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Investigation of tattoo pigments by Raman spectroscopy

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As a result of the increase in the practice of tattooing, the US Food and Drug Administration has identified a need for improved analytical methods to detect the pigments and potential impurities in the inks. Raman spectroscopy allows for nondestructive identification of compounds and is commonly used in art, archaeology, and forensics; however, the technique has only limitedly been applied to the identification of tattoo pigments. In this study, approximately 30 inorganic, organometallic, and organic pigments were evaluated with Raman spectroscopy by using 532, 633, and 780-nm lasers. Individual optimization of the instrumental parameters was performed for each pigment in order to enhance spectral quality. This research highlights the need for multiple laser interrogation, as the spectra of some pigments were difficult to obtain by using a particular wavelength due to interferences from absorption or fluorescence. However, by using these multiple wavelengths, all pigments could be identified by their unique spectral features. A spectral library of the pigments was created for each laser wavelength and then challenged with pigments from multiple manufacturers. All pigments were identified correctly, and the method is poised to be an effective, noninvasive means for qualitatively identifying tattoo pigments. Published 2017. This article is a U.S. Government work and is in the public domain in the USA.

Keywords: Raman spectroscopy; Tattoo inks; Pigments; Spectral library

Introduction

Tattoos are formed when ink containing insoluble pigments dispersed in an aqueous medium, often containing alcohol, glycerine, and/or witch hazel, is injected between the dermis and epidermis of the skin. Tattooing has been performed since ancient times and is currently used as an art form, as permanent makeup, and in medical applications.[1] Worldwide, an estimated 120 million people have tattoos[2] with approximately 12% of Europeans[3] and 24% of surveyed US citizens having a tattoo.[4]

Regulation of the practice of tattooing and tattoo inks varies worldwide. In the United States, the pigments used in tattoo inks are considered by the Food and Drug Administration (FDA) to be colour additives[5,6] and must be listed in the Code of Federal Regulations.[7] Currently, no colour additives have been approved by the FDA for use in injections such as tattooing. The practice of tattooing in the United States is regulated by state and local jurisdictions with adverse events monitored by the FDA.[1] While reported adverse events have generally involved microbial contamination,[8] such as the August 2015 recall of grey inks with *Mycobacterium*,[9] awareness of knowledge gaps on tattoo ink components and long-term risks is growing.[3] One concern is the overlap in tattoo pigments that are also used in the printing, plastics, coatings, textiles, and paint industries.[1] The Council of Europe 2008 Report proposed labelling requirements, chemicals that should not be used due to potential toxicity, and maximum allowed concentrations of impurities (e.g. heavy metals and polycyclic aromatic hydrocarbons) that could potentially address this concern.[10] Despite these guidelines, regulations for each country vary and research is needed to improve detection methods to identify tattoo ink pigments and impurities as highlighted in the European Commission's Joint Research Centre 2016 Report.[3]

Raman spectroscopy could contribute to this area of research, as the spectral bands are specific to the chemical composition and structure of individual compounds. While pigment identification has been performed by using infrared spectroscopy (IR), Raman has the additional ability to identify carbon and some inorganic compounds that are challenging to detect with IR.[11] Raman spectroscopy has been used to identify pigments in a variety of disciplines including art,[12] archaeology,[13–15] and forensics,[16–19] but research on tattoo pigment identification is limited. Raman spectroscopy has been used to investigate black tattoos on a 1000-year-old mummy[20] and for detection of components, including aromatic amines, in red tattoo inks and skin biopsies following reported allergic reactions.[21] Additional studies have focused on spectral library development, including that by Fremout and Saverwyns using micro-Raman spectroscopy at 785 nm to evaluate

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approximately 300 synthetic organic pigments[22] and that by Burgio and Clark with a 1064-nm Fourier transform Raman instrument to develop a database of pigments, minerals, and media.[23] A study by Poon *et al*. focused on *in situ* pigment analysis of a model system of tattoos in pig skin, and the authors were able to identify most of the expected six pigments; however, the authors noted that simple evaluation after smearing tattoo inks onto glass slides led to high fluorescent backgrounds when interrogating with a 632.8-nm He–Ne laser.[24] Further, Miranda performed a detailed evaluation of 14 pigment standards and tattoo inks from US, Chinese, and Brazilian manufacturers in order to identify the best analytical methods, including Raman, for qualitative classification.[25]

These investigations have demonstrated the utility of Raman spectroscopy as a powerful method for the evaluation of pigments used in tattoo inks. However, a study focused on Raman spectroscopy to create pigment spectral libraries at different excitation wavelengths has yet to be peer reviewed. Therefore, this work focused on Raman spectroscopy with 532, 633, and 780-nm lasers for the analysis of pigments in tattoo inks and then incorporated these spectra into searchable databases based on laser wavelength. Unique fingerprints for tattoo pigments, which allow for better characterization of these materials, especially carbon black and those pigments with fluorescence, were obtained. This work provides a general overview for choosing the best laser wavelength for the identification of pigments used in tattoo inks.

Experimental

Standards

Thirty-three pigment standards as well as iron (II) oxide (goethite) and iron (III) oxide (hematite) were obtained from FDA's Office of Cosmetics and Colors, Dick Blick Art Materials (Galesburg, IL), and Sigma-Aldrich (St Louis, MO). Pigments were chosen to provide a number of chemical compositions and uses in tattoo inks. Details of each pigment name, abbreviation, Colour Index (CI) number,[26] chemical class (based on CI structural groups), and pigment class according to chemical moiety[27] are given in Table 1. All pigment standards were powders except PR5, a historical sample synthesized in-house, which was a liquid. For the Raman investigations,

Table 1. Pigment names, abbreviations, CI numbers, chemical classes, and pigment classes for compounds evaluated

Pigment name	Abbreviation	CI number[26]	Chemical class[b]	Pigment class[27]
Activated Charcoal	Act Char	None	Inorganic	Carbon
Carbon	Carbon	None	Inorganic	Carbon
Pigment Black 7	PBk7	77266	Inorganic	Carbon
Pigment Black 8[a]	PBk8	77268	Inorganic	Carbon
Pigment Black 10	PBk10	77265	Inorganic	Carbon
Pigment Black 9[a]	PBk9	77267	Inorganic	Carbon, $Ca_3(PO_4)_2$, $CaCO_3$
Pigment Black 11	PBk11	77499	Inorganic	Carbon, FeO, Fe_2O_3
Pigment Red 101	PR101	77491	Inorganic	Mixture of metal oxides
Pigment White 6	PW6 (anatase)	77891	Inorganic	Titanium dioxide
Pigment White 6	PW6 (rutile)	77891	Inorganic	Titanium dioxide
Pigment Green 7	PG7	74260	Organometallic	Phthalocyanine
Pigment Blue 15	PB15	74160	Organometallic	Phthalocyanine
Pigment Red 5	PR5	12490	Monoazo	Naphthol AS
Pigment Red 22	PR22	12315	Monoazo	Naphthol AS
Pigment Red 170	PR170	12475	Monoazo	Naphthol AS
Pigment Red 175	PR175	12513	Monoazo	Benzimidazolone
Pigment Red 176	PR176	12515	Monoazo	Benzimidazolone
Pigment Red 185	PR185	12516	Monoazo	Benzimidazolone
Pigment Orange 5	PO5	12075	Monoazo	β-Naphthol
Pigment Orange 36	PO36	11780	Monoazo	Benzimidazolone
Pigment Orange 62	PO62	11775	Monoazo	Benzimidazolone
Pigment Yellow 74	PY74	11741	Monoazo	Monoazo yellow
Pigment Yellow 120	PY120	11783	Monoazo	Benzimidazolone
Pigment Yellow 151	PY151	13980	Monoazo	Benzimidazolone
Pigment Yellow 154	PY154	11781	Monoazo	Benzimidazolone
Pigment Violet 32	PV32	12517	Monoazo	Benzimidazolone
Pigment Brown 25	PBr25	12510	Monoazo	Benzimidazolone
Pigment Orange 13	PO13	21110	Disazo	Disazo pyrazolone
Pigment Orange 16	PO16	21160	Disazo	Diarylide yellow
Pigment Yellow 14	PY14	21095	Disazo	Diarylide yellow
Pigment Yellow 83	PY83	21108	Disazo	Diarylide yellow
Pigment Red 122	PR122	73915	Indigoid	Quinacridone
Pigment Violet 23	PV23	51319	Oxazine	Oxazine

[a]Suspected mislabelling from company due to findings in Raman spectra with regard to components; see text for details.
[b]Based on CI structural groups.

small portions (~0.05 g) of each powder pigment were placed on individual, labelled glass microscope slides (Fisher Scientific, Pittsburgh, PA). For PR5, a small volume (~5 μl) was streaked onto a glass slide.

Instrumentation

Raman spectra were acquired on a DXRxi Raman imaging microscope (Thermo Fisher Scientific, Madison, WI) controlled with the corresponding OMNIC™xi Raman imaging software. Prior to sample evaluation, automated alignment and calibration for each laser and grating pair were done to ensure proper instrument performance. The slide with each pigment sample was fitted onto the sample stage of the microscope, and the sample was focused by using a combination of bright-field and dark-field illumination. The sample was then interrogated with three lasers: 532-nm diode laser with a full range grating [5 cm^{-1} full width at half maximum (FWHM) nominal resolution and 50 to 3550 cm^{-1}], 633-nm helium–neon laser with a full range grating [5 cm^{-1} (FWHM) nominal resolution and 50 to 3550 cm^{-1}], and 780-nm diode laser with a high-resolution grating [2 cm^{-1} (FWHM) nominal resolution and 50 to 1800 cm^{-1}]. A 50-μm pinhole aperture was employed, and samples were interrogated via the 10× microscope objective. The instrumental parameters (laser power, exposure time, and number of scans) were optimized for each sample in order to minimize fluorescence while obtaining a high-intensity spectrum with limited spectral noise. The 532-nm laser was operated in the range of 0.2–3.3 mW, with exposure time between 0.003 and 0.06 s and at 10–90 scans. The 633-nm laser was operated in the range of 0.5–7.8 mW, with exposure time between 0.001 and 4.0 s and at 5–90 scans. The 780-nm laser was operated in the range of 0.3–10 mW, with exposure time between 0.005 and 2.0 s and at 10–90 scans.

Data processing

Spectra were obtained for each pigment with the three lasers and analysed by using OMNIC for dispersive Raman software (v. 9.2, Thermo Scientific) to evaluate spectral quality, identify band positions, and create searchable spectral databases. The spectral databases were created with the raw spectra (i.e. no baseline correction or normalization) and separated into three libraries based on laser wavelength. OMNIC Specta (v. 2.0) was equipped with manufacturer libraries and was employed for spectral searching during multicomponent analyses. Additionally, spectra were graphed by using GraphPad Prism software v. 5.02 (La Jolla, CA) for ease of visualization and in order to compare the effectiveness of different lasers for the identification of each pigment. For consistency, and considering that some Raman bands were broader or contained shoulders, all wavelength values were reported to the nearest integer.

Results and discussion

While reference standards consisting of pure, laboratory certified materials were desirable for creating the most precise and accurate spectral libraries, such tattoo pigment standards were generally unavailable or cost-prohibitive to acquire. With this in mind, the spectral libraries described herein are accurate with regard to the samples analysed, but there may be minor deviations (e.g. impurities) from pure standards. This diversity actually adds to the library by including pigment samples used by the tattoo industry. In order to evaluate the spectra from these samples, the tattoo pigments are discussed based on their chemical and pigment classes. The inorganic pigments investigated were carbon blacks, carbon–salt and carbon–metal oxide mixtures, and metal oxides. The organometallic pigments analysed were copper–phthalocyanine complexes. The organic pigments studied were monoazo, disazo, indigoid, and oxazine compounds subclassified as naphthol AS, benzimidazolone, β-naphthol, monoazo yellow, disazo pyrazolone, diarylide yellow, quinacridone, and oxazine (Table 1).[26]

Carbon black pigments

Carbon blacks are insoluble pigments derived from the combustion of hydrocarbons and are commonly used in tattoo inks as a source of black shades and to darken other ink shades. These pigments are not amenable to detection with analytical techniques used for the identification of soluble pigments, such as HPLC-photodiode array, powder X-ray diffraction (XRD), or analysis by IR spectroscopy. Raman spectroscopy is uniquely suited for the detection of these pigments due to the physical origin of the Raman vibrations. Previous studies in our laboratory[28] have been used to identify carbon black in a number of matrices, including cheese and candies, both directly on the sample and following sample treatment.

In this study, samples of various carbon black pigments were evaluated to create our spectral libraries. Full Raman spectra are illustrated in Fig. S1 with representative spectra for PBk7 shown in Fig. 1a. For the carbon pigments, the 532-nm excitation wavelength yielded the best spectra. While the 633 and 780-nm excitation wavelengths also gave appropriate spectra, the lower intensity and baseline deviations for the 633-nm laser and the higher noise and poorer signal for the 780-nm laser made these wavelengths less desirable for the identification of carbon black pigments.

Raman spectra for all carbon black samples acquired with the 532-nm laser are illustrated in Fig. 1b. These spectra had the typical, amorphous carbon bands at ~1350 and 1580 cm^{-1}, which are indicative of the D 'disorder' and G (E_{2g}) 'graphitic' natures of these pigments respectively. The spectra also had generally unresolved, overlapping bands between 2400 and 3300 cm^{-1} containing the second-order D band and C–H vibrations.[29–31] As discussed by Robertson[32] and Jawhari *et al.*,[30] these bands can be indicative of the order in the material. This can be seen when comparing the PBk7 and PBk10 spectra. The PBk7 spectrum had two broad and relatively unresolved bands, while the PBk10 spectrum had three sharper bands. The overlapping D and G bands in the PBk7 spectrum were indicative of a highly disordered, amorphous carbon, while the three sharper bands in the PBk10 spectrum indicated that PBk10 is more ordered. Additionally, the band at ~2700 cm^{-1} in the PBk10 spectrum was attributed to a second-order D band, which further indicated a more ordered structure.[31]

Interestingly, when evaluating the PBk8 and PBk9 spectra (Fig. 1b), it was clear that the PBk9 spectrum contained only the carbon bands previously discussed, while the PBk8 spectrum contained additional bands at 275, 710, and 1085 cm^{-1}. From a multicomponent spectral library analysis, it was determined that the PBk8 spectrum was a mixture of carbon black and calcium carbonate, as the three additional bands were fully attributable to calcium carbonate. We observed that the PBk8 composition matches the labelled composition of PBk9 (Table 1) and vice versa, with the note that neither pigment spectrum had characteristic calcium phosphate bands. As these two samples were

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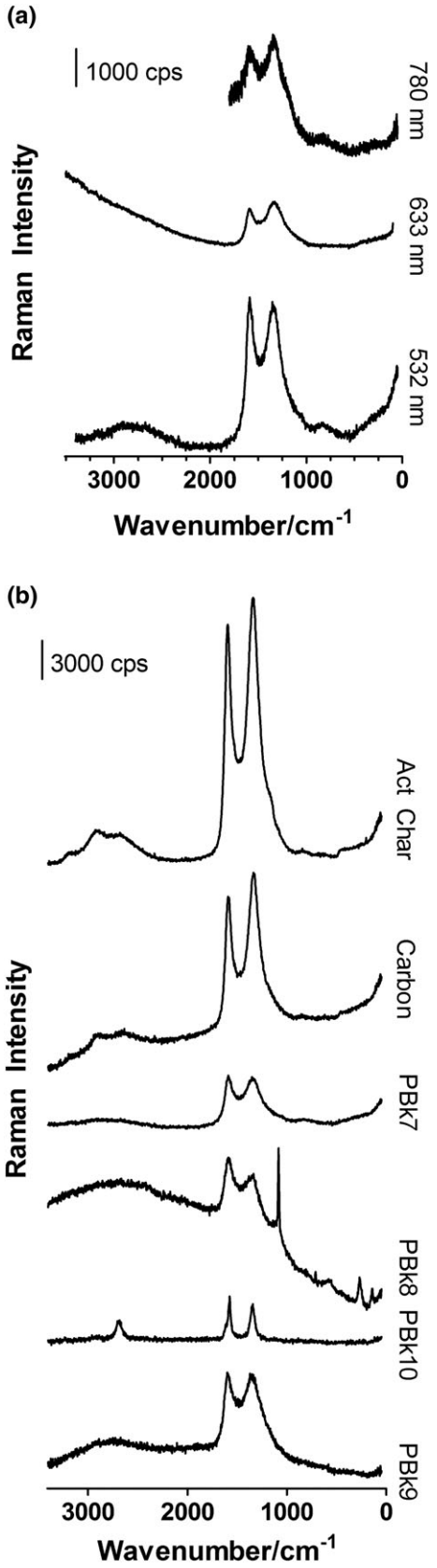

Figure 1. Carbon spectra (offset for visual clarity). (a) Spectra of PBk7 at three excitation wavelengths and (b) Raman spectra at 532 nm for each sample.

acquired from the same location, it is possible that these bottles were mislabelled prior to our receipt. This finding was further indicative of the difficulties of creating spectral libraries with nonreference materials, as well as the ability of Raman spectroscopy to obtain compositional information.

Iron and titanium oxide pigments

Iron oxides and titanium oxides are important components of tattoo inks. Iron oxides (e.g. hematite and magnetite) and oxyhydroxides (primarily goethite) are commonly employed to create red, black, yellow, or brown-hued inks, and pigment shade is dependent on the specific form employed. Titanium dioxide is used as the main source of white colour and for lightening other ink shades due to its high scattering ability, low absorption of light, and high refractive index compared with other white pigments[33] such as CI Pigment White 21 (barium sulfate) and CI Pigment White 18 : 1 (calcium carbonate). Further, the rutile form is more commonly used than the anatase form due to the strong photochemical activity of anatase when subjected to UV light.[34]

It has been previously shown that iron oxides and oxyhydroxides can be challenging to identify with Raman spectroscopy due to spectral dependence on and variance with laser power density (i.e. laser power combined with laser spot size). Specifically, deFaria *et al.*[35] did an extensive study that showed that sample degradation occurred under exposure to high laser powers with bands characteristic of hematite appearing upon degradation of other iron oxides. Additionally, Hanesch studied the transformations of iron oxides at low laser powers (0.01–1 mW) and concluded that, while transformations may occur, identification of different forms of iron oxides was still possible.[36] In general, these transformations were dependent on the stability of the iron oxide form, the laser power, and the exposure time. Thus, by careful selection of these parameters, it could be possible to identify the iron oxides used in tattoo inks. To ensure that the pigments were not degraded during analysis, pure component materials [e.g. iron (II) oxide (goethite) and iron (III) oxide (hematite)] were first analysed and used to set instrument-operating parameters (data not shown). Using these settings, the full Raman spectra for the oxide pigments investigated in this study were obtained and are illustrated in Fig. S2. All excitation wavelengths yielded good spectra, with the 633-nm laser giving the best spectra (good signal, low noise, and low baseline variations). This was especially true for the iron oxides and mixed oxides. Representative spectra at 633-nm excitation are illustrated in Fig. 2 for PBk11 [a mixture of carbon, iron (II) oxide, and iron (III) oxide], PR101 (a mixture of metal oxides), PW6 (rutile), and PW6 (anatase).

The Raman spectrum for PR101 had predominant bands at 225, 290, 405, 495, 605, and 1310 cm^{-1}, which corresponded well with the bands previously published[35] for hematite. The Raman spectrum for PBk11 contained a multitude of bands (225, 290, 345, 500, 650, and 1326 cm^{-1}) at lower wavenumbers. These likely corresponded to multiple iron oxide forms including α-Fe_2O_3 (hematite), γ-Fe_2O_3 (maghemite, broad bands at 345, 500, and 700 cm^{-1}), and FeO (wüstite, 650 cm^{-1}). Although low laser power ($\leq$3 mW) was used for these pigments, it was possible that transformation of the iron oxide compounds still occurred. Therefore, alternate methods, such as XRD, would be more appropriate for conclusively identifying iron oxide pigment forms. Finally, Fig. 2 illustrates the spectra for the two forms of titanium dioxide (PW6 rutile and PW6 anatase), and Raman

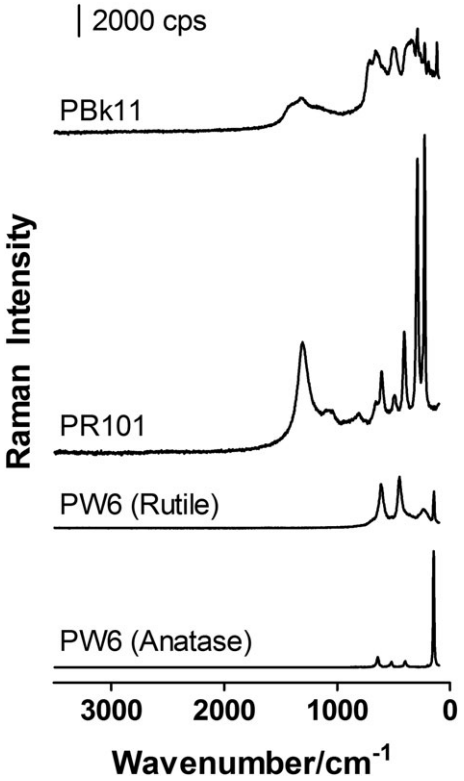

Figure 2. Oxide spectra (offset for visual clarity) acquired with 633-nm laser excitation.

spectroscopy was capable of distinguishing these two forms. Rutile exhibited Raman bands at 143, 237, 449, and 610 cm^{-1}, whereas anatase had a strong band at 145 cm^{-1} with weaker bands at 395, 517, and 641 cm^{-1}, both of which were consistent with previous investigations on titanium dioxide.[37]

Organometallic pigments

Copper phthalocyanine pigments are commonly used as green (e.g. PG7) and blue (e.g. PB15) tattoo pigments. Both organometallic pigments were easily identified with all three lasers (Fig. S3). However, the 532-nm laser yielded the best spectra due to the flat baselines; low baseline noise; and sharp, defined Raman bands. The observation that this 532-nm laser was best suited for phthalocyanine evaluation was supported by the research of Burgio and Clark on pigments, minerals, and media commonly used in artwork.[23] In their work, the challenge of evaluating copper-containing compounds was discussed, as these compounds can have an electronic adsorption band in the near-IR region and thus fluorescence and burning can occur when using excitation wavelengths in this region. However, as illustrated in Fig. 3, these pigments had comparable but not identical Raman spectra at 532 nm. The spectral similarities between PG7 and PB15 were not unexpected, as they have a similar molecular phthalocyanine backbone with PG7 being chlorinated on the outlying benzene rings of the compound. Despite the similarities, differences in their spectra allowed for spectral library matching. This was seen in the intense bands at 681 and 1494 cm^{-1} for PG7 and 1520 cm^{-1} for PB15 as well as unique bands at 1074, 1271, and 1494 cm^{-1} for PG7 and 594, 746, 1141, 1335, and 1447 cm^{-1} for PB15.

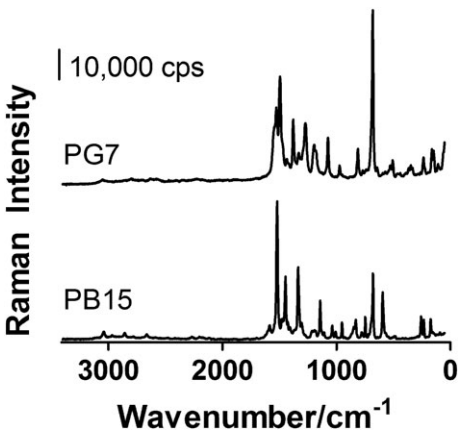

Figure 3. Phthalocyanine spectra (offset for visual clarity) acquired with 532-nm laser excitation.

Organic pigments

The organic pigments investigated in this study are classified as monoazo, disazo, indigoid, and oxazine pigments. These synthetic pigments impart a broad range of hues (yellow, orange, red, and violet) to tattoo inks and therefore are heavily used. Monoazo pigments contain the R^1-N=N-R^2 structural component with a wide variety of R^1 and R^2 functional groups, and the disazo compounds have two of these azo moieties. Indigoid molecules contain a quinacridone-like structure characterized by a heterocyclic ring containing nitrogen atoms, while oxazine molecules are characterized by a heterocyclic ring containing oxygen and nitrogen atoms. For these compounds, the 780-nm laser allowed the best Raman evaluations, as fluorescence dominated many of the spectra for the red, orange, and violet pigments when evaluated with the 532 and 633-nm lasers (Fig. S4 and S5).

Monoazo pigments comprised the largest group of pigments in this study and were further classified into subgroups based on their backbone structure or pigment class (Table 1). The main groups were monoazo yellow (PY74), β-naphthol (PO5), naphthol AS (PR5, PR22, and PR170), and benzimidazolone (PR175, PR176, PR185, PO36, PO62, PY120, PY151, PY154, PV32, and PBr25). Individual Raman spectra for these pigments are illustrated in Fig. 4, and detailed spectral analyses of many of these pigments can also be found in Fremout and Saverwyns.[22] These pigments can have complex spectra due to the variety of functional groups they contain. Also, those that are in the same pigment class can have only small spectral variations due to their highly similar structures (e.g. PO36 and PO62 share a similar backbone, with PO36 only containing its NO_2 moiety in a different position and an additional chlorine atom). Based on this, similarities were found, such as the azo N=N stretching vibrations that generally occurred between 1350 and 1580 cm^{-1}. Many of these pigments also exhibited benzene ring modes with the ring quadrant stretch vibration at ~1600 cm^{-1}, while the naphthol AS compounds exhibited a strong band at ~1365 cm^{-1} due to the naphthalene ring. Despite these similarities, the Raman spectra were distinct, with bands that vary in position and intensities allowing for identification through spectral library matching.[38,39]

The only sample that was nearly unidentifiable by Raman spectroscopy was PR5, which was the only liquid used in this study. Because PR5, PR22, and PR170 are similar in composition with PR22 and PR170 spectra containing distinct, intense bands, it was expected that PR5 would have similarly identifiable Raman bands

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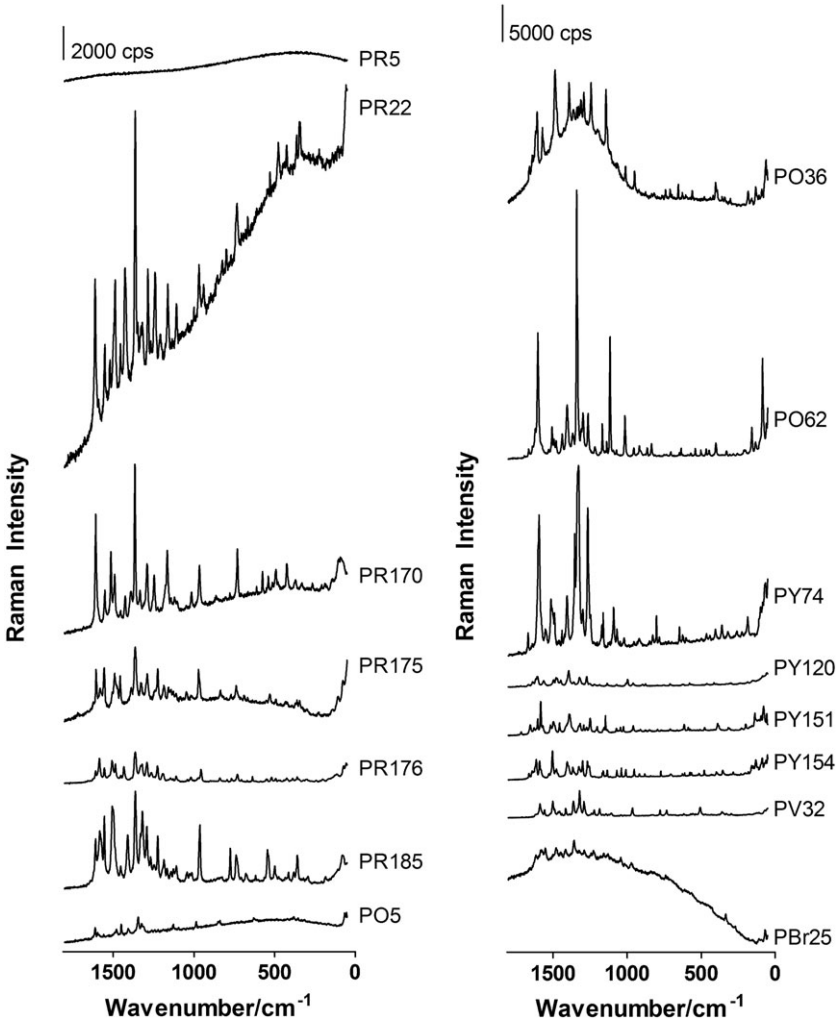

Figure 4. Monoazo spectra (offset for visual clarity) acquired with 780-nm laser excitation.

(Figs 4 and S4). The PR5 sample was prepared in-house, and its stability and purity were not established. As such, it was likely that the poor-quality PR5 spectrum may have been due to potential impurities that caused fluorescence or degradation of the liquid pigment over time.

Figure 5 illustrates the spectra for the disazo compounds, which consist of disazo pyrazolone (PO13) and diarylide yellow (PO16, PO14, and PY83) pigment classes. The spectra of the four disazo pigments contained one strong band between 1595 and 1601 cm^{-1} that belongs to aromatic ring vibrations. Additionally, these spectra had a strong band between 1275 and 1290 cm^{-1} that was attributed to the C–C bridge in a biphenyl group.[40,41] Characteristic of azo compounds, a Raman band for the N=N stretching vibration existed between 1350 and 1580 cm^{-1} in each spectrum.[38,39] Despite these similarities, the spectra for these pigments were distinct and could be identified via spectral matching. Figure 5 also illustrates the pigments in the indigoid (PR122) and oxazine (PV23) chemical classes. The spectrum of the heterocyclic quinacridone compound, PR122, had primary bands at 1645, 1593, and 1566 cm^{-1}, which agreed with previous studies.[42,43] Finally, the spectrum of PV23, the oxazine compound, had a distinct set of three intense bands (1425, 1384, and 1342 cm^{-1}) as well as weaker bands that could be used to identify this pigment, as was also shown by Bell *et al*. when identifying components in lilac paint samples.[44]

Evaluation of pigment libraries

Pigments from multiple manufacturers were evaluated to determine the robustness of the developed libraries. Spectra for PO62 sourced from two different manufactures ($N = 2$) are illustrated in Fig. S6. Using spectral intensity normalization, the bands were nearly visually identical in both position and height, and the spectra were strongly correlated with each other (i.e. spectrum A had an 86% match to library spectrum B). Similar evaluations were performed for PG7 ($N = 2$), PR22 ($N = 3$), PR170 ($N = 4$), PO5 ($N = 2$), PO36 ($N = 4$), PY74 ($N = 2$), PBr25 ($N = 2$), PO13 ($N = 2$), PO16 ($N = 2$), PR122 ($N = 3$), and PV23 ($N = 2$). For each pigment series, results similar to those for PO62 were obtained with good band wavenumber agreement and normalized band intensity as well as strong correlation upon comparison to the library spectrum. This study, while limited, reinforces the ability to use the developed libraries for correctly identifying pigments from different sources.

Using multiple laser wavelengths for evaluating pigments was advantageous, as various classes of compounds performed well at different wavelengths. Specifically, 532 nm was best for the carbon and phthalocyanine pigments; 633 nm was best for the metal oxide pigments; and 780 nm was best for the organic pigments, especially the red pigments for which fluorescence could hinder Raman detection. For ease of comparison, Table 2 gives an overview of the

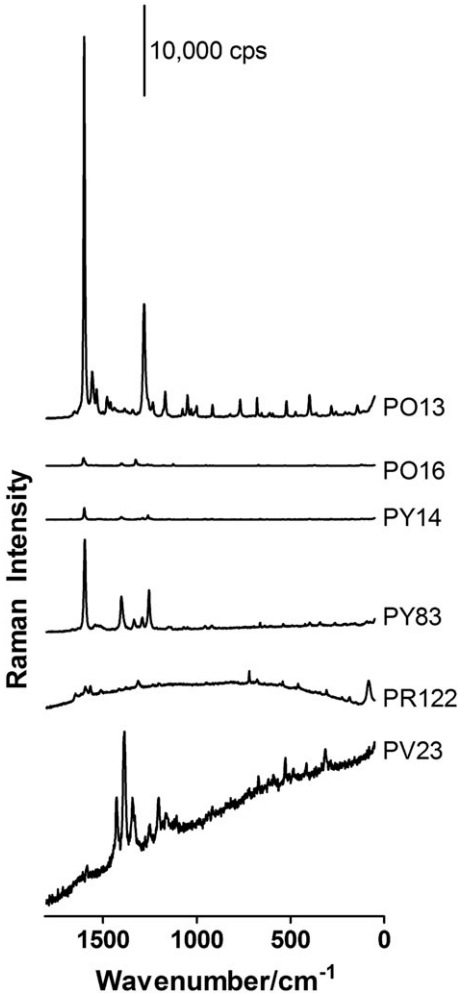

Figure 5. Disazo, indigoid, and oxazine spectra (offset for visual clarity) acquired with 780-nm laser excitation.

laser performance for each pigment evaluated in this study. In summary, the 532-nm laser provided good spectra for 18 pigments, acceptable spectra for 6 pigments, and unacceptable spectra for 9 pigments. The 633-nm laser provided good spectra for 17 pigments, acceptable spectra for 14 pigments, and unacceptable spectra for 2 pigments. The 780-nm laser exhibited the best overall performance by providing good spectra for 23 pigments, acceptable spectra for 9 pigments, and 1 pigment (the liquid, in-house PR5) that could not be identified. However, the 780-nm laser had the worst signal intensity and spectral noise for several pigments, particularly the carbon black pigments. If the pigments present in a tattoo ink were known (e.g. from reported formulations), then Table 2 could be used to select the best laser and associated spectral library. If no knowledge was available, then the 532-nm laser would be expected to perform best for pigments or tattoo inks that are visually yellow, green, blue, violet, and black. For visually red and orange tattoo inks, which suffer from fluorescence, longer wavelength lasers (633 and/or 780 nm) would be appropriate.

It is important to note that challenges may arise when attempting to perform spectral matches of pigments that have very similar structures and are present at low concentrations in tattoo inks. In this case, the evaluator will need to be cognizant of minor band differences to see if there are multiple match values for similar compounds. When a pigment composition is unknown, it is essential to use other analytical techniques to confirm the Raman identification. Current work consists of a market survey with comparison of the Raman spectral library identification to liquid chromatography-UV–Vis and powder XRD analyses in order to create a multi-analytical methodology capable of identifying the pigments used as components of tattoo inks.

Table 2. Pigment spectral performance for each laser (532, 633, and 780 nm)

Pigment	532 nm	633 nm	780 nm
Act Char	Y	Y	Y
Carbon	Y	Y	Y
PBk7	Y	OK[a]	OK[a]
PBk8	Y	OK[a]	OK[a]
PBk10	Y	Y	OK[a]
PBk9	Y	OK[a]	OK[a]
PBk11	OK[a]	Y	OK[a]
PR101	OK[a]	Y	Y
PW6 (rutile)	Y	Y	Y
PW6 (anatase)	Y	Y	Y
PG7	Y	Y	OK[ab]
PB15	Y	Y[b]	Y[b]
PR5	N[ab]	Ok[ab]	N[ab]
PR22	N[b]	OK[ab]	Y[b]
PR170	N[b]	Y[b]	Y
PR175	OK[ab]	OK[a]	Y
PR176	N[ab]	OK[a]	Y
PR185	N[ab]	OK[a]	Y
PO5	OK[ab]	OK[ab]	OK[ab]
PO36	OK[a]	Y[b]	Y[b]
PO62	Y	Y	Y
PY74	Y	Y	Y
PY120	OK[ab]	OK[ab]	Y
PY151	Y[b]	OK[a]	Y
PY154	Y	OK[ab]	Y
PV32	N[a]	OK[ab]	Y
PBr25	Y[b]	Y[b]	OK[ab]
PO13	N[ab]	Y	Y
PO16	N[b]	OK[ab]	Y[a]
PY14	Y[b]	Y[a]	Y[a]
PY83	Y	Y	Y
PR122	N[b]	N[ab]	OK[ab]
PV23	Y[b]	N[ab]	Y[ab]

[a]Low intensity and/or noisy spectrum even after optimization.
[b]High fluorescence, heating, and/or curved baseline.
Y, yes, good spectrum with appropriate band intensity/low noise for database.
OK, okay, acceptable spectrum but may hinder high correlation matching.
N, no, unacceptable spectrum (too low band intensities and/or too curved baseline).

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Please contact the corresponding author for inquiries regarding spectra availability in a digital format.

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