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Study of laser-induced plant fluorescence lifetime imaging technology for plant remote sensing monitor

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ABSTRACT

Plant fluorescence is too susceptible to interference and unsuitable for plant remote sensing monitor. A plant fluorescence lifetime imaging technology based on plant remote sensing monitor is presented to solve this problem. The technology can be used to analyse plant physiological information for environmental surveillance. The beam expander system was used to expand laser beam at a certain specific wavelengths, and the living plant chlorophyll fluorescence can be excited by this laser beam. Many of the same plant fluorescence signals were excited by continuous laser pulses, and furthermore the start delay time of Intensification Charge Coupled Device was adjusted consecutively in time resolved measurement method. Finally, a complete set of plant continuous fluorescence image was collected by Intensification Charge Coupled Device. The deconvolution can be used to retrieve the high-precision plant fluorescence lifetime of single pixel point in plant continuous fluorescence image. If the plant fluorescence lifetime values from all of pixel points were retrieved, the distribution map of plant fluorescence lifetime can be drawn. The fluorescence lifetime properties of living plants have already been studied preliminarily. The study indicates that the distribution map of plant fluorescence lifetime can more effectively reflect plant chlorophyll content than fluorescence intensity dose. The plant fluorescence lifetime was related to its chlorophyll content in a certain range, and there is a complex and subtle relationship between plant fluorescence lifetime and its physiological information.

1. Introduction

It's convenient for traditional fluorescence detection technologies using fluorescence intensity as measured parameter to study the plant physiological status. The fluorescence is extremely weak, and it is also susceptible to interference from ambient light, fluorescence scattering angle and excitation light intensity. Thus it is not suitable for remote sensing detection. The ambient light in environment contains a large number of photons with the same wavelength as chlorophyll fluorescence. The ambient light with this wavelength will penetrate optical filter together with the chlorophyll fluorescence in measurement task, and enter the photosensitive surface of detector. Therefore, the measured intensity by the detector is not exactly equivalent to the fluorescence intensity from the plant; moreover, the number of fluorescence photons emitted from the plant to all directions is not the same, so the number of fluorescence photons (fluorescence intensity) received by the detector may vary at different locations. The capacity of emitting fluorescence in the actually measured plant is fixed, so it is difficult for the fluorescence intensity to reflect the capability of plant emitting fluorescence; in addition, the fluorescence intensity is proportional to the excitation light intensity, and there are actually a large number of tiny particles in the air which can seriously affect the intensity distribution of the excitation light, thus the measured chlorophyll fluorescence intensity will be interfered. The fluorescence lifetime indicates the attenuation time of fluorescent photons, which is not interfered by external ambient light, fluorescence scattering angle and excitation light intensity, and it has the advantage of strong stability. Moreover, the chlorophyll fluorescence lifetime imaging technology can remotely detect the spatial distribution of plant physiological on a large scale.

The technology of laser-induced plant fluorescence lifetime analysis is based on the theory of plant photosynthesis. It is a novel type of plant measurement technology using chlorophyll fluorescence as a probe to study the subtle influence of external factors on plant physiological status [1–3]. This technology will gradually become a hot topic in the field of environmental and agriculture monitoring. The measurement method of laser-induced fluorescence lifetime has two forms: fluorescence lifetime scanning technology and Laser-Induced Fluorescence (abbreviated as LIF) lifetime imaging technology. A converged laser beam is used to irradiate the plant and excite it to produce a fluorescent signal in the first method. However, this technology cannot be applied

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to in vivo detection, because the convergent beam will cause plant tissue damage [4,5]. Chlorophyll fluorescence lifetime imaging technology can not only achieve non-destructive detection of living plants, but also get the distribution of fluorescence lifetime in specific regions of plant tissues. The divergent laser source of an appropriate wavelength is used to irradiate the plant samples to produce fluorescence, and to detect the plant fluorescence attenuation properties, so as to obtain its physiological status from chlorophyll fluorescence lifetime distribution map. Plant tissue will not be damaged by the divergent laser in model of continuous irradiation. In addition, fluorescence LiDAR, as a high resolution laser remote sensing technology, has been widely used in environmental monitoring. In 2011, the first plant fluorescence map of the earth was released by the US National Aeronautics and Space Administration (NASA) [6].

The measured chlorophyll fluorescence is produced spontaneously from the interior of the chloroplast, and compared with remote sensing technology based on scattering or absorption, it has greater distinguishing ability. Therefore, the chlorophyll fluorescence imaging can highly distinguish between the chlorophyll molecules and other molecules in the surrounding environment. In addition, chlorophyll fluorescence lifetime is not affected by the intensity of excitation light, ambient light and detector voltage [7,8]. Because of these properties, the chlorophyll fluorescence lifetime imaging technology has a good application prospect in the field of remote sensing detection.

Nevertheless, the chlorophyll fluorescence is weak, and fluorescence lifetime is very short. And the laser beam is very easy to stimulate or damage the plant tissue, so the collecting of chlorophyll fluorescence lifetime map in long distance is a challenging task. It is difficult for traditional instruments and methods to achieve it. In recent years, the single photon counting technology has greatly improved in time response and counting rate. Base on this, it possible to synchronize the transmitted laser pulse with chlorophyll fluorescence signal acquisition in the long-distance. The collecting of fluorescence lifetime map has been preliminarily developed internationally [9–11]. In this article, laser pulse is used to trigger the Intensification Charge-Coupled Device (abbreviated as ICCD) in the time-resolved measurement method [12–14], and then the chlorophyll fluorescence data in pixel array can be collected. The pixel array data contains a large number of time channels. The image processing technology is used to process data in all time channels successively, and the fluorescence lifetime distribution of the whole shooting area can be obtained finally. In the future, this technology will be applied to laser-induced fluorescence lifetime LiDAR (LIFL-LiDAR) to monitor the growth state of terrestrial plants in a wide range.

2. Basic principles of laser-induced chlorophyll fluorescence lifetime imaging

2.1. Measurement principle of laser-induced chlorophyll fluorescence lifetime

Laser beam is usually expanded in laser-induced fluorescence lifetime imaging technology, and then irradiates the plant to excite the chlorophyll fluorescence, whose signal is recorded by ICCD. The fluorescence signal $m_{ij}(t)$ received at any pixel of ICCD is actually the convolution of laser-induced chlorophyll fluorescence signal $y_{ij}(t)$ and ICCD response function $irf(t)$ (here i, j denote the transverse and vertical coordinates of pixels in the images taken by ICCD respectively); $y_{ij}(t)$ is the convolution of the laser pulse function $p(t)$ and the fluorescence attenuation function $g_{ij}(t)$. Here, $g_{ij}(t)$ is defined as

$$g_{ij}(t) = A \cdot \exp(-t/\tau). \tag{1}$$

where τ is fluorescence lifetime value, A represent initial fluorescence intensity. Therefore, the fluorescent signal collected by any pixel of ICCD can be expressed as

$$m_{ij}(t) = irf(t) \otimes y_{ij}(t) = [irf(t) \otimes p(t)] \otimes g_{ij}(t) = srf(t) \otimes g_{ij}(t). \tag{2}$$

This shows that the fluorescence attenuation function $g_{ij}(t)$ shall be obtained first prior to extracting the lifetime value of the fluorescence from Eq. (2). Because the quartz glass does not produce fluorescence, the quartz glass was used to reflect laser pulses. The reflection laser pulse signal recorded by ICCD can represent the system response function $srf(t)$ [15]. Afterwards, fluorescence attenuation function $g_{ij}(t)$ can be obtained by the deconvolution of $m_{ij}(t)$ and $srf(t)$, then to retrieve fluorescence lifetimeτ according to Eq. (1). In addition, the chlorophyll fluorescence lifetime has the following property:

$$\tau = \frac{1}{k_r + k_{nr}}. \tag{3}$$

Among them, k_r is the rate of fluorescence emission, k_{nr} is the rate of non-radiation (photosynthesis) process [16].

2.2. Chlorophyll fluorescence lifetime imaging technology

A set of Continuous Fluorescence Intensity Images taken by ICCD are called CFII data. Each data image in the CFII data is a cross-section of many fluorescent signals, and the CFII data can also be considered as a pixel array with a large number of time channels, as shown in the left of Fig. 1. Each pixel is a time channel that can record a group of discrete fluorescence data. The discrete fluorescence data was fitted, and the smooth fluorescence signal curve can be got, as shown in the right of Fig. 1. The distribution of discrete fluorescence signals in the region can be obtained by continuous shooting of the fluorescence in the plant

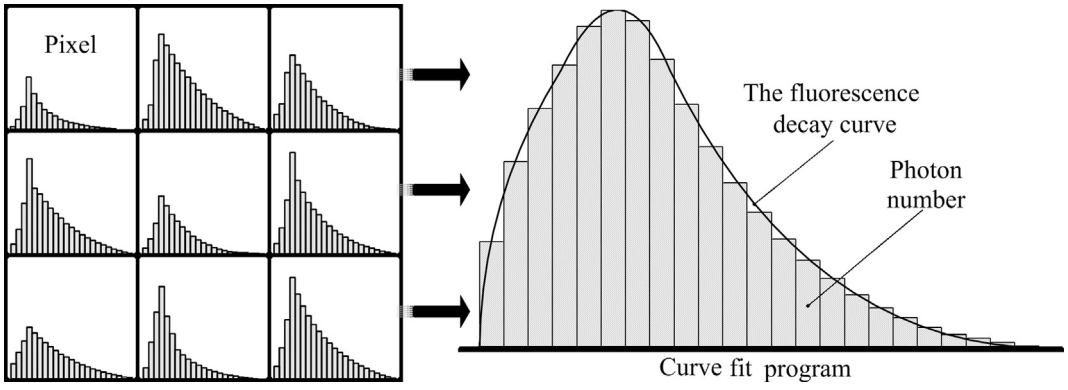

Fig. 1. The signals fitting for all of the pixels in CFII data.