Measurement and chemometrics analysis of laser induced fluorescence from the solution/suspension of pollens and other biological materials

Bannu[1], Naohiro Manago[1], Yasuto Kaba[1], Shuji Miyazawa[1], Takeshi Yokozawa[2], Jun Izawa[3], and Hiroaki Kuze[1]
[1] CEReS, Chiba University, [2] INC/IHI Corporation, [3] IHI Corporation

Abstract
Laser-induced fluorescence (LIF) spectra are measured for various fluorescing materials of biological origin. Suspension of sugi (cedar) pollen, hinoki (Japanese cypress) pollen, tryptophan, and solution of nicotinamide adenine dinucleotide (NAD) and green tea beverage are irradiated with laser beams of wavelength 266 and 351 nm. It is found that the spectral signatures are often similar for different materials, but occasionally it is possible to discriminate the fluorescing species on the basis of the observed spectral features. Chemometrics analysis is carried out for a mixture of hinoki pollen and NAD to study the possibility of identifying constituents.

1. Introduction

The method of laser-induced fluorescence (LIF) can be applied in many fields to identify particular species having minute concentrations. It is known [1,2] that when illuminated with ultra-violet (UV) laser beam, many aerosols of biological origin yield LIF signals, while those of non-biological origin give no or very limited response. Thus, the fluorescence lidar based on a UV laser beam can be useful for detecting biological aerosol particles. In this work, we study the LIF spectra that are produced when various biological materials are excited using laser pulses with wavelength 266 and 351 nm. In order to attain good signal-to-noise ratio, we employ suspension or solution of the materials, and the Raman intensity from liquid water is exploited for quantitative evaluation of the LIF intensities. We also apply the chemometrics analysis [3,4] to study the feasibility of identification of different species from the LIF signal obtained from a mixture of different species.

2. Experiment

Several materials are studied here as biological fluorescence substances; sugi (cedar, *Cryptomeria japonica*) pollen, hinoki (Japanese cypress, *Chamaecyparis obtusa*) pollen, Nicotinamide Adenine Dinucleotide (NAD), Tryptophan (all materials so far are manufactured by Wako Pure Chemical), and green tea beverage. The samples are mixed with purified water in petri dish and poured into a quartz cell (Starna, light path length 20 mm, width 10 mm, and capacity 7 ml). The cell is fixed using a handmade holder (Fig. 1). This holder transmits the LIF signal through a collimator lens that focuses it on a quartz fiber connected to a compact spectrometer (Ocean Optics, USB4000: wavelength range 200 - 850 nm, optical resolution 0.9 nm FWHM, 16 bit sampling depth). The calibration of the spectrometer sensitivity is carried out by means of deuterium and halogen lamps. The holder has a reflecting mirror so as to increase the detected light intensity. The wavelengths of UV lasers employed are 266 nm (Continuum, Minilite, 4ω of Nd:YAG, 20 mW, 10 Hz) and 351 nm (Photonics Industries, DC30-351, 3ω of Nd:YLF, 180 mW, 1 kHz). A short-pass filter is used to cut the unwanted second harmonic, and in some cases, a long-pass filter is placed between the collimator lens and the quartz fiber to cut the scattered laser light. During the laser irradiation, the solution/suspension in the quartz cell is stirred using a magnetic stirrer (IKA, Lab Disk) to prevent sedimentation. Also, the irradiation time duration is minimized to prevent the degradation of the samples.

Fig. 1 Quartz cell holder used for the LIF measurements. The right panel shows the bright feature of the cell during the UV laser irradiation.

The LIF spectrum is measured for each sample to establish a database. The water Raman signal serves as a standard for measuring the LIF intensity. In order to study the capability of identifying different species on the basis of LIF spectra, chemometrics analysis is tested on a mixture between sugi pollen suspension and NAD solution when illuminated at 351 nm. Both simulation and measurement are performed. In the measurement the concentration of each sample is adjusted with volume mixing ratio of sugi:NAD = 50:50 and 20:80. In this latter case (20:80) the LIF peak strengths become nearly the same (2000 count /10 s).

3. Results and analysis

Figures 2 and 3 show typical LIF spectrum observed for (a) sugi pollen, (b) hinoki pollen, (c) tea beverage, (d)

NAD, and (e) tryptophan samples when irradiated at 266 and 351 nm, respectively. It is noted that each spectrum is normalized so that the area of the water Raman peak becomes unity. The sample concentration has been chosen so as to obtain reasonable comparison with the water Raman signal. In Fig. 2, the largest LIF signal is found for tryptophan, ranging from around 300 to 450 nm, with a peak wavelength at around 350 nm. The peak intensities and spectral ranges for sugi, hinoki and tea are more or less similar, while somewhat longer wavelength range is observed for NAD. In Fig. 3, significant LIF intensities are observed for sugi and hinoki pollen, while the LIF intensities are much weaker for other species.

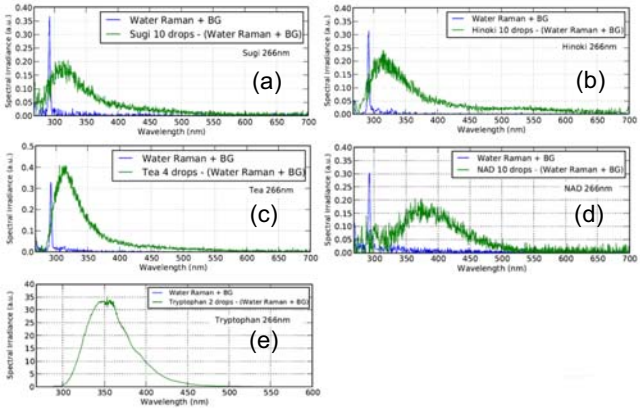


Fig. 2 LIF spectra observed for 266 nm excitation.

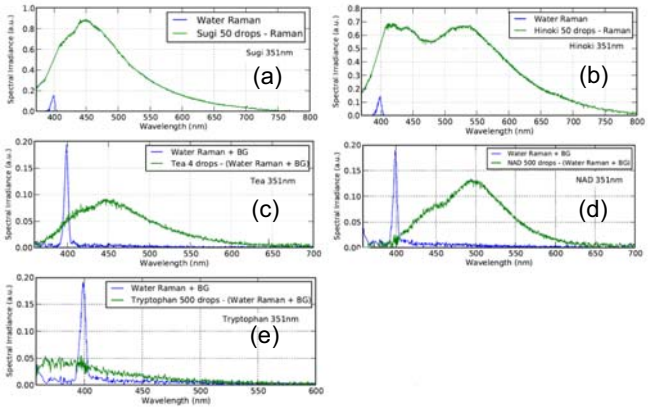


Fig. 3 LIF spectra observed for 351 nm excitation.

Among various methods of chemometrics [3,4], here we employ the method of multivariate curve resolution-alternating least squares (MCR-ALS) to analyze the LIF spectrum of mixed samples. Fig. 4 shows the results obtained for the mixture of sugi pollen and NAD excited at 351 nm. Figure 4(a) shows the reference spectra of sugi pollen and NAD, measured separately. Figure 4(b) and 4(c), on the other hand, compare the experimental curve and the theoretically analyzed curve for the volume mixing ratio of 50:50 and 20:80, respectively. As seen from these results, the assumption of linear mixture holds fairly well, though slight discrepancy is found between the experimental and simulation results, due presumably to the uncertainty associated with the estimation in the mixing ratio of the two species.

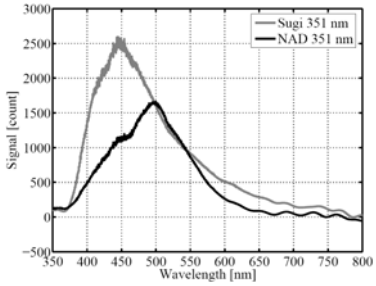
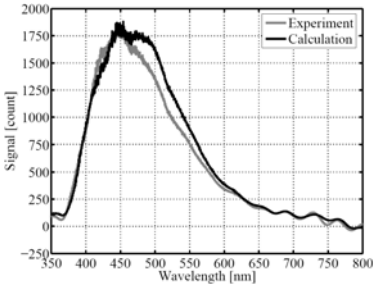
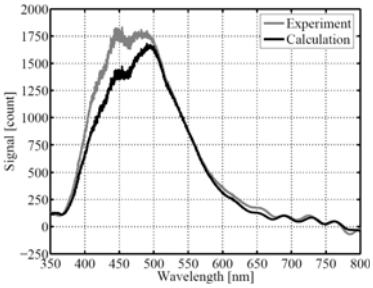


Fig. 4(a): reference spectra for sugi and NAD (separate measurement).



(b) Mixture spectra of sugi and NAD with mixing ratio of 50:50.



(c) Mixture spectra of sugi and NAD with mixing ratio of 20:80.

4. Conclusion

We have studied the LIF spectra of various materials of biological origin using UV laser irradiation at 266 and 351 nm. Chemometrics analysis has been implemented on a mixture of two different species that show different spectral features. The results obtained from the present laboratory experiments will be useful for the future development of stand-off, UV lidar measurement of biological aerosol particles.

References

[1] O. Farsund, G. Rustad, I. Kasen, T.V. Haavardsholm, Required spectral resolution for bioaerosol detection algorithms using standoff laser-induced fluorescence measurements, Sensors Journal, IEEE, 10(3), 655-661 (2010).
[2] R.G. Pinnick, S.C. Hill, P. Nachman, G. Videen, G. Chen and R.K. Chang, Aerosol fluorescence spectrum analyzer for rapid measurement of single micrometer-sized airborne biological particles, Aerosol Science and Technology 95-104 (1998).
[3] R. Tauler, Multivariate curve resolution applied to second order data. Chemom. Intell. Lab. Syst. 30, 133-146 (1995).
[4] T. Azzouz, and R. Tauler, Application of multivariate curve resolution alternating least squares (MCR-ALS) to the quantitative analysis of pharmaceutical and agricultural samples. Talanta. 74, 1201-1210 (2008).