Hyperspectral imaging of stone biofilms at the macroscopic scale

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Abstract. The ability to observe traces of biological material on buildings and stone artworks is of particular importance in understanding how to best deal with them and maybe, in the future, even make use of biofilms for conservation science. We have identified hyperspectral imaging as a viable method for the efficient analysis of such biological materials. Thanks to the high throughput of our approach based on an interferometric method based on Fourier Transform, we were not only able to detect traces of biofilms on stone samples, but also to map in which areas these were found to have higher biological activity.

1 Introduction

The state of conservation of historical buildings can be altered by the presence on the surface of several species of microorganisms in the form of sub-aerial biofilm. Traditionally, this is considered negative for the substrates, leading to biodeterioration. However, recent empirical evidence has highlighted that it may have a neutral or even a protective role and the topic is still up for debate. The research on the role of biofilms calls for new methods to investigate the presence and activity of even traces of biological materials on colonized surfaces. In this context, we suggest using a multimodal imaging approach based on hyperspectral imaging (HSI). The method combines optical imaging with spectroscopy to obtain spatially resolved spectral information of a sample. This is done by creating the so-called data-cubes, in which each element, or pixel, also carries the information about the reflectance or fluorescence spectrum at that specific location. Generally, HSI systems are based on either spectral or spatial filters that limits the throughput of acquisition.

2 Experimental setup

We propose the use of an interferometric approach based on Fourier Transform. Our HIS system in composed by a compact birefringent interferometer, called TWINS [1], coupled with a monochrome camera [2] in a macro configuration. The working principle of the system consists in the creation and inference of two replicas of the light coming from the sample, that have gained orthogonal polarization by passing through a polarizer and a relative phase delays as they travel through two wedges of birefringent material. During the acquisition, the two wedges are shifted, making the overall thickness of the birefringent material to vary.

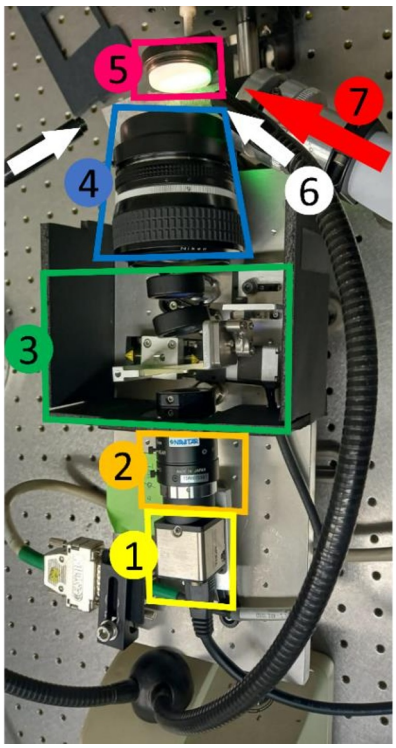

Fig. 1. Picture of the experimental setup, showing 1) the CMOS camera, 2) the detection objective, 3) the TWINS system, 4) the 50 mm f/1.2 Nikon objective, 5) the sample, 6) the illumination for reflectivity acquisition, 7) the fluorescence excitation.

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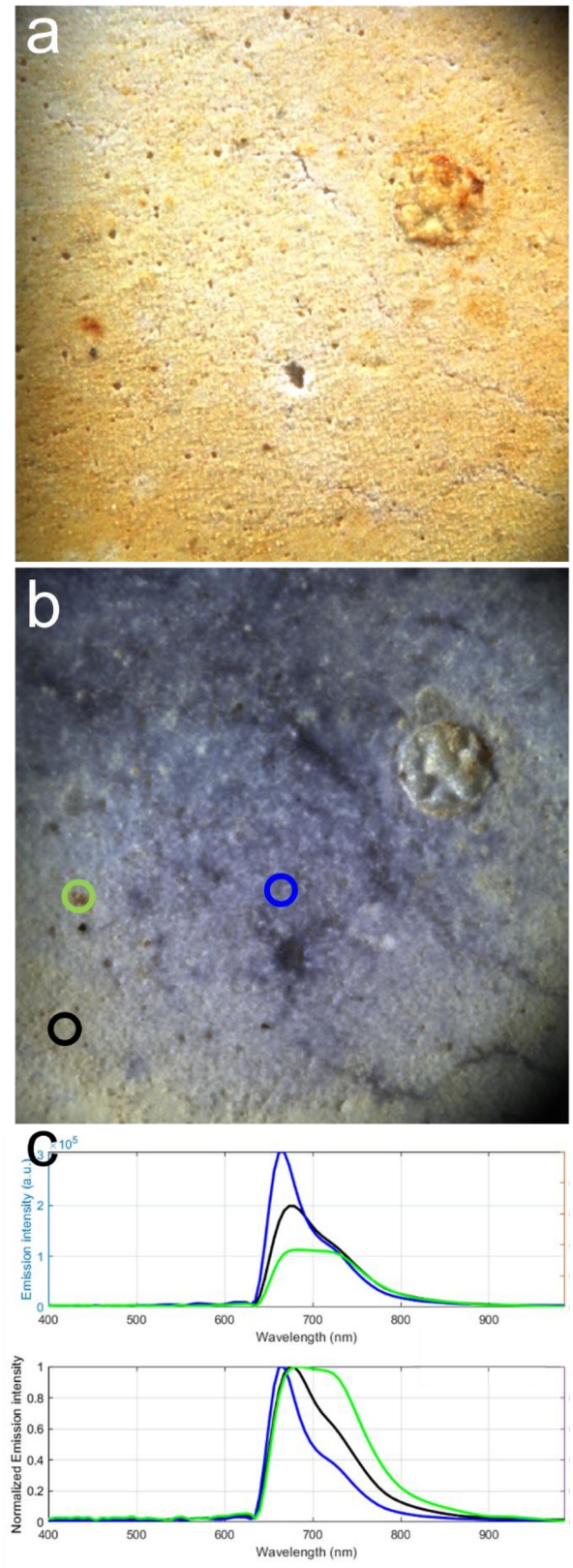

Fig. 2. Reflectivity and fluorescence false colour maps of the same area. a) Reflectivity map in false colour (R: 760nm-710nm; G: 710nm-660nm; B: 660nm-610nm) of the carbonatic stone, on which the biofilm can be hardly see. b) Fluorescence map in false colour (R: 730nm-710nm; G: 710nm-690nm; B: 690nm-660nm) showing a higher spatial variability associated to a different fluorescent emission. c) Fluorescence spectra corresponding to the circled areas in b), showing the difference in peak intensities for the different areas.

The two replicas indeed interfere at the camera with varying phase delay. The acquired temporal data-cube can then be transformed into a spectral one using a Fourier Transform process. The technique provides high robustness, flexibility and throughput thanks to the absence of any type of filter. Indeed, the acquisition time is also strongly reduced compared to standard HSI imaging, and the dose delivered to biological samples is considerably lower, even when performing HSI in fluorescence modality. Moreover, the system can be easily coupled to standard microscopic [3] or photographic objectives enabling hyperspectral analysis at variable spatial scales, ranging from microscopic to macroscopic to far field. In this particular version, the HSI camera is coupled to a 50 mm, f/1.2 photographic Nikon objective as shown in Fig. 1, which delivers a field of view of 1.5 cm and a resolution <10 µm. For reflectivity studies, the sample is illuminated with 2 halogen lamps and the results are balanced by using a Lambertian reference. For fluorescence experiments, a LED source emitting at 630 nm is used, and a high-pass filter at 650 nm is placed in the detection path.

3 Results

Carbonatic stone samples that have been colonized in laboratory with a cyanobacterium (phototroph), simulating the sub-aerial biofilm formation on stone surfaces, were analysed with the HSI macro camera. The measurements allowed for the mapping of the distribution of photosynthetic cyanobacterial pigments on the substrate by reflectance and fluorescence data performed subsequently on the very same field of view. More specifically, the fluorescence data have highlighted the presence of biofilms even in areas where they were not visible in the reflectance images (Fig. 2a). Figure 2c shows the fluorescence spectra of the highlighted areas in Fig. 2b, characteristic of the chlorophyll emission. In particular, it was possible to identify areas where the peak at 710 nm is more pronounced than the one at 680 nm, representative of areas of higher biological activity. Indeed, our approach has proved well suited for the mapping of traces of bio-films on such porous surfaces and for the identification of areas with different photosynthetic activity. More in general, our HSI macro camera can be used for the screening of biological activity to detect variations in physiology and stress conditions of vegetation.

References

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