Measurement of Biophoton Emission in Plants – An Alternative Monitoring System for Stress Factors

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- delayed fluorescence and biophoton imaging are non invasive tools to monitor stress

Abstract

Biophoton emission (BE) or autoluminescence imaging is a method to measure stress status of plants in a non-invasive way similar to delayed fluorescence. Both are extremely weak light emissions which can be used to monitor the physical state of a plant. Here we show the difference between both methods and state parameters to perform these measurements using the NightSHADE plant imaging system.

Introduction

Delayed fluorescence is a weak light emitted by healthy pre-illuminated plants (for review[1]). In our previously published application note AN985_003 examples of fungal infections and drought affecting delayed fluorescence[2] are shown. Whereas delayed fluorescence is based on the amount of intact chlorophyll and decreases in the case of stress, the source of biophotonic emission is unknown and increases in stressed parts of the plant.

The ultraweak biophoton emission is generated in response to stress such as wounding, salt stress or pathogen attacks. Its intensity is usually in a range less than 1000 photons/s*cm^{3}[3,4] and correlates with the second burst of ROS, which various plants show in R-gene mediated resistance and hypersensitive reaction responses[5,6]. Although biophoton generation is not dependent on ROS[7] its imaging still offers a nondestructive and facile method to investigate pathogen related processes. The molecular mechanism underlying this light emission is unknown[7]. Biophoton imaging can be performed once delayed fluorescence has faded away.

Delayed fluorescence and biophotonic emission act both as an indicator for the physiological state of a plant and vary based on environmental conditions. Both measurements require an imaging system with deeply cooled and very sensitive CCD (charge coupled device) cameras[3] such as the Berthold NightSHADE LB985 IK-models. We used modified parameters as described by Gould[8] et al., Bennett[7] et al. and Flor-Henry[3] et al. to test our system for its ability to perform delayed fluorescence and biophoton imaging. We show that the camera system of the NightSHADE *in vivo* imaging system is sensitive enough to easily detect these low emissions.

Experimental Procedures

LED afterglow

Low photon emissions such as DF and BE are measured after a short dark adaption of the plants to ensure that all prompt fluorescence of chlorophyll is gone. Furthermore an absolute light-tight dark chamber is needed for detection. In addition the NightSHADE offers LED-panels (470 nm, 660 nm, 730 nm and white LEDs) to excite chlorophyll followed by the detection of light emission up to hours. It has to be assured that during measuring time no afterglow of these LED panels is interfering with the light detection of the ultraweak photon emissions. However, since it is well-known that some LEDs will glow after switching off the electrical power, mirror foil was used to determine how long this afterglow is detectable. The measurement of remaining light was performed 5, 10, 20 and 60 seconds after switching of the LED panels. Afterglow was than detected for

60 s with a 2x2 pixel binning.

Delayed Fluorescence Measurement

Parameters were used as defined by Gould et al.[8] for detecting delayed fluorescence in *Arabidopsis thaliana*. *Carpinus betulus* leaves were illuminated with 35 µE for 10 min. The light intensity just above the sample was determined using the Einsteinmeter LI-250 (Licor). Setting the LED channels of 470 nm, 660 nm and 730 nm to 35 µE/m²*s each. After turning off the light a 10 s or alternatively a 3 s delay was inserted before the measurement. DF was than detected for 60 s with a 2x2 pixel binning. A black and white photo was taken with 0.1 s exposure time, 10% intensity after the DF measurement (Figure 1).

Biophotonic emission

After the measurement of delayed fluorescence the leaves were kept in absolute darkness for 30 minutes due to the observation that BE is masked by DF for 5-10 minutes[3]. An image was taken with 10 min detection time using an 8x8 pixel binning. A black and white photo was taken with 0.1 s exposure time, 10% intensity after the DF measurement.

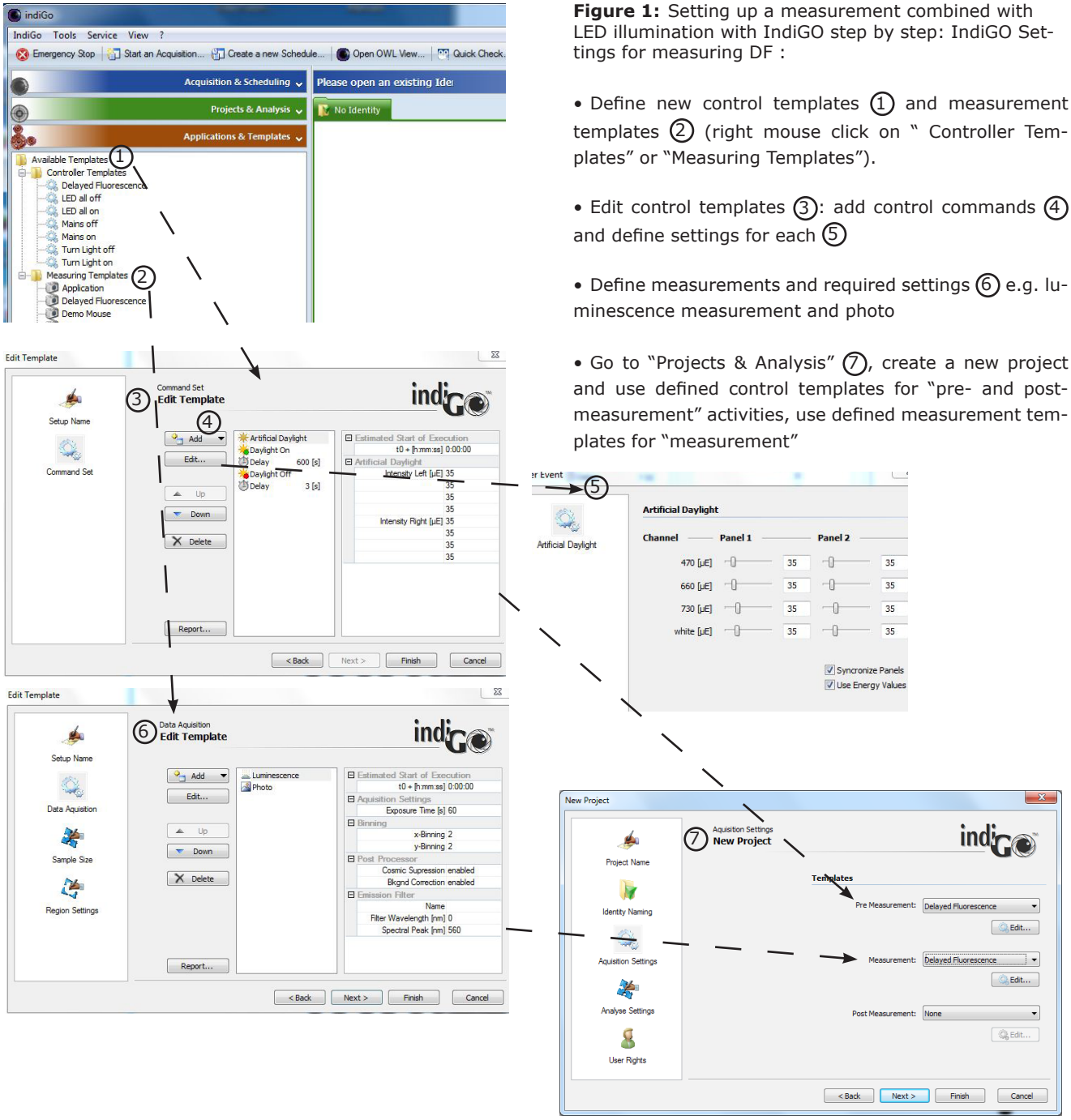

Figure 1: Setting up a measurement combined with LED illumination with IndiGO step by step: IndiGO Settings for measuring DF :

- Define new control templates ① and measurement templates ② (right mouse click on " Controller Templates" or "Measuring Templates").
- Edit control templates ③: add control commands ④ and define settings for each ⑤
- Define measurements and required settings ⑥ e.g. luminescence measurement and photo
- Go to "Projects & Analysis" ⑦, create a new project and use defined control templates for "pre- and post-measurement" activities, use defined measurement templates for "measurement"

Photographic Image

A Canon G11 digital photo camera was used to take a multi-colour image of the leaves. The area of the highest biophotonic emission was marked by standardizing retrieved photos and measurement-images to the same size using Photoshop Elements v4.

Material

- NightSHADE LB 985 IKflu, serial number 4001
- indiGO software v. 2.0.1
- LED panels
- Light-meter LI-250 (LI-COR)
- Photoshop Elements v4 (Adobe)
- Canon G11 digital camera

RESULTS

LED Afterglow

White LEDs show an afterglow after switching of the electrical power for up to 60 s covering the DF signal (Figure 2). Blue (470 nm), red (660 nm) or IR-red (730 nm) LEDs do not show this effect (data not shown).

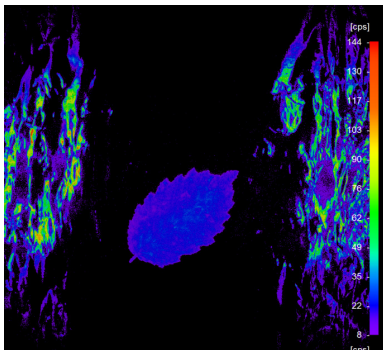

Figure 2: LED afterglow can be seen at the left and right side of the image

DF Image

DF was measured after a 10 min light exposure with 35µE/m²*s. Turning off the LEDs took about 2 s. Together with the 3 s delay time measurement of DF was started around 5s after turning off the light resulting in strong signals, showing a 140% higher intensity than a 10 s delay (data not shown). Furthermore increasing the LED intensity up to 140µE/m²*s did not result in a higher DF signal (data not shown) indicating that the system is already saturated at the 35 µE/m²*s irradiation. As shown in figure 3B healthy leaves show DF over the whole leaf area, reflecting homogenous chlorophyll levels.

BE Image

Both leaves show emission with much lower intensities than for the DF measurement (fig 3C). The highest intensity is detectable in an area where the leaf has damaged, but not complete dry tissue (fig 3D). This occurs along the border of healthy green and dry brown areas of the leaf.

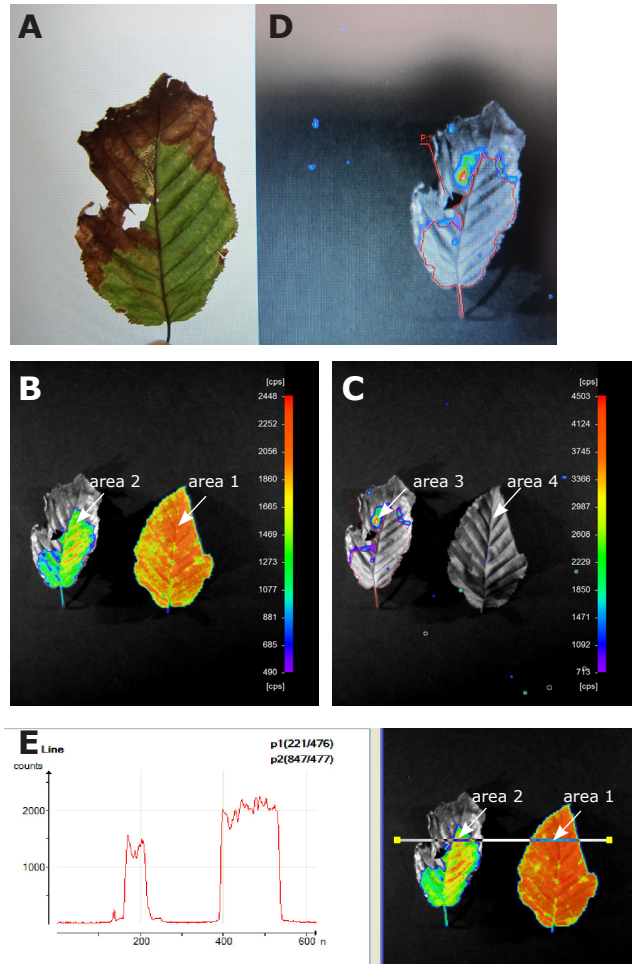

Figure 3: For DF and BE measurements a healty leaf and a brownish leaf (A) are used. B. DF is much stronger in a healthy leaf (right) than in the brownish, damaged leaf (left). The BE signal can only be seen in the part of the damaged leaf which is slightly brown, probably, indicating that this area still consist of living but highly stressed cells. E shows how counts in peak ares were determined using the line tool (see table 1).

Conclusion

DF and BE imaging are useful methods to monitor the stress status of a plant in a non-invasive way. DF is a long-known and well-studied process of photosynthetic organisms[1]. Photon emission results

	Counts (Peak)	Detection time	Binning	CPS (Peak)	Adjusted to pixel binning 1x1
DF (area 2)	1500	60s	2x2	25	6.25
DF (area 1)	2000	60s	2x2	33.3	8.3
BE (area 4)	500	600s	8x8	0.83	0.013
BE (area 3)	5000	600s	8x8	8.3	0.13

Table 1: Signal heights in healthy and damaged parts of leaves for DF and biophotons (see Figure 3). DF signal is higher in healthy areas (area 2) whereas biophoton emission is high in damaged areas (area 3)

from excited chlorophyll following the transfer of plants into the dark. Therefore this post-illumination emission offers a fast and simple way to determine the status of the chlorophyll and the photosystem in plants. Furthermore the level of DF is under a robust circadian control[8]. This means DF also provides a simple and high-throughput way to measure circadian rhythms without the need of reporter genes.

Here we have shown that our *in vivo* plant imaging system NightSHADE is capable of measuring DF. Using the settings of Gould et al.[8] we could easily detect DF and its intensity was dependent on the chlorophyll status of the plant (figure 3). In addition the NightSHADE offers LED panels, which can be used to excite the photosystem, but noticeably DF cannot be performed using white LEDs due to their long afterglow up to 60 s. According to Gould et al. the signal of delayed fluorescence is gone after 60 s and therefore not detectable anymore. We could verify this short lifetime of DF. In our experiments it was already dramatically reduced after 10 s.

In contrast to DF emission the source of BE, also called ultra-weak bioluminescence is unknown, although Birtic et al. could show that lipid oxidation is involved in the generation of BE[9]. Using the NightSHADE system we found the biophoton emission being significantly increased in brown and damaged parts of a leaf. The increase in BE emission has been observed in response to pathogen infections, salt and osmotic stress, mechanical damage and wounding[3]. Biophoton emission seems to be induced by the same conditions inducing ROS production, making it an internal marker of oxidative stress[9]. In our experiments we were able to confirm that DF emission was relatively strong compared to the levels of BE. Taking different detection times and pixel binning settings into consideration (table 1), a correction factor of 160 has to be applied to compare both types of measurements. When comparing the highest peaks of DF (area 2) and BE (area 3) respectively, the emission of DF is about 50 times higher than that of biophotons.

In summary DF and BE measurements show a great potential as universal, high-throughput methods to monitor circadian rhythm, pathogen infestation, R-gene dependent resistance responses, drought and other stresses. Furthermore it has been claimed that BE monitoring might be a non-pertubing measurement of the lipid oxidation status of plants and other organisms[9]. The NightSHADE is a well-suited instrument to perform all of these measurements and detect such low light intensities due to its sensitive CCD-camera. In our experiments we programmed the IndiGO software to perform both measurements in an automatic way with a specified delay time (figure 1). Intensities of DF and BE were easily determined using the analysis function of the software.

Literature

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