Highlights

- A multi-technique approach is used to study 14th-century polypytch
- Lapis lazuli confirms wealthy commission of the painting
- No original varnish, but coatings due to later restorations are found
- Traditional egg tempera and simple stratigraphy are the secrets of the exquisite pictorial quality

Microchemical and microscopic characterization of the pictorial quality of egg-tempera polyptych, late 14th century, Florence, Italy

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Abstract

This paper explores the added value of micro-chemical and microscopic approaches to gather scientific evidence that can technically explain the pictorial quality of an egg-tempera painting, and underpin assessments that otherwise would be based on naked eye observations only. Demonstration is here provided via the interdisciplinary investigation of the original technique used by Giovanni del Biondo in the polyptych *Annunciation and Saints* (1385 ca), Galleria dell'Accademia, Florence, Italy. The exquisite surface appearance makes this panel painting remarkable compared to artworks by coeval artists. Imaging techniques (UV, IR and IR false color), non-invasive single spot techniques (XRF and FORS spectrometry) and analytical investigations on eight selected micro-samples (ATR-FTIR, GC/MS and Py/GC-MS, ESEM-EDS) were combined to retrieve the palette and identify organic binding media and superficial coating

layer. Stratigraphic and micro-chemical data confirmed the use of a relatively simple egg-tempera technique applied on a ground made of gypsum mixed with animal glue, without complex stratigraphic superimposition of preparation and pictorial layers. Various pigments were identified, among which the precious lapis lazuli. While Py/GC-MS highlight that the coating is made of dammar resin and honey mixed with animal glue, the results allow us to state that the painting was not intentionally varnished by Giovanni del Biondo. These outcomes shed a new light on the technical knowledge of this polyptych, and prove how challenging is the attempt to categorize egg-tempera recipes used by ancient painters at the turn of the 14th century.

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Keywords

Paint cross section; GC/MS; ESEM-EDS; ATR-FTIR; egg tempera; coating

1. Introduction

In the professional practice, art historians and scholars of artistic techniques refer to the pictorial quality among the main observable features of a painting, to infer the technical procedure followed by the artist. The appearance of exquisite painted surfaces as they are seen with the naked eye can sometimes lead to the incorrect idea that they were created by using a sophisticated and elaborated technique, and/or mixing particular or even unusual painting materials. If not underpinned by scientific evidence, such approach can affect not only the assessment of the painting itself, but also the interpretation of its value in the context of the historical evolution of the painting techniques.

It is this latter key aspect that this paper aims to address based on the results of combined micro-chemical and microscopic investigations to study the *Annunciation and Saints* by Giovanni del Biondo (1356-1398 ca), which is

currently exhibited at the first floor of the Galleria dell'Accademia in Florence, Italy (Figure 1). The altarpiece was made for the Cavalcanti chapel in the sacristy of the Florentine church of Santa Maria Novella and it was probably commissioned by Andreola Acciaioli, widow of Mainardo Cavalcanti who died in 1380.

This large polyptych (406 cm x 377 cm) is dated around 1385, in a period when it is still debated whether and what binding media other than egg yolk were used in tempera panel paintings.

In this regard, the *Annunciation and Saints* is remarkably different from those paintings produced by coeval artists active in Florence, and even if compared with the rest of Del Biondo's production. Although this master is known for his adherence to the instructions given by Cennino Cennini in his *Libro dell'Arte* [1] and other technical textbooks [2], the surface of this painting looks matte rather than glossy. It seems to recall peculiar effects of medieval illumination and mural paintings, instead of traditional egg yolk tempera.

Figure 1 (a) Giovanni del Biondo, *Annunciation and Saints*, 1385, Galleria dell'Accademia, Florence; detailed images (white squares) on (b) Angel Gabriel and (c) Saints in the left panel, where the pictorial quality of the painting is appreciable. In the right panel, the yellow squares show the areas zoomed in Figure 2 where the paint surface was covered under the twisted columns of the wooden carpentry.

The good condition of most of the painted surface is a further proof of the durable quality of the technique used by the artist.

According to the official records, the Opificio delle Pietre Dure (OPD) of Florence carried out a restoration from 1971 to 1982, which only involved the wooden structure of the panel. Whereas, we can only hypothesize that few and soft interventions on the surface were executed between 1800 and 1827, when the painting was transported from Santa Maria Novella to the Convent of San Marco in Florence (1808-1810), and then was transferred to Galleria dell'Accademia before 1827.

The limited amount of interventions and retouching helped to preserve a nearly original painted surface. In early 1980s, Umberto Baldini, at that time OPD Director, commented with appreciation the degree of conservation of the painting, specifically mentioning the lateral areas of the painted surface that was protected by the twisted columns of the carpentry [3].

In late February 2013 the twisted columns were temporarily removed to expose the painted surfaces along the edges of the three panels (Figure 2). The visual inspection of the exposed surfaces does not provide a clear evidence of an oil/resin-based varnish, except for a brown glue-like coating. This coating is distributed over the painted surface but not under the twisted columns, and no mention about when this coating was applied is found in the archival documents. Therefore, it is more likely that the coating is due to past restorations, presumably carried out after the addition of the wooden frame.

In this context, the two main scientific questions were related with (1) the original materials and technical procedure used by Giovanni Del Biondo to obtain such an uncommon exquisite effect of the painted surface; and (2) the composition of the brownish external layer.

Figure 2 Details of the paint surface, on the bottom side of the right panel of *Annunciation and Saints*, after and before the removal of the twisted columns (cf. Figure 1 for location).

To this purpose an integrated analytical procedure was followed in line with the practice increasingly used across the heritage science community [4]. Imaging techniques including UV fluorescence (UV), IR reflectography (IR) and IR false color (IRFC) [5,6] were implemented to retrieve a comprehensive visualization of the external layer, palette, retouches and later additions. Portable Fiber Optics Reflectance Spectroscopy (FORS) [7] and X-Ray Fluorescence (XRF) [8,9] spectrometry provided both molecular and elemental in-situ characterization of distributed areas sampled over the painted and gilded layers. Based on the results obtained by the non-invasive approach eight micro-fragments were sampled from pre-existing *lacunae* in the right and central panels, by collecting both preparation and painted layers and documenting the surface at high magnification with digital portable microscopy (DM). Optical microscopy (OM) and Environmental Scanning Electron Microscope coupled with Energy Dispersive X-ray (ESEM-EDS) analyses in cross-section aimed to identify the stratigraphy and inorganic composition of all the layers. Fourier Transformation Infrared Attenuated Total Reflection spectrometer (ATR-FT-IR) [10,11] and a gas chromatograph (GC) equipped with a single golden quadruple mass spectrometer (MS) operated in Electron Ionization (EI) mode were used to elucidate the nature of the organic compounds. Lastly, pyrolysis (PY), using

hexamethylazane as derivatiser, followed by GC/MS, was exploited to confirm and/or supplement the data related on the organic constituents obtained by the GC/MS analysis of the paint fragments [12].

The above workflow therefore aimed to gather the scientific evidence that, at micro-scale, could justify the macro-scale quality observable with the naked eye. This allowed us to contextualize correctly the *Annunciation and Saints* in respect of tempera techniques used in Florence at the turn of the 14th century.

2. Materials and Methods

2.1 Imaging techniques, portable XRF and FORS

UV Images were acquired with a camera (Canon EOS 400D) supplied with gelatin filter series (Kodak Wratten No. 2) coupled with a glass filter (B + W Digital UV / IR). A tungsten filament lamp of 300W, covered with a gelatin filter (Kodak Wratten 87C) was employed for the acquisition of the IR images. These images were processed in Raster graphic editor: IR channel was substituted to B (Blue) channel in RGB images, resulting in the so-called InfraRed False Color images (IRFC).

A portable XRF spectrometer (ALPHA series 4000, InnovX) equipped with micro X-ray tube and a tantalum anode was used for a 2-step analysis, in two different excitation energies: 30KeV - 6.5 uA - 2mm aluminium filter for the determination of heavy elements and 15KeV - 7uA - 0.1 mm aluminium filter for light elements. The beam diameter was 4 mm, the shooting area was approximately 155 mm^2 and the acquisition time was 120 s. *Kα* emission has been considered for the qualitative evaluation of the elements, while only for mercury and lead we considered *Lα* emissions.

FORS measurements were performed with an Ocean Optic (*mod. HR2000*) spectrophotometer equipped with optical fibers and a tungsten lamp as a light source. A measurement head with illumination at 0° and signal collection at 45° allowed acquisition of reflectance spectra from an area of approximately 2 mm^2. Each spectrum is the average of 30 scans. A plate of Spectralon® with 99% of reflectance served as reference. The identification of pigments was performed by comparing the obtained spectra with an ICVBC homemade database.

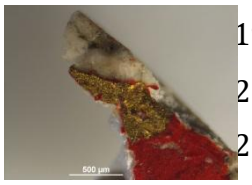
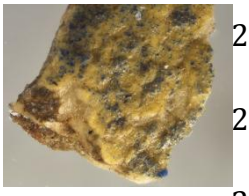
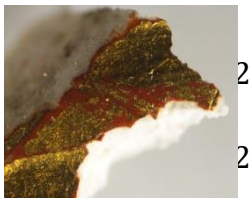
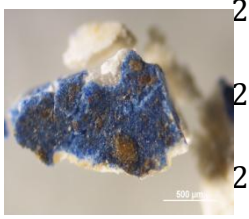
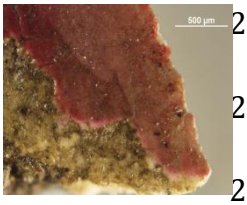
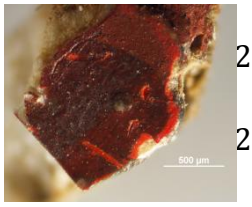
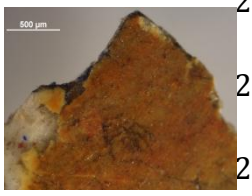
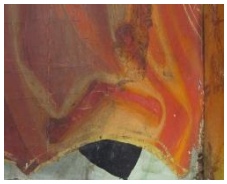
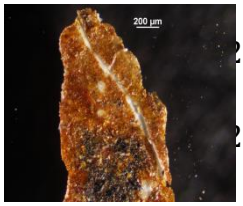
2.2 Samples, sample preparation and optical microscopic techniques

Eight fragments were sampled from the edges of existing lacunae in the central and right panels of the polypytch, in close collaboration with the restorers of Galleria dell'Accademia (Table 1). After a complete photographic documentation of the samples taken, cross sections were prepared embedding the fragments in epoxy resin (*Epofix resin*, Struers). After curing, the cross sections were polished using silicon carbide paper with different grind sizes (P200, P400, P800, P1200) so as to allow transversal observation and analysis of the different layers.

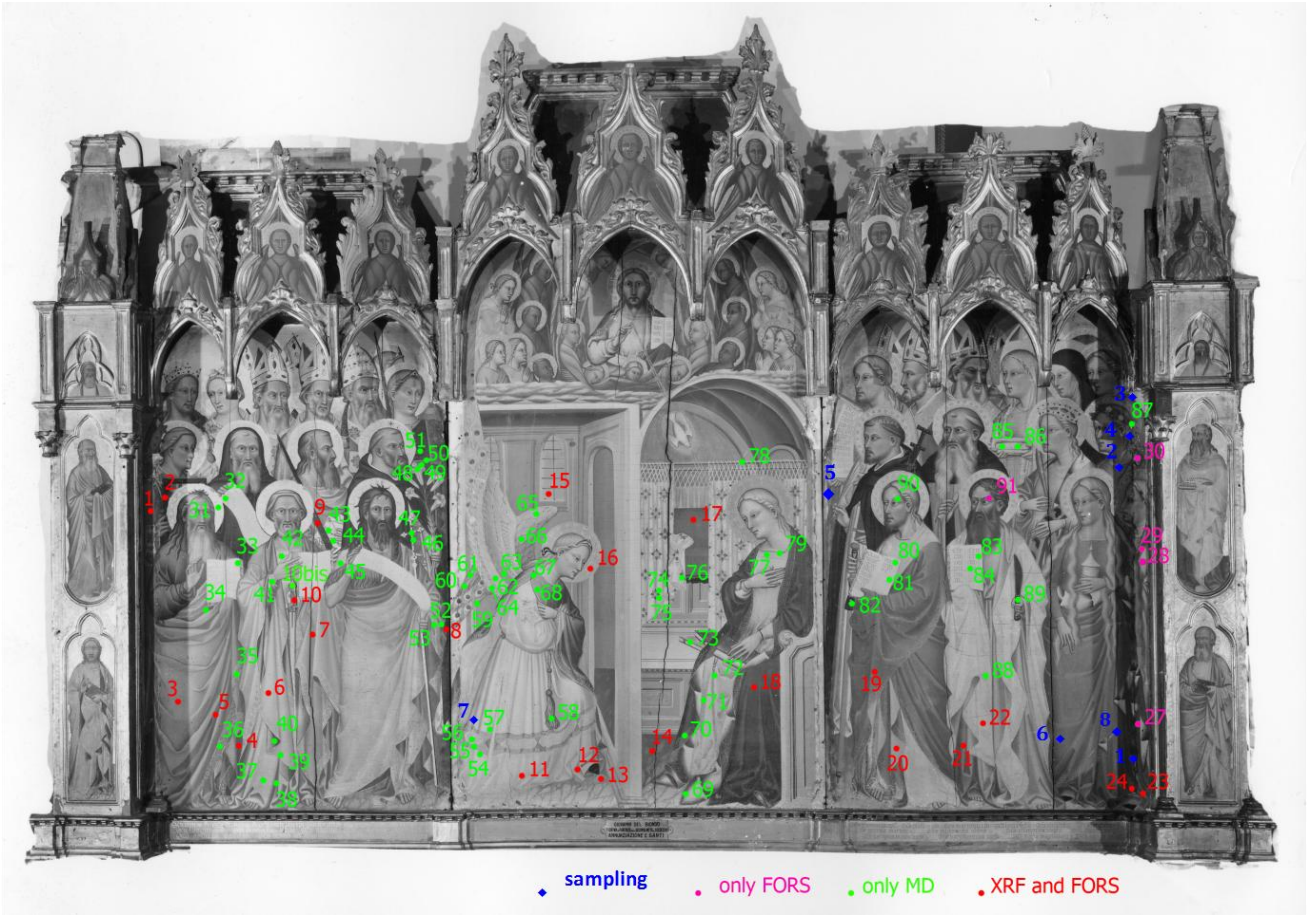
Under VIS and UV light, orthoscopic observations of cross sections were performed with an optical microscope Zeiss Axioplan equipped with objectives from 4x to 200x. The cross-sections were observed and photographed with a Nikon Eclipse 600 light microscope, equipped with an UV source (λ_{exc} 330-380 nm), to characterize their morphology, identify the stratigraphic sequence and detect materials fluorescent under UV light.

2.3 ESEM-EDS

A Quanta200 FEI/Philips Electron Optic microscope equipped with an EDS microanalysis system was used for ESEM measurements. Operating in *low vacuum* mode, it was possible to perform SEM measurements without metallizing the samples. Images were constructed from backscattered electrons. The elemental composition of the different layers was obtained with a primary electron beam of 25 keV, pressure 0.5 Torr, lifetime >50s, and CPS≈2,000; the acquisition software is equipped with a ZAF correction procedure for semi-quantitative analyses of the X-ray peaks.

	Sampling area		Fragment	
S1 Right Panel		gilding on red painted layer		199 200 201
S2 Right Panel		Green		202 203 204
S3 Right Panel		Gilding decoration		205 206
S4 Right Panel		Blue		207 208 209
S5 Right Panel		Pink		210 211 212
S6 Right Panel		Red		213 214
S7 Central Panel		Orange		215 216 217
S8 Right Panel		Coating		218 219 220

221 **Table 1** Description and pictures of the sampling points over the painting and the
222 collected fragments (OM documentation).



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224 **Figure 3** Giovanni Del Biondo, *Annunciation and Saints*. The red spots coincide with the areas analyzed with both XRF and FORS, while the pink ones
225 mark the supplementary FORS spot analyses. Green spots indicate the areas photographed with digital microscope (MD) and the blue spots are the
226 eight areas from which the samples (see Table 1) were taken.

2.4 Mid-ATR-FTIR

A commercial spectrometer *Spectrum System 2000 Perkin Elmer* coupled with a single bounce diamond ATR cell, Specac Golden Gate *GS 10500* was used for the Mid-ATR-FTIR analysis. Spectra were registered in the range of 4000–500 cm^{-1} with spectral resolution of 2 cm^{-1}. Each sample spectrum was the average of 32 scans. When necessary, the baseline adjustment, smoothing and normalisation were performed with *Spectrum 5.3, Perkin Elmer Inc.* software package.

Before the analysis, each layer of the sample was manually separated, with the aid of a scalpel under the microscope aiming to separate the different levels. Nevertheless, peaks of the ground strongly overlapped with those of binder and pigments. This represented a limitation of FTIR which provided information on the class of the organic compounds only, and not enough discrimination to identify pigments. For the data interpretation the spectra were compared with those of reference materials obtained from the same instrument. In addition, electronic spectra databases, such as IRUG [13], and spectra libraries on books were used [10,14].

2.5 GC/MS and Py/GC/MS

The analytical procedure based on GC/MS used for the analysis of lipids, terpenoid resins, proteinaceous materials and saccharides has been already reported in literature and is nowadays well regarded technique across the heritage science community for detection and identification of organic components of paintings [15,16,17]. The procedure involves an Omix C4 tip step for the separation and purification of the proteinaceous from the saccharide materials and clean-up purification with PTFE filter and a double-exchange resin of the saccharide fraction.

The different fraction were analyzed by means of a 6890N GC System gas chromatograph equipped with PTV injector that was coupled with a 5975 single golden quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer was operated in the EI positive mode (70 eV) and the mass range was from 50 to 750 m/z. Chromatograms were acquired both in total ion chromatogram (TIC) mode and selected ion monitoring (SIM) mode [15].

Five microliters of hexamethyldisilazane (derivatisation agent) were added to a few μg of samples into a cup and then the sample was inserted into the chamber of the EGY/PYP3030D pyrolyzer (FRONTIER LAB). The pyrolisis temperature was 550°C and the interface temperature 280° C. The pyrolisis chamber was connected through a PTV injector to a 6890N GC System gas chromatograph coupled with a 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA) single quadrupole mass spectrometer [18].

3. Results and Discussion

3.1 Preparation layer

Samples S1 and S4 provided information on the preparation layer of the painting. In both samples SEM-EDS analysis of the ground highlighted the presence of calcium (Ca) and sulphur (S) consistent with the presence of gypsum ($CaSO_4.2H_2O$) (Figure 4). This result matches with the FTIR determination on selected samples. Strontium (Sr), as minor component, was also observed in the EDS spectra. This chemical element is sometimes found in the grounds and it can be considered an impurity that may be present both as vicariant of calcium in the gypsum or as Celestine ($SrSO_4$) [19]. In this case the crystal morphology suggests that strontium would be present as Celestine as an impurity of the gypsum.

The BSE image of sample S4 (Figure 4a) clearly shows the presence of two different ground layers, the lower made of *gesso grosso* (coarse-grained) and the upper made of *gesso fine* (fine-grained). This is in agreement with Cennini's description concerning the preparation technique of the ground which was commonly used by painters at that time in Florence [1,20].

The painted layer is well separated from the ground, although there is no clear evidence for the presence of *imprimatura* as a thin layer applied to reduce the absorbency of the preparation.

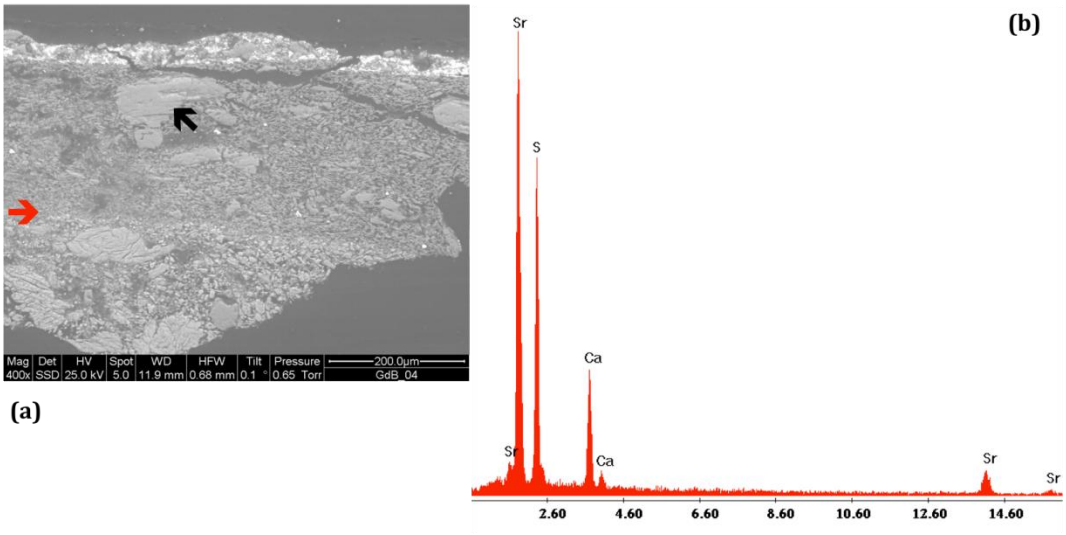


Figure 4 (a) Backscattered electron image (BSE) of sample S4. The red arrow indicates the interface between the *gesso grosso* and *gesso fine* layers within the ground. (b)EDS spectrum of the point indicated by the black arrow in picture a). The elemental composition suggests that the particle is made of Celestine ($SrSO_4$).

In FTIR spectra of grounds, the strong bands of gypsum made difficult to identify other materials (Figure 5). The main gypsum peaks are: a double broad peak from 1100 to 1250 cm^{-1} and two narrow peaks at 600 and 700 cm^{-1} (SO_4^{2-}) asymmetric stretching and bending vibration, respectively), a broad double peak from 3400 to 3450 cm^{-1} and a narrow double at 1620 and 1690 cm^{-1} (deformation vibration and stretching vibration of O-H water bond). Due to the co-presence of organic materials, SO_4^{2-} forms a single broad peak at ≈ 1087 cm^{-1}. The gypsum O-H deformation vibration peak from 3200 to 3500 cm^{-1} is broader and more similar to that of animal glue (Figure 5b). The spectrum of ground selected from sample S1 is directly compared with the animal glue reference spectrum, highlighting the following similarities in peak patterns: broad peak from 3300 to 3400 cm^{-1}; double peak at 2852 and 2925 cm^{-1} and the triple pattern from 1447 to 1640 cm^{-1} which is most probably related with the amide bonds I and II [10,11].

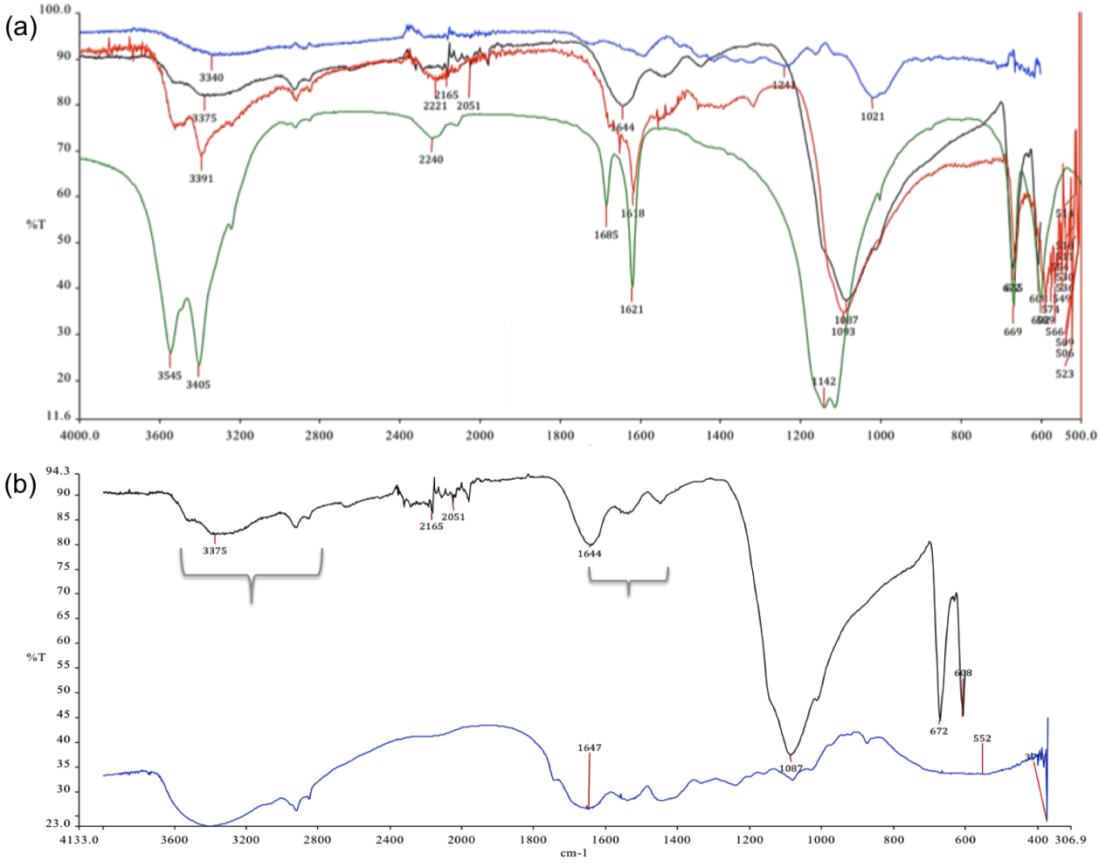


Figure 5 FT-IR spectra: (a) black: sample S1, preparation layer; blue: sample S4, preparation layer; red: sample S6, preparation layer; green: gypsum reference spectrum. (b) black: sample S1, preparation layer; blue: animal glue reference spectrum.

Because the FTIR analyses only provide a preliminary organic characterization of the sample, GC/MS analysis was crucial to unveil the nature of the binder. A fraction of the ground of sample S1 was scraped by scalpel, separating it from the pictorial layer and submitting it to the analytical procedure. For the identification of the proteinaceous materials, the relative percentage content of the amino acids (Table 2) was compared with a reference data set of 101 samples [12]. The relative percentage of the amino acid content of sample S1 (ground) is reported in Table 2 and the amino acid Single Ion Monitoring (SIM) chromatogram in Figure 6a.

%	casein	egg	animal glue	Sample S1/ground
Ala	5	7.7	12.3	12.3
Gly	3	4.8	29.4	28
Val	7.6	7.7	3.9	4.4
Leu	11.9	11	4.7	5.5
Ile	6.6	6.7	2.5	2.3
Ser	5.8	10.3	3.8	2.5
Pro	11.5	5.7	12.4	28.7
Phe	5.9	6.4	2.8	3.8
Asp	8.5	12.6	6.6	3.6
Glu	22.2	15	9.9	6.5
Hyp	0	0	7.7	2.4
µg tot	0.19	0.19	0.19	1.2

Table 2 The relative percentage content of quantified amino acids in sample S1, compared with the average content of reference materials (casein, egg and animal glue).

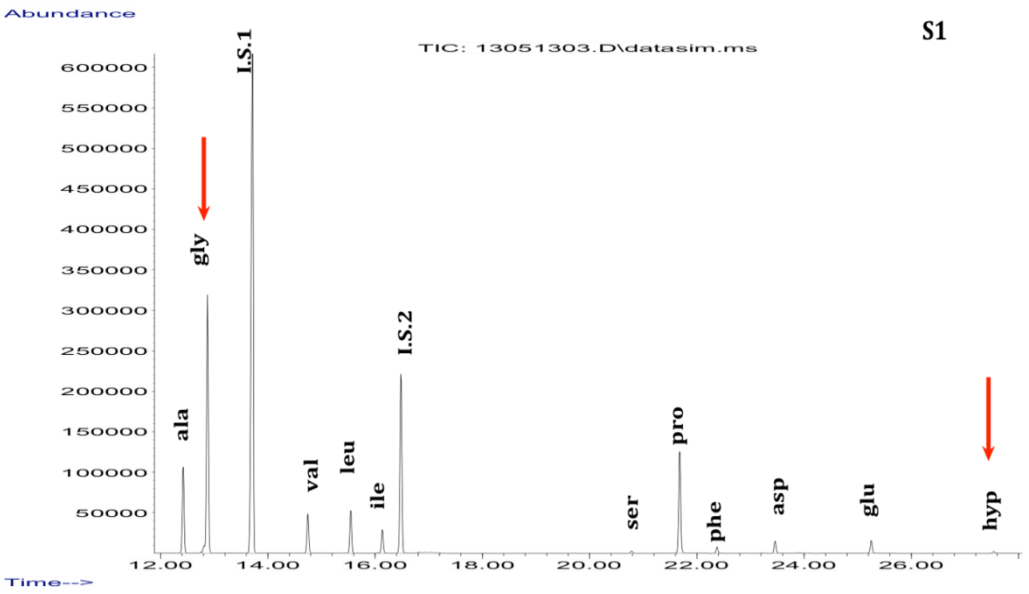
The amino acid content of sample S1, and particularly the concentration of hydroxyproline (Hyp) and glycine (Gly) which are markers of animal glue, indicates the use of the latter as the ground binder (Figure 6a and Table 2). Indeed, Principal Component Analysis (PCA analysis, plotted in Figure 6b) locates the sample S1 into the animal glue cluster.

3.2 Painting layer - pigments

The pigments used for the principal colours of the painting are lapis lazuli, minium, cinnabar, lead tin yellow and lead white.

Table 3 and Table 4 summarize ESEM-EDS analyses of all the seven painted samples (see also Table 1), and results of FORS and XRF in-situ analysis on the various areas of the painting (see also Figure 3).

(a)



(b)

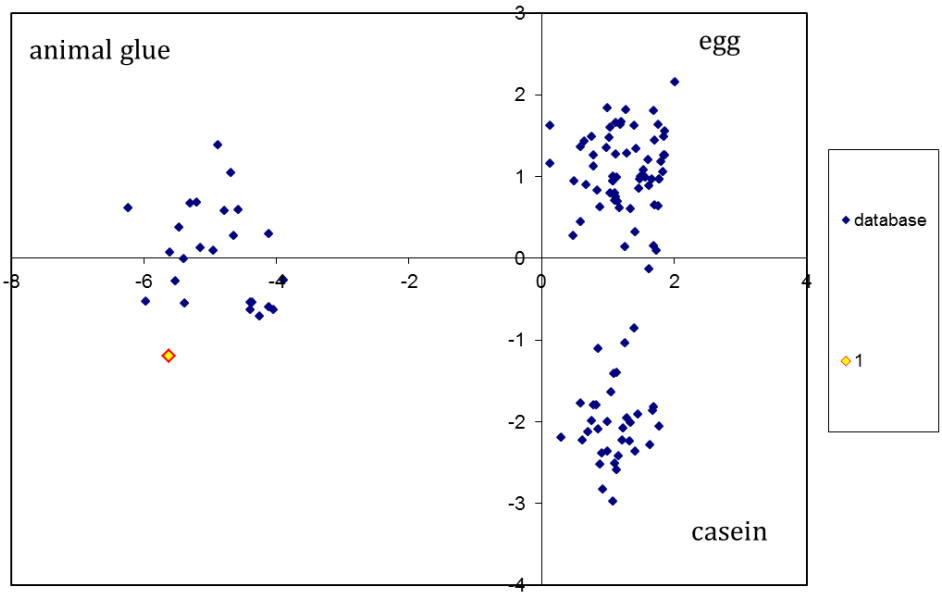


Figure 6. a) GC/MS chromatogram of the protein fraction of sample S1 (ground), acquired in the SIM mode, I.S.1, I.S.2, internal injection standard hexadecane and internal derivatization standard norleucine, respectively. (b) PCA score plot of amino acidic relative percentage content (yellow dot), compared with the reference samples database (blue dots).

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Colour	Sample	ESEM/EDS	Point	FORS
Blue	4	Si, Al, Na, Pb	18	Lapis lazuli\lead white
			19	Lapis lazuli\lead white
Green	2	Si, Al, Na, Sn, Pb	4	Lapis lazuli\lead-tin yellow
			5	Lapis lazuli\lead-tin yellow
			14	Lapis lazuli\lead-tin yellow
			21	Lapis lazuli\lead-tin yellow
Red	1	Hg, Au	1	Cinnabar
	6	Hg, Pb	12	Minium\Cinnabar\ Lead white
			24	Minium\Cinnabar\ Lead white
			17	Cinnabar
Yellow\Orange	7	Pb, Sn	3	Lead-tin yellow
			20	Lead-tin yellow
Black		//	2	//
		//	9	//
Gold	1	Au	10	//
	3	Au, Fe, Al, Si, Mg	15	//
			16	//

353
354 **Table 3** ESEM-EDS and FORS analyses of the painted samples. For location of the areas
355 sampled over the painting see red spots in Figure 3.
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		XRF						
		Fe	Cu	Ag	Sn	Hg	Au	Pb
Color	**Point**							
Blue	18	*	traces					****
	19	*						****
Green	4	*			*			****
	5	*			*			****
	13	**	*					****
	14	**			traces			****
	21	traces			*			****
Red	1	*				****		**
	12	traces			traces			****
	23	traces			traces		*	****
	24	*				****		***
	17	*				****		traces
Yellow	3	*			**			****
	20	traces			*			****
Black	2	****	traces	***				*
	9	****		***				*
Gold	15	***					****	traces
	16	***					****	traces

Table 4 XRF in-situ analyses of the areas of the painting the location of which is marked by red spots in Figure 3.

In IRFC images, almost all the blue and green areas of the painting appear with the characteristic light red color of lapis lazuli $(Na, Ca)_8(AlSiO_4)_6(S, SO_4, Cl)_{1-2}$ (Figure 7b).

The predominant presence of lapis lazuli is also confirmed by the FORS spectra of the representative blue points 18 (Madonna's robe) (Figure 7) and 19 (Figure 3) (robe of a Saint on the right panel), which are almost identical to the reference spectra either in ICVBC database or in the on-line IFAC database [21] (Figure 8). EDS analysis of one green sample (S2) and a blue one (S4) revealed the presence of aluminium (Al) and silicon (Si) compatible with lapis lazuli composition (Table 3). In sample S4, lead (Pb) presence is due to lead white ($2PbCO_3 \cdot Pb(OH)_2$), while in sample S2 the detected lead (Pb) and tin (Sn) correspond with lead tin yellow (Pb_2SnO_4) (Table 3 and Table 4).

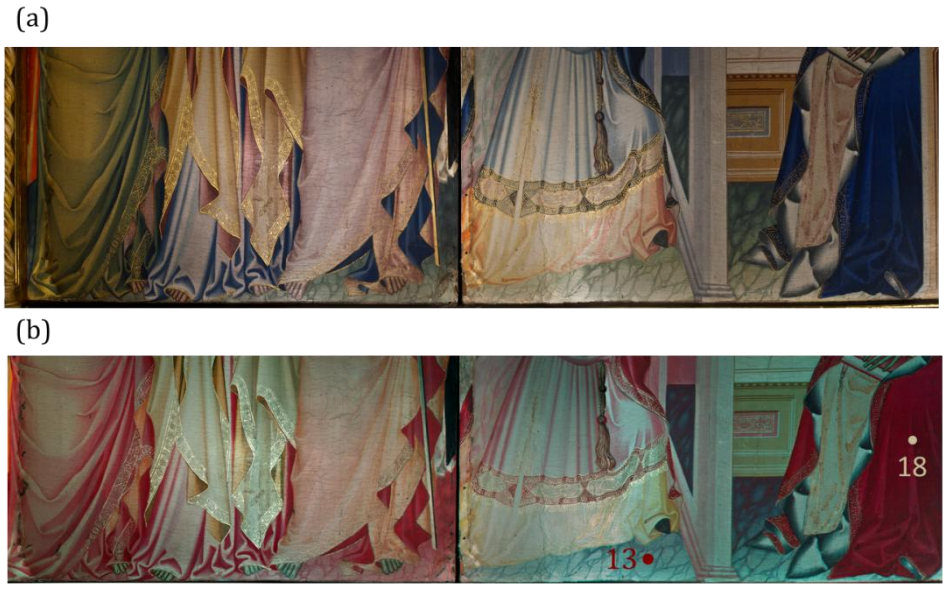


Figure 7 Left and central parts of the polypytch. (a) VIS image, (b) IR false color image. The numbers indicate the XRF and FORS spot analyses (compare with Figure 3).

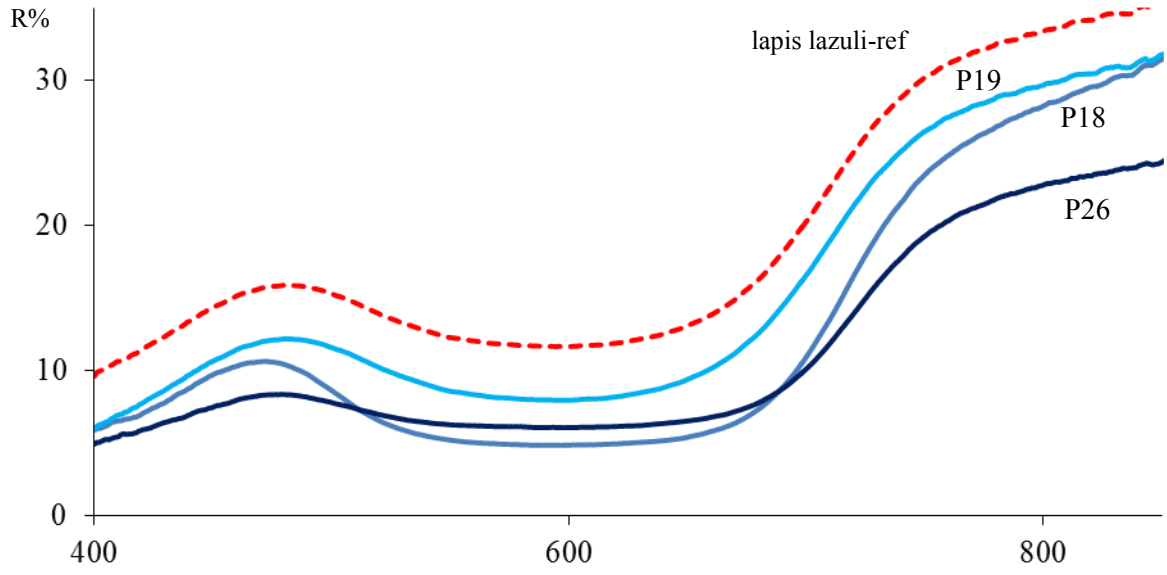


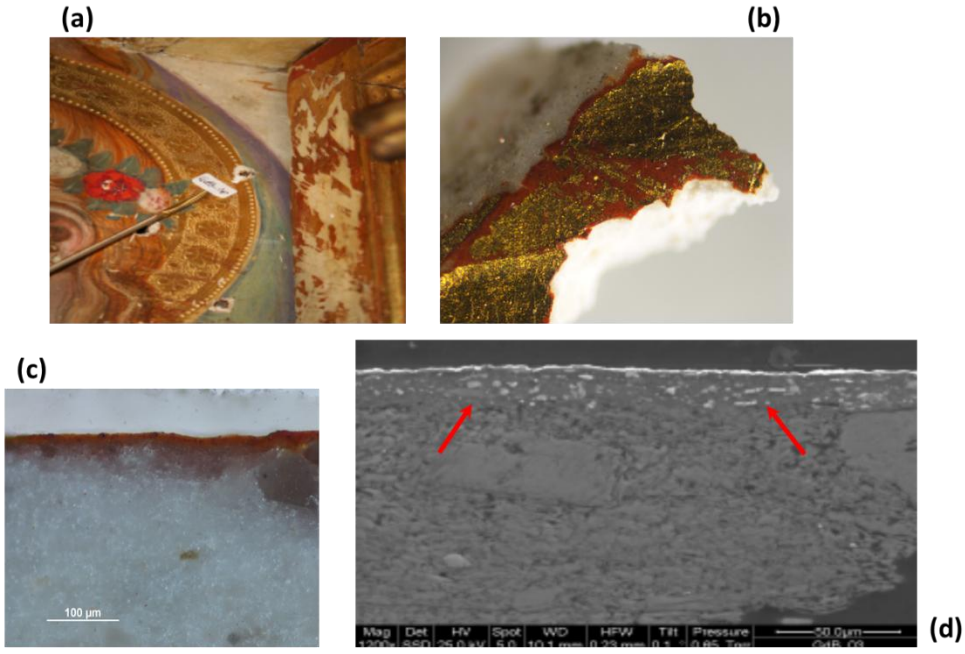
Figure 8 FORS spectra of blue points P18, P19 and P26. The red dashed curve is the reference spectrum of lapis lazuli (ICVBC database).

Based on imaging and spot analyses, no copper-based blue and green pigments were detected across the three panels. The only exception was some portions of the marble-like floor in the central panel, underneath Virgin Mary and the Announcing Angel. In IR false color image, these portions appear light blue (point n. 13, Figure 7) and not bright red, characteristic of lapis lazuli. XRF elemental analysis on the green floor of the right panel (point n. 13, Table 4) revealed a large quantity of lead (Pb) and minor amount of copper (Cu), while tin (Sn) was absent. Since the data are limited, there is no clear evidence for the use of a copper based pigment.

For the red color, Giovanni del Biondo used mainly pure cinnabar (HgS), as well as a mixture of cinnabar with minium (Pb_3O_4) intentionally applied to generate particular effects, as it is possible to observe in St Mary Madgalene's robe (Figure 2).

As expected, XRF analysis was useful for the characterization of metal leaves applied on the painting surface. Gold (Au) was detected in two gilded points (15, 16) and silver (Ag) was detected in two black areas (2, 9) (Figure 3 and Table 4). The strong signal of iron (Fe) in XRF spectrum of area 9 along with the strong signal of Fe and aluminosilicates (Si, Ca, S, Al, Mg) in ESEM-EDS spectrum of the gilded sample S3 can be related to the red layer, shown in Figure 9d, which, most probably, is *Armenian bole* .

Complementarily to the above micro-chemical information, the observations made under the optical and scanning electronic microscopes highlighted the extreme simplicity of the microstratigraphy. This is the most relevant evidence we have retrieved from the micro-stratigraphic investigation, as it is a common feature characterizing all the painted areas of the polyptych (Figure 9c-d are reported as a representative cross-section). Del Biondo did not use complex superimpositions of different layers to obtain specific pictorial effects in the painting.



412

413 **Figure 9** Sample 3. (a) Sampling area. (b) Picture of sample S3, 4x. (c) Cross section
414 (magnification 20x). (d) Backscattered image of the cross section of sample 3). The
415 white line on the top corresponds with the gold leaves and the red arrows indicate the
416 borderline between preparation and the layer between gilding and the preparation.
417

418 ***3.3 Painting layer – organic binder***

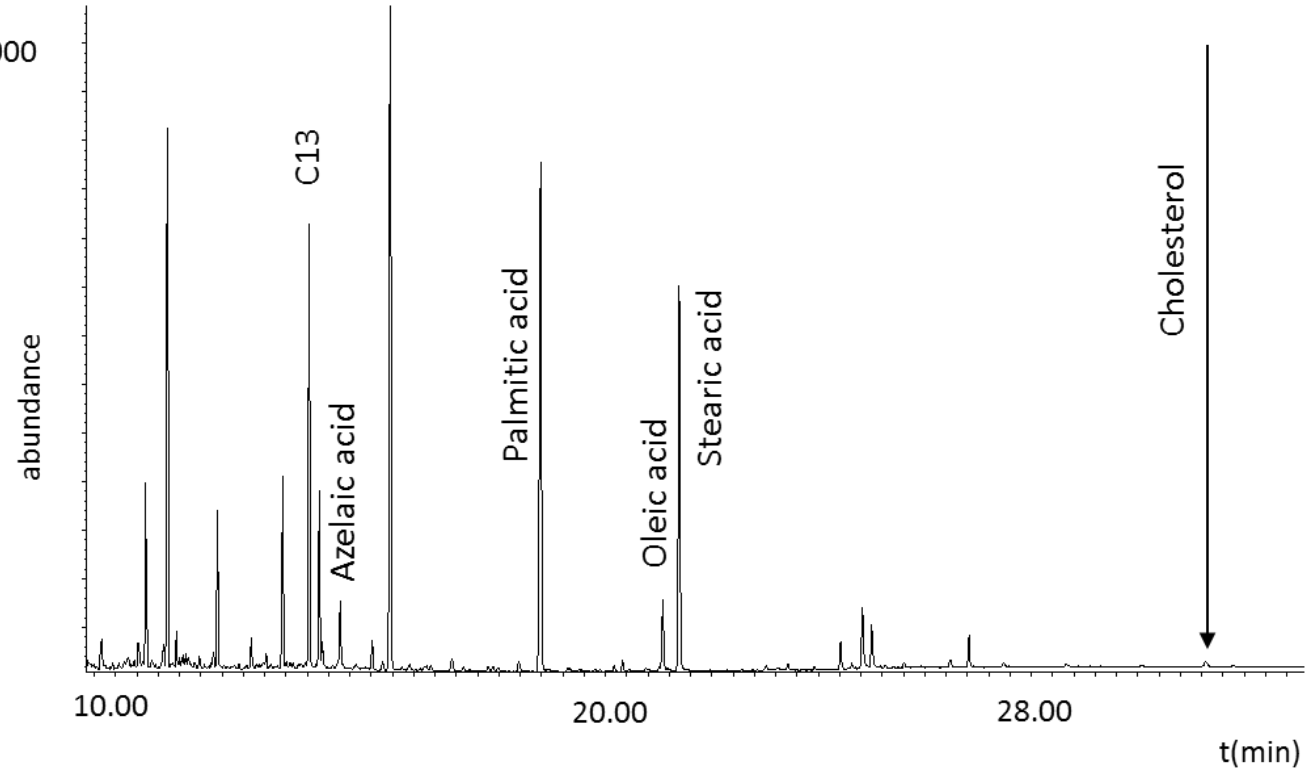
419 The GC/MS lipid resinous fractions were analyzed in order to characterize
420 the glycerolipids sources and investigate the presence of natural resin. With this
421 purpose, four samples (S1, S4, S5 and S6) were further sampled under the
422 microscope with the use of a scalpel, in order to isolate the paint layer and avoid
423 contamination from both the preparation and superficial layers. The study and
424 quantification of the monocarboxylic and dicarboxylic acids [12] have been
425 performed in order to define weather the polyptych is an oil-based painting or
426 not. Figure 10 shows the chromatogram of the pictorial layer of sample S1 as an
427 example, while in Table 5 the characteristic ratio values of the samples indicate
428 the absence of siccative oil (being the azelaic over palmitic acid ratio (A/P) <1
429 and the sum of dicarboxylic acids (ΣD%) < 10) [16]. The sum of dicarboxylic
430 acids of S1, S1_gilding, S4, S5, S6, ranges between 4 and 11 %, and the azelaic
431 over palmitic acid ratio is less than 0.2; this is consistent with the lipid fraction of
432 the egg yolk [17]. Moreover cholesterol or trace of it, has been found in each
433 sample. The Py/GC/MS analysis has been performed in order to confirm the

presence of this proteinaceous material. The presence of the hexadecanonitrile and octadecanonitrile in sample S6, which are known egg pyrolysis markers [18], allow us to state that the painting technique used by Giovanni del Biondo to paint the *Annunciation and Saints* was egg yolk tempera.

Description	**A/P**	**P/S**	**O/S**	**ΣDic.%**	**µg tot**
S1	0.01	1.02	0.02	7	3.06
S1_gilding	0.00	0.09	0.01	4	4.01
S4	0.01	1.08	0.01	6	3.09
S5	0.01	1.02	0.03	8	3.06
S6	0.00	1.01	0.01	7	3.02
S8	0	0.05	0	3.06	7.02
S6_coating	0.01	1.01	0.01	9.05	8.08

Table 5 Characteristic fatty acid ratios of sample S1, S1_guilding, S4, S5, S6, S8, and S6_coating along with the sum of dicarboxylic acid (ΣDic.%) and their total content in the sample (µg tot). Notation: azelaic acid (A); palmitic acid (P); stearic acid (S); azelaic over palmitic acid ratio (A/P); palmitic over stearic acid ratio (P/S); oleic over stearic acid ratio (O/S); sum of dicarboxylic acids (ΣDic.).

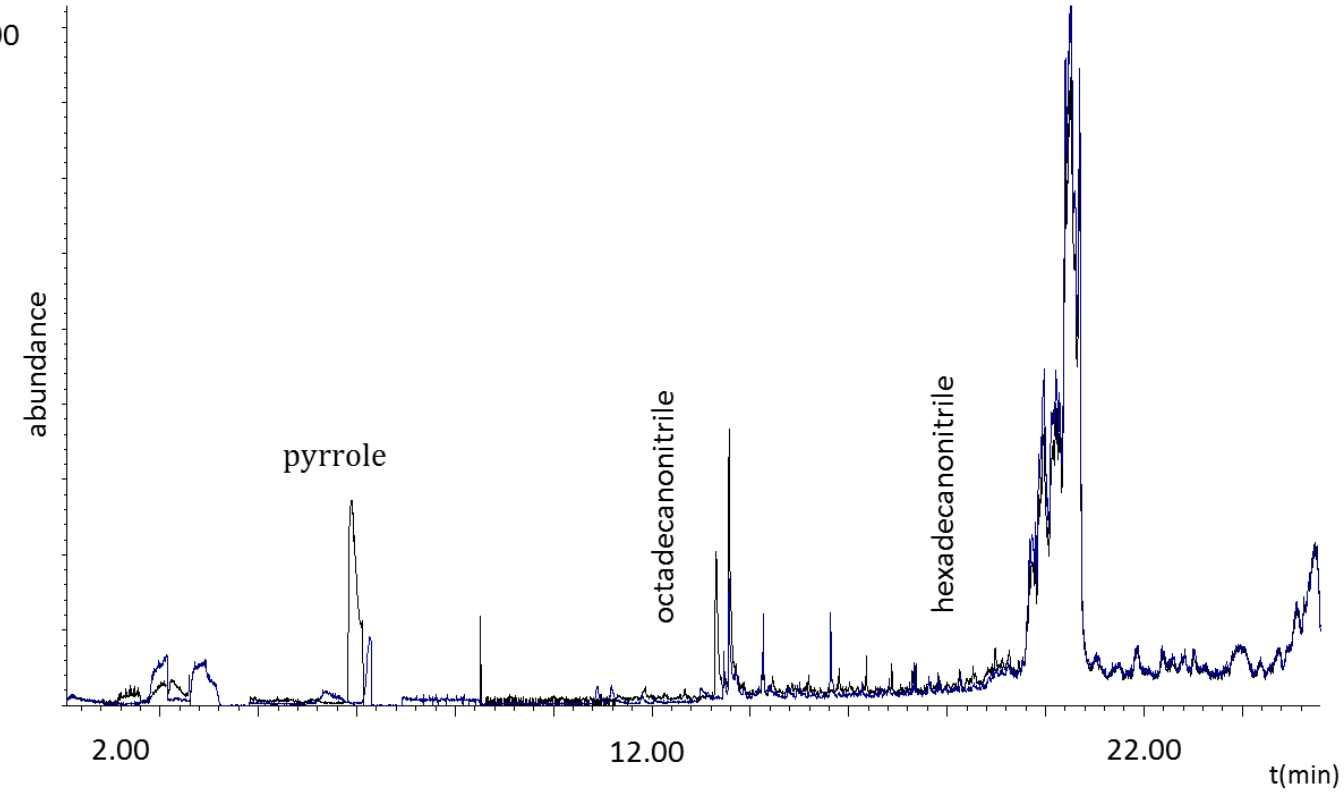
446 **(a)**


2600000
abundance
C13
Azelaic acid
Palmitic acid
Oleic acid
Stearic acid
Cholesterol
10.00
20.00
28.00
t(min)

447

448

449 **(b)**



450

451 **Figure 10** (a) GC/MS chromatogram of S1, acquired in TIC mode, internal injection standard: hexadecane, and internal derivatization standard: N,O-
452 bis(trimethylsilyl) trifluoroacetamide (BSTFA). (b)Pyrogram of S7, pyrrole (animal glue marker), octadecanonitrile and hexadecanonitrile (egg
453 markers). Pyrrole presence is due to the interfering gypsum.
454

3.4 Superficial layer

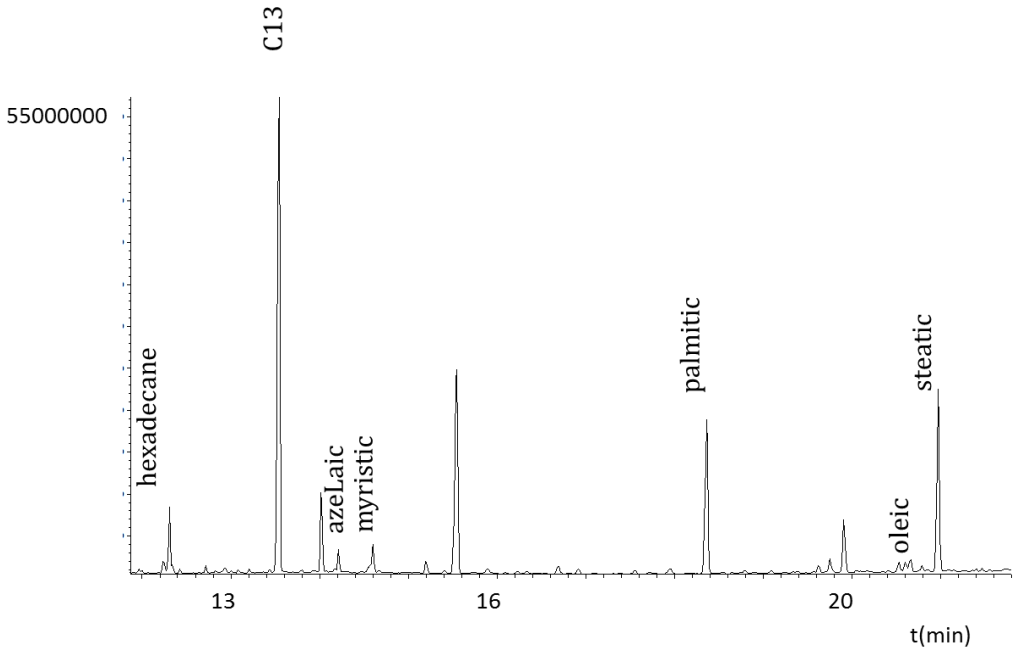
To identify the unknown superficial brown layer (see Figure 2), two samples, S6 and S8 taken from the right panel were selectively sampled and analyzed with GC/MS, while S7 taken from the central panel was analyzed with Py/GC/MS.

The GC/MS analysis of the lipid fraction of samples S6 and S8 evidenced a total content of dicarboxylic acids less than 10% (Figure 11a; Table 5) and the A/P ratio equal to 0, suggesting the absence of a lipid source such as egg. The GC/MS analysis of the proteinaceous fraction of samples S8 and S6, (Figure 11 and Figure 12b), the SIM chromatogram of S6 and the PCA analysis applied to the relative percentage content of the amino acid (Figure 11c) confirmed that the proteinaceous material of the coating is animal glue.

The analysis of the saccharide fraction of sample S6 revealed a content of other materials above the quantification limit of the analytical procedure. According to the literature, the abundant peaks of glucose and fructose at a rate ≈1:1 [15, 16] indicates the presence of honey. It is likely that honey was mixed with the animal glue to plasticize the mixture composing the coating layer which is now observed across the painted surface. This superficial layer should have been applied during a later restoration intervention of unknown dating. This interpretation is not only corroborated by the absence of the coating over the areas protected by the twisted columns of the carpentry, but also by the fact that the coating fills the cracks of the painted surface, as observed at naked eye and with the digital microscopy.

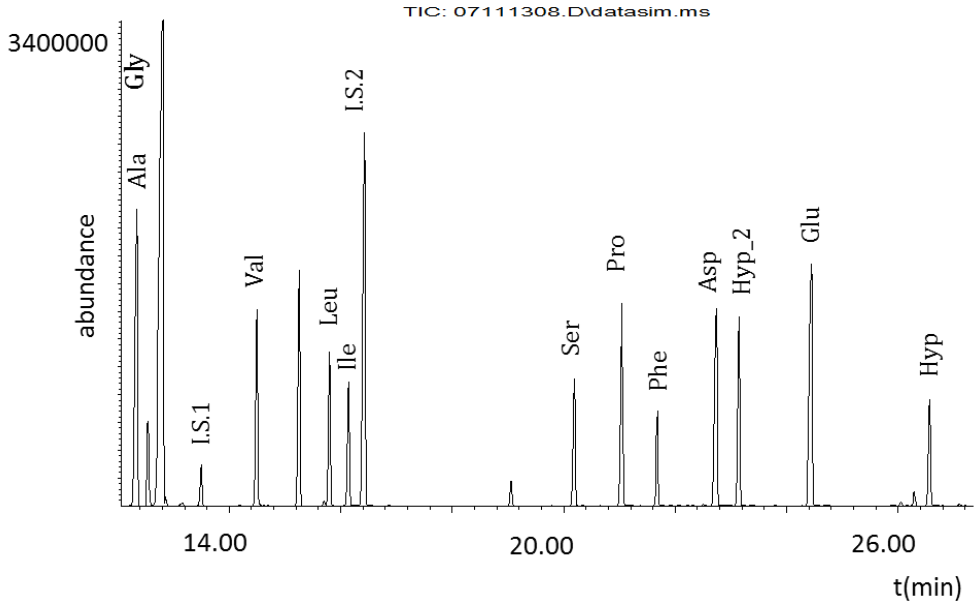
With regard to sample S7, no siccative oil was detected, while animal glue was found predominant (cf. section 3.1; Figure 6b). The Py/GC/MS analysis of this sample also revealed the presence of a triterpenic resin (Figure 12). The peaks recorded in Py/GC/MS are associated with the characteristic dammar compounds dammaredienol and ursonic together with dammaradienone and oleanonic acid [21].

(a)



Sample	A/P	Σ Dic.%	µg tot
6	≈0	7.2	3.2
8	≈0	3.6	7.2

(b)



(c)

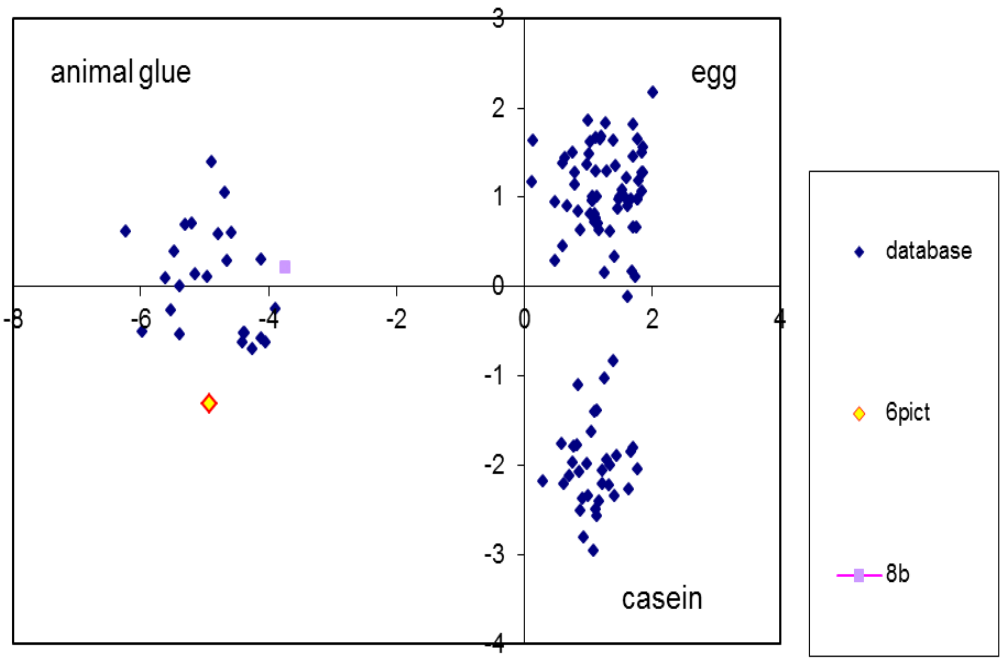


Figure 11 (a) Lipid fraction of S6, GC/MS chromatogram acquired in the TIC mode, hexadecane (internal injection standard), and tridecanoic acid (C13, internal derivatization standard with the characteristic ratio values. (b) Amino acid fraction of S6; GC/MS chromatogram acquired in SIM mode, I.S.1 is hexadecane (internal injection standard) and I.S.2 is norleucine (internal derivatisation standard). (c) PCA score plot of amino acidic profiles and of sample S6 and S8, and of the reference paint materials database.

This micro-chemical result suggests that we found here evidence of another restoration treatment which presumably was applied at a later stage and that we cannot date in absence of archival records. UV imaging, in fact, highlighted areas of resinous varnish over other areas of the polyptych, such as that representing the two saints of the left pillar of the carpentry.

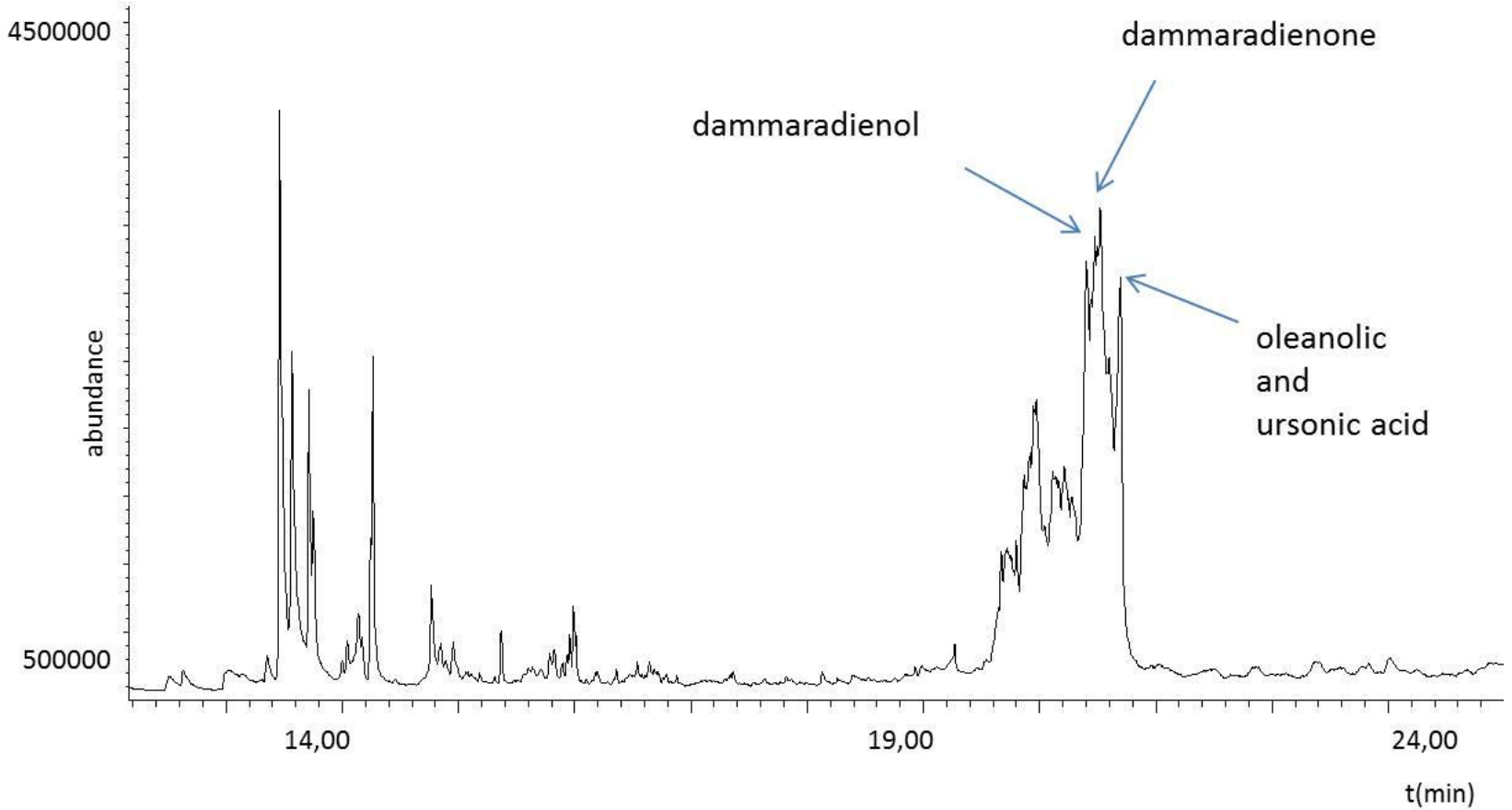


Figure 12 Pyrogram of the lipid fraction of S7, TIC mode with evidenced the main markers of the triterpenic resin: oleanonic acid, ursonic acid, dammaradienol and dammaradienone.

4. Conclusions

The integration of imaging techniques, FTIR, XRF, FORS and GC/MS enabled us to identify the organic and inorganic materials of del Biondo's painting.

In-situ imaging and spot analyses provided data related to the inorganic pigments and some hints about the organic ones in a distributed sample of points of the painted surface. FTIR was used both to characterize inorganic materials and to highlight the presence of organic substances. GC/MS allowed precise identification of the different proteinaceous, lipid, resinous and saccharidic materials.

The elemental analysis carried out by ESEM-EDS revealed the predominant presence of gypsum, while the BSE images showed that two gypsum layers – coarse and fine, respectively – compose the ground. GC/MS and Py/GC/MS analyses evidenced that the animal glue was mixed with the gypsum for the preparation layer. The blue color is almost exclusively lapis lazuli, mixed either with lead white or lead tin yellow, while cinnabar and minium were used for the red color. As well known in the literature [19, 23], lapis lazuli was by far the most expensive blue pigment at that time. In this context the extensive use of such precious material is a further sign of the wealthy commission of this polyptych by Andreola Acciaioli to commemorate her husband Mainardo Cavalcanti.

GC/MS analyses of the sample selected for the characterization of the paint binder proved the use of a pure egg tempera technique. Therefore, hypotheses by which this master exploited unusual organic binders or additives in his painting recipe cannot be supported. On the contrary, our results demonstrate that the exquisite appearance of this polyptych is owing to Giovanni del Biondo's expertise in the use of traditional egg tempera. Simple paint stratigraphy, pure colors and bespoke use of pigment mixtures to obtain specific pictorial effects are the key elements that justify the macro-scale beauty of the polyptych that we can appreciate with the naked eye.

As a consequence, the microchemical and microscopic findings of our research shed a new light on the technical knowledge of the *Annunciation and Saints*, and the perception of its value in the history of the tempera techniques. In

an historical perspective, the *Annunciation and Saints* can be regarded as a masterpiece demonstrating that the evolution of egg tempera at the turn of 14th century also relied on careful implementation of this well-established technique and not necessarily in its modification or alteration by adding different materials or changing the method to apply the layers.

These conclusions are better contextualized if compared against the analytical results from the diagnostic investigations that were made in the last decades to study del Biondo's and coeval artists' panel paintings belonging to the National Gallery of London. A thoroughly review of the data which was recently undertaken by the authors in the framework of EUFP7 CHARISMA grant (the detailed discussion of which is anyway beyond the scope of this paper) confirms that these artworks such as, for instance, Agnolo Gaddi's *Coronation of the Virgin* [24], were painted by using more elaborated pigment mixtures, with associated multi-layer structure of the stratigraphy. This scientific evidence therefore enhances the importance of the discovery made on the Florentine polyptych and presented in this paper, i.e. that such an exquisite painted surface relies on very simple painting procedure.

There is, anyway, another important element to account for. The artist seemed to have not considered varnishing necessary to reach the final pictorial effect. Our data do not provide elements to prove that del Biondo varnished the painting. On the contrary, the coatings which were found over the painted surface not protected by the carpentry are due to later maintenance or restorations. Whilst it is uncertain when these interventions were undertaken, there is no doubt that the exquisite quality of this polypytch is still preserved after centuries of exposure.

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