SPECTRAL IMAGE MEASUREMENT OF CHLOROPHYLL FLUORESCENCE USING THE OXYGEN A BAND: APPLICATION TO RICE FIELD AND FOREST

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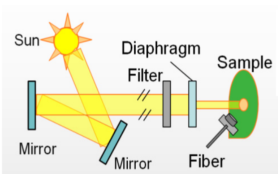
ABSTRACT

We report on a stand-off system that enables the observation of vegetation fluorescence under both laboartaory and field conditions. For field applications, the system enables the spectral measurement using a CCD spectrometer, together with the two-dimensional measurement of the fluorescence intensity distribution by means of a cooled CCD camera. A narrow-band optical filter centered at 760 nm, the wavelength of the oxygen A-band, is employed with the CCD camera to exploit the "solar blind" wavelength for the floresence measurement under daylight conditions. The most difficult aspect of the fluorescence detection is the quantitative separation between the fluorescence signal around 740 nm and the large reflection signal of vegetation leaves in the near infrared (NIR) spectral region. For realizing this separation, we have developed a LED-based light source, which is mostly free from the NIR radiation by utilizing a cyan filter. Under laboratory or night-time outdoor situations, this novel light source makes it possible to observe pure vegetation fluorescence without hinderance from large NIR reflection. In addition, the laser-induced fluorescence methodology is employed for obtaining the spectral shapes of fluorescence signals. The elimination of visible part of radiation, on the other hand, can lead to the measurement of the "pure" reflectance signal free from the effect of fluorescence. The combination of these spectral information makes it possible to implement the detailed analysis of solar radiation induced fluorescence signals. We describe the application of the system to rice field and forest monitoring recently performed in the framework of CEReS joint research.

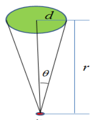
COLD MIRROR MEASUREMENT OF VEGETATION FLUORESCENCE

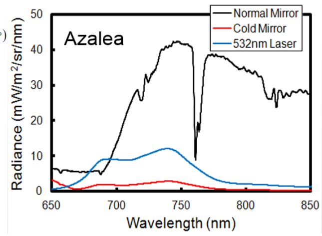


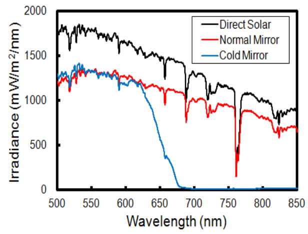
$r = 15\text{mm}, d = 4\text{mm}$

$\tan\theta = \frac{d}{r} = 0.267\ (\theta = 14.94°)$

$\Omega(sr) = \frac{d^2\pi}{r^2} = 0.223$







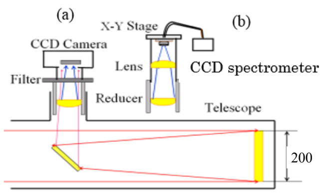
➢ A laser emitting at 532 nm was employed for stimulating LIF: in this case, nearly intrinsic fluorescence spectrum can be observed without any background.

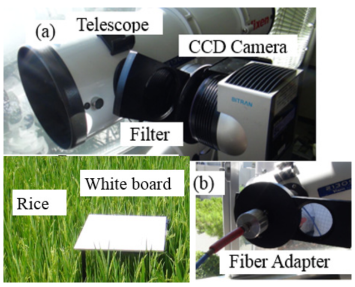
➢The results with normal and cold mirror pairs are compared in the dark room.
➢ For wavelengths longer than ~690 nm, reflectivity of the cold mirror pair is nearly negligible.

➢ A pair of cold mirrors is employed for eliminating the near-infrared wavelength, so that nearly pure fluorescence signal can be observed.
➢ Each cold mirror reflects the visible wavelength with 95% reflectivity, while the reflection of NIR wavelength is reduced to around 0.25% after double reflection.
➢ As seen from this figure, it is found that the intensity of solar radiation induced fluorescence (SRIF) is around 10% that of the NIR reflection.

STAND-OFF FLUORESCENCE DETECTION SYSTEM: (A)CCD CAMERA AND (B) CCD SPECTROMETER

QE65Pro Spectrometer
wavelength range
500 – 880 nm; SN 1000:1





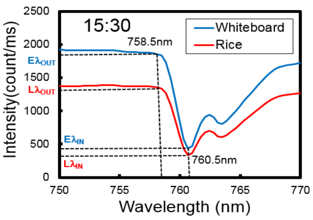
➢ The light scattered off the vegetation sample is collected with a telescope.
➢ The intensity distribution is obtained using a filter and a cooled CCD camera.
➢The corresponding spectral measurement can also be attained by means of a CCD spectrometer.

Collaborative campaign (August 4-7, 2015, Graduate School of Agriculture, Kyoto University)

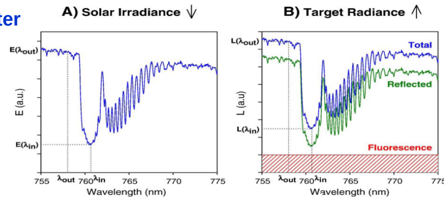
Thermal Imager

Portable Fluorescence System (PAM -WALZ)

Detection with a CCD spectrometer 15:30 on Aug. 5, 2015



Diurnal change of fluorescence Intensity



M. Meroni *et al.*, RSE, 2009.

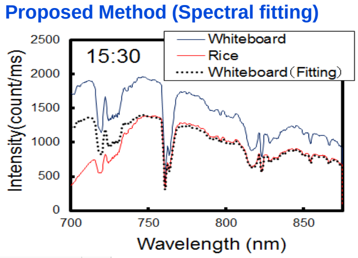
$$L(\lambda_{in}) = \frac{r(\lambda_{in}) \cdot E(\lambda_{in})}{\pi} + F(\lambda_{in})$$

$$L(\lambda_{out}) = \frac{r(\lambda_{out}) \cdot E(\lambda_{out})}{\pi} + F(\lambda_{out})$$

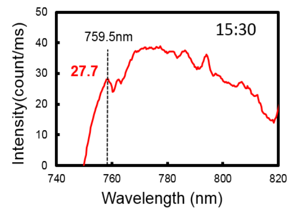
$$F(\lambda_{in}) = \frac{E(\lambda_{out}) \cdot L(\lambda_{in}) - L(\lambda_{out}) \cdot E(\lambda_{in})}{E(\lambda_{out}) - E(\lambda_{in})} \quad (1)$$

The rice spectra were recorded with a stand-off distance of 25 m using a CCD (QE65PRO) spectrometer (Kyoto, on Aug. 5, 2015) . Conventionally, Eq.(1) can be employed to calculate the fluorescence intensity, $F(\lambda_{in})$, where stands for the wavelength inside the oxygen A-band (760.5 nm). The radiance observed at the vegetation position is denoted as $L(\lambda_{in})$ and $L(\lambda_{out})$, where λ_{out} (=758.5 nm) is the wavelength just outside the A-band. The corresponding irradiance values observed with a white board nearby the vegetation sample is denoted as $E(\lambda_{in})$ and $E(\lambda_{out})$.

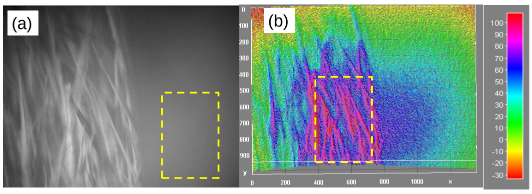
Proposed Method (Spectral fitting)



Spectral intensity around the oxygen A-band observed for vegetation leaves and whiteboard.

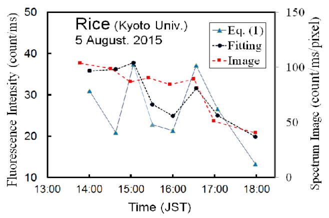


By multiplying the reflectance spectrum of whiteboard with the NIR reflectance of rice (ρ = 0.715), the fluorescence spectrum can be extracted as the difference between the two curves. The intensity at 759.5 nm is compared with the calculation based on Eq. (1).



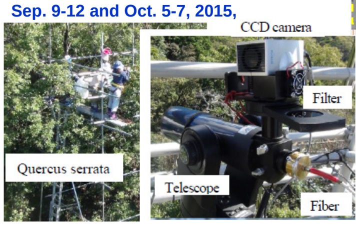
(a) Bitmap image obtained with the narrow-band filter centered at the oxygen A-band. (b) Fluorescence intensity calculated with the spectral fitting.

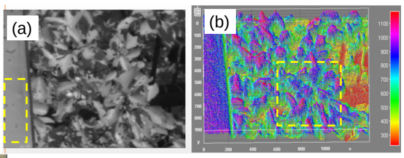
Rice fluorescence distribution observed using a cooled CCD camera equipped with a narrow-band (1 nm FWHM) filter centered at 760.68 nm. (a) Bitmap image of rice (left half) and white-board (WB, right half) placed side by side. (b) Color coded image (Image-J). By subtracting $\rho\times$WB from the rice image, one can extract the two-dimensional distribution of fluorescence intensity.



Chlorophyll fluorescence has been measure at the wavelength of oxygen A-band with three different approaches. The result of spectral fitting approach agreed fairly well with the conventional approach based on Eq. (1). Also, similar temporal change has been found for the bitmap image analysis.

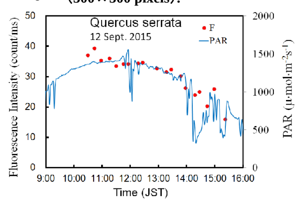
Forest measurement (Quercus serrata, Q.S) Yamashiro-site of Forestry Res. Inst. Sep. 9-12 and Oct. 5-7, 2015,





(a) Whiteboard pixels (200×500 pixel) are used to estimate the NR reflectance of Q.S. tree leaves.

(b) Fluorescence intensity is extracted by subtracting $\rho\times$WB from the image intensity in the designated area (500×500 pixels).

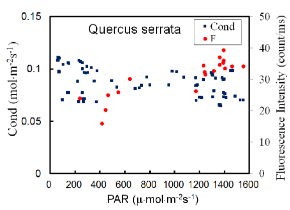


Measurement of solar radiation induced fluorescence (SRIF) from Quercus serrata (Konara) leaves was made at the Yamashiro-site of FFPRI during the two periods of Sep. 9-12 and Oct. 5-7, 2015. The telescope system was installed on a 30 m-tall CO_2 tower, from which the targets were measured with stand-off distance of 10-100 m.

The decrease of SRIF intensity was observed with the decrease in PAR (photosynthetically active radiation).

Conclusion

○ Fluorescence measurement in the laboratory
・With cold mirrors to remove NIR radiation
→ pure fluorescence spectra can be observed with no influence of NIR reflection (similar to LIF)
○ Outdoor SRIF observation of rice or forest canopy
・Stand-off measurement using oxygen A-band
→Alternative use of a compact spectrometer/a CCD camera
→Spectral measurement of SRIF
→SRIF image capture with a narrow-band filter
→Extraction of SRIF by subtracting $\rho\times$WB (white-board spectrum multiplied with NIR reflectance)



Comparison of SRIF intensity with the stomatal conductance observed using a portable photosynthesis system (6400-XT).

Reference

M. Meroni *et al.*, Remote sensing of solar-induced chlorophyll fluorescence: review of methods and applications, Remote Sens. Environ. 113, pp.2037-2051 (2009)