The effect of washing and blood enhancement reagents on the use of Raman spectroscopy for human blood identification

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ABSTRACT

Raman spectroscopy has been demonstrated to have the ability to identify body fluids commonly found in forensic investigations. This study aimed to further scrutinize Raman spectroscopy's ability to identify human blood in simulated evidence samples. Blood stains on a variety of fabrics were laundered and subsequently enhanced using commonly used blood enhancement reagents. Stains were also extracted from their substrate and analyzed. It was determined that Leuco Crystal Violet (LCV) and Coomassie blue create too much interference to identify the blood while on the fabric. However, it was possible to subtract the Luminol signal from the spectra, thereby providing a usable blood signature. Further to this, by extracting the fluids from their substrates, even post enhancement, it was possible to identify blood treated with Luminol and LCV. This study further explored the ability of Raman to identify human blood, not simply on laboratory clean samples, but on simulated evidence samples, thereby highlighting the utility of this technique and potential use in forensic casework.

INTRODUCTION

Many of the current methods for identifying human body fluids can be costly, in terms of both time and money, give varying levels of accuracy, and consume the sample, preventing further analysis. Raman spectroscopy has been an area of focus for the development of a non-destructive technique for body fluid identification. Raman spectroscopy has gained interest from the forensic science community due to its broad applications, requiring no reagents for preparation, as well as its non-destructive nature (Virkler & Lednev, 2010). Raman spectroscopy can be performed on minimal amounts of sample, making it ideally suited for work with trace evidence. The development of portable Raman spectrometers has also contributed to the technique's growing popularity, as they allow for analysis to be performed in the field as opposed to waiting for laboratory results (Eckenrode et al., 2001). Therefore, investigation of this method on simulated evidence samples is fully warranted requires exploration.

OBJECTIVE

To determine if Raman spectroscopy is capable of identifying blood on simulated evidence samples, including:

1. A variety of fabric types
2. At varying dilutions and volumes
3. Post blood enhancement using commercial reagents
4. Post extraction of the fluids from the fabrics

METHOD & MATERIALS

Following IRB approval, venous blood was gathered from volunteers into sterile vacutainer EDTA vials and stored at 4°C. Using a Thermo Scientific DXR Raman Microscope equipped with a 780-nm wavelength laser and the OMNIC software, Raman spectroscopy was performed on the human blood samples under a variety of conditions. These conditions included five fabrics (black and white cotton, black and white polyester, and denim), a dilution series (from 1:10 to $1:10^6$, both dry and wet), and after laundering and treatment with three enhancement reagents (Leuco Crystal Violet (LCV), Coomassie blue, and Luminol). A method of extracting the stain from the fabrics was also tested. Baseline corrections for fluorescence were performed as necessary in OMNIC. Spectral subtractions were performed using the GRAMS/AI 7.0 software. A library reference spectrum was created for human venous blood, then using OMNIC software, compared to the obtained spectra to obtain a match score.

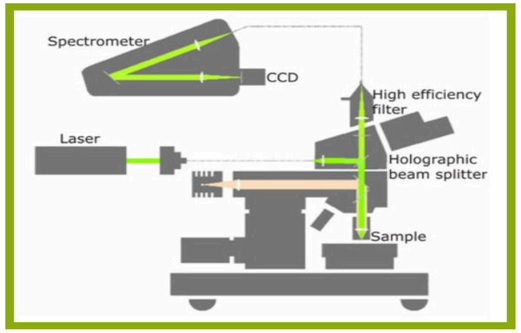

Figure 1: A diagram demonstrating Raman spectroscopy. The laser hits a sample focused under the microscope, and is scattered. That scattered light is then gathered and read by the spectrometer, which gives the spectrum.

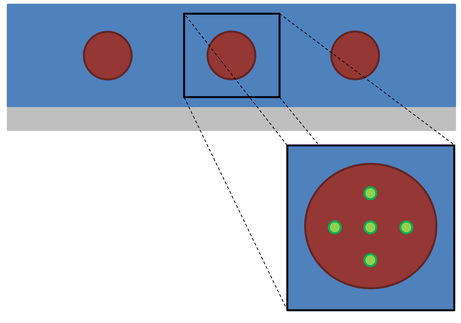

Figure 2: Example of a microscope slide with samples on a fabric used in this research. Also shown in enlarged section is the typical sampling pattern used to collect spectra from five different areas of the stains.

RESULTS

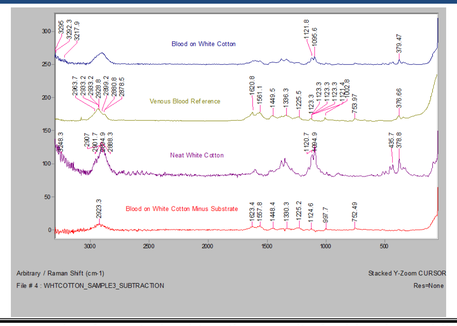

Figure 3: Example of spectral subtraction results for blood on white cotton.

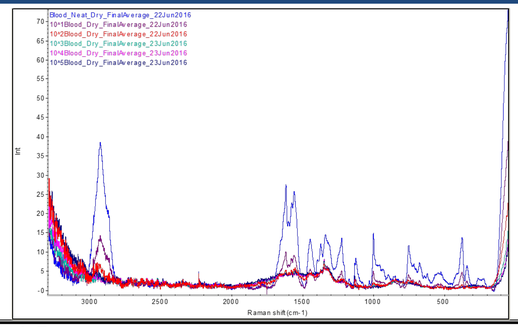

Figure 4: Final averaged spectra of dried dilution series.

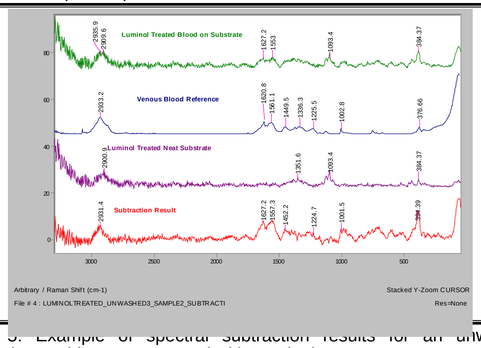

Figure 5: Example of spectral subtraction results for an unwashed bloodstain on white cotton treated with Luminol.

	WC	BC	WP	BP	D
10	40.97	33.23	37.62	33.85	38.62
50	36.30	33.79	35.57	33.08	38.95
100	39.60	44.68	38.48	33.10	37.70
150	42.81	42.12	37.15	42.62	41.01
200	38.32	39.19	35.56	39.62	39.90
250	41.73	37.69	35.22	40.76	42.03
1:10	37.29	32.55	34.48	31.99	31.11
1:100	29.52	29.32	30.46	29.48	29.56

Figure 6: Table showing average match scores for blood on White Cotton (WC), Black Cotton (BC), White Polyester (WP), Black Polyester (BP), or Denim (D) at the volume in microliters (neat blood) or of 150 microliters of the shown dilution.

DISCUSSION

This study revealed the utility of Raman spectroscopy for blood identification in a variety of simulated evidence samples.

Objective 1:
The substrate analysis revealed that blood could be detected on the white cotton and polyester, but not on the black cotton and polyester or denim.

Objective 2:
Dilution testing determined that while blood could be detected down to a dilution factor of 1:100 when dry, only neat blood was detected while still wet.

Objective 3:
The enhancement testing revealed that any of the laundered samples gave no blood signal after being washed. The unwashed samples treated with Luminol were the only samples to give a blood signal on the fabric, while the Luminol and LCV samples matched to the human blood reference post-extraction.

Objective 4:
The extraction results showed that while blood was detected post-extraction, the match scores were still low. Three matches did not give the blood reference as the highest match, but still had it in the top ten matches. It was also noted that small fibers were found in the samples under the microscope, suggesting the possibility of interference from the fabrics.

CONCLUSIONS

These results have demonstrated that Raman spectroscopy is capable of detecting the presence of human blood in simulated evidence samples after laundering and enhancement. They also show that while extracting the fluids from the substrates may be an alternative to spectral subtraction, there will need to be more research to find a method to obtain more robust signals.

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