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Near-infrared excitation Raman analysis of Underlying colorants on redyed fabric

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Forensic analysis of fabric is often critically important to establish a relationship between a suspect and a crime scene or demonstrate the absence of such connections. Most of commercially available fabric is colored with primarily organic colorants. These dye substances are highly fluorescent, which limits the use to conventional Raman spectroscopy for the analysis of the colorant content of fabric. At the same time, elucidation of the chemical composition of dyes in fabrics can be used to advance the importance of this physical piece of evidence for forensic research. Our recent findings showed that near-infrared excitation Raman spectroscopy (NIeRS) could be used to overcome this limitation. However, it remains unclear to what extent NIeRS could be used to identify the presence of several different colorants on fabric, as well as utilize for the analysis of dyes on fabric contaminated with paints. In this study, we utilized a hand-held NIeRS instrument to ex-amine re-colored cotton fabric and cotton fabric with household paints applied on it. Our results indicate that NIeRS coupled with chemometrics highly accurately identify the presence of several colorants on cotton. We also found that the presence of paint fully obscures the ability of NIeRS to extract the information about the dye content of the fabric. These results expand our understanding of the use of NIeRS in the forensic analysis of colored fabric.

Introduction

Fabric together with other physical pieces of evidence is often found at crime scenes.[1] Forensic analysis of fabric can be used to detect traces of illicit drugs, explosives and other important substances.[2,3] Microscopic examination of fabric can be also utilized to establish a connection between a suspect and a crime scene. Such analysis is primarily done by pattern recognition and visual comparison of fiber pigments.[4] Although straightforward and unexpensive, this approach is highly subjective.[1] Therefore, there is an on-going search for alternative techniques that can provide more accurate information about the fabric material.[5–7]

Raman spectroscopy (RS) is a powerful technique that can be used for non-invasive and non-destructive analysis of structure and composition of samples of interest.[5–7] RS is based on the phenomenon of inelastic light scattering, which takes place upon the excitement of molecules in the sample to higher vibrational and rotational states.[8] Consequently, inelastically scattered photons will have energies that correspond to certain molecular vibrations present in the sample. Acquisition and analysis of such photons allows for structural and compositional analysis of samples. Several research groups showed that RS has successfully been used to identify urine, sweat, semen, blood, hair colorants, and gunshot residue.[9–17] There is also a growing body of evidence that RS could be used to identify the origin of highly vulnerable historical fabrics that have dyes or pigments.[5,18,19] However, utilization of conventional RS for analysis of colored material is a challenging task. Primarily due to high fluorescence of organic dyes that are commonly used on nearly all types of materials including fabric and plastics. This limitation can be overcome by surface-enhanced effect discovered by Van Duyne.[20] In this case, nanostructures should be applied to the sample. If their electronic absorbance matches the excitation wavelength, coherent oscillations of conductive electrons are taken place on nanostructured surfaces.[21–26] These coherent oscillations, also known as localized surface plasmon resonances (LSPRs), determine 10^6–10^8 enhancement of Raman scattering.[27] This allows for both suppression of dye fluorescence and identification of colorants present at nearly single-molecule levels. Although this approach is broadly used in art conservation science, it is not ideal due to sample contamination with nanostructures.[28–30]

Our group recently found that utilization of near-infrared (NIR) excitation ($\lambda = 830$ nm) in RS (NIeRS) could be used to overcome dye fluorescence.[31] Most of commercially available dyes that have plant, insect or synthetic origin exhibit strong fluorescence in green–red parts of electromagnetic spectrum. This limits the use of excitation wavelength in this spectral region for RS. Utilization of NIR excitation allows to minimize

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dye fluorescence and, consequently, acquire excellent signal-to-noise Raman spectra. We also demonstrated that if coupled with chemometrics, NIeRS enabled highly accurate identification of more than 20 different dyes present on cotton. However, it remains unclear whether NIeRS can be used to detect the presence of two colorants on fabric. It is also unclear whether NIeRS could be used to probe the colorant content of fabric contaminated with household substances such as paint. In this study, we utilized a hand-held NIeRS spectrometer to answer these questions. For this, cotton fabric dyed with red colored was re-dyed afterwards with several different dyes. In parallel, we applied paint that is used to paint house walls on fabric colored by green and red dyes. Next, NIeRS spectra were acquired from all samples and analyzed using partial-squared least discrimination analysis (PLS-DA).

Experimental

Fabric materials

A 100% cotton canvas was purchased from Fruit of the Loom. Dyes (Kool Krafts, ASIN: B08N5HVXS8) were purchased from the Mosaiz Store. All dyes were from the same batch; we used different colours of the same brand. Red, blue, yellow, orange, green and purple dyed fabric samples were prepared by soaking the cotton in the corresponding liquid dye for 1 h. The samples were then rinsed under cold water until the water ran clear. The dyed cotton was then dried completely before scanning. To re-colour the fabric, the second dye was applied on the coloured fabric; soaked for 1 h and dried. Paint was purchased in the local supplies store and applied on the coloured cotton followed by complete drying.

Near-infrared excitation Raman spectroscopy (NIeRS)

NIeRS spectra were collected using a hand-held Agilent Resolve spectrometer equipped with an 830 nm laser. The instrument had a beam diameter of roughly 2 mm. Spectra were acquired with 1 s acquisition time and 495 mW laser power. The instrument performed an automatic baseline subtraction for every taken spectrum. In total, ~25 spectra were taken for every dyed (red, blue, yellow, orange, green and purple) and re-coloured blue-on-red (BonR), green-on-red (GonR), orange-on-red (OonR), purple-on-red (PonR), and yellow-on-red (YonR) fabric. The spectra were collected from different locations on the fabric sample.

Spectral processing and statistical analysis

Spectral processing was done in Matlab (Mathworks) equipped with PLS_Toolbox (Eigenvector Research, Inc., Manson, WA). All spectra were MSC normalized and smoothed (SalGol). For PLS-DA, the model was used with derivative (SalGov) (order: 2, window: 15 pt, tails: polyinterp) and mean centering. All data were used for training the model; cross-validation was then used for the prediction of accuracies of spectra assignments to their classes (reported in Tables 1 and 2). Spectra classes were assigned based on the colour or re-dyed colour. Kruskal–Wallis ANOVA was used to show significant differences between peak intensities. The number of latent variables (LVs) varied for each model.

Table 1 PLS-DA confusion matrix that demonstrates prediction accuracy of blue-on-red (BonR), green-on-red (GonR), orange-on-red (OonR), purple-on-red (PonR), and yellow-on-red (YonR) spectra together with the NIeRS spectra acquired from red fabric (Red)

Class	BonR	GonR	OonR	PonR	YonR	Red
Predicted as BonR	25	0	0	0	0	0
GonR	0	25	0	0	1	0
OonR	0	0	25	0	0	0
PonR	0	0	0	25	0	0
YonR	0	0	0	0	24	0
Red	0	0	0	0	0	25
Unassigned	0	0	0	0	0	0
Accuracy, %	100	100	100	100	96	100

Results and discussion

NIeRS spectrum acquired from cotton fabric colored by a red dye exhibited vibrational bands at 648, 828, 1227, 1278, 1331, 1353, 1413, 1469, and 1611 cm^{-1} that originated from the dye itself and peaks at 379, 437, 494, 518, 566, 610, 723, 900, 968, 995, 1095, 1118, 1149, 1237, 1289, 1336, 1378, 1475 and 1602 cm^{-1} that could be assigned to cellulose, Fig. 1. We also found that NIeRS spectra acquired from red-colored cotton re-dyed with blue dye exhibited the vibrational fingerprint of a blue dye. Specifically, we observed vibrational bands at 602, 749, 1181, 1265, 1407, and 1541 cm^{-1}. At the same time, in this NIeRS spectrum, we observed the vibrational bands typical for the red dye. Specifically, we observed an increase in the intensity of vibrational bands at 1409 and 1565 cm^{-1}, Fig. 1. Thus, we can conclude that although the vibrational signature of re-dyed fabric is dominated by the colorant applied last, NIeRS can be used to detect the underlying dye.

The same conclusions can be made for red fabric re-colored with green and yellow dyes. We also observed an increase in the intensity of 1409 and 1565 cm^{-1} in NIeRS spectrum acquired from the re-dyed fabric, Fig. 1. Significantly different spectroscopic changes were observed in NIeRS spectra acquired from cotton sample first colored with red dye and then re-dyed with

Table 2 PLS-DA confusion matrix that demonstrates prediction accuracy of paint-on-green (PonG), paint-on-red (PonR), and paint-on-blank (PonB) spectra together with the NIeRS spectra acquired from blank, red-, and green-dyed cotton fabric

Class	PonG	PonR	PonB	Green	Red	White
Predicted as PonG	12	15	9	0	0	0
PonR	12	10	15	0	0	0
PonB	0	0	1	0	0	0
Green	0	0	0	24	0	0
Red	0	0	0	0	24	0
White	0	0	0	0	0	25
Unassigned	0	0	0	0	0	0
Accuracy, %	52	40	4	100	100	100

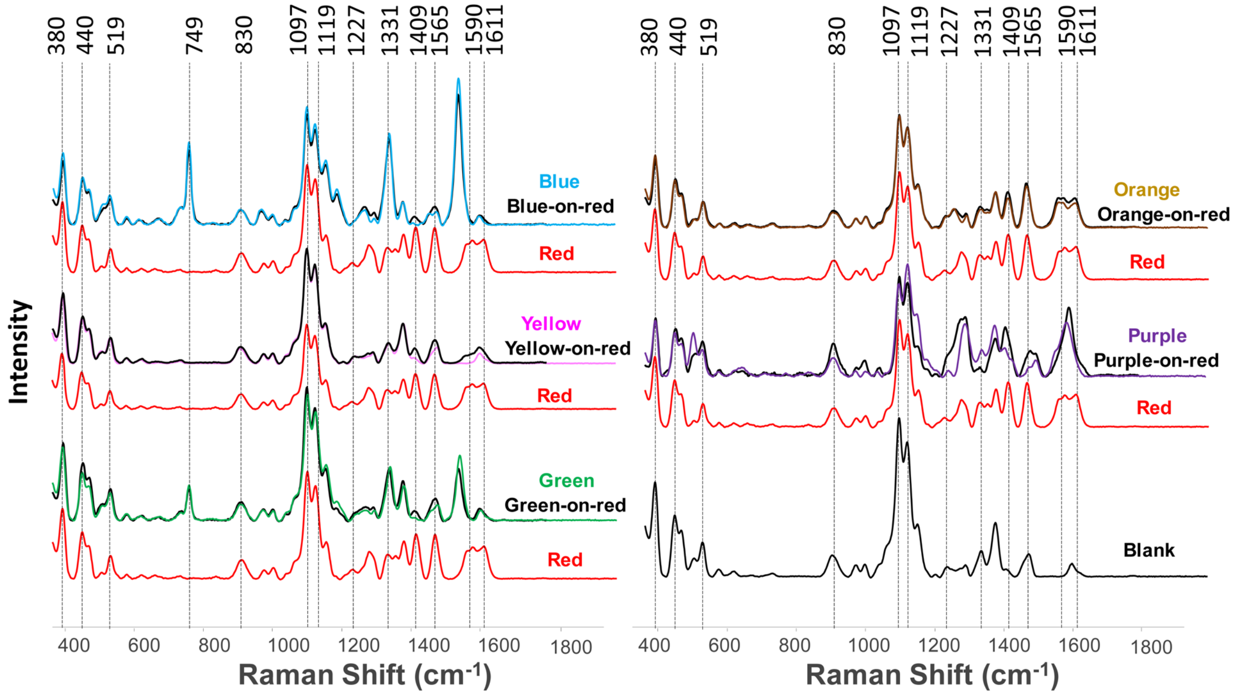

Fig. 1 NIeRS spectra acquired from blue-on-red (BonR), green-on-red (GonR), orange-on-red (OonR), purple-on-red (PonR), and yellow-on-red (YonR) spectra together with the NIeRS spectra acquired from red and blank fabric.

orange colorant. Specifically, we only observed a small increase in the intensity of 1331 cm^{-1}, as well as 1590 and 1611 cm^{-1}.[31] Finally, re-dying of red cotton with purple colorant resulted in drastic changes in the intensity of most of vibrational bands, including 968, 1149, 1237, 1289, 1336, 1378, 1475 and 1602 cm^{-1}. These results confirm the stated above conclusion that NIeRS can be used to reveal the information about the underlying colorant if the fabric was re-dyed afterwards.

To further explore the accuracy of this conclusion, we utilized PLS-DA. For this, 25 spectra were acquired from each sample. PLS-DA model allowed for highly accurate identification of acquired spectra, Fig. 2 and Table 1. Specifically, in all cases except yellow-on-red (YonR) spectra the accuracy of identification of 100%. In the same of YonR, 1 out of 25 spectra was mis-predicted as green-on-red (GonR). Thus, we can conclude that PLS-DA enabled highly accurate identification of re-dyed fabric with ~100% accuracy.

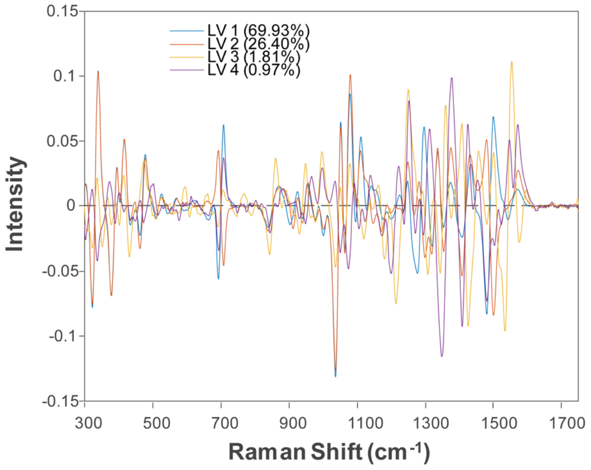

Fig. 2 Loading plot of the three predictive components (PC) in the NIeRS spectra acquired from dyed and re-dyed cotton.

Next, we investigated the extent to which paint could obscure the accuracy of NIeRS-based identification of dyes on fabric. For this, NIeRS spectra were acquired from blank, red-, and green-dyed cotton fabric contaminated with paint, Fig. 3. We found that in the collected spectra, the vibrational fingerprint of the paint was dominated. Specifically, we found two strong bands at ~450 and 600 cm^{-1} together with less intense bands at 820, 860 and 1450 cm^{-1} in all acquired spectra. We also observed vibrational bands that could be assigned to cotton, Fig. 3. Our results also showed that NIeRS spectra of blank and red cotton contaminated with paint were very similar if not identical. Thus, we can conclude that the presence of paint fully obscures the readability of colorants present on fabric. At the same time, in the NIeRS spectrum acquired from the green cotton contaminated by paint, we observed two vibrational bands centered at 749 and 1541 cm^{-1} that corresponded to the green dye. Thus, we can conclude that NIeRS could potentially identify the presence of some colorants on fabric contaminated by paint.

To further investigate this, we performed PLS-DA on the acquired NIeRS spectra, Fig. 4 and Table 2. We found that PLS-DA model was able to identify with 100% accuracy NIeRS spectra acquired from blank, red-, and green-dyed cotton fabric. However, no statistically significant accuracy was found in classification of spectra acquired from fabric contaminated

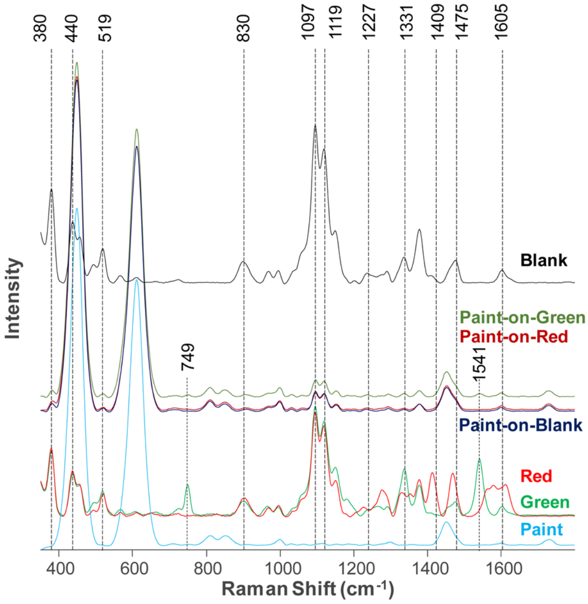

Fig. 3 NIeRS spectra acquired from blank (paint-on-blank), red- (paint-on-red), and green- (paint-on-green) dyed cotton fabric contaminated with paint, as well as reference spectra of paint, blank, red and green fabric.

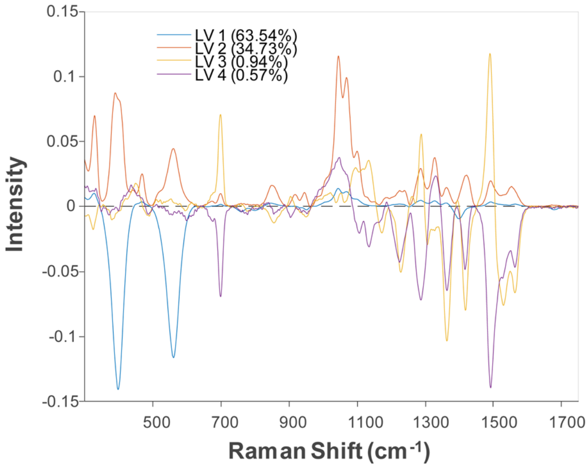

Fig. 4 Loading plot of the three predictive components (PC) in the NIeRS spectra acquired from dyed and re-dyed cotton contaminated with paint.

with paint. Based on this, we can conclude that the presence of paint poses substantial complications in the NIeRS-based identification of dyes on cotton.

In our previous study, we demonstrated that some household contaminants could obscure the readability of SERS-based identification of dye content on hair.[32] However, the gentle removal of such contaminants by water allowed for restoration of the accuracy of SERS-based sensing of dyes. One can expect that a similar approach could be used to remove paint from the colored fabric. Elucidation of the most preservative and efficient approaches for the paint removal is the subject for the separate study that is currently performed in our laboratory.

Conclusions

Our results show that NIeRS coupled with PLS-DA enabled highly accurate identification of the presence of several colorants on cotton. We also found that the presence of paint fully drastically lowers the ability of NIeRS to reveal the information about the dye content of the fabric. These results expand our understanding of the use of NIeRS in the forensic analysis of coloured fabric.

Conflicts of interest

There are no conflicts to declare.

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