Investigating the Effects of Substrate Color on the Detection of Ruhemann's Purple Using Confocal Raman Microscopy

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INTRODUCTION

Ninhydrin has long been the primary reagent for visualizing latent fingerprints on paper in forensic casework. However, the color of the underlying paper or printed background can reduce contrast and degrade the quality of developed impressions, especially on printed or dark-colored substrates where friction ridge detail may be obscured. Because fingerprint examination relies on human interpretation, the visual contrast and clarity of ridge detail can directly influence examiners' judgments.

Raman spectroscopy provides high sensitivity and spectral resolution for analyzing pigments and dyes and is increasingly used in forensic science as a non-destructive analytical tool. In this study, we evaluate whether Raman spectroscopy can objectively detect Ruhemann's purple, the ninhydrin reaction product, on color-dyed paper and assess its potential as a complementary spectroscopic approach for latent fingerprint examination.

MATERIALS & METHODS

Sample preparation and ninhydrin treatment

Colored copy papers were used as porous substrates to evaluate RP Raman signals on different background colors. Latent fingerprints were deposited with a commercial latent print pad, and 10 µL bovine blood stains were smeared on each substrate and dried for 24hrs at room temperature.

Fingerprints and bloodstains were then developed with a 0.5% (w/v) ninhydrin working solution in acetone (400 µL per sample) following the Tennessee Bureau of Investigation protocol and air-dried at room temperature without additional heating to avoid additional variables.

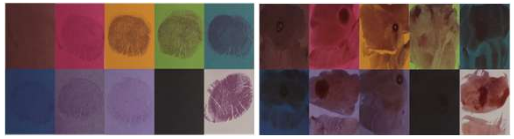

Figure 1. Ruhemann's purple developed on fingerprint residue (left), and Ruhemann's purple developed with ninhydrin on a blood sample. (right)

Raman Spectroscopic Measurements

Raman spectra were collected using an inVia™ Inspect confocal Raman microscope (Renishaw, UK) equipped with a 100× objective and either 532 nm or 785 nm excitation, over the spectral range of 200–2200 cm^{-1}. Five spectra (N = 5) were acquired per sample using a 10 s exposure with three accumulations, with laser power optimized for each sample. Spectra were baseline-corrected and processed using Spectragryph (v1.2.16.1, Oberstdorf, Germany).

RESULTS & DISCUSSION

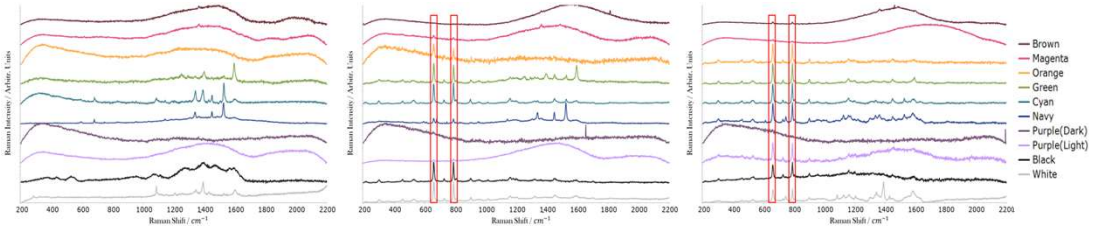

Figure 2. Raman spectra acquired using a 532 nm laser: background (left), Ruhemann's purple (RP) developed on fingerprint residue (center), and Ruhemann's purple developed on a bloodstain (right).

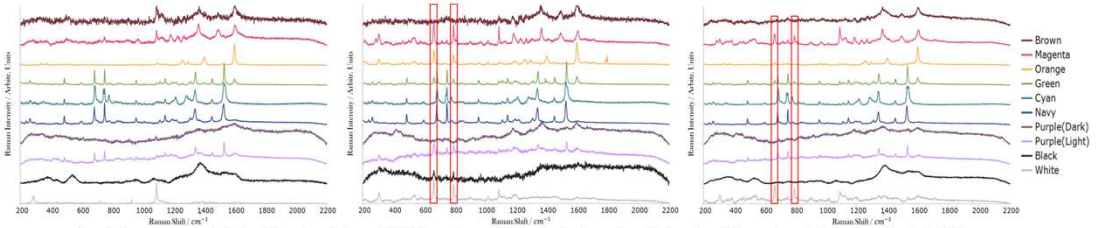

Figure 3. Raman spectra acquired using a 785 nm laser: background (left), Ruhemann's purple (RP) developed on fingerprint residue (center), and Ruhemann's purple developed on a bloodstain (right).

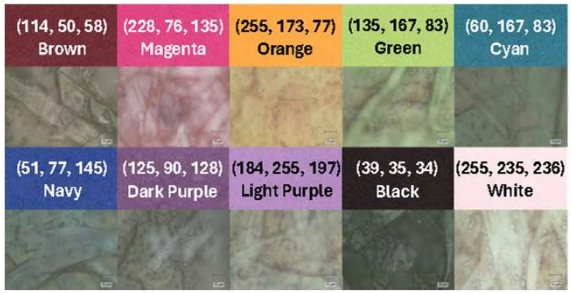

Figure 4. Background color photographs acquired under fluorescent lighting (ISO 640, 1/90 s, f/1.8, white balance 4100 K) and corresponding sample surface images recorded at 100× magnification using a Raman microscope (white light intensity 100%, A-stop 100, F-stop 100). RGB values were measured from the photographs to represent the background color of each paper.

Effect of laser power

- Laser power influenced both sample degradation and the signal-to-background ratio (SBR). GM1
- Higher laser powers were generally acceptable on brighter papers but resulted in visible damage on darker substrates.
- Increasing laser power did not necessarily enhance RP bands but could amplify background signals, so power needed to be optimized for each substrate.

Excitation wavelength and fluorescence interference

- At 532 nm, RP peaks were observable on most substrates, and blood-containing samples showed peaks and bands in the 1100–1700 cm^{-1} region consistent with blood-specific bands.
- At 785 nm, spectra were frequently dominated by paper and dye signals, and RP bands were often absent or obscured.

Dark-colored substrates

- On brown and dark purple papers, strong fluorescence and elevated baselines at both wavelengths obscured RP bands.
- Dark-colored papers included black, brown, magenta, cyan, navy, and dark purple (**Figure 4**) and provided poor visual contrast, so friction ridge details and blood stains were not clearly visible.
- On black substrates, locating RP spots was challenging even under microscopic examination.

Table 1. RP detection outcome and optimized laser power (%) for ninhydrin treated fingerprint residues (FPR) and blood (BL) on different colored paper substrates GM3

	532nm				785nm			
	FPR	%	BL	%	FPR	%	BL	%
Brown	🔴	-	🟡	-	🔴	-	🔴	-
Magenta	🟡	0.05	🟡	100	🟢	100	🟢	50
Orange	🟢	0.05	🟢	100	🟢	100	🟡	10
Green	🟢	1	🟢	100	⚪	100	⚪	10
Cyan	🟢	5	🟢	10	🔴	10	🔴	5
Navy	🟢	5	🟢	5	🔴	5	🔴	5
Dark Purple	🔴	-	🔴	50	🔴	50	🔴	5
Light Purple	🟡	5	🟢	100	🟢	100	🔴	50
Black	🟢	0.5	🟢	1	🟡	1	🔴	5
White	🟢	5	🟢	100	🟢	100	🟢	10

🟢 Green: Strong RP signal
🟡 Yellow: RP signal is detectible, but very weak
🔴 Red: No detection of RP
⚪ Grey: Unsure where the signals came from

CONCLUSIONS

- Laser power must be optimized for each substrate color and sample type; in this study, increasing laser power did not guarantee enhanced RP signals but increased background interference.
- RP signals were detectable on several dark-colored papers, indicating that Raman measurements can provide clearer friction ridge detail for fingerprint examination even when visual contrast is poor.
- At 532 nm, RP peaks and blood-specific bands were clearly observed across background colors, whereas 785 nm spectra were dominated by paper and dye signals rather than RP or blood.
- Overall, these results show that Raman spectroscopy can complement conventional ninhydrin processing by providing objective confirmation of RP, but further studies and careful consideration of practical implementation are required before routine casework use.

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Slide 1

GM1 Do you mean the signal-to-noise ratio (SNR)?
Monjardez, Geraldine, 2026-02-01T18:07:50.633

GM2 Since both Dr. Yu and I are co-authors on the poster, you don't need to include us in the acknowledgments section.
Monjardez, Geraldine, 2026-02-01T18:13:08.874

GM3 Instead of using X and O, which can be difficult to see for some people, you could use a neutral color table and a color code. Green for detected, orange for very weak and red for not detected. I understand that you may not have time to re-do it before tomorrow, but keep that in mind for future poster presentations.
Monjardez, Geraldine, 2026-02-01T18:17:10.728