A FEASIBILITY STUDY FOR THE REMOTE SENSING OF BIODETERIOGENS ON MURAL PAINTINGS

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Abstract
This paper presents a study devoted to investigate the feasibility of non-destructive remote sensing of biological growth on mural paintings by means of the fluorescence lidar technique. The latter is a technique that can be used to carry out fluorescence measurements without any direct contact with the investigated surface and independently from external environmental conditions like full sunlight. The study initially addressed the investigation of the effects of low-fluence pulsed UV laser radiation on different types of paint layers, prepared either with a fresco or a secco techniques and using different pigments and binders. To irradiate the samples we used a tripled-frequency Nd:YAG laser, emitting at 355 nm, and fluences ranging from 0.1 mJ/cm² to 1 mJ/cm². Different analytical techniques - colorimetry, optical microscopy, Fiber Optical Reflectance Spectroscopy (FORS), Attenuated Total Reflectance (ATR) microscopy and gas chromatography/mass spectrometry (GC/MS) - were applied to the irradiated samples to compare the morphological and physico-chemical properties before and after laser irradiation. The outcomes of this study were used to arrange a second experiment focused on fluorescence lidar measurements on model samples inoculated with different concentrations of biodeteriogens, specifically a green alga (Apatococcus sp.) and a cyanobacterium (Chroococcus sp.). The results demonstrated the feasibility of the remote detection of cyanobacteria and algae on painted surfaces at a pre-visual stage of development by means of the fluorescence lidar technique using a laser fluence of 1 mJ/cm² at the target.

Introduction
Fluorescence-based techniques are widely applied in several scientific fields as a diagnostic tool to detect the presence of photoautotrophic biodeteriogens. The method is essentially based on the detection of the typical fluorescence emission of chlorophyll a, which occurs in the red region of the spectrum. The same principle can be exploited to detect biodeteriogens, from a distance, on monuments by means of the fluorescence lidar technique. The latter is a remote sensing technique which permits to carry out fluorescence measurements without any direct contact with the investigated surface and independently from external environmental conditions like full sunlight. This technique has already been applied successfully to stone cultural heritage to detect biodeteriogens in the course of several field experiments [1]. The technique, in fact, is particularly suitable to operate on site on monumental surfaces, since it does not require the use of scaffolds or lifts and can work independently from most external environmental conditions. The use of the fluorescence lidar technique for non invasive diagnostics on mural paintings,
however, has not yet been thoroughly studied up to now, despite the several advantages it could offer for operation on extended surfaces often difficult to be reached. This study is thus devoted to investigate the feasibility of non-invasive detection of photoautotrophic biodeteriogens on mural paintings by means of the fluorescence lidar technique. To do this, we preliminarily investigated the effects of low-fluence pulsed UV laser radiation, as that typically used in fluorescence lidar applications, on different types of mural paintings. These were prepared either with a fresco or a secco techniques and using different pigments and binders. Different analytical techniques - colorimetry, optical microscopy, Fiber Optical Reflectance Spectroscopy (FORS), Attenuated Total Reflectance (ATR) microscopy and gas chromatography/mass spectrometry (GC/MS) - were used to compare the morphological and physico-chemical properties of the model samples before and after laser irradiation and to identify sustainable parameter settings (laser fluence, number of pulses, etc.) for the subsequent fluorescence lidar measurements. In the second phase of this study we investigated the feasibility of remote detection of biological growth on mural paintings: for this purpose we carried out fluorescence lidar measurements on model samples inoculated with different concentrations of biodeteriogens, specifically green algae and cyanobacteria, simulating in-field experimental conditions (15-m distance from the samples and full sunlight). Fluorescence lidar data were also compared with FORS data as for sensitivity and limits of application.

In the following sections we report a concise description of materials and methods used for the two experiments and a summary of main results.

Materials, instrumentation and methods

We used two sets of model samples: a set of model samples prepared with the a fresco technique and a second set of model samples prepared with the a secco technique. The first set, prepared with the a fresco technique, was made of six model samples. For the paint layer of the samples three pigments were used: bianco San Giovanni (white), yellow ochre and ultramarine blue so that, in the whole, we had two model samples for each different pigment. The second set, prepared with the a secco technique, was made of four model samples, each of them containing white lead and a different binder: animal glue and whole egg, whole egg, skimmed milk, egg-oil tempera.

The model samples used for the measurements to test the effects of low-fluence laser irradiation are reported in Table 1. A number of 2-cm diameter test areas was delimited on each model sample: a fresco model samples were divided into 9 test areas each (so that we had 18 test areas, in the whole, available for each type of pigment). The a secco model samples were divided into 15 test areas each. The study initially investigated of the effects of low-fluence UV laser radiation on the model samples. To irradiate the samples we used a Q-switched tripled-frequency Nd:YAG laser (Continuum, Minilite II), emitting 8 mJ at 355 nm. Typical pulse duration was 5 ns, maximum repetition frequency was 16 Hz. To adjust the laser fluence impinging on the sample we used a variable attenuator placed at the laser output. The variable attenuator was used to finely adjust the laser fluence on the sample up to 1 mJ/cm². These are typical fluence values used for fluorescence lidar remote sensing applications.

Each area was irradiated with a different combination of laser fluence and number of laser pulses. Laser fluence values were varied between 0.1 mJ to 1 mJ, whereas number of pulse ranged between 1 and 500 pulses. Each model sample had also one test area left as a control. In addition, for each type of pigment and/or binder one test area was irradiated with a higher laser fluence (two-order of magnitude higher) in order to induce visible damage on purpose.

We used several analytical techniques to characterise the model samples and compare their morphological and physico-chemical properties before and after laser irradiation. Specifically, we measured the samples properties before and after laser irradiation using the following techniques:

- Optical microscopy,
- Colorimetry,
- Fiber optical reflectance spectroscopy (FORS),
- Attenuated total reflectance (ATR) microscopy,
- Gas chromatography/mass spectrometry (GC/MS).
Table 1 – List of the model samples irradiated using low-fluence laser pulses at 355 nm.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Substrate</th>
<th>Pigment</th>
<th>Pigment composition</th>
<th>Binder</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>a fresco</td>
<td>Plaster (mortar and natural fiber)</td>
<td>Bianco San Giovanni</td>
<td>Calcium carbonate</td>
<td>-</td>
<td>10 cm x 10 cm, (9 test areas)</td>
</tr>
<tr>
<td>a fresco</td>
<td>Plaster (mortar and natural fiber)</td>
<td>Bianco San Giovanni</td>
<td>Calcium carbonate</td>
<td>-</td>
<td>10 cm x 10 cm, (9 test areas)</td>
</tr>
<tr>
<td>a fresco</td>
<td>Plaster (mortar and natural fiber)</td>
<td>Yellow ochre</td>
<td>Hydrated iron oxide</td>
<td>-</td>
<td>10 cm x 10 cm, (9 test areas)</td>
</tr>
<tr>
<td>a fresco</td>
<td>Plaster (mortar and natural fiber)</td>
<td>Yellow ochre</td>
<td>Hydrated iron oxide</td>
<td>-</td>
<td>10 cm x 10 cm, (9 test areas)</td>
</tr>
<tr>
<td>a fresco</td>
<td>Plaster (mortar and natural fiber)</td>
<td>Ultramarine blue</td>
<td>Polysulphide sodium aluminosilicate</td>
<td>-</td>
<td>10 cm x 10 cm, (9 test areas)</td>
</tr>
<tr>
<td>a fresco</td>
<td>Plaster (mortar and natural fiber)</td>
<td>Ultramarine blue</td>
<td>Polysulphide sodium aluminosilicate</td>
<td>-</td>
<td>10 cm x 10 cm, (9 test areas)</td>
</tr>
<tr>
<td>a secco</td>
<td>Plaster (mortar and sand)</td>
<td>Lead white</td>
<td>Lead carbonate</td>
<td>Animal glue + Whole egg</td>
<td>10 cm x 15 cm, (15 test areas)</td>
</tr>
<tr>
<td>a secco</td>
<td>Plaster (mortar and sand)</td>
<td>Lead white</td>
<td>Lead carbonate</td>
<td>Whole egg</td>
<td>10 cm x 15 cm, (15 test areas)</td>
</tr>
<tr>
<td>a secco</td>
<td>Plaster (mortar and sand)</td>
<td>Lead white</td>
<td>Lead carbonate</td>
<td>Skimmed milk</td>
<td>10 cm x 15 cm, (15 test areas)</td>
</tr>
<tr>
<td>a secco</td>
<td>Plaster (mortar and sand)</td>
<td>Lead white</td>
<td>Lead carbonate</td>
<td>Egg-oil tempera</td>
<td>10 cm x 15 cm, (15 test areas)</td>
</tr>
</tbody>
</table>

Optical microscopy, colorimetry, FORS and ATR microscopy were used to characterise both a fresco and a secco model samples. GC/MS was instead applied only to the a secco model samples in order to determine the complex organic components abundantly present in the painting layer containing binders and to detect possible degradation products induced by laser irradiation.

The second experiment consisted of fluorescence lidar measurements carried out on a fresco model samples inoculated with different concentrations of biodeteriogens. The model samples had dimensions of about 3 cm x 3 cm. We selected two types of biodeteriogens, chosen amongst those frequently found in green patinas of outdoor monuments: a green alga (Apatococcus sp.) and a cyanobacterium (Chroococcus sp.). These organisms can be easily found both on stone monuments and on frescoed surfaces. The isolated cultures were inoculated on a fresco model samples using concentrations ranging from 2.1x10^4 to 2.1x10^6 cells/cm^2, depending on the species. The model samples were prepared with the same three types of pigments used for the previous experiment: bianco San Giovanni (white), yellow ochre and ultramarine blue. A picture of the inoculated model samples is shown in Fig. 1: here a fresco model samples with a bianco San Giovanni paint layer are inoculated with different concentrations of green algae ((a) control, (b) 5.1 x 10^4 cells/cm^2, and (c) 1.0 x 10^6 cells/cm^2). It is to be noted that the presence of biodeteriogens on the inoculated model samples could not be detected by the naked eye, except for the highest concentrations examined (approx. > 3x10^5 cell/cm^2) on bianco San Giovanni and yellow ochre model samples.

The instrumentation we used to carry out remote fluorescence measurements was an in-house developed fluorescence lidar. The system featured the laser source described above as an excitation source. The fluorescence emitted by the samples was collected by a 25-cm diameter f/4 Newtonian telescope and fed to an optical fiber bundle. This was coupled to the entrance slit of a spectrometer with a 150 gg/mm grating providing a nominal spectrometric linear resolution at 435.8 nm of 0.51 nm/pixel. The spectrometer exit was coupled with an intensified gated 512x512 pixel CCD detector. Long-pass optical filters were used to reject the laser backscattered radiation and spectrometer higher orders. Data acquisition and storing were controlled via personal computer.
Results

In general, optical microscopy observation and spectroscopic measurements (FORS, ATR, colorimetry) did not point out any significant modification in the examined *a fresco* test areas irradiated using laser fluences between 0.1 mJ to 1 mJ at 355 nm. Results were independent from the number of laser pulses applied to the test area (up to 500 pulses). Higher fluences (88 mJ/cm$^2$, 1000 pulses) could induce a detectable damage only on the yellow ochre sample that showed a colour change in the irradiated area. Fig. 2 shows the reflectance spectra acquired with FORS instrumentation on yellow ochre model sample after irradiation with the highest laser fluence (88 mJ/cm$^2$). FORS spectra referring to the test area irradiated with a 88 mJ/cm$^2$ fluence are labeled 004 (cyano) and 009 (dark green) in the figure. In these two spectra the typical reflectance feature of yellow ochre (goethite) at about 600 nm tends to disappear and assumes the typical behaviour of a more intense wide absorption band, typical of dark ochres (hematite). This spectral variation is reflected in the change of colour from yellow to brownish, and is due to the loss of water molecules in the mineral lattice (iron oxy-hydroxide) and the subsequent formation of iron oxide, typical of natural red/brown earth pigments.

Fig. 2. FORS spectra of the yellow ochre sample after 355-nm laser irradiation (fluence: 88 mJ/cm$^2$).
A secco model samples as well did not show any significant change in their spectroscopic features after laser irradiation using laser fluences between 0.1 mJ to 1 mJ at 355 nm, except for the sample containing animal glue and egg as a binder, where some discrepancies were found in the amino-acid content after laser irradiation. On the contrary, irradiation using a fluence of 88 mJ/cm² produced visible damage after only 2 laser pulses. Thus, the examined a secco model samples were found more sensitive to laser irradiation than the a fresco samples. This behaviour of the a secco samples, however, could be also attributed to the presence of lead white in the paint layer. The GC/MS measurements, actually, did not point out remarkable change in the organic composition of the samples, even after irradiation using the 88 mJ/cm² fluence, neither the presence of degradation products. Such fluence values, however, induced a clear modification in the colour of the paint layer which turned darker, as pointed out also by the colorimetric and FORS measurements. Hence, the modifications induced by laser irradiation at this high fluence value should be ascribed to the inorganic component of the paint layer. This is consistent with other experimental data concerning lead-white containing samples and reported in other studies on laser ablation [2,3].

The outcomes of these measurements were used to arrange the experimental conditions of the subsequent experiment devoted to investigate the feasibility of remote detection of biodeteriogens on a fresco samples. Fluorescence measurements were remotely acquired on the samples inoculated with different concentrations of green algae. The model samples were placed at a distance of 15 m from the sensor. The area measured on the samples surface by the sensor corresponded to a 1.5-cm diameter spot. The laser fluence on the sample was set to 1 mJ/cm². Measurements were acquired in full sunlight. Fluorescence spectra obtained on the samples prepared with bianco San Giovanni paint layer are shown in Fig. 3. The green algae concentrations range from 2.1x10⁴ cells/cm² (dilution: 1:100) to 2.1x10⁶ cells/cm² (dilution: 1:0). Measurements were carried out soon after the inoculation of green algae (few hours). The spectra show the typical fluorescence band at about 700 nm due to the chlorophyll a contained in the green algae. The wide fluorescence band at shorter wavelengths is due to the paint layer. Each fluorescence spectrum was accumulated over 100 laser pulses. Similar results were obtained also on the other inoculated model samples (yellow ochre, ultramarine blue). A set of measurements were also conducted on a fresco model samples inoculated with cyanobacteria. Fluorescence spectra acquired on these samples showed, besides the fluorescence band at 700 nm due the chlorophyll a, an additional fluorescence peak at 660 nm which can be attributed to phycocyanin.

![Fig. 3. Fluorescence lidar spectra acquired on bianco San Giovanni samples inoculated with green algae.](image-url)
Point reflectance spectra acquired on the same model samples (bianco San Giovanni) by means of an optical fiber (FORS measurements, see [4] for details on the technique) are shown in Fig. 4. The spectra show the typical absorption peak at about 680 nm due to the chlorophylls contained in the green algae. As for the other two types of model samples, yellow ochre and ultramarine blue, the detection of biodeteriogens was possible only on the yellow ochre samples inoculated with high concentrations of green algae (>10⁶ cells/cm²). In the case of the ultramarine blue samples, actually, the intense absorption of this pigment in the red prevented the detection of the biodeteriogens on the basis of the chlorophyll absorption peak, which is in fact placed in the same spectral region.

Fig. 4. FORS spectra of the bianco San Giovanni samples inoculated with green algae.

Conclusions

Optical microscopy, FORS, colorimetry and ATR spectroscopic measurements, carried out on a fresco model samples exposed to different laser fluences at 355 nm, did not show any significant modifications after laser irradiation using fluence values between 0.1 mJ/cm² and 1 mJ/cm², independently from the number of laser pulses applied (up to 500 pulses) A similar behaviour was found for a set of a secco model samples (lead white and different type of binders) using the same range of laser fluences. In general, however, a secco model samples were found to be more sensitive to laser irradiation than a fresco samples, as pointed out by some test measurements carried out using a higher laser fluence (88 mJ/cm²). In the latter case, in fact, all the a secco model samples showed a remarkable change in the colour of the paint layer, as detected also by colorimetric and FORS measurements carried out soon after laser irradiation.

In the second part of the experiment a fluorescence lidar was used to detect remotely the presence of biodeteriogens on a fresco model samples. Fluorescence spectra, acquired from a distance of 15 m from the sample, in full sunlight, showed the typical fluorescence band at about 700 nm which is due to the chlorophyll a. Concentrations as low as 2.1x10⁴ cells/cm² could be detected in these experimental conditions on samples inoculated with green algae and using a laser fluence of 1 mJ/cm² at the sample. FORS measurements were also carried out on the same model samples inoculated with green algae to investigate the feasibility of their detection on different types of paint layers. This technique as well was successful in the detection of biodeteriogens, although in some cases the absorption bands of the pigment present in the paint layer can remarkably interfere and make it difficult the observation of the absorption band due to the chlorophylls contained in the biodeteriogens.
References


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