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1064 nm DISPERSIVE RAMAN MICRO-SPECTROSCOPY FOR THE IN-SITU IDENTIFICATION OF ORGANIC RED COLORANTS

FULL PAPER

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Raman spectra of red colorants were obtained in-situ using a dispersive Raman microscope equipped with a 1064 nm laser source and InGaAs array detector. By employing near-IR excitation, the fluorescence of organic colorants is significantly reduced compared to visible wavelengths. Spectra presented include alizarin crimson, lac dye, cochineal, brazilwood, dragon's blood, sandalwood, logwood, and safflower. Analyses of dry pigments versus those applied in binding media are also presented. This work demonstrates that using 1064 nm excitation in a dispersive Raman system may allow in-situ characterization of artists' materials, including organic colorants, which are challenging to analyze using visible excitation wavelengths because of interfering fluorescence.

1   Introduction

Raman spectroscopy has been widely applied to problems in conservation and archaeometry, and pigment identification is becoming a routine part of technical examination using this technique.[1-3] Establishing a palette for an artifact, a body of work, or a particular artist through pigment analysis can assist with assigning artistic authorship and play an important role in technical art history research. *In-situ* pigment identification techniques are strongly preferred for artworks for which sampling is impractical or disallowed, for example, for illuminated manuscripts where paint and ink layers are generally very thin.[2,4] Material analyses on such objects must be performed non-invasively, and visible dispersive Raman micro-spectroscopy can accomplish this *in-situ*, with no sampling requirements. This represents one of the technique's most important attributes. Indeed, the suitability of visible dispersive Raman micro-spectroscopy for non-invasive, non-destructive pigment analysis on works of art, including works on paper or parchment, is well established.[1,2,5-8]

Natural organic colorants of either plant or animal origin were important traditional sources of red hues,[9] and have therefore long been

targets of investigation. Although it has demonstrated great utility in the identification of traditional inorganic and modern synthetic organic pigments, Raman spectroscopy has yet to find broad application in the identification of traditional organic colorants.[4] When excited using visible radiation, these colorants typically fluoresce strongly, obscuring vibrational information that would allow unambiguous identification. Additionally, many organic colorants are highly tinting and, since very little pigment achieves the desired color saturation, are present on artifacts in low concentration.[10] These limitations have often frustrated the routine application of Raman spectroscopy to the *in-situ* identification of organic red colorants on cultural heritage objects.

The main disadvantage of using visible dispersive Raman techniques to examine organic red colorants on manuscripts stems from the strong fluorescence of organic pigments and/or binding media that has been noted by numerous researchers.[1-4,10-21] While visible dispersive Raman spectroscopy has been used successfully to identify organic red colorants such as alizarin, unfortunately the approaches are often invasive,[17,22] requiring sample removal. More recently, attempts to characterize red organic colorants via Raman spectroscopy have focused on surface enhancement techniques to overcome the obstacle posed by fluorescence.[10,12-14,17-20,22] While these methods allow collection of high quality spectra, disadvantages include the need for extracting colorants from complex sample matrices, the preparation of specialized substrates, the sensitivity of the resulting Raman spectra on sample preparation conditions such as extraction solution pH or chemical additives,[12,13,22] and, most importantly, the need to either remove samples from[10,14] or deposit metal nanoparticles directly onto,[14] the artifact under examination. These difficulties prevent routine application of SERS for materials from which samples cannot be taken. There remains, therefore, a need to develop an *in-situ* methodology for the identification of organic red colorants that makes use of dispersive Raman spectroscopy while avoiding fluorescence. This is the focus of the presented work.

In this study, Raman spectra of red colorants were obtained *in-situ* using a dispersive Raman microscope equipped with a 1064 nm laser source. Due to the ν^4-dependence of Raman scattering, the total Raman signal intensity is reduced by the use of 1064 nm near-IR radiation as compared to shorter wavelength visible sources. However, the near-IR source prevents excitation of target molecules into an excited electronic fluorescing state,[23] iso-

lating the Raman effect. It is shown here that many traditional red colorants can be identified both as dry pigments and in binding media using near-IR dispersive Raman spectroscopy.

2   Materials and Methods

Colorant samples were obtained from the Getty Reference Collection, an in-house repository of reference artists' materials containing, among other standards, a wide variety of pigments and dyes from numerous sources and suppliers. Colorants examined include the scale insect colorants cochineal and lac dye, and the plant-based colorants alizarin crimson, brazilwood, dragon's blood, sandalwood, logwood, and safflower. The pigment sources/manufacturers are provided in Table 1. Samples were analyzed under three conditions: (1) in dry powder form, (2) mixed with gum arabic and painted out onto clean glass slides, and (3) mixed with gum arabic and painted out onto a paper sample (Arches watercolor paper, neutral pH cotton with gelatin size). In the Raman analysis of organic red colorants, the pure chemical chromophore sometimes can give a different spectrum than the colorant in a complex matrix.[19] Therefore, in order to provide a spectroscopic database for identifying these pigments *in-situ* on artistic artifacts, complete colorants (i.e. those likely containing materials or impurities other than the chemical chromophore) were examined in this work without purification.

Colorants were analyzed using a Renishaw InVia dispersive Raman microscope which employs a Leica DMLM microscope equipped with a CCD camera to enable imaging of particles examined. During analysis, laser light was focused on individual particles of each colorant. The scattered Raman signal was collected through the microscope objective (50x-L; N.A. = 0.50, 8 mm working distance) and transmitted to the spectrometer through a notch filter and onto a detector. All spectra were acquired using the WiRE 2.0 software package (Renishaw), and analyzed using Grams AI (Thermo Scientific) and Igor Pro (Wavemetrics). The system is equipped with two excitation sources, both of which are employed in this study. The primary excitation source used in this work is a 1064 nm, 35-50 mW Nd:YAG laser, used with a 600 line/mm grating and a liquid nitrogen-cooled Princeton Instruments OMA-V InGaAs linear array detector. The sampled spot size[24] for this source is expected to be 2.6 μm in diameter. For the 1064 nm source, a relatively high power can be used without causing photodecomposition,[23] and the full ~35-50 mW power was therefore typically employ-
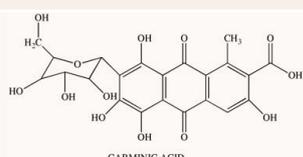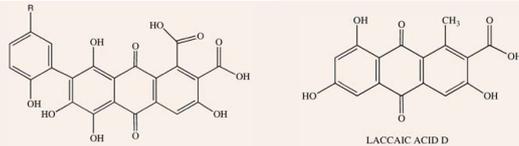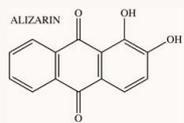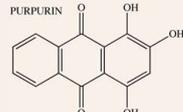
Colorant	Manufacturer / Source (Catalog Number)	Color Index52	Principal chromophores[10,15,27,53]
Carmine naccarat	Kremer Pigmente (4201)	Natural red 4 C.I. 75470	CARMINIC ACID
Carmine lake (cochineal alum mordant)	Oaxaca, Mexico	Natural red 4 C.I. 75470	
Lac dye	Kremer Pigmente (3602)	Natural red 25 C.I. 75450	LACCAIC ACID A: R=(CH₂)₂NHCOCH₃ B: R=(CH₂)₂OH C: R=CH₂CH(NH₂)COOH E: R=CH₂NH₂ LACCAIC ACID D ALIZARIN
Alizarine crimson dark	Kremer Pigmente (2361)	Pigment red 83 C.I. 58000:1	
Madder lake dark	Kremer Pigmente (37210)	Natural red 8 C.I. 75330 + 75420 + 75340 + 75350 + 75370 + 75410	PURPURIN
Madder lake pale	Kremer Pigmente (37214)	Natural red 8 C.I. 75330 + 75420 + 75340 + 75350 + 75370 + 75410	
Brazilwood	Kremer Pigmente (36160)	Natural red 24 C.I. 75280	BRAZILIN BRAZILEIN
Dragon's blood	Kremer Pigmente (37000)	C.I. 75200 + 75210	DRACORHODIN DRACORUBIN
Logwood	Kremer Pigmente (36110)	Natural black 1 C.I. 75290 + 75291	HAEMATOXYLIN HAEMATEIN
Sandalwood	Kremer Pigmente (36180)	Natural red 22 C.I. 75540	SANTALIN A SANTALIN B
Safflower	Verfmoolen 'De Kat' Zandaam, NL (spelled 'Safflour' on sample jar)	Natural red 26, C.I. 751500 Natural yellow 6	CARTHAMIN
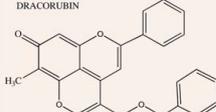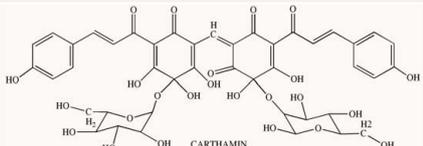
Table 1: Organic colorants examined in this work.

ed. All spectra taken using 1064 nm excitation were calibrated against the 219.1 cm^{-1} sulfur Raman line. A visible excitation source was also available and used to provide comparison spectra. This source is a 785 nm, 1000 mW diode laser, which employs a 1200 lines/mm grating and Renishaw Peltier-cooled CCD array detector. The power is limited to ~10 mW or less to avoid sample degradation when the visible source is used. All

spectra taken using 785 nm excitation were calibrated using the 520.5 cm^{-1} silicon Raman line.

Spectra collected in this study were compared to literature references where available. Both dispersive and FT instruments have been used in the study of organic red colorants. Most dispersive instruments employ excitation wavelengths less than 800 nm while FT instruments employ near-IR 1064 nm sources. For clarity, the term "visible dispersive" will be used to describe the former, and "FT-Raman" will be used to describe the latter. In contrast to these commonly used techniques, this paper presents the use of a 1064 nm source in a dispersive instrument, and this will be referred to as "1064 nm dispersive" spectroscopy. Additionally, as noted above, surface enhanced Raman spectroscopy (SERS), a technique which uses particular sample-preparation methods to enhance Raman signal and which can in theory be used with either dispersive or FT systems, has also been applied recently. In practice, most experimentalists couple SERS sample preparation to a visible excitation source, and references using this technique will be described simply as "SERS" studies.

3   Results and Discussion

In sections 3.1 and 3.2 below, the Raman signatures of insect-derived and plant-derived dry colorants, respectively, are presented. Spectra of selected colorants in binding media are presented in section 3.3. Peak positions in sections 3.1 and 3.2 are reported along with the relative intensity of the band using standard subjective notation (i.e. very weak (vw), weak (w), medium (m), strong (s), very strong (vs), broad (br) and shoulder (sh)); these designations are not directly comparable between different colorants. In some cases designations of very, very weak (vvw) and very, very strong (vvs) were added to better describe the observed range of peak intensities.

For each dry colorant examined, between 5-40 spectra were collected to ensure that Raman features are consistently observed and are representative of the total sample. Due to inhomogeneities in the samples, not all Raman features are discernable in every spectrum collected, and the peaks listed in each section are those most commonly seen and judged to be most useful for the *in-situ* identification of these colorants. Representative spectra of each colorant and relevant collection parameters are presented in Figures 1-3 and the figure captions, respectively.

In general, the 1064 nm dispersive Raman spectra have been corrected for the detector response by subtracting detector noise and correcting for inhomogenity across the array using a multipoint baseline correction. Additionally, for clarity, all data have been smoothed using a 5 point binomial algorithm. In those examples where a 785 nm spectrum is shown with the 1064 nm spectrum, the two spectra are presented on the same ordinate scale to facilitate comparison. Ordinate scales are not generally comparable between colorants.

3.1   Scale Insect-Derived Colorants: Dry Powder Spectra

Carmine/cochineal. "Carmine" can refer to pigments prepared from either cochineal or kermes, but since cochineal carmine is the more commonly encountered pigment,[25] the term carmine will here be used only in reference to cochineal-based pigments. Cochineal is a red dye extracted from "scarlet grain,"[26] the small scale insect *Dactylopius coccus* (previously known as *Coccus cacti*)[27] from Mexico, Central and South America (though Polish and Armenian species also exist).[25,26,28-30] The chromophore in these pigments is carminic acid.[25,26,29,31,32] The finest quality carmine colorants are naccarat carmines.[32]

Representative spectra of two cochineal pigments are shown in Figures 1a and 1b, where it can be seen that use of 1064 nm dispersive Raman consistently yields identifiable peaks with good signal-to-noise ratios. Spectra taken of the cochineal pigments using visible dispersive Raman, in contrast, are usually dominated by fluorescence, though certain individual particles can produce Raman spectral features. As an example for comparison, a spectrum of carmine naccarat acquired using the 785 nm source is provided in Figure 1a (upper trace), with features at 467 (vw-w), 1109 (vw-w), 1252 (w), 1312 (w-m), and 1428 cm^{-1} (vw). This carmine naccarat trace is typical of the 785 nm spectra of all of the insect-based red colorants examined in this work in terms of the intensity of the fluorescence and the weakness of the peaks that are occasionally discernable.

Spectra taken of carmine naccarat using 1064 nm excitation (Figure 1a, lower trace), in contrast, contain more prominent features than those taken using visible excitation. For the dry pigment, bands are visible at ~430 (w), 470 (w), 1249 (w-m), 1313 (m), 1433 (w), 1492 (w) and 1528 cm^{-1} (w). Similarly, spectra taken of cochineal lake using 1064 nm excitation (Figure 1b) include
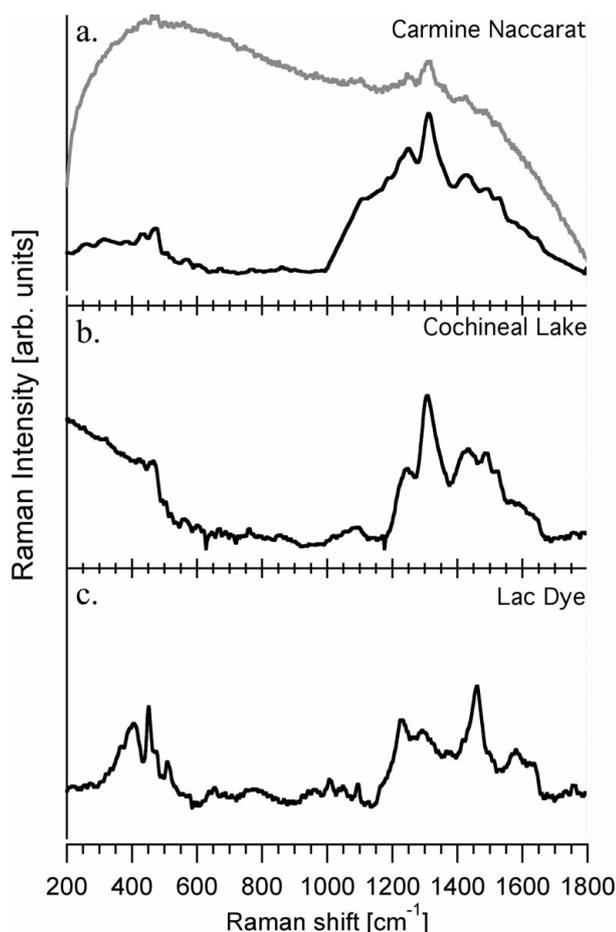
Figure 1: Representative spectra of scale insect derived colorants examined in this work: (a) carmine naccarat acquired using 785 nm excitation (upper trace) versus 1064 nm excitation (lower trace); (b) cochineal lake acquired using 1064 nm excitation; and (c) lac dye acquired using 1064 nm excitation. All 1064 nm spectra use 35-50 mW for a single 60 s acquisition, while that at 785 nm excitation uses ~10 mW for a single 10 s acquisition.

bands centered at 1245 (w), 1312 (m), 1434 (vw), 1488 (w), and ~1523 cm^{-1} (vw). Clearly, the Raman signatures of these cochineal colorants are more easily discerned using the 1064 nm source, facilitating identification of these species based on the features listed above.

The normal 780 nm Raman,[11] SERS[10,13,19] and FT-Raman[13] spectra, including spectral assignments,[13] of carminic acid have been reported. Reported spectra for the pure chromophore, however, differ somewhat from the spectra of historical cochineal containing pigments.[11,19] In general, the carmine spectra collected in this work agree more closely with the FT-Raman work than recent SERS measurements. For example, the band observed in this work centered between 1311-1315 cm^{-1} clearly agrees well with the value of 1314 cm^{-1} reported by Burgio,[11] but may be shifted by as much as 10 cm^{-1} from SERS measurements, which report a strong feature between 1291-1308 cm^{-1} in one study,[13] and 1304 (cochineal lake) or 1319 (carmi-

nic acid) in another.[19] The reasons for these differences are unclear, and may be due to in part the differing excitation wavelengths employed (1064 nm for this work, FT work, and one SERS report versus 632 nm in another SERS study).

Lac dye. Another scale insect used to produce red pigments is *Tachardia (Laccifer) lacca*, associated with fig trees of India and Asia, and used in the production of lac dye.[25-27,33] These insects were either collected from the wild or cultivated in south and southeast India, and imported to western cultures.[26] The main coloring agents of lac dye are the forms of laccaic acid.[25,34]

A representative spectrum of lac dye using 1064 nm dispersive Raman is shown in Figure 1c. As with the cochineal pigments, using the near-IR source results in significantly better spectra than the visible source. Under visible excitation (spectrum not shown), the only Raman peak consistently visible is a broad, very weak feature at 1465 cm^{-1}. Raman spectra taken using 1064 nm excitation (Figure 1c), in contrast, contain several features: 406 (w), 455 (m), 475 (w, sh), 511 (w-m), 961 (vw), 1010 (w-m), 1049 (w), 1095 (w), 1230 (m, br), and 1460 cm^{-1} (m-s). Features are also occasionally discernable at 1580 (vw), and/or 1753 cm^{-1} (vw).

In contrast to carmine, there is good agreement between the spectra collected in this work, SERS[10,19,20] and visible dispersive (633 nm) Raman[19] measurements of lac dye. For this particular pigment, visible excitation at 633 nm, SERS sample preparation (using 633 or 785 nm excitation), or 1064 nm excitation all result in identifiable spectra, but collection parameters appear to be technique-dependent. For example, the collection time reported for a 633 nm-visible dispersive Raman spectrum (120 s for 8 acquisitions) is more that 15 times those for the 1064 nm excitation spectrum reported in this work (60 s for a single acquisition).[19] In this case, the fluorescence suppression afforded by use of 1064 nm excitation in a dispersive system allows a dramatic reduction in collection time.

3.2 Plant-Derived Colorants: Dry Powder Spectra

Madder/alizarin. Madder colorants are extracted from the roots of a variety of plants in the Rubiaceae family,[28,35] most commonly from *Rubia tinctorum L.* from western Asia.[36] The primary chromophores in madder pigments are the anthra-

quinones alizarin (1,2-dihydroxyanthraquinone), purpurin (1,2,4-trihydroxyanthraquinone), and pseudopurpurin (1,2,4-trihydroxy-anthraquinone-3-carboxylic acid) among others;[18,26,27,34,36,37] of the chromophores, alizarin is considered the most important.[12,26] The pigment alizarin crimson is a lake* obtained from synthetic alizarin (which was first synthesized in the 1870s),[29,36] without the presence of the purpurin found in traditional madder lake pigments.

Figure 2a compares representative spectra of alizarin crimson using both 785 nm and 1064 nm excitation sources. As with the scale-insect based colorants, excitation at 1064 nm greatly suppresses fluorescence of the organic material. Alizarin appears to be among the strongest Raman scatterers of the organic red colorants examined in this study, and spectra taken using the 785 nm laser source consistently contain peaks at 482 (w), 843 (w), 1190 (vw), 1291 (w), 1327 (m), 1357 (vw), and 1481 cm^{-1} (w-m). However, all of these Raman bands exist over a strong fluorescence background (Figure 2a, upper trace). Spectra of alizarin crimson using 1064 nm excitation (Figure 2a, lower trace) display features at 243 (m), 488 (m-s), 660 (w), 847 (m), 908 (w), 1025 (w), 1047 (w), 1165 (w), 1193 (m-s), 1223 (w-m), 1296 (m-s), 1328 (m-s), 1358 (m), 1481 (vvs), 1519 (m), 1577 (w), and/or 1637 cm^{-1}(m). While alizarin may be identifiable using either source, near-IR excitation produces significantly less fluorescence than visible excitation and may allow the identification of lower concentrations of alizarin colorants.

Two madder lake pigments, madder lake dark and madder lake pale from Kremer Pigmente, were also examined, though spectra are not shown here. The alizarin spectra discussed above is expected to be partially reproduced for these species since madder pigments typically contain alizarin along with purpurin. Spectra taken of madder lake dark using 1064 nm excitation occasionally displays features, but these are typically too weak in intensity to be diagnostic. These results suggest that madder lakes would need to be particularly high in alizarin, a strong Raman scatterer under our experimental conditions, in order to be identified using 1064 nm dispersive Raman spectroscopy.

Literature spectra of alizarin and/or madder colorants acquired using SERS techniques,[10,12,14,18-20,22] visible dispersive Raman using 458 nm or 785

nm excitation[3,17,22] and FT-Raman,[11,12] have been reported, and the most important Raman features have been assigned.[3,12,17,22] The spectra reported in this work generally agree with these literature values to within a few wavenumbers (±4 cm^{-1}). However, discrepancies with the SERS spectra are observed for the bands centered near 1220, 1480, and 1630 cm^{-1}. These features have been ascribed in the literature to C-C-C or C-H bending modes (1220 cm^{-1}),[12,17] C-O and C-C stretching (1480 cm^{-1}),[12,17] and C=O stretching (1630 cm^{-1}).[3,12,17,22] Published intense features in some SERS spectra[10,19,22] appear to be red-shifted from our dispersive 1064 nm measurement of the peaks listed above by 5-20 cm^{-1}. These apparent differences may simply result from the use of differing excitation wavelengths, which can result in intensity variation, or from selective peak enhancement in SERS measurements. It is also possible that alizarin chemisorption on the SERS surface[19] may occur non-isotropically. If the more electronegative side of the molecule, i.e. the side containing three of the four oxygen-containing moieties (see Table
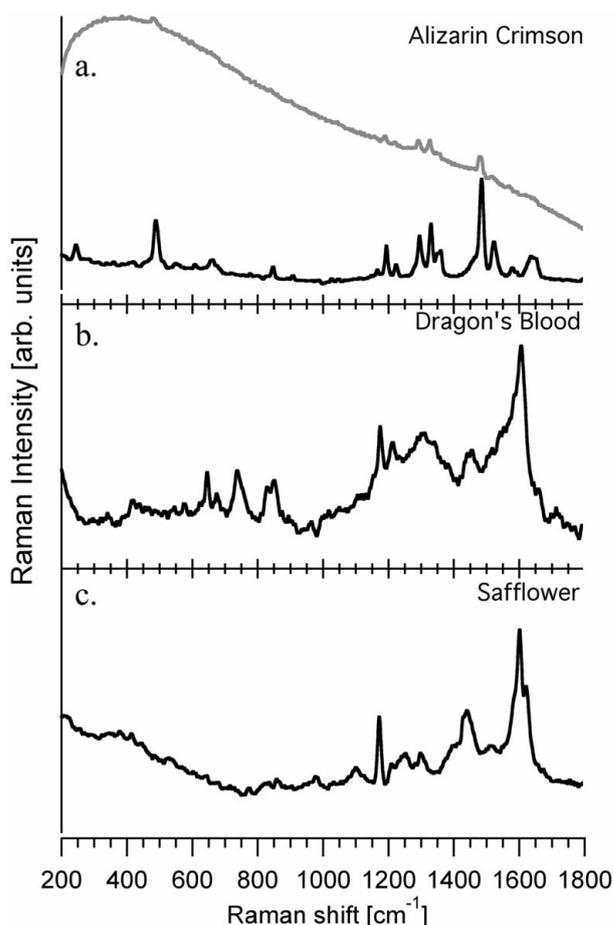

Figure 2: Representative spectra of: (a) alizarin crimson acquired using 785 nm excitation (upper trace) versus 1064 nm excitation (lower trace); (b) dragon's blood acquired using 1064 nm excitation; and (c) orange-red safflower particles acquired using 1064 nm excitation. All 1064 nm spectra use 35-50 mW for a single 60 s acquisition, while that at 785 nm excitation uses ~10 mW for a single 10 s acquisition.

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* Elemental analysis of the Kremer sample analyzed for this work indicates the presence of aluminum, phosphorous, sulfur, calcium, and barium that are likely parts of a complex laking substrate.

1), are preferentially oriented towards the metal SERS surface, the bonds in this area may effectively share electron density with the metal, lowering the vibrational frequencies of the affected bonds. The likely assignments of the shifted peaks support the notion that the carbonyl and alcohol functionalities may be experiencing this type of effect. However, orientational analysis via, e.g. Raman polarization experiments,[38] would be required to verify this hypothesis, and is beyond the scope of this work.

Dragon's blood. This boldly named pigment takes its moniker from legends describing an epic battle between an elephant and either a basilisk or dragon. A tree is said to have grown on the battle site, the fruit of which provides resin used to make the pigment.[15,39] In reality, the material is a resin extracted from Asian *Daemonorops (Calamus) draco* plants.[27,33] While the complete chemical composition of dragon's blood pigments can vary based on geographical origin,[15] and can include several flavonoid or xanthone components,[27] the main colorants in dragon's blood are dracorubin and dracorhodin.[15,40] This pigment is particularly susceptible to thermal damage in the visible region.[39]

A representative spectrum of dragon's blood is shown in Figure 2b. This colorant displays only fluorescence when interrogated using visible excitation, but spectra taken of the same material using 1064 nm excitation contain many well defined features: 417 (vw), 645 (w-m), 674 (w), 737 (m), 828 (m, sh), 848 (m-s), 1174 (m), 1211 (w), 1302 (w, br), 1452 (m), 1543 (w, sh), and 1606 cm^{-1} (s). While dragon's blood remains enigmatic under visible irradiation, the fluorescence suppression afforded by the near-IR source significantly improves the resulting spectra, rendering this colorant identifiable *in-situ*.

FT-Raman spectroscopy has previously been employed to analyze dragon's blood, assign the most important spectral features, and differentiate between several sources of the colorant.[15,39,40] In general, the peak positions observed in this work agree with these literature reports to within approximately ±3-4 cm^{-1}. As has been noted for other pigments examined, our dispersive 1064 nm system consistently matches 1064 nm FT-Raman results, underscoring the importance of the excitation source on the reproducibility of spectral data.

Safflower. A yellow or red dye, safflower is extracted from the flowers of *Carthamus tinctorius* from southern Europe and Asia.[27,30,33] Safflower is

also sometimes referred to as 'bastard saffron,' and has historically been confused with true saffron, though safflower and saffron have different plant sources.[27] Safflower has been found on Egyptian cloths dating to 1050 BC, and was introduced in Europe in the nineteenth century, and to the United States in the early twentieth century.[27,34] The main dyestuff is carthamin, and the colorant is most commonly found in the red form.[34]

The powdered safflower used in this work appears red-orange rather than pure red; a representative spectrum is presented in Figure 2c. As was the case with dragon's blood, the use of 1064 nm excitation is particularly useful for this colorant, since examination under 785 nm excitation produces only very strong fluorescence. When the excitation is switched to the 1064 nm source (Figure 2c), bands are visible at 1172 (s), 1252 (vw-w), 1300 (vw-w), 1441 (m), 1601 (s), and 1621 cm^{-1} (w, sh). Bands are also sometimes discernable at 382 cm^{-1} (vw) or 525 cm^{-1} (vw). Again, the fluorescence suppression of the near-IR source results in dramatically improved spectra, permitting identification of this colorant.

The Raman literature of safflower generally concerns safflower oil,[41-44] which may not be directly comparable to the spectrum reported here. Based on comparison between the peak positions observed in this work and characteristic Raman group frequencies, possible assignments for the safflower peaks observed here include: 1176 cm^{-1} (substituted aromatic[45,46]), 1302 cm^{-1} (CH_2 in-phase twist[45,46]), 1448 cm^{-1} (CH_2 bend[45]), 1603 cm^{-1}/1623 cm^{-1} (C=C ring stretch doublet).[46] The spectrum presented here may therefore serve as an initial reference spectrum of an orange-red safflower colorant for use in identifying safflower pigments *in-situ* on cultural heritage artifacts. Additional reference spectra of red versus yellow safflower pigments are also of interest.

Logwood. Logwood is extracted from the heartwood of the Central American redwood tree *Haematoxylum campechianum L.*,[27,30,47] and was commonly employed in the seventeenth century for watercolor painting.[27,48] Logwood contains two chromophores, haematin and hematoxylin.[27] Hematoxylin is the main colorant in the original red extract,[32] but is oxidized during dye preparation to haematin.[10,27] Modification of the extent to which this occurs allows the preparation of a range of colors from red to blue-black.[27,47] The sample of logwood used in this work is very dark, appearing macroscopically blue-black, though individual particles often appear to have some red character.
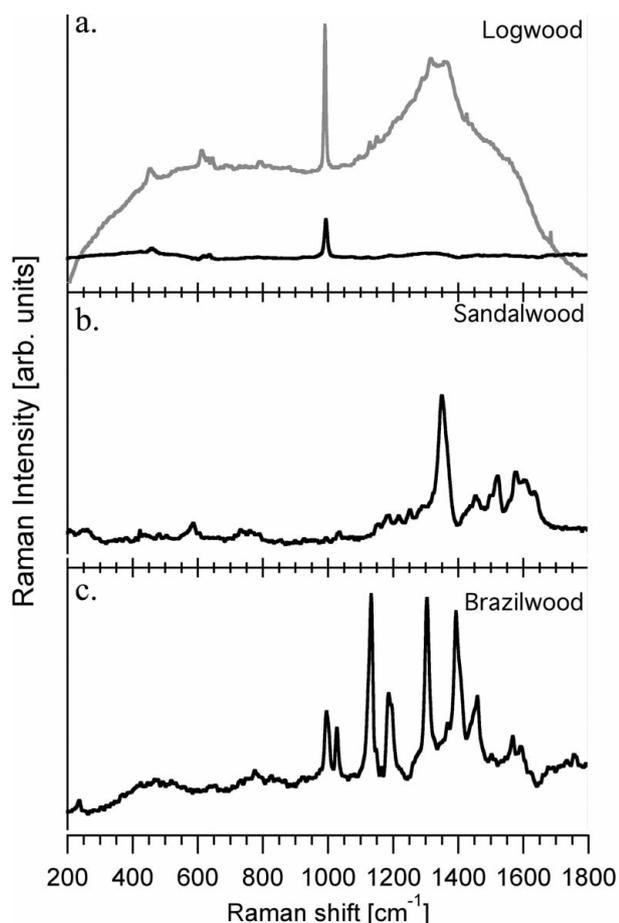
Figure 3: Representative spectra of: (a) logwood acquired using 785 nm excitation (upper trace) versus 1064 nm excitation (lower trace); (b) sandalwood acquired using 1064 nm excitation; and (c) brazilwood acquired using 1064 nm excitation. All 1064 nm spectra use 35-50 mW for a single 60 s acquisition, while that at 785 nm excitation uses 5 mW for a single 10 s acquisition.

For the dry logwood pigment, the differences in spectral quality between the 785 nm and 1064 nm sources, shown in Figure 3a, are less dramatic than with other colorants examined. Spectra taken using 785 nm laser light (Figure 3a, upper trace) contain peaks centered at 457 (w), 612 (w), 632 (w), 644 (vw), and 990 cm^{-1} (s). When the sample is interrogated using 1064 nm excitation (Figure 3a, lower trace), the same peaks are observed at 461 (w-m), 616 (w), 630 (w-m), and ~994 cm^{-1} (s). Of these, the feature at 616 cm^{-1} is not observed in all spectra; some spectra also contain a weak peak at ~318 cm^{-1}.

The current Raman literature regarding logwood is sparse.[10,49] Leona *et al.*[10] have published SERS spectra for the colorants expected in logwood samples, haematein and haematoxylin. Of the reported haematein and haematoxylin Raman bands, only the relatively weak signatures at 609 and 321 cm^{-1} (both associated with haematoxylin) appear to be present in logwood spectra reported here, appearing slightly shifted, with the former

present at 612 cm^{-1} in the 785 nm spectra and 616 cm^{-1} in the 1064 nm spectra, and the latter present at 318 cm^{-1} in the 1064 nm spectra. The other peaks reported here for logwood may therefore represent signatures of the complete pigment, perhaps including an unidentified substrate/lake,* and warrant further consideration.

Sandalwood. Sandalwood is produced from *Pterocarpus santalinus*, a woody plant from India, Sri Lanka, and Asia, and contains the chromophore santalin.[27] Santalin can be used as either as an adulterant or as a lake pigment. For the lake pigments, the color can be adjusted from brown to red to purple based on the laking anion.[27] The sample used here appears to be raw sandalwood with no laking anion based on elemental analysis, and is macroscopically red-orange.

Figure 3b shows a representative spectrum of sandalwood using 1064 nm dispersive Raman. Under 785 nm excitation, a broad, weak band is consistently visible centered at 1348 cm^{-1} (spectrum not shown). As with the other colorants examined in this work, excitation using 1064 nm excitation results in significantly less fluorescence and more identifiable Raman peaks (Figure 3b). Bands are located at 587 (w), 1034 (vw), 1182 (w), 1217 (vw), 1252 (w), 1350 (s), 1455 (w), 1501 (w), 1520 (m), and 1603 cm^{-1} (w-m, br). In some samples, the broad feature at 1603 cm^{-1} appears to be separable into three peaks at 1576 cm^{-1} (w, sh), 1605 cm^{-1} (vw) and 1639 cm^{-1} (vw). Literature spectra of this colorant could not be located for comparison to the spectra reported here. The reported Raman features for sandalwood therefore represents a reference spectrum, in this case of a red-orange form of the pigment, which may be of use in identifying sandalwood pigments *in-situ* using near-IR excitation.

Brazilwood. Brazilwood is an exotic red-purple dye extracted from European, South American, and Asian woods, primarily of the genus *Caesalpinia*, which was used throughout the Middle Ages.[27,33,34,50,51] The main colorants in brazilwood are brazilin and brazilein.[50] Brazilin is the main constituent of the woods themselves, and is oxidized in air to the brazilein form found as a colorant.[34]

A representative 1064 nm dispersive Raman spectrum of a powdered brazilwood sample is shown in Figure 3c. As with alizarin, using the 785 nm laser

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* Elemental analysis of the sample analyzed for this work indicates the presence of sulfur, sodium, calcium, and potassium that are likely parts of the laking substrate.

source (spectrum not shown) results in observation of very weak peaks over a strong fluorescence background, at 997 (vw), 1030 (vw), 1134 (w), 1187 (w), 1307 (w-m), and 1395 cm^{-1} (w-m). In contrast, spectra taken using 1064 nm excitation (Figure 3c) display features at 240 (vw), 776 (vw), 996 (w), 1009 (m), 1030 (m), 1132 (s), 1148 (vw), 1186 (m), 1195 (w, sh), 1304 (m-s), 1368 (vw), 1393 (m), and 1458 cm^{-1} (w). Very weak features are occasionally visible at 1567 or 1597 cm^{-1}. As with the other colorants examined, near-IR excitation substantially reduces fluorescence for this colorant, allowing identifying features to be clearly observed over the background.

Visible (633 nm) dispersive Raman,[19] FT-Raman[50,51] and SERS analysis (using 633 or 785 nm excitation) [10,19] of brazilwood,[19,51] and of the individual chromophores,[10,50] have been reported in the literature. These reports have additionally assigned the most important Raman features associated with brazilwood.[50,51] As was the case with lac dye, while visible dispersive Raman can be used to identify this colorant, the reduced florescence in the near-IR spectrum greatly improves the resulting spectrum and allows the collection of a high quality spectrum in less time than would be required with visible excitation.

3.3 Binding Media Effects

The effect of mixing the colorant with the binding medium gum arabic on the Raman spectrum was also explored. These experiments were undertaken to determine the suitability of using dispersive 1064 nm Raman spectroscopy to examine realistic samples *in-situ*. Spectra were collected of each colorant in gum arabic using the 1064 nm dispersive Raman, and examples which demonstrate the range of observed effects are presented here. For some colorants, the spectral quality appears to be largely unaffected by the addition of the binding medium, with the crystallinity of the specific particle examined, or the Raman scattering cross-section, having the greatest impact. As an example, the signal-to-noise ratio in the spectrum of alizarin crimson appears relatively unchanged (Figure 4a) regardless of substrate or the presence/absence of the gum arabic. In this example, using near-IR excitation avoids fluorescence sufficiently to allow unambiguous identification even in the presence of a binding medium. In other instances, the presence of the binding medium significantly reduces the intensity of the observed vibrational modes. This effect is clear in the spectra of carmine naccarat (Figure 4b), where increased fluorescence masks the Raman signature of these species. In

the most complex sample of this colorant, that in gum arabic on a paper substrate (top trace), only a single broad, weak feature at ~1315 cm^{-1} is present in the Raman spectrum collected using 1064 nm excitation. It should be noted that this is significantly better than the spectrum at 785 nm for this sample, which is dominated by fluorescence, but nonetheless implies that only the strongest feature in the cochineal spectrum may be observable in real samples. The ability of Raman microscopy to selectively focus on particles within the sample can be employed to avoid areas of heavy media to attenuate this influence during *in-situ* examination of cultural heritage objects.

Lastly, in one case, adding binding medium appears to lower the thermal damage threshold of the colorant. This effect was observed with logwood,
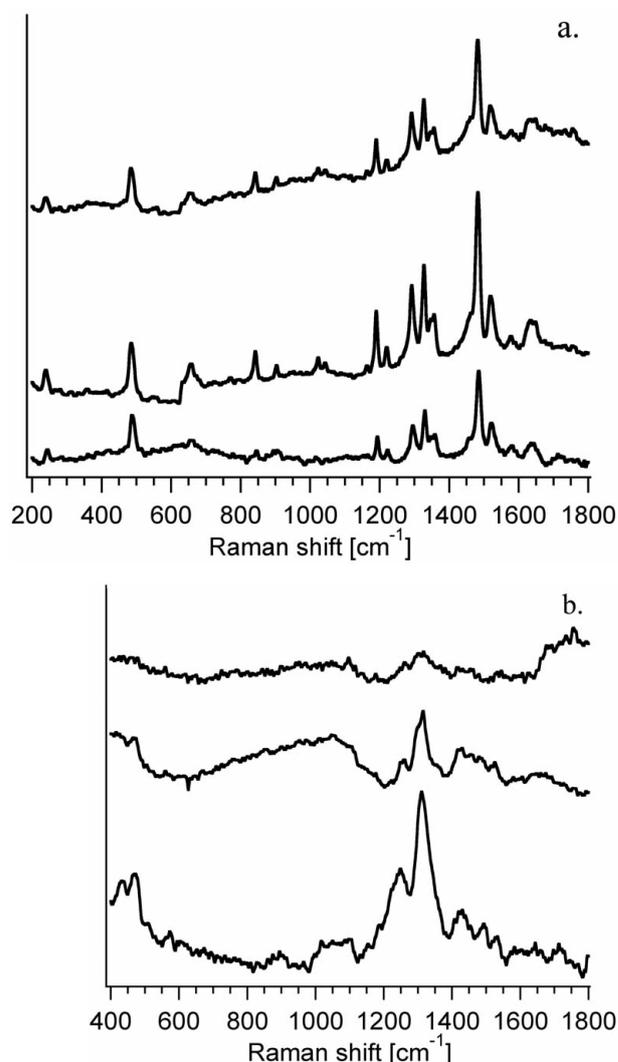

Figure 4: Effect of binding media on (a) alizarin crimson, and (b) carmine naccarat. All spectra are taken using 1064 nm excitation, 35-50 mW power, in a single 60 s acquisition and are only corrected for detector inhomogeneity. In both cases the bottom trace is of dry powder samples, the middle trace is of the colorant mixed with gum arabic and painted out onto glass slides; and the top trace is of the colorant mixed with gum arabic and painted out onto a paper substrate.

of which the sample used in this work is a particularly dark example. For the sample of logwood in gum arabic on glass, using up to 50% power (17-25 mW) results in a fluorescence-dominated spectrum with no features. Using the next available power setting for this instrument (100% power, ~35-50 mW), however, results in sample degradation within a second or two of exposure. In contrast, the spectrum of the dry powder presented in Figure 3, also taken using 100% power, results in no observable change to the particle under examination. This implies that there may be some specific instances where the 1064 nm dispersive technique may not be able to overcome the challenges of analyzing fluorescent natural organic colorants.

3.4 Application to Cultural Heritage Objects

The use of dispersive 1064 nm Raman spectroscopy can be particularly useful for works where only an *in-situ* technique can be used. Figure 5a shows a Peruvian print early 18th century (Getty Research Institute, Gutierrez 497). The red feather in the headpiece was examined using 1064 nm dispersive Raman. The spectrum resulting from a single 60 s acquisition at <5 mW power (Figure 5b, bottom trace) is readily identified as alizarin when compared to the spectrum of alizarin crimson powder (Figure 5b, top trace). This indicates the use of alizarin or a high alizarin-content madder lake pigment for this work. Such a conclusion may not be able to be drawn from data collected using visible excitation, which are usually dominated by florescence at the powers typically used to examine manuscripts. Again, it should be noted that very low power was used in this examination of a work of art, since damage to artifacts is always possible with exposure to laser light. This example clearly illustrates the potential utility of dispersive Raman spectroscopy in the near-IR spectral range for the *in-situ* identification of organic red colorants on art objects from which samples cannot be taken.

4 Conclusions

Overall, the work presented here clearly demonstrates that dispersive 1064 nm Raman spectroscopy may be used to identify traditional plant- and animal-based organic reds colorants, since it largely avoids the fluorescence often encountered using visible excitation. Using this technique, works of art may be examined *in-situ* when sampling is not practical or permissible. While the technique does have limits, for example the difficulty in identifying madder lakes with low alizarin content,
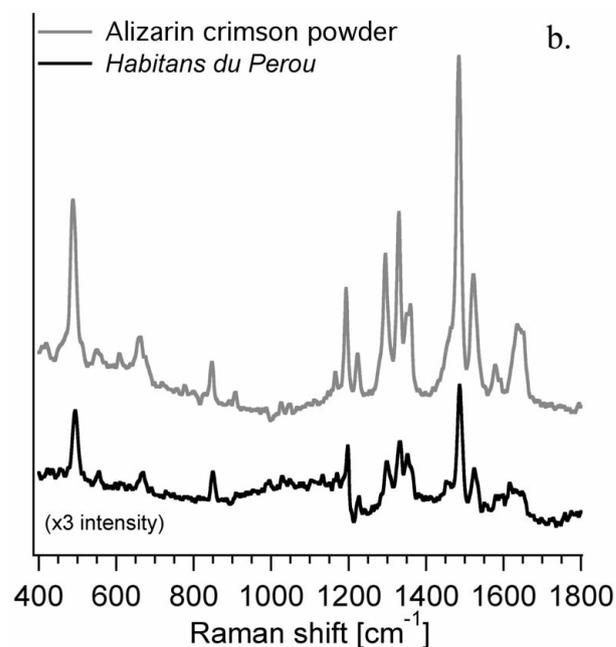

Figure 5: (a) Mattieu Ogier print, *Habitans du Pérou*, (15.2 × 9.2 cm; from *Voyage autour du monde, commencé en 1708 et fini en 1711 par le Capitaine Woodes Rogers, vol. 5, p. 471* by Woodes Rogers) from which in-situ spectra of an organic red colorant were taken, and (b) Raman spectrum of the organic red colorant (bottom trace) versus a reference sample of alizarin crimson powder (top trace).

it provides a complementary or alternative technique to other methods commonly used to identify organic red colorants. As such, dispersive near-IR Raman may be made part of a non-destructive analytical protocol for *in-situ* analysis of organic or florescent pigments.

5   Acknowledgements

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