A multidisciplinary diagnostic approach for the restoration of the inside surfaces decorative plaster of the vault of the late seventeenth-century Sant'Angelo Church in Monopoli (Bari, Italy)

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Abstract
The metropolitan church of Sant'Angelo (Monopoli, Bari - Italy), rebuilt in 1675 (Bellifemine, 1979) on the pre-existing medieval church, shows numerous forms of decay; the church is decorated with stuccoes and painted plaster dating back to the first half of the eighteenth century. The liturgical space has been heavily altered by extensive biological formations and sulfation that covered decorations, and by surface gaps, due to copious infiltration of rainwater caused by the absence of maintenance of the church since 1920 and also the deprivation of the roof since 1972. The diagnostic of degradation was supported by mineralogical, petrographic, chemical, and biological investigations performed with optical UV/VIS and electron microscopy, visible spectrometry. Biological samples, scraped and collected from the church vault, were duplicated and isolated by spread plating on plate count agar medium. The total DNA was extracted and the PCR products were sequenced and DNA similarity check was performed using the Gene Bank and EMBL databases. The various specialist analyses foreseen in the diagnostic project, preliminary to the restoration intervention, have highlighted important correlations between the technologies and the materials used in the re-editions and formal enrichments, datable between the end of the eighteenth century and the first half of the nineteenth century, as well as the disasters suffered over time, remedial actions and the long phase of abandonment of the church starting from the Second World War. Finally, the use of infrared thermography and the detection of the decorative apparatus with a 1:5 scale 3D laser scanner, accompanied by monitoring the physical and microclimatic conditions of the environment, helped to define in detail the structural criticalities connected to the interface between the wall structure and the decorative apparatus for timely restoration. Scanning electron microscopy evidenced the presence of fungal and bacterial colonies whose characterization is currently in progress.

Key words
multidisciplinary diagnostic approach, plaster degradation, biological degradation, infrared thermography, SEM-EDS, plasters, potassium alum, 16S rDNA sequencing, bacterial and fungal community

State of art

The 18th century-Church of Sant'Angelo del Borgo, of medieval origins, "rebuilt on the street level in the year 1675", received renovations in different periods between 1741 to 1940 that involved together with the old structures its enrichment through a painted stucco-and-plaster decorative apparatus, to the unity of space and volumes following the architectural taste of the epoch (Intini et al., 2004). The damages caused by WWII and the state of semi-abandonment have further increased the disarray, not only from the structural perspective but also from that of the decorative apparatus made of stucco. In a report of 1949, alongside a remarkable set of cracks which forces the building to be shut, and its side to be shored up to the walls of the opposite block, also the presence, on the inner side of the decorated vault, of "broad humidity stains and recurring separations of stuccoes and plaster due to the relevant seepages of rainwater" are described. In the end of 1970, a municipal decree follows up on this matter and bans the church to worship, with a subsequent securing intervention. The vault deprived of its covering for forty years caused a general worsening of the state of conservation of the internal decorations.

A remarkable amount of the pathologies of decay was related to the presence of water that has, in different ways, affected the painted surfaces, the frames, and the modeled parts made of slaked lime and stucco causing important colonization and, consequently biodeterioration by bacterial and fungal colonies. Since 2015, the Superintendence of archaeology, fine arts, and landscape of the Metropolitan City of Bari started restoration work aimed primarily at making the building safe and secondly at restoring the decorative apparatus of the deteriorated internal surfaces. (Bellifemmine, 1979, 1985; Pirrelli, 2009). A diagnostic plan has been put at the very basis of the surveys and has oriented the work towards a conservative approach to the issue, operating incisive choices in regards with the remotion of the aforementioned additions and the supplementing of the gaps, differentiated according to their role being 'lack' or 'loss'. A multidisciplinary approach was the basis in order to recover the building: architectural approach to verify the stability of the walls, petrographic-mineralogical approach to acknowledge the decorative apparatus entirety and of the different pictorial updates that have taken place in time, biological approach to identify the colonizers responsible of decay and restoration approach to carry out recovery interventions on the asset. This complex work involved different professional skills and their work is reported in this article.

Material and Methods

Petrographic-Mineralogical approach

Four samples of painted plasterwork (CSA1, 4, 6, 9) and a sample of efflorescence (CSA8) from spindle sectors of the pavilion vault of the altar, and a fragment of canopy draping (CSA5) were taken to investigate their structure and composition.

Samples CSA1, 4-6 and 9 were dried at 105 °C for 3 hours and then cooled and embedded in epoxy resin under vacuum for the preparation of thin sections.

Petrographic analysis was conducted on thin sections under a Carl Zeiss Axioskop 40 Pol polarizing microscope (POM). The petrographic description included the compositional and textural aspects of the painted plasters was carried out according to the guidelines of the UNI 11176 standard.

The scanning electron microscope (SEM) used in this research was a 50XVP LEO, operated at 15 kV, 500 pA probe current, about 25,000 cps output as average count rate on the whole spectrum, counting time 50 s and 8.5 mm as working distance. Energy dispersive spectrometric (EDS) microanalyses were conducted using an X-Max (80 mm^2) SDD detector and AZtec software

(Oxford Instruments) for X-ray maps and the correction of X-ray intensity was performed following Pouchou and Pichoir (1991). Different Micro-Analysis Consultants Ltd. (U.K.) mineral standards were used to check the accuracy of the analytical data. The thin sections of the samples were coated with graphite for SEM investigations.
The sample of efflorescence (CSA8) was analysed by X-ray powder diffraction analysis (XRPD), using a Philips X'Pert Pro X-Ray diffractometer under the following working conditions: Cu–Kα Ni-filtered radiation, 40 kV, 40 mA, divergence slit 1°, anti-scatter slit 0.5°, receiving slit 0.2 mm, step angle of 0.02°, 2θ from 2° to 65°, measuring time 1 s per step. The diffraction peaks of the XRPD spectra were identified by comparison with the Joint Committee on Powder Diffraction International Centre for Diffraction Data (JCPDS–ICDD) diffraction chart and the crystalline phases were detected.

Partial Results and Discussion

CSA1: POM of the thin section shows a layered plaster adhering to the calcarenite blocks of the vault (Fig. 1). Layer A is homogeneous and approximately 1 cm thick. The aggregate texture is seriated, with a predominant grain size in the medium sand class (0.25-0.5 mm). The aggregate consists almost entirely of calcareous sand and modest amounts of quartz and feldspars. The texture of the binder varies from micro- to cryptocrystalline. EDS microanalysis of the binder-enriched portion shows the use of lime putty. The primary pores (Figure 1) are less frequent than the shrinkage cracks developed during the setting of the lime plaster.
Layer B is approximately 1 mm thick and has the same composition and microstructures as layer A. On layer B, two paint layers with a total thickness of approx. 0.3 mm can be observed, which were partially damaged by sampling and preparation. Both are lime-based, but in the former case the presence of siliceous clasts is detected, with a maximum size of and around 50 µm (Figure 1). Microanalysis shows that some of these are quartz, while the largest part consists of minute glassy fragments (SiO_2 = 66.5 wt%; K_2O = 15 wt%; BaO = 7.5 wt%; Al_2O_3 = 2 wt%; CoO = 1 wt%; FeO_{tot} = 1.5 wt%; As_2O_3 = 1.5 wt%). This composition can be referred to as *azzurro di smalto* or *smaltino* (Borgia and Seccaroni, 2005; Casoli et al., 2007). Sulphur was detected along the outer surface and at some points in the open porosity of the plaster, as an indicator of the presence of gypsum.

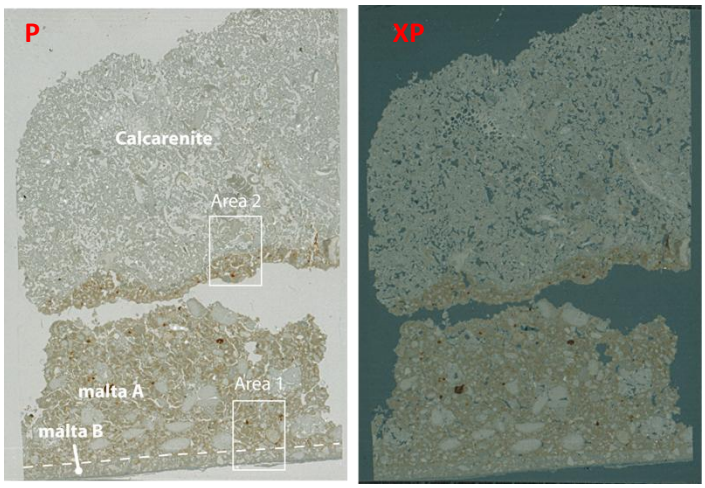

Figure 1 – CSA1, Thin section scans at polarizer only (P) and cross-polarizers (XP) and position of the analysed areas at SEM-EDS

CSA4: The structure is layered. On a plaster similar to the previously described mortar A, two sequences of paint layers occur. The first, approximately 0.1 mm thick, is based on lime putty,

while the second is composed of more or less gypsum-rich layers. From the EDS microanalysis, the use of lime putty as a binder in layer A can be observed.
CSA5: The three analysed fragments are layered, with a total thickness of approximately 0.3 mm. The two innermost layers show a laminated structure and are based on lime and gypsum. In layer B, rare quartz inclusions and clay minerals can be recognised. Layer C, on the other hand, is potassium alum (aluminum potassium sulfate dodecahydrate), with very fine quartz sand and iron oxides. On the outer surface there is gypsum as an alteration of the underlying alum.
In fragment 2 the layers are not always adherent each other. EDS analyses shows compositions comparable to those of fragment 1, although gypsum is present in A and B. The stratigraphic sequence of fragment 3 is enriched by a layer of gypsum and a layer of lime before the alum layer.

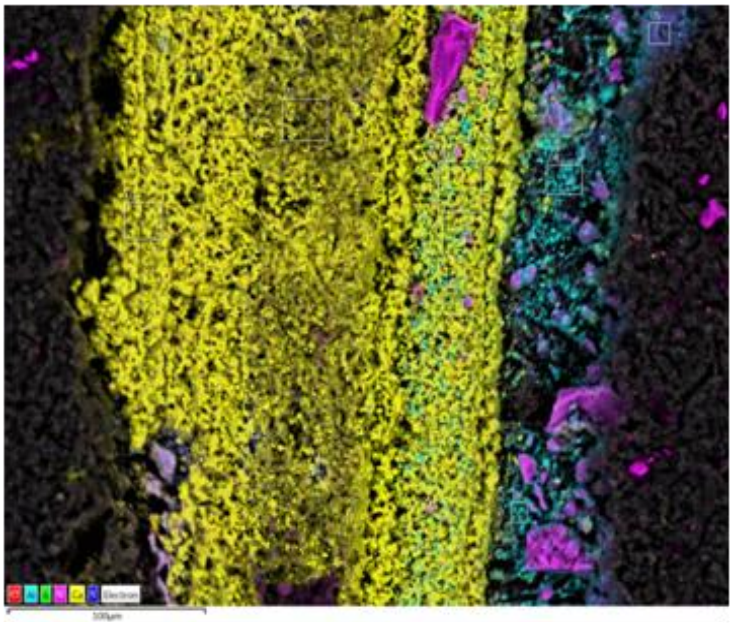

Figure 2 – CSA5, fragment 1: chemical map and portions analysed by EDS microanalysis. In the legend the elements considered in the map.

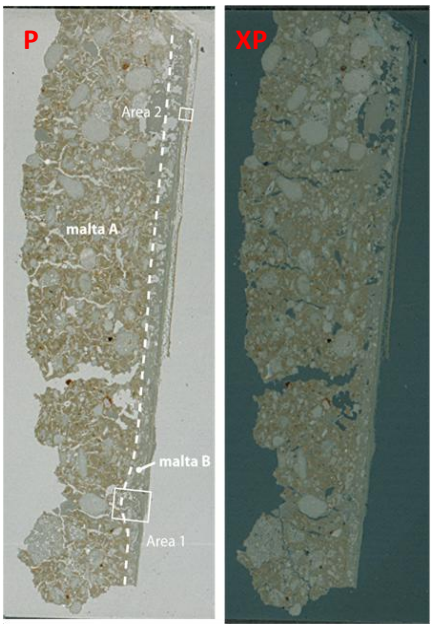

Figure 3 – CSA6, Thin section scans at plane polarized (P) and cross polarized (XP) light and location of the analysed areas at SEM-EDS.

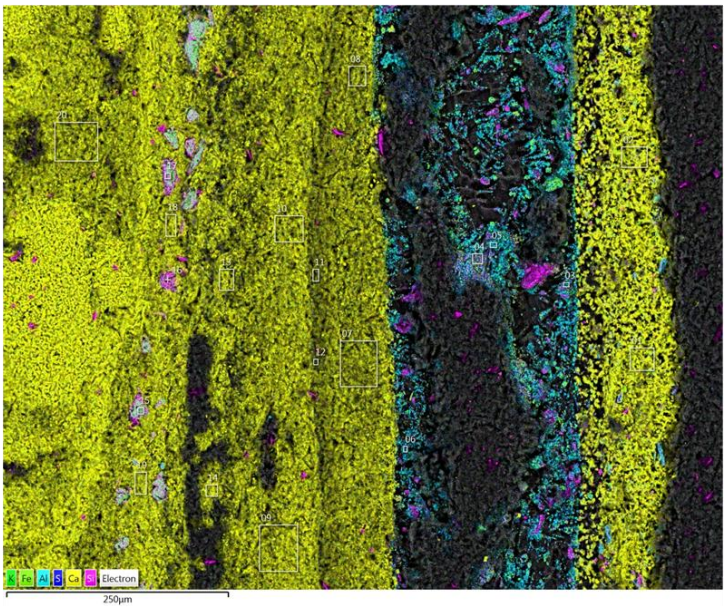

Figure 4 – CSA6, area 2: Chemical map and portions analyzed by EDS microanalysis (see Fig. 1). In legend the elements considered in the map.

CS6: The sample is stratified, with a sequence of paint layers reconstructed between areas 1 and 2 (Figure 2). Layer A shows similar composition and texture to those recognised in sample CSA1 and CSA4. Layer B is approximately 1 mm thick and reflects the characteristics of layer A. In the innermost layer, EDS microanalysis shows that the pigments are minute fragments of *azzurro di smalto*, as in sample CSA1. A lime wash coloured with copper sulphide overlays the blue layer and is followed by three other white lime washes (Figure 3). The outermost layer is alum, with non-negligible concentrations of copper and iron (FeO_{tot} = 0.7 ÷ 12.9 wt%; CuO = 0.6 ÷ 1.8 wt%).
CSA8: XRPD analysis revealed gypsum and traces of calcite as components of efflorescence.
CSA9: Two layers of mortar can be recognised, to which different paint films are adhered. Mortar A shows a mixed composition of lime putty and gypsum of the binder-enriched part. Relics of uncalcined gypsum are observed. The binder of mortar B, on the other hand, consists of lime. Limestone sand is present as an aggregate in both mortars, with a seriated arenaceous texture. The porosity differs in the two mortars: less present and rounded in A and diffuse and branched in B. The thickness of the surface layer C is approximately 0.25 mm. Five layers of 0.05 mm can be distinguished in it, the first composed of lime and the subsequent ones composed of lime and gypsum. On the outer surface there is gypsum as alteration product. In area 2 the surface laminae are articulated differently. The first three lime washes are followed by the alum layer already observed in CSA1 and CSA6. Barium sulphate pigments can be distinguished in this layer. The microcrystalline calcite encrustation covering it shows small concentrations of iron, chlorine, sulphur and barium, while on the outer surface an efflorescence of gypsum occurs.

The aggregate of layers A and B of the samples CSA1, CSA4 and CSA6 and the layers B and C of CSA9 is similar in both particle size and composition. These are lime mortars with arenaceous texture. In the case of CSA9, layer A has a mixed binder of lime and gypsum. CSA5, CSA6 and CSA9 show layers of potassium alum. In CSA1 and CSA6 a lime wash pigmented with *azzurro di smalto* (Borgia and Seccaroni, 2005) can be recognized. *Azzurro di smalto* was preferred to lapislazuli or azzurrite as pigment to be used with lime, because less reactive. This artificial pigment was quite diffused from 15th century and in 19th century was substituted with cobalt blue and synthetic ultramarine (Casoli et al., 2007). Other chromophores are iron oxy-hydroxides (CSA5) and copper based phase (CSA6).
The layers with potassium alum show an alteration in gypsum, caused by infiltrated meteoric water flowed over the surface of wall finishes, probably according to the following reaction:
$KAl(SO_4)_2 \cdot 12H_2O_{(s)} + 2Ca^{2+}_{(aq)} + 4HCO_3^-{}_{(aq)} \rightarrow 2CaSO_4 \cdot 2H_2O_{(s)} + KAlCO_3(OH)_{2(s)} + H_3O^+_{(aq)} + 3HCO_3^-{}_{(aq)} + 5H_2O$
Lower solubility of gypsum compared to that of K-dawsonite may explain its prevalence as degradation product.

Biological Approach
Sampling and bacterial strains isolation collected from inner surfaces by delicately scraping off material using a scalpel into sterile tubes according to the Italian Cultural Heritage Ministry Recommendation n.3/1980 and touching using sterile and dry swabs surfaces was carried out. Bacterial strains were isolated by dissolving sample swabs in 500 µL of sterile Ringer's solution, vortexing for 10 minutes and pipetting 100 µL aliquot into 5 mL of Plate Count Broth (PCB); after 24h incubation at 30°C, the culture was used for inoculation on Plate Count Agar (PCA) plates that were further incubated at 30°C for 7 days. Colonies showing a different morphology and

appearance were transferred to sterile PCA culture plates to obtain pure cultures. After that, the colonies were grown 24h at 30°C on BHI medium (Oxoid). Subsequently, bacteria were plated on sporulation medium and incubated another 4-5 days. After staining the microbiological material with amido black for 60 s and carbolfuchsin for 20 s, light microscope observations were carried out. The total DNA was extracted and the PCR products were sequenced and DNA similarity check was performed using the Gene Bank and EMBL databases. The same procedure was used for the fungal colonies identification.
Vegetative cells and sporulation test results were observed using a standard light microscope, after slide preparation and staining, and, in order to confirm the presence on biological colonizers on the deteriorated wall surfaces, SEM analyses (with the same instrument used for the Petrographic-Mineralogical approach), after silver-coating, were carried out.

Partial results and Discussion

The degradation state of the church was showy noticeable, and the attention was focused on the biodeterioration of the internal surfaces. Figure 5 highlights the state of decay and the sampling areas.

Figure 5 – State of decay and the sampling areas

From the sampling zones #1 - #4 the presence of fungal colonies emerged while the samplings #5- #6 showed the presence of bacterial colonies. Table 1 reports the biological identification while figures 6 and 7 show the SEM images. Notably, all isolated bacteria strains were gram-positive.

Table 1 Biological Colonizers on the vault wall of the church
and the identity percentage to the closest species

Sample	*Species*	*(%)*
#1, # 4	***FUNGAL COLONIES***	
	Penicillium Italicum	89
	Beauveria Pseudobassiana	77
	Cladosporium spp	92
	Alternaria alternata	100
	Parengyodontium album	100
#5, # 6	***BACTERIAL COLONIES***	
	Brevibacillus non-reactive	97.9
	Bacillus cereus 2	96.6
	Bacillus mycoides	13.8
	Bacillus firmus	1.7

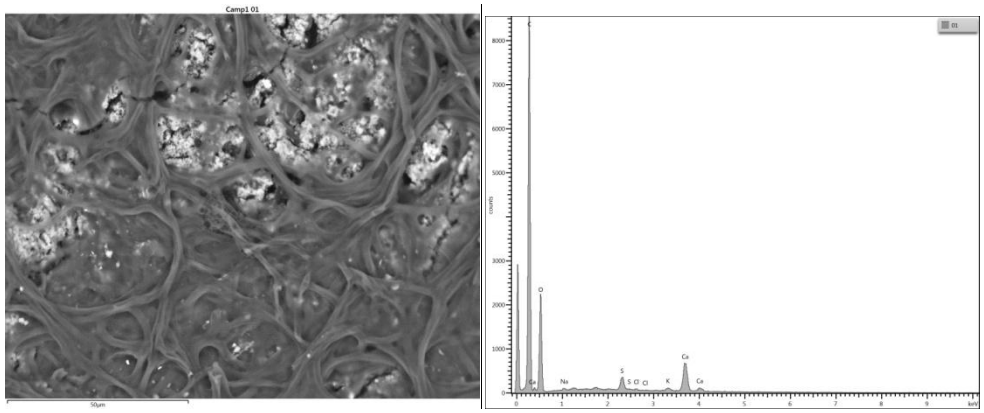

Figure 6 – Fungal colonies and relative EDS analysis

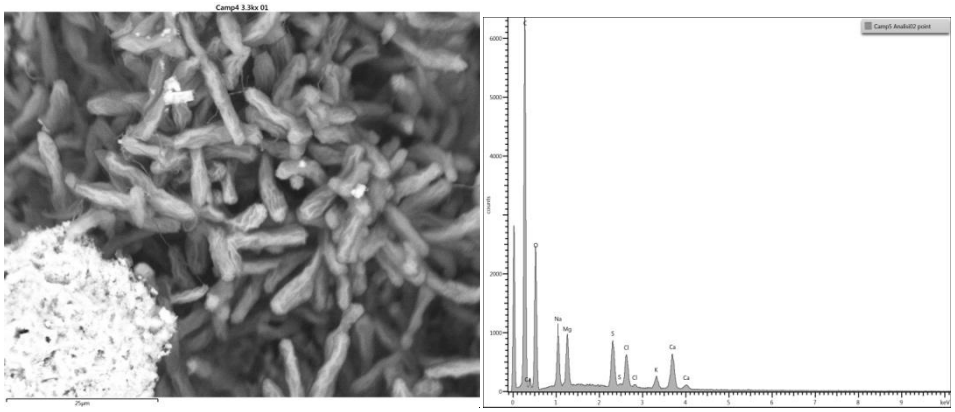

Figure 7 – Bacterial colonies and relative EDS analysis

Weathering of heritage starts as soon as a stone is extracted from its quarry and placed in a building. Climatic agents and microclima (wetness, variation of temperature) contribute gradually to the aging of all materials used, which surfaces can be broken down into smaller particles until the constituent minerals. At the same time, starts the bio-colonization: bacteria are assumed to be the first colonizers; subsequently, other organisms in succession develop, replace them and finally, thanks to the climatic agents and the activity of micro- and macro-organisms, a well-developed soil layer is formed, able to support, also the vascular plant communities (Ariño, et al. 2002, 2009). In this colonization process temperature and moisture must not be limiting factors (Di Carlo et al., 2016).

The prevalence of Bacillus species may be due to the specific environmental conditions, very similar in all sampling sites of the church, and their ability to produce endospores, dormant forms allowing bacteria to survive unfavourable conditions e.g., low nutrition, as the carbonate stone constituting the church walls is favourable for bacterial settlement (Li et al., 2018, Nicholson et al. 2000). The diffusion of microorganisms is linked to air movement (Mulec t al, 2008; Northup et al. 1994) but also it is related to presence of organic matter produced by the pigeons that had settled in the abandoned building. Organic matter, indeed, is as source of carbon for heterotrophic organisms. The identification of bacterial and fungal colonies on the stone material is a problem not limited to the prevention and restoration of aesthetic damages, but it can also be important from a sanitary point of view. Sporigenic bacterial and fungal species can be of health concern for visitors. In particular, the genus Bacillus produces toxins in self-defence, but if produced in excess in an area with poor ventilation, it can cause problems for the most fragile subjects (From et al., 2005).

The restoration approach

Before the restoration and to evaluate the state of conservation of the decorations and the plasters and to secure the duration of the works, a campaign of structural and relief diagnostic has been undertaken through the employment of laser scanner 3D tools, that has allowed to associate the structural deformations to the exact collapses of the decorative apparatus (not show) and which, associated to photogrammetry, resulting into 3D-models, to analyse the enveloping architecture and the low- and high-relief details, reaching a zooming detail equivalent to 1:5 on the scale. This was instrumental to monitor, during the intervention, the addition choices on the 'gaps' by measuring their quantitative incidence. The 3D model has also allowed the restitution of the different chromatic phases and the reconstruction of the missing parts, so that they could be kept documented. A campaign through infrared-thermography tools has allowed to evaluate exact collapses undetectable to the naked eye by critically checking upon the detected gradients through a continuous monitoring of the physical-chemical parameters inside the main body of the church.
In addition and in order to pursue the principle of conservation of the unrelated elements (superimposed) several samples have been executed to attempt a reduction of the chromatic alterations through the application of: a) Agar-Agar mixed with demineralized water, b) Compresses of EDTA in solution with demineralized water in Sepiolite suspension, c) Japanese paper-supported Carbogel, d) Anionic exchanger resins, d) EDTA and Ammonium Carbonate solution-imbued paper pulp compresses. The samples have been executed onto the carbonated surfaces of the stuccoes and of the base-coats of the painted areas.
For the stabilization of the body of the stuccoes which had been exposed from the processes of cracking and fracturing, two products were tested: the acrylic resin Acril ME in a 2.5% water-based solution and limewater (makes the limewater notably rich in calcite). In order to compensate for the loss of broad surfaces we have first proceeded by stabilizing the emerged areas with limewater and then, by reestablishing the compromised layer with a slake lime-based mortar (aged three months), calcium carbonate and yellow cocciopesto (comes from an artisanal furnace) with a 0.1 grain, checking with samples the final effect and the visual interference with the adjacent surfaces.

Conclusion

The multidisciplinary approach of this work has made it possible to better understand the problems existing on the building also caused by restoration operations that are not always adequate but probably linked to the historical period in which they took place. Advanced diagnostic techniques have allowed the restorers to intervene in a timely and conservative manner.
The future intervention planned will be the eco-cleaning by using natural and environmentally friendly substances in substitution of chemicals.

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