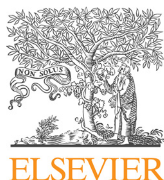
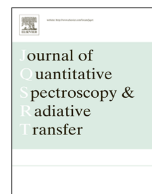
Journal of Quantitative Spectroscopy & Radiative Transfer 153 (2015) 4–12



Contents lists available at ScienceDirect

Journal of Quantitative Spectroscopy & Radiative Transfer

journal homepage: www.elsevier.com/locate/jqsrt



Photophoretic trapping-Raman spectroscopy for single pollens and fungal spores trapped in air

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form
4 November 2014
Accepted 6 November 2014
Available online 15 November 2014

Keywords:
Optical trapping
Raman spectroscopy
Bioaerosols
Pollen
Fungal spores
Single particle trapped in air

ABSTRACT

Photophoretic trapping-Raman spectroscopy (PTRS) is a new technique for measuring Raman spectra of particles that are held in air using photophoretic forces. It was initially demonstrated with Raman spectra of strongly-absorbing carbon nanoparticles (Pan et al. [44] (Opt Express 2012)). In the present paper we report the first demonstration of the use of PTRS to measure Raman spectra of absorbing and weakly-absorbing bioaerosol particles (pollens and spores). Raman spectra of three pollens and one smut spore in a size range of 6.2–41.8 μm illuminated at 488 nm are shown. Quality spectra were obtained in the Raman shift range of 1600–3400 cm^{-1} in this exploratory study. Distinguishable Raman scattering signals with one or a few clear Raman peaks for all four aerosol particles were observed within the wavenumber region 2940–3030 cm^{-1}. Peaks in this region are consistent with previous reports of Raman peaks in the 1600–3400 cm^{-1} range for pollens and spores excited at 514 nm measured by a conventional Raman spectrometer. Noise in the spectra, the fluorescence background, and the weak Raman signals in most of the 1600–3400 cm^{-1} region make some of the spectral features barely discernable or not discernable for these bioaerosols except the strong signal within 2940–3030 cm^{-1}. Up to five bands are identified in the three pollens and only two bands appear in the fungal spore, but this may be because the fungal spore is so much smaller than any of the pollens. The fungal spore signal relative to the air-nitrogen Raman band is approximately 10 times smaller than that ratio for the pollens. The five bands are tentatively assigned to the CH_2 symmetric stretch at 2948 cm^{-1}, CH_2 Fermi resonance stretch at 2970 cm^{-1}, CH_3 symmetric stretch at 2990 cm^{-1}, CH_3 out-of-plane end asymmetric stretch at 3010 cm^{-1}, and unsaturated $=$CH stretch at 3028 cm^{-1}. The two dominant bands of the up-to-five Raman bands in the 2940–3030 cm^{-1} region have a consistent band spacing of 25 cm^{-1} in all four aerosols. Finally we discuss improvements to the PTRS that should provide a system which can trap a higher fraction of particle types and obtain Raman spectra over a larger range (e.g., 200–3600 cm^{-1}) than those achieved here.

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1. Introduction

Biological particles (bioaerosols) in the atmosphere include primary biological aerosol particles (PBAP) such as bacteria, fungal spores, plant pollens, and small fragments of plants or fungi, and secondary bioaerosols such as those formed by ozone-initiated polymerization of terpenes. PBAP can transmit diseases of humans (e.g., inhalation anthrax) or plants (e.g., smuts, rusts); act as allergens (e.g., pollens, or dried proteins from cat saliva); affect climate by absorbing and/or emitting light; and change clouds and precipitation patterns by acting as condensation nuclei. There is a need for improved methods to rapidly characterize atmospheric bioaerosols.

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http://dx.doi.org/10.1016/j.jqsrt.2014.11.004
0022-4073/Published by Elsevier Ltd.

Several optical techniques which require no reagents have been developed and used for bioaerosol detection, partial characterization, or in some cases identification, depending upon the type of bioaerosol, the technique or combination of techniques used. For air samples collected onto a surface these techniques include optical and electron microscopy [1]; fluorescence microscopy and spectroscopy; Raman spectroscopy; and combinations of Raman spectroscopy and imaging [2]. Optical and electron microscopy provide information on the size, shape, and surface structure of a particle. X-ray fluorescence, which is often available with electron microscopes, provides information on the atomic composition. The combination of Raman spectroscopy with optical imaging offers information on types of chemical bonds and types of chemicals and biochemical molecules in particles. Instruments have been developed for particles in air samples which pass through an instrument, where the particles are not collected but are carried through the instrument in flowing air. Some of these instruments measure single particle elastic scattering, and/or single particle fluorescence, and/or laser- or spark-induced breakdown spectroscopy (LIBS/SIBS) and some are commercially available [3]. Laser induced fluorescence (LIF), especially dual-wavelength UV-LIF has been demonstrated for near-real time detection and partial classification of bioaerosols particles [4–9]. The technique was shown to be capable of differentiating pollens from various plant species [9,10]. Fluorescence clustering analysis, especially when it is combined with the dual-wavelength UV-LIF, appears promising for rapid airborne bioaerosol characterization [11,12]. When excited at a visible wavelength, e.g., 488 nm, 515 nm, 633 nm, or 780 nm, most pollens and fungal spores have one or several fluorescence humps in a wide spectral range, e.g., 500–800 nm. These relatively structureless fluorescence spectra usually lack discriminating signatures for chemical characterization of bioaerosols. LIBS, which characterizes the elemental composition of a particle, has also been used to study a pollen particles one-at-a-time [13]. Bioaerosols tend to be highly complex, and their fluorescence, Raman, or breakdown spectra may depend upon how a sample is grown, washed, dried, stored, and/or processed in the atmosphere by sunlight, ozone, etc.

Here we are particularly interested in methods which can be used to measure samples one-at-a-time as particles flow in air or are stably trapped in air. Raman scattering can be far more informative than elastic scattering or fluorescence. Pollens are a special case where the elastic scattering used in optical microscopy can in many cases be used for identification. Raman spectroscopy is well suited for bioaerosol characterization and even possible identification in cases where the composition of all the relevant aerosol particles that could reach a sampler in some location is known. Raman spectra of many materials can be so sensitive to the chemical constituents (e.g., proteins, DNA, RNA, fatty acids, fats, cellulose, sporopollenin, chitin, lignin), and their molecular structures that in many cases these spectra can be used as "fingerprints" to identify chemical species and even biological species [14–16]. Also, how these Raman spectra change as bioaerosols or other particles are modified by their environment is also of interest. A pollen, plant or fungal spore typically contains many thousands of molecules and numerous chemical functional groups. Their Raman spectra exhibit Raman bands (structures or peaks) that are related to vibrational modes of individual chemical groups. For example, in low spectral resolution or not-well-resolved Raman spectra of pollens, Raman bands attributed to different vibrational modes of sporopollenin (a carotenoid-like aliphatic polymer) consisting of aromatic or conjugated side chains dominate the spectra [17–25]. They have peaks at $\sim$600 cm^{-1} from aromatic ring deformation [18]; $\sim$1000 cm^{-1} from breathing mode of the trigonal ring [18–19]; $\sim$1080 cm^{-1} from the C–C skeletal vibrations [18–19]; $\sim$1600 cm^{-1} from ring stretches of phenyl structures [17–18]; $\sim$1440 cm^{-1} from C–H_2 deformation [17–19]; and $\sim$2900 cm^{-1} from CH_2 and CH_3 stretches [18]. Exemplar protein bands are at $\sim$1650 cm^{-1} from C=O stretch of the amide I system [18–20]; $\sim$1520 cm^{-1} from N–H stretch of the amide II; $\sim$1300 cm^{-1} from N–H and C–H deformation of the amide III [18–20]. Phenylalanine may have Raman bands at $\sim$600, 1000, and 1600 cm^{-1} [22]. Tryptophan and tyrosine have a band at $\sim$1166 cm^{-1} [23]. Nucleic acids may also have Raman bands at $\sim$820 cm^{-1} from C–O–P–O–C in RNA backbone; $\sim$1166 cm^{-1} from guanine; $\sim$1360 cm^{-1} from adenine and guanine; $\sim$1565 cm^{-1} from adenine and guanine. Cytosine and uracil have weak vibrations at $\sim$790 cm^{-1} [18–20]. Therefore, Raman spectra can be used for characterization of chemical compositions in bioaerosols. On the other hand, some of Raman bands have similar Raman shifts, because common molecular groups are included in different pollens. And the same chemical species (functional group) may have a series of Raman bands in different locations, e.g., triolein and trilionlenin have Raman bands at 970, 1065, 1081, 1121, 1302, 1440, and 1656 cm^{-1}[22]; oleic acids have bands at 1036, 1302, and 1440 cm^{-1}; carotenoid have bands at 1522, 1189, 1156 cm^{-1} [25], etc. Therefore, compared with chemical characterization, it is more challenging to achieve pollen/spore identification using specific Raman bands. For some studies of Raman spectra of pollens and spores the particles are laid on a substrate and spectra are recorded using a Raman spectrometer (e.g., [25,26]). In those studies, a Raman excitation laser beam was scanned across each micro-size (15–60 μm) pollen particle with a spatial resolution of 1 μm. About 16 weak Raman bands were indentified for each of the 15 pollens in a Raman shift range of 400–1600 cm^{-1}. The results showed some promise for pollen classification, and some Raman bands have been identified as signatures of pollen components. Guedes et al. [27] measured Raman spectra of 34 different airborne pollens using a Raman spectrometer toward the ambitious goal of developing a "pollen Raman spectra database". Each of the pollen spectra shows up to 20 Raman bands superposed on the top of a strong fluorescence background in the Raman shift range of 500–1800 cm^{-1}. Most bands are broad and very weak; only a few have a sharp peak. Similar spectral features (approximately the same number of Raman bands and the same fluorescence interference) in the spectral range of 500–1800 cm^{-1} were also reported in an earlier study of

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