Chemical-structural analysis of wooden painted specimens by clinical multi-slice computed tomography (MSCT) and surface-enhanced Raman scattering (SERS)

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Abstract – The results of Multi-slice X-ray Computed Tomography (MSCT) and Surface-Enhanced Raman Scattering (SERS) study performed on representative organic and inorganic ancient pigments painted on wooden support are presented and discussed. Clear Raman spectra were acquired for organic pigments such as a madder lake, overcoming their fluorescence and weak Raman scattering drawbacks. MSCT Hounsfield Units (HU) measurements show the ability to differentiate the materials, building a volumetric view and, with appropriate algorithms, to segment the layers of the specimen using suitable reconstructions. All these results in combination allowed a complete chemical-physical characterisation of painted pigments on wood samples.

I. INTRODUCTION

Pigments have mainly been used in the past to decorate works of art. Their identification and characterisation provide essential information for dating, authentication, and conservation treatment of these objects and studying art history in general [1,2]. Over time, many analytical techniques have been employed for the analysis of dyes and pigments in cultural heritage. The methods routinely used in the identification of inorganic pigments are generally not fully applicable to the organic ones: X-ray fluorescence because of the lack of an elemental signature, Fourier transform infrared spectroscopy because of the interference of binders and extenders [3]. Moreover, the identification of pigments has required relatively large samples (0.5–5 mm in diameter) for analysis by high-performance liquid chromatography [4]. Conventional micro-Raman spectroscopy has been used mainly due to its non-destructiveness feature. However, the Raman spectra are generally affected by the intense luminescence of dyes and several pigments, especially of organic origin, show weak Raman activity besides a strong fluorescence that prevents their identification [5]. Recently, new approaches can be adopted, such as Surface-enhanced Raman scattering (SERS) and X-ray computed tomography (CT). SERS can address the difficulties associated to fluorescence background since the presence of noble metal nanoparticles induces a giant

amplification of the Raman signal [6] and allows investigating samples as small as 25 μm in diameter.

On the other hand, clinical computed tomography based on multi-slice technology (MSCT), used for decades in the medical field, thanks to its non-invasive and non-destructive features, can be successfully applied to the study of cultural heritage artefacts as well as for the localisation of more significant structures over or under the painting's surface [7]. Generally, in the medical field, MSCT is very also used for densitometric measurements, expressed in Hounsfield Units (HU). Thus, full information about the density values of the different structures and tissues of the human body and their variations can be estimated. Furthermore, by MSCT scans, it is possible to obtain two main images reconstruction: 2D images through Multi-planar reconstruction (MPR) and 3D images by the volume rendering technique (VRT) reconstruction approach. VRT images are based on several 3D filters calibrated on densities (measured in HU) of various human body tissues (e.g. bones and lungs). This permits to medical doctors to navigate inside a human body virtually. By VRT reconstruction, it is possible to select one density and virtually erase the others. In this way, an object can be virtually "dissected" and studied from every angle. [8]. In this paper, an extension of clinical CT and its dedicated software (designed for medical use) was performed to investigate the microstructure of wooden polychromy. We present our experience scanning two wooden tablets painted with ancient pigments according to Cennino Cennini's instructions [9] and the subsequent processing of this data. SERS analysis was carried out both on pure pigments whose nature is known (they represent known references) and then on ancient pigments painted on wooden supports. In this way, we discriminated the different pigments and identified their typical contribution.

II. MATERIALS AND METHODS

A. Samples preparation

Two chestnut oak (Quercus prinus) wooden tablets, measuring 33x14 cm and painted with most used ancient pigments were analysed. Inorganic and organic pigments from Zecchi Firenze, such as red ochre, natural ultramarine, white lead, cinnabar, madder lake, malachite, red lead and azurite were chosen. Rabbit skin glue tempera as a binder was utilized, and two types of wooden support were made: Bologna plaster preparing and 'incamottatura', widely used on wooden paintings from XI to XVI century, which consist in a layer of linen canvas applied on the wooden support to simplify the colour expanse.

B. Multi-layer Computed Tomography (MSCT)

In our experiments, a Somatom Sensation 16 MSCT (Siemens Healthcare) with a gantry opening of 70 cm was used (Fig.1). The acquisition was performed setting the following parameters: 62 mA, 120 kV, slice thickness of 0.6 mm and B60f smooth Kernel. Images were evaluated by using MPR, VRT and Siemens SYNGO Software. On MPR images, Region of Interest (ROI) for measuring HU values of pigments were selected. During VRT imaging post-processing, conventional filters applied in medical computed tomography were used. The choice was based on filters which have densitometric characteristics close to those of our materials like the one usually used for skin and bones.

MSCT allows performing scans in a very short time. Images of organs are acquired in 1 second, those of heart in 5 seconds and that of the whole body in less than 10 seconds [10]. In this work, we scanned the two specimens in 12 seconds.

Fig. 1 – Wooden tablets, painted with the most used ancient pigments are placed on the CT scanner bed for the MSCT scanning

We remark that MSCT applied to a cultural heritage object allows to 1) view structures; 2) understand the implementation methods; 3) recognize subsequent restoration; 4) study the conservation state of manufacture and materials (for example, tunnels caused by xylophagous insects are perfectly visible) and 5) in case of wooden artefacts, to identify the types of wood used. However, the most important innovation that follows from this method is the possibility to estimate the different densities of materials which constitute the analysed samples [12]. The measurement of the electron density is made by using the Hounsfield scale, expressed

in HU, which includes 2001 different shades of grey, from black to white. The HU scale is a linear transformation of the original linear attenuation coefficient measurement into one in which the radio-density of distilled water, at standard pressure and temperature, is defined as 0 HU, while the radiodensity of air, at standard pressure and temperature (STP), is defined as -1000 HU; finally, radiodensity of bones is set to +1000 HU. For any material with linear attenuation coefficient μ, the corresponding HU value is therefore given by:

$$HU = \frac{\mu_x - \mu_{water}}{\mu_{water} - \mu_{air}} \times 1000 \qquad (1)$$

The Hounsfield unit is a relative parameter, where water is taken as a reference substance (Fig. 2).
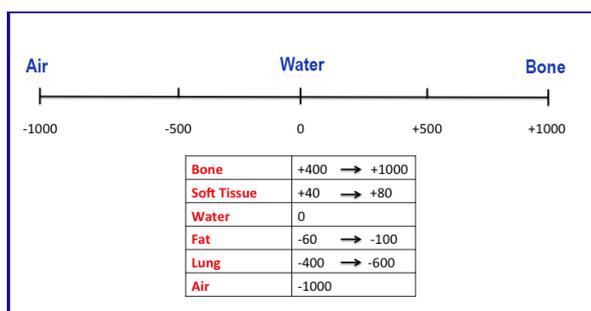
Fig. 2 – Hounsfield Unit scale

C. Surface-enhanced Raman scattering (SERS)

Gold films were obtained spraying Au colloids on glass and silicon substrates. The synthesis of Au colloids was carried out by ablating inside water a high purity target, using a focused nanosecond pulsed laser beam (laser excitation line at 532 nm) [11]. SERS spectra were acquired after soaking the gold substrates into the pure alizarin and purpurin solutions at different concentration levels (between 10^{-3} and 10^{-5} M) for 1h; then rinsed with deionised water and left to dry in air. Precise spectra of molecules were recorded on the substrates. Moreover, the homogeneity of the substrates was checked by performing measurements on the surface area of about 60x60 μm². Moreover, preliminary SERS measurements were carried out on pieces of the wooden tablets painted with madder lake and ochre.

Raman spectra were collected by the Horiba XploRA apparatus. The laser excitation is the 532 nm line emerging from a diode laser and an integration time of 80 sec was fixed for each measurement, with an accumulation time of 3.

III. RESULTS AND DISCUSSION

From the MPR (Multi-planar) images, HU values of different pigments on the wooden support have been estimated (Fig. 3a). Moreover, the graph in Fig. 3b displays the density in g/cm³ and the corresponding Hounsfield number for each pigment.
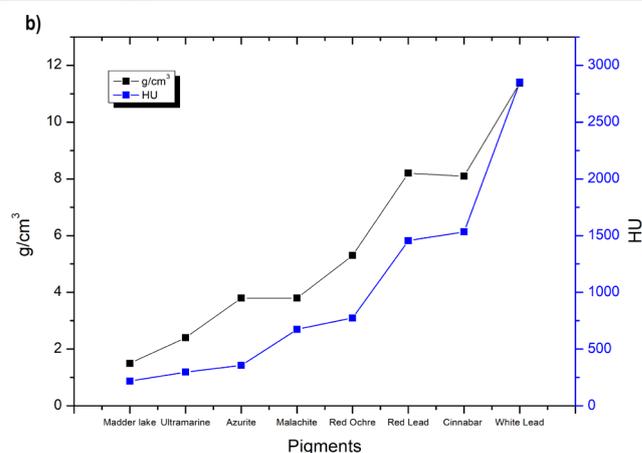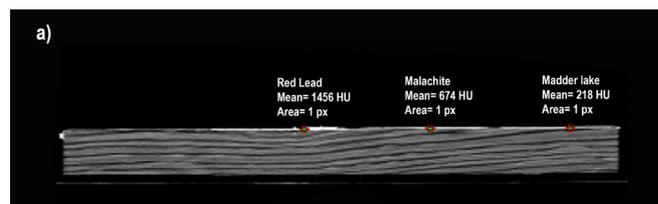
Fig. 3 – MSCT MPR reconstruction: HU values measurements of the different pigmentson the wooden support.

Tab. 1 – Pigments HU values, density and chemical formula

Pigments	Chemical formula	Density (g/cm³) [12]	HU Values
Madder lake	C14H8O4 or C14H8O5	1.5	218
Ultramarine	$Na_7Al_6Si_6O_{24}S_3$.	2.4	297
Azurite	$Cu_3(CO_3)_2(OH)_2$.	3.8	356
Malachite	$Cu_2CO_3(OH)_2$	3.8	674
Red Ochre	$\propto$-Fe_2O_3	5.3	775
Red Lead	$2PbO \cdot PbO_2$	8.2	1456
Cinnabar	HgS	8.2	1534
White Lead	$2\,PbCO_3 \cdot Pb(OH)_2$	11.37	2852

Table 1 shows as each pigment have its characteristics X-Ray densitometric values in HU. It is observable how

pigments with higher density values also have higher HU values. Considering the HU scale, we know that metal is collocated in the positive range of HU values starting from 1500 HU. In this case, most of the pigments are inorganic and made with metallic oxide. For example, white lead is made by basic lead carbonate, which has an HU value of about 2852 HU. It is the higher value found on samples. Red lead is also made by lead but, because its different chemical formula, it has a value of 1456 HU.

Going backwards until you reach 0 HU, which corresponds to water density, we could find cinnabar with 1534 HU values; red ochre with 775 HU; malachite with 674 HU; azurite with 356 HU; natural ultramarine with 297 HU and, finally the madder lake which is characterized by HU. All these values certainly follow density trend, because the higher density value corresponds to the higher HU value and so for the lowest values. The graph showing the correlation between the density (g/cm^3) and HU values are reported in Fig. 3b.

We outline that some materials have similar density values and similar HU ones. This is the case of cinnabar and red lead: they have both density values of 8.2 and HU values around 1500 HU. Otherwise, azurite and malachite have density values of 3.8 g/cm^3, but they have different HU values. This is probably due to the inhomogeneities of the pigment layer which measure less than 0.6 mm (CT slice thickness). Azurite is less opaque than malachite and probably an area with less concentration of azurite was measured. Azurite shows a 356 HU value while malachite 674 HU. Finally, we found that the estimated madder lake values are not realistic. This is explained considering that madder lake is an organic pigment and that X-Ray interactions depend on the Z number of the atomic elements. However, attenuation coefficients values are dependent upon the absorption and scattering of the incident radiation caused by several different mechanisms. Considering the x-ray energy used in our CT investigation (120keV), there is a competition between Compton scattering (incoherent scattering, for Z less than 20) and photoelectric absorption that increases for Z>20.

Moreover, the madder lake value obtained is influenced by the presence of calcium sulphate hydrate (bologna plaster) used during the sample preparation, which has a density value of around 2.9 g/cm^3.

In this work, we have chosen medical VRT filters calibrated on the human body and characterised by density values like the materials specimens investigated. For example, with skin and bone VRT filter, it has been possible to divide the sample into white areas (pigments based on lead and mercury); blue colour zones (wood) and peach colour zones (linen canvas and organic substances) (Fig. 4a). At a glance, a virtual map of all the materials that are on the samples was obtained. On the other hand, the second filter used, bones filter, highlights pigments only (see Fig. 4b), especially those with

densitometric HU values more near bone HU value (+1000 HU) (see tab. 1). The third filter used was that generally adopted for the pulmonary system evaluation, because wood is a very porous material, with a lot of air inside (see Fig. 4c). In this case, densitometric values are on the negative range of the HU Scale (air HU values are -1000).

Chestnut oak wood HU value of around -300 HU was measured. Densitometric measurements were performed on tree-rings too. ROIs were taken on latewood and earlywood, respectively: latewood -157 HU; earlywood -459 HU. Latewood has a value nearest to 0 (water HU value) which is higher if compared to that of earlywood nearest to -500 HU, lung HU values, and to the value of air equal to -1000 HU. Moreover, this agrees with the microstructural features of the growth rings, which expect latewood more compact and strength than earlywood [13]. The arithmetic average between these two values (-157 HU and -459 HU) results around -300 HU, which corresponds to the total HU values of chestnut oak.
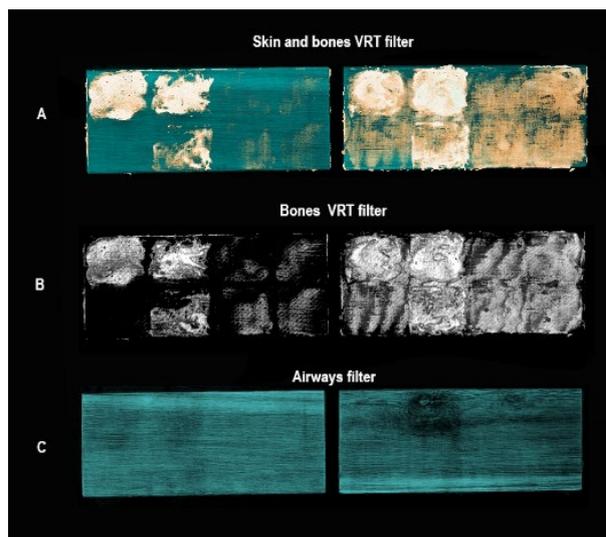
Fig.4 – MSCT VRT filters: a) skin (left) and bones (right) filter; b) bones filter; c) airways or pulmonary filter.

In the second phase, we carried out Raman measurements to identify the nature of the pigments. No Raman signal was collected, therefore surface-enhanced Raman scattering (SERS) was used to overcome such drawbacks. Raman cross-sections of molecules adsorbed on artificially roughened noble metal surfaces show dramatic enhancements because of the robust amplification of the incident field produced by the excitation of the localised plasmon resonance modes corresponding to the metallic nanostructure.
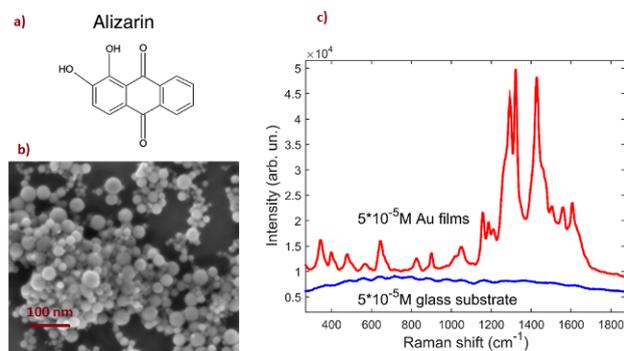
Fig. 5 – a) Chemical structure of alizarin; b) SEM images of the synthesised gold film; c) Raman spectra of alizarin adsorbed on the surface of the gold nanostructured substrate.

Fig. 6–Raman spectra of (a) madder lake and ochre on painted tablets covered with gold nano colloids and then dried in air.

Here, SERS behaviour of two dyes of interest in the cultural heritage field: alizarin and purpurin adsorbed on gold nanostructured substrates are analysed. Their molecular structures differ by the presence of another hydroxy group in purpurin allowing their identification from standard Raman measurements, but identification from SERS measurements may not be accessible due to adsorption mechanism on the substrate together with the presence of different chemical isomers that can play a role in their SERS behaviour. As an example, in Fig. 5 are shown: a) the chemical structure of alizarin; b) the surface morphology (studied by scanning electron microscopy (SEM) of the gold nanostructured film used for SERS, characterized by islands (agglomerates) with smooth edges; c) Raman spectra acquired on the surface of substrates soaked in the alizarin aqueous solution. The spectrum acquired on the bare glass substrate shows no evidence of Raman peaks related to the presence of the dye. On the contrary, the Raman spectra obtained on the surface of the gold-covered substrates show clear Raman contributions typical of the investigated dye.

The Raman spectra acquired on the commercial pigments were a reference for the subsequent measurements made on the painted tablets.

SERS approach allowed the identification of madder lake and ochre Raman features [14], directly on a wooden support (previously prepared using Firenze Zecchi pigments) covered with gold colloid and then air-dried before to carry out Raman measurements (see Fig. 6).

On the overall, from the results reported and discussed in this work, it is evident the possibility to obtain qualitative information about materials on the specimens. In detail, MPR reconstruction allows us to discriminate the different pigments using HU values which corresponds to X-Ray coefficient attenuation. It is, in fact, well known that each material has its own density [15]. This type of discrimination could also be extended to any other kind of materials found on an artistic object.
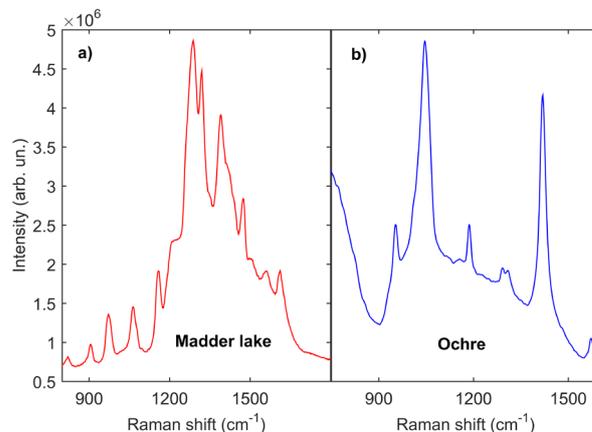
In this case, it was applied on the wooden support too. Moreover, VRT filters could be used to virtual dissect an object without compromising its integrity. It permits to have a look to details that, in some cases, are not visible and to have a complete map of all the materials that are on the artefacts.

Of course, there are some limitations, primarily related to the minimum slice thickness that could be set (0,6 mm in our case), but new Siemens scanners have a spatial resolution of 0,2 mm, and we think that in future it could be less. Another limitation could be the gantry opening because the maximum available on the market it is 78 cm in diameter. In this case, this method can be applied to small and medium-size objects, which is a considerable part of artefacts. Moreover, the widespread presence of this instrumentation all-around national and international territory makes it possible to carry out this type of analysis in any city, in short time (only 12s scan time) and during weekends. Improving this method could be an innovative, valuable and useful application for Cultural Heritage investigations.

Ultimately, because of the evidence reported in this work, combining this analysis to conventional non- destructive spectroscopic ones (FTIR and SERS characterization), a clear panorama about inorganic and organic materials on the objects of interest in cultural heritage could be obtained.

IV. CONCLUSIONS

For the first time, application and results of clinical MSCT to pigments investigation painted on wooden support was reported. The presented results show how MSCT is a valuable and promising diagnostic method for painted objects investigation. MPR and VRT post-processing techniques are adaptable to pigments analysis and have great potential to differentiate various colours. Combining X-Ray densitometric evidence to SERS

results, it is possible to identify and characterize both inorganic and organic materials present on the objects. This method is going to be refined and applied to more prospective studies.

REFERENCES

[1] L. Bonizzoni, S. Bruni, M. Gargano, V. Guglielmini, C. Zaffino, A. Pezzotta, A. Pilato, T. Auricchio, L. Delvaux, N. Ludwig, "Use of integrated non-invasive analyses for pigment characterization and indirect dating of old restorations on one Egyptian coffin of the XXI dynasty", Microchemical Journal, Vol. 138 (2018) 122-131.

[2] C. Denoël, P. Roger Puyo, A. M. Brunet, N. P. Siloe, "Illuminating the Carolingian era: new discoveries as a result of scientific analyses", Heritage Science, Vol. 6, Issue 1 (2018), article 28.

[3] M. Mantler, M. Schreiner, "X-ray Fluorescence Spectrometry in Art and Archaeology", X-ray Spectrometry, Vol. 29, Issue 1 (2000) 3-17.

[4] J. Wouters, A. Verhecken, "The coccid insect dyes: HPLC and computerised diode-array analysis of dyed yarns", Stud. Conserv. 34, (1989) 189.

[5] L.H. Oakley, S.A. Dinehart, S.A. Svoboda, K.L. Wustholz, "Identification of organic materials in historic oil paintings using correlated extraction less surface-enhanced Raman scattering and fluorescence microscopy.", Anal. Chem. 83 (2011) 3986.

[6] E. Fazio, F. Neri, A. Valenti, P.M. Ossi, S. Trusso, R.C. Ponterio, "Raman spectroscopy of organic dyes adsorbed on pulsed laser deposited silver thin films", Appl. Surf. Sci. 278 (2013) 259–264.

[7] C. Parisi, C. Pelosi, U. Santamaria, P. Pogliani, G. Agresti, S. Longo, "The conservation project of a liturgical object: The case of infant Jesus of Prague in the church of Saint Mary of providence at Macchia Giarre (Italy)", European Journal of Science and Theology, Vol. 12, Issue 2, (2016) 235-244.

[8] S. Longo, E. M. Mormina, F. Granata, D. Mallamace, M. Longo, S. Capuani, "Investigation of an Egyptian mummy board by using Clinical Multislice Computed Tomography", Studies in Conservation, Vol. 63, Issue 7 (2018) 383-390.

[9] C. Cennini, "Il Libro dell'Arte", a cura di Fabio Frezzato, edited by Neri Pozza Ed. (2009)

[10] L.W. Goldman, Principles of CT: Multislice CT, Journal of Nuclear Medicine Technology, 36(2) (2008) 57 -68.

[11] M. Tommasini, C. Zanchi, A. Lucotti, E. Fazio, M. Santoro, S. Spadaro, F. Neri, S. Trusso, E. Ciusani, U. de Grazia, M. Casazza, P.M. Ossi, "Laser synthesized nanoparticles for therapeutic drug monitoring", Advances in the Application of Lasers in Materials Science, edited by Springer, Vol. 274, Chapter 12 (2018).

[12] Mineralogy Database http://webmineral.com/

[13] S. Berti, A. Crivellaro, M. Nocetti, L. Rescic, L. Sozzi, "Conoscere il legno", edited by CNR IVALSA, Italy, (2007) Chapter 3.

[14] M.A. García-Bucio, E. Casanova-González, J.L.Ruvalcaba-Sil, E. Arroyo-Lemus, A. Mitrani-Viggiano, "Spectroscopic characterisation of sixteenth-century panel painting references using Raman, surface-enhanced Raman spectroscopy and helium-Raman system for in situ analysis of Ibero-American Colonial paintings", Phil.Trans. R. Soc. A 374, 20160051, (2016).

[15] J. H. Hubbel and S. M. Seltzer "X-Ray Mass Attenuation Coefficients", NIST Standard Reference Database 126, (2004).