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**THE MULTI-ANALYTICAL APPROACH TO CULTURAL
HERITAGE STUDY. TWO CASE STUDIES OF ITALIAN
RENAISSANCE PANEL PAINTING**
(S.S.D. CHIM/12)

PhD Candidate

Dott.ssa Tiziana Concetta Cavaleri

PhD Course's Coordinator

Prof. Andrea Luigi Facci

Tutor

Prof.ssa Claudia Pelosi

Co-tutor

Prof. Giuseppe Calabrò

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PREMISE

This PhD project was conducted at the Centro per la Conservazione ed il Restauro dei Beni Culturali *La Venaria Reale* (CCR) in collaboration with the University of Tuscia. In 2019, the two institutions signed an agreement to implement collaboration in research and training for the conservation and preservation of cultural heritage. Within this agreement, they collaborate on promoting and developing research activities, enhancing their knowledge and technical-scientific possibilities, and promoting and organizing professional training activities in sectors of mutual interest.

The CCR is one of the three national, strategic poles for the conservation, research, and higher education on cultural heritage. It is in a section of a former royal residence of the House of Savoy, a masterpiece of Baroque architecture in Venaria Reale, just outside Turin, recognized as a UNESCO World Heritage Site.

The CCR's General Coordination and External Relations are guided by a team of specialized art historians. The structure hosts nine Conservation Laboratories specializing in the conservation of various types of cultural heritage materials and objects, from paintings on canvas to panel paintings and wooden sculptures, wooden furniture, stone materials and wall paintings, textiles and leather, glass, metal, and ceramic artifacts, paper, photography, and contemporary art. Moreover, the CCR hosts Scientific Laboratories that operate on various fronts, from the scientific analysis of objects of archaeological, historical, and artistic significance to cutting-edge, applied research through grant-based national and international projects. Furthermore, the CCR has a Higher Education and Study School that organizes various training activities, and it hosts the master's degree in Conservation and Restoration of Cultural Heritage of the University of Turin.

The CCR, with its unique structure, represents a dynamic hub where numerous significant research projects emerge from engaging discussions with conservators, museum curators, art historians, scientists, and all the other professionals involved. These discussions, which focus on issues related to the study, conservation, and enhancement of artworks, lead to fruitful collaborations at national and international levels, forming a vibrant community of professionals dedicated to cultural heritage.

Scientific analysis conducted at CCR relies on a wide range of instrumental techniques. The aim is to support conservation treatments and increase current scholarship in art and archaeology, especially regarding artists' materials and manufacturing techniques of cultural heritage artifacts. Analytical plans are tailored to the questions raised by a multi-professional team, including conservators, curators, and art historians. They may involve different analytical steps and instrumental techniques depending on the specific objectives of each research project or conservation treatment. The analytical approach typically combines spot and imaging techniques and noninvasive and micro-invasive methods following specific scientific protocols. Scientific analysis often relies on a systematic comparison with reference materials prepared in the laboratory as accurate replicas of cultural heritage objects according to historical recipes and current knowledge of the materials and techniques used in a given place and time.

The two case studies presented in this PhD dissertation concern the scientific analysis of two panel paintings of the Italian Renaissance: one is the *Madonna with Child and Two Saints* from Palazzo Madama - Museo Civico di Arte Antica (Turin, Italy) painted by Defendente Ferrari and dating back to around 1505-1510; the other one is the *Madonna della Tenda* from Musei Reali Torino (MRT, Inv. 271, Turin, Italy) historically attributed to Raffaello Sanzio and subsequently recognized as a 16th-century copy after the Master. Both these artworks were the subject of multi-year projects of study and conservation coordinated by the CCR team and conducted with the valuable scientific collaboration of various research institutions. Specifically, eight scientific collaborations were launched within this PhD project, and sixteen different analysis methods were applied to integrate the CCR scientific equipment and guarantee a deeper understanding of the artworks.

This dissertation provides two concrete examples of paintings with very different problems, underlining the difficulties encountered and the analytical strategies adopted to overcome some instrumental limitations, in order to obtain reliable results and give concrete support to the conservators' team who dealt with the conservation treatment of the artworks.

STRUCTURE OF THE DISSERTATION

The core of this PhD project is the scientific analysis conducted on two panel paintings from the Italian Renaissance at the CCR in Venaria Reale for contributing to the conservation and preservation of these invaluable artworks. Based on these two case studies and many years of experience in this area, the dissertation proposes an ideal software designed for conservation scientists as a helpful tool for managing and interpreting data from diagnostic campaigns.

Chapter 1 provides a technical-artistic description that introduces understanding the various creative phases of a panel painting, with references to ancient sources, materials used by the artists, and the most well-known technical expedients. This description is followed by a summary of the most common degradation problems of panel paintings, problems from which all those questions that scientific investigation must answer usually arise. This first chapter also intends to share technical-artistic terminology with the reader, which will be widely adopted in chapters 2 and 3.

Chapters 2 and 3 deal in detail with the scientific analysis of the paintings, one for each case study. These chapters describe the research objectives and the issues to investigate relating to each painting. The scientific instruments and methods adopted are reported; the analytical strategies used to respond comprehensively to the research aims are presented and discussed. Finally, the results obtained from the interpretation and cross-referencing of the data are discussed following the stratigraphy of the work, that is, explaining what the scientific analysis definitively allowed to understand about the chemical composition, degradation, and structure of the painting layer by layer and which analytical techniques or strategies provided the data valuable for each interpretation.

Chapter 4 reports some considerations on the multi-analytic approach and data management. Starting from the experience of the two case studies and the hurdles encountered in data management during the interpretation of the results, the chapter presents a proposal for an ideal software designed for the operator in charge of cross-referencing and interpreting all the data from a diagnostic campaign.

Chapter 5 reports final observations and illustrates some research perspectives deriving from observations and reflections on these and previous case studies conducted at the CCR.

Chapter 1

INTRODUCTION

1.1 The Italian Renaissance panel painting: an introduction to the execution techniques and technical terminology

From antiquity to the 16th century, the wooden panel was the primary support of European pictorial works before being almost entirely replaced by painting on canvas.

Materials and techniques adopted in the art of panel painting can be traced in ancient sources and treatises such as *De Diversis artibus* by Teophylus Presbiter (12th century) and *Il Libro dell'arte* by Cennino Cennini (14th – 15th century). Over the years, these recipe books became a fundamental reference point for Renaissance workshops, which were active in Italy in the 15th and 16th centuries. Nowadays, these sources represent historical documentation of extreme value to understand the Medieval and Renaissance works of art from a material and technical point of view. Despite their importance, this documentation is very scarce compared to the enormous vastness, variety, and technical complexity of artworks that have come down to us. However, we can find many examples of more systematic studies conducted in recent times on specific artists [1-2] or specific topics, such as texts on the construction techniques of supports [3], the preparation and diffusion of pigments [4], and so on.

This complexity of materials and techniques is hugely relevant even if we try to focus on the Italian Renaissance panel painting, to which both artworks presented in the case studies of this dissertation belong. Just think about the number of workshops present in Italy in those years, each one guarding their recipes (formulas for creating specific colors or mediums), the way and number of tools for underdrawings, painting, and gilding, the technical complexity of all those Italian masterpieces which have marked, for various reasons, the history of art. Furthermore, we should consider that Renaissance materials and techniques have roots in previous centuries, evolving in certain aspects and marking a significant involution for others.

Therefore, a detailed examination of all the technical and material aspects of panel paintings from the Italian Renaissance would require immense research, which is beyond the scope of this dissertation. Nonetheless, to aid readers in understanding the text and the terminology used – particularly in chapters 2 and 3, which focus on the case studies –, a brief description of the

best-known materials and execution techniques adopted in that epoch is presented. This description will start from the wooden panel preparation to the outermost layers, guiding readers through the process of creating a panel painting.

The wooden supports: from the cut to the assembling and reinforcing the structure

The artists' first step was to carefully choose the wooden panels, considering the wood species – in Italy, mainly poplar, but also spruce and lime in Veneto, or walnut in Lombardy – and the type of cut to obtain from the tree trunk. After a long seasoning, the trunk was cut into boards longitudinally, thus obtaining boards of different qualities. The poplar trunk could generally provide a panel with a maximum width of around 80 cm; for larger paintings, it was essential to cut and join several panels (or planks), assembling them in different ways. The choice of central cuts avoided wood bending and cracks over time, depending on the type of wood chosen and its thickness. However, it is worth noting that, with respect to the Medieval epoch, the Renaissance sees the substantial abandonment of some construction precautions even in the careful selection of the cuts of the boards intended for painting.

The tools used for working the boards were axes, saws, and planes, methods and tools that remained almost unchanged from the 14th century until the last century when electrically powered machinery was introduced.

As for the methods of assembling the different boards, we range from glued live joints, dating back to the 12th century, to pins of several types. From the 13th to the 14th century, we can find the use of blind pins, i.e., inserted into the thickness of the two boards and of different shapes, with polygonal or circular sections. Further on, we can find double dovetails, i.e., dowels of harder wood inserted into the back joint as reinforcement or on the recto, but not visible because they are underneath the preparation. Rarer are the half-plane or mortise-and-tenon joint systems, i.e., joints along the entire edge of the board or dovetail joints.

The reinforcement systems of the structures are also critical, with the crosspieces playing a pivotal role. From the 12th century onwards, they have been applied to support differently. The oldest are certainly those fixed using sturdy nails, forged by hand, placed flush from the front before treating the surface with canvas and priming and then folding them onto the crosspiece. The head of the nail was sometimes protected by disks of cloth, parchment, wax, or tin sheets. Another type of nail, sometimes used to secure decorative elements, was the bell or eyelet nail,

which consisted of a folded hook that passed through the thickness of the board and came out in the shape of a ring. The sleepers were generally of a certain thickness and of wood different from that used for the panels (usually a harder wood), and the choice varied depending on the region and the school. In Italy, however, the wood used for crosspieces or frames was usually fir, oak, or chestnut.

Preparation phases: incamottatura, ground, underdrawing, imprimitura, and engravings

To counteract the characteristic hygroscopicity of the wood, which causes continuous shrinkage and swelling of the fibers, boards were prepared, first by applying a coat of animal glue to make the wood waterproof and less absorbent. Then, pieces of parchment, canvas, or both could be applied as an elastic layer capable of defending the painting from the support movements: the so-called *incamottatura*. The *incamottatura* could interest the entire surface to be painted, sometimes including the frame, or be localized only in some regions of the painting, more subject to the movements of the wood, such as the joints between the boards. Parchment underneath and canvas on top led to a double *incamottatura*. Towards the 14th century and on, the use of canvas alone was preferred, and, at times, it was limited to some canvas strips on the joints of the boards, testifying to a progressive simplification. This can be intended as an involution since the *incamottatura* compensated the wood's shrinkage and swelling for the thermo-hygrometric variations of the environment, which could have particularly harmful repercussions on the pictorial layers executed in egg tempera, but even more dramatically on the backgrounds covered with thin leaves and burnished and punched metal pieces that would have run the risk of tearing.

Subsequently, various layers of gypsum mixed with animal glue were applied in different grindings, from the coarsest to the thinnest, until a perfectly compact white surface was obtained: these layers are commonly called *ground* layers. The surface obtained had to be carefully sanded and polished, and any holes plugged, to allow the artist to trace the drawing – the so-called underdrawing.

The *underdrawing*, a crucial step in the artistic process, was often created with versatile tools such as charcoal or a brush dipped in ink, or a combination of both. These tools, with their unique properties, allowed for a freehand drawing, giving the artist the freedom to express their creativity.

Furthermore, artists mastered creating a full-scale drawing and transferring it to a panel to replicate recurring patterns, decorations, and designs: the *preparatory cartoon*. The preparatory cartoon allowed the artist to solve technical and artistic problems before executing the artwork on its definitive support. It was convenient when working with precious materials (such as gold) or techniques that imposed a rigorous process, limiting the possibility of the artist making modifications (such as egg tempera).

There were two precise methods of transferring cartoons onto a panel. The first was called *spolvero* (pouncing) and consisted of making dense and minute holes alongside the drawing outlines and then applying the pulverized charcoal with a rag (so letting the charcoal pass through the holes). The second was called *calco* (stylus tracing or incision) and consisted of transferring the drawing outlines onto the panel after having rubbed the back of the cartoon with charcoal or black powder, then impressing the outlines with an iron stylus, and finally removing the cartoon.

In addition to the underdrawing, the *engravings* played a significant role in the artistic process. They were usually executed directly on the ground layer with a *stilo* that served as a practical tool for precisely delimiting the contours of specific areas, such as the haloes and the backgrounds to be gilded, or for outlining architecture. In the Renaissance, the perspective played a crucial role: figures were represented in a well-defined space, giving the idea of a natural environment. Therefore, the preparatory drawing also involved the construction of the scene, which could be obtained through perspective lines from a point: the vanishing point. These lines could be guided, drawn with the help of a straightedge, and traced with a dry medium.

These setting phases could be followed by applying a thin and semi-transparent *imprimitura*, often composed of white lead diluted in a binder, and used to fix the underdrawing.

Gilding techniques: guazzo, missione, and shell gold

To obtain the *guazzo* gilding, the artists used gold (or silver) leaves applied to a clay-based ground, which, starting from the 13th century, generally consisted of red bole. However, the color and composition of the bole could vary depending on the effect desired. Gold was then burnished to become shiny and decorated with a punch, with tips variously shaped like rosettes, circles, and diamonds impressed on the table. Guazzo gilding was conducted before painting

the rest of the work, and it is the technique used for gold background paintings like the well-known masterpieces by Giotto.

A different technique was the *missione* gilding, named after the oil-resin adhesive used to apply gold to paintings that had already been completed and not subjected to burnishing. The *missione* was a mixture of resins and oils that made the gold foil adhere, practically gluing it to the surface. It was used to deliberately differentiate some parts from the *guazzo* gilding, which was then polished. In fact, with the *missione*, gold cannot be burnished, and many times, the artists used different techniques in the same artifact to differentiate the visual effects on different areas.

Another gilding technique was the so-called *shell gold*, which is a way of painting with gold: it consisted of grinding gold, mixing the powder with natural gums, and applying it with a brush. Its name is derived from the preservation of the fine powder in the valve of a mollusc.

Painting techniques: pigments, binders, and technical expedients in the use of colours

The palette of the Italian Renaissance is a fascinating array of diverse pigments such as lapis lazuli, azurite, smalt for the blue, malachite, verdigris, green earth for the green, cinnabar, minium, red ochre, and red lakes for the red, yellow ochre, orpiment, lead-tin yellow (*giallorino*), Naples yellow for the yellow tones, and then gold, carbon black, calcium carbonate and lead white.

By pictorial technique, we usually mean the type of organic binder used to dilute and apply the powder pigments. The pictorial technique mainly used during the Middle Ages was the egg yolk-based tempera. For egg tempera, the recipes were different according to various authors and treatises: Cennini recommends an egg yolk with an equal quantity of pigment diluted in water by weight [5]. Vasari suggests a beaten egg with additions of fig latex obtained from chopped fig branches: this juice, which has a milky appearance due to the presence of resin, wax, and rubber, gives the tempera the property of delaying the drying of the colors [6]. Another recipe is an egg yolk plus egg white whipped until stiff (from which the liquid settled at the bottom was obtained) and a spoonful of vinegar. Egg tempera gave the colors particular glaze, conferring the paintings a unique charm and a specific resistance over time.

It was also possible to make the tempera greasier by adding a few drops of drying oil (linseed or walnut oil); from this so-called fat tempera, we moved later and gradually to the use of oil on wood and then on canvas.

Each painting technique allowed for a different rendering and use of colours, affecting, for example, the way of applying the brushstrokes. Different artists have experimented with these over the centuries, and from these experimentations, various technical-pictorial expedients for rendering the colours, volumetrics, and landscapes were born.

For example, we can find colours obtained from overlaying various paints. The flesh tones, for example, could be obtained by applying the *verdaccio*, a layer of green preparation that gave the typical incarnation tone. This operation was an expedient already used by the ancients, who applied the green or grey base first and then the warm tones.

With the arrival of the Renaissance, whose task was imitating and, therefore, painting nature realistically, the way of applying brushstrokes evolved profoundly. It moved from the combination of colours and the application of flat backgrounds to the use, for example, of thinner brushstrokes with thin hatching and later arriving at light and nuanced semi-transparent glazes. These technical-pictorial expedients served to render the figures more realistic and more human, with the folds of the robes outlining the volume of the body underneath. Moreover, particular attention also begins to be paid to the preciousness of the materials and to the embroidered and gilded fabrics, which become to be painted in an increasingly realistic way.

The Renaissance painters, in their quest for realism and depth, began to experiment with new techniques. They introduced oil into their compositions, using it for specific colours or to separate the preparatory layer – which would hold the initial drawing – from the subsequent painting layers. This innovative use of oil, especially in glazes, allowed for the vibrant rendering of the bright and precious shades of some pigments such as red lakes and verdigris.

Concerning varnishes, i.e., the final application of a layer of transparent natural resin, it was not much appreciated in our tradition. Cennini [5] states that the varnish annuls any other tempera and that, in any case, one must wait a long time before applying any substance.

1.2 Panel paintings and degradation factors

Besides studying ancient materials and techniques with which the artwork was created, the study of degradation factors plays a pivotal role in the scientific and technical investigation of artwork since they deeply affect its materiality and influence its state of preservation. In particular, the integrity of panel paintings is influenced by factors linked to the environment in which they are preserved and by phenomena determined by precise physical laws, which concern the type of wood used, the cut, and the seasoning. As for the materials and execution techniques, these degradation problems can also vary greatly, resulting in an extensive and varied series of cases. Therefore, an exhaustive description of all the degradation factors and issues would require dedicated work, which goes beyond the scope of this dissertation.

However, it is considered proper to provide the reader with a summary of the primary degradation factors and subsequent issues linked to panel paintings, which are often attempted to be investigated through scientific analysis and also provide reference terminology. One can refer to [3] for an insight into this topic.

Wood shrinkage

Being a biological material with a highly anisotropic structure, wood undergoes shrinkage in response to variations in environmental parameters and this movement can occur with different relationships between the parts, causing deformations such as warping – the most common deformation – scything, arching, or twisting.

Construction defects

Construction defects can affect the preservation of the painting. For example, using a very knotty plank, where the broken knots have not been repaired in a workmanlike manner, or the choice of support already originally attacked by insects xylophagous and therefore deteriorated can lead to important issues. Even the reuse supports, i.e., the "recycled" panels – created for other projects and then recovered and readapted to new needs – were subject to degradation: operations such as lengthening, widening, or resection inevitably created dangerous weakenings or conditioning.

Biological attack

It is a very common degradation factor: in addition to xylophagous insects, fungi and bacteria can also attack the wood leading to irreparable – sometimes even structural – damage.

Dimensional changes

In the 15th century, some transformations occurred to adapt medieval formats to Renaissance canons. These operations involved severe damage to the artworks, causing the loss of part of the painting and architecture and the tampering of the original crossings. Those transformations created further damage due to the wooden additions and the anchors created to connect the added parts, which were so invasive that they seriously affected the preservation of the works.

Moreover, dimensional changes occurred over time to adapt the panel paintings to the furniture in churches and civil buildings, following the evolution of the taste and style of the various epochs.

Past conservation treatments

Past conservation treatments significantly impacted the preservation history of panel paintings. Today's conservators often have to remedy damage caused by previous conservation treatments.

These treatments could be localized or involve the entire work. Among the former, we can mention the case of aggressive cleaning, which often took place on the flesh tones removing part of the original layers while leaving out the cleaning of the remaining parts of the painting. Moreover, we can find localized overpainting, often conducted in correspondence of area or pigment particularly degraded, to revive the color.

Among the total interventions, there is undoubtedly the application of multiple layers of varnishes on the front of the painting, but also interventions on the support, such as thinning the panel from the back or transporting the painting from the original panel to canvas support. This invasive practice often occurred when a biological attack profoundly damaged the wooden panel.

1.3 The scientific analysis of panel paintings: interdisciplinarity, research, and tailored multi-analytical approach

It's heartening to see the increasing adoption of scientific analysis in the study and conservation of cultural heritage. This sector is becoming increasingly interdisciplinary, and each professional – curators, conservators, scientists, and art historians – plays a fundamental role in defining the artwork's study and research program and identifying all the questions to investigate. Multiple and complex questions regarding material, technical, historical, and stylistic aspects can arise around the object, especially during conservative treatments, representing a pivotal moment for exchanging knowledge.

The interdisciplinary nature of the working team, fostering the exchange of skills, is also the foundation for developing new analytical techniques and research topics. An example is undoubtedly that of the CNR's researchers, who, working closely with artworks and curators, develop increasingly cutting-edge instrumentation [7-9]. Similarly, many university research groups are involved in developing new instruments or protocols for studying and analyzing cultural heritage [10-15]. In addition to attempting to answer the questions raised, these innovative instruments and protocols take into account today's needs, such as the instrument's portability, the speed of acquisition, and the noninvasiveness of the analysis. Think, for example, of the accessibility of museums and the importance of rapid data acquisition when exhibited works of art cannot be transferred and must be analyzed in situ. Another effect of the team's interdisciplinarity is the rapid development of imaging and mapping technologies compared to spot techniques in the last few years [7, 8, 16, 17]. Images and maps are far more easily readable than graphs and spectra and transferable in a broader context among professionals with different backgrounds.

Today, it is necessary to adopt a multi-analytical approach to study and thoroughly understand the artwork. That is, to resort to various analyses that complement each other – each one providing a piece of information. This allows for leveraging each analytical technique's full potential and complementarity to provide more coherent data on the artwork's material and structure. Awareness of the potential of a multi-analytic approach is so widespread that its use is not confined to a specific epoch or artifact but is applied across the entire spectrum of cultural heritage. This approach has proven effective in various contexts, from the artifacts of Ancient Egypt [18-21] to ethnographic items [22], from illustrated manuscripts [23] and Renaissance panel paintings [10, 24-27] to contemporary art [28, 29].

Given the common view that integrating results from different analyses is necessary for a more in-depth investigation, it is unsurprising that even technological development in heritage science is moving towards combining two or more techniques in a single investigation [30-35].

Using more techniques has an impactful consequence for the operator: it inevitably leads to collecting a large amount of data of different types – from very high-resolution images to numbers to spectra – whose management with various software can be complex. Moreover, once these data have been acquired and processed, the operator must be able to interpret them, cross-reference the results from the various analyses, and present them with a common terminology that is understandable to the entire work group. Interpretation is a very delicate phase of this process: in addition to studying sources – where we can find a possible interpretation of the data collected –, experience also plays a fundamental role, that is, having studied and investigated artworks with similar characteristics by the same author or from the same period. Similarly, reading and comparing texts closer to us, i.e., scientific publications on comparable artworks, is very helpful in comparing results.

Scientific publications on panel paintings show that using some analytical techniques is pivotal, especially those that can analyze the wooden panel and all its characteristics, such as the wood grain or the tunnels of woodworms, internal voids, or fillings. For this purpose, alongside traditional X-ray radiography (XR) [1, 2, 24, 25], pulse compression thermography (Pu-CT) has also been tested in recent years, proving particularly effective [36-38]. However, while specific analytical techniques are undeniably crucial, not all artworks can be approached with the same analytical plan. Each work of art is a unique entity, heavily influenced by its nature, materiality, and history, necessitating a tailored approach. These unique characteristics significantly influence its state of preservation, adding complexity to the conservation process and explicitly defining the questions that scientific analysis must answer.

These specificities underline the crucial role of the scientific team in refining, integrating, or designing tailored analytical plans and methods, which can reveal specific data and add an essential piece to the artifact's knowledge. Recognizing the need for tailored analytical plans is a hallmark opportunity in this area. It enables the publication of case studies describing the specifics of the artwork, the analytical difficulties, and the analytical strategies used to answer the most challenging questions, with the final aim of creating a wealth of valuable experience for approaching artworks with the same or similar problems, fostering shared knowledge among the community.

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Chapter 2

THE NORTHERN-ITALY RENAISSANCE IN A PANEL BY DEFENDENTE FERRARI: A COMPLETE STUDY WITH A MULTI-ANALYTICAL INVESTIGATION

2.1 Introduction

This chapter deals with the scientific study carried out with an interdisciplinary and multi-analytical approach on the panel painting named *Madonna with Child and two Saints* by the illustrious painter Defendente Ferrari (Chivasso, c. 1480/1485 – Torino, c. 1540). The painting was originally located in the Collegiate Church of Carmagnola (province of Torino, Italy) and is currently in the collections of Palazzo Madama – Museo Civico di Arte Antica, in Torino. In 2017, with the financial support of Compagnia di San Paolo, the painting was transferred to the Centro per la Conservazione ed il Restauro dei Beni Culturali *La Venaria Reale* (CCR) for study and conservation purposes.

The activities performed on this painting were part of a more extensive research and conservation project by the CCR, which began in 2014 and focused on the artist and his circle. Defendente Ferrari was one of the most important painters of the Piedmontese Renaissance in the early 16th century. From the research carried out so far, it emerged that the cartoon of the Virgin and Child used for the preliminary concept of this painting was greatly appreciated since it was adopted in the artist's workshop for other contemporary and subsequent works [1].

The conservation treatment of this painting required the design of a specific, multi-analytical diagnostic campaign. This campaign was based on the use of standard and innovative methods to answer different questions. To accomplish this, we conducted the diagnostic campaign at CCR in close collaboration with several research institutions. This collaborative effort led to the production of a scientific publication within the framework of this PhD program [2], reinforcing our shared commitment to conservation research.

It is worth mentioning that we carried out part of this study during the COVID-19 pandemic. Luckily, we had collected most of the experimental data before, and subsequently, we

conducted most of the data processing and integration via virtual meetings. Despite the number of collaborators, it was possible to remotely plan several discussions so as to involve, at best, all the disciplines (science, art history, conservation).

2.2 Research aims

The *Madonna with Child and Two Saints* from Palazzo Madama – Museo Civico di Arte Antica (Torino, Italy) is a panel painting by Defendente Ferrari, dating back to around 1505-1510.

The artwork depicts the Virgin Mary and her Child in between two Saints. The Child is in the middle, his right hand raised in the gesture of blessing while holding the globe in the left one. The Virgin turns her gaze towards the Child – her head slightly tilted to the left. On the sides, two Saints wear luxurious armors, decorated with gold and jewels. The fiery red color of the drape on the Virgin's throne is the same as the cape resting on the left shoulder of the Saint on the left, holding the white crusader banner of the Theban Martyrs, as well as in the breastplate of the Saint on the right, holding a feather. Each character is meticulously detailed, from the folds of their garments to the expressions on their faces. An architectural background frames the scene, in which two lateral pilasters, surmounted by a capital, are visible (Figure 2.1a).

Before the last conservation treatment carried out at CCR, the painting showed a global, general yellowing, possibly due to laid-up varnishes, coupled with various forms of alteration and degradation, which compromised the satisfactory legibility of the artwork. These faults mainly affected the colors and volumes of Virgin's mantle and dress, where numerous retouches were present, resulting in a duller and flattened tone wherever present. On the slightly embossed panels, innumerable holes were evident on the front and back, evidence of previous entomotic attacks. Besides, various deformations were visible on the painting surface, such as cracks and slippage of color, dents, and hollows due to woodworm tunnels running under the preparatory layers. Within the halos of the Virgin and Child were nails and residues of metal foil as well as several holes, giving evidence of previous application of relief metal aureoles for devotional purposes. Moreover, dark spots were visible on the side of Virgin's face near the holes, particularly in the neck and the veil areas, where a ripple and a bubble deformed the surface, suggesting that the painting might have been exposed to a localized heat source (Figure 2.1b). Also, on some surface areas (e.g., near the Saint on the left; Figure 2.2a), a glimpse of a very tenacious brown-to-greyish patina was perceivable, probably partially removed and integrated (Figure 2.2b) during a previous restoration intervention [1].



a

b

Figure 2.1 *Madonna with Child and Two Saints* by Defendente Ferrari, before the last conservation treatment carried out at CCR: a) photograph in visible diffuse light; b) detail in visible raking light.

Considering the complex state of preservation of the artwork, the scientific analysis first aimed at better understanding the numerous forms of degradation, starting by studying the original material and techniques and the possible overlaid materials applied during previous conservation treatments.

In particular, the issue of the blue color of the Virgin's garment – in such an advanced state of alteration as to appear strongly blackened – was of extreme importance since this form of degradation seems to be quite recurrent not only in other works by the same author [3, 4] but

also in other artworks painted by different authors of the same period [5, 6]. Therefore, the study of this aspect aimed at gathering valuable data for understanding and possibly solving a diffuse problem.



Figure 2.2 Detail of the face of the Saint on the left: *a*) brown-greyish patina; *b*) magnification on previous retouching.

Another aim of the scientific analysis was to study the artist's unique executive strategies, also recognizable in other artworks [1, 4, 7], by detecting the materials and techniques adopted. One of the most interesting aspects from a technical and artistic point of view is the use of the *dotted* technique, a practice applied to render the nuances, volume, and flesh tones of the figures. This technique, which relies on the application of extremely thin dots on the paint surface using an unknown tool, showcases the artist's skill and precision. Another unique technical expedient is using thin, black cross-hatched strokes drawn to shape the clothes and aptly define the related volumes and folds, further demonstrating the artist's mastery.

2.3 Analytical strategy, experimental, and methods

A diagnostic campaign was set up with a multi-analytical approach to meet the research aims. Complementary techniques were selected to cross-reference the data and obtain more reliable results. Among the adopted methods, some analytical techniques with specific potential were selected to respond primarily to specific objectives.

For a preliminary interdisciplinary study of the painting, the wooden support, and the state of preservation, grazing visible light photographs, images in the UV, Vis, and NIR bands, and digital X-ray radiography (XR) were acquired.

A high-resolution Short-Wave Infrared reflectography (HR SWIR) was acquired with a prototype scanning system in the 1000-1700 nm range for insight into the underdrawing and the particular cross-hatched traits.

Imaging and spot techniques, known for their complementarity, were combined in order to map the different regions of the painting and to reconstruct the pictorial palette: in particular, the macro X-ray fluorescence (MA-XRF) was applied with a novel prototype system; no hyperspectral imaging system (HSI) was available for this study, but it was possible to apply the recently developed method of hypercolorimetric multispectral imaging (HMI). Fiber optic reflectance spectroscopy (FORS) in the 380-2200 nm range and portable Raman spectroscopy were used for a punctual characterization of pigments.

Some micro-samples were removed and analyzed with optical microscopy under Vis and UV light and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM/EDS) to study the painting stratigraphy.

Binders and varnishes were analyzed using Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectroscopy (GC/MS). Knowing the nature of the binder was immediately considered essential to understanding the dotted technique and the different renderings of the colors. Since no method available for this study could directly analyze organic compounds on the cross sections, FTIR and GC-MS were applied to selective samples.

Concerning the garment painted in azurite, nowadays characterized by severe darkening, this aspect was of particular value because we needed to evaluate the reversibility of the darkening process and the possibility of partially recovering the garment's volume and color to plan a correct conservation treatment. To investigate the nature of the pigment, binder, and degradation forms in depth, GC-MS, micro-Raman (μ -Raman), and micro-X-ray diffraction (μ -XRD) were performed.

Overall, the analytical plan adopted for this artwork involved the use of traditional and state-of-the-art techniques and the collaboration of the following research institutes and universities: the Department of Economics, Engineering, Society and Business Organization (DEIM) of the University of Tuscia for the HMI analyses, the Department of Earth Sciences of the University

of Torino for the μ -Raman and μ -XRD analyses, the Department of Chemistry and Industrial Chemistry of the University of Pisa for the GC-MS, the section of Catania of the CNR-Istituto di Scienze del Patrimonio Culturale for the MA-XRF, the ICCOM-CNR unit of Pisa for the portable Raman spectroscopy, the *Aldo Pontremoli* Department of Physics of the University of Milan for the HR SWIR reflectography.

The analytical plan and strategies were carefully designed within the framework of this PhD to ensure the highest quality of research. The process involved: conducting comprehensive analyses, coordinating the entire scientific team, cross-referencing and interpreting all results to pursue the research goals.

All the methods applied followed the general well-established rule based on the analysis conducted in subsequent phases. All the non-invasive imaging and spot techniques preceded the application of micro-invasive methods to minimize the number of samplings. Of course, the removal of samples was conducted only where strictly necessary and via a preliminary and specific authorization of the supervisory organizations.

In this analytical process, the outcomes of imaging analyses were used as a guide to select the most representative spots of analysis. Once all the images were acquired, multi-layer Adobe Photoshop (*.psd) files were composed by overlapping the various bands and maps (e.g., UVF, MA-XRF) and used to better visualize and cross-reference the imaging data and select the spots of analysis.

All methods adopted are described below. Figure 2.3 shows the localization of spot analyses and sampling sites; Table 2.1 lists the studies carried out on each spot or sampling site.

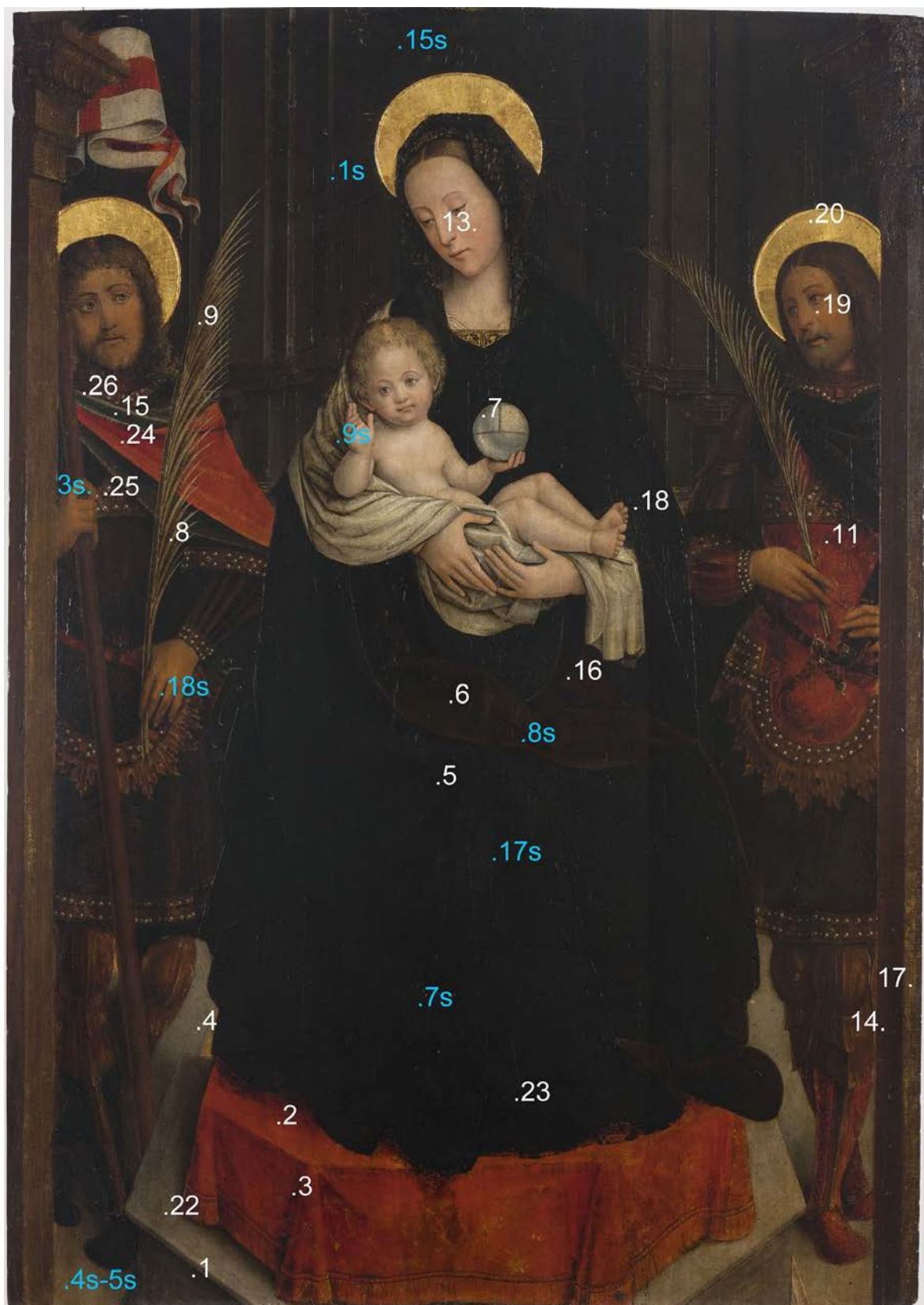


Figure 2.3 The painting after the last conservation treatment carried out at CCR with the spots of non-invasive analysis (in white) and sampling sites (in blue) localized. For the details of the analyses, see Table 2.1.

Table 2.1 List of the analyses carried out for each spot or sampling site (see Figure 2.3).

SPOT NUMBER AND SURFACE COLOUR	NON-INVASIVE SPOT ANALYSES
1 greyish	FORS
2 red	RAMAN, FORS
3 dark red	FORS
4 green-grey	FORS
5 blue	FORS
6 brown-green	FORS
7 light blue	FORS
8 light yellow	RAMAN, FORS
9 brown	FORS
11 red	FORS
13 pinkish flesh tone	FORS
14 brown	FORS
15 green	FORS
16 red	FORS
17 gold	FORS
18 blue	FORS
19 flesh	FORS
20 gold	FORS
22 light blue	FORS
23 black over blue	RAMAN, FORS
24 red	RAMAN, FORS
25 white	RAMAN, FORS
26 light yellow	RAMAN, FORS
SPOT NUMBER AND SURFACE COLOUR*	MICRO-INVASIVE ANALYSES
1s dark greyish	FTIR
3s dark flesh tone	FTIR
4s greyish light blue	FTIR
5s greyish light blue	GC/MS
7s darkened blue	OM, SEM/EDS, GC/MS
8s browned green	OM, SEM/EDS, GC/MS
9s whitish	FTIR
15s dark grey	FTIR
17s darkened blue	μ -RAMAN, μ -XRD
18s dark flesh tone	GC/MS

**Missing numbers refer to failed sampling or non-representative analyses.*

2.3.1 UV-Vis-NIR imaging techniques

For visible diffuse light photography (Vis), lighting was achieved by placing two 800W Ianiro Varibeam Halogen lamps to the right and left of the object, at an angle of approximately 45° to the normal to the surface, and with the aid of umbrellas for light diffusion. For grazing light photography (g-Vis), lighting was attained by positioning one lamp to the left of the object at a maximum angle of approximately 15° to the surface. Photographs were taken with a Nikon D810 Full-Frame DSLR camera (CMOS 7360 x 4912 pixels). The UV-induced visible fluorescence (UVF) was acquired with a Nikon D810 full-frame DSLR camera (CMOS 7360 x 4912 pixel) and a Hoya UV-IR Cut filter, irradiating the painting with two UV Labino® lamps (365 nm-emission peak). The Near-Infrared reflectography (NIR) was acquired with a Nikon D810 full-frame DSLR camera (CMOS 7360 x 4912 pixel) modified to extend the sensor sensitivity range and with a B+W 093 (87c) filter, illuminating the painting with Ianiro Varibeam Halogen 800 W lamps with indirect diffused light. For all acquisitions, image processing was carried out using Adobe Lightroom and Adobe Photoshop software, and a color correction was included through a 24-color ColorChecker Classic reference in the field of view.

2.3.2 High-resolution short-wave infrared reflectography (HR-SWIR)

HR-SWIR was performed with a spherical scanning system [8] based on an InGaAs camera (Xenics Xeva 1.7-640, 640×512 elements, spectral sensitivity: 1000–1700 nm) mounted on a spherical motorized head with a linear stage for refocusing. Two thousand two hundred images were acquired and merged to obtain a final image with a spatial resolution of 20 pixels/mm. Two halogen lamps with indirect and diffusing screens were used for the lighting.

2.3.3 X-radiography (XR)

The XR study was carried out with an innovative radio-tomographic apparatus, specifically designed to examine large-size artworks (up to 4 m wide and 3 m high). This apparatus was developed by the National Institute of Nuclear Physics (INFN) and the Department of Physics of the University of Torino in cooperation with CCR. The collaboration was part of the ‘*neu_ART*’ research project, which aimed to advance the field of art conservation through the development of new technologies. While the X-ray source is allowed to move vertically, the linear detector can move both horizontally and vertically. This allows for the sequential

collection of different radiographs by modulating the height of the X-ray source and the position of the detector [9, 10, 11].

2.3.4 Macro X-ray fluorescence (MA-XRF)

MA-XRF was performed with a mobile LANDIS-X scanner, used in situ at the CCR and developed by the 'XRAYlab' of the Istituto di Scienze del Patrimonio Culturale of the National Research Council (ISPC-CNR) and by the Laboratori Nazionali del Sud of the National Institute of Nuclear Physics (LNS-INFN), sections of Catania.

This scanning system can analyze large pictorial surfaces (up to 110 x 70 cm²) in short times (2 hours for covering the total area; lateral resolution of the imaging: 1000 µm) [12]. Based on the size of the painting and its pictorial details, the XRF imaging can be performed at different lateral resolutions (from a few dozen - micro XRF - up to few hundreds µm - MA-XRF) by simply placing the surface at varying distances from the measuring detector. A real-time technology allows continuous scanning of the surface, thus providing images in 'live' mode of each chemical element composing the pictorial layers and its distribution on the whole support. The primary X-ray beam, generated by a Rh-anode tube with 50 kV maximum voltage and 0.6 mA maximum current, is focused on the sample with a polycapillary optics.

A time list mode (TLIST), implemented in a multi-detector system, is used to detect the fluorescence radiation, with a ms acquisition time/pixel and high counting statistics. Two 50-mm² silicon drift detectors (SDD) are used, with a spectral energy resolution of 160 eV at 5.9 keV and a fast-fitting procedure (max processing speed: 7000 spectra/s), which can be moved through an XYZ-axis system (110 x 70 x 20 cm³) generating on-the-fly elemental images. Movement on the Z-axis accounts not only for a dynamical correction of the detector/surface distance (in case the surface is not flat), but also for allowing an investigation of the sample stratigraphy even at the deeper layers [13, 14, 15, 16, 17].

The full area of the painting (consisting of 794 x 655 pixels) was analyzed with a scanning speed of 80 mm/s, a dwell time/pixel of 12 ms and a lateral resolution of 1 mm. The measurement time was 1.7 hours, and the primary X-ray beam voltage was set at 50 kV with a current of 600 µA.

2.3.5 Hypercolorimetric multispectral imaging (HMI)

The HMI study was performed with a 36-megapixel reflex Nikon D800 digital camera modified to operate in the 300-1000 nm extended range, combined with suitable flash lamps and optical filters [18, 19, 20]. The SpectraPick Profilocore® software processed the HMI acquisitions into seven monochromatic images in TIFF files (plus an Adobe RGB color image) corresponding to seven spectral bands: UV reflected, the three visible bands R-G-B, and three near-infrared bands IR1, IR2, IR3. Therefore, we obtained seven images of the artwork after the calibration procedure centered at 350, 450, 550, 650, 750, 850, and 950 nm, together with the RGB output.

We used the PickViewer software to process the imaging data of the seven calibrated bands, obtaining colorimetric measurements, false-color images, PCA, cluster analysis, and spectral similarity mapping.

2.3.6 Fiber optics reflectance spectroscopy (FORS)

FORS analyses were carried out with a compact and portable system based on two spectral analyzers (a MCS 601 UV and a MCS 611 NIR 2.2 Zeiss Multi Channel Spectrometer system), a CLH 600 tungsten halogen lamp, 1 mm diameter optical fibers, and a Labsphere Spectralon Wavelength Calibration Standards white reference. Spectra were acquired in reflectance mode, within a 380–2200 nm spectral range, with a spectral resolution of about 2.5 nm in the Vis range and 18 nm in the NIR range, and with a 0°/2 x 45° optical geometry.

Spectra were interpreted by comparison with published literature and spectral libraries available at the CCR [21].

2.3.7 Raman spectroscopy (RAMAN)

In situ Raman measurements were carried out with a portable i-Raman® Plus instrument (BWTEK, USA), equipped with a diode laser emitting at 785 nm. Spectra were acquired in the 60-3300 cm⁻¹ range, with a spectral resolution of 3.5 cm⁻¹ @ 614 nm, exposure time of 10 s/acquisition, and laser power < 2.5 mW [22].

2.3.8 *Fourier transform infrared spectroscopy (FTIR)*

FTIR spectroscopy analysis on selective micro-samples was performed with a Bruker Vertex 70 FTIR spectrometer, coupled with a Bruker Hyperion 3000 IR microscope, and equipped with a Hg-Cd-Te (MCT) detector. Tiny specimens (few hundreds of μm across) scraped from the artwork surface were analyzed as bulk in transmission mode while compressing in a diamond cell, with a 15x objective. Spectra (resulting from the sum of 32 scans) were collected in the $4000\text{--}650\text{ cm}^{-1}$ range, with a 4 cm^{-1} resolution.

2.3.9 *Preparation of cross sections, optical microscopy (OM), and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDS)*

Multi-layered samples were removed from the artwork to prepare the cross sections in the CCR laboratories. First, samples were observed and photographed under visible light using an Olympus SZX10 stereomicroscope equipped with an Olympus Color View I digital camera. Then, the cross sections were obtained by embedding each sample within a double layer of epoxy resin. After removing the excess resin, the sample surface was finely polished using Struers abrasive cloths of progressively finer grits to expose the paint stratigraphy, thus enabling observation at high magnifications and scientific analysis with various instrumental techniques. Cross sections were observed and photographed under visible and ultraviolet light using an Olympus BX51 minero-petrographic microscope equipped with an Olympus DP71 digital camera. Image acquisition and processing were performed using analySIS FIVE proprietary software. Moreover, they were observed and analyzed with a Zeiss EVO60 scanning electron microscope equipped with a lanthanum hexaboride (LaB6) cathode and a silicon drift detector (SDD) and coupled with a 40 mm² Oxford Ultim Max EDS microprobe for semi-quantitative elemental analysis. Samples were coated with a nanometric layer of carbon and analyzed in high vacuum mode, using an accelerating voltage of 20 kV and a pressure of 60 Pa.

2.3.10 *Gas chromatography mass spectrometry (GC/MS)*

GC/MS analyses on selective samples were conducted to characterize the paint binders and varnishes. The protocol applied is based on a set of extraction and chemolysis steps aimed at producing an amino acidic fraction for the identification of proteins, a lipid-resinous fraction

for the characterization of lipids, resins, and waxes. Data were collected with a 6890N gas chromatograph (Agilent Technologies) equipped with a PTV injector and coupled with a 5975 Mass Selective Detector (Agilent Technologies). The analytical protocol and technical details are reported in the literature [23].

2.3.11 Micro-Raman (μ -Raman) and micro-X-ray diffraction (μ -XRD)

μ -Raman and μ -XRD data were collected on two samples coming from a single sampling site. μ -Raman analysis was carried out with an inVia Raman Microscope (Renishaw; filter: edge; integration time: 2 spectra/5 seconds), with an excitation line of 532 nm (green) and a 600 mesh/mm sieve (hole 200; slit 300). Data were collected directly on the extracted sample, by using a 100 X objective and D0.6 and D1 attenuating filters, in order to avoid any phase alteration induced by the laser energy.

μ -XRD data were collected on a Rigaku-Assing 'SMARTLAB XE' with $\text{CuK}\alpha$ radiation, equipped with CBO (Cross Beam Optics; parallel beam) and micro-area CBO-*f* optical devices, a 0.5 mm incident slit and a HyPix-3000 area detector in standard energy-mode and 2D scan-mode (vertical position). Diffraction data were collected on unpowdered samples, in the 3-70° 2 Theta range (step: 0.02°; speed: 0.5°/min) for 3 hours, and interpreted with the 'DIFFRAC PLUS, EVA software, Application 7.0.0.1' (2001), through direct comparison with proper databases (JCPDS-ICCD; ICSD; PCPDFWIN).

2.4 Results and discussion

The results reported hereafter are not listed technique by technique but interpreted and shown following the layering of the artwork – from the wooden support to the outer paint layers and varnishes, up to the layers of non-original materials applied during past treatments.

This approach to the discussion underscores the importance of integrating the diagnostic data from the different applied methods, ensuring the reliability of the results, and avoiding those partial interpretations that could be supported by one method but disclaimed by the other. However, for clarity of reading, each piece of information in the discussion is referred to the analytical technique/techniques from which it has been deduced.

2.4.1 *The wooden support and the resizing*

The painting's XR (Figure 2.4a), coupled with technical photography and direct observations, was fundamental for analyzing the wooden support in terms of assembly technique, past restorations, and state of preservation.

The panel is made of three thick planks of poplar wood, which are tangentially cut and vertically oriented. As shown in the detail of Figure 2.4b, the two parallel lines along the junction indicate the assembling using half-lap joints. The defects and numerous knots scattered over the surface suggest a relatively low wood quality.

By thoroughly observing the panel, it was evident that the artwork underwent a height resizing, which was also confirmed by the related analytical outcomes. Its upper and lower margins were resected throughout its entire thickness, affecting the figurative apparatus. Above the capitals of the side pillars, a curved double-incision (highlightable in g-Vis) defines an element painted in a greenish tone (probably a tent or a curtain), which was evidently resected with the upper part of the artwork. The hypothesis of the resizing is also further corroborated by the radiographic images, which highlight the presence of wooden pins inserted in correspondence with the junctions. One of these pins is very close to the left top edge (Figure 2.4b). In fact, between the left axis and the central one, the upper pin is close to the upper margin, whereas between the central axis and the right one, the pin is close to the lower edge.

The support shows clear signs of an intervened thinning, which left the underlying woodworm galleries on its back and edges uncovered. It is evidence of the total absence of traces related to ancient crossbars or any containment systems on the support, which were evidently lost during thinning.

The XR highlights another step of the history of the panel: two areas of marked unevenness are observed towards the lateral margins, at the same level as the faces of the Saints. Such unevenness could be perhaps related to nails initially inserted (and sealed) but later removed, connected to the crossbar on the back, or rather to the frame on the front [1].

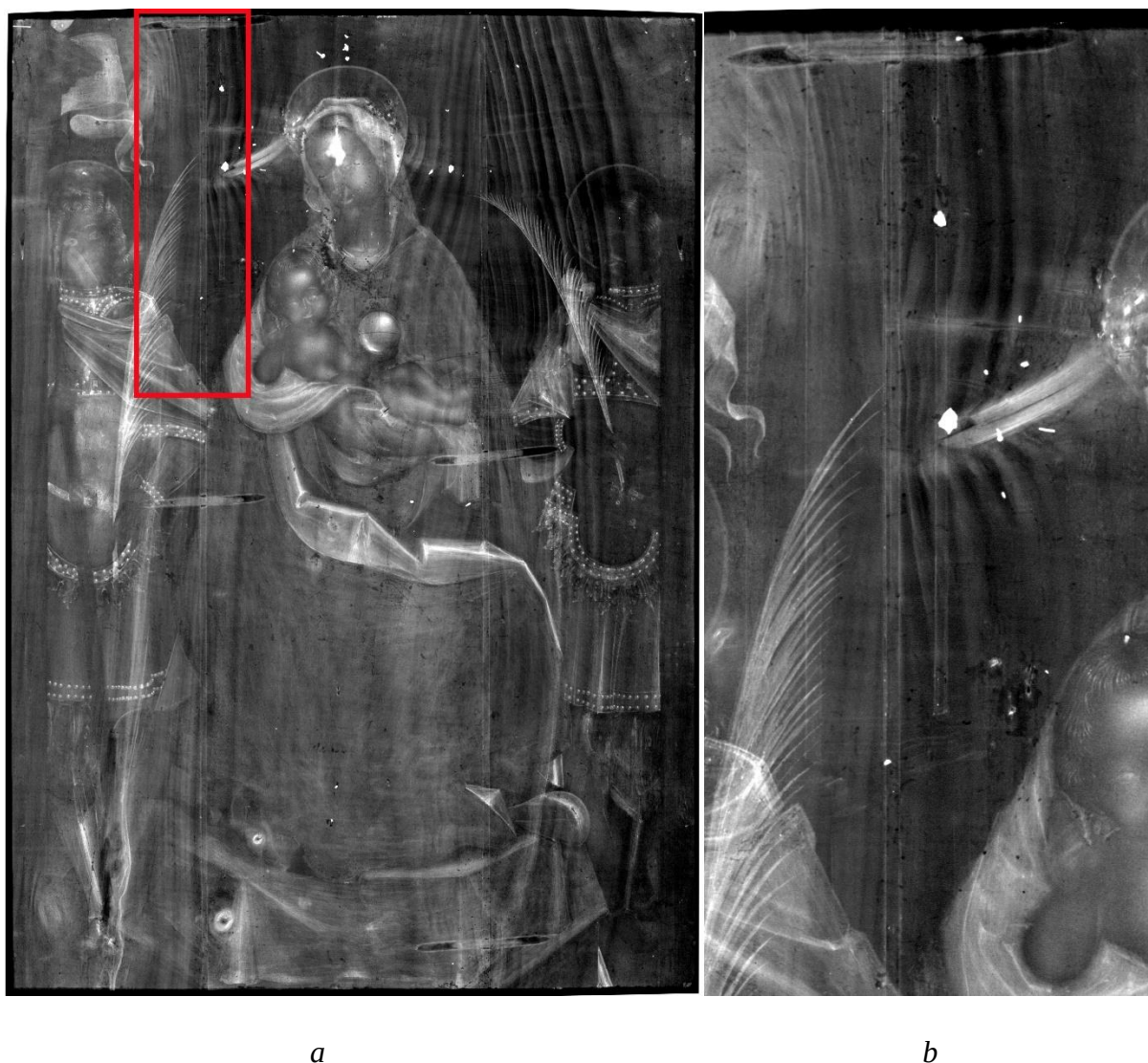


Figure 2.4 XR of the painting: *a*) image of the entire panel; *b*) detail of the red square where it is possible to notice one pin too close to the edge, indicating a previous resizing and the two parallel lines running from top to bottom showing the presence of half-lap joints to assemble the planks.

2.4.2 Preparation layer, incisions, and underdrawings

Combining the MA-XRF maps and SEM/EDS results on cross sections enabled us to confirm and map the presence of a thick, gypsum-based preparation layer containing K and other Si/Al impurities, which was applied directly onto the supporting panel. Such a layer was probably applied using an animal glue-based binder and aptly smoothed to accommodate the upper pictorial layers, as described in the literature [24]. The presence and distribution of Ca and K related to the thick gypsum preparation layer are well evident in the MA-XRF maps (Figure

5a) in those regions where they are not covered by Pb-based pigments (i.e., lead white, the signals of which dominate on the flesh tones: see the blue areas in Figure 2.5a).

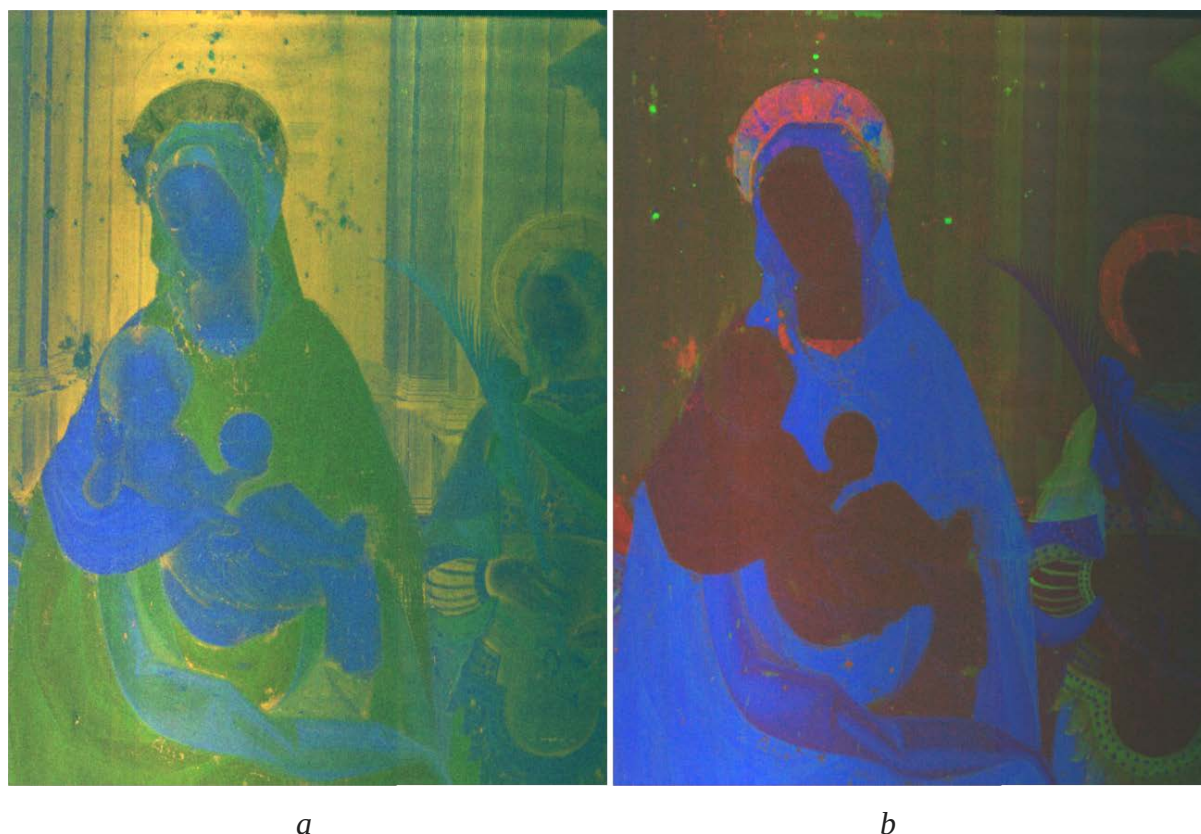


Figure 2.5 MA-XRF RGB correlation maps of: *a*) Ca (red), K (green), Pb (blue); *b*) Au (red), Fe (green), Cu (blue). According to the additive synthesis of colors, yellow results from the synthesis of the R and G channels, magenta from that of R and B (in both cases, indicating the co-presence of the two corresponding elements).

HR-SWIR reflectography (Figure 2.6) – coupled with a systematic and close inspection of the artwork surface – was fundamental for highlighting those techniques used by the artist to prepare his work, particularly the different means used to draw the preliminary sketches. Due to its spectral range, HR-SWIR proved to be fundamental for the visualization of potential underdrawings [8, 25] – not an easy task, considering the presence of azurite in the Virgin's garment and the many other darkened portions of the painting. This method allowed for observing that many details (i.e., the main architectural parts of the background, the contours of the figures, the volume of the drapery, the shoes, and part of the armor of the warrior Saints)

were preliminarily rendered by using construction lines, engraved directly in the preparation layer with a metal tip (incisions). In some cases, these lines were combined with carbon-based ink underdrawings.

The straight comparison of g-Vis and HR-SWIR images allowed for identifying numerous engravings: following the direction of the engraved architectural lines, the central vanishing point was identified, located in the Virgin's neck, thus evidencing an exact perspective design (Figure 2.7). The images collected near the top of the Virgin's halo (Figure 2.8) highlighted the sharp superposition between the underdrawings used to mark the circular incisions and the related architectural lines. Grazing light also allowed for identifying incisions performed with metal tips of different sizes, such as in the pedestal area. However, slight variations concerning the final version are also observed in the Virgin's garment and the construction lines of the pedestal, thus confirming the hypothesis of cardboard being used to transfer the engraved preparatory drawing and the following reworking made directly on the panel as underdrawing.



Figure 2.6 HR-SWIR of the painting.

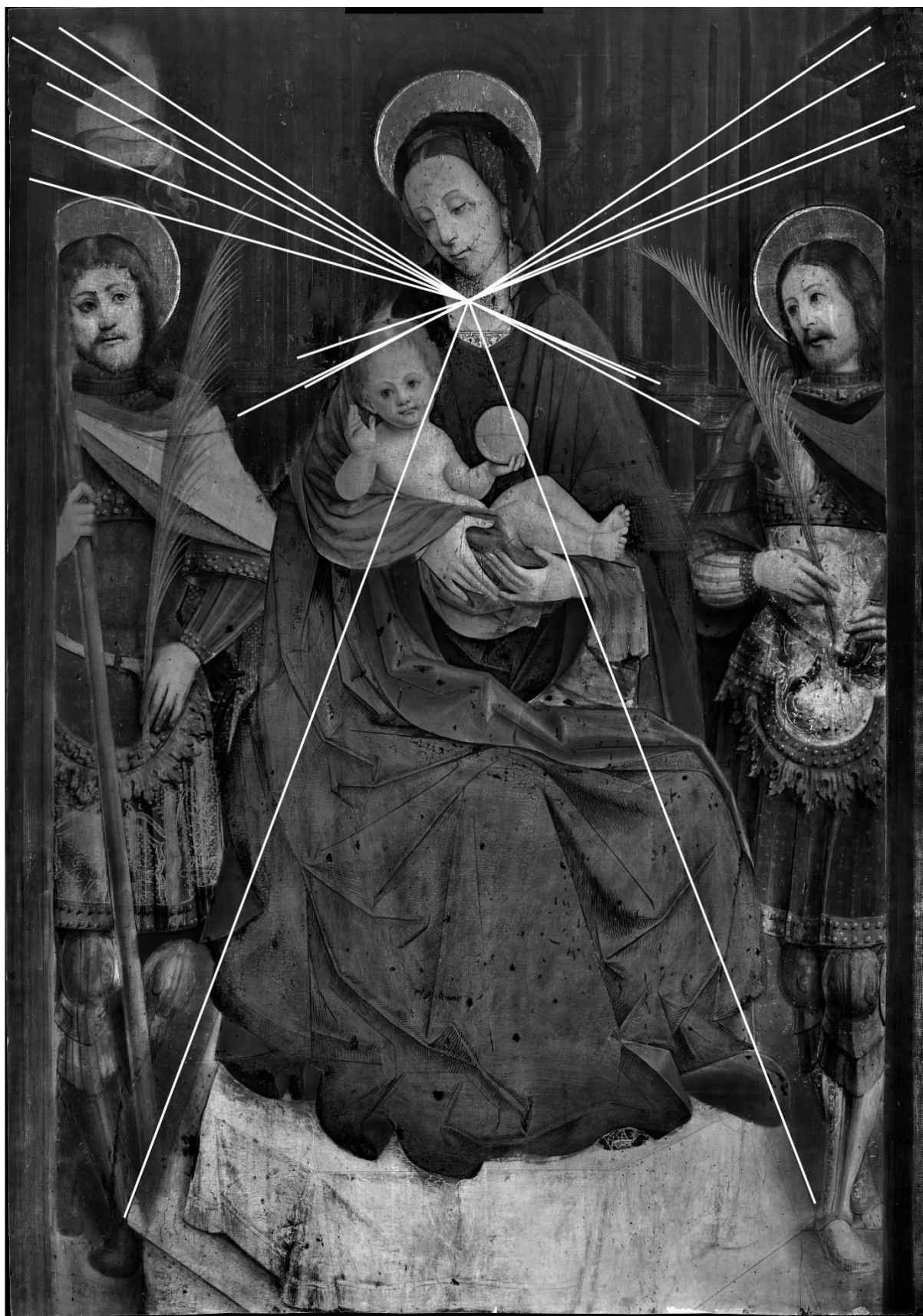


Figure 2.7 HR-SWIR image of the painting with the vanishing point lines overlaid.



Figure 2.8 Detail of the area around the Virgin's halo: *a*) g-Vis; *b*) HR-SWIR.

Although there is no evidence of a preparatory drawing transfer from a cartoon – e.g., pouncing, squaring, or using a paper rubbed with charcoal – the HR-SWIR shows a precise and detailed underdrawing somewhere visible to the naked eye, because of the particular painting technique. This precision proves that the artist fully mastered the graphical techniques used in this panel – from the first touches up to the final retracing of the contours. Draperies and shadows are rendered with elaborate hatching, showing different features concerning the desired effect: single, curved, crossed, and even hook-shaped lines alternate with the other, each with different thicknesses and saturation (Figure 2.9).

The underdrawings generally show two distinct saturation levels. A lighter gray trace is linked to the anatomical details, for which the use of a dry medium – such as charcoal, probably together with carbon-based ink – is possible (see, for example, the face and hand of the Child: Figure 2.10). A second type of saturation, with darker gray brushstrokes, can instead be observed in areas where the hatching occurs and for outlining the shadows. In the light of this result, it seems that the painter, after a first and preliminary setting of the underdrawings with a light trace, went over the exact details again by retracing and darkening these lines with thicker strokes in some specific areas (see, for example, the eyes in Figure 2.11), thus defining their possible shading.



Figure 2.9 HR-SWIR details of the Virgin garment: hatching is made in different ways of shadowing and defining the draperies.



Figure 2.10 The Child's face showing the different types of underdrawings: ink-based and dry medium.

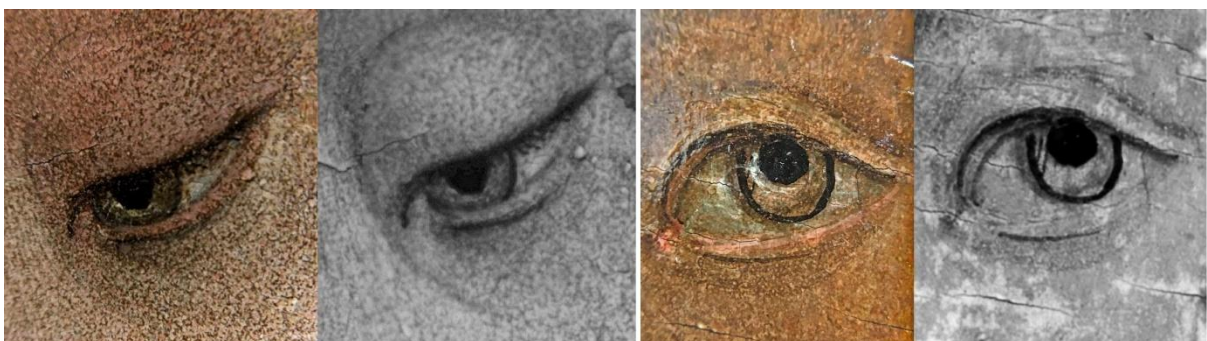


Figure 2.11 Details of the eyes of the Virgin and of the Saint on the left, Vis and HR-SWIR. A double brushstroke trace is present with different saturation, lighter for all the underdrawings, darker for the outlines retracing.

2.4.3 *Pigment palette*

Interpreting and cross-referencing the results from imaging and mapping techniques with noninvasive and micro-invasive analyses made it possible to reconstruct the artist's rich palette. The diagnostic power and complementarity of the techniques adopted played a fundamental role in suggesting or identifying the pigment palette, instilling confidence in the results. As shown in Table 2.2, the noninvasive (imaging, mapping, and spot) techniques allowed for advancing most of the hypotheses on the pigments present in the different spots of analysis, which were subsequently integrated with the micro-invasive outcomes.

Azurite and indigo were identified in the blue areas, green copper-based pigments and possible copper resinate occur in the green portions, red lakes (probably madder) and cinnabar were recognized in the red areas, whereas umber, gold, yellow ocher, and lead-tin yellow type I appear in the yellow ones; the white is mainly rendered with lead white, while the use of bone black and carbon-based black was detected for the black.

Specifically, azurite was preliminary hypothesized from the dark bluish response in the IRFC image obtained by the HMI software (Figure 2.12), the signal of Copper in the MA-XRF map, and the FORS spectrum that - despite having low reflectance values due to the darkening of the paint layer - was characterized by an absorption band centered at 640 nm [26]. However, the use of this pigment was confirmed by μ -XRD analysis performed on a selective sample (sample 17s, Figure 2.13) that unequivocally detected the typical diffraction pattern of azurite. An in-depth study of azurite about the degradation of the Virgin's blue garment is reported later.

The presence of indigo was preliminarily suggested by the red hue in the IRFC image obtained by the HMI software (see Figure 2.12). This hypothesis was further validated by the FORS spectrum, which shows an inflection between 690 and 730 nm and a maximum absorbance at about 660 nm (Figure 2.14): these reflectance features are consistent with the reference spectra of indigo [26, 27], providing a robust validation of our findings.

Table 2.2 Hypotheses on the pigments in the different analysis spots from the lonely noninvasive (imaging, mapping, and spot) techniques.

SPOTS	HYPOTHESIS OF COMPOSITION FROM THE SYNERGY OF THE NONINVASIVE (IMAGING, MAPPING, AND SPOT) ANALYSES	
	Main components	Other components
1 greyish light blue	Lead white and indigo, with umber	Ochres/earths and copper-based pigment
2, 24 red	Cinnabar	Lead white, ochres/earths, copper-based pigment
3 dark red	Red lake, over cinnabar	Ochres/earths, bone black, copper-based pigment
4 green-grey	Lead white, black bone, copper-based pigment	Ochres/earths, umber
5, 18 blue	Azurite	Ochres/earths
6 brown-green	Copper resinate	Lead-Tin yellow, ochres/earths, bone black
7, 22 light blue	Indigo and lead white	
8, 26 light yellow	Lead-tin yellow type I	Lead white
9 brown	Lead-tin yellow type I	Bone black
11 red	Red lake and lead white, over cinnabar	Copper-based pigment
13 pink	Lead white and cinnabar	
14 brown	Umber	Bone black
15 green	Copper-based green	Bone black, cinnabar
16 red	Red lake	Azurite, cinnabar, lead white, bone black
17, 20 gold	Gold, over bole	
19 flesh	Lead white and cinnabar	Ochres/earths, bone black
23 black over blue	Carbon-based black	
25 white	Lead white	

The MA-XRF maps for the green and brown areas of the painting initially suggested the use of green copper-based pigments. In particular, for the green details, the hypothesis was also based on the FORS spectra, where a large absorption band between 600 and 900 nm compatible with a generic copper-based green pigment was recorded [28]. As regards the brown areas, the iconography, even before other analyses, provided further helpful suggestions for characterization. The brown appearance of some details that were usually painted green – such as the inner lining of the Virgin's robe – could only suggest the use of copper resinate, whose sensitivity to oxidation and degradation phenomena is well attested [29, 30]. Subsequently, the observation and analysis using Vis-UV OM and SEM/EDS of a cross section from a brown area highlighted the presence of a copper-based green layer with a glassy appearance (sample 8s, see Figure 2.3 for the sampling site). Although not sufficient for complete identification [29], the glassy appearance of the green allowed us to support the hypothesis on the use of copper resinate. An in-depth analysis of copper resinate degradation is illustrated later.

The pink-reddish color of UVF suggested the presence of red lakes in some darkened red areas, further validated by the FORS spectrum (Figure 2.15), which exhibits an inflection in the spectrum at around 600 nm [31]. The observed darkening of the surface ascribable to the lake degradation [32] affects the quality of the data, preventing unambiguous discrimination of the exact nature of the dye – whether madder or cochineal. However, the maximum at about 502 nm suggests that the former is more likely [26, 33], providing a strong validation of the initial suggestion.

The use of cinnabar in some vivid red areas was preliminary suggested by the MA-XRF signals of mercury and sulfur and subsequently confirmed by in situ Raman analyses that allowed for identifying the typical bands at 251 and 342 cm^{-1} (Figure 2.16a).

The presence of lead-tin yellow type I was confirmed by the Raman peaks at 77, 107, 126, and 194 cm^{-1} (Figure 2.16b) [34, 35] in correspondence with evident signals of lead and tin in the MA-XRF maps. MA-XRF was also fundamental for mapping the distribution of lead white (see Figure 2.5a), identified by the typical Raman signal at 1051 cm^{-1} . Due to the Au intense signal, MA-XRF was decisive for the gold leaf identification (see Figure 2.5b).

Lastly, the carbonaceous nature of the black areas was confirmed by the Raman bands at 1350 and 1584 cm^{-1} while the use of bone black was hypothesized based on the XRF signal of phosphorus.



Figure 2.12 IRFC image of the painting obtained with the HMI software starting from the Adobe RGB (Vis) and the IR3 calibrated band (950 nm centered) images. In the RGB color space, the blue channel (B) is transferred into the green (G), the green into the red (R) and the red channel is substituted by the IR3.

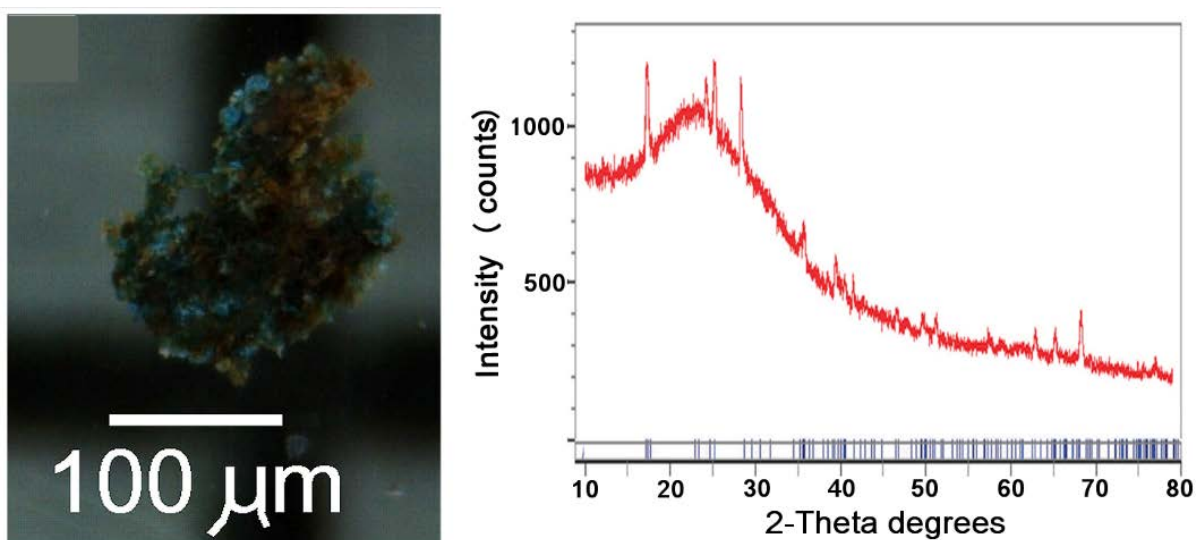


Figure 2.13 Sample 17s (approx. 200 μm across) from the Virgin's blue garment observed under an optical microscope and the related μ-diffraction pattern, in which the typical reflections of azurite can be appreciated.

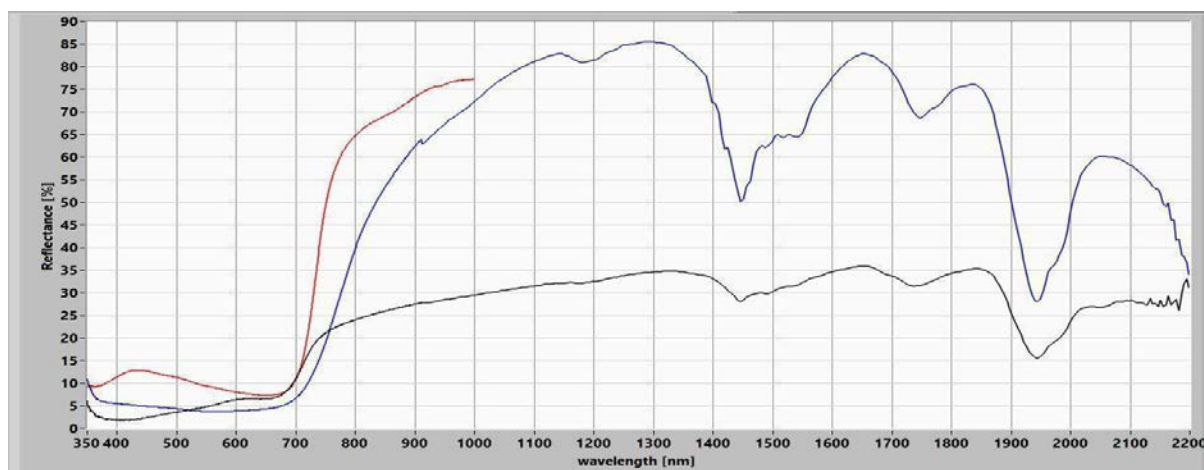


Figure 2.14 FORS spectra acquired on the gray-blue colour of the base of the throne (black curve, spot n° 22 in Figure 2.3) compared with reference mock-ups of indigo blue lake in linseed oil (blue curve) and of a mixture of indigo lake and lead white (red curve) from the CCR database.

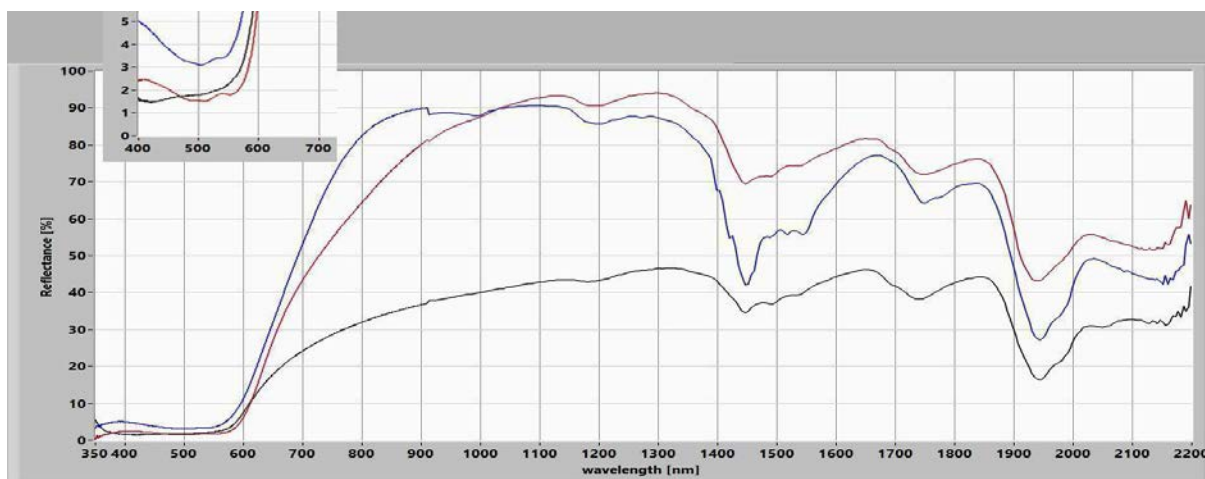


Figure 2.15 FORS spectra acquired on the Saint's red armor on the right (black curve; spot n° 11 in Figure 2.3), compared to the CCR database reference mock-ups made of madder lake (blue curve) and cochineal (red curve) in gum Arabic binder painted over a gypsum-based layer.

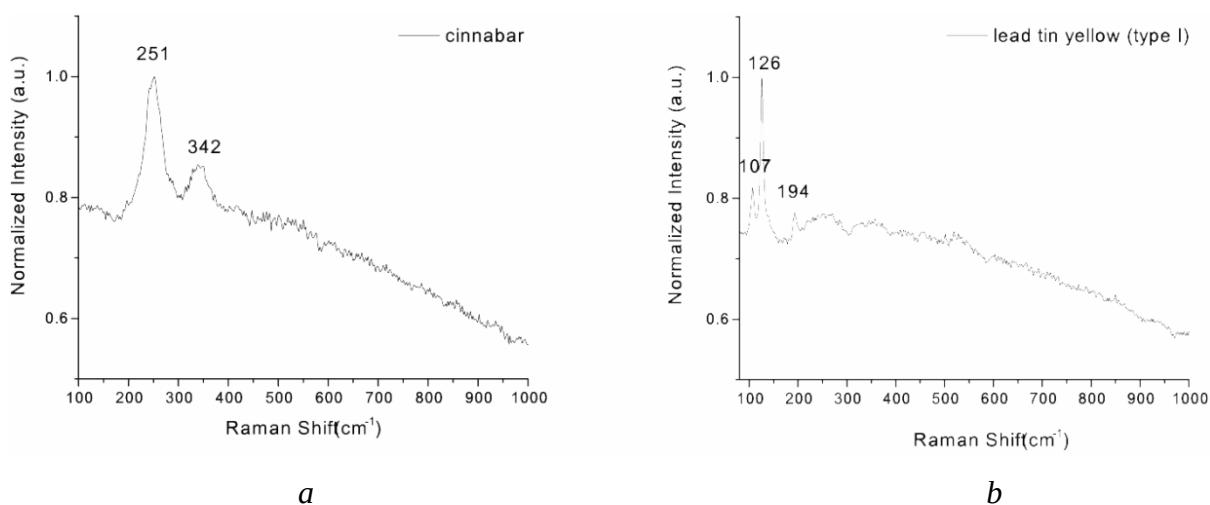


Figure 2.16 Raman spectra acquired on: *a*) the red stole of the Saint on the left, spot n° 24 in Figure 2.3; *b*) the light-yellow palm leaf on the left, spot n° 8 in Figure 2.3.

2.4.4 Painting technique and preservation issues

In the following paragraphs, the main characters and areas of the painting are introduced, while the pigments and binders used to depict them and the degradation phenomena observed are discussed. The various spots of analysis and sampling sites are shown in Figure 2.3; the performed analyses are reported in Table 2.1.

2.4.4.1 The Virgin's colors and a focus on the blackening of the azurite-based garment and the browning of the green

The clothing of the Virgin Mary consists of a red apron – a triangular small portion of which is still visible under her left hand – with a golden collar in the foreground, and of a large blue garment with a green inner lining. All the hues of these clothes are nowadays moderately to strongly darkened, as a result of different and (maybe) irreversible forms of degradation. The diagnostic campaign's results showed that the Virgin's flesh tones (spot n° 13) were rendered by a mixture of lead white and cinnabar in different proportions, probably with small amounts of calcite. In the whiter tones of her eyes and the shaded area of her neck, tiny amounts of Cu-based pigments were added to lead white. The red apron (spot n° 16) is obtained mainly with a red lake, added with tiny amounts of other pigments (probably cinnabar, lead white, azurite, and bone black). Unfortunately, such a technical choice contributed to the degradation and chromatic alteration of this area, due to the particular sensitivity of this lake [36, 37, 38].

Close observation and analyses revealed that the golden collar is made of gold leaf (spot n° 10). The halo was originally made with the gold leaf technique, embellished with a slight relief profile made in *pastiglia* and with thick engraved rays: however, the MA-XRF maps of Au, Fe, Cu (see Figure 2.5b) and Zn (Figure 2.17) showed that it was largely reworked with more modern pigments during previous restorations.

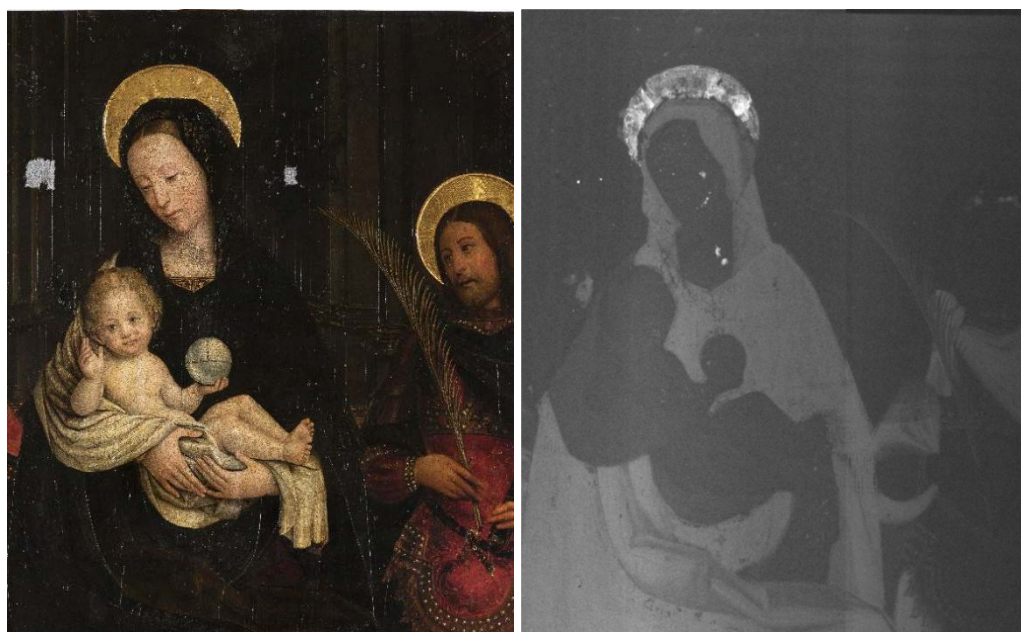


Figure 2.17 Detail of the painting in visible diffuse light and MA-XRF map of Zn.

The Virgin's blue garment, affected by a severe darkening, is made of azurite [a copper-alkali carbonate: $\text{Cu}_3(\text{CO}_3)_2(\text{OH})_2$] (spots n° 5 and 18), which is unique in that it contains no white lead but only iron oxide impurities – a feature also confirmed by the MA-XRF maps (see Figure 2.5b). Despite the general chromatic flattening, some black lines of carbonaceous nature (spot n° 23) used for outlining this garment can still be appreciated, probably made with a brush before (under the pigment strokes) and after being painted.

After applying the noninvasive methods, two small samples (a few hundred μm across), one representative of the stratigraphy and the other selective of the superficial paint layer, were extracted from the blue garment and studied with optical microscopy, SEM-EDS, $\mu\text{-XRD}$, $\mu\text{-Raman}$, and GC/MS. The stratigraphic study was performed on the sample n° 7s (Figure 2.18) using optical microscopy and SEM/EDS, which revealed the presence of three layers, namely (from bottom to top): 1) the gypsum-based preparation layer; 2) a thin (around 10 μm) black layer, possibly made of carbon-based black; 3) the blue azurite-based paint layer. In layer 3, azurite is coarse with well-preserved and intensely blue-colored grains (even > 20 μm across), displaying no evident darkening. Also, brown-to-reddish areas are observed among the interstices of the blue grains, possibly related to the organic binder.

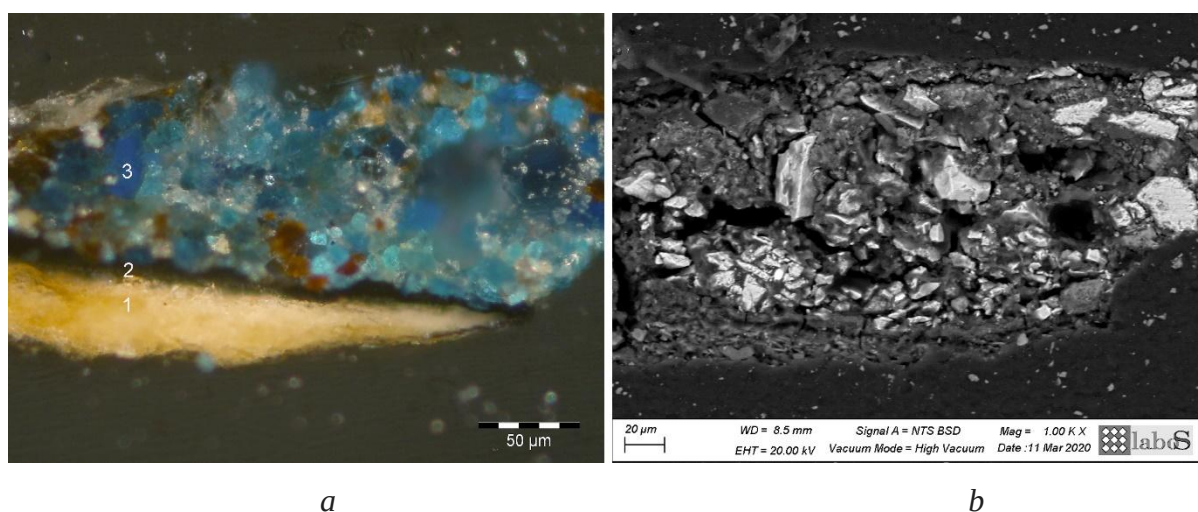


Figure 2.18 Cross section of sample 7s, from the Virgin's blue garment: *a*) Vis-OM; *b*) SEM/BSD. The numbers in *a*) indicate the layers recognized through the investigations: 1) the gypsum-based preparation layer; 2) a thin (around 10 μm) black layer, possibly made of carbon-based black; 3) the blue azurite-based paint layer with coarse, well-preserved, and intensely blue-colored grains (even > 20 μm across) and no evident darkening. The brown-to-reddish areas among the interstices of the blue grains are possibly related to the organic binder.

The μ -XRD and μ -Raman analyses were performed on the selective sample n° 17s (Figure 2.19). The first unequivocally confirmed that the constituent pigment is azurite (see Figure 2.13); μ -Raman, on the other hand, provided more interesting results concerning the preservation of the pigment and its decay. Besides assessing its nature, the surface of the sample, observed with a transmitted-light optical stereo-microscope, confirmed the integrity and good preservation of the azurite grains (Figure 2.19a-b) – thus proving that the darkening should not be related to a partial, progressive transformation of azurite (blue) to tenorite (a black Cu oxide: CuO) – an occurrence at times reported in the literature [39, 40]. No evidence of this latter compound is observed either by μ -XRD or μ -Raman. However, the diffuse presence on the artwork surface of a dark patina (and other reddish areas related to the binder) appears in some microscopic images (Figure 2.19c). μ -Raman spectra of these black areas show strong bands (at 1350 and 1550 cm^{-1} ; Figure 2.19d) related to the presence of a carbon film – possibly in the form of scarcely crystalline graphite. This patina could be responsible, at least in part, for the observed darkening process and might be related to prolonged exposure to fumes produced by candle burning. Such a hypothesis is consistent with the history of the painting, which also shows clear evidence of heating damage occurring in the areas close to the Virgin's neck (see Figure 2.1b).

The GC/MS analysis performed on the same bulk sample proved that the binder, which appears slightly browned in cross section, is based on egg white. The amino acidic fraction contains amino acids above the quantitation limit of the analytical procedure (LOQ)¹. The relative percentage content of amino acids was subject to principal component analysis with a dataset of egg, casein, and animal glue reference samples. The resulting score plot (Figure 2.20) indicates that egg is the likely binder in this sample. The lipid resinous fraction shows fatty acids at the detection limit of the procedure² and the absence of dicarboxylic acids, as visible in the chromatogram shown in Figure 2.20. This indicates that oxidized egg yolk lipids are not detected. The presence of proteins and the absence of oxidized lipids suggest the use of egg white to paint the blue mantle.

Considering all these outcomes, the encountered darkening of the Virgin's garment can thus be explained, with all due probability, by the contextual actions of several interacting

¹ LOD: 0.14 μg ; LOQ: 0.27 μg (confidence level 95%).

² LOD: 0.62 μg ; LOQ: 1.01 μg (confidence level 95%).

parameters. The coarse texture of the azurite grains made it possible for the surface to retain high amounts of the organic binder – aged and browned over time. The thick mixture of coarse-grained azurite and binder also favored other superficial deposits' adhesion (and possible sorption), such as the black carbonaceous powders derived from candle burning. Using such coarse-grained azurite was not accidental: artists knew that a more severe grinding of the pigment grains would have caused a loss in intensity of the blue color. Azurite had to be coarse-grained for the resulting hue to resemble lapis lazuli, which was far more precious and expensive [41].

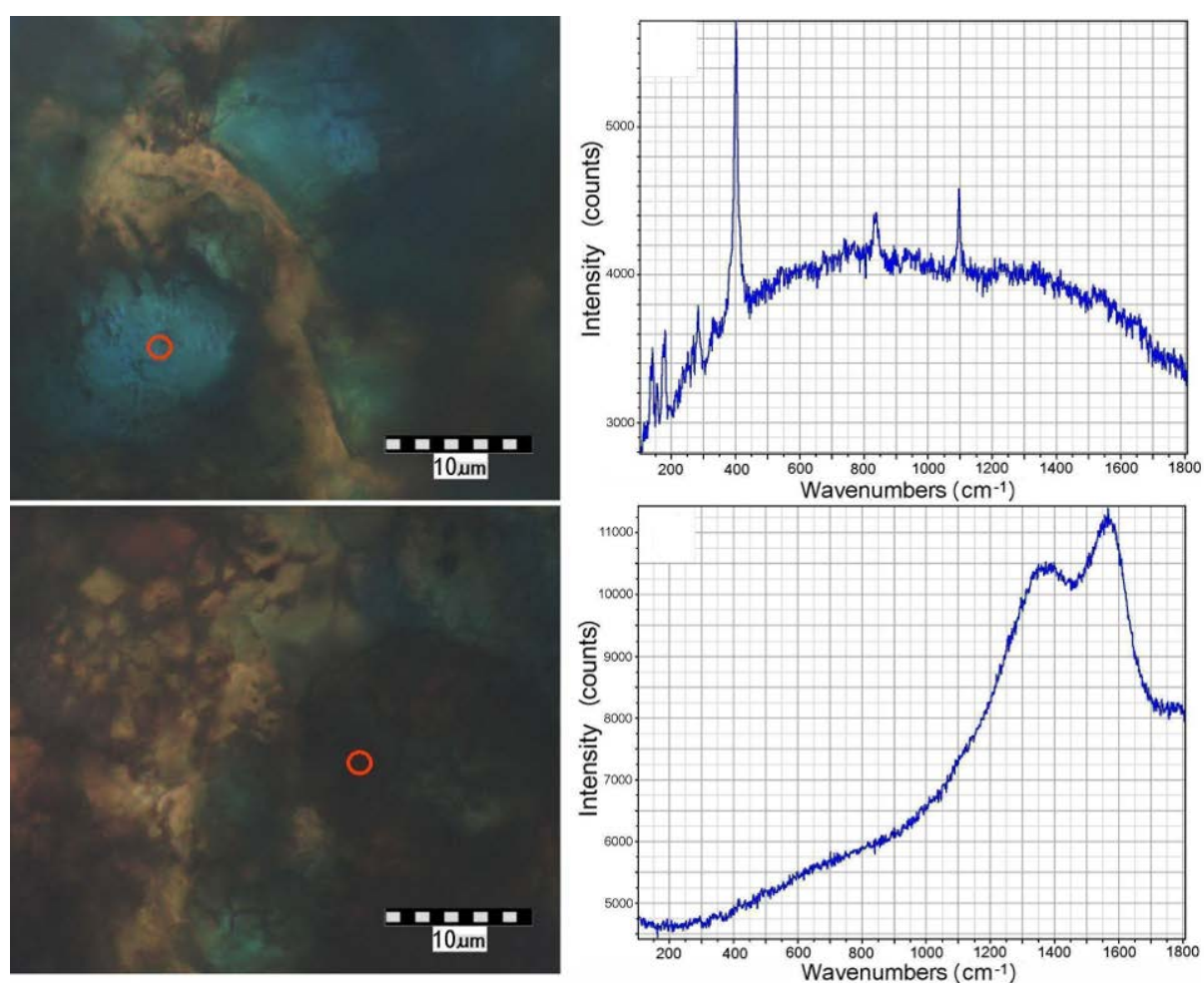


Figure 2.19 Sample 17s (approx. 200 μm across), extracted from the Virgin's blue garment. Top: OM image in transmitted light of the crystalline blue pigment of azurite and relative μ -Raman spectrum collected on the red circle, where the typical bands of azurite are observed. Bottom: OM image in transmitted light of the blackish patina homogeneously covering most

of the sample surface (the reddish area, on the left, is related to the organic binder) and relative μ -Raman spectrum collected on the red circle, showing the typical bands of carbon.

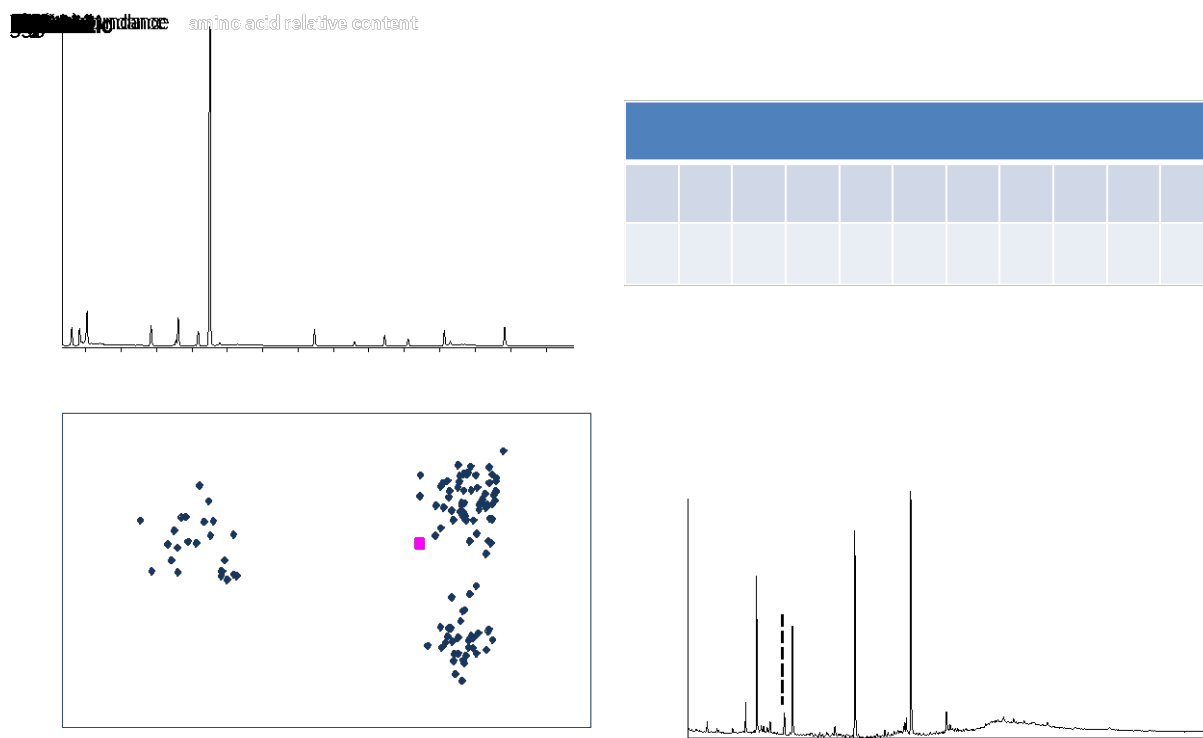


Figure 2.20 Sample 17s from the Virgin's blue garment, GC/MS results. Top left: chromatographic profile of the amino acidic fraction (i.s. internal standard; all compounds are TBDMS derivatives). Top right: relative amino acid percentage content. Bottom left: Principal Component Analysis Score Plot. Bottom right: chromatographic profile of the lipid-resinous fraction (i.s. internal standard; all compounds are TMS derivatives).

As far as the green inner lining of the garment is concerned, its original tone was probably an iridescent, intense, and vibrant green hue – obtained with the technical expedient of overlapping several semi-transparent glazes. The paint stratigraphy was studied on sample 8s, a tiny specimen extracted from this area (Figure 2.21). Analyses revealed the presence of six layers, namely (from bottom to top): 1) a light green draft, 40 μm thick, made of a mixture of azurite, lead-tin yellow, and particles of red ochre; 2) a thinner copper resinate layer, of about 25 μm ; 3) a thicker layer of copper-based green and lead-tin yellow, about 50 μm thick; 4) a thin layer of lead-tin yellow, about 15 μm ; 5) a very thin superficial glaze of copper resinate, about 10 μm thick, with traces of lead-tin yellow, and finally 6) a layer of natural resin subsequently identified as a restoration varnish.

The paint binder of this part contains egg, based on the detection of amino acids above the quantitation limit with a chromatographic profile similar to that of reference sample of egg paint layers (Figure 2.22). Unfortunately, an analytical interference prevented us from drawing any conclusion on the content of lipids and terpenoid resins. The presence of copper resinate on the surface, known for its sensitivity to different degradation processes [29, 30], would explain the poor state of preservation of the inner lining of the Virgin's garment, as well as the irreversible chromatic flattening of its once green tone. A similar decay also characterizes the palms branches of the martyrdom of the two Saints, obtained in the lighter parts of the leaves with shades of yellow and copper resinate, and umber earth for the stem.

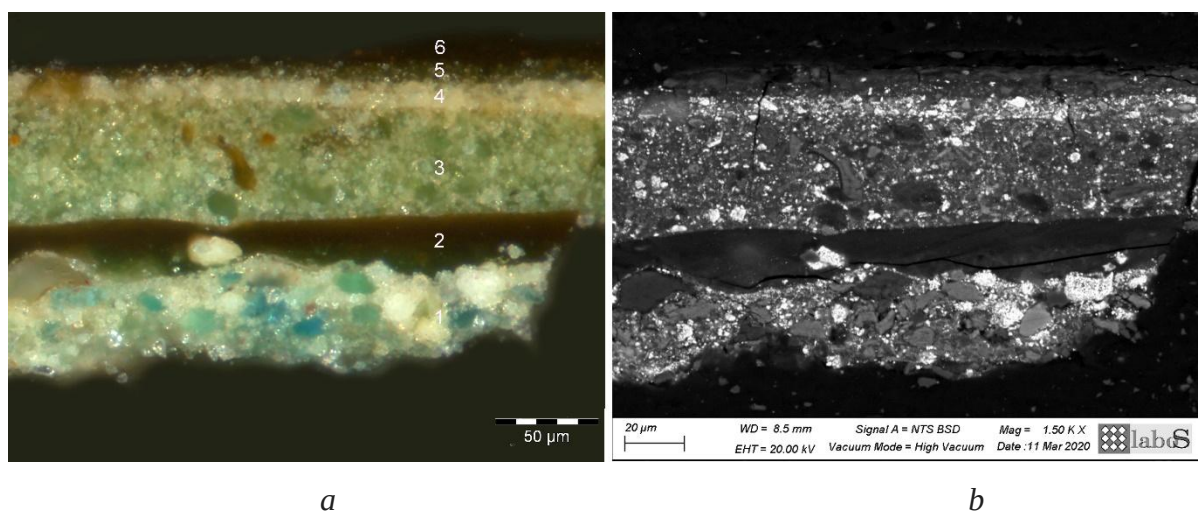


Figure 2.21 Cross section of sample 8s from the green inner lining of the Virgin's garment: *a*) Vis-OM; *b*) SEM/BSD. The numbering in *a*) refers to the layers identified through the analyses: 1) light green layer made of azurite, lead-tin yellow, and small amount of red ochre, ca. 40 µm thick; 2) copper resinate layer, ca. 25 µm; 3) layer of copper-based green and lead-tin yellow, ca. 50 µm; 4) layer of lead-tin yellow, ca. 15 µm; 5) superficial glaze of copper resinate with traces of lead-tin yellow, ca. 10 µm; 6) layer of natural resin.

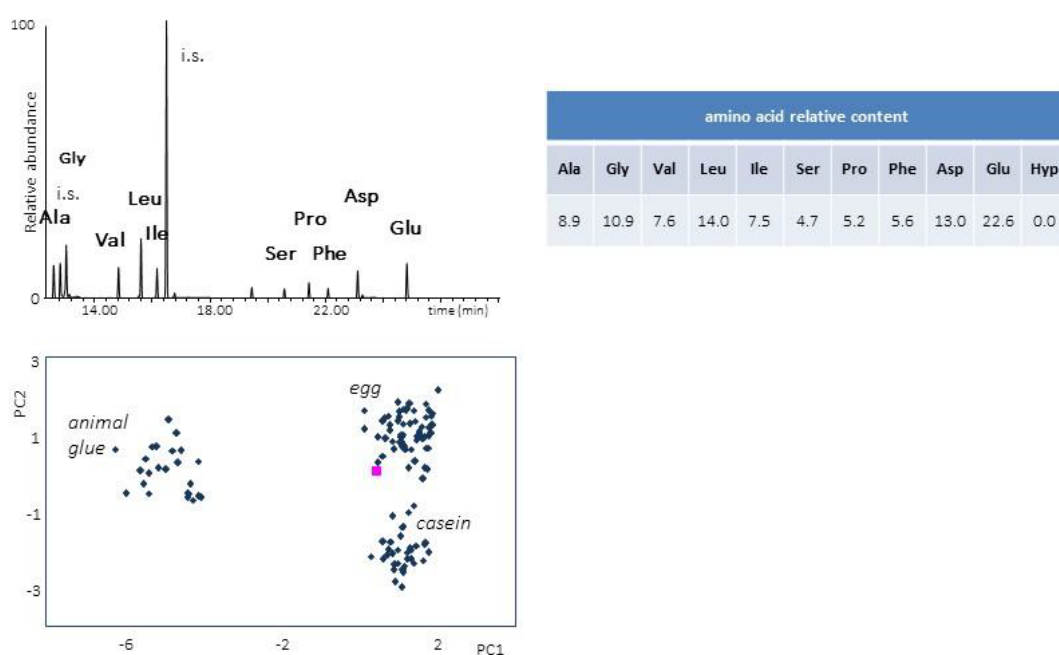


Figure 2.22 Sample 8s, from the green inner lining of the Virgin’s garment. GC/MS results.

2.4.4.2 The Child, the throne, and the two Saints

Analyses revealed that the white stole wrapping the Child is rendered with lead white. The same pigment, aptly mixed with indigo, is also used to render the light blue color of the sphere in his left hand (spot n° 7) as well as the gray-blue tone of the throne (spot n° 22); for the latter, umber was also added in the shaded areas (spot n° 1).

The red drape covering the throne is rendered with red lake, embellished by a decoration of superposed gold leaf that is clearly visible in the shaded areas (spot n° 3) while, in others (spot n° 2), is applied onto a cinnabar layer. Although just a few traces of this decoration remain nowadays, they are enough for reconstructing the original ornamental motif, which included interweaving lozenges, tangles, flowers, and animals (doves and, perhaps, rabbits), organized in a sophisticated pattern that was successfully reused in other artworks of the same author’s circle to adorn similar red cloths [1, 4, 7].

Close observation and analyses revealed the presence of a mission gold decoration, initially used to embellish several details of the armor of the Saints but nowadays reduced to a few fragments, presumably due to previous aggressive cleaning treatments. The artist realized the

Saint's halos in gold leaf (spot n° 20). In their hands, both Saints carry a palm leaf, in which we found umber earth for the stem (spot n° 9) and lead-tin yellow type I to render the light yellowish tone of the brighter parts of the leaves (spot n° 8).

Regarding the Saint on the left, the artist used cinnabar for the external side of his red mantle (spot n° 24), copper-based green for the internal (spot n° 15), and lead-tin yellow type I (spot n° 26) for the borders. The pearls on the dress were rendered with lead white (spot n° 25).

The Saint on the right wears a red cuirass, rendered with red lake and lead white over a cinnabar layer (spot n° 11). For both Saints, the artist obtained the flesh tones by mixing lead white and cinnabar (spot n° 19).

2.4.4.3 The background

From the analysis outcomes, the architectural details in the background and the pillars in the foreground on the lateral margins of the painting (spot n° 14) are made of an umber-based color, applied directly onto the preparation layer. This interpretation comes mainly from the MA-XRF, which revealed the presence of iron and manganese, ascribable to umber, and calcium and potassium, ascribable to the preparation layer.

Our meticulous analyses and close observation have led us to confidently detect the use of shell gold for gilding the exposed parts of the pillars (spot n° 17).

The data collection on the grey-greenish tone of the floor has allowed us to confidently hypothesize using a mixture of lead white, bone black, and a copper-based pigment (spot n° 4).

After applying noninvasive methods, we selected the area of the background for further study of the binders' nature, as well as to investigate the brownish patina covering most of the surface of the painting. For the latter, please refer to the paragraph 2.4.5.

The sample extracted from the background (sample 5s, Figure 2.23) is a bulk sample containing the paint layer and the superficial brownish patina. In the sample, fatty and dicarboxylic acids and amino acids were detected above the detection limit, revealing the presence of both a drying oil – most likely linseed oil - and egg. Given the presence of drying oil, as it can be inferred by the relatively high content of azelaic acid, we cannot conclude which fraction of the egg (white, yolk, whole) was used by the artist. As a comparison, the FTIR analysis of a sample containing mainly the brownish patina shows the spectral features of a proteinaceous medium (sample 4s,

Figure 2.24) – egg white, based on the GC/MS data. A shoulder in the 1700-1735 cm^{-1} range is visible, which could be due to minor amounts of lipid and/or resinous materials. On these bases, we hypothesized that the brownish patina is mainly made of egg white, and the paint layer underneath is based on linseed oil or a mixed medium technique entailing the use of linseed oil and egg.

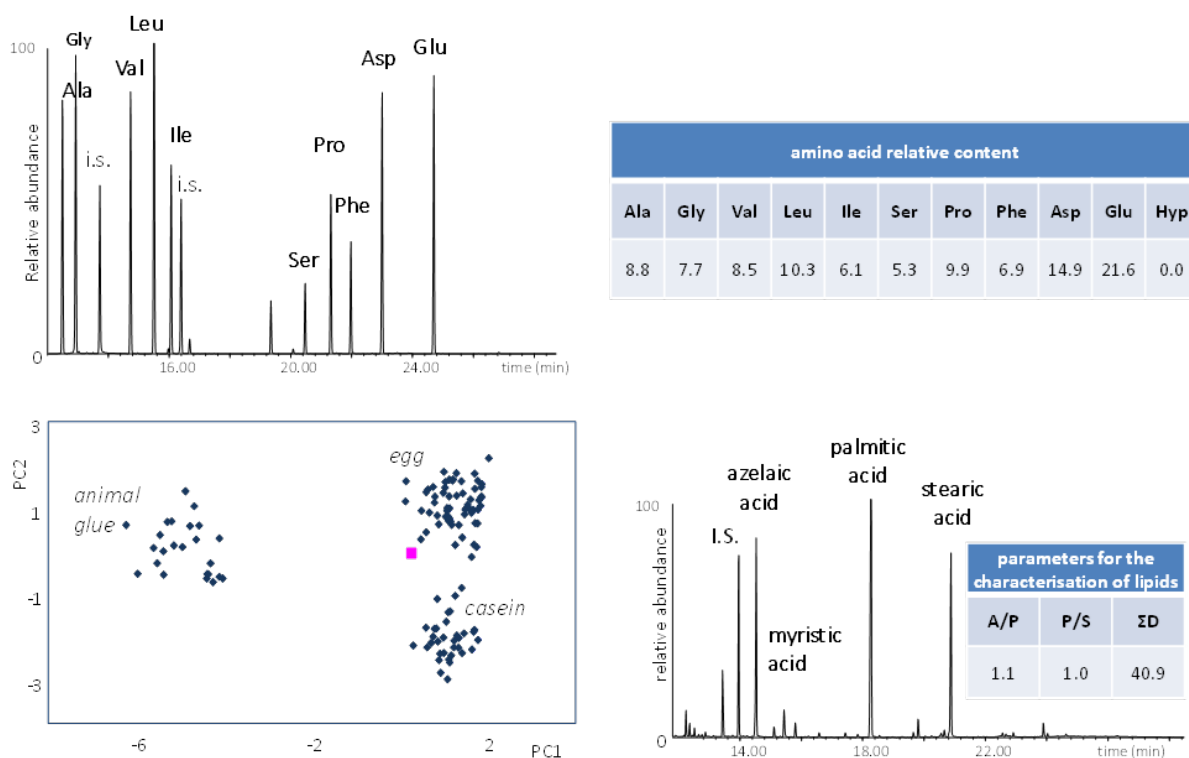


Figure 2.23 Sample 5s, from the grey-greenish background. Top left: chromatographic profile of the amino acid fraction (i.s. internal standard; all compounds are TBDMS derivatives). Top right: relative amino acid percentage content. Bottom left: Principal Component Analysis Score Plot. Bottom right: chromatographic profile of the lipid-resinous fraction with parameters used for the lipid characterization (i.s. internal standard; A: azelaic acid; P: palmitic acid; ΣD : sum of the relative content of dicarboxylic acids; all compounds are TMS derivatives).

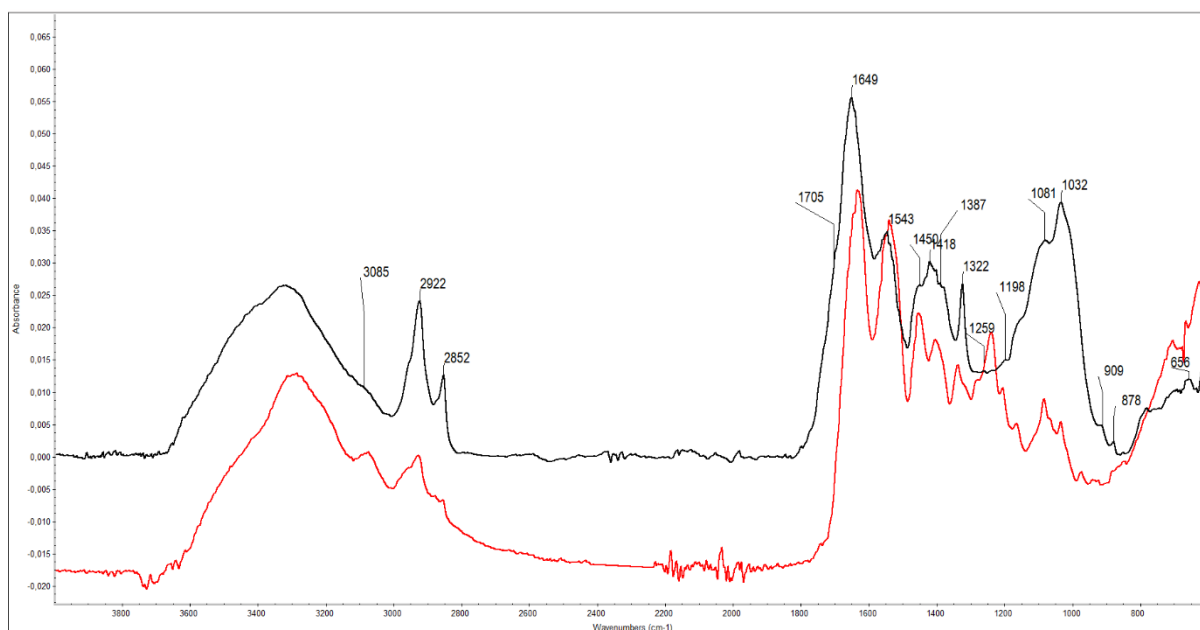


Figure 2.24 FTIR spectrum of sample 4s, from the superficial brown patina (black line) compared to a reference of proteinaceous substance. Assigned bands: 3085 cm^{-1} : N–H stretching band; 1649, 1543, 1450 cm^{-1} : C=O stretching band; calcium carbonate, silicate, oxalates, and a natural resin or aged drying oil are also identified.

2.4.4.4 The flesh tones: a focus on the dotted technique

The *dotted* technique involves the painstaking application of small pigment droplets on the artwork surface *via* a preliminary nebulization. When observed in detail – at high magnifications – a peculiar, dotted effect is thus perceived. When observed by a certain distance, on the other hand, such an effect causes the related backgrounds to acquire consistency and brightness. This technique is reserved only for some regions of the painting and can be found also in other works by Defendente Ferrari and his circle [1, 4, 7]. In the *Madonna with Child and Two Saints*, the *dotted* technique is used to render, for example, the Virgin’s flesh tones: the related macro-photographs show the compact juxtaposition of small dots of various colors applied on a lighter background (Figure 2.25a). A similar rendition can also be found on the hand of one of the Saints (Figure 2.25b).

Unlike other areas analyzed in the painting, the GC/MS analysis of the organic fraction of a bulk sample coming from the *dotted* flesh tones (sample 18s, Figure 2.26) revealed a different composition. The lipid fraction showed the presence of fatty and dicarboxylic acids above the LOQ, with a molecular profile in agreement with that of egg lipids. The amino acidic fraction

also shows signals above the LOQ, and the presence of hydroxyproline identifies animal glue (collagen). The Principal Component Analysis of the amino acid relative content indicates a mixture of animal glue and another proteinaceous medium. Combining the data on this sample with the others, we could conclude that the egg (whole egg or egg yolk) is the binder of the background, that is, the lighter tone paint layer, and animal glue is possibly the binder used for the pictorial binder of the darker dots applied on top.



Figure 2.25 Details of the *dotted* technique on: *a*) the Virgin's eye; *b*) the hand of the Saint on the left.

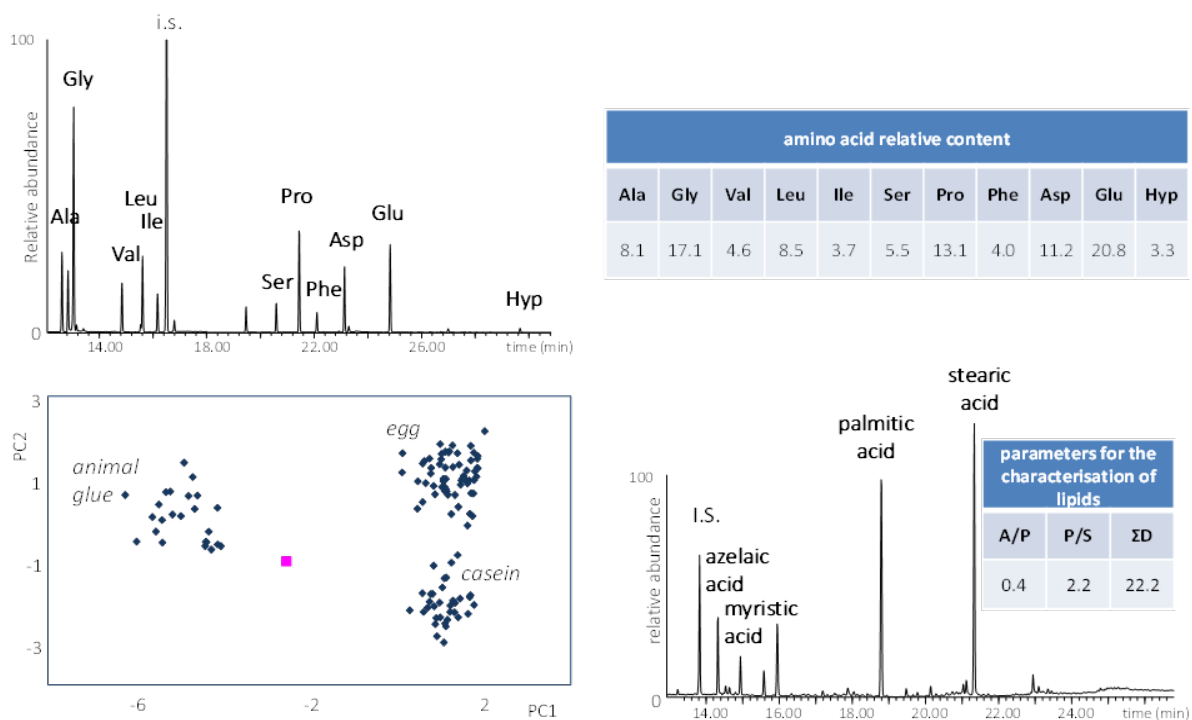


Figure 2.26 Sample 18s, from the flesh tones of the Saint on the left. Top left: chromatographic profile of the amino acidic fraction (i.s. internal standard; all compounds are TBDMS derivatives). Top right: relative amino acid percentage content. Bottom left: Principal Component Analysis Score Plot. Bottom right: chromatographic profile of the lipid-resinous fraction with parameters used for the lipid characterization (i.s. internal standard; A: azelaic acid; P: palmitic acid; ΣD : sum of the relative content of dicarboxylic acids; all compounds are TMS derivatives).

2.4.5 Original finishing and previous interventions varnishes

By close observation, a relatively uniform and firmly cracked patina of brown-to-grayish color is well-visible over the artwork's surface, especially in some lighter areas, such as the skin of the Saint on the left (see Figure 2.2a). The patina's craquelure mainly follows that of the underlying pictorial layer, suggesting a close relationship between these two layers. Therefore, the layer that today appears brown-to-grayish was probably applied by the artist over the pictorial layer. Based on this observation and FTIR and GC/MS data related to samples collected from the background, we can hypothesize that the patina is an original egg-white based varnish which had the function of protecting and increasing the color saturation of the painting [42].

The statistical treatment of HMI images allowed for mapping the distribution of this patina: Figure 2.27 shows the spectral similarity map based on the reflectance spectra of the face of the Saint on the left, where the patina is most evident. The patina is absent only on the Virgin's face and Child and in those areas where deeper lacunae are evident, probably due to an ancient and quite aggressive localized restoration, which likely insisted on the light-colored regions, causing a partial removal of the original patina.

Moreover, several overlaying varnishes were applied to the above during previous conservation treatments. The most superficial one shows coarse brushstrokes, clearly visible in UVF (Figure 2.27). The intense and greenish UVF suggested an aged natural resin was used, as confirmed by FTIR (sample 1s, Figure 2.28).

Finally, the UVF images had a fundamental role during the conservation treatment performed at the CCR for monitoring and controlling an efficient removal of the overlaying varnish layers while preserving the ancient, and likely original, patina.

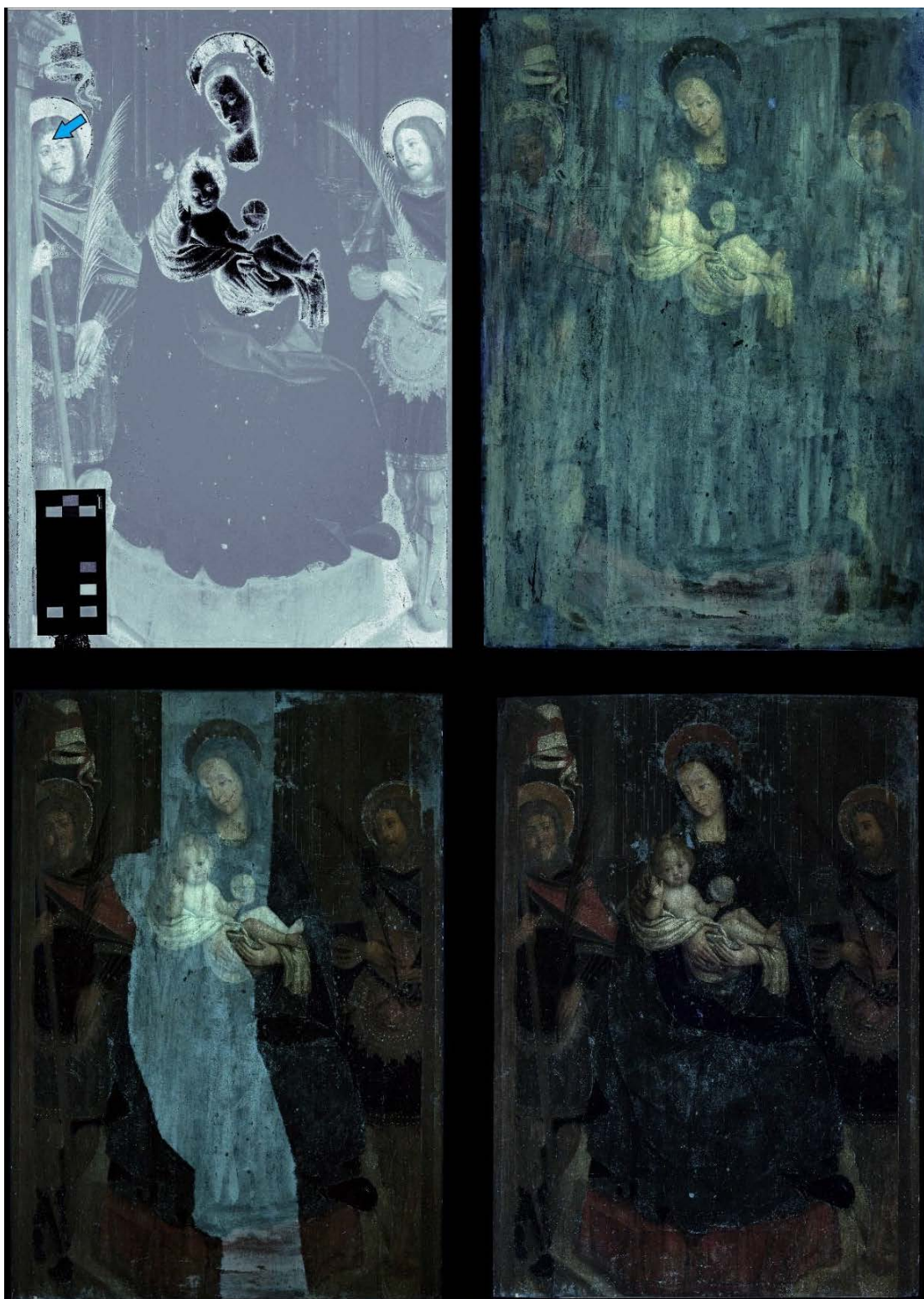


Figure 2.27 Top left: HMI, spectral similarity map of the brown patina registered on the face of the Saint on the left (blue arrow): the black areas indicate the absence of the patina's spectral signature. Top right and bottom: UVF images acquired before and during the recent conservation treatment carried out at CCR.

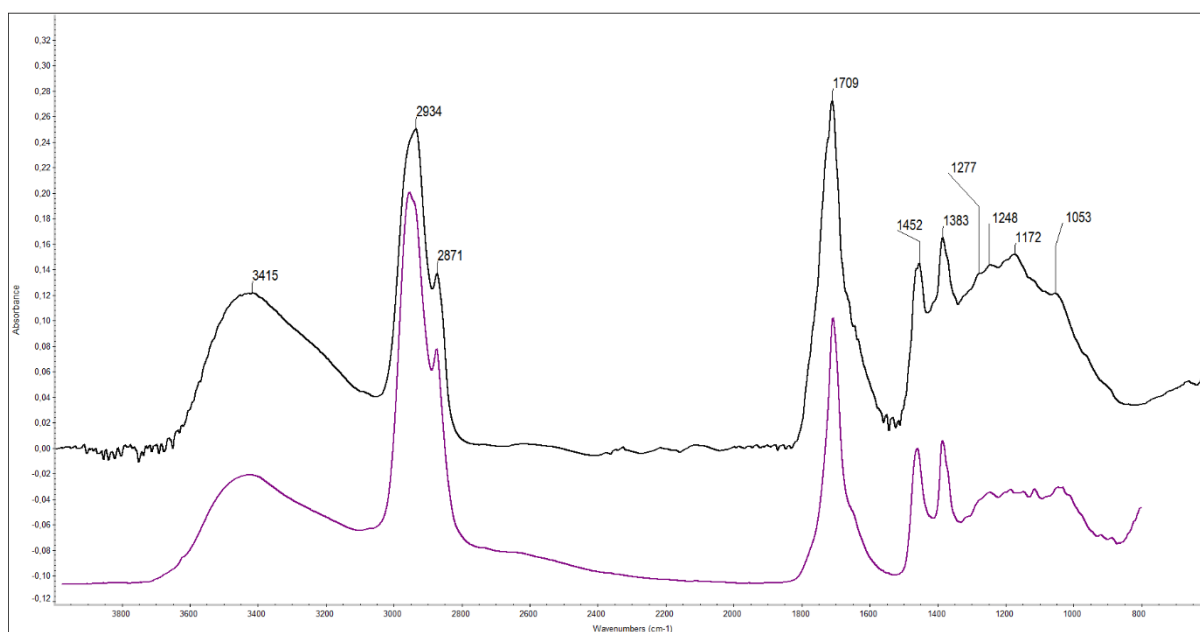


Figure 2.28 FTIR spectrum of sample 1s, from the superficial varnish in the background (black line) compared to a reference of natural resin (green line). Assigned bands 2934–2871 cm^{-1} : C–H stretching bands; 1709 cm^{-1} : C=O stretching band; 1452–1383 cm^{-1} : C–H bending band; 1277–1053 cm^{-1} : C–O and C–O–C stretching band.

2.5 Conclusions

The multi-analytical investigation of the *Madonna with Child and Two Saints* by Defendente Ferrari has unearthed crucial insights into the artist's painting technique. These findings were instrumental in guiding the recent conservation treatment at the CCR's laboratories. The investigation delved into specific and challenging conservation issues, such as the potential cleaning of the Virgin's blue garment and the removal of ancient varnishes while preserving the artwork's original finishes and materials. It also shed light on technical aspects, including the nature of the binders, the rendering of the dotted flesh tones, and the techniques used for drawing and rendering the volume of the figures' cloths.

Interpreting and cross-referencing the vast amount of analytical data collected with the various techniques achieved solid and consistent outcomes.

The identification and mapping of the superimposed varnishes based on natural (aged) resin, which contributed to a general yellowing of the whole artwork, supported the planning of their removal.

The detection, below these superficial varnishes, of an ancient (and likely original) varnish based on egg white led to the decision to preserve it despite its browned appearance.

Once the nature of the varnishes was identified, the UVF images played a pivotal role in this conservation treatment. They facilitated the monitoring and control of the selective removal of the overlaying varnish, underscoring their significant contribution to the process.

The study of the binders, conducted on four regions of the painting characterized by different textures and states of preservation, has provided valuable insights. It revealed that the artist was adept at using several materials (egg, linseed oil, and animal glue) and employed them in different ways to achieve various aesthetical effects, underscoring the importance of this research in understanding the artist's technique.

The binder of the green inner lining of the Virgin's garment – obtained with the technical expedient of overlapping several semi-transparent glazes and whose original tone, nowadays severely damaged and browned, was probably an iridescent, intense, and vibrant green hue – contains egg. This result is based on the detection of amino acids above the quantitation limit with a chromatographic profile similar to that of the reference sample of egg paint layers, but, unfortunately, an analytical interference prevented us from drawing any conclusion on the possible content of lipids and terpenoid resins.

The background and floor region, characterized by a greyish-light blue color, was painted with a linseed oil binder or a mixed medium technique involving linseed oil and egg.

Adopting an animal glue-based binder was found exclusively on the flesh tones rendered with the dotted technique. This piece of information is fascinating. The hypothesis is that Defendente used a narrow tube to spray the dark colors, a sort of “mouth spray gun”. The choice of animal glue is probably due to technical reasons: unlike oil or egg tempera, an aqueous binder based on animal glue can be diluted to obtain a thin paint that could be sprayed. This result is particularly relevant because the above described painting technique is recurrent in many of the paintings by Defendente Ferrari, and it has also been used by other contemporary artists, such as his colleague and collaborator Gerolamo Giovenone, during the first decade of the 16th century.

The study of the blackening of the Virgin's blue garment highlighted that the artwork suffered heating damage, evident in the area close to the Virgin's neck. While the coarse grains of the azurite pigment, used for the blue hue of the garment, showed an excellent state of preservation (with no evident degradation to tenorite), a carbon coating – possibly due to prolonged exposure to fumes produced by candle burning – homogeneously covered most of the garment surface. Such evidence initially oriented the conservation treatment towards an extremely cautious superficial cleaning. However, the black carbon film was ingrained on the surface of the browned (due to aging) egg white binder of the azurite particles. As a result, any attempt aimed at thinning this coating would have also caused the partial removal of some original pictorial material. After considering all collected analytical evidence, the decision was made to accept the partial non-recoverability of this garment's original aspect – to be achieved through a respectful restoration. This acceptance underscores the conservation challenges and the need for understanding and support in such operations. Since similar chromatic alterations (and color flattening) are also found in other contemporary artworks by the same author (in which azurite is pure and not mixed with lead white), the adopted diagnostic protocol and the ensuing results might act as a guide for future and analogous operations.

While the conservation treatment could not fully restore the artwork's original colors and garment volumes, the SWIR reflectography played a pivotal role in recovering its legibility. The related image demonstrates an excellent contrast, accurately depicting the transparency of azurite in the SWIR range and the significant absorption of the black pigment used for creating volumes and shadows. This significant recovery led the curator of Palazzo Madama – Museo Civico d'Arte Antica in Torino to plan the painting's exhibition alongside the SWIR image.

This study was conducted during the COVID-19 pandemic. Although we carried out most of the analyses before its spread, we performed the subsequent processing and integration of the data during the pandemic through virtual meetings, thanks to the active cooperation and synergy of all the researchers involved. In all phases of analysis and discussion of the results, what was missing most was the possibility of close observation of the work. However, this experience has underscored the potential of digital tools, such as a digital twin with very high-resolution images, for the remote study of the painting and for the purposes of fruition and valorization. This experience also demonstrated how developing specific software for data management and visualization – aimed at promptly handling the examination, integration, and exchange of analytical data coming either from imaging or spot techniques – would be extremely useful for complex case studies like the one presented here.

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Chapter 3

MULTI-ANALYTICAL STUDY OF A 16th-CENTURY PANEL PAINTING COPY AFTER RAPHAEL: TRACING THE HISTORY OF PAST TREATMENTS

3.1 Introduction

This chapter presents the multi-analytical investigation conducted on the *Madonna della Tenda*, a panel painting from Musei Reali Torino (MRT, Inv. 271, Torino, Italy) historically attributed to Raffaello Sanzio and subsequently recognized as a 16th-century copy after the Master.

In anticipation of the temporary exhibition *Sulle tracce di Raffaello nelle Collezioni Sabaude* (MRT, October 2020 – June 2021), focused on the critical fortune of the Raffaello's school and old master paintings in the Savoy collection, the painting was carried to the Centro per la Conservazione ed il Restauro dei Beni Culturali *La Venaria Reale* (CCR) where a program of artistic-historical study, scientific investigation, and conservation treatment was carried out. The painting's surface was particularly yellowed, suggesting the presence of several layers of varnish and, likely, overpainting. The back featured a centimetric hollow engraved in the wood, which provoked a thinning of the wooden support and deformations and fractures on the front.

Before and during the conservation treatment of the painting, the need for in-depth scientific analysis emerged to answer the numerous questions about the original materials and techniques, the state of preservation, and the nature of past treatments.

Considering all the aspects of the work of art to be addressed, the timing of the exhibition, the resources, and the availability of analytical instrumentation, the CCR team deemed it necessary to launch scientific collaborations. These collaborations were aimed at applying a specific multi-analytical protocol, including an *ad hoc* sampling method, that was essential for a thorough analysis of the painting's full thickness. The result of the team's efforts was the production of two scientific publications within the framework of this PhD program [1, 2].

3.2 Research aims

The investigated work is titled *Madonna della Tenda* (Inv. 271, MRT, Torino, Italy) and depicts the Virgin and Child with Saint John (Figure 3.1). It is made on a 79 × 56 cm poplar panel with an average thickness of about 2.5 cm. The painting had been historically attributed to Raffaello Sanzio and subsequently identified as a copy after the Master, dated around 1525–1550, the original version displayed at the Alte Pinakothek in Munich, Germany [3].

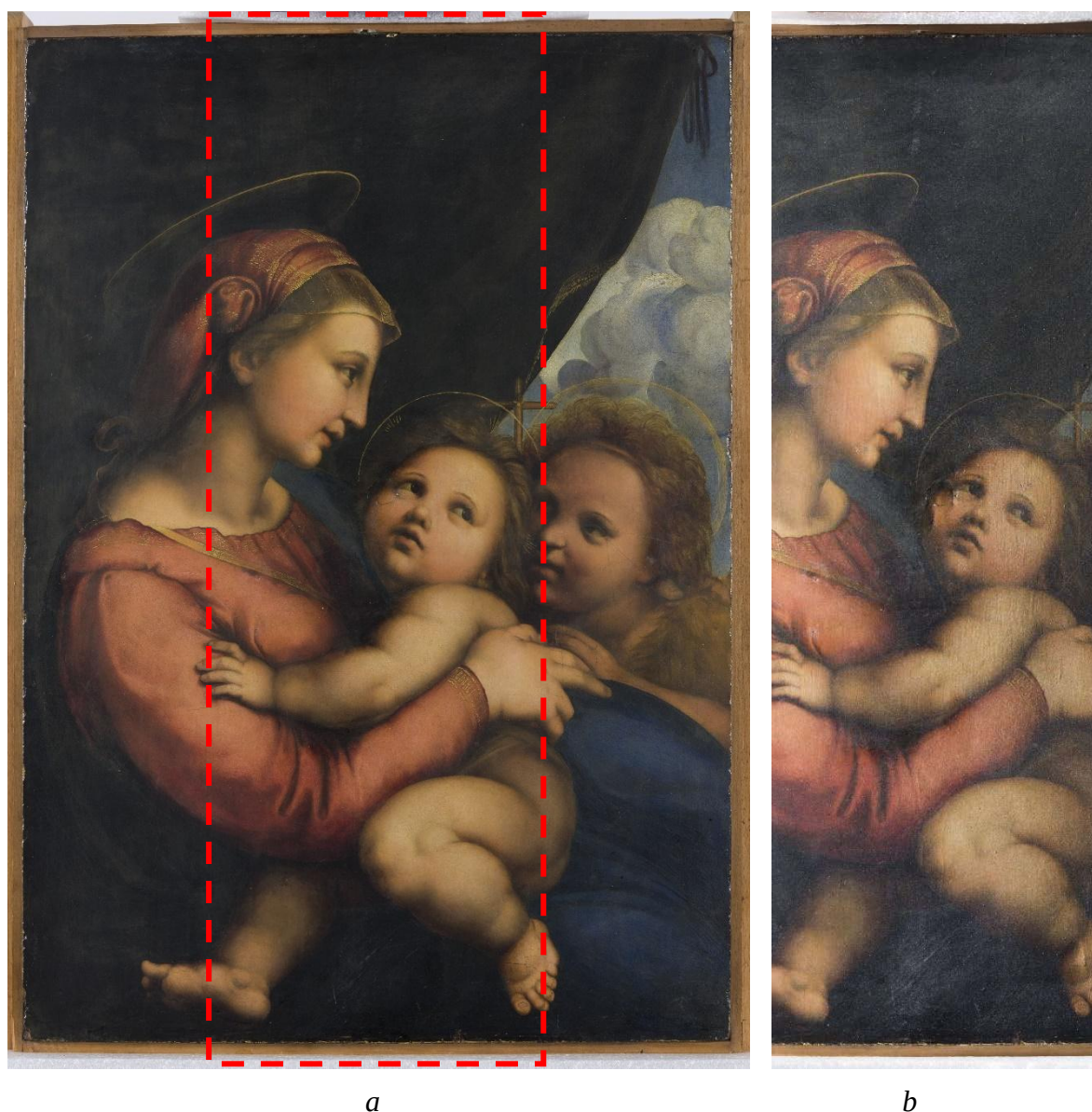


Figure 3.1 The panel painting *Madonna della tenda* (MRT, Inv. 271, Torino, Italy) before the last conservation treatment carried out at CCR: a) photograph in visible diffuse light; b) detail of the dotted region in visible raking light.

A black ink handwritten inscription on the back reports the words “*Quadro del divino Rafaele Sanzio D’ Urbino*” (Figure 3.2). The panel shows visible signs of several changes of ownership and relocation for restoration purposes – from the Porporati seal of Piosasco to traces of inventory numbers and the Milan customs wax seal. Interestingly, the back features also a centimetric hollow, which was caused at first analysis by the loss of a knot and subsequently widened: the edges of the hollow are dug and engraved, showing traces of adhesive substances on the internal walls (Figure 3.2).

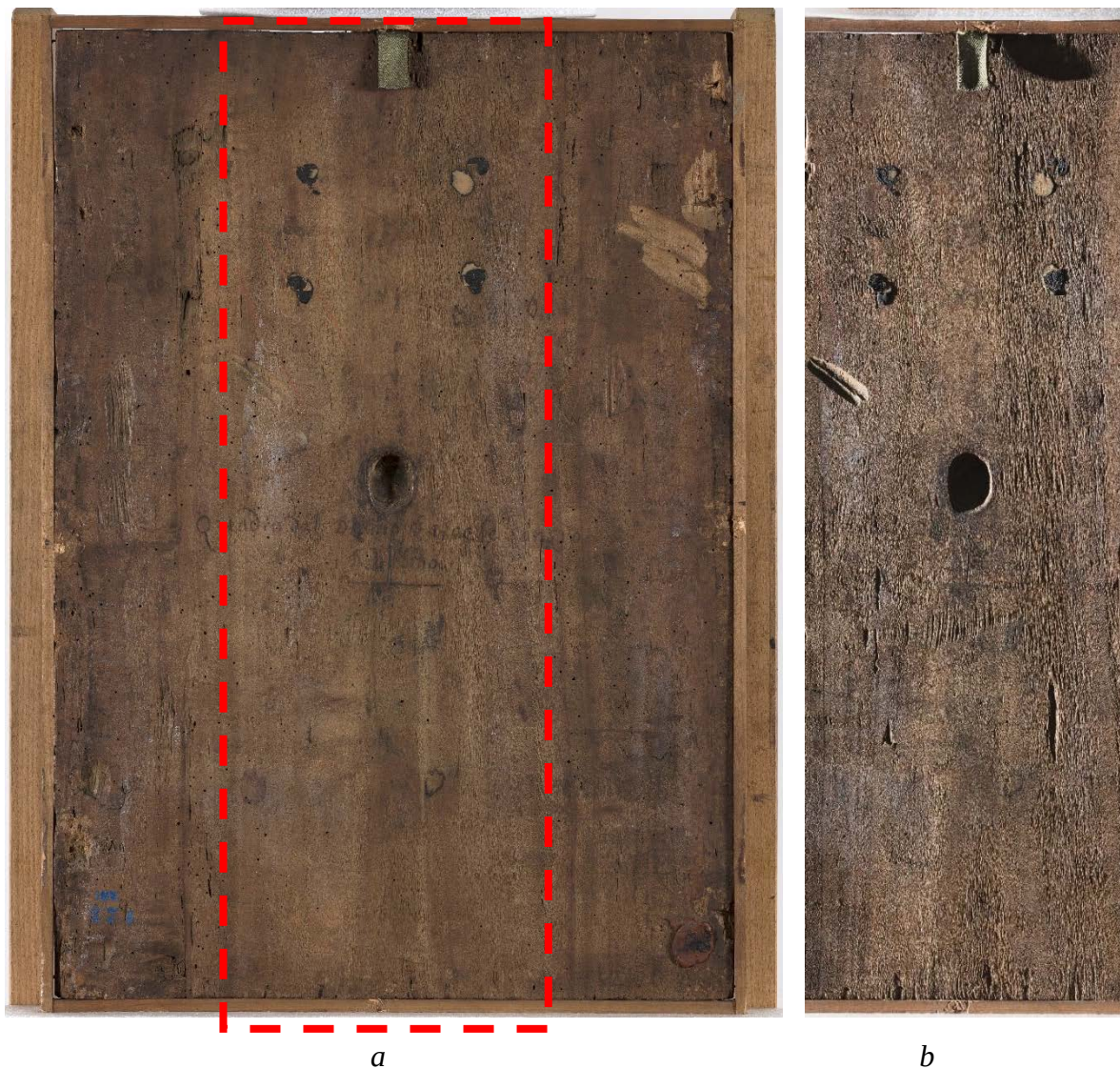


Figure 3.2 Back of the painting: a) photograph in visible diffuse light; b) detail of the dotted region in visible raking light.

As reported by the museum curators, in correspondence to the hollow, the wooden support was so thin that it provoked deformations and fractures on the front of the painting (see Figure 3.1b). As mentioned, the yellowing on the front suggested the presence of several overlaying varnishes, while the uneven painting technique – with very full-bodied brushstrokes in some areas, as in the Virgin's blue mantle and the clouds (Figure 3.3a-b), and much thinner and delicate brushes in correspondence with the flesh and red tones (Figures 3.3c-d) – suggested the presence of overpainting. For a comprehensive and detailed technical description of the painting, one can refer to the contribution of the CCR's multidisciplinary group in the exhibition catalog [3].

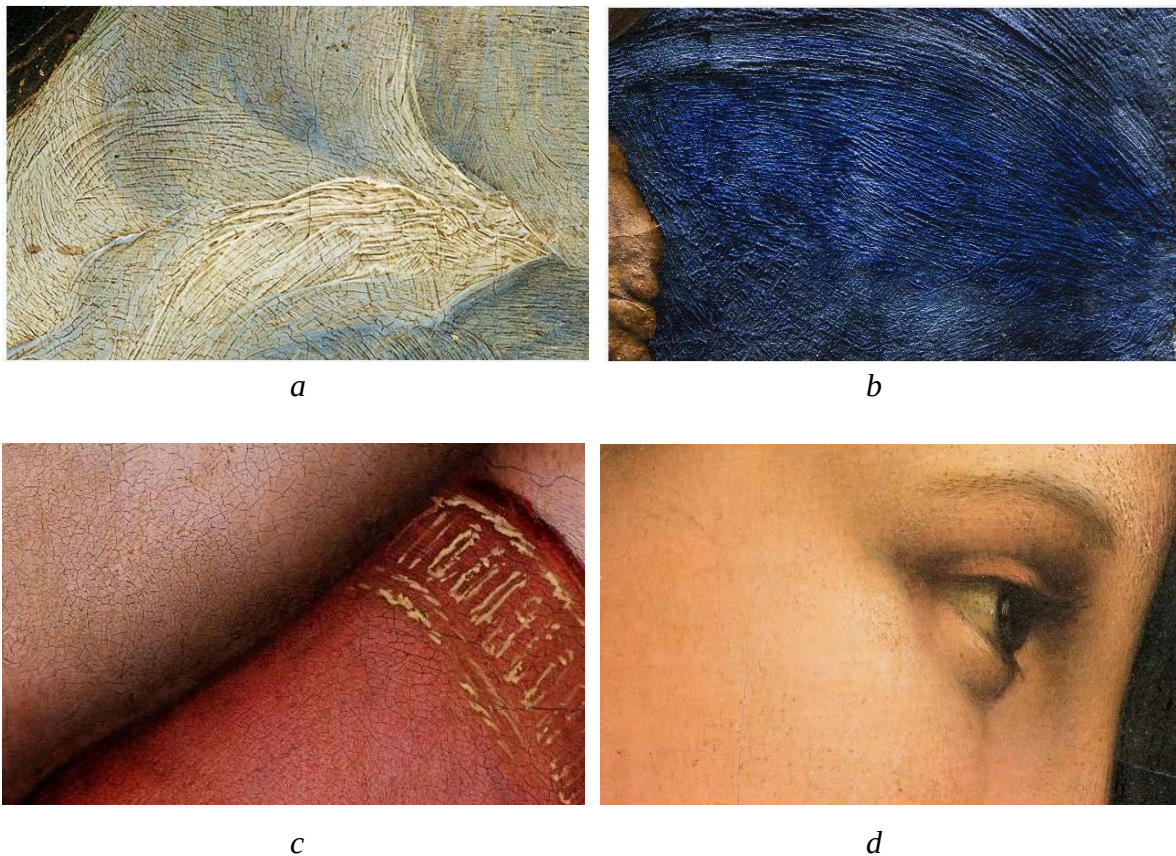


Figure 3.3 Details of the different brushstrokes and uneven painting techniques: *a*) on the clouds, *b*) on the Virgin's blue mantle near the Child's left foot; *c*) on the Child's flesh tone and the Virgin's red robe in correspondence with her right sleeve; *d*) on the Virgin's flesh tone.

Based on the evidence described, the first goal of the research was to scientifically investigate the preservation state of the wooden support and painting layers, the latter having visible issues near the Child's right eye, precisely in correspondence with the hollow on the back surface of the panel. Therefore, the scientific team's task was to go in-depth into the nature of this hollow, revealing possible preservation problems linked to it, using appropriate analytical methods.

Another goal of the investigation was to determine the exact number and composition of the overlaying varnishes, the original paints, and the overpainting, whose presence was suggested by the yellowed and uneven surface aspect, thus supporting with scientific data the conservators in designing the conservation treatment.

3.3 Analytical strategy, experimental, and methods

Answering the research aims required developing a tailored multi-analytical plan and launching specific scientific collaborations. The adopted methods were selected based on their complementarity, potential, and specificity.

To deepen the study of the wooden panel, its state of preservation, and the nature of the hollow present on the back, a combination of X-ray radiography (XR) and pulse compression thermography (PuCT) was planned. Both are non-destructive imaging techniques whose effectiveness in investigating the morphological and physical features of a panel painting's inner structure is well known [4-6]. In this case, PuCT was used as a replacement for a more common CT scan since the CT instrumentation available at CCR was not suitable for the dimensions of the artifact [7], and the "gentle" heating of the PuCT was considered suitable for the study of the artwork. The XR was performed using traditional radiographic films to obtain high-resolution images.

The XR was coupled to high-resolution infrared reflectography in the NIR and SWIR bands, known to gain insight into preparatory phases such as underdrawings and engravings [8-10].

To investigate the original palette and map the overpainting, imaging and spot techniques, known for their complementarity, were combined. In particular, macro X-ray fluorescence (MA-XRF) was applied with a novel prototype system and combined with the recently developed hypercolorimetric multispectral imaging (HMI) method. After the image data acquisition, all the images and maps were aligned in a multi-layer Adobe Photoshop file (with

*.psd format) to cross-reference the results and use them as a guide to select the subsequent spots of analysis.

Fiber optic reflectance spectroscopy (FORS) in the 360-1000 nm range was used for a punctual characterization of pigments and lakes present on the surface. No hyperspectral imaging system (HSI) nor Raman spectroscopy were available for this study.

The removal and analysis of a few multi-layered samples were planned to deepen the study of the original materials and techniques through optical microscopy and SEM/EDS and detect the presence of possible previous localized treatments or overpainting.

The following strategy was adopted to distinguish and study the chemical composition of the varnish layers, whose presence was already suggested by the intense yellowing of the painting's surface and then observed in the cross sections under UV light. The in-depth study of organic compounds in paintings is still very challenging due to the complex composition of natural resin and synthetic varnishes and the reduced thickness of the varnish layers. In this regard, some pioneering works reported using time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging to study paint cross sections and identify simultaneously mineral pigments and organic components like binders and varnishes. The technique provides sub-micrometer resolution mass spectrometry images and high mass resolution, but the instrumentation is expensive and still needs to be widely available. Moreover, sample preparation requires great care and setting up the analytical conditions and data interpretation are very difficult, especially when mixtures of different organic substances are analyzed [11, 12]. Therefore, to shed light on the composition of the various varnish layers, we preferred to rely on a separation technique with a high response specificity, such as pyrolysis gas chromatography mass spectrometry (Py-GC/MS), complemented by Fourier transform infrared spectroscopy (FTIR) on selective samples – as FTIR analyses provide preliminary information on the classes of organic and inorganic compounds functional to the chromatographic analyses. As described in section 3.3.9, a tailored sampling method was designed for this scope.

All the methods applied followed the general well-established rule based on the analysis conducted in subsequent phases, that is, using noninvasive imaging and spot techniques before applying microinvasive methods to minimize the number of samplings and enhance their representativeness.

All the methods adopted are described below. Figures 3.4 and 3.5 show the localization of spot analyses and sampling sites; Table 3.1 lists the studies carried out on each sample.

This analytical plan was designed in the frame of this PhD. All the analyses were carried out at CCR with the following scientific collaborations: the Department of Economics, Engineering, Society and Business Organization (DEIM) of the University of Tuscia for the HMI analyses, the Department of Informatics, Modeling, Electronics, and Systems Engineering (DIMES) of the University of Calabria for the PuCT, the section of Catania of the CNR Istituto di Scienze del Patrimonio Culturale (CNR ISPC) for the MA-XRF, the Aldo Potremoli Department of Physics of the University of Milan for the XR and HR SWIR reflectography, and the Department of Chemistry of the University of Torino for the Py-GC/MS analyses.

Conducting part of the analyses, coordinating the entire scientific team, cross-referencing and interpreting all results, occurred within the scope of this PhD.



a



b

Figure 3.4 The painting during the conservation treatment conducted at CCR: *a*) front of the painting in Vis diffuse light photograph with spots of FORS analysis (in white) and sampling sites (in red); *b*) back of the painting in Vis diffuse light photograph with spots of FORS analysis (in white) and sampling sites (in red). For the details of the micro-invasive analyses, see Table 3.1.

Table 3.1 List of micro-invasive analyses (sampling sites reported in red on Figures 3.4a-b).

SPOT NUMBER AND SURFACE COLOUR	MICRO-INVASIVE ANALYSES
1s dark green	FTIR
2s dark blue	FTIR, OM, SEM/EDS
3s wood	FTIR, GC/MS
4s dark green	FTIR, OM, SEM/EDS, GC/MS
5s dark red	FTIR, OM, SEM/EDS
6s flesh tone	OM, SEM/EDS

3.3.1 UV-Vis-NIR imaging techniques

For visible diffuse light photography (**Vis**), lighting was achieved by placing two 800W Ianiro Varibeam Halogen lamps to the right and left of the object, at an angle of approximately 45° to the normal to the surface, and with the aid of umbrellas for light diffusion. For grazing light photography (**g-Vis**), lighting was attained by positioning one lamp to the left of the object at a maximum angle of approximately 15° to the surface. Photographs were taken with a Nikon D810 Full-Frame DSLR camera (CMOS 7360 x 4912 pixels). The UV-induced visible fluorescence (**UVF**) was acquired via a Nikon D810 full-frame DSLR camera (CMOS 7360 × 4912 pixel) and a Hoya UV-IR Cut filter, irradiating the painting with two UV Labino® lamps (365 nm-emission peak). The near-infrared reflectography (**NIR**) was performed using a Nikon D810 full-frame DSLR camera (CMOS 7360 × 4912 pixel), modified to extend the sensor sensitivity range up to 1000 nm, and with a B+W 093 (87c) filter, illuminating the painting using Ianiro Varibeam Halogen 800 W lamps with indirect diffused light. Image processing was carried out in Adobe Lightroom and Adobe Photoshop (Version C6S) and included a color correction conducted by inserting a 24-color ColorChecker Classic reference in the field of view.

3.3.2 High-Resolution Shortwave Infrared Reflectography (HR-SWIR)

HR-SWIR reflectography was performed with a spherical scanning system [13] based on an InGaAs camera (Xenics Xeva 1.7-640, 640×512 elements, spectral sensitivity: 1000–1700 nm) mounted on a spherical motorized head, with a linear stage for refocusing. A total of 2200 images were acquired and merged to obtain a final image with a spatial resolution 20 pixel/mm. Two halogen lamps, with indirect and diffusing screens, were used for the lighting.

3.3.3 X-ray Radiography (XR)

XR was performed using the traditional RX film technique, a General Electric Eresco 42MF4 X-ray source (200kV max, 4.5 mA), and AGFA D4DW plates of 30×40 cm in size. In addition to the exposure tests, six plates were exposed to cover the entire painting's surface with an adequate overlap, setting the tube tension to 43 kV for 1 min. The painting was positioned vertically in front of the X-ray source at a 150 cm distance. The plates were then developed in a darkroom at a temperature of 20 °C for 6 min, and subsequently digitized and merged using Adobe Photoshop.

3.3.4 Pulse-Compression Thermography (PuCT)

The here-employed PuCT setup was the same used in other works, see for example [5, 14, 15]. In particular, a National Instrument 1433 Camera Link Frame Grabber and National Instrument PCI-6711 AWG board was used to acquire thermograms from a Xenics Onca-MWIR-InSb IR (320×256 pixels, 3.6–4.9 μm) at 40 FPS and to modulate the on/off state of a TDK Lambda GEN 750 W power supply, respectively. In this way, 8 LED chips (6500 K, 4500 LM) were driven at 200 W so as to emit light following a Legendre code pseudo-noise excitation of order equal to 31 for an overall heating stimulus duration of 62 s – the reader is referred to [16, 17] for an in-depth mathematical explanation on the correct use of such code in PuC applications. The LED system and the thermal camera were placed on the same side at about 50 cm from the inspected surface, so that the resulting inspection was performed in reflection mode. A desktop computer was used to store the data using an in-house routine developed in LabVIEW.

As for the setup, both the camera and the LED were moved after each measurement – the limited field of view and relatively low pixel resolution of the thermal camera are used because

the whole painting could not be inspected otherwise. A mosaic was then reconstructed by sticking each measurement next to the others. Finally, all the reported results are shown using a grey colormap, where the darker pixels are related to higher temperature/emissivity.

3.3.5 Macro X-ray Fluorescence (MA-XRF)

MA-XRF was performed via a mobile X-ray scanner, used in situ at the CCR and developed by the XRAYlab of the Institute of Cultural Heritage Sciences, National Research Council (ISPC-CNR), and by the South National Laboratories, Institute of Nuclear Physics (LNS-INFN), Catania, Italy. The scanning system allows for analyzing large painting surfaces up to $110 \times 70 \text{ cm}^2$. The time taken for inspecting the whole painted area was 2.5 h, with a lateral resolution of $1000 \text{ }\mu\text{m}$ at the maximum scanning speed of 100 mm/s [18]. This innovative real-time technology allows for a continuous scanning of the surface to be performed, instead of using the conventional step-by-step mode. All the images of the chemical elements composing the painting layers are elaborated in live mode, and its distribution on the support is visualized in real-time during the scanning. The primary X-ray beam is generated by an Rh-anode tube (50 kV maximum voltage and 0.6 mA maximum current) and it is focused on the sample by a polycapillary optics coupled with the X-ray source. A time list mode (TLIST), implemented in a multi-detector system composed of two 50 mm^2 silicon drift detectors (SDD) with a 160 eV spectral energy resolution at 5.9 keV, is used to detect the emitted fluorescence radiation. The detection system allows for a time/pixel in the ms range and high counting statistics. A fast-fitting procedure (max processing speed: 7000 spectra/s) is applied to all pixel spectra generating on-the-fly elemental images. During the scanning, the movement of the z-axis adjusts the measuring head/surface distance, monitored in real-time by a laser sensor, to follow, if present, the non-flat surface of the sample [19-23]. The whole area of the painting (consisting of c.a. 537×802 pixels) was analyzed with a scanning speed of 70 mm/s , a dwell time/pixel of 15 ms, and a lateral resolution of 1 mm. The measurement time was 1.8 h, and the primary X-ray beam voltage was set at 50 kV, with a current of $600 \text{ }\mu\text{A}$.

3.3.6 Hypercolorimetric multispectral imaging (HMI)

HMI is a portable multispectral system developed by the Italian company Profilocolore® consisting of a Nikon D800 modified full-frame camera and two proprietary softwares, one

based on AI for calibration and the other for processing the calibrated images [24]. The camera is equipped with two bandpass filters whose spectral transmittances are complementary. The lighting is obtained by two modified flashes controlled by the camera through triggers. The combination of these filters with the Bayer matrix of the camera sensor allows to acquire the spectral range between 300 and 1000 nm with just two photo shoots, subsequently separated into 7 bands by the calibration software (UV; the B, G, R bands of the Vis; IR1 at 750 nm; IR2 at 850 nm and IR3 at 950 nm) providing imaging data with a radiometric accuracy of 95% and colorimetric error $\Delta E < 2$. To obtain the calibrated images, it is necessary to include acquired scene white radiometric references and a Colorchecker from the Natural Colour System® catalog. The white references must perimeter the scene and include the Colorchecker. The processing software allows for calculating the reflectance values at the seven calibrated wavelengths of each pixel of the image, comparing them with a database of pigments' spectral signatures, elaborating calibrated false color images, and applying chromatic and spectral similarity algorithms and other tools.

3.3.7 Fiber optics reflectance spectroscopy (FORS)

The FORS setup employed an Ocean Optics HR2000+ES spectrophotometer, an Ocean Optics HL-2000-FHSA halogen lamp, and a Labsphere Spectralon Wavelength Calibration Standards white reference. The spectral resolution is about 0.5 nm. The system includes two fiber optics, one single and one bifurcated, with a 400- μ m diameter, equipped with an Ocean Optics RPH-2 anodized aluminum fiber support, enabling reflection measurements at a 90° or 45° angle to the bearing surface. Spectra were acquired in reflectance mode, within a 360-1000 nm spectral range, with 2x45/0 optical geometry. Spectra were interpreted by comparison with published literature and spectral libraries available at the CCR scientific laboratories [25].

3.3.8 Optical microscopy in visible and ultraviolet light (Vis-UV OM) and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDS)

Four multi-layered samples were taken from the painting, observed, and photographed under visible light using an Olympus SZX10 stereomicroscope equipped with an Olympus Color View I digital camera. After being arranged as cross sections, the samples were photographed under Vis and UV light using an Olympus BX51 minero-petrographic microscope equipped with an Olympus DP71 digital camera. In both cases, image acquisition and processing were

performed using the analySIS FIVE proprietary software (Version 2005). The cross sections were then analyzed in back-scattered electron mode (BSE) using a Zeiss EVO60 scanning electron microscope equipped with a lanthanum hexaboride (LaB6) cathode and a silicon drift detector (SDD), coupled with a 40 mm² Oxford Ultim Max EDS microprobe for semi-quantitative elemental analysis. They were coated with a nanometric layer of carbon and analyzed in high vacuum mode, using an accelerating voltage of 20 kV and pressure of 10⁻⁵ Pa.

3.3.9 *Selective sampling*

The selective sampling was based on the different UV fluorescence of the varnishes observed during the cleaning tests conducted by the conservator and in the cross sections. Therefore, different from the usual protocol, the sampling was conducted under a UV lamp, taking the scrapings with a scalpel and delicately scratching the surface of the painting to remove the different varnishes observed. The transition from one varnish layer to the other was checked during the sampling by observing the surface's UV fluorescence change. Four micro-samples representative of four layers of varnish were thus possible.

3.3.10 *Fourier-transform infrared (FTIR) spectroscopy*

FTIR analysis on selective samples was performed with a Bruker Vertex 70 FTIR spectrometer coupled with a Bruker Hyperion 3000 infrared microscope and equipped with a mercury cadmium telluride (MCT) detector. Scrapings were analyzed as a bulk in transmission mode through a 15x objective, upon compression in a diamond cell. Data were collected in the 4000-650 cm⁻¹ spectral range, at a spectral resolution of 4 cm⁻¹, as the sum of 32 scans. Spectra were interpreted by comparison with published literature and spectral libraries available at the CCR.

3.3.11 *Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS)*

The selective samples of varnishes removed from the painting were analyzed by Py-GC/MS using a micro-furnace EGA/PY-3030D pyrolyzer interfaced with a 6890N Network GC System gas chromatograph equipped with HP-5 cross-linked 5% Ph Me Silicone capillary

column (30 m, 0.25 mm i.d., 0.25 μ m film thickness) and a 5973 Network Mass Selective Detector mass spectrometer. Samples were derivatized with the Thermally Assisted Hydrolysis and Methylation method (THM) using tetramethylammonium hydroxide (TMAH) in an aqueous solution at a concentration of 25% by weight. Pyrolysis was carried out at 500°C for 12 s. The interface temperature of the pyrolizer was 300 °C and the temperature of the GC injector was kept at 280 °C. For the gas chromatographic separation, the following temperature program was used: 2-minute isotherm at 50 °C, ramp of 10 °C/min up to 300 °C, isotherm at 300 °C for 15 minutes. The mass spectra, obtained by electron impactation at 70 eV, were recorded in the range 40-650 m/z. The carrier gas used is helium, with a flow rate of 1.0 ml/min. All instruments were controlled by Enhanced Chem Station (ver. 9.00.00.38) software. The mass spectra assignment was done with the Wiley 138 and NIST2008 libraries and by comparison with literature data.

3.4 Results and discussion

In this section, the results are reported and discussed not technique by technique but divided into macro topics following the stratigraphy of the work – from the wooden support and its internal structure to the most external finishes, all of which are of significant value.

For each topic, the results relating to the execution technique and the conservation aspects of the painting are described in a comprehensive manner, highlighting the types of analysis and methodologies that contributed most to obtaining this result.

3.4.1 *Wooden Support: the hollow on the panel's back and preservation issues*

Our initial findings, in line with the expert technical observation of the conservator, were significant. They revealed that the panel is crafted from a single poplar wood board, identifiable by the characteristic radiographic pattern of the wood grain (Figure 3.5). The hollow on the back of the panel, with its engraved edges, is treated with organic compounds, likely serving an insulating function. This was initially detected by the UVF (Figure 3.6) and later confirmed by Py-GC/MS as colophony and beeswax.

In correspondence with the hollow, the combined results of XR and PuCT made it possible to detect the original presence of a small knot, largely removed. In XR, the wood grain is particularly evident in the side portions but much less noticeable in the central stripe (see Figure

3.5). In PuCT, the widening of the wood grain inside the thickness of the board can be observed around the hollow. Moreover, a tau-shaped element is visible near the hollow using both techniques. Figure 3.7 shows a series of thermograms at increasing time instants obtained using PuCT. The thermal signature from deeper layers becomes visible as time elapses – the painting layer tends to disappear at a time value equal to 6 s, while the details belonging to the inner wooden panel simultaneously appear more and more clear. Interestingly, the tau-shape, hereafter referred as tau, thermal signature is visible from $t = 6$ s (see red arrows) to $t = 15$ s. In addition, a series of vertical strips become evident during the same time interval (yellow arrow); these correspond to the wood grains, which are also visible in the XR analysis (see Figure 3.5).



Figure 3.5 XR of the painting showing the pattern of wood grains of the support (much less noticeable in the central stripe) and the numerous woodworm tunnels following the pattern of the wood fibers.

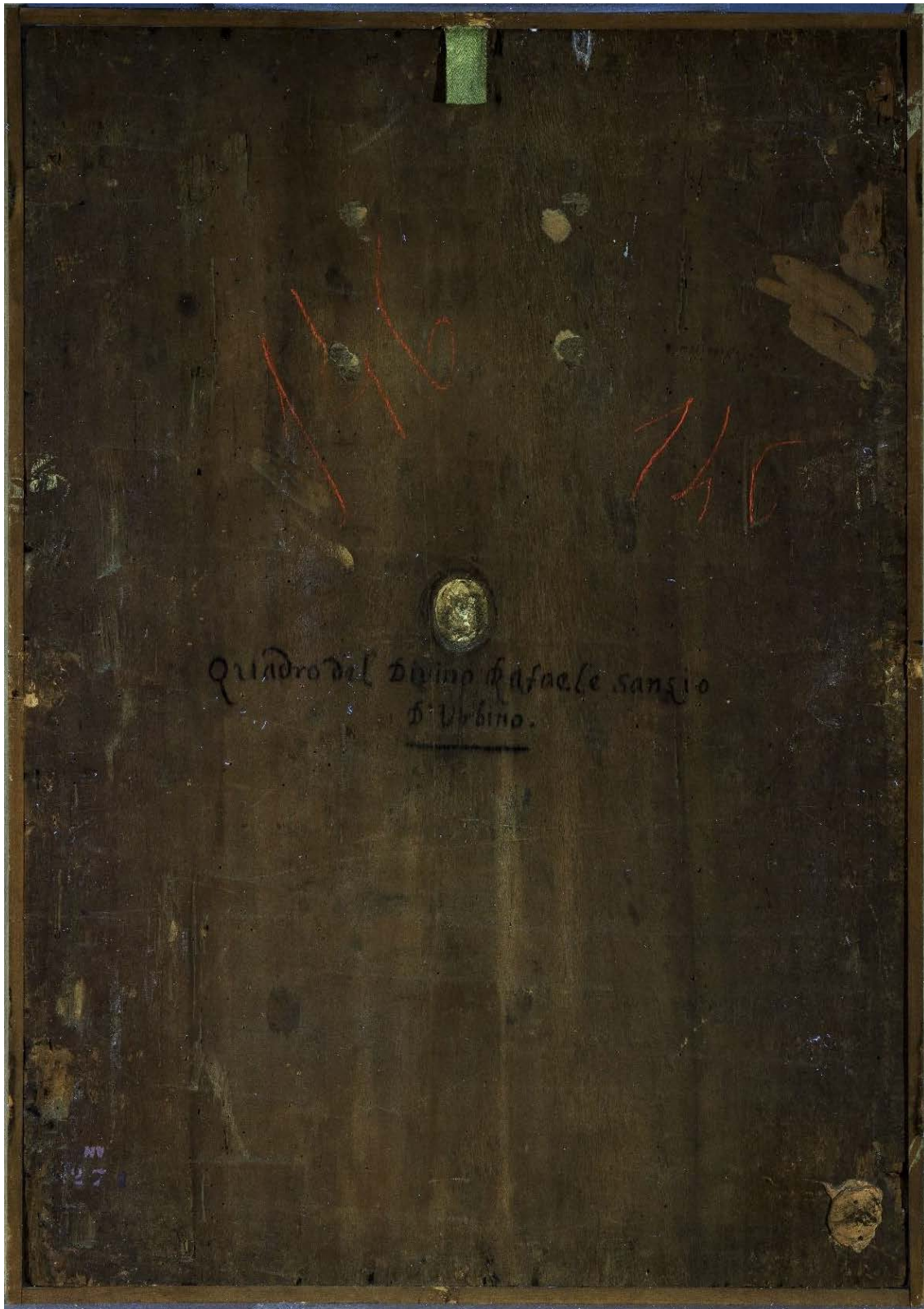


Figure 3.6 Back of the painting, UVF.

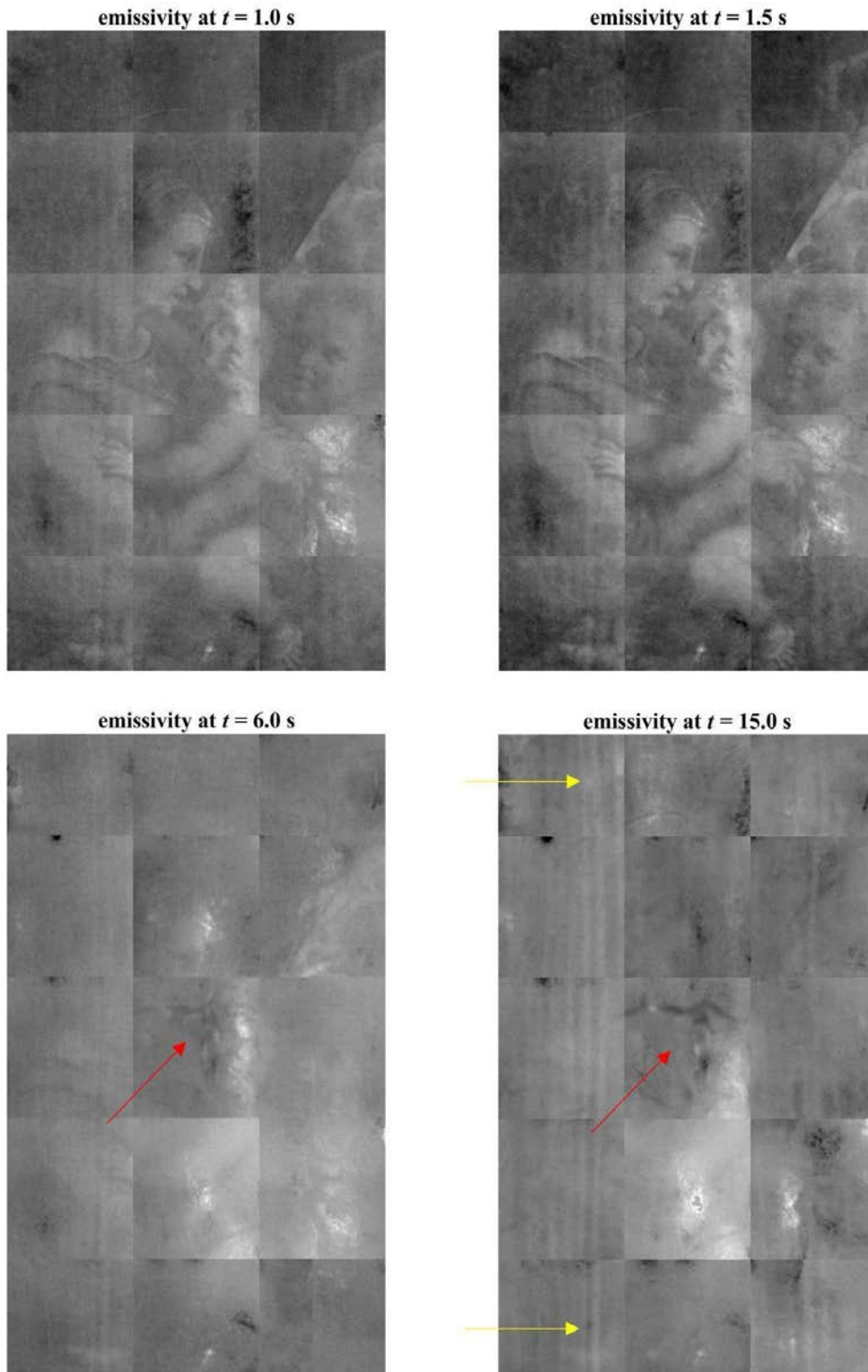


Figure 3.7 A series of thermograms at different time instants obtained by PuCT. A tau-shaped thermal signature is visible, starting from $t = 6$ s (red arrow). As a reference, the yellow arrows point out a single vertical wood grain signature. Note that other quasi-parallel grains are also well defined all across the inspected surface.

The depth of the tau from the inspected surface, i.e., the painted surface, can be inferred by comparing the thermal contrast obtained from a pixel area of the tau, and that obtained from an area of the wood grain. These 4×4 pixels areas are highlighted using red- and green square markers respectively, while orange has been used to mark the sound area employed as a reference for computing the thermal contrast (see Figure 3.8b), with the thermal contrast defined as:

$$\begin{aligned} Contrast_{\tau_{\text{tau}}}(t) &= \frac{\tau_{\text{tau}}(t) - \text{sound}(t)}{\text{sound}(t)}, \\ Contrast_{\text{wood}}(t) &= \frac{\text{wood}(t) - \text{sound}(t)}{\text{sound}(t)}, \end{aligned} \tag{1}$$

with $\text{wood}(t)$, $\tau_{\text{tau}}(t)$, and $\text{sound}(t)$ being the averaged temperature at the selected grain, tau, and sound areas, respectively.

The line plots in Figure 3.8a show the obtained contrasts within the evaluated time interval; the two maxima are reached at about 12 s, indicating that the tau is located at the same depth of the wood grain. In other words, the tau is a feature belonging to the support panel.

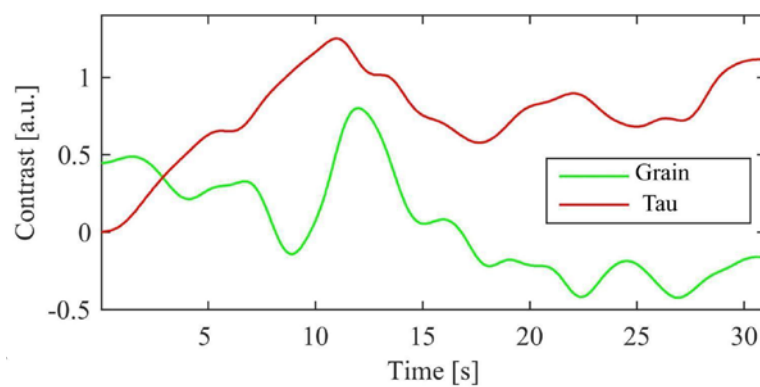
Interestingly, the tau signature appears darker than the surrounding area (see Figure 3.8b) where the thermogram at 12 s is imaged, meaning that its temperature is higher than that of the other pixels. Commonly, this happens when a material with a lower thermal capacity is found, a good example being delaminations with air trapped within the preparatory layer and the wood support. This suggests that the tau is potentially a cracked and unfilled area of the wood support.

The tau is located geometrically close to the hollow area visible from the back surface of the panel. It is worth investigating whether more information on the nature of the tau can be inferred using the PuCT analysis of the back surface of the panel. To this aim, Figure 3.9a shows a series of thermograms at different time instants, whereby both the hollow wood area and the wood grains appear clearly. To gain more insight into the back surface of the support, Figures 3.9b-c depict the emissivity at $t = 4$ s and the time-phase feature at $t = 1$ s, respectively. Both the imaged features show an advanced state of degradation of the inspected surface, where woodworm holes are also visible, especially in Figure 3.9c.

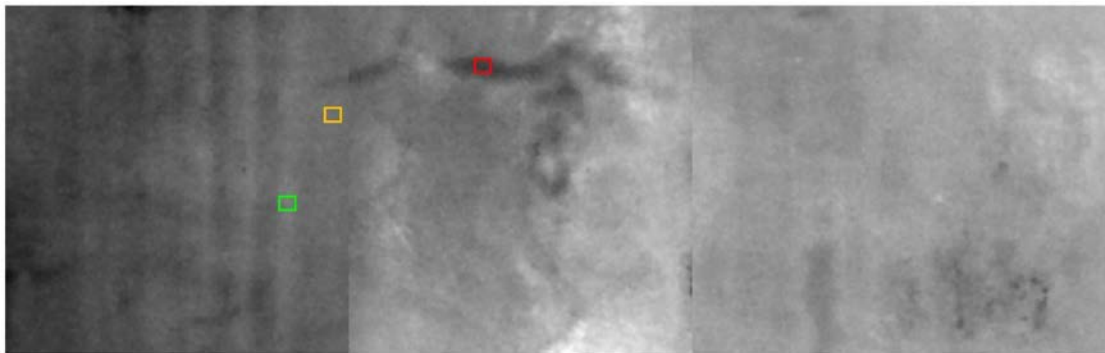
Interestingly, the number of visible grains is considerably higher at both the left- and right-end sides, compared to that visible near the hollow part. Moreover, the most visible wood grains near the hollow follow a smooth trajectory around it, suggesting/confirming that the hollow

part could have been a wood knot. This indicates, in turn, that the tau might have been generated due to the fragile nature of this part of the panel support, which is further confirmed by the potential loss of the knot.

The hollow is so deep that it reaches a few millimeters from the painted surface. As a visible result, in correspondence with it, the wooden support is so thin that it has caused deformations and fractures on the painting film; moreover, in correspondence with the surface along the tau, other points of fragility were found, such as the one on the Virgin's chin (see Figure 3.1b).



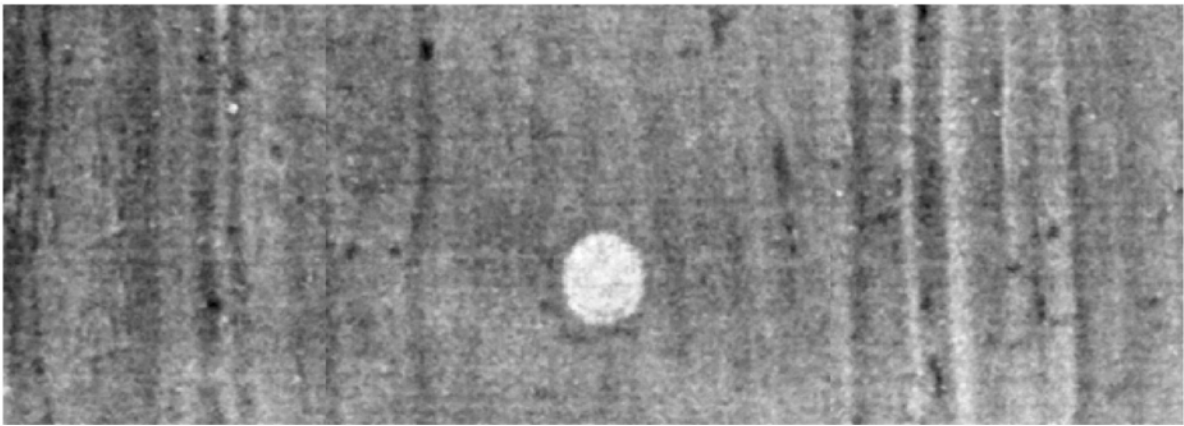
a



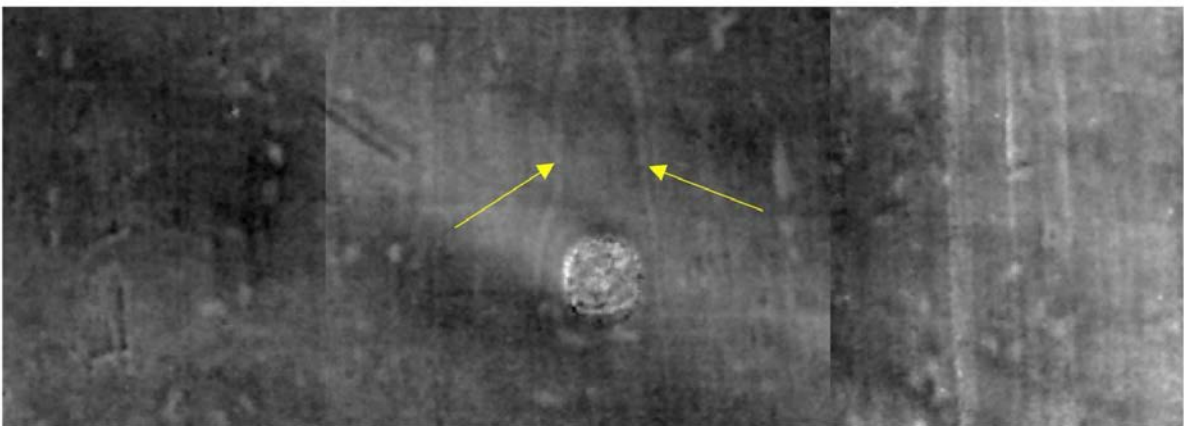
b

Figure 3.8 Results from the PuCT analysis: *a*) thermal contrast for the grain (green) and tau (red) pixel areas as a function of time. A maximum is reached for both quantities at $t = 13$ s; *b*) a thermogram showing the investigated area at $t = 13$ s, marked to highlight the regions employed for computing the thermal contrast, i.e., tau within the red square, wood grain within the green square, and sound within the orange square.

a



b



c

Figure 3.9 Results from the PuCT analysis: *a*) a series of thermograms at different time instants acquired from the back panel surface; *b*) one of the thermograms, for a single instant $t = 4$ s; *c*) image obtained using the time-phase feature, with yellow arrows pointing towards the wood grain surrounding the hollow area.

In many cases, as observed in XR (see Figure 3.5), the numerous woodworm tunnels follow the pattern of the wood fibers and are visible as a darker and less radio-opaque trace due to the lack of wooden material, whereas other vertical signs are more radio-opaque: figure 3.10 shows an example of these signs in correspondence to the green curtain, which is painted with a copper-based green pigment.

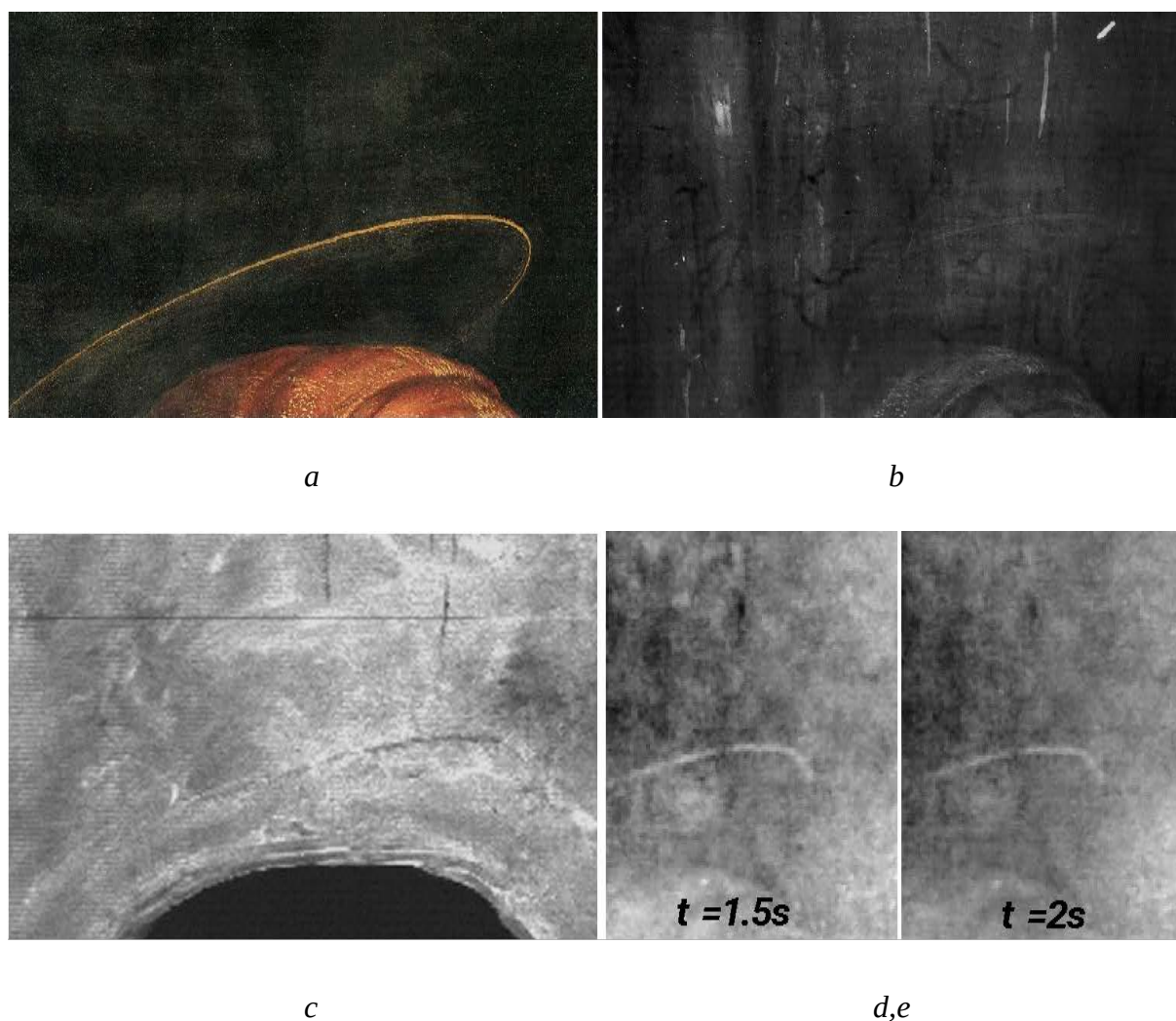


Figure 3.10 Detail of the green curtain painted with a copper-based green pigment; *a*) Vis light photograph; *b*) XR showing the woodworm tunnels and the more radio-opaque vertical lines; *c*) MA-XRF distribution map of copper showing the lower Cu-signal along the vertical lines; *d,e*) PuCT thermograms at $t = 1.5\text{ s}$ and $t = 2\text{ s}$ when the dark vertical lines became whiter.

In the regions of the more radio-opaque lines, a lower signal of copper is detected, as evidenced by the greyscale MA-XRF distribution map of copper (Figure 3.10c), where the lines appear

less bright, while no other elemental differences are detected using the same technique. In the same area, a series of thermograms at different time instants acquired from the front panel surface using PuCT show these vertical lines as dark lines, becoming whiter as time elapses, suggesting that these have been filled and/or restored (Figures 3.10d-e). Therefore, these lines could be cracks filled with some radio-opaque compound, not better identified, that probably involves only the upper layers: in correspondence with such signs, in fact, the painting exhibits some micro-cracking, suggesting that they are probably on the surface of the painting and not inside the wooden panel, as is the case for the tunnels caused by worms.

Overall, investigating the presence and nature of the hollow on the painting's back surface led to a better understanding of the features of the wood grain, the woodworm tunnels, and other peculiar characteristics of the wooden support. The results are summarized in Table 3.2, which also shows the synergy and complementarity of the methods used for this scope.

Table 3.2 Summary of the XR, PuCT, and MA-XRF results relative to the wooden support.

Wooden Support	XR	PuCT	MA-XRF
Wood grains (general view)	The wood grain, typical of poplar wood, is particularly visible in both the left- and right-end sides of the panel, and are much less noticeable in the central slice containing the hole.	The number of visible grains is considerably higher at both the left- and right-end sides, compared to those visible near the hole.	Not detectable
Wood grains around the hole	Not evident	The wood grain inside the thickness of the board widen around the hole: the most visible wood grains near the hollow follow a smooth trajectory around it, suggesting/confirming that the hollow part could have been a wood knot.	Not detectable
Tau-shaped element near the hole	Clearly visible	Clearly visible; it is located at the same depth of the wood grain, thus belonging to the support; it appears darker than the surrounding area (its temperature is higher than that of the other pixels/it has lower thermal capacity, so it is potentially a cracked and unfilled area of the wood support).	Not detectable
Woodworm tunnels	Visible as radio-transparent traces in the central part of the panel: in many cases they follow the pattern of the wood fibers.	Visible	Not detectable
Other vertical signs (area of the green curtain)	Visible as more radio-opaque vertical traces, since filled and/or restored.	Visible as dark lines, becoming whiter as time elapses, in the thermograms acquired at different times from the front panel surface, Figure 3.10-suggesting that these have been filled and/or restored; filler probably involves only the upper layers.	Only visible in the Cu-distribution map (a lower signal in the distribution of copper make the vertical signs visible).

3.4.2 Preparation phases: from the ground to the underdrawings

The scientific analysis of the painting allowed for an understanding of the layering and composition of the preparation phases.

As observed in the multi-layer samples – an example of which is reported in Figures 3.11a-b, showing the sample coming from the Virgin's blue mantle –, the wooden panel is prepared with white layers of gypsum-based plaster (ground); the binder for this layer is animal glue, as confirmed by FTIR (Figure 3.12). The ground is followed by a thin layer of a yellow-orange imprimatura made of lead white and ochres and applied with a brush. Moreover, the imprimatura layer is remarkably transparent, allowing the artist to see the drawing during the composition. A description of these technical aspects is reported in the contribution by the CCR team in the exhibition catalog [3].

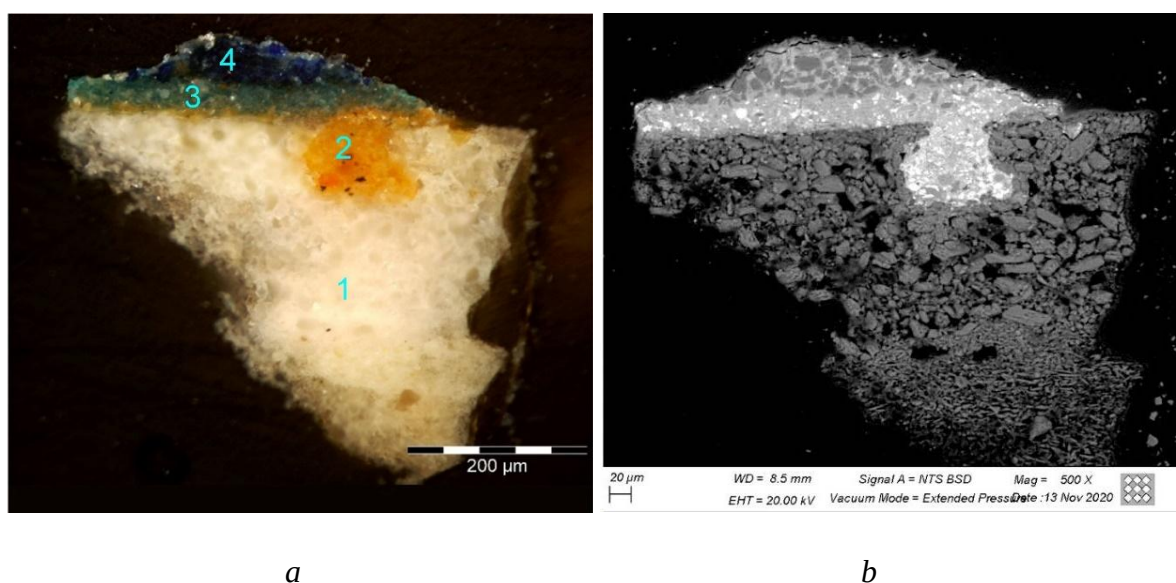


Figure 3.11 Microscopic images of the cross section from the Virgin's blue mantle: *a*) OM in visible reflected light; *b*) SEM/BSE. The images show a yellow-orange rounded inclusion of pigmented imprimatura (layer 2) in the gypsum-based ground (layer 1). Above this, two painting layers – the first in azurite and the second in lapis lazuli (layers 3 and 4 respectively) – render the color of the blue mantle.

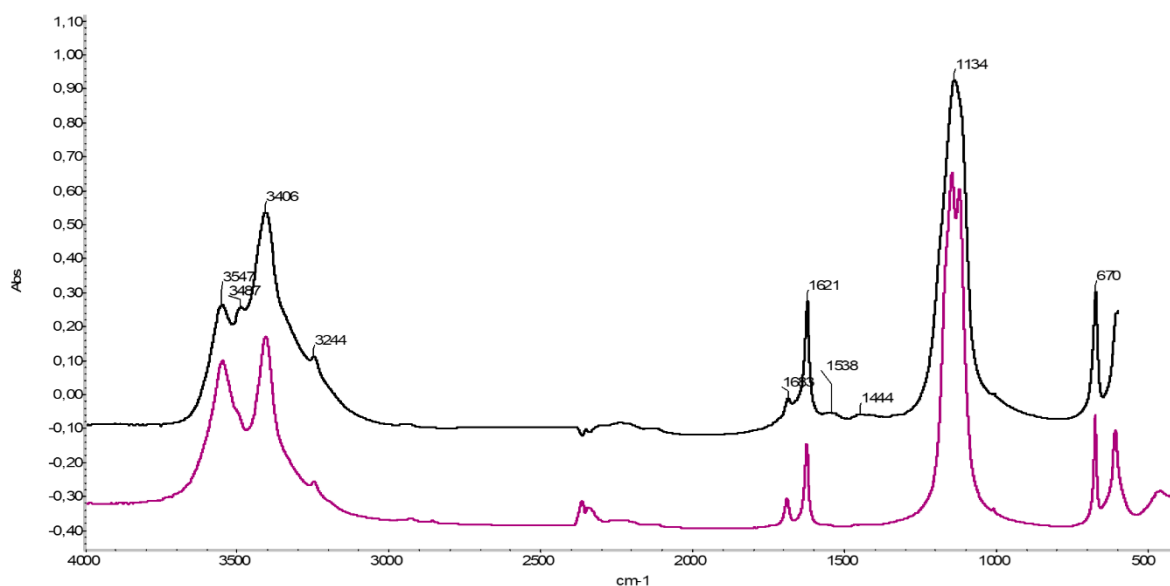


Figure 3.12 In black: FTIR spectrum of a scraping selectively removed from the white ground (sampling site 2s in Figure 3.4). In the sample, the spectral features of gypsum were detected (reference spectrum in purple), whereas the signals at 1538 and 1444 cm^{-1} were assigned to a protein substance.

Interestingly, observing the painting's XR at high magnification, we noticed numerous spots of more radiopaque material that look like widespread, small, and irregular white spots (Figure 3.13). Comparing the cross section's images of the blue mantle with the radiographic details, it was possible to attribute the presence of these spots to bubbles formed during the preparation and mixing of the plaster; these bubbles, during the smoothing of the ground layer, are opened and can be filled by the imprimatura, thus showing very radiopaque and diffuse dots on the whole surface of the painting. The average size of these inclusions corresponds to approximately 100 μm .

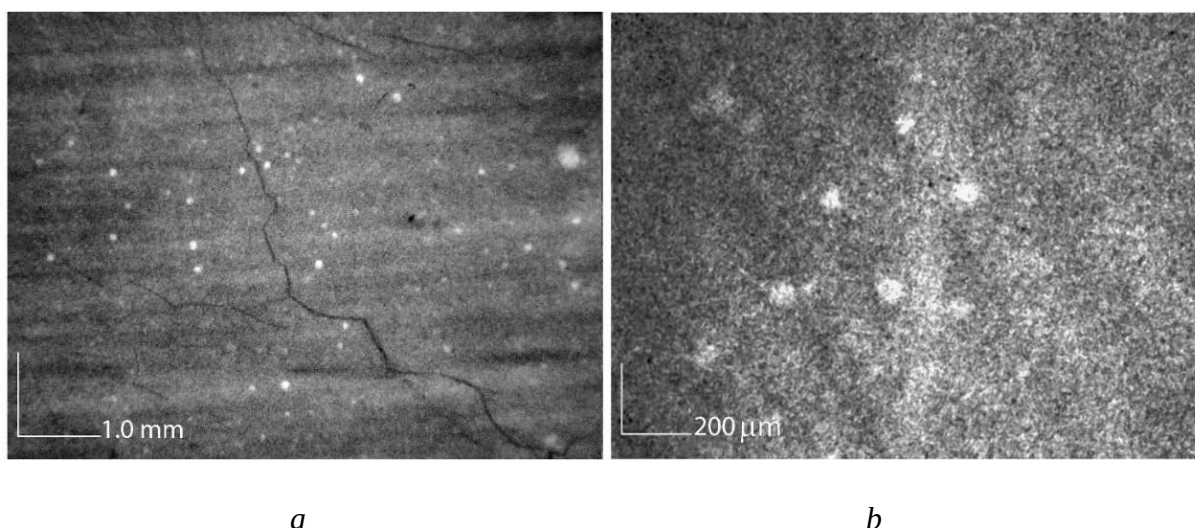
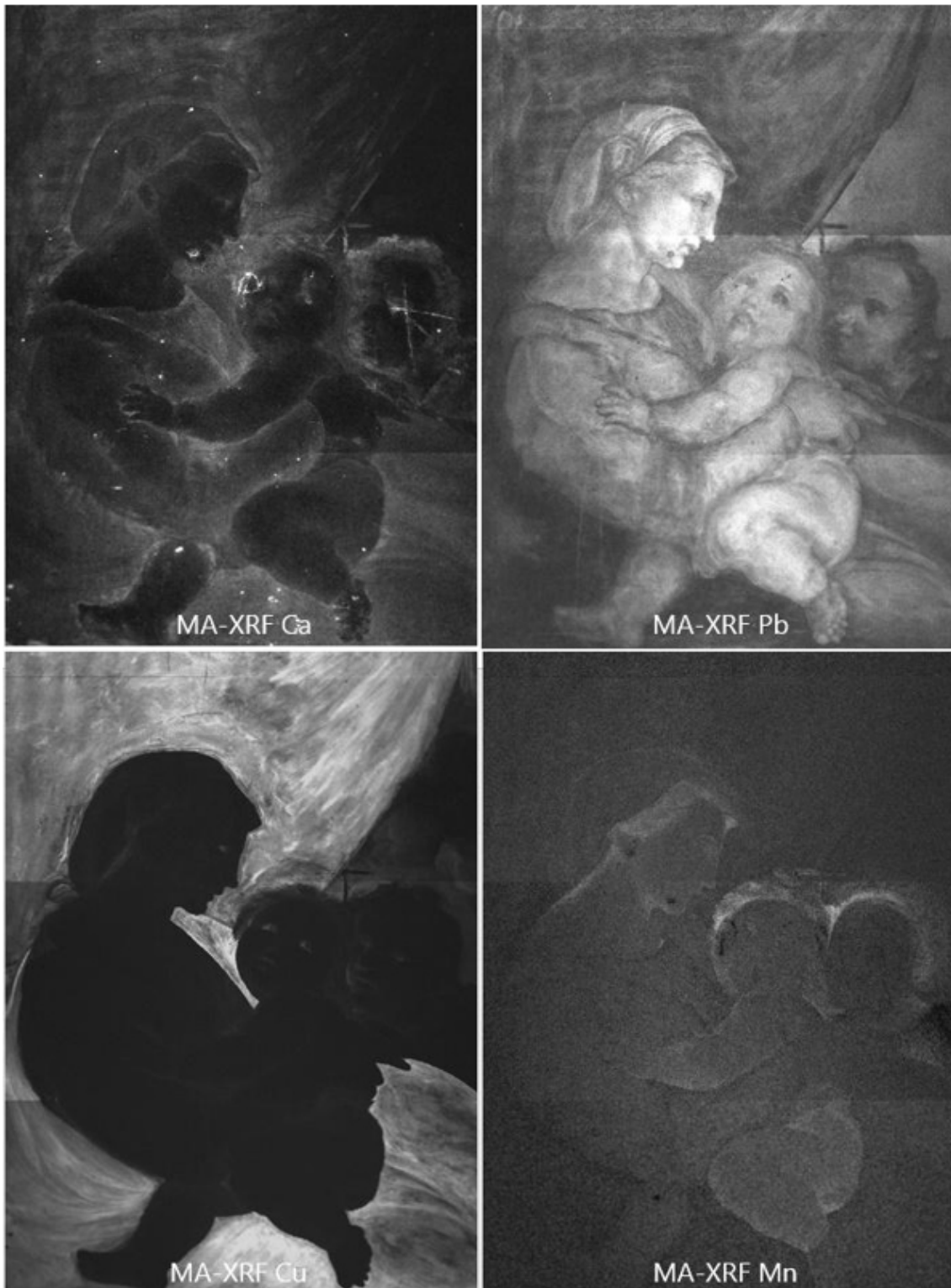
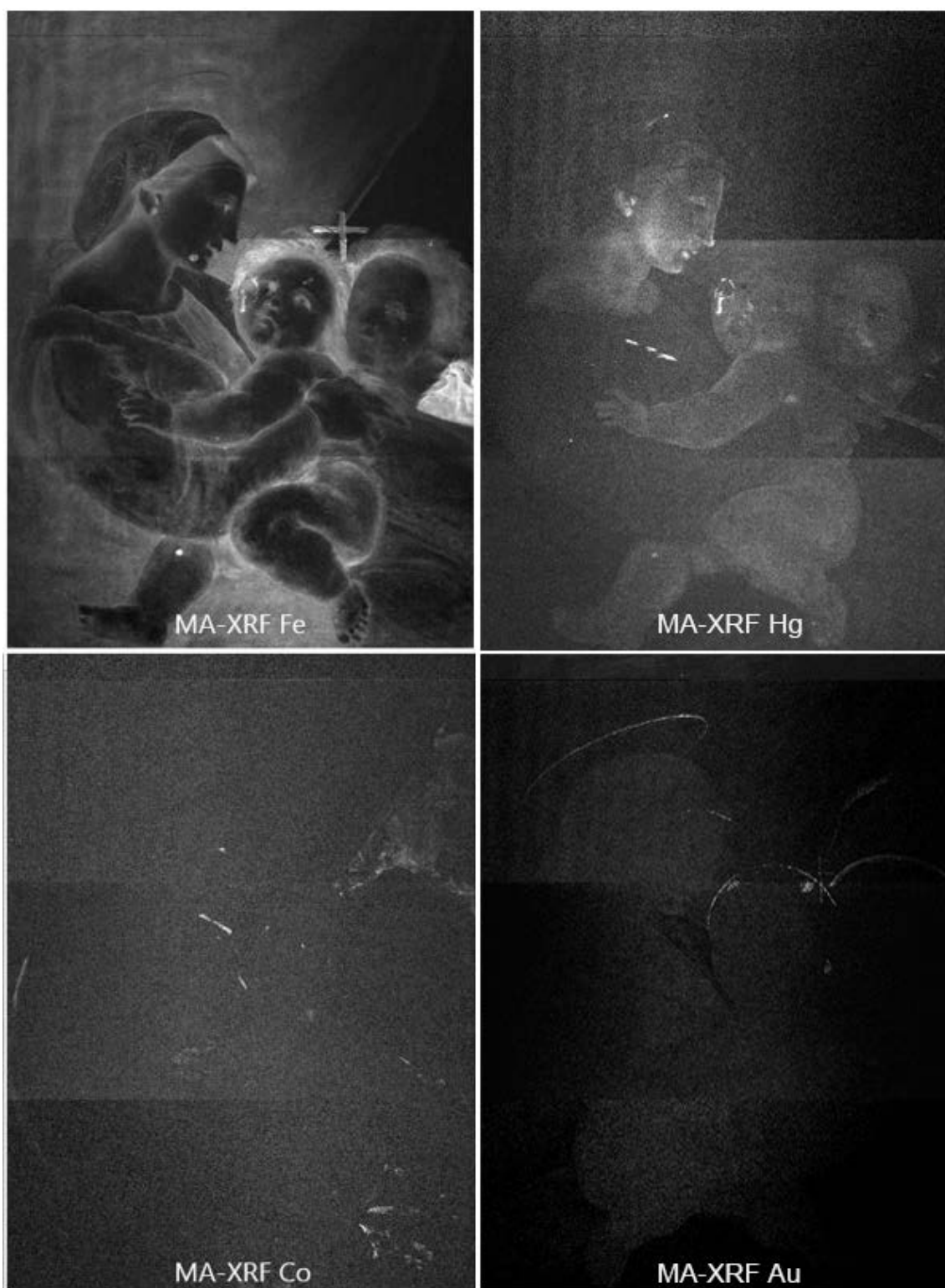


Figure 3.13 Enlarged radiographic details of the painting at *a)* 55x and *b)* 220x. The white spots' size is fully compatible with the imprimitura inclusions in the ground layer shown in Figure 3.11.

Figure 3.14 illustrates the distribution maps of the primary chemical elements identified in the painting using the MA-XRF technique. The comprehensive distribution of lead, iron, and calcium across the entire support provides strong support for the hypothesis of the use of lead, iron, and calcium-based pigments in the preparation layers (ground and imprimitura), as confirmed by the micro-invasive analyses. However, it's important to remember that the information provided by MA-XRF is integrated throughout the entire stratigraphy, so the detected signals of lead, iron, and calcium may originate from the preparatory layer and the upper layers' pigments or compounds. For instance, the lead map suggests that the artist employed lead white pigment not only for the imprimitura but also for the characters' skin tones and the highlighting effect on the robes and curtain. Similarly, iron-based pigments, likely ochres, were used for the brown tones, such as the characters' hair. Moreover, it is essential to remember that, due to the pigments' chemical nature, the painting's stratigraphy can make detecting low-energy fluorescence lines difficult, especially when the low Z elements, such as calcium, are present in the deeper layers. Indeed, the fluorescence emitted by the low Z elements can be almost completely absorbed in the upper layers composed of heavier elements, such as lead or copper. This absorption effect is visible in the maps of Figure 3.14, which show how the calcium and iron signals coming from the preparation are strongly absorbed in correspondence with the lead white-based areas of paint. In contrast, the calcium

signal, for example, is evident in small regions affected by past restorations, such as grouting or fillings.





b

Figure 3.14 MA-XRF distribution maps of: *a*) calcium (Ca), lead (Pb), copper (Cu), and manganese (Mn); *b*) iron (Fe), mercury (Hg), cobalt (Co), and gold (Au).

As shown in Figure 3.15, the infrared reflectography was crucial for revealing and studying the underdrawing. The NIR image (Figure 3.15a) provides a view of the primary traces, but its ability to penetrate all the pigment layers is limited. In contrast, HR-SWIR reflectography (Figure 3.15b) is capable of highlighting all the details, even in areas with darker or copper-based pigments. It reveals a highly detailed, clean, and skillful stroke in the underdrawing, which was created using a brush and a fluid carbon black ink. Additionally, it allows for the hypothesis of a preparatory cartoon transferred using punched dots and carbon powder. Some of these punched dots are faintly visible in correspondence to the underdrawing (Figure 3.16), likely due to their removal with a brush and their dilution in the primary underdrawing traces completed freehand. The artist further refined the drawing by adding shading and other indications, which served as a guide during the painting phase for executing the drapery of the clothes and details of the figures.

Besides the traces of underdrawing made with black ink, signs of metal point engravings and needle points are also present due to the construction of the figures' haloes, made using a compass (Figure 3.17), presumably drawn on the plaster layer. As reported in the technical study of the artwork [3], the haloes were subsequently gilded and then repainted with gold during past conservation treatments. The traces of these haloes are also visible as a more intense signal in the MA-XRF distribution maps of lead, probably due to the greater thickness of imprimatura inside the groove left by the compass. On the other hand, in the distribution maps of calcium and iron, the form of the haloes appears as a less intense signal due to their absorption in the upper layer characterized by the presence of gold.

Overall, the micro-invasive OM and SEM/EDS analyses on the four multi-layered samples confirmed and detailed what was hypothesized from the non-invasive imaging techniques regarding the preparation phases of the painting. The results are summarized in Table 3.3, showing the complementarity of the analytical methods used.



a



b

Figure 3.15 Infrared reflectography image comparison: *a*) NIR and *b*) HR-SWIR, showing the difference in revealing the underdrawings made with a brush and a carbon black ink.

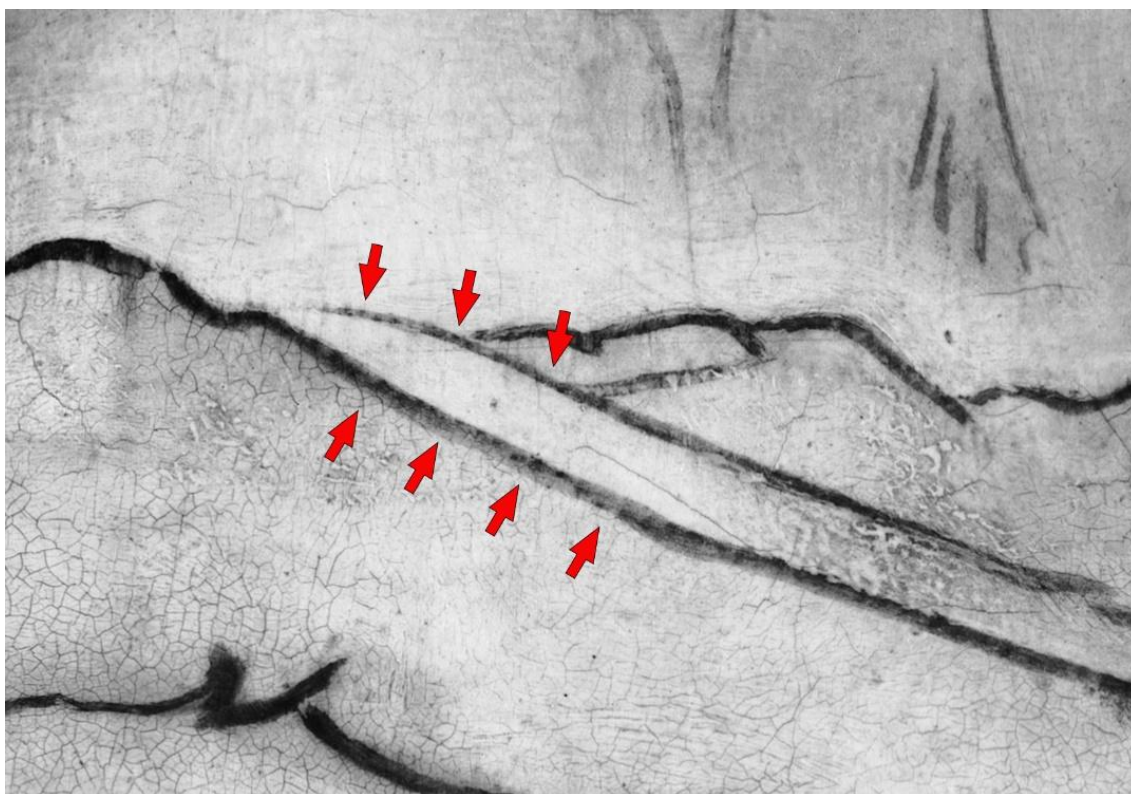


Figure 3.16 HR-SWIR detail showing the traces of punched dots, indicated by the red arrow, in correspondence to the neck of the Madonna.



Figure 3.17 XR detail, where the signs of metal point engravings and needle points are evident in the tracing of the two haloes by means of a drawing compass.

Table 3.3 Summary of the OM, SEM/EDS, MA-XRF, XR, and HR-SWIR results relative to the preparation phases.

PREPARATION PHASES	OM and SEM/EDS	MA-XRF	XR	HR-SWIR
Ground layer	White layer made of plaster, detected in the four samples		Not evident	Not evident
Imprimitura	Yellow-orange layer made of lead white and ochres, detected in all the four samples	Detected the use of calcium-, lead-, and iron-based pigments for the preparation phases (ground and imprimitura not distinguishable between each other).	Visible as radiopaque brushstrokes in some areas, also not in correspondence with lighter areas of paint; imprimitura inclusions in the plaster layer are visible at high magnification as a widespread presence of small, irregular white spots.	Not evident
Underdrawing	Black carbon-based grains in sample n.5	Not detectable	Visible in correspondence to the previously engraved haloes of the figures.	Limited punched dots and a complete underdrawing made with black ink and brush are visible.
Engravings	Hardly detectable with this technique; not present in the samples analyzed	Evident as a footprint in the Ca-, Pb-, and Fe-distribution maps (more intense signal in the Pb-distribution map; less intense signal in the Ca- and Fe-distribution map).	Visible in correspondence to the figures' haloes made with a drawing compass.	Not evident

3.4.3 The paint layers: materials and techniques

The cross-reference of data collected from UV-Vis-NIR imaging techniques, MA-XRF, HMI, FORS, FTIR, UV-Vis OM, and SEM/EDS allowed for shedding light on the material and artistic technique of the painting. Especially, MA-XRF provided the first crucial information, namely the distribution of the chemical elements over the surface of the work. For the maps of the most significant chemical elements, see Figure 3.14. As discussed in 3.4.2, calcium, lead, and iron signals are omnipresent and distributed fairly uniformly, suggesting they come (also) from the preparatory layers. Other chemical elements – such as copper, manganese, and cobalt – are localized on delimited areas of paint, suggesting their signals come more likely from the pigments of the paint layers. All other observations followed from these chemical maps: when the MA-XRF analysis was not clarifying, such as for the green curtain and blue mantle resulting both as copper-based pigments, the information from HMI and the spectral reflectance factor collected via FORS led to an improved pigment characterization. Finally, the analysis of the cross sections added further decisive data.

This process allowed for detecting the possible use of lead white, gold, azurite, smalt, lapis lazuli, copper-based green, copper resinate, cinnabar, red and yellow ochres, lead-tin yellow, and madder lake, all pigments compatible with 16th-century manufacturing. Proceeding by color fields, the results of the investigations on flesh tones, the red of the Virgin's robe, the blue of her mantle, and the green of the curtain are reported below.

3.4.3.1 The Virgin's red robe

Focusing on the Virgin's red robe (Figure 3.18a), the MA-XRF maps indicated the presence of a lead-based pigment, likely lead white, with few amounts of iron (Figure 3.18b). However, we noticed that the iron signal, attributable to ochres and majorly distributed in the shadows, was too low to be responsible for that intense red. At the same time, the intense pink UV fluorescence after removing the outermost layers of varnish (Figure 3.18c) suggested the presence of some organic or lake pigments [26-29]. The following data were analyzed and discussed hereafter to deepen the study of this red color.

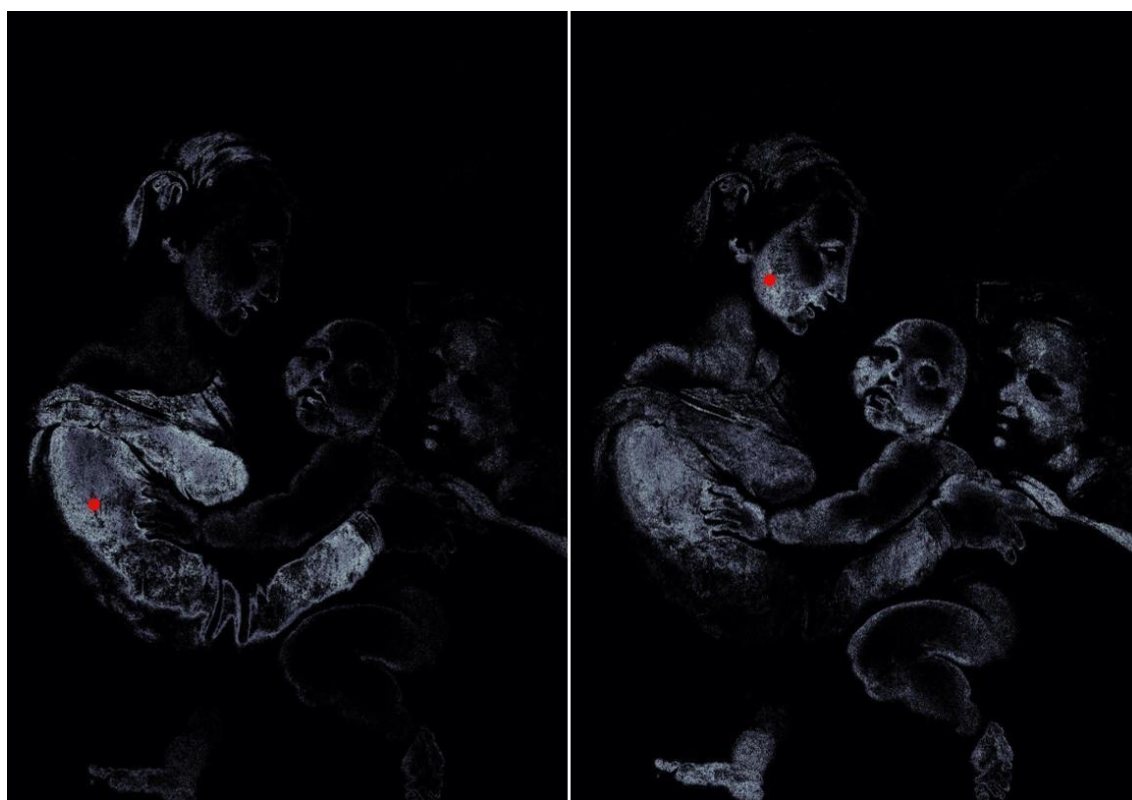


Figure 3.18 Detail of the Virgin's red robe: *a*) diffuse visible light photograph; *b*) MA-XRF distribution map of iron; *c*) UVF image after removing the outermost layers of varnish.

The chromatic similarity map obtained through HMI processing software showed the distribution of the red colour in the robe (Figure 3.19a: the white pixels have high similarity among them, and the black pixels have no similarity); as for the material composition, no match compatible with the MA-XRF results was found in the database supplied by Profilocolore.

The FORS analysis was decisive in our research, as it detected the spectral behavior typical of a red lake, a key finding that contributes significantly to our understanding of the robe's color. In fact, in the spectrum, the two absorption peaks within the ranges 505-515 nm and 540-550 nm (Figure 3.20) could suggest the use of a madder lake [30-32].

Thanks to the intense pink UV fluorescence, the red lake is also visible in the multi-layered sample from the red robe (Figure 3.21a). Cross-referencing also the data from the UV-Vis OM and SEM/EDS analysis, we deduced that the artist rendered the color with a first layer of red lake, lead white, and minor amounts of red ochre, and outer glazing of red lake alone (layers 3 and 4, respectively). In addition, we detected in the sample the presence of a yellow imprimatura made of lead white and yellow ochres with rare grains of red ochres (layer 2). Interestingly, several black grains at the bottom of this layer probably come from the underdrawing and show EDS signals of phosphorus and calcium, suggesting the use of a bone's black. The outermost layers of the sample, mainly organic, will be discussed later.



a

b

Figure 3.19 Result of the HMI chromatic similarity tool applied to the painting in the points highlighted by the red dots: *a*) red robe of the Virgin; *b*) dark flesh tone of the Virgin's face.

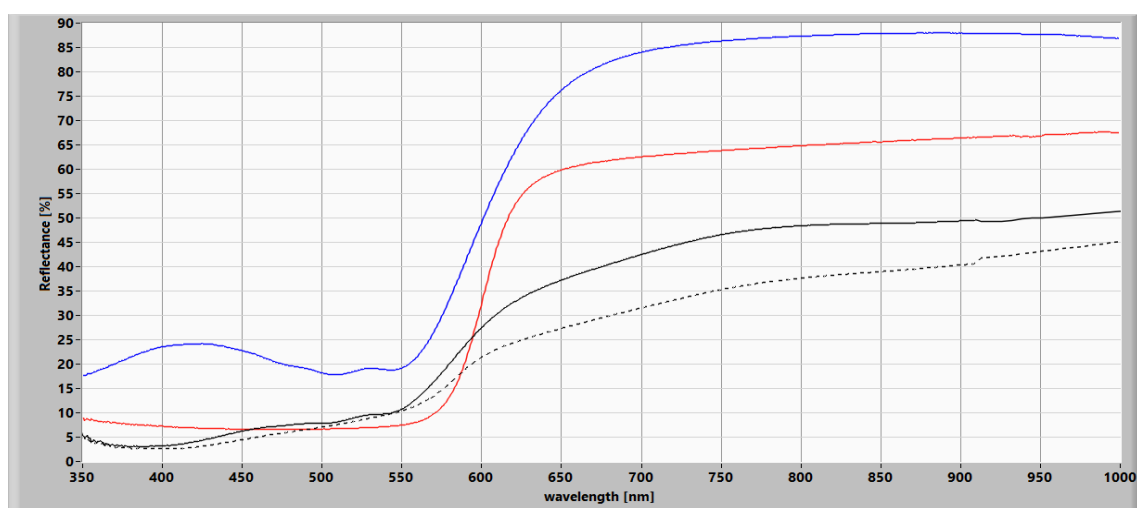
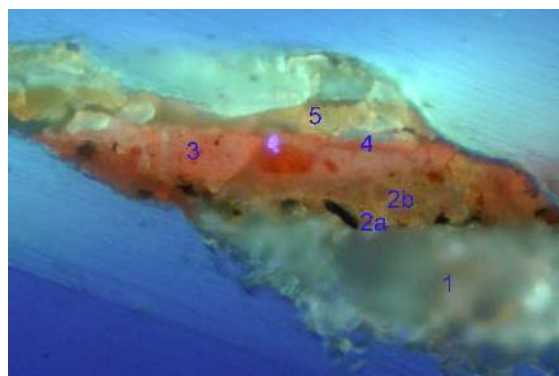


Figure 3.20 FORS spectra of the Virgin's red robe (black curve) and her neck (dashed curve) compared to the database reference spectra of madder lake (blue curve) and cinnabar (red curve): spots of analysis, reported in white in Figure 3.4, are respectively n° 8 and 16.



a

b

Figure 3.21 Microscopic images of a cross section from the Virgin's red robe: *a*) Vis OM; *b*) UV OM. The images show the red color obtained by the artist with a first layer of red lake, lead white, and minor amounts of red ochre, and an outer glazing of red lake alone (layers 3 and 4, respectively). Underneath, a yellow imprimatura made of lead white and yellow ochres with rare grains of red ochres (layer 2) is laid over the gypsum-based ground (layer 1).

3.4.3.2 *The flesh tones*

As far as flesh tones are concerned, the data collected and reported hereafter were interpreted as follows.

MA-XRF maps indicated the presence of lead white as the majority element, with mercury and minor amounts of iron (see Figure 3.14).

Thanks to the HMI chromatic similarity tool, we could differentiate the darker zone of the Virgin's face from the lighter cheek and forehead (see Figure 3.19b).

The FORS spectrum collected on a darker zone of the flesh tone was interpreted as compatible with the co-presence of cinnabar and red lake (see Figure 3.20, dashed curve and references).

Subsequently, the comprehensive study under Vis-UV OM and the SEM/EDS analyses of a multi-layered sample from the Virgin's neck allowed for deepening the chemical composition of the flesh tone (Figure 3.22).

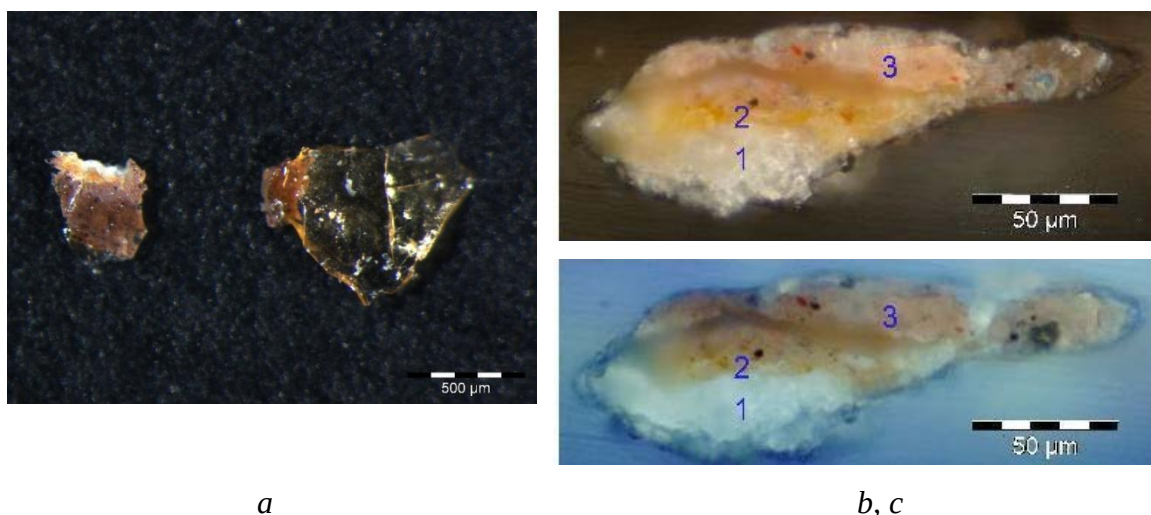


Figure 3.22 Microscopic images of the multi-layer sample from the Virgin's neck: *a*) the image shows that the sample broke into two fragments during the sample preparation; the left one was embedded for the cross section; *b*) Vis OM and *c*) UV OM images of the cross section show the flesh hue obtained by the artist by mixing lead white with minor amounts of ocher and cinnabar and rare grains of red lake (layer 3); the sample also shows the presence of the yellow imprimatura (layer 2) above the gypsum-based ground (layer 1).

Overall, no overpainting was observed on the flesh tones.

3.4.3.3 *The Virgin's blue mantle*

As a first clue, the MA-XRF maps on the Virgin's blue mantle indicated the presence of copper as a significant chemical element, suggesting the presence of azurite, with only some tiny cobalt-based details (see Figure 3.14).

At the same time, the IRFC image showed that most brushstrokes over the blue surface have a red false color, which cannot be associated with azurite: this pigment turning red in IRFC seems to alternate with, and more often cover, a further underlying paint layer that has instead a dark false color attributable to azurite (Figure 3.23a).

The application of HMI allowed for mapping the two primary pigments – azurite and a possible lapis lazuli – whose distribution appears almost complementary (Figure 3.23b,c). However, it could not distinguish the smalt-based detail indicated by MA-XRF.

On the brushstrokes of the blue mantle characterized by a false red color, FORS analyses made it possible to detect typical spectral features of lapis lazuli, supporting the hypothesis of a pigment of natural origin. In Figure 3.24, the reference spectra of lapis lazuli and artificial ultramarine are reported for comparison with the spectrum of the blue mantle. As reported in the literature [33], the absorption band at 600 nm occurs almost unchanged in both the natural and synthetic versions of ultramarine blue. However, it is flatter in the synthetic version, i.e., its reflectance starts rising at longer wavelengths. Moreover, the reflectance in the natural version shows an inflection point in the 700–720 nm range, as in the spectrum of the mantle, which accounts for a reddish note that significantly contributes to the global color, while in the synthetic version, the reflectance rises at longer wavelengths depending on the inflection point located in the 725–740 nm range [33].

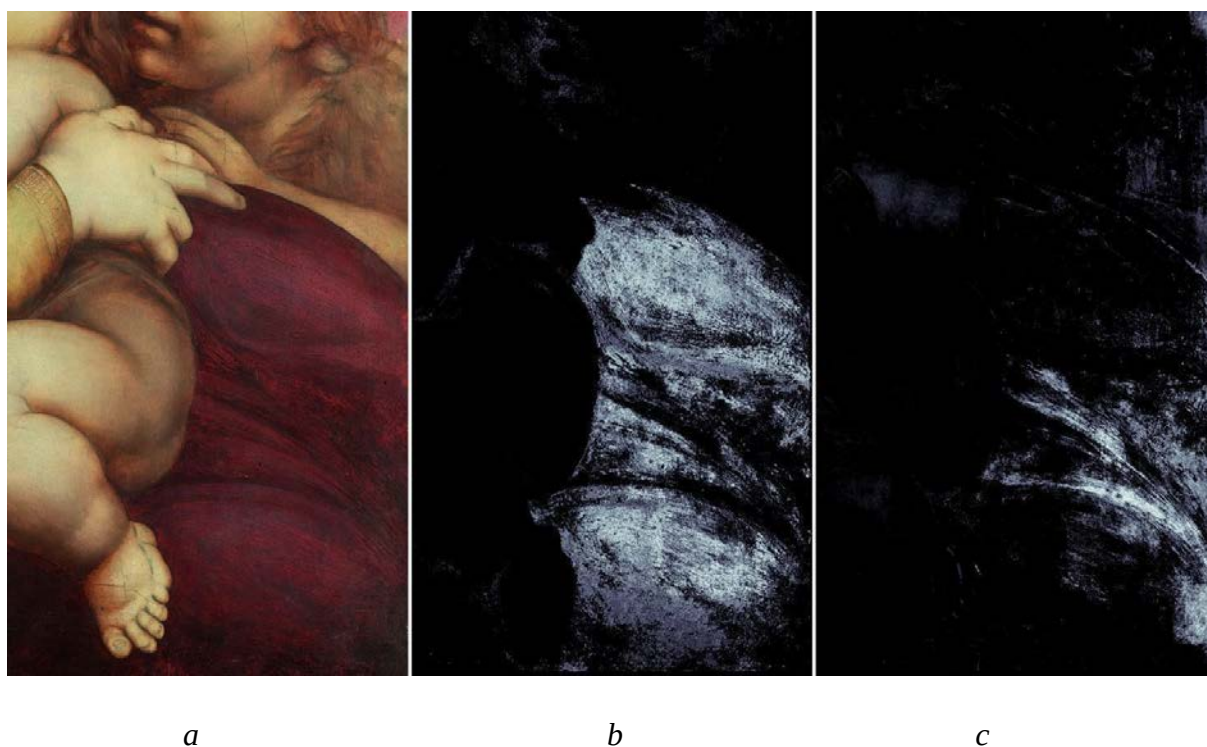


Figure 3.23 Detail of the Virgin's blue mantle: *a*) IRFC; *b*) and *c*) are HMI spectral similarity maps indicating respectively the localization of lapis lazuli and azurite.

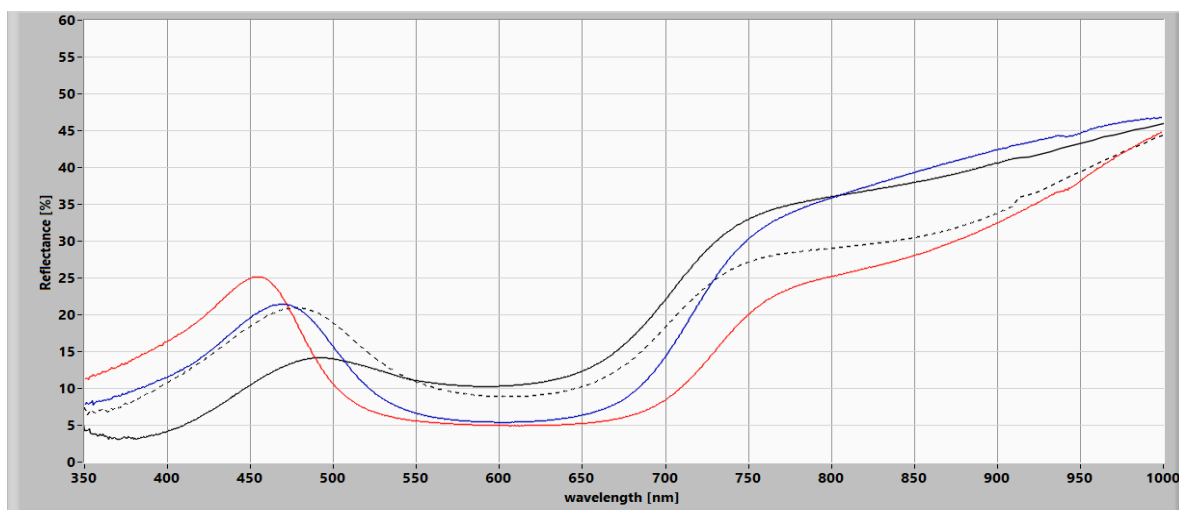


Figure 3.24 FORS spectra of the Virgin's blue mantle (dashed curve) and the blue sky (black curve) compared to reference spectra of lapis lazuli (blue curve) and artificial ultramarine (red curve). Spots of analysis, reported in Figure 3.4, are respectively n° 22 and 21.

The Vis-UV OM and SEM/EDS analysis of a multi-layered sample from the blue mantle (see Figure 3.11) provided a thorough insight, especially on the outer blue hypothesized as lapis lazuli: its presence above another blue layer and the coarseness of the brushstrokes (see Figure 3.3b) could suggest a restoration overpainting. In the cross section, the outer blue presents a coarse and irregular grain, a characteristic more compatible with an original natural lapis lazuli than a modern synthetic blue [33, 34]. It is laid over the azurite layer, but the two layers are very consistent with each other. The absence of any dividing lines is another fact that allowed the team to exclude the presence of overpainting and consider both layers as original. Moreover, FTIR analysis of a scraping from the superficial blue paint on a single blue grain confirmed the presence of silicates – referring to lapis lazuli – and detected the use of a drying oil as a binder (Figure 3.25).

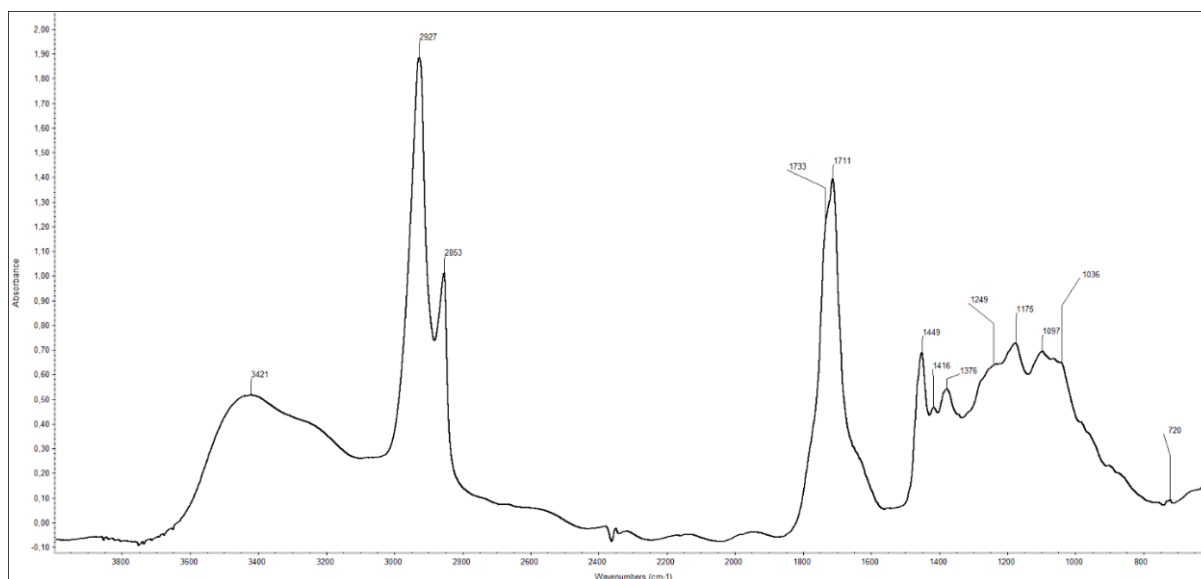


Figure 3.25 FTIR spectrum of a scraping selectively removed from the original blue layer (sampling site n° 2s in Figure 3.4), analysis of a blue grain: spectral features of an aged drying oil were detected at 2927, 2853, 1733, 1711, 1449, 1376, 1249, 1175 cm^{-1} ; signals at 1097 and 1036 cm^{-1} were assigned to silicates, attributable to lapis lazuli.

Based on these results, all the pigments detected in the Virgin's blue mantle were considered original since they are compatible with the painting's time.

3.4.3.4 The blue sky and the clouds

In the area of the sky, FORS analyses also allowed for the detection of the typical spectral features of lapis lazuli (see Figure 3.24). This detection was based on the comparison of the sky's spectral features with the reference spectra of lapis lazuli in the database, supporting the hypothesis of a pigment of natural origin.

In correspondence with the small cobalt-based details localized via MA-XRF on the clouds (see Figure 3.14), FORS spectra allowed for recording the typical absorption with three sub-bands in the 550-650 nm range due to the Co(II) ion [35], also suggesting the presence of a natural smalt, rather than a synthetic cobalt blue, for comparison with reference spectra of the database (Figure 3.26).

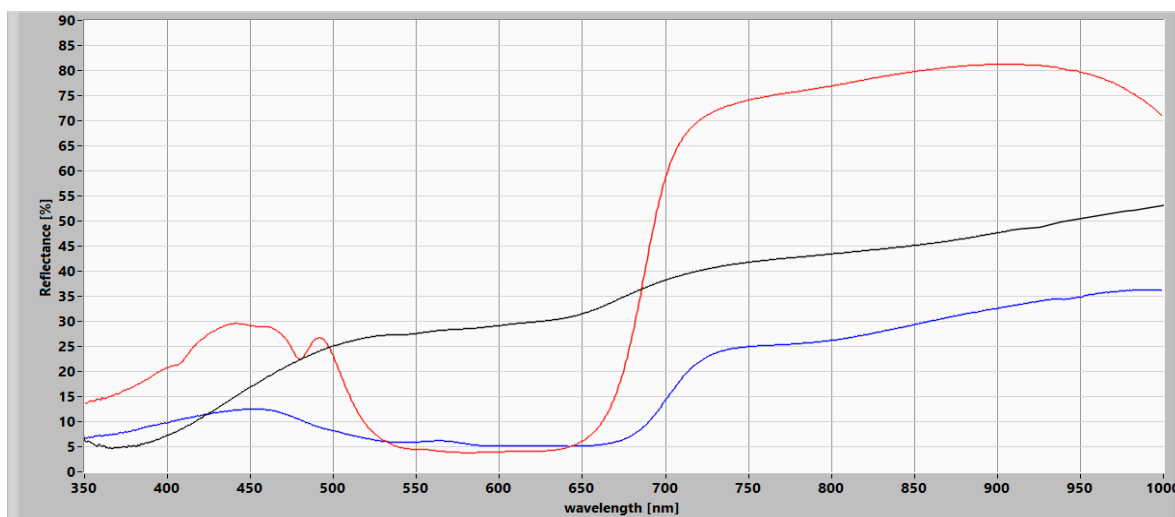


Figure 3.26 FORS spectra of the light blue cloud (black curve, spot of analysis n° 5 in Figure 3.4) compared to reference spectra of smalt (blue curve) and synthetic cobalt blue (red curve).

Based on this interpretation, all the pigments detected in the sky and clouds were considered original since they are compatible with the painting's time.

3.4.3.5 *The green curtain*

Before the conservation treatment, the color of the curtain against the background of the painting appeared dark brown, although iconography would have suggested it to be green. Based on the outcomes of the non-invasive analyses, we first hypothesized the presence of copper resinate, whose sensitivity to oxidation and degradation phenomena is well-attested [36, 37].

From an analytical point of view, the MA-XRF maps indicated copper as the leading chemical element, and FORS data were compatible with a generic copper-based green pigment [38].

A multi-layered sample from the green curtain provided the most exciting data for trying to understand both the chemical composition of the green and the history of previous conservation treatments that the work underwent over the years. Vis-UV OM and SEM/BSE images of the cross section (Figures 3.27 and 3.28) showed that the green copper-based layer, identified by EDS, has a glassy aspect with rare discrete green particles. This layer (layer 4) lies on a background of carbon black containing small amounts of ochres and lead white (layer 3).

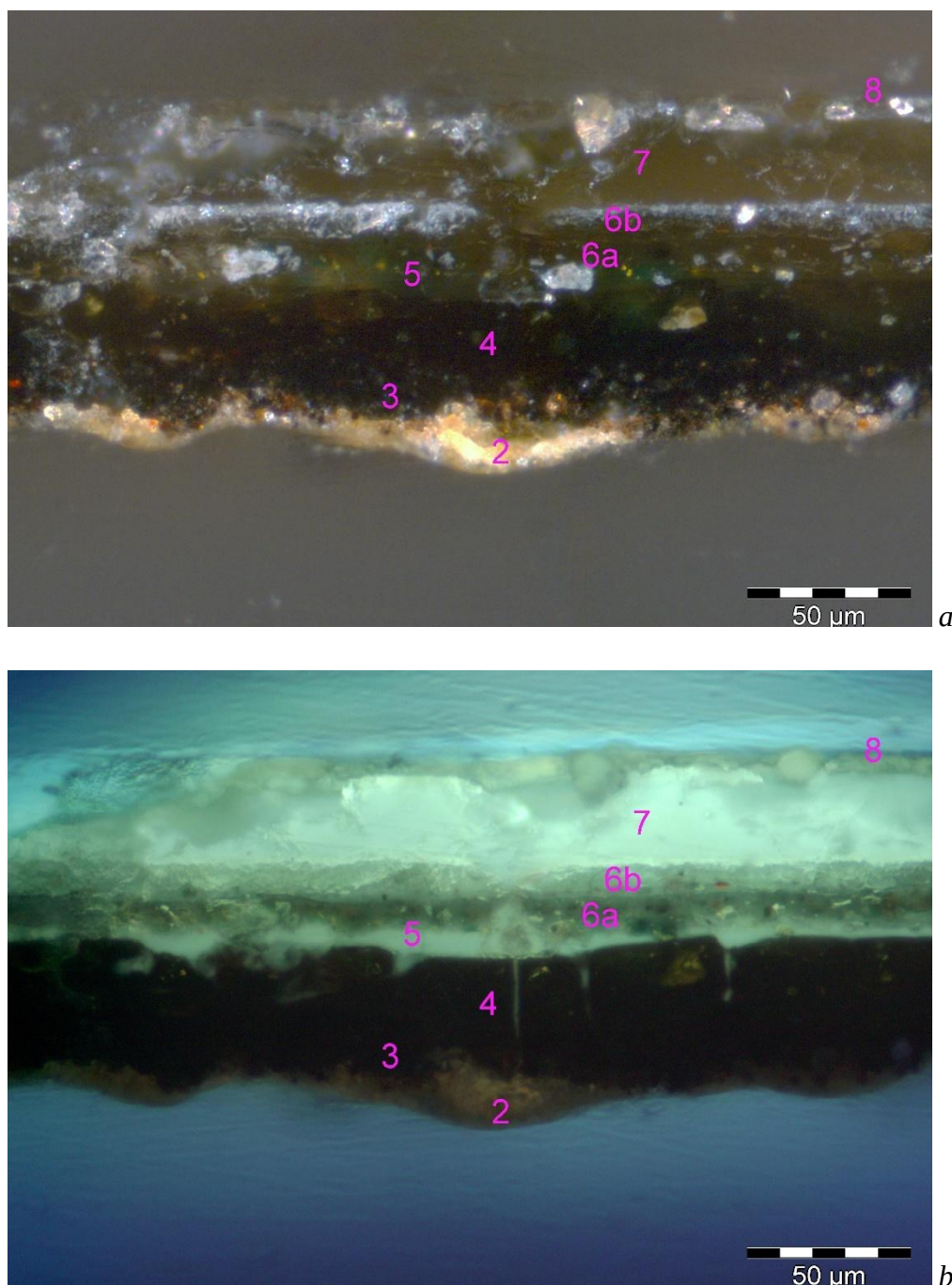


Figure 3.27 Microscopic images of the cross section from the green curtain: *a*) Vis OM; *b*) UV OM. The numbering indicates the identified layers: 2) the yellow imprimatura made of lead white and yellow ochres with rare grains of red ochres; 3) a carbon black-based background layer containing small amounts of ochres and lead white; 4) the green copper-based layer with the glassy aspect; 5) organic layer; 6a and 6b) organic layers with rare grains containing chrome, lead, iron, and copper; 7) and 8) organic layers. In this sample, the gypsum-based ground (identified in all other samples as layer 1) is missing; this can happen when sampling is not deep enough to include all layers.

Although not sufficient for a complete identification [36], the glassy aspect of the green (Figure 3.28) led us to speculate that it could be copper resinate, which would explain the bad preservation of the curtain. Therefore, both layers 3 and 4 were interpreted as original.

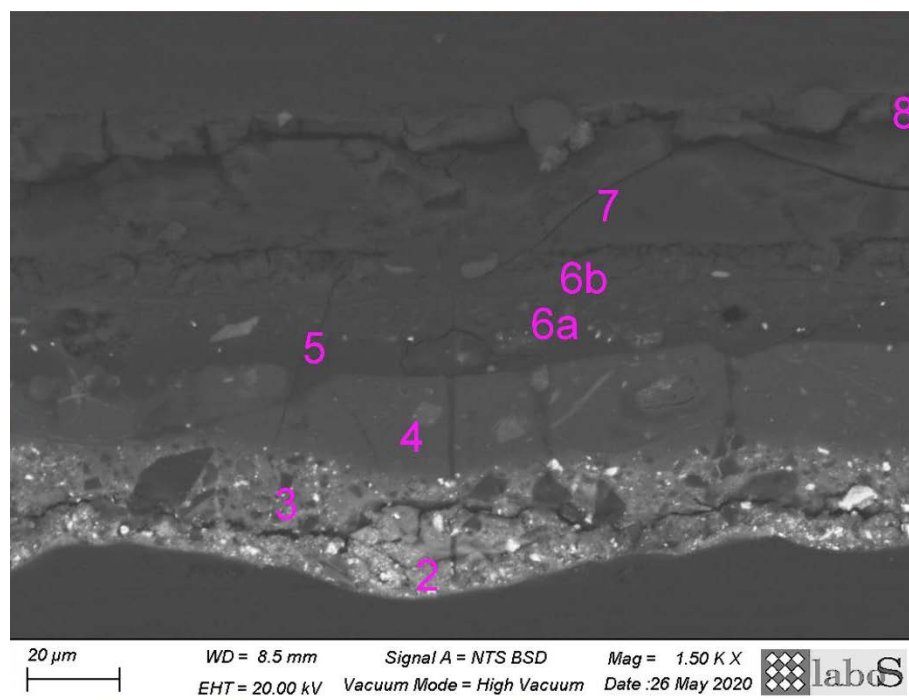


Figure 3.28 SEM/BSE image of the cross section from the green curtain (same of Figure 3.27), showing the glassy aspect of the copper green-based paint layer (layer 4).

The chemical composition of the organic layers on the top of the sample will be discussed later. However, it is worth noting that the green curtain is the only area where the cross section undoubtedly indicates the presence of overpainting. In fact, EDS analysis of layers 6a and 6b – so named because they look like two coats of the same paint – allowed for detecting the presence of rare grains containing chrome, lead, iron, and copper diffused within a mostly organic matrix (Figure 3.29).

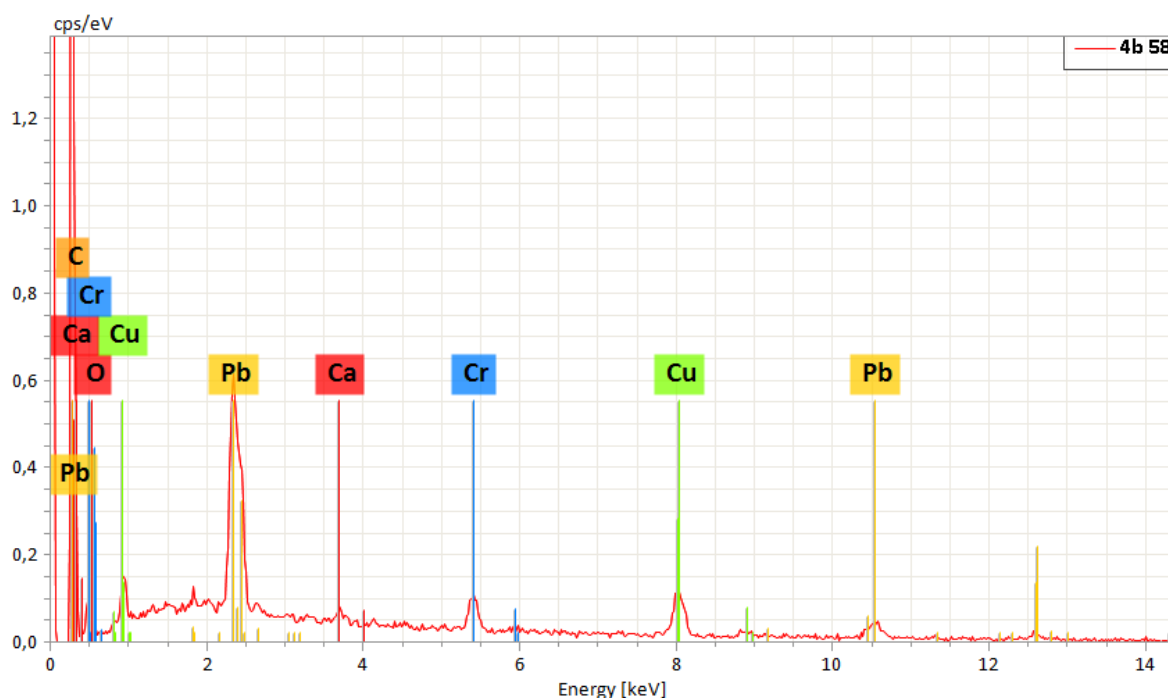


Figure 3.29 EDS spectrum of a yellow grain containing lead and chrome (interpreted as chrome yellow) in layer 6a of the cross section from the green curtain. The signal of copper was attributed to a small amount of copper resinate in the organic matrix of the layer.

As shown in Figure 3.30, FTIR analysis of a scraping selectively removed from layers 6a and 6b indicated the presence of Prussian blue and natural resin. That led to interpreting those layers as resin-based, slightly colored overpainting containing Prussian blue and chrome yellow, therefore a green translucent, undoubtedly modern overpainting. Lead chromate, or chrome yellow, was introduced as a pigment between 1804 and 1809 [34]. In the following decades of the nineteenth century, the terms “chromium green” and “cinnabar green” indicate a composite pigment where Prussian blue is precipitated on chromium yellow. This mixture makes a beautiful green but degrades over time, making it unsuitable for the fine arts [34].

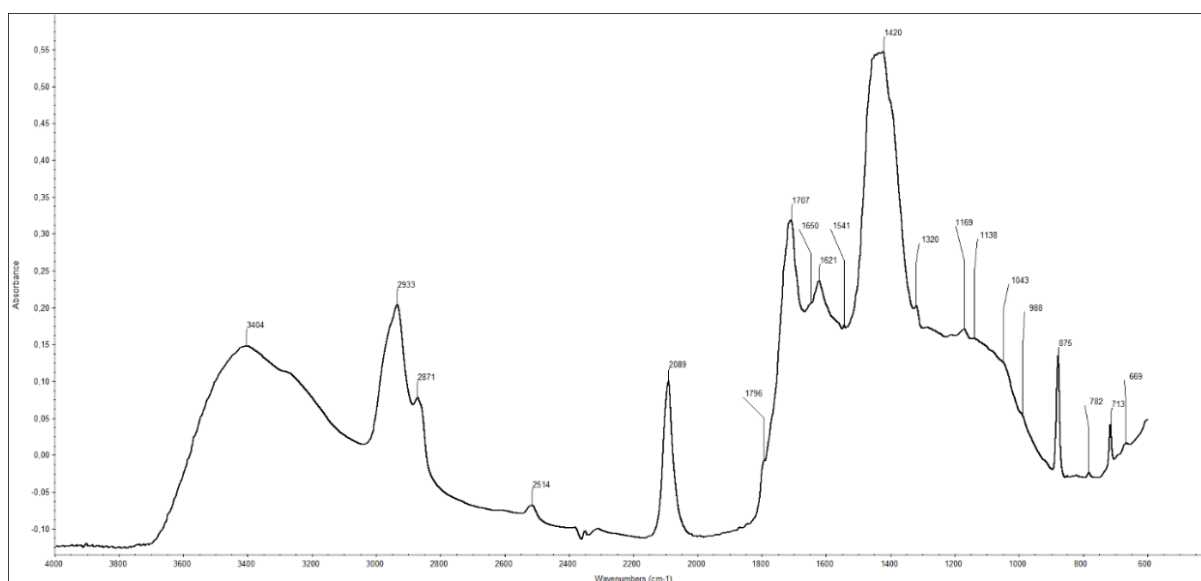


Figure 3.30 FTIR spectrum of the bluish grains selectively removed from the overpainting on the green curtain (layers 6a and 6b in Figure 3.28): diagnostic band for Prussian Blue at 2089 cm^{-1} was detected, 2514, 1791, 1420, 875, 713 cm^{-1} were assigned to calcite, 1171, 1043 cm^{-1} to silicates, 2933, 2871, 1707 cm^{-1} to natural resin, and 1621, 1319 cm^{-1} to oxalates; 1541 cm^{-1} signal was attributed to zinc stearate.

3.4.4 Original finishing and previous intervention varnishes

As shown in Figure 3.31, the artwork before the conservation treatment conducted at CCR presented a glossy appearance and an intense UV fluorescence, indicating the presence of thick layers of varnish over the surface.

By studying the cross sections, it was possible to reconstruct the layering and number of the original and overlaying varnishes. In particular, the cross section from the Virgin's red robe under UV light (see Figure 3.21) allowed for counting three layers of varnish (numbered as layers 5, 7, and 8) over the painting layer, revealing a stratigraphy similar to the one of the green curtain (see Figure 3.27) except for layer 6. The latter, present only in the green curtain, is laid between the innermost layer of varnish (layer 5) and the following one (layer 7) and, as described above, was identified as overpainting.

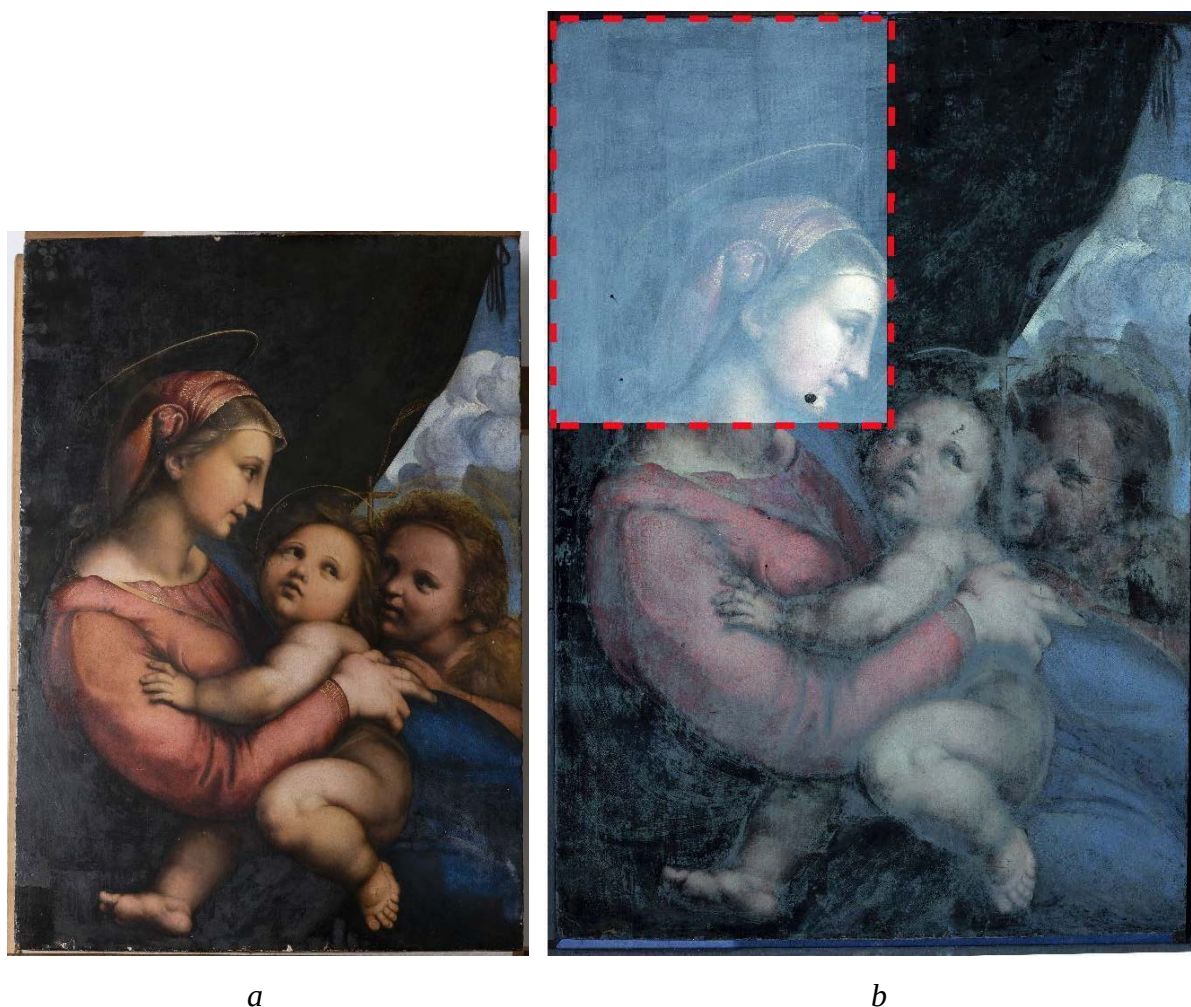


Figure 3.31 The painting during the progressive removal of non-original varnishes: *a*) Vis light photograph; *b*) UVF, with a red box in the upper left documenting the initial state.

As described in 3.3.9, the varnish scrapings for FTIR and Py-GC/MS were selectively sampled with a scalpel in correspondence with the green curtain and with the aid of the UV light.

These FTIR analyses provided preliminary data about the chemical composition of the varnish layers, and Py/GC-MS was decisive for the identification.

Although the thickness of the layers did not allow a truly selective sampling of the varnishes, Py-GC/MS analyses pointed out differences in the composition of the various layers, thus allowing the stratigraphy to be linked to the painting's conservation history. Table 3.4 provides a comprehensive list of all compounds identified in the Py-GC/MS curves shown in Figure 3.32.

Table 3.4 Lists of compounds identified by Py-GC/MS. Due to methylation with TMAH acidic groups are detected as methyl esters and hydroxy groups as alkoxy derivatives.

Peak No.	Retention time (min)	Assignment	Layers of varnishes and overpainting numbered as in Figure 3.27			
			n. 5	n. 6	n. 7	n. 8
1	5.2	1,2,3-trimethoxypropane	X	X	X	X
2	13.4	Octanedioic acid dimethyl ester	X	X	X	
3	14.7	Nonanedioic acid dimethyl ester	X	X	X	X
4	16.7	Tetradecanoic acid methyl ester	X	X	X	X
5	17.7	Pentadecanoic acid methyl ester	X	X		X
6	18.8	Hexadecanoic acid methyl ester	X	X	X	X
7	20.4	9-octadecenoic acid methyl ester	X			X
8	20.7	Octadecanoic acid methyl ester	X	X	X	X
9	22.7	Unidentified compound (m/z 69, 137, 157, 169, 185, 201, 327)	X			
10	22.7	Dehydroabietic acid methyl ester	X	X		
11	23.5	7-methoxydehydroabietic acid methyl ester	X	X		
12	23.6	7-methoxytetrahydroabietic acid methyl ester	X	X	X	
13	24.1	15-hydroxy-7-oxodehydroabietic acid methyl ester	X	X		
14	24.8	7,15-dimethoxytetrahydroabietic acid methyl ester	X	X	X	
15	31.1	3,4-seco-olean-18-en-3,28-dioic acid methyl ester	X	X		
16	32.1	Moronic acid methyl ester (3-oxo-olean-18-en-28-oic acid methyl ester)	X	X		
17	32.1	Unidentified compound (m/z 151, 189, 219, 482)	X	X		
18	32.4	3-oxo-18 α H-olean-12-en-28-oic acid methyl ester	X	X		
19	32.7	Unidentified compound (m/z 145, 271, 435, 451, 510)	X	X		
20	33.0	Unidentified compound (m/z 145, 165, 189, 233, 453, 496)	X	X		
21	33.3	Unidentified compound (m/z 112, 189, 428, 465, 524)	X	X		

22	33.6	Unidentified compound (m/z 189, 421, 496)	X	X		
23	33.6	Unidentified compound (m/z 465, 511, 524)	X	X		
24	14.3	Dodecanoic acid methyl ester		X	X	X
25	15.0	Longicamphenylone		X	X	
26	15.2	3,4-dimethoxybenzoic acid methyl ester		X		
27	19.7	Unidentified compound (m/z 102, 146, 256)		X	X	X
28	20.2	Unidentified compound (m/z 88, 102, 132, 242, 256)		X	X	X
29	20.5	Unidentified compound (m/z 102, 116, 169, 256, 270)		X		
30	21.5	Unidentified compound (m/z 102, 146, 284)		X	X	X
31	23.2	Unidentified compound (m/z 102, 146, 312)		X	X	X
32	3.3	3-methylenecyclohexene			X	
33	5.1	Cyclohexanone			X	
34	5.6	Hexanoic acid methyl ester			X	
35	6.0	2-methylcyclohexanone			X	
36	6.5	2-methylenecyclohexanone			X	
37	8.9	Octanoic acid methyl ester			X	
38	9.1	Pentanedioic acid dimethyl ester			X	
39	9.7	2,3-dimethoxytoluene			X	
40	10.3	Nonanoic acid methyl ester			X	
41	10.7	Hexanedioic acid dimethyl ester			X	
42	11.5	Unidentified compound (m/z 55, 57, 69, 71, 83, 85, 97, 111, 125, 153, 154)			X	X
43	11.8	Unidentified compound (m/z 55, 57, 69, 71, 83, 85, 97, 111, 125, 154)			X	X
44	12.1	Heptanedioic acid dimethyl ester			X	
45	12.9	Unidentified compound (m/z 55, 69, 83, 96, 114, 128, 139, 141, 151, 154 182, 183)			X	
46	13.0	Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene (longifolene)			X	
47	16.8	Methylcyclohexanone dimer			X	
48	16.9	Methylcyclohexanone dimer			X	

49	17.0	Methylcyclohexanone dimer			X	X
50	17.1	Methylcyclohexanone dimer			X	X
51	21.0	Methylcyclohexanone trimer			X	
52	21.3	Methylcyclohexanone trimer			X	
53	21.7	Methylcyclohexanone trimer			X	
54	22.2	Methylcyclohexanone trimer			X	
55	27.0	Methylcyclohexanone tetramer			X	
56	27.6	Methylcyclohexanone tetramer			X	
57	18.5	9-hexadecenoic acid methyl ester				X
58	20.4	14-methoxyhexadecanoic acid methyl ester				X
59	20.5	15-methoxyhexadecanoic acid methyl ester				X
60	22.4	Eicosanoic acid methyl ester				X
61	24.1	Docosanoic acid methyl ester				X
62	25.6	Tetracosanoic acid methyl ester				X
63	26.3	Squalene				X
64	27.0	Hexacosanoic acid methyl ester				X
65	27.7	1-methoxyoctacosane				X
66	28.5	Octacosanoic acid methyl ester				X
67	29.4	1-methoxytriacontane				X
68	31.7	1-methoxydotriacontane				X

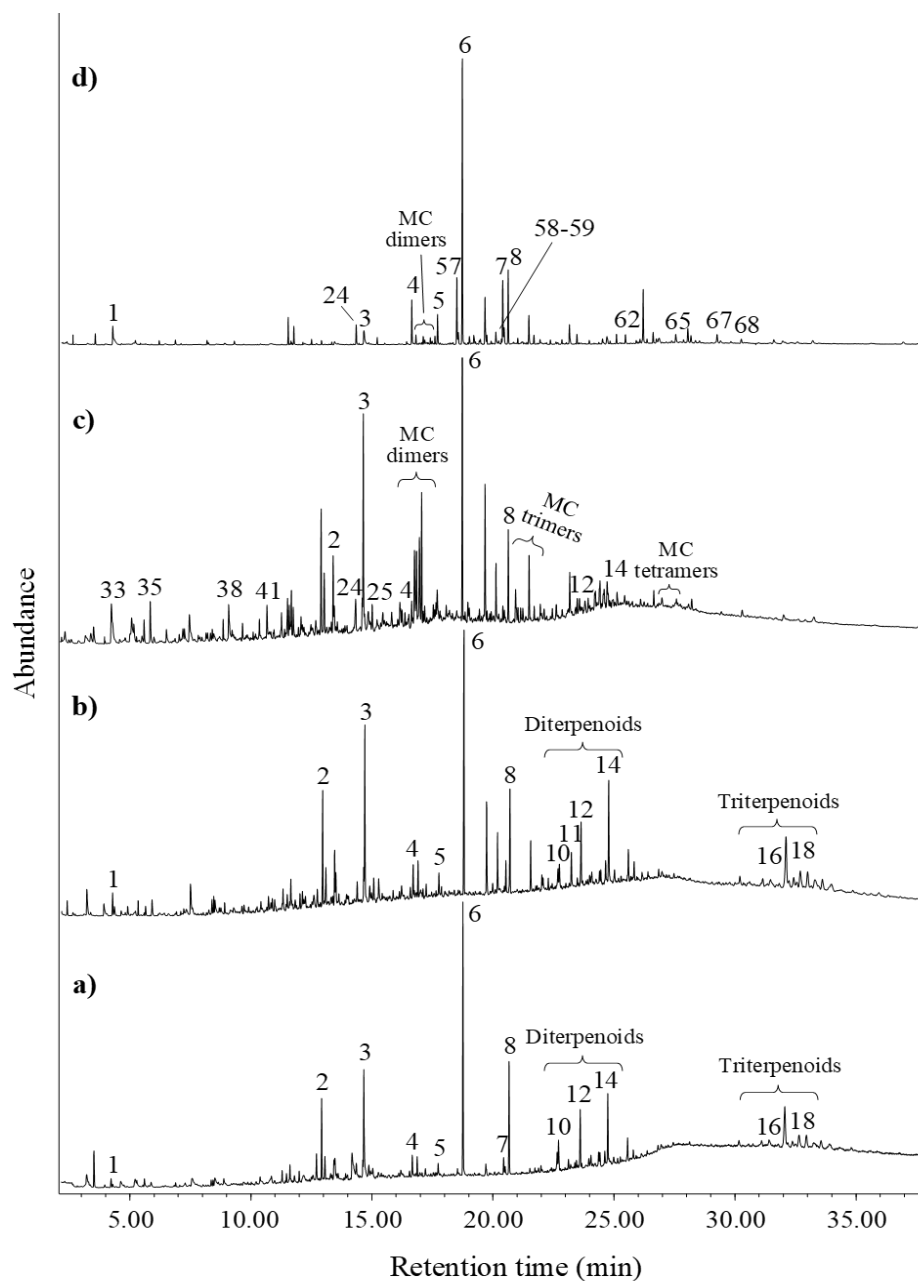


Figure 3.32 Py-GC/MS analysis of the three layers of varnish and the layer of overpainting: *a)* the oldest varnish; *b)* the overpainting; *c)* the intermediate varnish; *d)* the most recent varnish. Peak numbers refer to Table 3.4. MC: methylcyclohexanone.

Starting from the inside and moving toward the surface, the oldest varnish (layer 5 in Figure 3.27) is oleoresinous, based on siccative oil, combined with di- and triterpenic resins (Figure 3.32a). The main marker compounds of siccative oils are the methyl esters of suberic acid (octanedioic acid, peak n. 2 of Table 3.4), azelaic acid (nonanedioic acid, peak n. 3), myristic acid (tetradecanoic acid, peak n. 4), palmitic acid (P, peak n. 6), stearic acid (S, peak n. 8) and traces of oleic acid (peak n.7). The A/P ratio, based on peak areas, is 0.7 and confirms the siccative nature of the oil. The P/S ratio is 2.3, a value close to the upper limit found in the literature for linseed oil (i.e. 2.5 [39, 40]) and in a range more characteristic of walnut oil [39, 41, 42]. The diterpenoids identified in the pyrograms are markers of Pinaceae resins like colophony. More specifically the three relatively intense peaks eluted between 22 and 25 minutes of retention time are assigned to dehydroabietic acid (peak n. 10), 7-hydroxydehydroabietic acid (peak n. 11), 7-hydroxytetrahydroabietic acid (peak n. 12), 15-hydroxy-7-oxodehydroabietic acid (peak n. 13) and 7,15-dihydroxytetrahydroabietic acid (peak n. 14) [43]. At higher retention time, between 30 and 35 minutes, several peaks point to the presence of less volatile components. Although the interpretation of mass spectra corresponding to these peaks was not easy due to coelution of more species, the methyl esters of moronic acid (3-oxo-olean-18-en-28-oic acid, peak n. 16) and 3-oxo-18 α H-olean-12-en-28-oic acid (peak n. 18), that are characteristic triterpenoids contained in mastic were identified [44]. Another chromatographic peak was tentatively assigned to 3,4-seco-olean-18-en-3,28-dioic acid, which may result from the oxidation of moronic acid by breaking the bond near the carbonyl in position 3 according to the Norrish reaction.

Other peaks remain unknown. Although they can all be referred to compounds with a molecular weight similar to that of oxidized triterpenes, it has not been possible to identify them with certainty, nor to establish whether they are products of oxidation of the mastic resin or additional components of the varnish.

The oldest varnish is followed by a layer of similar composition but it is clearly recognizable in the stratigraphy (layer 6) because of the presence of some compounds that lower the UV fluorescence. As already described in section 3.4.3.5, this layer was detected only on the area of the green curtain and is actually a modern overpainting containing rare particles of Prussian blue and chrome yellow dispersed in an oleoresinous matrix. The relative intensities of the fatty acids, diterpenic markers, and triterpenic markers (Figure 3.32b) of the overpainting are the same as the varnish below (Figure 3.32a) and also the A/P and P/S values, which are 0.8 and

2.3 respectively, demonstrate that the oleoresinous components of these first two layers have a similar composition.

The varnish above (layer 7) is still oil-based but with low colophony content and no mastic. Compounds assigned to monomers, dimers, trimers, and tetramers of a ketone resin have been identified (Figure 3.32c) [45, 46].

Even if the peaks of ketone resin markers have relatively high intensity, these are probably contaminations of the most superficial varnish layer (layer 8), in which, in addition to traces of ketone resin and oil, beeswax was detected as the main component (Figure 3.32d). In fact, long-chain fatty acids (peaks n. 60, 61, 62, 64, 66) and long-chain alcohols (peaks n. 65, 67, 68) have been identified, as well as characteristic hydroxyacids of beeswax such as 14-hydroxyhexadecanoic acid and 15-hydroxyhexadecanoic acid (peak n. 59) [47, 48]. The P/S and A/P ratios calculated for this layer of the painting, equal to 3.4 and 0.2 respectively, confirm that the fatty acid profile results from a combination of different components, with a predominance of beeswax and a lower presence of drying oil. Overall, the Py-GC/MS analyses indicate that the outermost layer of varnish is a waxy ketone varnish which itself exhibits a layering of its two components, with the ketone resin localized at the interface with the underlying varnish layer and the waxy component stratified on the surface exposed to the air.

3.5 Conclusions

The multi-analytical investigation described in this chapter was conducted on the *Madonna della Tenda* panel painting from Musei Reali Torino to scientifically support the conservation treatment performed on the artwork from 2018 to 2020 at CCR in Venaria Reale.

The main goals of the research were to analyze the preservation of the wooden support and paint layers, significantly compromised by a deep hollow engraved from the back of the panel, and to determine the exact number and composition of the overlaying varnishes, the original paints, and the overpainting, whose presence was suggested by the yellowed and uneven surface aspect.

This research demonstrated the high potential of combining non-invasive imaging techniques such as PuCT, XR, MA-XRF, and IR reflectography for investigating the wooden support and all the preparatory phases of the painting, from the ground to the underdrawing. This analysis step allowed for an in-depth study of certain aspects of the artistic technique and the artwork's

state of preservation, primarily referring to the nature of the hollow found on the panel back surface.

The combination of XR and PuCT was beneficial for studying the wood grain characteristics and detecting defects, such as tunnels, grouting, and cracks in the panel support. PuCT allowed for an inner view of the board's thickness, clearly observing the wood grain widening around the hollow, confirming that the hollow area could have been a wood knot. Moreover, XR and PuCT allowed for the detection of a tau-shaped element near the hollow: PuCT aided in its localization in the thickness of the board, confirming it as an element belonging to the wood panel. The tau's analytical answer in both techniques suggested that this part was a cracked and unfilled area, presumably generated due to the fragile nature of this part of the board. Again, concerning points of fragility and the state of the preservation of the panel, a clear view of the woodworm tunnels was possible using both techniques: in the high-resolution XR image, the tunnels visible as radio-transparent traces follow the pattern of the wood fibers in most of the cases. In parallel, other signatures – this time more radiopaque – are visible as vertical lines in XR; in PuCT these become whiter as time elapses, suggesting that they have been filled during restoration, and probably involve only the upper layers. In the area of the green curtain, painted mainly with copper green, they are also visible in the MA-XRF distribution map of copper due to the slightly lower signal of this element.

The combination of MA-XRF, XR, and HR-SWIR reflectography was particularly effective for studying the preparatory phases of the painting, such as the ground layer, engravings, imprimatura, and underdrawing. Particularly, MA-XRF helped to detect the presence of calcium-, lead- and iron-based pigments on the entire painting and to attribute them to the preparatory phases, although it did not allow for a stratigraphic distinction between the ground layers – made of plaster and animal glue – and the thin imprimatura layer made of lead white and ochres. Interestingly, XR aided in recognizing, in some areas of the painting, the radiopaque brushstrokes of the imprimatura and also, at high magnifications, the slight, more radiopaque imprimatura inclusions in the plaster layer due to the opening and filling of the bubbles during the smoothing of the plaster layer: the average size of the inclusions turned out to be compatible with the one detected and sampled in one of the cross sections. Moreover, the combination of the two X-ray techniques helped study the signs of the metal point engravings in correspondence to the figures' haloes made using a drawing compass, suggesting that they were probably drawn on the plaster layer.

The presence of limited punched dots and a complete underdrawing, made with black ink and a brush, was clearly defined and studied through HR-SWIR reflectography: the combination with XR helped in understanding the process of carrying out the preparatory phases of the painting.

This case study also demonstrated that combining UV-Vis-NIR imaging techniques, MA-XRF, HMI, and FORS is effective for studying the paint layers, preliminary to any detection of overpainting. MA-XRF analysis provided crucial information on the chemical composition and distribution of the pigments. The HMI processing software, through spectral or chromatic similarity maps, revealed the distribution of colors over the painting, enabling the formulation of hypotheses about the pigments. Vis-NIR FORS played a decisive role in characterizing many materials, such as lapis lazuli, azurite, smalt, and red lakes. The integration of these complementary techniques provided solid data about the artwork materials in a non-invasive way.

Furthermore, this study underscores the continued importance of removing and analyzing multi-layer samples. This process remains a necessary step to gather decisive data on pigments and varnish layering, particularly in the context of supporting conservation treatment.

In this work, observing and comparing the cross sections under UV light was fundamental to distinguish the different varnishes and overpainting applied over time on the surface of the painting. These layers showed slightly different UV fluorescence: this was crucial during the cleaning tests for removing the non-original varnishes and during the conservation treatment itself. Moreover, the comparison among the cross sections allowed for an immediate understanding of the presence of overpainting in the green curtain: while the other samples showed the same layering of varnishes, an extra layer – between the first and the second varnish – was detected in the green curtain.

The difference in UV fluorescence was likewise exploited to selectively remove the scrapings for the micro-invasive chemical analyses, setting up an ad hoc method of selective sampling.

The Py-GC/MS of the scrapings made it possible to reveal the chemical composition of the varnishes. The oldest varnish, for instance, is oleoresinous and consists of siccative oil, diterpene resin, and mastic. Above is another oleoresinous varnish based on siccative oil and diterpene resin but without mastic. Finally, the most recent varnish is based on ketone resin and beeswax. The variety of organic components in each varnish and the reduced thickness of the layers, from about 10 to 25 μm , proved the effectiveness of the applied methodology.

Regarding the overpainting in the green curtain, the combination of information from OM, SEM/EDS, Py-GC/MS, and FTIR led to the identification of a mixture of oleoresin and modern pigments, i.e., yellow chrome and Prussian blue. The applied methodology proved to be highly effective in studying this overpainting, providing valuable insights into the artist's intentions and the work's history.

Such information was crucial for shedding light on the work's history and past conservation treatments.

After the study and conservation activities at CCR, the work went on display at the temporary exhibition *Sulle tracce di Raffaello nelle collezioni Sabaude* in the Discovery Section of the Musei Reali Torino in Torino, from October 30th, 2020, to June 2nd, 2021. All the collected data, especially those on the underdrawing, constitute a significant opportunity and study tool for all the disciplines still involved in this research, as well as for a technical, stylistic comparison with the original Raphael masterpiece displayed at the Alte Pinakothek in Munich, Germany.

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Chapter 4

CONSIDERATIONS ON THE CASE STUDIES AND THE MULTI-ANALYTICAL APPROACH: THE NEED FOR OPEN-SOURCE SOFTWARE FOR DATA MANAGEMENT IN RESULT INTERPRETATION

Works of art, from a technical and material perspective, are complex objects that require, as we have seen, a multidisciplinary and multi-analytical approach for their understanding. This approach assumes peculiar features and value when scientific analysis is conducted during the conservation treatment of an artwork, as in the two case studies presented.

The conservation treatment phase is a crucial part of the art preservation process, representing a pivotal moment of knowledge. It is during these operations that numerous questions emerge, not only from the technical observation of the artifact but also from the interaction process with its materials, which is gradually defined. Therefore, each artwork necessitates tailored analytical plans and strategies, underscoring the value of scientific analysis in the conservation treatment of art.

The development of an analytical plan often represents a compromise between the ideal analytical plan, which may be entirely based on innovative and state-of-the-art methods, the techniques available to a laboratory, and those that can be made available through scientific collaborations or funded research programs. To give a concrete example, eight scientific collaborations were activated at the CCR to study the paintings presented in this dissertation, and sixteen different methods of analysis, including imaging and spot techniques, were adopted.

Unlike when a work of art is studied in a museum (without any treatment being envisaged), the analytical plan for a work of art destined for conservative treatment must inevitably address more issues, including the crucial role of scientific support in guiding the conservator's operations. During the conservative treatment, works of art undergo gradual changes in their appearance, underscoring the necessity of repeated analyses during the work. For example, it is often necessary to repeat some analyses before and after the removal of overpainting or to

take multiple shots of a painting in UV-induced visible fluorescence during the removal of the non-original varnishes to document the work's progress.

These and other aspects make the diagnostic campaign very demanding regarding the number of analyses and types of methods to apply – as well as the skills required to conduct the analyses and interpret results. Consequently, a large amount of data is acquired during a complete survey, requiring management capacity primarily at the IT level.

From this point of view, the CCR has adopted a very efficient system to manage and archive data, assigning a code number to each artwork. Regarding the photographic documentation and scientific data, to each artwork is dedicated:

- 1) one folder containing all data from the spot analyses and the most significant images from the photographic and multiband imaging campaign, both conducted at CCR, and all the 'extra' data, that is, coming from scientific collaborations;
- 2) one folder with all the high-resolution images from the photographic and multiband imaging campaign, conducted at CCR, including the shots taken during the conservation treatment – asked by the conservators – that document the work's progress;
- 1) one folder copy of the latter with the same images but in low resolution, strategically designed to facilitate viewing from various PCs, ensuring accessibility and ease of use for all users.

To give an idea of the data volumes, the scientific folder for the *Madonna della Tenda* painting presented in this dissertation contains 24 GB of data, and the high-resolution photos folder is 15 GB.

While the CCR has excellently addressed the challenge of data storage and accessibility through Google Drive, there is a pressing need for better file management while interpreting results.

In this regard, some technical considerations are reported below, illustrating the complex process of data cross-referencing that, based on the methods' complementarity, is functional in interpreting the diagnostic campaign's results.

In the end, a proposal for ideal software for data management during the interpretation process is presented based on a critical analysis of the hurdles encountered.

4.1 Cross-referencing and interpreting data from a multi-analytical diagnostic campaign: specifications and hurdles in data management

The first output of the analysis from a diagnostic campaign is raw data. Data processing with proprietary software is the first step in allowing data visualization, comparison, and cross-referencing with other data or analysis, which is mandatory for a thorough and reliable result. For a photograph, the processing consists, for example, of applying color correction; for an XRF spectrum, of assigning the chemical elements to the peaks; and for a FORS spectrum, of recognizing the absorption bands, also through comparison with reference databases.

Most of the time, when the data comes from a scientific collaboration, it arrives already processed, i.e., the operator with their specific skills has already processed it to make it available and usable on non-proprietary software. An example is the elemental maps from the MA-XRF campaign that can be shared in image format (e.g., *.tiff).

Once all data are processed, the operator can start the interpretation process, which includes cross-referencing the data acquired on the same analysis spot and the imaging outcomes, based on the well-known advantage of exploiting the methods' full ability and complementarity.

The methods of an analytical plan can provide data of different format and scale. As for the scale, they can regard a single spot of the painting, show a high magnification of a precise point, or represent the whole artwork. As for the format, we can obtain digital photographs, maps, spectra, lists of numbers (e.g., colorimetry), graphs (e.g., dendrochronology), or analogic plates like those used for traditional radiography.

This variety in format and scale represents the first hurdle in managing data during the results' interpretation process because it implies working with multiple points of view – macro and micro – and with various files and software in parallel.

As described in this dissertation, once the artwork's images and maps from a diagnostic campaign have been acquired, they are aligned and assembled into a multi-layer Adobe Photoshop file (in .psd format, or .psb for larger images, with sizes of up to 300 GB and more than 30,000 pixels) to allow a more straightforward comparison. To make an example, the aligning and overlaying of grazing light, XR, and HR SWIR of the painting by Defendente Ferrari allowed us to detect the engravings, the vanishing point, and the underdrawings made with different tools and in subsequently steps, leading to a thorough understanding of the *modus operandi* adopted by the artist in the painting's preparation phases.

The microscope images, being on a completely different scale from the whole painting, cannot be superimposed on the *.psd file. However, they are aligned and compared in another *.psd file (one or more for each cross section, depending on the sample region analyzed and the magnification level used), as cross-referencing data from different analyses is also beneficial on a micro-scale. An example is when we need to characterize a single minute grain of pigment in a mixture of fine-grain pigments. The various images of the cross section – under UV light, visible light, and backscattered electron (BSE) – must be perfectly aligned to allow their comparison, even if they are usually managed with different software and can be acquired at different magnification levels. However, since all information contributes to the characterization, we need to observe the exact grain of pigment under visible light to observe its color, UV fluorescence, and gray level in the BSE image. In the case study of the painting copy after Raphael, this process was fundamental to hypothesizing the presence of tiny grains of lead chrome yellow in the dark green overpainting layer: the hypothesis has been based on the visible color of the grains and the presence of chrome and lead in the EDS spectra – the dark UVF, in this case, was referred to the co-presence of copper resin and Prussian blue in the same layer.

These examples show that, regardless of scale, image superposition is paramount, given the complementary nature of the methods adopted.

Equally crucial is the precise placement of the spots of analysis and sampling sites on the painting, especially when analyzing a brushstroke of a few microns or capturing images in video microscopy. As observed in the case studies, once the survey with spot techniques is completed, and every time it is repeated during the work, a further level is added to the multi-layer file to fix the spots of analysis, using, for example, a text level.

Data cross-referencing becomes further complicated when data from spot and imaging methods must be compared. The case studies show that this is frequent and the cross-referencing allows for avoiding serious interpretation errors. Below are some examples testifying to the advantage of interpreting spot analyses in light of the imaging techniques' results:

- 1) detection of organic compounds by FTIR analysis. Considering the limits of the technique – firstly, the threshold sensitivity, and secondly, the fact that not all classes of compounds can be detected by the analysis – it is more than appropriate to integrate the FTIR analysis with the observation of the painting's UV-induced visible fluorescence (UVF) in correspondence with the sampling site, especially when other

methods are unavailable. FTIR spectrum may reveal only inorganic material and not the presence of organic compounds because of a low amount in the sample (below the detectability threshold). In this case, noting an intense UVF can, at least, suggest the presence of organic compounds, since most of them emit UVF, indicating the need to go in-depth with the investigation;

- 2) non-invasive characterization of red lakes in the presence of cinnabar. As shown in the case studies, FORS is an effective technique for detecting the absorption bands of the red lakes, even if they are more complex to detect in pigment mixtures (the precise identification of lakes implies using micro-invasive analyses). Other methods, such as portable XRF, are limited in detecting lakes, whereas they can detect mercury and sulfur, suggesting the presence of cinnabar. Therefore, observing the painting's UVF in correspondence with that paint region can be decisive because red lakes usually emit an intense pink UVF even when mixed with cinnabar.

These examples demonstrate that cross-referencing spot and imaging data is not just a matter of documentation, i.e., of reporting the analysis spots on the painting' photograph. On the contrary, reading the 'content' of imaging and spot data in parallel can add value to the interpretation and knowledge of the painting's materiality. Nonetheless, from a practical perspective, the contextual reading of multiple spectra and images, with the consequent use of different software, represents a further hurdle for the operator.

4.2 Structure and functions of an ideal software designed for data management and interpretation

Based on the operator's needs, who has to collect and interpret the data of a multi-analytical diagnostic campaign, and starting from the experience of the two case studies presented, the structure and functions of an ideal software designed for data management and interpretation are presented in the following.

Figure 4.1 shows a hypothetical user interface that is divided into two parts. When opening a new project (i.e., the file created with this software), the left part is dedicated to the 'macro-scale' images, that is, all the images or maps representing the whole painting or big portions of it. The images are perfectly aligned and overlaid and it is possible to zoom in and out the images to the desired level: zooming one image, all the other overlaid will follow the zooming, keeping the alignment between each other. You can click on the icons from the panel with the

acronyms to activate the desired layer and make it visible (as you would in Photoshop with the upper layer).

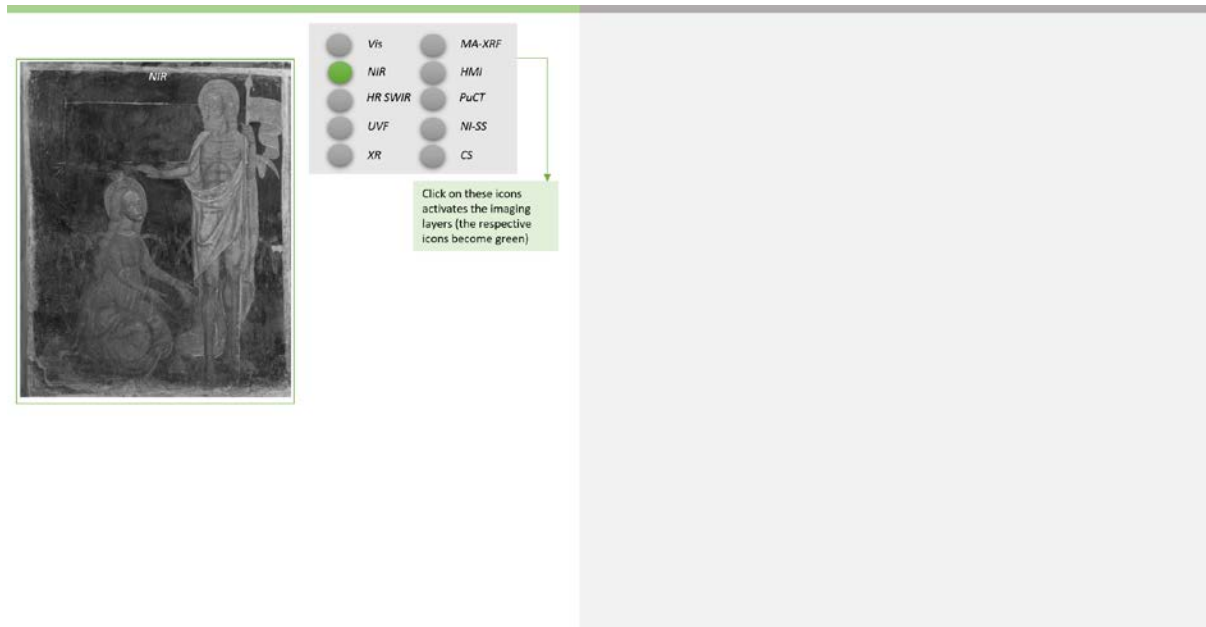


Figure 4.1 Proposal of the software user interface designed for data management and interpretation from a diagnostic campaign. The window is divided into two parts: the left half is dedicated to the ‘macro-scale’ image, the right half is dedicated to the contents of popups (see after). From the panel with the acronyms, it is possible to activate the desired level.

The legend with the acronyms of the analyses should be filled in at the beginning by the operator based on the methods adopted in the diagnostic campaign. By way of example, Figure 4.1 and the following show some acronyms of the techniques used in the two case studies presented, plus the acronyms to indicate macro-categories of analysis:

- Vis: visible diffuse light photography
- NIR: near-infrared reflectography
- HR SWIR: high resolution shortwave infrared reflectography
- UVF: ultraviolet-induced visible fluorescence
- XR: X-ray radiography
- MA-XRF: macro-X ray fluorescence
- HMI: hypercolorimetric multispectral imaging
- PuCT: Pulse-Compression Thermography

- NI-SS: non-invasive spot analyses and analyses on selective samples
- CS: micro-invasive analyses on cross sections

The macro-category called NI-SS contains the following techniques:

- VM: video-microscopy
- XRF: X-ray fluorescence spectroscopy
- FORS: fiber optics reflectance spectroscopy
- RAMAN: Raman spectroscopy
- FTIR: Fourier transform infrared spectroscopy

while the CS category contains:

- GC-MS: gas chromatography mass spectroscopy
- Vis-UV OM: optical microscopy in visible and ultraviolet light
- SEM/EDS: scanning electron microscopy with energy-dispersive X-ray spectroscopy.

This subdivision could be unusual at first glance because it groups some micro-invasive analyses (those on selective samples) with non-invasive spot analyses while keeping the micro-invasive analyses on cross sections separately. The reason for this subdivision (between NI-SS and CS) is to ensure practicality for the operator. In fact, in interpretation, the data on a selective sample often integrates those from non-invasive spot analyses, as illustrated in the following examples.

Figure 4.2 shows the aspect of the NI-SS layer. It is a transparent layer that reports the numbering and placement of the analysis points. These numbers, therefore, appear superimposed on the selected image (in the example of Figure 4.2, on the infrared reflectography). The numbers are represented excessively large in this simulation only to facilitate description. In reality, they must be positioned precisely (to the millimeter) on the actual spot of analysis or sampling. Therefore, when the image is enlarged or reduced, the size of the numbers must be re-proportioned, and the spots must move in a manner integral to the images.

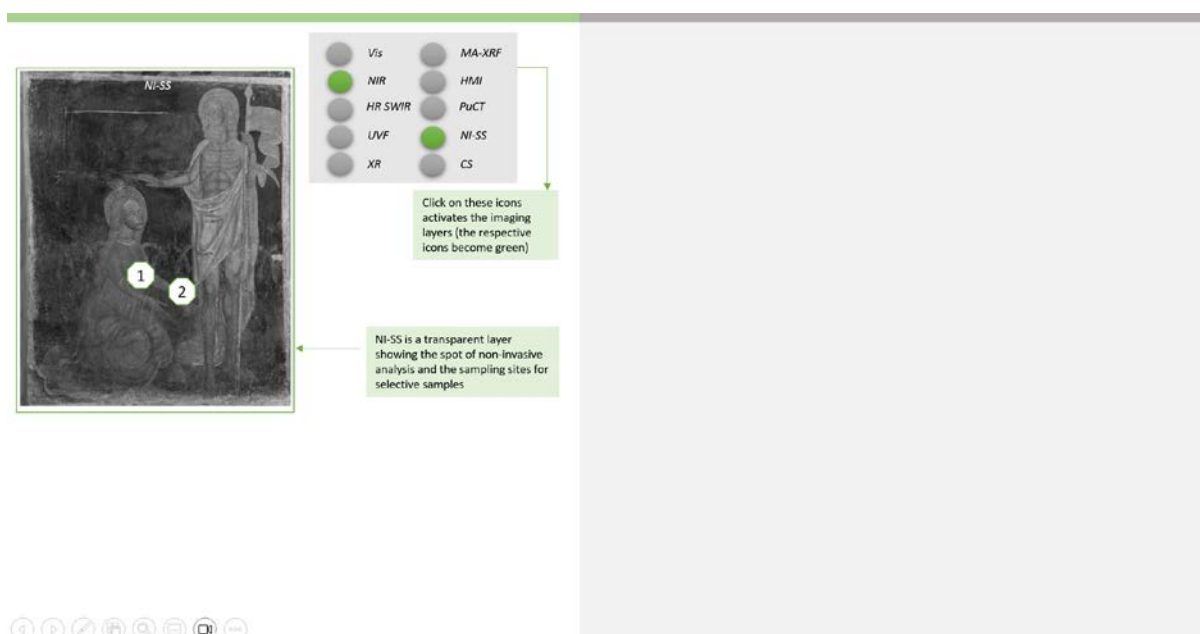


Figure 4.2 User interface showing, by way of example, the NI-SS transparent layer (reporting the spots of non-invasive analysis and selective sampling) over the NIR image of the painting. Once activated the layer, the respective icon becomes green.

As shown in Figure 4.3, by clicking on each spot of the NI-SS level, a type 1 popup (popup_1) is activated. This popup shows on the right the data of the analyses carried out on the selected spot (spot n° 2 in the example), a box note for each technique, and a box note for the complete interpretation of results from the data cross-referencing. The example reported is of a light green area: here, the VM image allows for observing white grains and green grains, indicating the presence of a mixture of pigments, and the FORS analysis allows for recognizing the typical spectral curve of green earth. In addition, the FTIR analysis of a selective scraping of the green paint allows for detecting the presence of oil, thus providing information on the pictorial binder and contributing to the knowledge of the paint. Even if not shown in the figure, a possible GC-MS analysis of the same sample would further contribute to completing and deepening the information on the green paint. For this reason, this method is also part of the NI-SS group.

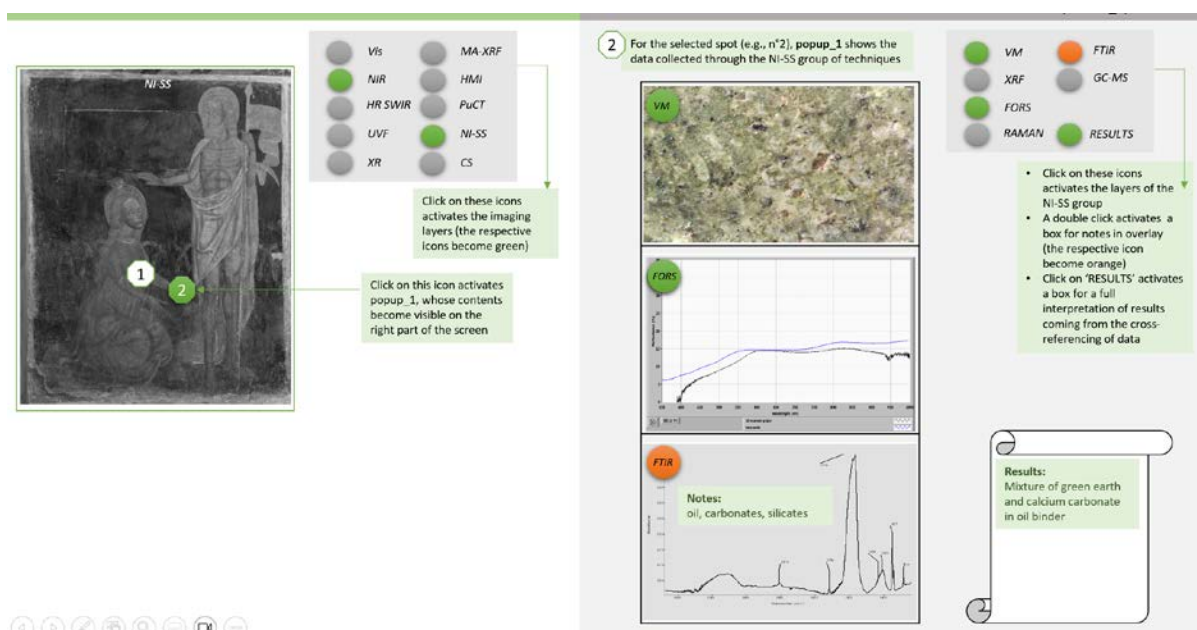


Figure 4.3 The user interface shows the content of popup_1 in the right part of the window, activated for the site n° 2. Green icons indicate the activated layers (images and analytical data selected); orange icons indicate that the note box has become available for the operator.

The cross-referencing and interpretation of the data obtainable from a cross-section follow a significantly different process. For this reason, a separate section was designed for the analysis group called CS, which allows easy comparison between the data on a micro-scale without losing sight of the sampling site and, therefore, the overall vision.

As shown in Figure 4.4, clicking on the CS icon activates a transparent layer reporting the sampling sites of the cross sections, superimposed over the selected image. In this case, the sampling sites numbered 3 and 4 are shown over the XR image of the painting. A type 2 popup (popup_2) is activated by clicking on one of the sampling sites, which become green (Figure 4.5). Popup_2 opens on the left the images of the cross section, and on the right the data from the analyses conducted on the same sample, a box note for each technique, and a box note for the complete interpretation of results from the data cross-referencing.

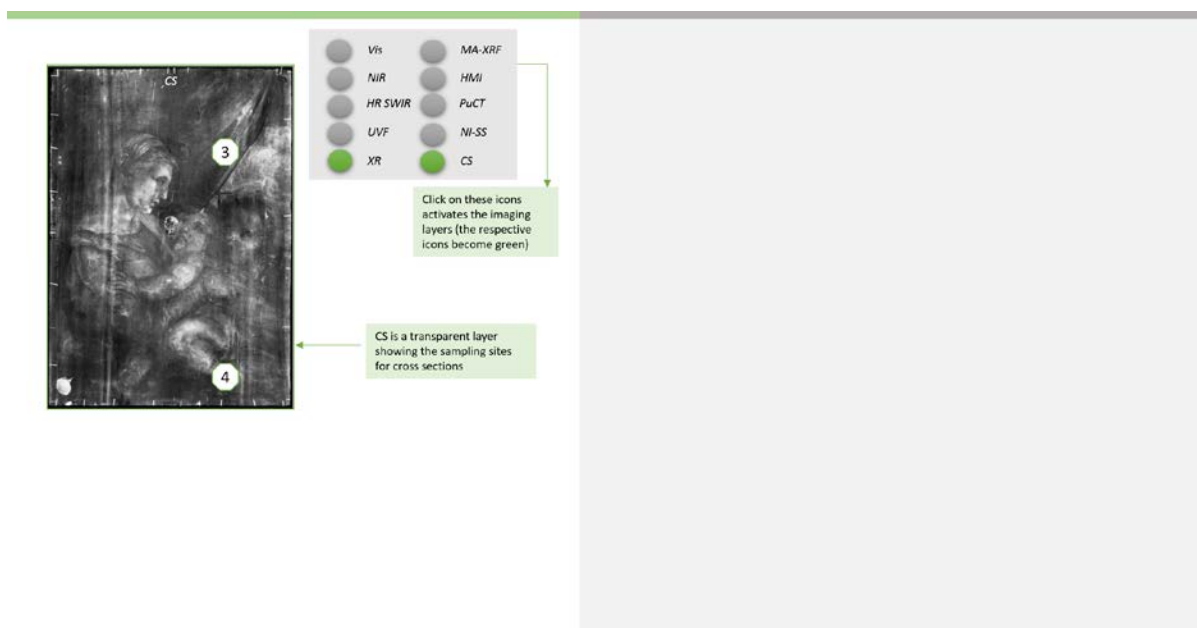


Figure 4.4 The user interface shows the CS transparent layer (reporting the sampling sites of the cross sections) over the XR image of the painting.

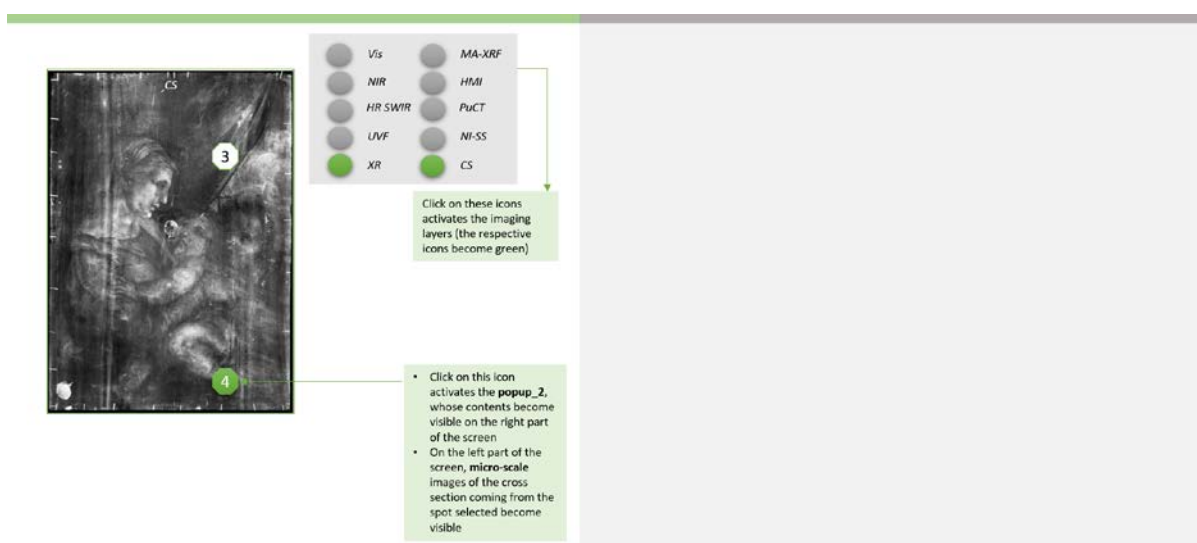


Figure 4.5 This example shows the transparent CS layer (reporting the sampling sites of the cross sections) over the painting's XR. Clicking on each sampling site activates a type 2 popup in the right half of the window (see Figure 4.6 for the contents).

Figure 4.6 shows an example of a cross section where, for each layer of the sample, the chemical information from the EDS is compared to the color of the grains (Vis OM) and the FTIR spectrum of a scraping selectively removed from that layer. This leads to a complete

description of the layer – pigments and binders, which can be reported in the note box labeled ‘Results’. From this screen, it is always possible to return to observe the painting at the sampling site and how it responds in the various analysis bands without losing the overall vision.

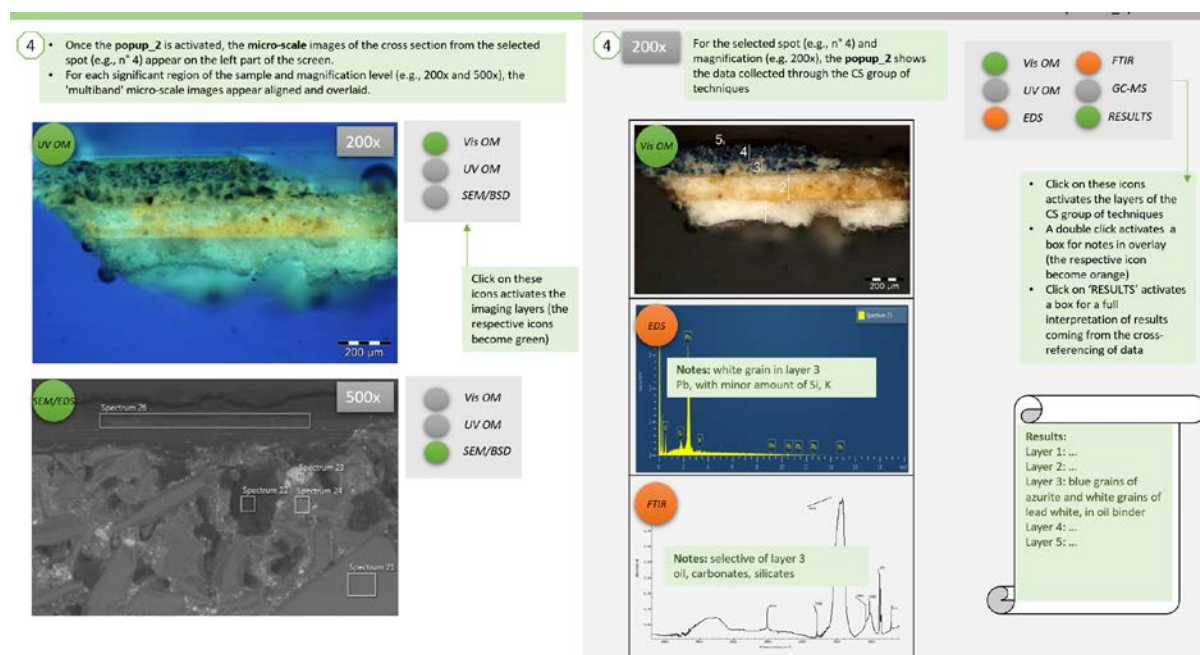


Figure 4.6 The image shows that popup_2 has been activated by clicking on sampling site n° 4. On the left: the cross section images appear; in each box, the images of the same sample region, captured at the same magnification (200x and 500x in the example), appear; the green icons on the panels show which layer is active. On the right: data from the analyses become visible (green icons), and a box note becomes available for the operator (orange icon).

Considering all the aspects and functions of this ideal software, it is easy to understand how a single project containing all images, maps, spectra, and notes may become a very ‘heavy’ file, thus necessitating very high-performance and expensive computers.

To overcome this problem, the software should allow users to navigate through the images, zooming in and out on them, without downloading them completely (i.e., without cluttering up the computer’s memory), as well as happens, for example, when we use Google Maps.

Moreover, the open-source nature of the software presents a unique opportunity to foster collaboration among the laboratory’s internal staff and other collaborating bodies in scientific

analysis. This collaborative platform could ignite exciting interdisciplinary exchanges, thereby enhancing the interpretation and communication of results.

With a user-friendly and simplified interface, communication or meetings with the artworks' owners and the Soprintendence will be straightforward and efficient.

Finally, the open-source software will enable the archiving and collection of all the diagnostic campaign data in a single project for sharing with the scientific community. This practical feature ensures the project's long-term usefulness, allowing for the revisiting of data interpretation following new research or discoveries, and ensuring the data's relevance over time.

4.3 Notes on the preprocessing of data required by the software

Using this software necessitates some precautions on the operator's part, such as preprocessing data. For spectral data such as XRF, FORS, EDS, and FTIR, spectra must be preprocessed by assigning the chemical elements, absorption bands, or signals to the peaks (or spectral curves), eventually using reference databases (see the examples in Figures 4.3 and 4.6). Regarding the micro-scale images, the operator can load the most representative images of the cross section, i.e., images of a specific sample region and at a particular level of magnification (see the examples in Figure 4.6 where the 200x and 500x magnification levels are shown). The operator will likely take more photos and images than he will use for interpretation. However, by knowing the functions of the software, he will be able to take them into account also during data acquisition, for example, by capturing OM and SEM images in the same region of the sample (software like Zeiss ZEN core for SEM systems, for example, provide automatic sample alignment starting from an optical microscope image).

The macro-scale images must be rectified with respect to a guide image (for example, orthophotography) to guarantee a perfect alignment. This step is, however, foreseen for a good interpretation of the data; therefore, using this software would not create additional work for the user but rather optimize it. To address this latter issue, a multi-institutional endeavor was recently made within the framework of this PhD to develop methodologies for the co-registration of images and maps from different techniques. The focus is mainly on the XR images acquired with the CCR scientific equipment, with the final goal of facilitating data comparison and alignment [1]. While the image deformation problem of photographic

techniques and multispectral imaging can be solved through photogrammetry and orthorectification [2], manual post-processing of the images is commonly performed for other methods such as XR and MA-XRF. The X-ray apparatus at the CCR enables the acquisition of digital radiographs of large artworks, i.e., up to $2 \times 3 \text{ m}^2$, using a fine-moved linear detector along the horizontal axis [3]. However, due to the geometry and type of cone beam used, a certain deformation is induced at the radiograph edges, affecting the overall quality of the final mosaic, especially in the case of large-format paintings. A geometric correction model was developed as a result of this study, similar to the lens correction function in Adobe Photoshop. This research was a collaborative effort among the CCR, the DEIM department of the University of Tuscia, the Department of Informatics, Modelling, Electronics and Systems Engineering (DIMES) of the University of Calabria, the Department of Applied Science and Technology of the Polytechnic University of Turin, the Department of Physics of the University of Turin, and the INFN Turin Division. The preliminary results were presented in 2023 at the International Conference on Analytical Techniques in Art and Cultural Heritage, Technart 2023, in Lisbon.

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Chapter 5

FINAL REMARKS AND RESEARCH PERSPECTIVES

The case studies presented in this dissertation regarded two panel paintings of the Italian Renaissance – the *Madonna with Child and Two Saints* from Palazzo Madama Museo Civico di Arte Antica (Torino, Italy) painted by Defendente Ferrari and dating back to around 1505-1510, and the *Madonna della Tenda* from Musei Reali Torino (MRT, Inv. 271, Torino, Italy) historically attributed to Raffaello Sanzio and subsequently recognized as a 16th-century copy after the Master.

The scientific research conducted on these artworks within a multiprofessional work team showed that the multi-analytical approach and design of tailored analytical plan and strategy are fundamental to answer the issues that can arise during the study and conservation treatments of panel paintings.

The most challenging questions that we had to answer through scientific analysis regarded the internal structure of the wooden panels – especially the nature of the hollow present on the back of the painting copy after Raphael and the preservation issues linked to it –, the identification of localized overpainting, the chemical characterization of the thin layers of original and not original varnishes which were observed in the cross sections, and the understanding of the numerous forms of degradation of the artworks – with a particular focus on the blackening of the Virgin's blue garment in the painting by Defendente. Understanding these aspects was fundamental to supporting the conservators with scientific data in their operational choices during the recent conservation treatments of the two paintings.

Furthermore, the research has unveiled novel findings on the execution technique of the two paintings. It has revealed the underdrawings, engravings, perspective lines, and the vanishing point, while also hypothesizing the tools used by the artists. The study has also shed light on some of the technical expedients employed by Defendente Ferrari, particularly his unique method of rendering flesh tones with 'dotted' and black crossed strokes for shades, volumes, and lines.

To address these case studies, a reflection on the potential and limits of the methods available at CCR was necessary and eight scientific collaborations were launched. Overall, sixteen different methods of analysis, including imaging and spot techniques, were adopted.

The demanding issues posed by the conservators became opportunities to test state-of-the-art methods and combinations of techniques, leading to the publication of three scientific papers.

For instance, combining traditional XR and the recently developed PuCT method allowed for understanding the nature of the hollow in the painting copy after Raphael, detecting the trajectory of the wood grain and confirming the presence of an old wood knot, as well as of a tau-shaped element supposed to be a cracked and unfilled area of the wooden support near the hollow. This data has led to a better understanding of the artwork's preservation state, even if the reason why this hollow was so deeply dug and enlarged remains a mystery. PuCT has proven to be an effective technique in studying the internal structure of the wooden panel, representing an alternative to the more usual CT scan.

The combination of Vis-UV OM and SEM/EDS on cross sections and FTIR and Py/GC-MS analyses on selective scrapings allowed for recognizing a historical localized overpainting in the *Madonna della tenda* and for revealing the presence and chemical composition of three different layers of varnishes overlaid. This information was extremely precious during the recent conservation treatment of the painting, where the modern varnishes were removed and the historical overpainting maintained – a green paint made of Prussian blue and yellow chrome, likely used to refresh the damaged green color of the tent. Nowadays, observing the varnishes of a painting in a cross section under UV OM is a consolidated technique, but precisely identifying the organic compounds in the cross section is highly complicated and requires sophisticated techniques. For this reason, in our research, we have developed a selective sampling method (that followed the removal and analysis of the stratigraphy) with which the varnishes were taken layer by layer based on their different UVF. Although this method was beneficial for this research, it has limitations because it cannot guarantee maximum sample selectivity. Developing analytical methodologies capable of directly analyzing organic compounds in the cross section would be crucial for better understanding the artworks and guiding the conservation treatments.

Research into the blackening of the Virgin's blue robe in Defendente Ferrari's painting also provided valuable information for conservative treatment. The combined application of OM, SEM-EDS, μ -Raman, μ -XRD, FTIR, and GC-MS made it possible to verify the excellent

condition of the azurite particles (by excluding a transformation into tenorite) and to observe the presence of carbonaceous particles on the surface and of an egg white binder which has browned probably due to aging. By demonstrating that the pictorial layer, although blackened, is composed of original material (partially degraded), any attempt to remove or thin this layer was excluded. However, using the HR SWIR, an exceptional recovery of the artwork's readability was obtained. This method made it possible to detect all the hatchings and crossed lines with which the artist rendered the shadows and volumes, especially for the blue robe, which still appears flat and dark. After the conservation treatment, the HR SWIR image was displayed alongside the painting in a temporary exhibition. Considering the excellent results obtainable with this technique, it would be desirable to increase museum awareness by promoting and disseminating these interesting contents and including them in exhibitions.

Again regarding Defendente's artistic technique, the GC-MS analysis of the pictorial binders on various spots of the artwork allowed us to understand that the artist mastered the use of several materials, i.e., egg, linseed oil, and animal glue, and employed them in different ways to achieve various aesthetical effects. Of particular interest was the discovery of the adoption of an animal glue-based binder found exclusively on the flesh tones rendered with the 'dotted' technique. The hypothesis is that Defendente used a narrow tube to spray the dark colours, a sort of 'mouth spray gun.' The choice of animal glue is probably due to technical reasons: unlike oil or egg tempera, an aqueous binder based on animal glue can be diluted to obtain a thin paint that could be sprayed. Since this technical expedient is a recurrent parameter in many of the paintings by Defendente Ferrari and has also been used by other contemporary artists – such as his colleague and collaborator Gerolamo Giovenone during the first decade of the 16th century –, it would be desirable in the future to apply a similar analytical plan to analyse and compare other artworks.

Finally, regarding data management, to which Chapter 4 is dedicated, the experience of these case studies has undoubtedly demonstrated that developing open-source software for data management and interpretation would be desirable. This tool would not only benefit the operator in charge of collecting and interpreting the results of a diagnostic campaign but also open up exciting possibilities for exchanging analytical data and reflections with the scientific community and communicating results in a broader interdisciplinary context.

I. LIST OF FIGURES

Figure 2.1 Madonna with Child and Two Saints by Defendente Ferrari, before the last conservation treatment carried out at CCR: a) photograph in visible diffuse light; b) detail in visible raking light.

Figure 2.2 Detail of the face of the Saint on the left: a) brown-greyish patina; b) magnification on previous retouching.

Figure 2.3 The painting after the last conservation treatment carried out at CCR with the spots of non-invasive analysis (in white) and sampling sites (in blue) localized. For the details of the analyses, see Table 2.1.

Figure 2.4 XR of the painting: a) image of the entire panel; b) detail of the red square where it is possible to notice one pin too close to the edge, indicating a previous resizing and the two parallel lines running from top to bottom showing the presence of half-lap joints to assemble the planks.

Figure 2.5 MA-XRF RGB correlation maps of: a) Ca (red), K (green), Pb (blue); b) Au (red), Fe (green), Cu (blue). According to the additive synthesis of colors, yellow results from the synthesis of the R and G channels, magenta from that of R and B (in both cases, indicating the co-presence of the two corresponding elements).

Figure 2.6 HR-SWIR of the painting.

Figure 2.7 HR-SWIR image of the painting with the vanishing point lines overlaid.

Figure 2.8 Detail of the area around the Virgin's halo: a) g-Vis; b) HR-SWIR.

Figure 2.9 HR-SWIR details of the Virgin garment: hatching is made in different ways of shadowing and defining the draperies.

Figure 2.10 The Child's face showing the different types of underdrawings: ink-based and dry medium.

Figure 2.11 Details of the eyes of the Virgin and of the Saint on the left, Vis and HR-SWIR. A double brushstroke trace is present with different saturation, lighter for all the underdrawings, darker for the outlines retracing.

Figure 2.12 IRFC image of the painting obtained with the HMI software starting from the Adobe RGB (Vis) and the IR3 calibrated band (950 nm centered) images. In the RGB color space, the blue channel (B) is transferred into the green (G), the green into the red (R) and the red channel is substituted by the IR3.

Figure 2.13 Sample 17s (approx. 200 μm across) from the Virgin's blue garment observed under an optical microscope and the related μ -diffraction pattern, in which the typical reflections of azurite can be appreciated.

Figure 2.14 FORS spectra acquired on the gray-blue colour of the base of the throne (black curve, spot n° 22 in Figure 2.3) compared with reference mock-ups of indigo blue lake in linseed oil (blue curve) and of a mixture of indigo lake and lead white (red curve) from the CCR database.

Figure 2.15 FORS spectra acquired on the Saint's red armor on the right (black curve; spot n° 11 in Figure 2.3), compared to the CCR database reference mock-ups made of madder lake (blue curve) and cochineal (red curve) in gum Arabic binder painted over a gypsum-based layer.

Figure 2.16 Raman spectra acquired on: a) the red stole of the Saint on the left, spot n° 24 in Figure 2.3; b) the light yellow palm leaf on the left, spot n° 8 in Figure 2.3.

Figure 2.17 Detail of the painting in visible diffuse light and MA-XRF map of Zn.

Figure 2.18 Cross section of sample 7s, from the Virgin's blue garment: a) Vis-OM; b) SEM/BSD. The numbers in a) indicate the layers recognized through the investigations: 1) the gypsum-based preparation layer; 2) a thin (around 10 μm) black layer, possibly made of carbon-based black; 3) the blue azurite-based paint layer with coarse, well-preserved, and intensely blue-colored grains (even > 20 μm across) and no evident darkening. The brown-to-reddish areas among the interstices of the blue grains are possibly related to the organic binder.

Figure 2.19 Sample 17s (approx. 200 μm across), extracted from the Virgin's blue garment. Top: OM image in transmitted light of the crystalline blue pigment of azurite and relative μ -Raman spectrum collected on the red circle, where the typical bands of azurite are observed. Bottom: OM image in transmitted light of the blackish patina homogeneously covering most of the sample surface (the reddish area, on the left,

is related to the organic binder) and relative μ -Raman spectrum collected on the red circle, showing the typical bands of carbon.

Figure 2.20 Sample 17s from the Virgin's blue garment, GC/MS results. Top left: chromatographic profile of the amino acidic fraction (i.s. internal standard; all compounds are TBDMS derivatives). Top right: relative amino acid percentage content. Bottom left: Principal Component Analysis Score Plot. Bottom right: chromatographic profile of the lipid-resinous fraction (i.s. internal standard; all compounds are TMS derivatives).

Figure 2.21 Cross section of sample 8s from the green inner lining of the Virgin's garment: a) Vis-OM; b) SEM/BSD. The numbering in a) refers to the layers identified through the analyses: 1) light green layer made of azurite, lead-tin yellow, and small amount of red ochre, ca. 40 μm thick; 2) copper resinate layer, ca. 25 μm ; 3) layer of copper-based green and lead-tin yellow, ca. 50 μm ; 4) layer of lead-tin yellow, ca. 15 μm ; 5) superficial glaze of copper resinate with traces of lead-tin yellow, ca. 10 μm ; 6) layer of natural resin.

Figure 2.22 Sample 8s, from the green inner lining of the Virgin's garment. GC/MS results.

Figure 2.23 Sample 5s, from the grey-greenish background. Top left: chromatographic profile of the amino acidic fraction (i.s. internal standard; all compounds are TBDMS derivatives). Top right: relative amino acid percentage content. Bottom left: Principal Component Analysis Score Plot. Bottom right: chromatographic profile of the lipid-resinous fraction with parameters used for the lipid characterization (i.s. internal standard; A: azelaic acid; P: palmitic acid; ΣD : sum of the relative content of dicarboxylic acids; all compounds are TMS derivatives).

Figure 2.24 FTIR spectrum of sample 4s, from the superficial brown patina (black line) compared to a reference of proteinaceous substance. Assigned bands: 3085 cm^{-1} : N-H stretching band; 1649, 1543, 1450 cm^{-1} : C=O stretching band; calcium carbonate, silicate, oxalates, and a natural resin or aged drying oil are also identified.

Figure 2.25 Details of the dotted technique on: a) the Virgin's eye; b) the hand of the Saint on the left.

Figure 2.26 Sample 18s, from the flesh tones of the Saint on the left. Top left: chromatographic profile of the amino acidic fraction (i.s. internal standard; all compounds are TBDMS derivatives). Top right: relative amino acid percentage content. Bottom left: Principal Component Analysis Score Plot. Bottom right: chromatographic profile of the lipid-resinous fraction with parameters used for the lipid characterization (i.s. internal standard; A: azelaic acid; P: palmitic acid; Σ D: sum of the relative content of dicarboxylic acids; all compounds are TMS derivatives).

Figure 2.27 Top left: HMI, spectral similarity map of the brown patina registered on the face of the Saint on the left (blue arrow): the black areas indicate the absence of the patina's spectral signature. Top right and bottom: UVF images acquired before and during the recent conservation treatment carried out at CCR.

Figure 2.28 FTIR spectrum of sample 1s, from the superficial varnish in the background (black line) compared to a reference of natural resin (green line). Assigned bands 2934–2871 cm^{-1} : C–H stretching bands; 1709 cm^{-1} : C=O stretching band; 1452–1383 cm^{-1} : C–H bending band; 1277–1053 cm^{-1} : C–O and C–O–C stretching band.

Figure 3.1 The panel painting *Madonna della tenda* (MRT, Inv. 271, Torino, Italy) before the last conservation treatment carried out at CCR: a) photograph in visible diffuse light; b) detail of the dotted region in visible raking light.

Figure 3.2 Back of the painting: a) photograph in visible diffuse light; b) detail of the dotted region in visible raking light.

Figure 3.3 Details of the different brushstrokes and uneven painting techniques: a) on the clouds, b) on the Virgin's blue mantle near the Child's left foot; c) on the Child's flesh tone and the Virgin's red robe in correspondence with her right sleeve; d) on the Virgin's flesh tone.

Figure 3.4 The painting during the conservation treatment conducted at CCR: a) front of the painting in Vis diffuse light photograph with spots of FORS analysis (in white) and sampling sites (in red); b) back of the painting in Vis diffuse light photograph with spots of FORS analysis (in white) and sampling sites (in red). For the details of the micro-invasive analyses, see Table 3.1.

Figure 3.5 XR of the painting showing the pattern of wood grains of the support (much less noticeable in the central stripe) and the numerous woodworm tunnels following the pattern of the wood fibers.

Figure 3.6 Back of the painting, UVF.

Figure 3.7 A series of thermograms at different time instants obtained by PuCT. A tau-shaped thermal signature is visible, starting from $t = 6$ s (red arrow). As a reference, the yellow arrows point out a single vertical wood grain signature. Note that other quasi-parallel grains are also well defined all across the inspected surface.

Figure 3.8 Results from the PuCT analysis: a) thermal contrast for the grain (green) and tau (red) pixel areas as a function of time. A maximum is reached for both quantities at $t = 13$ s; b) a thermogram showing the investigated area at $t = 13$ s, marked to highlight the regions employed for computing the thermal contrast, i.e., tau within the red square, wood grain within the green square, and sound within the orange square.

Figure 3.9 Results from the PuCT analysis: a) a series of thermograms at different time instants acquired from the back panel surface; b) one of the thermograms, for a single instant $t = 4$ s; c) image obtained using the time-phase feature, with yellow arrows pointing towards the wood grain surrounding the hollow area.

Figure 3.10 Detail of the green curtain painted with a copper-based green pigment; a) Vis light photograph; b) XR showing the woodworm tunnels and the more radio-opaque vertical lines; c) MA-XRF distribution map of copper showing the lower Cu-signal along the vertical lines; d,e) PuCT thermograms at $t = 1.5$ s and $t = 2$ s when the dark vertical lines became whiter.

Figure 3.11 Microscopic images of the cross section from the Virgin's blue mantle: a) OM in visible reflected light; b) SEM/BSE. The images show a yellow-orange rounded inclusion of pigmented imprimitura (layer 2) in the gypsum-based ground (layer 1). Above this, two painting layers – the first in azurite and the second in lapis lazuli (layers 3 and 4 respectively) – render the color of the blue mantle.

Figure 3.12 In black: FTIR spectrum of a scraping selectively removed from the white ground (sampling site 2s in Figure 3.4). In the sample, the spectral features of gypsum were

detected (reference spectrum in purple), whereas the signals at 1538 and 1444 cm^{-1} were assigned to a protein substance.

Figure 3.13 Enlarged radiographic details of the painting at a) 55x and b) 220x. The white spots' size is fully compatible with the imprimitura inclusions in the ground layer shown in Figure 3.11.

Figure 3.14 MA-XRF distribution maps of: a) calcium (Ca), lead (Pb), copper (Cu), and manganese (Mn); b) iron (Fe), mercury (Hg), cobalt (Co), and gold (Au).

Figure 3.15 Infrared reflectography image comparison: a) NIR and b) HR-SWIR, showing the difference in revealing the underdrawings made with a brush and a carbon black ink.

Figure 3.16 HR-SWIR detail showing the traces of punched dots, indicated by the red arrow, in correspondence to the neck of the Madonna.

Figure 3.17 XR detail, where the signs of metal point engravings and needle points are evident in the tracing of the two haloes by means of a drawing compass.

Figure 3.18 Detail of the Virgin's red robe: a) diffuse visible light photograph; b) MA-XRF distribution map of iron; c) UVF image after removing the outermost layers of varnish.

Figure 3.19 Result of the HMI chromatic similarity tool applied to the painting in the points highlighted by the red dots: a) red robe of the Virgin; b) dark flesh tone of the Virgin's face.

Figure 3.20 FORS spectra of the Virgin's red robe (black curve) and her neck (dashed curve) compared to the database reference spectra of madder lake (blue curve) and cinnabar (red curve): spots of analysis, reported in white in Figure 3.4, are respectively n° 8 and 16.

Figure 3.21 Microscopic images of a cross section from the Virgin's red robe: a) Vis OM; b) UV OM. The images show the red color obtained by the artist with a first layer of red lake, lead white, and minor amounts of red ochre, and an outer glazing of red lake alone (layers 3 and 4, respectively). Underneath, a yellow imprimitura made of lead white and yellow ochres with rare grains of red ochres (layer 2) is laid over the gypsum-based ground (layer 1).

Figure 3.22 Microscopic images of the multi-layer sample from the Virgin's neck: a) the image shows that the sample broke into two fragments during the sample preparation; the left one was embedded for the cross section; b) Vis OM and c) UV OM images of the cross section show the flesh hue obtained by the artist by mixing lead white with minor amounts of ocher and cinnabar and rare grains of red lake (layer 3); the sample also shows the presence of the yellow imprimatura (layer 2) above the gypsum-based ground (layer 1).

Figure 3.23 Detail of the Virgin's blue mantle: a) IRFC; b) and c) are HMI spectral similarity maps indicating respectively the localization of lapis lazuli and azurite.

Figure 3.24 FORS spectra of the Virgin's blue mantle (dashed curve) and the blue sky (black curve) compared to reference spectra of lapis lazuli (blue curve) and artificial ultramarine (red curve). Spots of analysis, reported in Figure 3.4, are respectively n° 22 and 21.

Figure 3.25 FTIR spectrum of a scraping selectively removed from the original blue layer (sampling site n° 2s in Figure 3.4), analysis of a blue grain: spectral features of an aged drying oil were detected at 2927, 2853, 1733, 1711, 1449, 1376, 1249, 1175 cm⁻¹; signals at 1097 and 1036 cm⁻¹ were assigned to silicates, attributable to lapis lazuli.

Figure 3.26 FORS spectra of the light blue cloud (black curve, spot of analysis n° 5 in Figure 3.4) compared to reference spectra of smalt (blue curve) and synthetic cobalt blue (red curve).

Figure 3.27 Microscopic images of the cross section from the green curtain: a) Vis OM; b) UV OM. The numbering indicates the identified layers: 2) the yellow imprimatura made of lead white and yellow ochres with rare grains of red ochres; 3) a carbon black-based background layer containing small amounts of ochres and lead white; 4) the green copper-based layer with the glassy aspect; 5) organic layer; 6a and 6b) organic layers with rare grains containing chrome, lead, iron, and copper; 7) and 8) organic layers. In this sample, the gypsum-based ground (identified in all other samples as layer 1) is missing; this can happen when sampling is not deep enough to include all layers.

Figure 3.28 SEM/BSE image of the cross section from the green curtain (same of Figure 3.27), showing the glassy aspect of the copper green-based paint layer (layer 4).

Figure 3.29 EDS spectrum of a yellow grain containing lead and chrome (interpreted as chrome yellow) in layer 6a of the cross section from the green curtain. The signal of copper was attributed to a small amount of copper resinate in the organic matrix of the layer.

Figure 3.30 FTIR spectrum of the bluish grains selectively removed from the overpainting on the green curtain (layers 6a and 6b in Figure 3.28): diagnostic band for Prussian Blue at 2089 cm⁻¹ was detected, 2514, 1791, 1420, 875, 713 cm⁻¹ were assigned to calcite, 1171, 1043 cm⁻¹ to silicates, 2933, 2871, 1707 cm⁻¹ to natural resin, and 1621, 1319 cm⁻¹ to oxalates; 1541 cm⁻¹ signal was attributed to zinc stearate.

Figure 3.31 The painting during the progressive removal of non-original varnishes: a) Vis light photograph; b) UVF, with a red box in the upper left documenting the initial state.

Figure 3.32 Py-GC/MS analysis of the three layers of varnish and the layer of overpainting: a) the oldest varnish; b) the overpainting; c) the intermediate varnish; d) the most recent varnish. Peak numbers refer to Table 3.4. MC: methylcyclohexanone.

Figure 4.1 Proposal of the software user interface designed for data management and interpretation from a diagnostic campaign. The window is divided into two parts: the left half is dedicated to the 'macro-scale' image, the right half is dedicated to the contents of popups (see after). From the panel with the acronyms, it is possible to activate the desired level.

Figure 4.2 User interface showing, by way of example, the NI-SS transparent layer (reporting the spots of non-invasive analysis and selective sampling) over the NIR image of the painting. Once activated the layer, the respective icon becomes green.

Figure 4.3 The user interface shows the content of popup_1 in the right part of the window, activated for the site n° 2. Green icons indicate the activated layers (images and analytical data selected); orange icons indicate that the note box has become available for the operator.

Figure 4.4 The user interface shows the CS transparent layer (reporting the sampling sites of the cross sections) over the XR image of the painting.

Figure 4.5 This example shows the transparent CS layer (reporting the sampling sites of the cross sections) over the painting's XR. Clicking on each sampling site activates a type 2 popup in the right half of the window (see Figure 4.6 for the contents).

Figure 4.6 The image shows that popup_2 has been activated by clicking on sampling site n° 4. On the left: the cross section images appear; in each box, the images of the same sample region, captured at the same magnification (200x and 500x in the example), appear; the green icons on the panels show which layer is active. On the right: data from the analyses become visible (green icons), and a box note become available for the operator (orange icon).

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Table 2.1 List of the analyses carried out for each spot or sampling site (see Figure 2.3).

Table 2.2 Hypotheses on the pigments in the different analysis spots from the lonely noninvasive (imaging, mapping, and spot) techniques.

Table 3.1 List of micro-invasive analyses (sampling sites reported in red on Figures 3.4a-b).

Table 3.2 Summary of the XR, PuCT, and MA-XRF results relative to the wooden support.

Table 3.3 Summary of the OM, SEM/EDS, MA-XRF, XR, and HR-SWIR results relative to the preparation phases.

Table 3.4 Lists of compounds identified by Py-GC/MS. Due to methylation with TMAH acidic groups are detected as methyl esters and hydroxy groups as alkoxy derivatives.

III. SUMMARY OF THE LEARNING ACTIVITIES

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Y. Z. Helvaci, R. Bordalo, G. Chiari, M. Achladianakis, K. Zervaki, H. Koksalan, T. Cavaleri, M. Gulmini, R. Giustetto, A Case of Discontinuity and Evolution of the 16th Century Cretan Byzantine Painting: Venetian Influences and the Choice of Raw Materials, 13° iCCS International Congress of Cretan Studies, Agios Nikolaos, Creta, Greece, 2022

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J. J. Lucejko, T. Cavaleri, P. Buscaglia, E. Ferraris, M. Borla, I. Degano, F. Modugno, Chemical characterization of a reddish coating on Egyptian coffins, Convegno tematico AIAR 2022 La Sostenibilità nei beni culturali, Padova, 2022

Training and PhD Schools attended

Doctoral Summer School, Excellence in heritage science for conservation and collection care research and access, IPERION HS, HSAcademy, E-RHIS, University of Bologna, July 13-16th, 2021

Training school, From Imaging to Spot Analysis. School of UV-Vis-NIR Spectroscopy, Multiband and Hyperspectral Imaging for Applications to Cultural Heritage, CCR La Venaria Reale September 21-24th, 2021

Courses and Webinars attended

Le tecniche omiche per lo studio dei beni culturali, online on the CCR digital lab platform, June 19th-20th, 2023, 8 hours

Analisi multivariata di dati spettroscopici. Strategie chemiometriche di avanguardia per la scienza della conservazione, online on the CCR digital lab platform, June 5th-6th, 2023, 6 hours

Lecture #10: Towards cathedrals of digital data and multidisciplinary knowledge in heritage science, online on the IPERION HS platform, June 22nd, 2023

Lecture #8: X-ray imaging in cultural heritage: the past to the present, online on the IPERION HS platform, April 20th, 2023

Lecture #4: Past, present and future of citizen heritage science, online on the IPERION HS platform, December 15th, 2022

Hyperspectral and multispectral imaging in heritage science, online on the IPERION HS platform, April 5th, 2022

Informatica applicata ai beni culturali, Prof. Lanteri University of Tuscia, remote classes, 64 hours