



## **Fast and non-invasive phenotyping of plant health/stress status using a LED induced chlorophyll fluorescence transient imager**

Henk Jalink, Wageningen UR Greenhouse Horticulture, The Netherlands

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**Forschungszentrum Jülich, Germany**  
**ICG-3: Phytosphere**  
**Jülich Plant Phenotyping Centre (JPPC)**  
**Website: <http://www.jppc.de>**

**<http://www.plantphenomics.com/phenotyping2009>**

# Fast and non-invasive phenotyping of plant health/stress status using a LED induced chlorophyll fluorescence transient imager

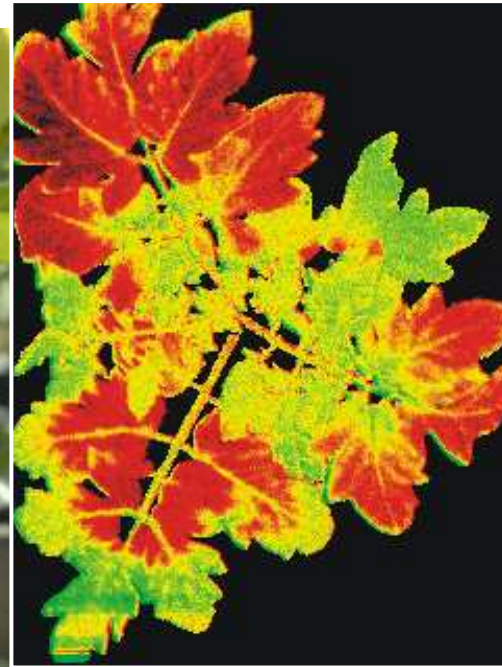
Henk Jalink

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What is needed:

- Technologies to characterize plant performance and dynamics
- High throughput techniques to measure plant at desired scenarios



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What is needed:

- Technologies to characterize plant performance and dynamics
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Objective:

- Develop an imaging sensor that monitors the health and dynamic response of whole plants

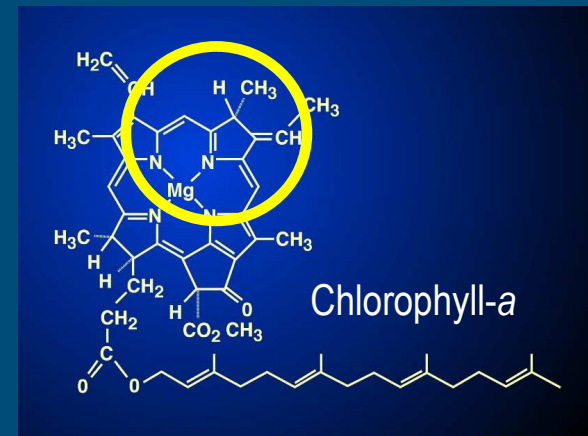
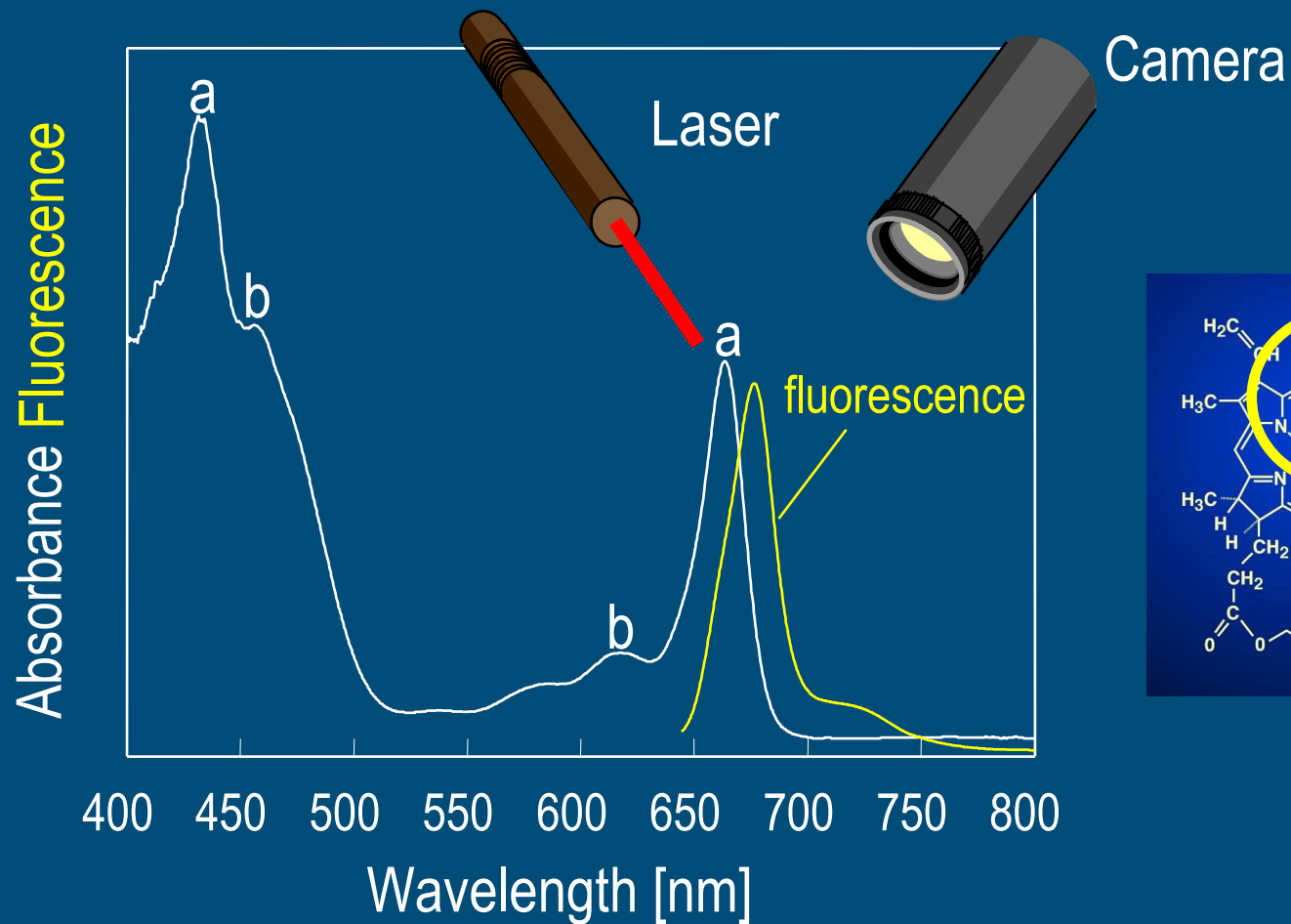
## Advantage of chlorophyll fluorescence:

- only plant tissue is visible; no background

LED imager



# Spectral properties of chlorophyll



# Plant recognition using color



Easy for computer to recognize the plant



Difficult for computer to recognize the plant

# Color and fluorescence image of a plant



Difficult for computer to recognize the plant



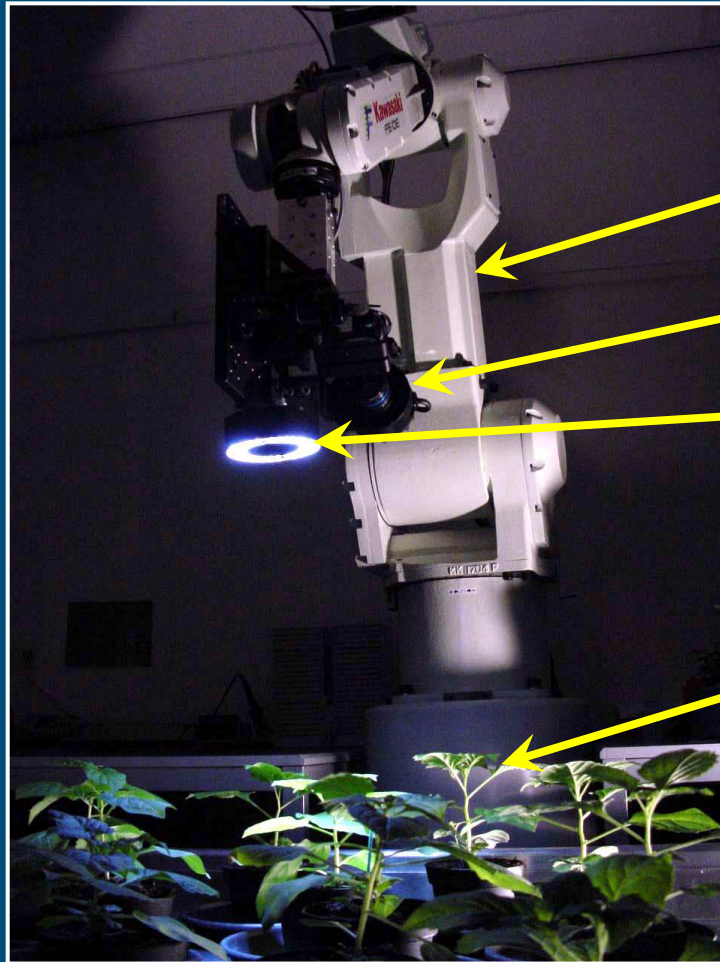
Easy for computer to recognize the plant on fluorescence

# Color and fluorescence image of a plant



Integration  
time of 0.1 s

# MIPS™ robot arm with colour and CF camera



Robot arm for scanning plants

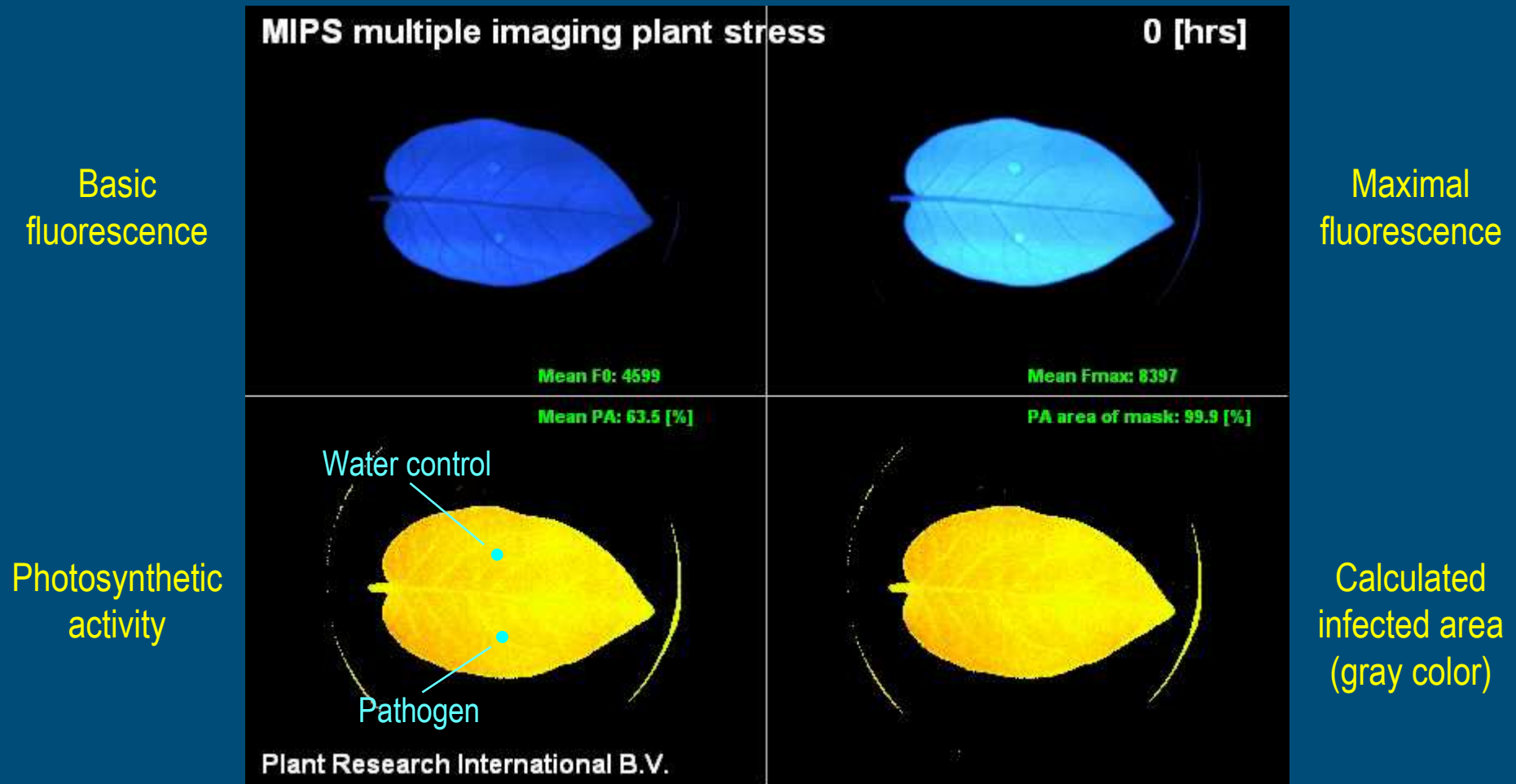
MIPS™ camera with laser for CF image

Light source for colour image

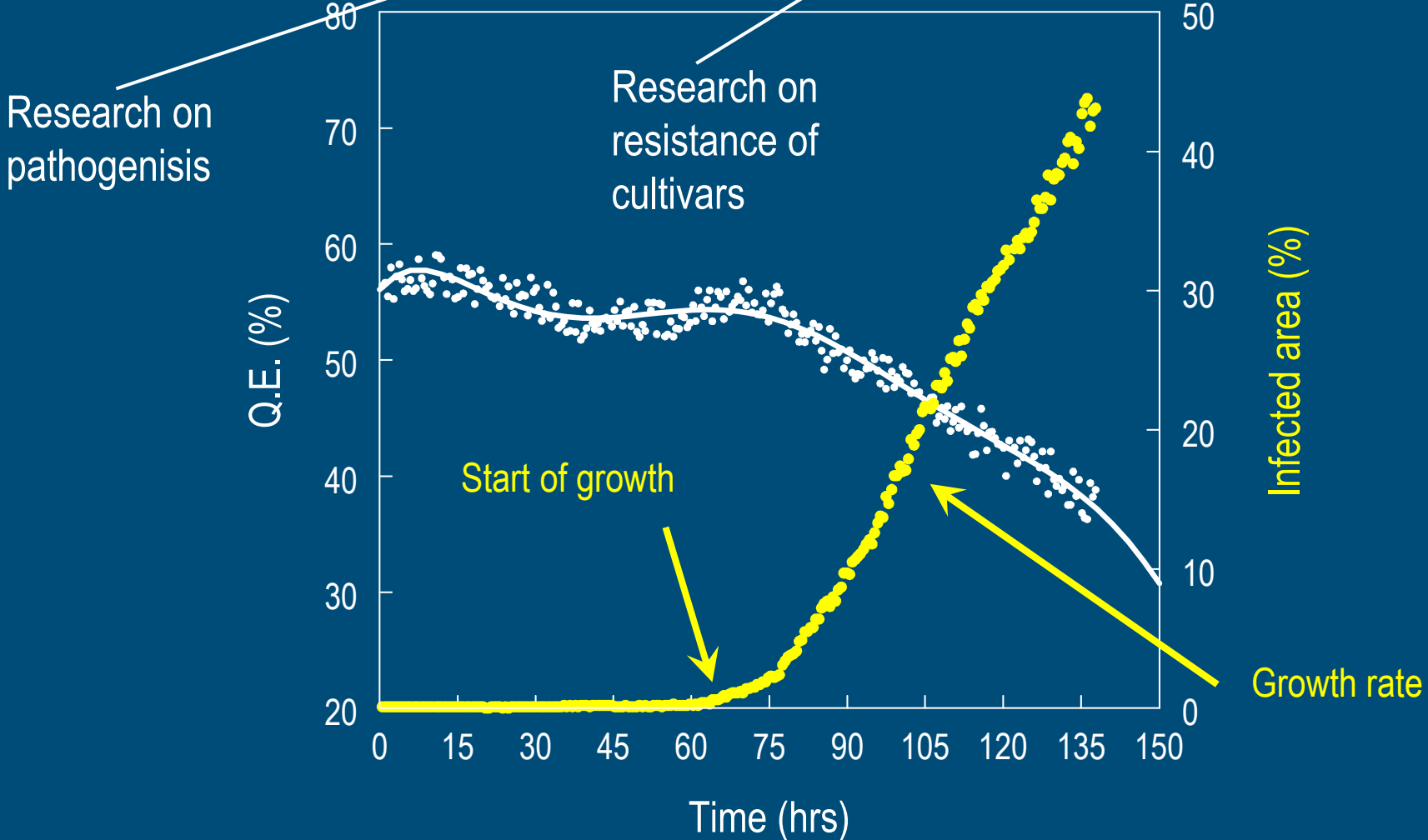
Table with plants



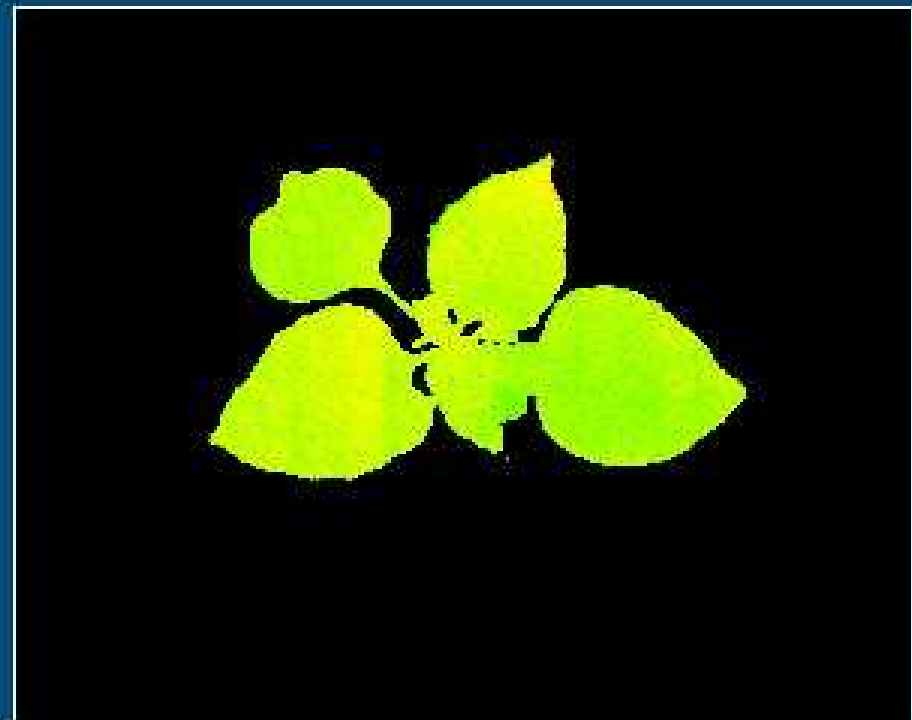
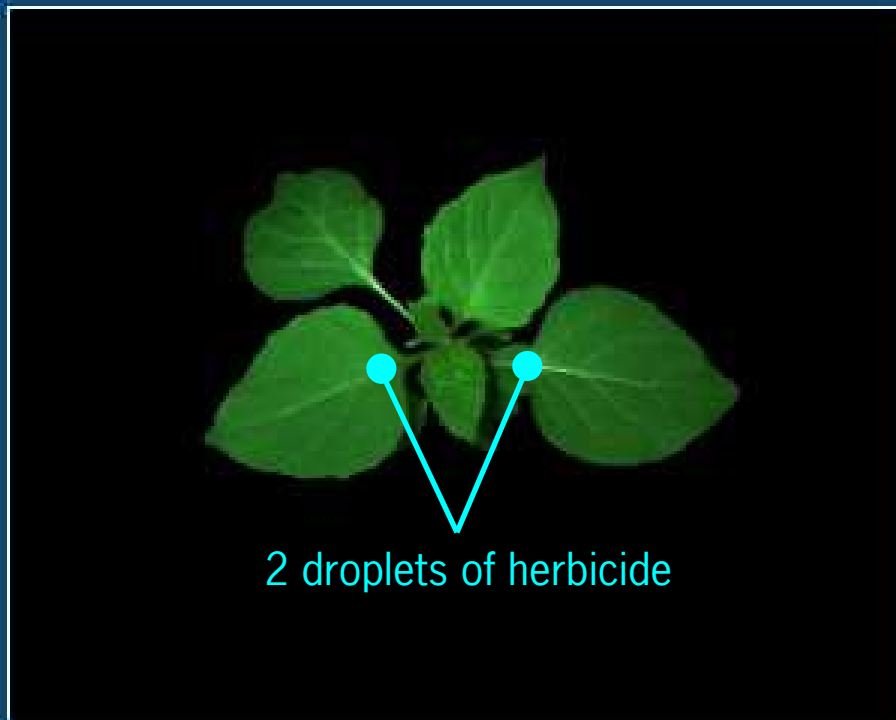
# Outgrowth of *Phytophthora* on potato leaf in petri dish



# Outgrowth of *Phytophthora* on potato leaf



# Herbicide formulation testing on PA



0 hrs

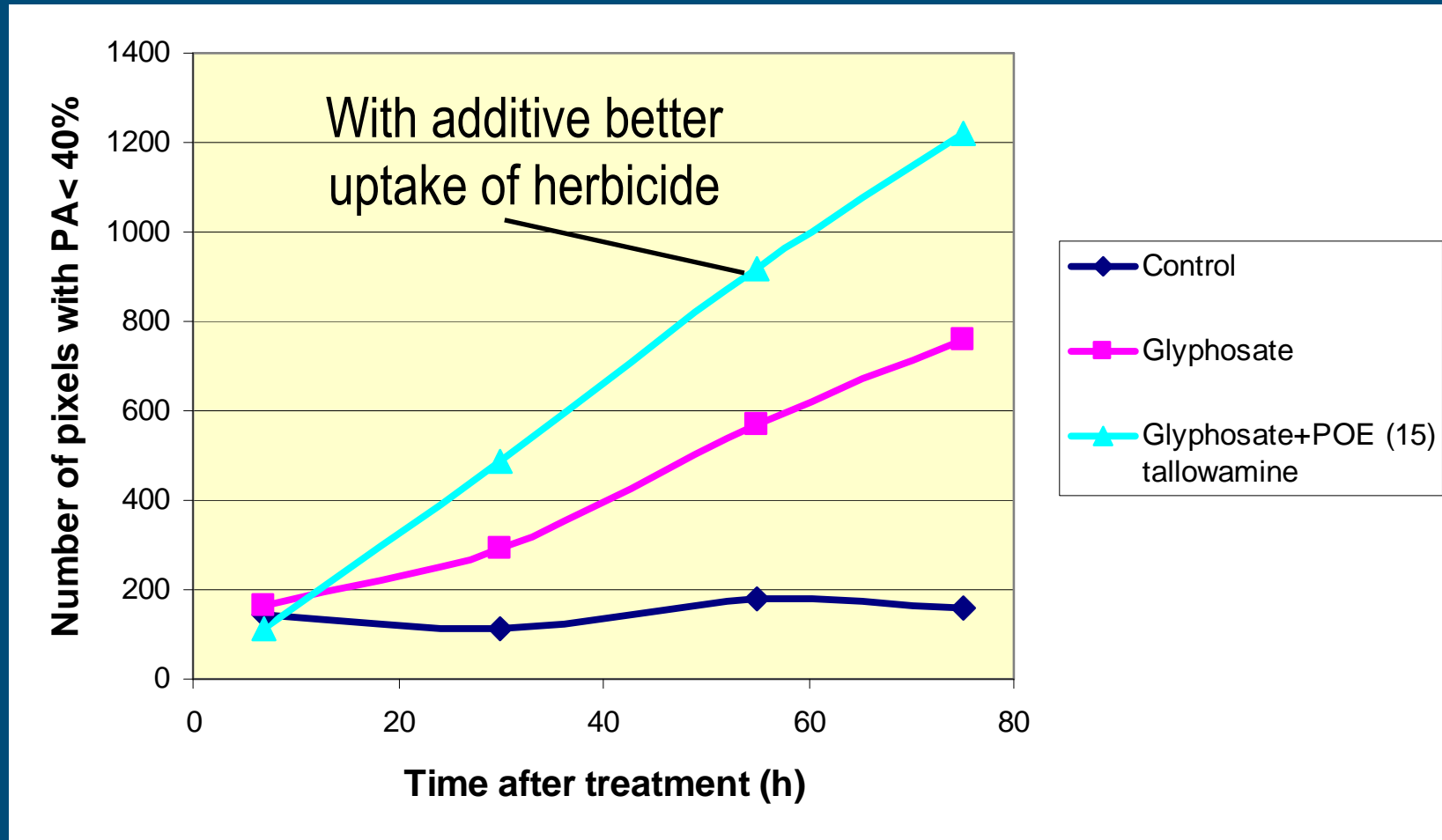


0 hrs



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# Counting pixels with PA < 40%



## Some conclusions

- MIPS system is too slow for high throughput and screening: about 20 sec
- MIPS can not measure at elevated light levels
- Needed: a fast methodology that is able to saturate the photosynthesis within a sec

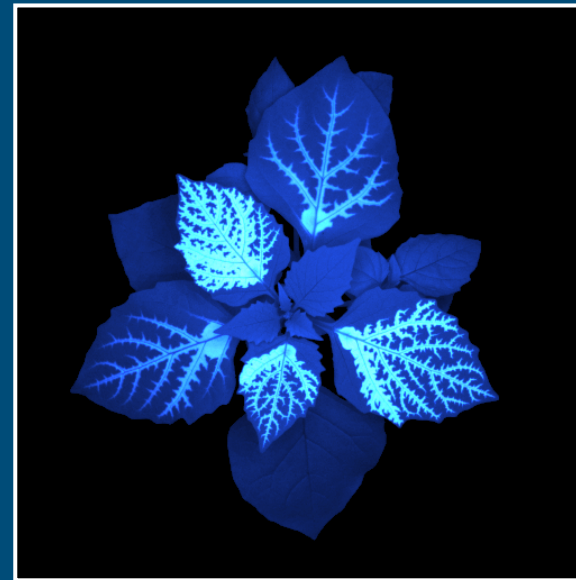
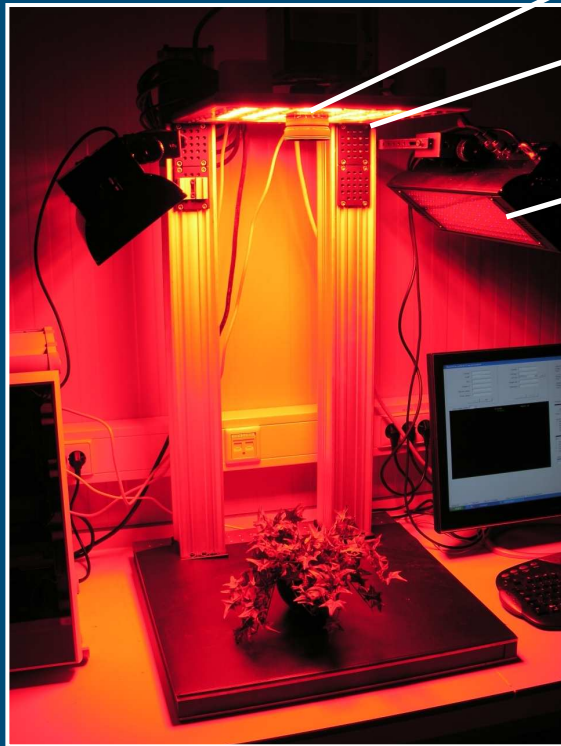
# LED Induced Fluorescence Transient Imager for Measuring Stress

CCD-camera – 14 bit

LED's 5000 Watt

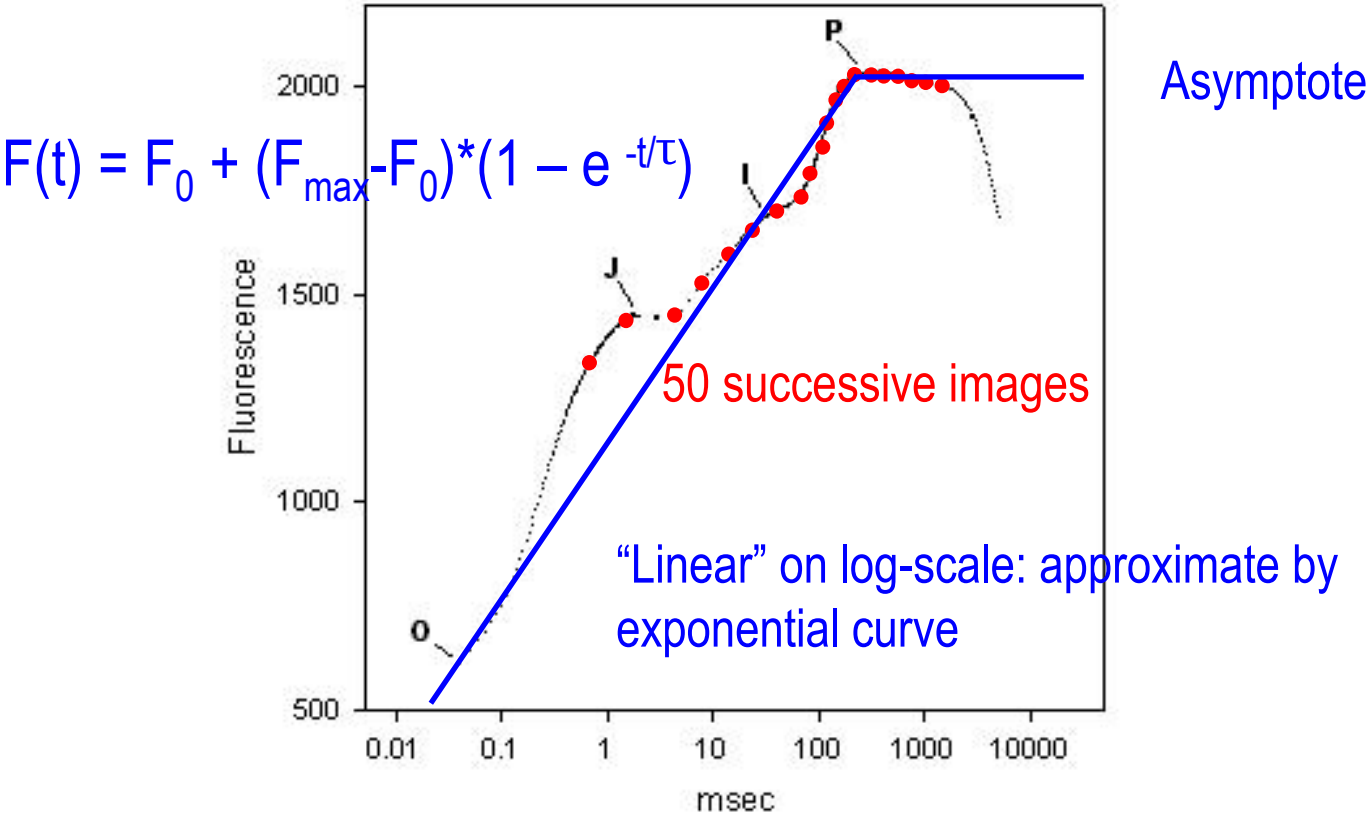
1000  $\mu\text{mol}/\text{m}^2\text{s}$  at 40x40  $\text{cm}^2$

Background light



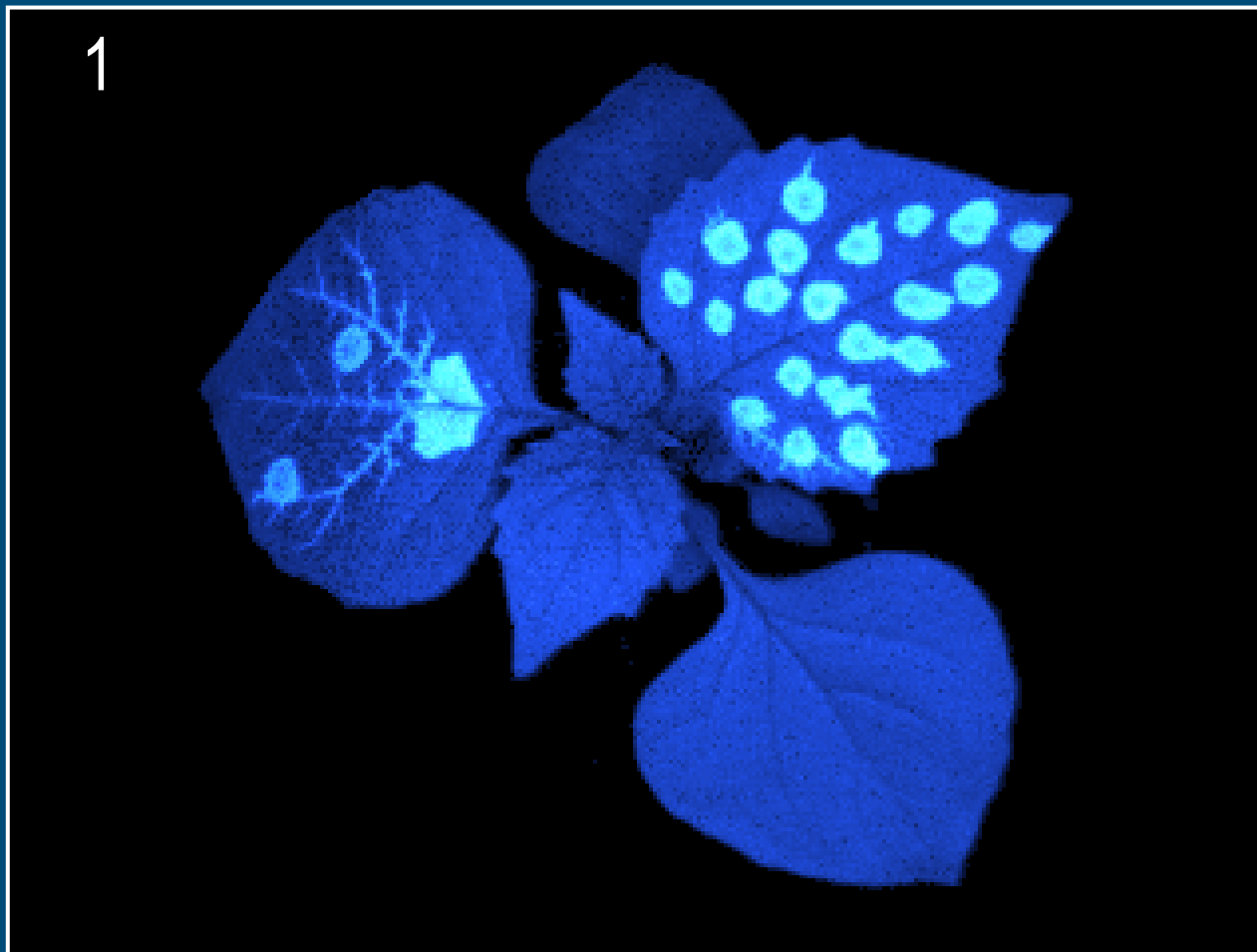
# Methodology of the LED Induced Fluorescence Transient Imager

## CF induction (Kautsky) curve of a corn leaf



Sinsawat, 1999 PhD thesis ETH

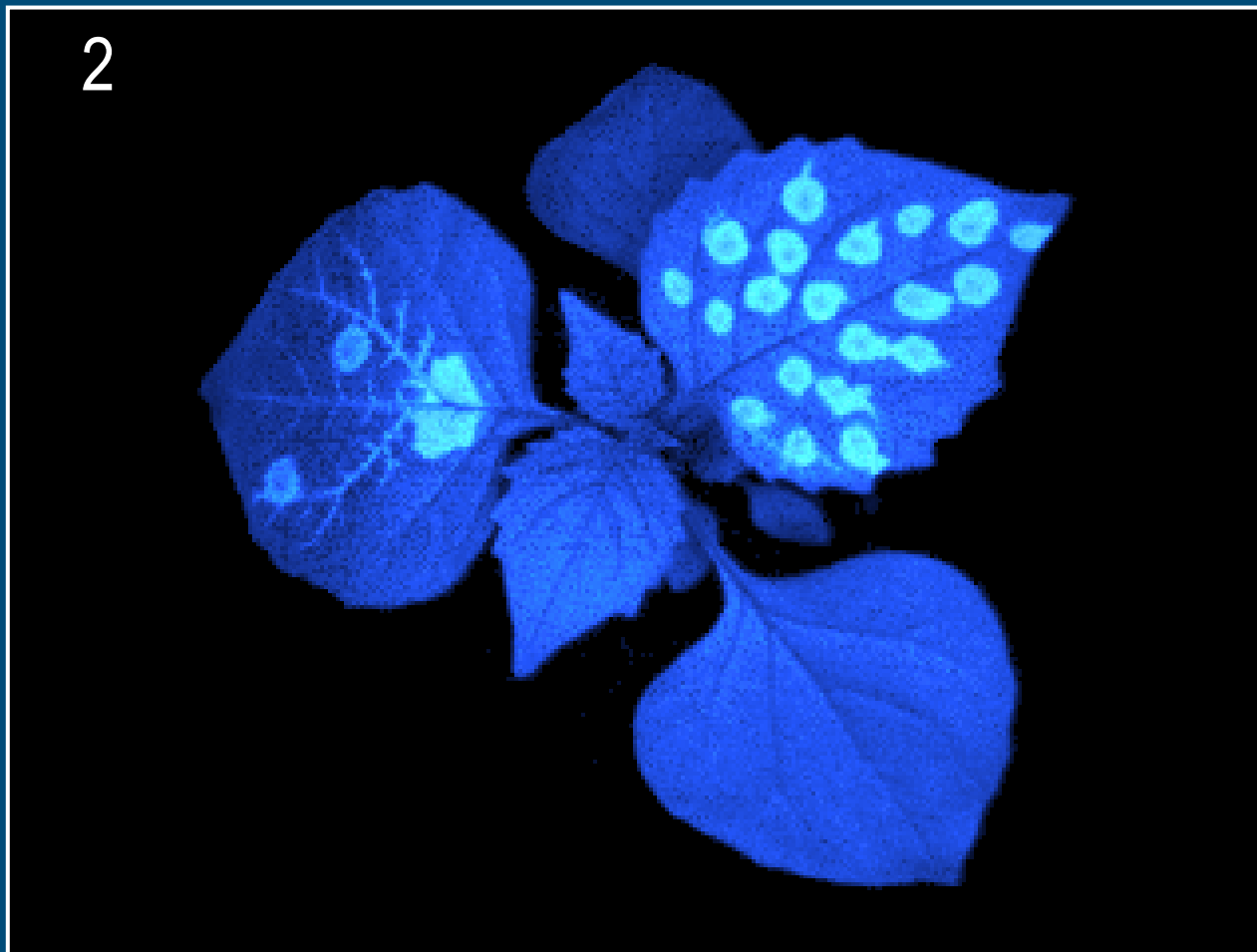
# Led pulse of 15 ms; good contrast



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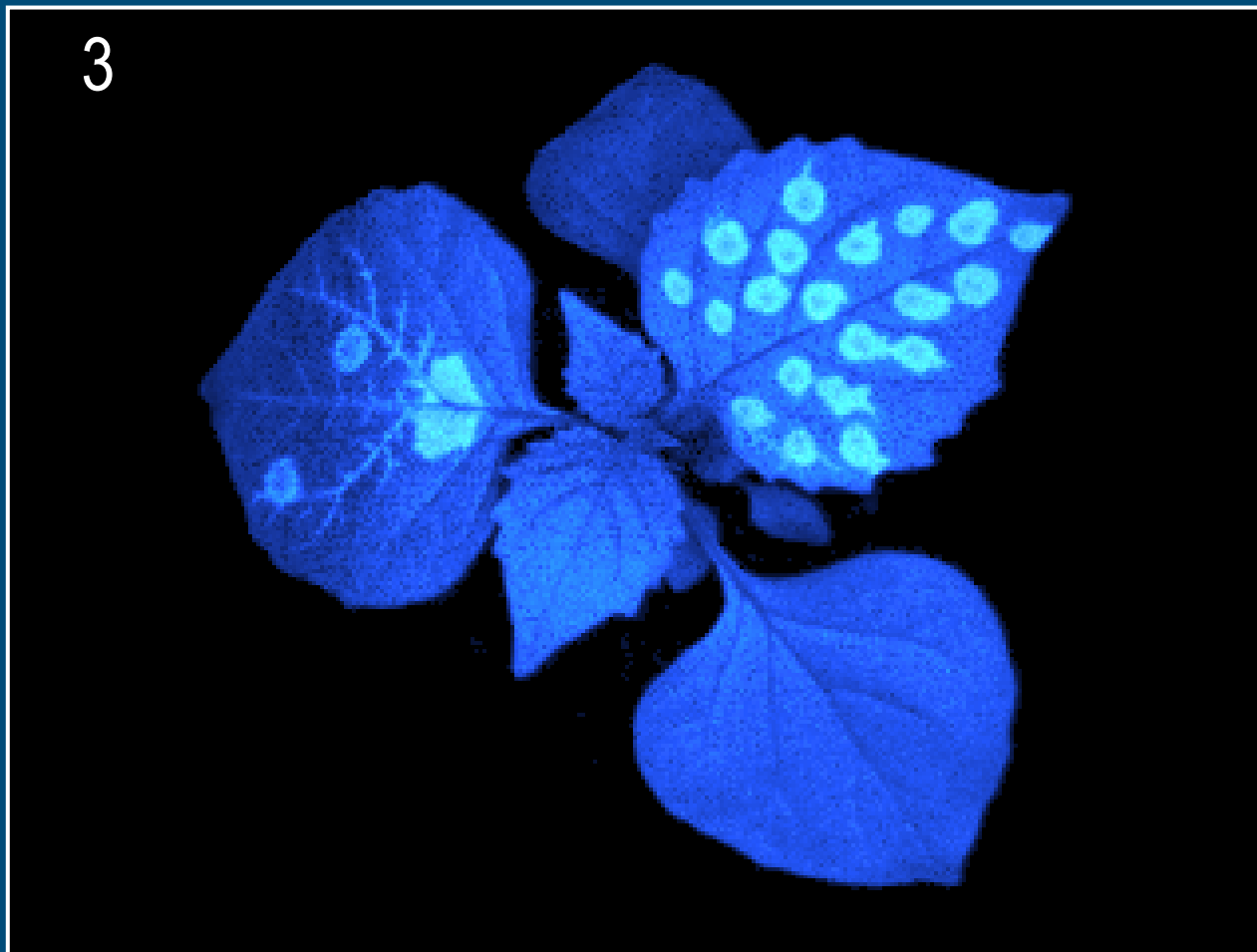
# Led pulse of 15 ms



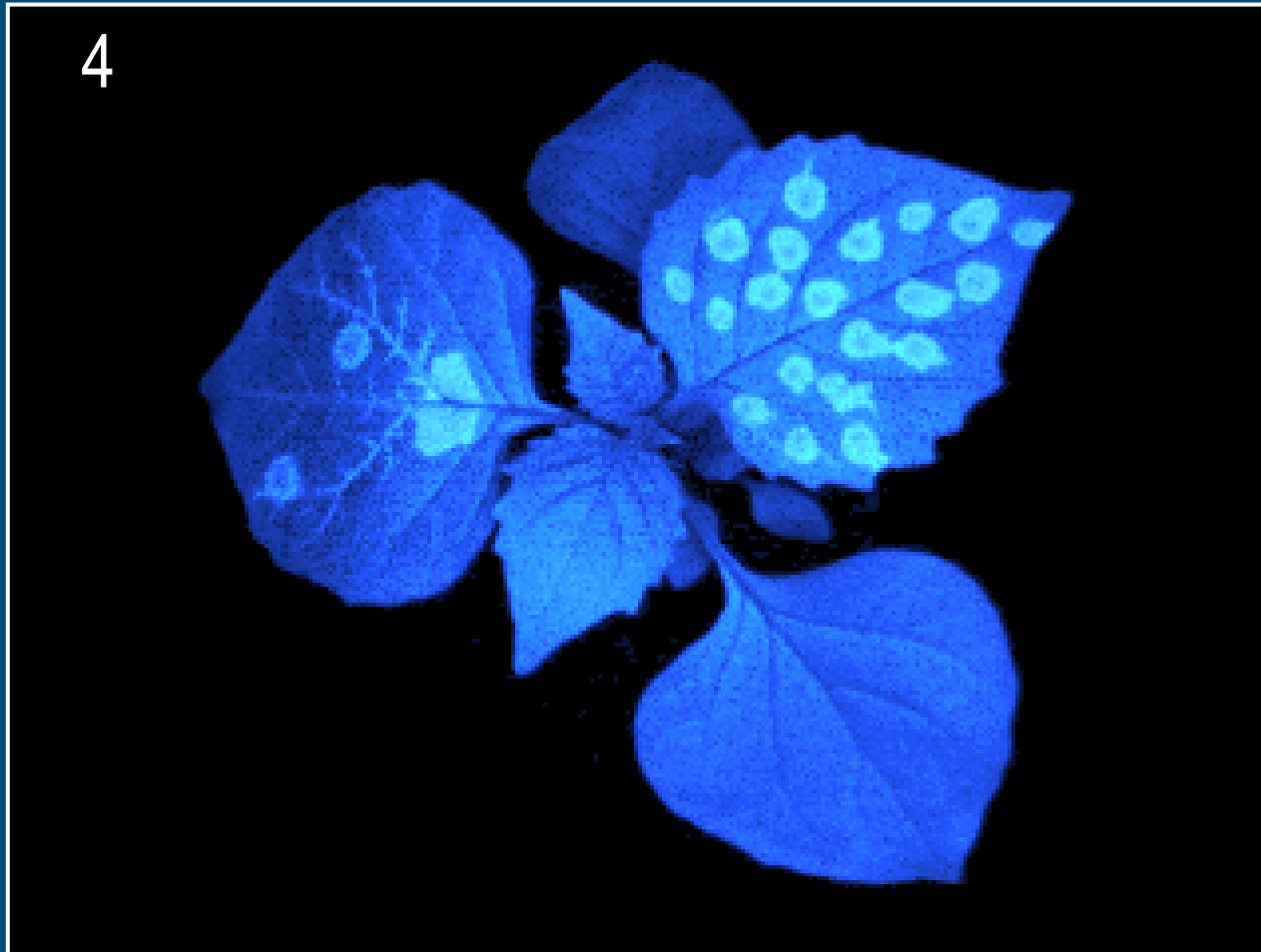
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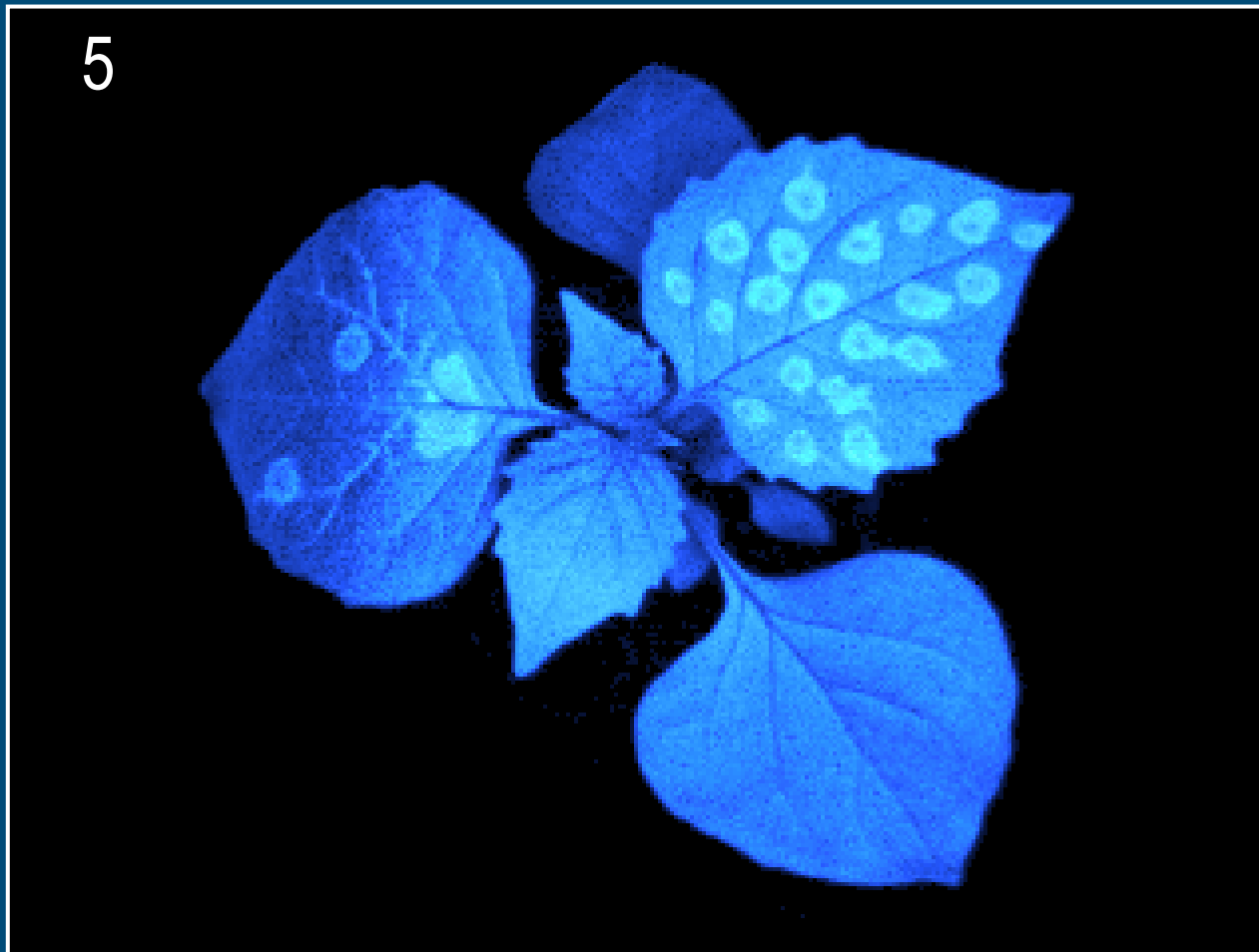
# Led pulse of 15 ms



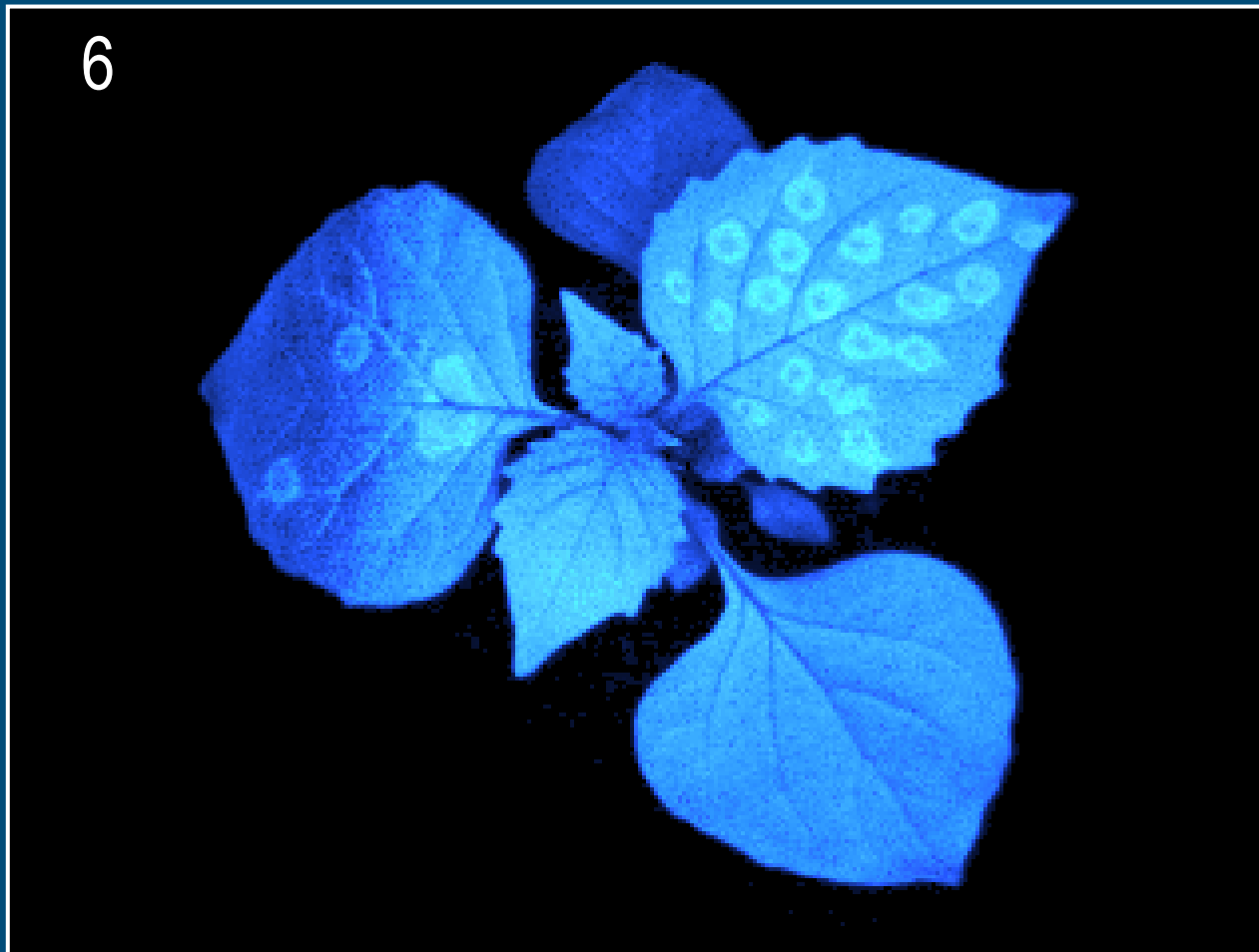
# Led pulse of 15 ms



# Led pulse of 15 ms



# Led pulse of 15 ms



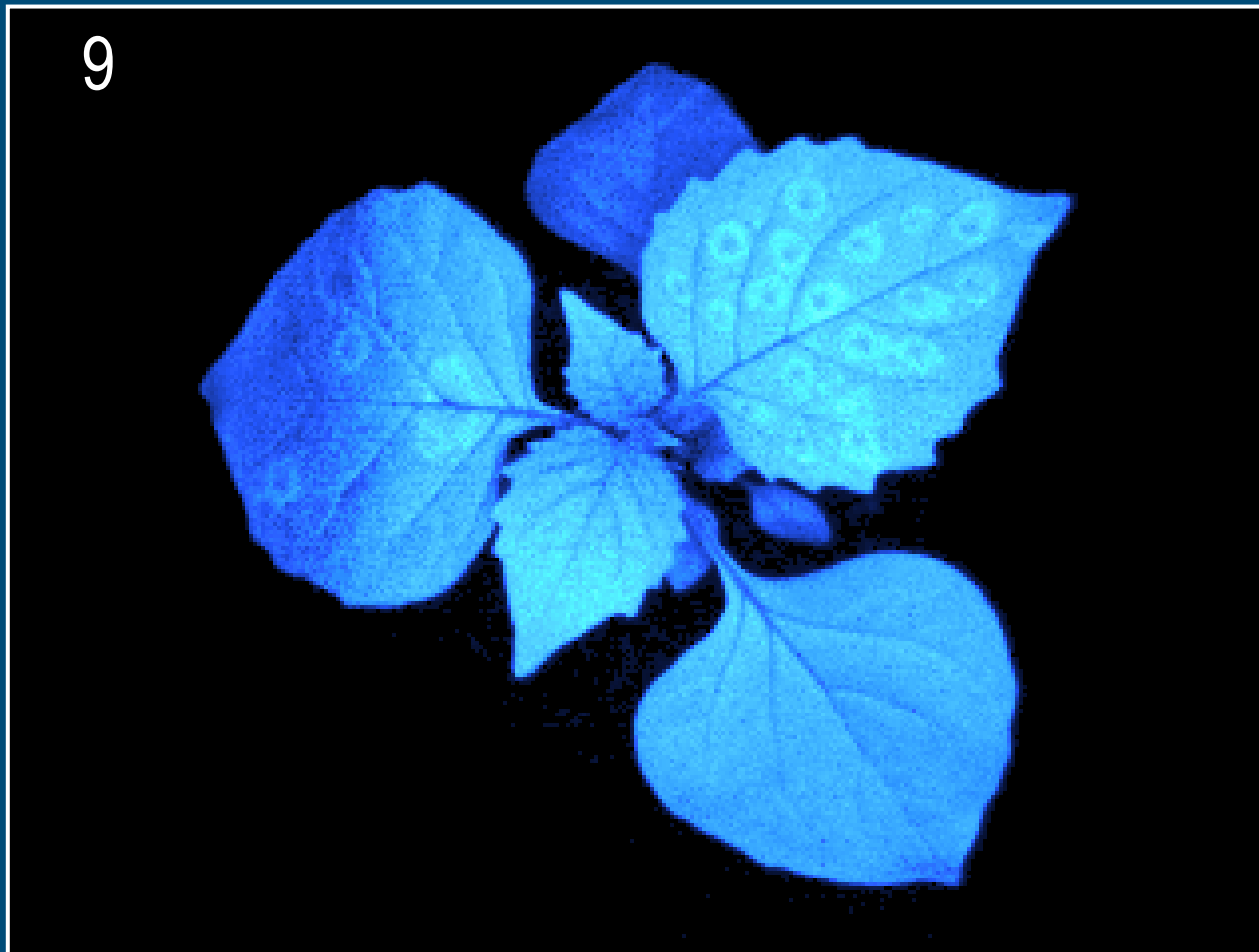
# Led pulse of 15 ms



# Led pulse of 15 ms



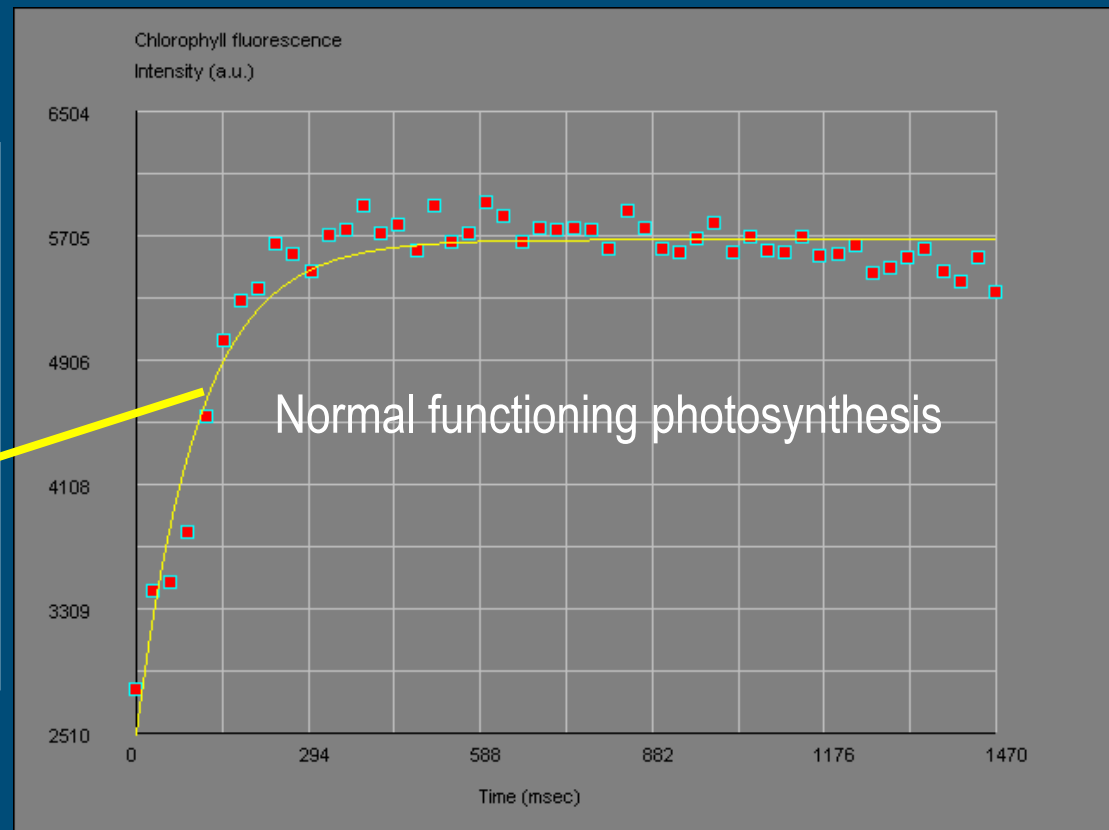
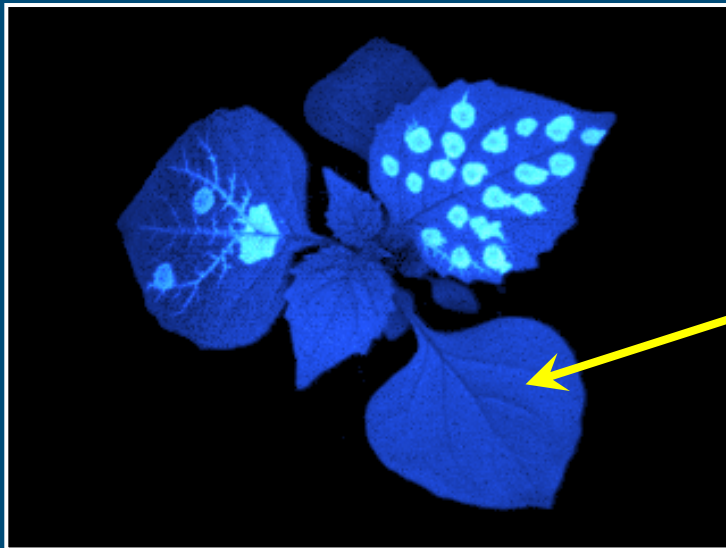
# Led pulse of 15 ms



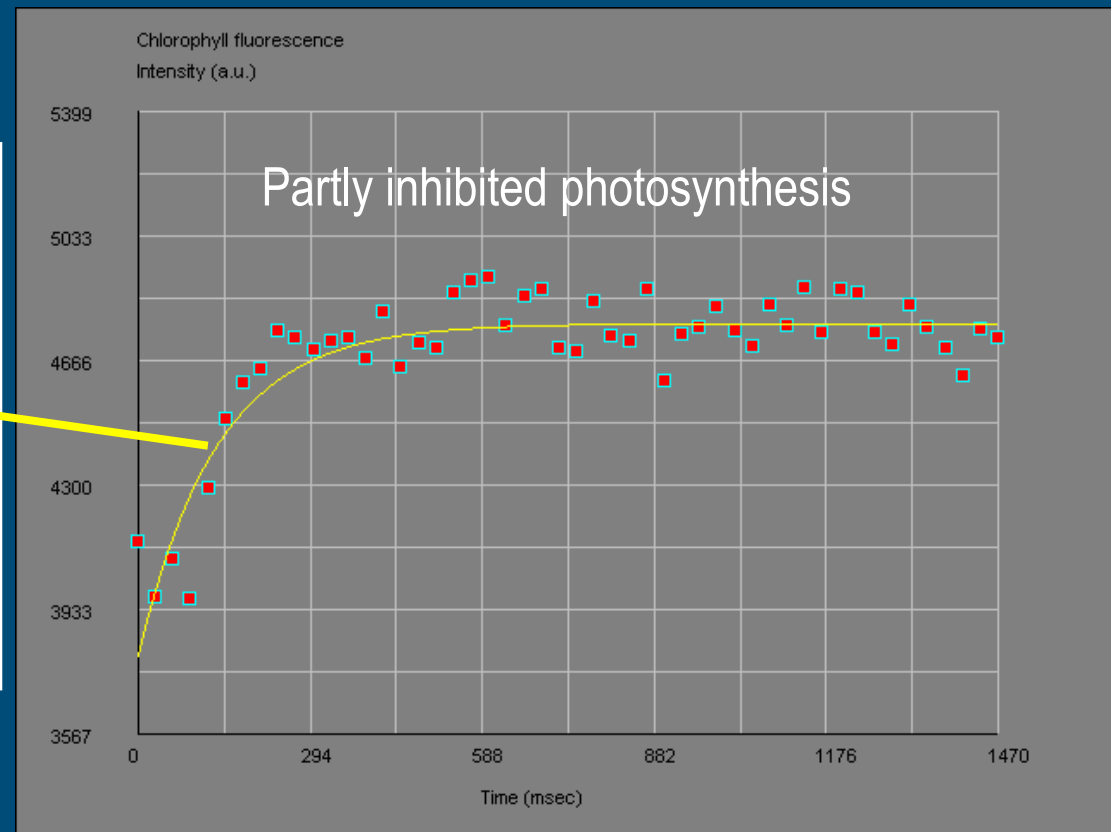
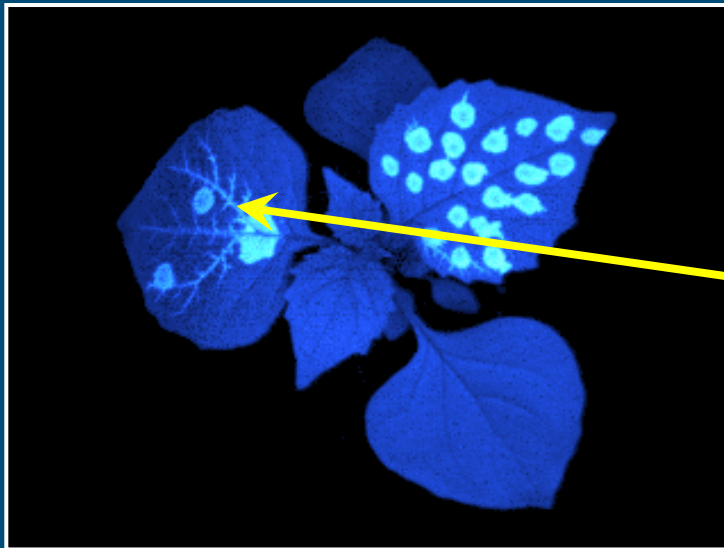
# Hardly any contrast due to saturation of photosynthesis



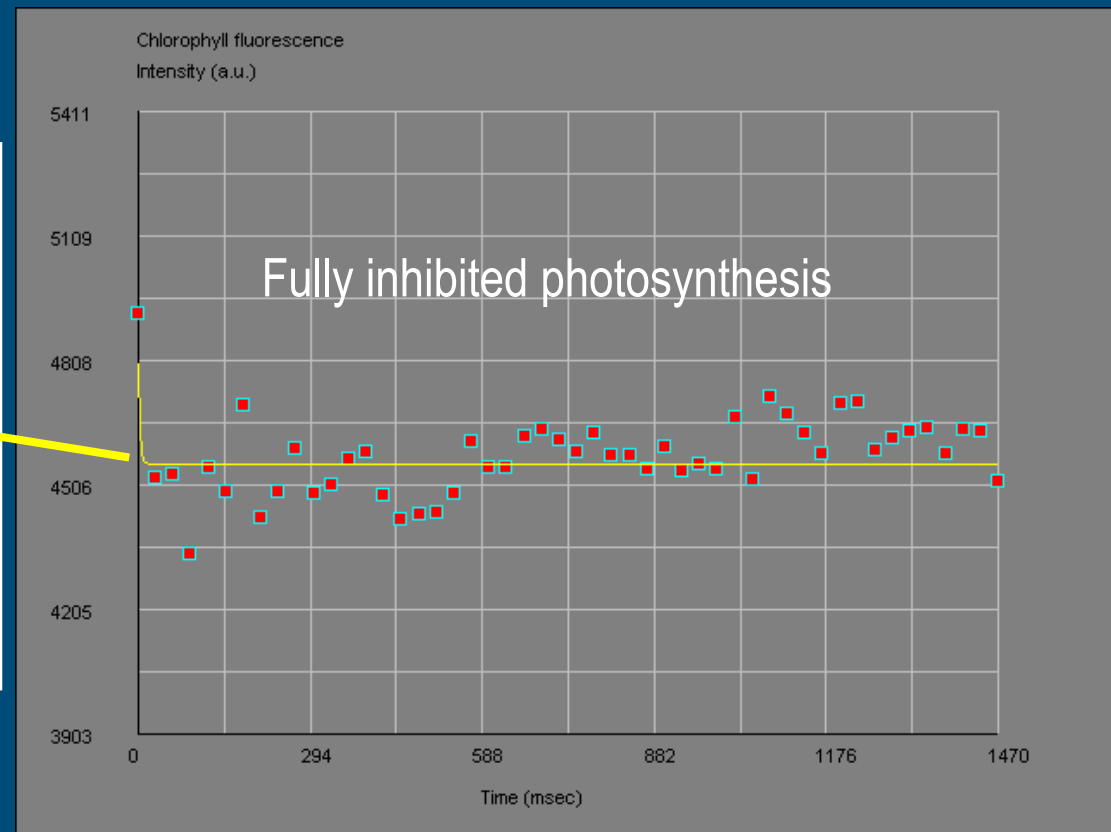
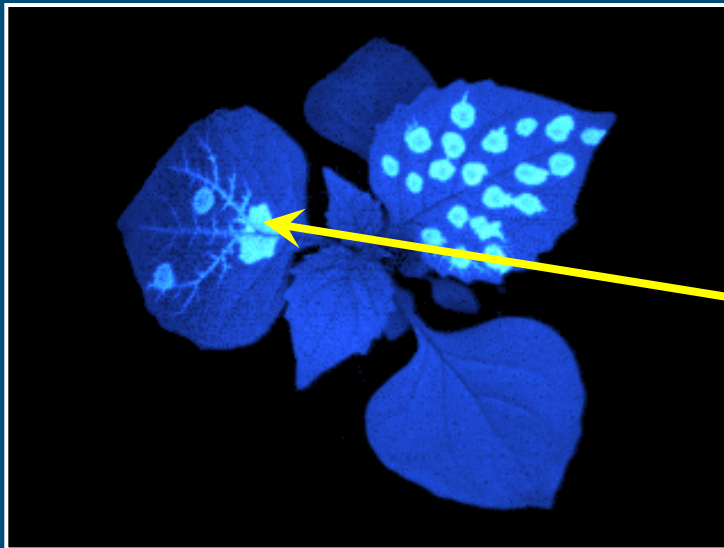
# Time response of healthy tissue



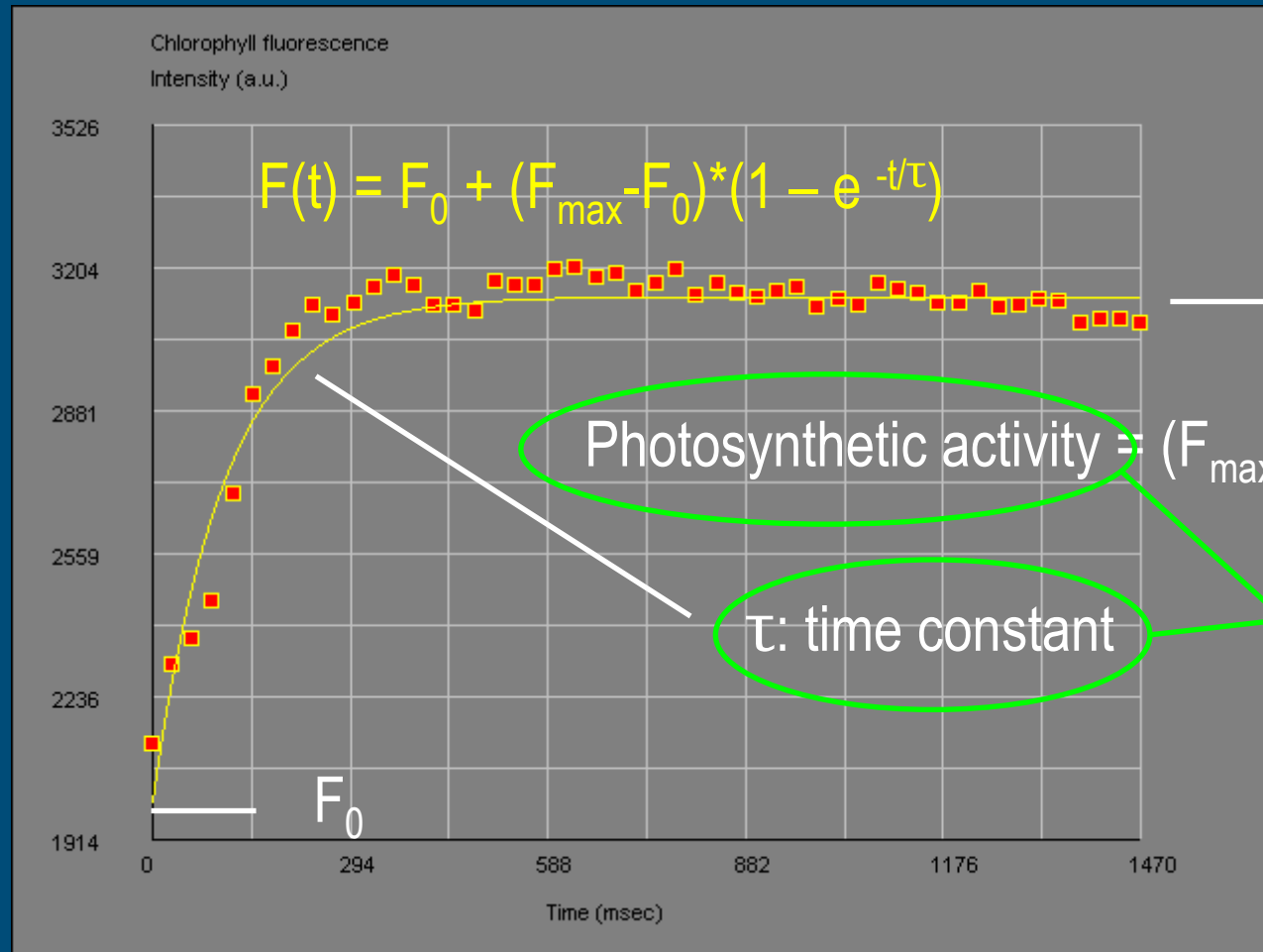
# Time response of partly inhibited photosynthesis



# Time response of fully inhibited photosynthesis



# Creating images of PA and $\tau$ by fitting each pixel

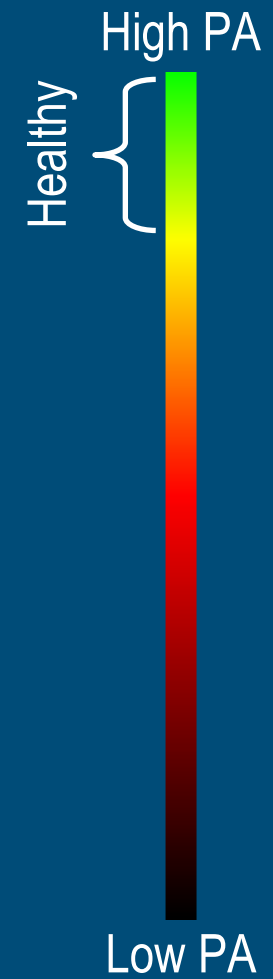
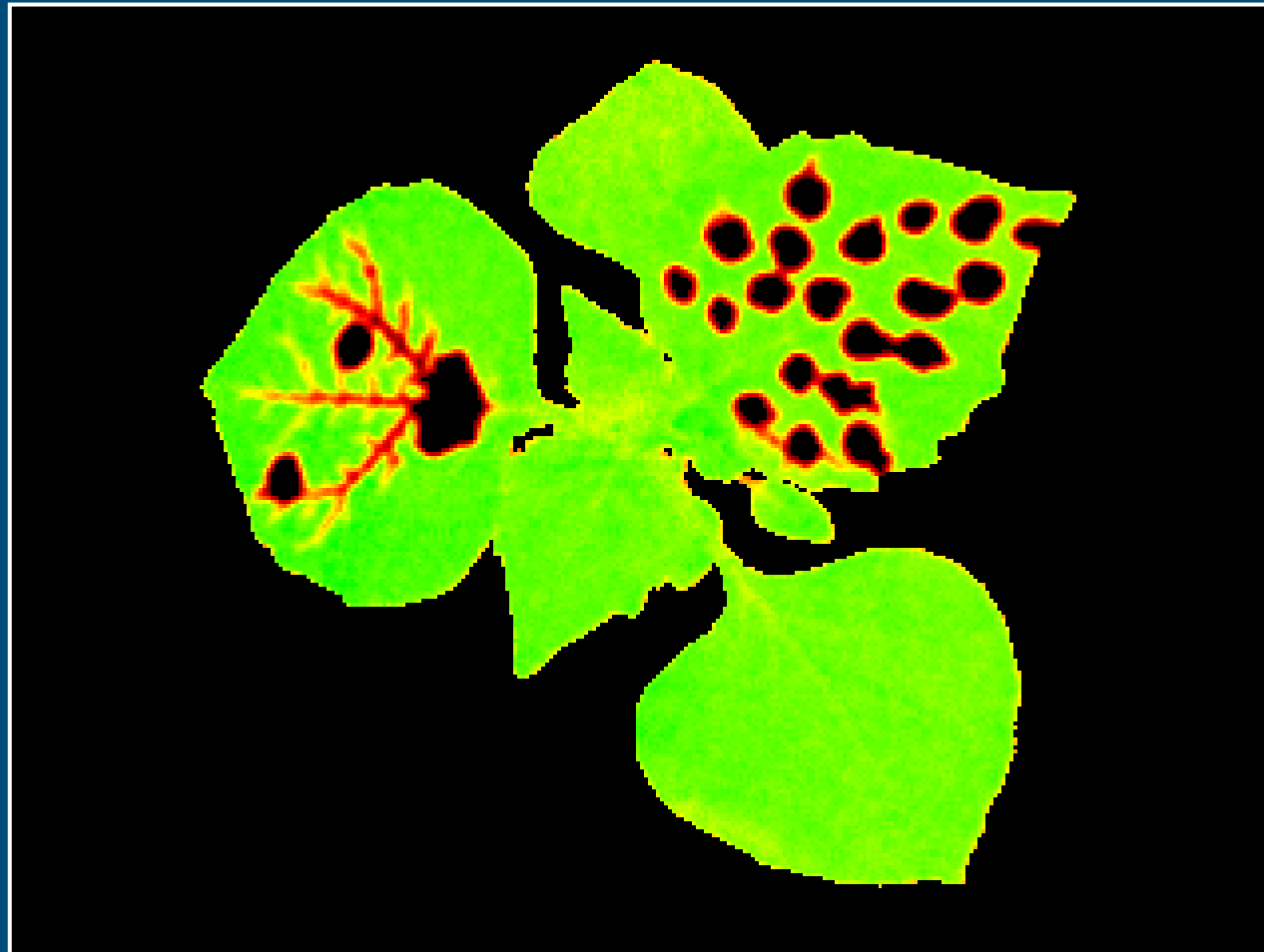


$F_{\max}$

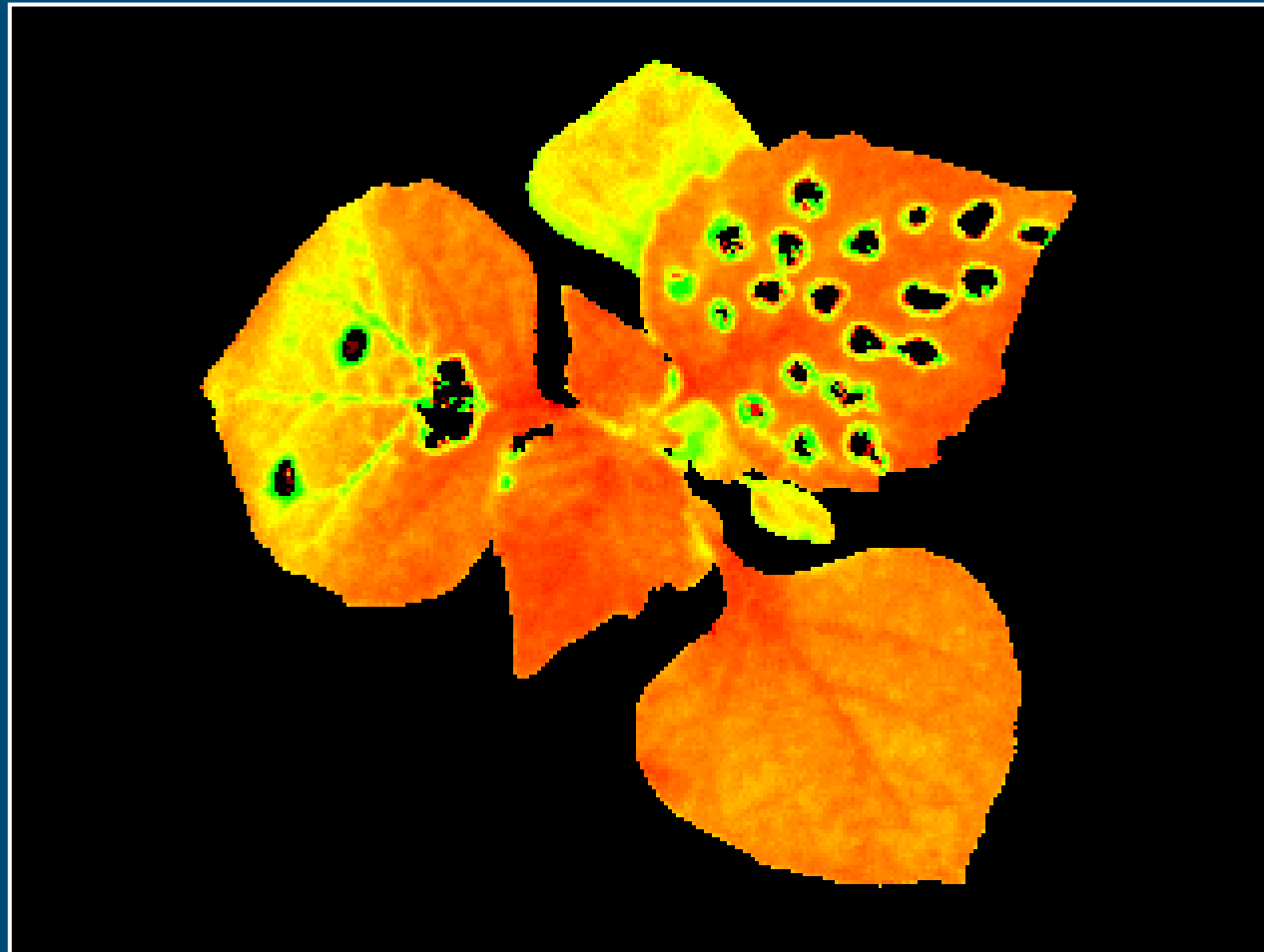
$(F_{\max} - F_0) / F_{\max}$

Two independent  
parameters from  
which images can  
be calculated

# Image of the photosynthetic activity, PA



# Image of the time constant, $\tau$

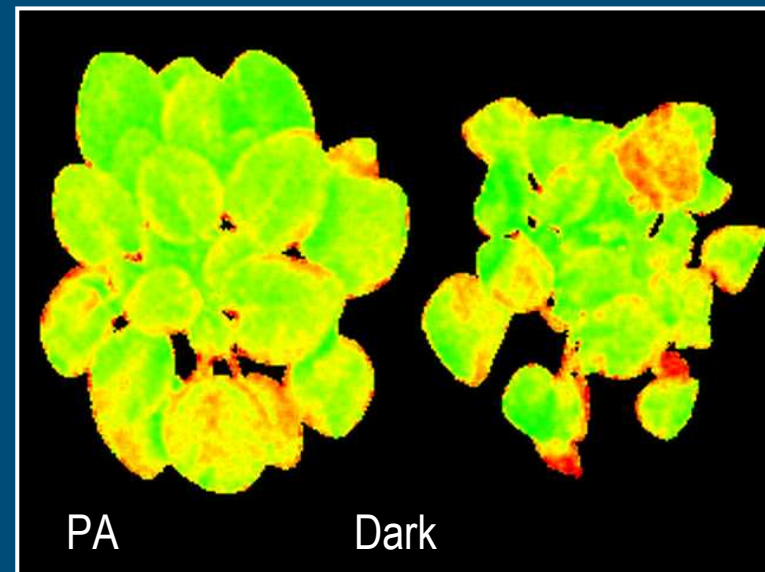
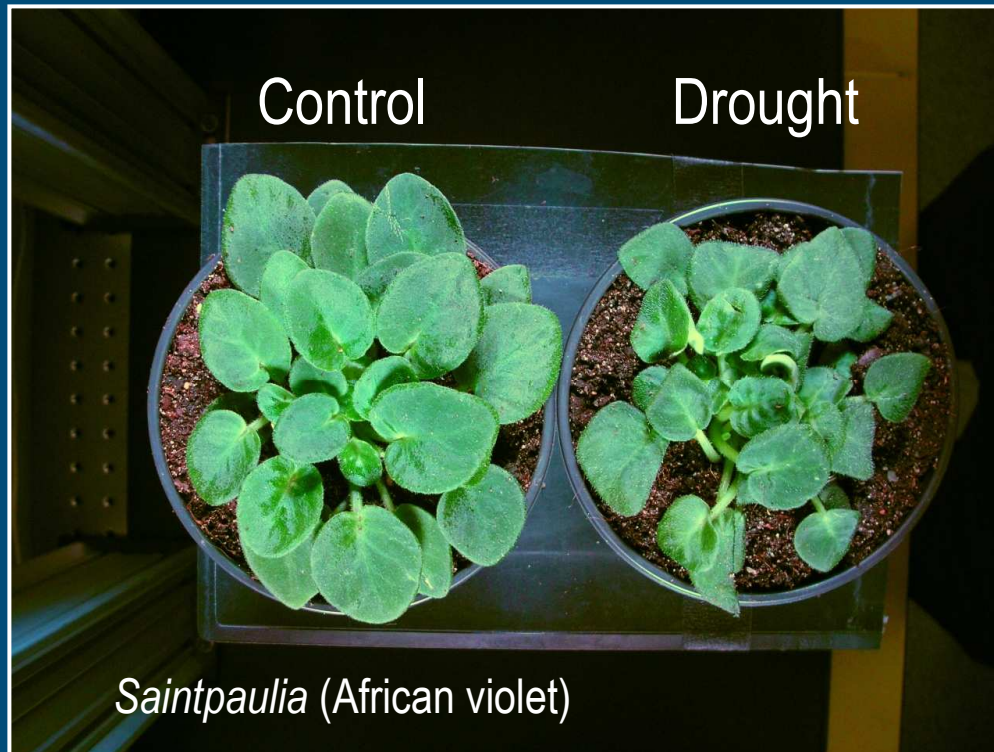


# Robotized LED imager for phenotyping at different scenarios

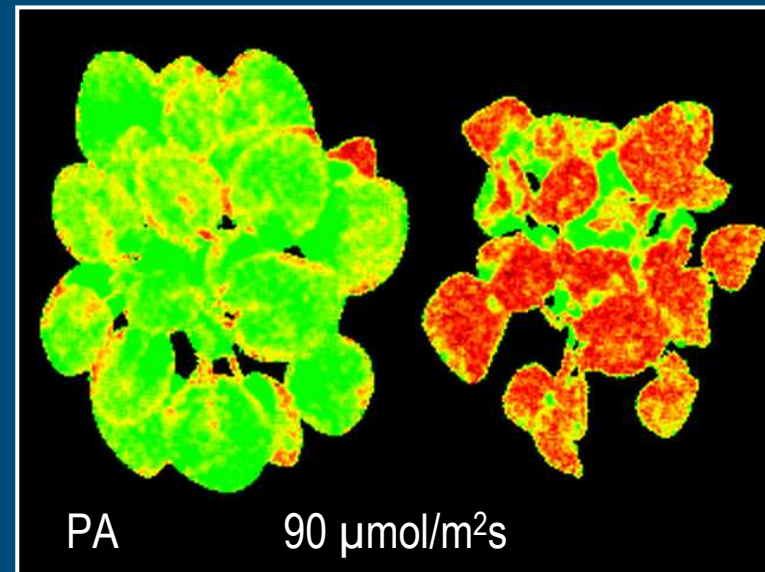
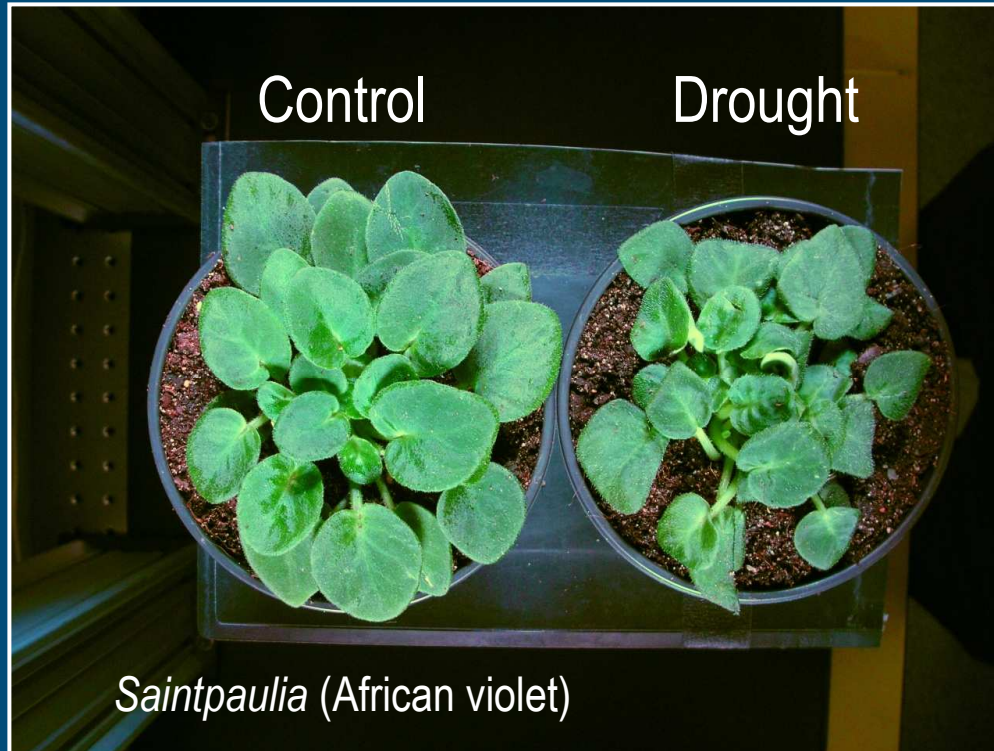


- LED imager on robot arm
- Climate room
- Fully programmable
- 30 images within one second
- Calculation of PA- and  $\tau$ - image
- Growth of projected leaf area
- Can run for weeks

# Drought stress: measurement in the dark (adaptation)



# Drought stress: measurement in the light (adaptation)



# Black nightshade stressed and healthy

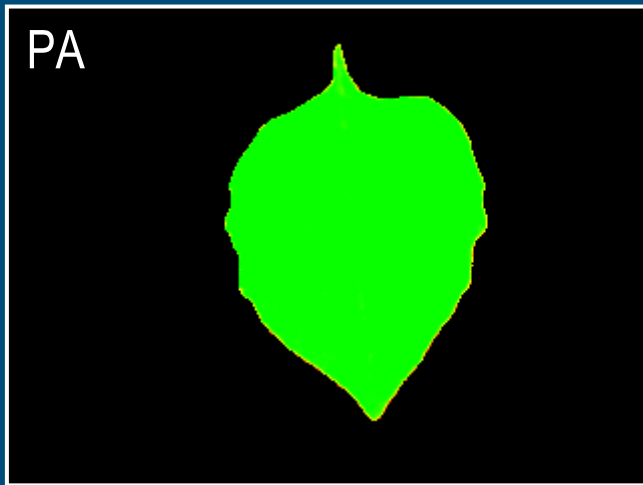


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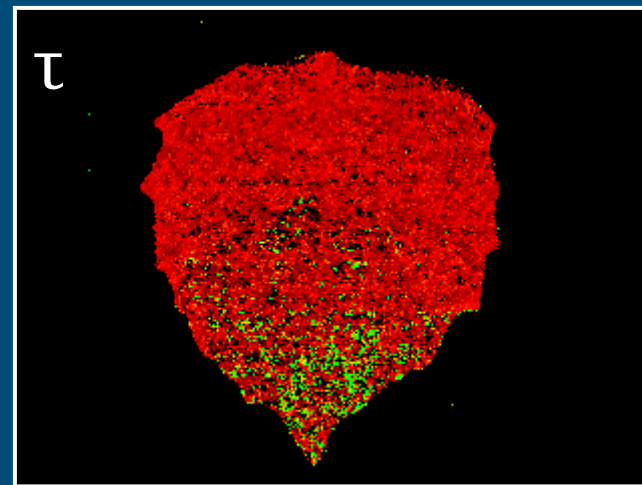
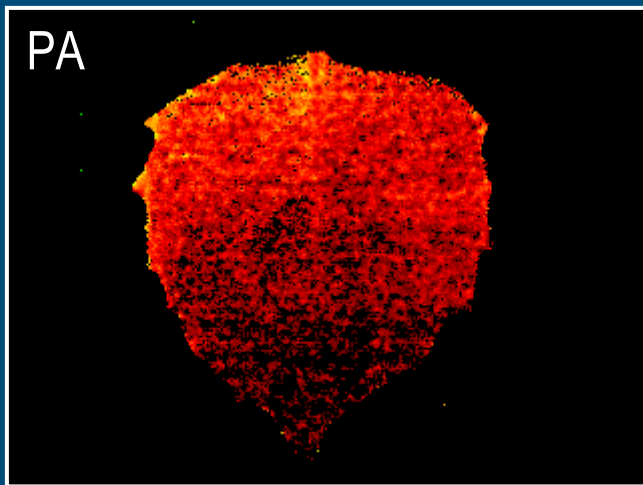
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# Black nightshade healthy and heavily stressed

Healthy



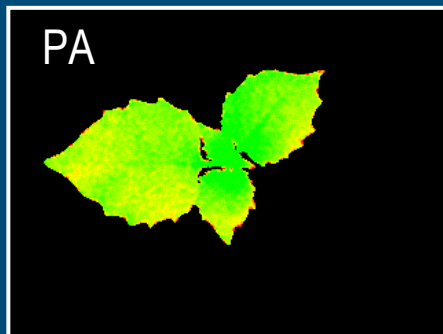
Stressed



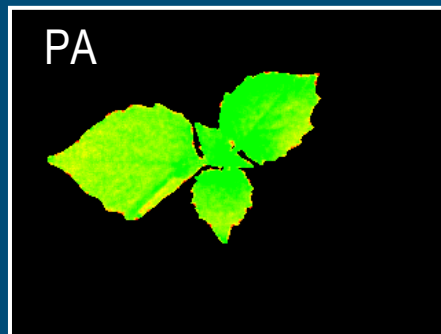
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# Wilting of a detached leaf of black nightshade plant



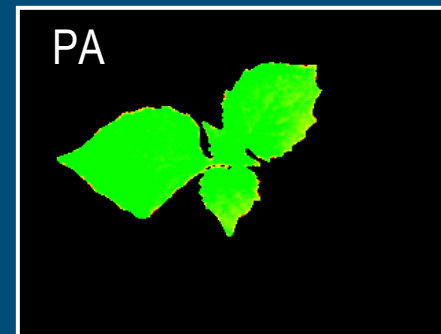
t=0



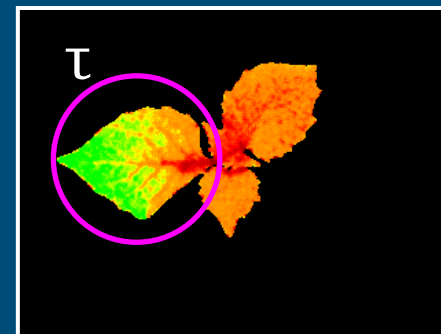
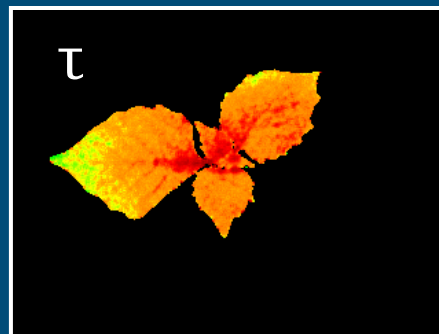
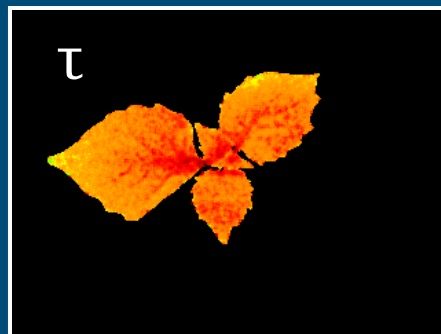
t=15 min.



t=30 min.

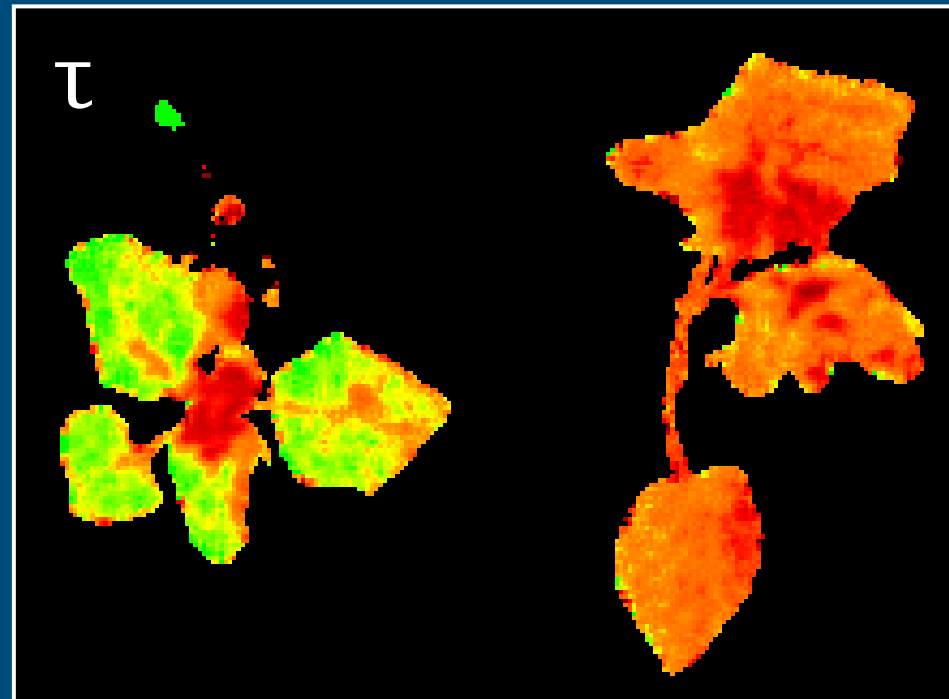


t=60 min.



At t=1 min. a leaf is detached

# Screening potato genotypes on salinity stress



Stressed

Control



# Summary

- Stress of whole plants can be visualized on PA and  $\tau$
- Changes in PA- and  $\tau$ -image can depend on the type of stress
- Only stress that influences the photosynthetic apparatus like diseases, herbicides, light stress and drought
- Non-invasive, quantitative, accurate and objective
- Plants can be monitored continuously at different scenarios
- Within a time frame of 300 ms: high throughput

# Future development

- New camera system for fluorescence and reflection imaging
- Camera with 8 position filter wheel
- White LED's for reflection imaging
- Pulsed high intensity red LED's for imaging Kautsky curve
- LED's for fluorescence imaging of chlorophyll and anthocyanins content

# Time for discussion

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<http://www.greenhousehorticulture.wur.nl>



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