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Pollen Raman spectra database: Application to the identification of airborne pollen

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ABSTRACT

Raman microspectroscopy allows a non-destructive identification of airborne particles. However, the identification of particles such as pollen is hindered by the absence of a spectral library. Although reference spectra of pollen have been published before, they have always been limited to a certain number of species.

In this work, Raman spectra of 34 pollen types are presented and were used to build a pollen spectra primary library. Afterward, the applicability of this database for detecting and identifying pollen in airborne samples was tested. Airborne pollen samples collected during April, May and August were compared with blank pollen spectra by means of Hit Quality Index. Although a much larger library would be required, our results showed that all first hits correspond to the same blank pollen species of the questioned sample from the air. This possibility is an innovative idea and a promising line of investigation for future RAMAN technology development in the area of aerobiology.

1. Introduction

Pollen grains are the male gametophyte of seed plants being produced as part of the sexual reproduction cycle. They are biological inert particles being considered seasonal air pollutants, since pollen is only dispersed into the atmosphere during the flowering season. Pollen identification has been widely used in many areas of research such as Environmental Monitoring, Agriculture, Medicine and Forensic Sciences.

The pollen structure, morphology and the exine pattern are genetically stables between different species, providing a good taxonomic parameter [1,2]. Also, the size of pollen grains and the exine characteristics are the most influential variables for the discrimination of the different pollen types [2]. The combinations between these features enable the taxonomic distinction of pollen at the family, genus and seldom at the species level. Along the years light microscopy has been the primary method used for pollen identification and quantification. However, this method is time consuming and requires an experienced palynologist, being also liable to the observer subjectivity [3].

Pollen grains present several differences at its chemical composition level, for instance in the pollen wall [4–6], that can allow its identification. Therefore, during the last years, the chemical and spectroscopic examination of pollen has become increasingly important and improvements in the use of these techniques have resulted in a reduction in sample consumption. The introduction of the Raman instrumentation has provided additional impulse for the adoption of Raman spectroscopic techniques in pollen identification, characterization and also classification in situ without prior preparation (purification, extraction and contrast medium). Additionally this technique offers high flexibility and good chemical and structural specificity, high spatial resolution, short acquisition times for analysis and can be used in a non-destructive and minimally invasive manner on single pollen. Applications vary from fundamental studies to applied research in areas of defense and security and in monitoring of environmental pollution. Some limitations of the technique have been very commonly achieved by Raman variations, such as surface-enhanced Raman spectroscopy, coherent anti-stokes Raman spectroscopy, resonance Raman, and UV Raman spectroscopy [7].

The chemical–structural characterization of several pollen grains by Raman spectroscopy has been carried by several authors [8–11], and the works include the vibrational assignments of signals frequently found in Raman spectra of pollen specimens.

Raman spectra of biological material are very complex, because they consist of signals from all molecules present in cells [12], and pollen grains confirm this rule. However this complexity of biomolecules also has its advantages since the obtained spectra allow

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identification [13]. Some works performed on a single pollen using Raman spectroscopy showed that molecular vibration of pollen grains vary among plant-types and therefore allowed the pollen identification [9,10].

One important application of pollen identification, using optic microscopy, is related to the elaboration of airborne pollen calendars. The monitoring of airborne pollen levels give important information of the presence and prevalence of allergenic pollen in the atmosphere of a sampling area that can trigger respiratory allergies (commonly known as pollinosis). They are associated to an allergic response of patients to the pollen grains of several anemophilous trees, grasses and weeds that are released into the atmosphere during plant flowering season. This is a general health problem worldwide, affecting life quality of earth inhabitants. Moreover, different species present different allergenicity levels and therefore detailed airborne pollen identification is very important to depict the real risk of allergen exposure.

The examination of airborne pollen using Raman spectroscopy is hindered by the absence of a spectral library database of pollen. The identification of this material will necessitate generally a large, comprehensive database of reference spectra. Such a database is currently not available, especially on a genus level, of which there are numerous possibilities.

In this work, we present a comprehensive library of Raman spectra of pollen, which can be regarded as the precursor of a larger pollen database. This preliminary database of pollen was used to assay the possibility of identify airborne pollen, something that was never done before. Airborne samples collected during April, May and August of 2012 were examined and the pollen identification was performed based on the comparison between the questionable Raman spectra and the library of Raman spectra obtained from blank pollen samples.

The problem of incompleteness of a spectral library is well known; however, it will be shown that a close examination of the spectrum make it possible to identify the pollen particles at a species level.

2. Material and methods

2.1. Samples

Several blank pollen samples and ten airborne samples collected during different months were selected for the Raman microspectroscopy analyses.

Thirty-four pollen types belonging to different plant species, that were considered as reference pollen samples, were analyzed (Table 1). This pollen was collected in the city of Porto, northwest of Portugal, in public gardens and sidewalks, during the flowering period. In each plant, flowers/catkins were randomly collected from all quadrants in different branches until a $30 \times 10\ cm^2$ box was filled. After removal of other flower parts, the anthers were dried at 27 °C during 24 h, gently crushed and after that the pollen thus released was passed through different grades of sieves to obtain pure pollen which was stored at −20 °C.

Airborne pollen sampling, including pollen, was performed using a Burkard Cyclone sampler (Burkard Manufacturing Co Ltd. Hertfordshire, UK) consisting of a continuous volumetric sampler based on a single reverse-flow miniature cyclone with an air flow rate of 16.5 l/min. The samples were collected daily directly into a 1.5-ml Eppendorf vial and stored at −20 °C.

2.2. Procedure

A Horiba Jobin-Yvon LabRaman spectrometer interfaced to an Olympus optical microscope with 100× objective lens was used for the pollen characterization. The excitation wavelengths of 632.8 nm from a HeNe laser (20 mW) and grating with 1800 lines mm^{-1} were used. A slit of 300 μm was used and the incident beam perpendicular to the plane of the sample is focused through the microscope lens, which also collects the Raman scattered radiation in back-scattering geometry. The Raman signal is detected on a cooled charge-coupled device (CCD) detector.

Extended scans were performed on the spectral range 200–3100 cm^{-1}. The time of acquisition and the number of accumulations varied in order to obtain an optimized spectrum for each analyzed particle at spectral resolutions near 1 cm^{-1}. Within the same pollen type, 3–6 spectra were obtained to avoid differences due to variability and microscale heterogeneity between pollen grains and for Library construction and search the optimum spectra for each pollen type analyzed was chosen. Prior to each reference measurement, the instrument was calibrated on the internal Si reference standard ($520.6 \pm 0.1\ cm^{-1}$).

Pollen samples were labeled according to the plant species or genus, which is a general reference on the classification and were divided into two groups: (i) trees and shrubs; (ii) grasses and weeds (Table 1). Both groups contain the most common pollen types found in the atmosphere [14–16]. The different blank pollen grains and airborne particles were placed on a glass slide for analysis. Blank pollen grains were used as a bulk powder while airborne samples were obtained of the Ependorff vial.

2.3. Pre-processing and library search

Both reference and test spectra were pre-processed in the same way: baseline correction was performed prior to the application of a denoise function–denoise algorithm, Labspec 6, Horiba Scientific – to reduce noise and enhance the spectrum quality without losing subtle spectral information. The spectra were then normalized to constant area, where the area under the curve is set to 100 (a.u.). The spectra were limited to the fingerprint region: 400–1800 cm^{-1}.

Pre-processed spectra of the reference samples, corresponding to different pollen species were added to a database, using the KnowItAll software from Bio-Rad. The 10 test spectra-airborne samples – were then evaluated against the library. The Hit Quality Index (HQI) was used to rank the results of a spectral search. The HQI, which is scaled between 0 and 1000, indicates how well each spectrum from the database matches the test spectrum. Two different algorithms – Euclidean distance and Correlation, KnowItAll, Bio-Rad – were used to evaluate the HQIs and rank them for each test spectra. The spectral search is performed on the whole spectral range 400–1800 cm^{-1}.

3. Results and discussion

Some examples of Raman spectra and relative intensities of the pollen studied are listed in Fig. 1 (all spectra can be consulted on on-line supplementary material). Although fluorescent background are found in some of the spectra, all of them have distinct bands in the functionality region between 1000 and 1700 cm^{-1} and bands in the CH stretching region near 3000 cm^{-1}. At lower wavenumbers, several vibrations appear, these being more frequent in the group of grasses and weeds pollen. No features were found between 1800 and 2700 cm^{-1}.

As a result of a spectral survey we observe the presence of bands in the CH stretching region near 3000 cm^{-1}, with common spectral features between 2850 and 2970 cm^{-1} assigned to CH_2 and CH_3 stretching [7], probably in lipids and sporopolenin [8], a very weak band at 3060 cm^{-1} assigned to C=C–H aromatic stretching [7]. A band at ca. 1650 cm^{-1} ascribed to the amide I band [7,8,10] is also present, however was not detected on *Chamaerops humilis* pollen (Fig. 1). A strong band at ~1600 cm^{-1} is also a common feature on