Colour Metrology for Colourimetric Holographic Biosensor Application

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Abstract

A novel methodology is developed to identify colour information of the holographic pH sensor using modern smartphones by leveraging its integrated LED light source and CCD camera. This study addressed the difference in colour appearance of the same sample inspected with different devices. The colour metrology solution includes standardizing the lighting and perceiving condition, calibrating reference colour standards under standard colour measurement conditions, and calibrating the holographic sensor's colour appearance taken by smartphone device by using calibrated reference colour standards. Results after calibration demonstrated a significant improvement in consistency, differences in HUE of biosensor captured by different smartphones can be improved by up to 50%. This confirms that the smartphone, as a measuring tool, coupled with this metrology solution is able to accurately and reliably measure the colour of holographic biosensors, offering a more consistent colourimetric analysis.

Keywords: colour metrology, HUE, holographic biosensor

1. Introduction

Holographic pH sensors are colourimetric sensors that change colour based on analyte concentration selectively and non-consumptively. As a non-contact sensor, it is useful for providing reliable, real-time and noninvasive monitoring of the analyte, such as pH, lactate, and glucose. It quantifies the analytes by reflecting light in certain wavelengths which depends on the concentration of the measurement target. Changes in pH lead to the expansion or contraction of the gel matrix, resulting in a change in gel volume, which correspondingly shifts the fringe distance between each adjacent layer at different pH concentrations. Due to Bragg's condition, this, in turn, affects the resultant diffraction wavelength of incident white light [1]. As such, the colour observed will change accordingly. However, accurately determining the sensor's colour remains an obstacle to precisely quantifying the characteristics of the analyte solution.

Well-developed smartphones are a highly prevalent type of device that can be utilized for colour recognition. A smartphone has an integrated LED light source and various cameras, providing an option to capture pictures with high actability and availability regardless of location and time. Leveraging on the components readily available in a smartphone, it is a suitable tool that comprises both a light source and measurement tools for the holographic biosensor

colour response. Colourimetric analysis using smartphones is not uncommon. It has been performed on biochemical liquid analytes and solid samples in recent years with the aid of RGB extraction applications, proposing that using smartphones for colourimetry is highly feasible and reliable [2][3]. In 2022, Cebrián et al. also discussed colour correction methods for digital pictures based on a linear fit using least squares [4]. Calibration results confirmed that this proposed correction method is suitable for colour correction across different devices, significantly reducing variance between devices.

However, two key issues arise when using smartphones as the colour capture and recognition tool in our context, namely experimental condition dependence (angle, lighting) and device dependence. Khalili et al. reported a smartphone-based colour quantification algorithm [5] in order to standardise and improve the determination of holographic pH sensor similar to our application. They deployed an angled setup, and used reference colour patches and gray scale to characterize the individual camera response. They converted RGB to CIEL*a*b* to conclude the colours. Different to [5], we have proposed a co-axis measurement scheme to tackle experimental angle condition dependence in the Bragg grating structure based holographic sensor, and a image colour RGB to HUE calibration method to tackle device dependence. Hence, in this paper, we aim to discuss how the co-axis measurement and RGB to HUE calibration methods were used to reduce the impact of the dependencies for colour recognition results.

2. Method

2.1 Co-axis Measurement Scheme

Given that the colour reflected by the holographic biosensor is wavelength sensitive due to its layered structure that reflects light at wavelength matching with the Bragg condition (Figure 1), observing from different angles will result in a difference in wavelength and thereby colour. Hence, a co-axis measurement scheme is applied to minimise the effect on results due to the observation angle. This technology improves the consistency of measurement data by reducing the effects of angle dependency.

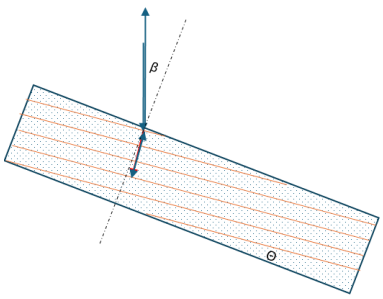

Figure 1: Fabricated Bragg grating structure in the gel

The Ag layers are not parallel or perpendicular to the sides of the gel. This separates the specular reflection of the surface of the gel from the biosensor Bragg's reflection. Θ is the angle of the fabricated Bragg pattern in the gel. The light incidents into the gel surface with a tilt angle of β. Under idea co-axis measurement, the light enters the gel and interacts with the Bragg grating at a normal angle. The reflected light will exit the gel at the same angle (β) as the incident light. The tilt angle β is determined at $\sin(\beta) = n \sin(\Theta)$ where n is the refractive index of the gel. In our experimental setup, n is 1.5, Θ is 6.6º, and β is 10º.

In the actual co-axis measurement setup, the smartphone is positioned directly about 40cm above the biosensor, making use of the close proximity of the integrated LED light and camera. Given that they are integrated side by side with less than 2 cm distance in between, the slight angle between incident and reflected light (less than 3º) can be neglected as it would cause a minimal variation on the biosensor measurement. Figure 2 shows the co-axis measurement setup configuration. The figure is not to scale. The biosensor gel has a diameter of approximately 1 mm and a thickness of 30 µm. Given 40 cm distance, the divergence angle of the light reaching the biosensor gel is less than 0.15°, allowing the incident light to be reasonably approximated as parallel when illuminating the gel. Under this set-up, the reflected light will now be approximately at the same angle as the incident light, hence, the observation and light source are almost co-axis to the biosensor. By using this setup, the consistency in lighting and observation angle will be ensured, causing minimum difference in the measurement configuration even with different smart phones. Pictures are captured using only the smart phone LED flash to minimize ambient light interference.

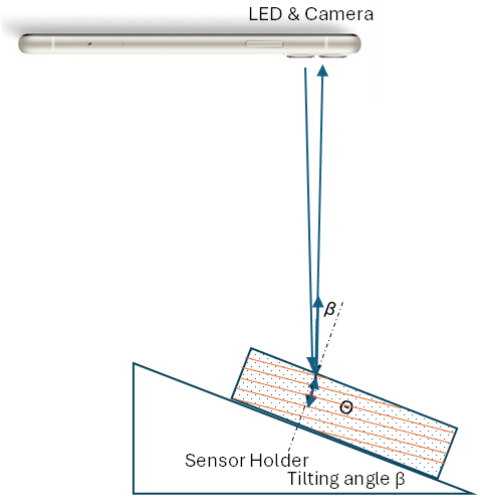

Figure 2: Co-axis measurement setup

2.2 Colour Calibration for picture taken by smartphone device

Colour can be quantified in various types of colour space such as CIE-L*a*b, CIE-xyz, RGB, HSV, and so on. The biosensor has a grating-based structure, which means the sensor has a very selective wavelength reflection characteristic. The reflection wavelength shift due to pH value change will cause colour change of the biosensor. Considering the characteristics of the biosensor, namely wavelength selectivity, using HUE as the indicator of colour appearance analysis appears more suitable, as it is independent of saturation and brightness and solely depends on the wavelength of reflection.

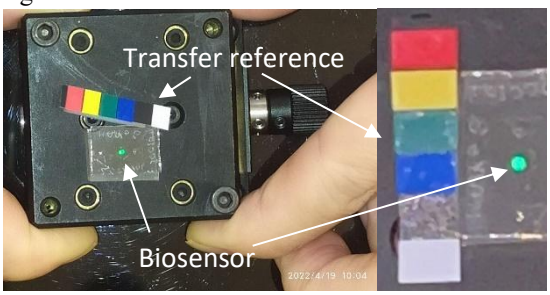

Figure 3: Photos of holographic biosensors taken by 2 different models of smartphones

Pictures are then captured with LED light as the only light source, obtaining sets of pictures when the biosensor is immersed in a solution of different pH values, appearing in different colours respectively. However, the light source and camera sensors of each smartphone are highly unlikely to be identical. The reference colour block is used and placed right beside the biosensor gel, taken together with the biosensor in the same picture (Figure 3), and provides reference colour value. These pictures will then go through calibration process.

Since a co-axis scheme is applied when taking pictures of the holographic biosensor, the smartphone will be located directly above the biosensor, different from the standard colour measurement geometry conducted previously to identify the device differences. Hence, some adaptations have to be made to ensure that the colour reference can be utilised consistently.

2.3 HUE under different angle

Since co-axis measurment is a condition different from standard measuremend condition, we need to study the HUE difference between the standard colour measurement condition and the co-axis condition. The SpyderCheckr reference was measured at standard colour measurement condition by using spectrophotomer. Its HUE was calculated to be 354.2. We then measured the SpyderCheckr reference under co-axis setup (Figure 2) with different tilting angles to determine the variation caused by the angles. In this setup, we used a D50 white light source and a smartphone camera. The tilting angle of the SpyderCheckr reference is gradually increased from 0° to 55°. The purpose is to determine the angle at which the reference shown in the picture will have the most stable HUE values, and the most stable position for the reference block beside the biosensor.

Figure 4 shows how the angles affect the HUE value of SpyderCheckr red reference. We found that the variation of HUE is less than 1 when the tilting angle is between 10° to 55°. The variation of HUE value of the reference is minimum when positioned at an angle of 30°. SpyderCheckr yellow and green references show the similar characteristic across tilting angle. There is a deviation of HUE from the pictures taken under co-axis condition from the HUE by spectrophotometer under standard colour measurement condition.

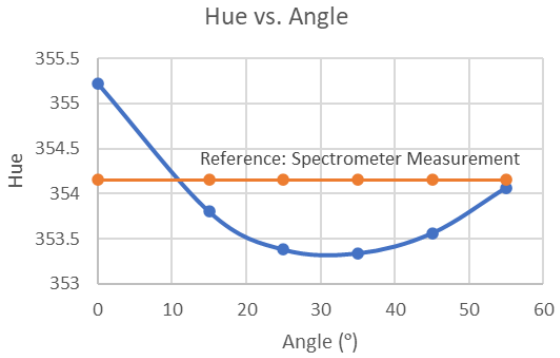

Figure 4. Change of HUE over the change of angle

Hence, we concluded that measurement by smartphone under co-axis condition with 30° tilting angle provides a stable HUE value. HUE values from spectrophotometer calibration can be used as the reference value for calibration process under co-axis condition. This deviation will be accounted for the calibration of the biosensor by using the references.

2.4 Transfer reference for biosensor

As the holographic biosensor is less than 1 mm in diameter, SpyderCheckr colour reference is too big to be put beside it. A small transfer reference is used beside the biosensor for the picture taking (Fig. 3). This reference is too small to be placed into the spectrophotometer for measurement. Hence, we will use the SpyderCheckr colour reference which is calibrated by the spectrophotometer and reflection standard to calibrate the transfer reference and then the transfer reference to calibrate the biosensor.

2.5 Verification of colour calibration method for pictures

In order to test the feasibility of calibrating the transfer reference with the SpyderCheckr, we have done tests with coloured paper, which can be verified and measured using a spectrophotometer and reflection standard. Coloured papers and SpyderCheckr reference are placed under a set-up in

reference to the standard colour measurement geometry [6]. A D50 white light is used as a light source located directly above the sample. The phone is levelled using the built-in application 'Compass'. The phone is then tilted to 45° using the leveller in Compass to capture pictures.

The camera sensor difference is tested by taking pictures of various samples under the same lighting conditions. Photos of paper sample and SpyderCheckr reference taken by 3 different models of smartphones under the standard colour measurement geometry are shown in Figure 5. Calibration for the colour of SpyderCheckr reference in the picture is by adjusting the RGB values of the SpyderCheckr reference in the picture according to their respective value measured with spectrophotometer and reflection standard, by multiplying R, G and B values with 3 factors respectively. These 3 factors are then multiplied to calibrate the R, G and B values of the colour paper sample, respectively. Calibrated HUE value for the colour paper is obtained from its calibrated RGB values. If the calibrated HUE value of the colour paper sample has equivalent result with its HUE value calculated from measured RGB with spectrophotometer and reflection standard, it proves that the colour calibration method for pictures can yield an accurate and reliable result. Hence, an accurate and consistent measurement of the biosensor can be determined by using the calibrated transfer reference.

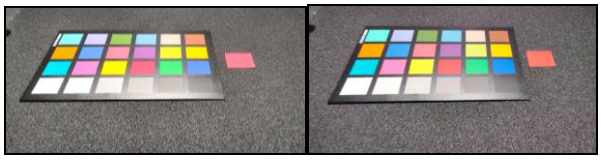

Figure 5: Photos of paper sample together with SpyderCheckr reference taken by different smartphones under the standard colour measurement geometry.

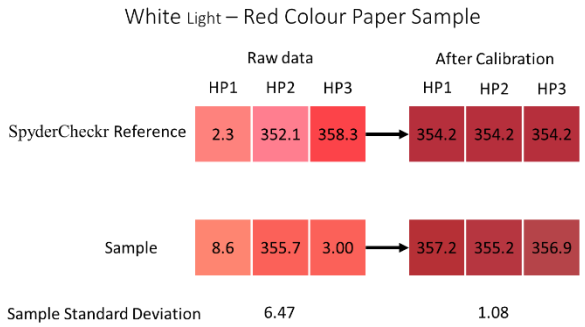

Figure 6: Effect before and after calibration of red paper sample with SpyderCheckr reference.

In the verification, triplicate measurements were carried out for each sample with the same smart phone. The spread for the triplicate measurement is insignificant compared with the differences between that of the different smart phones. Average values were calculated for each sample with each smart phone, with captured red values depicted in Fig 6. The HUE value of the red, yellow and green coloured paper sample measured by spectrophotometer and reflection standard is 356.92, 46.46 and 112.79 respectively. Figure 6 shows the effect before and after calibration for the red colour paper sample by using colour calibration for pictures. The original HUE values of the SpyderCheckr red reference taken by 3 different smartphone devices are 2.3, 352.1, 358.3, respectively. The original HUE values of the red colour paper taken by 3 different handphone devices are 8.6, 355.7, 3.00, respectively. The HUE value of the SpyderCheckr red reference and red coloured paper sample from measurement by spectrophotometer is 354.2 and 356.92, respectively. Table 1 shows the standard deviation and absolute deviation summary for the 3 tested colours, green, red and yellow. Both standard deviation of the HUE values of the same colour paper sample by different devices and absolute deviation of the average HUE by 3 handphone devices from the HUE by spectrophotometer measurement are improved significantly after the calibration. The results showed accuracy of the colour of the paper sample taken by smartphone devices can be significantly enhanced by using SpyderCheckr reference.

Table 1: Verification of HUE calibration for coloured paper

Sample	Standard deviation of HUE captured by 3 smartphone devices		Absolute difference between average HUE calculated from images and calibrated value from spectrophotometer measurement	
	Before calibration	After calibration	Before calibration	After calibration
Red paper	6.47	1.08	5.49	0.47
Yellow paper	2.41	1.34	11.22	3.79
Green paper	12.78	5.51	19.93	10.02

The uncertainty in this calibration comes from the uncertainty of the SpyderCheckr reference reflectance spectrum measurement and the uncertainty in converting the spectrum to HUE. The SpyderCheckr reflectance spectrum measurement in our setup has an expanded uncertainty of 0.5% estimated at a level of confidence of approximately 95 % with a coverage factor $k = 2$. This uncertainty reflectance spectrum would convert to less than 0.00012 standard uncertainty in colour x and y according to uncertainty estimation in colour measurement [7]. The 0.00012 standard uncertainty in x and y would convert to less than 1 uncertainty in R, G and B. Then RGB of SpyderCheckr reference from reflectance spectrum measurement will be applied to adjust the RGB of SpyderCheckr reference in pictures taken by handphone devices and the same factors to RGB of colour paper in pictures. As the value of RGB from pictures has

resolution of 1, we calculated HUE values based on RGB values with ±1 in each component, and found that the ±1 uncertainty in R, G and B after calibration would cause 0.7, 0.8 and 1.5 standard uncertainty in HUE for red, yellow and green colour paper, respectively. Hence, the expanded uncertainty in HUE would be 1.4, 1.6 and 3, for red, yellow and green colour paper, respectively. Similarly, the uncertainty of the reflectance spectrum measurement and resolution in RGB for the colour paper would also cause the expanded uncertainty in HUE to be 1.4, 1,6 and 3, for red, yellow and green colour paper, respectively.

The equivalence of two independent measurement results is compared by computing the Normalised Error ratio (E_N ratio), where $E_N = (L_1 - L_2)/\sqrt{{U_1}^2 + {U_1}^2}$, where L_1 and L_2 are two independent measurement results, U_1 and U_2 are the expanded uncertainty associated with the two independent measurements. If the results are equivalent, the E_N ratio should be within the range ±1, on approximately 95 % of occasions.

Table 2 shows the E_N ratio of the verification of red, yellow and green paper colours by handphone devices and spectrophotometer. E_N ratio check shows that the HUE values of red colour sample taken by the 3 handphone devices after calibration by the SpyderCheckr reference agree to its HUE value directly calibrated by spectrophotometer within the measurement uncertainty. However, E_N ratios for yellow and green colour samples exceed ±1, indicating the uncertainty in the colour calibration by the SpyderCheckr reference for the pictures taken by handphone devices is underestimated.

Table 2: E_N ratio of the verification

	Red	Yellow	Green
HP1	0.14	1.25	-2.66
HP2	-0.87	1.43	-3.49
HP3	-0.01	2.36	-0.94

2.6 Analysis of residue error in calibration

The colour calibration for red samples in the pictures always has least deviation in HUE after calibration than yellow and green. We suspect that due to the difference in reflectance spectra of the different colours, a simple RGB ratio calibration can't fully compensate the error caused by the difference in spectral response of handphone devices, especially for yellow and green colours. We ran a simulation to study this phenomenon. A set of deviation factors were added to the CIE colour-matching function to simulate the sensor difference across smartphones. The factors were multiplied to the x, y and z functions, respectively. The same D50 illuminant and colour paper reflectance spectrum were used to determine the respective XYZ values by multiplying the various adjusted colour-matching functions.

Table 3 shows the simulated absolute differences due to camera sensor variation with arbitrary error factors of 0.9, 0.95 and 1.1 for XYZ, respectively. It shows that HUE value of red paper sample is the least affected with error in the CIE colour-matching function, and green is the most affected. This explains why the calibrated value of red-coloured samples has the smallest standard deviation in experiments and the smallest E_N ratio in the verification.

Table 3: Colour paper HUE values with and without arbitrary error factors for camera sensor.

HUE	Without error factors	With error factors	Absolute Difference in HUE
Red	356.9	356.4	0.5
Yellow	46.5	51.7	5.2
Green	112.8	132.6	19.8

Table 4: Biosensor HUE values with and without arbitrary error factors for camera sensor.

HUE	Without error factors	With error factors	Absolute Difference in HUE
Red	0	0	0
Yellow	42.6	46.1	3.5
Green	108.3	120.0	11.7

We also studied the effect of camera sensor difference on biosensor colour response measurement. A typical biosensor reflectance spectrum was used in the calculation. It was found that with the same error factors applied to the camera sensors, the absolute difference of HUE induced at the biosensor (Table 4) is much smaller than colour paper sample (Table 3). This is because of different mechanism of colour generation in colour paper and the biosensor. The colour of the colour paper arises from the selective absorption of specific wavelengths of light by pigments or dyes in the material. The remaining, unabsorbed wavelengths are transmitted or reflected, and these wavelengths determine the perceived colour. Hence the reflectance spectrum of the colour paper in our simulation is broadband. However, the biosensor has a narrow band reflection spectrum due to its Bragg grating structure. When multiplying the reflection spectrum with x, y, z functions with errors, the colour paper with broadband reflection spectrum would induce larger variations. Therefore, we expect that colour calibration for biosensors would generate better result than colour calibration for tested colour papers.

With the above verification and analysis, we conclude that the colour calibration method for pictures is applicable to red

samples and we expect that the red transfer reference can be calibrated by SpyderCheckr reference and be used to calibrate the biosensor.

3. Biosensor Experimental Results

Firstly, we performed the same calibration for the transfer reference with SpyderCheckr colour board. Co-axis measurement scheme is used to take pictures of biosensors by integrated LED light source and CCD camera in smartphones. The HUE value of the small reference is identified from its RGB values calibrated by SpyderCheckr RGB values from spectrometer measurement.

Images of the biosensor alongside small references (Figure 3) were then captured with the reference positioned at a 30° angle from the horizontal. As the biosensor has a narrow band reflectance spectrum, HUE values are directly used in calibration process as it is a better representation of the wavelength-selective biosensor. We obtain the original values of transfer reference and biosensor from the pictures. A linear HUE shift method is applied here to adjust the transfer reference HUE to the calibrated HUE in the previous step. Then the same shift is applied to the HUE value of biosensors from the image to obtain the calibrated of the biosensor. As HUE colour wheel has a circular nature, 360 is equivalent to 0 in the process.

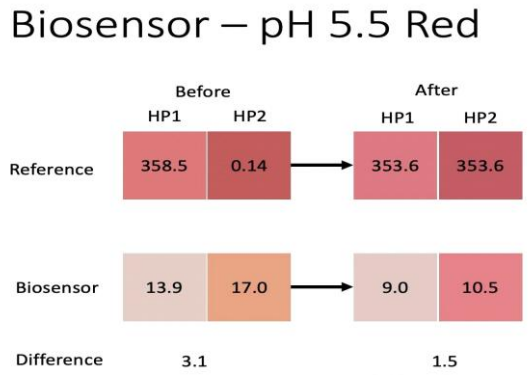

Figure 7: Effect before and after calibration of biosensor with SpyderCheckr reference.

The experiments were carried for the biosensor being immersed in solutions of pH 5.5 to appear red colour. Pictures were taken by 3 different smartphones. However, due to the limit of optical zoom capability and image quality for the tiny biosensor, only pictures by 2 smartphones were processed. Figure 7 shows the colours (HUE) of biosensor taken by the 2 smartphones before and after calibration. The colour difference in HUE taken by different handphones after calibration is decreased from 3.1 to 1.5 for red. This results have shown at least significant improvement in accuracy and consistency of HUE identification by using different handphone devices. The standard uncertainty in transfer reference red calibration was estimated to be 0.7. The resolution of RGB in the picture of biosensor and transfer standard will cause another standard uncertainty of 0.7. We would expect the combined uncertainty in HUE calibration for the biosensor to be 1, and the expanded uncertainty to be 2, estimated at a level of confidence of approximately 95 % with a coverage factor $k = 2$. The confidence level refers to the probability that the true value of the measurand lies within the stated uncertainty interval. A coverage factor of 2 corresponds to a confidence level of approximately 95%, assuming a normal distribution and a coverage interval of ±2 standard deviations (2σ). This analysis follows the Guide to the expression of uncertainty in measurement [8].

4. Discussion and Conclusion

In this study, we explored the application of modern smartphones on holographic pH sensors to achieve accurate colourimetric analysis for real-time, non-invasive monitoring of analytes such as pH, lactate and glucose. We validated the method's effectiveness for colourimetric holographic biosensors in scenarios displaying red colour. The results highlight the potential of using smartphones as versatile tools for colour recognition, leveraging the integrated LED light sources and CCD cameras to capture and analyse colour changes in biosensors. The co-axis measurement scheme adopted significantly reduced angle-dependent variations, and the colour metrology solution demonstrated a large improvement in colour consistency, reducing device-dependent variations. These approaches addressed the key issues and established a reliable method for enhancing the consistency and accuracy of quantifying the analyte concentration based on colourimetric responses.

Future work will focus on improving the measurement methodologies to ensure broader applicability and higher precision in colourimetric analytical and monitoring contexts. In summary, the application of smartphones for holographic pH sensors colour detection with color metrology solutions offers a promising avenue for portable, accurate and efficient analyte monitoring.

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