ADVANCES IN THE ANALYSIS OF RED LAKE PIGMENTS FROM 15\textsuperscript{TH} AND 16\textsuperscript{TH} C. PAINTINGS USING FLUORESCENCE AND RAMAN SPECTROSCOPY

Austin Nevin, Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, Milano, 20133, Italy

Iacopo Osticioli, Daniela Comelli, Antonietta Gallone Galassi, Gianluca Valentini, Rinaldo Cubeddu, Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, Milano, 20133, Italy

Abstract

An ongoing challenge in the analysis of paintings and paint cross-sections is the identification of organic pigments. The focus of this work is the application of fluorescence imaging, fluorescence lifetime imaging and micro- Raman spectroscopy for the characterisation of organic red pigments in easel and wall paintings. Results of the analysis of model samples of a variety of red lake pigments with fluorescence spectroscopy and Raman spectroscopy highlight significant spectral differences for natural red-lake pigments. The in-situ analysis of wall paintings using non-destructive spectrally-resolved fluorescence imaging and fluorescence lifetime imaging techniques has highlighted the presence of red-lake based pigments, some of which are barely visible to the naked eye. However, while fluorescence may provide clear indications of the presence of lake pigments, the identification of original materials on the basis of fluorescence spectra alone is particularly challenging due to the heterogeneity of painting and paint layers and the presence of conservation treatments. Therefore, non-destructive analysis of historical paint cross-sections using environmental Scanning Electron Microscopy (SEM-EDX) and micro-Raman spectroscopy has been performed. Novel applications of micro-Raman spectroscopy coupled with Subtracted Shift Raman Spectroscopy (SSRS) for the subtraction of fluorescence from historical paint cross-sections of highlight that sufficiently well-defined spectra may allow the identification of original materials. The non-destructive analysis of organic historical cross-sections has highlighted the presence of anthraquinone-based organic red dyes bound to an Aluminium-based mordant.

Introduction

The identification of the origin, preparation and use of red lake pigments has direct implications on the understanding of artists’ methods, historical trade routes and, fundamentally, on the conservation of paintings [1-4]. Red lake pigments are based on natural chromophores which are found in plants (for example brazilwood and madder) and insects (lac, kermes and cochineal); pigments prepared directly from the extracted dyes or following the extraction of dyed cloth can be found in a range of different hues which depend on the preparation of the lakes using different recipes and ingredients. The detection of lake pigments may be a particular challenge as the lakes are often found mixed with other pigments, and, in addition, can be fugitive; indeed there are many examples of paintings which have altered due to the bleaching of sensitive red lakes.

Few non-invasive or non-destructive methods exist for the analysis of lake pigments. Instead routine analysis usually involves the extraction of chromophores followed by chromatographic separation [5]. As many lake pigments are strongly fluorescent, fluorescence spectroscopy has been successfully applied to the detection of major dyes in solution [6], using fibre-optic fluorescence spectroscopy for the detection of lake pigments in paintings [7] and using micro-spectrofluorimetry for the analysis of paint cross-sections [8]. Fluorescence imaging has also been successful in the detection of the presence of lake pigments on various laboratory samples [9].

While micro-Raman spectroscopy has recently been suggested for the analysis of various organic red pigments in paint and in varnishes [8], the detection of Raman scattering can be compromised, often completely, by competing fluorescence, and this accounts for the limited number of studies based micro-Raman spectroscopy for the analysis of red lakes and the employment of Fourier Transform Raman spectroscopy for their identification [9-12]. Various methods for correcting for luminescence signal have been proposed including Subtracted Shifted (SS) Raman Spectroscopy [13], which has recently been
applied to the analysis of textile samples [14]. SERS is still the most common Raman technique for analysing organic colorants [15, 16] and it has recently been suggested for the analysis of extracted pigment particles which can be removed from cross-sections [16, 17]. In addition, novel methods for enhancing Raman signal have found applications in the detection and identification of anthraquinone reds and other organic dyes in paint; with the development of Surface enhanced metal nano-particles [15, 16], extracts or various types of sample including fabrics pastels and paints has been convincingly demonstrated.

This work focuses on two different analytical methods for the assessment and identification of red-lake pigments found in paintings. The first approach is based on non-invasive analysis of wall paintings in-situ, and proposes spectroscopic analysis based on hyperspectral fluorescence imaging [18]. The second non-destructive approach is based on the analysis of historical cross-sections of wall paintings using optical microscopy, elemental and molecular analysis. The paint samples analysed are cross-sections from the collection of the Archivio Gallone (www.archiviogallone.fisi.polimi.it). Micro-Raman spectroscopy has been employed in combination with SS for the analysis of cross-section samples of significant paintings. Further, in order to better understand the morphology and elemental composition of the lake pigments in samples, Scanning Electron Microscopy combined with Energy Dispersive X-ray Spectroscopy (SEM-EDX) has also been employed for analysis of the same cross-sections.

Through the integration of results from the two different approaches, it is possible to better understand the original composition of the red organic pigments

Materials

Various cross-sections of paintings from the Archivio Gallone were analysed. In this work, analysis from two cross-sections has been included. The first sample (A) is from the gilded decoration of the red garments worn by the kneeling figure of Salome from the scene of Herod’s Banquet on the south wall of the Baptistry of Catiglione Olona, painted by Masolino di Panicale in 1435. The second sample (B) is from the blue drapery worn by Saint Andrew from Leonardo da Vinci’s Last Supper, painted between 1494-98. The wall painting was executed using a mixture of media including oil and egg-based binders in the refectory of the complex of Santa Maria delle Grazie Milan.

Methods

Analysis was carried out in-situ of wall paintings by Masolino di Panicale depicting the life of John the Baptist (1435) in the Baptistry of Castiglione Olona, Varese. For this work, analysis focussed on the assessment of the fluorescence detected on the red-dress worn by Salome (seen in Figure 1), which fluoresces bright pink following illumination with a wood lamp [21].

Fluorescence hyperspectral imaging

The hyper-spectral fluorescence imaging system is based on two home-made filtered lamps emitting UV radiation with a maximum emission at approximately 365 nm and on a tunable liquid crystal filter (VariSpec, Cambridge Research Instruments) coupled to a cooled CCD (Retiga 2000, QImaging) for collecting fluorescence images at selected different spectral bands in the visible range [21]. The apparatus has been used to record the UV fluorescence spectrum at each point of a surface under analysis.

Optical microscopy and Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

Polished cross sections were examined using a Leica DMRE microscope equipped with dark-field illumination and a Leica digital camera. Photomicrographs of the samples were captured at different magnifications. The same cross-sections were analysed using an Environmental Scanning Electron Microscope Zeiss EVO 50 EP equipped with an Oxford INCA 200 - PentaFet LZ4 energy dispersive X-ray spectrometer. The energy dispersive X-ray qualitative spectra from representative areas of the sample were registered from 0 to 20 kV and at 1–3×10⁻⁷ A.
**Micro-Raman spectroscopy**

Micro Raman spectroscopy was employed for the analysis of small areas on the surface of the polished cross sections. The technique was carried out using a Renishaw 2000 spectrometer coupled to a CCD detector cooled by Peltier effect with a solid state diode laser emitting at 785 nm. Using a 50X objective, the laser was focused on different points of the sample with a spot size of approximately 5 x 5 µm², an incident power lower than 3 mW and a typical integration time of 100 seconds. Instrumental calibration was performed using the characteristic Raman-stokes line of Silicon at 520 cm⁻¹ as a reference; the system is characterised by a spectral resolution close to 4 cm⁻¹. Carminic acid was purchased from Sigma.

SSRS were performed subtracting two Raman spectra acquired by moving the diffraction grating position. The applied shift was choosen on the base of to the full width at half-maximum of the weak Raman bands in the spectra [13].

**Results and discussion**

1. **In situ Hyperspectral Fluorescence Imaging of wall paintings by Masolino di Panicale**

![Figure 1](link)

Figure 1 detail of Salome (left), under UV illumination (centre) and the fluorescence spectrum (right)

A comparison of the fluorescence spectrum measured in situ with published fluorescence spectra of red lakes [6] suggests the presence of an insect-based anthraquinone lake, characterized by a measured emission maximum between 630 - 650 nm, which is bathochromically shifted with respect to the emission of madder (alizarin-purpurin) lakes which is observed between 600 - 615 nm. The band which has a maximum at approximately 480 nm is ascribed to the protective treatment was applied over the surface of the wall paintings during restoration. While fluorescence spectroscopy of the complex surface may provide important information regarding the presence of different materials, and in this case the red lake pigment which is applied in different concentrations as paint, the information provided is insufficient to identify the exact nature of the pigment. Therefore, historical cross-sections from the same painting and, for comparison, a cross-section of a sample from the Last Supper have been analysed using non-destructive spectroscopic techniques.
2. Analysis of cross-sections containing red-lake pigments

Figure 2 Cross section A - sample from the sleeve of Salome’s red drapery covered by a metal leaf

Visual examination of the cross-section from Masolino’s wall painting reveals the presence of a (1) metal-leaf decoration over (2) a thick lead white and oil-based mordant. Below the white layer, (3) a dark red lake layer which is approximately 25 μm in thickness covers another (4) thinner layer of lighter-red. Within layers (3) and (4), the red particles are less than 10 μm in diameter and are amorphous shame and uniform hue [19]. SEM-EDX has confirmed the presence of a tin-leaf based decoration, and high concentrations of Al in the lake particles, along with traces of K and P, suggesting the preparation of dye from insects (due to P) using an alum-based mordant.

Figure 3 Cross-section B- sample of the blue drapery worn by Saint Andrew

The precious blue ultramarine is found here in a two layers; (1) an upper 30 μm layer of the finely ground pure pigment (10 μm) is found over (2) a thicker layer of approximately 70 μm which contains fine particles of lead white and inclusions of a dark red lake pigment, some of which are large and irregular in shape, reaching up to 40 μm in length. A (3) thin (max 25 μm) layer of lead white bound in oil, which covers the surface of the wall, covers the plaster ground [20]. SEM-EDX analysis suggests the presence of an alum-based red lake due to the presence of K and Al, although strong signal from the surrounding copper aluminium silicate ultramarine pigments is also detected. The lake particles also contain trace concentrations of P, suggesting the extraction of the pigment from insects and not from fibres.
**Micro Raman spectroscopy**

![Figure 4 SS Raman spectra from (1) cross section A, (2) cross section B, and (3) carminic acid reference](image)

The Raman spectra measured directly from a 5 x 5 μm spot from the red particles within the blue layer in the cross section from the Last Supper (B) is very similar to that observed in the cross-section from Masolino’s painting (A), and many bands observed in the carminic acid reference (3) correspond to those observed in the analysed cross sections. Both spectra (1) and (2) are characterised by a distinct band at 1315 cm\(^{-1}\) with a shoulder between 1220 -50 cm\(^{-1}\) and further less intense but well defined bands at 1430 and 1600 cm\(^{-1}\). A Raman spectrum with similar features was measured in the lighter red layer. The relatively intense band at 1315 cm\(^{-1}\) is characteristic of carmine-based lakes and is ascribed to the carbonyl complexed with Al, and for this reason this band is not found in carminic acid which is not complexed with a metal ion. Raman bands in the spectral region between 1150 and 1250 cm\(^{-1}\) are assigned to the stretching vibrations of the aromatic rings, whereas the signals between 1400 and 1600 cm\(^{-1}\) are sensitive to the stretching vibration of the C=O group. A survey of published Raman spectra of red lakes \([16, 15, 22]\) suggests that the observed spectrum relates to the presence of a carmine lake, based on kermesic and/or carminic acid, and not alizarin/purpurin as would have been observed for a madder based lake, or laccaic acids which would be associated with the use of lac.

**Conclusions**

Analysis using fluorescence and Raman spectroscopy of paintings and paint samples provides complementary information regarding their composition. However, information yielded through the use of these non-destructive techniques does not allow the confirmation of the origin of a lake (for example whether the lake is from kermes of cochineal.) For this purpose, other micro-destructive methods (HPLC, for instance) would be useful. Nonetheless, the techniques demonstrated are powerful not only for the localisation of materials on a paint surface using fluorescence imaging, but also for the differentiation between anthraquinone and other types of lake pigments which is possible through the use of micro Raman spectroscopy combined with automatic SS methods. Future work will focus on the analysis of other cross-sections in order to assess if spectral differences can be ascribed to differences in preparation and origin of lake pigments found in cross-sections.

**References**


