



## PAPER

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# Correcting the effect of the detection angular on laser-induced chlorophyll fluorescence

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**Keywords:** laser-induced fluorescence, angular distribution, fluorescence ratios, intensity correction

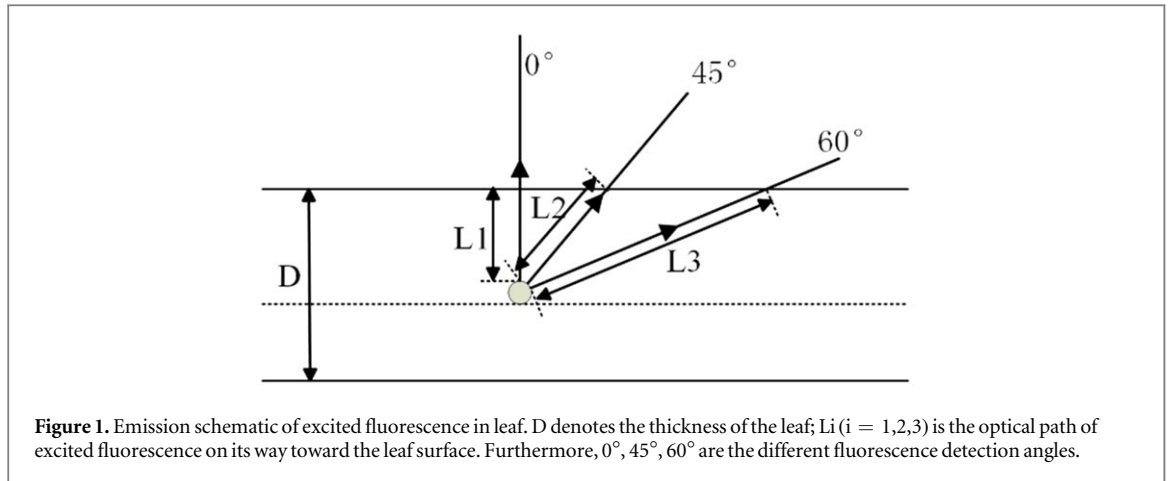
## Abstract

Chlorophyll fluorescence has been widely used to monitor vegetation growth status and quantitative remote sensing monitoring of vegetation biochemical content. Therefore, it is significant to accurately measure the fluorescence information. In this study, the variation in fluorescence intensity of vivo vegetation leaf with the change in detection angle on the basis of laser-induced fluorescence technology was discussed. Experimental results demonstrated that the relationship between the emission fluorescence intensity and detection angles could be explained by the cosine expression. Then, two-step fluorescence intensity correction method was proposed. Firstly, the fluorescence intensity was corrected based on the changing of cosine expression. Secondly, the fluorescence ratio calculated based on the corrected fluorescence intensity. Results demonstrated that the effect of detection angles on fluorescence signals can be efficiently eliminated compared to the traditional spectral ratio method. Findings of this study may be valuable in promoting the applications of laser-induced fluorescence in remote sensing to achieve accurate chlorophyll fluorescence information for quantitative monitoring of plant nutrient stress.

## 1. Introduction

Laser-induced fluorescence (LIF) has elicited extensive attention in technological applications and scientific investigations as soon as it is proposed, and its uniqueness can be regarded as a fluorescent material fingerprint [1, 2]. The fluorescence spectral shapes and intensities are strongly dependent on the fluorophore properties [3]. LIF also exhibits superior sensitivity compared to other methods [4, 5]. Thus, LIF as a prime technology has been comprehensively utilized in chemical, biological, and medical fields to measure DNA sequences, fluorophore electronic structures, and complex biological macromolecules [6–10]. In recent decades, research interest in monitoring vegetation that uses LIF in remote sensing has also increased [11–14]. LIF technology can monitor vegetation capacity in the changes and transitions of the natural environment by biophysiological activities and plant productivity [15–19].

Subhash and Mohanan [20] studied rice leaf red chlorophyll fluorescence spectra based on laser-induced fluorescence technology and the fluorescence ratios can be used for monitoring rice nutrient stress. McMurtrey *et al* [21] applied chlorophyll fluorescence spectrum to distinguish nitrogen fertilization levels in field corn. The capability of LIF for monitoring crops status has been investigated, and they found that fluorescence intensity ratio  $F_{685}/F_{730}$  (the fluorescence intensity at 685 nm divided by that at 730 nm) is sensitive for the changing of chlorophyll concentration when chlorophyll concentration does not significant reduction [3, 22, 23]. Gameiro *et al* [24] used LIF technology as a fast and non-destructive mean to analyze the water stress of *Arabidopsis*. Gu *et al* [25] discussed the effect of flooding and waterlogging on the fluorescence characteristics. Anderson *et al* [26] analyzed the performance of LIF spectra for the assessment of the crop yield of cowpea (*Vigna unguiculata* (L) Walp), and found that the fluorescence characteristics can be efficiently applied in analyzing the change in photosynthetic activity. In addition, Yang *et al* [12, 19] thoroughly discussed the performance of fluorescence



parameters combined with multivariate analysis in the monitoring of N stress in paddy rice. What is more, leaf nitrogen concentration monitoring on the basis of the combination reflectance spectrum with fluorescence parameters was also investigated [27, 28].

In order to acquire the accurate fluorescence information, some influencing factors of fluorescence intensity must be discussed in the application of fluorescence signals. Apostol *et al* [29, 30] investigated the effect of the excitation wavelengths on the fluorescence characteristics in the remote detection of the nitrogen status of crops. The effect of temperature and laser on the fluorescence signals was also discussed by investigators [31]. In addition, our previous research analyzed the effect of the incidence angle on chlorophyll fluorescence signals based on LIF-Lidar. In this situation, the incidence direction of excitation light is consistent with the direction of the received signal. The changing of emission fluorescence intensity with incidence angles can be described by using the cosine description [32]. However, few of researches considered the changing of detection angles (DAs). Therefore, correcting the angular distribution of vivo vegetation leaf fluorescence intensity is critical for the development of LIF technology in remote sensing from the leaf scale.

Unlike various applications of fluorescence [33–35], few investigations have been conducted on the angular distribution of fluorescence intensity of vivo vegetation leaf. In this study, the fluorescence spectrum of vivo vegetation leaf was measured at different DAs by using an improved version of the existing fluorescence measuring system with different vegetation varieties. Then, the variation in fluorescence intensity with the change in DAs was analyzed. Lastly, the performance of fluorescence ratio calculated based on the corrected fluorescence intensity by using cosine expression for eliminating the effect of DAs on fluorescence signals was analyzed.

## 2. Materials and experiment

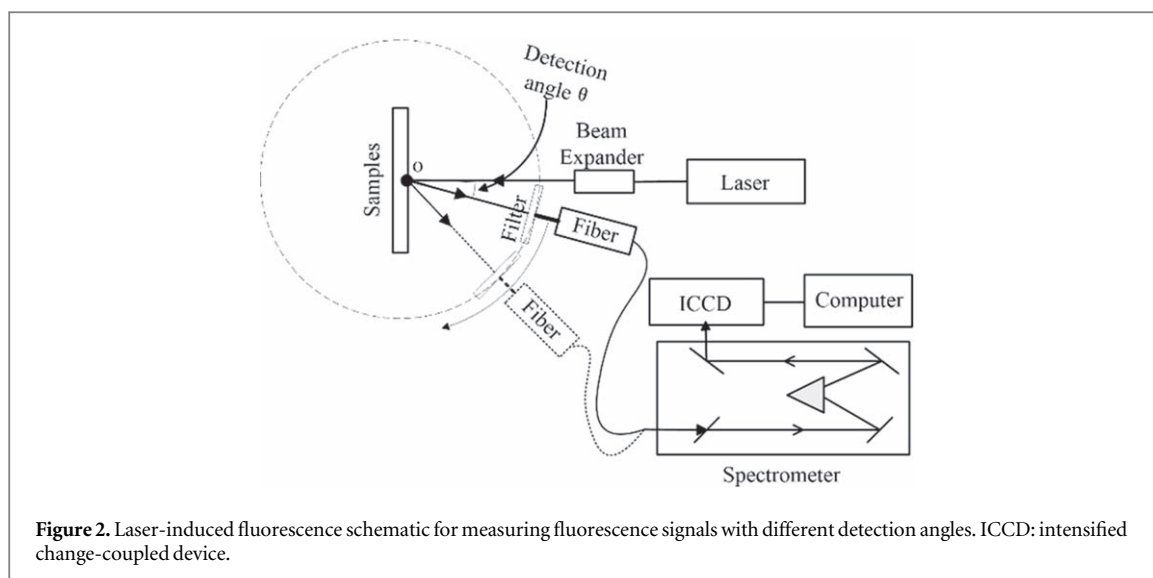
### 2.1. Theory

Fluorescence refers to the phenomenon when fluorophore transfers part or all of its absorbed energy at longer wavelengths after exposure to photons of a certain wavelength [36]. Previous investigations have indicated that the excited fluorescence will be released in all directions with the same radiation quantity [37]. The pathway on its way toward the leaf surface is different which can be illustrated in figure 1.

Figure 1 shows the optical path of the variation in excited fluorescence with the change in DAs in the leaf. The leaf thickness is ranged from 100 to 300  $\mu\text{m}$ . The fluorescence intensity of vegetation leaf can be described as a function of DAs. Related investigations have demonstrated that the emission fluorescence will be re-absorbed on its way toward the leaf surface. In addition, the optical paths increase with DAs. According to previous researches [13], the angular distribution of the fluorescence intensity can be assumed similar to the distribution of cosine. However, the effect of reabsorption characteristics and internal structure on the fluorescence signal, the angular distribution of fluorescence intensity can be assumed as [13, 32, 38]:

$$I(\theta) = a \cdot \cos(b \times \theta) + c \quad (1)$$

where  $\theta$  is the DA that is projected between the normal and the detection direction,  $a$ ,  $b$  and  $c$  are the parameters that describe vegetation characteristics. Thus,  $b = 0$  indicates that the fluorescence intensity is unaffected by the DAs. then, parameters  $a$  and  $c$  are related to the chlorophyll concentration and the excited light energy.  $b = 1$  and  $c = 0$  show equation (1) is complete cosine expression. Given that the fluorescence intensity depends on the chlorophyll concentration of the vegetation leaf, parameter  $a$  will be associated with the leaf chlorophyll



concentration and the depth of penetration. In this study, the coefficient of determination ( $R^2$ ) in the fitting was used to analyze the performance of the cosine expression.

## 2.2. Laser-induced fluorescence system

A schematic for measuring fluorescence intensity varying with DAs is illustrated in figure 2. To ensure the same observational position when measuring the fluorescence intensity of different DAs, the leaf sample was placed above the rotation axis of a rotator. The receiving fibres and rotating platform were fixed together by a steel plate that could move around the rotation axis of the rotator. Furthermore, the optical axis of the receiving system and the rotation axis of rotator were retained in the same plane and orthogonality when detection angle changed. The excitation source was a 355 nm frequency-tripled Nd:YAG laser with output maximum peak energy and the width per pulse being 30 mJ and 5 ns, respectively. Surelite I-20 included a Q-switch which was used to control the output energy. Q-switch demonstrates the ratio of the total energy stored to the energy lost per unit time in the chamber. The value of Q-switch was set to 340 and the output power of per pulse was 1.5 mJ in this study which will not damage the sample after the experiment. Then, the laser light through a  $5\times$  beam expander. Emission fluorescence was measured using a spectrograph (SP-2558) and ICCD (PI-MAX). In addition, a long-pass filter of 355 nm was positioned between the sample and fibre to eliminate the effects of light reflected from the sample. The fluorescence spectrum acquired ranged from 650 nm to 800 nm with a sampling interval of 0.5 nm.

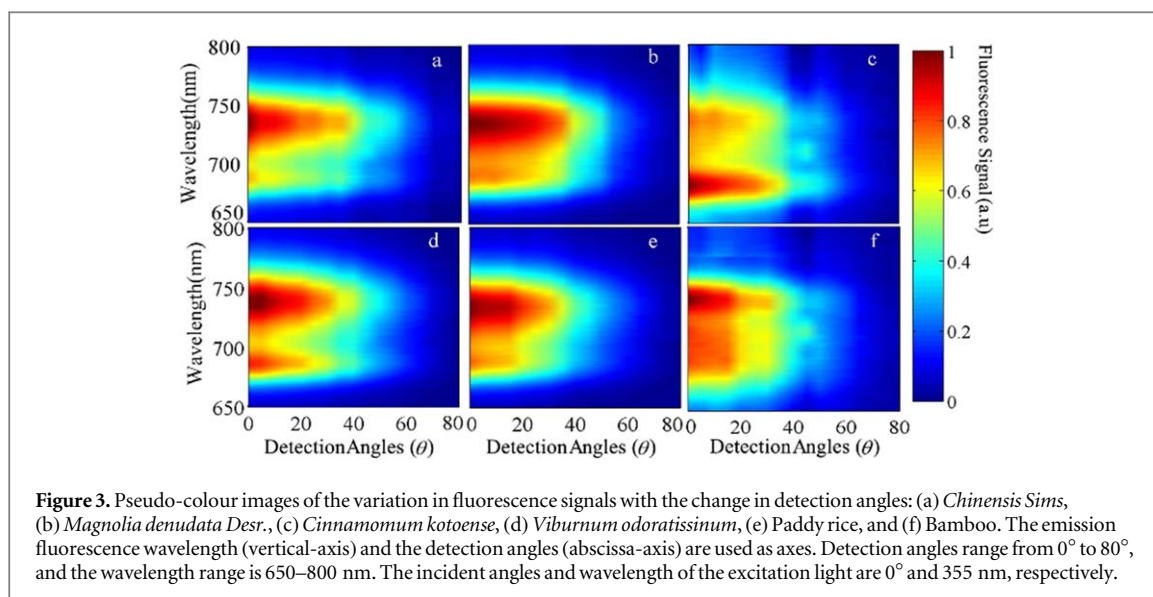
## 2.3. Materials

Six types of vivo vegetation leaf (including *Chinensis Sims*, *Magnolia denudata Desr.*, *Cinnamomum kotoense*, *Viburnum odoratissimum*, Paddy rice, and Bamboo) were used in our experiments for demonstrating the use of the cosine expression to analyze the relationship between the fluorescence intensity and DAs. The samples were collected from Jiangnan Plain, China, which is located in the subtropical zone. The latitude and longitude of the area are  $29^{\circ}26'-31^{\circ}22'N$  and  $111^{\circ}45'-115^{\circ}05'E$ , respectively. All leaves were destructively sampled by random cutting and stored in the freezer of  $-20^{\circ}C$  to keep the vivo of leaf and the stability of the composition.

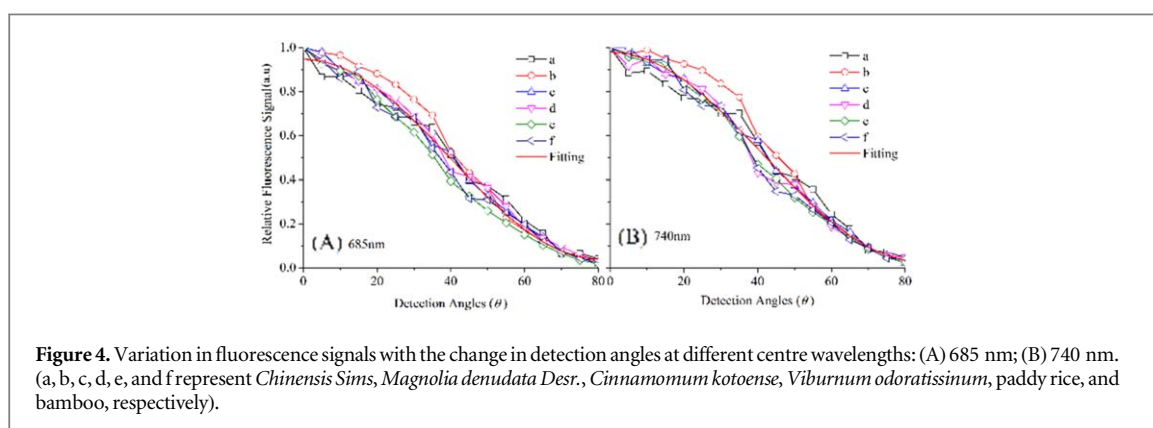
## 3. Results and discussion

### 3.1. Fluorescence spectra

For each measurement, a single leaf of vivo vegetation was flattened completely and laid flat on top of the measurement platform. In the measuring process, the optical axis of exciting light was perpendicular to the measurement platform. The fluorescence spectrum of different DAs was acquired by rotating the receiving system with a sampling interval of  $5^{\circ}$ . In addition, the distance between fibres and targets is about 2.1 m. The range between fibres probe and excited light axis is about 2.5 cm. Thus, the initial angle was about  $0.68^{\circ}$  and approximates  $0^{\circ}$ . In figure 3, pseudo-colour images of the vegetation leaf relative fluorescence intensities (photon numbers) were used to demonstrate the variable relationship between the fluorescence intensity and DAs.



**Figure 3.** Pseudo-colour images of the variation in fluorescence signals with the change in detection angles: (a) *Chinensis Sims*, (b) *Magnolia denudata Desr.*, (c) *Cinnamomum kotoense*, (d) *Viburnum odoratissimum*, (e) Paddy rice, and (f) Bamboo. The emission fluorescence wavelength (vertical-axis) and the detection angles (abscissa-axis) are used as axes. Detection angles range from  $0^\circ$  to  $80^\circ$ , and the wavelength range is 650–800 nm. The incident angles and wavelength of the excitation light are  $0^\circ$  and 355 nm, respectively.



**Figure 4.** Variation in fluorescence signals with the change in detection angles at different centre wavelengths: (A) 685 nm; (B) 740 nm. (a, b, c, d, e, and f represent *Chinensis Sims*, *Magnolia denudata Desr.*, *Cinnamomum kotoense*, *Viburnum odoratissimum*, paddy rice, and bamboo, respectively).

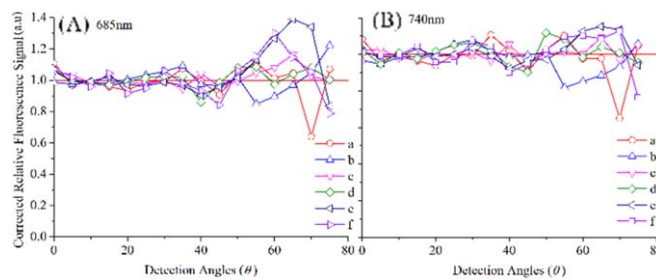
According to Saito *et al* [39], fluorescence signals of vegetation leaf are mainly contributed by the chlorophyll, and the spectrum ranges from 650 to 800 nm. The centre wavelengths of fluorescence peaks are 685 and 740 nm, and these peaks are contributions of the centre pigment of Photosystem II and antennae chlorophyll of Photosystem I, respectively. Figure 3 shows the variation in emitted fluorescence intensity with the change in DAs for different vegetation species. The fluorescence intensities (at 685 and 740 nm) of these plants were consistent as the DAs changed. The fluorescence intensities excited by the 355 nm laser decreased with the increase in DAs. Figure 3 further demonstrates that the fluorescence intensity decreased to half of its original value when DAs exceeded  $40^\circ$ . These findings will be useful for the application of fluorescence in quantitative monitoring of plant nutrient stress.

### 3.2. Relationship between fluorescence signals and detection angles

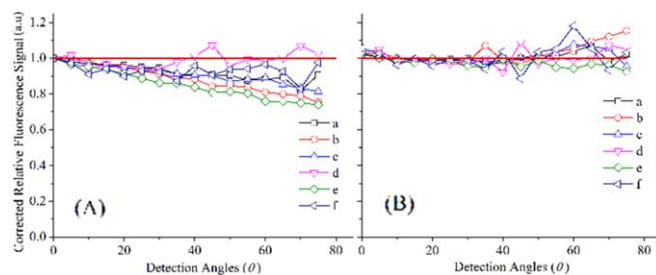
The fluorescence signals of characteristic wavelengths at 685 and 740 nm were discussed separately in quantitatively analyzing the changes in the fluorescence intensity as a function of DAs. Fluorescence signals of different DAs were normalized to  $I(0^\circ) = 1$  in determining the angular distribution of fluorescence intensity. Notably, the fluorescence intensity changed following a function of DAs (figure 4), and six types of species exhibited the same results. Equation (1) was used to fit the average value of fluorescence intensities of the six types of leaves at 685 and 740 nm. The results demonstrated that the  $R^2$  was 0.946, and RMSE was equal to 2.07% at 685 nm (figure 4(A)) the  $R^2$  was 0.956, and RMSE was equal to 3.14% at 740 nm (figure 4(B)). Therefore, the experimental results demonstrated that the correlation between fluorescence intensity and DAs was in accordance with the cosine expression.

### 3.3. Correction of fluorescence signals

Then, the effect of DAs on the fluorescence intensity was eliminated to a certain extent by using the cosine expression. The corrected fluorescence intensity changing with DAs is shown in figure 5.



**Figure 5.** Correction of fluorescence signals based on the cosine expression at different centre wavelengths: (A) 685 nm; (B) 740 nm. ((a)–(f) represent *Chinensis Sims*, *Magnolia denudata Desr.*, *Cinnamomum kotoense*, *Viburnum odoratissimum*, paddy rice, and bamboo, respectively).



**Figure 6.** Correction of fluorescence signals based on (A) the ratio of fluorescence F740/F685 (the fluorescence signal at 740 nm divided by that at 685 nm); (B) the ratio of corrected fluorescence using cosine expression ((a)–(f) represent *Chinensis Sims*, *Magnolia denudata Desr.*, *Cinnamomum kotoense*, *Viburnum odoratissimum*, paddy rice, and bamboo, respectively).

Figure 5 shows the changing of corrected fluorescence intensity by using the cosine expression with the DAs. The results demonstrated that the effect of DAs on the fluorescence intensity can be eliminated within 10% error for all vegetation varieties when the DAs less than  $50^\circ$ . However, the error increases as the angle increases when the DAs more than  $55^\circ$ . The main reason is that the fluorescence signal decreases as the DAs increases, resulting in a lower signal-to-noise ratio (SNR). In addition, excited fluorescence would be re-absorbed by the chlorophyll on its way toward the leaf surface [40]. Figure 1 demonstrates that the optical path of excited fluorescence on its way towards the leaf surface was added with the increase in DAs. Thus, the cosine expression exhibited the potential for reducing the effect of the DAs on the chlorophyll fluorescence.

In order to efficiently eliminated the effect of the DAs on the fluorescence intensity, the fluorescence ratio (F740/F685: the fluorescence intensity at 740 nm divided by that at 685 nm) was used based on the corrected fluorescence intensity using the cosine expression. Then, the variation of fluorescence ratio with DAs was shown in figure 6(B). In addition, the traditional fluorescence ratio based on the fluorescence intensity without corrected was also conducted figure 6(A).

Figure 6 is the changing of fluorescence ratio (F740/F685) with DAs. Figure 6(a) is the fluorescence ratio based on the fluorescence intensity without corrected by using the cosine expression. The fluorescence ratio decreases as the DAs increases. For the reflectance, the spectral ratio can efficiently eliminate the effect of the geometrical factors on spectral information. However, the mechanism of LIF is different to the reflectance. LIF is that the chlorophyll absorbs a photon of light with sufficient energy to excite an electron within the molecule to a higher energy state. In addition, the emission spectra from the molecule will be re-absorbed by the chlorophyll pigment and internal structure of leaf on its way toward the leaf surface. Relative researches demonstrated that the fluorescence emitted between 680 and 695 nm was more strongly reabsorbed by the chlorophyll pigment in the upper layer leaf cells than the fluorescence emitted between 730 and 750 nm [41]. Therefore, chlorophyll pigment had little influence on the fluorescence peak at 740 nm.

Figure 6(b) is the fluorescence ratio based on the corrected fluorescence intensity changes with DAs. It can be found that the effect of the DAs on fluorescence signals can be efficiently eliminated by using the fluorescence ratio calculated based on the fluorescence intensity corrected by the cosine expression. The actual mechanism was still difficult to determine in the present work. However, the possible interpretation is that the cosine expression can eliminate the influence of reabsorption on the fluorescent signal to some extent. In addition, the emission fluorescence might be affected by the internal organizational structure due to the scattering and the pigment composition of the vegetation due to the absorption. Therefore, a detailed mechanism needs to be



further studied. By comparison, it is found that the fluorescence ratio calculated by the corrected fluorescence signal is superior to that calculated directly by the fluorescence signal. The cosine expression exhibited the potential for efficiently reducing the effect of the DAs on the chlorophyll fluorescence, which can provide reference for the further application of LIF technology in quantitative monitoring of agricultural biochemical concentration.

## 4. Conclusion

This investigation presents the angular distribution characteristics of vivo vegetation leaf fluorescence intensity induced by an ultraviolet laser (excitation wavelength is 355 nm). Numerical and experimental results demonstrate that the variation in excited fluorescence intensity with the change in DAs is in accordance with the cosine expression. Then, the fluorescence ratio calculated based on the fluorescence intensity corrected by the cosine expression exhibited better performance for eliminating the effect of DAs on fluorescence intensity than that calculated directly by the fluorescence intensity without cross corrected. The determination of the cosine expression that governs fluorescence intensity distributions will be valuable for correcting the intensity information of LIF to achieve accurate quantitative monitoring of plant nutrient stress and assessment of crops productivity. The proposed fluorescence intensity corrected method can potentially promote LIF for monitoring vegetation status and improving the accuracy of fluorescence intensity retrieved.

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