

**RAMAN SPECTROSCOPY OF ARTISTS' MATERIALS:
ADVANCES IN CHARACTERISATION AND ANALYSIS**

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Abstract

Cultural heritage research and conservation practice seek to preserve our cultural heritage. Understanding the composition of the materials that comprise the artwork or cultural object is critical to inform collections management and preservation treatments; however methods of analysis are constrained to those that are either non-destructive or can obtain the desired information from micro-samples in order to retain the integrity of the object. Raman spectroscopy is an ideal characterisation technique for characterising cultural materials as it is non-destructive, requires relatively little sample preparation and utilises short measurement times. Micro-Raman is especially useful for examining micro-samples and painting cross sections due to its spatial resolution being sufficiently high to target individual pigment grains.

Despite the non-destructive, micro-sample analysis advantages, there are limitations to the use of Raman spectroscopy in the cultural heritage conservation context. Firstly, fluorescence is frequently observed in cultural materials. Secondly, with the large number of compositionally complex and heterogenous materials encountered in conservation, there is a need for advanced methods that can deal with large sample datasets. These methods are needed to facilitate examination of both non-spatial data and spatial (imaging) data, to extract the maximum amount of information possible from the limited sample available. It is the aim of this thesis to demonstrate how Raman micro-spectroscopy can provide new and useful information about paintings using the following strategies.

(1) Creation of a reliable pigment database supported by X-ray diffraction data to confirm the structural identity of each pigment.

At the time this work was started, a spectral library for pigment identification was necessary to have a comprehensive set of pigment reference spectra against which to compare unknowns. A Raman spectral pigment reference library was developed comprising over 180 samples from the National Gallery of Victoria's pigment collection. Samples were validated using X-ray Powder Diffraction (XRPD) prior to Raman analysis. This Raman spectral pigment library can also

support future identification of materials and artworks, alongside other Raman spectral databases that are now available.

(2) Utilisation of the database in conjunction with longer wavelengths to mitigate fluorescence effects

In the presence of fluorescent components, Raman analysis is hampered. To mitigate the impact of fluorescence observed at 514nm, the efficiency of 830nm wavelength incident irradiation was examined. It was found effective and used to answer five research questions (case studies) regarding authenticity and art historical practice and to inform attribution, provenance studies and conservation treatments:

- a) Mock-up paintings were prepared to trial the experimental methodology. The overpaint in a 16th century portrait miniature was identified as Zinc white pigment, indicating the overpaint was applied after the mid-19th century.
- b) An Australian Impressionists artist's catalogue from 1889 was examined and the inks found to contain Prussian blue and vermilion as the main pigments, with a minor addition of minium and perhaps a lake pigment, providing insight into the artist's technical methods.
- c) Overpainted cracks in Tom Roberts' iconic painting *Shearing the Rams* were suspected to have been due to the type of pigment used. The main pigment was vermilion, which is not known to cause cracking, so the cause of cracking is now believed to be due to the high ratio of binding medium in the paint.
- d) *The Finding of Moses* (1712) was reattributed to Giambattista Tiepolo (1696-1770), after Prussian blue was identified as a key component of the paint layer.
- e) The organic blue colour in an Indian Palampore was dissimilar to indigo but matched a published spectrum of indigo on silk, highlighting the importance of local structure and bonding on subtle features in Raman spectra.

(3) Identified a practical method of Surface Enhanced Raman Spectroscopy for conservation to increase the Raman signal and make it visible over the photoluminescent background.

This work reviewed several SERS substrate configurations, then prepared and evaluated SERS substrates prepared by a) colloidal Ag nanoparticles, b) Ag coated nanospheres, c) Ag foil etching and d) electroless deposition of Ag on a Cu coupon. The ease of production and reproducibility were used to select the most practical substrate for SERS analysis in conservation being SERS

substrates prepared using an electroless deposition method. The selected substrate was used to identify dammar as the varnish used on an important Italian Renaissance painting by Tiepolo with the outcomes published in 2008.

(4) Developing new methods of data analysis for managing complexity in spectra and large data sets

Multivariate analysis techniques have been used to analyse spectral datasets in numerous fields and provide an excellent opportunity to enhance the analysis of large Raman spectra datasets in conservation. Principal components analysis (PCA) and hierarchical clustering analysis (HCA) methods were used to visualise the data structural relationships amongst Raman spectra of natural and synthetic resins. It has been demonstrated that the two most utilised natural resins, dammar and mastic, are able to be distinguished from one another by PCA and HCA of their Raman spectra, irrespective of their supplier and the naturally occurring sample variance.

This work also shows, using PCA and HCA, that the synthetic cyclohexanones resins Ketone N and Laropal K 80 are indistinguishable whilst the other synthetic painting varnish cyclohexanone, MS2A, is easily separable. The synthetic resins were found to be quite homogeneous in composition with little variability in their Raman spectral response compared to a very much greater degree of variance was observed within the natural resins: amber, copal, colophony and sandarac.

Finally, a multivariate image analysis method, assembling the data into a 3D data-cube and using PCA and clustering techniques, was developed. The method for assembling and analysing the spectral 3D data cubes was achieved using prepared samples of known pigments in binder.

The technique was used in the analysis of an Italian renaissance painting. PCA and clustering methods were applied to SEM-EDS elemental maps of Ti, Sn, Si, Pb, Mn, Mg, K, Fe, Cu, Ca, Al, S (corrected for Pb) and O, to develop a compositional map of the materials used and indicate their sequence in the layered construction of the painting. Secondly, using Raman maps of spectral intensity collected at 830 nm to mitigate fluorescence, and using the spectral database, vermilion, lead-tin yellow type 1 and a blue-green pigment consistent with terre-verte or another green silicate pigment were found in the paint layer. The ground layer was found to contain anhydrite with large gypsum inclusions. The identification of these components has led to the attribution of a previously anonymous painting to Dosso Dossi with the outcomes published in 2008, receiving 70 citations until 2019.

Multivariate methods developed here have been further applied in published research in both conservation and non-conservation applications, which is noted in this thesis.

Declaration

This is to certify that:

- i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,
- ii. due acknowledgement has been made in the text to all other material used,
- iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Deborah Lau

Preface

This thesis was commenced in 1998 and undertaken in a part time capacity due to full time work and family commitments. A number of intermittent periods of leave of absence were taken, and while almost completed (including the completion seminar presented to the University of Melbourne School of Physics), my candidature lapsed in good standing due to overwhelming work pressures. It is now finally presented for examination. All experimental work towards the thesis was carried out during enrolment in the degree.

Some elements of the work were carried out in collaboration with conservators at the National Gallery of Victoria. The art historical research questions were raised by them and I evaluated their suitability to be answered using Raman spectroscopic techniques of analysis. In situations where it was agreed Raman spectroscopy is an appropriate analytical strategy, any samples removed from artworks were collected by the collaborating conservator.

Two publications were produced in the period 2007-8. For both publications, my contribution was identification of the suitability of the technique to answer an art historical question, development of the experimental protocol, performing the analysis, interpretation and assembly of the results, preparing the draft for publication and undertaking the publication process.

Application of surface-enhanced Raman spectroscopy (SERS) to the analysis of natural resins in artworks (Journal of Raman Spectroscopy, 2008), is co-authored by Michelle Livett and Steven Prawer, who provided advice and discussion regarding the theory of Raman Spectroscopy, reviewed and edited the draft publication. John Payne (National Gallery of Victoria) collected and provided varnish samples.

Multispectral and hyperspectral image analysis of elemental and micro-Raman maps of cross-sections from a 16th century painting (*Analytica Chimica Acta*, 2008), is co-authored by Carl Villis, who collected and mounted samples from the painting and provided background and context for the work. Scott Furman and Michelle Livett provided advice and discussion regarding Raman maps. All reviewed and edited the draft publication. Matt Glenn from CSIRO Minerals provided technical assistance with set-up for collecting the ESEM elemental maps.

No work towards this thesis has been submitted for other qualifications.

No funding was provided to support this work. However, samples were generously provided by the National Gallery of Victoria., and ancillary sample materials and access to instrumentation were provided by the CSIRO divisions of Minerals and Materials Science and Engineering and Monash School of Chemistry.

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I wish to sincerely acknowledge the longstanding patience of all those around me who have endured the many years taken to write this thesis. My deepest appreciation goes to my supervisors, Associate Professor Michelle Livett and Professor Steven Prawer from University of Melbourne and John Payne from the National Gallery of Victoria for continuing to support my candidature when there appeared to be no ending. More than a decade ago Dr Kerry Nugent instructed me in the use of the Dilor Raman spectrometer at the University of Melbourne and spent many hours in the lab with me and the crossword page. To my work colleagues, Drs Scott Furman and Fiona Scholes at CSIRO, I am grateful for numerous discussions in Raman theory and experiment, and constant encouragement and Dr Ian Madsen for access to x-ray diffraction facilities. Many thanks go to Drs Neil Sims and Glenn Newnham for providing access to and guidance with technical aspects of imaging software. Thank you to all the current and former staff in the Conservation department of the National Gallery of Victoria, particularly Carl Villis, Cobus van Breda, Michael Varcoe-Cocks, Marika Strohschnieder, Briony Pemberton and Bronwyn Cosgrove. Finally, thank you to my wonderful family, especially Matt, Amelia and Lucinda, who have all grown in the years that passed while this continued amidst the rest of life.

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Chapter 1: Context and Introduction

Materials of cultural importance or historical significance play a unique role in modern society and have educational, spiritual, historical, cultural, or aesthetic value. Caretakers have a responsibility for ensuring the preservation of these artefacts or structures. Conservators are the professionals that directly undertake the preservation of cultural heritage objects, using approaches that can be interventive (such as treatment or restoration) or non-interventive. Non-interventive conservation utilises principles of preventive action and seeks to increase the longevity of an object by indirect means such as modifying the environment. Alongside the imperative to preserve cultural heritage materials for future generations, the fundamental knowledge of artists' materials and techniques is also significant. It informs the knowledge of practices and technical approaches used by an artist, informing treatment approaches and may provide evidence used for dating and authentication. Thus, understanding the material and chemical structure of an artwork is of importance in preserving cultural heritage.

Conservation practice requires a detailed understanding of the material composition of the object or structure in order to determine appropriate preservation strategies. Decisions regarding storage conditions and display, handling or transport requirements are directly influenced by the knowledge of the constituent material chemistry and its susceptibility to external influences; in particular, response to temperature, humidity, light and pollutants. Guidelines for the museum environment published over 30 years ago (1) have been adhered to rigorously and describe optimal museum environment parameters, with recommendations for temperature and humidity values of $50\% \pm 5$ RH and $21^{\circ}\text{C} \pm 2$ based on a scientific understanding of materials degradation and deterioration. As energy costs for maintaining strict parameters have increased a review has occurred and new guidelines (2) are providing some flexibility.

Restoration treatments undertaken on an object may be assisted through understanding the structural material composition. This can inform the appropriate choice of solvents, films, pigments, fillers, coatings or other materials that may be used in treatments and will be in intimate compatible contact with the object. If the structural material of an object has degraded, then knowing the identity of the degradation product may be an indicator of the processes involved.

An artwork's value has the potential to change dependent on attribution to an artist. Being able to attribute a painting to an artist relies on developing a considerable body of knowledge of the materials and working techniques known for use by the artist and is a corroboration of provenance, connoisseurship and materials knowledge. There are some extensive undertakings in this area; for example, research into Van Gogh's studio practice is being undertaken by the Van Gogh Museum in the Netherlands (3) and that into the work of Rembrandt by the Rembrandt Research project (4). Research associated with both projects have identified paintings, attributed or de-attributed on the basis of the materials used in the construction of the painting, resulting in a significant effect on their monetary and perceived value.

Provenance is the history of ownership of an object, supported by documentary records or treatment records and may be assisted further by dating techniques. Dating is based on knowledge of the use of certain materials within specific time frames, or after the date of their discovery or invention. For paintings this is most often based on knowledge of the period when pigments were in widespread use by artists, or when first manufactured and thus their first possible availability. For example, the white pigment lead white was introduced as early as 500-1500 BCE, zinc white (zinc oxide) in 1834, lithopone (a mixture of zinc sulfide and barium sulfate) came into use in 1874, titanium dioxide (anatase) used since 1923 and titanium dioxide (rutile) from 1947 (5).

Although the application of paint layers may be able to be dated using the dates of manufacture as an indicator, there may be numerous possible explanations for the presence of a particular paint layer or pigment component in a painting. A sample could come from areas previously over-painted or restored at a date later than the original construction. Dating an area of a painting due to the use of a particular pigment requires a significant understanding of the use of the pigment within the entire paint layer since the pigment and its use may be restricted to certain areas for any number of reasons, including later additions by the artist or later additions or restorations by someone other than the artist.

Research into artists' materials and techniques can be enhanced by knowledge of the chemical and material structure. Understanding the palette and technique of the period or individual comprise a reference base against which other works can be compared and provides some insight into how the painted image has come to be the one that is perceived.

Conservation treatments and studies of artists' methods and materials rely on fully substantiated corroborative evidence and objective information. Scientific materials analysis can provide

further certainty. Indeed, scientific analysis has been applied to paintings and other artwork since the early part of last century. As advances in techniques of non-invasive non-destructive analysis are made, these new approaches help answer specific questions for artworks not able to be answered previously.

1.1. Material and chemical composition of artworks

The chemistry and material composition of cultural heritage objects may comprise any conceivable combination of natural or synthetic materials known to humankind. They may be permanent or ephemeral. Objects created by experimental visual and performance artists may even contain biomaterials and interact with living systems that are transient and continually evolving (6).

In galleries around the world, the conservation profession falls into several different disciplines. Larger conservation laboratories are typically divided into areas incorporating works on paper, textiles, photographs, metals, paintings, objects and frames. Specialising in a discipline of conservation allows conservation professionals to develop a range of skills, relevant to the types of objects they will encounter in their area.

The work in this thesis will focus primarily on the analysis of materials encountered on paintings; ground layer media, pigments, binding media, additives and varnish coatings. Tradition has influenced the materials and techniques used by painters with many adhering to a generalised method for painting construction on canvas wall paintings.

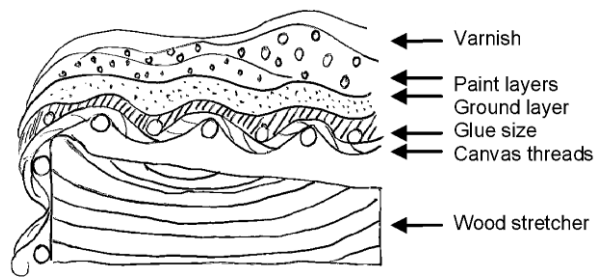


Figure 1. Schematic of a cross section through a traditional canvas painting.

Many historical wall and easel paintings are constructed with a traditional sequence of layers as shown in Figure 1. A painting support (wood panel or canvas) is first covered by an animal glue size layer to seal and protect the fibres, and then a preparatory layer called the ground or gesso layer is applied. The gesso may be comprised of chalk (calcite) or gypsum also bound with animal glue; the composition is somewhat dependent on the location and period. There may be one or more ground layers, sometimes additional ones tinted with colour to provide tonality to the upper paint layers. These preparatory layers form a protective barrier from the deleterious effects of the oil paint layers. Paint layers are typically comprised of pigment, an oil binding medium and may contain fillers or modifiers. Paint layers are applied over the ground layer(s) and the painting, which may be followed by a final varnish layer. While the entire thickness is variable, historical paintings are generally found to be between 200 – 400 μm in cross sectional thickness including the ground layer. Paint layers themselves may be approximately 20 – 100 μm . The layer sequence and thickness can vary, but often follows the pattern described above.



Figure 2. Paint cross section layer structure. Sample from: Sebastiano Ricci (currently attributed to) Italian 1659-1734. *The finding of Moses*. c.1720. Oil on canvas. 234.5 cm x 308.0 cm. National Gallery of Victoria, Melbourne. Melbourne Felton Bequest, 1959.

Figure 2 shows an example of a small cross section removed from the painting *The Finding of Moses*, from the dark blue lining of the page's jacket, just above the dog's hind quarters in the lower far-right hand side of the painting, with the traditional sequence of ground layer, paint layers and varnish. This painting and Raman pigment analysis are more extensively described in Chapter 2 but shown here is the illustrative layering structure revealed by cross sections taken from paintings. When a micro paint sample is removed from a painting and prepared as a cross section it usually only contains the layers above the canvas, as the glue size layer is often so intimately bound to the canvas it is retained on the substrate when the paint is sampled.

1.2. Instrumental analysis and conservation science

Before the advent of modern scientific analyses, understanding the materials and techniques used by an artist relied mainly on connoisseurship, a subjective appraisal based on art historical knowledge. Instrumental techniques for characterisation and analysis can contribute to a more accurate knowledge of material composition and there are many techniques routinely used as the physical and chemical structure of artworks is virtually unbounded. X-ray, vibrational

spectroscopy and chromatography techniques are all used. Most often used are scanning electron microscopy with energy dispersive x-ray analysis (SEM-EDS), Fourier transform infra-red (FTIR) spectroscopy, X-ray diffraction (XRD), X-ray fluorescence (XRF) and Raman spectroscopy. A summary of the most commonly used sample presentations and characterisation information is presented in Table 1. Many other techniques have been used such as polarised light microscopy (PLM), laser-induced breakdown spectroscopy (LIBS), laser-induced fluorescence (LIF), particle-induced X-ray emission / gamma-ray emission (PIXE/PIGE), particle-induced (PIGE), but these are not used as frequently.

Table 1. Analytical techniques most often used in conservation science

Technique	Material category	Sample presentation	Information provided
SEM-EDS	Inorganic /visual	Micro-sample in chamber	Elemental composition & visual
XRD	Crystalline organic or inorganic	Powder or in-situ	Crystalline phase
FTIR	Organic (mostly)	Various accessories on instrument	Chemical composition via IR spectrum
XRF	Inorganic	Powder or in-situ (handheld instruments)	Elemental composition
LC/GCMS	Organic	Dissolved in solvent	Chemical via mass spectrum
Raman spectroscopy	Inorganic and organic	On microscope stage	Chemical via Raman spectrum

Availability of or access to instrumentation is a common driver for the relative use of different techniques, and this also corresponds with the types of analytical techniques used by materials researchers in non-conservation laboratories. Since a large proportion of conservation science analytical research is undertaken in partnership between conservators and scientists in research laboratories, it is understandable that the most commonly used and understood techniques for general materials microanalysis are utilised.

The value of many artworks makes sampling an issue because the integrity of a piece must be considered. Australian codes of ethics and practice have been developed by the Australian Institute for the Conservation of Cultural Material (7). Even large objects may be disfigured by sampling if they are in pristine condition, and there is a sound argument against the practice of sampling as it is not a sustainable practice for the lifetime of an object. If the benefits of sampling can be justified, then sample size is limited to the smallest physically manageable piece that can provide good quality data. Non-destructive analytical techniques such as SEM-EDS, FTIR, XRD, XRF and Raman spectroscopy have the advantage that a single sample may be re-analysed using multiple techniques. Raman micro-spectroscopy has spatial resolution in the order of a few microns (discussed in more detail in section 1.4) often providing the ability to identify the composition of individual pigment grains.

A paint cross section is a useful method of sample presentation since it provides both structural information (organisation of the paint layers) and an opportunity for chemical analysis of the exposed face. To prepare a painting cross section, a painting is sampled from an inconspicuous location with the aid of a stereo microscope using a scalpel to remove a small flake through all the paint layers, right down to the ground layer and sometimes even the supporting substrate. The flake is mounted in resin and microtomed or carefully hand-ground using successive grades of abrasive paper to reveal the cross-section face. In other instances, small flakes may be removed and analysed without being prepared as cross sections. Alternatively, a cotton swab may be gently rubbed over a surface to pick up a few pigment grains. Paint flakes may be collected from under the covered section of a frame or found as detached flakes in the bottom of a frame if they correspond with the region of interest.

In-situ characterisation is non-invasive and non-destructive, and a number of techniques have been developed to characterise materials without the need for sampling. Some smaller artworks that are small enough to sit on a microscope stage may be analysed without sampling.

1.3. The Raman Effect

Light scattering phenomena are many and varied and exist on a range of scales from macroscopic to molecular. When light impinges on an object the light may be transmitted, absorbed, scattered,

diffracted and/or reflected. Reflected light is, for the most part, elastically scattered with the same wavelength as the incident irradiation, known as Rayleigh scattering. Following the predictions of several groups, CV Raman in 1928, observed a fundamental property of light, now utilized as a key analytical spectroscopic technique for measuring molecular vibrational frequencies – the Raman Effect where a small percentage of light is scattered from a surface *inelastically* (i.e., with a small change in wavelength from the incident radiation), occurring with a much-reduced likelihood of 10^{-6} .

Figure 3 illustrates different processes resulting from light-matter interactions including the Raman scattering process. The ground vibrational state (S_0) refers to the resting energy level of a molecule's covalent bonding electron configuration. An incident photon may cause a distortion in the electron distribution and elevation to an excited or virtual state. Although the energy levels are quantized, the virtual state is not a stationary energy state and immediate relaxation to a lower level results with the emission of a photon. Upon photonic excitement the energy level can be elevated and return with no net energy change. When the light is elastically scattered and no shift in the wavelength of light is observed and this is *Rayleigh* scattering. Raman *Stokes* scattering involves a shift from ground state to a higher virtual state and descent to a higher, but normal vibrational state. Some of the energy of the photon is lost and so when emitted, the photon has less energy and a correspondingly increased wavelength and a negative wavenumber shift. Raman *anti-Stokes* is the transition from a higher normal vibrational state to virtual state and relaxation back to ground state with the incident photon increasing in energy and decreased in wavelength resulting in a positive wavenumber shift. Stokes and anti-Stokes Raman shift are of equal magnitude equivalent to the vibrational energy difference between (8).

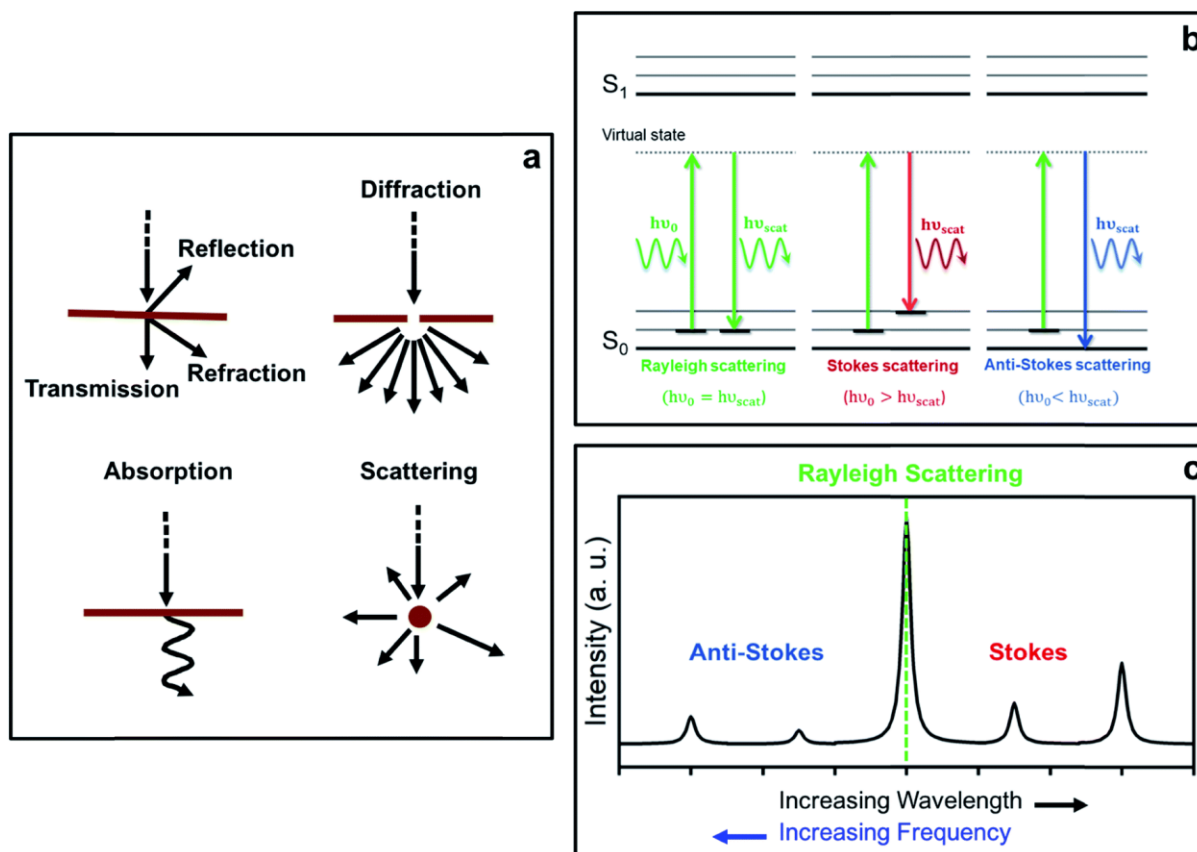


Figure 3. (a) Different processes resulting from light-matter interactions, (b) Rayleigh and Raman Stokes and anti-Stokes scattering processes, and (c) Raman spectrum with relative intensities of Rayleigh, Raman Stokes and anti-Stokes. Reproduced from (9)

The relative intensities of Stokes and anti-Stokes scattering are not equal, and the Boltzmann distribution describes the relative number of molecules in each vibrational energy level. At thermal equilibrium there are always more molecules in lower vibrational energy levels, therefore the Stokes Raman intensity will always be greater than the anti-Stokes intensity.

Raman spectra thereby measure the magnitude of shift (in arbitrary units) across a specified range from the frequency of incident irradiation, typically measured in wavenumbers. Wavenumber is a unit of frequency in atomic, molecular, and nuclear spectroscopy and is the number of waves in a wave train over a specified unit of distance. In vibrational spectroscopy it is usually measured in units of reciprocal centimetres (cm^{-1}).

Once light has penetrated the sample matrix, multiple internal reflections, internal scattering or absorption events may occur and depending on the sample's propensity to absorb a particular wavelength (described by the absorption coefficient of the material), with sufficient photonic flux, the sample may heat as a consequence of the absorbed energy. Sample heating is an important practical consideration as it may lead to thermal degradation.

Each unique chemical or molecule exhibits, because of its unique molecular structure and thereby electron cloud and vibrational states around each atom, an individual absorption and scattering profile which facilitates its identification. The competing electronic transitions within a chemical structure are represented in the relative intensities of the peaks in the spectrum.

In practice a Raman spectrometer attached to a microscope, as shown in Figure 4, can be used to direct incident laser irradiation onto a sample. The spectral output resulting from the interaction of incident laser irradiation with the sample is a combination of Rayleigh and Raman modes, but can also include interference such as luminescence. Rayleigh scattered light is separated from characteristic Raman scattered light using holographic notch filters and diffraction gratings. The frequency and intensity of the remaining Raman shifted light can be captured by a CCD detector and recorded as the Raman spectrum.

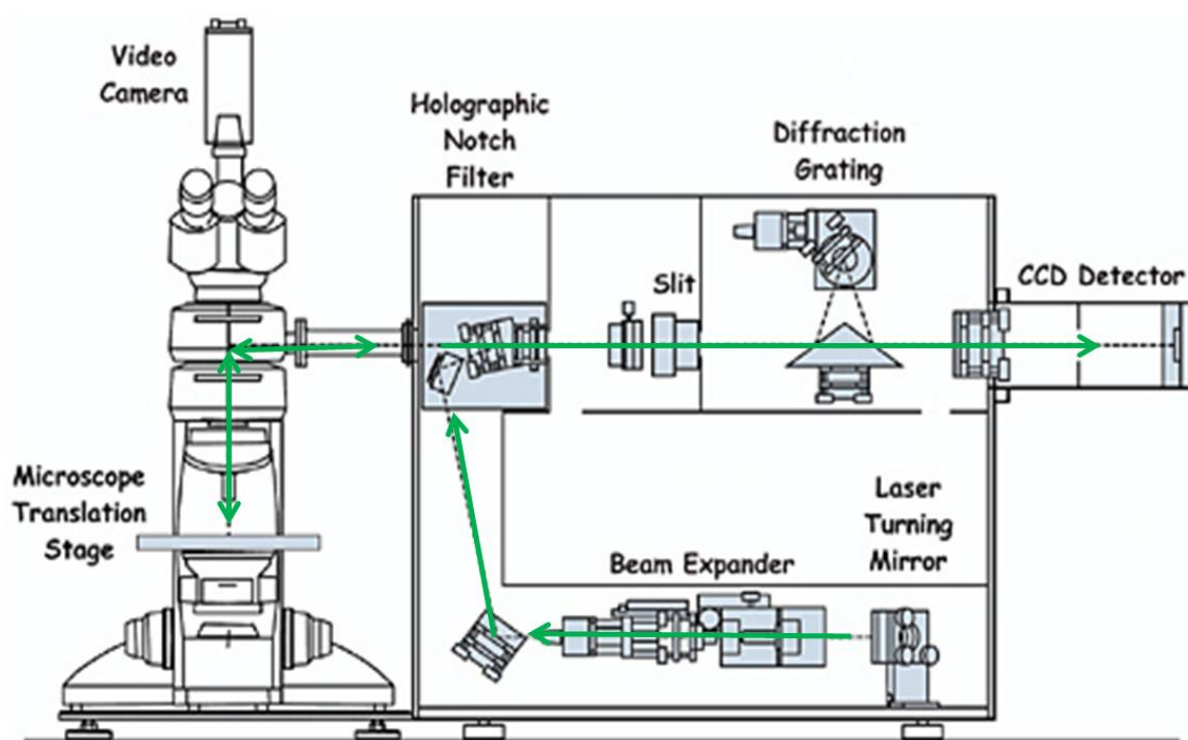


Figure 4. Schematic of Raman micro-spectrometer reproduced from (10). Laser enters at benchtop level directly behind the laser turning mirror.

Selection Rules for Raman

Polarisability (α) is a measure of the relative ease with which an electron cloud may be distorted from its normal shape by a nearby electric field. A bond must have an anisotropic polarisability in order to be Raman active. Polarisability is not simply scalar as it may respond to an electric field differently in each of x,y and z directions and is thereby described with a polarisability rank 2 tensor (a 3 x 3 matrix). Many crystalline materials are more easily polarised in one direction so the orientation of crystalline grains can influence the resulting spectrum and subsequently material identification when using a reference database.

Scattering response (I_s) varies with wavelength to the fourth power so using lower wavenumber laser sources increases Raman scattering intensity:

$$I_s \propto \lambda^{-4} \quad \text{Equation 1-1}$$

A balance must be achieved because although an increase in ν_0 (the incident laser excitation frequency) increases scattering efficiency, the possibility of exciting fluorescence also increases. Also, because of increased absorption, heating and thermal degradation can occur in sensitive materials resulting in chemical alteration, both damaging the sample and preventing identification of the original sample material.

The intensity of Raman scattering can be described by

$$I_R = \frac{2^4 \pi^3}{45 \times 3^2 \times c^4} \times \frac{h I_L N (\nu_0 - \nu)^4}{\mu \nu (1 - e^{-h\nu/kT})} [45(\alpha_a')^2 + 7(\gamma_a')^2] \quad \text{Equation 1-2}$$

Where

- c = speed of light (m/s)
- h = Planck's constant (J.s)
- I_L = excitation intensity
- N = number of scattering molecules
- ν = molecular vibrational frequency (Hz)
- ν_0 = laser excitation frequency (Hz)
- μ = reduced mass of the vibrating atoms
- k = Boltzmann's constant (J/K)
- T = absolute temperature (K)
- α_a' = mean value invariant of the polarisability tensor

γ_a' = anisotropy invariant of the polarisability tensor

From equation 1-2 above (8) it can be seen Raman scattering intensity is directly proportional to the number of molecules being illuminated and demonstrates Raman spectroscopy is useful for quantitative analysis. I_R is also proportional to the excitation intensity so an increase in irradiation power (greater numbers of photons) increases Raman scattering.

Another consideration is the volume of material sampled by interaction with the beam. With UV irradiation, only a very shallow layer is sampled because of high absorption and so limits the Raman scattering response. The limited availability of lasers, optics and lenses for UV is a practical issue.

The spectroscopic response is similar to that described by the Beer-Lambert law used in absorption spectrometry and Raman intensity can be described in related terms:

$$I_R = (I_L \sigma K) PC \quad \text{Equation 1-3}$$

With I_R = measured Raman intensity (photons/second)
 I_L = laser intensity (photons/second)
 σ = absolute Raman cross section ($\text{cm}^2/\text{molecule}$)
 K = measurement parameters
 P = sample pathlength (cm)
 C = concentration (molecules per cm^3)

1.4. Spatial resolution and interaction volume

Although collecting Raman spectra from a sample surface may be thought of as a surface measurement technique, the dimensions of many pigment grains in a paint layer are of an order similar to the wavelengths of light used to study them. It is therefore important to consider the spatial resolution that can be achieved in a Raman microscope experiment.

A Raman spectrum at any point in a paint layer is contributed to by all Raman active (and fluorescent) material within the beam interaction volume. Materials within the interaction volume will be in the correct position to be first irradiated and then allow Raman scattering (I_s) to be collected. Interaction volume is governed by the geometry of the focussed beam, primarily

the beam diameter, which provides the lateral (x, y) resolution, and the depth of focus (confocality parameter), which indicates the spatial resolution in the z direction.

The beam profile of most argon ion lasers is Gaussian and when focussed using the microscope objective, forms a waist at its narrowest point with radius w_0 , and depth of focus b , as shown in Figure 5. The diabolo shape is only valid in air/vacuum (optical index = 1); in a solid, the shape is modified by the change in optical index and the absorption.

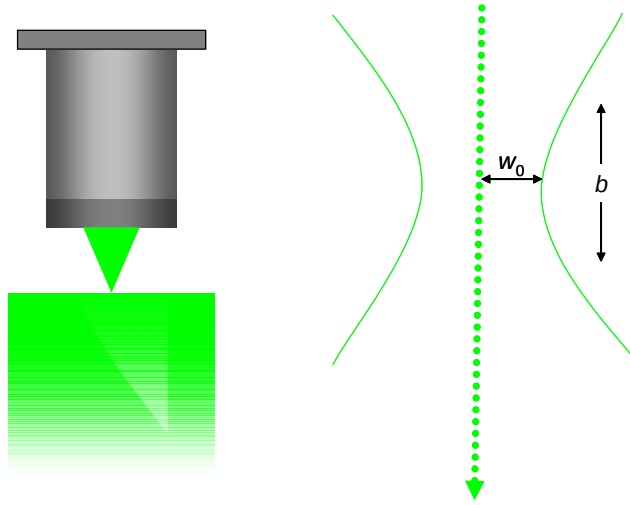


Figure 5. Illustration of microscope lens and beam profile in air/vacuum

The minimum theoretical beam diameter can be calculated from diffraction theory using the relationship

$$w_0(\mu m) = \frac{0.61 * \lambda(nm)}{NA}$$

where NA is the numerical aperture of the lens. For example, the 50 x objective lens used in most of the work presented in this thesis has $NA = 0.75$, so using the Argon ion laser 514 nm emission line gives a theoretical spot diameter of 0.8 μm . An 830 nm emission line gives a theoretical spot diameter of 1.4 μm .

The depth of focus b , can be calculated using (11)

$$b = \frac{2.\pi.w_0^2}{\lambda}$$

which, for the same lens and 514 nm laser line, is 2 μm .

Although the depth of focus of the laser may extend beyond the dimensions of many pigment grains, in practice it may be limited by the opacity of the material (and influenced by porosity, density and extinction coefficient).

1.5. Raman techniques for archaeology and artworks

Raman is more accessible than in the 1990's and instruments are increasingly installed in analytical laboratories enabling the opportunity for work on artefacts and works of art. Figure 6 indicates the growth in publications on paintings and cultural artefacts examined using Raman spectroscopy techniques over the last 2 decades.

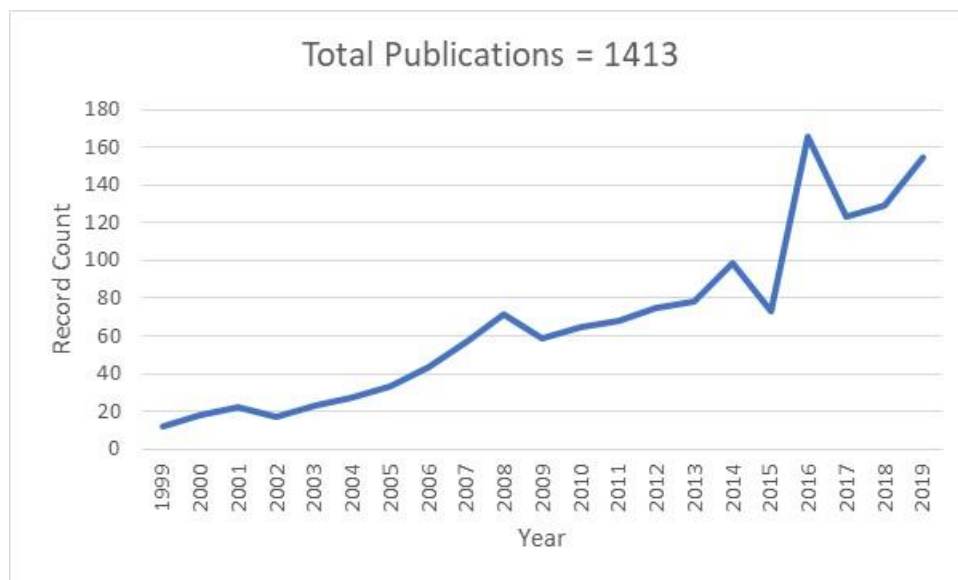


Figure 6. Decadal growth in the number of publications on the analysis of paintings using Raman spectroscopy. Thompson Reuters Web of Science. (Keywords: Raman, paintings, paintings + pigments, artworks, cultural conservation)

Three instrumental configurations are potentially useful for analysing artworks with Raman spectroscopy.

a) Fibre optic probe

Raman spectroscopy can be performed at the macro scale using fibre optic probes which apply the advantage of non-destructive, in-situ analysis to larger objects. Although it is not utilised extensively it has been reported for the identification of yellow pigments in a 17th century Italian painting (12) and Fourier transform (FT) fibre optic Raman has been used to examine pigments in several paintings through the varnish layer (13). Hand crafted wallpaper was examined during its restoration process in 2004 and the date of manufacture estimated at 1828-1830 based on the pigment composition (14).

b) Portable or Mobile Raman

A portable or mobile instrument has similar advantages to fibre optic instruments with the additional benefit of facilitating the analysis of objects in-situ, permitting analysis in a gallery or museum space and eliminating the need to transport the artwork. A comparison of the suitability of 5 mobile Raman spectrometers was undertaken with the following: Renishaw RA100, Renishaw Portable Raman Analyser RX210, Ocean Optics RSL-1, Delta Nu Inspector Raman and Mobile Art Analyser – MartA. It was found that despite the advantages of portability they performed less effectively than laboratory based instruments (15).

Mobile instruments have been evaluated for the analysis of a range of museum objects and painted surfaces. Challenges observed include fluorescing varnish layers, ambient sunlight, handling and manipulation issues (16). Portable Raman has been successfully used for the in-situ examination of medieval wall paintings (17) mortars and pigments (18) and to monitor cleaning and consolidation (19).

There are many small portable hand-held Raman analysers on the market from companies including Sci-Aps¹, BWTEK², Ocean Optics³, Rigaku⁴ and Metrohm⁵ and seem ideal for museological purposes. However, since there is no control over the power output, there is a risk of damage to samples/works unless the material structure is already known. For this reason, there is a general reluctance to use these instruments in the conservation community. In suitable circumstances they may be used, for example, Roman glass specimens that have little chance of being thermally damaged (20).

c) Micro-Raman

Micro-Raman is the most commonly used technique, whereby the small sample is presented on a microscope stage and the incident laser light is directed from the laser source through the microscope optics to the sample. Raman scattered light is collected through the microscope optics and to a detector. Because of the experimental advantages of micro-Raman, it has gained wide acceptance for use in archaeological and museological contexts over the last decade.

Raman is well suited for the characterisation of cross sections as the spatial resolution attainable with the microprobe allows identification of individual pigment grains (larger than 1 μm) in paint layers. Fluorescence is the single most challenging aspect encountered when examining paintings and artworks using Raman spectroscopy. Fluorescence swamps the detector and often renders the relatively small Raman scattered signal undetectable amidst the dominant fluorescent signal.

Paintings with media such as an oil or acrylic binder can be analysed (as presented in chapters 2 and 5), but generally with a lower success rate because the binding medium completely surrounds the pigment grains and oil containing binders fluoresce strongly. Many wall paintings have the additional disadvantage of being varnished which, in theory, microscope confocality should eliminate but in practice it does not because scattering of the incident laser beam irradiates the adjacent fluorescent layers.

¹ <http://sciaps.com/portable-raman-spectrometers/>

² <http://bwtek.com/technology/raman/>

³ <http://oceanoptics.com/product/idraman-mini/>

⁴ <http://www.rigakuanalytical.com/products/handheld-raman-spectrometer-portable-raman-spectrometer-progeny/>

⁵ <http://www.metrohm.com/en/products/spectroscopy/raman-analyzers/>

Numerous studies have been undertaken around the world, but the University College, London chemistry department which works in close collaboration with the British Museum pioneered work in this area. Robin Clark and his co-workers have published over a hundred articles in the field, compiling reference databases and examining western and eastern manuscripts, paintings, ceramics, papyri, icons and polychromes (painted wooden sculptures, often depicting religious icons) and wall paintings (5, 21-29).

A majority of published work has concentrated on pigment identification for two main reasons. First, colouration of an image or object contributes to the main visual impact, so art historical interest is mostly focussed on the use of colouring materials and identifying their composition. Secondly the fluorescence of binding media, organic resins and coatings make the collection of Raman spectra challenging. Techniques for reducing fluorescence such as using longer wavelength laser lines (eg: NIR $\lambda_0 = 1064\text{nm}$) are reported to be successful (30-33).

1.6. Raman Spectral Libraries

Materials analysis with vibrational spectroscopy is most commonly performed by spectral comparison with known reference materials. This has been the standard technique for Raman pigment identification from its beginnings. The first pigment database of historical pigments was published in 1997 both on-line (34) and as a journal article (35). This was later supplemented in 2001 with an FT-Raman library (36) with additional pigments and related materials that could not be collected in the first publication.

For pigment identification in this work, it was necessary to have a comprehensive set of pigment reference spectra (spectral library) against which to compare unknowns.

Databases of pigment reference spectra were limited at the start of this research. To enable spectral matching and gain familiarity with the type of spectral features and spectral characteristics that can be expected to be encountered, a database of over 180 pigments and paints was compiled. Pigments were verified with x-ray powder diffraction (XRPD) to confirm their identity prior to Raman analysis.

Most pigments are sold with the only information being the colour name, which provides little information regarding the material and chemical content of the pigment. Each sample examined in this work was analysed using XRPD to verify the composition. Thus, the Raman spectrum could be referenced to the actual chemical composition rather than the pigment label, which as found in many cases, can be misleading or incorrect. It is not always clear whether published Raman spectral libraries validate the composition of samples before collecting Raman spectra. For example, University College London (35) do not confirm that material composition has been verified, whilst the Caltech-based RRUFFTM Project mineralogical database (37) do and publish Raman spectra alongside XRD and chemical data.

The spectra in this current work are presented as a practical, visual aid for the identification of pigments using Raman spectroscopy. A question often posed in relation to pigment identification in a paint layer cross section is “which pigment is responsible for the colour?” Collecting suitable Raman spectra to provide an identification can be a laborious and often unsuccessful undertaking due to interference from fluorescence which diminishes the signal to noise ratio. Providing an overview of the range of spectra for a particular colour can be a useful indicator as to which areas of the spectrum should be focussed on for collection purposes.

More recent on-line spectral libraries of pigments have been developed, such as the Romanian Database of Raman Spectroscopy (38) with 35 pigment standards. The RRUFFTM Project mineralogical database (37) contains thousands of mineral Raman spectra, proving to be a useful resource since many historical pigments are of mineral origin. The Infrared and Raman Users Group (IRUG) is a spectral database dedicated to conservation, which includes Raman spectra in addition to its original focus on Infrared spectra. It currently contains reference spectra for 105 minerals and inorganic pigments and 248 organic dyes and organic pigments (39).

The option to use different irradiation laser sources depends on instrumentation available in the laboratory. However, most spectral libraries are collected at 514.5, 488 and 633, 1064 nm suggesting these are currently the most commonly available and frequently utilised wavelengths.

In Appendix 1, Raman pigment reference spectra are presented in graphical format with the major peaks indicated in order to provide an overview of the spectral character. Spectral features were visually examined to assist with the interpretation of spectra and identification of pigments from artworks as presented in chapter 2. Where a visual spectral match was observed in the library, data from the relevant reference spectra was overlaid for confirmation.

Resonance Raman occurs when the energy of the incident radiation coincides with the electronic transition of the analyte, with the excitation resulting in structural changes that may enhance or reduce Raman scattering of certain vibrational modes. Changes in bond length and/or force constant during the electronic excitation influence the polarisability and hence Raman intensity. This can be useful for signal detection of specific molecules or materials, whose scattering signal may be increased by several orders of magnitude. The implication for analysis of many coloured materials used by artists is that their Raman spectrum can differ markedly in terms of maximum relative intensity and wavenumbers can be moderately shifted. The combination of resonance effects and sample variability therefore makes the use of large reference databases and broad comparison necessary.

1.7. Opportunities for advances in characterisation and analysis

Fluorescence of binding media and varnishes is a well-known and utilised feature in conservation. UV-induced fluorescence imaging of varnish coatings and organic materials is a common painting examination technique used to reveal where coatings and paint layers are located over the surface. When illuminated with long wavelength UV or UV_A, natural resins fluoresce a milky yellow-green. Linseed oil exhibits a blueish haze, shellac a bright orange while acrylic binders and synthetic resins do not generally fluoresce. Raman analysis of organic binding media is experimentally challenging as fluorescence is frequently encountered. In view of the prevalence of fluorescence in organic painting materials, it can be seen why Raman of binding media materials is not as successful as for pigments, although there has been limited work in this area. Vandenabeele *et al* (31) have sought to classify some organic media (resins, proteins, polysaccharides and fatty acids) on the basis of unique vibrational modes, although this technique was not applied to “unknown” samples.

Fluorescence of resins and oils is the main challenge for Raman spectroscopists working with painting materials so Raman spectroscopy has, for the most part, not been useful in situations where these materials occur. Attempts to minimise the problem include: use of purified sample, photo-bleaching, longer incident wavelengths or FT-Raman (8).

Common techniques to reduce sample fluorescence are to use longer excitation wavelengths or photo-bleaching (quenching by laser irradiation for some time before spectral collection). These approaches have proven unsuccessful for some organic artists' materials and surface-enhanced Raman spectroscopy (SERS) provides an alternative opportunity to obtain Raman spectra not previously collectable as presented in chapter 3.

The scientific analysis of valuable objects requires specialised skills for data collection and interpretation of results. Through the work presented here, it is demonstrated how the development of novel methods for collecting Raman spectra and chemometric techniques for processing spectra, made possible valuable materials characterisation, previously unattainable.

1.8. Overview and objectives of this work

This thesis presents new developments and approaches to the examination of artworks using Raman spectroscopy that aim to overcome the major existing limitations inherent in the application of the technique for examining artists' materials. The problem of fluorescence is illustrated in Figure 7, where the overall intensity is extremely high but decreases away from the incident wavelength, and characteristic spectral features cannot be discerned.

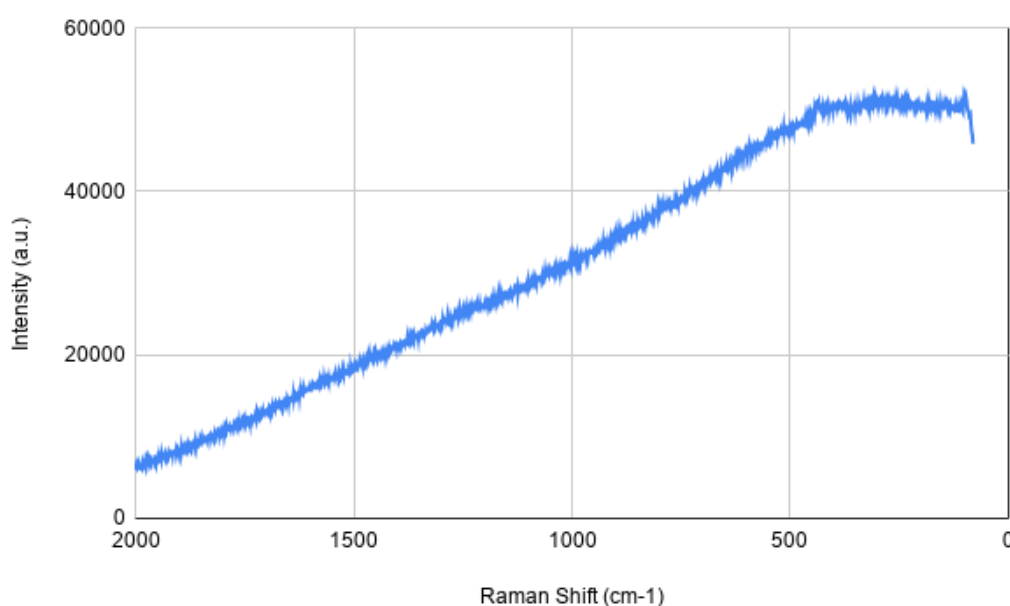


Figure 7. Raman spectrum of oil-based painting sample exhibiting high background fluorescence

Because of the advantages specific to analysis with Raman spectroscopy, such as chemical specificity, high spatial resolution and its ability to examine samples or objects non-destructively, use of this technique for identifying and studying historical materials has flourished in recent years. Despite its benefits, there are issues that complicate Raman spectral analysis of cultural materials. The primary distinction is that cultural materials have unique sampling and analytical restrictions due to the inherent value of the artwork. Samples are not only limited in availability and size, but complex in their structure, often extremely heterogeneous and contain interfering fluorescent components.

Raman identification of materials generally relies on spectral comparison with libraries or databases of known materials. While there are many commercial Raman spectral databases for chemicals, polymers and laboratory substances, at the time of analysis there were very few spectral databases for the types of materials encountered in paintings. Where they did exist, cross validation of composition and identity using alternate techniques was not always evident. It was therefore necessary to compile a reference set of known and validated pigment material for use in this thesis. A discussion of pigments and presentation of a pigment database is presented in Appendix 1.

Raman spectroscopic analysis of artworks is undertaken to answer technical questions relating to the materials used in the artwork. Five case studies requiring knowledge of the pigment identity are presented in chapter 2. Spectra compiled in the pigment database are used to assist with identification of pigments in cross sections and small artworks.

Historical painting varnishes are comprised of various naturally occurring resins which are very fluorescent; so much that their auto-fluorescence across the painting surface is often used as a quick, non-specific method of indicating the presence of a natural resin on a painting surface. Identification of natural resins using Raman has not been possible using visible wavelengths because of the extremely high fluorescence signal. In chapter 3 Surface Enhanced Raman Spectroscopy (SERS) is presented as a method to overcome this limitation. Methodologies for preparing an inexpensive, easy to prepare SERS substrate are reviewed and compared for use in the context of a conservation laboratory. An efficient SERS substrate is identified and used to characterise varnish removed from a painting.

Understanding the overall spectral similarities and differences between both natural and synthetic resins used as painting varnishes is important when interpreting the identity of a resin since similar spectra may be misinterpreted. In chapter 4 discusses use of multivariate analysis of painting varnish material spectra to examine spectral relationships and differentiation within and between spectra using unsupervised clustering. Two multivariate analysis techniques, Hierarchical clustering and Principal Components Analysis (PCA) were used to visualise overall similarities and differences.

Having examined the application of multivariate data analysis to the analysis of Raman spectra from individual samples, chapter 5 is an extension of the concept applied to spectra derived from image pixels of Raman maps. Since Raman spectral images are produced by the sequential collection of individual Raman spectra across a sample surface, each point in the image (pixel) can be considered a single sample and the entire image dataset can be treated using the same multivariate statistical approaches as described for individual samples. Work presented in chapter 5 extends the principles of PCA and classification of non-spatial data from individual non-spatial samples as examined in chapter 4, to the spatial analysis of 2D Raman image data across painting cross sections.

This technique enabled understanding of the complex nature of layers in a painting cross section, providing insights into the sequence of use and application of materials by the artist as a diagnostic feature. Specific pigments located within the paint layer were identified. A mid-preparatory layer called *imprimatura* was characterised and the sequence and composition of materials were used to aid attribution of a valuable Renaissance painting.

Chapter 2: Raman spectroscopic analysis of selected artworks

2.1 Introduction

This chapter presents five individual case studies demonstrating the multiple challenges typically encountered when attempting to identify pigments to aid conservation treatments and art historical research. As discussed in chapter 1, challenges include:

- 1) Not being able to sample an artwork for ethical reasons or an institutional requirement.
- 2) Spectral interference due to the presence of fluorescent components, commonly varnish and/or binding media and
- 3) Variability in object type and the chemical environment of the pigment.

Through each of these case studies, the identification of pigments has informed art historical studies and conservation treatment or storage decisions. Pigment identity may be used for dating and attribution, understanding technical production methods of an artist, or determining provenance. Conservation strategies are informed by knowledge of pigments and the material structure of an artwork. These case studies were selected as diverse real-life examples of how Raman analysis can be used to support conservation treatment and management decision making and are used to test and examine the advantages and limitations provided by the technique.

Fluorescence describes electronic transitions from higher to lower excited singlet states of molecules that occur in the nanosecond time frame. Figure 8 illustrates the fluorescence process. As described in chapter 1, the ground vibrational state (S_0) refers to the resting energy level of a molecule's covalent bonding electron configuration. When sample molecules are exposed to incident light with energy that matches a possible electronic transition within the molecule, absorption can promote ground state electrons to a higher energy orbital and an excited state (S_1). Electrons are generally elevated from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) (40). As electrons relax back from the excited state to the ground state, radiation of a lower energy is emitted.

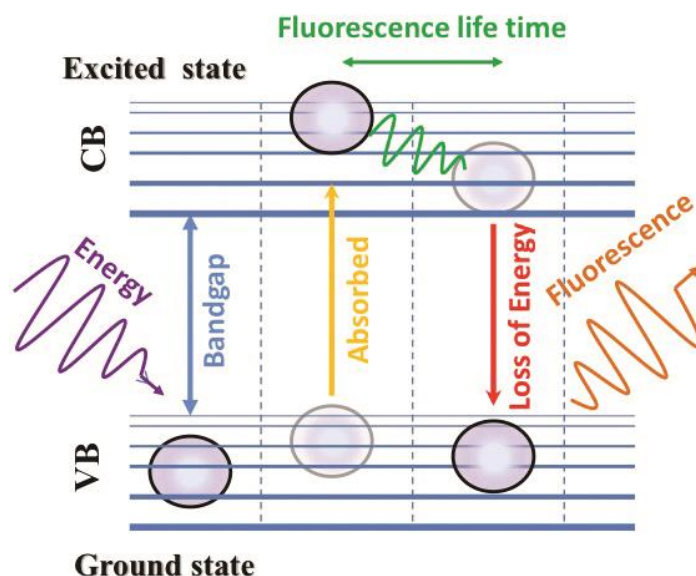


Figure 8. Illustration of fluorescence process. VB denotes valence band, CB denotes conduction band.
Reproduced from (41)

It is undesirable if the wavelength of incident radiation utilised for Raman analysis fits the absorption profile of a sample and promotes fluorescence and overwhelms the Raman signal. As introduced in chapter 1, this is one of the most commonly reported interferences encountered when analysing samples from real artworks (42, 43), which can be mitigated via the use of longer wavelengths. In the Raman spectroscopic analysis of a ‘noli me tangere’ painting, Hibberts *et al* (44) reported that bulk excised paint specimens fluoresced strongly, and the presence of a surface varnish coating caused extensive background fluorescence. It was found that in most cases, bulk excised samples fail to provide useful Raman spectra.

For the case studies examined in this chapter, nearly all samples fluoresced far too strongly when excited at 514 nm, either due to the binding medium or admixed compositional components, to be able to collect useful spectra. It is significant that only one published study reports the identification of pigments that are bound within a fluorescing oil binding medium (from an oil painting on canvas). In this instance, Aibeo *et al* (45) used an exciting line of 785 nm to reduce fluorescence.

Techniques such as quenching or bleaching were not considered appropriate for samples of this nature as they cause irreversible change in the composition of the material. In these processes the sample is subjected to continual irradiation, until fluorescence is quenched due to having

destroyed the fluorescent species. However, this is not a useful technique if the material to be characterised is altered. It is therefore only useful when fluorescence is due to an impurity in the sample. Lowering laser power further was not feasible since extremely low powers were already being used and collection times would have increases to an impractical time frame. As such, the strategy in this chapter was to reduce fluorescence through use of a longer excitation line, such as 1064, 830 or 782 nm which are often available on laboratory-based instruments.

Although 1064 nm is sufficiently low in energy to reduce fluorescence in most cases, the signal is reduced considerably because the scattering cross section is inversely proportional to the fourth power of the excitation wavelength as illustrated in Figure 9. This necessitates longer scans and use of higher laser powers than used for visible excitation, posing greater risk to the sample. Furthermore, pigments containing iron or copper, which have electronic absorption bands in the near-infrared region, can fluoresce or thermally degrade when irradiated at 1064 nm.

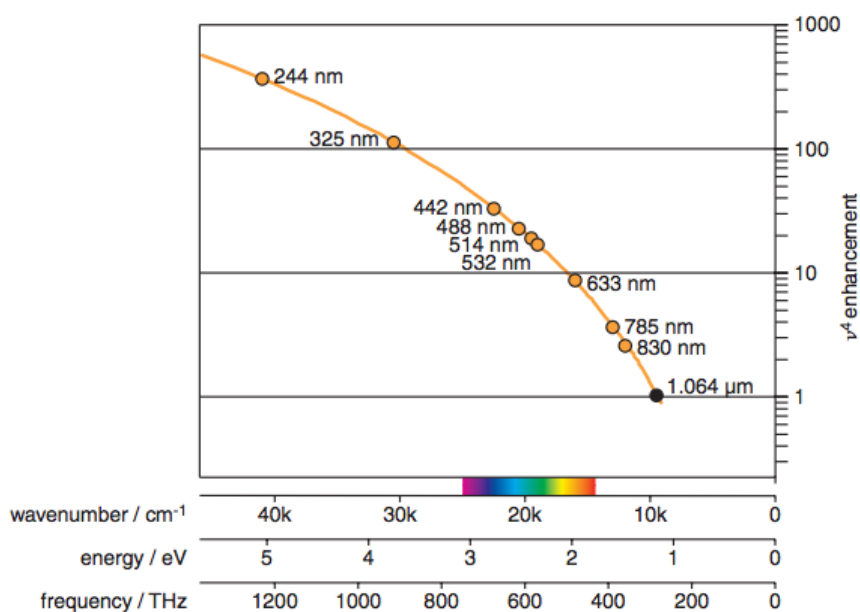


Figure 9. Raman scattering efficiency as a function of excitation wavelengths. Intensity at 1.064 μm is normalised to have an excitation efficiency of 1 (35).

Raman spectroscopy performed at 1064 nm is most often performed using an FT-Raman spectrometer, which is an adaptation of an FTIR spectrometer. The spatial resolution of FT-Raman systems is generally greater than 5 μm . In this work 1064 nm was not used as such instrumentation was not easily accessible.

Figure 9 compares relative intensity differences between laser lines available in commercially available instruments. Experimental efficiency needs to consider the entire optical path (e.g., lenses, mirrors and detector) and experimental process. Whilst Raman scattering efficiency is greatest for blue to violet laser lines, UV Raman spectroscopy suffers from many disadvantages including high cost, practical challenges such as adjusting the focus with a microscope, and more degradation of the illuminated materials. Optimisation of the technique for artists' materials (experimental efficiency) depends on many factors as discussed, so it is impractical to select an incident line simply because of its high Raman scattering efficiency.

This chapter presents five case studies undertaken because knowledge of the pigment composition was important to provide historical or contextual information. The first two examples are small artworks, which were, because of their size, too precious to allow sampling and required the development of an experimental approach ensuring no damage occurred to the work, even at the microscopic level, during analysis. These small artworks (portrait miniatures and a painted catalogue from an exhibition of Streeton paintings) were of a suitable size and shape to fit on the microscope stage. In these studies, mock samples were prepared and examined using materials expected to be found on the artworks according to literature available at the time (painting support, pigments and binding medium) to establish that damage would not occur during the analysis.

Case studies three and four present examination of micro painting cross section samples from two different oil paintings with pigments bound in fluorescent oil. Case study five involves examination of dyes and colourants on a historical Indian textile matrix.

2.2 Case studies

2.2.1 *Portrait miniatures*

Portrait miniatures (limnings) are an artistic derivation of the decorations found in illuminated manuscripts and developed as a distinct art form around the 16th century. Many are glazed and housed in complicated, delicate lockets, which are easily damaged if removed. The housing is integral to the painting and often impossible to remove without damage. Analysis through the glazing is of obvious benefit as the integrity of the locket or frame can be retained and the painting is not exposed to many potentially harmful effects and most obviously the risk associated with removal.

Portrait miniatures were originally painted on parchment, commonly with an opaque ground layer, which has come to be known as a carnation layer. An image was usually built up with successive applications of transparent colour by way of pigment bound in water, sometimes with additives such as gum arabic, sugar, honey and ox gall.

Towards the beginning of the 18th century ivory replaced parchment as the preferred support. The carnation layer was abandoned, and the luminosity of the ivory was exploited with paint that contained more additives. The oiliness of the ivory surface was addressed by adding more surfactants such as eel, carp or ox gall, and gum was added with increasing quantities of sugar and honey. With the development of photography in the late 19th century, portrait miniatures experienced a decline in favour (Cobus Van Breda, personal communication 1998).

Much work has been published on the analysis of pigments in illuminated manuscripts with Raman microscopy (46-55) and since portrait miniatures are painted in a comparable manner, using paints which are pigment-rich and contain relatively little binding medium, it was expected analysis of these works would be possible at 514.5 nm. Raman is ideally suited for examination of these objects, with the limitations imposed by the sensitive nature of the work, and the probable need to analyse through the cover glass of the locket.

This study examined two “mock-up” paintings in order to develop the experimental protocols and test the influence of a glass cover glazing, and then two portrait miniatures from the collection of the National Gallery of Victoria over which there are questions of attribution and authentication. Different instruments with different wavelengths were accessed. For one of the

paintings, identification of the white pigment used in over-painted areas would assist with determining the date of the restoration.

2.2.2 Part 1 – Mock ups

2.2.2.1 Methodology and experimental

The two mock-ups shown in Figure 10 were painted by NGV paper conservator (Cobus Van Breda, 1998) using materials and techniques consistent with pre and post 18th century practice. Miniature 1 was painted on fine parchment pasted to card. Miniature 2 was painted on ivory attached to card. Facial features were painted with gummy washes (using gum Arabic) with additives of sugar and ox-gall. These two miniatures were examined with and then without a domed cover glass to ascertain any risks associated with the technique, especially thermal susceptibility of the paint film to the laser irradiation parameters, and to ascertain whether the underlying support would contribute to the spectra.



Figure 10. Mock-up Paintings 1 and 2. Each measured approximately 30 mm in diameter

The portrait miniature mock-ups were first examined (both with glazing and without) on the microscope stage of a Dilor XY confocal Raman microscope with 100 x objective lens using Argon ion laser excitation of 514.5 nm operating at power 0.5 – 2 mW with collection times of 2-5 mins.

2.2.2.2 Results and discussion

Considering the nature of the binding medium, fluorescence was not expected and was indeed found to be insignificant. The quality of spectra obtained from the mock-ups was comparable in

intensity and resolution to that obtained from reference spectra of dry powder pigments. No evidence of spectral contribution from the support was observed.

In mock-up 1, spectra taken from a region in the red background matched with French red lake. These are shown in Figure 11. Spectra collected from the white area in the face matched with the database reference spectrum for lead white Figure 12. The blue of the jacket in mock-up 2 was found to be ultramarine blue Figure 13 and the reddish tones in the complexion were found to be vermilion, Figure 14.

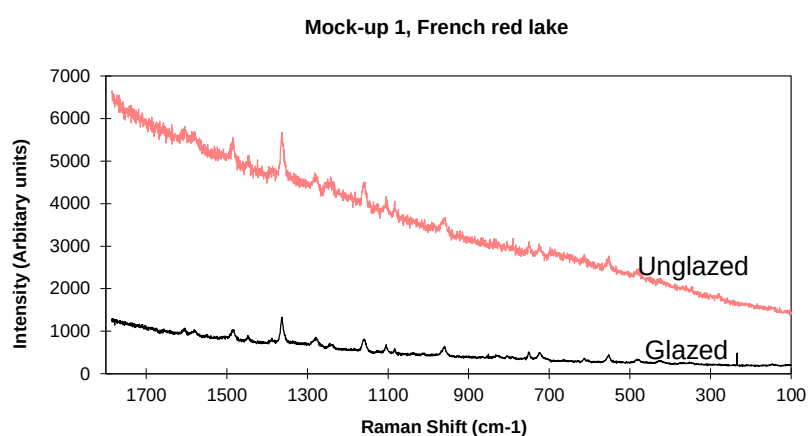


Figure 11. Mock-up 1 painting; spectra collected in red background area found to be French red lake, both glazed (black) and unglazed (red). 514.5 nm, 0.5 mW, 5 mins.

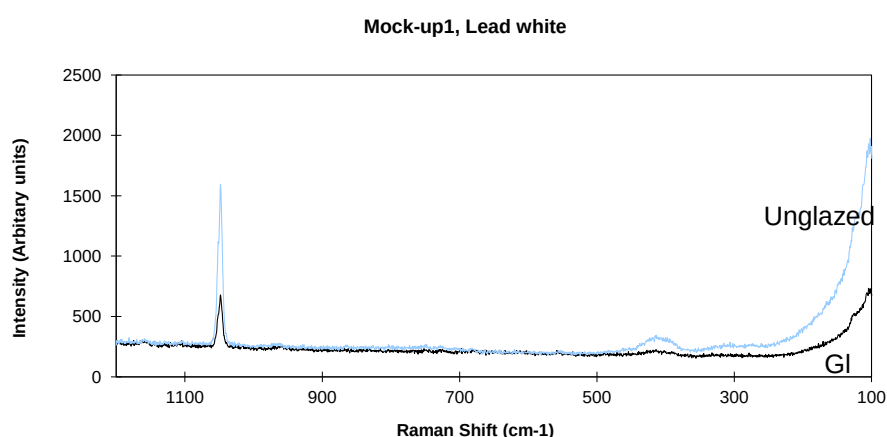


Figure 12. Mock-up 1 painting; spectra collected in white face area found to be lead white, both glazed (black) and unglazed (blue). 514.5 nm, 2 mW, 2 mins.

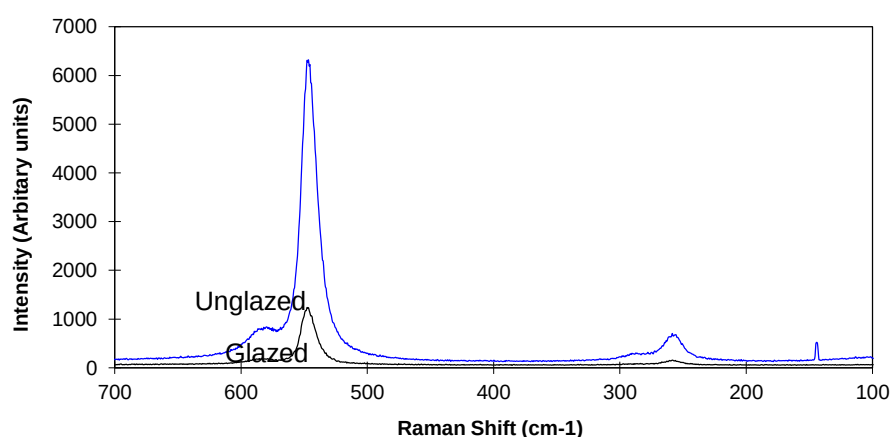


Figure 13. Mock up 2 painting; spectra collected in blue jacket area found to be ultramarine blue, both glazed (black) and unglazed (blue). 514.5 nm, 2 mW, 2 mins.

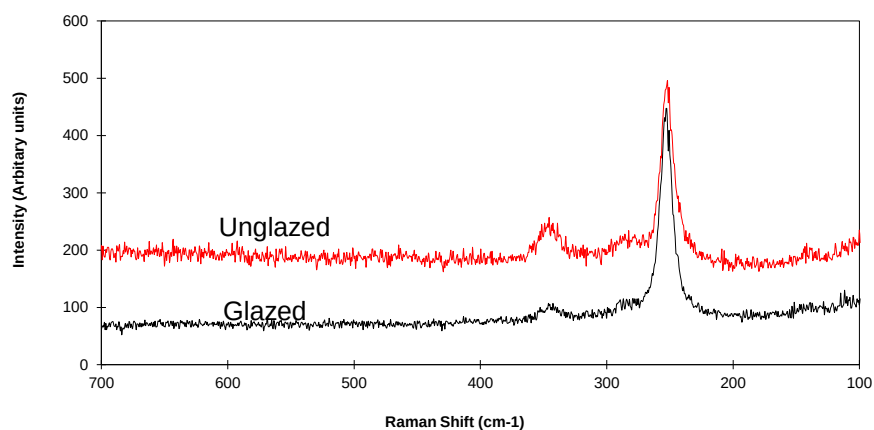


Figure 14. Mock up painting; spectra collected in red tones of complexion area found to be vermilion, both glazed (black) and unglazed (red). 514.5 nm, 0.5 mW, 2 mins.

The two paintings were analysed with and without glazing and their response compared. The glazing did not affect the spectral character but merely reduced the overall intensity of the spectrum, the consequence of which could potentially (where conditions permit) be overcome by increasing the laser power or collecting data for longer periods.

This exercise demonstrated successful identification of pigments in the mock portrait miniatures suggesting analysis of portrait miniatures from the collection should be possible without causing damage using the described experimental conditions, with no interfering contribution from the underlying substrate, and with sufficient intensity through cover glasses to provide satisfactory spectra for pigment identification.

2.2.3 Part 2 – Portrait Miniature Paintings

Portrait of a Lady wearing Elizabethan costume is a portrait miniature said to be of Lady Arabella Stuart, 16th century (Figure 15). Original conservation examination found the format, support, technique, hat and dress to be consistent with attribution to the 16th century. Questions were raised about the provenance of the work when a 1997 examination under UV_A radiation found some of the white used in the lacework detail on the surface brocade in the central panel (bodice) of the costume fluoresced in the manner of Zinc white (ZnO), a pigment not available until 1790, suggesting it could be a later addition. Identification of the white over-paint was important to clarify the apparently inconsistent application dates.



Figure 15. *Portrait of a Lady wearing Elizabethan costume*. Diameter 107 mm, National Gallery of Victoria, Melbourne

Portrait of a Man depicts a gentleman in a bright blue jacket and white frilled neck-piece (Figure 16). Very little is known about this work and it was hoped pigment identification would assist with assigning an attribution. It is thought to be from the 19th century.



Figure 16. *Portrait of a Man*. 37 x 31mm. National Gallery of Victoria, Melbourne

2.2.3.1 *Methodology and experimental*

Both paintings were first examined at 514.5 nm in the same manner as the mock-ups. Fluorescence in some regions, especially the brocade, was observed and necessitated subsequent examinations with a higher wavelength laser. A second examination was made using a Renishaw Micro Raman Spectrometer 2000 (University of Melbourne, School of Chemistry) using a long working distance (8 mm) 50× lens. A diode laser operating at 1.5 mW incident power and 782 nm was used; 3 – 16 spectral accumulations were collected with collection time for each being 10 – 300 s. Examination was inconclusive with no spectral signature discernible above the fluorescent baseline. A third examination of the white over-paint was made using a Renishaw inVia confocal microscope system (CSIRO, Clayton). Specimens were illuminated through a 50× short working distance objective lens with 830 nm excitation from a diode laser, operating at incident power of 1.5 mW.

2.2.3.2 *Results and discussion*

2.2.3.2.1 *Portrait of a Lady wearing Elizabethan costume*

Two areas on the hipline of the dress were analysed with 514.5 nm. One in an area where the original paint was lost but had been replaced with colour (over-paint), and the other an area of the blue which was representative of the original coloured region. The blue pigment in the dark blue-grey primary layer was also examined. All blue pigment areas identified as ultramarine due to a strong band at 548 cm^{-1} as shown in Figure 17.

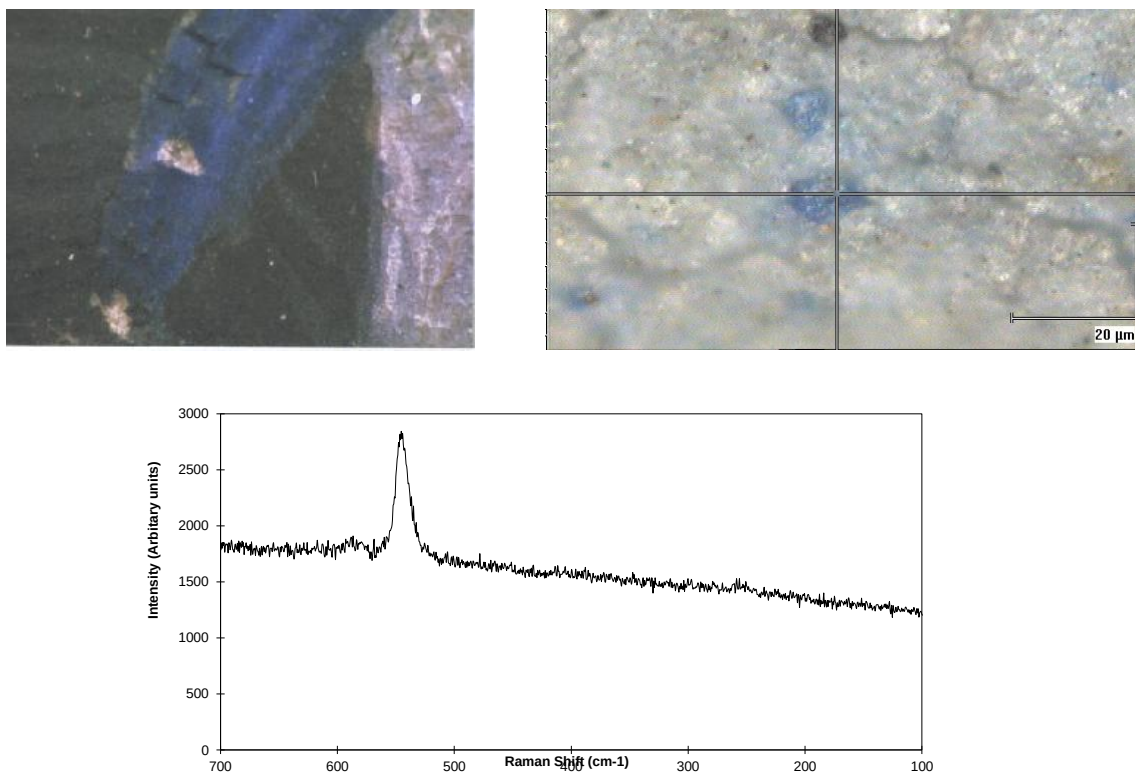


Figure 17. a) Left: Blue pigment grain examined in blue-grey paint of bodice b) Right: Retouching blue on the hipline of the dress. c) Below: Example of sample spectrum with band at 548 cm^{-1} indicating the pigment is ultramarine. 830 nm, 1.5 mW, 5 min.

Detail of the white brocade in the bodice of the dress is shown in Figure 18. Painted in what appear to be three distinct layers, the primary layer is a background of blue-grey paint, the next layer a mid-grey and the final, and the uppermost layer is a white over-paint.



Figure 18. Detail of brocade in the bodice of the dress

All three layers in the bodice were examined. The mid-grey layer and blue-grey layer were highly fluorescent, but a small peak was evident at 1049 cm^{-1} indicating lead white in both (spectra not shown). The white over-paint, suspected to be Zinc white, fluoresced too greatly to be identified at either 514.5 or 782 nm. Examination of the brocade with 830 nm was successful, however, and although the spectrum exhibited a very strong fluorescent baseline signal, the signature peak at 438 cm^{-1} facilitated a match with Zinc white from the collected library as shown in Figure 19.

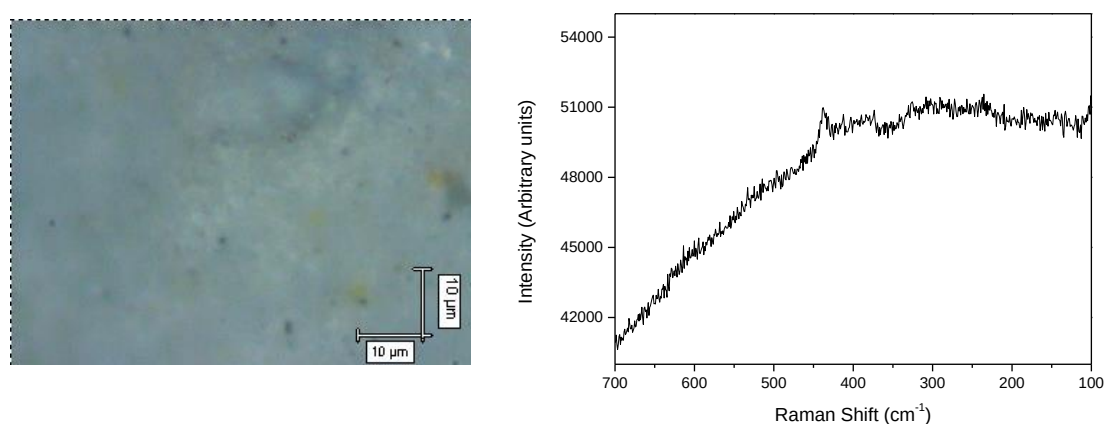


Figure 19. Image of white area in brocade and Raman spectrum collected with 830 nm incident irradiation. 438 cm^{-1} band indicates zinc white. 830 nm, 1.5 mW, 5 min.

2.2.3.2.2 *Portrait of a Man*

The nature of the paint on this painting was not what would normally be expected as the paint surface appeared very shiny, becoming more obvious under the microscope as the viewing light was reflected specularly. During examination by the conservator, it was noted, "...The artist who painted this work has mixed much more gum than usual with the blue pigment" (56). Analysis of the gummy regions (which occurred over most of the painting) at 514.5 and 782 nm resulted in high fluorescence and no Raman spectra were able to be collected. The painting was not available to be examined with 830 nm.

It was possible to examine one small region with 782 nm, where the white highlight in the eye was not gummy, and a spectrum was obtained with a band at 960 cm^{-1} . This corresponds with the symmetric stretch of the $(\text{PO}_4)^{3-}$ anion at 961 cm^{-1} and suggests the use of $\text{Ca}_3(\text{PO}_4)_2$, bone white (35).

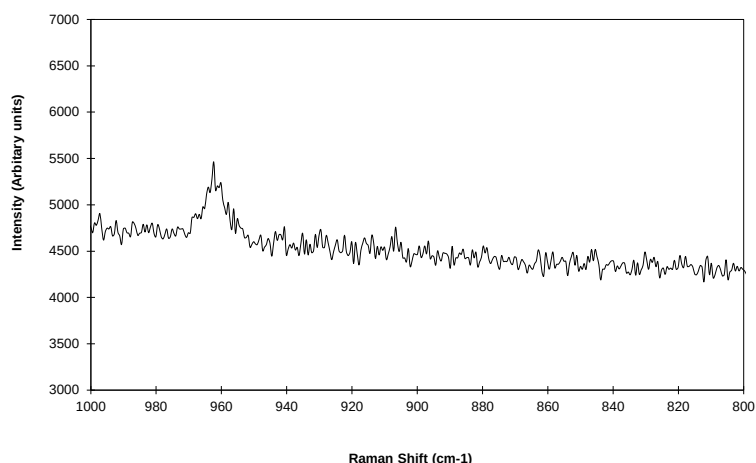


Figure 20. Spectrum 782 nm taken from white highlight in the eye. 960 cm^{-1} band corresponds to bone white. 830 nm, 1.5 mW, 5 min.

In summary, it was found that it is possible to examine portrait miniature paintings without destructive sampling by placing the entire object on the microscope stage. It is also possible to examine through the cover glass, which only attenuates the Raman signal. This is unproblematic as long as there is sufficient signal to noise, and the signal may be improved by longer collection times or increased power. Fluorescence was observed in gummy areas of the painting *Portrait of a Man* and in the brocade in *Portrait of a Lady wearing Elizabethan costume* when examined at 514.5 nm. Fluorescence was reduced by using a 782 nm laser to examine the grey areas of the brocade but was still evident in the white over-paint. Using 830 nm was successful and the white over-paint was found to be Zinc white. Since Zinc white was not used widely until the late 18th century, with widespread use beginning in the 1830-40s, the evidence provided by this study supports the belief that the white brocade overpaint was applied at a much later date than the original painting. This additional knowledge provided by pigment identification contributes to a more comprehensive understanding of the provenance and history of the artefact, and that the brocade overpaint is not contemporary with the original painting.

2.3 Streeton catalogue

2.3.1 Introduction

In 1889 the Australian Impressionists, also known as the Heidelberg School, held an exhibition of paintings made in an impressionist style. The name *9 by 5 Impression Exhibition* refers to the fact that many of the paintings were painted on cigar box lids collected from tobacconists that are boards measuring approximately 9 x 5 inches. However just as many were painted on other boards and canvas. The exhibition attracted enormous interest and was quite controversial due to the style of the artworks. It was also the first exhibition of its type in Australia, with the artists responsible for planning and presenting the event themselves.

In preparation for an Australian Impressionists exhibition planned for early 2007 in the National Gallery of Victoria (NGV), a series of works were examined. Artefacts included an original catalogue from the renowned 1889 exhibition. The catalogue was printed by the artists, so the material composition and methods of preparation were of considerable interest. Characterisation of the pigments in the ink used to print the catalogue is reported here.

The image on the cover of the catalogue (Figure 21) is a photolithograph. Standard lithographic printing ink of the time would have been used and the image taken from a zinc plate. The text inside was printed using a letterpress. Type was handcast and individual characters were handset into lines. The ink is olive green in colour and since the artists in this exhibition designed the catalogue themselves, would have specified the colours to be used in the printing.

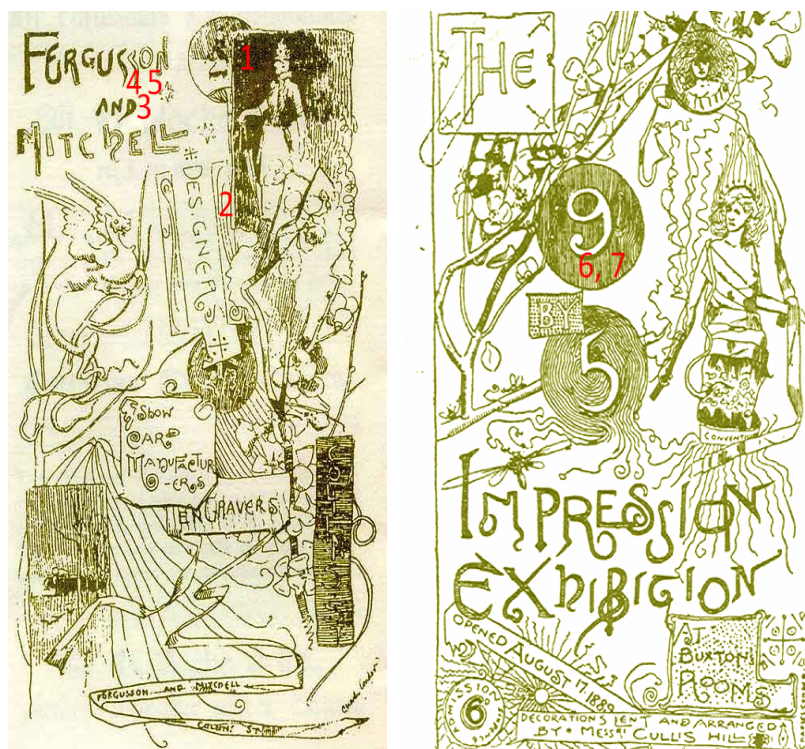


Figure 21. Catalogue with overlaid red numbers indicating the points examined on back cover (LHS) and front cover (RHS). Inside the catalogue is not shown.

Printing ink in the 19th century was mostly a simple formulation of painting pigments and oil binder, very similar in composition to an oil painting. The oil used for lithography printing ink was known as litho varnish, which is linseed oil heated to increase thickness by partial cross-linking. Sometimes resin was added to increase the thickness. Ink was prepared by grinding the pigment and oil together using a glass muller. Pigment was continually added until the ink became very stiff.

The cover is printed with a forest-olive green. A visual stereo microscopic examination of the olive-green ink in the catalogue revealed it is a mixture of a number of different coloured pigments, notably a blue and one or more yellow/orange pigments. A treatise on lithography produced at the time discussed the preparation of green inks and indicated the, likely pigment

composition to be lithography ink containing thickened linseed oil as the binding medium, possibly with pigments including Prussian blue (iron (III) hexa-cyanoferrate (II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{--}16\text{H}_2\text{O}$), yellow ochre/ goethite ($\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ + clay + silica) and vermilion (mercury(II) sulfide, HgS).

2.3.2 Methodology

The catalogue of the 9 by 5 Impression Exhibition 1889 is made by photo-lithograph and letterpress on hand-made paper. It measures 17.7 x 10.6 cm (closed). It was designed by Charles Conder (England, 1868–1909), who lived in Australia from 1884–90. It was printed by Fergusson & Mitchell, printers of Melbourne who were active from 1857– (1890s).

A preliminary investigation to establish the effect of examination on the catalogue was explored using samples provided by the NGV that were prepared in their laboratory by their painting conservator. Equivalent samples of ink were prepared according to the documented recipes for inks of the era, and painted onto filter paper and thin card, then examined to ensure no damage would occur using the instrument conditions normally encountered.

Examination of linseed oil bound pigments was unsuccessful using using a 514 nm laser, due to intense fluorescence, so 830 nm was used. Raman spectroscopy was performed using a Renishaw in-Via Raman spectrometer with 830 nm incident laser. Conditions: 1% intensity (1.5 mW), 1 second, 10 accumulations, 50× objective lens (short working distance). The entire catalogue could be placed on the microscope stage of the instrument and was examined in-situ as shown in Figure 22.



Figure 22. Streeton catalogue inside the Raman spectrometer

2.3.3 Results and discussion

A clear area of paper devoid of pigment was examined at point 5 and no Raman signal was detected. The absence of a spectrum from the paper suggests that any signal from coloured areas was entirely attributable to the ink. A summary of sample locations and spectra are presented in Table 2. The location of spots 1-7 are indicated in Figure 23. Spots 8-11 were collected from the inside of the catalogue and were not photographed.

Table 2. Sample locations and Raman spectra

Spot Number	Sample Location	Identified Pigment
1	Back Cover, Heavily pigmented area	Inconclusive
2	Back Cover, Middle of thin line, orange grain	Prussian Blue, Vermilion
3	Back Cover, Bright orange grain	Minium, Prussian Blue
4	Back Cover, Blue area	Inconclusive
5	Back Cover, White paper	Inconclusive
6	Front Cover, Base of "9" on image	Prussian Blue
7	Front cover, Homogenous green	Highly fluorescent, inconclusive
8	Inside, Reddish particle	Vermilion
9	Inside, Yellow particle	Vermilion and Prussian Blue
10	Inside, Dark green	Vermilion and Prussian Blue
11	Inside, Dark red grain	Highly fluorescent, suggests red lake

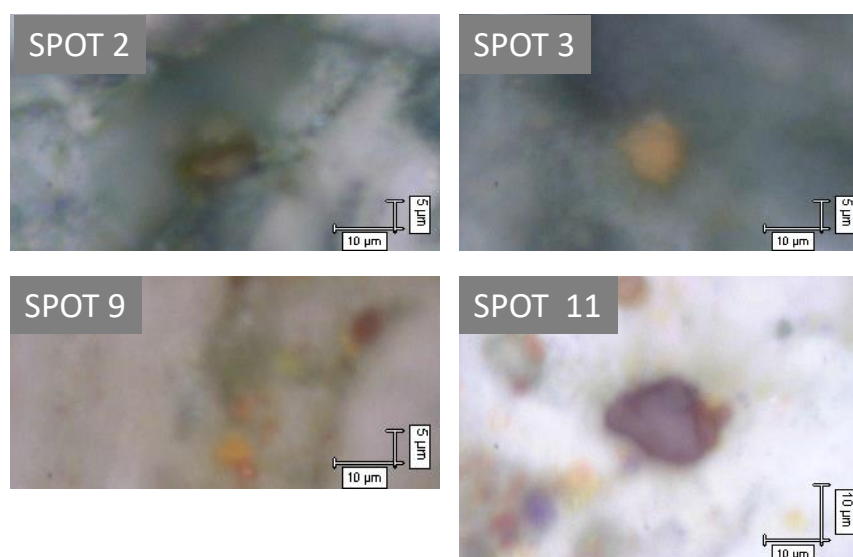


Figure 23. Examples of analysed pigment grains

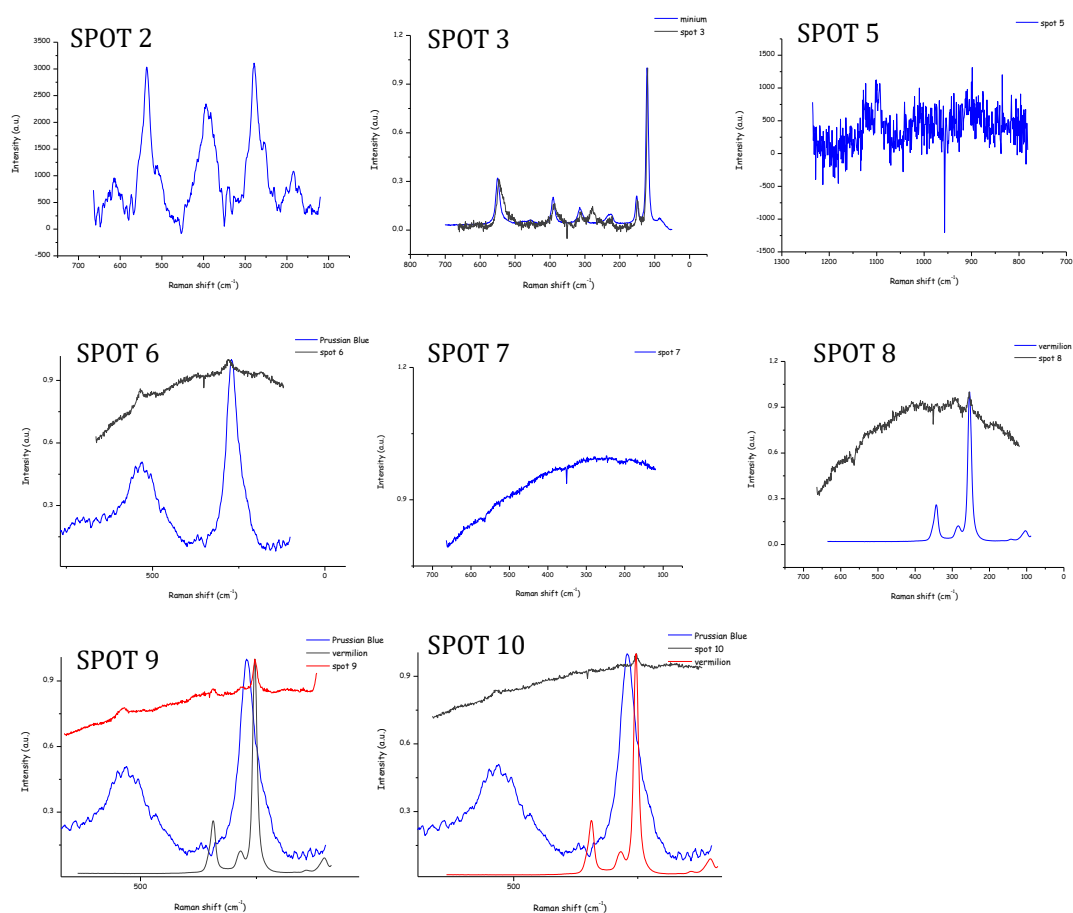


Figure 24. Raman spectra from locations in Table 2. X axis is Raman shift (cm^{-1}), Y axis is intensity (arbitrary units). 830 nm, 1.5 mW, 5 min.

Sample spectra are plotted here without processing to illustrate the quality of spectra collected and were compared with reference spectra from the NGV pigment database. Spectra collected in blue areas matched reference spectra of Prussian blue, with bands⁶ at 271 (s), 530 (m), 2084 (sh), and 2147 (m) cm^{-1} and was consistent in all blue areas, so most of the examination was directed at identification of the more sparsely distributed yellow/red/orange grains.

Vermilion was present in locations; 2, 8, 9 and 10, matching with reference spectrum bands at 343 (m), 284 (w), 253 (vs) and 104 (w) cm^{-1} .

Minium (dilead (II) lead (IV) oxide, Pb_3O_4) was detected only in location 3, matching with reference spectrum bands at 122 (vs), 151(m), 226 (m) 315 (m), 390(m), 456 (w), 476 (w) and 550 (s) cm^{-1} .

Spot 1 was inconclusive. Also problematic were the dark red pigment grains at positions 7 and 11 which were highly fluorescent, even at 830 nm. Resolvable Raman spectra could not be collected, as illustrated by the spectrum for spot 7 in Figure 24. The glassy red visual appearance of the grain at spot 11 coupled with the highly fluorescent response suggests a lake pigment.

This investigation indicated the use of Prussian blue and vermilion as the main pigments in the ink of the catalogue, with a minor addition of minium and perhaps a lake pigment. Richmond's recipes for the preparation of olive ink specifically dictate the use of blue mixed with orange, which is what is observed here. Richmond makes mention of the use of yellow and red to produce an orange colour; however, he specifically mentions the use of Chrome yellow or Yellow Lake and does not refer to vermilion, minium or red lake at all. No evidence of Chrome yellow or Yellow Lake was found. From previous work, it is known that the artists of the Australian Impressionist school used vermilion extensively and the incorporation of this pigment into the ink suggests their contribution to the formulation.

Identification of these specific pigments confirms that the recipes and documentation of the era were fundamentally adopted by this group of artists however they were modified by the addition of pigments used extensively in their own work to achieve specific colouration. It suggests this

⁶ Bands are described as: Strong (s), medium (m), weak (w) or shoulder (sh).

group of artists were as exacting with the visual appearance of the catalogue as they were with their paintings.

2.4 Shearing the Rams

2.4.1 Introduction

Shearing the Rams is an iconic Australian painting in the National Gallery of Victoria. Painted by Tom Roberts (1856 - 1869) around 1888–1890, it is painted with oil binder on canvas, later adhered to composition board, measuring 122.4 x 183.3 cm. Significant cleaning and conservation treatments were undertaken in 2006. X-radiograph images indicate important changes in the main composition made by the artist with a change in the posture and face of the central figure (Figure 25). He is understood to have been altered the central figure from a more upright and clean-shaven figure to one leaning further down with a bushy beard.

The X-radiograph of the painting also shows the central poles appearing very light, which is due to attenuation of the X-rays in transmission. It has been thought that the arrangement of light areas of the painting may have been different during the composition of the work, and the poles may have been painted a lighter colour, which would have been done using lead white. The attenuation in the X-radiograph due to heavy metals provides very little information about the elemental composition since the attenuation could be due to any number of heavy metal containing pigments, for example mercury, lead, antimony or tin. Understanding the pigment composition is important for understanding the painting technique.

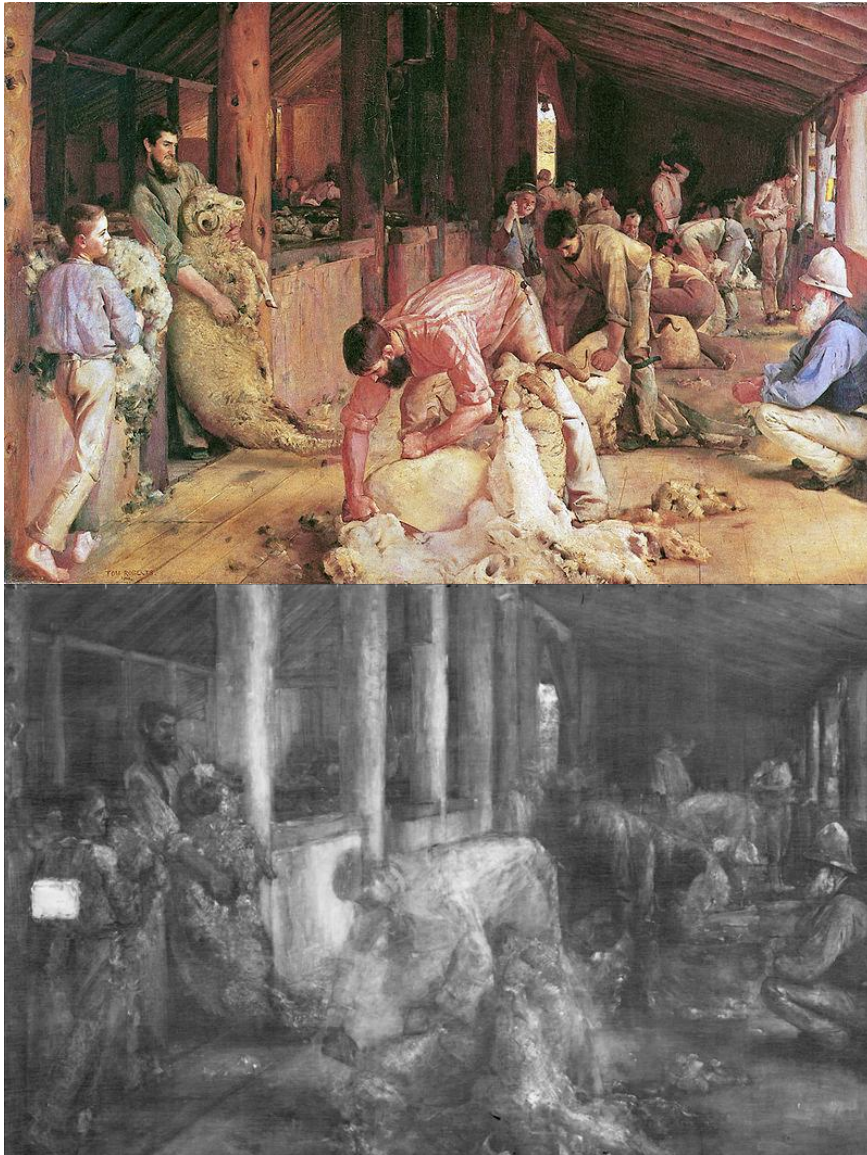


Figure 25. *Shearing the Rams*, National Gallery of Victoria, Melbourne (above) and X-radiograph (below)

The painting has significant cracks (up to a few mm in width) in the paint layer, especially around the centre of the painting in the poles above the central figure. The prominence of the cracks has led to the artwork previously being over-painted in that region, to reduce the visual impact of the distracting cracks.

The key questions were to understand the composition of the primary underlying paint layer and in particular the red pigment which was believed to be a factor contributing to the presence of the drying cracks and the subsequent motivation for repainting.

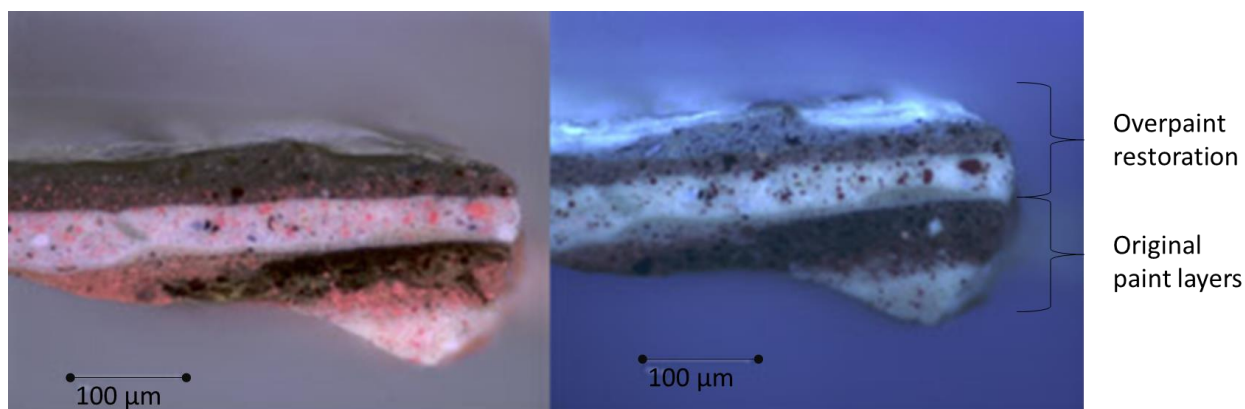


Figure 26. Cross section from region on central poles. Visible light illumination (left) and in UV illumination (right). Magnification 200 $\times$.

Figure 26 shows the cross section taken from the largest areas of over-paint on the central poles. This is a layer thought to be restoration (seen in the middle of cross section below varnish and above the brown original paint) and appears to have a fluorescence similar to resin.

2.4.2 Method

Cross section samples were taken and prepared by the paintings conservator. Small samples were excised through all layers using a microsurgery scalpel aided visually with a stereomicroscope. Paint samples were placed on the top of a half-filled cavity of already cured polyester resin in a cross-section mould, then the top half was filled with resin. Samples were ground to reveal the cross section with successively finer grade abrasive paper and cloth, finishing with 10,000 grit. Raman spectroscopy was performed using a Renishaw in-Via Raman spectrometer with 830 nm incident laser. Conditions: 1% intensity (1.5 mW), 10 second, 10 accumulations, 50 $\times$ objective lens (short working distance).

2.4.3 Results and discussion

Raman spectra were collected from individual red particles in the original brown paint layer. Coincident bands between 200 and 400 cm^{-1} indicate the red pigment is vermillion as shown in Figure 27, whilst the orange-red colour of the pigment grains observed in the microscope images also supports the identification.

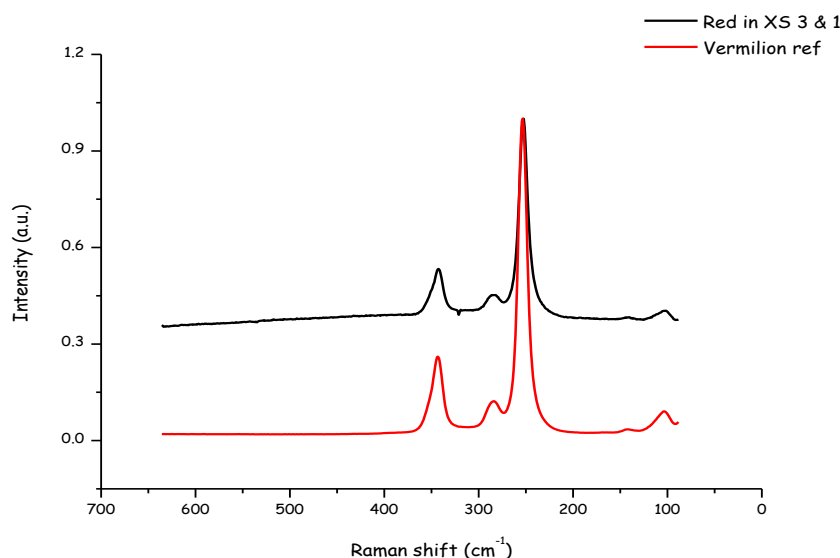


Figure 27. Raman spectrum of red pigment and reference spectrum of vermilion. 830 nm, 1.5 mW, 5 min.

The presence of vermilion (HgS) detected by Raman spectroscopy means the lightness observed in the central poles in the X-radiograph of the painting is, in considerable part, due to the high concentration of mercury in the paint layer and not lead, antimony or tin.

There is no evidence in the literature that vermilion promotes drying cracks, so the presence of drying cracks is not believed to be associated with the extensive use of vermilion to paint the central poles. The main contributing factor is reported to be the low ratio of binding medium to pigment, which is the most likely explanation for the cracks appearing in the central poles of this painting.

2.5 *The Finding of Moses*

2.5.1 *Introduction*

The Finding of Moses (Figure 28) is an oil on canvas painting in the National Gallery of Victoria that was, from its purchase in 1959 until 2010, attributed to Sebastiano Ricci and dated to the early-mid 18th century. Conservation work was performed to repair damages to the painting and research undertaken to better establish the painting's origins. For several decades scholars have proposed the painting is by Tiepolo, for example Antonio Morassi included the painting in his biography G. B. Tiepolo: His Life and Work, published in 1955.



Figure 28. *The Finding of Moses* (234.5 x 308.0 cm), National Gallery of Victoria, Melbourne

2.5.2 Method

Cross section samples as illustrated in Figure 29 were taken and prepared by the painting conservator, from the dark blue of lining of the page's jacket, just above the dog's hind quarters in the far-right hand side of the painting, showing the sequence of ground layer, paint layers and varnish.

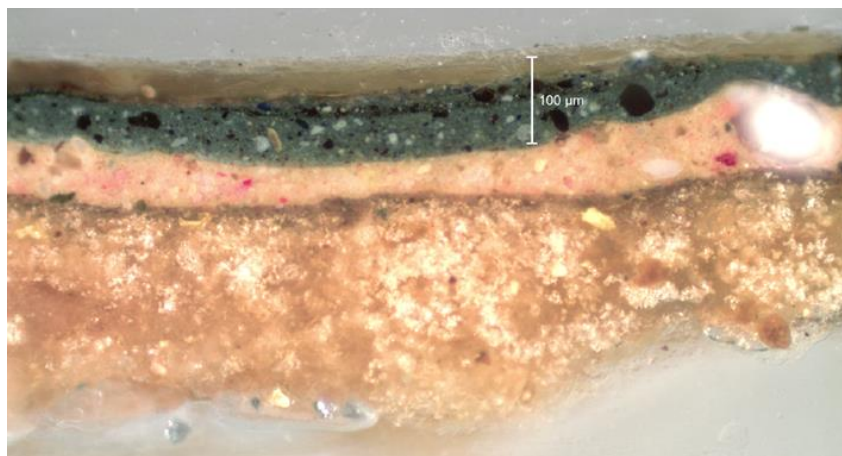


Figure 29. Cross section sample, dark blue of the lining of Page's jacket, just above the dog's hind quarters removed from *The Finding of Moses*. Magnification x 100

Small samples were excised through all layers using a microsurgery scalpel aided visually with a stereomicroscope. Paint samples were placed on the top of a half-filled cavity of already cured polyester resin in a cross-section mould, then the top half was filled with resin. Samples were ground to reveal the cross section with successively finer grade abrasive paper and cloth, finishing with 10,000 grit. A Renishaw in-Via Raman spectrometer was used with 830 nm incident diode laser. Conditions: 1% intensity (1.5 mW), 1 - 10 second scans, 3 accumulations, 50× objective lens (short working distance).

2.5.3 Results and discussion

It was quite difficult to see the pigment particles in this sample under the Raman microscope since they are illuminated coaxially and reflectance was problematic (especially the glaze/coating layers). It was possible to see the larger particles and navigate from them. As shown in Figure 30, the spectrum from the largest blue particle in dark blue paint layer with the main band at 2152 cm^{-1} corresponded with Prussian blue. The homogenous paint layer adjacent to the particle also contained Prussian blue.

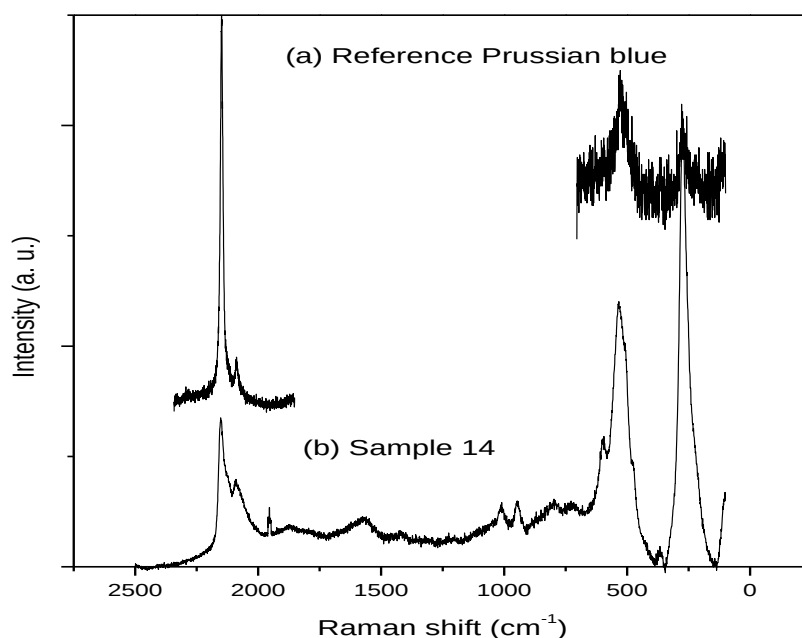


Figure 30. (a) Reference spectrum of Prussian blue and (b) spectrum from blue in painting cross section. 830 nm, 1.5 mW, 5 min.

The current attribution of the painting to Sebastiano Ricci rests on a dating to around 1712, when that painter was active in London. 1712 is too early for widespread use of Prussian blue as it does not come into wide usage in Venetian painting until the late 1720s or early 1730s. The presence of Prussian blue is important in the context of this painting because it is more consistent with a later dating or attribution.

Further to the attribution, the identification of Prussian blue in the painting will assist in the choice of in-painting pigments for retouching and, together with the stylistic interpretation of the techniques used by the painter, contributes to evidence for re-attribution of this work.

2.6 Indigo on fibre

2.6.1 Introduction

A palampore is an Indian hand painted decorative cloth, of cotton or linen fabric, such as may be used for a decorative wall hanging or bed cover. Palampores were painted using a kalam pen to apply mordant in the pattern or intricate design, and then dying in a bath - the colour only attached where the mordant was applied. Successive applications of colour and additional hand colouring of intricate detail made it a lengthy process and the decorated cloths were very valuable at the time of production. They are now very rare and even more valuable as many were made into garments, and their production was later replaced by printing technologies (57). The fragile palampore examined in this study (shown in Figure 31) has been stylistically dated to 1720 and is made of cotton.



Figure 31. *Coromandel coast Palampore*. 17th century. 321.5 x 244.0 cm. National Gallery of Victoria, Melbourne. Purchased 1947

The palampore fibres were identified microscopically as cotton by their flattened twisted ribbon morphology (Figure 34). Cotton fibres are cellulosic, comprised of linear polymer chains of about 1000 – 3000 glucose monomers, each with three hydroxyl groups making it an extremely hygroscopic material.

Colours used for palampores in general were natural dyes such as woad or natural indigo for blues. For reds and related shades, artists used madder. When used with alum as a mordant it gave pink and red, and with iron mordants produced violet shades (58).

Indigo is insoluble in water but dying with indigo is carried out in aqueous solution. Indigo must first be converted to the reduced form (clear – yellow), leucoindigo (Figure 32), before it can solubilise in water making it available for attachment to cellulose fibres. The dye is absorbed into the fibres and on exposure to air, O_2 oxidises the colourless leucoindigo back to blue coloured water insoluble indigo. In a vat dying process the cloth must be removed from the dye bath and the excess solution squeezed out for this to occur (59).

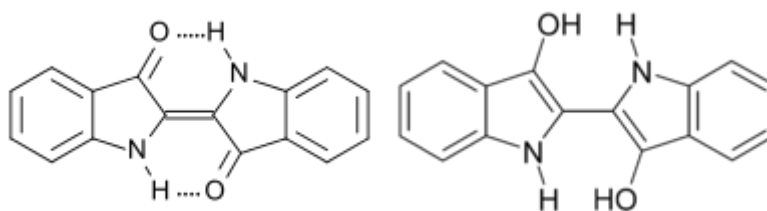


Figure 32. Chemical structure of indigo (left) and leucoindigo (right)

2.6.2 Method

Small samples of thread collected adjacent to existing damaged areas in the fabric are shown in Figure 33.



Figure 33. Stereo microscope image of the fibres from the palampore. Magnification 10x

Sample Colours:

1. Red
2. Tan
3. Brown (expected to be similar to sample 2)
4. Blue
5. Dark blue – from an area of mid and dark blues.

Samples were supported on an aluminium sheet for analysis. A Renishaw in-Via Raman spectrometer was used with 830 nm incident diode laser. Conditions: 1% intensity (1.5 mW), 1 - 10 second scans, 3 accumulations, 50× objective lens (short working distance).

For SEM-EDS examination, an FEI Quanta 400 field emission environmental scanning electron microscope (ESEM), operating under high vacuum conditions, was used to examine fibres mounted on standard 25 mm SEM stubs using electrically conducting, double-sided, adhesive carbon tape. Both secondary electron and backscattered electron imaging were performed with beam energy of 15 kV and a probe current of ~300 pA.

2.6.3 Results and discussion

The red fibres were too fluorescent to enable a Raman spectrum to be collected and the spectra from the tan and brown fibres did not exhibit any bands other than those from the cellulose of the fibre at xxx cm⁻¹.

SEM-EDS of the red and brown fibres showed they were most likely organic in composition, being comprised mainly of C and O. It did not indicate the identity of the mordant – even if one was used in these coloured areas. SEM-EDS of the blue fibres is shown in Figure 32 and suggests they were also mainly organic in composition, with the predominant signal being C and O. There was only a low signal from inorganic components, and they were not typical of an inorganic blue colourant.

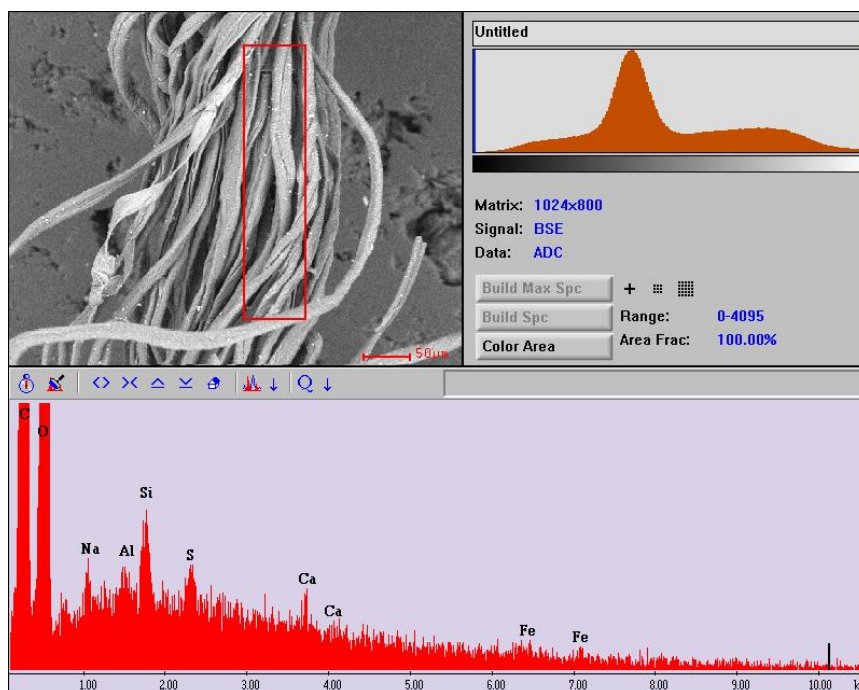


Figure 34. SEM-EDS spectrum of the blue fibres in sample 5

The blue fibres (Figure 35) provided suitable Raman spectra. They were quite fluorescent but satisfactory signal to noise was observed above the fluorescent baseline. A comparison with the reference spectra for indigo collected in the Raman reference library (Appendix 1), did not produce a satisfactory identification.

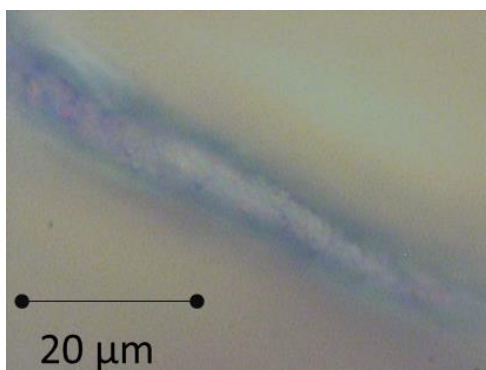


Figure 35. Blue fibre from sample 5 viewed under Raman microscope. Visible light, 500x

There are many published instances of indigo being identified in artworks and textiles, with some variation in the relative intensities and positions of bands. The reference spectra for a natural and synthetic indigo are presented in Appendix 1 and their spectra are very similar to each other. However, the spectra for the dry pigment do not correspond with Raman spectra from the fibres of the palampore.

A comparison of the spectral bands in the fibre and the indigo reference samples are shown in Table 3. Assignment is based of bands measured from indigo powder measured in a capillary (60).

Table 3. Raman bands observed in the palampore fibre sample compared to reference indigo

Palampore	NGV Reference pigments #155 & #181 (cm ⁻¹)	Indigo powder in capillary @1064 nm (cm ⁻¹) (60)	Assignment (60)
	1692	1701	ν C=O, ν C=C
		1625	ν C=C
		1582	ν C=O, ν C=C
	1561	1571	ν C—C
		1482	ν C—C
	1458	1460	ν C—C
	1434	1365	δ N—H
	1331	1310	ν C—C
	1273	1248	δ C—H
	1212	1224	δ C—H
		1190	ν C—C
		1121	
	1103	1147	δ C—H
	1079	1015	δ C—H
		921	ν C—C
		868	
	720	758	γ C—H
		674	δ C—C
		598	δ C=O
	563	544	wx
	511	310	wx
		275	wx
		286	wx

Symbols for vibrations ν , stretching; δ , deformation; β , bending; ζ , rocking; γ , wagging; τ , twisting; σ , scissoring. wx = Vibration of the five-membered ring.

There are two possible effects that may lead to differences between indigo spectra collected from the different samples. Firstly, change in the planarity of the indigo molecule occurs when it is fixed to a substrate such as a fibre (61). The molecular stereochemistry (and thus vibrational modes) are different in a fibre-bonded molecule of indigo compared to a sample of dry pigment powder which would be expected to result in a modified spectral response.

In addition, some resonance modes could be activated due to the modified stereochemistry and may cause the relative intensities of bands to change with the excitation line (62). A comparison of the main observed bands and their dependence on the excitation line is shown in Table 4.

The combined effect of these factors offers an explanation as to why there are observable differences between the reference samples of pigment indigo, the blue fibre sample and some published spectra.

Table 4. Reported main Raman spectral bands for indigo

Occurrence	Year	Excitation line (nm)	Reported main bands (cm ⁻¹)
Illustrated manuscript (51)	2009	632.8, 532	550, 604, 1585
16th century Portuguese Ferreirim Masters oil paintings (63)	2009	785.2	545, 1584
Modern pigments on purportedly medieval miniatures (53)	2009	632.8	252, 545, 598
Thai manuscripts (64)	2008	532	253, 546, 1575
16th- to 18th-century Islamic manuscripts (65)	2008	632.8	546, 599, 1575
Ancient Chinese artefacts (66)	2007	785	252, 545, 1574
Studies of synthetic Maya pigments (67)	2007	1064	544, 1310, 1365, 1582

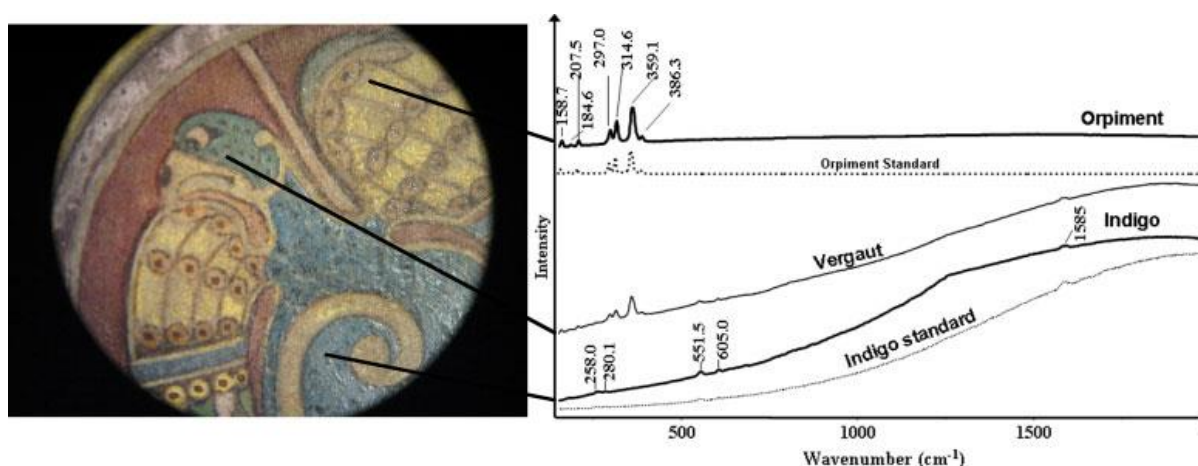


Figure 36. Raman spectrum of indigo from the Book of Kells. Reproduced from (51)

An illustrative example (Figure 36) of a published spectrum is the indigo from The Book of Kells (51) with bands at 1585, 605, 551, 280 and 259 cm⁻¹.

There is, however, consistency observed between the spectra of indigo when the same sample form is measured with the same wavelength. The indigo powder reference spectra from the NGV

pigment collection (Appendix 1) demonstrate an excellent match with other published spectra for dry powder indigo, both natural and synthetic as shown in Figure 37.

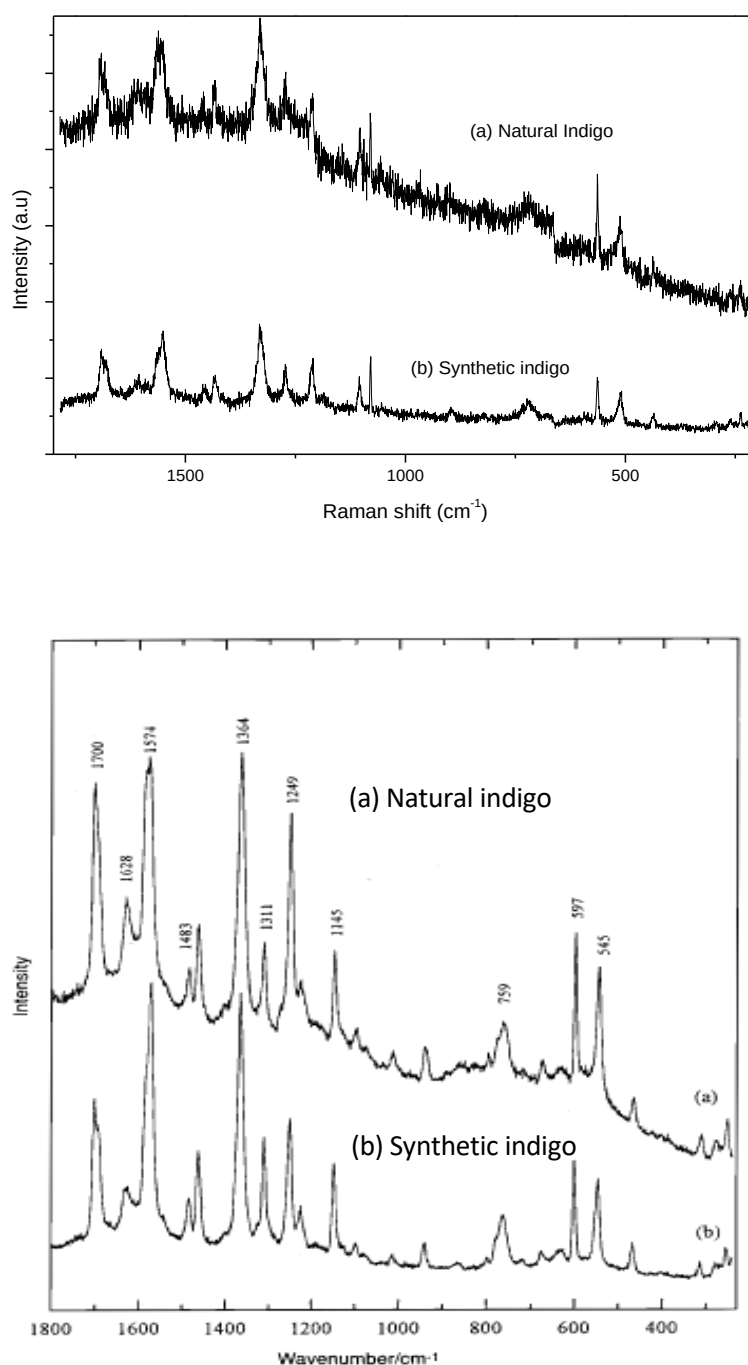


Figure 37. Indigo powder reference spectra. Above: from NGV pigment collection. Below: compared with Raman spectra of (a) a natural indigo powder and (b) a synthetic indigo powder (below) from (61). All collected with 514.5 nm excitation

Spectra from several spots in the palampore blue fibre were collected. Examples are Samples 4 and 5 shown in Figure 38.

The identity of the blue colourant in the palampore was able to be confirmed by comparison with a published spectrum of indigo on fibre. Cheng *et al* (66) examined a sample from the silk fabric collections of the Qing dynasty (1644 – 1911B.C.) For their analysis, the blue dye was not extracted from the silk textile, but the dyed textile was examined directly under the microscope with the 785 nm excitation line. They also examined modern silk fabric, which was dyed with traditional indigo dyeing technique as a reference. Figure 38 shows the Raman spectrum. Cheng *et al* observed bands at 173, 252, 264, 544, 598, 674, 755, 1222, 1310, 1358, 1460 and 1572 cm^{-1} , in good agreement with the positions and relative intensities of the bands from the palampore samples.

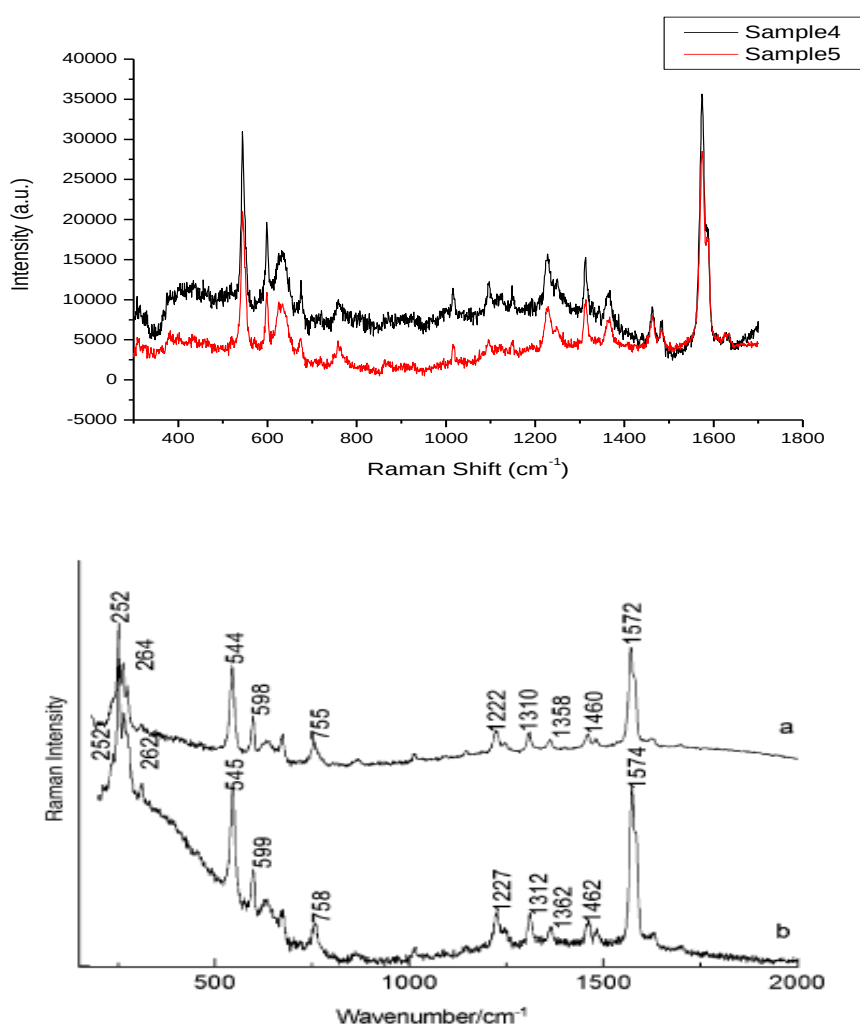


Figure 38. Above: Indigo spectra from 2 sample spots in the palampore fibre. Below: published spectra of (a) indigo adsorbed on Qing dynasty silk textile and (b) indigo adsorbed onto modern silk. Reproduced from (66)

In summary, an elemental analysis of the blue fibres in the palampore using SEM-EDS showed an absence of an inorganic colourant, thereby suggesting an organic colourant was used. Raman spectra did not directly match with the reference library spectra for powder indigo but were an excellent match in position and relative intensity with published reference spectra for indigo adsorbed onto textile fibres. The importance of local bonding structure and molecular structure interaction is highlighted as an important consideration for the identification of pigments that are not in a particulate form.

2.7 Conclusions

From a theoretical perspective, the mitigation of fluorescence by using longer wavelength incident lines is a relatively straightforward approach. In practical terms, and when using Raman spectroscopy to analyse artworks, challenges arise from numerous issues.

Taking complete artworks from a gallery collection to a laboratory for analysis requires significant administrative work often involving working with the gallery registration department, insurance and conservator's time to accompany the artwork. Each of the instruments accessed in this study had only one or two laser lines available, and at the time of analysis it was unusual for Raman spectrometers to have multiple lines available,

830 nm is effective at reducing fluorescence whilst promoting sufficient Raman scattering intensity to allow spectral identification in most cases, but in situations where fluorescence is not an issue, 514.5 nm would be preferred to maximise Raman scattering efficiency, reduce collection times, minimise absorption and thus reduce the risk of thermal damage. From these studies, the most successful outcomes for the examination of fluorescent samples were obtained using an 830 nm incident laser irradiation so the work here demonstrates the most effective and practical approach is to plan to utilise a longer wavelength if available.

The five case studies presented here demonstrate the importance of pigment identification for assistance with attribution, provenance studies and to aid in decisions regarding conservation treatments.

1. In the portrait miniature “*Portrait of a Lady Wearing Elizabethan Costume*”, the identification of Zinc white pigment dated the overpaint to being applied after the mid-19th century, much later than the original painting dated from the 16th century.
2. The inks in Streeton’s catalogue, printed by artists in the Heidelberg School, use of Prussian blue and vermilion as the main pigments in the ink of the catalogue, with a minor addition of minium and perhaps a lake pigment. Identification of these specific pigments confirms that ink recipes documented from the era were used as a foundation, but the recipes were augmented by pigments used extensively by the artists in their paintings.
3. Overpainted changes on Tom Roberts’ iconic painting “*Shearing the Rams*” occur predominantly in the centre of the painting, around the central poles. It was thought that the cracks may have been due to the type of pigment used. The main pigment was found to be vermilion which is not known to cause cracking, so the cause is now believed to be due to the high ratio of binding medium to pigment used in the paint preparation.
4. *The Finding of Moses* was purchased by the National Gallery of Victoria in 1959 and originally attributed to Sebastiano Ricci, dating to around 1712 when that painter was active in London. Prussian blue was identified as a key component of the paint layer (in the lower right region of the painting) but since it does not come into wide usage in Venetian painting until the late 1720s or early 1730s, it substantiates the stylistic attribution of this painting to 18th-century master Italian painter Giambattista Tiepolo (1696-1770). Additionally, the identification of the pigment assisted in the choice of in-painting pigments for retouching.
5. The colours used to decorate the cotton fibres of an Indian Palampore could not be identified using SEM-EDS as they were predominantly organic. Although the Raman spectrum of the blue fibres did not correspond with indigo pigment in particulate form, it did match with a published spectrum of indigo on silk highlighting the importance of local structure and bonding on the Raman spectral character.

As with many samples encountered from artworks and artefacts, fluorescence is the major obstacle to non-destructive or in-situ analysis using Raman spectroscopy. Here, a longer excitation line of 830 nm has been shown to overcome fluorescence, and analysis could be

performed on sample types that, at the time, were not being widely examined or reported in the literature; cross section samples taken from oil paintings.

Utilisation of longer wavelengths continues to be successful and is utilised in more recent work published in 2019. Artesani *et al* (68) used Raman to analyse pigments in micro-samples from Russian Avant-garde modern paintings successfully using 785 nm. In other research, pigments in cross sections from Byzantine icons, dated from the 14th to 19th centuries, were analysed using 785 nm (69). In the examination of a Yugoslavian 20th century painting, strong fluorescence was observed with both 532 nm and 632 nm lasers but was minimal for the longer wavelength red excitation (70). The paucity of more recently published Raman spectroscopy research utilising shorter wavelength incident irradiation to examine painting materials confirms longer wavelength selection is a critical factor in the success of conservation material and pigment identification.

Chapter 3: Analysis of resins with SERS

3.1 Introduction

A less well recognised method for overcoming fluorescence in artwork samples analysed using Raman spectroscopy is Surface Enhanced Raman Spectroscopy (SERS). With the adsorption of the analyte onto metal nanoparticles or metal surfaces with atomic scale to nano-meter scale roughness, significant enhancement of Raman scattering can be achieved. SERS techniques have been used to study many chemical species through enhancing the Raman response by many orders of magnitude over the fluorescence.

The phenomenon was first reported in the 70s and rapidly developed in the 80's when sub mono-layer detection was reported, and recently reports of enhancement factors of 10^{16} are quoted (71) with single molecule detection (72). SERS enhancement is generally recognised as improving the Raman cross section by several orders of magnitude. This has enormous potential for the development of experimental methods for the Raman identification of:

1. Trace levels of materials;
2. Analytes that are poor Raman scatterers or
3. Materials which exhibit fluorescence when irradiated in the visible region.

Fluorescence is generally quenched when a molecule is in direct contact with a metal surface due to energy transfer to the metal (73), although the size and morphology of the metal has the potential to either increase or decrease fluorescence. Additionally, the suppression or enhancement of fluorescence is affected by orientation of the fluorophore's molecular dipolar moment, and whether the fluorophore emission spectrum coincides with the plasmon resonance spectrum of the SERS substrate (74).

Conducting or semi-conducting materials with dimensions near or below the wavelengths of light exhibit unique optical properties and have been the subject of much research interest over the last few decades. They can be utilised in many ways: optical filters, waveguides, optical switches biosensors, optical trapping (tweezing), optical bio-labels, "spasers" (surface plasmon

amplification by stimulated emission of radiation) and much attention is directed to their use as SERS substrates (75).

The unique optical properties of metallic nanoparticles lead to an exciting array of colours. Long before the advent of any scientific understanding of their physico-chemical properties, they were exploited in the manufacture of artistic and decorative objects. Colloidal gold has been used as the colourant in red or ruby glass and used in stained glass and decorative glassware. Silver and copper nanoparticles have been found in lustre glazes of medieval and renaissance pottery (76). The famous Lycurgus cup dating from the 4th century in Rome, now housed in the British Museum exhibits the remarkable properties of appearing red when viewed in transmitted light and green when viewed in reflected light (Figure 39). It has been shown to contain 70nm nanoparticles of an Au/Ag alloy with approximately 70/30 volume fraction (77).



Figure 39. *Lycurgus cup* in transmitted and reflected light (images from the British Museum)

Sampling a varnish or resin coating from paintings is of less concern than removing irreplaceable paint samples because the removal and reapplication of painting varnishes is a frequent treatment. Varnish coatings are not usually considered integral to the artwork and are often removed and reapplied many times over the lifetime of a painting. Sampling methodology is easily performed by wiping a solvent dipped cotton swab over the surface of a painting to dissolve a small amount onto the swab which can then be extracted into a small volume of solvent.

The case study presented in this chapter was originally published in 2008 and at the time, it was the first research to report the use of SERS for organic materials in conservation analysis. SERS has not been used to examine resins or binding media but has been predominantly applied to the analysis of red lake pigments such as alizarin, lac and carmine (78-82). The analysis of amino acids and small peptides with SERS indicates it is of use for examining archaeological materials and residues (83).

This chapter explores how metal nanoparticles and nano-structured surfaces can mitigate fluorescent materials encountered in artworks using SERS. This chapter presents an introduction to SERS, a literature review of current techniques for producing SERS surfaces and particles; then describes the experimental production and evaluation of SERS substrates and finally the preferred substrate is used in the analysis and identification of resins.

3.2 The SERS enhancement phenomenon

The efficiency of SERS enhancement relies on the coincidence of the plasmon resonance frequency maxima of the metal and the frequency of the laser line used for Raman interrogation. The size, shape, surface topography composition, layering and arrangement of the metal nanoparticles or metal surface features all influence SERS enhancement and the materials used in production must also be compatible with the characterisation of resins in organic solvents.

The behaviour of these systems is a result of a resonance condition of the conduction electrons of the metal, known as surface plasmon resonance. In a bulk metal, density waves of conduction electrons oscillate in response to the oscillating electric field of irradiating electromagnetic radiation. The surface plasmon resonance is a collective oscillation of electrons near the surface of the metal and is different from the ordinary plasma frequency. Because of their surface area to volume ratio and disconnection from the dampening effect of the bulk, small metallic particles exhibit surface plasmon resonance, shown in a schematic by Peiris (84) in Figure 40, a phenomenon which is also observed in nano-structured or roughened metal surfaces.

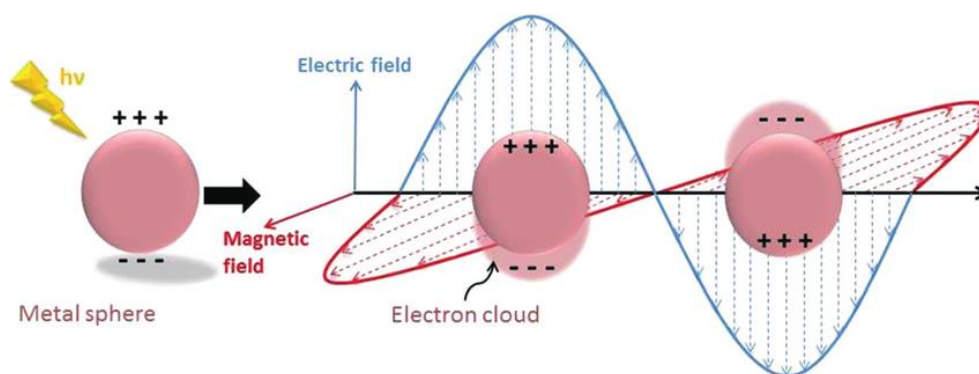


Figure 40. Dipolar plasmon resonance of small metallic particles induced by incident electromagnetic wave. Reproduced from Peiris, 2015.

Plasmon resonance in the visible spectrum is mostly observed in metallic particles or surfaces of Au, Ag and Cu (the coinage metals) although many other metals and semi-conductors have been investigated for their plasmon resonance effects.

The first observation of the SERS effect was in 1974 with pyridine molecules adsorbed onto an electrochemically roughened silver electrode (85) The dramatic increase in Raman signal was initially attributed to an increased surface area provided by roughening but later found to be a true surface enhancement process. Since then, many fundamental studies have been performed to understand the mechanism of enhancement, but as yet the understanding of this mechanism is incomplete.

Some understanding of the SERS process is provided by considering the classical theory of light scattering. Upon irradiating a small metallic particle, an incident light field may induce an oscillation dipole, μ . Light is re-emitted (scattered) with the frequency of the dipole oscillation. The dipole moment is comprised of multiple harmonic frequency components, each of which may be described by:

$$\mu(t) = \mu^0 \cos(2\pi\nu t)$$

where ν is the dipole oscillation frequency (scattering frequency) and μ^0 is the maximum induced dipole moment for a given frequency component of μ . If the incident electric field (E_{inc}) is not too large the induced dipole moment can be simplified to

$$\mu(t) = P \cdot E_{inc}(t)$$

with P being the polarisability of the molecule (78). Thereby the Raman intensity, which is proportional to the square of the induced dipole μ , can be influenced by E_{inc} , the electric field, or P , the molecular polarisability. These are generally considered as two contributing aspects: the “electromagnetic effect” and the “chemical effect” and several models have been derived to explain each.

3.2.1 *Electromagnetic models*

Electromagnetic models rely on understanding the exact geometry of the system being modelled. As will be described later in this chapter, there are many geometric configurations which produce SERS enhancement successfully and preparation of surfaces produces a great deal of uncontrolled variety (through slight variations in experimental conditions) resulting in unpredictable surface or particle morphology.

Most theoretical models predict the properties approximated for discrete particles (the Rayleigh approximation) however practical experience has shown that most systems are random or fractal arrangements (clusters) of nanoparticles, or regularly patterned nano-structured surfaces. For these arrangements the electrostatic approximation does not hold, and the Lorentz-Mie or electrodynamic formalism is required. Other models exist for concentric spheres, prolate spheroids and ellipsoids. The discrete dipole approximation has been applied to random structures such as clumps of colloid material. These often display “hot spots”, areas of extremely intense Raman enhancement, due to an advantageous constructive influence of local electromagnetic field effects.

Electromagnetic effects include the concentration of electromagnetic field lines at points of high curvature (8) and the excitation of surface plasmons, oscillations of conduction band electrons, in the metal corresponding with the irradiating frequency. The metal structure and surface topography of the metal surface influence the surface plasmon resonance frequency. The plasmon resonance produces the enhancement of the Raman shifted light resulting in an amplification of the Raman response. Electromagnetic effects are distance dependent and

decrease further from the metal surface. The enhancement field strength for a molecule on a metal sphere is approximately $(a/r)^{10}$ where a is the metal sphere radius and r is the molecule-sphere centre distance. SERS enhancement has been reported at up to 20 nm from the metal surface (86).

3.2.2 Chemical models

Chemical enhancement is the response of the analyte to being in close proximity to the metal surface. Adsorption on the metal surface can induce charge transfer in the analyte, producing an effect analogous to resonance Raman scattering. Pelletier ascribes chemical enhancement being due to the overlap of metal and adsorbate wave-functions (8). The adsorption induced charge redistribution transfers excitation to the adsorbed molecule. In all explanations, the chemisorption of the analyte to the metal induces a change in the electron distribution around the molecule (either through direct charge transfer or molecular stereochemical alteration) resulting in a resonance Raman response due to alteration of molecular polarisability.

It may seem counterintuitive that Raman scattering intensity is increased above photoluminescence, however when fluorescent analyte molecules are adsorbed onto a metallic surface the fluorescence is quenched by transfer of energy from the excited state to the metal. The coupling of surface plasmon modes of the metal to the near field of the emitting molecule and are distance dependent, so molecular adsorption onto a metal will decrease the lifetime of fluorescence to zero (87).

3.3 Review of current SERS technologies for substrate production

SERS relies on the plasmon resonance of the metal, which is governed by the nano-morphological features of the surface and inter-particle proximities. So, in effect, the SERS efficiency is ultimately reliant on the morphological structure of the substrate. Success in producing an effective surface depends on an ability to design those features into the material being used. Experimentally there are many ways to prepare SERS-active substrates and new methods are being developed continually, falling into two main categories: nanoparticles and nano-structured films or surfaces. Some of these are generalised and represented in Figure 41. This section is a review of current methodologies for substrate production, enabling selection of

the most suitable method (with respect to material selection and ease of production) for the analysis of natural resins used as painting varnishes.

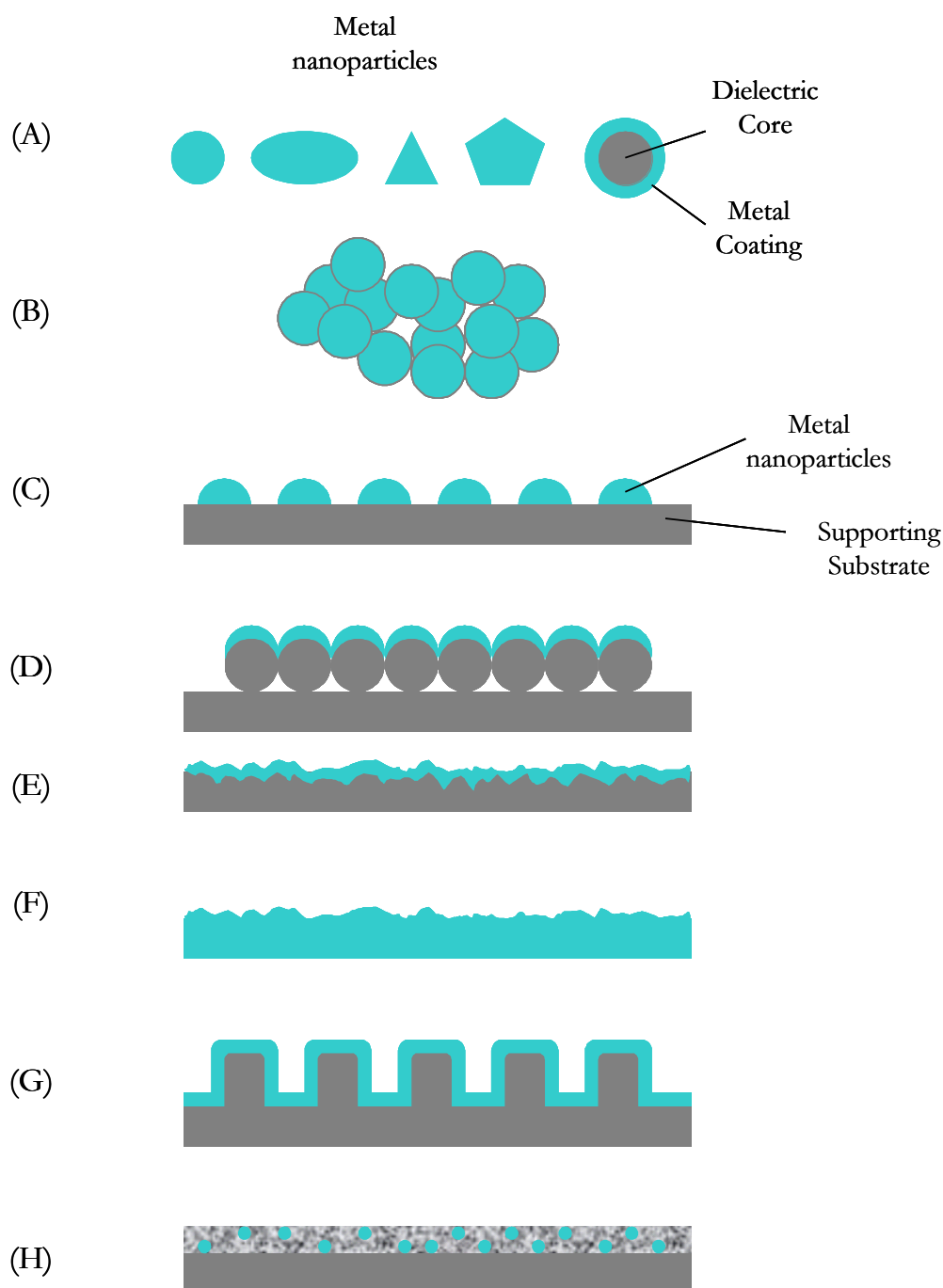


Figure 41. Representations of a range of SERS active supports. (A) Isolated metal nanoparticles and metal coated nano-spheres (B) Cluster of metal nanoparticles (C) Metal nanoparticles on supporting substrate (D) Metal coating over dielectric nanospheres (E) Metal coating over roughened surface (F) Electrochemically roughened metal surface (G) Metal coating over regularly structured surface (H) Film with embedded nanoparticles

3.3.1 Colloids and particles

Individual metallic nanoparticles are produced as colloids and the most common colloidal preparation is reduction of metal ions in solution with a reducing agent such as sodium citrate (88), sodium borohydride (89), sodium formaldehydesulfoxylate (SFS) (90) hydrogen peroxide (91) or ascorbic acid (92). Controlling the conditions of formation (rate of reduction, temperature, additional ionic species, pH etc) greatly influences particle shape and size distribution as do post-treatments such as annealing or photo-exposure. Silver colloidal hydrosols are most often used, followed by gold.

The spectrum of plasmon resonance is dependent on the geometry of the particle and the illuminating wavelength. Mock *et al* (93) showed that specific geometrical shapes give distinct spectral responses. In that study triangular particles appeared mostly red, pentagon shaped particles green and spherical particles blue when viewed under a conventional dark-field microscope with magnification 1000 x. Particle sizes were in the range 40 to 120nm. The scattered light spectral maxima could be blue shifted by heating triangular particles; as the corners became more rounded with heating, scattered light maximum intensity moved from red through orange to yellow/green.

The production and use of colloidal solutions have the advantage of being relatively easily prepared and characterised by their absorption spectrum. Particles produced in this manner will always exhibit some degree of diversity with respect to size and shape so if particle uniformity is necessary, then the process requires size fractionation using centrifugation or nanofiltration (94).

The use of water-in-oil (w/o) microemulsions is an elegant way of controlling the volume of metal salt solution that is reduced, with micelles limiting the dimensions of the metal nanoparticles and improving monodispersity. Preparation of iron, platinum, cadmium, palladium, silver, copper, nickel and gold nanoparticles are influenced by temperature, light, nature of the metal salt, surfactants and reaction conditions as found in simpler colloid reduction methodologies (95). Simple ultrasonic treatment of metal ion solutions have resulted in reduction and this has been used in conjunction with electrolysis to produce gold nanorods (96).

3.3.1.1 Metal-coated nanoparticles

Metal nanoshell structures have been used extensively to investigate correlation of predicted plasmon resonance against experimental observation. By controlling the diameter of the core and

the thickness of the metal shell the plasmon resonance can be “tuned” from the near UV to near IR (91, 97). Changes in the refractive index of the core or surrounding matrix influence the position of the surface plasmon extinction maximum (98).

Preparation of silver coated latex spheres in solution is described by Mayer et al (99) who use a range of chemical reductants to coat latex particles with silver. Deposition of silver from solution onto silica spheres using ultrasound reduction has been demonstrated (100), vacuum deposition of silver onto silica nanospheres (101), laser induced photoreduction at 514.5nm was used to coat silica nano-particles with silver (102) and Ag can be incorporated into silica microsphere outer shells simultaneously as silica colloid is prepared with the Stöber process (103).

3.3.1.2 *Clusters*

The formation of aggregates by way of disordered metallic nanoclusters has some distinct analytical advantages for SERS. The discrete dipole approximation has been used to demonstrate sharp plasmon resonances occurring in localized regions of the cluster (also called “hot spots”) at frequencies different from the plasmon resonance of the metal (104). Due to random constructive interferences they provide remarkable enhancement at those points in the cluster. The occurrence of hot spots is random and unpredictable, and it has not been established how much they contribute to the overall observed enhancement produced by a given metallic surface area.

3.3.2 *Surfaces*

3.3.2.1 *Metal islands on a dielectric support*

One of the simplest methods of substrate production is to vapour deposit silver (or other metal) directly onto glass or a similar substrate. On a smooth surface such as glass, deposition builds by addition of metal atoms around nucleation points on the surface and individual islands develop. Prolonged deposition of more than about 10 – 40 nm leads to coalescing of the islands and a continuous surface with very little surface definition, so layers below 40 nm are necessary to retain the correct surface roughness. SERS activity has been demonstrated with this simple technique by evaporating a thin 4 nm film of Ag islands onto a glass microscope slide (105).

3.3.2.2 *Metal coatings over nanostructured substrates*

Silver deposition may also be over a textured surface such as an oxide layer, a patterned substrate or an array of glass or polymer nanospheres. Substrates include filter paper (106), cellulosic and

polymer filter membranes, zeolite crystal films (107), glass and quartz. A suspension of polymer (108) or diamond (109) nanoparticles may be applied over a surface, then metal vapour deposited over the nano-particles, generally to a thickness of 50 – 100 nm. Oxidised aluminium foils which displayed nanometer scale roughness have been sputter coated with gold to produce SERS active films (110).

Electron-beam lithography provides control over the dimensions of regularly patterned features and fabrication can be performed with a lift-off technique or plasma etching (111). Control over regularly patterned surface characteristics is useful for modelling studies (112) as the plasmon resonance of thin films is dominated by the structure or patterning of the surface, surface/interface imperfections and the material (113).

Work by Jensen *et al* (114) utilised nanosphere lithography techniques. Silver was vapour deposited over nanosphere 2D arrays, which were then removed to reveal triangular arrays of silver nanoparticles in the spaces unprotected by the spheres. High aspect ratio nanopillar structures may be prepared by etching polysilicon substrate then coating with a thin layer of Ag (115). In a similar experiment, etched nanopillars coated with gold showed the density of nanopillars correlated with the intensity of Raman enhancement (116).

3.3.2.3 *Etched metal surfaces*

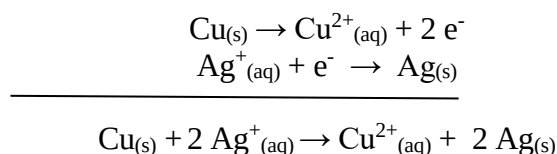
Roughness may be induced on Ag surfaces by acid etching (117-119). Reported advantages are the reversibility of the surface, ie: the ability to desorb the analyte from the surface with washing. Additional stability of the analyte/Ag-foil complex under prolonged irradiation was noted and attributed thermal transfer away from the analyte, not achievable with Ag colloid on filter paper or nanoparticles supported on non-conducting substrates.

Roughening of smooth Ag films has produced SERS active substrates through plasma oxidation-reduction cycles (120). Laser ablation of pure gold and silver in water produced colloids and roughened metal substrates that were SERS active (121).

3.3.2.4 *Electroless deposition*

Using the technique for electroless metal plating (122), it is possible to deposit Ag^+ from solution onto a metal that is preferentially oxidised. Copper is a convenient base for silver deposition as it is readily available in many forms and relatively inexpensive. Deposition from aqueous

solution onto printable circuit board (PCB) was performed recently by Nie (123) by means of the galvanic displacement of copper metal with silver from a solution of silver nitrate:



Standard Redox Potentials for silver and copper are:



Since a species with a low standard reduction potential tends to reduce a species with a high standard reduction potential, the deposition of Ag from solution is spontaneous on a metallic copper surface.

Silver has also been deposited onto the surface of small particles, fine copper powder (124) and colloidal silica spheres (125).

Electrolessly produced surfaces are randomly rough (self-affine fractal) and have some advantages over regularly roughened surfaces or monodisperse nanoparticles. Surface plasmon modes span a broad range of wavelengths, while periodic clusters or surfaces have a relatively narrow band of plasmon resonance activity so these random surfaces are expected to be useful for a wide range of incident wavelengths. Given that the resonance condition for periodic structures is narrow and specifically restricted by geometry which must be designed into the metal intentionally (or occur by chance through irregularities) fractals provide many more opportunities or the observation of the plasmon resonance condition essential for SERS.

In a disordered arrangement of particles, areas known as “hot-spots” can occur but understanding the influences that create these areas is still in its infancy. For clusters of small particles, “hot spots” occur in few localised regions with respect to the overall volume of the cluster and should occur within this random surface structure which appears structurally similar to clusters through having adjacent randomly distributed nanoparticles.

The development of a non-periodic metallic nano-structured material can take advantage of (a) the probability that hot-spots will occur in a random or “fractal” structure and (b) the fact that all visible wavelengths are absorbed maximally due to the diversity of particle arrangements present.

Further theoretical considerations provide an explanation for the efficiency of a random, electrolessly deposited surface. Optical absorption and SERS excitation spectra become independent of the size of the fractal as the microstructure is retained as the size of the material is increased (126).

Morphology-dependent resonances (MDRs) can result from total internal reflection within microcavities which further contribute to field enhancement effects. Microcavities producing large intensity enhancements resulting from confinement are thought to contribute to field enhancement through the Smith-Purcell effect whereby light is emitted when a high voltage electron beam is aligned along a metal diffraction grating surface in a direction perpendicular to the diffraction grating rulings (127). Shalaev (128) reported enhancement in fractal-microcavity composites of at least 10^{12} and considered 10^7 due to the fractal colloid and 10^5 due to the microcavity – a significant contribution to enhancement.

3.3.2.5 *Silver embedded in films*

Silver particles suspended in a silica matrix produced by the sol-gel process provide a durable support for field applications. Silver ions incorporated in the silica matrix are photo- or chemically reduced and remain incorporated in the silica film (129-131).

Nanoparticle film deposition can be coupled with photolithography, micro-contact printing and dip-pen nanolithographic patterning techniques to produce well-ordered structures on surfaces (132), while deposition conditions such as immersion time, concentration and temperature can be modified to control particle character and surface morphology (133).

3.3.2.6 *Other methods*

Commercially available products can also be efficient. Microscopic silver coated glass beads (Conduct-O-Fil[®])⁷ were found to be better SERS enhancers than silver colloid or laboratory

⁷ Conduct-O-Fil[®]: Potters Industries Inc. PO Box 840 Valley Forge, Philadelphia, USA. Australian distributor PQ Corporation, PO Box 659, 9 – 13 Rhur St, Dandenong, Victoria, Australia

prepared silver coated glass beads (134). Silver powders with particle size 2 – 3.5 μm (reagent grade) have been shown to have equivalent enhancement to evaporated thin films of silver (135).

3.3.3 *Improvement of response with chloride*

Additional enhancement of SERS on silver, gold and copper colloids has been achieved with chloride, perchlorate and nitrate anions (136). Enhancement with chloride is not attributed to a change in the morphology of the colloidal aggregate, but rather to the formation of a metal-adsorbate charge transfer complex, which is demonstrated by increase in NIR absorbance in the absorbance spectrum.

3.3.4 *Sample preparation*

Experimental methodologies for dosing the SERS substrate with the analyte depend on factors including the amount of analyte available and the substrate configuration. If there is a large volume of analyte available, suitable techniques for using a solid surface substrate may be immersion of the substrate in the analyte solution (137) or by incubation/immersion, rinsing and drying (110). A simple dip and dry can be effective (138).

For a colloid in solution, the analyte may be simply admixed and analysed as a liquid sample in a melting point capillary tube (91) or solution cell (88). The metal particles can be removed from the solution, rinsed and dried then analysed (135).

Other application methods can be used for small quantities of analyte. A small volume (1 – 10 μL) of analyte applied to a surface substrate can be studied by simply dropping it onto the SERS surface then drying (101). Dried deposits on a non-wetting surface are often accumulated in a saturation concentrated ring (known as the “coffee-ring” effect). A sample solution may be dropped onto a hydrophobic surface and analysis performed on the external ring that results on drying. It is described as “Drop Coating Deposition Raman” (DCDR) (139, 140) and is recommended for trace protein analyses.

3.4 Evaluation of SERS substrates for resin analysis

From the preceding section (4.2) it is evident there are numerous methods from which it is possible to select SERS substrates. Four types were chosen based on practical reproducibility and ease of preparation, to evaluate their suitability for natural resin analysis;

1. Silver colloid
2. Silver coated polystyrene nanospheres
3. Etched silver foil
4. Electroless deposition of silver on copper.

Comparative SERS enhancement of each of the substrates was first evaluated using Rhodamine 6G (R6G), which is a common analyte for verifying SERS activity (141-147). Without SERS activity, R6G dye fluoresces far too strongly for a normal Raman spectrum (NRS) to be collected, but with successful SERS enhancement the dye spectrum becomes clearly identifiable.

3.4.1 Colloidal Ag preparation

3.4.1.1 Experimental

Preparation of colloidal Ag followed the method described by Lee and Meisel (88). 90 mg of AgNO_3 was dissolved in 500 mL deionised water and brought to boiling point. 10 mL of 1% sodium citrate was added as a reducing agent and the solution allowed cool to room temperature. At this point, the solution appeared clear. Although not described by Lee and Meisel, it was found the solution could be photo-reduced by exposure to daylight (on a window sill) resulting in a grey-green opaque colour. Alternatively, when the clear solution was kept in the dark for 3 months, a slow transition occurred and the colour changed from pale straw yellow through darker yellow and to orange over the time span. The rate of colour change and colloidal development seems directly related to time and intensity of photo-exposure. Darkening of the quickly reduced solution is attributable to aggregation and growth of reduced metallic Ag nano-particles which appear black/grey, suspended in solution.

The Raman Spectrometer used was a Renishaw 2000 with an incident laser of 633 nm operating at 10% (15mW) power. Collection time was 10 s, microscope objective 50x (numerical aperture 0.75 and a working distance of 0.37mm).

A drop of colloid solution was dried onto a microscope slide and 10^{-3} M R6G solution applied over the colloid spot and dried. The surface was inhomogeneous when viewed with 500 x magnification. This was consistent with the variability in the intensity of spectral response observed in different areas of the drop as the dye and colloid were not uniformly distributed over the surface of the spot.

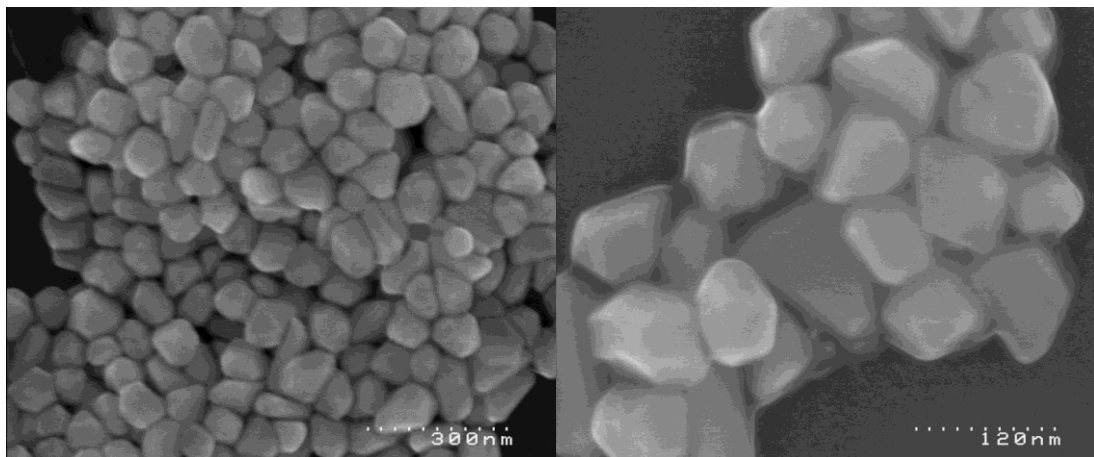


Figure 42. FEG-SEM images of dried Ag colloid (Lee-Meisel preparation)

The colloid solution was examined with Field Emission Gun Scanning Electron Microscope (FEG-SEM) using an in-lens Hitachi S5000. A drop of the colloid solution was dried onto freshly cleaved pyrolytic carbon for high-resolution imaging. Figure 42 shows the colloid aggregated into clusters, but particle size appears monodisperse, with particle size approximately 70 to 80 nm. Particle shapes range from almost spherical to triangular.

Figure 43 shows the comparison between Raman spectra collected from R6G solution dropped onto and dried on a microscope slide (Normal Raman Spectroscopy, NRS), and R6G dried onto colloid solution as described above (with SERS). It shows that the Raman spectrum of R6G is present only if Ag colloid is used. There is no Raman activity evident in the spectrum of R6G when on glass alone and this demonstrates the production of an effective SERS substrate.

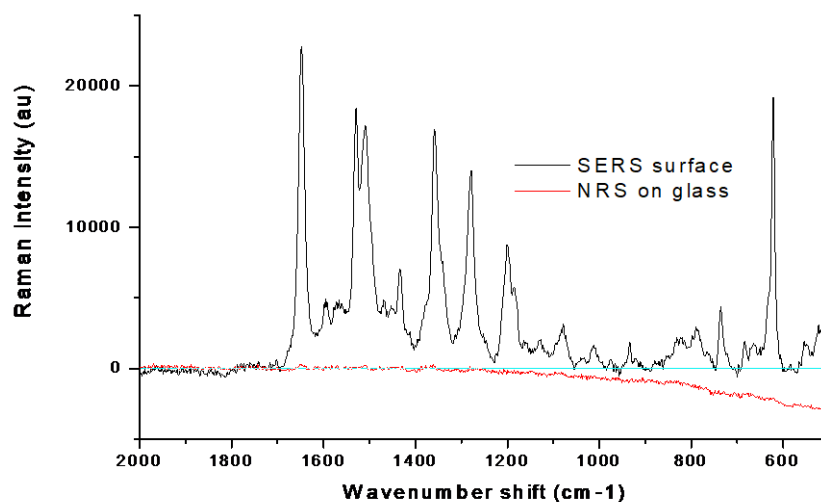


Figure 43. Raman Spectrum of rhodamine 6G with and without SERS enhancement

3.4.2 *Ag coated polystyrene nanospheres*

3.4.2.1 *Experimental*

A structured surface was prepared using 90 nm polystyrene nano-spheres deposited in a 2D array on a glass microscope slide. The spheres are polystyrene and dispersed in water which, when dried on a microscope slide without treatment, dry into a large clump in the centre due to surface tension. To overcome the effect of surface tension, the sphere dispersion was mixed with ethanol in the ratio 1:1 and applied to the microscope slide surface with the desired result of producing a thin mono-layer of spheres over the surface. Once dried, slides were sputter coated with approximately 20 nm of Ag using a Polaron sputter coater for 1 minute.

A drop of R6G solution was placed on the surface to dry. The sample was examined with the conditions described in section 3.4.1.1, and activity was observed with a similar SERS enhancement response to that shown in Figure 43.

3.4.3 *Foil etching*

5mm squares of Ag foil (The Gold Leaf Factory International P/L, Frankston, Australia) were immersed in 30% nitric acid for approximately 5 minutes, until a milky white surface developed. Samples were rinsed in deionised water and air-dried.

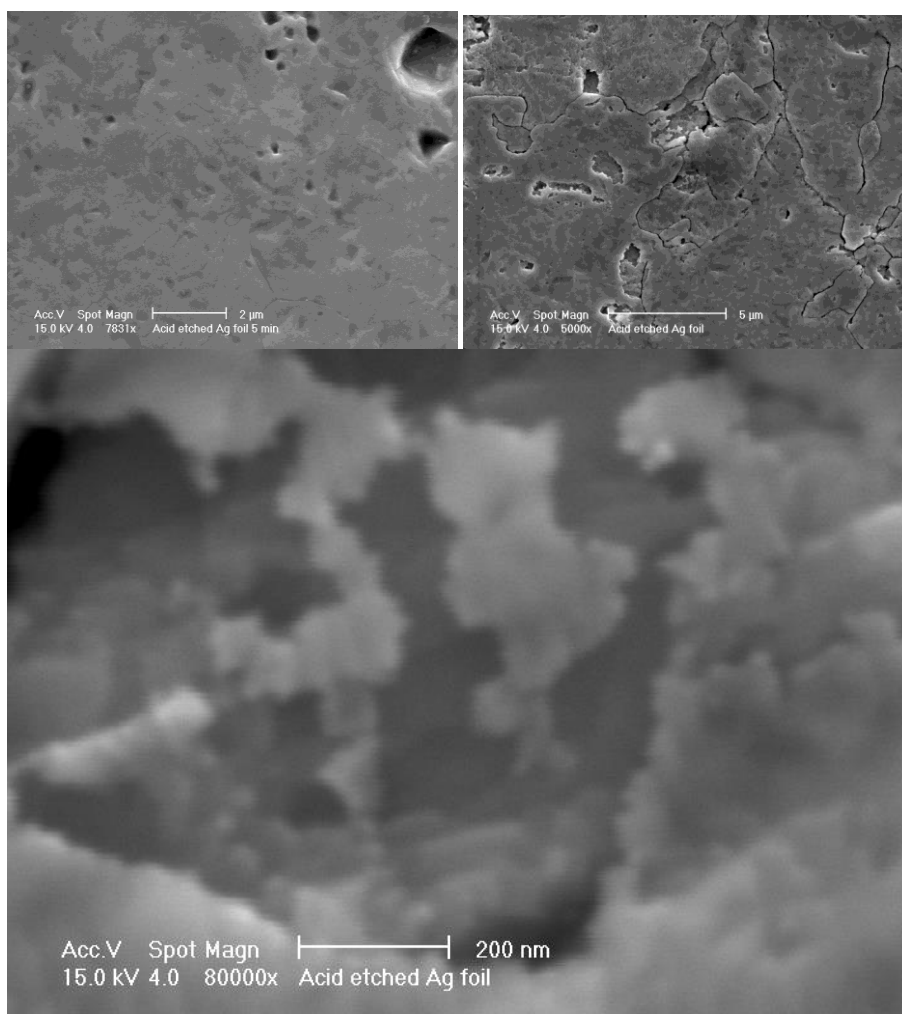


Figure 44. Acid etched Ag foil - surface detail

Examination at high magnification with SEM,

Figure 44, shows the surface structure exhibiting roughness in the 10-100 nm scale required for SERS enhancement.

The sample was examined with the conditions described in section 3.4.1.1, and activity was observed with a similar SERS enhancement response to that shown in Figure 43.

3.4.4 Electroless deposition of Ag on Cu

3.4.4.1 Experimental

Surfaces were prepared by etching copper foil (100 micron thickness) in 20% HNO₃ (aq) briefly to remove surface oxide. Immersion in 2×10^{-2} M AgNO₃ (aq) for a few seconds formed a thin coating of Ag nano-crystals, which appeared black. Prolonged immersion lead to the growth of reflective, metallic Ag crystals, and substantial oxidation of the underlying surface causing the reduced Ag layer to detach from the surface. After development of the Ag crystallite layer, the surface was rinsed in deionised water to halt the reduction of Ag⁺ on the surface, allowed to air dry and stored in a desiccator. SEM imaging is shown in Figure 45.

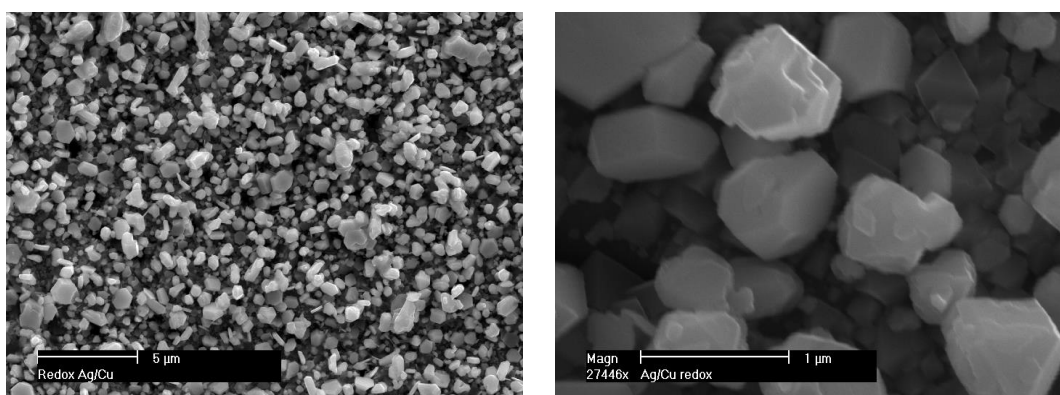


Figure 45. SEM images of Ag deposited on Cu

A drop of R6G solution was placed on the surface to dry. The sample was examined with the conditions described in section 3.4.1.1, and activity was observed with a similar SERS enhancement response to that shown in Figure 43.

3.4.4.2 Evaluation and comparison of SERS substrates

Use of the colloid in solution was dismissed as an option for analysis of resins. The drop of dried colloid, although equally as effective in producing SERS enhancement, was discounted for the variability in response over the surface of the dried drop, and the expected lack of reproducibility from drop to drop. As discussed in 3.4.1.1, the surface was inhomogeneous when examined under the microscope and there was variability in the intensity of spectral response observed in different areas of the drop as the dye and colloid were not uniformly distributed over the surface of the spot.

Etched foil was relatively easy to produce. It was somewhat more difficult to manipulate as the small squares, once etched, were much thinner than the original foil and folded into useless shapes very easily. This made washing and drying difficult.

Silver deposited over polyspheres was an effective and reproducible surface. However, this method was rejected as resins applied to the surface dissolved in organic solvent may swell the supporting polystyrene spheres and the Ag surface could be disrupted.

None of the above disadvantages apply to electroless deposition, which also demonstrates the following advantages:

1. The surface can be washed easily to remove adsorbates from the surface after production ensuring Ag surface is free for the analyte to adsorb to
2. Thermal degradation of analyte is reduced due to a larger volume of conducting material to dissipate thermal energy
3. The production process is relatively quick and inexpensive – worth taking into consideration as SERS surfaces are most easily used if disposable.

Comparative enhancements were not made for the different types of SERS substrates prepared here as the surfaces had dramatically different surface contact angles. The dried colloid, silver over polyspheres and acid etched foil were hydrophobic while the surface prepared by electroless deposition was extremely hydrophilic. Because the surface coverage of the applied analyte differed, the amount of analyte in the interrogation area responsible for the Raman signal was not directly comparable. SERS substrates prepared by electroless deposition were considered the optimal choice for the study of resins as follows.

3.5 SERS of natural resins

3.5.1 Origins, use and chemistry of natural resins

Natural resins are a product of many plants. After injury to the bark, a sticky liquid exudes which hardens on drying as a protective mechanism. Resins are collected easily and have been used throughout history as adhesives, coatings, inks, incense, medicine, soaps and many other products. They can also be added to paint binding media to alter handling properties, as in

megilps⁸. The dried product can even be carved into small artefacts or jewellery. As a coating or lacquer for artworks, natural resins have been used extensively and are still popular as a traditional material even though synthetic alternatives are available. Modern synthetic resins have been developed with a view to reducing the problems of yellowing, embrittlement and irreversibility associated with the ageing process of natural resin varnishes.

Because of their biological origins, natural resins are variable and dependent on the source but are commonly comprised of compounds known as terpenoids. Terpenoids are a widespread chemical class in nature with compounds made up from units of the C₅ compound isoprene illustrated in Figure 46. Essential oils of plants are mostly monoterpenoids (C₁₀ compounds) and sesquiterpenoids (C₁₅ compounds) and well recognised examples include camphor, menthol and thymol. Natural resins contain mono-, sesqui-, di- (C₂₀) and triterpenoids (C₃₀). Most are plant exudates although a few derive from insects, for example shellac.

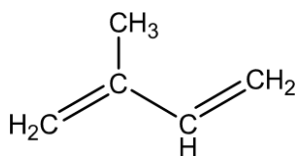


Figure 46. Chemical structure of isoprene

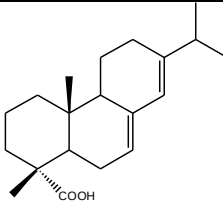
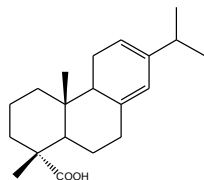
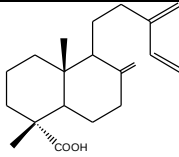
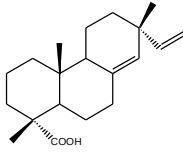
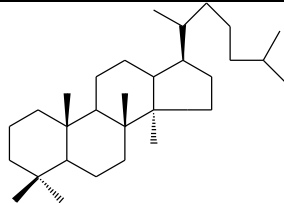
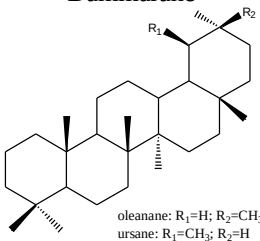
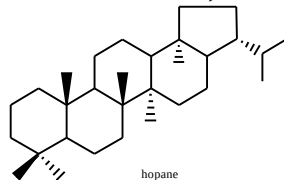
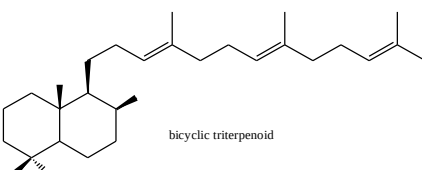
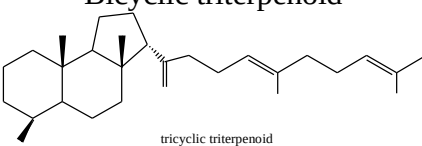
Natural resins are applied as solutions, in solvents such as alcohol or spirits, or in oils. Turpentine and alcohol are the most common solvents for spirit-based varnishes. Distillation of pine resin produces oil of turpentine, a mixture of mostly monoterpenes, which is used as a thinner or solvent. The relatively non-volatile resin that remains after distillation of the volatiles is colophony, also known as rosin. Sandarac is another diterpenoid resin, which has been used since the 16th century, particularly as a varnish ingredient in the Middle Ages. Other diterpenoids used as ingredients in varnish recipes include copal, Venice turpentine, Canada balsam and amber, a fossil resin.

⁸ A megilp is a “buttery” paint medium made by mixing mastic with turpentine and blending with linseed oil 148. Gettens RJ, Stout GL. *Painting Materials. A Short Encyclopaedia*. New York: Dover; 1966.

Dammar and mastic are favoured as painting varnishes. Dammar is said not to have been used as a varnish resin before 1829 but is now the most often used natural resin. Mastic has been used from the 16th century until present times (148).

Natural resins are comprised of a mixture of compounds and the characteristics of the mixture need to be retained for identification. An experimental approach of purification and identification of key components would therefore be unsuitable.

In this study, two diterpenoid resins, sandarac and colophony, and the two most commonly encountered triterpenoid varnish resins, dammar and mastic were examined.

	Diterpenoid Resins		Triterpenoid Resins	
	Colophony	Sandarac	Dammar	Mastic
Family of plant origin	Pinaceae	Cupressaceae	Dipterocarpaceae	Anacardiaceae
Genus	Pinus spp	Tetraclinis articulata	In over 500 species	Pistacia spp
Examples of chemical structure of major components	 abietic acid Abietic acid  laevopimaric acid Laevopimaric acid	 iso-communic acid Iso-communic acid  sandaracopimaric acid Sandaracopimaric acid	 dammarane Dammarane  oleanane: $R_1=H$; $R_2=CH_3$ ursane: $R_1=CH_3$; $R_2=H$ Oleanane: $R_1=H$; $R_2=CH_3$ Ursane: $R_1=CH_3$; $R_2=H$  hopane Hopane  bicyclic triterpeneoid Bicyclic triterpeneoid  tricyclic triterpeneoid Tricyclic triterpeneoid	

3.5.2 *Experimental*

3.5.2.1 *Preparation of resin solutions*

Sandarac and colophony were obtained from Kremer Pigmente (Germany). Mastic was supplied by Jack Harrison (JNH), conservation materials supplier, Melbourne, Australia, through the National Gallery of Victoria. A second sample of mastic and dammar were supplied by Langridge artists' materials suppliers, Melbourne, Australia.

10% w/v solutions of each were prepared in mineral of turpentine (source) or 95% ethanol (source) depending on solubility. Sandarac, colophony and mastic were prepared in ethanol and dammar was prepared in mineral turpentine.

Samples of natural resin material are from “bulk” supplies, meaning they are the raw material from which varnishes are prepared. In normal practice the resin granules or pearls are dissolved in a suitable solvent and often there is a sediment or undissolved fraction that is removed by filtration to produce a clear varnish solution. These samples did not produce any undissolved material.

SERS surfaces were prepared by electroless deposition of Ag or Cu as described in section 3.4.4.

For analysis a 5 μL sample of resin solution was applied to a clean SERS substrate and oven dried at 40°C for 10 minutes. SERS spectra were recorded on a Renishaw In-Via micro-spectrometer with Argon ion excitation at 514.5 nm and an electrically cooled CCD detector. Collection was performed through the 50 x objective lens. Laser power was 5 mW and collection time 20 seconds with 2 accumulations over the range 200 to 3000 cm^{-1} .

3.5.2.2 *SERS Analysis*

A single example of mastic (Figure 47) shows the comparison between SERS-enhanced Raman spectra and unenhanced normal Raman spectra.

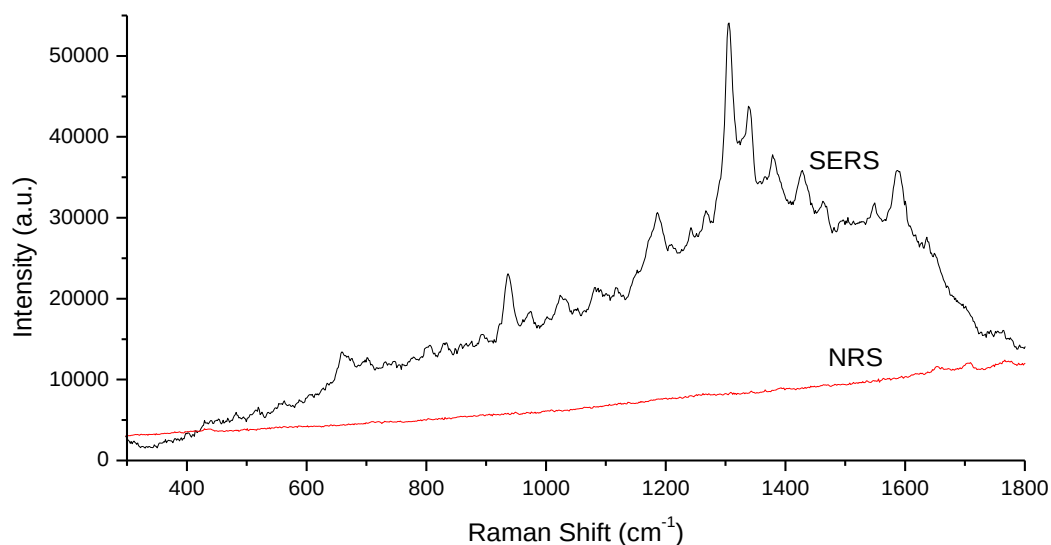


Figure 47. Untreated Raman spectra of mastic with SERS enhancement and on glass (NRS)

The differences between NRS and SERS spectra arise because chemical enhancement mechanisms modify the charge distribution within a molecule and thereby influence the vibrational modes, and dramatically increase the Raman signal from only the portion of the molecule in direct contact with the metal surface. Electromagnetic mechanisms are responsible for enhancement extending further away from the point of contact. Therefore, the SERS spectrum is not a uniform enhancement of the NRS spectrum derived through overall resonance amplification of vibrational modes. Importantly, conformational constraints imposed by attachment of the analyte to the surface (chemical enhancement) are believed to alter vibrational modes from the non-enhanced state through charge transfer.

The Raman peaks and their vibrational assignments for the natural resin samples are listed in Table 6 with overall spectra illustrated in Figure 48. Wavenumber positions and intensities are of a typical singly collected spectrum for each sample. Spectra were collected from several areas over the sample, and some variability in the quality of spectra was observed. In areas where the film was so thin that it was not visible, it is thought that lyophobic repellence of the metal surface may have prevented sufficient surface contact between the analyte solution and the surface. Vibrational frequency positions and peak intensity ratios did not vary within a sample, only the overall signal to noise of the spectrum.

Published data for triterpenoids (149) indicate strong bands at 1380 cm^{-1} with weaker bands at 530 cm^{-1} and these were observed in the samples of dammar and mastic.

These bands were absent in the samples of diterpenoids, colophony and sandarac. Bands indicative of diterpenoids (1303 , 977 and 740 cm^{-1}) were not found in the diterpenoid samples examined with SERS. The band at 1303 cm^{-1} was present in colophony only and the band at 977 cm^{-1} in sandarac only, with the band at 740 cm^{-1} absent.

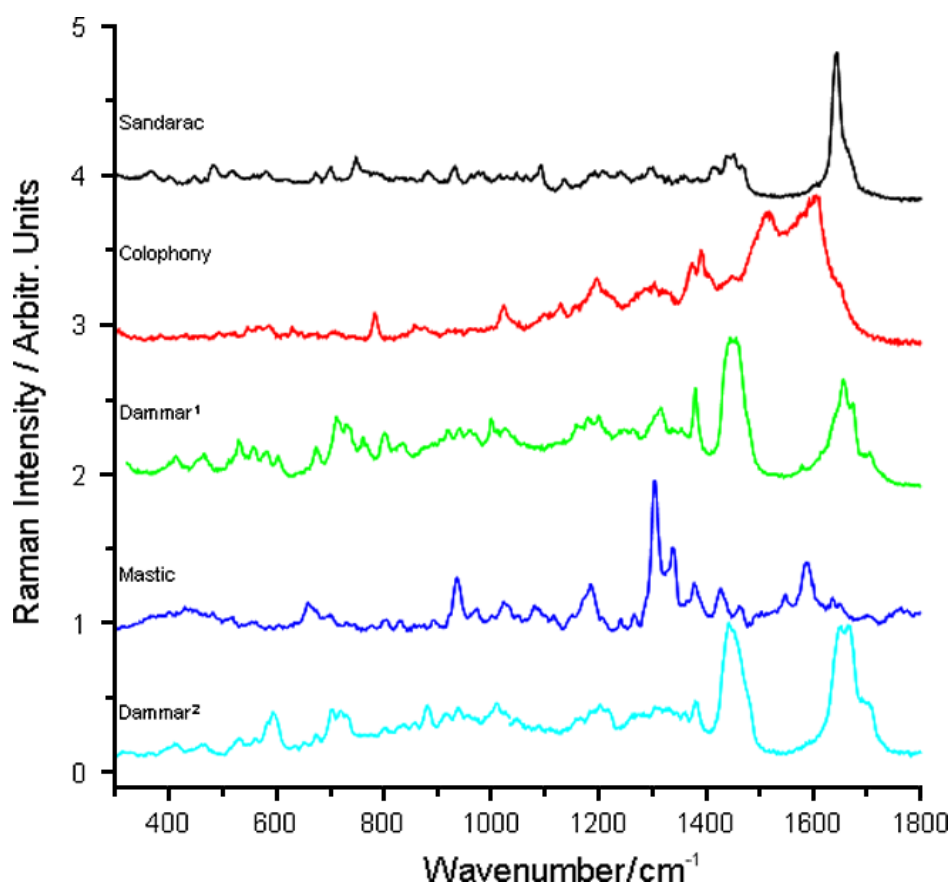


Figure 48. Normalised SERS spectra of mastic (2 sources), dammar, colophony and sandarac. Normalisation range 0-1.

Bands consistent in dammar and mastic SERS spectra are at approximately 1700 cm^{-1} , attributable to $\nu(\text{C}=\text{O})$; 1653 cm^{-1} due to $\nu(\text{C}=\text{C})$ conjugated with $\text{C}=\text{C}$ or $\text{C}=\text{O}$; 1380 cm^{-1} due to $\delta(\text{CH}_2)$, $\delta(\text{CH}_3)$; 938 cm^{-1} $\rho(\text{C}-\text{H})$, 802 cm^{-1} from $\text{C}-\text{C}$ ring breathing and 559 cm^{-1} due to

bending of (C-C-O) groups. Vibrational modes are ν , stretching; δ , deformation and ρ anti-symmetric deformations or rocking. These bands are not evident in diterpenoids.

Bands consistent and unique to the diterpenoids samples sandarac and colophony are at approximately 1299 cm^{-1} $\delta(\text{CH}_2)$ and 1193 cm^{-1} from C-C ring breathing. Ongoing work into the variability within and between resin types would obviously be beneficial in identifying further bands that could be used as indicators of resin identity.

3.5.2.3 Identification of unknown samples

Although a full understanding of the attachment mechanisms for the analyte-SERS substrate complex is not yet available, the spectra are reproducible for the same reference material. This allowed the comparison of SERS spectra collected from unknowns to be compared with SERS reference spectra for identification. The distinctive spectra observed for natural resin samples suggest identification of unknown resins from painting varnish may be possible.



Figure 49. *The Banquet of Cleopatra* (1743-1744), Giambattista Tiepolo, oil on canvas, 250.3 x 357.0 cm, National Gallery of Victoria, Melbourne, Felton Bequest, 1933.

Figure 49 shows the canvas painting *The Banquet of Cleopatra* which underwent major conservation and restoration treatment in 2003 (150, 151). SERS was used to characterise the original resin varnish of the painting that was removed during the conservation treatment.

Cotton swabs wetted with acetone were used to remove micro samples of the painting varnish. Resin from the dried swab was extracted with mineral turpentine and the solution analysed using the same SERS methodology as previously described.

Figure 50 shows the varnish sample compared with the reference spectrum of dammar.

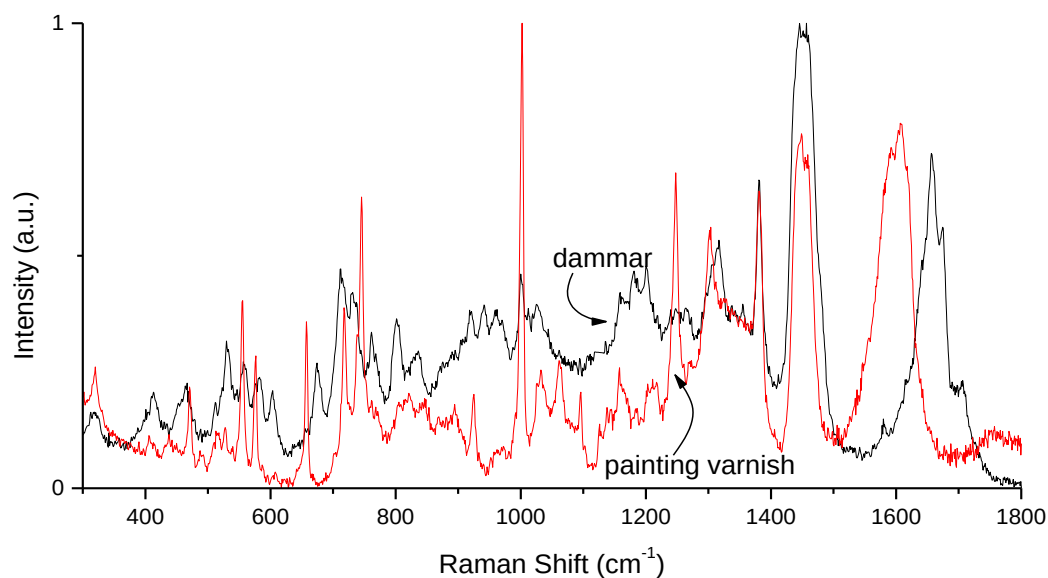


Figure 50. Overlaid SERS spectra of varnish from "The Banquet of Cleopatra" (red) and the closest spectral match, dammar (black).

Table 6 compares the observed vibrational modes from the varnish sample with those of sandarac, colophony, mastic and dammar.

Table 6. Vibrational modes for natural resin SERS spectra. Approximate assignments from refs (31, 32, 152).

Sandarac (cm ⁻¹)	Colophony (cm ⁻¹)	Mastic (cm ⁻¹)	Mastic ² (cm ⁻¹)	Dammar (cm ⁻¹)	Banquet of Cleopatra (cm ⁻¹)	Vibrational mode – approximate assignment
		1763 w				
		1702 m, sh	1699 s, sh	1703 s, sh		$\nu(\text{C=O})$
				1674 s, sh		$\nu(\text{C=O})$
1666m,sh			1667 vs			$\nu(\text{C=O})$
		1650 w	1650 vs	1655 vs		$\nu(\text{C=C})$ conjugated with C=C or C=O
1643 s						$\nu(\text{C=C})$ conjugated with C=C or C=O
		1635 mw				$\nu(\text{C=C})$ conjugated with C=C or C=O
					1617 w, sh	$\nu(\text{C=C})$ conjugated with C=C or C=O
	1604 ms				1607 s	$\nu(\text{C=C})$ conjugated with C=C or C=O

		1587 ms			1590 m, sh	$\nu(\text{C}=\text{C})$ conjugated with $\text{C}=\text{C}$ or $\text{C}=\text{O}$
				1579 w		$\delta(\text{CH}_2), \delta(\text{CH}_3)$
		1546 mw				$\delta(\text{CH}_2), \delta(\text{CH}_3)$
	1514 ms					$\delta(\text{CH}_2), \delta(\text{CH}_3)$
1469 m, sh		1462 mw			1458 m, sh	$\delta(\text{CH}_2), \delta(\text{CH}_3)$
			1453 s, sh	1451 vs	1448 s	$\delta(\text{CH}_2), \delta(\text{CH}_3)$
1446 m			1443 vs			$\delta(\text{CH}_2), \delta(\text{CH}_3)$
		1428 m				$\delta(\text{CH}_2), \delta(\text{CH}_3)$
1415 mw, sh						$\delta(\text{CH}_2), \delta(\text{CH}_3)$
	1390 m					$\delta(\text{CH}_2), \delta(\text{CH}_3)$
		1378 m	1381 m	1380 s	1380 s	$\delta(\text{CH}_2), \delta(\text{CH}_3)$
	1372 m, sh					$\delta(\text{CH}_2), \delta(\text{CH}_3)$
		1348 ms				$\delta(\text{CH}_2), \delta(\text{CH}_3)$
				1316 m		$\delta(\text{CH}_2), \delta(\text{CH}_3)$
			1308 m, br			<i>Twisting</i> (CH_2)vib
	1302 w, br	1304 s			1301 m	<i>Twisting</i> (CH_2)vib
1297 m						<i>Twisting</i> (CH_2)vib
		1267 mw				$\delta(\text{CH}=\text{CH})$
				1252 w	1247 m	$\delta(\text{CH}=\text{CH})$
1242 m		1240 mw				$\delta(\text{CH}=\text{CH})$
1208 m			1203 m	1200 mw	1202 w	$\delta(\text{CH}=\text{CH})$
1191 m	1196 w, br					$\delta(\text{CH}=\text{CH})$
		1186 m			1184 w	$\delta(\text{CH}=\text{CH})$
				1178 mw		$\nu(\text{C}-\text{C})$ ring
				1158 mw	1156 m	$\nu(\text{C}-\text{C})$ ring
					1144 w	
1136 m					1138 w	$\nu(\text{C}-\text{O}-\text{C})$ asym
		1117 mw				$\nu(\text{C})$
						$\nu(\text{C})$
	1130 w				1126 w	$\nu(\text{C}-\text{O}-\text{C})$ asym
1092 ms					1096 mw	$\nu(\text{C}-\text{C})$ ring
		1084 mw				$\nu(\text{C}-\text{C})$ ring
1065 w					1062 mw	$\nu(\text{C}-\text{C})$ ring
1047 mw						$\nu(\text{C}-\text{C})$ ring
					1032 mw	$\nu(\text{C}-\text{C})$ ring
	1023 mw	1026 mw		1025 mw		$\nu(\text{C}-\text{C})$ ring
1017 mw						$\nu(\text{C}-\text{C})$ ring
			1010 m			$\nu(\text{C}-\text{C})$ ring
				997 m	1002 vs	$\nu(\text{C}-\text{C})$ ring
976 m, br		970 mw				$\nu(\text{C}-\text{C})$ ring
				959 m		
930 ms						$\rho(\text{C}-\text{H}), \text{cis, trans}$
		935 m	939 w	941 m		$\rho(\text{C}-\text{H}), \text{cis, trans}$
					925 m	
			916 w	920 m		$\rho(\text{C}-\text{H}), \text{cis, trans}$
		893 mw			894 mw	
882 m			881 m			$\rho(\text{C}-\text{H}), \text{cis, trans}$
			855 w			$\nu(\text{C}-\text{C}-\text{OH})$
		828 mw		834 w		$\nu(\text{C}-\text{C}-\text{OH})$
		802 mw	802 w	801 mw	805 m	$\nu(\text{C}-\text{C})$ ring
	782 mw					$\nu(\text{C}-\text{C})$ ring
				760 mw		$\nu(\text{C}-\text{C})$ ring
747 ms					745 m	$\nu(\text{C}-\text{C})$ ring

			730 m	731 m		$\nu(\text{C-C})$ ring
			719 m		717 m	
				711 m		
			703 m			
690 m						
670 m			674 w	673 mw		
		657 mw	651 w		657 m	
				602 mw		
			593 m			
580 m				582 mw		
					576 mw	
		562 w	561 w	556 mw	555 m	$\delta(\text{C-C-O})$
			529 w	531 mw	528 w	$\delta(\text{C-C-O})$
516 m		519 w				$\delta(\text{C-C-O})$
481 m		482 w				$\delta(\text{C-C-O})$
					470 mw	$\delta(\text{C-O})$ in plane def
			464 w	462 mw		$\delta(\text{C-O})$ in plane def
443 m						$\delta(\text{C-O})$ in plane def
		429 w				
			410 w	413 mw		
399 m						
364 m			364 vw			$\delta(\text{C-C-C})$
			325 vw			
				316 mw	319 mw	
302 m						

Vibrational modes are ν , stretching; δ , deformation and ρ anti-symmetric deformations or rocking. v= very, s = strong, m = medium, w = weak, sh = shoulder

Ageing of natural resins through oxidation and hydrolysis is expected to induce structural change resulting in altered vibrational modes. Some effects of resin ageing on the Raman spectra have been investigated in diterpenoids, *Pinus pinea* and *Pinus halepensis* (149) and have shown that the intensity of bands at 1649 cm^{-1} associated with abietic acid and 1611 cm^{-1} associated with dehydroabietic acid indicate levels of degradation in diterpenoid samples. Dehydroabietic acid is found in relatively fresh resins. A spectral feature centred at 1440 cm^{-1} , with three main bands at 1470 , 1443 and 1433 cm^{-1} (assigned to CH_2 and CH_3 deformation modes) is thought to change shape with degradation of the sample. In the same study, triterpenoid resin (*Pistacia* spp) spectra were examined and spectral features specifically related to degradation were not able to be identified.

Triterpenoid resins are expected to exhibit bands at 1380 and 530 cm^{-1} . The band at 1380 cm^{-1} is strong in both the dammar and painting samples. It is moderate in mastic and not evident in the diterpenoid samples. The band at 530 cm^{-1} is weak in all spectra from dammar, mastic and the painting sample. The overall comparison of the painting sample spectrum with other natural resin

spectra matched most closely with dammar in terms of peak positions and the overall pattern of the spectrum.

Some dissimilarity observed between the sample spectrum and the reference spectrum of dammar is to be expected, with some difference attributable to chemical degradation due to ageing and some due to natural variation in the source of the resin reference material and possibly deposition and contamination of the varnish on the painting. Identification as dammar was independently verified with GC-MS analysis (153).

3.6 Conclusions and further outcomes

SERS was demonstrated to have promise as a method for investigating natural resins using 514.5 nm irradiation. By overcoming fluorescence normally encountered when examining resin samples with Raman spectroscopy, new opportunities exist for the spectroscopic examination and identification of materials available in limited quantity such as those from artworks. An evaluation of SERS surface production methodologies has shown that electroless silver deposited onto copper sheet is a surface preparation technique that can be duplicated easily. This is a straightforward and inexpensive method that can be duplicated for use in conservation applications.

Through creation of SERS reference libraries, the identification of natural resins with 514.5nm incident irradiation is possible, with the understanding that ageing and contamination of painting surfaces may influence the structural composition of natural resin materials and introduce spectral variability and thereby affect the degree of certainty with which identification can be achieved.

In 2008, work in this chapter was published in the *Journal of Raman Spectroscopy* titled *Application of surface-enhanced Raman spectroscopy (SERS) to the analysis of natural resins in artworks* (154). At the time of publication, the journal had an impact factor of 3.5. The article has shown its interest to the scientific community having been cited 28 times.

The method of analysis presented in this work has been recognised by most citing authors as the principal example of how SERS is an efficient technique to overcome the inherent fluorescence

issue associated with binding media and varnishes. The year after publication it was cited as an example of recent advances in linear and non-linear Raman spectroscopy (155). Casadio *et al* (156) and Serafini and Ciccola (157) cite this work in demonstrating that the application of SERS can be extended beyond the analysis of dyestuff, to the successful analysis of resins.

Results were further built on by other researchers who utilised the peak assignments of Raman spectra of natural resins presented in the paper. Nevin *et al* applied the knowledge to ageing studies of natural resins and identified specific markers, which change with ageing in FTIR and Raman spectra (158). Agnoletto *et al* used the peak assignments to understand that the varnish applied during the 1984 restoration of Jacopo Tintoretto's painting "Wedding Feast at Cana" had a natural origin (159). Daher *et al* (160) developed signal treatment and statistical analysis methods for processing FT-Raman and FT-IR spectra of natural organic substances of Cultural Heritage interest. The authors used peak assignments from this paper to propose a new objective discriminating methodology to identify resins, oils, animal glues, and gums.

More recently in 2019, Gagliano Candela (161), further extended the use of SERS by developing a new sampling methodology. Gel-Transfer Surface Enhanced Raman Spectroscopy (GT-SERS) is based on direct contact with an Ag-gel matrix. Small circular discs of Ag-gel matrix shape are applied to the surface of the artwork. Micro-fragments of sample from the artwork are attached by mechanical adhesion. The Ag-gel matrix portions are placed on a glass slide and after drying can be analysed.

This paper is recognised as the first reporting of this technique to analyse fluorescent painting varnish with SERS and in the 2019 book "Nanotechnologies and Nanomaterials, Nanotechnologies and Nanomaterials for Diagnostic, Conservation and Restoration of Cultural Heritage" (157), is the key article used to describe how SERS can be used for the analysis of resins.

Chapter 4: Multivariate analysis of organic artists' materials

4.1 Introduction

As shown above, Raman spectroscopy is an important tool for characterising artworks and archaeological objects with a unique combination of practical and analytical advantages. The Raman spectra of inorganic materials tend to be relatively simple with clear, well resolved bands derived from fewer vibrational modes, making the characterisation of pigments a process that can sometimes even be done by eye; for example, the two D & G bands of carbonaceous black pigments. There are only two or three characteristic bands in pigments from metal oxides and carbonates such as chromium oxide, viridian, iron oxides, chrome yellow, titanium and lead white leading to easy visual recognition.

Raman and FTIR spectra of organic materials are far more complex in nature since they contain numerous vibrational modes with overlapping bands and subtle differences in peak height and ratio. Even if the problem of Raman fluorescence of some organic materials can be mitigated with techniques such as SERS, the difficulty of peak assignment and spectral comparison remains.

In addition to the fact that spectra of organic materials are much more complex than inorganic materials, naturally derived organic materials have spectra that are further influenced by the variability of the source through growing conditions, processing and susceptibility to degradation by ageing.

Although achieving a match between a single sample and reference material is possible using standard library search software that utilises peak height and position information, there can be subtle differences within and between groups of materials that are not obvious by inspection and require a more sophisticated statistical treatment. Importantly, multivariate data analysis provides tools for understanding relationships within the data that are not easily observed by simple spectral inspection. It can reveal and explore hidden data structures such as the degree of similarity or dissimilarity between spectra and indicate the specific spectral features which are responsible, thereby providing a greater measure of confidence in the identification of the species of interest.

Vibrational spectra are multivariate, so understanding the relationships between individuals and groups of samples can be achieved through the use of multivariate data analysis techniques. The differences within and between groups of data, in this case spectra, are more easily recognised visually using abstracted or geometric representations of the data in projected space. Multivariate analysis is an excellent tool for this application, used to translate the inherent variance between samples or groups of samples into visually comprehensible representations.

This chapter presents an investigation of the application of multivariate techniques for the analysis of Raman spectra. Through examples of organic artists' materials, the research elucidated in this chapter aims to demonstrate how multivariate approaches can be useful in examining the relationships between types of resins used as painting varnishes and assist in the interpretation of Raman spectra from them.

4.2 Multivariate data analysis

Multivariate data refers to data having many variables associated with a single sample or measurement. In the same way that measurements of parameters such as pH, temperature, colour, absorbance, elasticity or any other physical property can be used together to characterise a sample, so can a Raman spectrum, which has a Raman intensity measurement for each of a range of wavenumbers.

In a case with only two or three variables, they may be represented using 2D or 3D plots, so that samples with similar co-ordinates are located close together in the plot. In situations where there are more than three variables (spectra typically contain hundreds or thousands of single wavelength measurements to form a spectrum) then matrix transforms of the data are necessary to reduce the dimensionality to one that can be interpreted. There are many approaches and techniques available, only some of which will be used here, but all are used as methods for data reduction to achieve the aims of; data description, discrimination/classification and regression/prediction.

Due to the large number of multivariate data analysis approaches, an understanding of the use and application of different methods is useful. They can be categorised into three main groupings dependent on their purpose (162).

4.2.1 Data description

Data description is also described as “explorative data structure modelling”, and covers determination of means, standard deviations and other descriptive statistics. Principal Components Analysis (PCA, described in more detail later) is the approach most frequently used to explore data structure and correlation of one variable with another. One of the first multivariate techniques to be adopted by chemists was factor analysis, an extension of the PCA approach which has its origins in the behavioural and social sciences (163) whereby the PCs identified are related to underlying causative factors. It was developed for the analysis of large data sets and is well suited to dealing with the large volumes of numerical data that are collected and handled in psychometrics. Since being applied to psychometrics, factor analysis has been readily adopted in chemometrics, biometrics and related fields.

Factor analysis allows the identification of underlying variables, or factors, that explain the pattern of correlations within a set of observed variables. Factor analysis is often used for data reduction to identify a small number of factors that explain most of the variance observed in a much larger number of manifest variables and can also be used to generate hypotheses regarding causal mechanisms or to screen variables for subsequent analysis (for example, to identify collinearity prior to performing a linear regression analysis). In summary, factor analysis can:

1. Indicate the number of factors that influence the data
2. Identify which factors are influential
3. Indicate similarity or dissimilarity between samples by grouping into clusters
4. Identify additional influential factors
5. Predict new data based on previously observed results (164).

PCA is also referred to as *eigenanalysis* or alternatively *principal factor analysis* (PFA) because of the connotation of the word “component” for chemists. This technique will be referred to as PCA throughout this work.

4.2.2 Discrimination and classification

These techniques are used to demonstrate the separation of data into groups or clusters and provide a process for the further classification of new samples or individuals in relation to the established groups. Classification based on *a priori* knowledge of the class description is

“supervised” classification or discriminant analysis. “Training” samples whose identities are known are used to define the class structure. Unknown samples may be compared against the class to see whether they belong.

“Unsupervised” techniques (eg: K-means, Isocluster, Hierarchical Cluster Analysis (HCA)) rely only on the information contained in the data, and use a range of partitioning methods to define the grouping arrangement (165, 166).

4.2.3 *Regression and prediction*

Regression is a method for defining the relationship between two (or more) variables (X and Y). The model derived through regression is used to predict one or more dependent variables (Y) based on the knowledge of an independent variable (X). Regression methods commonly used are Principal Component Regression (PCR), Partial Least Squares Regression (PLS) and Multiple Linear Regression (MLR).

4.2.4 *PCA*

The treatment of spectral data with PCA requires that the data is formatted into an X-matrix, with n objects and p variables. In other applications the objects may be observations or experiments but here they are the individual resin samples. The variables are the observations and may be parametric or non-parametric (distribution free) and here they are the intensity measurements recorded at each wavenumber. Any number of variables may be used, provided that they adequately describe the difference between samples.

PCA is a projection method that allows visualisation of the information contained in the X-matrix through representation in a Cartesian co-ordinate system of dimension p . Through mathematical deconstruction which will not be discussed here, but is well covered by Malonowski (164), the data matrix is decomposed into eigenvectors and eigenvalues. These “abstract” factors have no meaning themselves other than a being a mathematical transformation of the experimental data. The primary eigenvector passes through the greatest number of data points and accounts for the greatest proportion of the variance. Successive axes are orthogonal to those already located and account for the next greatest amount of variance, a procedure which is repeated until all the variance is accounted for.

Each sample has a score on each PC and score plots display the value of the score for that PC. Similarity in a sample results in a close score along a PC and therefore, overall relationships between samples are demonstrated by examination of score plots.

While a score plot depicts the sample relationships, a loading plot indicates relationships between variables and their contribution to each PC and describes the reasons behind the object distribution. Spectroscopic data sets often comprise matrices of hundreds and thousands of variables (wavenumbers) and although the loading plots from these data are not useful for describing the relationships between variables, the plots can indicate selective variables as diagnostic spectral bands whereby the largest loadings for each PC correspond to the most important diagnostic features of the spectrum.

In section 4.5.2.1 of this chapter, PCA is used as described above to visualise the overall spectral relationships between natural and synthetic resin samples and identify the bands which are responsible for the majority of differences between groups of spectra.

4.2.5 HCA

Hierarchical Cluster Analysis is an alternate visualisation technique for examining relationships between samples based on spectroscopic data and organises the data into a branched assemblage (e.g. dendrogram) based on the degree of similarity or dissimilarity. The diagram in Figure 51 represents the concept of clustering into specified numbers of clusters. Samples may be combined one by one sequentially, until all samples are accounted for and only one cluster remains, so HCA may produce from 1 to n clusters depending on the operator's requirements for representing similarity.

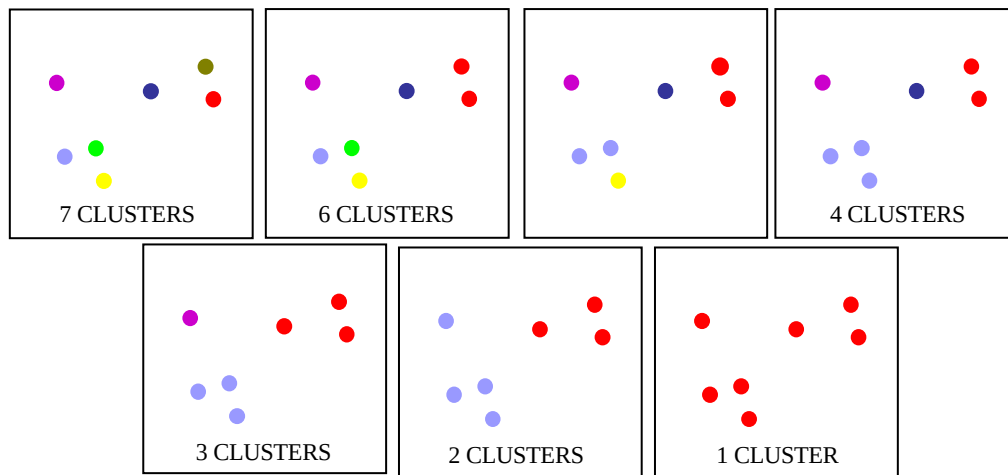


Figure 51. Representation of sequential clustering

There are various algorithms for calculating the similarity between samples, such as average linkage, complete linkage, single linkage, weighted average, unweighted pair group, median function and Ward function (166).

Average linkage clustering uses the average distance between all pairs of objects in the cluster. Complete linkage clustering uses the furthest point in the cluster. Single linkage clustering calculates the distance between two clusters as the two closest objects in the cluster. Weighted average calculates the average using the size of the cluster as a weighting factor. Centroid function calculates the average point in multidimensional space and can be used as a median function when weighting due to the size of the cluster is included. Ward's linkage uses an analysis of variance approach to evaluate the distances between clusters by minimising the Sum of Squares (SS) of any two (hypothetical) clusters that can be formed at each step.

The HCA procedure processing output provides different visual representations of the relationships; an agglomeration schedule, distance (or similarity) matrix, cluster membership or graphically as dendrograms or icicle plots. The results are most often viewed as a dendrogram where the samples with high similarity are connected within in the same cluster. The hierarchy of partitioning is used to explore similarity and dissimilarity, and the distance along the branching structure provides an index of measure.

4.3 Multivariate data analysis in art and archaeological applications

Although established, the application of chemometric techniques for spectroscopic data exploration is still gaining momentum. Chemometrics developed in the 1960s and 70s from availability of and access to computing resources, and from which the allied fields of bioinformatics and cheminformatics have grown (167). Most activity is still evident in the biomedical science field where it is used in conjunction with genomics and popular spectroscopic techniques. It receives most attention when coupled with FTIR results. The early years of conservation science research tended to lag somewhat behind that of other fields and meant there had been relatively little work using multivariate techniques until around 2010. PCA is the most used technique by far, as the following examples demonstrate.

Multivariate analysis has been used in conservation research studies, most of which treat the data from X-ray or chromatographic analytical techniques. Roman bricks from 18 archaeological sites of the Lagoon of Venice were classified in order to recognise the age of a brick by its elemental composition (168). Factor analysis of mass spectroscopic data was used to classify amber dependent on its geographic origins (169). In work by another group, amber was geographically classified with hierarchical cluster analysis and PCA of py-GC data and 18 IR absorption bands (170). The classification of copper corrosion types in natural patinas on archaeological bronzes (Cu-Sn alloys) was assisted by PCA treatment of EDS data (171). PCA and artificial neural networks (ANNs) were used to classify Chinese neolithic potteries and show they belong to three categories, the Yellow River Valley (YR) region, the Yangtse River Valley (YV) region and other regions (OR). The authors found the ANNs were more successful than PCA (172). PCA of diffuse reflectance near-infrared (NIR) spectra has been used for discriminating areas involved in the sulfation process of calcareous stones. NIR spectra of standard mixtures containing $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, $\text{CaSO}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$, and CaCO_3 were recorded. Discrimination between the two reaction products $\text{CaSO}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ was obtained (173). In a study of minor and trace element chemistry of pottery sherds determined by EDS, PCA found two clusters in the data set which correspond with the local sedimentary raw materials near the sites (174) by observing the spatial segregation between the clusters in a plot of the first and second principal component scores.

Some examples where the analysis of organic painting media have been facilitated by PCA include the anion exchange chromatographs of reference plant gums which were analysed with PCA to form a training set of polysaccharide clusters: samples from a watercolour painting and polychrome artwork were treated similarly and the gum identified (175); characterisation of drying oils and proteinaceous binders was achieved by PCA performed on relative percentages of amino acids while the identification of the drying oils was based on ratios of fatty acids (176); fibre optic FTIR data treated by PCA was used to classify a limited set of pigments and binding media in painted layers (177).

The identification of modern organic pigments was performed by comparison of the Raman spectra of samples from the painting “Faubourg” by the Belgian surrealist painter Paul Delvaux with reference spectra from coloured pencils using a principal component searching algorithm. Using data reduction, the algorithm reduced the number of points for each spectrum to 150 and compared spectra by Euclidian distance after pre-treating data with baseline correction, linear interpolation and standardisation, thereby showing the effectiveness of the technique for spectral matching (178).

Using a Raman fibre optic probe to collect spectra directly from a painting surface increases the sample target area and the resulting spectrum is often a mixture of pigment particles. Soft (multivariate curve resolution method, MVCR) and hard (analyser of pigments, ADP) modelling methods were developed to better analyse mixtures of materials. (179). The ADP filtered the raw Raman spectrum and reproduced the original as a series of Lorentzian shapes that were compared with a spectral database of pure Raman spectra. The identification was not effective in the presence of strong fluorescence effects showing fluorescence is problematic for this technique when dealing with real samples.

Far fewer articles exist describing the use of clustering techniques such as HCA for the analysis of data structure of Raman spectra in conservation, despite the evolution of spectroscopic and statistical data treatment over the last decade (156).

4.4 Exploration of varnish resins with PCA and HCA

From the review of current published literature, it is apparent that despite the analytical potential held by multivariate techniques, they have not yet been applied extensively to the Raman characterisation of painting varnishes, which still typically rely on chromatographic methods with long preparation and running times. In the experiments presented in this chapter, a group of common natural and synthetic resins used as painting varnishes were examined with PCA and HCA in order to examine the benefits of the application of multivariate analysis for painting resin analysis with Raman spectroscopy.

4.5 Painting varnishes

Painting varnishes are the transparent coating applied over the paint film and play a role in protection as well as having an important influence on the visual appearance of the image. Degradation due to UV exposure, temperature and other environmental influences leads to yellowing of the coating material which significantly affects the way the image is represented. With all tonality shifted toward yellow, whites become yellowish, blues become greenish and pinks or reds become orange and the entire mood of the painting may alter from that which was originally intended by the artist. Degradation of the surface coating can also lead to cracking or a hazy opacity, also having the effect of obstructing the image of the painting underneath.

Where warranted, paintings that have a degraded surface may have the varnish removed using solvent assisted removal. Solvent removal involves wiping the surface with a solvent saturated swab or application of a solvent containing gel, which can soften the varnish layer sufficiently so that the softened varnish can be removed mechanically by wiping with a clean cotton swab. Techniques for cleaning can involve chelating or complexing materials; solvents applied in gels, emulsions, pastes and poultices or resin soaps and enzymes. Obviously, the resistance of the paint film below needs to be carefully tested with any cleaning treatment before progressing to prevent any undesirable action on the paint film. After the removal of a yellowed or degraded coating, paintings are usually re-varnished with a new coating of natural or synthetic resin.

Surface coatings for paintings are thought to have been introduced as a protective coating for protecting water soluble or friable surfaces (148). Easel paintings may even use mediums used for paint binders, but without any pigment or filler (honey, gum Arabic, egg white or mixtures with resins). Paintings described as “oiled” or “oiled out” refer to wiping the dry surface with a drying oil such as that which may be used for a paint binder; e.g. walnut, poppy or linseed.

Specific varnish recipes were recorded as early as the eleventh century and the first were “oil varnishes” which were prepared by boiling painting oils with natural resins such as amber, rosin, mastic or sandarac. First mentioned in 16th century and more widely used in the 17th century, “spirit varnishes”, or “essential oil varnishes” are prepared without oil, and are a solution of the natural resin in a volatile solvent such as turpentine or alcohol. The absence of oil leads to a more brittle film (less durable), however the absence of oil leads to easier removal from the surface of a painting as the cross-linked fatty acid chains of the oil reduce solubility. The most common resins used during that period were thought to be mastic, sandarac and rosin. In the 19th century, dammar was introduced and was thought to be more stable in comparison to other resins. It continues to be in use today with dammar and mastic currently the most popular natural resins (180).

The chemistry of the natural resins used in this experiment was described earlier in chapter 3 so the following discussion only concerns the chemistry of the most commonly used synthetic painting varnish resins. The earliest synthetic resins, such as poly (vinyl acetate) (PVAc) from the 1930s, were never originally developed as surface coatings specifically for paintings, however, they soon became useful in that context. In the late 1930s (181) and through the 1950s (182) synthetic resins based on polymerised acrylates became available and are still used. Poly acrylates are formed by polymerisation of acrylate monomers as shown in Figure 52, through polymerisation through the carbon to carbon double bond. Paraloid B 66, B 67, B 72 and B 82 are produced by Rohm and Haas and are extensively used in conservation as films, adhesives and coatings. Paraloid B 67 is an isobutyl methacrylate homopolymer (PiBMA) and B 72 is a methyl acrylate – ethyl methacrylate copolymer (MA/EMA). B 67 and B 82 are less frequently encountered and so not investigated here. With ageing, these types of films can cross-link extensively and thereby become difficult to remove. They have become less desirable as painting varnishes as a result of their irreversibility upon ageing.

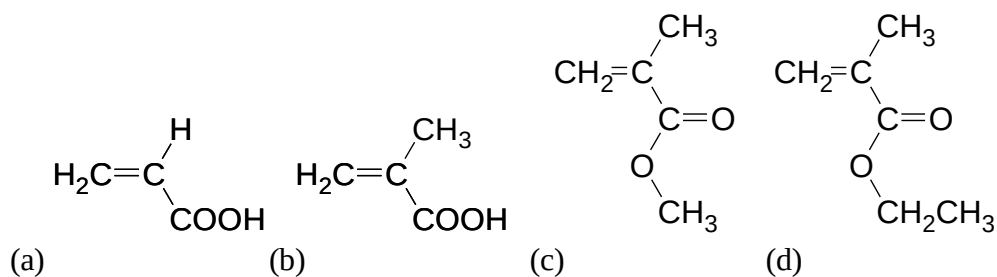


Figure 52. Acrylic acid (a) methacrylic acid (b) methyl methacrylate (c) and ethyl methacrylate (d)

Ketone resins were first patented in the 1920s (183). From the 1950s, products such as Laropal K 80 and Ketone resin N were developed and began to be used as painting varnishes. Both Laropal K 80 and Ketone resin N are currently produced by BASF and are identical in composition although manufactured differently; Laropal K 80 is produced in a continuous process and Ketone resin N in a discontinuous one (180, 184). De la Rie observed that the products are similar with FTIR and gas chromatography, which found an identical GC fingerprint for these two resins. There is less understanding of the exact chemistry of these resins because of commercial restrictions but a proposed structure based on a series of likely aldol condensation, dehydration and oxidation reactions is shown in Figure 53.

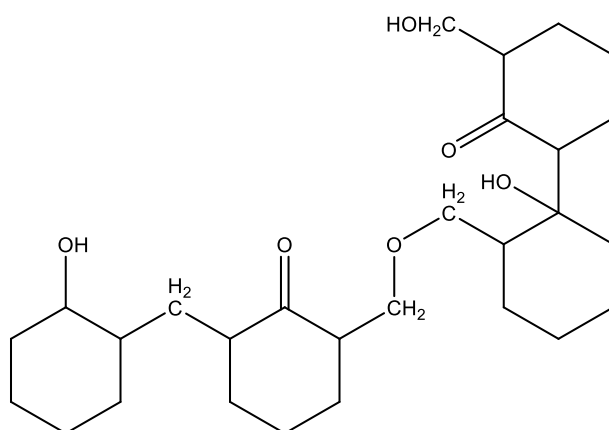


Figure 53. Theoretical structure of cyclohexanone resin (182)

MS2A is another cyclohexanone developed from the reduced form of an earlier product, MS2, produced by Howards in the UK from the 1930s (182).

4.5.1 Methodology

The purpose of this exercise was to explore and illustrate the extent of difference within and between types of natural and synthetic resins. Natural resins are expected to exhibit a greater degree of natural variability, being derived from a natural botanical source, while synthetic resins are more chemically homogeneous due to reproducible production methodologies. The variability demonstrated by samples from a particular type of resin will have direct implications for the process of identification by spectral matching. Where natural resin types have looser or overlapping groupings, they may be more prone to misidentification. An overall representation of spectral similarity can demonstrate resin types that are chemically and spectrally close.

The sample set consisted of 5 synthetic and 6 natural resin types. The natural resins dammar and mastic were obtained from 3 different suppliers to ensure different sources of the same type of resin were incorporated. Details of the resin samples are listed in Table 7.

Table 7. Synthetic and natural resins examined

Category	Resin	Source	Chemistry
Synthetic	Ketone Resin N	Jack Harrison (artist's materials)	Cyclohexanone
Synthetic	Laropal K80	Lascaux	Cyclohexanone
Synthetic	MS2A	Linden Chemicals	Cyclohexanone
Synthetic	Paraloid B67	Rhom and Haas	Isobutyl methacrylate homopolymer (PiBMA)
Synthetic	Paraloid B72	Rhom and Haas	Acrylic/methacrylic copolymer
Natural	Amber	Kremer pigmente	
Natural	Colophony	Kremer pigmente	
Natural	Copal	Kremer pigmente	Diterpenoid
Natural	Dammar	Kremer pigmente	Triterpenoid
Natural	Dammar	Jack Harrison (artist's materials)	Triterpenoid
Natural	Dammar	Langridge (artists materials)	Triterpenoid
Natural	Mastic	Kremer pigmente	Triterpenoid
Natural	Mastic	Jack Harrison (artist's materials)	Triterpenoid
Natural	Mastic	Langridge (artists materials)	Triterpenoid
Natural	Sandarac	Kremer pigmente	Diterpenoid

Raman spectra of each raw resin were recorded on a Renishaw In-Via micro-spectrometer with 300 mW diode laser at 830 nm and an electrically cooled CCD detector. As shown in chapter 2, 830 nm incident irradiation can mitigate fluorescence from binding media and varnishes. Collection was performed through a 50 x objective lens. Laser power was set at 10% (15mW). For each spectrum, 3 accumulations of 10 s scans over the range 100 to 2000 cm^{-1} were collected (2303 wavenumber points). The spectra of 5 separate granules of each resin sample were collected. Spectra were inspected for cosmic ray spikes as collected and where found and removed manually using baseline-to-baseline averaging. Spectra were saved in .spc format using the Renishaw WIRE 2 software program. All spectra were imported into Origin v7 in .txt format to check for consistency in the number of collected data points.

Spectra were imported into The Unscrambler v 9.7, Camo, Norway. The Unscrambler provides three methods to estimate the prediction error: test set validation, cross validation and leverage correction. Samples were subjected to PCA analysis with full cross validation and data centering. PCA was performed both with and without spectral correction to evaluate the influence of spectral correction on the output. Correction involved 3-point smoothing, baseline correction and

normalisation (rescaling to the range between 0-1). A simple linear baseline correction was used to reduce the additional variance that an operator may introduce with an interactive polynomial or cubic spline correction.

Data were re-formatted in Origin v7 and saved as a .csv matrix before being imported into a separate program for HCA, which was performed using PC-ORD for Windows v 4.25. One outlier sample, dammar JNH_0, which was identified by the PCA treatment, was not included in the HCA treatment.

4.5.2 Results and discussion

4.5.2.1 PCA

The 5 replicate Raman spectra from each of the 15 natural and synthetic resin samples are shown in Figure 54 to illustrate the variance observed between individual spectra from different granules within one resin type.

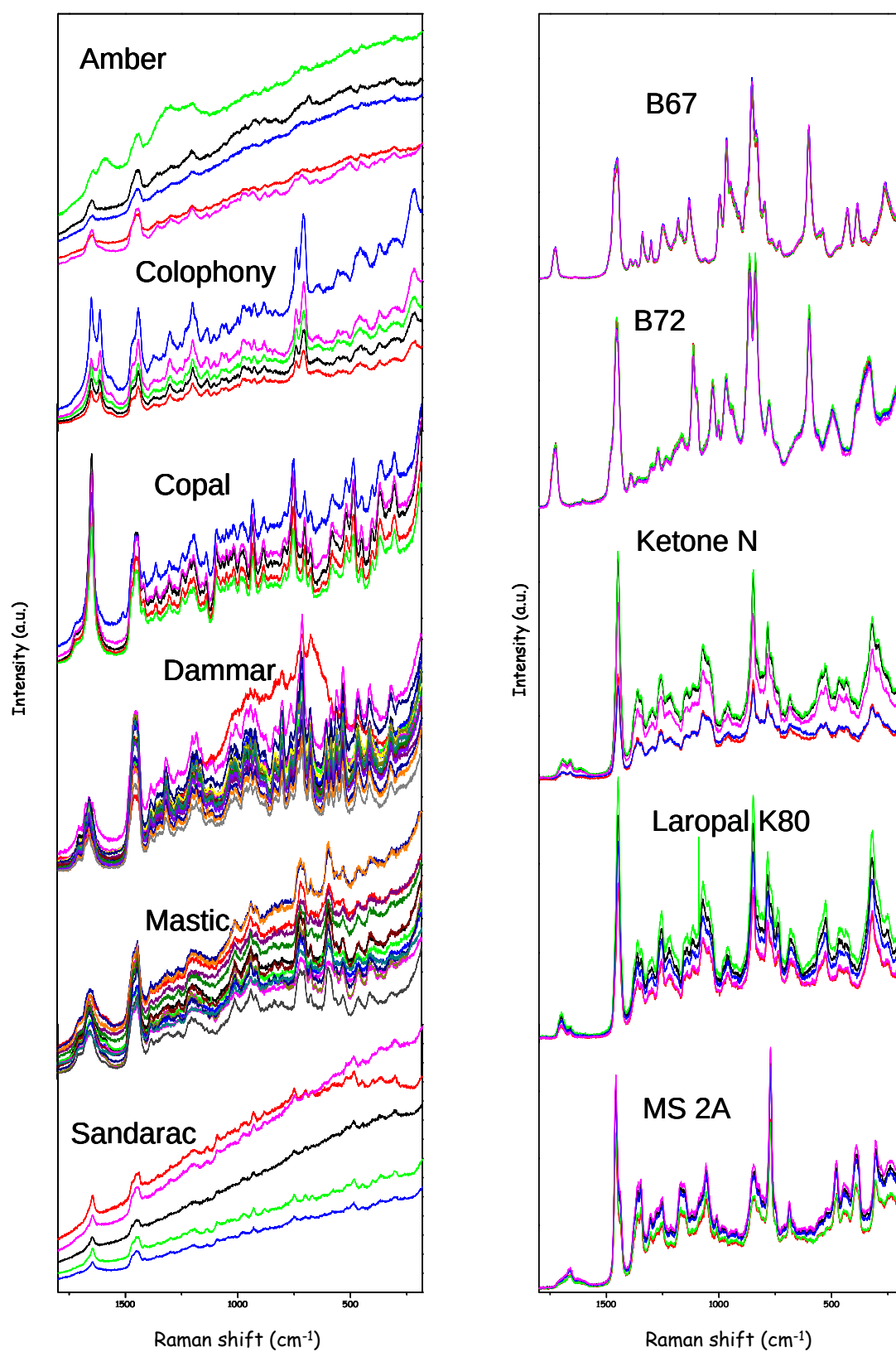


Figure 54. Overlaid Raman spectra from individual natural and synthetic resin samples. Dammar and mastic from 3 sources are presented together. Plot range: 1800 – 180 cm⁻¹

The synthetic resins were found to be extremely homogeneous in composition with little spectral variance within a resin type. The natural resin spectra were generally more variable within a resin type, which might be expected for naturally derived materials. The differences in amber related primarily to the baseline profile (due to inherent fluorescence) rather than peak positions or relative peak heights. A consistently strong spectral feature common to all the resins examined here (both synthetic and natural) is a band occurring at approximately $1410 - 1480\text{ cm}^{-1}$ due to $\delta(\text{CH}_2)$, $\delta(\text{CH}_3)$ vibrational modes. A band at $1600 - 1740\text{ cm}^{-1}$ was observed in the synthetic resins with low intensity, and much higher intensity in the natural resins. This band is attributable to $\nu(\text{C}=\text{C})$ conjugated with $\text{C}=\text{C}$ or $\text{C}=\text{O}$ modes which appear more in the di- and tri-terpene natural resin structures than the synthetic resin structures described previously.

Comparison of the differences between resin types overall, by visual inspection of 75 spectra simultaneously, as shown in Figure 55, is challenging. Ordination of the spectral data with PCA was used to visualise and explore the data structure and relationships.

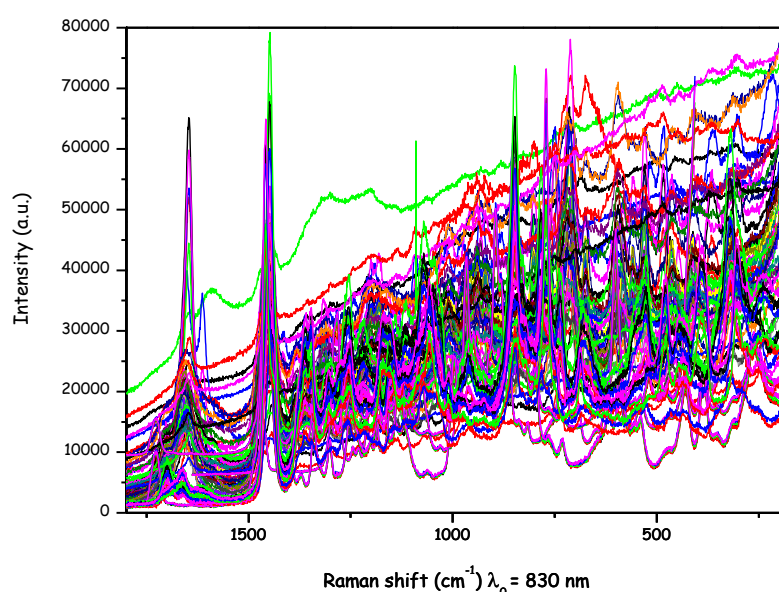


Figure 55. Overlaid corrected Raman spectra from all 75 natural and synthetic resin samples. Plot range: $2000 - 240\text{ cm}^{-1}$

A scores plot of all the PCA transformed spectra is shown in Figure 56. The plot shows the overall relationships between the entire set of samples. The two types of acrylic resins are very tightly clustered because of consistency in the sample composition and homogeneity, which is possible in synthetic products. In contrast, the amber and sandarac spectra are spread quite

loosely. The higher variance could be attributable to differences in the source, as the sample material is made from beads of natural resin that are not expected to be compositionally identical, with individual differences in trees and the growing environment. One dammar sample is an outlier in the plot, but it was not removed at this stage as it gives some indication of the experimental sample spread, which may be observed. A PCA transform was initially performed on untreated data and, although not presented here, only showed the major grouping arrangements and was not able to separate the dammar and mastic or other spectrally similar samples satisfactorily. Differences in spectral acquisition are believed to be due to sample presentation; geometry and illuminance conditions during the spectroscopic measurement, scattering and fluorescence. This demonstrated the importance of appropriate spectral pre-treatment such as baseline correction, normalisation and smoothing prior to PCA treatment.

Table 8. Tabulated eigenvalues (calibration) for 20 PCs for the resin samples

Eigenvalue	Explained variance
PC_00	0
PC_01	42.4
PC_02	68.4
PC_03	77.5
PC_04	84.8
PC_05	89.0
PC_06	92.0
PC_07	94.1
PC_08	95.7
PC_09	97.2
PC_10	98.3
PC_11	98.7
PC_12	99.0
PC_13	99.3
PC_14	99.5
PC_15	99.6
PC_16	99.7
PC_17	99.7
PC_18	99.8
PC_19	99.8
PC_20	99.8

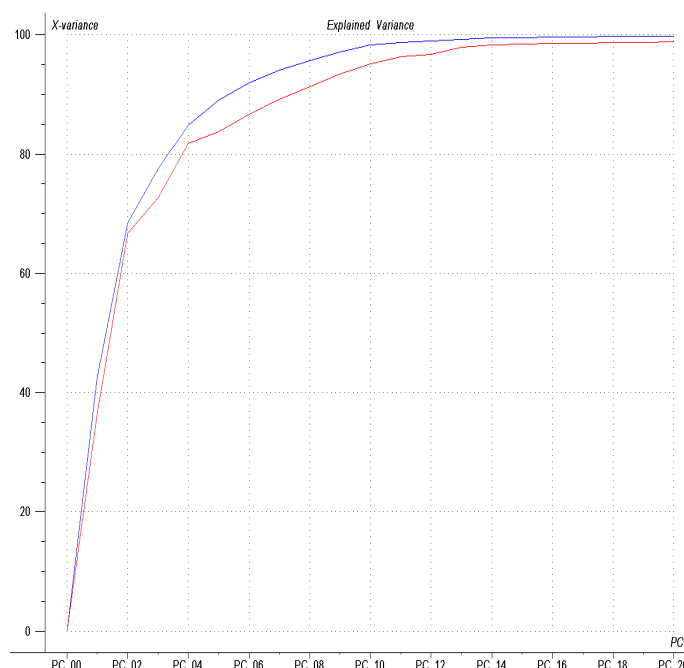


Figure 57. Eigenvalues for 20 PCs for resin dataset. Calibration values shown in blue, validation in red

The results of the PCA analysis are shown numerically in Table 8 and graphically in Figure 57, indicating 10 PCs, which account for 98% of the variance in the data. The remainder of the variance can be considered to be noise. Thus 10 PCs can be considered to sufficiently represent the information contained in the dataset. In practical terms, Eigenvalues may be used to indicate the number of components or significantly contributing factors or variables in a mixture. In this exercise the wavenumbers are the variable parameter, so there are approximately 10 wavenumber positions (out of the 2303 collected), which comprise the majority of the overall variance.

The loadings plots for the first 4 PCs are shown in Figure 58. Loadings indicate the influence of each variable (wavenumber) on a particular PC and can be used to indicate the underlying factors contributing to the PC. Here it can be seen that the major influences acting on the first, third and fourth PCs are the position and intensity of particular and specific peaks, while the major

influence on the second PC is the gently sloping rise of a baseline curve. The position of individual peaks is discussed in more detail in the following section.

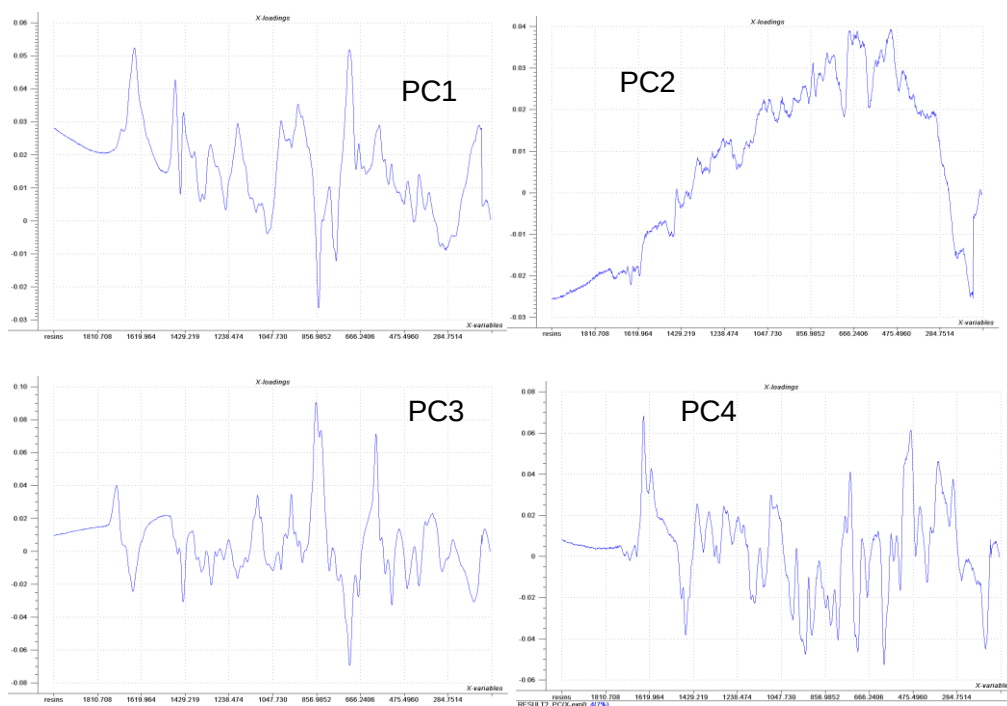


Figure 58. Single variable loadings plots for PCs 1–4

The cyclohexanone resins grouped together and were distinctly separated from the natural resins. An expanded region showing only cyclohexanone resins; MS2A, Ketone N and Laropal K 80 is shown in

Figure 59.

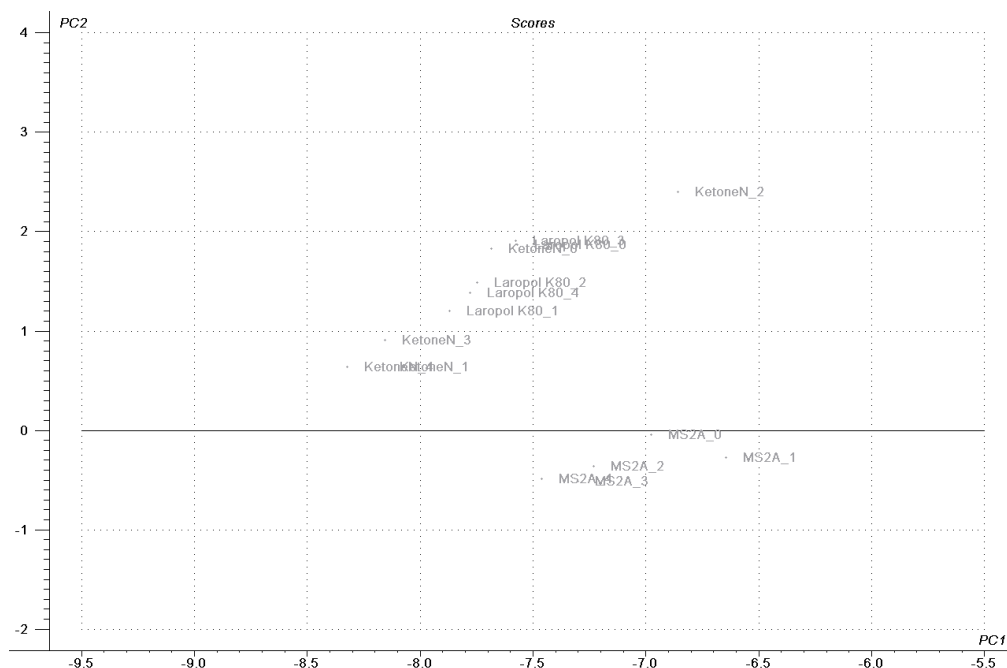


Figure 59. Scores plot of PC1 vs PC2 for cyclohexanone samples

They appear to form 2 reasonably tight groups. Ketone N and Laropal K 80 are near one another and MS2A is clearly distinguishable as a separate compound. This provides further evidence that Ketone N and Laropal K 80 are the same compound even though they are manufactured using different processes.

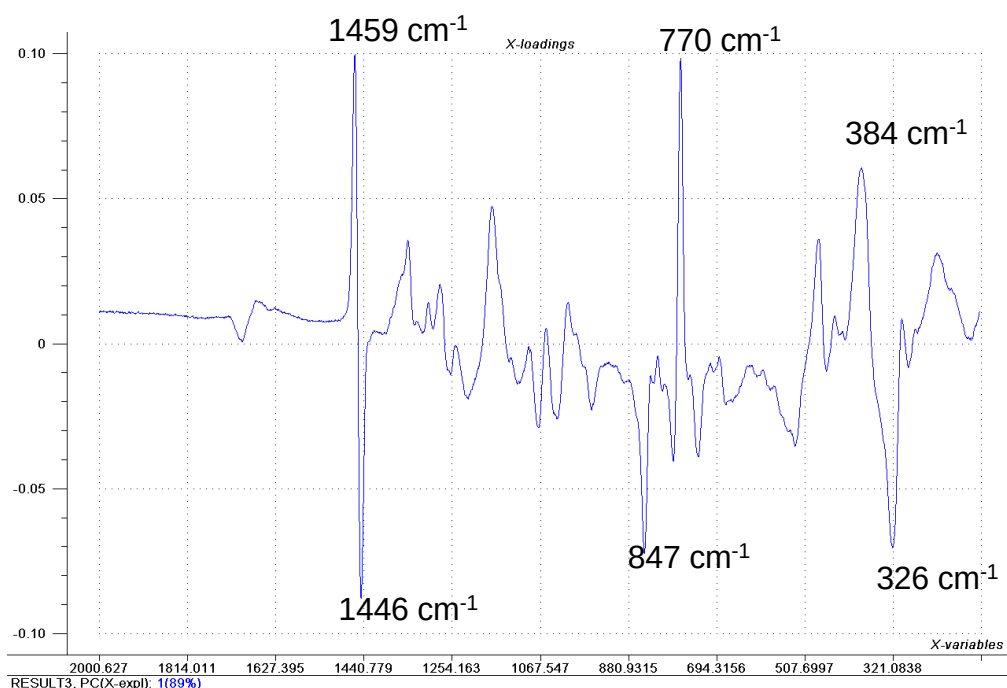


Figure 60. Loadings plot of PCA of cyclohexanone resins

PCA of only the cyclohexanones produced the loadings plot shown in Figure 60. This is not simply a difference (subtraction) plot but represents the wavenumbers responsible for overall variance between samples. A high magnitude in the loading plot indicates the position of wavenumbers, which have greatest contribution to the variance. The vibrational modes responsible for spectral differentiation between MS2A, Laropal K 80 and Ketone N are listed in Table 9.

Table 9. Vibrational modes separating MS2A from Laropal K 80 and Ketone N

Wavenumber position (cm ⁻¹)	Group	Vibrational mode
1459	R-(CH ₂) ₄ -O	CH ₂ def
1446	R-(CH ₂) ₄ -O	CH ₂ def
847	C-O-C	symm str
770	CH-(CH ₃) ₂	skeletal vib
384		unassigned
326		unassigned

A plot of only dammar and mastic samples is shown in

Figure 61. The plots are presented both including the outlier sample JNH_O and re-calculated without the outlier to demonstrate how different the outlier is from either of the 2 sample groups, although the individual spectrum (Figure 53) appears to contain the same peak positions. The high baseline and low signal to noise ratio in the individual spectrum contributes variance, which overwhelms the intra-sample relationships. This is a limitation of this approach because any sample with inconsistency in the shape of the fluorescent baseline will have that inconsistency represented as spectral variance.

Baseline subtraction in Raman spectra is generally done manually on an individual basis because the shape of the fluorescence can vary from sample to sample, and even at different points in the same sample. Cubic spline interpolation, polynomial subtraction or point-to-point are the usual options in proprietary software programs. An approach where the running minimum of wavenumbers (20 or 30) is subtracted from the spectrum is useful in some cases, but the expanse of overlapped peaks in the region approximately from 370 to 1500 cm^{-1} means this is not possible for natural resin samples.

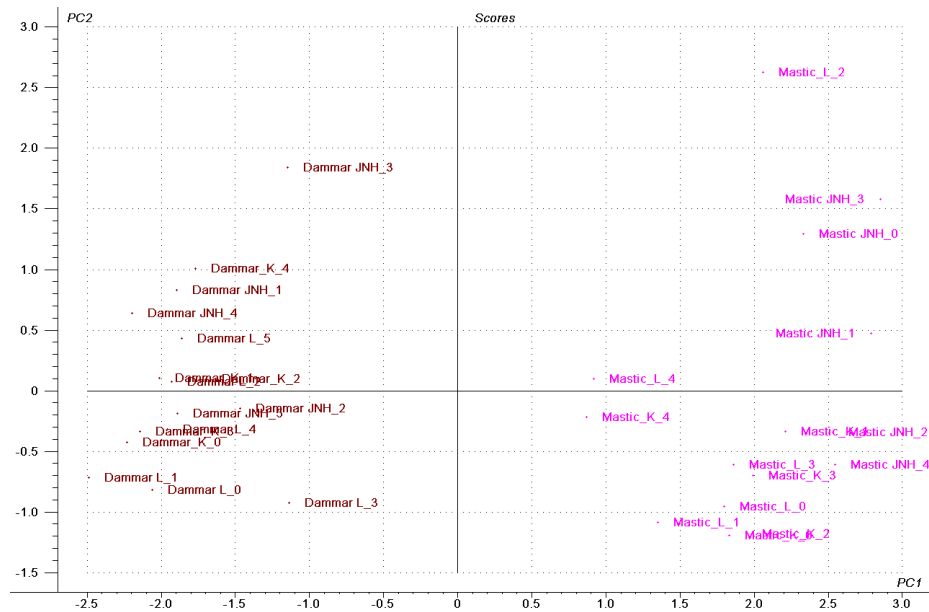
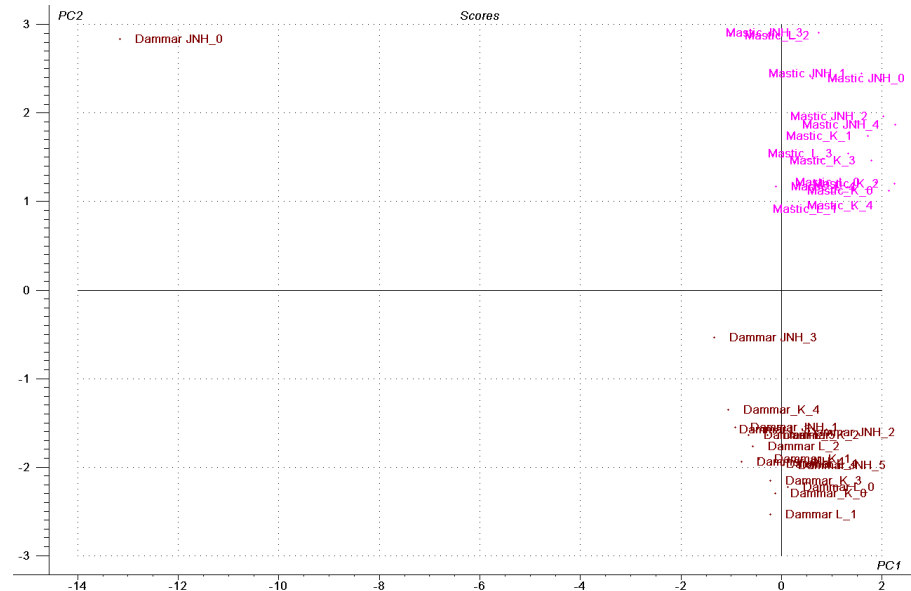


Figure 61. Scores plot of PC1 vs PC2 for dammar and mastic including outlier (above) and recalculated without outlier (below)

Mastic is a separate cluster, easily distinguishable from dammar. The source or supplier does not appear to have an influence the group placement, so this technique is therefore unable to distinguish between suppliers although it does satisfactorily distinguish between resin types due to the measurable difference in chemical structure that translates into a spectral response.

The wavenumbers with the greatest influence on the first PC of the model are shown in the loadings plot circled in red (Figure 62a). As indicated in Figure 62b, the peak especially at 594 cm^{-1} but also 528, 698, 798, 853 and 1640 cm^{-1} (wherever the magnitude of the loading is high) indicates the magnitude and position of wavenumbers, which are responsible for the distinction between mastic and dammar.

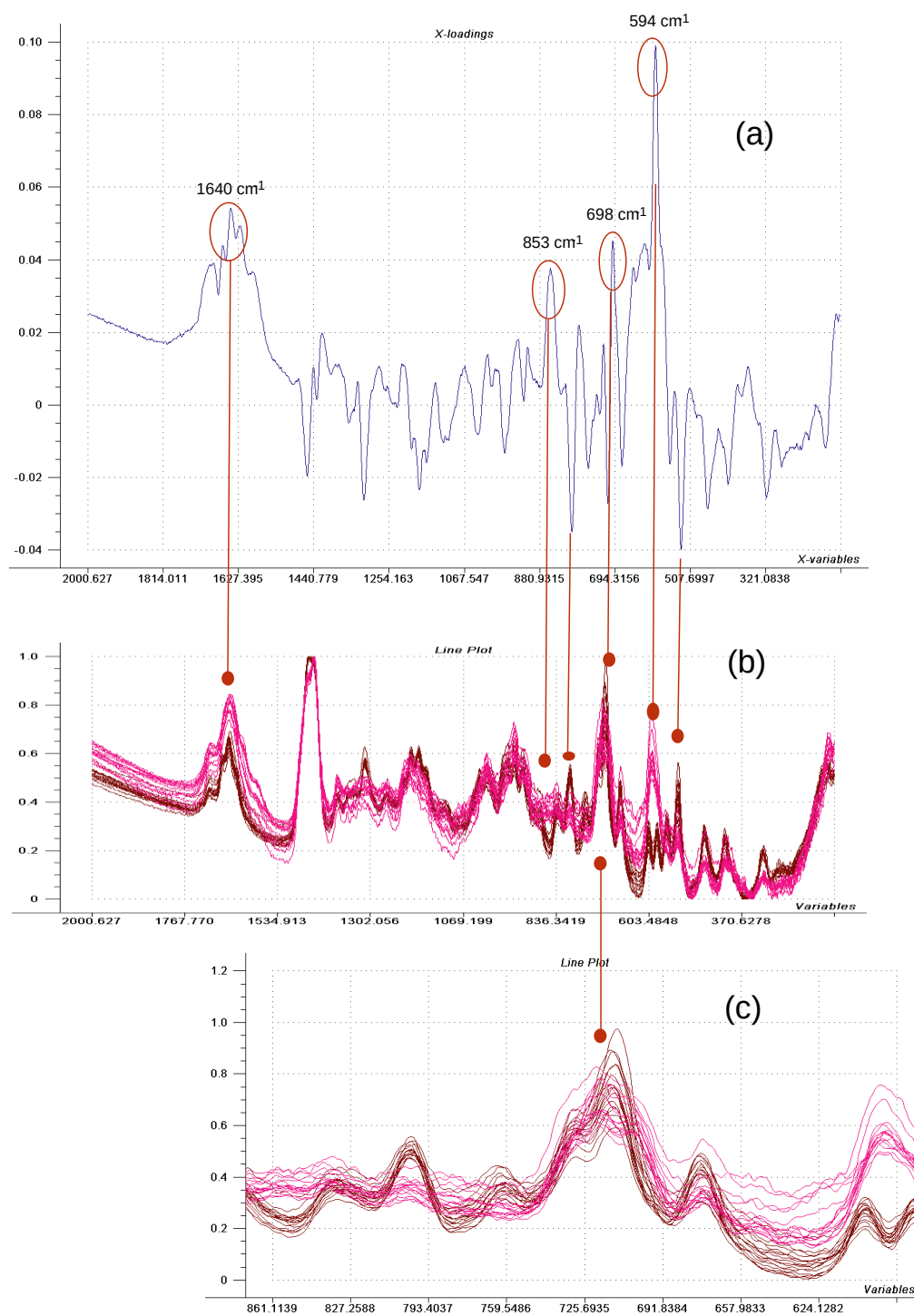


Figure 62. Loadings plot for the first PC (a), overlaid dammar and mastic spectra (b) and expanded region of dammar and mastic spectra (c)

Since most of the variation in the data can be explained by these variables, this data reduction technique suggests these wavenumbers could be used as indicators in a screening process, eliminating the need to measure the entire spectrum from 2000 to 100 cm^{-1} . In practice it is unlikely that an analyst would choose to measure selected or individual wavenumbers, but rather a reduced wavenumber range. In applications where numerous spectra may need to be collected, a reduced spectral range, which incorporated the regions of maximal loadings would be sufficient and still achieve the same result with shorter collection times. Here, the region to be covered would be 500 – 1800 cm^{-1} .

4.5.2.2 HCA

HCA is an alternative technique to visualise clustering and examine grouping structure. It was performed on the Raman spectra of the natural and synthetic resins to evaluate their range of similarity and variability. A dendrogram groups samples hierarchically, according to the similarity of the overall spectral character with the horizontal axis of the dendrogram representing the distance or dissimilarity between clusters. The vertical axis represents the objects and clusters.

The first dendrogram shown in

Figure 63 was performed on uncorrected Raman spectra. The mastic samples were grouped unsatisfactorily as they were split into two groups placed either side of the dammar, copal and colophony groups. A second analysis was then performed on the data matrix treated in the same manner as for PCA. After smoothing, baseline correction and normalisation, the HCA result is shown as a dendrogram in Figure 64. Sandarac and copal were somewhat mixed however this was considered acceptable since we now know these samples have higher variance as shown by PCA, and all the remaining samples were grouped satisfactorily.

The dendrogram clearly shows the acrylic resins as being distinct from each other and all other resins. The cyclohexanones are grouped according to their composition successfully; Ketone N and Laropal K 80 are together and the other cyclohexanone, MS2A, is a separate group but related at the 50% level.

The resins dammar and mastic are quite close but have about 20% of the information separating them. The two resin types are therefore distinguishable with this approach even though they are both tri-terpenoids. The dendrogram is an excellent representation of the data structure as the single sample types are effectively grouped at the lowest branching level.

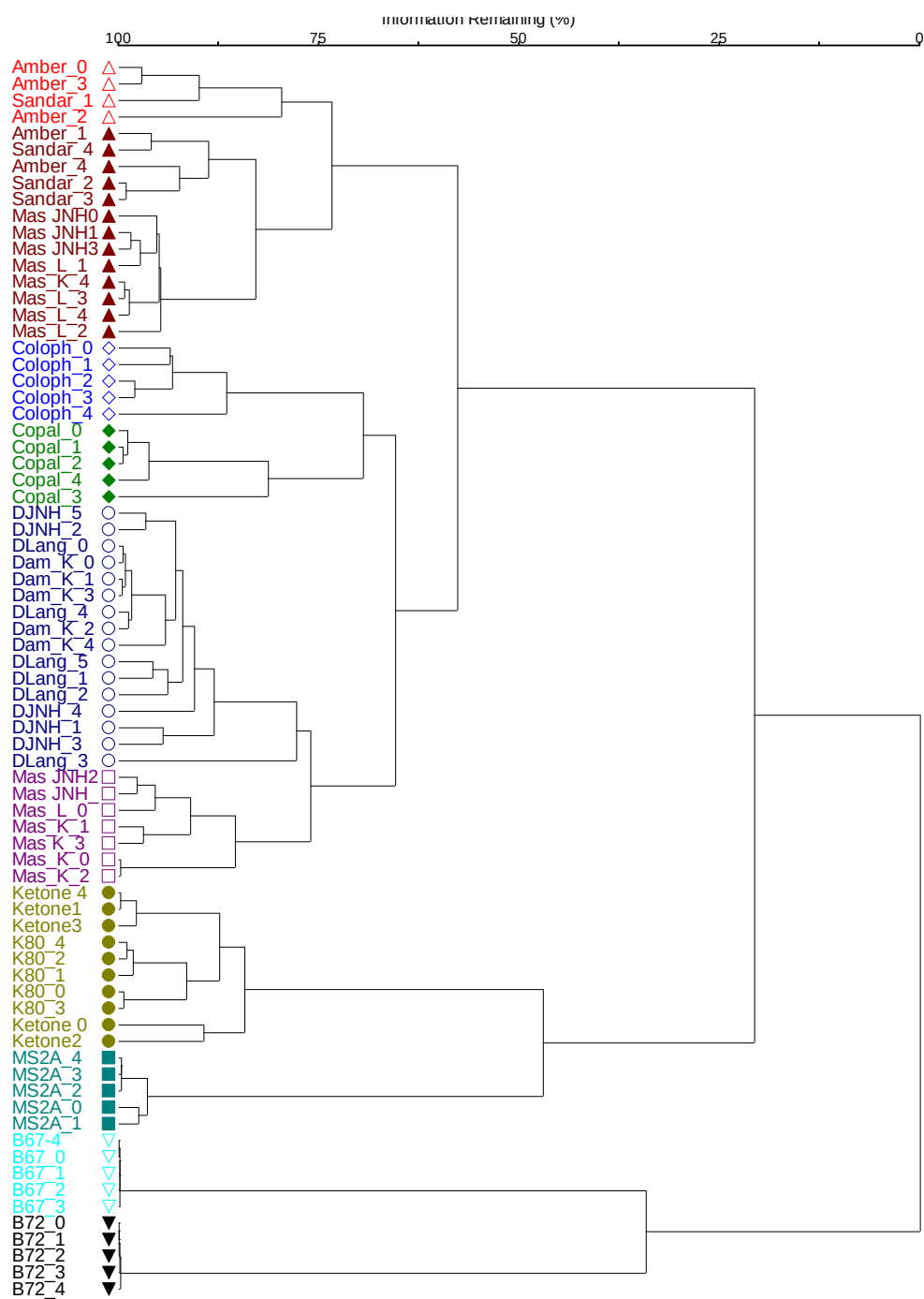


Figure 63. HCA dendrogram of uncorrected Raman spectra

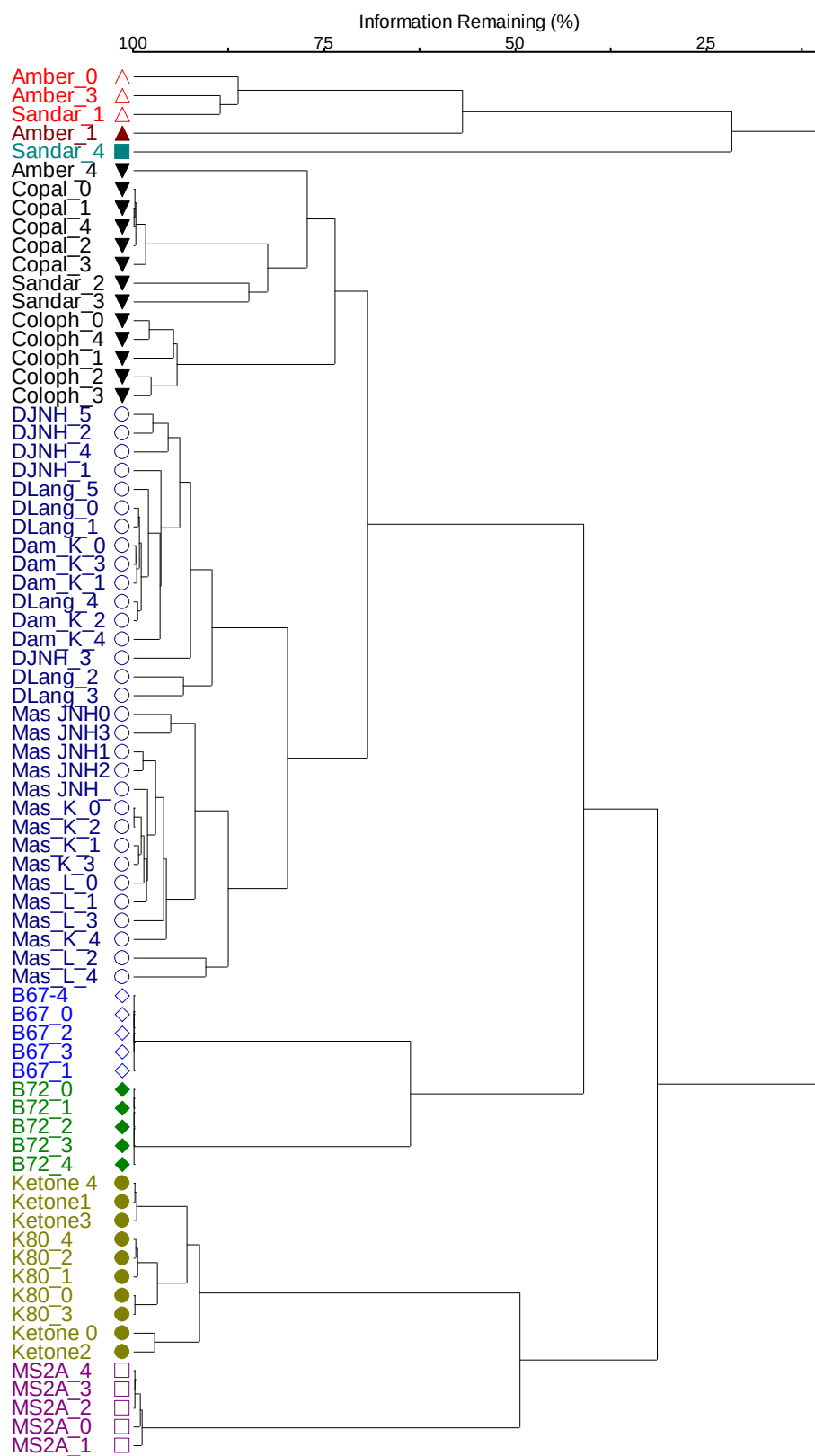


Figure 64. HCA dendrogram of corrected Raman spectra

Through the treatment of spectra as multivariate data sets, it is possible to evaluate whether there is a discernible spectral difference between two groups of chemically similar material with very similar spectra and aid the decision about whether the difference is attributable to individual sample variance or not. Inspection of the factors accounting for the majority of variance between the samples can indicate which factors are responsible for those differences. Because the variables are wavenumbers in a spectrum, it is possible to infer which chemical groups are responsible for the observable differences.

As previously discussed, discrimination between the vibrational spectra of natural resins is often a difficult prospect and this is acknowledged by Creagh (185) who states “Identification of the binding medium by FTIR is sufficient only if the general category of the binding medium is needed, e.g. drying oil, wax, resin, gum or a proteinaceous material. The technique cannot be used to characterize these compounds further, with certainty”. As shown, the use of multivariate classification techniques such as PCA and HCA can visually assist this process and classify further than the general category.

It is clear that spectral contribution from any other material would render this technique ineffective as the spectral character and subsequent variance would be attributable to other factors. However, in instances where pigment sits on the surface of the painting support with minimal or no measurable binding media, such as in watercolours or illuminated manuscripts, this may be a viable technique.

4.5.3 *Conclusions*

In this chapter the application of the multivariate data analysis techniques PCA and HCA have been used to explore the data structural relationships amongst Raman spectra of natural and synthetic resins. It has been demonstrated that the two most utilised natural resins, dammar and mastic, are able to be distinguished from one another by PCA and HCA of their Raman spectra, irrespective of their supplier and the naturally occurring sample variance. An exploration of samples from other sources and suppliers would be beneficial in understanding a broader sample selection spread, however the resins of two Australian suppliers and one German are a useful starting comparison.

It has also been shown, using PCA and HCA, that the synthetic cyclohexanones resins Ketone N and Laropal K 80 are indistinguishable while the other synthetic painting varnish cyclohexanone, MS2A, is easily separable. The synthetic resins were found to be quite homogeneous in composition and consistent in their Raman spectral response compared to a very much higher degree of variance observed within the natural resins: amber, copal, colophony and sandarac.

MS2A went out of production in 2014 and has now been redeveloped by an Australian manufacturer for the conservation market (186). Quality control and product standards are important for chemical manufacture and the techniques developed here have demonstrated the feasibility for multivariate analysis and Raman spectroscopy to monitor production of new resins.

Methods for Raman spectral processing that were developed by the author in this chapter have subsequently been applied to other spectroscopic characterisation techniques in conservation. PCA was applied to X-ray absorption spectra and used to identify green copper phase pigments in an Egyptian artefact. Data projection using PCA was used to explore spectral data variability within the dataset and to illustrate the relationship between the spectra and copper speciation. A resulting training set of the reference materials were compared with samples to suggest the identity of green pigments that were unable to be characterised by other instrumental techniques (187).

The method has been applied to the analysis of Near Infrared (NIR) spectra, whereby PCA projections were used to visualise the variance between different sample locations on petroglyphs (a form of Rock Art created by engraving the surface of rocks to create the image) (188), and to explore whether there were observable differences in spectra collected over a 7-year time period. A transition in spectra over that time was not observed.

The spectral processing method developed here has also been applied to FTIR analysis of adhesives used in conservation repair. The grouping structure of FTIR spectra of adhesives illustrated the variance observed within and between types of archaeological pottery adhesives (189). Furthermore, in a later study of the pottery collection, cellulose nitrate was found as a component in PVA adhesive formulations and demonstrated using PCA (190). The work was valuable in revealing adhesive formulations, knowledge that was used to inform the preservation management of pottery collections.

Chapter 5: Multivariate Raman image analysis

The previous chapter discussed the application of multivariate data analysis to the analysis of Raman spectra from individual samples, the subsequent exploration of relationships between the samples, and influence of associated variables. Raman spectral images may be produced by the sequential collection of individual Raman spectra across a sample surface, with each point in the image (pixel) considered to be a single sample so the entire image dataset can be treated using the same multivariate statistical approaches as described for individual samples. The work presented in this chapter extends the principles of PCA and classification of individual samples to the analysis of Raman image data collected from painting cross sections.

5.1 The multivariate image

A grey-scale image can be thought of as univariate, where each pixel in the image has a single value, which is the brightness value. In a bitmap image, the scale from black to white extends from 0 = black to 255 = white. The single variable is the grey-scale value. In a red-green-blue (RGB) colour image, each pixel has a specific intensity value of each of the three colours and appears as a composite of all three, with intensity values for each colour component; red, green and blue (Figure 65). Each pixel has three variables. The relative proportions of all three define the colour that is seen. The variables, RGB, are also referred to as spectral bands, or simply as bands.

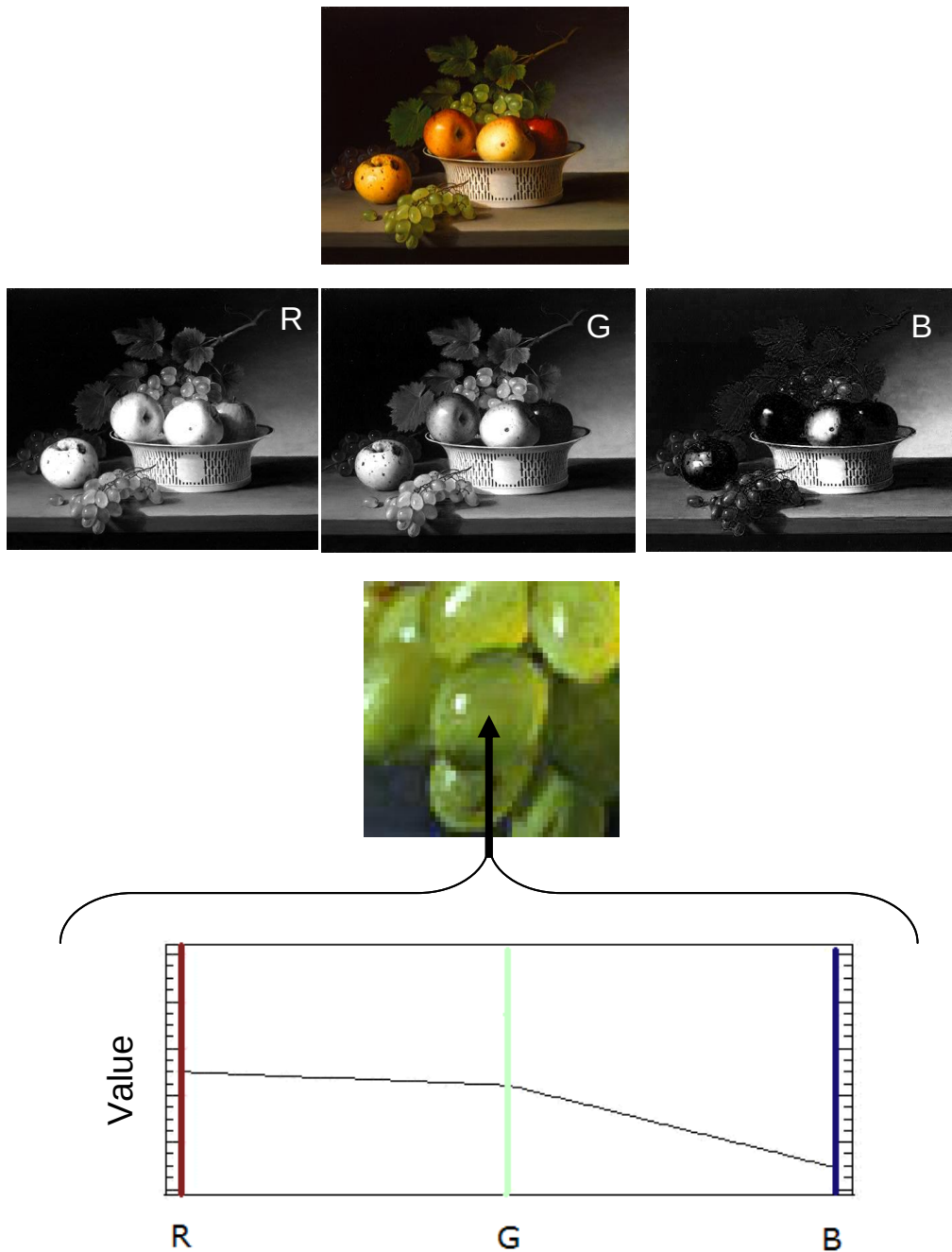


Figure 65. Full colour and single band images (RGB) of a coloured image and the spectral profile of the pixel in the middle of a grape

A spectral map consists of pixels with x and y co-ordinates. Rather than a value for three bands (RGB) as in a colour image, a Raman map image contains an entire Raman spectrum at each pixel in the image. Each pixel contains the data of hundreds or thousands of wavenumbers = variables = bands (z). The compilation of all the data into a single 3-D data cube (x,y,z) is represented in Figure 66 which comprises the entire multivariate dataset.

Because the approaches used in this area use methodologies from the fields of statistics, spectroscopy, geospatial mapping and chemistry, the differences in terminology can be confusing. Multivariate refers to the multiple variables (the intensity values (z) at n Raman wavelengths. Data in each (x,y) pixel can be treated using the multivariate statistical approaches of PCA and clustering techniques. In geospatial imaging, the process is called multi or hyperspectral imaging with “multi” referring to several variables and “hyper” indicating hundreds or thousands of variables.

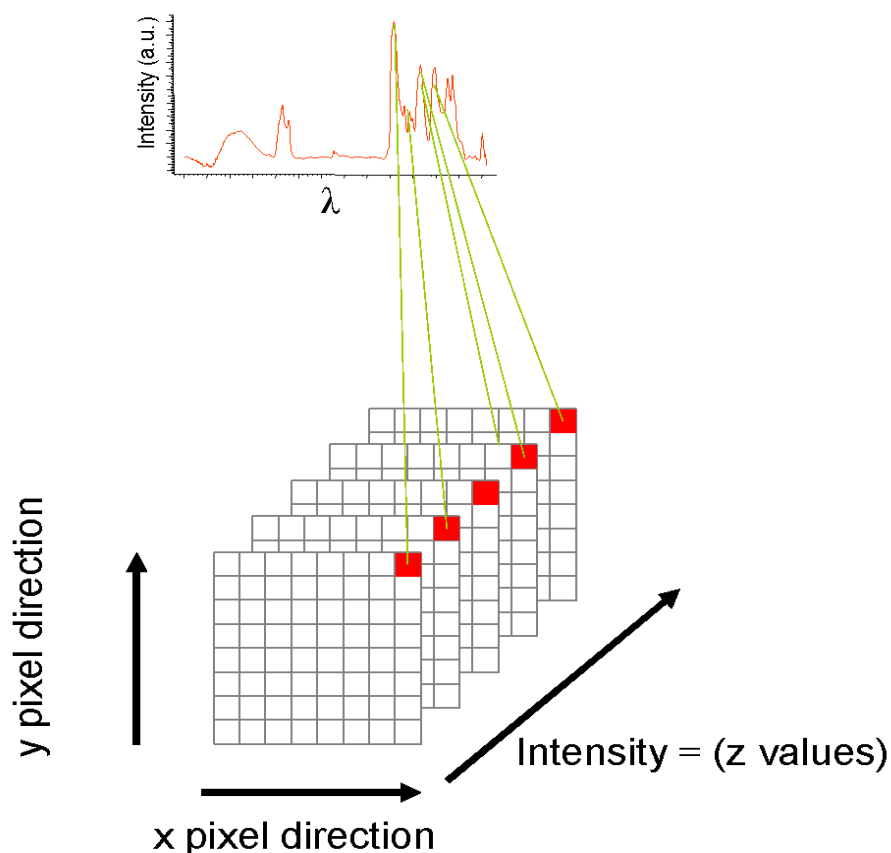


Figure 66. Schematic diagram of the values used in compilation of a spectral data-cube for multivariate image analysis

This technique has evolved in the fields of remote sensing; telemetry and geospatial analysis, which are growing rapidly. The discipline is constantly developing new data processing and data mining tools to improve detection and classification of terrain, minerals, vegetation and weaponry. Remote sensing has even been used to locate buried ceramic and obsidian artefacts using visible and NIR reflectance spectroscopy amidst backgrounds of soil, rock and vegetation

(191). Multivariate imaging on a microscopic scale using the vibrational spectroscopic techniques of FTIR or Raman spectroscopy is gaining popularity in biomedical imaging and pharmaceutical or product development (192, 193).

Microscopic multivariate imaging techniques were not prevalent in the conservation literature at the time this work was started, but there were several examples of multivariate imaging being used on a macroscopic scale. Fluorescence imaging has been coupled with PCA to examine fluorescent organic binders and pigments in fresco paintings in Italy (194). Paintings by El Greco were examined using diffuse reflectance and fluorescence spectra to identify painting pigments (195). A number of blue pigments were located and identified in paintings from the Australian War Memorial in a study by Kubik, whereby a library of pigments was created and reflectance spectra from the visible and NIR regions were compared with the library to map pigment distribution (196). Bio-deteriorated (due to blue-green algae) calcarenite from the balcony of a cloister was examined with multivariate image analysis and PCA of Fiber Optics Reflectance Spectroscopy (FORS) in the visible and NIR range 400 – 2200 nm to detect bio-pitting corrosion underneath protective covering layers (197). An example of smaller-scale imaging in the conservation realm is the use of multivariate imaging using spectral reflectance to examine historical documents covering an area of 125 x 125 mm. The technique collected 70 bands with pixel dimensions 60 μm x 60 μm (198).

Overall, in 2007 – 2008, no published research was found, which examines the application of multispectral imaging to Raman micro-spectroscopy in the field of cultural heritage and conservation, but the advantages of the technique are equally applicable and were explored in this chapter. Image analysis of spectral data incorporates a plethora of options for data treatment, but those with the greatest utility for Raman spectral data sets are:

1. *Unsupervised classification* groups pixels together based on their degree of statistical similarity. There are several methods used for partitioning; in this chapter K-means and Isodata are used. K-means randomly distributes K (an operator defined number) centroids, then iteratively clusters the pixels into the nearest class. Each iteration calculates a new mean and then re-calculates the alignment. This process continues until there is no change in pixel assignment, or until the maximum number of iterations is reached (166, 199). Isodata utilises a similar principle, but the number of centroids is specified as a range, and classes are iteratively merged, split or deleted in order to achieve

the minimum standard deviation threshold or until the maximum number of iterations is reached (199).

2. *Spectral matching (supervised classification)* involves matching spectra in an image with a known reference spectrum of a material. A reference spectrum may be derived from the image itself, or from a reference library. Important considerations with this approach are the intensity scaling of the image and reference spectra, and the wavenumber spacing if a different reference spectrum is used. If the spectral reference is derived from within the image, it may be a single pixel spectrum, or a group of pixels, which comprise a selected area of the image representing a single constituent.
3. There are several methods for evaluating the distance between the reference and sample spectra; Parallelepiped, Minimum Distance, Mahalanobis Distance, Maximum Likelihood and Spectral Angle Mapper (SAM).
4. *Data transform*, such as PCA treatment whereby the transform function is used to produce a new set of uncorrelated bands, which contain the data variance which can be represented in sequential images. The first PC contains the majority of the variance; the second contains the next greatest amount and so on. The last bands are usually due to noise and therefore do not contain compositional information.
5. *Intensity mapping* is the map of the intensity of a particular wavelength value. Typically, it is the intensity of a characteristic peak of a compound of interest, so it maps the spatial distribution of the compound but is reliant on the compound being known or previously identified in the sample. It also requires that there is no interference or contribution to intensity from adjacent peaks.

Using a laboratory prepared sample of pigment in synthetic binder, each of the above-described approaches for image analysis were implemented and are described in this chapter.

5.2 Method

A mixture of equal proportions of 0.2 g titanium dioxide, 0.2 g ultramarine and 0.2 g azurite were mixed then added to 10 mL Struers Epofix epoxy mounting resin which was mixed with hardener mixed according to manufacturers' instructions. The resin was cured at room temperature for 24

hours and wet/dry ground with 500, 800, 1000 and 4000 grit abrasive SiC paper to reveal the embedded pigment particles.

Two Raman maps of the exposed surface were collected, one at 514.5 nm and a second at 830 nm, using the proprietary software for the Renishaw Raman, WIRE 2.0 and saved in .txt format. The map data were exported to Origin7 where it was converted to a single column of ASCII data so it could be read and processed using the geospatial software program ENVI 4.2. PCA and cluster analysis were performed on each set of map data collected at 514.5 nm and 830 nm.

5.3 Results and discussion

Optical images of the mapped areas are shown in Figure 67 with mapped points indicated by the green crosses. A high proportion of binder was used in the pigment dispersion so that any effects of binder on the interpretation of map data could be evaluated. Epoxy was chosen as it does not fluoresce at 514.5 nm and cures within a manageable timeframe. Each mapped area was chosen so as to include the three pigments included in the mixture, for the purposes of evaluating the mapping and multivariate data treatment. The large white particle is titanium dioxide, the dark blue is ultramarine and the paler blue is azurite.

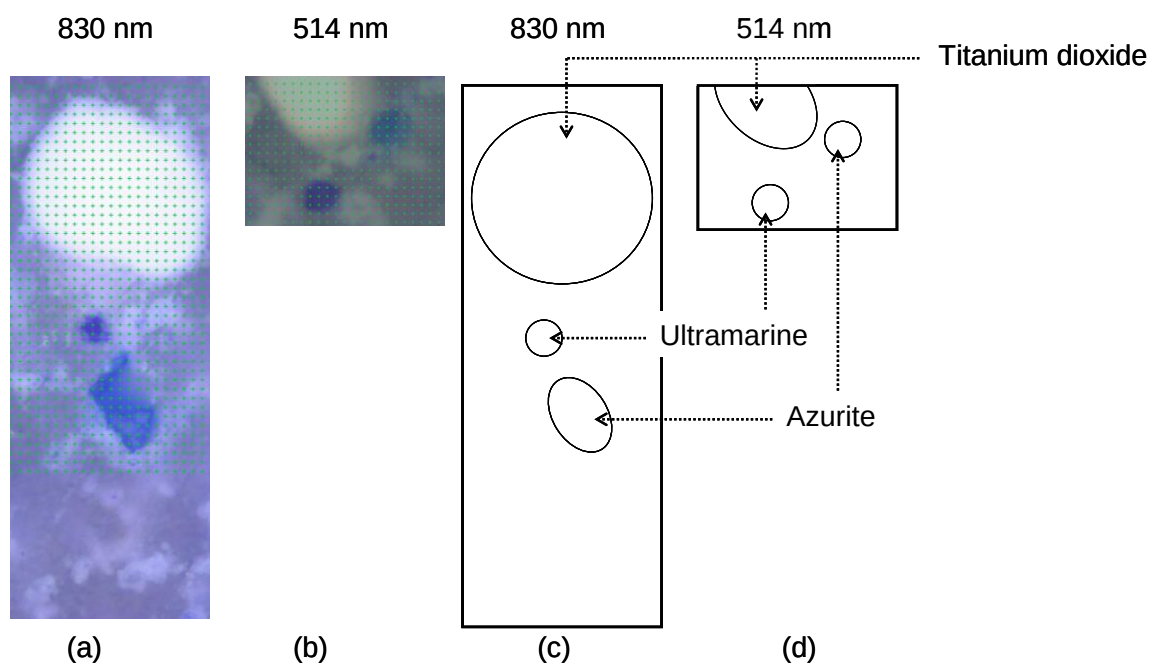


Figure 67. Resin bound pigment samples. Raman maps were collected at 514 nm (a) and at 830 nm (b). Mapped points are indicated by green crosses. Pigment grain locations in maps were collected at 514 nm (c) and at 830 nm (d)

Individual spectra from the 514 nm map of the sample and the 830 nm map of the sample are shown in Figure 68 and Figure 69 respectively. Representative spectra of the binder were collected in the area away from the pigment grains and are shown in Figure 70. The binder does not exhibit a Raman spectrum when irradiated with the 514 nm laser, but with 830 nm it has an identifiable spectral profile which corresponded with a commercial library spectrum (name) of epoxy resin.

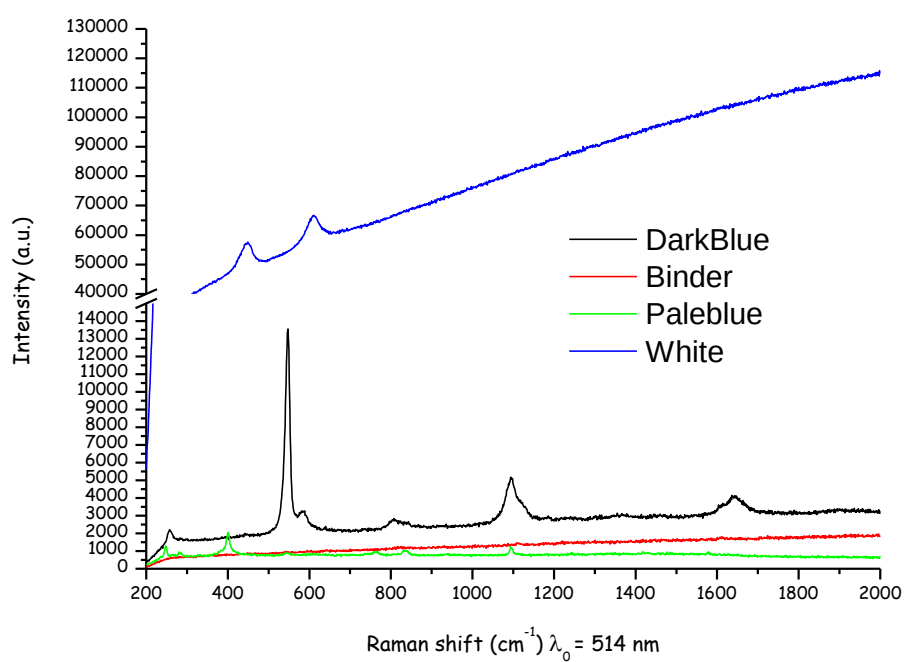


Figure 68. Individual pigment and binder spectra for map collected at 514 nm

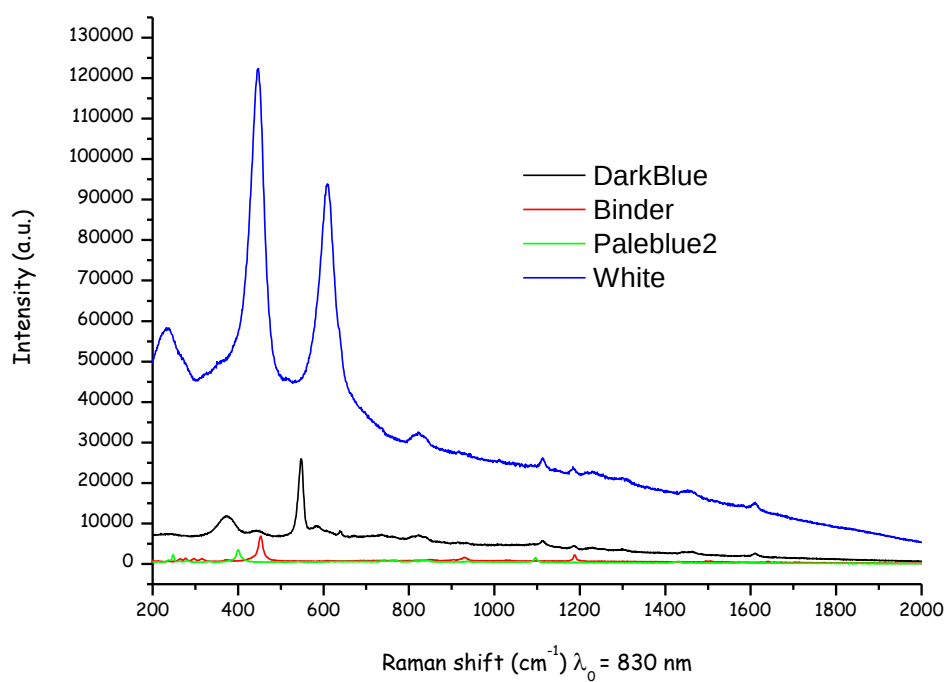


Figure 69. Individual pigment and binder spectra for map collected at 830 nm

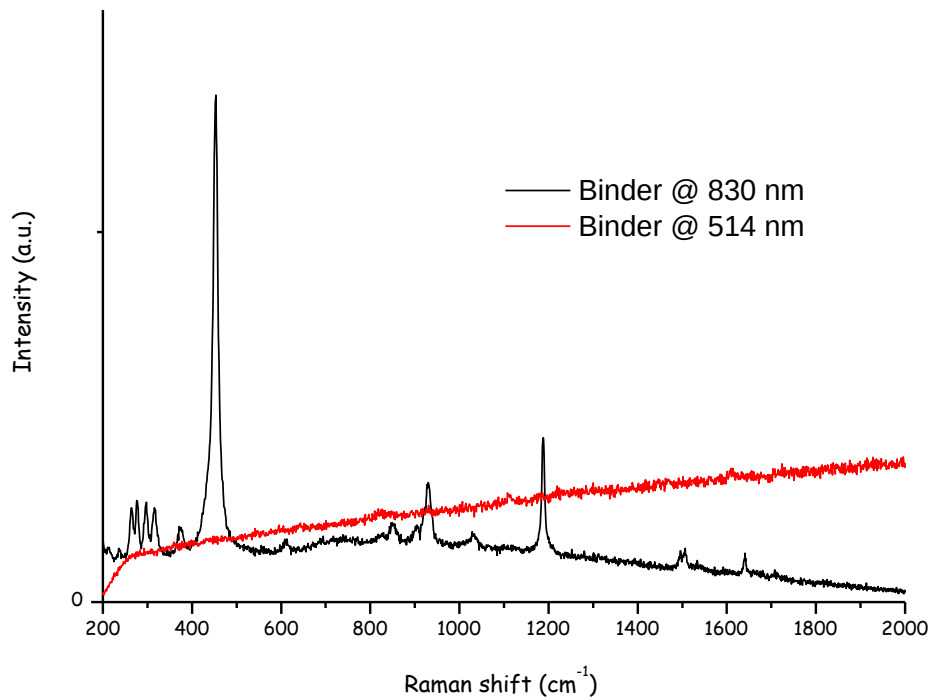


Figure 70. Comparative Raman spectra of binding media at 514 nm and 830 nm

On inspection of each of the individual spectra in the map collected at 830 nm, evidence of bands corresponding with epoxy were observed in most areas across the sample face, except for the centre of the large white pigment grain. Auto-fluorescence across the sample was evident at 830 nm but not at 514 nm which produced a high degree of variability due to elevation of the spectral baseline.

In order to remove the contribution of auto-fluorescence to spectral variance, a linear baseline correction was performed on all spectra. The final data matrix formats (x,y,z) were:

$$514 \text{ nm} = (23 \times 43 \times 575) = 568,680 \text{ matrix values}$$

$$830 \text{ nm} = (24 \times 17 \times 2303) = 994,896 \text{ matrix values.}$$

5.3.1 Unsupervised classification

The results of unsupervised classification using Isodata (5 – 10 class) and K-means (10 class) are presented in Figure 71. Overall, both approaches produce an almost identical identification and

distribution of classes. The variance in auto-fluorescence within the titanium dioxide particle was greater than the variance between the blue pigments and so the blue particles were not distinguished from one another, whereas the differences in spectral intensity from the centre toward the outside edge of the particle are expressed as 4 to 5 separate classes.

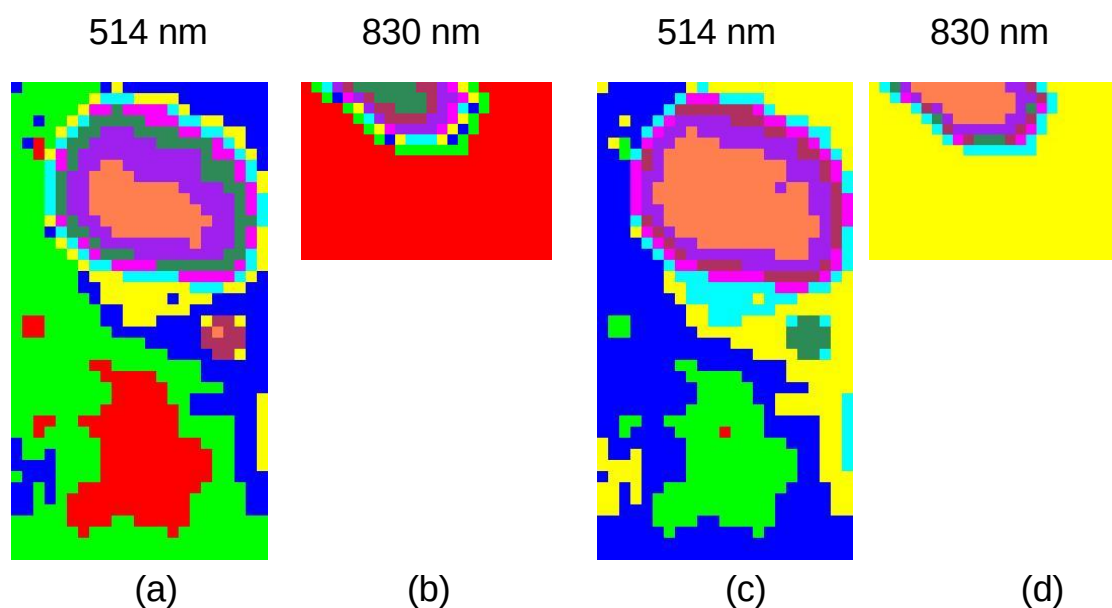


Figure 71. Classification outcomes for Isodata (a and b) and K-means (c and d) treatments of Raman maps collected at 830 nm and 514 nm

The pixel classes do not exactly correspond with the blue pigment particles in the 514 nm map, and completely fail to recognise ultramarine and azurite in the 830 nm map. This illustrates that an unsatisfactory representation of the pigment distribution is likely to result if untreated data are used since spectral variance generated within a single pigment can be greater than the spectral variance between different pigment particle types.

In order to eliminate the effect of small intensity differences observed due to compositional variance being overwhelmed by the substantial differences in intensity from titanium dioxide, the spectra were re-scaled to a fixed minimum and maximum value (normalised). Each individual spectrum was normalised over the range 0 - 1 using the following equation:

$$I_n = (I - I_{\min}) / (I_{\max} - I_{\min})$$

Where I is the intensity value, I_n is the normalised intensity value, $I_{\min}$ the minimum intensity value for the spectrum and $I_{\max}$ the maximum intensity value for the spectrum.

5.3.1.1 Comparison of un-normalised and normalised individual pigment spectra

Figure 72, Figure 73 and Figure 74 are comparative maps of raw and normalised intensity for the sample mapped at 830 nm, at band 1939 = 453 cm^{-1} . The spectral profile below each map is for the pixel indicated by the position of the red marker in the sample. The maximum intensity for titanium dioxide is above 8×10^4 counts, whereas the maximum intensity of the major peak for epoxy resin is only approximately 1500 counts. This illustrates the importance and effect of normalisation so that spectral variance is attributable to spectral shape rather than being dominated by intensity.

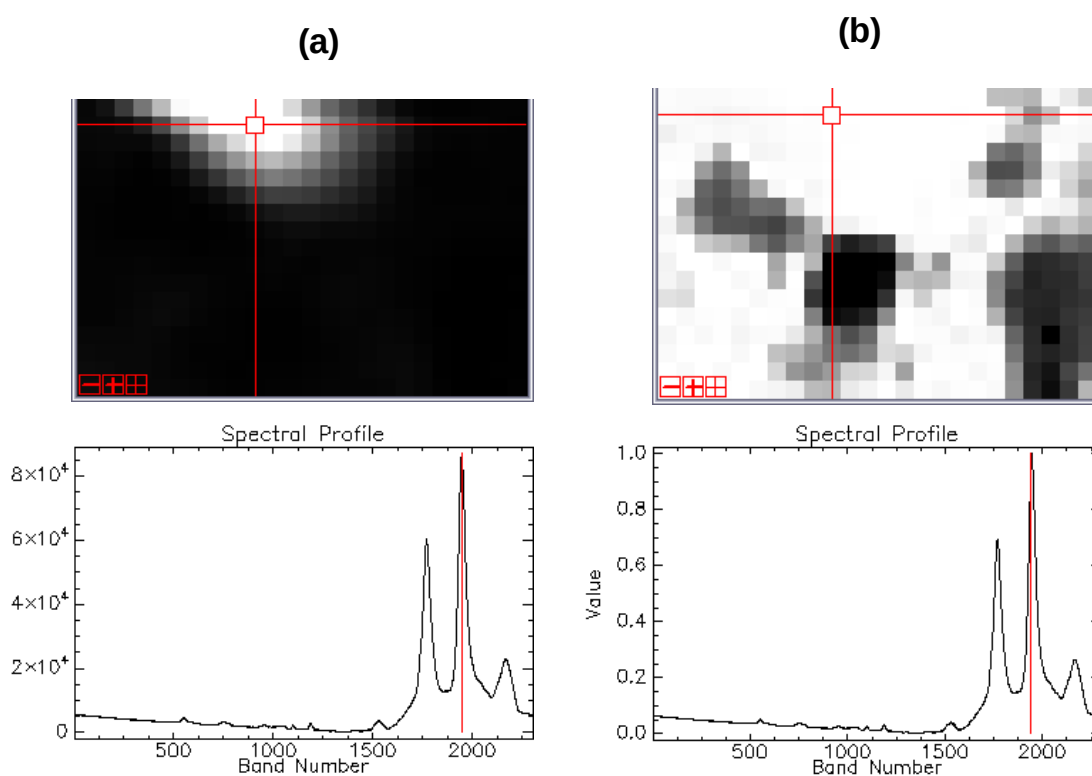


Figure 72. (a) Raw and (b) normalised 830 nm map of intensity at band 1939 = 453 cm^{-1} . The spectral profile below each map is for the pixel indicated by the red marker located in the titanium dioxide pigment particle

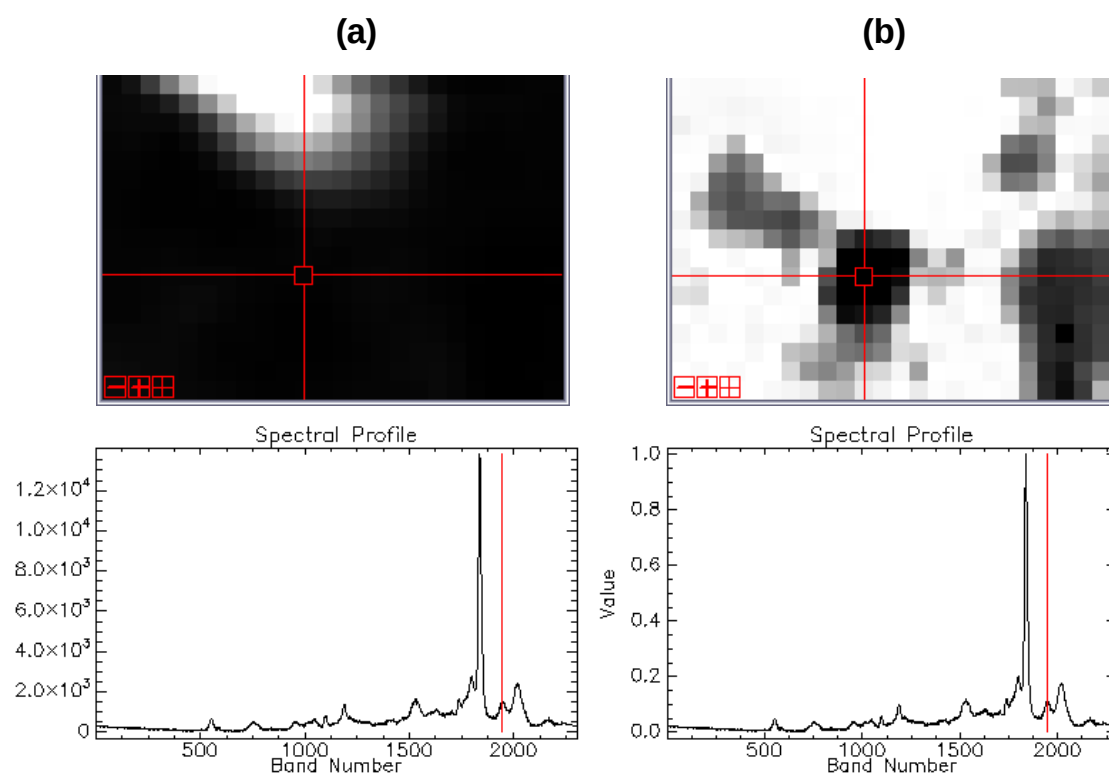


Figure 73. (a) Raw and (b) normalised 830 nm map of intensity at band 1939 = 453 cm⁻¹. The spectral profile below each map is for the pixel indicated by the red marker located in the ultramarine pigment particle

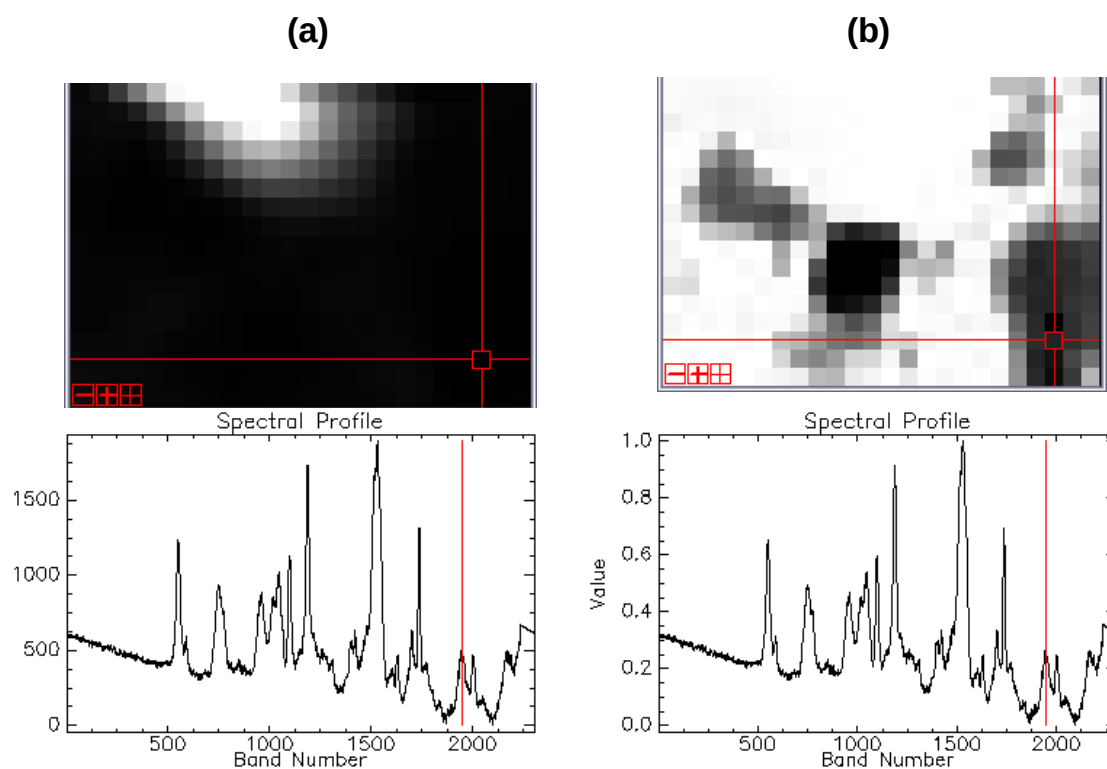


Figure 74. (a) Raw and (b) normalised 830 nm map of intensity at band 1939 = 453 cm^{-1} . The spectral profile below each map is for the pixel indicated by the red marker in the embedding resin area of the sample

5.3.1.2 Normalised clustering results

K-means clustering was repeated after normalisation and the result is shown in Figure 75. Classification into 10 classes identified the titanium dioxide particle, ultramarine and azurite. The 5 class outcome failed to identify the azurite particle as the spectral profile in that area is dominated by the resin spectrum. This indicates that in areas where there is high contribution to a spectrum from the surrounding resin, unsupervised techniques are unlikely to be able to differentiate pigments that have minor spectral features, which exhibit small degrees of departure from the spectra in the surrounding matrix.

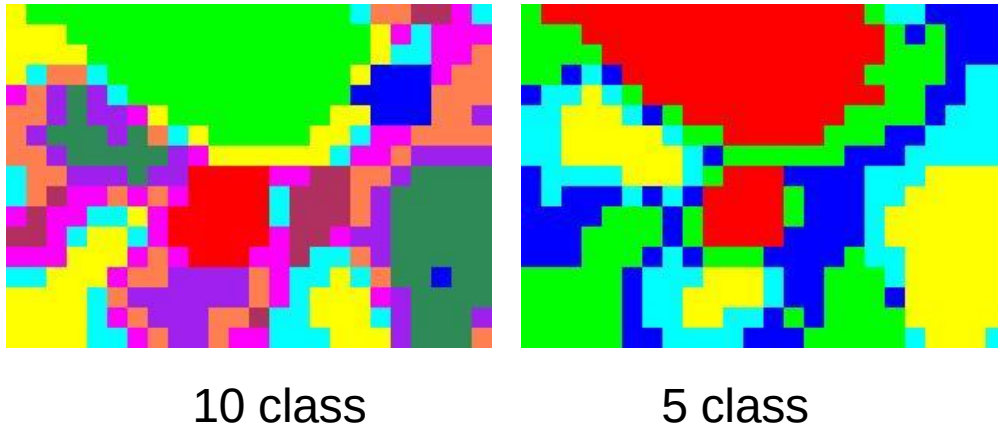


Figure 75. K-means classification of 830 nm map after spectral normalisation

5.3.2 PCA

The PCA transformed data are shown in Figure 76 showing that about 5 PCs account for the majority of variance in both samples. While some of the principal components relate directly to pigment grains, others relate to spectral variance observed in the particle edge regions. Pre-treatment normalisation reduces noise, which can be seen in

Figure 77 whereby the large pigment grain image retains its integrity.

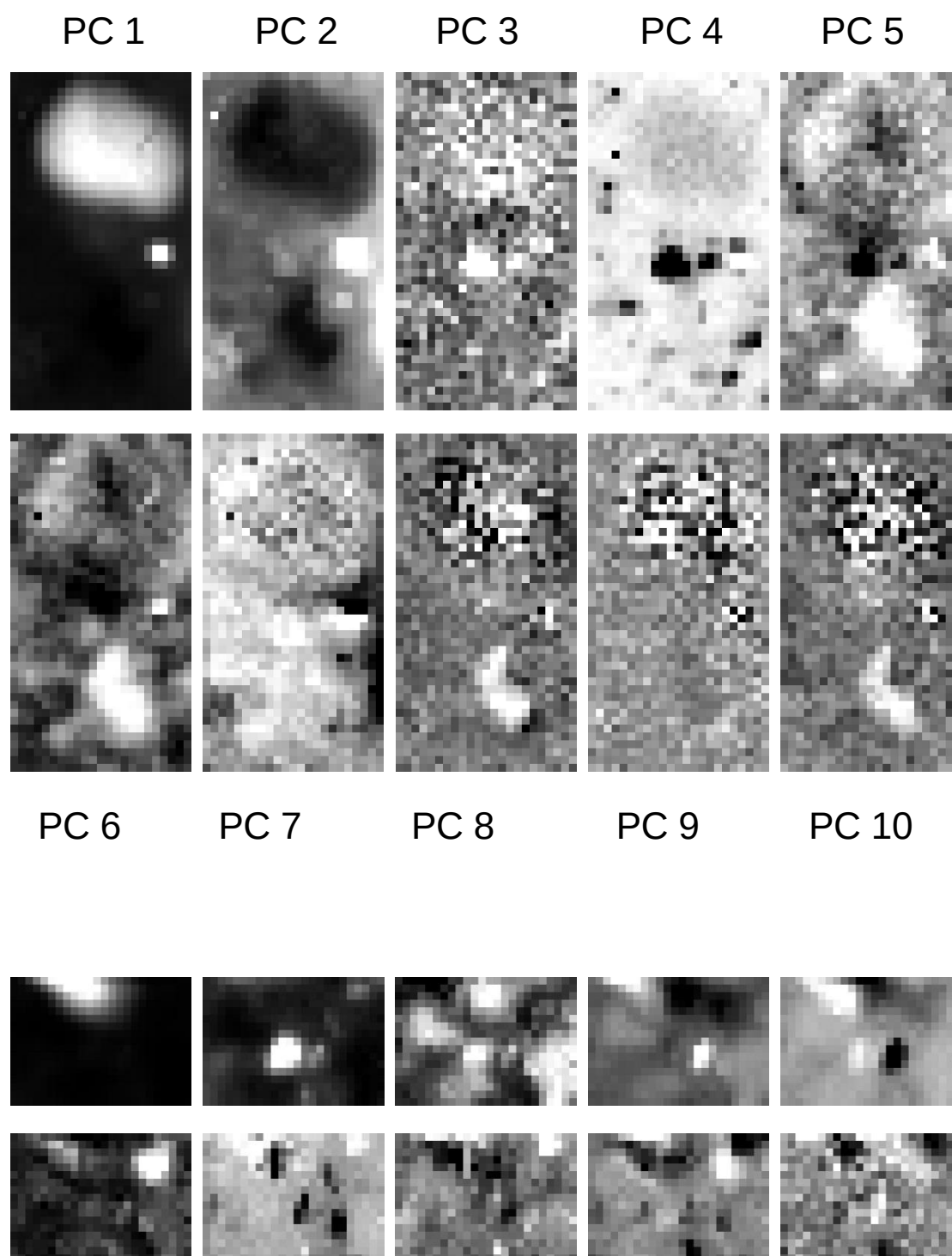


Figure 76. PCA transform outcomes for Raman maps of pigments in epoxy collected at (a) 514 nm and (b) 830 nm

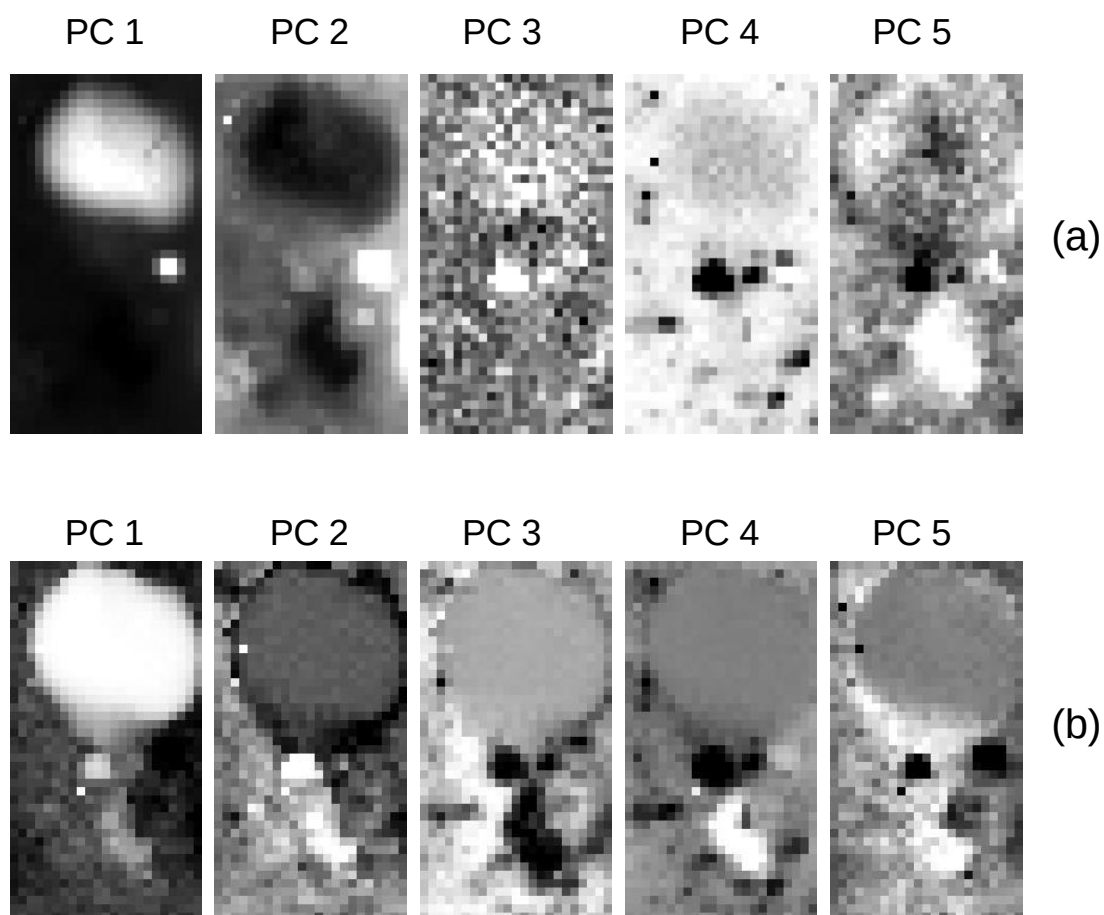


Figure 77. Comparison of PCA transform of Raman maps collected at 514 nm. (a) Raw spectra and (b) normalised spectra

5.3.2.1 *Mapping peak intensity*

This exploration of unsupervised clustering shows it has great potential for the examination of painting cross sections, but only when the variance in spectra is managed by

1. Minimising the variance due to fluorescence which will affect the spectral baseline across the mapped area
2. Treating spectra to account for differences in the Raman scattering cross section (intensity due to the pigment alone) of different pigments
3. Considering the use of an excitation line, which does not introduce substantial contribution to the spectra from the binding media.

In situations where the paint binder in a cross section is linseed oil, which is fluorescent at 514 nm, cross section examination needs to be performed at 830 nm. Another approach to visualising the distribution of pigments is by mapping peak intensity (peak intensity to spectral baseline) of an individual characteristic peak. This approach is demonstrated as follows on cross sections from a sixteenth century renaissance painting.

5.3.2.2 *Introduction*

For much of the 15th and 16th centuries, the city-state of Ferrara was considered one of the greatest centres of culture, learning and the arts. Dosso Dossi is considered Ferrara's most important painter of the 16th century, and one of the most enigmatic. Dosso was primarily a painter of allegorical, historical or religious subjects, and although he is known to have painted portraits, none of the few currently attributed to him are undisputed. The National Gallery of Victoria's *Portrait of a Youth* (datable to around 1515, accession no. 1587-5) has been attributed to an anonymous north Italian painter since its arrival in Melbourne in 1966. However, a major technical examination of the painting revealed a significant number of similarities between the materials and techniques used, and Dosso's known working methods and materials. Raman spectral mapping was used to characterise the preparatory layers and pigments in the painting in order to establish if the character of the pigments and gesso preparatory layers in *Portrait of a Youth* are consistent with other Dosso paintings. This work was published in 2008 (200) and the first page is illustrated in Appendix 4; *Multispectral and hyperspectral image analysis of elemental and micro-Raman maps of cross-sections from a 16th century painting*.

5.3.2.3 *Methods and materials*

Three maps were collected over different areas of cross-section S II (Figure 78):

1. Map S IIa: Pigmented layer above the imprimatura.
2. Map S IIb: Through all paint and preparatory layers.
3. Map S IIc: Extended examination through the ground layer.

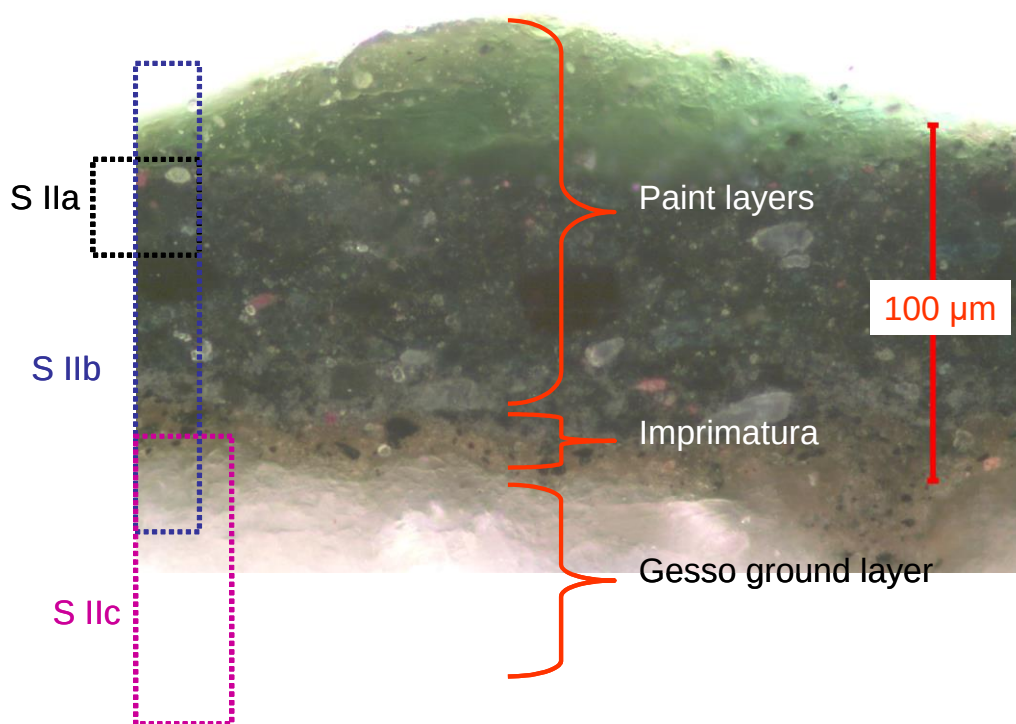


Figure 78. Optical image of cross-section S II with locations of Raman mapped areas S IIa, S IIb and S IIc

It was not possible to collect a single map over the entire cross-section due to instability in the spectral response when the mapping time exceeded several hours.

Raman maps of S IIa and S IIb were collected using a Renishaw inVia confocal microscope system. Specimens were illuminated through a 50× short working distance objective lens with 830 nm excitation from a diode laser, operating at incident power of 1.5 mW with a spot size of approximately $2 \times 4 \mu\text{m}$. Spectra were collected over the range $100\text{--}1200 \text{ cm}^{-1}$. Each spectrum was averaged over five scans corresponding to a collection time of approximately 50 s per map point (pixel).

The Raman map of S IIc was collected using a Renishaw Ramascope confocal microscope system operating in line-scan mode. Specimens were illuminated through a 50× short working

distance objective lens with 830 nm excitation from a 300 mW diode laser, operating at a power setting of 25%. Spectra were collected over the approximate range of 800–1200 cm^{-1} . Each spectrum was averaged over two scans.

Raman map spectra were pre-processed using a Visual Basic routine⁹ to smooth, end-to-end baseline correct and bin/2. ENVI 4.2 was used to compile data into a single 3D data cube with x and y being the pixel position, and z being the Raman intensity at each wavenumber. Maps of the overall distribution of a single pigment were constructed by plotting the intensity (from the baseline) of the main peak.

5.3.2.4 *Results and discussion*

The PCA and clustering techniques for imaging, developed earlier in this chapter (section 5.3), were applied to SEM-EDS elemental maps of the painting cross section. Detailed methods and results are not presented in this chapter which focuses on the Raman analysis, but can be found in the publication which documents this study (200). Elemental maps were collected for the elements Ti, Sn, Si, Pb, Mn, Mg, K, Fe, Cu, Ca, Al, S (corrected for Pb) and O, and were assembled into a single 3D data cube with x and y being the pixel position, and z being the relative abundance of each element. PCA imaging as described in section 5.3.2 was applied to determine the minimum number of eigen-images to include in the compositional map. Clustering methods from 5.3.1.2 were used to build the compositional map of the cross section, which is complementary to the Raman compositional mapping presented as follows.

As a preliminary screening step, all individual spectra in the maps were briefly surveyed before processing to identify those containing Raman peaks. Individual spectra from areas of interest in the maps were matched with library references for identification. Thousands of reference spectra of minerals were searched, including the pigment reference spectra in Appendix 1 and published spectral databases of pigments and minerals (34, 35, 37).

⁹ Visual Basic routine written by Scott Furman, CSIRO Division of Materials Science and Engineering, Bayview Avenue, Clayton, 3169.

5.3.2.4.1 Map S IIa – pigmented layer above the imprimatura

The Raman spectra and maps from S IIa are shown in Figure 79. It is interesting to note that the measured distribution of a pigment does not correspond exactly with the visual observation of the sample, which supports the use of mapping as a method for evaluating composition, which is devoid of an analyst's visual interpretation and expectation. Around some pigment grains the strongest Raman scattering occurred adjacent to the visual centre of the pigment grain, which may be due to the bulk of the grain sitting just below the surface and adjacent to the exposed face. Good quality spectra were collected for the yellow, orange-red and blue-green pigments in the layer.

The orange-red pigment was identified as vermilion, mercury(II) sulfide, HgS with bands at 252vs ; 282w(sh) ; 343m cm^{-1} . The map shows discrete pigment grains with no observable background intensity, consistent with their appearance as isolated larger grains.

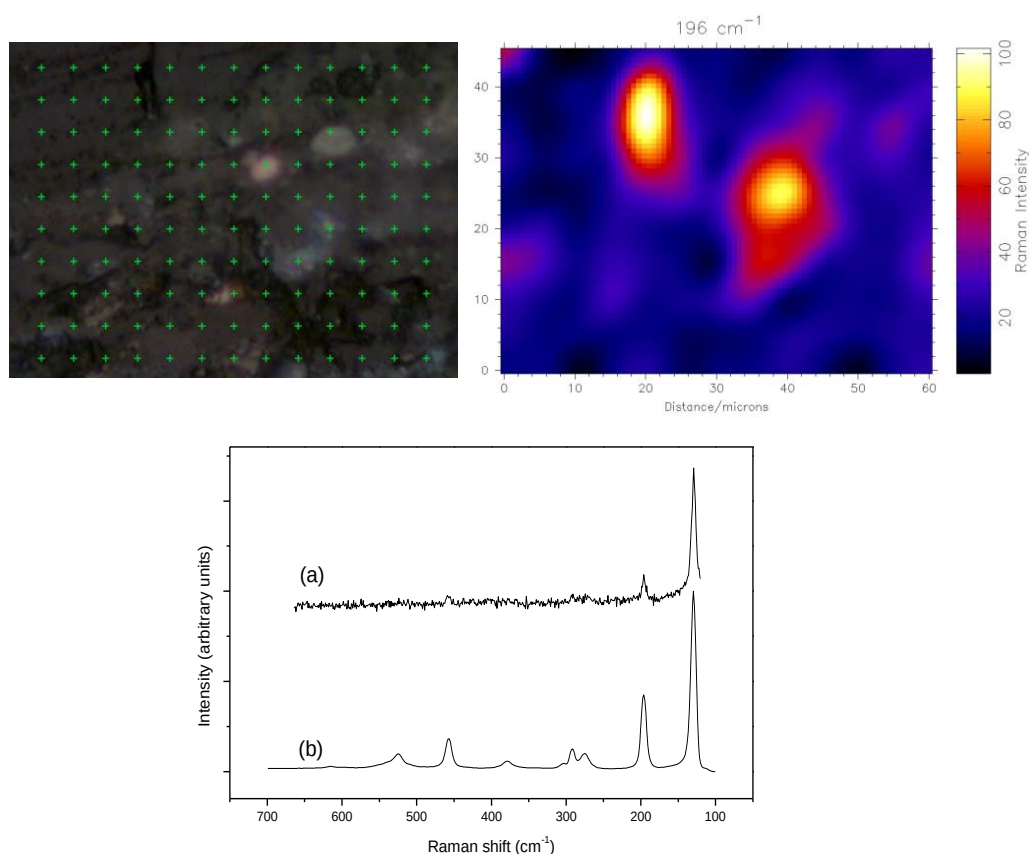


Figure 79. Optical image of S IIa (Top left) and Raman map of intensity at 196 cm^{-1} (Top right). Raman spectra of (a) yellow pigment grain; (b) lead-tin yellow type 1 reference sample [16]. $\lambda_0 = 830\text{ nm}$, 1.5 mW (Bottom)

Lead-tin yellow type 1, lead(II) stannate Pb_2SnO_4 was identified from its spectral match with bands at 129vs; 196s; 275w(br); 291w; 303w; 379w; 457m; 525w cm^{-1} . The map of lead-tin yellow (from the peak at 129 cm^{-1}) shows some discrete grains, but also a low intensity contribution distributed over the surface, which corresponds with the white light observation of small particles of yellow evenly distributed throughout the paint layer.

Raman spectra from the blue-green pigment (Figure 80) exhibit several identifiable broad bands, however a satisfactory identification could not be made. A significant number of the best matches were with silicates (Table 10) suggesting the pigment may have a silicate-type chemistry. The broadness of the peaks indicates significant disorder in the crystalline structure. A main band was observed at 717 cm^{-1} , and others, as identified from fitting peaks in the baseline corrected spectrum, are listed in Table 11. Bands at 502, 547 and 593 cm^{-1} are believed attributable to the Si-O bending vibration, at 661 cm^{-1} an Al-O stretch, 716, 749 and 831 cm^{-1} to the Al-OH vibrations of surface hydroxyls, 936 and 984 cm^{-1} to the in-plane bending of surface hydroxyls, and 1084–1107 cm^{-1} to the perpendicular Si-O vibrations (201, 202). Raman spectra of terre verte (green earth) pigments are quite variable and this blue-green may fall into that classification.

5.3.2.4.2 *Map S IIb – through all cross-sectional layers and Map S IIc – extended through ground layer*

The high spatial resolution afforded by ESEM-EDS mapping makes it an excellent tool for exploring the composition and spatial relationships of components within paint layers. It is somewhat limited, however, since the chemical composition must be inferred by the elemental composition. This is particularly evident in the current examination of the ground layer, which contains Ca, S and O, which could be attributable to either gypsum or anhydrite, based on the elemental evidence alone.

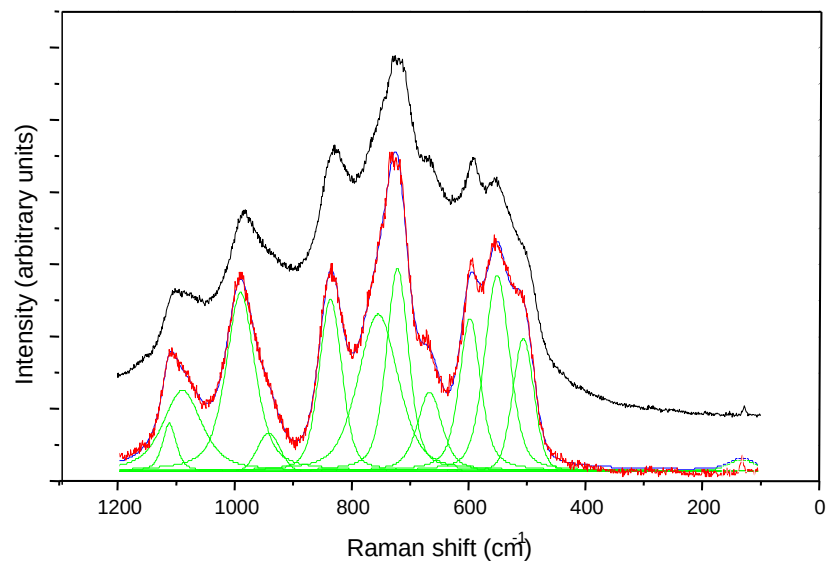


Figure 80. Raman spectra of blue-green pigment grain in S IIa: (black) untreated spectrum; (red) curve-fitted baseline corrected spectrum; (green) fitted curves. 830 nm, 1.5 mW.

Table 10. Library matches with blue-green pigment

Mineral	Ideal chemistry
Magnesioriebeckite	$\text{Na}_2(\text{Mg}_3\text{Fe}^{3+}_2)\text{Si}_8\text{O}_{22}(\text{OH})_2$
Georgbakiite	$\text{Cu}_5\text{O}_2(\text{Se}^{4+}\text{O}_3)_2\text{Cl}_2$
Magnesite	MgCO_3
Spinel	MgAl_2O_4
Zinnwaldite	$\text{K}(\text{Al},\text{Fe},\text{Li})_3(\text{Si},\text{Al})_4\text{O}_{10}(\text{OH})\text{F}$
Annite	$\text{KFe}^{2+}_3(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH},\text{F})_2$
Galaxite	$\text{Mn}^{2+}\text{Al}_2\text{O}_4$
Magnesiohornblende	$\text{Ca}_2[\text{Mg}_4(\text{Al},\text{Fe}^{3+})]\text{Si}_7\text{AlO}_{22}(\text{OH})_2$
Arfvedsonite	$\text{NaNa}_2(\text{Fe}^{2+}_4\text{Fe}^{3+})\text{Si}_8\text{O}_{22}(\text{OH})_2$
Brazilianite	$\text{NaAl}_3(\text{PO}_4)_2(\text{OH})_4$

Table 11. Curve fit parameters from Raman spectrum of blue-green particle

Centre (cm^{-1})	Width, FWHM (cm^{-1})	Height (intensity)	Gaussian (%)
502	42	4933	82
547	51	7301	63
593	43	5707	43
662	50	2955	47
717	45	7551	55
750	81	5847	56
831	46	6412	65
937	45	1411	100
985	58	6653	45
1084	79	3008	40
1107	28	1771	45

The Raman spectrum of blue-green pigment grain in S IIa (Figure 80) is fitted by Gaussian bands as labelled in Table 11. Raman theory indicates a Lorentzian form of Raman bands, however the distribution of a Lorentzian series can give a Gaussian shape as shown by the red fitted curve with negligible residual.

In a study of four other Dosso paintings (203), the ground layer of each was found to be over 100 μm thick. XRD powder diffraction performed on two paintings found a combination of gypsum, the dihydrate of calcium sulfate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and anhydrite, an anhydrous calcium sulfate (CaSO_4). In other studies, ground layers of Dosso paintings have been characterised using

elemental analysis, and the presence of calcium and sulfur has sometimes led to the assumption that the gesso was prepared with gypsum (204). Elemental analysis was consistent with these previous studies in that it also found calcium and sulfur in the ground layer, which may be attributable to either anhydrite or gypsum.

However, Raman spectra from the ground layer did not match gypsum, which has bands at 181w, 414m, 493w, 619vw, 670vw, 1007vs and 1132m cm^{-1} . In this sample the ground layer is identified as anhydrite (CaSO_4), which has bands at 1161m, 1129m, 1087vw, 1017vs, 674m, 325w, 607vw, 497w, 414vw, 231w and 161vw cm^{-1} (37).

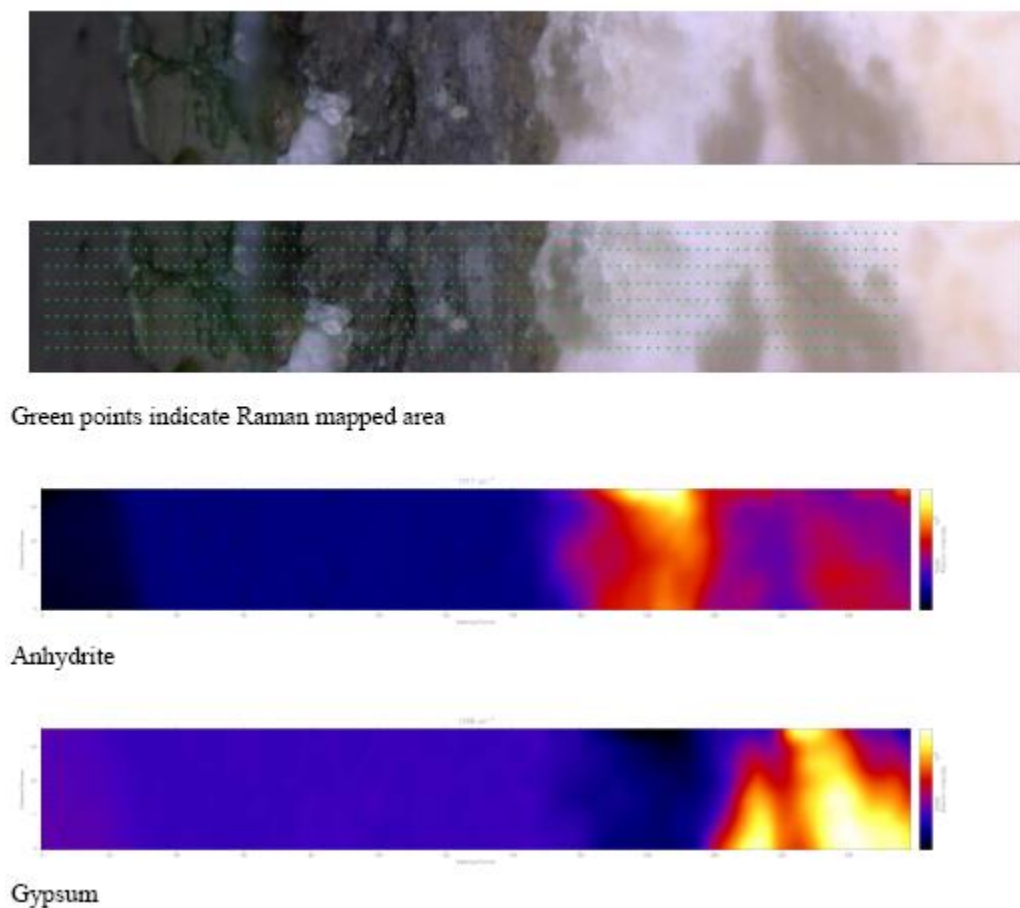


Figure 81. Raman maps of S IIb

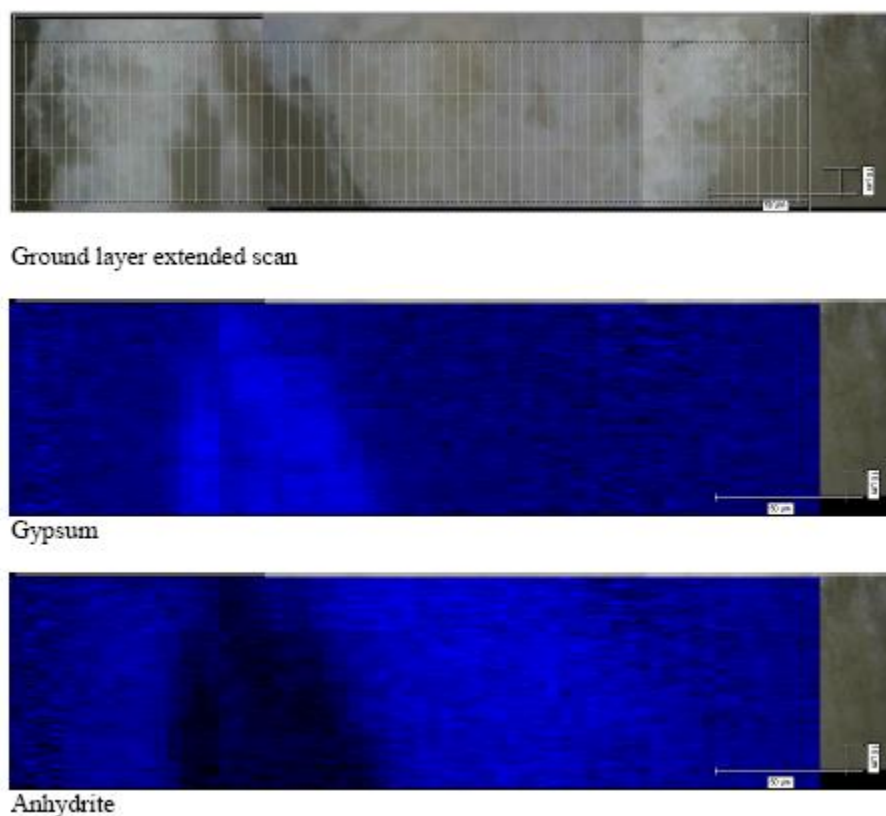


Figure 82. Raman maps of S IIc

Raman maps of anhydrite and gypsum shown in Figure 81 and Figure 82, show two large particles (up to $40 \times 80 \mu\text{m}$) of gypsum in the ground layer. Anhydrite extends evenly throughout the layer except for the large inclusions of gypsum, so there is no evidence for the use of a compositionally layered preparatory technique in the gesso of this painting. The occurrence of anhydrite together with gypsum in a painting of this period is believed to indicate a particular method of roasting gypsum to $300\text{--}400^\circ\text{C}$ and the preparation of ground layers known as '*gesso grosso*' and '*gesso sottile*' (205), which are known to occur in northern Italian paintings of this time.

Vermilion, lead-tin yellow type 1 and a blue-green pigment consistent with terre-verte or another green silicate pigment were found in the paint layer. The ground layer has been identified as anhydrite with large gypsum inclusions. Attribution of this painting to Dosso is therefore

supported by the identification of components in the paint and ground layers of the painting, consistent with works unquestioned in their attribution to him.

5.4 Conclusions

This chapter developed a new PCA and clustering methodology for multivariate image analysis. The method for assembling and analysing the spectral 3D data cubes was developed using Raman maps of known pigments prepared in binder was applied to both Raman spectra and SEM-EDS elemental map spectral profiles.

This image analysis methodology was used to study a painting cross section with the intention of informing attribution. Firstly, the PCA and clustering methods were applied to elemental maps of Ti, Sn, Si, Pb, Mn, Mg, K, Fe, Cu, Ca, Al, S (corrected for Pb) and O, to develop a compositional map of the materials used and indicate their sequence in the layered construction of the painting. Secondly, using Raman maps of spectral intensity collected at 830 nm to mitigate fluorescence, and using the spectral library (appendix 1) vermilion, lead-tin yellow type 1 and a blue-green pigment consistent with terre-verte or another green silicate pigment were found in the paint layer. The ground layer was found to contain anhydrite with large gypsum inclusions. The identification of these components has led to the attribution of a previously anonymous painting to Dosso Dossi.

This work has identified the following issues specific to the analysis of painting cross sections:

1. The nature of the binding medium in the painting will affect the outcome. If it is fluorescent, this necessitates the use of a longer wavelength, which may also collect the binding medium spectrum. The presence of the binding medium spectrum reduces the ability of unsupervised classification techniques to discriminate between materials. If a shorter wavelength can be used, or there is no contribution from the resin to the spectrum, classification is more successful.
2. Clustering and PCA are very much influenced by the overall variance between spectra, which is dominated by the differences in overall intensity between spectra. The implementation of spectral normalisation pre-treatment reduces the variance to emphasise band position and shape, the effective spectral characteristics, which represent composition.

3. PCA is useful to identify areas of interest in a sample and represent their spatial distribution. It is also significantly influenced by differences in spectral intensity and requires data pre-treatment by normalisation.
4. Intensity mapping is a useful way of illustrating the distribution of specific pigments by using single band intensity representation. It is less influenced by the other spectral components unless they overlap the band of interest.
5. Due to the variety and nature of the materials encountered in painting layers, it is not possible to establish a single, generic approach to multivariate analysis of micro-Raman spectral maps, but a combination of methods enhances the information which may be extracted from the large quantity of data available in the sample dataset. The method requires collection of high-quality spectra which is the most important criterion, and as shown in earlier chapters, the choice of laser source, high sensitivity and resolution of the spectrometer and detector, as well as quality of the optical path are critical prerequisites.

Since its 2008 publication, the article in *Analytica Chimica Acta* with impact factor 5.3, has contributed significant value to the scientific community having been cited by 70 authors.

The work in this paper has been used to validate the technique of micro-Raman spectral imaging and further extensions have been made by Aramendia *et al* (206) who present a novel quantification method using Raman imaging and Laser Induce Breakdown Spectroscopy (LIBS) for the analysis of cultural heritage materials. The authors found good agreement between the quantitative results from the independent analyses of the Raman image data and the LIBS data. Ropret *et al* (207, 208) extended the scale of non-destructive Raman mapping for larger objects. By using mirrors to direct the beam through a horizontal exit on the Raman micro-spectrometer, larger objects could be studied. Staniszewska *et al* (209) used FTIR maps created by hierarchical cluster analysis and Raman maps constructed by using univariate analysis to demonstrate their degree of utility for the study of paint cross sections

Techniques for macro imaging also reference the work in this paper. Mencaglia *et al* (210) developed a novel Raman scanner capable of performing point-to-point mapping of relatively large surface of paintings using an excitation wavelength of 1064 nm, a high efficiency probe in order to collect the back-scattered light, long depth-of-field optics and an autofocus system.

Colantonio et al (211) used spectral reflectance measurements between 300nm and 1000nm to obtain degradation maps of the painting layers that could be used to inform sampling points.

Sciutto et al (212) developed a strategy for multivariate treatment of XRF spectral and hyperspectral data sets, able to account for inter-correlation among variables which can be important for elemental analyses. XRF spectral profiles were analysed directly, without computation of derived parameters, by means of principal component analysis (PCA). Maybury et al (213) used hyperspectral imaging over the wavelength range 380–1000 nm to improve the speed of pigment identification across manuscript folios and artwork. It was noted that the technique must be used in conjunction with a technique such as Raman spectroscopy for cross-validation.

The methods developed in this paper have been directly applied to multivariate imaging in other fields of materials analysis. I have applied the same Raman spectroscopy and X-ray elemental mapping imaging methods to characterise the composition of corrosion products on aerospace aluminium and visualise their distribution across the metal surface (214).

Other authors have referenced this paper in recognising the importance of developing multi and hyperspectral microscopic imaging methods; Zhu et al (215) developed methods for fluorescence and transmission spectral imaging of pollen and Lin et al (216) developed transmission and fluorescence imaging to identify species of intestinal fungi.

The work presented in the paper was, in 2016, still recognised as an advanced development in the field of Raman imaging in overviews by Centeno (217) and Bersani and Lottici (218).

Chapter 6: Overall conclusions and future work

Raman spectroscopy continues to be an excellent characterisation technique for examining cultural materials due to its ability to analyse non-destructively with relatively little sample material preparation utilising short measurement times. Micro-Raman is especially useful for examining painting cross sections due to its high spatial resolution however spatial resolution is diminished with increasing wavelength. Raman spectroscopy has a variety of modes of analysis but the success of any of these is potentially impacted by laser-induced fluorescence which is frequently encountered in artworks and materials from cultural collections. Moreover, with the large number of natural and synthetic materials encountered in conservation, identification of large sample and reference datasets has required the development of new techniques of visualisation and information extraction.

Work presented in this thesis has identified and developed two methodologies to mitigate the effects of fluorescence (using longer wavelengths and SERS) and methodologies to improve the interpretation and understanding of non-spatial and spatial multivariate Raman data, that are specifically applicable to conservation applications.

At the time this work was started, a spectral library for pigment identification was necessary to have a comprehensive set of pigment reference spectra against which to compare unknowns. A Raman spectral pigment reference library was developed comprising over 180 samples from the National Gallery of Victoria's pigment collection. Samples were validated with XRPD prior to Raman analysis, which enabled the identification of pigments throughout this study. This Raman spectral pigment library can also support future identification of materials and artworks, alongside other Raman spectral databases that are now available.

To mitigate the impact of fluorescence observed at 514nm, use of a longer 830nm wavelength incident irradiation proved successful and the methodology was used to answer five research questions regarding authenticity and art historical practice for assistance with attribution, provenance studies and to aid in decisions regarding conservation treatments.

1. In the portrait miniature *Portrait of a Lady Wearing Elizabethan Costume*, the identification of Zinc white pigment dated the overpaint to being applied after the mid-19th century, much later than the original painting dated from the 16th century.

2. The inks in Streeton's catalogue comprises Prussian blue and vermillion as the main pigments in the ink of the catalogue, with a minor addition of minium and perhaps a lake pigment, confirming that ink recipes documented from the era were used as a foundation, with the recipes augmented by pigments used extensively by the artists in their paintings.

3. Overpainted changes on Tom Roberts' iconic painting "*Shearing the Rams*" correspond with cracks in the painting and may have been due to the type of pigment used. The main pigment is vermillion, which is not known to cause cracking, so the cause is now believed to be the high ratio of binding medium in the paint.

4. *The Finding of Moses* was originally attributed to Sebastiano Ricci, dating to around 1712. Prussian blue was identified as a key component of the paint layer but since it does not come into wide usage in Venetian painting until the late 1720s or early 1730s, it substantiates the stylistic attribution of this painting to 18th-century master Italian painter Giambattista Tiepolo (1696-1770).

5. The colours used to decorate the cotton fibres of an Indian Palampore matched with a published spectrum of indigo on silk highlighted the importance of local structure and bonding on subtle features in Raman spectra.

A practical, accessible and inexpensive SERS method suitable for conservation was developed. A SERS substrate using electrolessly deposited silver on a copper coupon was used to identify dammar as the varnish used on an important Italian Renaissance painting by Tiepolo with the outcomes published in 2008. The article has been cited 28 times since publication in the period up to 2019 demonstrating the contribution made to the research field.

Multivariate analysis techniques were developed for data analysis of samples of both natural and synthetic resins, and clustering techniques were developed to visualise the complex relationships between sample types.

Finally, multivariate data analysis techniques were developed and applied to develop Raman compositional images. Pigment maps of painting cross sections assisted with the attribution of another Italian renaissance painting to Dosso Dossi with the outcomes published in 2008, receiving 70 citations until 2019.

The purpose of Conservation Science is to support and enable effective conservation practices by informing treatments, storage and display, made possible by understanding materials and composition. Future possible research directions in Raman spectroscopy for the examination of artists' materials that would enable improvements in conservation practices could be made in the following areas:

1. Raman spectral libraries should be broadened to encompass more materials.

Conservation analytical laboratories usually have in-house spectral libraries with collection conditions that replicate the intended sample collection but are often limited by the availability of reference sample material. Having the broadest range of possible pigments and binding media collected under similar conditions can provide greater confidence in the result. Collection of spectra from known and controlled mixtures and mock-ups is especially useful, as demonstrated in chapter 2.2.1, which undertook an examination of portrait miniatures. Ultimately, the in-house collection of comprehensive libraries of individual and mixed materials that is supported by the availability and accessibility of expansive published libraries (both in terms of material type and incident wavelength) would be of great benefit to the conservation science community.

2. As instrumentation evolves and the range of incident laser lines increases, the need for spectral libraries increases. Reference and commercially available libraries are often collected at wavelengths different to that of the laboratory instrument. This was recognised by Marucci *et al* (219) who collected reference spectra of 32 different pigments and colourants mostly used in Medieval manuscripts. Five different wavelengths 488 nm, 532 nm, 632.8 nm, 785 nm and 830 nm were used as excitation sources.

Disparate Raman spectral libraries are available in different formats including journal publications, commercial databases, websites and searchable general materials databases. If the

abundance of distributed information could be aggregated and digitally unified it would provide an expansive reference collection for conservators and conservation researchers who may not be as familiar with the distributed breadth of information available. It would reduce analysis and research timeframes and support improvements in accurate spectral matching. Integration of the Raman spectral library developed in this thesis with other available libraries, both commercial and public access, could create a valuable “meta-library”.

3. New methods for identifying Raman spectra could be developed, as current methods rely on the availability of a match being available in a library or database. Throughout this research the approach taken was to compare collected spectra with those of known reference materials. If a sample is not included in a database or library, identification is made more difficult, hence the need for expansive libraries and databases. However, it could be possible to develop machine-learning methods, or sophisticated modelling systems that predict the composition of the material from known parameters.

4. As newer geospatial mapping techniques and Geographic Information Systems (GIS) are developed, their improved spectral processing power can be applied to the visualisation of Raman maps and the improvement of materials identification. This could be especially valuable for the identification of mixed materials, especially organic pigments and media with complex spectral profiles.

5. Data fusion, also known as data integration, combines the information from two or more sources making it more informative than from either source alone. The fusion or integration of data from different mapping techniques that are complementary to Raman would build additional information into each pixel of a Raman mapped image. Mapping with Time of Flight -Secondary Ion Mass Spectroscopy (TOF-SIMS), SEM-EDS, XRF, FTIR and perhaps Raman at different wavelengths all provide information at a similar spatial resolution. Inclusion of elemental data would provide greater compositional differentiation between pixels, further enhancing the chemical signature of each analysed point in a map. Advances in instrumentation that combine SEM-EDS and Raman have been made already by companies such as Renishaw (220) and Nanounity (221), however data collected from different instruments could also be fused with appropriate image registration.

6. 2D imaging such as presented in chapter 5 of this thesis provides substantially greater detail about the composition of a painting structure than single point analysis, with insights into overall pigment mixtures, composition, heterogeneity and distribution within separate paint layers. 3D imaging could provide even greater insights into understanding the distribution and composition more broadly. Highly confocal 3D imaging Raman microscopes are now available commercially and this technique is yet to be explored for the examination of painting cross sections.

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Appendix 1: Raman pigment library

1 Introduction

As described in chapter 1, a Raman spectral pigment library was developed from the National Gallery of Victoria Painting Conservation Department pigment collection. This collection was accumulated from artists' materials suppliers (artists' colourmen) or donated materials that came from artists or conservators working in private practice in Victoria and comprises over 180 samples. Some pigments, for example, azurite or lapis lazuli, had multiple sample sources.

Samples were analysed with both XRD powder diffraction (where listed in the following table) and Raman spectroscopy. Pigment history, and production methods are described here along with the experimental results.

2 Experimental

2.1 XRD analysis

As sample quantities were limited, a few milligrams (with volume approximating a rice grain) of each pigment type were dispersed in ethanol and spread over a "zero" (oriented silicon) collection plate. Samples were analysed with a Philips Analytical X-ray BV with PC-APD Diffraction, collection time was 300s, spinning on, Angle: 5-90° and Mo target.

Qualitative analysis was performed with major and minor constituents identified by matching the measured diffraction patterns against those from the International Centre for Diffraction Data (ICDD) Joint Committee on Powder Diffraction Standards (JCPDS) database.

2.2 Raman analysis

The quantity of pigment was limited so samples were prepared in a way that would minimise the amount being consumed. Small (3 x 3 mm) squares of double sided adhesive tape were used to

adhere a small volume of pigment powder to a microscope slide. This had the advantage of immobilising the grains and reducing contamination between samples held on the same slide. The pigment grains were analysed with the Raman microscope by focussing on either one or a small clump of grains, depending on the grain size.

Instrumentation was a Dilor Confocal Raman Spectrometer with a DILOR XY Modular Laser Raman Spectrometer equipped with an Olympus optical microscope and nitrogen-cooled multichannel CCD camera. The objective lens was 50x, illuminated with the 514.5 nm line of a Coherent Innova Model 70 Ar⁺ laser operating with an output power of 1 -2 mW. Between 3 and 10 multiple scans were performed on each sample and counts added to provide the final spectrum.



Appendix Figure 1. Dilor XY Raman Spectrometer in the School of Physics, University of Melbourne

Spectra were collected in the proprietary *.mso file format using the Dilor software and modified in Excel to generate an x-y dataset (wavenumber vs intensity) for each spectrum. Origin 7.0 was used to remove cosmic ray lines before plotting. Smoothing or baseline corrections were not performed to preserve inherent fluorescence and the Raman scattering efficiency of the pigment, with low signal to noise resulting in a noisy baseline.

3 Results and discussion

The nature and production of pigments has changed throughout the course of history with implications for their chemical composition. Historical pigments are predominantly naturally

sourced minerals that are mined and ground before use and considering their source, they are expected to contain varying amounts of admixed and ancillary minerals. Organic materials came from other natural sources, for example plant dyes and squid ink (sepia). The earliest synthetic pigment, Egyptian blue, was produced around 2500 BCE with later manufactured pigments such as verdigris, vermilion and lead-tin yellow produced through antiquity and the Middle Ages. The industrial revolution saw a vast expansion in the range of pigments developed with organic and organometallic compounds providing colourants with greater colouring power and replacing the use of toxic, heavy metal-based pigments. Impurities from production process as well as admixtures and fillers may be expected in all artificially produced pigments.

Heterogeneity is expected in pigment samples derived from both natural and synthetic origins however the Raman spectroscopic identification of pigment type is generally based on identification of the single primary colourant within the pigment. While an overall visual observation of colour may be attributable to a combination or mixture of pigments, the identity of an individual pigment is attributable to its individual molecular structure and the identification, through spectral matching or observation of a key vibrational mode, provides the confirmation of pigment identity. The following descriptions of pigment composition and production methods provide a basis for the composition that may be observed during analysis.

3.1.1 Blue

3.1.1.1 Azurite

Azurite is a mineral blue composed of basic copper (II) carbonate ($2\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$). The name originates from the Persian *lazhward*, meaning “blue” and was used extensively throughout the renaissance and Middle ages declining in popularity from about 1800 as manufactured pigments became more widely available. It is mined from natural deposits of copper, found in the upper oxidised layers in association with other copper minerals such as malachite. A nineteenth century recipe describes artificial preparation of blue verditer (the manufactured counterpart) by dissolving copper metal in nitric acid, precipitating the copper with quicklime (CaO), decanting the liquid and grinding with more quicklime to convert the green precipitate to blue (222-224).

3.1.1.2 *Cerulean Blue*

The name derives from the Latin *caeruleum*, meaning the sky or heavens, and the colour itself is often referred to as sky blue. It was introduced by artists' colourmen Rowney in 1860 after development by Höpfner in 1821 and is still a popular pigment.

Cobalt (II) stannate ($\text{CoO} \cdot n\text{SnO}_2$) is prepared by mixing aqueous solutions of cobaltous chloride and potassium stannate. The precipitate is thoroughly washed and calcined with silica and calcium sulfate.

3.1.1.3 *Cobalt blue*

The discovery of cobalt blue originates from research into the blue colour of smalt early in the 18th century by the Swedish chemist Brandt. As an initiative of the French government at the end of the 18th century for research into the improvement of artists' colours, Cobalt (II) oxide-aluminium oxide ($\text{CoO} \cdot \text{Al}_2\text{O}_3$) was discovered in 1802 and reported by Thenard in 1803. His experiments involved roasting alumina with cobalt arsenate or cobalt phosphate. It is still in use as an artist's pigment.

3.1.1.4 *Egyptian blue*

Calcium copper (II) silicate ($\text{CaCuSi}_4\text{O}_{10}$) is the first artificially prepared pigment. It was used extensively from the early fourth dynasty in Egypt (3200 BC) until the Roman period ended in Europe. It was made by heating limestone (or other calcium containing compound), a copper mineral or compound (eg copper ores, malachite, bronze scraps) and silica (sand) to approximately 850 - 1000°C. The process is similar to those used to make blue glazes for pottery and pigment making may have originated as a consequence (225). The chemical structure of Egyptian blue is identical to that of the natural mineral cuprorivaite, investigated with XRD (226, 227). Raman identification of Egyptian blue is well documented (228, 229).

3.1.1.5 *Lazurite (Ultramarine)*

Historically, natural ultramarine was one the most expensive and esteemed pigments, most prominent in illuminated manuscripts and Italian panel paintings where it was used to mark the significance of the work in area such as the robes of religious figures. It has a rich, royal blue colour ($\lambda_{\text{max}} \approx 600 \text{ nm}$). The blue mineral lazurite ($3\text{Na}_2\text{O}_3 \cdot 3\text{Al}_2\text{O}_3 \cdot 6\text{SiO}_2 \cdot 2\text{Na}_2\text{S}$) of the sodalite group is extracted from the precious stone lapis lazuli, mined in the Middle Ages in the mountains of Afghanistan and then later in the 19th century from Siberia. The natural pigment was always prohibitively expensive leading to a competition to encourage an artificial product. Jean Baptiste

Guimet was awarded a prize of six thousand francs for developing a process for artificial production of ultramarine in 1828 (224, 230).

Other sodium alumino-silicates with related chemistry are known as ultramarine green, violet, pink and red, in reference to their hue. The key chromophores in ultramarine green have been investigated with Raman and shown to be S_3^- and S_2^- (231). Ultramarine violet and pink contain S_3^- and S_2^- together with a third uncertain chromophore (232).

Ultramarine blue is easily characterised using Raman spectroscopy. The Resonance Raman (RR) effect has been shown at a number of wavelengths: 457.9, 488.0, 514.5, 568.2 and 647.1 nm (233).

3.1.1.6 Prussian blue

Prussian blue (ferric ferrocyanide, $KFe[Fe(CN)_6]$ and $Fe_4[Fe(CN)_6]$) is described as the first artificially produced modern pigment. Its accidental discovery was reported by a German Chemist, Stahl, sometime during the years 1704 to 1707. Until publication of the method in Philosophical Transactions of 1724 the method was kept secret. “To an alkali calcined with bullock’s blood, dissolved and brought to boiling point, a solution of alum and ferrous sulphate was added while also boiling. During the effervescence which followed the mixture turned green and after it had been allowed to stand it was strained. The residual greenish precipitate turned blue as spirit of salt (hydrochloric acid) was poured on it. The pigment was then left to stand and washed several times with water the next day, after which it was filtered and dried under gentle heat” (224). It is still a popular pigment and its use continues today although since about 1970 it is often replaced by phthalocyanine blue (234).

3.1.1.7 Smalt

Smalt is a sometimes deep blue, sometimes pale blue-grey coloured pigment, a cobalt containing ground glass ($K, Co, (Al).n(SiO_2)$). Use is documented before the 16th century and ceases after the 18th century. It was a relatively inexpensive pigment prepared by fusing potash with saffer (cobalt ore heated to form an oxide and mixed with silica). The deep blue glass was vitrified and plunged into cool water to make it friable. It was then ground and washed. The quality varies with the intensity of blue colour (224).

3.1.2 *Black*

3.1.2.1 *Bone black/ivory black*

Carbonising bones or the waste trimmings of ivory produces these two blacks. Bones (that had been boiled to remove glue or fat) or ivory were packed into lidded clay containers and placed in a kiln to char. The residue is ground, washed and dried. Beginning in around the 4th century BC, the production of ivory black ceased around 1929 though manufacture of bone black continues today. Bone black is sometimes still marketed under the name ivory black and contains approximately 10% carbon, 84% calcium phosphate and 6% calcium carbonate (148).

3.1.2.2 *Vine black/fruit stone black*

Also known as charcoal black and carbon black, vine black is prepared by charring small branches, twigs or vine clippings. Fruit stones commonly used are cherries, peaches or dates. Cork, almond, walnut and coconut shells have also been used to produce blacks in this manner (224).

3.1.2.3 *Mars black*

Magnetite (iron (II) di-iron(III) oxide, Fe_3O_4) was manufactured from the middle of the nineteenth century and is still marketed today. It is thermally reactive and rapidly transforms to Fe_2O_3 with laser irradiation (35).

3.1.2.4 *Lamp black*

Lamp black was an easy pigment for artists to produce themselves, but also produced commercially. Special rooms were constructed which collected the soot on a porous material as it passed through a chimney. It derives from the partially unburnt hydrocarbons of the fuel oil or wax (224).

3.1.3 *Green*

3.1.3.1 *Chrome green and viridian*

Chrome green: Chromium (III)-oxide (Cr_2O_3) and viridian: Chromium (III)-oxide dihydrate ($\text{Cr}_2\text{O}_3 \cdot 2\text{H}_2\text{O}$) were both introduced in the early 19th century. Other rarer chrome containing pigments are documented but not well recognised. Anhydrous chrome oxide is a dull olive green and more opaque than the bright green dihydrate. Chromium oxide is still widely available while viridian is less so. There are a number of methods for preparation of chromium oxide;

1. Calcining mercury chromate
2. Calcining a mixture of potassium chromate and sulphur
3. Calcining neutral potassium chromate with ammonium chloride. The product was washed, dried and ignited
4. Current methods generally involve sodium, potassium or ammonium dichromate being reduced with sulphur or carbonaceous material.

Viridian was produced in 1859 by calcining boric acid and potassium bichromate, the product being thoroughly washed. Later variations of the procedure describe different ratios of the two components. Boron is known to be combined in the product in some cases (235).

Chrome green is also the name given to a green pigment made from the mixture of Prussian blue and chrome yellow (236). This mixture is sometimes known as Brunswick green.

3.1.3.2 Chrysocolla

Chrysocolla is a mineral green copper silicate ($\text{CuSiO}_3 \cdot n\text{H}_2\text{O}$). Despite historical accounts of production, it has not been identified in any paintings. It is more likely it was used in watercolours which are subjected to pigment analysis far less frequently.

3.1.3.3 Cobalt green

Cobalt green Cobalt(II)-oxide-zinc(II)-oxide ($\text{CoO} \cdot n\text{ZnO}$) is also known as zinc green or Rinman's green and was developed by the Swedish chemist, Rinman. A process for making a compound of cobalt and zinc in 1780 was published in the journal of the Stockholm Academy of Sciences. In the book, *The Chemistry of Paints and Painting*, the production is described as using nitrates of zinc and cobalt being mixed and "precipitated with an alkaline carbonate" and then strongly heated. The product was washed and ground.

Historical accounts of the pigment "chrome green" in the late eighteenth and early nineteenth centuries often refer to it as a mixture of cobalt blue and chrome yellow. Use of the pigment in the form discovered by Rinman is thought to coincide with the availability of zinc oxide which was not manufactured on a large scale until the mid nineteenth century. It is still in use (224).

3.1.3.4 Emerald green

Copper(II)-acetoarsenite $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot 3 \text{Cu}(\text{AsO}_2)_2$ was developed during the period 1814-1960s and is also known as Paris green, discovered while trying to improve Scheele's green. It

has a brilliant blue-green to green hue but is no longer available because of toxicity. Discovery credited it to two different sources. German paint manufacturer Sattler mixed verdigris with an arsenic compound and a pleasant green colour was formed. With the help of pharmacist Russ he produced a product called Schweinfurt green, kept as a trade secret until 1822. Mitis from Vienna was also credited with discovery between 1798 and 1812 although it may be Scheele's green – the reliability of the source is unknown (237).

Windsor and Newton discontinued stocking it in the 1960s, replacing it with a mixture of azo yellow and phthalo green, called Windsor emerald. Other colour suppliers produce colours called emerald green that are composites of newer less toxic pigments.

3.1.3.5 Malachite and green verditer

Malachite ($\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$) is a natural mineral green often found in association with chrysocolla and azurite. Green verditer (also $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$) is artificially produced green basic copper carbonate.

Malachite has been used in Egyptian paintings of the fourth dynasty and was important mainly in the 15th and 16th centuries. Although recognised as a pigment, it is not often identified on artworks. In a report of its use in a fifteenth century illuminated parchment, it could not be identified using Raman but FTIR was successful (48).

3.1.3.6 Scheele's green

Scheele's green is Copper (II) arsenite $\text{Cu}(\text{AsO}_2)_2$ compounds of varying composition, depending on the method of production. Colours range from pale yellow-green to deep green. While researching arsenic, Swedish chemist Scheele discovered Scheele's green in 1775 and published his findings in 1778. Synthesis involved mixing solutions of copper sulfate and arsenic trioxide (dissolved with potassium carbonate) to produce a precipitate of copper arsenite. It became unpopular as a pigment largely due to darkening with time. It was rarely used after the late 1870s (although there is no exact date for discontinuation of use), gradually replaced in use by Emerald green (237).

3.1.3.7 Terre-verte

Also known as green earth, it is a naturally derived complex of aluminosilicate minerals, predominantly the clay minerals glauconite and celadonite $\text{K}[(\text{Al}, \text{Fe}^{\text{III}}), (\text{Fe}^{\text{II}}, \text{Mg})](\text{AlSi}_3, \text{Si}_4)\text{O}_{10}(\text{OH})_2$ with layered silicate structure similar to mica. As

mineral sources are not always pure, other minerals are often found in association, eg; montmorillonites, chlorites, kaolinites and ferric oxides (238). Preparation involves grinding the mined material. Sources of the late eighteenth century were Italy, France and Cyprus (224). It appears in medieval paintings as under-paint for shadows and flesh tones and declines in favour after the Renaissance (239).

3.1.3.8 *Verdigris*

Neutral verdigris is copper acetate, $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ while basic verdigris contains more $\text{Cu}(\text{OH})_2$ and H_2O and is known as $\text{Cu}(\text{OH})_2 \cdot (\text{CH}_3\text{COO})_2 \cdot 5 \text{H}_2\text{O}$. It is the oldest manufactured green, produced in association with winemaking and earlier accounts of production describe suspending copper plates above sour red wine in earthenware pots. After several days, the plates were scraped collecting the corrosion product which formed. The refined variety, neutral verdigris, was made by mixing crude basic verdigris from the process above, with distilled vinegar then filtering and drying to promote conversion of the bluer copper hydroxide to the greener copper acetate. It was used frequently from antiquity through the Middle Ages, Renaissance and Baroque. Verdigris was the most vibrant green available until the development of the chrome and arsenic greens of the 19th century. The colour “green copper resinate” is made by mixing well ground verdigris and Venice Turpentine which is used as a glaze, often over lead white (224).

3.1.4 *Red/orange/brown*

3.1.4.1 *Alizarin*

Alizarin is one of the main chromophores of the natural plant dye, madder (*Rubia tinctorum*). It has a prominent history of use as a fabric dye and “lake” pigment. It is one of a group of anthraquinone colourants, being 1,2-dihydroxyanthraquinone and when used as a pigment is attached to a base, commonly alum. Synthesised in 1868 the synthetic product has nearly completely replaced the use of the natural variety (224).

3.1.4.2 *Litharge*

Litharge is tetragonal lead (II) oxide, PbO . Both massicot and litharge are lead monoxide, but the names reflect differences in the source, crystallography and slightly different properties. It is the crystalline oxide formed from direct oxidation of molten lead in reverberatory furnaces. Analysis with Raman spectroscopy can lead to misidentification if it is not performed with possible

thermal degradation products in mind (240-242). It is not considered a pigment usually, rather it is used as a paint and varnish drier and is an intermediary in the production of red lead (148).

3.1.4.3 Mars red

The Mars colours (Mars yellow, Mars Orange, Mars Red and Mars Violet) are artificially prepared iron oxides (ochres) formed from the combination of a soluble iron salt with alum (aluminium sulfate) together with an alkali such as lime (Ca(OH)_2) or potash (KOH). The proportion of alum determines the depth of yellow colouration, and heating to various degrees develops the colours of brown, orange, red and violet (148).

3.1.4.4 Realgar

Realgar is a natural orange-red arsenic sulfide mineral (As_2S_2). It is found in nature, often in mineral deposits in association with orpiment, a yellow arsenic sulfide. Its name comes from Arabic *Rahj al ghār* meaning powder of the mine and although not found in paintings as frequently as orpiment, it is found on Eastern miniatures and illuminated manuscripts with orpiment (148).

3.1.4.5 Red, yellow and brown earths/ochres

Anhydrous iron(III)-oxide (Fe_2O_3), hematite, is one of the oldest pigments known to man, used in cave paintings and other prehistoric artefacts. It is still widely used today. As it is a naturally occurring pigment, it is often found in association with clays and silica in surface deposits around the world. Ochres are variable with hues ranging from pale yellow through deep red to violet and brown depending on the hydration and resultant crystalline morphology. Heating develops the brown colour by partially hydrating the oxide (148).

3.1.4.6 Red lead

Lead(II,IV)-oxide (Pb_3O_4) also known as minium in the seventeenth century, is a bright scarlet red produced by roasting litharge (PbO) at about 480°C . This must be done carefully as overheating decomposes the product back to PbO . There are some instances where red lead has discoloured due to degradation from environmental conditions aggravated by humidity and air pollution (148). It has been used since antiquity as a pigment and in recent times as an anti-corrosive primer for structural metals and a priming paint for decorators.

3.1.4.7 Vermilion

Mercuric sulfide (HgS) occurs naturally as the mineral cinnabar and was produced artificially since the early medieval period. The dry method of preparation is described as an amorphous mixture of mercury and sulfur, heated in a pan and the sublimation product collected on earthenware pots or cylinders. A wet method for production was known as early as the seventeenth century. Mercury and sulfur are ground together in water and at the end of the grinding process, caustic potash is added causing the black amorphous mass to become red. The product is then washed and dried to remove soluble sulfur compounds (224).

Cinnabar is particularly susceptible to thermal degradation during analysis using Raman spectroscopy, and the laser power when more than only a few mW, often results in a black spot where irradiation has decomposed the sample. This relates to a material phase change from red α -HgS with a hexagonal crystal structure to metacinnabar (α' -HgS), or amorphous HgS, both of which are black structural isomers (243).

3.1.4.8 Brown ochre and Umber

Brown ochre and umber are iron oxides. Iron oxides are discussed in comments on red and yellows.

3.1.4.9 Vandyke brown

Vandyke brown is a transparent colour, usually with over 90% organic content being humic material such as brown coal, peat or soil. It is believed to have originated in Germany, giving rise to its other common names Cassel Earth and Cologne Earth and is prepared by milling (after drying) the finely divided material that comes as a wet mass from digging pits. Earliest dates of use are possibly late sixteenth century and it is still in use. The name refers to the seventeenth century Flemish painter, Anthony van Dyck and is supposedly the brown used in his pictures (244).

3.1.5 Yellow

3.1.5.1 Cadmium yellow, orange and red

Cadmium sulfide (CdS) and slight chemical variations produce yellow ranging in colour from very light to deeper orange which sometimes contain zinc in solid solution (Cd,ZnS) and red through the substitution of sulfur for selenium (CdS,Se). Cadmium mercury oranges (Cd,HgS) and reds are also variants on the basic structure. There are two main types of the yellow; 1) the

compound CdS and 2) the lithopone type which is co-precipitated cadmium sulfide and barium sulfate. Cadmium yellow lithopone contains about 38% cadmium sulphide with the advantage of reduced manufacturing cost.

Stromeyer, in 1818, discovered metallic cadmium and recommended the new yellow sulfide as a pigment. However, due to scarcity of the metal, the pigment was not available for commercialisation until the mid-1840s. It is still a popular yellow pigment. The colour is not only dependent upon composition and crystallinity, but on pigment grain size and paler varieties often have pigment grains very much (up to fifty times) smaller than the darker varieties (148).

3.1.5.2 Chrome yellow and orange

Chrome yellows range in colour from medium yellow to a red-orange; medium yellows are nearly pure lead chromate. Chrome yellow exists as PbCrO_4 and $\text{PbCrO}_4 \cdot \text{PbSO}_4$, chrome orange $\text{PbCrO}_4 \cdot \text{PbO}$. As with cadmium yellows and oranges, colour depends on crystal size – the lighter, more yellow variety has finer grains, the darker has larger more rectangular crystals (148).

3.1.5.3 Cobalt yellow

Also known as aureolin, cobalt yellow is potassium cobaltinitrite $\text{K}_3[\text{Co}(\text{NO}_2)_6] \cdot \text{H}_2\text{O}$. It was discovered by Fisher in 1848 and introduced as a pigment in 1861. It is prepared from the precipitation of a cobalt salt in acid solution with concentrated potassium nitrite. Thorough washing of the precipitate is necessary to ensure stability of the pigment (148).

3.1.5.4 Indian yellow

Indian yellow is magnesium euxanthate (magnesium or calcium salt of euxanthic acid) $\text{C}_{19}\text{H}_{16}\text{O}_{11}\text{Mg} \cdot 5 \text{H}_2\text{O}$. It was discovered in the east in the mid fifteenth century and traditionally the pigment was made in Monghyr, a city in Bengal, from the urine of cows fed only on mango leaves. The dried extract was powdered and washed producing a deep, rich yellow colour that was reportedly lightfast. The malnourishment of the animals led to the prohibition by law of pigment production in 1908. A synthetic substitute exists (it is found in the Colour Index as Natural Dye No 75320) but it is not used in current times (245).

3.1.5.5 Lead-tin yellow

Lead tin yellows are divided into two types: I and II, based on their crystallography and silicon content. Type I is Pb_2SnO_4 , a paler yellow than type II which is $\text{PbSn}_{1-x}\text{Si}_x\text{O}_3$ and generally used in different locations in earlier periods. They have a complicated history, used between the

thirteenth and eighteenth centuries, Type II may have originated from the glass colouring industry where lead and tin were used as opacifiers and colourants. It seems to have been used earlier, in Italian and Bohemian paintings of the “trecento” (before 1400) and in paintings of the “International Court Style” and is said to have fallen out of use around the mid fifteenth century (246). Lead tin yellow type I comes from Northern Italy, perhaps as a by-product of ceramic colourants and is found in paintings of the sixteenth century, often in association with type II which reappears at this time (247).

3.1.5.6 Massicot and Litharge

Massicot is orthorhombic lead (II) oxide, PbO and has the same stoichiometry as litharge, but a slightly different crystalline structure resulting in a paler yellow colour. Massicot is made by roasting white lead at about 300°C while litharge (or “flake” litharge) results from the oxidation of molten lead (148).

3.1.5.7 Naples yellow

Lead (II) antimonite, is generally referred to as a structural analogue of the mineral bindheimite $\text{Pb}_2\text{Sb}_2\text{O}_7$ although other structural formulae can be encountered in the literature $\text{Pb}_3(\text{SbO}_4)_2$, $\text{Pb}_3(\text{SbO}_3)_2$, $\text{Pb}_8(\text{SbO}_4)_2$, $\text{Pb}(\text{SbO}_4)_2$ and PbSb_2O_4 . On the basis of this, a proposed formula is $\text{Pb}_{2-3}\text{Sb}_{1-2}\text{O}_7$ which would be expected from the dependence of ratios of lead and antimony used, other ingredients and the temperature and time of calcination. Differences in colour are observed with composition, and range from deep yellow to orange yellow (248).

It originates from the ceramic industry in Egypt and has been identified in cake form with other pigments of that era. Use in wall paintings has a limited date (1700 – 1850), occurring after lead-tin yellows and declining in use with the advent of lead chromate and cadmium sulfide yellows. Despite this, it is still used as a ceramic glaze colourant where it may be encountered currently.

3.1.5.8 Orpiment

Orpiment, also known as King’s yellow, is a naturally occurring mineral yellow arsenic sulfide (As_2S_3). The name comes from Latin, *auripigmentum* meaning gold colour or paint. It is most commonly found in association with Realgar (As_2S_2) and Pararealgar (As_4S_4). It does not occur in Western easel paintings often, mainly found in Asian, Byzantine and Persian paintings (148).

3.1.5.9 Strontium, barium and calcium chromates

Strontium chromate (SrCrO_4) and barium chromate (BaCrO_4) are both known as lemon yellow. In chemistry and colour, strontium yellow is much like barium yellow and is prepared by mixing solutions of strontium chloride with an alkali solution of chromate or dichromate. It was known to chemists in the early 1790s but may not have been used as a pigment until after 1809. Strontium chromate pigment is frequently applied to metals in primer paint layers so that if the paint film becomes damaged, moisture will release soluble chromate to act as a corrosion inhibitor on the bare metal surface.

Barium chromate is prepared by precipitation of barium chloride with potassium chromate or dichromate. It was considered as a yellow-green pigment for porcelain before introduction as a pigment around the middle of the nineteenth century. Calcium chromate (CaCrO_4) was first produced at the start of the nineteenth century, but it is not mentioned in terms of being used in paints until the 1860s. It is not known to be used as a pigment, but an extender for other yellow pigments such as chrome yellow (249).

3.1.5.10 Yellow ochre

Ochres have been used universally as pigments from earliest history and are still used extensively. Along with golden ochre, red ochre and brown ochre, yellow ochre is a natural earth pigment consisting predominantly of silica and clay. Red ochre is coloured by hematite (Fe_2O_3) while yellow is attributable to goethite ($\text{FeO} \cdot \text{OH}$). Artificial iron based yellows and reds are now common, and known as Mars Colours (148).

3.1.5.11 Zinc yellow

There are two commonly known zinc chromate yellows; zinc potassium chromate ($\text{K}_2\text{O} \cdot 4\text{ZnCrO}_4 \cdot 3\text{H}_2\text{O}$) corresponding with CI Pigment Yellow 36, No. 77955, and basic zinc yellow with composition $\text{ZnCrO}_4 \cdot 4\text{Zn}(\text{OH})_2$ referred to as CI Pigment Yellow 36, No. 77956 (250).

As with many pigments, there is some uncertainty about the exact dates of production and introduction as a pigment but is not mentioned in technological encyclopaedias before 1850. “Zinc chrome green” was a pigment mixture of zinc yellow and Prussian blue and a further mixture known as “Victoria green” was a mixture of zinc chrome green and hydrated chrome oxide. Zinc yellow is still in production (249).

3.1.6 White

3.1.6.1 Barium sulfate

Barium white, BaSO_4 , derives from both natural and synthetic sources. The natural product (barytes) began use as an artists' pigment after 1782 when Guyton de Morveau provided a report into natural alternatives to lead white. Synthetic Blanc Fixe dates from around 1832 and is prepared from native barytes, which is almost never found naturally as a pure mineral. There are a number of patents representing many methods of preparation. One uses a soluble barium salt and a soluble sulfate to precipitate Barium sulfate, another mixes barium chloride (hydrated) with sulfuric acid. Yet another describes the use of barium carbonate dissolved in hydrochloric acid then reacted with sulfuric acid to form barium sulfate.

In earlier years, it was often used as an extender with lead white and is still used as a base or mordant for organic lake pigments. It is also encountered in the synthetic white pigment lithopone, which is a mixture of BaSO_4 and ZnS . Although use of barium sulfate has declined in recent years, both natural and synthetic products are still available (251).

3.1.6.2 Calcium carbonate whites

Chalk (CaCO_3) is a natural mineral, calcite, occurring in deposits world-wide which originate from sedimentary deposits of marine organisms (*Foraminifera*). Its use as a pigment is limited as it is somewhat translucent and does not have the whitening power of lead or titanium white. It is used extensively as filler in cheaper paints and as a ground layer applied in gesso. Gesso is usually a mixture of animal glue and gypsum (sometimes chalk) applied before the painting of the image commences.

Bone white ($\text{Ca}_3(\text{PO}_4)_2$) and shell white (CaCO_3) were used infrequently as an alternative to lead white when reactivity of pigments was a problem. They are reported to have been used in the seventeenth and eighteenth centuries. Bone white was prepared from calcined bones while shell white was prepared by finely grinding bird eggshells or sea shells. Because the chemical composition of chalk and shell white is identical, they are unable to be distinguished by analytical techniques easily (148).

3.1.6.3 Gypsum

Gypsum, calcium sulfate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), is most often encountered in the ground layers of paintings as part of the preparation layers known as gesso. Gypsum can also be used as a filler

in paints to add bulk and change the handling properties of the paint or reduce cost. It is mined from mineral deposits and occurs all over the world but most importantly in Europe, the United States and Canada. Use of calcium whites occurs from antiquity to the present day (148).

3.1.6.4 Lead white

Basic lead carbonate ($2 \text{Pb}(\text{CO}_3)_2 \cdot \text{Pb}(\text{OH})_2$) was the only white pigment used in European easel paintings until the nineteenth century. Traditional methods of manufacture involved suspending coils or plates of lead above vinegar in barrels or pots. These would be set in beds of dung to provide heat, and the lead periodically removed and beaten to remove the white product after a few weeks.

The risks associated with use by a painter while painting were far less than those encountered during the production of the pigment and the subsequent grinding with oil to make paints. Acknowledging its potential for toxicity it is almost completely replaced by the more modern, less toxic whites introduced in the eighteenth century and Titanium white of the early twentieth century (224).

3.1.6.5 Titanium white

Because of its non-toxicity, opacity and cost titanium white, TiO_2 , has almost completely replaced the use of other white pigments worldwide. The three main polymorphs of TiO_2 found are anatase (tetragonal), brookite (orthorhombic) and rutile (tetragonal). Each has a unique electronic band structure and consequently, Raman spectrum (252). It is manufactured as either pure titanium dioxide (anatase or rutile) or as a coating on a base of barium or calcium sulphate. Production is by the sulfate process or more recent and more compact chloride process. The sulfate process results in either anatase or rutile, depending on the additives used prior to calcination. The chloride process produces the rutile form only.

Although discovered earlier as part of research into recovering iron from titaniferous ores, a commercial product was available in limited quantities in Norway and the United States from about 1916-18. Use in paints may have been limited to house paints in the earlier years, and artists colour makers did not accept it widely into their products until the early 1930s. The most notable and often quoted date of introduction is that by Windsor and Newton, 1928-1934; however there are accounts of French colour makers' introduction as early as 1925 (148).

3.1.6.6 Zinc white

Also widely recognised as Chinese white, zinc white (ZnO) was known for many years before general use as a pigment at the end of the eighteenth century. It is most often found in watercolours. There are documented references to the use of Zinc white as a possible pigment alternative to lead white as early as 1739 and 1783, however it was not until the 1830-40s that there was genuine interest for its use in paints. Use continues today.

The 'indirect process' uses the smoke derived from molten zinc heated to 150°C and collected in a series of chambers. This is also known as the French process. Another processes, the American or direct process involves heating zinc ore. The third group of production methods involve precipitation or hydrolysis of solutions of zinc salts and the product is known as “precipitated zinc white” (253).

3.2 Visual observations

Although not apparent without magnification, many of the naturally derived pigments (not synthetically manufactured) were found to be quite heterogeneous at the microscopic level.

The sampling approach (bulk or micro-analytical) affects the representation of components of the sample. If a bulk analysis such as powder XRD is chosen, the composition is more representative of the overall mixture and will indicate the various proportions of components. If a micro-analytical approach is taken whereby individual grains are selected, identification of pigments in paint relies on the detection and selection of representative coloured pigment grains.

Some pigment grains, especially those of naturally derived materials, were found to be in the order of up to several microns. Others, such as manufactured Cd and Cr based pigments were sub-micron in dimension. The visual appearance of cross sectioned paint layers is in keeping with the pigment grain dimensions and so paint layers can appear granular and particulate or smooth. Raman identification with an interaction volume of 1 – 2 μm does not have sufficient spatial resolution to select individual smaller pigment grains if they are part of a mixture, and it is much easier in practice to target larger single colourant particles.

3.3 XRD results

The main identified crystalline phases are tabulated as follows. A full Reitveld quantitative analysis was not considered necessary as detailed quantitative information is of limited use for art historical questions, considering how paints are expected to be mixed or can be easily contaminated; however minor phases were identified and are listed in parenthesis. In most samples the mineralogical composition was consistent with the expected phases for the pigment name, for example sample Green52 was labelled chrysocolla but XRD identified malachite.

Pigment Common Name	Pigment Chemistry	Source	Source Location	NGV Pigment reference number	Raman Spectrum File Name	Raman Spectral Range (cm ⁻¹)	Raman Spectrum Plot	XRD PDF Match Number	XRD Match description
Azurite (extra fine)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1021	Germany	NGV#38	blue38.dx	100-1800	Raman spectra of blues: Part 1	11-682 (33-1161)	Azurite (Quartz)
Azurite (extra fine)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1021	Germany	NGV#38	blue38b.dx	3190-3600	Raman spectra of blues: Part 1	11-682 (33-1161)	Azurite (Quartz)
Azurite (standard)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1020	Germany	NGV#39	blue39.dx	100-1800	Raman spectra of blues: Part 1	11-682	Azurite
Azurite (standard)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1020	Germany	NGV#39	blue39b.dx	3190-3600	Raman spectra of blues: Part 1		
Azurite (standard)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1028	Germany	NGV#40	blue40.dx	100-1800	Raman spectra of blues: Part 1		
Azurite (standard)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1028	Germany	NGV#40	blue40b.dx	3190-3600	Raman spectra of blues: Part 1		
Azurite (dark extra fine)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1026	Germany	NGV#41	blue41.dx	100-1800	Raman spectra of blues: Part 1	11-682 (33-1161)	Azurite (Quartz)
Azurite (dark extra fine)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1026	Germany	NGV#41	blue41b.dx	3190-3600	Raman spectra of blues: Part 1	11-682 (33-1161)	Azurite (Quartz)
Azurite (dark standard)	basic copper (II) carbonate, 2CuCO ₃ .Cu(OH) ₂	Kremer#1025	Germany	NGV#42	blue42.dx	100-1800	Raman spectra of blues: Part 1	11-682	Azurite

Azurite (dark standard)	basic copper (II) carbonate, $2\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$	Kremer#1025	Germany	NGV#42	blue42b.dx	3600-3190	Raman spectra of blues: Part 1	11-682	Azurite
Cerulean blue	Wustite, Cassiterite (cobalt (II) stannate, $\text{CoO} \cdot n\text{SnO}_2$)	Schminke#487	Germany	NGV#151	blue151.dx	100-1300	Raman spectra of blues: Part 1		
Cerulean blue	Wustite, Cassiterite (cobalt (II) stannate, $\text{CoO} \cdot n\text{SnO}_2$)	Schminke#487	Germany	NGV#151	blue151b.dx	100-1300	Raman spectra of blues: Part 1		
Cerulean Blue	Wustite, Cassiterite (cobalt (II) stannate, $\text{CoO} \cdot n\text{SnO}_2$)	A Thorn	Melbourne, Australia	NGV#170	blue170.dx	1850-2300	Raman spectra of blues: Part 1		
Cerulean blue	Wustite, Cassiterite (cobalt (II) stannate, $\text{CoO} \cdot n\text{SnO}_2$)	JNH	Melbourne, Australia	NGV#5	blue5.dx	100-1260	Raman spectra of blues: Part 1	29-514 (41-1145)	Cobalt tin oxide (cassiterite)
Cobalt substitute blue	Barite and non-crystalline additive	Schminke#496	Germany	NGV#150	blue150a.dx	100-700	Raman spectra of blues: Part 1	24-1035	Barite, syn
Cobalt substitute blue	Barite and non-crystalline additive	Schminke#496	Germany	NGV#150	blue150b.dx	100-1800	Raman spectra of blues: Part 1	24-1035	Barite, syn
Cobalt aluminate	$\text{CoO} \cdot \text{Al}_2\text{O}_3$	JNH	Melbourne, Australia	NGV#6	blue6.dx	100-700	Raman spectra of blues: Part 1	10-458	Cobalt Aluminium Oxide
Lapis lazuli best quality	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	Kremer#1053	Germany	NGV#44	blue44.dx	100-700	Raman spectra of blues: Part 1		
Lapis lazuli Crystalline	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	Kremer#1054	Germany	NGV#46	blue46.dx	100-700	Raman spectra of blues: Part 1		
Lapis lazuli good quality	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	Kremer#1052	Germany	NGV#45	blue45.dx	100-700	Raman spectra of blues: Part 1		
Lapis lazuli grey-blue	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	Kremer#1050	Germany	NGV#47	blue47.dx	100-700	Raman spectra of blues: Part 1		

Lapis lazuli medium quality	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	Kremer#1051	Germany	NGV#43	blue43.dx	100-700	Raman spectra of blues: Part 1		
Lightproof blue	Barite and non-crystalline colourant	Schminke#498	Germany	NGV#147	blue147.dx	100-1800	Raman spectra of blues: Part 1	24-1035	Barite, syn
Manganese Cerulean Blue		Schminke#492	Germany	NGV#149	blue149.dx	100-1300	Raman spectra of blues: Part 1		
Natural indigo blue	indigotin, $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_2$	Ashill colour studio	UK	NGV#155	blue155.dx	100-1800	Raman spectra of blues: Part 2		non-crystalline
Synthetic indigo	indigotin, $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_2$	Supplied by Cornelissen via A Thorn	Melbourne, Australia	NGV#181	blue181.dx	100-1800	Raman spectra of blues: Part 2		
Paris (Prussian) blue	iron (III) hexacyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	Schminke#493	Germany	NGV#152	blue152a.dx	1850-2300	Raman spectra of blues: Part 2	1-239	Iron Cyanide
Paris (Prussian) blue	iron (III) hexacyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	Schminke#493	Germany	NGV#152	blue152b.dx	100-700	Raman spectra of blues: Part 2	1-239	Iron Cyanide
Paris (Prussian) blue	iron (III) hexacyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	Kremer#4250	Germany	NGV#153	blue153a.dx	1850-2340	Raman spectra of blues: Part 2	1-239	Iron Cyanide
Paris (Prussian) blue	iron (III) hexacyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	Kremer#4250	Germany	NGV#153	blue153b.dx	100-700	Raman spectra of blues: Part 2	1-239	Iron Cyanide
Prussian blue	iron (III) hexacyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	Supplied by Cornelissen via A Thorn	Melbourne, Australia	NGV#178	blue178a.dx	100-700	Raman spectra of blues: Part 2		
Prussian blue	iron (III) hexacyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	Supplied by Cornelissen via A Thorn	Melbourne, Australia	NGV#178	blue178b.dx	1955-2438	Raman spectra of blues: Part 2		

Prussian blue	iron (III) hexa-cyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	JNH	Melbourne, Australia	NGV#3	blue3a.dx	1850-2340	Raman spectra of blues: Part 2	22-215	Cobalt cyanide hydrate
Prussian blue	iron (III) hexa-cyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	JNH	Melbourne, Australia	NGV#3	blue3b.dx	100-700	Raman spectra of blues: Part 2	22-215	Cobalt cyanide hydrate
Prussian blue	iron (III) hexa-cyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	JNH	Melbourne, Australia	NGV#3	blue3c.dx	100-700	Raman spectra of blues: Part 2	22-215	Cobalt cyanide hydrate
Antwerp (Prussian) blue	iron (III) hexa-cyanoferrate(II) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 14\text{-}16\text{H}_2\text{O}$	A Thorn	Melbourne, Australia	NGV#169	blue169.dx	1850-2340	Raman spectra of blues: Part 2		
Pthalo-mona blue	copper phthalocyanine	Cornelissen	UK	NGV#154	blue154.dx	100-1800	Raman spectra of blues: Part 2		non-crystalline
Pthalo-mona blue	copper phthalocyanine	JNH	Melbourne, Australia	NGV#16	blue16.dx	100-1800	Raman spectra of blues: Part 2	11-893	Beta-copper phthalocyanine
Purpurite	purpurite ($\text{Mn,Fe}(\text{PO}_4)$)	Kremer#1096	Germany	NGV#68	blue68.dx	100-1260	Raman spectra of blues: Part 2	37-478 (33-1161)	Purpurite (Quartz)
Smalt (extra fine)	Cobalt (II) silicate, $\text{CoO} \cdot n\text{SiO}_2$	Kremer#1001	Germany	NGV#34	blue34.dx	100-1300	Raman spectra of blues: Part 2		
Smalt (grind unknown)	Cobalt (II) silicate, $\text{CoO} \cdot n\text{SiO}_2$	Kremer	Germany	NGV#32	blue32.dx	100-1300	Raman spectra of blues: Part 2		
Smalt (standard)	Cobalt (II) silicate, $\text{CoO} \cdot n\text{SiO}_2$	Kremer#1000	Germany	NGV#35	blue35.dx	100-1300	Raman spectra of blues: Part 2		
Smalt (standard)	Cobalt (II) silicate, $\text{CoO} \cdot n\text{SiO}_2$	Kremer#1000	Germany	NGV#35	blue35a.dx	100-1300	Raman spectra of blues: Part 2		
							Raman spectra of blues: Part 2		
Ultramarine	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	JNH	Melbourne, Australia	NGV#14	blue14.dx	100-700	Raman spectra of blues: Part 2	17-479	Lazurite-c
Ultramarine	Lazurite, S3- and S2- in a sodium alumino-silicate matrix $\text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Sn}$	Kremer #4500	Germany	NGV#143	blue143.dx	100-700	Raman spectra of blues: Part 2	17-479	Lazurite-c

Ultramarine	Lazurite, S3- and S2- in a sodium alumino-silicate matrix Na ₈ [Al ₆ Si ₆ O ₂₄]Sn	Kremer #4503	Germany	NGV#144	blue144.dx	100-700	Raman spectra of blues: Part 2	17-479	Lazurite-c
Ultramarine	Lazurite, S3- and S2- in a sodium alumino-silicate matrix Na ₈ [Al ₆ Si ₆ O ₂₄]Sn	Schminke#490	Germany	NGV#146	blue146.dx	100-700	Raman spectra of blues: Part 2	17-479	Lazurite-c
Ultramarine	Lazurite, S3- and S2- in a sodium alumino-silicate matrix Na ₈ [Al ₆ Si ₆ O ₂₄]Sn	JNH	Melbourne, Australia	NGV#14	blue14a.dx	100-700	Raman spectra of blues: Part 2		
Ultramarine ash	Lazurite, S3- and S2- in a sodium alumino-silicate matrix Na ₈ [Al ₆ Si ₆ O ₂₄]Sn	Kremer#1058	Germany	NGV#48	blue48.dx	100-700	Raman spectra of blues: Part 2		
Antimony	Antimony (Sb)	Kremer #1094	Germany	NGV#69	black69.dx	100-700	Raman spectra of blacks: Part 1		
Black iron oxide	Iron oxide, magnetite, (Fe ⁺² Fe ⁺³ 2O ₄)	JNH	Melbourne, Australia	NGV#28	black28.dx	100-700	Raman spectra of blacks: Part 1		
Carbon black	Amorphous carbon	Conservation Support Systems	USA	NGV#100	black100.dx	1100-1800	Raman spectra of blacks: Part 1		non-crystalline
Furnace black	Amorphous carbon	Degussa	Frankfurt	NGV#184	black184.dx	700-1800	Raman spectra of blacks: Part 1		non-crystalline
Galena	Lead sulfide (PbS)	Kremer #1090	Germany	NGV#74	black74.dx	100-700	Raman spectra of blacks: Part 1		
Galena	Lead sulfide (PbS)	Kremer #1090	Germany	NGV#74	black74A.dx	700-1300	Raman spectra of blacks: Part 1		
Ivory black	Amorphous carbon	Schminke#723		NGV#101	black101.dx	700-1800.	Raman spectra of blacks: Part 1		non-crystalline
Ivory black	Amorphous carbon	Kremer #1200	Germany	NGV#73	black73.dx	700-1800	Raman spectra of blacks: Part 1		non-crystalline
Lamp Black	Amorphous carbon	JNH	Melbourne, Australia	NGV#27	black27.dx	700-1800	Raman spectra of blacks: Part 2		non-crystalline
Pyrite, Standard	Iron sulfide (FeS ₂)	Kremer #1092	Germany	NGV#71	black71.dx	100-700	Raman spectra of blacks: Part 2		
Vine black	Amorphous carbon	Schminke#722		NGV#102	black102.dx	700-1800	Raman spectra of blacks: Part 2		non-crystalline

China(?) Green		A Thorn	Melbourne Australia	NGV#180	green180.dx	100-700	Raman spectra of greens: Part 1		
Cobalt green	see XRD analysis	A Thorn	Melbourne Australia	NGV#179	green179.dx	100-700	Raman spectra of greens: Part 1	5-664	Zincite
Epidote	Calcium/Iron aluminosilicate	Kremer #1033	Germany	NGV#63	green63.dx	100-1300	Raman spectra of greens: Part 1	17-514 (33-1161)	Epidote (Quartz)
Fuchsite (coarse)		Kremer #11424	Germany	NGV#60	green60.dx	100-1000	Raman spectra of greens: Part 1	33-1161, 29-1499	Quartz, Montmorillonite-21A
Fuchsite (fine)		Kremer #11420	Germany	NGV#58	green58.dx	100-1000	Raman spectra of greens: Part 1	33-1161, 29-1499	Quartz, Montmorillonite-21A
Fuchsite (medium)		Kremer #11422	Germany	NGV#59	green59.dx	100-1000	Raman spectra of greens: Part 1	33-1161, 29-1499	Quartz, Montmorillonite-21A
Green Jasper	Sedimentary siliceous rock	Kremer #1120	Germany	NGV#51	green51.dx	100-700	Raman spectra of greens: Part 1	33-1161 (17-521, 9-439)	Quartz (Celadonite, Glauconite)
Labelled Chrysocola	Malachite (CuCO ₃ .Cu(OH) ₂)	Kremer #1035	Germany	NGV#52	green52.dx	100-1800	Raman spectra of greens: Part 1	41-1390 (33-1161)	Malachite, syn (Quartz)
Malachite (natural, dark)	Malachite (CuCO ₃ .Cu(OH) ₂)	Kremer #1032	Germany	NGV#55	green55.dx	100-1800	Raman spectra of greens: Part 2	41-1390 (33-1161)	Malachite, syn (Quartz)
Malachite (natural, dark, extra fine)	Malachite (CuCO ₃ .Cu(OH) ₂)	Kremer #1033	Germany	NGV#33	green33.dx	100-1300	Raman spectra of greens: Part 2	41-1390	Malachite, syn
Malachite (natural, extra fine)	Malachite (CuCO ₃ .Cu(OH) ₂)	Kremer #50	Germany	NGV#50	green50.dx	100-1800	Raman spectra of greens: Part 2	41-1390	Malachite, syn
Malachite (standard)	Malachite (CuCO ₃ .Cu(OH) ₂)	Kremer #1030	Germany	NGV#49	green49.dx	100-1800	Raman spectra of greens: Part 2	41-1390	Malachite, syn
Oxide of chromium	Chromium(III)-oxide (Cr ₂ O ₃)	JNH	Melbourne Australia	NGV#11	green11.dx	100-700	Raman spectra of greens: Part 2	38-1479	Eskolaite, syn
Permanent green	Nitrosonaphthol	A Thorn	Melbourne Australia	NGV#175	green175.dx	100-1800	Raman spectra of greens: Part 2		non-crystalline
Permanent green deep		Schminke#513	Germany	NGV#94	green94.dx	100-700	Raman spectra of greens: Part 2		non-crystalline
Phthalokinone green light		Cornelissen via A Thorn	Melbourne Australia	NGV#171	green171.dx	100-700	Raman spectra of greens: Part 2		non-crystalline
Sap green		Le franc and Bourgeois via A Thorn	Melbourne Australia	NGV#176	green176.dx	100-1260	Raman spectra of greens: Part 2		
Terre verte		JNH	Melbourne Australia	NGV#1				33-311, 24-1035	Gypsum, Barite
Verdigris	copper acetate, Cu(CH ₃ COO) ₂	Kremer #4445	Germany	NGV#145	green145.dx	100-1800	Raman spectra of greens: Part 3		

Viridian	Chromium(III)-oxide dihydrate (Cr ₂ O ₃ .2H ₂ O)	JNH	Melbourne Australia	NGV#15	green15.dx	100-700	Raman spectra of greens: Part 3		
Viridian, Glowing	Chromium(III)-oxide dihydrate (Cr ₂ O ₃ .2H ₂ O)	Schminke#507	Germany	NGV#95	green95.dx	100-700	Raman spectra of greens: Part 3		
Burnt Sienna, Semi-transparent	hematite (iron oxide, Fe ₂ O ₃)	JNH	Melbourne Australia	NGV#13	brown13.dx	100-700	Raman spectra of browns: Part 1	33-664	Hematite
Burnt Umber	hematite (iron oxide, Fe ₂ O ₃)	JNH	Melbourne Australia	NGV#26	brown26.dx	100-700	Raman spectra of browns: Part 1	33-664 (33-1161)	Hematite (Quartz)
Burnt Umber, Cyprus, Brownish	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4071	Germany	NGV#140	brown140.dx	100-700	Raman spectra of browns: Part 1	33-664, 33-1161	Hematite, Quartz
Burnt Umber, Cyprus, Red	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4073	Germany	NGV#138	brown138.dx	100-700	Raman spectra of browns: Part 1	33-664 (33-1161)	Hematite (Quartz)
English Red, light	hematite (iron oxide, Fe ₂ O ₃)	Schminke#649	Germany	NGV#88	brown88.dx	100-700	Raman spectra of browns: Part 1	33-664	Hematite
Indian Red	hematite (iron oxide, Fe ₂ O ₃)	JNH	Melbourne Australia	NGV#12	brown12.dx	100-700	Raman spectra of browns: Part 1		
Indian red	hematite (iron oxide, Fe ₂ O ₃)	Schminke#651	Germany	NGV#90	brown90.dx	100-700	Raman spectra of browns: Part 1		
Raw Umber	hematite (iron oxide, Fe ₂ O ₃)	JNH	Melbourne Australia	NGV#19	brown19.dx	100-700	Raman spectra of browns: Part 1	29-713 (33-664, 33-1161)	Goethite (Hematite, Quartz)
Raw umber, greenish	hematite (iron oxide, Fe ₂ O ₃)	Schminke#682	Melbourne Australia	NGV#87	brown87.dx	100-700	Raman spectra of browns: Part 1	24-1035, 34-140, 29-713 (33-1161, 33-664)	Barite, Chromite, Goethite (Quartz, Hematite)
Red Jasper	Sedimentary siliceous rock	Kremer#1130	Germany	NGV#64	brown64.dx	100-700	Raman spectra of browns: Part 1		
Terra Pozzuoli	hematite (iron oxide, Fe ₂ O ₃)	Schminke#655	Germany	NGV#89	brown89.dx	100-700	Raman spectra of browns: Part 2	33-664	Hematite
Venetian red	hematite (iron oxide, Fe ₂ O ₃)	JNH	Melbourne Australia	NGV#7	brown7.dx	100-700	Raman spectra of browns: Part 2		
Venetian red	hematite (iron oxide, Fe ₂ O ₃)	Kremer#4051	Germany	NGV#85	brown85.dx	100-700	Raman spectra of browns: Part 2	33-311 (33-1161)	Gypsum, (Hematite)

Alizarin Crimson	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	Cornelissen	UK	NGV#156	red156.dx	100-1800	Raman spectra of reds and violets: Part 1		non-crystalline
Alizarin crimson		JNH	Melbourne, Australia	NGV#9	red9.dx	100-1800			non-crystalline
Alizarin crimson	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	JNH	Melbourne, Australia	NGV#9	red9a.dx	100-1800			non-crystalline
Alizarin crimson	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	JNH	Melbourne, Australia	NGV#9	red9b.dx	100-1800			non-crystalline
Alizarin crimson	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	JNH	Melbourne, Australia	NGV#9	red9c.dx	100-1800			non-crystalline
Alizarin crimson	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	JNH	Melbourne, Australia	NGV#9	red9d.dx	1230-1760			non-crystalline
Alizarin Light	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	Kremer #2360	Germany	NGV#123	red123.dx	100-1800			non-crystalline
Alizarin madder lake deep	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	Schminke#367	Germany	NGV#121	red121.dx	1230-1760			non-crystalline
Alizarin madder lake deep	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	Schminke#367	Germany	NGV#121	red121a.dx	100-1800			non-crystalline
Brown Madder		Sennelier	France	NGV#86	red86.dx	100-1800	Raman spectra of reds and violets: Part 1		non-crystalline
Brown Madder		Sennelier	France	NGV#86	red86a.dx	100-1800	Raman spectra of reds and violets: Part 1		non-crystalline
Cadmium Red Dark		Schminke#361	Germany	NGV#128	red128.dx	100-700	Raman spectra of reds and violets: Part 1		
Cadmium Red Light		Schminke#360	Germany	NGV#127	red127.dx	100-700	Raman spectra of reds and violets: Part 1		
Cadmium Red Light		Cornelissen via A Thom	Melbourne, Australia	NGV#173	red173.dx	100-700	Raman spectra of reds and violets: Part 1		

Cadmium Vermilion		JNH	Melbourne, Australia	NGV#8	red8.dx	100-700	Raman spectra of reds and violets: Part 1		
Cobalt Violet dark		JNH	Melbourne, Australia	NGV#2	viol2.dx	100-1260	Raman spectra of reds and violets: Part 1	13-503 (33-432)	Cobalt Phosphate (Cobalt phosphate hydrate)
French Red Lake	1,2 dihydroxyanthraquinone C ₁₄ H ₈ O ₄	Poggi, 1996		NGV#156	red158.dx	100-1800	Raman spectra of reds and violets: Part 1		non-crystalline
Manganese Violet		Kremer #4535	Germany	NGV#142	viol142.dx	100-1260	Raman spectra of reds and violets: Part 1		
Napthol crimson		A Thorn	Melbourne, Australia	NGV#156	red163.dx	100-1800	Raman spectra of reds and violets: Part 2		non-crystalline
Permanent red (quinacridone)		Windsor and Newton via A Thorn	Melbourne, Australia	NGV#177	red177.dx	100-1800	Raman spectra of reds and violets: Part 2		non-crystalline
Permanent Red 1		Schminke#369	Germany	NGV#124	red124.dx	100-1800			non-crystalline
Permanent Red 2		Schminke#370	Germany	NGV#125	red125a.dx	100-1800			non-crystalline
Permanent Red 3		Schminke#370	Germany	NGV#125	red125b.dx	100-1800			non-crystalline
Permanent Red 4		Schminke#372	Germany	NGV#126	red126.dx	100-1800			non-crystalline
Pink Colour (Cr Al stannate)		Kremer #1015	Germany	NGV#65	red65.dx	100-1800	Raman spectra of reds and violets: Part 2		
Red Lead, Minium		Kremer #4250	Germany	NGV#129	red129.dx	100-700	Raman spectra of reds and violets: Part 2	41-1493	Minium
Translucent Iron Oxide Red		Kremer #5240	Germany	NGV#96	red96.dx	100-700	Raman spectra of reds and violets: Part 2	33-664 (29-713)	Hematite (Goethite)
Ultramarine red		Schminke#357	Germany	NGV#141	red141.dx	100-700	Raman spectra of reds and violets: Part 2		
Ultramarine Violet		Schminke#497	Germany	NGV#148	viol148.dx	100-700	Raman spectra of reds and violets: Part 2	44-1458, 17-749, 33-1161	Lazurite-O, Lazurite-C, Quartz
Vermilion	HgS	A Thorn	Melbourne, Australia	NGV#168	red168.dx	100-700	Raman spectra of reds and violets: Part 2	42-1408	Cinnabar

Vermilion	HgS	Kremer #1061	Supplier:Germany; Pigment: Monte Amiata, Italy	NGV#36	red36.dx	100-700	Raman spectra of reds and violets: Part 2	6-256	Cinnabar(syn)
Vermilion	HgS	Kremer #1061	Supplier:Germany; Pigment: Monte Amiata, Italy	NGV#36	red36a.dx	100-700	Raman spectra of reds and violets: Part 2	6-256	Cinnabar(syn)
Vermilion (synthetic)	HgS	Kremer #1062	Germany	NGV#37	red37.dx	100-700	Raman spectra of reds and violets: Part 2		
Alizarin pure (dye)	1,2 dihydroxyanthraquinone C14H8O4	Kremer #9410	Germany	NGV#106	yell106.dx	100-700			non-crystalline
Cadmium Orange	Cadmium sulfide (CdS)	Schminke#231	Germany	NGV#111	yell111.dx	100-700			non-crystalline
Cadmium Primrose Yellow		JNH	Melbourne, Australia	NGV#17	yell17.dx	100-700	Raman spectra of yellows and oranges: Part 1	40-835	Cd Zn Sulfide
Cadmium Yellow		JNH	Melbourne, Australia	NGV#18	yell18.dx	100-700	Raman spectra of yellows and oranges: Part 1	40-835, 24-1035	Cd Zn Sulfide, Barite
Cadmium Yellow deep	Cadmium sulfide (CdS)	Schminke#229	Germany	NGV#104	yell104.dx	100-700	Raman spectra of yellows and oranges: Part 1	41-1049 (24-1035)	Greenockite (Barite)
Cadmium Yellow deep	Cadmium sulfide (CdS)	Schminke#229	Germany	NGV#104	yell104a.dx	100-700	Raman spectra of yellows and oranges: Part 1	41-1049 (24-1035)	Greenockite (Barite)
Cadmium yellow deep	Cadmium sulfide (CdS)	Supplied by Cornelissen via A Thorn	Melbourne, Australia	NGV#162	yell162.dx	100-700	Raman spectra of yellows and oranges: Part 1		
Cadmium Yellow lemon	Cadmium sulfide (CdS)	Schminke#226	Germany	NGV#117	yell117.dx	100-700	Raman spectra of yellows and oranges: Part 1	40-835, 24-1035	Cd Zn Sulfide, Barite
Cadmium Yellow light	Cadmium sulfide (CdS)	Schminke#227	Germany	NGV#109	yell109.dx	100-700	Raman spectra of yellows and oranges: Part 1	41-1049 (24-1035)	Greenockite (Barite)
Chrome orange	PbCrO4	Schminke#213	Germany	NGV#108	yell108.dx	100-1260	Raman spectra of yellows and oranges: Part 1		
Chrome Yellow	PbCrO4	A Thorn 1997	Melbourne, Australia	NGV#182	yell182.dx	100-1300	Raman spectra of yellows and oranges: Part 1		
Chrome yellow deep	PbCrO4	Schminke#211	Germany	NGV#107	yell107.dx	100-1260	Raman spectra of yellows and oranges: Part 1	8-209	Crocoite

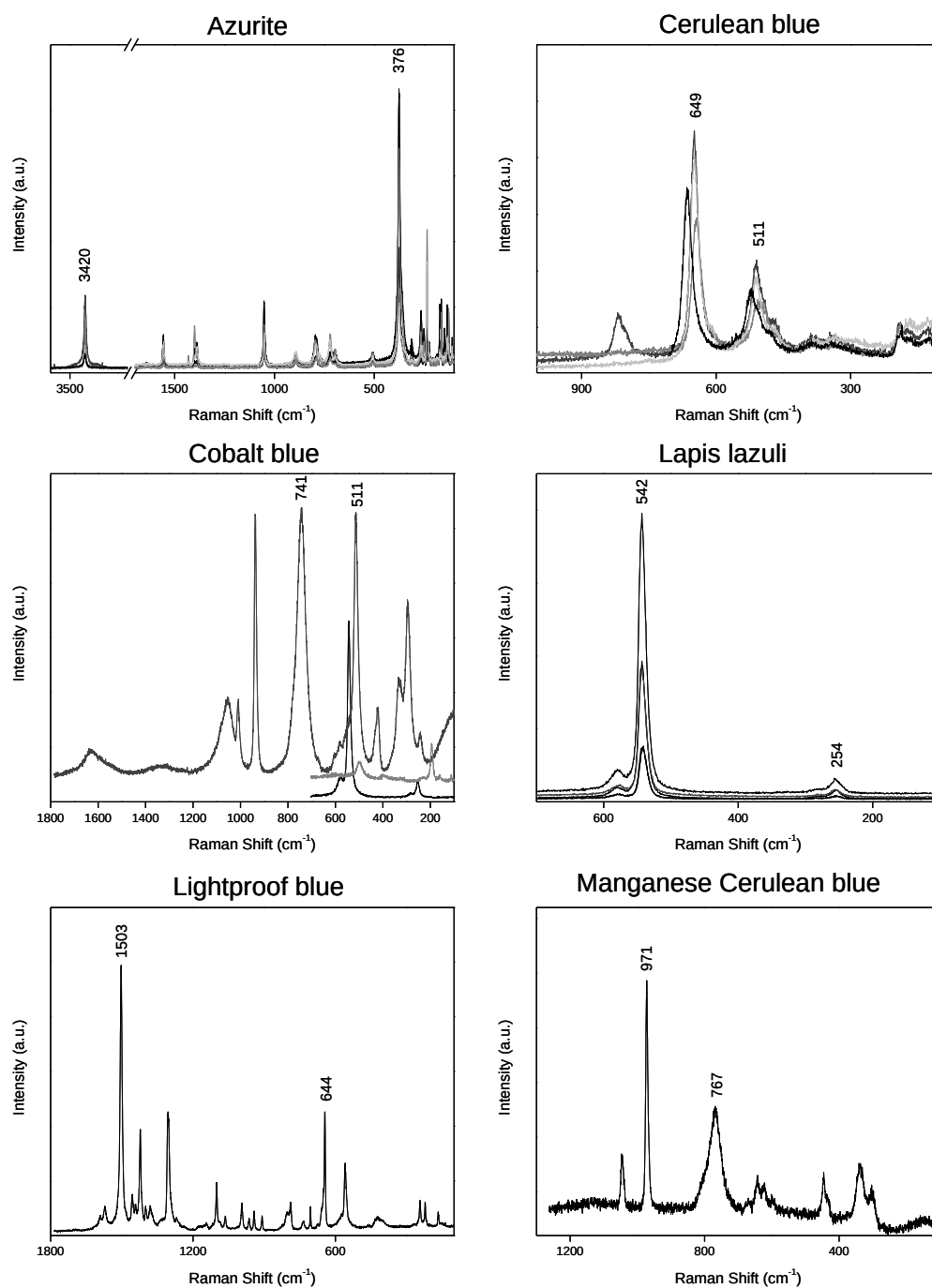
Chrome Yellow light	PbCrO ₄	Schminke#209	Germany	NGV#105	yell105.dx	100-1260	Raman spectra of yellows and oranges: Part 1	8-209	Crocoite
Chrome yellow-orange	PbCrO ₄	A Thorn	Melbourne, Australia	NGV#161	yell161.dx	100-1300	Raman spectra of yellows and oranges: Part 1		
Genuine Naples Yellow light	Pb ₂ -3Sb ₁ -2O ₇	Cornelissen	UK	NGV#118	yell118.dx	100-700	Raman spectra of yellows and oranges: Part 1	42-1355	Bindheimite
Genuine Naples Yellow light	Pb ₂ -3Sb ₁ -2O ₇	Cornelissen	UK	NGV#118	yell118a.dx	100-700	Raman spectra of yellows and oranges: Part 1	42-1355	Bindheimite
Genuine Naples Yellow, reddish	Pb ₂ -3Sb ₁ -2O ₇	Kremer #4313	Germany	NGV#136	yell136.dx	100-700	Raman spectra of yellows and oranges: Part 1	42-1355 (33-1161)	Bindheimite (Quartz)
Genuine orange madder		Supplied by Cornelissen via A Thorn	Melbourne, Australia	NGV#164	yell164.dx	100-1800	Raman spectra of yellows and oranges: Part 1		non-crystalline
Gold Ochre		Schminke#621	Germany	NGV#92	yell92.dx	100-700	Raman spectra of yellows and oranges: Part 1	33-1161, 29-713 (14-164, 33-664)	Quartz, Goethite (Kaolinite, Hematite)
Hansa yellow, Permanent Yellow, PY6		Kremer #2332	Germany	NGV#110	yell110.dx	1710-1180	Raman spectra of yellows and oranges: Part 1		non-crystalline
Hostaperm orange PO60		Kremer #2355	Germany	NGV#120	yell120.dx	100-1800	Raman spectra of yellows and oranges: Part 2		non-crystalline
Indian yellow	magnesium euxanthate	Schminke#243	Germany	NGV#103	yell103.dx	1180-1710	Raman spectra of yellows and oranges: Part 2	24-1035	Barite
Isindolor orange PO61		Kremer #2380	Germany	NGV#112	yell112.dx	100-1800	Raman spectra of yellows and oranges: Part 2		non-crystalline
Italian Gold Ochre	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4042	Germany	NGV#134	yell134.dx	100-700	Raman spectra of yellows and oranges: Part 2		
Lead tin yellow dark		Kremer #1011	Germany	NGV#31	yell31.dx	700-1300	Raman spectra of yellows and oranges: Part 2	24-589 (41-1445)	Lead tin oxide (cassiterite)
Lead tin yellow light		Kremer #1010	Germany	NGV#57	yell57.dx	700-1300	Raman spectra of yellows and oranges: Part 2	24-589 (41-1445)	Lead tin oxide (cassiterite)

Lemon Yellow	Strontium chromate (SrCrO ₄) and barium chromate (BaCrO ₄)	A Thorn 1997	Melbourne, Australia	NGV#167	yell167.dx	100-1300	Raman spectra of yellows and oranges: Part 2		
Naples Yellow deep	Pb ₂ -3Sb ₁ -2O ₇	A Thorn 1997	Melbourne, Australia	NGV#165	yell165.dx	100-700	Raman spectra of yellows and oranges: Part 3	42-1355 (39-834)	Bindheimite (Lead antimony oxide)
Permanent yellow deep		Schminke#240	Germany	NGV#113	yell113.dx	100-1800	Raman spectra of yellows and oranges: Part 3		non-crystalline
Permanent yellow lemon		Schminke#237	Germany	NGV#114	yell114.dx	100-1800	Raman spectra of yellows and oranges: Part 3		non-crystalline
Permanent yellow light		Schminke#239	Germany	NGV#115	yell115.dx	1180-1710	Raman spectra of yellows and oranges: Part 3		non-crystalline
Raw Sienna	hematite (iron oxide, Fe ₂ O ₃)	JNH	Melbourne Australia	NGV#10	yell10.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713, 33-1161 (14-164, 33-664)	Goethite, Quartz (Kaolinite, Hematite)
Realgar		Kremer #1080	Germany	NGV#30	yell30.dx	100-700	Raman spectra of yellows and oranges: Part 3	24-77, 24-75	Realgar, Orpiment
Sienna, Italian, Natural	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4040	Germany	NGV#132	yell132.dx	100-700	Raman spectra of yellows and oranges: Part 3		
Sienna, Italian, Natural	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4040	Germany	NGV#132	yell132b.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713 (14-164)	Goethite (Kaolinite)
Sienna, Italian, Natural	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4040	Germany	NGV#132	yell132c.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713 (14-164)	Goethite (Kaolinite)
Sienna, Italian, Natural	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4040	Germany	NGV#132	yell132d.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713 (14-164)	Goethite (Kaolinite)
Sienna, Italian, Natural	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4040	Germany	NGV#132	yell132e.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713 (14-164)	Goethite (Kaolinite)
Sienna, Italian, Natural, brownish	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4041	Germany	NGV#133	yell133a.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713	Goethite
Sienna, Italian, Natural, brownish	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4041	Germany	NGV#133	yell133b.dx	100-1000	Raman spectra of yellows and oranges: Part 3	29-713	Goethite
Sienna, Italian, Natural, brownish	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4041	Germany	NGV#133	yell133c.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713	Goethite

Sienna, Italian, Natural, brownish	hematite (iron oxide, Fe ₂ O ₃)	Kremer #4041	Germany	NGV#133	yell133d.dx	100-700	Raman spectra of yellows and oranges: Part 3	29-713	Goethite
Spanish Gold Ochre		Kremer #1150	Germany	NGV#62	yell62.dx	100-700	Raman spectra of yellows and oranges: Part 3	5-586 (29-713, 41-1487)	Calcite, syn (goethite, Graphite)
Strontium Yellow	Strontium chromate (SrCrO ₄)	A Thorn 1997	Melbourne, Australia	NGV#172	yell172.dx	100-1300	Raman spectra of yellows and oranges: Part 4		
Yellow Ochre	hematite (iron oxide, Fe ₂ O ₃)	A Thorn 1997	Melbourne, Australia	NGV#166	yell166.dx	100-700	Raman spectra of yellows and oranges: Part 4		
Yellow Ochre	hematite (iron oxide, Fe ₂ O ₃)	A Thorn 1997	Melbourne, Australia	NGV#166	yell166a.dx	100-700	Raman spectra of yellows and oranges: Part 4		
Yellow Ochre 1		JNH	Melbourne, Australia	NGV#21	yell21.dx	100-700	Raman spectra of yellows and oranges: Part 4	29-713 (33-1161)	Goethite (quartz)
Yellow Ochre 2		JNH	Melbourne, Australia	NGV#22	yell22.dx	100-700	Raman spectra of yellows and oranges: Part 4	29-713 (33-1161)	Goethite (quartz)
Zinc Yellow	zinc potassium chromate (K ₂ O.4ZnCrO ₄ .3H ₂ O)	A Thorn 1997	Melbourne, Australia	NGV#183	yell183.dx	100-1300	Raman spectra of yellows and oranges: Part 4	8-202	Potassium zinc chromium oxide hydrate
Gypseous spar	Gypsum, calcium sulphate dihydrate, CaSO ₄ .2H ₂ O	Kremer #1180	Germany	NGV#78	w78.dx	100-1300	Raman spectra of whites	33-311	Gypsum
Gypseous spar, extra fine	Gypsum, calcium sulphate dihydrate, CaSO ₄ .2H ₂ O	Kremer #1181	Germany	NGV#79	w79.dx	100-1300	Raman spectra of whites	33-311	Gypsum
Huntite	Talc, hydrous magnesium silicate, Mg ₃ Si ₄ O ₁₀ (OH) ₂	Used by Rover Thomas		NGV#80	w80.dx	100-700	Raman spectra of whites	29-1493	Talc-2M
Lead White	basic lead (II) carbonate, 2PbCO ₃ .Pb(OH) ₂	Kremer #4600	Germany	NGV#91	w91.dx	100-1300	Raman spectra of whites	13-131	Hydrocerussite
Mountain crystal	Quartz, SiO ₂	Kremer #1140	Germany	NGV#77	w77.dx	100-1300	Raman spectra of whites	33-1161	Quartz
Titanium white	titanium dioxide, TiO ₂	JNH	Melbourne, Australia	NGV#23	w23.dx	100-700	Raman spectra of whites	21-1276	Rutile, syn
Zinc white	zinc oxide, ZnO	JNH	Melbourne, Australia	NGV#25	w25.dx	100-1300	Raman spectra of whites	36-1451, 19-1458	Zincite, Hydrozincite

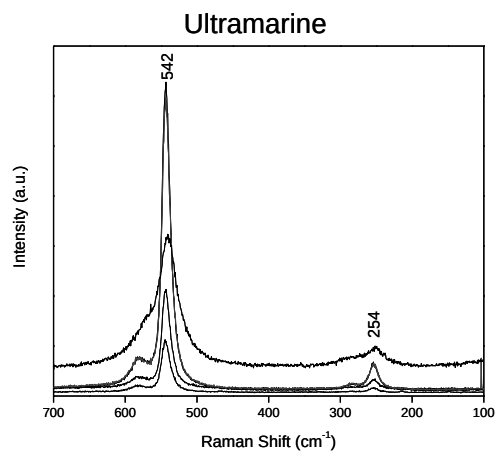
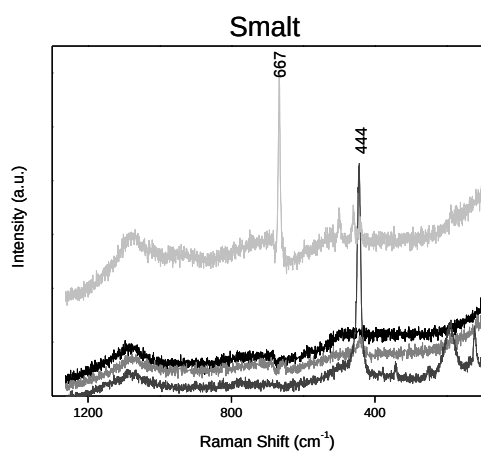
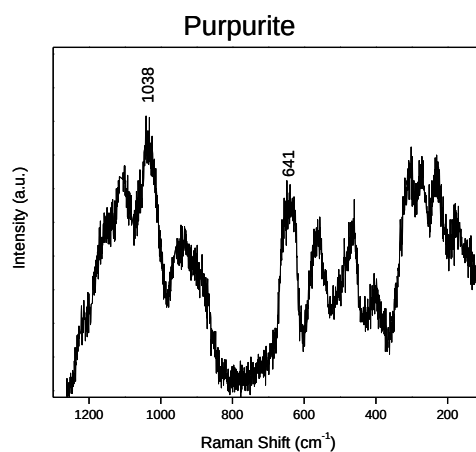
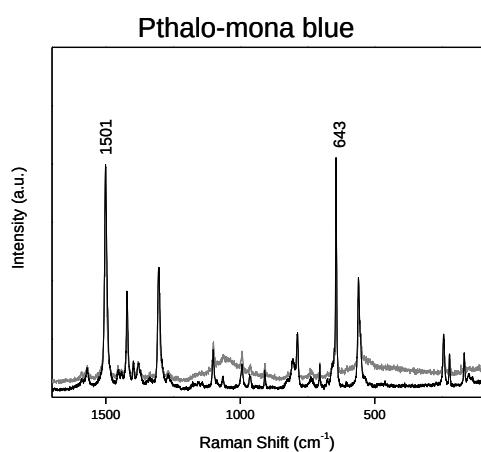
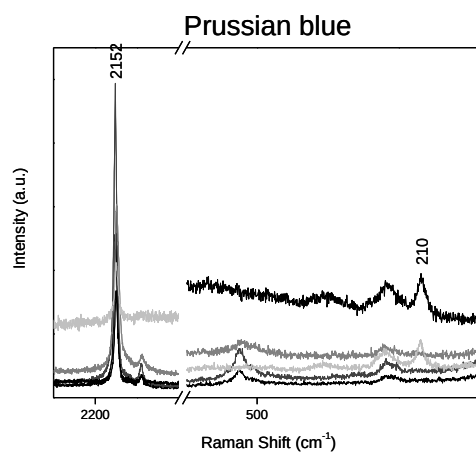
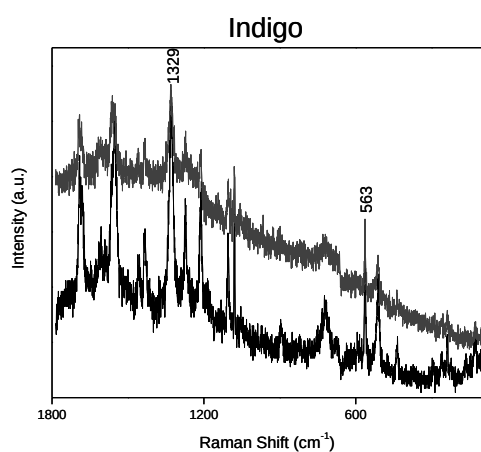
3.4 Raman Spectra

The collected Raman spectra were plotted and are presented here, arranged into their major colour group and alphabetically within the group. Spectra were collected only in the region where spectral features occurred, so x-axis wavenumbers are not equivalent for all samples.



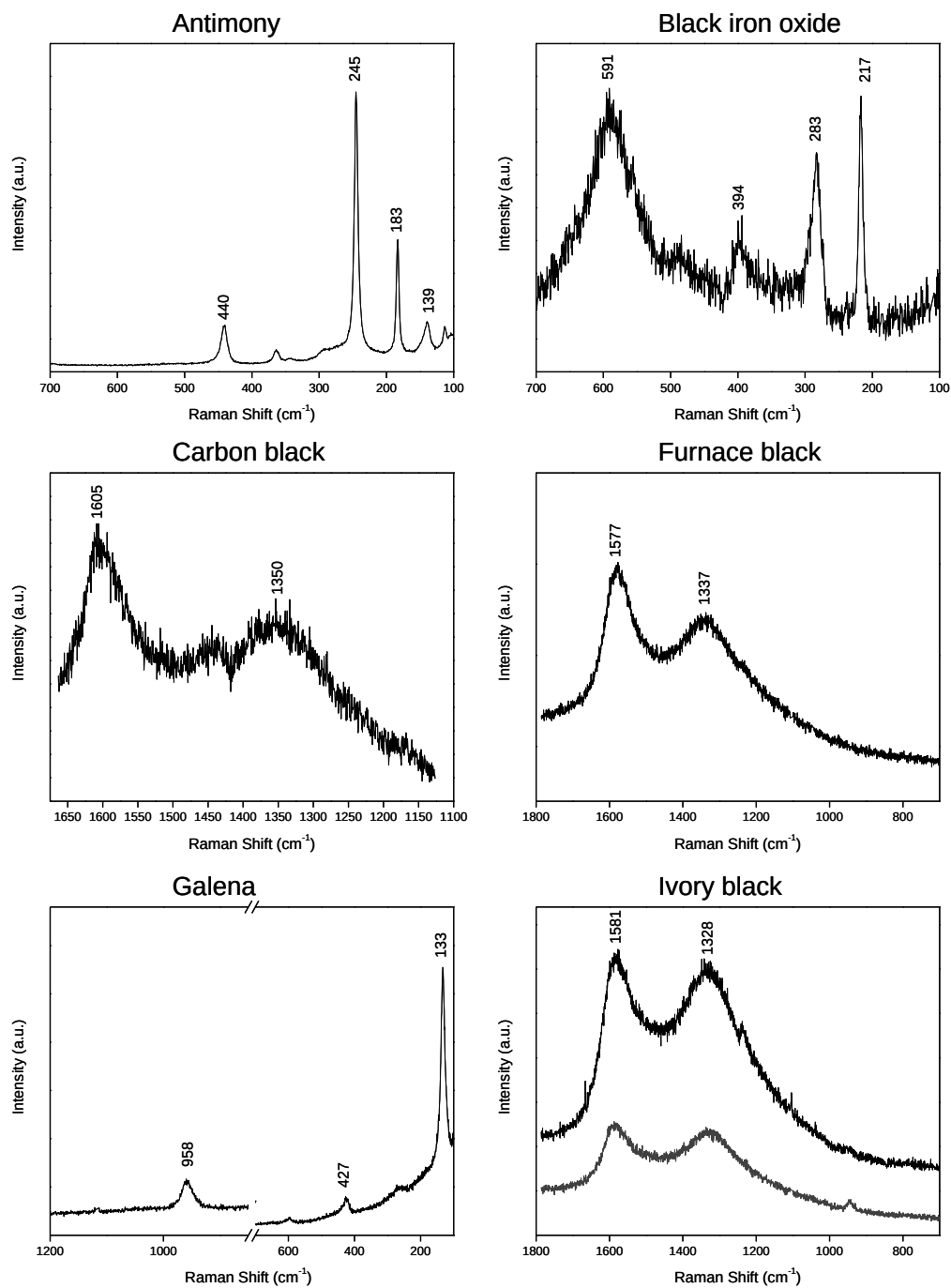
Raman spectra of blues: Part 1

Azurite (NGV#38, 39, 40, 41, 42); Cerulean blue (NGV#5, 151, 170); Cobalt blue (NGV#6, 150a, 150b); Lapis lazuli (NGV#43, 44, 45, 46, 47), Lightproof blue (NGV#147), Manganese Cerulean Blue (NGV#149)



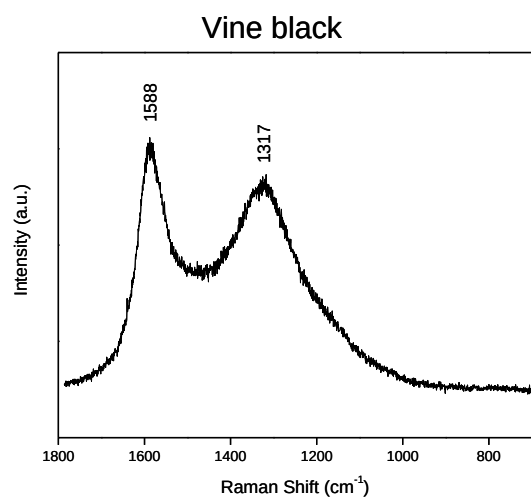
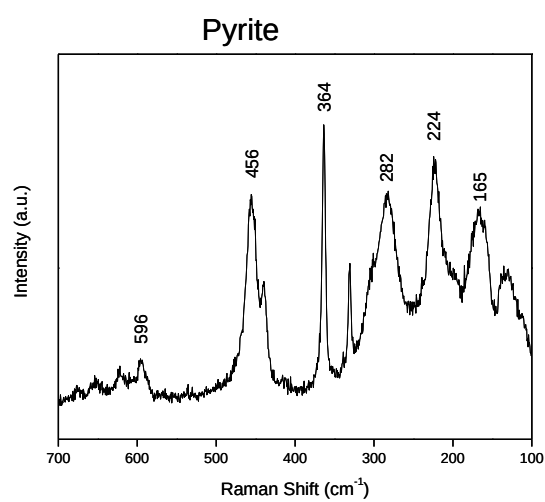
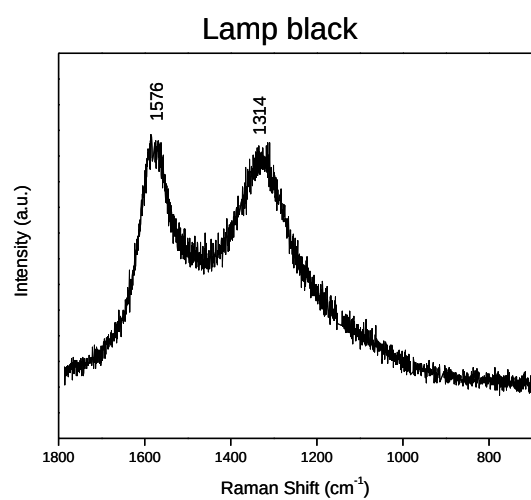
Raman spectra of blues: Part 2

Indigo (NGV#155, 181); Prussian blue (NGV#3, 152, 153, 169, 178); Pthalo-mona blue (NGV#16, 154); Purpurite (NGV#68); Smalt (NGV#32, 34, 35, 36); Ultramarine (NGV#14, 48, 143, 144, 146)



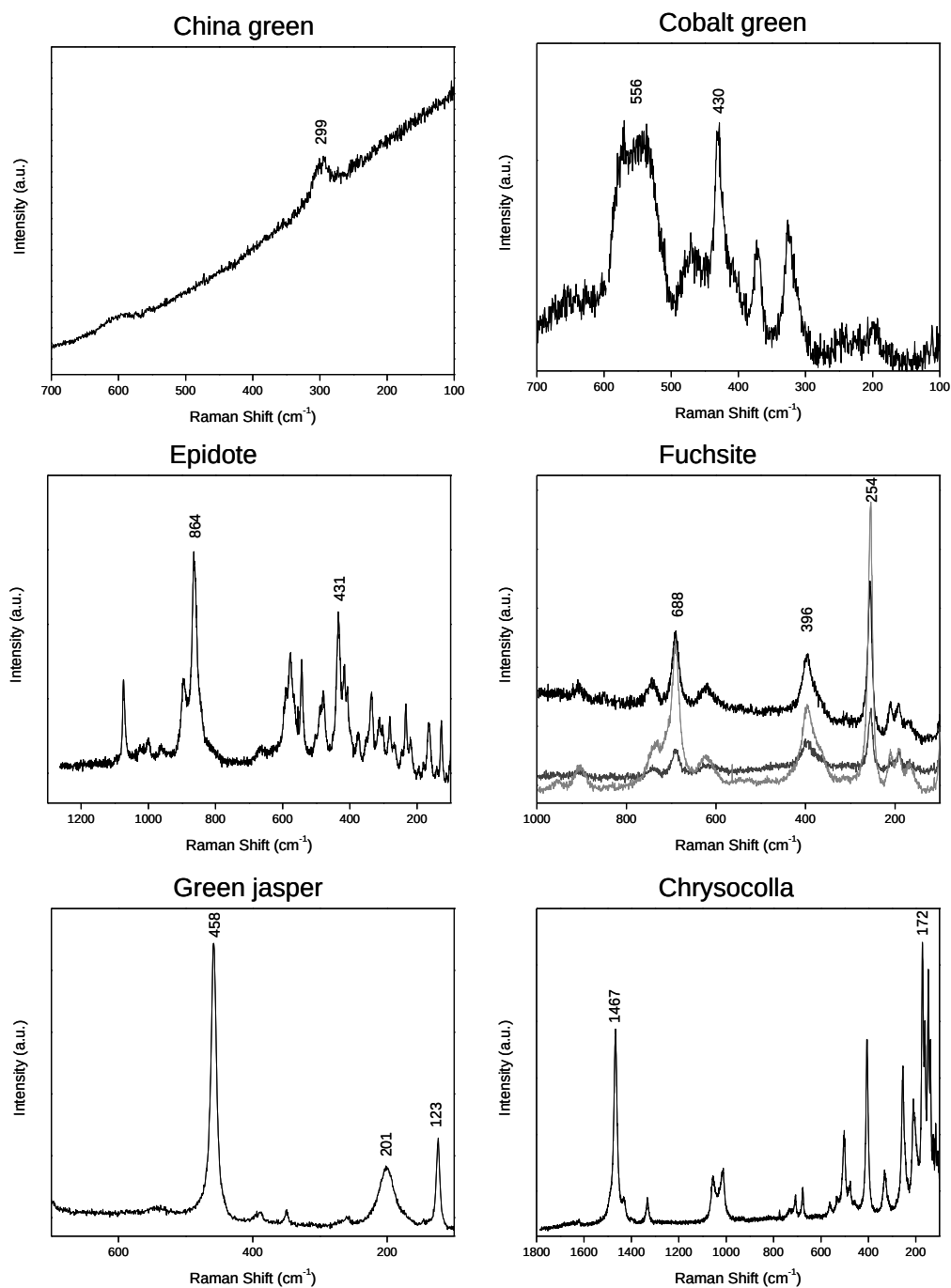
Raman spectra of blacks: Part 1

Antimony (NGV#69); Black iron oxide (NGV#28); Carbon black (NGV#100), Furnace black (NGV#184), Galena (NGV#74); Ivory black (NGV#73, 101)



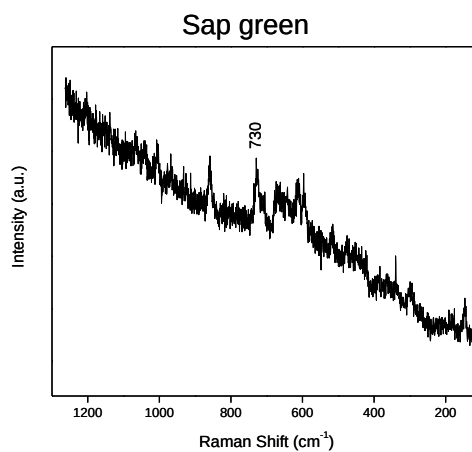
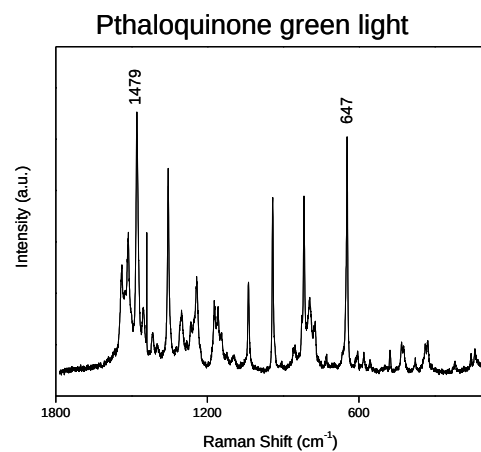
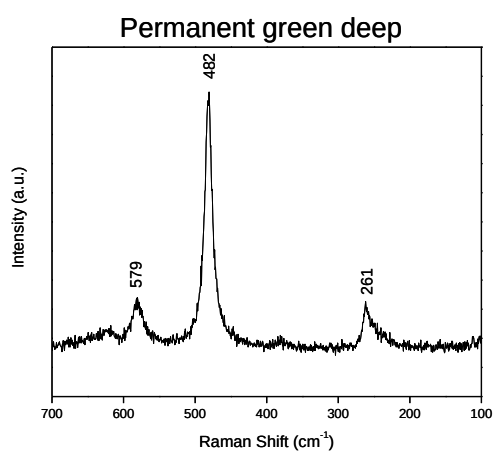
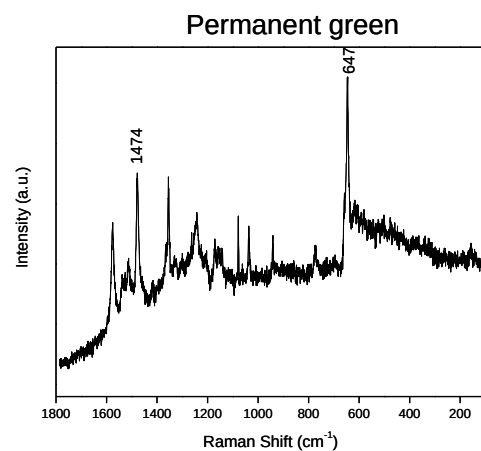
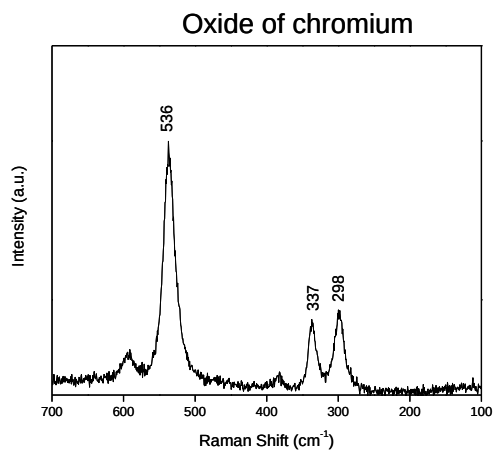
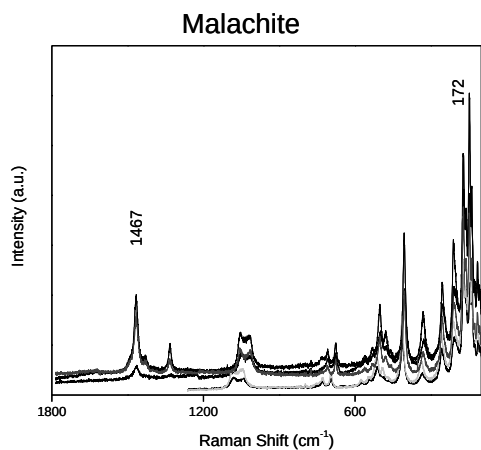
Raman spectra of blacks: Part 2

Lamp Black (NGV#27); Pyrite (NGV#71);
Vine black (NGV#102)



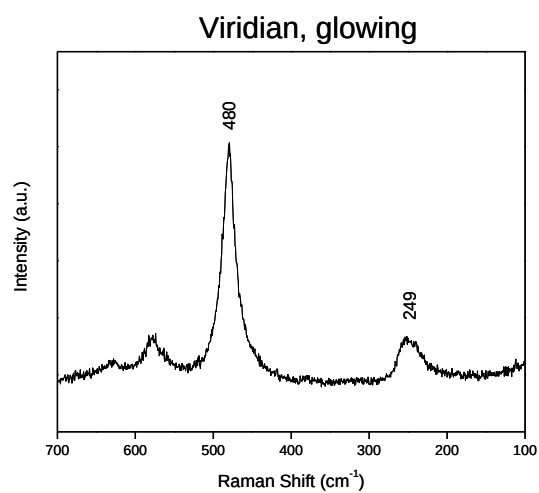
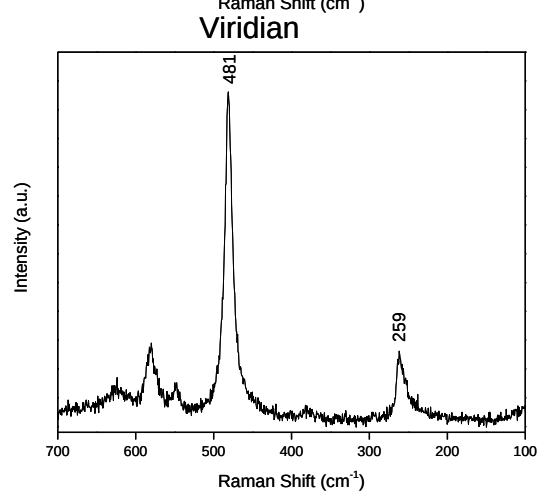
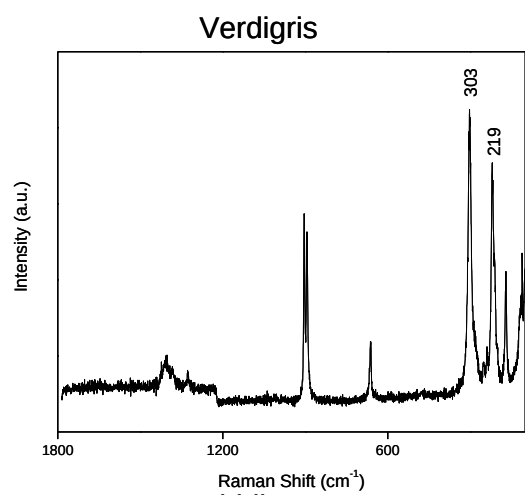
Raman spectra of greens: Part 1

China Green (NGV#180); Cobalt green (NGV#179), Epidote (NGV#63); Fuchsite (NGV#58, 59, 60), Green Jasper (NGV#51); Labelled Chrysocolla (NGV#52)



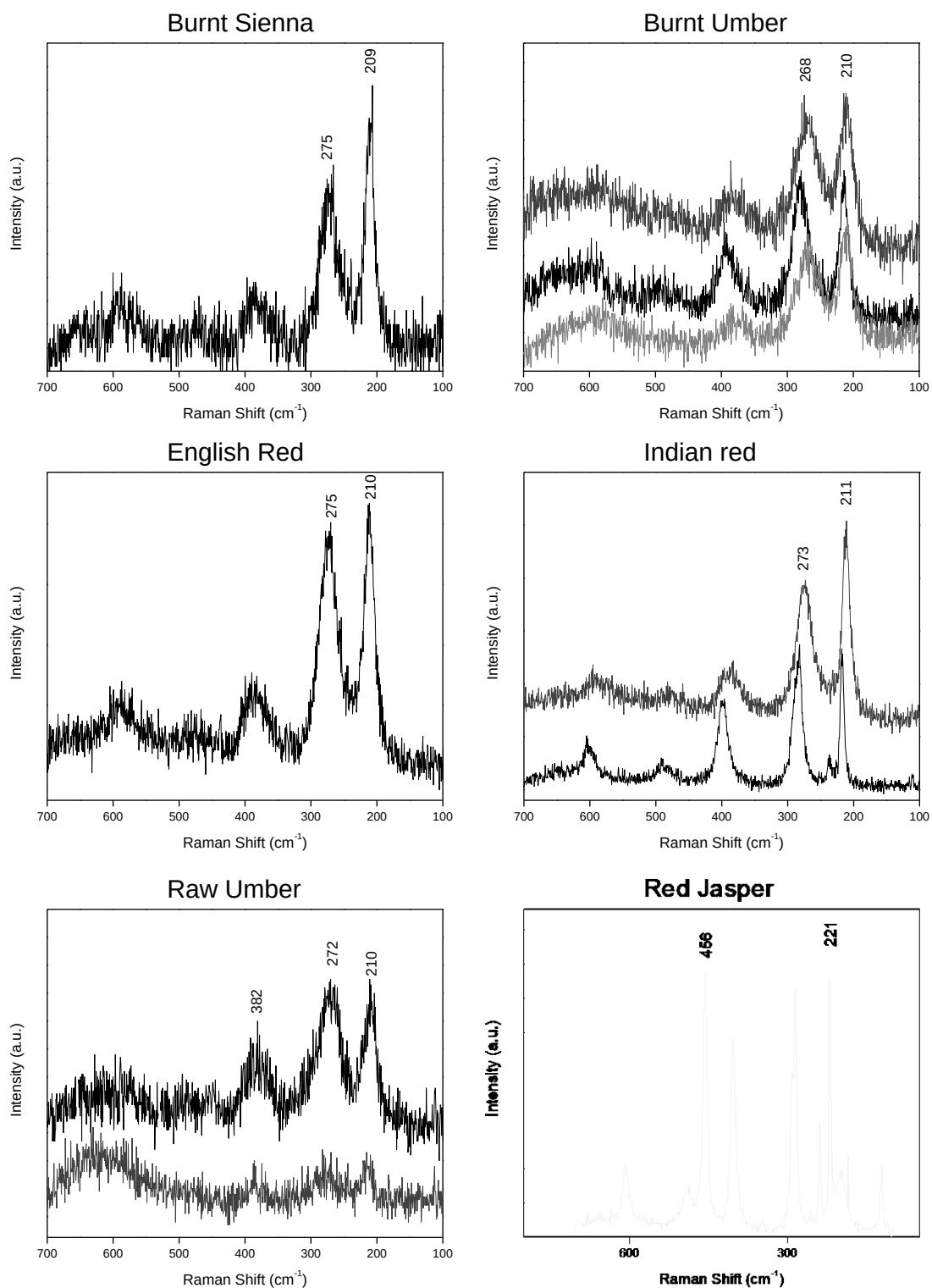
Raman spectra of greens: Part 2

Malachite (NGV#33, 49, 50, 55); Oxide of chromium (NGV#11); Permanent green (NGV#175); Permanent green deep (NGV#94); Pthaloquinone green light (NGV#171), Sap green (NGV#176)



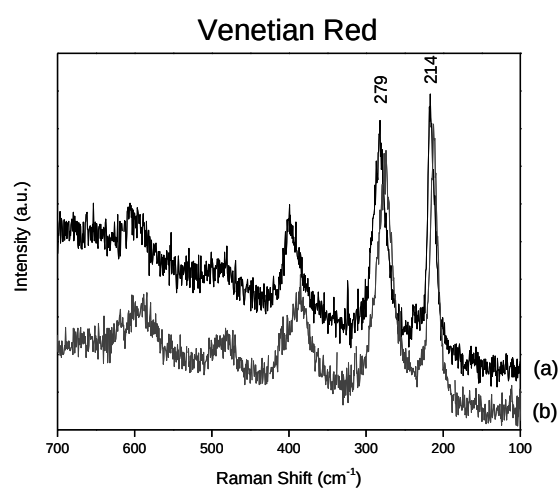
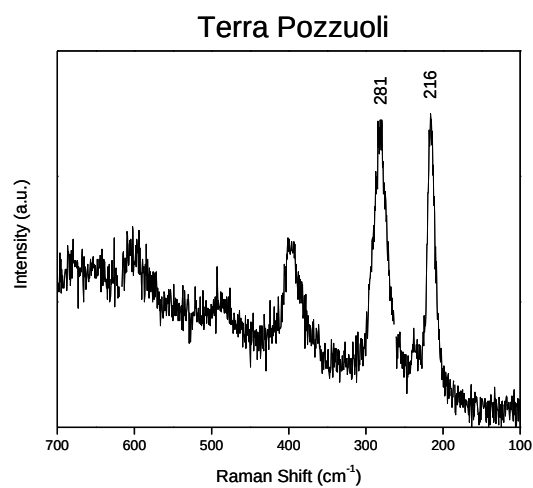
Raman spectra of greens: Part 3

Verdigris (NGV#145); Viridian (NGV#15);
Viridian, Glowing (NGV#95)



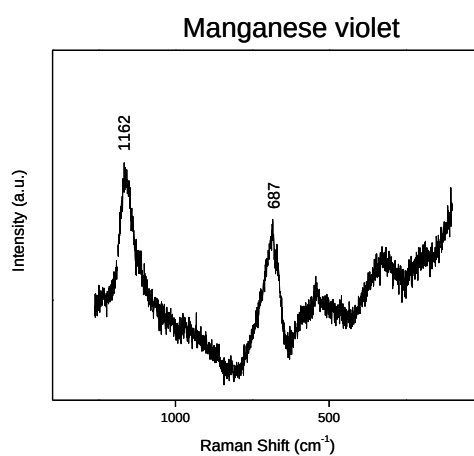
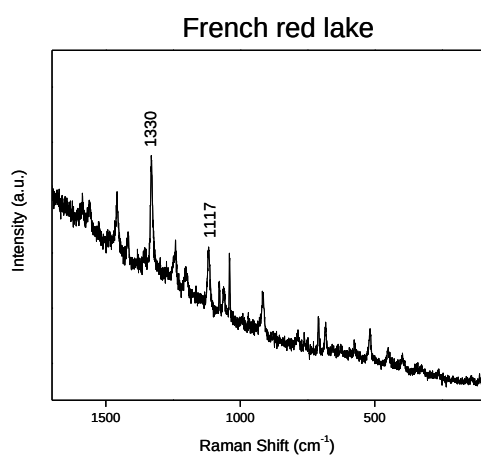
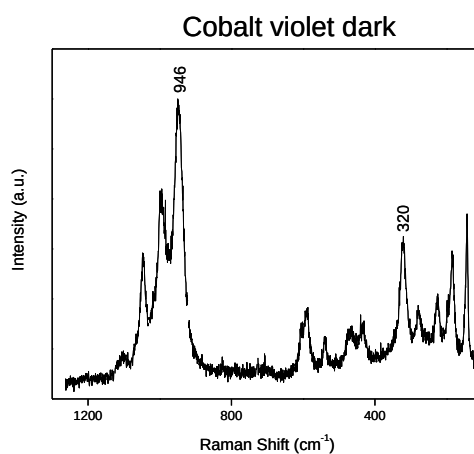
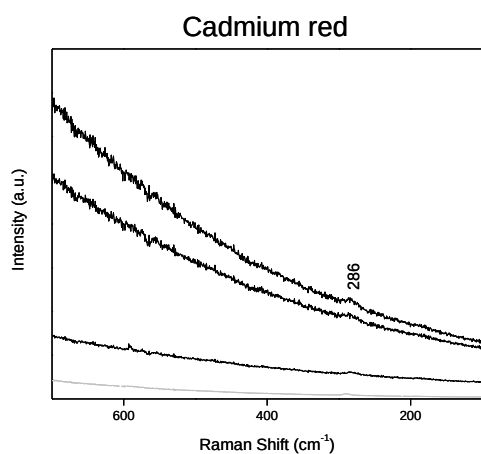
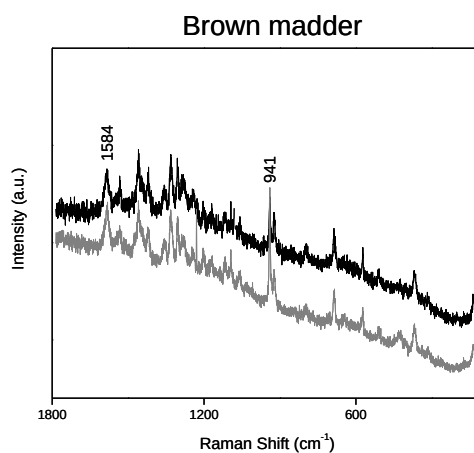
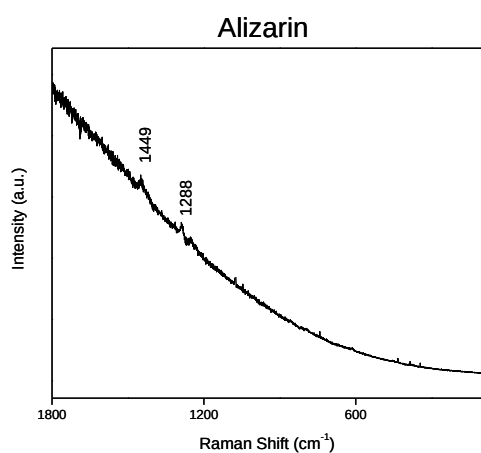
Raman spectra of browns: Part 1

Burnt Sienna (NGV#13); Burnt Umber (NGV#26, 138, 140); English Red (NGV#88); Indian Red (NGV#12, 90); Raw Umber (NGV#19, 87); Red Jasper (NGV#64)



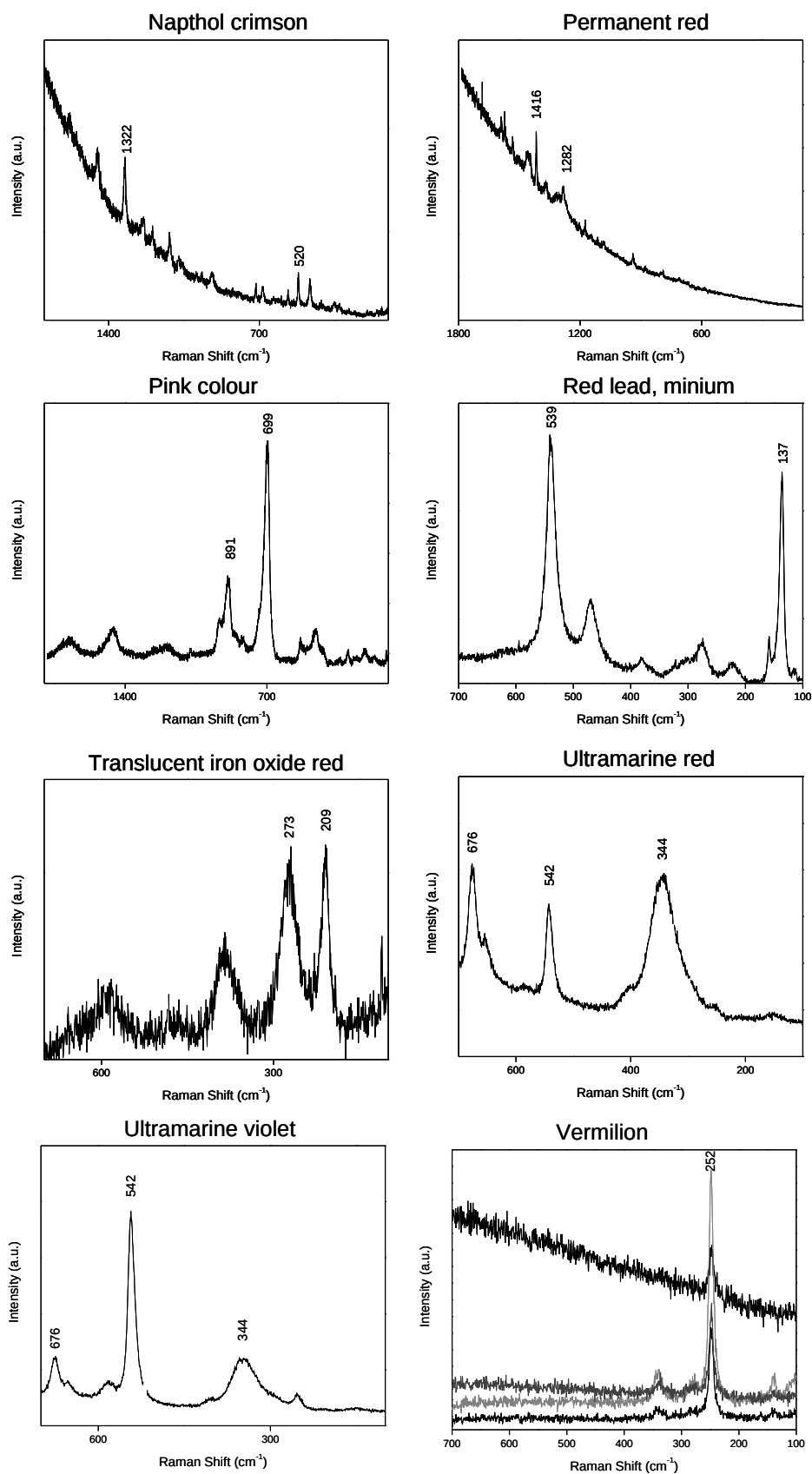
Raman spectra of browns: Part 2

Terra Pozzuoli (NGV#89); Venetian red
(NGV#7, 85)



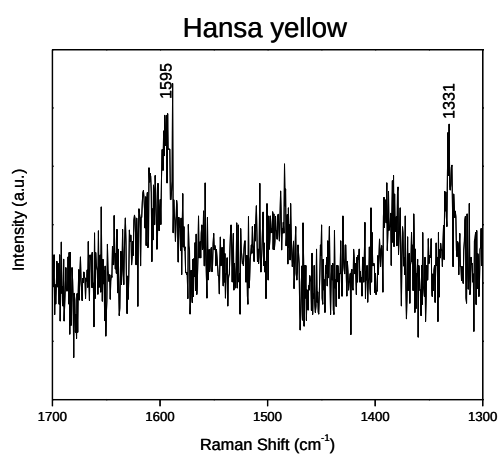
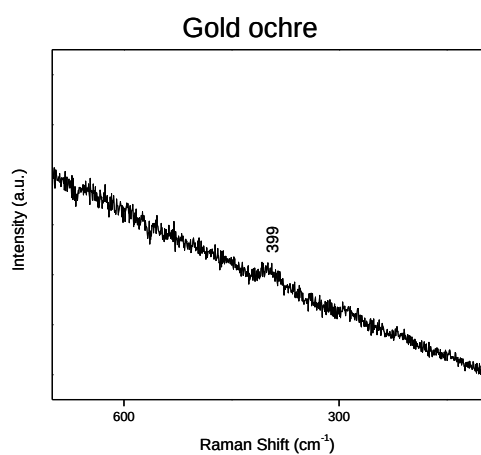
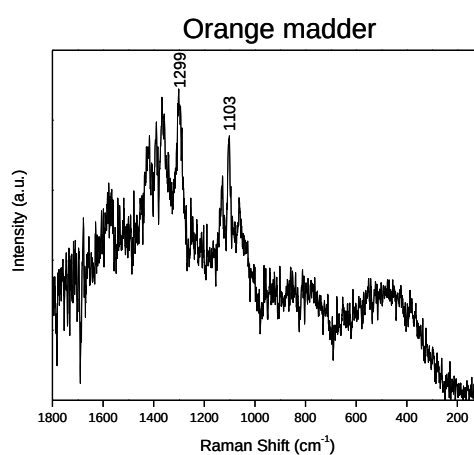
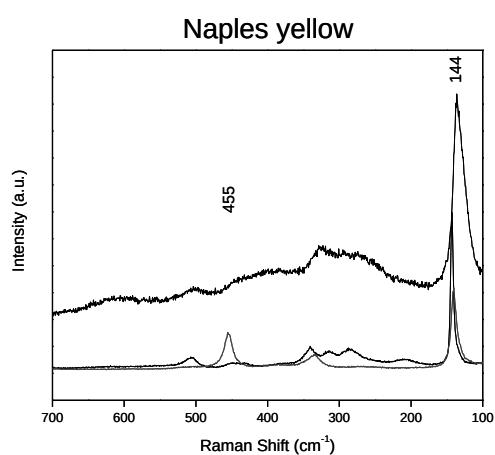
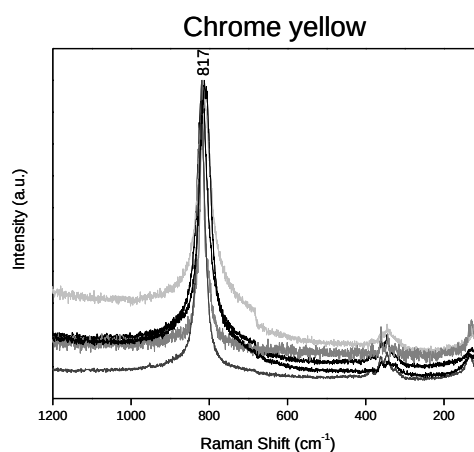
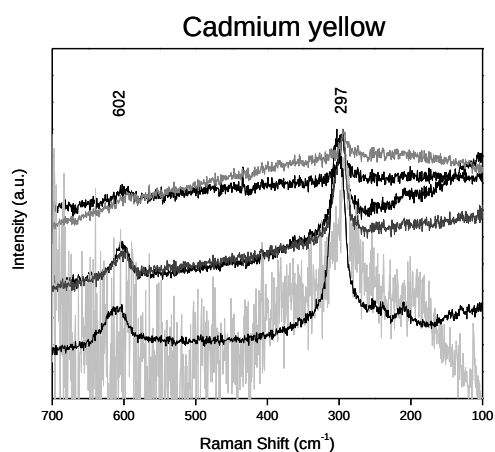
Raman spectra of reds and violets: Part 1

Alizarin (NGV#156); Brown Madder (NGV#86, 86a); Cadmium (NGV#8, 127, 128, 173); Cobalt Violet (NGV#2); French Red Lake (NGV#156); Manganese Violet (NGV#142)



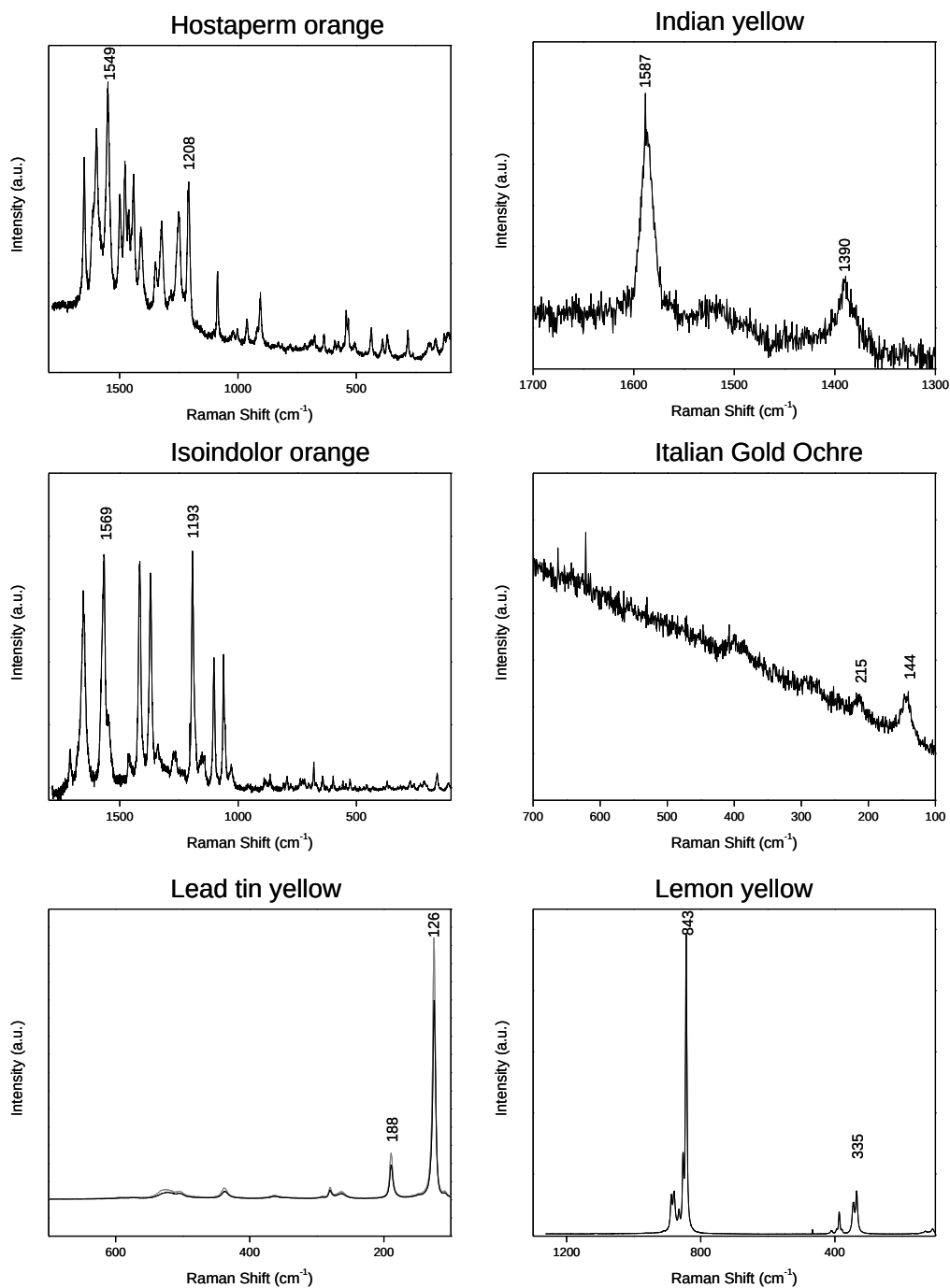
Raman spectra of reds and violets: Part 2

Naphthol crimson (NGV#156); Permanent red (NGV#177); Pink Colour (NGV#65); Red Lead, minium (NGV#129); Translucent Iron Oxide Red (NGV#96); Ultramarine red (NGV#141); Ultramarine Violet (NGV#148); Vermilion (NGV#36, 36a, 37, 168)



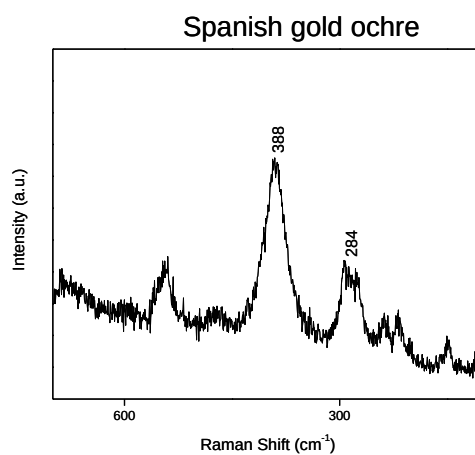
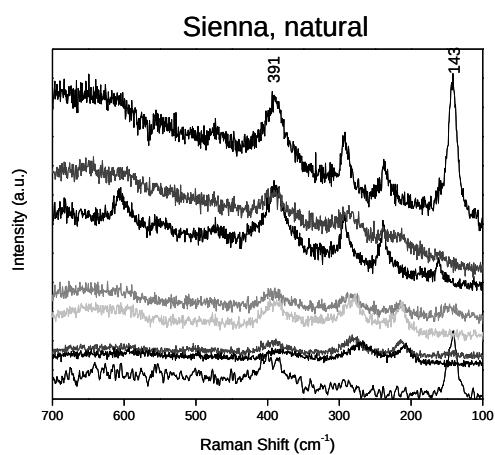
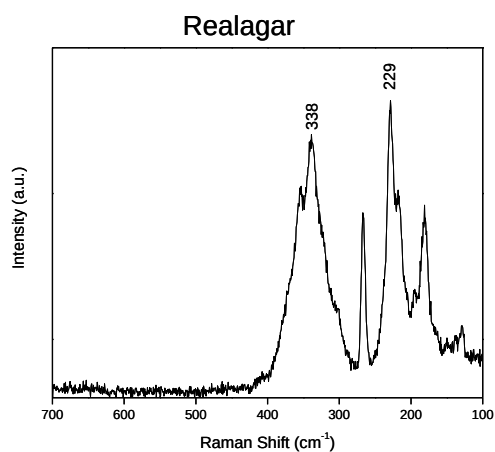
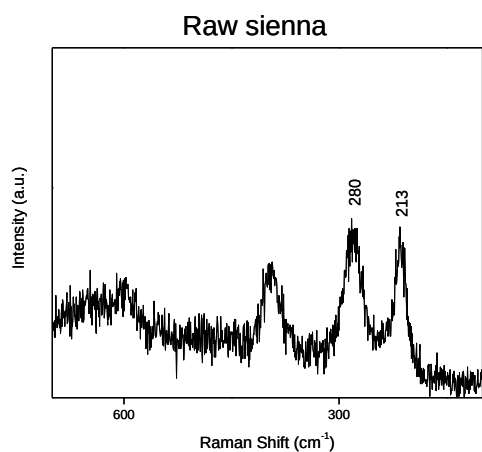
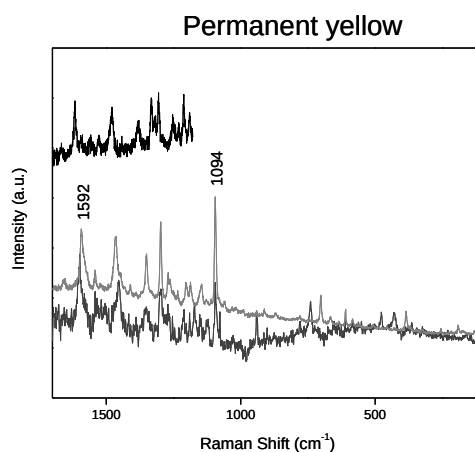
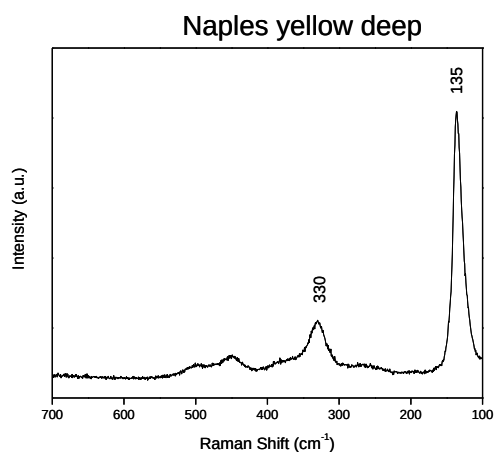
Raman spectra of yellows and oranges: Part 1

Cadmium Yellow (NGV#17, 18, 104, 109, 117, 162); Chrome Yellow (NGV#105, 107, 108, 161, 182); Genuine Naples Yellow light (NGV#118, 118a, 136); Genuine orange madder (NGV#164); Gold Ochre (NGV#92); Hansa yellow (NGV#110)



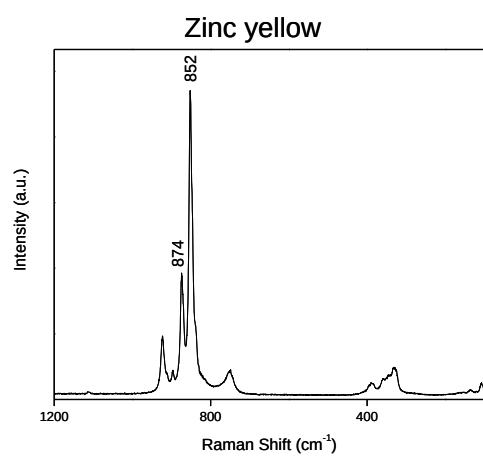
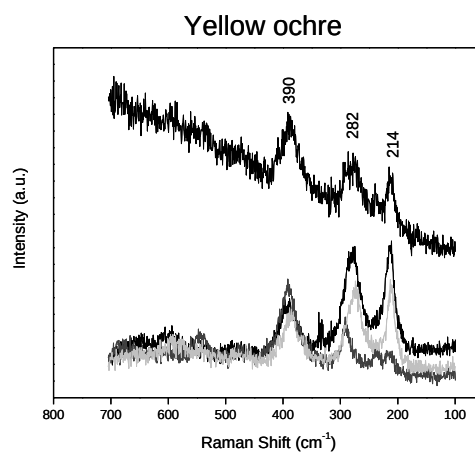
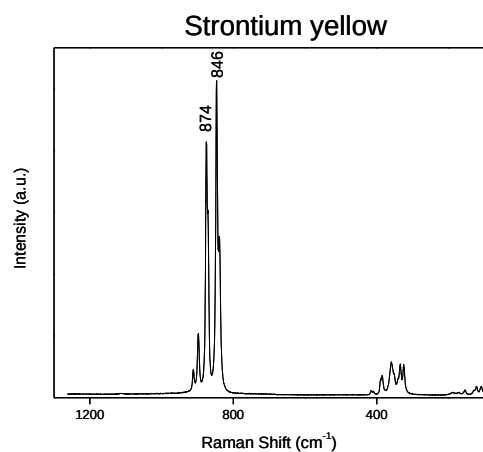
Raman spectra of yellows and oranges: Part 2

Hostaperm orange (NGV#120); Indian yellow (NGV#103); Isoindolor orange (NGV#112); Italian Gold Ochre (NGV#134); Lead tin yellow (NGV#31, 57); Lemon Yellow(NGV#167)



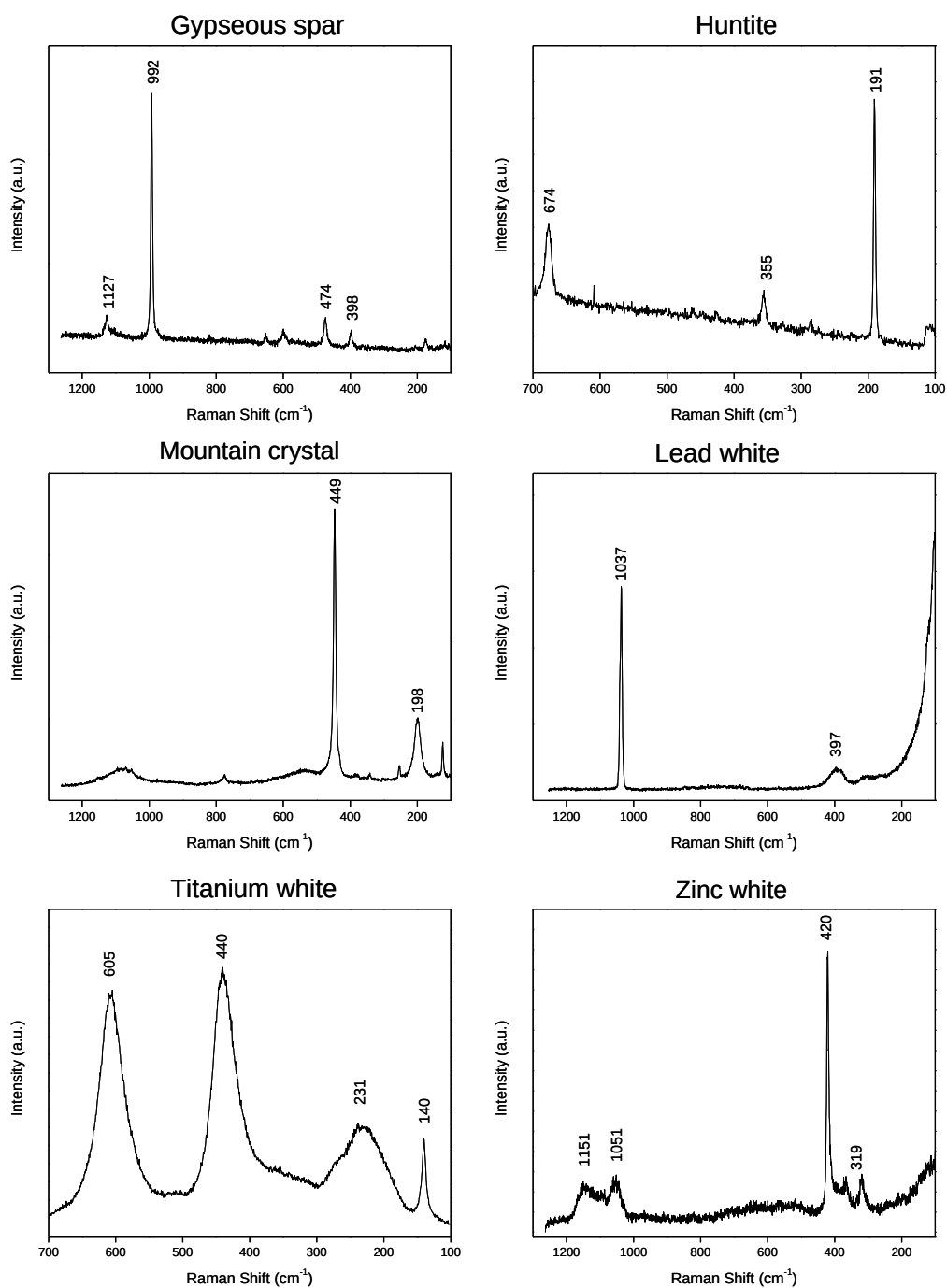
Raman spectra of yellows and oranges: Part 3

Naples Yellow deep (NGV#165); Permanent yellow (NGV#113, 114, 115); Raw Sienna (NGV#10); Realgar (NGV#30); Sienna Natural (NGV#132, 132b, 132c, 132d, 133a, 133b, 133c, 133d); Spanish Gold Ochre (NGV#62)



Raman spectra of yellows and oranges: Part 4

Strontium Yellow (NGV#172); Yellow Ochre (NGV#21, 22, 166, 166a); Zinc Yellow (NGV#183)



Raman spectra of whites

Gypseous spar (NGV#78); Huntite (NGV#80); Mountain crystal (NGV#77); Lead White (NGV#91), Titanium white (NGV#23); Zinc white (NGV#25)

Appendix 2: IDL code for spectral normalisation

IDL code for spectral normalisation developed in conjunction with Glenn Newnham, CSIRO Division of Sustainable Ecosystems, Bayview Avenue, Clayton, 3169.

```
pro lau

infile = 'input file location - name'
envi_open_file, infile, r_fid=fid
envi_file_query, fid, ns=ns, nl=nl, nb=nb
dims = [-1, 0, ns-1, 0, nl-1]
print, 'ns:', ns, 'nl:', nl, 'nb:', nb

outdat=fltarr(ns,nl,nb)

    for i=0,nb-1 do begin

        dat = envi_get_data(fid=fid, dims=dims, pos=i)
        outdat[:,*,i] = (dat-min(dat)) / (max(dat)-min(dat))

    endfor

ENVI_WRITE_ENVI_FILE, outdat, out_name=infile+'_normalised.img'

end
```

Appendix 3: Page 1 of publication from chapter 3

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Application of surface-enhanced Raman spectroscopy (SERS) to the analysis of natural resins in artworks

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Surface-enhanced Raman spectroscopy (SERS) is rapidly growing as an analytical technique for the detection of extremely low concentrations of analytes. The analysis of natural resins from artworks is often restricted by sample size constraints in general, and Raman spectroscopy in particular is hampered by fluorescence when using visible irradiation wavelengths. This work demonstrates that SERS is able to overcome interference from fluorescence in such samples using the incident wavelength 514.5 nm, to allow collection of SERS spectra from extremely small samples. Characterisation of the natural resin surface coating from a painting by Tiepolo is discussed. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: natural resins; surface-enhanced Raman spectroscopy (SERS); conservation; art and archaeology

INTRODUCTION

Through knowledge of the chemical and structural composition of materials comprising artworks, informed decisions can be made with respect to storage, treatment, restoration, transport or display, thereby assisting in attribution, provenance and authentication. Varnishes and surface coatings are used extensively on paintings and other artworks to afford a measure of protection to the surface as well as to improve the aesthetic quality of the surface by providing visual saturation and depth. Current analytical procedures for identifying organic media in binders or coatings of artworks are often restricted by the constraint of limited sample material. They include Fourier transform infrared (FTIR) analysis^{1,2} and chromatographic techniques: high-pressure liquid chromatography (HPLC), and gas chromatography (GC), often coupled with mass spectrometry (MS).^{3–7} Most of these techniques are relatively time consuming to perform and can require extensive derivatisation or pre-treatment of sample material before analysis.

For many years now, Raman spectroscopy has been used as a tool for identification of materials encountered in art and archaeology. The most often discussed advantage is the possibility for non-destructive or minimally destructive analysis (using microscopic samples) and little or no sample preparation. The most commonly encountered application of Raman spectroscopy is the identification of pigments used to colour artworks, which is generally successful only

in situations where there is no overwhelming fluorescence from either the pigment itself or the surrounding binding medium. Indeed, Raman spectroscopy has not been used extensively for the analysis of natural organic media such as natural or synthetic resins because of their tendency to fluoresce. Fluorescence itself can be a feature used to indicate natural varnishes on paintings using UV examination.⁸

Much work exists documenting the Raman identification of pigments^{9–15} (these are but a few) but there are only a handful of Raman studies of organic media encountered in artworks.^{16–18} The authors of these works overcome the problem of fluorescence by using longer wavelength excitation (Brody *et al.*¹⁶ used 1064 nm and Vandenberg *et al.*¹⁸ used 780 nm) in conjunction with FT Raman instruments, an alternative that is not always available in practice. Using a longer wavelength incident laser line has the added disadvantage of reducing the Raman scattering intensity, which is proportional to the excitation wavenumber in the fourth power.¹⁹ Surface-enhanced Raman spectroscopy (SERS) is another option for analysing materials that usually exhibit fluorescence with lower wavelength (visible) excitation, but without diminishing the Raman signal.

Recent technological developments in Raman spectroscopy using surface-enhanced techniques have shown that SERS is eminently suitable for detection of small concentrations of analytes. This is based on the unique feature of some small metallic particles or nano-scale rough metal surfaces to augment the Raman response of analytes in contact with the metal surface, producing an SERS effect. The most effective are the coinage metals: silver, gold and copper, although others have been shown to be successful.

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Multispectral and hyperspectral image analysis of elemental and micro-Raman maps of cross-sections from a 16th century painting

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ABSTRACT

Spectroscopic imaging is well suited to the study of micro-samples from art works, where the sample material is limited and the maximum amount of information needs to be obtained. In this study, a new approach to imaging elemental data from energy dispersive X-ray analysis maps was used in conjunction with micro-Raman spectroscopic imaging to characterise the paint layers within micro-samples. Cross-sections from the 16th century painting *Portrait of a Youth* were found to contain vermilion, lead-tin yellow type 1 and a blue-green pigment consistent with terre-verte. The mid-preparatory layer (imprimatura) contains a high proportion of elements and mineral inclusions that indicates a clay-type composition. The ground layer was identified as anhydrite with large gypsum inclusions. The pigments and composition of the preparatory layers are consistent with those used by Italian Renaissance artist Dosso Dossi.

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1. Introduction

1.1. Analytical techniques

Analytical limitations imposed by working with precious art-works demand the constant development of techniques to extract the maximum possible information from samples when they are taken. Although, sampling for cross-section analysis is micro-destructive, it can be justified as it provides the opportunity to obtain information regarding paint layers and their structure, and paint composition including binders, particle size and the nature of pigments, fillers and additives. If the analytical method is non-destructive, then further com-

positional information may be obtained by a complementary technique.

Where analysis of cross-sections is performed at individual points on a cross-sectional face, it is conceivable that other points of interest may be overlooked. Microanalytical maps and compositional images provide an opportunity to explore a sample surface more extensively.

Ongoing advances in instrumentation have meant micro-analytical maps of artists' materials can now be produced with a growing range of instrumental techniques. Fourier transform infrared spectroscopy (FTIR) [1], time of flight secondary ion mass spectroscopy (TOF-SIMS) [1–3], particle-induced X-ray emission (PIXE) [4] and X-ray fluorescence (XRF) [5] can all

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