



Task

Rapid Multiplex Fluorescence Screening in Plants

Solution

The UVP GelStudio automated digital imaging system equipped with a 12MP camera.

Taking Root – Integrating Area Imagers into Plant Screening Pipelines

Introduction

Recombinant DNA strategies have made it possible to develop fluorescent organisms for numerous research applications. As molecular technologies advance, the rapidity in which one can create a transgenic ‘glowing’ organism has increased dramatically such that one can go from concept to experimentation in as little as a few weeks. This progress has concomitantly increased the potential scale of the experiment size. This creates a practical problem for researchers - how does one quickly screen many fluorescent samples? In general, researchers have two options, an area imager or a microscope.

Area imagers with fluorescence capability have become a useful tool in the screening pipeline for several reasons 1) area imagers are plug and play and require very little tweaking to capture an image in contrast to microscopy; 2) the resolution of an area imager is sufficient for screening purposes and saves time when used in parallel with microscopy; 3) area imagers are generally less expensive than fluorescent microscopes and have a lower barrier to entry in terms of expertise; 4) area imagers require less space in a laboratory environment; 5) and perhaps most importantly, Analytik Jena Studio imagers are capable of a range of applications beyond in vivo fluorescence-based screening including gel imaging, immunoblotting, and colony counting.

Below we demonstrate the ease in which researchers can perform multiplex fluorescent imaging of *Mesorhizobia spp.* in *Acmispon wrangelianus* root nodules with the GelStudio 12MP Imager.

Methods:

Rhizobia and Plant Materials

sfGFP (ex 485 nm, em 510 nm) and mScarlet (ex 569 nm/ em 594 nm) fluorescent *Mesorhizobia spp.* were prepared by A. Montoya and S. Porter at Washington State University, Vancouver. These rhizobia were introduced to *Acmispon wrangelianus*. After formation of root nodules, plant roots were screened for fluorescence on the Gel Studio 12 MP imager.

Imaging of Root Nodules using the Analytik Jena GelStudio 12 MP

Root nodules were screened by separating root tissue from canopy tissue and placing the root tissue directly on a black acrylic platen on the GelStudio 12 MP imager floor. Auto-focusing and auto-exposure were used to setup the instrument for sample image capture. For each sample, a white light image was captured without using an emission filter. For sfGFP, we used blue LED excitation, with a 535 nm filter with a 50 nm bandpass (P/N 38-0340-01). For mScarlet, we used green LED 605 nm filter with a 50 nm bandpass (P/N 38-0220-01). Each fluorescent image was captured in <4 seconds.

Area Density Analysis of Root Nodules using Analytik Jena VisionWorks

Using our Area Density Analysis Tool in VisionWorks v9.1, users can analyze their images using drawing tools, such as rectangle, circle, and freeform. The freeform drawing tool was used to measure the fluorescence from each root nodule since their shapes and sizes are heterogeneous (Figure 1, Panel A). Regions of interest (ROIs) were drawn using the white light image, to prevent selection-bias of the margins of the root nodule that might occur when only viewing a fluorescent signal. Within VisionWorks software, the ROIs can be saved, exported, and applied to other images. Therefore, the white light ROIs were applied to the sfGFP image, and the mScarlet image, so that the measured ROIs were consistent across samples. The fluorescent signal was then evaluated using our Report Tool.

In our Report Tool, several data are generated. For the purposes of quantifying and comparing root nodule fluorescence signal, Mean Density was used in this analysis, which is automatically computed and accounts for background signal (Equation 1).

Equation 1: $\text{Mean Density} = \text{Mean Raw Density} - \text{Mean Background Density}$

Mean density is defined as the mean of intensities of all pixels within Target ROIs (Mean Raw Density), minus the mean intensity of all pixels within background ROIs (Mean Background Density). ‘Density’ implies ROI ‘area’ and is automatically considered in the computation. Nonetheless, ROI area is separately reported in the Report Tool if users want access to this value.

To background subtract root autofluorescence, at least three background regions were used to define the Mean Background Density, which included a combination of autofluorescent root tissue and autofluorescent root nodules, that were otherwise devoid of sfGFP or mScarlet signal. Importantly, Mean Raw Density values approximated one another between these two background tissue types and differences were statistically insignificant (data not shown). The Mean Background Density is then automatically subtracted from all Mean Raw Density ROIs. Again, the result of this computation is automatically reported as Mean Density in the Report Tool. Finally, all the data can be easily exported as .xlsx files to be read into third-party analysis software.

Results and Concluding Remarks

To help visualize the spread of the mScarlet+ root nodules versus the sfGFP+ root nodules, we compared non-zero log-transformed fluorescent Mean Density between sfGFP and mScarlet using box and whisker plots. The spread of the sfGFP+ root nodules is higher, but the medians approximate one another. Using a heatmap, we can compare at-a-glance the distribution of sfGFP+, mScarlet+, double positive, or no fluorescent root nodules in a given sample (Figure 1C) — these values are further summarized into categories in Figure 1D.

Because imaging and analysis is easily performed with the GelStudio 12 MP imager and VisionWorks software, this screening pipeline can be scaled to many samples quickly. This helps researchers identify trends in datasets and support hypothesis-generating/exploratory research studies.

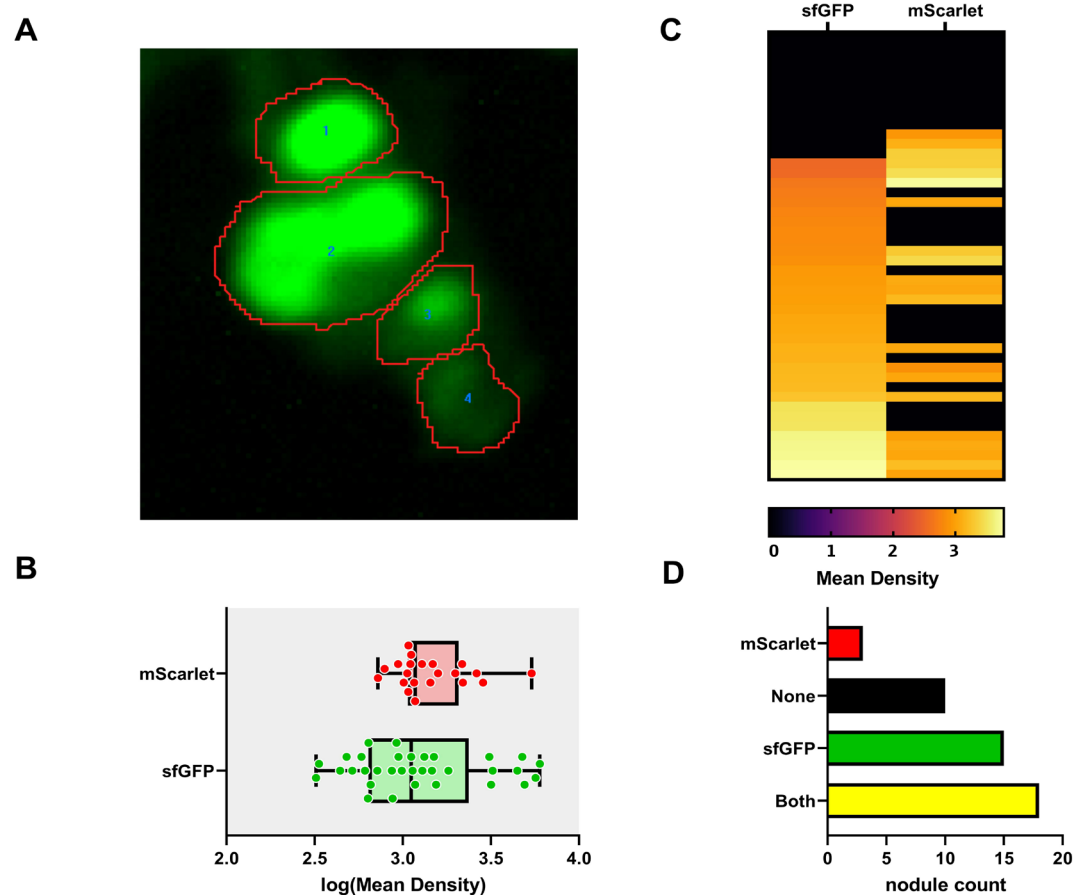


Figure 1: Screening for in vivo plant fluorescence of root nodule rhizobia using the GelStudio 12 MP Imager.

- An example sfGFP positive root nodules is displayed. sfGFP positive root nodules were captured using blue LED excitation and the 535bp50 emission filter. The Area Density Tool in VisionWorks was used to draw freeform regions of interest (ROIs), to analyze root nodule fluorescence. Summary data from one plant is provided in Figure 1B-D.
- Box and whisker plot representation of non-zero log transformed Mean Density fluorescent signal for sfGFP and mScarlet fluorescence.
- Heatmap representation of 46 root nodules. Values represent log transformed Mean Density fluorescent intensities.
- A bar graph summary describes distribution of root nodules into None, mScarlet+, sfGFP+, or Both (mScarlet+/sfGFP+) categories. Graphical figures were prepared in Graphpad version 8.4.3.

Overall, the GelStudio 12 MP imager can be integrated in research pipelines where large volumes of samples need to be analyzed. While this does not replace the use of higher-resolution imaging, such as microscopy, an area imager can be used in parallel to reduce time wasted at the microscope.

Finally, our VisionWorks software enables researchers to generate presentation and publication quality images, as seen in the composite images in Figure 2. While imaging root nodules in plant tissue as seen here is relatively straightforward, some applications require more advanced technology for deep tissue imaging or faint signals. In this case, we recommend users explore other imagers in our advanced imager portfolio such as the ChemStudio and iBox Scientia. For additional support, researchers should contact our Applications Support Team at support@us.analytik-jena.com.

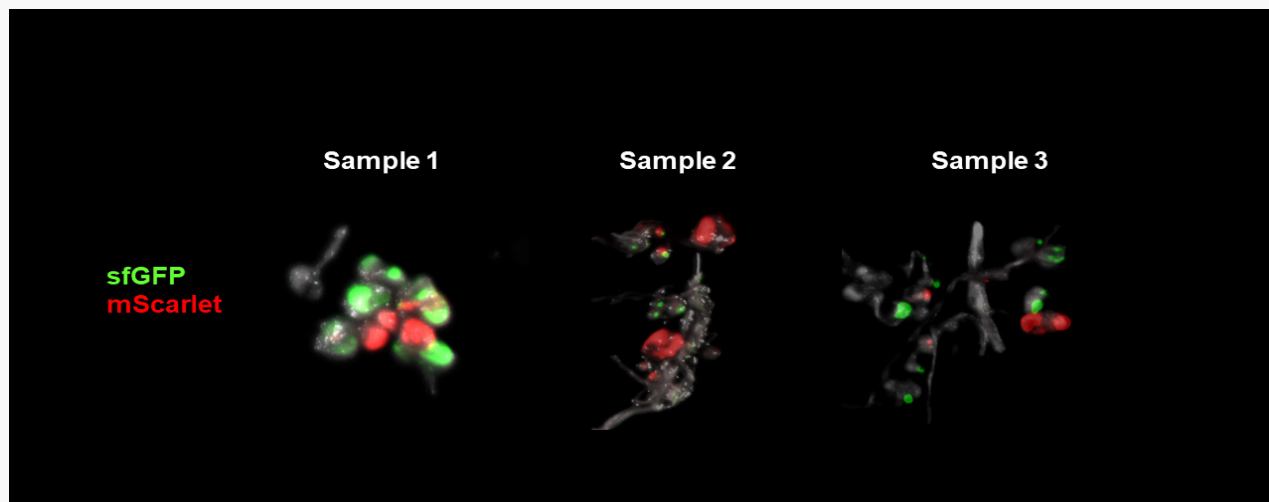


Figure 2: Composite Fluorescent Images of Root Nodules using the GelStudio 12 MP Imager. Using VisionWorks software version 9.1, white light, sfGFP, and mScarlet images were composited using the Merge Tool. Three representative samples from a root nodule screen are displayed.



My research investigates the evolutionary genetics of cooperation of rhizobia symbionts to understand what type of selection maintains cooperation, and how environmental factors and uncooperative strains impact the evolution of host-microbe cooperation. Using fluorescent markers, we can track strains in vivo that differ in the presence or absence of symbiosis genes or differ in their quality as nitrogen fixers, to determine if hosts preferentially associate with certain strains or if any strains are cheating the host or other symbionts.

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