Analysis of Biofilms from Roman Catacombs by Laser Induced Fluorescence and Multivariate Analysis

L. Caneve, F. Colao, V. Spizzichino
ENEA, UTAPRAD-DIM, CR Frascati, Via Enrico Fermi 45, 00044 Frascati (RM), Italy

Abstract

Biofilms grown on tufa and mortar surfaces from Roman catacombs have been analyzed by Laser Induced Fluorescence (LIF). Different results have been obtained depending on the analyzed area of the samples. Well distinguishable emission bands have been pointed out in the fluorescence spectra revealing the presence of different contributions.

Principal Component Analysis (PCA) and Spectral Angle Mapper (SAM) have been applied to the data set of the resulting emission spectra to discriminate among the developed microbial communities.

Introduction

The laser-based techniques are largely and successfully used as diagnostic tools in the field of cultural heritage [1]. Their increasing application, both for conservation and restoration actions, is mainly due to the offered advantages of minimum invasiveness, in situ applicability, remote capability of measurement and high sensitivity.

Laser Induced Fluorescence (LIF) technique, in particular, has been used in several applications as in the monitoring of the protective treatment on stone surfaces [2], to identify some acrylic resins of interest for artworks [3], or to detect cultures of fungal and bacterial strains [4].

The formation of biofilms from microorganisms under favourable conditions of humidity and temperature is well known and their detection and identification are very important to prevent the deterioration processes of monuments.

In this work, some biofilms from Roman catacombs have been analyzed by LIF technique. Principal Component Analysis (PCA) and Spectral Angle Mapper (SAM) have been applied to identify the origin of the different contributions to the resulting emission spectra and to discriminate among the developed microbial communities.

Experimental

The studied biofilms were collected in Roman catacombs sited along the ancient via Appia (Rome, Italy), where the relative humidity is higher than 90%, the temperature is constant in the range 15.6–18 °C and the photosynthetic photon flux density (PPFD) is below 2.5 μmol photon m^{-2} s^{-1}. The samples were maintained in wet and completely dark conditions in order to reproduce hypogeal environment and, thus, to preserve their features. Also the temperature was controlled at appropriate values.

The Laser Induced Fluorescence (LIF) of the biofilms has been measured with a scanning hyperspectral lidar fluorosensor developed at the ENEA Center of Frascati. The radiation source is a diode pulsed Nd:YAG laser working at 355 nm with a time duration of 10 ns at a fluence from 1 to 50mJ/cm^2. All the mechanical and optical elements have been installed in an aluminum box of 58×43×36 cm^3 for less than 15kg, allowing for an easy transfer of the system and its operation from scaffoldings until a distance of 10 m.

Results

Fluorescence emission bands from the analyzed samples have been detected with good results. LIF spectra, as can be observed in figure 1, depend on the particular area under investigation and appear as the superimposition of several contributions, due to the presence of complex microbial colonies in the same sample.

Each resulting component, pointed out from the deconvolution of the spectra, can be ascribed to one of the several pigments (such as Chlorophyll *a*, *b*, *c* and carotenoids) characterizing the microorganisms here present.

Moreover, the effect of the substrate on the nature of the developed microorganisms seems to be relevant. Spectra from mortar and tufa, in fact, differ on shape and intensity. Furthermore, distinct biofilms sampled on mortar present very different emission bands, while those on tufa are very similar.

The Principal Component Analysis performed on the LIF spectra of biofilms was able to make a distinction also between samples characterized by spectra very similar, as in the case of the biofilms developed on tufa (fig.2). The samples on mortar are well separated by PCA, too. The PCA put in evidence a partial overlap of some results relative to biofilms from different surfaces, suggesting the presence of the same microorganisms. SAM analysis applied to the whole data set allows us to determine the surface areas where the same microorganism has grown, starting from a reference fluorescence spectrum.

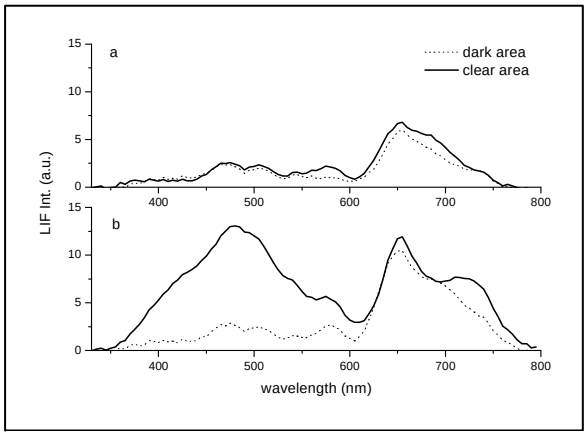

Fig.1; LIF spectra of biofilms from dark area (dot) and clear area (solid) on: (a) tufa and (b) mortar.

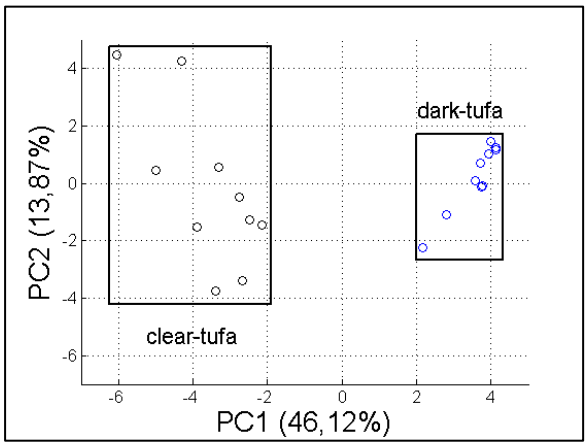

Fig.2; Principal component analysis on LIF spectra of dark and clear biofilms on tufa.

Conclusions

Multivariate analysis has been applied to LIF spectra and permitted to discriminate and classify different microbial communities developed on Roman hypogeal surfaces.

The authors would like to thank Prof. Albertano and her team for supplying samples.

References

1) C. Fotakis, Lasers for Art's sake!, *Opt. Photon. News,* 6(5), (1995), 30-35
2) G. Ballerini, S. Bracci, L. Pantani, P. Tiano, Lidar remote sensing of stone cultural heritage: detection of protective treatments*, Opt. Eng.*, 40, (2001), 1579-1583
3) L. Caneve, F. Colao, R. Fantoni, L. Fornarini, Laser induced fluorescence analysis of acrylic resins used in conservation of cultural heritage, *Proc. OSAV'2008, The 2nd Int. Topical Meeting on Optical Sensing and Artificial Vision St. Petersburg, Russia***,** 12-15 May 2008, 57-63, ISBN 978-5-7577-0333-6
4) V. Raimondi, L. Palombi, G. Cecchi, D. Lognoli, M Trambusti, I. Gomoiu, Remote detection of laser-induced autofluorescence on pure cultures of fungal and bacterial strains and their analysis with multivariate techniques, *Opt. Commun.*, 273(1), (2007), 219-225