# Trans-illumination – a Solution for Excitation of GFP Expressing Plants in Petri Dishes for *In Vivo* Imaging

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- Testing trans-illumination vs epi-illumination of plates with agar used for growing plants
- Background reduction is possible by using trans-illumination

## Abstract

This application note aims to show that excitation of GFP-transformed plants in petri dishes filled with agar can be easily performed by Trans-illumination, with no reflection on the plastic material as it would happen with Epi-illumination. Background light intensities are in the range of 800-1000 RLU at 470 nm and 10% intensity setting of the trans-illuminator. Such values are relative low compared with the dynamic range of used 16-bit CCD camera.

## Introduction

*In vivo* imaging of plants became a common research tool for gene expression, studies of circadian rhythm, etc. all over the world. Plants are transfected either with luciferase or photoproteins like GFP (Figure 1), YFP or dsRED. Epi-illumination (from above) is easy to perform with plants not kept in petri dishes (Fig.1). However, epi-illumination of petri dishes causes problems due to reflection of the illumination devices seen on the surface of petri dishes. Trans-illumination (from below) could also excitate transfected plants but in this case the light of the trans-illuminator shines directly into the camera. Therefore the blocking factor of the emission filter and the overlap of the emission spectra of trans-illuminators and the emission filters are the critical factors.

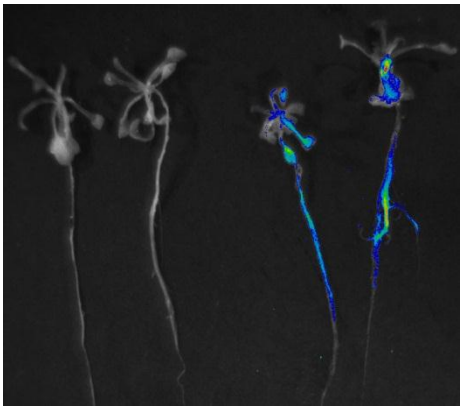

**Figure 1:** GFP-transformed (right) and non-transformed (left) *Arabidopsis thaliana* seedlings, epi-illuminated with gooseneck in NightOWL LB 983.

In a preliminary experiment (Figure 2) we obtained GFP excitation of GFP expressing *Arabidopsis thaliana* seedlings as a proof of principle, but the background was very high when we used a 315 nm trans-illuminator. This was the reason for starting the optimization of trans-illumination.

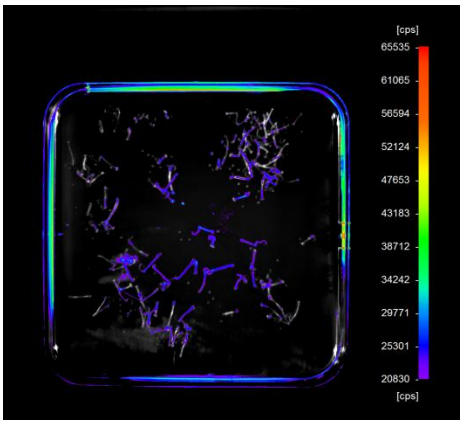


**Figure 2:** GFP-transformed *Arabidopsis thaliana* plants trans-illuminated with a 315 nm trans-illuminator.

## Experimental Procedures

Excitation of GFP can be performed within a range between approx. 340 to 480 nm. In this range two trans-illuminators are available at BERTHOLD TECHNOLOGIES, at 365 nm (#42602) and 470 nm (#42604) peak emission. The intensity of these trans-illuminators can be changed between 0 and 100% in 10%-steps.

Different Petri dishes were used, 12 x 12 cm dishes from Greiner-bio-one (#688102) and 9 x 9 cm dishes from Falcon (#351112). Agar (8 g/L) and growth media (8 g/L agar, 2,15 g/L MS-salt obtained from Duchefa, 0,5 g/L MES, adjusted to pH 5,7 with KOH) were filled into the dishes at four different heights of 1, 2, 3 and 4 mm.

The trans-illuminators were positioned in the optical axis of NightSHADE LB 985 IkLu, manufactured by BERTHOLD TECHNOLOGIES. Holes in the base plate allow easy positioning. An interference emission filter with central peak at 520 nm and a half band width of 10 nm (#39805) was attached in front of the lens (Xenon 25 mm, f 0,95 from Schneider)

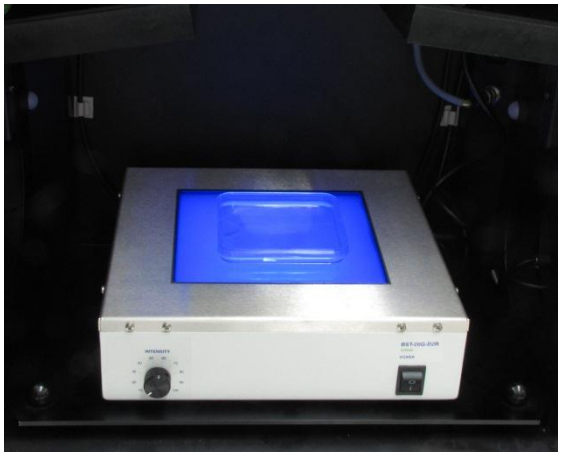

**Figure 3:** Principle of test procedure. The 470 nm trans-illuminator was positioned in the optical axis of NightSHADE LB 985 IKlu.

Intensities of light were measured in relative light units (RLU = cps in this case) with indiGO™ software v. 2.0 from BERTHOLD TECHNOLOGIES. Background intensities were obtained by visual view on the screen, when only black and no more false colour was seen.

## Material

- LB985 NightSHADE IkLu (Berthold Technologies)
- Trans-illuminators 365nm and 470nm (Berthold Technologies)
- Petri dishes 12x12 (Greiner-bio-one); 9x9 (Falcon)
- Emission Filter 520/10 nm

## Results

First it was observed, that the lid on the petri dish and the interference emission filter does not change the optical properties (not shown). Furthermore there is no difference of background between the two different petri dishes, although the 9x9 cm petri dish shows higher intensities at the side walls (Figure 7). There is also no difference between pure agar and agar with growth media, but a small effect can be seen regarding the agar height (Figure 4).

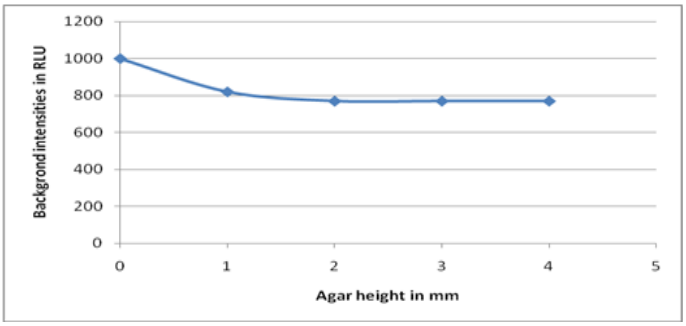


**Figure 4:** Effect of agar height. Background intensities were measured at 0,1s exposure time with a 470 nm trans-illuminator, 10% intensity

There is a linear dependency between background intensities and exposure time. The 365 nm trans-illuminator shows roughly 5-times higher background (Figure 5).

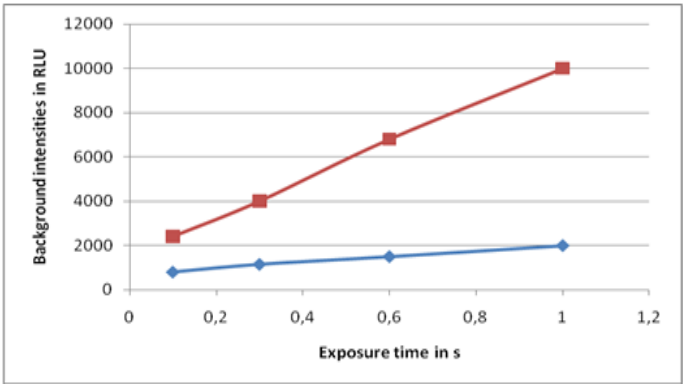


**Figure 5:** Effect of exposure time on empty petri dishes; red line 365 nm trans-illuminator, blue line 470 nm trans-illuminator. The intensity of the trans-illuminators is set to 10 %.

There is also a linear dependency between background intensities and trans-illuminator intensity (Figure 6).

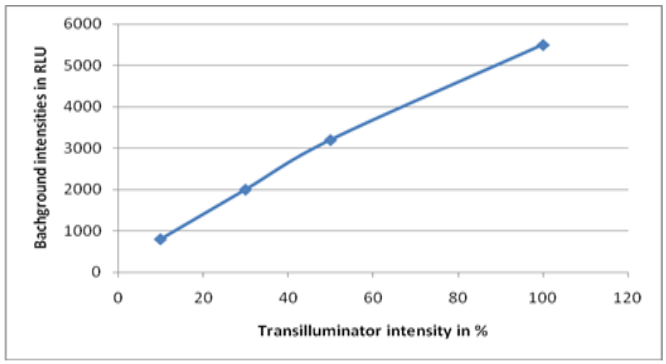


**Figure 6:** Effect of trans-illuminator intensity at 470 nm. The exposure time is set to 0,1s.

The intensity across the trans-illuminator is very homogenous. Repeated measurements after hours and days give same values ± 10 RLU. Droplets of 100 nmol/L FITC can be easily detected (Figure 7).

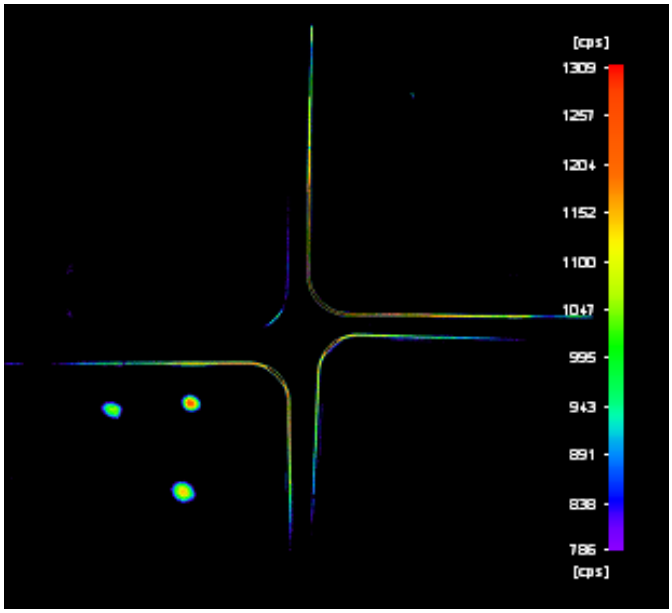


**Figure 7:** A 100 nmol/L FITC solution is pipetted into the lower left Petri dish, trans-illuminated at 470 nm, 0,1s exposure time and 10% trans-illuminator intensity.

## Conclusion

The linear dependency of background intensities against exposure time and trans-illuminator intensity shows very clearly that the limiting factor of trans-illumination is the blocking factor of the used interference-emission filter. High quality interference filters have a blocking factor of around $10^5$. This means, when 100 000 photons hit the interference filter, one photon passes.

The 470 nm trans-illuminator shows much lower background than a 315 nm or 365 nm trans-illuminator.

There is definitely no overlapping of emission spectrum of the 315 and 365 nm trans-illuminator and 520 nm emission filter, which was the reason that they have been tested as well. It could be proofed that there is also no overlapping with the 470 nm trans-illuminator, otherwise the FITC droplets could not have been detected. FITC and GFP have a very similar excitation/emission spectrum.

With a 470 nm trans-illuminator model from BERTHOLD TECHNOLOGIES set to 10% intensity, all GFP-transfected plants in petri dishes filled with agar can be detected, which show higher emission intensities than 800-1000 RLU. Compared with luminescence, luciferase expressing plants can be detected, if the signal intensities are higher than 30 RLU. But for screening and clone-picking of GFP expressing seedlings in Petri dishes the trans-illumination method could be an alternative.

### Acknowledgement

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