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.

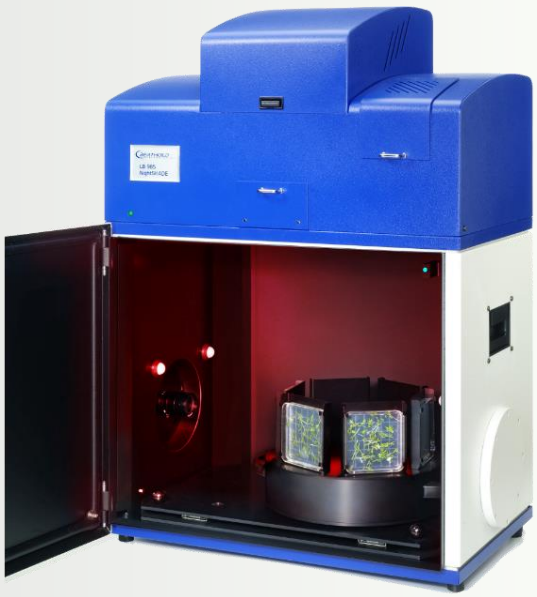
Line	Fluorophore	Promoter	Subcellular location	Comments	References
YC3.6	CYT YFP (cpVenus)/ CFP (ECFP) +NES	UBQ10	Cytoplasm	FRET line to measure Ca^{2+} fluxes	[4, 5]
YC3.6 - PM	YFP (cpVenus)/ CFP (ECFP)	UBQ10	Plasma membrane	FRET line to measure Ca^{2+} fluxes	[4, 5]
YC3.6 - NUC	YFP (cpVenus)/ CFP (ECFP) +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.*

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

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.

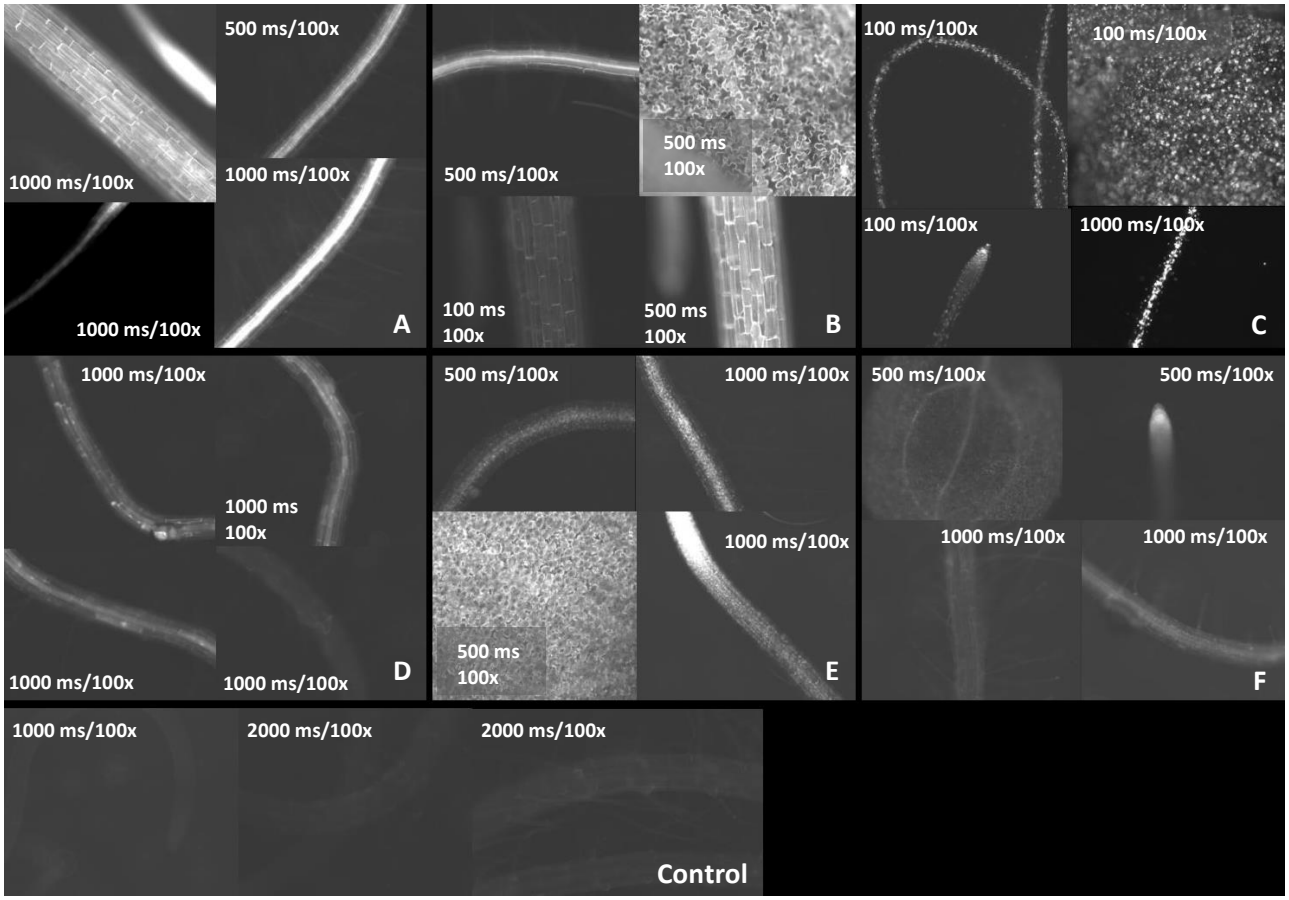


***Figure 1**. Wide field fluorescence microscopy pictures of roots and leaves of Arabidopsis seedlings expressing YFP or GFP. A: YC3.6-CYT; B: YC3.6-PM; C: YC3.6-NUC; D: roGFP-CYT; E: roGFP-PLA; F: roGFP-MIT. Pictures of 5 days old seedlings were taken using FITC filter settings with different magnifications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

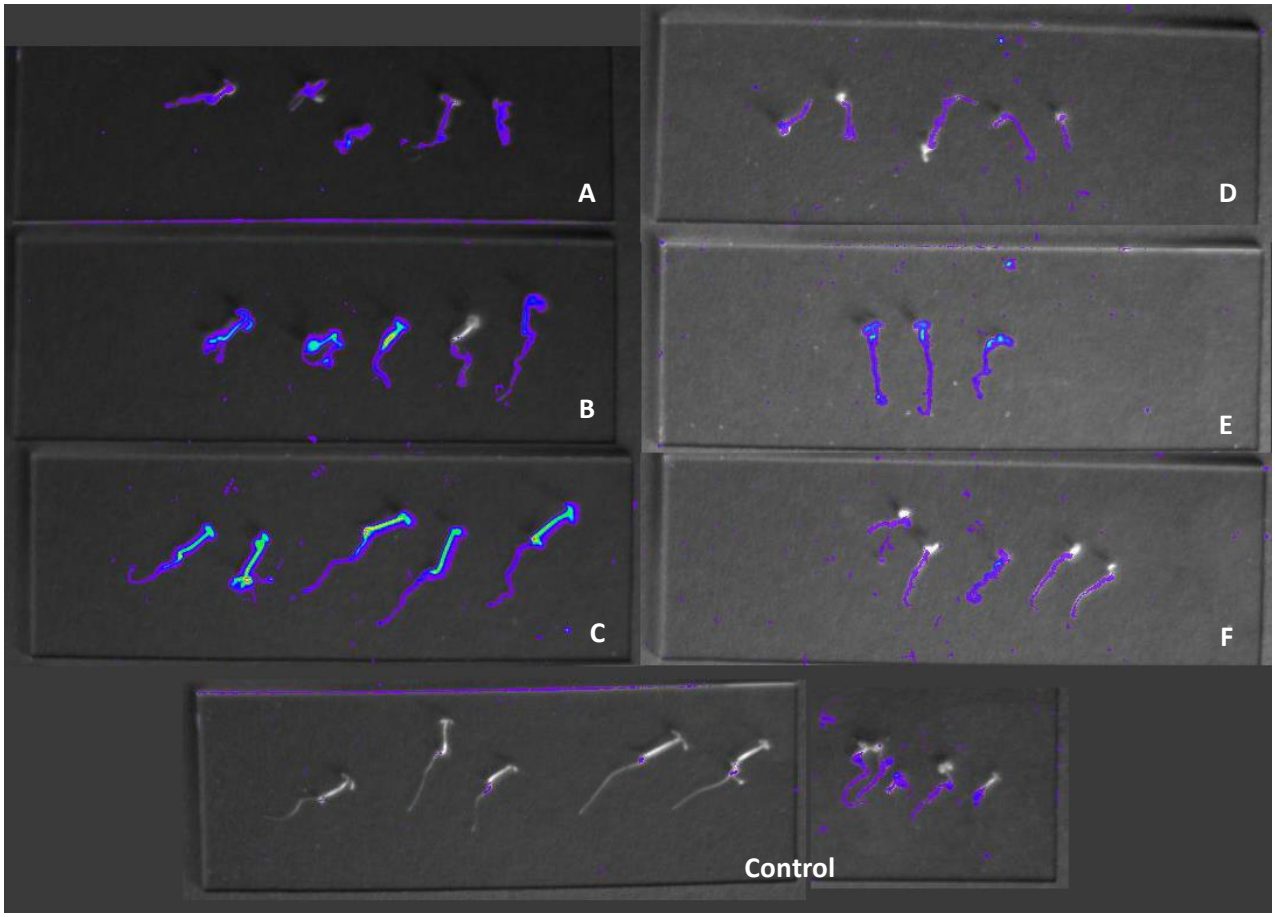


***Figure 2**. Fluorescence pictures of roots and leaves of Arabidopsis seedlings expressing YFP or GFP acquired with the NightSHADE. A: YC3.6-CYT; B: YC3.6-PM; C: YC3.6-NUC; D: roGFP-CYT; E: roGFP-PLA; F: roGFP-MIT. Images of the seedlings were acquired with a 5 s exposure time with excitation filter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

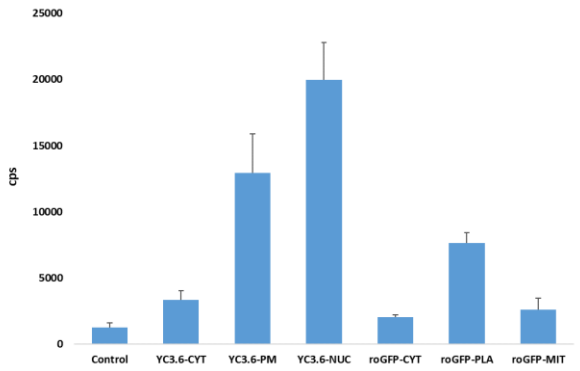


***Figure 3**. Quantification of the fluorescence of images acquired with the NightSHADE. Images of the seedlings were acquired with a 5 s exposure time with excitation filter 475/20 and 520/10 emission filter. Bars are the average of 3-5 seedlings and error bars represent the SEM.*

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

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