



LemnaTec Bonit

softwaremodule for the LemnaTec Scanalyzer System

Video densitometry for the Evaluation of

GFP-Expression

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Biological background

The green fluorescent protein (GFP) has become a valuable tool in gene sciences.

Found in the deep sea medusa *Aequorea Victoria* it so far represents the only known natural occurring fluorescent protein.

If illuminated with blue light (489 nm) the protein will emit a green fluorescence at 511 nm. Usually modifications of the original GFP-gene are used which result in enhanced properties and different fluorescent wavelengths (e.g. BlueFP and YellowFP).



Picture 1: Aequorea Victoria

Besides a wide variety of biological applications the LemnaTec Scanalyzer technology can be used for video densitometrical measurements of the fluorescence emitted by GFP-expressing organisms. This is achieved by the application of digital image analysis.

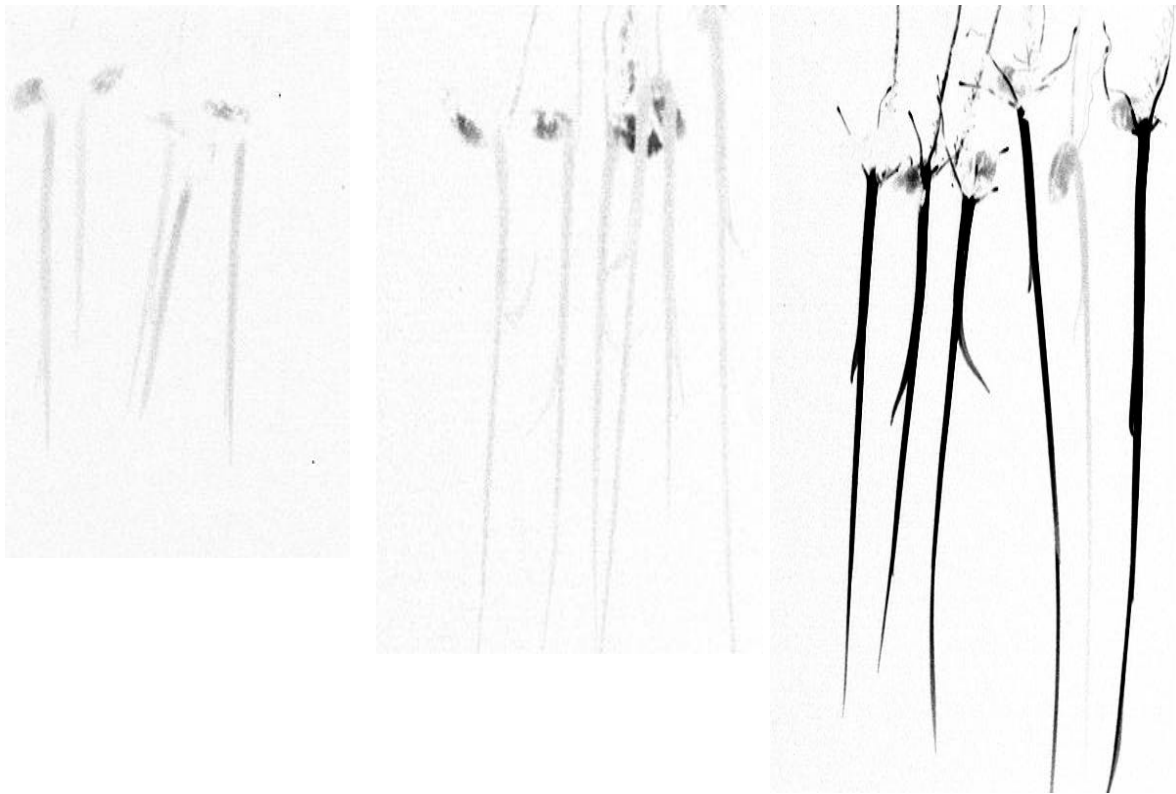
Hardware

The LemnaTec Scanalyzer is the source for excellent digital images. At the same time special requirements for an application in a laboratory (e.g. GLP) environment are met.

For the acquisition of true-colour images a high-resolution digital camera is available. Together with the reflex suppressing illumination this forms the fundament for precise and reliable image analysis – a digital precision image.

Especially for the video densitometry the LemnaTec Scanalyzer is equipped with an optional GFP-imaging kit.

For the excitation of the fluorescence a cold-light source and a blue-filter are used.

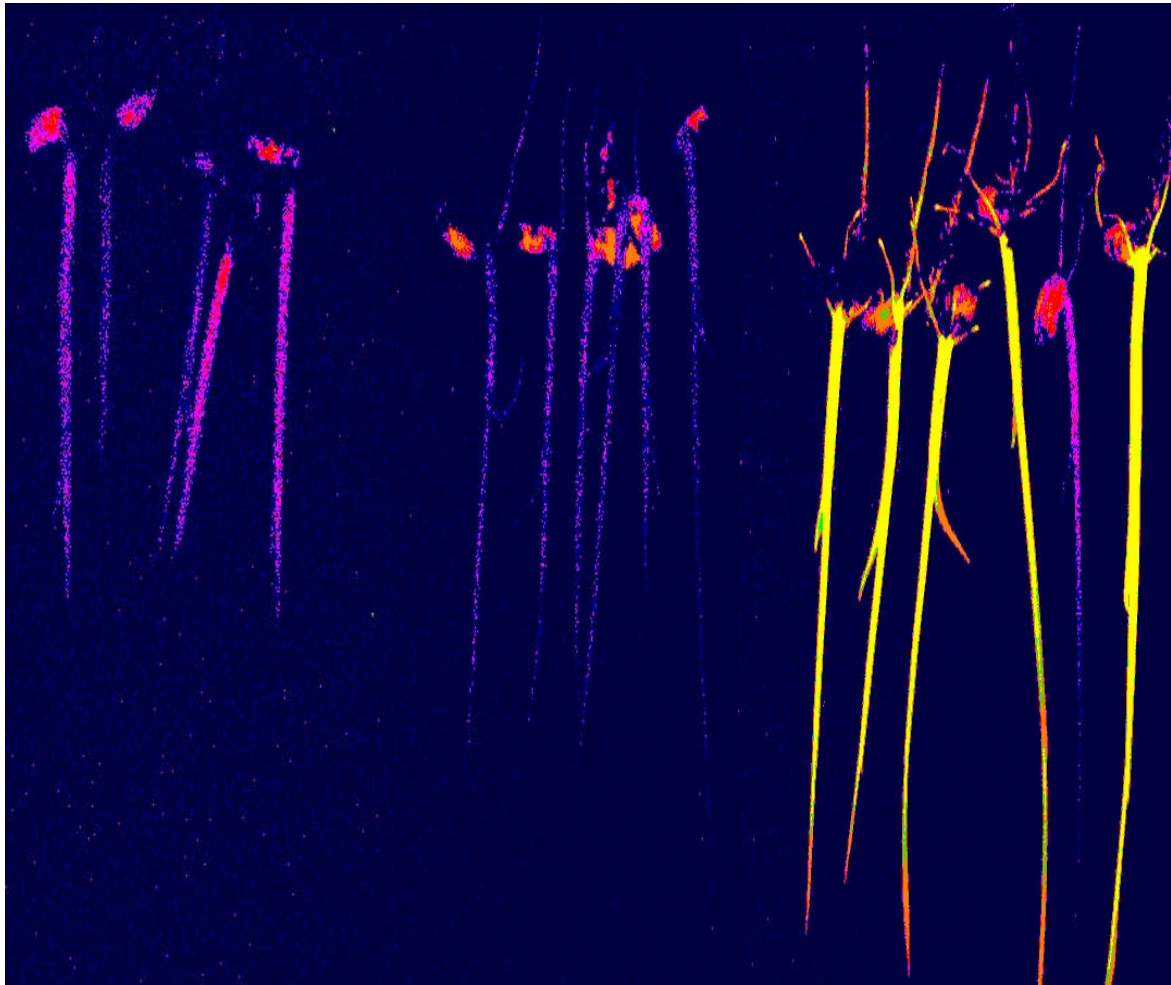


Picture 2: Inverted original images of fluorescent objects

A high sensitive camera system with an adapted filter is used as a detector for the fluorescent light emitted by GFP (see pic.2). Due to the low intensities a highly sensitive detection is crucial for this technology. This is often done by using expensive cooled CCD chip cameras. However LemnaTec utilises modern cost-effective camera technology combined with image amplifying software avoiding these costly devices.

Software

The LemnaTec software ascertains the intensity of the fluorescence on base of the brightness of pixels (pic. 2) and visualizes it by a false coloured image. By doing so even slight differences in intensity become evident at first sight. A quick and convenient estimation of the GFP distribution in the organism is enabled as well.



intensity



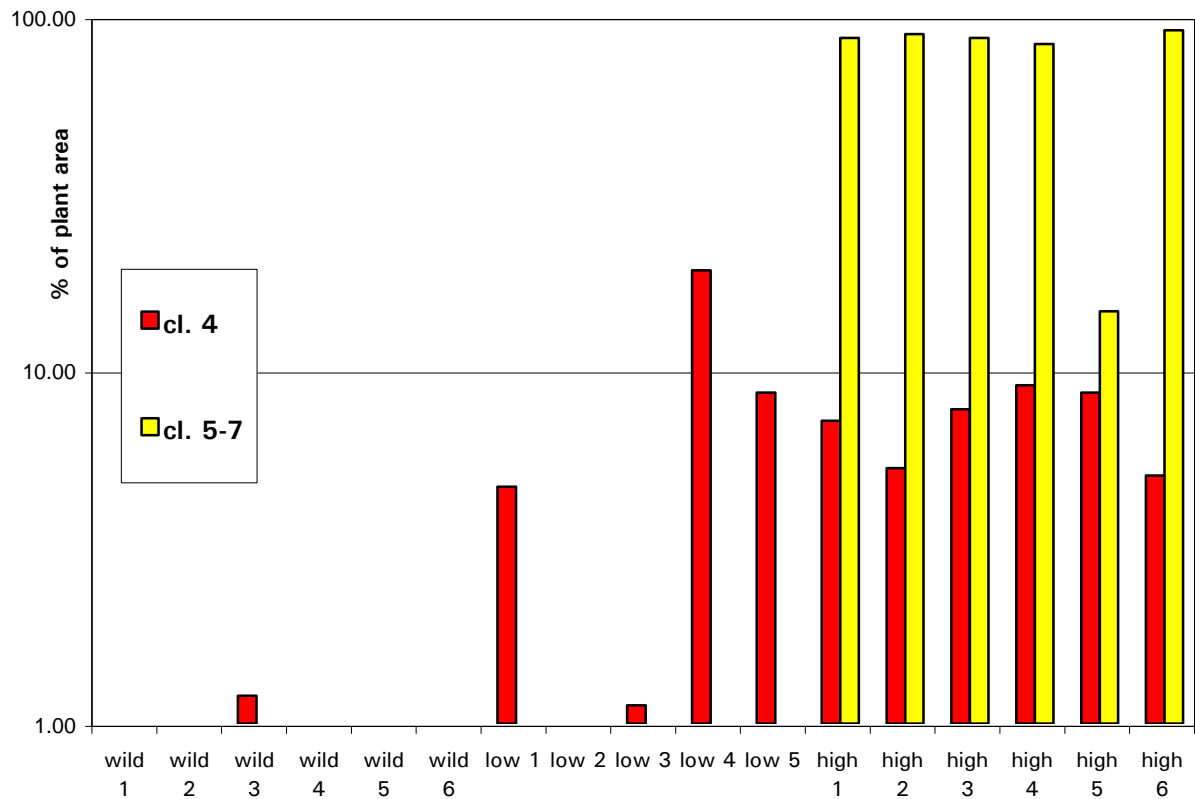
high

low

Picture 3: False coloured image of the intensity of fluorescence

By scaling to the objects area a value is determined, that stands in good correlation to the expression of GFP in the sample.

Finally the data can be exported as MS-Excel[®] file, HTML file or as standard text. Thus in short term a fast and reliable determination of the GFP expression in whole measurement series is made possible (see pic. 4).



Picture 4: Classes of intensity in percent of the organisms area

The LemnaTec Software is consequently designed to measure a high amount of samples in a short time. It can be combined with the LemnaTec Scanalyzer HTS to master even biggest screening tasks. For the desktop version of the Scanalyzer a barcode reader is available, to even rise the efficiency of sample handling.

Despite the ease of handling no compromise is made in regard to data validity. The software features GLP conform determination of data by a password secured user management and an explicitly guided sample handling.

The raw-data –in this case the digital image– is stored together with all the GLP relevant data and is never altered nor changed during the processing.

In combination with the Scanalyzer the software forms an economical and valid system for the semi-quantitative evaluation of the expression of GFP.