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On the use of Laser-Induced Fluorescence for biological agent detection

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Summary. — The uncontrolled spread of biological agents, such as bacteria and viruses, could be hazardous for human health. Despite the interest in detecting their presence, the common methods are usually impracticable, since the diagnosis methods are time-consuming, could be invasive or require a lot of consumables which make the cost unsustainable. A good candidate to perform biological agent detection is the Laser-Induced Fluorescence (LIF) spectroscopy since the emitted spectra are characteristic of the chemical composition of the agent. For this study, some tests with this technique on some biological agents have been performed and the classification and detection performances have been evaluated.

1. – Introduction

The development of fast, specific, and sensitive new instruments to detect the presence of biological agents, able to allow their classification, is of great interest in several biological, medical, and physical research fields and applications. The recent pandemic due to SARS-CoV-2 demonstrated and highlighted how much present diagnostic systems are time-consuming and unsuitable for fast and real-time monitoring. In fact, they usually require sampling, long times of analysis, and are not fully automatised [1-4] Optical and laser-based techniques demonstrated to be powerful instruments to develop fast, sensitive, and specific tools for the investigations of a medium. Lidar, DIAL, LIBS, LIF, Raman and optical spectroscopy are some of the most common laser-based techniques used for a huge range of applications (from chemical [5] to metal analysis [6], plasma diagnostics [7], water quality control, pollution/aggressive monitoring [8], CB

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agent detection [9, 10], etc.). These methods are based on observing how a specific light-matter interaction phenomenon occurs in the presence of specific molecules, atoms or as a function of the properties of the medium. Moreover, the recent development in the realm of machine learning, deep learning tools, and many other advanced data analysis approaches allowed to increase the performances of those methods in significant ways. An investigated and interesting method to detect and classify biological agents is the Laser-Induced Fluorescence (LIF), and more specifically, LIF spectroscopy. The main idea of LIF spectroscopy for biological agents is based on the principle that different bio-agents (bacteria, viruses, fungi, etc.) have a different chemical composition which reflects on the emitted fluorescence spectra, making the shape of the spectrum a finger-print? specific of the agent. Moreover, if the shape of the spectrum is specific of the agent, the fluorescence intensity is strongly correlated to its concentration [11]. Several studies are present in the literature about the possibility to detect and classify biological agents, and between them, it is worth mentioning the great work done by Duschek and his research group [13]. They demonstrated not only the capability of LIF to perform accurate classification between bacteria, pollen and fungi, but also the possibility to use this technology in very challenging situations, developing a remote sensing LIF with distances over 100 m. Also advanced approaches, such as the multiwavelength LIF and the time-resolved LIF, have been analysed and it has been shown that they can lead to high-performance improvements [12-15]. This paper reviews the work of the authors about the use of LIF spectroscopy to classify viruses [15], highlighting the methodologies used, the preliminary results achieved, the limits observed, the possible implementations, and the future challenges.

2. – Materials and methods

In this work, the authors analysed the possibility to discriminate among different types of viruses. Six different samples have been prepared. Four samples contained pure viruses: Hepatitis A, Coxsackie A7, Coxsackie A9 and Coxsackie B4. One sample contained the blank, *i.e.*, the solvent (Phosphate Buffered Saline, PBS) where the viruses were dispersed. The last sample contained a pool of viruses containing Rotavirus, Hepatitis A, Echovirus type 1, and Astrovirus. The laser used to induce the fluorescence is a Q-switched diode-pumped solid-state laser with a central wavelength of 266 nm, with a pulse frequency rate of 10 kHz and an average power of 25 mW. The laser beam crosses the quartz cuvette (mainly transparent to UV radiation) and interacts with the sample, involving the emission of scattering and fluorescence radiation (UV and visible). Orthogonally to the laser beam direction, an optical system compounded by different convex lenses and a band-stop filter is used to collect a large portion of the emitted fluorescence and to filter the elastic scattering. The radiation is then collimated into the spectrometer which records and stores the spectra. The system is placed inside a box to exclude optical noise from the external optical background (sunlight, etc.). All the spectra are recorded using an exposure time of the spectrometer equal to 60 s, and 20 spectra for each sample have been recorded. Even if small, the background radiation has been removed from each spectrum. The background has been acquired before each measurement, by measuring a spectrum with the laser turned off. The use of LIF as an instrument to detect and classify biological agents needs specific algorithms. The use of supervised machine learning algorithms demonstrated to ensure the best performances in terms of classifications. Supervised algorithms are based on two steps: training and testing. During the training, the algorithm needs a database where each spectrum is

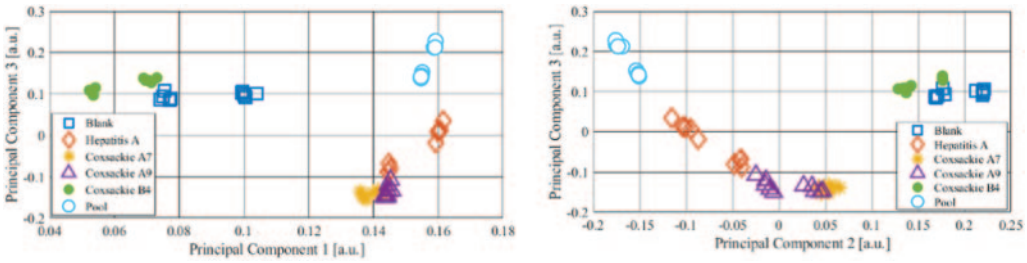

Fig. 1. – Results: principal component 1 and 3 (panel on the left), 2 and 3 (panel on the right).

associated with the class. During this phase, the algorithm searches the best way to classify each spectrum, maximising the classification performances of the spectra in the database. Once the algorithm is trained and validated, it is possible to use the algorithm to classify new spectra which are not in the database. Three different algorithms have been used to test their classification capabilities: the Support Vector Machine (SVM), the Classification And Regression Trees (CART) and a simple feed-forward Neural Networks (NN). For the three algorithms, the spectra have been adimensionalized by the sum of spectral intensities, making the spectra insensitive to the concentration of the agents (true only for pure samples). In the case of the NN, the inputs are the spectra without any other type of pre-processing, while for both SVM and CART the Principal Component Analysis (PCA) has been applied.

3. – Results

In general, those spectra have some small features which are different and that could be used to perform classification. A more performant graphical way to observe spectral differences between the agents is by plotting the Principal Components (PC) of the spectra calculated by the PCA (fig. 1). The PCA calculates the best linear combinations of the adimensional spectral intensities which maximise the variance between the spectra. Figure 1 shows the spectra in the space of the first three PCs, and the great differentiation between the various classes can be observed. The pool is well separated by the other agents, with the Hepatitis A which is the nearest agent. This should be expected since in the pool there is also Hepatitis A. The Coxsackie A7 and A9 are quite close, and it is expected too since they should have a similar chemical composition. The blank is near the Coxsackie B4, even if the separation is still good to allow a good classification, as shown in the next paragraph. The three supervised classification algorithms have been tested and demonstrated to be able to classify the samples with great performances. In fact, all three algorithms returned 100%of true positive and negative rates. Only in the case of a very coarse CART, some errors arise. Specifically, the coarse CART confused a blank with a Coxsackie B4 (false alarm), a Coxsackie A7 with a Coxsackie A9 (wrong agent) and a Coxsackie B4 with a blank (missed alarm). However, using a “deeper” machine learning tool allowed to reach a perfect classification.

4. – Discussion and conclusion

In this short paper, the authors reviewed their last results obtained with the laser-induced fluorescence to detect and classify viruses in pure samples. The preliminary studies are promising since they demonstrated the capability to classify viruses in samples

with high accuracy, with an automatable and fast device (analysis time colud be few minutes up to half an hour). Despite the interesting results, there is a long way ahead. At first, the capability to classify a virus, or a specific biological agent in general, in a fluorescent background should be investigated, since in many applications it is expected to have particles (proteins, pollen, etc.) which are fluorescent and that will interfere with the measurements and the classification. The smallest detectable concentration for each agent should be investigated, as well as the development of libraries and algorithms which are specific for each application. Also, the development and implementation of more specific techniques, such as multiwavelength and time-resolved techniques, and hybrid approaches (LIF + Raman, LIF + LIBS, etc.) should be evaluated. Finally, although the application can be used for clinical analyses, the presence of a high background noise due to the biological material should be evaluated and an in-depth analysis phase will be required before understanding its real applicability.

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