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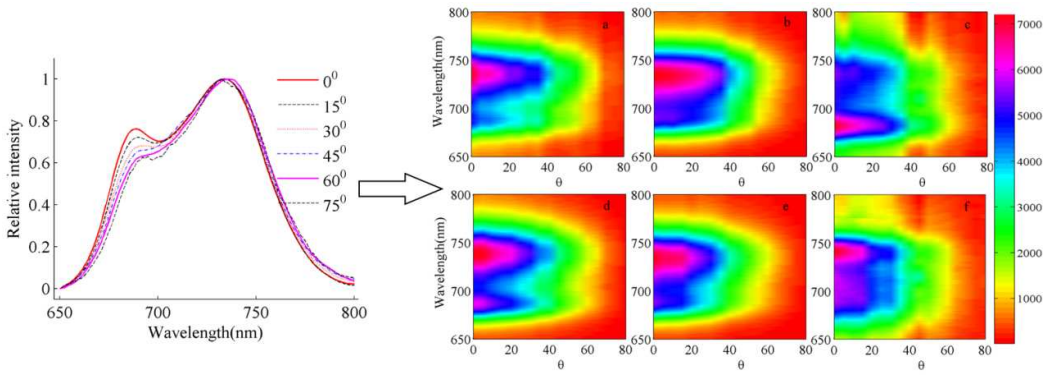
The PCIFSD were firstly utilized in plant species analysis. Plant species can be effortless distinguished using PCIFSD in this paper.



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ARTICLE

Vegetation identification based on characteristics of fluorescence spectral spatial distribution

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Abstract: To differentiate and analyze plant types and species, a spectral identification approach is proposed founded on the characteristics of fluorescence spectral spatial distribution. Pseudo-color images of fluorescence spectral spatial distribution outperforming steady-state fluorescence spectra are constructed to serve as individual fingerprints for plant types and species, which can be utilized to accurately discriminate different plant types and species especially the different species of the same family. The introduced method provides a more reliable and stabilized means for identifying and analyzing plant species in the fields of vegetation-ecology and remote sensing. Stability and reliability are validated by using the spatial distribution of fluorescence spectra measurements of paddy rice and dracaena sanderiana at two different incident angle of excitation light source in an additional experiment.

Introduction

To specifically distinguish and identify various vegetation types and species is one of the most significant purposes in the development of the global remote sensing technology [1,2]. Once the plant species are acquired, a background understanding of its growth pattern, both geographically and temporal, can be employed to correlate the spectra detections with factors including canopy cover[3,4] or total green biomass[5,6] and the effects of environmental stress[7,8]. Thereby, identification of plant species is important for assessing capacity of vegetation in the changes and transitions of the natural environment through biophysiological activities.

In the last few decades, many techniques of passive and active remote sensing[9-12] have been presented to detect the vegetation types and species. Spectral reflectance measurements of plant region mainly afford only frank information associating with how much of a region being scanned is wrapped by plant containing chlorophyll. These may be very valuable information, especially for those interested in the changing of detecting regional plant. However, further significant utilizing of the detecting data is depended on the capacity to accurately distinguish and identify vegetation species or vegetation groups. Thus, all of these reasons prompt investigators to search for new technologies. A remote sensing technique has been investigated by Emmett et al.[13] in the past many years which employed the laser-induced fluorescence (LIF) characteristics of vegetation as a likely approach to identify different vegetation types and species. The feasibility of the LIF as an active remote sensing has been verified by use aircraft [14]. Thus, it could be established from satellites in a fashion similar to passive multi-spectral reflectance scanners. Whereas, it is usually insufficient for identifying varieties of plant species, especially those that contained the same family, that traditional LIF technology depending on measurements of single-photon emission fluorescence spectra. To compensate for these limitations and flexibly to traditional LIF technologies, time-resolved fluorescence[15-16] and fluorescence decay measurements[17-19] have been studied. These approaches will greatly improve the ability of discrimination of different plant species, but the application of fluorescence decay is restricted by the complicacy of the deconvolution of the time response, and the time-resolved fluorescence is limited due to the signal to noise ratio (SNR), etc.

The LIF spectral intensities and shapes of vegetation change over angle were mentioned by Saito et al. to analyze the number of constituents and the interior structure of the leaves[20]. Thus, in this paper, the pseudo-color image of the fluorescence spectral spatial distribution (PCIFSD) of vegetation is proposed by employing the changes of the intensities and shapes of LIF spectra and utilizing this approach to identify and distinguish different plant species. Compared with the ordinary LIF spectra, more information contained in the two-dimensional false-color fluorescence spectral image of plant species which including both the relative fluorescence intensity and the shapes of the LIF spectra. This method has the advantage of high sensitivity and high discrimination for detecting the plant types or species, which is making classification more reliable and accurate.

Experimental

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Samples

Six kinds of the typical vegetation (including camphora officinarum, scindapsus aureus, cinnamomum kotoense, dracaena sanderiana, paddy rice, bamboo) were collected from the Jianghan Plain which locates in the subtropical zone. The latitude and longitude are 29.970° N and 113.880° E, respectively.

Table 1 The plant species used in this study

Label	Sample	Class
a	Camphora officinarum	Dicots
b	Scindapsus aureus	Monocots
c	Cinnamomum kotoense	Dicots
d	Dracaena sanderiana	Monocots
e	Paddy rice	Monocots
f	Bamboo	Monocots

Principles

The major fluorescence parameters of vegetation include fluorescence quantum efficiency, fluorescence intensity, fluorescence lifetime, and the fluorescence quenching, etc. However, the fluorescence intensity varies with wavelength is one of the most significant indices and it can be presented as follows: [21-23]

$$I(\lambda,\lambda_{ex},\theta) = I_0(\lambda_{ex})a^*(\lambda_{ex})\omega(\theta)\varphi c\int_0^R e^{-(\beta_{\lambda_{ex}}+\beta_\lambda)r}dr \quad (1)$$

Where $I(\lambda,\lambda_{ex},\theta)$ is the emission fluorescence intensity; $I_0(\lambda_{ex})$ is the intensity of excitation light source; *c* is the concentration of fluorophore in the leaf; $a^*(\lambda_{ex})$ is the specific absorption coefficient of chlorophyll at incident light wavelength; θ is the angle of fluorescence emission which is between the normal line and the acceptance direction; $\omega(\theta)$ is the angle coefficient; φ is the fluorescence efficiency at detection wavelength; $\beta_{\lambda ex}$ and β_λ are the extinction coefficients at incident light and detection wavelength; λ_{ex} and λ are the excitation wavelength and the emission wavelength, respectively.

The exponential function is integrated over the leaf thickness *R*. Thus, the principle shows that it is possible to classify the taxonomically similar plant species by using the characteristics of fluorescence spectral spatial distribution.

LIF Instrument

Fig. 1 shows the schematic of the experimental setup, which can be divided into three main parts: the excitation source part, optical receiver system, and the signal acquisition assembly. To ensure the same observational position when the instrument received angle (θ) changes, the sample was placed at the above rotation axis of a rotator. The receiving fiber optics and rotating platform were fixed together by a steel plate which could move around the rotation axis of rotator. In addition, the optical axis of receiving is also through the rotation axis of rotating platform.

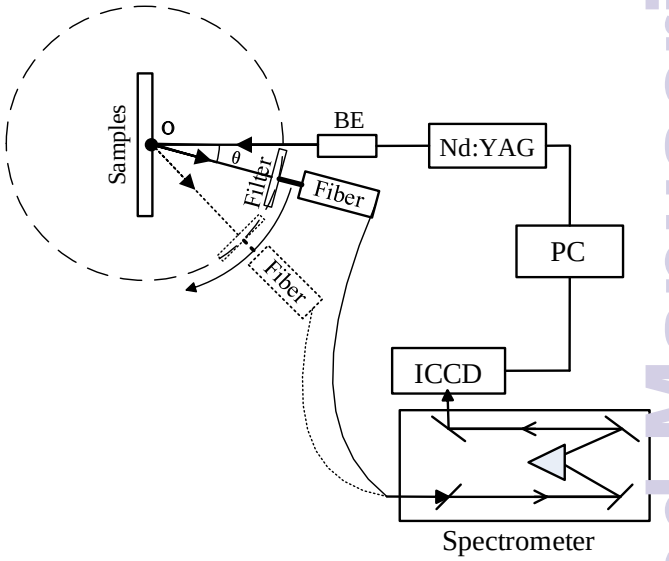

Fig. 1 Schematic of LIF system. PC is a personal computer, BE is a 10 times of beam expander, ICCD is intensified charge coupled device.

The excitation light source is an Nd: YAG, the repetition frequency is 20 Hz with output power and width per pulse are 1.5mJ and 5ns, respectively. The fiber optics with a diameter of 200 μm is employed to gather the fluorescence signal. The fluorescence excited was transmitted into the fiber optics and then focused through a 0.05mm slit entered the spectrometer. The fluorescence signal varies with wavelength was acquired by utilizing intensified charge-coupled device (ICCD), and the data were stored by using a person computer (PC). In order to eliminate the reflected light from the laser entering the fiber optics, an additional 355nm cut-off filter was placed in front of the fiber optics. In this paper, the scans range of spectrometer is ranging from 650nm to 800nm and the sampling interval is 0.5nm.

Fluorescence spectra measurement

Before measurement, samples were stored in the darkroom to keep the stability of the composition. In addition, in order to show the repeatability of the approach, all of the fluorescence spectra are repeated measure more than once. Fluorescence spectra acquired by the LIF instrument (Fig. 1) were stored in a PC and processed with MATLAB. Before analysis, wavelet transform[24] and the moving window polynomial fitting[25] were utilized to denoised and smoothened for these fluorescence spectra, respectively.

Results and discussion

Fig. 2 is the fluorescence spectra of different plant species at 0° of fluorescence emission angle which is the direction of the maximum fluorescence intensity[20]. It can be known from Fig. 2,

the fluorescence spectra (ranging from 650nm to 800nm) of all vegetation reveal two main bands center at 685nm and 740, respectively. The two peaks are attributed to the center pigment (CPI) of Photosystem II and antennae chlorophyll (CPa) of Photosystem I, respectively[26]. It can be found that the six kinds of plant species, especially Camphora officinarum, Scindapsus aureus, and Bamboo are difficult to distinguish just by using the information of LIF spectra (Fig. 2).

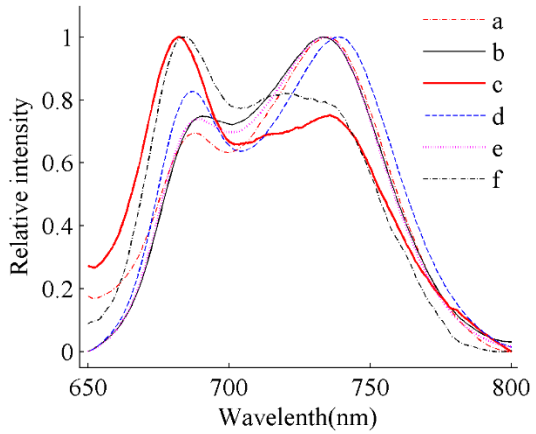

Fig. 2 Normalized LIF spectrum of vegetation with the excitation wavelength is 355nm. The fluorescence emission angle is 0 °. (a) Camphora officinarum, (b) Scindapsus aureus, (c) Cinnamomum kotoense, (d) Dracaena sanderiana, (e) paddy, and (f) bamboo.

The fluorescence spectrum of the plant varying with angle was obtained by measuring the fluorescence intensity at different fluorescence emission angles. Thus, the intensities and shapes of the steady-state LIF spectra of the plant species related with the angle of fluorescence emission. As shows in Fig. 3, it gives an example that the shapes of fluorescence spectra of scindapsus aureus change over angle. In this illustration, the angles of acceptance were set to 0°, 15°, 30°, 45°, 60°, and 75° respectively. This change demonstrates that the shapes of the fluorescence spectrum in the spatial distribution will vary with angle, which can be also known from Eq. (1). These variations in the fluorescence spectra rely on the plant species, which can be conveniently utilized to discriminate different plant species.

Pseudo-color images which are employing angle and fluorescence wavelength as axes are utilized to demonstrate the spatial distribution of LIF spectra. Zero angle on the *x*-axis corresponds to the initial measuring position. As shown in Fig. 4, the angle range of fluorescence emission along the abscissa is 80°, with a sampling interval of 5°. What's more, the longitudinal axis of PCIFSDs constructed at 355 nm excitation wavelength has wavelengths ranging from 650 nm to 800 nm with a 0.5 nm sampling interval. Thus, LIF spectra should be measured at 17 different angles of fluorescence emission for each sample of the plant species to build an intact PCIFSD. In order to improve the SNR and avoid the effect of laser energy fluctuation on the fluorescence intensity, the fluorescence spectrum of each sample has been repeatedly measured 5 times. Thus, less than 1 minute is required for each sample when repetition rate of excitation light source is 2[illegible] Hz. The pseudo-color images demonstrating the difference in the characteristic of fluorescence spectral spatial distribution as well as the shapes of fluorescence spectra over angle can be served as unique fingerprints for plant species (Fig. 4). These PCIFSDs of plant are quite different from each other, demonstrating that the fluorescence spectral spatial distribution based approach can effectively distinguish different plant types and species.

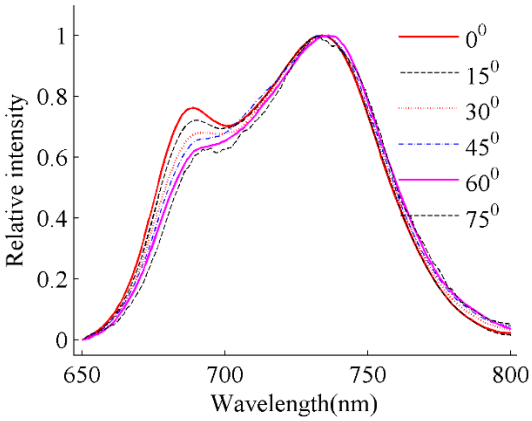

Fig. 3 The normalized LIF spectra of scindapsus aureus with different angles. The angle of fluorescence emission were set to 0°, 15°, 30°, 45°, 60°, and 75° respectively.

A series of LIF spectra, which is associated with the angle, can be acquired along the vertical axis. In addition, the variations in the relative fluorescence intensity of certified emission wavelength can be also measured along the horizontal axis. Compared with that in LIF, the difference among the PCIFSDs of different plant species is more distinct. Plant species samples (e.g., (a) Camphora officinarum (b) Scindapsus aureus, and (f) Bamboo) can be effortless distinguished by using this approach even though they cannot be discriminated by traditional LIF.

In order to show the stability and reliability of the provided approach in discriminating plant types and species, the PCIFSDs of other samples of paddy rice and dracaena sanderiana are acquired at different incident angle (90° and45°) of excitation light source in an additional experiment. Paddy rice and dracaena sanderiana at two different incident angle all have similar PCIFSDs (Fig. 4e and Fig. 4g, Fig. 4d and Fig. 4h), respectively. The results demonstrate that LIF fluorescence properties of plant species could be as unique fingerprint to distinguish different plant types and species. It also provides an evidence of the repeatability of the experiment.

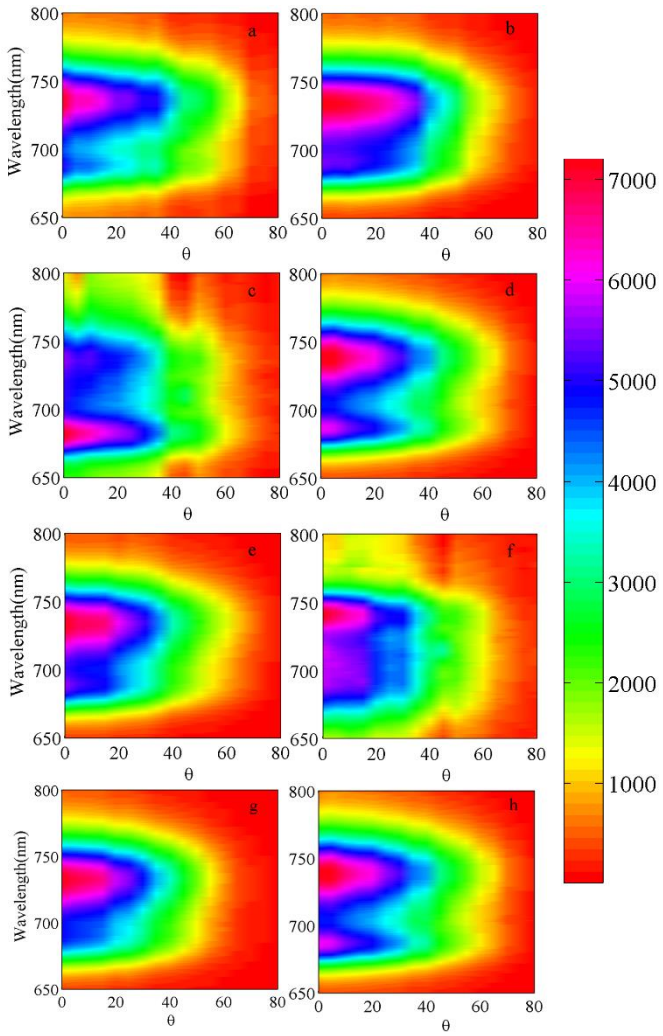

Fig. 4 PCIFSDs of (a) Camphora officinarum, (b) Scindapsus aureus, (c) Cinnamomum kotoense, (d) Dracaena sanderiana, (e) Paddy rice, (f) Bamboo. The y-axis and x-axis are the emission wavelength and the angle of fluorescence emission, respectively. The wavelength ranges from 650 nm to 800 nm, and the angle range is from 0° to 80°. In addition, the incident angle and wavelength of excitation light source are 0° and 355 nm, respectively. (g) Paddy rice, (h) Dracaena sanderiana with incident angle of excitation light source is 45°.

Conclusions

In conclusion, there is certain correlation between the intensities and shapes of LIF spectra and angle (Fig. 3), thus proposing a new method for differentiating various plant species. Compared with traditional LIF spectra and total luminescence spectroscopy, PCIFSDs are proposed to improve the discrimination capabilities. The proposed method superior to the steady-state fluorescence spectra method, which will be effortless for the classification and analysis of plant species. The reliability and reproducibility of the approach are showed by analyzing PCIFSDs of paddy rice and dracaena sanderiana at two different incident angles in an additional experiment. The approach may be also further promoted by utilizing smaller sampling interval in the pseudo-color images. Created to propose peculiar fingerprints for plant types and species, this technique provides biologist and botanist with a more reliable and rapid tools of identifying and discriminating plant types and species.

Acknowledgements

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