# Exploring plant *in vivo* imaging: A comprehensive guide

Improve your plant imaging skills and tackle common challenges with this expert guide into the latest techniques and technologies

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# Introduction

**Crop yield loss is a substantial problem across the globe, brought about by a wide range of biotic and abiotic stresses from drought and heat to pathogens and pollutants. In an era of climate change, such stresses are predicted to increase in intensity, threatening food security and making the study of plant pathogens, stress and basic plant biology a high priority for research.**

Understanding natural plant circadian rhythms and the impacts of stress upon gene expression can aid the development of strategies to mitigate negative impacts upon plant health and, by extension, crop yields. Nevertheless, this understanding is hard-won and often requires real-time whole-plant imaging that is not a feature, for example, of conventional fluorescence microscopes.

The latest *in vivo* plant imaging systems are set to transform plant science research, offering numerous advantages over existing technologies that will be of great interest to researchers in both academia and industry. Whether for plant breeding, crop development, seed manufacturing or agricultural biotechnology in general, whole-plant multi-position imaging under controlled environmental conditions offers a powerful technology solution.

In this eBook, we take a look at several applications of Berthold Technologies' NightSHADE evo *In Vivo* Plant Imaging System to help you understand how this modular,

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easy-to-use optical imaging system enables sensitive luminescence and fluorescence monitoring in plant tissues, seedlings and whole plants in a controlled growth environment. From monitoring the progression of plant disease and understanding responses to environmental stress, to tracking how circadian rhythms affect plant gene expression, learn how the latest *in vivo* plant imaging systems facilitate real-time, multi-position, whole-plant analysis.

**NightSHADE *in vivo* imaging to decode circadian rhythms in plants**

The circadian clock in plants regulates many aspects of growth and development at the molecular level throughout the day/night cycle. Understanding this regulation requires tools that can assess gene expression dynamically over long periods of time and in a controlled environment. In this Application Note, understand how the NightSHADE evo offers several tools for investigating circadian rhythms in the model plant *Arabidopsis thaliana*. This includes quantification of gene expression via bioluminescent reporter genes and measurement of hypocotyl length in seedlings using the lateral camera position, all under carefully controlled lighting conditions.

**Optimizing the analysis of circadian rhythms**

Research on circadian rhythms in general benefits from the ability to monitor gene expression in real time. However, multicellular organisms like plants have circadian clocks that are variable at both the tissue and individual cell level, leading to noisy or inaccurate data. In this Application Note, discover how the NightSHADE evo was harnessed for robust circadian experiments in several *Arabdopsis thaliana* reporter lines, reducing luminescence variation between replicates and improving data quality.

**Harnessing NightSHADE imagery for advanced plant pathogen detection**

Plant pathogens can cause significant yield losses in food crops, threatening food security as well as farmers' livelihoods. In this Application Note, learn how Berthold Technologies' NightSHADE evo imaging system enables the localization of a fungal pathogen in the leaves of infected wheat and an assessment of the extent of the infection. Furthermore, understand how imaging the delayed fluorescence of chlorophyll using this technology can reveal the state of health of the plant's photosynthetic systems in the areas infected by the fungus.

**Illuminating plant stress – delayed fluorescence observations**

Delayed fluorescence (DF) is an indicator of the physiological health of plants and can be used to understand the impacts of various stress factors. It can be measured non-invasively without the need for special labeling or specimen treatment. In this Application Note, learn how DF imaging using the NightSHADE evo easily detected the effects on DF of both fungal infection and mild drought stress (just two days). Plus, understand how the ability to image the whole plant in a single image with the charge-coupled device (CCD) camera is more informative than methods focusing on a part of the plant such as a single leaf.

**Exploring stress signals through combined delayed fluorescence and ultra-weak photon emission**

DF and ultra-weak photon emission are natural properties of plants that vary according to a broad range of stress signals, from drought and heat to pathogens and pollutants. They can be studied non-invasively in physiologically relevant conditions to understand plant health. Using the NightSHADE evo, discover from this Application Note how highly stressed cells in leaves turning brown were clearly detectable using both these properties.

**Spotlight on seedling fluorescence – NightSHADE versus microscope detection**

Basic plant research benefits from the ability to image fluorescence in many different subcellular compartments of the plant. In this Application Note, learn how the NightSHADE evo compared favourably with fluorescence microscopes in its ability to detect medium- or high-intensity fluorescence in all *Arabidopsis* cell lines tested irrespective of subcellular compartment.

Application Note

# USING THE NIGHTSHADE IN VIVO PLANT IMAGING SYSTEM TO STUDY THE CIRCADIAN CLOCK

## Abstract

The circadian clock regulates many different biological functions in most eukaryotes and some prokaryotes. The study of these functions requires tools that can dynamically assess gene expression over long periods of time and in a controlled environment. This application note describes how the NightSHADE In Vivo Plant Imaging System was used to monitor the activity of the CCA1 and LHCB promoters and hypocotyl elongation in controlled lighting conditions.

## Introduction

The alternance between day and night is of great importance for most living beings because many factors that are important for life can change dramatically between day and night: temperature, availability of food, presence of predators, and others. Responding to the differences between day and night requires many physiological, biochemical, and developmental processes to be synchronized with light and darkness cycles. The circadian clock is an endogenous 24-h timer that is found in most eukaryotes and even some prokaryotes (photosynthetic bacteria). It is responsible for synchronising biological processes with the day/night cycle [1]. In plants, many aspects of growth and development are regulated by the circadian clock and light signalling.

Studying the influence of the circadian clock on the regulation of gene expression requires tools that allow to assess gene expression dynamically over long periods of time (at least several days) in a controlled growth environment and in a non-destructive way. This is possible with the combination of reporter constructs with firefly luciferase (LUC) under the control of promoters of interest and sensitive imaging systems, such as the NightSHADE *In Vivo* Plant Imaging System.

In addition to reporter genes, the measurement of growth rate of the seedlings and the hypocotyl [2, 3] is another approach to investigate the circadian clock that can be performed using in vivo imaging.

In this application note we show two examples of the use of in vivo imaging for the study of the circadian clock: monitoring of the expression of genes involved in circadian rhythms for several days, and the measurement of hypocotyl lenghts.

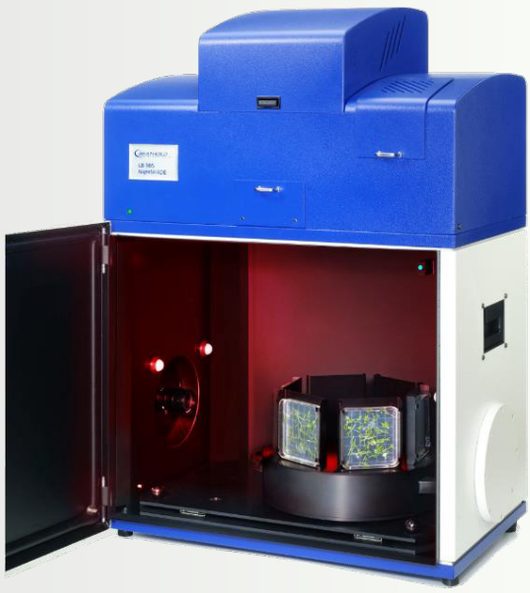
**Rainer Kembügler, Charles Schmidt, Bernd Hutter & Francesc Felipe**

**Berthold Technologies – www.berthold.com**

# The Berthold Technologies NightSHADE evo LB 985N In Vivo Plant Imaging System

The NightSHADE evo LB 985N In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



## Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System, from Berthold Technologies.
- Transgenic *A. thaliana* lines (prCCA1::LUC, CS9382; prCAB::LUC, CS9381; Col-0, CS28168), from TAIR stock center.
- IndiGO™ software, from Berthold Technologies, for image acquisition and quantification of bioluminescence.
- ImageJ software, from NIH, to measure hypocotyl lengths.

After stratification, seeds were germinated [2] and grown on plates containing MS medium.

Seedlings were entrained under short day conditions (8 h light/16 h dark) for 7 days (bioluminescent reporter experiment) or 3 days (hypocotyl elongation experiment). Light was provided by cool white fluorescents, 50 µmol $m^{-2}$ $s^{-1}$.

For the bioluminescent reporter experiment, seedlings were sprayed with luciferin and imaged in Luminescence mode every hour for 3 days under dim constant light using the CCD camera of the NightSHADE.

For the hypocotyl elongation experiment, seedlings were imaged in Photo mode using the CCD camera of the NightSHADE in the side position (Fig. 1), every 4 hours. The indiGO™ software was used to control the CCD camera, the rotor, and light/dark conditions. Images were imported in the ImageJ software to measure hypocotyl lengths.

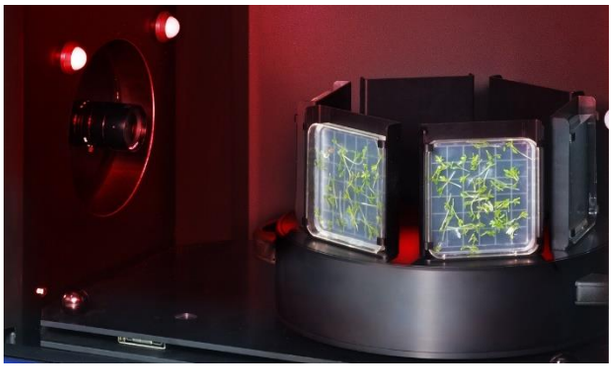


***Figure 1**: Rotating plattform inside the NightSHADE holding square plates to be imaged using the side position of the CCD camera.*

## Results and discussion

The signal corresponding to the activation of the promoter of CCA1 (Circadian Clock Associated 1) started at a very high level in the first data point available (2 h after starting the first light period) but declined quickly (Fig. 2). Several peaks were visible around t = 11 h, t = 23 h, t = 26 h, t = 50 h, and t = 72 h, and valleys around t = 20 h, t = 24 h, t = 42 h and t = 67 h.

Similarly, the signal of CAB (chlorophyll a/b-binding protein, also known as LHCB, light-harvesting chlorophyll a/b-binding protein) showed peaks around t = 11 h, t = 22 h, t = 30 h, t = 59 h and t = 72 h, and valleys around t = 19 h, t = 24 h, t = 45 h and t = 67 h. The amplitude of the changes was maximal in the first 24 h cycle and smaller in the following cycles.

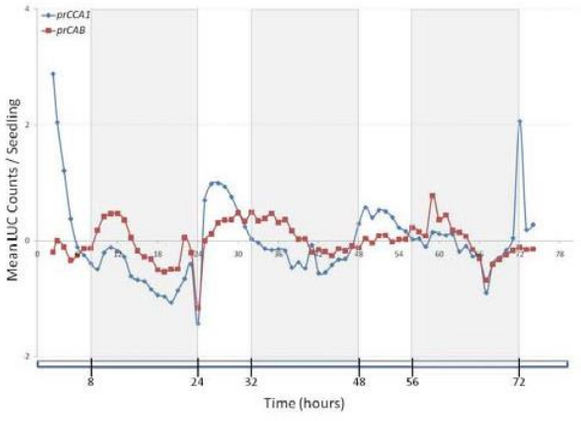



***Figure 2**: Bioluminescent signal corresponding to the activation of the promoter of CCA1 (blue) and CAB (red). Seedlings (prCCA1::LUC & prCAB::LUC) were entrained under 8-h-light (cool white fluorescents, 50 µmol m-2 s-1) / 16-h dark photoperiods for 7 days, sprayed with luciferin and imaged for bioluminescence every hour for 3 days under dim constant light in the NightSHADE.*

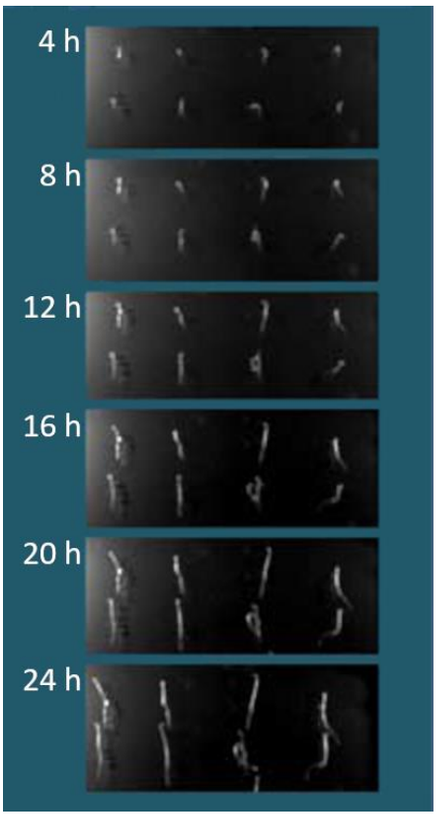



***Figure 3**: A. thaliana (Col-0) seedlings were grown as described and imaged in the NightSHADE every 4 hours. Only the top-half of the plate is shown in each image.*

CCA1 is involved in the central feedback loop in Arabidopsis and is thought to act around dawn to activate CAB gene expression [4]. Thus, the coincidence in time of most peaks and valleys in the signal of CCA1 and CAB is consistent with the activation of CAB by CCA1. In this experiment it is not always possible to see the change in CCA1 signal preceding that of CAB. However, it should be noted that in this experiment both signals are not measured in the same seedlings, but each line expresses a different construct. This could explain timing of the expression being not exactly as expected.

Elongation of the hypocotyl after 24 h was evident (Fig. 3) and easy to measure using ImageJ on the images acquired with indiGO™ (data not shown).

## Conclusions

The NightSHADE evo in vivo plant imaging system offers various tools for the investigation of the circadian clock, which are presented in this application note: first, the quantification of the signal of bioluminescent reporter genes in seedlings of different lines of *A. thaliana*. Second, the measurement of hypocotyl length in seedlings using the lateral camera position combined with the ImageJ software and third, the control of lighting conditions during the whole experiment (several days). The combination of all three features constitutes a very powerful toolbox for the study of the expression of genes under the control of the circadian clock or the influence of photoperiod or light quality and quantity.

## References

1. Gould, P.D., Diaz, P., Hogbe, H., et al. (2009). Delayed fluorescence as a universal tool for the measurement of circadian rhythms in higher plants. The Plant Journal 58, pp. 893-901. https://doi.org/10.1111/j.1365-313x.2009.03819.x
2. Nozue, K., Covington, M.F., Duek, P.D., et al. (2007). Rhythmic growth explained by coincidence between internal and external cues. Nature 448, pp. 358-361. https://doi.org/10.1038/nature05946

3. Cai, Y., Liu, Y., Fan, Y., et al (2023). MYB112 connects light and circadian clock signals to promote hypocotyl elongation in Arabidopsis. Plant Cell 35(9), pp. 3485-3503. https://doi.org/10.1093/plcell/koad170
4. Wang, ZY, Kenigsbuch, D, Sun, L, et al (1997). A Myb-related trnascription factor is involved in the phytochrome regulation of an Arabidopsis Lhcb gene. Plant Cell 9(4), pp. 491-507. https://doi.org/10.1105/tpc.9.4.491

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Application Note

# IMPROVED EXPERIMENTAL SETUP FOR ANALYSIS OF CIRCADIAN RHYTHMS USING THE NIGHTSHADE

## Abstract

Endogenous biological clocks drive daily rhythms enabling organisms to anticipate environmental changes as well as to coordinate and adapt their physiology in a synchronized manner. Research on circadian rhythms benefits from real-time monitoring of reporter lines in which the promoter of a gene of interest drives the expression of luciferase (pGENE::LUC+) in combination with sensitive imaging systems [1]. However, in multicellular organisms, circadian clocks are naturally variable at individual, tissue as well as cellular level [2, 3], culminating in noisy or inaccurate data. Therefore, robustness is required to accurately address key questions in circadian biology. For this purpose, we developed a simple protocol for circadian rhythms experiments with Arabidopsis thaliana reporter lines using the NightShade LB 985. Our experimental setup improves data quality, reduces luminescence variation between replicates and highly correlates with modelling predictions.

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## Introduction

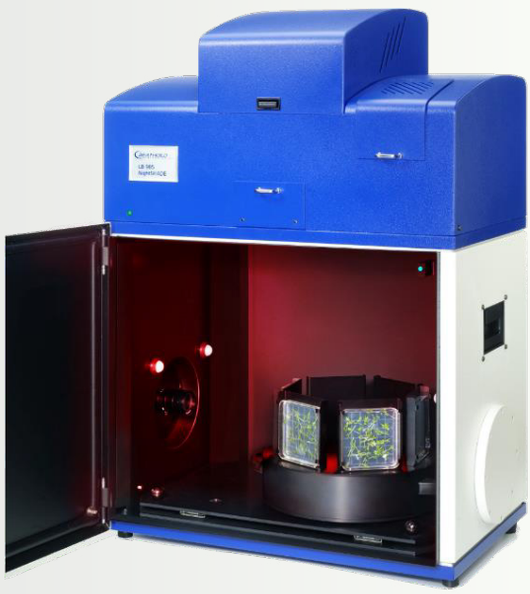
The circadian clock enables plants to anticipate as well as to respond to environmental variations and thus, improves their fitness [4, 5]. Both, environmental and metabolic signals feed into the clock, which is comprised of a network of genes and keep it synchronized with day/night cycle. In return, the clock controls various pathways and ensures they get activated at the appropriate time of the day.

Light is one of the so-called Zeitgebers, which can reset the clock. In this work, we looked at the effect of light on the complex system of clock-genes in Arabidopsis thaliana to explore the mechanisms by which the plant clock adapts to day length variation.

# The Berthold Technologies NightShade LB 985 In Vivo Plant Imaging System

The NightShade LB 985 In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



## Materials and Methods

Arabidopsis seedlings bearing pCCA1::LUC+ construct were used for circadian rhythm analysis. Eight-day-old seedlings entrained in short days (8 h light, 16 h darkness) were transferred into the NightSHADE chamber for luminescence recording during eight additional days. Further details about the device, CCD camera, growth condition, light sources and luciferin manipulation can be found at https://www.berthold.com/en/bioanalytic/products/in-vivo-imaging-systems/nightshade-lb985/ and https://doi.org/10.1016/j.jtbi.2017.03.005 [6].

Luminescence measurements were performed in 10–15 pooled seedlings using top-housed CCD camera in darkness during 600 s (binning 1x1, high gain) after dark-adaptation for 120 s prior to photon acquisition. Plant culture was performed in horizontally oriented plates and placed around 20 cm below the camera for enhanced signal acquisition (Fig. 1). Manual focus of CCD camera is required.

## Results

Dark-adaptation for 120 s prior to photon counting prevented the incidence of chlorophyll auto-fluorescence, such as demonstrated by the lack of signal in Col-0 wild-type in contrast to reporter seedlings (Fig. 1). The *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene encodes a master regulator of the central circadian oscillator [7]. This gene is highly expressed at dawn and repressed through dusk over the night [7]. The activity of the *CCA1 promoter::LUC+* was recorded during eight days. The period of the oscillations was 23.910 ± 0.004 h (Fig. 2). Robustness of rhythms is shown by the Relative Amplitude Error (Fig. 3). In addition to 24 h cycles, this experimental setup allowed to accurately test mathematical predictions of light inputs to the plant circadian clock in a large range of entrainment cycles, such as 8, 13 and 16 h [6].

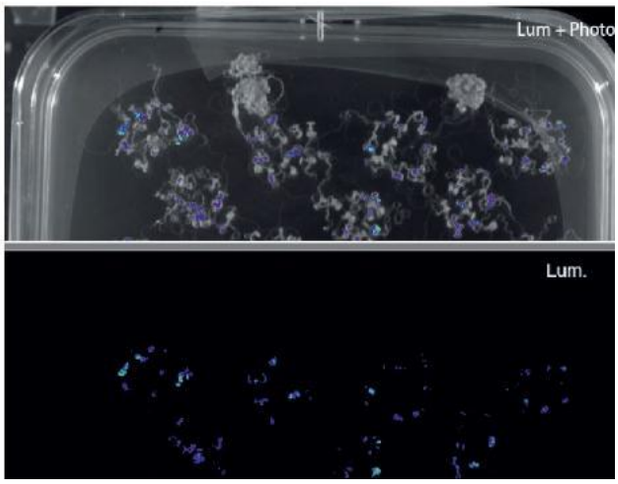



***Figure 1.*** *A. thaliana pCCA1::LUC+ (glowing) and Col-0 wild type (negative control) seedlings 7 h after light onset (when luminescence measurements started).*

## Conclusions

We developed a significantly improved robust protocol for circadian experiments using the NightShade LB 985. The results of our experiments with Arabidopsis thaliana reporter lines agree with previous reports in that the light-sensitive Arabidopsis clock gene network provides the plant with the ability to adapt to seasonal changes in day length. In addition, our experimental setup reduces luminescence variation between replicates and highly correlates with modelling predictions, thus, improving data quality.

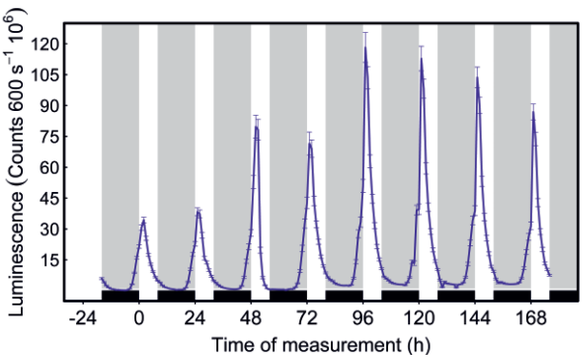



***Figure 2.*** *Luc reporter activity of A. thaliana pCCA1::LUC+ seedlings under short-day cycles (8 h light/16 h dark).*

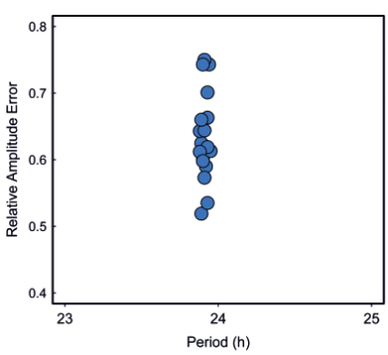



***Figure 3.*** *Relative Amplitude Error demonstrating the robustness of the circadian oscillations.*

## References

1. Millar, A.J., et al. Plant Cell, 1992. 4(9): p. 1075-1087.
2. Endo, M. Curr Opin Plant Biol, 2016. 29: p. 44-49.
3. Endo, M., et al. Nature, 2014. 515(7527): p. 419-422.
4. Green, R.M., et al. Plant Physiol. 2002. 129: p. 576-584.
5. Creux, N., Harmer, S. Cold Spring Harb Perspect Biol. 2022. 11(9). a034611.
6. De Caluwe, J., et al. J Theor Biol, 2017. 420: p. 220-231.
7. Alabadi, D., et al. Curr Biol, 2002. 12(9): p. 757-761.

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Application Note

# PLANT PATHOGEN MONITORING WITH THE NIGHTSHADE USING IMAGING OF PROMPT AND DELAYED FLUORESCENCE

## Abstract

Plant pathogens cause important yield losses in food crops, which makes the study of plant diseases an area of research of high priority. In this application note, imaging of the prompt fluorescence of a transgenic pathogen is combined with imaging of the delayed fluorescence of chlorophyll to follow the infection and asses its effects using the NightSHADE In Vivo Imaging System. Results show a good correlation between both imaging methods and suggest that the combination of both imaging methods can be a useful tool for the study of plant diseases.

## Introduction

Plant pathogens are the cause of many diseases. The impact of those diseases varies depending on the specific pathogen and plant, but crop yield loss is one of the most important ones: pathogens and pests cause global yield losses from 8 to 41% in food crops, depending on crop and country [1] It is expected that the frequency and severity of plant disease outbreaks will increase as a result of climate change [2]. This makes the study of plant pathogens an area of research of high priority.

This application note provides 2 examples of methods useful for studying plant pathogens: Firstly, infection monitoring using fluorescence-labelled pathogenic fungi that enable visualisation of infected areas through prompt fluorescence imaging.; and secondly, the assessment of plant health based on the delayed fluorescence of chlorophyll, which is an indicator of the state of the photosynthetic system [3].

## Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System (Berthold Technologies).
- Excitation filter 475/20.
- Emission filter 520/10.
- indiGO™ image analysis software (Berthold Technologies).

The pathogen fungus to be used in the experiment was transfected with the fluorophore ZSgreen, and wheat was infected with this transgenic fungus.

For imaging, two leaves, one with a high level of infection and one with a low level of infection were

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fixed on a clipboard and transferred to the imaging chamber of the NightSHADE.

For imaging of delayed fluorescence, leaves were illuminated for 30 s with the LED panels, light was turned off, and the image was immediately acquired in Luminescence mode without filters, with 20 s exposure time and 4x4 pixel binning.

For imaging of prompt fluorescence of ZSgreen, the filters above were used, and imaging was performed in Fluorescence mode with a 2 s exposure time and 90% lamp energy.

A picture in Photo mode was also acquired and is used in the figures to show the position of the fluorescence in the leaves.

The intensity of fluorescence was quantified using the indiGO™ image analysis software and expressed in counts per second (cps).

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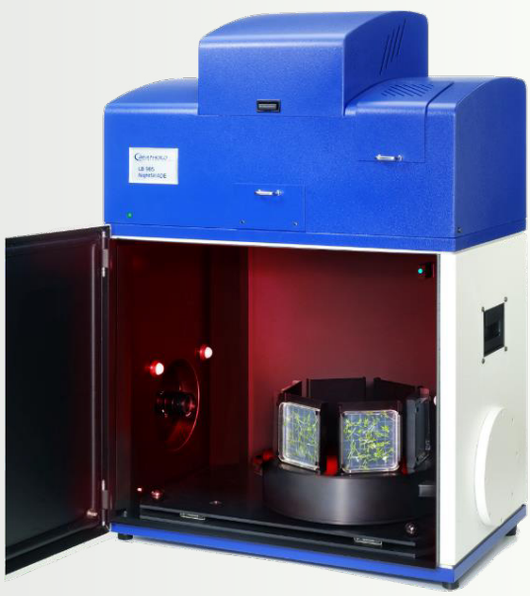

## Results

In the image showing the prompt fluorescence from the transfected fungus (Fig. 1, left), the left leaf shows high levels of prompt fluorescence close to the tip, with lower levels of fluorescence around the right edge and in the central part of the leaf. The right leaf shows no fluorescence coming from the fungus, which indicates low or no infection. In the image showing the delayed fluorescence of chlorophyll (Fig. 1, right), the left leaf shows no delayed fluorescence near the tip and around the left edge, and reduced levels of delayed fluorescence in the central part of the leaf, but also areas of very low ZSgreen fluorescence (below 300 cps), indicating low but detectable infection. These areas show a significant reduction in delayed fluorescence (highlighted by red ovals). The right leaf displayed high levels of delayed fluorescence on the entire surface, except for the tip. Horizontal lines with high (left image) or low (right image) fluorescence are artifacts caused by the sample holder.

Overall, results show a good correlation between the position of the prompt fluorescence of the fungus and the reduction of delayed fluorescence of chlorophyll.

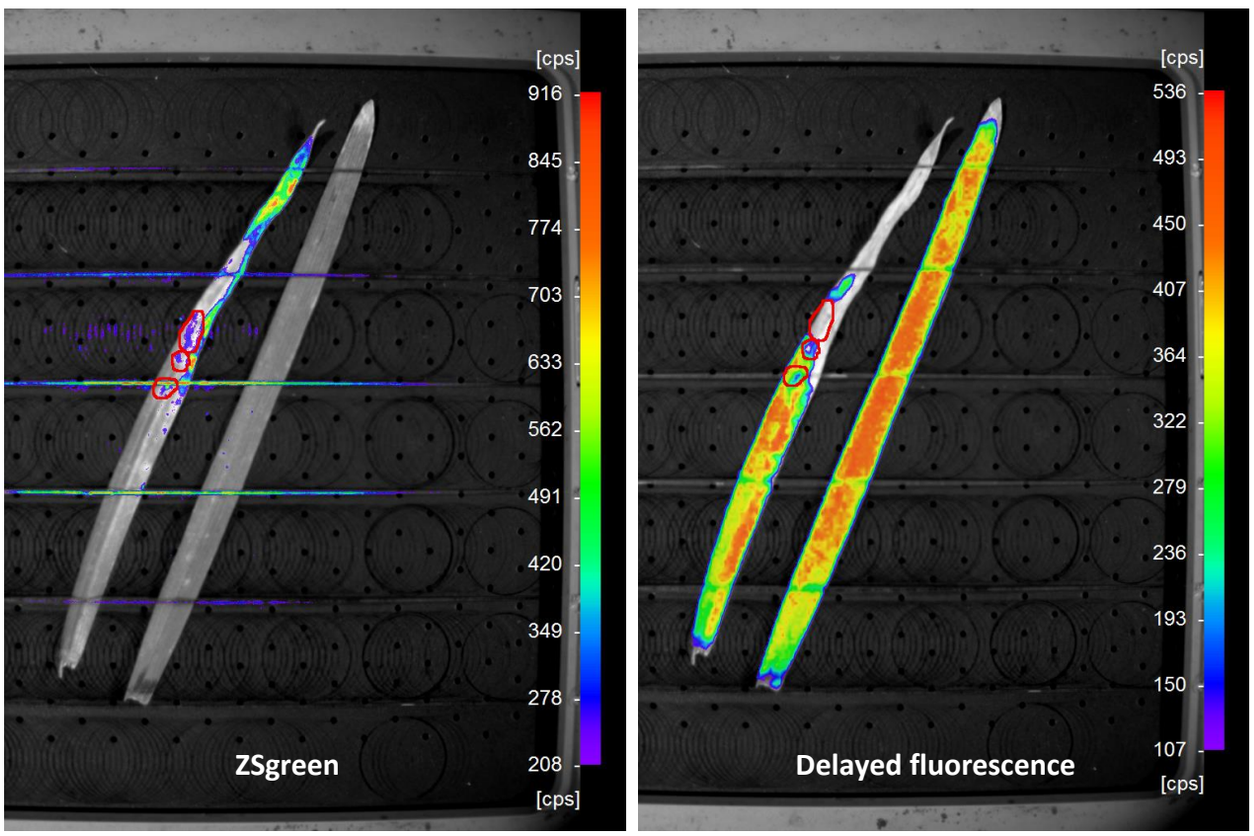


***Figure 1***. *Prompt fluorescence of ZSgreen (left) and delayed fluorescence of chlorophyll (right). Red ovals mark areas with low levels of ZSgreen fluorescence and reduced delayed fluorescence.*

## Conclusions

Imaging the prompt fluorescence of fungal pathogens expressing ZSgreen with the NightSHADE permits localisation of the pathogen in the leaves of infected wheat and an assessment of the extent of infection. Furthermore, imaging the delayed fluorescence of chlorophyll with the NightSHADE shows a significant reduction in the health of the plant´s photosynthetic system in areas infected by the fungus.

The fluorescence spectra of ZSgreen partially overlaps with those of chlorophyll. While the results obtained in this application note were good, labelling the pathogen with other fluorophores without spectral overlap with chlorophyll might improve the performance of the assay.

As a conclusion, the combination of imaging of the prompt fluorescence of transgenic pathogens with imaging of the delayed fluorescence of chlorophyll using the NightSHADE In Vivo Plant Imaging System can be a useful method to study plant diseases.

## References

1. Ristaino, J.B., Anderson, P.K., Bebber, D.P., et al. (2021). The persistent threat of emerging plant disease pandemics to global food security. PNAS 118(23), e2022239118. https://doi.org/10.1073/pnas.2022239118
2. Singh, B.K., Delgado-Baquerizo, M., Egidi, E., et al. (2023). Climate change impacts on plant pathogens, food security and paths forward. Nature Reviews Microbiology 21, 640-656. https://doi.org/10.1038/s41579-023-00900-7
3. Lichtenthaler, H.K. and Rinderle, U. (1988). The Role of Chlorophyll Fluorescence in the Detection of Stress Conditions in Plants. CRC Critical Reviews in Analytical Chemistry, 19, S29-S85. http://dx.doi.org/10.1080/15476510.1988.10401466

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Application Note

# MONITORING OF THE EFFECTS OF STRESS IN PLANTS USING DELAYED FLUORESCENCE AND THE NIGHTSHADE IN VIVO IMAGING SYSTEM

## Abstract

Delayed fluorescence is an indicator of the physiological state of plants and can be used to study the effects of stress factors. Because it is a natural process of plants, it can be measured in a non-invasive way without special labelling or treatment of the specimens being studied.. In this application note, DF imaging with the NightSHADE was tested with plants under mycotic infection and under drought conditions. The effects of both stress factors on DF were easily detectable with the CCD camera of the NightSHADE.

## Introduction

Preilluminated and undamaged plants release light particles known as delayed fluorescence (DF). DF arises from the radiative deactivation of secondary excited chlorophyll molecules within the antenna complexes of Photosystem II (PS II). These excitations are generated through backward electron-transfer reactions occurring on both the donor and acceptor sides of PS II [1].

DF is coupled with the processes of forward photosynthetic activities, and this means that it is informative about plant physiological states and plant-environment interactions. DF is affected by many factors, such as nutritional status of the plant, salt stress, chilling stress, heat stress, drought stress, acid rain, herbicides, metals, and others [2]. And, as it is a natural process of plants, it can be measured without any special labelling or treatment of the specimens to be studied, and in a non-invasive way.

In this application note we test the imaging of DF with the NightSHADE In Vivo Plant Imaging System under two different conditions: fungal infections and drought.

## Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System (Berthold Technologies).
- 24-well plates (Greiner Bio-One).
- indiGO™ image analysis software (Berthold Technologies).

For the fungal infection test, discs were cut from tomato leaves from uninfected plants or from plants 8 days post infection with a fungus. Discs were inserted into the wells of a 24-well plate and illuminated for 30 s with the LED panels. Immediately after switching the light off, images were acquired in the NightSHADE in Luminescence mode with an exposure time of 20 s and 4x4 pixel binning.

For the drought stress test, two groups of soybean plants were used: 50% of the plants were kept dry, and the other 50% were watered. All of them were imaged before starting the experimental period of 2 days to measure their initial (baseline) DF. For DF imaging, plants were illuminated for 30 s with the LED panels. Immediately after switching the light off, images were acquired in the NightSHADE in Luminescence mode with an exposure time of 30 s and 4x4 pixel binning. After 2 days, all plants were imaged using the same settings.

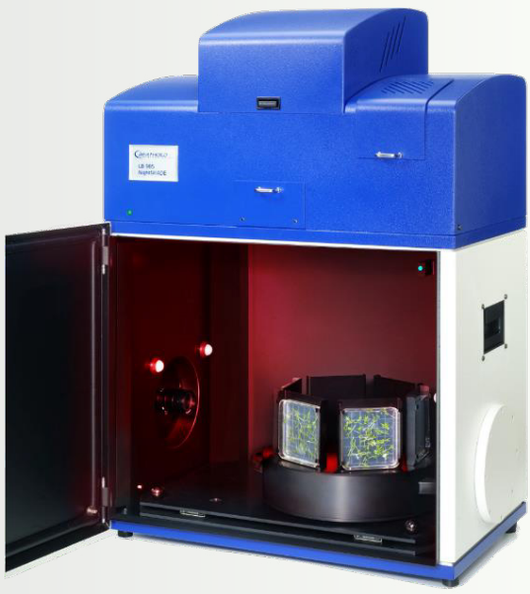
In all cases, pictures in Photo mode were also acquired and are used in the figures to show the position of the fluorescence in the leaves.

Images were analysed using the indiGO™ image analysis software and fluorescence intensity was expressed in counts per second (cps).

## The Berthold Technologies NightSHADE evo LB 985N In Vivo Plant Imaging System

The NightSHADE evo LB 985N In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



## Results

The effects of both stress factors tested (mycotic infection or drought stress) had clearly visible effects on DF in both experiments.

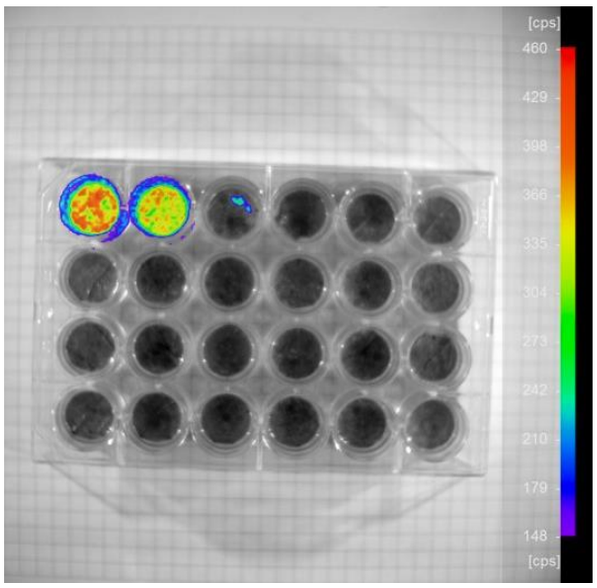



***Figure 1**. Delayed fluorescence of tomato leaves, untreated (wells A1 and A2) or after 8 days of fungal infection (rest of the wells). Image was acquired with 20 s exposure time and 4x4 pixel binning.*

In the image of tomato leaves (Fig. 1), uninfected leaves (wells A1 and A2) showed strong signals of DF, as expected from healthy plants. However, infected leaves showed very low DF (well A3) or no detectable DF (rest of the wells), indicating a heavily damaged photosynthetic system as a consequence of the mycotic infection.

The images of the drought stress test (Fig. 2) also show a clear effect, but not as pronounced. DF content is reduced in most leaves, with leaves with high DF content (approx. 3500-4500 cps) dropping to a medium level (2000-2400 cps) and leaves with medium DF content (approx. 2500 cps) dropping below 1000 cps after two days of drought.

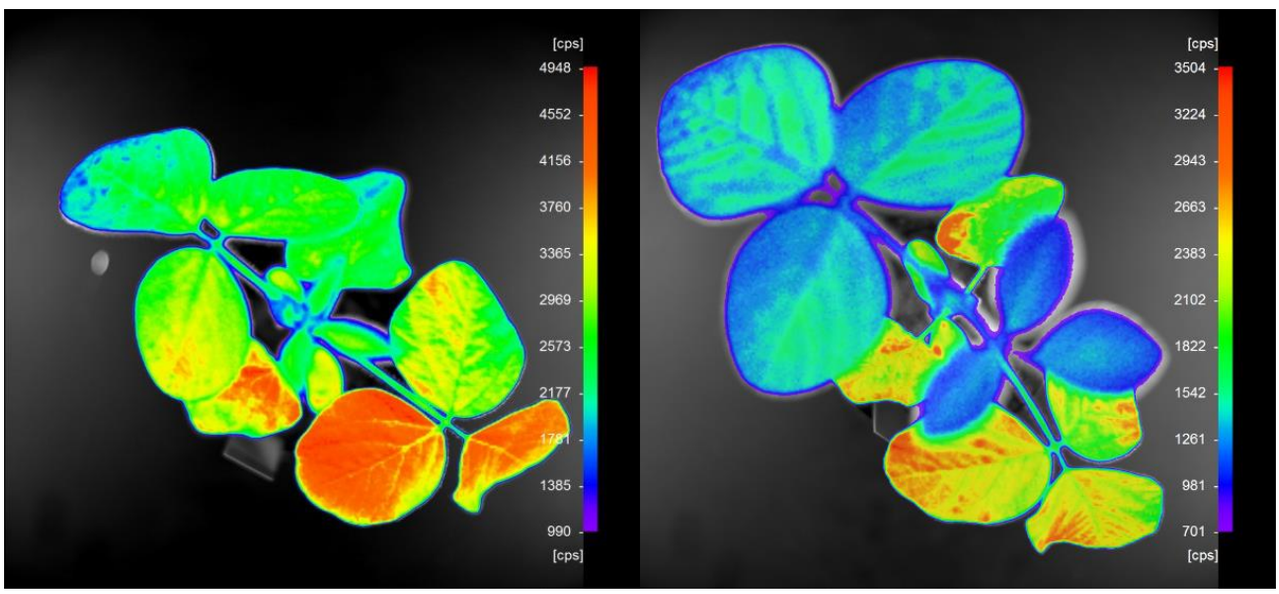



***Figure 2**. Delayed fluorescence in soybean plants after 2 days watered (left) or dry (right). Both pictures are from the same plant. Images were acquired with a 30 s exposure time and 4x4 pixel binning.*

## Conclusions

Imaging of DF is a straightforward and non-invasive method to assess the effect of different types of stress factors. Using the NightSHADE evo In Vivo Imaging System, even the effect on DF of mild stress factors, such as 2 days of drought stress, could be easily detected. The ability to image the whole plant in a single image is more informative than methods performing the measurement in a smaller part of the plant, such as a single leaf. Imaging settings can be adjusted if necessary to recognise lower DF values (by increasing the exposure times and pixel binning).

In conclusion, the NightSHADE In Vivo Plant Imaging System is a valuable tool to assess the effect of different types of stress factors using DF imaging.

## References

1. Goltsev, V., Zaharieva, I., Chernev, P. and Strasser, R. (2014). Delayed Chlorophyll Fluorescence as a Monitor for Physiological State of Photosynthetic Apparatus. Biotechnology & Biotechnological Equipment, 23:sup1, 452-457. https://doi.org/10.1080/13102818.2009.10818461
2. Guo, Y., and Tan, J. (2013). Applications of Delayed Fluorescence from Photosystem II. Sensors (Basel) 13(12): 17332–17345. https://doi.org/10.3390%2Fs131217332

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Application Note

# COMBINING DELAYED FLUORESCENCE AND ULTRAWEAK PHOTON EMISSION TO MONITOR STRESS RESPONSES IN PLANTS USING THE NIGHTSHADE

## Abstract

Understanding the effects of stress factors on plants can help developing strategies to mitigate their effects on plant health and crop yield. Delayed fluorescence and ultra-weak photon emission are natural properties of plants. They can be studied non-invasively and in physiologically relevant conditions and respond to a broad range of stress factors. In this application note, imaging of delayed fluorescence and of ultra-weak photon emission are used to assess the stress status of leaves turning brown using the NightSHADE In Vivo Plant Imaging System. Results show a good correlation between these imaging methods and indicate that the combination of both imaging methods using the NightSHADE can be a useful tool for the study of stress factors in plants.

## Introduction

Plants are exposed to many stress factors, such as drought, salt, heat, cold, pathogens, contaminants, and others, - all of which can have a negative effect on plant health and crop yield. Studying the effect of stress factors and the mechanisms of defence of the plant against them can help developing strategies to mitigate their effects on plant health and crop yield.

Many methods are available for the study of effects stress factors in plants, but some of them are invasive or require transformation or excessive manipulation of the plant. Methods based on the measurement of natural properties of the plant, requiring minimal manipulation, are more physiologically relevant and easier to perform. Delayed fluorescence (DF) is a natural property of plants which informs about the status of the photosynthetic system, and affected by many factors, such as nutritional status of the plant, salt stress, chilling stress, heat stress, drought stress, acid rain, herbicides, metals, and others [1]. However, it's not the only available method.

Ultraweak photon emission (UPE, also known as biophoton emission or low-level chemi-luminescence) is the emission of photons at a very low intensity ($10^{1}$-$10^{3}$photons·sec$^{-1}$·cm$^{-2}$, or $10^{-16}$ to $10^{-18}$ W·cm$^{-2}$ [2]), that seems to happen in all living systems [3]. UPE originates from the oxidative metabolic reaction in microbial, plant and animal cells, and it is generally considered that

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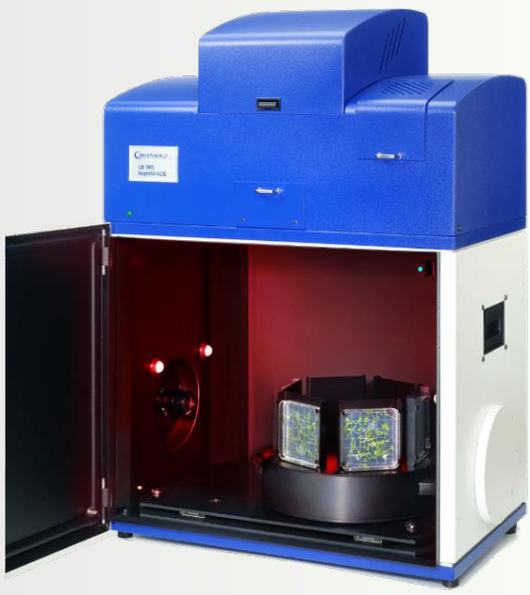
electronically excited species formed during the oxidative metabolic processes are solely responsible for it [4]. UPE is also a non-invasive method, based on natural properties of the cells, and has been demonstrated to respond to many different stress factors in plants, animals, and humans (see [4] and [5] for reviews). Hence, it can be a good alternative or complement to DF for the study of the effects of stress factors.

In this application note, DF and UFE are used to look for the stress signs in leaves turning brown, and the performance of both methods is compared.

## The Berthold Technologies NightSHADE evo LB 985N In Vivo Plant Imaging System

The NightSHADE evo LB 985N In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



### Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System with LED panels (Berthold Technologies).
- Powershot G11 digital camera (Canon).
- Leaves from *Carpinus betulus*.
- IndiGO™ image analysis software (Berthold Technologies).

Leaves from *Carpinus betulus* (one healthy, one brownish) were illuminated with the LED panels for 10 minutes with the 470 nm, 660 nm, and 730 nm channels (35 uE·mm$^{-2}$·s$^{-1}$ in each channel). White LEDs must be avoided because they exhibit afterglow that could mask DF and UPE. LEDs were then turned off and, after a short delay (3 and 10 s were tested), DF images were acquired in Luminescence with 60 s exposure time and 2x2 pixel binning.

After acquiring the DF images, leaves were kept in absolute darkness for 30 minutes to let DF fade out (DF has been described to mask UFE for 5-10 minutes [x]). Then, UFE images were acquired in Luminescence mode with an exposure time of 10 minutes and 8x8 pixel binning.

As last step, a black-and-white picture was taken in Photo mode with 0.1 s exposure time and 10% illumination intensity. DF and UPE images were overlaid over this picture for spatial reference. Colour pictures of the leaves were also taken using the Canon camera.

Images were analysed using indiGO™ image analysis software. Emission intensity was determined using the line tool and expressed as counts per second (cps). In order to make results of DF and UPE comparable, cps values were corrected for the pixel binning: cps were divided by 4 for 2x2 pixel binning and by 64 for 8x8 pixel binning.

## Results

Healthy leaves show high levels of DF in the (fig. 1A, right), accompanied with undetectable levels of UPE (fig. 1B, right). Brownish leaves show reduced levels of DF in some areas, and undetectable levels of DF in others (fig. 1A, left). Areas with detectable levels of DF correspond with areas of de leaf that stay green (fig. 1C). UPE is undetectable in most of the surface of the leaf: neither green areas nor brown areas show detectable levels of UPE. However, UPE is high in a damaged area that seems to be transitioning from green to brown (fig. 1C, red box), and it can also be detected in other green-to-brown transition areas, but at lower levels.

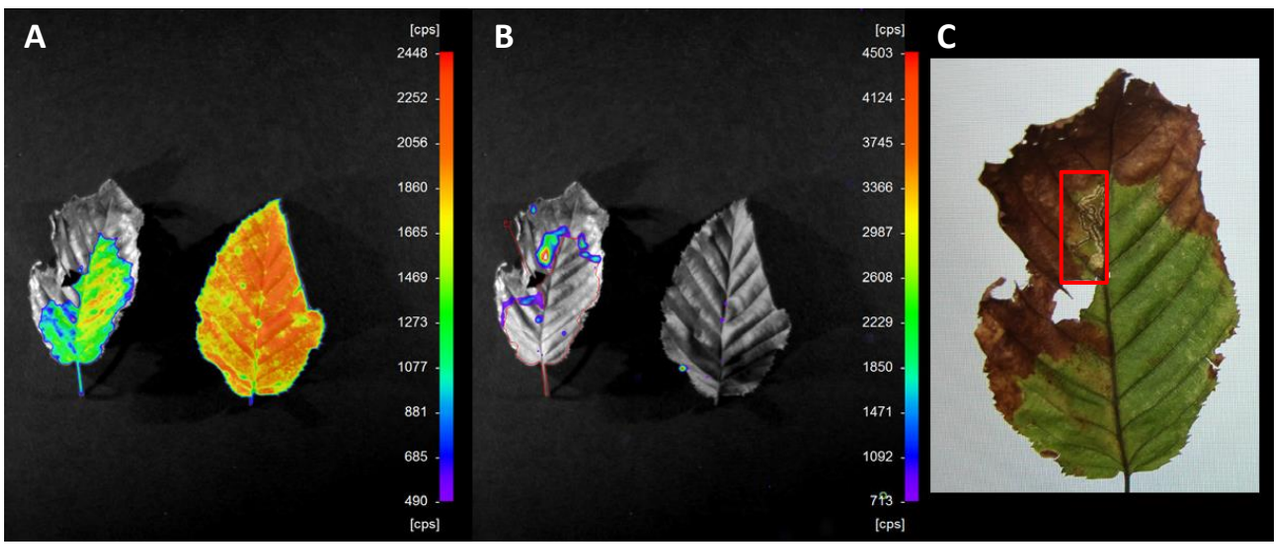



***Figure 1**. A) DF emission of a healthy leaf (right) and a brownish leaf (left). Image was acquired with 60 s exposure time and 2x2 pixel binning. B) UPE of a healthy leaf (right) and a brownish leaf (left). Image was acquired with 10 min exposure time and 8x8 pixel binning. C) Picture of the brownish leaf taken with a standard digital camera (Canon Powershot G11); the box marks an area showing high UPE.*

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In order to have quantitative data, light intensity was quantified using the indiGO™ image analysis software. Emission intensity was determined with the line tool, drawing a line that crossed the most representative areas of both leaves (fig. 2, right), including the damaged area of the brownish leaf (fig. 1C, red box). The resulting intensity graph of the DF is shown in figure 2 (left). Results were expressed in counts per second (cps) and corrected for the binning factor (table 1). The quantification shows that, while the effects of leaf damage are visible in the values of DF, with a reduction of a 25%, the effect is much more evident in UPE, which increases by almost 10 times. The combination of low DF and high UPE in those areas is indicative of high levels of stress.

| | DF | UPE |
|---|---|---|
| **Healthy area** | 8.300 | 0.013 |
| **Damaged area** | 6.250 | 0.125 |

***Table 1**. Quantification of delayed fluorescence (DF) and ultraweak photon emission (UPE) using the line tool of the indiGO™ software. Results expressed as counts per second and corrected for the binning factor.*

The quantification also shows how weak UPE is compared to DF: 5 times lower in damaged areas, and more than 600 times lower in healthy areas.

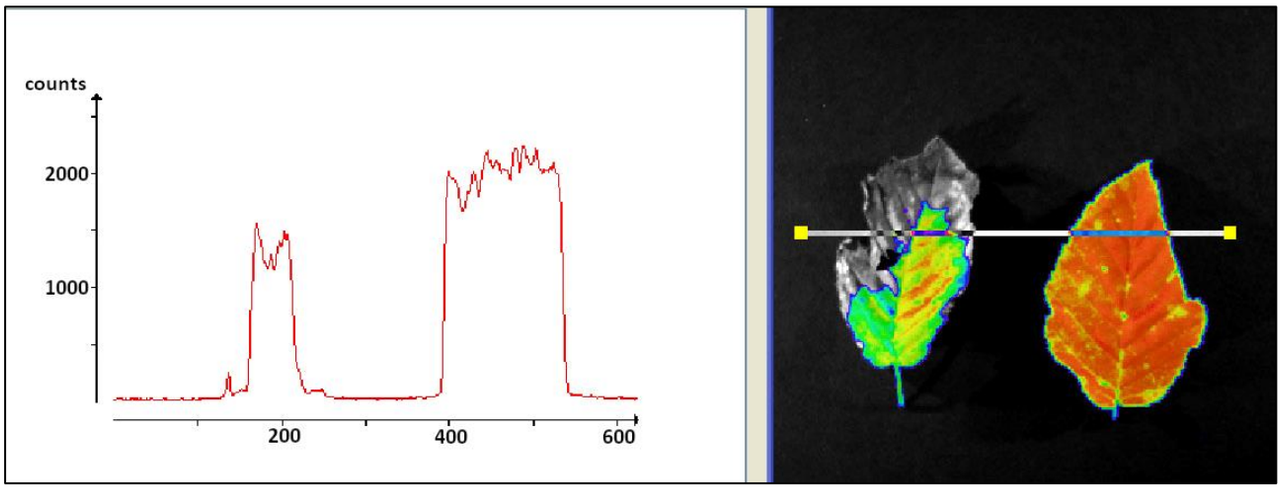


***Figure 2**. Quantification of delayed fluorescence (DF) using the line tool of the indiGO™ software. The graph (left) represents the quantification of the intensity in the areas crossed by the line (left).*

## Conclusions

At the settings tested in NightSHADE, the UPE values were almost undetectable in the healthy leaf, but very clearly detectable in the damaged areas of the brownish leaf. On the other hand, the decrease in DF was clearly visible. The low levels of DF and high levels of UPE in areas of the brownish leaf transitioning from green to brown indicate that cells in those areas are highly stressed.

The results shown in this application note demonstrate that the NightSHADE evo In Vivo Imaging System is suitable for the study of the effects of stress factors by imaging DF and UPE in plants.

## References

1. Guo, Y., and Tan, J. (2013). Applications of Delayed Fluorescence from Photosystem II. Sensors (Basel) 13(12): 17332–17345. https://doi.org/10.3390%2Fs131217332
2. Kobayashi, M. (2003). Spontaneous ultraweak photon emission of living organisms-biophotons-phenomena and detection techniques for extracting biological information. Trends in Photochemistry and Photobiology 10: 111–135.
3. Devaraj, B., Usa, M., and Inaba, H. (1997). Biophotons: ultraweak light emission from living systems. Current Opinion in Solid State and Materials Science 2(2): 188-193. https://doi.org/10.1016/S1359-0286(97)80064-2
4. Cifra, M. and Pospíšil, P. (2014). Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications. Journal of Photochemistry and Photobiology B: Biology, 139: 2-10. https://doi.org/10.1016/j.jphotobiol.2014.02.009
5. Du, J., Deng, T., Cao, B., et al. (2023). The application and trend of ultra-weak photon emission in biology and medicine. Frontiers in Chemistry 11: 1140128. https://doi.org/10.3389%2Ffchem.2023.1140128

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Application Note

# DETECTION OF FLUORESCENCE FROM DIFFERENT SUBCELLULAR LOCATIONS OF SEEDLINGS: NIGHTSHADE VS MICROSCOPE

## Abstract

Detecting fluorescence with an in vivo imaging system has many advantages over fluorescence microscopes for plant research, such as the large field of view, but performance can be lower. In this application note, the performance of the NightSHADE In Vivo Plant Imaging System for detecting fluorescence from different subcellular areas was compared with that of fluorescence microscopes. Fluorescence could be detected irrespective of the subcellular compartment, but performance was borderline for lines exhibiting low levels of expression. This makes the NightSHADE evo a good solution in cases in which fluorescence has medium or high intensity and a wide field of view is desired.

## Introduction

The broad range of fluorescent labels available provides a very valuable toolbox for molecular and cellular biology research. Using different fluorophores to label different molecules, it is possible to monitor different cell types, organelles, or processes, and this has enabled important advances in plant research (reviewed in [1], [2] and [3]).

Fluorescent imaging is usually performed using a microscope, but in vivo imaging systems offer several advantages over microscopes, such as the ability to quickly image and analyse the whole plant or even many plants in a short time, which is valuable for many applications. The sensitivity of in vivo imaging systems is generally lower than that of fluorescence microscopes, mainly due to the fact that the excitation light is spread over a large area and the camera is far away from the sample. This may raise concerns about its suitability to image fluorophores expressed in some cellular compartments.

In this application note, the ability of the NightSHADE evo in vivo plant imaging system to detect fluorescence from different subcellular locations is tested and compared to the performance of various fluorescence microscopes.

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## Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System (Berthold Technologies) with excitation filter 475/20 and 520/10 emission filter.
- Eclipse 90i widefield microscope (Nikon) with CoolSNAP ES camera (Photometrics) and FITC filter settings.
- MZFLII stereomicroscope (Leica), with GFP filter settings.

The plant lines used, including the fluorophore its subcellular location, are detailed in Table 1.

Seedlings were grown on MS or phytoagar plates for 5 days; then, they were placed on a glass slide for imaging.

Imaging with the NightSHADE evo: images of the seedlings were acquired with a 5 s exposure time in Fluorescence mode using the filters above and overlaid with an image acquired in Photo mode. Fluorescence was quantified using the indiGO™ software using a scale of 100-2000 and automated peak search based on noise detection. For samples without detectable peaks, an area was manually defined around the seedling. Fluorescence was expressed as cps (counts per second)/mm$^2$.

Wide field microscopy settings: images of seedlings were taken at a 100x, 200x or 400x magnification with 100, 500, 1000 or 2000 ms detection time. Images were analysed using the MetaMorph® Software.

| Line | Fluorophore | Promoter | Subcellular location | Comments | References |
|---|---|---|---|---|---|
| **YC3.6** | CYT YFP (cpVenus)/ CFP (ECFP) +NES | UBQ10 | Cytoplasm | FRET line to measure $Ca^{2+}$ fluxes | [4, 5] |
| **YC3.6 - PM** | YFP (cpVenus)/ CFP (ECFP) | UBQ10 | Plasma membrane | FRET line to measure $Ca^{2+}$ fluxes | [4, 5] |
| **YC3.6 - NUC** | YFP (cpVenus)/ CFP (ECFP) +NLS | UBQ10 | Nucleus | FRET line to measure $Ca^{2+}$ fluxes | [4, 5] |
| **roGFP - CYT** | redox-sensitive GFP | CaMV-35S | Cytoplasm | | [6] |
| **roGFP - Plastid** | redox-sensitive GFP | CaMV-35S | Chloroplast | | [6] |
| **roGFP - MIT** | redox-sensitive GFP | CaMV-35S | Mitochondria | | [7] |
| **Control** | - | | | | |

***Table 1**. Description of A. thaliana lines used in the comparison.*

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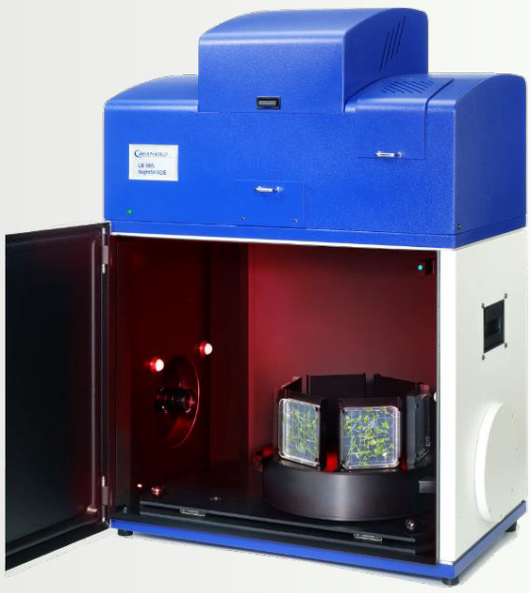
The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



## Results

To have a reference to compare the results of the NightSHADE evo, pictures of the seedlings expressing GFP or YFP were acquired with the Nikon widefield fluorescence microscope. Fluorescence was visible in all lines expressing GFP or YFP. YC3.6-NUC (Fig. 1 C) and YC3.6-PM (Fig. 1 B) showed the strongest signal, which was detectable with detection times as short as 100 ms, followed by YC3.6-CYT (Fig. 1 A) and roGFP-Plastid (Fig. 1 E), which showed strong signal with detection times of 1000 ms, whereas roGFP-MI (Fig. 1 F) and roGFP-CYT (Fig. 1 D) emitted weak signals. Very weak autoluminescence was visible in the controls with long detection times (1000-2000 ms), but intensity was in all cases clearly lower than in the weakest fluorescent lines. Interestingly, fluorescence of the two lines expressing fluorescence in the cytoplasm, CY3.6-CYT and roGPF-CYT, is quite different, with CY3.6-CYT showing much stronger fluorescence than roGFP-CYT; this suggests that fluorescence intensity is more dependent on the promoter driving expression (UBQ10 vs CaMV-35S) or

expressed protein (YFP vs GFP) than on subcellular location. However, subcellular location does indeed play a role: while roGFP-Plastid and roGFP-MIT share promoter and fluorophore, fluorescence intensity is clearly stronger if the fluorophore is expressed in chloroplasts than if it's expressed in mitochondria.

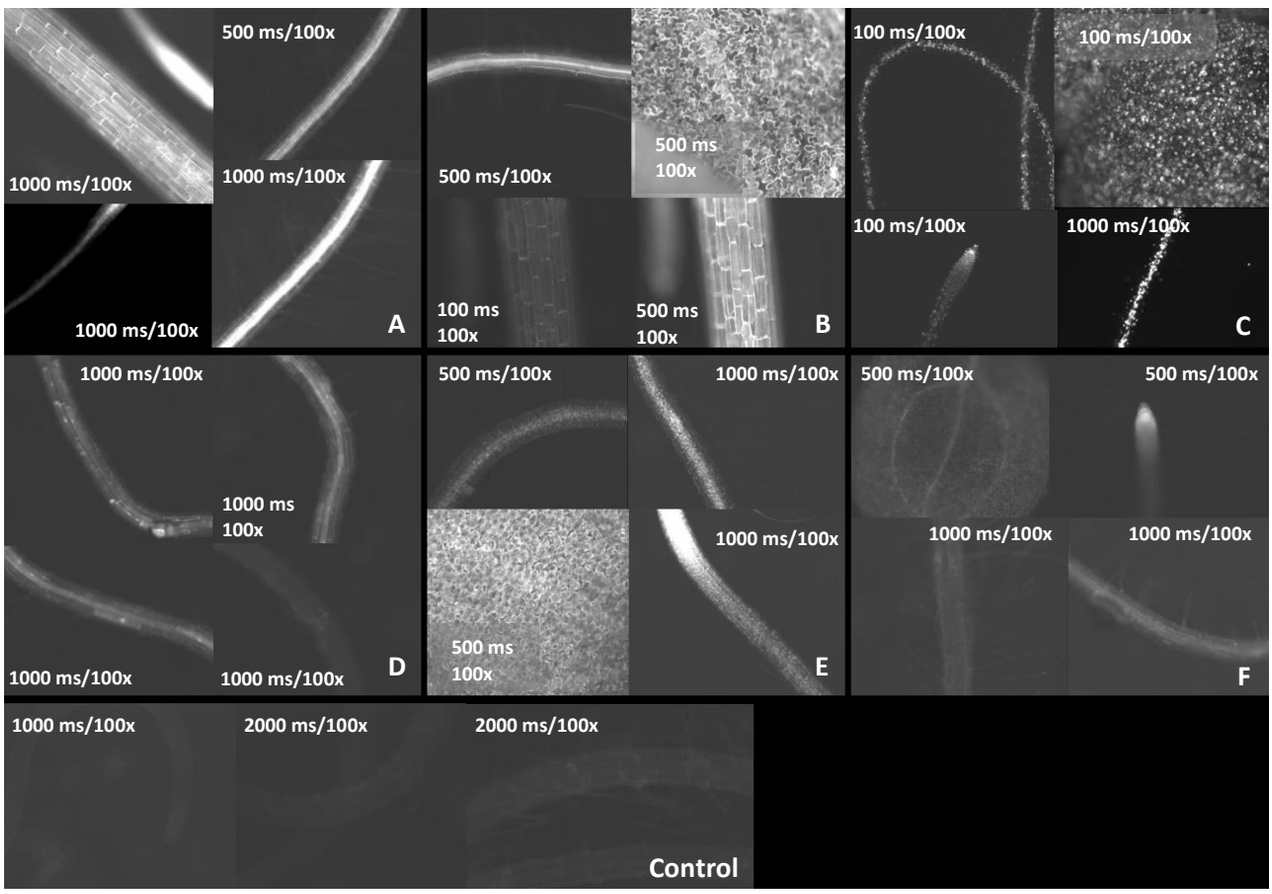



***Figure 1**. Wide field fluorescence microscopy pictures of roots and leaves of Arabidopsis seedlings expressing YFP or GFP. A: YC3.6-CYT; B: YC3.6-PM; C: YC3.6-NUC; D: roGFP-CYT; E: roGFP-PLA; F: roGFP-MIT. Pictures of 5 days old seedlings were taken using FITC filter settings with different magnifications and detection times.*

Quantifying the fluorescence of images acquired with the NightSHADE using indiGO™ provides similar results to the Nikon microscope images (Fig. 2). In this case, the fluorescence of the YC3.6-NUC and YC3.6-PM lines showed the strongest signal, followed by roGFP plastid, which showed a strong signal, and roGFP-MI and roGFP-CYT with weak signals. Fluorescence of roGFP-CYT appears to be stronger than of roGFP plastid in the microscopic images but gives lower intensity values when quantified in the NightSHADE data. This could be due to the individual characteristics of the seedling selected for the microscopic images. Furthermore, differences seen between the controls and the lines with low fluorescence were smaller to the differences seen in images acquired with the Nikon microscope.

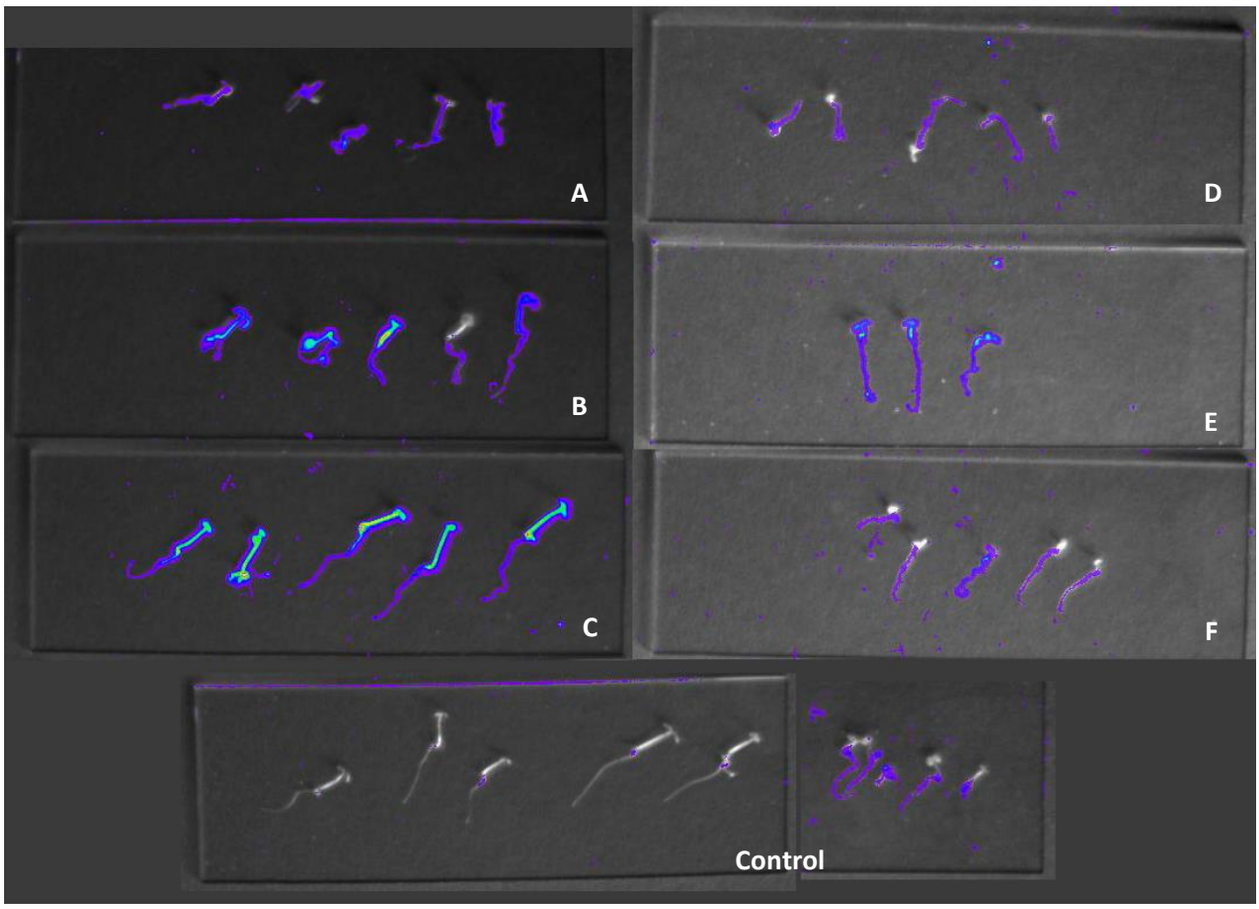



***Figure 2**. Fluorescence pictures of roots and leaves of Arabidopsis seedlings expressing YFP or GFP acquired with the NightSHADE. A: YC3.6-CYT; B: YC3.6-PM; C: YC3.6-NUC; D: roGFP-CYT; E: roGFP-PLA; F: roGFP-MIT. Images of the seedlings were acquired with a 5 s exposure time with excitation filter 475/20 and 520/10 emission filter.*

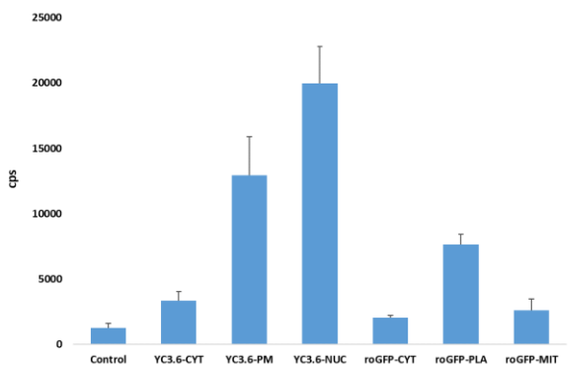



***Figure 3**. Quantification of the fluorescence of images acquired with the NightSHADE. Images of the seedlings were acquired with a 5 s exposure time with excitation filter 475/20 and 520/10 emission filter. Bars are the average of 3-5 seedlings and error bars represent the SEM.*

With the exception of one seedling in the roGFP-MIT image, fluorescence for the rest of seedlings from the control, roGFP-CYT and roGFP-MIT lines was too low to be identified using the automated peak search of indiGO™, and quantification was performed by manually defining the areas to be quantified.

No fluorescence was visible when observing the seedlings under the Leica stereomicroscope (data not shown).

## Conclusions

Performance of the NightSHADE evo was much higher than of that of the stereomicroscope, which failed to detect fluorescence in any of the seedlings. Compared to the widefield microscope, the NightSHADE was able to detect fluorescence in all lines expressing GFP or YFP, irrespective of the subcellular compartment in which it was expressed. The differences between lines with low fluorescence intensity and the controls was smaller in the NightSHADE than in the widefield microscope.

Taking everything into account, the high throughput of the NightSHADE, large field of view and its ability to detect fluorescence in all lines, irrespective of the subcellular compartment where fluorescence is expressed, make it a good solution for screening of seedlings expressing GFP or YFP. However, caution has to be taken if expression levels are low, as performance is lower than in the widefield microscope.

## References

1. Berg, R.H., and Beachy, R.N. (2008). Fluorescent Protein Applications in Plants. Methods in Cell Biology 85, pp 153-177. https://doi.org/10.1016/S0091-679X(08)85008-X
2. Wenzislava, C., Caragea, A.E., Goldstein, R.S., and Thomas, B. (2011). Glow in the Dark: Fluorescent Proteins as Cell and Tissue-Specific Markers in Plants. Molecular Plant 4(5), pp. 794-804. https://doi.org/10.1093/mp/ssr059
3. Yagi, N., Yoshinari, A., Iwatate, R.J., et al. (2021). Advances in Synthetic Fluorescent Probe Labeling for Live-Cell Imaging in Plants. Plant and Cell Physiology 62(8), pp. 1259-1268. https://doi.org/10.1093%2Fpcp%2Fpcab104
4. Nagai T., Yamada S., Tominaga T., et al. (2004). Expanded dynamic range of fluorescent indicators for $Ca^{2+}$ by circularly permuted yellow fluorescent proteins. PNAS 101(29), pp. 10554-10559, https://doi.org/10.1073/pnas.0400417101
5. Krebs M., Held K., Binder A., et al. (2012). FRET-based genetically encoded sensors allow high-resolution live cell imaging of $Ca^{2+}$ dynamics. Plant Journal 69(1), pp. 181-192. https://doi.org/10.1111/j.1365-313x.2011.04780.x
6. Schwarzländer M., Fricker M.D., Müller C., et al. (2008). Confocal imaging of glutathione redox potential in living plant cells. Journal of Microscopy 231(2), pp. 299–316. https://doi.org/10.1111/j.1365-2818.2008.02030.x
7. Jiang K., Schwarzer C., Lally E., et al. (2006). Expression and characterization of a redox-sensing green fluorescent protein (reduction-oxidation-sensitive green fluorescent protein) in Arabidopsis. Plant Physiology 141(2), pp. 397-403. https://doi.org/10.1104/pp.106.078246
8. Costa A., Drago I., Behera S., et al. (2010). $H_2O_2$ in plant peroxisomes: an in vivo analysis uncovers a $Ca^{2+}$ dependent scavenging system. Plant Journal. 62(5), pp. 760-72. https://doi.org/10.1111/j.1365-313x.2010.04190.x
9. Kircher S., Gil P., Kozma-Bognar L., et al. (2002). Nucleocytoplasmic Partitioning of the Plant Photoreceptors Phytochrome A, B, C, D and E is Regulated Differentially by Light and Exhibits a Diurnal Rhythm. Plant Cell 14(7), pp. 1541-1555. https://doi.org/10.1105/tpc.001156

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# Featured products

## NightSHADE evo LB 985N *In Vivo* Plant Imaging System by BERTHOLD TECHNOLOGIES GmbH & Co. KG

The NightSHADE evo LB 985N *In Vivo* Plant Imaging System is a user-friendly and modular optical imaging system designed for the *in vivo* analysis of plants. Featuring a light-tight cabinet and a cooled charge-coupled device (CCD) camera, this instrument facilitates precise monitoring of luminescence and fluorescence in tissues, seedlings, and entire plants.

**Key features:**

• Versatile imaging: NightSHADE's smart chamber ensures full compatibility for many applications. The user can configure it to suit their needs with a multi-position camera, easy sample handling, and direct chamber access.

• Environmental control: NightSHADE facilitates standardized experiments by enabling precise control of key conditions – temperature and daylight simulation, and also humidity if the system is placed in an appropriate environmental chamber.

• Optical flexibility: Achieve high-quality results across various experiments with NightSHADE's flexible optical setup and sensitivity.

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