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Advances in Root Phenotyping

Malcolm Bennett

ROOT ZONE BREEDING MEETING
May 2020

EPSRC
Engineering and Physical Sciences Research Council

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Future Challenges facing Agriculture

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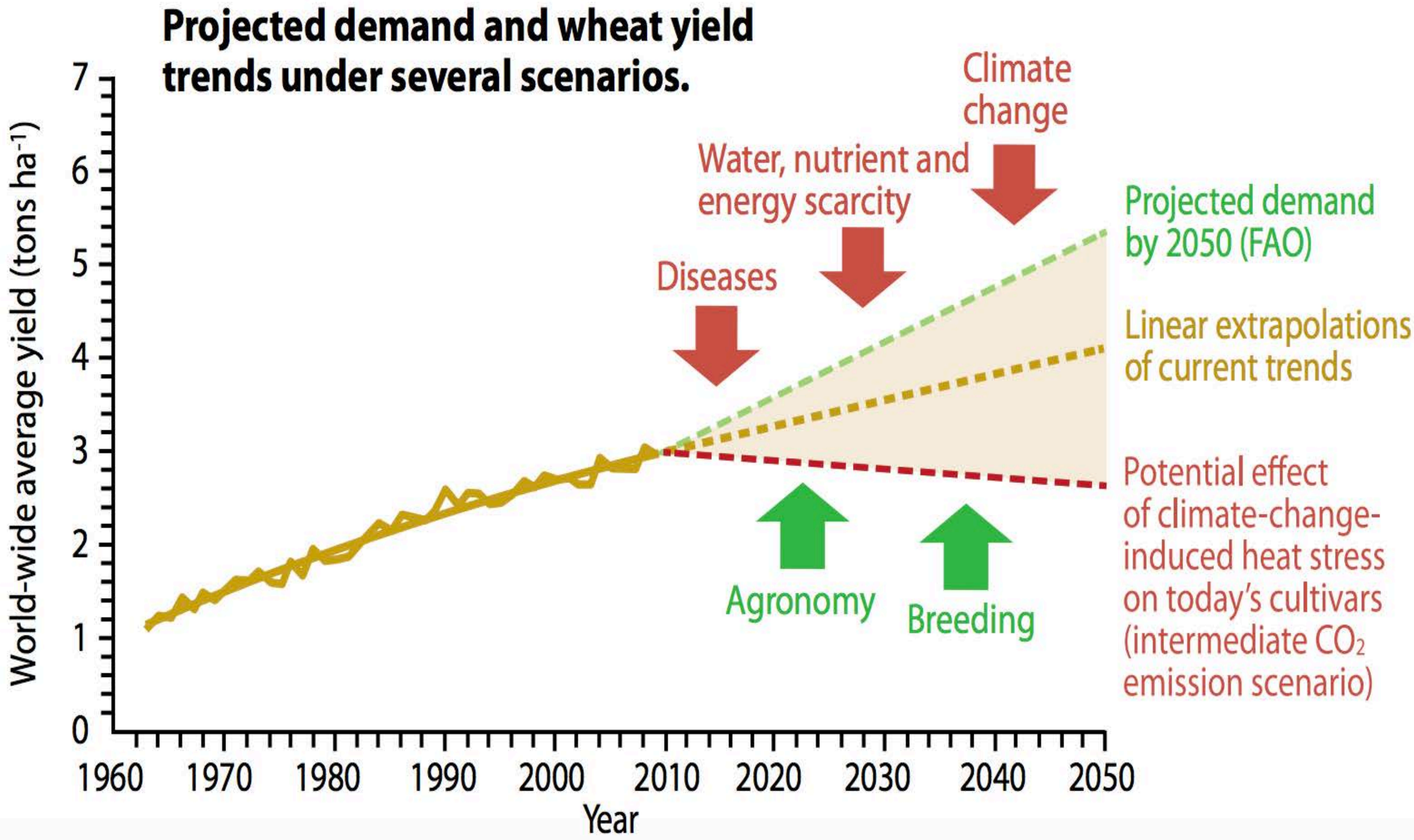

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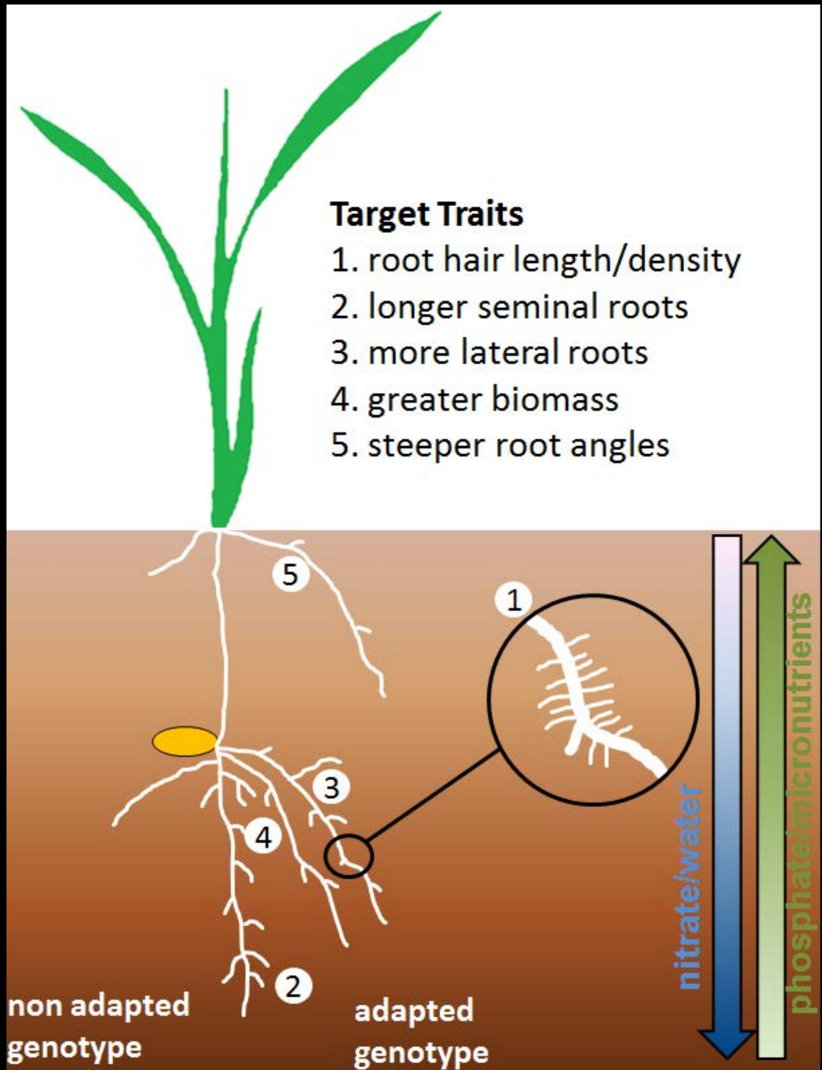
Uncovering the 'Hidden Half' of Plant traits

Root Angle
Bennett *et al* (1996)
Science **273** 948-950
Band et al (2012)
PNAS **109**, 4668-473
Huang *et al* (2018)
Nature Comms **9**:2346

Root Hairs
Giri *et al* (2018)
Nature Comms **9** 1408
Bhosale *et al* (2018)
Nature Comms **9** 1409
Dindas *et al* (2018)
Nature Comms 9 1174
Schoeners et al (2018)
Current Biology 5 722

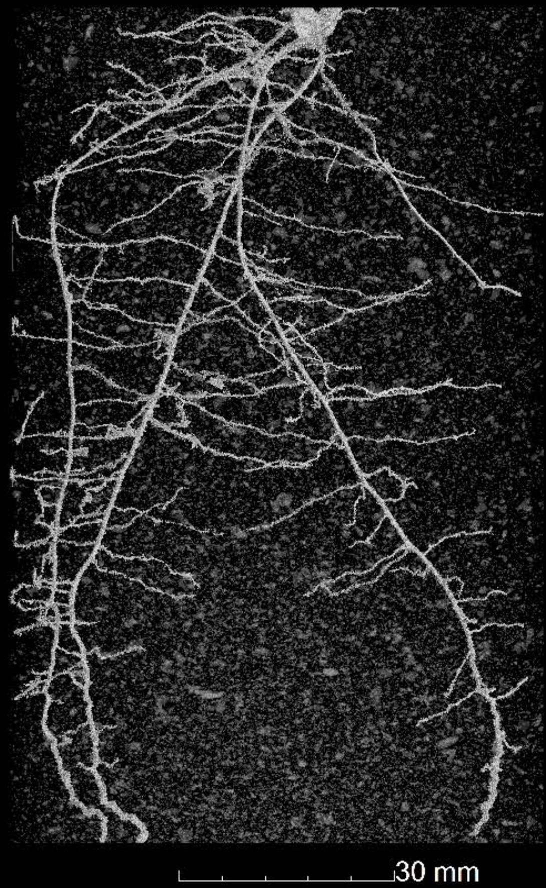


Root Branching
Casimiro *et al* (2001)
Plant Cell **13**:843
De Smet et al (2007)
Development **134**:681
Swarup et al (2008)
Nature Cell Biology **10**:946
Peret et al (2012)
Nature Cell Biol **10**, 991
Bao et al (2014)
PNAS, **111**, 9319
Goh et al (2016)
Development **143**, 3363
Orman-Ligeza et al (2018)
Current Biology, 10.2139
Orosa-Puente et al (2018)
Science, 362, 1407-1410

Advances in Root Phenotyping

3 topics covered in today's seminar include

- Field phenotyping for root traits
- Imaging root traits for plants grown in controlled environments
- New innovations in root phenotyping



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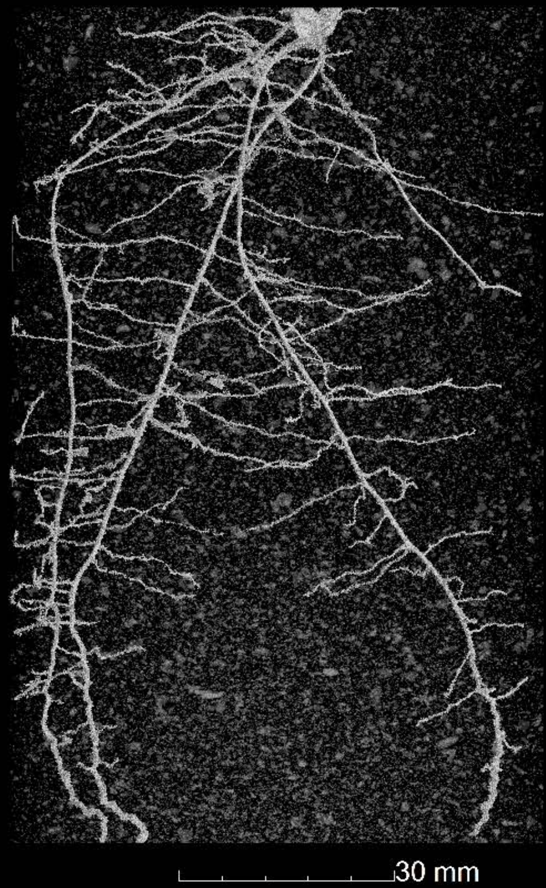
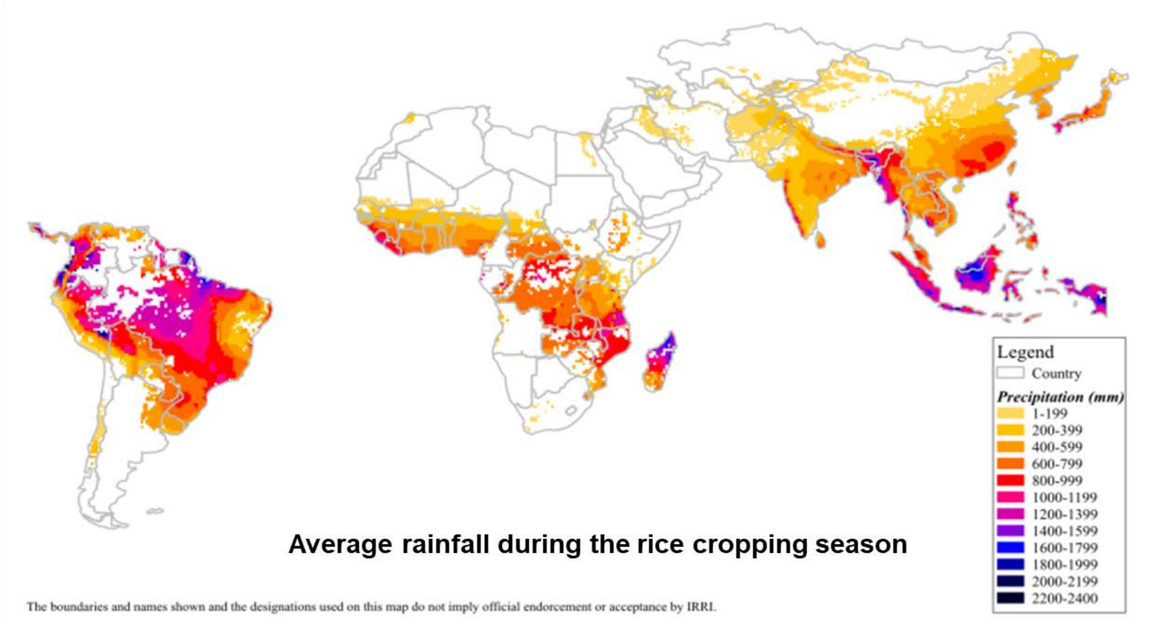


Drought stress threatens global rice production

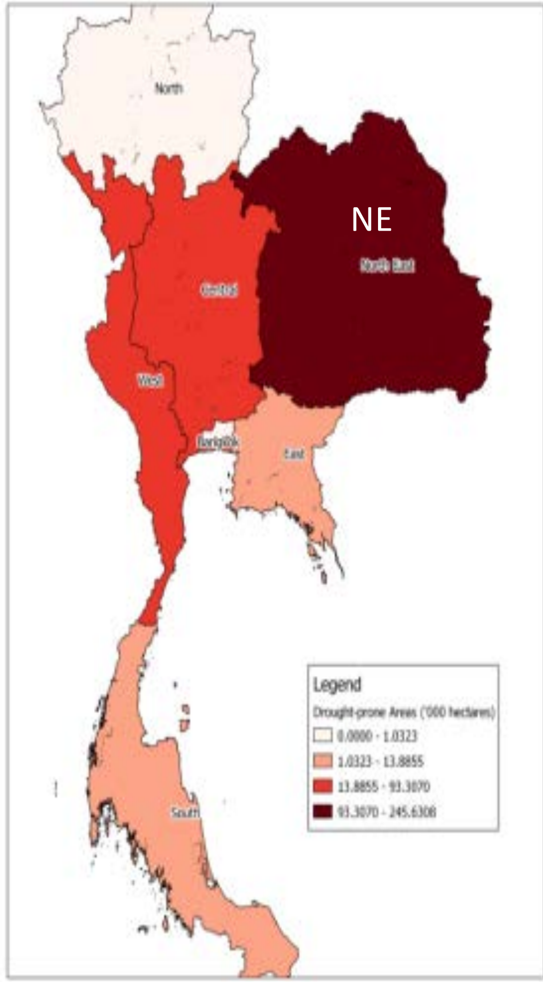


Adam Sparks, IRRI

Nepal

IRRI

Ubon, Thailand



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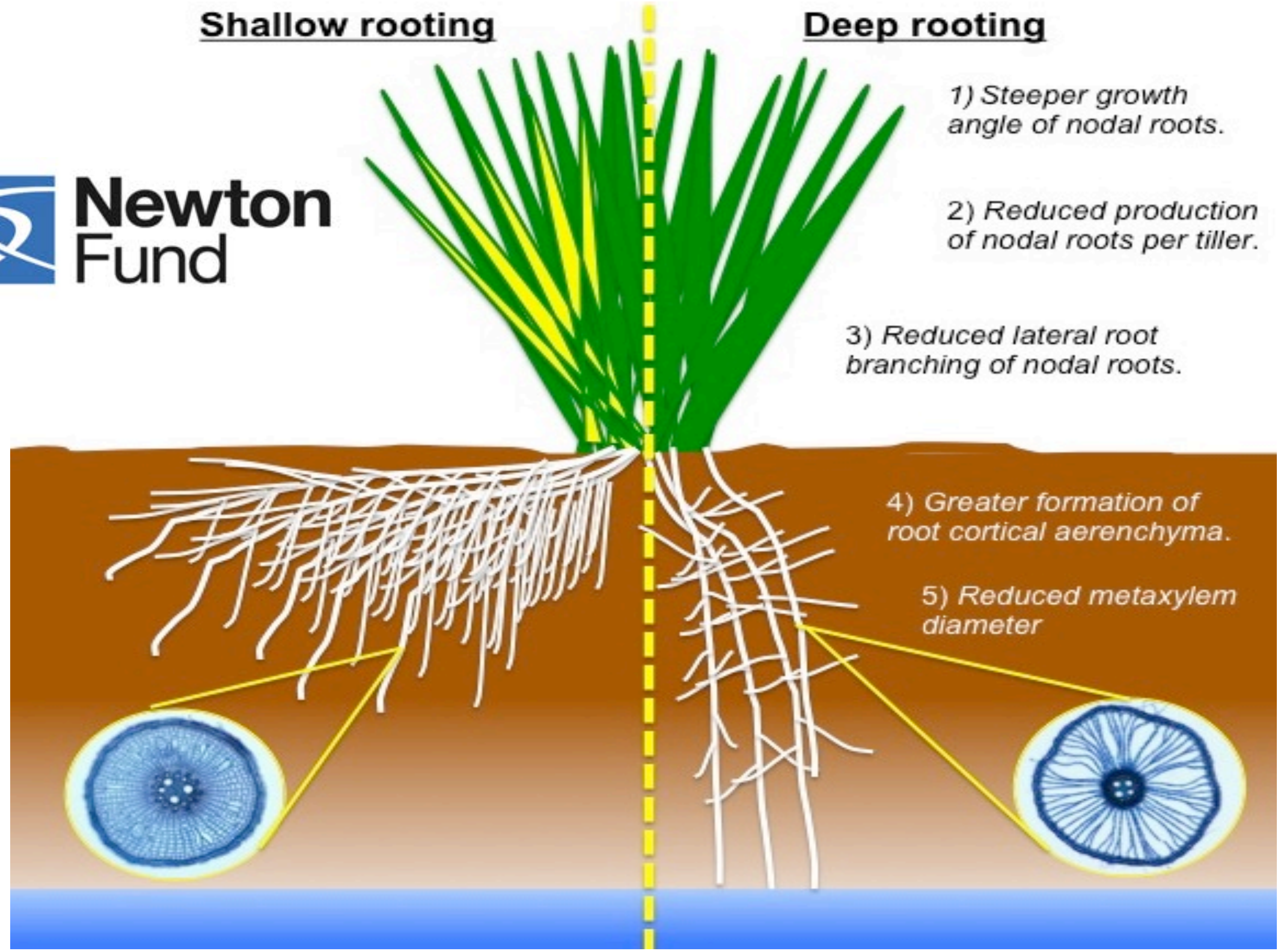
Rhizo-rice: a novel root ideotype to improve drought tolerance

Newton Fund



Do *Rhizo-rice* phenotypes improve drought tolerance in the field?

Field experiments

KDML105 CSSL introgression lines

'Isophenic lines'

Thai/ Southeast Asian panel
(210 sequenced genotypes selected from the 3k set - 3 subsets by maturity group)

Physiology study

IRRI

Natural drought environments

BBSRC bioscience for the future · Newton Fund · NSTDA · BIOTEC a member of NSTDA · IRRI · The University of Nottingham · PENNSTATE

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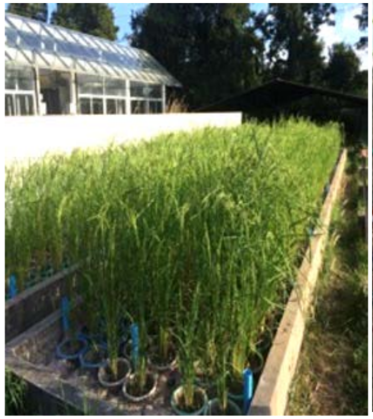
Root Phenotyping Approaches Employed in Rhizo-Rice

Method

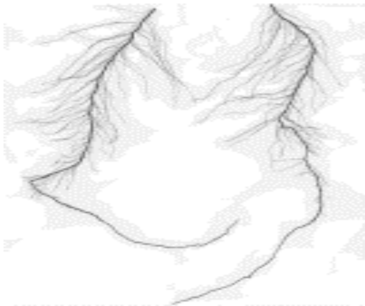
Shovelomics	Monolith	Soil Coring	Basket	Box
				

Measurements

Nodal root Angle, number & diameter

DiRT

Lateral Root Density & length



WinRHIZO

% aerenchyma, xylem number & diameter

LAT

Root growth rate
Root length density
Plant height
Tiller number
% deep roots
Canopy temperature
NDVI
DTF
Biomass
Grain yield
Sap bleeding rate
Soil and ambient conditions

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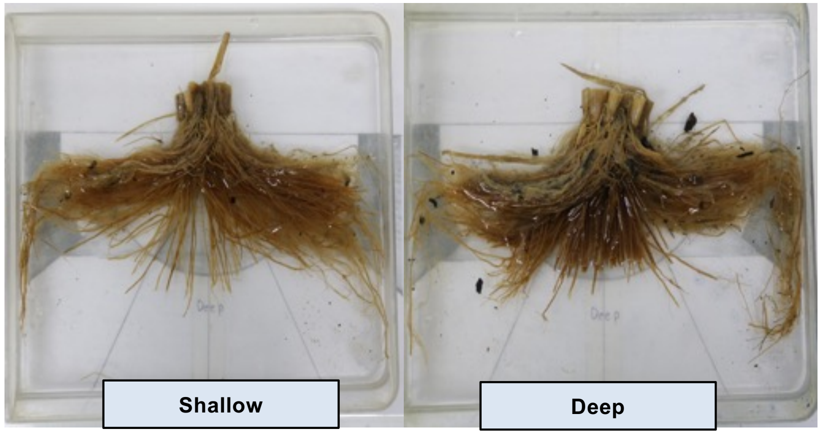
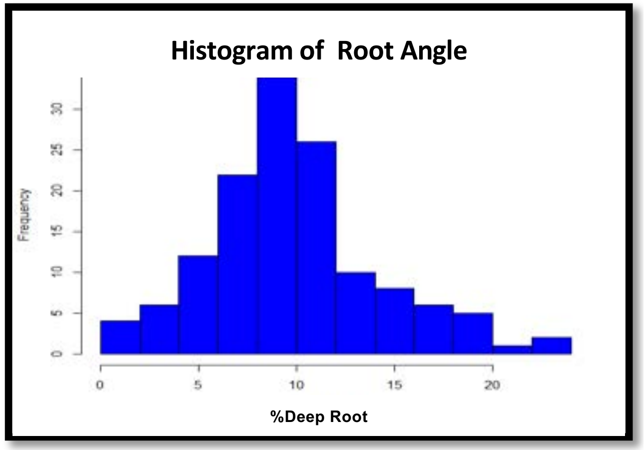
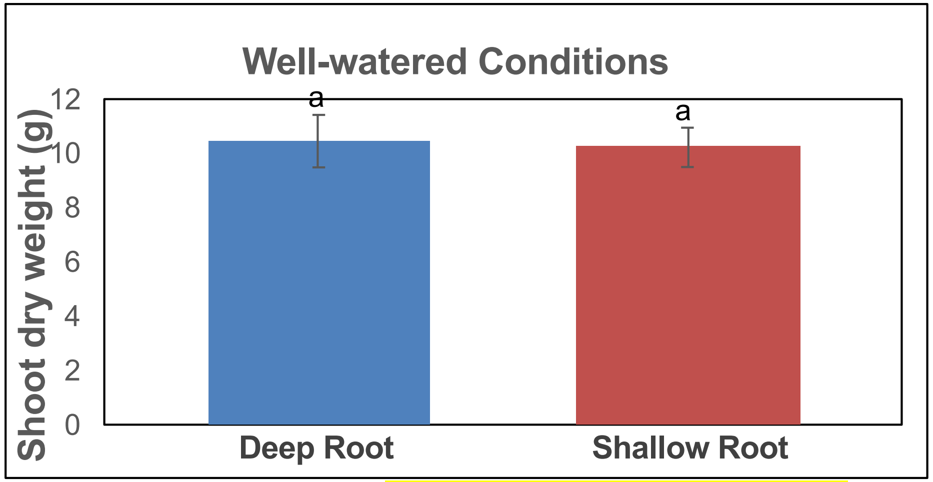
Steep root angles improve drought tolerance



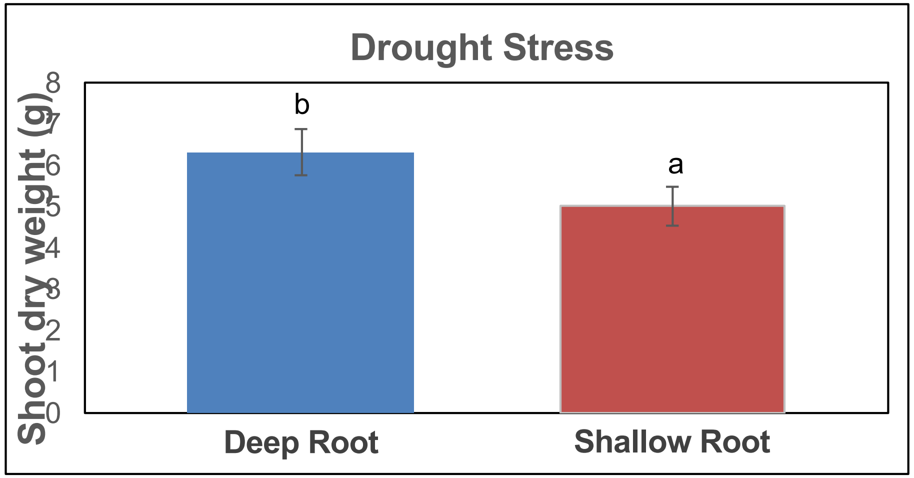
Shallow vs. Deep Root Growth Angle

Shallow	Deep
CSSL23	CSSL28
CSSL71	CSSL36
CSSL81	CSSL41
CSSL111	CSSL80
CSSL118	CSSL50
CSSL120	CSSL62
CSSL127	CSSL100





F = 0.360 P-value = 0.850



F = 3.246 P-value = 0.079

Drought stress reduced shoot biomass ~45%. Under drought stress, lines with deeper roots had 21% more shoot biomass

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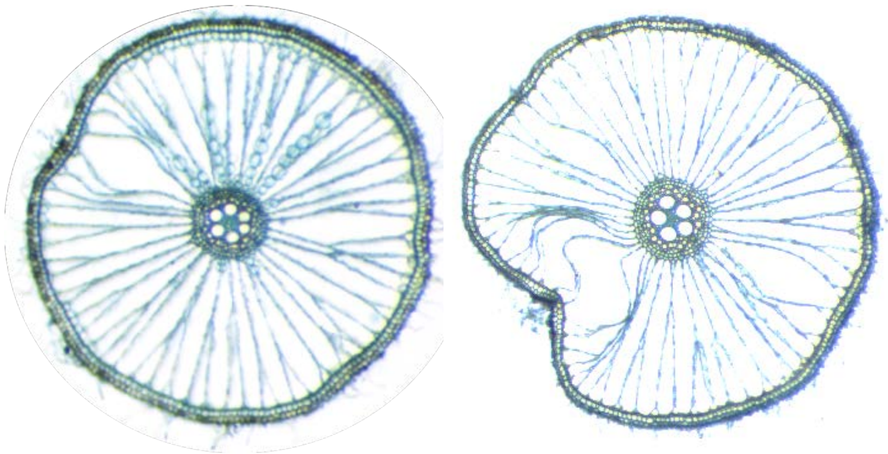
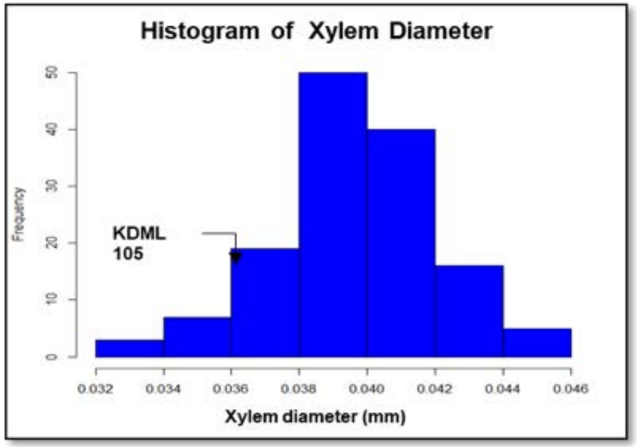
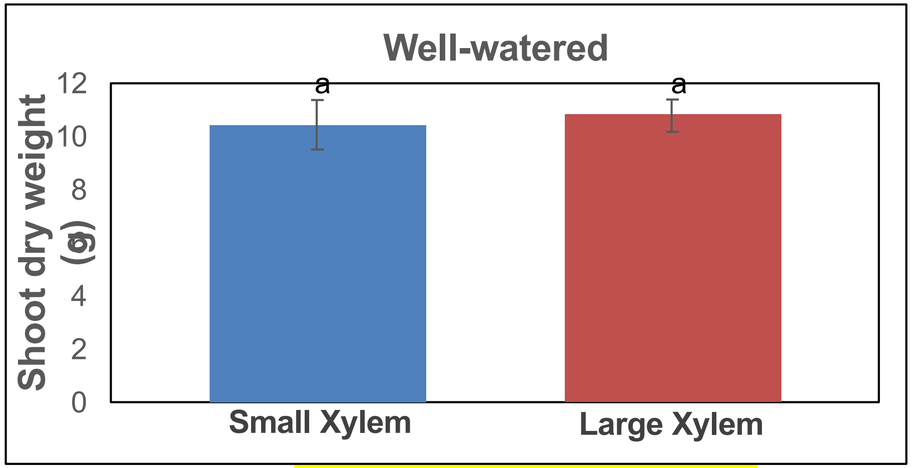
Small xylem vessels improve drought tolerance



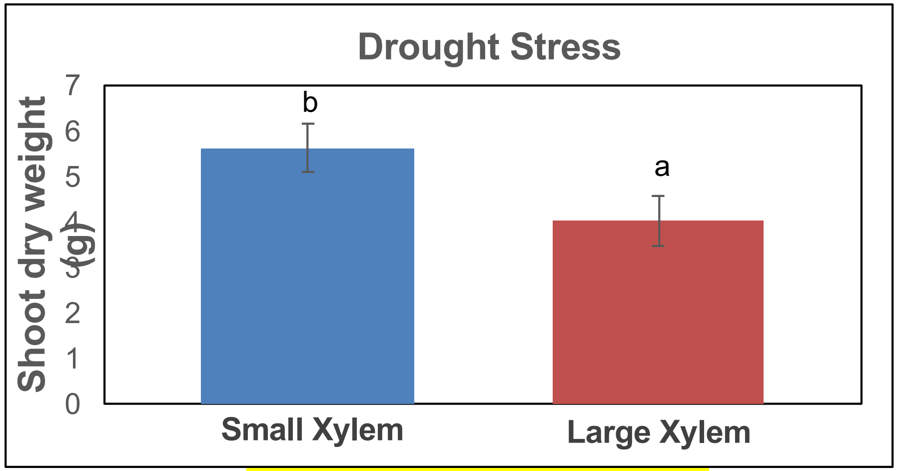
Small vs. Large Xylem Diameter

Small Xylem	Large Xylem
CSSL18	CSSL9
CSSL37	CSSL10
CSSL51	CSSL13
CSSL56	CSSL35
CSSL131	CSSL48
CSSL133	CSSL124
CSSL139	CSSL135





F = 0.092 P-value = 0.763



F = 4.430 P-value = 0.041*

Drought reduced shoot biomass by 56%. Lines with smaller xylem size had 24% more shoot biomass under drought.

Acknowledgements

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ROOTS
Soil Carbon Capture

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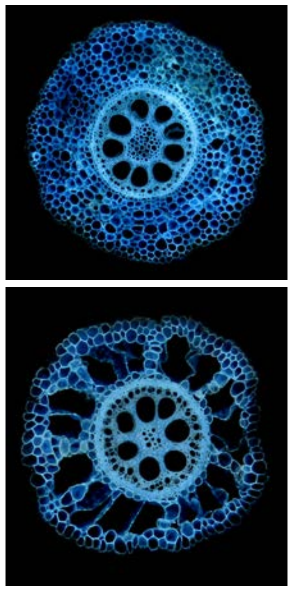
DEEPER: AN INTEGRATED PLATFORM FOR DEEPER ROOTS

Penn State, Wisconsin, Georgia & Nottingham

Technology Summary

Development of platform for breeding maize with deeper roots that sequester more carbon and are more efficient in utilization of nitrogen and water.



Proposed Targets

Metric	State of the Art	Proposed
Non-destructive detection of root depth	none	root depth in 2 min/plant
Excavate and image root crowns	Shovelomics 3 plants/ person/h	Root Robot /DIRT3D: 30 plants/person/h
3-D anatomical image collection and analysis	none	50-100 samples/day
Model root penetration of hard soil	none	*SimRoot/Deep*
Selection of deep roots	none	Prediction with GS models

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Soil Carbon Capture

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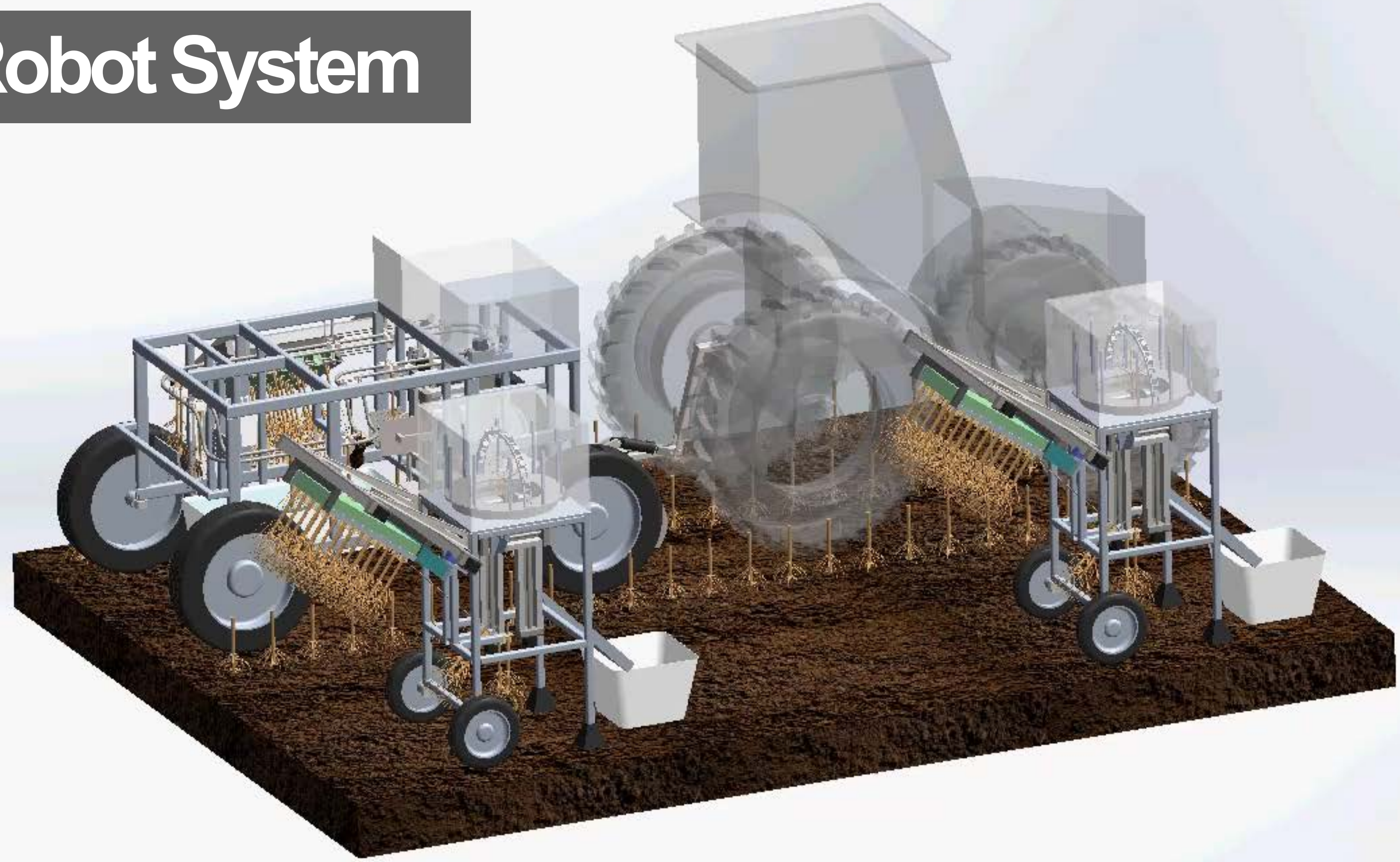
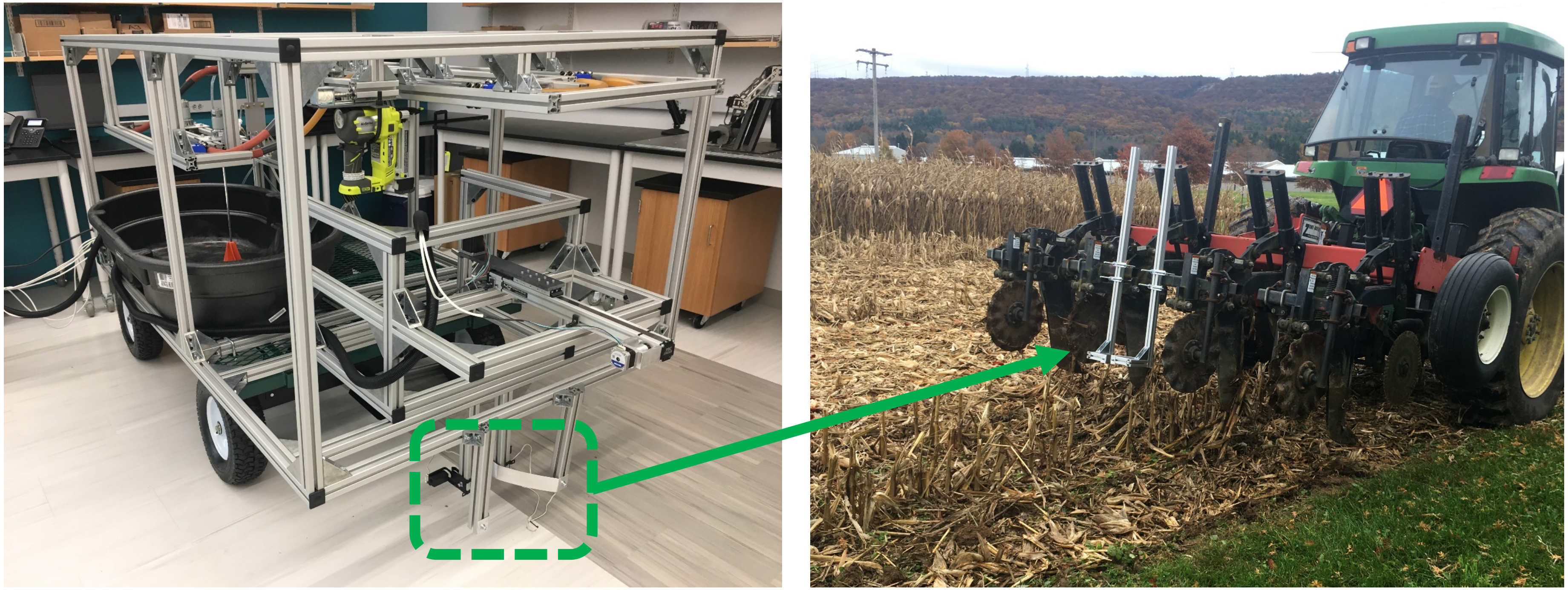
RootRobot System



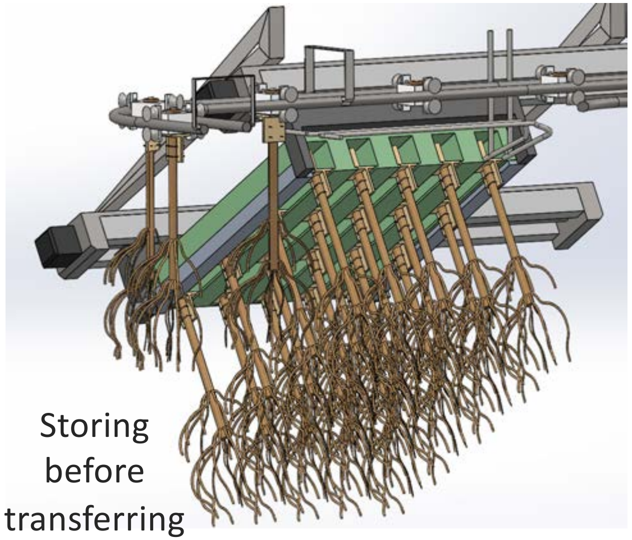
RootRobot System

RootRobot System

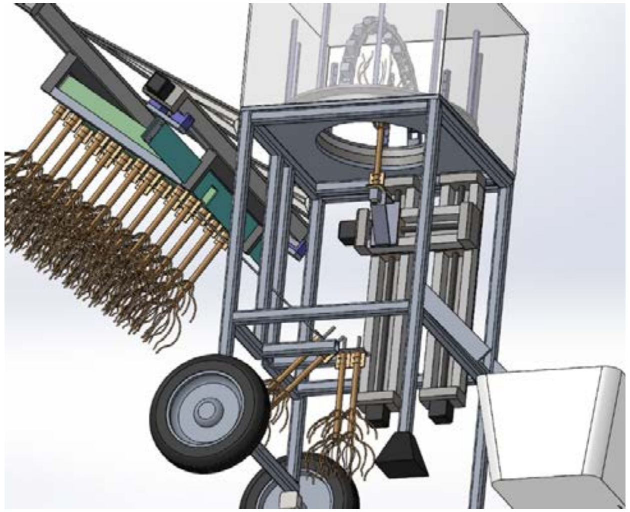


Field test for stalk guide bars
(Stalks stay straight up after being deeply tilled and guided)

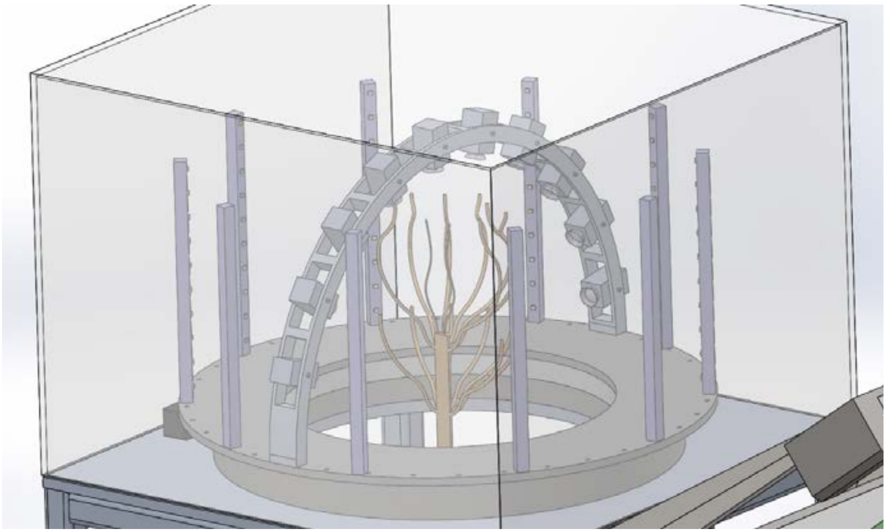
RootRobot System



Storing before transferring



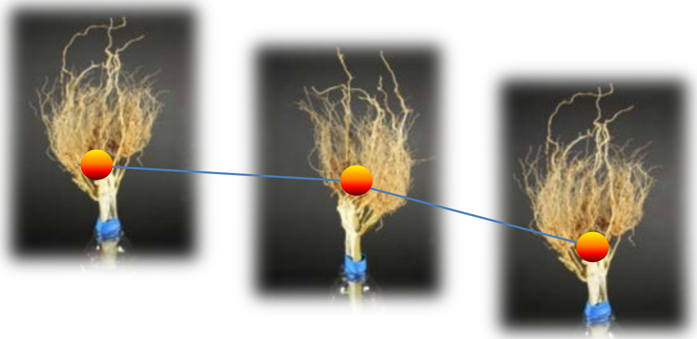
Transferring to imaging unit



Root in imaging chamber

Image Analysis Using DIRT3D

a. Imaging Camera

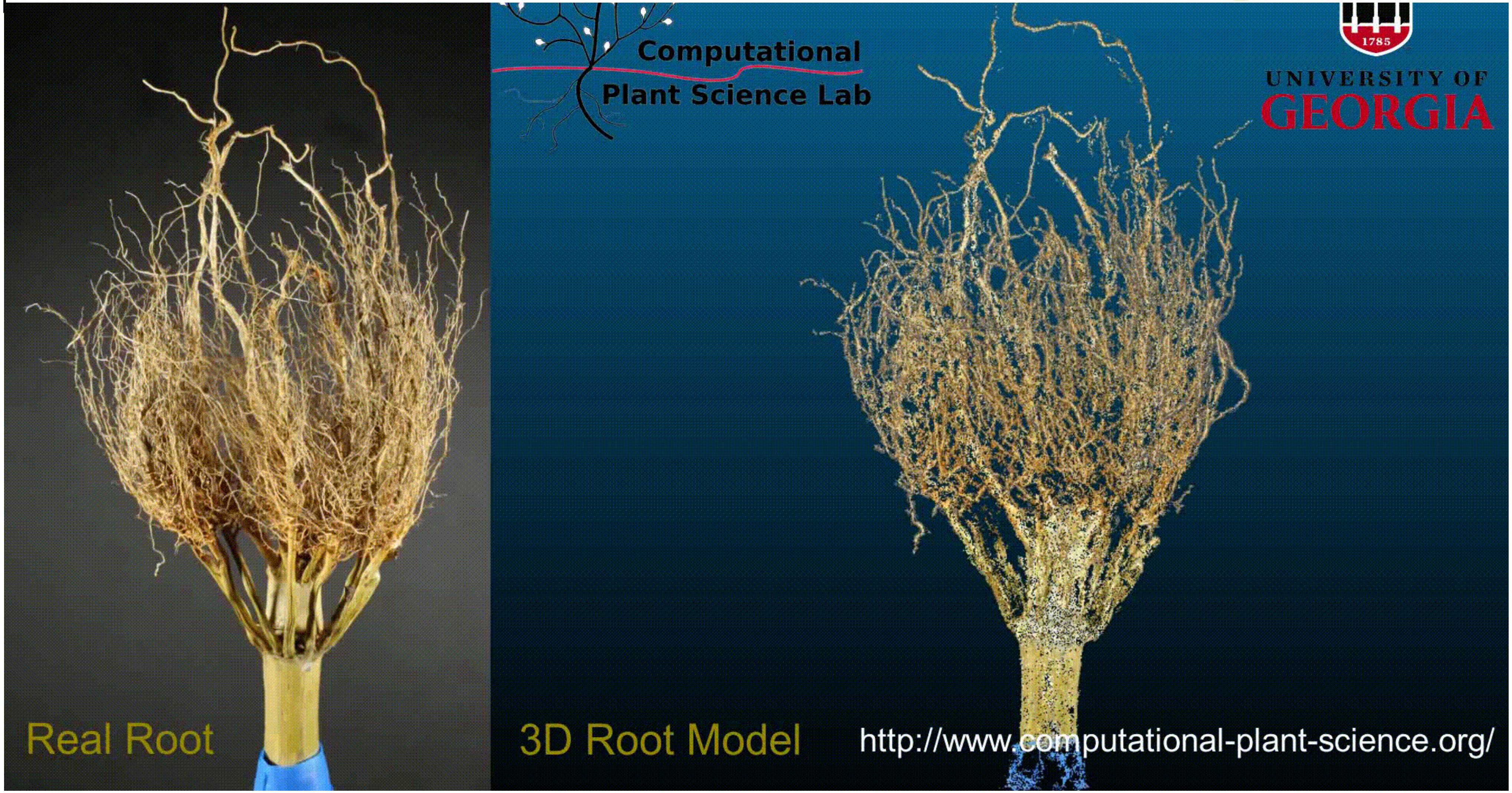


b. Feature matching

c. Root imaging

d. Computation

3D fine root models vs. Real roots



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3D root phene decomposition

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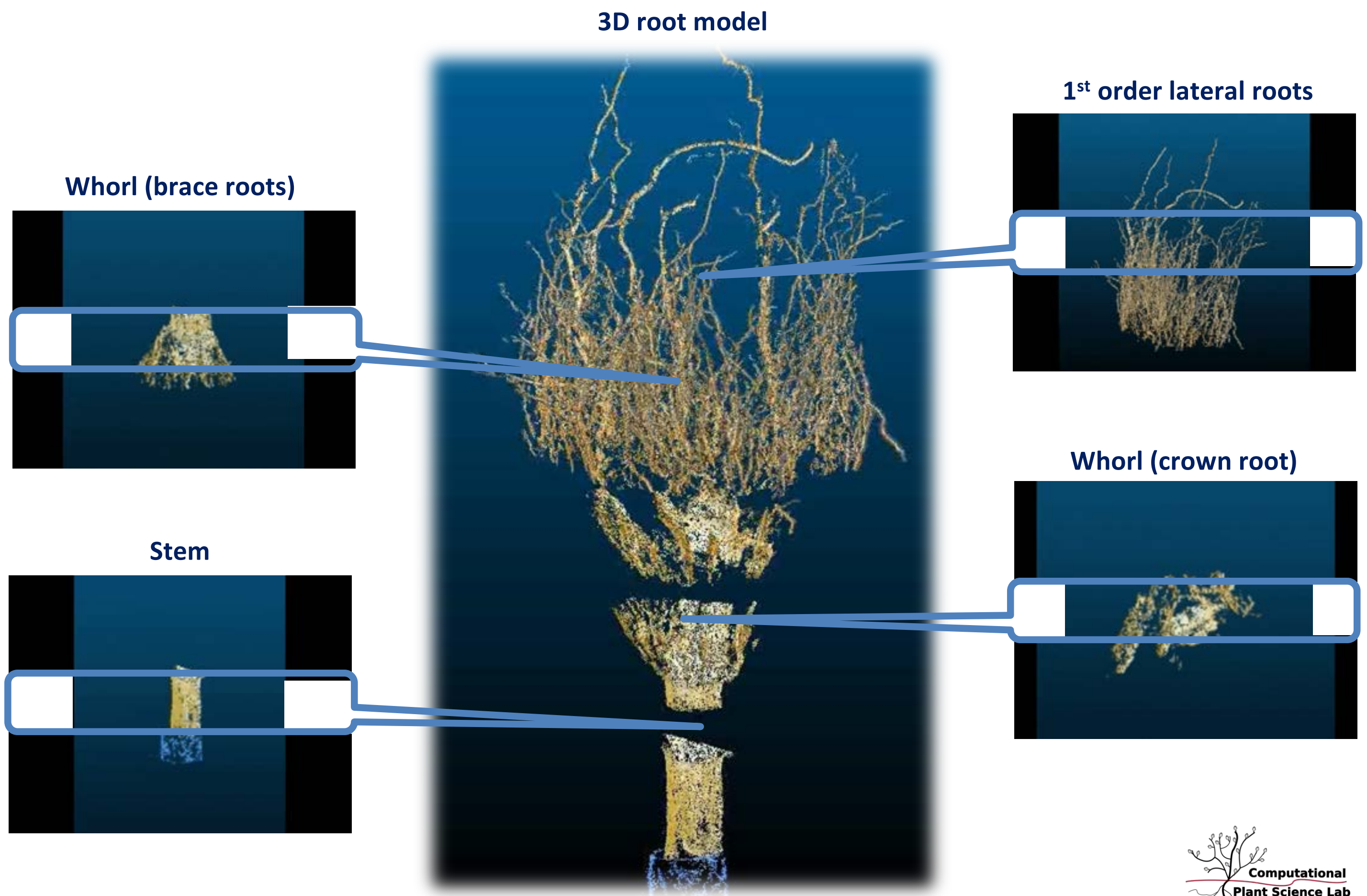
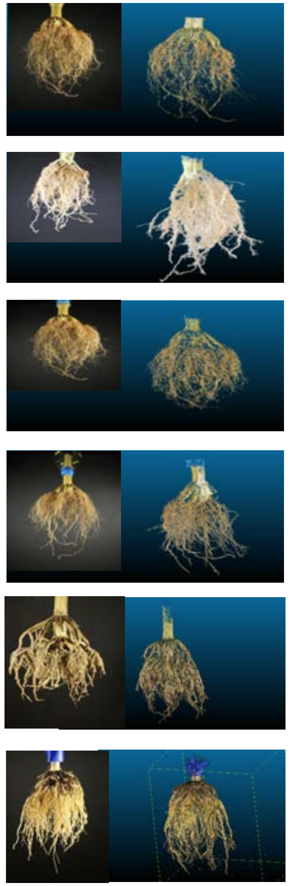
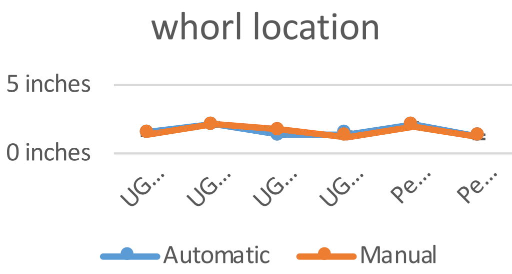
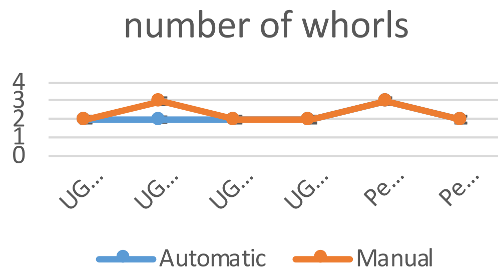


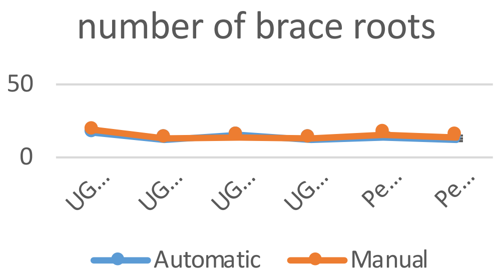
Computational Plant Science Lab

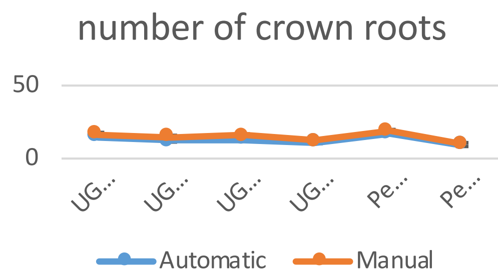
3D phene validation

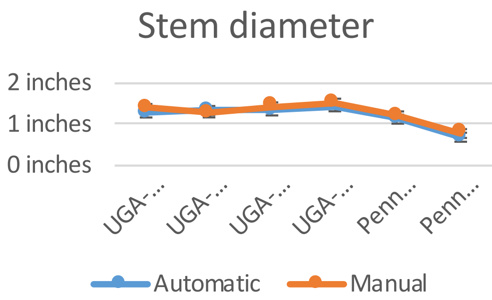


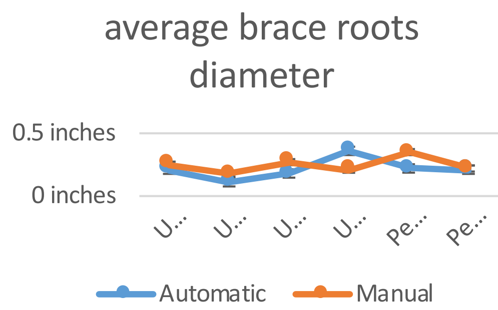












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DEEPGENES discovery pipeline

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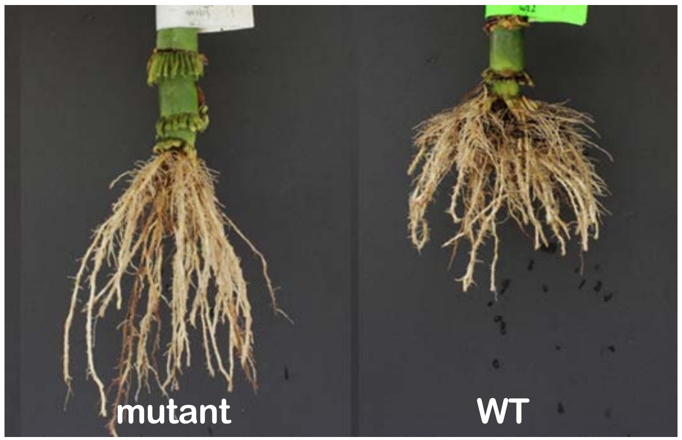
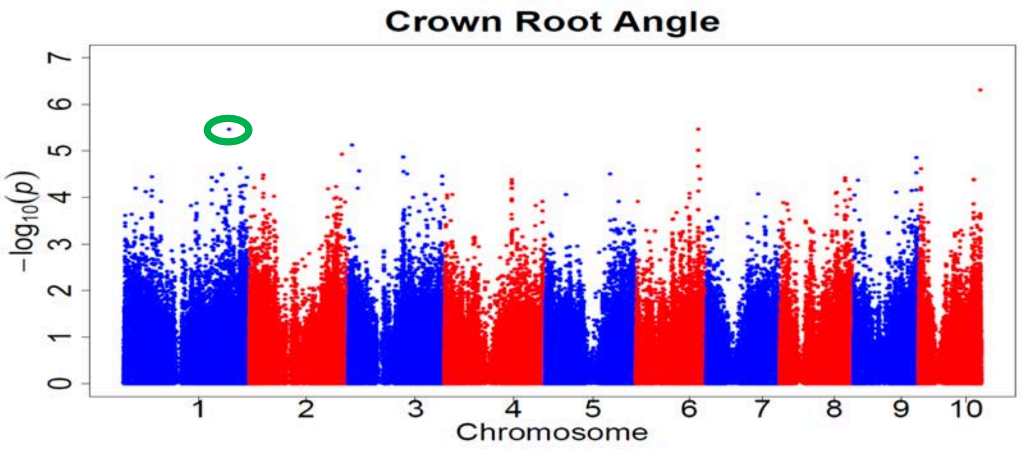
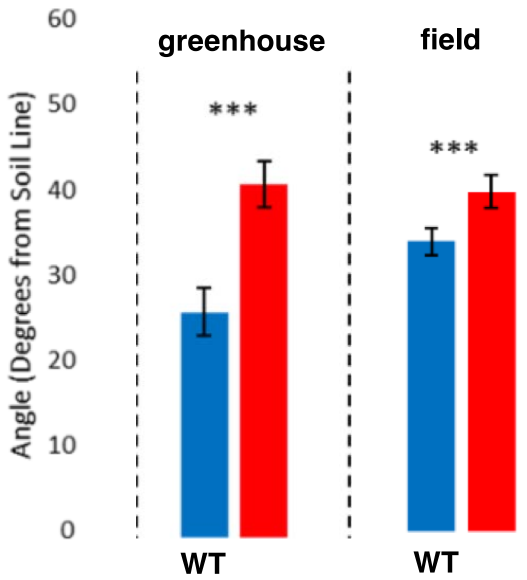
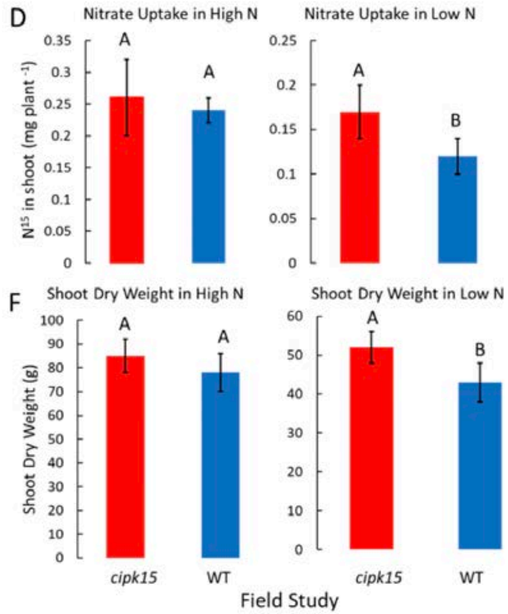
Example: Crown root angle

Variation in WiDiv Association Panel









Research of Hannah Schneider

ROOTS Soil Carbon Capture

US-UK DEEPER Team

arpa-e U.S. DEPARTMENT OF ENERGY

PennState

Jonathan Lynch
Kathy Brown
Paul Heinemann
Dana Choi
Molly Hanlon
Hannah Schneider
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Suxing Liu
Chris Cotter

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Riccardo Fusi
Eze Benson

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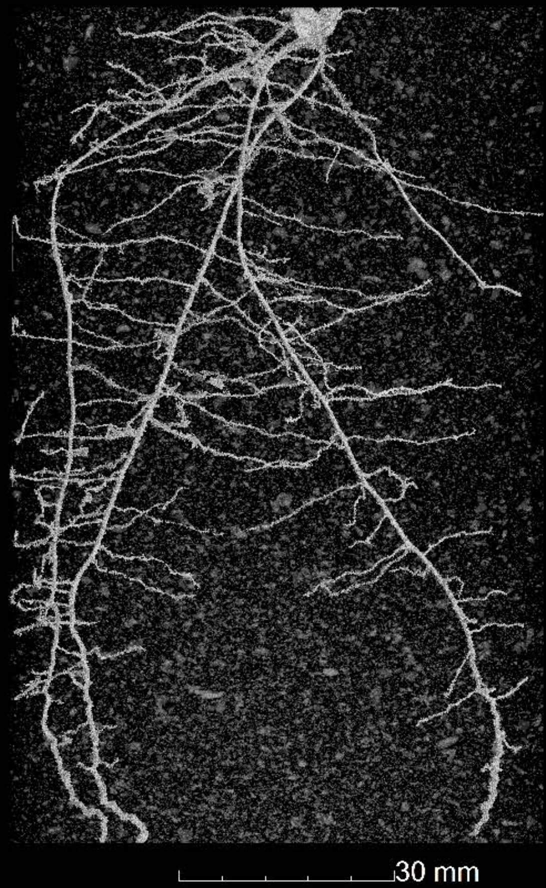
Advances in Root Phenotyping

3 topics covered in today's seminar include

- Field phenotyping for root traits
- Imaging root traits for plants grown in controlled environments
- New innovations in root phenotyping



Imaging root traits for plants grown in controlled environments

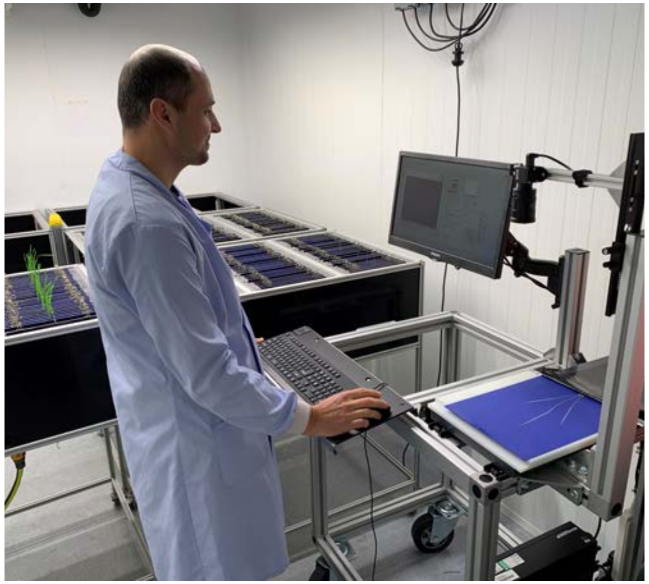
- Soil free 2D imaging of root systems
- Aeroponic-based root phenotyping
- Rhizotron-based root imaging
- High throughput imaging of roots in pots
- High content 3D X-ray microCT root imaging
- Imaging roots throughout a crops life cycle

2D Seedling Root Phenotyping

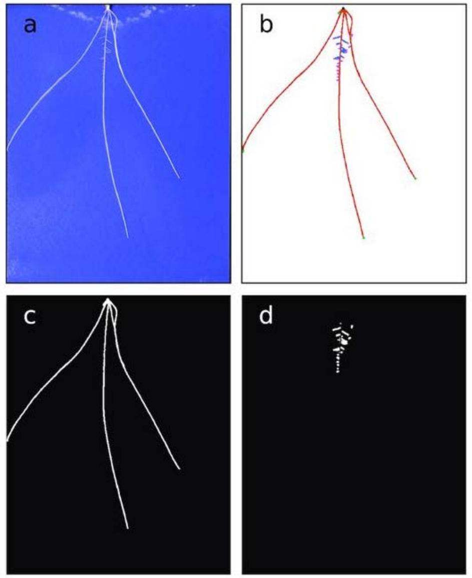
Growth room
- 600 plants in growth pouches
- 2 weeks growth
- Gravity fed automatic watering

Atkinson et al., 2015

Imaging
- Mobile imaging station with machine vision camera
- QR code system for automatic plant identification and image tagging



Software
- RootNav 2.0
- Fully automatic image analysis via deep multi-task convolutional neural network



Pound et al., 2019

RootPhAir: Root Phenotyping in Air

Plants grown in aeroponics, roots hanging in air
Roots are naturally separated, imaging made easy

GrowScreen-Rhizo 1 – phenotyping root and shoot traits of plants grown in soil-filled rhizoboxes

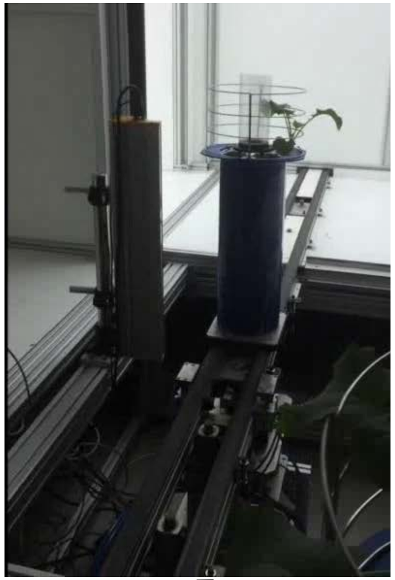
Throughput: up to 240 plants – 60 min

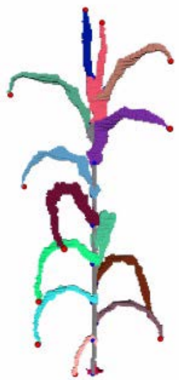
Nagel et al. 2012, Functional Plant Biology

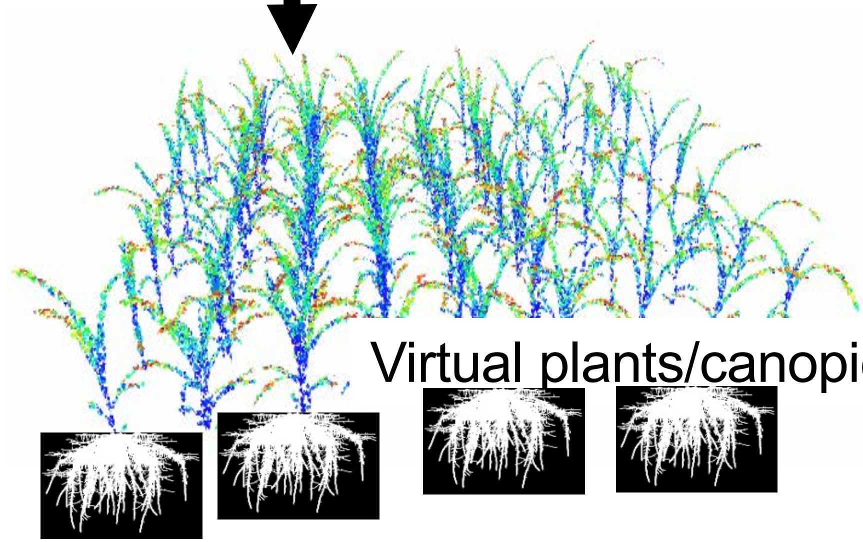
Multi-scale phenomics, **Controlled conditions**

2400 plants

1800 rhizotrons







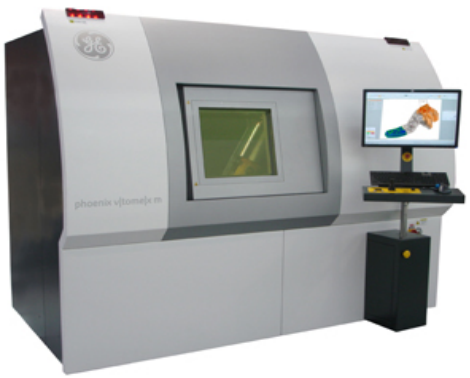
Virtual plants/canopies

Montpellier, Dijon, Angers, Toulouse

Tardieu et al 2017 Current Biol

Root systems of plants in 3D

Hounsfield Facility
3D X-ray imaging

Small CT	Medium CT	Large CT
		
		
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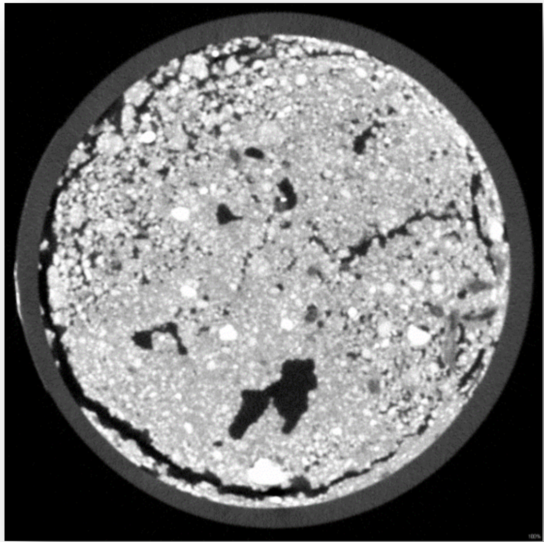

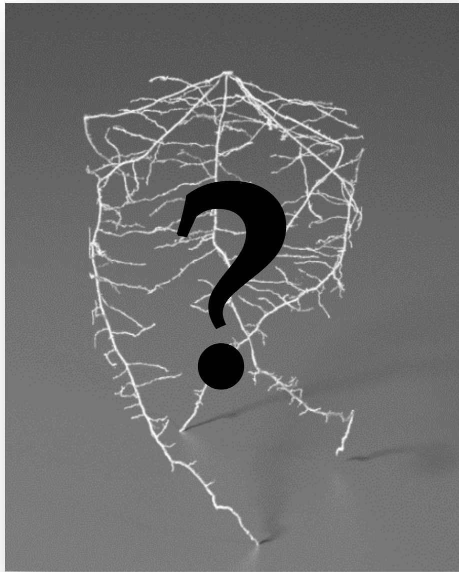

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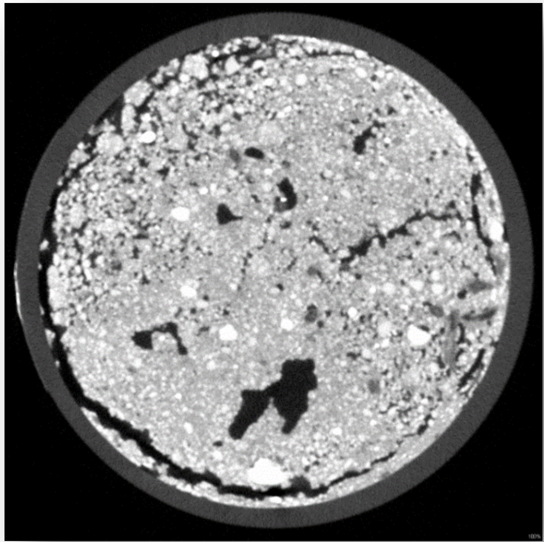
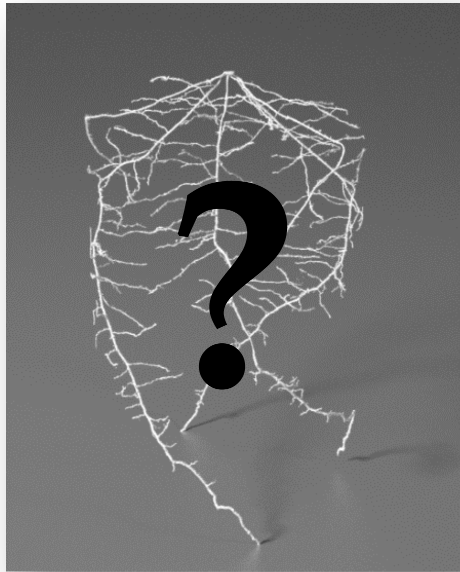
Building a Root Phenotyping Pipeline using CT Imaging

European Research Council
Established by the European Commission

CT-based Root Phenotyping Pipeline

Acquisition	X-ray CT Imaging
Recovery	Recovery of Root Material
Quantification	Quantification of Root Traits
Data	Data Management
Simulation	Modelling and Simulation



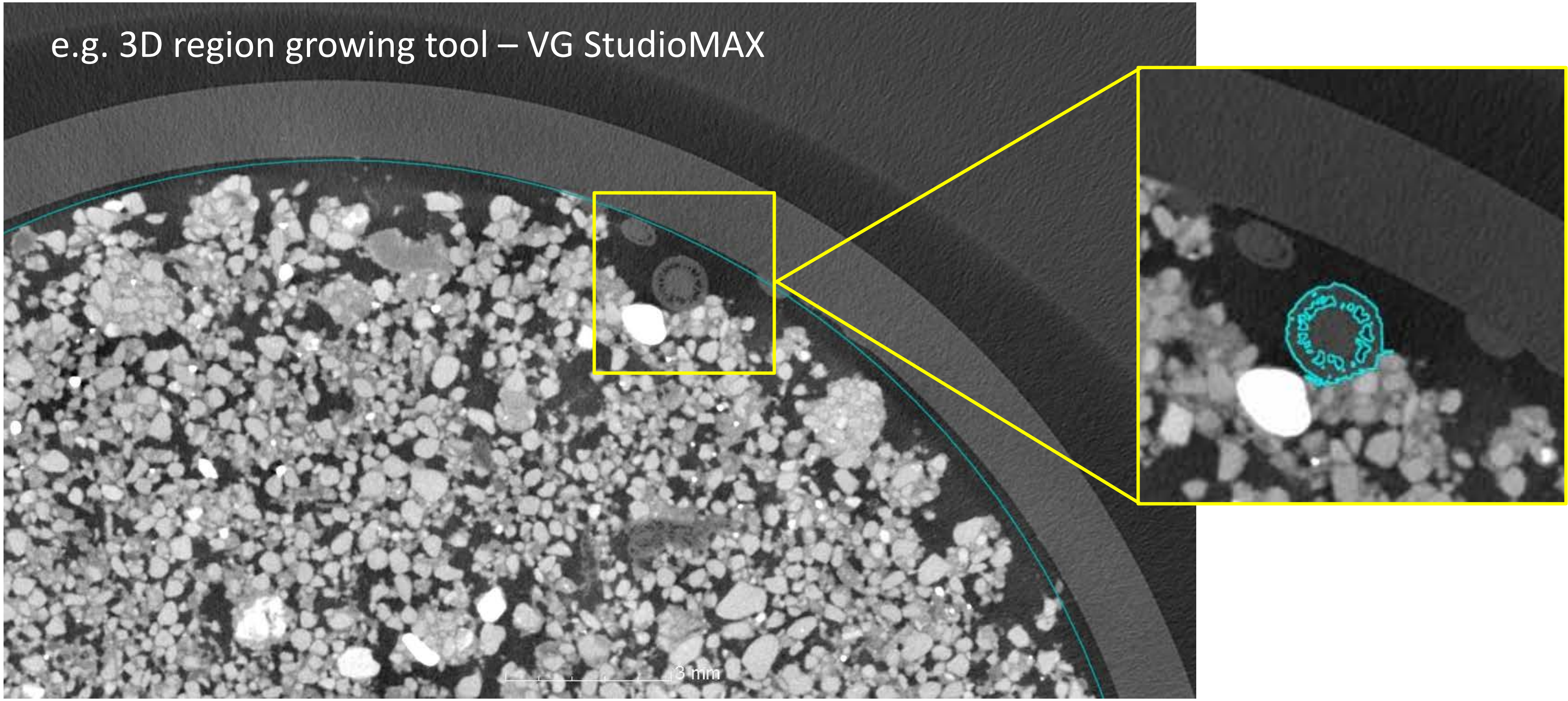


Commercial software

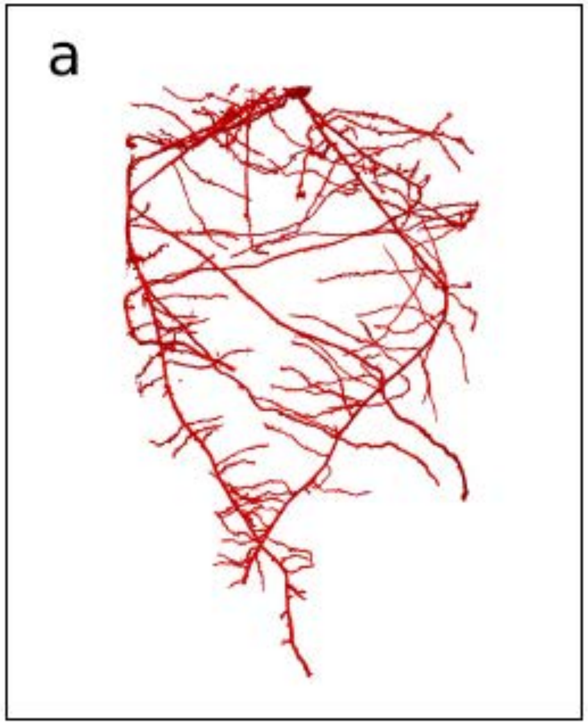
- Volume Graphics VG StudioMax
- Avizo Fire

Open Source

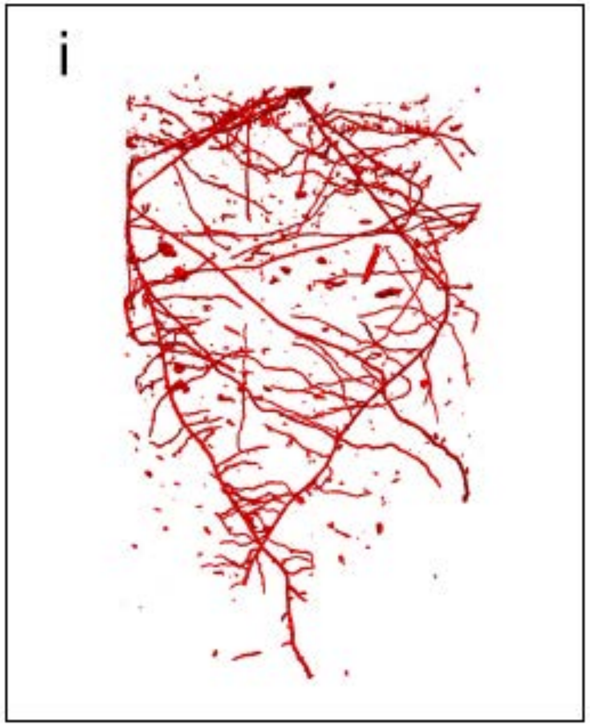
- RooTrak (Mairhofer et al. 2012)
- Root1 – ImageJ Plugin (Flavel et al. 2017)
- Deep learning approaches (Soltaninejad et al. 2020)

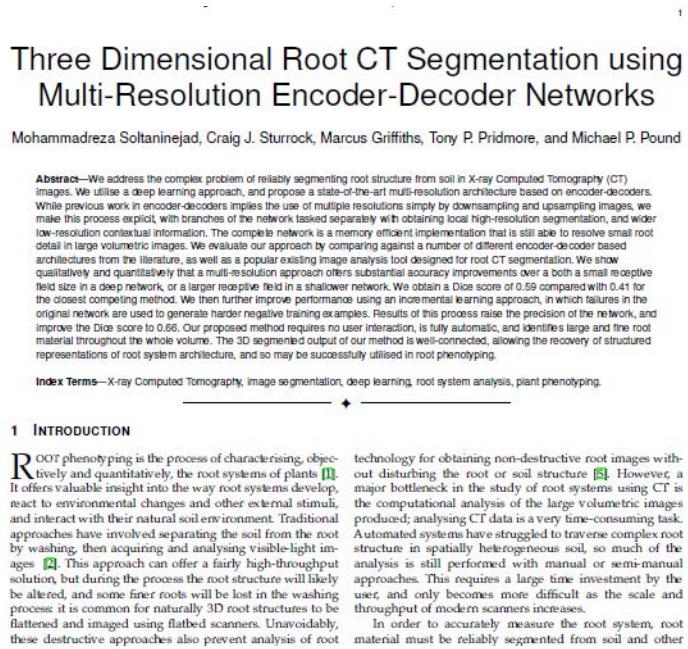

e.g. 3D region growing tool – VG StudioMAX
3 mm

Training Image



Output Image





Three Dimensional Root CT Segmentation using Multi-Resolution Encoder-Decoder Networks

Mohammadreza Soltaninejad, Craig J. Sturrock, Marcus Griffiths, Tony P. Pridmore, and Michael P. Pound

Abstract—We address the complex problem of reliably segmenting root structure from soil in X-ray Computed Tomography (CT) images. We utilise a deep learning approach, and propose a state-of-the-art multi-resolution architecture based on encoder-decoders. While previous work in encoder-decoders implies the use of multiple resolutions simply by downsampling and upsampling images, we make this process explicit, with branches of the network tasked separately with obtaining local high-resolution segmentation, and wider low-resolution contextual information. The complete network is a memory efficient implementation that is still able to resolve small root detail in large volumetric images. We evaluate our approach by comparing against a number of different encoder-decoder based architectures from the literature, as well as a popular existing image analysis tool designed for root CT segmentation. We show qualitatively and quantitatively that a multi-resolution approach offers substantial accuracy improvements over a both a small receptive field size in a deep network, or a larger receptive field in a shallower network. We obtain a Dice score of 0.59 compared with 0.41 for the closest competing method. We then further improve performance using an incremental learning approach, in which failures in the original network are used to generate harder negative training examples. Results of this process raise the precision of the network, and improve the Dice score to 0.66. Our proposed method requires no user interaction, is fully automatic, and identifies large and fine root material throughout the whole volume. The 3D segmented output of our method is well-connected, allowing the recovery of structured representations of root system architecture, and so may be successfully utilised in root phenotyping.

Index Terms—X-ray Computed Tomography, image segmentation, deep learning, root system analysis, plant phenotyping.

1 Introduction

ROOT phenotyping is the process of characterising, objectively and quantitatively, the root systems of plants [1]. It offers valuable insight into the way root systems develop, react to environmental changes and other external stimuli, and interact with their natural soil environment. Traditional approaches have involved separating the soil from the root by washing, then acquiring and analysing visible-light images [2]. This approach can offer a fairly high-throughput solution, but during the process the root structure will likely be altered, and some finer roots will be lost in the washing process: it is common for naturally 3D root structures to be flattened and imaged using flatbed scanners. Unavoidably, these destructive approaches also prevent analysis of root technology for obtaining non-destructive root images without disturbing the root or soil structure [5]. However, a major bottleneck in the study of root systems using CT is the computational analysis of the large volumetric images produced; analysing CT data is a very time-consuming task. Automated systems have struggled to traverse complex root structure in spatially heterogeneous soil, so much of the analysis is still performed with manual or semi-manual approaches. This requires a large time investment by the user, and only becomes more difficult as the scale and throughput of modern scanners increases.

In order to accurately measure the root system, root material must be reliably segmented from soil and other

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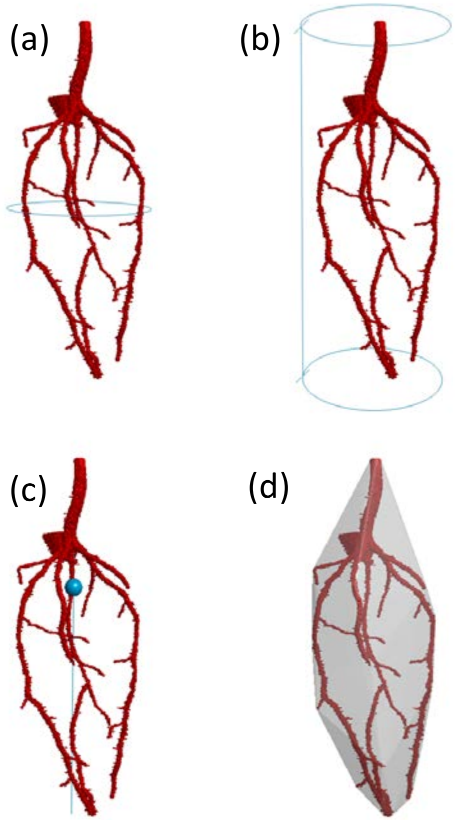
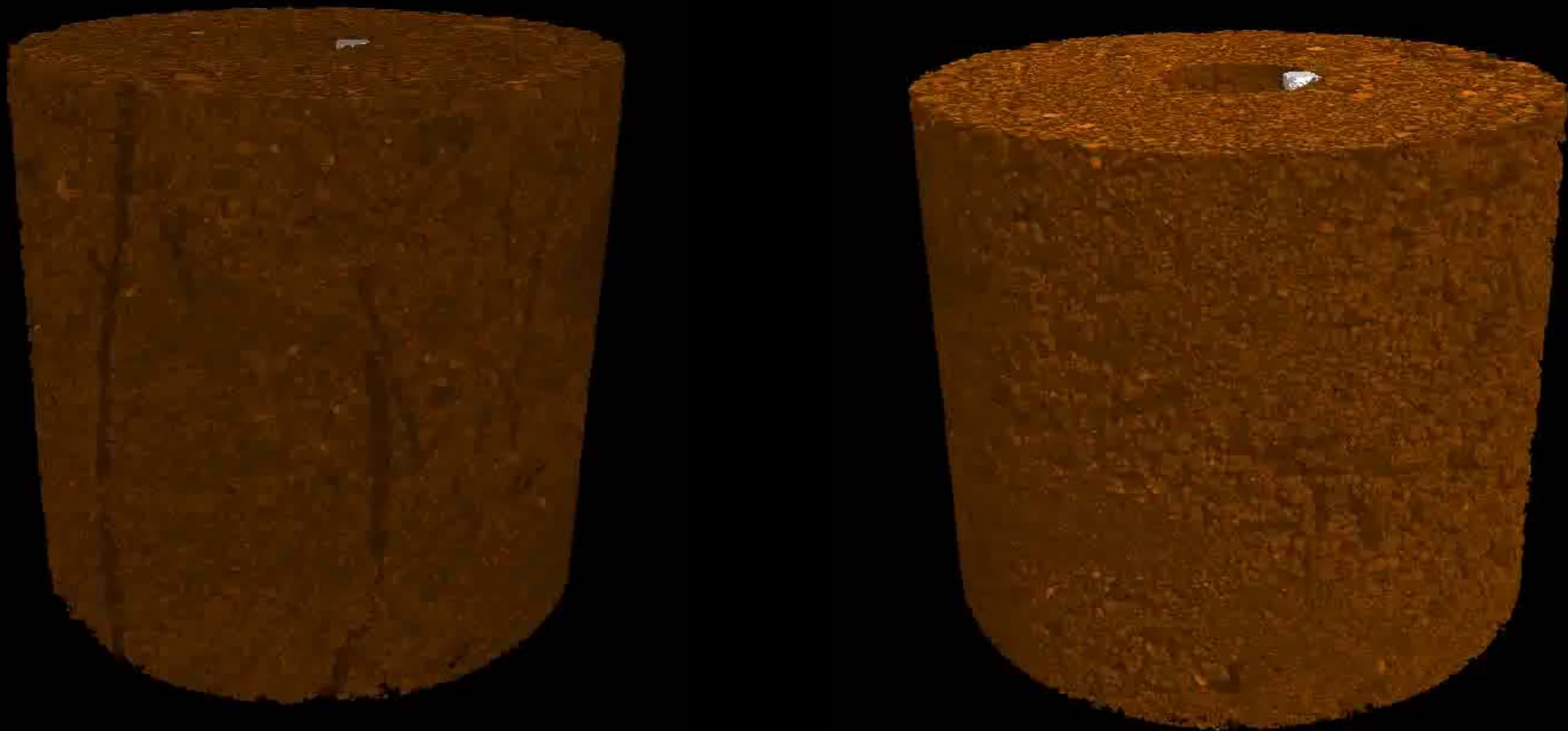
doi: http://dx.doi.org/10.1101/713859



- **Maximum Width (a)**
- Minimum Width
- Minimum Width / Maximum Width
- **Maximum Width / Maximum Depth (b)**
- Narrowness Index
- **Centroid (c)**
- Root Count
- Tip Count
- Median Number of Roots
- Maximum Number of Roots
- Median / Maximum Number of Roots (Bushiness Index)
- **Convex Hull (volume of soil explored) (d)**

Root development is highly plastic

Hydropattening promotes branching for roots in contact with moisture



Bao et al (2014) *PNAS* 111, 9319

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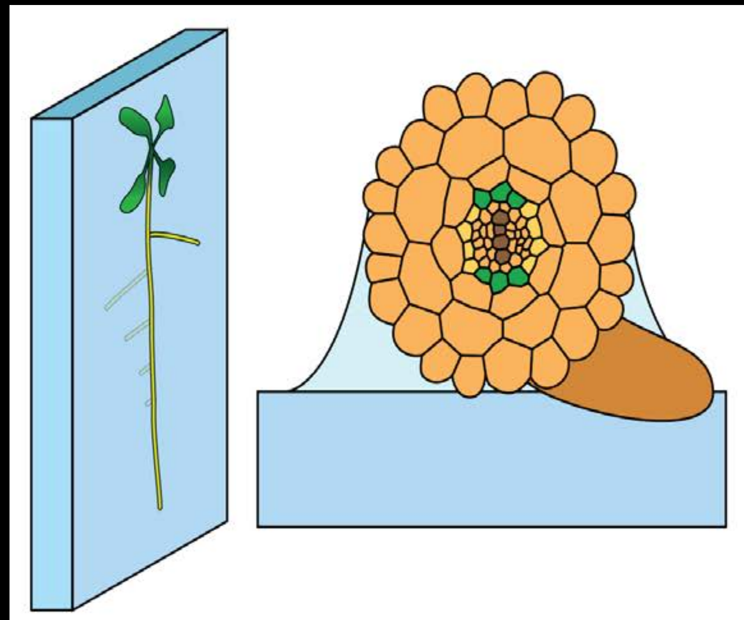
Lateral root development is influenced by its hydraulic landscape



Orosa-Puente et al (2018)
Science 362, 1407-1410

Von Wangenheim et al (2020)
Nature Plants 6 (2), 73-77

Imaging root growth throughout a crops life cycle

Hounsfield Facility
3D X-ray imaging

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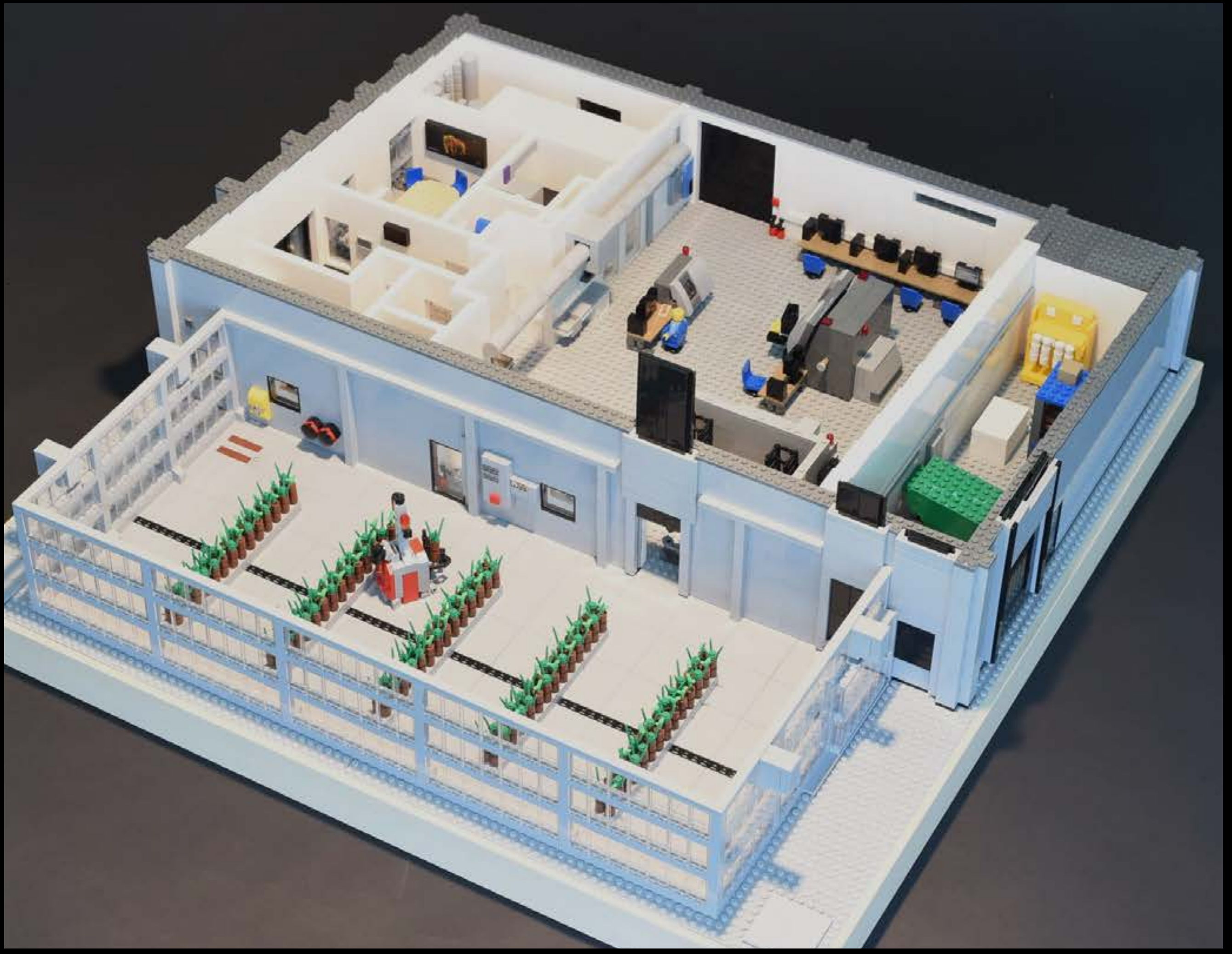

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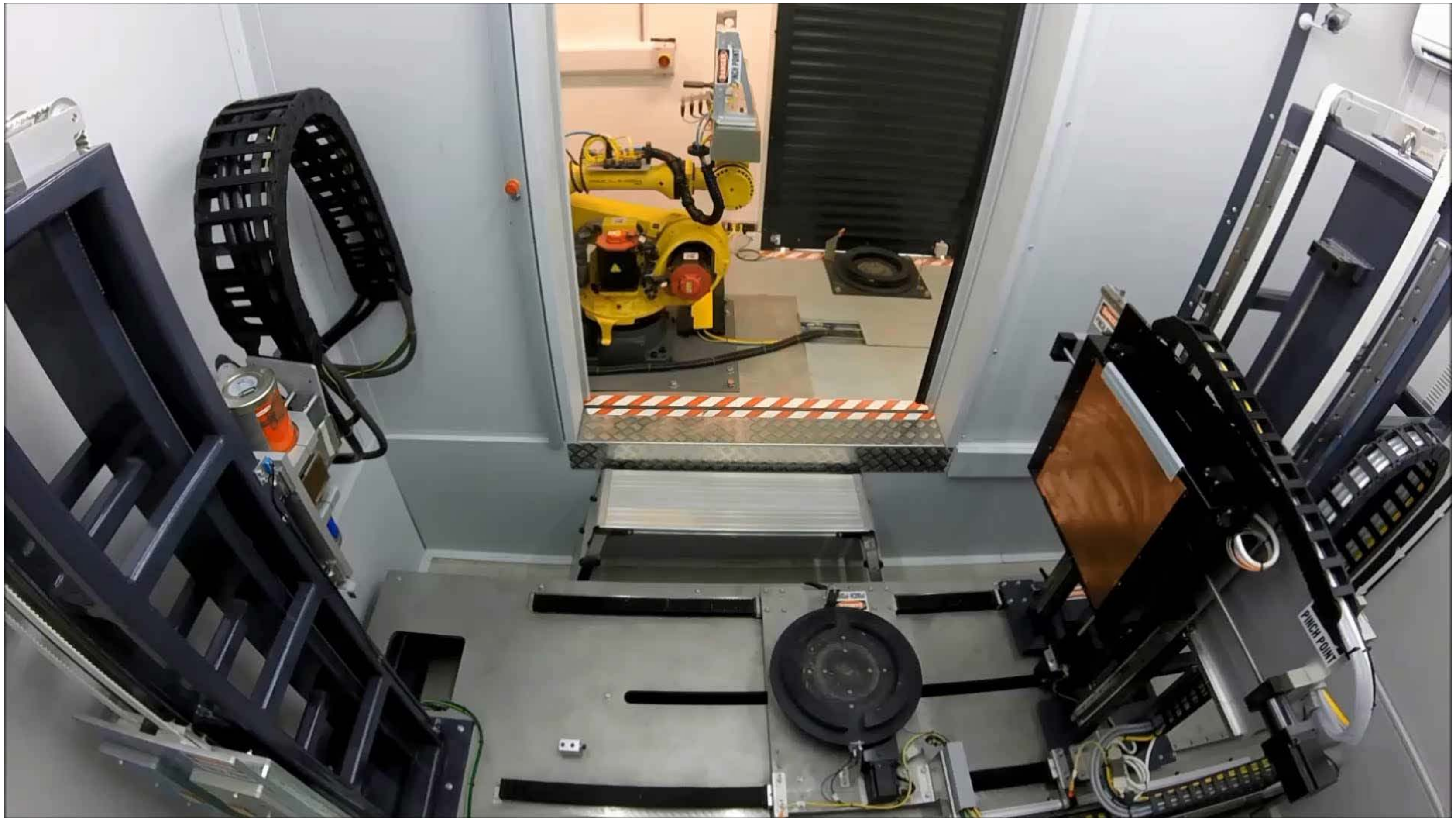
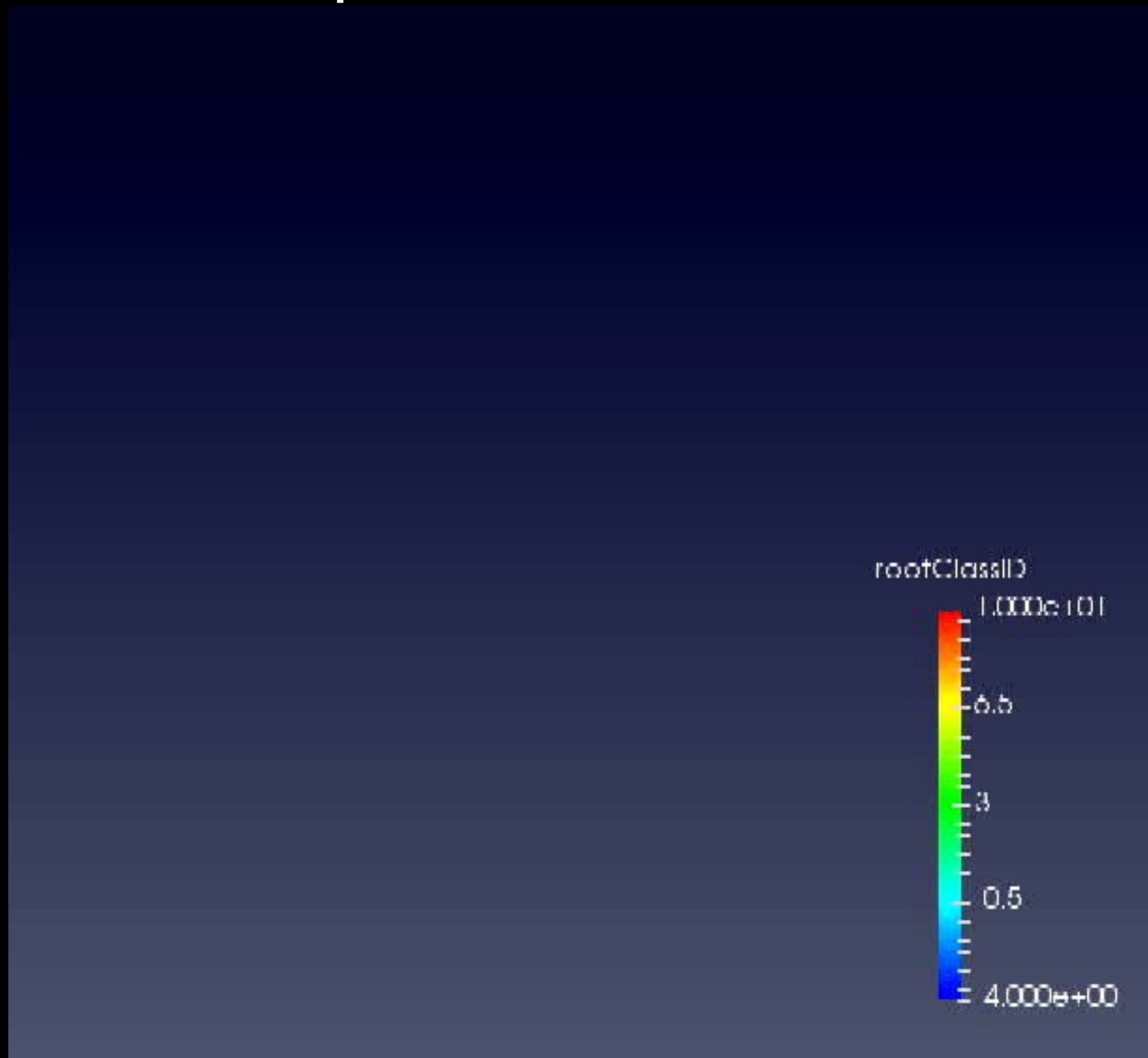


Imaging Wheat Root Development: *from start to finish*



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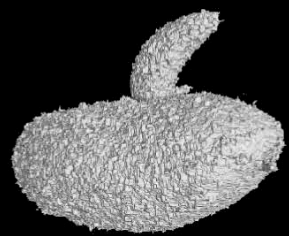

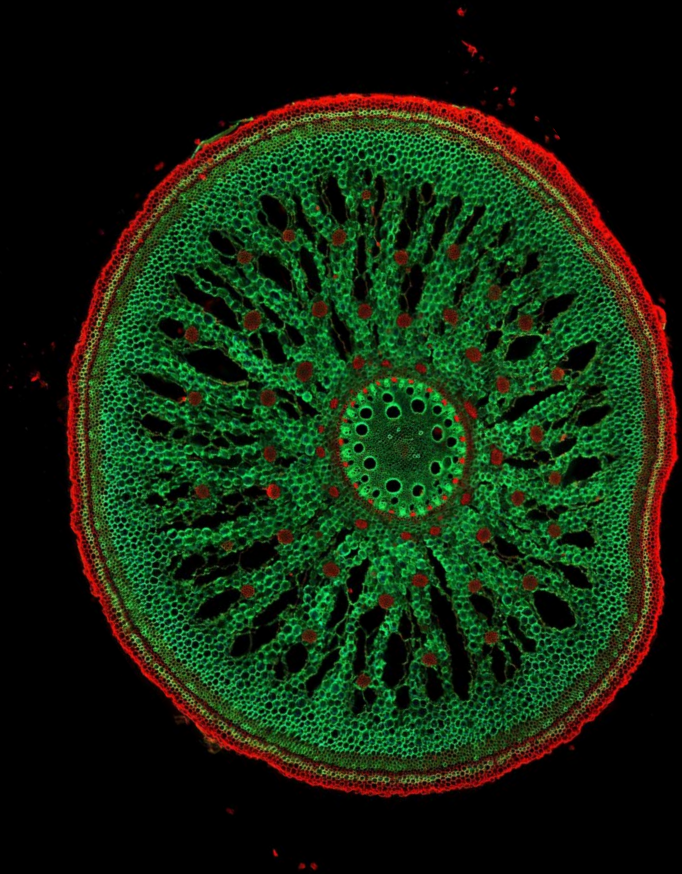

Hidden Half....of Date Palm

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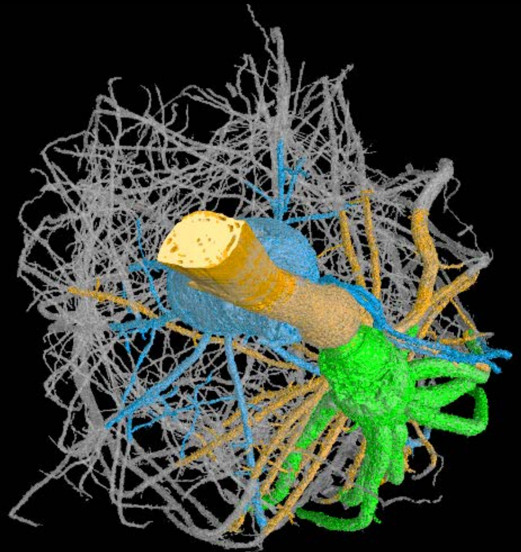

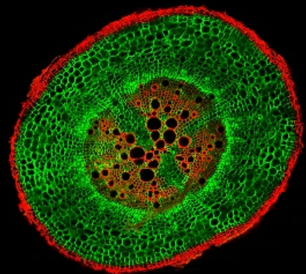

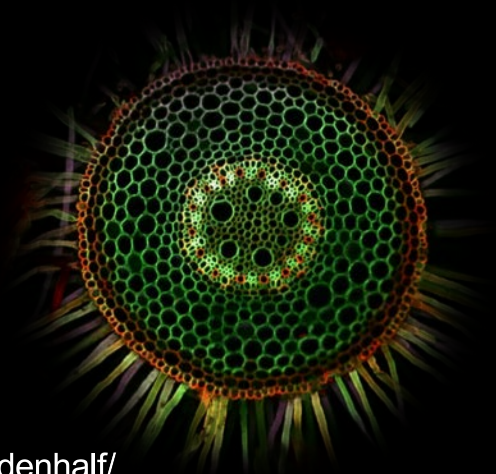

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3D X-ray imaging

Brian Atkinson
Jonathan Atkinson
Malcolm Bennett
Jasmine Burr-Hersey
Daniela Dietrich
Susie Lydon
Sacha Mooney
Emily Morris
Craig Sturrock

The Hidden Half

www.nottingham.ac.uk/hiddenhalf/

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ERC FUTURE ROOTS Team

Hounsfield Facility

3D X-ray imaging

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Imaging root traits for plants grown in controlled environments

- Soil free 2D imaging of root systems
- Aeroponic-based root phenotyping
- Rhizotron-based root imaging
- High throughput imaging of roots in pots
- High content 3D X-ray microCT root imaging
- Imaging roots throughout a crops life cycle

European Plant Phenotyping Network

EPPN 2020

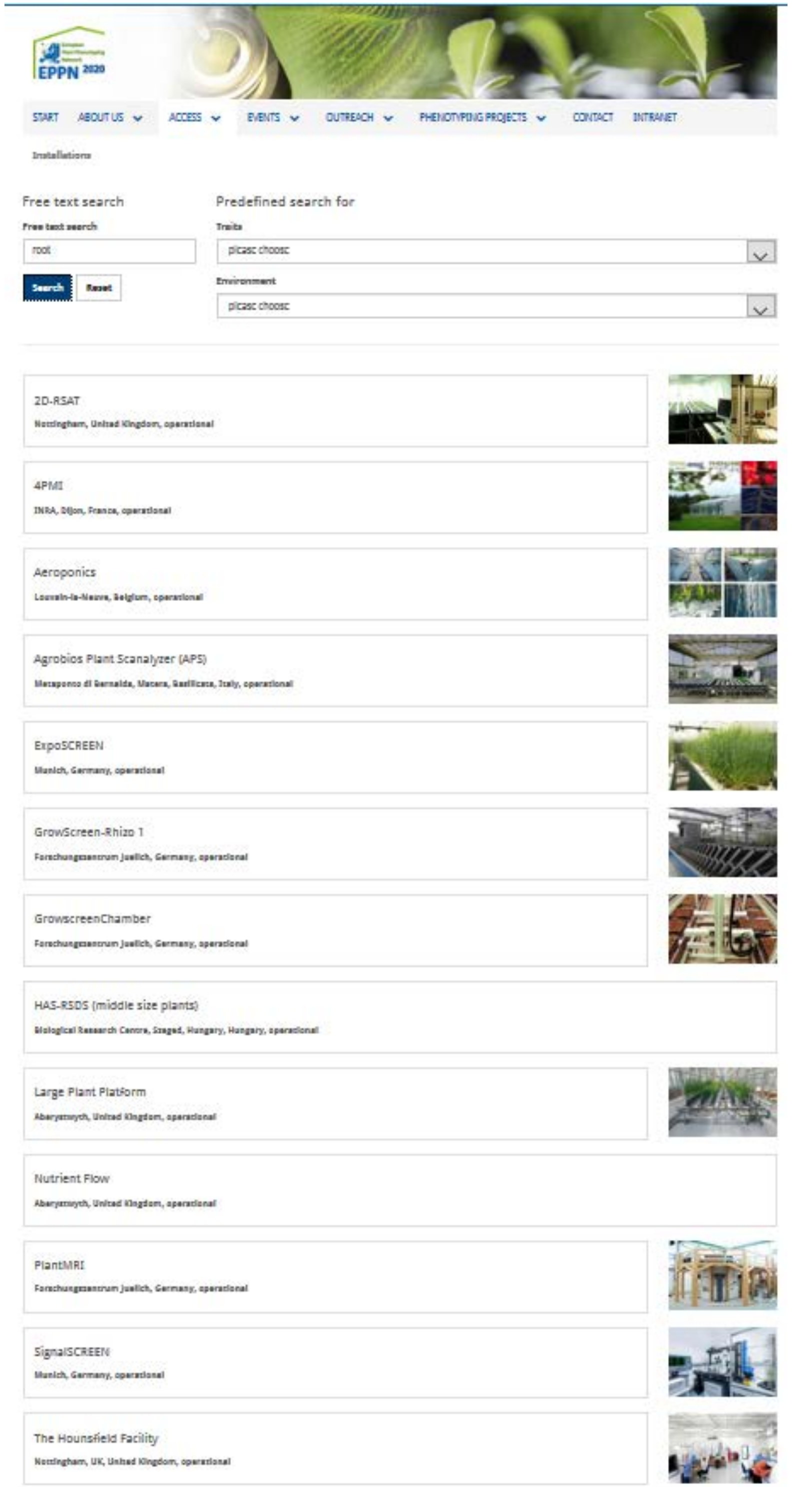
2017- April 2021
Access to 31 facilities in Europe

- Based on a simple application procedure
- Full cost of projects covered by the project, including travel
- 20% access funds for non-European labs
- Calls every 6 months

European Plant Phenotyping Network
EPPN 2020

Phenotyping root traits

- Essential element of installations providing access
- 13 facilities address different aspects of root phenotyping



EPPN 2020

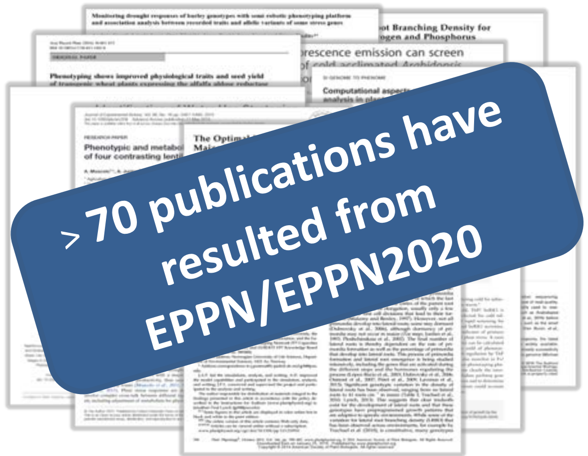
European Plant Phenotyping Network

Final call will be opened in June 2020

EPPN^{2020} users

Five calls have been launched already

- Applications from 34 different countries
- 124 projects are ongoing
- Capacity for up to 150 experiments



https://eppn2020.plant-phenotyping.eu/

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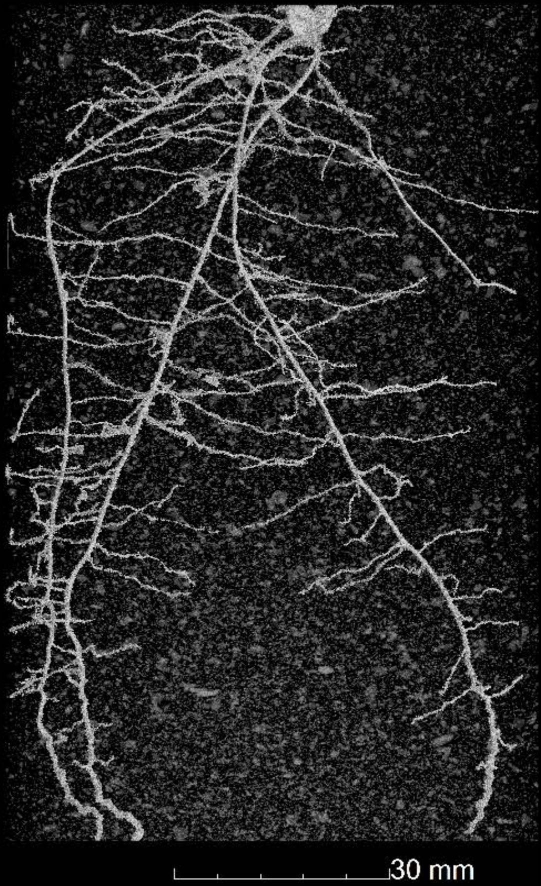
Advances in Root Phenotyping

3 topics covered in today's seminar include

- Field phenotyping for root traits
- Imaging root traits for plants grown in controlled environments
- New innovations in root phenotyping



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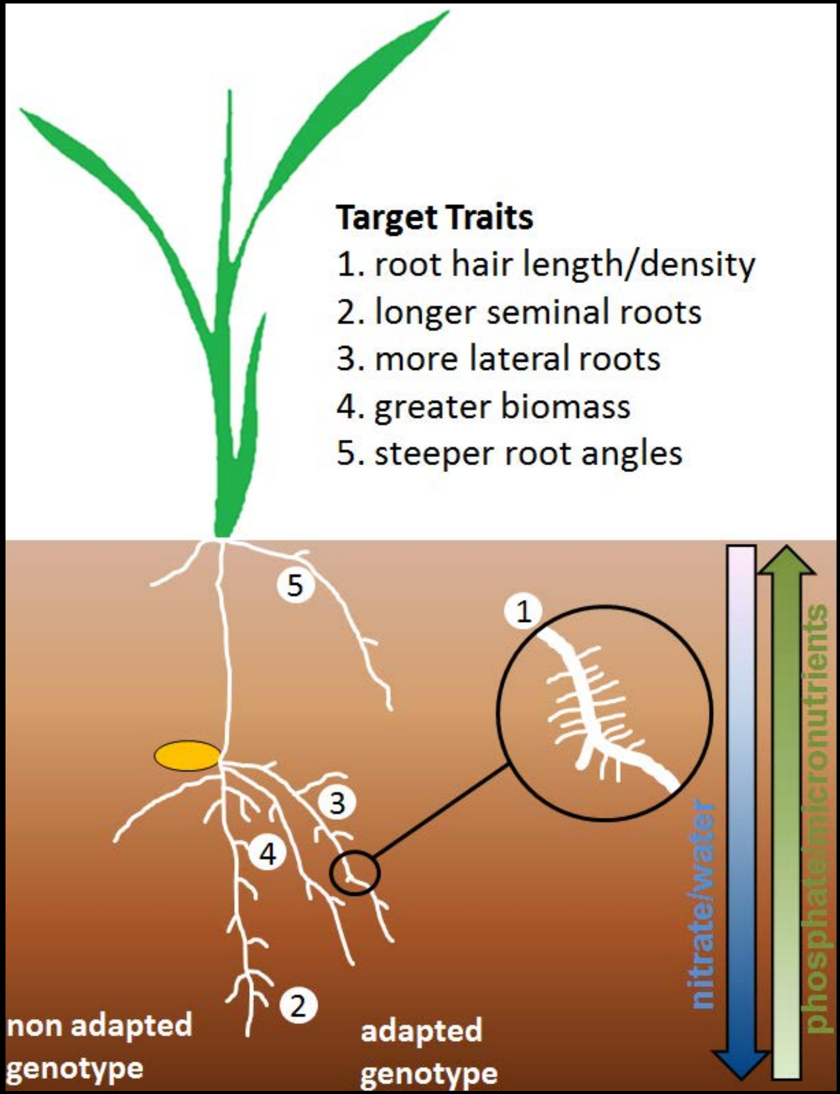
*Redesigning Root **Architecture***



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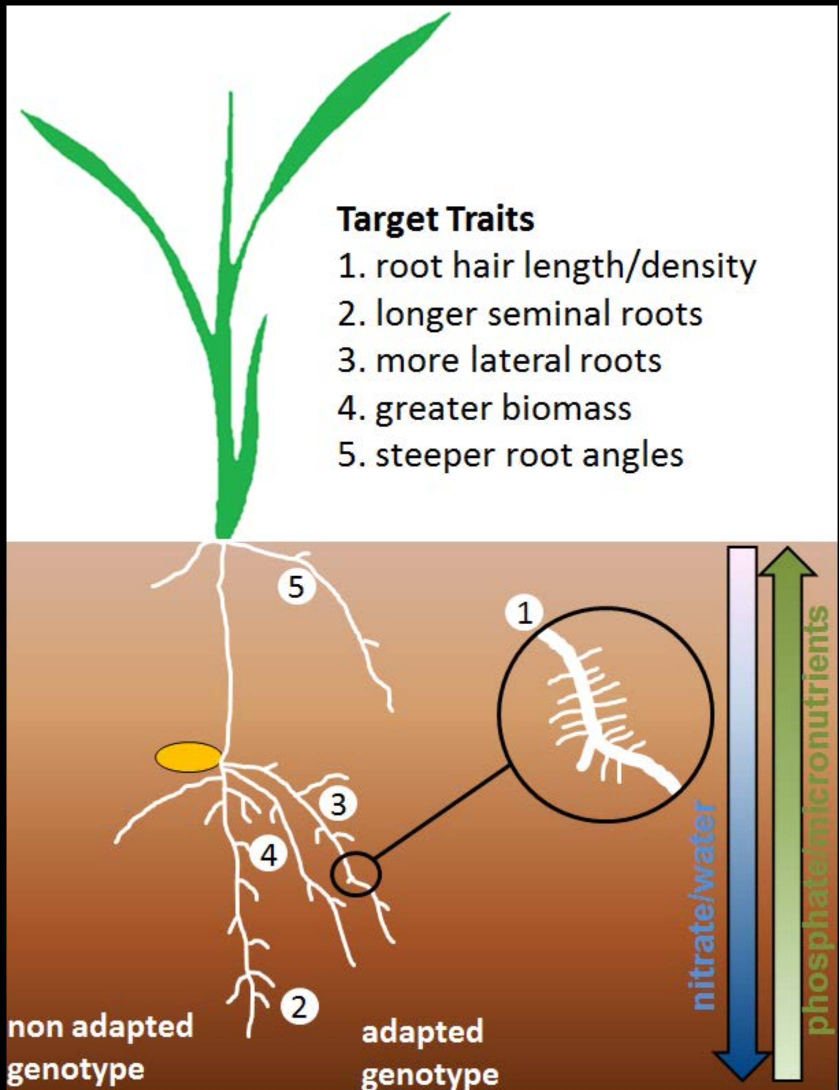
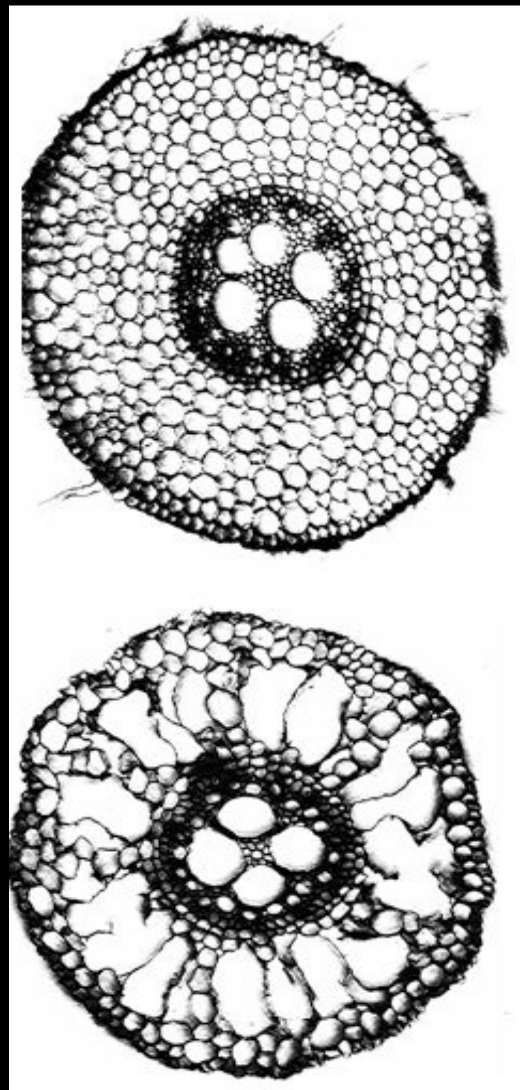
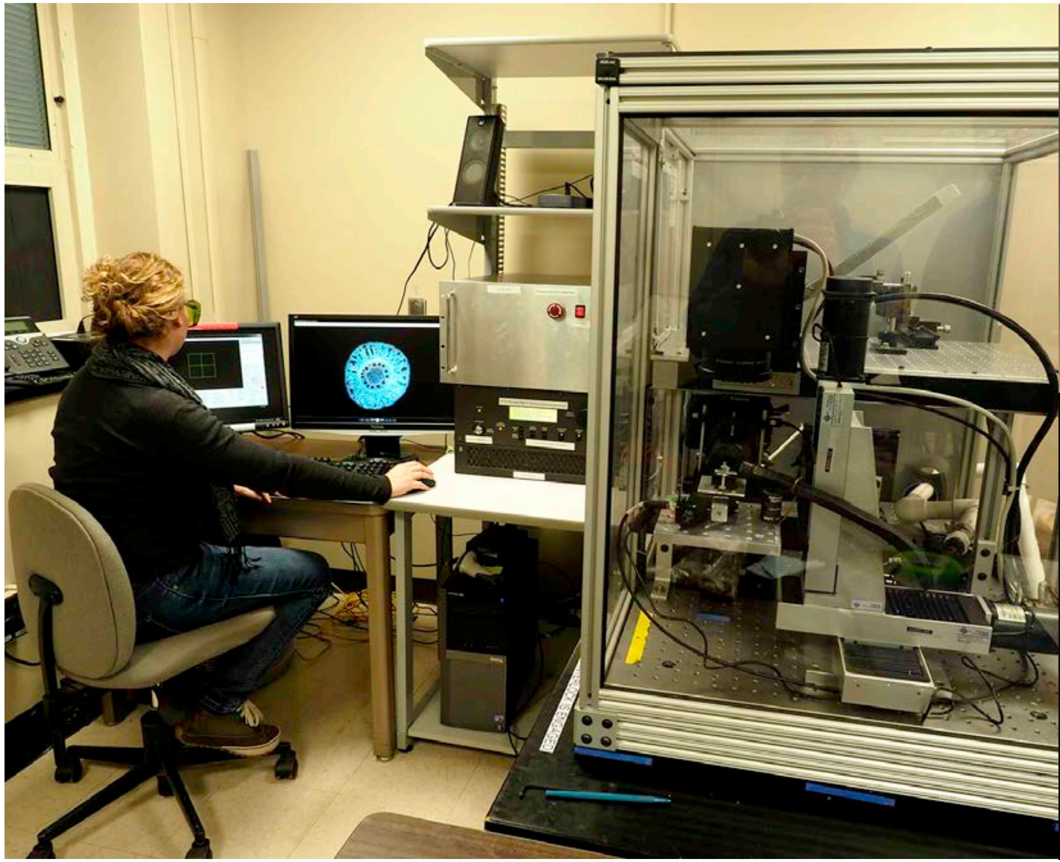
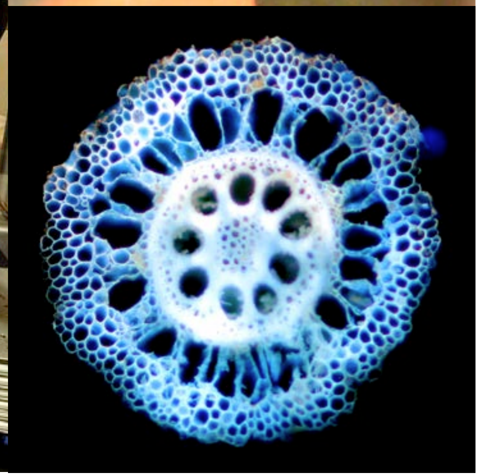
Redesigning Root ***Anatomy***





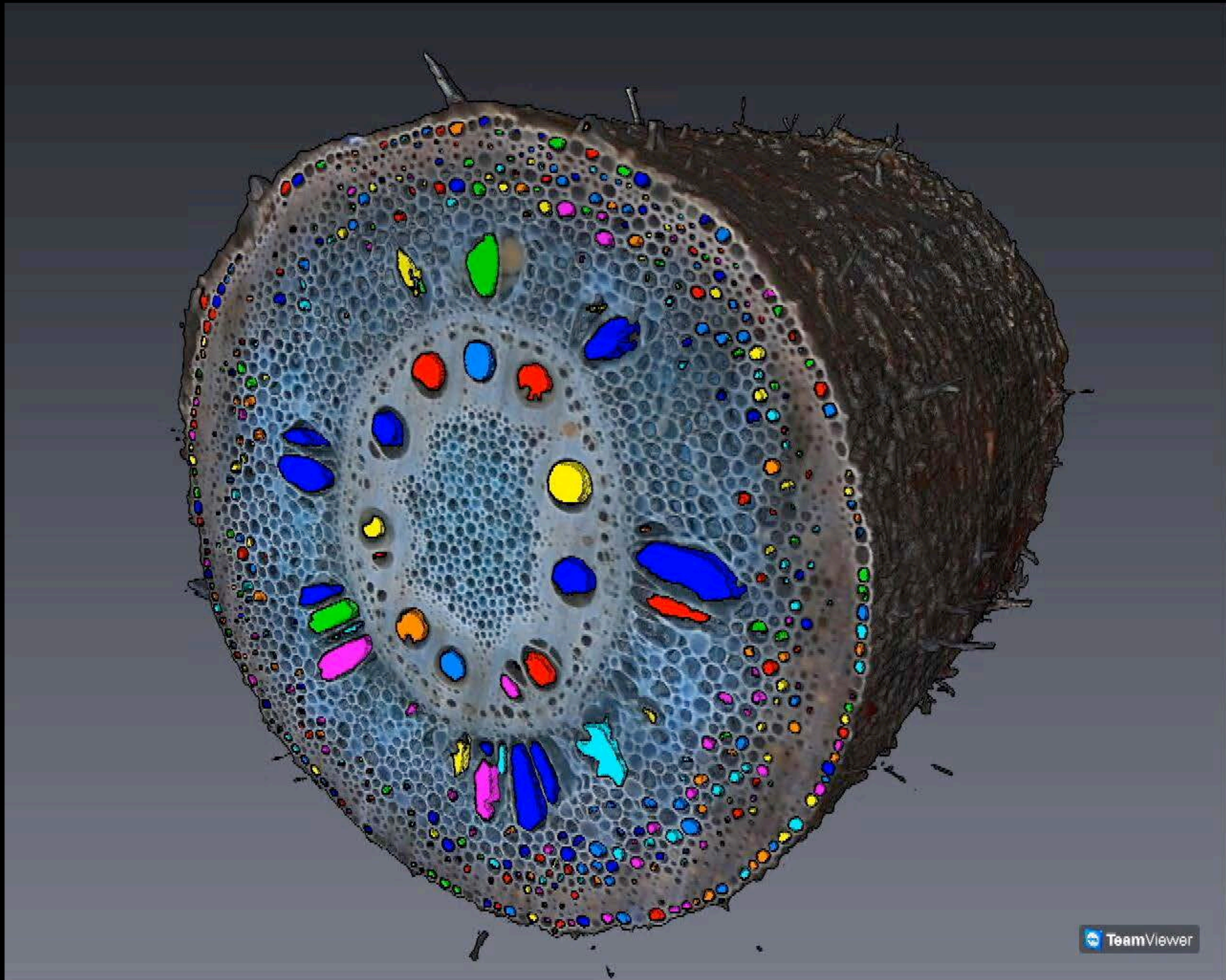
Phenoyping Root Anatomical Traits Using Laser Ablation Tomography (LAT)





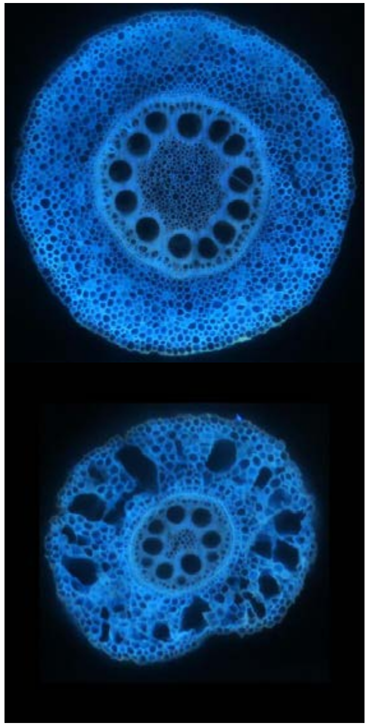
Jonathan Lynch
Penn State
Nottingham

Laser Ablation Tomography

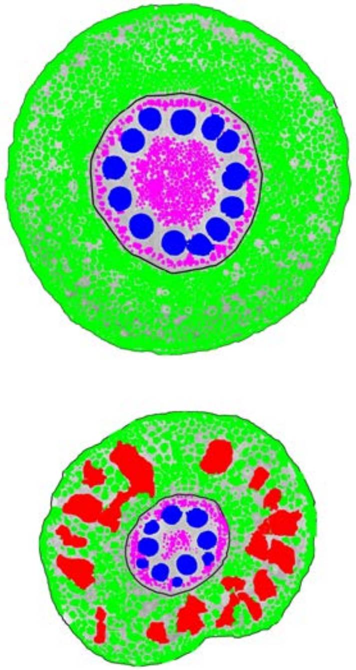


Jenn Yang

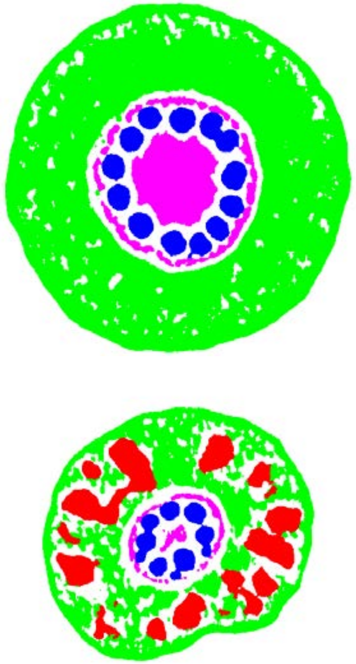
Automated image analysis with neural networks



Input Image



Ground Truth



Network Output

Ezenwoko Benson & Tony Pridmore, Nottingham

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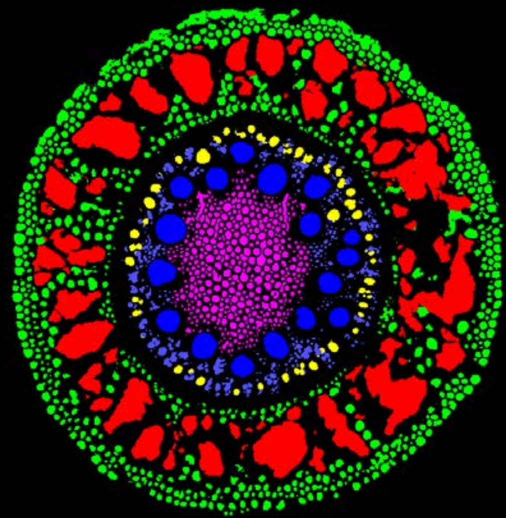
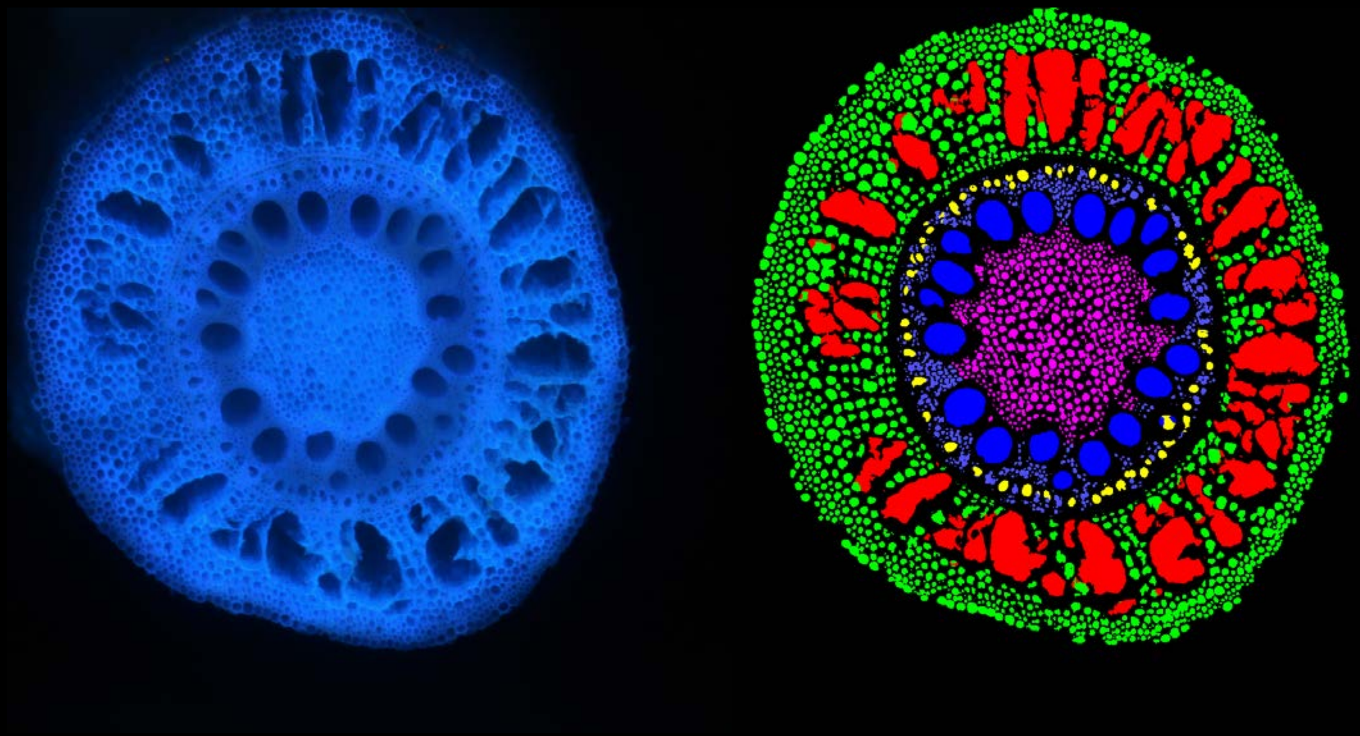
Automated analysis of maize LAT images

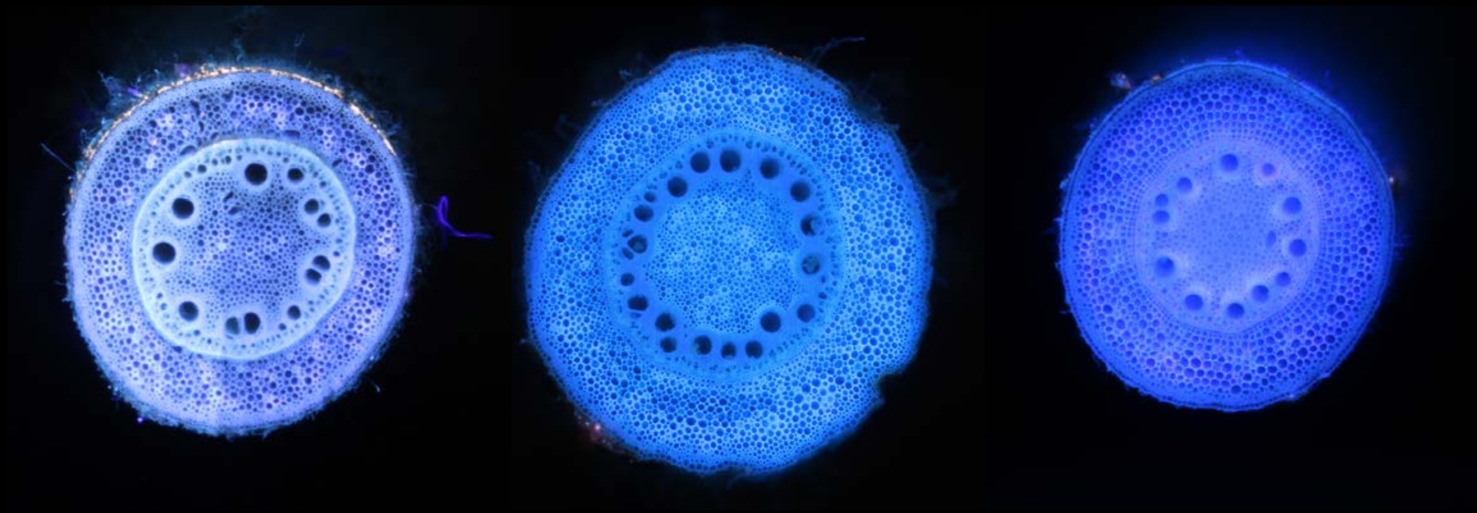


Automated analysis of maize LAT images



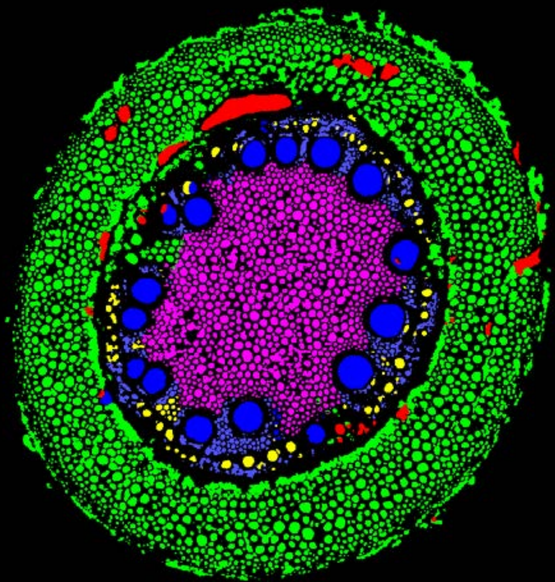
Which plant specie is this root section from?

Which plant specie is this root section from?

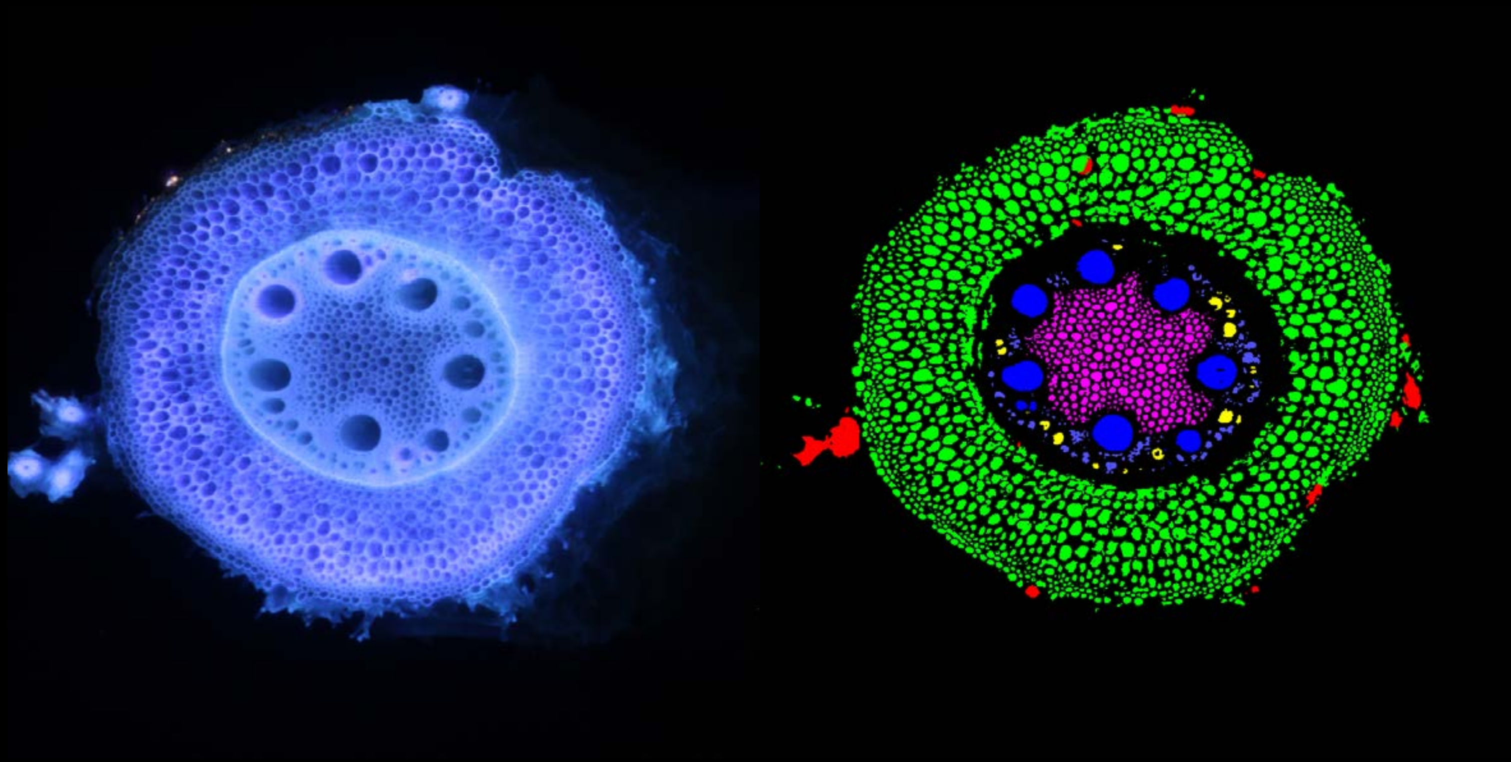


Pearl Millet | Maize | Sorghum

Transfer Learning enables classification of Pearl Millet tissues

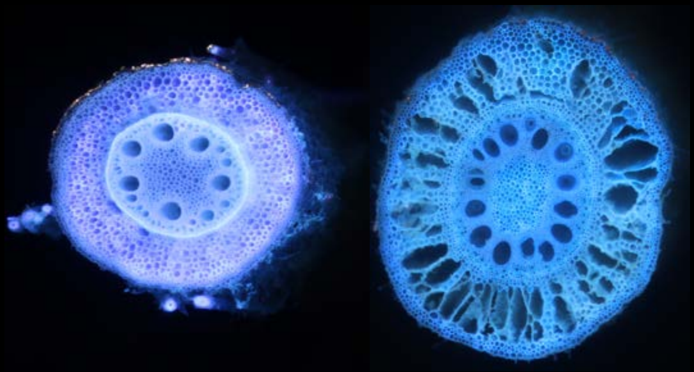


Transfer Learning enables classification of Pearl Millet tissues

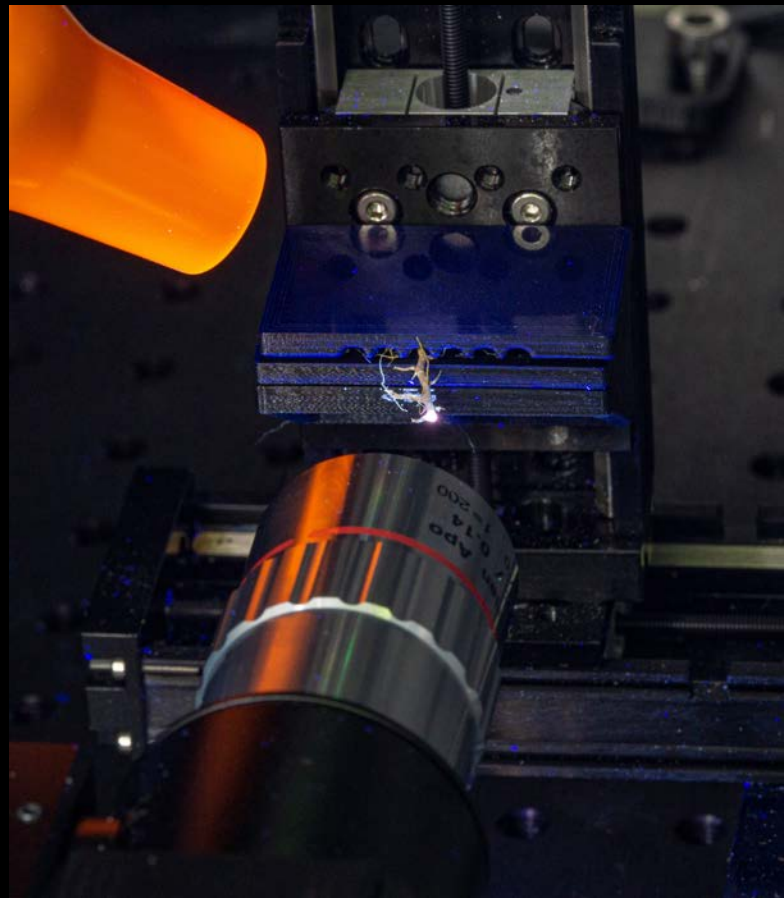


How can we improve CNN performance?

- Higher quality images
  - Better images with the new LAT2.0 will give clearer boundaries and expose smaller anatomical traits
- Transfer Learning
  - Increases the accuracy of a CNN on an unseen domain



LAT2.0



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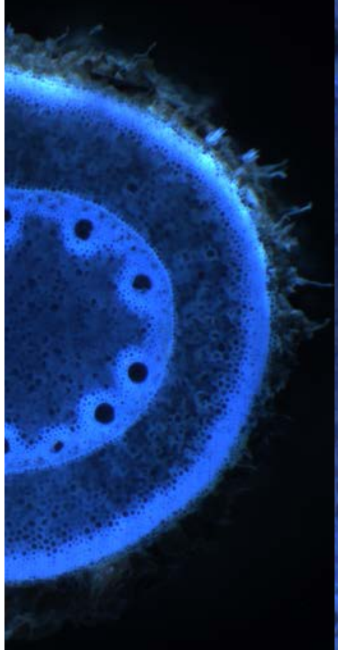
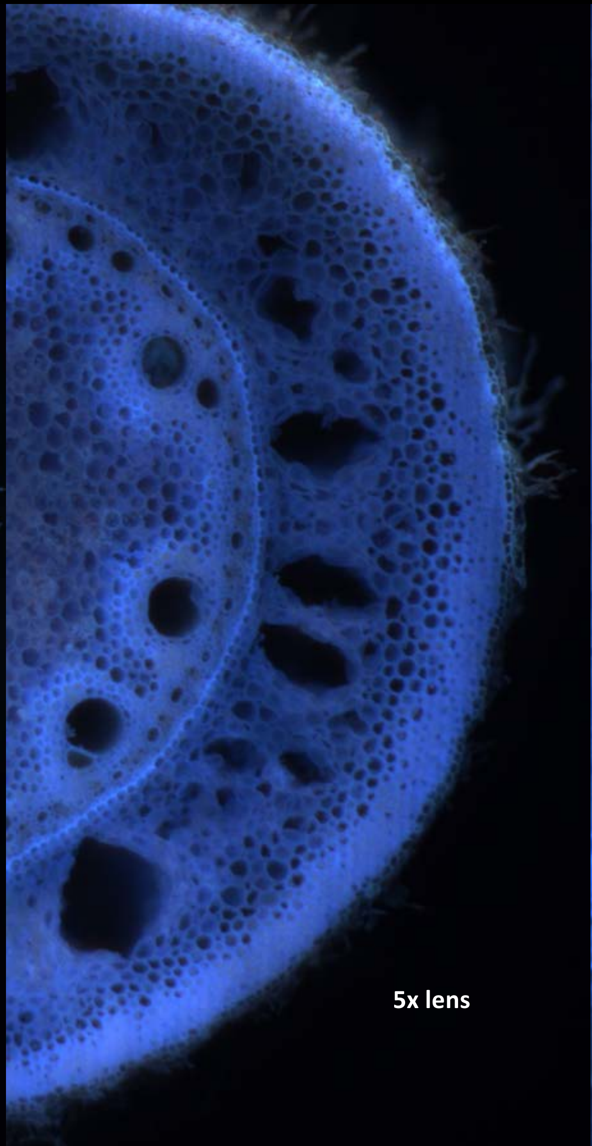
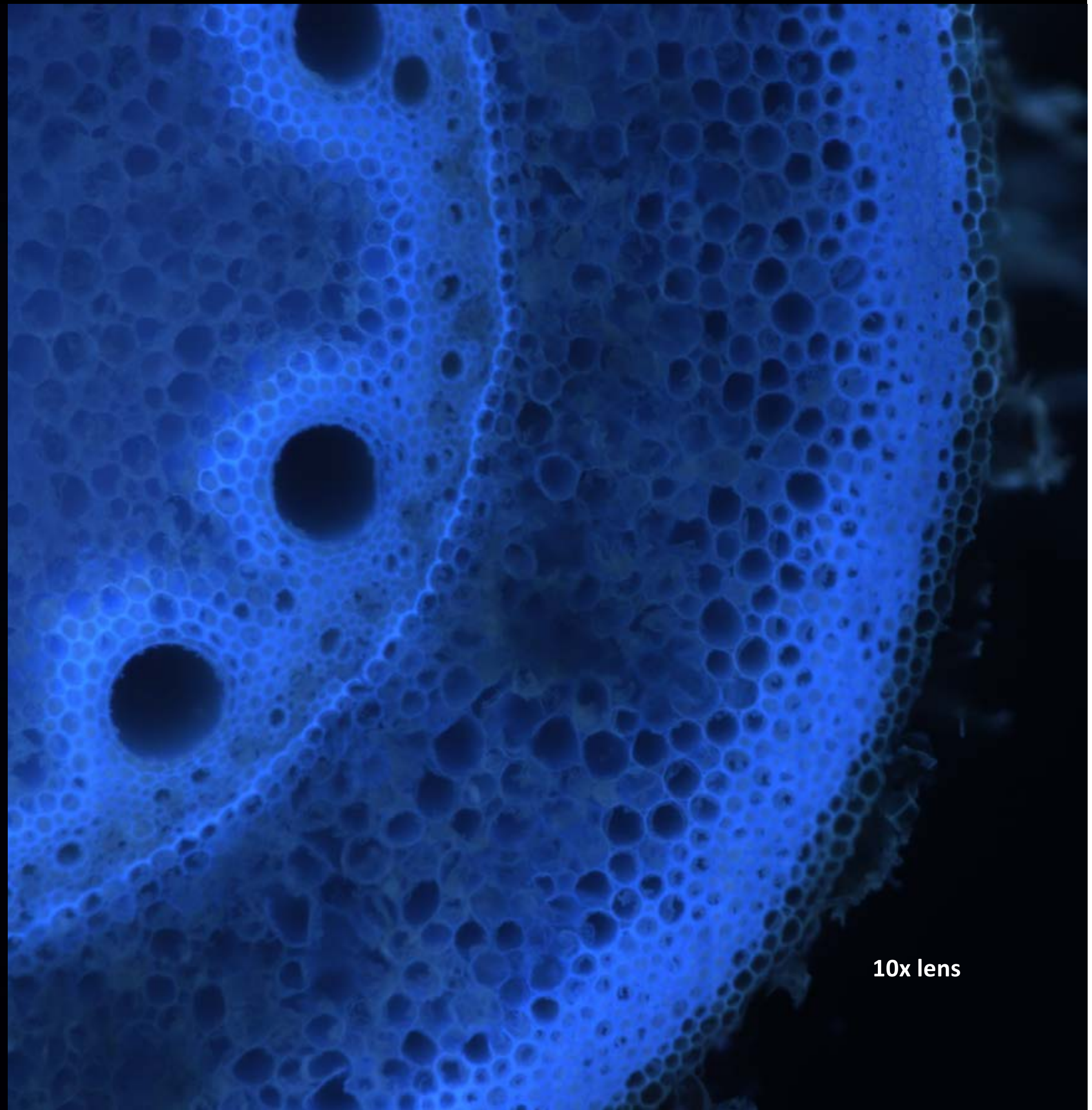


Pearl Millet adventitious root, laser illumination, 2x lens



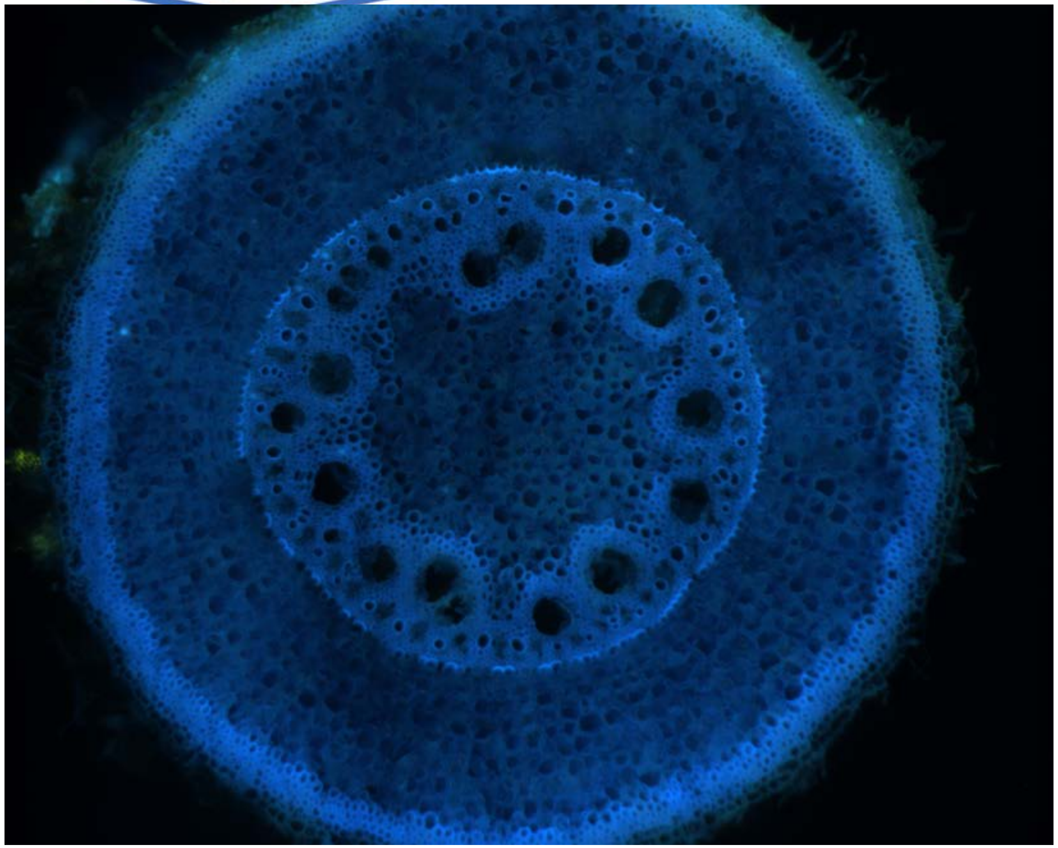
5x lens



10x lens

Phenotyping root traits in the field using shovelomics

ANATOMICS: Accelerated breeding of stress tolerant crops with novel root anatomical traits



Anatomics in pearl millet:
improving yield and drought tolerance of a major dietary staple food in the Sahel

ISRA, Nottingham, IRD and Penn State

3 year project (2019-2021)

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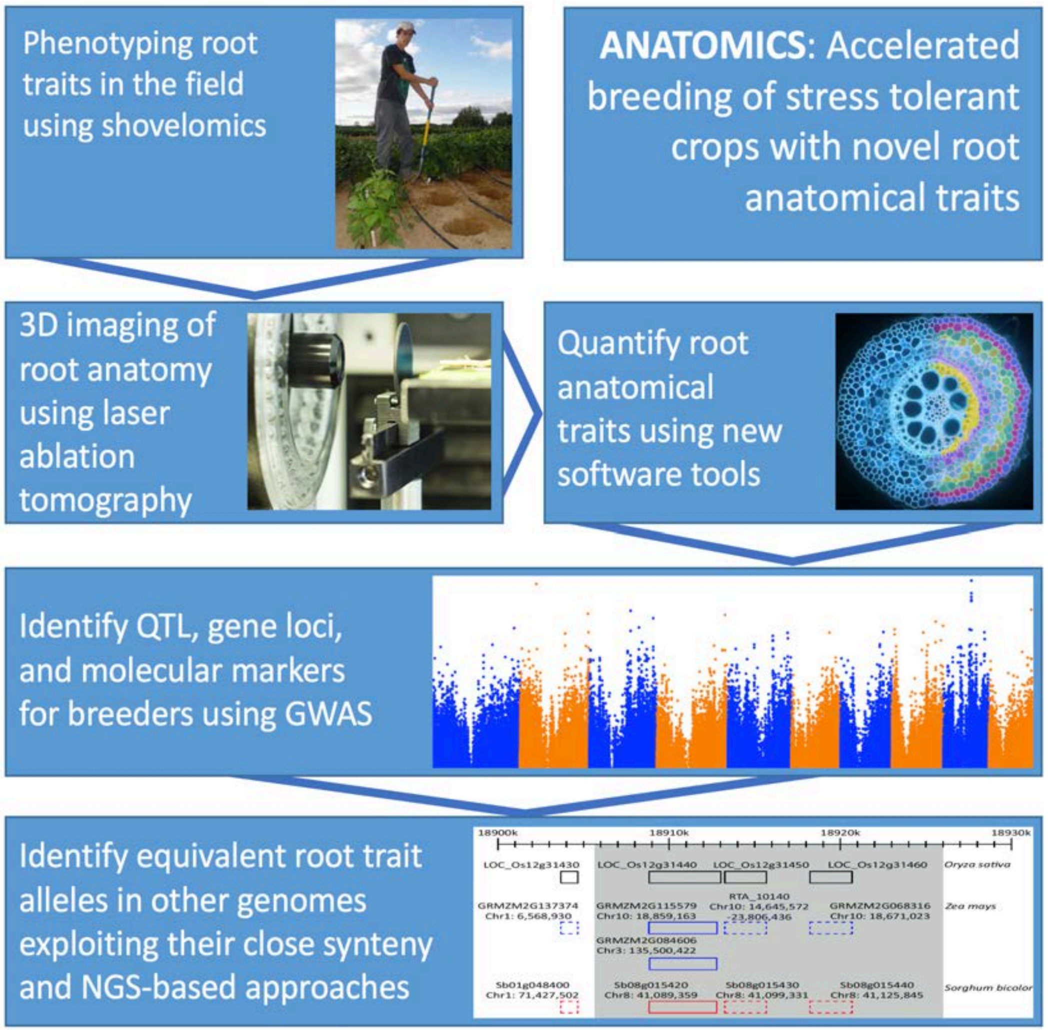

Anatomics in pearl millet:
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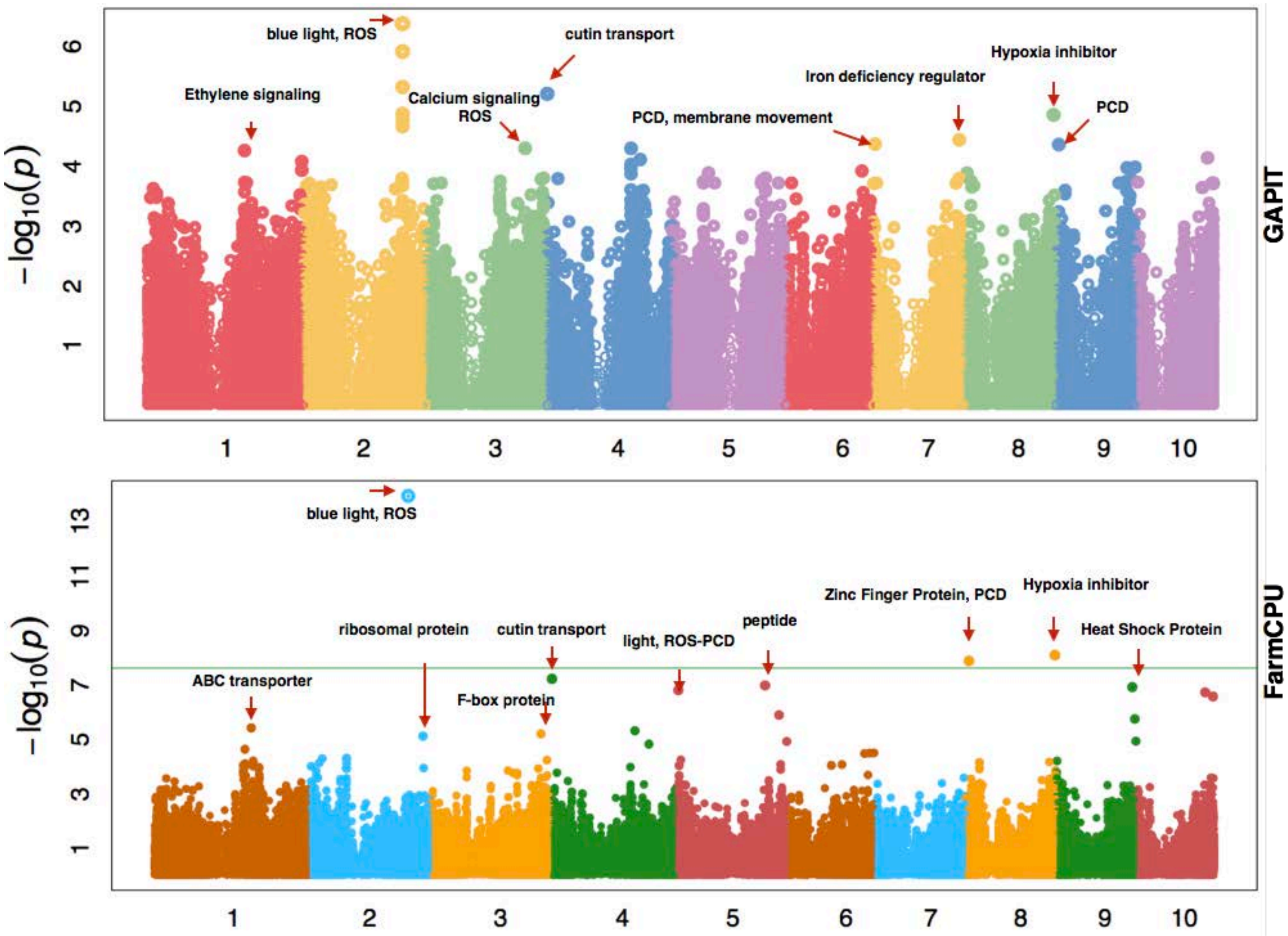
ROOTS
Soil Carbon Capture

arpa·e
U.S. DEPARTMENT OF ENERGY

GWAS Mapping of Root Cortical Aerenchyma Trait Genes

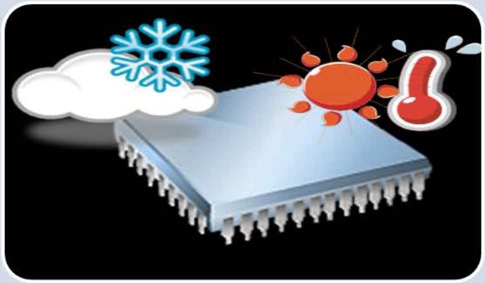


Key themes covered in today's seminar

- Root Phenotyping techniques like LAT and CT are helping *uncover the hidden half of crop performance*
- Multidisciplinary approaches are key to uncovering new insights about root growth & development
- Active engagement and uptake of approaches from other disciplines and disruptive technologies is critical
- Pan-European initiatives such as EPPN2020 and EMPHASIS are helping pool tools and resources

EMPHASIS Vision: Empowering plant science to enable sustainable plant production, agricultural industry and food security.

EMPHASIS objectives:



Develop an integrated pan-European infrastructure of instrumented facilities available to the user community

Link data acquisition to a European-level data information system and modelling

Develop, evaluate and disseminate knowledge and novel technologies providing innovative opportunities for academia & industry

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Rooting 2021

9th International Symposium on Root Development

University of Nottingham 24-28th May 2021

Invited speakers include:
Anna Amtmann (Glasgow)
Xavier Draye (UC Louvain)
Caroline Gutjahr (TUM, Munich)
Karin Ljung (SLU Umea)
Charles Melnyk (SLU Uppsala)
Bert de Rybel (VIB Ghent)

Sessions:
1. Hormones and root system architecture
2. Root morphogenesis
3. Responses to abiotic constraints
4. Nutrient and water uptake
5. Root biotic interactions
6. Grafting / re-generation
7. Root phenomics

Registration opens Jan 2021

www.rooting2020.com

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Tomato stem collected at soil surface level, 30 µm sections, combined laser illumination and brightfield

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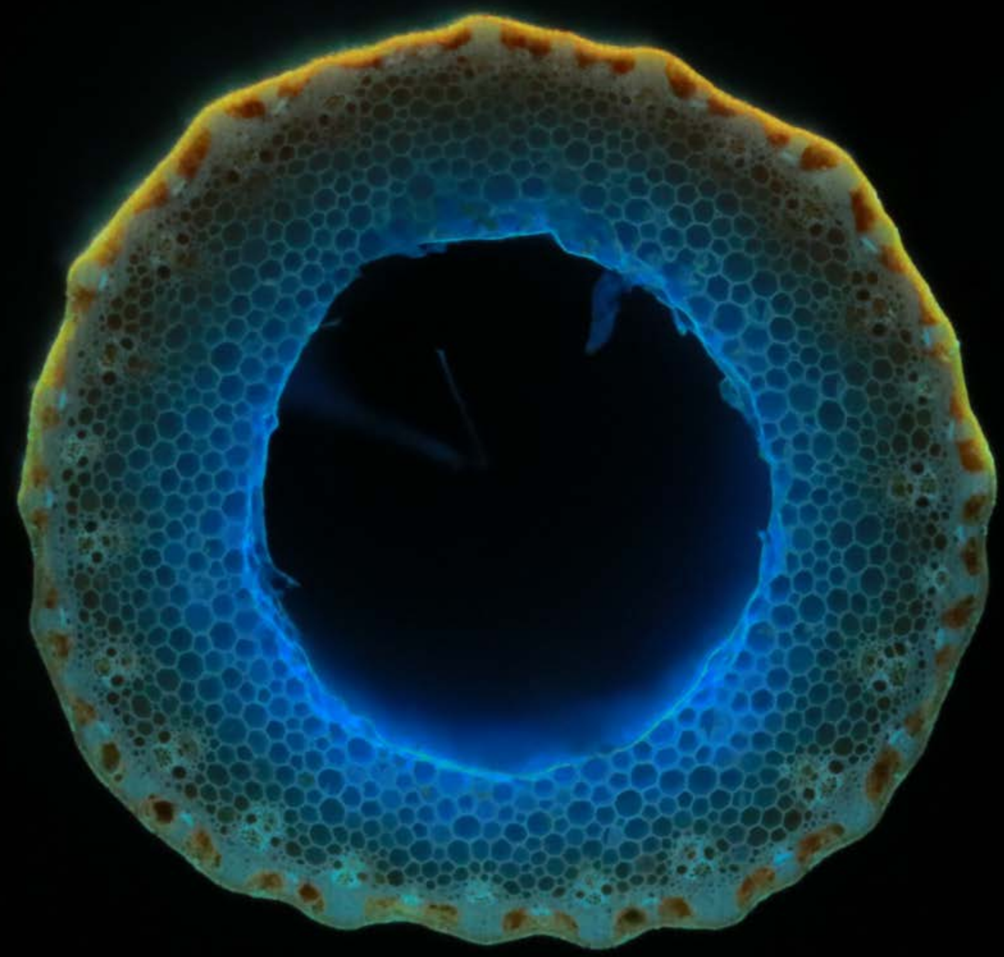

Wheat stem, single section, laser illumination

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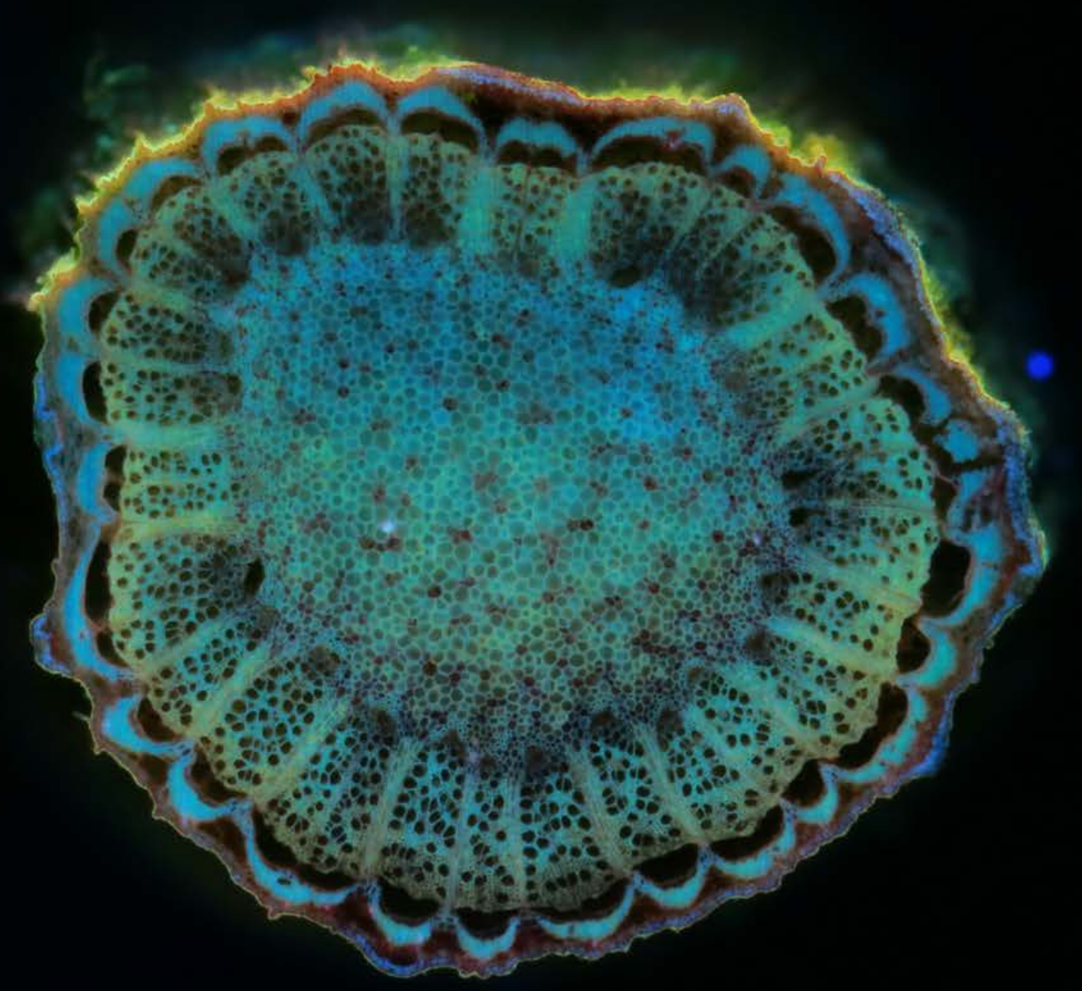

Woody branch, single section laser illumination

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Wheat ears and seed, single section, combined laser illumination and brightfield