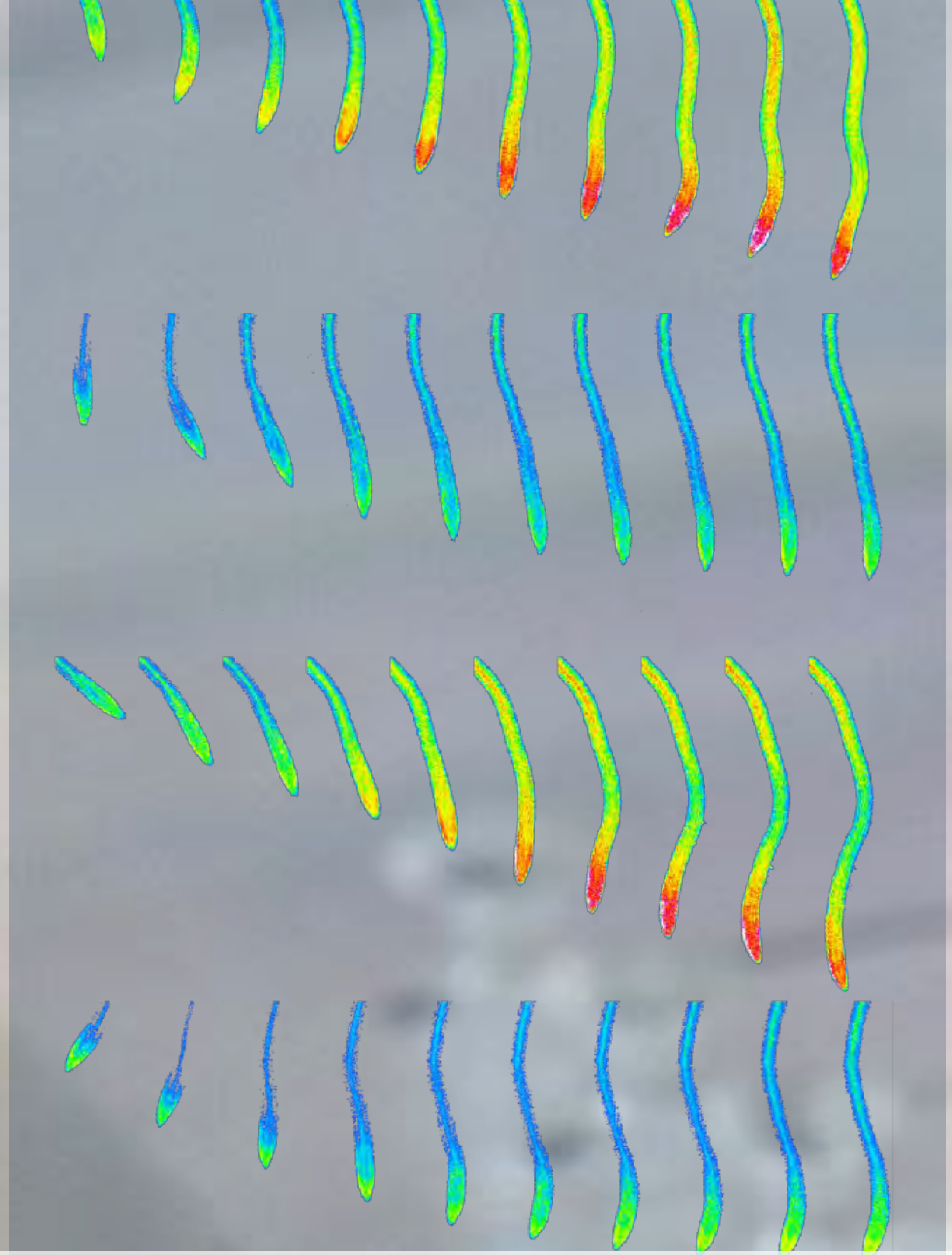


NANOSENSORS AND MICROFLUIDICS

Techniques for **kinetic plant phenotyping** at the microscale

Guido Grossmann

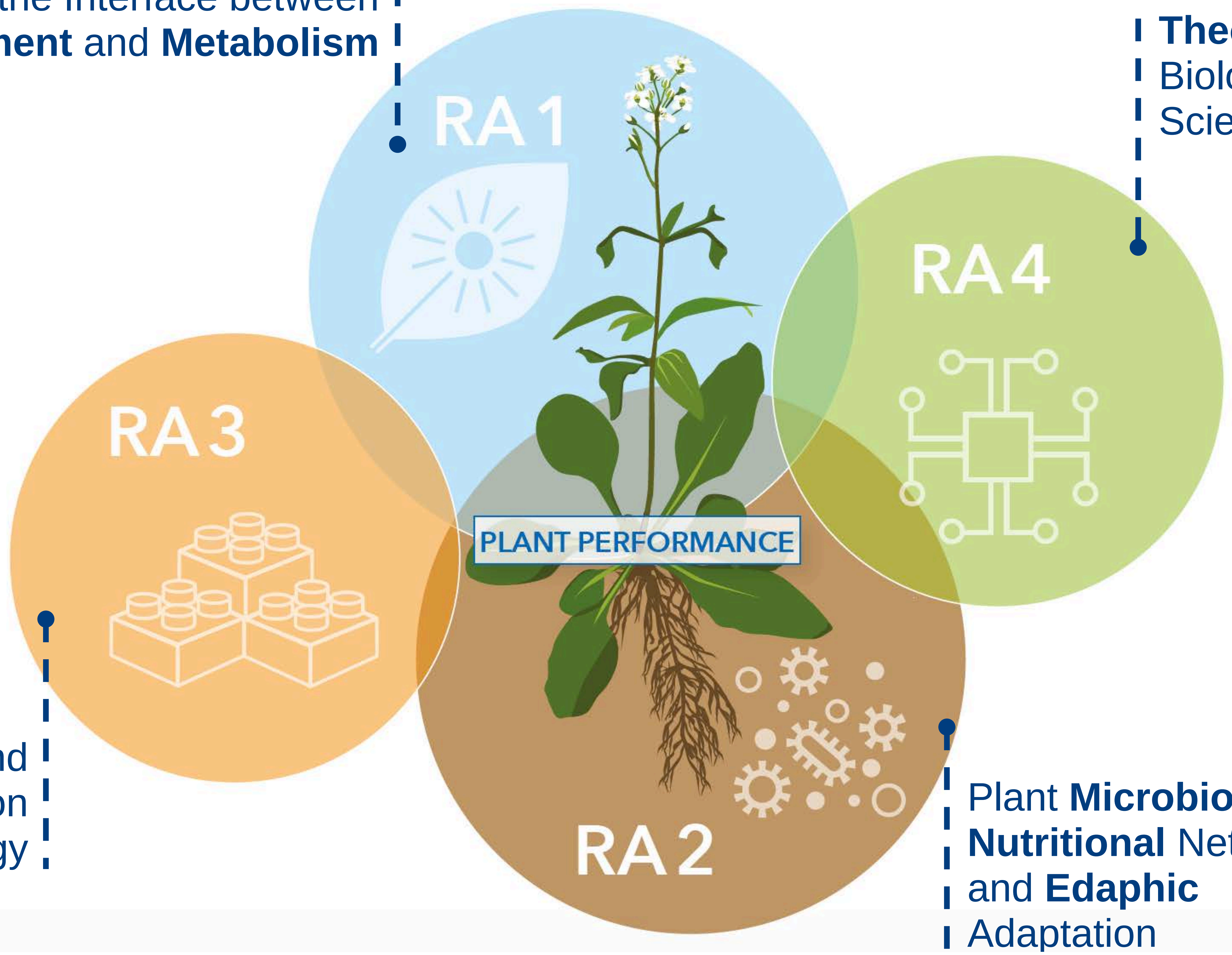
Institute for Cell and Interaction Biology - HHU Düsseldorf
Cluster of Excellence in Plant Sciences - CEPLAS

Cluster of Excellence in Plant Sciences

SMART Plants for Tomorrow’s Needs

Optimizing Plant Performance by Mapping the Interface between **Development** and **Metabolism**

Theoretical Plant Biology and **Data** Science



Synthetic and Reconstruction Biology

Plant **Microbiota** **Nutritional** Networks and **Edaphic** Adaptation

Heinrich Heine Universität Düsseldorf

JÜLICH FORSCHUNGSZENTRUM

Universität zu Köln

Max-Planck-Institut für Pflanzenzüchtungsforschung

More than 50 labs are members of CEPLAS

www.ceplas.eu

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Academic cooperation & technology transfer

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ibr
INNOVATION BY RESEARCH

Dr. Günter Strittmatter
CEPLAS Technology Transfer and Cooperation Management

CEPLAS - Germany‘s Research Triangle for molecular plant sciences

Nanosensors and Microfluidics

Techniques for **kinetic plant phenotyping** at the microscale

Kinetic phenotyping: probing plant performance under changing environmental conditions

Guido Grossmann

Kinetic phenotyping: probing plant performance under changing environmental conditions

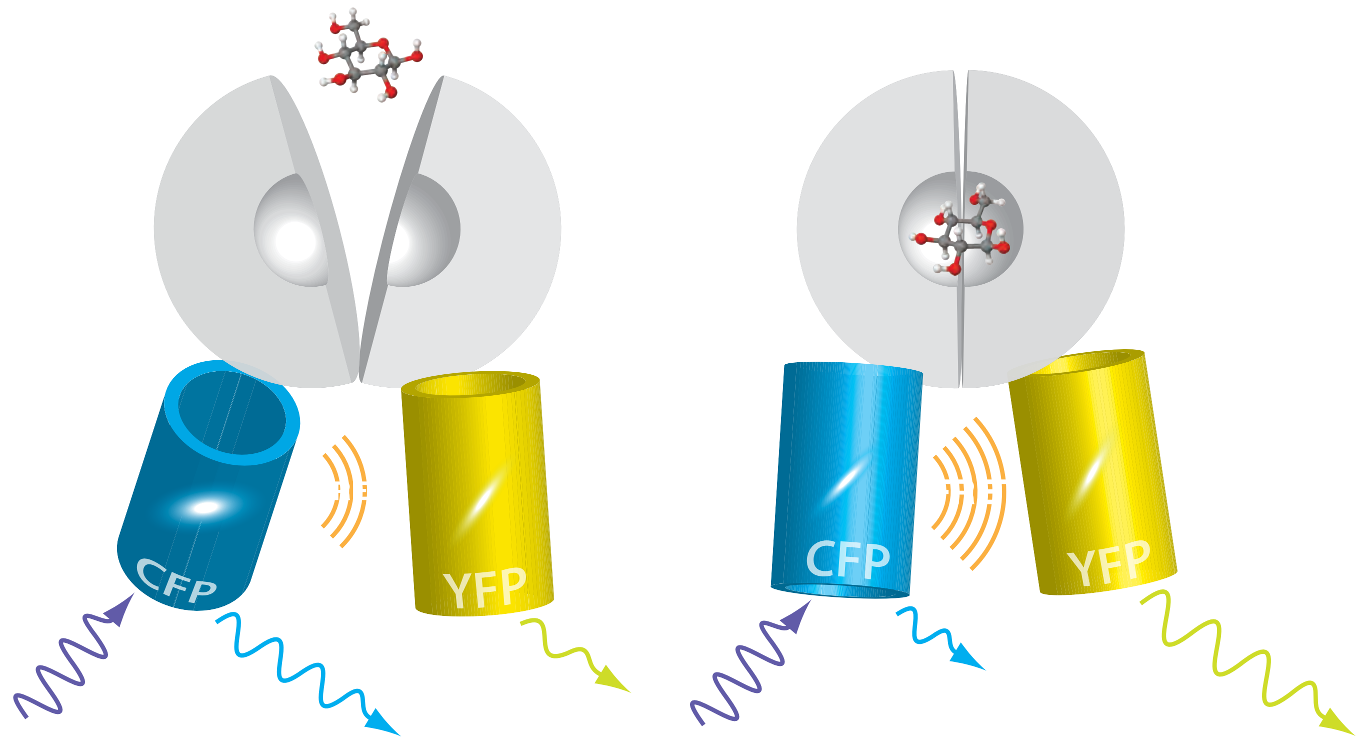
Guido Grossmann

Kinetic phenotyping: probing plant performance under changing environmental conditions

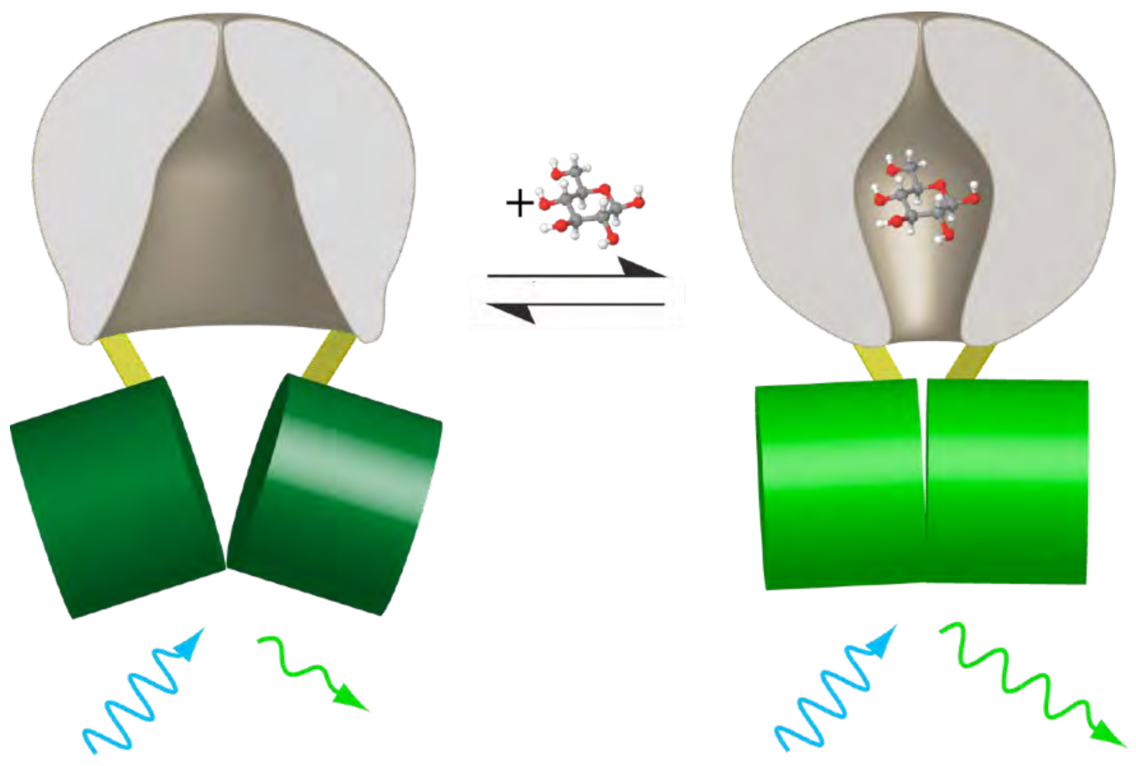
Guido Grossmann

Biosensors for dynamic and quantitative imaging of small molecules

Ratiometric design



Intensiometric design

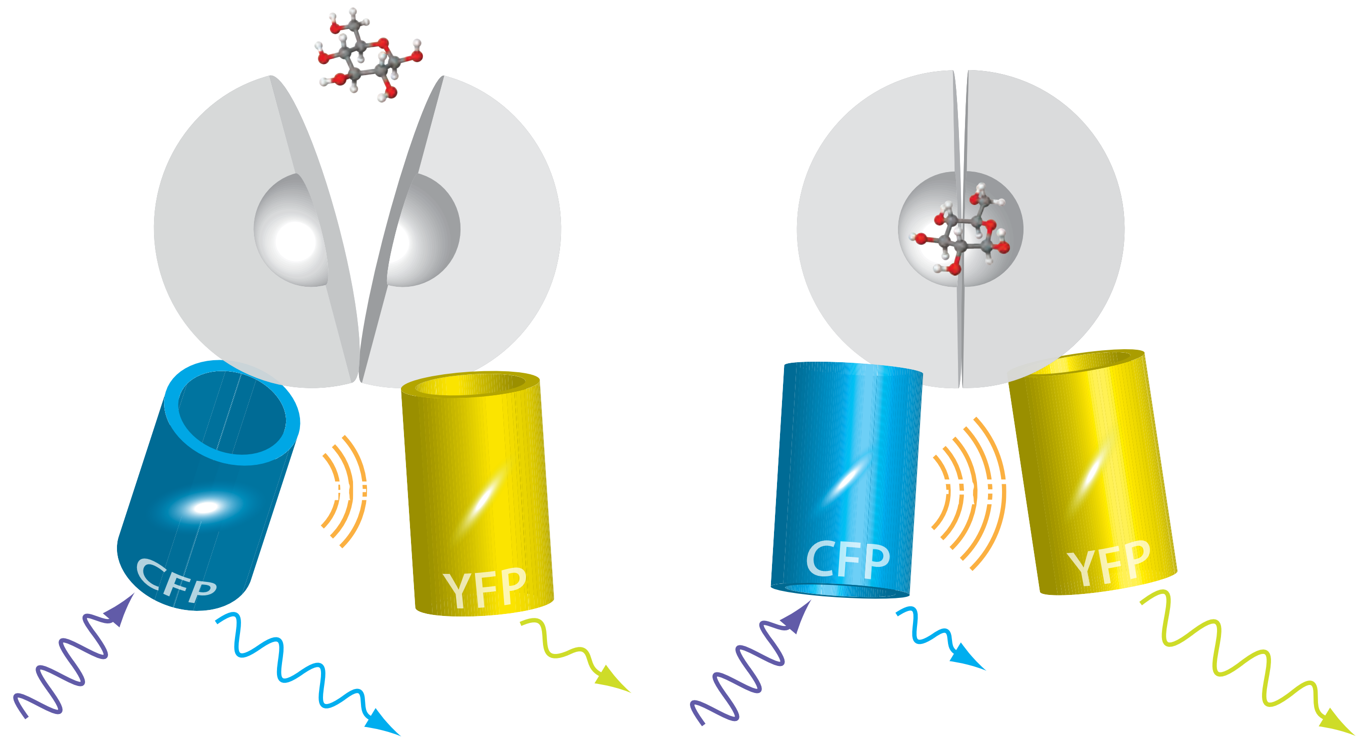


- minimally invasive; genetically encoded
- dynamic measurements
- subcellular targeting
- qualitative or quantitative (req. calibration)

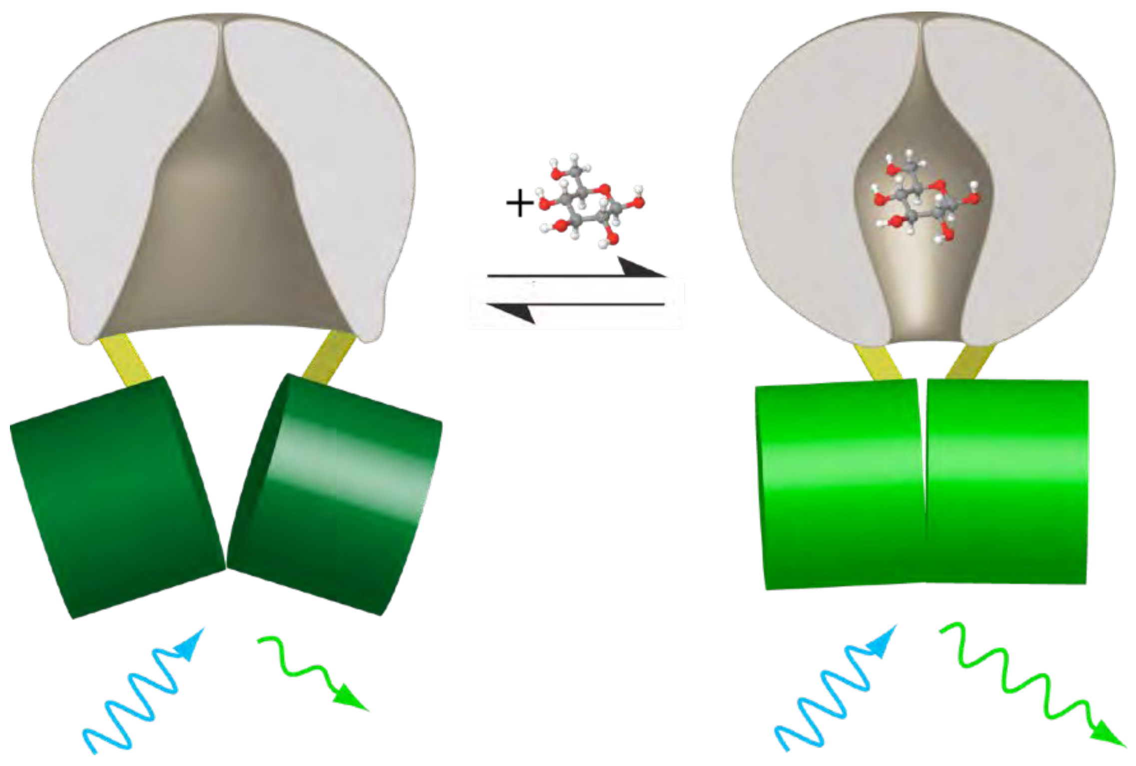
Grossmann et al. 2011 *Plant Cell*

Biosensors for dynamic and quantitative imaging of small molecules

Ratiometric design



Intensiometric design



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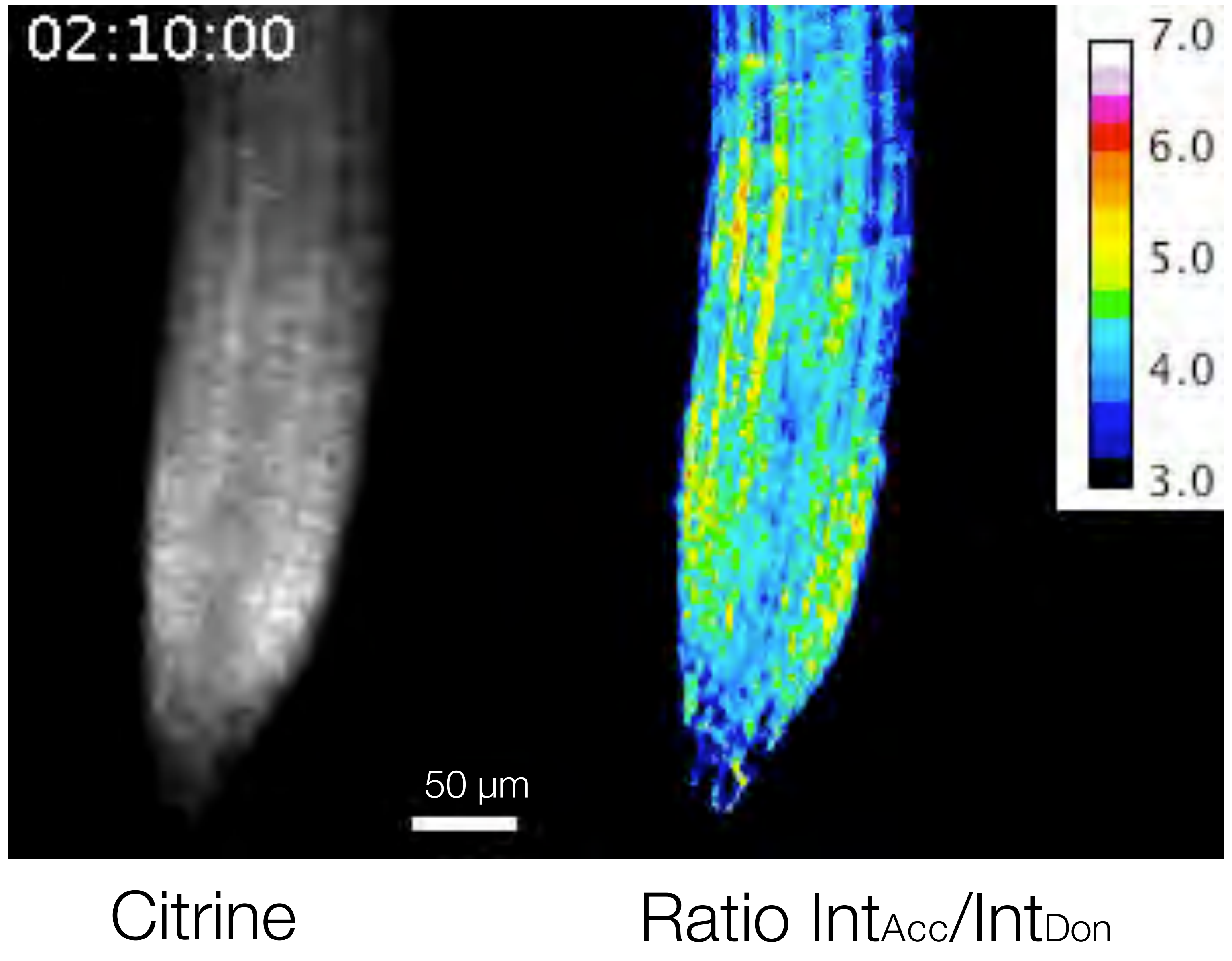
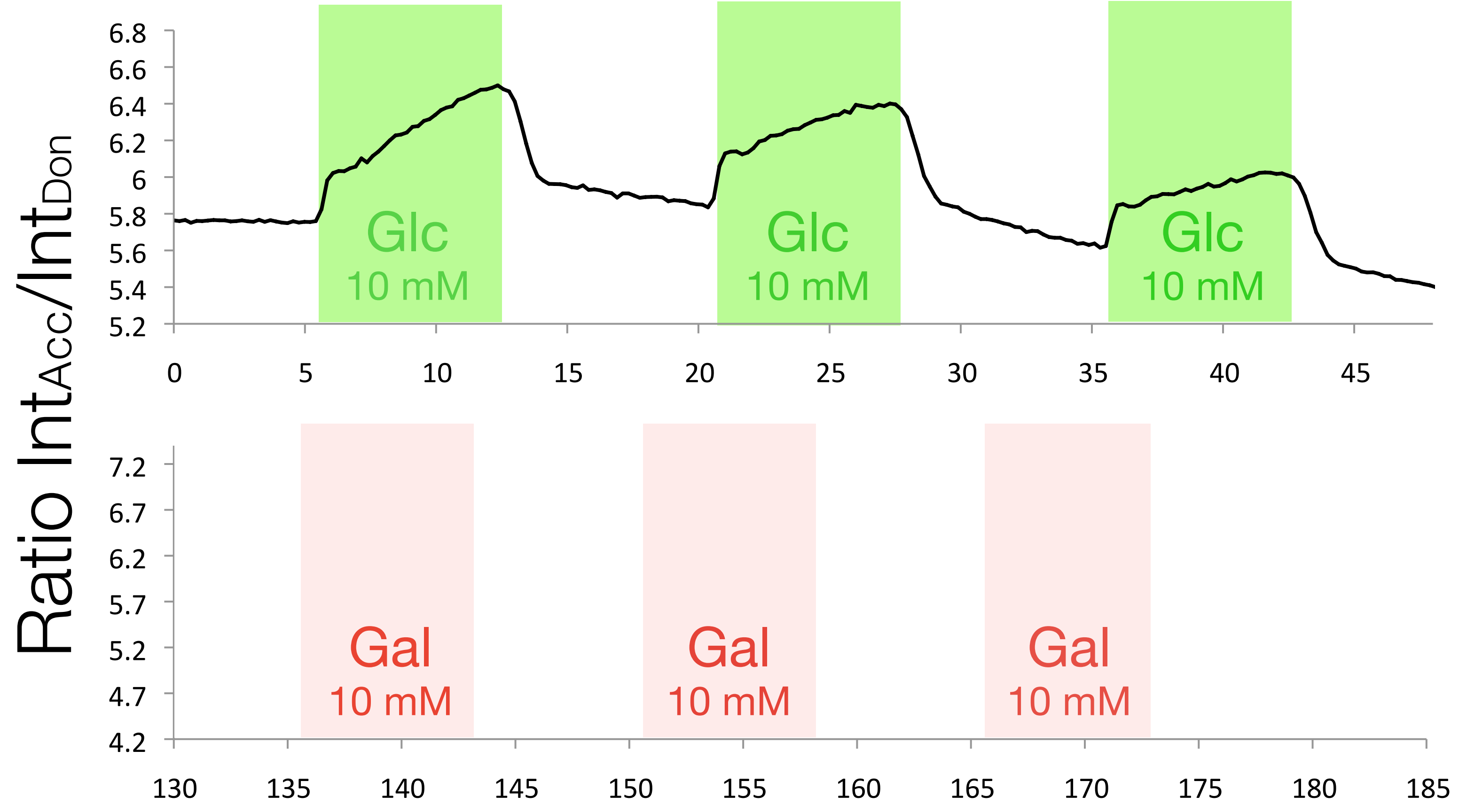
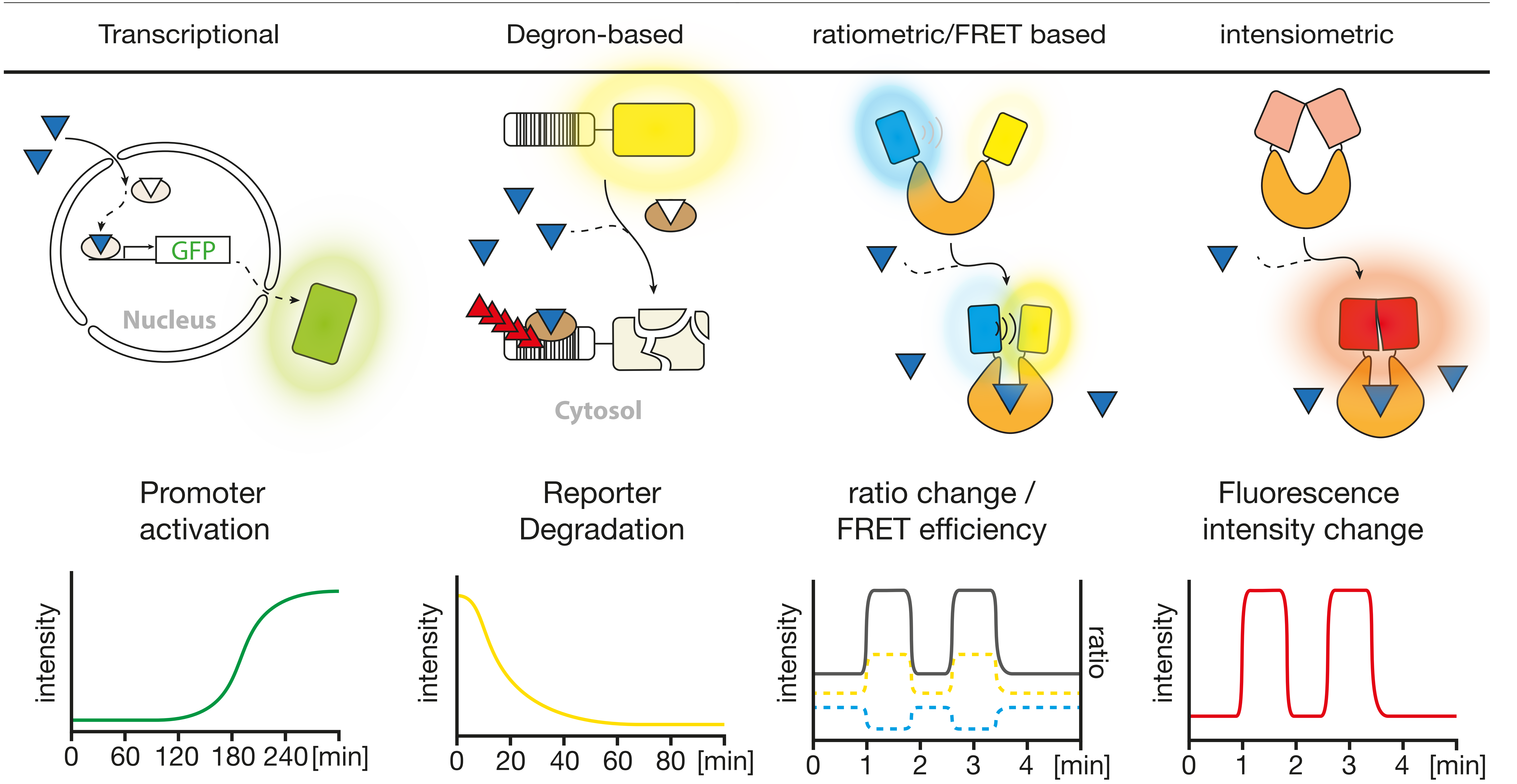




Roots expressing genetically encoded FLIPglu FRET sensor

Grossmann et al. 2011 *Plant Cell*

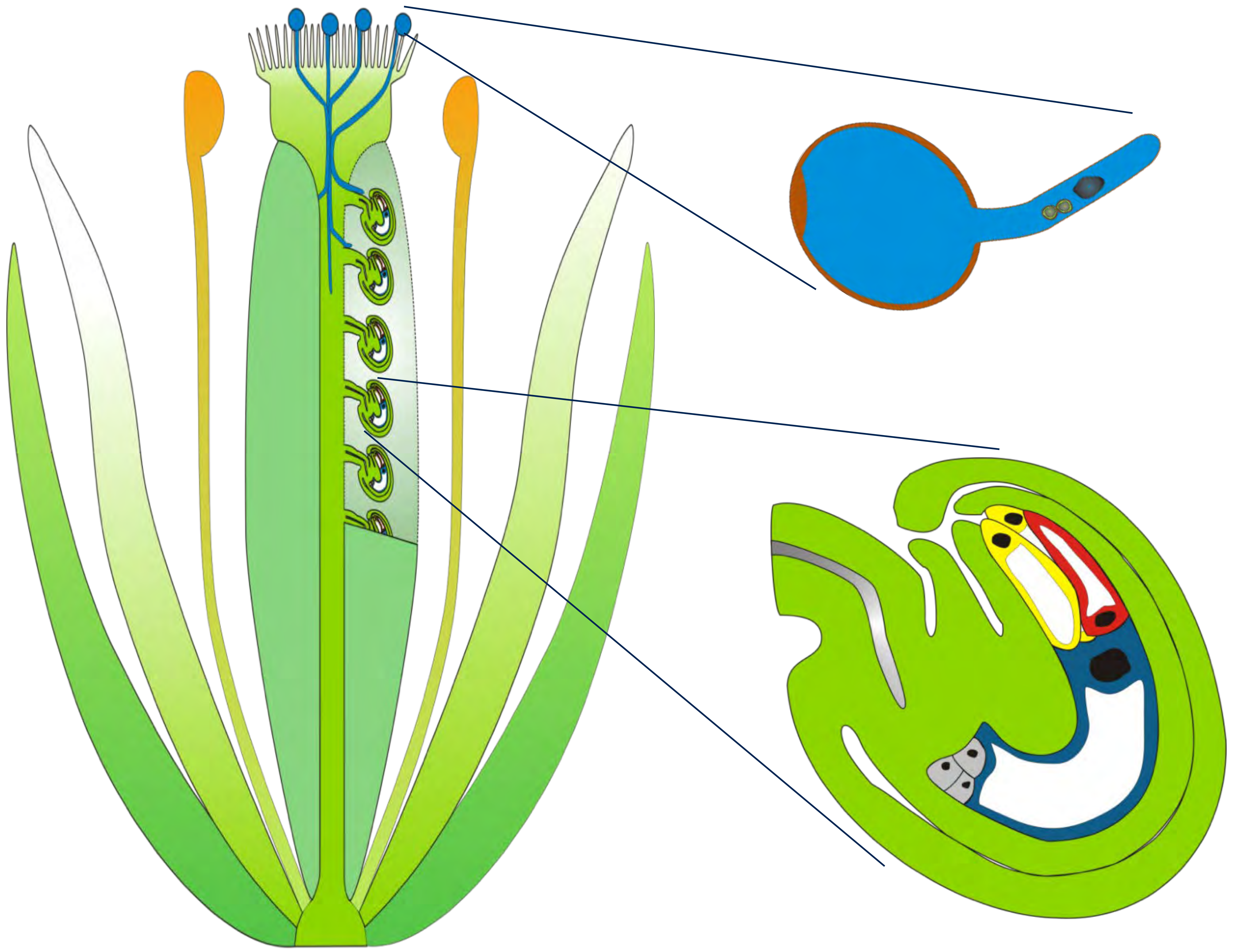
Technologies for microscopic imaging of molecular dynamics

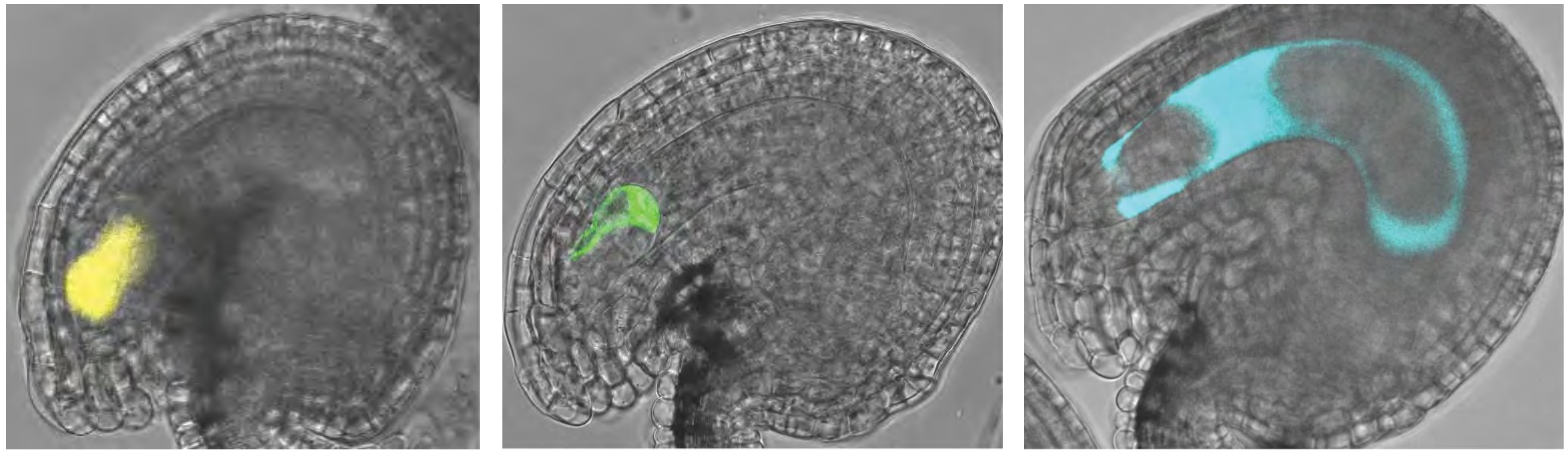


Uslu & Grossmann, *Curr Opin Plant Biol* 2015

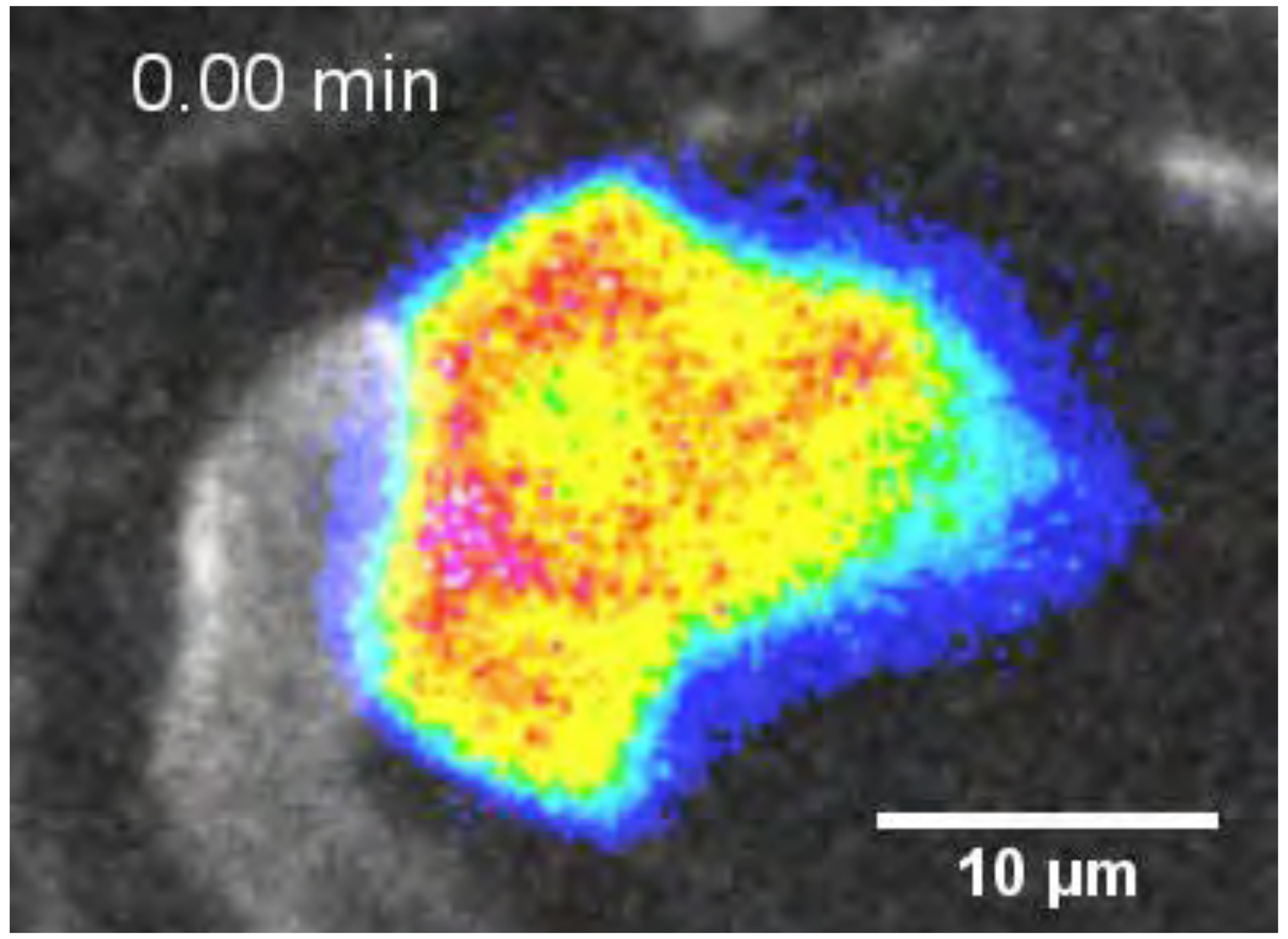
Calcium imaging during double fertilization

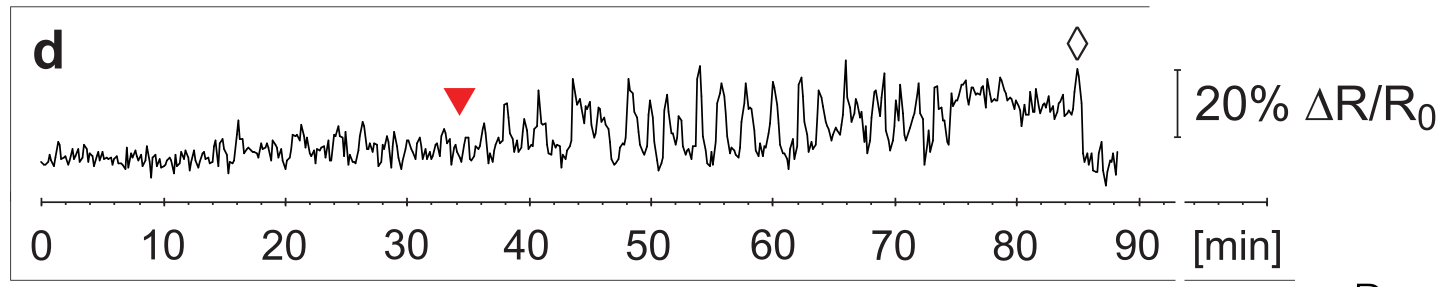
Male and female gametophytes in Arabidopsis





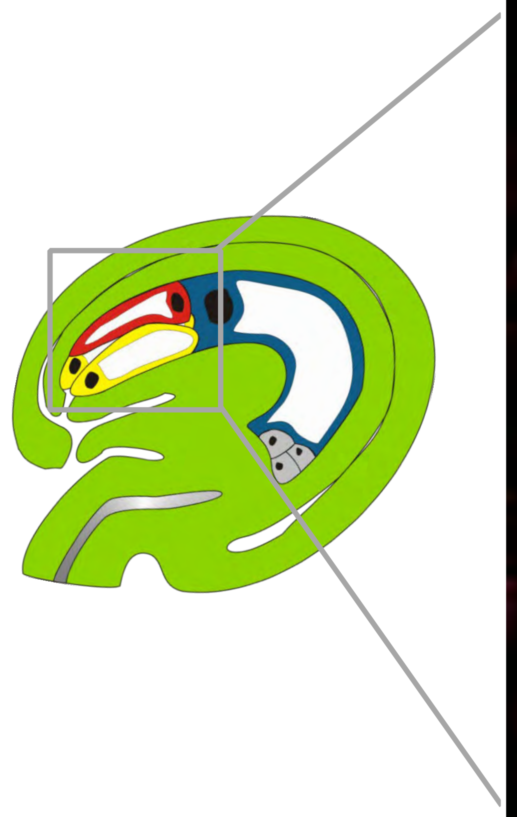
Synergids expressing CerTNL15

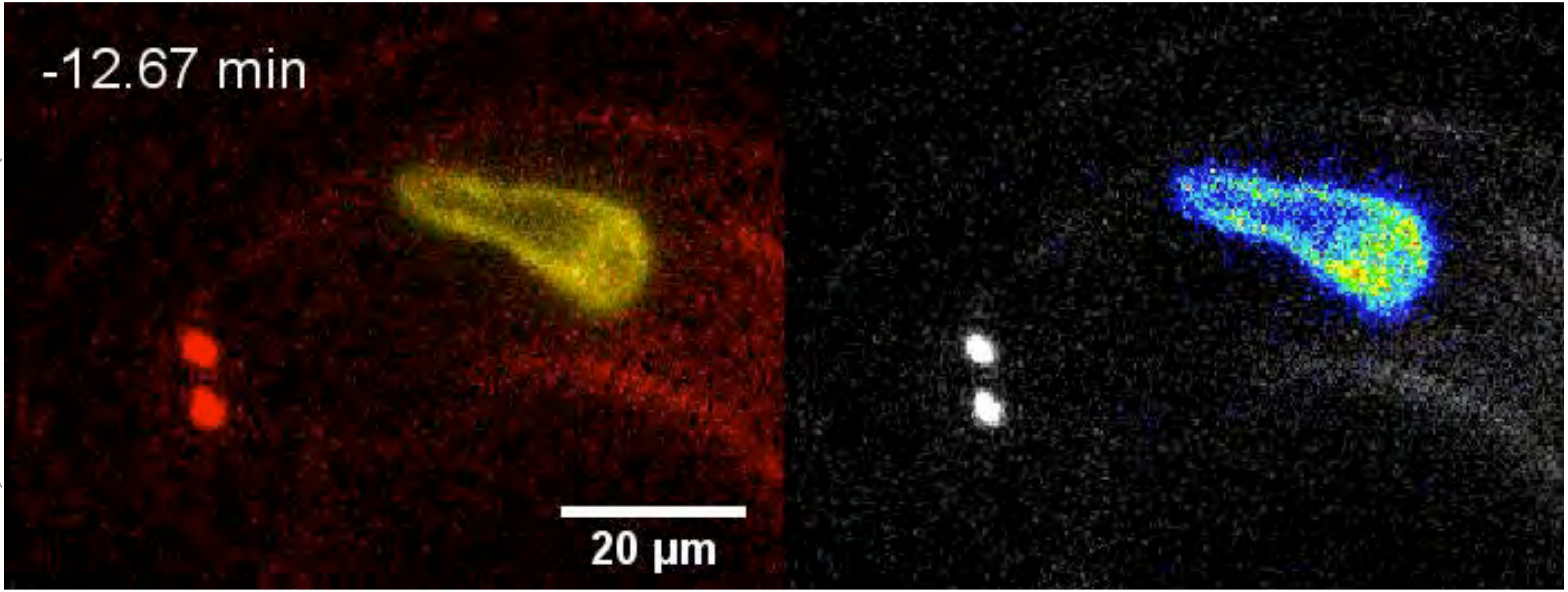


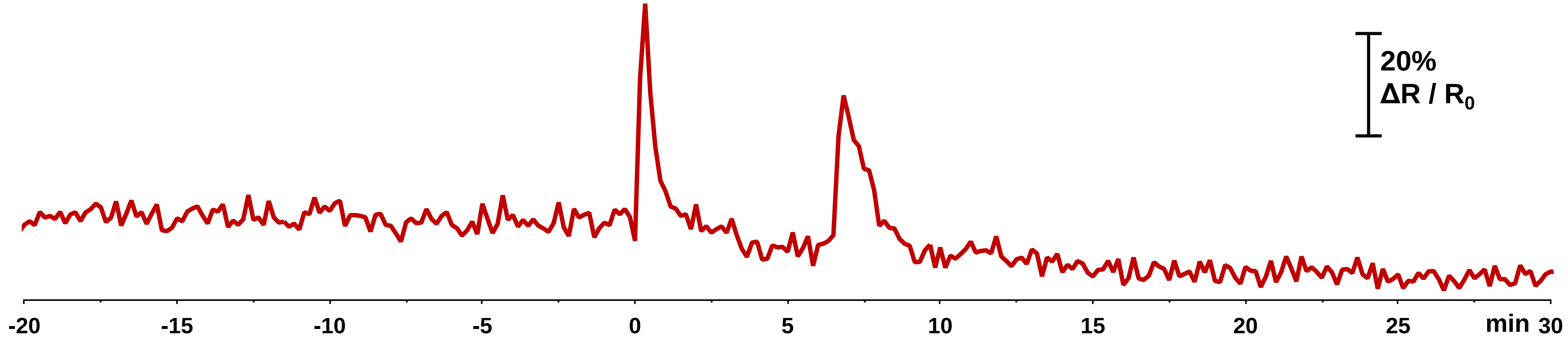


Denninger et al., 2014 *Nat Commun*

Calcium waves in the egg cell upon sperm cell arrival and gamete fusion



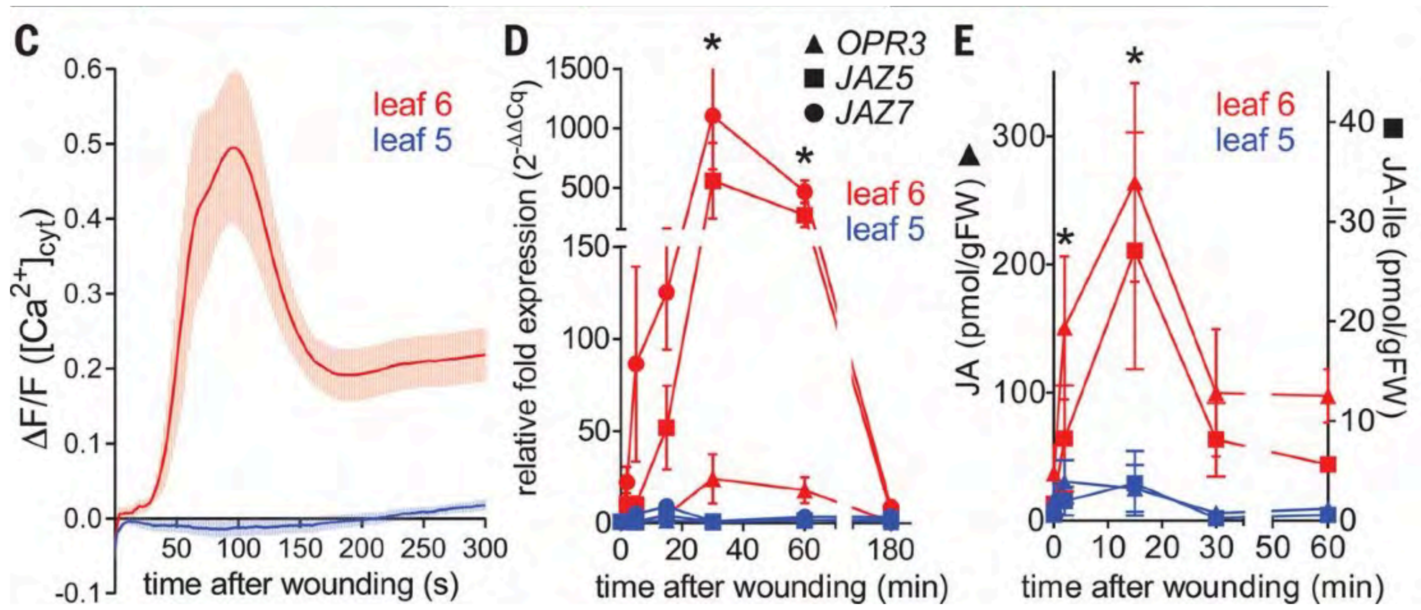




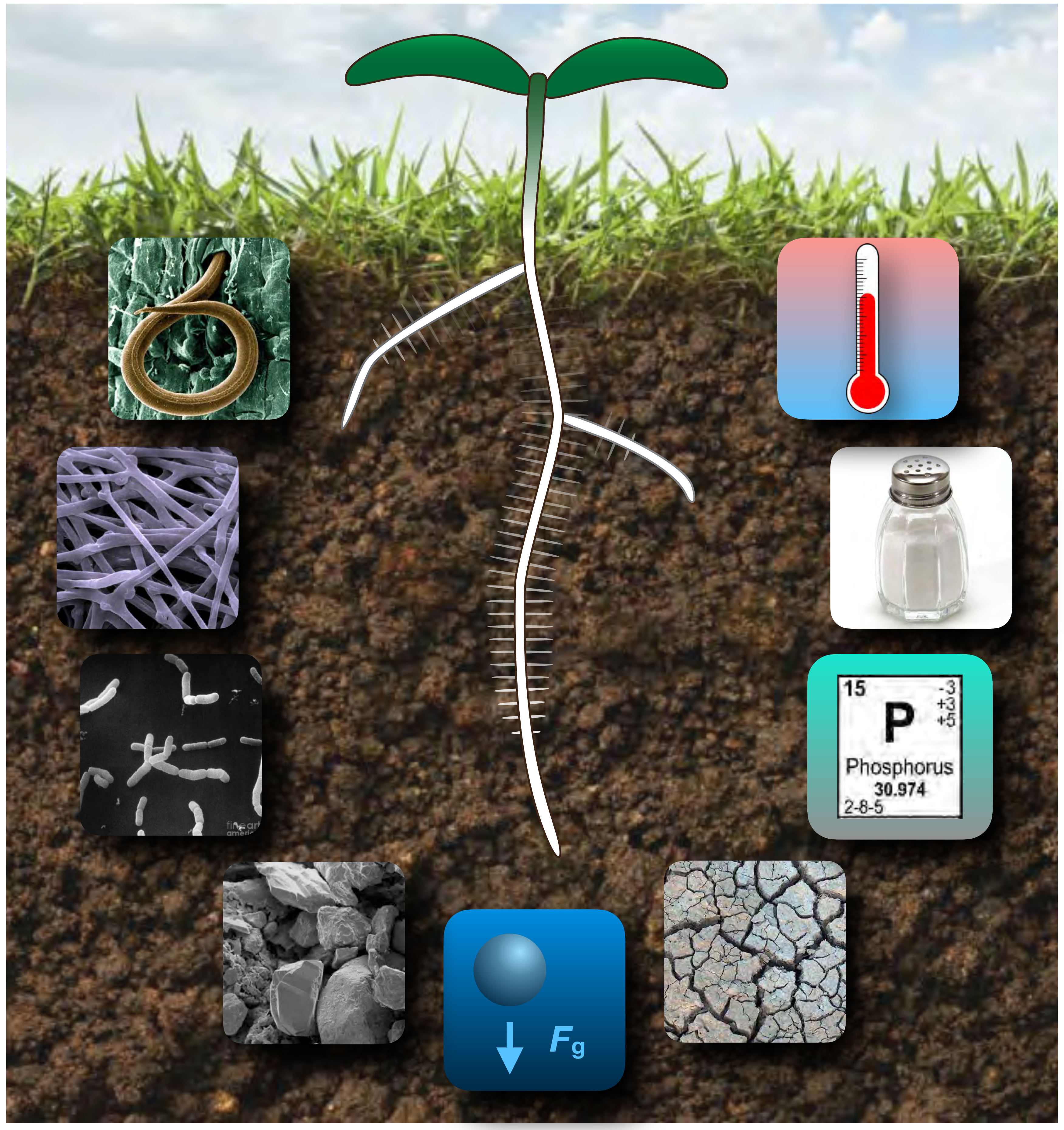
Denninger et al., 2014 *Nat Commun*

Successful defense is a matter of timing

Herbivory triggers Ca^{2+} waves towards younger leaves



Toyota et al. 2018 *Science*

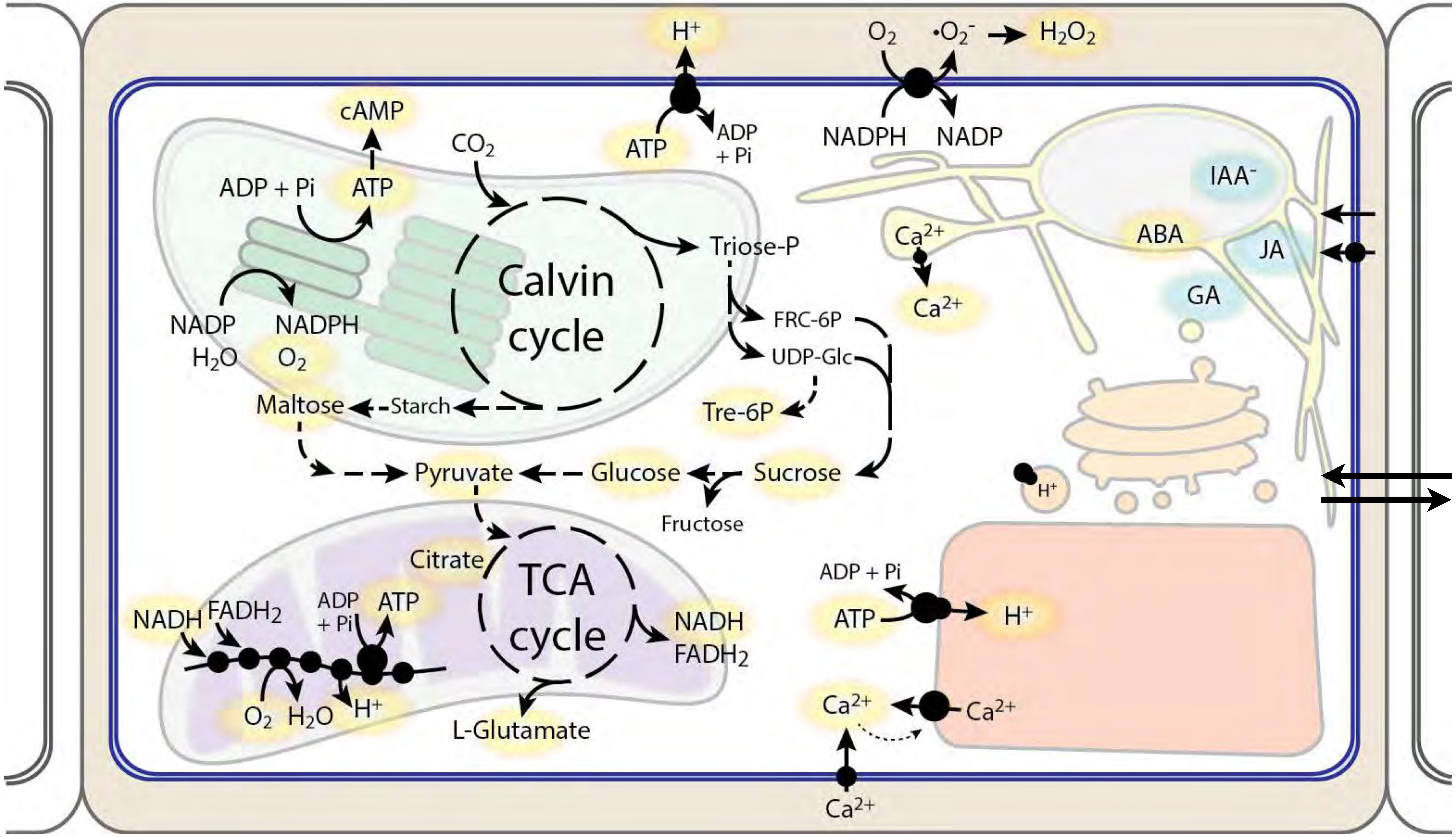


Time is critical for *any* successful acclimatization

Goal: understanding the mechanisms that determine the <u>kinetics</u> of molecular dynamics?

The ever-expanding Biosensor toolbox

… for a broad spectrum of nutrients, metabolites and signaling molecules.



K
Mg
P
Zn
(Fe)
B_{12}
…

- https://www.molecular-physiology.hhu.de/en/resources

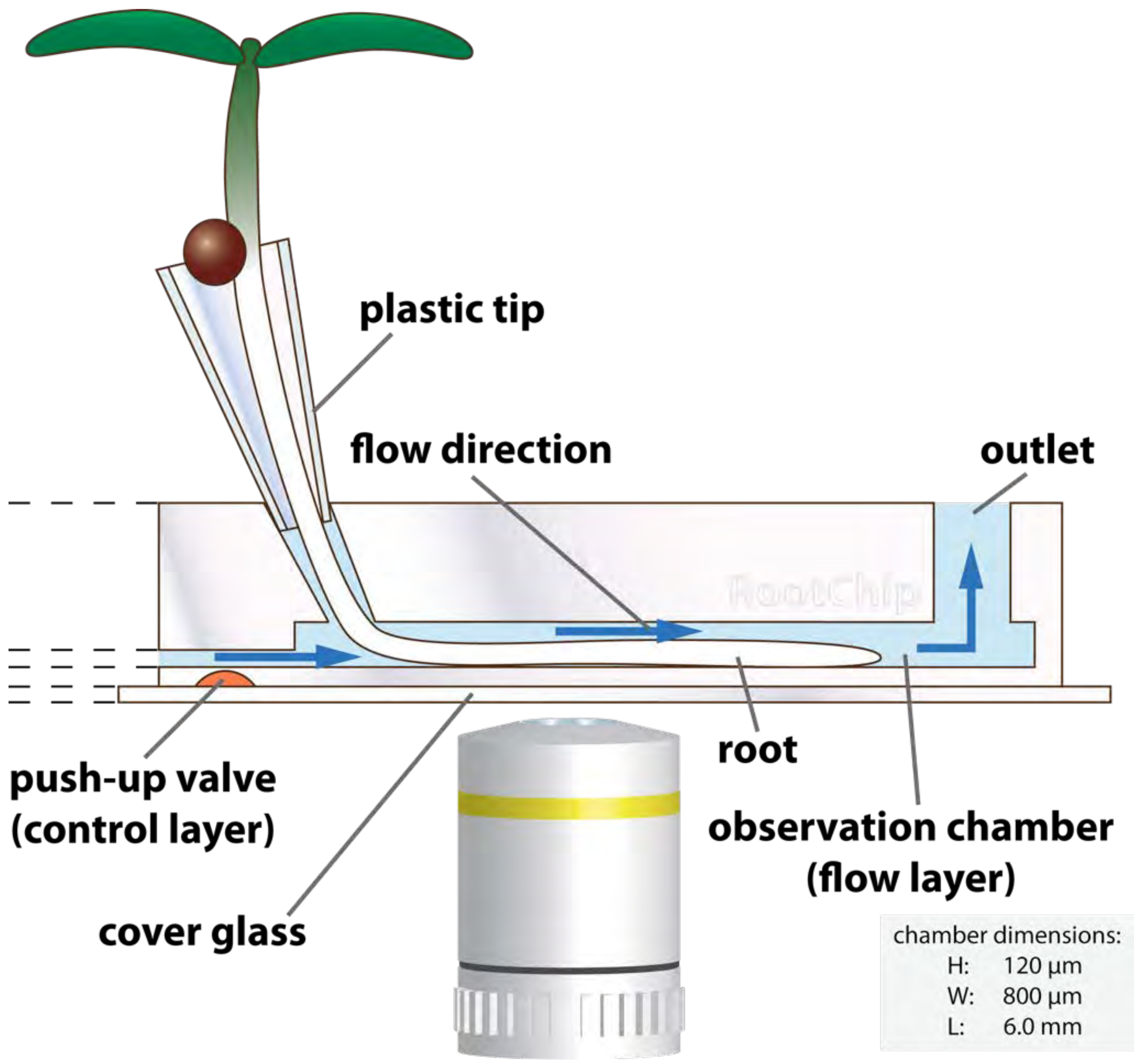
modified from Uslu & Grossmann, *Curr Opin Plant Biol* 2015

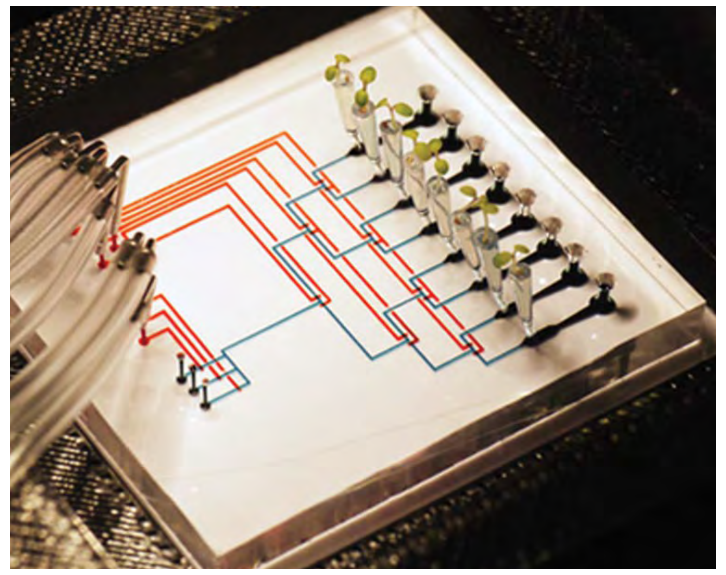
Devices for precision control of the root microenvironment

RootChip

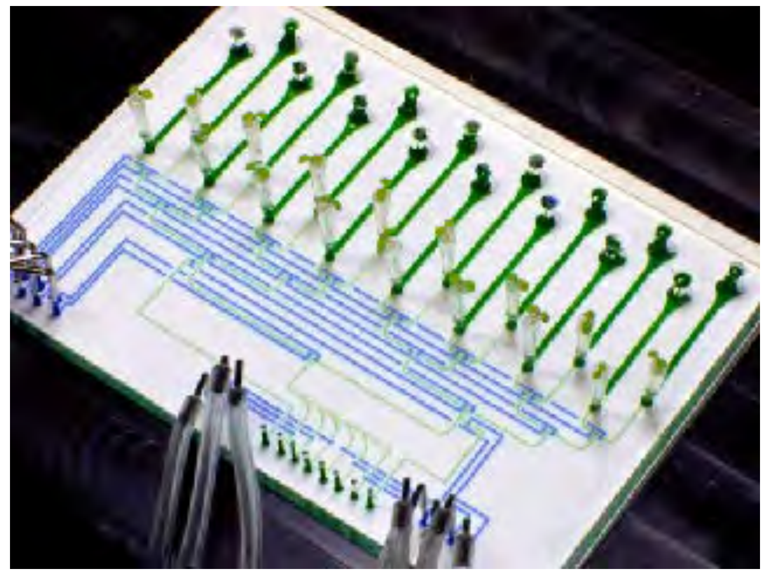
microfluidic perfusion and imaging platform for roots

- Individually controllable micro-perfusion chambers
- Root growth on chip = no specimen handling
- Parallelization & automation

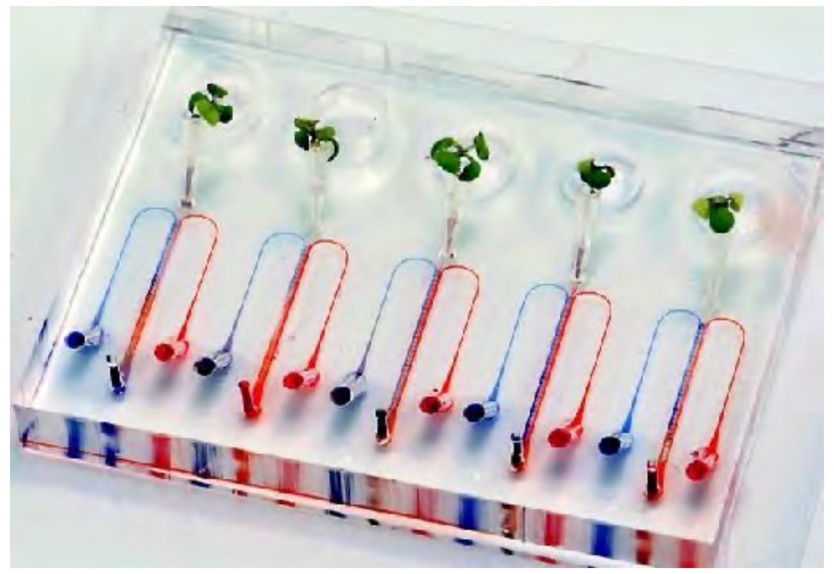




RootChip
Grossmann et al. 2011 *Plant Cell*
Grossmann et al. 2012 *JoVE*
Lanquar et al. 2013 *New Phytol*



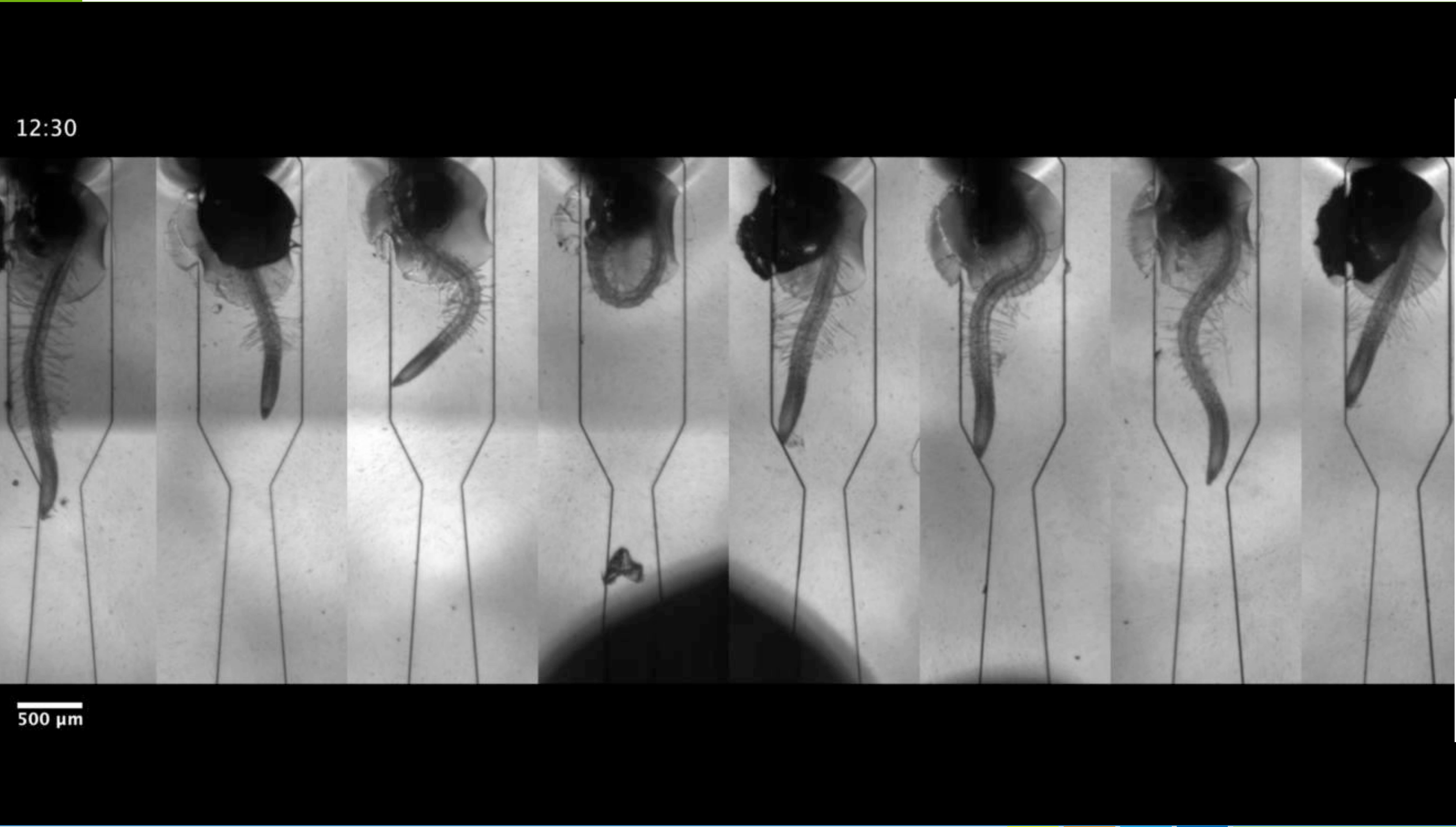
RootChip16
Jones et al. 2014 *eLife;*
Denninger et al. 2014 *Nat Commun*
Keinath et al. 2015 *Mol Plant*
Souza et al. 2017 *Plant Phys*
Xing et al. 2017 *PNAS*
Brost et al. 2019 *Plant J*

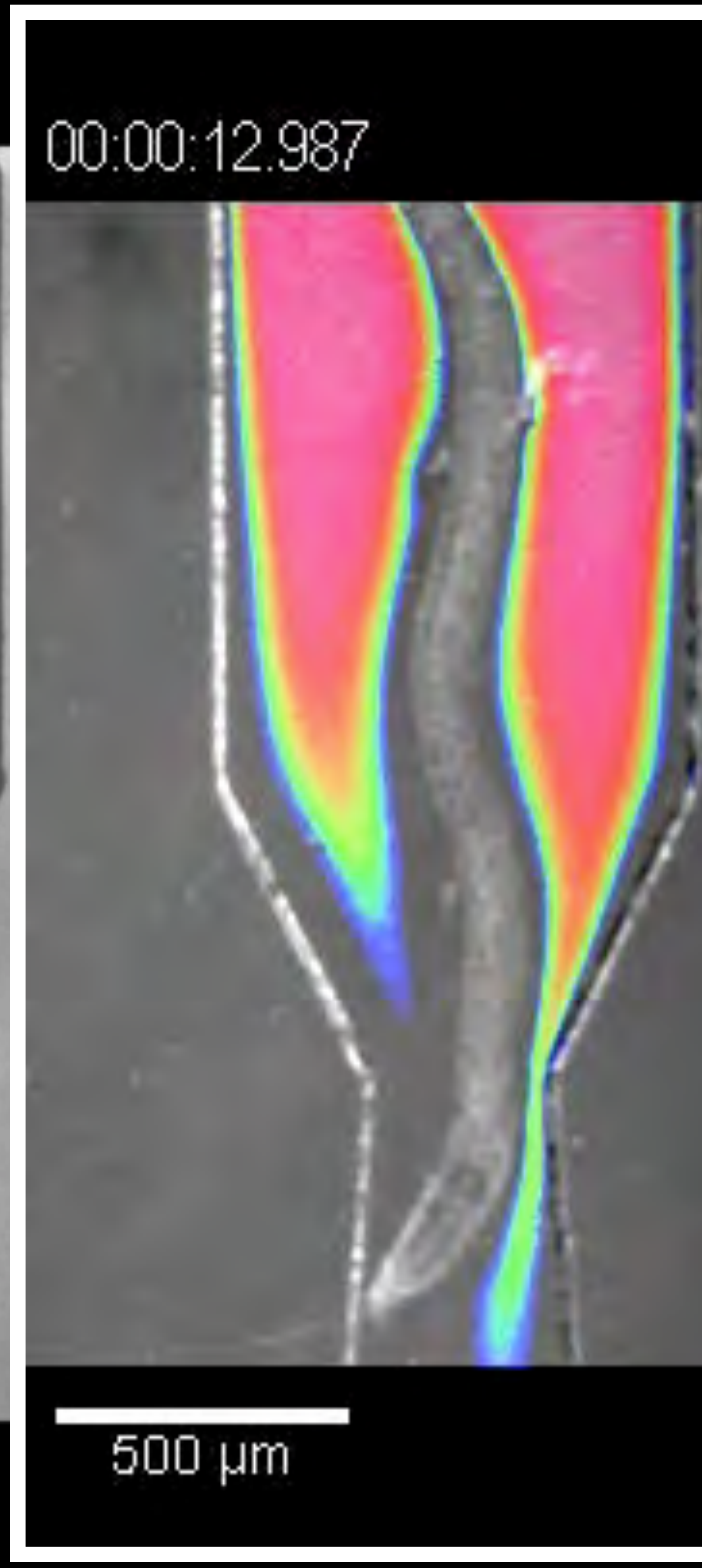


dfRootChip
Stanley, Shrivastava et al. 2018 *New Phytol*
Stanley et al. 2018 *bio-protocol*

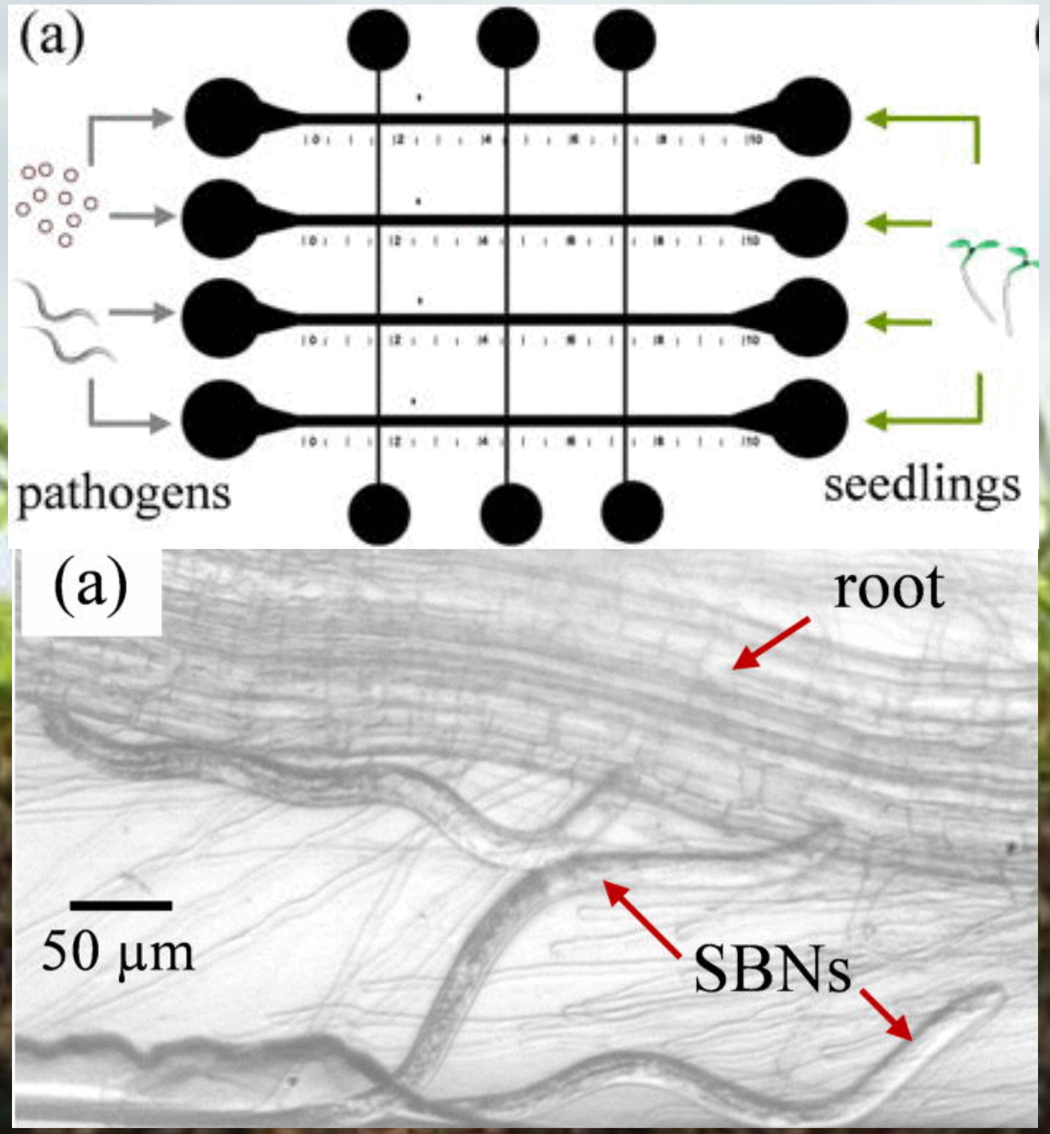
RootChip8S
Denninger, Reichelt et al 2019 *Curr Biol*
Guichard et al. 2020 *Meth Cell Biol*
Rizza et al. 2021 *PNAS*

RootChip - microfluidic technology for precision control of the root microenvironment

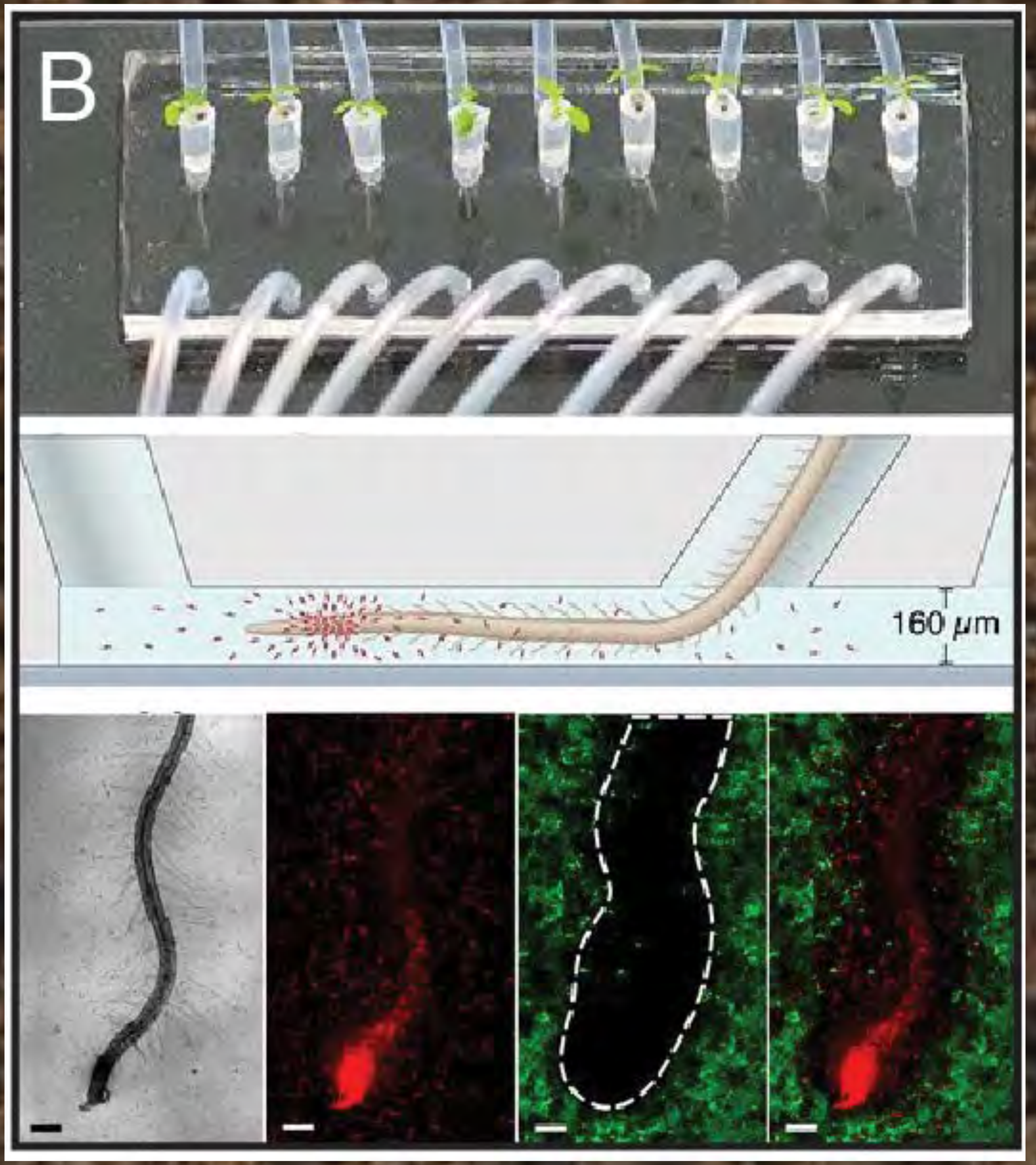




Microdevices for microscopic access to the „rhizosphere“

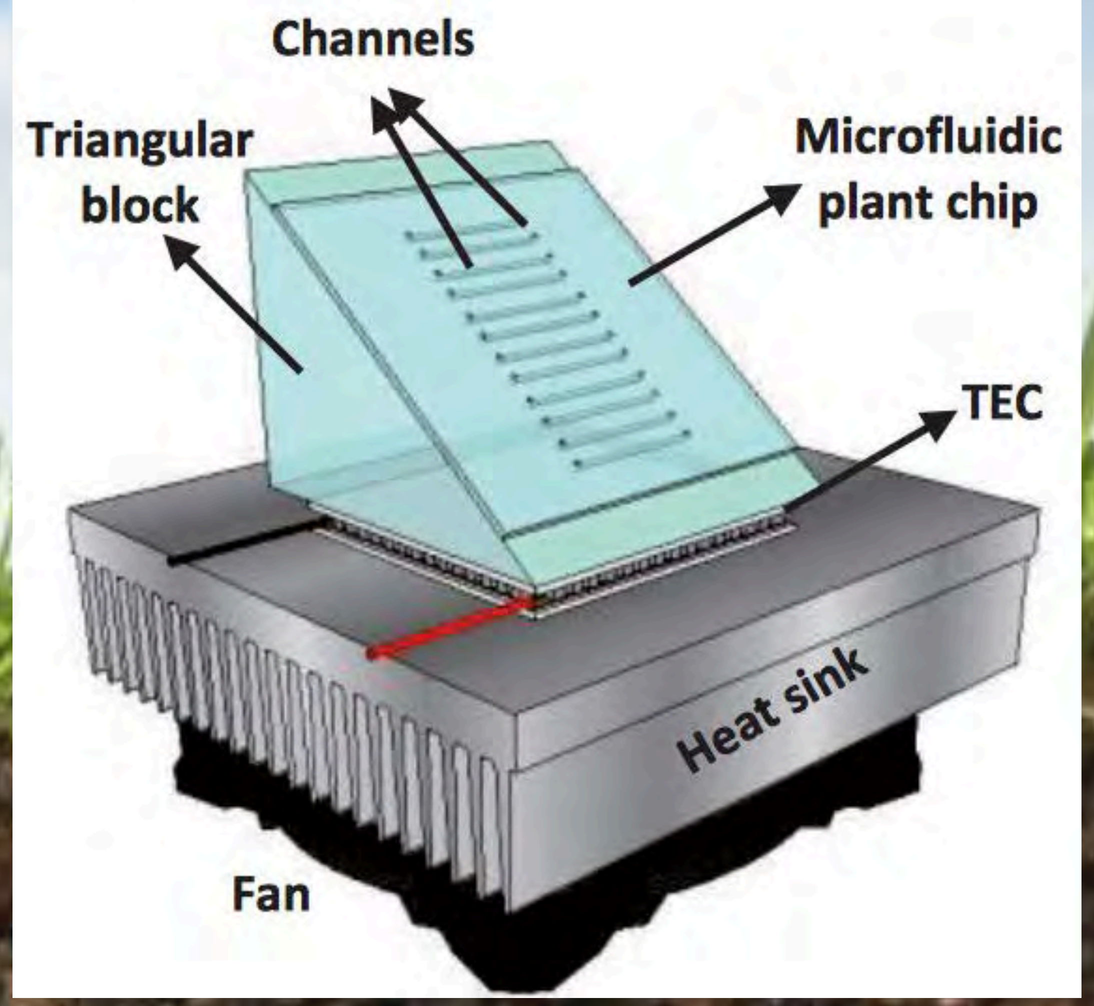


Parashar & Pandey 2011 *Appl Phys Lett*

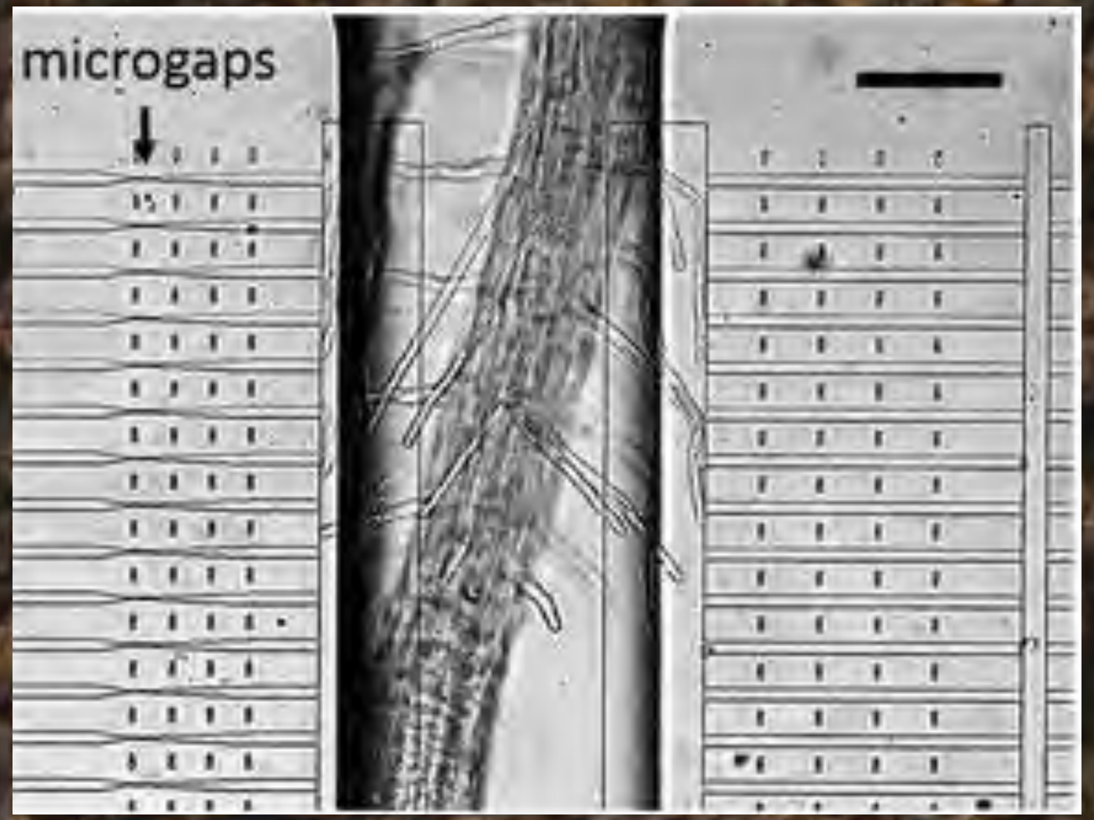


Massalha et al. 2017 *PNAS*

Sakai et al 2019 *Plant Meth.*

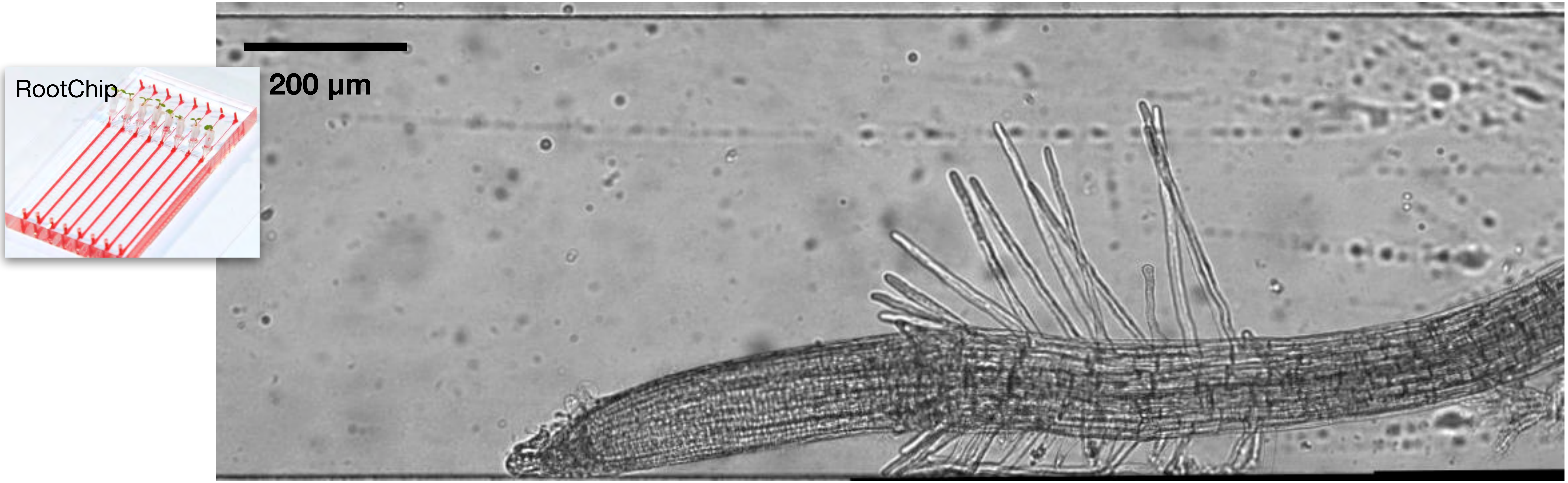


Wang et al 2017 *IEEE*



Yanagisawa et al. 2017 *Sci. Rep.*

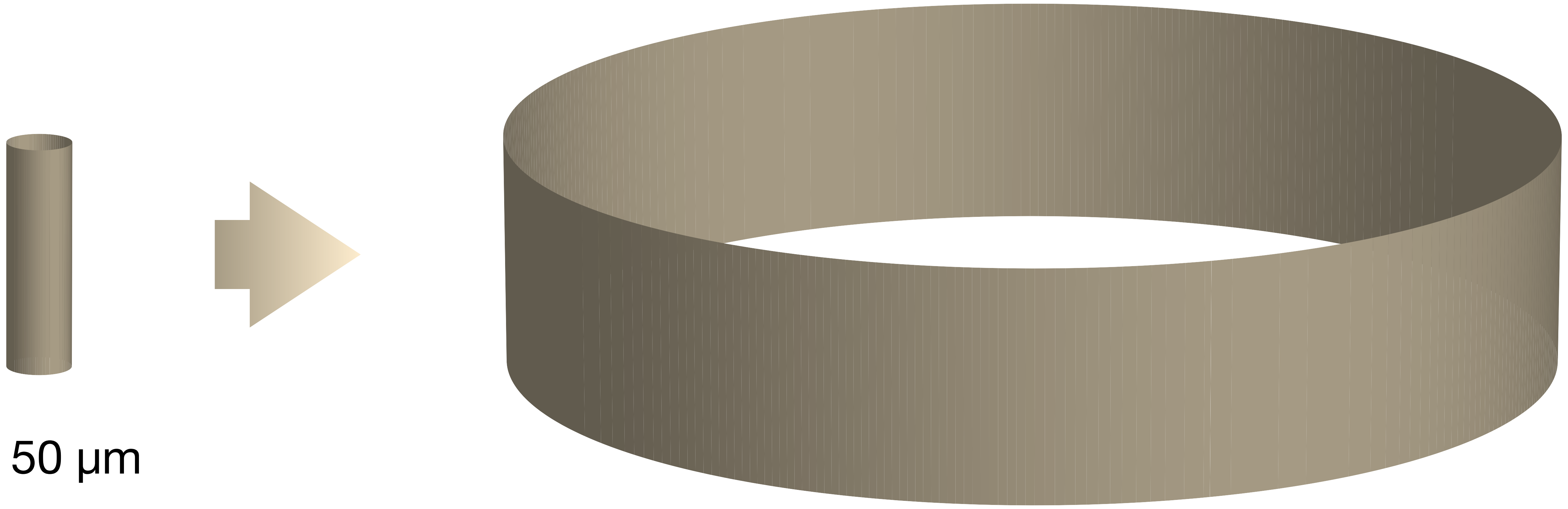
Adaptive response: root hairs enlarge the accessible substrate volume



Arabidopsis root grown in the RootChip-8S

max. length: ~800 µm

credit: Vanessa Fuchs



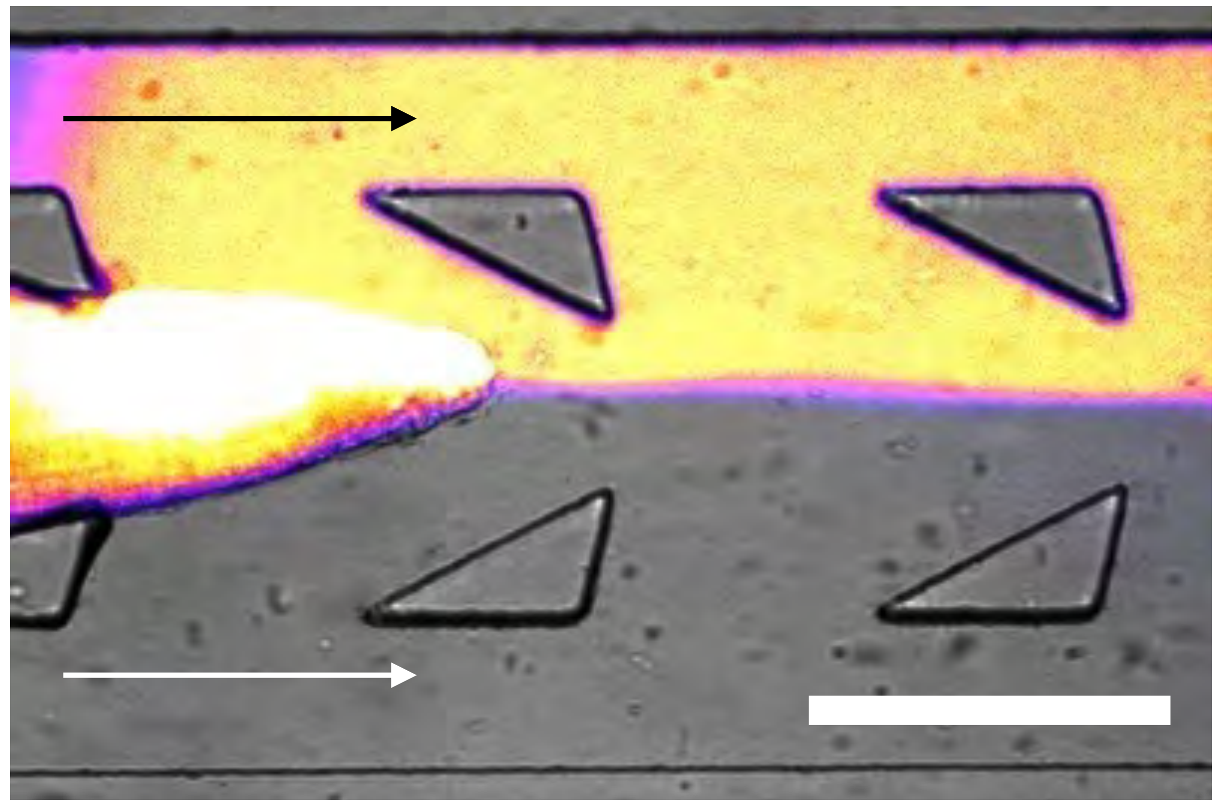
r_n= 50 µm

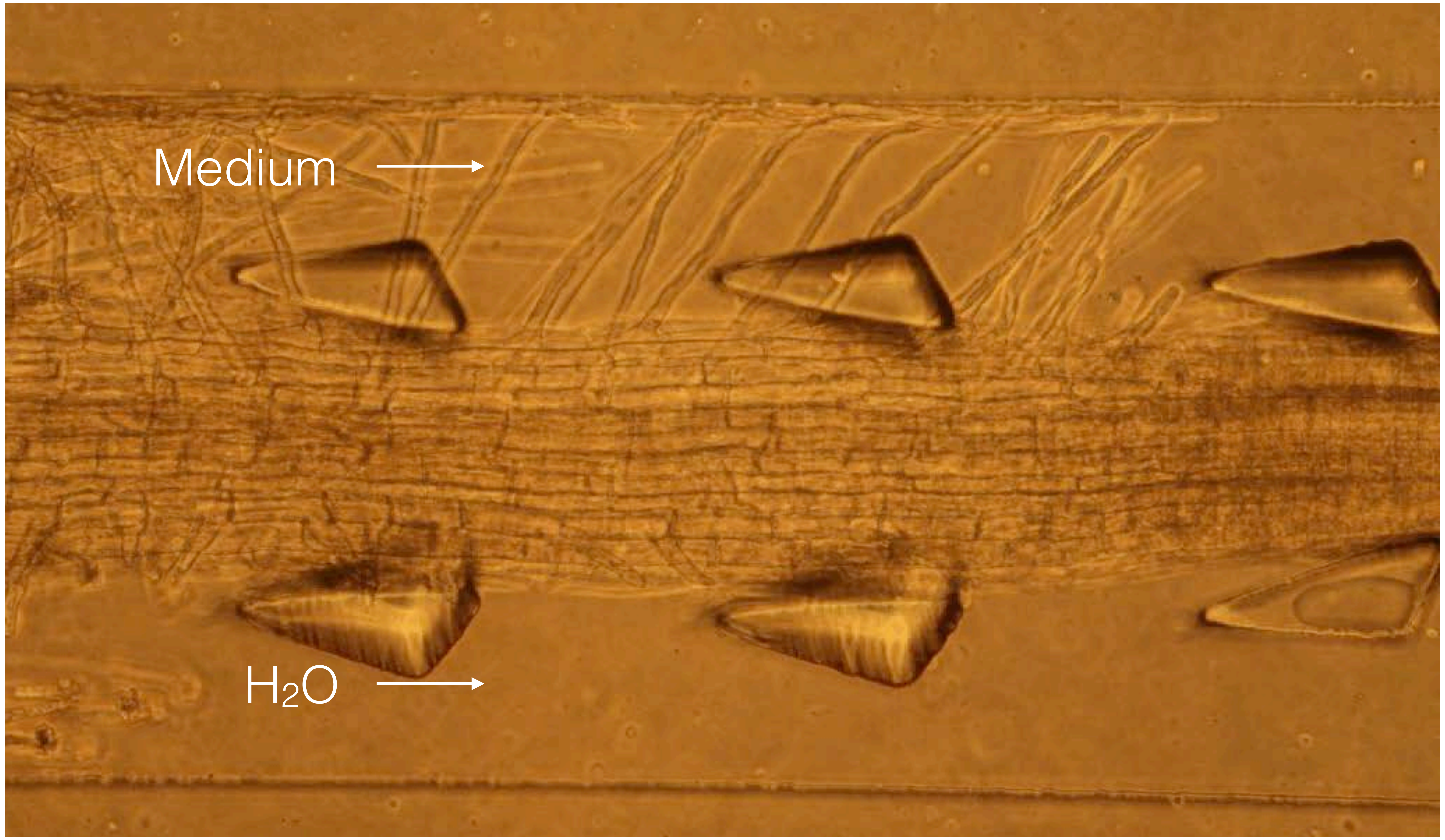
r_h= 800 µm

V_h= 256* V_n

Using microfluidics to create asymmetric microenvironments for roots

Is root hair growth coordinated or cell-autonomously regulated?



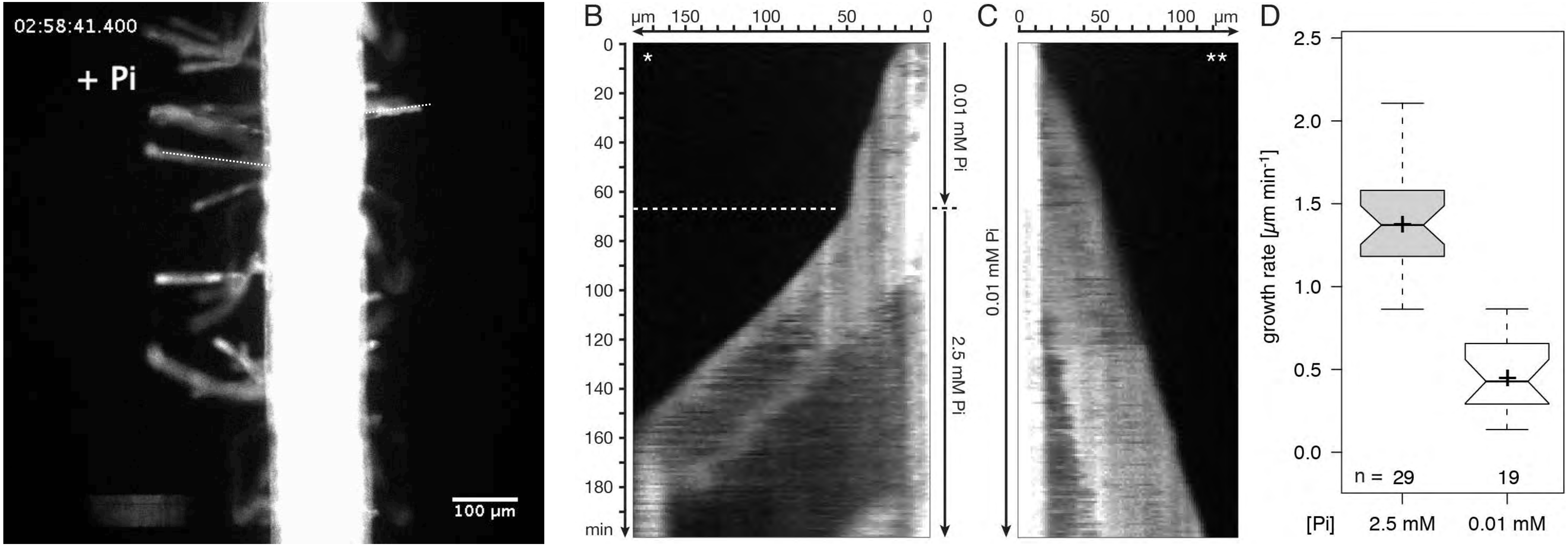


Claire Stanley
ETHZ

Jagriti Shrivastava

Stanley, Shrivastava et al. 2018 *New Phytol*

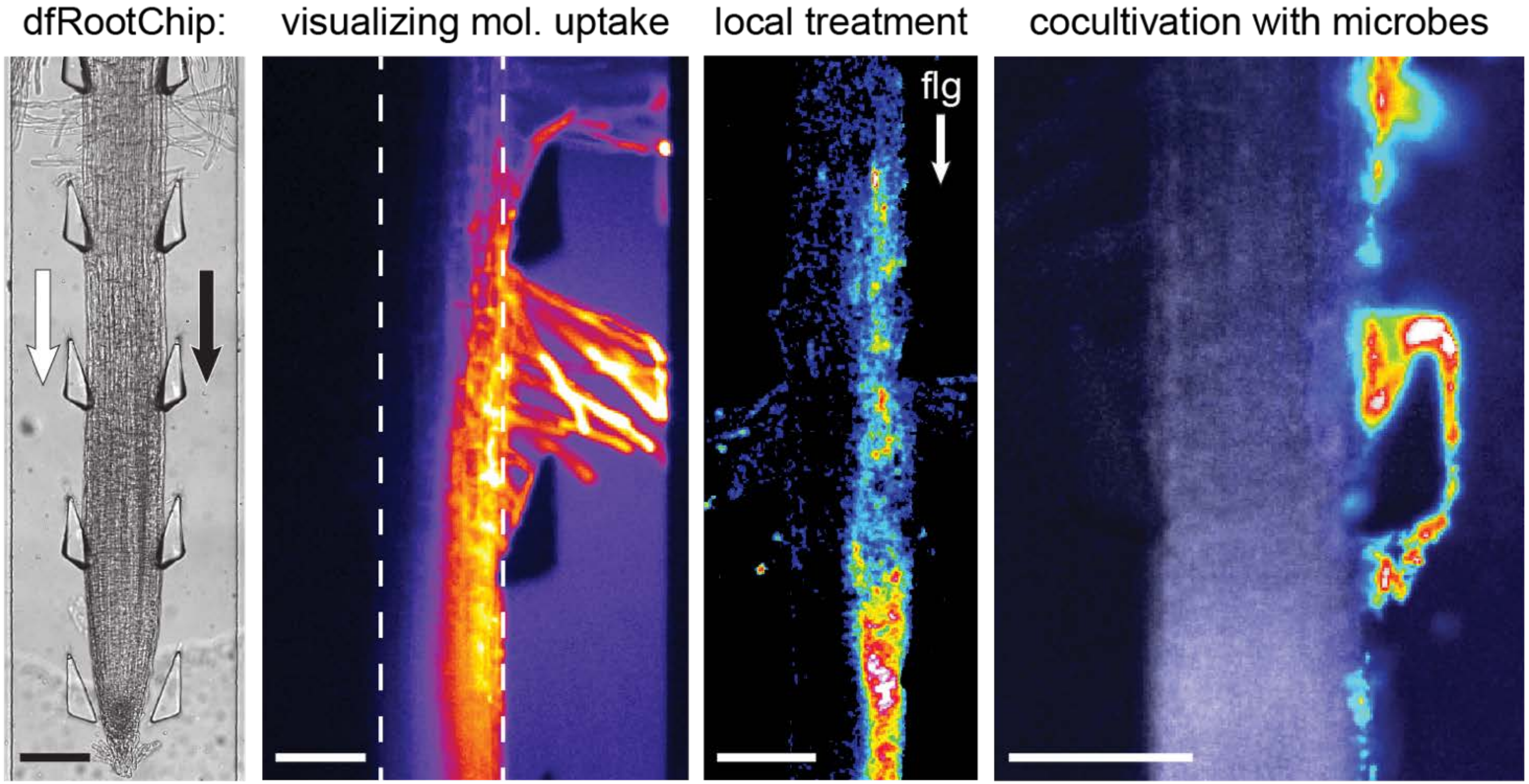
Root hairs respond to changing nutrient availability within minutes



Rapid **cell-autonomous** adjustment of growth rate through **direct** regulation of the tip growth machinery

Stanley, Shrivastava et al. 2018 *New Phytol*

Asymmetric growth conditions in the dual-flow-RootChip



fluorescein staining

Ca^{2+} response to flg22

inoculation with GFP-expressing P. fluorescens

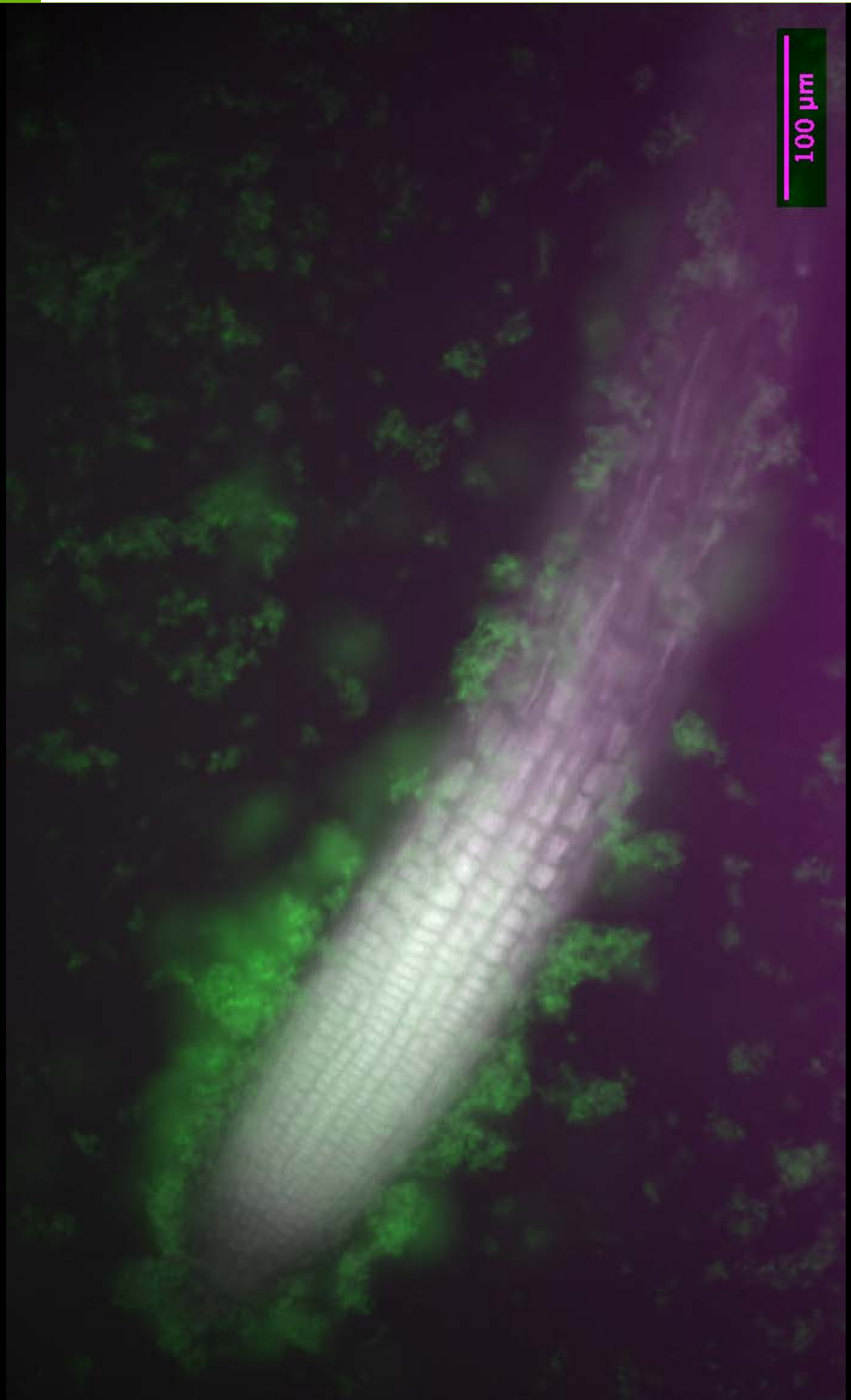
Stanley, Shrivastava et al. 2018 *New Phytol*

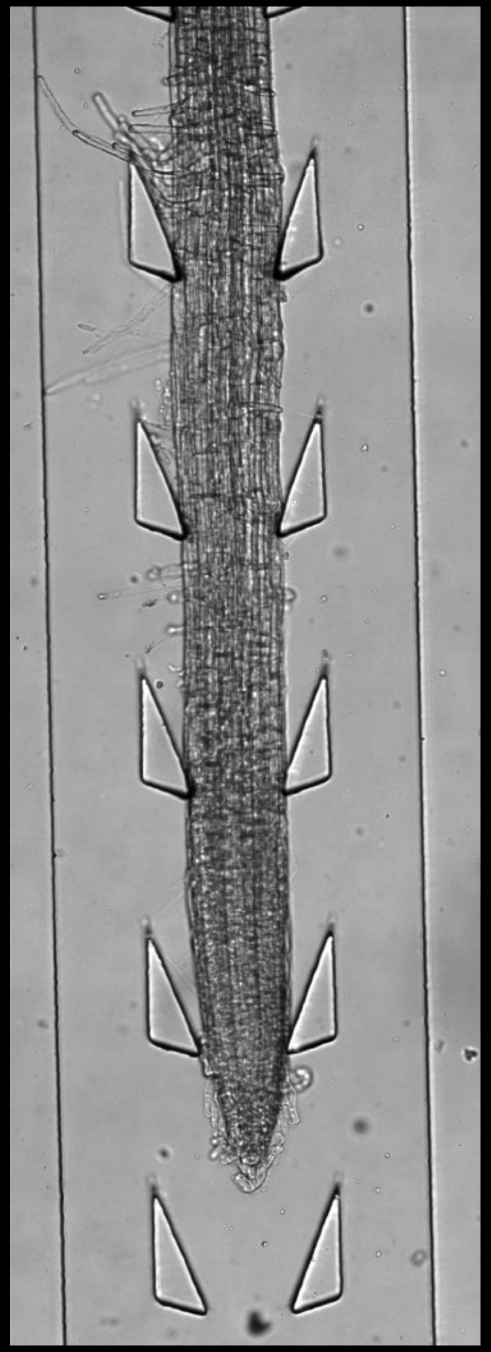
Tracking root-bacteria interactions and Ca^{2+} signaling *in planta*

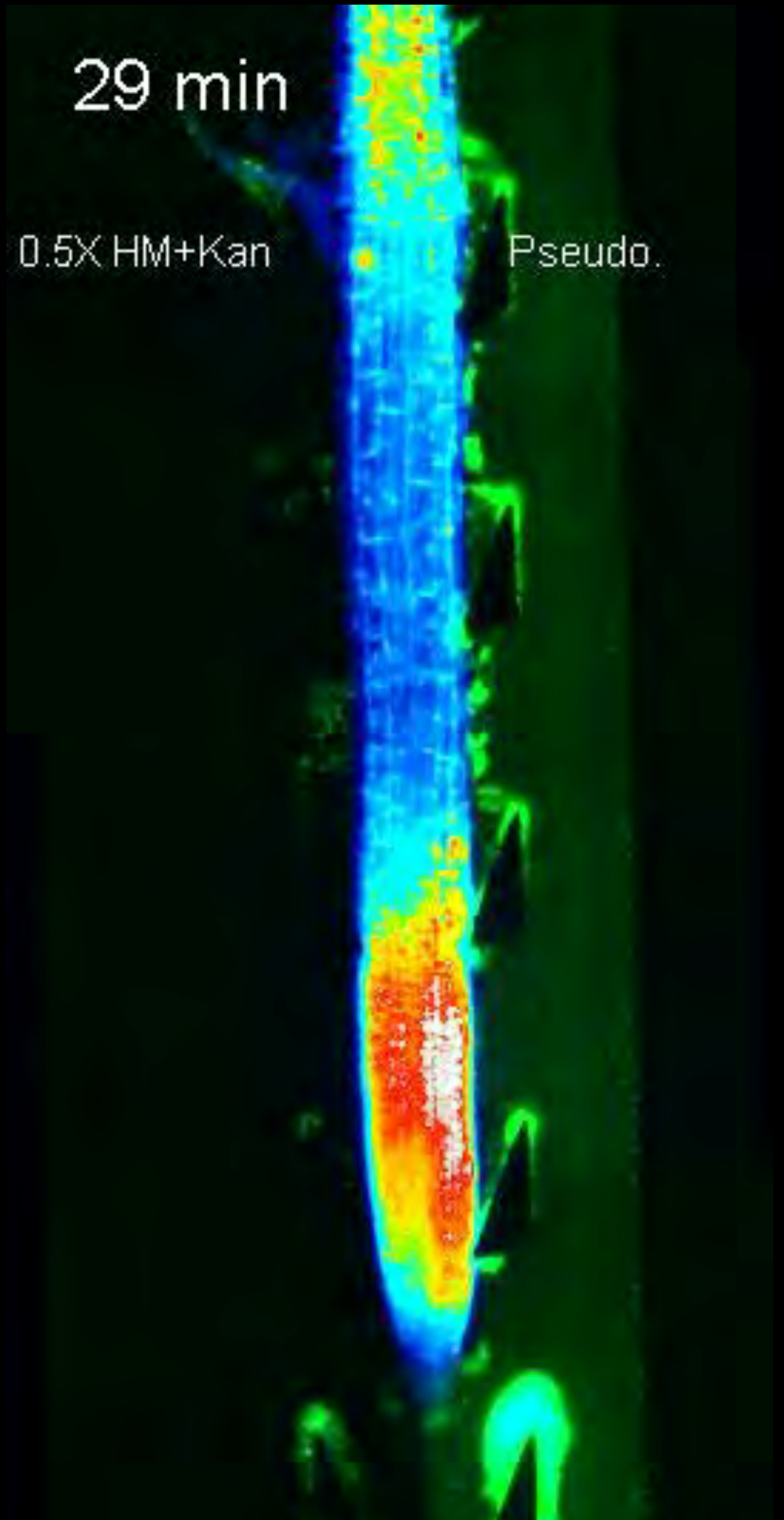
Arabidopsis thaliana vs *Bacillus sp.*

credit: Christian-Frederic Kaiser

Tracking root-bacteria interactions and Ca^{2+} signaling *in planta*







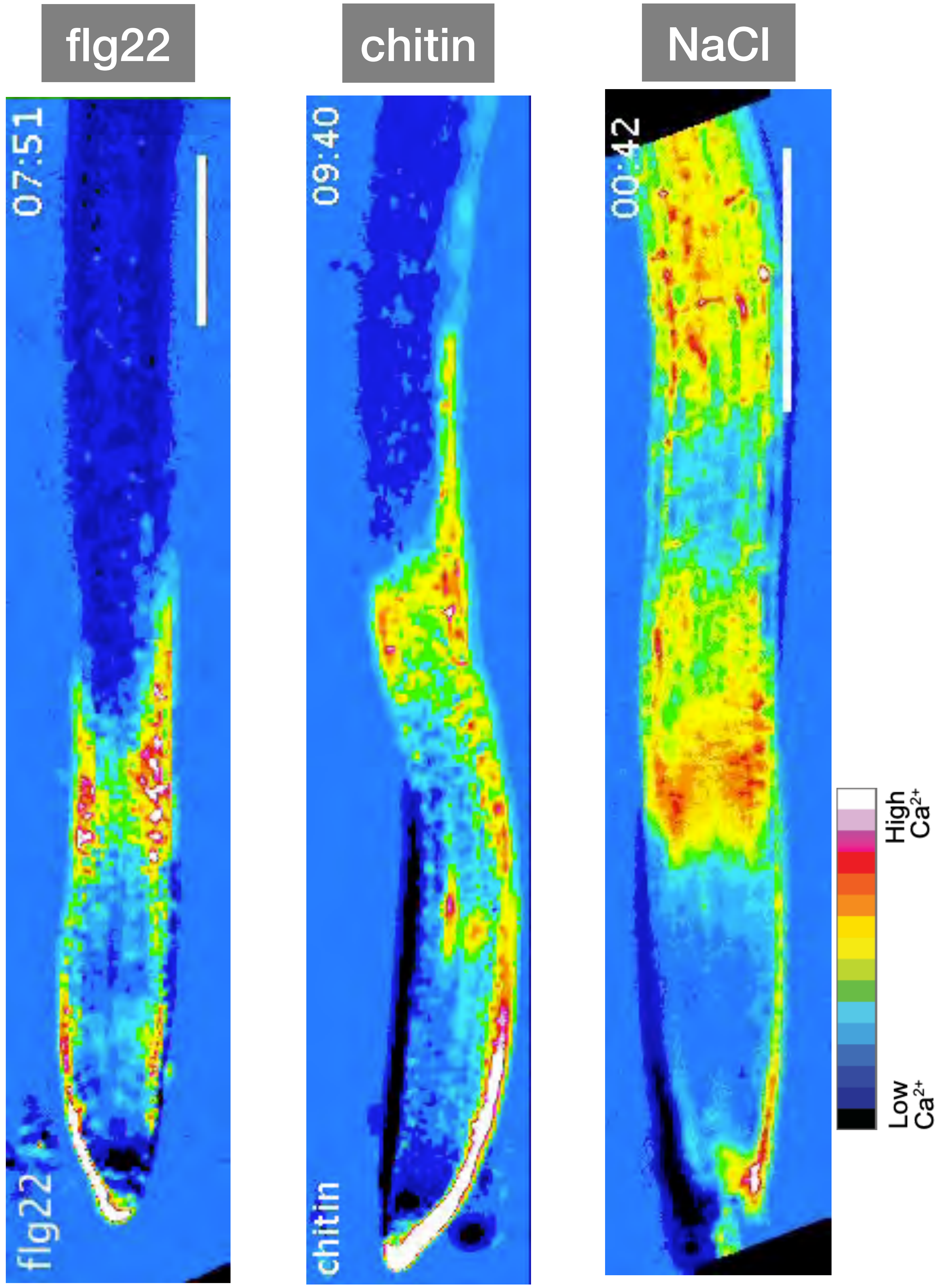
RGECO1-expressing root inoculated with GFP-expressing *Pseudomonas fluorescens* (gift by Cara Haney)

Jagriti Shrivastava

credit: Christian-Frederic Kaiser

How do plants perceive and respond to changing environmental conditions?

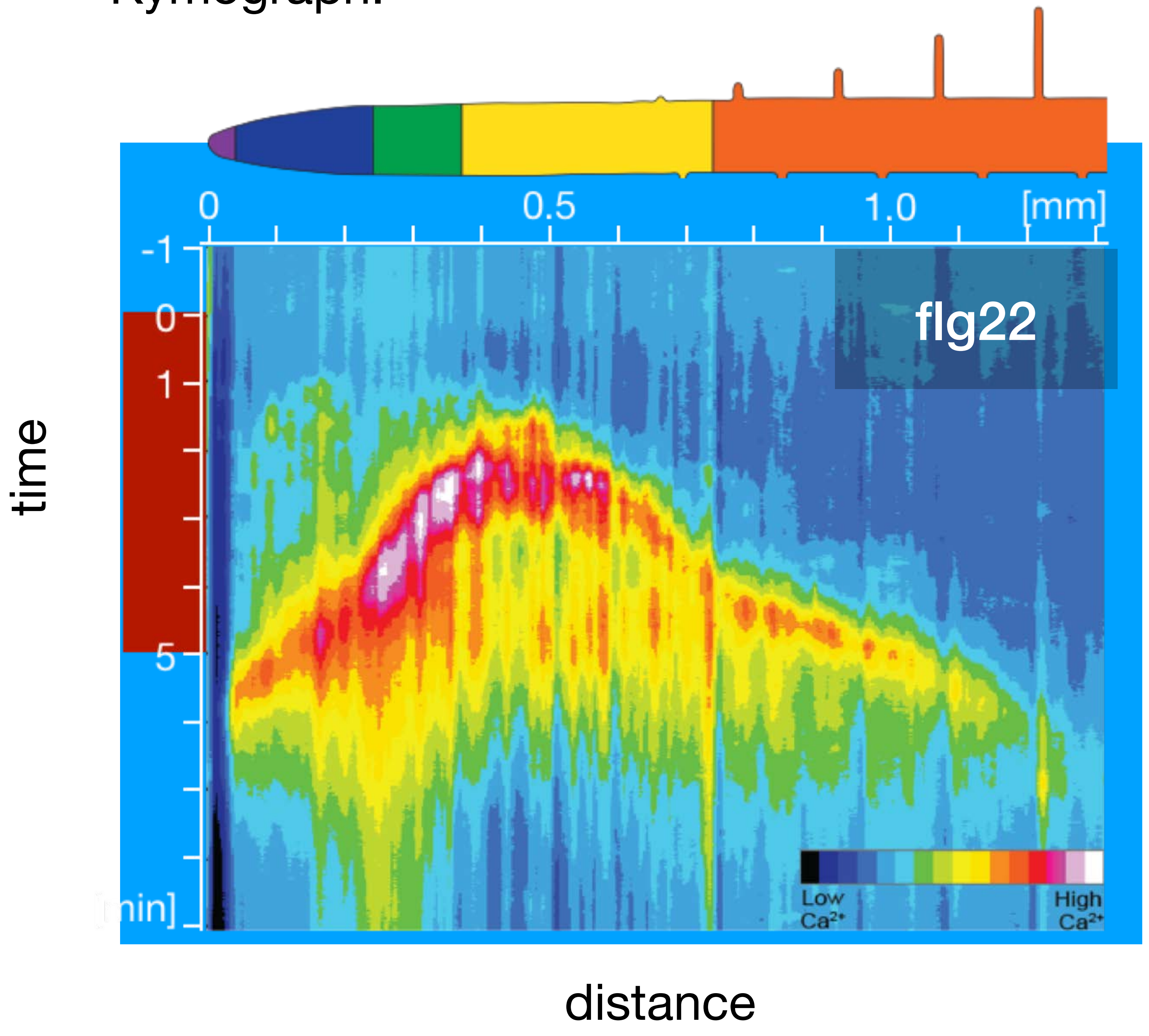
Ca^{2+} signaling as readout for the perception of stress conditions



Normalized (ΔF/F) Ca^{2+} response of **R-GECO1** to different elicitors.

Scale 200 µm, time int. 1.5s

Kymograph:

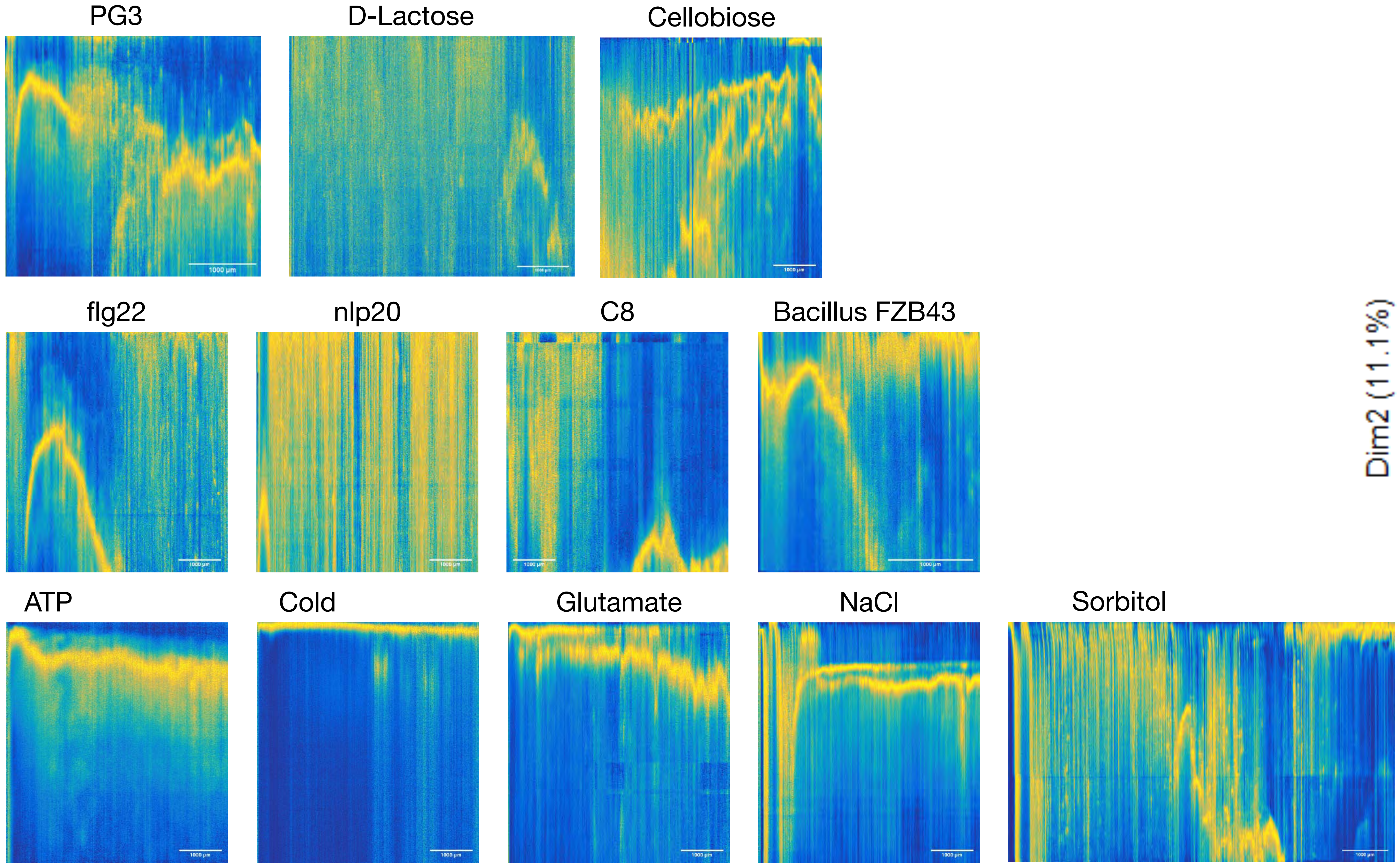


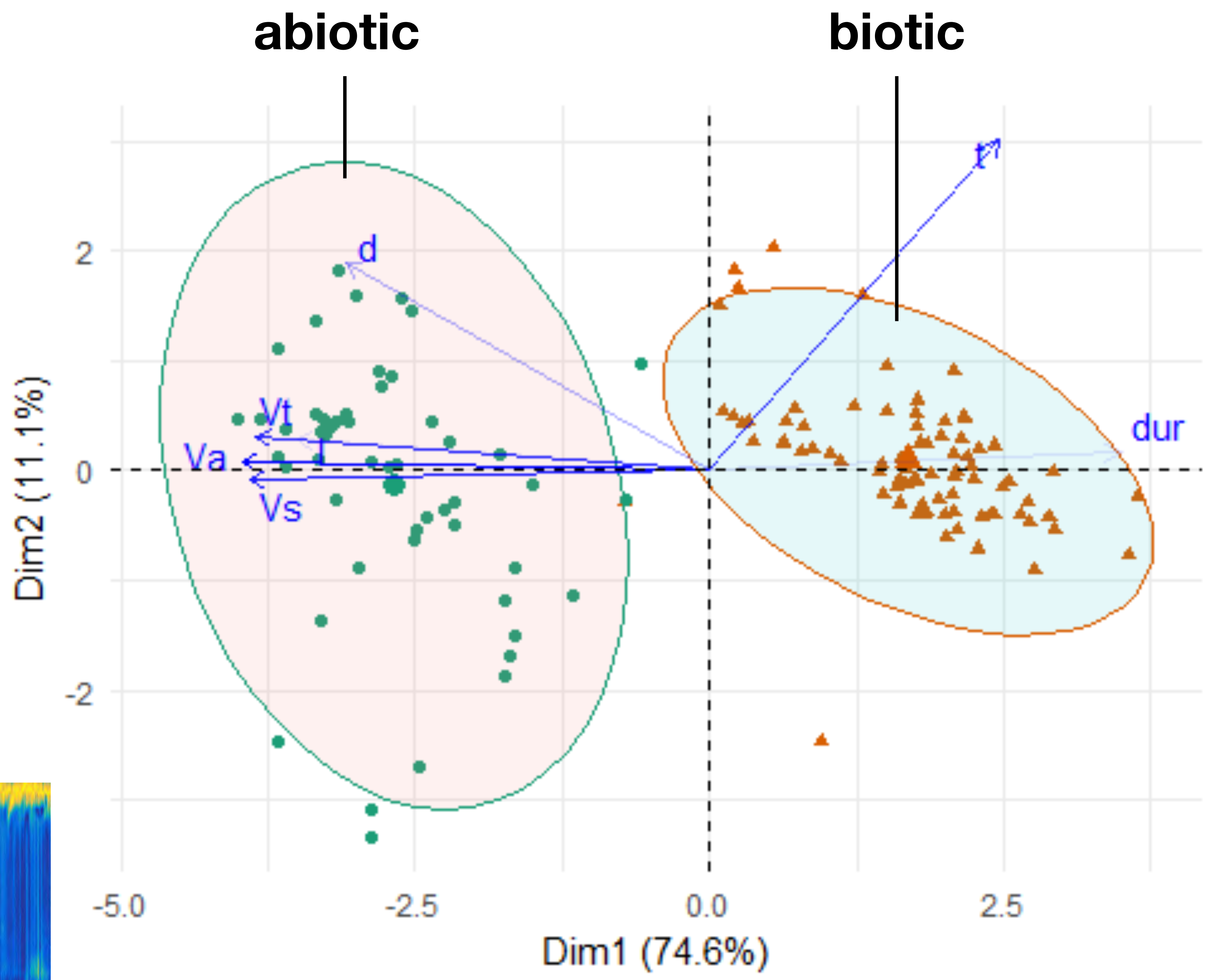
...

Keinath et al. 2015 *New Phytol*

'Fingerprinting' of different abiotic/biotic elicitors

Towards a calcium signaling atlas





Milan Zupunski

TAKE HOME

NANOSENSORS AND MICROFLUIDICS

Techniques for **kinetic plant phenotyping** at the microscale

SENSORS enable quantitative imaging of molecular uptake and signaling dynamics in living plant tissue.

MICRODEVICES provide microscopic access and precise control over the plant microenvironment.

KINETIC PHENOTYPING digital phenotyping at high spatiotemporal resolution probing dynamic (sub-)cellular responses to changing conditions.

THANK YOU

Current team members:
Claudia Franken-Stemmler
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Marjorie Guichard
Christian-Frederic Kaiser
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Daša Wernerová
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Till Franz
Aylin Haas
Janos Löffler
Jagriti Shrivastava

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Wolf Frommer, HHU
Karin Schumacher, U. Heidelberg
Matias Zurbriggen, HHU

ICB Institute of **Cell** and **Interaction** Biology

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Exzellenzcluster CellNetworks
DLR
Schlumberger Foundation
Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg
DFG
Heisenberg-Programm
Deutsche Forschungsgemeinschaft

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Cluster of Excellence on Plant Sciences

CEPLAS II
Cluster of Excellence on Plant Sciences

Opportunities for Cooperation in PPPs

Technology transfer and cooperation management

From lab to field

- Technology Transfer Officer
- Active Scouting
- Establishing an Entrepreneurial Mindset
- CEPLAS Entrepreneurship Training Module (Design Thinking, Business Model Generation, IPR Protection, Start-up lectures)

Targets for Cooperations with Companies

From the CEPLAS point of view

- Integration of know-how/perspectives not present in CEPLAS; sustainable interaction with mutual benefit
- Participation in novel educational approaches
- Support of application relevant projects: integration of know-how and financial support
- Economic exploitation of research results; generation of ROI for public investment in research
- Opening career opportunities for scientists

Possible Elements of Cooperation

- Joint research projects in fields of mutual interest
- Sponsoring: CEPLAS Graduate School, Postdoctoral Fellows, Junior Research Groups
- Mentoring: early career researchers
- Internships
- Participation in teaching programs
- Membership in Steering Bodies
- Joint program for support of start-up foundations

Commercial exploitation rights according to contribution of partners

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