MSc Chemistry
Analytical Sciences

Master Thesis

Egg-Tempera techniques in late 14th century in Florence:
An integrated investigation of the polyptych `Annunciation and Saints` by Giovanni del Biondo (Galleria dell’ Accademia, Florence)

by

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ABSTRACT

This research work investigates the painting technique adopted by Giovanni del Biondo in the polyptych *Annunciation and Saints* (1385), exhibited at Galleria dell’Accademia, in Florence, in Italy. The research interest of this painting is its peculiar matt appearance, remarkably different form those painted by coeval artists, active in Florence.

In February 2013, the twisted columns of the carpentry were temporarily removed. Below, it was revealed a clean painted surface, free of any brownish layer that was noticed in the rest of the painting surface. Integrated protocol by using different methodologies was followed both studying the superficial layer covering and identifying the materials in the different layers of the painting in order to discriminate the original painting technique from any possible restoration executed in the past.

Imaging techniques (i.e. UV fluorescence, IR and IR false colour) aimed to retrieve a general perspective concerning the external layer, palette, retouches and later additions, were combined with X-ray diffraction (XRF) and Fiber optics Reflectance Spectroscopy (FORS) in order to obtain information about the composition of the pictorial layers in a non-invasive way. As further step, eight micro-fragments were sampled from pre-existing *lacunae* in the right and central panels, collecting both the preparation and painted layers and documenting the surface at high magnification with digital portable microscopy (DM). Optical microscopy (OM) and ESEM-EDX analyses in cross-section aimed to the identification of both the constituting materials of preparation and the pictorial layers; organic compounds were analysed by means of FT-IR spectroscopy, Gas Chromatography/Mass Spectrometry (GC/MS) and pyrolysis-GC/MS analysis.

According to the data obtained, the preparation layer is composed by gypsum (CaSO$_4$.2H$_2$O) with some crystals of celestine (SrSO$_4$) mixed with animal glue. Indeed two preparation layers are distinguishable by the granulometry of gypsum (*gesso grosso* and *gesso fine*).

In the panel, all the blue areas are composed by lapis lazuli, which was mixed either with lead white or lead tin yellow in the various bluish and
greenish hues. The only green area showing a different material is located in the bottom side of the central panel, where a copper-based green pigment was found. In the predella, the only blue area not consisting of lapis lazuli, is the robe of the Saint on the left side of the left part, as well as the retouched areas of the Madonna’s robe. For the red colour, cinnabar was used and, for some special effects, as in the robe of St Mary Magdalene minium was added to cinnabar.

The binding medium of the pictorial layer is egg yolk based, as confirmed by FT-IR and GC/MS analyses.

The gold leaves were applied on red Armenian Bole for the ground or directly on the pictorial layer for the decoration of mantles. Silver leaves, heavily tarnished, used for some details such as swords were also applied on Armenian bole.

The external layer is a mixture of animal glue and honey. The mixture of triterpenic dammar resin and animal glue, detected by py-GC/MS analysis can be linked with the old restoration executed in the beginning of 18th century.
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Chapter 1

Introduction

In this essay we investigated a hypothesis related to the painting technique, as well as the conservation conditions, of the polyptych panel painting “Annunciation and Saints” by Giovanni Di Niccolò Del Biondo (14th century), exhibited in Galleria dell’Accademia in Florence. In order to face this challenge, this essay compiles and employs interdisciplinary methodologies and techniques. Imaging and micro-destructive techniques provided the concluding results after they were compared with already published data, and filtered through the art historical methodology. This integrated methodology also provided the opportunity to evaluate potential and limitations of the non-invasive approach to the complex case of old paintings.

This thesis starts with a theoretical part, which includes a brief description of painting techniques adopted by Italian artists and the materials they used in Renaissance (Chapter 2). The following chapter offers biographical information about Giovanni del Biondo and a short description of his painting “Annunciation and Saints”. In Chapter 4, we can find a review of analytical techniques –characteristics and limitations- that have been applied on old paintings, so far. In Chapter 5 there is an extensive description of the methodology and experiments conducted in the frame of this thesis. Ultimately, the experimental data are discussed in Chapter 6, and concluded in Chapter 7.

1.1 The aim of the Research

The aim of this research was the characterisation of the technique used by Giovanni del Biondo in “Annunciation and Saints”. This painting offered the possibility to explore the challenging research topic of egg-tempera in the transition from 14th to 15th century, as well as the issues related to varnish in Italian tempera paintings and stability of coatings. In detail, we aimed to:

- define what makes the painting surface matt; through the identification of the organic binders and of the brownish cover layer,
- identify the pigments,
➢ study the inorganic composition of the ground layer,
➢ describe and analyse the metal leaves and
➢ examine the case of an old – not recorded - restoration.

1.2 Challenges and Methodology

The design of an analytical methodology is inextricably linked with the nature of the material to be analysed. Therefore, in order to map the limitations, the risks and the advantages of a method, it is important firstly to look in detail the physicochemical characteristics of the artefact.

During Renaissance, in Italy, the multi-layered panel paintings had been developed on canvas or wooden supports stabilized with linen. This structure was enriched with a preparation (i.e. calcium sulphate salts mixed with a proteinaceous binding medium). On the top of it, they designed the under-drawing, right before the application of the pictorial layer (inorganic pigments and organic pigments dispersed in protein-based or in drying oil-based binders). Ultimately, an external varnish layer (i.e. terpenoids or polysaccharide materials) protected the painting and turned it glossier.

The analytical methodology is not only challenged by the co-existence of various different classes of substances - distributed in separated micro-layers-, but also by the fact that through the centuries the materials integrate and usually undergone to deterioration processes, thus altering their chemical composition. These facts reduce the analytical sensitivity and sensibility; and require careful pre-treatments and layer separation. The last and very important condition is the uniqueness of paintings and the consequent rigid restrictions in sampling, touching or altering the original appearance of the artefact. When art is under study, the main objective is to obtain as much data as possible avoiding or limiting the invasive methods, in order always to preserve the original view of the object.

Therefore, due to the complicity and uniqueness of paintings, integrated analytical methods are suggested. This strategiesm, combining both invasive and non-invasive techniques, accurately separate and detect micro-quantities of a wide range of inorganic and organic substances.
1.3 Contribution

In general, the extensive analysis of painting layers and materials offer important information for both restorers and art historians.

- **Restoration/Curating**

An optimum restoration and curating of a painting requires 1) the scientific confirmation of the authenticity of the painting, 2) the localization and differentiation of the original parts from the latter additions and retouching, 3) the identification of the chemical composition of the existing materials, 4) their structural arrangement and the level of their deterioration through the centuries. This information allows the restorers to design an efficient restoration plan, which not only rebuilds the original view of the work, without damaging it, but also to shields it from the environmental deteriorating parameters in order to avoid any future alterations.

- **Art history**

Chemical analysis in the service of old paintings offer precious data that can be collected and categorised in databases, which help art historians to map the technological evolution related with the art history.

Art historical questions concern the way the artists developed their painting techniques, each period; how they achieved the favourable hues, the light, the glossy effects, and the protection of their work from the time. Only few decades ago, chemistry and analytical sciences initiated interdisciplinary research along with Art History, Conservation and Archaeology. Untill then, only authors, historians, archaeologists and collectors recorded the chronology of the compounds used in old artefacts. Since the last years, analytical sciences has been challenging or confirming this data, and hence more accurate and detailed databases were built, through the years. This offered a more explicit view of the history of Art and Technology, and enabled the identification and dating of the innovations of old Masters.

Particularly, the polyptych Annunciation and Saints gives a unique opportunity for a further investigation in the evolution of the egg–Tempera technique from the 14th to 15th century in Florence. This is because the matte surface of the painting constitutes an exceptional case for this period, as the coeval paintings executed in Florence render a strong glossy effect.
Chapter 2

Panel Paintings – Italian Renaissance

2.1 Development of Panel Paintings

In Italy, panel paintings - paintings executed on wood - rank among the most significant artefacts of Western art from the 6th until the 16th century, when canvas predominated as a support. Despite the various techniques and materials used for their development around that time, all the paintings show almost the same compositional layers.

The seasoned and smoothed wooden planks were sealed with animal glue with a treating process, called sizing. Above this, wiped –in animal glue- linens or parchments were placed in stripes for better stabilizing the panel and for preventing the colour absorption. After linens were dried, one or more gypsum layers (CaSO₄·2H₂O), put on the top, made the surface plane and homogenous. Above the gypsum layer, the drawing was occured with a mixture of bone black or carbon black and an organic medium (i.e egg). Ultimately, the old Masters painted the surface with pigments dispersed into an organic binder (egg, animal glue, oil). Frequently, artists finished their work with natural resins, proteinaceous or saccharide mixtures to provide a glossier appearance.

2.2 Painting layers and materials

2.2.1 Wooden Panel

Wood had been continuously used as support in paintings, from the old antiquity until the end of 19th century. It was a low-cost, strong and relatively light material that could be easily curved and planed smooth for serving the best drawing surface. In Italy, wooden panels were the most popular supporting media, from 12th until the end of 15th century, when canvas predominated, because it was lighter and less expensive.

In Renaissance, Italian painters used local or sometimes Dalmatian wood such as poplar as well as chestnut, walnut or oak. Wooden panels were fabricated either by a single piece or with different pieces of wood joined
together both mechanically -with crossbars and nails-, and chemically -with organic adhesives. As Cennini recorded, artists strongly coated the wooden panels with crossbars, glued textiles, linen or parchments for stabilizing the preparation layer (Cennini, 1975). With this pre-treatement, even when the panel cracked, the linen coating kept strongly the wooden joints and the preparation remained untouched.

2.2.2 Preparation layer

Artists pre-treated the wooden panel aiming to a stable, flat and homogenenous surface. In Italy, they coated the wood with one or more gypsum layers mixed with natural organic glues (usually collagen extracted from animal tissues) -10 or 15% (Gettens & Mrose, 1954). This mixture is called preparation or ground layer and appears to be a thick and white water paint.

The word *gesso* has an Italian origin and refers to the naturally occurring mineral gypsum \(\text{CaSO}_4\cdot2\text{H}_2\text{O}\), which was processed with heat, before mixing with the organic medium (Feller, 1986). In Italy, as Cennini mentioned in his book, *Il libro dell’ arte* (1975), painters preferred to burn gypsum at 300-650 °C. The result was a mixture of dihydrate calcium sulphate salt with the soluble anhydrite and was called *gesso grosso* -coarse gypsum. Its coarse particles have enough body to hide and smoothen the roughly carved mouldings and hence coarse gypsum served as foundation. For finely finishing the surface, Italians soaked the burnt gypsum in water, for about one month, and produced the *gesso sottile* –fine gypsum. This product is a calcium sulfate dihydrate salt, free of any water insoluble impurities, such as iron (Fe) and aluminium (Al) salts. It is a white inert that has a fine texture and shrinks down extensively when dries. For this reason it could hardly conceal the carvings, while it could offer a smooth and delicate finishing (Gettens & Mrose, 1954).

According to Feller’s (1986) bibliographic research, the most documented compositions of *gesso grosso* and *gesso sottile* in Florence and Siena, in early and late Renaissance, are the following. *Gesso grosso*: 100% anhydrite or 75% anhydrite and 25% dihydrate or 50:50 anhydrite: dihydrate and *gesso sottile*: 100% dihydrate or 75% dihydrate and 25% anhydrite.
The elimination of the naturally existing irregularities in panels was necessary for facilitating the development of the initial drawing, and for applying the colour homogeneously. Additionally, the seasoned preparation prevented the panel to absorb liquids, such as glues and pigments, and made pigments to dry through evaporation and not absorption. As a result, the colours were glossier and more vivid.

2.2.3 Pictorial layer

2.2.3.1 Pigments

In Renaissance, the commerce roots bridged Europe and Asia, and the painters had in their disposal a wide variety of natural colouring minerals and semi-precious stones such as vermillion, azurite and lapis lazuli.

The systematic scientific study of pigments started in the beginning of 19th century, when the doctor John Haslam, analysed samples taken from a wall painting in Westminster, dated back to 14th century (Best et al., 1992). Until today, the study of pigments has offered data related to the chemical composition of pigments used in each period of time. This information 1) helps us to recognise any possible old or recent conservation work in old paintings and 2) allows curators to reproduce identical hues, with cheaper materials, for restoration purposes.

Artists were concerned about the compatibility of every pigment, its durability, absorption, alteration; the effects of impurities and the colour permanence. For these reasons, they preferred pigments, which were stable and inert with other pigments, moisture, air, and light. Among the inorganic compounds used - oxides, sulphides, carbonates, sulphates, phosphates, and silicates of the heavy metals- simple oxides are the most stable and preserved their colour without fading.

The most popular inorganic and organic pigments used in Renaissance – from 14th to 17th century- are listed in Table 2.1.
2.2.3.1.1 Lapis Lazuli

Lapis Lazuli \(((\text{Na, Ca})_8(\text{AlSiO}_4)_6(\text{SO}_4, \text{S, Cl})_2)\) had been of the most precious pigments used in European paintings from the early medieval times until 17th century. It is a bright blue natural pigment obtained from the semi-precious mineral lazurite found in Kokcha River valley in Badakhshan, an area that is now part of Afghanistan.

It was the only exotic semi-precious pigment being carried for so long distance, in the Medieval and Renaissance times. Because of the transfer cost and its far origin, lapis lazuli had the same, and even higher price than gold, in European markets.

Its blue colour is caused by the presence of the active group \(\text{S}_3^-\), which is responsible for strong absorption at 600nm. Lapis lazuli was in the first place among the most popular renaissance pigments, as it was found in the worldwide famous masterpieces of the Italian Renaissance. Because of being significantly expensive, lapis lazuli mainly used for decorating the most important saints of
Christianity as well as Madonna and Christ. For instance, Italian painters saved this pigment for colouring the mantles of Madonna and Christ, whereas for the other blue sites they frequently used azurite, as it was less expensive and easier to find. Some examples are Botticelli’s “Madonna con Bambino e S. Giovannino”, and Leonardo da Vinci’s “The virgin and Child with Saint Anne” (Bersani et al., 2008; Spring, 2012).

In the early medieval years, the pigment was being simply produced by grinding and washing the mineral yielding a dark blue colour. Based on Cennini’s description, after 1200, they finely grinded the stone and they mixed the produced powder with wax and resin to form a paste. Afterwards, they mixed the paste with potassium carbonate solution until the sedimentation of blue particles (Hoeniger, 1991). After repeating this process for several times, the most intense blue particles could be extracted.

2.2.3.1.2 Manufactured Pigments

Red lead or “minium” (Pb₃O₄) was first manufactured the same period when lead was discovered. Its colour is bright red and it was available for use in water solutions. Artists produced the red pigment by heating the white lead until the removal of carbon dioxide in the form of gas. Extended heating or mixing with nitric acid turned the white lead into the brown lead peroxide or into a light violet dust. Depending on the manufacturing way, red lead had either crystalline or amorphous structure.

Lead white (2PbCO₃•Pb(OH)₂) was artificially prepared with metallic lead and vinegar. Its use dates back to the very early years, and it was the most popular white pigment in Europe, until in 19th century, when a tin –based white pigment (SnO₂) was also introduced. Lead white is a basic lead carbonate soluble in nitric and acetic acid, which, in particular conditions may turns darker when exposed to the atmospheric air it shows inertness towards light or organic coatings.

Lead tin yellow (Pb₂SnO₄) is a pale yellow pigment, used between 14th and 17th century. Cennini first called it giallorino and the diminutive ending –ino, described the pale colouring profile of the pigment (Feller, 1986). For its
preparation, artists roasted white lead until the production of lead monoxide. Afterwards they added tin and produced two types of lead tin yellow. Type I (Pb$_2$SnO$_4$) was the most commonly used in Renaissance, and type II was a tin oxide containing additional silicon (Roy, 1993). It does not react with alkalis; it is light, inert. Type I, due to its increased compatibility with the pigments had been frequently mixed with verdigris, azurite or lapis lazuli for producing green hues.

Cinnabar (HgS) was a red pigment extracted from natural rock, and vermilion –with the same chemical formula- was produced synthetically. Although crushed mineral could be used as a pigment, men from the early times knew how to make the chemical reaction between mercury and sulphur in order to produce the red artificial Vermillion (Gettens, 1966). For cinnabar production, the only procedure needed was to finely crush and grind the mineral. For the production of vermilion artists followed complicate chemical processes. Referring to chemical and physical properties, the only difference between the natural and the synthetic pigment, is the size of the particles. The natural pigment contains coarser particles and the synthetic ones show smaller and equal-sized crystals.

2.2.3.2 Gilding

Gilding is the application of significantly thin metal foils on prepared solid surfaces, such as wood, stone or metal. This method was extensively used in old paintings for carrying out the details of the drawing. Gold, due to its metallic effects under the light, represented the transcendental light and the divine substance in the medieval times (Lucas, 1992).

In Renaissance, artists used either pure gold and metal alloys (gold/silver) or double leaves made up by gold over a silver or tin layer (Pinna et al., 2009). Pure silver was used less often because it easily gets tarnished due to formation of silver sulfide (Ag$_2$S).

Gilding techniques are divided into three general categories: mordant gilding, water gilding, and gilding with mission. In all three techniques the wooden panel was treated in the same way: gesso (gypsum or calk mixed with
glue) application on the panel and when the mixture was still dry, they re-wetted the surface with a sizing usually made of animal glue and water.

In mordant gilding, a gypsum-organic layer was applied on the substrate, followed by a second layer with bole, finely grinded in a very dilute solution of animal glue. Bole was primarily polished with a dry cloth and afterwards washed with water. While bole was still wet, the painter polished the surface with an agate tool, adding progressively the gold leaves and removing the gold in excess (Matteini & Moles, 1989). Bole layer was either white or coloured. White bole was made with kaolin, whereas red bole - Armenian bole- (Gettens, 1966) was natural and consisted of ferruginous aluminium silicate. Mordant gilding offered a shinning warm reddish surface, due to the metallic reflection of gold leaves and the dark red colour emerged through the partially transparent leaves. This technique was used for large decorations.

Gilding with mission served for executing the small decorative details – punching- of the painting. The ground layer was an adhesive medium, consisting of linseed oil mixed with finely grinded pigments. Artists applied the gold leaves above this layer while it was not completely dry. Water gilding was used for very fine details - punching decoration- or for a bright finish of the painting frame. Similarly to previous techniques, the surface has to be smoothened, and fully wetted with a water size made of gelatin dissolved in hot water. When the surface was still wet, the painter added the gold leaves and removed the excess where it was needed.

### 2.2.4 Organic Binders

Old painters mixed the preparation layer and pigments with organic binders. In the first case, binders served as primers, and in painting layers as pigment adhesives, which formed the appropriate matrix where colour was stabilized.

The most used primers were animal glues, and they were extracted from animal tissues of skins or bones. As it was mentioned before, the role of priming was to shield the preparation layer form any liquids, and hence to preserve the vivid colours in painting layer.
Organic binders mixed in paint layer served for dispersing the grinded pigments and for making glossier optical effects. The binders had to meet the following conditions: i) to be compatible with the pigment without dissolving it, ii) to be transparent, iii) to dry fast and form a stable, elastic, but not sticky film and iv) to be stable during time (Colombini & Modugno, 2009).

Painters tried various natural organic materials to disperse their pigments. Proteins (egg, animal glue, casein or milk), polysaccharide gums (arabic, tragacanth and fruit tree gums) and drying oils (linseed, walnut and poppy seed oils) were the most frequently used materials, in Renaissance. The name of the painting technique depended on the type of the binder used:

- Proteinaceous Tempera (ie. egg tempera).
- Polysaccharide Tempera.
- Tempera grassa – where the binding medium is a mixture of drying oil and a proteinaceous material.
- Oil painting.
- Encaustic technique, where the pigment was dispersed in hot beeswax.

**Proteinaceous Tempera**

According to literature, most of paintings in early Italian Renaissance were executed with the proteinaceous tempera technique. Milk – an aqueous emulsion of proteins and lipids-, and particularly its protein, casein, was rarely used compared to egg or animal glue (Colombini & Modugno, 2004). Whole egg, or egg yolk are the most documented materials from the early medieval years until the 16th century, when the development of oil paintings started flourishing. Dried egg yolk contains a consistent fraction of lipids – 41% lipids and a low content of polyunsaturated fatty acids, 1.5% - and strongly kept pigment granules together, preventing the colour spread out. Additionally, egg yolk dries fast to a strong but also flexible, water-resistant, albuminlus matrix, which provides significant mechanical resistance. For these reasons, paintings prevented the extended damages and preserved their glossy and fresh view,
even after six or seven centuries. Particularly, as Thompson (1956) mentions in his book, *The Materials and Techniques of Medieval Painting*, paintings in egg tempera show less damage after five hundred years, than oil paintings after three hundred years.

As proteins are quick-drying media, the dispersed colours in then did not blend, and the artists had a sharp and translucent result. Nevertheless, in 15th century, humanism was the predominant inspiration source, and consequently a natural interpretation of the world was required. Therefore, the glossy proteinaceous *Tempera* –reflecting the divine and the supernatural idea- gradually replaced by siccative oils, which dry slowly and offer a blended result with softer edges and natural colour hue (Sultan, 1999).

### 2.2.5 Varnishes

Painters varnished their works with organic components for protecting them against dirt and mechanical damage. This coating creates a water-resistant shield and extends the painting lifespan. Apart from the protecting role, varnishes strongly altered the appearance of the painting, as they made the colours glossier, darker, more intense and more saturated (Colombini, 2000).

Colour glossing depends on the degree of the surface roughness, which influences the proportion of the specular light reflection (De la Rie, 1987). In rough areas the incident light does not penetrate the painting layer, and the diffused reflecting light is whiter, resulting in less vivid colour hues. Contrary, the smoother the surface the more penetrating the incident light is and consequently the glossier the colours are. Given that varnishing results in smoother surfaces, the colours are more saturated and stronger. They also show darker hues, as the refractive index of varnishes is rather high (De la Rie, 1987).

Varnishing dates back in 11th century, when the first recipe for oil varnish was described by Theofilus (De la Rie, 1987). Until 19th century a variety of natural and synthetic transparent materials was used.

Oil-based varnishes were mainly manufactured by boiling natural resins - sandarac, rosin or mastic – with drying oils -linseed oil and walnut oil. Cennino Cennini in his book, *Il Libro dell’ Arte*, mentioned that *corsivo*, -coating material
appeared in 14th century- was a sandarac solution in linseed oil (De la Rie, 1989). The same period, painters mixed either egg whites with honey and gums (aka arabic gam) or animal glue with proteinaceous, saccharide and resinous materials. In Italy, in 16th century, artists discovered the adhesive properties of mastic and Venice Turpentine when are dissolved in volatile solvents, such as turpentine oil. More recently, in 19th century, painters and conservators used solutions with dissolved dammar resin -Diptocarpaceae family- or gum mastic in turpentine oil or animal glue.
Chapter 3

The polyptich ``Annunciation and Saints``

3.1 Giovanni di Niccolò del Biondo

Giovanni di Niccolò del Biondo (1356-1399) was an Italian Master of the Gothic and Early Renaissance period, who lived and flourished in Florence. According to Offner, Casentino and Steinweg (1969) the oldest evidence related to Giovanni del Biondo dates back to 1356, when the painter acquired the citizenship of Florence. He specialised in religious paintings and frescoes such as the frescoes in Strozzi Palace (1353 and 1357), the panel painting *Martyrdom of St Sebastian and scenes from his life* (1370) in Museo dell’Opera del Duomo in Florence, the panel painting *Madonna and Child* (1377) in Gallery of Siena and the *Rinuncini Polyptych* (1379) in Santa Croce Church (Kemp, 2000).

3.2 The Polyptich: ``Annunciation and Saints``

The polyptich ``Annunciation and Saints`` (*Polittico con l’Annunciazione e santi*) was painted in 1385 for decorating the altar of the Cavalcanti Chapel in Santa Maria Novella, church in Florence. At the end of 17th century, it was transferred in Galleria dell’Accademia in Florence, where it remained until today.

It is a poplar panel painting with dimensions 406 cm x 377 cm, with gilding decorations and is composed of two main parts. The triptych panel with twisted wooden columns depicts the Annunciation, in Madonna’s house, surrounded by numerous Saints. Below the panel, predella presents the Flagellation, Crucifixion and Resurrection of Christ.

The good condition of most of the painting proves the refined technique used by Giovanni Del Biondo, who realized his artworks looking at the quality of the painting and its durability in time. According to technical reports in museum archives, in a period between 1971 and 1982 only the wooden frame had been restored by Opificio delle Pietre Dure. This intervention was lead by Baldini’s suggestion, who highlighted that the twisted columns had preserved those
surfaces from alteration, dust deposition and human interventions, thereby saving the former intensity and luminosity.

Figure 3.1. Giovanni Del Biondo, Annunciation and Saints, 1285, Galleria dell’Accademia, Florence
Chapter 4

Scientific methodology for the investigation of old paintings

For the analytical scientists, who study artefacts, the main challenge is the development of a reliable method in order to accurately answer the question posed. The most important premise is the level of intervention in the work of art. In most of the cases, the old paintings are of “unique” importance, hence sampling is limited to micro samples or sometimes is even prohibited. Therefore, usually, it is required to follow non-invasive strategies and occasionally very sensitive destructive methods.

4.1 Non-invasive analysis

Non-invasive methods provide both qualitative and quantitative identification of organic and inorganic compounds. The molecular identification is based on the interaction between the particles of the artefact and a wide range of radiation from NIR to UV and to X and gamma rays, while keeping the artefact totally intact.

For area examinations, five regions of the electromagnetic spectrum are of special interest:
- X-rays (0.01-10 nm) fluorescence
- Near or long-wave Ultraviolet radiation (320-400 nm) for UV-fluorescence and reflected UV study.
- Visible range (400-780 nm) for colour and documentation by photographic emulsion and digital photography.
- Infrared (780-3000nm) radiation and reflectograms for superficial study and imaging of under drawing
- Near-Infrared (720-900 nm) for depth examination of paintings

The non-invasive techniques are separated in two general categories:
- Imaging Spectroscopy, the data is modified images
- Punctual Spectroscopy, the data is spectra
Depending on the type of the analysis they provide, punctual techniques are further split in other two categories: Molecular and Elementary analysis. According to literature, the most reliable and popular non-invasive techniques applied in paintings are listed in Figure 4.1.

![Figure 4.1. Categorization of the most popular non-invasive techniques applied in the study of paintings.](image)

The last thirty years, scientist developed portable devices, which apply some of the abovementioned principals. These devices allow researchers to conduct in-situ analysis, when 1) it is rather difficult to transfer a painting, because of its size, or when 2) it is prohibited to remove a painting form the museum, or church etc., due to its unique historical value.

The most popular analytical methods –adjusted in portable device- for the in-situ analysis of paintings are listed in Table 4.1

<table>
<thead>
<tr>
<th>Technique</th>
<th>type of information</th>
<th>Sensitivity</th>
<th>Immunity to interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raman</td>
<td>molecular</td>
<td>good</td>
<td>good</td>
</tr>
<tr>
<td>FT-IR</td>
<td>molecular</td>
<td>fair</td>
<td>poor</td>
</tr>
<tr>
<td>PLM</td>
<td>molecular</td>
<td>fair</td>
<td>excellent</td>
</tr>
<tr>
<td>XRF</td>
<td>elemental</td>
<td>good</td>
<td>good</td>
</tr>
<tr>
<td>FORS</td>
<td>molecular</td>
<td>fair</td>
<td>poor</td>
</tr>
</tbody>
</table>

*Table 4.1. The most popular analytical techniques -adjusted in potable devices- for the in-situ analysis of paintings.*
If the analytical scope is the characterisation of all the painting components, its complicated and non-homogeneous nature should be strongly considered.

**Figure 4.2.** Multilayer painting fragment (Giovanni Del Biondo, *Annunciation and Saints*, Galleria dell’Accademia, Florence. (a) and (b) images captured with a Zeiss Axioplan Optical Microscope, (c) a general scheme of a canvas painting.

**source:** http://vxartnews.com/

In **Figure 4.2**, the (a) and (b) images and the scheme (c) illustrate the complexity of the painting fragments. Every layer ranges from 1-200μm and consists of various compounds, both organic and inorganic (Joseph et al., 2010).

Another parameter that should be considered is the penetration depth of each method. Techniques, such as FORS and UV Fluorescence Imaging, interact only with the top layer, while Infrared Imaging, Infrared Reflectography, XRF and X-rays Radiography penetrate the painting and meet some of the deeper layers. Another influential parameter is the principal of the method: XRF carries out elementary identification without elucidating the molecular structure, while FTIR spectroscopy detects the bond vibrations. All these facts highlight the wide range of analytical approaches and justify the need for combining techniques, which have different principles, sensitivity and penetration depths.

Nevertheless, to what extend we can study the painting technique with only non-invasive methods?

According to literature, numerous studies of paintings were based only in non-invasive strategies such as: Van der Snickt et al., 2008; Vagnini et al., 2009;
Calza et al., 2009; Pages-Camagna et al., 2010; Butti et al., 2013. The scope of these analyses was to identify the pigments, the metal leafing, to study the varnish or to visualize the under-drawing. However, their methodology did not meet the requirements for a precise elementary analysis of the bottom layers. Even in studies that carried out the deep penetrating IR Reflectrography and X-rays Radiography, the visualisation or the elementary analysis of the preparation layer was impossible.

Furthermore, non-invasive techniques show limitations when it comes to the detection of organic materials. Researchers who combined non-invasive and micro-destructive techniques came up with this conclusion: Regarding to inorganic materials, the reliability of non-invasive techniques is fairly the same reliable with that of destructive techniques. However, when it comes to the elucidation of the organic compounds, non-invasive methods are not sufficient (Milliani et al., 2007). As Cucci et al., (2012, p. 87) mentioned: “all the above-mentioned techniques (non-invasive) could be used in a complementary manner in order to reach the desired level of knowledge on the object under analysis”.

Consequently, in the case of paintings and artefacts, non-invasive analysis plays a crucial role, given that it is important to preserve the works of art the most intact we can. Nevertheless, the non-invasive techniques 1) show low sensitivity in organic analysis and 2) several times due to the plethora of co-existent compounds, the matrix effects lead to not well-resolved peaks. Thereby, micro-destructive methods are important when we need to reach all the layers separately and to detect every single component of the painting.

4.2 Integrated analysis: Non-invasive combined with destructive techniques.

For the analysis of a painting the destructive methodology can be separated in two paths: 1) the preparation of cross-sections and their analysis with non-destructive or micro-destructive techniques, and 2) the analysis of fragments with micro-destructive techniques.

Cross-section stratigraphic analysis exposes all the painting layers. When we study cross-sections under the microscope, we can visualise and measure the
thickness of the different layers. Under the UV light, we also can localise the fluorophore organic compounds, within the layers.

![Image](image.jpg)


For instance, in *Figure 4.3b*, in layer 2 the greenish fluorescence is weaker than in layer 0 (Prati et al., 2012). This means that the organic presence in layer 0 is stronger than in layer 2. For higher sensitivity and quantitative analysis, we can apply non-invasive techniques on cross sections, such as ESEM-EDX (inorganic elementary analysis) and FT-IR (molecular analysis); also, destructive ones such as immunoassays (organic analysis).

ATR-FTIR, GC/MS, pyrolysis GC/MS, HPLC and LC/MS are of the reliable micro-destructive techniques for the detection of organic compounds, in painting fragments (Colombini & Modugno, 2004; Colombini et al., 2010; Pitthard et al., 2006; Gimeno-Adelantado et al., 2000; Andreotti et al., 2006; Assimopoulou & Papageorgiou, 2005; Ribechini et al., 2009; Chiavari, et al., 1993). Immunoassays and ELISA methods have also been used (Leo et al., 2009; Palmieri et al., 2011), but still they need optimization.

A challenging condition met in organic analysis is the abundant presence of inorganic compounds, which strongly interfere and overlap the organic signals. Particularly, in FTIR, GC/MS and HPLC, the isolation of the layer of interest could be a solution for the problem of low sensitivity. Most of the times, for GC/MS analysis, it is not only required the mechanical separation of layers, but also the chemical separation of the different classes of the organic compounds (Lluveras et al., 2010). An example is the protocol developed by
Lluveras et al., (2010) for the simultaneous identification of lipids, proteins and sugars in painting fragments.

To conclude, integrated methodologies, which combine both destructive and non-destructive analysis, meet the requirements for an accurate and sensitive detection of every material in a painting. Usually, in literature, the general analytical schemes include a preliminary observation of the painting with Imaging Spectroscopy, -usually combined with non-invasive punctual techniques; and afterwards micro-destructive techniques, for strengthening the assumptions made so far. For instance, Menu et al., (2011) carried out VIS, UV imaging and XRF in-situ analysis. Afterwards, they studied cross-sections with XRD, ESEM-EDX and they concluded with FTIR and GC/MS analysis of the fragments. In this way, they achieved the elementary analysis of pigments and preparation as well as the detection of the organic binders and coating. Other literature research that combined XRD, XRF, FORS, cross-section and FTIR, in a similar way, are Burgio et al., (2000), Rosi et al,(2009) Zięba-Palus & Borusiewicz, (2006), Van der Snickt et al., (2008), Hochleitner et al., (2003), Appolonia et al., (2009).
Chapter 5

The tools for the study of the polyptich
``Annunciation and Saints`` by Giovanni del Biondo

An integrated method, combining both non-invasive and micro-destructive analyses was carried out. CNR-ICVBC and the department of Chemistry and Industrial Chemistry in Pisa (Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Italy) offered the instrumentation for the analysis.

In the beginning, we carried out UV, IR, IR False Colour Imaging, FORS and XRF, at Galleria dell’ Academia. Afterwards, eight micro-fragments were collected for 1) the cross sections study, with ESEM-EDX and fluorescence microscope and 2) for analysing the organic compounds with FTIR, GC/MS and py-GC/MS. The experimental methodology is depicted in Table 5.1.

Non-destructive analysis: Imaging Spectroscopy

- Ultraviolet (UV) fluorescence
- IR photography
- IR false colour

The electromagnetic radiation from Near Infrared (1100nm) to Ultraviolet (320 nm) provided a preliminary multispectral investigation of the cover layer and pigments. Furthermore, this investigation was also useful for taking the appropriate decision on the number and the location of sampling.

Given that UV radiation interacts only with the external layer, UV Imaging captured any possible fluorescence emission only by the top layer (cover). IR radiation, as penetrates the top layers and goes deeper, offered a black and white view of the under-drawing. Ultimately, false colour IR photography was used for a preliminary discrimination of pigments. Under the IRFC, metals and organic materials reflect the light dissimilarly and render colours that are different than the ones are shown under the Vis light. Consequently, with IRfc, pigments with different composition -though with the same colour under VIS- can be easily separated.
Non-invasive punctual analysis

- FORS: Fiber Optics Reflectance Spectroscopy (VIS)
- XRF: X-ray Florescence Spectroscopy

FORS offered a superficial analysis, while XRF penetrated the painting around 300 μm (Longoni et al., 1998). XRF spectra provided only inorganic elementary analysis of pigments and gilding. FORS carried out molecular analysis, and it offered data also for the organic pigments.

Invasive analysis

- Cross-Section/ESEM-EDX
• FTIR
• GC-MS, py-GC/MS

The painting fragments were re-sampled three times for 1) the preparation of cross sections, 2) the FTIR experiments and 3) the GC/MS and py-GC/MS analysis. Cross-sections were studied with an optical microscope under both UV and VIS light for 1) the detailed examination of the nature and the thickness of the compositional layers, 2) the study of the distribution of the particles of pigments and 3) the identification of the gilding technique. Furthermore, we studied the cross-sections with ESEM-EDX, for a qualitative and quantitative inorganic examination of all the layers, separately.

We also performed FTIR spectroscopy, in order to characterise the organic class of the materials and the nature of the preparation layer. Lastly, GC/MS was carried out for the elucidation of the organic compounds served as external coating and binders.

5.1 Non invasive analysis

5.1.1 Imaging Spectroscopy

Imaging Spectroscopy offers two-dimensional images captured in different ranges of wavelengths. This technique was initially applied in artefacts, by Rawlings in 30s -at the national Gallery of London (Bacci, 2000).

The imaging data results from the reflected/absorbed or emitted electromagnetic radiation, by the components of the painting. The wavelength of this radiation depends on the molecular composition of each material. High-resolution digital cameras using light wavelengths - ranging from Ultraviolet to Infrared and X-rays- offer a non-invasive imaging of either the surface or deeper layers (Bitossi et al., 2005). The various electromagnetic areas penetrate the surface at a different level; they also interact differently with every component.

These instrumentations offer a fast, non-invasive in situ analysis, as the apparatuses are transportable. The principal of their function is based on recording a set of images at different wavelengths in order to register the
intensity of the reflected light, as a function of location (pixel) and wavelength (Rebollo et al., 2013). The various light do not overheat the surface and therefore the method is totally safe. Various lends and zoom applications can be used, as well as the images can be acquired with high definition, regardless the size of the work.

5.1.1.1 UV Fluorescence Imaging

Ultraviolet (400nm-10nm) fluorescence photography requires the same equipment as the ordinary photography. The only difference is the radiation sources, which are 1) gas discharge lamps, such as mercury high and low pressure lamps, 2) fluorescent tubes, 3) metal halides lamps and 4) Xenon arcs (De la Rie, 1982). When UV irradiates a painting surface, one part of the energy is absorbed by the to top layer, while the other part is reflected. The absorbed radiation is re-emitted, as fluorescence wavelengths belong in Vis area. This method is able to identify only the fluorophore substances, and discriminate them based on their different chemical composition. In Figure 5.1, the second column summarizes the colour hues rendered by various popular old natural pigments under UV light.

*Figure 5.1.* The first column depicts the colours of fifteen pigments under the Vis light, and the second column shows their colours under the UV light.

The indispensable condition for pigment identification is the absence or the removal of any organic cover layer. The poor penetration ability of UV light leads to its strong absorption by the external layer as well as the quench of any fluorescence coming from the lower layers (Bitossi et al., 2005). If the cover layer is removed, then UV can offer data related with pigments. Furthermore, under UV, images give information about later additions or restored areas. This is because these areas render black colour under the UV light.

For example, in UV image Figure 5.2.b, the black spots represent the repainted blue areas, where the original lapis lazuli was replaced by another blue pigment (Pelagotti et al., 2008).

If paintings are not varnished, UV photography meets pigments and pictorial binders (Comelli et al., 2008; Mansfield et al., 2002). Thanks to fluorescence, we can identify the three main classes of the organic pictorial binders: 1) drying oils render strong yellow fluorescence, 2) animal glues show a whitish fluorescence, 3) while egg tempera a weak bluish hue (Creagh & Bradley, 2000).

When the painting is varnished, UV can be used for a preliminary examination of the cover layer, and its conservation state (Thoury et al., 2007). Depending on the chemical composition of the cover layer, the captured fluorescence render different hues. Usually, strong yellowish fluorescence corresponds to old natural varnishes, indicating that the painting had not been
recently restored (De la Rie, 1982).

The main limitation of this technique is its high sensitivity. This allows even traces of active impurities to highly fluorescence, and consequently to quench the compounds of favour. For instance, the pigment verdigris quenches the fluorescence of mastic and dammar resins (De la Rie, 1982). The same holds for certain ochres, umbers and sienna earths. Therefore, the detection of compounds on the base of a characteristic fluorescence hue, sometimes can be very unreliable.

5.1.1.1.1 Experimental Part: Set Up and data acquisition

Imaging (VIS)

The camera was positioned perpendicularly to the surface of the painting, while the two light sources were located in this way so as to reach the least light reflection caused by the glossy cover of the painting.

A camera Canon EOS 400D -with a RGB colour filter and a sensor 22,2 x14,8mm- was used for the acquisition of the images after removing the internal low-pass filter. The effective resolution was 10.10 megapixels and the images were recorded in RAW with the sensitivity of ISO 100. The white balance and the exposure control were carried out with the reflection standard Spectralon 99% and a Macbeth chart. The lens of the camera was EF 28mm f/2.8, which turned to 50 mm due to the conversion factor.

For the imaging, after removing the internal low-pass filter, we used a digital filter (B + W UV / IR Blocking 486) for excluding the ultraviolet and infrared parasite radiation.

The images were taken with aperture f / 9.0 which is the best compromise for achieving a good field depth and the least influence of chromatic aberrations and geometric distortion.

Imaging (UV)

The same camera (Canon EOS 400D) was located perpendicularly to the painting surface. The only difference between the two methods is the filter of the camera. For the UV imagining there is a need of blocking the reflected UV radiation and therefore we used a gelatin filter series (Kodak Wratten No. 2), which absorbs the radiation below 390 nm. The gelatine filter was coupled with
a glass filter (B + W Digital UV / IR), which blocks the parasite radiation from 486 nm to IR, which is generated by the mercury powder existing in the lamp bulbs.

5.1.1.2    IR Imaging

5.1.1.2.1    Near IR photography

IR radiation ranges from 0.78 μm to 500 μm and is divided into three general areas: Near IR, Mid IR and Far IR. The various materials interact differently with each wavelength, and this property is used for analytical purposes.

When Near Infrared (0.78μm-2.5μm) applied on paintings, visualizes the layers under the surface. Haze and pigments, which appear opaque under the Vis light, reflect or transmit Near IR. As a result, they are invisible and leave the under-drawing layer exposed. The transparency of top layers depends on the chemical composition of pigments, the sensitivity of the detector, and the materials used for the under-drawing. This part of the painting, which is untouched by later hands, offers precious information about the original drawing and facilitates the conservation.

Van Asperen de Boer (1968) introduced the use of NIR by applying the Kubelka-Munk theory (Walmsley et al., 1994). Since then, IR photography has revealed not only the primal drawing, but also gave answers 1) on how it relates to the other painting layers, 2) what later changes the painter did on the drawing, 3) what areas of the painting were not underdrawn, 4) which parts of the painting were restored, and 5) which materials were used for the restoration.
For example, Figure 5.3 shows images taken in the frame of the study of Giovanni del Biondo’s painting. In NIR picture (0.7-1 μm), it is evident that the painter drew the book before the left hand of the saint.

The reason why several pigments reflect/transmit Near IR is unknown and it seems that transparency under this IR portion is an inherent physical property that cannot be predicted (Bendiganavale, & Malshe, 2008). However, over the last decades, it was empirically proved that most of the old paintings are NIR transparent, and particularly, their transparency increases from 1 to 2.5 μm. Particularly, the transparency increases in white and reddish layers, and in several brown and grey pigments.

Hue differences under NIR have been noticed also in pigments that render the same colour in Vis, such as blue lapis lazuli and azurite. In 1930,
Marie Farnsworth was the first to discover that in a black-and-white IR photography, azurite appears black, whereas ultramarine is far lighter (Hoeniger, 1991). In 2005, Delaney et al., (2005) studied the NIR reflection of various colours using several different wavelengths. They photographed and compared the various hues of lapis lazuli and azurite reference samples and they proved that it is possible to discriminate them with NIR imaging. Furthermore, after examining the NIR images in different wavelengths, they concluded on the best wavelength for separating these two pigments. Figure 5.4 lists the various hues under the NIR light; around 90 μm, their discrimination is more efficient.

As Near infrared radiation is not visible to human eyes, it can be traced by CCD or CMOS sensors, which are applied on regular cameras (Creagh, & Bradley, 2000). The painting surface transmits or reflects the IR radiation and is recorded on a film sensitized up to a wavelength of 900 nm. This technique requires filters, which exclude the Vis radiation, and prohibits any superimposition of the Vis and IR radiation. The long-pass filter - gelatine filters, such as Wratten 87 and 87 C, or glass filters, such as Schott RG 780 (Mairinger, 2004) - is positioned in front of the camera lenses, providing the characteristic black-and-white profile of the picture. Most of the time, calibration procedures are being applied for correcting the non-uniform images, so as to make them clearer, better focused, without dark shadows and chromatic aberrations. As the resolution of infrared video cameras is rather low, only small areas can be recorded. With the use of a computer software, each single frame collected is
digitised and corrected of distortions and is then assembled into an entire picture.

### 5.1.1.2.1.1 Experimental Part: Set Up and data acquisition

For the imaging, we used tungsten filament lamps of 300W, which allow a strong light emission in IR. Also, the lamps emit heat and for this reason cannot be kept in front of the painting surface for long time. The lenses were covered with a gelatin filter (Kodak Wratten 87C), which excludes the radiation around 850nm at approximately 50%.

### 5.1.1.2.2 IR False Colour Imaging

IR false colour photography identifies and discriminates pigments with Vis and NIR radiation. Similar to the black and white NIR films, the characteristic absorption and reflection of pigments are displayed on the colour infrared film, and they differentiate on a basis of colour contrasts. The significant advantage of this technique is that pigments that render the same colour in Vis are distinguishable with IR false colour, even without having identical chemical composition. For this reason, in old paintings, IR false colour photography has been used frequently for the rapid separation of the very expensive lapis lazuli from azurite. In infrared false colour images, lapis lazuli renders a light cherry colour – as it strongly reflects IR –, while azurite appears dark blue.

The IR colour film is sensitive up to 900 nm and its emulsion consists of three layers that are sensitive to the green, red and IR wavelengths respectively. After excluding the blue and yellow light, which affects all three layers, the overlapping of Vis and NIR is achieved through imaging processing. In the final picture, the painting surface renders only three colours, which are not the real ones. For this reason, the technique is called infrared false colour photography. Pigments with the same colour in Vis, and different chemical composition, reflect NIR differently, and consequently, render distinguishable false colours.

False colours rendered by the most popular pigments in Renaissance are listed below in *Table 5.1* (Doumas, 2008).
To conclude, IR false colour photography is an easy operating technique, which provides a fast and preliminary analysis of the old pigments. Given that this technique is able to distinguish pigments of the same colour, it is also able to visualise retouching and any later alterations on the painting.

### 5.1.1.2.2.1 Experimental Part: Set Up and data acquisition

**IR False-Colour Imaging**

The false-colour digital images were obtained by superimposing the B (Blue) and G (Green) colour image (RGB) to the infrared (IR). The overlap is acheived later, through a program of image processing. The interpretation of the data was based on the pigments electronic database *Pigments through the Ages* (Douma, curator 2008).

<table>
<thead>
<tr>
<th>Pigment</th>
<th>VIS</th>
<th>IR-FAULSE</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnabar (vermilion)</td>
<td>Red</td>
<td>Yellow</td>
<td>dark blue</td>
</tr>
<tr>
<td>Red lead (Minium)</td>
<td>red</td>
<td>Orange, brown yellow</td>
<td></td>
</tr>
<tr>
<td>Red Lake</td>
<td>Red</td>
<td>orange</td>
<td>no</td>
</tr>
<tr>
<td>Red Ochre (Fe)</td>
<td>Red</td>
<td>Yellow brown</td>
<td>Dark red</td>
</tr>
<tr>
<td>Lapis lazuli</td>
<td>Blue</td>
<td>red</td>
<td>dark blue</td>
</tr>
<tr>
<td>Azurite</td>
<td>Blue</td>
<td>Deep blue</td>
<td>no</td>
</tr>
<tr>
<td>Malachite</td>
<td>Green</td>
<td>blue</td>
<td>no</td>
</tr>
<tr>
<td>Yellow Lead-tin</td>
<td>Yellow</td>
<td>Pale white</td>
<td>no</td>
</tr>
<tr>
<td>Yellow ochre</td>
<td>Yellow</td>
<td>Blue</td>
<td>----</td>
</tr>
<tr>
<td>White lead</td>
<td>White</td>
<td>White</td>
<td>Pale white</td>
</tr>
<tr>
<td>Carbon black</td>
<td>Black</td>
<td>Black</td>
<td>----</td>
</tr>
</tbody>
</table>

*Table 5.1.* The behaviour of various pigments under IR and UV light.
5.1.2 Punctual Analysis

5.1.2.2 X-rays Fluorescence (XRF)

The portable X-rays fluorescence device offers a fast, in situ and true multi-element analysis – around eighty elements. It measures the total element concentration and the analysis is independent on the compound. The lowest detection limits range from 1 to 10 ppm and the precision of the method depends strongly on the homogeneity of the sample. The small layer beam that is used for shooting the X-rays on the sample’s surface allows a punctual analysis, on a penetration level depending on X-rays energy.

X-rays radiation wavelengths range from 0.01 to 10 nm and the corresponding energies from 100 eV to 100 keV. One of the phenomena caused by X-radiation is the photoelectric effect and the consequent secondary X-rays fluorescence (XRF) emission by the analysed atoms.

In photoelectric phenomenon, when an X-ray photon of sufficient energy strikes an atom, it dislocates an electron from one of the inner shells such as the K shell. Afterwards, the atom fills the gap in two ways: either with an electron from the next lower-energy shell (L) or with an electron from a shell two quantized energy levels lower (M). In this way the atom becomes an ion and in the first case the result of the transition is the emission of Kα X-rays, while in the second case it is the emission of Kβ X-ray.

In this phenomenon, the emission of secondary X-rays does not depend on the compounds but only on atoms. Due to the fact that the incident X-rays interact only with the inner shells of the atoms and not with the bonding orbitals, the emitted fluorescence X-rays are characteristic for each atom. The intensity of each fluorescence line varies according to the quantity of the element, while it is not noticeably affected by the chemical state of the analysed atoms. Consequently, while, with XRF we can conduct an indirect compound detection, we are not able to obtain any information related with its chemical structure. The most powerful portable XRF systems detect elements of medium–high atomic number (z>15) at concentrations up to a few ppm (Moioli & Seccaroni, 2000). The limitation in elemental detection is related to the atomic
number \((z)\) of the elements and to the type of X-rays tubes used as primary radiation sources.

The low \(z\) elements –Ar, Ca, Cl– give strongly only the K peaks, while L peaks cannot be clearly seen due to their low energy. The high \(z\) elements (Hg, Pb) give only L peaks, while their K peaks have so high energy that they do not appear within the spectral range; the middle \(z\) elements might show both K and L peaks. The XRF technique does not detect organic compounds, as their fluorescence photons have too low energy to be transmitted through air and cannot be detected with silicone detectors.

The X-ray sources operate at cathode-to-anode with voltages ranging from 10 to 100 kV and currents ranging from 1 µA to 10 mA. Depending on the p-XRF device and the analytical scope, we can use X-rays tubes either of low power or of high power. The low-weight, low-power tubes work at rather low voltages and are unable to excite the K-lines of elements such as Ag and Sn; while the high-power tubes are heavier due to the shielding and the cooling system, but provide a wider detection range.

Although, XRF peaks do not correlate with compounds, they depend on the chemical environment of the analysing atom. Because of the first condition, any pre-treatment of the sample would not result in any sensitivity increase. This enhances the efficiency of p-XRF as an in situ and non-invasive analytical technique. However, the homogeneity of the elementary concentration significantly affects the intensities. The reason rests on that the peak intensity of each element is the ratio of its concentration to the overall composition of the analysing area. Therefore, the matrix effects –including any interactions among the elements- leads to intensities reductions. Mantler and Schreiner (2000) in following table summarized the matrix effects appearing because of the coexistence of various pigments dispersed in a binding media in the pictorial layer of a painting.
In this table, the \( a_M \) factor is the arithmetical matrix interference, calculated by Mantler and Schreiner (2000). The ideal situation is when \( a_M \) is zero and the intensity counted by the instrument corresponds to the real one. Any reduction occurred by matrix effects obstructs the identification of the true elementary concentration in the sample and leads to false quantitative results.

### Portable XRF for pigments identification

Over the last forty years, portable XRF devices have been used for the pigments identification in old and contemporary paintings. Frahm and Doonan (2012) carried out a literature research on how p-XRF is used in art and archaeological studies. They concluded that most of the times, portable XRF devices were used in the laboratory, and only one fifth for in situ analysis in museums or excavation sites. On the other hand, in an older research, Craig et al., (2007) compared the sensitivity of a portable-XRF in situ analysis and conventional XRF instrument in the laboratory in the same obsidian samples. The authors concluded that even if the portable device measured fewer elements, it provided a high-resolution data at a low analytical cost and short time frame.

The last decades, numerous research studies have demonstrated that the most straightforward and frequent uses of the XRF method are pigments and metal leafing identification on a wide range of materials, such as paintings,
glasses and ceramics (Frahm, & Doonan, 2012; Craig et al, 2007; Ferrero et al., 1999; Pappalardo et al., 2004; Klockenkämper et al., 2000; Szökefalvi-Nagy et al., 2004; Bronk et al., 2001; Desnica & Schreiner, 2006).

Only for painting pigments analysis, we meet XRF either interpreting the primal role or being a part of a long integrated study (Sawczak et al., 2009; Aucouturier & Darque-Ceretti, 2007; Ricci et al., 2004; Rosi et., 2009). The aforementioned studies proved the reliability of XRF in establishing the provenance of inorganic materials of the pictorial layer and in characterizing any unoriginal part existing due to restoration.

The X-rays absorption rate depends on the surface’s nature, volume, density and the atomic numbers of its compositional elements. Furthermore, it depends on the power of the X-rays source, which defines how penetrating is the incident radiation on the surface. The thickness of the painting influences the sensitivity of the method and should be strongly considered while deciding the energy of the beam generator. In paintings, the penetration depth is limited to the first two or three layers, while the first layer –varnish- is invisible. Usually, the pictorial layer ranges from a few micrometres up to 1 mm or more. All these parameters make the spectra interpretation complicated, as the interferences from the ground layer are consequent. Therefore, the correct interpretation of spectra requires considerable experience and knowledge of the painting technique, as most of the time overlapping or increased noise will obstruct the interpretation.

Another limitation is that XRF cannot detect directly organic pigments. Given that, most of the old organic pigments consist of at least one heavy metal, we can detect them, indirectly. XRF provides sensitive detection of heavy elements, as they strongly absorb the radiation. Therefore, for example, it is possible to discriminate cinnabar and red lead, based on the detection of Hg or Pb, respectively.

All in all, p-XRF combines the advantages of a non-destructive and a sensitive multi-elemental in situ analysis. It offers a fast qualitative and quantitative identification of medium and heavy metals, which allows us to map indirectly the pigments of a painting. Despite the matrix effects, which increase
the background noise, -mainly due to inorganic components- it is enough sensitive for carrying out an entire and reliable elementary analysis.

5.1.2.2.2 Experimental Part: Set-Up and data acquisition and interpretation

A portable XRF spectrometer (ALPHA series 4000, Innov -X) with micro X-ray tube and a tantalum anode was used for the in situ analysis. The portable instrument allows the detection of elements with atomic number higher than silicon (Z > 14). The analysis was carried out in two steps, with two different excitation energies: 30KeV - 6.5 uA - 2mm aluminium filter for the determination of heavy elements and 15KeV - 7uA - 0.1 mm aluminium filter for the determination of light elements. The beam diameter was 4 mm, the shooting area was approximately 155 mm² and the acquisition time was 120 s. Generally, the Ka emission has been considered for the qualitative evaluation of the elements; only for mercury and lead we used La emissions.

The spectrometer is equipped with a Si- PIN detector (FWHM < 230 eV at 5.95 keV Ka line of Mn) cooled by a Peltier cell.

During the analysis, the apparatus was placed on the painting surface, keeping the distance of the tube and the detector constant. The detector was located at a fixed angle within the head of the instrument, and was protected by a Kapton window. The constancy of the geometric conditions ensures the
reproducibility of the analysis and therefore the comparability of the data. The spectra were digitised with the use of software installed in a small touch-screen computer, located on the top of the portable device. The interpretation of the spectra was carried out with the same software used for the acquisition.

The following table —used as a literature database for the data interpretation— lists the chemical composition and the XRF signals corresponding to the most popular pigments used in Renaissance, in Italy.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Element</th>
<th>Pigment</th>
<th>Chemical Composition</th>
<th>XRF band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Hg</td>
<td>Cinnabar</td>
<td>HgS</td>
<td>Hg Lα 9.95; Lβ 11.87; Ly 13, 83</td>
</tr>
<tr>
<td>Red</td>
<td>Organic</td>
<td>Lake</td>
<td>C₃H₆O₁₃</td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>Fe</td>
<td>Red Ochre</td>
<td>Fe₂O₃</td>
<td>Fe Kα 6.40; Kβ 7, 96</td>
</tr>
<tr>
<td>Red</td>
<td>Pb</td>
<td>Red lead</td>
<td>Pb₃O₄</td>
<td>Pb Lα 10, 50; Lβ 12, 62; Ly 14, 76</td>
</tr>
<tr>
<td>Blue</td>
<td>Li₄SiO₄</td>
<td>Lapis Lazuli</td>
<td>(Na, Ca)₄(Al₂O₃)₄(SiO₄)₂</td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td>Cu</td>
<td>Azarite</td>
<td>Cu₅(CO₃)₃(OH)₂</td>
<td>Cu Kα 8.04; Kβ 8.90</td>
</tr>
<tr>
<td>Yellow</td>
<td>Pb-Sn</td>
<td>Yellow Lead-Tin</td>
<td>Pb-Sn</td>
<td>Pb Lα 10, 50; Lβ 12, 62; Ly 14, 76</td>
</tr>
<tr>
<td>Yellow</td>
<td>Cu</td>
<td>Yellow Ochre</td>
<td>Fe₂O₃H₂O</td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>Cu</td>
<td>Malachite</td>
<td>Cu₃CO₃(OH)₂</td>
<td>Cu Kα 8.04; Kβ 8.90</td>
</tr>
<tr>
<td>Green</td>
<td>Cu</td>
<td>Verdigris</td>
<td>Cu₃(CH₃COO)₂·Cu(OH)₃·5H₂O</td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>Fe</td>
<td>Green Earth</td>
<td>(K₂N₂)[Fe₃(AL₉Mg₆(Si₂Al)₁₂O₃₁₀(OH)₁₀·10H₂O) and MnO₃</td>
<td>Fe Kα 6.40; Kβ 7.06</td>
</tr>
<tr>
<td>Brown</td>
<td>Fe</td>
<td>Earth</td>
<td>Fe₂O₃(Al₂O₃)·SiO₂·H₂O and MnO₃</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>Pb</td>
<td>Lead white</td>
<td>2PbCO₃Pb(OH)₃</td>
<td>Pb Lα 10,50; Lβ 12, 62; Ly 14, 76</td>
</tr>
<tr>
<td>Black</td>
<td>Organic</td>
<td>Carbon Black</td>
<td>Carbon, coil</td>
<td></td>
</tr>
</tbody>
</table>

*Table 5.3.* XRF bands of the most popular pigments in Renaissance

### 5.1.2.3 Fiber Optics Reflectance Spectroscopy (FORS)

Reflectance spectroscopy with optical fibers (FORS) is a non-invasive spectrophotometric technique, which provides a superficial and a punctual - both inorganic and organic- investigation of the artefact. The analysis is based on measuring the ratio between the intensity of the radiation reflected from the analysing surface —reported as percentage of scattered light- and the intensity from a white reference surface.
A hemispherical probe (measuring geometry $45^\circ/0^\circ/45^\circ$) is used for 1) sending the light -generated by a tungsten lamp- and for 2) collecting the reflected light. The irradiating bundle is sent through optical fibers perpendicular to the investigated area, while the collected backscattered radiation is sent to the sensors through two separate fibers located at $45^\circ$ in respect to the studied surface. The obtained reflectance spectrum, in Vis, is a graph, where the intensity of the backscattered radiation ($y$-axis) is reported as function of the wavelength of the applied radiation ($x$-axis). Afterwards, the spectra are re-processed using the Kubelka-Munk theory for counting the scattering and absorption coefficients of the references (Depuis & Menu, 2006). We can conduct both quantitative and qualitative pigment analysis, considering a linear relationship between the aforementioned optical properties and the pigment volume concentration.

In painting study, one of the most important roles of FORS is the discrimination of lapis lazuli and azurite. Lapis lazuli can be identified by XRF only indirectly –through the absence of heavy metals related to the other blue pigments. On the contrary, FORS by interacting with the painting surface with both Vis and NIR offers a sensitive analysis of this pigment.

Ultimately, the FORS is an easily transportable technique offers rapid and reliable analysis. Therefore, it enables the acquisition of a large number of spectra over the entire surface of the painting. In this way, not only we can focus on the painting's details, but also, after gathering all the spectra together, we can achieve a detailed overall perspective of the palette.

5.1.2.3.1 Experimental Part: Set Up, data acquisition and interpretation

A tungsten lamp and the detector Ocean Optics (mod. HR2000) coupled with optical fibers were used to obtain the spectra. The irradiation proceeded at $45^\circ$ and the signal collection at $0^\circ$. The result was the acquisition of a spectrum from an area of about 2mm of diameter for each single spot. Each spectrum is the average of 30 scans. The reference was a plate of barium sulphate with 99% of reflectance. The identification of pigments was performed with the visual
comparison of the obtained spectra with both CNR-ICVBC and IFAC databases (source: http://fors.ifac.cnr.it/info.php).

![Image](image_url)

**Figure 5.7.** Giovanni dell'Blondo, *Annunciation and Saints*, Galleria dell' Academia, portable FORS Ocean Optics (mod. HR2000)

## 5.2 Sampling

### 5.2.1 Non-invasive sampling

The decision whether the areas are to be irradiated or sampled depends both on the analytical capability of each method and on the nature of our research questions. The scope of the XRF and FORS analyses was the identification of the palette and the determination of the gilding technique. Considering the characteristics of each method, we came up with the following sampling map:
The Table 5.4 lists the analysed points along with their location and a brief description.

<table>
<thead>
<tr>
<th>XRF/FORS Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left panel, shoulder of San Lawrence (saint on the left side of the painting, second row), red dot covered by the column. Height in respect to the node: 3 cm from the middle of the node and is fitted with central point to 1 cm</td>
</tr>
<tr>
<td>2</td>
<td>Left panel, point on San Lawrence’s grid behind the halo of the front saint</td>
</tr>
<tr>
<td>3</td>
<td>Left panel, point on the central area of the yellow robe of the first saint on the left side, first row.</td>
</tr>
<tr>
<td>4</td>
<td>Left panel, first saint on the left side, first row. Blue area within the crinkles below the pink crinkle.</td>
</tr>
</tbody>
</table>

**Table 5.4.** XRF-FORS studied areas and their brief description.
<table>
<thead>
<tr>
<th>XRF/FORS Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Left panel, first saint on the left, first row. Blue crinkle, slightly below the decoration with red triangles.</td>
</tr>
<tr>
<td>6</td>
<td>Left panel, light yellow point within San Peter’s robe, above the second red crinkle on the left side.</td>
</tr>
<tr>
<td>7</td>
<td>Left panel, dark yellow point on the dark fold of the cloth of San Peter, below the hand holding the book</td>
</tr>
<tr>
<td>8</td>
<td>Left panel, point uncovered, on the left pink sleeve of Saint John the Baptist’s cloth.</td>
</tr>
<tr>
<td>9</td>
<td>Left panel, black point on Saint Paul’s sword, close to Saint Peter’s halo.</td>
</tr>
<tr>
<td>10</td>
<td>Left panel, point in the middle of Saint Peter’s keys.</td>
</tr>
<tr>
<td>11</td>
<td>Central panel, light yellow point, on the folds of Michael Archangel’s cloth.</td>
</tr>
<tr>
<td>12</td>
<td>Central panel, bright orange point point, close to Michael Archangel’s foot.</td>
</tr>
<tr>
<td>13</td>
<td>Central panel, point on a marble cluster of the green floor, close to Michael Archangel’s foot.</td>
</tr>
<tr>
<td>14</td>
<td>Central panel, point on a marble vein of the green floor, on the top, close to Madonna.</td>
</tr>
<tr>
<td>15</td>
<td>Central panel, golden leaf of the bottom left part of the window.</td>
</tr>
<tr>
<td>16</td>
<td>Central panel, gilded halo of Michael Archangel.</td>
</tr>
<tr>
<td>17</td>
<td>Central panel, red blanket.</td>
</tr>
<tr>
<td>18</td>
<td>Central panel, the Madonna’s blue, below Her left hand.</td>
</tr>
<tr>
<td>19</td>
<td>Right panel, yellow robe of the Saint in the first row, close to Madonna.</td>
</tr>
<tr>
<td>20</td>
<td>Right panel, green robe of the second Saint of the first row.</td>
</tr>
<tr>
<td>21</td>
<td>Right panel, light yellow cloth of the second Saint, first row.</td>
</tr>
<tr>
<td>22</td>
<td>Right panel, St Mary Magdalene, light yellow point on the bottom right of the cloth (uncovered area).</td>
</tr>
</tbody>
</table>

**Table 5.5** XRF FORS studied areas and their brief description
### 5.2.2 Sampling for micro-destructive analysis

In the case of artworks, sampling is rather challenging due to the irreplaceability of the artefact, which has to be altered the least possible.

The aim of sampling is summarized below:

- The stratigraphic analysis (cross-section) in order to observe – under the fluorescence microscope and ESEM- all the painting layers in detail and to define their chemical composition.

- The identification of the chemical composition of the ground layer and the class of the organic compounds serving as binding media and cover layer, with the use of FTIR spectrometer and GC/MS analysis.

We detached deep samples – containing all the layers from the preparation to the painted/cover layer- to prepare cross-sections. As mentioned on the third chapter, the panel of the painting -not recently restored- is covered by an unknown brownish layer. After the removal of the wooden lateral joints, an area free of this unknown layer was revealed. Both the covered and the uncovered area were sampled. Furthermore, for the more sensitive identification of the cover layer, we scratched superficially the painting and collected a brownish dust.

For the study of gilding, we sampled an isolated gilded layer and a deep fragment – carrying gilding on the top. The fragments were sampled from the central panel (S7), from the left side of the right panel (S5) and from the right side of the right panel (S1, S2, S3, S4, S6). Each fragment contains a different

<table>
<thead>
<tr>
<th>XRF/FORS Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Right panel, St Mary Magdalene, dark orange abraded area on the bottom right of the cloth.</td>
</tr>
<tr>
<td>24</td>
<td>Right panel, St Mary Magdalene, dark orange point, abraded area on the bottom right of the cloth.</td>
</tr>
<tr>
<td>25</td>
<td>Predella, central panel, repainted area of Madonna’s blue robe</td>
</tr>
<tr>
<td>26</td>
<td>Predella, central panel, blue point on Madonna’s shoulder, close to the central gilded star.</td>
</tr>
</tbody>
</table>

Table 5.6 XRF FORS studied areas and their brief description
colour. The location of each sample is depicted in Figures 5.9 and 5.10, and the description of the sampled areas is summarized on Table 5.7.

Figure 5.9. Panel Painting, sampling areas

Figure 5.10. Panel painting, sampling areas

Images of the eight painting fragments and a brief description of the each sampling area are listed in Table 5.7a and 5.7b.
All samples -except for gdb_8- contained all the layers, and hence both inorganic and organic compounds. The co-existence of different types of compounds eliminates the sensitivity of FTIR and GC/MS techniques and results in strongly interfered spectra. Therefore, for a reliable analysis, we re-sampled the fragments, and isolated the layers of interest. The re-sampling was carried out under the microscope with the use of tiny and delicate metallic tools. The inevitable human error prohibited the 100% discrimination of layers. For this in most of the FTIR spectra, the ground layer overlapped the rest layers. Contrary, in GC/MS spectra, the peaks were resolved well, because we performed a chemical sample pre-treatment, aiming to separate the compounds according to
5.3 Micro-destructive Analysis

5.3.1 Mid-IR Spectroscopy: Fourier Transformation Infrared Attenuated Total Reflection (ATR-FTIR)

Infrared spectroscopy interacts with the molecular vibrational motions. There are two types of vibrations that cause IR absorptions: stretching – displacement of the atoms along the axis of the bond-, and bending – change of the bond angles, which are linked to the same atom. From these vibrations, the ones that can be observed are these, which change the electric dipole moment of the molecule. After the IR radiation, different groups of atoms absorb different amounts of energy resulting in transition of stretching and bending vibrations. The energy emitted from these transitions gives a signal, which is transformed into a spectrum. The peaks of the spectrum offer information about the kind of atoms are bonded or grouped in an unknown compound.

Table 5.7b. Painting fragments and the description of sampling areas.
The ATR, or Attenuated Total Reflectance technique, is especially useful for the strongly absorbing samples, which cannot be measured by infrared transmission. The ATR works well for this type of samples, since the intensity of the reflected light decays exponentially with distance, and hence makes the method insensitive to sample thickness. This property is out of most importance when it comes to painting framgets, which are multi-layered. According to the principal of the technique, an IR beam shoots the sample, which afterwards reflects a light. This light is reflected between the surface of the sample and the body of the crystal. In our instrumentation the ATR crystal used was diamond, which shows increased durability, chemical inertness, high refractive index and leads to only one light reflection.

The polished side of the diamond is positioned on the top, where we stabilize the sample. Afterwards, we set the current, which generates the IR beam. The infrared beam inserts in the diamond at an angle of 45°-which is an angle greater than the critical one- and it is totally reflected at the sample-diamond interface. The light, which reaches the sample surface is called evanescent wave and its penetration depth depends on the wavelength of the light and the properties of the sample and the diamond. After the reflection, the light reaches the detector and then the signal is digitized.

An important limitation of this method when it is applied in painting fragments is related with the strong overlapping of the organic materials as well as the inorganic pigments, due to the strong presence of the preparation layer. Furthermore, when we use Mid-IR, we lose information corresponding to inorganic oxides of various popular pigments, such as red lead (Pb₃O₄), lead tin yellow (Pb₂SnO₄) and cinnabar (HgS). Particularly, oxides, hydroxides and sulphides of heavy elements give peaks below 400 cm⁻¹ (Far-IR), while Mid-IR ranges from 400 to 4000 cm⁻¹. Another important pigment of Renaissance is lapis lazuli, which also cannot be detected. This is because the strong peak of the ion SO₄²⁻ -gypsum- overlaps the silicate bonds.

Another limitation is the very low sensitivity of this method in elucidating the molecular structure. Given that, this method is capable only to detect characteristic molecular groups and bonds, we can only identify the class of the
organic or inorganic compound without reaching to tell, with certenity, which is the compound in the sample.

Because of these limitations, it is important to isolate the painting layers before the scanning so as to reach the least interference. Furthermore, we should carefully choose the IR range depending on what is our analytical scope, so as to reach the highest sensitivity. Lastly, in the case of organic characterisation, even if we eliminate sufficiently the overlapping effect, we should combine FTIR analysis by other more sensitive techniques such as GC/MS or HPLC.

### 5.1.1.2 Experimental Part: Set Up, sample pre-treatment, data acquisition and interpretation

ATR-FTIR analysis was performed with a commercial spectrometer *Spectrum System 2000 Perkin Elmer* coupled with a single bounce diamond ATR cell, Specac Golden Gate *GS 10500*. Spectra were obtained, over the range of 4000–3700cm⁻¹ at a spectral resolution of 2cm⁻¹. 32 scans have been recorded and the resulting interferogram averaged. Spectra were co-added to improve the signal to noise ratio. A background single-beam spectrum of the clean ATR crystal has been first collected and the whole data set was baseline-corrected so the spectra had not been processed separately. The baseline adjustment, smoothing and normalisation were performed with the use of the *Spectrum 5.3, Perkin Elmer Inc* software package.

In order to increase the peak resolution, we separated the pictorial and the preparation layer and we scanned them separately. Nevertheless, we could not avoid the strong overlapping caused by preparation. This disabled the accurate direct detection of pigments and organic compounds. Therefore, FTIR was limited to identify only the class of the binding media and cover layer, and to give only supplementary information for the pigments.

FTIR spectra were compared with spectra of reference materials, obtained from the same instrument. For further accuracy of the data interpretation, we also used the available reference spectra libraries on books of Derrick et al. (1999) and of Nyquist and Kagel (1972).
5.3.1 Cross-Section (Stratigraphic Analysis)

The most common practice for studying the painting stratigraphy is the preparation of cross-sections embedding of the fragments in resins. Cross-sections, observed under Vis and UV light and studied with ESEM-EDX analysis, offered precious information regarding to the nature, the chemical structure and the thickness of each layer, the state of ageing, the level of interaction and mixing of the different layers, the pigments particles and their distribution within the binding medium. Hence, cross-section are out of most importance for defining the painting technique, the level of degradation and ultimately, to categorise any possible old restoration that was occurred.

5.3.1.1 Experimental Part: Preparation of cross-sections and Fluorescence Microscope Set up

5.3.1.1.1 Preparation of cross-sections

The method of flat embedding in a silicon rubber mould was used for the preparation of cross-sections. The samples were positioned into the wells, which had been half-filled with Epofix resin and half-filled with mount medium. Wee placed the samples in this way so as to expose its entire side on the edge of the block.

After the cooling down of the resin blocks, we polished carefully all their sides, in water conditions, for making them planar and parallel. This offered highly reflecting surfaces, which could be clearly observed under the microscope. Given that, our samples did not contain water sensible components, water was used for making the polishing faster. We used three different polishing surfaces; at first a coarse abrasive paper in order to reach carefully the sample, secondly an abrasive paper with finer grades for reducing the scratched areas and lastly –in dry conditions- a smooth cloth to create a fine and shining surface free of spots and finger marks.

5.3.1.1.2 Fluorescece Microscope Set Up

The Zeiss Axioplan Optical Microscope, equipped with objectives from 4x to 50x, was used for cross section observation, under visible and UV light. A
Nikon filter cube B2A (λ_{exc}=330-350 nm; λ_{em}>420 nm) was used for the observation under the UV light.

### 5.3.2 Environmental Scanning Electron Microscope coupled with Energy Dispersive Detector (ESEM-EDX)

The ESEM-EDX technique is a versatile tool that provides a sensitive and selective visualization and identification of the inorganic materials in stratigraphies. This method is widely used in paintings, because it can analyse all the painting layers separately, without requiring any further sample pre-treatment.

An electron beam scans the stratigraphy and interacts with its atomic structure resulting in two types of electron scattering: elastic and inelastic. In the first case, electrons, after colliding with atoms, change direction -without changing energy-, and produce the backscattered electrons (BSE). Contrary, inelastic scattered electrons lose energy after the interaction with the sample, and produce secondary electrons (SE’s), which follow various directions.

The backscattered electrons depend strongly on the atomic number: the heavier the elements, the larger the number of the ejected backscattered electrons. For this reason, as the signal comes brighter with heavier elements and darker with the lighter ones, Backscattered Electron Imaging offer a high compositional contrast within cross-section layers.

This last property offers an efficient discrimination between pigments and the binder. This is because most of the old pigments contain heavy metals, while binders are organic compounds. Furthermore, BSE visualizes the stratigraphy, the morphological structure of layers as well as any interaction occurring among them. The nature of the surface of cross-sections affects BSE images; the more planar is the surface the higher is the image contrast and therefore the sharper is the visualization of particles, at higher magnification. When ESEM is coupled with EDX, provides an inorganic semi-quantitative analysis. The obtained spectra show the elementary distribution within the layers.
5.3.2.1 **Experimental Part: Set Up**

The elemental composition of the different paint layers was performed with an acceleration voltage of 25 keV, pressure 0.5 Torr, lifetime >50s, CPS ≈ 2,000, equipped with a ZAF correction procedure for bulk specimens was used for semi-quantitative analyses of the X-ray peaks.

5.3.3 **GC/MS and py-GC/MS**

5.3.3.1 **Organic materials of old paintings**

In Renaissance, the natural organic materials used as binders and varnishes - egg, casein, collagen, derivatives from animal skins or animal bones, siccative oils, blood, honey, plant gums, honey, plant resins, beeswax - were complex mixtures, mainly consisting of proteins, triglycerides, sterols, aliphatic esters, aliphatic alcohols, polysaccharides, fatty acids and terpenoids (Table 5.7).

<table>
<thead>
<tr>
<th>Organic compounds in paintings</th>
<th>Materials</th>
<th>Painting technique-the use of the organic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Whole egg, egg yolk, egg white, casein</td>
<td>Binder of the pictorial layer: Egg tempera, Casein tempera</td>
</tr>
<tr>
<td>Protein</td>
<td>Rabbit, fish glue</td>
<td>Binder of the preparation layer, gilding’s glue</td>
</tr>
<tr>
<td>Drying Oil</td>
<td>Linseed, poppy and walnut oil</td>
<td>Binder of the pictorial layer: Oil painting</td>
</tr>
<tr>
<td>Mixture of protein + oil</td>
<td>egg, casein, animal glue with drying oils</td>
<td>Binder of the pictorial layer: Tempera Grassa</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>Plant gums, honey, starch</td>
<td>varnish, minor compounds of binders</td>
</tr>
<tr>
<td>Terpenoids (Resins)</td>
<td>Dammar, mastic, copal, colophony, shellac</td>
<td>varnish, minor compounds of binders</td>
</tr>
</tbody>
</table>

*Table 5.7. Organic materials used in paintings by old Masters.*

**Proteinaceous materials**

All the different types of collagen are fibrous proteins containing thin fibres of amino acids covalently bonded in a specific sequence forming three poly-peptidic α-chains. These α-chains are arranged in a conformation of a triple helix with a strong hydrogen bond between the hydroxyl group of the hydroxyproline and hydrogens of glycine’s amino group. The most abundant
amino acid in collagen is glycine as it accounts for approximately 26% of the total amino acidic content.

In old paintings they used both the two parts of egg – the white part, glair, and the yolk. Both parts contain asparagine, glutamine, and leucine. Particularly, the chemical composition of glair reads as 50% ovalbumin – glycoprotein- , 15% conalbumin –glycoprotein- and 3% lysozyme and the remaining part of albumen contains salts, lecithin, and carbohydrates (Colombini & Modugno, 2009, p. 238). The proteins in egg yolk are phosvitin (phosphoprotein), a- and b-lipovitellins (lipo-proteins) and count for 4%. Fatty acids count for 41% and lecithin for 9%. The safest way to differentiate the two parts of egg is the detection of cholesterol, which is absent in egg white, while it counts for 9% in egg yolk.

The principal protein content of milk is casein (26%), which is a phosphoprotein complex. B-lactoglobulin and a-lactalbumin are presentl, in significant abundance and lipids count for 26% and sugars for 39% (Colombini & Modugno, 2009, p. 238).

Oils

Siccative oils had been used as dispersing media of pigments, or as diluters of saccharide and resin materials. In siccative oils, the concentration of the polyunsaturated FAs is rather high and their drying properties are linked with the number of conjugated double bonds in acyclic chains (Colombini & Modugno, 2009). Particularly, only if the percentage of double bonds in FAs is over 65%, the oil can be considered siccative oil. The drying oils that were mainly used in paintings are linseed oil, poppy seed and walnut oil.

Natural Resins

Natural resins served either as pigment binders, or as varnishes. Among their compounds, the most characteristic ones are terpenics. The most important diterpenoid resins come from the Pinaceae family -such as Pine resins and Venice turpentine- and the Cupressaceae family -such as Sandarac, Juniper resins. And the most popular triterpenoid resins were mastic – Anacardiaceae family- and dammar -Dipterocarpaceae family.
Their complicated composition can be found in detail in the page 14 of the book *Organic mass spectrometry in art and archaeology*, written by Colombini and Modugno, first published in 2009.

**Polysaccharides**

Polysaccharides –such as honey, plant gums and starch- are poly-monosaccharide chains held with glycoside bonds. The most used plant gums in paintings were the Arabic gum, the tragacanth gum, the fruit tree gum and the guar gum, ext. The plant gums have differences in their molecular masses. We can discriminate each species based on the detection of their characteristic monosaccharide. Anna Lluveras et al. (2012), categorized the most popular polysaccharides and plant gums, through a systematic scheme, which was used as a database for our data interpretation.

**5.3.3.2 GC/MS and py-GC/MS analysis and challenges**

The challenges and limitation of the organic analysis are not related only to the complicated nature of painting fragments, but also to the very small quantity of the sample. For an efficient analysis, the micro/nano quantities require very low detection limits. Furthermore, the various co-existent compounds interact each other, resulting in their chemical alteration and the production of new materials, sometimes unknown. Lastly, the analysis is also strongly interfered by degradation compounds –due to oxidation, polymerisation- formed as a result of ageing, restoration treatment, and pollution.

**5.3.3.3 Experimental Part: GC/MS and py-GC/MS**

In order to overcome the above-mentioned limitations, we applied a highly sensitive analytical method with very low detection limits –trace analysis- and high peak resolution. We carried out GC/MS after the pre-treatment of every sample, based on the protocol published by Lluveras, Andriotti et al., (2010). Because of this pre-treatment, it was possible the simultaneous analysis of natural waxes, lipids, aminoacids, terpenoids and polysaccharide materials in the same microsample, without the interference of the existing inorganic media.
even in high concentrations. We reached higher sensitivity with a complementary pyrolysis-GC/MS analysis, which is a fast and single analysis. Contrary to GC/MS, it does not need any sample pre-treatment and therefore, it is time saving and reduces the risk of contamination.

5.3.3.3.1 **Principal of py-GC/MS**

The principal of py-GC/MS is based on the thermal degradation of large molecules into small moieties (pyrolysates), which, afterwards, are GC separated and detected in the mass analyser. Particularly, the sample is introduced into a quartz capillary and then is vaporized in a pyrolyser with very high temperature controlled by a calibrated resistor based on the Joule effect. Lastly, in very high temperature, the resulting pyrolysates reach the GC injector. The main drawback of this method is the high retention of the less volatile compounds - the polar ones-, such as proteins, hydrocarbons, in pre-column or in GC column. In order to face these problems, hexamethyldisilazane was used, for the production of derivatives, which are more volatile and hence easier to be detected.

5.3.3.3.2 **Sampling and Sample pre-treatment**

For increasing the resolution and the sensitivity of the method, we separated manually the three general layers -preparation, painted layer and external cover. A brief description of all fragments used and the analytical scope are summarized in Table 5.8 and 5.9.
We followed a pre-treatment procedure that was published by Anna Lluveras et al. in 2012 (Figure 5.11). The protocol included extractions, C4 cleanup tests (C4 stationary phase placed on pipet tip) hydrolysis and derivatizations for the separation of each sample in three general fractions: amino acid, lipid/resinous and saccharide fraction. Each fraction was injected separately, in GC/MS after subjected to hydrolysis and derivatization procedures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>Analysis</th>
<th>Analytical scope</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Red sample: preparation layer and pictorial layer</td>
<td>GC/MS</td>
<td>Binder identification of the preparation and pictorial layer</td>
</tr>
<tr>
<td>S4</td>
<td>Blue sample: pictorial layer without the external cover</td>
<td>GC/MS</td>
<td>Binder identification of the pictorial layer</td>
</tr>
<tr>
<td>S5</td>
<td>Pink sample: pictorial layer + external cover layer</td>
<td>GC/MS</td>
<td>Binder identification of the pictorial layer</td>
</tr>
<tr>
<td>S6</td>
<td>External cover layer above threed pictorial layer of S6</td>
<td>GC/MS and py-GC/MS</td>
<td>External cover layer identification</td>
</tr>
</tbody>
</table>

*Table 5.8.* Painting fragments analysed with GC-MS and py-GC-MS.
In a more detail, the most important steps of the protocol are:

-Ammonia extraction
At the beginning, we extracted the sample with ammonia in order to solubilize the proteins and the saccharides, and separate them from the insoluble inorganic salts. Together with the proteinaceous and polysaccharide compounds, also the free organic acids were extracted. The resulting residue, containing insoluble organic and inorganic species is kept for saponification.

- **Amino acid fraction**

  We extracted the polysaccharide/proteinaceous fraction with diethyl ester (III) and we obtained an acidic solution, which contained proteins, peptides, saccharides, soluble salts, and free organic acids. Afterwards, we purified the acidic solution with an Omix C4 tip (IV). With this purification we also, isolated the proteinaceous fraction, which was hydrolysed and derivatised. The internal derivatisation standard was norleucine. After these steps we injected 2μmL of the purified proteinaceous fraction in GC/MS.

- **Saccharide fraction**

  The purified polysaccharide fraction obtained from step IV, was hydrolysed in microwaves. This turned polysaccharides into saccharides and simplified the chromatogram. The internal standard of derivatisation was mannitol. Afterwards we cleaned the solution with strong cation-exchange resins, in order to remove the insoluble materials (VI). The eluted aqueous fraction, containing purified aldoses and uronic acids, was mercaptalised and then siliated in order to obtain one peak for each saccharide and each uronic acid. Two μmL of the silated saccharide solution were injected in GC/MS.

- **Lipid-resinous fraction**

  The extract – from step III, contained drying oils, waxes and resins. We saponificated/salificated the extract, in order to remove the OH moieties (XII). After the saponification, we extracted the solution with n-hexane/diethyl ether, for the isolation of fatty acids. We derivatised the solution, in order to obtain single peaks in the chromatogram. The internal standard of the derivatisation was tridecanoic acid dissolved in iso-octane. In the end, we injected 2μmL of the derivatised solution in GC/MS. The obtained chromatogram gave single peaks.
corresponding to acidic and neutral terpenoid compounds, sterols, alcohols, alkanes, monocarboxylic acids, dicarboxylic acids, and hydroxy acids.

5.3.3.3.3 **GC/MS and py-GC/MS Apparatuses**

**GC/MS**

A 6990N GC system gas chromatographer (Agilent Technologies), equipped with a PTV injector was coupled with a 5975 mass pole mass spectrometer. The mass spectrometer was operated in EI positive mode (70 eV). The chromatograms obtained were both TIC (total ion chromatograph) and (single ion mode) SIM. The GC-MS data acquisition conditions are reported in literature (Lluveras et al., 2010).

**Py-GC/MS**

Analyses were performed with a multi-shot pyrolyzer EGY/PY-3030D of FRONTIER LAB. The temperature of pyrolysis was 550 °C and the interface temperature 280 °C, split 1:5. The reagent used was a hexamethylazane solution diluted 1:4 in acetone.

5.3.3.3.4 **GC/MS Analysis**

Given the complicated and multiple steps of the samples pre-treatment, the risk taken in every step we should calculate the risk taken in every step. For minimizing the individual errors and their cumulative effects we carried out a series of experiments such as corrections, calibration curves; run of blancs, standards and internal standards. These experiments are described in detail in chapter 9.5 *Analysis and Quantitation* in the book: *Organic mass spectrometry in art and archaeology* (Colombini and Modugno, 2009). The concentrations of blanks, standards and internal standards are mentioned in literature (Lluveras et al., 2010).

The calibration curve of each amino acid, sugar and lipid compound was performed based on standards. In SIM chromatograms, we corrected the area of each fraction and we calculated their correct relative concentration percentage with the use of the slope and intercept of the calibration curves. Particularly, we
corrected the areas with the use of both internal standard 1 – injection internal standard– and internal standard 2 –derivatisation internal standard-. We also performed this calculation regarding to the recovery of the day. In order to control the daily state of the instrument and compare it with calibration curves, we injected every day one or more standards.

### 5.3.3.3.5 Detection and Quantification

**Amino acids**

For the identification of the proteinaceous materials, at first, we compared the peaks of solutions of 11 amino acids with the peaks of the 11 amino acid derivatives of a multi-standard solution. Afterwards, we calculated the integrated areas for each amino acid peak regarding to alanine. In this way, we built a database with the relative percentage content of quantified amino acids in casein, egg and animal glue (Table 5.10).

<table>
<thead>
<tr>
<th></th>
<th>Ala</th>
<th>Gly</th>
<th>Val</th>
<th>Leu</th>
<th>Ile</th>
<th>Ser</th>
<th>Pro</th>
<th>Phe</th>
<th>Asp</th>
<th>Glu</th>
<th>Hyp</th>
<th>DL µg</th>
<th>QL µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>casein</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>11</td>
<td>6.6</td>
<td>5.8</td>
<td>11.5</td>
<td>5.9</td>
<td>8.5</td>
<td>22.2</td>
<td>0</td>
<td>0.19</td>
</tr>
<tr>
<td>egg</td>
<td>7.7</td>
<td>4.8</td>
<td>7.7</td>
<td>11</td>
<td>6.7</td>
<td>10.3</td>
<td>5.7</td>
<td>6.4</td>
<td>12.6</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>animal glue</td>
<td>12.3</td>
<td>29.4</td>
<td>3.9</td>
<td>4.7</td>
<td>2.5</td>
<td>3.8</td>
<td>12.4</td>
<td>2.8</td>
<td>6.6</td>
<td>9.9</td>
<td>7.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Table 5.10.* The relative percentage content of quantified amino acids in casein, egg and animal glue.

According to Table 5.10, animal glue can easily differentiate from the other two proteinaceous materials based on the glycine and hydroxiprolime detection. In contrast with egg yolk, egg white consists of high concentration of leucine, proline and phenylaline, but does not have cholesterol. For this reason, lipid analysis is crucial for the differentiation of egg white and egg yolk. Casein, due to the strong peak of glutaminic acid and proline, can easily differentiate from egg; while leucine presence is similar in both materials.

The 11 amino acids and their mass to charge ratios are depicted in Table 5.11.
Table 5.11. 11 amino acids and their m/z in SIM chromatograms.

One reliable way to identify the proteinaceous materials in unknown samples is to perform the principal component analysis (PCA). While this software offers n-dimensional information on amino acid components, allows us to focus on three orthogonal variables - the principal components - without significant loss of variance. Particularly, a reference data set of 101 samples containing egg, casein and animal glue - the three principal components - served as a database. The unknown samples were distributed graphically among these three components, depending on their chemical composition. In this way, we could easily differentiate and group all our samples. The strict condition when performing PCA is that the sum of the quantified amino acids of every sample has to be over the LOQ, which is 0.3.

Fatty acids

The evaluation of the characteristic ratios of dicarboxylic fatty acids allows us to distinguish between the different triglycerides sources (drying oils or egg) and also to determine the kind of drying oil (poppyseed, walnut, linseed). The evaluation parameters are:

- A/P (azelaic over palmitic acid ratio),
- P/S (palmitic over stearic acid ratio),
- ΣD (sum of dicarboxylic acids).

The values of cholesterol and dicarboxylic fatty acidic ratios corresponding to different reference samples are plotted in Table 5 (Colombini & Modugno, 2009). Based on this reference database, we evaluated our results and we concluded with certainty on the lipid materials in Giovanni del Biondo's painting.
The A/P parameter contributes to distinguish the drying oils and egg lipids. This is because the amount of dicarboxylic acids resulting from the ageing of egg lipids is far smaller than from the ageing of drying oils. Hence, quantitatively, the categorization is as follows:

- \( \text{A/P} > 1 \rightarrow \text{drying oil} \)
- \( \text{A/P} < 0.3 \rightarrow \text{egg lipids (egg tempera)} \)
- \( 1 < \text{A/P} < 0.3 \rightarrow \text{tempera grassa (mixture of egg and drying oil)} \)

The P/S is a reliable parameter for discriminating the drying oils, as these monocarboxylic acids are less affected by ageing or treatment.

- \( \text{P/S}: 1.4-2.4 \rightarrow \text{linseed oil} \)
- \( \text{P/S}: 2-4.5 \rightarrow \text{walnut oil} \)
- \( \text{P/S}: 2.5-3.5 \rightarrow \text{egg} \)

The ageing state of the lipid component can be assessed by the oleic to stearic acid (O/S). The double bonds of the oleic acid falls easily into oxidation, and in old paintings the ratio ranges from 0.1 to 0.2.

The sum of dicarboxylic fatty acids (ΣD%):

- \( \Sigma D\% > 20 \rightarrow \text{drying oil is the only binding medium} \)
- \( \Sigma D\% < 10 \rightarrow \text{no drying oils} \)

In SIM chromatograms, the m/z values of the fatty acids of interest are shown in the Table 6.

**Table 5.12.** Presence of cholesterol and ratios of monocarboxylic and dicarboxylic fatty acids in different reference painting samples (Colombini & Modugno, 2009).
Cholesterol was detected in the total ion chromatogram (TIC) at m/z: 129,458. As it was mentioned before, cholesterol detection allows us to choose between egg yolk and egg white.

**Polysaccharides**

After the derivatisation of the polysaccharide fraction, we obtained simple chromatograms where only one peak is shown for every uronic acid and every monosaccharide. The quantification of each monosaccharide in every sample requires the same calculations and corrections as for the amino acid calculation. The standard solutions of monosaccharides and uronic acids that were used are mentioned in detail in literature (Lluveras et al., 2010).

A dataset published in the book of Colombini and Modugno, *Organic mass spectrometry in art and archaeology*, (2009), in page 21, gives the saccharide composition of the most popular saccharide materials in old paintings (*Table 5.14*).

We compared our experimental data with this reference table and we worked with the decisional schemes for the gum identification, published by
Lluveras et al., (2012). After this data elaboration we successfully interpretated the sugar chromatograms of our painting fragments.
Chapter 6

Results and Discussion

The opportunity to study this painting using a range of non-invasive and micro-destructive methods allowed an evaluation of the potential and limitations of the selected methods, with regards to the problems posed by the complex mixtures in Renaissance paintings. The results obtained from non-invasive analyses are discussed in comparison with results from a micro-sampling approach including ESEM-EDS for the identification of inorganic pigments, GC/MS and FTIR for the identification of binders.

6.1. Preparation layer

The white ground consists of a fine (top) and coarse-grained (bottom) gypsum layer, as BSE images show in Figure 6.1. In these images, layers are clearly distinguishable, due to the different intensity of the electron back scattering radiated by the various elements and particles. The only cross-section with only one gypsum layer, –fine gypsum -, is sample S2. For this, the most probable reason is the small sampling depth.
The X-ray microanalysis (EDX) of all samples, showed the predominant presence of Ca, S and Sr (Figure 6.2), which correspond to calcium sulphate and celestine (SrSO₄). In the coarse layer of S3 and S6, we observed aggregated crystals composed by Fe, Ba, Zn, Fe and Si. During gypsum preparation, the abovementioned elements usually replace Ca, because they have the same molar mass and charge with it. For this reason, they are frequently detected gypsum as gypsum impurities.
The following FTIR spectra (Figure 6.3, 6.4 and 6.5) correspond to samples S1, S4 and S6. The area of diamond noise ranges from 1800 to 2400 cm\(^{-1}\). In all spectra we can clearly see the characteristic gypsum absorption peaks. Particularly, a double broad peak from 1100 to 1250 cm\(^{-1}\) and two narrow peaks at 600 and 700 cm\(^{-1}\) reflect the SO\(_4^{2-}\) asymmetric stretching and bending vibration, respectively. A rather broad double peak from 3400 to 3450 cm\(^{-1}\) and a narrow double at 1620 and 1690 cm\(^{-1}\), correspond to the deformation vibration and stretching vibration of O-H water bond.

In Figure 6.6, spectra of samples 1, 4 and 6 are gathered together and compared with gypsum reference spectrum. The peak shifting and the alteration of peak shape are due to the ATR-FTIR apparatus and the interference of other compounds. For instance, an ATR accessory effect that can be seen in all spectra is shifting of the asymmetric stretching vibration of SO\(_4^{2-}\) at around 1090 cm\(^{-1}\).
When SO$_4^{2-}$ signal is not overlapped, it shows a double broad peak. In our experimental spectra, SO$_4^{2-}$ has a single broad shape, which proves the presence of interfering compounds. Since the studied samples contained only the preparation, we assumed that the interfering compound is the organic binder. Also, the alteration of the double peak at 3405 and 3545 cm$^{-1}$ can be attributed to the organic binder (Figure 6.3).

**Figure 6.3.** Black: S$_1$ preparation layer. Blue: Gypsum reference spectra. ATR-FTIR. Spectrum System 2000 Perkin Elmer with Specac Golden Gate GS 10500.

**Figure 6.4.** Black: S$_4$ preparation layer. Blue: Gypsum reference spectra. ATR, Spectrum System 2000 Perkin Elmer with Specac Golden Gate GS 10500.
6.1.1 Organic binder

As it was explained in paragraph 2.3 Organic Binders, during Renaissance, in Italy, painters used proteinaceous animal glues for priming the preparation layer. FTIR and GC/MS analysis were used to confirm the animal glue presence in “Annunciation and Saints”.

In FITR spectra, organic peaks are overlapped, because in preparation the concentration of the binder is significantly lower compared with that of gypsum. Nevertheless, we can trace the organic presence following the split/altered gypsum peaks. Thereby, as FTIR is not sensitive in organic compounds -when it comes to painting fragments-, we chose this technique for the identification of exclusively the class of the organic binder.
Figures 6.7 and 6.8 depict FTIR spectra of samples 1 and 5. Both of them show a double narrow peak in organic C-H bond area and a double broad peak from 3200 to 3500 cm\(^{-1}\), corresponding to O-H bond. In Figure 6.9, we can see the O-H deformation and stretching vibration peaks in reference spectra of gypsum and animal glue. By comparing Figure 6.9 with Figures 6.7 and 6.8, we noticed that O-H deformation vibration peak of sample 1 and 5 is more similar to that of animal glue reference spectra. Furthermore, in Figure 6.7, the triple peak pattern from 1447 to 1640 cm\(^{-1}\) is similar to the amide peaks I and II, and the two narrow gypsum peaks at 1617 and 1650 cm\(^{-1}\) (Figure 6.9c) are split (Figures 6.8, 6.5, 6.3). Also, our fragments gave peaks below 1010 cm\(^{-1}\) (i.e. Figure 6.5). In this area, the detected peaks 956, 871, 806, 711 and 667 cm\(^{-1}\) correspond to C-H aromatic bending vibrations or bending vibrations of alkenes C-H. While in the same area, the peaks at 670 and 608 cm\(^{-1}\), correspond to gypsum (Figures 6.3, 6.4 and 6.7). All these facts prove that gypsum was mixed with an organic compound.

The binder is not an acid, ester, ketone or an aldehyde, as we have not detected the carbonyl narrow peak ranging from 1670 to 1820 cm\(^{-1}\). In Figure 6.10, S1 spectrum is put together with the animal glue reference spectrum and we noticed the following similar peak patterns: 1) broad peak from 2300 to 2400 cm\(^{-1}\), 2) double peak at 2852 and 2925 cm\(^{-1}\) and 3) triple pattern from 1447 to 1640 cm\(^{-1}\). All the above-mentioned FTIR data led us to conclude that the organic binder is a proteinaceous material and more probably animal glue.
Figure 6.8. S5 preparation layer. ATR-FTIR, Spectrum System 2000 Perkin Elmer with Specac Golden Gate GS 10500.

Figure 6.9. Gypsum and animal glue reference peaks. (a) gypsum reference spectrum: O-H deformation vibration, (b) animal glue reference spectrum: O-H deformation vibration, (c) gypsum reference spectrum: O-H stretching vibration. ATR-FTIR, Spectrum System 2000 Perkin Elmer with Specac Golden Gate GS 10500.

Figure 6.10. S1 preparation layer and animal glue reference spectra. ATR-FTIR, Spectrum System 2000 Perkin Elmer with Specac Golden Gate GS 10500
After the ATR-FTIR experiments, we carried out GC/MS in order to confirm our hypothesis that the binder is animal glue.

The red sample S1 was taken from the uncovered area of Mary Magdalene’s robe -uncovered area- and it was analysed without isolating the preparation layer.

**Figure 6.11.** Entire sample S1 containing a large quantity of preparation layer and a thin red painted layer. (a) Sampling from an existing hole-red robe of St Mary Magdalene, (b) picture taken with the fluorescence microscope, magnitude 4x.
We elaborated the GC/MS data based on the procedure described in paragraph 5.3.4.3.5 Detection and Quantification. The amino acid contents of S1 and references samples are depicted in Figure 6.12c. The captured hydroxiproline and glycine show high concentration in S1. Given that these compounds are animal glue markers -according to reference data-, we can safely state that animal glue is the binder of the ground layer. This is also confirmed, graphically, in the PCA score plot -Figure 6.12b, where S1 is distributed in animal glue group.

Hydroxiproline was also detected, at a lower rate, in samples 5, 6, 7, which contained only the pictorial and cover layer. The presence of hydroxiproline was either due to the remaining preparation layer, or due to the co-existent cover layer. The amino acid and fatty acid analysis of these samples are discussed in paragraphs 6.3.2. Organic Binder and 6.6 Coating.
6.2 Under-drawing

The under-drawing was visualized with IR imaging. The following figures render the under-drawing of predella (Figure 1b) and of the bottom part of the panel (Figure 6.13).

The following figures demonstrate under-drawing details, which reveal the way in which Giovanni del Biondo drew the painting. For instance, in Figure 6.14, we can see that the book was drawn before the left hand.

In Figure 6.15a and 6.15b, the grey line indicates the initial drawing, which is larger than the final one. Similarly, in Figure 6.15c, the pale grey line above the fingers shows where was initially drawn the finger.
In Figure 6.16, the Saint’s thumb is rather blurred, indicating the multiple corrections occurred around this area.

![Image](image1.png)

**Figure 6.15.** IR images. (a) and (b) Board Panel_right part. (c) Predella_left part

The line of under-drawing is black, thick and not fragmented. This means, that, more likely, the painter used a homogeneous mixture with charcoal dispersed in an unknown liquid.

### 6.3 Pictorial Layer

#### 6.3.1 Palette

IR false colour imaging, XRF, FORS, FTIR, and ESEM-EDX were used for the identification of pigments.

IR false colour imaging offered a preliminary identification of pigments and visualized the retouched areas. FORS and XRF portable devices were applied in twenty-seven points, both in panel (25 points) and in predela (2 points). After
the non-destructive analyses, we sampled the painting from the far right and central part of the panel. We separated the samples in three fragments. The one fragment was studied with FTIR; the second was used for cross-sections and ESEM-EDX analysis, and the last one for GC/MS analysis.

**Blue and Green colour**

The most popular blue pigments in Renaissance are azurite and lapis lazuli. In "Annunciation and the Saints" the major blue pigment is lapis lazuli, (Na, Ca)₈(Al₂SiO₄)₆(S, SO₄, Cl)₁₋₂, mixed with lead tin yellow (Pb₂SnO₄), white lead or black pigment. The lapis lazuli presence was observed in IR false colour imaging and confirmed by FORS spectroscopy and EDX analysis. Particularly in Figures 6.17b and 6.18b, the bright red colour corresponds to lapis lazuli according to IRfc database (Douma, 2008). The only area of the panel not painted with lapis lazuli is on the right part of the pavement (Figure 6.17b). In this IRfc image, the light green-blue area proves the use of a different blue or green pigment mixed with traces of lapis lazuli -bright red lines pointed by arrows.

**Figure 6.17.** Left and central part of the panel. (a) VIS, (b) IR false colour. The red spots indicate the areas XRF and FORS analysis. The red arrows point the lapis lazuli stripes.
The XRF data of points 13, 14, 18, 19 and 21 (Figures 6.17 and 6.19) are summarised in Table 6.1. The predominant presence of Pb correlates with lead white and lead tin yellow. Due to the penetration depth of X-rays, the strong signal of Sr and Ca is related with celestine (SrSO₄) and gypsum (CaSO₄.2H₂O) respectively and, Fe signal is gypsum impurity.

Figure 6.18. Right part of the panel, (a) VIS, (b) IR false colour. The red spots indicate the areas of XRF and FORS analysis.
The Pb and Sn detection –point 14 and 21- suggests lead tin yellow, while in the same area, the bright red colour is due to lapis lazuli (Figure 6.18).

None of the green or blue points of Table 6.1 gave copper (Cu) signal, except for point 13, which –as it shown in Figure 6.17b- lies on the blue-green area of the pavement. The copper identification could recommend azurite \( \text{Cu}_3(\text{CO}_3)_2(\text{OH})_2 \), vedigris \( \text{Cu}(\text{OH})_2\cdot(\text{CH}_3\text{COO})_2 \cdot 5\ \text{H}_2\text{O} \) or malachite \( 2\text{CuCO}_3\cdot\text{Cu}\ (\text{OH})_2 \). In the light greenish-blue point 13, the strong presence of lead (Pb) is not combined with tin (Sn), and therefore there is no lead tin yellow \( \text{Pb}_2\text{SnO}_4 \) mixed with a blue pigment. This means that the pigment in point 13 has to be pure green and consequently, we rejected the hypothesis about azurite. This also agrees with the fact that the FORS spectrum of point 13 is not similar to azurite reference spectra (Figure 6.19 ). Based on these facts, the most probable assumption is that a green copper based pigment (vedigris or malachite) was mixed with lead white \( 2\text{PbCO}_3\cdot\text{Pb}(\text{OH})_2 \).

Regarding to the rest two green points, the S-shaped feature in their FORS spectra are lower and less curved compared with that of lapis lazuli reference spectrum. This is because of the organic external cover and of the co-existing lead tin yellow. Also, in Figure 6.19b, the curve of point 14 is very low,
and this can be explained by the possible presence of an organic black pigment – which is undetectable by FORS or XRF analysis.

![Figure 6.19](image.png)

Figure 6.19. Spectra of the green points 13, 14, 21. (a, b, c), (d) reference spectra of lapis lazuli (green curve) and azurite (blue curve). FORS-Ocean Optics (mod. HR2000, VIS-NIR).

The initial assumption about lapis lazuli is confirmed by FORS spectra of points 18 and 19 - Figures 6.20a and 6.20b-, which are identical to lapis lazuli reference spectrum: 1) broad maximum peak at around 470 nm, 2) minimum broad peak at around 600 nm and 3) an upward curve, which begins at around 740 nm. Since XRF spectra shows strong lead presence in point 18 and 19, we concluded that in these areas lapis lazuli was mixed with lead white (Figure 6.20).
The green colour of points 4 and 5 is a mixture of lapis lazuli and lead tin yellow: FORS spectra of the abovementioned points are identical to lapis lazuli reference spectra (Figure 6.21) and XRF analysis of these points gave strong signal in Pb and Sn (Table 6.2).
The image of cross-section of sample 2 visualises the mixture of lapis lazuli with lead tin yellow (Figure 6.22c).

EDX detected of Al, Si and Na, which are components of lapis lazuli (Figure 6.22). However, these elements are hardly detectable by FTIR due to gypsum overlapping. Particularly, the characteristic silicate peaks of lapis lazuli in FTIR are: Si-H (800-959 cm\(^{-1}\)), Si-O (1000 cm\(^{-1}\)) and Si–O–Si (1084 cm\(^{-1}\)). The strong broad peak of gypsum at 1084 cm\(^{-1}\) overlaps these peaks and the result is a broad split triple peak in FTIR spectrum of S2 (Figure 6.23).
The yellow pigment of S2 (Figures 6.22a and 22c) is lead tin yellow, as EDX analysis gave strong signals to Pb and Sn (Figure 6.22d). With FTIR it was impossible to detect this pigment, because lead and tin oxides give peaks below 600 cm\(^{-1}\).

In Figure 6.24c, the cross-section image of sample 4 shows a mixture of blue and white pigments. The blue particles are very similar to the ones of lapis lazuli as they are depicted in Figure 6.22c. This was confirmed by EDX, which detected Al, Na and Si (Figure 6.24d).
The very strong Pb peak in EDX (*Figure 6.24*) proves that lead white was mixed with lapis lazuli. Although, lead oxides are undetectable in NIR range, the FITR spectrum of S4 shows some peaks that could be attributed to lead white. Generally, in layers painted with lead white, the organic binder interacts with the lead carbonate salts and produces a saponification product. This lead soap displays stretching bands between 1450 and 1560 cm\(^{-1}\). Nevertheless, since in this area, bonding stretching and bending vibration of H-C, N-H, C=C and amide bonds give also peaks, we can only assume that the peak -indicated by the red arrows (1454 cm\(^{-1}\))- corresponds to the lead soap (*Figure 6.25*). Furthermore, the characteristic area of the in-plane bending modes of CO\(_{3}^{2-}\) appear at around 700 cm\(^{-1}\), while the 800-900 cm\(^{-1}\) zone stands for the out-of plane bending modes of CO\(_{3}^{2-}\). Hence, in *Figure 6.25*, some of the peaks shown in these areas can be attributed to the CO\(_{3}^{2-}\) ions of lead white. Lastly, the red arrow at 1018 cm\(^{-1}\) points the double shifted gypsum peak (990 and 1010 cm\(^{-1}\)), that more likely was overlapped by the silicate bonds of lapis lazuli.

*Figure 6.25. S4, blue pictorial layer. ATR (Specac Golden Gate GS 10500)-FTIR (Spectrum System 2000 Perkin Elmer)*

In IR false colour images (*Figure 6.26d, e, f*), the blue areas in predella – except for the ones pointed out by red arrows- render from pale red to bright red colour. As the only blue pigment that has bright red colour in IRfc is lapis lazuli, we can conclude with certainty the use of lapis lazuli (Douma, 2008). The various red hues can be either explained by the different concentrations of lapis lazuli or because of being mixed with a white pigment.
To conclude, based on the experimental data, Giovanni del Biondo used lapis lazuli for the blue and greenish areas, apart from 1) the pavement on the bottom of the panel and 2) the robe of the Saint on the left side of predella. In Figure 6.26e, the dark blue spots on Madonna’s robe are a retouching; this will be discussed later in paragraph 6.5 Retouching.

Lapis lazuli is a silicon-based pigment and is strongly overlapped by gypsum in NIR spectroscopy. Furthermore, lead oxides are undetectable in near IR range. Contrary, ESEM-EDX analysis offered strong and well-separated peaks of the inorganic elements of lapis lazuli, lead white and lead-tin yellow pigments.

**Red colour**

On the bottom right corner of the panel, there is the borderline of the organic cover (Figure 6.27).
Aiming to study the red hues, we carried out XRF, FORS and GC/MS analysis in both the covered and uncovered area. In Figure 6.28 the three black and white images depict the irradiated and sampled points: points 24, 27 and sample 1 from the covered area –deep red-, sample 6 and point 23 from the uncovered area –light red.
In Figure 6.29, the XRF spectra of red points 23 and 24 show different composition. Point 23 – covered area - consists of only Pb (Figure 6.29a), while Point 24 – uncovered area - consists of both Pb and Hg (Figure 6.29b).
ESEM-EDX and FORS results agreed with the ones of XRF (Table 6.2 and Figure 6.31). ESEM-EDX detected Hg in sample 1 and Pb in sample 6. Although, it was difficult to identify the pigments with FORS due to interferences, the spectra of covered points 24 and 27 are almost identical with each other and different from that one of the uncovered point 23. This allowed us to assume that the composition of the uncovered layer is different from that one of covered layer. Consequently, based on all these data, we concluded that the covered red layer is
a mixture of cinnabar and minium (Pb₃O₄), while the uncovered layer is composed only by minium.

![Graphs of Point 23, Point 24, and Point 27](image)

**Figure 6.31.** (a) red covered point 23: minium (Pb) with lead yellow, (b) red uncovered point 24: cinnabar (Hg), (c) red covered point 27: cinnabar (Hg). FORS-Optics (mod. HR2000), VIS-NIR

On the bottom side of the panel, the covered red point 12 was studied with FORS and XRF. The FORS spectrum of point 12 is almost identical to FORS reference spectra of minium, and does not show any similarity with that one of cinnabar. Furthermore, XRF analysis gave strong signal only for Pb and not for Hg. Hence, the painter here used only minium.
With XRF and FORS, we studied other two covered red points from the upper part of the panel: point 1 and 17. XRF analysis of both areas showed strong Hg (cinnabar) presence and gave a weak signal for lead (minium). Similarly, the two FORS spectra are a lot similar to FORS reference spectrum of cinnabar.

The red colour in predella was studied only with imaging techniques. The red arrows in Figure 6.34 point all the red areas, which –in IRfc- render the typical yellow of the mercury-based cinnabar (Douma, 2008).
All in all, Giovanni del Biondo used cinnabar and, for some special effects, such as the robe of St Mary Madgalene, he mixed it with minium.

**Yellow colour**

The yellow points studied with XRF and FORS are plotted in Figure 21.
In Figure 6.36, we summarized the XRF spectra and data of all the yellow points that were studied. Given the predominant presence of lead and the high concentration of tin, we concluded that the artist interpreted the yellow colour with lead-tin yellow.

**Dark areas**

Ultimately, we studied two glossy, dark points on the top left corner of the painting (Figure 6.37a)
We carried out only XRF analysis, because dark areas do not give any signal in FORS spectroscopy. In both analysis, the strong signal of Ag can be linked with silver leaves, which explain the metallic effect shown in microscope pictures 6.37b and 6.37c. Also, in these pictures, within the silver leaves, we observed red details, which could be part of the preparation. Given that iron detected in high concentration, we could conclude that the preparation of silver leafing is armenian bole (paragraph 2.2.2 Gilding). Although, carbon-based pigments cannot be detected with XRF, the non-mat profile of these dark areas strengthens the assumption about the use of silver leaves instead of carbon black.

6.3.2 Binding Medium

In order to identify the chemical composition of the organic binder of the pictorial layer, we carried out GC/MS, py-GC/MS and FTIR spectrometry.
GC/MS

Binder’s characterisation was based on both the statistical evaluation of the amino acid distribution and of three selected fatty acid ratios: azelaic/palmitic acid, palmitic/stearic acid and oleic/stearic acid.

The analysed samples were:

- Sample 1 (S1): preparation layer + small amount of the red pictorial layer
- Sample 4 (S4): blue pictorial layer
- Sample 5 (S5): pink pictorial layer
- Sample 6 (S6): red pictorial layer
- Sample 7 (S7): orange pictorial layer

**Sample 1**

S1 is a red sample taken from an uncovered area in the right panel (Figure 6.38b). This sample is the only analysed fragment that contained the preparation layer.

![Image](image.png)

*Figure 6.38. Sample (S1), (a) fluorescence microscope, magnitude 4x. (b) sampling from an existing hole- red robe of St Mary Magdalene. (c) wider view of the sampling area on the right panel. (d) Cross section of Sample 1, magnitude 4x.*
As it was mentioned before, in Renaissance, in Italy, painters used animal glue in preparation and egg (yolk or glair) or siccative oils and resins in pictorial layer (Colombini & Modugno, 2009). Furthermore, as it was described in paragraph 5.3.4.5. Detection and Quantification, we built a table based on the amino acid content of reference materials, so as to safely categorise each of our samples (Figure 6.39c). Based on this data, in paragraph 6.1.2. Organic binder, we proved that the amino acid profile of S1 matches with that of animal glue, as it contained high concentration of hydroxiproline and glycine (Figure 6.39a).

The lipid analysis of S1 detected cholesterol, which indicates the presence of egg yolk (Figure 6.40c). Taking into consideration that Cennini (1975) mentioned that egg was never used in preparation, we linked the detection of cholesterol with the painted layer. Nevertheless, in PCA score plot (Figure 6.39b) S1 is not distributed between egg and animal glue (Figure 6.39b). This can be explained due to the significantly larger quantity of preparation compared to the thin red painted layer (Figure 6.38d).
Meanwhile, in fatty acid analysis the sum of dicarboxylic acids (ΣD) was lower than 10% (Figure 6.40bii). According to reference table (Figure 6.40bi), published by Colombini and Modugno in 2009, no siccative oils were used in the painting.
Sample 4

S4 is a blue fragment –only pictorial layer- taken from an already existing hole (Figure 6.41c).

The results of the lipid analysis showed that the sum of dicarboxylic acids was less than 10% (Figure 6.41d), which means that no siccative oils were used in painted layer. Furthermore, cholesterol was detected at a rate lower than the detection limit; more probably, due to the very small quantity of the sample. This fact did not allow us to carry out further analysis.

<table>
<thead>
<tr>
<th>Linseed oil</th>
<th>Walnut oil</th>
<th>Poppy oil</th>
<th>Egg</th>
<th>Tempera grassa</th>
</tr>
</thead>
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<td>2.2-3.0</td>
<td>&gt;3</td>
<td>2.7-3.2</td>
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<td>&gt;1</td>
<td>&gt;1</td>
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<tr>
<td>SD</td>
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<td>&gt;40</td>
<td>&gt;40</td>
<td>&lt;25%</td>
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<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<table>
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<tr>
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<th>P/S</th>
<th>O/S</th>
<th>Σ Dic. %</th>
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<td>1.2</td>
<td>0.2</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Figure 6.40b. (i) Literature ratios of fatty acids content in three different drying oils (linseed oil, walnut oil, poppy oil), in egg and in egg mixed with drying oils (Colombini & Modugno, 2009). (ii) The experimental fatty acid ratios of sample 1 as well as the sum of di-carboxylic acid content in sample 1 (ΣDic.%).

Figure 6.41. (a) S4 fluorescence microscope magnitude 4x, (b) sampling area on the right panel, (c) sampling area from an already existing hole.
Sample 5 was taken from a covered area, from an existing hole, on the left side of the right panel. The analysed fragment contained both the painted and the cover layer. Hydroxiproline was not detected, while the strong signal of glycine indicates the presence of animal glue *(Figure 6.44 and the table of 6.45)*.
Given that, artists in that period did not use animal glue in painted layer, we assumed that glycine detection was due to interferences from the preparation layer or from the co-existing coating.

Furthermore, in lipid fraction analysis we found that the sum of dicarboxylic fatty acid content is 7.9%. According to Colombini and Modugno (2009) this rate indicates the absence of any siccative oils. Additionally, based on the reference table (Figure 7b), this rate more likely proves the presence of egg, as it is less than 25%. This last hypothesis was confirmed by the PCA amino acid score plot, in which S5 is distributed between egg and animal glue (Figure 6.45). Unfortunately, we detected cholesterol at a rate lower than the detection
limit, and therefore we cannot state with certainty whether the painter used the egg yolk or not.

SS: pictorial layer/ lipid-resinous fraction

Sample 6_pict

Sample six was taken from a covered area, from an already existing hole. The analysed fragment consisted of the painted and the cover layer.

Similarly to sample 5, the strong signals of hydroxyproline and glycine (Figure 6.48, 6.49) can be attributed to the interfering preparation layer, or to the co-existent coating. The composition of the cover layer will be discussed in detail in paragraph 6.6 Coating.
The GC/MS lipid fraction analysis did not give any data about cholesterol; most probably due to the insufficient derivatisation of the sample in step XV of the pre-treatment protocol (paragraph 5.3.4.3.2. Sampling and Sample pre-treatment). Similarly to the other samples, the sum of dicarboxylic acids was detected below 10% (Figure 6.50b) and therefore no siccative oils were used. We also carried out a complementary py-GC/MS analysis of the same fraction and we detected only traces of cholesterol. This is probably because in this
sample the concentration of inorganic materials was higher, and the analysis was less sensitive. Nevertheless, based on Colombini and Modugno (2009) the fatty acid pattern in pyrogram -particularly the strong signal of glycerol- is ascribable to the egg’s lipid profile. Based on all these, we can safely confirm the used of egg in the painted layer.

S6.pict: pictorial layer/ lipid-resinous fraction

<table>
<thead>
<tr>
<th>Linseed oil</th>
<th>Walnut oil</th>
<th>Poppy oil</th>
<th>Uovo</th>
<th>Tempora grassa</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/S</td>
<td>&lt;2</td>
<td>&gt;3</td>
<td>2.7-3.2</td>
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<tr>
<td>A/F</td>
<td>&gt;1</td>
<td>&gt;1</td>
<td>&lt;0.1</td>
<td>0.5-1</td>
</tr>
<tr>
<td>SD</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&lt;25%</td>
<td>20-40%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coles ter oil</th>
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<th>No</th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
</table>

**Figure 6.50.** (a) GC/MS chromatogram acquired in TIC mode, internal injection standard, hexadecane, and internal derivatisation standard, N-C-bis(trimethylsilyl)tri[fluoroacetoacetid (BSTFA), (b) literature ratios of fatty acids content in three different drying oils (linseed oil, walnut oil, poppy oil), in egg and in egg mixed with drying oils, and the experimental fatty acid ratios of Sample 6 as well as the sum of di-carboxylic acid content in Sample 6 (%Dic.%).

S6.pict: pictorial layer, py-GC/MS, lipid-resinous analysis

**Figure 6.51.** Pyrogram of lipid/resinous analysis of sample 6.

**Sample 7**

Sample 7 was taken from a covered area on the central panel -Archangel Gabriel’s robe-, from an already existing hole (**Figure 6.52b**). The analysed fragment consisted of both the painted and the cover layer.
We carried out GC/MS and py-GC/MS analysis and we detected glycine, hydroxyproline and pyrrole (m/z 67), which are markers of animal glue. Additionally, we detected traces of octadecanonitrile (m/z: 222/236) and hexadecanonitrile (m/z: 194, 208), which are markers of egg (Figure 6.53).

**Figure 6.52.** S7, (a) sampling area on the central panel, from a covered area - Archangel Gabriel’s robe-, (b) sampling from an already existing hole, (c) fluorescence microscope, magnitude 4x.

**Figure 6.53.** (a) GC/MS chromatogram acquired in SIM mode, I.S.1, I.S.2, internal injection standard hexadecane and internal derivatisation standard norleusine, respectively. (b) Pyrogram of S7, pyrrole (animal glue marker), octadecanonitrile and hexadecanonitrile (egg markers) were detected.
We assumed that animal glue detection is related with the interfering preparation layer and the co-existent coating. As it was mentioned before, the composition of coating is discussed in paragraph 6.6. *Coating*. The lipid fraction analysis traced siccative oils in concentration below LOD, while we captured terpenic compounds, which are related with the cover layer (6.6. *Coating*).

Furthermore, no odd hydrocarbon or even hydroxy acid were captured, therefore no waxes were used in the painting (Bonaduce & Colombini, 2004).

**ATR-FTIR spectroscopy**

As it was discussed in paragraph 5.3.1 *Mid-IR Spectroscopy*, FTIR can offer information only related to the class of the compounds, without differentiating compounds of the same class.

![FTIR spectra](image)

*Figure 6.54.* FTIR spectra range: 4000-2800 cm⁻¹. (b) sample 2, green painted layer, (c) sample 3, gliding, (d) reference spectra of gypsum (blue curve) and egg yolk (red curve).

In the beginning, we separated manually the painted from the white preparation layer of each sample. Nevertheless, the remaining preparation highly overlapped the peaks corresponding to the painted layers. Because of this, these spectra show significant similarities with the reference FTIR spectrum of gypsum (*Figure 6.6*). Consequently, we traced the organic compounds only by the alteration/split of gypsum peaks - as it was discussed in 6.1.1*Organic Binder*. For instance, the gypsum narrow double peak at 3404 and
3545 cm\(^{-1}\) transformed in a broader double peak (Figure 6.54a, b and c) with a shape more similar to the single broad peak from 3200 to 3600 cm\(^{-1}\), which corresponds O-H deformation vibration of the organic materials in egg yolk (Figure 6.54d).

![Figure 6.54](image)

**Figure 6.55.** (a) FTIR spectra, (reference spectra of gypsum, 1800-1000 cm\(^{-1}\), (b) sample 1, red painted layer, (c) sample 2, blue painted layer, (d) sample 4, green painted layer, (e) sample 4, green pictorial layer, (f) reference spectra of egg yolk.

Furthermore, we observed the broadening of the narrow double peak of gypsum at 1620 and 1690 cm\(^{-1}\). This alteration gave to the peak a form similar to the one of the single broad peak (1580 to 1685 cm\(^{-1}\)) shown in the reference spectra of egg yolk and corresponds to the amide bond I. Additionally, from 1520 to 1630 cm\(^{-1}\), the triple pattern in Figures 6.55b, 15d and 15e, is a lot similar to the peak pattern of the amide bond II at 1540 cm\(^{-1}\), due to the N–H bending motions and the C–N stretching vibration.

Ultimately, as it was mentioned in paragraph 6.1.2 Organic binder, the absence of any narrow peak from 1670 to 1820 cm\(^{-1}\), allows us to exclude the classes of acids, esters, ketones and aldehydes.

To conclude, with FTIR, we confirmed the presence of a proteinaceous material, without characterising the compound. Nevertheless, the experimental spectra have a lot similar peak patterns with the reference spectrum of egg yolk. GC/MS analysis detected cholesterol and hydroxiproline in almost all samples,
while did not capture any siccative oils. Based on these experiments and on historical and scientific reference data bases, we came up with the following conclusion: egg yolk was used as a binder in the painted layer, while the detected animal glue is due to the interference of the preparation and the co-existent cover layer.

6.4 Gilding

For the study of gilding technique we carried out XRF, ESEM-EDX, IR false imaging and GC-MS analysis. The location and XRF data of three different areas decorated with metal leaves are depicted in Figure 6.56.

![Figure 6.56](image)

**Table 6.6.** Sampling areas and elementary qualitative analysis of gilding points 10, 15 and 16.

<table>
<thead>
<tr>
<th>Point</th>
<th>Colour</th>
<th>XRF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>10</td>
<td>Gold</td>
<td>*</td>
</tr>
<tr>
<td>15</td>
<td>Gold</td>
<td>*</td>
</tr>
<tr>
<td>16</td>
<td>Gold</td>
<td>**</td>
</tr>
</tbody>
</table>
Point 10, belongs to Saint Peter’s key, which is composed by silver (Ag), while gold (Au) is totally absent (Figure 6.56, XRF data, point 10). In Figure 6.56.10b, we can see the support of silver leaves. Since, this layer is, mainly, composed by Fe, probably the painter, used armenian bole (Fe and clay oxides). The detected Sr and Ca traces are celestine impurities of the preparation. Points 15 and 16 belong to the gilded window and the gilded halo depicted in Figures 6.5615a and 16a. The gold peaks of points 15 and 16 are shown in XRF spectra in Figure 6.57. Apart from gold, we also detected Fe and Sr, which means that also in these two areas, the painter used armenian bole.

ESEM-EDX and GC/MS analyses were carried out for the gilding study in two fragments: S1, fine gilding decoration of Maria Madeleine's robe (Figure 6.58) and S3, fine gilding decoration of the halo of the Saint above Maria Madeleine (Figure 6.61).
All the images of S1 (Figure 6.58 and 6.59) render a homogenous red colour, which lies below a thin black layer and above the white preparation layer. Based on ESEM-EDX analysis this red layer consists of Hg. The thin black layer, probably, served as the gilding's preparation.

In Figure 6.59, image (b) is a magnified view of S1 cross-section and image (a) is S1 BSE analysis (400x). Arrow i indicates the gilding layer (shown as a white line) and arrow ii points on the black layer. The third arrow in image (b) points on the black layer.

Figure 6.58. Sample 1. (a) Sampling point 1. Zeiss Axioplan Optical Microscope (b) 4x, (c) 4x Cross sections, Zeiss Axioplan Optical Microscope (d) 4x, (e) 10x, (f) 20x.

Figure 6.59. Sample 1. (a) Backscattered Image, Microscope Quanta200- EDX, 400x. The arrow i indicates the golden leaf and the arrow ii indicates the black binding medium between the golden leaf and the red pigment. (b) Zeiss Axioplan Optical Microscope, 20x. The red arrow indicates the black binding medium.
We isolated the gilded/painted part of S1 and we studied it with GC/MS in order to characterise the black preparation. The lipid analysis captured cholesterol, while the sum of dicarboxylic acids counted below 10%. Furthermore, amino acid analysis detected hydroxiproline, but only under the detection limit (Figure 6.60). This is explained by the very small quantity of the sample. Given that no siccative oils, sugars and resins were detected, we concluded that the black thin layer is a proteinaceous material. Furthermore, since the gilded fragment also contained the painted layer, more probably, the cholesterol signal is linked with the painted and not with the black layer. Lastly, as the sample was free of preparation, we could assume that the detected hydroxiproline indicates that the black layer consists of animal glue. However, this can be only an assumption, as hydroxiproline was detected below the detection limit.

**S1 gilding:** gilding / amino acid fraction analysis.

![Figure 6.60](image.png)

*Figure 6.60.* Gilding analysis of sample. (a) GC/MS chromatogram acquired in SIM mode. I.S.1.: internal injection standard, hexadecane and I.S.2.: internal derivatisation standard: norleusine. Detected traces of animal glue.

All in all, based on the abovementioned data and facts, the gilding technique of the decoration on Madeleine’s robe is *mission (paragraph 2.2.3.2 Gold leafing)*. According to this technique, the painter applied a proteinaceous layer –probably animal glue-, and while it was still wet, he added the fine gold leaves on the top.

EDX analysis of sample 3 detected aluminosilicates and iron (Fe, Si, Ca, S, Al, Mg), which can be linked with *armenian bole*. In *Figure 6.61d*, the red arrows point the broader line between *armenian bole* and the fine ground gypsum layer. With a closer look on *Figure 6.61c and 6.61d*, we observed that there is no
intermediate layer between the gold leaves and *armenian bole*. This means that, in this area, gilding was applied directly on the bole.

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**Figure 6.61.** Sample 3. (a) Sampling area. (b) Cross section, Zeiss Axioplan Optical Microscope, 4x. (c) Cross section, Zeiss Axioplan Optical Microscope, 20x. (d) Backscattered image, Microscope Quanta200-EDX, 1200x.

### 6.5 Retouching

With imaging techniques, we localized the recently retouched areas, and in some cases we studied the nature of the restoration materials (i.e. pigments etc.). As it was mentioned in Chapter 3, the only part of the painting being restored is predella. In this paragraph, we examine some of the restoration works and we discuss the restoration materials and techniques that were used.

**Predella: Left part**

On the left side, the gilded background is retouched with the typical Florentine method, *selezione cromatica dell’oro*, introduced by Baldini (1978) (*Figure 1*). According to this method, yellow ochre mixed with minium and black pigment –water soluble-, is applied with brush strokes that follow the lines of the drawing. This restoration method aims to smoothen the contrast between the damaged area and the rest –preserved- gilded area.
As it was discussed in paragraph 6.3 Pictorial layer, lapis lazuli is the pigment of almost all the bluish and greenish areas of predella. IR false colour images revealed the characteristic light red hue of lapis lazuli (Doumas, 2008). In IR false colour images of Saint’s robe (Figure 6.63a and 6.63b), we observed light dark blue spots on the red surface. This colour heterogeneity proves later intervention on the original work. According to the electronic database, the blue pigment azurite renders blue hues -from light to dark blue, depending on its concentration- in IR false colour images (Doumas, 2008). Based on this fact, we can only assume that azurite was used for this particular restoration work.

Figure 6.62. Predella, left part. Vis (a), IRc (b), IR (c), UV (d). Restored area –around the shoulder-, with the method of “selezione cromatica dell’oro”, which was introduced by Umberto Baldini.

Predella, Central Part

Figure 6.64. Predella: central part. Saint on the left side. IRc (a) and Vis (b) images. Saint on the right side. IR false colour (c) and Vis (d) images. The light blue areas on the cloth indicate restoration.
In Figure 6.65b and 6.65c, we can see the restored areas on Madonna’s robe. The light red colour shown in IR false colour image 6.65c proves that the robe was painted with lapis lazuli (Doumas, 2008). In IR image 6.65b, lapis lazuli renders a light grey colour, due to its high reflectance for infrared light (Figure 6.65b). Most probably, the restoration pigment is azurite. Our assumption is based on that 1) azurite renders blue colour in IR false colour, similar to this one in Figure 6.65c and that 2) azurite appears black in IR black and white images, as can be seen in Figure 6.65b (Delaney et al., 2005). In Figure 6.65e, we can see the restoration technique made with parallel brush movements.

We further studied this retouching with XRF and FORS spectrometry. We selected one point from the retouched area (point 25) and one point from the untouched one (point 26). By comparing the FORS spectrum of point 26 and the reference spectrum of lapis lazuli, we observed the similar S-feature with an inflection point around 480 nm and 700 nm. This confirms our initial assumption about the use of lapis lazuli. Referring to the restoration pigment, although, our expectation for a strong XRF peak in Cu – predominant metal in azurite- fell out, FORS spectra of point 25 (Figure 6.66b) is a lot similar to azurite. This
contradiction in our data could be explained by the fact that point 25 is somewhere between the retouched and the untouched area. Lastly, we should highlight that the hypothesis about azurite agrees with literature. The historical and scientific data of the studied paintings of Renaissance explained the extended use of azurite, as a restoration pigment in older times, due to its significantly lower cost than that of lapis lazuli (2.2.1.1 Lapis Lazuli). Nevertheless, it is important to carry out further experiments in order to strengthen our hypothesis.

![Graphs and Tables]

**Figure 6.6.** FORS-Optics (med. HR2000), VIS-NIR spectra of Point 26 (a), Point 25 –restored area– (b) and FORS reference spectra of lapis lazuli (green curve) and azurite (blue curve) (c). XRF data of points 25 and 26 (d). By comparing the FORS reference spectra and the FORS spectra of point 25 and 26, we conclude that the blue cloth was originally interpreted with lapis lazuli, while, we assume that the cloth was partially retouched with azurite in later years.

### 6.6 Coating

The characterisation of the brownish coating is necessary for understanding the original painting technique as well as for identifying any latter restoration work. According to technical reports in Opificio delle pietre dure, the painting was not recently restored. An integrated method including UV imaging, GC/MS and py-GC/MS, was used for the identification of the coating materials.
Under UV light, the covered areas rendered bluish fluorescence (*Figure 6.67d* and *6.67e*). Given that UV interacts only with the superficial layer, we can conclude that the coating is an organic material, or mixture of organic components. In images of *Figure 6.67*, we pointed the border line of coating – red arrows –, which separates the bluish cover layer on the left and the non fluorescent uncover painting stripe -longitudinally the wooden column- on the right.

![Figure 6.67.
Imaging of the right panel. Boarder line of coating, pointed by the red arrows. (a) VIS, (b) IR, (c) IR false colour, (d) UV, (e) detail of the UV image d.](image)

After imaging, we sampled fragments containing either only the cover layer or all the layers of the painting (*Table 6.4*).
6.6.1. GC/MS amino acid and lipid fraction analysis

GC/MC and py-GC/MS analysis of all the eight fragments captured hydroxiprolone and glycine (animal glue markers) in average and high rates (Figure 6.68). According to literature, animal glue was used frequently as a diluter of various materials served for varnishing, such as sugars, terpenes etc. (Colombini & Modugno, 2009).

![Table 6.4. Samples containing the cover layer and analyzed by GC/MS and py-GC/MS](image)

<table>
<thead>
<tr>
<th>Samples:</th>
<th>Analysis</th>
<th>Samples:</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>pictorial layer + coating</td>
<td>GC/MS</td>
<td>S5</td>
<td>GC/MS and py-GC/MS</td>
</tr>
<tr>
<td>S6_pict</td>
<td>GC/MS</td>
<td>S6</td>
<td>GC/MS</td>
</tr>
<tr>
<td>S7</td>
<td>GC/MS and py-GC/MS</td>
<td>S8</td>
<td>GC/MS</td>
</tr>
<tr>
<td>S8_b</td>
<td>GC/MS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 6.4.** Samples containing the cover layer and analyzed by GC/MS and py-GC/MS

**Figure 6.68.** Score plot of amino acid profiles obtained in GC/MS analysis of samples from the collection of paint reference materials of Opificio delle Pietre Dure (●), containing egg, casein and animal glue as binders, and of S6_pict, S8b, S1, S5, S6, S8. (b) The calculated-corrected relative percentage content of each amino acid in all the samples and the total amino acid content in all samples.

<table>
<thead>
<tr>
<th>Ala</th>
<th>Gly</th>
<th>Val</th>
<th>Leu</th>
<th>Ile</th>
<th>Ser</th>
<th>Pro</th>
<th>Phe</th>
<th>Asp</th>
<th>Glu</th>
<th>Hyp</th>
<th>µg tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>casein</td>
<td>3</td>
<td>3</td>
<td>7.6</td>
<td>11.9</td>
<td>6.6</td>
<td>5.8</td>
<td>11.5</td>
<td>5.9</td>
<td>8.5</td>
<td>22.2</td>
<td>0</td>
</tr>
<tr>
<td>egg</td>
<td>7.7</td>
<td>4.8</td>
<td>7.7</td>
<td>11</td>
<td>6.7</td>
<td>10.3</td>
<td>5.7</td>
<td>6.4</td>
<td>12.6</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>animal glue</td>
<td>12.3</td>
<td>29.4</td>
<td>3.9</td>
<td>4.7</td>
<td>2.5</td>
<td>3.0</td>
<td>12.4</td>
<td>2.8</td>
<td>6.6</td>
<td>9.9</td>
<td>7.7</td>
</tr>
<tr>
<td>S1</td>
<td>12.3</td>
<td>28</td>
<td>4.4</td>
<td>5.5</td>
<td>2.3</td>
<td>2.5</td>
<td>28.7</td>
<td>3.8</td>
<td>3.6</td>
<td>6.5</td>
<td>2.4</td>
</tr>
<tr>
<td>S5</td>
<td>11.4</td>
<td>19.4</td>
<td>12.3</td>
<td>15.2</td>
<td>7</td>
<td>4.6</td>
<td>19.2</td>
<td>5.5</td>
<td>3.2</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>S6</td>
<td>8.9</td>
<td>13.2</td>
<td>12.4</td>
<td>10.5</td>
<td>4.6</td>
<td>5.4</td>
<td>30.7</td>
<td>5.1</td>
<td>4.9</td>
<td>4.1</td>
<td>0.4</td>
</tr>
<tr>
<td>S8</td>
<td>10.6</td>
<td>26.5</td>
<td>4</td>
<td>6.4</td>
<td>2.2</td>
<td>6</td>
<td>24.5</td>
<td>4.4</td>
<td>7.7</td>
<td>7.3</td>
<td>0.5</td>
</tr>
<tr>
<td>S6_pict</td>
<td>8.7</td>
<td>15.3</td>
<td>2.9</td>
<td>4.4</td>
<td>1.9</td>
<td>2.6</td>
<td>19.4</td>
<td>1.1</td>
<td>6.7</td>
<td>11.2</td>
<td>25.7</td>
</tr>
<tr>
<td>S8b</td>
<td>7.7</td>
<td>12.7</td>
<td>4.7</td>
<td>4.9</td>
<td>3.5</td>
<td>11.9</td>
<td>7.5</td>
<td>3.5</td>
<td>8.2</td>
<td>18.5</td>
<td>16.8</td>
</tr>
</tbody>
</table>
Given that hydroxirpoline and glycine were also detected in S8b and S6 - samples containing only the cover layer-, we presumed that coating consists of animal glue. In detail, in Figure 6.68, the glycine content ranges from 13 to 28 units, and S1 -preparation layer- and S8 -cover layer- were the samples with the strongest signal. Hydroxiproline is rather high also in sample S6_pict, which contained the painted and the cover layer. Lastly, in S8_b -coating sample- we also detected hydroxiproline and in S7 -painted and cover layer-, py-GC/MS captured pirrole, which is animal glue marker (Figure 6.53b).

Regarding to the lipid content, we summarised the results of lipid analysis of all samples in Table 6.5.

<table>
<thead>
<tr>
<th>Samples</th>
<th>A/P</th>
<th>P/S</th>
<th>O/S</th>
<th>Σ Dic.%</th>
<th>µg tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>1.2</td>
<td>0.2</td>
<td>7.4</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>1.8</td>
<td>0.1</td>
<td>6.4</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>1.2</td>
<td>0.3</td>
<td>7.9</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1.1</td>
<td>0.1</td>
<td>7.2</td>
<td>3.2</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>3.6</td>
<td>7.2</td>
</tr>
<tr>
<td>8b</td>
<td>0.2</td>
<td>1.1</td>
<td>0.1</td>
<td>11.4</td>
<td>2.4</td>
</tr>
<tr>
<td>6_pict</td>
<td>0.1</td>
<td>1.1</td>
<td>0.1</td>
<td>9.5</td>
<td>8.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Linseed</th>
<th>Walnut oil</th>
<th>Poppy oil</th>
<th>Egg</th>
<th>Tempera grassa</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/S</td>
<td>&lt;2</td>
<td>2.2-3.0</td>
<td>&gt;3</td>
<td>2.7-3.2</td>
</tr>
<tr>
<td>A/P</td>
<td>&gt;1</td>
<td>&gt;1</td>
<td>&gt;1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Σ D</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&lt;25%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 6.5. (a) Data of the lipid fraction of all the samples analyzed with GC/MS. (b) GC/MS lipid analysis of samples from the collection of paint reference materials of Opificio delle Pietre Dure.

Given that the sum of dicarboxylic acids is below 10%, we concluded that no siccative oils are present in any of the layers (Colombini & Modugno, 2009). Similarly to animal glue, siccative oils were not only used as binders but also as diluters of saccharide or terpenic materials. Consequently, the absence of siccative oils strengthens the assumption about animal glue serving as the coating diluter.
6.2.2. GC/MS Saccharide and py-GC/MS Resin analysis

Sample 6

![Image](image.jpg)

**Sample 6: saccharide fraction**

<table>
<thead>
<tr>
<th>xylene</th>
<th>arabinose</th>
<th>rhamnose</th>
<th>fucose</th>
<th>galacturonic acid</th>
<th>glucoron acid</th>
<th>glucose</th>
<th>mannose</th>
<th>galactose</th>
<th>fructose</th>
<th>µg tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>98.7</td>
<td>0.4</td>
<td>0.9</td>
<td>1.0</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**Figure 6.69.** (a) GC/MS chromatogram of S6 acquired in SIM mode. I.S.1 derivatisation standard, mannitol. Ketose peaks (*). (b) The percentage composition of monosaccharides in S6. (c) Database with the percentage composition of plant gums and honey firstly published by Colombini & Modugno (2009) in the book *Organic mass spectrometry in art and archaeology* (p.21).

We analysed the saccharide fraction of S6 with GC/MS. The obtained SIM chromatogram is depicted in Figure 6.69a, where we can see the strong peak of glucose. Figure 6.69b is a table with the corrected monosaccharide content of S6, where the predominant components are glucose and fructose. Figure 6.69c is a literature database with the monosaccharide percentage composition of the most popular saccharide materials used for varnishing in Renaissance. This table was first published by Colombini and Modugno (2009), in the book *Organic mass spectrometry in art and archaeology* (p.21). According to this table, the saccharide content of S6 matches with that one of honey.
Sample 8_b

The GC/MS chromatogram of the saccharide fraction of S8_b is depicted in Figure 6.70b. According to the reference table (Figure 6.69c) and the decisional schemes of sugar analysis published by Lluveras et al., (2012) the saccharide profile of S8_b (Figure 6.70b) does not match with any of the plant gums. Furthermore, the total amino acid content of S8_b is 34.3μg, which is significantly larger, compared to the saccharide content, which is only 0,4μm (Figures 6.68b and 6.70b). Based on these facts, we assumed the main component of this sample is animal glue, while it also contains sugars as contaminants.

Sample 8_b: saccharide fraction

<table>
<thead>
<tr>
<th>xylose</th>
<th>arabinose</th>
<th>rhamnose</th>
<th>fucose</th>
<th>galacturonic acid</th>
<th>glucuronic acid</th>
<th>glucose</th>
<th>mannose</th>
<th>galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>2.8</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>17.3</td>
<td>74.1</td>
</tr>
</tbody>
</table>

Figure 6.70. (a) GC/MS chromatogram of S9_b, acquired in SIM mode. I.S.1 derivatisation standard, manitol. (b) The percentage composition of monosaccharides in S8_b.
Sample 7

In the pyrogram (Figure 6.71a) we can see the markers of the natural triterpenic resins: Dammaradienol (m/z 189, 426), Dammaradienone (109, 424), Oleanonic acid, Ursonic acid (203, m/z) (Colombini et al., 2000).

In Renaissance, artists used extensively natural triterpenoid resins for varnishing and these resins belong to two different botanical origins; dammar resin, extracted from the Hopea tree and mastic Pistacia lentisca. The characteristic compounds of dammar resins are the dammarenic acid and the ursonic. Mastic resins consist of moronic acid, masticadienoic and isomasticadienoic acid, while oleanonic acid is present in both resins (Colombini et al., 2000). Given that the detected dammaradienone and dammaradienol are not present in mastic resins, it is more probable that the coating consists of raw dammar resin. As it was mentioned before, artists mixed resins with siccative oils or glues in order to dissolve and stabilise the coating. As no siccative oils were detected, and the amino acid analysis of S7 (free of preparation) captured pyrrole (Figure 6.53), we concluded that the cover layer of this fragment is a mixture of dammar resin with animal glue.
6.7. Non invasive in situ-investigation versus micro-sampling

This study demonstrated that the quantity and the quality of the information that can be obtained using the present in-situ, non-invasive multi-technique approach, make it an important and valuable tool for the technical study of the complex painting stratigraphy.

With regard to the inorganic characterization, the comparison of the results with those obtained through micro-sampling shows a remarkable convergence as summarized in Table 6.4.

<table>
<thead>
<tr>
<th>Inorganic Characterisation</th>
<th>Non-invasive</th>
<th>Micro-destructive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Painted layer Blue</td>
<td>lapis lazuli (IRfc, FORS)</td>
<td>lapis lazuli (ESEM-EDX)</td>
</tr>
<tr>
<td>Blue</td>
<td>cinnabar/minium (XRF, FORS)</td>
<td>cinnabar/minium (ESEM-EDX)</td>
</tr>
<tr>
<td>White</td>
<td>lead white (XRF, FORS)</td>
<td>lead white (ESEM-EDX)</td>
</tr>
<tr>
<td>Yellow</td>
<td>lead-tin yellow (XRF, FORS)</td>
<td>lead-tin yellow (ESEM-EDX)</td>
</tr>
<tr>
<td>Gilding Preparation layer</td>
<td>gold leaves (XRF)</td>
<td>gold leaves (ESEM-EDX)</td>
</tr>
<tr>
<td>Preparation layer</td>
<td>gypsum, seestine (XRF)</td>
<td>gypsum, seestine (ESEM-EDX)</td>
</tr>
</tbody>
</table>

Table 6.4. Comparison between results obtained by the non-invasive and micro-destructive approach.

In evaluating the performance of the non-invasive methods, it is relevant to note that possible limitations are not only related to low sensitivity or low specificity of the exploited techniques but also to the restricted spectral range offered by the non-invasive set-up. For instance the commonly used X-rays generators do not detect elements with atomic number lower than 14, thus not permitting the identification of lapis lazuli (sodium aluminium silicate cage containing sulphur anions). Another limitation arises from the so-called matrix effect, when working directly on the paint surface. This effect particularly prohibits quantitative XRF investigations, where every layer of the stratigraphy screens X-rays both of excitation and of fluorescence leading to the loss of
proportionality between intensity and concentration. Nevertheless, signal intensity can be used to obtain relative comparison among sampling points having similar bulk, such as points on the painted layer.

The analytical problems are more complicated when characterising organic compounds, such as binders and coatings, because the spectroscopic techniques (such as FTIR) provide data only on functional groups and are therefore less specific for these compounds. In our study, in FTIR, the significant matrix effects mainly caused by gypsum resulted in overlapped absorption bands of the organic materials that could not be properly resolved. However, in spite of this inconvenience, it is possible to trace the organic presence studying the shifting of peaks of gypsum peaks. However, for the precise identification of organic molecules from paintings, only chromatographic methods on micro-samples are capable. Despite that, this work demonstrated that a spectroscopic characterization applied on painting fragments combined with the in situ non-invasive UV imaging could be of great benefit for enhancing the capabilities of the chromatographic analysis of coating and binders.

The great advantage of non-invasive methods is that no contact or sampling is necessary and therefore the painting can be examined without any limitation. This overcomes the main restriction of the micro-destructive techniques, where data is obtained from only a single sampling site that is determined not only by choice, but also by the opportunity to sample close to an edge of the painting or the edge of an already damaged area. Furthermore, the large number of measurements of the non-invasive approach can provide statistical validation of the data, so that even small features, such as CO$_3^{2-}$ stretching bands of lead white in IR, or overlapped silicate peaks of lapis lazuli, can be considered reliable. Nevertheless, micro sampling remains obligatory when we need to precisely identify the organic compounds in a painting.
Chapter 7

Conclusions

This study demonstrated that the quantity and the quality of the information that can be obtained using the present integrated approach make it an important and valuable method for the technical study of the complex painting materials. The sufficient calibration of portable devices, along with the optimum sampling and the sensitive micro-destructive techniques, offered reliable imaging data and spectra.

The preparation layer, studied by FT-IR, ESEM-EDX and GC/MS analysis is a mixture of animal glue with gypsum (CaSO$_4$·2H$_2$O), and is composed of two layers distinguished by the granulometry of gypsum (*gesso grosso* and *gesso fine*). ESEM-EDX also detected selestine (SrSO$_4$) and gypsum impurities such as aggregated crystals of Fe, Ba, Zn, Fe, Si. In FTIR spectra, the characteristic peak pattern of gypsum was overlapped from 3200 to 3500 cm$^{-1}$ and 1450 to 1550 cm$^{-1}$, by a proteinaceous material. In GC/MS chromatograms, the strong signals of hydroxiproline and glycine indicated the presence of animal glue in preparation.

The palette was preliminary studied by IR false colour imaging and punctually by FORS, XRF and EXEM-EDX analysis. According to the combined data, all the blue areas are composed by lapis lazuli mixed either with lead white or lead tin yellow. The only green area painted with a different pigment is on the bottom of the central panel, where a copper-based green pigment was detected. In *predella*, the only blue areas not consisting of lapis lazuli, is the robe of the Saint on the left side, as well as the retouched areas of Madonna’s robe, which were painted with azurite. The red colour was interpreted with cinnabar, and for some special effects, such as the robe of St Mary Madgalene, it was mixed with minium.

According to FT-IR and GC/MS analysis, the binder of the painted layer is egg yolk. In GC/MS chromatograms, and py-GC/MS pyrograms, we detected the egg yolk fatty acids and cholesterol. The detected hydroxiproline in
samples, containing only the painted layer, is interference from the neighbouring layers.

The gold leaves, on mantles and halos, were applied either on red *armenian bole* or directly on the painted layer. The GC/MS analysis of the gilding sample, on Saint Mary Magdaleine’s mantle, traced animal glue. Because of this, we assumed that the painter used the *mission* technique for this decoration. Silver leaves, heavily tarnished, used for some details, such as swords, were also applied on *armenian bole*.

The visual inspection (UV imaging) did not provide a clear evidence of oil/resin-based varnish, except for a glue-like coating. Indeed, GC/MS sugar analysis detected honey and animal glue. Such coating seems to be due to past restorations, presumably carried out after the addition of the twisted columns. Indeed the surfaces behind the latter are quite clean and not affected by this coating, thereby supporting the hypothesis of past restorations (during the transfer of the painting) rather than an original treatment of the painted surface. Py-GC/MS analysis of a unique sample taken from the central panel, identified a mixture of trierpenic dammar resin and animal glue. Also, this coating can be attributed to an old restoration, executed after the beginning of 18th century.

In predella there are areas integrated with the method of *selezione chromatica*, such as the blue Madonna's robe, initially painted with lapis lazuli and retouched probably with azurite. This can be attributed to an old predella restoration, between 1700 and 1827.

The outcomes of this multidisciplinary diagnostic work conducted on the polyptych *Annunciation and Saints* enhanced the painting skills of Giovanni del Biondo. At this stage of our investigation we can robustly state that del Biondo represents a generation of painters who brought *egg-tempera* technique to a high level of quality at the turn of 14th century. The investigation of the painting will be continued, in order to contextualise it in the broader European artistic environment of the 14th -15th century. Further chromatographic analysis of coating and organic pigments will be carried out, in order to achieve a deeper view of the painting technique and of the special matt effect of the painting’s surface.
The study of old paintings is of the most challenging analytical task, due to the complexity, the dimensions and the uniqueness of the work. According to the techniques known and studied so far, the presence of organic materials is significantly weaker than that of inorganic materials. Based on this and given that micro-sampling techniques are limited in analysing only few fragments, it is out of most importance to work on the optimisation of portable devices/non-invasive techniques, and especially those who carry out organic characterisation.
REFERENCES

Articles


**Books**


**INTERNET**

