Supplementary Information

Chromaeus: A Browser-Based Analytical Platform for Quantitative Colorimetric Measurement in Digital Chemistry Applications

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Methods

Colorimetric conversion algorithms and metric computation

To ensure rigorous colorimetric analysis, raw pixel data extraction and color space conversions were implemented using standardized algorithms rather than operating system-dependent libraries. For every defined ROI, the application first extracts the mean red (R), green (G), and blue (B) values from the canvas pixel buffer. These values (8-bit integers, 0-255) are normalized to the range [0, 1] (R', G', B') prior to transformation.

The application computes metrics across three complementary color spaces, each utilizing specific mathematical models:

(i) HSV (Hue, Saturation, Value): To decouple chromaticity from illumination intensity, the cylindrical HSV model is calculated based on the hexagonal cone projection. The Value (V) and Saturation (S) are derived as follows:

$$V = max(R', G', B')$$

$$S = \frac{max\left(R', G', B'\right) - min\left(R', G', B'\right)}{max\left(R', G', B'\right)}$$

Hue (H) is calculated as the angular displacement on the color wheel (0-360°), normalized based on the dominant color channel.

(ii) CMYK (Cyan, Magenta, Yellow, Key): Unlike device-specific ICC profiles used in printing, Chromaeus employs the standard geometric subtractive model. This approach is preferred for analytical applications as it often yields superior linearity with analyte concentration. The Key (Black) component is calculated as the complement of the maximum RGB brightness:

$$K = 1 - max\left(R', G', B'\right)$$

The remaining components (C, M, Y) are calculated proportionally to the inverse of their respective RGB channels, normalized by ($1-K$) {Tiuftiakov, 2021 #8}.

(iii) CIE $L*a*b*$: Conversion to the perceptually uniform CIE $L*a*b*$ space requires a two-step transformation to account for the non-linear gamma encoding of standard digital images (sRGB). First, normalized RGB values are linearized using the inverse gamma correction function to remove the sRGB transfer characteristic:

$$C_{linear} = \begin{cases} \dfrac{C'}{12.92}, & C' \leq 0.04045 \\ \left(\dfrac{C' + 0.055}{1.055}\right)^{2.4}, & C' > 0.04045 \end{cases}$$

where C' represents R', G', or B'. The linearized RGB values are then transformed into XYZ tristimulus values using the standard sRGB-to-XYZ matrix. Finally, XYZ values are converted to $L*a*b*$ coordinates relative to the CIE Standard Illuminant D65 (Daylight, 6500K), using the standard transformation function $f(t)$ involving the cube root of the luminance ratios (Y/Y_n). This rigorous implementation ensures that $L*$ (Lightness), $a*$ (Red-Green), and $b*$ (Blue-Yellow) metrics accurately reflect human color perception, independent of device gamma settings.

Preparation and characterization of PVP-capped green silver nanoparticles

Green-colored silver nanoparticles (AgNPs) were synthesized following the procedure adapted from {Mendis, 2016 #11;Ghorbanizamani, 2025 #10}. Briefly, freshly prepared aqueous sodium borohydride ($NaBH_4$, 1.0 mM) was employed as the primary reducing agent, while hydrazinium sulfate (2.0 mM) acted as a co-reductant and stabilizer in the presence of trisodium citrate (10 mM). The mixture was magnetically stirred for 10 min at room temperature, after which silver nitrate ($AgNO_3$, 1.0 mM) was added dropwise under continuous stirring. To ensure reproducibility, $NaBH_4$ solutions were prepared immediately prior to use due to their high reactivity.

For capping, 50 µL of polyvinylpyrrolidone (PVP, 1-6 mg/mL, MW = 29 kDa) was mixed with 450 µL of the pre-synthesized AgNP solution and stirred for 30 min at ambient temperature. Excess, non-bound PVP was removed by centrifugation at $8000 \times g$ for 10 min (room temperature), and the pellet containing PVP-stabilized AgNPs was resuspended in distilled water. The stability of the synthesized AgNPs was followed via UV-Vis analysis by doing daily measurements for 7 days (n=3).

The optical properties of the synthesized green-colored AgNPs were assessed using UV-Vis spectrophotometry (Perkin Elmer Lambda 35), scanning from 340 to 700 nm to identify the characteristic plasmon resonance bands {Ghorbanizamani, 2025 #10}. To evaluate surface functionalization, Fourier-transform infrared spectroscopy (Perkin Elmer Spectrum Two ATR-FTIR) was performed, highlighting the characteristic vibrational bands of PVP that confirmed successful capping of the nanoparticles. All measurements were conducted in triplicates {Ghorbanizamani, 2025 #10}.

Optimization of the AgNP-H_2O_2 colorimetric reaction

The response of AgNPs to the addition of different concentrations of H_2O_2 solutions was followed by UV-Visible spectrometry. Briefly, 1.0 mL of AgNPs was mixed with 200 µL of different concentrations of H_2O_2 (0, 0.5, 1.0, 5.0, 10, 20, 30, 40, 50, and 100 µM), and the mixture was vortexed and then incubated at 37°C for half an hour. The absorbance changes for the AgNPs colored solutions were recorded at 416 nm. The obtained data were used to determine the calibration curve, detection range, limit of detection (LOD), and other analytical parameters. All experiments were performed in triplicates (n=3).

The reaction conditions were optimized for pH, temperature, and incubation time. To investigate the pH effect, similar reactions were applied at pH values ranging from pH2 to pH10 after addition of 200 µL of H_2O_2 (40 µM) to 1.0 mL AgNPs solution and incubated for 30 minutes at 37°C, the response of the reaction to various pH environments was recorded by UV-Vis spectra. To find the optimized reaction time and temperature, the reaction containing 40 µM H_2O_2 (200 µL) and 1.0 mL AgNPs was carried at different times (15, 30, 45, and 60 min), and different temperatures (25, 30, 35, 40, 45 and 50°C), respectively before recording UV-Vis spectra. The PVP concentration was optimized in the same conditions with varying concentrations of PVP (1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg/mL).

Results

Digital metrology validation

Accurate digital color metrology relies on a combination of image fidelity, correct color encoding, resolution integrity, and reliable interpretation of pixel-level data. In computational colorimetry, colors are represented using various color spaces, the most widely adopted being RGB (Red, Green, Blue), HSV (Hue, Saturation, Value), CIE Lab, and CMYK (Cyan, Magenta, Yellow, Black) {Phuangsaijai, 2021 #31;Sirisathitkul, 2025 #30}. These color spaces are designed to approximate human visual perception through mathematically defined transformations. For example, the canonical conversion from sRGB to CIE Lab involves an intermediate CIE XYZ space, where the CIE76 color difference, $\Delta E*76$, is computed as described in the method section. This metric provides a perceptual measure of color accuracy, where values of $\Delta E<1$ indicate imperceptible differences to the human eye. Additionally, pixel resolution, defined as dots per inch (DPI), affects the sampling density of a digital image, influencing spatial clarity but not always its colorimetric integrity, particularly when color sampling is averaged from larger homogeneous regions {Tiuftiakov, 2021 #32}.

Fig. S1A demonstrates additive (RGB) and subtractive (CMYK) color models through overlapping color diagrams and the perceptual color wheel. In additive mixing, the primary colors Red, Green, and Blue combine to generate secondary colors Cyan, Magenta, and Yellow, while simultaneous combination of RGB produces white, highlighting the light-based color model used in digital imaging. Conversely, in CMYK (subtractive) systems used in printing, color generation is based on light absorption rather than emission, where black represents a chromatic mixture of CMY pigments. The central color wheel gamut visually represents the spectral range of visible colors, indicating how the digital RGB model is confined to a subset of the human perceptible spectrum, conforming to ISO 12647 color reproduction standards {Bao, 2025 #34;Fay, 2024 #33}.

Fig. S1B visualizes color representation using RGB and HSV models, both processed by Chromaeus. The RGB color cube represents color intensity as Cartesian coordinates along R, G, and B axes, each ranging from 0 to 255 (8-bit depth). This numerical representation aligns with ISO/IEC 11664-1 standards for digital color encoding. In contrast, the HSV model visualizes Hue (H), Saturation (S), and Value (V) in a perceptually structured form, where hue is angular (0°-360°), saturation is radial (0-1), and brightness is vertical (0-1). HSV enables intuitive processing of saturation and shade, particularly in chromatic pattern detection {Bao, 2025 #34;Fay, 2024 #33}. The mathematical conversion from HSV to RGB used in Chromaeus follows:

$$R', G', B' = V \cdot (1 - S),\ V \cdot (1 - S \cdot f),\ V \cdot (1 - S \cdot (1 - f))$$

Where:

$$H' = \frac{H}{60},\ i = \lfloor H' \rfloor,\ f = H' - i$$

Final conversion to RGB space (8-bit normalized):

$$R = 255 \cdot R',\ G = 255 \cdot G',\ B = 255 \cdot B'$$

Chromaeus internally converts all extracted RGB values into the perceptually uniform CIE L*a*b* color space for ΔE (CIE76) evaluation. Following ISO/CIE 11664-4:2019 standards, RGB values are first converted to CIE XYZ using:

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = M \cdot \begin{bmatrix} R' \\ G' \\ B' \end{bmatrix}$$

Where M is the sRGB-D65 conversion matrix:

$$M = \begin{bmatrix} 0.4124564 & 0.3575761 & 0.1804375 \\ 0.2126729 & 0.7151522 & 0.0721750 \\ 0.0193339 & 0.1191920 & 0.9503041 \end{bmatrix}$$

The XYZ values are then converted to CIE Lab (L*, a*, b*) using:

$$L^* = 116 f\left(\frac{Y}{Y_n}\right) - 16$$

$$a^* = 500\left[f\left(\frac{X}{X_n}\right) - f\left(\frac{Y}{Y_n}\right)\right]$$

$$b^* = 200\left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right]$$

Where:

$$f(t) = \begin{cases} t^{1/3}, & t > 0.008856 \\ 7.787t + \dfrac{16}{116}, & \text{otherwise} \end{cases}$$

These transformations enable Chromaeus to convert raw image pixels into perceptually accurate colorimetry suitable for ΔE_{76} comparisons. The ΔE_{76} metric, defined in ISO/CIE 11664-6, quantifies the Euclidean distance between two colors in Lab space, as described by the formal in the method section. Values of $\Delta E < 1$ indicate no perceptible difference to the human eye, while $\Delta E < 2$ is considered acceptable in digital imaging workflows {Ripoll, 2023 #13;Minaker, 2021 #12}.

Fig. S1C illustrates how image resolution, measured in dots per inch (DPI), affects spatial density of pixels over a fixed physical surface. At low DPI, individual pixel boundaries are coarsely segmented, whereas at high DPI, pixel boundaries become visually seamless, producing smoother tone transitions and finer gradations. Although DPI directly influences spatial resolution, it does not necessarily affect color accuracy in large homogeneous regions, as Chromaeus relies on statistical pixel averaging rather than edge-dependent sampling.

These conceptual and mathematical foundations presented in Figure S3 support the subsequent experimental design and confirm that Chromaeus operates using robust, ISO-compliant, and perceptually accurate color science methods.
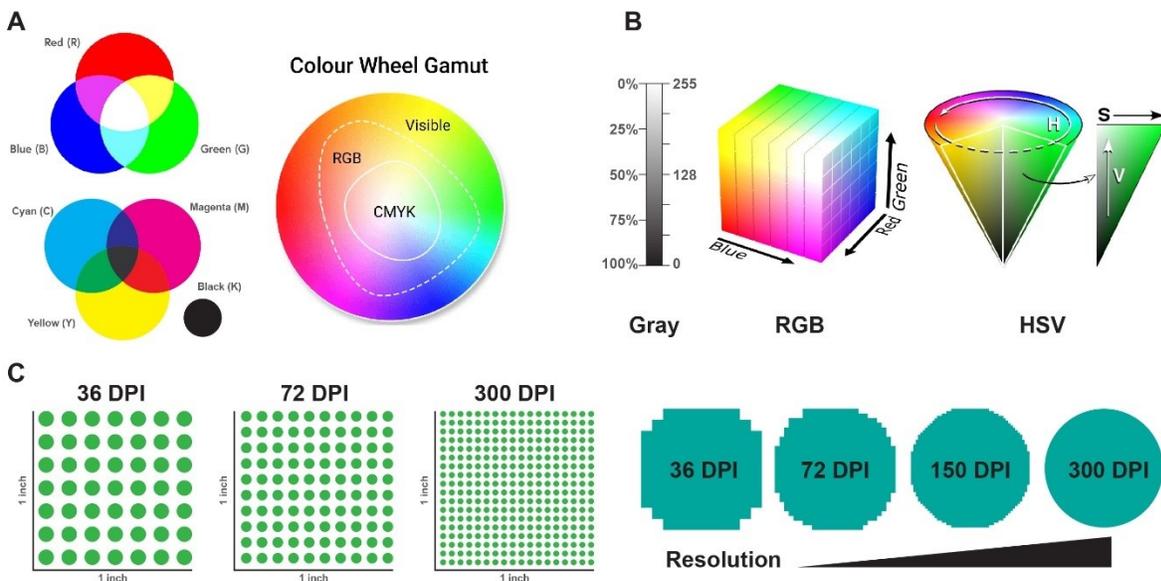

Fig. S1. Conceptual visualization of digital color models and resolution. (**A**) Additive (RGB) and subtractive (CMYK) color models with the visible gamut. (**B**) Representation of Grayscale, RGB cube, and HSV cone showing how digital colors are encoded and

interpreted. (**C**) Effect of resolution (DPI) on pixel density, illustrating how lower DPI reduces spatial clarity but does not necessarily affect colorimetric accuracy in homogeneous color regions.

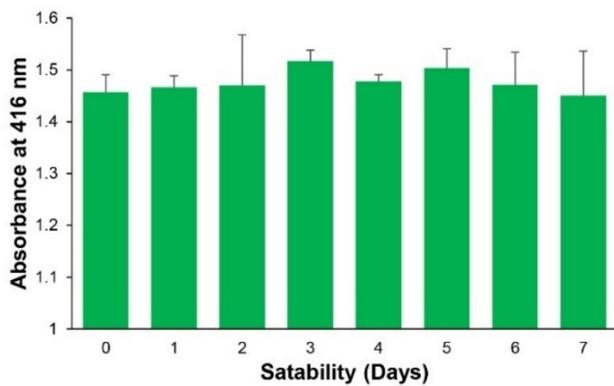## Stability of synthesized green-colored AgNPs

Fig. S2. Stability profile of PVP-AgNPs over seven days, showing consistent absorbance values at 416 nm, confirming minimal aggregation and strong colloidal stability.

Reaction optimization for H_2O_2 detection

The color shifting of green AgNPs by hydrogen peroxide occurs through oxidative dissolution, electron transfer, and surface modification reactions, which collectively weaken the surface plasmon resonance (SPR) responsible for the characteristic green coloration {Avenido, 2023 #23;Ismillayli, 2024 #22}. To ensure optimal performance for both spectrophotometric and image-based digital quantification, we optimized key reaction parameters including pH, temperature, incubation time, and PVP concentration. Each condition was evaluated using both full spectral scans (Fig. S3) and the corresponding residual absorbance at 416 nm (Fig. S3), which directly reflects the extent of nanoparticle color shifting.

Effect of pH

pH had a pronounced influence on color shifting kinetics. As shown in Fig. S3A, highly acidic conditions (pH 2-4) induced rapid and aggressive collapse of the SPR band, suggesting strong oxidative dissolution of AgNPs, but also increasing nonspecific nanoparticle degradation and instability. Conversely, under alkaline conditions (pH 9-10), the SPR peak was attenuated more gradually, indicating reduced oxidative efficiency of H_2O_2 likely due to decomposition into oxygen and water via base-catalyzed pathways {Molleman, 2017 #26;Qin, 2018 #25;Sequeira, 2021 #24}. The most favorable behavior was observed at pH 6-7, where the SPR band showed a smooth, progressive decline in intensity while preserving peak symmetry and spectral shape. This reflects controlled color shifting behavior that is both chemically meaningful and analytically suitable for digital quantification. The corresponding bar plot confirmed that residual absorbance values at pH 6-7 provided the best dynamic range while maintaining stability and reproducibility, making this range the optimal operational window for further experiments.

Effect of temperature

Temperature accelerated the color shifting rate in accordance with reaction kinetics, but excessive heat introduced structural instability. As shown in Fig. S3B, room temperature (25°C) produced slow and incomplete color shifting, whereas moderate heating (35-40°C) resulted in a stronger and more controlled reduction in plasmon intensity without compromising spectral definition. At temperatures ≥45°C, spectral broadening and baseline elevation indicated potential PVP desorption or nanoparticle destabilization, suggesting that chemical color shifting was accompanied by thermal degradation. Based on both spectral trends and quantitative absorbance data, 35-40°C was determined as the optimal temperature range, providing rapid yet stable nanoparticle color shifting while preserving spectral clarity for reliable digital extraction.

Effect of reaction time

Time-dependent monitoring revealed a sigmoidal color shifting pattern. As shown in Fig. S3C, minimal changes were observed during the first 10-15 min, followed by a rapid decline in SPR absorbance between 15 and 30 min, and finally a plateau beyond 45 min. The absorbance bar chart confirmed that 30 min provided a strong color shifting response while avoiding over-degradation or signal saturation. This time window also aligns well with practical image-based workflows, ensuring that color changes are chemically driven rather than occurring due to prolonged aggregation or sample instability.
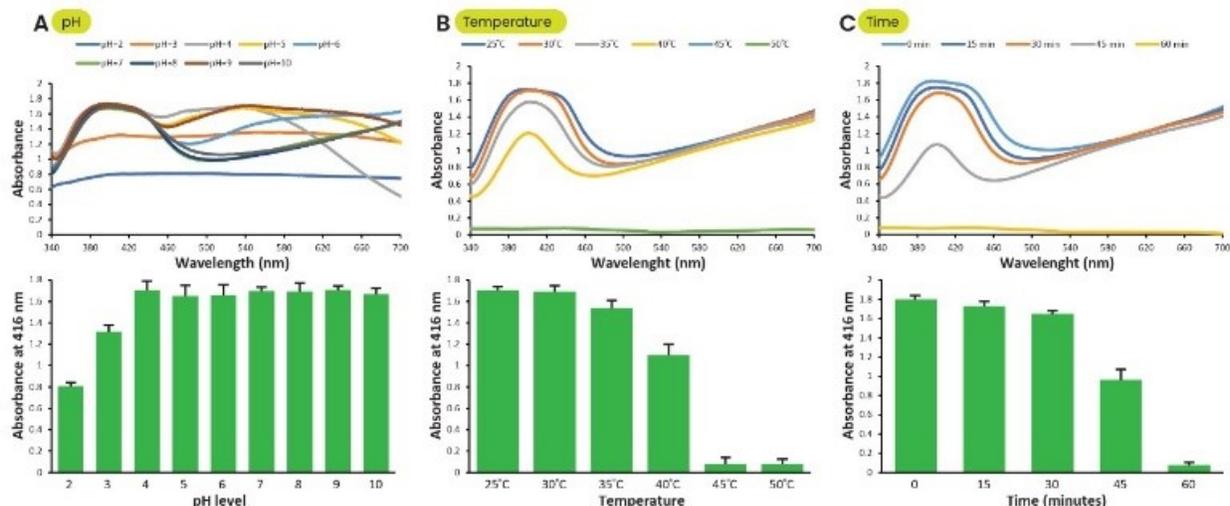
Fig. S3 Optimization of AgNP-H_2O_2 colorimetric reaction showing UV-Vis spectra (top) and corresponding absorbance at 416 nm (bottom) under varying (**A**) pH levels (2-10), (**B**) temperature (25-50°C), and (**C**) reaction time (0-60 min). All measurements were performed in triplicate (n = 3).

Effect of PVP concentration

The concentration of PVP influenced both the stabilization of nanoparticles and the optical clarity of the sensing system. As shown in Fig. S4, increasing PVP concentration from 1.0 to 6.0 mg/mL improved nanoparticle dispersion and led to higher SPR absorbance values, reflecting reduced aggregation and enhanced stabilization {Zein, 2022 #21}. However, excessively high PVP concentrations may hinder H_2O_2 access to the particle surface due to stronger steric shielding. Based on the absorbance trends, 4-5 mg/mL PVP provided the optimal balance between stability and reactivity and was selected for all subsequent experiments.

The trends observed across pH, temperature, time, and stabilizer concentration confirm that color shifting under optimized conditions is predictable, monotonic, and luminance-driven, supporting meaningful digital quantification using colors and brightness-based parameters such as RGB-G, HSV-V and CIELAB-L*. This controlled color fading behavior is critical for image-based calibration, allowing Chromaeus to reliably capture chemically driven changes rather than artifacts of aggregation, lighting, or instability.
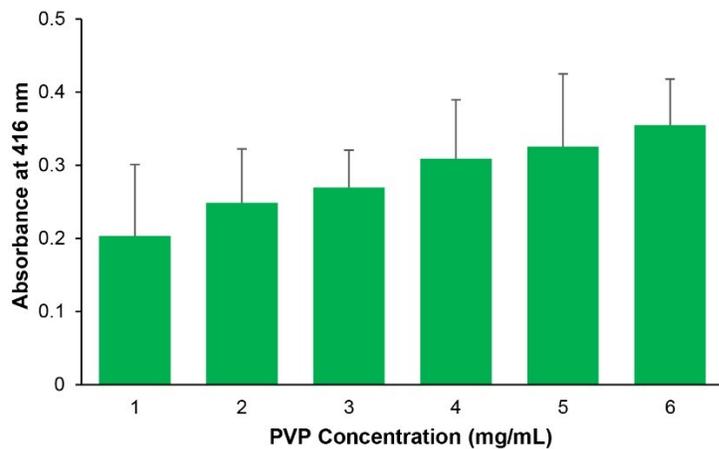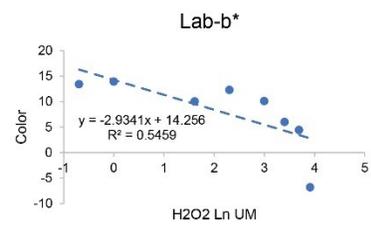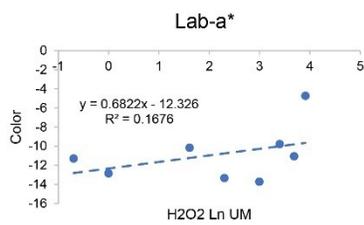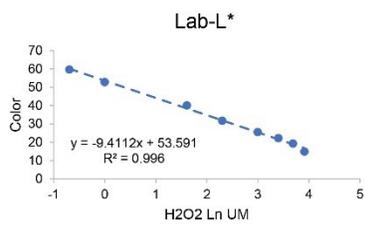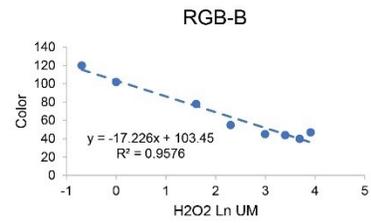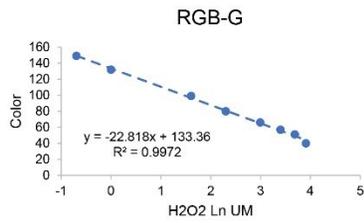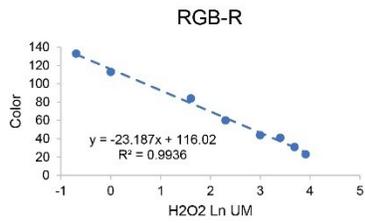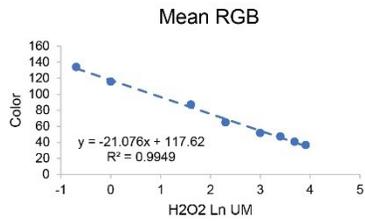
Fig. S4. Effect of PVP concentration (1-6 mg/mL) on SPR intensity at 416 nm.

Chromaeus extracted parameters

Fig. S5. Calibration curves calculated from various color parameters obtained from Chromaeus using the AgNPs based assay for H_2O_2 measurement.

Mean RGB

$y = -21.076x + 117.62$
$R^2 = 0.9949$

RGB-R

$y = -23.187x + 116.02$
$R^2 = 0.9936$

RGB-G

$y = -22.818x + 133.36$
$R^2 = 0.9972$

RGB-B

$y = -17.226x + 103.45$
$R^2 = 0.9576$

HSV-H

$y = 15.673x + 91.325$
$R^2 = 0.6141$

HSV-S

$y = 0.0492x + 0.202$
$R^2 = 0.6439$

HSV-V

$y = -0.0872x + 0.5214$
$R^2 = 0.9991$

Lab-L*

$y = -9.4112x + 53.591$
$R^2 = 0.996$

Lab-a*

$y = 0.6822x - 12.326$
$R^2 = 0.1676$

Lab-b*

$y = -2.9341x + 14.256$
$R^2 = 0.5459$

References