Zealquest AI Netherlands

Zealquest Group Company

## High-Throughput Dynamic Phenomics Analysis System for Plant Seedlings

# DynaPlant® Seedlings

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In traditional botanical research, experimental methods were often limited to studying the static state of plant phenotypes at a single time point, reflecting the cumulative effects of all biological processes up to that point. However, plant growth and development are a series of spatiotemporal-specific processes. Only by observing dynamic changes can we gain a more comprehensive understanding of the spatiotemporal specificity of plant growth and development regulation.

DynaPlant® Seedlings is a commercial instrument platform for dynamic imaging analysis of plant seedlings, capable of performing kinetic analysis at the minute scale. This precision is achieved through unique image recognition and analysis algorithms, a long-range 3D moving platform with micron-level positioning accuracy, and hardware-optimized shooting control programs. The high-throughput imaging capability makes the system suitable for large-scale screening of mutant libraries, ecotype libraries, and the establishment of phenomics. The automatic control of various experimental conditions makes the system truly suitable for basic research, enabling in-depth studies of molecular mechanisms.

## Multiple Condition Controls

- Growth in Dark
- Adjustable Light Source
- Light Intensity
- Photoperiod
- Weightlessness
- Gravity Redirection
- Temperature 12 ~ 42 ± 0.2 °C
- Humidity 50 ~ 85% ± 7%
- $O_2$ 3 ~ 21%
- $CO_2$ 400 ~ 5000 ppm
- $C_2H_4$ 0 ~ 20 ppm
- Compound treatments

## Multiple Detection Methods

- Macro Morphological Phenotypes
- Wide - angle Morphological Phenotypes
- Chemiluminescence
- Fluorescence Detection

## Multiple Data Analysis Types

- Seed Germination Rate
- Root Growth Rate
- Hypocotyl/Stem Growth Rate
- Cotyledon Angle
- Root Tip Changes
- Dynamic Luminescence Quantification

## Applications

- Plant hormone responses
- Plant phototropism/gravitropism
- Plant developmental biology
- Plant and atmospheric environmental interactions
- Abiotic stress (heat, cold, salt, hypoxia resistance, etc.)
- Dynamic changes in plant gene expression regulation
- Plant circadian rhythms and their regulatory dynamics
- Plant skotomorphogenesis and photomorphogenesis

**High-throughput and high-precision imaging**

In an experiment, the phenotypes of hundreds of seedlings are detected. By integrating a high - resolution camera, a macro lens, and a high - precision three - dimensional stage, continuous detection of samples with high spatial and high temporal (in minutes) resolution can be achieved.

**Multimodal imaging technology**

Infrared morphological phenotype detection, unaffected by changes in cultivation light. It also has chemiluminescence and fluorescence imaging modes.

**Environmental control and instantaneous change**

Multiple environmental condition control, and can be quickly changed according to the settings, phenotype detection under complex treatment conditions.

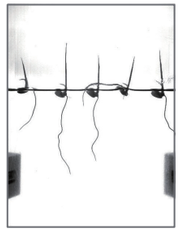
**Automation and batch analysis**

Customized fully automatic unattended shooting; automated batch analysis, directly outputting statistical curves.


## High-Throughput Detection

DynaPlant® Seedlings adopts the OctAdapter® eight - way rotating sample holder system. Different functional culture modules with different magnifications can be replaced through standard interfaces. it is suitable for the cultivation and growth of various seedlings and improves the detection throughput of samples shot simultaneously.


Arabidopsis


Rice


Soya bean


Cabbage


OctAdapter® sample holder system

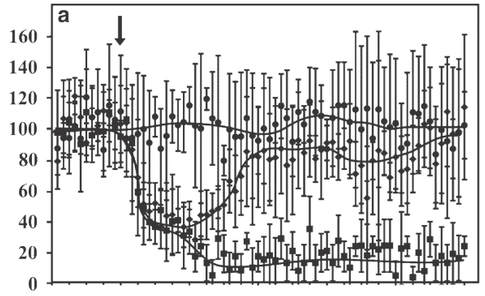
| Macro Standard Petri Dish | Wide-Angle Standard Petri Dish | Wide-Angle Large-Size Petri Dish or Pot |
|---|---|---|
| 10×7.5mm Field of view<br>200 ~ 1000 Arabidopsis seedlings | 100×100 mm maximum size<br>16 Petri Dishes | 100×300 mm maximum size<br>8 Dishes or 8 Pots<br>8 ~ 40 Rice / Soybean |

## High-Precision Detection

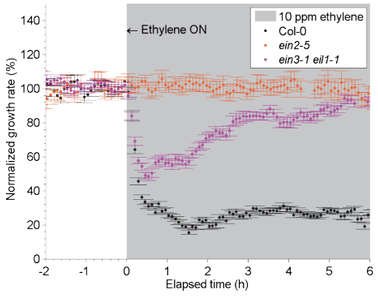
High Spatial Resolution: When measuring fine changes in growth parameters, DynaPlant® Seedlings can achieve both a high resolution of 1.07 μm/pixel (close to the typical resolution of a 10x microscope objective) and a wide field of view of 10×7.5 mm (100 times the typical field of view of a 10x microscope objective) using a customized 65-megapixel camera and a high numerical aperture telecentric macro lens. Additionally, its exceptional lens distortion control ensures high measurement accuracy.

High Temporal Resolution: Through the high-speed, high-precision 3D direct-drive displacement system, DynaPlant® Seedlings can simultaneously ensure high spatial and temporal resolution during high-throughput sample

detection, accurately measuring rapid changes in plant seedling growth rates at the minute scale with minimal error. Compared to internationally reported technologies, DynaPlant® Seedlings provides higher precision results.




Binder et al., 2007




DynaPlant measurement results
(unpublished data)

## Infrared Imaging

When performing phenotypic detection with traditional visible light illumination sources, it is impossible to photograph samples that must be kept in the dark, such as etiolated seedlings of Arabidopsis thaliana. In addition, the changes in lighting conditions caused by the photoperiod will lead to significant differences in image morphology, making it impossible to conduct batch analysis uniformly.

DynaPlant® Seedlings can solve the above problems by using a 940 nm infrared illumination source and an infrared detection camera. Plants are not sensitive to 940 nm infrared light, so it can be used to observe the growth of plants in the dark. The infrared detection camera is not sensitive to visible light, and the changes in the light and dark cycles of the cultivation light source will not affect the imaging.

| Ordinary RGB Imaging |
| --- |
| White-light illumination + ordinary RGB camera<br>cannot photograph in a dark environment<br>the imaging effect is different<br>when the light is turned on/off |

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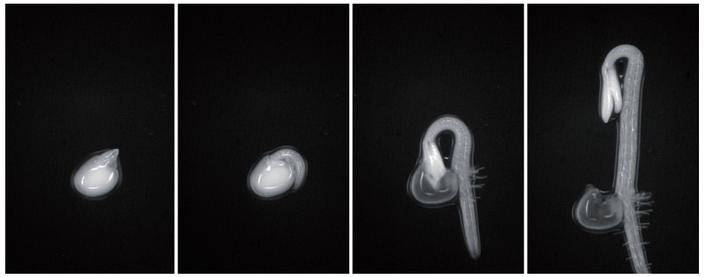
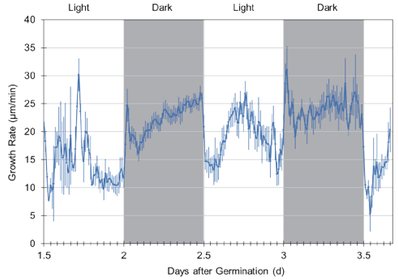
| --- |
| White-light illumination + ordinary RGB camera<br>cannot photograph in a dark environment<br>the imaging effect is different<br>when the light is turned on/off |



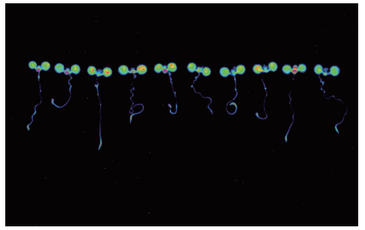
Arabidopsis sprouts in the dark



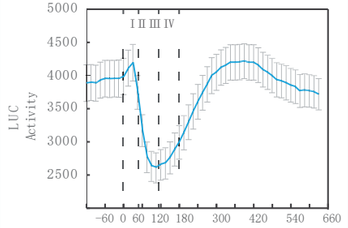

Periodic rhythm of hypocotyl growth rate
in soybean seedlings (unpublished data)

## Chemiluminescence Imaging

DynaPlant® Seedlings can not only analyze the morphological phenotypes of plants but also conduct continuous real - time quantitative detection of chemiluminescence such as Luciferase. The chemiluminescence imaging component is equipped with a high - sensitivity cooled CCD camera and an F/0.8 large - aperture lens, which can detect weak signals and draw curves of luminescence intensity changes. It is suitable for research such as the kinetics of gene expression regulation and biological circadian rhythms, etc .



IAA17-LUC
Auxin Distribution




Changes of luminescence intensity of IAA17-LUC after experimental treatment conditions are applied (Huang et al., Phenomics, 2022)

DynaPlant® Seedlings allows users to customize experimental procedures and freely combine one or more experimental conditions.

## illumination

DynaPlant® Seedlings is equipped with an independent light source module for each culture dish to ensure the consistency of lighting conditions. Users can either use the standard four-in-one strip light source or a customized light source. The standard light source module enables the setting of monochromatic light or mixed light, light intensity, and photoperiod.

| | | | | |
|---|---|---|---|---|
| Four-in-one light | Simulated Sunlight | Blue Light (450 nm) | Red Light (660 nm) | Far - Red Light (730 nm) |
| Light source control | Monochromatic light sources or mixed light sources, with adjustable light ratios and photoperiods. | | | |
| Max light intensity* | 220 μmol·m$^{-2}$·s$^{-1}$ standard white light** | 260 μmol·m$^{-2}$·s$^{-1}$ standard blue light | 120 μmol·m$^{-2}$·s$^{-1}$ standard red light | 120 μmol·m$^{-2}$·s$^{-1}$ standard far-red light |
| Custom light sources | UV-B and other wavelengths | | 600μmol·m$^{-2}$·s$^{-1}$ Customizable max light intensity** | |

Measured at the typical growth position of the sample (7 cm directly below the light source)

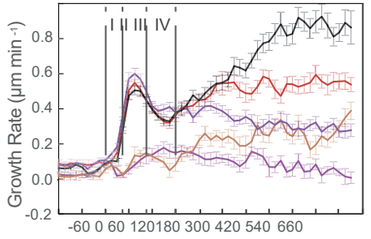
** Equivalent illuminance: 13000 lx

*** Only supports single-channel light sources

### Application Case: Shade Avoidance Response in Arabidopsis Green Seedlings

By quantitatively changing the ratio of red to far-red light, Arabidopsis can exhibit a shade avoidance response, resulting in changes in hypocotyl growth rate. Compared to traditional kinetic research methods (Cole et al., Plant Journal, 2011), DynaPlant® Seedlings not only obtains cumulative growth curves but also precisely measures growth rate changes as low as 0.1 μm/min, clearly distinguishing between the rapid and long-term response phases of the shade avoidance response.

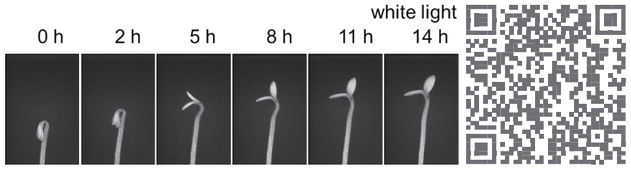

Arabidopsis green seedlings



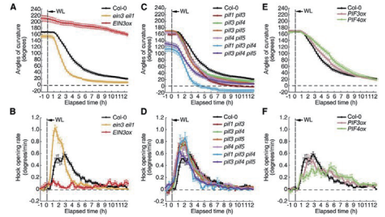

Changes in the growth rate of hypocotyls of green Arabidopsis thaliana seedlings induced by shade (Huang et al., Phenomics, 2022)

### Application Case: Shade Avoidance of Arabidopsis thaliana Green Seedling Hypocotyls

By quantitatively changing the ratio of red to far-red light, Arabidopsis can exhibit a shade avoidance response, resulting in changes in hypocotyl growth rate. Compared to traditional kinetic research methods (Cole et al., Plant Journal, 2011), DynaPlant® Seedlings not only obtains cumulative growth curves but also precisely measures growth rate changes as low as 0.1 μm/min, clearly distinguishing between the rapid and long-term response phases of the shade avoidance response.




The apical hook of Arabidopsis thaliana opens after exposure to light



Zhang et al., Plant Cell, 2018, 30(9): 1971-1988

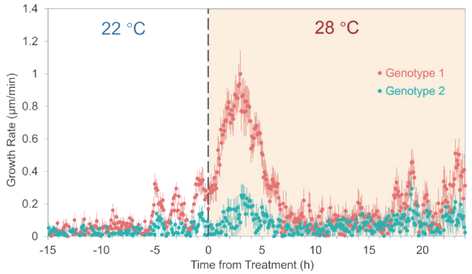
## Temperature and Humidity

Temperature and humidity directly affect physiological processes of plants such as photosynthesis, respiration, and transpiration, thus influencing plant growth and development. DynaPlant® Seedlings provides a temperature and humidity control system that can control the temperature and humidity in the entire chamber, providing a suitable growth environment during long - term continuous detection and can change the settings regularly according to the program.

| 12 ~ 42°C | ±0.5°C | ±0.2°C | 50 ~ 85°C | ±7% |
|---|---|---|---|---|
| Temperature Range | Temperature Uniformity | Temperature Accuracy | Humidity Range | Humidity Accuracy |

### Application Case: Rapid Response of Arabidopsis thaliana Seedlings to High Temperature

Literature reports that high temperature (28°C) makes the growth rate of Arabidopsis thaliana green seedling hypocotyls faster, while low temperature (16°C) makes it slower. DynaPlant® Seedlings can accurately measure the growth rate of hypocotyls while quickly changing the culture environment temperature, distinguishing the rapid response process of seedlings to temperature changes.




Rapid response of the growth rate of Arabidopsis thaliana seedling hypocotyls to high temperature (Unpublished data)

## GAS

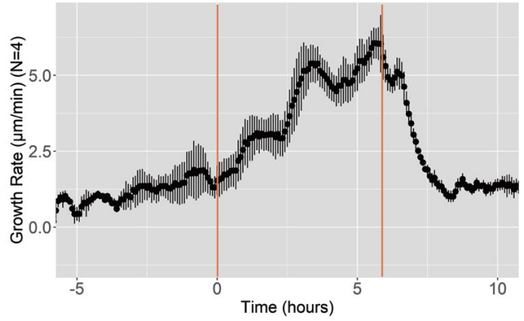
DynaPlant® Seedlings has a gas concentration control component. By real - time measuring the concentrations of oxygen, carbon dioxide, and ethylene and connecting to external corresponding gases, and using a gas flow controller to control gas on - off, it can achieve rapid treatment and removal of hypoxic environments, carbon dioxide, and ethylene. It can also customize the detection and concentration control of other gas components according to user experimental requirements.

| | Concentration Range | Detection Resolution | Target Concentration Control Precision |
|---|---|---|---|
| $O_2$ | 3~21% | 0.1% | ± 5% |
| $CO_2$ | 400~5000 ppm | 1 ppm | ± 5% |
| $C_2H_4$ | 0~20 ppm | 0.1 ppm | ± 5% |

### Application Case: Rapid Response of Rice Coleoptile Growth Rate to Ethylene Concentration

Ethylene can promote the growth of rice coleoptiles. By applying ethylene treatment and removing ethylene treatment, the system can accurately measure the rapid response of rice coleoptile growth rate to ethylene concentration.

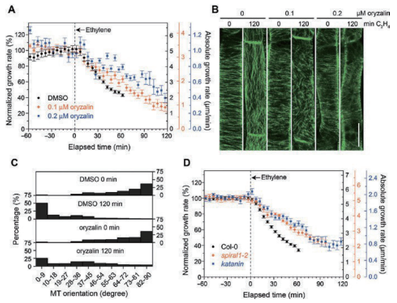
Rice seeds and coleoptiles




Unpublished data (Mean±S.E.)

### Application Case: The correlation between ethylene-induced inhibition of primary root growth and microtubule rearrangement in Arabidopsis.

Wang et al. reported the necessity of ethylene - induced microtubule rearrangement for its rapid inhibition of Arabidopsis thaliana root elongation. Traditional research methods can only photograph the root phenotypes after long - term ethylene treatment, reflecting the cumulative effect during the entire treatment period. In order to detect the rapid root response after ethylene treatment, the author used the DynaPlant® Seedlings analysis platform to measure the kinetics of root elongation after ethylene treatment. The system can measure the root growth rate as low as 0.1 μm/min, so it can distinguish slight changes in ethylene sensitivity between different genotypes or treatments.



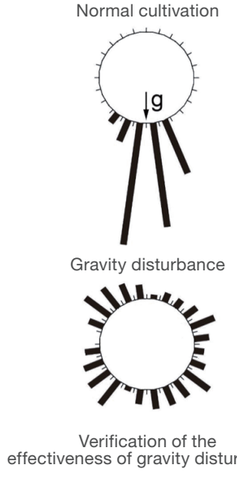

Wang et al., J Integr Plant Biol, 2018, 60(9): 864-877

## Gravity

DynaPlant® Seedlings is also equipped with a rotation control component, which can fix or continuously change the angle of samples in the vertical direction for gravity - related experiments.

Directional rotation mode: Rotate the culture dish to a fixed angle according to the program settings for gravitropism/negative gravitropism research. The angle control accuracy is better than 0.1°.

Gravity disturbance mode: Continuously rotate at a low speed to disrupt the direction of gravity, simulating a weightless condition.



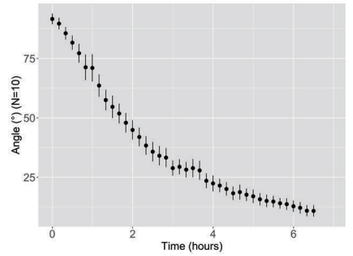

Verification of the effectiveness of gravity disturbance

Gravity component

### Application Case: Dynamic changes in the growth orientation of Arabidopsis root tips

Plants perceive gravity signals and produce tropic growth to maximize the utilization of light, soil moisture, and nutrients. By controlling the gravity module of DynaPlant® Seedlings to regularly and quantitatively change the orientation of plant seedlings, the kinetic process of plant gravitropism responses can be studied, revealing the details of rapid changes.

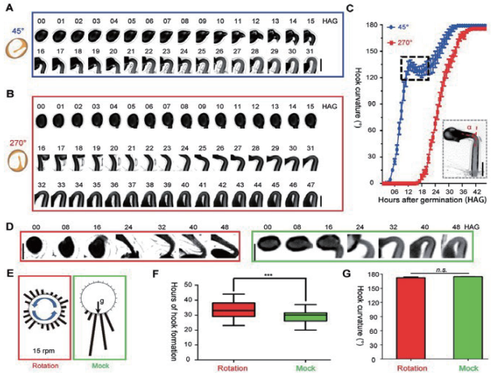
The main root of Arabidopsis




Changes in root tip orientation

### Application Case: Relationship between Arabidopsis Radicle Gravitropic Bending and Apical Hook Formation

Peng et al. proposed a new model for the early initiation stage of the apical hook: during hypocotyl elongation after seed germination, a subtle but discernible growth asymmetry randomly emerges, which is subsequently amplified by the establishment of an asymmetric auxin response, leading to the formation of the apical hook structure. Using the DynaPlant® Seedlings analysis platform, the authors conducted detailed tracking and analysis of the initiation and formation stages of the apical hook in Arabidopsis seedlings. They also applied gravity disturbance treatments using the gravity module, demonstrating that root gravitropic bending can promote but is not essential for apical hook formation.

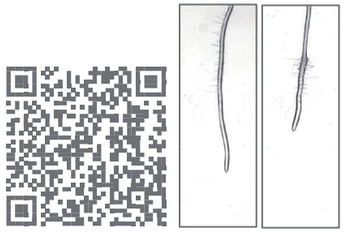



Peng et al.,J Integr Plant Biol,2022,64(1):5-22

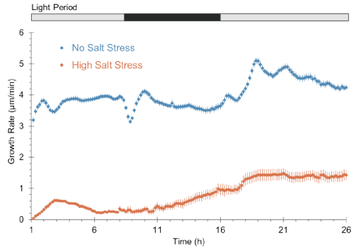
By combining the multiple imaging methods and processing conditions provided by DynaPlant® Seedlings, users can design new experiments according to their research directions and conduct research in various fields such as abiotic stress (resistance to high temperature, low temperature, salt, hypoxia, etc.), plant developmental biology, and chemiluminescence-labeled detection.

## The response and recovery process of Arabidopsis primary roots to high-concentration NaCl stress

High salt stress severely inhibits root growth in plant seedlings, leading to the formation of swollen nodes and affecting root hair development. Using DynaPlant® Seedlings, the precise measurement of root tip growth rates clearly reveals the dynamic process of growth inhibition and adaptive recovery under high-concentration NaCl treatment. This provides a novel approach for studying the molecular mechanisms of salt stress signal transduction.



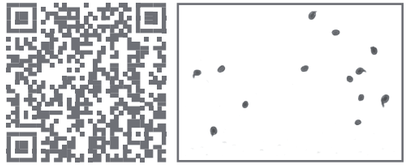
No stress High salt stress



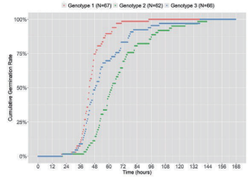

Main Root Growth Rate Curve

## Seed Germination Rate

DynaPlant® Seedlings can automatically analyze seed morphology, judge the germination time of seeds in continuous imaging, and draw cumulative germination rate curves or germination rate histograms at a certain moment.



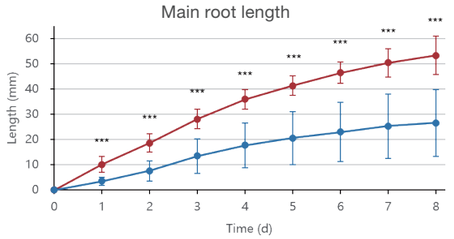
Germination of Arabidopsis seeds

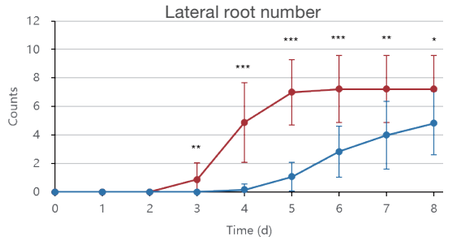


Cumulative Germination Rate Curve
(Unpublished Data)

## Analysis of Differences between Chinese Cabbage Varieties

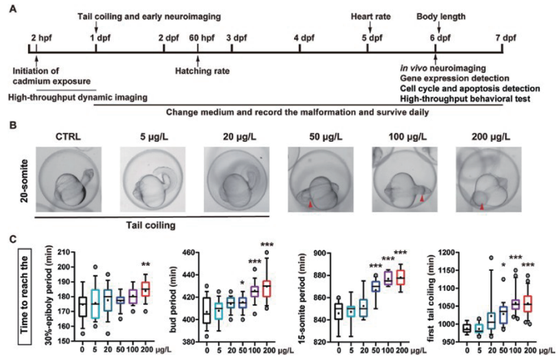
By dynamically detecting the growth and development of the main roots and lateral roots of different Chinese cabbage varieties, the differences between Chinese cabbage varieties can be analyzed.




## Zebrafish Embryo Development Research

Relying on its highly flexible and modular imaging function, DynaPlant® Seedlings can also be flexibly applied to other fields, such as zebrafish embryo development research. Xu et al. utilized the advantages of zebrafish in high - throughput imaging and behavioral testing to explore the potential developmental neurotoxicity of cadmium at environmentally relevant levels from the perspectives of neurobehavior and neuroimaging.

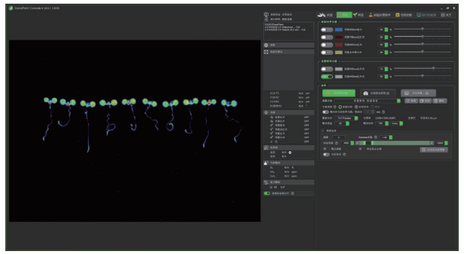



Xu et al. , Chemosphere, 2022, 291: 132802.

The author used the DynaPlant® Seedlings imaging platform to study the damage of environmental cadmium to the early development of zebrafish.

## Professional and User-Friendly Software System

DynaPlant® Seedlings comes with a graphical shooting control console software that adjusts all setting parameters. Before shooting, users can quickly set up experimental steps via the computer, enabling real-time autofocus, tracking plant growth, and adjusting the camera's field of view during shooting, ensuring precision during long-term high-resolution imaging.



The DynaPlant® Seedlings analysis software allows users to view experimental settings and run logs, process acquired images, perform automated batch analysis on large numbers of images, and generate calculation results and charts.

## Software Control

- Full - graphical operation interface
- Support for autofocus
- Support for automatic tracking of seedling growth
- Control the on - off and brightness of each light source channel in the software
- Control the concentration of each gas channel in the software
- Control the temperature and humidity of the culture environment in the software
- Set all experimental treatment conditions and treatment times before shooting for unattended automatic shooting tasks
- Multi - level log recording and exception handling mechanism to record all environmental variable parameters during shooting
- View and control the shooting operation status from remote computers and mobile devices
- Provide a background mode to adjust all background parameters

## Seedling Automation Analysis

- Root growth rate
- Root bending angle change rate
- Root hair position distribution
- Root hair length change rate
- Seed germination rate
- Hypocotyl growth rate of seedlings
- Cotyledon angle change rate of seedlings

## Other Analysis Functions

- Batch automatic analysis
- Multithreaded tasks
- Provide a visual graphical interface to display image analysis results and statistical curves
- Export raw data and calculation results into EXCEL spreadsheet files
- Export statistical charts into JPG image files

## Chemiluminescence Imaging Analysis

- Quantitatively detect the chemiluminescence intensity of Luciferase
- Analyze the dynamic changes of reporter gene expression
- Measure length, area, profile brightness curves, with
- ROI and masking functions, and can generate false - color images

## Publications (Partial)


- Zhang X, Ji Y, Xue C, et al. Integrated regulation of apical hook development by transcriptional coupling of
- EIN3/EIL1 and PIFs in Arabidopsis. Plant Cell, 2018, 30(9): 1971-1988.
- Wang Y, Ji Y, Fu Y, et al. Ethylene-induced microtubule reorientation is essential for fast inhibition of root elongation in Arabidopsis. J Integr Plant Biol, 2018, 60(9): 864-877.
- Jiang Y, Yang C, Huang S, et al. The ELF3-PIF7 interaction mediates the circadian gating of the shade response in Arabi- dopsis. iScience, 2019, 22: 288-298.
- Zhao N, Zhao M, Tian Y, et al. Interaction between BZR1 and EIN3 mediates signalling crosstalk between brassinos- teroids and ethylene. New Phytol, 2021, 232(6): 2308-2323.
- Xu Y, Zhao H, Wang Z, et al. Developmental exposure to environmental levels of cadmium induces neurotoxicity and activates microglia in zebrafish larvae: From the perspectives of neurobehavior and neuroimaging. Chemosphere, 2021, 291: 132802.
- Huang S, Yang C, Li L. Unraveling the dynamic integration of auxin, brassinosteroid and gibberellin in early shade-in- duced hypocotyl elongation. Phenomics, 2022, 2(2):119-129.
- Peng Y, Zhang D, Qiu Y, et al. Growth asymmetry precedes differential auxin response during apical hook initiation in Arabidopsis. J Integr Plant Biol, 2022, 64: 5-22.
- Aizezi Y, Shu H, Zhang L, et al. Cytokinin regulates apical hook development via the coordinated actions of
- EIN3/EIL1 and PIF transcription factors in Arabidopsis. J Exp Bot, 2022, 73(1): 213-227.
- Yang C, Zhu T, Zhou N, et al. PIF7-mediated epigenetic reprogramming promotes the transcriptional response to shade in Arabidopsis. EMBO J, 2023, 42(8): e111472.
- Shi L, Lin K, Su T, Shi F. Abscisic acid inhibits cortical microtubules reorganization and enhances ultraviolet-B tolerance in Arabidopsis thaliana. Genes, 2023, 14(4):892.