CHAPTER 6

ACCURATE ESTIMATION OF CHLOROPHYLL IN TEA LEAVES USING A SMARTPHONE-BASED FLUORESCENCE SENSOR

The design of a smartphone-based fluorescence sensing tool has been discussed. Details about the optical system developed for converting the smartphone in to a fluorescence system has been illustrated and the utility of the phone's inbuilt sensors and android applications for sensitive fluorescence signal detection have been discussed. The usability of the developed platform has been demonstrated for monitoring of chlorophyll content in plant leaves. A 3D printed compact optical set-up has been designed to couple it with the phone to record the emitted fluorescence signal intensity from the sample and the usability of the developed set-up has been demonstrated for estimation of chlorophyll in fresh tea leaves.

6.1 Introduction

Chlorophyll estimation is important to monitor the growth of plants as it aids in converting solar energy into chemical energy for the growth of plants. A decrease in total leaf chlorophyll concentration affects the overall efficiency of photosynthetic processes that subsequently affect the growth of the plant [1, 2]. Chlorophyll content in leaves, abiotic stress, calcium level, nitrogen concentrations etc., are some of the important parameters that determine the overall health condition of plants. Also, the presence of nitrogen content [3], biotic stress, as well as abiotic concerns such as light, dehydration, and pigment inhibiting herbicide damage in plants, can be estimated by measuring the chlorophyll content in plant leaves [3, 4]. The estimation of chlorophyll

in plants is thus a vital parameter as far as monitoring and regulating agricultural and environmental activities are concerned [5, 6]. There are various methods available for quantification of chlorophyll content, such as spectrophotometry, high-performance liquid chromatography (HPLC), fluorimetry etc. The involvement of bulky instruments and time-consuming sample preparation steps make these methods unsuitable for the common citizen. Again, the majority of absorption-based techniques for chlorophyll estimations are performed with the laboratory spectrophotometers, making the measurement process inappropriate [7, 8] for in-field applications. Among the various commercially available tools, Minolta's SPAD 502 (Soil Plant Analysis Development) is being widely used to assess the chlorophyll concentrations in leaves [9–11]. The primary limitation of the SPAD 502 system is that it only calculates the chlorophyll content within a specific region on the leaf by estimating the absorbance at one spot of the leaf under investigation. Multiple measurements at various regions of the leaf are generally done to estimate the average chlorophyll concentration, which is again a time-consuming process. Fluorescence signal analysis of chlorophyll is another useful technique to estimate its concentration in plant leaves [12]. Also, this parameter of plant can be studied through analyzing the transmission optical spectra [13], or by capturing the plant leaf color in digital photographs. The images can be captured by in-built high-resolution cameras of a smartphones and the chlorophyll concentration can be estimated by analyzing the images with a custom-designed android application [14]. Nowadays, a smartphone is integrated with various in-built sensors, high-resolution cameras up to 108 megapixels, and high processing software, which allows users to perform image processing within the phone itself. The inbuilt camera sensor of the phone has been exploited for the detection of various elements of plants [15, 16]. Recently, the whole-plant tissue nitrogen content in floriculture crops has been estimated by performing image-processing of the recorded images of the plant leaves using a smartphone camera [17]. The nitrogen content in leaves can also be estimated from the leaf color. Various smartphone-based applications are readily available which can determine the nitrogen content present in rice leaves by capturing the images of different regions of rice leaves and comparing the RGB (red, green and blue) color values on a standard leaf color chart (LCC) on the same application itself [18]. Apart from smartphones, sophisticated device like google glass has also been used to detect chlorophyll content in plants by sending the captured images to the internet server to estimate the chlorophyll amount present in plants [19]. The specifications of the camera vary largely from phone to phone, which affects camera-based measurements. The external light condition also affects the quality of the captured photographs. Also, the CMOS camera of a smartphone is only sensitive in the wavelength range of 400-700 nm. Beyond this limit, fluorescence analysis of plant chlorophyll cannot be performed using a smartphone's camera. On the other

hand, the ALS (Ambient Light Sensor) of the phone is sensitive to a wide spectral range (350-1000 nm). Absorption based plant chlorophyll estimation is done by sensing the transmitted modulated signals from the chlorophyll samples with the ALS of a phone [20]. The basis for a sensitive evaluation of plant chlorophyll is the detection of fluorescence emission [21]. Upon illumination of the chlorophyll sample with an optical light signal in the wavelength range 470-500 nm, it emits a fluorescence signal in the range 650-700 nm. The emitted fluorescence signal intensity depends on the molar chlorophyll concentration in the sample [22]. This specific optical property of the leaves can be utilized to estimate the overall chlorophyll content in plant leaves.

Present work illustrates the design of a cost-effective, portable and user-friendly smartphone-based fluorescence sensing system has been used to monitor the chlorophyll concentration in plant leaves. The selected plant leaf for this work is the tea leaf, as it is one of the highly consumed beverages in the world. Chlorophyll is a highly significant pigment in tea leaves and non-fermented teas, and its proportion affects the final color of green tea extract [23]. The designed sensing system has been utilized for the estimation of chlorophyll concentration of tea leaves. The detailed chlorophyll quantification process has been explained in the following sections.

6.2 Materials and methods

6.2.1 Chlorophyll extraction procedure and preparation of standard chlorophyll samples

Prior to the experimental examination, all glassware were cleaned with pure hydrochloric acid (HCl) and distilled water to remove any possible chemical contamination. The chemicals that were used for extraction of chlorophyll content from the tea leaves was ethanol. At first, 80% ethanol (v/v) was prepared in distilled water [24]. Chlorophyll extracts were prepared by weighing 700 mg of fresh tissue of tea leaves and adding 80% ethanol or acetone in a 10 mL glass tube till the tissue gets immersed. The prepared sample was kept at -18°C for 3 hours. After freezing for 3 hours, the suspended leaves were crushed in a mortar and finally filtered the suspension through a membrane filter paper (grade1, Cat No. 1010-125). Also, a wide range of standard samples with varying chlorophyll concentrations from 1 to 12.7 mg/g was prepared by serially diluting the freshly extracted raw chlorophyll sample by adding a proportionate amount of ethanol or acetone. The images of the extracted chlorophyll from tea leaf samples are shown in figure 6.1. The chlorophyll content present in the prepared samples were measured using a standard chlorophyll meter (SPAD 502) and spectrometer based absorbance equations [25].

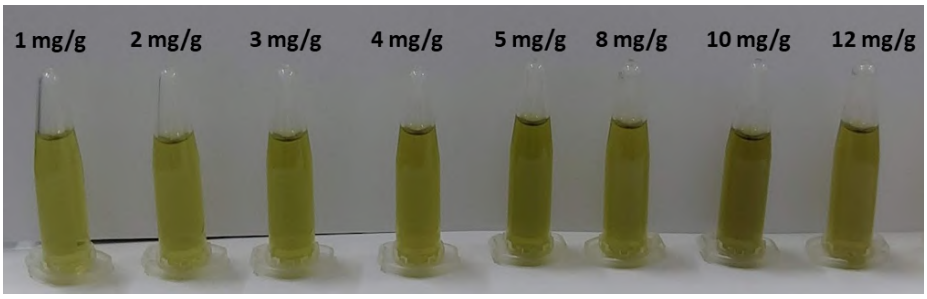

Figure 6.1: Images of extracted chlorophyll samples from tea leaves.

6.2.2 Fluorescence analysis of tea leaf chlorophyll samples

Under white light condition, a dilute solution of leaf chlorophyll in organic solvent appears green in colour (left inset of figure 6.2). The wavelengths in transmitted and reflected light from chlrophyll sample correspond to bands of blue and red as these wavelengths are strongly absorbed by chlrophyll, whereas mid-range wavelengths that correspond to green light are relatively weakly absorbed. However, the solution will appear dark red when viewed at right angles to the incident light signal due to the energy that is re-emitted as fluorescence (right inset of figure 6.2). The

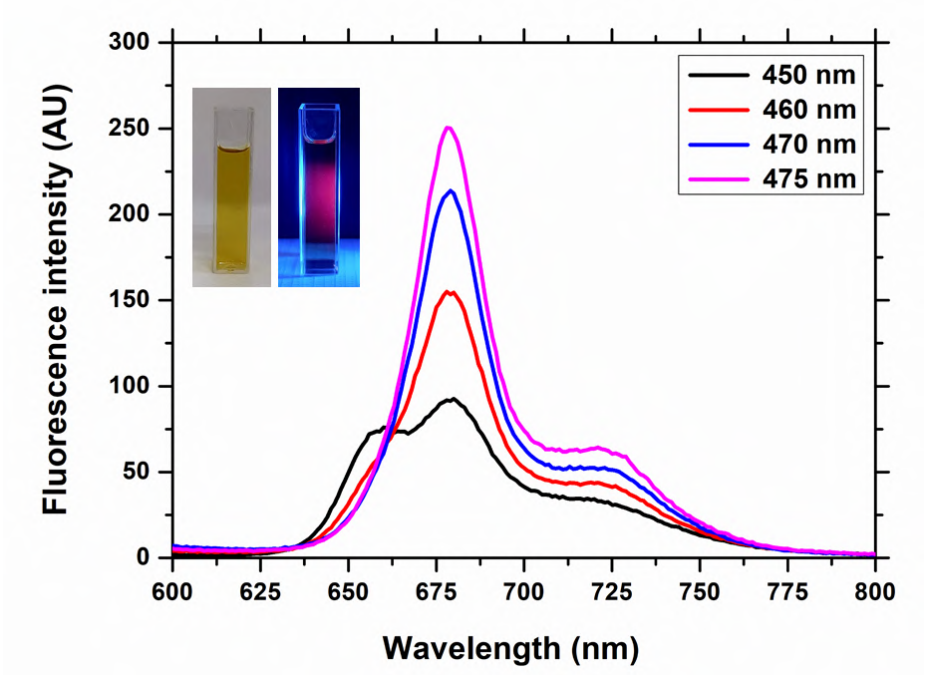

Figure 6.2: Images of extracted chlorophyll samples from tea leavesFluorescent spectra of tea leaf chlorophyll sample excited at 450, 460, 470, and 475 nm. Inset (from left to right) image of extracted tea leaf chlorophyll sample under natural light condition and fluorescent image of extracted tea leaf chlorophyll sample under blue light at 475 nm.

chlorophyll can absorb energy from incident photon. This absorbed energy can be released as heat, fluorescence radiation, or by activating photosynthetic activities.

When the chlorophyll is extracted from leaf, it loses its ability to transfer energy to other molecules and instead releases a large portion of its energy as fluorescence emission when excited by a certain wavelength of light. The emitted fluorescence intensity by plant leaf is strongly associated with chlorophyll concentration present in the leaves [26]. The emission of chlorophyll fluorescence is mostly observed in the spectral region of 650-750 nm upon excitation with light of wavelength 350-500 nm [3]. The chlorophyll sample (12 mg/g) has been excited with 4 different optical sources, each with a different peak emission wavelength ranging from 450 to 475 nm while maintaining the same output optical power, in order to investigate the excitation wavelength for which the maximum fluorescence emission intensity from the tea leaf can be observed. For an excitation source irradiated at 475 nm, the maximum emitted fluorescence intensity has been observed at 679 nm as shown in figure 6.2. Under natural light condition the extracted chlorophyll sample does not emit any fluorescence emission as shown in left inset of figure 6.2 while under blue light condition the chlorophyll sample emits red fluorescence signal as shown in right inset of figure 6.2.

6.2.3 Design of the experimental sensing setup

The schematic diagram for the proposed smartphone-based chlorophyll sensing setup is shown in figure 6.3(a). In the designed sensing setup, the embedded ambient light sensor (ALS) of the phone has been used as a detector to record the intensity of the fluorescent signals. An LED of peak emission wavelength 475 nm has been used as an optical source and is powered from the smartphone's internal battery through a USB-OTG cable. A Plano Convex Lens (7 mm diameter, Edmund Optics 32-404) with a focal length of 11 mm has been used to collimate the light signal emerging from the LED. The collimated light beam passes through the sample housed in a quartz cuvette (45 mm $\times$12.5mm $\times$12.5mm), having an optical path length of 10 mm. The fluorescence signal emitted from the chlorophyll sample has been detected at a right angle to the direction of the incoming light signal by the ALS of the phone. To eliminate the excitation wavelength, a 670 nm band-pass optical filter has been placed in the signal's optical path to the ALS of the phone. The fluorescence signal emitted from the sample is focused using a focusing lens (6 mm diameter, Edmund Optics 45-690) and finally coupled to the ALS of the smartphone. Moto One Power (Android 8.1 Oreo) smartphone (Motorola Inc.) has been used for the present sensing investigations. Almost all the smartphones companies use Avago APDS 9930 proximity and ambient light sensor chip inside the smartphone sensors [27]. In the visible spectral region, ALS is extremely sensitive. Fluctuations in the intensity measurements can be observed when a highly intense collimated light beam from the

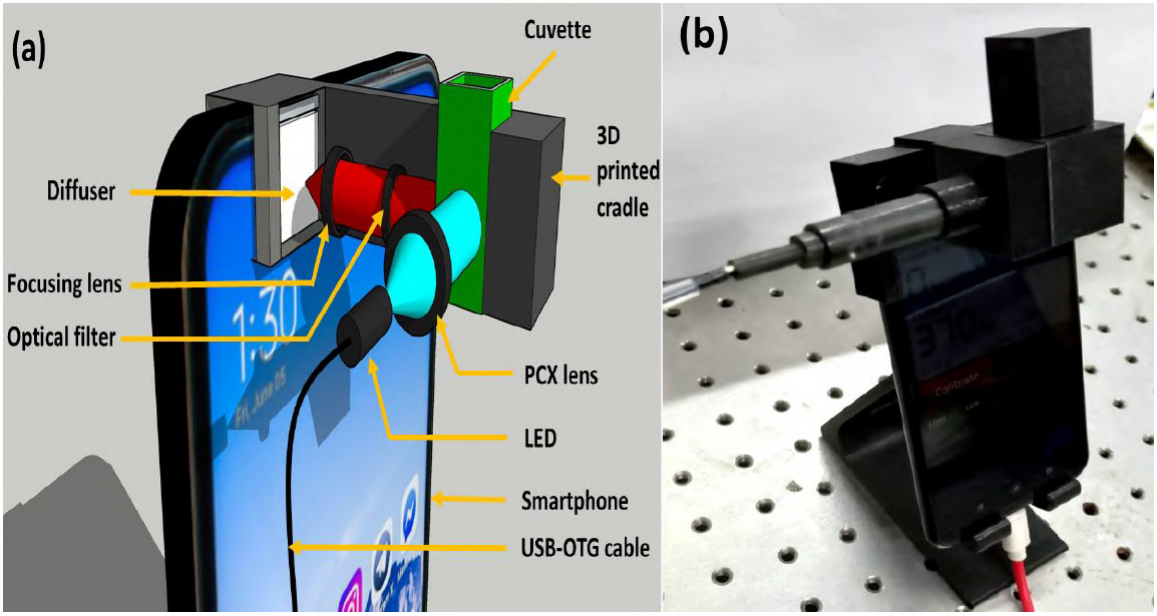

Figure 6.3: (a) Schematic of smartphone based sensor, (b) photo image of the designed sensor

LED is focused on the photosensitive portion of the ALS. Eventually, the noise level associated with the intended optical signal will also rise. This can be obviated by using a 1 mm thick ground glass diffuser (Model no. HO-DF-50S-32, Holmarc optics, India) in the optical path between the focusing lens and the smartphone's ALS. The diffuser would reduce possible errors that may cause due to intensity fluctuations in the transmitted modulated signal. All the optical components are arranged in a custom-made black 3D printed cradle, which can be attached to the phone easily so that external light cannot interfere any measurements. The photo image of the designed sensor is shown in figure 6.3(b).

6.2.4 Android applications workflow

One freely available android application, "Light Meter" App, has been used to measure the transmitted modulated fluorescence signal intensity by the smartphone. In this application, the fluorescence intensity recorded by the ALS is converted into a "LUX" unit. The application further calculates the average value by measuring the least and greatest variations in dispersed lux output over a certain duration of time. Another open-access application, "stanXY", has been used to plot the fluctuations of dispersed lux intensity with the chlorophyll concentrations. The sensor response curve between the dispersed lux irradiance with chlorophyll concentration variations for different samples was first obtained using this application. This characteristic calibration curve has subsequently been used to estimate the chlorophyll concentration from an unknown sample. Thus, using these two open-access android application

platforms, one can easily estimate chlorophyll concentration with the designed smartphone sensor.

6.2.5 Fluorescence based sensing of plant chlorophyll using smartphone

At first, the designed sensing system has been calibrated for different chlorophyll samples of known concentration. Following the procedure described in the section 6.2.1, twelve different samples in the range 1 mg/g – 12 mg/g have been prepared in the laboratory. The fluorescence signal from these samples were initially recorded by the standard fluorescence spectrometer (Agilent Cary Eclipse) at an excitation wavelength of 475 nm. The peak signal intensity at 679 nm from the samples are found to be increased with chlorophyll concentration in the samples as shown in figure 6.4(a). The characteristic plot between the peak fluorescence intensities at 679 nm vs chlorophyll concentrations is shown in figure 6.4(b). The linear fitted graph with regression coefficient (R^2) value of 0.955 implies a good degree of linearity between fluorescence intensity and chlorophyll concentration.

6.2.6 Smartphone based measurement and analysis

The chlorophyll content present in the reference samples were initially measured using a standard chlorophyll meter (SPAD-502) prior to the measurement with the designed smartphone sensor. The fluorescence signal intensities of these samples upon excitation with a light signal at 475 nm wavelength were recorded by designed smartphone sensor. The emitted fluorescence intensity recorded by the ALS in the designed setup has been determined by calculating the mean value of the distributed lux irradiance of all the prepared samples. Figure 6.5 (a) shows the characteristic curve of the normalized fluorescence signal intensity variations with the chlorophyll concentration of the samples. The linear flitted curve with a regression value of $R^2 = 0.988$ indicates again that the designed sensor exhibits a fairly linear response with chlorophyll concentration in the sample. This results is in agreement with the results obtained with the standard fluorescence spectrometer discussed in section 6.2.5. The residual versus fitted graph given in figure 6.5(b) confirms the validity of the regression model. Following calibration equation has been obtained from the regression analysis between chlorophyll concentration and the normalized intensity recorded by designed sensor:

$$Chlorophyll\ concentration\ = \frac{Normalized\ intensity\text{-}0.19683}{0.06803} \tag{6.1}$$

Using the above calibration equation, one can measure the chlorophyll concen-

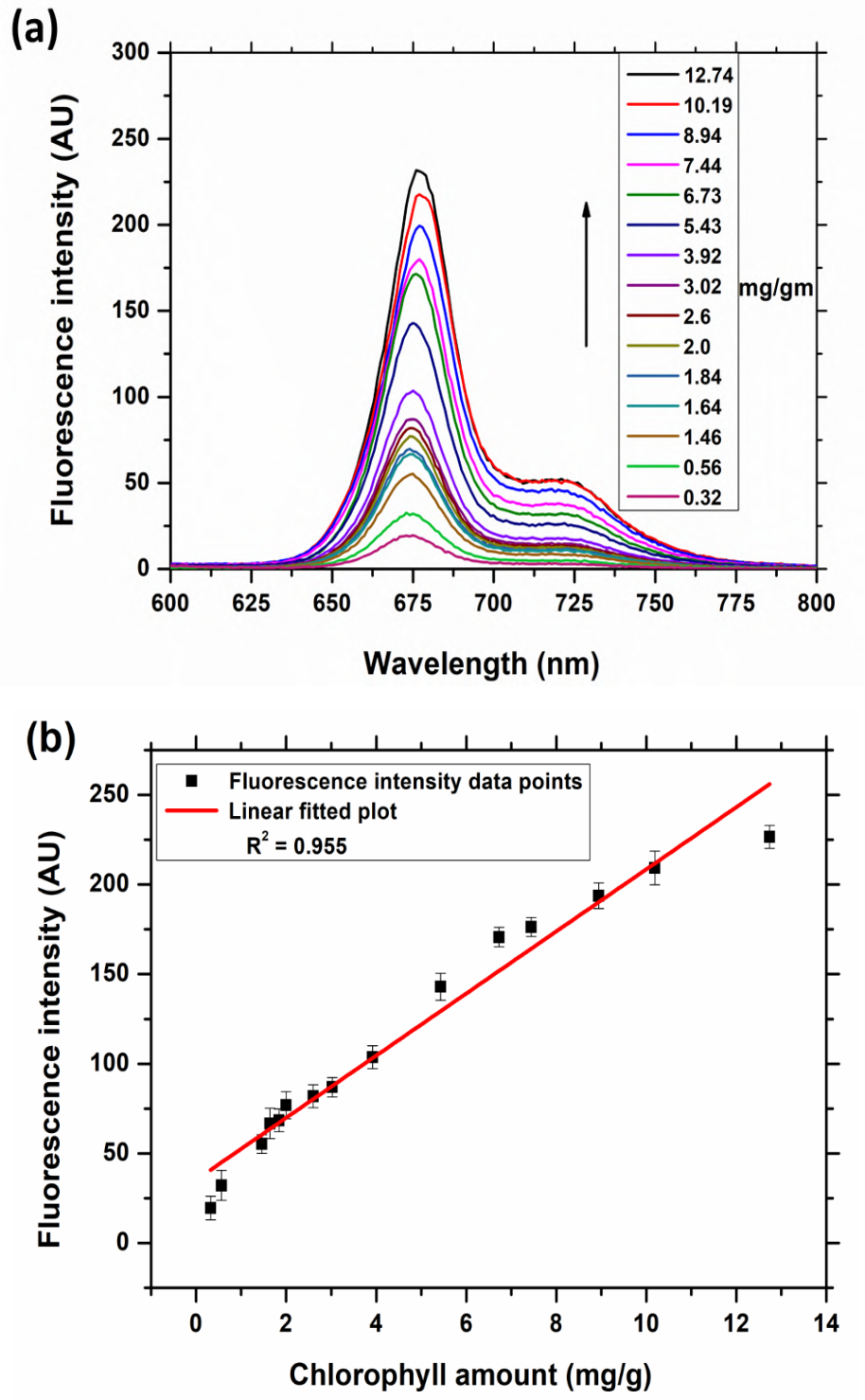

Figure 6.4: (a) Fluorescence spectra and (b) calibration curve at 679 nm of the plant chlorophyll samples obtained with the fluorescence spectrometer

tration of unknown plant sample. To evaluate the uncertainty in measurements of the designed platform, the fluorescence intensity emitted by a range of chlorophyll samples with concentrations 0.5-10 mg/g have been recorded. Each sample has been measured for ten consecutive times and the standard deviation values have been plot-

ted with the sample concentrations shown in figure 6.5(c). The maximum uncertainty value in the measurements was found to be 0.070 AU. This low level of uncertainty suggests that with the proposed smartphone sensor, the chlorophyll concentration as low as 0.5 mg/g can be estimated reliably and accurately.

6.3 Evaluation of sensor parameters

Sensitivity: A sensor's sensitivity is associated with a change in output per unit change in input [28]. It indicates the gradient of the calibration graph. Based on the characteristic sensor response curve shown in figure 6.5(a), the sensitivity of the developed sensing instrument is estimated to be 0.068 AU.g/mg.

Limit of detection: The limit of detection (LoD) is the lowest concentration of an analyte that can be consistently detected by designed sensor [29]. The LoD is determined according to the recommendations of the International Conference on Harmonization [30], which is given by:

$$LoD = \frac{3.3\sigma}{S} \tag{6.2}$$

where, S is the slope of the calibration curve and σ is the standard deviation of the regression line's y-intercept. By inserting the values of S and σ from the calibration curve into equation 6.2, the LoD of the developed sensor is found to be 0.0618 mg/g.

Resolution: The proposed sensing system's resolution is determined by the ALS resolution, which is 0.01 lux or 1.06×10^{-4} AU and it can be calculated as follows [31]:

$$Resolution(R) = \frac{1}{S} \times R_{ALS} \tag{6.3}$$

where, R_{ALS} is resolution of the ALS used in present work and S is the sensitivity of the designed sensor. By putting the values of R_{ALS} and S in equation 6.3, the resolution of the developed sensor is estimated to be 1.55×10^{-4} mg/g.

Accuracy and precision: Three reference test samples with chlorophyll concentrations of 1.05 mg/g, 6.21 mg/g, and 12.1 mg/g were used to validate the accuracy and precision of the developed smartphone sensing tool. Sensor precision and accuracy are commonly stated as %Bias and %RSD. The following formulae are used to calculate these two parameters.

$$\%Bias = \frac{Known\ chlorophyll\ conc.\text{- }Measured\ chlorophyll\ conc.}{Known\ chlorophyll\ conc.} \times 100 \tag{6.4}$$

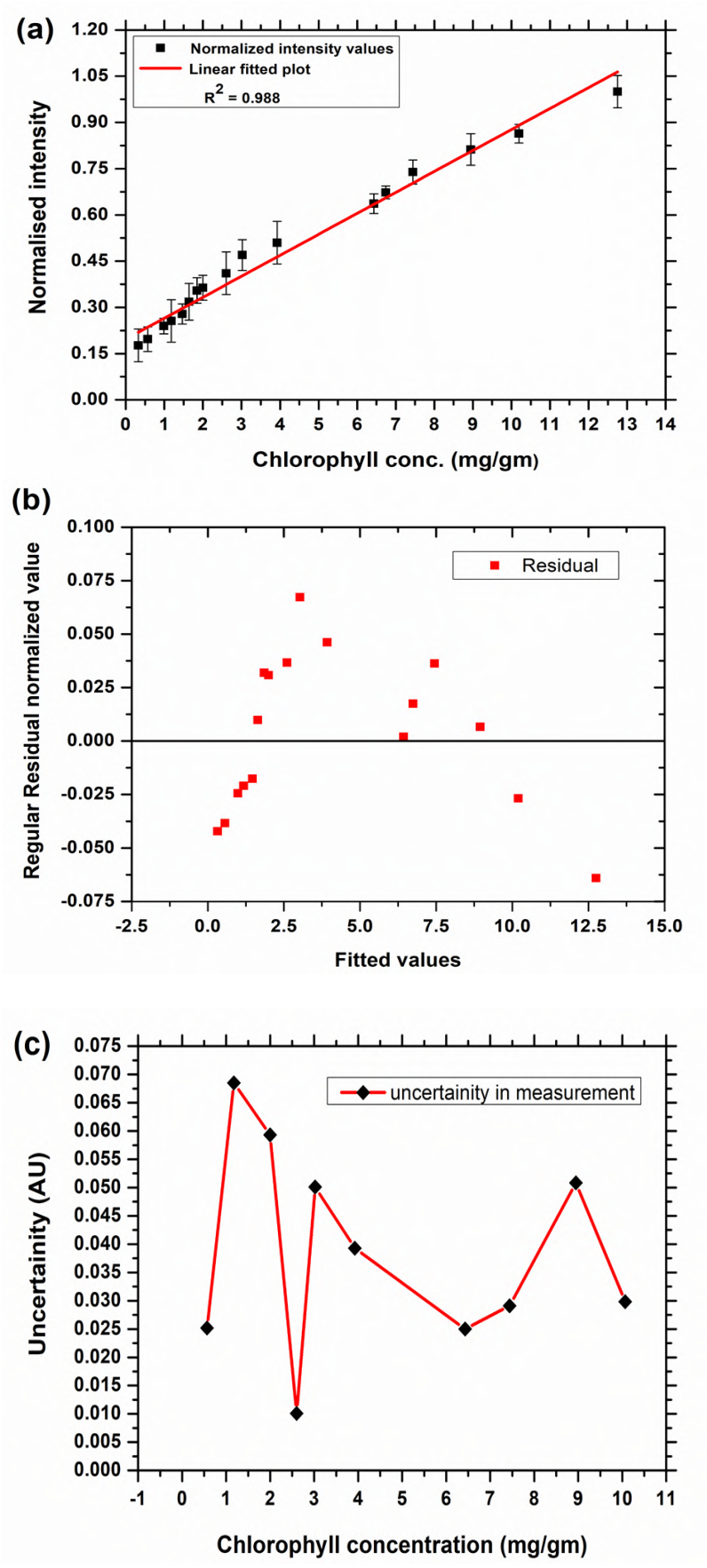

Figure 6.5: (a) Smartphone sensor response curve for standard chlorophyll solutions of different concentrations (b) residual plot of sensor response curve, and (c) the uncertainty found in ten times measurements for the range 0.5-10 mg/gm chlorophyll samples.

$$\%RSD = \frac{Standard\ Deviation}{Mean} \times 100 \tag{6.5}$$

The accuracy and precision have been calculated by using the above equations for measurements of each of the three considered samples for five consecutive times by the designed smartphone sensor. The average bias accuracy and the average RSD are estimated to be 0.85 % and 1.33 %, respectively. The low %Bias and low %RSD indicate the degree of precision and accuracy of the proposed platform.

6.4 Evaluation of the designed sensor for field collected samples

In the final step, the field applicability of smartphone sensor has been evaluated with field-collected tea leaves samples. Tea leaves samples from eight different tea states of Assam have been collected and subsequently the chlorophyll content present in the samples have been extracted following the procedure described in section 6.2.1. The chlorophyll content present in the different samples have been measured with the commercial chlorophyll meter (SPAD 502). The intrinsic fluorescence emission intensity from these samples were recorded by a standard laboratory grade fluorescence spectrometer and then by the developed smartphone sensor. From the recorded data chlorophyll concentration present in the field collected tea samples were measured and compared with the standard chlorophyll meter data. Figure 6.6 shows comparison of the experimental data recorded by the three tools- considered for the present investigation. The error bar shown in the figure is the standard deviation obtained for each set of measurement. The maximum variations in chlorophyll measurement detected by the designed smartphone sensor is found to be less than 4%, than the results measured by its commercial counterpart. A factorial two-way ANOVA (Analysis of Variance) test [32] was performed to compare the three sets of findings acquired by the chlorophyll meter, standard fluorescence spectrometer and the proposed smartphone-based chlorophyll sensing tool. The effect of these three tools on the results has a significant effect in terms of factor $F(1, 64) = 80.36$, significant value $p = 0.00$, and partial eta squared $\eta^2 = 3.93$, indicating that there is no significant difference in the measurements recorded by the three instruments. The data suggest that the designed smartphone-based sensing platform can be used reliably for the estimation of chlorophyll content in plants.

It is envisioned that the proposed sensing technique could emerge as a potentially affordable analytical tool for estimation of chlorophyll content with excellent precision and repeatability characteristics. The overall cost of the designed sensing tool is approximately $100 (8000.00 INR) excluding the cost of the smartphone. Further,

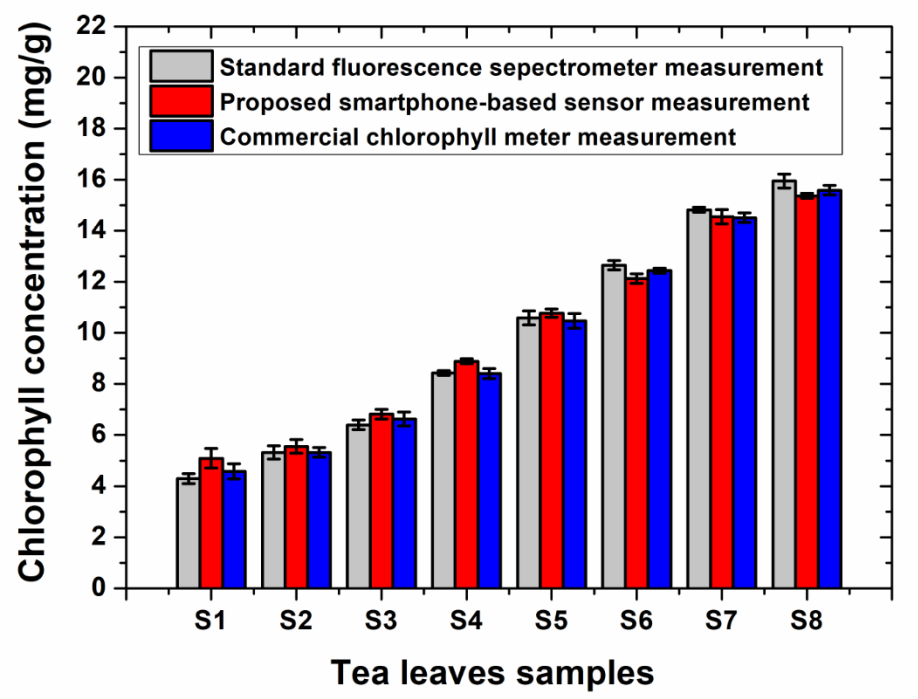

Figure 6.6: Bar graph representation of the comparison of chlorophyll measurement by the fluorescence spectrometer, designed smartphone sensor and the commercial counterpart.

with the present sensing platform, it is possible to share the in-field chlorophyll data anywhere in the world through the mobile broadband network. The suggested sensing system may even be utilized by an untrained individual to monitor the health of the plants within the personal smartphone itself. Table 6.1 summarizes the performance comparison of the designed sensing platform with its commercial counterpart and the similar work reported elsewhere [33].

6.5 Summary

In summary, a cost-effective, robust, and user-friendly smartphone-based fluorescence spectrometer capable of measuring chlorophyll content in plant leaves has been demonstrated. The device has been developed using the inbuilt ambient light sensor of the smartphone with some simple optical components like lens, filter and LED. The LED in the sensing set-up has been powered from the internal battery of the smartphone which reduces the cost of the external power supply. A good degree of correlation between chlorophyll content and the intrinsic fluorescence emission intensity has been observed while comparing the experimental results of the designed sensor with the standard fluorescence spectrometer. The use of relatively less chemical for the present study makes it a user convenient method. The chlorophyll measurement of field-collected tea leaves agrees well with the commercial chlorophyll meter.

Table 6.1: Performance comparison of the proposed smartphone chlorophyll meter with the commercial counterpart and the previously reported work.

Specifications	**SPAD 502 meter**	**SmartFluo**	**Proposed smartphone chlorophyll meter**	**Remarks**
Hardware	It consists of two LEDs, one photodiode, LCD panel, memory chip, analog to digital converter, Bluetooth interface and external battery.	It consists of external LED, voltage circuit, cuvette, transparency filters, mirror, battery, 3D printed holder, smartphone.	It consists of one LED, two lenses, optical filter, sample holder, and smartphone	In the proposed setup the smartphone battery is used to power the LED. No external power supply and electronic sensor is required. Hence less component is needed for the development of the proposed sensor.
Detector	Photodiode (measures optical density difference at two specific wavelengths)	CMOS sensor of smartphone (captures the fluorescence images of the chlorophyll sample)	ALS of smartphone (measures the emitted fluorescence intensity from the sample)	ALS of smartphone (measures the emitted fluorescence intensity from the sample)
LoD	Unknown	10 μg/L	0.0618 mg/g	The proposed sensor is highly sensitive.
Operational range	-9.9 to 199.9 SPAD units	1-50μg/L	1-12 mg/g	The proposed sensor has a wide range of tea chlorophyll estimation. This range can be tuned according to the plant sample.
Cost	$2542	$73	$70	The designed sensor is cost-effective.

With such significant correlation, the chlorophyll estimation can be done more reliably and accurately within the phone itself. It is envisioned that the proposed smartphone-based sensing system might be an innovative and cost-effective way to produce low-cost chlorophyll meters for better crop yield, which is urgently required in the agriculture sector.

References

[1] Kalaji, H. M., Schansker, G., Brestic, M., Bussotti, F., Calatayud, A., Ferroni, L., Goltsev, V., Guidi, L., Jajoo, A., Li, P., et al. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynthesis Research*, 132 (1):13–66, 2017.

[2] Muñoz-Huerta, R. F., Guevara-Gonzalez, R. G., Contreras-Medina, L. M., Torres-Pacheco, I., Prado-Olivarez, J., and Ocampo-Velazquez, R. V. A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. *sensors*, 13(8):10823–10843, 2013.

[3] Intaravanne, Y. and Sumriddetchkajorn, S. Android-based rice leaf color analyzer for estimating the needed amount of nitrogen fertilizer. *Computers and Electronics in Agriculture*, 116:228–233, 2015.

[4] Martín-Tornero, E., Martín-Merás, I. D., Mansilla, A. E., Lopes, J. A., and de Jorge Páscoa, R. N. M. Geographical discrimination of grapevine leaves using fibre optic fluorescence data and chemometrics. determination of total polyphenols and chlorophylls along different vegetative stages. *Microchemical Journal*, page 107647, 2022.

[5] Guanter, L., Zhang, Y., Jung, M., Joiner, J., Voigt, M., Berry, J. A., Frankenberg, C., Huete, A. R., Zarco-Tejada, P., Lee, J.-E., et al. Global and time-resolved monitoring of crop photosynthesis with chlorophyll fluorescence. *Proceedings of the National Academy of Sciences*, 111(14):E1327–E1333, 2014.

[6] Peng, S., Garcia, F., Laza, R., Sanico, A., Visperas, R., and Cassman, K. Increased n-use efficiency using a chlorophyll meter on high-yielding irrigated rice. *Field Crops Research*, 47(2-3):243–252, 1996.

[7] Marinho, O. R., Lima, M. J., Rocha, F. R., Reis, B. F., and Kamogawa, M. Y. A greener, fast, and cost-effective smartphone-based digital image procedure for quantification of ethanol in distilled beverages. *Microchemical Journal*, 147: 437–443, 2019.

[8] Aminot, A. and Rey, F. Standard procedure for the determination of chlorophyll a by spectroscopic methods. *International Council for the Exploration of the Sea*, 112:25, 2000.

[9] Chang, S. X. and Robison, D. J. Nondestructive and rapid estimation of hardwood foliar nitrogen status using the spad-502 chlorophyll meter. *Forest Ecology and Management*, 181(3):331–338, 2003.

[10] Pagola, M., Ortiz, R., Irigoyen, I., Bustince, H., Barrenechea, E., Aparicio-Tejo, P., Lamsfus, C., and Lasa, B. New method to assess barley nitrogen nutrition status based on image colour analysis: comparison with spad-502. *Computers and electronics in agriculture*, 65(2):213–218, 2009.

[11] Hu, H., Zhang, G., and Zheng, K. Modeling leaf image, chlorophyll fluorescence, reflectance from spad readings. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, 7(11):4368–4373, 2014.

[12] Maxwell, K. and Johnson, G. N. Chlorophyll fluorescence—a practical guide. *Journal of experimental botany*, 51(345):659–668, 2000.

[13] Mackinney, G. Absorption of light by chlorophyll solutions. *Journal of biological chemistry*, 140(2):315–322, 1941.

[14] Vesali, F., Omid, M., Kaleita, A., and Mobli, H. Development of an android app to estimate chlorophyll content of corn leaves based on contact imaging. *Computers and Electronics in Agriculture*, 116:211–220, 2015.

[15] Li, Z., Paul, R., Ba Tis, T., Saville, A. C., Hansel, J. C., Yu, T., Ristaino, J. B., and Wei, Q. Non-invasive plant disease diagnostics enabled by smartphone-based fingerprinting of leaf volatiles. *Nature plants*, 5(8):856–866, 2019.

[16] Petrie, P. R., Wang, Y., Liu, S., Lam, S., Whitty, M. A., and Skewes, M. A. The accuracy and utility of a low cost thermal camera and smartphone-based system to assess grapevine water status. *biosystems engineering*, 179:126–139, 2019.

[17] Adhikari, R., Li, C., Kalbaugh, K., and Nemali, K. A low-cost smartphone controlled sensor based on image analysis for estimating whole-plant tissue nitrogen (n) content in floriculture crops. *Computers and electronics in agriculture*, 169: 105173, 2020.

[18] Tao, M., Ma, X., Huang, X., Liu, C., Deng, R., Liang, K., and Qi, L. Smartphone-based detection of leaf color levels in rice plants. *Computers and electronics in agriculture*, 173:105431, 2020.

[19] Cortazar, B., Koydemir, H. C., Tseng, D., Feng, S., and Ozcan, A. Quantification of plant chlorophyll content using google glass. *Lab on a Chip*, 15(7):1708–1716, 2015.

[20] Gaviria-Palacio, D., Guáqueta-Restrepo, J. J., Pineda-Tobón, D. M., and Pérez, J. C. Fast estimation of chlorophyll content on plant leaves using the light sensor of a smartphone. *Dyna*, 84(203):234–239, 2017.

[21] Meroni, M. and Colombo, R. Leaf level detection of solar induced chlorophyll fluorescence by means of a subnanometer resolution spectroradiometer. *Remote Sensing of Environment*, 103(4):438–448, 2006.

[22] Buschmann, C., Langsdorf, G., and Lichtenthaler, H. Imaging of the blue, green, and red fluorescence emission of plants: an overview. *Photosynthetica*, 38(4): 483–491, 2000.

[23] Ošt'ádalová, M., Tremlová, B., Pokorná, J., Král, M., et al. Chlorophyll as an indicator of green tea quality. *Acta Veterinaria Brno*, 83(10):103–109, 2015.

[24] Wellburn, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of plant physiology*, 144(3):307–313, 1994.

[25] Ignat, T., Schmilovitch, Z., Feföldi, J., Bernstein, N., Steiner, B., Egozi, H., and Hoffman, A. Nonlinear methods for estimation of maturity stage, total chlorophyll, and carotenoid content in intact bell peppers. *Biosystems engineering*, 114(4):414–425, 2013.

[26] Lamb, J. J., Eaton-Rye, J. J., and Hohmann-Marriott, M. F. An led-based fluorometer for chlorophyll quantification in the laboratory and in the field. *Photosynthesis research*, 114(1):59–68, 2012.

[27] Hussain, I. and Bowden, A. K. Smartphone-based optical spectroscopic platforms for biomedical applications: a review. *Biomedical Optics Express*, 12(4): 1974–1998, 2021.

[28] Nakra, B. and Chaudhry, K. *Instrumentation, measurement and analysis*. Tata McGraw-Hill Education, 2003.

[29] Armbruster, D. A. and Pry, T. Limit of blank, limit of detection and limit of quantitation. *The clinical biochemist reviews*, 29(Suppl 1):S49, 2008.

[30] Guideline, I. H. T. et al. Validation of analytical procedures: text and methodology. *Q2 (R1)*, 1(20):05, 2005.

[31] Hussain, I., Ahamad, K. U., and Nath, P. Low-cost, robust, and field portable smartphone platform photometric sensor for fluoride level detection in drinking water. *Analytical chemistry*, 89(1):767–775, 2017.

[32] St, L., Wold, S., et al. Analysis of variance (anova). *Chemometrics and intelligent laboratory systems*, 6(4):259–272, 1989.

[33] Friedrichs, A., Busch, J. A., Van der Woerd, H. J., and Zielinski, O. Smartfluo: A method and affordable adapter to measure chlorophyll a fluorescence with smartphones. *Sensors*, 17(4):678, 2017.