Learning from the past, intervening in the present: the role of conservation science in the challenging restoration of the wall painting *Marriage at Cana* by Luca Longhi (Ravenna, Italy)

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### Abstract

The paper discusses the case study of the *Marriage at Cana*, a sixteenth century wall painting located in Ravenna and executed by Luca Longhi. A multi-analytical approach based upon OM, SEM–EDS, μ-Raman, μ-FTIR and biological analyses was selected to investigate the painting technique and the state of preservation of the artwork, compromised by a severe alteration. Data demonstrated that the artwork was executed with a dry painting technique: a siccative oil was used as binder, while indigo, lead white, carbon black, ochres, vermilion and red lead were identified as pigments. Biological analyses clearly allowed identifying *Eurotium halophilicum* as the fungus responsible for the white patina compromising the painted surface and, according to this result, Biotin T was selected as the most effective biocide to stop the biological attack. The precarious conditions in which the painting was, attributable to previously performed interventions and to the conservation environment, laid the groundwork for a challenging restoration conducted in 2016. Scientific analyses better clarified the kind of materials employed in the execution on the artwork, as well as how the previous restoration was carried out; furthermore, analytical data methodologically supported phases of the intervention like cleaning, filling of the lacunae and pictorial retouching, as products were selected on the basis of their affinity to original materials and painting technique. This study will hopefully encourage reflections on how a synergic dialogue between conservation science and restoration can represent an important reference point for interventions to be conducted with scientific criteria and suitable methodology, in the light of the shared vision and common goal of transferring patrimony to future generations.

**Keywords:** Conservation science, Restoration, Wall painting, Multi-analytical study, Painting technique, Cultural heritage biodeterioration, Lacuna treatment, Luca Longhi

### Introduction

In October 2013, an international consortium of 16 institutions was brought into being by ICCROM in Rome, venue for noteworthy reflections about the role of science in the field of cultural heritage conservation. Among the key issues, the ICCROM Forum on Conservation Science highlighted the crucial requirement of fostering the integration and enhancing the impact of this discipline within the cultural heritage sector: “Seeking sustainable solutions through collaboration and sharing” was identified as a priority for the future [1], with the invitation to look for solution-orientated applied research to be...
developed in synergy with end-users, in order to solve primary issues in heritage conservation.

The case study of the wall painting *Marriage at Cana* stands as a tangible attempt to fit into this research priority: conservation scientists and restorers were called to operate in strong interaction, with the shared goal of properly identifying key issues to be addressed and clear-cut questions to be answered for the work to be conducted with scientific criteria and suitable methodology.

Adorning the wall of the Sala Dantesca of the Biblioteca Classense in Ravenna (Italy), the *Marriage at Cana* (Fig. 1) was executed by Luca Longhi (1507–1580), famous portrait painter of the Romagna area, in 1579–1580. By virtue of its impressive dimension, as it is placed over a surface area of around 40 m², and relevant historic-artistic features like the organisation of the scene, the great number of figures and the variety of their movements, allowing a direct comparison with paintings of the same subject made by Masters as Leonardo da Vinci and Paolo Veronese, this painting is considered Longhi’s last and greatest work of art [2, 3].

However, starting from just a few decades after its making, the painting was affected by a series of unfortunate events and oversights that greatly impacted upon its conservation. A conspicuous flood occurred in 1636, resulting in the loss of the lower portion of the painting, but no intervention was carried out until 1779 by the restorer Francesco Zannoni [4]. Due to the worsening of the state of conservation of the painting, partly ascribable to the increasing degree of humidity within the wall, various requests of intervention were raised in the nineteenth century and, finally, in 1882, another restoration work took place [2, 3]. It was, however, decided not to detach the wall painting by reasons of its critical conditions of conservation, the artwork’s dimension and the high cost estimate. The humidity coming from the wall was, thus, reduced opening a series of small arches below the painting [5] and, in 1884, the restorer Filippo Fiscali also carried out a cleaning of the pictorial surface [6].

Nevertheless, these operations were not conclusive. The ever-increasing issues related to humidity resulted in a further restoration in the 1970s and the intervention was undertaken by Ottorino Nonfarmale [4]. It started with the removal of the retouching applied during previous restorations, while a further attempt of decreasing the humidity of the masonry by heating and aerating the room was being performed. However, the extremely precarious state of conservation in which the painting was left no room for other solutions than its detachment. The restoration report [4] specifically refers to the use of the so-called “stacco” technique, although it was possible to remove only a very thick layer of plaster and roll it around a cylinder to move it to the laboratory and carry out the transposition on a new support. According

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1 Data from the Notary Archive of Ravenna, notary deeds by Girolamo da Porto, 1579.
to the description, a double canvas layer was applied on the whole surface with an organic product to protect the pictorial layer (“facing”) and then the “stacco” was performed. First, the back of the wall painting was put on a triple support of canvases and acrylic resin (“backing”); then, it was placed on a panel of anodized aluminium, selected for its adequate rigidity, non-deformability and, if necessary, reversibility. The wall painting was glued to the aluminium panel by acrylic resin mixed with inorganic compounds as calcium carbonate and silicates and, last, the double layer of canvas on the surface was removed by water. After the intervention, the wall painting was re-located on its original place in the Biblioteca Classense, slightly detached from the wall to avoid further humidity-related problems. A reconstruction of the structure of the painting after its detachment is provided in Fig. 2.

In the last 30 years, the surface of the painting has been progressively damaged by the presence of a whitish patina, which has led to a gradual fading of the chromatic tones and to a loss of legibility of the artwork (Fig. 3).

The loss of almost 70 cm related to the flood occurred in 1636 represented a particularly harmful situation for the painting that could have led to its collapse. Moreover, the surface showed some lacunae with a vertical trend that affected the entire height of the painting, whose morphology was consistent with the cracks present in the wall before the detachment intervention. In addition, the numerous colour losses of the pictorial layer, though relatively small, were in sharp contrast with the well preserved original portions. Lastly, on the surface there also were some swellings probably due to previous consolidation operations carried out by
injections, as well as canvas residues ascribable to the detachment, especially localised in the lower part. For the aforementioned reasons, it was decided to set up a further restoration intervention in 2016. In order for it to be conducted with a scientific-based methodology, analyses were carried out to gain information on the artwork's material composition and explore the painting technique, with the prime aim of characterising the complex structure generally distinguishing polychrome objects, made of both organic and inorganic compounds. On the occasion of the scheduled restoration, micro-samples were collected from different areas of the painted surface and studied with a multi-analytical approach based upon an integration of optical microscopy (OM), scanning electron microscopy with energy dispersion spectroscopy (SEM–EDS), Fourier transform infrared microspectroscopy (µ-FTIR) and Raman microspectroscopy (µ-Raman), in accordance with the established analytical approach validated by previous studies on wall paintings [7–10]. Since the restorers hypothesised the whitish patina to be ascribable to a decay mechanisms presumably deriving from biological activity—decreasing readability of the surface, altering the original colours and creating a particular risky situation for the painting—biological analyses were also performed for the exact characterisation of the patina itself, in order to identify the microbial species covering the artwork and, thus, helping in the choice of the most suitable biocides for the treatment of the biological attack. The results gathered from the analyses played, therefore, a fundamental role in the methodological choices and materials adopted during the restoration. For the first time, evidence was gathered on the materials used by Longhi for the making of Marriage at Cana, providing information on the artist's painting technique. Furthermore, data related to previous restoration materials and biological decay allowed to understand the reasons underpinning the bad state of conservation of the painting, supporting the restorers in selecting the most suitable products to be used for the removal of the biological attack and the integration of the lacunae.

**Experimental studies**

In Fig. 1 sampling points of the analysed samples are shown. Two micro-fragments (MC1 and MC2) were collected from original portions of the artwork and prepared in cross-section to investigate the painting technique. Sample MC3 was taken from a restored area, in order to obtain a cross-section and study the backing of the painting. MC4 was scratched from the surface to gain insights on the possible presence of organic matter on the surface of the artwork. The analytical protocol selected for the pictorial samples (MC1, MC2, MC3) is explained in the following sections “Preliminary operations: documentation and preparative”, “Optical microscopy (OM)”, “Scanning electron microscopy coupled with energy dispersion system (SEM-EDS)”, “Raman microscopy (µ-Raman)” and “Fourier transform infrared microscopy (µ-FTIR)”. For characterising the whitish patina, three samples were collected following a well-established procedure described in “Biological analyses” section.

**Preliminary operations: documentation and preparative**

Preliminary visual inspection was performed on all samples by an Olympus SZ61 stereo-microscope with fixed ocular magnification of 10× and objective magnification up to 4.5×, associated with an Olympus Soft Imaging Solutions GmbH model SC100 camera. Pictorial samples (MC1, MC2 and MC3) were embedded first in a KBr pellet and then in polyester resin, following an established methodology [11]; samples were all dry-polished using abrasive papers of decreasing grain, in order to expose the stratigraphic section avoiding the loss of layers. Surface sample (MC4) was partly treated with 3 M HCl to reduce the presence of calcium carbonate.

**Optical microscopy (OM)**

Following embedding and polishing procedure, stratigraphic sections were all observed and documented by using an Olympus BX51M optical microscope with a 10× fixed magnification ocular and objectives of 5×, 10×, 20×, 50× magnification was used for observation of the samples and their photographic documentation; under VIS light an Olympus U25LBD filter was used, while the UV source was an Olympus U-RFL-T. Images were acquired by Primoplus software and an Olympus DP70 scanner directly connected to the microscope.
Scanning electron microscopy coupled with energy
dispersion system (SEM–EDS)
A SEM Quanta Inspect S FEI, equipped with a Philips
New XL-30 microprobe was used for analysing cross-
sectioned samples, to investigate micro-textural features
of the layers and perform preliminary elemental inves-
tigation. Analyses were carried out in high vacuum on
areas and spots for the characterisation of the pigments
within the cross-sections at 30 kV, with a tungsten fila-
ment current of 100 μA, 100 s of acquisition and 10 mm
working distance.

Raman microscopy (µ-Raman)
Raman measurements were carried out on cross-sect-
tioned pictorial samples, in order to provide in-depth
spectroscopic characterisation of the pigments. Spec-
tra were collected with a Bruker Senterra Microscope
equipped with a BX40 Olympus microscope (20× and
50× objective lens) fitted with a 785 nm laser and a CCD
detector (1024 × 256 pixel, thermoelectrically cooled to
−70 °C). The analyses were carried out with a 1 mW laser
power, in the 50–2600 cm⁻¹ spectral region and with a
resolution of 3 cm⁻¹.

Fourier transform infrared microscopy (µ-FTIR)
Fourier transform infrared microscopy analyses were
carried out with a Nicolet iN™ 10MX microscope
coupled to a mercury cadmium tellurium (MCT) detector
cooled with liquid nitrogen to reduce heat-related
electrical noise. This was used in the attenuated total
reflection (ATR) mode with single-point acquisition on
cross-sectioned samples MC1, MC2, MC3 and MC4, and
in diamond cell mode on sample MC4, after treatment
with 3 M HCl to reduce the presence of calcium carbon-
ate, interfering in the measurement. ATR investigations
of the sections were made with a conical germanium
crystal. The spectra were taken in a range from 4000 to
675 cm⁻¹, with a spectral resolution of 4 cm⁻¹, using vari-
ables of aperture and number of scans dependent on the
type of analysis and information being sought.

Biological analyses
In three different areas of the artwork showing biologi-
cal contamination (see Fig. 1), samples were collected fol-
lowing established techniques [12–14] with (i) cotton dry
swabs for cultural analysis and ATP assay, wiping across
a 100 cm² area, (ii) sterile membranes of nitrocellulose
(0.45 μm pore-size, Millipore; 47 mm in diameter), gently
pressed for ten seconds over the white spots, then imme-
diately transferred to the surface of 6 cm Petri dishes con-
taining dichloran 18% glycerol (DG18) agar [15] and (iii)
removable transparent adhesive tape (Fungi Tape; Sci-
cientific Device Lab., Glenview, Illinois, USA; 1 mm thick,
n.745), gently pressed over spots, to collect eventual
fruiting structures and spores. Each swab of microbial
growth was immersed in a sterile vial containing 5 mL
of Ringer’s solution, and homogenized by vortexing. Ali-
quets (100 μL) of homogenate were spread on each 9 cm
Petri dishes (2 plates per sample) containing separately
DRBC (dichloran rose bengale agar) and DG18 agar, a
selective low water activity (aw) medium for xerophilic
moulds. Plates were incubated respectively for 7 and
14 days at 20 °C in the dark. Nitrocellulose membrane on
DG18 plates were incubated at 20 °C in the dark. After
colony appearance, membranes were removed and por-
tions of the colony were picked up for isolation in pure
culture on DG18 agar plates.

Fungal isolates were identified to genus level using bio-
metric and microscopic features. Swabs for ATP quan-
tification were analysed following a modified method
described by Rakotonirainy and colleagues [16]: ATP was
extracted incubating each swabs in Glo Lysis buffer (Pro-
mega) for 20’ at 25 °C and brief vortexing. The quantifica-
tion of ATP contained in the suspension was performed
immediately after extraction by using the ENLITEN ATP
Assay System (Promega, USA, Madison) and a Lumi-
nometer (Pbi SPA-Milan). An additional swab wiped
across a 100 cm² not contaminated area was used as a
negative control.

Fungi tapes were aseptically mounted on a glass slide
with a drop of fluorescein diacetate (FDA) solution
(20 mg of FDA in 1 mL of phosphate buffer pH 7.3).
FDA staining was used for observations of active struc-
tures using an inverted epifluorescent microscope (Nikon
eclipse T2000) equipped with a FITC filter (blue excita-
tion wavelength: 495 nm). Active structures (positive
staining) were assessed by the presence of a greenish flu-
orescence emanating from the cytoplasm of spores and
hyphae, due to the liberation of fluorescein by enzymatic
(hydrolytic) cleavage. Samples stained with FDA were
observed after 20 min of incubation in the dark at 20 °C.
All slides were examined at 400 and 600× magnification.
Micrographs were acquired using a digital camera con-
ected to a PC equipped with NIS Elements software
(Nikon).

The efficacy of three different biocide products in
aqueous solution against the causal agent of the mural
painting deterioration was evaluated by in vitro test. A
preliminary in vitro test was run using a modified ver-
sion of the Italian UNI-NORMAL Reference 38/93 [17].
The commercial name, the main active principles and the
concentration used in the tests are reported in Table 1.
The causal fungal organism was grown for 2 weeks on a
DG18 agar plate culture. Form the margin of the colo-
nies, 6 mm individual mycelial plugs were transferred in
the centre of new 9 cm DG18 agar plates. On each plate,
for any product, cellulose sterile filter disks, soaked at the three different concentration plus one soaked just with water, were plated at 2.5 cm from the fungal plug, on the axis of an imaginary cross with the plug in the middle. For any product three replicates were considered. After 2 weeks, the biocides’ inhibition activity was calculated by summing the distances from the plug to the margins of the growing colony on all four axes of the aforementioned imaginary cross (Fig. 4). Selected concentration of the same products tested in vitro (Table 1) were applied with a brush over small portions (10 × 10 cm) of the painting characterized by a homogeneous white fungal contamination, using a Japanese tissue for a better spread of the products. Two additional area of the same size were considered as controls, being the one untreated and the other treated with water. After 1 week, for any treated and untreated area, one half of each area were sampled with sterile swabs for ATP assay and the other half with sterile nitrocellulose membranes for cultural analysis, as described before.

Results and discussion
This part of the article will be divided into four sections, respectively focusing on: the study of pictorial technique “Painting technique”; the identification of the restoration products used on the occasion of previous interventions “Identification of restoration products”; the current state of conservation, centred upon biological alteration “State of conservation: focus on biological analyses”; insights into the restoration intervention undertaken in 2016, to highlight how analytical data supported decision-making “The restoration of 2016”. Overall results for each sample are provided in Table 2, while data from analyses performed on each sample are presented in Additional file 1: Tables S1–S3. The interpretation of Raman and FTIR spectra was performed by comparison with references from the literature [18–25].

Painting technique
The characterisation of the painting technique was carried out based on the data obtained from OM, SEM–EDS, μ-FTIR and μ-Raman analyses performed on the cross-sections of samples MC1 (pink) and MC2 (azure). Detailed description of micro-stratigraphies and analytical data obtained for each sample are reported in Additional file 1: Tables S1, S2.

Over a non-original beige layer made of acrylic resin and cellulose (n.1)—ascribable to the detachment intervention carried out by Ottorino Nonfarmale, whose characterisation will be discussed in detail in the following section “Identification of restoration products”—samples MC1 and MC2 both show the presence of a first brown layer (n.2), made of fine dark grains in a homogeneous matrix. SEM–EDS measurements performed on

Table 1 Commercial name, active principle and selected concentration of the tested products

| Commercial product | Active principle                                      | Concentration for in vitro tests (%) | Concentration applied on the surface |
|--------------------|-------------------------------------------------------|--------------------------------------|--------------------------------------|
| Biotin T           | Octylisothiazoline; ammonium quaternary salt          | .5                                   | ✔                                    |
| Biotin T           | Octylisothiazoline; ammonium quaternary salt          | 1.5                                  |                                     |
| Biotin T           | Octylisothiazoline; ammonium quaternary salt          | 3                                    | ✔                                    |
| Bio104             | Octylisothiazoline; ammonium quaternary salts         | .5                                   | ✔                                    |
| Bio104             | Octylisothiazoline; ammonium quaternary salts         | 1                                    |                                     |
| Bio104             | Octylisothiazoline; ammonium quaternary salts         | 2                                    |                                     |
| BC50               | Ammonium quaternary salts                             | 1                                    | ✔                                    |
| BC50               | Ammonium quaternary salts                             | 2                                    |                                     |
| BC50               | Ammonium quaternary salts                             | 4                                    |                                     |

In the last column, selected concentration of the same products applied on the painted surface are reported.

Fig. 4 Example of in vitro test: inhibition activity is determined by summing the four distances from plug to the margins.
the layer showed the presence of relatively high contents of Pb, followed by lower Fe, Ca, Si, and C. FTIR bands at 1395 cm$^{-1}$, 871, 711 cm$^{-1}$ and at 838 and 3521 cm$^{-1}$ allowed identifying the presence of both calcite and lead white [21]; bands related to aliphatic CH stretching (2924 and 2853 cm$^{-1}$) and the ester peak (1729 cm$^{-1}$) characterised the binder as an oil [21]. Lead carboxylates (1538 cm$^{-1}$) were also detected, ascribable either to degradation product or formed as a result of siccative pigment used for accelerating the drying of vegetable oils [22, 23]. Raman analyses performed on the brown layer (n.2) identified carbon black (1330, 1595 cm$^{-1}$) and red lead (121, 550 cm$^{-1}$) [18–20] as pigments (Additional file 1: Tables S1, S2).

In both MC1 and MC2 samples, pictorial layers are superimposed to the brownish one. Sample MC1 shows a homogeneous and relatively thick (50 μm) light pink pictorial layer (n.3), made of calcium carbonate (FTIR bands at 1408 and 873 cm$^{-1}$) and lead white (836 cm$^{-1}$). The binder can be interpreted as a drying oil for the presence of the aliphatic CH absorptions at 2923 and 2852 cm$^{-1}$ associated to the ester peak at 1729 cm$^{-1}$. The broad band at 1042 cm$^{-1}$ is related to silicates, while the bands at 1633 and 1319 cm$^{-1}$ are indicative of calcium oxalate, such as weddellite [24]. The origin of this compound may be due to the oxidation of the organic matter present in the pictorial or coating layers or due to biological mechanisms, such as the metabolism or the mineralisation and collapse of microorganisms. Lead carboxylates (1539 cm$^{-1}$) are also found. Raman analyses were performed on the yellow grains in the layer, but analysis failed to produce a spectrum; considering the presence of Fe in SEM–EDS and silicates demonstrated by μ-FTIR analysis, it can be hypothesised that these yellow grains are yellow ochres.

Table 2  Samples collected from the artwork, with details of performed analyses and summary of achieved results

| Sample  | Colour     | Photo | Analyses                                                                 | Results                                                                 |
|---------|------------|-------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
| MC1     | Pink       |       | Cross-section analysed by SEM–EDS, μ-FTIR and μ-Raman                   | 4—calcium oxalate                                                       |
|         |            |       |                                                                         | 3—calcium carbonate, lead white, yellow pigment (maybe ochre), lead carboxylate. Binder: oil |
|         |            |       |                                                                         | 2—calcium carbonate, lead white, red lead, carbon black, lead carboxylate. Binder: oil |
|         |            |       |                                                                         | 1—acrylic resin and cellulose-based compound                              |
|         |            |       |                                                                         | 0—canvas                                                                  |
| MC2     | Blue       |       | Cross-section analysed by SEM–EDS, μ-FTIR and μ-Raman                   | 7—indigo, lead white, calcium carbonate, calcium oxalate. Binder: oil |
|         |            |       |                                                                         | 5, 6—calcium carbonate, lead white, silicates, lead carboxylates, indigo. Binder: oil |
|         |            |       |                                                                         | 4—calcium carbonate, lead white, silicates, lead carboxylates, oil       |
|         |            |       |                                                                         | 3—red lead, vermilion, in a siliceous matrix                              |
|         |            |       |                                                                         | 2—calcium carbonate, lead white, carbon black, red lead, lead carboxylates. Binder: oil |
|         |            |       |                                                                         | 1—acrylic resin, cellulose-based compound                                |
| MC3     | Restoration area | | Cross-section analysed by SEM–EDS and μ-FTIR | 3—calcium carbonate                                                       |
|         |            |       |                                                                         | 2—acrylic resin and cellulose-based compound                              |
|         |            |       |                                                                         | 1—calcium carbonate, animal glue, lead white                              |
| MC4     | White patina |       | Sample treated with HCl and analysed by μ-FTIR                        | Acrylic resin and a cellulose based compound                              |
As sample MC2 was taken from a border area between the blue painted building on the background and the orange dress of a character, the micro-stratigraphy of the sample shows the presence of both colours. SEM–EDS analyses carried out on the orange layer (n.3) highlighted the presence of relatively high lead contents, and further investigation by μ-Raman allowed characterising the pigment as red lead for the bands at 121, 151, 224, 313, 390, 549 cm⁻¹. A few red particles were analysed as well, and the bands at 254 and 343 cm⁻¹ recognised vermilion [18–20]. Lead was identified as the main component of greyish layers n.4, n.5 and n.6 under SEM–EDS inspection, with small contents of iron, magnesium, and silicon were also found, possibly related to earth-based pigments; calcium was also detected. μ-FTIR analysis demonstrated the presence of a carbonate matrix (1398 cm⁻¹), made both of both calcium (875 cm⁻¹) and lead (838 cm⁻¹) carbonates. Peaks in the range 1000–1100 cm⁻¹ are related to silicates, confirming the use of earth-based pigments, while the intense and strong bands of the aliphatic CH (2922 and 2853 cm⁻¹) and the weak peak of the ester (1732 cm⁻¹) identify the binder as a drying oil. Raman analyses allowed to identify on layers n.5 and n.6 the presence of indigo due to the characteristic doublet at ~1575 and 1585 cm⁻¹ [19, 20]. SEM–EDS and μ-FTIR analyses showed that the azure layer (n.7) was made of calcium carbonate (1396 and 872 cm⁻¹) and lead white (838 cm⁻¹), with drying oil as a binder (2921, 2851, 1728 cm⁻¹). Lead carboxylates (1536 cm⁻¹) and calcium oxalate (1631 and 1316 cm⁻¹) were found as well. The pigment was identified by μ-Raman as indigo, a natural organic dye with characteristic bands at 1585, 1573, 1311, 599, 541, 252 cm⁻¹.

Identification of restoration products
The cross-section of sample MC3, taken from a restored portion of the painting, was analysed by means of OM and μ-FTIR (Additional file 1: Table S3). The first layer (n.1), applied over the canvas intended to support and hold the painting after the detachment, is mainly composed of calcium carbonate (FTIR peaks at 1396, 873 cm⁻¹); the presence of lead white (1078, 3637 cm⁻¹) was also detected. Furthermore, the occurrence of proteinaceous material can be hypothesised for the aliphatic CH absorptions (2923 and 2851 cm⁻¹) and the weak peaks around 1653 and 1592 cm⁻¹, ascribable to the amide I and II [21]. The overlying layer (n.2) is composed of a cellulose matrix, as indicated by the strong absorption at 1022 cm⁻¹ [25] mixed with another organic compound, identifiable as an acrylic resin for the characteristic bands in the aliphatic CH range (2870, 2927, 2959 cm⁻¹), the strong ester absorption at 1728 cm⁻¹, and the weak peaks at 1491 and 1384 cm⁻¹ [21]. In this layer, the presence of pigments was not detected. The most suitable hypothesis is that it functioned as waterproof and adhesive structure, onto which the original detached pictorial layers had been attached. Achieved data are, thus, in good agreement with the summary of the detachment procedure adopted by Ottorino Nonfar- male in the 1970s, reported in [4] (see “Introduction”).

FTIR analyses performed on sample MC4, scratched from the painted surface, showed the presence of an organic compound, recognisable for the bands in the 2850–3000 cm⁻¹ range. The intense peak at 1722 cm⁻¹ and the ones around 1460, 1375, 1236, 1142 cm⁻¹ are consistent with an acrylic resin, like Paraloid B72 or Acril 33. The band at about 1630 cm⁻¹, associated with the strong absorption of the OH bond visible over 3000 cm⁻¹ and the one at 1020–1050 cm⁻¹, indicates the presence of a cellulose-based compound [25] (Fig. 5).

Hence, it can be hypothesised that the “organic material able to avoid humidity” [6] placed on the painted surface when “facing” operations were being performed, was an acrylic resin applied on the wall painting to consolidate the pictorial layer prior to the detachment, as well as to avoid that the subsequent removal of the glue (used for “facing” operations) with hot water could compromise the pictorial surface, made with an oily binder.

State of conservation: focus on biological analyses
The study of the state of conservation was mainly focussed upon in-depth biological characterisation of the species grown on the painted surfaces, as the restorers hypothesised the whitish patina to be ascribable to a decay mechanism presumably deriving from biological activity.

Sampling procedure using sterile swabs followed by serial dilution produced on agar media only few and heterogeneous colonies. Direct plating nitrocellulose membrane on DG18 plates produced after 7 days many slow-growing white colonies, slightly depressed in the middle. All colonies were of similar morphological and biometric features. After re-isolation on DG18 agar, white colonies were observed to grow at a rate of 6 mm per week. As agar medium get drier (about 2–3 weeks after inoculation), colonies developed cleistothecia that were globose, white to cream in colour, 150–200 mm in diameter. Conidial production is not observed on agar plate. All these features are typical of the species Eurotium halophilicum (An. Aspergillus halophilicus). This peculiar organism is an obligate xerophilic fungus with high tolerance to water stress. The minimum water activity for its germination and growth of this 0.675, and growth does not occur above 0.935 [26]. Recently, it has been associated with paper and books biodeterioration in museums, libraries or archives [13, 27]. In vivo it
is present only in its anamorphic state, while in vitro it shows only the teleomorphic state. Its growth seems to be supported by organic and synthetic dust [13].

Optical microscopic observations of adhesive tape samples revealed the presence of fungal structures, conidiophores and conidia, typical of *A. halophilicus* (Fig. 6a, b). Observations of fungal elements trapped on the adhesive tape showed only conidial states. Fungal structures stained with FDA, observed with an

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**Fig. 5** a) µ-FTIR spectrum acquired on sample MC4, showing the characteristic peaks of acrylic resin and those related to cellulose compounds (~1000, 1634 cm⁻¹); b) acrylic resin standard (Paraloid B72 by phase)

**Fig. 6** a) *Aspergillus*-like conidial head and b) spores captured by Fungi Tape; c) active spores stained with FDA emitting green light observed under epifluorescent microscope; d) ATP assay on contaminated and uncontaminated areas; e) in vitro Agar diffusion test; f) results of the in vivo ATP assay
epifluorescent microscope using blue filter, fluoresced green showing an enzymatic activity (Fig. 6c).

ATP assay results are reported in Fig. 6d. Samples 1–3 refer to contaminated area, negative control refers to uncontaminated area.

Several biocides were tested on laboratory cultures, with the aim of identifying which typology was the most effective. Combining the results of the analytical findings, which highlighted the presence of acrylic resin both on the surface and in depth, biocides which required an organic solvent were excluded. The retention of the organic solvent and its penetration through the layers of the painting could have compromised the stability of the work by damaging the adhesives used in the restoration. In addition, the volatility of this kind of solvents could have acted on the final protective product causing bleaching and compromising the colors.

The choice of the biocide to be used has thus fallen on a product soluble in deionized water, which is an ideal solvent for several reasons. First, it would inhibit the growth of the fungus by itself (being an obligate xerophilus); moreover, it would have had no negative interactions neither with the painting technique nor with the acrylic resin used in the various restoration phases; finally, it would have helped to remove the residues of adhesive of a protein nature and the fragments of canvas found on the surface.

Following the culturing tests, three products in aqueous solution (Biotin T, Bio 104 and BC50) were identified and firstly tested in vitro at different concentrations. Results are reported in the graph in Fig. 6e, where a lower value means a higher inhibition activity.

The three biocides were then tested in vivo, with the addition of two further tests: a blank (related to a contaminated area but not treated with biocides) and an “only deionized water” test. Each test was carried out by brush spread with interposition of Japanese paper (a total of 8 defined and numbered areas). After a sufficiently long time, with the same methods (cotton dry swabs, sterile membranes and adhesive tapes) new samples were collected for the examination of efficiency through the same cultivation, microscopic and biochemical methods described above.

The results are reported in Fig. 6f. For all the products the inhibition activity is higher at lower concentration. As expected, the only deionized water test inhibited the viability of the fungus. However, the application of water alone without the addition of the biocide could have facilitated the attack of more hydrophilic fungi. Thus, on the basis of the results obtained with the cultivation and ATP analyses, it was decided for an aqueous solution with Biotin T biocide at 0.5% concentration, which proved to be the most effective.

Finally, few residual colonies (about 0.1–0.2 CFU/cm²) of E. halophilicum are observed after treating with Biotin T and BIO 104 at low and high concentration. More colonies are observed after treating with BC50 at low concentration (0.5 CFU/cm²) and in untreated area (0.9 CFU/cm²). Sample from the area treated with water shows the presence of some Cladosporium sp. colonies but not E. halophilicum.

The restoration of 2016

Information gained on the pictorial technique and surface alteration played a fundamental role in the selection of the most suitable procedures and materials to be employed for the restoration of the wall painting carried out in 2016.

Since analyses had demonstrated the presence of both E. halophilicum fungal species and acrylic resin on the surface, all biocides needing an organic solvent for their dilution had to be excluded: on the one hand, the retention of an organic solvent and its penetration through the layers of the painting could have compromised the stability of the artwork by damaging the adhesives used in the “backing” operation; on the other hand, the volatility of these solvents could have acted on the final protective layer causing its whitening and, thus, compromising the colours. A biocide that could be dissolved in deionized water was, thus, selected, as it would have inhibited the growth of the fungus without negative interactions either with the painting technique or the acrylic resin. Taking all this into account, to counteract the formation of the fungal species on the painted surface, an aqueous solution of Biotin T biocide was laid upon it, with Japanese paper functioning as a support. In order to guarantee a longer-lasting effect, a more diluted solution of biocide (0.5%) was first applied, followed by a second application of a more concentrated product (3%).

Results obtained by analyses carried out for the identification of the painting technique played an important role in selecting the most suitable methodology and products to be used in the cleaning of the pictorial surface, as well as in the following filling of the lacunae and retouching operations. All selected methodological approaches and materials did not compromise the original painting and they did not interact with the support the artwork had been put on after the detachment.

Regarding the cleaning, as the painting had been executed by using an oily binder, a first dry, scalpel-aided mechanical removal of the fragments of canvas adhering to the painted surface, residues of the “facing” protective system applied on the occasion of the detachment during the 1970s restoration, was carried out. Then, the glue deposits were cleaned by using deionized water, by means of localised and controlled treatments. Last,
cleaning of the whole pictorial surface from the acrylic resin identified through FTIR measurements was made by using butyl-acetate, a non-polar solvent meeting two fundamental requirements: (a) not being excessively volatile, to avoid surface bleaching and (b) having a poor penetrating power to avoid interactions with the original layers of the painting.

Concerning the filling of the lacunae, it was appointed after preliminary on site tests with different types of stuccoes made of Bologna gypsum and Aquazol 200, a poly(2-ethyl-2-oxazoline) water-based adhesive. A study aimed at evaluating the effectiveness of this material was conducted by Julie Arslanoglu, comparing Aquazol with other water-based adhesives commonly used in the consolidation of the painted layers, such as PVA, animal gelatine and sturgeon glue. Results demonstrated that Aquazol behaves as a weak adhesive and can, therefore, be used where only moderate adhesive forces are required, as in the case of delicate polychromies [28]. It was, thus, decided to use a 10% water-based solution of Aquazol 200, in a 1:2 ratio to gypsum, as it showed adequate cohesion and adherence to the surface. As it is a water-soluble adhesive, the reversibility of Aquazol is also granted, avoiding the interaction with the oily binder of the artwork.

The subsequent phase of the intervention was the selection of the most suitable solution to be adopted for appointing retouches able to provide aesthetic connection between the well-preserved upper portion of the painting and the lower area, most damaged. The intervention methodology developed for the reconstruction of the missing parts, differentiated according to the areas to be treated, can be considered particularly innovative compared to the traditional approach, with particular reference to the solution adopted for the lower portion of the painting [29]. This area was gradually retouched in undertones by the addition of several overlapping layers, to enhance the appraisal of the entire artwork and to avoid a strong and unaesthetic contrast compared to the pristine upper part. Here, where needed, the pictorial retouching of small lacunae was performed in undertones compared to the original portions of the wall painting. Specifically, tone on tone integrations were also executed in correspondence of the vertical cracks and lacunae in the central portion of the painting (Fig. 7). For retouching, Laropal A81 was used as a pigment binder, a urea-aldehyde resin highly resistant to aging and with close optical properties to those of natural resins. Given its low molecular weight, it has low viscosity, excellent leveling power and high gloss, making it particularly suitable for oily painted surface [30].

Conclusions
Analyses carried out on the painting Marriage at Cana allowed an in-depth characterisation of the painting’s original materials, making technique and state of conservation.

On the one hand, data provided the first comprehensive characterisation of Longhi’s Marriage at Cana, considered his greatest and last artwork and being of
utmost importance for the Biblioteca Classense and the Romagna region, providing new insights on materials and techniques able to enhance the actual knowledge of this painter and his working technique. The artwork was executed with a dry painting technique, with a siccative oil as binder. Original plaster was not found on the cross-sections, the lower beige layer being made of acrylic resin plus a cellulose-based compound ascribable to the “backing” operations after the painting had been detached. Furthermore, OM documentation showed the presence of canvas residues on the back of the samples, referable to the “backing” procedures as well. Data seem, therefore, to suggest that the painting was actually torn (strappato) and not detached (staccato) from the wall, removing only the pictorial film and the preparatory layer. Last, all the identified materials, both pigments and binders, like indigo, red lead, carbon black, calcium carbonate, lead white, ochres and vermilion, are quite common in art history and no modern pigments were detected.

On the other hand, information achieved on the materials applied during previous interventions, with specific reference to that carried out in 1970s, and the patina covering the surface, both responsible for the precarious state of conservation of the work of art, strongly supported in setting the basis for the intervention of 2016. Data obtained by biological studies and FTIR analysis suggest that the surface patina is due to the presence of fungal species Eurotium halophilicum. There currently are no specific literature data on nutritional citations of this species, but it is mainly found on the covers of the books, without a particular preference among covers in cloth, parchment or cardboard. It rather seems, therefore, that the common denominator is represented by the occurrence of organic compounds (i.e. protective, consolidating product or resins) associated with these covers. It can, thus, be hypothesised that a jointed action occurred, as the presence of the acrylic resin identified by FTIR measurements could have triggered the growth of the fungus. Although no literature data are available on nutritional preferences of Eurotium halophilicum, the presence of biological attack is often found on synthetic materials, where they do not provide a carbon source but in some still not fully understood way interact with biological species [31, 32].

Taking these results into account, Biotin T was selected as the most effective biocide and the biological attack was stopped. Moreover, to avoid the onset of further biological attacks, canvas residues attributable to previous detachment operations were carefully removed from the painted surfaces. Materials and methods to be employed in filling of the lacunae and retouching were also carefully selected on the basis of achieved data on the painting technique, in order to avoid the risk of further damaging the delicate polychromies, highly deteriorated and made with a dry, more sensitive, medium.

The discussed study case stands, thus, as an explicative example of how a restoration intervention can represent a fundamental occasion of confrontation among specialists from different research field, working together for answering specific questions aimed at effectively preserving the artwork for the future.

**Supplementary information**

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### Authors’ contributions

FF designed the work, carried out OM, µ-FTIR and µ-Raman analyses and elaborated the data; SF supervised the research and the preparation of the manuscript; MM performed biological analyses and interpreted the data; CRM and ADB carried out the restoration; MV co-ordinated research activities and made Laboratory’s facilities available. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Analysed samples (cross-sections) are stored at the Department of Cultural Heritage, University of Bologna-Ravenna campus, within the Conservation Science Laboratory. The wall painting analysed for this study is currently displayed and curated at the Biblioteca Classense, Ravenna, Italy.

### Competing interests

The authors declare that they have no competing interests.

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