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Surface-Enhanced Raman Analysis of Underlaying Colorants on Redyed Hair

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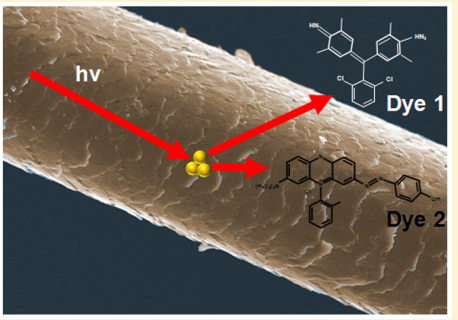
Supporting Information

ABSTRACT: Forensic examination of hair evidence can help with establishing a connection between a suspect and a crime scene or demonstrate the absence of such connections. Currently, it is primarily done by a subjective microscopic examination which can only elucidate the species of origin and, if human, the part of the body the hair came from. Several years ago, surface-enhanced Raman spectroscopy (SERS) was proposed for advanced forensic analysis of hair (Kurouski, D.; Van Duyne, R. P. In situ detection and identification of hair dyes using surface-enhanced Raman spectroscopy (SERS). *Anal. Chem.* **2015**, *87*, 2901−2906. DOI: 10.1021/ac504405u). It was shown that SERS could be used to determine whether hair was dyed or not and even reveal what commercial hair colorant was used. Expanding upon those findings, we show that SERS is capable of probing the original colorant even if hair was redyed afterward. Specifically, we were able to detect and identify the underlaying blue semipermanent colorant on hair redyed by both black semipermanent and black permanent colorants. We also demonstrate that original black permanent colorant could be detected by SERS if the hair was recolored by blue semipermanent dye. However, it could not if the hair was recolored by another (blue or black) permanent dye. We also provide experimental evidence that SERS can be used to detect the dye on hair colored more than two months prior to its spectroscopic examination. These experimental findings substantially expand capabilities of SERS in forensics.



According to the report published in 2009 by the U.S. National Academy of Sciences, current forensic examination of hair lacks appropriate scientific bases.[1] Mostly, this is because such analysis primarily relies on a subjective microscopic comparison of hair found at the crime scene with a sample of suspect's hair. The experienced forensic hair examiner can provide answers on the following questions: Is the physical evidence a hair or a fiber? Is the hair human or from some other animal? If human, what part of the body did the hair come from and what was the biogeographical ("racial") origin of hair?[2] Of course, such a subjective microscopic comparison is often inconclusive. For instance, upon a review of approximately 342 cases in which FBI laboratories reported a hair match, 268 of these cases used hair evidence against the defendants. Of those 268, 258 contained flawed testimony. This includes 32 defendants sentenced to death. Fourteen defendants have been executed or died in prison.[3] These findings catalyzed development of more accurate and reliable techniques for advanced forensic examination of hair evidence. DNA analysis of hair is highly specific; however, it can be done only if the hair bulb remained intact. Liquid chromatography and mass spectrometry enable detection of warfare agents and numerous abused drugs in hair such as amphetamine and heroin.[4,5] However, these analytical techniques are destructive and require large amounts of sample.

Raman spectroscopy (RS) is a noninvasive and nondestructive technique that can be used for confirmatory structural analysis of samples. During the past decade, several research groups developed RS for analysis of body fluids,[6−8] plant disease diagnostics,[9−11] bone fragments,[12] inks,[14] and explosives.[13,14] The Raman scattering can be amplified by coherent oscillations of conductive electrons, also known as localized surface plasmon resonances (LSPRs).[15−18] LSPRs can be induced on the surface of noble metal nanostructures upon their illumination by electromagnetic radiation. This spectroscopic technique is known as surface-enhanced Raman spectroscopy (SERS).[19] In addition to the single-molecule sensitivity, SERS has the advantage of fluorescence quenching. These advantages make SERS ideal for confirmatory structural analysis of various samples of biological origin.[20−22]

In 2015, Kurouski and Van Duyne explored the potential of SERS for detection and identification of artificial hair dyes.[23] It has been found that SERS could be used to (1) determine whether hair was dyed or not, (2) reveal whether a permanent or semipermanent colorant was used, and (3) distinguish the commercial brands that were utilized to dye hair. Later, Lednev group confirmed these findings using Fourier-transform infrared (FTIR) spectroscopy.[24] Nevertheless, many questions

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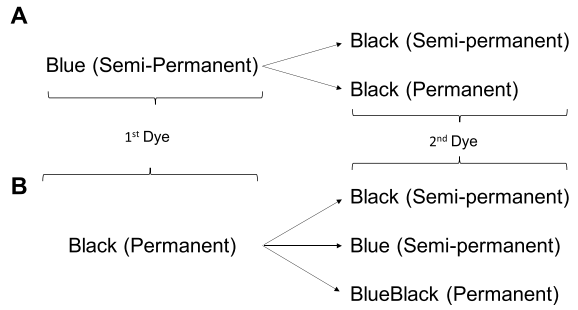
about forensic analysis of dyes on hair remained unanswered. For instance, can the chemical structure of original colorant be revealed if the hair was redyed afterward? Also, can SERS be used to detect and identify dyes on hair that was colored more than two months ago (a typical time period for dying hair)?

In this study, we further expand practical application of SERS for forensic analysis of hair. We investigated whether the original colorant on hair could be identified if the hair was redyed afterward. To answer this question, we performed two sets of experiments. In one, hair was first dyed by a blue semipermanent dye (BLU^{SP}) and then redyed by another semipermanent dye, black semipermanent (BLK^{SP}), Scheme 1, A; Table 1. The same BLU^{SP} hair was also redyed by a permanent black dye (BLK^{P}). This set of redying procedures would let us answer the questions if the original semi-permanent colorant could be detected if hair was redyed by both semipermanent and permeant dyes of a different color afterward. In the second experiment, hair was first dyed by a black permanent dye (BLK^{P}) and then redyed by semi-permanent dyes (black semipermanent (BLK^{SP}) and blue semipermanent (BLU^{SP})), Scheme 1, B; Table 1. Finally, we redyed BLK^{P} by another blue-black permanent dye $BLBK^{P}$. This set of experiments would let us answer the questions if the original permanent colorant could be detected if hair was redyed by both semipermanent and permeant colorants afterward.

Scheme 1. Hair Redying Procedures of Underlying Blue Semi-Permanent Dye (A) and Underlying Black Permanent Dye (B)[a]



[a]Arrows indicate the second dyeing process.

Table 1. Description, Abbreviation, and Commercial Name of the Colorants Used in Our Study[a]

dye description	abbreviation	commercial name
blue (semipermanent) dye on hair sample	BLU^{SP}	Ion Color Brilliance Sky Blue
black (semipermanent) dye on hair sample	BLK^{SP}	Ion Color Brilliance Blackest Black
black (permanent) dye on hair sample	BLK^{P}	Ion Color Brilliance Jet Black
blue-black (permanent) dye on hair sample	$BLBK^{P}$	Ion Color Brilliance Blue Black

[a]An addition of (D) to the end of the abbreviation signifies the dye alone (e.g. BLU^{SP}(D)).

EXPERIMENTAL SECTION

Materials. Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), hexadecyltrimethylammonium bromide (CTAB, 99%), sliver nitrate ($AgNO_3$, 99%), sodium borohydride ($NaBH_4$, 99%), and L-ascorbic acid (AA, 99%) were purchased from Sigma-Aldrich (St. Louis, MO); hydrochloric acid (HCl, 36.5−38.0%) was purchased from Avantor (Center Valley, PA). Ethanol was purchased from Decon Laboratories (King of Prussia, PA). All chemicals were used as received without purification.

Hair Samples. Undyed hair samples were collected from anonymous donors in barbershops of College Station, TX, and used in experiments without any special preparations. The samples were taken by individuals who had no prior history of dyeing their hair.

Colorants and Dying Procedures. Hair dyes (Table 1) were purchased from a local supply store (Sally Beauty Supply). Samples of human hair were dyed in 50 mL falcon tubes for ∼2 h and then extensively washed with Millipore water until no dye was visually observed in the rinsing water. Permanent colorants (BLK^{P} and $BLBK^{P}$) were premixed in a 1:1 ratio with Salon Care 20 Volume reduction agent, deposited on hair, and dyed for ∼2 h. Washing procedure was identical to semipermanent dyes. This procedure was repeated with the overlaying dye.

Nanorod Synthesis. Gold nanorods (AuNRs) were synthesized based on a seed mediated, CTAB-assisted growth procedure reported by El-Sayed et al.[25] with modifications. Specifically, the seed solution was prepared by the addition of $HAuCl_4$ (10 mM, 0.25 mL) into CTAB (0.1 M, 9.75 mL) in a 25 mL flask with gentle stirring (200 rpm). Then, a freshly prepared ice-cold $NaBH_4$ solution (0.01 M, 0.6 mL) was then added quickly into the above-mentioned solution, followed by rapid stirring (500 rpm) for 2 min. The yellow color changed immediately to brown, indicating the formation of gold seeds. These seeds were aged for 2 h to allow the hydrolysis of unreacted $NaBH_4$. In a AuNR growth process, $HAuCl_4$ (25 mM, 0.5 mL) and $AgNO_3$ (10 mM, 0.25 mL) were mixed with CTAB (0.1 M, 25 mL) in a 50 mL flask. HCl (1.0 M, 0.1 mL) was then added, followed by the addition of AA (78.8 mM, 0.175 mL). Finally, 30 μL of the seed solution was added into the growth solution. The solution was gently stirred (200 rpm) for 10 s and left undisturbed for 12 h. Subsequently, the resulting products were precipitated, centrifuged (11 000 rpm, 10 min), and washed twice with ethanol. The obtained purified AuNRs were finally dispersed in 10 mL of ethanol for further use. Absorbance spectrum of the AuNRs, which was collected on Hitachi U-4100 UV−vis−NIR spectrophotometer, revealed two maxima located at ∼520 and 766 nm (see Figure S1 in the Supporting Information).

Spectroscopy. SER spectra were collected on a confocal inverted microscope (Nikon, Model TE-2000U) with 20× dry Nikon objective (NA = 0.45). A solid-state laser (Necsel SLM-785.0-FS-01) was used for 785 nm excitation. The signal was collected in a backscattering geometry and sent to a spectrometer (Princeton Instruments, IsoPlane-320) equipped with a 600 groove/mm grating. 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 the CCD (PIX-400BR). All data were processed using GRAMS/AI 7.0 (Thermo Galactic, Salem,

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NH). Spectra shown are raw spectra; no smoothing or baseline correction was applied.

RESULTS AND DISCUSSION

The SER spectrum of hair dyed with BLU^{SP} (Figure 1) exhibited peaks at 884, 919, 967, 1043, 1094, 1151, 1208, 1230, 1310, 1344, 1376, 1390, 1400, 1444, 1472, 1508, 1583, 1617, and 1640 cm^{-1} (Table 2). These peaks perfectly match the SER spectrum of dye itself (BLU^{SP} (D)). This confirms our previously reported results that SERS can be used to detect and identify semipermanent colorants on hair.[23] Next, the same hair sample was redyed by BLK^{SP} dye ($BLU^{SP} \rightarrow BLK^{SP}$).

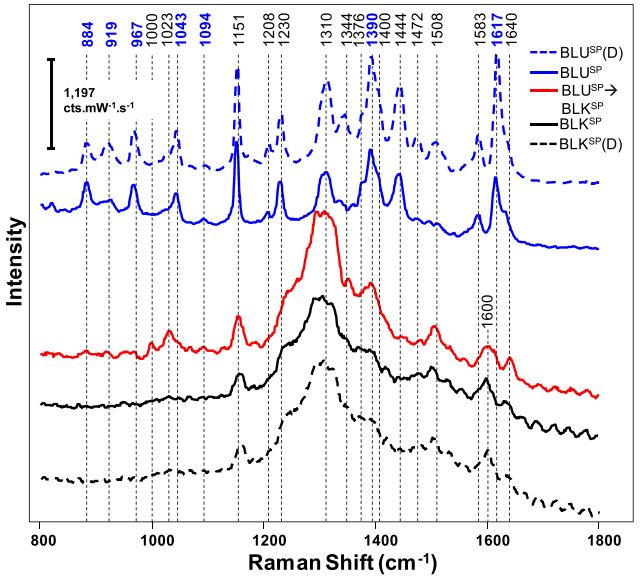

Figure 1. SER spectra of hair colored by BLU^{SP} dye (BLU^{SP} (D)) and redyed afterward by BLK^{SP} ($BLU^{SP} \rightarrow BLK^{SP}$). The corresponding spectra of BLK^{SP} on hair, the dye itself (BLK^{SP} (D)), BLU^{SP}, and the dye itself (BLU^{SP}(D)) are also included.

Table 2. Colorants and Corresponding Bands in Their Raman Spectra

dye	vibrational bands
BLU^{SP}	884, 919, 967, 1043, 1094, 1151, 1208, 1230, 1310, 1344, 1376, 1390, 1400, 1444, 1472, 1508, 1583, 1617, 1640
BLK^{SP}	1151, 1208, 1310, 1390, 1508, 1583, 1600, 1640
BLK^{P}	820, 868, 946, 1127, 1208, 1310, 1344, 1423, 1505, 1583, 1640
$BLBK^{P}$	820, 868, 946, 1004, 1332, 1208, 1310, 1328, 1425, 1508, 1598, 1640

Next, we investigated whether BLU^{SP} dye could be detected if hair was recolored by a black permanent dye (BLK^{P}). We collected SER spectra from $BLU^{SP} \rightarrow BLK^{P}$ hair samples and observed two groups of spectra with distinctly different profiles (Figure 2, I and II). Group I exhibited signals originating from the underlaying BLU^{SP} dye (967, 1043, 1151, 1230, 1390, 1444, and 1617 cm^{-1}) as well as from the second dye (BLK^{P}). At the same time, group II spectra had only vibrational bands

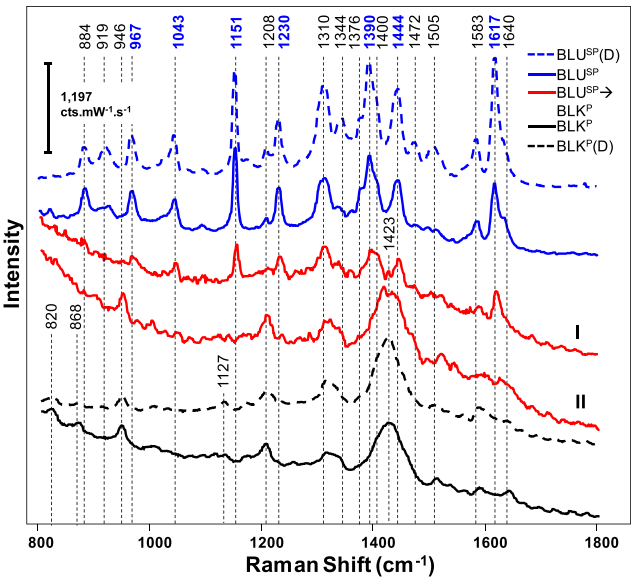

Figure 2. SER spectra of hair colored by BLU^{SP} dye (BLU^{SP} (D)) and redyed afterward by BLK^{P} ($BLU^{SP} \rightarrow BLK^{P}$). The corresponding spectra of BLK^{P} on hair, the dye itself (BLK^{P} (D)), BLU^{SP}, and the dye itself (BLU^{SP}(D)) are also included.

that were observed in BLK^{P} (946, 1208, 1310, 1344, 1423, 1472, 1505, 1583, and 1640 cm^{-1}). This experimental evidence suggests that dye distribution on hair may not be evenly uniform. Thus, some spots on hair remain uncovered by the second (BLK^{P}) dye. Presence of AuNRs on such uncovered spot on hair enables sensing of the underlaying colorant. At the same time, AuNRs located on the recolored parts of hair could sense only the second (BLK^{P}) dye. Although this experimental evidence indicates that SERS could detect the underlaying semipermanent colorant on hair that was redyed by the permanent dye, such detection may be challenging and likely should require an acquisition of more than one spectrum from hair sample.

In the next set of experiments, we explored a possibility of SERS-based detection of underlaying permanent dye on hair recolored by semipermanent dyes. First, we dyed hair by a black permanent dye (BLK^{P}) and then recolored it by the semipermanent colorant of the same color (BLK^{SP}), Figure 3. SERS analysis of the $BLK^{P} \rightarrow BLK^{SP}$ hair indicated presence of vibrational bands that could be assigned to both BLK^{SP} (1151, 1208, 1310, 1390, 1508, 1583, and 1640 cm^{-1}) and BLK^{P} (946 and 1415 cm^{-1}), Figure 3, indicating that SERS can be used to detect the underlaying BLK^{P} dye on the hair recolored by a semipermanent colorant of the same color.

To confirm this finding, BLK^{P} dyed hair was recolored by a blue semipermanent dye (BLU^{SP}), Figure 4. SERS analysis of $BLK^{P} \rightarrow BLU^{SP}$ hair sample (Figure 4) revealed presence of two distinctly different types of spectra. Spectra that could be assigned to the first group (group I) exhibited vibrational bands originating from both BLK^{P} and BLU^{SP}. Specifically, in these spectra, we observed vibrational bands at 946 and 1423 cm^{-1}, which confirmed detection of underlaying BLK^{P} dye. At the same time, spectra from the second group (group II) primarily exhibited vibrational bands that could be exclusively assigned to BLU^{SP}.

We performed Raman imaging of the single hair to unravel spatial distribution of these semipermanent and permanent

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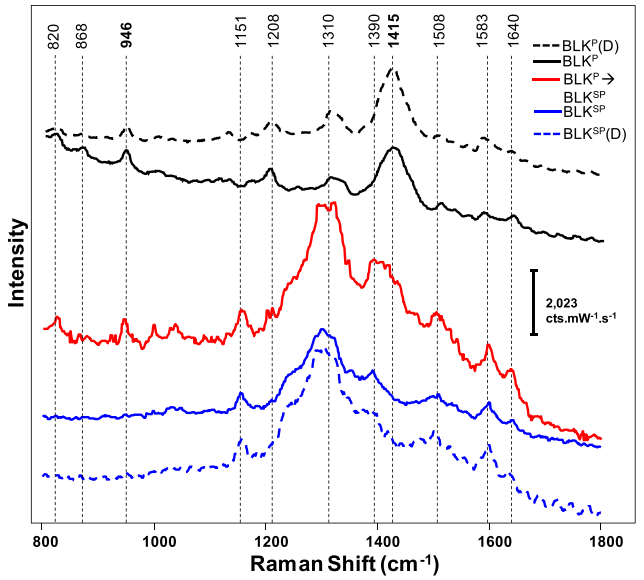

Figure 3. SER spectra of hair colored by BLK^{P} dye (BLK^{P} (D)) and redyed afterward by BLK^{SP} (BLK^{P} → BLK^{SP}). The corresponding spectra of BLK^{P} on hair, the dye itself (BLK^{P} (D)), BLK^{SP}, and the dye itself (BLK^{SP}(D)) are also included.

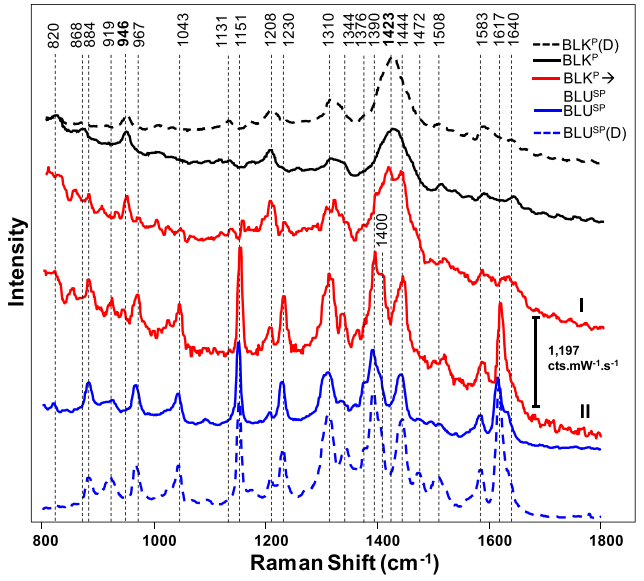

Figure 4. SER spectra of hair colored by BLK^{P} dye (BLK^{P} (D)) and redyed afterward by BLU^{SP} (BLK^{P} → BLU^{SP}). The corresponding spectra of BLK^{P} on hair, the dye itself (BLK^{P} (D)), BLU^{SP}, and the dye itself (BLU^{SP}(D)) are also included.

dyes (Figure S2). Similar to the single-point spectra, Raman mapping revealed two types of spectral patterns. Some of the collected spectra had only vibrational fingerprint of BLU^{SP}, whereas other spectra exhibited vibrational signatures of both BLK^{P} and BLU^{SP} dyes. Also, Raman mapping revealed that the intensity of 1423 cm^{-1} (BLK^{P} dye) in those spectra varied. In some of the collected spectra (spectra 3−4 and 8−10), the intensity of the 1423 cm^{-1} band was found to be lower than in the others (spectra 5−7). This experimental evidence further confirms our hypothesis about uneven distribution of colorants on hair. We can also conclude that although multiple spectra have to be acquired, SERS-based detection of underlaying permanent colorant on hair redyed by semipermanent dyes is feasible.

Of course, the confirmatory detection of the underlaying dye can be possible only if this dye and the applied afterward colorant have different chemical structures. In our previous study, we made a detailed investigation of chemical content of various commercially available permanent and semipermanent colorants and concluded that nearly all of them have their own unique chemical composition.[23] This chemical diversity of colorants is caused by IP-driven requirements for manufacturers to come up with a new dye formula for any new colorant. As a result, all colorants even if they have the same color have different chemical composition. Therefore, one can expect that our discovery can be broadly utilized for detection and identification of various semipermanent colorants if they were redyed by commercially available semipermanent and permanent colorants.

Oxidation of diaminobenzene derivatives, chemical components of all permanent dyes, results in a formation of large polyaromatic compounds known as Borowsky bases.[26,27] Such oxidation reactions are not structure-specific, leading to formation of polymeric products with various molecular masses. One can expect that this may cause appearance of similar oxidation products upon development of permanent colorants from different commercial brands. To test this hypothesis, we recolored BLK^{P} dyed hair by another permanent dye with a similar color (BLBK^{P}), Figure 5.

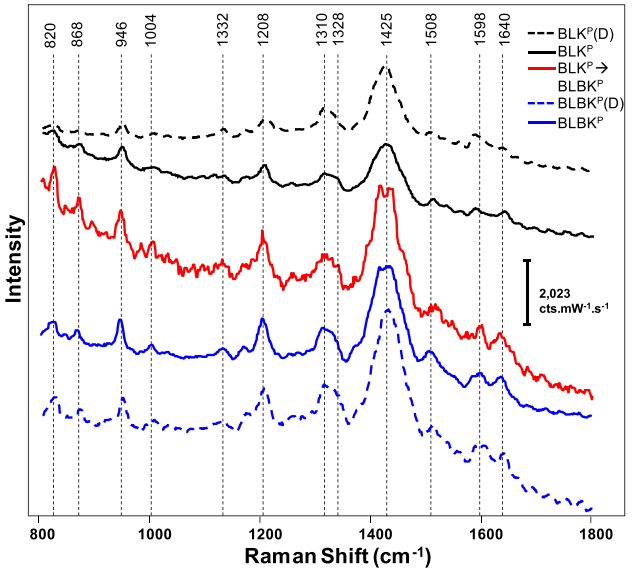

Figure 5. SER spectra of hair colored by BLK^{P} dye (BLK^{P} (D) and redyed afterward by BLBK^{P} (BLK^{P} → BLBK^{P}). The corresponding spectra of BLK^{P} on hair, the dye itself (BLK^{P} (D)), BLBK^{P}, and the dye itself (BLBK^{P}(D)) are also included.

We found that hair dyed with both BLK^{P} and BLBK^{P} dyes exhibited the same set of vibrational bands, making them indistinguishable by SERS on the recolored hair (BLK^{P} → BLBK^{P}).

Once dyed, hair is typically recolored with a periodicity of two months. This time period is determined by the growth rate of human hair. Therefore, a question to ask is whether SERS can be used to detect and identify dyes on hair colored more than two months ago. To answer this question, a volunteer

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from our laboratory dyed her hair by a BLU^{SP} colorant. The volunteer performed normal daily hygiene washing and combing her hair. Samples of hair were taken every week and analyzed by SERS. Our results indicate (Figure 6) that SERS is capable of sensing the colorant on hair that was applied as long as nine weeks (and possibly longer) prior to the spectroscopic analysis.

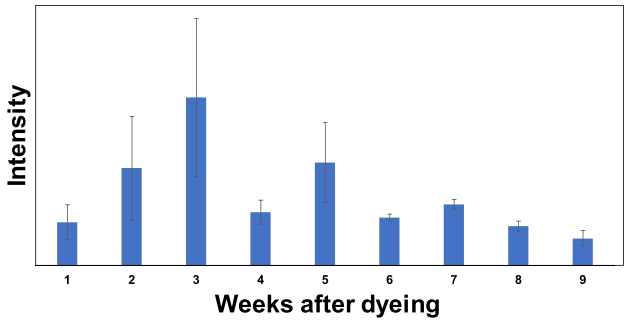

Figure 6. Change in the intensity of BLU^{SP} dye on hair during the nine-week period.

We also noticed that intensities of SERS signals had large fluctuations. Such signal fluctuations are very typical for SERS.[19] In our case, they can be attributed to uneven distribution of AuNRs on the surface of hair. One can envision that SER spectra collected from spots with high AuNR density on hair would exhibit high signal intensity. Spectra with low intensity, on the opposite, likely originated from areas on hair with low density of AuNRs. Thus, more robust experimental protocol has to be developed to enable uniform distribution of plasmonic material on hair. If this could be achieved, one can imagine that SERS-based detection of dyes on hair could allow for precise determination of postdying time intervals.

CONCLUSIONS

Using SERS, we were able to detect and identify the blue semipermanent dye on hair recolored by both black semipermanent and black permanent dyes. We also showed that original black permanent colorant could be detected by SERS if the hair was redyed by blue semipermanent dye. However, it could not if the hair was recolored by another (blue or black) permanent dye due to similarity of oxidation products of permanent dyes. We demonstrated that SERS can be used for confirmatory detection of dyes on hair that was colored more than two months prior to the analysis and washed daily during this time period.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b01021.

Absorption spectrum, micrograph of hair, and Raman spectra (PDF)

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Notes

The authors declare no competing financial interest.

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