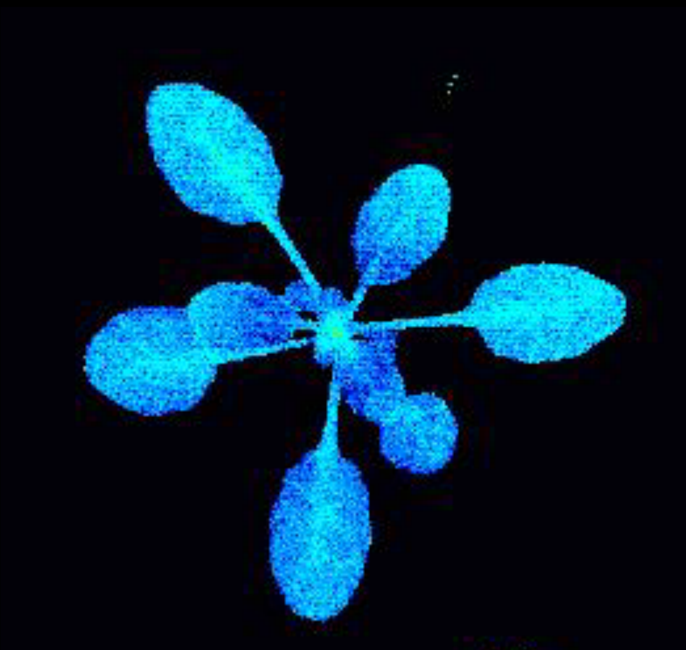
Next Generation Plant Phenometrics: Dynamic Photosynthetic Phenotyping



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1. We are in big trouble (energy and food)
2. We have to improve plants to be more productive in a changing environment.
3. We do not have millennia to do this!
4. This is BIG SCIENCE, and requires new thinking in both basic and applied science
5. Genomics et al. have revolutionized our understanding of plants.
6. The key limitation is phenotyping!
7. But not just any phenotyping… something much bigger.
8. That’s what we are doing.

Outline:

Motivations: Why we to understand photosynthesis *in situ*, and why we need special tools to do so?

Why we need to know the connections between genotype and phenoptype (performance)? Between genes, photosynthesis and productivity?

Why do we need to understand these processes in situ, under **dynamic environmental conditions**?

Our approach:

- Dynamic Environmental Phenometrics
  - The overall concept: integration of
    - phenometrics measurements
    - environmental controls

Proofs of concept (several examples demonstrating why our approach is so cool)

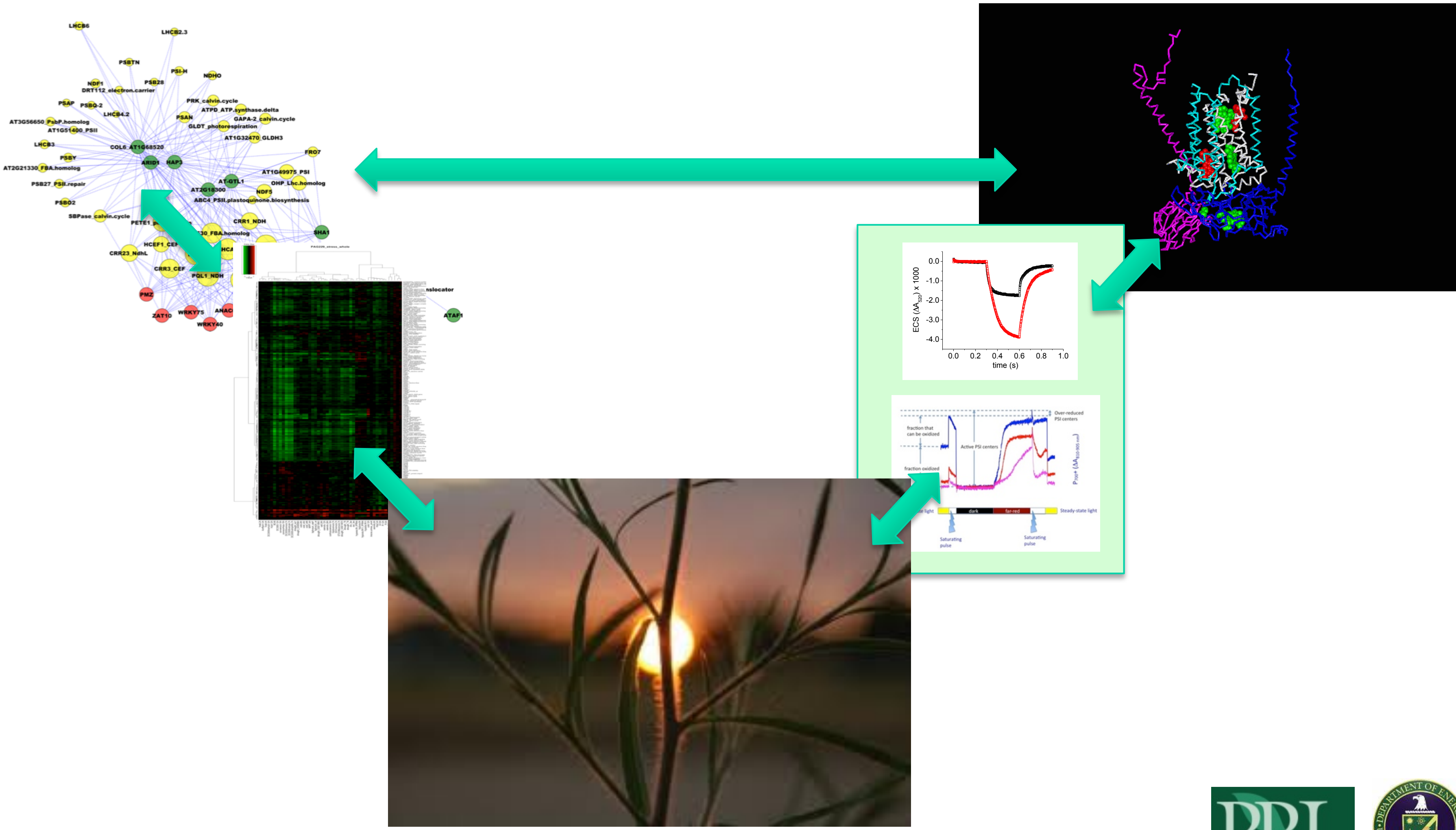
- including two more refined stories
- (probably punt and give up).

Next steps
- Moving towards crops plants
- 3D imaging
- scaling our system up to larger venues
- Possible applications to crop development

Big goal: make critical connections between genotype and phenotype: What makes photosynthesis more efficient and more robust?

A central focus of the PRL.

Phenometrics is a key enabling technology for this group.



“Phenometrics” aka next generation phenotyping

Enabling technology for high throughput, detailed phenotyping under dynamic conditions relevant to those experienced during appropriate production or natural growth.

Why Phenometrics is the next "big thing" in plant sciences

Dramatic advances allow us to rapidly and cheaply sequence the genome or transcriptome of any organism on the planet. (Other 'omics are catching up)

However, we do not know how these omic controls translate into performance, i.e. what is the connection between genotype to phenotype?

Laboratory is not the field!

We need to rapid and detailed identification of phenotypes under appropriate conditions.

Why is phenomics the 'last omic'?
Phenometrics is technically challenging

- Every phenotype measurement requires a different platform and technology.

Ultimately,

- Measurements must be made non-invasively
- Need to apply these techniques to many different environments, time ranges etc.
- Need to interpret the data (computational tools for high throughput analysis of phenotyping data is in its infancy.

Phenometrics should be problem-driven

- It is possible to develop sensors for almost any process
- The question is: which of these are important?
- Focus development on specific, important questions:

Our initial question is: What determines the efficiency and robustness of energy capture and storage in plants and algae?

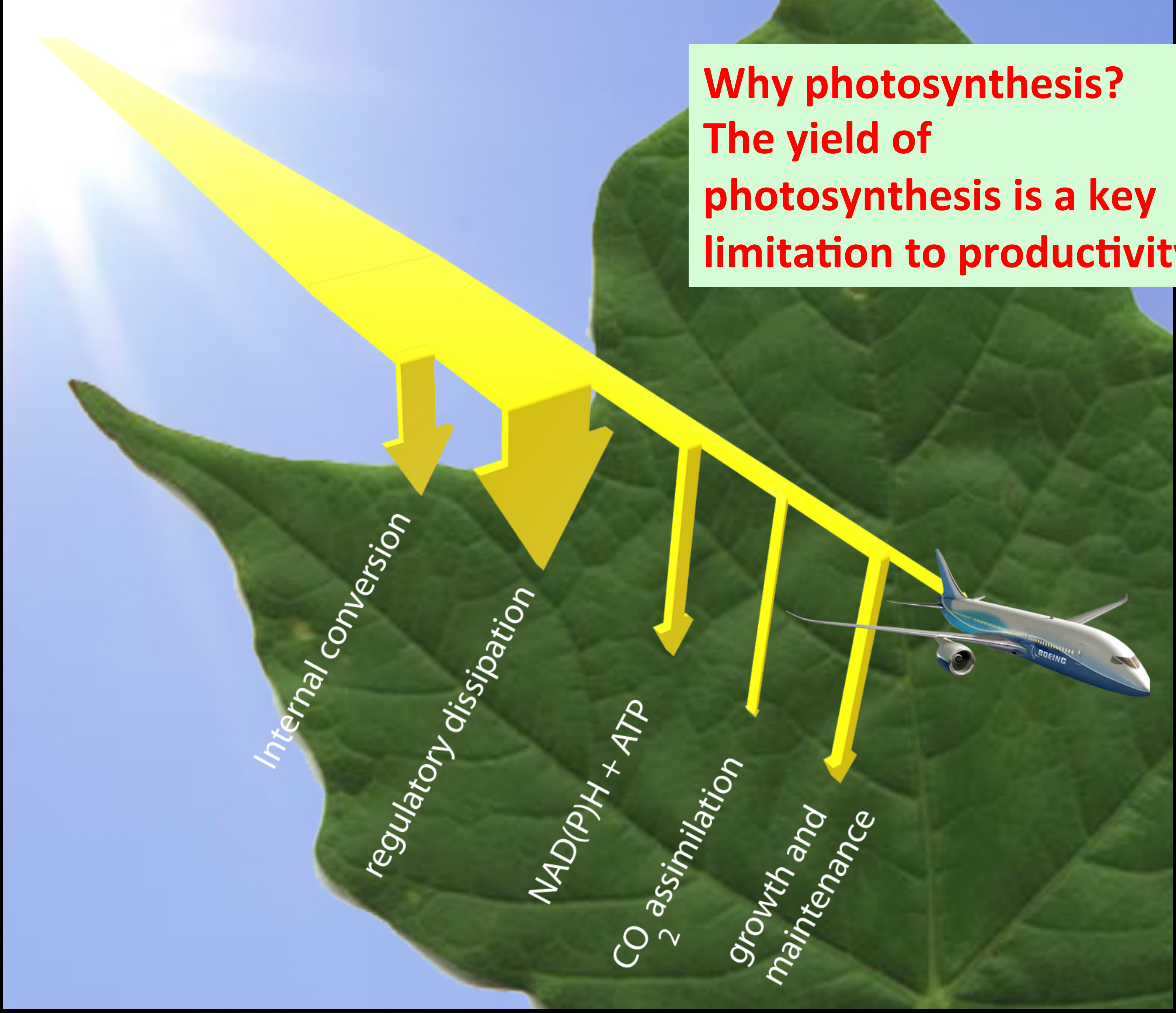
fuels (DOE)

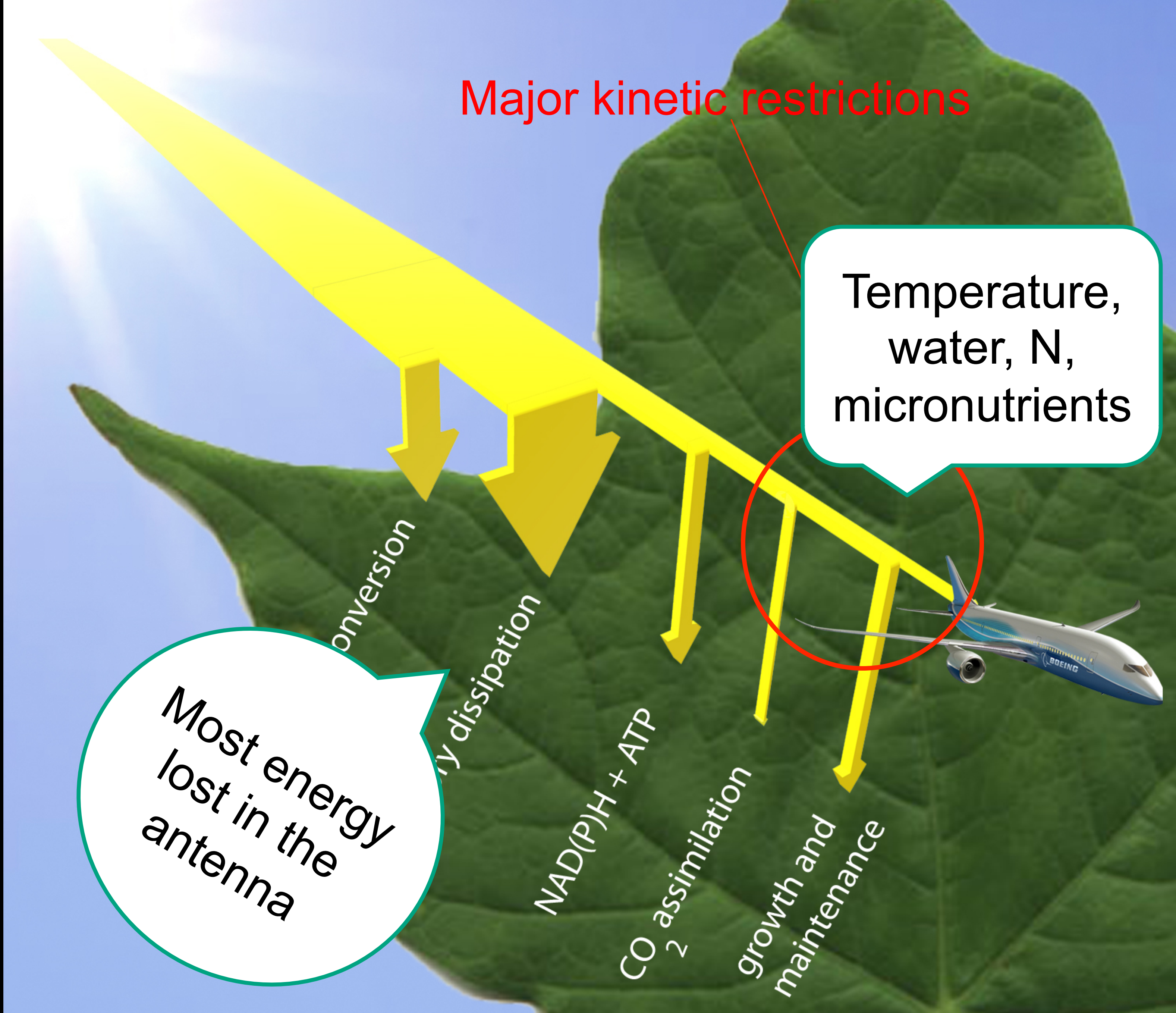
food (everyone else)

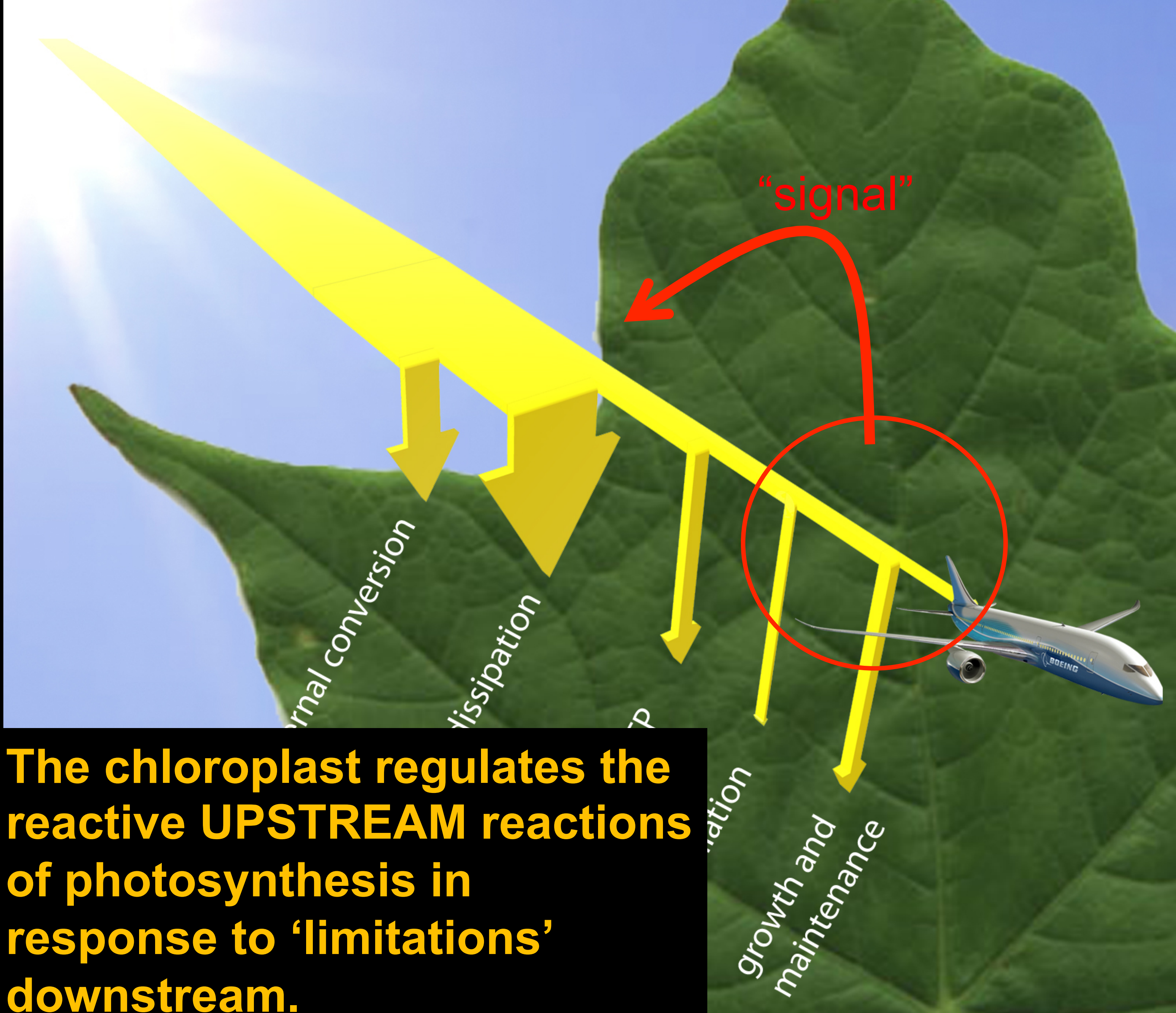
We start from photosynthesis.

There is room to expand this to other emerging aspects, especially climate change, agriculture, specialty products, etc. (End of slide show).

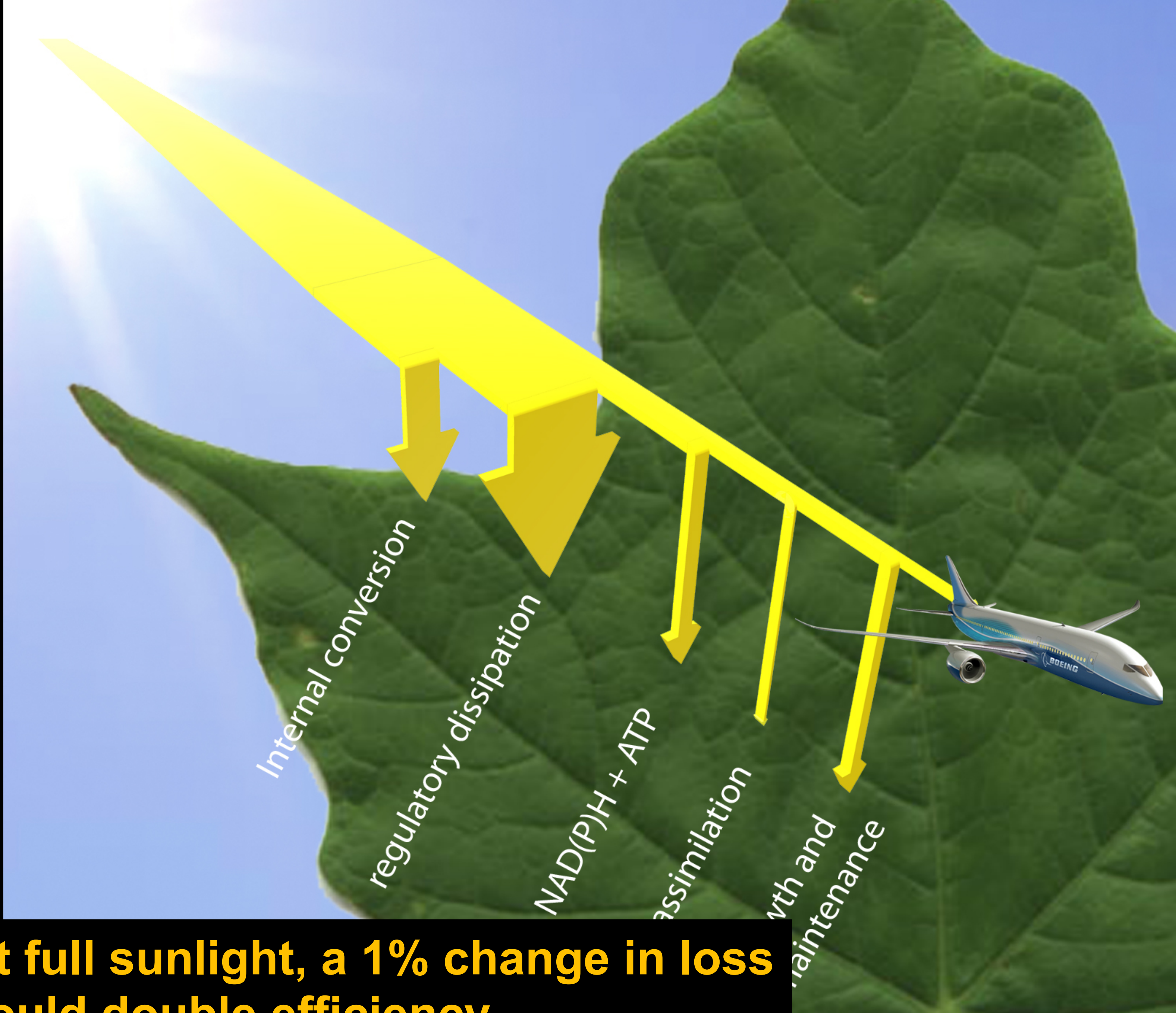
Why photosynthesis?
The yield of photosynthesis is a key limitation to productivity.




Major kinetic restrictions
Temperature, water, N, micronutrients
onversion
y dissipation
NAD(P)H + ATP
CO_2 assimilation
growth and maintenance
Most energy lost in the antenna

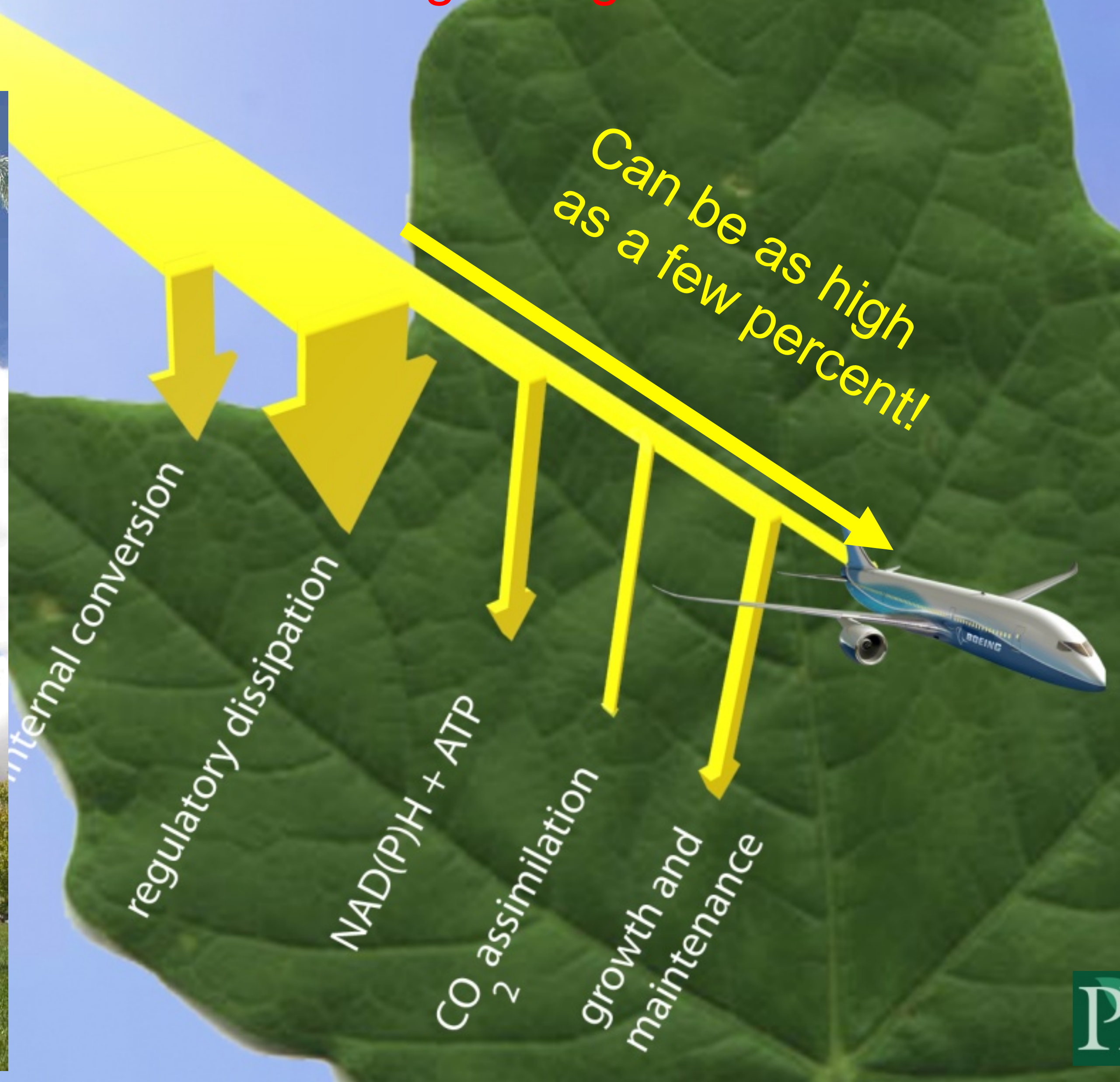


The chloroplast regulates the reactive UPSTREAM reactions of photosynthesis in response to 'limitations' downstream.



At full sunlight, a 1% change in loss could double efficiency.

There is large variation between species in energy efficiency, even under similar growing conditions.

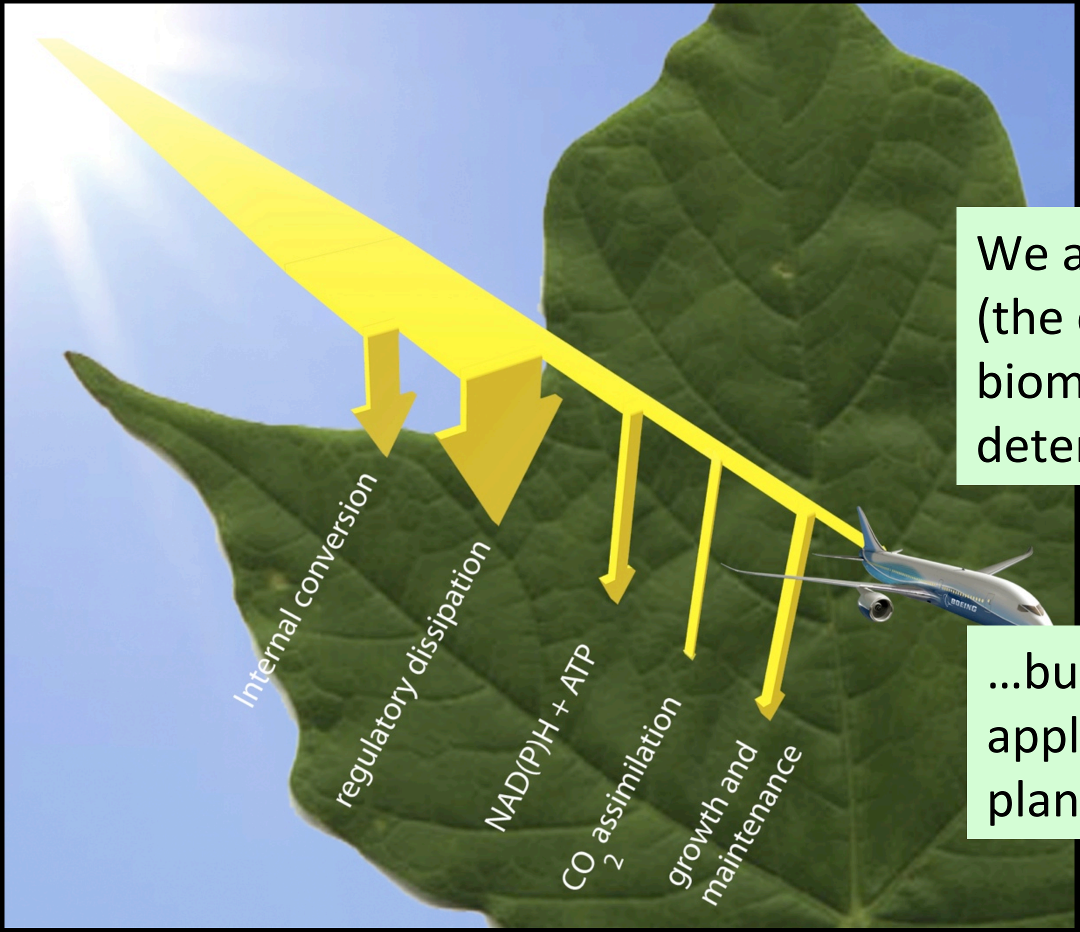


PRL

Why photosynthesis?

- key limiting factor for productivity
- linked to downstream processes
    - can be used as a monitor of plant status
- Opportunity: we have non-invasive measurements

6 technical and scientific hurdles for understanding and improving photosynthetic productivity using phenometrics



We are initially focused on yield (the capture of light energy in biomass), which is a key determinant of yield….

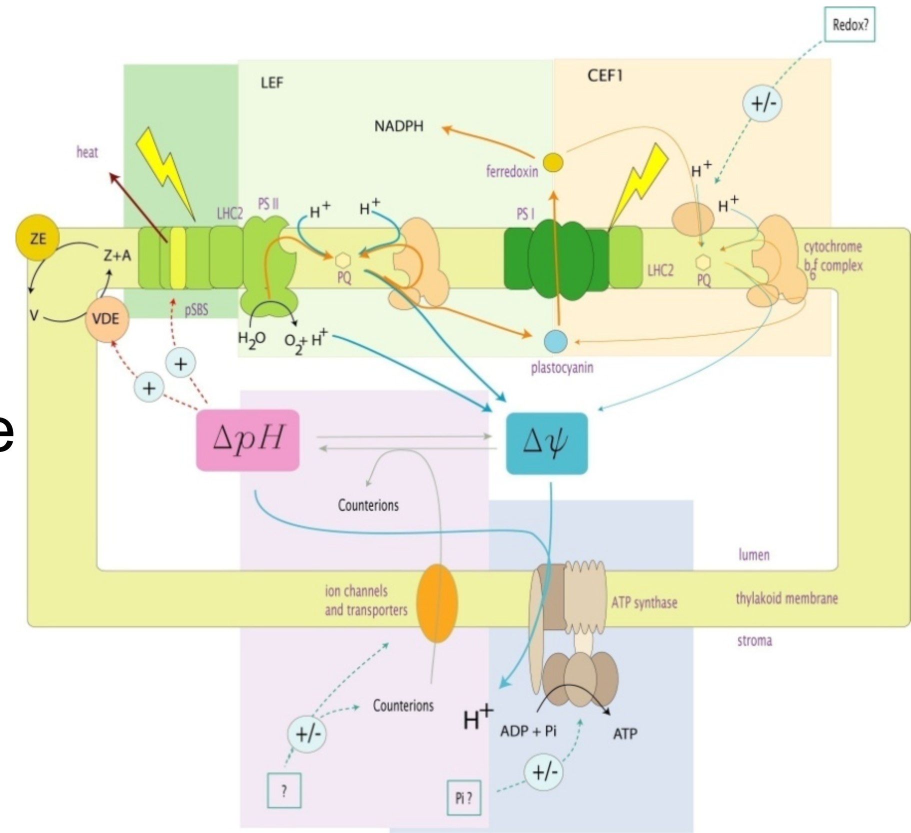
…but the tools should be applicable to many aspects of plant biology.

#1: The Measurement Problem

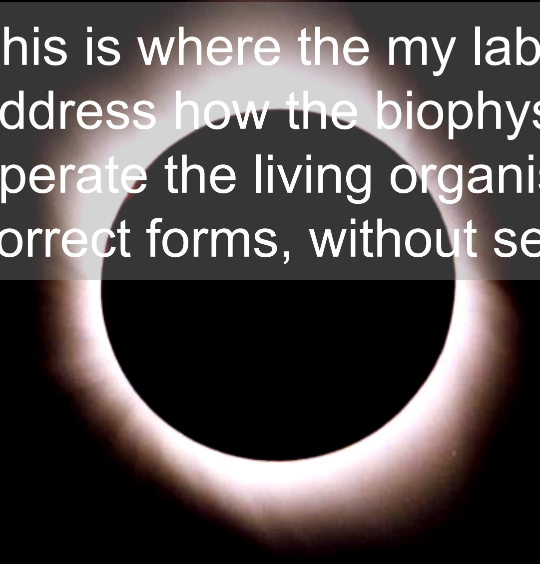
The key intermediates of photosynthesis (*pmf*, excitons etc.) cannot be isolated

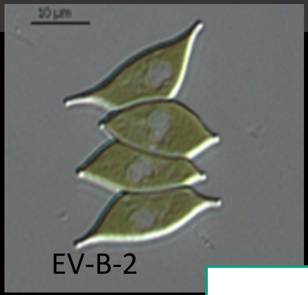
There is NO HOPE of isolating the key intermediates of photosynthesis (proton gradient, excitons etc)!

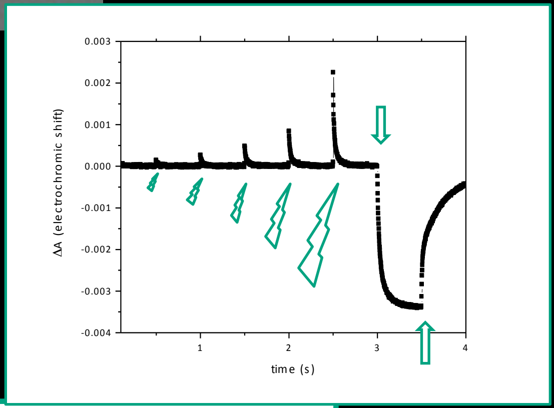
We want to probe these *in vivo* in a way that does not disturb the system.

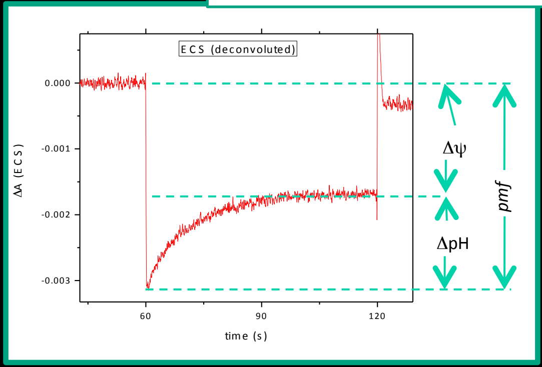


This is where the my lab came into this area, by developing the tools to address how the biophysical and biochemical machinery of photosynthesis operate the living organism to provide the correct amount of energy, in the correct forms, without self-destruction.



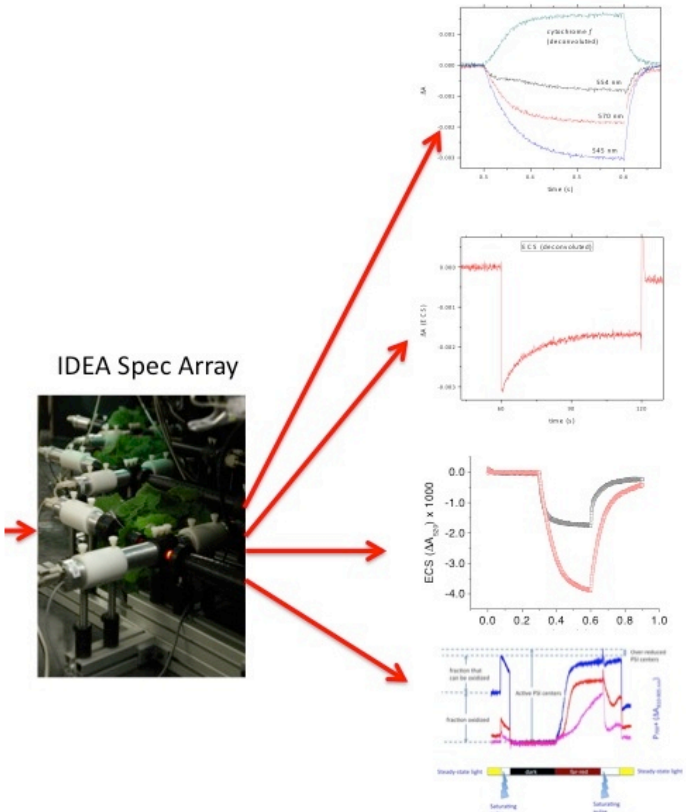


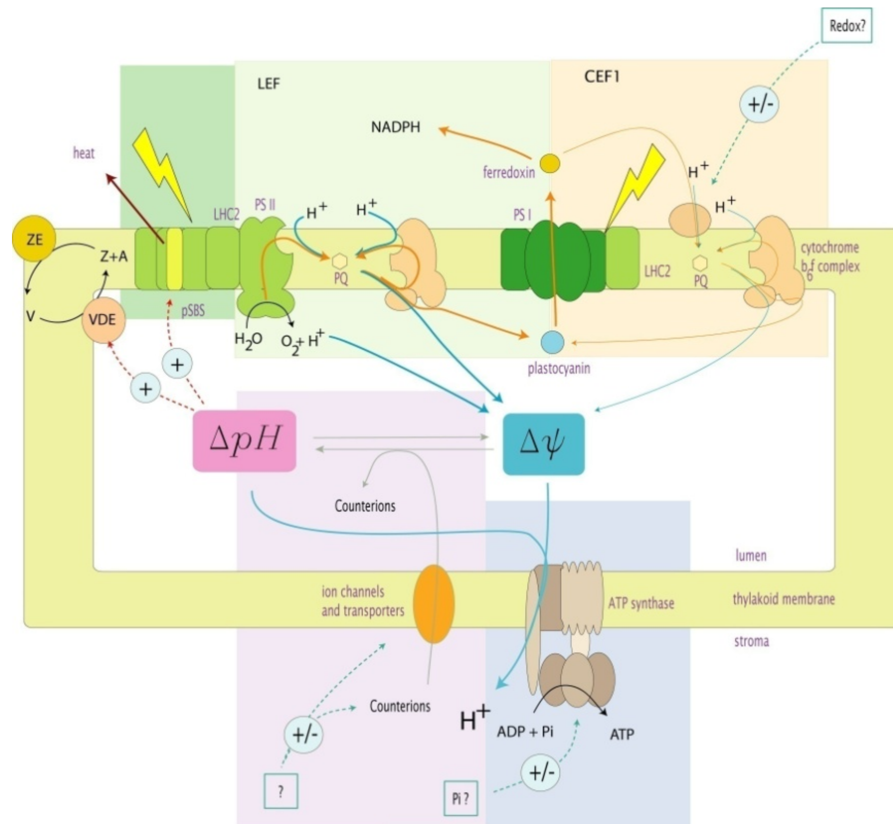




We can now measure many key photosynthetic energy capture processes under under growth conditions.

The fact that we can do this enabled other approaches, e.g. biochemical, genetics, genomics, metabolomics…





#2: "The Hyper-dimensional Problem (HDP)"

- Many factors influence bioenergy yield (genetic, biotic, developmental, and abiotic).

- These factors interact with each other

- All of them are likely to be critical

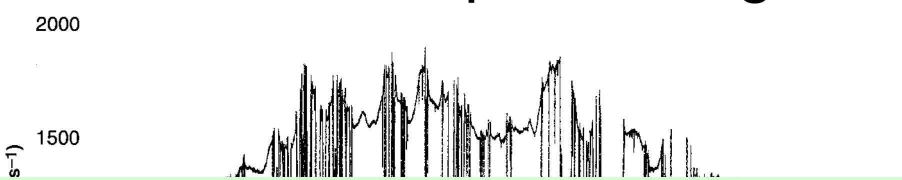
To get a true picture, we need to measure many things in many variants (cultivars, mutants) under many conditions, all the time. This is a 'hyper-dimensional' problem (HDP).

We need to make our photosynthesis measurements high throughput.

#3: The Dynamics Problem

Plants and algae have evolved to cope with unpredictable, fluctuating environmental conditions, but we study them under ‘static’ controlled conditions

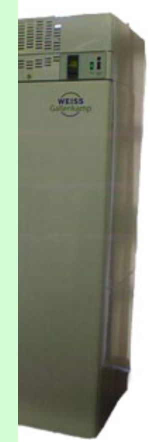
- OK for ‘reductionist’ experiments
- But, miss important regulatory factors



…not just light, but other conditions:

temperature,
wind
chemicals
nutrients
water
insects

…and don’t forget that these change over developmental time scales!



Examples: Various photosynthetic regulatory processes have been selectively disabled without effects under static conditions, but these have large effects under fluctuating conditions

Photoprotection (*npq1*, *npq2*, *npq4*)
Xanthophyles carotenoids (*npq2*)
Light distribution, phosphorylation (*stn7*, *stn8*)
Cyclic Electron Flow (*ndha-i*, *crr,2-2*, *pgr5*, *pgrl1*, *pifi*)
Ascorbate (*vtc2-2*)
a-tocopherol (*vte1, vte2*)
ATP synthase regulation (*cfq*)
Polyamines (*adc2-2,* putrescine)
Lipid composition (*fad4*, *fad7*, *act1,* and *fab1*)
PSII repair (Aro, Last, etc.)
…

#4: Functions of Genes of Unknown Function
Key plant traits can be hidden in laboratory conditions

•Many genes only function under non-laboratory conditions.

•Disruption of conserved genes often leads to ‘no visible phenotype’ under the limited conditions we study them in.

•We know important genes exists, but do not know what their functions are.

•We thus need a platform which approximates the conditions under which the cultures will be grown.

- If we do not get this right, we could spend huge time and money obtaining **precisely the wrong characteristics**.

#5: Standard Platform Problem

How can we compare data from different experiments if the conditions are so different?

Some of the myriad (and inappropriate) algal growth methods.

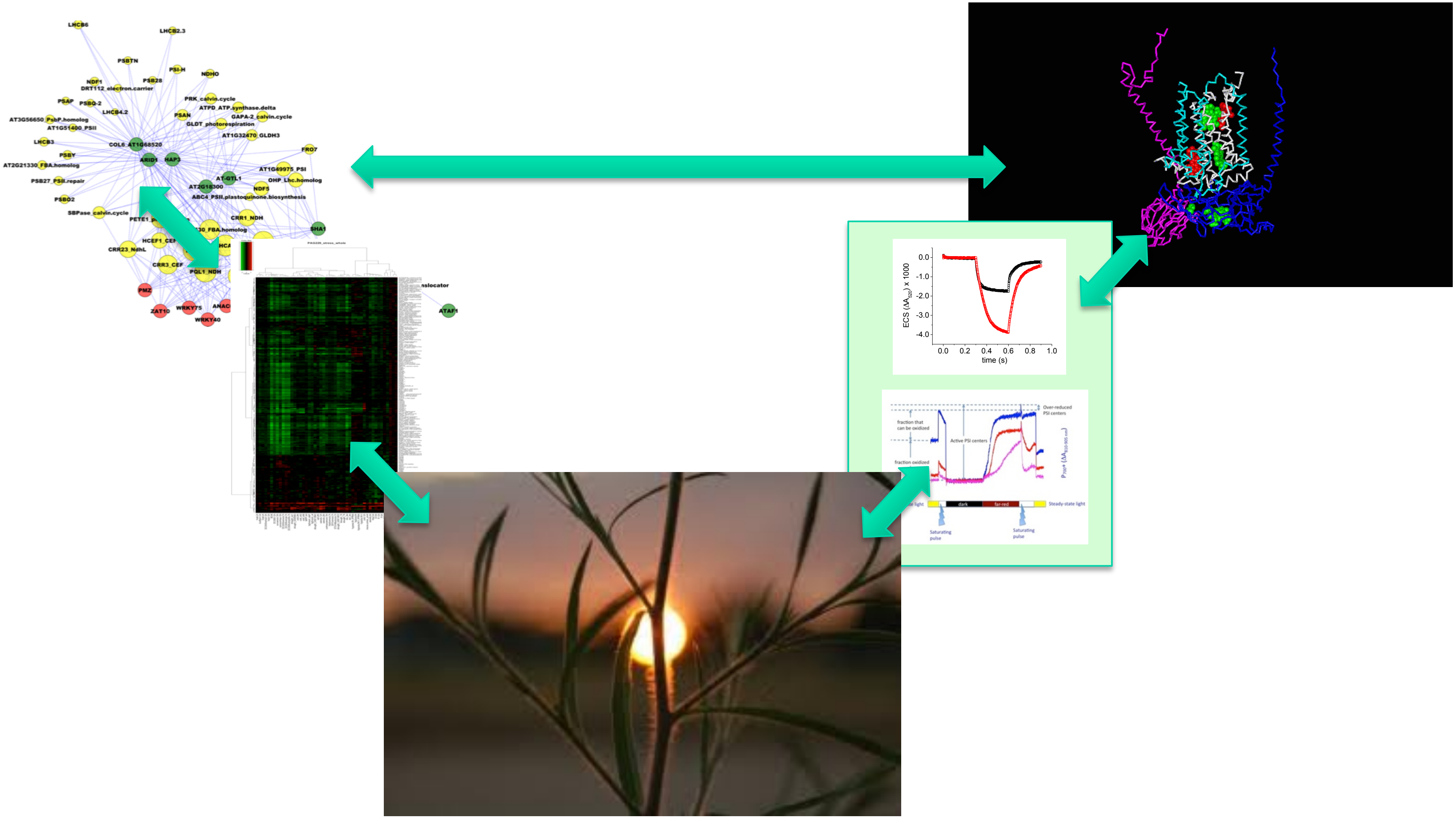
*None of these looks like the real thing.

*Comparisons cannot be made between groups

*Cannot probe photosynthesis in these.

#6: Tractability Problem:

If we succeed in addressing #1-#5, how do we make functional connections between huge phenotyping data sets and huge genotype data?



Approach: Develop transformative tools to study dynamic regulation of photosynthesis and apply to rapidly emerging, highly tractable genetics, metabolites and systems approaches.

Photosynthetic Phenomics Array (PPA) (plants, PlaNet project)

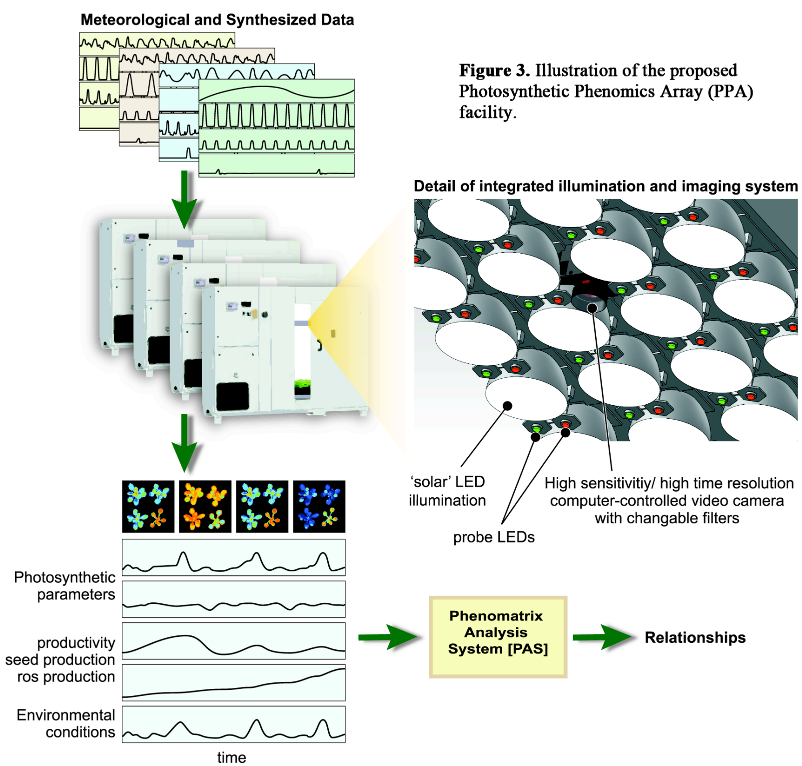
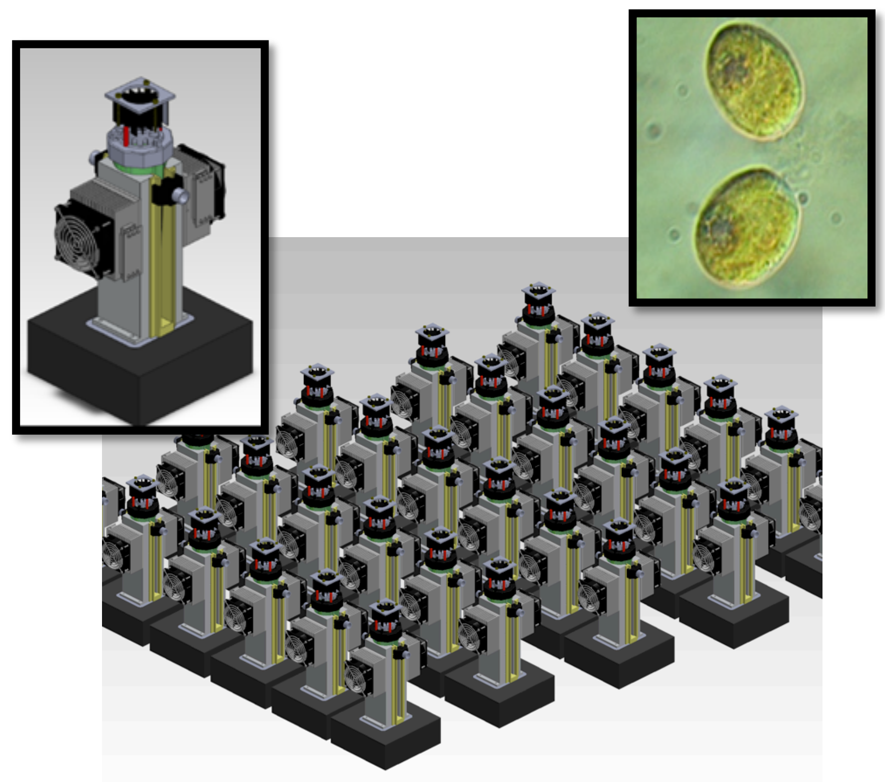
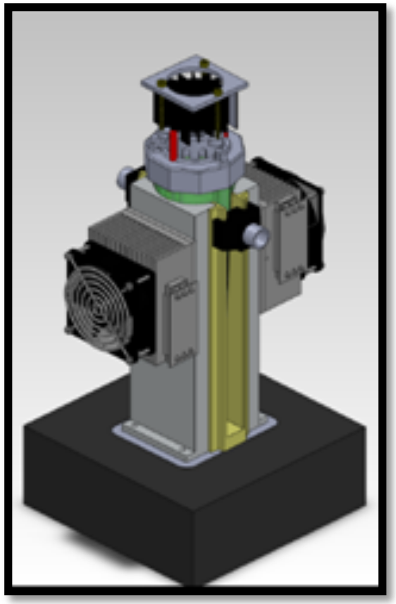


Figure 3. Illustration of the proposed Photosynthetic Phenomics Array (PPA) facility.

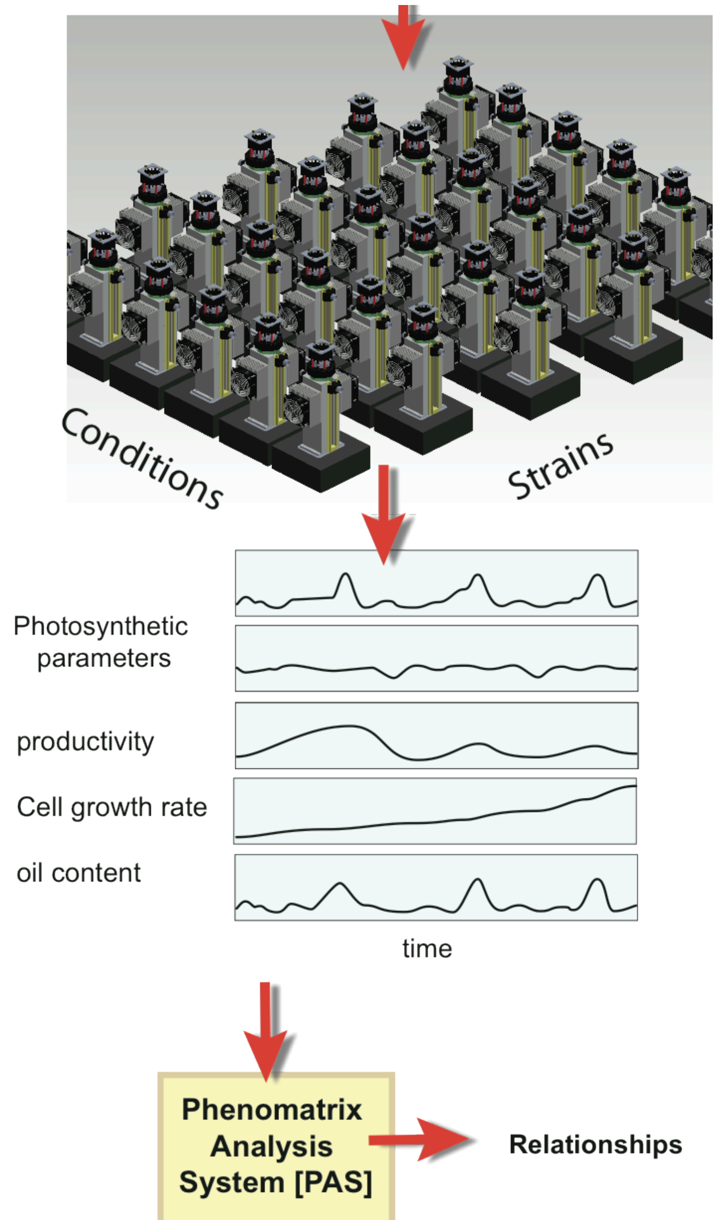
PBR Sensor Matrix (algae, NAABB)



Photobioreactor/Sensor Matrix



- Simulates conditions experienced in under field conditions
- High throughput mode
- Can measure photosynthetic and growth performance
- SOP at NAABB



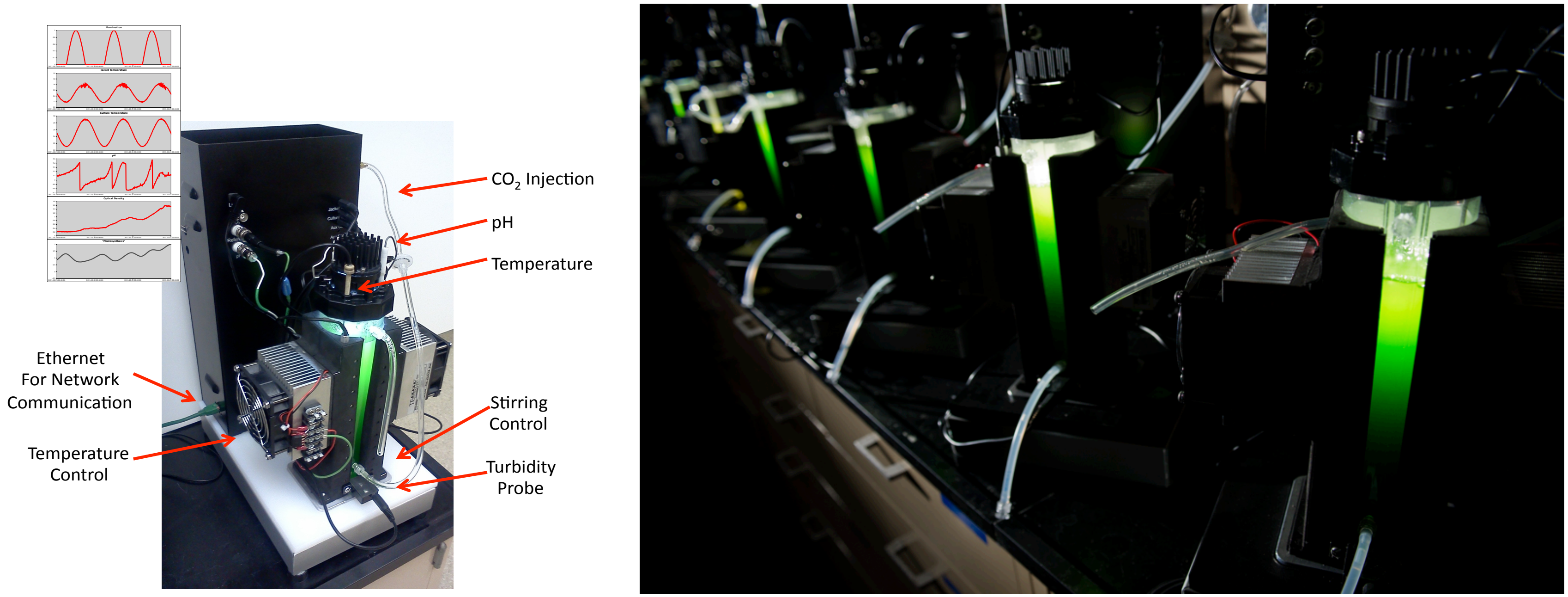


Figure 3. An ePBR matrix in action. Left panel shows a working ePBR unit and output data. The right panel is a photograph of part of a 20-unit ePBR matrix currently working in the Kramer lab.

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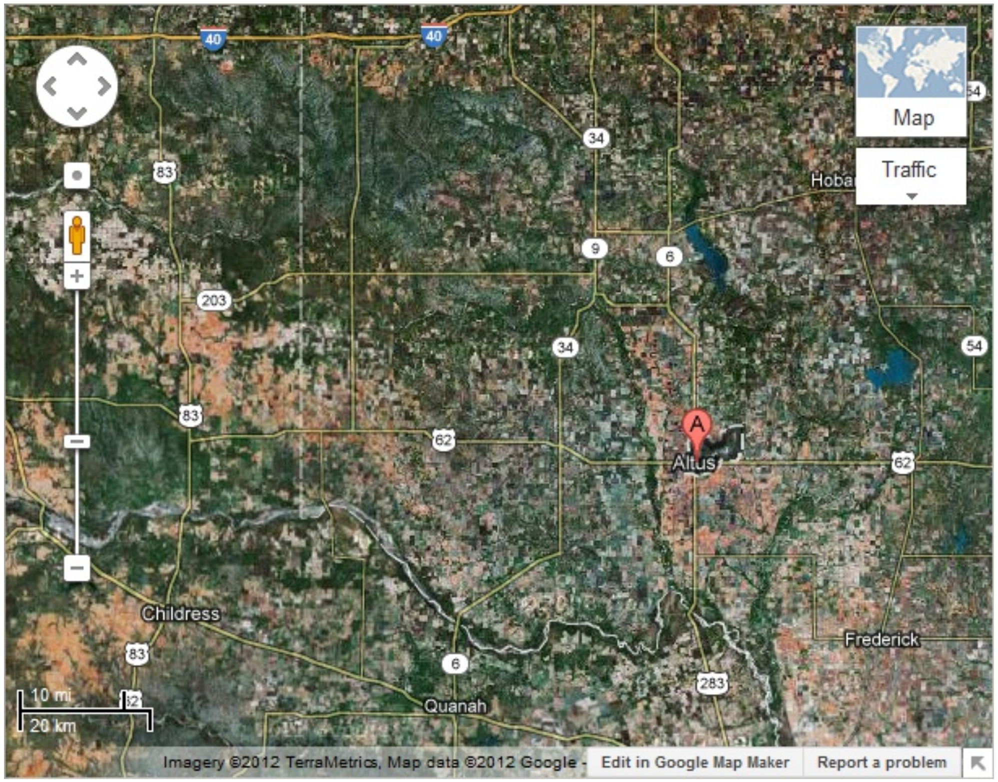
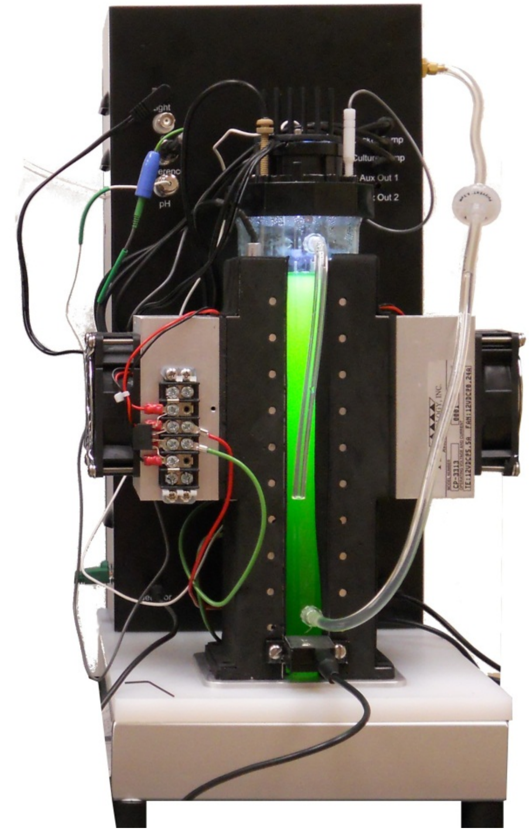
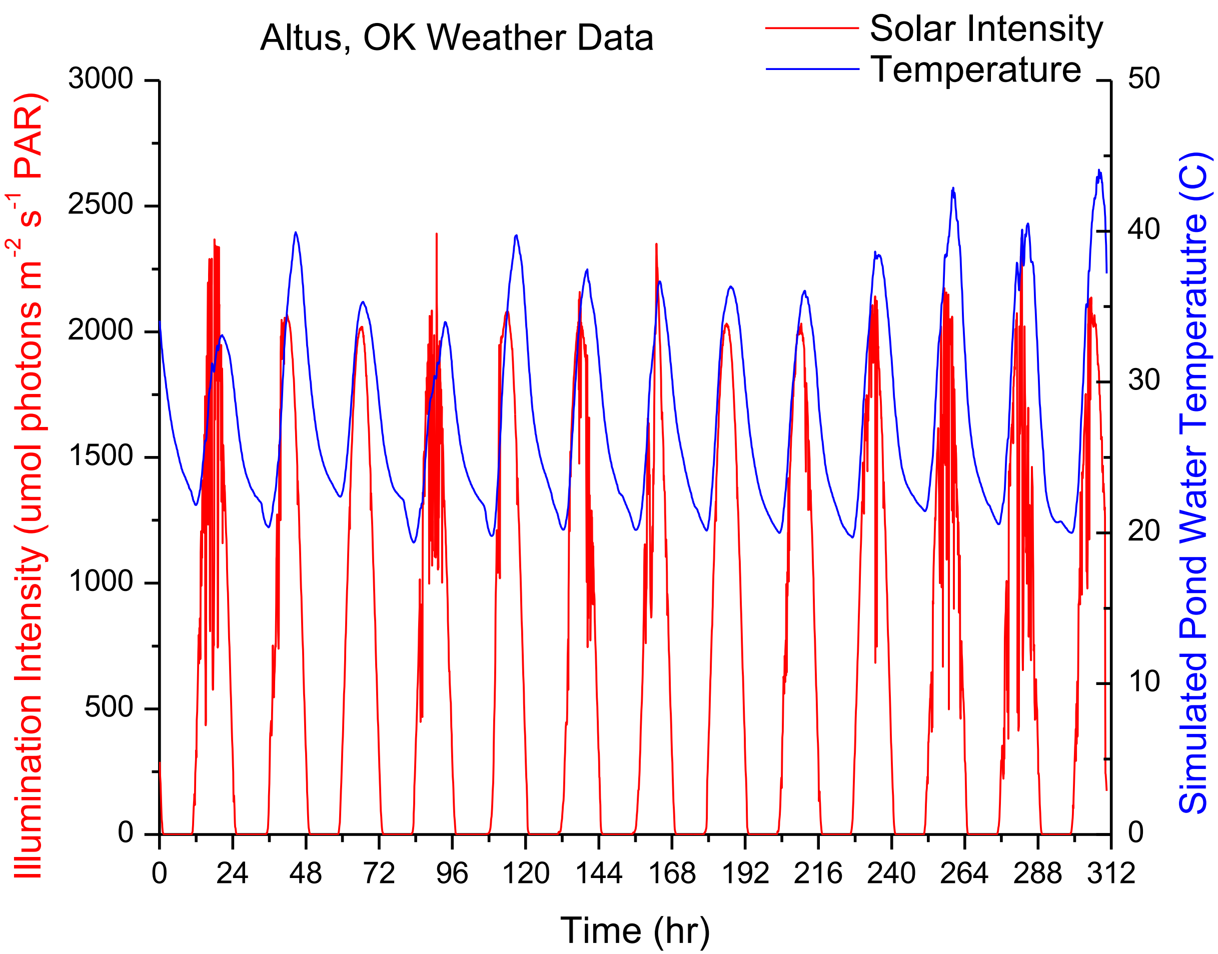
Replaying the Weather on the Lab Bench





Weather data from Altus, Ok weather archive was used to simulate a production pond. Water temperature simulation provided by Quentin Bechet and Benoit Guieysse at Massey University, New Zealand

Replaying the Weather on the Lab Bench

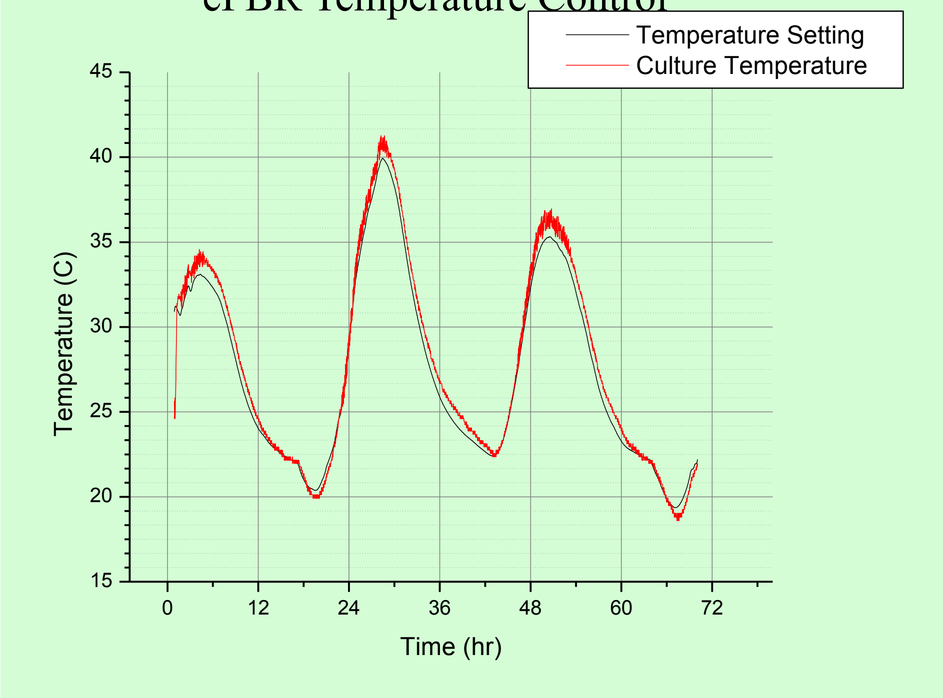


Weather contains a mix of sunny and cloudy days with daily temperature fluctuations of about 20C

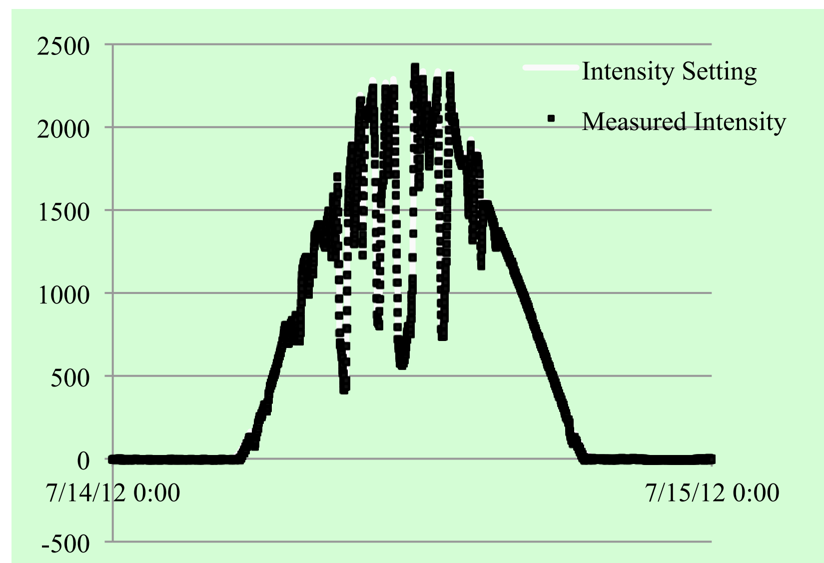
Replaying the Weather on the Lab Bench

The ePBRs are able to replicate the light intensity and temperature from the weather data file

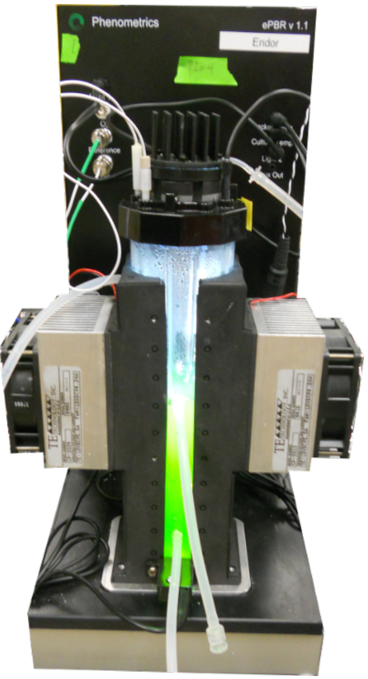
Accuracy and Precision of Updated ePBR Temperature Control

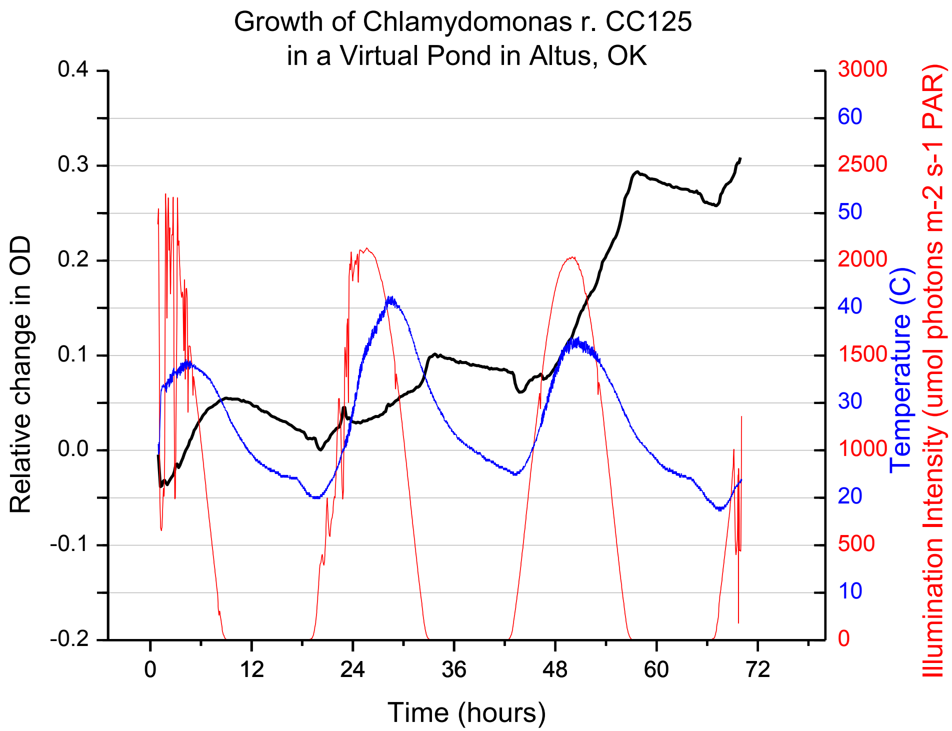


ePBR Light and Temperature



Replaying weather in ePBR





Growth of wild-type *Chlamydomonas reinhardtii* CC125 under simulated weather conditions.

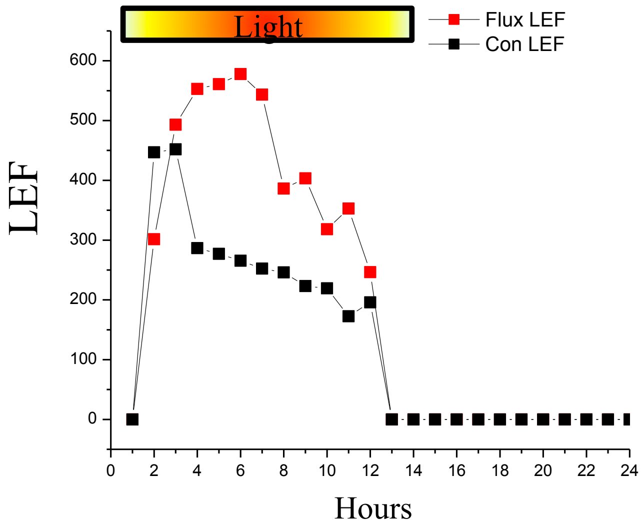
These light intensities would be considered lethal for flask and petri-dish growth (1) and the high temperatures are known to be stressful to *Chlamydomonas r.* (2)

However, in the ePBR column, CC125 was able to grow in spite of the light and temperature. The culture did not appear yellow after 3 days.

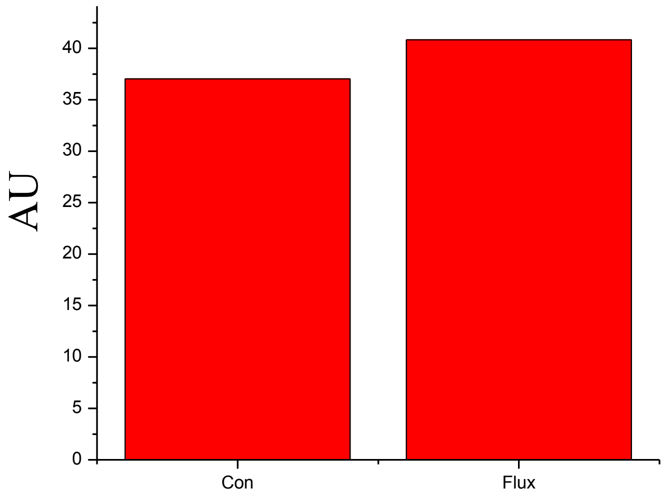
1: Förster, B., Osmond, C. B., Boynton, J. E., Gillham, N. W., J. Photochem. Photobiol. B-Biol. 1999, 48, 127–135.
2: Tanaka, Y., Nishiyama, Y., Murata, N., Plant Phys. 2000, 124, 441-450.)

Controlling environmental conditions matters!

Relative Linear Electron Flow Rate



Productivity / Integrated LEF



Problem:

While a small antenna may give high productivity to an entire culture, it will not compete well with large antenna wild types. Large antenna strains can “shade out” small antenna mutants.

Saturated and suffering from photoinhibition

Starved of light

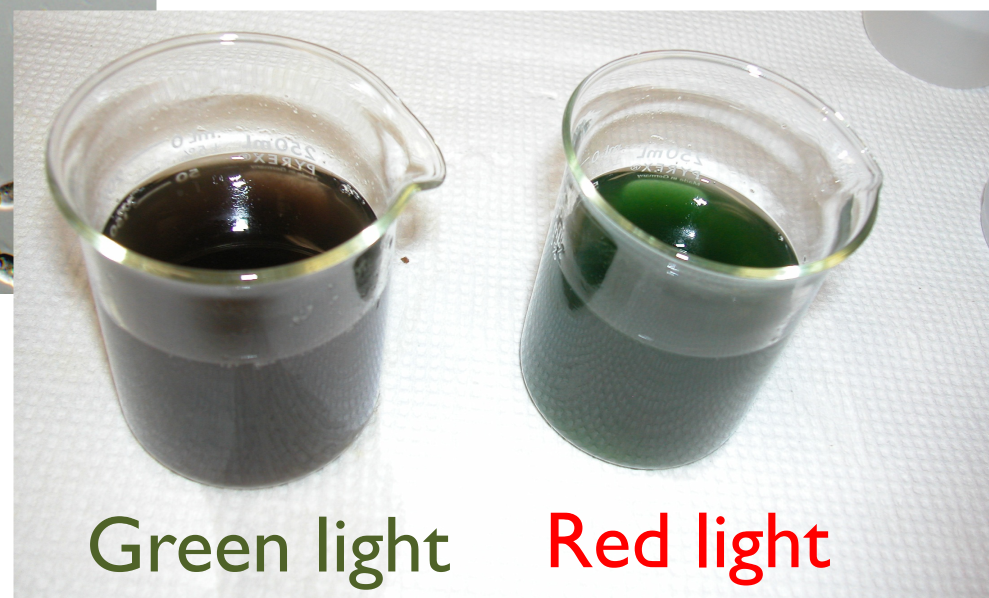
Approach:

Use ePBR array to find conditions and mutations under which small antenna strains outcompete the larger. Use these systems to identify the biophysical, biochemcial and genetic bases of these effects and address the question: What are the implications for the balance between efficient light capture and avoidance of photodamage?

Fremyella diplosiphon

Shows dramatic ight-dependent changes in pigmentation and morphology

Complementary chromatic adaptation (CCA)



Beronda Montgomery
Marco Agostoni

Ben Lucker
Christopher Hall

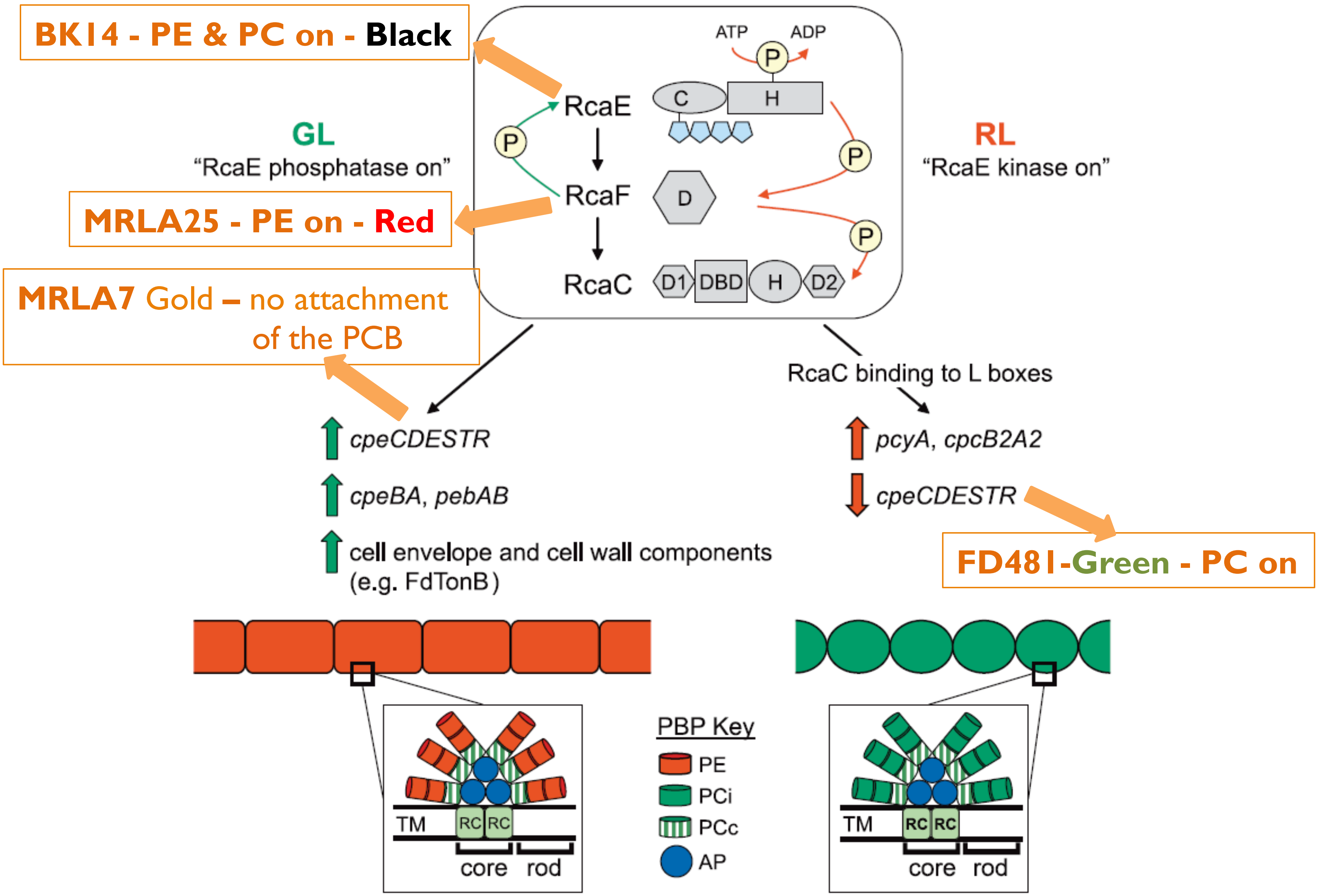
Well characterized mutants:

Black 14 = ΔrcaE,
receptor involved in controlling complementary chromatic adaptation

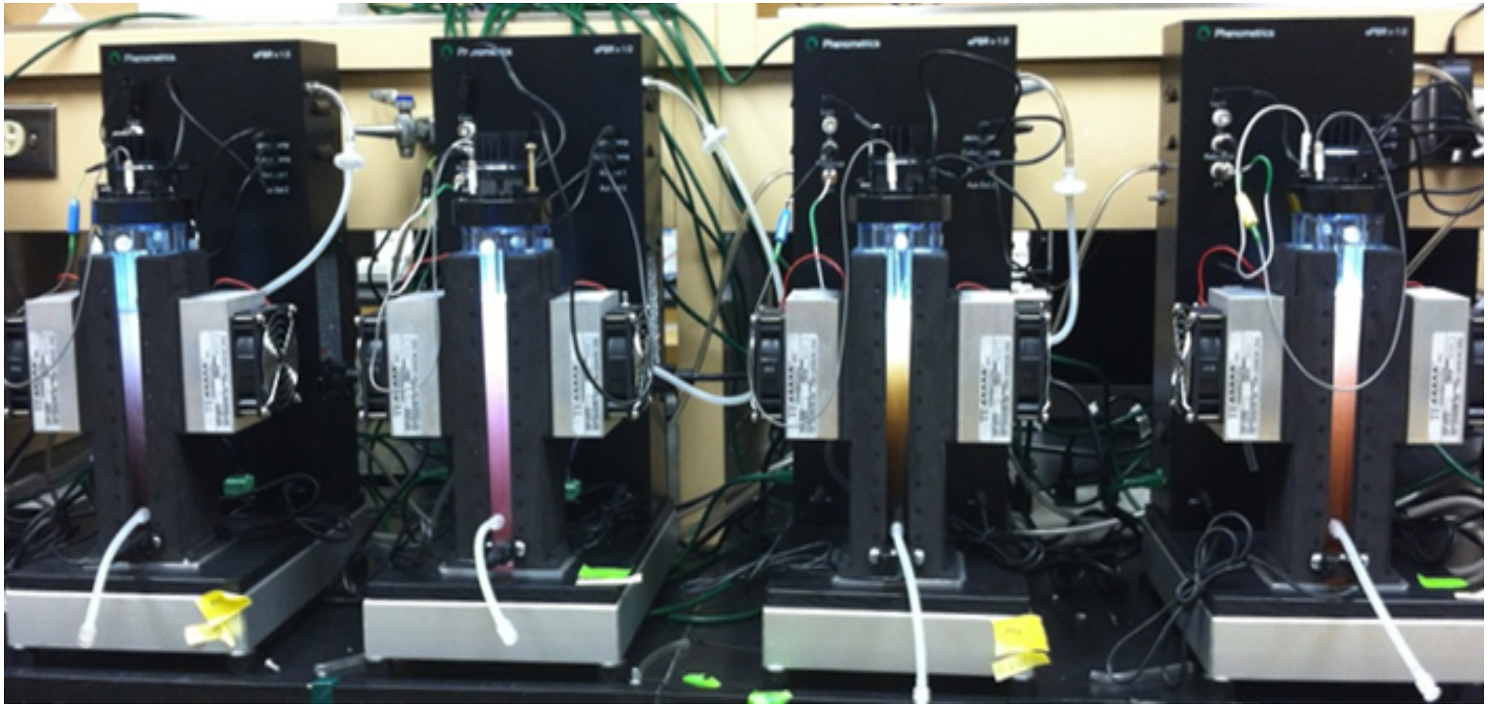
Green FD418G = ΔcpeR
cpeR is required for the synthesis of Phycoerythrin (PE) (transposase IS66)

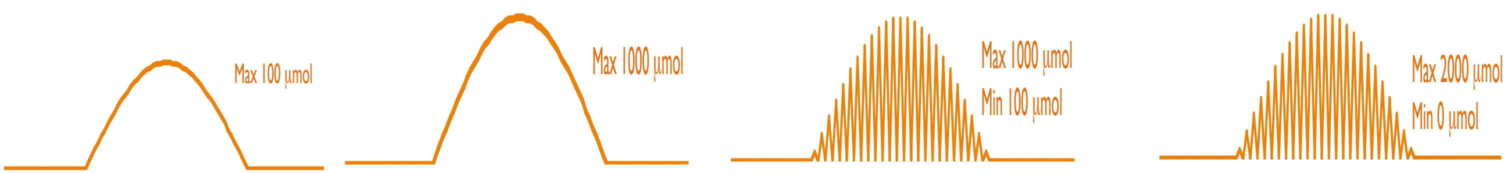
Red MRLA25 = ΔrcaF
RcaF acts downstream of RcaE required for the expression of the phycocyanin PC
(IS701 transposase)

Gold MRLA7 = ΔcpcF
cpcF catalyzes the attachment of the PCB chromophore
(IS701 transposase)


BK14 - PE & PC on - Black
MRLA25 - PE on - Red
MRLA7 Gold – no attachment of the PCB
GL
"RcaE phosphatase on"
RL
"RcaE kinase on"
ATP
ADP
P
C
H
RcaE
RcaF
D
RcaC
D1
DBD
D2
RcaC binding to L boxes
cpeCDESTR
cpeBA, pebAB
cell envelope and cell wall components (e.g. FdTonB)
pcyA, cpcB2A2
cpeCDESTR
FD481-Green - PC on
PBP Key
PE
PCi
PCc
AP
TM
RC
RC
core
rod
TM
RC
RC
core
rod

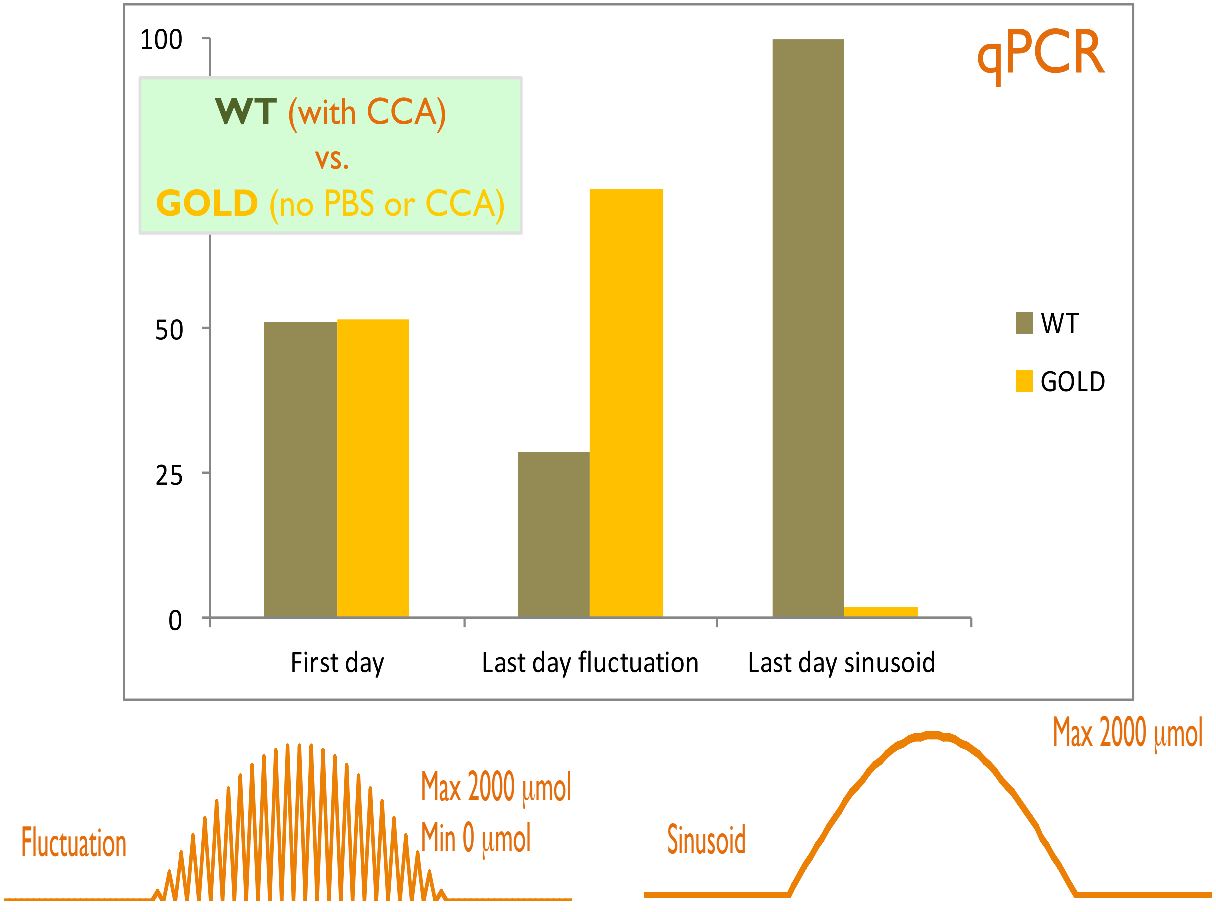
Different CCA mutants “win” under different dynamic light fluctuations



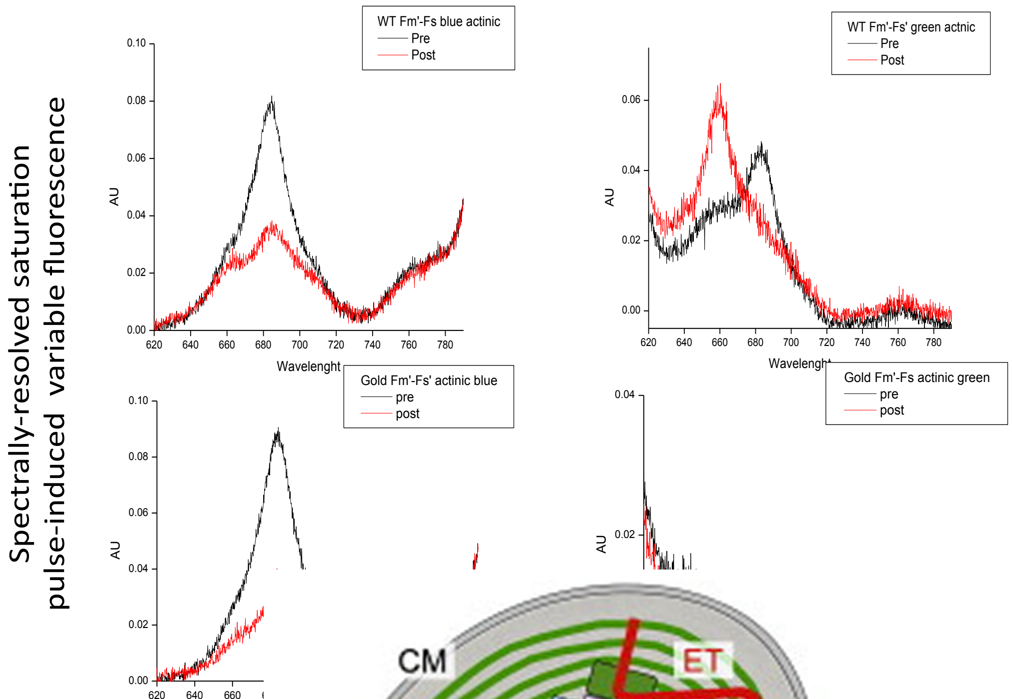


This is an excellent system to study the importance of antenna composition for balancing efficient light capture, photoprotection, “competitive shading”, etc.

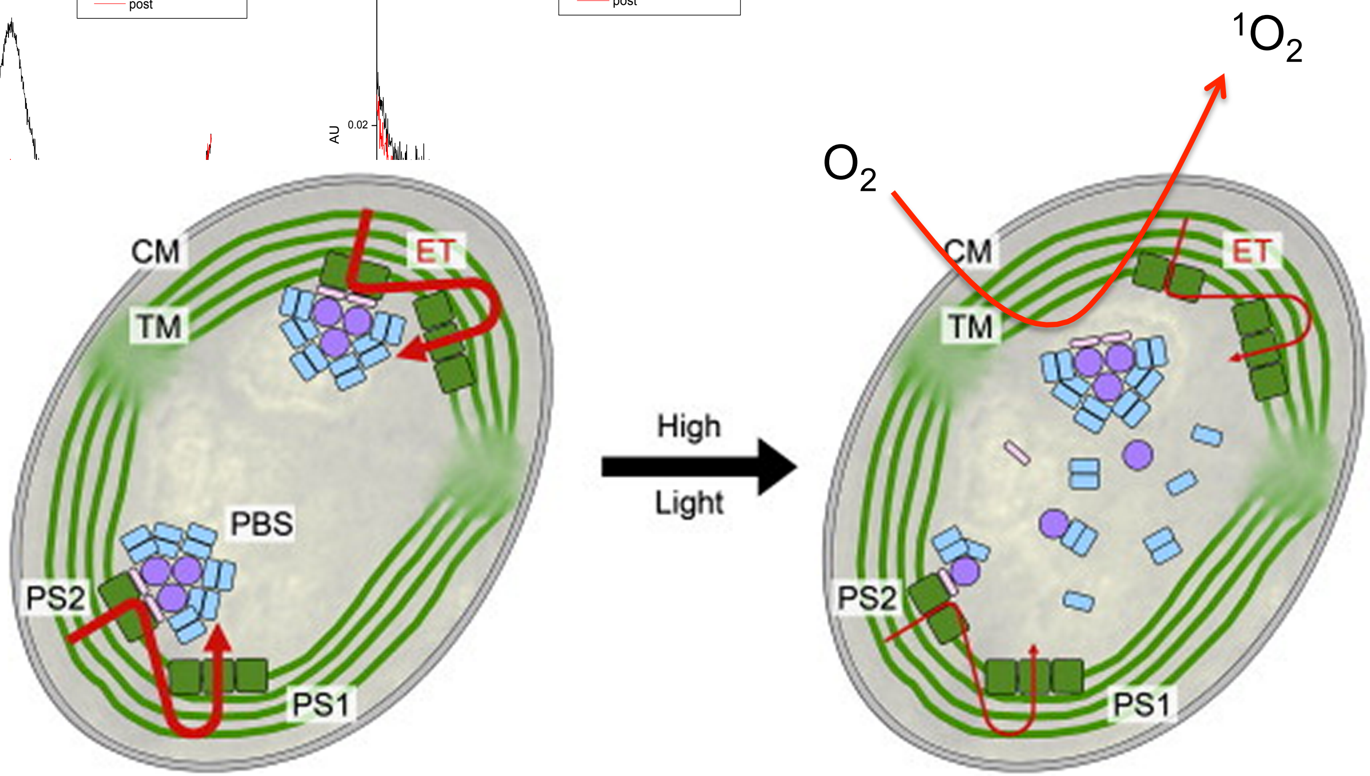
Low antenna "GOLD" beats Wt under fluctuating light



GOLD >>> WT **WT >>> GOLD**



Partially tested model: rapid changes in light leads to detachment of phycobisomes which leads to ROS.

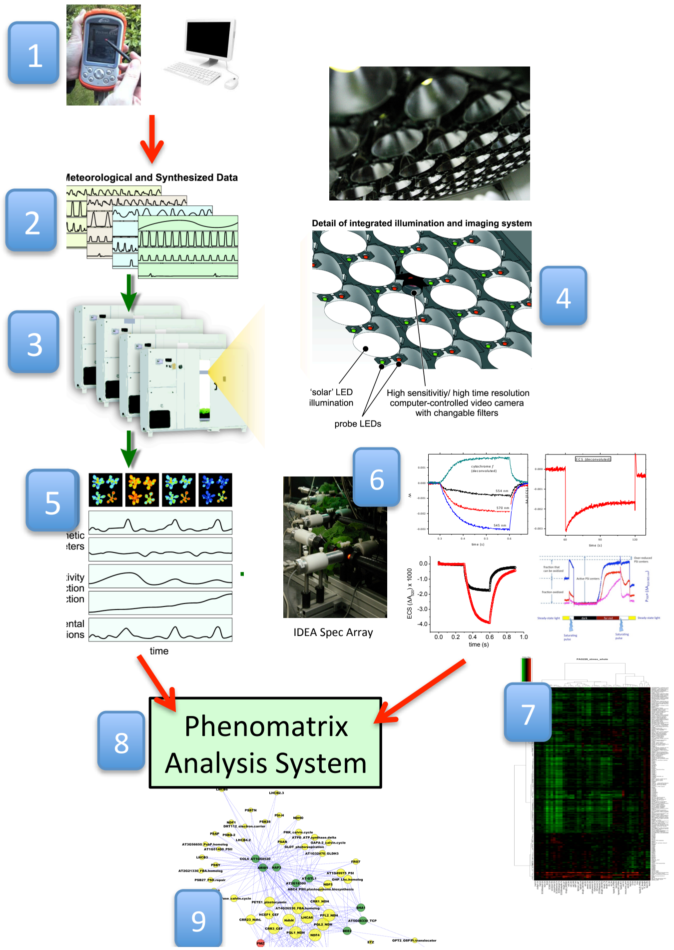


Both Wt and GOLD sh
exposed to strong (10
light for 30 min. In th
blue, probing variable
by chlorophyll antenn

Structural and functional alterations of cyanobacterial phycobilisomes induced by high-light stress

Eyal Tamary[a, b], Vladimir Kiss[a], Reinat Nevo[a], Zach Adam[b], Gábor Bernát[c], Sascha Rexroth[c], Matthias Rögner[c], Ziv Reich[a],

[a] Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

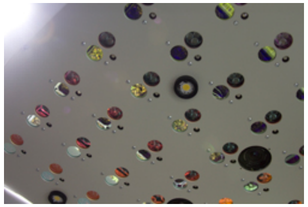


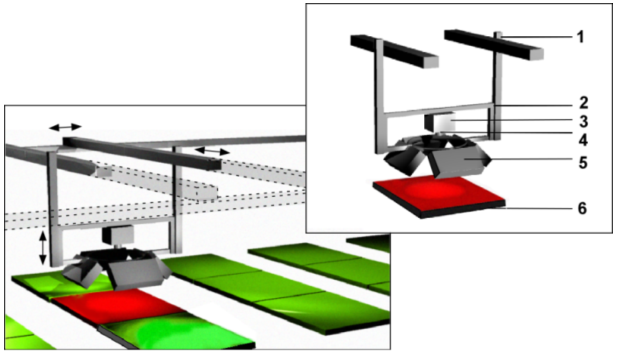
Our approach for plants:

4-dimensional in situ imaging, registration and Modeling (4D-ISIRM)

DEPI: Dynamic Environmental Photosynthetic Imaging Plantform (DEPI)

FluorCam (PSI/Qubit/Waltz)





Requires non-natural LED color

- Cannot grow and measure under same conditions
- Blocks/alters light under measuring conditions
- Limit of dynamic measurement range

Compare to current systems

3D imaging

One approach: move plants via conveyer system, rotate to image

Advantages:

Imaging plants individually dramatically simplifies 3D reconstruction (e.g. eliminates background clutter; provides continuous internal reference)

One imager can service many plants

Commercially available

Disadvantages (that we address)

Moving the plant

*imposes dynamic environmental changes

induces stress responses

misses dynamic responses

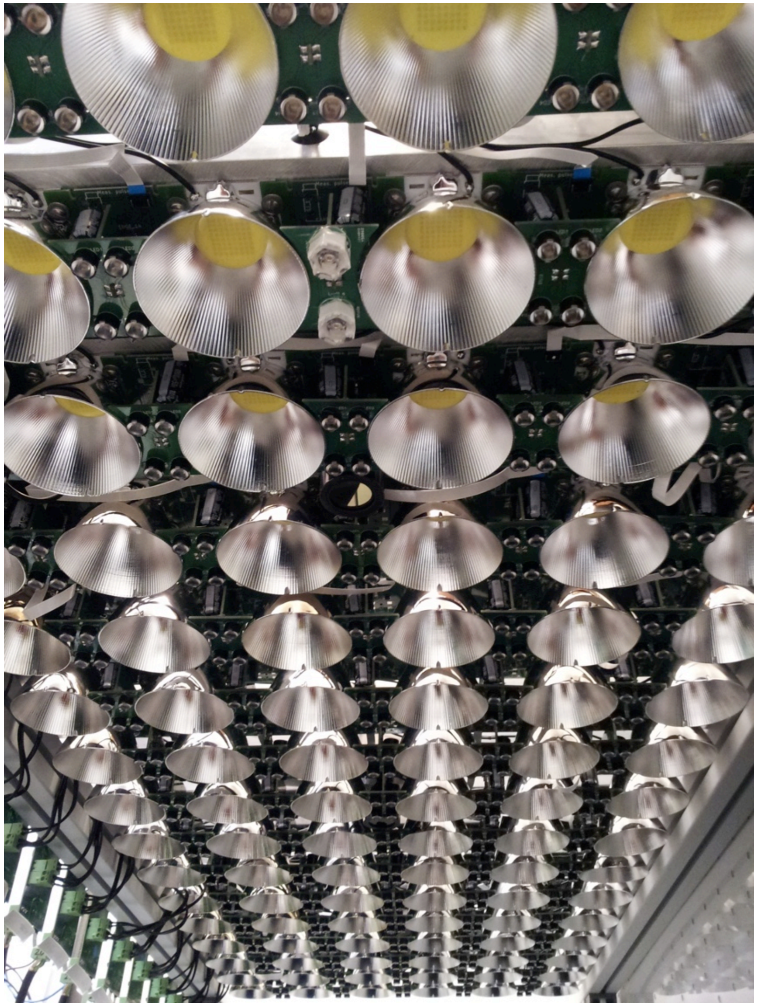
takes plant out of its canopy (development)

limits the type of container (scalable?)

limits image resolution and accuracy (time-sequence problem at least in our hands)

cannot give simultaneous measurements of other phenotypes(?)

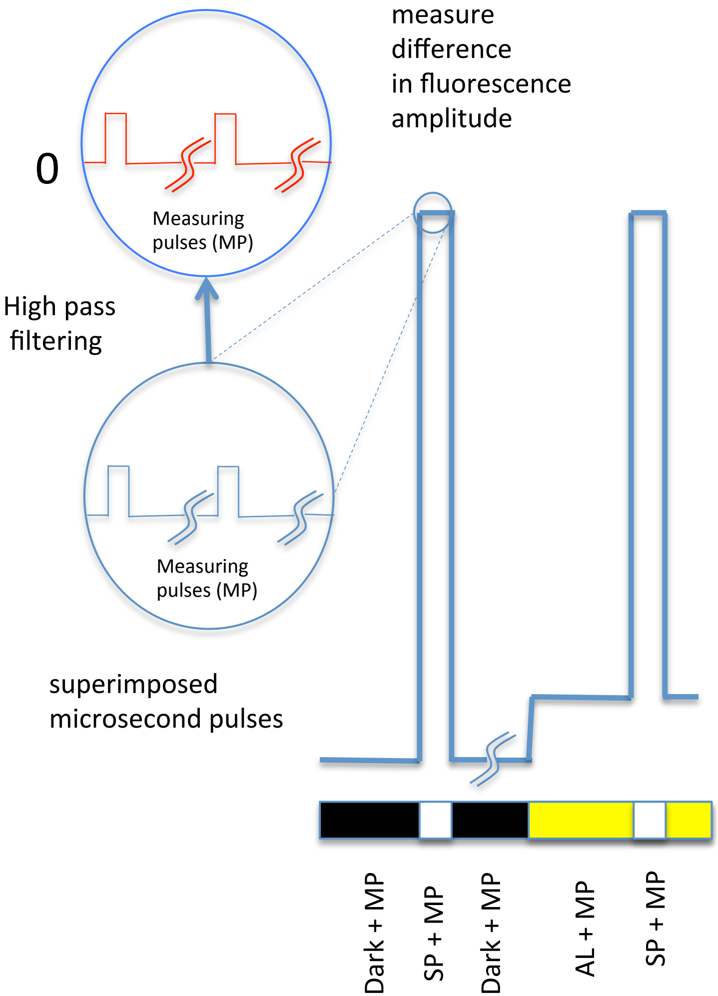
Field-deployable (?)



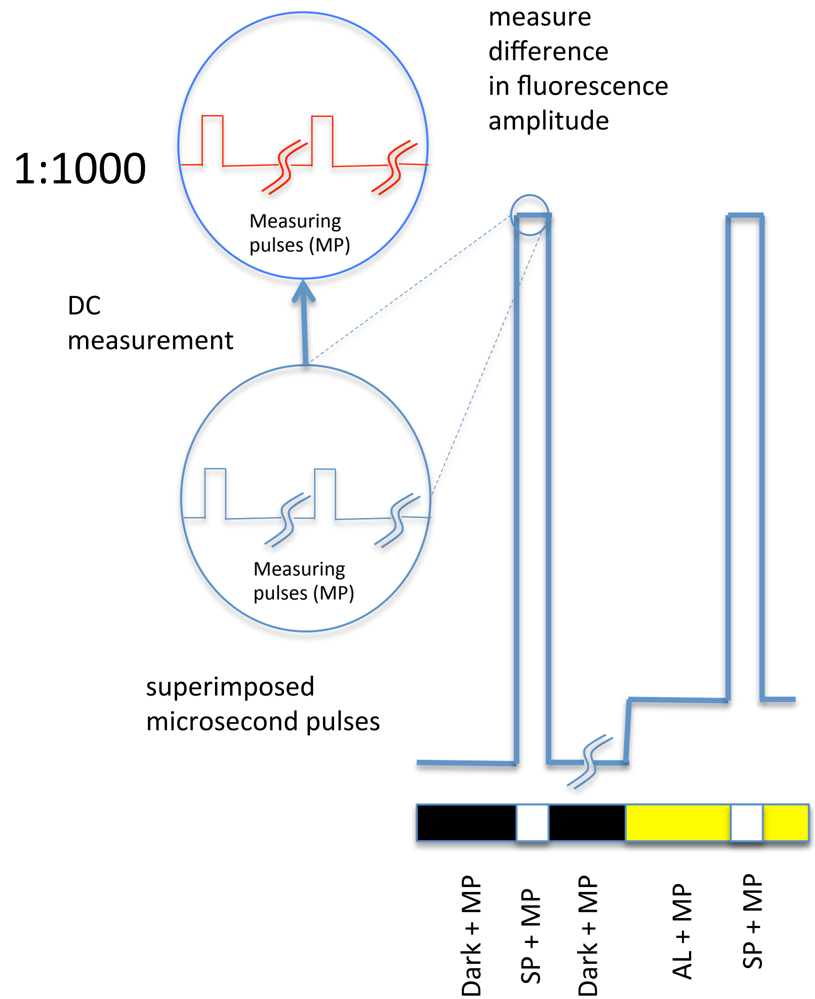
4D Dynamic Environmental Phenotype Imaging (4D-DEPI)

Specifically to non-invasively measure phenotypes continuously and dynamically (over rapid or developmental time scales) under conditions that mimic those in the field.

PAM measurement



FLUORCAM measurement

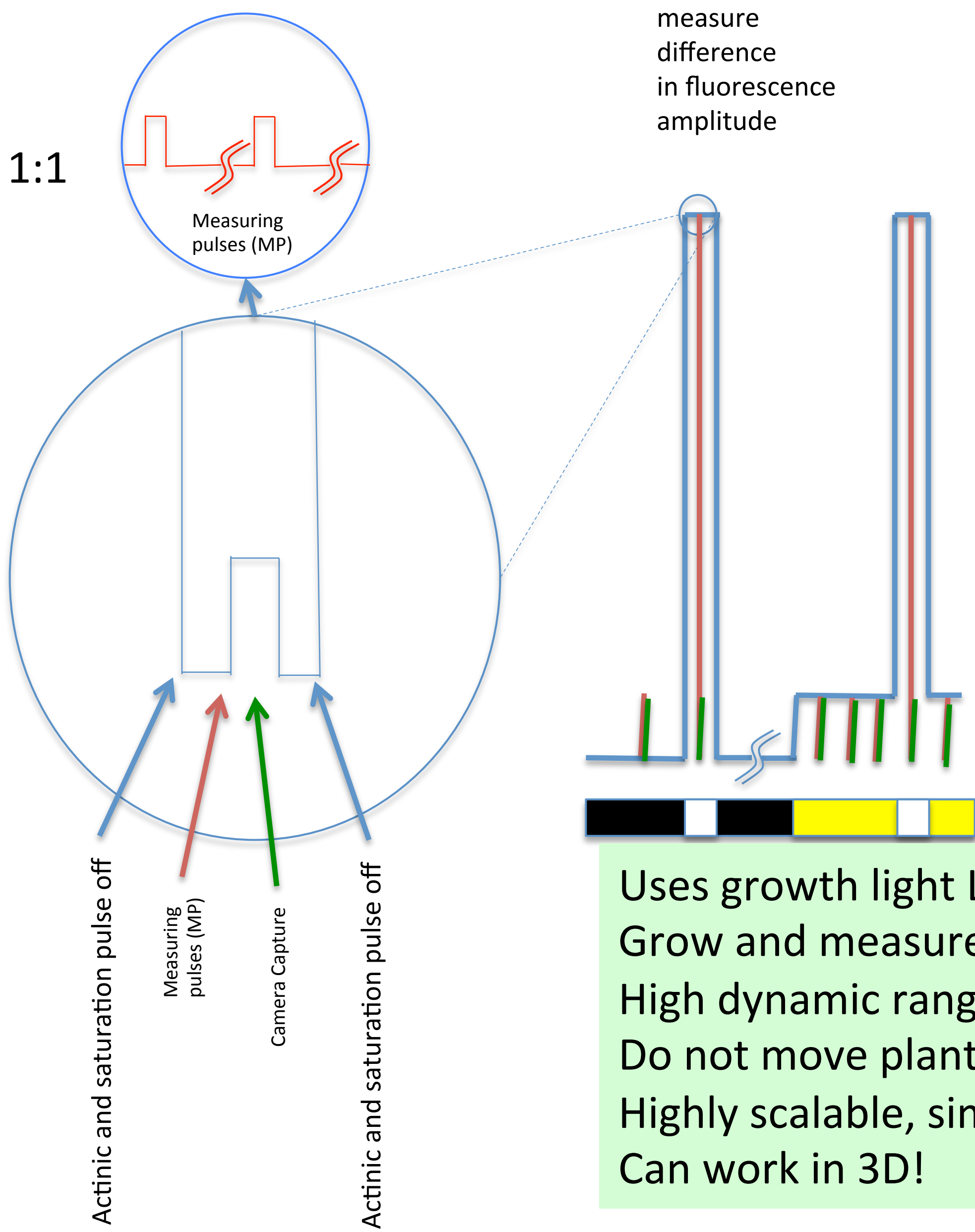


Requires non-natural LED color

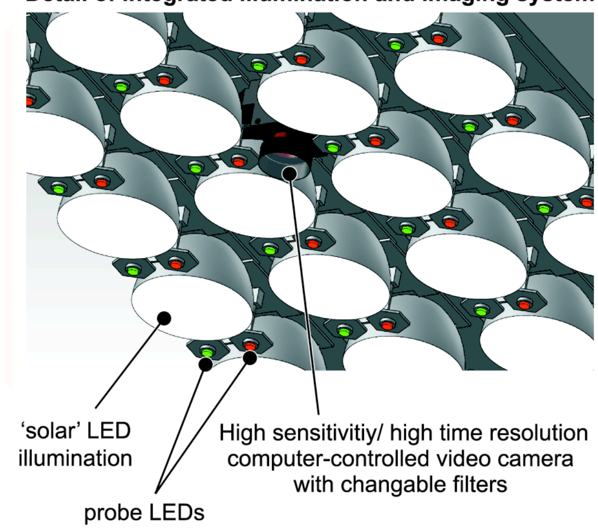
Cannot grow and measure under same conditions

Limit of dynamic range

Dynamic Environmental Photosynthesis Imager (DEPI) (simplified)



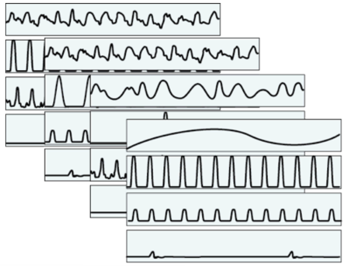
Detail of integrated illumination and imaging system



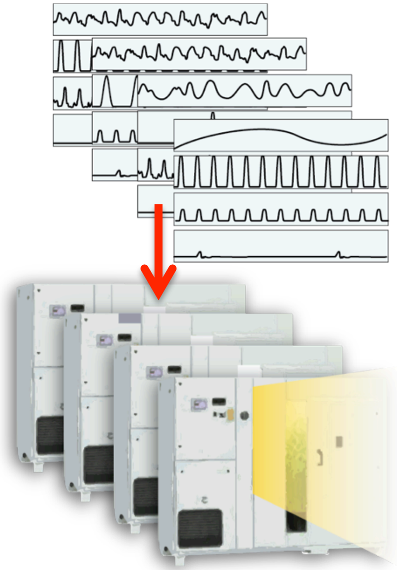
Uses growth light LED
Grow and measure under same conditions (dynamics!)
High dynamic range
Do not move plant
Highly scalable, simultaneous (high throughout)
Can work in 3D!

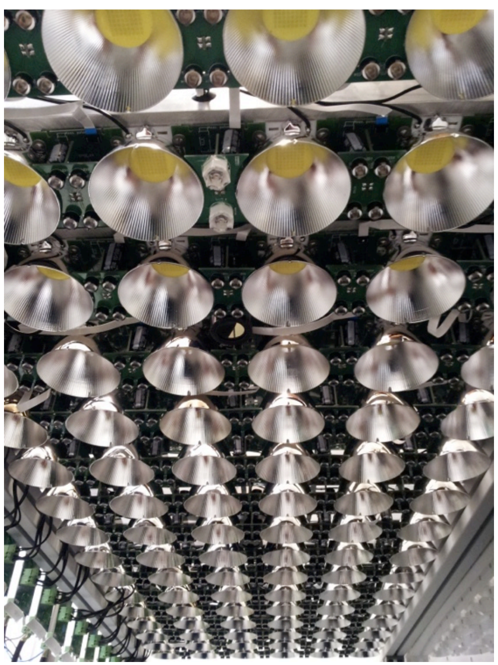
1: Measure or synthesis sets of environmental parameters:

Start with light, temperature, humidity



Component analysis



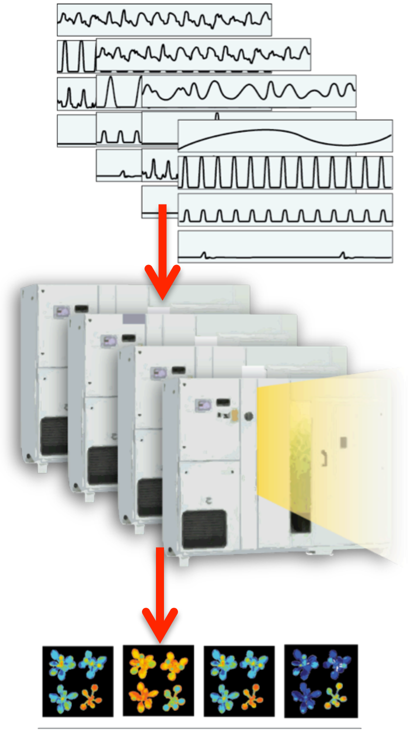


2: Replay dynamic* environmental parameter conditions reproducibly in MULTIPLE specially-designed chambers. (Approach the kinds of fluctuations seen in nature).

Start with light, temperature, humidity etc.

- VERY even light intensities
- Light intensities mimicking solar influx
  - High intensity (>2500 μmol m^{-2} s^{-1})
  - Fluctuating
  - Spectral (starting with White LEDs)**
- Temperatures (freezing, chilling, heat)
  - fluctuating from -10^{o}C to 45^{o}C
- Humidity

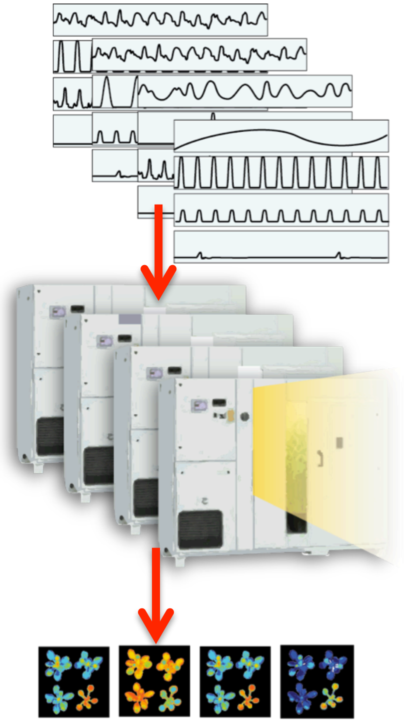
*key distinction



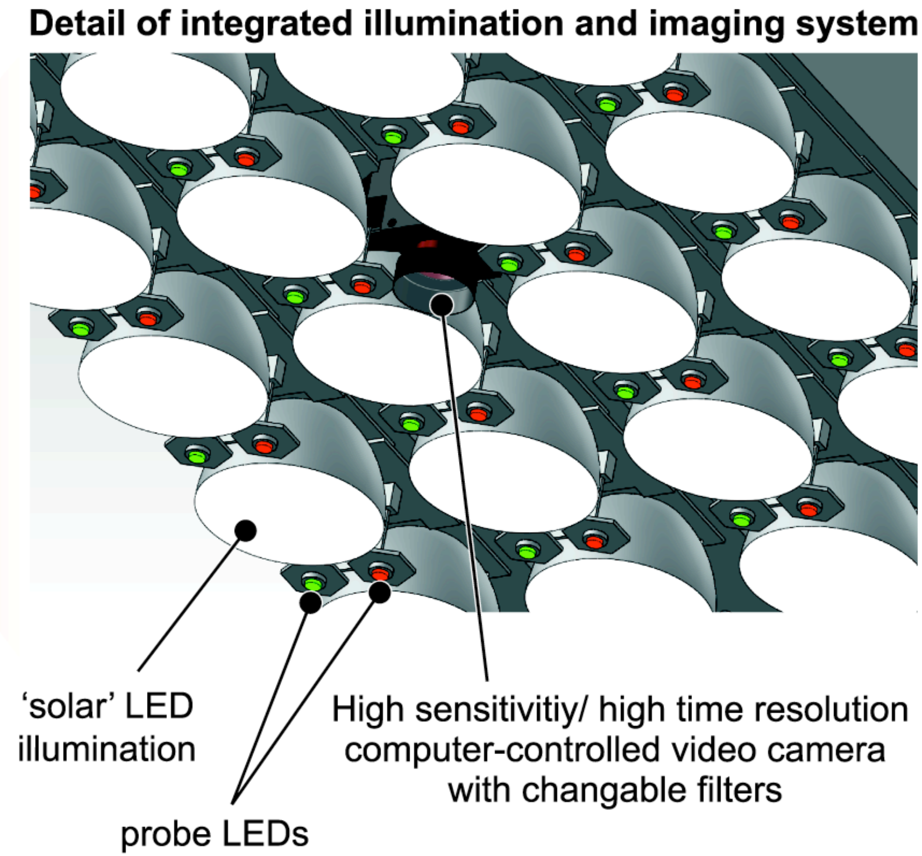
3: Measure key photosynthetic parameters overall **all plants continuously*** **to capture** **transient events**

Fluorescence
- Efficiency
- Photoprotection
- Redox states
- stomatal aperture

*key distinction



Sophisticated engineering problem



- Parallel imaging of fluorescence under continuous WHITE light
- Massive data collection
- High power LED system (80 KW pulses)
- Open source software/hardware

Extended Capability Matrix

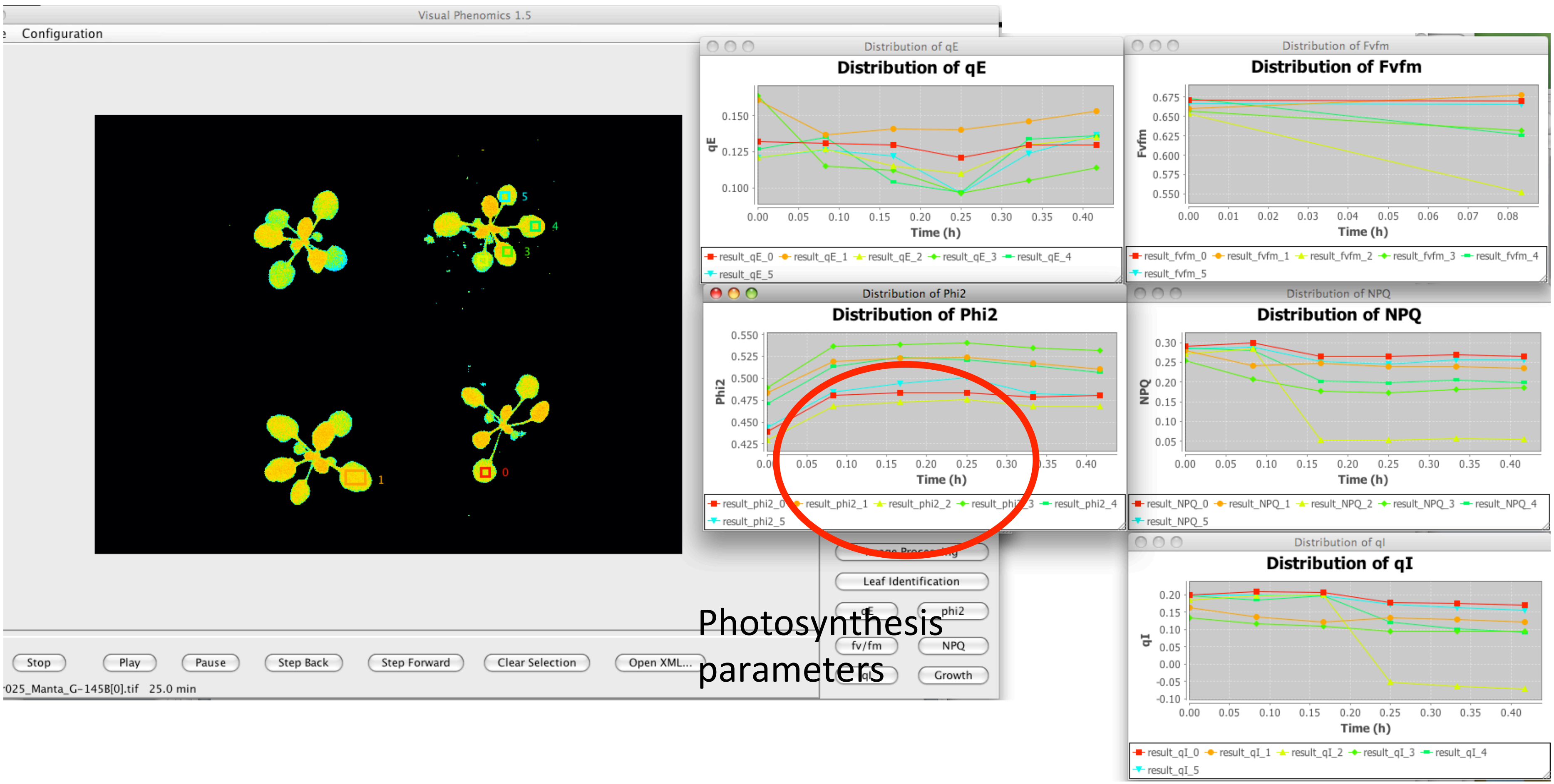
Freezing/high temperature

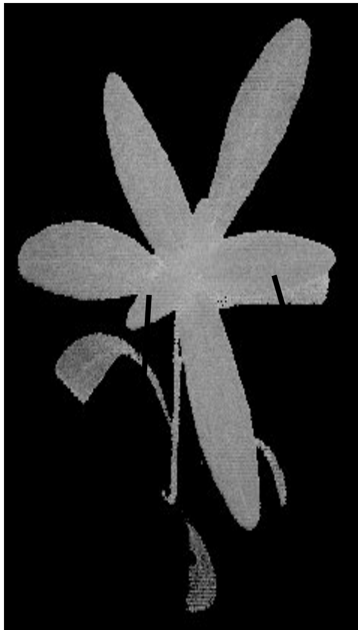
High CO_2/low CO_2

Light quality

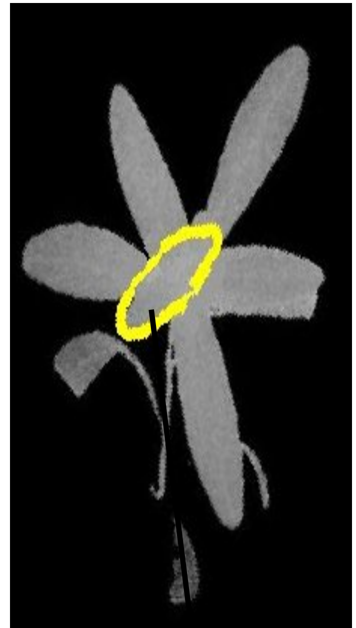
Chemicals/hormones

VisualPhenomics: workhorse of the system: primary analysis and visual representation of data

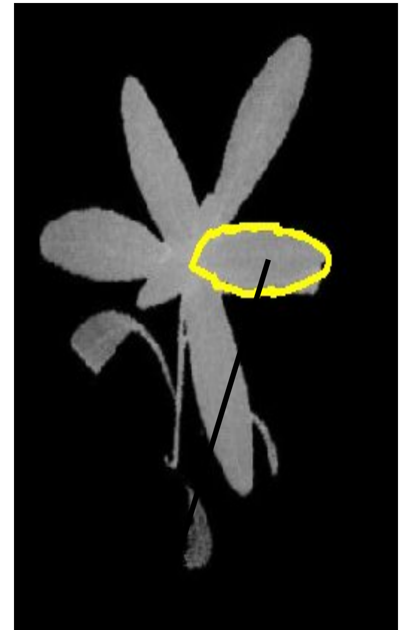




Original Image

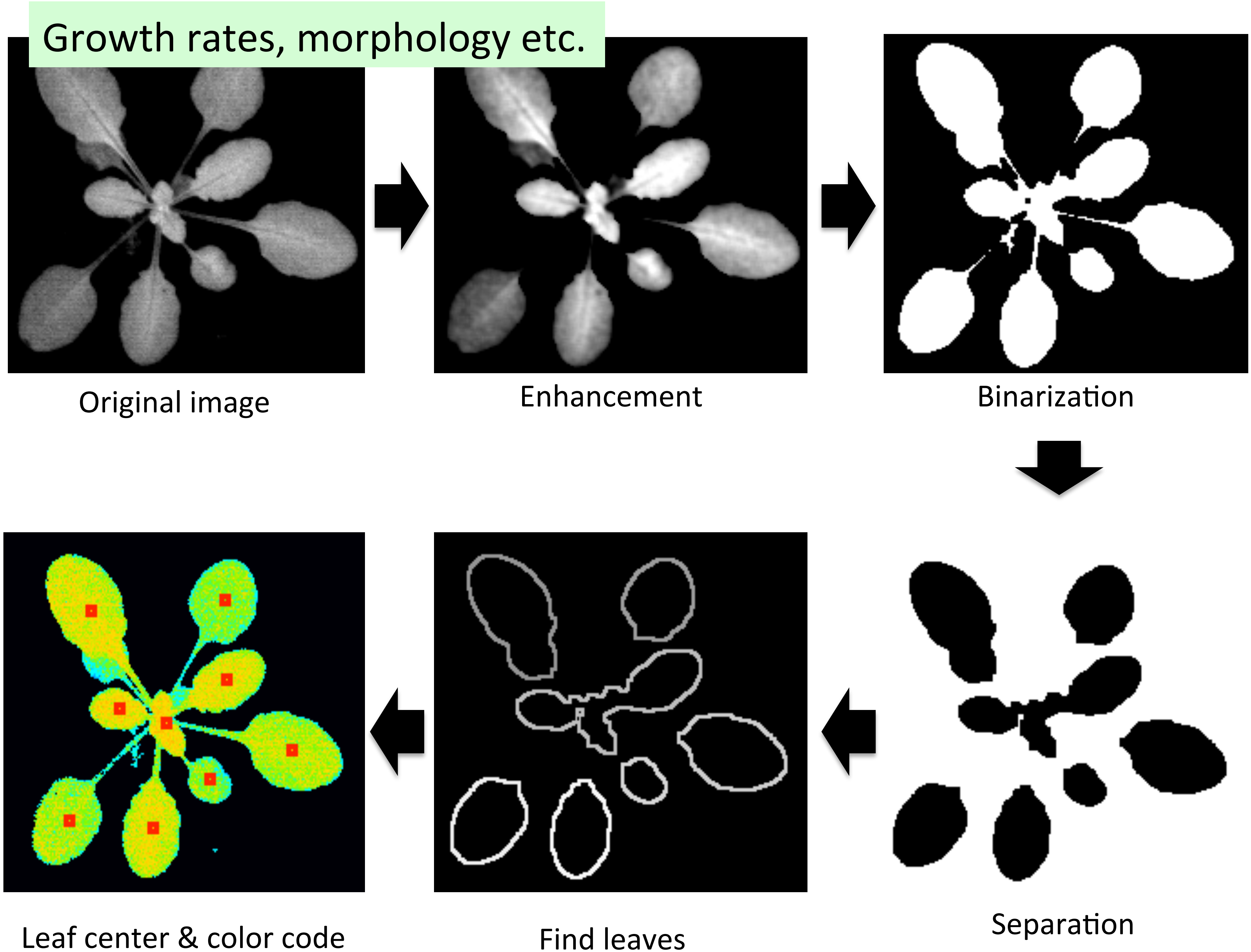


Center leaf Identification



Overlapped leaf Identification

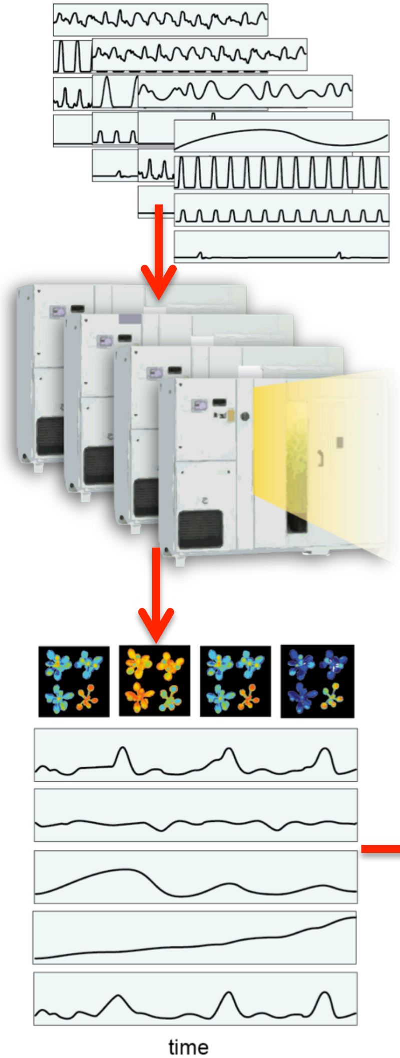
We applied the active appearance model (AAM) for leaf identification. AAM is a computer vision algorithm for matching a statistical model of object shape and appearance to a new image.



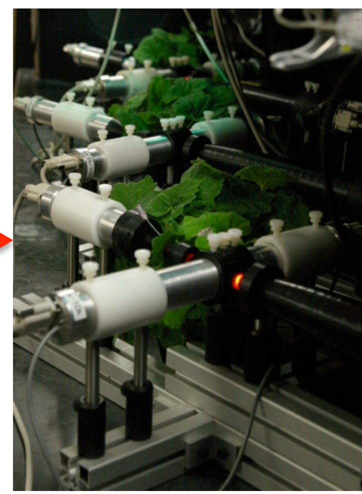
Growth rates, morphology etc.
Original image
Enhancement
Binarization
Separation
Find leaves
Leaf center & color code

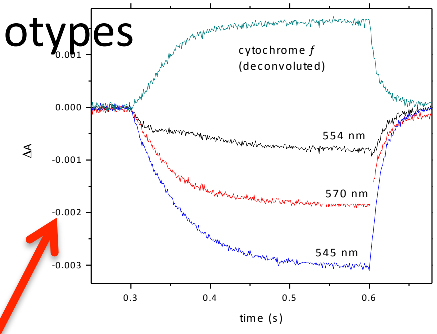
6: High resolution photosynthetic phenotypes

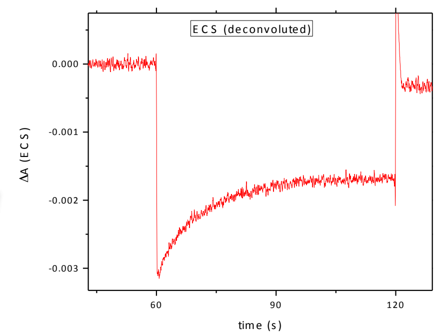
- Photochemistry
- Photoprotection
- Electron and proton transfer
- Gas exchange
- Metabolites (RFC instrument)

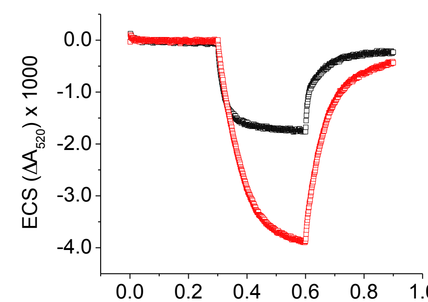


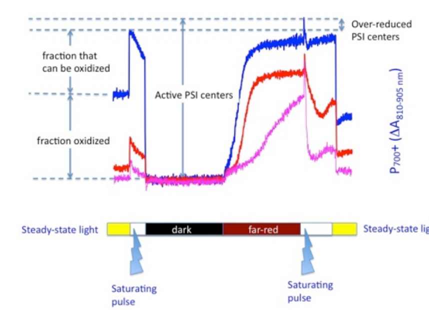
IDEA Spec Array





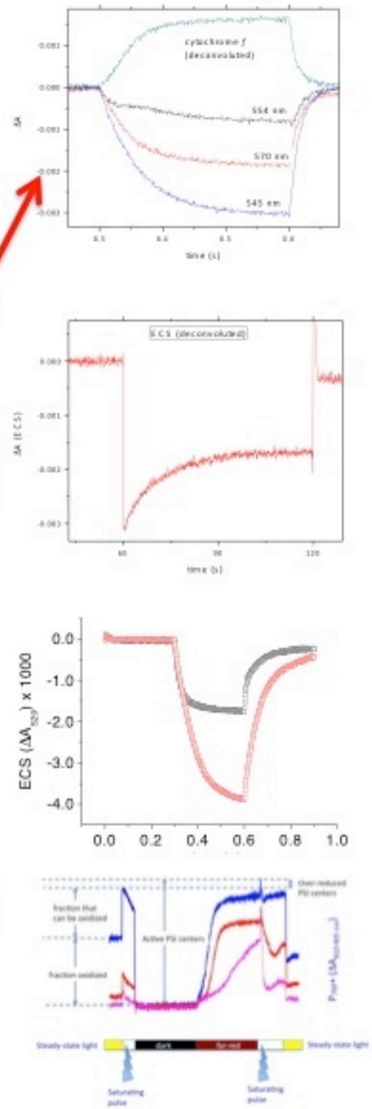


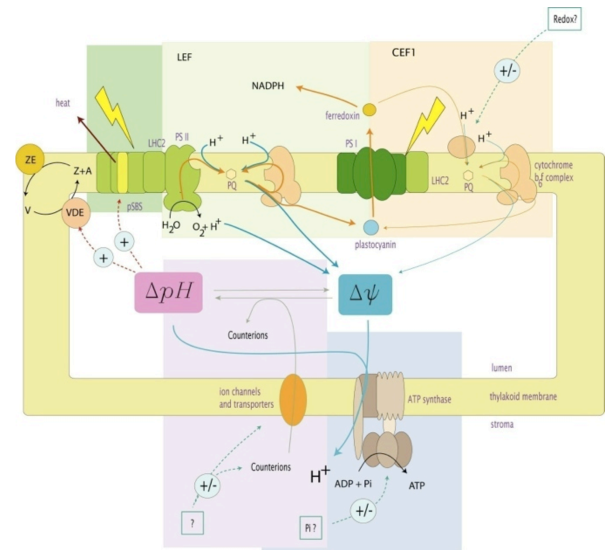




6. Dealing with large data sets,
A. Automated analysis of data into systems models.

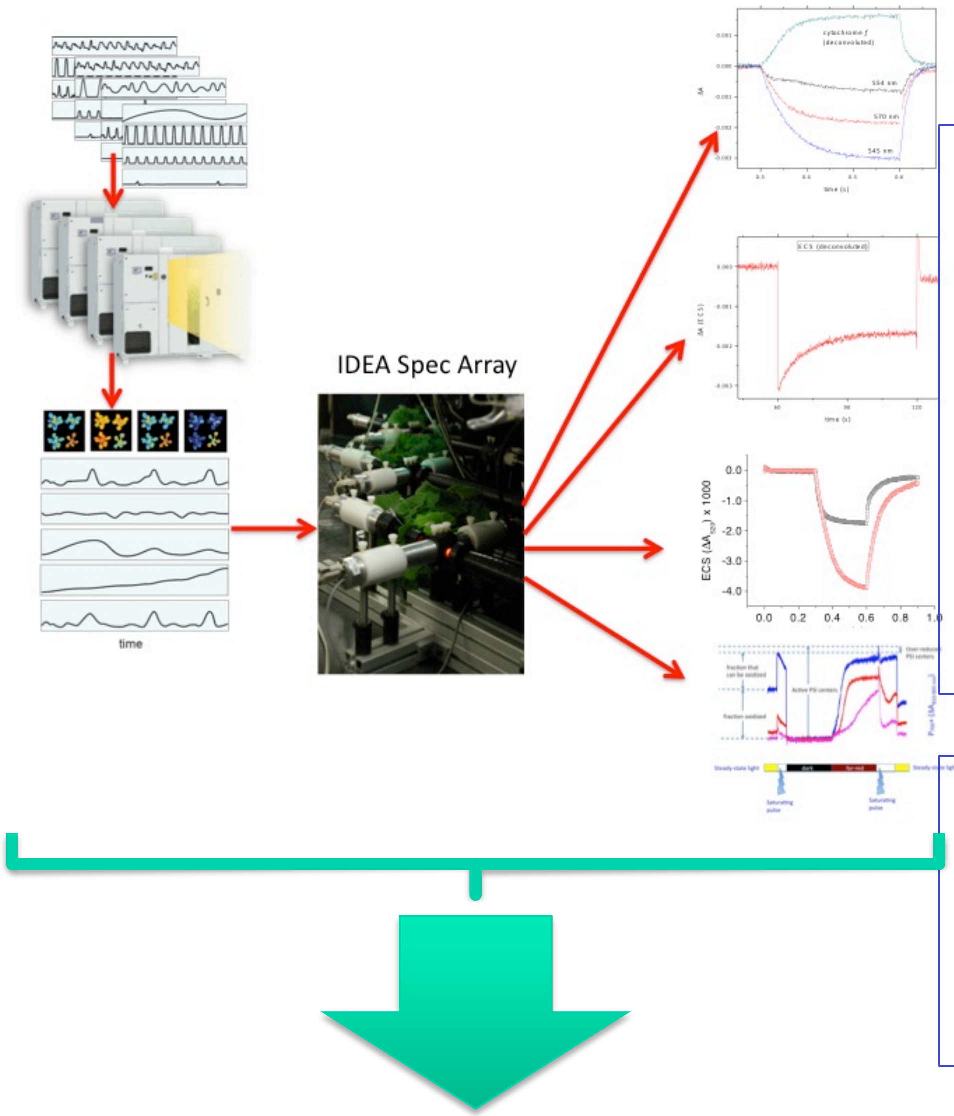
IDEA Spec Array





Kent Kovac (MSU)
Andrew Tomes (MSU)
Deserah Strand (MSU)
Jeffrey Cruz (MSU)

B: PhenoMath: Complex bioinformatics approaches



PAS

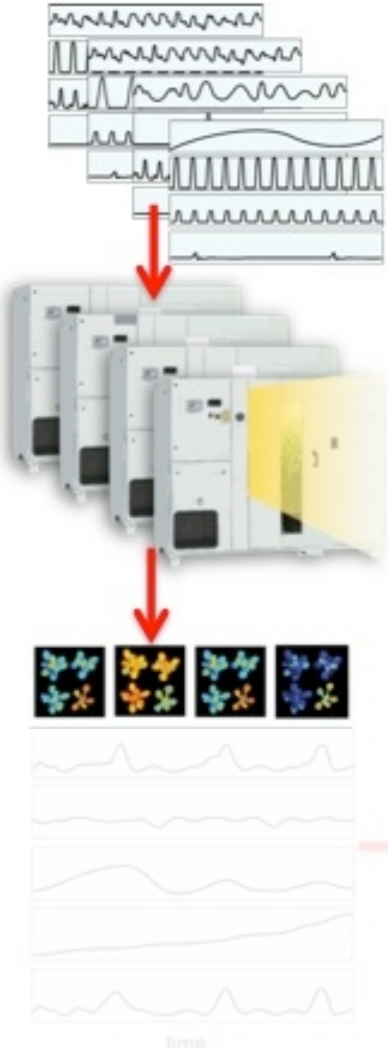
PhenoMatrix Analysis System (PAS)
Jin Chen

- Pattern mining for known mutants
  - Periodic condition patterns
  - Endpoint measurements of survival or productivity
  - Relationships (dynamic responses <-> known mutants)
- Search for the mined patterns in random mutants, ecotypes, etc
- identify new dynamic phenomena in random mutants

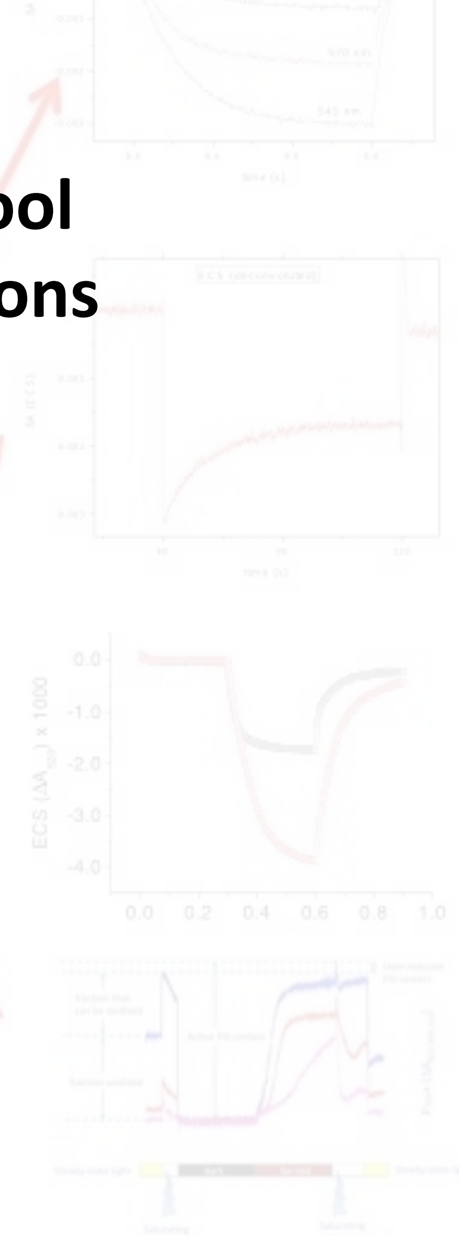
- Data mining
  - Pattern recognition
  - Clustering & Classification
- Statistics
  - Bayesian network

Jin Chen (PRL)
Yuhua Zhiao
Kent Kovac
Oliver Tessmer

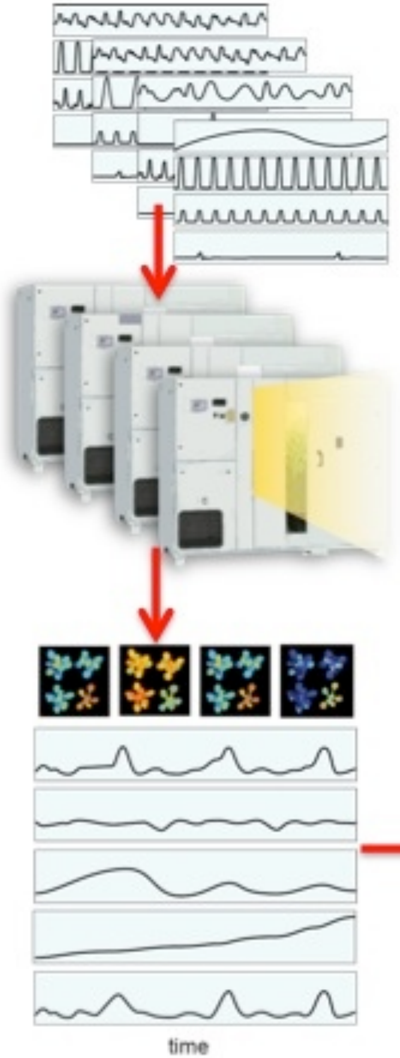
Parts of the facility can be used separately

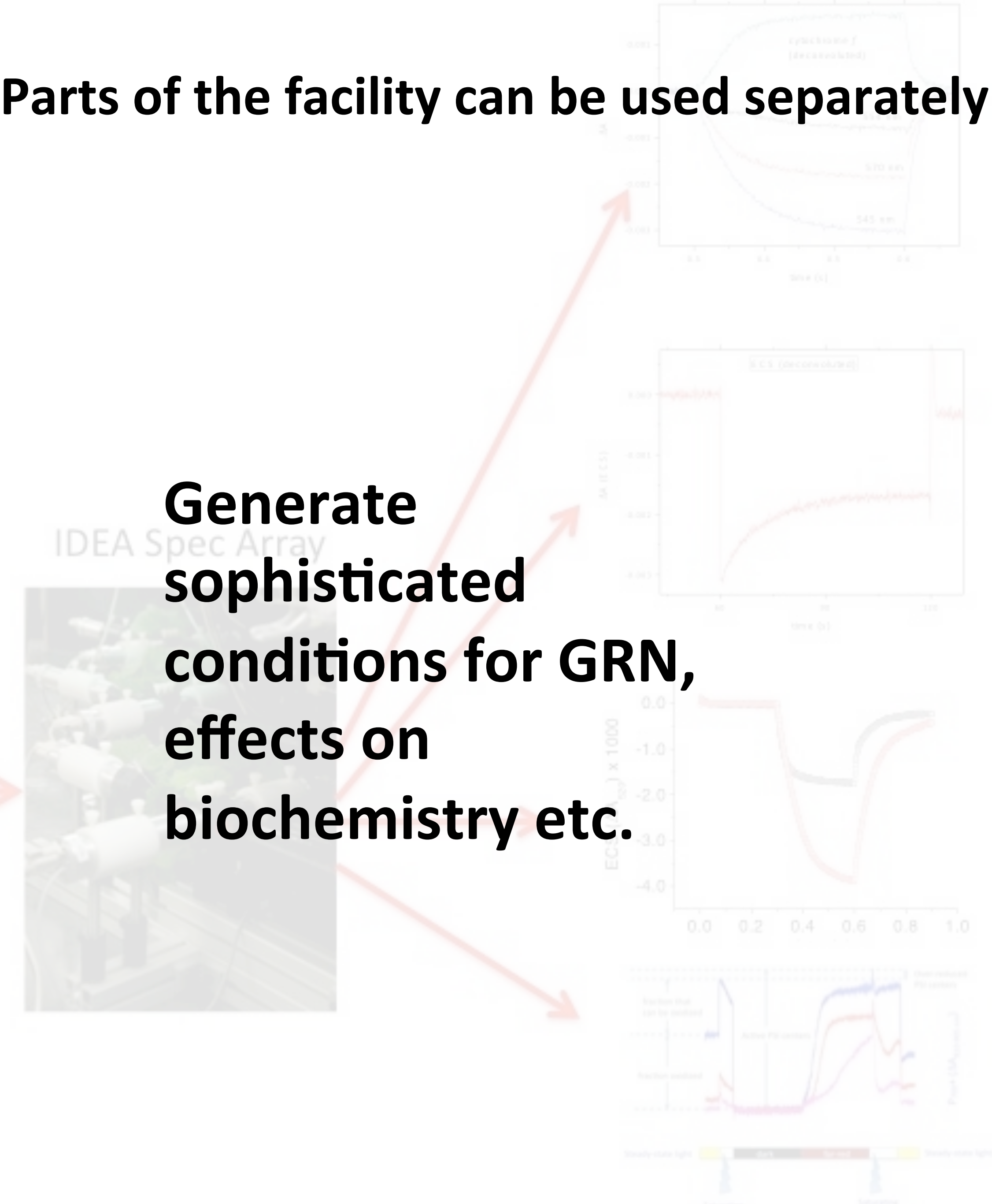


Rapid screening tool for genetic selections or condition assessment



Parts of the facility can be used separately



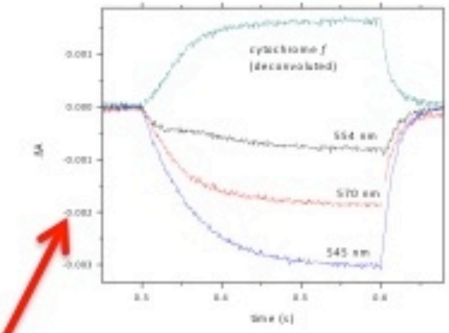


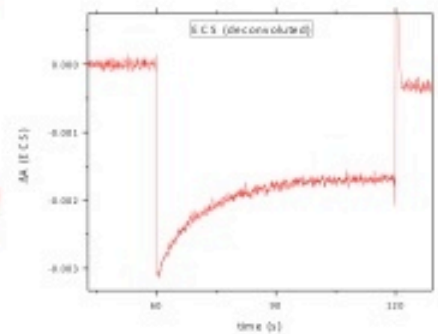
Generate sophisticated conditions for GRN, effects on biochemistry etc.

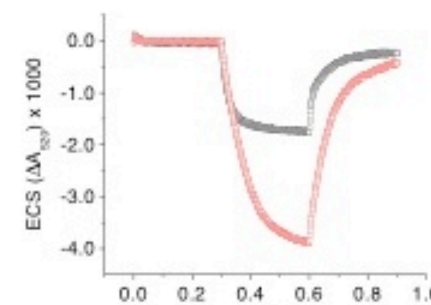
Powerful tools for characterization of photosynthetic phenotypes

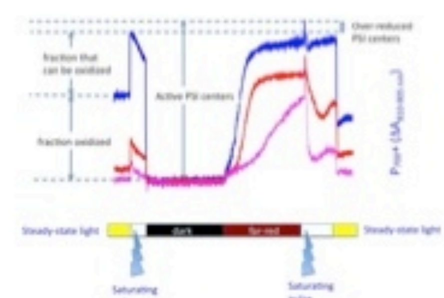
We now have 28 of these instruments, each one customized for specific measurements.

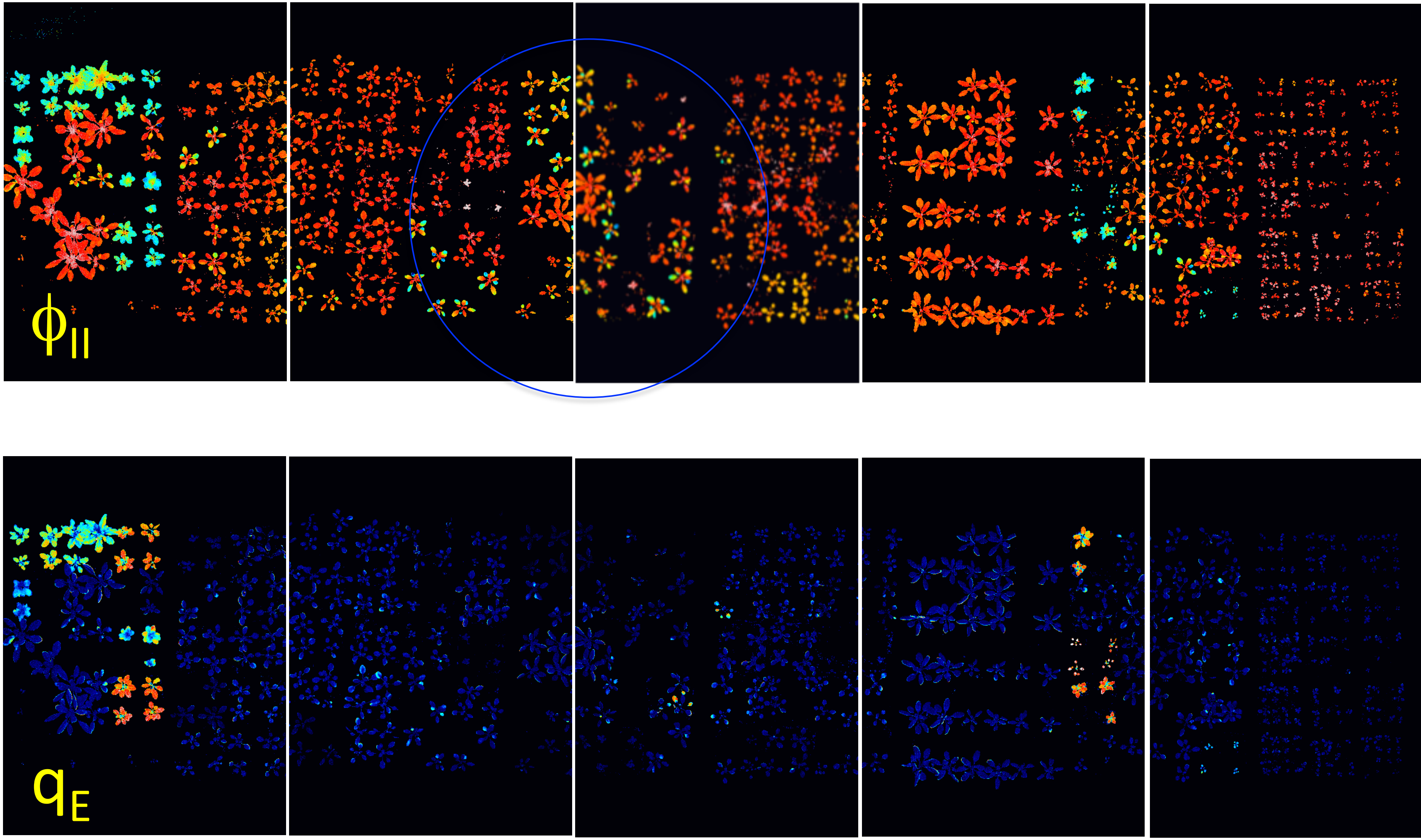
IDEA Spec Array

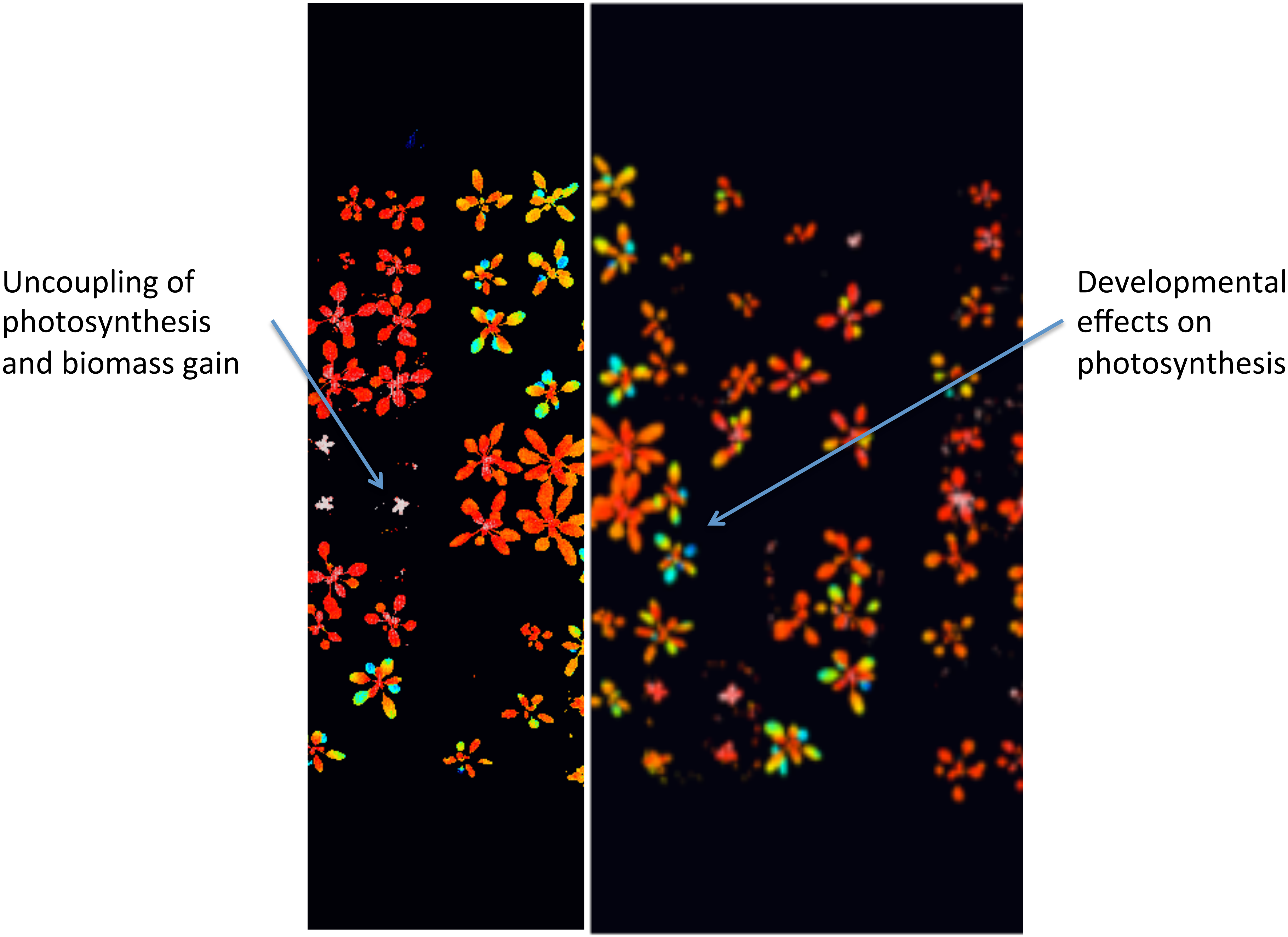






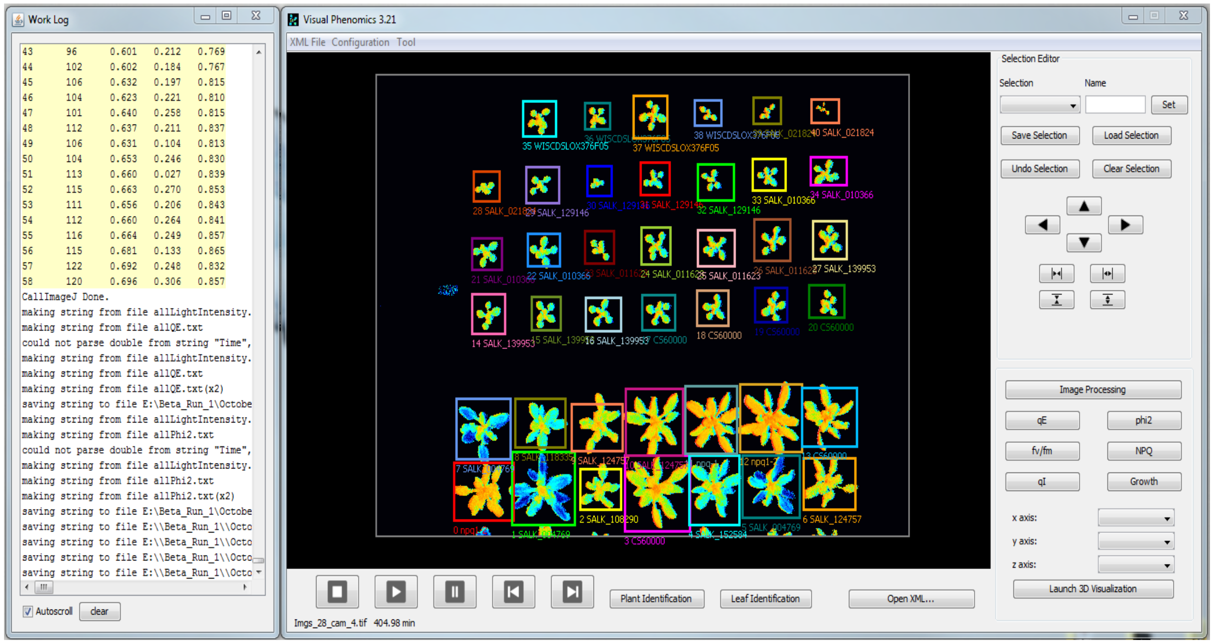



ϕ_{II}
q_E

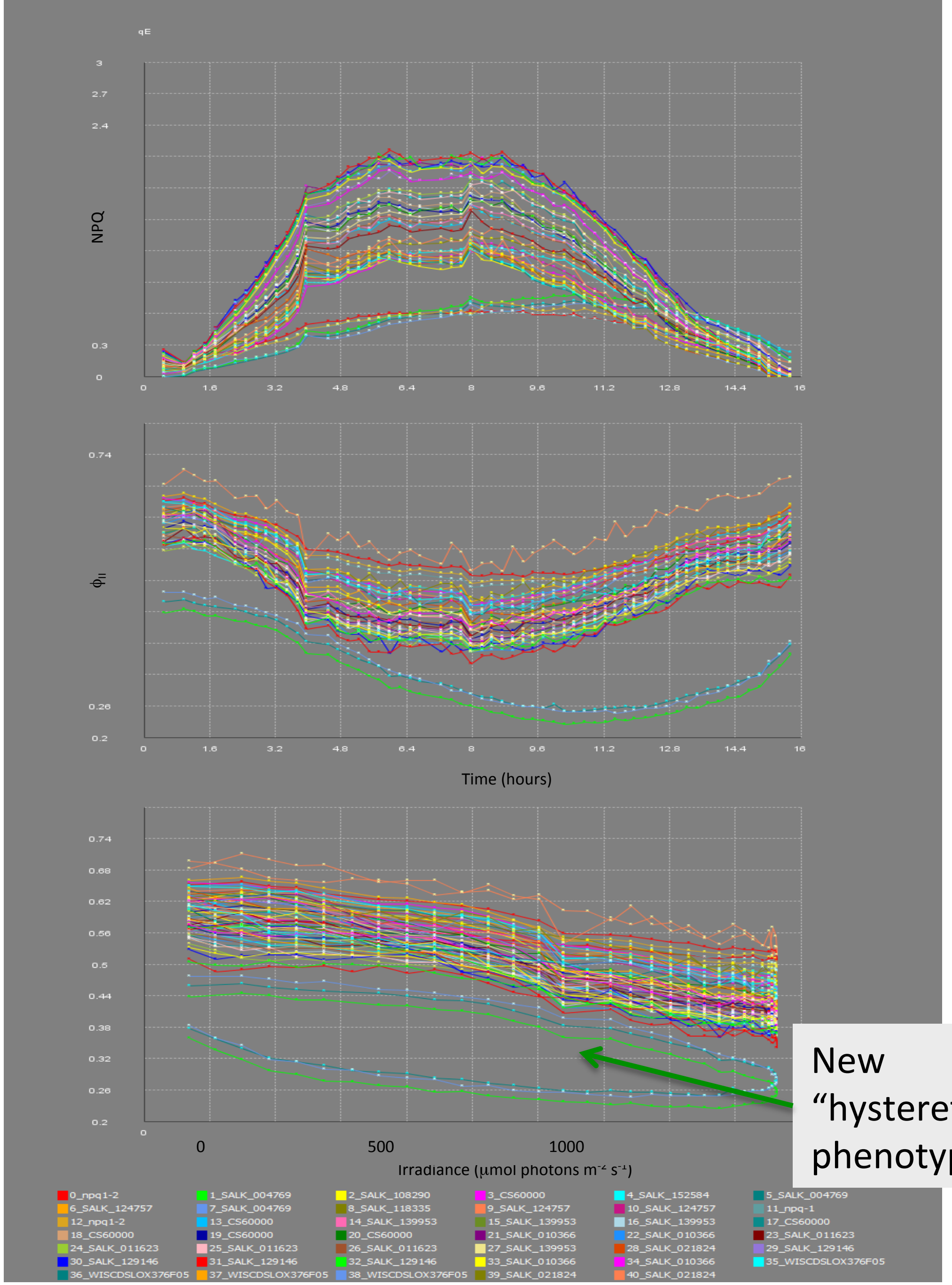

Uncoupling of photosynthesis and biomass gain
Developmental effects on photosynthesis

The functions of genes of unknown function

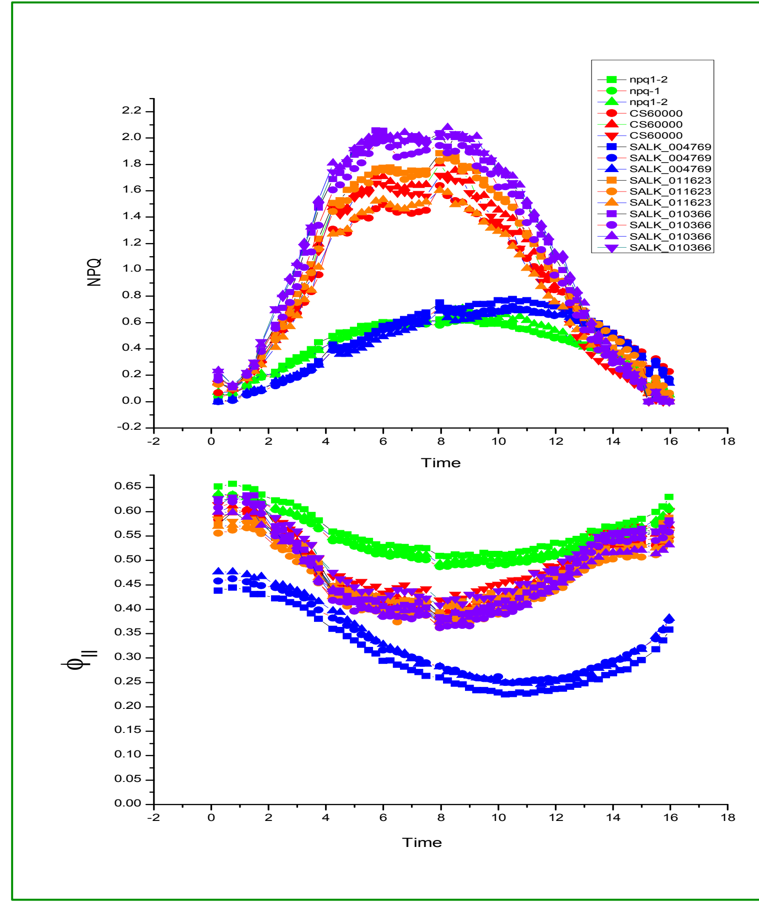
Linda Savage, Kent Kovac, Jeffrey A. Cruz, David M. Kramer, Robert Last



Question: how reliable is the data? Plant-plant variations, etc.



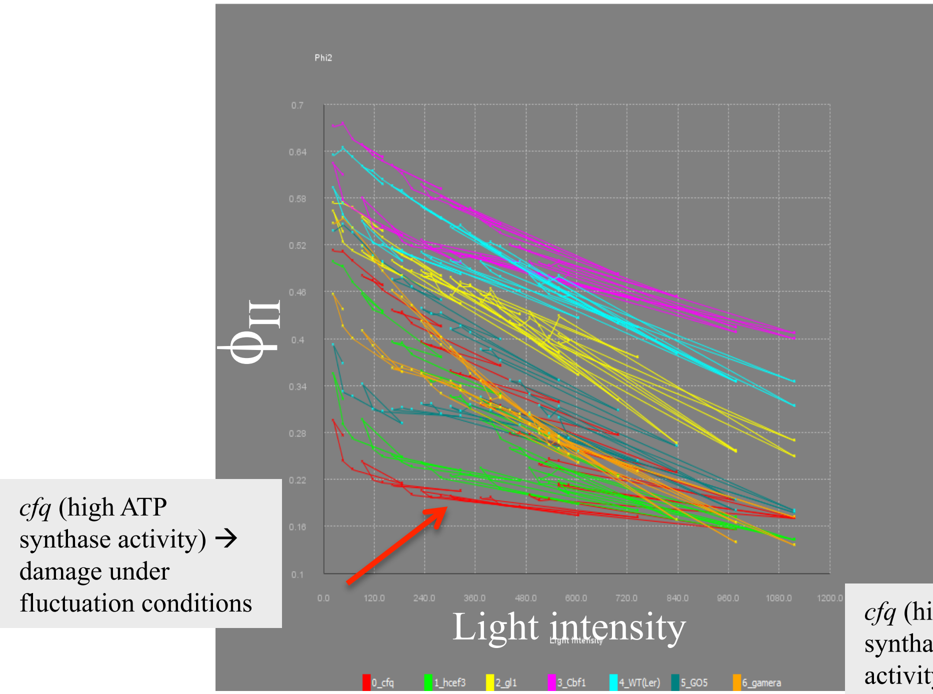
New "hysteretic" phenotype

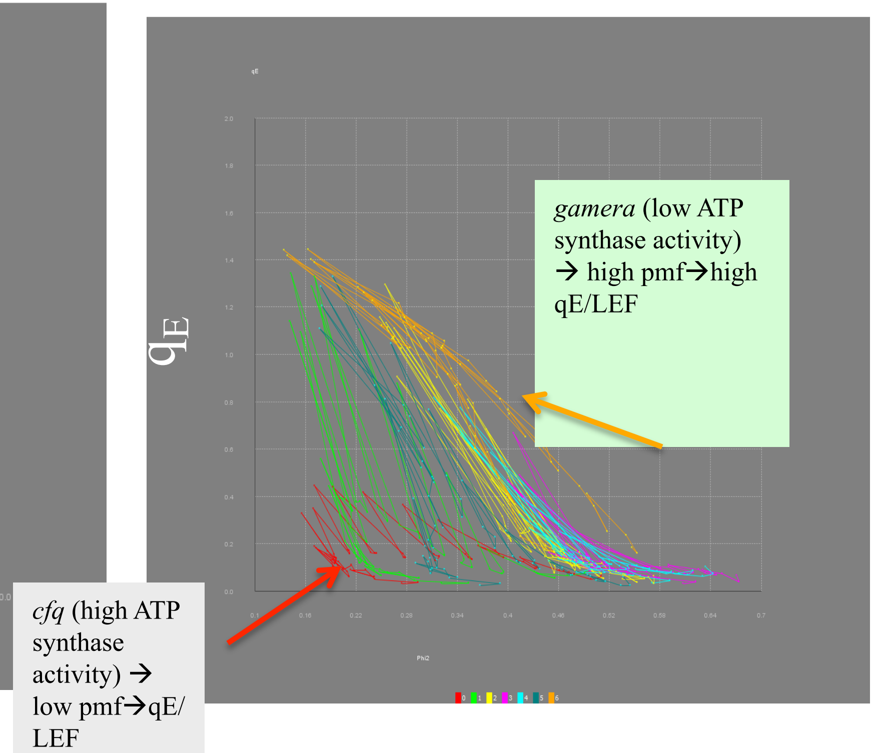


Important: Note the tight clustering of data from biological replicates grown in different chamber positions!

We can statistically distinguish better than 5% integrated variations between variants

Easily pick out mutants sensitive to environmental conditions using “hysteresis” analysis.

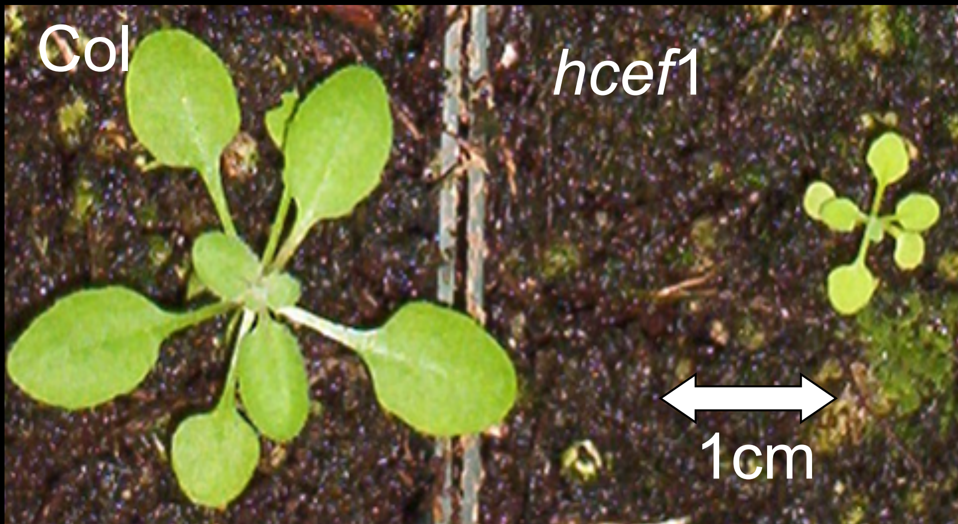


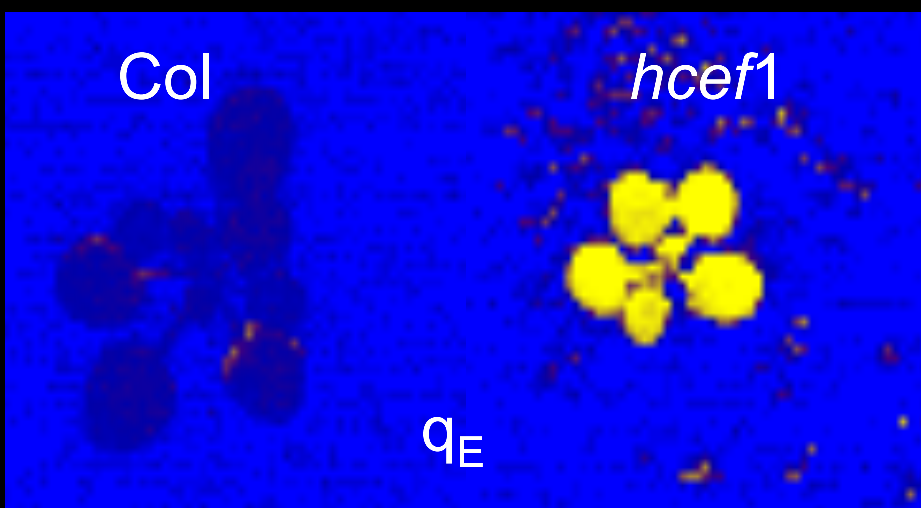


Hysteresis plot: ϕ_{II} vs. light reveals mutants damaged by fluctuating light

Altered relationships between photoprotection (qE) and PSII efficiency (ϕ_{II})

Allows sophisticated, multi-step selection screens for complex traits like cyclic electron flow.





~50,000 Arabidopsis EMS mutants

↓

High q_E (~400)

↓

High *pmf* ($ECS_{t,,}$ a-spec) (~390)

↓ *

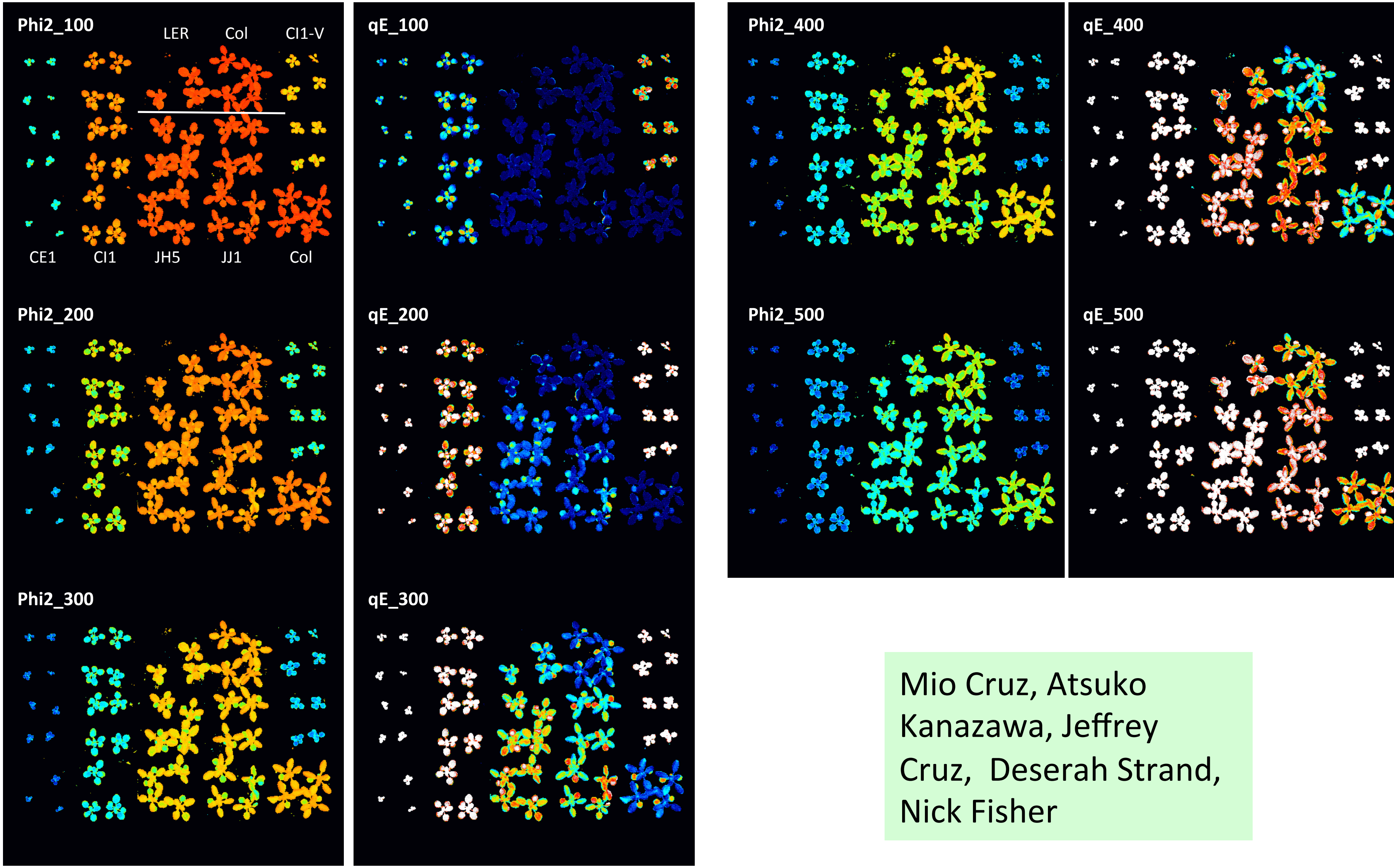
"normal" ATP synthase activity (5)

↓

hcef (5)

Livingston et al., (2010) Plant Cell

High Cyclic candidate mutant screening



Mio Cruz, Atsuko Kanazawa, Jeffrey Cruz, Deserah Strand, Nick Fisher

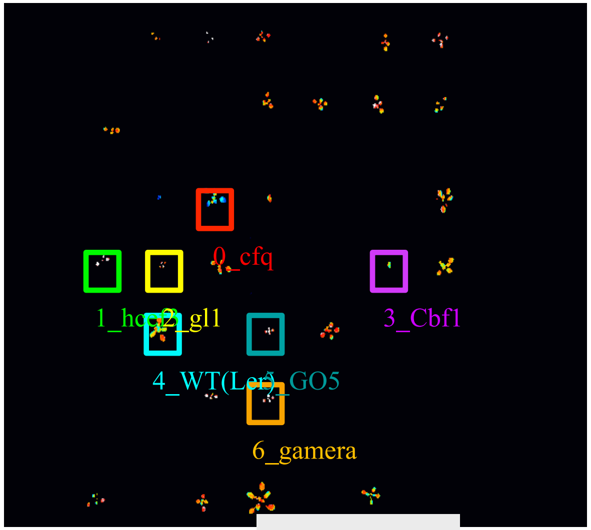
We are able to isolate mutants that differ in dynamic rather than static light intensity changes.

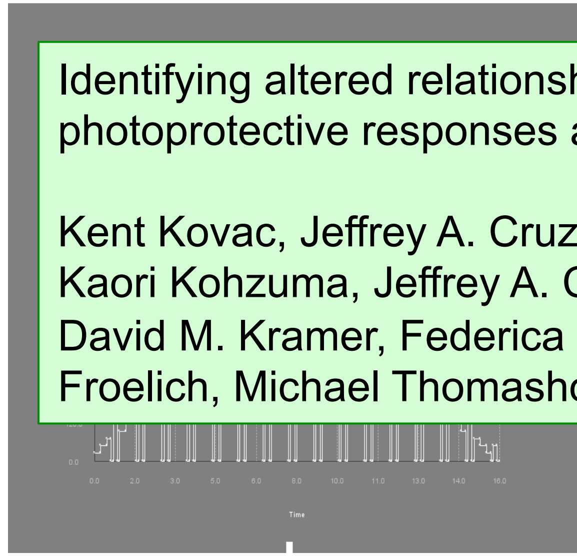
Conclusion:

It is very likely that such loci will affect performance in the field vs. controlled conditions. (More on this in the *cfq* story)

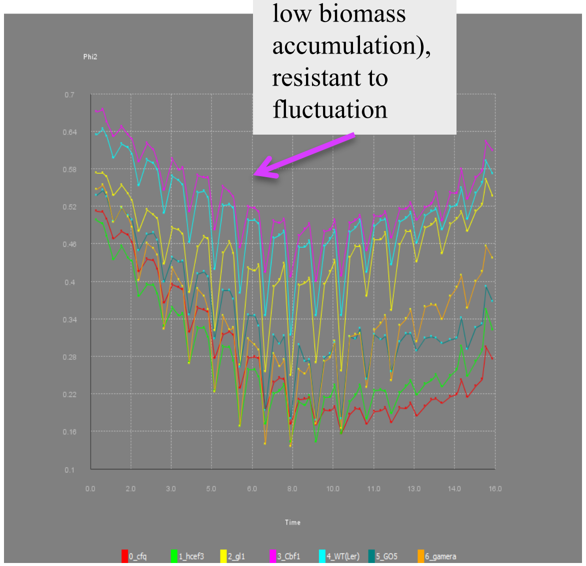
Identifying altered relationships between electron transfer, photoprotective responses and biomass accumulation

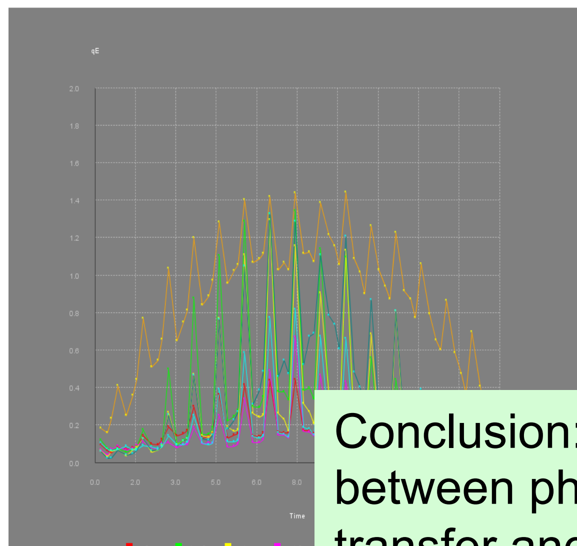
Kent Kovac, Jeffrey A. Cruz, Mio Cruz, Atsuko Kanazawa, Kaori Kohzuma, Jeffrey A. Cruz, Yuhua Jiao, Jin Chen, David M. Kramer, Federica Brandizzi, Jianping Hu, John Froelich, Michael Thomashow



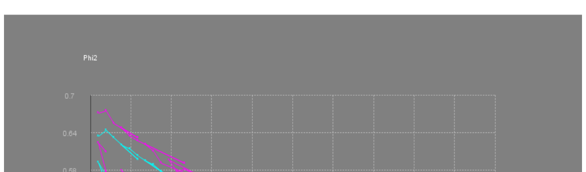


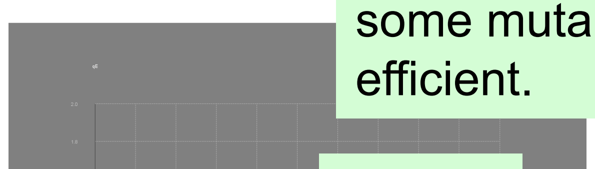
CBF2 (high ϕ_{II} low biomass accumulation), resistant to fluctuation



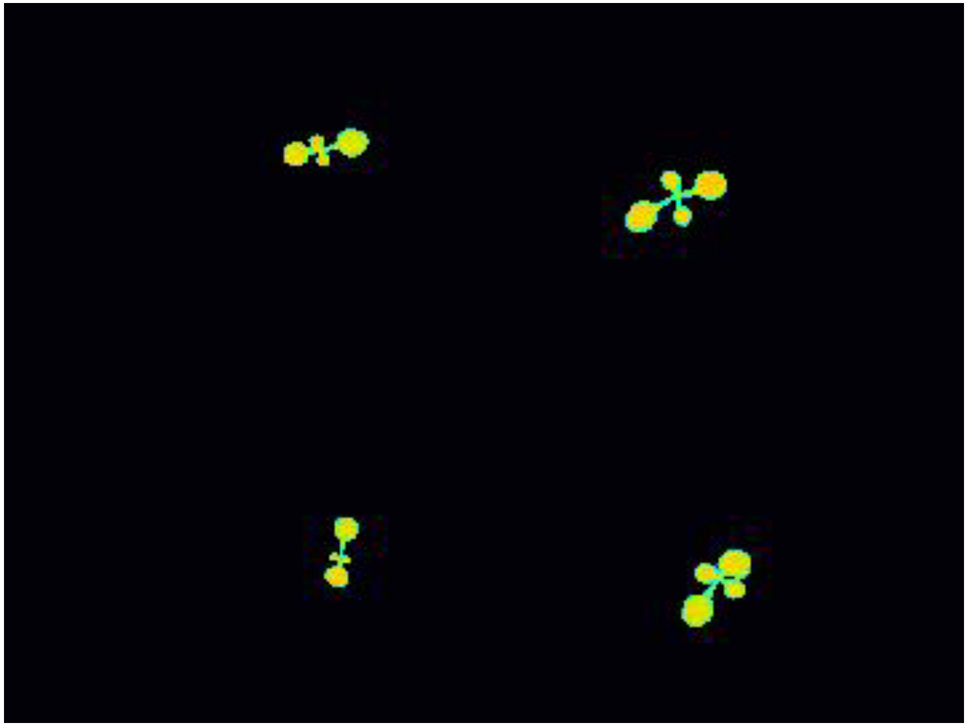


Conclusion: the relationship between photosynthetic electron transfer and biomass is not fixed: some mutants are more or less efficient.





Dynamic conditions and simultaneous growth and photosynthesis measurements



Conclusion: very clearly, photosynthesis changes ovder the course of development. (We knew that, but now we have a phenotyping handle on it.

Can increasing canopy light penetration enhance photosynthetic efficiency?

Elisabeth Ostendorf, Jin Chen, Jeffrey A. Cruz, David M. Kramer
(Co-funded by ARPA-E CECE)

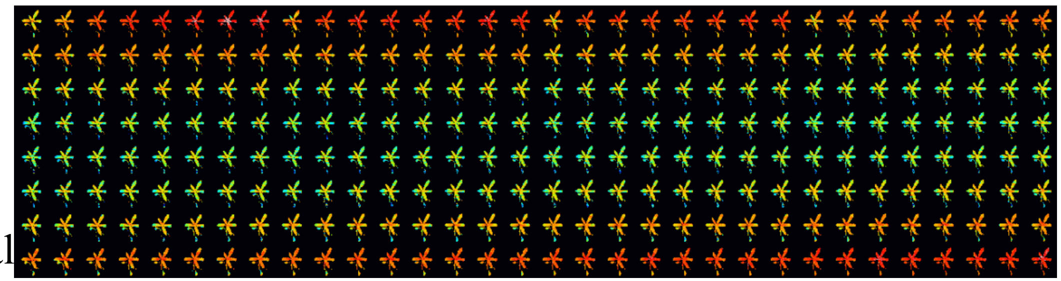
Variations in light intensity over a 16 hour day

F_V/F_M

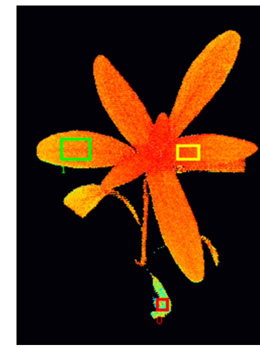
Φ_{II}, Quantum Yield of PSII

Sinusoidal

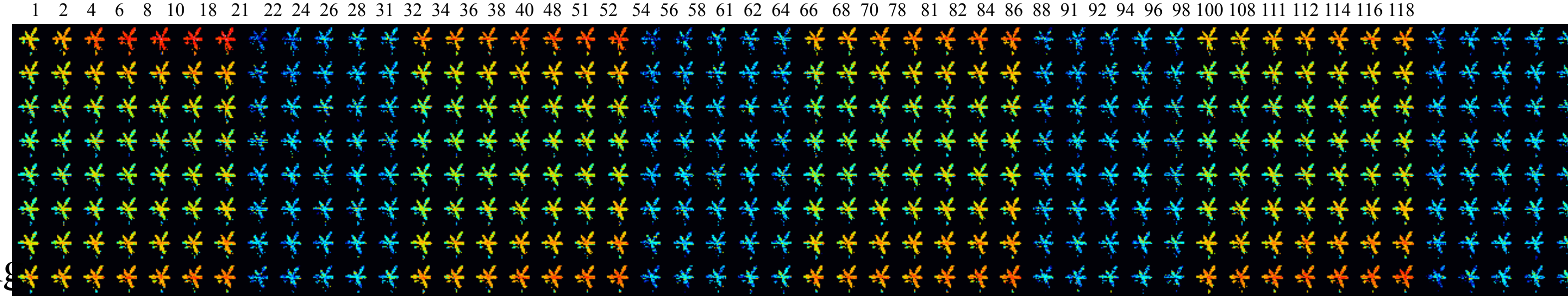
1 2 4 6 8 10 20 28 31 32 34 36 38 40 50 58 61 62 64 66 68 70 80 88 91 92 94 96 98 100 110 118



6-8A
8-10A
10A-N
N-2P
2-4P
4-6P
6-8P
8-10P

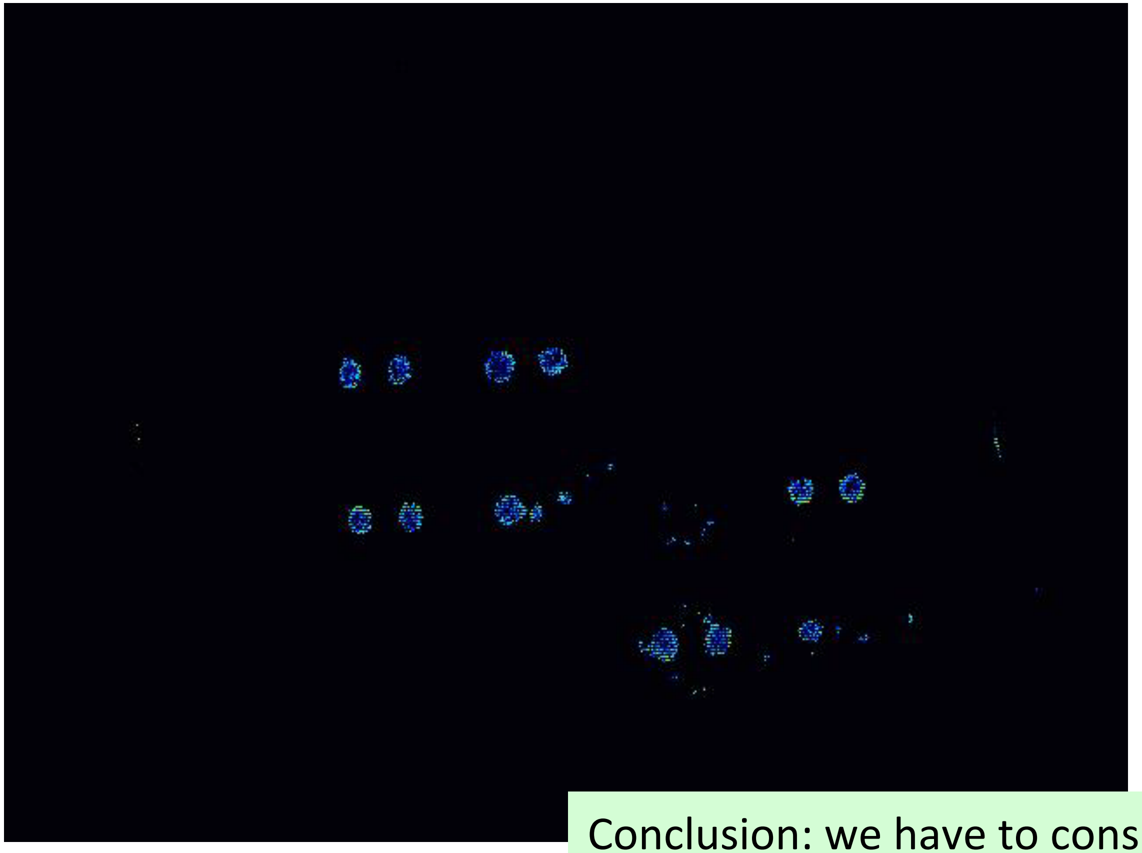


1 2 4 6 8 10 18 21 22 24 26 28 31 32 34 36 38 40 48 51 52 54 56 58 61 62 64 66 68 70 78 81 82 84 86 88 91 92 94 96 98 100 108 111 112 114 116 118



Fluctuating Sinusoidal

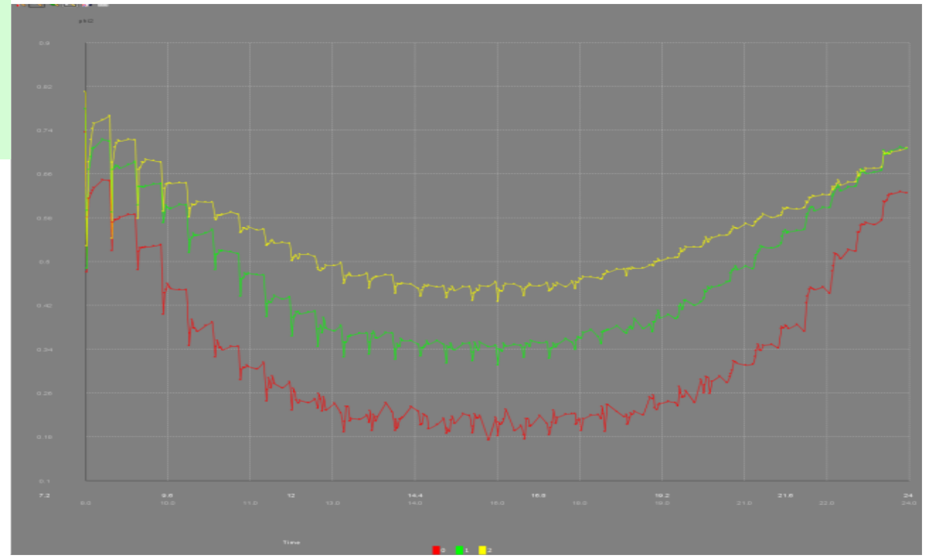
Integrated growth and photosynthesis in *Camelina:* full sun from seed or transferred from standard growth chamber.

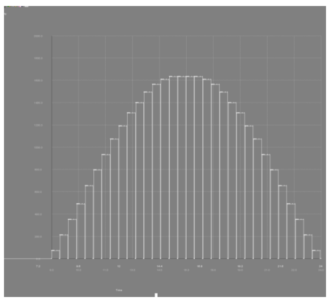


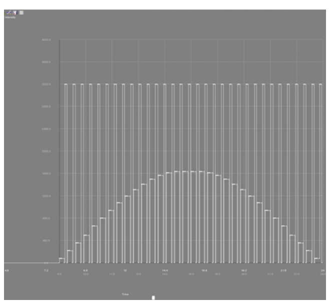
Conclusion: we have to consider the capacity of under-canopy leaves, not just the light. The capacity depends on the exposure of plants to fluctuations in light and other environmental conditions.

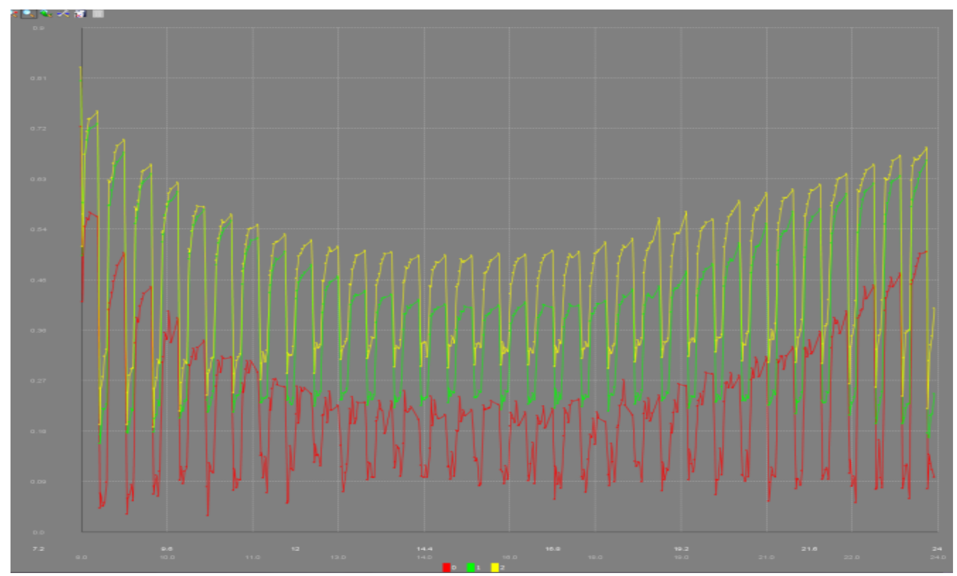
Conclusion: we have to consider the capacity of under-canopy leaves, not just the light. The capacity depends on the exposure of plants to fluctuations in light and other environmental conditions.

Φ_{II} vs. time









Photosynthetic Acclimation to Low Temperatures
G. Rudd Larson, Jeffrey A. Cruz, David M. Kramer, Michael Thomashow

The Importance of Spatial Heterogeneity for Photosynthetic Acclimation to Low Temperatures
G. Rudd Larson, Jeffrey A. Cruz, David M. Kramer, Kent Kovac, Yuhua Jiao, Jin Chen, Michael Thomashow

Dave, open up the REAL other slide show....

Mutants with altered chloroplast morphology are sensitive to fluctuating light.

Colloboration with Katherine Osteryoung and Siddhartha Dutta:

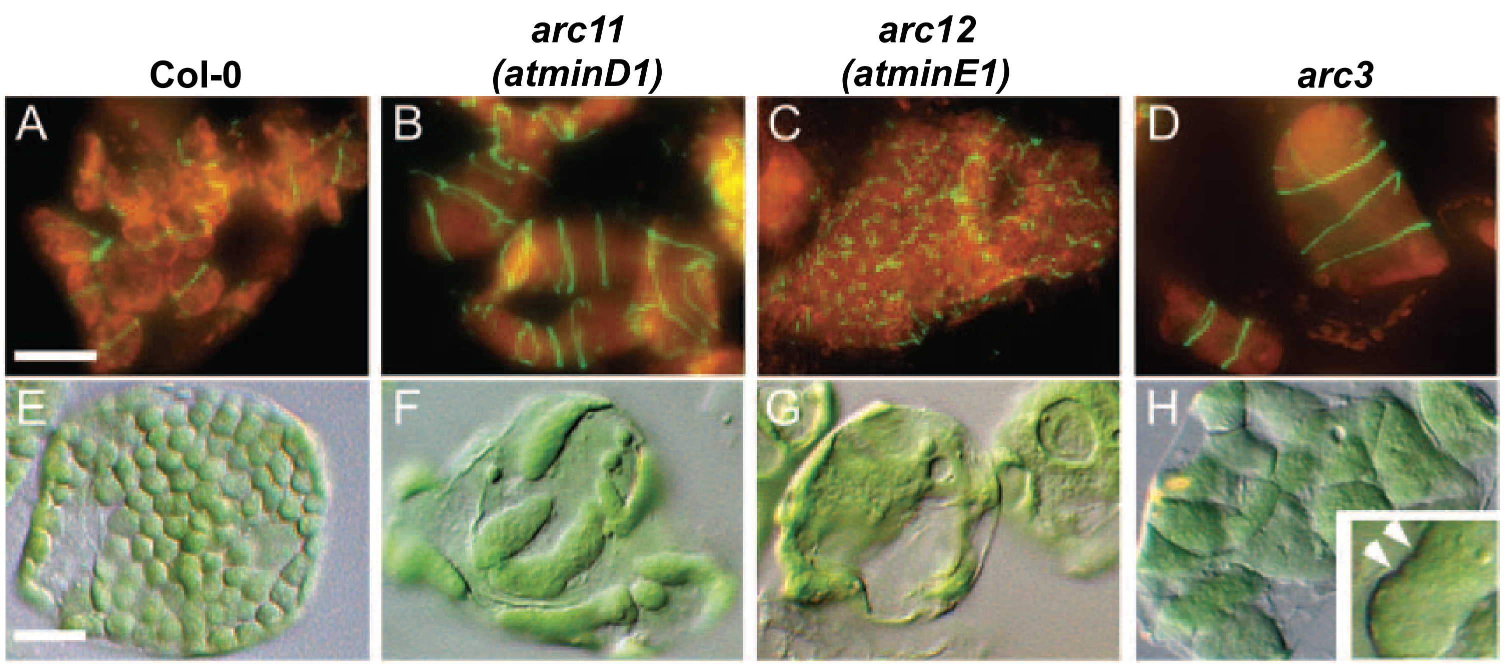
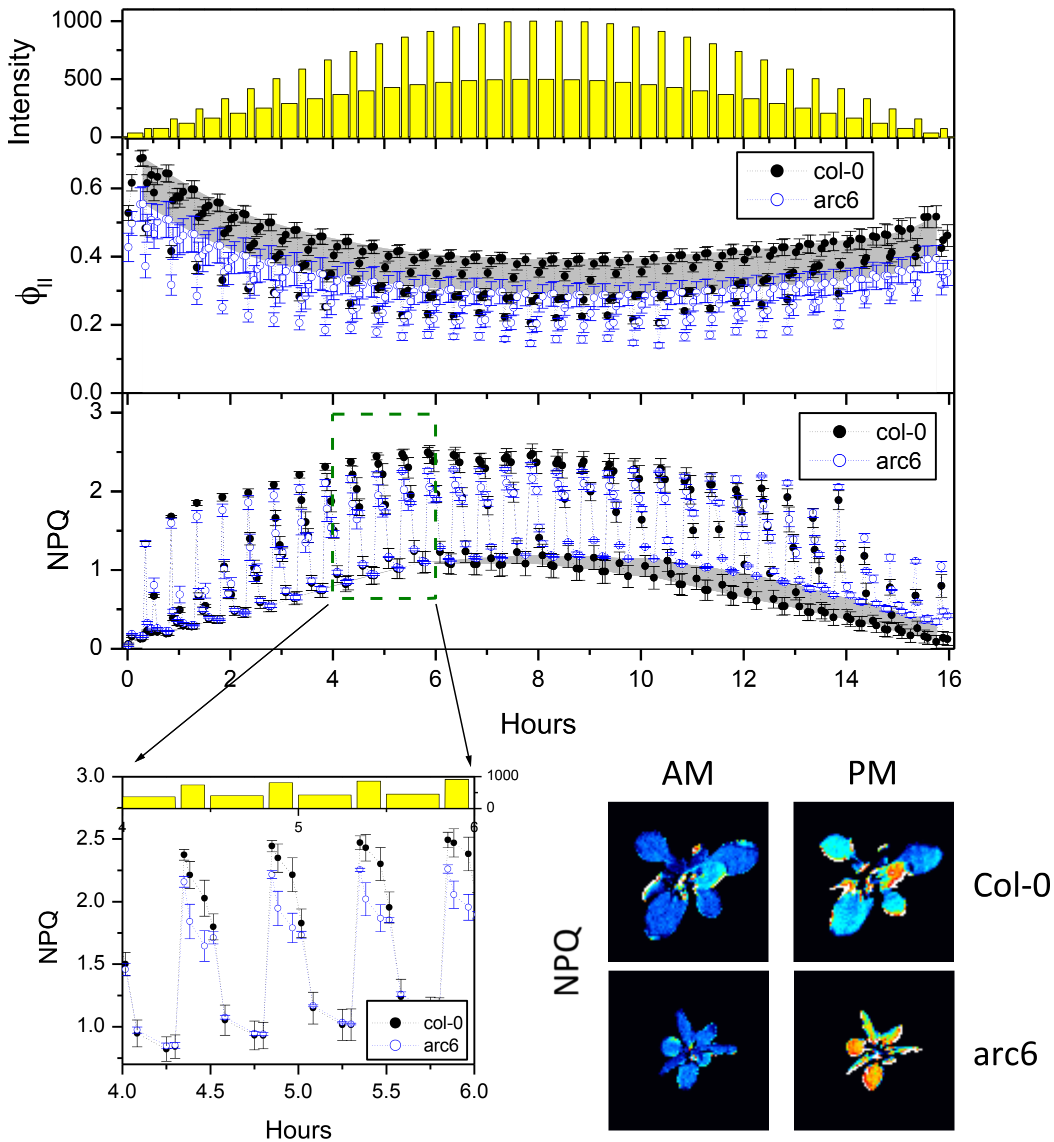


Fig. 3. Chloroplast and FtsZ morphology in mesophyll cells of cpMin mutants. (A-D) Immunofluorescence localization of FtsZ. Here and in other figures, merged images of FtsZ (green) and chlorophyll autofluorescence (red) are shown. In panel A, single Z rings are visible at the middle of the small chloroplasts in WT. Bar, 10 μm. (E-H) Chloroplast morphologies in the indicated plants. Inset in H shows multiple constrictions in *arc3.* Bar, 20 μm. \



Intensity
1000
500
0
0.6
0.4
0.2
0.0
col-0
arc6
NPQ
3
2
1
0
0
2
4
6
8
10
12
14
16
Hours
3.0
2.5
2.0
1.5
1.0
1000
0
4
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6
4.0
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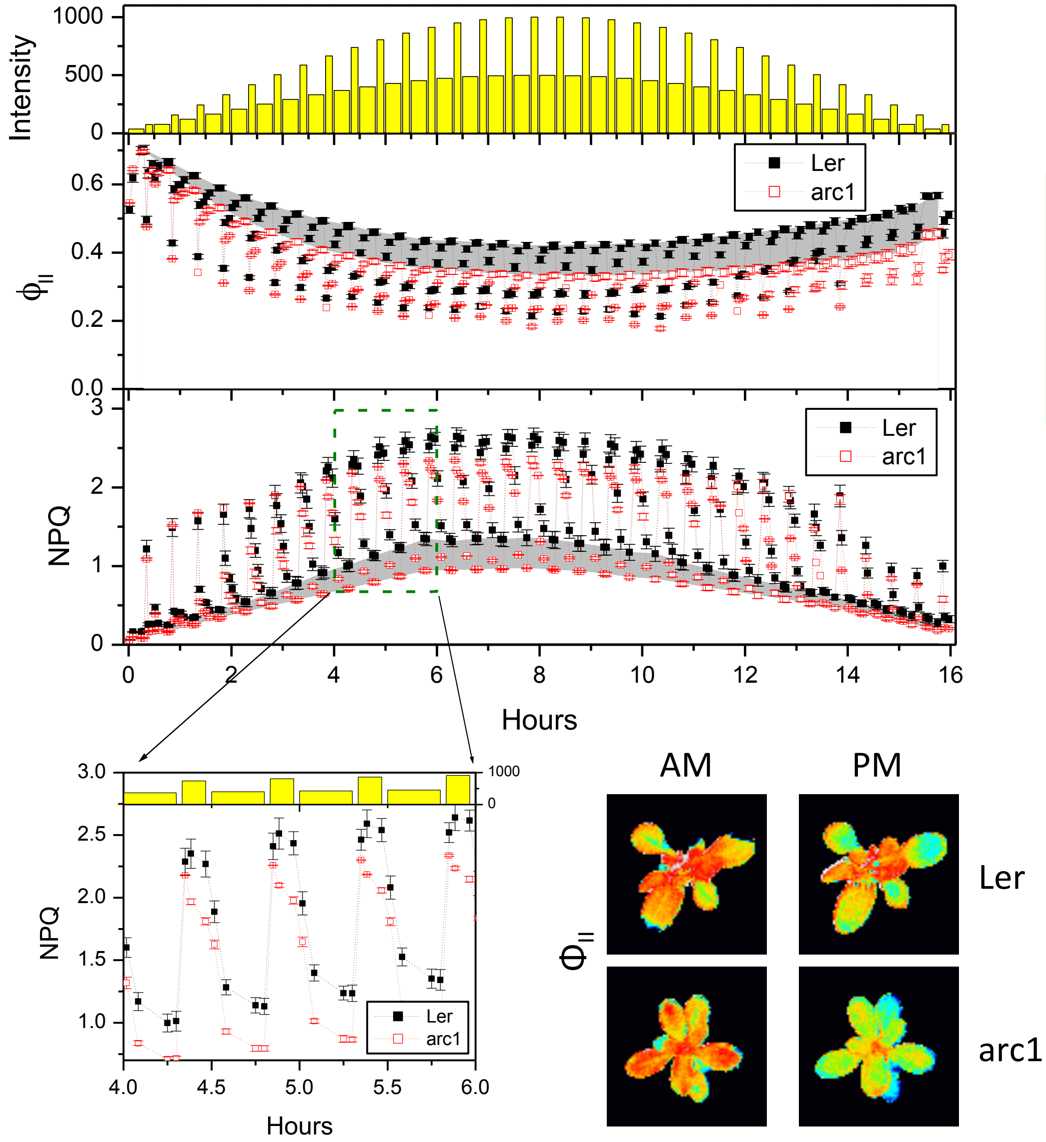
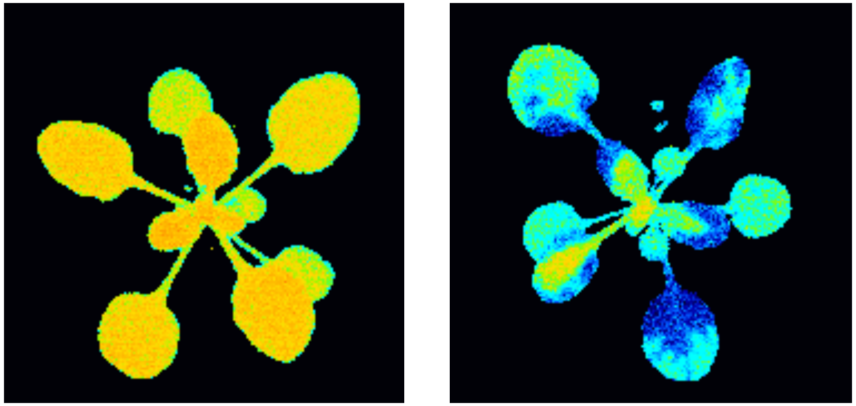
AM
PM
Col-0
arc6



Conclusions: We need to look at fluctuations not only over the short term, but also over hours or even days.

The connection between plant defense and photosynthesis

Approach: Dynamic Photosynthetic Phenometrics

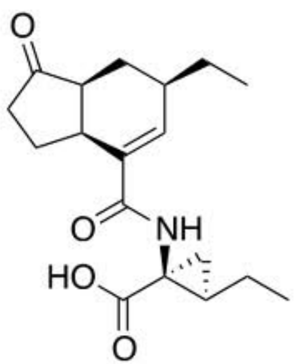


- Tools to study dynamic regulation of photosynthesis and growth
- Continuous monitoring of photosynthesis in multiple plants under changing environmental conditions

PRL has initiated the MSU Center for Advanced Algal and Plant Phenotyping (CAAPP)

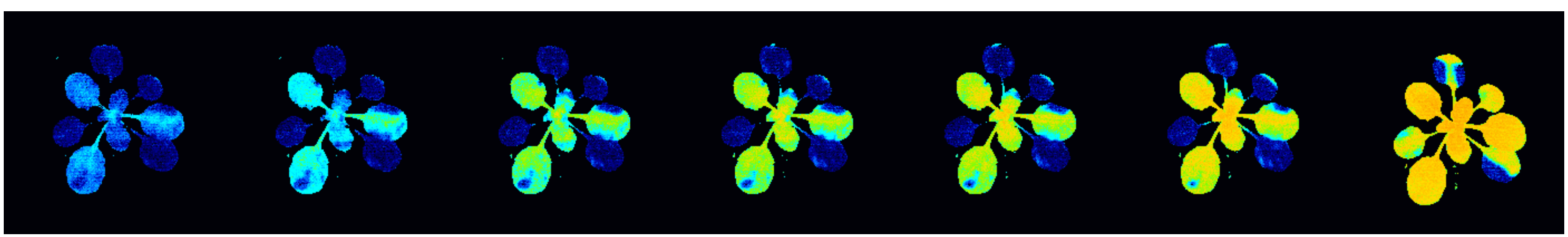
- Supported in part by DOE-BES

Effects of biotic stress on photosynthesis



- Demonstrate the importance of *in situ* monitoring of photosynthesis, in time scales ranging from seconds to weeks (developmental time scales)

- Demonstrate the importance of multiple simultaneous measurements to diagnose the effect of environmental perturbation on photosynthesis



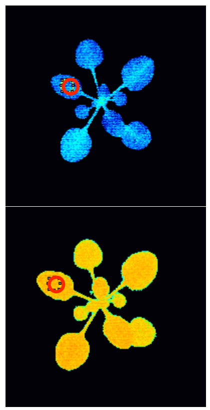
Time

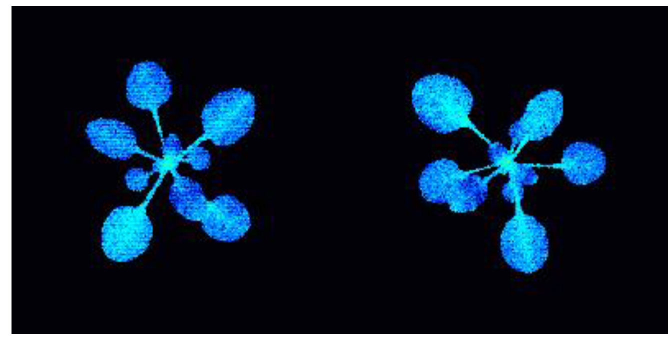
Activation of the JA pathway has transient and long-term effects on photosynthesis

Experimental Set up:

LOW
0.24

HIGH
0.56





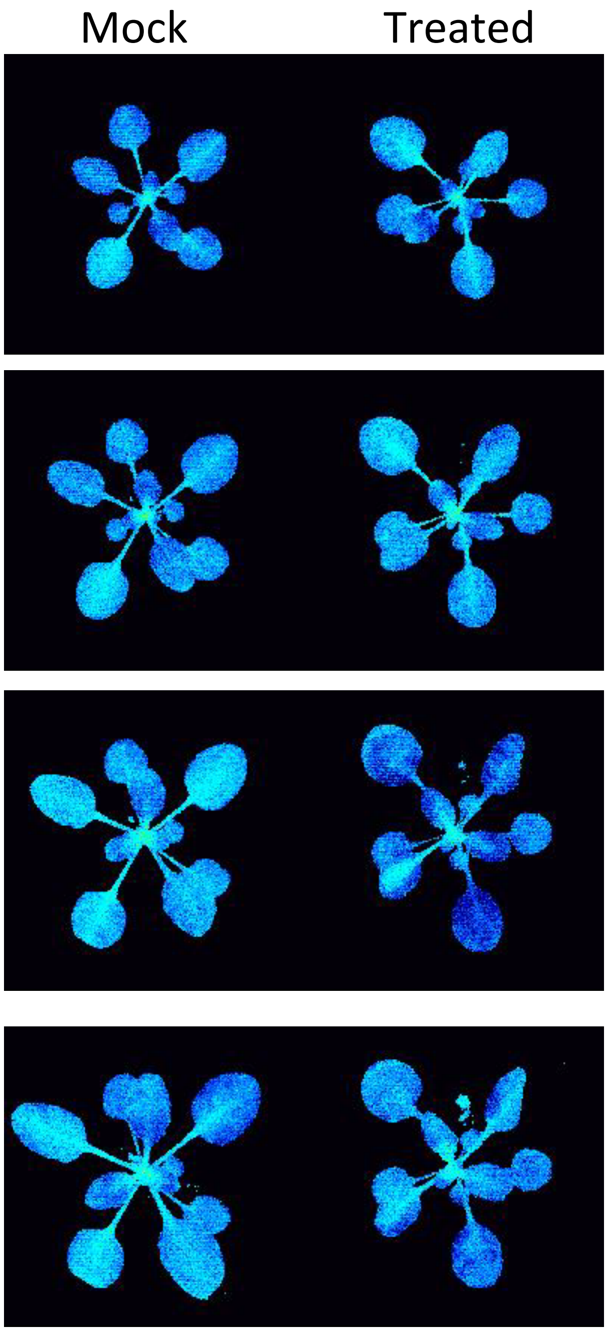
Mock +COR

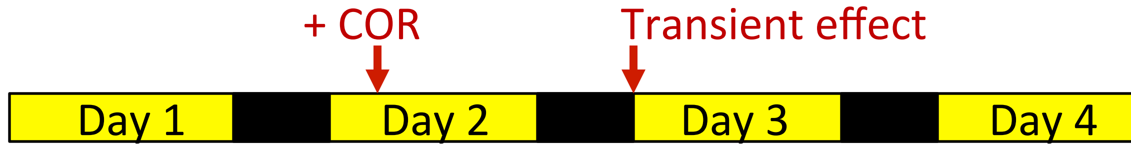
Results:

Two distinct coronatine-mediated effects are observed:

1. sharp, transient effect on photosynthetic induction
2. small but sustained (long-term) decrease in photosynthesis

Elham Attaran





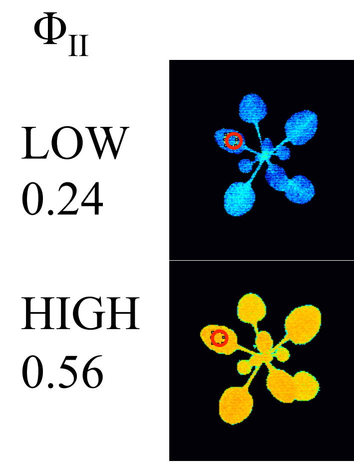
Day 1: Pre-treatment acclimation

- No differences

Day 2: COR treatment

- Still no differences

(Despite gene expression and growth suppression)



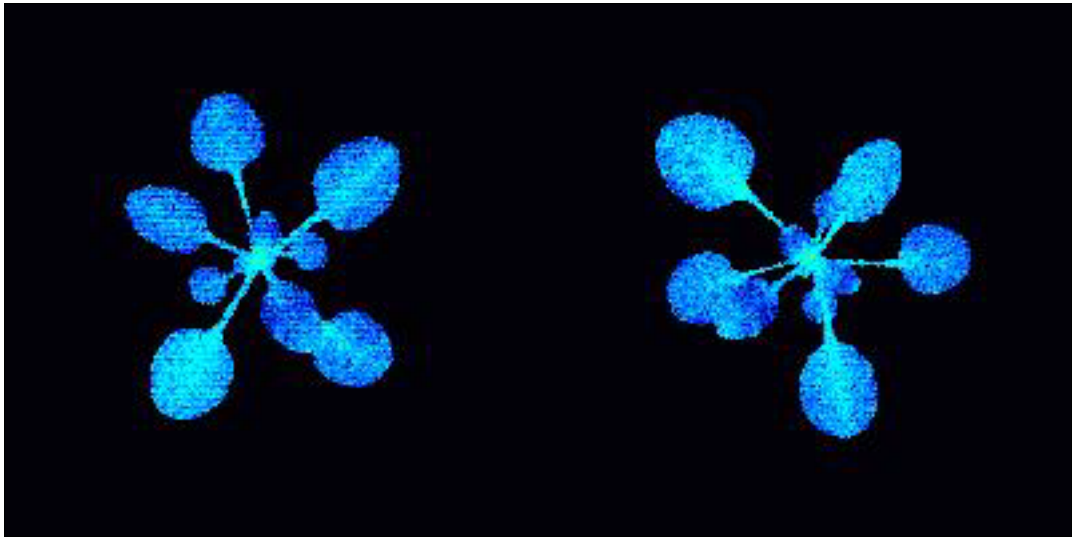
Day 3: Day after treatment

- Transient effect

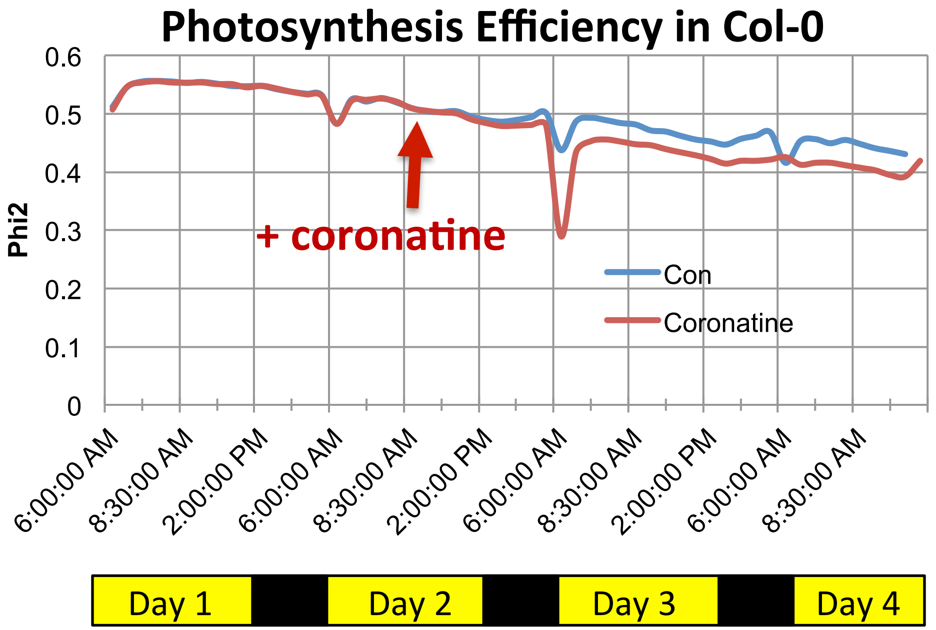
Day 4: 2nd day post treatment

- Transient effect reduced but not eliminated
- Long-term suppression of photosynthesis

Control

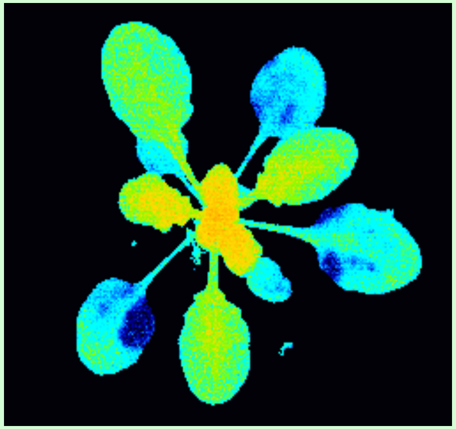


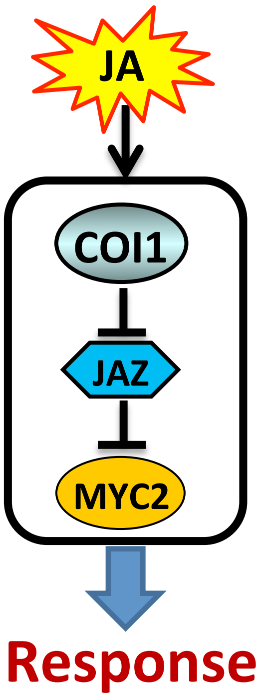
Treated

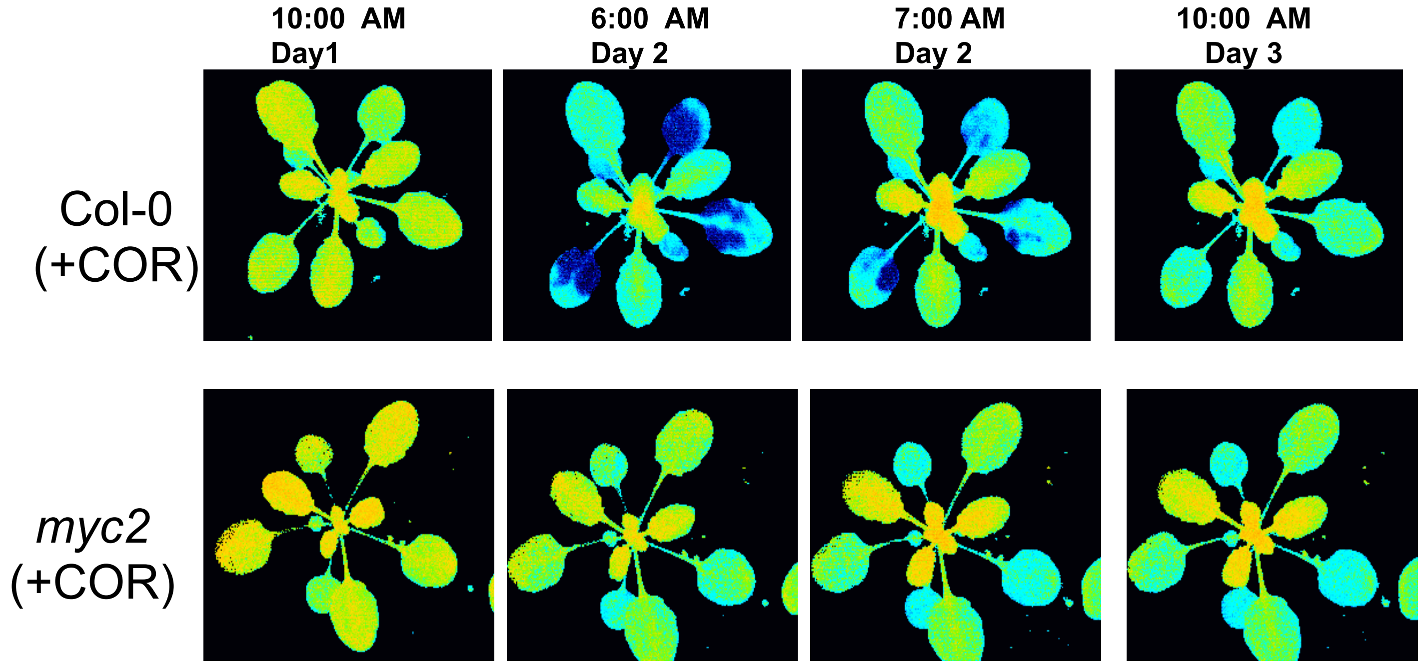


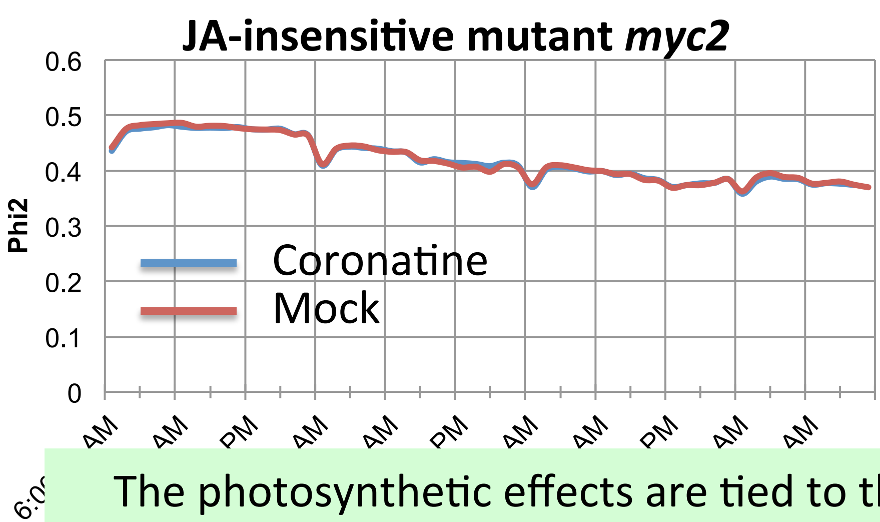
We captured a novel *transient* effect on photosynthesis

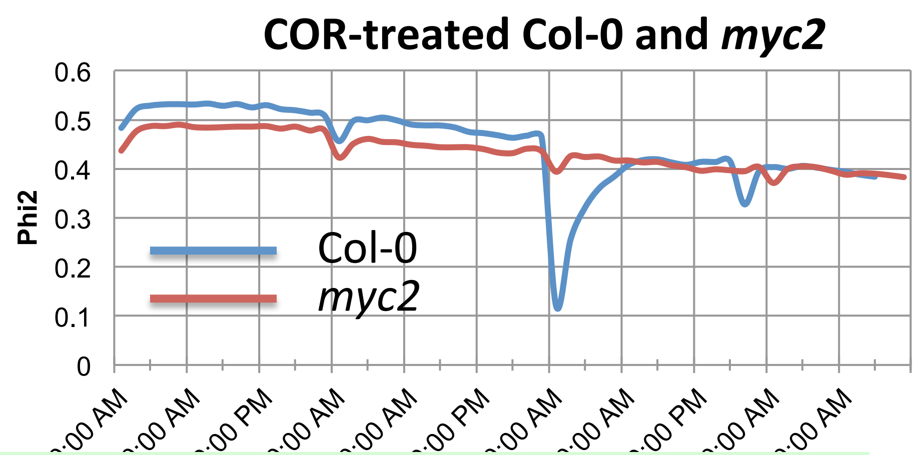
- ✓Effect was not seen in previous low time-resolution measurements
- ✓Moving plants from growth chamber to imaging chamber elicits physiological changes that may mask the effect
- ✓ Photosynthetic efficiency is heterogeneous, and affected by developmental stage
- ✓**Highlights the need for new technology to continuously monitor many plants in parallel, over the entire plant**







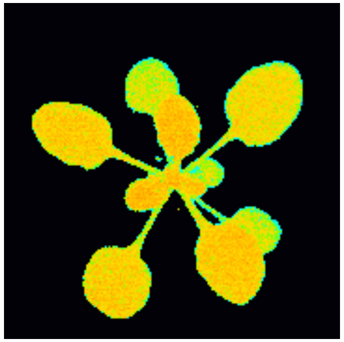




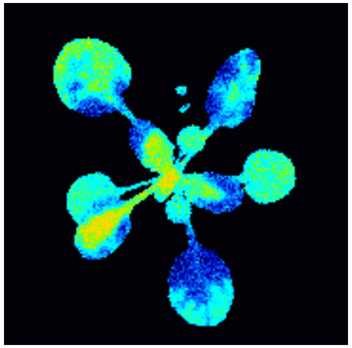
The photosynthetic effects are tied to the activation of defense signaling by MYC2.

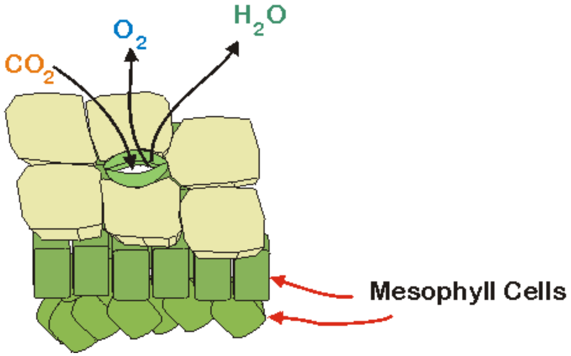
What is the physiology basis of the transient decrease in photosynthetic efficiency?

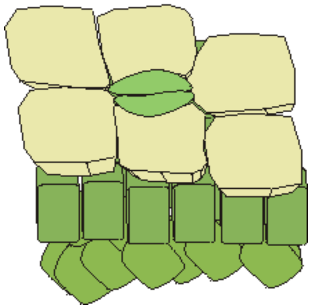
Control



Treated



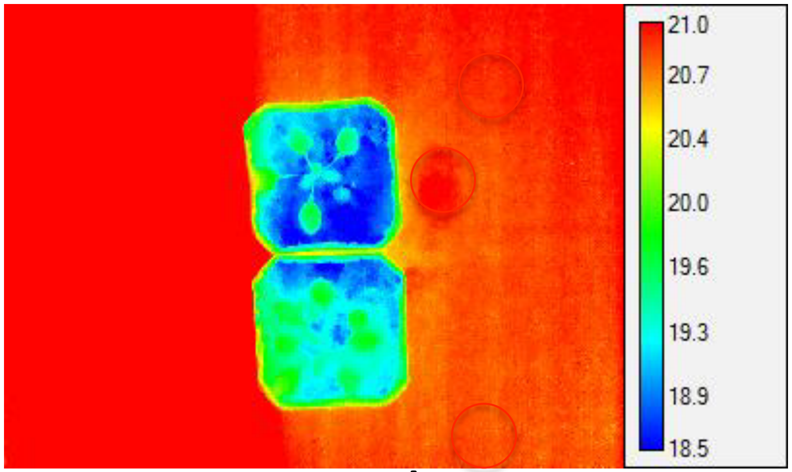




Approach: Test stomata regulation with rapid, non-invasive imaging methods.

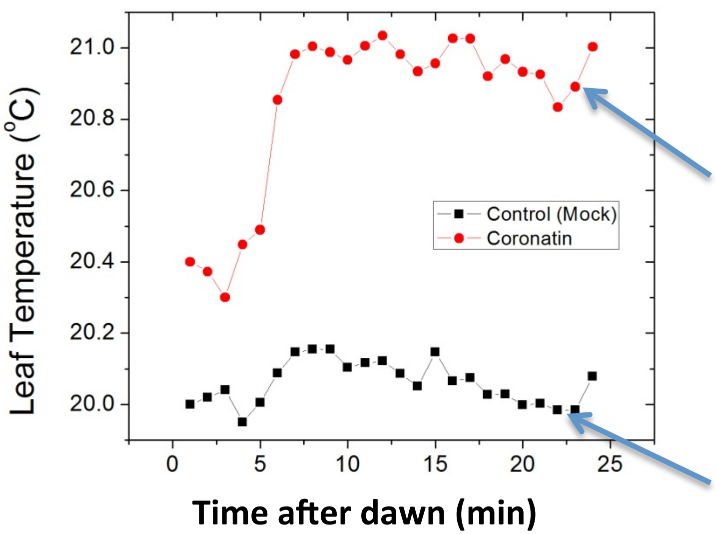
Effects of coronatine on stomatal opening at dawn

Control



Treated

- Thermal imaging as a rapid, time-resolved qualitative probe:
- Open stomata increase transpiration, decreasing leaf temperature.

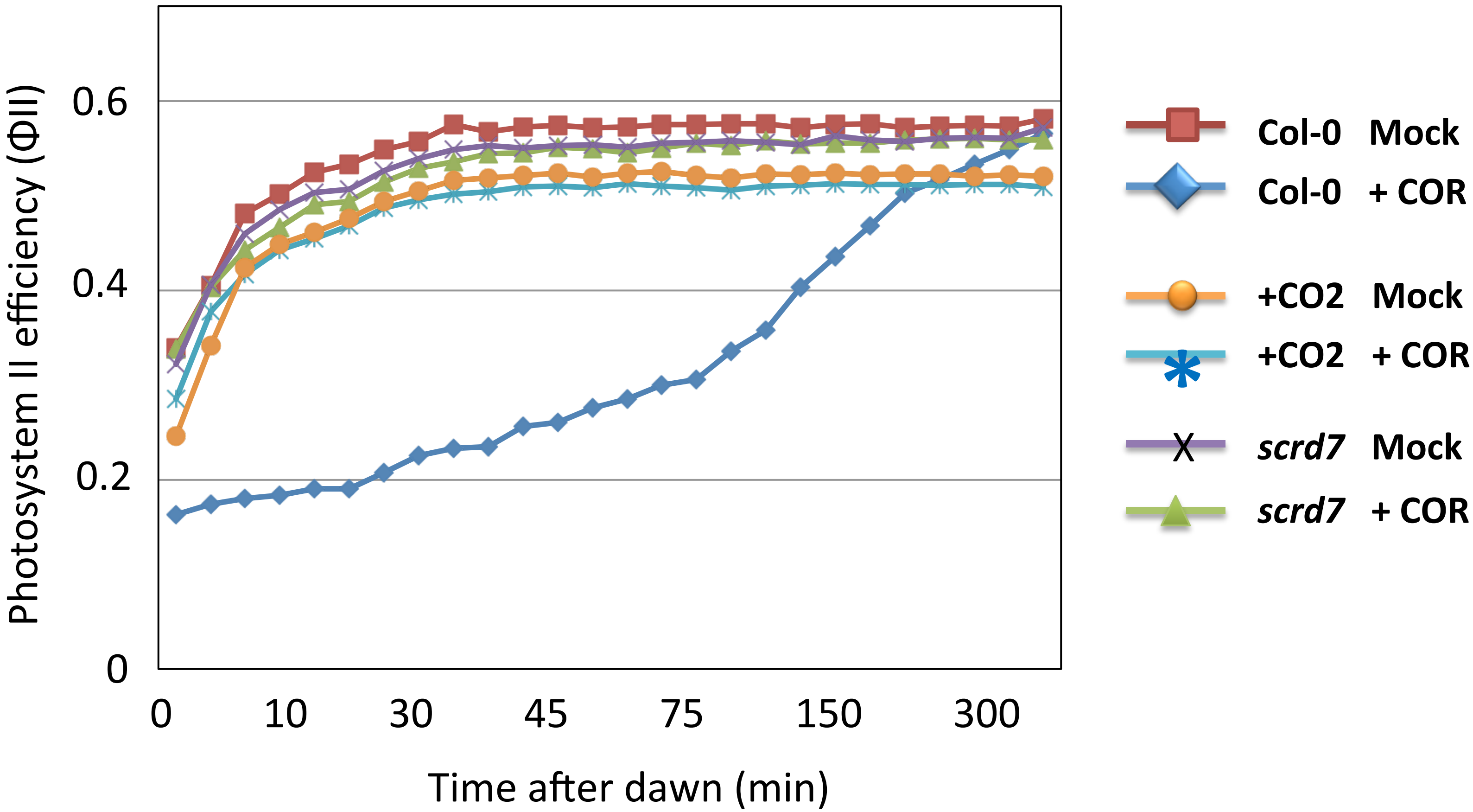


Coronatine: Sustained leaf heating by light (stomata remain closed)

Control: Transient leaf heating (stomata open in minutes)

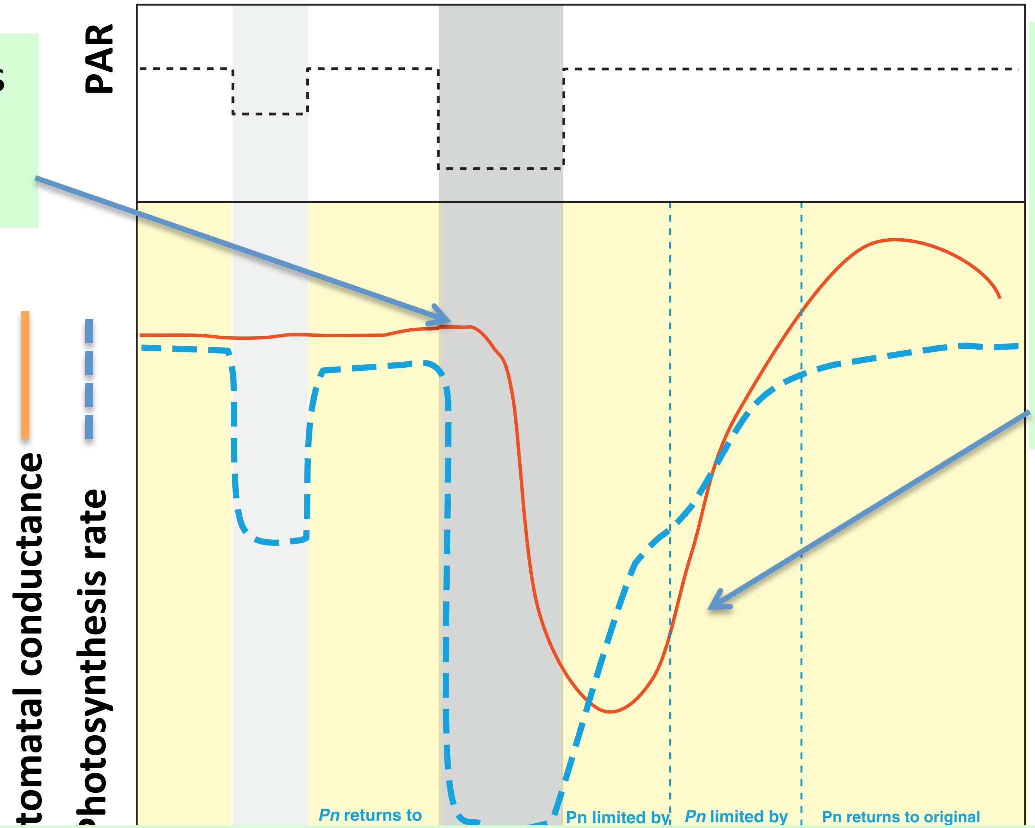
Is this the cause of the photosynthetic effect, or is it a response to it?

Coronatine promotes stomatal closure and a transient decrease in photosynthesis



Stomatal dynamics have already been identified as a target for improving photosynthetic efficiency

Stomatal closure is "too slow" to prevent water loss



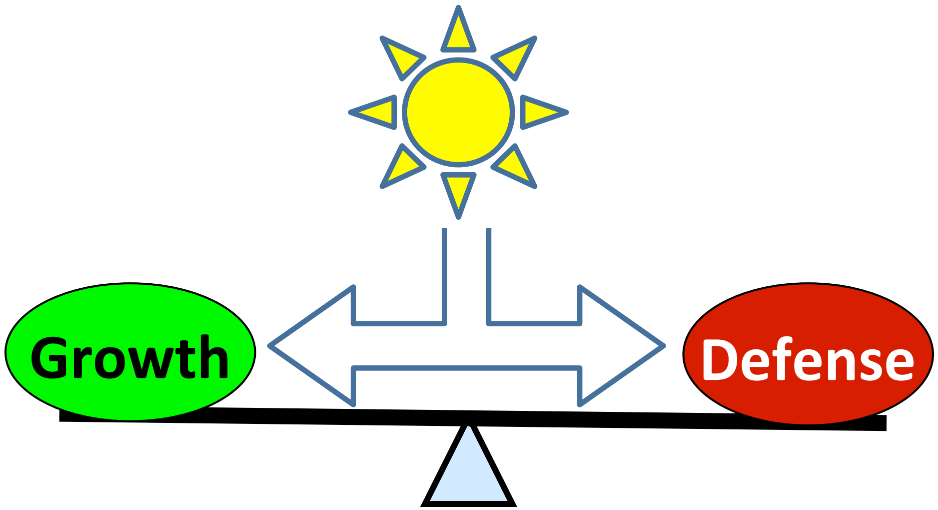
Recovery "too slow" to take advantage of high light -- leading to a loss of potential productivity

In the context of maximizing photosynthetic efficiency, stomatal behavior may not be "optimized" to respond to rapid changes in environment.

An energetic cost of defense responses

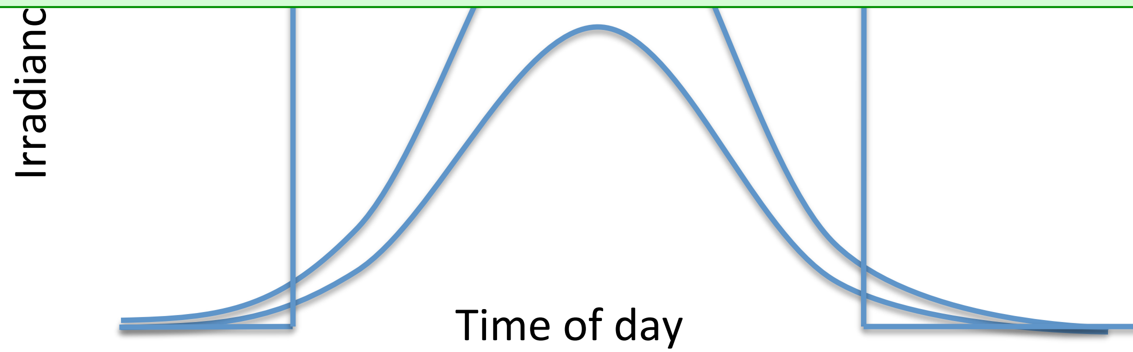
Defense responses impose an additional constraint on stomatal regulation of C assimilation vs. water loss

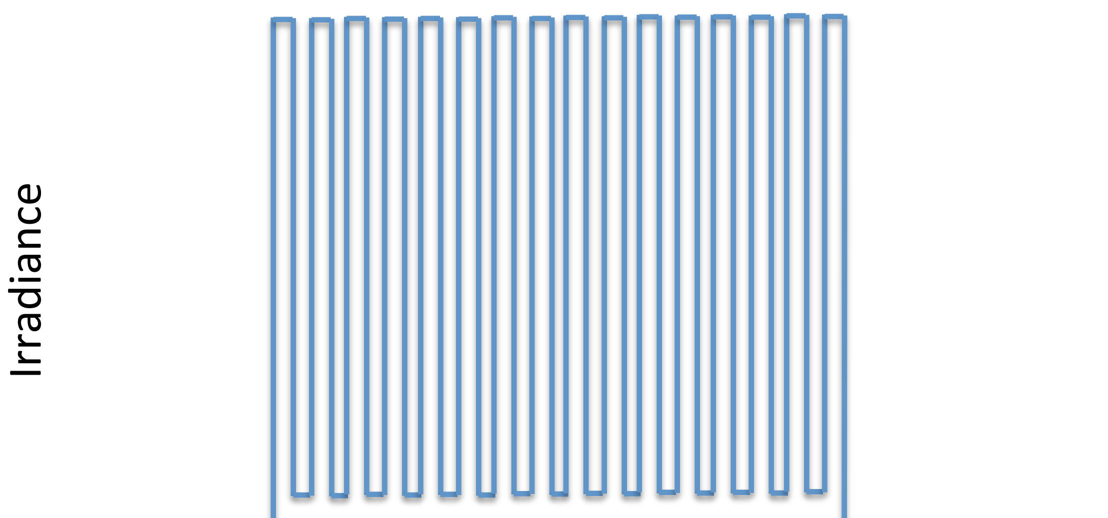
- Altered stomatal dynamics leads to substantial (but transient) loss of photosynthesis during induction
- Long-term suppression of Φ_{II} decreases light capture
- Suppression of growth decreases leaf surface area

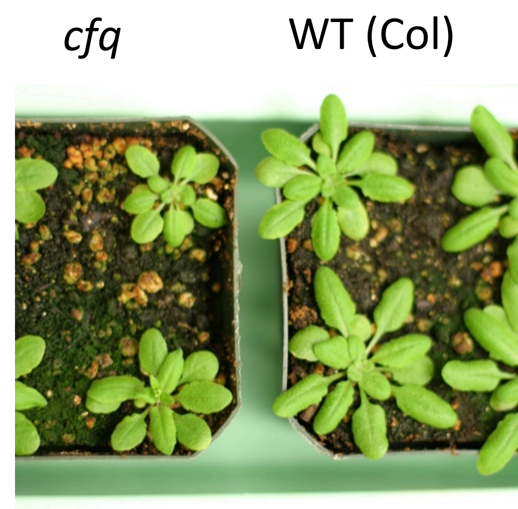


Taking the brakes off photosynthesis: Efficiency vs. safety

Atsuko Kanazawa, Kaori Kohzuma, Elisabeth Ostendorf, Jeffrey A. Cruz and David M. Kramer

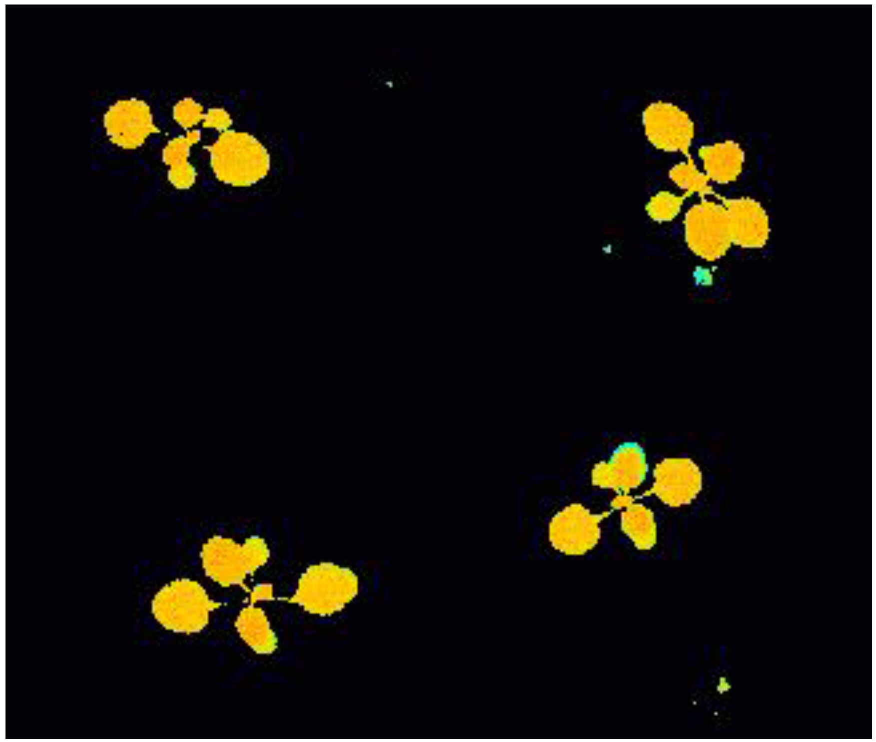




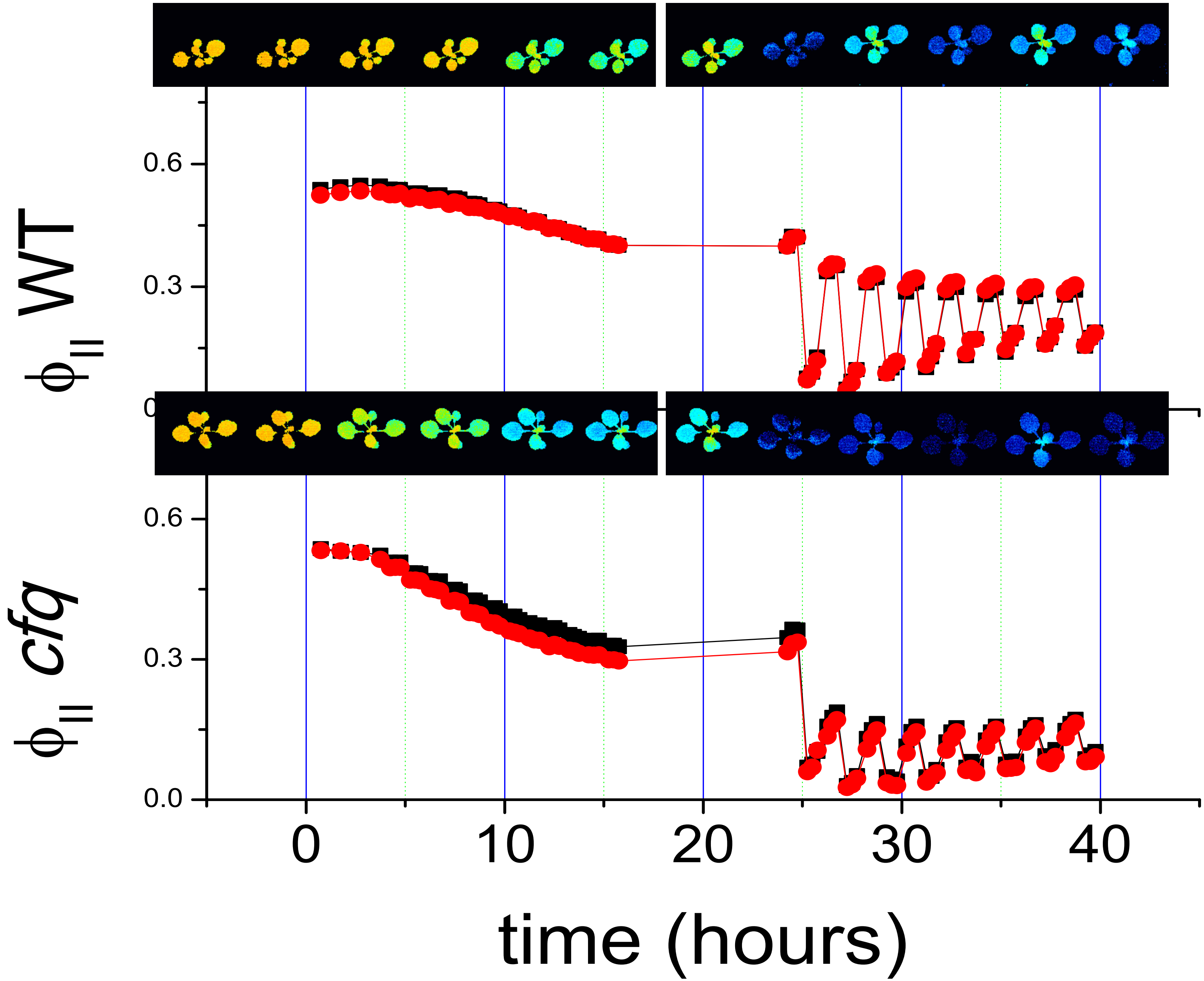


*real differences in biomass, not partitioning.

Dynamic regulation of photosynthesis

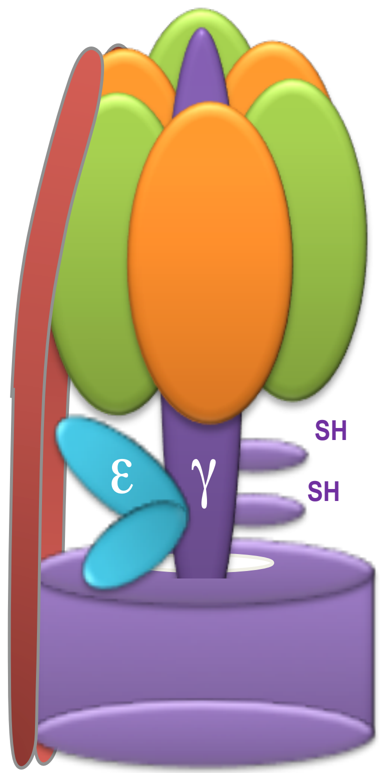


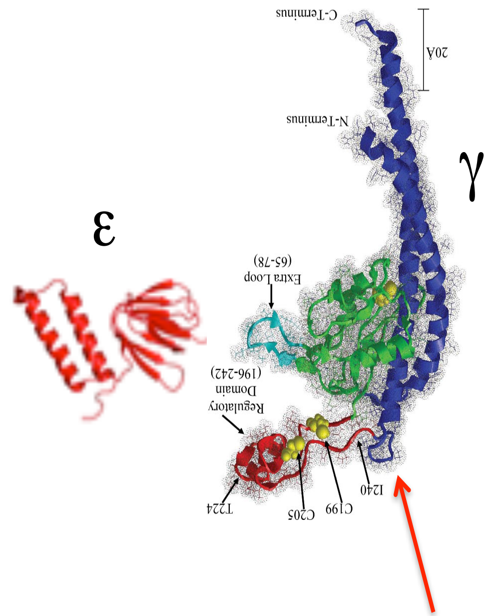
..removing the brakes from photosynthesis.



ϕ_{II} WT
ϕ_{II} cfq
0.6
0.3
0.0
0
10
20
30
40
time (hours)

cfq: (coupling factor quick reoxidation)



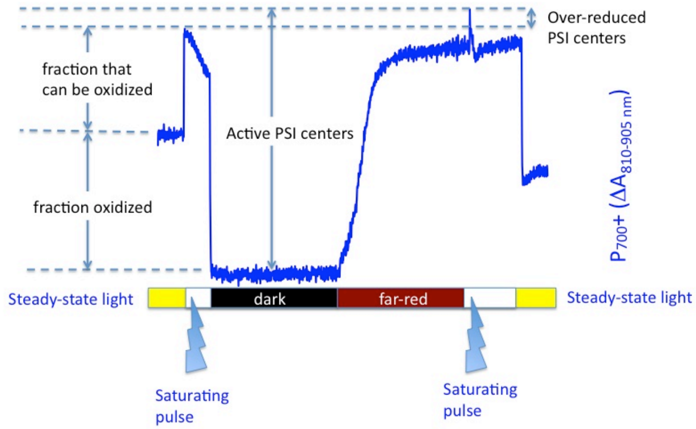


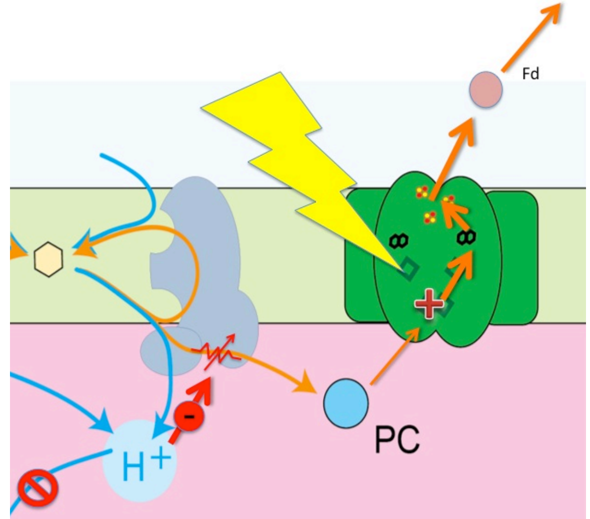
cfq (E244K)

Wu et al (2007) JBC.

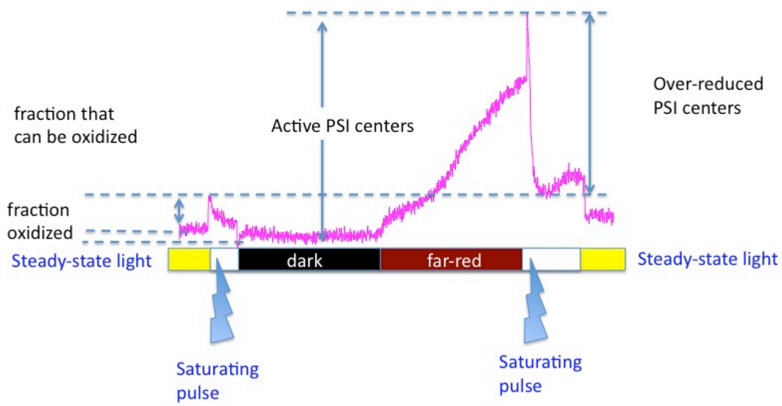
Dramatic consequences for in PSI

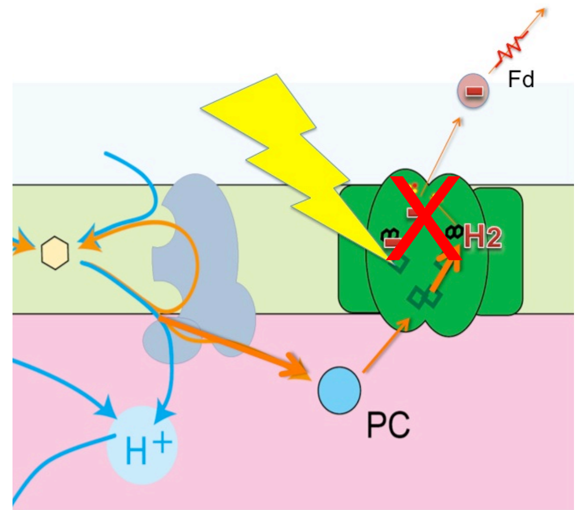
Col:



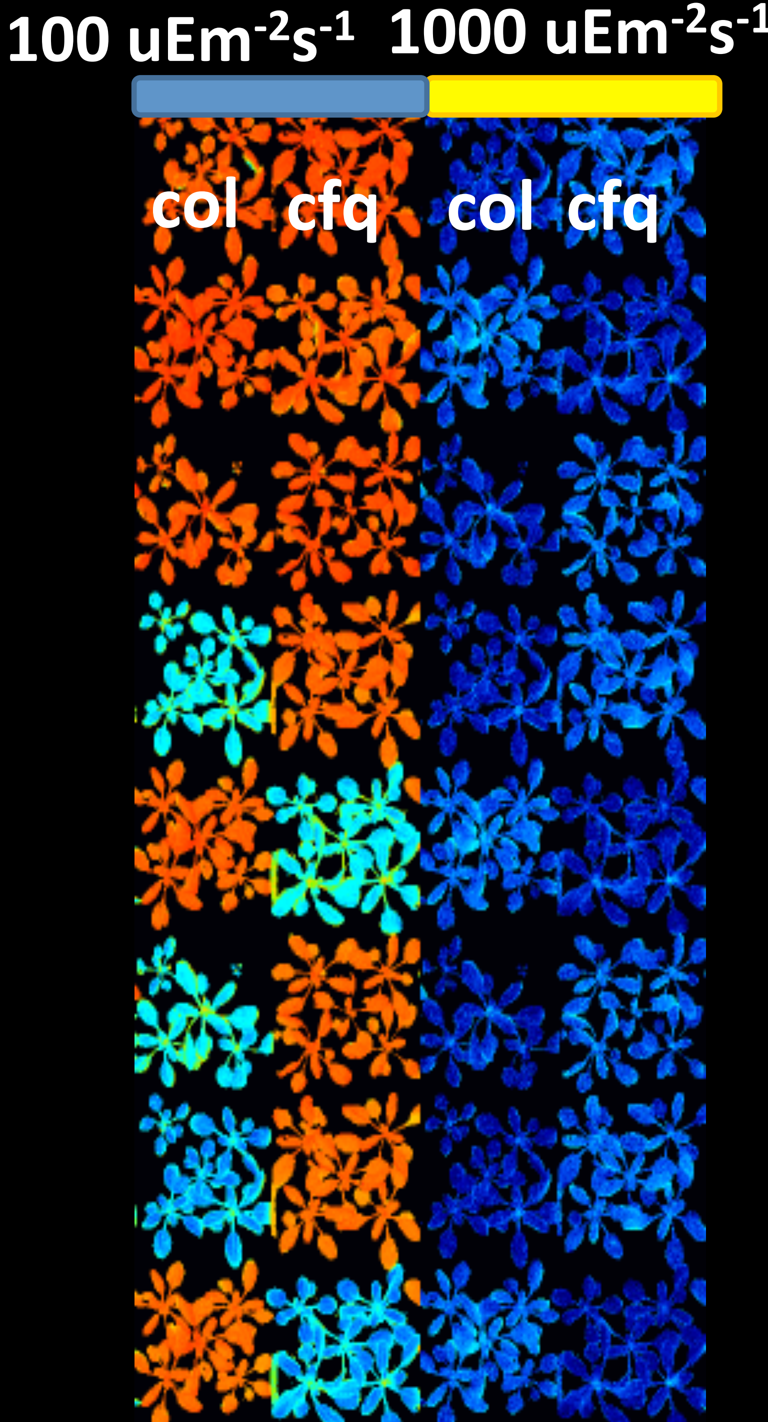


cfq:

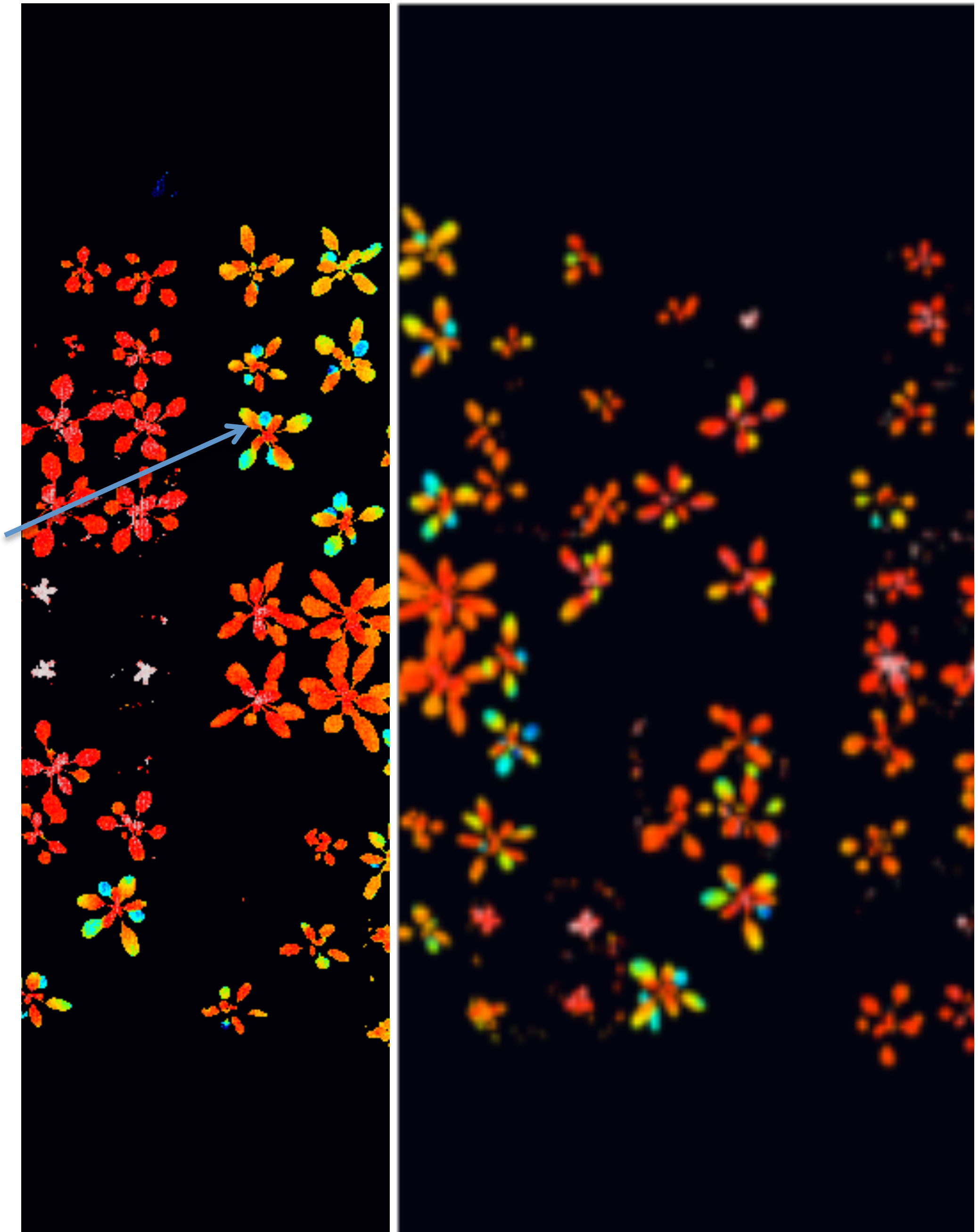




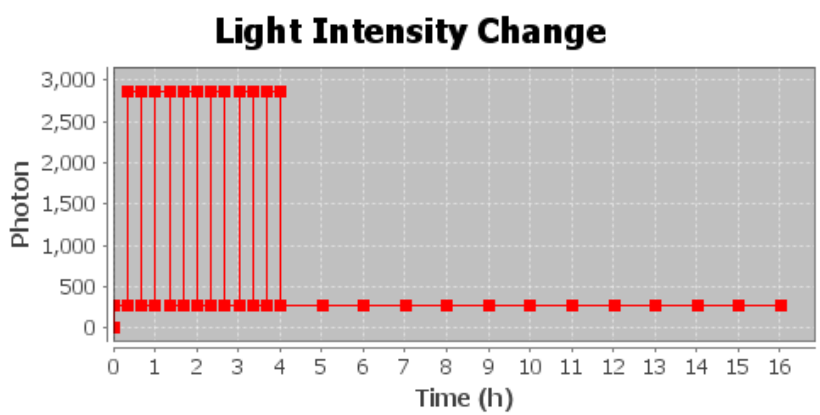
Over-reduction leads to specific, rapid damage to PSI
Implies a **new function** for control of electron transfer by the ATP synthase
Possible importance for cold stress?

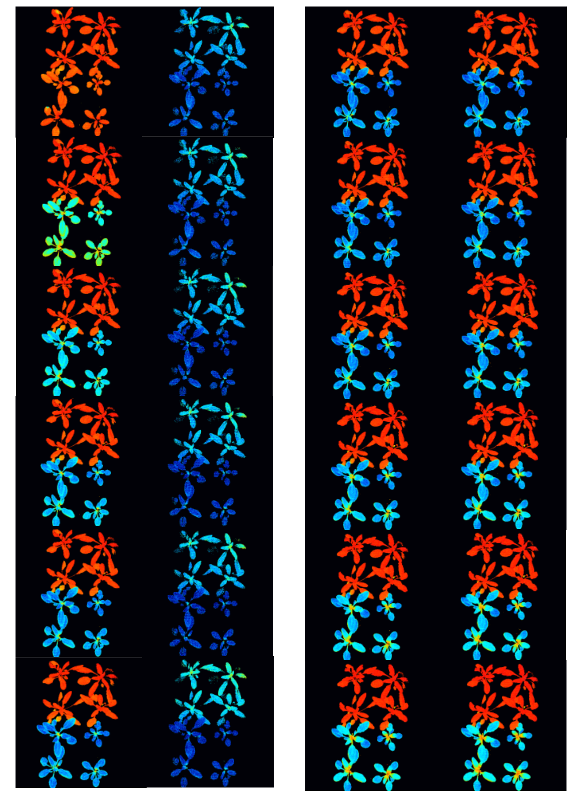

100 uEm-2s-1
1000 uEm-2s-1
col
cfq
col
cfq

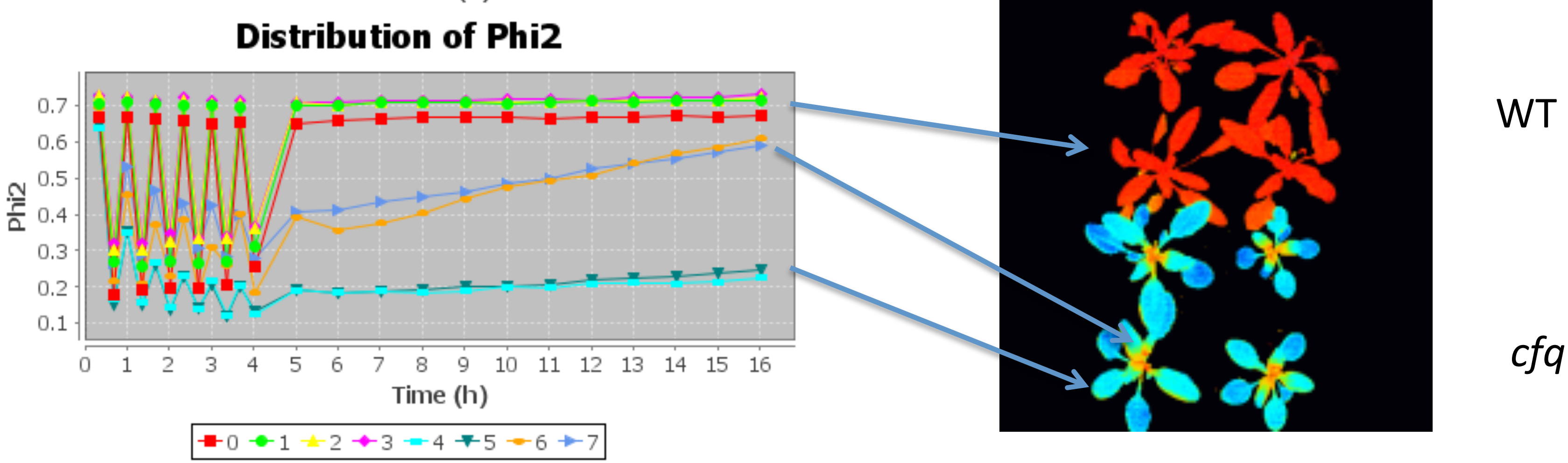
In contrast, *cfq* shows highly heterogeneous photosynthesis when grown under fluctuating conditions.

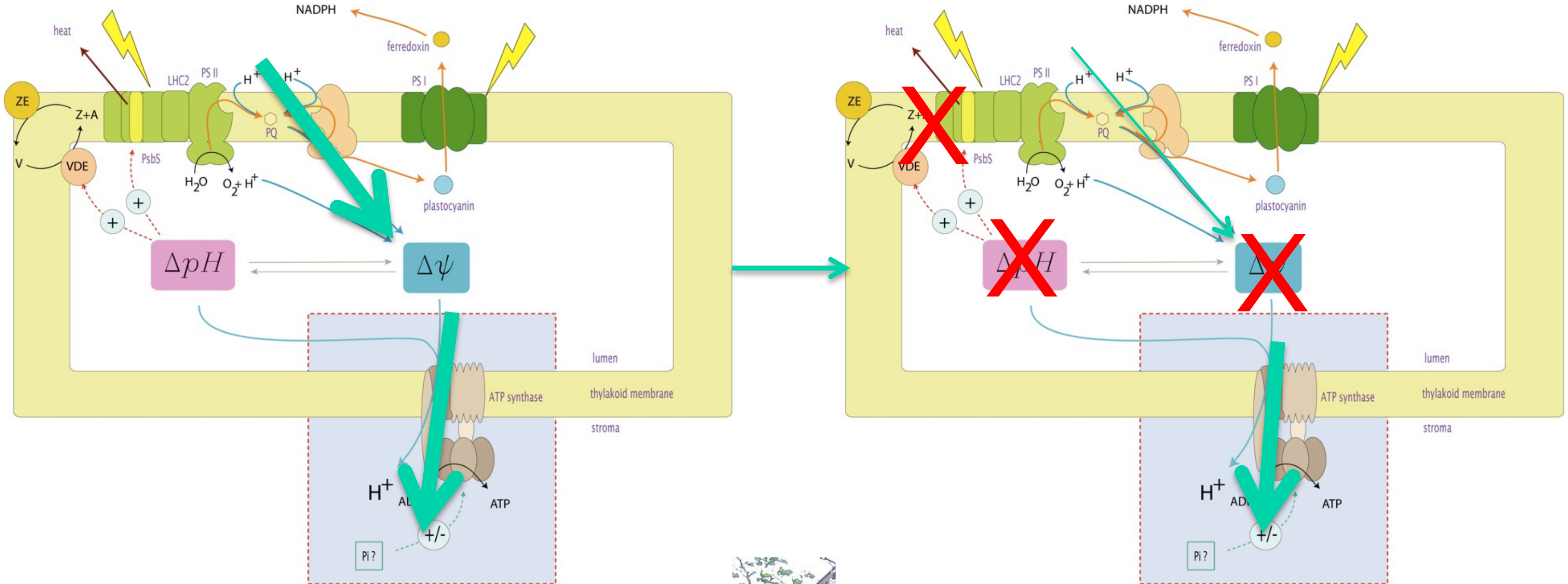


Photosynthetic heterogeneity in the cfq mutant is caused mainly by spatial differences in the **rate of recovery** from strong photoinhibition. We suspect that older leaves are programmed to not maintain photosynthetic apparatus.









1) We CAN make photosynthesis go faster!
2) But, doing so haphazardly can be bad (disabling the brakes lets the car go faster…for awhile)
3) Adding more dynamic range to regulation my be the key.
4) We absolutely must optimize under realistically dynamic conditions.
5) (re)development of photosynthesis may be an important target.

Dynamic Photosynthetic Phenometrics

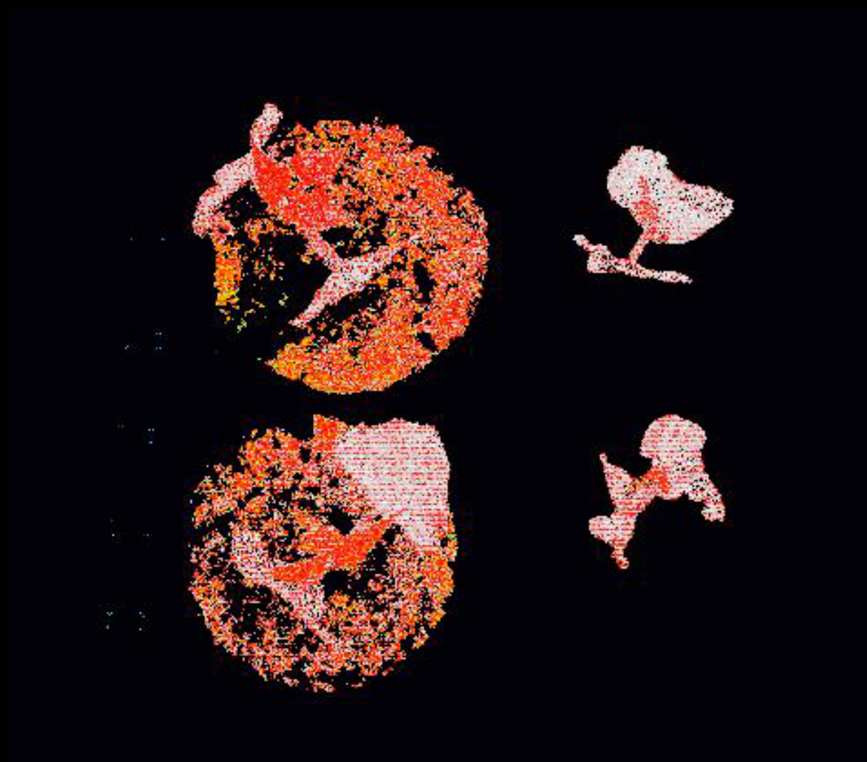
Dr. Jeffrey Cruz
Dr. Ben Lucker
Christopher Hall
Atsuko Kanazawa
Kaori Kohzuma
Deserah Strand
Rudd Larson
Elham Attaran

Robert Zegarac
Ben Wolf
Nathan Galbreath
Tim Conn
Lola Alvarez
Linda Savage
Kent Kovac
JonathanDelauter

r

Jin Chen
Juhua Jiao
Oliver Tessme

Beronda Montgomery
Marco Agostoni
Gregg Howe
Shen Yang He
Elham Attaran
Michael Thomashow
Yair Shachar-Hill
Federica Brandizzi
Jianping Hu
Ken Keegstra
Jonathan Walton
Peter Wolk
Robert Last
Thomas Sharkey
Kathy Osteryoung



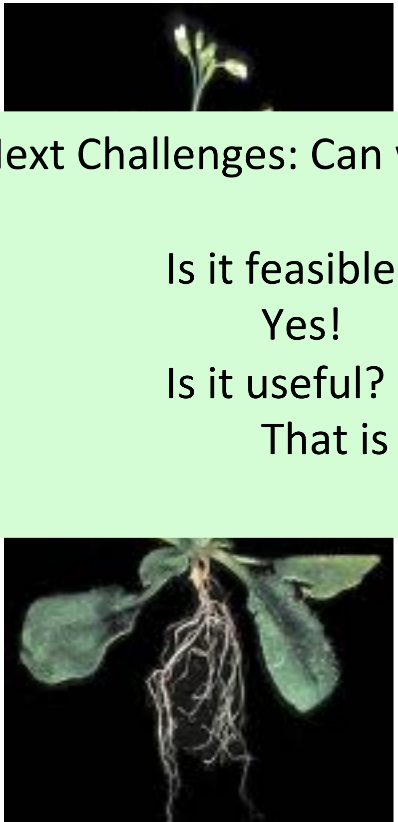
Next Challenges: Can we apply this to crop improvement?

Is it feasible to apply to crops?

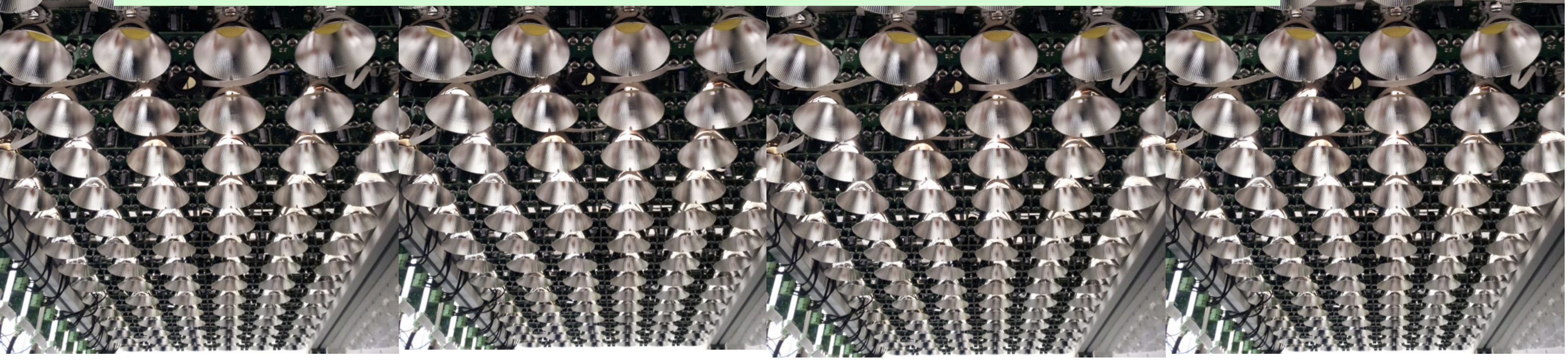
Yes!

Is it useful?

That is the question we want to answer!



Challenge 1: Scale: Our system can be arbitrarily scaled to larger areas with minimal "logistical" problems.

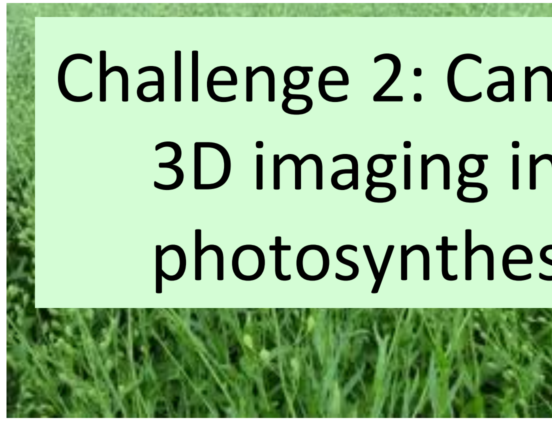


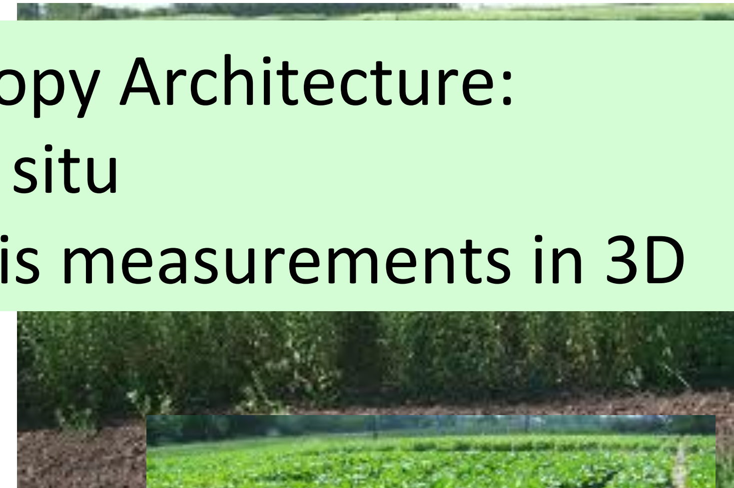
Challenge 2: Canopy Architecture:

3D imaging in situ

photosynthesis measurements in 3D





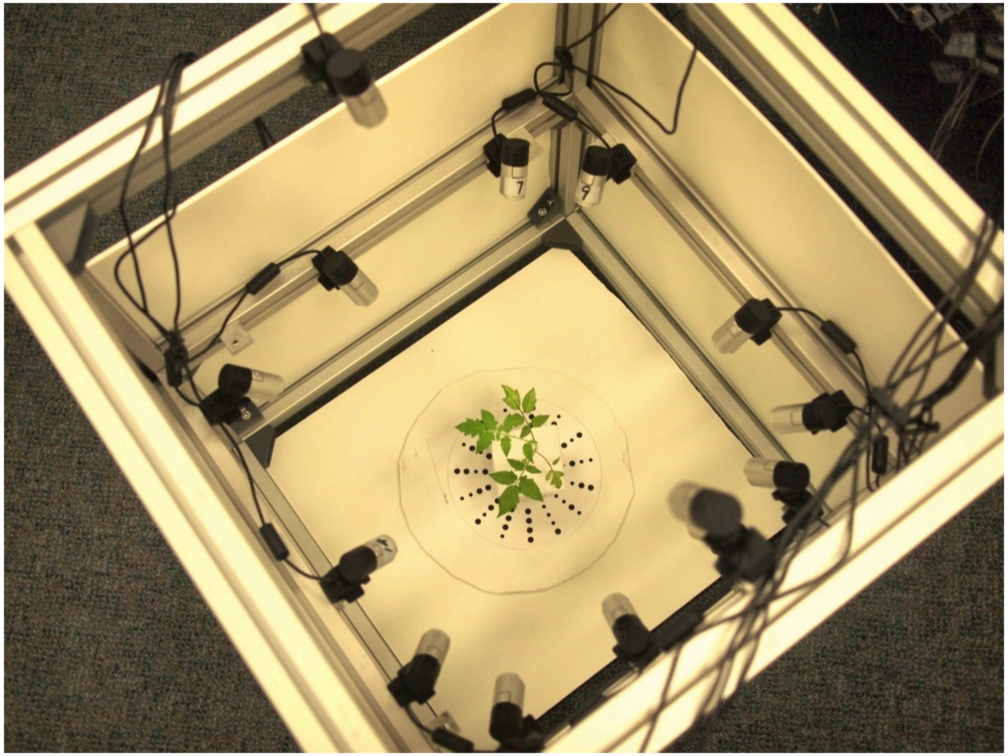



Our first step towards towards crop plants: in situ 3D imaging.

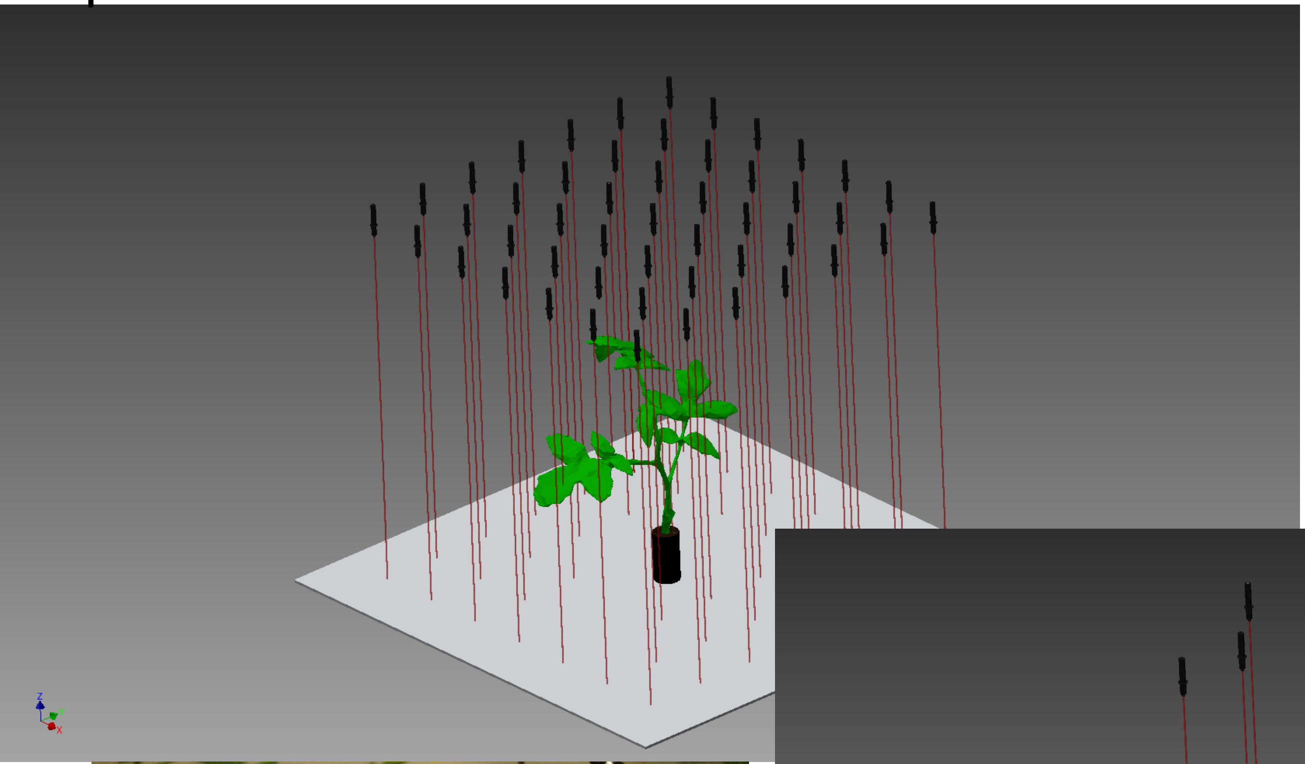
Rotate the plant

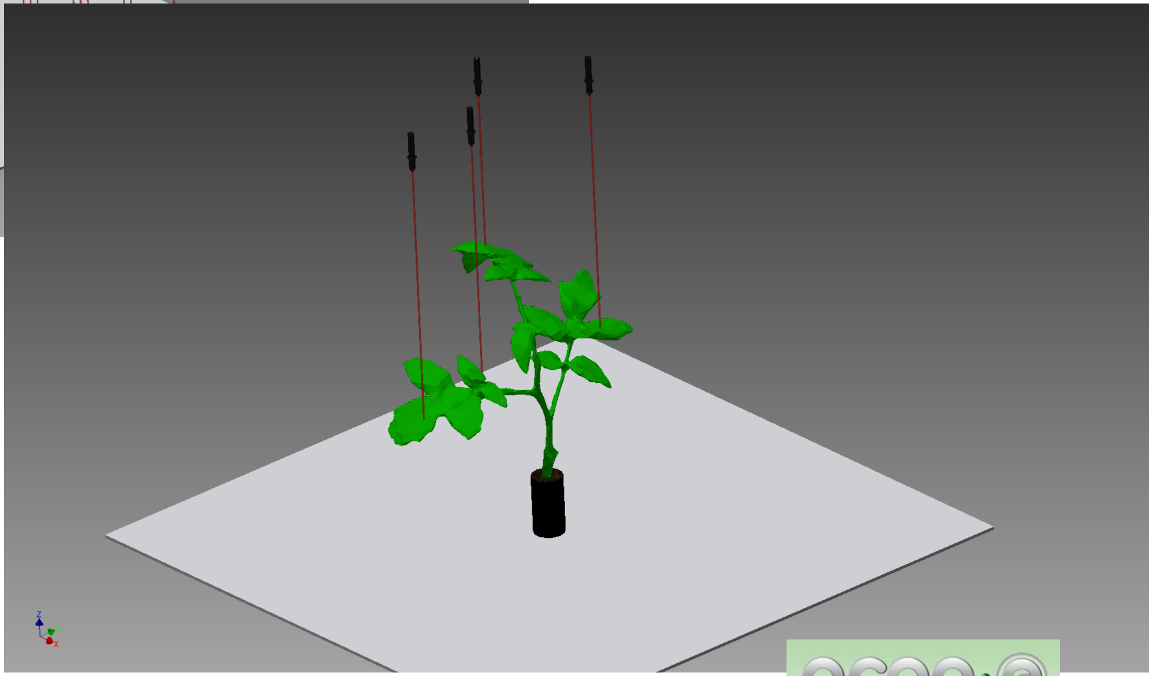
Swivel the camera

Use multiple cameras from different positions



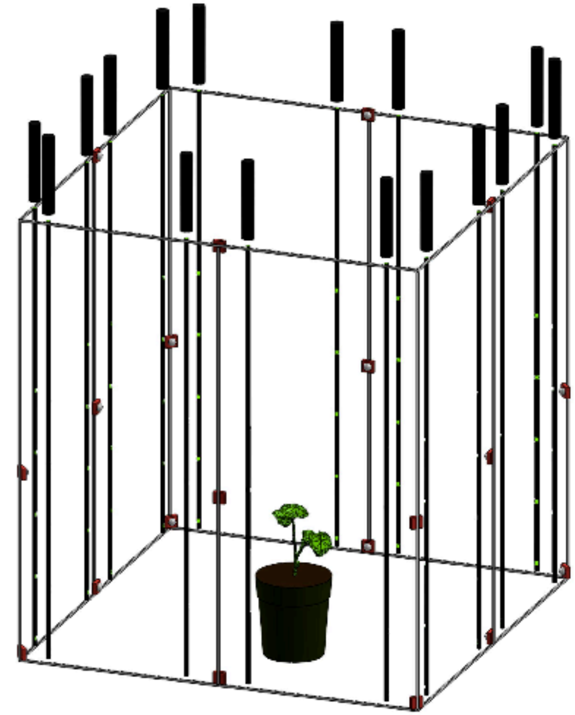
Laser- and light fiber fiduciary points define the Z-coordinates in X-Y space





arpa·e

Next step: Non-obtrusive matrix imaging



LeafDB

Definition: LeafDB is a functional leaf database for capturing, storing, extracting and sharing leaf shapes of many plants in 3D.

Why do we need LeafDB in plant 3D modeling?

- Leaf recognition
- Leaf reconstruction
- De-obscuring
- Plant 3D photosynthesis modeling

Fig 1. Leaf 3D image capture with NextEngine 3D scanner

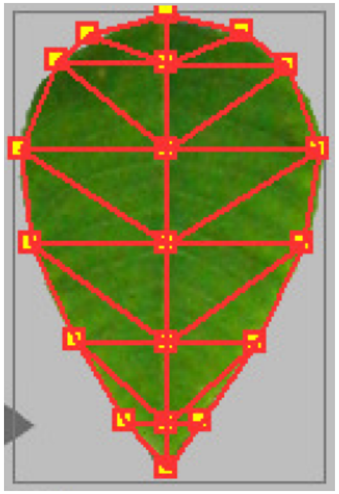


Fig 2. Leaf feature extraction with smooth curve fitting and triangulation

Excellent example of LeafDB (2D)

Plants is one of most difficult kinds of object to model due to their complex geometry and wide variation in appearance!

leafsnap

Leafsnap: An Electronic Field Guide

Leafsnap is the first in a series of electronic field guides being developed by researchers from Columbia University, the University of Maryland, and the Smithsonian Institution. This free mobile app uses visual recognition software to help identify tree species from photographs of their leaves.

Leafsnap contains beautiful high-resolution images of leaves, flowers, fruit, petiole, seeds, and bark. Leafsnap currently includes the trees of New York City and Washington, D.C., and will soon grow to include the trees of the entire continental United States.

This website shows the tree species included in Leafsnap, the collections of its users, and the team of research volunteers working to produce it.

Free for iPhone: Available on the App Store **and iPad:** Available on the iPad App Store

Japanese Flowering Crabapple

Malus floribunda

This species is native to Asia, but has been introduced into the United States for its beautiful floral display. These deciduous trees grow 4-7 m tall, producing copious amounts of white to pale pink flowers in the spring. The species name floribunda means "abundant flowers" in Latin.

Habitat: Planted as an ornamental, growing best in full sun.

Growth Habit: Deciduous tree, growing to about 4.5-7.6 m tall.

Bloom Time: Spring.

Longevity: Moderate.

Presence in US: CT DC KS LA MA MD NY OH OR PA

You Tube

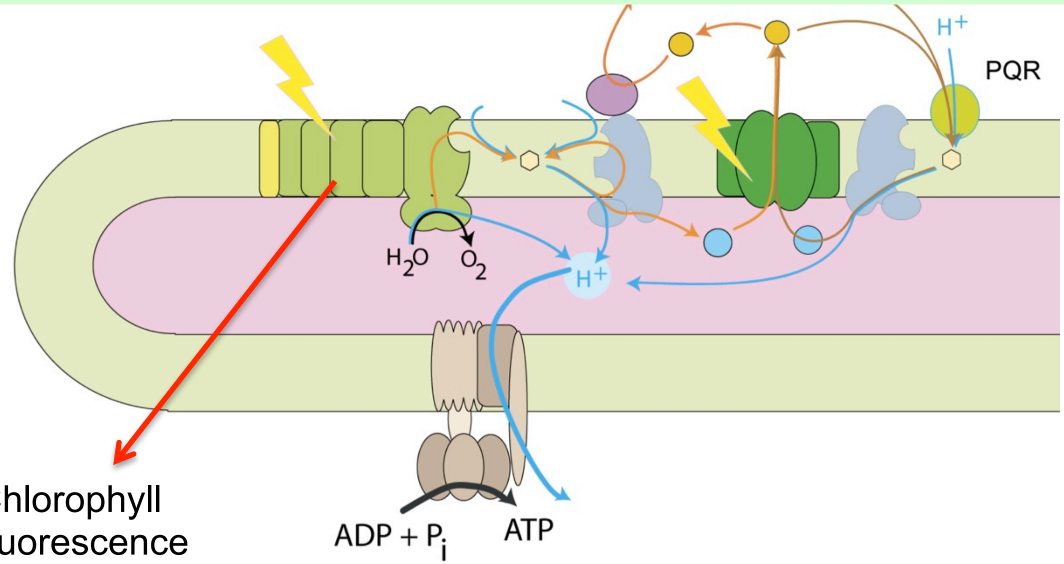
guardian.co.uk

How to distinguish one plant from another in a canopy?

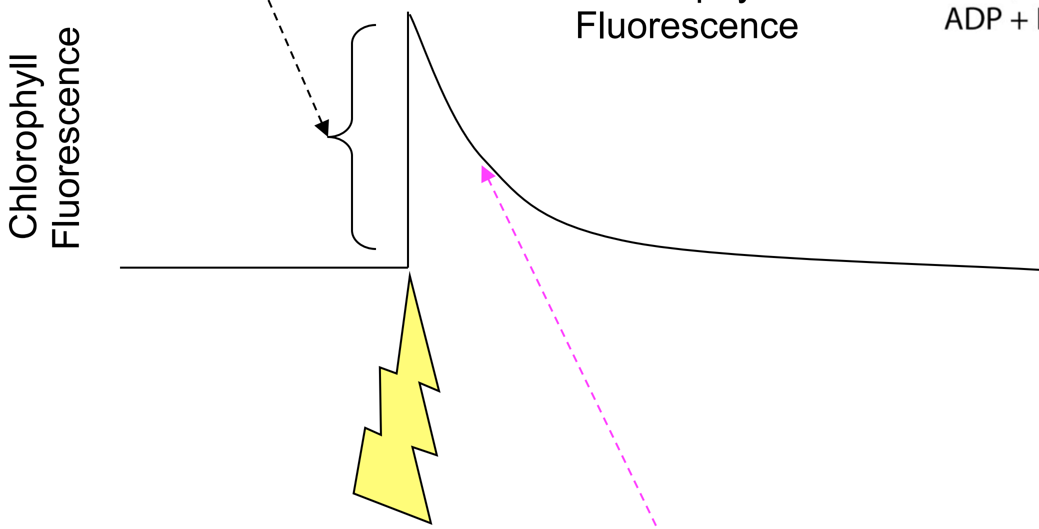
Plant model.

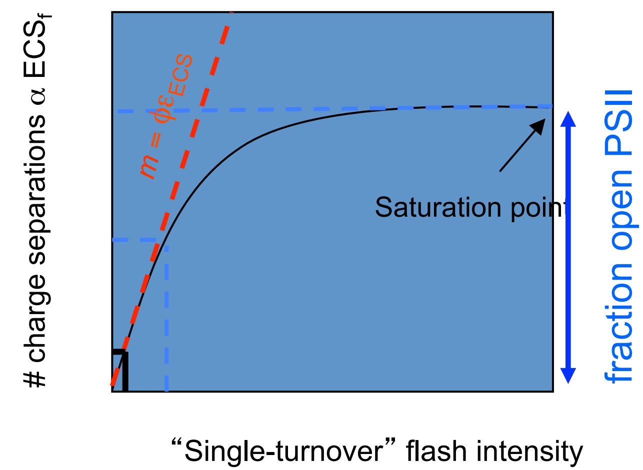
Time sequence (measure from seed to senescence…know the position and shape of leaves as they develop).

New assay can image photosynthesis without saturation pulses, in 3D, possibly in field



A function of the number excitations of PSI





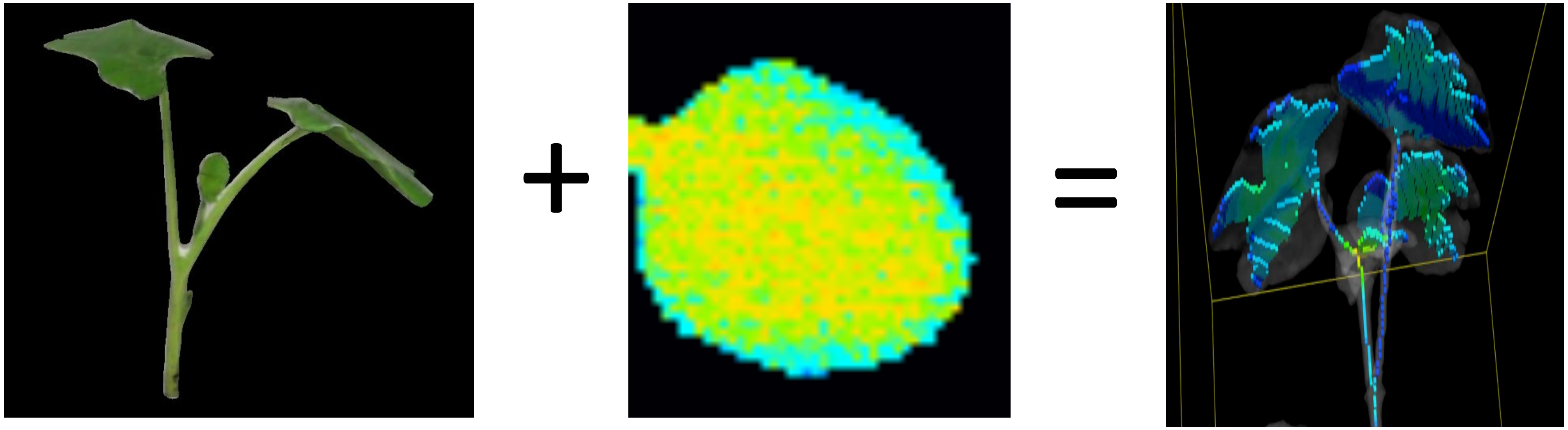
= μs flash

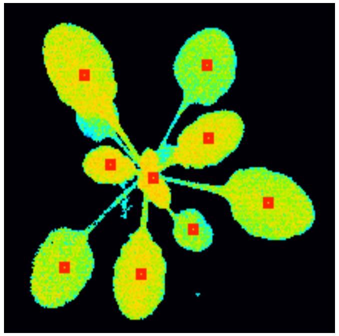
A function of the PSII turnover rate

3D image registration and modeling

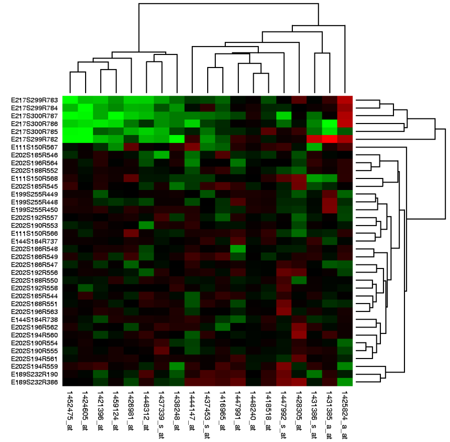
Definition: Image registration is the process of establishing point-by-point correspondence between two images of a scene.

Goal: To integrate the 3D image-derived information and photosynthesis information to model photosynthesis and productivity throughout the life of the plant



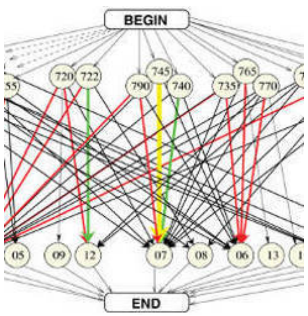


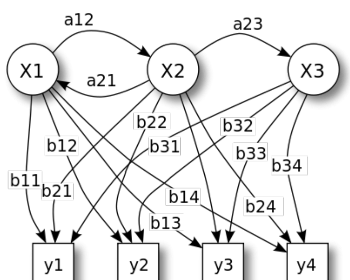
Phenotype data



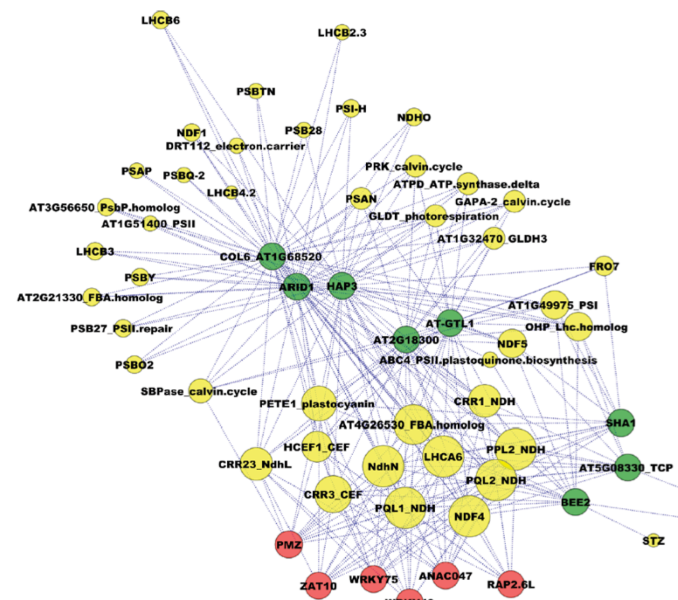
Genotype data

Challenge 3: (The big challenge): How to connect the measured phenotypes to performance in the field, and ultimately to genotype.





Probabilistic graphical models



Immediate short-term (achievable?) goal:

Can we better predict field performance outcomes from controlled (chamber/greenhouse) experiments?

Possible Approach:

1. Start with a library of plants the do well under
2. What conditions and measurements predict the observe outcomes
3. Expose to different dynamic environmental conditions
4. photosynthesis, HSR, growth, architecture, leaf shape etc.