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Review article

Lorenzo Lotto's painting materials: an integrated diagnostic approach

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ABSTRACT

This paper presents the results of a comprehensive diagnostic investigation carried out on five paintings (three wood panels and two paintings on canvas) by Lorenzo Lotto, one of the most significant artists of the Italian Renaissance in the first half of 16th century. The paintings considered belong to 1508–1522 period, corresponding to the most significant years of Lotto's evolution. A wide array of non-invasive (reflectance spectrometry and X-ray fluorescence) and micro-invasive analytical techniques (optical microscopy, scanning electron microscopy with energy dispersive spectroscopy, micro-FTIR spectroscopy, micro-Raman spectroscopy, gas chromatography coupled with mass spectrometry and high performance liquid chromatography coupled with photodiode array detection and mass spectrometry) were applied in order to provide a large set of significant data, limiting as much as possible the sampling. This study has proved that Lotto's painting palette was typical of Venetian practice of that period, but some significant peculiarities emerged: the use of two kinds of red lakes, the addition of calcium carbonate and colourless powdered glass, the latter frequently found in pictorial and ground layers. Moreover, the integrated investigation showed that Lotto's technique was sometimes characterized by the use of coloured priming and multi-layer sequences with complex mixtures. Chromatographic analyses allowed to identify in all specimens: azelaic, palmitic and stearic acids, generally referring to the presence of drying oils.
The extension of additional non-invasive examination to about 50 paintings by the same author, spanning from 1505 to around 1556, helped to verify the evolution in the use of some pigments, such as the yellow ones, where Pb-Sb yellow was used alongside Pb-Sn yellow.

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1. Introduction

Lorenzo Lotto (Venice, 1480 – Loreto, 1556 or 1557) was one of the most interesting painters of Venetian 16th century, besides Titian, Giorgione, the late Giovanni Bellini, and before the new generation of Veronese and Tintoretto, both for the elegance of his work and for his iconographic and chromatic innovations, with a special feel for opposite colour juxtapositions and for cold and saturated hues. His painting technique was investigated during the last years [1–2], but never with a complete approach including non-invasive examinations together with micro-invasive analyses over a broad range of works. Many features of Lotto's technique, taking into account literature and a first screening of new collected data (mainly non-invasive) have recently been discussed in non- technical essays in Italian [3]. More specific results have been published about almost each single painting placed in Marche [4,5] and Veneto regions [6], and about three works of Bergamo [7], but without detailed micro-chemical data.

We based this study on large paintings by Lotto, particularly three wood panels and two paintings on canvas. The three wood panels are: the polyptych of the church of St. Dominic (1508) and the *Transfiguration of Christ* (1511–12), both in Recanati (Museo Civico, Villa Colloredo Mels), and the polyptych of Ponteranica, near Bergamo (1522). The two paintings on canvas, dated 1521, are the altarpieces of the churches of St. Bernardino and of the Holy Spirit in Bergamo (Fig. 1, see also Figs. S1-S4, Supporting material).


Fig. 1. Lorenzo Lotto, *Holy Spirit* altarpiece (1521), Bergamo, Church of Santo Spirito. During the cleaning intervention.

The respect for conservation issues and the analytical needs suggested the application of an integrated approach based on the use of both non-invasive (first step) and micro-invasive techniques (second step) to investigate painting materials and techniques [8]. As a first step, a non-invasive in situ campaign was carried out on about 50 paintings of Lotto using portable instruments, in order to collect as much information as possible by minimizing the sampling. Spectroscopic techniques, like reflectance spectrometry in the visible range (vis-RS) and energy-dispersive X-ray fluorescence spectroscopy (ED-XRF) were chosen as informative first-step analyses, preceded by photographic, UV fluorescence and IR reflectography campaigns to identify the best areas to be sampled as well as to record conservative issues and some features about painting technique, like underdrawing and changes [5–7].

As a second step, after carefully collecting, a minimum set of micro-fragments on the 5 aforementioned paintings was analysed by several analytical techniques with the aim to obtain a characterization of the inorganic and organic components: optical microscopy, environmental scanning electron microscopy with energy dispersive spectroscopy (ESEM/EDX), micro-FTIR spectroscopy, micro-Raman spectroscopy, gas chromatography coupled with mass spectrometry (GC–MS), and high performance liquid chromatography coupled with high resolution time of flight mass spectrometry (HPLC–HR-ToF-MS).

2. Experimental

2.1. Instruments and methods

2.1.1. Energy dispersive X-ray fluorescence (ED-XRF)

X-ray fluorescence analysis (ED-XRF) was carried out using two Bruker Tracer III SD energy dispersive spectrometers, both operating at 40 kV, with X-Flash SDD detector and 4 mm diameter spot, one with an Ag target X-ray tube operating at 22 μA, the other with a Rh tube at 11 μA, with proper Al-Ti-Cu filter. They are both particularly sensitive to Sn and Sb K-lines. Bruker Artax software was used to elaborate spectra.

2.1.2. Reflectance spectrometry (vis-RS)

Reflectance spectroscopy measurements, mainly in the visible range (vis-RS), were obtained using a handheld spectrophotometer Minolta CM 2600d: 360–740 nm range, 10 nm acquisition step, integrating sphere included, UV source included, d/8 geometry, 3 mm diameter spot. This choice enables fast data acquisition and reliability in identifying spectra as tested on the field during many campaign of analyses on ancient and modern pigments, considering the typical broad bands of RS spectra and the little variability in the position of the features of the spectra (reflectance minima and shoulders) when pigment concentration changes, usually in the order of a few nanometres. RS instruments with higher spectral resolution (1 nm or lower) are usually not necessary to identify a large class of pigments. A wide personal reference database was used to interpret results, together with literature data. Colorimetric data in CIELab colour space were also acquired. Minolta SpectraMagic software and Excel were used to elaborate spectra.

2.1.3. Optical microscopy (OM)

An optical microscope Nikon TK-1270E was used in reflected light. Dark field observations were performed with fixed oculars of 10× and objectives with different magnifications (5, 10, 20 and 40×). Cross-section photomicrographs were recorded with an Olympus DP70 digital scanner camera directly connected to the microscope.

2.1.4. Scanning electron microscopy and environmental scanning electron microscopy coupled with energy dispersive X-Ray (SEM and ESEM/EDX)

A scanning electron microscope (Philips XL 20 SEM) equipped with an energy dispersive X-ray spectrometer (Phillips EDAX PV9900) with a light element detector type ECON was firstly used; at a later time an environmental scanning electron microscope (Philips Quanta FEI 200) equipped with an energy dispersive X-ray spectrometer (EDX) by Link Analytical Oxford (Link, UK), model 6103 was also used. The same samples already prepared for optical microscope observations were analysed; some of them were sprayed with gold depending on the methodology used (Au presence in some spectra is due to the gold metallization). A series of analyses were performed on each layer of the cross-sections. The elemental composition was carried out at 30 kV acceleration voltages, 100 s lifetime and 30 mm working distance. Samples were examined in low vacuum with a chamber pressure set at 23 Pa.

2.1.5. Micro-Raman spectroscopy (MRS)

Micro-Raman spectra were recorded with a Labram instrument from the Jobin Yvon-Horiba, equipped with a red 633 nm laser and a Peltier-cooled (−70 °C) CCD detector with 1024 × 256 pixels; 1 cm^{-1} spectral resolution of 1 μm spatial resolution. According to the intrinsic intensity of the spectrum recorded, the scanning time varied from 5 to 20 s and 5–20 scans with laser power (5 mW) attenuated to about 1/10. Olympus 50× and 100× long-distance objectives were used. Raman analyses were performed on both cross-sections and non-embedded micro-fragments of the same samples.

2.1.6. Fourier transform infrared spectroscopy with micro-attenuated total reflection (μ-FTIR-ATR)

Micro-FTIR spectra were taken in attenuated total reflectance (ATR) mode employing a ThermoNicolet "Continuμm" Nexus line micro-spectrophotometer, equipped with a MCT detector. A micro-slide-on ATR silicon crystal directly connected to the objective has been used. Infrared spectra were recorded in the 4000–650 cm^{-1} ranges; resolution 4 cm^{-1} and 120 scans. All spectra were collected on the micro-samples and are given in transmittance units after baseline correction.

2.1.7. Gas chromatography - mass spectrometry (GC–MS)

A Focus GC (Thermo Scientific) coupled to DSQ II (Thermo Scientific) with single quadrupole and split-splitless injector was used. The mass spectrometer was operated in the EI positive mode (70 eV). The carrier gas was used in the constant flow mode (He, purity 99.995%) at 20 mL/min. Fatty acid and amino acid analytical procedures - the basic methodology relied on the identification of fatty acids and amino acids in the same sample. Two chromatograms were therefore collected for each sample: the first one for fatty acid derivatives, the second for amino acid derivatives [9–10]. The internal standards employed were: heptadecanoic acid (50 μl of a 0.1 mg/ml solution *w*/*v*) for the analysis of fatty acids; norleucine (50 μl of a 0.1 mg/ml solution *w*/*v*), and norvaline (50 μl of a 0.01 mg/ml solution *w*/*v*) for the amino acids analysis, respectively; the analysis was conducted on 1 mg of paint samples. The chromatographic separation of searched components was performed by means of a fused-silica capillary column (RXI-5, Restek) with a 0.25 μm (30 m × 0.25 mm × 0.25 μm) methyl-silicone (5% phenyl) film and the injector was used in splitless mode. The sample was treated with 4 *N*-HCl in methanol (1 ml) and *n*-hexane (1 ml) for 2 h at 50 °C. The n-hexane phase, which contains fatty acid methyl-esters, was used for gas chromatographic analysis (1 μl). The separation of the methyl ester of fatty acids was achieved with this T gradient: isothermal conditions at 80 °C for 2 min, followed by 20 °C/min heating up to 280 °C and then isothermal conditions at 280 °C for 6 min (total run time: 18 min). The mass spectra were collected in Total Ion Current (TIC; 40–500 *m*/*z* fragmentation rate) mode. After evaporation to dryness of the methanol phase, the residues were dissolved in 6 N hydrochloric acid (2 ml) and hydrolysed in a screw-capped container for 5 h at 100 °C in an oil bath, under nitrogen atmosphere. After evaporation to dryness, the hydrolysed residues were esterified using 3 ml of 2 N HCl in propan-2-ol at 90 °C for 1 h. After cooling, the solvent was evaporated under vacuum and the residue of the paint was dissolved in 0.2 ml of dichloromethane and derivatized with 0.2 ml of trifluoroacetic anhydride at 60 °C during 1 h. After cooling, the solvent was evaporated under vacuum and the residue of the paint sample was dissolved in 0.2 ml of dichloromethane, then the solution was used for gas-chromatographic analysis (1 μl). The separation of *N*-trifluoroacetyl-*O*-2-propyl esters amino acids derivatives was achieved following this temperature program: isothermal conditions at 60 °C for 3 min, with 25 °C/min heating up to 260 °C and isothermal conditions at 260 °C for 6 min (total run time 17.00 min). The mass spectra were recorded in Selected Ion Monitoring (SIM; 140, 126, 154, 153, 139, 168, 182, 166, 164, 184, 180, 198, 91, 190 *m*/*z* fragments). A qualitative analysis was also performed in order to identify lipids and proteins contained in the painting, and the average amount of related fatty acids and amino acids was estimated.

2.1.8. High performance liquid chromatography coupled with high resolution time of flight mass spectrometry (HPLC–HR-ToF-MS)

Chromatographic separation was carried out with an Agilent 1200 HPLC system (Avondale, PA, USA) coupled to a High Resolution Time of Flight Mass Spectrometer (HR-ToF-MS) Agilent (SRA Instruments S.p.a.) via ElectroSpray Ionization (ESI) interface.

Reversed Phase separation was performed in a Gemini-NXC18 Chromatographic Column (100 cm × 2.0 mm, 3 μm) opportunely preceded by the correspondent C18 Guard Column, both by Phenomenex (Torrance, CA, USA). Isocratic elution was performed with a polar mobile phase compose of MeOH/H_2O (99:1 v/v). All the solvents were "HPLC gradient grade" from ROMIL (Dublin, Ireland) with the exception of the water, which was purified through the system Millipore MilliRo/MilliQ (Bedford, MA, USA) and further filtered with a High Performance Extraction Disk Cartridge by 3MTM EmporeTM. Chromatograms were acquired and processed by Mass Hunter software (Agilent Technologies). Organic binders were extracted from collected samples by sonication with 2 ml of isopropyl alcohol for 20 min. After this step, the solid phase was separated to be submitted to a second extraction with hexane and the isopropyl alcohol solution concentrated down to 0.5 ml under a soft nitrogen flux before injection. The second extract was subjected to a solvent change from hexane to isopropyl alcohol. During volume reduction down to 0.5 mL before injection.

2.2. Samples

On the basis of preliminary non-invasive investigation, a total of 16 micro-samples (Table 1) were collected from the five paintings, either by gently rubbing the colour from the surface, or by detaching a small portion to prepare the cross-sections according to current methodology [11].

3. Results and discussion

The main features emerged about supports, ground layers, pigments (organized by colour) and organic binders are discussed. A detailed

Table 1
Description of the painting samples investigated with different invasive and non invasive analyses.

Wood panel	Sample	Sampling location	Colour
St. Dominic polyptych (Recanati), 1508	PL1	Pope Gregory XII's cope	Dark green
	PL2	St. Lucia's robe	Orange
	PL3	St. Lucia's cloak	Ground
Transfiguration of Christ (Recanati), 1511–1512	TR1	Border, St. John's robe	Red and yellow
	TR2	St. Peter's robe	Blue
	TR3	St. Peter's cloak	Orange
	TR4	St. Elia's robe	Dark green
	TR5	St. Elia's robe	Pink
	TR6	Wood panel, back	–
	TR7	Right side, central part	White ground
Ponteranica polyptych (Ponteranica, Bergamo), 1522	PPB1	Virgin's lectern	Hazel

Canvas	Sample	Sampling location	Colour
St. Bernardino church altarpiece (Bergamo), 1521	PSB1	Tent	Green
	PSB2	Canvas, back	–
Holy Spirit church altarpiece (Bergamo), 1521	LPS1	St. Sebastian's robe	Orange
	LPS2	Sky	Blue
	LPS3	Cloud	Pink

synthesis of data obtained by integrated analyses carried out on some of the studied micro-samples is illustrated in Tables 2-5 and related images (Figs. 2-8, 10), with the associated non-invasive exams (Table 6, Fig. 9). More information is available in supporting material (Figs. S1-S13).

3.1. Supports

All examined panels were painted on wood axes arranged vertically and held together with long butterfly inserts on the *recto*, made evident by dilations and contractions. The wood species used in the panel of *Transfiguration of Christ*, as evidenced by SEM morphological investigations (sample TR6, Fig. 2), is the genus *Populus sp*., Salicaceae family, widely used by Lotto as a support for his works [12]. The altarpiece of St. Bernardino is painted on a fine and dense canvas, orthogonal mesh and rather high density, equal to about 20 × 22 threads/cm. A similar canvas was used for the Holy Spirit altarpiece, where the density was not determined because of lining.

3.2. Ground layers

The wood or canvas supports in Lotto's paintings were typically prepared with calcium sulphate dihydrate ("gesso") and animal glue [1–3,5,6]. In the samples PL1, PL2, PL3, TR1, TR4, TR5, PPB1, PSB1 and LPS3, Ca and S with Si traces were clearly identified by EDX (Tables 2-5). At the excitation energy applied, XRF was able to detect Ca, Sr (a substitute of Ca in some of its minerals, or due to impurities of celestine) and some amounts of Fe, sometimes present at impurity level. As known, in XRF the S line is hidden by Pb *M*-line, and could be seen only in lacunas where the possible priming and upper layers were absent.

Besides Ca and S, in the ground of St. Dominic polyptych small amounts of Si and Al are present (Table 3). Sometimes also traces of Fe, together with Si and Al were moreover detected (PL2 sample; Table 3, Fig. S5, Supporting material and PL3 sample, see Fig. S6, Supporting material) as well as in the ground of the Holy Spirit altarpiece (LPS3 samples; Table 5 S7, Supporting material), indicating the existence of a few particles of earths dispersed in the white ground,

Table 2
Stratigraphic sequence of the cross-sections by integrated microanalyses on samples TR1, TR2, TR3, TR4 (n.a. = not analysed), from the *Transfiguration of Christ* (Recanati), 1511–1512.

Samples	Layers	Maximum thickness (μm)	Typology	EDX, detected elements	Stratigraphic identification (OM + ESEM/EDX)	Micro Raman identifications
TR1 red	4	5	Light area	Pb, Hg, S, Si, Al, Ca	Lead white, vermillion *Si*—Al based particles, calcium carbonate	Lead white, vermillion, litharge, Indigo, calcite, saffron, gamboge?
	3	20		Pb, Si, Sn, Al, Ca	Lead-tin yellow in glassy matrix, calcium carbonate	
	2	45	Painting layers	Hg, S, Si	Vermillion, silica particles	Vermillion
	1	110	Priming	Pb, Si, Al, Sn, Ca, Fe	Lead-tin yellow in glassy matrix, lead white, calcium carbonate	Lead-tin yellow type I, cerussite, litharge, calcite particles
TR2 blue	4	55	Painting layers	Cu, Si	Azurite/chrysocolla?	Azurite, black carbon
	3	25		Pb, As, S, Hg, Ca, Al, Si, Fe, K	Arsenic sulphide, Pb-based pigments, red lake, vermillion, red ochre	Arsenic sulphide, vermillion, litharge
	2	10	Drawing		Carbon black particles	Black carbon
	1	30	Priming	Pb, Si, Ca, Sn	Lead white, lead-tin yellow in glassy matrix	Lead white, cerussite, lead-tin yellow type I, goethite
TR3 orange	1	50	Painting layers	Pb, As, S, Hg, Ca, Al, Si, Fe, K	Arsenic sulphide, Pb-based pigments, red lake, vermillion, red ochre	n.a.
TR4 green	5	10	Painting layers	Cu	Cu-based green	n.a.
	4	40		Pb, Cu, Al, Sn, Si, Ca	Cu-based green, lead-tin yellow in glassy matrix, calcium carbonate	
	3	15		Si, Pb, Ca, Al, Mg, K	Lead white, silicates	
	2	30		Pb, Cu, Si, Fe	Lead white, azurite, hematite	
	1	10	Priming	C	Organic compounds	
	0	50	Ground layer	S, Ca, Al, Si	calcium sulphate, Si/Al-based particles	

Table 3
Stratigraphic sequence of the cross-sections by integrated microanalyses on samples PL1, PL2 (n.a. = not analysed), from the polyptych of St. Dominic (Recanati), 1508.

Samples	Layers	Maximum thickness (μm)	typology	EDX, detected elements	Stratigraphic identification (OM + ESEM/EDX)	Micro Raman identifications
PL1 green	6	15	Painting layers	Pb, Cu, Sn, Si, Al, Ca	Cu-based green, lead-tin yellow in glassy matrix	n.a.
	5	15		Cu, Si	Cu-based green, chrysocolla?	
	4	50		Pb, Cu, Si, Sn, Ca	Lead-tin yellow in glassy matrix, Cu-based green, calcium carbonate	
	3	20		Pb, Sn, Si, Ca, Al, Na	Lead-tin yellow in glassy matrix	
	2	10		Pb, Cu, Si	Lead white in glassy matrix, Cu-based green	
	1	15	Priming	Pb, Sn, Ca, Al, Si, Na	Lead-tin yellow in glassy matrix, calcium carbonate, red lead	
	0	20	Ground layer	Ca, S, Si	Calcium sulphate, silica particles	
PL2 orange	3	25	Painting layers	Pb, As, S, Si, Ca, K, Fe, Zn, Cu, Al	Arsenic sulphides (orpiment/realgar), red ochre, Pb-based pigments, Zn Containing pigment	Orpiment, litharge, lead white, realgar, pseudobrookite, rutile, goethite, Calcite, carbon black, HgO
	2	10		Pb, Ca	Lead white, calcium carbonate	lead white, cerussite
	1	35		Pb, Sn, Cu, Si, Al, K, Fe, Ca	Lead white, azurite, lead-tin yellow, glassy particles, calcium carbonate	Azurite, litharge, lead white, goethite
	0	100	Ground layer	Ca, S, Si, Al, Fe	Calcium sulphate, silica particles	n.a.

coherently with XRF data. In a coeval work, the St. Bernardino altarpiece, the light brown ground contained calcium sulphate with earth (Table 4, Fig. S8, Supporting material). This choice for a yellow-brownish ground represents an exception, as far as we know today about Lotto's and Venetian painting in those years [3,13], and it could be due to an experimental approach towards canvas, a support that was gradually substituting wood in altarpieces of Northern Italy in the first decades of 16th century. Mg and Ca particles were also identified by ESEM-EDX in the ground of polyptych of Ponteranica (Table 4, Fig. S9, Supporting material).

Sometimes priming – white or coloured, depending on the area and on the desired effect – was added over the ground, at least locally. In particular, in the *Transfiguration of Christ* (Fig. 3a-b; Table 2) and in the St. Dominic's polyptych altarpiece, priming (Fig. 4a; Table 3) is characterized by the presence of Pb and Sn yellow particles related to lead tin yellow type I. White particles were also observed, sometimes containing Ca, so indicating a calcium carbonate presence (samples TR1, Table 2 and PL1, Table 3, Fig. S10, Supporting material, sample LPS1). Pb signal could apparently be increased thanks to a very common [14] addition of lead white (sample TR2, Table 2; sample PL1, Table 3, Fig. S10, Supporting material) in the priming. Raman analysis confirm lead-tin yellow type I presence in TR1 sample (Table 2, Fig. S11, Supporting material) in admixture with calcite, litharge and saffron (probably yellow gamboge).

In the priming of sample PPB1 (in Ponteranica polyptych) lead white, cerussite together with glassy particles were detected as in sample PSB1 (St. Bernardino church altarpiece) in which lead oxides, calcite and carbon black were identified (Table 4, Fig. S9, Supporting material).

In the priming of St. Sebastian's robe (in the Holy Spirit church altarpiece) together with lead-tin yellow type I, lead white and calcite, goethite was detected by Raman analysis (Sample LPS1, Table 5, Fig. S12, Supporting material).

Lead-tin yellow was probably mixed in order to accelerate the drying of siccative oil, as suggested for other coeval artists [15–16].

The saffron identification in the priming seems quite interesting (Fig. 7b) because it was never found with other techniques and not

Table 4
Stratigraphic sequence of the cross-sections by integrated microanalyses on samples PSB1, PPB1 (n.a. = not analysed), from the polyptych of Ponteranica, 1522.

Samples	Layers	Maximum thickness (μm)	Typology	EDX, detected elements	Stratigraphic identification (OM + ESEM/EDX)	Micro Raman identifications
PSB1 green	6	20	Varnish			
	5	20	Painting layers	Cu, Pb, Ca, Si, Sn	Cu-based green, lead-tin yellow in glassy matrix, calcium carbonate	Azurite, verdigris, lead-tin yellow type I, lead white, goethite, litharge, phthalocyanine on the surface
	4	45		Cu, Pb, Ca, Si, Sn, Cl, K	Cu-based green, lead-tin yellow in glassy matrix, Cl/K-based particles	Lead white and cerussite, verdigris
	3	40		Pb, Cu, Sn, Si, Ca, Fe	Cu-based green, lead-tin yellow in glassy matrix	Lead-tin yellow type I, lead white, cerussite, verdigris?
	2	35		Cu, Pb, Si, Sn, Ca, Al	Cu-based green, lead-tin yellow in glassy matrix	Lead-tin yellow type I, cerussite, verdigris, litharge, carbon black
	1	25	Priming	Pb, Si, Ca, Al, Mg, K, Fe	Lead white, calcium carbonate, glassy particles	Lead white, cerussite, calcite, carbon black, lead oxides
	0	95	Ground layer	Ca, S, Si, K, Fe, Mg, Al	Calcium sulphate, silicates	Gypsum
PPB1 hazel	4	20	Painting layers	Pb, C, Ca, Si, Fe, K	Lead white, red ochre	Particles of copper phthalocyanine on the surface
	3	25		Pb, Si, Fe, Ca, K, Cu, Sn, Al	Lead-tin yellow, led white, azurite, red ochre	Lead-tin yellow type I (strong band at 132 cm^{-1}), azurite, lead white
	2	30		Fe, Al, Si, K, Hg, S, Ca	Iron oxides, carbon black, vermillion	Hematite, carbon black
	1	95	Priming	Pb, Si, C, Ca, Al, Mg, K, Fe	Lead white, carbon black, glassy particles	Carbon black, lead white, cerussite
	0	275	Ground layer	Ca, S, Si, Mg, Fe, Mn	Calcium sulphate, calcium and magnesium carbonate, earth (raw umber?)	Gypsum

Table 5
Stratigraphic sequence of the cross-sections by integrated microanalyses on samples LPS1, LPS3 (n.a. = not analysed), from the Holy Spirit church altarpiece (Bergamo), 1521.

Samples	Layers	Maximum thickness (μm)	Typology	EDX, detected elements	Stratigraphic identification (OM + ESEM/EDX)	Micro Raman identifications
LPS1 orange	4	10	Painting layers	Fe, Pb, Si, As, Ca, S, K, Al, Na	Red ochre, arsenic sulphides, lead white	Hematite, magnetite, red sulphides
	3	45		Pb, Fe, Sn, Ca, Si,Mg, Al	Lead-tin yellow, calcite, red ochre	Lead-tin yellow type I, calcite, minium
	2	15		Cu, Pb, Al, Si, Ca, K	Azurite, lead white, red lake	Azurite, cerussite, litharge, massicot, calcite, carbon black
	1	5	Priming	Pb, Sn, Si, Fe, Ca	Lead white, lead-tin yellow, Si and Ca particles, iron oxides	Lead-tin yellow type I, lead white, calcite, goethite carbon black
LPS3 pink	2	10	Painting layer	Pb, Al, Si, Ca	Lead white, silicates	Cerussite, lead white, massicot, carbon black
	1	15	Priming	Pb, Ca	Lead white, calcite	Cerussite, carbon black
	0	25	Ground layer	Ca, S, Si, Al, Mg, K	Calcium sulphate, earth	Gypsum, organic red?

typically found in paintings of that age. In St. Dominic polyptych red lead particles were detected by using ESEM-EDX analysis (Table 3, Fig. S10, Supporting material) – as found in the later *Annunciation* of the Municipal Art Gallery in Jesi (about 1526) [4]. Sn signals, together with Pb, were widely detected by XRF analyses on many paintings by Lotto, not linked to yellow, orange or green areas, confirming the existence of diffused lead-tin yellow priming.

Of course, we don't know if Lotto's preference in these years, emerged by these new studies [3], of pale yellow priming (as Raphael did), regarded also aesthetic reasons, as can suggest the addiction of a saffron-based lake in order to presumably increase the yellow tone.

3.3. Blue pigments and violet colours

Lotto used lapis lazuli (vis-RS abs. band around 590–600 nm) or azurite (vis-RS abs. band around 640 nm), also in the same painting, to obtain different blue tones (Fig. 9a), both characterized by a very good quality, without impurities. In many cases the precious ultramarine blue, usually dedicated to the Virgin's cloaks and sometimes to the sky, was used above a layer of azurite, like happened in 15th century practice, probably to avoid a thick single layer of the more expensive ultramarine.

As vis-RS showed (see paragraph 3.6), small amounts of red lakes could have been added to the blue pigment to reach the desired blue hue. A violet tone is obtained using mixing larger amounts of red lake with azurite or lapis lazuli (Fig. 9a). The blue Cu-carbonate, mixed with yellow, was also used, rarely, to obtain some specific dark green or blue-grey shades. In the St. Peter's robe sample (*Transfiguration of Christ*: sample TR2) azurite particles were detected by Raman analysis (Fig. S13, Supporting material); Si and Cu rich particles were found by EDX analyses (Fig. 3b Table 2) probably related to the association azurite/chrysocolla, often together in natural copper mineralisation.

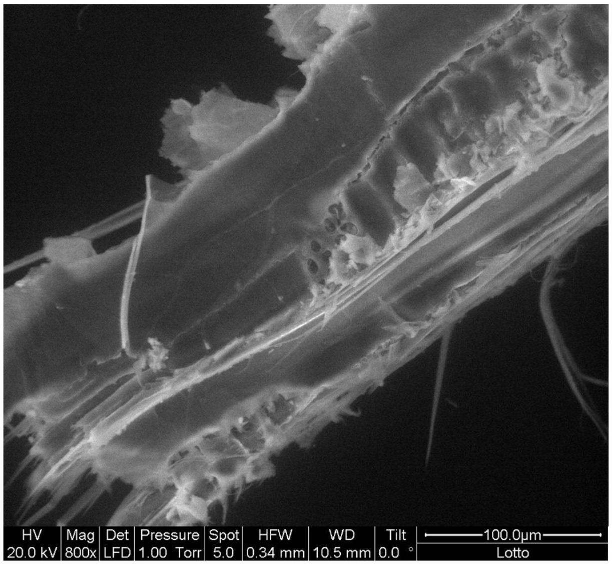

Fig. 2. SEM micrograph of wood sample TR6.

As the Raman analysis showed, the original blue layer in St. Lucy's robe (St. Dominic's polyptych) was realized mixing azurite with litharge, lead white and goethite. Probably lead-tin yellow and calcium carbonate were added together with glassy particles (first painting layer in PL2 sample: Figs. 4b and 6a-b; Table 3, see Fig. S5, Supporting material). The use of grinded glass to accelerate the drying of the oil was found also in other artists but mainly regarding red lake layers [15].

In the Holy Spirit church altarpiece, the first painting layer of St. Sebastian orange robe is made of azurite, lead white and red lake particles that was overpainted by two orange layers (LPS1 sample: Fig. 5a; Table 5, see Fig. S12, Supporting material). The blue underlayer can be considered a pentimento in respect to the final yellow colour.

As vis-RS and XRF confirmed, Co-containing blue glass, smalt blue, seemed to have been used by Lotto only in mural paintings, i.e. in the St. Vincent Ferrer, in Recanati (1513) [4], and in the frescoes of Credaro (Bergamo, 1525), while azurite was widely employed in the important mural cycle of Trescore Balneario near Bergamo (1524) [3]. Indigo was detected by Raman spectroscopy (Fig. 7a) in an inner red layer of the *Transfiguration* mixed with litharge, but it was never found with vis-RS in external layers, except for the predella of the late Mogliano altarpiece (1548) attributed to a Lotto's collaborator. This investigation confirmed that vis-RS is a very suitable tool to detect smalt blue in the painting surface [8] as well as indigo (or woad) even in small amounts, because of the indigotine specific strong absorbance band at about 660–670 nm and its characteristic shape [17].

3.4. Green pigments

In the *Transfiguration of Christ*, in St. Dominic's polyptych and in the St. Bernardino church altarpiece the verdigris presence was detected in the green area by Vis-RS. The band around 700–720 nm is frequently found where copper acetate is mixed with yellow pigments (like lead-tin one) or sometimes with lead white, as many comparative analyses (vis-RS and cross sections) revealed. The broad absorbance band of verdigris alone lies in the range 700–750 nm, and its reflectance peak, generally around 520 nm when pure, is shifted to a higher wavelength (about 580 nm) as a typical effect of aging, where possible alterations of binder, varnish, or of pigment itself may have occurred. For malachite, the reflectance peak is around 540 nm, and in old paintings also some shift can occur; its broad absorption band falls in the range 750–900 nm, far ahead in respect to verdigris [18]. So, the position of the peak can't be considered a good criterion to distinguish between the most common Cu-containing greens, while the absorption band is generally a good one. Verdigris is the green more diffusely used by Lotto

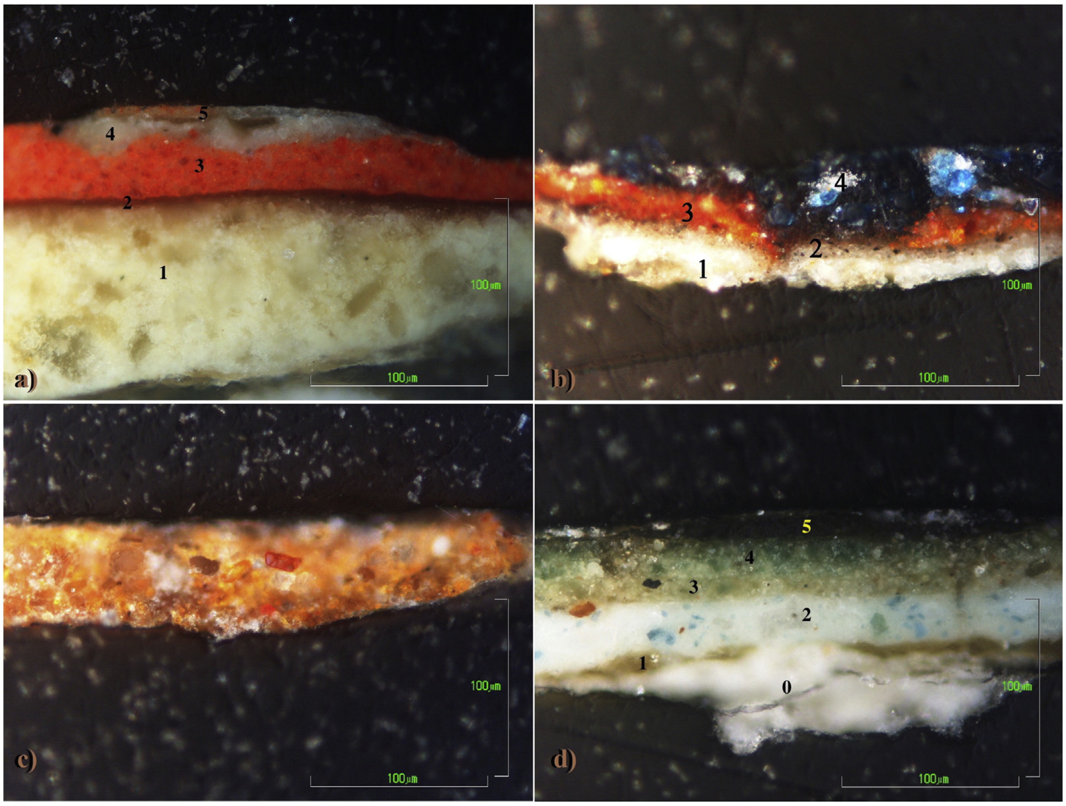

Fig. 3. Cross-sections of samples: a) TR1; b) TR2; c) TR3; d) TR4.

[2–3], as well as from the majority of 16th century Italian painters, allowing translucent glazes made of thin brushstrokes.

Cu-based green, probably referable to verdigris, usually mixed with lead-tin yellow type I and painted in many overlying layers up to four layer (PL1, TR4 and PSB1 samples: Tables 2-4; Figs. 3d, 4a and 5c) was inferred from EDX. The green pigments are very finely grinded (samples TR4 and PSB1) but sometimes are coarser (sample PL1). Raman detected verdigris on the sample PSB1 (Fig. S8, Supporting material). Green earth is rare, used apparently only in mixtures. Malachite, previously cited for two Lotto's works [3], was never detected in the collected samples, but old samples should be *re*-examined with modern analytical tools.

Phthalocyanine, a modern pigment, was also found in the surfaces of samples PSB1 and PPB1. The presence of blue and green phthalocyanines, modern synthetic organic pigments [19] (after ab. 1935), is related to modern restorations.

3.5. Yellow and orange pigments

Lead-tin yellow type I was commonly present in yellow and green areas, as well as in priming, as told, as confirmed by XRF, EDX and Raman (Fig. 8; see also Figs. S8, S11, Supporting material) analyses. In some works starting from 1530 to the last years of that century, also lead-antimony (Naples yellow) pigments was revealed, together with the previous lead-tin yellow or almost alone, by means of XRF analyses: they can be related to the “zalolin da vasarj” cited by Lotto in 1541 in his account book, known as the “Libro di spese diverse”. This yellow appeared to be a little darker, intense, than the lead-tin yellow, which exhibited instead a pale colour. The earliest use of a Pb-Sb yellow by Lotto regards a painting of 1505 [20], and it seems to be occasional, being the only occurrence until his 1530s works [3]. Some yellow particles detected by micro-Raman in many layers of the cross sections can be attributed to litharge, being the main peak at about 142 cm^{-1}.

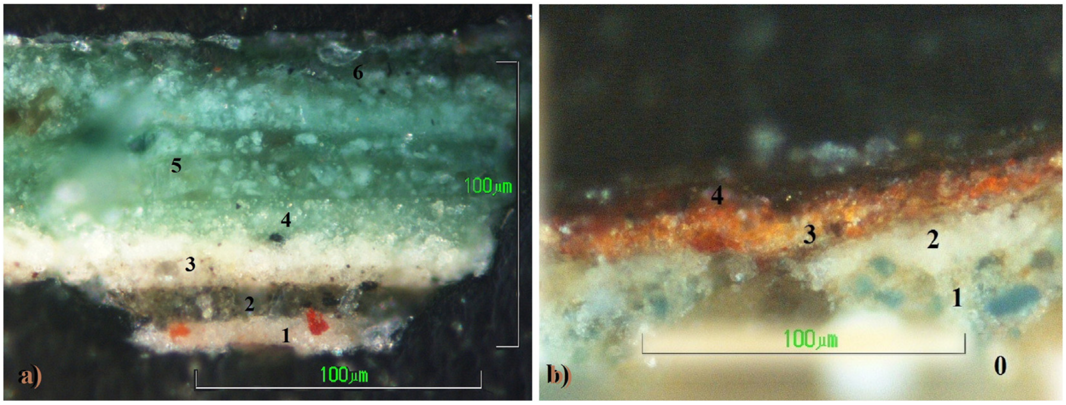

Fig. 4. Cross-sections of samples: a) PL1; b) PL2.

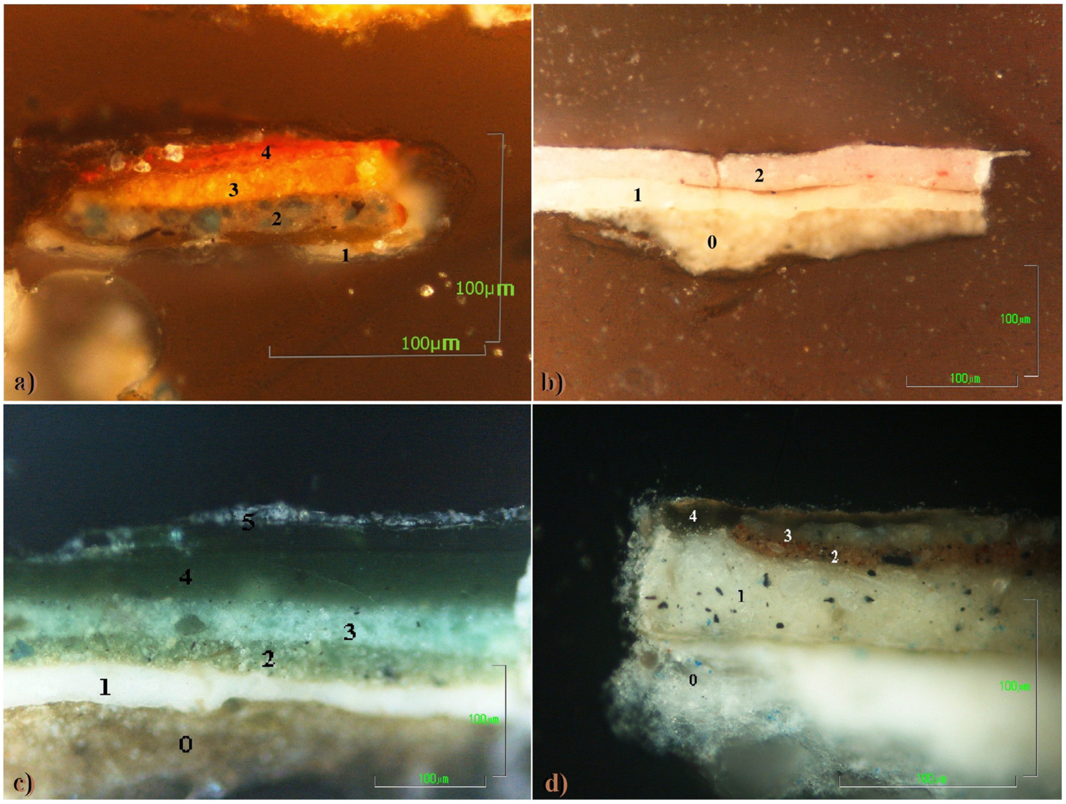

Fig. 5. Cross-sections of samples: a) LPS1; b) LPS3; c) PSB1; d) PPB1.

Apart a deliberate use of litharge yellow, its presence can be interpreted with the existence of some lead compounds not always stoichiometric or generated by the preparation procedure of the lead compounds.

As known, XRF cannot distinguish some Pb compounds like Pb oxides – minium and litharge. The presence of minium is certified by vis-RS (*S*-shaped spectrum, absorption band <540 nm, shoulder at about 580 nm) when it belongs to the surface layer: it was detected in two paintings investigated only by means of non-invasive exams, the *Deposition* of Municipal Art Gallery in Jesi (1511–1512) [4] and the Sedrina altarpiece (1542).

Arsenic sulphides (orpiment and/or realgar) were detected in orange areas, usually shaded and mixed with Pb-based pigments, red lake, vermilion and red ochre (Fig. 3a-b-c). In Holy Spirit's altarpiece, a complex stratification constituted the orange-yellow of St. Sebastian's robe (sample LPS1: Fig. 5a, Table 5; Fig. S12, Supporting material), where a mixture of lead-tin yellow, red ochre, litharge and minium was covered by a glazing containing red ochre, massicot and arsenic

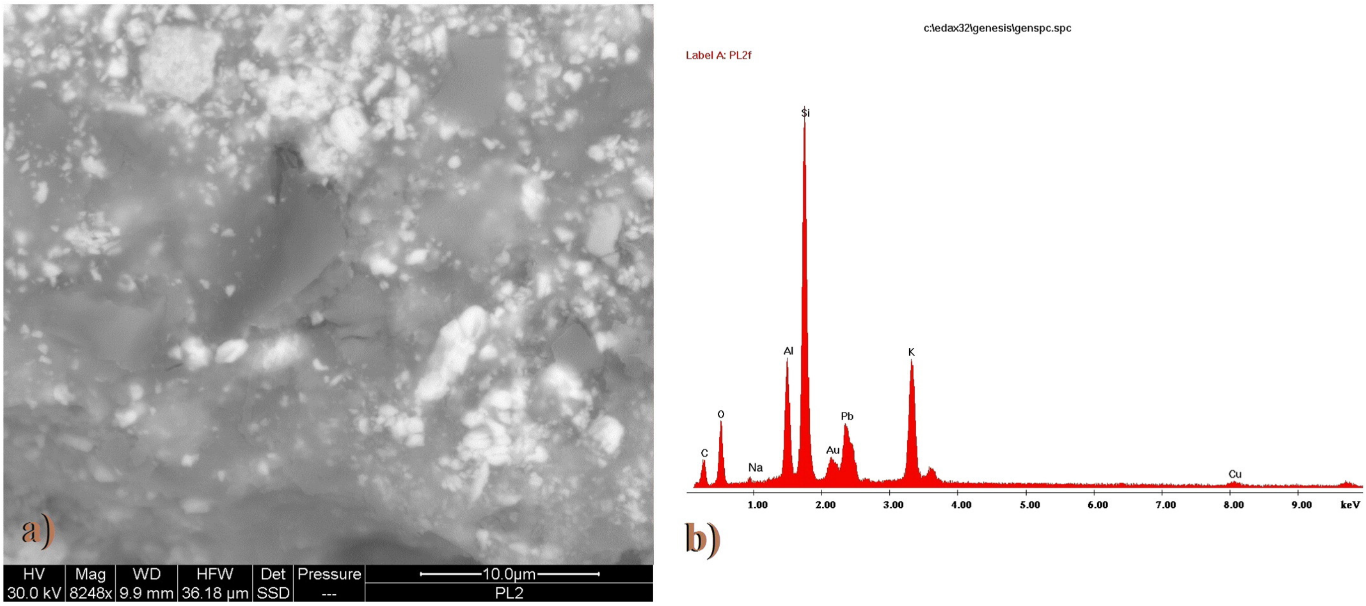

Fig. 6. Sample PL2: a) ESEM-BSE micrograph of glass fragment in layer 1; b) EDX spectrum.

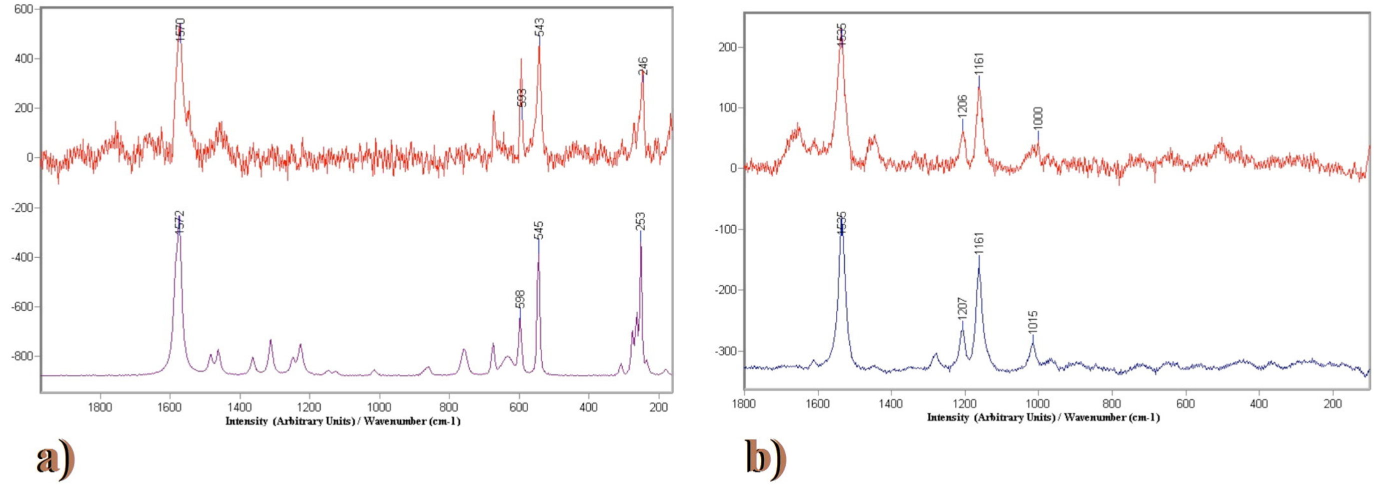

Fig. 7. Micro-Raman spectra: a) Indigo (top) on sample TR1, layer 2, compared to Clark database (bottom); b) Saffron (top) on priming of sample TR1, compared to standard.

sulphides. At non-invasive level, the detection of orpiment/realgar was inferred by XRF because of As content, but it could be confirmed by vis-RS only if they are not mixed with other pigments. In As-containing areas frequently RS spectra reported the presence of iron oxides, with the characteristic electronic transitions typical of Fe^{3+} ions band around 450 nm.

Gold was used only in a very few early paintings, like in the *Transfiguration* of Recanati.

3.6. Red pigments

Along with vermilion (HgS), used for bright layers and clearly detectable by XRF and vis-RS (*S*-shaped spectrum, absorption band <560 nm, shoulder at 600–620 nm), Lotto used red lakes, as vis-RS clearly stated. Red lake spectra, measured on not too dark areas, can be grouped into two sets, according to their absorbance bands, one with abs. bands at about 520–530 and 560–570 nm (sometimes, for darker samples, only a band edge around 580 nm can be seen), the other at 510 and 550 nm (Fig. 9b). The first set is usually related to “carmine-type” lake, extracted from a variety of coccid insects (kermes, cochineal, etc.); the second to a lake of vegetal origin, i.e. madder species, where the bands can be related to the contribution of purpurin. This preliminary distinction is supported by literature [21,22] and by analyses performed on broad collections of ancient samples as well as homemade samples of textiles and paintings derived from known dyes. The limitations in the possibility of distinguishing among different red lakes in paintings by means of reflectance spectrometry, partially suggested some years ago [23], are undergoing more in-depth studies. Sometimes these two lakes, carmine and madder, were used in different areas of Lotto's paintings or in alternate layers of the same area (i.e. in clothes), and together with glass [24]. Red lake particles presence was supposed by EDX analyses (samples TR2 and TR3; Table 2) because of the Al enrichment in admixture together with Ca, Si and K [25]. In several red layers red lake, red ochre and sometimes hematite particles were found mixed with vermillion (Fig. 3a-b-c; Table 2). In the Holy Spirit church altarpiece, red lake particles were added to lead white and calcium carbonate (LPS3 sample: Fig. 5b; Table 5) to realize the clouds.

A red compound with a large band at 300 cm^{-1} was detected by micro-Raman in sample PL2, its characterization is difficult: it can be presumably attributed to a glass compound generated by As and S [26], both detected in the same layers, where orpiment, litharge, Pb cromate and HgO are present (Fig. 4b; Table 3; Fig. S5, Supporting material).

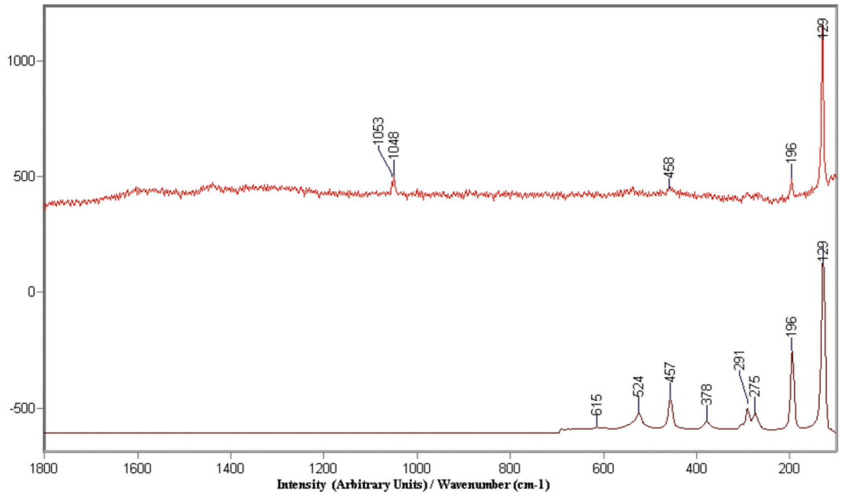

Fig. 8. Micro-Raman spectrum (top) of sample PSB1, layer 5, compared (bottom) to a standard of lead-tin yellow type I. Lead-tin yellow type I, lead white (cerussite and hydrocerussite) can be detected.

Table 6
Pigments detected by non-invasive analyses in the same area of the indicated samples. Vis-RS characteristics refer to reflectance spectra (M = max, m = absorption band, s = shoulder, w = weak). ^ indicates higher amounts, (…) stands for very low counts, n.a. = not analysed.

Samples	Vis-RS spectra characteristics (nm)	Vis-RS identification	XRF, detected elements	XRF identification (except ground layer)
TR1	460Mw, <570 m, 620–630 s	Vermilion, traces of red carmine-type lake (the lake is clearly detected in the shadow)	Ca, Fe, (Cu), Hg^, Pb^, Sr, Sn^	Cu-based pigment, lead-tin yellow, lead white
TR2	490 M, 560 m, 640 m	Azurite, small amounts of red carmine-type lake	Ca, Fe, Cu^, Pb^, Sr, Sn^	Cu-based pigment, lead-tin yellow, lead white
TR4	590 M, 700 m	Copper acetate (verdigris)	Ca, Fe, Cu^, (Hg), Pb^, Sr, Sn	Cu-based pigment, lead-tin yellow, lead white
PL1	560 M, 700 m	Copper acetate (verdigris)	Ca, Fe, Cu^, (Hg), Pb, Sr, Sn^	Cu-based pigment, lead-tin yellow
PL2	<420 m, 390sw, 590 s/M, 640 mw	Azurite, yellow pigment, traces of Zn-oxide (or Ti-oxide?)	Ca, Fe, Cu^, Zn?, As, Pb, Sr, Sn	Cu-based pigment, lead-tin yellow, *As*-based pigment
PSB1	560 M, 700-710 m	Copper acetate (verdigris)	Ca, Fe, Cu^, Pb, Sr, Sn	Cu-based pigment, lead-tin yellow, lead white
PPB1	450sw, 570 s, 460mw	Yellow-brown ochre, small amounts of blue/green pigment (azurite?)	Ca, Fe^, Cu^, Pb^, Sr, Sn^	Cu-based pigment, lead-tin yellow, lead white, iron oxide (ochre/earth)
LPS1	450sw, 590–600 s	Ochre	n.a.	n.a.
LPS3	460 M, 510 m, 530Mw, 540–550 m, 600–620 s	Madder-type lake	n.a.	n.a.

3.7. Brown, black and white pigments

The only detected black pigment was carbon black. Various iron oxides were used by Lotto; some of them are Mn-rich (raw umber), as XRF analyses indicate. Only in a few paintings also Zn-rich brown earths could be found, with a Zn/Fe ratio usually lower than 0.2 [27]. The presence of zinc in iron oxides pigments can be attributed to zincite, a mineral, which colour varies from yellow to red; it can be found in mines of the valleys over Bergamo (Val Brembana) [28]. Zn-rich earths were detected in brown areas of paintings by Giovanni Battista Moroni of Bergamo (1520/24–1579) as well as in some Venetian and Central Italian paintings [29].

In the polyptych of St. Dominic, Zn amounts were detected by XRF where Fe content is higher, and according to vis-RS spectra [30] zinc white (ZnO) appears to be absent. This can indicate a Zn-rich earth, also if a strong Fe-Zn correlation is absent (Zn/Fe ratio varies from 0.1 to 0.5); data were taken before and also after cleaning treatment [27].

In the layer 3 of the sample PL2 (Fig. 4b, Table 3, Fig. S5, Supporting material), taken before restoration, in the layer 3 containing orpiment/realgar, the intensity of Zn and Fe is the same. In this case the layer was not original, since rutile and pseudobrookite were detected by Raman analysis, which suggested that Zn could be related to galena (blende-sphalerite) or to the presence of zinc white used in previous restoration treatments.

This and other studies pointed out that the presence of orpiment/realgar, a pigment widely used in 16th century and scarcely ever in later paintings, is not a good criterion to consider an external layer as original: in this case it is introduced later, as well as in other areas of the same polyptych (central panel, St. Gregory's arms).

Lead white was found almost everywhere in the examined painting layers, but in lower amounts where arsenic sulphides were also present. Lead white exhibits a double Raman peak at 1054 and 1046 cm^{-1}, being a mixture of cerussite and hydrocerussite (Figs. 10; S5, S8, S9, Supporting material). The second one undergoes a slow carbonation process with time, confirming the aging of lead white.

Calcium carbonate was frequently employed (as a filler or extender) inside the layers, a process that seems quite typical of Lotto and perhaps not so infrequent at that age.

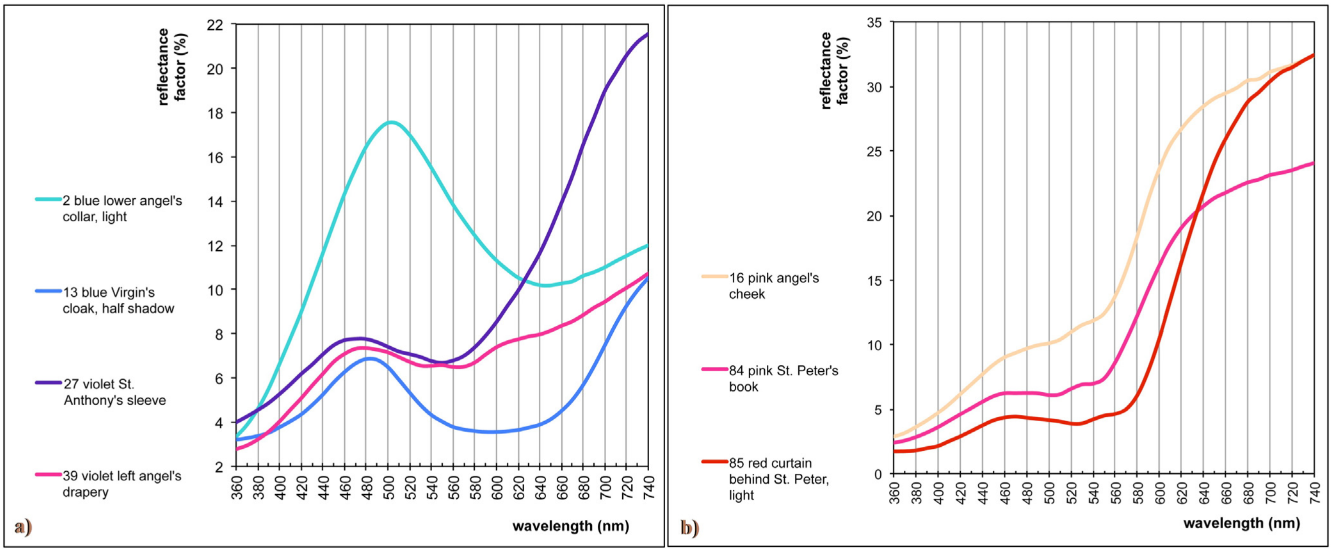

Fig. 9. Vis-RS spectra: a) Blue and violet colours from the San Bernardino altarpiece. Pigments identified: 2 = azurite, 13 = lapis lazuli, 39 = azurite with carmine-type red lake, 27 = lapis lazuli with madder-type lake; b) Red colours from the San Bernardino altarpiece. Curve 85 shows the presence of a carmine type lake (bands at 520–530 nm and 570 nm); curves 16 and 84 are related to madder-type lake (bands at ab. 510 and 550 nm).

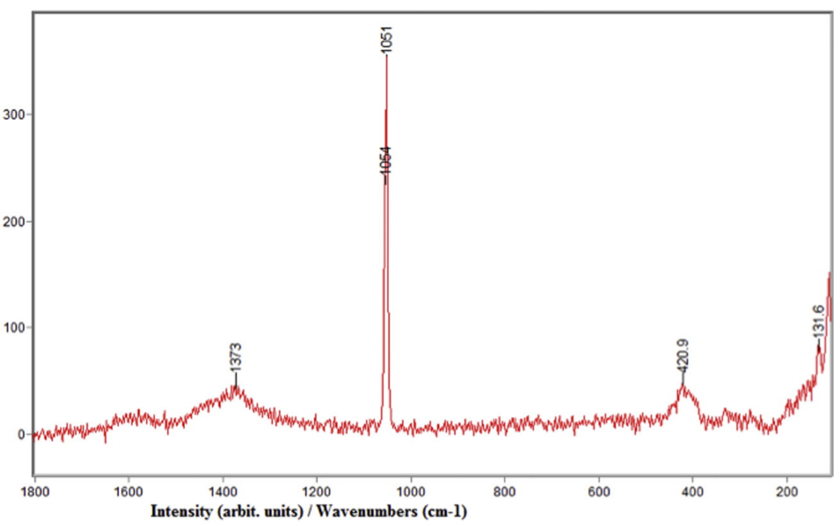

Fig. 10. Micro-Raman spectra of sample PSB1, layer 4: hydrocerussite (white lead) and cerussite.

3.8. Flesh tones

The flesh colour of female figures consisted of lead white coloured by vermilion, with particles of red lake in some cheeks areas, while red and brown ochres were added to lead white for males, as XRF and vis-RS could ascertain. Shadows were usually obtained using thin layers of brown earth.

3.9. Organic materials

The FT-IR spectra of the samples TR1, TR2 and TR3 (*Transfiguration of Christ*) show typical bands of lipidic/terpenic material (2924, 2851, 1708 cm^{-1}), and carboxylate (1522 cm^{-1}) (Fig. 11).

Samples collected were investigated also by GC–MS. The samples did not show relevant signal connected to amino acids, excluding the presence of proteinaceous binders. Lipidic fraction gas chromatograms of samples TR1, TR2 and TR3 are characterized by dicarboxylic acids, particularly azelaic acid, and saturated fatty acids, principally palmitic and stearic acids. The identification of the source of the lipid materials was based on the parameters A/P (ratio between azelaic acid and palmitic acid) and P/S (ratio between palmitic acid and stearic acid) [3]. Mills established this approach for the first time in 1996 [31]. The presence and the amount of azelaic acid, whose formation is due to the oxidation of unsaturated acids, suggests that the observed lipidic fraction can be attributed to siccative oil. Concentration ratios between palmitic/stearic acid revealed the presence of linseed oil [9].

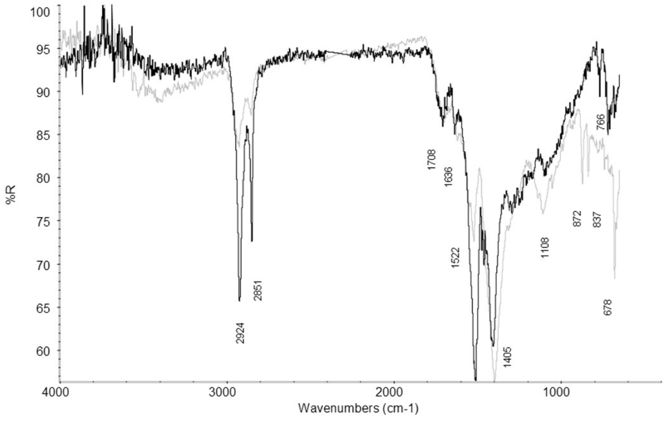

Fig. 11. FTIR spectra of sample TR1 (black line) and of a dammar resin sample (grey line).

Samples TR4, TR5, TR7 and PL1 were analysed by mean of HPLC-HR-ToF-MS in order to investigate the lipidic and resinaceous binders. In all examined samples azelaic, palmitic and stearic acids were detected, indicating the presence of siccative oil. In samples TR5 and TR7 the Azelaic acid/Palmitic acid (A/P) ratio perfectly confirmed this hypothesis since calculated values were respectively 1.1 and 2.4. Samples TR4 and PL1 showed instead an A/P ratio of 0.4–0.5.

The resins research was quite laborious due to the small amounts available for the analysis (with the exception of the sample TR7, significantly greater [32,33]. All the resins markers detected are reported in Table 7.

The first considerable result emerged from the study is that all the samples showed the same composition of the terpenic components. In the whole samples both diterpenic and triterpenic resins were detected. On the base of the detected compounds the triterpenic ones were identified as belonging to dammar. As this resin started to be employed in Europe from 1829, it is clear that it was not an original material but a later one, probably applied during a restoration intervention. The diterpenoids content revealed the use of sandarac or copal. Unfortunately, these two resins exhibit a similar chemical composition that made impossible (under the employed conditions) to distinguish them in a real sample.

The presence of *Pinaceae* resins (like colophony, Venice turpentine etc.) could be excluded in all samples since they are always marked by high content of abietic acid, which was not found in the analysed samples.

4. Conclusions

The chemical-physical investigations carried out in the present study allowed a comprehensive characterization of constitutive materials used for the execution of five paintings by Lorenzo Lotto, one of the most important artists of the Italian Renaissance in the first half of 16th century. The restoration of these paintings, corresponding to the most significant years (1508 to 1522) of Lotto's evolution, allowed to experiment the combination of a wide set of non-invasive and micro-invasive tools to assess the correct analytical procedure for the study of his technique, showing also limits and potentials of each single method. The followed protocol allowed to minimize the number of samples from each artwork.

Table 7
List of terpenic compounds identified in the samples by means of HPLC-HR-TOF-MS.

Dammar	Formula	*m*/*z* Molecular Ion	TR4	TR5	TR7	PL1
Ursonic acid, oleanonic acid	C30H46O3	$[M\text{-H}]^{-}$: 453,33,509	☐	☐	☐	☐
Oleanilic acid	C30H48O3	$[M\text{-H}]^{-}$: 455,34,864	☐	☐	☐	☐
11-Oxo oleanonic acid, 11- Oxo- ursonic acid	C30H44O4	$[M\text{-H}]^{-}$: 467,31,560	☐	☐	☐	☐
Lactonic oxidation product of dammar enolic acid	C27H42O4	$[M\text{-H}]^{-}$: 429,29,487			☐	☐
Shoreic acid	C30H50O4	$[M\text{-H}]^{-}$: 473,36,071			☐	☐
Nor-α-amyrone, Nor-β-amyrone	C29H46O	$[M + H]^{+}$: 411.36214		☐	☐	☐
Dammaradienol	C30H50O	$[M + H]^{+}$: 427.39344	☐	☐	☐	☐
Hydroxydammarenone, hydroxyhopanone	C30H50O2	$[M + H]^{+}$: 443.38836			☐	☐
3-Oxo-25,26,27-tisnor ammarano-24,20-lactone	C27H42O3	$[M + H]^{+}$: 415.32067				
3-oxo-25,26,27-trisnor dammarano-20,24-lactone			☐	☐	☐	☐
17-Hydroxy-11-oxo-nor-b-amyrone	C29H44O3	$[M + H]^{+}$: 441.33632				
17-Hydroxy-11-oxo-nor-a-amyrone			☐		☐	☐
Sandarac/copal	Formula	*m*/*z* Molecular Ion	TR4	TR5	TR7	PL1
Pimaric acid, Communic acid, Sandaraco pimaric acid	C20H30O2	$[\text{M-H}]^{-}$: 301,22,028	☐		☐	☐
Dehydroabietic acid	C20H28O2	$[\text{M-H}]^{-}$: 299,20,273	☐	☐	☐	☐
Agathalic acid	C20H30O3	$[\text{M-H}]^{-}$: 317,21,026			☐	
Agatholic acid	C20H32O3	$[\text{M-H}]^{-}$: 319,22,591	☐	☐	☐	☐
Agathic acid	C20H30O4	$[\text{M-H}]^{-}$: 333,20,713	☐	☐	☐	☐
Turolosic acid	C18H32O3	$[\text{M-H}]^{-}$: 295,22,787	☐	☐	☐	☐
Sandaracinic acid	C22H34O5	$[\text{M-H}]^{-}$: 377,22,995	☐		☐	☐
Sandaracinolic acid	C24H36O3	$[\text{M-H}]^{-}$: 371,2450	☐	☐	☐	☐
7-oxo-dehydroabietic acid	C20H26O3	$[\text{M-H}]^{-}$: 313,18,092	☐	☐	☐	☐
7-hydroxy-dehydroabietic acid	C21H30O3	$[\text{M-H}]^{-}$: 329,21,243	☐	☐	☐	☐
Sandaraco pimarinol	C20H32O	$[M + H]^{+}$: 289.25259		☐	☐	☐
Sandaraco resene	C22H36O2	$[M + H]^{+}$: 333.27881	☐	☐	☐	☐

The use of Lotto's typical preparatory ground on canvas and wood panels, made of gypsum, was confirmed. Sometimes calcium and magnesium carbonate, as well as earths, were also detected. Perhaps the introduction of earths inside the ground was believed to make the ground more elastic than a simple calcium sulphate one.

An opaque white or off-white priming layer was also found sometimes above the ground: white coloured when made by fine lead white pigment particles, pale yellow when made by a mixture of lead white and lead tin yellow (type I), occasionally mixed with a few black particles or coloured pigments, including red lead (minium) and saffron. Lead tin yellow and glassy particles were also added, probably in order to accelerate the drying oil.

The obtained results considerably increase information about Lotto's pictorial technique. Lotto's broad palette consisted mainly of traditional pigments, quite common for his period especially in Venice, such as lead white, azurite, lapis lazuli (ultramarine), verdigris, lead-tin yellow type I, litharge, realgar, orpiment, vermilion, red ochre, hematite, red carmine-type lake and madder lake, carbon black, more rarely red lead and yellow lake (saffron-based). Pigments were bound in an oil medium. Quite peculiar was, in some cases, the way he used them, in terms of mixtures and sequences, like for areas containing blue or red lake pigments.

The extension of non-invasive examinations to about 50 paintings by Lotto, spanning from 1505 to around 1556, helped to verify the evolution in the use of some pigments, like yellow ones, where Pb-Sb yellow was used alongside Pb-Sn, as confirmed by XRF. The presence of Zn in some paintings was also discussed.

In particular, reflectance spectroscopy (vis-RS) confirmed to be an invaluable tool to identify many pigments, especially blue, red and orange ones, also in some mixtures. It could distinguish two classes of red lakes, that Lotto used alternately also in the same painting, sometimes in the same area. The joined use of vis-RS and XRF allowed the characterization of blue pigments layering (ultramarine over azurite), of violet hues where red lakes were mixed with ultramarine and azurite and of green colours obtained mixing a blue pigment with a yellow one.

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