TRUE COLOR ANALYSIS OF PLANT PIGMENT BASED ON IMAGE REALIZATION STEGANOGAPHY TECHNIQUE

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Abstract

Digital steganography is the practice of concealing secret messages behind seemingly normal digital data that does not raise suspicion. This study introduces a novel method of genuine color analysis of plant pigments that resembles a thin film coated on paper chromatography, by analyzing digital images of plant pigments pixel by pixel, we can correlate the color data with the corresponding chromatogram, providing insights into the plant's biochemical composition, *referred to as Image Realization Steganography (IRS).* This is a mapping-based technique that hides the image cover file in an undetectable manner. The image cover is only intended for reference. The suggested method provides a cover-secret mapping that reveals the true color of a concealed plant pigment without affecting the reference image. Comparing the acquired data to IRS to estimate the true color of Wolffia globosa (L.) Wimm pigment was presented. The conclusions indicate that steganography is a viable option for determining the true color of plant pigments in the form of color codes, specifically RGB and CMYK.

Keywords: **Image Steganography; Image Realization Steganography; Wolffia globosa (L.) Wimm; Plant Pigment Paper Chromatography**

Introduction

The diverse colors of plants are attributed to various pigments that play crucial roles in photosynthesis and protecting plants from adverse environmental conditions (Lichtenthaler, 1987). Analyzing plant color is a fundamental tool in plant biology research and has applications in agriculture, food production, and medicine (Gitelson *et al*., 2003). Traditionally, plant color analysis has relied on complex and time-consuming chemical techniques such as spectrophotometry and chromatography (Markam, 1983), requiring expensive equipment and specialized expertise. However, rapid technological

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advancements have introduced novel methods for plant color analysis. This research leverages steganography techniques. Steganography involves embedding data within digital media like images, audio, or video without causing noticeable distortions (Johnson and Jajodia, 1998). Applying this technique to plant color analysis allows us to extract hidden color information directly from plant images, providing a faster, more accurate, and cost-effective method for field-based pigment assessment. Plant color analysis is pivotal in understanding plant biology as it reveals the molecular mechanisms behind pigment formation. This fundamental knowledge is essential for developing improved plant breeding techniques. Additionally, it can be applied to assess the quality of plant-based products such as vegetables, fruits, and herbs, as well as ensuring food safety. In this research, we employ Wolffia, a rapidly growing and small-sized plant, as our experimental model. Wolffia is also promising for applications in the food industry. By analyzing plant images at the pixel level, we can uncover hidden color patterns that indicate the types of pigments present. This technique offers a rapid and cost-effective alternative, *removing the reliance on complex equipment and reducing the use of hazardous chemicals.* Consequently, the objectives of this research are to develop a plant pigment analysis method using steganography.

Materials and Methods

Isolate and identify plant pigments using paper chromatography.

Paper chromatography is a technique used to separate mixtures based on the differential adsorption and solubility of the components on a stationary phase. In the case of plant pigments, the chromatography paper serves as the stationary phase, while a suitable solvent acts as the mobile phase.

Materials:

- Fresh plant samples (Wolffia)
- Ethanol (Jaitae, 2003)
- Chromatography paper
- Beakers
- Pencil
- Ruler
- Forceps
- Developing chamber
- Spray reagents (e.g., iodine solution, ninhydrin)

Methods:

Sample Preparation:

1. Grind the selected plant material into a fine powder using a mortar and pestle.
2. Extract the pigments by soaking the powdered sample in the chosen solvent.
3. Filter the extract to remove plant debris.

Spotting:

1. Using a capillary tube, apply a small spot of the extract onto a chromatography paper, approximately 2 cm from the bottom edge.
2. Allow the spots to dry completely before applying additional spots.

Development:

1. Place the spotted chromatography paper into a developing chamber containing the solvent.
2. Ensure the solvent level is below the spotted line.
3. Close the chamber and allow the solvent to ascend the paper until the desired height is reached.

Visualization:

1. Remove the chromatogram from the chamber and allow the solvent to evaporate.
2. Visualize the separated pigments under visible light or by using specific spray reagents (e.g., iodine for general visualization, ninhydrin for amino acids).

Calculation of Rf values:

1. Measure the distance traveled by the solvent front and the distance traveled by each pigment spot.
2. Calculate the Rf value for each spot using the formula:

Rf = distance traveled by pigment / distance traveled by solvent.

Data Analysis:

1. Compare the Rf values obtained with standard values for known pigments to identify the pigments present in the plant extract.
2. Document the results, including the Rf values, color of the pigment spots, and any observations.

Steganography techniques

A digital image is a finite collection of picture elements, known as pixels. Each pixel has a specific location and a corresponding color value. The color of each pixel is determined by a combination of the three primary colors: Red, Green, and Blue (RGB). This color value can be represented using binary, hexadecimal, or decimal formats. The number of bits used to represent each RGB component determines the color depth and, consequently, the image quality. Many image file formats utilize 8 bits to represent a single pixel, translating to approximately 2-3 bits per color component.This color information within the image can be manipulated to embed secret data, Forming the basic of image steganography. (Westfeld, (2010); Hussain and Hussain, (2013); Subhedar and Mankar, (2014)). By subtly modifying the least significant bits (LSBs) of pixel color values, it is possible to encode secret messages without significantly affecting the visual appearance of the image.

Steganography techniques can be classified based on the type of multimedia data used (e.g., images, audio, video) and the reversibility of the

Steganography techniques can be classified based on the type of multimedia data used (e.g., images, audio, video) and the reversibility of the process. Reversible techniques allow for the complete recovery of the cover object, while irreversible techniques may result in some loss of original data quality, Call Image Realization Steganography (IRS).

Image Realization Steganography (IRS) is a sophisticated and contemporary data hiding technique that leverages mathematical principles and image processing to embed secret information within digital images without introducing perceptible distortions to the resulting image (Bhattacharyya *et al*, 2009). IRS operates by constructing a mathematical model of the original image. Subsequently, secret data is embedded within this model by modifying certain parameters, such as altering coefficients in different domains (e.g., frequency domain or wavelet domain). Upon reconstructing a new image from-the modified model, the resulting image appears visually identical to the original, yet it covertly contains the embedded data. Figure 1.

Proposed Method

A Comparative Study of Wolffia Growth Under Different Light

ConditionsExperimental Setup

***Plant Material*:** An equal number of Wolffia plants were evenly distributed into three experimental groups: LED light, natural light, and a dark control.

***Growth Conditions*:** Each group was cultivated in separate beakers containing an equal volume of nutrient solution.

Experimental Duration: The experiment was conducted over a 15-day period.

Data Collection and Processing

Paper Chromatography: Paper chromatography was employed to separate the pigments present in the Wolffia plants.

Image Acquisition and Processing:

1. Standardized Imaging: Digital images of the chromatograms were captured under controlled lighting conditions, The process of converting a physical image into a digital one involves using a scanner with a light source and light-sensitive sensors to capture the image. The analog signals are then converted into digital signals using an analog-to-digital converter (ADC). The digital data is processed to enhance image quality, such as adjusting brightness, sharpness, and color correction. Finally, the digital image is saved in a digital file format for storage and use (Bentley Historical Library, 2012).

2. Image Enhancement: HDR tone mapping was applied to enhance color visualization without introducing artifacts.

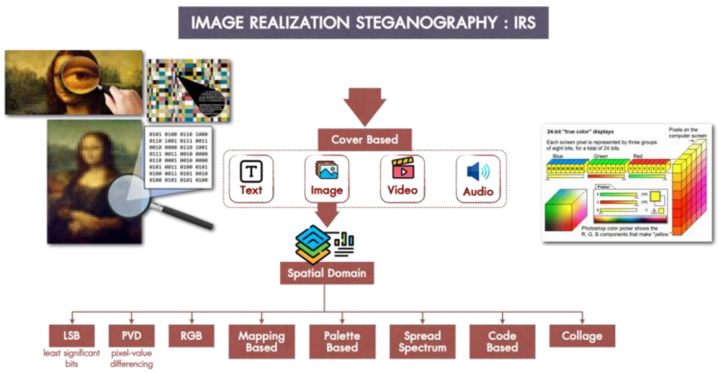

Figure 1. **classified based on the type of multimedia data used (e.g., images, audio, video) and the reversibility of the process**

3. Edge Detection: Edge detection algorithms were used to accurately delineate the boundaries of the pigment bands.

4. Color Edge Detection: Color edge detection was further applied to refine the color boundaries.

Data Analysis:

1. Rf Value Calculation: Retention factor (Rf) values were calculated for each pigment band.

2. Statistical Analysis: Kolmogorov-Smirnov test: This test was used to compare the distribution of Rf values among the three groups.Friedman test: This test was employed to compare the RGB and CMYK color values of the pigments.

3. Data Visualization: Line graphs were generated to visualize the trends in Rf values.

Steganography for Color Extraction

Color Value Extraction: Steganography techniques were utilized to extract precise RGB and CMYK color values from the chromatograms.

Data Analysis and Interpretation

Statistical Analysis: Statistical tests were conducted to determine if there were significant differences in Rf values and color values among the different light conditions.

Interpretation: The results of the statistical analysis were interpreted to understand the impact of light conditions on the pigment composition of Wolffia plants.

Results and Discussion

This section is divided into 3 parts, as follows:

Part 1: Image Realization Steganography (IRS).

The color profiles of the pigment bands observed in the paper chromatograms, as visualized through digital image analysis, exhibited a high level of similarity and continuity across the LED, natural, and dark conditions. Figure 2.

We employed steganography techniques within the spatial domain of the image. The process involved three steps: (1) HDR tone mapping enhanced color representation; (2) edge detection algorithms delineated color boundaries; and (3) color edge detection refined these boundaries. Figure 3.

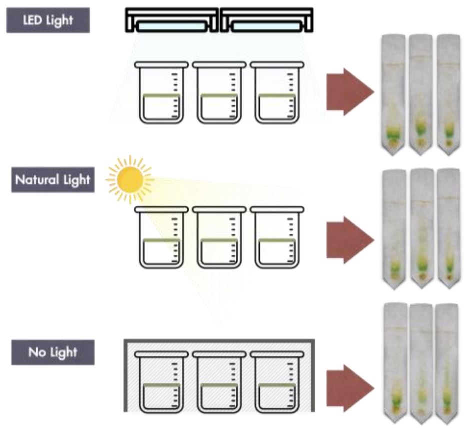

Figure 2. **Show a digital image obtained from the paper chromatography experiment**

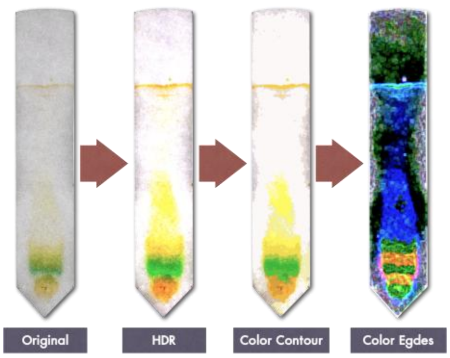

Figure 3. **Shows the IRS output image showcases HDR tone, color contour, and boundary features**

Table 1. **Show the distance measurement of the colorto obtain the Rf value**

Conditions	Diameter (cm)				
	Green	Yellow-Green	Yellow-Brown	Yellow	Orange
	Chlorophyll b	Chlorophyll a	Xanthophyll	Phaeophytin	Carotene
LED	0.83	1.24	1.77	4.23	7.23
	1.39	1.84	2.97	4.58	7.94
	1.07	1.49	2.05	5.25	7.3
Nature	1.01	1.35	2.06	5.4	6.95
	1.08	1.31	2.45	6.52	6.95
	1.25	1.77	2.94	5.68	7.38
Dark	1.07	1.35	2.02	6.73	7.62
	1.25	1.52	2.83	5.7	7.41
	1	1.45	2.12	5.94	7.34

Following IRS processing, the three images were aligned to determine the location of distinct colors. Figure 4. Measurements were taken from the origin to each color spot, allowing for the calculation of the relative retention factor (Rf) for each pigment under the three experimental conditions. Table 1-2 and Figure 5.

The retention factors of the pigments were found to be consistent across all three conditions. To statistically verify this observation, a Kolmogorov-Smirnov One Sample Test was performed, confirming that the retention factors were not significantly different at the 0.005 level significance.

Steganography allowed for a high degree of precision in determining the color values of plant pigments, which were subsequently expressed in RGB and CMYK color systems. Table 3-5 and Figure 6.

Friedman test analysis of RGB and CMYK values revealed no significant differences at a 0.05 significance level.

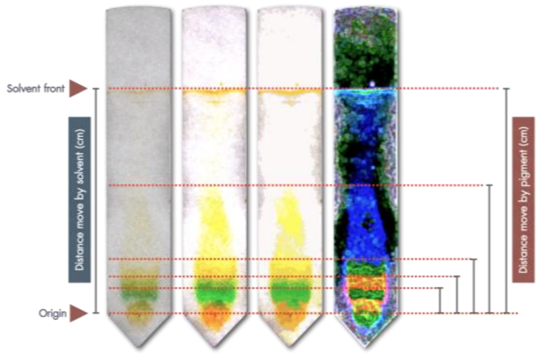

Figure 4. Shows identify the positions of different colors

Table 2. Shows the RF values were calculated forsubsequent statistical analysis to determine if there were significant differences

Conditions	Rf Value				
	Green Chlorophyll b	Yellow-Green Chlorophyll a	Yellow-Brown Xanthophyll	Yellow Phaeophytin	Orange Carotene
LED	0.11	0.17	0.24	0.59	1.00
	0.18	0.23	0.37	0.58	1.00
	0.15	0.20	0.28	0.72	1.00
Nature	0.15	0.19	0.30	0.78	1.00
	0.16	0.19	0.35	0.94	1.00
	0.17	0.24	0.40	0.77	1.00
Dark	0.14	0.18	0.27	0.88	1.00
	0.17	0.21	0.38	0.77	1.00
	0.14	0.20	0.29	0.81	1.00

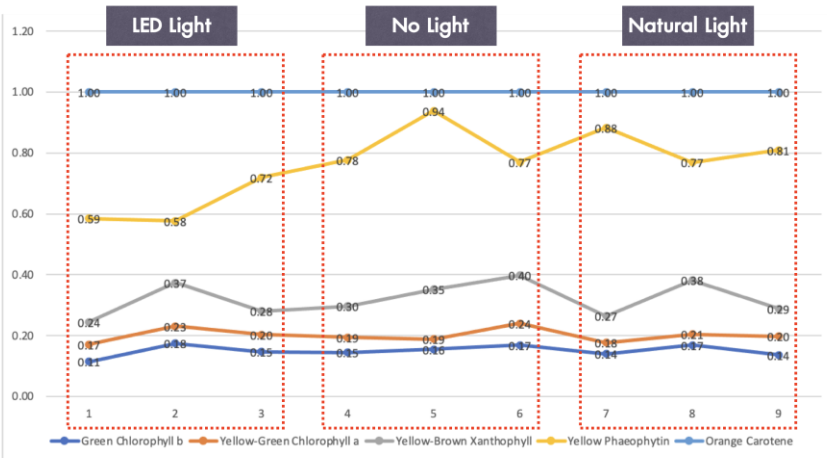

Figure 5. Shows visualize the trends in retention factors across different conditions, we plotted the data as line graphs

Table 3. Show numerical values for RGB and CMYK colors in LED Condition

Conditions	Plant Pigment	Color	Color System		Sample (color code) Sample-1	Sample-2	Sample-3
LED	Chlorophyll b	Green	RGB	R	1	1	1
				G	195	167	170
				B	1	0	1
			CMYK	C	75	82	82
				M	0	5	4
				Y	100	100	100
				K	0	1	1
	Chlorophyll a	Yellow-Green	RGB	R	148	145	179
				G	188	168	188
				B	1	0	1
			CMYK	C	48	49	35
				M	7	18	13
				Y	100	100	100
				K	0	1	0
	Xanthophyll	Yellow-Brown	RGB	R	254	254	254
				G	223	223	226
				B	1	1	1
			CMYK	C	2	2	2
				M	8	8	6
				Y	99	99	99
				K	0	0	0
	Phaeophytin	Yellow	RGB	R	255	253	253
				G	253	242	247
				B	155	154	142
			CMYK	C	3	2	3
				M	0	1	0
				Y	48	49	54
				K	0	0	0
	Carotene	Orange	RGB	R	255	254	254
				G	206	213	186
				B	1	8	1
			CMYK	C	1	2	0
				M	18	14	29
				Y	99	99	100
				K	0	0	0

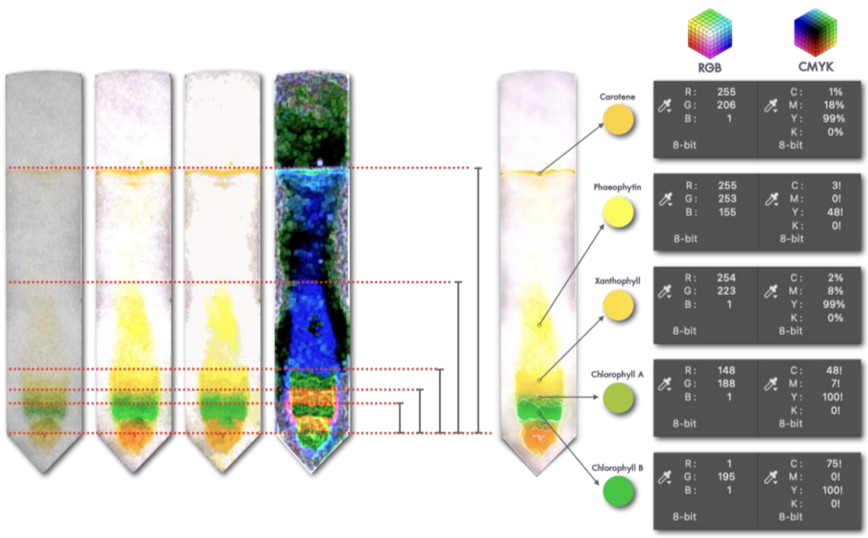

Figure 6. Shows color values of plant pigments in both RGB and CMYK color spaces

Based on our findings, we can conclude that steganography offers a reliable and precise method for determining the true color of plant pigments. However, it is important to note that our results are specific to the plant species examined in this study. Furthermore, our analysis revealed a remarkable consistency in the color of plant pigments across different experimental conditions, suggesting that the color of plant pigments is largely determined by the plant's genetic makeup and is likely a result of natural selection.

Table 4. Shows numerical values for RGB and CMYK colors in Nature Condition

Conditions	Plant Pigment	Color	Color System		Sample (color code)		
					Sample-1	Sample-2	Sample-3
Nature	Chlorophyll b	Green	RGB	R	1	1	1
				G	128	195	160
				B	1	0	0
			CMYK	C	70	75	83
				M	0	0	9
				Y	100	100	100
				K	0	0	0
	Chlorophyll a	Yellow-Green	RGB	R	139	145	125
				G	204	191	168
				B	1	0	0
			CMYK	C	51	49	57
				M	0	4	15
				Y	100	100	100
				K	0	0	0
	Xanthophyll	Yellow-Brown	RGB	R	254	253	253
				G	222	228	220
				B	1	0	1
			CMYK	C	2	3	2
				M	8	5	9
				Y	99	99	100
				K	0	0	0
	Phaeophytin	Yellow	RGB	R	253	255	254
				G	251	255	251
				B	132	182	190
			CMYK	C	4	2	2
				M	0	0	0
				Y	58	35	31
				K	0	0	0
	Carotene	Orange	RGB	R	254	255	254
				G	155	134	157
				B	1	1	1
			CMYK	C	0	0	0
				M	46	58	45
				Y	100	100	100
				K	0	0	0

Table 5. Shows numerical values for RGB and CMYK colors in Nature Condition

Conditions	Plant Pigment	Color	Color System		Sample (color code)		
					Sample-1	Sample-2	Sample-3
Dark	Chlorophyll b	Green	RGB	R	4	12	2
				G	162	224	171
				B	1	0	0
			CMYK	C	83	69	82
				M	8	0	3
				Y	100	100	100
				K	1	0	1
	Chlorophyll a	Yellow-Green	RGB	R	175	179	120
				G	191	202	202
				B	1	0	1
			CMYK	C	37	35	57
				M	11	4	0
				Y	100	100	100
				K	0	0	0
	Xanthophyll	Yellow-Brown	RGB	R	255	250	253
				G	226	206	219
				B	1	0	0
			CMYK	C	2	2	2
				M	6	17	10
				Y	99	100	100
				K	0	0	0
	Phaeophytin	Yellow	RGB	R	255	254	254
				G	253	251	253
				B	171	193	11
			CMYK	C	2	2	6
				M	0	0	0
				Y	40	30	95
				K	0	0	0
	Carotene	Orange	RGB	R	255	251	255
				G	190	173	207
				B	3	6	2
			CMYK	C	0	1	1
				M	27	36	17
				Y	100	100	99
				K	0	0	0

Conclusions

The conclusions indicate that steganography is a viable option for determining the true color of plant pigments in the form of color codes, specifically RGB and CMYK. However, it should be noted that our results are specific to the plant species examined in this study. Additionally, our analysis revealed a remarkable consistency in the color of plant pigments under different experimental conditions, suggesting that the color of plant pigments is primarily determined by the plant's genetic makeup and is likely a result of natural selection. This study investigates the correlation between the visible spectrum and the RGB and CMYK color spaces, aiming to determine color values from digital files.

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