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Uncover the mantle: rediscovering Gregório Lopes palette and technique with a study on the painting "Mater Misericordiae"

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Abstract Gregório Lopes (c. 1490–1550) was one of the most prominent painters of the renaissance and Mannerism in Portugal. The painting "Mater Misericordiae" made for the Sesimbra Holy House of Mercy, circa 1535–1538, is one of the most significant works of the artist, and his only painting on this theme, being also one of the most significant Portuguese paintings of sixteenth century. The recent restoration provided the possibility to study materially the painting for the first time, with a multianalytical methodology incorporating portable energy-dispersive X-ray fluorescence spectroscopy, scanning electron microscopy–energy-dispersive spectroscopy, micro-X-ray diffraction, micro-Raman spectroscopy and high-performance liquid chromatography coupled to diode array and mass spectrometry detectors. The analytical study was complemented by infrared reflectography, allowing the study of the underdrawing technique and also by dendrochronology to confirm the date of the wooden panels (1535–1538). The results of this study were compared with previous ones on the painter's workshop, and significant differences and similitudes were found in the materials and techniques used.

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1 Introduction

Gregório Lopes was the royal painter of Kings Manuel I and João III. The artist was nominated knight of the Order of Santiago in 1524. Recognized by his individualized style, differing from the common collectivist tendency of the time [1], the artist is one of the Portuguese painters with more assigned work and was the introducer of Antwerp Mannerism in Portugal [1]. His partnership work is also well known, having performed artistic contracts with other painters such as Gaspar Vaz, Garcia Fernandes and Cristóvão de Figueiredo, known as the Ferreirim masters in 1533–1534, for the execution of the Ferreirim Monastery altarpieces in Lamego. During his life, he also worked from north to south of Portugal, having paintings in Lisbon, Coimbra and Évora.

The painting "Mater Misericordiae" made for the Sesimbra Holy House of Mercy, circa 1535–1538, is one of the most significant works of the artist, and his only painting on this theme, being also one of the most significant Portuguese paintings from the sixteenth century by its iconographic representations of the royal family and other important characters of that time. “Mater Misericordiae” is protecting with her mantle the different social strata that are represented. The representatives of religious orders on the right are the Pope, the Cardinal, the Bishop, the Abbot and representatives of monastic orders; the representatives of secular society on the left are the Emperor, the empress, the king, the queen, the princess and the dowager, the warrior and a member of the people in the figure of a harvester [2] (Fig. 1).

The importance of Gregório Lopes work in the Portuguese painting leads to a publication, in 1999, by the Instituto Português de Conservação e Restauro [3], bringing to light the research on his painting materials and the characterization of the workshop palette. In this study, the techniques used to define the painter’s palette were microchemical analysis, X-ray fluorescence spectroscopy (XRF) and Fourier transform infrared spectrometry (μ-FTIR). The colors of the workshop palette characterized were: for the whites, lead white; for the browns, ocher; for the greens, malachite, copper resinate and verdigris; for the reds, red ocher, vermilion and possibly dye madder and kermes; for the yellows, lead–tin yellow and ocher; and finally, for the blues, azurite, and for black, bone black [4].

Azurite grains of irregular shape and oversized dimension, a particular characteristic of this painting workshop, were found in the blue color of the sky as well as in other blue layers in the studied paintings [4].

The ground layer of the paintings is made of calcium sulfates, more specifically of “*gesso grosso*” (coarse material having more anhydrite than gypsum) with grains of calcium carbonate, in accordance with the ground layers typology used by the Lisbon workshop painters [3, 5, 6].

Other studies of the Ferreirim masters oil paintings have shown similar results concerning ground layers [6] and type of pigments previously identified in the characterization of the artist palette, with the exception of indigo, detected in one sample [7]. In these paintings, lamp black

Fig. 1 Panel painting “Mater Misericordiae” (Baltic wood oil painting panel, dimensions: 180 × 217.5 cm), made for the Sesimbra Holy House of Mercy c. 1535–1538, assigned to the painter Gregório Lopes, with the representatives of monastic orders on the right and secular society on the left of the Virgin (photograph: Vanessa Antunes)

instead of bone black was found, and calcium carbonate grains were also found as fillers in certain colors such as the Virgin's mantle blue in the painting coronation of the Virgin, being also found in several panels the red madder dye [7, 8].

In the present study, the referred painting was analyzed through different techniques with the objective of identifying layers and pigments specificities. By comparing materials and its overlapping, it was possible to recognize novelties in the technical achievements of this painter. These results brought a new insight on the painter technique by using complementary microscopic techniques. To reach these aims, portable energy-dispersive X-ray fluorescence spectroscopy (EDXRF), scanning electron microscopy–energy-dispersive spectroscopy (SEM–EDS), micro-X-ray diffraction (μ-XRD), micro-Raman spectroscopy (μ-Raman) and high-performance liquid chromatography coupled to diode array and mass spectrometry detectors (HPLC/DAD/MS) techniques were used. X-ray fluorescence technique is one of the most significant techniques used in conservation of cultural heritage due to the noninvasive approach to the artwork. Portable EDXRF technique was firstly used in the determination of pigments elemental composition and trace elements that can provide relevant information on the painting materials [5, 9–11]. EDXRF elemental results, infrared reflectography (IRR) and macrophotography noninvasive approach have put in evidence the presence of more than one layer in the construction of the painting [4, 12]. The sampling of the painting was necessary to achieve our main goal of understanding the artist's painting method and palette, bringing a deep knowledge of materials and techniques. The investigation of organic and inorganic pigments and ground layers is the dominant issue in the painting analysis using Raman spectroscopy. This technique allows the identification of crystalline and amorphous compounds, and the discrimination of the mineral phases without the interference of matrix signal [7, 8, 13, 14]. Nevertheless, XRD was used as a complementary tool to the identification of crystalline compounds since the fluorescence effect in Raman spectroscopy may hamper the identification of the pigments characteristic bands. SEM–EDS provides morphological characterization and elemental distribution maps of the painting and ground layers [15–18]. The high resolution and sensibility in the identification of trace elements and organic and inorganic compounds strongly recommend the application of these techniques in the painting field. HPLC/DAD/MS was fundamental to the definition of the type of lakes used, since this was a relevant material in the artist's choice when constructing this painting.

2 Materials and methods

2.1 Sample collection and preparation

Collection of samples was carried out after thorough visual inspection of the painting and infrared reflectography and photography in order to analyze the state of conservation of the painting, and after preliminary elemental identification carried out in situ by portable energy-dispersive X-ray fluorescence spectroscopy (EDXRF). These procedures enabled the identification of the most favorable sampling areas for micro-X-ray diffraction (μ-XRD), micro-Raman (μ-Raman) and high-performance liquid chromatography with diode array detection (HPLC/DAD/MS). Analyses were carried first directly on the samples and afterward in mounted samples as cross sections in epoxy polymeric resin and polished with silicon carbide.

2.2 Description of the analytical and imaging techniques

2.2.1 Optical microscopy (OM)

Samples were studied by optical microscopy with a Leitz Wetzlar optical dark-field and bright-field microscope coupled to a digital camera Leica DC 500.

2.2.2 Dendrochronology

Dendrochronological analysis was performed by direct observation of the wooden support. The transversal cross section of each plank was prepared with a blade, to regularize and renew the surface in order to allow visualizing clearly the growth rings. The dating technique is based on obtaining macrophotographs calibrated on a semi-millimetric scale of the cross section of each plank [19] with a digital camera (CANON EOS 1100D). The measurement of the growth rings was taken in Image Analysis software (Analysis 2.1), through the sequential display of the photographs. The data obtained in this software were exported to TSAP Win Scientific 4.64 (Rinntech), and the dating was done through the standard chronologies of the database. The advantage of this method is to allow the visualization of growth rings measurements in the future and for further re-evaluations [20] and avoids the repeated handling of paintings.

2.2.3 Portable energy-dispersive X-ray fluorescence spectroscopy (EDXRF)

The portable EDXRF spectrometer consists on an Amptek Mini-X Rh X-ray generator (50 kV, 200 mA, 2.25 W) and an Amptek XR-100SDD silicon drift detector with a 25 mm^2 detection area and 500 mm thickness and a 12.5-µm Be window. The energy resolution is 140 eV at 5.9 keV [21]. The angle between the incident and the emitted beam is 90°. This geometry allows for a high background reduction due to Compton scattering [22]. The X-ray generator was operated at 30 kV and 15 µA during 120 s. Analyses were carried out in air. Spectra were acquired using DppPMCA software, and its deconvolution and evaluation was performed using WinAXIL software package by Canberra.

2.2.4 *Infrared reflectography (IRR)*

Infrared reflectography was performed with a high-resolution infrared reflectography camera (Osiris) with an InGaAs detector allowing a wavelength response from 900 to 1700 nm, and equipped with a 16 × 16 tile system which enables an image size of 4096 × 4096 pixels. The camera is equipped with a long-pass filter Schott RG850, allowing to transmit infrared wavelength and block the undesired shorter wavelength until 850 nm. The reflectograms were performed for 60 × 60 cm^2 painting's area. Reflectograms were recorded with a working distance (front of body camera to painting) of 125 cm, focus (front of body camera to lens) of 28 cm, an f/11 aperture and diffuse illumination at 1000 lux by reflectors with 2 × 1000 W Tungsten Halogen VC—1000Q Quartz Light. The final image, composed by several reflectograms, was ensemble in Photoshop CS5, with the Photomerge tool. All the images had a small treatment, adjusting levels and increase contrast.

2.2.5 *Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM–EDS)*

A Hitachi S-3700 N scanning electron microscope with a coupled Bruker XFlash 5010 SDD energy-dispersive detector operating at 20 kV was used to perform scanning electron microscopy imaging (backscattering mode) and elemental composition of the samples cross sections. Samples were analyzed in variable pressure mode at 40 Pa without carbon coating to be further analyzed by other analytical methods.

2.2.6 *Micro-X-ray diffraction (µ-XRD)*

A Bruker general area detection diffraction system (GADDS) microdiffractometer (Bruker AXS, D8 Discover) was used to perform micro-X-ray diffraction. This microdiffractometer is equipped with a two-dimensional HiStar gas-filled area detector, a Goebel mirror, a laser-video sample alignment system and a motorized XYZ stage. Diffraction data were registered using Cu Kα radiation, tube running at 40 kV, 40 mA, with the incident beam collimated to 1 mm diameter. XRD patterns were measured in the range 8°–70° 2θ, a step size of 0.02°, with a recording time of 1800 s for each step. International Centre for Diffraction Data Powder Diffraction Files (ICDD PDF) was used for the identification of crystalline phases using the Bruker EVA software. Samples were analyzed unmounted.

2.2.7 *Micro-Raman spectroscopy (µ-Raman)*

Raman analyses were undertaken using a Horiba-Jobin-Yvon XploRA confocal spectrometer, using a 785-nm excitation wavelength, with a maximum incident power of 0.2 mW. Using a 100× magnification objective with a pinhole of 300 µm and an entrance slit of 100 µm, the scattered light collected by the objective was dispersed onto the air-cooled CCD array of an Andor iDus detector by a 1200 lines/mm grating. Raman spectroscopy was performed in a range of 100–3000 cm^{-1}. Spectra deconvolution was performed using LabSpec (V5.78). The identification of pigments was made with Spectral ID™ [23].

2.2.8 *High-performance liquid chromatography with diode array detection (HPLC/DAD/MS)*

Lake extraction from the samples (<200 µg) was done according to the methodology proposed by Wouters et al. [24]. The sample was extracted during 4 h with 200 µL of an hydrofluoric acid solution [24], lyophilized and redissolved in 50 µL of methanol/water (1:1, *v*/*v*). After centrifugation, the supernatant was collected for injection in the LC/DAD/MS system. Analyses were carried out in a LCQ Fleet Thermo Finnigan mass spectrometer equipped with an electrospray ionization source and an ion trap mass analyzer. The conditions of the MS analysis were capillary temperature of 300 °C; source voltage of 5.0 kV; source current of 100.0 µA; and capillary voltage of −7.0 V in negative ion mode. Analyses were detected in full MS mode (100–800 *m*/*z*), and two segments were used: 10.0 V of source fragmentation from 0 to 12 min and 30.0 V of source fragmentation from 12 to 30 min. The mass spectrometer equipment was coupled to an HPLC system with autosampler and DAD. The analytical column was a reversed-phase Zorbax Eclipse XDB C_{18} (Narrow-Bore, particle size 3.5 µm, 150 mm × 2.1 mm). Column temperature was set at 30 °C, and tray temperature was set at 24 °C. The chromatographic separation was performed with the mobile phase at a flow rate of 0.2 mL min^{-1} and by injecting 20 µL of sample. The mobile phase was composed by 0.1 % (*v*/*v*) formic acid solution (A) and acetonitrile (B) using the following elution program: linear gradient from 0 to 63 % of solvent B (0–14 min) and from 63 to 90 % of solvent B (14–25 min); isocratic with 90 % of solvent B (25–30 min). The DAD detector was scanned from 200 to 800 nm.

3 Results and discussion

3.1 Panel characteristics

The wooden support of the panel is made of Baltic oak composed with eight boards placed horizontally. Two of them are not datable, but the dendrochronological study [8] gives *as terminus post quem* 1532–1538. This dating fits in the period of artistic activity of the painter Gregório Lopes, who died in 1550. The panel has been painted in its original frame, with a barbed and unpainted edge on the four sides. Joints have been re-glued and the panel has been thinned and cradled in a previous restoration.

The frame is a coarse imitation of an old model with a gilded molding on the inside and outside flat band with black paint.

3.2 Painting technique

The painting technique consists on the application of thin paint layers with barely visible brushwork. In general, the painting appears to have been painted from light to dark hues. The only exception was found in the green color of the king's mantle where a first dark layer was applied directly over the ground layer, having an overlapped brighter layer in the luminous area.

Shadows and bright areas were made by adding specific pigments and/or using different concentrations of the same materials. Vegetable charcoal was the main pigment used for drawing and was also chosen to make the darker hues of the different colors, composing, in mixture with each pigment, the shadow areas of each figure. In the brighter painting areas, pure colors and dyes were applied in different layers, such as in the red mantles, or pigments were mixed with lead white and/or lead–tin yellow.

Original chromatic layers vary between 1 and 6, putting in evidence a greater number of layers in colors contemplating a lake finishing. These glazed layers were particularly elaborated in the most important figures, as in the case for the red and green mantles of the Emperor or of the king.

It is possible to identify different stages during the construction of the drawing and painting. Ensuring better adhesion of painting layers to the ground and reflecting a specific vibrancy hue trough these layers, priming was specifically addressed to certain areas, such as the blue color in Virgin's mantle and the sky. Priming was also initially applied to the sky, firstly painted, according to the visible contour areas. The study of the contour areas of the painting composition suggests that the last layers of the painting were painted foremost in the following order: sky, Virgin's mantle, clothing of the figures, carnations and finally the details (Fig. 2).

Minute golden and silver representations were left to be finished at the end of the painting construction. The almost absence of underdrawing of these objects, such as the royal crowns, suggests that the artist had a great experience, showing this capacity by making miniature details and the art of imitating goldsmith work without actually using this precious material. These precious imitations were reserved for the characters of the foreground, closer to the observer, so as to be decorously contemplated the ability of the artist and its work as a miniaturist (Online Resource 1).

Pentimenti were recognized in specific areas such as the crown of the king. The crown was firstly painted straight over the head of the king and afterward was repositioned a little away from the king's head.

3.3 Ground layer, drawing and priming

The painting ground is made of *gesso grosso* containing calcium sulfates, with more anhydrite than gypsum, and also grains of calcite (Fig. 3). Characteristic Raman bands of anhydrite and calcite were found, respectively, around 1017 and 1088 cm^{-1}. This type of ground layer is frequent in Lisbon painting workshop and specifically addressing the previous studied work of Gregório Lopes [5, 6]. Calcium carbonate, present in the upper area of the ground, was possibly used as an extender [6, 25], being a possible reminiscence of the Flemish influence in the use of chalk ground layers. The existence of grains of Sr in the ground layer is also a frequent component of Portuguese calcium sulfate ground layers [6, 26]. The fact that we did not find large amounts of dolomite in the calcium sulfate might connect this painting to earlier works of the painter workshop, since in his latest works (1544) large amounts of this material can be found in the composition of the ground layers [5, 6].

Underdrawing has a first geometrical phase made by incision and dry charcoal to define the position of the characters (Fig. 4), a second phase of contour drawing and a last phase of finishing detail drawing. Underdrawings appear to have been made directly over the ground layer but also over the priming layer, having a significant organic content that can be observed in C element by SEM–EDS (Online Resource 2). This fact can indicate that the first priming was applied in some areas, and after that, the drawing was made or enhanced. In SEM–EDS analysis, we can find other elements mixed with charcoal. It seems that an organic binder

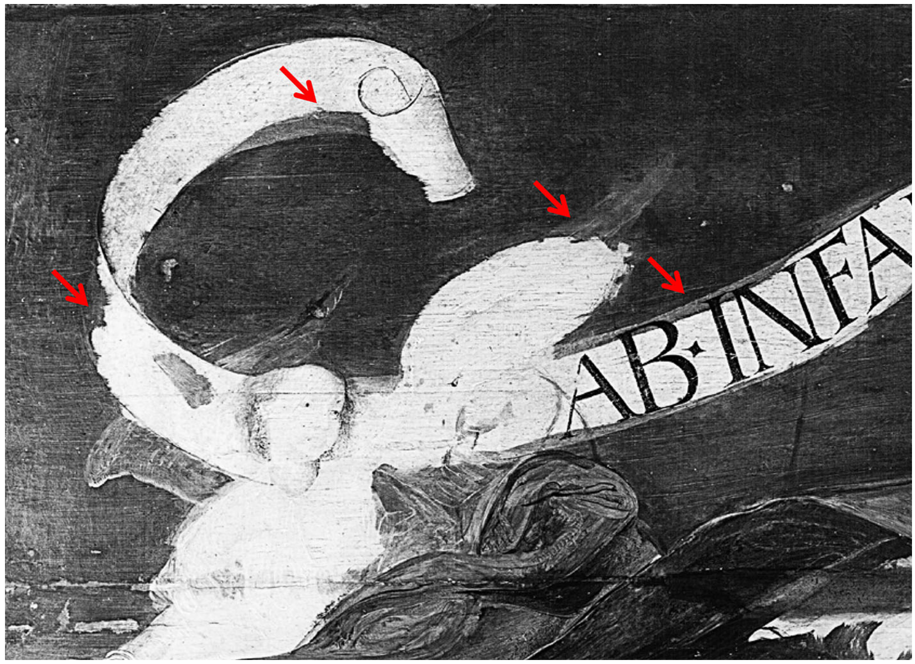

Fig. 2 Infrared reflectography detail of the painting “Mater Misericordiae” evidencing the last plans of the painting painted foremost in strong brushes and overlapping the drawing contours, indicated by *red arrows* (IRR: Sónia Costa)

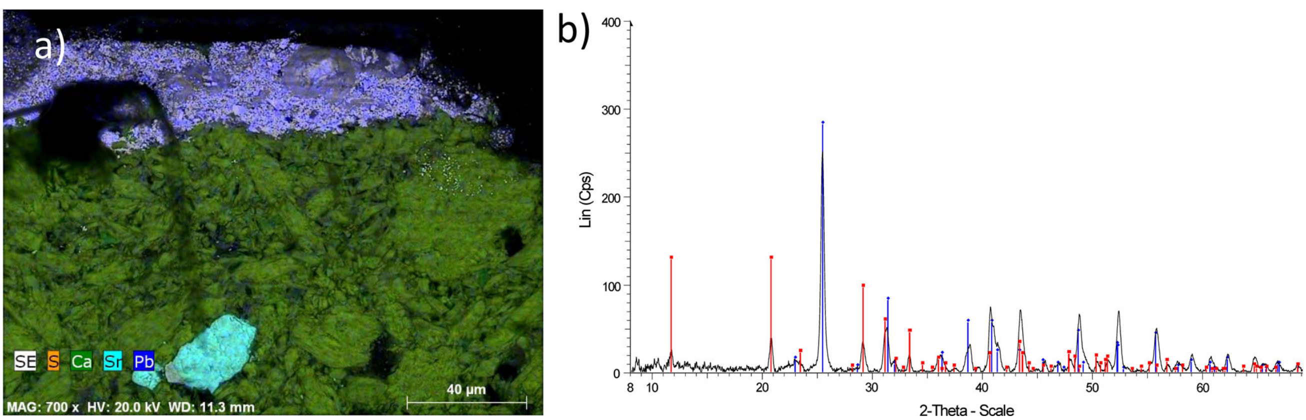

Fig. 3 **a** Elemental map combining the distribution of Sr, S, Ca and Pb in the sample 45-15-29 of the painting “Mater Misericordiae.” The combination of S and Ca elements corresponds to the compound calcium sulfate and Sr most likely to celestite; **b** diffractogram of sample 3 showing in *blue color* the anhydrite peaks and in *red color* the gypsum peaks

has been added, having also calcium and aluminum particles, that may be constituents of the drawing mixture. Connecting these results with information brought by Portuguese treatises, we find ink recipes, such as an ink used in parchment writing, with a mixture Caparosa (a black dye extracted from the plant *Ludwigia caparosa* or iron sulfate), oak galls (*galha*) and alum (*pedra hume*), among other organic materials [27], and also other recipes with Caparosa and brazilwood (*pau-brasil*) mixed with lime or ash resulting in a black color [28]. Efforts have been made to identify this pigment by LC/DAD/MS, but we were not well successful. The fact that SEM analysis evidenced an organic component in this layer is an indicator that probably the drawing was made by liquid means, applied with brush in the thicker areas or pen in thinner lines. This fact is verifiable in the IR reflectography where we can see the drop left by the painting brush or pen in the end of each line drawn (Fig. 4). In this examination, it was possible to find some redrawn areas by the painter, as in the case of the Virgin’s eyes, probably to match the print that inspired the painting, made to the frontispiece of the Lisbon Mercy Brotherhood’s commitment, printed in 1516 (Fig. 5a, Online Resource 3) [29]. It was also revealed the use of mechanical drawing such as drawing by decal, drawing by transferring pouncing patterns from perforated surfaces and stencil drawing, as in the case of the brocade imitation of the empress dress (Fig. 5b, c).

The priming layer is partial and not observed in all samples. This thin layer is transparent with the purpose to see the underdrawing. This layer was reserved to the

Fig. 4 **a** The noble dowager figure (photograph: Maria J. Francisco); **b** infrared reflectography detail of the noble dowager evidencing the underdrawing geometrical and free-hand traces *arrow 1* shows geometrical drawing and *arrow 2* shows free-hand brush drawing of the same figure (IRR: Sónia Costa)

brightest colors such as the sky or the Virgin's mantle. This esthetic option was probably to create lighter parts in the painting in order to highlight more important motifs. The fact that this priming was applied under the blue color with different mixtures and concentration is an important evidence. In the lighter colors, such as the sky, it was applied with the mixture of a small amount of finely ground vegetable charcoal. In the darker blue, such as the Virgin's mantle, lead white was applied with a small amount of ground vegetable charcoal and azurite to amplify the vibrancy of this combination as a final result (Online Resource 4). This technical option leads to other intentional criterion characteristic of this artist: the use of coarse grains of azurite [4] in and on the priming. Coarser ground material allowed to incorporate the vibrancy of the underlayer priming since it has a greater space between particles. This interparticle space allows to notice the underlayer vibration in the final result of the painting, bringing more light to this color.

In the areas where priming layer does not exist, we can verify by OM observation that there is a bigger absorption of the oil by the ground layer (Online Resource 5). This confirms the statement that lead white layer obstructs the penetration of oil on the ground layer and as a result acts as a barrier layer [25]. The probable barrier to the absorption of oil leads to technical measures such as polishing the ground layer with oil to make an easier drawing, as stated by Portuguese art treatises [30].

3.4 Pigments and painting layers stratigraphic technique

Blue hues were executed with overlapping layers of different white and blue colors. In the Virgin's mantle, we can see a first layer of white and blue probably made to achieve a brighter color. Stratigraphically, we have the first layer with coarser lead white mixed with a small amount of azurite in coarse grains. An intermediate layer only with

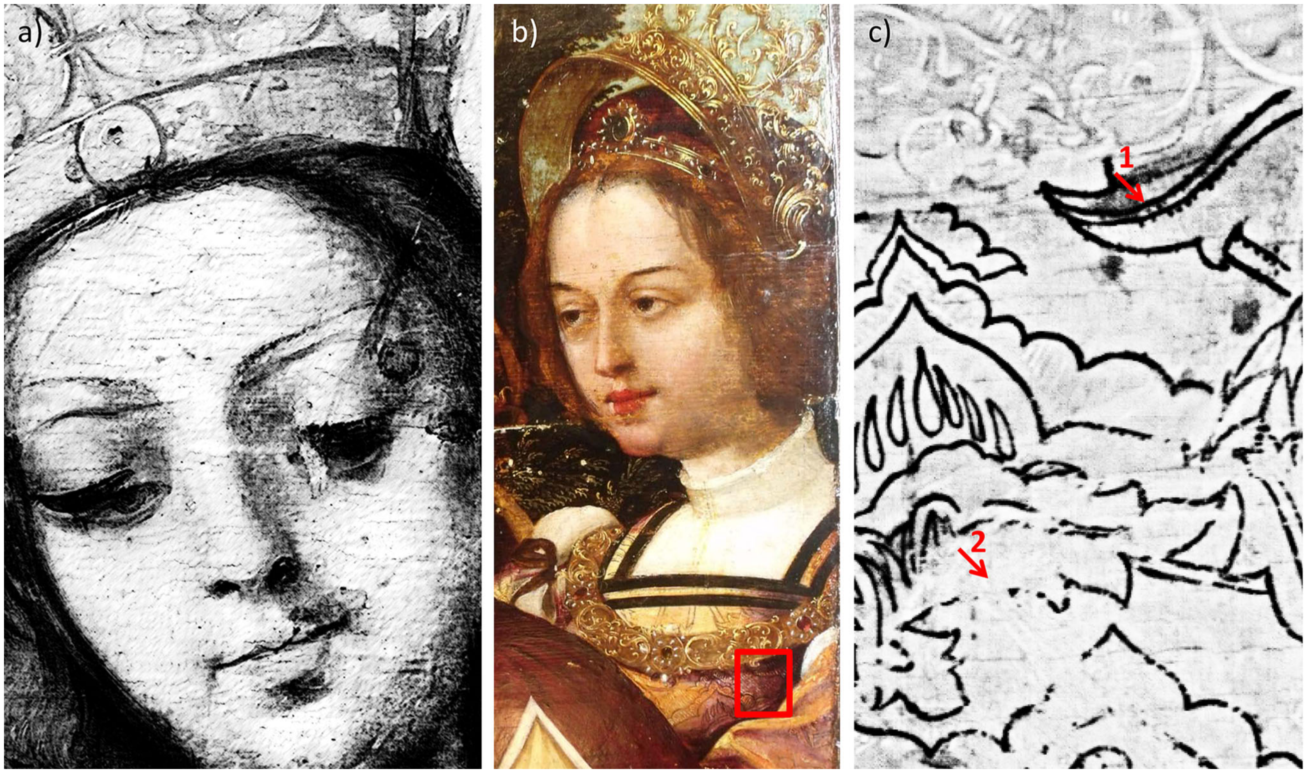

Fig. 5 **a** Infrared reflectography detail of the Virgin's face evidencing redrawn areas in the eyes and eyebrows (IRR: Sónia Costa); **b** the empress figure with a square indicating the pormenor represented in c) (photograph: Maria J. Francisco); **c** infrared reflectography detail of stencil drawing of the brocade imitation of the empress dress (IRR: Sónia Costa) *arrow 1* shows excesses of ink typical from stencil and *arrow 2* shows ink lack when painting by stencil

azurite particles mixed with a higher quantity of binder. The top layer has azurite with the same coarse granules mixed with lead white ground to a finer degree. The sky has a first layer containing lead white and vegetable charcoal. This layer is possibly a priming layer. Over this layer was applied another layer containing azurite and lead white. Characteristic Raman bands were found at 250, 400, 764, 844 and 1098 cm^{-1} for azurite, at 1050 cm^{-1} for lead white, and around 1324 and 1595 cm^{-1} for carbon black.

The use of coarser azurite particles also known as *azul de cabeça* (head blue) is characteristic of this painter [4]. This pigment was used in illumination and was described by the treatises as a great quality blue pigment with coarser granules that retained their vibrant color, being visually closest to ultramarine blue [27, 30–32].

Carnations are composed of more than one layer. These layers have different concentration and grinding of the same pigments and dye (cochineal) in order to make the flesh more brightened or more shadowed. Pigments found were lead white, lead–tin yellow, vermillion, mixed with vegetable charcoal in the shadow areas.

The flesh of the Emperor has two original layers over the ground. A first brighter layer with more white lead and lead–tin yellow than vermillion, being also the upper layer darkened with an organic component, confirmed as cochineal (ID 35 Table 1; Fig. 6).

In the Virgin's carnation, a first clearer finely ground layer was applied over the ground. Above it, a darker layer with coarser grains of vermillion pigment mixed with a red dye (possibly madder, kermes or most likely cochineal, in accordance with the results for the Emperor's carnation) seems to have been applied as a finishing glazing layer (Online Resource 6). Over these layers, we can observe by SEM–EDS the presence of Ca grains, which were confirmed by μ-Raman as calcite (Online Resource 6). These grains, kept in the most profound irregularities of the original layer, seem to be remains of a polish made with calcium carbonate with a polishing material (usually cloth or leather) on the faces of the figures in order to obtain the desired pale transparency of the flesh. The elimination of the irregularities left by the paintbrush and the brilliance left by the polishing made the flesh more real and technically distanced from the rest of the painting, highlighting these important parts. This polishing probably intentionally left the flesh layers very thin (Online Resource 6), thereby profiting from the superficial light brought by the white ground layer. This calcite polishing might be a workshop characteristic since it has not been noticed before in

Table 1 Summary table of the results and conclusions on the dyes used identified by HPLC/DAD/MS technique

ID	Color	r_t (min)	LC/DAD data (nm)	LC/MS data (*m/z*) ESI⁻	Identification	Possible dye source
6	Red light	15.01	245, 275, 490	**491**, 327	Carminic acid	Cochineal
9	Red shadow	15.00	245, 275, 312, 494	**491**, 447	Carminic acid	Polish cochineal (*Porphyrophora polonica* L.) Rose/Pink Madder?
		19.22	249, 272, 487	**329**, 285, 257, 213	Kermesic acid	
		20.31	252, 494	**299**, 255	Pseudopurpurin	
10	Green shadow	14.96	247, 275, 493	**491**, 447	Carminic acid	American cochineal (*Dactylopius coccus* Costa)
		19.18	245, 275, 492	**491**, 447, 357, 327	dcIV/dcVII	
12	Green shadow	14.96	247, 274, 487	**491**, 447	Carminic acid	Cochineal
16	Pink shadow	15.00	246, 273, 488	**491**, 327	Carminic acid	Polish cochineal (*Porphyrophora polonica* L.)
		19.25	249, 270, 489	**329**, 285, 257, 213	Kermesic acid	
28	Yellow shadow	15.77	249, 265sh, 342	–	Flavone-based chromophore	Yellow flavone-based lake
		15.99	248, 268sh, 345	–	Flavone-based chromophore	
		18.82	249, 267sh, 343	**285**, 241	Luteolin	
35	Carnation light	15.04	247, 272, 490	**491**, 327	Carminic acid	Cochineal
37	Brown shadow	15.03	246, 275, 315, 492	**491**, 447, 357, 327	Carminic acid	Cochineal + weld?
		15.95	248, 268sh, 346	**447**, 285	Luteolin–glucoside	
		16.68	248, 267sh, 342	**447**, 285	Luteolin–glucoside	
		18.79	248, 269sh, 341	**285**, 241	Luteolin	
40	White light	15.02	247, 275, 495	**491**, 327	Carminic acid	Cochineal
43	Purple shadow	15.02	247, 275, 490	**491**, 327	Carminic acid	Cochineal

Bold values indicate major ions

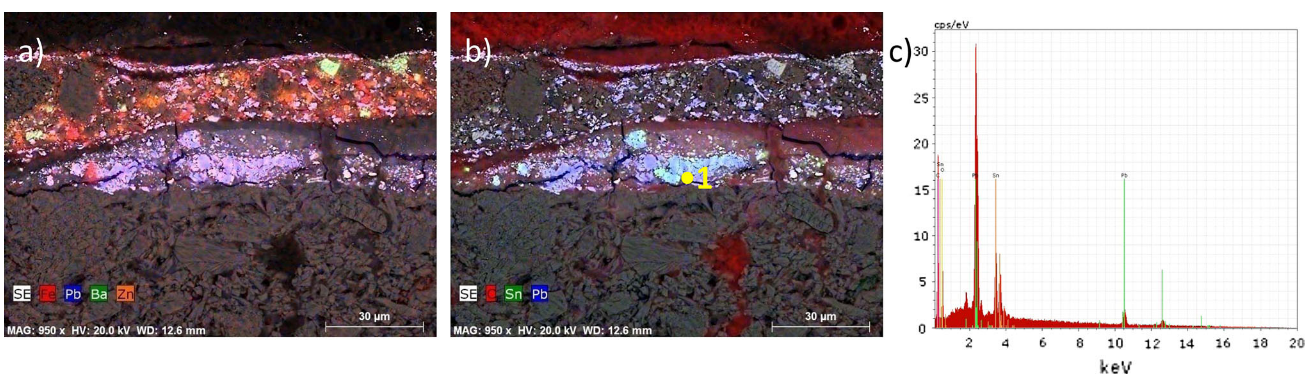

Fig. 6 **a** Combined map of elemental analysis by SEM–EDS of sample 45-15-35, carnation of the Emperor's face, with the elements Fe, Pb, Ba and Zn showing the superficial layer as a repaint; **b** combined map of elemental analysis by SEM–EDS of the same map with the elements Sn and Pb in the original carnation layer, *point 1* marks the position of the spectrum analysis; **c** EDS spectrum of the analysis made in *point 1* with the elements Pb and Sn suggesting the presence of lead–tin yellow in the composition of the original carnation

Portuguese painting. Portuguese treatises refer to polishing over a light layer of carnation made overall with fatty oil (oil thickened in the sun) mixed with lead white, polishing this layer with wet leather from gloves and finishing the carnation with vermillion and lacquer mixed in the same oil [27]. Lead white for polishing may have been replaced in this case by calcium carbonate.

Red hues exhibit different stratigraphic composition. The Cardinal's cope is built in three different layers, the first and second composed by vermillion (254, 288 and 344 cm^{-1}) and cerussite (1055 cm^{-1}), detected by µ-Raman and by µ-XRD, and finished with a final layer of cochineal (ID 6 Table 1). The Emperor's mantle is composed by six pictorial layers. Above the ground, a first bright red layer composed by vermillion (254, 288 and 344 cm^{-1}), vegetable charcoal (1330 and 1580 cm^{-1}) and cerussite (1055 cm^{-1}) were detected by μ-Raman. Grains composed by S, Al and Hg, and also grains mainly composed by C with Al, Pb, S, Ca with trace elements of K, P, Si and Mg were detected by EDS. These elements are often found in red dyestuff [33].These results suggest the mixing of red, white and black pigments with an organic dyestuff in the first thicker layer. This layer is overlapped by other thinner layer containing Ca, Ca–S with traces of Al and Pb grains, also with grains composed by Al and trace elements of S, P, Na, Mg, Si, K and Ca, suggesting the presence of a dyestuff as the matrix layer. This second layer is superimposed by three other darker organic layers, separated by small Ca grains, probably the remains of a polishing between each layer, as observed for the carnation. These last layers don't show visible grains, suggesting an exclusively organic matrix. Red glazing dyes contain an alum-based lake that can have vegetable origin, like madder (*Rubia tinctorum*), or animal origin, like cochineal or kermes. These tend to be the most common red lakes used, as stated by Iberian treatises (Online Resource 7) [27, 34–36]. As one of the most precious, rare and expensive dyes, cochineal was a symbol of power and prestige [37]. In the identification of red cochineal dyes, great care must be taken in the definition of its species since there are several types of this insect dye [38]. The pattern of the cochineal found in the Emperor's mantle seems to match the Polish cochineal (*Porphyrophora polonica* L.) (ID 9 Table 1). Other minor components found in cochineal-based lakes, like dcIV, dcVII and flavokermesic acid, were absent. The carminic-to-kermesic acid ratio of 78/22 (in percentage, measured at 275 nm) was also consistent with the composition of Polish cochineal [39]. This type of cochineal, produced in central Europe, was one of the most traded prior to sixteenth century [37]. Studies refer to the use of American cochineal after 1521 with the first shipments from Mexico [37]. Taking into consideration the dendrochronological data to the manufacture of this painting (1532–1538), the use of Polish cochineal in specific parts of the painting may reveal certain reservations of the painter or of the owner of the painting choices concerning the use of new materials to achieve the desired color. The chosen material (Polish cochineal) had a guaranteed quality proved by centuries of trading and advised by Portuguese treatises for oil painting [27]. It was reserved to depict the highest elite: the Emperor, evidencing the deference for this figure. Although in Portugal harvesting and marketing of kermes (*Grã*) was initially a privilege of the royal house, King Manuel I liberalized in 1499, its harvest and trade [40]. In this case, Polish and American cochineal seems to have been chosen over an identical Portuguese resource.

In the superficial layer of the Emperor's mantle pseudopurpurin, a chromophore usually found in madder dye was detected. Nevertheless, no other madder major components, like alizarin and purpurin, were detected. Rose/pink madder is a variant of madder lake, obtained by selective extraction and deposition of pseudopurpurin. Rose madder is mentioned in some sources, like the British colorman Georges Field, a noted manufacturer of this pigment in the early nineteenth century [41]. Due to the time period in which this lake was produced, it is very likely that this is a material from an intervention.

In the pink color of the angels dressing, besides vermillion at 254, 288 and 344 cm^{-1} and cerussite at 1055 cm^{-1}, detected by μ-Raman and by μ-XRD, Polish cochineal (*P. polonica* L.) (ID 16 Table 1) was also found. The absence of other minor components found in cochineal-based lakes, like dcIV, dcVII and flavokermesic acid and the carminic-to-kermesic acid ratio of 77/23 (in percentage, measured at 275 nm), is consistent with the composition of Polish cochineal [39].

The orange color of the Empress's dress is made of lead white, vermilion and minium, this last detected by μ-Raman since in EDS Pb element is impossible to distinguish chemically out from white or red lead, being this last, however, distinguished in SEM image by its coarse grains and also visible in OM image (Fig. 7a, b). This dress was made by stamping in black the brocade imitation in a first step and in a second step was applied the white–orange mixture of pigments as seen in figure (Fig. 7c).

The yellow color, such as the Emperor's sleeve, is composed of a lead white and lead–tin yellow type I matrix with some grains of vegetable charcoal, yellow ocher and calcite as detected by μ-Raman. In the finishing layer of this color, it was also found a yellow flavone-based lake [42] (ID 28 Table 1), being probably a yellow lake pigment, composed by a yellow dye absorbed in an inorganic mineral such as chalk [43], corresponding most probably to the calcite grains found in this layer. Also, simple compositions were made for this color in the aureole enclosing the Virgin's figure, made only of lead–tin yellow, as well as the angel tunic. Characteristic Raman bands for lead tin yellow type I were found around 129, 197 and 459 cm^{-1} and for yellow ocher around 299 and 390 cm^{-1}.

The green velvet color used in the Emperor's tunic is composed of a first layer of white lead and a copper pigment (azurite or malachite) having above two layers with organic matrix, the first being of a darker color and composed by Pb with Ca, Cu, Al, Mg and K. These are often found in both plant and insect dyestuff specimens [33]. American cochineal (*Dactylopius coccus* Costa) was identified in this sample (ID 10 Table 1). No evidence of kermesic/flavokermesic acid was found. Carminic-to-dcIV/dcVII ratio was 95/5 (in percentage, measured at 275 nm),

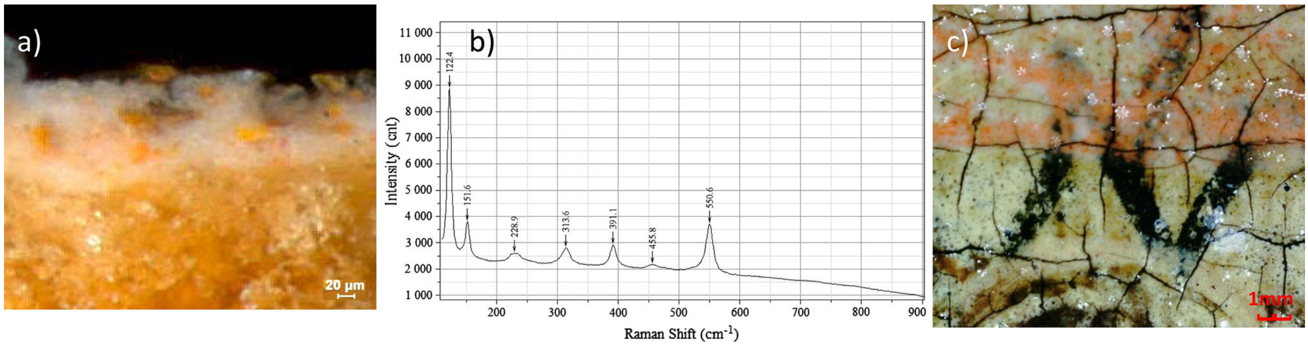

Fig. 7 **a** OM image of sample 45-15-29, *orange color* of the empress dress showing *orange* grains in a white matrix; **b** Raman spectrum of the same sample with red lead (minium); **c** detail of the empress dress stamping in *black* the brocade imitation under the *white–orange* mixture of pigments

consistent with the composition of American cochineal [39]. This cheaper cochineal was probably used since it was not intended to make a deep red color but a green color. Probably in this case, it was not needed such an expensive material to achieve deep green velvet texture and color.

The second layer presents a reddish color and is in majority composed by C. The presence of an organic layer without the presence of alum indicates shellac, a resin secreted by insects of the cochineal family used by the name of *lacra* [27, 31] and recommended for glazing by Portuguese treatises [27].

The green light color from the king's mantle has a first black layer with Sn and Pb, lead–tin yellow type I, grains of C possibly vegetable charcoal and also Ca, Cu and Al, being an alum-based dye probably cochineal, as identified in ID 12 Table 1); this material is also visible in the superimposed white greenish layer, although with coarser grains of lead white (Pb) mixed with type I lead–tin yellow. This black and white undermodeling (first modeling of the painting) is described in Portuguese treatises as glazing: "to glaze you shall do first with white & black, being high colors white, and black colors truly black," being finished with a glazing material, using verdigris in the case of green color [27] (Online Resource 8). The shadow area of green color from the king's mantle is only made with the first black layer, being probably the first to be painted, since it appears firstly in both light and shadow hues. In this layer was found cochineal (ID 12 Table 1) along with calcite, type I lead–tin yellow and vegetable charcoal.

The purple changing cloth color of the King's tunic has several superimposed layers. Over a layer of lead–tin yellow mixed with vegetable charcoal (C) pigment layer was applied an organic red brownish layer (shellac?), superimposed by a mixture of organic dye in a white matrix. In this matrix were detected cerussite, calcite, anhydrite and gypsum by μ-XRD, and EDS confirmed the presence in orange grains of Fe, Si, Pb, As, Al and Ca, suggesting the presence of a compound containing realgar (*rosalgar*) or red arsenic, although the nature of this pigment may not be determined solely by EDS analysis (Online Resource 9) [44]. The rounded shape and Al-rich red particle analyzed by EDS and trace elements of Na, Al and K suggest the presence of a dye prepared with alum (*pedra de hume*), since this was one of the fixative materials of some dyes [27, 31, 33], such as cochineal [27, 31], a dye found in this sample by HPLC/DAD/MS (ID 43 Table 1). The presence of Ca grains between the two layers suggests once more a polishing of the glazing before the final painting layer (Online Resource 9). The finishing of this purple changing cloth color was made by painting minute brushed yellow spots in the light areas to imitate a golden finishing of the cloth, as seen in figure (Online Resource 9c and 9d).

Minute golden object representations, such as the Pope's crosier, were made with lead tin yellow and lead white in a thick layer for the light areas, made over a previous yellow ocher (goethite) (Fe) layer in the shadow areas (Online Resource 10), as shown in several examples in Online Resource 11. This technique, according to Portuguese treatises, is called *perfilar* (profiling) and refers that in a first step the object is painted with red ocher (*almagra*) and minium (*zarcão*) and after drying can be retouched with lead tin yellow type I to obtain the light areas [27, 31].

Micro-Raman analysis has shown a first cerussite layer and a second minium and cerussite layer in the composition of the brown of the Pope's mantle. EDS has shown grains of Al–Si suggesting the presence of aluminosilicates and single grains of Si, probably quartz in these two bottom layers. These layers are followed by a third lead–tin yellow and vermillion layer, being finished with an organic coating, a glazing with a mixture of cochineal and probably weld, a yellow dye obtained from the plant dyer's rocket (ID 37 Table 1). The mantle is completed with detailed pearls and golden imitation lacework; finishing details were made as previously explained, as seen in Online Resource 12.

The gray color imitating silver in the pedestal cup-shaped which rests the Virgin has evidenced in the EDS analysis, confirmed by the XRF and Raman identification, a matrix of lead white mixed with other pigments in lesser quantity such as lead–tin yellow, ocher and vegetable charcoal also with cochineal (ID 40 Table 1); the base of the pedestal is darker, corresponding to a shadow zone, and it was constructed with a first black layer under the white used to model the images, as visible in images of some detail and worn out areas (Online Resource 13).

Finally, the phrase over the Virgin's head is written in black: "AB INFANTIA MEA CREVIT MECUM MISERACIO, ET AB UTERO MATRIS ME EGRESSA EST MECUM" (*For from my infancy mercy grew up with a father, and from my mother's womb it came forth with me*, book of Job 31:18). This color was made in two layers. A first layer was placed over the lead white bottom of the phylactery and is composed by an organic matrix with grains suggesting the presence of vegetable charcoal and other grains of Ca suggestive of the occurrence of calcite grains. These are similar components to the ones present in the ink used to make the underdrawing. The second layer was probably made to enhance the black tone, which is mainly composed by an organic matrix without visible grains. EDS analysis showed that the major elements found in this layer were Ca and P, having also Na, Si, Pb, Al and Mg, with trace elements of K (Online Resource 14). Although it was not possible to identify a dye in the black color, these elements are often found in organic dyestuffs used in paintings due to its provenance from living animals and/or plants [33].

The analytical results identified in the characterization of Gregório Lopes palette (Table 2) established the use of pigments and mixtures of pigments in the painting "Mater Misericordiae" (Table 3).

Table 2 "Mater Misericordiae" palette detected by μ-XRD, μ-Raman and HPLC techniques

"Mater Misericordiae" palette	μ-XRD	μ-Raman	HPLC
Lead–tin yellow type I	x	x	
Azurite	x	x	
Lead white	x	x	
Ocher	x	x	
Vegetable charcoal		x	
Animal charcoal			
Red ocher	x	x	
Vermillion	x	x	
Minium		x	
Cochineal			x
Madder			x
Yellow flavone-based lake			x
Weld (?)			x
Ground layer *gesso grosso* (>anhydrite < gypsum) + calcite (grains)	x	x	

4 Conclusions

Portuguese painting workshops have had their maximum autonomy between the period of 1450–1550 defining its own characteristics [45]. Being the first big overseas empire of the European renaissance, several Portuguese painters were trained in Flanders and several Flemish painters worked in Portugal [45]. But not even this strong Flemish influence has been dominant in what concerns to adapt Portuguese paintings to their own style and materials. It is the case of the ground layer matrix of the studied painting that is made of calcium sulfate instead of calcium carbonate, the typical ground layer material from northern Europe. This aspect evidences that the painter has ingrained the Portuguese traditions, particularly from the Lisbon painting workshop, where Gregório Lopes trained and where he worked in partnerships. The master of the Lisbon workshop, Jorge Afonso was the father in law of Gregório Lopes and followed the restricted rules of the Lisbon corporation working regime. Painting was still considered a mechanical job and one minor activity obeying rules that diminished creative liberty. It was the case of this painting, a specific commission that portrays the main figures of Portuguese and European cultures. The painter delivered the work by copying known portraits of the main figures, in order to clarify and spiritually commove the public, probably following a restricted contract, and putting his creation in technical issues such as the execution of the draperies, since textiles were one of the biggest visible forms of wealth in Portuguese society [46]. The novelty of new textiles–textures–draperies was very carefully painted with the finest materials and the thinnest layers overlapped to achieve a result close to reality. In addition, this Master virtuous work is visible in the jewelry details which distinguish him from other Portuguese painters.

Learned workshop practices are evident in this painting. The use of a wood from the Baltic (Baltic oak) reveals the following of Flemish traditions, in opposition to the use of calcium sulfate ground layers matrix, traditional from Portuguese school workshops, in particular Lisbon workshop. However, the use of calcium carbonate grains in the upper areas of the calcium sulfate ground layer may be related to remains of traditional Flemish technique.

Was also revealed as a novelty in the study of Lisbon workshop the use of a geometrical drawing to establish the main location of the figures, before the common use of a

Table 3 Main pigments and mixtures by color of the painting "Mater Misericordiae"

Colors	Main pigments by colors	Mixtures of pigments by colors
Yellow	Lead–tin yellow	Lead–tin yellow type I + lead white + ocher
	Yellow lake	Yellow flavone-based lake
	Ocher	Ocher
Blue	Azurite	Azurite + lead white + vegetable charcoal
White	Lead white	Lead white + vegetable charcoal
Brown	Lead–tin yellow	Lead–tin yellow type I + lead white + vermillion + vegetable charcoal + cochineal + weld?
	Vermillion	
	Vegetable charcoal	
Black	Vegetable charcoal	Vegetable charcoal
	Animal charcoal	Animal charcoal
Gray	Lead white	Lead white + vegetable charcoal + lead–tin yellow type I + ocher + cochineal
	Vegetable charcoal	
Green	Green Dye	Lead–tin yellow + lead white + cochineal
	Lead–tin yellow	
	Azurite	
Red	Vermillion	Vermillion + lead white + vegetable charcoal + calcite + gypsum + cochineal
	Minium	
		Vermillion + vegetable charcoal + minium + lead white
Flesh colors	Lead white	Lead white + vermillion + vegetable charcoal + lead–tin yellow type I + cochineal
	Vermillion	

dry contour drawing and of liquid brush hand drawing; mechanical drawing such as transferring pouncing patterns from perforated surfaces, stencil drawing and drawing by decal were also found. When comparing to other later works of the painter, the amount of free-hand drawing is lower in this painting probably due to the representation of important figures recognized by printing circulation. This aspect shows the painter's knowledge of the art in circulation at the time and the respect for the original portraits.

The coloring materials that were used and its transformation by the artist in the painting "Mater Misericordiae" also highlight parallels with the illumination painting. This is the case of the use of coarser grains of azurite, identified as a characteristic of the painter's technique or some mixtures of pigments or the overlapping of layers technique in the making of jewelry or drapery. The brightest colors were highlighted by the priming layer in more important issues. The cochineal was used to glaze carnations red, green, purple and gray colors, being referred in treatises as a common procedure for illumination, as stated by the Portuguese treatises. The use of different cochineals according to the importance of the color was an interesting achievement to future comparative studies. These glazes found in the painting were often polished with calcium carbonate, probably a novel characteristic of the painter's technique.

The painter's miniaturist style was combined with some materials used also in illumination paintings, confirming its knowledge also in this area, as reported by art history and treatises. These personalized working of details distinguished Gregório Lopes work from his other partnership painters, being distinguished in 1520 as a knight of Santiago Order. This distinction evidenced its technical achievements as a painter and its social recognition, being a major contribution for the ascension of Portuguese painter's ennoblement and to the autonomy of the painter profession.

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