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Review Paper

Spectrophotometry vs Digital Image Colorimetry: Analytical Challenges, Standardization, and Emerging Intelligent Frameworks

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Abstract
Colorimetric analysis provides a rapid and cost-effective approach for monitoring chemical reactions and determining analyte concentrations. This review examines the strengths and limitations of conventional spectrophotometric methods, known for high precision but high cost and low portability, against digital image colorimetry (DIC). DIC, utilizing smartphones and other portable imaging devices, offers advantages in portability, low operational cost, and alignment with Green Analytical Chemistry principles. However, its analytical performance is often influenced by illumination variability, differences between imaging devices, color-space selection, and calibration strategies. This paper reviews recent developments in DIC, including hardware platforms, color-space transformations, and advanced data processing approaches used to improve analytical performance. Furthermore, to evaluate analytical reliability, this review introduces a novel fuzzy logic-based qualitative reasoning framework. Using a Mamdani-type inference system, the model evaluates the complex interactions between four key variables: lighting stability, device consistency, color-space robustness, and calibration strength. The framework demonstrates that DIC can achieve a "Good" analytical reliability score when lighting is moderately controlled, color-space selection is robust, and calibration strategies are sufficiently strong. However, uncontrolled device variability remains a limiting factor preventing systems from reaching an "Excellent" reliability category, highlighting the ongoing need for methodological standardization in digital colorimetric technologies.

Keywords
Digital Image Colorimetry, Spectrophotometry, Smartphone-Based Sensing, Color Space Analysis, Green Analytical Chemistry, Analytical Reliability

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1. INTRODUCTION

Colorimetric analysis remains a cornerstone of analytical chemistry because of its low operational cost, rapid readout, and straightforward visual interpretation, making it particularly suitable for reaction monitoring, rapid screening, and field applications (Fan et al., 2021a; Mazur et al., 2024; Ko and Liao, 2023; Kiwfo et al., 2024; Eksperiandova et al., 2016; Kawamura et al., 2021). Over recent years, advances in imaging hardware, including smartphone CMOS sensors, flatbed scanners, WiFi scanner-assisted paper platforms, and RGBC modules on microcontroller-based systems, together with computational methods such as color-space transformations, image segmentation, nonlinearity correction, and machine learning, have driven the emergence of digital image colorimetry (DIC) as a practical alternative to conventional laboratory spectrophotometry, especially where portability, accessibility, and the principles of Green Analytical Chemistry are prioritized (Soares et al., 2023; Antela et al., 2023; Woolf et al., 2021; Wang et al., 2023; Hou et al., 2021; Joh et al., 2025).

Operationally, DIC converts the spectrally integrated signal captured by imaging devices into quantitative analytical readouts by extracting color channels and transforming them into more stable color representations (e.g., HSV and CIELAB) prior to calibration. The choice of color space and preprocessing steps strongly influences the linearity, sensitivity, and accuracy of the resulting concentration estimates (Phuangsaijai et al., 2021; Tiuftiakov et al., 2021; Woolf et al., 2021; Kwiek and Jakubowska, 2024; Joh et al., 2025). Key technical challenges that distinguish DIC from spectrophotometry include illumination variability, inter-device sensor response differences, signal nonlinearity introduced by camera gamma correction and scattering on solid substrates such as μPADs, as well as dependence on feature-extraction and image-processing strategies (Pires et al., 2024; Baker et al., 2025; Azhar et al., 2023; Zeng et al., 2021; Nuanjan et al., 2024).

A range of complementary mitigation strategies has been developed and validated in the literature. First, optical control using enclosed housings (dark boxes), spectrally stable LED illumination, and internal color reference cards can reduce illumination artifacts and improve measurement repeatability (Baker et al., 2025; Pires et al., 2024; Azhar et al., 2023; Zeng et al., 2021; Nguyen et al., 2025). Second, color space selection, for example, the use of Hue in HSV for assays dominated by hue shifts or a*/b* coordinates in CIELAB for perceptually uniform quantification, often yields more linear relationships with analyte concentration than raw RGB channels (Phuangsaijai et al., 2021; Tiuftiakov et al., 2021; Fay and Wu, 2024; Woolf et al., 2021; Kwiek and Jakubowska, 2024). Third, advanced calibration strategies, ranging from simple mathematical transforms such as $1/R$ and $\log(R_0/R)$ and polynomial fitting to data-driven models based on neural networks, support vector regression, and ensemble methods, can model sensor nonlinearities and inter-channel correlations to improve quantitative accuracy (Ji et al., 2020; Sáez-Hernández et al., 2025a; Joh et al., 2025; García-Miralles et al., 2024; Doğan et al., 2022). In addition, video-based acquisition and frame aggregation have been shown to stabilize kinetic signals and reduce transient noise arising from momentary illumination fluctuations (Bhatt et al., 2024; Woolf et al., 2021).

Material and platform innovations further extend DIC capabilities. Functional materials such as nanozymes, gold nanoparticles, and metal–organic frameworks (MOFs) incorporated into μPADs or colorimetric sensor arrays (CSAs) enhance signal intensity and selectivity, enabling detection limits approaching those of laboratory instruments for selected analytes while maintaining low reagent consumption (Martínez-Pérez-Cejuela et al., 2023; Liu et al., 2025; Pongkitdachoti and Unob, 2022; Velasco et al., 2025). For complex sample classification and multiplexed sensing, CSAs combined with chemometric methods (PCA, HCA) and machine learning classifiers produce characteristic color "fingerprints" that improve discrimination and adulteration detection relative to single-sensor approaches (Lyu et al., 2021; Dinmeung et al., 2023; Gomes et al., 2022; Liu et al., 2023; Liang et al., 2025). Studies published in Science and Technology Indonesia further demonstrate locally relevant implementations such as MOF-based colorimetric sensing and μPAD–smartphone integrations for biomarker detection, underscoring the method's applicability in regional contexts (Firdaus et al., 2023a; Sabarudin et al., 2025; Markus et al., 2023; Nath et al., 2025).

Despite these advances, the lack of methodological standardization remains a major barrier to broader adoption. The absence of consensus protocols for reporting acquisition conditions -such as device model, illumination spectrum, and imaging geometry-along with limited agreement on cross-device performance metrics (e.g., limits of detection, linear range, and robustness to environmental variation) and the lack of standardized calibration datasets, continues to impede reproducibility and interlaboratory validation (Baker et al., 2025; Woolf et al., 2021; Azhar et al., 2023; Zeng et al., 2021; Nuanjan et al., 2024). Furthermore, variations in imaging hardware and uncontrolled environmental conditions introduce additional uncertainty, reinforcing the need for harmonized validation frameworks (Nguyen et al., 2025; García-Miralles et al., 2024; Kiwfo et al., 2024). Addressing these gaps requires coordinated community efforts to define reporting standards, establish shared cross-device calibration repositories, and conduct multi-site validation studies that systematically benchmark DIC performance against reference spectrophotometric methods (Ko and Liao, 2023; Wang et al., 2023).

This review synthesizes recent developments in (i) acquisition platforms (smartphones, scanners, microcontrollers), (ii) sensor and μPAD/CSA design, (iii) data-processing strategies (color-space selection, nonlinearity correction, machine learning), and (iv) recommendations for methodological standardization. By highlighting practical guidance and recent evidence from both international and regional publications, this article aims to support researchers and practitioners in designing reproducible, device-agnostic DIC workflows suitable for food safety, environmental monitoring, and point-of-care testing.

2. PERFORMANCE COMPARISON: SPECTROPHOTOMETRY VS. DIGITAL COLORIMETRY

Historically, spectrophotometry has been regarded as the "gold standard" analytical technique because of its high accuracy, precision, and reproducibility, supported by well-established measurement protocols (Resende et al., 2023; García-Miralles et al., 2024; Woolf et al., 2021). In spectrophotometric instruments, a monochromator is used to isolate a specific wavelength of light, enabling precise measurement of absorbance and providing high sensitivity and selectivity for quantitative analysis (Wang et al., 2023; Joh et al., 2025).

Recent studies indicate that properly calibrated digital colorimetric systems can achieve comparable analytical performance in many applications. Wongthanyakram et al. (2023) compared portable analytical instruments and reported that, for rapid testing and screening purposes, the results were comparable to those obtained using conventional laboratory methods. Similar conclusions have been reported in other quantitative analytical applications, particularly in food and pharmaceutical analysis, where digital image analysis provided valid and more cost-effective results (Resende et al., 2023; Botelho et al., 2022; Mermer et al., 2022; Markus et al., 2023; Doğan et al., 2022). Recent neural network approaches enable digital cameras to reconstruct spectra with <2% error compared to spectrophotometers (García-Miralles et al., 2024; Joh et al., 2025; Wang et al., 2023), while smart devices enhance water monitoring (Meng et al., 2024; Zhang et al., 2024; Zeng et al., 2021; Nguyen et al., 2025).

Nevertheless, several studies also highlight important limitations of digital imaging approaches. In particular, digital colorimetric analysis may perform poorly under uncontrolled experimental conditions, such as low illumination or when device standardization is not possible (Fay and Wu, 2024; Baker et al., 2025; Zeng et al., 2021; Nuanjan et al., 2024). These limitations often arise from variability in ambient lighting con-

Table 1. Comparison of Analytical Performance of Spectrophotometric and Digital Colorimetric Methods for Selected Analytes

Analyte	Method (Platform)	LOD/LOQ	Linear Range (R^2)
Acrylamide	Digital Image Colorimetry (Smartphones)	20 μg kg^{-1}	R^2 = 0.9491 (Linear, R channel), R^2 = 0.9801 (Polynomial, h* channel)
Chromium	Digital Image-Based Colorimetry	–	R^2 = 0.9962
Iron	Digital Image-Based Colorimetry	–	R^2 = 0.9935

ditions and differences in camera sensors and device hardware, which can introduce inconsistencies in the measured color signals and reduce analytical reliability (Azhar et al., 2023; Woolf et al., 2021; Kwiek and Jakubowska, 2024).

The objective of digital colorimetry is therefore not to replace spectrophotometric instruments entirely, but to provide a practical and context-specific analytical alternative. While spectrophotometry remains the preferred technique for regulated laboratory measurements and certified analytical procedures, digital colorimetry can provide sufficient analytical accuracy for screening, monitoring, and field-based applications. These advantages include lower operational cost, improved portability, and simplified instrumentation, as summarized in Table 1.

3. HARDWARE PLATFORM FOR COLORIMETRIC DATA ACQUISITION

A literature review shows a clear evolution in colorimetric methods, progressing from complex laboratory instruments toward more accessible and portable analytical devices. This transition from conventional spectrophotometry has been facilitated by the development of various digital image acquisition platforms. Each platform presents distinct advantages and limitations in terms of cost, portability, and analytical reliability, as summarized in Table 2 and Figure 1.

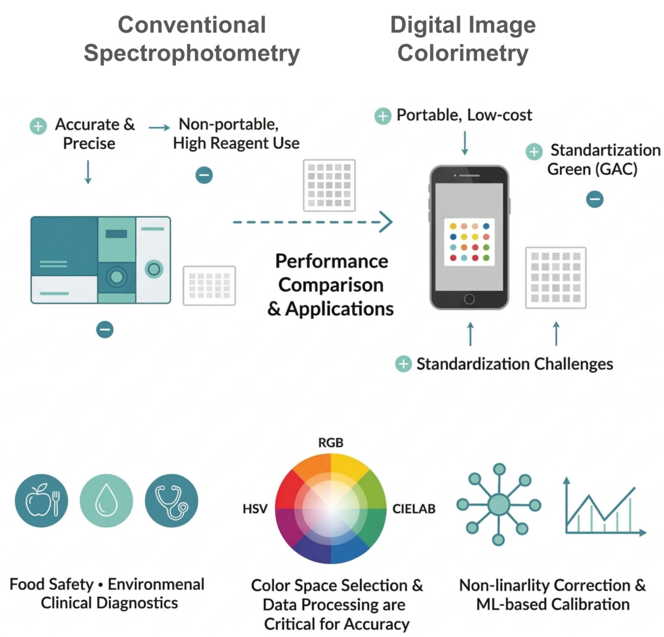

Figure 1. Conceptual Comparison Between Conventional Spectrophotometry and Digital Image-Based Colorimetry, Highlighting Key Attributes, Application Areas, and Factors Affecting Analytical Accuracy, Including Color-Space Selection and Data Processing

3.1 Smartphone as a Portable Detector

Among the available platforms, smartphones have become one of the most prominent and promising tools for digital colorimetric analysis (Soares et al., 2023; Ko and Liao, 2023; Kiwfo et al., 2024). Their widespread availability, together with high-quality Complementary Metal-Oxide-Semiconductor (CMOS) cameras, strong internal processing capabilities, and instant connectivity, enables smartphones to function as portable analytical devices (Baker et al., 2025; Bhatt et al., 2024; Wang et al., 2023; Markus et al., 2023; Mermer et al., 2022). Many researchers have adopted smartphone-based systems for point-of-care (POC) testing, where rapid analysis and accessibility are often prioritized over laboratory-level precision, with applications ranging from biomedical assays and environmental monitoring to food analysis (Gölcez et al., 2021; Doğan et al., 2022; Nath et al., 2025; Nguyen et al., 2025).

Smartphone-based applications have been reported across a wide range of analytical fields. Several studies have demonstrated quantitative determination of pharmaceutical compounds in dosage forms and synthetic urine, as well as the detection of antibiotics for on-site testing (Li et al., 2022; Mermer et al., 2022; James and Honeychurch, 2024). In the area of food safety, smartphones have been used to measure acrylamide levels in toast (Sáez-Hernández et al., 2022) and to evaluate other quality parameters, including phenolic compounds and flavonoids in food matrices (Gomes et al., 2022; Liang et al., 2025). Their application has also expanded into biomedical analysis, where smartphones serve as platforms for bioanalytical and diagnostic testing, (Bhatt et al., 2024; Markus et al., 2023; Destanoğlu et al., 2023; Nath et al., 2025), and into forensic science for the colorimetric characterization of bloodstains (Dinmeung et al., 2023). In environmental monitoring, smartphones have been used as detectors for single-use optical sensors designed to measure multiple analytes in water quality assessment (Wongthanyakram et al., 2023; Doğan et al., 2022; Peixoto et al., 2022; Zeng et al., 2021). Additional applications

Table 2. Comparison of Hardware Platforms Used in Conventional and Digital Colorimetric Analysis

Method	General Instrumentation	Output / Data	Key Advantages	Key Limitations
Spectrophotometry (conventional)	Benchtop spectrophotometer; monochromator or dedicated microplate reader	Absorbance at specific wavelengths	High analytical accuracy and well-established calibration workflows	High capital cost, limited portability, and higher energy/-maintenance needs
Portable photocolorimeter / reflectometer / scanner	Handheld photocolorimeter, reflectometer, desktop scanner	Broadband RGB channels or reflectance units	Portable, lower cost; suitable for field screening	Reproducibility varies between devices; dependent on instrument geometry
Digital image analysis (scanner / camera)	Flatbed scanners, CCD/CMOS cameras; controlled illumination or dark hood	RGB / HSV / CIELAB values from images; can be converted to absorbance with processing	Low cost, high throughput (microplates); can replace spectrophotometers for many assays if calibrated	Sensitive to illumination, gamma correction, and selected color space
Smartphone-based readers and accessories	Smartphone + optical accessories (hood, LED, lens) or 3D-printed spectrometer	Camera-derived RGB/HSV; sometimes smartphone spectrometer output	Highly portable, inexpensive, instant processing and connectivity	Device dependency, ambient light, color space variability; requires robust calibration
Paper-based tests with imaging (μPADs)	μPADs read by scanner or smartphone camera; sometimes with MOF/nanozyme amplification	Color zone intensity converted to concentration via image analysis	Very low sample/reagent use, disposable, suitable for on-site testing and green chemistry	Often lower intrinsic sensitivity; substrate interactions can deviate from Beer's law
Low-cost automated colorimeter	Custom microcontroller-based (Raspberry Pi) device and RGBC sensor	Direct digital color channel output (including clear channel)	Automated sampling, low cost, in-situ operation with R^2 comparable to spectrophotometers in studies	Sensor spectral response and calibration are required to match benchtop instruments

in food safety include the simultaneous determination of common food dyes in commercial products (Botelho et al., 2022) and broader digitalization of colorimetric sensing technologies for food quality monitoring (Mazur et al., 2024; Ko and Liao, 2023; Kiwfo et al., 2024).

Despite these advantages, several challenges remain. Analytical performance may be affected by hardware differences between smartphone models and by the need to control external lighting conditions. Variations in ambient light are a major source of measurement error in smartphone-based colorimetry (Soares et al., 2023; Zeng et al., 2021; Nuanjan et al., 2024). To address this issue, researchers often employ controlled imaging environments, such as 3D-printed dark boxes equipped with stable LED illumination, to standardize image acquisition (Baker et al., 2025; Gölcez et al., 2021; Yang et al., 2022). Software-based corrections also play an important role, including the use of reference color cards to enable real-time calibration and data normalization (Pires et al., 2024; Azhar et al., 2023; Kwiek and Jakubowska, 2024; Nguyen et al., 2025). Recent studies suggest that video recording can provide more stable analytical data than single-image acquisition. By analyzing multiple video frames, researchers can select the most stable images or perform real-time kinetic analysis of color changes. This approach helps reduce the influence of temporary lighting fluctuations and provides a more reliable dataset by monitoring dynamic color intensity changes over time (Bhatt et al., 2024; Woolf et al., 2021; Joh et al., 2025).

3.2 Scanners and Digital Cameras

Flatbed scanners represent one of the earliest low-cost platforms used in digital colorimetry and have often been applied as an alternative to commercial microplate readers (Costa et al., 2023; Hou et al., 2021; Zeng et al., 2021). A major advantage of scanners is their highly controlled illumination system. The internal light source, typically a cold-cathode fluorescent lamp or LED, together with the scanning mechanism, provides stable and uniform illumination while minimizing the influence of ambient lighting conditions (Martínez-Pérez-Cejuela et al., 2023; García-Miralles et al., 2024; Woolf et al., 2021).

These characteristics make scanners particularly suitable for analyzing planar analytical formats, especially microfluidic paper-based analytical devices (μPADs) (Martínez-Pérez-Cejuela et al., 2023; Hou et al., 2021; Velasco et al., 2025). Their high optical resolution enables detailed color analysis and surface characterization, supporting both qualitative pattern recognition and quantitative signal extraction (Woolf et al., 2021; García-Miralles et al., 2024). However, scanners are generally limited to two-dimensional analysis and lack the portability required for field applications (Costa et al., 2023; Zeng et al., 2021). Conventional digital cameras, including CCD and CMOS systems, have also been used in laboratory-based digital colorimetry. In most cases, however, they require carefully controlled imaging setups such as dark hoods or enclosed boxes to eliminate ambient light and maintain consistent imaging distance (Wongthanyakram et al., 2023; Azhar et al., 2023; Nuanjan et al., 2024). Even with these arrangements, issues such as lens distortion and non-uniform sensor response can still occur, requiring additional image-processing corrections and calibration strategies to improve analytical accuracy (Costa et al., 2023; Kwiek and Jakubowska, 2024; Nguyen et al., 2025).

3.2.1 Custom Microcontroller-Based Devices

Recent developments in open-source hardware have enabled the construction of custom colorimetric instruments at relatively low cost using microcontroller platforms such as Raspberry Pi and Arduino (Antela et al., 2023; Yang et al., 2022; Velasco et al., 2025). This "Do-It-Yourself" (DIY) approach allows researchers to design instruments that are specifically adapted to particular analytical tasks and field conditions, particularly for portable and field-deployable sensing applications (Wang et al., 2023; Kiwfo et al., 2024). These devices typically integrate an inexpensive RGB color sensor, such as an RGBC sensor, together with a controlled LED light source within a compact 3D-printed housing (Antela et al., 2023; Yang et al., 2022; García-Miralles et al., 2024). Smartphone DIC developments (Sáez-Hernández et al., 2025b) and Raspberry Pi systems (Antela et al., 2023), further enhance portability and enable integration with advanced data processing, including machine learning and real-time analysis (Joh et al., 2025; Liu et al., 2025).

By controlling both illumination and sensor geometry, microcontroller -based colorimeters can reduce the influence of ambient light variability commonly encountered in smartphone-based systems (Antela et al., 2023; Azhar et al., 2023; Zeng et al., 2021). Several studies have demonstrated that such devices can achieve strong analytical performance, including good linearity (R^2) and detection limits comparable to benchtop spectrophotometers in certain applications, while maintaining significantly lower instrument cost (Antela et al., 2023; Doğan et al., 2022; Markus et al., 2023; Nguyen et al., 2025).

4. ANALYTICAL PLATFORM AND SENSOR DESIGN

Image acquisition hardware must be integrated with an analytical platform capable of processing and interpreting colorimetric reactions. The design of the analytical platform is therefore a critical component of the overall analytical system, particularly in terms of reagent consumption, analytical reliability, and suitability for on-site applications. Figure 2 presents a schematic comparison of the two main analytical platforms used in digital colorimetric analysis.

4.1 Microfluidic Paper-Based Analytical Devices (μPADs)

Microfluidic paper-based analytical devices (μPADs) have significantly expanded the possibilities of point-of-care (POC) testing because of their cost-effectiveness and simple fabrication methods, including wax printing and molding. Moreover, their minimal reagent consumption at the microliter scale aligns them with Green Analytical Chemistry (GAC) principles, which aim to reduce environmental impact without sacrificing analytical performance (Mazur et al., 2024; Ko and Liao, 2023; Li and Feng, 2020). μPAD-based fluoride detection using smartphone readout (Li et al., 2025), smartphone-based multiplex detection systems (Li et al., 2022), and ML-enhanced sensor arrays for multiplexed sensing (Kim et al., 2026), together with recent developments in paper-based sensor arrays and smartphone-integrated platforms (Liang et al., 2025; Liu et al., 2025), collectively demonstrate strong alignment with Green Analytical Chemistry (GAC) principles (Mazur et al., 2024).

The analytical capabilities of μPADs can be further enhanced when combined with smartphone-based detection systems, enabling quantitative measurements to be performed outside conventional laboratory environments (Bhatt et al., 2024; Gölcez et al., 2021; Wang et al., 2023). This integration has been widely applied to the detection and quantification of various analytes, including compounds in food samples, environmental monitoring targets, and biomedical markers (Bhatt et al., 2024; Gomes et al., 2022; Nath et al., 2025; Peixoto et al., 2022). Despite these advantages, μPAD-based methods often exhibit lower sensitivity compared with solution-based analytical techniques. Additional challenges include controlling sample volume and maintaining consistent capillary flow rates within the paper matrix, which may lead to variability in reaction time and signal development (Tong and Hutcheson, 2021; Zeng et al., 2021).

To address these limitations, several studies have incorporated advanced materials into the paper substrate to im-

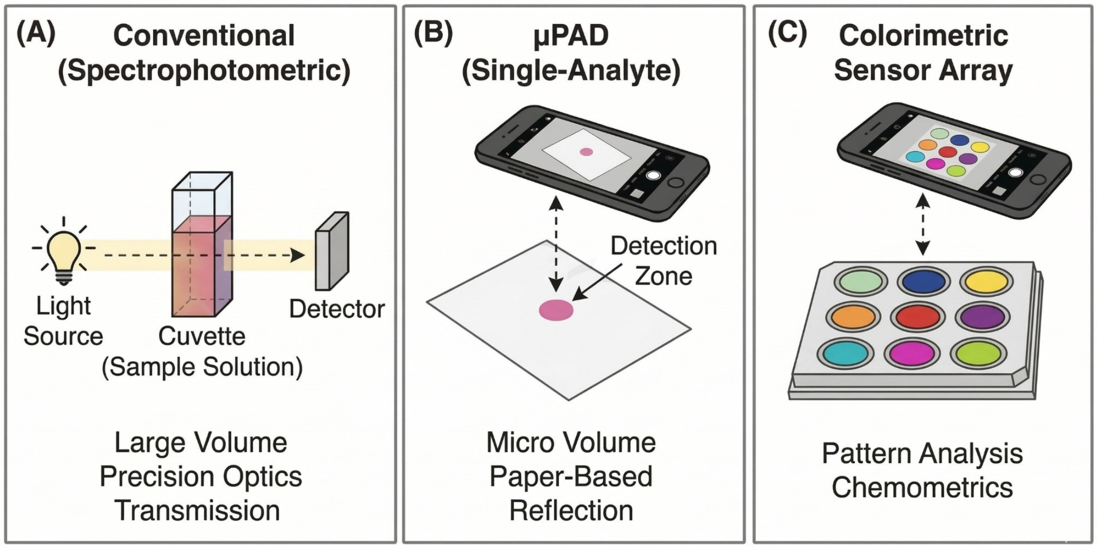

Figure 2. Schematic Comparison of Analytical Platforms Used in Colorimetric Analysis. (A) Reaction in a Cuvette for Conventional Spectrophotometric Measurement With Larger Sample Volume and Precision Optics. (B) A Single Reaction Zone On a μpad (Paper-Based Microfluidic Device) Enabling Micro-Volume Analysis with Reflective Reading. (C) A Colorimetric Sensor Array Consisting of Multiple Reaction Spots That Generate Distinct Color Patterns for Analyte Identification

prove analytical performance. For instance, nanozyme-based nanomaterials such as gold nanoparticles can catalyze colorimetric reactions and enhance signal intensity, while recent nanozyme-assisted sensor arrays further improve multiplexed detection performance (Liu et al., 2023; Pongkitdachoti and Unob, 2022). Similarly, materials such as metal–organic frameworks (MOFs) have been incorporated into μPAD systems to improve selectivity and sensitivity in the determination of phenolic compounds (Martínez-Pérez-Cejuela et al., 2023), and hybrid microfluidic–paper platforms continue to enhance analytical robustness and integration capabilities (Velasco et al., 2025; Wu et al., 2024).

4.2 Colorimetric Sensor Arrays

For complex samples, a single colorimetric reaction is often insufficient to provide reliable identification. As a result, colorimetric sensor arrays have been developed to improve analytical capability. These systems, often referred to as colorimetric "tongues" or "noses," do not target a single analyte specifically. Instead, they consist of multiple sensing elements that respond in a cross-reactive manner to different components in a sample, generating a characteristic color pattern or "fingerprint."

Such arrays can be constructed using materials including modified gold nanoparticles and natural plant pigments. These systems have demonstrated the ability to distinguish between similar compounds, such as sweeteners and non-sweeteners, or to evaluate the authenticity of products such as monofloral honey. Tasks of this type are difficult to achieve using a single sensor alone. Because the resulting color patterns are often complex, visual interpretation is generally insufficient. Instead, the image data are analyzed using chemometric techniques such as Principal Component Analysis (PCA) or Hierarchical Cluster Analysis (HCA), which enable the identification of subtle patterns, sample classification, and quantitative analysis of complex mixtures (Lyu et al., 2021; Gomes et al., 2022; Liu et al., 2023; Adampourezare et al., 2024).

One of the main advantages of colorimetric sensor arrays (CSAs) is their ability to perform high-throughput multiplex analysis, enabling the simultaneous detection and discrimination of multiple analytes within a single sample. This capability often surpasses that of single-target colorimetric sensors (Mazur et al., 2024; Liang et al., 2025; Liu et al., 2025). Their cross-reactive sensing strategy also facilitates the classification of complex mixtures, such as distinguishing sweeteners or verifying honey authenticity, which are challenging tasks for traditional single-analyte sensors (Lyu et al., 2021; Yang et al., 2023; Gomes et al., 2022). The integration of advanced nanomaterials and sensing platforms, including metal–organic frameworks (MOFs), can further improve CSA performance by enhancing sensitivity and selectivity for difficult analytical targets (Martínez-Pérez-Cejuela et al., 2023; Liu et al., 2023).

Through the combination of multiplex sensing, chemometric pattern recognition, and advanced functional materials, CSAs represent a versatile analytical platform for applications in food safety, environmental monitoring, and biomedical diagnostics (Mazur et al., 2024; Bhatt et al., 2024; Ko and Liao, 2023; Kiwfo et al., 2024; Khan et al., 2025). In addition, the integration of nanoparticle-based sensing systems with smartphone-assisted digital image colorimetry has enabled highly sensitive and portable detection platforms; for example, gold nanoparticle (AuNP)-based systems have demonstrated accurate Cr(III)/Cr(VI) detection in food-related matrices with limits of detection comparable to spectrophotometric methods (Firdaus et al., 2023a; Doğan et al., 2022; Markus et al., 2023).

5. DATA PROCESSING CHALLENGES: COLOR SPACES AND CALIBRATION

A significant challenge in digital image colorimetry (DIC) is the conversion of complex raw image color information into accurate, linear, and reproducible concentration values (Tiuftiakov et al., 2021; Woolf et al., 2021; Kwiek and Jakubowska, 2024; Joh et al., 2025). The choice of data processing strategy is therefore as important as the hardware platform itself, because different color space models and preprocessing approaches can produce substantially different analytical responses (Figure 3) (Phuangsaijai et al., 2021; García-Miralles et al., 2024).

5.1 Comparison of Color Space Models (RGB, HSV, CIELAB)

The conversion of raw image data into quantitative analytical signals represents a critical step in digital image colorimetry (DIC). The selection of an appropriate color space model can strongly influence analytical accuracy, linearity, and precision (Fay and Wu, 2024; Woolf et al., 2021; Kwiek and Jakubowska, 2024; Joh et al., 2025). To minimize the effects of ambient lighting variability and obtain more stable analytical signals, raw camera outputs are often transformed into alternative color spaces before quantitative analysis, often combined with preprocessing strategies such as image normalization and segmentation (Woolf et al., 2021; García-Miralles et al., 2024).

The HSV (Hue, Saturation, Value) model offers advantages in separating color information from brightness intensity. In this model, color is represented by Hue, color purity by Saturation, and brightness by Value (Phuangsaijai et al., 2021). This separation allows the Hue channel to be relatively robust against minor illumination fluctuations and shadow effects. For colorimetric assays involving pronounced hue changes, such as transitions between red and blue colors, the Hue channel often provides a stable and more linear response with respect to analyte concentration (Phuangsaijai et al., 2021; Doğan et al., 2022; Markus et al., 2023).

For applications requiring high perceptual consistency and improved analytical accuracy, the CIELAB color space (Lab*) is often considered one of the most reliable color representations. This color space is designed to be perceptually uniform and theoretically device-independent. It represents color using three coordinates: L* for lightness, a* for the green–red axis, and b* for the blue-yellow axis (Tiuftiakov et al., 2021). A key advantage of the CIELAB system is that numerical changes in the a* or b* coordinates correspond more closely to perceived color differences. As a result, colorimetric reactions that produce shifts along these axes often show stronger linear correlations with analyte concentration when analyzed in the CIELAB space, outperforming other color models in many cases (Phuangsaijai et al., 2021; Tiuftiakov et al., 2021; Zhbanova, 2020; Joh et al., 2025).

In contrast, the RGB (Red, Green, Blue) color model, which represents the default output of most cameras and scanners, presents several limitations for quantitative analysis. The R, G, and B channels are highly correlated and strongly influenced by variations in illumination intensity and brightness conditions (Fay and Wu, 2024; Zeng et al., 2021; Azhar et al., 2023). Furthermore, the RGB color space is perceptually nonlinear, meaning that equal numerical changes in RGB values do not correspond to consistent perceived color differences (Fay and Wu, 2024; Woolf et al., 2021). For this reason, RGB values are often used as the initial data source in digital colorimetric analysis but are typically transformed into alternative color spaces such as HSV or CIELAB to obtain more reliable analytical signals. A critical study by Fay and Wu (2024) highlights that the choice of color space should not be overlooked, as it can strongly influence the success or failure of a digital image colorimetric method.

5.2 Calibration and Non-linearity Correction

Another important challenge in digital image colorimetry (DIC) is the frequent nonlinearity between extracted color signals and analyte concentration. This behavior contrasts with the linear relationship typically observed in spectrophotometric measurements governed by the Beer–Lambert law (Soda et al., 2020). Such deviations may arise from several factors, including intrinsic nonlinearity in camera sensors, automatic gamma correction, light scattering effects from substrates, particularly in μPAD systems (Tong and Hutcheson, 2021), and complex reaction kinetics that affect signal development (Woolf et al., 2021; Zeng et al., 2021).

An essential step in addressing this issue is selecting an appropriate color space for data analysis, as predictive performance, calibration linearity, and measurement reliability are strongly influenced by this choice (Fay and Wu, 2024; Phuangsaijai et al., 2021; Tiuftiakov et al., 2021; Kwiek and Jakubowska, 2024; Joh et al., 2025). Although the RGB color space is the direct output of most imaging devices, it is often not optimal for quantitative applications because of its sensitivity to illumination variations and the strong correlation among its channels (Fay and Wu, 2024; Woolf et al., 2021). Consequently, alternative color representations are frequently used.

The HSV model can improve signal robustness by separating hue information from brightness intensity. In colorimetric reactions involving clear hue changes, the Hue channel can provide relatively stable signals even when illumination conditions vary slightly (Phuangsaijai et al., 2021; Doğan et al., 2022). For applications requiring higher analytical accuracy, the CIELAB color space is often preferred due to its perceptual uniformity. In this representation, changes in the a* (green-red) and b* (blue-yellow) coordinates often correspond more closely to actual color differences, which can result in improved calibration performance (Phuangsaijai et al., 2021; Tiuftiakov et al., 2021; Zhbanova, 2020).

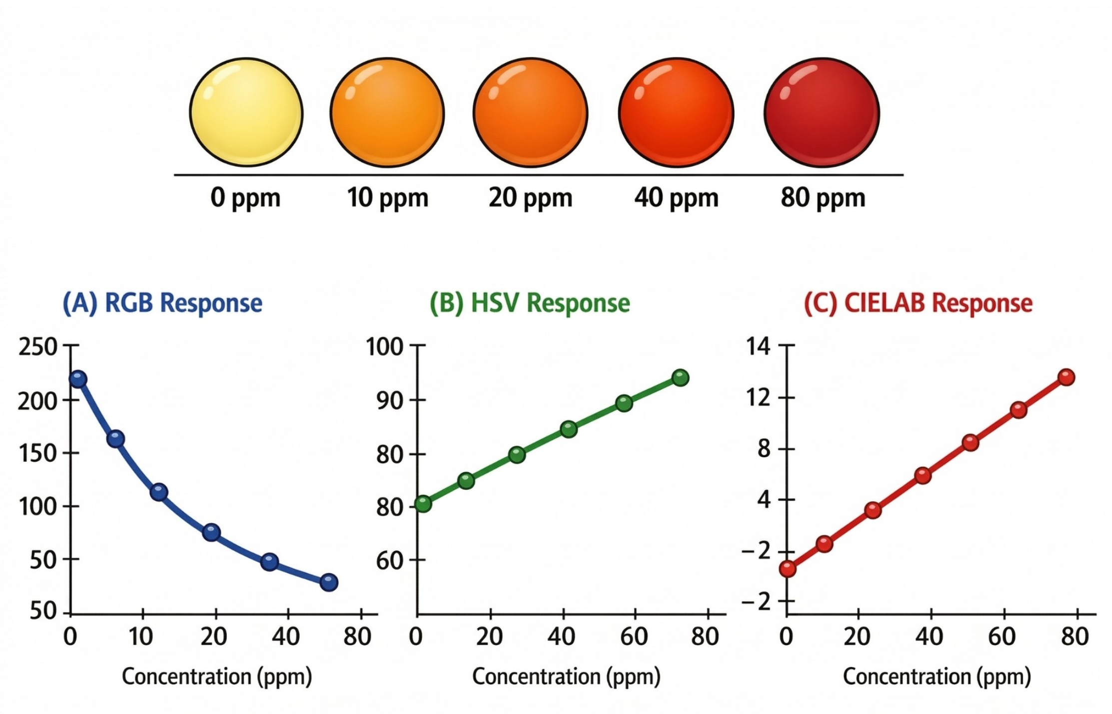

Figure 3. Comparison of Color-Space Responses to Analyte Concentration. (A) Relationship Between Concentration and Green Channel (G) Intensity in RGB, Showing a Non-Linear Response. (B) Relationship Between Concentration and Hue (H) in HSV, Showing Improved Linearity. (C) Relationship Between Concentration and A* Value in CIELAB, Showing the Highest Linearity

After selecting the appropriate color space, the extracted color values (such as R, G, B, H, or a*) typically require mathematical transformation to establish a reliable relationship with analyte concentration. In relatively simple systems, basic transformations such as $1/R$ or $\log(R_0/R)$, where R represents the channel intensity and R^0 is a reference value, can be applied to approximate linear behavior (Pires et al., 2024; García-Miralles et al., 2024).

For more complex and highly nonlinear responses, advanced regression techniques are often required. Polynomial regression models, including second- or third-order equations, are commonly used to fit calibration data (Ji et al., 2020). More recently, machine learning (ML) algorithms have been used to model complex relationships between multidimensional color inputs and analyte concentration (Sáez-Hernández et al., 2025b; Joh et al., 2025; Liu et al., 2025). These inputs may include multiple parameters derived from different color spaces.

Applying ML methods typically involves several stages, including data preprocessing to clean and normalize the input data, feature selection to identify relevant variables, model training using algorithms such as neural networks or support vector machines, and model validation to evaluate predictive performance (Kwiek and Jakubowska, 2024; García-Miralles et al., 2024). Open-source tools such as TensorFlow and scikit-learn facilitate the implementation of these approaches and provide accessible resources for researchers working in digital colorimetric analysis. Data-driven ML approaches can significantly improve calibration accuracy and robustness while addressing the limitations associated with traditional linear or polynomial fitting methods (Wang et al., 2023; Khan et al., 2025).

6. KEY APPLICATION AREAS OF DIGITAL COLORIMETRY

The portability, low cost, and ease of use of digital colorimetry have expanded its application into many analytical fields, particularly those that benefit from on-site testing capabilities. These characteristics make digital image colorimetry well suited for rapid screening and field-based measurements where conventional laboratory instruments may be impractical. An overview of several major application areas of digital colorimetric analysis is summarized in Figure 4.

6.1 Food Safety and Quality

The food and beverage sector represents one of the major application areas for digital colorimetric analysis (Mazur et al., 2024; Ko and Liao, 2023). Digital image colorimetry (DIC) has been widely applied to monitor both food safety and product quality, particularly due to its capability for rapid, on-site, and non-destructive analysis (Gomes et al., 2022; Yang et al., 2025). For example, rapid detection of process contaminants such as acrylamide in toasted products has been demonstrated, enabling direct quality monitoring during food processing (Sáez-Hernández et al., 2022). These analytical approaches have also

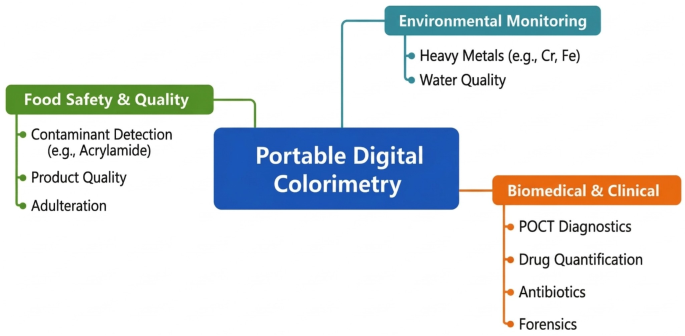

Figure 4. Major Application Areas of Digital Colorimetry

been used to determine heavy metal contaminants, including chromium and iron, in food products (Botelho et al., 2022; Nguyen et al., 2025).

In addition to contaminant detection, digital colorimetric methods can be used to monitor essential components or identify adulteration practices, such as the use of prohibited food dyes in commercial products (Botelho et al., 2022; Mahdi, 2023). Green digital image colorimetry (DIC) has been applied to the determination of pharmaceutical compounds such as aspirin and salicylic acid (Bharti et al., 2022; Elagamy et al., 2023), as well as dyes, heavy metals, and other food contaminants in food systems (Botelho et al., 2022; Resende et al., 2023; Peixoto et al., 2022; Yeerum et al., 2022).

In addition, AuNP-based smartphone colorimetric sensing enables accurate detection of Cr(III)/Cr(VI) in food contaminants, achieving low detection limits comparable to spectrophotometric methods (Firdaus et al., 2023b; Liang et al., 2025).

6.2 Environmental Monitoring

Field-deployable analytical methods are particularly valuable for environmental monitoring because sample collection, transportation to laboratories, and storage can introduce additional costs and increase the risk of sample degradation. Portable colorimetric systems enable on-site measurement of environmental contaminants, including heavy metals such as chromium and iron in water samples (Botelho et al., 2022; Doğan et al., 2022; Nguyen et al., 2025; Peixoto et al., 2022; Ponhong et al., 2025). In addition, digital colorimetric methods have been applied to evaluate various water quality parameters in environmental samples (Wongthanyakram et al., 2023; Phuangsaijai et al., 2021; Zeng et al., 2021; Thongkon et al., 2024; Kiwfo et al., 2024). The ability to perform measurements directly in the field allows faster responses to pollution events and supports more efficient long-term environmental monitoring, particularly when combined with smartphone-based sensing platforms and portable imaging systems (Wang et al., 2023; Woolf et al., 2021).

6.3 Biomedical and Clinical Applications

The point-of-care testing (POCT) sector represents another important application area for digital colorimetric analysis (Bhatt et al., 2024; Ko and Liao, 2023; Wang et al., 2023). In remote locations or low-resource settings where clinical laboratory facilities are limited, smartphones and portable imaging systems can function as practical diagnostic tools, enabling rapid and decentralized analysis Kiwfo et al. (2024) Reported applications include the quantification of pharmaceutical compounds in dosage forms or patient-derived samples, as well as the detection of antibiotics such as streptomycin for monitoring antimicrobial resistance in field conditions (Li et al., 2022; Peixoto et al., 2022; Nguyen et al., 2025). Additional applications have been reported in biomedical and clinical contexts, including serum protein quantification and enzymatic activity analysis (Markus et al., 2023; Destanoğlu et al., 2023; Nath et al., 2025), as well as in forensic science, where digital colorimetric analysis can assist in the characterization of bloodstains (Dinmeung et al., 2023), and in dental research for objective color matching of prosthetic materials (Akl et al., 2022). Beyond conventional assays, advanced paper-based and microfluidic platforms have demonstrated strong performance for clinical screening; for example, 3D-μPADs integrated with AuNPs have been reported for urinary albumin–creatinine ratio (ACR) detection with high accuracy (93%) and good linearity over 30-400 mg/g, making them suitable for chronic kidney disease (CKD) screening in resource-limited settings (Sabarudin et al., 2025; Velasco et al., 2025; Khan et al., 2025).

7. CHALLENGES, SOLUTIONS, AND FUTURE PROSPECTS

Despite its significant potential and rapid development, the widespread adoption of digital image colorimetry as a standard alternative to spectrophotometric analysis still faces several important challenges. These challenges relate mainly to analytical reliability, reproducibility, and methodological standardization. The major challenges and potential solutions identified in the literature are summarized in Table 3.

7.1 Key Challenges: Standardization and Reproducibility

One of the most frequently reported challenges in the literature is the lack of methodological standardization in digital colorimetric analysis (Bhatt et al., 2024; Woolf et al., 2021; Azhar et al., 2023; Kiwfo et al., 2024). Without appropriate standardization, results obtained using one device, for example an iPhone 14, cannot be directly compared with those produced by another device, such as a Samsung Galaxy S23, or by different laboratories.

This problem arises mainly from two sources. The first is inter-device variability. Different smartphone cameras use different image sensors, color filter arrays, and internal image-processing algorithms such as automatic white balance and gamma correction. The quality of sensor readout also varies between devices. Two different cameras may generate substantially different RGB values even when capturing the same color signal (Soda et al., 2020; Nguyen et al., 2025; García-Miralles et al., 2024).

The second factor is sensitivity to ambient lighting con-

Table 3. Summary of Key Challenges and Proposed Solutions in Digital Colorimetry

Key Challenges	Proposed Solutions
Ambient Lighting Variability	Hardware accessories (eg, 3D-printed dark box), internal LED light source
Inter-Device Variability (Sensors and Software)	Use of robust color spaces (eg, CIELAB, HSV), reference-based calibration
Non-Linearity of Color Response	Non-linear calibration models (eg, polynomial regression, machine learning)
Low Sensitivity (compared to spectrophotometry)	Incorporation with signal-amplifying materials (eg, nanozymes, MOFs)

ditions. Variations in illumination intensity, light angle, and color temperature, for example differences between fluorescent lighting and natural sunlight, can alter the colors recorded by a camera sensor. These variations can introduce significant analytical errors in digital colorimetric measurements (Soda et al., 2020; Zeng et al., 2021; Nuanjan et al., 2024; Kwiek and Jakubowska, 2024).

7.2 Hardware and Software Solutions

Addressing these challenges requires improvements in both hardware and software. From the hardware perspective, one practical solution is isolating the imaging sensor from uncontrolled ambient lighting. This can be achieved using low-cost accessories such as 3D-printed dark boxes or hoods that can be attached to smartphones (Fan et al., 2021b; Azhar et al., 2023; Zeng et al., 2021; Nguyen et al., 2025). These accessories maintain a consistent imaging distance and angle and usually include an internal light source, commonly a white LED, to provide stable and reproducible illumination conditions (Woolf et al., 2021; Gölcez et al., 2021).

From the software perspective, advanced data-processing approaches can improve measurement consistency. Analytical applications can perform internal color correction and calibration by including reference standards such as color calibration cards within the captured image (Pires et al., 2024; Azhar et al., 2023; Kwiek and Jakubowska, 2024). Computational approaches, including machine learning algorithms, can also be applied to compensate for sensor variability and nonlinear device responses (Sáez-Hernández et al., 2025b; Joh et al., 2025; García-Miralles et al., 2024; Doğan et al., 2022). Appropriate color-space selection, such as the use of the CIELAB model, can improve robustness against illumination variations and enhance calibration performance (Phuangsaijai et al., 2021; Woolf et al., 2021; Zhbanova, 2020).

7.3 Conceptual Decision Framework for Digital Colorimetry Reliability

Considerable progress has been achieved through hardware stabilization, improved color-space selection, and advanced calibration techniques (Fan et al., 2021a; Soares et al., 2023; Woolf et al., 2021; Doğan et al., 2022; García-Miralles et al., 2024). Despite these developments, the analytical reliability of digital image colorimetry remains dependent on the interaction of multiple experimental variables. Lighting stability, device consistency, color-space robustness, and calibration strategy jointly influence analytical performance (Baker et al., 2025; Fan et al., 2021b; Azhar et al., 2023; Zeng et al., 2021; Nguyen et al., 2025). These factors interact in nonlinear and context-dependent ways, making purely deterministic evaluation insufficient. This underscores the need for an interpretable evaluation framework that systematically captures the interactions among key experimental variables and enables transparent assessment of analytical reliability.

A qualitative reasoning framework based on fuzzy logic is proposed to represent these interactions. Fuzzy logic has been widely adopted for modeling uncertainty and nonlinear interactions in analytical and decision systems, particularly when precise deterministic relationships are difficult to establish (Zadeh, 1965; Mendel, 1995; Ross, 2010; Liu et al., 2025; Joh et al., 2025). This approach enables the representation of gradual transitions between states, making it well suited for complex experimental conditions in digital colorimetry, where multiple interdependent factors influence analytical performance simultaneously.

Fuzzy reasoning is particularly suitable for analytical systems operating under partially uncertain conditions (Khan et al., 2025). Experimental parameters can be expressed using linguistic variables such as low, medium, and high rather than rigid numerical thresholds. This reflects practical experimental conditions, where illumination may be partially controlled (Baker et al., 2025; Zeng et al., 2021), device variability may be moderate (Akl et al., 2022; Bhatt et al., 2024; Azhar et al., 2023), and calibration strategies may vary in complexity (Joh et al., 2025; Doğan et al., 2022). Gradual variations of this type are difficult to represent using binary decision rules, while fuzzy inference provides a more appropriate representation.

The framework considers four input variables: lighting stability (LS), device consistency (DC), color-space robustness (CSR), and calibration strength (CS). These variables correspond to the main analytical challenges discussed throughout this review. They include ambient illumination variability (Baker et al., 2025; Nuanjan et al., 2024), differences between imaging sensors (Akl et al., 2022; Bhatt et al., 2024; Nguyen et al., 2025), sensitivity of color-space models to illumination changes (Fay and Wu, 2024; Phuangsaijai et al., 2021; Kwiek and Jakubowska, 2024; Woolf et al., 2021), and the need for reliable calibration strategies (Tong and Hutcheson, 2021; Ji et al., 2020; García-Miralles et al., 2024; Doğan et al., 2022). The output variable is analytical reliability (AR), representing the expected analytical performance of a digital colorimetric system under specific experimental conditions.

Each variable is represented using triangular membership functions defined on a normalized scale from 0 to 10. Input variables are categorized into low, medium, and high, while calibration strength is defined as weak, moderate, and strong. Analytical reliability is expressed using four categories: poor, acceptable, good, and excellent, allowing a gradual transition between performance levels.

The inference process follows a Mamdani rule-based system, where expert knowledge is translated into interpretable analytical rules. Rule activation is evaluated using the minimum operator, and aggregation is performed using the maximum operator. Defuzzification is carried out using the centroid method to obtain a continuous reliability score. An illustrative evaluation shows that a system with moderately controlled illumination, moderate device consistency, high color-space robustness, and strong calibration achieves a reliability level within the "good" category. This reflects typical digital colorimetry conditions, where performance is sufficient for many applications but remains constrained by device variability. Full equivalence with laboratory spectrophotometric instruments still requires further methodological standardization (Antela et al., 2023; Woolf et al., 2021; Azhar et al., 2023).

This framework provides a structured interpretation of the interaction between experimental variables affecting digital colorimetric performance. It complements conventional calibration approaches by introducing an interpretable reasoning layer that supports method selection and system optimization. The conceptual structure of the framework is illustrated in Figure 5.

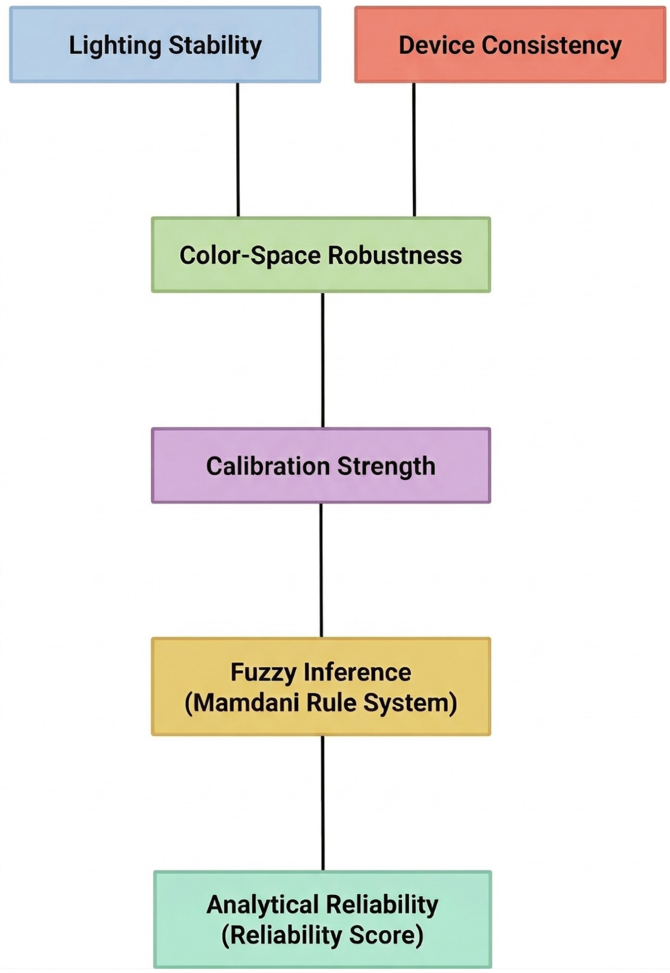

Figure 5. Conceptual Fuzzy Inference Framework for Evaluating Analytical Reliability in Digital Colorimetry Systems. The Framework Integrates Four Key Experimental Variables: Lighting Stability, Device Consistency, Color-Space Robustness, and Calibration Strength Into a Mamdani Type Fuzzy Inference System. These Variables Represent Major Sources of Uncertainty in Digital Colorimetric Measurements. The Inference Process Combines Fuzzy Rules Derived from Analytical Expertise to Produce an Overall Analytical Reliability Score Through Defuzzification

The fuzzy logic framework can also be interpreted as an explainable artificial intelligence layer for digital colorimetry systems. Data-driven models such as machine learning improve predictive performance but often lack interpretability. In contrast, fuzzy reasoning provides transparent rule-based logic that explicitly describes how experimental factors influence analytical reliability. Integration of fuzzy reasoning with data-driven calibration models may enable hybrid analytical systems that combine predictive capability with interpretable decision support (Mazur et al., 2024; Dinmeung et al., 2023; Joh et al., 2025; Liu et al., 2025; Wang et al., 2023).

7.4 Future Prospects

Digital colorimetry is expected to continue evolving through advances in three main areas: sensor materials, connectivity, and intelligent data processing. Integration of advanced sensing materials, including metal-organic frameworks (MOFs) (Martínez-Pérez-Cejuela et al., 2023) and nanozyme catalysts, into platforms such as μPADs is expected to reduce detection limits and improve analytical sensitivity. Performance may approach that of laboratory instruments in specific applications (Liu et al., 2025; Liang et al., 2025; Ganguly and Sengupta, 2025). Fabrication of complex sensor arrays on inexpensive substrates can support multiplex detection of several analytes simultaneously (Adampourezare et al., 2024; Yang et al., 2025). These systems produce characteristic analytical fingerprints for complex samples.

The conceptual foundation of digital image-based quantification can be traced back to early implementations using flatbed scanners, where color intensity values extracted from digital images were successfully correlated with analyte concentration, demonstrating the feasibility of low-cost image-based analytical systems (Soldat et al., 2009).

Integration of low-cost sensor devices with Internet of Things (IoT) technology may enable distributed analytical networks. Systems based on microcontroller platforms such as Raspberry Pi Antela et al. (2023) could support real-time environmental or industrial monitoring (Yang et al., 2025; Velasco et al., 2025). Data from multiple sensors can be transmitted to cloud platforms for continuous analysis and decision support (Wang et al., 2023).

Another emerging direction involves the use of video-based monitoring instead of single static images. Video acquisition provides dynamic datasets that capture the kinetic development of colorimetric reactions. Temporal data can reduce transient noise, allow selection of optimal analysis frames, and support reaction-rate analysis as an additional analytical parameter. These approaches improve measurement reproducibility and reduce environmental fluctuations (Soda et al., 2020; Woolf et al., 2021; Bhatt et al., 2024).

Analysis of complex datasets produced by video monitoring or sensor arrays increasingly relies on machine learning algorithms. These models can represent nonlinear relationships between multidimensional color signals and analyte concentrations. Analytical accuracy, precision, and robustness can improve through this approach (Joh et al., 2025; García-Miralles et al., 2024; Khan et al., 2025). Advances in sensing materials, connected analytical systems, and intelligent data analysis will shape the future development of digital colorimetric analysis.

Taken together, recent advances in sensor materials, low-cost imaging platforms, and data-driven modeling demonstrate that digital colorimetry is transitioning from a qualitative screening tool to a robust quantitative analytical approach with expanding applicability across environmental, biomedical, and industrial domains (Mazur et al., 2024; Soares et al., 2023; Wang et al., 2023; Joh et al., 2025; Liu et al., 2025). Future progress will depend on the integration of standardized acquisition protocols, intelligent calibration strategies, and connected analytical systems, enabling reliable, scalable, and field-deployable solutions that align with the principles of sustainable and accessible analytical chemistry (Ganguly and Sengupta, 2025; Kwiek and Jakubowska, 2024; Woolf et al., 2021).

8. CONCLUSIONS

Digital colorimetry has emerged as a practical, portable, and cost-effective alternative to conventional spectrophotometric analysis, aligning well with the principles of Green Analytical Chemistry. When appropriate calibration strategies and suitable color space models, such as CIELAB or HSV, are applied, this method can achieve analytical performance comparable to spectrophotometric methods in many applications. Nevertheless, challenges related to standardization and measurement reproducibility remain, driven primarily by variability between imaging devices and sensitivity to ambient lighting conditions. These limitations can be mitigated through careful experimental design, including the use of controlled imaging environments and computational calibration approaches. Looking forward, the integration of advanced sensing materials, intelligent data analysis, and connected analytical platforms will further expand the capabilities of digital colorimetric systems, supporting the development of decentralized analytical setups suitable for field-based chemical analysis.

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