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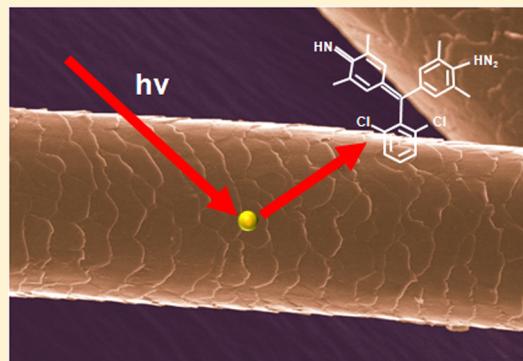
# In Situ Detection and Identification of Hair Dyes Using Surface-Enhanced Raman Spectroscopy (SERS)

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 Supporting Information

**ABSTRACT:** Hair is one of the most common types of physical evidence found at a crime scene. Forensic examination may suggest a connection between a suspect and a crime scene or victim, or it may demonstrate an absence of such associations. Therefore, forensic analysis of hair evidence is invaluable to criminal investigations. Current hair forensic examinations are primarily based on a subjective microscopic comparison of hair found at the crime scene with a sample of suspect's hair. Since this is often inconclusive, the development of alternative and more-accurate hair analysis techniques is critical. In this study, we utilized surface-enhanced Raman spectroscopy (SERS) to demonstrate that artificial dyes can be directly detected on hair. This spectroscopic technique is capable of a confirmatory identification of analytes with single molecule resolution, requires minimal sample, and has the advantage of fluorescence quenching. Our study reveals that SERS can (1) identify whether hair was artificially dyed or not, (2) determine if a permanent or semipermanent colorants were used, and (3) distinguish the commercial brands that are utilized to dye hair. Such analysis is rapid, minimally destructive, and can be performed directly at the crime scene. This study provides a novel perspective of forensic investigations of hair evidence.



Hair analysis has been used in forensic investigations, the cosmetics industry, and various medical fields for many years. In forensic analysis, it is primarily done by a microscopic comparison of hair found at the crime scene to a known hair sample.<sup>1,2</sup> In most cases, this comparison identifies the body area from where the hair came and the race of the individual. Based on microscopic similarities, hair evidence can be found to be "associated with", "non-associated with", or "inconclusive" with the known hair sample and, consequently, a suspect. However, such a subjective microscopic comparison cannot be always conclusive.<sup>3</sup> For example, according to a recent FBI study, of 80 hair samples that were concluded to be "associated with" by microscopic comparison, 12.5% of these samples actually came from different sources. DNA analysis of hair evidence can achieve very high accuracy in suspect identification, but its application is time-consuming and limited to hair samples with an intact bulb. Since there are hundreds of thousands of samples that have not yet been processed in the United States, alternative analytical methods to examine hair must be developed. Liquid chromatography and mass spectrometry can detect warfare agents and numerous abused drugs in hair, such as amphetamine and heroin.<sup>4,5</sup> However, these analytical techniques are destructive and require large amounts of sample.

Raman spectroscopy has been shown to be a highly efficient tool for the identification of a variety of samples relevant to forensic science, such as body fluids,<sup>6–9</sup> gunshot residues,<sup>10,11</sup> bone fragments,<sup>12</sup> explosives,<sup>13,14</sup> inks,<sup>15,16</sup> and illicit drugs.<sup>17</sup> Raman spectroscopy is particularly attractive for forensic

purposes, because it is rapid, noninvasive, nondestructive, provides confirmatory identification of analyzed samples, can be done in the field, and requires minimal sample size.<sup>18</sup> Identification is accomplished by comparison of the *in situ* acquired Raman spectrum with a commercially available library of chemical substances or previously obtained Raman spectra of the characterized materials.<sup>19</sup>

SERS amplifies the Raman signal of an analyte due to interactions of analyte molecules with a roughened noble-metal surface, which also substantially increases the detection limit.<sup>20–23</sup> For example, studies recently demonstrated that SERS can detect commonly abused drugs, such as cocaine,<sup>17</sup> down to microgram quantities. In addition, SERS has been shown to rapidly and accurately detect explosives, such as half-mustard agent and dinitrobenzenethiol.<sup>13,24</sup> There are also numerous examples of successful SERS applications in cultural heritage research.<sup>25–27</sup> Micrograms of the painting sample are required to identify the colorants used in a particular artwork and consequently authenticate its origin and historical significance, in addition to determining an artwork's state of conservation.<sup>28–30</sup> Recently, it was demonstrated that tip-enhanced Raman spectroscopy (TERS), which is an analogue of SERS, could detect dyes and iron gall ink directly on paper and a historical 19th century manuscript.<sup>31</sup>

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In this study, we investigate the ability of SERS to detect and identify artificial hair dyes *in situ*. We found that SERS can identify if the hair was colored or not, and distinguish whether permanent or semipermanent colorants were used. Finally, our results indicate that SERS can distinguish the commercial brands that are utilized to dye hair. This study demonstrates that SERS analysis of hair evidence provides valuable information about the artificial dye content, which can be utilized in forensic investigations and the cosmetics industry.

## 2. EXPERIMENTAL SECTION

**Materials.** Hair dyes: Ion Jet Black (No. 305730), Ion Black (No. 305052), Clairol Jet Black (share B22D, level 1 base ash), Ion Sky Blue (No. 305082), Color JamZ Huckleberry Blue, and N Rage Color Cobalt Blue were purchased from a local supply store (Sally Beauty Supply). Samples of human hair were dyed in Petri dishes using the aforementioned semipermanent colorants (all except Ion Jet Black (No. 305730)) for  $\sim 2$  h, and then extensively washed by Millipore water until no dye was visually observed in the rinsing water. Permanent colorant (Ion Jet Black (No. 305730)) was mixed in a 1:1 ratio with Salon Care 20 Volume reduction agent, prior to deposition on the hair sample. Hair was dyed for  $\sim 2$  h. Washing procedure was identical to semipermanent dyes. Basic Blue 77 was generously provided by Aashiana Dyestuffs, Ink (Bolingbrook, IL) and used as received. Hydrogen peroxide (30%) and 2-methyl-*p*-phenylenediamine sulfate were purchased from Sigma-Aldrich (St. Louis, MO). 2-Methyl-*p*-phenylenediamine sulfate (1 mM) was oxidized by hydrogen peroxide (1:1 molar ratio) overnight at room temperature.

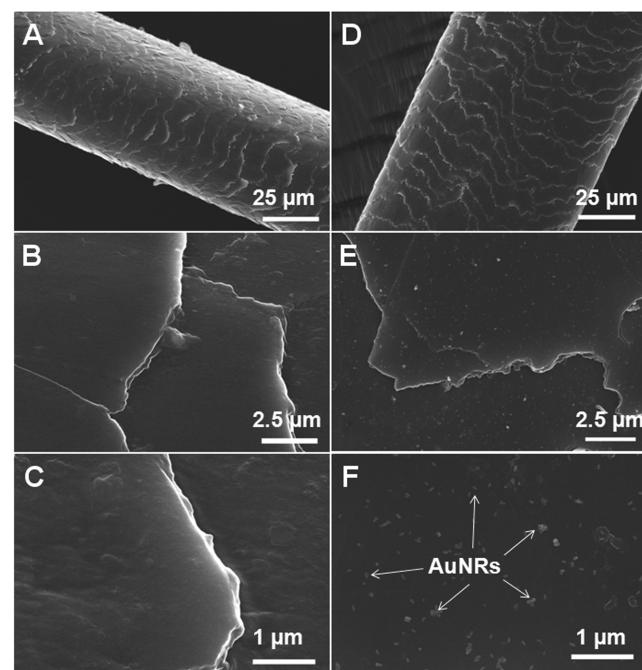
Gold nanorods (AuNRs) were synthesized in Catherine J. Murphy's laboratory (Chemistry Department, University of Illinois at Urbana-Champaign), as previously described.<sup>32</sup> On average, the synthesized AuNRs have dimensions of  $5\text{ nm} \times 30\text{ nm}$  (see Supporting Figure 1 in the Supporting Information). Prior to use, the excess cetyltrimethylammonium bromide (CTAB) capping agent was rinsed from the AuNRs by centrifugation in Millipore water at 5000g for 25 min. After the supernatant was removed, the pellet was redispersed in Millipore water. The washing procedure was repeated twice. The final solution of AuNRs (0.264 nM) that was used for this study was characterized with an Agilent Cary 5000 UV-vis/NIR spectrometer. Ultraviolet-visible light (UV-vis) absorption maxima were located at 521 and 714 nm (see Supporting Figure 2 in the Supporting Information).

**Spectroscopy.** Normal Raman (NR) and SER spectra were collected on a confocal inverted microscope (Nikon, Model TE-300) with 20 $\times$  dry Nikon objective (NA = 0.45). A diode-pumped solid-state laser (Spectra-Physics Millenia) was used for 532-nm excitation. It was also used to drive the tunable Ti:sapphire oscillator to generate 785-nm light. The laser spot size was  $2.7\text{ }\mu\text{m} \times 1.8\text{ }\mu\text{m}$ . The signal was collected in a backscattering geometry and sent to a spectrometer (Princeton Instruments, Model SP2500i) equipped with a 600 groove/mm grating and a slit entrance set to 100  $\mu\text{m}$ . Prior to entering the spectrograph, the Rayleigh scattering was filtered with a long-pass filter (Semrock, LP03-785RS-25). The dispersed light was then sent to a liquid nitrogen-cooled CCD (Action300i, Spec10 400B).

**Spectral Processing.** For all spectra shown, the raw intensity counts were divided by the power and acquisition time to normalize the spectral intensity, such that the intensity is reported in the units of analog to digital conversion units (or

ADU  $\text{mW}^{-1}\text{ s}^{-1}$ ). All data was processed using GRAMS/AI 7.0 (Thermo Galactic, Salem, NH). Spectra shown are raw spectra, no smoothing or baseline correction was applied.

**Scanning Electron Microscopy (SEM).** SEM images were taken on Hitachi, Model SU8030 microscope using a 10 kV acceleration voltage. Prior to imaging, hair samples were coated with 9 nm of osmium, using an osmium plasma coater, to increase the sample conductivity.



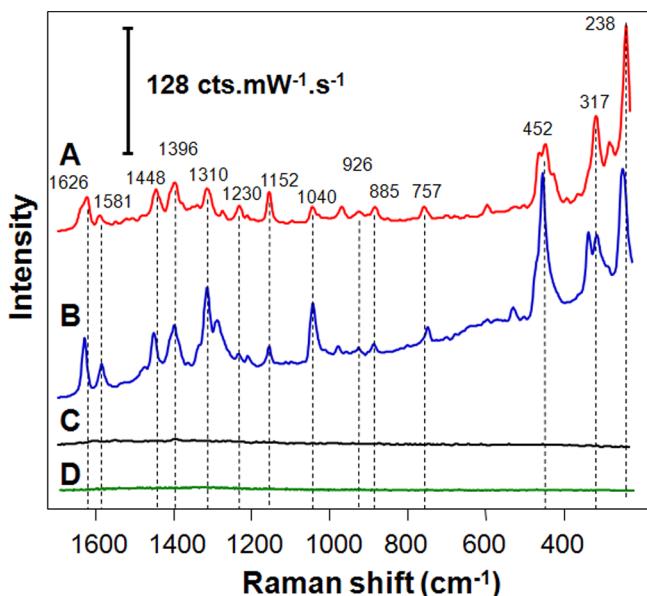
**Figure 1.** SEM images of (A–C) undyed human hair and (D–F) dyed human hair (Ion Blue Sky) with adsorbed AuNRs (marked by white arrows) on its surface.

## 3. RESULTS AND DISCUSSION

All hair colorants can be arbitrarily divided into three groups based on their dye content: (1) monodyes, which are colorants that are based on just one chemical dye; (2) polydyes, which contain a mixture of chemical dyes; and (3) reactive colorants, which require dye oxidation to generate the color. Together with the chemical dye components currently available, commercial colorants contain numerous ingredients, such as lauryl, cetearyl, myristyl, and stearyl alcohols, fragrance, sodium sulfide, ammonia, and detergents. To demonstrate that SERS can detect the dye component on colored hair, even in the presence of other ingredients, we dyed human hair with a monodye "Ion Blue Sky" (Ion Professional Products, Inc.), which is a widely sold dye brand in numerous countries around the world. This is a blue semipermanent dye, and is one of the most commonly used colorants by teenagers and artists. After the hair was extensively washed to remove any unbound dye, a drop ( $20\text{ }\mu\text{L}$ ) of AuNRs was placed on the dyed hair. SEM examination of the hair surface exposed to AuNRs showed no visual morphological changes in the hair morphology (see Figures 1A and 1D). Instead, we found that the AuNRs adsorbed on the hair surface at various aggregation states, including monomers, dimers, trimers, and other ensembles (see Figures 1C and 1F). Illumination of the adsorbed AuNRs using laser radiation leads to a drastic ( $10^6$ – $10^8$ ) amplification of the

Raman scattering from dye molecules present on the AuNRs surface.

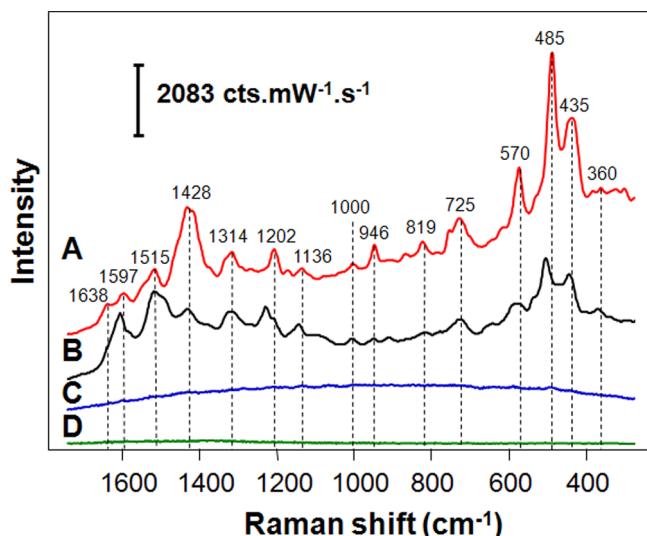
The SER spectrum of hair dyed with "Ion Blue Sky" (Figure 2) exhibited peaks at 1626, 1581, 1448, 1396, 1310, 1230, 1152, 1040, 926, 885, 757, 452, 317, and 238 cm<sup>-1</sup>. These peaks perfectly match the Raman spectrum of Basic Blue 77, a chemical dye of "Ion Blue Sky". This indicates that SERS directly detects the dye present on the colored hair. It should be noted that NR spectroscopy was not able to detect the dye on the hair (Figure 1C). Detection and identification of the artificial hair dye does not interfere with the signal from the natural hair pigments, which are located in the hair cortex and therefore inaccessible by SERS (Figure 1D).



**Figure 2.** SER spectrum of human hair colored with Ion Blue Sky (trace A), normal Raman (NR) spectrum of Basic Blue 77 (trace B), NR spectra of human hair colored with Ion Blue Sky (trace C), and undyed hair (trace D).  $P = 0.36 \text{ mW}$ ,  $\lambda = 785 \text{ nm}$ .

Black and brown permanent or reactive dyes are some of the most commonly used colorants by people of all age groups. Dyeing properties of such colorants are based on oxidation of *p*-phenylenediamine and its derivatives. Bandrowski and Erdmann reported that the oxidation of *p*-phenylenediamine in weakly alkaline solution leads to a formation of a compound  $C_{18}H_{18}N_6$ , known as Bandrowski's base.<sup>33</sup> Dolinsky et al. used chromatography to demonstrate that, besides Bandrowski's base, at least five other products are formed, with colors varying from yellow to dark brown.<sup>34</sup>

We used SERS to detect and identify dyes in hair colored with permanent or reactive dyes. For this, human hair was dyed with "Ion Jet Black" and extensively washed to remove any unbound dye. SER spectrum of hair dyed with "Ion Jet Black" permanent dye showed peaks at 1638, 1597, 1515, 1428, 1314, 1202, 1136, 1000, 946, 819, 725, 570, 485, 435, and 360 cm<sup>-1</sup> (see Figure 3). To demonstrate that the observed peaks correspond to the oxidation products of 2-methyl-*p*-phenylenediaminesulfate, the major component of "Ion Jet Black" (Ion Professional Products, Inc.), this chemical was oxidized by hydrogen peroxide. The SER spectrum of the 2-methyl-*p*-phenylenediaminesulfate oxidation products almost perfectly matches the SER spectrum of hair colored with "Ion Jet Black".

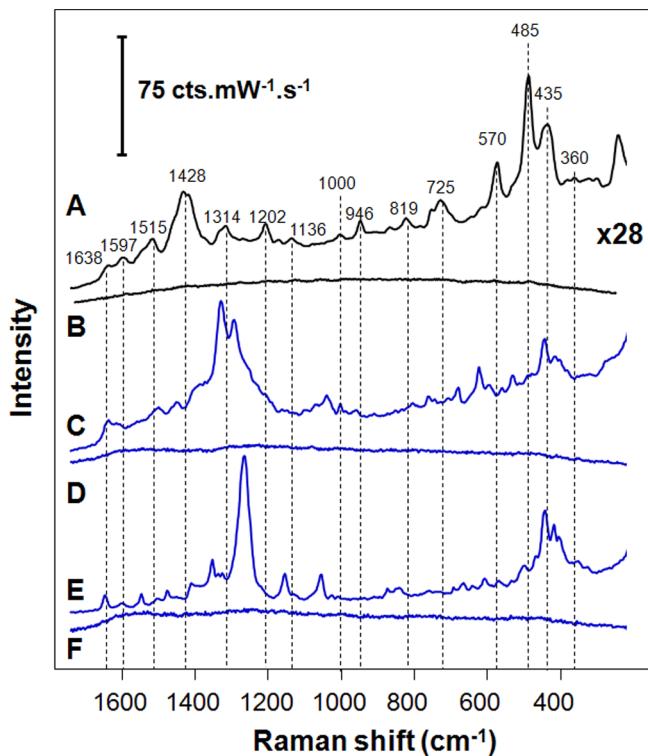


**Figure 3.** SER spectra of human hair colored with Ion Jet Black (trace A) and oxidation products of 2-methyl-*p*-phenylenediaminesulfate (trace B), NR spectra of human hair colored with Ion Jet Black (trace C), and undyed hair (trace D).  $P = 1.5 \text{ mW}$ ,  $\lambda = 785 \text{ nm}$ .

More-specific band assignment requires chromatographic separation of the reaction products, which is beyond the scope of the current work. It should be noted that NR spectroscopy was not able to reveal any presence of permanent dye on hair (Figure 3C); only some fluorescence was observed.

Based on these experimental results, we can conclude that SERS is capable of detecting and identifying chemical dyes of both semipermanent and permanent hair colorants directly on hair. A pertinent question to ask is whether SERS can be utilized to distinguish hair colored with semipermanent dye from hair colored with permanent dyes of the same color (for example, black). To address this question, we dyed hair with semipermanent black colorant of the same brand (Ion Professional Products, Inc.). To investigate whether black colorants from different companies can be distinguished, we also dyed hair with a different commercial brand, Clairol (Clairol Jet Black, Procter and Gamble Co.). Acquired SER spectra were compared to the aforementioned SER spectrum of hair colored with the permanent dye "Ion Jet Black" (see Figure 4).

The SER spectra acquired from both hair samples colored with semipermanent colorants have different Raman bands, compared to the SER spectrum of hair colored with a permanent black dye (Figure 4). This indicates that SERS is capable of distinguishing hair that has been colored with permanent and semipermanent colorant of the same color. Moreover, our results indicate that SERS can distinguish between commercial brands that are utilized for hair coloring (see Figure 4). This is primarily due to the different combinations of chemical dye components present in each of the brands. Ion Black's coloring properties are based on all of the following dyes: Blue 2, Yellow 4, Violet 1, and Basic Blue 99. Clairol Jet Black contains Blue 377, Dispersive Violet 7, Dispersive Black 9, Yellow 2, Blue 2, and Red 3. Detailed assignment of vibrational bands in SER spectra to a particular chemical dye is beyond the scope of the current work. However, we can conclude that SERS can identify the commercial brand that was used to color the hair, as long as the commercial formulas are different.

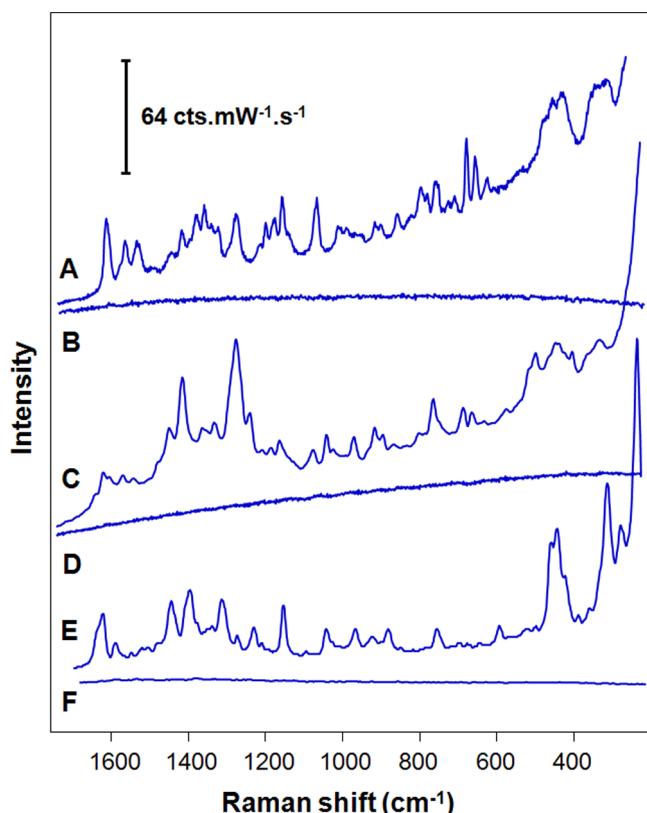


**Figure 4.** SER spectra of hair colored with permanent Ion Jet Black (trace A), semipermanent Ion Black (trace C), and Clairol Jet Black black colorants (trace E) and the corresponding normal Raman spectra (traces B, D, and F, respectively).  $P = 1.5 \text{ mW}$  (traces A and B) and  $0.4 \text{ mW}$  (traces C–F),  $\lambda = 785 \text{ nm}$ .

To further demonstrate that SERS is able to distinguish hair colored with the same color of different commercial brands, we dyed human hair with blue semipermanent colorants of different brands: Color JamZ Huckleberry Blue (Beyond the Zone, Inc.), N Rage Color Cobalt Blue (N Range, Co.), and Ion Blue Sky (see Figure 5). All of these colorants are monodyes. Specifically, Acid Blue 62 is used in Color JamZ Huckleberry Blue, N Rage Color Cobalt Blue is based on Basic Blue 7, and Ion Blue Sky is based on Basic Blue 77. Based on our results, it can be concluded that SERS can distinguish commercial brands that were utilized for hair coloring. No signal was observed in the NR spectra acquired from the colored hair in this set of experiments. This further proves the above-discussed concept that different commercial brands can be identified. Confirmatory identification of a particular commercial colorant and its assignment to a specific chemical dye would require the creation of a spectroscopic library of such organic molecules,<sup>35</sup> which is beyond the scope of the current study.

Previous applications of SERS to art conservation science have demonstrated that confirmatory dye identification can be achieved with just micrograms of colorants and millimeters of fabric samples.<sup>25–27</sup> In this study, we further demonstrated the high sensitivity of SERS: colorant can be detected on a hair sample that is only a fraction of a millimeter in length. Practically, the hair sample must be, at a minimum, the size of the laser beam ( $\sim 2.5 \mu\text{m}$ ) to successfully detect the artificial hair colorant. On average, people who artificially dye their hair will apply the dye every two months. Analysis of a hair sample that was taken from an individual who colored their hair using several permanent dyes (Ion Professional Products, Inc. series)

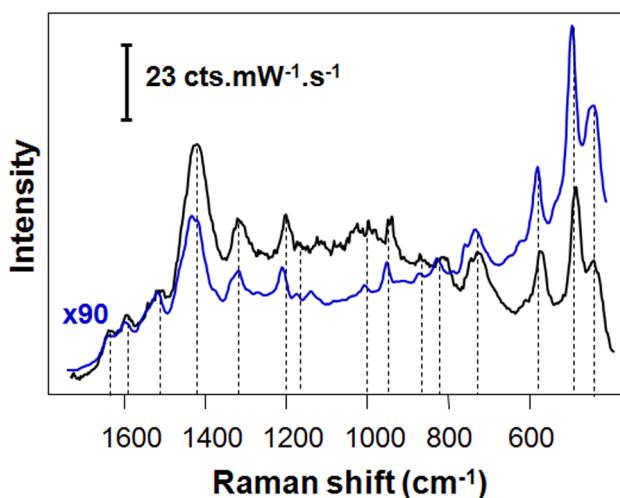
about one month prior to the SERS study revealed a strong signal from the dyes (see Supporting Figure 3 in the Supporting Information). Therefore, one can expect that artificial dyes can be detected with SERS on hair that was colored more than 4 weeks prior to the analysis. A detailed examination of the colorants fading on hair dyed with both permanent and semipermanent dyes, as well as determination of factors that may accelerate or delay this process, requires additional studies that are beyond the scope of this manuscript.



**Figure 5.** SER spectra of hair colored with blue semipermanent colorants: Color JamZ Huckleberry Blue (trace A), N Rage Color Cobalt Blue (trace C), and Ion Blue Sky (trace E).  $P = 1.5 \text{ mW}$ ,  $\lambda = 785 \text{ nm}$ . Corresponding NR spectra are shown in traces B, D, and F.  $P = 1.5 \text{ mW}$ ,  $\lambda = 785 \text{ nm}$ .

**Rapid Examination of Dyed Hair in the Field.** Portable Raman spectrometers offer a unique opportunity to perform forensic examinations of hair directly at the crime scene. We utilized a Snowy Range CBEx Portable Raman spectrometer to demonstrate that information about the hair dye content can be acquired in the field. A strand of hair colored with a permanent colorant “Ion Jet Black” was immersed into solution of AuNRs and SER spectra were acquired using the same excitation wavelength (785 nm) (see Figure 6).

Our results indicate that high signal-to-noise-ratio SER spectra of a single-colored hair can be acquired in the field within minutes, using AuNRs and a commercially available portable Raman spectrometer. We found that all vibrational bands visible in SERS spectra acquired with a confocal Raman spectrometer are evident in the SER spectrum collected in the field using the portable Raman spectrometer. The observed difference in the spectrum background is most likely caused by background fluorescence.



**Figure 6.** (Top) Photograph of Snowy Range CBEx portable Raman spectrometer utilized for the on-field spectral acquisition. (Bottom) SER spectra collected from a single hair colored with a black permanent dye “Ion Jet Black” using Snowy Range CBEx portable Raman spectrometer (black trace) and confocal Raman spectrometer (blue trace).

#### 4. CONCLUSIONS

In the current manuscript, we have demonstrated that surface-enhanced Raman spectroscopy (SERS) can detect and identify dyes on artificially colored hair. SERS can identify whether hair was artificially dyed or not, and determine whether permanent or semipermanent colorants were used. Our results indicate that SERS is capable of distinguishing the commercial brands that were utilized to dye hair, as long as their commercial formulas are different. Finally, this confirmatory and minimally invasive forensic analysis of hair evidence can be easily performed in the field.

#### ■ ASSOCIATED CONTENT

##### ■ Supporting Information

Supporting Figures 1, 2, and 3. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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##### Notes

The authors declare no competing financial interest.

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