Investigating the relationship between passive and active fluorescence

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Introduction

Chlorophyll fluorescence as a probe to photosynthesis is used to examine photosynthetic performance and stress in plants. It became evident that under some circumstances fluorescence emissions in photosynthetic organisms could be correlated to their photosynthetic rates(Baker, 2008). According to excitation light, the chlorophyll fluorescence is classified into passive and active. Passive fluorescence (solar-induced fluorescence, SIF) offers one of the most powerful ways to non-destructively quantify plant photosynthetic and dissipation activity from local, region to global scale. However, the strong dependence to the incoming light makes SIF signal difficult to monitoring plant functional status, such as stress detection. In contrast, in active fluorescence detecting techniques, a constant modulated artificial light is applied as the excitation light. The weak and modulated light allows detecting fluorescence signal without affecting by ambient illumination whereas the SIF signal is. The active fluorometers only record the fluorescence information excited by the measuring light. Hence, active fluorescence provides a direct approach to monitoring the plant physiology, but it is limited to small scale due to the use of artificial light.

Pulse amplitude modulation (PAM) fluorometer is the most common tool to acquire active fluorescence in physiological and ecophysiolgical studies. It is widely believed that PAM or PAM like active fluorometers measure the relative chlorophyll fluorescence quantum yield. The assumptions made here are that the added measuring light is too weak to affect plant physiology and the fluorescence yield (FY, the ratio between the emitted fluorescence photons and plant absorbed photons) remains under the illumination of the added light.

The information and knowledge from active fluorescence techniques are used to build the understandings of remote sensing observations and also photosynthesis process. Although the measuring light is much weaker comparing to the ambient illumination, the fluorescence yield theoretically changes during the effect of measuring light not matter how small it is. It is undiscovered what are the consequences of the neglected small change. The widely used active fluorescence has not been fully understood and its relationship with SIF are still unclear. A detailed explanation of the active fluorescence measurements is essential to consolidate active and passive fluorescence to interpret photosynthesis activity.

The passive technique allows the measurement of the fluorescence yield(Amoros‐Lopez et al., 2008). The FY is calculated from the spectroradiometric measurements as the ratio of the Fluorescence Photon Flux Density (FPFD) over the Photosynthetic Photon Flux Density.

Measuring SIF and PAM fluorescence simultaneously, the relative FY from passive fluorescence measurements offers a way to interpret the active fluorescence measurement. Further, a better interpretation of plant physiology through SIF from remotely sensed data will benefit from this study. Lastly, the theoretical foundation of SCOPE model will be tested with these measurements. To pursue this goal, both the passive fluorescence and active should be detected more accurately, and more innovative tools will be performed.

The Short Term Scientific Missions (STSMs) has the potential to improve our understanding about active fluorescence measurement and its link to SIF. Meanwhile, collaborating with the group in the Forschungszentrum Jülich, gives the opportunity to access to some specific and advanced instruments, and learn new techniques on fluorescence detecting.

Experiments and methods

Description of the work carried out during the STSM

The mission included field work and model simulation with the support of Professor Uwe Rascher's group in IBG-2, Jülich, Forschungszentrum, Germany. The field experiment was carried out in the Campus Klein Altendorf (CKA) located close to Bonn, Germany.

The first period of the STSM was carried out the field experiment and last about 1 month following with data process and model simulation. In order to comparing passive fluorescence with active fluorescence, the constant area on leaf was measured by using PAM and SIF-Box. We studied the diurnal cycle of several plants, including corn, soybean and sugar beet.

Two advanced instruments were used in this mission. The passive fluorescence detecting system, so called SIF-Box, uses a QE-Pro (Ocean Optics) hyperspectrometer to obtaining incoming radiance and apparent reflected radiance from plant. It allows the measurement of the passive fluorescence automatically. The spectrometer has two channel connecting with two fiber. One channel (REF) measures the sun irradiance over the reference panel. Another fiber points towards the vegetation and measures the reflected radiance (VEG). By opening and closing the shutters of the single spectrometer, we are capable of alternatively measuring: 1) irradiance (REF); 2) reflected radiance (VEG); and 3) dark

current (DC). The system also includes a Peltier element and stabilizes the temperature inside the compartment at 20 °C. This keeps the DC of the spectrometer, which is dependent on operating temperature, at a stable level. (For more details, see (Burkart et al., 2012))

Using an integrating sphere (1200C-SL, Labsphere, North Sutton, US) with known radiance, we measured both channels of the SIF-Box at different integrating time. Additionally, a DC measurement was taken with each integrating time so that the digital numbers measured for each pixel at different integrating time could be translated into physical radiance (radiance in mW sr^{-1} m^{-2} nm^{-1}).

For acquiring active fluorescence, the Monitoring-PAM fluorometer or MONI-PAM (Walz, Effeltrich, Germany) was used. It facilitates continuous measurements of the chlorophyll fluorescence from constant area. The MONI-PAM comprise up to 7 emitter-detector units (MONI-head/485). Each MONI-head/485 represents an independent fluorometer. All the data are transferred using USB, RS232 or Ethernet communication. A PAM fluorometer uses a modulated fluorescence excitation light, so called measuring light. This light passes a short-pass filter and the photodiode detector of the fluorometer was protected by far-red cut-off (long-pass) filter. Normally, the measuring light used in PAM fluorometer can be adjusted to several probing flashes of micron second level length and variable intensity. For instance, the measuring light intensity of the MONI-PAM includes between 1 and 5 probing flashes of 8 µs length, and photon flux densities between 0.1 and 1 µmol$m^{-2}s^{-1}$ at 5 Hz, or between 1 and 15 µmol$m^{-2}s^{-1}$ at 100 Hz. (For more details, see(Porcar-Castell, Pfündel, Korhonen, & Juurola, 2008))

In one day, the leaf properties, such as the chlorophyll content, the water content, can be assumed as constant. The driver of the fluorescence yield or the passive is only the incoming light and the temperature. If the measurement light is kept unchanged, the active fluorescence is also affected by the same environmental factors. In this case, passive and active fluorescence are comparable since both of them can be expressed as the function of the fluorescence yield.

In this study, Moni-PAM was used to acquire the active fluorescence with the frequency of 10 minutes. Meanwhile, the passive fluorescence was obtained with the SIF-box (Fig.1). SIF-box measures passive at the frequency of 30 seconds and worked from 7:00 to 19:00 (local time). Moni-PAM worked continually even in the night and it allows obtaining the maximum fluorescence of the dark-adapted leaf.

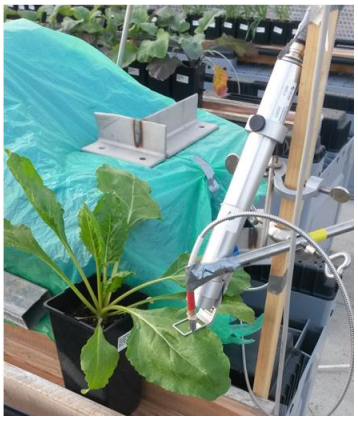

Figure 1. The measuring of passive and active fluorescence

Several plants were investigated in the period from August 6th to August 26th. However, the detection of SIF is highly critical of the weather. Negative fluorescence or abnormal observations were found in the raining and cloudy days.

Table 1. the description of measurements

Date	Plants	Instrument (active)	Starting end	End time
Aug 13	Corn	Moni-PAM	11:00	12:30
Aug 14	Corn	Moni-PAM	10:30	17:00
Aug 19	Soybean	Mini-PAM	10:00	17:00
Aug 21	Corn	Moni-PAM	7:00	19:00
Aug 22	Sugar beet	Moni-PAM	7:00	19:00
Aug 23	Sugar beet	Moni-PAM	7:00	12:00
Aug 25	Soybean (treated with DCMU)	Mini-PAM	14:00	16:00
Aug 26	2 leaves on the one sugar beet plant	Moni-PAM	9:00	14:00
Aug 26	Sugar beet (one treated with DCMU, another as a reference)	Moni-PAM	14:00	17:00

Solar-induced fluorescence measurements

The method to detect passive fluorescence emission based on the in-filling method named FLD was presented in (Plascyk, 1975) and (Plascyk & Gabriel, 1975).The SIF signal, which contributes less than 3% of the reflected light energy near infrared part of the spectrum, is too weak to detect under natural light. However, reduced by the atmospheric absorption, the solar spectrum at ground level has many dark

lines whose band widths are between 0.1nm and 10 nm (which are so-called Fraunhofer lines). The most important bands in the ChlF emission region, apart from Hα, are two of the oxygen absorption bands: O_2-A at 760 nm and O_2-B at 687 nm. Fluorescence signal is highlight and plant reflection signal is weak inside Fraunhofer Line, which makes it possible to extract SIF (for a review of techniques, see Meroni et al., [2009]).

Based on the assumption that the fluorescence emission and ground reflectance standard are lambert, the vegetation apparent radiance $L(\lambda)$ at band λ is composed by two parts: Reflected energy of incoming light and fluorescence emitted by vegetation:

$$L(\lambda) = \frac{E(\lambda)R(\lambda)}{\pi} + F(\lambda) \quad (1)$$

Where $F(\lambda)$ is the fluorescence at band λ, $R(\lambda)$ is the actual reflectance which is fluorescence free, $E(\lambda)$ is solar incident irradiance interacting with the plant.(Moya et al., 2004)

The original FLD method relies on two flux measurements, one inside and one outside a Fraunhofer line. The magnitude of SIF is deduced by comparing the signal measured inside the dark line with the signal measured in a nearby wavelength that contains the entire solar background irradiance.(Meroni et al., 2009) SIF signal is calculating like the Eq.2 by assuming that the reflectance and fluorescence are constant between inside and outside the selected Fraunhofer line.

$$F = \frac{E_{out}L_{in} - E_{in}L_{out}}{E_{out} - E_{in}} \quad (2)$$

Where L_{in} and L_{out} are the target radiance in and out of the O_2-A Fraunhofer Line. E_{in} and E_{out} represent the solar irradiance in and out of the O_2-A Fraunhofer Line.

To overcome the limitations given by FLD assumptions, Maier et al. proposed the use of three bands FLD method in which the single reference band is replaced by the combination of two bands out of the absorption line, so called 3FLD (Maier, Günther, & Stellmes, 2003).

In this study, both FLD, 3FLD and other two new methods were used. Among these four methods, 3FLD has been widely proven. In this report, all the passive fluorescence was retrieved by using 3FLD method. The comparison and evaluation of retrieval methods will come with a detail report.

Results

During a sunny day, the incoming radiation intensity changes in a similar pattern which is increasing from morning to midday and decreasing after that time. Moni-PAM equipped with a PAR sensor can easily measures the incoming light intensity. Meanwhile, the PAR also can be calculated from the incoming irradiance obtained by the SIF-box. The wavelength chosen for PAR calculation was 400 nm - 700 nm. Here, only one day (August 22nd) measurements were presented.

In the Figure 2, it shows the PAR measured by both PAM and SIF-box, and also the passive fluorescence and active fluorescence. It should be noted that all of them come from the raw data. In the Figure 3, the rejection of outlier data was done. The abnormal data mainly caused by the shade of the surroundings and the unstable weather condition. The principle of the rejections is applying the PauTa rules in the leaf reflectance measurements. The data with the reflectance is out of the range $[\xi - 3\sigma, \xi + 3\sigma]$ (ξ is the average reflectance and σ is the standard deviation) was eliminated.

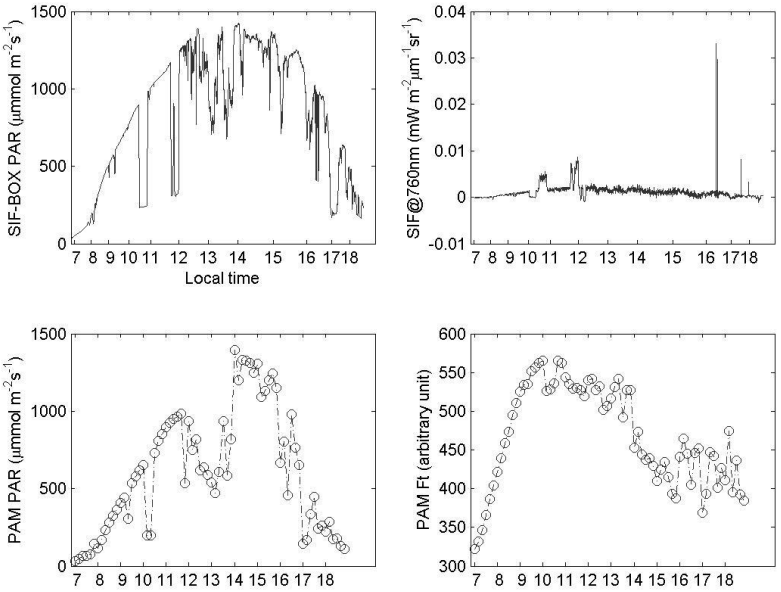

Figure 2. PAR measurements calculated from incoming spectrum (upper, left) and measured by PAM (lower, left). The passive fluorescence calculated from incoming spectrum (upper, right) and the active fluorescence measured by PAM (lower, right);

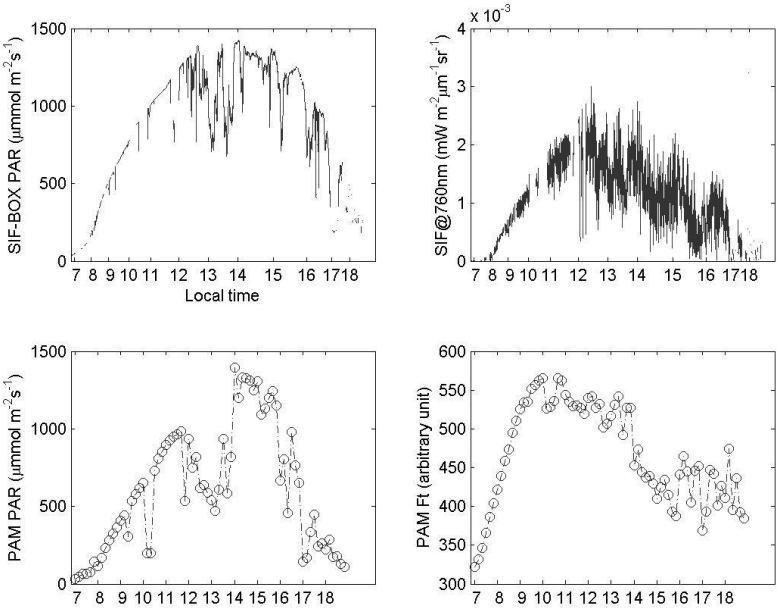

Figure 3. After the rejection of the abnormal data. PAR measurements calculated from incoming spectrum (upper, left) and measured by PAM (lower, left). The passive fluorescence calculated from incoming spectrum (upper, right) and the active fluorescence measured by PAM (lower, right).

SIF-box and PAM measure at different frequencies. In order to comparing these two kinds of fluorescence signal, we selected the SIF-box measurements which measured in the same time with every PAM measurement. The average of ten SIF-box measurements nearby the PAM measurements was taken to reduce the error.

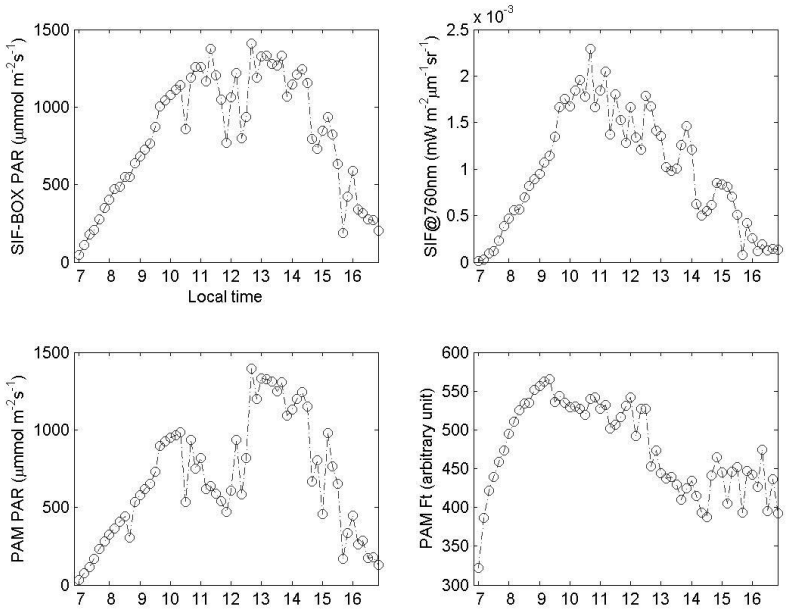

Figure 4. The diurnal PAR measurement from SIF-BOX (upper, left) and from MONI-PAM (lower, left); active fluorescence (lower, right) and passive fluorescence at 760 nm (upper, left)

Both the passive and active fluorescence are close related to the incoming light. In the morning, the plant was getting active, the fluorescence increases. However, the response behaviors of active and passive fluorescence are different. For the active fluorescence, the incoming light and the other environmental factors affect the plant's physiology status. Plants adapt the current environment condition by adjusting the fractions of light used in photochemistry, fluorescence and heat dissipation. The difference between the passive and active fluorescence is that the active fluorescence is induced by constant light, while the passive fluorescence technique detect the signal induced by the solar light. In this case, the incoming light can affect the passive fluorescence signal in two different ways. One is changing in fluorescence emission efficiency, which is the yield of fluorescence. Another more direct way is changing in the light can be used by plant which is photosynthetically active radiation.

Future plan and collaboration

From the data, we successfully obtained PAM measurements and passive fluorescence. However, the effect of measuring light and what does the PAM measures still remains unclear. In the next step, we used model to simulate Ft and compared to the filed measurement and also fluorescence yield. The leaf properties are needed to modeling the active fluorescence, and the samples were collected and preserved in the laboratory at Jülich. The leaf samples will be analyzed with the help of the host institution. The model simulation can provide a straight-forward comparison between Ft and the fluorescence yield. Besides, evidences came from the filed measurement will introduced to offer explanation to support the understanding of the active fluorescence measurement. Further experiments has been planned in November cooperated with the host. Instead of comparing active fluorescence with SIF at 760nm, the whole fluorescence emission spectrum will be collected by using FluoWAT and compared with PAM measurement.

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