figure 4.1 illustrates the complete design and assembly of the apparatus used in this process. By utilizing this apparatus images of the PADs can be captured under uniformly illuminated fields from various angles (0° - 360° , with a difference of 45°) while maintaining a constant distance of 15 cm between the smartphones and PADs. The position of the PADs and smartphones remained fixed, while the light sources can be moved at a constant angle of 45° , to ensure consistent distance between the smartphones and PADs for all test groups, while varying the illumination angle.

4.2 DEVELOPMENT OF AN USER-FRIENDLY DISPOSABLE BIOMICROSYSTEM FOR ON-SITE GLUCOSE DETECTION

This study explores a smartphone-based spot detection framework for glucose in a rapid, simple, and affordable paper-based analytical device (PAD), which employs machine-learning algorithms to estimate various glucose concentrations. Herein, two different surface functionalization methods were chosen with chitosan(C) and without chitosan (WC) for the color change analysis. Being a biopolymer, chitosan improves the analytical performance of PADs when used with a chromogenic agent. Moreover, the influence of the illumination conditions and camera optics on the professed color of glucose strips was observed by choosing various illumination conditions and different smartphones.

4.2.1 Introduction

Paper-based analytical devices (PADs) have received a significant attention as cutting-edge and affordable analytical tools for point-of-care testing (POCT) devices because they meet all of the ASSURED standards recommended by the World Health Organisation (WHO)(Nilghaz et al., 2015). Chemiluminescence, colorimetric, electrochemiluminescence, fluorescence, and electrochemical are a few of the detection methods that can be used for the sensitive, selective, and quick detection(Kishnani, Park, et al., 2021). Among all the detection techniques, colorimetric detection is one of the most approachable detection techniques. It works on the reaction of the analyte of interest, which produces colour at the detection zone for the ease of visual readout. The concentration of the analyte directly influences the intensity of its colour. The application area of colorimetric analysis is vast, *viz.* urinary creatinine detection(Lewińska et al., 2021), blood analysis(Komatsu et al., 2020), salivary biomarkers(Pomili et al., 2021), metal ion sensing(Ratnarathorn et al., 2012), and other bio sensing applications(Gupta et al., 2019). The colour intensity of the biosensors are directly related to its sensitivity, which can be enhanced through the surface modification of the biosensors. These surface modification can be achieved by using various strategies including incorporation of functional nanoparticles, metal oxides etc(Gupta et al., 2017)(Kishnani, Mondal, et al., 2022)(Yadav et al., 2022)(Gupta & Pal, 2018b)(Antibody Immobilization for ZnO Nanowire Based Biosensor Application, 2014). The information produced by the colour intensity can be collected through the various colour spaces, *viz.* RGB (Red-Green-Blue), HSV (Hue-Saturation-Value), and $L^*a^*b^*$ (where 'L*' stands for Lightness, 'a*' and 'b*' stands for exclusive colours of human vision (*viz.* Green-Red, Blue-Yellow)). Over the past few decades, numerous researchers have dedicated their efforts to reduce the occurrence of misinterpretations resulting from visual inspections. Their primary goals have been to provide both qualitative and quantitative results and develop robust methods for detecting target zones. For example, researchers all over the globe adopted several technologies for the enhancement of the colour signals, such as polyaniline nanoparticles and red blood cell membrane were used for enhancing the selectivity of the colorimetric urine glucose sensor(T. Lee et al., 2021). In another work, silver nanoparticles were used for the colorimetric sensing of copper ions in the water sample(Ratnarathorn et al., 2012). In recent times, smartphones have gained significant attention as a transformative platform for implementing point-of-care testing (POCT). This is primarily due to their compact size, ease of use, and electronic capabilities, which offer a wide range of functionalities compared to traditional detection devices. Smartphones possess features such as robust network connectivity, Bluetooth, Wi-Fi, powerful computing capabilities, high-resolution cameras, and high-quality displays as default functions. As a result, smartphone-based instrumentation has been widely explored for various applications, including colorimetry-based analyses(Mutlu et al., 2017)(Biswas et al., 2021), bioluminescence(Lopreside et al., 2021), and fluorescence(Placer et al., 2022).

Being a susceptible process, colorimetric results are heavily affected by the surrounding illumination conditions and the camera specification. To counter these problems, unconventional algorithms were previously implemented for the qualitative and quantitative analysis and it was found that the grouping of machine learning (ML) and colour spaces escalated the signal(W.

Castro et al., 2019). The conversion of one colour space into the another can also improve the performance such as converted RGB colour space into the colour spaces of L*a*b* and HSV for the improvement of the performance(Bae et al., 2015)(H. Kim et al., 2017)(H. K. Kim & Kim, 2017)(S. D. Kim et al., 2017)(Nelis et al., 2020). A fully integrated detection platform was developed for the rapid sampling, transmission, and data processing, combined with polyacrylonitrile nanosheets (PAN-NSS) colour sensor and deep convolutional neural network (DCNN) for the detection of nitrite in food through an application (APP) as a visual monitoring platform(Z.-J. J. Huang et al., 2022). In another work, the colour difference maps were used as an impression to differentiate the samples of honey; based on its origin, pre- and post-processing of the data. A range of fitting accuracy was obtained by using SVM (100%), partial least square discriminant analysis (88%), and linear discriminant analysis (86%)(Chaharlangi et al., 2021). Further, an attempt was made for the colour detection by using LED lights of different smartphones by gaining stable colour information by avoiding interference of external light source(D. Kim et al., 2020).

In this proposed work, glucose concentration is determined through low-cost paper-based colorimetric analysis via spot detection for two cases with chitosan (C) and without chitosan (WC) and machine-learning classifiers were seamlessly integrated into the system to enable its unrestricted use under ambient light conditions. By incorporating machine learning, the platform was simplified for its wider usage in smartphone-based biosensors. To train the machine learning classifiers and mitigate the effects of illumination variances and camera optics, a dataset was created using different light sources and five distinct smartphones. Additionally, to eliminate the influence of ambient light, colour images were captured within a closed container. For making it user-friendly and predict the most accurate data at the commercial level, the image data set was taken at various angles (0°-360° with the difference of 45° angle) of incident light. Because each angle of incident light has different illumination in its region of interest, the angle variation of incident light can change the RGB values of the region of interest. To achieve an analytical approach, a range of machine learning classification models were employed. These machine learning algorithms enabled the classification of different concentrations and prediction of new data. The performance of these models was evaluated and compared for both cases C and WC. In addition to machine learning algorithms, various colour spaces and image processing techniques were utilized. Transforming the colour information helped in amplifying the signals, thereby enhancing the overall analysis process.

4.2.2 Materials and Methods

To fabricate disposable paper-based biosensor Whatman No. 4 filter paper with a diameter of 125 mm, obtained from GE Healthcare Life Sciences. DI water (Ultra Gold, specific conductivity of less than 1, acquired from OLC Pvt Ltd, India. Glucose oxidase (GOx) was obtained from *Aspergillus niger* Type X-S, in the form of lyophilized powder with a solid concentration of 100,000-250,000 units/g. Potassium Iodide (KI) was purchased from Sigma-Aldrich, meeting the ACS reagent standard with a minimum purity of 99.0%. Horseradish Peroxidase (HRP) was purchased from Sigma Aldrich, Type VI, with a minimum activity of ≥250 units/mg and solubility of 0.1M PBS at a concentration of 10mg/ml. D-Glucose was obtained from Thermo Fisher Scientific Private Limited, India. Phosphate buffered saline (PBS) was purchased from Sigma-Aldrich, India, as water-soluble tablets with a pH range of 7.2-7.6 (1 tablet per 200ml). Chitosan (85% deacetylated powder) was acquired from Alfa Aesar (Thermo Fisher Scientific). Glacial acetic acid meeting the ACS reagent standard with a minimum purity of 99.7% was obtained from Sigma Aldrich. Several smartphone models were used in the experiment: Realme 8, Nokia C20 Plus, Samsung M51, iPhone 11, and Samsung Galaxy M30. Unless stated otherwise, all chemicals were used in their highest analytical grade without any additional purification.

(a) Fabrication of PAD

The design patterns were created using the computer aided design software (SOLIDWORKS 2020). The microfluidic device design featured two concentric circles with a

sample zone measuring 4 mm. The region between the outer edge of the inner circle and the inner edge of the outer circle was utilized as a boundary to establish a hydrophobic barrier. For a comprehensive description of the fabrication process, previous publication by the group can be referred (Kishnani, Kumari, et al., 2022).

(b) Image Capturing

A dataset was created using multiple smartphones under different illumination conditions and angles, simulating the adverse effects under controlled illumination conditions. The light sources used included White (W), Yellow (Y), room condition, and sunlight (S), either individually or in combination. Various illumination conditions were created, such as W, Y, YW, S, room condition (with light, without light, with concentrated flash), closed container (with flash), and sunlight (with and without shade), to capture images of each PAD for concentration ranges of 10mM, 20mM, 30mM, and 40mM. We aimed to encompass a wide range of illumination conditions that a normal person might encounter, although additional illumination sources could be included to expand the dataset. Images of the PADs were captured under uniformly illuminated fields from various angles (0° - 360° , with a difference of 45°) while maintaining a constant distance of 15 cm between the smartphones and PADs by utilizing the apparatus shown in Figure 4.1. The position of the PADs and smartphones remained fixed, while the light sources moved at a constant angle of 45° , to ensure consistent distance between the smartphones and PADs for all test groups, while varying the illumination angle. Since individuals have different styles of taking pictures under different angles and lighting conditions, we aimed to minimize errors by capturing images under all possible angle and illumination conditions to enhance the dataset for machine learning.

As smartphones are equipped with distinct cameras, optics, and imaging software, even under controlled illumination conditions, the resulting images exhibit diversity. Table 4.1 provides the technical specifications of the smartphones used for capturing the images of the PADs for further analysis. The positioning and height of the smartphones were kept consistent while capturing images of each PAD for all the illumination conditions and concentrations. The automatic mode of the embedded imaging software was used to adjust settings such as colour, temperature, ISO, exposure time, and shutter speed. With four concentrations and various illumination conditions, a total of 132 images were obtained for each device, resulting in a dataset of 660 images. This dataset was then transferred to a computer for further processing using MATLAB (R2021b, MathWorks Inc.). The region of interest (ROI), where the color change occurs due to the reaction of the reagent, needed to be extracted. This involved application of several image-processing methods sequentially; including resizing, grayscale conversion, thresholding, binarization, clearing borders, area filtering, noise reduction, selection of the region of interest, and masking. The extracted ROI was then overlaid onto the original images to extract features for machine learning purposes.

Table 4.1 : Technical specification of smartphones used to capture the image for machine learning

S. No	Smartphone Brand	Resolution	Aperture Size	Megapixels
1.	Samsung Galaxy M30	1080*2340	1/1.9	13
2.	iPhone 11	828*1792	1/1.8	12
3.	Realme 8	1080*2400	1/1.8	64
4.	Samsung M51	1080*2400	1/1.8	64
5.	Nokia C20 Plus	720*1600	-	8

(c) Data Analysis

Original RGB images were collected under a homogeneously illuminated field at various angles by smartphone as shown in Figure 4.1. All images were cropped, and ROI was extracted by several image-processing methods. Figure 4.2 depicts the flowchart illustrating the sequential steps involved in image processing. After the complete image processing, the cropped image of ROI has the shape of the bounding box, which is further used for the conversion of RGB into HSV, $L^*a^*b^*$, NTSC (National Television Standards Committee) colour space, XYZ, and greyscale colour spaces. Further, following features were determined for all the filter data *viz.* mean, skewness, kurtosis, entropy, intensity and grey level co-occurrence matrix. The reason for choosing HSV colour space is; its robustness towards the change in external lighting, and L^* , a^* , b^* colour space is used for its usefulness for image definition and colour boosting.

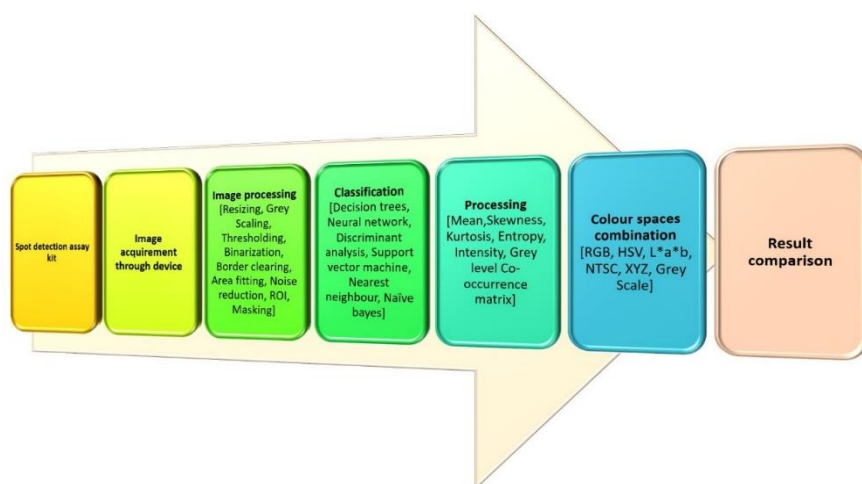


Figure 4.2 : Flowchart of image processing steps with machine learning classification

Raw images were captured from smartphone and pre-processed before data analysis for homogenization. The captured images were first resized to a dimension of 250*250 pixels. Afterwards, the resized images were converted to grayscale, removing the hue and saturation information, while retaining the luminance component. Further, it is required binarization of the image; in this process, pixels of the image are separated in binary pixel collection (black and white) for the segmentation into foreground and background. Binarized images contain noise (white dots in the black region) which was removed by noise reduction. Noise-free image masked through the ROI and carved. Figure 4.3 shows pre-processing of a 10 mM concentration image under the white light for both the case C and WC.

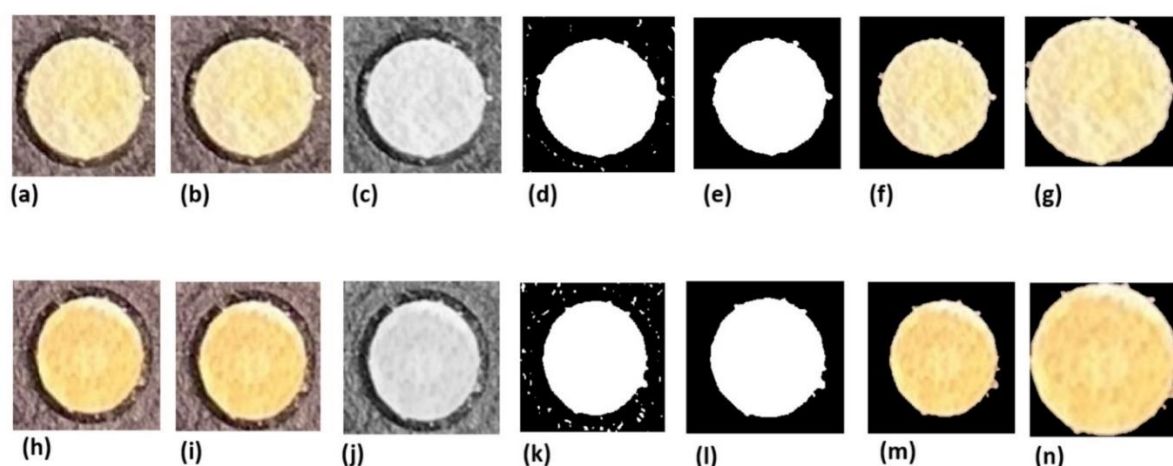


Figure 4.3 : Steps for image processing of 10 mM concentration under white light. Figure 3 show images for the WC case, while parts (h–n) show images for the C case. The raw image captured from the smartphone is

given in (a, h) resized to 250×250 (b, i) converted into greyscale, (c, j) binarized in (d, k) noise removal in (e, l) masked in (f, m) carved mask region of interest in (g, n)

(d) Machine Learning Classification

The classification learners available in MATLAB apps (Version R2021b) were utilized to assess the functioning of the device and recognise the correlation between colour change and concentration. Supervised learning techniques were employed to analyze the training data and build a training model. The MATLAB app provided several classification algorithms including Decision Trees (DT), Naive Bayes (NB), Discriminant Analysis (DA), K-Nearest Neighbor (KNN), Neural Network (NN), and Support Vector Machine (SVM).

These algorithms were executed using data obtained from typical dilution tests. K-fold cross-validation with $k = 10$ was applied for training and validation to prevent overfitting. Among these classifiers, supervised learning methods are commonly used. They involve automatic learning tasks that infer a function based on categorized training data. SVM, specifically, is a supervised non-parametric classification method that performs binary classification by constructing hyperplanes or sets of hyperplanes in a high-dimensional space (W. Castro et al., 2019). The classification process involved utilizing the optimal hyperplane, which separates the datasets, along with support vectors that are neighboring data points to the hyperplane. In SVM algorithms, kernel functions such as quadratic, linear, radial basis function, Gaussian, and cubic kernel were applied to transform the training data. This transformation allows for the creation of a non-linear decision surface that can be represented as a linear equation in higher-dimensional spaces (Y. Zhang & Wu, 2012). K-nearest neighbors (KNN) relies on the concept of utilizing the number of nearest neighbors to make predictions. The optimal value for the parameter k , which represents the number of nearest neighbors to consider, is crucial and should be carefully selected for optimal performance (Nugrahaeni & Mutijarsa, 2017). Decision trees are a supervised classification method that utilizes a hierarchical structure composed of branches, internal nodes, and leaf nodes. The data is partitioned into two branches at each internal node, based on specific conditions or features. The process continues until reaching the leaf nodes, which represent the final outcomes or predicted class labels. Decision trees provide a clear and interpretable representation of the decision-making process within the data (J. Ali et al., 2012) (Liu et al., 2015).

(e) Smartphone Application: “Micro Glucometer”

The smartphone-based Android app was developed, including all the essential functionality for image gathering and subsequent analysis using a machine-learning algorithm. The "Micro-Gluco meter" program also does pre-processing of acquired images to remove unwanted noises and normalization of the extracted features to increase result accuracy. Being an Android-based mobile application, “Micro-Gluco meter” performs three critical functions of a quantitative diagnostic assay: image acquisition, image processing and analysis, and test result display (illustrated in Figure 4.4). Following were the features of the camera, which had been in consideration while capturing images in auto image capturing mode *viz.* mode, ISO, white balance, exposure duration, shutter speed etc. In this app, a basic registration of the patient has been required, which is saved in phone memory up to the limit of 1000 entries. After the registration, images can be taken directly from the camera or from previously taken images. After acquiring the image, a cross-verification will be done for the region of interest (ROI). If ROI is not captured properly, then the image can be taken again for perfect ROI. After getting the perfect images, these images are processed in four phases: which is as follows, Pre-processing (white balance adjustments, light gradient corrections, and colour masking removal), ROI segmentation (i.e., exact identification of colorimetric signals regardless of variances), picture feature extraction, and ultimately feature analysis to estimate the sample value.

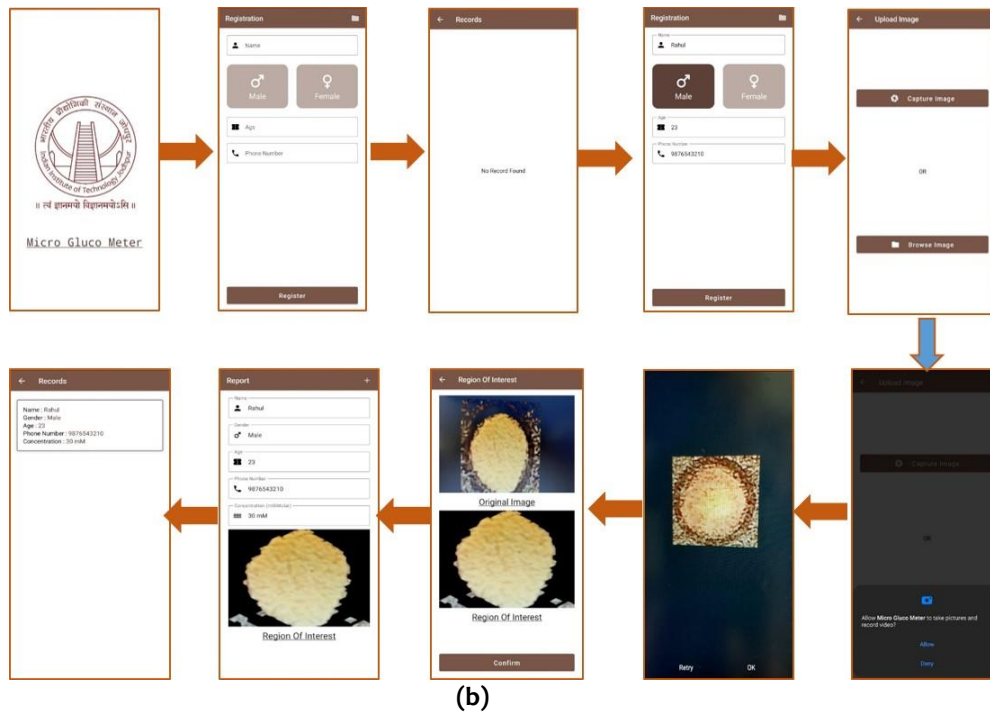
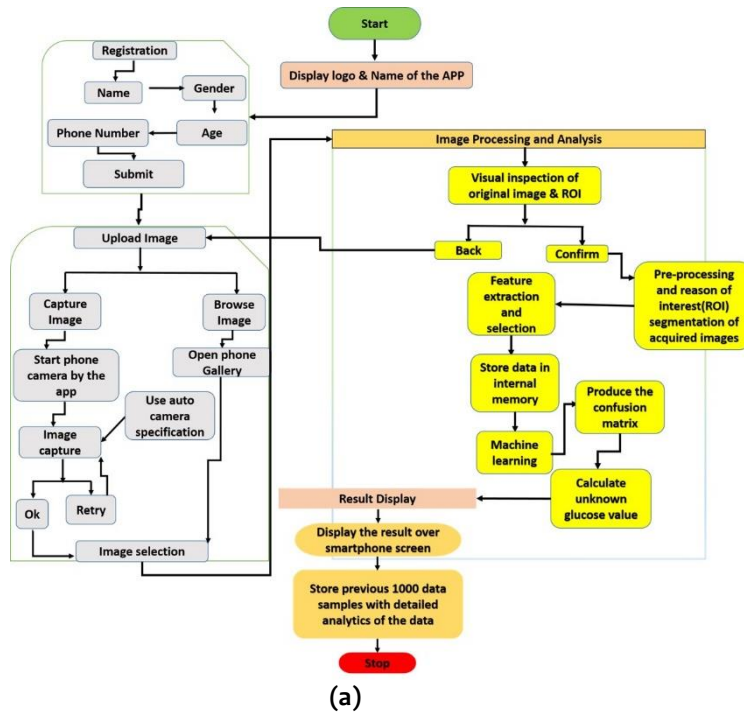


Figure 4.4: (a) Flowchart and (b) schematic representation of the “Micro Glucometer” app

Further, after the removal of unwanted noises, the ROI is identified using a segmentation technique to extract image attributes (such as grayscale intensities and RGB values, HSV, $L^*a^*b^*$, NTSC, and XYZ). These obtained visual features were analysed through machine learning for feature analysis. Further, classification and training of the algorithm were done, followed by feature extraction. Results were generated and displayed as the glucose concentration over the phone display and stored in the phone memory. These generated data can be stored in the phone memory for up to 1000 entries, which can be further checked. The uniqueness of this exercise is no requirement to adjust light-balance and camera optic settings to be performed via user. Because the image taken for training purposes is captured at all the possible atmospheric illumination conditions with five different types of smart phones.

4.2.3 RESULTS AND DISCUSSION

(a) Machine-learning classification results

The machine learning process was performed using the MATLAB app (Version R2021b). The app was trained with images obtained from standard experiments. Multiple models were used for training, including Decision Trees (Fine, Medium, Coarse), Naive Bayes (Gaussian, Kernel), Discriminant Analysis (Linear), K-Nearest Neighbor (Fine, Medium, Coarse, Cosine, Cubic, Weighted), Neural Network (Narrow, Medium, Wide, Bilayer, Three-layered), Support Vector Machine (Cubic, Quadratic, Linear, Fine Gaussian, Medium Gaussian, Coarse Gaussian), Subspace Discriminant, Subspace KNN, Boosted Trees, Bagged Trees, SVM Kernel, Logistic Regression Kernel, and RUSBoosted Trees.

Table 4.2 : Data analysis for all concentrations without chitosan using different machine learning algorithm

Model Type	Accuracy (%)	Prediction Speed (obs/sec)	No. of fully connected layers	Layer size	Activation	Box constraint level	Multiclass method
Linear Discriminant	90.2%	13000	-	-	-	-	-
Quadratic SVM	91.7%	7600	-	-	-	1	One-vs-One
Cubic SVM	92.7%	1400	-	-	-	1	One-vs-One
Narrow Neural Network	92.3%	10000	1	10	ReLU	-	-
Medium Neural Network	91.7%	15000	1	25	ReLU	-	-
Wide Neural Network	91.8%	15000	1	100	ReLU	-	-
Bilayered Neural Network	90.0%	14000	2	10, 10	ReLU	-	-

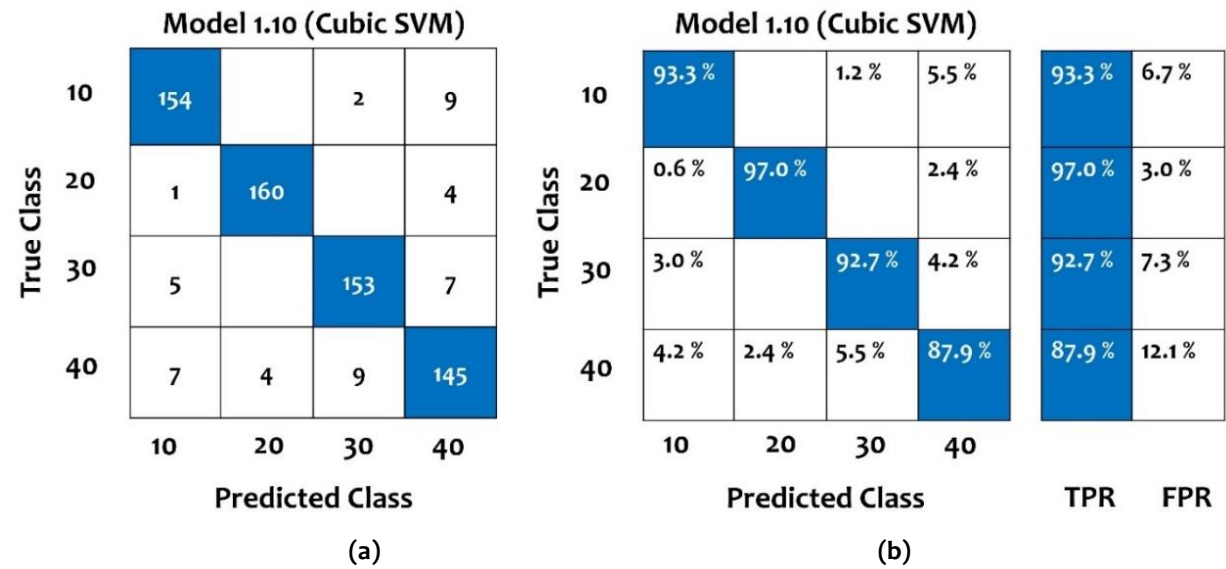
Table 4.3 : Data analysis for all concentrations with chitosan using different machine learning algorithm

Model Type	Accuracy (%)	Prediction Speed (obs/sec)	No. of fully connected layers	Layer size	Activation	Box constraint level	Multiclass method
Linear Discriminant	94.1%	13000	-	-	-	-	-
Quadratic SVM	93.9%	8600	-	-	-	1	One-vs-One
Cubic SVM	93.0%	8900	-	-	-	1	One-vs-One
Subspace Discriminant	90.9%	2600	-	-	-	-	-

Narrow Neural Network	92.3%	16000	1	10	ReLU	-	-
Medium Neural Network	92.1%	13000	1	25	ReLU	-	-
Wide Neural Network	93.0%	14000	1	100	ReLU	-	-
Bilayered Neural Network	90.3%	15000	2	10, 10	ReLU	-	-
Trilayered Neural Network	91.1%	16000	3	10, 10, 10	ReLU	-	-

In the present work, k=10 has been used to prevent overfitting for K-fold cross-validation, for initial training and validation purpose. Among these classifiers, few were selected based on their accuracy over 90% for the samples WC (Table 4.2) and C (Table 4.3). The classifiers Cubic SVM and Narrow Neural Network achieved the highest accuracy for the sample WC, up to 92.7% and 92.3%, respectively. For the samples C, classifiers Linear Discriminant and Quadratic SVM showed the highest accuracy, up to 94.1% and 93.9%, respectively.

Further, Table 4.2 and Table 4.3 provides data comparison for all concentrations using different machine learning algorithms, certain classifiers with low data accuracy were excluded from table to select the best results.



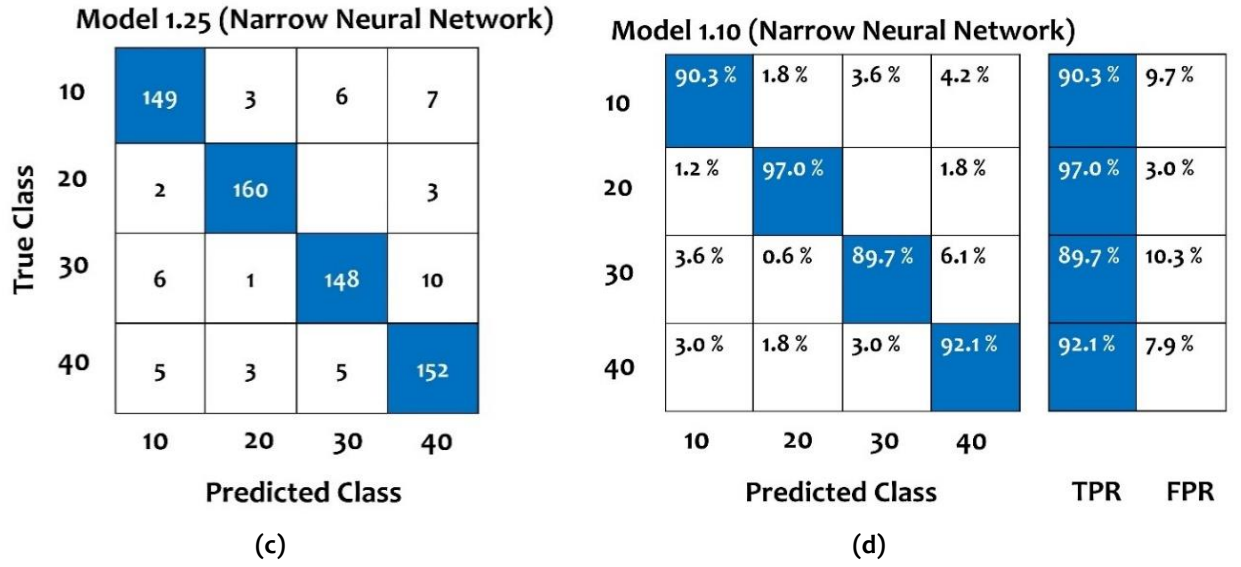


Figure 4.5 : Confusion matrices and True/False negative rates of the sample without chitosan for the classifier, cubic SVM (a, b), and narrow neural network (c, d)

The confusion matrices are shown in Figure 4.5 (a, c) for the better understanding of the classification performance. The results obtained through classifier cubic SVM and narrow neural network, are shown in these matrices for the class without chitosan PAD. Actual class and predicted class are the two dimensions of a confusion matrix. Each row and column of the matrix represent instances of an actual class and instances of a predicted class. The relationship between predicted class (C_j) and the actual class (C_i) is displayed in a tabular format. The confusion matrices offers useful data for accessing the effectiveness of the classifiers. The confusion matrix can be used to determine the experimental outcomes in terms of true positive (TP_i), true negative (TN_i), false positive (FP_i) as referred in Eq.(4.3), and false negatives (FN_i) as shown in Eq.(4.4). Where the element N_{ij} represents the samples that belong to class (C_i) but are classified as class (C_j). The information derived from the confusion matrices; several performance measures can be defined. These include accuracy Eq.(4.5), precision Eq.(4.6), recall Eq.(4.7), and F-measure Eq.(4.8). These performance measures were determined based on the number of classification errors and correct predictions made by the classifier. The mentioned terms "true" and "false" indicate, whether the samples are correctly or incorrectly classified. The term true positive refers to the number of samples from class i that are correctly classified as positive Eq.(4.1). While, the term true negative denotes samples that do not belong to class i , but correctly classified as not i Eq.(4.2). The term false positive represents samples that do not belong to class i , but wrongly classified as class i Eq.(4.3), while false negative represents a certain number of samples from class i that wrongly classified as any class except class i Eq.(4.4).

$$TP_i = N_{ij} \quad (4.1)$$

$$TN_i = \sum_{j,k \neq i} N_{jk} = \sum_{j=1}^n \sum_{k=1}^n N_{jk} - (TP_i + FP_i + FN_i) \quad (4.2)$$

$$FP_i = \sum_{k \neq i} N_{ki} = \sum_{k=1}^n N_{ki} - TP_i \quad (4.3)$$

$$FN_i = \sum_{k \neq i} N_{ik} = \sum_{k=1}^n N_{ik} - TP_i \quad (4.4)$$

Accuracy, in the context of a multiclass classifier, is a measure that quantifies the proportion of correctly predicted instances out of the total number of instances. It represents the overall correctness of the classifier's predictions Eq.(4.5).

$$Accuracy = \frac{\sum_{i=1}^n TP_i}{\sum_{i=1}^n \sum_{j=1}^n N_{ij}} \quad (4.5)$$

The ratio of true positive and all the positive samples predicted by classifier of class (C_i) defined as precision Eq.(4.6).

$$Precision_i = \frac{TP_i}{TP_i + FP_i} \quad (4.6)$$

The ability of a classifier to select exact positive samples from the targeted class can be defined as a true positive rate or recall Eq.(4.7).

$$Recall_i = \frac{TP_i}{TP_i + FN_i} \quad (4.7)$$

The F-measure, also known as the F1 score, is a metric that combines the precision and true positive rate (recall) into a single value. It is defined as the harmonic mean of precision and recall Eq.(4.8).

$$F - Measure_i = 2 \times \frac{Precision_i \times Recall_i}{Precision_i + Recall_i} \quad (4.8)$$

It can be observed in Figure 4.5 (b). 20mM sample shows a higher accuracy (97%) in comparison to the average accuracy (92.7%). The reason behind this phenomenon can be easily observed in Figure 4.5 (a), in cubic SVM out of 165 samples, four samples were predicted as 40mM, and one sample was predicted as 10mM. The same can be observed in Figure 4.5 (c, d), 20mM sample shows a higher accuracy (97%) in comparison to the average accuracy (92.3%), because in narrow neural network out of 165 samples, three samples were predicted as 40 mM and two samples were predicted as 10mM.

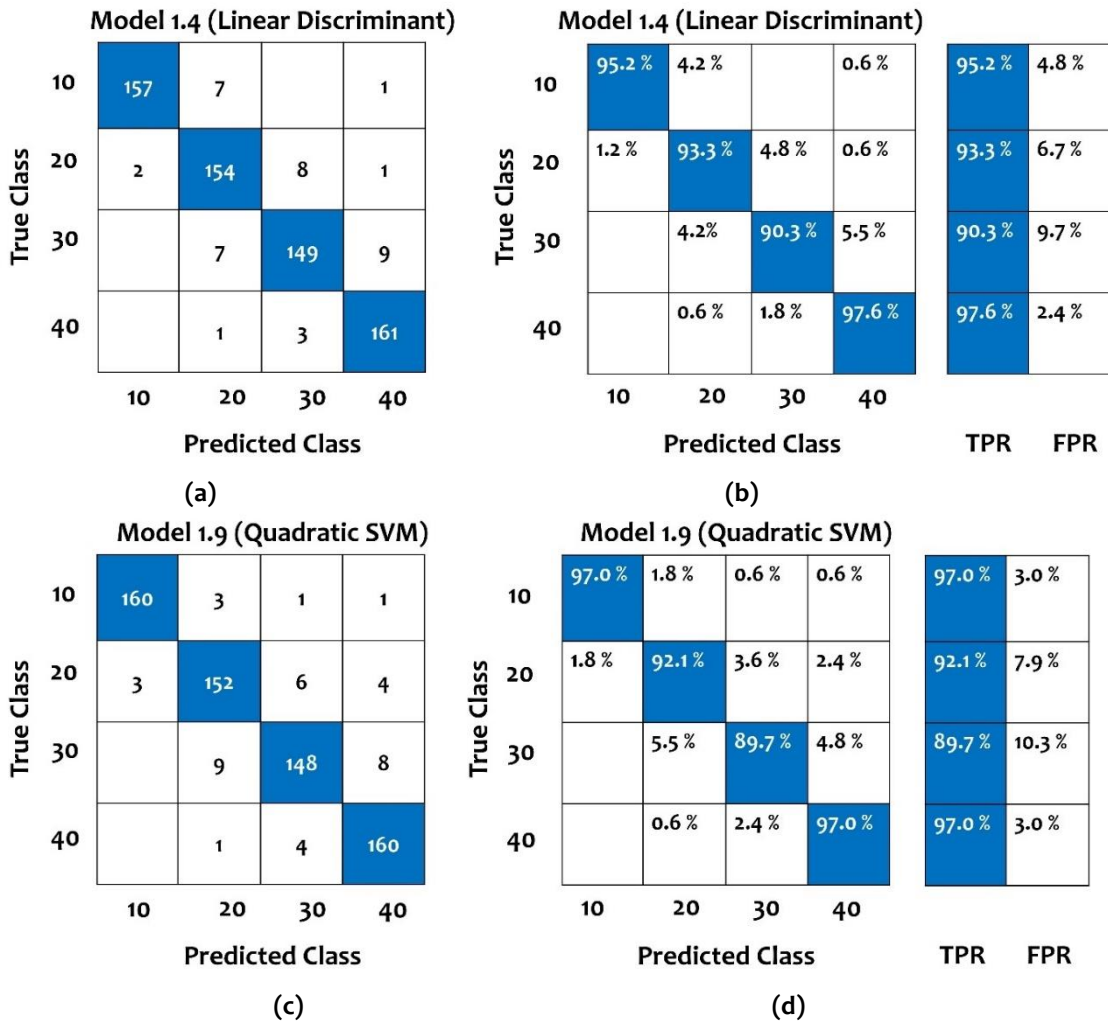


Figure 4.6: Confusion matrices and True/False negative rates of the sample having chitosan for the classifier, linear discriminant (a, b), and Quadratic SVM (c, d) respectively

Further, the confusion matrices given in Figure 4.6 (a, c), shows the performance of the linear discriminant and Quadratic SVM in the class of with chitosan PAD, respectively. The confusion matrix is a tabular representation that illustrates the relationship between the predicted class labels and the true class labels. It can be seen clearly in Figure 4.6 (b) sample of 40mM shows a higher accuracy (97.6%) in comparison to the average accuracy (94.1%). The reason behind this can be easily seen in Figure 4.6 (a), out of 165 samples, three samples were predicted as 30mM, and one sample was predicted as 20mM for linear discriminant. The same phenomena can be observe in Figure 4.6 (c, d) sample of 40mM shows a higher score (97%) in comparison to the average score (93.9%), because in Quadratic SVM classifier out of 165 samples, four samples were predicted as 30mM, and one sample predicted as 20mM.

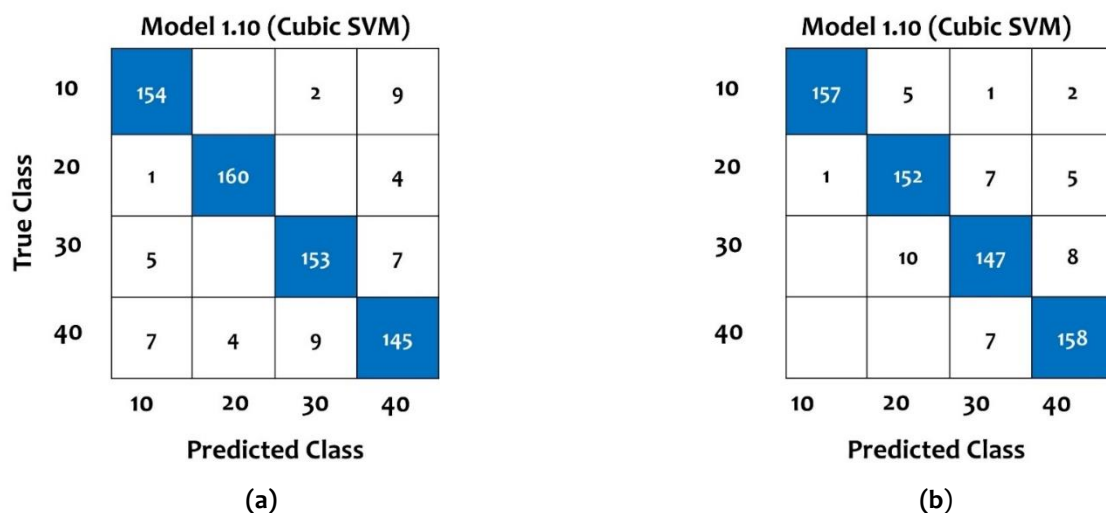


Figure 4.7 : Confusion matrices (a) without and (b) with chitosan for the Cubic SVM classifier

The performance of the classifiers was evaluated for four different concentrations (10, 20, 30, and 40mM) in terms of precision and accuracy for two kinds of the case without chitosan and with chitosan; among all the classifiers, cubic SVM and narrow neural network shows higher accuracy for without chitosan case. While, linear discriminant and quadratic SVM shows higher accuracy for with chitosan case. Therefore, it is necessary to choose a single classifier for the proper functionalization, which can be used universally for both case at high accuracy. From Tables (2) and (3), it can observe that cubic SVM shows near about the same accuracy (92.7% and 93 %) for both case. So, the cubic SVM can be chosen as an ideal classifier for this case. Figure 4.7 shows comparison confusion matrices for cubic SVM for both cases, and Table 4.4 shows comparison of few recent works.

Table 4.4 : Recent work published in the domain of colorimetric sensing using machine learning

S. No	Classifier	Accuracy	Colour Space	Features	Reference
1.	Kernel support vector machine	88.2%	RGB	Mean, contrast, homogeneity, Energy, variance, correlation, entropy	(Y. Zhang & Wu, 2012)
2.	Random forest	92%	RGB, HSV, L*a*b*	Mean values	(Thakur et al., 2021)

3.	Support vector machine	90%	RGB	Calibration	(Bao et al., 2018)
4.	Cubic support vector machine	92.7%	RGB, HSV, L*a*b*, NTSC, XYZ, Grey scale	Mean, skewness, kurtosis, entropy, intensity, grey level co-occurrence matrix	This reported work
	Narrow neural network	92.3%			
	Linear discriminant	94.1%			
	Quadratic SVM	93.9%			

4.2.4 Summary

The present study introduces a distinct approach for using machine-learning classifiers to detect glucose levels (with concentration range 10-40 mM) based on the colour change in PADs. The classifiers were trained using raw images captured under various illumination conditions, encompassing a wide range of user conditions and different smartphones with varying camera optics. This approach enhances the robustness of the analysis platform by accounting for different atmospheric lighting conditions and camera characteristics. Additionally, to ensure user-friendliness, images were captured under all possible directional illumination conditions. The combination of machine learning methods and different smartphones at various illumination and directional conditions proved effective in achieving high classification accuracy. The linear discriminant classifier demonstrated the highest accuracy for the detection reagent chitosan. However, for both detection reagents, the cubic SVM classifier achieved the highest overall accuracy, reaching approximately 93%. The findings suggest that the proposed platform is capable of accurately determining glucose levels in samples, regardless of the incident light's colour, direction, time, location, and camera optics. To the best of our knowledge, this study is the latest report on integration of machine-learning classifiers within a smartphone-based platform for glucose detection under different directional illumination conditions. Further improvements can be achieved by expanding the dataset with diverse smartphones and incorporating more data.

4.3 RESEARCH OUTCOMES

- Kishnani, V., & Gupta, A. (2023). Predictive Framework Development for User-Friendly On-Site Glucose Detection. *ACS Applied Bio Materials*, 6(10), 4336-4344, Doi: [10.1021/acsabm.3c00532](https://doi.org/10.1021/acsabm.3c00532)