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Geneviève Massonnet,[1] Ph.D.; Patrick Buzzini,[1] M.Sc.; Georg Jochem,[2,3] Ph.D.; Michael Stauber,[4] Ph.D.; Tiernan Coyle,[5] M.Sc.; Claude Roux,[6] Ph.D.; Jane Thomas,[6] B.Sc (Hons); Henk Leijenhorst,[7] M.Sc; Zita Van Zanten,[7] B.Sc; Ken Wiggins[8]; Charlotte Russell,[8] B.Sc.; Souad Chabli,[9] Ph.D.; and Avner Rosengarten,[10] M.Sc.

Evaluation of Raman Spectroscopy for the Analysis of Colored Fibers: A Collaborative Study

ABSTRACT: A collaborative study on Raman spectroscopy was carried out by members of the ENFSI (European Network of Forensic Science Institutes) European Fibres Group (EFG) on three dyed fibers: two red acrylics and one red wool. Raman instruments from six different manufacturers were tested as well as nine different laser wavelengths ranging from blue ($\lambda = 458$ nm) to near infrared-NIR ($\lambda = 1064$ nm). This represents the largest comparison study of Raman analytical parameters carried out on identical fiber samples. For the chosen fiber and dye samples, red lasers ($\lambda = 633$ and 685 nm) gave the poorest spectral quality whereas blue (458 nm), green (514 nm) and near infrared lasers (785, 830 and 1064 nm) provided average results. Blue (488 nm) and green lasers (532 nm) globally gave the best quality spectra. Fluorescence problems were often encountered with some of the excitation wavelengths and therefore a flexible Raman instrument equipped with different lasers can be recommended to measure forensic fiber samples. The instrument should also be equipped with a Raman microscope in order to be able to focus on a single fiber. This study shows that Raman spectroscopy usually enables the identification of the main dye present in a colored fiber; however, minor dye components are much more difficult to detect. SERRS (Surface Enhanced Resonance Raman Scattering) techniques give an improvement of the dye's spectral intensity but no spectral improvement was observed for the two red acrylic and red wool fibers tested.

KEYWORDS: forensic science, Raman spectroscopy, comparison study, laser wavelength, fibers, acrylic, wool, dyes, dye identification, SERRS

Raman spectroscopy has already been applied to different types of forensic science samples like inks (1–3), automotive paints (4–5), drugs (6–7), lipsticks (2,8), explosives (9–10), gunshot residues (11) and automotive lenses (4).

When considering fiber analysis, Raman spectroscopy was mainly used for the analysis of man-made fibers (12–15). This research shows that the polymeric classes and sometime sub-classes can be determined when undyed fibers are considered. Edwards et al. (16) were able to distinguish between some vegetable fibers using Raman spectroscopy based on the amount of cellulosic material present.

[1] Ecole des Sciences Criminelles ESC, Institut de Police Scientifique IPS, UNIL-Bâtiment de Chimie, CH-1015 LAUSANNE-DORIGNY, Switzerland.
[2] Kriminaltechnisches Institut, Landeskriminalamt Sachsen, Neuländer Strasse 60, 01129 Dresden, Germany.
[3] Bundeskriminalamt, KT33, Thaerstrasse 11, 65193 Wiesbaden, Germany.
[4] Landeskriminalamt Nordrhein-Westfalen, KTI, Völklinger Strasse 49, 40221 Düsseldorf, Germany.
[5] Forensic Alliance Ltd., F5 Culham Science Centre, Abingdon, OX14 3ED, UK.
[6] Centre for Forensic Science, UTS, Sydney, PO Box 123, Broadway, NSW 2007, Australia.
[7] Netherlands Forensic Institute, PO Box 24044, 2490 AA Den Haag, The Netherlands.
[8] London Laboratory, Forensic Science Service, 109 Lambeth Road, London SE1 7LP, England.
[9] Laboratoire de Police Scientifique, 3 Quai de l'Horloge, Paris 75001, France.
[10] Fibers & Polymers Laboratory, Israel Police National HQ, Haim Bar-Lev Road, Jerusalem 91906, Israel.
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Jochem and Lehnert (17) analyzed pigmented polyacrylonitrile (PAN) and cellulose viscose fibers. They noticed that for highly pigmented fibers, the signal of the polymer matrix is totally hidden by the spectra of the main pigment present.

Cotton fibers dyed with synthetic and natural indigo were analyzed by Coupry et al. (18). All the samples showed similar Raman spectra that could be attributed to indigo blue ($C_{16}H_{10}N_2O_2$, C.I. Vat Blue 1). White (19) used SERRS techniques with some success to identify some reactive dyes present in cotton fibers. Thomas et al. (20) analyzed blue, black and gray cotton fibers dyed with reactive dyes. Raman spectroscopy usually enables the identification of the main dye present.

Some red and blue acrylic fibers were analyzed by Bourgeois and Church (21) using an FT-Raman instrument. The Raman spectra obtained contained both information about the polymer and the dye but spectral subtraction was necessary in order to identify the dye present.

To make a comprehensive analysis of dyes, it is often necessary to use several analytical techniques. Color is one of the most distinguishing features of fibers and textiles. The most common techniques used in forensic science for the analysis of fiber dyes are microspectrophotometry (MSP) which provides a non-destructive and objective measurement of color, but with little information about the specific dye used (22), and thin layer chromatography (TLC) which provides information about the dyes used but is semi or completely destructive (23). Other techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been tested for use (24–25). Both methods have value for the dye industry in separating dyes but there are a number of practical problems in developing suitable methods for forensic fiber dye analysis (i.e., variety of dye classes, organic extraction solvent not easily compatible with HPLC column or with the CE aqueous buffer).

Because Raman microscopic examination requires no sample preparation and does not damage samples, further analysis can be carried out on the same fiber using other techniques. Only a small sample is needed and it may have dimensions no larger than the focal spot (approximately 1 μm), depending on the aperture of the objective and the selected wavelengths of the laser. This capability of obtaining, in a non-destructive way, molecular information on a microsample, makes Raman microscopy a very powerful technique. The purpose of this study was to investigate Raman spectroscopy as a possible means of examining fiber dyes (in powder form and in fiber matrices) and to compare different analytical conditions and instrumentation. A limitation of Raman spectroscopy so far has been the large variety of parameters, designs and sampling procedures which has rendered a clear evaluation of the technique difficult until now.

In this collaborative study from the European Fibres Group (EFG), Raman spectra were recorded with nine different laser sources to observe the influence of the excitation wavelength on the spectral quality. Raman spectra were evaluated on nine dye powders and three colored fibers (two red acrylics and one red wool). The possibility to identify dyes by *in situ* analysis of colored fibers was also evaluated.

The main limitation of Raman spectroscopy is fluorescence that sometimes completely obscures the Raman spectrum. This fluorescence can, in some instances, be reduced, or removed mathematically using post-processing, by changing the laser wavelength (17) or by using SERRS techniques which generally showed an increase of the signal and a quenching of the fluorescence (2,19,26,27). SERRS spectra were also recorded in this survey.

Materials and Methods

Dyes and Fiber Samples

The reference fiber set consisted of two red acrylics (samples 1*a* and 3) and one red wool (sample 1*b*, see Table 1). Both acrylics were distinguishable by their polymer types: sample 1*a* was a copolymer of polyacrylonitrile and vinyl acetate (PAN/VA) and sample 3 a copolymer of polyacrylonitrile and methyl acrylate (PAN Crumeron™). For one of the dyed acrylic fibers (sample 3), an undyed reference fiber was also available (sample 2, PAN Crumeron™). Each of the red fibers were dyed using three different but known dyes (samples 4 to 11, see Table 1). For six of the nine dyes, their corresponding Colour Index (C.I.) names were known. Five of them were basic (3 red and 2 blue) and 1 acid (violet). The relative concentration of these dyes in the different fibers was unknown.

Sample Preparation

Fiber dyes—Dye powders were deposited directly onto a support (glass microscope slide, aluminium foil or holder) or diluted in water and then dried on the support.

Fiber samples—Fiber samples were generally analyzed in situ without sample preparation. The fibers were fixed on different supports, i.e. glass microscope slides, double side adhesive tape, aluminium foil or gel-pads used for shoeprint lifting.

Some fibers were also mounted dry or in XAM™ mounting media on a glass microscope slide, under glass or quartz coverslips.

Raman Spectroscopy

Different laser excitation wavelengths were compared and tested. Raman instruments from six manufacturers (instrument codes I to VI, see Table 2) were available to us. It should be emphasis that this study does not intend to be an exhaustive review/comparison of Raman instruments on the market but it reports the results of a collaborative study investigating the value of Raman spectroscopy for fiber examination mainly based on the laser excitation wavelength used.

Instrument I was not designed for fiber examination but for documents analysis. Most of the instruments, with only one exception (instrument VI, FT-Raman with a NIR laser emitting at $\lambda = 1064$ nm), were equipped with a microscope.

As the same manufacturer can use different laser configurations depending on their customer needs, the wavelength configurations are also described in Table 2.

Measurements were carried out using the optimum analytical parameters (measurement time, laser power, accumulation, etc.) of each instrument for the nine dyes and the three fiber samples. Spectra were recorded from 200 to 500 cm^{-1} until 2000 to 3500 cm^{-1} depending on the instrument. The magnification of the microscope objectives varies between the different Raman microscopes from 10 to 100x.

Surface Enhanced Resonance Raman Scattering (SERRS) Spectroscopy

Four participants used SERRS methods on the given dye and fiber samples. Usually one drop (or 0.1 μL) of poly-L-lysine (0.01%), then one drop (or 0.1 μL) of silver colloid were applied directly on the diluted dried dyes or on the colored fibers.

One participant measured the dyes in solution (3 mg dyes/10 mL ethanol/distilled water until an approximate dye concentration of

TABLE 1—*Fiber samples and their corresponding dyes.*

Fiber	Composition	Color	Corresponding Dyes and Number	Colour Index Name (C.I)
1a	PAN/VA	Red	Rouge Astrazon GTL-N (4)	Basic Red 18:1
			Rouge Maxillon GRL (5)	Basic Red 46
			Bleu Astrazon FGRL (6)	Basic Blue 159
1b	WOOL	Red	Bordeaux Lanaset B (7)	—
			Bleu Lanaset B (8)	—
			Rouge Polaire 10B (9)	Acid Violet 54
2	PAN Crumeron™	Undyed	None	None
3	PAN Crumeron™	Red	Jaune-or Astrazon GLE (10)	—
			Rouge Astrazon FBL (11)	Basic Red 46
			Bleu Astrazon FGGL (12)	Basic Blue 41

PAN Polyacrylonitrile, VA Vinyl acetate, Crumeron™ polyacrylonitrile trademark from the company ZOLTEC (Hungary). Sample 2 was used as reference.

TABLE 2—*Raman spectrometers tested and their corresponding laser wavelengths.*

Manufacturers, Models	Laser Wavelengths [nm]	Instrument Code	Microscope
Foster + Freeman, FORAM 685-2	685	I	Yes
Foster + Freeman, FORAM 685-2	685	I	Yes
Foster + Freeman, FORAM 685-2	685	I	Yes
Foster + Freeman, FORAM 685-2	685	I	Yes
Renishaw, RM 1000	514, 785	II a)	Yes
Renishaw, RM 1000	514, 633, 785	II b)	Yes
Renishaw, RM 1000	514, 633, 785, 830	II c)	Yes
Horiba Jobin Yvon, LabRAM	458, 488, 514, 633, 785	III a)	Yes
Horiba Jobin Yvon, LabRAM	488, 532, 633, 785	III b)	Yes
Jasco, Ventuno 21	532	IV	Yes
ThermoNicolet, Almega	532, 785	V	Yes
Bruker, FT-Raman RFS 100/S	1064	VI	No

10^{-5} M), 1000 μL of silver colloid was added to the dye solutions (50 μL) followed by 75 μL poly-L-lysine (0.01%).

Results

Raman in Situ Analysis of Fiber Dyes

Raman spectra of the nine given dyes were measured under the best analytical conditions for each instrument and at all the available wavelengths.

Results show that identical dyes measured with different instruments, but with the same excitation wavelength, are similar. On the other hand, identical dyes measured with different excitation wavelengths induce different Raman response: some of the laser wavelengths induce fluorescence problems and spectral differences (mainly in the relative intensities of Raman bands) are often encountered. Figure 1 shows an example of dye 6 (C.I. Basic blue 159) measured with instrument IIc) at four different excitation wavelengths: $\lambda = 514$, 633, 785 and 830 nm. For this specific blue dye, fluorescence was observed with both the red ($\lambda = 633$ nm) and the NIR ($\lambda = 785$ nm) lasers. Spectral differences (modification of the relative intensities of some Raman bands) are visible between the green ($\lambda = 514$ nm) and the NIR lasers ($\lambda = 830$ nm).

The quality of the Raman spectra from the nine dyes was evaluated using three classification criteria:

- No spectra: No spectral information obtained (for example due to fluorescence problems).
- Poor spectra: Spectra of poor quality with only few Raman bands of low intensities.
- Good spectra: Well-defined Raman bands, good spectral quality.

Figure 2 shows examples of spectra belonging to the three chosen categories measured with instrument type IIc): Good spectra (dye 10, $\lambda = 830$ nm), Poor spectra (dye 12, $\lambda = 830$ nm), No spectra (dye 8, $\lambda = 633$ nm).

Figure 3 shows an overview of the relative percentages of dye spectra in each category according to the laser excitation wavelengths. The numbers on the top of the columns represent the total

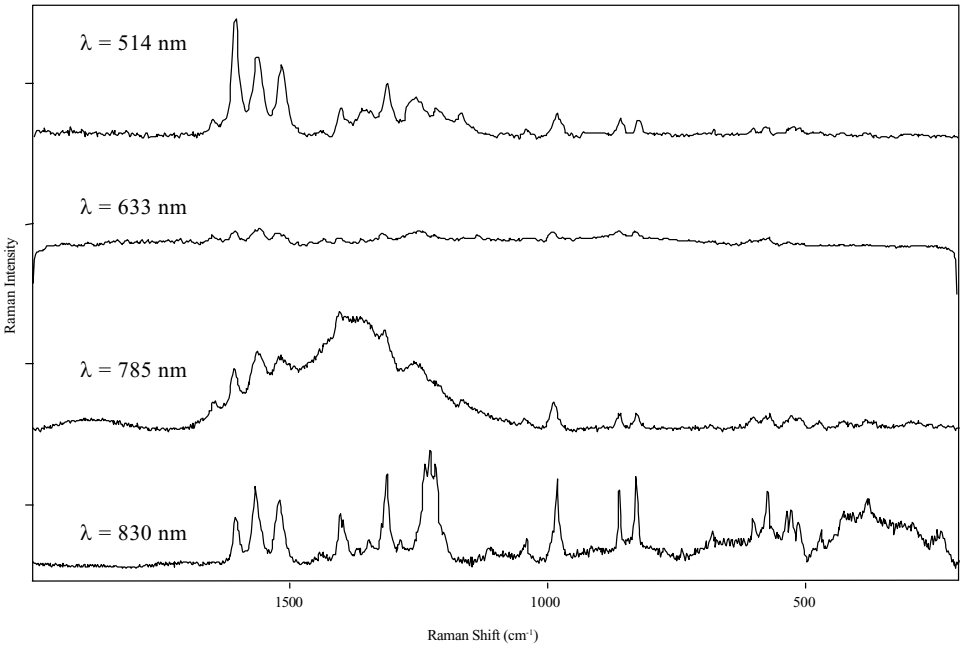

FIG. 1—*Dye 6 (Bleu astrazon FGRL—C.I. Basic Blue 159) measured with instrument IIc) at different excitation wavelengths (from the top to the bottom $\lambda = 514$, 633, 785 and 830 nm).*

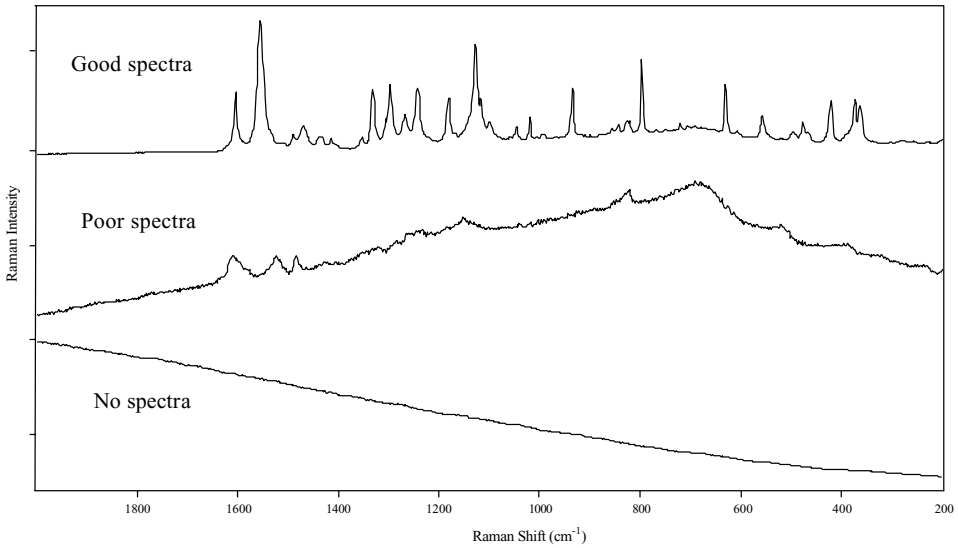

FIG. 2—*Example of spectra belonging to the three chosen categories measured with instrument IIc): Good spectra (dye 10, λ = 830 nm), Poor spectra (dye 12, λ = 830 nm), No spectra (dye 8, λ = 633 nm).*

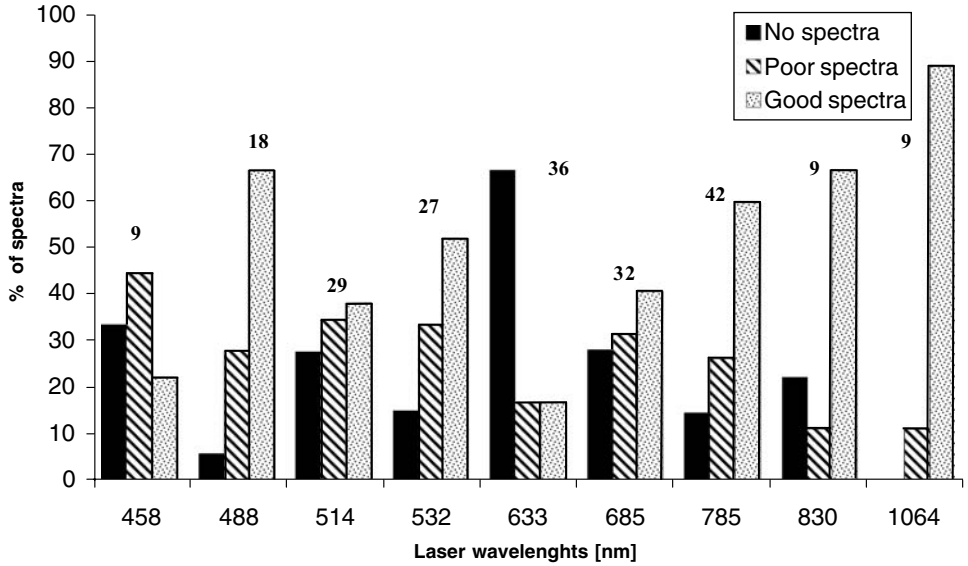

FIG. 3—*Quality of the dye (samples 4 to 12) Raman spectra according to the excitation wavelengths using three classification criteria: no spectra, poor spectra and good spectra. The numbers on top of the column represent the total amount of spectra received at each specific wavelength.*

number of spectra for each laser wavelength (i.e., 36 spectra were recorded at λ = 633 nm and only 9 at λ = 458, 830 and 1064 nm). This global evaluation shows that for the tested dyes the red laser at 633 nm is giving the poorest results (no spectra for 67% of the measurements). Lasers at 488, 532, 785, 830 and 1064 nm gave more than 50% "good spectra" whereas the other red laser at 685 nm as well as the blue at 458 nm and the green at 514 nm gave less than 50% "good spectra."

Spectra recorded with instrument type I usually showed broader Raman bands of a lower intensity than those obtained with the other instruments.

When the Raman bands obtained are of weak intensities, data treatment was used in order to ameliorate the resulting spectra (i.e. baseline correction or signal amplification). Figure 4 shows the raw spectra of dye 9 (C.I. Acid Violet 54) measured with three different instruments type I (λ = 685 nm, laboratories A, B and C). These Raman spectra are very similar.

Figure 5 shows the same spectra after modifications of the raw data: the amplification of the Raman signal is clearly visible but significant differences appear, especially between 500 and 900 cm^{-1} when comparing these modified spectra. This shows that great care must always be taken when manipulating all types of spectral data.

Raman in Situ Analysis of Fibers

Raman spectra of the three red fibers (samples 1*a*, 1*b* and 3) were recorded with the different instruments under optimized analytical conditions and at all the possible wavelengths. As for the dyes, results show that Raman spectra of each individual fiber measured with different instruments but with the same excitation wavelength correspond. On the other hand, the same fiber measured with different lasers induces different Raman response.

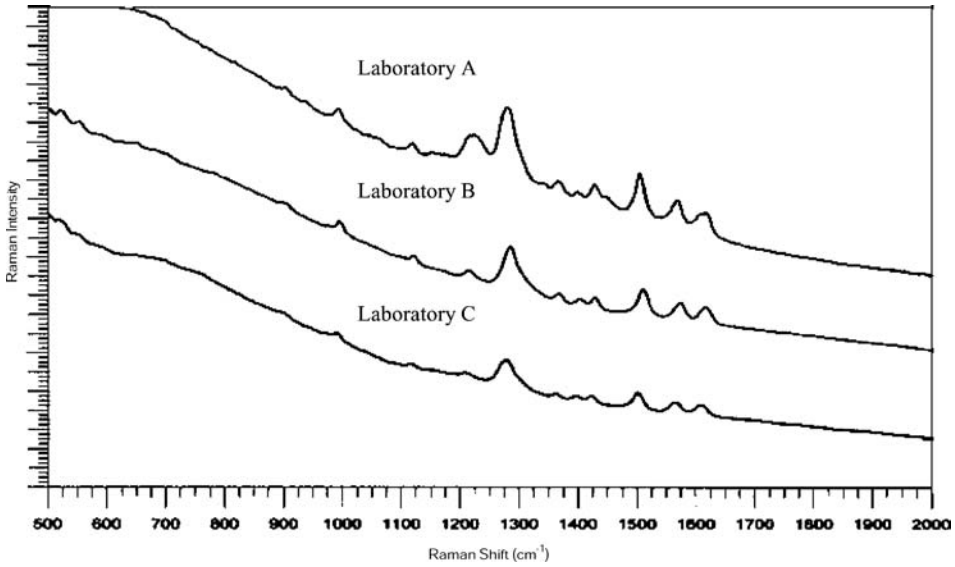

FIG. 4—*Raman spectra of dye 9 (C.I. Acid Violet 54) measured with three different instruments type I (λ = 685 nm, laboratories A, B and C), raw data.*

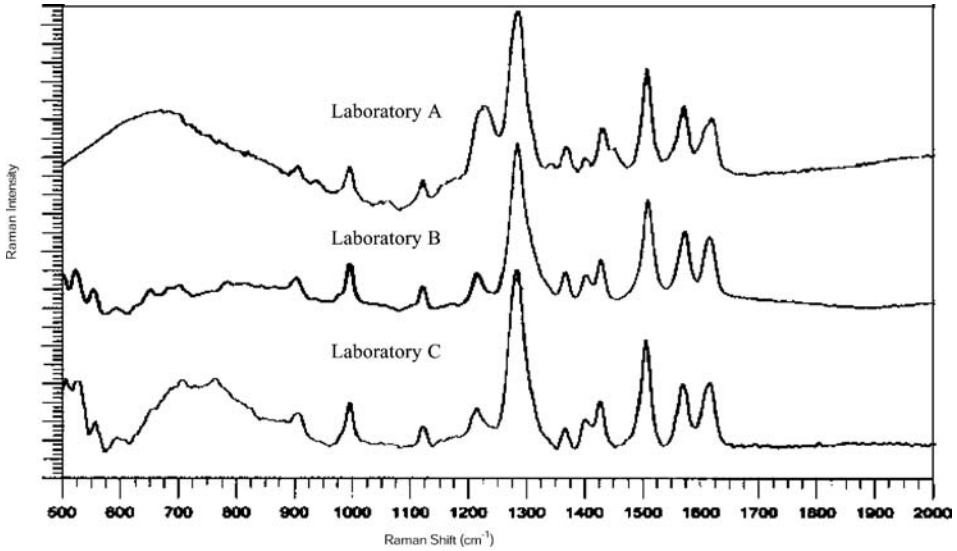

FIG. 5—*Raman spectra of dye 9 (C.I. Acid Violet 54) measured with three different instruments type I (λ = 685 nm, laboratories A, B and C) and after data treatment.*

The quality of all the fiber spectra was evaluated according to the excitation wavelengths of the lasers using the same classification criteria as describe in the previous dyes section (no spectra, poor spectra and good spectra). Figure 6 shows the quality of the Raman spectra obtained for this red fiber set (sample 1*a*, 1*b* and 3). The numbers on top of the column indicate the total amount of spectra at each specific wavelength. This global evaluation shows that the red lasers at λ = 633 and 685 nm give no Raman response for the red fiber samples (1*a*, 1*b* and 3). Only three lasers at λ = 458, 488 (blue) and 532 (green) gave more than 50% of good quality spectra whereas the NIR lasers (785, 830 and 1064 nm), as well as the blue laser at 514 nm, gave less than 50% of good spectra.

It was noticed that the sample preparation influences the quality of the spectra obtained. When the fibers are analyzed *in situ*, fixed on a support (aluminium foil, double sided adhesive tape, glass slide or gel-pads used for shoeprint lifting) the support did not influence the result. Difficulties were encountered when the fibers are mounted. XAM™ mounting media gives a Raman spectrum that contributes to the Raman spectrum of the fiber. Glass coverslips give a Raman signal when working with the NIR lasers. Quartz coverslips can be used instead as they do not interfere with the fiber signal when using the NIR lasers.

Identification of the Dyes in the Raman Spectra of the Three Red Fibers

The Raman spectra of the three colored fibers were compared to the spectra of their corresponding dyes in order to determine if an identification of these dyes is possible.

Figure 7 shows the Raman spectrum of the red PAN/VA (sample 1*a*) and its three corresponding dyes (instrument IIb), λ = 514 nm). Most of the Raman bands correspond to the red dye 5 (Rouge Maxillon GRL, C.I. Basic Red 46), the presence of the two

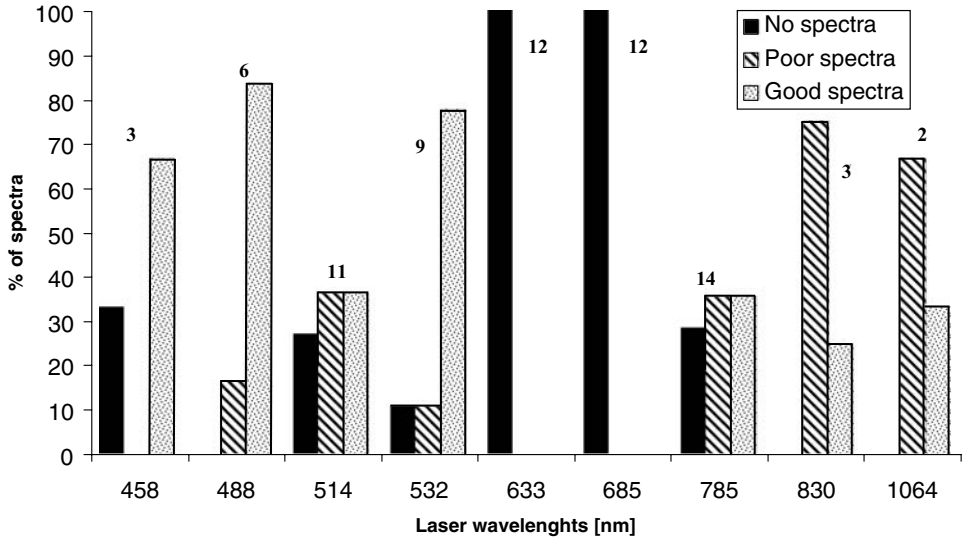

FIG. 6—*Quality of the red fibers (samples 1a, 1b and 3) Raman spectra according to the excitation wavelengths using three classification criteria: no spectra, poor spectra and good spectra. The numbers on top of the column represent the total amount of spectra received at each specific wavelength.*

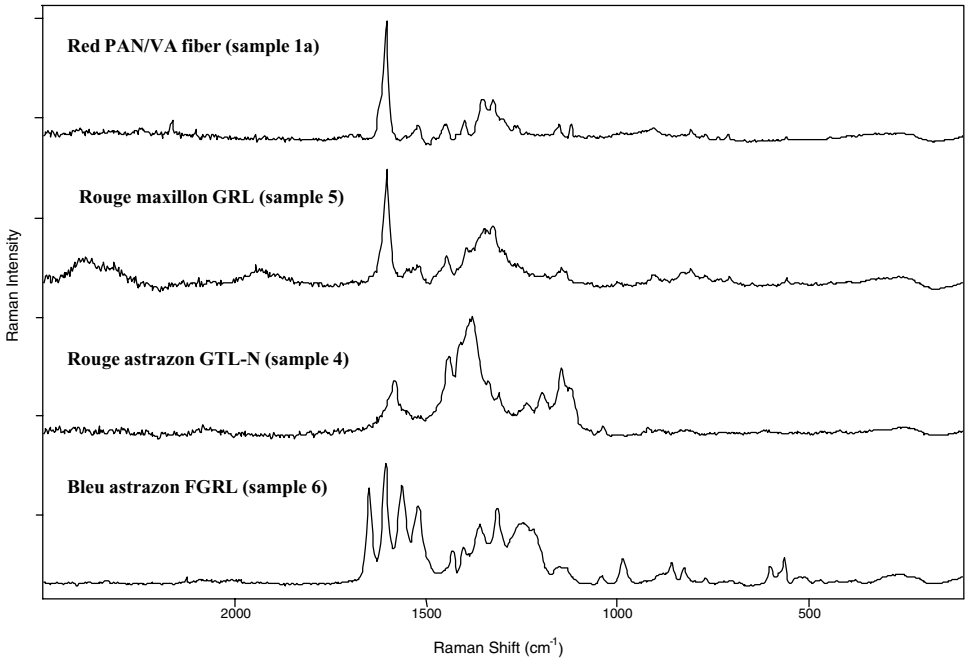

FIG. 7—*Raman spectra of the red PAN/VA fiber (sample 1a) and its three dyes: dye 5 (Rouge maxillon GRL, C.I. Basic Red 46), dye 4 (Rouge astrazon GTL-N, C.I. Basic Red 18:1) and dye 6 (Bleu astrazon FGGL, C.I. Basic Blue 159). These spectra were measured with instrument IIb) at* $\lambda = 514$ *nm.*

other dyes (dye 4—Rouge Astrazon GTL-N, C.I. Basic Red C.I. 18:1 and dye 6—Bleu Astrazon FGRL, C.I. Basic Blue 59) is not visible in the resulting fiber spectrum.

Figure 8 illustrates the Raman spectrum of the red wool (sample 1*b*) with its three dye compounds (instrument V, $\lambda =$ 532 nm). The resulting fiber spectrum corresponds to dye 7 (Bordeaux Lanaset B) and, once again, the contribution of the two other dyes is not seen (dye 8—Bleu lanaset B and dye 9 and Rouge polaire 10B, C.I. Acid Violet 54).

Figure 9 shows the Raman spectrum of the red PAN (sample 3) with its three dye compounds as well as the spectrum of the corresponding undyed PAN (instrument IIb), $\lambda = 514$ nm). Most of the Raman bands correspond to the dye 11 (rouge Astrazon FBL, C.I. Basic Red 46). Two bands from the undyed PAN are also present (corresponding bands are indicated by "$*$" on Fig. 9). The contribution of the two other dyes is not seen (dye 10—Jaune-or astrazon GLE and dye 12—Bleu astrazon FGGL, C.I. Basic Blue 41).

Table 3 is a summary of the dyes identified in the three red fibers (samples 1*a*, 1*b* and 3) for each instrument. The corresponding wavelengths are mentioned in brackets. No dye identification was possible with the instrument type I (laser: 685 nm) as all three fiber types were fluorescent and gave no Raman response.

Dyes 5 and 11 (both C.I. Basic Red 46) used in the two red acrylic fibers (samples 1*a* and 3) were easily identified by different instrument types (II, III, IV and V) and with different lasers wavelengths.

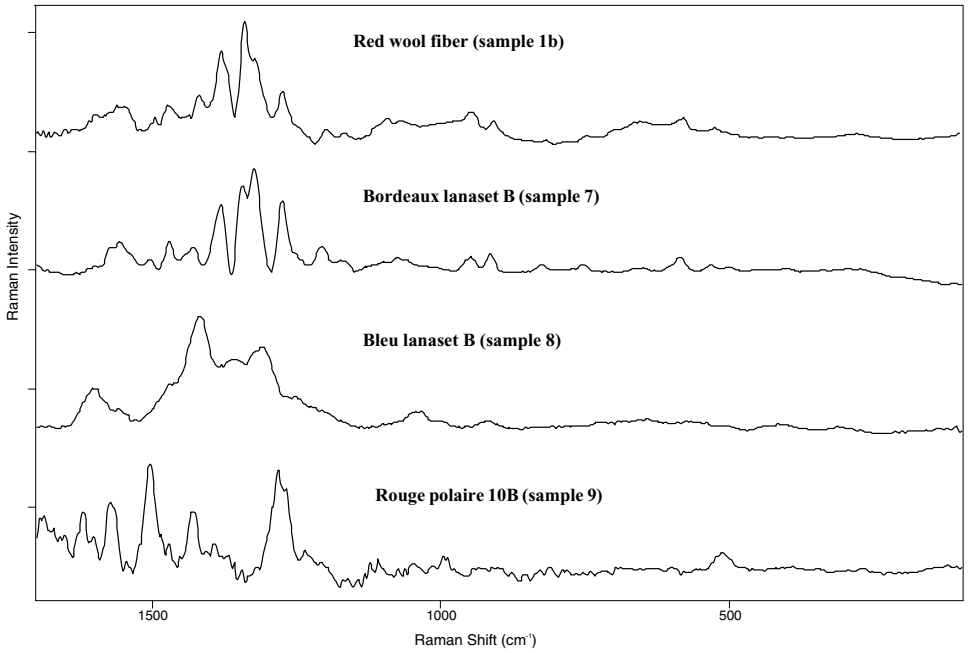

FIG. 8—*Raman spectra of the red wool fiber (sample 1b) and its three dyes: dye 7 (Bordeaux lanaset B), dye 8 (Bleu lanaset B) and dye 9 (Rouge polaire 10B, C.I. Acid Violet 54). These spectra were measured with instrument V at* $\lambda = 532$ *nm.*

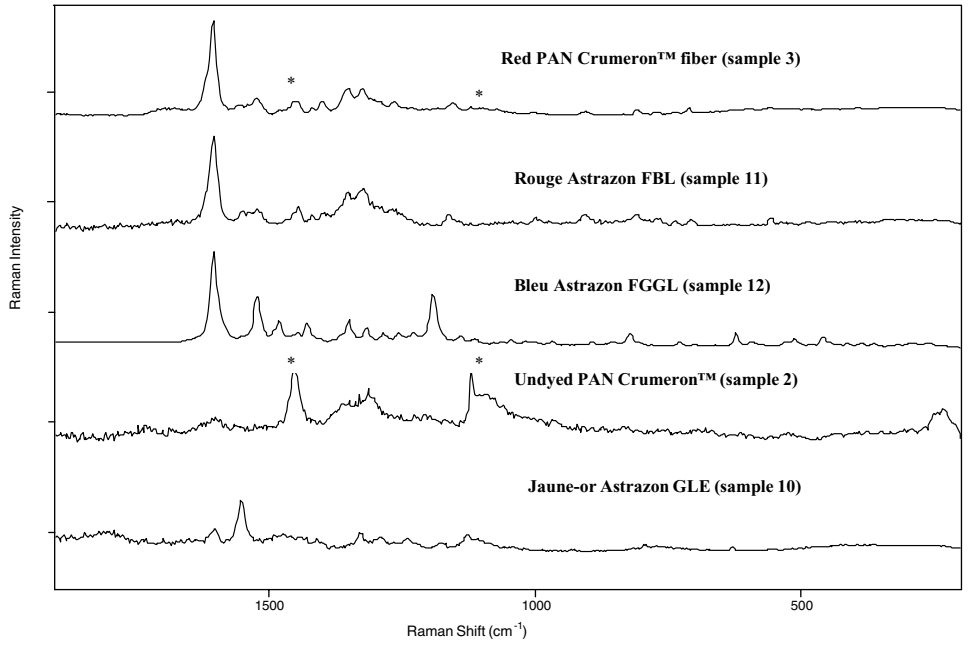

FIG. 9—*Raman spectra of the red PAN Crumeron*^{TM} *fiber (sample 3), dye 11 (Rouge Astrazon FBL, C.I. Basic Red 46), dye 12 (Bleu Astrazon FGGL, C.I. Basic Blue 41), undyed PAN Crumeron*^{TM} *(sample 2) and dye 10 (Jaune-or Astrazon GLE). These spectra were measured with instrument IIb) at* $\lambda = 514$ *nm.*

The two red acrylic fibers (1a and 3) give very similar Raman response for several laser wavelengths (see Fig. 10 for $\lambda = 514$ nm, instrument IIb)) as in both samples the dominant dye compound is identical (Basic Red 46).

Nevertheless, when using NIR lasers at 785 or 830 nm it was possible to differentiate these two red acrylic samples (see Fig. 11 for $\lambda = 830$ nm, instrument IIc)).

Fluorescence problems were encountered with the red wool fiber (sample 1*b*) with several excitation wavelengths ($\lambda = 514$, 633, 685, 785 and 830 nm) and no dye identification was possible with the instruments II b) and c). Only four Raman instruments III a), IIIb), V and VI were able to identify the dye 7 Bordeau Lanaset B in sample 1*b* (laser at 488 and 532 nm). This sample was not measured with the instrument type VI (NIR 1064 nm).

In summary, if the Raman spectra of the red fibers are of good quality, the main dye can easily be identified given that the corresponding dye spectra are available. The presence of the two other minor dyes is impossible or very difficult to detect. In fact,

TABLE 3—*Information concerning the dyes identified in each fiber sample with the different Raman instruments. The laser wavelength used for the identification is given in bracket as well as the corresponding sample number for the dyes and the fibers. If the C.I. name of the dye was known it is given instead of the commercial name.*

Instrument Code	Red PAN/VA [1a]	Red Wool [1b]	Red PAN [3]
I	—	—	—
I	—	—	—
I	—	—	—
I	—	—	—
II a)	?	?	?
II b)	Basic Red 46 [dye 5, 514 nm]	—	Basic Red 46 [dye 11, 514nm] & Basic Blue 41 [dye 12, 785 nm]
II c)	Basic Red 46 [dye 5, 514/830 nm]	—	Basic Red 46 [dye 11, 514/830 nm]
III a)	Basic Red 46 [dye 5, 458/488/514 nm]	Bordeau Lanaset B [dye 7, 488]	Basic Red 46 [dye 11, 458/488/514 nm]
III b)	Basic Red 46 [dye 5, 532 nm]	Bordeau Lanaset B [dye 7, 488]	Basic Red 46 [dye 11, 532 nm]
IV	Basic Red 46 [dye 5, 532 nm]	Bordeau Lanaset B [dye 7, 532]	Basic Red 46 [dye 11, 532 nm]
V	Basic Red 46 [dye 5, 532 nm]	Bordeau Lanaset B [dye 7, 488]	Basic Red 46 [dye 11, 532 nm]
VI	?	?	?

—: Identification of the dye impossible.
?: No information provided concerning the dye identification.

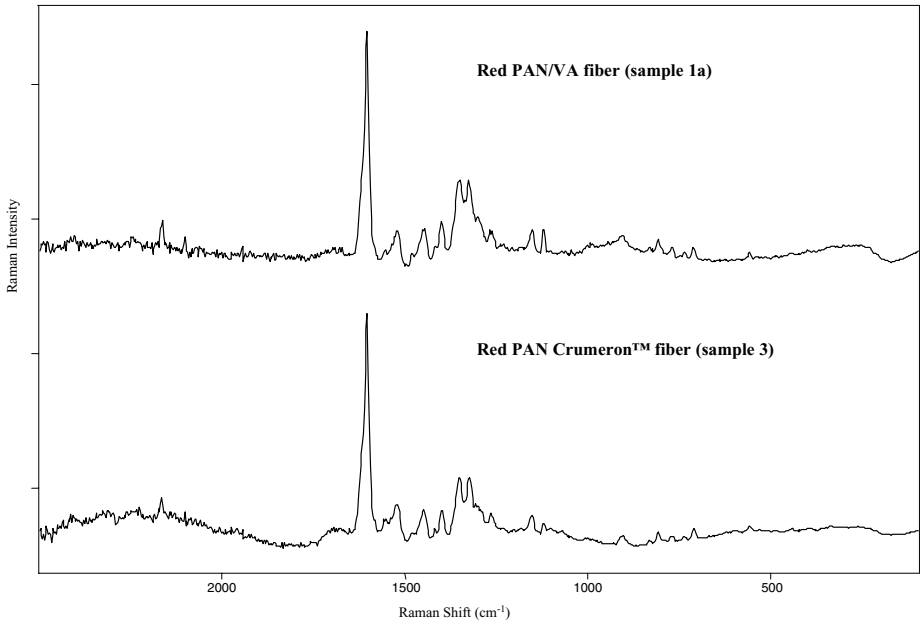

FIG. 10—*Comparison of the Raman spectra of the two red acrylic fibers (sample 1a, PAN/VA and sample 3 PAN Crumeron^{TM}). These spectra were measured with instrument IIb) at* $\lambda = 514$ *nm.*

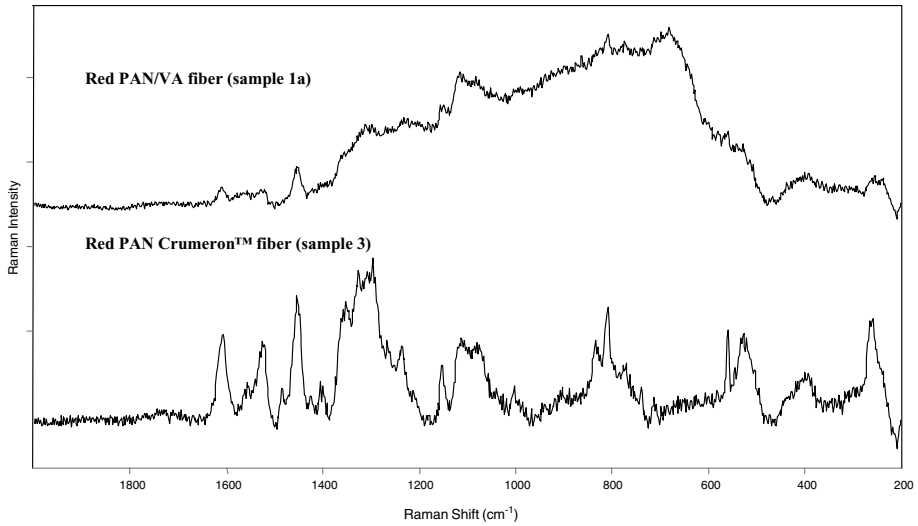

FIG. 11—*Comparison of the Raman spectra of the two red acrylic fibers (sample 1a, PAN/VA and sample 3 PAN Crumeron^{TM}). These spectra were recorded with instrument IIc) at* $\lambda = 830$ *nm.*

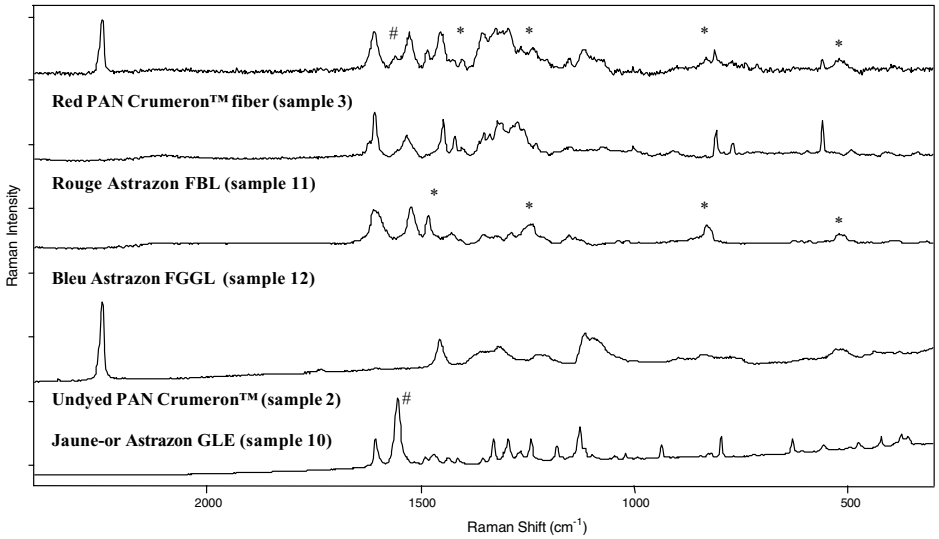

FIG. 12—*Raman spectra of the red PAN Crumeron™ fiber (sample 3), dye 11 (Rouge Astrazon FBL, C.I. Basic Red 46), dye 12 (Bleu Astrazon FGGL, C.I. Basic Blue 41), undyed PAN Crumeron™ (sample 2) and dye 10 (Jaune-or Astrazon GLE). These spectra were measured with instrument IIb) at* $\lambda = 785$ *nm.*

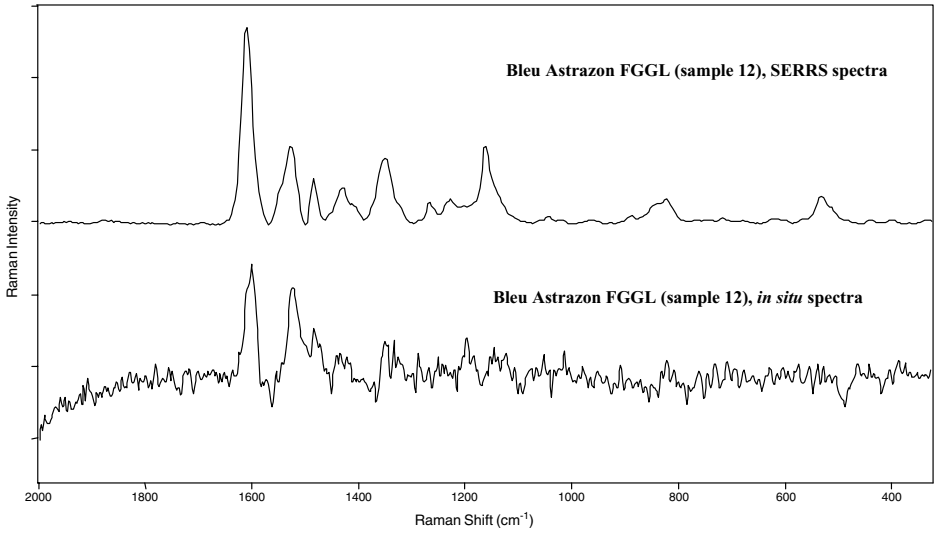

FIG. 13—*Raman spectra of the blue dye Bleu Astrazon FGGL (C.I. Basic Blue 41, sample 12) measured in situ on dye powder and with SERRS technique in solution. These spectra were measured with instrument V at* $\lambda = 532$ *nm.*

only one laboratory mentioned the detection of a second dye in the acrylic sample 3: the presence of Basic Blue 41 (dye 12) was assessed with an excitation wavelength of 765 nm (instrument IIb)). Figure 12 shows that even if the main contribution to the PAN fiber spectrum (sample 3) comes from the dye 11 (rouge Astrazon FBL, C.I. Basic Red 46), at least four bands correspond to the blue dye 12 (bleu Astrazon FGGL, C.I. Basic Blue 41, corresponding bands are indicated by "$*$" on Fig. 12). Even the contribution of the yellow dye 10 (jaune-or Astrazon GLE) is visible at about 1550 cm^{-1} (corresponding band is indicated by "#" on Fig. 12).

Finally, for two instruments no information was provided concerning the dye identification. Instrument VI is an FT-Raman without microscope (1064 nm) and the fibers were difficult to measure. Moreover, the Raman bands observed for the two acrylic fibers (1*a* and 3) were almost all generated by the PAN (polyacrylonitrile) polymer. For the second one (instrument II a)), no spectral evaluation was given; however, as for the results obtained by the other instruments type II , the C.I. Basic Red 46 could be identified in the two red acrylic spectra (samples 1a and 3, laser at 514 nm).

SERRS Techniques

Only four laboratories performed SERRS techniques on the given dyes and fiber samples. One laboratory found no amelioration of the Raman spectra at all (instrument I) whereas the three other laboratories found a great amelioration of the dye spectra (instruments I, II c) and V). Figure 13 shows the Raman spectra of dye 12 (Bleu Astrazon FGGL, C.I. Basic Blue 41) with and without SERRS (instrument type V at 532 nm). SERRS spectra were recorded with the dye in solution as the in situ spectra were measured directly on the dye powder. Figure 13 shows a great amelioration of the dye spectra measured under SERRS conditions.

With respect to the three red fiber samples, no amelioration of the Raman spectra was detected.

TABLE 4—*Combined evaluation of the spectral quality obtained for the 9 dye powders and the 3 colored fibers according to the laser wavelengths.*

Spectra Quality (Dyes and Fibers)	Laser Spectral Domain (color) and Wavelengths [nm]
"No spectra" in the majority of the cases for either fibers or dyes	Red (633, 685 nm)
Less than 50% "good spectra" for either fibers or dyes	Blue (458 nm), green (514 nm), NIR (785, 830, 1064 nm)
More than 50% "good spectra" for both fibers and dyes	Blue (488 nm), Green (532 nm)

Discussion

Raman in Situ Analysis of Fiber Dyes

The dyes were analyzed either in-situ or diluted, mostly in water, then dried on a support. It was noticed that the dilution procedure reduces fluorescence problems. It is also generally easier to focus on the diluted and dried dyes than on the dye powders.

The quality of the spectra obtained depends greatly on the laser wavelengths used. This phenomenon was already observed by other authors like Jochem and Lehnert (17). With the red laser at 633 nm very often fluorescence problems occurred and for 67% of the measurements no spectra were obtained for the samples chosen in this study. The other red laser at 685 nm as well as the blue lasers at 458 and 514 nm gave less than 50% "good spectra." All the other excitation wavelengths (488, 532, 785, 830 and 1064 nm) gave more than 50% "good spectra."

Raman in Situ Analysis of Fibers

For fibers, the sample's preparation influences the Raman spectra obtained. No particular difficulties were encountered when the fibers where fixed on different supports but spectral interference was encountered when the fibers were mounted in XAM as well as when glass coverslips were used with NIR lasers. In this case, the use of quartz coverslips can avoid spectral interference.

The choice of the excitation wavelengths influences the spectral quality of the colored fiber measured. The selected three fibers were globally difficult to measure and fluorescence problems were often encountered. Both red lasers at 633 and 685 nm induced fluorescence of all the samples. Fluorescence problems were also mentioned with undyed fibers at 633 nm by Keen et al. (13). NIR lasers (785, 830 and 1064 nm) as well as the green laser at 514 nm gave less than 50% "good spectra." Only three lasers at 458, 488 (blue) and 532 (green) gave more than 50% "good spectra."

Global Evaluation of all the Excitation Wavelengths Tested on Dyes and Fibers

The evaluation of the spectral quality was carried out on dyes and fibers independently. Table 4 shows a global evaluation of the laser wavelengths tested on the total sample set (dyes and fibers). This table shows that the red lasers (633, 685 nm) very often gave fluorescence problems. Nevertheless, the limited number of samples tested does not allow generalizing the results to all the fiber samples encountered in forensic science cases. Keen et al. (13) also found similar fluorescence problems on different fiber matrices with the 633 nm excitation wavelength. The blue (458 nm), green (514 nm) and all the NIR lasers (785, 839, 1064 nm) gave average quality spectra whereas only two lasers produced good quality spectra for both fibers and dyes: a blue laser at 488 nm and a green laser at 532 nm. It can be noted that even if the two green lasers have rather close excitation wavelengths (18 nm difference), the 514 nm laser gave significantly poorer results than the 532 nm laser.

Identification of the Dyes in the Raman Spectra of the Three Red Fibers

Globally, if the Raman spectrum of the red fibers is of good quality, the main dye can easily be identified given that the corresponding dye spectrum is available. Dye 5 was identified in the red acrylic fiber 1*a*, dye 7 in the red wool 1b and dye 11 in the red acrylic 3. On the other hand, the two other minor dyes present in each of these fibers are either impossible or very difficult to detect. These results are in accordance to those obtained on pigmented fibers where only the main pigment was easily detected (17).

With respect to the two red acrylic fibers, the Raman signal of the polymer is weak in comparison to the signal of the dye and only the main Raman bands of the polymer are visible in the spectra. For the red wool, the Raman spectrum only shows the bands of the main dye.

SERRS Techniques

Generally, an amelioration of the dye spectra was obtained using SERRS techniques. This shows that a particular interaction between the dyes and silver colloid does exist.

On the other hand, no spectral amelioration was observed for the three colored fiber samples.

Using SERRS technique, an important enhancement of the signal and a quenching of the fluorescence was expected: it was the case for the dyes but not for the red fibers considered in this work. Therefore, the difficulty comes from the fibers matrices themselves and from the absence of direct contact between the dyes and the silver colloid for these fibers.

In previous studies, SERRS applications undertaken on dyes in solution (26,27) and on dyed and pigmented items, such as inks (28) and lipstick traces (8), succeeded because the colored molecules could easily be in contact with the silver colloid. Successful applications of SERRS on dyed cotton were reported too (2, 19). Cotton fibers are quite porous and often dyed with reactive dyes, which are covalently bonded with the cotton, so quite accessible for the colloid. More studies are needed in order to understand better the mechanism of interaction between SERRS and a given substrate.

Conclusion

This collaborative research of the European Fibres Group (EFG) is the largest study ever realized on colored fibers (two red acrylics and one red wool) and on dye powders with Raman instruments from six manufacturers. Nine different laser wavelengths ranging from blue ($\lambda = 458$, 488 nm), green ($\lambda = 514$, 532 nm), red ($\lambda = 633$, 685 nm), and near infrared (NIR, $\lambda = 785$, 830, 1064 nm) were available.

As the quality of the spectra obtained depends greatly on the laser wavelengths, this is a very important parameter to consider when choosing a Raman instrument. The results of this survey indicate that red laser at 633 and 685 nm very often showed fluorescence problems and they cannot be recommended for this sample set.

Blue (458 nm), green (514 nm) and near infrared lasers (785, 830 and 1064 nm) provided average results. Blue (488 nm) and green lasers (532 nm) globally gave the best quality spectra.

As fluorescence phenomena are difficult to predict but often encountered, a flexible Raman instrument with different laser wavelengths can be recommended taking into account the large variety of different combination of fiber types and dyes in forensic fiber examinations. The instrument should also be equipped with a microscope in order to be able to focus on a single fiber.

Globally, Raman spectroscopy enabled the identification of the main dye present in a fiber sample given that the spectrum obtained is of good quality (no fluorescence) and the corresponding dye spectra is available. On the other hand, the two other minor dyes present in each of these fibers were either impossible or very difficult to detect. This result shows some limitations of Raman spectroscopy and indicates that further analysis (or perhaps the use of multivariate analysis) is recommended to fully characterize dye mixtures.

On our sample set, the Raman signal of the fiber matrices was weak in comparison to the signal of the main dye and only the main bands of the acrylic polymer were visible in the resulting Raman spectra.

The SERRS technique showed an important enhancement of the signal and a quenching of the fluorescence for the dye samples but no spectral improvement for this set of red acrylic and red wool fibers. More studies are needed in order to understand better the mechanism of interaction between SERRS and a given substrate.

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Additional information and reprint requests:
Geneviève Massonnet, Ph.D.
Ecole des Sciences Criminelles ESC
Institut de Police Scientifique IPS
UNIL-Bâtiment de Chimie
CH-1015 LAUSANNE-DORIGNY
Switzerland
E-mail: Genevieve.Massonnet@unil.ch