

PE^{PRO+} explorer Plant



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 **PhenoVation**
Life Sciences

DESCRIPTION

The PlantExplorer has a 12.1-megapixel high-resolution CMOS-camera to image the efficiency of photosynthesis. By using the two individual blue led strings a saturation and measuring pulse of $7000 \mu\text{mol m}^{-2} \text{s}^{-1}$ can be produced.

Software

The control software is installed on the internal computer to capture different images using pre-defined protocols. These pre-defined protocols can be changed by the user using the user-interface. A combination of light sources and filters will provide a range of different images.

Within the control software there is a feature to control the system remotely. By using the remote-control software, the system can be easily integrated into a phenotyping system later on. The top part of the PlantExplorer can be detached from the base and so be integrated into an automated plant phenotyping system.

The frame / Building quality

The PlantExplorer is constructed from a solid aluminum frame and all the components (light sources, camera, computer, filter-wheel, etc) are integrated into this frame so the user does not have to assemble the system.

The frame is made from powder coated aluminum and all the critical components are in a air / dust free environment. The system is cooled by thermal bridges so dust and moist can not harm the internal components.

Safety

On the door there is an induction sensor that monitors if the door is closed. Because of the high intensity lights during the measurements it is not recommended to look into the lights. When the door is opened the light will automatically switch off. Active cooling on the outside of the system will make sure the system will not overheat. The cooling is temperature controlled to switch on when certain temperatures are reached.

Maintenance

The system hardly needs any maintenance except cleaning the inside of the cabinet. There is a possibility for a maintenance contract so PhenoVation will calibrate the system on a yearly basis.



LIGHT SOURCES

Saturation Pulse / Measuring Lights

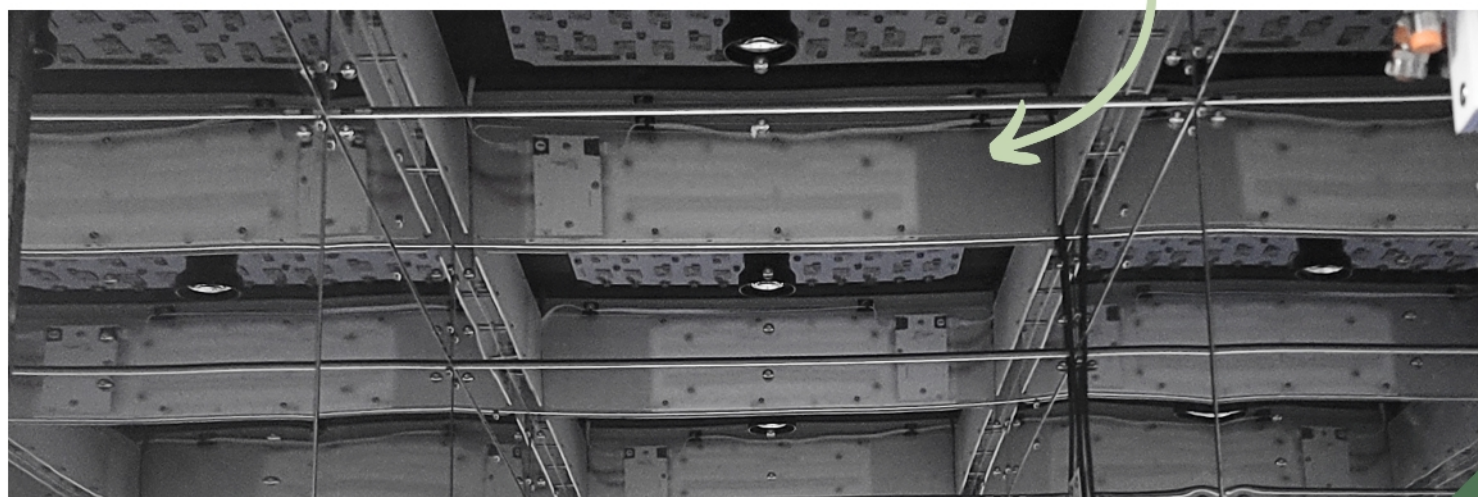
The PlantExplorer has two strings of high intensity Blue LEDs. One string is used as the saturation light for the PAM PSII measurement and one string is used as the measuring light for the PAM PSII measurement. Each string of Blue LEDs can produce up to $7000 \mu\text{mol} / \text{m}^2 / \text{s}^{-1}$ of light.

Actinic light

Within the PlantExplorer there are different LEDs that can be used for Actinic light. The following LEDs are incorporated:

SPECTRUM	USED FOR
450 nm	Actinic light
660 nm	Actinic light
White 3000K	Actinic light
735 nm	Actinic light / PSII relaxation

Every spectrum can be controlled from 0 - 100% and a combined intensity of $800 \mu\text{mol} / \text{m}^2 / \text{s}^{-1}$ can be reached.



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FILTER WHEEL & CAMERA

Incorporate filters

Twelve filter wheel positions are available inside the filter wheel of the PlantExplorer. The following filters are standard:

FILTER	USED FOR
769 nm	NIR Imaging
732 nm	Fluorescence
710 nm	Red Edge
640 nm	Red Imaging
550 nm	Green Imaging
540 nm	Anthocyanin
475 nm	Blue Imaging
1 CUSTOM	
2 CUSTOM	
3 CUSTOM	
4 CUSTOM	
5 CUSTOM	

The five CUSTOM positions can be chosen by the customer. These can be in the wavelength range of 400nm -800nm. If higher wavelengths are required the excitation LEDS also need to be adjusted, this is a possibility to change.

Automated Focusing system

The camera system can be focused by using the user-interface. In the user interface there is a button NEAR and FAR. By pressing on these buttons the focusing distance can be changed and nearly every object can be focus from 10 cm up to 70cm distance from the lens. Also the depth of field of the camera is already high, up to 40 centimeters.



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PRINCIPLES OF PSII MEASUREMENT

1. Chlorophyll Fluorescence

- a. Direct CF imaging
- b. PAM Imaging (Pulsed Amplitude Modulation)
- c. Kautsky OJIP Induction Curve Imaging

a) Direct chlorophyll fluorescence imaging is used to determine the amount of chlorophyll in seeds and plants. The used intensity of the blue LEDs can be varied by the user combined with the integration time of the camera to obtain high quality fluorescence images. These chlorophyll fluorescence images are used for correlating chlorophyll with maturity status of seeds and physiological status of plants. Furthermore, these images are used in the analysis software for thresholding objects from the background.

b & c) For imaging photosynthesis, two basic measuring principles can be applied: Pulsed Amplitude Modulation and Kautsky. These two measuring principles can be selected independently of each other. a) During short pulses of measuring light with amber LEDs F_0 is being determined by capturing images during the measuring pulse. A saturating pulse is applied for capturing the maximum fluorescence yield, F_m . From this data different photosynthesis parameters are calculated like F_v/F_m . Actinic light is turned on to measure F_t with the PAM method and applying saturation and short measuring pulses, F_m is being measured in the light. This yields for instance $\phi PSII$.

c) The Kautsky principle can be applied as a second method to capture chlorophyll fluorescence images of the OJIP induction curve like F_0 , F_i and F_m and calculate different photosynthesis parameters. Before each induction curve is being imaged, the background image is being captured and subtracted from the images captured during the induction curve.

Dark adapted:

Plants are measured when they are dark adapted. This yields F_0 and F_m . From this data the parameter that correlates with the maximum efficiency of photosystem II, F_v/F_m , is calculated and presented as an image.

Measured parameters: For PAM F_0 and F_m are being imaged for dark adapted plants. For Kautsky the induction curve is being imaged. This yields F_0 , F_i and F_m images of the OJIP induction curve (F_i depending on used frame rate).

Light adapted:

The same measuring procedure is used for plants in the light to measure parameters that correlate with the effective efficiency of photosystem II, $\phi PSII$, ABS and ETR image. Actinic light provides control over the applied spectrum and intensity.

Kinetic analysis:

For time resolved kinetic analysis plants are first dark adapted. After the adaptation procedure plants are first being measured in the dark. Then they are continuously illuminated by LEDs. The camera measures the plant when the plant is adapting to the light intensity: induction analysis. Then the actinic light is turned-off and the recovery is measured in time. Images of F_0 , F_m , F_m' , F_t and F_0' are being recorded in time and F_v/F_m , F_v'/F_m' , $\phi PSII$, NPQ, q_N , q_P , ETR are being calculated.



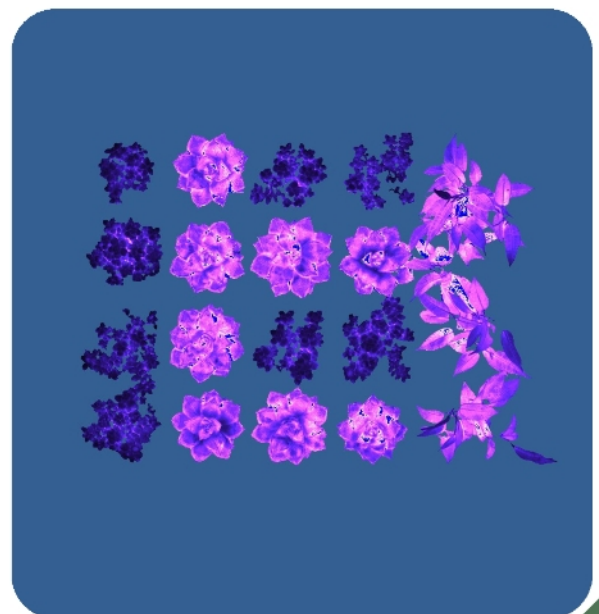
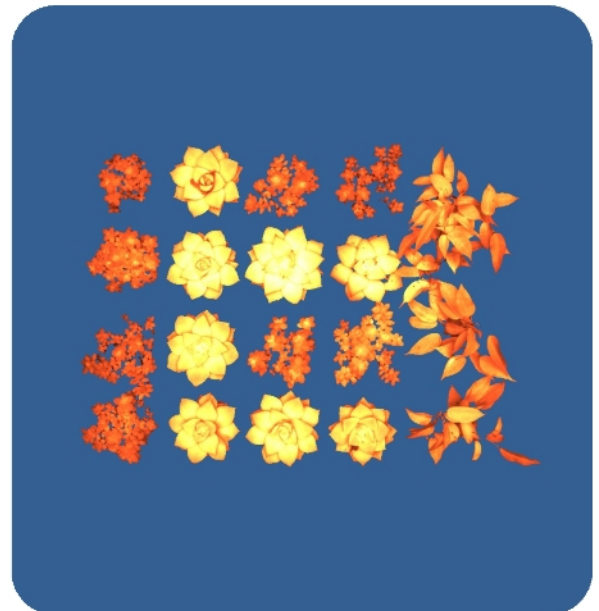
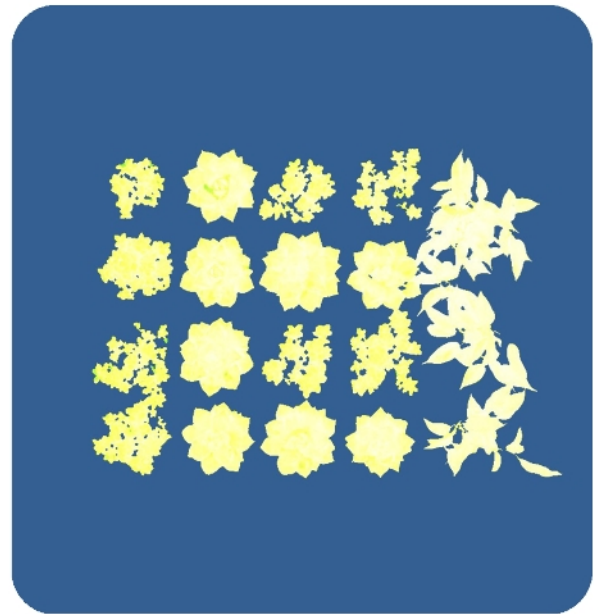
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Highlights

- PAM- and Kautsky-principle chlorophyll fluorescence imaging
- Single leaf (macro) and whole plant imaging both at 12.1 Mp
- Provides: F_0 , F_m , F_v , F_0' , F_s' , F_m' , F_v' , F_t
- Kinetic chlorophyll fluorescence images are provided from light adaptation and dark adaptation protocols
- High signal-to-noise ratio of chlorophyll fluorescence images
- Blue 450 nm LED measuring light source used for measuring F_0 and so on;
- pulse width: 10 – 100 μ s / DC
- Blue 450 nm LED saturating light source used for measuring F_m and so on;
- pulse width: 10 – 100 μ s / DC
- LED light source with 730 nm far-red for measuring F_0' etc.
- Measuring distance between plant and camera ranges over a distance from 10cm– 70cm
- Effective imaging area of 50x50 cm^2
- High quality Mp lens, with broad band visible and NIR coating
- No visible lens distortion, no correction needed



Acquisition protocols

- $F_v/F_m=(F_m-F_0)/F_m$ image that correlates with the maximum quantum yield of PSII photochemistry
- $\phi\text{PSII}=F_q'/F_m'=(F_m'-F_s')/F_m'$ image that correlates with the effective quantum yield of PSII photochemistry
- $\text{NPQ}=(F_m-F_m')/F_m'$ image that correlates with non-photochemical quenching
- ABS=image of the absorption coefficient of chlorophyll
- ETR = image that correlates with the electron transport rate.
- Chfl image: the chlorophyll fluorescence image at relative long exposure time for thresholding plant material from background
- Light adaptation curves
- Dark adaptation curves
- Delivered images by controller software of PlantExplorer in raw data 16-bit format
- F_0 minimum chlorophyll fluorescence image in dark adapted state
- F_m maximum fluorescence image in dark adapted state
- F_s' steady state minimum chlorophyll fluorescence image in light adapted state
- F_t' instantaneous chlorophyll fluorescence image during light adaptation
- F_m' steady state maximum fluorescence in light state
- F_m' maximum fluorescence during light adaptation
- F_0' minimum chlorophyll fluorescence image during dark relaxation after applying farred
- $F_{\text{background}}$ for all fluorescence images
- R_{NIR} reflectance image at NIR wavelength, 760nm
- R_{Red} reflectance image for red at 660nm



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MULTI SPECTRAL MEASUREMENTS

The following spectral measurements / calculations are done with the PlantExplorer;

MEASUREMENT	CALCULATIONS
NIR Imaging	Chlorophyll Index
Red Edge Imaging	Anthocyanin Index
540nm Green	Color images
Red Imaging	HSV
Green Imaging	NDVI
Blue Imaging	RGB Ratio

Pixel to Pixel

All the images are made with the same camera system. In this way all the images from the PSII measurements and Multispectral measurements are pixel to pixel. Because of this every individual pixel can be compared.

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